Direct observation of the ultrafast dynamics of ionized biomolecules in aqueous solution

Muhammad Shafiq Mohd Yusof

2021

Muhammad Shafiq Mohd Yusof (2021). Direct observation of the ultrafast dynamics of ionized biomolecules in aqueous solution. Doctoral thesis, Nanyang Technological University, Singapore. <https://hdl.handle.net/10356/154805>

<https://hdl.handle.net/10356/154805>

<https://doi.org/10.32657/10356/154805>

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0).

Downloaded on 26 Apr 2023 18:49:44 SGT

**NANYANG
TECHNOLOGICAL
UNIVERSITY**

SINGAPORE

**DIRECT OBSERVATION OF THE ULTRAFAST DYNAMICS
OF IONIZED BIOMOLECULES IN AQUEOUS SOLUTION**

MUHAMMAD SHAFIQ BIN MOHD YUSOF

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

2021

DIRECT OBSERVATION OF THE ULTRAFAST DYNAMICS OF IONIZED BIOMOLECULES IN AQUEOUS SOLUTION

MUHAMMAD SHAFIQ BIN MOHD YUSOF

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

A thesis submitted to the Nanyang Technological
University in partial fulfilment of the requirement for the
degree of Doctor of Philosophy

2021

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research done by me except where otherwise stated in this thesis. The thesis work has not been submitted for a degree or professional qualification to any other university or institution. I declare that this thesis is written by myself and is free of plagiarism and of sufficient grammatical clarity to be examined. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

13th December 2021

.....

Date

NTU NTU NTU NTU NTU NTU NTU NTU
NTU NTU NTU NTU NTU NTU NTU NTU
NTU NTU NTU NTU NTU NTU NTU NTU
NTU NTU NTU NTU NTU NTU NTU NTU

.....

Muhammad Shafiq Bin Mohd Yusof

Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it of sufficient grammatical clarity to be examined. To the best of my knowledge, the thesis is free of plagiarism and the research and writing are those of the candidate's except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

13th December 2021

.....

Date

NTU NTU NTU NTU NTU NTU NTU
NTU NTU NTU NTU NTU NTU NTU
NTU NTU NTU NTU NTU NTU NTU
NTU NTU NTU NTU NTU NTU NTU

.....

Loh Zhi Heng

Authorship Attribution Statement

This thesis contains material from two papers published in the following peer-reviewed journals / from papers accepted at conferences in which I am listed as an author.

Chapter 3 is published as M. S. Bin Mohd Yusof, Y. L. Lim, and Z.-H. Loh. Ultrafast Vibrational Wave Packet Dynamics of the Aqueous Tyrosyl Radical Anion Induced by Photodetachment. *Phys. Chem. Chem. Phys.* **23**, 18525–18534 (2021). DOI: 10.1039/D1CP02975D.

The contributions of the co-authors are as follows:

- Assoc. Prof. Loh Zhi-Heng provided the initial project direction and edited the manuscript drafts.
- I prepared the manuscript drafts, and the manuscript was reviewed by Mr. Lim Yong Liang.
- Mr. Lim Yong Liang and I performed sample preparation, conducted the experiments, theoretical calculations, and analysis of the data at the School of Physical and Mathematical Sciences.
- All authors discussed the project.

Chapter 4 is published as M. S. Bin Mohd Yusof, T. Debnath, Z.-H. Loh. Observation of Intra- and Intermolecular Vibrational Coherences of the Aqueous Tryptophan Radical Induced by Photodetachment. *J. Chem. Phys.* **155**, 134306 (2021). DOI: 10.1063/5.0067335

The contributions of the co-authors are as follows:

- Assoc. Prof. Loh Zhi-Heng provided the initial project direction and edited the manuscript drafts.
- I prepared the manuscript drafts, and the manuscript was reviewed by Dr. Tushar Debnath.
- Dr. Tushar Debnath aided with the initial setting up of the experimental pump-probe setup.

- Shafiq*

• • • • •

.....

Muhammad Shafiq Bin Mohd Yusof

Abstract

In biological systems, biomolecules such as amino acids play important roles as building blocks of proteins and precursors to several neurotransmitters. The amino acids and their radicals are involved in several biological processes, and they are therefore of interest. The development of ultrashort pulses allows scientists to study and elucidate many of these ultrafast processes. In this thesis, we present the ultrafast dynamics of photoionized or photodetached biomolecules in aqueous solution by utilizing few-femtosecond laser pulses in transient absorption pump-probe spectroscopy. Our experimental results are complemented with density functional theory (DFT) calculations.

Chapter 1 will introduce the ultrafast processes that accompany the ejection of electrons from biomolecules in aqueous solution. The photoionization or photodetachment of neutral or anionic biomolecules, respectively, will reveal ultrafast dynamics such as structural rearrangement due to the difference in the equilibrium molecules geometries between the unionized and ionized species. To investigate these ultrafast dynamics, we utilize few-femtosecond transient absorption spectroscopy to probe the radical species formed. Our motivations for studying the ultrafast dynamics of ionized biomolecules in aqueous solution will be discussed.

In Chapter 2 of this thesis, we present the experimental procedure employed in our studies. We describe the generation of few-cycle laser pulses spanning the visible to near-infrared (NIR), their temporal compression by a combination of chirped mirrors and pulse shaping, the upconversion of the visible-NIR pulses into the ultraviolet, their temporal characterization, and their application to optical pump-probe spectroscopy. In addition, we describe the processing of our raw data to obtain the time-resolved differential absorption spectrum $\Delta A(\lambda, t)$.

Chapter 3 presents the ultrafast vibrational wave packet dynamics of photodetached aqueous tyrosinate dianion (Tyr^{2-}). Photodetachment of the tyrosinate dianion yields the tyrosyl radical anion ($\text{Tyr}^{\bullet-}$), an important intermediate in biological redox reactions, although studies of its ultrafast dynamics are limited. Here, we utilize femtosecond optical pump-probe spectroscopy to investigate the ultrafast structural reorganization dynamics that follow the photodetachment of the tyrosinate dianion in aqueous solution. Photodetachment of the tyrosinate dianion leads to vibrational wave packet motion along seven vibrational modes that are coupled to the photodetachment process, as evident from the large displacement vectors being localized on the phenoxide sidechain, from which the electron is photodetached. This result is comparable to the recent study on the photodetachment of phenoxide anion in aqueous system. Moreover, DFT calculations reveal that the photodetachment is accompanied by the elongation of the hydrogen bond between the radical and the surrounding water molecules, evident from the equilibrium geometry of the microhydrated Tyr^{2-} dianion and $\text{Tyr}^{\bullet-}$ anion.

Chapter 4 presents the ultrafast vibrational wave packet dynamics of the photodetached aqueous tryptophan anion (Trp^-). Photodetachment of the tryptophan anion yields the tryptophan radical ($\text{Trp}^{\bullet}$), which is known to play an important role in numerous redox reactions in biology, although studies of its ultrafast dynamics are limited. Here, we employ femtosecond optical pump-probe spectroscopy, probing in both the ultraviolet and the visible, to elucidate the ultrafast structural rearrangement dynamics that accompany the photodetachment of the aqueous tryptophan anion. Photodetachment of the tryptophan anion reveals eight unique vibrational modes that are coupled to the photodetachment process. This result is supported by DFT calculations, which reveal large displacement vectors localized on the indole sidechain, from which the electron is photodetached. Aside from intramolecular vibrational modes, our results also point to the involvement of intermolecular modes that drive solvent rearrangement about the indole sidechain.

Chapter 5 presents the ultrafast dynamics of the photodetached phenylalaninate anion (Phe^-) in aqueous solution. Photodetachment of the phenylalaninate anion yields the phenylalanine radical ($\text{Phe}^\bullet$), which has been proposed to participate in biological processes such as long-range electron transport. However, not much about the ultrafast dynamics of $\text{Phe}^\bullet$ radical has been explored. Moreover, the absorption spectrum of $\text{Phe}^\bullet$ radical has not been reported thus far. Here, we employ femtosecond optical pump-probe spectroscopy to elucidate the spectroscopic observation and ultrafast coherent dynamics of the aqueous $\text{Phe}^\bullet$ radical. The differential absorption spectrum of photodetached aqueous phenylalaninate anion reveals an absorption band peaked at 308 nm, assigned to the $\text{Phe}^\bullet$ radical. In addition, coherent vibrational motion is launched in the $\text{Phe}^\bullet$ radical, giving rise to pronounced modulations of the differential absorption signal as a function of pump-probe time delay. Fourier transform analysis of the vibrational wave packet dynamics reveals the contribution from nine vibrational modes, assigned with the aid of DFT calculations. The first overtone of the vibrational mode with the largest contribution to the differential absorption modulation is also detected; the dephasing time is more than one order of magnitude shorter for the overtone frequency (0.13 ± 0.07 ps) than the fundamental frequency (5.5 ± 0.7 ps). The vibrational modes observed are coupled to the photodetachment process and are observed even though some of them involve only sub-0.1-pm changes in bond lengths according to DFT calculations. Finally, we employ *ab initio* simulations to explore the decarboxylation pathway previously suggested for $\text{Phe}^\bullet$ radical. Our simulations suggest that decarboxylation of an isolated $\text{Phe}^\bullet$ radical proceeds *via* a direct dissociation pathway. However, the inclusion of explicit water molecules of hydration leads to the emergence of a dissociation barrier, thus suppressing ultrafast decarboxylation on the picosecond timescale, consistent with the experimental results. Our study yields the hitherto unobserved spectroscopic signature of the aqueous $\text{Phe}^\bullet$ radical, demonstrates the feasibility of employing time-domain vibrational wave packet spectroscopy to identify the vibrational modes

that drive structural reorganization following photoionization or photodetachment of a biomolecule in aqueous solution, and reveals the role of hydration structure on governing the extent of molecular fragmentation in aqueous solution.

Chapter 6 presents the ultrafast proton transfer dynamics of the phenol radical cation ($\text{PhOH}\bullet^+$) in aqueous solution. The photoionization of phenol (PhOH), the sidechain of tyrosine that is known to participate in proton-coupled electron transfer reactions, yields the $\text{PhOH}\bullet^+$ radical cation. However, detecting the $\text{PhOH}\bullet^+$ radical cation has been challenging as it undergoes rapid proton transfer with one of the surrounding water molecules to form the phenoxyl radical ($\text{PhO}\bullet$), a process that has been suggested to occur within ~ 0.2 ps. Here, we aim to elucidate the lifetime of the $\text{PhOH}\bullet^+$ radical cation by utilizing the visible-NIR pump – near-UV probe transient absorption spectroscopy. The photoionization of aqueous phenol reveals wave packet dynamics involving two vibrational modes that are unique to the phenolic O–H bond, the O–H wag and bend, associated with the $\text{PhOH}\bullet^+$ radical cation. Time-domain analysis affords a short dephasing time of 0.18 ± 0.02 ps and 0.16 ± 0.02 ps for the $\text{PhOH}\bullet^+$ radical cation O–H wag and bend respectively, suggestive of ultrafast proton transfer occurring on the ~ 0.2 ps timescale. *Ab initio* calculations of the reduced potential energy surface reveal that the proton transfer process proceeds *via* an initial contraction of the intermolecular oxygen-oxygen bond (i.e. $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$) distance between the $\text{PhOH}\bullet^+$ radical cation and the neighboring proton-accepting water molecule. This $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ contraction leads to a barrierless proton transfer process, hence explaining its ultrafast nature. Wave packet propagation simulations reveal that the lifetime of $\text{PhOH}\bullet^+$ radical cation is ~ 0.2 ps to form the proton-transferred product, in good agreement with the estimated lifetime of the $\text{PhOH}\bullet^+$ radical cation obtained from the time-domain analysis. These results suggest that photoionization can be harnessed to produce photoacids that undergo ultrafast proton transfer.

Chapter 7 summarizes the work presented in this thesis, identifies some of the limitations of these studies, and suggests some future work that can further expand on the current work.

Acknowledgements

As I was writing this section of my thesis, it is coming to the end of my four-year Ph.D. journey in NTU. I reminisce all the people, events, the ups and downs and lessons I have learnt from this long and arduous journey.

I would like to express my deepest appreciation to my Ph.D. supervisor – Assoc. Prof. Loh Zhi Heng – who gave me the chance to work in his research group twice during my undergraduate years, and eventually accepting me as his Ph.D. student. He is the best advisor that anyone could ask for. Since the first day I joined the research group, he had helped me in bridging my knowledge in physics and guided through a relatively new (to me) field of Chemistry that had not been taught in school. He was always patient with me, and I have learnt an abundance of knowledge from him. The main memories about my supervisor are the late night in the lab, either trying to figure out why the experiments are not working out as intended or chatting about life, and my first ever trip to the USA for a research attachment at Linac Coherent Light Source (LCLS) at Stanford University. That was my first time travelling to USA, and I will always be grateful for the opportunity to experience how a fully functioning free-electron laser lab worked (when you have a large grant) and meeting prominent experts in the field of ultrafast spectroscopy. This field was daunting at first, but I have learnt to grow to love and appreciate the beauty of ultrafast spectroscopy (though there are more to explore).

It will be incomplete without mentioning the people that I had worked alongside in the research group and have given valuable advice throughout my time in graduate school. First, I would like to thank my mentor during my honor's project, Mr. Low Pei Jiang, who introduced me to the fundamentals of laser and workings of a laser lab. He was really knowledgeable in the field, and really trained me to work efficiently in the laser lab. Secondly, I must also thank

Dr. Tushar Debnath, who took on the role of my mentor after Mr. Low Pei Jiang left to pursue his Ph.D. studies. Dr. Debnath guided me in the first year of my Ph.D. journey through the tryptophan and tyrosine studies. We worked alongside with the phenoxide study too. Many thanks to the former and current members of the research group – Dr. Wei Zhengrong, Dr. Ronald Ulbricht, Dr. Xu Ce and Dr. Woo Kyung Chul – for all their valuable guidance and advice, and the wonderful group sashimi feast we had every year. Special mention to my Thesis Advisory Committee (TAC) members, Assoc. Prof. Tan Howe Siang and Prof. Ricky Ang Lay Kee from Singapore University of Technology and Design (SUTD), for their valuable advice.

Next, I want to give thanks to all the undergraduates, Master students and collaborators that had helped with the research work. Thanks to Mr. Lim Yong Liang for his contributions to the tyrosine work that was performed before my Qualifying Examination (QE). Thanks to Miss Siow Jing Xuan, Miss Yang Ningchen and Miss Chan Wei Xin for their contributions to the phenylalanine work that worked relentlessly to figure out whether phenylalanine radical dissociates. Thanks to Miss Lowe Bethany Elizabeth for her contributions to the phenol work, that gave us so much agony when performing the analysis and calculations. I would like to thank Dr. Song Hongwei and Prof. Yang Minghui from Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, for their wonderful contributions to the phenol study by performing the theoretical calculations. Thanks to other students – Miss Ng Zi Xuan, Mr. Perrett Samuel Fridolin, Mr. Teo Hong Kang – for their unwavering support and hard work to contribute to the research group.

My Ph.D. journey will not be sane without the friends I had during my Ph.D. journey, most prominently Mr. Teo Hong Xiang Ronald and Mr. Seah Wee Kiong Jeffery. They are the people who I hung out since the first day of Ph.D. and provided laughter and joy to the mundane life of Ph.D. We thoroughly enjoyed each other's company as we went for several trips together, most notably to Japan in 2019. On the day I am writing this acknowledgement, we

just had a discussion to travel together again once the pandemic has eased. I would also like to express my thanks to all my secondary school, junior college, national service and university friends for always been supportive of my Ph.D. journey, though it had sometimes been difficult to meet up due to the hectic schedule.

Most importantly, none of this could have happened without my family. My mother and sister had always been supportive of my choices in my life. Ever since my father passed away when I was young, my mother had to take up the role as the breadwinner of the family, to support me and my siblings. We had experienced some ups and downs, but we managed it to ride out of the storm. This thesis stands as a testament to your unconditional love and encouragement. Lastly, to my late father who did not have the opportunity to see me grow up to the person I become, I hope you are watching over me as I move on to the next chapter of my life and be proud of what I have achieved.

Table of Contents

ABSTRACT.....	1
ACKNOWLEDGEMENTS	6
TABLE OF CONTENTS	9
ABBREVIATIONS LIST.....	13
CHAPTER 1 INTRODUCTION.....	15
1.1. Photoionization of Biomolecules.....	15
1.1.1. Photoionization of Biomolecules in Aqueous Solution	15
1.1.2. Ultrafast Structural Rearrangement of Ionized Biomolecules	18
1.2. Ultrafast Optical Spectroscopy	20
1.2.1. Transient Absorption Spectroscopy	21
1.3. Objective	22
1.4. Organization	23
CHAPTER 2 FEMTOSECOND TIME-RESOLVED TRANSIENT ABSORPTION SPECTROSCOPY	36
2.1. Few-Cycle Pulse Generation	37
2.2. Generation and Characterization of Ultrashort Laser Pulses	38
2.3. Sample Preparation	41
2.4. Differential Absorption	42
2.4.1. Visible Probe.....	43
2.4.2. Near-UV Probe	45
2.4.3. UV Probe	48
2.4.4. Differential Absorption Measurement	50

**CHAPTER 3 ULTRAFAST DYNAMICS OF PHOTODETACHED
TYROSINATE DIANION IN AQUEOUS SOLUTION.....54**

3.1. Introduction.....	54
3.2. Materials and Methods.....	57
3.2.1. Theoretical Calculations	57
3.2.2. Experimental Method.....	58
3.3. Results	62
3.3.1. Theoretical Calculations	62
3.3.2. Transient Absorption of Photodetached Aqueous Tyrosinate Dianion	64
3.3.3. Vibrational Wave Packet Dynamics of Tyrosyl Radical Anion	67
3.4. Discussion.....	73
3.4.1. Global Fit Analysis	73
3.4.2. Vibrational Wave Packet Dynamics of Tyrosyl Radical Anion	77
3.5. Conclusion	83

**CHAPTER 4 ULTRAFAST DYNAMICS OF PHOTODETACHED
TRYPTOPHAN ANION IN AQUEOUS SOLUTION.....93**

4.1. Introduction.....	93
4.2. Materials and Methods.....	97
4.2.1. Theoretical Calculations	97
4.2.2. Experimental Method.....	98
4.3. Results	103
4.3.1. Theoretical Calculations	103
4.3.2. Transient Absorption of Photodetached Aqueous Tryptophan Anion.....	105
4.3.3. Vibrational Wave Packet Dynamics of Tryptophan Radical	110
4.4. Discussion.....	116
4.5. Conclusion	121

**CHAPTER 5 ULTRAFAST DYNAMICS OF PHOTODETACHED
PHENYLALANINATE ANION IN AQUEOUS SOLUTION137**

5.1. Introduction.....	137
5.2. Materials and Methods.....	141
5.2.1. Theoretical Calculations	141
5.2.2. Experimental Method.....	143
5.3. Results	147
5.3.1. Transient Absorption of Photodetached Aqueous Phenylalaninate Anion.....	147
5.3.2. Vibrational Wave Packet Dynamics of Phenylalanine Radical	151
5.3.3. Theoretical Calculations	155
5.4. Discussion.....	160
5.4.1. Transient Absorption of Photodetached Aqueous Phenylalaninate Anion.....	160
5.4.2. Vibrational Wave Packet Dynamics of Phenylalanine Radical	163
5.4.3. Theoretical Calculations	168
5.5. Conclusion	171
 CHAPTER 6 ULTRAFAST PROTON TRANSFER OF PHENOL RADICAL	
CATION IN AQUEOUS SOLUTION	182
6.1. Introduction.....	182
6.2. Materials and Methods.....	185
6.2.1. Theoretical Calculations	185
6.2.2. Experimental Method.....	188
6.3. Results	192
6.3.1. Transient Absorption of Ionized Aqueous Phenol.....	192
6.3.2. Vibrational Wave Packet Dynamics of Ionized Phenol.....	196
6.4. Discussion.....	201
6.4.1. Transient Absorption of Ionized Aqueous Phenol.....	201
6.4.2. Vibrational Wave Packet Dynamics of Ionized Phenol.....	208
6.4.3. Theoretical Calculations	211
6.5. Conclusion	218
 CHAPTER 7 SUMMARY AND FUTURE WORK.....	234

7.1. Summary.....	234
7.2. Future Work.....	237
APPENDIX.....	243
Appendix A.....	243
Appendix B	246
Appendix C	249
Appendix D.....	256

Abbreviations List

B3	Becke
BBO	Beta-Barium Borate
CASPT2	Complete Active Space Perturbation Theory
CPA	Chirped Pulse Amplification
DFG	Difference Frequency Generation
DFT	Density Functional Theory
DNA	Deoxyribonucleic Acid
DOPA	3,4-dihydroxyphenylalanine
ESIPT	Excited-State Intramolecular Proton Transfer
ESPT	Excited-State Proton Transfer
ESPTS	Excited-State Proton Transfer to Solvent
FFT	Fast-Fourier Transform
FSRS	Femtosecond Stimulated Raman Spectroscopy
FTIR	Fourier-Transform Infrared
FWHM	Full-Width Half-Maximum
GFP	Green Fluorescent Protein
GVD	Group Velocity Dispersion
HOMO	Highest Occupied Molecular Orbital
ISRS	Impulsive Stimulated Raman Scattering
LYP	Lee, Yang and Parr
MICS	Multiple Independent Comb Shaping
MIIPS	Multiphoton Intrapulse Interference Phase Scan

MM	Molecular Mechanics
MRCI	Multireference Configuration Interaction
NHE	Normal Hydrogen Electrode
PCET	Proton-Coupled Electron Transfer
PCM	Polarizable Continuum Model
PEC	Potential Energy Curve
PES	Potential Energy Surface
Phe	Phenylalanine
PT	Proton Transfer
QCy9	Quinone Cyanine 9
QM	Quantum Mechanics
RMSE	Root Mean Square Error
SHE	Standard Hydrogen Electrode
SHG	Second Harmonic Generation
SLM	Spatial Light Modulator
SOMO	Singly Occupied Molecular Orbital
TA	Transient Absorption
TD-DFT	Time-dependent Density Functional Theory
TIPS	Trapped Ion Photodissociation Spectroscopy
Trp	Tryptophan
Tyr	Tyrosine
UV	Ultraviolet
UVR	Ultraviolet Resonance Raman
VIE	Vertical Ionization Energy

Chapter 1

Introduction

1.1. Photoionization of Biomolecules

The photoionization of biomolecules in an aqueous environment is a universal phenomenon that accompanies the interaction of high-energy radiation with biological matter. Many of these chemical reactions involve ions, electrons and / or radicals, which have garnered many interests in this field of science. This forms the basis of many radiation chemistry and radiation biology.^{1,2}

1.1.1. Photoionization of Biomolecules in Aqueous Solution

Biomolecules play a vital role in many photochemical reactions in biological materials, such as proteins and deoxyribonucleic acid (DNA). Both DNA and proteins are important, but in our study, we focus on amino acids as they are commonly found in proteins and polypeptides. The photochemical reactions of these amino acids and their residues in proteins play an important role in nature.³⁻⁸ For instance, amino acids such as tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) (Fig. 1.1) are chromophores due to the present of aromatic groups that are able absorb light at a certain frequency and they are involved in many chemical reactions that are essential to the function of biological systems, which include biological events such as cell apoptosis. In many of these chemical reactions, the radicals are key intermediate of several

Figure 1.1. Structure of **(a)** tryptophan, **(b)** tyrosine and **(c)** phenylalanine. The carbon, hydrogen, oxygen and nitrogen atoms are in gray, white, red and blue, respectively.

biological processes.⁹⁻¹³ For this reason, these biomolecules and their reactive intermediate species have been of interest.¹⁴⁻²⁰

The radicals of these biomolecules are of key interest as the vertical ionization potential of these biomolecules are comparably lower than the vertical ionization potential of liquid water. For instance, the vertical ionization potential of Trp, Tyr and Phe in aqueous solution are 7.3 eV, 7.8 eV and 8.7 eV respectively.²¹ The relatively low vertical ionization potential is attributed to the presence of an aromatic ring with weakly-bound π -electrons, hence, the loss of an electron is more likely to occur from the aromatic ring. On the other hand, the vertical

ionization potential of liquid water is 11.2 eV,²²⁻²⁵ significantly higher than the vertical ionization potential of these biomolecules. Hence, selective photoionization or photodetachment of these biomolecules in aqueous solution is possible *via* strong-field multiphoton ionization or photodetachment.

In addition, the photoionization or photodetachment of these biomolecules would yield the corresponding hydrated electron. The ground-state hydrated electron absorption curve is displayed in Fig. 1.2. The ground-state hydrated electron has an absorption band spanning from ~300 nm to 1000 nm, with maximum absorption at 715 nm with a molar extinction coefficient, $\epsilon_{715\text{nm}}$, of 19687 M⁻¹ cm⁻¹.^{26,27} The dynamics of the hydrated electron had been extensively studied previously, in which the electron is injected into the conduction band of liquid water,^{28,29} followed by vibrational cooling.³⁰⁻³⁶

Furthermore, the absorption and dynamics of these radical species have also been studied previously. The tryptophan radical has several absorption bands: 580 nm ($\epsilon_{580\text{nm}} = 3000 \text{ M}^{-1} \text{ cm}^{-1}$), 510 nm ($\epsilon_{510\text{nm}} = 2300 \text{ M}^{-1} \text{ cm}^{-1}$) and 325 nm ($\epsilon_{325\text{nm}} = 3670 \text{ M}^{-1} \text{ cm}^{-1}$).³⁷⁻⁴⁶ tyrosyl radical has a main absorption band at 410 nm ($\epsilon_{410\text{nm}} = 2750 \text{ M}^{-1} \text{ cm}^{-1}$) and a vibronic band at 390 nm ($\epsilon_{390\text{nm}} = 1700 \text{ M}^{-1} \text{ cm}^{-1}$),⁴⁷⁻⁵⁰ and the radical produced from the photoionization of phenylalanine has maximum absorption bands at 307 nm ($\epsilon_{307\text{nm}} = 4700 \text{ M}^{-1} \text{ cm}^{-1}$) and 318 nm ($\epsilon_{318\text{nm}} = 9000 \text{ M}^{-1} \text{ cm}^{-1}$).⁵¹⁻⁵⁵ For the photoionization of phenylalanine, note that the two absorption bands observed have been suggested to be associated with the benzyl radical that is formed from the photoionization-photoinduced dissociation of the phenylalanine in aqueous solution,^{53,54} and the absorption spectrum of the phenylalanine radical is hitherto unobserved. However, not many studies have been performed to study the ultrafast dynamics of these radical species in aqueous system. The ultrafast dynamics of these radical species will be discussed further in their respective chapters.

Figure 1.2. The molar absorption coefficient of the ground-state hydrated electron (ϵ_e) as a function of wavelength, with a maximum absorption at 715 nm ($\epsilon_{715\text{nm}} = 19687 \text{ M}^{-1} \text{ cm}^{-1}$).^{26,27}

1.1.2. Ultrafast Structural Rearrangement of Ionized Biomolecules

The photoionization or photodetachment of these biomolecules leads to the ultrafast structural rearrangement due to the difference in the equilibrium geometries between the unionized and ionized species. This difference in equilibrium geometries results in a non-zero displacement in the potential energy curve of the ionized species with respect to the unionized species. The potential energy curve of the unionized, ionized and ionized excited state is illustrated in Fig. 1.3. For a particular vibrational mode, the ionizing pump will ionize the biomolecule, which launches the vibrational wave packet, which is defined as a coherent superposition of stationary states, vertically into the potential energy curve of the ionized species, which results in certain vibrational eigenstates to be populated *via* the Franck-Condon principle. The Franck-Condon principle states that transitions between electronic states correspond to vertical lines on an

Figure 1.3. Vibrational wave packet generation by ionization of a biomolecule. The strong-field ionization laser pulse (red arrow) launches the vibrational wave packet into the ionized species potential energy curve. The ionized species is subsequently probed (blue, green and orange arrows) to the excited state. Wave packet motion involves normal modes Q_i , whose minima are displaced relative to the unionized species.

potential energy diagram. The basis of this principle is that electronic transitions occur on a timescale that is faster than the vibrational period of the molecule. The coherent superposition of vibrational eigenstates results in the wave packet to oscillate back and forth within the potential energy curve of the ionized species during the course of the vibration. This structural rearrangement would give rise to vibrational wave packet motion along normal modes, which reveal themselves as energy and amplitude modulation in the differential absorption, $\Delta A(\omega, t)$, spectrum as the ionized species is probed. Fourier-transformation of these modulations give rise to a set of vibrational frequencies, and these frequencies belong to the modes that are

coupled to the photoionization or photodetachment process. This would only be possible with ultrashort laser pulses with very short time resolution. This would provide further insights into the fundamental chemistry of such radicals.⁵⁶⁻⁵⁸

Recent work on the ultrafast structural rearrangement of photodetached phenoxide ion in aqueous solution reveals 12 different vibrational frequencies observed associated with the phenoxyl radical, which are coupled to the photodetachment process, compared to the gas-phase measurement, which reveals only one Franck-Condon-active vibrational mode.⁵⁹ *Ab initio* calculations reveal that the hydrogen bonding interaction between the phenoxyl radical and the surrounding water molecules in an aqueous system plays an important role in resulting in a larger displacement between the potential energy curve between the phenoxide ion and phenoxyl radical as compared to the gas phase. Thus, it is concluded that the solvent also plays a pivotal role in the ultrafast structural rearrangement process.⁶⁰

Such studies open the possibility of utilizing these ultrashort laser pulses to observe these high frequency vibrational modes, which usually occur at timescale on ~ 10 to 100 fs.⁶¹ Limited studies have been performed due to the lack of time resolution required to observe these vibrational modes that are coupled to the photoionization or photodetachment process and the large photon energies that are required to drive single-photon photoionization or photodetachment.

1.2. Ultrafast Optical Spectroscopy

Ultrafast optical spectroscopy utilizes an array of ultrashort light pulses, with femtosecond duration, to study the photoinduced dynamics in atoms, molecules, nanomaterials, and solids. This field in science has experienced tremendous growth. It began from a simple experiment involving high-speed photography to capture the different phases of the motion of a galloping

horse, performed by Eadweard Muybridge in 1878 with a time scale of only millisecond (10^{-3} s), to the development of mode-locked laser⁶²⁻⁶⁴ and chirp pulse amplification (CPA) techniques^{65,66} to generate femtosecond (10^{-15} s) laser pulses and more intricate spectroscopic methods to study these fast photoinduced dynamics. Such high-time resolution laser pulses enable us to observe ultrafast dynamics such as vibrational and rotational motions or understanding the kinetics between two reacting species.

1.2.1. Transient Absorption Spectroscopy

One of the most commonly used ultrafast optical spectroscopy methods is transient absorption (TA) spectroscopy, which involves a pump-probe experiment. A general schematic diagram of a pump-probe experiment used in TA spectroscopy is shown in Fig. 1.4. Typically, a pump-probe experiment requires at least two ultrashort light pulses that are incident on a sample in which they are spatially overlapped. The first one, which is the pump, perturbs the sample, either by exciting the sample to generate an excited state or ionizing the sample to generate the radical species. The second one, which is usually a weaker broadband probe, is delayed with respect to the pump, t , and measures the relative absorption changes of the sample at different time delay, Δt .

Typically, the pump pulse is modulated by introducing an optical chopper in its path to measure the differential absorption, ΔA . Thus, we can compare the changes in the probe pulse characteristics induced by the pump pulse by directing the probe pulse into a spectrograph, where the probe pulse is dispersed, and its spectrum is recorded with an array of detectors. This allows the measurement of the delay-dependent differential absorption spectrum, $\Delta A(\omega, t)$, where ω is the probe frequency. $\Delta A(\omega, t)$ can be obtained by the following expression

Figure 1.4. General schematic diagram of a pump-probe experiment used in a transient absorption measurement, where t is delayed time between pump and broadband probe.

$$\Delta A(\omega, t) = -\log \frac{I_{pump\ on}(\omega, t)}{I_{pump\ off}(\omega, t)}, \quad (1.1)$$

where $I_{pump\ on}(\omega, t)$ and $I_{pump\ off}(\omega, t)$ are the intensity of the probe spectra detected by the spectrograph when the pump is on and off, respectively.^{67,68} Hence, $\Delta A(\omega, t)$ indicates the change in absorption by the sample at different time delay when the pump is on and off. TA spectroscopy has been widely known to be very versatile, where it has been used in many areas in the field of science. TA spectroscopy has been utilized to understand the dynamical processes in biomolecules,⁶⁹⁻⁷¹ nanostructures,⁷²⁻⁷⁴ and solids.^{75,76}

1.3. Objective

Our study explores the ultrafast dynamics of photoionized or photodetached biomolecules in aqueous solution with strong-field ionizing laser pulses. This thesis presents the ultrafast vibrational wave packet dynamics of the radical species, and these modes are coupled to the photoionization or photodetachment process as the radical undergoes structural rearrangement. Our experimental results are complemented by *ab initio* calculations to obtain the equilibrium

geometries and vibrational frequencies of these biomolecules and their respective radical species. The observed vibrational frequencies and modes are then assigned with the aid of the *ab initio* calculations. Other *ab initio* calculations are performed to study the behavior of certain radical species such as the short lifetime of phenol radical cation and the dissociation behavior of phenylalanine radical.

Such studies will offer a glimpse into the earliest fundamental quantum dynamics of these biomolecules that follow the interaction of ionizing radiation with biological matter. This would give us insights on the behavior of the biomolecule in a biological process, that occurs at very short timescale, and eventually, to understand the roles of these biomolecules in many biological processes.

1.4. Organization

This thesis will outline the study on the ultrafast dynamics of ionized or photodetached biomolecules in aqueous solution *via* few-femtosecond transient absorption pump-probe spectroscopy.

Chapter 2 describes the femtosecond TA spectroscopy method (i.e. pump-probe) along with the experimental setup utilized in our studies. The chapter describes the various features to generate and characterize sub-femtosecond laser pulses that allow the elucidation of these vibrational wave packets coupled to the photoionization or photodetachment process. In addition, this chapter will present the derivation to obtain the differential absorption, $\Delta A(\lambda, t)$, spectrum from the experimental raw data.

Chapter 3 investigates the ultrafast vibrational wave packet dynamics of photodetached tyrosinate dianion in aqueous solution by utilizing the visible-NIR pump – UV probe transient absorption spectroscopy. The study aims to investigate the structural rearrangement in aqueous

solution and comparing with the recent study with the photodetachment of aqueous phenoxide ion, with the aid of density functional theory (DFT) calculations. This is studied by utilizing vibrational wave packet to study the displacement vector involved in structural rearrangement in aqueous phase.

Chapter 4 discusses the findings on the ultrafast vibrational wave packet dynamics of photodetached tryptophan anion, using the visible-NIR pump – visible probe, near-UV probe and UV probe transient absorption spectroscopy. Similar to the study on the photodetached tyrosinate dianion, the study aims to explore the structural rearrangement of the tryptophan radical, which is complemented with DFT calculations, by identifying vibrational modes that are involved in the structural rearrangement in aqueous solution.

Chapter 5 examines the ultrafast dynamics of photodetached phenylalaninate anion in aqueous solution by applying the visible-NIR pump – UV probe transient absorption spectroscopy. The study aims to explore the vibrational wave packet dynamics of photodetached phenylalaninate anion, the photodetachment-induced-dissociation pathway of phenylalanine radical in an aqueous solution that has been proposed by previous studies,^{53,54} and elucidate the spectroscopic signature of the phenylalanine radical. The study is complemented by *ab initio* calculations by performing constrained optimized geometry calculations to further understand the dissociation behavior of phenylalanine radical.

Chapter 6 explores the ultrafast dynamics of ionized phenol in aqueous solution by employing the visible-NIR pump – near-UV probe transient absorption spectroscopy. This study aims to investigate the ultrafast photoionization-induced proton transfer process of phenol in aqueous system by elucidating the short-lived phenol radical cation. It has been suggested from previous studies that the phenol radical cation undergoes rapid proton transfer with one of the surrounding water molecules, which occur at sub-picosecond timescale (~200 fs).^{77,78} The study intends to elucidate the short-lived species. In addition, isotopic

studies are performed with deuterated phenol in D₂O. The study is supported by *ab initio* calculations by performing 2D constrained optimized geometry calculations and wave packet propagation simulations, to rationalize the ultrafast proton transfer process.

Chapter 7 summarizes the main findings in our studies and proposes some future work that would improve our existing studies.

References

1. Hart, E. J., Radiation Chemistry of Aqueous Solutions. *Annu. Rev. Nucl. Sci.* **1965**, *15* (1), 125-150.
2. Garrett, B. C.; Dixon, D. A.; Camaioni, D. M.; Chipman, D. M.; Johnson, M. A.; Jonah, C. D.; Kimmel, G. A.; Miller, J. H.; Rescigno, T. N.; Rossky, P. J.; Xantheas, S. S.; Colson, S. D.; Laufer, A. H.; Ray, D.; Barbara, P. F.; Bartels, D. M.; Becker, K. H.; Bowen, K. H.; Bradforth, S. E.; Carmichael, I.; Coe, J. V.; Corrales, L. R.; Cowin, J. P.; Dupuis, M.; Eisenthal, K. B.; Franz, J. A.; Gutowski, M. S.; Jordan, K. D.; Kay, B. D.; LaVerne, J. A.; Lymar, S. V.; Madey, T. E.; McCurdy, C. W.; Meisel, D.; Mukamel, S.; Nilsson, A. R.; Orlando, T. M.; Petrik, N. G.; Pimblott, S. M.; Rustad, J. R.; Schenter, G. K.; Singer, S. J.; Tokmakoff, A.; Wang, L.-S.; Zwier, T. S., Role of Water in Electron-Initiated Processes and Radical Chemistry: Issues and Scientific Advances. *Chem. Rev.* **2005**, *105* (1), 355-390.
3. Westerlund, K.; Berry, B. W.; Privett, H. K.; Tommos, C., Exploring Amino-Acid Radical Chemistry: Protein Engineering and de novo Design. *Biochim. Biophys. Acta Bioenerg.* **2005**, *1707* (1), 103-116.
4. Kovacic, P., Protein Electron Transfer (Mechanism and Reproductive Toxicity): Iminium, Hydrogen Bonding, Homoconjugation, Amino Acid Side Chains (Redox and Charged), and Cell Signaling. *Birth Defects Res. C Embryo Today* **2007**, *81* (1), 51-64.
5. Morozova, O. B.; Kiryutin, A. S.; Sagdeev, R. Z.; Yurkovskaya, A. V., Electron Transfer between Guanosine Radical and Amino Acids in Aqueous Solution. 1. Reduction of Guanosine Radical by Tyrosine. *J. Phys. Chem. B* **2007**, *111* (25), 7439-7448.

6. Morozova, O. B.; Kiryutin, A. S.; Yurkovskaya, A. V., Electron Transfer between Guanosine Radicals and Amino Acids in Aqueous Solution. II. Reduction of Guanosine Radicals by Tryptophan. *J. Phys. Chem. B* **2008**, *112* (9), 2747-2754.
7. Bogdanov, A. M.; Mishin, A. S.; Yampolsky, I. V.; Belousov, V. V.; Chudakov, D. M.; Subach, F. V.; Verkhusha, V. V.; Lukyanov, S.; Lukyanov, K. A., Green Fluorescent Proteins are Light-Induced Electron Donors. *Nat. Chem. Biol.* **2009**, *5* (7), 459-461.
8. Winkler, J. R.; Gray, H. B., Could Tyrosine and Tryptophan Serve Multiple Roles in Biological Redox Processes? *Philos. Trans. A Math. Phys. Eng. Sci.* **2015**, *373* (2037), 20140178.
9. Reeve, A. E.; Hopkins, T. R., Photochemical Reaction between Protein and Nucleic Acids: Photoaddition of Tryptophan to Pyrimidine Bases. *Photochem. Photobiol.* **1980**, *31* (4), 297-304.
10. Creed, D., The Photophysics and Photochemistry of the Near-UV Absorbing Amino Acids - I. Tryptophan and its Simple Derivatives. *Photochem. Photobiol.* **1984**, *39* (4), 537-562.
11. Sherin, P. S.; Snytnikova, O. A.; Tsentalovich, Y. P., Tryptophan Photoionization from Prefluorescent and Fluorescent States. *Chem. Phys. Lett.* **2004**, *391* (1), 44-49.
12. Igarashi, N.; Onoue, S.; Tsuda, Y., Photoreactivity of Amino Acids: Tryptophan-Induced Photochemical Events *via* Reactive Oxygen Species Generation. *Anal. Sci.* **2007**, *23* (8), 943-948.
13. Jensen, R. L.; Arnbjerg, J.; Ogilby, P. R., Reaction of Singlet Oxygen with Tryptophan in Proteins: A Pronounced Effect of the Local Environment on the Reaction Rate. *J. Am. Chem. Soc.* **2012**, *134* (23), 9820-9826.
14. Bleifuss, G.; Kolberg, M.; Pötsch, S.; Hofbauer, W.; Bittl, R.; Lubitz, W.; Gräslund, A.; Lassmann, G.; Lendzian, F., Tryptophan and Tyrosine Radicals in Ribonucleotide

- Reductase: A Comparative High-Field EPR Study at 94 GHz. *Biochemistry* **2001**, *40* (50), 15362-15368.
15. Pogni, R.; Baratto, M. C.; Teutloff, C.; Giansanti, S.; Ruiz-Dueñas, F.; Choinowski, T.; Piontek, K.; Martinez, A. T.; Lendzian, F.; Basosi, R., A Tryptophan Neutral Radical in the Oxidized State of Versatile Peroxidase from *Pleurotus eryngii*: A Combined Multifrequency EPR and Density Functional Theory Study. *J. Biol. Chem.* **2006**, *281*, 9517-26.
 16. Gregersen, J. A.; Tureček, F., Mass-spectrometric and Computational Study of Tryptophan Radicals (Trp + H)[•] Produced by Collisional Electron Transfer to Protonated Tryptophan in the Gas Phase. *Phys. Chem. Chem. Phys.* **2010**, *12* (41), 13434-13447.
 17. Lundeen, R. A.; Janssen, E. M. L.; Chu, C.; McNeill, K., Environmental Photochemistry of Amino Acids, Peptides and Proteins. *Chimia* **2014**, *68* (11), 812-817.
 18. Joshi, R., Charge Transfer Reactions from Tryptophan and Tyrosine to Sulfur-Centered Dimer Radical Cation in Aqueous –Sulfuric Acid Medium: A Pulse Radiolysis Study. *J. Phys. Org. Chem.* **2016**, *29* (6), 281-287.
 19. Davis, I.; Koto, T.; Terrell, J. R.; Kozhanov, A.; Krzystek, J.; Liu, A., High-Frequency/High-Field Electron Paramagnetic Resonance and Theoretical Studies of Tryptophan-Based Radicals. *J. Phys. Chem. A* **2018**, *122* (12), 3170-3176.
 20. Müller, P.; Ignatz, E.; Kiontke, S.; Brettel, K.; Essen, L.-O., Sub-Nanosecond Tryptophan Radical Deprotonation Mediated by a Protein-Bound Water Cluster in Class II DNA Photolyases. *Chem. Sci.* **2018**, *9* (5), 1200-1212.
 21. Roy, A.; Seidel, R.; Kumar, G.; Bradforth, S. E., Exploring Redox Properties of Aromatic Amino Acids in Water: Contrasting Single Photon vs Resonant Multiphoton Ionization in Aqueous Solutions. *J. Phys. Chem. B* **2018**, *122* (14), 3723-3733.

22. Nishizawa, K.; Kurahashi, N.; Sekiguchi, K.; Mizuno, T.; Ogi, Y.; Horio, T.; Oura, M.; Kosugi, N.; Suzuki, T., High-Resolution Soft X-Ray Photoelectron Spectroscopy of Liquid Water. *Phys. Chem. Chem. Phys.* **2011**, *13* (2), 413-417.
23. Kurahashi, N.; Karashima, S.; Tang, Y.; Horio, T.; Abulimiti, B.; Suzuki, Y.-I.; Ogi, Y.; Oura, M.; Suzuki, T., Photoelectron Spectroscopy of Aqueous Solutions: Streaming Potentials of NaX (X = Cl, Br, and I) Solutions and Electron Binding Energies of Liquid Water and X^- . *J. Chem. Phys.* **2014**, *140* (17), 174506.
24. Perry, C. F.; Zhang, P.; Nunes, F. B.; Jordan, I.; von Conta, A.; Wörner, H. J., Ionization Energy of Liquid Water Revisited. *J. Phys. Chem. Lett.* **2020**, *11* (5), 1789-1794.
25. Winter, B.; Weber, R.; Widdra, W.; Dittmar, M.; Faubel, M.; Hertel, I. V., Full Valence Band Photoemission from Liquid Water Using EUV Synchrotron Radiation. *J. Phys. Chem. A* **2004**, *108* (14), 2625-2632.
26. Hare, P. M.; Price, E. A.; Bartels, D. M., Hydrated Electron Extinction Coefficient Revisited. *J. Phys. Chem. A* **2008**, *112* (30), 6800-6802.
27. Torche, F.; Marignier, J.-L., Direct Evaluation of the Molar Absorption Coefficient of Hydrated Electron by the Isosbestic Point Method. *J. Phys. Chem. B* **2016**, *120* (29), 7201-7206.
28. Ambrosio, F.; Guo, Z.; Pasquarello, A., Absolute Energy Levels of Liquid Water. *J. Phys. Chem. Lett.* **2018**, *9* (12), 3212-3216.
29. Ambrosio, F.; Miceli, G.; Pasquarello, A., Electronic Levels of Excess Electrons in Liquid Water. *J. Phys. Chem. Lett.* **2017**, *8* (9), 2055-2059.
30. Crowell, R. A.; Bartels, D. M., Multiphoton Ionization of Liquid Water with 3.0–5.0 eV Photons. *J. Phys. Chem.* **1996**, *100* (45), 17940-17949.

31. Pépin, C.; Goulet, T.; Houde, D.; Jay-Gerin, J. P., Observation of a Continuous Spectral Shift in the Solvation Kinetics of Electrons in Neat Liquid Deuterated Water. *J. Phys. Chem. A* **1997**, *101* (24), 4351-4360.
32. Silva, C.; Walhout, P. K.; Yokoyama, K.; Barbara, P. F., Femtosecond Solvation Dynamics of the Hydrated Electron. *Phys. Rev. Lett.* **1998**, *80* (5), 1086-1089.
33. Vilchiz, V. H.; Kloepper, J. A.; Germaine, A. C.; Lenchenkov, V. A.; Bradforth, S. E., Map for the Relaxation Dynamics of Hot Photoelectrons Injected into Liquid Water *via* Anion Threshold Photodetachment and Above Threshold Solvent Ionization. *J. Phys. Chem. A* **2001**, *105* (10), 1711-1723.
34. Pshenichnikov, M. S.; Baltuška, A.; Wiersma, D. A., Hydrated-Electron Population Dynamics. *Chem. Phys. Lett.* **2004**, *389* (1), 171-175.
35. Mostafavi, M.; Lampre, I., An Overview of Solvated Electrons: Recent Advances. 2010; Vol. 105, pp 21-58.
36. Elkins, M. H.; Williams, H. L.; Shreve, A. T.; Neumark, D. M., Relaxation Mechanism of the Hydrated Electron. *Science* **2013**, *342* (6165), 1496-1499.
37. Bent, D. V.; Hayon, E., Excited State Chemistry of Aromatic Amino Acids and Related Peptides. III. Tryptophan. *J. Am. Chem. Soc.* **1975**, *97* (10), 2612-2619.
38. Bryant, F. D.; Santus, R.; Grossweiner, L. I., Laser Flash Photolysis of Aqueous Tryptophan. *J. Phys. Chem.* **1975**, *79* (25), 2711-2716.
39. Redpath, J. L.; Santus, R.; Ovadia, J.; Grossweiner, L. I., The Oxidation of Tryptophan by Radical Anions. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **1975**, *27* (2), 201-204.
40. Baugher, J. F.; Grossweiner, L. I., Photolysis Mechanism of Aqueous Tryptophan. *J. Phys. Chem.* **1977**, *81* (14), 1349-1354.

41. Solar, S.; Getoff, N.; Surdhar, P. S.; Armstrong, D. A.; Singh, A., Oxidation of Tryptophan and N-methylindole by $\text{N}_3^\bullet$, $\text{Br}_2^{\bullet-}$, and $(\text{SCN})_2^{\bullet-}$ Radicals in Light- and Heavy-Water Solutions: A Pulse Radiolysis Study. *J. Phys. Chem.* **1991**, *95* (9), 3639-3643.
42. Kandori, H.; Borkman, R. F.; Yoshihara, K., Picosecond Transient Absorption of Aqueous Tryptophan. *J. Phys. Chem.* **1993**, *97* (38), 9664-9667.
43. McGimpsey, W. G.; Görner, H., Photoionization of Indole, N-Methylindole and Tryptophan In Aqueous Solution upon Excitation at 193 nm. *Photochem. Photobiol.* **1996**, *64* (3), 501-509.
44. Stevenson, K. L.; Papadantonakis, G. A.; LeBreton, P. R., Nanosecond UV Laser Photoionization of Aqueous Tryptophan: Temperature Dependence of Quantum Yield, Mechanism, and Kinetics of Hydrated Electron Decay. *J. Photochem. Photobiol. A* **2000**, *133* (3), 159-167.
45. Tsentalovich, Y. P.; Snytnikova, O. A.; Sagdeev, R. Z., Properties of Excited States of Aqueous Tryptophan. *J. Photochem. Photobiol. A* **2004**, *162* (2), 371-379.
46. Larson, B. C.; Pomponio, J. R.; Shafaat, H. S.; Kim, R. H.; Leigh, B. S.; Tauber, M. J.; Kim, J. E., Photogeneration and Quenching of Tryptophan Radical in Azurin. *J. Phys. Chem. B* **2015**, *119* (29), 9438-9449.
47. Bent, D. V.; Hayon, E., Excited State Chemistry of Aromatic Amino Acids and Related Peptides. I. Tyrosine. *J. Am. Chem. Soc.* **1975**, *97* (10), 2599-2606.
48. Petersson, L.; Gräslund, A.; Ehrenberg, A.; Sjöberg, B.-M.; Reichard, P., The Iron Center in Ribonucleotide Reductase from Escherichia coli. *J. Biol. Chem.* **1980**, *255*, 6706-12.
49. Ghanem, R.; Xu, Y.; Pan, J.; Hoffmann, T.; Andersson, J.; Polívka, T.; Pascher, T.; Styring, S.; Sun, L.; Sundström, V., Light-Driven Tyrosine Radical Formation in a

- Ruthenium–Tyrosine Complex Attached to Nanoparticle TiO₂. *Inorg. Chem.* **2002**, *41* (24), 6258-6266.
50. Oldemeyer, S.; Franz, S.; Wenzel, S.; Essen, L.-O.; Mittag, M.; Kottke, T., Essential Role of an Unusually Long-Lived Tyrosyl Radical in the Response to Red Light of the Animal-like Cryptochrome aCRY. *J. Biol. Chem.* **2016**, *291* (27), 14062-14071.
51. Mittal, J. P.; Hayon, E., Electronic Absorption Spectrum and Decay Kinetics of the Benzyl Radical in Solution. *Nat. Phys. Sci.* **1972**, *240* (97), 20-21.
52. Christensen, H. C.; Sehested, K.; Hart, E. J., Formation of Benzyl Radicals by Pulse Radiolysis of Toluene in Aqueous Solutions. *J. Phys. Chem.* **1973**, *77* (8), 983-987.
53. Mittal, L. J.; Mittal, J. P.; Hayon, E., Biphotonic Photodissociation of Phenylalanine in Aqueous Solution at 20°C. *Chem. Phys. Lett.* **1973**, *18* (3), 319-322.
54. Mittal, L. J.; Mittal, J. P.; Hayon, E., Primary Processes in the Photochemistry of Phenylalanine and Derivatives in Aqueous Solution. Biphotonic Photoionization and Photodissociation Reactions. *J. Am. Chem. Soc.* **1973**, *95* (19), 6203-6210.
55. Bent, D. V.; Hayon, E., Excited State Chemistry of Aromatic Amino Acids and Related Peptides. II. Phenylalanine. *J. Am. Chem. Soc.* **1975**, *97* (10), 2606-2612.
56. Ayuso, D.; Kimura, M.; Kooser, K.; Patanen, M.; Plésiat, E.; Argenti, L.; Mondal, S.; Travnikova, O.; Sakai, K.; Palacios, A.; Kukk, E.; Decleva, P.; Ueda, K.; Martín, F.; Miron, C., Vibrationally Resolved B 1s Photoionization Cross Section of BF₃. *J. Phys. Chem. A* **2015**, *119* (23), 5971-5978.
57. Wei, Z.; Li, J.; Wang, L.; See, S. T.; Jhon, M. H.; Zhang, Y.; Shi, F.; Yang, M.; Loh, Z.-H., Elucidating the Origins of Multimode Vibrational Coherences of Polyatomic Molecules Induced by Intense Laser Fields. *Nat. Commun.* **2017**, *8* (1), 735.

58. Ando, T.; Shimamoto, A.; Miura, S.; Iwasaki, A.; Nakai, K.; Yamanouchi, K., Coherent Vibrations in Methanol Cation Probed by Periodic H_3^+ Ejection after Double Ionization. *Commun. Chem.* **2018**, *1* (1), 7.
59. Kim, J. B.; Yacovitch, T. I.; Hock, C.; Neumark, D. M., Slow Photoelectron Velocity-Map Imaging Spectroscopy of the Phenoxide and Thiophenoxide Anions. *Phys. Chem. Chem. Phys.* **2011**, *13* (38), 17378-17383.
60. Debnath, T.; Mohd Yusof, M. S. B.; Low, P. J.; Loh, Z.-H., Ultrafast Structural Rearrangement Dynamics Induced by the Photodetachment of Phenoxide in Aqueous Solution. *Nat. Commun.* **2019**, *10* (1), 2944.
61. Dantus, M.; Bowman, R. M.; Zewail, A. H., Femtosecond Laser Observations of Molecular Vibration and Rotation. *Nature* **1990**, *343* (6260), 737-739.
62. Hargrove, L. E.; Fork, R. L.; Pollack, M. A., Locking of He–Ne Laser Modes Induced by Synchronous Intracavity Modulation. *Appl. Phys. Lett.* **1964**, *5* (1), 4-5.
63. Yariv, A., Internal Modulation in Multimode Laser Oscillators. *J. Appl. Phys.* **1965**, *36* (2), 388-391.
64. Keller, U., Recent Developments in Compact Ultrafast Lasers. *Nature* **2003**, *424* (6950), 831-838.
65. Strickland, D.; Mourou, G., Compression of Amplified Chirped Optical Pulses. *Opt. Commun.* **1985**, *56* (3), 219-221.
66. Backus, S.; Durfee, C. G.; Murnane, M. M.; Kapteyn, H. C., High Power Ultrafast Lasers. *Rev. Sci. Instrum.* **1998**, *69* (3), 1207-1223.
67. Cerullo, G.; Manzoni, C.; Lüer, L.; Polli, D., Time-Resolved Methods in Biophysics. 4. Broadband Pump–probe Spectroscopy System with Sub-20 fs Temporal Resolution for the Study of Energy Transfer Processes in Photosynthesis. *Photochem. Photobiol. Sci.* **2007**, *6* (2), 135-144.

68. Polli, D.; Ler, L.; Cerullo, G., High-Time-Resolution Pump-Probe System with Broadband Detection for the Study of Time-Domain Vibrational Dynamics. *Rev. Sci. Instrum.* **2007**, 78 (10), 103108.
69. Ruban, A. V.; Berera, R.; Iliaia, C.; van Stokkum, I. H. M.; Kennis, J. T. M.; Pascal, A. A.; van Amerongen, H.; Robert, B.; Horton, P.; van Grondelle, R., Identification of a Mechanism of Photoprotective Energy Dissipation in Higher Plants. *Nature* **2007**, 450 (7169), 575-578.
70. Berera, R.; van Grondelle, R.; Kennis, J. T. M., Ultrafast Transient Absorption Spectroscopy: Principles and Application to Photosynthetic Systems. *Photosynth. Res.* **2009**, 101 (2-3), 105-118.
71. Madsen, M. M.; Jensen, F.; Thgersen, J., The Primary Photo-Dissociation Dynamics of Amino Acids in Aqueous Solution: Breaking the C α -bond. *Phys. Chem. Chem. Phys.* **2020**, 22 (4), 2307-2318.
72. Tamaki, Y.; Furube, A.; Murai, M.; Hara, K.; Katoh, R.; Tachiya, M., Dynamics of Efficient Electron–Hole Separation in TiO₂ Nanoparticles Revealed by Femtosecond Transient Absorption Spectroscopy under the Weak-Excitation Condition. *Phys. Chem. Chem. Phys.* **2007**, 9 (12), 1453-1460.
73. Wu, K.; Chen, J.; McBride, J. R.; Lian, T., Efficient Hot-Electron Transfer by a Plasmon-Induced Interfacial Charge-Transfer Transition. *Science* **2015**, 349 (6248), 632-635.
74. Kedawat, G.; Sharma, I.; Nagpal, K.; Kumar, M.; Gupta, G.; Gupta, B. K., Studies of Ultrafast Transient Absorption Spectroscopy of Gold Nanorods in an Aqueous Solution. *ACS Omega* **2019**, 4 (7), 12626-12631.
75. Glinas, S.; Rao, A.; Kumar, A.; Smith, S. L.; Chin, A. W.; Clark, J.; van der Poll, T. S.; Bazan, G. C.; Friend, R. H., Ultrafast Long-Range Charge Separation in Organic Semiconductor Photovoltaic Diodes. *Science* **2014**, 343 (6170), 512-516.

76. Borja, L. J.; Zürich, M.; Pemmaraju, C. D.; Schultze, M.; Ramasesha, K.; Gandman, A.; Prell, J. S.; Prendergast, D.; Neumark, D. M.; Leone, S. R., Extreme Ultraviolet Transient Absorption of Solids from Femtosecond to Attosecond Timescales [Invited]. *J. Opt. Soc. Am. B* **2016**, *33* (7), C57-C64.
77. Gadosy, T. A.; Shukla, D.; Johnston, L. J., Generation, Characterization, and Deprotonation of Phenol Radical Cations. *J. Phys. Chem. A* **1999**, *103* (44), 8834-8839.
78. Oliver, T. A. A.; Zhang, Y.; Roy, A.; Ashfold, M. N. R.; Bradforth, S. E., Exploring Autoionization and Photoinduced Proton-Coupled Electron Transfer Pathways of Phenol in Aqueous Solution. *J. Phys. Chem. Lett.* **2015**, *6* (20), 4159-4164.

Chapter 2

Femtosecond Time-Resolved Transient

Absorption Spectroscopy

As discussed in Chapter 1, a typical transient absorption (TA) spectroscopy method (i.e. pump-probe) usually involves two ultrashort light pulses – a pump and a broadband probe – to monitor light-matter interaction. The pump pulse perturbs the sample at $t = 0$ to generate the transient species. The weaker broadband probe pulse is then subsequently directed at the sample at different time delay, Δt , to monitor the ultrafast dynamics of the transient species such as measuring the change in the absorbance. With a probe pulse with ultrashort time resolution, we are able to observe ultrafast dynamics ($< 10^{-12}$ s) of chemical and atomic dynamics such as the kinetics and molecular vibrations.

This chapter discusses the experimental procedure to study the ultrafast dynamics of ionized or photodetached biomolecules in aqueous solution. Since our studies involve the photoionization or photodetachment of biomolecules, the pump pulse functions as an ionizing pump, hence a high peak intensity ($> 10^{13}$ W cm⁻²) is required for multiphoton ionization or photodetachment. In addition, this chapter will discuss the methods utilized to generate and characterize these sub-picosecond laser pulses that allow us to observe and elucidate the ultrafast vibrational wave packet dynamics of the ionized or photodetached biomolecules in aqueous solution.

2.1. Few-Cycle Pulse Generation

The transient absorption pump-probe experiment is performed with a chirped-pulse-amplified Ti:sapphire laser (Coherent, Legend Elite Duo-USX). The laser system has an output of ~ 4 -mJ, 30-fs pulses at 1-kHz repetition rate and 800-nm center wavelength, with a bandwidth of 50 nm. The pulses undergo self-phase modulation in a helium-filled hollow-core fiber before they are temporally compressed by chirped mirrors (Ultrafast Innovations GmbH, PC1332). The compressed pulses are incident on a 80T/20R beamsplitter (Layertec 131191), with 80% of the transmitted beam (~ 0.6 mJ) being directed to a $4f$ visible-NIR pulse shaper with a 128-element spatial light modulator (SLM) (Biophotonics Solutions, FemtoJock-P) for further pulse compression. The $4f$ visible-NIR pulse shaper performs adaptive pulse compression of the visible-NIR pulse to correct for any higher-order dispersions introduced by the various optical components in the laser beams' path to ensure that clean, transform-limited pulses are produced daily. The spectrum of the output visible-NIR pulse is truncated and has a typical wavelength range of 520 nm to 750 nm. The visible-NIR spectrum is shown in Fig. 2.1. The transform-limited laser pulses, which is defined as the shortest pulse duration that can be attained for a given optical spectrum, are obtained through the multiphoton intrapulse interference phase scan (MIIPS) technique to measure the spectral phase at the sample, followed by using the SLM to introduce an oppositely signed phase to the laser pulses, thus compressing it to the transform-limited duration.¹ Subsequently, a second-order interferometric auto-correlation trace is produced by multiple independent comb shaping (MICS) technique.² The transform-limited pulses, which function as the strong-field ionizing pump, are directed to the sample. The ionizing pulse is then focused onto the sample by a 50-cm focal length spherical mirror. The peak intensity of the ionizing pump is typically $\sim 10^{13}$ W cm⁻².

Figure 2.1. The visible-NIR ionizing pump spectrum ranges from 520 nm to 750 nm.

2.2. Generation and Characterization of Ultrashort Laser Pulses

Dispersion of the laser pulses are inevitable when laser pulses propagate through optical materials and reflecting off from dielectric surfaces. This phenomenon affects the pulse duration of the laser pulses and introduces a frequency chirp. It is imperative to characterize and control the dispersion of these laser pulses to get the shortest pulse duration (i.e. transform-limited pulse) on a daily basis. In general, the group velocity dispersion (GVD), is given as the following

$$GVD = \frac{\lambda^3}{2\pi c} \left(\frac{d^2 n(\lambda)}{d\lambda^2} \right), \quad (2.1)$$

where λ is the wavelength, c is the speed of light and $n(\lambda)$ is the wavelength-dependent refractive index. Optical materials like fused silica have a positive GVD, resulting in positive chirping, which is described as the red-shift of leading edge of an ultrashort pulse. This is

illustrated in Fig. 2.2. Conversely, optical materials such as chirped mirrors have negative GVD, resulting in a blue-shift of leading edge of an ultrashort pulse. In order to obtain transform-limited laser pulses, the dispersion must be compensated by several methods. In our experimental setup, the laser output from the helium-filled hollow core fiber^{3,4} is pre-compensated with several chirp mirrors. Subsequently, MIIPS combined with high-order dispersion compensation by means of a SLM pulse shaper yields transform-limited pulse.

MIIPS is a single-beam pulse shaping method to characterize the ultrashort laser pulse and compensate the higher-order dispersions as the laser pulses propagate through the different optical materials in the setup. This higher-order dispersions distort the phase profile of the laser pulse. In order to correct this distortion in the phase profile, MIIPS introduces a calibrated reference phase function to measure the spectral phase distortions in the frequency domain. The phase spectrum of the second harmonic generation (SHG) produced from the laser pulses is then used to measure the second derivative of the phase. As the reference phase is scanned, a MIIPS trace is retrieved and the difference in phase is calculated. This difference in phase is then used to correct the distortions in the phase and compensate the pulse to achieve the transform-limited ~ 5 fs pulses on a daily basis, which generates the maximum SHG signal.¹

To characterize the transform-limited pulse, a second-order interferometric auto-correlation is performed to obtain an auto-correlation trace. A second-order auto-correlation function is given as

$$\begin{aligned}
 I_2(\tau) &= \int_{-\infty}^{+\infty} |[\mathbf{E}(t) + \mathbf{E}(t - \tau)]|^2 dt \\
 &= \int_{-\infty}^{+\infty} 2\mathbf{E}^4(t) + 6\mathbf{E}^2(t)\mathbf{E}^2(t - \tau) \\
 &\quad + 4\mathbf{E}^3(t)\mathbf{E}(t - \tau) + 4\mathbf{E}(t)\mathbf{E}^3(t - \tau) dt, \tag{2.2}
 \end{aligned}$$

Figure 2.2. An ultrashort laser pulse propagating through an optical material that have a positive dispersion ($GVD > 0$), resulting in positive chirping, where the red component travels faster than the blue component.

where $I_2(\tau)$ is the intensity of the second-order auto-correlation intensity and $\mathbf{E}(t)$ and $\mathbf{E}(t - \tau)$ are the time-domain electric field of the two pulses, where the second pulse is delayed by τ with respect to the first pulse. When $\tau = 0$, i.e. when the two pulses are in coherent superposition, then the intensity of the second-order auto-correlation at the peak is given as

$$I_2(\tau = 0) = 2^4 \int \mathbf{E}^4(t) dt, \quad (2.3)$$

while the intensity of the second-order auto-correlation of the background, far from the superposition ie. $\tau \rightarrow \pm \infty$ is given as

$$I_2(\tau \rightarrow \pm \infty) = 2 \int \mathbf{E}^4(t) dt. \quad (2.4)$$

Thus, a second-order auto-correlation trace gives an 8:1 ratio from the peak to the background. A second-order interferometric auto-correlation trace of the visible-NIR ionizing pump pulse is shown in Fig. 2.3, which is consistent with the 8:1 ratio.⁵ The second-order interferometric auto-correlation trace reveals a pulse duration of full-width at half-maximum (FWHM) of ~ 5 fs.

Figure 2.3. A second-order interferometric auto-correlation trace of the visible-NIR ionizing pump spectrum, measured in a 10- μm -thick, $\theta = 37.0$ deg Type I BBO crystal, reveals a pulse duration with FWHM of ~ 5 fs.

2.3. Sample Preparation

Amino acids generally have low solubility in water at its isoelectronic point,⁶⁻⁸ hence they are dissolved in sodium hydroxide at pH 14 to produce the anionic form. On the other hand, phenol is dissolved in water due to its relatively high solubility.

A slit nozzle (Metaheuristics, Type L) is used in conjunction with a peristaltic pump (Cole-Parmer, 07528-10) and a pulse dampener to produce a microjet of the solution for experiments. The volume flow rate of 92 mL min^{-1} translates a vertical flow rate of approximately 8.5 mm ms^{-1} . The relatively high vertical flow rate ensures that fresh volume of sample is exposed to each ionizing pump pulse. By employing spectral interferometry, the liquid microjet path length is found to be $7\text{-}\mu\text{m}$, sufficient for producing a sizeable differential absorption signal ($\Delta A \sim 0.1$), while minimizing the temporal broadening of the pulses in the

liquid jet due to dispersion. In addition, a microjet is required to limit the group-velocity mismatch between the pump and probe such that it has a negligible effect on the time resolution.

Static UV-visible measurements are performed with a double-beam UV-visible-NIR spectrophotometer (Agilent Technologies, Cary 300) with a 1-mm path length quartz cuvette by measuring absorbance. The spectrophotometer has a wavelength range from 190 to 800 nm.

2.4. Differential Absorption

To obtain the ΔA spectrum, an optical delay stage line, driven by a piezo translation stage (Physik Instrumente, N-664.3A) is in the ionizing pump's path to provide a variable time delay between pump and probe pulses. A synchronized optical chopper in the ionizing pump's path before the sample allows modulation of the ionizing pump at a frequency of 0.5 kHz. An achromatic half-waveplate (B. Halle, RAC4.2.15 L) is introduced to allow for optical beam polarization adjustments, allowing for polarization anisotropy measurements if needed. The pump and probe are focused onto the sample, in which the probe is then focused into a spectrograph (Princeton Instruments, Acton SP2300). The spectrograph is equipped with a grating and a 1024-pixels silicon photodiode array detector (Stresing, S8381-1024Q) with a read-out rate of 1 kHz, which allow simultaneous acquisition of the probe spectra obtained with and without the ionizing pump pulse. The silicon photodiode array detectors are interfaced into desktop computers for automated acquisition of data. The reference pulse, that is split off from the probe by utilizing a beamsplitter, is focused to an identical spectrograph, which is equipped with similar grating and photodiode array at 1-kHz repetition rate. This method reduces noise interference caused by shot-to-shot fluctuations of the probe pulse.

2.4.1. Visible Probe

To generate the visible probe, the transform-limited visible-NIR pulses produced from the 4f-pulse shaper is split off by a 90T/10R beamsplitter (Layertec, 130945) to produce the strong-field ionizing pump and visible-NIR probe, hence both the pump and probe pulses have a similar range in wavelength and pulse duration. The visible-NIR probe spectrum is similar to ionizing pump spectrum, as shown in Fig. 2.1. The visible-NIR pulse is split off by a reflective neutral density beamsplitter (Newport, FRQ-ND05) into the probe and reference pulses. The reflected visible-NIR probe pulses are then focused onto the sample by a 15-cm focal length off-axis parabolic reflector (Thorlabs), which results in a typical beam waist ($1/e^2$) of $\sim 35 \mu\text{m}$. The peak intensity of the visible-NIR probe is typically $\sim 10^{11} \text{ W cm}^{-2}$.

The visible-NIR probe is then directed and focused into a 300-nm spectrograph (Princeton Instruments, Acton SP2300), equipped with a 150-grooves per mm grating blazed at 800 nm. The pump-probe experimental setup for visible-NIR pump – visible-NIR probe experiment is shown in Fig. 2.4.

Figure 2.4. Experimental pump-probe setup with visible-NIR probe. The visible-NIR pulses are directed to a $4f$ visible-NIR pulse shaper. The laser pulses are then split off by a 90T/10R beamsplitter to produce the visible-NIR ionizing pump (in red) and probe (in orange). The visible-NIR pump and probe are then focused on to the sample, where the probe is then directed to the spectrograph.

2.4.2. Near-UV Probe

To generate the near-UV probe, the remaining output from the chirped mirror compressor after the 80T/20R beamsplitter (Layertec 131191) is directed to a 50- μm -thick Type I beta-barium borate (BBO) crystal (Casix, $\theta = 29.0^\circ$) to generate the broadband near-UV probe *via* SHG. A pair of wedges (Femtolasers, OA124) and UV fused-silica windows are inserted to compensate for the dispersion to produce a broadband near-UV probe, ranging from 360 nm to 470 nm. The broadband near-UV probe spectrum is shown in Fig. 2.5(a). A pair of dichroic beamsplitters (Layertec, 109433) is introduced after the BBO crystal to eliminate residual fundamental beam. The near-UV pulse is then split off by a reflective neutral density beamsplitter (Newport, FRQ-ND05) to produce the probe and reference pulses. The reflected near-UV probe is then focused onto the sample by a 15-cm focal length off-axis parabolic reflector (Thorlabs), which results in a typical beam waist ($1/e^2$) of $\sim 40\ \mu\text{m}$. The peak intensity of the near-UV probe pulse is typically $\sim 10^{10}\ \text{W cm}^{-2}$.

The near-UV probe is then directed and focused into a 300-nm spectrograph (Princeton Instruments, Acton SP2300) equipped with a 600-grooves per mm grating blazed at 500 nm. The cross-correlation of the pump and probe, by measuring the difference frequency generated (DFG) beam by utilizing a 10- μm thick Type I BBO crystal (Casteck, $\theta = 29.2^\circ$) yields an instrument response of 12 fs at FWHM. The cross-correlation trace is displayed in Fig. 2.5(b). The pump-probe experimental setup for visible-NIR pump – near-UV probe experiment is shown in Fig. 2.6.

Figure 2.5. (a) The broadband near-UV probe spectrum, with a wavelength range of 360 nm to 470 nm, generated *via* SHG. (b) The pump-probe cross-correlation, measured *via* difference frequency mixing in a 10- μm -thick, $\theta = 29.2$ deg Type I BBO crystal, reveals a FWHM of 12 fs.

Figure 2.6. Experimental pump-probe setup with UV probe. The visible-NIR fundamental pulse is split off by an 80T/20R beamsplitter, where the transmitted pulse function as the ionizing pump pulse (in red). The reflected pulse is directed to a BBO crystal to generate the UV-probe (in blue) *via* SHG, which subsequently directed to a pair of dichroic beamsplitters to eliminate residual fundamental beam. The visible-NIR pump and UV probe are then focused on to the sample, where the probe is then directed to the spectrograph.

2.4.3. UV Probe

To generate the UV probe, the remaining output from the chirped mirror compressor after the 80T/20R beamsplitter (Layertec 131191) is telescoped down by a factor of 3, with a pair of concave and convex mirrors, and subsequently directed to a 10- μm -thick Type I BBO crystal (Castech, $\theta = 44.0^\circ$) to generate the UV probe *via* SHG. A pair of wedges (Femtolasers, OA124) and UV fused-silica windows are inserted to compensate for the dispersion to produce a broadband UV probe, ranging from 290 nm to 370 nm. The UV probe spectrum is displayed in Fig. 2.7(a). Several dichroic beamsplitters (Layertec, 106163) are introduced after the BBO crystal to eliminate residual fundamental beam. The UV pulse is then split off by a reflective neutral density beamsplitter (Edmund Optics, 88271) to produce the UV probe pulse and reference pulse. The reflected UV probe is then focused onto the sample by a 15-cm focal length off-axis parabolic reflector (Thorlabs), which results in a typical beam waist ($1/e^2$) of $\sim 40\ \mu\text{m}$. The peak intensity of the UV probe pulse is typically $\sim 10^{10}\ \text{W cm}^{-2}$.

Similarly, the UV probe is directed and focused into a 300-nm spectrograph (Princeton Instruments, Acton SP2300) equipped with a 600-grooves per mm grating blazed at 500 nm. The cross-correlation of the pump and UV probe, by measuring the DFG beam by utilizing a 10- μm thick Type I BBO crystal (Castech, $\theta = 37.0^\circ$) yields an instrument response of 24 fs at FWHM. The cross-correlation trace is displayed in Fig. 2.7(b). The pump-probe experimental setup for visible-NIR pump – UV probe experiment is similar to the setup shown in Fig. 2.6.

Figure 2.7. (a) The broadband UV probe spectrum, with a wavelength range of 290 nm to 370 nm, generated *via* SHG. **(b)** The pump-probe cross-correlation, measured *via* difference frequency mixing in a 10- μm -thick, $\theta = 37.0$ deg Type I BBO crystal reveals a FWHM of 24 fs.

2.4.4. Differential Absorption Measurement

In a typical pump-probe experiment, the intensity of the probe is measured by the spectrograph. As mentioned in the experimental section, the probe is split off by a beamsplitter to yield the probe pulse and the reference pulse. Both the intensity of the probe and reference pulses are measured simultaneously by two spectrographs, equipped with similar grating and photodiode array to obtain the differential absorption, $\Delta A(\lambda, t)$, signal as a function of probe wavelength, λ , and time delay, t .

The intensity of the reference spectrum, $I_{reference}(\lambda, t)$, and the probe spectrum is recorded. Since the ionizing pump is modulated by the optical chopper, the spectrograph records the intensity of the probe spectrum when the pump is on, $I_{pump\ on}(\lambda, t)$, and when the pump is off, $I_{pump\ off}(\lambda, t)$. Hence, a difference in transmittance signal, $\Delta T(\lambda, t)$, in terms of intensity of the probe and reference is obtained by the following expression.

$$\begin{aligned}\Delta T(\lambda, t) &= \frac{I_{pump\ on}(\lambda, t)}{I_{reference}(\lambda, t)} - \frac{I_{pump\ off}(\lambda, t)}{I_{reference}(\lambda, t)} \\ &= T_{ionized}(\lambda, t) - T_{unionized}(\lambda, t)\end{aligned}\tag{2.5}$$

where $T_{ionized}(\lambda, t)$ and $T_{unionized}(\lambda, t)$ are the transmittance of the ionized and unionized species respectively. The normalized differential transmittance, $\frac{\Delta T(\lambda, t)}{T(\lambda, t)}$, can be obtained in terms of transmittance as given in the following equation.

$$\begin{aligned}\frac{\Delta T(\lambda, t)}{T(\lambda, t)} &= \frac{T_{ionized}(\lambda, t) - T_{unionized}(\lambda, t)}{T_{unionized}(\lambda, t)} \\ &= \frac{T_{ionized}(\lambda, t)}{T_{unionized}(\lambda, t)} - 1\end{aligned}\tag{2.6}$$

Since absorbance, A , has a logarithmic relationship with transmittance, as shown in equation (2.7).

$$A = -\log T \quad (2.7)$$

hence, the differential absorption, $\Delta A(\lambda, t)$, which is the difference between the absorbance of the ionized species, $A_{ionized}(\lambda, t)$, and unionized species, $A_{unionized}(\lambda, t)$, can be obtained in terms of differential transmittance by using equation (2.6) to obtain the following equation.

$$\begin{aligned} \Delta A(\lambda, t) &= A_{ionized}(\lambda, t) - A_{unionized}(\lambda, t) \\ &= -\log T_{ionized}(\lambda, t) + \log T_{unionized}(\lambda, t) \\ &= -\log \left(\frac{T_{ionized}(\lambda, t)}{T_{unionized}(\lambda, t)} \right) \\ &= -\log \left[\frac{\Delta T(\lambda, t)}{T(\lambda, t)} + 1 \right] \end{aligned} \quad (2.8)$$

By using this relationship, we are able to obtain the $\Delta A(\lambda, t)$ signal of the photoionized or photodetached biomolecules from the measurement of the change of the intensity of the probe, which will be explored in the next few chapters. Such single-shot spectral referencing method reduces fluctuations of the probe light, and improves the signal-to-noise ratio of our ΔA signal.⁹

References

1. Dantus, M.; Lozovoy, V.; Pastirk, I., MIIPS Characterizes and Corrects Femtosecond Pulses. **2007**, *43*, 101-104.
2. Pestov, D.; Lozovoy, V. V.; Dantus, M., Multiple Independent Comb Shaping (MICS): Phase-Only Generation of Optical Pulse Sequences. *Opt. Express* **2009**, *17* (16), 14351-14361.
3. Nisoli, M.; Silveshi, S. D.; Svelto, O. In *Generation of High-Energy 10-fs Pulses by a New Pulse Compression Technique*, Summaries of Papers Presented at the Conference on Lasers and Electro-Optics, 1996; pp 189-190.
4. Nisoli, M.; De Silvestri, S.; Svelto, O.; Szpöcs, R.; Ferencz, K.; Spielmann, C.; Sartania, S.; Krausz, F., Compression of High-Energy Laser Pulses below 5 fs. *Opt. Lett.* **1997**, *22* (8), 522-524.
5. Narimousa, M.; Sabaeian, M.; Mousavi Ghahfarrokhi, S. M.; Panahi, O., Second-Order Interferometric Autocorrelation for Measuring Group Velocity Dispersion and Pulse Broadening of Femtosecond Pulses. *Appl. Opt.* **2018**, *57* (18), 5011-5018.
6. Liu, L.-F.; Yang, L.-L.; Jin, K.-Y.; Xu, D.-Q.; Gao, C.-J., Recovery of L-Tryptophan from Crystallization Wastewater by Combined Membrane PSeparation and Purification Technologyprocess. *Sep. Purif. Technol.* **2009**, *66* (3), 443-449.
7. Tseng, H.-C.; Lee, C.-Y.; Weng, W.-L.; Shiah, I. M., Solubilities of Amino Acids in Water at Various pH Values under 298.15K. *Fluid Ph. Equilibria* **2009**, *285* (1), 90-95.
8. Nelson, D. L.; Cox, M. M.; Lehninger, A. L., *Principles of Biochemistry*. 7th edition ed.; W.H. Freeman: New York, 2017.
9. Dobryakov, A. L.; Kovalenko, S. A.; Weigel, A.; Pérez-Lustres, J. L.; Lange, J.; Müller, A.; Ernsting, N. P.; N., T. A.; W., G.; M., J.; C., B.; M., C., Femtosecond

Pump/Supercontinuum-Probe Spectroscopy: Optimized Setup and Signal Analysis for Single-Shot Spectral Referencing. *Rev. Sci. Instrum.* **2010**, *81* (11), 113106.

Chapter 3

Ultrafast Dynamics of Photodetached

Tyrosinate Dianion in Aqueous Solution

3.1. Introduction

The photoionization of biomolecules in an aqueous system involves the interaction of high-energy radiation with biological matter to yield the corresponding radical species and the hydrated electron. The formation of these reactive species in turn triggers a plethora of chemical reactions that form the basis of radiation chemistry and radiation biology.¹⁻³ The investigation of radiation damage involving redox-active amino acids, such as tyrosine, tryptophan, and phenylalanine, is particularly pertinent due to their facile oxidation, which is in turn afforded by their relatively low ionization potentials. In the gas phase, the vertical ionization energies of redox-active amino acids are $\sim 1 - 2$ eV lower than those with aliphatic side chains.^{4,5} In aqueous solution, the lowest vertical ionization energies of tyrosine, tryptophan, and phenylalanine have been measured to be 7.8 eV, 7.3 eV, and 8.8 eV, respectively.⁶ These vertical ionization energies are downshifted by $\sim 0.6 - 0.7$ eV relative to the gas-phase values⁵ due to the stabilization of the radical cation final state by the polar solvent environment.

In this chapter, we focus on tyrosine (Tyr), an amino acid with a phenolic side chain. Tyrosine participates in biological activities such as protein phosphorylation,⁷⁻¹⁰

deoxyribonucleic acid (DNA) repair,¹¹⁻¹⁴ and photoreception.¹⁵ A key intermediate in many of these processes is the tyrosyl radical (Tyr•), whose involvement in proton-coupled electron transfer (PCET) has been the subject of extensive experimental and theoretical studies.^{11,13-19} Early microsecond flash photolysis and nanosecond laser experiments have elucidated the formation of the tyrosyl radical *via* the triplet state of tyrosine and its subsequent bimolecular reactions.²⁰⁻²² Ultraviolet resonance Raman (UVR)R^{23,24} and Fourier-transformed infrared (FTIR) spectroscopies²⁵ have identified the characteristic vibrational frequencies of the tyrosyl radical, both in aqueous solution and in a peptide matrix.²⁶⁻²⁸

While numerous studies have unraveled the dynamics of the hydrated electron produced by the photoionization of aqueous solutes,²⁹⁻³⁵ investigations of the ultrafast dynamics of the accompanying radical species are relatively limited. Here, building upon our earlier work on the photodetachment of aqueous phenoxide ion,³⁶ we aim to elucidate the ultrafast vibrational wave packet dynamics of the tyrosyl radical anion (Tyr•⁻) triggered by the photodetachment of the tyrosinate dianion (Tyr²⁻; Fig. 3.1) in aqueous solution. When driven by an ultrashort laser pulse, photodetachment triggers ultrafast vibrational wave packet dynamics involving vibrational modes along which the equilibrium geometries before and after electron ejection are displaced.³⁶ Vibrational modes that appear in the wave packet dynamics therefore encode structural reorganization. In our experiment, vibrational coherences will be detected by near-ultraviolet transient absorption spectroscopy, performed with ~10-fs time resolution. This time-domain approach complements energy-resolved measurements of the Franck-Condon progression by photoelectron spectroscopy. For amino acids in aqueous solution, however, their Franck-Condon progression is obfuscated by the ~1-eV-wide, inhomogeneously broadened valence photoelectron bands.⁶

Figure 3.1. Structure of the tyrosinate dianion (Tyr^{2-}). The carbon, hydrogen, oxygen and nitrogen atoms are in gray, white, red and blue, respectively.

Aside from its relevance to radiation chemistry and biological redox reactions, the choice of the tyrosine for the present study is also motivated by several practical considerations. First, the estimated vertical ionization energy of the tyrosinate dianion (~ 7.1 eV)^{6,37} is lower compared to those of liquid water (11.2 eV)³⁸⁻⁴¹ and aqueous hydroxide (9.2 eV).⁴² When driving photodetachment in the strong-field limit, the lower vertical ionization energy of the tyrosinate dianion favors its photodetachment and minimizes contributions from the photodetachment of aqueous hydroxide and the solvent to the transient absorption signal. Second, the dianion form is more water-soluble (60.0 mM at pH 13) than the neutral form (2.7 mM at the isoelectronic point, which is the pH the amino acid has no net charge),⁴³ hence further increasing the contribution from the photodetachment of tyrosinate dianion to the transient absorption signal. Third, the absorption signature of the tyrosyl radical at ~ 400 nm^{22,44,45} is located away from the strong absorption peak of the hydrated electron byproduct at ~ 715 nm,^{46,47} thus enabling a clear view of the tyrosyl radical absorption spectrum evolving in time due to vibrational wave packet motion.

In this chapter, we present the ultrafast vibrational wave packet dynamics induced by the photodetachment of the tyrosinate dianion in aqueous solution. We identify the vibrational modes of the tyrosyl radical anion along which structural reorganization occurs upon photodetachment. Our experimental results are complemented by density functional theory (DFT) calculations of vibrational frequencies of the aqueous tyrosyl radical anion. This study provides further insights into the ultrafast structural dynamics that accompany the interaction of ionization radiation with biological matter.

3.2. Materials and Methods

3.2.1. Theoretical Calculations

The calculated equilibrium geometries and vibrational spectroscopic data of tyrosinate dianion and tyrosyl radical anion are performed and obtained with the GAUSSIAN 16 program.⁴⁸ DFT method is employed where the hybrid exchange functional of Becke (B3)⁴⁹ and the correlation functional of Lee, Yang and Parr (LYP)⁵⁰ are used. The split-valence triple- ξ with d-orbital and diffuse functions on heavy atoms (carbon, nitrogen and oxygen) and p-orbital and diffuse functions on hydrogen atoms, 6-311G++(d,p) atomic basis set is employed. Two models of the tyrosinate dianion and tyrosyl radical anion are constructed. The first model is the isolated tyrosinate dianion and tyrosyl radical anion, while the second model is the microhydrated version. The microhydrated species includes explicit water molecules hydrogen bonded to the oxygen atom of the phenolic sidechain. Furthermore, a polarizable continuum model (PCM) is included for both models to mimic the interaction of the amino acid and radical with the surrounding water solvent.⁵¹ The water molecules present in the microhydrated species are fully relaxed during the geometry optimization. Each geometry optimization is followed by

vibrational frequency calculation to verify the optimized geometry corresponds to a local minimum.

3.2.2. Experimental Method

L-tyrosine (Sigma-Aldrich, $\geq 98\%$ HPLC) and sodium hydroxide (VWR Chemicals, 98.9% pellets) are used as received. Since L-tyrosine has relatively low solubility in water (2.7 mM at isoelectronic point),⁴³ L-tyrosine is dissolved and sonicated in sodium hydroxide solution to produce a tyrosinate dianion solution with concentration of 150 mM at pH 14. Given the phenol sidechain pK_a of 10.07,⁵² we expect $> 99.9\%$ deprotonation of the phenolic OH at pH 14. Thus, tyrosine exists in our experiment in the dianionic form.

The experimental procedure has been described in detailed in Chapter 2. In summary, the transient absorption pump-probe experiment is performed with a chirped-pulse-amplified Ti:sapphire laser system (Coherent, Legend Elite Duo-USX), with an output of 4.5-mJ, 30-fs pulses at 1-kHz repetition rate and 800-nm center wavelength, with a bandwidth of 50 nm. The pulses undergo self-phase modulation in a helium-filled hollow-core fiber before they are temporally compressed by chirped mirrors (Ultrafast Innovations GmbH, PC1332). The compressed pulses are incident on a beam splitter (Layertec, 131191), with 80% of the transmitted beam (~ 0.6 mJ) being directed to a 4f visible-NIR pulse shaper with a 128-element spatial light modulator (SLM) (Biophotonics Solutions, FemtoJock-P) for further pulse compression, which truncates the wavelength range of the visible-NIR from 520 nm to 750 nm, with a center wavelength of 630 nm (1.97-eV photon energy). The visible-NIR spectrum is shown in Fig. 3.2(a). Transform-limited laser pulses with full-width half-maximum (FWHM) duration of 5.2 fs are obtained by using multiphoton intrapulse interference phase scan (MIIPS) to measure the spectral phase at the sample, followed by using the SLM to introduce an

oppositely signed phase to the laser pulses, thus compressing it to the transform-limited duration.⁵³ Subsequently, a second-order interferometric auto-correlation trace is recorded by multiple independent comb shaping (MICS).⁵⁴ The second-order interferometric auto-correlation trace is shown in Fig. 3.3(a). The transform-limited pulses, which function as the strong-field ionizing pump, are directed to the sample. The ionizing pulse is then focused onto the sample by a 50-cm focal length spherical mirror. A maximum pulse energy available for experiments is 25 μJ , with a beam waist ($1/e^2$) of 120 μm , resulting in a peak intensity of $2.0 \times 10^{13} \text{ W cm}^{-2}$ for the photodetachment process. An optical delay stage line, driven by a piezo translation stage (Physik Instrumente, N-664.3A) in the ionizing pump's path provide a variable time delay between pump and probe pulses in 5-fs steps.

The remaining output from the chirped mirror compressor passes through a pair of wedges (Femtolasers, OA124), followed by a 50- μm -thick Type I beta-barium borate (BBO) crystal (Casix, $\theta = 29.0^\circ$) to generate the UV probe *via* second harmonic generation (SHG). The broadband near-UV probe spans 360 nm to 470 nm. The broadband near-UV probe spectrum is shown in Fig. 3.2(b). A pair of dichroic beamsplitters (Layertec, 109433) is introduced to eliminate residual fundamental beam. The near-UV beam is then split off by a reflective neutral density beamsplitter (Newport, FRQ-ND05) to produce the probe and reference pulses. The reflected near-UV probe is focused onto the sample by a 15-cm focal length off-axis parabolic reflector (Thorlabs, MPD369H-F01). The focused near-UV probe at the sample target has a beam waist ($1/e^2$) of 42 μm , with a pulse energy of 17 nJ. The peak intensity of the near-UV probe is $5.2 \times 10^{10} \text{ W cm}^{-2}$.

A slit nozzle (Metaheuristics, Type L) is used in conjunction with a peristaltic pump (Cole-Parmer, 07528-10) and a pulse dampener to produce a microjet of the tyrosinate dianion solution for experiments. The volume flow rate of 92 mL min^{-1} translates to a vertical flow rate

Figure 3.2. (a) The visible-NIR ionizing pump spectrum ranges from 520 nm to 750 nm, with a center wavelength of 630 nm. (b) The near-UV probe spectrum ranges from 360 nm to 470 nm, generated *via* SHG.

of approximately 8.5 mm ms^{-1} . The relatively high vertical flow rate ensures that fresh volume of tyrosinate dianion solution is exposed to each ionizing pump pulse. A liquid jet of $7\text{-}\mu\text{m}$ thickness is produced, which yields a sizeable differential absorption signal ($\Delta A \sim 0.1$), while minimizing the temporal broadening of the pulses in the liquid jet due to dispersion. In addition, a thin liquid jet limits the group-velocity mismatch between the pump and probe to $< 1 \text{ fs}$, making its effect on the time resolution negligible.

A synchronized optical chopper in the ionizing pump's path before the sample allow modulation of the ionizing pump at a frequency of 0.5 kHz. The probe beam that is transmitted through the microjet is focused into a 300-nm spectrograph (Princeton Instruments, Acton SP2300), equipped with a 600-grooves per mm grating blazed at 500-nm and a 1024-pixels silicon (Si) photodiode array detector (Stresing, S8381-1024Q) with a read-out rate of 1 kHz, which allow simultaneous acquisition of the probe spectra obtained with and without the ionizing pump pulse. The reference near-UV pulses are focused to an identical spectrograph, which is equipped with similar grating and photodiode array at 1-kHz repetition rate.

Figure 3.3. (a) A second-order interferometric auto-correlation trace of the visible-NIR ionizing pump spectrum, measured in a 10- μm -thick, $\theta = 37.0$ deg Type I BBO crystal, reveals a pulse duration with full-width half-maximum (FWHM) of 5.2 fs. (b) The pump-probe cross-correlation, measured *via* difference frequency mixing in a 10- μm -thick, $\theta = 29.2$ deg Type I BBO crystal, reveals a FWHM of 12 fs.

Referenced detection reduces noise caused by shot-to-shot fluctuations of the probe beam. The Si photodiode array detectors are interfaced to desktop computers for automated data acquisition.

The focal spot size of the probe beam is $\sim 3\times$ smaller than that of the ionizing pump beam, hence minimizing the effect of spatial averaging on the observed ultrafast dynamics. The cross-correlation of the pump and probe, by measuring the difference frequency generated (DFG) beam by utilizing a 10- μm thick Type I BBO crystal (Casteck, $\theta = 29.2^\circ$) yields an instrument response of 12 fs at FWHM. The cross-correlation trace is displayed in Fig. 3.3(b).

Static UV-visible measurements are performed with a double-beam UV-visible-NIR spectrophotometer (Agilent Technologies, Cary 300) with a 1-mm path length quartz cuvette by measuring absorbance. The spectrophotometer has a wavelength range from 190 to 800 nm.

3.3. Results

3.3.1. Theoretical Calculations

The equilibrium geometries of the isolated and microhydrated tyrosinate dianion and tyrosyl radical anion are displayed in Fig. 3.4. The equilibrium geometry of the microhydrated tyrosyl radical anion includes three water molecules hydrogen bonded to the oxygen atom of the phenoxide sidechain, which has a distance of 2.84 – 2.90 Å. The hydrogen donor-acceptor distance is consistent with the typical hydrogen bonding distance of 2.70 – 3.00 Å.^{55,56} Furthermore, the equilibrium geometry corresponds to a local minimum in terms of energy and produce real vibrational frequencies. The full vibrational frequencies and assignments of the tyrosyl radical anion are displayed in the Appendix A. Note that the numbered assignments denote benzene ring modes in the Wilson notation.^{57,58}

Figure 3.4. The equilibrium geometries of **(a)** the tyrosinate dianion, **(b)** the tyrosyl radical anion, **(c)** the microhydrated tyrosinate dianion and **(d)** the microhydrated tyrosyl radical anion with PCM solvation. The microhydrated species includes three water molecules directly hydrogen bonded to the oxygen atom of the phenoxide sidechain. The carbon, hydrogen, oxygen and nitrogen atoms are in gray, white, red and blue respectively.

3.3.2. Transient Absorption of Photodetached Aqueous Tyrosinate Dianion

Firstly, a power-dependence measurement is performed to determine the photon order of the strong-field photodetachment process of the tyrosinate dianion in aqueous solution. For an N -photon photodetachment process, the differential absorption signal, ΔA , scales with intensity of the ionizing pump, I , as shown in the following relationship

$$\Delta A \propto I^N. \quad (3.1)$$

The photon-order measurement is performed by using a visible-NIR probe, which has a spectral range of 520 – 750 nm (similar to the spectrum of the ionizing pump). The photon-order measurement measures the absorption of the ground-state hydrated electron at 720 nm at a pump-probe time delay of 5 ps. Since the hydrated electron is produced alongside upon the photodetachment of tyrosinate dianion and hydroxide ion, the ground-state hydrated electron absorption signal is proportional to the extent of the photodetachment process.

Several factors are taken into consideration in order to determine the photon order accurately. During the photodetachment of the aqueous tyrosinate dianion, there is a possibility that electrons are produced from both the tyrosinate dianion and hydroxide ion, even though the photodetachment of the solute is more likely to occur due to its significantly lower vertical ionization potential. In our study, the ΔA against ionizing pump intensity spectrum is recorded for both aqueous tyrosinate dianion and aqueous sodium hydroxide under similar experimental conditions. A difference spectrum, $\Delta\Delta A$, is then obtained, which corresponds to solely the ΔA signal from the photodetachment of tyrosinate dianion. The $\Delta\Delta A$ signal is then fitted with the following expression.

$$\Delta\Delta A = \frac{\alpha * I^N}{1 + \left(\frac{I}{I_{sat}}\right)^N}, \quad (3.2)$$

where α is the amplitude, N is the photon order and I_{sat} is the saturation intensity. The converged fit when $N = 3$ yields $\alpha = (6.90 \pm 0.06) \times 10^{-42} \text{ cm}^6 \text{ W}^{-3}$ and $I_{sat} = (1.67 \pm 0.01) \times 10^{13} \text{ W cm}^{-2}$. The value of N indicates that the photodetachment of tyrosinate dianion in aqueous environment occurs *via* a three-photon process. A log-log plot of $\Delta\Delta A$ against pump intensity is shown in Fig. 3.5.

The differential absorption, $\Delta A(\lambda, t)$, spectrum of the photodetached aqueous tyrosinate dianion is displayed in Fig. 3.6(a) as a function of pump-probe time delay. The multiphoton photodetachment of tyrosinate dianion yields the tyrosyl radical anion ($\text{Tyr}^{\bullet-}$) and the corresponding hydrated electron. $\text{Tyr}^{\bullet-}$ has an absorption band peaking at 413 nm and a vibronic transition at 400 nm, consistent with previous studies of the tyrosyl radical in aqueous solution.^{22,45} In addition, the increase in ΔA signal at longer wavelength beyond 430 nm is due to the absorption of the ground-state hydrated electron, which spans 400 – 1000 nm.^{46,47}

Previous studies have shown that 4.68-eV (265 nm) photoexcitation of tyrosine to the S_1 ($\pi\pi^*$) state yields the tyrosyl radical *via* a two-photon process involving the lowest-energy triplet state of tyrosine as an intermediate.²² In the present study, however, we eliminate the possibility that the tyrosyl radical anion is formed *via* the electronically excited states of the tyrosinate dianion. This is evident from the pump-power dependent measurements that show the photodetachment occurs directly *via* a three-photon process (Fig. 3.5). While the vertical ionization energy of the tyrosinate dianion is unknown, we note that tyrosine and phenol have identical vertical ionization energies of 7.8 eV.⁶ As such, we expect the vertical ionization energies of tyrosinate dianion and phenoxide to be similar; the latter is reported to be 7.1 eV,³⁷ with the photoelectron band extending to <6-eV binding energy. Considering that the bottom

Figure 3.5. Photon-order measurement for strong-field photodetachment of tyrosinate dianion. Log-log plot of $\Delta\Delta A$ vs. pump intensity after subtraction of the response from aqueous sodium hydroxide.

of the conduction band of liquid water resides ~ 1 eV below the vacuum level,⁵⁹ the total energy input ~ 5.9 eV that is associated with the three-photon photodetachment process is sufficient to remove an electron from the tyrosinate dianion and inject it into the conduction band of liquid water.

Global fitting of the time-resolved ΔA spectra over the 370 – 455 nm probe wavelength range and 120 fs after time-zero to the function

$$\Delta A(\lambda, t) = \Delta A_0(\lambda) + \Delta A_1(\lambda)(e^{-t/\tau_{d1}} - e^{-t/\tau_{r1}}) + \Delta A_2(\lambda)(e^{-t/\tau_{d2}} - 1), \quad (3.3)$$

where $\Delta A_0(\lambda)$, $\Delta A_1(\lambda)$ and $\Delta A_2(\lambda)$ are spectral amplitudes, τ_{r1} and τ_{d1} reflect the time constants associated with the rise and decay of the hydrated electron population, respectively, and τ_{d2} is an additional decay component. To avoid contributions from coherent artifacts, the global fitting does not include the vicinity of time-zero. For the photodetachment of an NaOH-

Figure 3.6. (a) Time-resolved differential absorption, $\Delta A(\lambda, t)$, spectrum of photodetached aqueous tyrosinate dianion as a function of pump-probe time delay. **(b)** Time traces at 413 nm of photodetached aqueous tyrosinate dianion (in red) and photodetached aqueous sodium hydroxide (in blue). Note the offset that is present in the photodetached aqueous tryosinate dianion time trace, which indicates the immediate formation of tyrosyl radical upon photodetachment. In addition, global fit analysis on both time-traces from 120 fs is performed, where the best-fit line is shown in black.

only aqueous solution, we obtain $\tau_{r1} = 0.42 \pm 0.01$ ps and $\tau_{d1} = 54 \pm 1$ ps, while for the photodetachment of tyrosinate dianion, we obtain $\tau_{d2} = 0.93 \pm 0.02$ ps. The time traces of photodetached tyrosinate dianion and photodetached sodium hydroxide at 413 nm with the respective fit function is shown in Fig. 3.6(b).

3.3.3. Vibrational Wave Packet Dynamics of Tyrosyl Radical Anion

The time-resolved $\Delta A(\lambda, t)$ spectrum of photodetached aqueous tyrosinate dianion reveals amplitude modulations as a function of time delay, suggesting the presence of vibrational wave

packet dynamics. We exclude contributions from impulsive stimulated Raman scattering (ISRS) of tyrosinate dianion to the observed wave packet dynamics because the $\Delta A(\lambda, t)$ signal and time-domain oscillations disappear when the ionizing pump power is attenuated to suppress the strong-field photodetachment. Furthermore, the absorption spectrum of aqueous tyrosinate dianion does not overlap with the probe spectral range. This is evident from the absorbance spectrum of 3 mM tyrosinate dianion solution at pH 14, shown in Fig. 3.7, which has a maximum absorption at 293 nm.⁶⁰

To analyze the vibrational wave packet dynamics quantitatively, we compute the spectral first-moment, $\langle E(t) \rangle$, time trace given as

$$\langle E(t) \rangle = \frac{\int_{E_i}^{E_f} E \Delta A(E, t) dE}{\int_{E_i}^{E_f} \Delta A(E, t) dE}, \quad (3.4)$$

where E represents the probe photon energy, t is the pump-probe time delay and $\Delta A(E, t)$ is the differential absorbance as a function of probe photon energy and pump-probe time delay. E_i and E_f are defined as the spectral range that is used to compute the spectral first-moment – for this case, E_i and E_f is 2.83 eV (438 nm) and 3.17 eV (391 nm) respectively. The resultant $\langle E(t) \rangle$, after removing the slow population dynamics, is shown in Fig. 3.8(a); a zoomed-in view of $\langle E(t) \rangle$ is shown in the inset of Fig. 3.8(a).

The spectral first-moment time trace encodes the vibrational wave packet, and this can be seen from the oscillatory features of the first-moment time trace. These oscillatory features arise from vibrational wave packet motion of the tyrosyl radical anion. Wave packet motion along the vibrational mode Q_i requires photodetachment to project the vibrational probability density of Tyr^{2-} vertically onto the potential energy curve of $\text{Tyr}^{\bullet-}$, and for the potential energy surfaces of Tyr^{2-} and $\text{Tyr}^{\bullet-}$ to be displaced along Q_i . From the Franck-Condon region of the $\text{Tyr}^{\bullet-}$ potential, temporal evolution of the coherent superposition of vibrational eigenstates

Figure 3.7. Static UV-visible absorption spectrum of 3 mM tyrosinate dianion in aqueous sodium hydroxide (pH 14), measured with a 1-mm path length quartz cuvette. The absorption maximum is at 293 nm.

leads to vibrational wave packet motion. This wave packet in turn modulates the probe transition energy of the $\text{Tyr}^{\bullet-}$ anion radical, giving rise to the observed oscillations in the first-moment time trace. It is important to note that vibrational wave packet motion can be initiated by such a displacive mechanism only when the equilibrium geometries of Tyr^{2-} and $\text{Tyr}^{\bullet-}$ are displaced along the given vibrational mode Q_i . The vibrational modes can be identified by their characteristic frequencies, in turn obtained from the fast-Fourier transform (FFT) power spectrum of the first-moment time trace, as shown in Fig. 3.8(b), in red. These frequencies and their relative FFT powers are summarized in Table 3.1.

A control experiment with aqueous sodium hydroxide (pH 14) under similar photodetachment conditions confirms that the oscillatory features observed in the first-moment time trace originate from photodetached tyrosinate dianion. The FFT power spectrum of photodetached aqueous sodium hydroxide is shown in Fig. 3.8(b), in blue. Here, the only

Figure 3.8. (a) First-moment, $\langle E(t) \rangle$, time trace of photodetached aqueous tyrosinate dianion (in black) with fit function (in red). The zoomed-in view of $\langle E(t) \rangle$ time trace from 0.25 ps to 1 ps is shown in the inset. (b) FFT power spectra of photodetached aqueous tyrosinate dianion (in red) and photodetached aqueous sodium hydroxide (in blue). The peaks correspond to vibrational frequencies associated with tyrosyl radical anion. Note that the oxygen and nitrogen vibrational frequencies are observed at 1554 cm^{-1} and 2328 cm^{-1} respectively. The zoomed-in view of FFT power spectra from 0 cm^{-1} to 250 cm^{-1} is shown in the inset.

observed frequencies are 1554 cm^{-1} and 2328 cm^{-1} , which correspond to the stretching frequencies of oxygen (O_2) and nitrogen (N_2) stretching frequencies respectively.^{61,62} These frequencies also present in the FFT power spectrum of the photodetached tyrosinate dianion, are due to ISRS of N_2 and O_2 , which in turn arise from the experiments being performed in air. The estimated vibrational frequency of the hydroxyl radical produced by photodetachment of OH^- is $\sim 3880 \text{ cm}^{-1}$, which corresponds to the O–H stretching.⁶³ Since this frequency is beyond the high-frequency cut-off of our experiment, we conclude that the remaining seven vibrational frequencies reported in Table 3.1 are associated with the tyrosyl radical anion.

Aside from FFT, the first-moment time trace can also be analyzed in the time domain by fitting it to a sum of exponentially damped cosine functions

$$\langle E(t) \rangle = \sum_{i=1}^n A_i e^{-\frac{t}{\tau_i}} \cos(\omega_i t + \phi_i), \quad (3.5)$$

where A_i , τ_i , ω_i and ϕ_i are the amplitude, dephasing time, vibrational frequency and phase of the vibrational mode Q_i respectively. The fit parameters are summarized in Table 3.1. Compared to FFT, time-domain analysis directly furnishes the dephasing times of the vibrational modes. The calculated vibrational frequencies reported in Table 3.1 are those of the microhydrated model, the assignments should be considered tentative due to the presence of multiple calculated vibrational frequencies in the vicinity of each of the experimentally observed frequency. Although including explicit water molecules of solvation does not shift the vibrational frequencies that are higher than 200 cm^{-1} appreciably ($\sim 1\%$ or less).

Table 3.1. Experimental FFT power, time-domain analysis results, and calculated frequencies of the tyrosyl radical anion in aqueous solution and their assignments. The calculated frequencies are based on the equilibrium geometry of the microhydrated tyrosyl radical anion. Numbered assignments denote benzene ring modes in the Wilson notation.^{57,58}

Experimental							Calculated Freq. (cm ⁻¹)	Assignment
FFT Analysis			Time-Domain Analysis					
Freq. (cm ⁻¹)	Power (norm.)	Freq. (cm ⁻¹)	Amplitude (meV)	Dephasing Time (ps)	Phase (π -rad)			
476	6.0×10^{-2}	483 $\pm$ 1	0.45 $\pm$ 0.06	0.90 $\pm$ 0.14	1.06 $\pm$ 0.04	482	CCC bend (6a)	
825	1.9×10^{-1}	816 $\pm$ 2	7.29 $\pm$ 0.84	0.19 $\pm$ 0.01	0.77 $\pm$ 0.04	832	CCC bend (1)	
978	6.8×10^{-2}	1000 $\pm$ 3	2.19 $\pm$ 0.46	0.25 $\pm$ 0.03	0.74 $\pm$ 0.07	994	CCC trigonal bend (19a)	
1166	9.1×10^{-2}	1172 $\pm$ 1	0.41 $\pm$ 0.06	1.30 $\pm$ 0.37	0.54 $\pm$ 0.06	1185	Benzene CH bend (8a)	
1211	1.3×10^{-1}	1210 $\pm$ 1	1.03 $\pm$ 0.13	0.65 $\pm$ 0.10	0.30 $\pm$ 0.05	1240	Benzene CH bend / CC stretch (13)	
1516	1	1515 $\pm$ 1	2.01 $\pm$ 0.09	0.77 $\pm$ 0.06	1.69 $\pm$ 0.02	1520	CO stretch (20a)	
1606	1.9×10^{-1}	1601 $\pm$ 1	1.11 $\pm$ 0.09	0.73 $\pm$ 0.10	0.60 $\pm$ 0.03	1626	CC stretch (9a)	
1552	8.1×10^{-1}	1554 $\pm$ 1	0.40 $\pm$ 0.03	3.67 $\pm$ 0.55	0.45 $\pm$ 0.01		Oxygen	
2324	7.1×10^{-1}	2328 $\pm$ 1	0.47 $\pm$ 0.03	4.01 $\pm$ 0.45	0.56 $\pm$ 0.01		Nitrogen	

3.4. Discussion

3.4.1. Global Fit Analysis

The formation and subsequent decay of the hydrated electron is modeled by sequential first-order kinetics (see the second term in Eq. 3.3). Hydrated electron formation involves the trapping of the electron that is initially injected into the conduction band by photodetachment⁶⁴ followed by vibrational cooling; this entire process can be modeled by first-order kinetics.²⁹⁻³⁵ Since the hydrated electron depopulation occurs *via* geminate recombination, a process that exhibits nonexponential decay behavior,^{29,65} the exponential decay constant that is extracted from our analysis should be regarded as merely an approximation to the decay of the hydrated electron population by geminate recombination and observed within a very narrow (<4 ps) time-delay window. Moreover, in view of an earlier finding that electron-ion recombination dynamics involving solutes in aqueous solution is considerably slower than the diffusion limit,⁶⁶ which suggests negligible recombination between tyrosyl radical anions and hydrated electrons on the few-picosecond timescale in our experiments, we employ the same time constants as those obtained from aqueous NaOH in our analysis. While a detailed analysis of the geminate recombination dynamics lies beyond the scope of the present work, a future study that varies the concentration of the Tyr²⁻ dianion solute and extends the pump-probe measurements to >0.1 ns time delays would allow one to disentangle the relative contributions of the geminate recombination channels that involve the hydroxyl radicals and the tyrosyl radical anions.

Extrapolating the $\Delta A(\lambda, t)$ time traces (Fig. 3.6(b)) collected for the photodetached tyrosinate dianion to time zero reveals an offset $\Delta A_0(\lambda)$, which indicates the formation of the tyrosyl radical anion immediately upon strong-field photodetachment (Fig. 3.9, in red). This

Figure 3.9. (a) The wavelength-dependent offset values, $\Delta A_0(\lambda)$, obtained from the global fitting of photodetached aqueous sodium hydroxide (in blue) and photodetached aqueous tyrosinate dianion (in red) time traces. The fitting errors are comparable to the size of the symbol. **(b)** The wavelength-dependent $\Delta A_2(\lambda)$ value obtained from the global fitting (in red) and ΔA spectrum of photodetached aqueous tyrosinate dianion at 2 ps (in blue).

offset is noticeably smaller for the time traces for photodetached aqueous sodium hydroxide obtained under similar experimental conditions (Fig. 3.9(a), in blue), consistent with the fact that the hydroxyl radical produced by the photodetachment of aqueous sodium hydroxide, does not exhibit any appreciable absorption in the >400 -nm region ($\epsilon_{OH} = 30 \text{ M}^{-1} \text{ cm}^{-1}$ at 400 nm).⁶⁷ The wavelength-dependent offset $\Delta A_0(\lambda)$ that is obtained for the tyrosinate dianion corresponds to the absorption spectrum of the tyrosyl radical anion at the Franck-Condon region, generated immediately upon photodetachment, prior to vibrational cooling. Compared to the $\Delta A(\lambda, t)$ spectrum for the tyrosyl radical reported in the literature,^{22,44,45} the additional broadening of $\Delta A_0(\lambda)$ towards the long-wavelength side is suggestive of a vibrationally excited tyrosyl radical anion that is produced immediately upon photodetachment. On the other hand, $\Delta A_0(\lambda)$ of the sodium hydroxide shows a gradual increase with decreasing probe wavelength, following the absorption spectral signature of the hydroxyl radical.⁶⁷

Global analysis using Eq. 3.3 gives $\tau_{d2} = 0.93 \pm 0.02$ ps. Based on previous experimental results that reveal the stability of tyrosyl radical with respect to dissociation,⁶⁸ we exclude the possibility that the decay of the ΔA signal arises from photofragmentation. The appearance of the decay associated spectrum $\Delta A_2(\lambda)$ is suggestive of the decay of the ΔA signal in the long-wavelength region ($\lambda > 420$ nm) and the sharpening of the 400-nm and 413-nm absorption maxima of the $\text{Tyr}^{\bullet-}$ radical spectrum. As such, we ascribe τ_{d2} to vibrational relaxation. A similar analysis performed on photodetached phenoxide anions in aqueous solution yields a vibrational relaxation timescale of 1.02 ± 0.03 ps,³⁶ suggesting a small but noticeable increase in the vibrational relaxation due to the amino acid backbone. Due to the limited range of time delays (~ 4 ps) sampled in our experiments, however, we cannot rule out the possible existence of slower vibrational relaxation pathways.

Since $\Delta A_1(\lambda)$ tracks the formation of the hydrated electron and its subsequent decay by geminate recombination, it should follow the absorption spectrum of the hydrated electron. Using the relation

$$\Delta A_1(\lambda) = \varepsilon_e(\lambda)c_e L, \quad (3.6)$$

where $\varepsilon_e(\lambda)$ is the molar absorption coefficient of the hydrated electron, c_e is the initial concentration of hydrated electrons produced by strong-field photodetachment of both the tyrosinate dianion and the sodium hydroxide, and L is the sample path length. By plotting a graph of $\Delta A_1(\lambda)$ as a function of ε_e (Fig. 3.10), we can obtain the concentration of hydrated electron, c_e . Using the relation in Eq. 3.6, the gradient obtained from the linear fit is $c_e L = (3.08 \pm 0.02) \times 10^{-5}$ M cm. Since $L = 7 \pm 1$ μm , we obtain $c_e = 44 \pm 6$ mM.

To obtain an estimate of the concentration of the tyrosyl radical anion, we employ the relation

Figure 3.10. Plot of $\Delta A_1(\lambda)$ as a function of molar extinction coefficient of hydrated electron, ε_e , and the linear fit function, in red. The gradient of the linear fit function corresponds to the product of the concentration of hydrated electron, c_e and the sample path length, L .

$$\Delta A_0(\lambda) = [\varepsilon_{\text{Tyr}}(\lambda)c_{\text{Tyr}} + \varepsilon_{\text{OH}}(\lambda)c_{\text{OH}}]L, \quad (3.7)$$

where ε_{Tyr} (ε_{OH}) and c_{Tyr} (c_{OH}) are the molar absorption coefficients and initial concentrations of the vibrationally hot tyrosyl radical anion (vibrationally hot hydroxyl radical), respectively. In the absence of the molar absorption coefficients of the vibrationally hot species, we employ the molar absorption coefficients of the vibrationally relaxed species at 410 nm, reported in the literature for tyrosyl radical ($\varepsilon_{\text{Tyr}} = 2750 \text{ M}^{-1} \text{ cm}^{-1}$)⁶⁹ and hydroxyl radical ($\varepsilon_{\text{OH}} = 23 \text{ M}^{-1} \text{ cm}^{-1}$),⁶⁷ to obtain $c_{\text{Tyr}} = 30 \pm 10 \text{ mM}$ and $c_{\text{OH}} = 14 \pm 8 \text{ mM}$. This result suggests that, under our experimental conditions, the photodetachment of tyrosinate dianion (150 mM) dominates over that of hydroxide ion, and the photodetachment yield of tyrosinate dianion is ~20%.

3.4.2. Vibrational Wave Packet Dynamics of Tyrosyl Radical Anion

The vibrational modes observed from the spectral first-moment time trace would only be observed when, for a given vibrational mode, there is a non-zero displacement between the potential energy curves of tyrosinate dianion and tyrosyl radical anion. The seven vibrational frequencies that are associated with the tyrosyl radical anion are the vibrational modes coupled to the photodetachment process. From Table 3.1, the majority of the vibrational modes have dephasing times on the order of ~ 1 ps, comparable to those observed for other molecules in the condensed phase.^{70,71} It is interesting to compare the photodetachment-induced vibrational wave packet dynamics of the tyrosyl radical anion and the phenoxyl radical. The vibrational wave packet dynamics triggered by the photodetachment of aqueous phenoxide is characterized by an FFT power spectrum that is strongly peaked at 527 cm^{-1} .³⁶ This vibrational frequency, assigned to the benzene CCC bend, also dominates the Franck-Condon progression observed in the gas-phase photodetachment spectrum of phenoxide.⁷² On the other hand, the largest FFT power observed in the photodetachment-induced vibrational coherence of the tyrosyl radical anion is associated with the C–O stretch at 1515 cm^{-1} (Fig. 3.8(b), in red); in comparison, the FFT power of the corresponding CCC bending mode in the tyrosyl radical anion, downshifted to 483 cm^{-1} , is $>10\times$ weaker. Aside from changes in the relative FFT power, the vibrational modes at 816 and 1000 cm^{-1} of the tyrosyl radical anion also exhibit markedly shorter dephasing times that are $\sim 3\times$ shorter than those for the corresponding modes of the phenoxyl radical. This faster dephasing is suggestive of anharmonic coupling of these benzene ring modes to the amino acid backbone of tyrosyl radical anion.

The displacement vectors of the vibrational modes that contribute to the first-moment time trace are largely localized on the phenoxide moiety, which are shown in Fig. 3.11. This

Figure 3.11. Vibrational modes of tyrosyl radical anion coupled to the photodetachment, corresponding to the calculated frequencies **(a)** 482 cm^{-1} **(b)** 832 cm^{-1} **(c)** 994 cm^{-1} **(d)** 1185 cm^{-1} **(e)** 1240 cm^{-1} **(f)** 1520 cm^{-1} **(g)** 1626 cm^{-1} . The blue arrows indicate the direction and magnitude of the displacement of the atoms.

result can be rationalized by considering the electronic character of the orbital from which the electron is ejected. Strong-field processes preferentially eject the most weakly bound electrons

from molecules. Within a molecular orbital picture, this implies that the electron that lies in the highest occupied molecular orbital (HOMO) of the tyrosinate dianion has the highest probability of being removed by strong-field photodetachment. The calculated HOMO is primarily localized on the phenoxide sidechain, which is shown in Fig. 3.12. Removal of an electron from the HOMO therefore leads to vibrational wave packet motion encoding primarily the structural rearrangement of the phenoxide sidechain, as seen from the few-picometer changes in the C–O and C–C bond lengths of microhydrated tyrosinate dianion and tyrosine radical anion (Table 3.2). In addition to the phenoxide moiety, the HOMO also bears a significant albeit minor contribution from the α and β carbon atoms, which explains the calculated changes in the C–C bond distances involving the amino acid backbone; the C–C stretching character is present in the vibrational modes with frequencies 832, 1240 and 1626 cm^{-1} . While the change in the C–O bond length is comparable to that calculated at the same level of theory for the photodetachment of phenoxide, and changes in the C–C bond lengths differ from those calculated for phenoxide,³⁶ suggesting that the amino acid backbone influences the relative contributions of the various vibrational modes to the structural rearrangement. This result in turn provides a possible explanation for the quantitatively different appearance of the FFT power spectra associated with vibrational wave packet motion of the phenoxyl radical³⁶ and the tyrosyl radical anion (Fig. 3.8(b), in red).

Structural changes that are induced by photodetachment are not confined to the covalent framework of the amino acid itself. According to the DFT calculations, the intermolecular O $\cdots$ O distance increases from 2.69 – 2.70 Å in the microhydrated tyrosinate dianion to 2.84 – 2.89 Å in the microhydrated tyrosyl radical anion. These O $\cdots$ O distances lie within the range of 2.7 – 3.0 Å that is characteristic of hydrogen bonding.^{55,56} The increase in the intermolecular O $\cdots$ O distances can be attributed to the weakening of the hydrogen bond caused by the removal of electron density on the phenoxide oxygen. The large changes in the

Figure 3.12. HOMO of the microhydrated tyrosinate dianion.

intermolecular distance upon photodetachment should trigger coherent hindered translational motion, typically overdamped in liquid water.^{73,74} Our DFT calculations predict that these intermolecular motions are characterized by vibrational frequencies of $\sim 130 - 160 \text{ cm}^{-1}$ (Fig. 3.13), coinciding with a band observed in the low-frequency region of the FFT power spectrum (Fig. 3.8(b), inset). The list of low vibrational frequencies is shown in Table 3.3. Future investigations will aim to elucidate the ultrafast solvation dynamics that accompany the photodetachment process.

Table 3.2. Results of DFT calculations, showing selected bond lengths of the tyrosinate dianion and the tyrosyl radical anion, and the difference bond lengths in aqueous solution and bond lengths of phenoxide and phenoxyl radical from a previous study.³⁶

Bond	Bond Length / pm		Difference / pm	Bond Length / pm		Difference / pm
	Tyrosinate Dianion	Tyrosyl Radical Anion		Phenoxide	Phenoxyl Radical	
C1–C2	155.2	156.3	1.1			
C2–C3	155.7	158.8	3.1			
C3–C4	151.2	148.5	-2.7			
C4–C5	140.1	141.9	1.8	141.7	144.8	3.1
C5–C6	139.5	137.1	-2.4	139.3	137.2	-2.1
C6–C7	141.3	144.4	3.1	139.8	141.1	1.3
C7–C8	141.7	144.5	2.8	139.8	141.1	1.3
C8–C9	139.1	136.8	-2.3	139.3	137.2	-2.1
C9–C4	140.3	142.3	2.0	141.7	144.8	3.1
C7–O10	132.9	127.3	-5.6	132.6	126.8	-5.8
O10–O23	269.8	289.0	19.2			
O10–O24	269.7	289.0	19.3			
O10–O25	268.9	283.8	14.9

Figure 3.13. Low frequency intermolecular vibrational modes of microhydrated tyrosyl radical anion **(a)** 130 cm^{-1} , **(b)** 134 cm^{-1} and **(c)** 158 cm^{-1} .

Table 3.3. Calculated low frequency vibrational modes of microhydrated tyrosyl radical anion.

Calculated Frequency / cm^{-1}		Assignment
1	16	Intermolecular bending
2	22	Intermolecular bending
3	26	Intermolecular bending
4	31	Intermolecular bending
5	34	Intermolecular bending
6	38	Intermolecular bending
7	62	Intermolecular bending
8	70	Intermolecular bending
9	74	Intermolecular bending
10	130	Translation
11	134	Translation
12	158	Translation

3.5. Conclusion

In conclusion, femtosecond strong-field photodetachment of the tyrosinate dianion in aqueous solution results in vibrational wave packet dynamics of the tyrosyl radical anion product. The wave packet motion is resolved into seven different vibrational modes and assigned with the aid of DFT calculations. These vibrational modes are observed because they participate in ultrafast structural rearrangement upon photodetachment, consistent with their displacement vectors being largely localized on the phenoxide sidechain, from which the electron is photodetached. Compared to previous experimental results for the aqueous phenoxyl radical, prepared by the strong-field photodetachment of aqueous phenoxide anion, we find that the presence of the amino acid backbone in the tyrosine radical anion changes the relative FFT intensities and decreases the dephasing times of certain vibrational modes. Moreover, the DFT calculations of the microhydrated tyrosinate dianion and the tyrosyl radical anion suggests that photodetachment is accompanied by the elongation of the hydrogen-bonded $\text{O}\cdots\text{O}$ distances by ~ 0.2 Å. Solvation dynamics driven by photoionization or photodetachment will be a subject of future investigations. The observation of these photodetachment-induced vibrational coherences of the tyrosyl radical anion in aqueous solution offers a glimpse into the elementary structural dynamics that accompany the interaction of ionizing radiation with biological molecules. These results suggest that our experimental approach can be extended to other redox-active amino acids such as tryptophan and phenylalanine as well as to larger biomolecules.

References

1. Hart, E. J., Radiation Chemistry of Aqueous Solutions. *Annu. Rev. Nucl. Sci.* **1965**, *15* (1), 125-150.
2. Garrett, B. C.; Dixon, D. A.; Camaioni, D. M.; Chipman, D. M.; Johnson, M. A.; Jonah, C. D.; Kimmel, G. A.; Miller, J. H.; Rescigno, T. N.; Rossky, P. J.; Xantheas, S. S.; Colson, S. D.; Laufer, A. H.; Ray, D.; Barbara, P. F.; Bartels, D. M.; Becker, K. H.; Bowen, K. H.; Bradforth, S. E.; Carmichael, I.; Coe, J. V.; Corrales, L. R.; Cowin, J. P.; Dupuis, M.; Eisenthal, K. B.; Franz, J. A.; Gutowski, M. S.; Jordan, K. D.; Kay, B. D.; LaVerne, J. A.; Lymar, S. V.; Madey, T. E.; McCurdy, C. W.; Meisel, D.; Mukamel, S.; Nilsson, A. R.; Orlando, T. M.; Petrik, N. G.; Pimblott, S. M.; Rustad, J. R.; Schenter, G. K.; Singer, S. J.; Tokmakoff, A.; Wang, L.-S.; Zwier, T. S., Role of Water in Electron-Initiated Processes and Radical Chemistry: Issues and Scientific Advances. *Chem. Rev.* **2005**, *105* (1), 355-390.
3. Wardman, P., The Importance of Radiation Chemistry to Radiation and Free Radical Biology (The 2008 Silvanus Thompson Memorial Lecture). *Br. J. Radiol.* **2009**, *82* (974), 89-104.
4. Cannington, P. H.; Ham, N. S., He(I) and He(II) Photoelectron Spectra of Glycine and Related Molecules. *J. Electron Spectros. Relat. Phenomena* **1983**, *32* (2), 139-151.
5. Close, D. M., Calculated Vertical Ionization Energies of the Common α -Amino Acids in the Gas Phase and in Solution. *J. Phys. Chem. A* **2011**, *115* (13), 2900-2912.
6. Roy, A.; Seidel, R.; Kumar, G.; Bradforth, S. E., Exploring Redox Properties of Aromatic Amino Acids in Water: Contrasting Single Photon vs Resonant Multiphoton Ionization in Aqueous Solutions. *J. Phys. Chem. B* **2018**, *122* (14), 3723-3733.

7. Gmeiner, B. M. K.; Seelos, C. C. C., Tyrosine Phosphorylation Blocks Tyrosine Free Radical Formation and, hence, the Hormonogenic Iodination Reaction. *Free Radic. Biol. Med.* **1996**, *21* (3), 349-351.
8. Grangeasse, C.; Cozzone, A. J.; Deutscher, J.; Mijakovic, I., Tyrosine Phosphorylation: An Emerging Regulatory Device of Bacterial Physiology. *Trends Biochem. Sci.* **2007**, *32* (2), 86-94.
9. Hunter, T., Tyrosine Phosphorylation: Thirty Years and Counting. *Curr. Opin. Cell Biol.* **2009**, *21* (2), 140-146.
10. Getz, L. J.; Runte, C. S.; Rainey, J. K.; Thomas, N. A., Tyrosine Phosphorylation as a Widespread Regulatory Mechanism in Prokaryotes. *J. Bacteriol.* **2019**, *201* (19), e00205-19.
11. Morozova, O. B.; Kiryutin, A. S.; Sagdeev, R. Z.; Yurkovskaya, A. V., Electron Transfer between Guanosine Radical and Amino Acids in Aqueous Solution. 1. Reduction of Guanosine Radical by Tyrosine. *J. Phys. Chem. B* **2007**, *111* (25), 7439-7448.
12. Warren, J. J.; Winkler, J. R.; Gray, H. B., Redox Properties of Tyrosine and Related Molecules. *FEBS Lett.* **2012**, *586* (5), 596-602.
13. Weinberg, D. R.; Gagliardi, C. J.; Hull, J. F.; Murphy, C. F.; Kent, C. A.; Westlake, B. C.; Paul, A.; Ess, D. H.; McCafferty, D. G.; Meyer, T. J., Proton-Coupled Electron Transfer. *Chem. Rev.* **2012**, *112* (7), 4016-4093.
14. McCaslin, T. G.; Pagba, C. V.; Hwang, H.; Gumbart, J. C.; Chi, S.-H.; Perry, J. W.; Barry, B. A., Tyrosine, Cysteine, and Proton Coupled Electron Transfer in a Ribonucleotide Reductase-Inspired Beta Hairpin Maquette. *Chem. Commun.* **2019**, *55* (63), 9399-9402.
15. Lacomat, F.; Espagne, A.; Dozova, N.; Plaza, P.; Müller, P.; Brettel, K.; Franz-Badur, S.; Essen, L.-O., Ultrafast Oxidation of a Tyrosine by Proton-Coupled Electron Transfer

- Promotes Light Activation of an Animal-like Cryptochrome. *J. Am. Chem. Soc.* **2019**, *141* (34), 13394-13409.
16. Stubbe, J.; Nocera, D. G.; Yee, C. S.; Chang, M. C. Y., Radical Initiation in the Class I Ribonucleotide Reductase: Long-Range Proton-Coupled Electron Transfer? *Chem. Rev.* **2003**, *103* (6), 2167-2202.
 17. Hammes-Schiffer, S., Proton-Coupled Electron Transfer: Classification Scheme and Guide to Theoretical Methods. *Energy Environ. Sci.* **2012**, *5* (7), 7696-7703.
 18. Migliore, A.; Polizzi, N. F.; Therien, M. J.; Beratan, D. N., Biochemistry and Theory of Proton-Coupled Electron Transfer. *Chem. Rev.* **2014**, *114* (7), 3381-3465.
 19. Hammes-Schiffer, S., Proton-Coupled Electron Transfer: Moving Together and Charging Forward. *J. Am. Chem. Soc.* **2015**, *137* (28), 8860-8871.
 20. Feitelson, J.; Hayon, E., Electron Ejection and Electron Capture by Phenolic Compounds. *J. Phys. Chem.* **1973**, *77* (1), 10-15.
 21. Feitelson, J.; Hayon, E.; Treinin, A., Photoionization of Phenols in Water. Effects of Light Intensity, Oxygen, pH, and Temperature. *J. Am. Chem. Soc.* **1973**, *95* (4), 1025-1029.
 22. Bent, D. V.; Hayon, E., Excited State Chemistry of Aromatic Amino Acids and Related Peptides. I. Tyrosine. *J. Am. Chem. Soc.* **1975**, *97* (10), 2599-2606.
 23. Johnson, C. R.; Ludwig, M.; Asher, S. A., Ultraviolet Resonance Raman Characterization of Photochemical Transients of Phenol, Tyrosine, and Tryptophan. *J. Am. Chem. Soc.* **1986**, *108* (5), 905-912.
 24. Ayala, I.; Range, K.; York, D.; Barry, B. A., Spectroscopic Properties of Tyrosyl Radicals in Dipeptides. *J. Am. Chem. Soc.* **2002**, *124* (19), 5496-5505.

25. Hameka, H. F.; Jensen, J. O., Calculations of the Energies and Geometries of the Ground and First Excited States of Phenylalanine and Tyrosine. *J. Mol. Struct.* **1993**, 288 (1), 9-16.
26. Barry, B. A.; Chen, J.; Keough, J.; Jenson, D.; Offenbacher, A.; Pagba, C., Proton-Coupled Electron Transfer and Redox-Active Tyrosines: Structure and Function of the Tyrosyl Radicals in Ribonucleotide Reductase and Photosystem II. *J. Phys. Chem. Lett.* **2012**, 3 (4), 543-554.
27. Pagba, C. V.; McCaslin, T. G.; Veglia, G.; Porcelli, F.; Yohannan, J.; Guo, Z.; McDaniel, M.; Barry, B. A., A Tyrosine–Tryptophan Dyad and Radical-Based Charge Transfer in a Ribonucleotide Reductase-inspired Maquette. *Nat. Commun.* **2015**, 6 (1), 10010.
28. Pirisi, K.; Nag, L.; Fekete, Z.; Iuliano, J. N.; Tolentino Collado, J.; Clark, I. P.; Pécsi, I.; Sournia, P.; Liebl, U.; Greetham, G. M.; Tonge, P. J.; Meech, S. R.; Vos, M. H.; Lukacs, A., Identification of the Vibrational Marker of Tyrosine Cation Radical using Ultrafast Transient Infrared Spectroscopy of Flavoprotein Systems. *Photochem. Photobiol. Sci.* **2021**, 20 (3), 369-378.
29. Crowell, R. A.; Bartels, D. M., Multiphoton Ionization of Liquid Water with 3.0–5.0 eV Photons. *J. Phys. Chem.* **1996**, 100 (45), 17940-17949.
30. Pépin, C.; Goulet, T.; Houde, D.; Jay-Gerin, J. P., Observation of a Continuous Spectral Shift in the Solvation Kinetics of Electrons in Neat Liquid Deuterated Water. *J. Phys. Chem. A* **1997**, 101 (24), 4351-4360.
31. Silva, C.; Walhout, P. K.; Yokoyama, K.; Barbara, P. F., Femtosecond Solvation Dynamics of the Hydrated Electron. *Phys. Rev. Lett.* **1998**, 80 (5), 1086-1089.
32. Vilchiz, V. H.; Kloepper, J. A.; Germaine, A. C.; Lenchenkov, V. A.; Bradforth, S. E., Map for the Relaxation Dynamics of Hot Photoelectrons Injected into Liquid Water *via*

- Anion Threshold Photodetachment and above Threshold Solvent Ionization. *J. Phys. Chem. A* **2001**, *105* (10), 1711-1723.
33. Pshenichnikov, M. S.; Baltuška, A.; Wiersma, D. A., Hydrated-Electron Population Dynamics. *Chem. Phys. Lett.* **2004**, *389* (1), 171-175.
 34. Mostafavi, M.; Lampre, I., An Overview of Solvated Electrons: Recent Advances. 2010; Vol. 105, pp 21-58.
 35. Elkins, M. H.; Williams, H. L.; Shreve, A. T.; Neumark, D. M., Relaxation Mechanism of the Hydrated Electron. *Science* **2013**, *342* (6165), 1496-1499.
 36. Debnath, T.; Mohd Yusof, M. S. B.; Low, P. J.; Loh, Z.-H., Ultrafast Structural Rearrangement Dynamics Induced by the Photodetachment of Phenoxide in Aqueous Solution. *Nat. Commun.* **2019**, *10* (1), 2944.
 37. Ghosh, D.; Roy, A.; Seidel, R.; Winter, B.; Bradforth, S.; Krylov, A. I., First-Principle Protocol for Calculating Ionization Energies and Redox Potentials of Solvated Molecules and Ions: Theory and Application to Aqueous Phenol and Phenolate. *J. Phys. Chem. B* **2012**, *116* (24), 7269-7280.
 38. Winter, B.; Weber, R.; Widdra, W.; Dittmar, M.; Faubel, M.; Hertel, I. V., Full Valence Band Photoemission from Liquid Water Using EUV Synchrotron Radiation. *J. Phys. Chem. A* **2004**, *108* (14), 2625-2632.
 39. Nishizawa, K.; Kurahashi, N.; Sekiguchi, K.; Mizuno, T.; Ogi, Y.; Horio, T.; Oura, M.; Kosugi, N.; Suzuki, T., High-Resolution Soft X-Ray Photoelectron Spectroscopy of Liquid Water. *Phys. Chem. Chem. Phys.* **2011**, *13* (2), 413-417.
 40. Kurahashi, N.; Karashima, S.; Tang, Y.; Horio, T.; Abulimiti, B.; Suzuki, Y.-I.; Ogi, Y.; Oura, M.; Suzuki, T., Photoelectron Spectroscopy of Aqueous Solutions: Streaming Potentials of NaX (X = Cl, Br, and I) Solutions and Electron Binding Energies of Liquid Water and X⁻. *J. Chem. Phys.* **2014**, *140* (17), 174506.

41. Perry, C. F.; Zhang, P.; Nunes, F. B.; Jordan, I.; von Conta, A.; Wörner, H. J., Ionization Energy of Liquid Water Revisited. *J. Phys. Chem. Lett.* **2020**, *11* (5), 1789-1794.
42. Winter, B.; Faubel, M.; Vácha, R.; Jungwirth, P., Behavior of Hydroxide at the Water/Vapor Interface. *Chem. Phys. Lett.* **2009**, *474* (4), 241-247.
43. Carta, R.; Tola, G., Solubilities of L-Cystine, L-Tyrosine, L-Leucine, and Glycine in Aqueous Solutions at Various pHs and NaCl Concentrations. *J. Chem. Eng. Data* **1996**, *41* (3), 414-417.
44. Gräslund, A.; Sahlin, M.; Sjöberg, B. M., The Tyrosyl Free Radical in Ribonucleotide Reductase. *Environ. Health Perspect.* **1985**, *64*, 139-149.
45. Oldemeyer, S.; Franz, S.; Wenzel, S.; Essen, L.-O.; Mittag, M.; Kottke, T., Essential Role of an Unusually Long-Lived Tyrosyl Radical in the Response to Red Light of the Animal-like Cryptochrome aCRY. *J. Biol. Chem.* **2016**, *291* (27), 14062-14071.
46. Hare, P. M.; Price, E. A.; Bartels, D. M., Hydrated Electron Extinction Coefficient Revisited. *J. Phys. Chem. A* **2008**, *112* (30), 6800-6802.
47. Torche, F.; Marignier, J.-L., Direct Evaluation of the Molar Absorption Coefficient of Hydrated Electron by the Isosbestic Point Method. *J. Phys. Chem. B* **2016**, *120* (29), 7201-7206.
48. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J.

- J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. *Gaussian 16 Rev. C.01*, Wallingford, CT, 2016.
49. Becke, A. D., Density-Functional Exchange-Energy Approximation with Correct Asymptotic Behavior. *Phys. Rev. A* **1988**, 38 (6), 3098-3100.
 50. Lee, C.; Yang, W.; Parr, R. G., Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density. *Phys. Rev. B* **1988**, 37 (2), 785-789.
 51. Tomasi, J.; Mennucci, B.; Cammi, R., Quantum Mechanical Continuum Solvation Models. *Chem. Rev.* **2005**, 105 (8), 2999-3094.
 52. Nelson, D. L.; Cox, M. M.; Lehninger, A. L., *Principles of Biochemistry*. 7th edition ed.; W.H. Freeman: New York, 2017.
 53. Lozovoy, V. V.; Pastirk, I.; Dantus, M., Multiphoton Intrapulse Interference. IV. Ultrashort Laser Pulse Spectral Phase Characterization and Compensation. *Opt. Lett.* **2004**, 29 (7), 775-777.
 54. Pestov, D.; Lozovoy, V. V.; Dantus, M., Multiple Independent Comb Shaping (MICS): Phase-Only Generation of Optical Pulse Sequences. *Opt. Express* **2009**, 17 (16), 14351-14361.
 55. Jeffrey, G. A., *An Introduction to Hydrogen Bonding* Oxford University Press: New York, 1997.
 56. Gilli, P.; Pretto, L.; Bertolasi, V.; Gilli, G., Predicting Hydrogen-Bond Strengths from Acid–Base Molecular Properties. The pK_a Slide Rule: Toward the Solution of a Long-Lasting Problem. *Acc. Chem. Res.* **2009**, 42 (1), 33-44.

57. Wilson, E. B., The Normal Modes and Frequencies of Vibration of the Regular Plane Hexagon Model of the Benzene Molecule. *Phys. Rev.* **1934**, *45* (10), 706-714.
58. Grace, L. I.; Cohen, R.; Dunn, T. M.; Lubman, D. M.; de Vries, M. S., The R2PI Spectroscopy of Tyrosine: A Vibronic Analysis. *J. Mol. Spectrosc.* **2002**, *215* (2), 204-219.
59. Ambrosio, F.; Guo, Z.; Pasquarello, A., Absolute Energy Levels of Liquid Water. *J. Phys. Chem. Lett.* **2018**, *9* (12), 3212-3216.
60. Lundblad, R. L.; Macdonald, F., *Handbook of Biochemistry and Molecular Biology* Fifth edition ed.; CRC Press: 2018.
61. Odhner, J. H.; Romanov, D. A.; Levis, R. J., Rovibrational Wave-Packet Dispersion during Femtosecond Laser Filamentation in Air. *Phys. Rev. Lett.* **2009**, *103* (7), 075005.
62. Salter, R.; Chu, J.; Hippler, M., Cavity-Enhanced Raman Spectroscopy with Optical Feedback CW Diode Lasers for Gas Phase Analysis and Spectroscopy. *Analyst* **2012**, *137* (20), 4669-4676.
63. Loh, Z.-H.; Doumy, G.; Arnold, C.; Kjellsson, L.; Southworth, S. H.; Al Haddad, A.; Kumagai, Y.; Tu, M.-F.; Ho, P. J.; March, A. M.; Schaller, R. D.; Bin Mohd Yusof, M. S.; Debnath, T.; Simon, M.; Welsch, R.; Inhester, L.; Khalili, K.; Nanda, K.; Krylov, A. I.; Moeller, S.; Coslovich, G.; Koralek, J.; Minitti, M. P.; Schlotter, W. F.; Rubensson, J.-E.; Santra, R.; Young, L., Observation of the Fastest Chemical Processes in the Radiolysis of Water. *Science* **2020**, *367* (6474), 179-182.
64. Ambrosio, F.; Miceli, G.; Pasquarello, A., Electronic Levels of Excess Electrons in Liquid Water. *J. Phys. Chem. Lett.* **2017**, *8* (9), 2055-2059.
65. Klopfer, J. A.; Vilchiz, V. H.; Lenchenkov, V. A.; Germaine, A. C.; Bradforth, S. E., The Ejection Distribution of Solvated Electrons Generated by the One-Photon

- Photodetachment of Aqueous I^- and Two-Photon Ionization of the Solvent. *J. Chem. Phys.* **2000**, *113* (15), 6288-6307.
66. Peon, J.; Hess, G. C.; Pecourt, J.-M. L.; Yuzawa, T.; Kohler, B., Ultrafast Photoionization Dynamics of Indole in Water. *J. Phys. Chem. A* **1999**, *103* (14), 2460-2466.
 67. Czapski, G.; Bielski, B. H. J., Absorption Spectra of the $\bullet\text{OH}$ and $\text{O}\bullet^-$ Radicals in Aqueous Solutions. *Radiat. Phys. Chem.* **1993**, *41* (3), 503-505.
 68. Noble, J. A.; Aranguren-Abate, J. P.; Dedonder, C.; Jouvet, C.; Pino, G. A., Photodetachment of Deprotonated Aromatic Amino Acids: Stability of the Dehydrogenated Radical Depends on the Deprotonation Site. *Phys. Chem. Chem. Phys.* **2019**, *21* (42), 23346-23354.
 69. Petersson, L.; Gräslund, A.; Ehrenberg, A.; Sjöberg, B.-M.; Reichard, P., The Iron Center in Ribonucleotide Reductase from Escherichia coli. *J. Biol. Chem.* **1980**, *255*, 6706-12.
 70. Dean, J. C.; Rafiq, S.; Oblinsky, D. G.; Cassette, E.; Jumper, C. C.; Scholes, G. D., Broadband Transient Absorption and Two-Dimensional Electronic Spectroscopy of Methylene Blue. *J. Phys. Chem. A* **2015**, *119* (34), 9098-9108.
 71. Rafiq, S.; Scholes, G. D., Slow Intramolecular Vibrational Relaxation Leads to Long-Lived Excited-State Wavepackets. *J. Phys. Chem. A* **2016**, *120* (34), 6792-6799.
 72. Kim, J. B.; Yacovitch, T. I.; Hock, C.; Neumark, D. M., Slow Photoelectron Velocity-Map Imaging Spectroscopy of the Phenoxide and Thiophenoxide Anions. *Phys. Chem. Chem. Phys.* **2011**, *13* (38), 17378-17383.
 73. Palese, S.; Schilling, L.; Miller, R. J. D.; Staver, P. R.; Lotshaw, W. T., Femtosecond Optical Kerr Effect Studies of Water. *J. Phys. Chem.* **1994**, *98* (25), 6308-6316.
 74. Fecko, C. J.; Eaves, J. D.; Tokmakoff, A., Isotropic and Anisotropic Raman Scattering from Molecular Liquids Measured by Spatially Masked Optical Kerr Effect Spectroscopy. *J. Chem. Phys.* **2002**, *117* (3), 1139-1154.

Chapter 4

Ultrafast Dynamics of Photodetached

Tryptophan Anion in Aqueous Solution

4.1. Introduction

The interaction of high-energy radiation with biological matter results in the ionization of biomolecules in an aqueous environment. The subsequent chemical reactions that involve radicals, ions, and hydrated electrons represent some of the most elementary processes in radiation chemistry and radiation biology.¹ Given their relatively low ionization potentials, the study of radiation damage involving amino acids with redox-active sidechains, such as tryptophan, tyrosine, and phenylalanine, is particularly important. The vertical ionization energies (VIE) of these redox-active amino acids are typically $\sim 1 - 2$ eV lower than the other amino acids.^{2,3}

In this study, we focus on tryptophan (Trp), one of the essential amino acids that serves as a building block in protein biosynthesis and is also a precursor to several molecules of biological importance, such as serotonin^{4,5} and melatonin.^{6,7} In addition, tryptophan also plays a vital role in many photochemical reactions in Nature,^{8,9} such as the reaction of singlet oxygen with tryptophan residues in proteins¹⁰⁻¹² and in phototriggered cell apoptosis.¹³⁻¹⁶ While the oxidation of tryptophan residues often leads to deleterious effects on protein function,¹⁷ the oxidized form of tryptophan – either the tryptophan radical (Trp•) or the tryptophan radical

cation ($\text{Trp}^{\bullet+}$), depending on the pH of the aqueous medium – also serves as a key intermediate in several biological and environmental processes.¹⁸⁻²⁴ Examples of such processes include electron transfer in deoxyribonucleic acid (DNA) photolyase,²⁵⁻²⁷ oxidation of cytochrome *c* by cytochrome *c* peroxidase,²⁸⁻³⁰ radical transport in ribonucleotide reductase,³¹⁻³³ and protection of enzymes against oxidative damage during catalysis.^{34,35} In addition, the tryptophan radical and radical cation also form the basis of various photochemical radical initiation schemes,³⁶⁻³⁸ employed to shed light on radical formation and transport mechanisms in biological systems.

We are interested in exploring the ultrafast structural rearrangement dynamics that follow the ejection of an electron from tryptophan in aqueous solution. The ionization potential of aqueous tryptophan has been determined to be 7.3 eV,³⁹ significantly lower than the ionization potential of liquid water (11.2 eV).⁴⁰⁻⁴³ As such, intense laser fields can be harnessed to selectively ionize tryptophan over liquid water *via* strong-field multiphoton ionization. The ionization of tryptophan yields the tryptophan radical cation and an accompanying electron that is injected into the conduction band of liquid water. This conduction-band electron subsequently undergoes trapping and solvation to yield the hydrated electron, a process that has been the subject of numerous ultrafast spectroscopy studies.^{44,45} The hydrated electron has a broad absorption band that spans 400 – 1000 nm, assigned to the transition from the *s*-like ground state to the *p*-like excited state, with the absorption maximum at 715 nm ($\epsilon_{\text{max}} = 19687 \text{ M}^{-1} \text{ cm}^{-1}$).^{45,46}

The aqueous tryptophan radical cation exhibits absorption maxima at 580 nm ($\epsilon_{\text{max}} = 3000 \text{ M}^{-1} \text{ cm}^{-1}$) and 335 nm ($\epsilon_{\text{max}} = 4750 \text{ M}^{-1} \text{ cm}^{-1}$),⁴⁷ similar to those observed for the indole radical cation;^{48,49} CASSCF/CASPT2 calculations reveal that the visible and UV absorption bands of the indole radical cation originate predominantly from the π_3 (HOMO–2) $\rightarrow \pi_5$ [singly occupied molecular orbital (SOMO)] and π_5 (SOMO) $\rightarrow \pi_6^*$ (LUMO) transitions,

respectively.⁵⁰ In aqueous solutions with pH exceeding its pK_a value of ~ 4.2 ,^{47,51,52} deprotonation of the indole N–H in $\text{Trp}^{\bullet+}$ becomes thermodynamically favorable. The visible and UV absorption maxima of the resultant $\text{Trp}^{\bullet}$ radical are blueshifted to 510 nm ($\epsilon_{\text{max}} = 2300 \text{ M}^{-1} \text{ cm}^{-1}$) and 325 nm ($\epsilon_{\text{max}} = 3670 \text{ M}^{-1} \text{ cm}^{-1}$), respectively.^{47,49,52-58} Aside from its electronic absorption spectrum, the resonance Raman spectrum of the $\text{Trp}^{\bullet+}$ radical cation recorded with 514.5-nm irradiation has also been reported.⁵⁹ The resonance Raman spectrum is dominated by peaks in the $\sim 1100 - 1600 \text{ cm}^{-1}$ region, corresponding to modes that are largely localized on the indole radical cation sidechain.

Following our recent work on the observation of coherent vibrational motion in aqueous phenoxide induced by photodetachment,⁶⁰ our strategy for elucidating the vibrational modes that drive structural rearrangement after electron ejection is based on the observation of vibrational wave packet dynamics. Electron ejection by an ultrashort laser pulse triggers wave packet dynamics involving vibrational modes along which the equilibrium geometries before and after electron ejection are displaced. As such, vibrational modes that appear in the wave packet dynamics represent vibrational modes along which structural reorganization occur. We detect coherent vibrational motion in the tryptophan radical by transient absorption spectroscopy, detecting its absorption signature in the UV and the visible. This time-domain approach is in-principle complementary to measurements of the Franck-Condon progression in the valence photoelectron spectrum, although such energy-resolved measurements are unfeasible for molecules in aqueous solutions because their valence photoelectron bands are inhomogeneously broadened by $\sim 1 \text{ eV}$.³⁹

In this chapter, we report the observation of coherent vibrational dynamics induced by the strong-field photodetachment of the aqueous tryptophan anion Trp^- (Fig. 4.1). Since the experiments are performed in alkaline solution, the starting material, $(\text{C}_8\text{H}_5\text{NH})\text{CH}_2\text{CH}(\text{NH}_2)(\text{COO}^-)$, yields the tryptophan radical,

Fig. 4.1. Structure of the tryptophan anion. The carbon, hydrogen, oxygen and nitrogen atoms are in grey, white, red and blue respectively.

$(\text{C}_8\text{H}_5\text{NH}\cdot^+)\text{CH}_2\text{CH}(\text{NH}_2)(\text{COO}^-)$, upon photodetachment. For the remainder of this chapter, we refer to this radical product as $\text{Trp}\cdot$, not to be mistaken for the tryptophan radical observed in previous studies, i.e., $(\text{C}_8\text{H}_5\text{N}\cdot)\text{CH}_2\text{CH}(\text{NH}_3^+)(\text{COO}^-)$, in which the indole N–H is deprotonated. Probing the UV and the visible absorption bands of the $\text{Trp}\cdot$ radical, we identify both intramolecular and intermolecular vibrational modes that participate in structural rearrangement upon photodetachment of the Trp^- anion. Finally, we present the results of density functional theory (DFT) calculations, which aid the assignment of the vibrational frequencies that contribute to the observed wave packet dynamics.

4.2. Materials and Methods

4.2.1. Theoretical Calculations

The equilibrium geometries, vibrational normal modes and vibrational frequencies of the tryptophan anion and the tryptophan radical are obtained from DFT calculations, performed with the GAUSSIAN 16 program.⁶¹ The calculations employ the hybrid exchange functional of Becke (B3)⁶² and the correlation functional of Lee, Yang and Parr (LYP)⁶³ and the 6-311G++(d,p) split-valence, triple- ξ basis set. Two models of the tryptophan anion and tryptophan radical are constructed. The first model is the isolated tryptophan anion and tryptophan radical, while the second model is the microhydrated version. The microhydrated species includes a water molecule hydrogen bonded to the hydrogen of the indolyl N-H group to account for the direct interaction of the tryptophan anion or radical with the surrounding solvent. The model is further hydrated with two additional water molecules. This microhydration configuration was chosen because photodetachment is expected to significantly perturb the charge density of the indole side chain. Furthermore, a polarizable continuum model (PCM) is included for both models to mimic the interaction of the amino acid and radical with the surrounding water solvent⁶⁴. The water molecule present in the microhydrated species is fully relaxed during the geometry optimization process. All geometry optimizations are followed by vibrational frequency calculation to verify the optimized geometry corresponds to a local minimum.

4.2.2. Experimental Method

L-tryptophan (Sigma-Aldrich, $\geq 98\%$ HPLC) and sodium hydroxide (VWR Chemicals, 98.9% pellets) are used as received. Due to the low solubility of L-tryptophan in water (55.8 mM at its isoelectronic point),⁶⁵ L-tryptophan is dissolved and sonicated in sodium hydroxide to produce a tryptophan anion solution with concentration of 400 mM at pH 14. Given the pK_a of the carboxyl group of 2.38,⁶⁶ we expect $> 99.9\%$ deprotonation of the carboxyl group at pH 14. Thus, tryptophan exists in our experiment in the anionic form.

The experimental procedure has been described in detailed in Chapter 2. In summary, the transient absorption pump-probe experiment is performed with a chirped-pulse-amplified Ti:sapphire laser system (Coherent, Legend Elite Duo-USX), with an output of 4.5-mJ, 30-fs pulses at 1-kHz repetition rate and 800-nm center wavelength, with a bandwidth of 50 nm. The pulses undergo self-phase modulation in a helium-filled hollow-core fiber before they are temporally compressed by chirped mirrors (Ultrafast Innovations GmbH, PC1332). The compressed pulses are incident on a beam splitter (Layertec, 131191), with 80% of the transmitted beam (~ 0.6 mJ) being directed to a $4f$ visible-NIR pulse shaper with a 128-element spatial light modulator (SLM) (Biophotonics Solutions, FemtoJock-P) for further pulse compression, which truncates the wavelength range of the visible-NIR from 500 nm to 750 nm, with a center wavelength of 611 nm (2.03-eV photon energy). The visible-NIR spectrum is shown in Fig. 4.2(a). Transform-limited laser pulses with full-width half-maximum (FWHM) duration of 5 fs are obtained by using multiphoton intrapulse interference phase scan (MIIPS) to measure the spectral phase at the sample, followed by using the SLM to introduce an oppositely signed phase to the laser pulses, thus compressing it to the transform-limited duration.⁶⁷ Subsequently, a second-order interferometric auto-correlation trace is recorded by multiple independent comb shaping (MICS).⁶⁸ The second-order interferometric auto-

Figure 4.2. (a) The visible-NIR ionizing pump spectrum ranges from 500 nm to 750 nm, with a center wavelength of 611 nm. (b) A second-order interferometric auto-correlation trace of the visible-NIR ionizing pump spectrum, measured in a 10- μm -thick, $\theta = 37.0$ deg Type I BBO crystal, reveals a pulse duration with FWHM of 5 fs

correlation trace is shown in Fig. 4.2(b). The transform-limited pulses, which function as the strong-field ionizing pump, are directed to the sample. The ionizing pump is then focused onto the sample by a 50-cm focal length spherical mirror. A maximum pulse energy available for experiments of 28 μJ , with a beam waist ($1/e^2$) of 120 μm , resulting in a peak intensity of $2.5 \times 10^{13} \text{ W cm}^{-2}$ for the photodetachment process. An optical delay stage line, driven by a piezo translation stage (Physik Instrumente, N-664.3A) in the ionizing pump's path provide a variable time delay between pump and probe pulses in 5-fs steps.

To study the ultrafast dynamics of photodetached aqueous tryptophan anion in the visible-NIR region, the primary visible-NIR pulse is split off by a 90T/10R beamsplitter (Layertec, 130945) to produce the strong-field ionizing pump and visible-NIR probe. The broadband visible-NIR probe spectrum has a similar spectral range as visible-NIR ionizing pump (Fig. 4.2(a)). The probe is then split by a reflective neutral density beamsplitter (Newport, FRQ-ND05) to produce the visible-NIR probe and reference pulses. The reflected

visible-NIR probe is then focused onto the sample by a 15-cm focal length off-axis parabolic reflector (Thorlabs, MPD369H-F01). The focused visible-NIR probe has a beam waist ($1/e^2$) of 35 μm , with a pulse energy of 27 nJ, which has a peak intensity of $2.6 \times 10^{11} \text{ W cm}^{-2}$. An achromatic half-waveplate (B. Halle, RAC4.2.15 L) is introduced in the visible-NIR probe pulse path for independent control of the polarization of the probe, to acquire isotropic signal when necessary.

For experiments that utilize the UV (near-UV) probe, the remaining 20% output from the chirped mirrors is directed to a 10- μm -thick (50- μm -thick) Type I beta-barium borate (BBO) crystal (CASTECH), cut at $\theta = 44.0^\circ$ ($\theta = 29.0^\circ$), to generate the UV (near-UV) probe *via* second harmonic generation (SHG). The broadband UV (near-UV) probe pulses span 290 – 360 nm (360 – 470 nm) (Figs. 4.3(a) & 4.4(a) respectively). Dichroic mirrors (Layertec, 106163 for UV and Layertec, 109433 for near-UV) are introduced to eliminate the residual fundamental beam. The UV (near-UV) pulse is then split off by a reflective neutral density beamsplitter (Edmund, 88271) to produce the UV (near-UV) probe and reference pulses. The reflected UV (near-UV) probe is focused onto the sample by a 15-cm focal length off-axis parabolic reflector (Thorlabs, MPD369H-P01). The focused UV (near-UV) probe has a beam waist ($1/e^2$) of 80 μm (44 μm) and a pulse energy of 150 nJ (300 nJ), corresponding to a peak intensity of $6.4 \times 10^{10} \text{ W}\cdot\text{cm}^{-2}$ ($7.7 \times 10^{11} \text{ W}\cdot\text{cm}^{-2}$). The cross-correlation of the vis-NIR pump and UV (near-UV) probe is measured by difference frequency generation (DFG) in a 10- μm -thick Type I BBO crystal (CASTECH, $\theta = 37.0^\circ$). These measurements yield an instrument response of 24 fs (12 fs) at full-width at half-maximum (FWHM) (Figs. 4.3(b) & 4.3(b) respectively).

A slit nozzle (Metaheuristics, Type L) is used in conjunction with a peristaltic pump (Cole-Parmer, 07528-10) and a dampener to produce a microjet of tryptophan anion solution for experiments. The volume flow rate of 92 mL min⁻¹ translates to a vertical flow rate of

Figure 4.3. (a) The UV probe spectrum ranges from 290 nm to 370 nm. (b) The pump-probe cross-correlation trace measured *via* difference frequency mixing in a 10- μ m-thick, $\theta = 37.0$ deg Type I BBO crystal, reveals a FWHM of 24 fs.

approximately 8.5 mm ms^{-1} . The relatively high vertical flow rate ensures that fresh volume of tryptophan anion solution is exposed to each ionizing pump pulse. A liquid jet of 7- μ m thickness is produced, which yields a sizeable differential absorption signal ($\Delta A \sim 0.1$), while minimizing the temporal broadening of the pulses in the liquid jet due to dispersion. In addition, a thin liquid jet limits the group-velocity mismatch between the pump and probe to $< 1 \text{ fs}$, making its effect on the time resolution negligible.

A synchronized optical chopper in the ionizing pump's path before the sample allow modulation of the ionizing pump at a frequency of 0.5 kHz. The visible-NIR probe transmitted through the microjet is focused into a 300-nm spectrograph (Princeton Instruments, Acton SP2300), equipped with a 150-grooves per mm grating blazed at 800-nm while the UV probe is focused into a 300-nm spectrograph (Princeton Instruments, Acton SP2300), equipped with a 600-grooves per mm grating blazed at 500-nm. In both cases, the spectrographs are equipped with a 1024-pixels silicon (Si) photodiode array detector (Stresing, S8381-1024Q) with a read-out rate of 1 kHz, which allow simultaneous acquisition of the probe spectra obtained with and

Figure 4.4. (a) The near-UV probe spectrum ranges from 360 nm to 570 nm. (b) The pump-probe cross-correlation trace measured *via* difference frequency mixing in a 10- μ m-thick, $\theta = 37.0$ deg Type I BBO crystal, reveals a FWHM of 12 fs.

without the ionizing pump pulse. The reference pulses are focused to an identical spectrograph, which is equipped with similar grating and photodiode array at 1-kHz repetition rate. This method reduces noise interference caused by shot-to-shot fluctuations of the probe pulse. The Si photodiode array detectors are interfaced to desktop computers for automated data acquisition.

The focal spot size of the probe beam is $\sim 1.5 - 3\times$ smaller than that of the ionizing pump beam, hence minimizing the effect of spatial averaging on the observed ultrafast dynamics.

Static UV-visible measurements are performed with a double-beam UV-visible-NIR spectrophotometer (Agilent Technologies, Cary 300) with a 1-mm path length quartz cuvette by measuring absorbance. The spectrophotometer has a wavelength range from 190 to 800 nm.

4.3. Results

4.3.1. Theoretical Calculations

The equilibrium geometries of the isolated and microhydrated tryptophan anion and tryptophan radical species are displayed in Fig. 4.5. The equilibrium geometry of the microhydrated tryptophan radical includes an explicit water molecule hydrogen bonded to the hydrogen atom of the indolyl N-H group of the tryptophan radical, which has a distance of approximately 2.83 Å. The hydrogen donor-acceptor distance is consistent with the typical hydrogen bonding distance of ~ 3.00 Å.^{69,70} Furthermore, the equilibrium geometry corresponds to a local minimum in terms of energy and produce real vibrational frequencies. The full vibrational frequencies and assignments of the tryptophan radical are displayed in the Appendix B.

Figure 4.5. The equilibrium geometries of **(a)** the tryptophan anion, **(b)** the tryptophan radical, **(c)** the microhydrated tryptophan anion and **(d)** the microhydrated tryptophan radical with PCM solvation. The microhydrated species include a water molecule hydrogen bonded to the hydrogen of the indolyl N–H group. The carbon, hydrogen, oxygen and nitrogen atoms are in gray, white, red and blue respectively.

4.3.2. Transient Absorption of Photodetached Aqueous Tryptophan Anion

As illustrated in the previous chapter, a power-dependence measurement is performed to determine the photon order of the strong-field photodetachment process of aqueous tryptophan anion. For an N -photon photodetachment process, the differential absorption signal, ΔA , scales with the intensity of the ionizing pump, I , as shown in the following relationship.

$$\Delta A \propto I^N \quad (4.1)$$

In this study, the photon-order measurement is performed by using a near-UV probe, which has spectral range of 360 – 470 nm, which measures the absorption of the ground-state hydrated electron at 445 nm at a pump-probe time delay of 5 ps. Since the hydrated electron is produced alongside upon the photodetachment of tryptophan anion and hydroxide ion, the ground-state hydrated electron absorption signal is proportional to the extent of the photodetachment process.

Similarly, during the photodetachment of aqueous tryptophan anion, there is a possibility that electrons are produced from both the tryptophan anion and hydroxide ion, even though the photodetachment of the solute is more likely to occur due to its significantly lower vertical ionization potential. In our study, the ΔA against ionizing pump intensity spectrum is recorded for both aqueous tryptophan anion and aqueous sodium hydroxide under similar experimental conditions. A difference spectrum, $\Delta\Delta A$, is then obtained, which corresponds to solely the ΔA signal from the photodetachment of tryptophan anion. The $\Delta\Delta A$ signal is then fitted with the following function

$$\Delta\Delta A = \frac{\alpha * I^N}{1 + \left(\frac{I}{I_{sat}}\right)^N}, \quad (4.2)$$

where α is the amplitude, N is the photon order and I_{sat} is the saturation intensity. The best fit when $N = 3$ yields $\alpha = (6.49 \pm 0.17) \times 10^{-43} \text{ cm}^6 \text{ W}^{-3}$ and $I_{sat} = (2.46 \pm 0.05) \times 10^{13} \text{ W cm}^{-2}$. The value of N indicates that the photodetachment of tryptophan anion in aqueous environment occurs *via* a three-photon process. A log-log plot of $\Delta\Delta A$ against pump intensity is shown in Fig. 4.6.

Previous studies have shown that 4.68-eV (265-nm) photoexcitation of tryptophan to the S_1 ($\pi\pi^*$) state yields the $\text{Trp}^\bullet$ radical *via* delayed electron ejection.^{48,52,53} However, we eliminate the possibility that the $\text{Trp}^\bullet$ radical is formed in our experiments *via* the dense manifold of the electronic excited state of the tryptophan anion. This is evident from the pump-power dependence measurements, which show that photodetachment occurs *via* a three-photon process (Fig. 4.6). The vertical ionization energy of aqueous tryptophan has been reported to be 7.3 eV, with the photoelectron band extending to ~ 6 -eV binding energy.³⁹ Considering the ~ 1 -eV vertical electron affinity of liquid water,⁷¹ the total energy input of 6.09 eV from the three-photon process is sufficient to eject an electron from the tryptophan anion and inject it into the conduction band of liquid water.⁷² The conduction-band electron eventually undergoes electronic relaxation and vibrational cooling to form the hydrated electron.^{44,73-78} In comparison, resonance-enhanced photodetachment *via* the manifold of electronically excited states, with a low-energy tail extending to ~ 4.0 eV (Fig. 4.7), would involve two-photon excitation, contradicting the measured photon order. Moreover, $\text{Trp}^\bullet$ formation *via* delayed electron ejection from an electronically excited state is unlikely to occur in the presence of an intense laser field because the strong-field photodetachment of any electronically excited population, which occurs within the ~ 5 -fs envelope of the intense laser pulse, proceeds more rapidly than delayed electron ejection.

The time-resolved differential absorption (ΔA) spectra, collected in the visible-NIR, near-UV, and UV spectral ranges after the photodetachment of the aqueous tryptophan anion,

Figure 4.7. Static UV-visible absorption spectrum of 1mM tryptophan anion in aqueous sodium hydroxide (pH 14), measured with a 1-mm path length quartz cuvette. The maximum absorption is at 280 nm.

are shown in Figs. 4.8(a), 4.8(b), and 4.8(c), respectively. The ΔA spectra in the visible-NIR reveal the growth of a pronounced absorption band at ~ 700 nm, characteristic of the hydrated electron absorption. This absorption band appears with a time constant of 0.35 ± 0.01 ps (Fig. 4.9, in red), comparable to the timescales for hydrated electron formation reported in the literature^{44,73-78} as well as the time constant of 0.36 ± 0.01 ps for hydrated electron formation observed in aqueous NaOH (Fig. 4.9, in blue) thus providing further support for the assignment of the absorption band to the hydrated electron. The sub-picosecond dynamics observed with visible-NIR thus primarily reflects the formation of the hydrated electron from the conduction-band electron. The visible absorption signature of the $\text{Trp}^\bullet$ radical, previously observed at ~ 580 nm,^{47,49,52,55,56} appears to be masked by the broad absorption band of the hydrated electron. This result can be rationalized based on the molar extinction coefficient of the

Figure 4.8. Time-resolved differential absorption ($\Delta A(\lambda, t)$) spectra of photodetached tryptophan anion in aqueous solution as a function of pump-probe time delay with **(a)** visible-NIR, **(b)** near-UV, and **(c)** UV probe pulses. Each upper panel shows the averaged ΔA spectrum over time delays of 2 – 3 ps; averaging is performed to minimize the effect of spectral oscillations.

hydrated electron ($11792 \text{ M}^{-1} \text{ cm}^{-1}$)⁴⁶ being $\sim 4\times$ larger than that of the $\text{Trp}^\bullet$ radical ($3000 \text{ M}^{-1} \text{ cm}^{-1}$).⁴⁷

Tuning the probe spectrum to the near-UV, the ΔA spectra show a valley at $\sim 380 - 430 \text{ nm}$, with the ΔA signal rising to the short- and long-wavelength regions. The increase in ΔA at probe wavelengths $> 430 \text{ nm}$ is due to the hydrated electron absorption, prominently observed in the visible-NIR probe spectral range (Fig. 4.8(c)). The increase in ΔA

Figure 4.9. Time traces at 580 nm of photodetached aqueous tryptophan anion (in red) and photodetached aqueous sodium hydroxide (in blue). Global fit on both time traces is shown in black.

for probe wavelengths <380 nm, on the other hand, is suggestive of UV absorption by a photodetachment product.

The ΔA spectra obtained with UV probe pulses exhibit a prominent absorption peak at ~ 334 nm. Below ~ 305 nm, the ΔA signal is negative due to the ground-state bleaching; the static absorption peak of the Trp^- anion at 280 nm extends to ~ 310 nm (Fig. 4.7). The observed peaks in the ΔA spectra are similar to the previously reported UV absorption feature of the $\text{Trp}^\bullet$ radical ($\lambda_{\text{max}} = 335$ nm, $\varepsilon_{\text{max}} = 4750 \text{ M}^{-1} \text{ cm}^{-1}$).⁴⁷ The molar extinction coefficients of the byproducts – the hydrated electron ($1387 \text{ M}^{-1} \text{ cm}^{-1}$)⁴⁶ and the hydroxyl radical ($75 \text{ M}^{-1} \text{ cm}^{-1}$),⁷⁹ the latter produced *via* the photodetachment of the aqueous hydroxide medium – are not only smaller in comparison, but they are also broad and featureless in the 300 – 350 nm region. As such, the UV differential absorption feature originates mainly from the $\text{Trp}^\bullet$ radical. The observed absorption maximum at 334 nm is consistent with the $\text{Trp}^\bullet$

radical bearing an indole radical cation;⁴⁷ the N–H deprotonated Trp• radical species, on the other hand, exhibits an absorption maximum at 325 nm.⁴⁷ This result rules out the indole N–H moiety undergoing deprotonation on the few-picosecond timescale probed in the present experiments.

4.3.3. Vibrational Wave Packet Dynamics of Tryptophan Radical

The vibrational wave packet dynamics of tryptophan radical can be obtained from the time traces of the $\Delta A(\lambda, t)$ spectrum of photodetached aqueous tryptophan anion. We note that there is no evidence of impulsive stimulated Raman scattering (ISRS) of tryptophan anion since the $\Delta A(\lambda, t)$ signal and time-domain oscillations disappear when the ionizing pump power is attenuated to suppress the strong-field photodetachment process. Furthermore, the UV-vis absorption spectrum of aqueous tryptophan anion does not overlap with both probe spectral range. This is evident from the absorbance spectrum of 1 mM tryptophan anion solution at pH 14, shown in Fig. 4.7, which has a maximum absorption at 280 nm.⁶⁶

The time-resolved ΔA spectra in the UV reveal amplitude modulations as a function of time delay, suggesting the presence of vibrational wave packet dynamics. As a means of analyzing the wave packet dynamics quantitatively, we compute the spectral first-moment time trace, given as

$$\langle E(t) \rangle = \frac{\int_{E_i}^{E_f} E \Delta A(E, t) dE}{\int_{E_i}^{E_f} \Delta A(E, t) dE}, \quad (4.3)$$

where E represents the probe photon energy, t is the pump-probe time delay and $\Delta A(E, t)$ is the differential absorbance as a function of probe photon energy and pump-probe time delay.

E_i and E_f define the spectral range that is used to compute the spectral first-moment. E_i and E_f correspond to 3.54 eV (350 nm) and 3.76 eV (330 nm), respectively. The same procedure is repeated for the time-resolved ΔA spectra collected in the visible-NIR, with E_i and E_f set to 2.07 eV (600 nm) and 2.25 eV (550 nm), respectively. After removing the slow population dynamics, the resultant $\langle E(t) \rangle$ traces obtained in the UV and the visible-NIR spectral regions are shown in Figs. 4.10(a) and 4.10(b), respectively; zoomed-in views that show $\langle E(t) \rangle$ up to a time delay of 1 ps are shown in the insets.

The observed oscillations in the $\langle E(t) \rangle$ traces report on vibrational wave packet motion of the Trp• radical. Wave packet motion along a particular vibrational mode Q_i arises when firstly, photodetachment projects the vibrational probability density of Trp⁻ anion onto the potential energy curve of Trp• radical and secondly, the two potential energy curves are displaced along Q_i . The ensuing temporal evolution of the coherent vibrational superposition leads to vibrational wave packet motion. This motion in turn modulates the probe transition energy of the Trp• radical, thus giving rise to the observed oscillations in the $\langle E(t) \rangle$ trace. Since wave packet motion requires the potential energy surfaces of Trp⁻ anion and Trp• radical to be displaced, the vibrational modes that participate in wave packet motion also encode structural rearrangement induced by photodetachment. These vibrational modes can be identified by their characteristic vibrational frequencies, in turn obtained from fast-Fourier transform (FFT) of $\langle E(t) \rangle$. The FFT power spectra of $\langle E(t) \rangle$ retrieved in UV and visible-NIR are shown in Figs. 4.10(c) and 4.10(d), respectively; the insets show the low-frequency ($<300 \text{ cm}^{-1}$) regions. The frequencies and their relative FFT powers are summarized in Table 4.1. Note that the relative FFT powers not only depend on the extent of structural change induced by photodetachment, they are also determined by the displacement between the probe-initial (Trp•) and probe-final (Trp•*) states as well as the time resolution of the experimental setup. A larger displacement

Figure 4.10. First-moment, $\langle E(t) \rangle$, time trace of the photodetached aqueous tryptophan anion in the (a) UV and (b) visible-NIR region with the respective fit function. The inset shows the enlarged $\langle E(t) \rangle$ from 0.25 ps to 1 ps. FFT power spectra of the $\langle E(t) \rangle$ traces of photodetached aqueous tryptophan anion and photodetached aqueous sodium hydroxide (green) in the (c) UV (blue) and (d) visible-NIR (red) region. The inset shows the enlarged FFT power spectra for the frequency range of 0 – 300 cm^{-1} . Note that the oxygen and nitrogen vibrational frequencies are observed at 1557 cm^{-1} and 2329 cm^{-1} respectively.

between the electronic states that are coupled by the probe transition leads to a larger oscillation amplitude. At the same time, a higher time resolution also allows the oscillations to be resolved more readily, resulting in a larger measured oscillation amplitude.

Remarkably, the $\langle E(t) \rangle$ trace in the visible-NIR (Fig. 4.10(b)) appears to report on the vibrational wave packet dynamics of the Trp• radical, even though its absorption signature in the visible-NIR is obfuscated by the hydrated electron absorption. To ascertain that the observed vibrational frequencies originate from the Trp• radical, we performed a set of control experiments with aqueous NaOH (pH 14) under similar photodetachment conditions. The resultant FFT power spectrum (Figs. 4.10(c) and 4.10(d), in green), unlike that obtained with the Trp⁻ anion sample, only reveals vibrational frequencies 1557 cm⁻¹ and 2329 cm⁻¹, assigned to the stretching frequencies of O₂ and N₂, respectively.^{80,81} These frequencies, which appear due to the experiments being performed in air, are also present in the FFT power spectrum of the Trp• radical observed in the UV.

Aside from FFT, the first-moment time trace can also be analyzed in the time domain by fitting it to a sum of exponentially damped cosine functions

$$\langle E(t) \rangle = \sum_{i=1}^n A_i e^{-\frac{t}{\tau_i}} \cos(\omega_i t + \phi_i), \quad (4.4)$$

where A_i , τ_i , ω_i and ϕ_i are the amplitude, dephasing time, vibrational frequency and phase of the vibrational mode Q_i respectively. The fit parameters are summarized in Table 4.2 in the UV and visible-NIR region respectively. Compared to FFT, time-domain analysis directly furnishes the dephasing times of the vibrational modes. The calculated vibrational frequencies reported in Tables 4.1 and 4.2 are those of the microhydrated model.

Table 4.1. Experimental and calculated vibrational frequencies of the tryptophan radical in aqueous solution and their assignments. The calculated frequencies are based on the equilibrium geometry of the microhydrated tryptophan radical.

Experimental FFT Analysis			Calculated Frequency (cm ⁻¹)	Assignment
Frequency (cm ⁻¹)	FFT Power (norm.)			
	UV	Visible-NIR		
753	8.3×10^{-1}	2.0×10^{-1}	766	Indole CH out-of-plane bend
874	7.6×10^{-2}	4.4×10^{-2}	875	Indole CH out-of-plane bend
1008	1	6.6×10^{-1}	1033	Indole in-plane deformation
1323	5.3×10^{-2}	6.7×10^{-2}	1349	Indole CC stretch
1360	1.4×10^{-1}	1.4×10^{-2}	1373	Indole CC and CN stretch
1554	1.6×10^{-1}	1	1553	Indole CC stretch / Indole NH wag
1614	3.5×10^{-2}	3.3×10^{-2}	1610	Indole CC stretch
3071	—	1.8×10^{-2}	3046	Indole NH stretch
1554				Oxygen
2331				Nitrogen

Table 4.2. Experimental time-domain analysis results, and calculated frequencies of the tryptophan radical in aqueous solution and their assignments in the UV and visible-NIR regions. The calculated frequencies are based on the equilibrium geometry of the microhydrated tryptophan radical.

Time-Domain Analysis										Calc. Freq. (cm ⁻¹)	Assignment
UV				Visible-NIR							
Freq. (cm ⁻¹)	Amplitude (meV)	Dephasing Time (ps)	Phase (π-rad)	Freq. (cm ⁻¹)	Amplitude (meV)	Dephasing Time (ps)	Phase (π-rad)				
758 ± 1	7.32 ± 0.23	1.40 ± 0.08	1.51 ± 0.01	757 ± 1	0.07 ± 0.01	1.23 ± 0.10	0.36 ± 0.02	766	Indole CH out-of-plane bend		
879 ± 1	4.85 ± 0.37	0.71 ± 0.08	0.96 ± 0.03	879 ± 1	0.04 ± 0.01	0.79 ± 0.12	0.11 ± 0.04	875	Indole CH out-of-plane bend		
1012 ± 1	6.80 ± 0.20	1.80 ± 0.14	0.70 ± 0.01	1012 ± 1	0.11 ± 0.01	1.39 ± 0.09	0.10 ± 0.01	1033	Indole in-plane deformation		
1337 ± 6	2.80 ± 1.07	0.53 ± 0.23	0.01 ± 0.20	1332 ± 4	0.23 ± 0.06	0.28 ± 0.04	0.17 ± 0.11	1349	Indole CC stretch		
1361 ± 2	4.66 ± 1.01	0.79 ± 0.21	1.58 ± 0.06	1368 ± 3	0.16 ± 0.06	0.36 ± 0.08	1.38 ± 0.09	1373	Indole CC and CN stretch		
1526 ± 8	7.40 ± 3.44	0.22 ± 0.07	1.37 ± 0.14	1553 ± 2	1.09 ± 0.08	0.18 ± 0.01	0.01 ± 0.03	1553	Indole CC stretch / Indole NH wag		
1578 ± 2	1.20 ± 0.35	1.43 ± 0.55	1.25 ± 0.06	1604 ± 3	0.01 ± 0.01	1.50 ± 1.17	1.77 ± 0.11	1610	Indole CC stretch		
—	—	—	—	3068 ± 3	0.08 ± 0.01	0.39 ± 0.08	1.14 ± 0.06	3046	Indole NH stretch		
1550 ± 1	4.84 ± 0.99	0.86 ± 0.19	1.31 ± 0.06	1557 ± 1	0.10 ± 0.01	1.18 ± 0.10	1.48 ± 0.02		Oxygen		
2331 ± 1	1.02 ± 0.15	5.17 ± 1.78	0.50 ± 0.02	2329 ± 1	0.07 ± 0.01	2.70 ± 0.15	1.43 ± 0.01		Nitrogen		

4.4. Discussion

The vibrational modes observed from the spectral first-moment time trace would only be observed when, for a given vibration mode, there is a non-zero displacement between the potential energy curves of tryptophan anion and tryptophan radical. Excluding the vibrational frequencies of oxygen (1557 cm^{-1}) and nitrogen (2329 cm^{-1}), the remaining eight vibrational frequencies are associated with the vibrational modes of tryptophan radical coupled to the photodetachment process. From Table 4.2, the majority of the vibrational modes have a dephasing time on the order of $\sim 1\text{ ps}$, comparable to those observed for other molecules in the condensed phase.^{82,83}

The vibrational modes that contribute to the $\langle E(t) \rangle$ time trace have displacement vectors that are largely localized on the indole sidechain (Fig. 4.11). This result is consistent with the highest occupied molecular orbital (HOMO) residing on the indole moiety (Fig. 4.12). As such, the ejection of the HOMO electron by strong-field photodetachment alters the electronic configuration of the π covalent bonds within the indole ring, thus driving structural rearrangement predominantly on the indole ring. This observation is supported by the changes in the C–C and C–N bond lengths within the indole sidechain (Table 4.3), in particular, the C4–C5 and C5–N13 bonds, which upon photodetachment change by $+4.8\text{ pm}$ and -4.9 pm , respectively. The present results are also in line with the previously observed vibrational wave packet dynamics induced by the strong-field photodetachment of the tyrosinate dianion.⁸⁴ There, the vibrational modes activated in the tyrosyl radical anion are primarily localized on the phenoxyl sidechain, where the HOMO of the tyrosinate dianion resides. In the present work,

Fig. 4.11. Displacement vectors of the vibrational modes of the tryptophan radical that are coupled to the photodetachment process. The blue arrows represent the magnitude and direction of the atomic displacements. The calculated vibrational frequencies are **(a)** 766 cm^{-1} **(b)** 875 cm^{-1} **(c)** 1033 cm^{-1} **(d)** 1349 cm^{-1} **(e)** 1373 cm^{-1} **(f)** 1553 cm^{-1} **(g)** 1610 cm^{-1} **(h)** 3046 cm^{-1} .

the sub-10-fs time resolution that is available with visible-NIR probing also makes it possible

Fig. 4.12. HOMO of the microhydrated tryptophan radical.

to resolve the high-frequency N–H stretch at $\sim 3070\text{ cm}^{-1}$.

Aside from vibrational modes with displacement vectors that are localized on the indole sidechain, the *ab initio* calculations reveal a marked contraction in the intermolecular N $\cdots$ O distance between the indole sidechain and the microhydrating water molecule from 2.872 Å in the tryptophan anion to 2.749 Å in the tryptophan radical; both distances are consistent with the typical hydrogen bonding distance of 2.70 – 3.00 Å.⁶⁹ Going from the Trp[–] anion to the Trp[•] radical, the Mulliken charge on the indole ring increases from +0.253 to +0.539, and the Mulliken charge localized on the indolyl N–H moiety increase from +0.246 to +0.376. Hence, the decrease in the N $\cdots$ O distance by $>0.1\text{ Å}$ probably arises from the indole sidechain acquiring a formal positive charge upon photodetachment, which in turn strengthens the hydrogen bond between the indolyl N–H bond (the hydrogen bond donor) and the microhydrating water molecule (the hydrogen bond acceptor). The large change in the

Table 4.3. Results of DFT calculations, showing selected bond lengths of the microhydrated tryptophan anion and the microhydrated tryptophan radical, and changes in bond lengths upon photodetachment.

Bond	Bond Length / pm		Difference / pm
	Tryptophan Anion	Tryptophan Radical	
C1–C2	155.2	155.6	0.4
C2–C3	155.5	158.2	2.7
C3–C4	150.1	147.8	-2.3
C4–C5	137.5	142.3	4.8
C5–N13	138.0	133.1	-4.9
C6–N13	137.5	140.3	2.8
C6–C7	140.0	138.3	-1.7
C7–C8	139.0	140.2	1.2
C8–C9	141.1	140.1	-1.0
C9–C10	138.9	139.4	0.5
C10–C11	140.7	140.2	-0.5
C4–C11	144.3	143.7	-0.6
C6–C11	142.6	141.7	-0.9
N13–H22	102.4	104.2	1.8
N13–O27	287.2	274.9	-12.3

intermolecular distance is likely to trigger coherent hindered translational motion. According to the theoretical calculations, these modes are characterized by frequencies of 128, 240, and 265 cm^{-1} (Fig. 4.13), in good agreement with the features observed in the $<300\text{-cm}^{-1}$ region of the FFT power spectra (inset of Figs. 4.10(c) and 4.10(d)). Similar coherent solvent reorganization dynamics was recently observed for a Fe(II)–Ru(III) transition metal complex in water following metal-to-metal charge transfer photoexcitation; there, femtosecond X-ray scattering resolved coherent hindered translational motion between the solute and the solvent

Fig. 4.13. Low frequency intermolecular vibrational modes of microhydrated tryptophan radical **(a)** 128 cm^{-1} , **(b)** 240 cm^{-1} and **(c)** 265 cm^{-1} .

with a characteristic frequency of $\sim 180\text{ cm}^{-1}$.⁸⁵ Future studies employing D_2O as solvent could potentially verify the proposed assignment of the observed low-frequency modes to intermolecular hindered translation, although we note that the frequency is expected to downshift by only $\sim 5\%$ upon isotopic substitution due to the similar masses of H_2O and D_2O .

Finally, we employ *ab initio* calculations to address the apparent stability of the indole radical sidechain with respect to N–H deprotonation. The $\text{p}K_{\text{a}}$ value of the indole radical cation is previously found to be ~ 4.3 ,^{49,51,86} consistent with the observation of the deprotonated indole radical at pH 7.^{48,49} As such, one might expect the tryptophan radical anion ($\text{Trp}^{\bullet-}$), in which the indole side chain exists in the deprotonated form, to be produced under our experimental conditions (pH 14). The result of a relaxed potential energy scan is shown in Fig. 4.14. In the scan, the N–H bond length is varied in the range of $0.8 - 3.8\text{ \AA}$ while allowing all the other degrees of freedom to optimize. The potential energy curve reveals that the N–H bond is bound by a $>1\text{-eV}$ -deep well. The large dissociation energy suppresses spontaneous deprotonation of the indole radical cation. Instead, deprotonation likely involves a diffusion-limited bimolecular

Fig. 4.14. Potential energy curve as a function of the indole N–H bond length for the microhydrated tryptophan radical.

reaction of the $\text{Trp}^\bullet$ radical with the OH^- anion, which occurs on timescales beyond the few-picoseconds probed in the present experiments.

4.5. Conclusion

In conclusion, femtosecond strong-field photodetachment of the tryptophan anion in aqueous solution followed by probing in either the UV or the visible-NIR results in the coherent vibrational motion of the tryptophan radical product. Even though the visible absorption feature of the $\text{Trp}^\bullet$ radical is obfuscated by the $\sim 4\times$ more intense hydrated electron absorption, the $\langle E(t) \rangle$ time trace computed about the previously reported visible absorption maximum of the $\text{Trp}^\bullet$ radical at ~ 580 nm nevertheless reveals oscillatory features that encode vibrational wave packet dynamics. Analysis of the wave packet dynamics observed in both the UV and the visible reveals contributions from multiple distinct frequency components, which are assigned

with the aid of DFT calculations that include explicit water molecules of hydration. The appearance of these vibrational modes signals their participation in the photodetachment-induced ultrafast structural rearrangement dynamics. The displacement vectors of the vibrational modes that encode intramolecular structural rearrangement are primarily localized on the indole sidechain, a result that can be rationalized in terms of the strong-field electron ejection from the indole-centered HOMO perturbing the indole π covalent framework. In addition, both experiment and theory suggest the existence of intermolecular structural rearrangement, mainly along the hindered translational mode involving a water molecule hydrogen-bonded to the indole N–H moiety. This intermolecular vibration is characterized by low-frequency modes that appear below 300 cm^{-1} in the experimental FFT power spectra and the *ab initio* calculations.

We do note the limitation of our study. The absorption band of the tryptophan radical in the visible region ($\sim 580\text{ nm}$) is not apparent due to the overlap with the absorption signal of the ground-state hydrated electron. Future work can be performed to suppress the absorption signal of the ground-state hydrated electron by performing a detrapping experiment. The detrapping experiment can be performed by introducing a third pulse, which is a long UV pulse, centered at $\sim 400\text{ nm}$, to suppress the absorption signal of the hydrated electron by exciting the hydrated electron to the conduction band of liquid water. This will result in the depletion of the population of the ground-state hydrated electron that interferes with the absorption of Trp• radical at 580 nm . Moreover, Trp• radical does not absorb strongly at $\sim 400\text{ nm}$, thus this will reduce the interference of the detrapping 400 nm pulse with the Trp• radical dynamics. Therefore, the absorption band associated with the hydrated electron would become less prominent and Trp• radical would persist longer and the absorption band at 580 nm associated with Trp• radical would become more prominent.^{87,88}

Our study of the ultrafast coherent vibrational dynamics of the aqueous Trp• radical species provides insight into the ultrafast structural reorganization dynamics triggered by the interaction of ionizing radiation with molecules of biological importance. Although hitherto unobserved in our studies of photodetached tyrosinate and tryptophan anions in aqueous solution, our experimental approach can also elucidate possible ultrafast fragmentation dynamics following photoionization or photodetachment,⁸⁹⁻⁹⁵ including those that accompany charge migration.⁹⁶⁻¹⁰¹

References

1. Garrett, B. C.; Dixon, D. A.; Camaioni, D. M.; Chipman, D. M.; Johnson, M. A.; Jonah, C. D.; Kimmel, G. A.; Miller, J. H.; Rescigno, T. N.; Rossky, P. J.; Xantheas, S. S.; Colson, S. D.; Laufer, A. H.; Ray, D.; Barbara, P. F.; Bartels, D. M.; Becker, K. H.; Bowen, K. H.; Bradforth, S. E.; Carmichael, I.; Coe, J. V.; Corrales, L. R.; Cowin, J. P.; Dupuis, M.; Eienthal, K. B.; Franz, J. A.; Gutowski, M. S.; Jordan, K. D.; Kay, B. D.; LaVerne, J. A.; Lyman, S. V.; Madey, T. E.; McCurdy, C. W.; Meisel, D.; Mukamel, S.; Nilsson, A. R.; Orlando, T. M.; Petrik, N. G.; Pimblott, S. M.; Rustad, J. R.; Schenter, G. K.; Singer, S. J.; Tokmakoff, A.; Wang, L.-S.; Zwier, T. S., Role of Water in Electron-Initiated Processes and Radical Chemistry: Issues and Scientific Advances. *Chem. Rev.* **2005**, *105* (1), 355-390.
2. Cannington, P. H.; Ham, N. S., He(I) and He(II) Photoelectron Spectra of Glycine and Related Molecules. *J. Electron Spectros. Relat. Phenomena* **1983**, *32* (2), 139-151.
3. Close, D. M., Calculated Vertical Ionization Energies of the Common α -Amino Acids in the Gas Phase and in Solution. *J. Phys. Chem. A* **2011**, *115* (13), 2900-2912.
4. Fernstrom, J. D., Role of Precursor Availability in Control of Monoamine Biosynthesis in Brain. *Physiol. Rev.* **1983**, *63* (2), 484-546.
5. Schaechter, J. D.; Wurtman, R. J., Serotonin Release varies with Brain Tryptophan Levels. *Brain Res.* **1990**, *532* (1), 203-210.
6. Murch, S. J.; KrishnaRaj, S.; Saxena, P. K., Tryptophan is a Precursor for Melatonin and Serotonin Biosynthesis in in vitro Regenerated St. John's Wort (*Hypericum perforatum* L. cv. Anthos) Plants. *Plant Cell Rep.* **2000**, *19* (7), 698-704.
7. Paredes, S. D.; Barriga, C.; Reiter, R. J.; Rodríguez, A. B., Assessment of the Potential Role of Tryptophan as the Precursor of Serotonin and Melatonin for the Aged Sleep-

- wake Cycle and Immune Function: Streptopelia Risoris as a Model. *Int. J. Tryptophan Res.* **2009**, 2, 23-26.
8. Reeve, A. E.; Hopkins, T. R., Photochemical Reaction between Protein and Nucleic Acids: Photoaddition of Tryptophan to Pyrimidine Bases. *Photochem. Photobiol.* **1980**, 31 (4), 297-304.
 9. Sherin, P. S.; Snytnikova, O. A.; Tsentalovich, Y. P., Tryptophan Photoionization from Prefluorescent and Fluorescent States. *Chem. Phys. Lett.* **2004**, 391 (1), 44-49.
 10. Igarashi, N.; Onoue, S.; Tsuda, Y., Photoreactivity of Amino Acids: Tryptophan-Induced Photochemical Events *via* Reactive Oxygen Species Generation. *Anal. Sci.* **2007**, 23 (8), 943-948.
 11. Jensen, R. L.; Arnbjerg, J.; Ogilby, P. R., Reaction of Singlet Oxygen with Tryptophan in Proteins: A Pronounced Effect of the Local Environment on the Reaction Rate. *J. Am. Chem. Soc.* **2012**, 134 (23), 9820-9826.
 12. Janssen, E. M. L.; Erickson, P. R.; McNeill, K., Dual Roles of Dissolved Organic Matter as Sensitizer and Quencher in the Photooxidation of Tryptophan. *Environ. Sci. Technol.* **2014**, 48 (9), 4916-4924.
 13. Creed, D., The Photophysics and Photochemistry of the Near-UV Absorbing Amino Acids - I. Tryptophan and its Simple Derivatives. *Photochem. Photobiol.* **1984**, 39 (4), 537-562.
 14. Lee, G. K.; Park, H. J.; Macleod, M.; Chandler, P.; Munn, D. H.; Mellor, A. L., Tryptophan Deprivation Sensitizes Activated T Cells to Apoptosis prior to Cell Division. *Immunology* **2002**, 107 (4), 452-460.
 15. Richard, D. M.; Dawes, M. A.; Mathias, C. W.; Acheson, A.; Hill-Kapturczak, N.; Dougherty, D. M., L-Tryptophan: Basic Metabolic Functions, Behavioral Research and Therapeutic Indications. *Int. J. Tryptophan Res.* **2009**, 2, 45-60.

16. Engin, A.; Engin, A. B., *Tryptophan Metabolism: Implications for Biological Processes, Health and Disease*. Springer International Publishing: Cham, 2015.
17. Ehrenshaft, M.; Deterding, L. J.; Mason, R. P., Tripping up Trp: Modification of Protein Tryptophan Residues by Reactive Oxygen Species, Modes of Detection, and Biological Consequences. *Free Radic. Biol. Med.* **2015**, *89*, 220-228.
18. Himo, F.; Eriksson, L. A., Theoretical Study of Model Tryptophan Radicals and Radical Cations: Comparison with Experimental Data of DNA Photolyase, Cytochrome *c* Peroxidase, and Ribonucleotide Reductase. *J. Phys. Chem. B* **1997**, *101* (47), 9811-9819.
19. Bleifuss, G.; Kolberg, M.; Pötsch, S.; Hofbauer, W.; Bittl, R.; Lubitz, W.; Gräslund, A.; Lassmann, G.; Lendzian, F., Tryptophan and Tyrosine Radicals in Ribonucleotide Reductase: A Comparative High-Field EPR Study at 94 GHz. *Biochemistry* **2001**, *40* (50), 15362-15368.
20. Pogni, R.; Baratto, M. C.; Teutloff, C.; Giansanti, S.; Ruiz-Dueñas, F.; Choinowski, T.; Piontek, K.; Martinez, A. T.; Lendzian, F.; Basosi, R., A Tryptophan Neutral Radical in the Oxidized State of Versatile Peroxidase from *Pleurotus eryngii*: A Combined Multifrequency EPR and Density Functional Theory Study. *J. Biol. Chem.* **2006**, *281*, 9517-26.
21. Lundeen, R. A.; Janssen, E. M. L.; Chu, C.; McNeill, K., Environmental Photochemistry of Amino Acids, Peptides and Proteins. *Chimia* **2014**, *68* (11), 812-817.
22. Joshi, R., Charge Transfer Reactions from Tryptophan and Tyrosine to Sulfur-Centered Dimer Radical Cation in Aqueous –Sulfuric Acid Medium: A Pulse Radiolysis Study. *J. Phys. Org. Chem.* **2016**, *29* (6), 281-287.
23. Davis, I.; Koto, T.; Terrell, J. R.; Kozhanov, A.; Krzystek, J.; Liu, A., High-Frequency/High-Field Electron Paramagnetic Resonance and Theoretical Studies of Tryptophan-Based Radicals. *J. Phys. Chem. A* **2018**, *122* (12), 3170-3176.

24. Müller, P.; Ignatz, E.; Kiontke, S.; Brettel, K.; Essen, L.-O., Sub-Nanosecond Tryptophan Radical Deprotonation Mediated by a Protein-Bound Water Cluster in Class II DNA Photolyases. *Chem. Sci.* **2018**, 9 (5), 1200-1212.
25. Li, Y. F.; Heelis, P. F.; Sancar, A., Active Site of DNA Photolyase: Tryptophan-306 is the Intrinsic Hydrogen Atom Donor Essential for Flavin Radical Photoreduction and DNA Repair in vitro. *Biochemistry* **1991**, 30 (25), 6322-6329.
26. Brettel, K.; Byrdin, M., Reaction Mechanisms of DNA Photolyase. *Curr. Opin. Struct. Biol.* **2010**, 20 (6), 693-701.
27. Aubert, C.; Vos, M. H.; Mathis, P.; Eker, A. P. M.; Brettel, K., Intraprotein Radical Transfer during Photoactivation of DNA Photolyase. *Nature* **2000**, 405 (6786), 586-590.
28. Mauro, J. M.; Fishel, L. A.; Hazzard, J. T.; Meyer, T. E.; Tollin, G.; Cusanovich, M. A.; Kraut, J., Tryptophan-191 → Phenylalanine, A Proximal-Side Mutation in Yeast Cytochrome *c* Peroxidase that Strongly Affects the Kinetics of Ferrocycytochrome *c* Oxidation. *Biochemistry* **1988**, 27 (17), 6243-6256.
29. Sivaraja, M.; Goodin, D.; Smith, M.; Hoffman, B., Identification by ENDOR of Trp191 as the Free-Radical Site in Cytochrome *c* Peroxidase Compound ES. *Science* **1989**, 245 (4919), 738-740.
30. Volkov, A. N.; Nicholls, P.; Worrall, J. A. R., The Complex of Cytochrome *c* and Cytochrome *c* Peroxidase: The End of the Road? *Biochim. Biophys. Acta Bioenerg.* **2011**, 1807 (11), 1482-1503.
31. Stubbe, J.; Nocera, D. G.; Yee, C. S.; Chang, M. C. Y., Radical Initiation in the Class I Ribonucleotide Reductase: Long-Range Proton-Coupled Electron Transfer? *Chem. Rev.* **2003**, 103 (6), 2167-2202.

32. Reece, S. Y.; Stubbe, J.; Nocera, D. G., pH Dependence of Charge Transfer between Tryptophan and Tyrosine in Dipeptides. *Biochim. Biophys. Acta Bioenerg.* **2005**, *1706* (3), 232-238.
33. Greene, B. L.; Kang, G.; Cui, C.; Bennati, M.; Nocera, D. G.; Drennan, C. L.; Stubbe, J., Ribonucleotide Reductases: Structure, Chemistry, and Metabolism Suggest New Therapeutic Targets. *Annu. Rev. Biochem.* **2020**, *89* (1), 45-75.
34. Gray, H. B.; Winkler, J. R., Hole Hopping through Tyrosine/Tryptophan Chains Protects Proteins from Oxidative Damage. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 10920–10925.
35. Winkler, J. R.; Gray, H. B., Could Tyrosine and Tryptophan Serve Multiple Roles in Biological Redox Processes? *Philos. Trans. A Math. Phys. Eng. Sci.* **2015**, *373* (2037), 20140178.
36. Chang, M. C. Y.; Yee, C. S.; Stubbe, J.; Nocera, D. G., Turning on Ribonucleotide Reductase by Light-Initiated Amino Acid Radical Generation. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101* (18), 6882-6887.
37. Pizano, A. A.; Lutterman, D. A.; Holder, P. G.; Teets, T. S.; Stubbe, J.; Nocera, D. G., Photo-Ribonucleotide Reductase $\beta 2$ by Selective Cysteine Labeling with a Radical Phototrigger. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *109*, 39-43.
38. Olshansky, L.; Greene, B. L.; Finkbeiner, C.; Stubbe, J.; Nocera, D. G., Photochemical Generation of a Tryptophan Radical within the Subunit Interface of Ribonucleotide Reductase. *Biochemistry* **2016**, *55* (23), 3234-3240.
39. Roy, A.; Seidel, R.; Kumar, G.; Bradforth, S. E., Exploring Redox Properties of Aromatic Amino Acids in Water: Contrasting Single Photon vs Resonant Multiphoton Ionization in Aqueous Solutions. *J. Phys. Chem. B* **2018**, *122* (14), 3723-3733.

40. Winter, B.; Weber, R.; Widdra, W.; Dittmar, M.; Faubel, M.; Hertel, I. V., Full Valence Band Photoemission from Liquid Water Using EUV Synchrotron Radiation. *J. Phys. Chem. A* **2004**, *108* (14), 2625-2632.
41. Nishizawa, K.; Kurahashi, N.; Sekiguchi, K.; Mizuno, T.; Ogi, Y.; Horio, T.; Oura, M.; Kosugi, N.; Suzuki, T., High-Resolution Soft X-Ray Photoelectron Spectroscopy of Liquid Water. *Phys. Chem. Chem. Phys.* **2011**, *13* (2), 413-417.
42. Kurahashi, N.; Karashima, S.; Tang, Y.; Horio, T.; Abulimiti, B.; Suzuki, Y.-I.; Ogi, Y.; Oura, M.; Suzuki, T., Photoelectron Spectroscopy of Aqueous Solutions: Streaming Potentials of NaX (X = Cl, Br, and I) Solutions and Electron Binding Energies of Liquid Water and X^- . *J. Chem. Phys.* **2014**, *140* (17), 174506.
43. Perry, C. F.; Zhang, P.; Nunes, F. B.; Jordan, I.; von Conta, A.; Wörner, H. J., Ionization Energy of Liquid Water Revisited. *J. Phys. Chem. Lett.* **2020**, *11* (5), 1789-1794.
44. Pépin, C.; Goulet, T.; Houde, D.; Jay-Gerin, J. P., Observation of a Continuous Spectral Shift in the Solvation Kinetics of Electrons in Neat Liquid Deuterated Water. *J. Phys. Chem. A* **1997**, *101* (24), 4351-4360.
45. Hare, P. M.; Price, E. A.; Bartels, D. M., Hydrated Electron Extinction Coefficient Revisited. *J. Phys. Chem. A* **2008**, *112* (30), 6800-6802.
46. Torche, F.; Marignier, J.-L., Direct Evaluation of the Molar Absorption Coefficient of Hydrated Electron by the Isosbestic Point Method. *J. Phys. Chem. B* **2016**, *120* (29), 7201-7206.
47. Solar, S.; Getoff, N.; Surdhar, P. S.; Armstrong, D. A.; Singh, A., Oxidation of Tryptophan and N-methylindole by $N_3^\bullet$, $Br_2^{\bullet-}$, and $(SCN)_2^{\bullet-}$ Radicals in Light- and Heavy-Water Solutions: A Pulse Radiolysis Study. *J. Phys. Chem.* **1991**, *95* (9), 3639-3643.

48. Bent, D. V.; Hayon, E., Excited State Chemistry of Aromatic Amino Acids and Related Peptides. III. Tryptophan. *J. Am. Chem. Soc.* **1975**, *97* (10), 2612-2619.
49. McGimpsey, W. G.; Görner, H., Photoionization of Indole, N-Methylindole and Tryptophan In Aqueous Solution upon Excitation at 193 nm. *Photochem. Photobiol.* **1996**, *64* (3), 501-509.
50. Błoch-Mechkour, A.; Bally, T.; Sikora, A.; Michalski, R.; Marcinek, A.; Gębicki, J., Radicals and Radical Ions Derived from Indole, Indole-3-carbinol and Diindolylmethane. *J. Phys. Chem. A* **2010**, *114* (25), 6787-6794.
51. Posener, M. L.; Adams, G. E.; Wardman, P.; Cundall, R. B., Mechanism of Tryptophan Oxidation by Some Inorganic Radical-Anions: A Pulse Radiolysis Study. *J. Chem. Soc., Faraday Trans. 1* **1976**, *72*, 2231-2239.
52. Baugher, J. F.; Grossweiner, L. I., Photolysis Mechanism of Aqueous Tryptophan. *J. Phys. Chem.* **1977**, *81* (14), 1349-1354.
53. Bryant, F. D.; Santus, R.; Grossweiner, L. I., Laser Flash Photolysis of Aqueous Tryptophan. *J. Phys. Chem.* **1975**, *79* (25), 2711-2716.
54. Redpath, J. L.; Santus, R.; Ovadia, J.; Grossweiner, L. I., The Oxidation of Tryptophan by Radical Anions. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **1975**, *27* (2), 201-204.
55. Kandori, H.; Borkman, R. F.; Yoshihara, K., Picosecond Transient Absorption of Aqueous Tryptophan. *J. Phys. Chem.* **1993**, *97* (38), 9664-9667.
56. Stevenson, K. L.; Papadantonakis, G. A.; LeBreton, P. R., Nanosecond UV Laser Photoionization of Aqueous Tryptophan: Temperature Dependence of Quantum Yield, Mechanism, and Kinetics of Hydrated Electron Decay. *J. Photochem. Photobiol. A* **2000**, *133* (3), 159-167.

57. Tsentalovich, Y. P.; Snytnikova, O. A.; Sagdeev, R. Z., Properties of Excited States of Aqueous Tryptophan. *J. Photochem. Photobiol. A* **2004**, *162* (2), 371-379.
58. Larson, B. C.; Pomponio, J. R.; Shafaat, H. S.; Kim, R. H.; Leigh, B. S.; Tauber, M. J.; Kim, J. E., Photogeneration and Quenching of Tryptophan Radical in Azurin. *J. Phys. Chem. B* **2015**, *119* (29), 9438-9449.
59. Shafaat, H. S.; Kim, J. E., Resonance Raman Analysis of the Tryptophan Cation Radical. *J. Phys. Chem. Lett.* **2014**, *5* (17), 3009-3014.
60. Debnath, T.; Mohd Yusof, M. S. B.; Low, P. J.; Loh, Z.-H., Ultrafast Structural Rearrangement Dynamics Induced by the Photodetachment of Phenoxide in Aqueous Solution. *Nat. Commun.* **2019**, *10* (1), 2944.
61. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. *Gaussian 16 Rev. C.01*, Wallingford, CT, 2016.
62. Becke, A. D., Density-Functional Exchange-Energy Approximation with Correct Asymptotic Behavior. *Phys. Rev. A* **1988**, *38* (6), 3098-3100.

63. Lee, C.; Yang, W.; Parr, R. G., Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density. *Phys. Rev. B* **1988**, 37 (2), 785-789.
64. Tomasi, J.; Mennucci, B.; Cammi, R., Quantum Mechanical Continuum Solvation Models. *Chem. Rev.* **2005**, 105 (8), 2999-3094.
65. Liu, L.-F.; Yang, L.-L.; Jin, K.-Y.; Xu, D.-Q.; Gao, C.-J., Recovery of L-Tryptophan from Crystallization Wastewater by Combined Membrane PSeparation and Purification Technologyrocess. *Sep. Purif. Technol.* **2009**, 66 (3), 443-449.
66. Lundblad, R. L.; Macdonald, F., *Handbook of Biochemistry and Molecular Biology* Fifth edition ed.; CRC Press: 2018.
67. Lozovoy, V. V.; Pastirk, I.; Dantus, M., Multiphoton Intrapulse Interference. IV. Ultrashort Laser Pulse Spectral Phase Characterization and Compensation. *Opt. Lett.* **2004**, 29 (7), 775-777.
68. Pestov, D.; Lozovoy, V. V.; Dantus, M., Multiple Independent Comb Shaping (MICS): Phase-Only Generation of Optical Pulse Sequences. **2009**, - 17 (- 16), - 14361.
69. Jeffrey, G. A., *An Introduction to Hydrogen Bonding* Oxford University Press: New York, 1997.
70. Gilli, P.; Pretto, L.; Bertolasi, V.; Gilli, G., Predicting Hydrogen-Bond Strengths from Acid–Base Molecular Properties. The pKa Slide Rule: Toward the Solution of a Long-Lasting Problem. *Acc. Chem. Res.* **2009**, 42 (1), 33-44.
71. Ambrosio, F.; Guo, Z.; Pasquarello, A., Absolute Energy Levels of Liquid Water. *J. Phys. Chem. Lett.* **2018**, 9 (12), 3212-3216.
72. Ambrosio, F.; Miceli, G.; Pasquarello, A., Electronic Levels of Excess Electrons in Liquid Water. *J. Phys. Chem. Lett.* **2017**, 8 (9), 2055-2059.
73. Mostafavi, M.; Lampre, I., An Overview of Solvated Electrons: Recent Advances. 2010; Vol. 105, pp 21-58.

74. Crowell, R. A.; Bartels, D. M., Multiphoton Ionization of Liquid Water with 3.0–5.0 eV Photons. *J. Phys. Chem.* **1996**, *100* (45), 17940-17949.
75. Silva, C.; Walhout, P. K.; Yokoyama, K.; Barbara, P. F., Femtosecond Solvation Dynamics of the Hydrated Electron. *Phys. Rev. Lett.* **1998**, *80* (5), 1086-1089.
76. Vilchiz, V. H.; Klopfer, J. A.; Germaine, A. C.; Lenchenkov, V. A.; Bradforth, S. E., Map for the Relaxation Dynamics of Hot Photoelectrons Injected into Liquid Water *via* Anion Threshold Photodetachment and above Threshold Solvent Ionization. *J. Phys. Chem. A* **2001**, *105* (10), 1711-1723.
77. Pshenichnikov, M. S.; Baltuška, A.; Wiersma, D. A., Hydrated-electron Population Dynamics. *Chem. Phys. Lett.* **2004**, *389* (1), 171-175.
78. Elkins, M. H.; Williams, H. L.; Shreve, A. T.; Neumark, D. M., Relaxation Mechanism of the Hydrated Electron. *Science* **2013**, *342* (6165), 1496-1499.
79. Czapski, G.; Bielski, B. H. J., Absorption Spectra of the $\bullet\text{OH}$ and $\text{O}\bullet^-$ Radicals in Aqueous Solutions. *Radiat. Phys. Chem.* **1993**, *41* (3), 503-505.
80. Odhner, J. H.; Romanov, D. A.; Levis, R. J., Rovibrational Wave-Packet Dispersion during Femtosecond Laser Filamentation in Air. *Phys. Rev. Lett.* **2009**, *103* (7), 075005.
81. Salter, R.; Chu, J.; Hippler, M., Cavity-Enhanced Raman Spectroscopy with Optical Feedback CW Diode Lasers for Gas Phase Analysis and Spectroscopy. *Analyst* **2012**, *137* (20), 4669-4676.
82. Dean, J. C.; Rafiq, S.; Oblinsky, D. G.; Cassette, E.; Jumper, C. C.; Scholes, G. D., Broadband Transient Absorption and Two-Dimensional Electronic Spectroscopy of Methylene Blue. *J. Phys. Chem. A* **2015**, *119* (34), 9098-9108.
83. Rafiq, S.; Scholes, G. D., Slow Intramolecular Vibrational Relaxation Leads to Long-Lived Excited-State Wavepackets. *J. Phys. Chem. A* **2016**, *120* (34), 6792-6799.

84. Bin Mohd Yusof, M. S.; Lim, Y. L.; Loh, Z.-H., Ultrafast Vibrational Wave Packet Dynamics of the Aqueous Tyrosyl Radical Anion Induced by Photodetachment. *Phys. Chem. Chem. Phys.* **2021**, 23 (34), 18525-18534.
85. Biasin, E.; Fox, Z. W.; Andersen, A.; Ledbetter, K.; Kjær, K. S.; Alonso-Mori, R.; Carlstad, J. M.; Chollet, M.; Gaynor, J. D.; Glowina, J. M.; Hong, K.; Kroll, T.; Lee, J. H.; Liekhus-Schmaltz, C.; Reinhard, M.; Sokaras, D.; Zhang, Y.; Doumy, G.; March, A. M.; Southworth, S. H.; Mukamel, S.; Gaffney, K. J.; Schoenlein, R. W.; Govind, N.; Cordones, A. A.; Khalil, M., Direct Observation of Coherent Femtosecond Solvent Reorganization Coupled to Intramolecular Electron Transfer. *Nat. Chem.* **2021**, 13 (4), 343-349.
86. Jovanovic, S. V.; Steenken, S.; Simic, M. G., Kinetics and Energetics of One-Electron-Transfer Reactions Involving Tryptophan Neutral and Cation Radicals. *J. Phys. Chem.* **1991**, 95 (2), 684-687.
87. Hee Son, D.; Kambhampati, P.; Kee, T. W.; Barbara, P. F., One-photon UV Detrapping of the Hydrated Electron. *Chem. Phys. Lett.* **2001**, 342 (5), 571-577.
88. Son, D. H.; Kambhampati, P.; Kee, T. W.; Barbara, P. F., Delocalizing Electrons in Water with Light. *J. Phys. Chem. A* **2001**, 105 (36), 8269-8272.
89. Weinkauf, R.; Aicher, P.; Wesley, G.; Grotemeyer, J.; Schlag, E. W., Femtosecond versus Nanosecond Multiphoton Ionization and Dissociation of Large Molecules. *J. Phys. Chem.* **1994**, 98 (34), 8381-8391.
90. Shemesh, D.; Gerber, R. B., Classical Trajectory Simulations of Photoionization Dynamics of Tryptophan: Intramolecular Energy Flow, Hydrogen-Transfer Processes and Conformational Transitions. *J. Phys. Chem. A* **2006**, 110 (27), 8401-8408.
91. Schwell, M.; Jochims, H.-W.; Baumgärtel, H.; Dulieu, F.; Leach, S., VUV Photochemistry of Small Biomolecules. *Planet. Space Sci.* **2006**, 54 (11), 1073-1085.

92. Vorsa, V.; Kono, T.; Willey, K. F.; Winograd, N., Femtosecond Photoionization of Ion Beam Desorbed Aliphatic and Aromatic Amino Acids: Fragmentation *via* α -Cleavage Reactions. *J. Phys. Chem. B* **1999**, *103* (37), 7889-7895.
93. Lockyer, N. P.; Vickerman, J. C., Multiphoton Ionization Mass Spectrometry of Small Biomolecules with Nanosecond and Femtosecond Laser Pulses. *Int. J. Mass Spectrom.* **1998**, *176* (1), 77-86.
94. Plekan, O.; Feyer, V.; Richter, R.; Coreno, M.; Prince, K. C., Valence Photoionization and Photofragmentation of Aromatic Amino Acids. *Mol. Phys.* **2008**, *106* (9-10), 1143-1153.
95. Noble, J. A.; Aranguren-Abate, J. P.; Dedonder, C.; Jouvet, C.; Pino, G. A., Photodetachment of Deprotonated Aromatic Amino Acids: Stability of the Dehydrogenated Radical Depends on the Deprotonation Site. *Phys. Chem. Chem. Phys.* **2019**, *21* (42), 23346-23354.
96. Weinkauff, R.; Schanen, P.; Yang, D.; Soukara, S.; Schlag, E. W., Elementary Processes in Peptides: Electron Mobility and Dissociation in Peptide Cations in the Gas Phase. *J. Phys. Chem.* **1995**, *99* (28), 11255-11265.
97. Weinkauff, R.; Schlag, E. W.; Martinez, T. J.; Levine, R. D., Nonstationary Electronic States and Site-Selective Reactivity. *J. Phys. Chem. A* **1997**, *101* (42), 7702-7710.
98. Remacle, F.; Levine, R. D.; Schlag, E. W.; Weinkauff, R., Electronic Control of Site Selective Reactivity: A Model Combining Charge Migration and Dissociation. *J. Phys. Chem. A* **1999**, *103* (49), 10149-10158.
99. Belshaw, L.; Calegari, F.; Duffy, M. J.; Trabattoni, A.; Poletto, L.; Nisoli, M.; Greenwood, J. B., Observation of Ultrafast Charge Migration in an Amino Acid. *J. Phys. Chem. Lett.* **2012**, *3* (24), 3751-3754.

100. Calegari, F.; Ayuso, D.; Trabattoni, A.; Belshaw, L.; De Camillis, S.; Anumula, S.; Frassetto, F.; Poletto, L.; Palacios, A.; Decleva, P.; Greenwood, J. B.; Martín, F.; Nisoli, M., Ultrafast Electron Dynamics in Phenylalanine Initiated by Attosecond Pulses. *Science* **2014**, *346* (6207), 336-339.
101. Trabattoni, A.; Galli, M.; Lara-Astiaso, M.; Palacios, A.; Greenwood, J.; Tavernelli, I.; Decleva, P.; Nisoli, M.; Martín, F.; Calegari, F., Charge Migration in Photo-Ionized Aromatic Amino Acids. *Philos. Trans. A Math. Phys. Eng. Sci.* **2019**, *377* (2145), 20170472.

Chapter 5

Ultrafast Dynamics of Photodetached

Phenylalaninate Anion in Aqueous Solution

5.1. Introduction

Phenylalanine (Phe) is one of the essential amino acids that serves as a precursor in the biosynthesis of tyrosine, 3,4-dihydroxyphenylalanine (DOPA), dopamine, norepinephrine, and epinephrine.^{1,2} Being an amino acid with an aromatic sidechain, along with tryptophan and tyrosine, its ionization potential is $\sim 1 - 2$ eV lower than those of amino acids with aliphatic sidechains.^{3,4} As such, radiation damage of phenylalanine induced by ionizing radiation is expected to be facile. While studies of radiation-molecule interaction by pulse radiolysis⁵ and *ab initio* simulations⁶ have uncovered the pathways for the hydroxylation of phenylalanine in aqueous solution, little is known about the spectroscopic signature or the ultrafast dynamics of the phenylalanine radical (Phe•). Earlier nanosecond to microsecond UV flash photolysis studies of phenylalanine in aqueous solution revealed parallel photodissociation and photoionization pathways (Scheme 5.1).⁷⁻⁹ The transient absorption spectra collected at the earliest time delay of 20 ns revealed the absorption signature of either the benzyl radical photoproduct (at acidic or neutral pH values) or the phenylalanine triplet state (at alkaline pH values), with no sign of the Phe• radical.⁹ Unlike the well-established spectroscopic signatures of the tyrosyl¹⁰⁻¹² and tryptophan radicals,¹³⁻¹⁹ the Phe• radical species has thus been elusive, presumably due to its rapid decarboxylation.

Scheme 5.1. Photodissociation pathways of the phenylalaninate anion upon interaction with radiation. The first pathway involves the photodetachment of the phenylalaninate anion followed by subsequent decarboxylation to form the 2-phenylethanamine radical, CO_2 , and the hydrated electron. The second pathway involves the photodissociation of the phenylalaninate anion to form the benzyl radical and the glycine radical.

Our study of the ultrafast structural rearrangement dynamics of the $\text{Phe}^\bullet$ radical is primarily motivated by the recent report of long-lived coherent electron motion in gas-phase phenylalanine, triggered by attosecond photoionization.²⁰ There, sudden ionization of the inner-valence orbitals of phenylalanine by an attosecond extreme ultraviolet pulse, followed by probing with a 4-fs near-infrared pulse, led to oscillations in the yield of the 2-phenylethanamine dication $[(\text{PhCH}_2-\text{CH}-\text{NH}_2)^{2+}]$ as a function of time delay. Over the pump-probe time delay range of $\sim 10 - 35$ fs, the dication yield oscillates with a frequency of 0.234 PHz (7790 cm^{-1}). The origin of the oscillation is attributed to coherent electron motion as this oscillation frequency lies beyond the calculated highest fundamental vibrational frequency of the $\text{Phe}^\bullet$ radical (3730.9 cm^{-1}).²¹ It is surprising that an electronic coherence survives in a molecule with many vibrational degrees of freedom, over multiple oscillation periods without any noticeable sign of decoherence. Nevertheless, the experimentally observed oscillation frequency is in good agreement with the 0.25-PHz frequency obtained from

theoretical simulations of electron dynamics, and the experimental observation of an electronic coherence was recently extended to tryptophan, for which a beat frequency of 0.252 PHz (8390 cm^{-1}) was found.²² We note, however, that the interpretation of the experimental results thus far does not consider the coupling of the coherent electron motion to vibrational wave packet dynamics that will invariably be triggered by impulsive ionization. In anticipation of the extension of these attosecond experiments into the condensed phase, our goal is to identify the vibrational modes along which vibrational wave packet dynamics will be launched upon the impulsive ejection of electrons from aqueous phenylalanine.

Further motivation for investigating the Phe• radical in aqueous solution comes from the recent suggestion that it participates in long-distance hole transport in proteins.^{23,24} This proposal appears to be counterintuitive, given that the oxidation potential is significantly higher for phenylalanine (3.22 V vs. SHE) than tryptophan (1.62 V vs. SHE) or tyrosine (2.12 V vs. SHE),²⁵ thus making reversible oxidation – required for long-range hole transport – unfavorable. A combination of nanosecond flash photolysis measurements and *ab initio* simulations was recently employed to show that the presence of amide groups in a peptide environment stabilizes the arene radical, hence presumably facilitating the oxidation of phenylalanine residue.²⁶ This study, along with earlier flash photolysis studies of phenylalanine,^{7,8} however, did not reveal a clear spectroscopic signature of the Phe• radical. The unknown electronic absorption spectrum of the Phe• radical prevents its spectroscopic identification and hampers further studies aimed at elucidating the role of the Phe• radical in long-range biological electron transfer.

In this chapter, we employ a combination of optical pump-probe spectroscopy and *ab initio* simulations to investigate the ultrafast dynamics of the Phe• radical in aqueous solution. The Phe• radical is prepared by using sub-6-fs laser pulses to drive the strong-field

Figure 5.1. Structure of the phenylalaninate anion. The carbon, hydrogen, oxygen and nitrogen atoms are in gray, white, red and blue, respectively.

photodetachment of the aqueous phenylalaninate anion (Fig. 5.1). Subsequent probing in the UV for pump-probe time delays extending to 10 ps reveals the differential absorption (ΔA) spectrum of the Phe• radical. The ΔA signal oscillates as a function of time delay due to vibrational wave packet dynamics. Following our earlier work on the ultrafast photodetachment of aqueous phenoxide, we identify the observed vibrational frequencies with vibrational modes along which structural rearrangement occur following photodetachment.²⁷ The experimental results are complemented by *ab initio* calculations of the vibrational frequencies of the Phe• radical, hydrated to different extents by explicit water molecules of solvation, as well as its potential energy curve along the decarboxylation reaction coordinate.

5.2. Materials and Methods

5.2.1. Theoretical Calculations

The optimized equilibrium geometries and calculated vibrational frequencies of the phenylalaninate anion and the phenylalanine radical are obtained with the GAUSSIAN 16 program.²⁸ DFT calculations employ the hybrid exchange functional of Becke (B3),²⁹ the correlation functional of Lee, Yang and Parr (LYP),³⁰ and the 6-311G++(d,p) split-valence triple- ξ basis set, which comprises d-orbital polarization and diffuse functions on the heavy atoms (carbon, oxygen and nitrogen) and p-orbital polarization and diffuse functions on the hydrogen atoms. Two types of molecular models are used in the calculations. The first model is the isolated phenylalaninate anion or the phenylalanine radical, whereas the second model includes explicit water molecules of hydration. In the case of the microhydrated models, several water molecules are directly hydrogen-bonded to the carboxylate ($-\text{CO}_2$) and amine ($-\text{NH}_2$) groups of the amino acid (Fig. 5.2). The polarizable continuum model (PCM) is included in both models to mimic the interaction of radical species with the dielectric environment of the surrounding solvent.³¹ The water molecules present in the microhydrated species are fully relaxed during the geometry optimization process. Each geometry optimization is followed by vibrational frequency calculation to verify the optimized geometry corresponds to a local minimum.

To investigate the possible decarboxylation of the phenylalaninate anion upon photodetachment, constrained geometry optimization calculations are performed on the phenylalanine radical while varying the C–C bond length between the carboxylate carbon (C1) and the α -carbon (C2). The C1–C2 distance is varied between 1.5 – 5.0 Å, in increments of 0.05 Å. The plot of the potential energy as a function of C1–C2 distance yields the potential

Figure 5.2. Models of the microhydrated phenylalanine radical. The hydrogen bonds are indicated as blue dashed lines.

energy curve (PEC) along the C1–C2 elongation coordinate. These PEC calculations are performed for the isolated and the various microhydration models of the phenylalanine radical, all with the inclusion of PCM. Geometry optimizations are also performed to locate the transition states of $\text{Phe}(\text{H}_2\text{O})_3^\bullet$, $\text{Phe}(\text{H}_2\text{O})_{6a}^\bullet$, and $\text{Phe}(\text{H}_2\text{O})_9^\bullet$ with respect to decarboxylation. The energies of the transition states computed in this manner are within ~ 0.1 meV of those found by constrained geometry optimization, thus suggesting that the transition states obtained by constrained geometry optimization are first-order saddle points.

5.2.2. Experimental Method

Phenylalanine (Sigma-Aldrich, reagent grade $\geq 98\%$) and sodium hydroxide (Schedelco, Grade AR) are used as received without further purification. Since phenylalanine has relatively low solubility in water (85 mM at its isoelectronic point),³² phenylalanine is dissolved in sodium hydroxide solution to produce a phenylalaninate solution with a concentration of 400 mM. Given that the pK_a of the carboxyl group is 2.18,³³ we expect $>99.9\%$ deprotonation of the carboxyl group at pH 14. Thus, phenylalanine exists in our experiment in the anionic form.

The experimental procedure has been described in detailed in Chapter 2. In summary, the transient absorption pump-probe experiment is performed with a chirped-pulse-amplified Ti:sapphire laser system (Coherent, Legend Elite Duo-USX), with an output of 4.3-mJ, 30-fs pulses at 1-kHz repetition rate and 800-nm center wavelength, with a bandwidth of 50 nm. The pulses undergo self-phase modulation in a helium-filled hollow-core fiber before they are temporally compressed by chirped mirrors (Ultrafast Innovations GmbH, PC1332). The compressed pulses are incident on an 80T/20R beam splitter (Layertec, 131191), with 80% of the transmitted beam (~ 0.6 mJ) being directed to a $4f$ visible-NIR pulse shaper with a 128-element spatial light modulator (SLM) (Biophotonics Solutions, FemtoJock-P) for further pulse compression, which truncates the wavelength range of the visible-NIR from 530 nm to 750 nm, with a center wavelength of 642 nm (1.93-eV photon energy). The visible-NIR spectrum is shown in Fig. 5.3(a). Transform-limited laser pulses with full-width half-maximum (FWHM) of 5.6 fs are obtained by using multiphoton intrapulse interference phase scan (MIIPS) to measure the spectral phase at the sample, followed by using the SLM to introduce an oppositely signed spectral phase to the laser pulses, thus compressing it to the transform-limited duration.³⁴ Subsequently, a second-order interferometric auto-correlation trace is recorded by multiple independent comb shaping (MICS).³⁵ The second-order interferometric

auto-correlation trace is shown in Fig. 5.4(a). The transform-limited pulses, which function as the strong-field ionizing pump, are directed to the sample. The ionizing pulse is then focused onto the sample by a 50-cm focal length spherical mirror. A maximum pulse energy available for experiments of 34 μJ , with a beam waist ($1/e^2$) of 100 μm , resulting in a peak intensity of $3.6 \times 10^{13} \text{ W cm}^{-2}$ for the photodetachment process. An optical delay stage line, driven by a piezo translation stage (Physik Instrumente, N-664.3A) in the ionizing pump's path provide a variable time delay between pump and probe pulses. An achromatic half-waveplate (B. Halle, RAC4.2.15 L) is introduced in the ionizing pump pulse's path to allow the acquisition of isotropic signal when necessary.

The remaining output from the chirped mirror compressor passes through a pair of wedges (Femtolasers, OA124), and subsequently telescoped down by a factor of 3, with a pair of concave and convex mirrors. This is followed by a 10- μm -thick Type I beta-barium borate (BBO) crystal (Casteck, $\theta = 44.0^\circ$) to generate the UV probe *via* second harmonic generation (SHG). The broadband UV probe spans 290 nm to 370 nm. The broadband UV probe spectrum is shown in Fig. 5.3(b). Four dichroic beamsplitters (Layertec, 106163) are introduced to eliminate residual fundamental beam. The UV beam is then split off by a reflective neutral density beamsplitter (Edmund Optics, 88271) to produce the UV probe and reference pulses. The reflected UV probe is then focused onto the sample by a 15-cm focal length off-axis parabolic reflector (Thorlabs, MPD369H-P01). The focused UV probe has a beam waist ($1/e^2$) of 40 μm , with a pulse energy of 100 nJ, which has a peak intensity of $1.6 \times 10^{11} \text{ W cm}^{-2}$.

A slit nozzle (Metaheuristics, Type L) is used in conjunction with a peristaltic pump (Cole-Parmer, 07528-10) and a pulse dampener to produce a microjet of the phenylalanine anion solution for experiments. The volume flow rate of 92 mL min^{-1} translates to a vertical flow rate of approximately 8.5 mm ms^{-1} . The relatively high vertical flow rate ensures that

Figure 5.3. (a) The visible-NIR ionizing pump spectrum ranges from 530 nm to 750 nm, with a center wavelength of 642 nm. (b) Broadband UV probe spectrum, with a wavelength range of 290 nm to 370 nm, generated *via* SHG.

fresh volume of phenylalanine anion solution is exposed to each ionizing pump pulse. A liquid jet of 7- μm thickness is produced, which yields a sizeable differential absorption signal ($\Delta A \sim 0.01$), while minimizing the temporal broadening of the pulses in the liquid jet due to dispersion. In addition, a thin liquid jet limits the group-velocity mismatch between the pump and probe to <1 fs, making its effect on the time resolution negligible.

A synchronized optical chopper in the ionizing pump's path before the sample allows modulation of the ionizing pump at a frequency of 0.5 kHz. The UV probe beam that is transmitted through the microjet is focused into a 300-nm spectrograph (Princeton Instruments, Acton SP2300), equipped with a 600-grooves per mm grating blazed at 500-nm and a 1024-pixels silicon (Si) photodiode array detector (Stresing, S8381-1024Q) with a read-out rate of 1 kHz, which allow simultaneous acquisition of the probe spectra obtained with and without the ionizing pump pulse. The reference UV pulses are focused to an identical spectrograph, which is equipped with similar grating and photodiode array at 1-kHz repetition rate. This method

Figure 5.4. (a) A second-order interferometric auto-correlation trace of the visible-NIR ionizing pump spectrum, measured in a 10- μm -thick, $\theta = 37.0$ deg Type I BBO crystal, reveals a pulse duration with FWHM of 5.6 fs. (b) The pump-probe cross-correlation, measured *via* difference frequency mixing in a 10- μm -thick, $\theta = 37.0$ deg Type I BBO crystal reveals a FWHM of 24 fs.

reduces noise interference caused by shot-to-shot fluctuations of the probe pulse. The Si photodiode array detectors are interfaced to desktop computers for automated data acquisition.

The focal spot size of the probe beam is generally $\sim 3\times$ smaller than that of the ionizing pump beam, hence minimizing the effect of spatial averaging on the observed ultrafast dynamics. The cross-correlation of the pump and UV probe, by measuring the difference frequency generated (DFG) beam by utilizing a 10- μm thick Type I BBO crystal (Casteck, $\theta = 37.0^\circ$) yields an instrument response of 24 fs at FWHM. The cross-correlation trace is displayed in Fig. 5.4(b).

Static UV-visible measurements are performed with a double-beam UV-visible-NIR spectrophotometer (Agilent Technologies, Cary 300) with a 1-mm path length quartz cuvette by measuring absorbance. The spectrophotometer has a wavelength range from 190 to 800 nm.

5.3. Results

5.3.1. Transient Absorption of Photodetached Aqueous Phenylalaninate Anion

As illustrated in previous chapters, a power-dependence measurement is performed to determine the photon order of the strong-field photodetachment process of the phenylalaninate anion in aqueous solution. For an N -photon photodetachment process, the differential absorption signal, ΔA , scales with intensity of the ionizing pump, I , as shown in the following relationship

$$\Delta A \propto I^N. \quad (5.1)$$

In this study, the photon-order measurements are performed by using a UV probe, which measures the absorption of the radical at a pump-probe time delay of 1 ps and at a probe wavelength of ~ 308 nm, corresponding to the maximum absorption of the Phe $\bullet$ radical. Hence, the absorption signal observed is proportional to the photodetachment yield.

To determine the photon order of the photodetachment process accurately, one must consider that electrons can originate from both the phenylalaninate and hydroxide anions during the photodetachment of the alkaline phenylalaninate solution. To account for the contribution from the photodetachment of hydroxide anions, we record the ΔA spectrum against the pump intensity for both the aqueous phenylalaninate and aqueous sodium hydroxide solutions under similar experimental conditions. The difference ΔA spectrum, i.e., the $\Delta\Delta A$ spectrum, is then obtained, which in principle corresponds to solely the ΔA signal from the photodetachment of phenylalaninate anion, shown in Fig. 5.5. The $\Delta\Delta A$ signal is fit to the function

$$\Delta\Delta A = \frac{\alpha * I^N}{1 + \left(\frac{I}{I_{sat}}\right)^N}, \quad (5.2)$$

where α is the amplitude, N is the photon order and I_{sat} is the saturation intensity. The best fit when $N = 3$ yields $\alpha = (9.80 \pm 0.46) \times 10^{-41} \text{ cm}^2 \text{ W}^{-1}$ and $I_{sat} = (1.15 \pm 0.02) \times 10^{13} \text{ W cm}^{-2}$. The value of N indicates that the photodetachment of phenylalaninate anion in aqueous environment occurs *via* a three-photon resonant enhancement process. A log-log plot of $\Delta\Delta A$ against pump intensity is shown in Fig. 5.5.

Previous studies of phenylalanine in alkaline medium have shown that 4.68-eV (265-nm) photoexcitation to the S_1 ($\pi\pi^*$) state yields the $\text{Phe}^\bullet$ radical *via* delayed electron ejection from the T_1 state. The $\text{Phe}^\bullet$ radical then undergoes decarboxylation to form the 2-phenylethanamine radical and CO_2 .⁷⁻⁹ In the present study, we exclude the possibility of photodetachment occurring *via* the S_1 or T_1 state. Instead, our pump-power dependence measurement reveals a three-photon photodetachment process (Fig. 5.5). Given the vertical ionization energy of aqueous phenylalanine (8.7 eV)²⁵ and the vertical electron affinity of liquid water (~ 1 eV),^{36,37} the total energy input provided by the three-photon process (5.8 eV) is insufficient to eject an electron from the aqueous phenylalaninate anion and inject it into the conduction band of liquid water, even when one considers that the broad photoelectron band of aqueous phenylalanine extends to ~ 7.5 -eV binding energy.²⁵ As such, we believe that strong-field photodetachment proceeds under our experimental conditions *via* a (3 + 1) process involving an intermediate Phe^- anion excited state (inset in Fig. 5.5).

The intermediate excited state that is accessed by the three-photon excitation of Phe^- is likely to be its L_a state,^{38,39} which is related to the $^1B_{1u}$ excited state of benzene. In the far-UV absorption spectrum of the aqueous Phe^- anion, the electronic transition to the L_a state appears as a shoulder at 212 nm (5.85 eV).³⁸ This transition energy is well-matched to the total energy

Figure 5.5. Photon-order measurement for strong-field photodetachment of phenylalanine anion. Log-log plot of $\Delta\Delta A$ vs. pump intensity after subtraction of response from aqueous sodium hydroxide. The solid line is a fit to a three-photon process. The $(3 + 1)$ resonance-enhanced multiphoton photodetachment process of phenylalaninate anion through the L_a excited state (Phe^{-*}) is shown as inset.

input of 5.8 eV from three-photon photoexcitation, thus lending support to the results of the pump-power dependence study. From this L_a excited state, the absorption of an additional photon leads to photodetachment and the concomitant injection of an electron into the conduction band of liquid water. While nominally a $\pi \rightarrow \pi^*$ transition, our time-dependent density functional theory (TD-DFT) calculations suggest that the transition to the L_a state also involves the promotion of the electron in the highest occupied molecular orbital (HOMO), localized primarily on the amino acid backbone (Appendix C, Section C1).

The differential absorption (ΔA) spectrum of the photodetached aqueous phenylalaninate anion collected as a function of pump-probe time delay is shown in Fig. 5.6(a). The ΔA signal exhibits pronounced amplitude modulations as a function of time delay (Fig. 5.6(b)). In addition, close examination of the ΔA spectrum reveals structure in the 290 –

Figure 5.6. (a) Isotropic differential absorption (ΔA) spectrum of the photodetached aqueous phenylalaninate anion as a function of pump-probe time delay. (b) Time traces at 310 nm of the photodetached aqueous phenylalaninate anion (in red) and the photodetached aqueous sodium hydroxide (in blue). The former shows pronounced modulations due to vibrational wave packet dynamics. (c) Time traces at 310 nm, obtained from a scan extended to 10-ps time delay. The black lines show the results from global fitting.

320 nm region, reminiscent of a vibronic progression, while the spectral region beyond >320 nm is featureless. Strong-field multiphoton photodetachment of the Phe^- anion yields the $\text{Phe}^\bullet$ radical and an injected electron in the conduction band of liquid water. The conduction-band electron undergoes trapping³⁷ and subsequent vibrational relaxation to yield the hydrated electron.⁴⁰⁻⁴⁵ The featureless, broadband absorption spectrum of the hydrated electron spans

the UV to the NIR and is peaked at 715 nm.⁴⁶ The structure observed in the ΔA spectrum thus cannot be ascribed to the hydrated electron. Instead, we attribute the spectral features to the Phe• radical absorption.

Aside from the pronounced amplitude modulations, the time-resolved ΔA signal also exhibits a gradual decay as a function of pump-probe time delay (Fig. 5.6(c)). To extract the timescale relevant to the observed decay dynamics, we employ global fitting of the spectrally resolved $\Delta A(\lambda, t)$ signal to the expression

$$\Delta A(\lambda, t) = \Delta A_0(\lambda) + \Delta A_1(\lambda)e^{-t/\tau_d}, \quad (5.3)$$

where $\Delta A_0(\lambda)$ is the offset spectrum and $\Delta A_1(\lambda)$ is the spectral amplitude that is associated with the decay constant τ_d . Performing the global fit over a wavelength range of 290 – 320 nm and a time delay range of 0.15 – 10 ps yields $\tau_d = 1.54 \pm 0.01$ ps.

5.3.2. Vibrational Wave Packet Dynamics of Phenylalanine Radical

First-moment vibrational wave packet dynamics analysis is performed from the time traces of the $\Delta A(\lambda, t)$ spectrum of the photodetached aqueous phenylalaninate anion to furnish the vibrational modes that are coupled to the photodetachment process. We note that there is no evidence of impulsive stimulated Raman scattering (ISRS) of phenylalanine since the ΔA signal and time-domain oscillations disappear when the ionizing-pump power is attenuated to suppress the strong-field photodetachment. Furthermore, the UV absorption spectrum of aqueous phenylalaninate anion does not overlap with the probe spectral range. This is evident

from the absorbance spectrum of 40 mM phenylalaninate anion solution at pH 14, as shown in Fig. 5.7, which has a maximum absorption of 258 nm.³³

The pronounced modulations in the ΔA spectra as a function of time delay (Fig. 5.6(b)) encode vibrational wave packet dynamics. We quantitatively analyze the wave packet dynamics by computing the spectral first-moment time trace $\langle E(t) \rangle$, given as

$$\langle E(t) \rangle = \frac{\int_{E_i}^{E_f} E \Delta A(E, t) dE}{\int_{E_i}^{E_f} \Delta A(E, t) dE}. \quad (5.4)$$

In the above expression, E represents the probe photon energy, t is the pump-probe time delay and $\Delta A(E, t)$ is the differential absorbance as a function of probe photon energy and pump-probe time delay. E_i and E_f define the spectral range over which the spectral first-moment is computed; in this case, E_i and E_f is 3.90 eV (318 nm) and 4.13 eV (300 nm), respectively. The resultant $\langle E(t) \rangle$ trace is shown in Fig. 5.8(a), with the inset showing a zoomed-in view of $\langle E(t) \rangle$ from 0.1 ps to 1 ps.

The oscillatory nature of the $\langle E(t) \rangle$ trace arises from vibrational wave packet motion, involving vibrational modes for which the potential energy curves of the Phe^- anion and the $\text{Phe}^\bullet$ radical are displaced. Following vertical photodetachment, the initial vibrational probability density of the Phe^- anion that is projected onto the $\text{Phe}^\bullet$ radical potential energy curve evolves as a coherent superposition of vibrational eigenstates of the $\text{Phe}^\bullet$ radical, giving rise to wave packet motion. This wave packet motion modulates the transition energy of the probe pulse to the probe final state, $\text{Phe}^{\bullet*}$, thus resulting in the observed oscillations in the $\langle E(t) \rangle$ trace. This time-domain approach to the identification of vibrational modes that are coupled to photodetachment is complementary to measurements of the Franck-Condon progression in the valence photoelectron spectrum. We note that energy-resolved measurements, however, would not be feasible for molecules in aqueous solutions due to the

Figure 5.7. Static UV-visible absorption spectrum of 40 mM phenylalaninate anion in aqueous sodium hydroxide (pH 14), measured with a 1-mm path length quartz cuvette. The absorption maximum is at 258 nm.

extensive inhomogeneous broadening (~ 1 eV) associated with the multiple hydration configurations in aqueous solutions.^{25,47} Fast-Fourier transform (FFT) of the $\langle E(t) \rangle$ trace yields the vibrational frequencies (Fig. 5.8(b), in red), which in turn can be used to identify the vibrational modes that participate in wave packet motion. Table 5.1 summarizes the vibrational frequencies, their relative FFT powers, and their vibrational mode assignments obtained from DFT calculations (see below).^{48,49}

A control experiment performed with aqueous NaOH (pH 14) yields the vibrational frequencies of N_2 (2329 cm^{-1}) and O_2 (1555 cm^{-1}) only (Fig. 5.8(b), in blue);^{50,51} these originate from impulsive stimulated Raman scattering of N_2 and O_2 molecules in air, in which the experiments are performed. The photodetachment of hydroxide ion yields the hydroxyl radical ($\bullet OH$) and the accompanying hydrated electron. The hydroxyl radical has only one vibrational mode at 3880 cm^{-1} , which corresponds to the O–H stretching.⁵² Since this frequency is beyond

(a)

Figure 5.8. (a) Residual first-moment, $\langle E(t) \rangle$, time trace of photodetached aqueous phenylalanine anion (in black) with fit function (in red). The zoomed-in view of $\langle E(t) \rangle$ time trace from 0.1 ps to 1 ps is shown in the inset. (b) FFT power spectra of photodetached aqueous phenylalaninate anion (in red) and photodetached aqueous sodium hydroxide (in blue). Note that the oxygen and nitrogen vibrational frequencies are also observed at 1555 cm⁻¹ and 2329 cm⁻¹ respectively.

the high frequency cut-off of our experiment, the result strongly suggests that the vibrational frequencies listed in Table 5.1 originate from the Phe• radical.

Aside from FFT, the first-moment time trace can also be analyzed in the time domain by fitting it to a sum of exponentially damped cosine functions,

$$\langle E(t) \rangle = \sum_{i=1}^n A_i e^{-\frac{t}{\tau_i}} \cos(\omega_i t + \phi_i) \quad (5.5)$$

where A_i , τ_i , ω_i and ϕ_i are the amplitude, dephasing time, vibrational frequency and phase of the vibrational mode Q_i respectively. The fit parameters are summarized in Table 5.1. Compared to FFT, time-domain analysis directly furnishes the dephasing times of the vibrational modes. The calculated vibrational frequencies reported in Table 5.1 are those of the microhydrated model, Phe(H₂O)₄•.

5.3.3. Theoretical Calculations

The equilibrium geometries of the isolated and microhydrated phenylalanine radical are shown in Fig. 5.9. The equilibrium geometry of the microhydrated phenylalanine radical consists of two water molecules hydrogen bonded to the carboxyl and amino group of the radical species respectively. The equilibrium geometry of the phenylalanine radical shows a dissociation of the carboxyl group (i.e., decarboxylation). This geometry corresponds to a local minimum in terms of energy and produce real vibrational frequencies. The complete list of vibrational frequencies of the Phe• radical, modeled with different microhydration configurations, is tabulated along with their assignments in the Appendix C (Table C1).

The calculated PECs are obtained with the isolated phenylalanine radical (Phe•) and several models of the microhydrated phenylalanine radical with different number of explicit water molecules, which is shown in Fig. 5.10(a). The isolated phenylalanine radical and the five models of microhydrated phenylalanine radical are shown in Fig. 5.10(b). The PECs are obtained by subtracting all data points with the minimum energy, which is the equilibrium geometry of the phenylalanine radical. A summary of the PEC calculations for the photodissociation of the phenylalanine radical is displayed in Table 5.2, where the table summarizes the equilibrium bond length of C1–C2 of the isolated and microhydrated phenylalanine radicals, the dissociation barrier height which refers to the minimum of the potential energy curve, the bond dissociation energy which is the energy difference between the potential energy when the C1–C2 bond length is 5 Å and the minimum of the potential energy curve, and the excess energy which is the energy difference between the potential energy at the Franck-Condon region and the dissociation barrier height.

Figure 5.9. The equilibrium geometries of **(a)** the phenylalanine radical and **(b)** the microhydrated phenylalanine radical. The microhydrated species includes the carboxylate ($-\text{COO}$) and amine ($-\text{NH}_2$) groups of the phenylalanine radical hydrogen bonded to two water molecules. The carbon, hydrogen, oxygen and nitrogen atoms are in gray, white, red and blue respectively.

Figure 5.10. (a) Calculated PECs along the C1–C2 bond length elongation coordinate for the isolated Phe• and the various microhydrated configurations. The Franck-Condon region is indicated by the shaded grey region. The PECs are displaced by 0.75 eV from Phe• to Phe(H₂O)₃• and 0.5 eV between Phe(H₂O)₃• to Phe(H₂O)₁₀•. (b) Equilibrium geometries of the various microhydrated Phe• radicals.

Table 5.1. Experimental FFT power, time-domain analysis results, and calculated frequencies of the phenylalanine radical in aqueous solution and their assignments. The calculated frequencies are based on the equilibrium geometry of the microhydrated phenylalanine radical, Phe(H₂O)₄•. Numbered assignments denote benzene ring modes in the Wilson notation.^{49,50}

Experimental							Calc. Freq. (cm ⁻¹)	Assignment
FFT Analysis		Time-Domain Analysis				Phase (π-rad)		
Freq. (cm ⁻¹)	Power (norm.)	Freq. (cm ⁻¹)	Amplitude (meV)	Dephasing Time (ps)				
486	5.6 × 10 ⁻²	479 ± 2	0.98 ± 0.15	0.64 ± 0.12	0.88 ± 0.05		512	Ring deformation [16b]
750	3.7 × 10 ⁻²	736 ± 6	3.98 ± 1.12	0.17 ± 0.03	1.00 ± 0.08		755	NH ₂ wag
776	1.7 × 10 ⁻²	805 ± 6	0.52 ± 0.29	0.50 ± 0.28	1.18 ± 0.18		837	Ring breath [6a]
1003	1	1004 ± 1	4.23 ± 0.07	5.47 ± 0.73	1.76 ± 0.01		1015	Benzene trigonal bend [12]
1032	7.5 × 10 ⁻²	1031 ± 1	1.46 ± 0.08	2.83 ± 0.68	1.79 ± 0.02		1048	Benzene trigonal bend [19a]
1208	7.0 × 10 ⁻²	1208 ± 1	2.48 ± 0.13	0.98 ± 0.11	1.83 ± 0.02		1221	Chain CH ₂ wag
1282	1.3 × 10 ⁻³	1269 ± 5	0.54 ± 0.18	0.71 ± 0.41	1.95 ± 0.15		1299	Benzene CC stretch [1 (19a, 12, 7a, 20a, 8a, 12, 6a)]
1319	9.9 × 10 ⁻⁴	1329 ± 5	0.36 ± 0.16	0.87 ± 0.71	0.28 ± 0.16		1354	Chain CH ₂ wag / NH ₂ twist
1601	2.3 × 10 ⁻³	1604 ± 2	0.30 ± 0.07	2.09 ± 1.41	0.69 ± 0.07		1640	Benzene CC stretch [9a] / NH ₂ wag
2005	1.9 × 10 ⁻³	1971 ± 22	1.61 ± 1.22	0.13 ± 0.07	0.58 ± 0.24		–	Overtone of 1003 cm ⁻¹
1555	4.3 × 10 ⁻²	1555 ± 1	0.80 ± 0.05	7.37 ± 3.21	1.81 ± 0.01			Oxygen
2327	4.4 × 10 ⁻²	2329 ± 1	0.90 ± 0.06	15.38 ± 8.04	0.96 ± 0.01			Nitrogen

*Numbers in parentheses refers to minor contributions of phenyl vibrational modes to the main vibrational mode, in descending

order of importance.

Table 5.2. Optimized C α -CO₂ bond length, bond dissociation energy, dissociation barrier height, and excess energy available for dissociation obtained from DFT constrained geometry optimization calculations.

	C1–C2 Bond Length / Å	Dissociation Barrier Height¹ / eV	Bond Dissociation Energy² / eV	Excess Energy³ / eV
Phe•	3.478	0.006	0.006	0.554
Phe(H ₂ O) ₃ •	2.046	0.062	0.027	0.232
Phe(H ₂ O) _{6a} •	1.950	0.193	0.192	-0.007
Phe(H ₂ O) _{6b} •	1.997	0.128	0.116	0.138
Phe(H ₂ O) ₉ •	1.899	0.304	0.232	-0.123
Phe(H ₂ O) ₁₀ •	1.905	0.320	0.231	-0.135

¹ The dissociation barrier height is referenced to the minimum of the potential energy curve.

² The bond dissociation energy is the energy difference between the potential energy when the C1–C2 bond length is 5 Å and the minimum of the potential energy curve.

³ The excess energy refers to the energy difference between the potential energy at the Franck-Condon region and the dissociation barrier height. A positive excess energy implies that dissociation from the Franck-Condon region is energetically favorable.

5.4. Discussion

5.4.1. Transient Absorption of Photodetached Aqueous Phenylalaninate Anion

The Phe• radical that is initially produced by vertical photodetachment exists as a vibrationally hot state in the Franck-Condon region. As such we tentatively assign $\tau_d = 1.54 \pm 0.01$ ps obtained from the global fit to the timescale for vibrational relaxation, which gives rise to changes in the absorption spectrum due to a combination of changing Franck-Condon factors and non-Condon effects. Another possible explanation for the observed decay dynamics is the depopulation of the Phe• radical by fragmentation, for example, *via* the decarboxylation pathway that was previously observed from nanosecond laser and microsecond flash photolysis studies of the Phe[−] anion in alkaline medium.⁷⁻⁹ While our experimental data does not provide any direct evidence that rules out the possibility of decarboxylation occurring in the phenylalanine radical – indeed this has been shown in studies of gas-phase photodetached carboxylate anions to be the dominant dissociation pathway^{53,54} – we note that both anion photoelectron spectroscopy and *ab initio* calculations have shown that microhydrated carboxyl radicals are stable with respect to decarboxylation.⁵⁵ Consistent with previous studies of microhydrated carboxyl radicals, results from our DFT calculations suggest that decarboxylation does not occur on the picosecond timescale (see below).

The offset spectrum $\Delta A_0(\lambda)$ represents the differential absorption spectrum in the limit of large pump-probe time delays. It is of interest because it encodes the hitherto unobserved absorption spectrum of the Phe• radical. However, the retrieved $\Delta A_0(\lambda)$ (Fig. 5.11(a), in red) comprises absorption contributions from the Phe• and OH• radicals, the latter produced by the photodetachment of hydroxide anions OH[−] present in the alkaline medium, as well as hydrated

Figure 5.11 (a) The wavelength-dependent offset values, $\Delta A_0(\lambda)$, obtained from the global fitting of the photodetached aqueous phenylalaninate anion (in red) and photodetached aqueous sodium hydroxide (in blue) time traces. The fitting errors are comparable to the size of the symbol. **(b)** The molar extinction coefficient, ϵ_{Phe} , of the phenylalanine radical as a function of wavelength.

electrons, produced by the photodetachment of both Phe^- and OH^- anions. To obtain the absorption spectrum of the $Phe^\bullet$ radical, it is necessary to remove the contributions from the $OH^\bullet$ radical and the hydrated electron that originate from the OH^- anion. This is done by performing a control experiment on a NaOH-only sample under identical photodetachment conditions. Employing the same global fitting procedure on the NaOH-only data yields the offset spectrum $\Delta A_0^{NaOH}(\lambda)$ (Fig. 5.11(a), in blue). Taking the difference between the offset spectra gives the absorption spectrum that is associated with the $Phe^\bullet$ radical, i.e., $\Delta A_0^{Phe}(\lambda) = \Delta A_0(\lambda) - \Delta A_0^{NaOH}(\lambda)$, which is shown in Fig. 5.12(a), in red. It shows a vertical offset, most likely due to the absorption of hydrated electrons (Fig. 5.12(b), in blue, dashed line) produced by the photodetachment of the Phe^- anion, not accounted for by $\Delta A_0^{NaOH}(\lambda)$. From the value of the offset [$\Delta A_0^{Phe} = (2.8 \pm 0.1) \times 10^{-3}$] and the molar extinction coefficient of the hydrated

Figure 5.12 (a) The wavelength-dependent offset values, $\Delta A_0(\lambda)$, obtained from the global fitting of photodetached aqueous phenylalaninate anion (in red) after taking the difference between the offset spectra of the aqueous phenylalaninate anion and aqueous sodium hydroxide. The residual vertical offset at long wavelength is most likely due to the absorption of the hydrated electron (in blue, dashed line). **(b)** Second-derivative spectrum of the molar extinction spectrum of the aqueous phenylalanine radical, showing the locations of the main peak and the vibronic bands. **(c)** A linear fit to the transition energies of the vibronic bands reveals a spacing of $482 \pm 15 \text{ cm}^{-1}$ for the vibronic progression.

electron at 327 nm ($\epsilon = 1286 \text{ M}^{-1} \cdot \text{cm}^{-1}$), along with the path length of the microjet ($L = 7 \pm 1 \text{ } \mu\text{m}$), we infer that photodetachment of Phe^- anions produces $3.1 \pm 0.5 \text{ mM}$ of hydrated electrons, and hence, $\text{Phe}^\bullet$ radicals. This corresponds to a photodetachment yield of $\sim 0.8\%$ if one neglects the geminate recombination between hydrated electrons and the $\text{Phe}^\bullet$ radicals on the few-picosecond timescale, an assumption that is supported by previous studies

of photoionized aqueous indole.⁵⁶ Given this initial concentration of Phe• radicals, the molar extinction coefficients for the Phe• radical can thus be determined (Fig. 5.11(b)). Aside from the main peak at 308 nm [$\epsilon_{\text{max}} = (3.3 \pm 0.7) \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$], the molar extinction spectrum of the Phe• radical exhibits a pronounced vibronic progression with a spacing of $482 \pm 15 \text{ cm}^{-1}$, which coincides with a benzene ring deformation mode (see below). The second derivative of the molar extinction coefficients of Phe• radical is shown in Fig. 5.12(b), and the linear fit of the vibronic spacing is shown in Fig. 5.12(c).

5.4.2. Vibrational Wave Packet Dynamics of Phenylalanine Radical

The FFT power spectrum is dominated by the peak at 1003 cm^{-1} , assigned to the benzene ring breathing mode (ν_{12}). This peak is $>10\times$ more intense than the next strongest peak, assigned to the benzene trigonal bending (ν_{19a}). The peak that appears at 2005 cm^{-1} is assigned, in the absence of a calculated vibrational frequency in that frequency range, to the first overtone of the ν_{12} mode. The emergence of the overtone indicates large-amplitude wave packet motion along the ν_{12} mode. Interestingly, time-domain analysis reveals damping times of $5.5 \pm 0.7 \text{ ps}$ and $0.13 \pm 0.07 \text{ ps}$ for the fundamental and overtone frequencies, respectively (Table 5.1). The markedly shorter damping time for the overtone reflects the stronger anharmonic coupling of higher vibrational levels to other vibrational modes, thus promoting more rapid dephasing.

The displacement vectors of vibrational modes that contribute to the wave packet (Fig. 5.13) involve both the phenyl sidechain and the amino acid backbone. To rationalize this result, we first recall that photodetachment of the Phe⁻ anion under our experimental conditions proceeds *via* its electronically excited L_a state, located 5.8 eV above the ground state and

Figure 5.13. Displacement vectors of the Phe• radical vibrational modes that are observed in the FFT power spectrum. The blue arrows indicate the direction and magnitude of the atomic displacements. The following vibrational modes are observed: **(a)** 512 cm^{-1} , **(b)** 755 cm^{-1} , **(c)** 837 cm^{-1} , **(d)** 1015 cm^{-1} , **(e)** 1048 cm^{-1} , **(f)** 1221 cm^{-1} , **(g)** 1299 cm^{-1} , **(h)** 1354 cm^{-1} and **(i)** 1640 cm^{-1} .

accessed *via* three-photon resonant excitation. Subsequent ejection of the electron from the L_a state by the absorption of an additional photon yields the Phe• radical. The hole density distribution of the L_a state thus reflects the electron density distribution that is removed by photodetachment. According to TD-DFT calculations, the electronically excited states located

~5.8 eV above the Phe^- ground state originate predominantly from transitions from the HOMO and HOMO-1 orbitals (Appendix C, Section C1). The HOMO and HOMO-1 orbitals are localized on the amino acid backbone and the phenyl ring, respectively (Figure 5.14). Removal of the electron density about these moieties by strong-field photodetachment thus triggers structural rearrangement involving both the amino acid backbone and the phenyl sidechain.

To gain further insight into the extent of structural reorganization induced by photodetachment, we examine the C-C, C-N, and C-O bond lengths of the optimized structures of the microhydrated Phe^- anion and $\text{Phe}^\bullet$ radical (Table 5.3). Interestingly, the phenyl C-C bond lengths undergo negligible changes (<0.1 pm) upon photodetachment. This lies in contrast to the few-picometer changes in the C-C bond lengths previously calculated for the phenoxide, tyrosinate dianion and tryptophan anion upon their photodetachment to yield the phenoxyl,²⁷ tyrosine radical anion⁵⁷ and tryptophan radical,⁵⁸ respectively. To resolve this apparent discrepancy, it should be noted that the FFT power that is associated with a particular vibrational mode Q_i depends on both the displacements between the Phe^- anion and the $\text{Phe}^\bullet$ radical potentials as well as between the $\text{Phe}^\bullet$ radical and $\text{Phe}^{\bullet*}$ probe final-state potentials. Both displacements must be non-vanishing for wave packet motion to be observable in $\langle E(t) \rangle$. In the case of the $\text{Phe}^\bullet$ radical, the ability to resolve sub-0.1-pm changes in the phenyl C-C bonds suggests that a large displacement must exist between the $\text{Phe}^\bullet$ and $\text{Phe}^{\bullet*}$ potential energy curves along mode Q_i .

We note that the experimentally measured vibrational frequencies are compared to those computed for the microhydration models of the $\text{Phe}^\bullet$ radical. As these models exhibit an equilibrium C1-C2 bond length (~1.9 – 2.0 Å; see below) that is elongated relative to that of the microhydrated Phe^- anion (~1.55 Å), it might seem counterintuitive that the vibrational wave packet dynamics at the Franck-Condon region can be assigned to the vibrational

Figure 5.14. HOMO (left) and HOMO-1 (right) orbitals of the microhydrated phenylalaninate anion, $\text{Phe}(\text{H}_2\text{O})_{10}^-$.

frequencies computed for a structure that is located away from the Franck-Condon region. According to classical trajectory calculations, elongation of the C1–C2 bond length from 1.55 Å to 2.0 Å requires ~40 fs, too long to serve as an impulsive excitation of any of the vibrational modes observed in the FFT power spectrum (Fig. 5.8(b), in red). That is, the observed wave packet dynamics is unlikely to originate from reaction-driven coherence about the equilibrium $\text{Phe}^\bullet$ radical geometry.^{59,60} Instead, we hypothesize that the vibrational modes that are coherently excited at the Franck-Condon region of the $\text{Phe}^\bullet$ radical are weakly coupled to C1–C2 bond elongation, such that the frequencies of the vibrational modes that participate in wave packet motion are relatively unaffected by C1–C2 bond elongation. In support of this, we note that the displacement vectors in Fig. 5.13 do not exhibit any C1–C2 stretching character. Moreover, mapping the observed vibrational modes of the $\text{Phe}^\bullet$ radical to the analogous modes of the 2-phenylethanamine radical, the latter being the decarboxylation product, reveals that the vibrational frequencies of the 2-phenylethanamine backbone are

Table 5.3. Selected bond lengths of the microhydrated phenylalaninate anion, $\text{Phe}(\text{H}_2\text{O})_{10}^-$ and the phenylalanine radical, $\text{Phe}(\text{H}_2\text{O})_{10}^\bullet$ and the differences in bond lengths between the two species.

Bond	Phenylalaninate	Phenylalanine	Difference / pm
	Anion	Radical	
	Bond Length / pm		
C1–C2	154.80	190.50	35.70
C2–C3	154.68	152.65	-2.03
C3–C4	151.28	151.38	0.10
C4–C5	139.98	139.87	-0.11
C5–C6	139.59	139.56	0.03
C6–C7	139.42	139.41	0.01
C7–C8	139.61	139.57	-0.04
C8–C9	139.39	139.37	-0.02
C9–C4	140.18	140.11	-0.07
C1–O10	126.31	121.59	-4.72
C1–O11	126.29	121.77	-4.52

relatively insensitive to decarboxylation and hence, C1–C2 bond elongation (Appendix C, Table C2). Future investigations that employ time-resolved femtosecond stimulated Raman spectroscopy (FSRS)^{61–63} or impulsive stimulated Raman spectroscopy (ISRS)^{64,65} can be used to identify vibrational modes, such as the CO_2 bend, which are sensitive to C1–C2 bond elongation. These modes can then be used to track the vibrational relaxation dynamics of the $\text{Phe}^\bullet$ radical from the Franck-Condon region to its equilibrium geometry. In the present work, rapid dephasing promoted by the strong anharmonic coupling between the C1–C2 elongation and, for example, the CO_2 bend suppresses the observation of vibrational wave packet dynamics along these modes.

Aside from intramolecular structural rearrangement, the DFT calculations also reveal large changes in the intermolecular distances between the microhydrating water molecules and the -NH_2 and -CO_2 groups of phenylalanine. For the amine group, the two intermolecular $\text{NH}_2\cdots\text{OH}_2$ distances decrease from 3.344 Å and 3.194 Å to 2.916 Å and 2.904 Å, respectively. The contraction of the $\text{NH}_2\cdots\text{OH}_2$ distances is consistent with the -NH_2 group gaining a partial positive charge upon photodetachment, resulting in a stronger electrostatic interaction between itself and the hydrogen bond-accepting H_2O molecules. The carboxylate group, on the other hand, loses its formal negative charge upon photodetachment. The weakened hydrogen bond between the carboxylate group and the hydrogen bond-donating water molecules is reflected in the increase in the intermolecular $\text{CO}_2\cdots\text{H}_2\text{O}$ bond distances from 2.634 Å and 2.648 Å to 2.825 Å and 2.832 Å, respectively. These large changes in the intermolecular distances favor the initiation of intermolecular vibrational wave packet motion involving hindered translation between the $\text{Phe}^\bullet$ radical and the water molecules of hydration. Based on our DFT calculations, these modes are found at $\sim 100 - 200 \text{ cm}^{-1}$. Future investigations will aim to elucidate coherent intermolecular vibrational dynamics that are associated with the solvation of the $\text{Phe}^\bullet$ radical.

5.4.3. Theoretical Calculations

DFT calculations were performed to assign the vibrational frequencies that appear in the vibrational wave packet dynamics. The calculated vibrational frequencies reported in Table 5.1 are those of the microhydrated $\text{Phe}(\text{H}_2\text{O})_4^\bullet$ model, in which the -NH_2 and -CO_2 functional groups are hydrogen-bonded to three water molecules, respectively. Our results suggest that explicit water molecules of hydration are necessary to accurately model the vibrational modes, both intramolecular and intermolecular, that involve the -NH_2 and -CO_2 groups. For example, the calculated NH_2 wag frequency is 784 cm^{-1} for the $\text{Phe}(\text{H}_2\text{O})_9^\bullet$ model with PCM, in good

agreement with the experimentally observed peak at 776 cm^{-1} in the FFT power spectrum. In contrast, calculations of the isolated Phe• radical, even with PCM, yields a frequency of 687 cm^{-1} .

The inclusion of microhydration in the DFT calculations is also important for explaining the apparent absence of the decarboxylation pathway (Scheme 1), even though it was inferred from earlier flash photolysis⁷⁻⁹ and pulse radiolysis⁵ studies of phenylalanine to be one of the dominant fragmentation pathways of the Phe• radical. Interestingly, the optimized geometry for the isolated Phe• radical with PCM shows a C1–C2 bond length (R_{CC}) of 3.478 Å , more than twice the equilibrium R_{CC} of the Phe[−] anion. Constrained geometry optimization while varying R_{CC} yields a potential energy curve that is effectively unbound with respect to C1–C2 elongation (Fig. 5.10(a)); the bond dissociation energy of only $\sim 6\text{ meV}$ is lower than the zero-point energy. For this potential energy curve, classical trajectory calculations show that it takes only 214 fs for R_{CC} to extend from the Franck-Condon region (1.557 Å) to 5.00 Å , where the bond is effectively broken (Fig. 5.15). Our experimental data, however, does not support such an ultrafast depopulation of the Phe• radical. To further investigate the origin of this discrepancy, we repeat the constrained geometry optimization on models of the Phe• radical with different degrees of microhydration.

The addition of explicit water molecules of hydration to the Phe• radical model leads to the emergence of a pronounced minimum at $R_{CC} \sim 1.9 - 2.0\text{ Å}$ in the calculated potential energy curves. Moreover, both the barrier to dissociation and the bond dissociation energy generally increase with the degree of hydration. Going from Phe(H₂O)₃• to Phe(H₂O)₁₀•, for example, the barrier height increases from 0.062 eV to 0.320 eV while the bond dissociation energy increases from 0.027 eV to 0.231 eV (Table 5.2). Notably, the Franck-Condon region goes from being destabilized with respect to the dissociation barrier to being stabilized as the

Figure 5.15. Classical trajectory calculations performed using the potential energy curve calculated for the isolated Phe• radical reveal a duration of 214 fs for the C1–C2 bond to extend from the Franck-Condon region (1.557 Å) to 5.00 Å.

degree of hydration increases. For example, the Franck-Condon region for Phe(H₂O)₃• is 0.232 eV above the dissociation barrier, whereas in the case of Phe(H₂O)₁₀•, the Franck-Condon region is 0.135 eV below the dissociation barrier. When the Franck-Condon region lies below the dissociation barrier, the initially produced wave packet is unable to surmount the barrier, hence suppressing direct C1–C2 dissociation. The potential energy curves obtained from these calculations of the microhydrated Phe• radical thus provide a possible explanation for the absence of decarboxylation on the few-picosecond timescale of our experiments. Given that DFT underestimates reaction barriers,⁶⁶ however, it is more appropriate to pay attention to how the potential energy curves (Fig. 5.10(a)) and the energetic parameters (Table 5.2) vary with the degree of microhydration than to the absolute values of the parameters; quantitative accuracy would require multireference configuration interaction (MRCI)⁶⁷ or complete active space perturbation theory (CASPT2),^{68,69} which however are impractical for the large

microhydrated Phe• radical. Consistent with the findings of previous *ab initio* studies, our results suggest that the inclusion of microhydration is needed to correctly reproduce the chemical stability of radical species in aqueous solution.^{47,70-74} It is important to note that energy residing within other vibrational modes, including those of the solvent bath, can promote the statistical fragmentation of the Phe• radical, albeit on timescales beyond the few picoseconds probed in the current experiments.

5.5. Conclusion

Femtosecond photodetachment of aqueous phenylalaninate anion *via* a strong-field (3+1)-multiphoton process leads to the formation of the hitherto unobserved Phe• radical. Analysis of the time-resolved ΔA spectra collected over $\sim 0.1 - 10$ -ps time delay yields the absorption spectrum of the aqueous Phe• radical. The absorption spectrum reveals a peak at 308 nm, with an estimated peak molar extinction coefficient of $(3.3 \pm 0.7) \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$, and a vibronic progression characterized by a spacing of $482 \pm 15 \text{ cm}^{-1}$. Pronounced modulations of the ΔA signal as a function of time delay are observed atop the Phe• radical absorption. These modulations are due to coherent vibrational wave packet dynamics. FFT of the first-moment time trace resolves the wave packet dynamics into ten vibrational frequencies, assigned with the aid of DFT calculations to nine vibrational modes, with one of the vibrational frequencies being a first overtone $2\nu_{12}$. Time-domain analysis yields a dephasing time of $0.13 \pm 0.07 \text{ ps}$ for the overtone, markedly shorter than the dephasing time of $5.5 \pm 0.7 \text{ ps}$ obtained for the fundamental frequency. Coherent vibrational motion along these modes is driven by the ultrafast structural rearrangement of the Phe• radical from the Franck-Condon region to its equilibrium geometry. Surprisingly, several vibrational modes that involve the breathing and stretching modes of the phenyl sidechain are observed, despite our DFT calculations showing

only sub-0.1-ps changes in the bond lengths between the optimized geometries of the Phe⁻ anion and the Phe• radical. Moreover, *ab initio* calculations of the potential energy curve along the C1–C2 elongation coordinate show that the isolated Phe• radical, with solvation modeled by PCM, is expected to undergo direct decarboxylation on the 0.2-ps timescale, contrary to the experimental result. The inclusion of explicit water molecules of hydration in the model, however, results in the emergence of a dissociation barrier. With nine water molecules hydrogen-bonded to the –NH₂ and –CO₂ moieties of the Phe• radical, direct dissociation becomes energetically unfavorable as the dissociation barrier rises above the potential energy of the Franck-Condon region. This result explains the absence of ultrafast decarboxylation on the few-picosecond timescale of our experiments and points to the importance of including explicit water molecules of solvation in theoretical models. The identification of the electronic absorption signature of the Phe• radical will facilitate the further exploration of the possible role played by the Phe• radical in mediating long-distance electron transport in biology. Moreover, the observation of vibrational modes that are coupled to electron ejection, including one that gives rise to an overtone frequency with a ~0.1-ps-long dephasing time, is pertinent to understanding the recently reported long-lived coherent electron dynamics launched by the attosecond photoionization of phenylalanine. Just as in the present femtosecond photodetachment study, attosecond photoionization is similarly expected to launch vibrational wave packet dynamics whose coupling to coherent electronic motion should be considered. Interestingly, the appearance of an overtone frequency component in our experiments suggests that one cannot exclude the possibility that the long-lived quantum coherence reported in ref. 20 is vibrational in origin. We believe that the present findings are pertinent to future studies of electronic coherences triggered by the attosecond photoionization of biomolecules in aqueous solution.

References

1. Bhagavan, N. V.; Ha, C.-E., Chapter 15 - Protein and Amino Acid Metabolism. In *Essentials of Medical Biochemistry (Second Edition)*, Bhagavan, N. V.; Ha, C.-E., Eds. Academic Press: San Diego, 2015; pp 227-268.
2. Komoda, T.; Matsunaga, T., Chapter 4 - Metabolic Pathways in the Human Body. In *Biochemistry for Medical Professionals*, Komoda, T.; Matsunaga, T., Eds. Academic Press: Boston, 2015; pp 25-63.
3. Cannington, P. H.; Ham, N. S., He(I) and He(II) Photoelectron Spectra of Glycine and Related Molecules. *J. Electron Spectros. Relat. Phenomena* **1983**, 32 (2), 139-151.
4. Close, D. M., Calculated Vertical Ionization Energies of the Common α -Amino Acids in the Gas Phase and in Solution. *J. Phys. Chem. A* **2011**, 115 (13), 2900-2912.
5. Wang, D.; Schuchmann, H.-P.; Sonntag, C. v., Phenylalanine: Its $\cdot\text{OH}$ and $\text{SO}_4^{\cdot-}$ -Induced Oxidation and Decarboxylation. A Pulse Radiolysis and Product Analysis Study. *Z. Naturforsch. B* **1993**, 48 (6), 761-770.
6. Galano, A.; Cruz-Torres, A., OH Radical Reactions with Phenylalanine in Free and Peptide Forms. *Org. Biomol. Chem.* **2008**, 6 (4), 732-738.
7. Mittal, L. J.; Mittal, J. P.; Hayon, E., Biphotonic Photodissociation of Phenylalanine in Aqueous Solution at 20°C. *Chem. Phys. Lett.* **1973**, 18 (3), 319-322.
8. Mittal, L. J.; Mittal, J. P.; Hayon, E., Primary Processes in the Photochemistry of Phenylalanine and Derivatives in Aqueous Solution. Biphotonic Photoionization and Photodissociation Reactions. *J. Am. Chem. Soc.* **1973**, 95 (19), 6203-6210.
9. Bent, D. V.; Hayon, E., Excited State Chemistry of Aromatic Amino Acids and Related Peptides. II. Phenylalanine. *J. Am. Chem. Soc.* **1975**, 97 (10), 2606-2612.

10. Bent, D. V.; Hayon, E., Excited State Chemistry of Aromatic Amino Acids and Related Peptides. I. Tyrosine. *J. Am. Chem. Soc.* **1975**, *97* (10), 2599-2606.
11. Gräslund, A.; Sahlin, M.; Sjöberg, B. M., The Tyrosyl Free Radical in Ribonucleotide Reductase. *Environ. Health Perspect.* **1985**, *64*, 139-149.
12. Oldemeyer, S.; Franz, S.; Wenzel, S.; Essen, L.-O.; Mittag, M.; Kottke, T., Essential Role of an Unusually Long-Lived Tyrosyl Radical in the Response to Red Light of the Animal-like Cryptochrome aCRY. *J. Biol. Chem.* **2016**, *291* (27), 14062-14071.
13. Redpath, J. L.; Santus, R.; Ovadia, J.; Grossweiner, L. I., The Oxidation of Tryptophan by Radical Anions. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **1975**, *27* (2), 201-204.
14. Baugher, J. F.; Grossweiner, L. I., Photolysis Mechanism of Aqueous Tryptophan. *J. Phys. Chem.* **1977**, *81* (14), 1349-1354.
15. Solar, S.; Getoff, N.; Surdhar, P. S.; Armstrong, D. A.; Singh, A., Oxidation of Tryptophan and N-methylindole by $N_3^\bullet$, $Br_2^{\bullet-}$, and $(SCN)_2^{\bullet-}$ Radicals in Light- and Heavy-Water Solutions: A Pulse Radiolysis Study. *J. Phys. Chem.* **1991**, *95* (9), 3639-3643.
16. Kandori, H.; Borkman, R. F.; Yoshihara, K., Picosecond Transient Absorption of Aqueous Tryptophan. *J. Phys. Chem.* **1993**, *97* (38), 9664-9667.
17. McGimpsey, W. G.; Görner, H., Photoionization of Indole, N-Methylindole and Tryptophan In Aqueous Solution upon Excitation at 193 nm. *Photochem. Photobiol.* **1996**, *64* (3), 501-509.
18. Stevenson, K. L.; Papadantonakis, G. A.; LeBreton, P. R., Nanosecond UV Laser Photoionization of Aqueous Tryptophan: Temperature Dependence of Quantum Yield, Mechanism, and Kinetics of Hydrated Electron Decay. *J. Photochem. Photobiol. A* **2000**, *133* (3), 159-167.

19. Larson, B. C.; Pomponio, J. R.; Shafaat, H. S.; Kim, R. H.; Leigh, B. S.; Tauber, M. J.; Kim, J. E., Photogeneration and Quenching of Tryptophan Radical in Azurin. *J. Phys. Chem. B* **2015**, *119* (29), 9438-9449.
20. Calegari, F.; Ayuso, D.; Trabattoni, A.; Belshaw, L.; De Camillis, S.; Anumula, S.; Frassetto, F.; Poletto, L.; Palacios, A.; Decleva, P.; Greenwood, J. B.; Martín, F.; Nisoli, M., Ultrafast Electron Dynamics in Phenylalanine Initiated by Attosecond Pulses. *Science* **2014**, *346* (6207), 336-339.
21. Calegari, F.; Ayuso, D.; Trabattoni, A.; Belshaw, L.; Camillis, S. D.; Frassetto, F.; Poletto, L.; Palacios, A.; Decleva, P.; Greenwood, J. B.; Martín, F.; Nisoli, M., Ultrafast Charge Dynamics in an Amino Acid Induced by Attosecond Pulses. *IEEE J. Sel. Top. Quantum Electron.* **2015**, *21* (5), 1-12.
22. Lara-Astiaso, M.; Galli, M.; Trabattoni, A.; Palacios, A.; Ayuso, D.; Frassetto, F.; Poletto, L.; De Camillis, S.; Greenwood, J.; Decleva, P.; Tavernelli, I.; Calegari, F.; Nisoli, M.; Martín, F., Attosecond Pump–Probe Spectroscopy of Charge Dynamics in Tryptophan. *J. Phys. Chem. Lett.* **2018**, *9* (16), 4570-4577.
23. Vargas, M.; Malvankar, N. S.; Tremblay, P.-L.; Leang, C.; Smith, J. A.; Patel, P.; Synoeyenbos-West, O.; Nevin, K. P.; Lovley, D. R.; Giovannoni, S. J., Aromatic Amino Acids Required for Pili Conductivity and Long-Range Extracellular Electron Transport in *Geobacter sulfurreducens*. *mBio* **2013**, *4* (2), e00105-13.
24. Feliciano, G. T.; Steidl, R. J.; Reguera, G., Structural and Functional Insights into the Conductive Pili of *Geobacter sulfurreducens* Revealed in Molecular Dynamics Simulations. *Phys. Chem. Chem. Phys.* **2015**, *17* (34), 22217-22226.
25. Roy, A.; Seidel, R.; Kumar, G.; Bradforth, S. E., Exploring Redox Properties of Aromatic Amino Acids in Water: Contrasting Single Photon vs Resonant Multiphoton Ionization in Aqueous Solutions. *J. Phys. Chem. B* **2018**, *122* (14), 3723-3733.

26. Nathanael, J. G.; Gamon, L. F.; Cordes, M.; Rablen, P. R.; Bally, T.; Fromm, K. M.; Giese, B.; Wille, U., Amide Neighbouring-Group Effects in Peptides: Phenylalanine as Relay Amino Acid in Long-Distance Electron Transfer. *ChemBioChem* **2018**, *19* (9), 922-926.
27. Debnath, T.; Mohd Yusof, M. S. B.; Low, P. J.; Loh, Z.-H., Ultrafast Structural Rearrangement Dynamics Induced by the Photodetachment of Phenoxide in Aqueous Solution. *Nat. Commun.* **2019**, *10* (1), 2944.
28. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. *Gaussian 16 Rev. C.01*, Wallingford, CT, 2016.
29. Becke, A. D., Density-Functional Exchange-Energy Approximation with Correct Asymptotic Behavior. *Phys. Rev. A* **1988**, *38* (6), 3098-3100.
30. Lee, C.; Yang, W.; Parr, R. G., Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density. *Phys. Rev. B* **1988**, *37* (2), 785-789.

31. Tomasi, J.; Mennucci, B.; Cammi, R., Quantum Mechanical Continuum Solvation Models. *Chem. Rev.* **2005**, *105* (8), 2999-3094.
32. Tseng, H.-C.; Lee, C.-Y.; Weng, W.-L.; Shiah, I. M., Solubilities of Amino Acids in Water at Various pH Values under 298.15K. *Fluid Ph. Equilibria* **2009**, *285* (1), 90-95.
33. Lundblad, R. L.; Macdonald, F., *Handbook of Biochemistry and Molecular Biology* Fifth edition ed.; CRC Press: 2018.
34. Lozovoy, V. V.; Pastirk, I.; Dantus, M., Multiphoton Intrapulse Interference. IV. Ultrashort Laser Pulse Spectral Phase Characterization and Compensation. *Opt. Lett.* **2004**, *29* (7), 775-777.
35. Pestov, D.; Lozovoy, V. V.; Dantus, M., Multiple Independent Comb Shaping (MICS): Phase-Only Generation of Optical Pulse Sequences. *Opt. Express* **2009**, *17* (16), 14351-14361.
36. Grand, D.; Bernas, A.; Amouyal, E., Photoionization of Aqueous Indole: Conduction Band Edge and Energy Gap in Liquid Water. *Chem. Phys.* **1979**, *44* (1), 73-79.
37. Ambrosio, F.; Miceli, G.; Pasquarello, A., Electronic Levels of Excess Electrons in Liquid Water. *J. Phys. Chem. Lett.* **2017**, *8* (9), 2055-2059.
38. Goto, T.; Ikehata, A.; Morisawa, Y.; Ozaki, Y., Electronic Transitions of Protonated and Deprotonated Amino Acids in Aqueous Solution in the Region 145–300 nm Studied by Attenuated Total Reflection Far-Ultraviolet Spectroscopy. *J. Phys. Chem. A* **2013**, *117* (12), 2517-2528.
39. Asher, S. A.; Ludwig, M.; Johnson, C. R., UV Resonance Raman Excitation Profiles of the Aromatic Amino Acids. *J. Am. Chem. Soc.* **1986**, *108* (12), 3186-3197.
40. Crowell, R. A.; Bartels, D. M., Multiphoton Ionization of Liquid Water with 3.0–5.0 eV Photons. *J. Phys. Chem.* **1996**, *100* (45), 17940-17949.

41. Pépin, C.; Goulet, T.; Houde, D.; Jay-Gerin, J. P., Observation of a Continuous Spectral Shift in the Solvation Kinetics of Electrons in Neat Liquid Deuterated Water. *J. Phys. Chem. A* **1997**, *101* (24), 4351-4360.
42. Silva, C.; Walhout, P. K.; Yokoyama, K.; Barbara, P. F., Femtosecond Solvation Dynamics of the Hydrated Electron. *Phys. Rev. Lett.* **1998**, *80* (5), 1086-1089.
43. Vilchiz, V. H.; Kloepper, J. A.; Germaine, A. C.; Lenchenkov, V. A.; Bradforth, S. E., Map for the Relaxation Dynamics of Hot Photoelectrons Injected into Liquid Water *via* Anion Threshold Photodetachment and Above Threshold Solvent Ionization. *J. Phys. Chem. A* **2001**, *105* (10), 1711-1723.
44. Elkins, M. H.; Williams, H. L.; Shreve, A. T.; Neumark, D. M., Relaxation Mechanism of the Hydrated Electron. *Science* **2013**, *342* (6165), 1496-1499.
45. Mostafavi, M.; Lampre, I., An Overview of Solvated Electrons: Recent Advances. 2010; Vol. 105, pp 21-58.
46. Torche, F.; Marignier, J.-L., Direct Evaluation of the Molar Absorption Coefficient of Hydrated Electron by the Isosbestic Point Method. *J. Phys. Chem. B* **2016**, *120* (29), 7201-7206.
47. Ghosh, D.; Roy, A.; Seidel, R.; Winter, B.; Bradforth, S.; Krylov, A. I., First-Principle Protocol for Calculating Ionization Energies and Redox Potentials of Solvated Molecules and Ions: Theory and Application to Aqueous Phenol and Phenolate. *J. Phys. Chem. B* **2012**, *116* (24), 7269-7280.
48. Gardner, A. M.; Wright, T. G., Consistent Assignment of the Vibrations of Monosubstituted Benzenes. *J. Chem. Phys.* **2011**, *135* (11), 114305.
49. Wilson, E. B., The Normal Modes and Frequencies of Vibration of the Regular Plane Hexagon Model of the Benzene Molecule. *Phys. Rev.* **1934**, *45* (10), 706-714.

50. Salter, R.; Chu, J.; Hippler, M., Cavity-Enhanced Raman Spectroscopy with Optical Feedback CW Diode Lasers for Gas Phase Analysis and Spectroscopy. *Analyst* **2012**, *137* (20), 4669-4676.
51. Odhner, J. H.; Romanov, D. A.; Levis, R. J., Rovibrational Wave-Packet Dispersion during Femtosecond Laser Filamentation in Air. *Phys. Rev. Lett.* **2009**, *103* (7), 075005.
52. Loh, Z.-H.; Doumy, G.; Arnold, C.; Kjellsson, L.; Southworth, S. H.; Al Haddad, A.; Kumagai, Y.; Tu, M.-F.; Ho, P. J.; March, A. M.; Schaller, R. D.; Bin Mohd Yusof, M. S.; Debnath, T.; Simon, M.; Welsch, R.; Inhester, L.; Khalili, K.; Nanda, K.; Krylov, A. I.; Moeller, S.; Coslovich, G.; Koralek, J.; Minitti, M. P.; Schlotter, W. F.; Rubensson, J.-E.; Santra, R.; Young, L., Observation of the Fastest Chemical Processes in the Radiolysis of Water. *Science* **2020**, *367* (6474), 179-182.
53. Lu, Z.; Continetti, R. E., Dynamics of the Acetyloxyl Radical Studied by Dissociative Photodetachment of the Acetate Anion. *The Journal of Physical Chemistry A* **2004**, *108* (45), 9962-9969.
54. Gibbard, J. A.; Continetti, R. E., Photoelectron Photofragment Coincidence Spectroscopy of Carboxylates. *RSC Adv.* **2021**, *11* (54), 34250-34261.
55. Xing, X.-P.; Wang, X.-B.; Wang, L.-S., Photoelectron Imaging of Doubly Charged Anions, $^{-}\text{O}_2\text{C}(\text{CH}_2)_n\text{CO}_2^{-}$ ($n = 2-8$): Observation of Near 0 eV Electrons Due to Secondary Dissociative Autodetachment. *The Journal of Physical Chemistry A* **2010**, *114* (13), 4524-4530.
56. Peon, J.; Hess, G. C.; Pecourt, J.-M. L.; Yuzawa, T.; Kohler, B., Ultrafast Photoionization Dynamics of Indole in Water. *J. Phys. Chem. A* **1999**, *103* (14), 2460-2466.
57. Bin Mohd Yusof, M. S.; Lim, Y. L.; Loh, Z.-H., Ultrafast Vibrational Wave Packet Dynamics of the Aqueous Tyrosyl Radical Anion Induced by Photodetachment. *Phys. Chem. Chem. Phys.* **2021**, *23* (34), 18525-18534.

58. Bin Mohd Yusof, M. S.; Debnath, T.; Loh, Z.-H., Observation of Intra- and Intermolecular Vibrational Coherences of the Aqueous Tryptophan Radical Induced by Photodetachment. *J. Chem. Phys.* **2021**, *155* (13), 134306.
59. Zhu, L.; Sage, J.; Champion, P., Observation of Coherent Reaction Dynamics in Heme Proteins. *Science* **1994**, *266* (5185), 629-632.
60. Kumar, A. T. N.; Rosca, F.; Widom, A.; Champion, P. M., Investigations of Amplitude and Phase Excitation Profiles in Femtosecond Coherence Spectroscopy. *J. Chem. Phys.* **2001**, *114* (2), 701-724.
61. McCamant, D. W.; Kukura, P.; Yoon, S.; Mathies, R. A., Femtosecond Broadband Stimulated Raman Spectroscopy: Apparatus and Methods. *Rev. Sci. Instrum.* **2004**, *75* (11), 4971-4980.
62. Kukura, P.; McCamant, D. W.; Yoon, S.; Wandschneider, D. B.; Mathies, R. A., Structural Observation of the Primary Isomerization in Vision with Femtosecond-Stimulated Raman. *Science* **2005**, *310* (5750), 1006-1009.
63. Frontiera, R. R.; Fang, C.; Dasgupta, J.; Mathies, R. A., Probing Structural Evolution along Multidimensional Reaction Coordinates with Femtosecond Stimulated Raman Spectroscopy. *Phys. Chem. Chem. Phys.* **2012**, *14* (2), 405-414.
64. Kuramochi, H.; Takeuchi, S.; Tahara, T.; H., H.; L., G. T., Femtosecond Time-Resolved Impulsive Stimulated Raman Spectroscopy using Sub-7-fs Pulses: Apparatus and Applications. *Rev. Sci. Instrum.* **2016**, *87* (4), 043107.
65. Kuramochi, H.; Takeuchi, S.; Yonezawa, K.; Kamikubo, H.; Kataoka, M.; Tahara, T., Probing the Early Stages of Photoreception in Photoactive Yellow Protein with Ultrafast Time-Domain Raman Spectroscopy. *Nat. Chem.* **2017**, *9* (7), 660-666.
66. Cohen, A. J.; Mori-Sánchez, P.; Yang, W., Insights into Current Limitations of Density Functional Theory. *Science* **2008**, *321* (5890), 792-794.

67. Szalay, P. G.; Müller, T.; Gidofalvi, G.; Lischka, H.; Shepard, R., Multiconfiguration Self-Consistent Field and Multireference Configuration Interaction Methods and Applications. *Chem. Rev.* **2012**, *112* (1), 108-181.
68. Andersson, K.; Malmqvist, P. Å.; Roos, B. O., Second-Order Perturbation Theory with a Complete Active Space Self-Consistent Field Reference Function. *J. Chem. Phys.* **1992**, *96* (2), 1218-1226.
69. Roca-Sanjuán, D.; Aquilante, F.; Lindh, R., Multiconfiguration Second-Order Perturbation Theory Approach to Strong Electron Correlation in Chemistry and Photochemistry. *WIREs Comput. Mol. Sci.* **2012**, *2* (4), 585-603.
70. Hanus, M.; Kabeláč, M.; Rejnek, J.; Ryjáček, F.; Hobza, P., Correlated *Ab Initio* Study of Nucleic Acid Bases and Their Tautomers in the Gas Phase, in a Microhydrated Environment, and in Aqueous Solution. Part 3. Adenine. *J. Phys. Chem. B* **2004**, *108* (6), 2087-2097.
71. Kim, S.; III, H. F. S., Microhydration of Cytosine and its Radical Anion: Cytosine $\cdot$ (H₂O)_n (n = 1–5). *J. Chem. Phys.* **2007**, *126* (6), 064301.
72. Belau, L.; Wilson, K. R.; Leone, S. R.; Ahmed, M., Vacuum-Ultraviolet Photoionization Studies of the Microhydration of DNA Bases (Guanine, Cytosine, Adenine, and Thymine). *J. Phys. Chem. A* **2007**, *111* (31), 7562-7568.
73. Khistyayev, K.; Golan, A.; Bravaya, K. B.; Orms, N.; Krylov, A. I.; Ahmed, M., Proton Transfer in Nucleobases is Mediated by Water. *J. Phys. Chem. A* **2013**, *117* (31), 6789-6797.
74. Walton, J. C., Dissociations of Free Radicals to Generate Protons, Electrophiles or Nucleophiles: Role in DNA Strand Breaks. *Chem. Soc. Rev.* **2021**, *50* (13), 7496-7512.

Chapter 6

Ultrafast Proton Transfer of Phenol

Radical Cation in Aqueous Solution

6.1. Introduction

Proton transfer reactions are ubiquitous in biological processes such as enzyme catalysis^{1,2} and the transport of ions through membranes.³ In addition, many biological redox reactions are facilitated by concomitant proton transfer (PT) and electron transfer, as in proton-coupled electron transfer.⁴⁻⁷ Beyond PT reactions that occur in the electronic ground state, proton transfer can also involve electronically excited states. Excited-state proton transfer (ESPT) plays an important role in bioluminescence,⁸⁻¹⁰ as exemplified by the green fluorescent protein (GFP).¹¹⁻¹³ Moreover, ESPT is also central to technologically important applications such as photoinitiated polymerization^{14,15} and photolithography.¹⁶

Being triggered by light, ESPT reactions are particularly amenable to investigation by ultrafast spectroscopy.^{7,17,18} Previous time-resolved studies have elucidated ultrafast excited-state intramolecular proton transfer (ESIPT) that either occurs ballistically¹⁹ or is mediated by skeletal vibrational modes that modify the proton donor-acceptor distance.^{20,21} Ballistic PT occurs on timescales as short as ~10 fs and exhibits pronounced H/D isotopic dependence whereas PT that is gated by structural deformations typically occur on timescales of ~100 fs. Complementing molecules that undergo ESIPT is the class of molecules known as photoacids,^{18,22,23} which transfer a proton to a neighboring solvent molecule upon

photoexcitation. Extensive studies of intermolecular excited-state proton transfer to solvent (ESPTS) have revealed a correlation between the PT rate and the acid dissociation constant of the electronically excited state, pK_a^* .²⁴ The strongest photoacids, dubbed “superphotoacids”, have $pK_a^* < -7$ and exhibit proton transfer timescales of ~ 100 fs, limited by the frequency of the intermolecular vibration between the proton donor and proton acceptor.²⁵⁻²⁸

Aside from photoexcitation, photoionization can also lead to proton transfer. *Ab initio* simulations and experiments performed on ionized size-selected clusters comprising the proton donor and a variable number of solvent molecules in the gas phase, have revealed spectroscopic signatures of the PT product.²⁹⁻³⁴ However, only a limited number of studies have shed light on the dynamics of PT induced by photoionization. Time-resolved ion yield measurements performed on the gas-phase phenol-ammonia complex, $\text{PhOH}^{\bullet+}\text{-NH}_3$ radical cation, suggest that PT begins with the contraction of the intermolecular $\text{O}\cdots\text{N}$ distance within ~ 0.1 ps followed by a ~ 1 -ps-long vibrational relaxation of the PT product.^{35,36} In the condensed phase, femtosecond soft X-ray absorption spectroscopy was recently revealed that the $\text{H}_2\text{O}^{\bullet+}$ radical cation, produced by the intense laser ionization of liquid water, undergoes PT on the ~ 50 -fs timescale.³⁷ Beyond liquid water, however, the PT dynamics of ionized molecules in aqueous solution remains largely unexplored. It is important to note that ultrafast PT bears implications for the chemical reactivity of radicals. For example, the standard reduction potential of the $\text{H}_2\text{O}^{\bullet+}$ radical cation (3.8 V)³⁸ is larger than that of the hydroxyl radical ($\text{OH}^\bullet$) PT product (1.8 V).³⁹ Within the context of radiation chemistry and radiation biology, the ultrashort lifetime of the $\text{H}_2\text{O}^{\bullet+}$ radical cation diminishes its potential for oxidative damage.

In this chapter, we employ optical pump-probe spectroscopy to resolve the ultrafast PT dynamics of the aqueous phenol radical cation, produced by the ionization of aqueous phenol (Fig. 6.1). A schematic diagram of the photoionization-induced proton transfer process

Scheme 6.1. Schematic diagram illustrating the photoionization of aqueous phenol to form the phenol radical cation ($\text{PhOH}^{\bullet+}$), followed by the proton transfer process between the $\text{PhOH}^{\bullet+}$ radical cation with a neighboring water molecule to form the proton-transferred product, phenoxyl radical ($\text{PhO}^{\bullet}$) and hydronium ion (H_3O^+). The phenol radical cation and phenoxyl radical has a maximum absorption at ~ 425 nm and 392 nm respectively.

between phenol radical cation and a neighboring water molecule is shown in Scheme 6.1. Our choice of phenol is motivated by its occurrence as the side chain of tyrosine (TyrOH), an amino acid whose redox activity is entwined with proton transfer.^{7,40} Oxidation of tyrosine requires a highly oxidizing potential of +1.46 V vs. NHE to give $\text{TyrOH}^{\bullet+}$,^{41,42} this potential decreases to +0.94 V when deprotonation occurs in concert with oxidation to yield $\text{TyrO}^{\bullet}$.⁴³ While trapped ion photodissociation spectroscopy (TIPS) has elucidated evidence of PT in gas-phase $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_n$ ($n \geq 3$),³⁰⁻³⁴ the timescale for proton transfer remains unknown. In agreement with the TIPS findings, a recent time-resolved photofragment ion spectroscopy investigation showed that $\text{PhOH}^{\bullet+}-\text{H}_2\text{O}$ does not undergo proton transfer.^{35,36} These experimental results are consistent with those obtained from *ab initio* calculations, which reveal that PT is barrierless for $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_3$ ^{33,44} but absent for $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})$.^{33,36,44,45} Our experiments reveal ultrafast dephasing of the vibrational modes of the aqueous $\text{PhOH}^{\bullet+}$ radical cation, suggestive of proton

Figure 6.1. Structure of the phenol. The carbon, hydrogen and oxygen atoms are in gray, white and red respectively.

transfer occurring on the ~ 0.2 -ps timescale. These results are supported by quantum mechanical wave packet trajectory calculations.

6.2. Materials and Methods

6.2.1. Theoretical Calculations

The equilibrium molecular geometries of phenol and the phenol radical cation, as well as their associated vibrational frequencies, are obtained from DFT calculations performed with the GAUSSIAN 16 package.⁴⁶ The calculations employ the hybrid exchange functional of Becke (B3),⁴⁷ the correlation functional of Lee, Yang and Parr (LYP),⁴⁸ and the 6-311G++(d,p) split-valence triple- ξ basis set. Three different models of phenol and the phenol radical cation are

employed in the simulations. The first model is the isolated molecule, the second model is the molecule with three explicit water molecules of hydration, i.e., $\text{PhOH}-(\text{H}_2\text{O})_3$ or $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_3$, whereas the third model is the molecule with five explicit water molecules of hydration, i.e., $\text{PhOH}-(\text{H}_2\text{O})_5$ or $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_5$. All three models include a polarizable continuum (PCM) to mimic the dielectric environment of the water solvent.⁴⁹ For $\text{PhOH}-(\text{H}_2\text{O})_3$ or $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_3$, the phenolic OH is directly hydrogen-bonded to all three water molecules, where one acts as the hydrogen bond acceptor and two act as hydrogen bond donors. For $\text{PhOH}-(\text{H}_2\text{O})_5$ or $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_5$, two additional hydrogen bond-accepting water molecules are coordinated to the proton-accepting H_2O in $\text{PhOH}-(\text{H}_2\text{O})_3$ and $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_3$. The C_s point group symmetry, with the PhOH moiety residing within the reflection plane, is imposed during geometry optimization. Each geometry optimization is followed by a vibrational frequency calculation to verify that the optimized geometry corresponds to a local minimum.

To investigate the proton transfer dynamics, we construct the reduced potential energy surface (PES) along the intramolecular phenol O–H distance coordinate and the intermolecular $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ distance coordinate involving the phenol oxygen atom (O_{Ph}) and the oxygen atom of the proton-accepting water molecule (O_{a}). Since $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_3$ does not support proton transfer, we employ $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_5$ instead for the PES scan. The $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ distance is varied between 2.00 – 3.50 Å in steps of 0.05 Å while the phenol O–H distance is varied between 0.80 – 1.80 Å in steps of 0.05 Å; in addition, phenol O–H distances of 1.90 Å, 2.00 Å, 2.20 Å, and 2.40 Å are also included in the PES scan. The PES scan is repeated for neutral phenol, $\text{PhOH}-(\text{H}_2\text{O})_5$, where the phenol O–H distance is varied from 0.79 – 1.49 Å and the $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ distance is varied between 2.04 – 3.54 Å, both with steps of 0.05 Å.

The resultant reduced PESs are fit by using the neural network method. The architecture of neural network is 2-20-40-1, giving a total of 941 parameters. The input layer consists of the two bond distances, the two hidden layers have 20 and 40 neurons, and the output layer has 1 potential energy. All fittings are carried out using the Levenberg–Marquardt algorithm⁵⁰ and employ the root-mean-square error (RMSE) to assess the quality of each fit. To avoid overfitting, the data set is randomly divided into two groups, with 90% of the points as the training set and 10% as the validating set. The fits with the two sets that give similarly small RMSEs are thought to be reasonable. The total RMSEs of the final neutral and cationic PESs are 1.462 meV and 3.068 meV, respectively.

Quantum dynamics simulations employ the 2D Hamiltonian

$$\hat{H} = -\frac{\hbar^2}{2\mu_r} \frac{\partial^2}{\partial r^2} - \frac{\hbar^2}{2\mu_R} \frac{\partial^2}{\partial R^2} + \hat{V}(r, R), \quad (1)$$

where r and R denote the phenol O–H and intermolecular O_{Ph}···O_a distances, respectively, μ_r and μ_R are their associated masses, and $V(r, R)$ is the PES obtained from our *ab initio* calculations. Proton transfer on the cationic PES is simulated based on the Condon approximation, in which the initial wave packet is obtained by diagonalizing the 2D Hamiltonian on the neutral PES and placed vertically on the cationic PES.⁵¹ The wave packet is then propagated using the split-operator method.⁵² The outgoing boundary conditions are imposed by applying a negative imaginary absorbing potential at the edge of the grid. The population decay of the phenol radical cation is calculated by evaluating the fraction of the wave packet that persists in the Franck–Condon region at different times.

6.2.2. Experimental Method

Phenol (Sigma-Aldrich, $\geq 99\%$) is used as received without further purification. Phenol is dissolved in distilled water to produce a phenol solution with a concentration of 200 mM (pH ~ 5.3). Deuterated phenol that is employed in the isotopic studies is prepared by dissolving phenol in deuterium oxide (D_2O) (Cambridge Isotope Laboratories, Inc., 99.9%) to yield C_6H_5OD (PhOD). Given that phenol has a pK_a of 9.99,⁵³ we expect $<0.01\%$ of phenol to be deprotonated. A similar percentage of phenol-OD to be deprotonated is expected as phenol-OD has a pK_a of 10.62.⁵⁴

The experimental procedure has been described in detailed in Chapter 2. In summary, the transient absorption pump-probe experiment is performed with a chirped-pulse-amplified Ti:sapphire laser system (Coherent, Legend Elite Duo-USX), with an output of 4.5-mJ, 30-fs pulses at 1-kHz repetition rate and 800-nm center wavelength, with a bandwidth of 50 nm. The pulses undergo self-phase modulation in a helium-filled hollow-core fiber before they are temporally compressed by chirped mirrors (Ultrafast Innovations GmbH, PC1332). The compressed pulses are incident on a beam splitter (Layertec, 131191), with 80% of the transmitted beam (~ 0.6 mJ) being directed to a 4f visible-NIR pulse shaper with a 128-element spatial light modulator (SLM) (Biophotonics Solutions, FemtoJock-P) for further pulse compression, which truncates the wavelength range of the visible-NIR from 520 nm to 750 nm, with a center wavelength of 636 nm (1.95-eV photon energy). The visible-NIR spectrum is shown in Fig. 6.2(a). Transform-limited laser pulses with full-width half-maximum (FWHM) duration of ~ 6.2 fs are obtained by using multiphoton intrapulse interference phase scan (MIIPS) to measure the spectral phase at the sample, followed by using SLM to introduce an oppositely signed phase to the laser pulses, thus compressing it to the transform-limited duration.⁵⁵ Subsequently, a second-order interferometric auto-correlation trace is recorded by

Figure 6.2. (a) The visible-NIR ionizing pump spectrum ranges from 520 nm to 750 nm, with a center wavelength of 636 nm. (b) Broadband near-UV probe spectrum ranges from 360 nm to 460 nm, generated *via* SHG.

multiple independent comb shaping (MICS).⁵⁶ The second-order interferometric autocorrelation time is shown in Fig. 6.3(a). The transform-limited pulses, which function as the strong-field ionizing pump, are directed to the sample. The ionizing pump is then focused onto the sample by a 50-cm focal length spherical mirror. A maximum pulse energy available for experiments of 25 μJ , with a beam waist ($1/e^2$) of 80 μm , resulting in a peak intensity of $4.7 \times 10^{13} \text{ W cm}^{-2}$ for the photoionization process. An optical delay stage line, driven by a piezo translation stage (Physik Instrumente, N-664.3A) in the ionizing pump's path provides a variable time delay between pump and probe pulses. An achromatic half-waveplate (B. Halle, RAC4.2.15 L) is introduced in the ionizing pump pulse's path to allow the acquisition of isotropic signal when necessary.

The remaining output from the chirped mirror compressor passes through a pair of wedges (Femtolasers, OA124), and subsequently directed to a 50- μm -thick Type I beta-barium borate (BBO) crystal (Casix, $\theta = 29.0^\circ$) to generate the UV probe *via* second harmonic generation (SHG). The broadband near-UV probe spans 360 nm to 470 nm. The broadband

Figure 6.3. (a) A second-order interferometric auto-correlation trace of the visible-NIR ionizing pump spectrum, measured in a 10- μm -thick, $\theta = 37.0$ deg Type I BBO crystal, reveals a pulse duration with FWHM of ~ 6.2 fs. (b) The pump-probe cross-correlation, measured *via* difference frequency mixing in a 10- μm -thick, $\theta = 29.2$ deg Type I BBO crystal, reveals a FWHM of 12 fs.

near-UV probe spectrum is shown in Fig. 6.2(b). A pair of dichroic beamsplitters (Layertec, 109433) is introduced to eliminate residual fundamental beam. The near-UV probe is then directed to a pair of chirped mirrors (Ultrafast Innovations, CM82) with a group delay dispersion (GDD) of $\sim -50 \text{ fs}^2$. The near-UV pulse is then split off by a reflective neutral density beamsplitter (Newport, FRQ-ND05) to produce the probe and reference pulses. The reflected near-UV probe is then focused onto the sample by a 15-cm focal length off-axis parabolic reflector (Thorlabs, MPD369H-F01). The focused near-UV probe has a beam waist ($1/e^2$) of $26 \mu\text{m}$, with a pulse energy of 100 nJ, which has a peak intensity of $7.4 \times 10^{11} \text{ W cm}^{-2}$.

A slit nozzle (Metaheuristics, Type L) is used in conjunction with a peristaltic pump (Cole-Parmer, 07528-10) and a pulse dampener to produce a microjet of aqueous phenol for experiments. The volume rate of 92 mL min^{-1} translates to a vertical flow rate of approximately 8.5 mm ms^{-1} . The relatively high vertical flow rate ensures that fresh volume of phenol solution

is exposed to each ionizing pump pulse. A liquid jet of 7- μm thickness is produced, which yields a sizeable differential absorption signal ($\Delta A \sim 0.1$), while minimizing the temporal broadening of the pulses in the liquid jet due to dispersion. In addition, a thin liquid jet limits the group-velocity mismatch between the pump and probe to <1 fs, making its effect on the time resolution negligible.

A synchronized optical chopper in the ionizing pump's path before the sample allow modulation of the ionizing pump at a frequency of 0.5 kHz. The probe beam that is transmitted through the microjet is focused into a 300-nm spectrograph (Princeton Instruments, Acton SP2300), equipped with 600-grooves per mm grating blazed at 500-nm and a 1024-pixels silicon (Si) photodiode array detector (Stresing, S8381-1024Q) with a read-out rate of 1 kHz, which allow simultaneous acquisition of the probe spectra obtained with and without the ionizing pump pulse. The reference near-UV pulses are focused to an identical spectrograph, which is equipped with similar grating and photodiode array at 1-kHz repetition rate. This method reduces noise interference cause by shot-to-shot fluctuations of the probe pulse. The Si photodiode array detectors are interfaced to desktop computers for automated data acquisition.

The focal spot size of the probe beam is generally $\sim 3\times$ smaller than that of the ionizing pump beam, hence minimizing the effect of spatial averaging on the observed ultrafast dynamics. The cross-correlation of the pump and probe, by measuring the difference frequency generated (DFG) beam, is generated by utilizing a 10- μm thick Type I BBO crystal (Casteck, $\theta = 29.2^\circ$) which yield an instrument response of 12 fs at FWHM. The cross-correlation trace is displayed in Fig. 6.3(b).

Static UV-visible measurements are performed with a double-beam UV-visible-NIR spectrophotometer (Agilent Technologies, Cary 300) with a 1-mm path length quartz cuvette by measuring absorbance. The spectrophotometer has a wavelength range from 190 to 800 nm.

6.3. Results

6.3.1. Transient Absorption of Ionized Aqueous Phenol

As illustrated in previous chapters, a power-dependence measurement is performed to determine the photon order of the strong-field photoionization process of phenol in aqueous solution. For an N -photon photodetachment process, the differential absorption signal, ΔA , scales with the intensity of ionizing pump, I , as shown in the following relationship.

$$\Delta A \propto I^N \quad (6.1)$$

In this study, the photon-order measurement is performed by using a near-UV probe, which measures the absorption of the ground-state hydrated electron at 425 nm at a pump-probe time delay of 1.5 ps. Hence, the absorption signal is proportional to the extent of the photoionization process. The ΔA against ionizing pump power spectrum is recorded for aqueous phenol. The ΔA vs. I signal is then fitted with the following expression

$$\Delta A = \frac{\alpha * I^N}{1 + \left(\frac{I}{I_{sat}}\right)^N}, \quad (6.2)$$

where α is the amplitude, N is the photon order and I_{sat} is the saturation intensity. The best fit when $N = 4$ yields $\alpha = (1.70 \pm 0.02) \times 10^{-58} \text{ cm}^8 \text{ W}^{-4}$ and $I_{sat} = (8.39 \pm 0.38) \times 10^{13} \text{ W cm}^{-2}$. The value of N indicates that the photoionization of aqueous phenol occurs *via* a four-photon process. A log-log plot of ΔA against pump intensity is shown in Fig. 6.4.

Previous studies have suggested that the UV photoexcitation of phenol, with photon energies spanning 4.6 – 6.2 eV, leads to its ionization either by autoionization from electronically excited states to the solvent continuum⁵⁷ or by O–H photodissociation along the repulsive $1^1\pi\sigma^*$ potential^{44,58} followed by charge transfer to solvent.⁵⁹ Our pump-power dependence measurements show that the photoionization of phenol occurs *via* a four-photon

Figure 6.4. Photon-order measurement for strong-field photoionization of phenol. Log-log plot of ΔA vs. pump intensity reveals a four-photon process for the photoionization of aqueous phenol.

process, corresponding to a total input energy of 7.8 eV (Fig. 6.4). Considering the vertical ionization energy of 7.8 eV for aqueous phenol⁶⁰ and the vertical electron affinity of ~ 1 eV for liquid water,⁵⁷ the 7.8-eV energy input is sufficient to eject an electron from phenol and inject it into the conduction band of liquid water. As such, we rule out any participation of intermediate electronically excited states in the multiphoton photoionization process.

The isotropic differential absorption (ΔA) spectrum of ionized aqueous phenol collected as a function of pump-probe time delay is shown in Fig. 6.5(a), whereas the time trace obtained at 425 nm is shown in Fig. 6.5(b). The increasing ΔA signal on the long-wavelength side of the probe spectral range is the absorption signature of the hydrated electron, formed from the conduction-band electron *via* electronic relaxation^{57,61} followed by vibrational cooling.⁶²⁻⁶⁸ In addition, the ΔA spectrum exhibits a pronounced absorption peak at 400 nm alongside a

Fig. 6.5. (a) Isotropic differential absorption (ΔA) spectrum of the ionized aqueous PhOH as a function of pump-probe time delay. (b) Time traces at 425 nm of the ionized aqueous PhOH (in red) and ionized liquid water (in blue). The former shows pronounced modulations due to vibrational wave packet dynamics. The black line shows the result from global analysis. (c) The wavelength-dependent $\Delta A_0(\lambda)$, $\Delta A_1(\lambda)$ and $\Delta A_2(\lambda)$ spectra obtained from global fitting are shown in red, blue and magenta respectively.

vibronic feature at 385 nm, indicative of the ${}^2B_1 \leftarrow {}^2B_1$ transition of the phenoxyl radical electronic ground state.^{69,70}

The absorption signature of aqueous $\text{PhOH}^{\bullet+}$ radical cation, produced by the vertical ionization of phenol, appears to be missing from the ΔA spectra. In n-dodecane, cyclohexane,

and 1,2-dichloroethane, the absorption maximum of the phenol radical cation is located at 430 nm, whereas the absorption maximum in n-butyl chloride is at 440 nm.⁷¹ These absorption maxima are broadened and red-shifted relative to the absorption maximum of the PhO• radical at 400 nm, recorded in the same solvents. In highly acidic aqueous solution (~14 M H₂SO₄), an absorption peak at 419 nm ($\epsilon_{\text{max}} = 1800 \pm 150 \text{ M}^{-1} \text{ cm}^{-1}$), with a linewidth of 50 nm, has been attributed to the PhOH•⁺ radical cation.⁷² The broad absorption feature of PhOH•⁺ radical cation in both aqueous and nonaqueous solvents is also consistent with the broad, featureless electronic absorption spectra of gas-phase PhOH•⁺-(H₂O)_n ($n = 1, 2$), for which proton transfer does not occur; in comparison, the electronic absorption spectra of gas-phase PhOH•⁺-(H₂O)_n ($n = 3 - 6$) show well-defined peaks that are characteristic of the PhO• radical proton transfer product. Interestingly, a recent study of aqueous phenol photoexcited at 200 nm attributed a weak and short-lived ΔA feature at 425 nm to aqueous PhOH•⁺.⁷⁰ Unfortunately, since the feature appears only within the 200-fs time resolution of the experimental setup, the decay constant of the feature, and hence presumably the lifetime of the phenol radical cation, could not be resolved. In our case, it is possible that the broad absorption signature of the phenol radical cation is concealed by the prominent absorption of the hydrated electron⁷² ($\epsilon = 3148 \text{ M}^{-1} \text{ cm}^{-1}$ at 425 nm).⁷³

To elucidate the timescales of the ultrafast dynamics encoded in the time-resolved ΔA spectra, we fit the ΔA time traces collected over the wavelength range of 365 – 450 nm and time delay range of 100 – 4455 fs to the function

$$A(\lambda, t) = \Delta A_0(\lambda) + \Delta A_1(\lambda)(e^{-t/\tau_{d1}} - e^{-t/\tau_{r1}}) + \Delta A_2(\lambda)(e^{-t/\tau_{d2}} - 1). \quad (6.3)$$

In the above expression, $\Delta A_1(\lambda)$ and $\Delta A_2(\lambda)$ are the decay associated difference spectra, $\Delta A_0(\lambda)$ is the offset, τ_{d1} and τ_{d2} are the decay time constants and τ_{r1} is the rise time constant. The global fit reveals that ΔA rises with a characteristic time constant $\tau_{r1} = 0.51 \pm 0.01 \text{ ps}$ and

decays with time constant $\tau_{d1} = 72 \pm 1$ ps; these time constants are consistent with those obtained from our experiments on pure liquid water, performed under identical experimental conditions. In the case of ionized phenol, an additional decay constant $\tau_{d2} = 1.8 \pm 0.1$ ps is found. The time traces of ionized aqueous phenol and ionized liquid water at 425 nm, along with their respective fit functions, are shown in Fig. 6.5(b). The decay associated difference spectra, $\Delta A_1(\lambda)$ and $\Delta A_2(\lambda)$, and the offset, $\Delta A_0(\lambda)$, are plotted in Fig. 6.5(c).

6.3.2. Vibrational Wave Packet Dynamics of Ionized Phenol

The time-resolved ΔA spectra also exhibit oscillatory features that encode vibrational wave packet dynamics. To analyze the wave packet dynamics quantitatively, we compute the spectral first-moment time trace $\langle E(t) \rangle$, given as

$$\langle E(t) \rangle = \frac{\int_{E_i}^{E_f} E \Delta A(E, t) dE}{\int_{E_i}^{E_f} \Delta A(E, t) dE}. \quad (6.4)$$

In the above expression, E represents the probe photon energy, t is the pump-probe time delay and $\Delta A(E, t)$ is the differential absorbance as a function of probe photon energy and pump-probe time delay. E_i and E_f define the spectral range over which $\langle E(t) \rangle$ is computed; in this case, E_i and E_f correspond to 2.95 eV (420 nm) and 2.88 eV (430 nm), respectively. To minimize possible contributions from coherent artifacts, $\langle E(t) \rangle$ is computed only from a time delay of 0.1 ps onward. The resultant $\langle E(t) \rangle$ trace is shown in Fig. 6.6(a), with the inset showing a zoomed-in view of $\langle E(t) \rangle$ in the range of 0.1 – 1 ps. Fast-Fourier transform (FFT) of the $\langle E(t) \rangle$ trace yields its FFT power spectrum (Fig. 6.6(b)), from which the frequencies of vibrational modes that contribute to $\langle E(t) \rangle$ can be identified. To aid the assignment of the

Figure 6.6. (a) First-moment time trace, $\langle E(t) \rangle$, of ionized aqueous PhOH and its time-domain fit. The inset shows the enlarged $\langle E(t) \rangle$ from 0.1 ps to 1 ps. (b) FFT power spectrum of the first-moment time trace of ionized aqueous phenol. The $\langle E(t) \rangle$ time trace and the FFT power spectrum of ionized aqueous PhOD are shown in (c) and (d), respectively. The peaks marked by an asterisk are assigned to the OH (OD) bend and wag modes of PhOH (PhOD). Note that the O₂ and N₂ vibrational frequencies, located at 1552 cm⁻¹ and 2334 cm⁻¹, respectively, are also observed due to the experiment being performed in open air.

observed FFT frequencies, experiments were also performed on the isotopomer, phenol-OD (PhOD), dissolved in D₂O. The ΔA of ionized aqueous phenol-OD is shown in Fig. 6.7. The $\langle E(t) \rangle$ time trace obtained for ionized aqueous PhOD and its corresponding FFT power spectrum are shown in Figs. 6.6(c) and 6.6(d), respectively. Table 6.1 summarizes the FFT

Figure 6.7. Isotropic differential absorption (ΔA) spectrum of the ionized aqueous phenol-OD as a function of pump-probe time delay.

frequencies and normalized FFT powers for the peaks observed in the FFT power spectra, with the corresponding calculated frequencies and assignment.

Our recent studies of the vibrational wave packet dynamics of radical species prepared by strong-field photoionization or photodetachment show that the displacive mechanism for the generation of vibrational coherences can be extended from photoionization^{74,75} to photodetachment.^{76,77} According to this mechanism, wave packet motion is launched along vibrational modes for which the equilibrium geometries of the species before and after electron ejection are displaced. Extending this analysis to the present work would require us to consider the equilibrium geometries of neutral PhOH and the $\text{PhOH}\cdot^+$ radical cation. However, since the time-resolved ΔA spectra recorded over 0.1 – 4 ps is dominated by the absorption signature of the $\text{PhO}\cdot$ radical, without any obvious sign of the $\text{PhOH}\cdot^+$ radical cation absorption, we expect the observed vibrational wave packet dynamics to originate primarily from the $\text{PhO}\cdot$ radical as well. Table 6.1 gives the assignments of the observed vibrational frequencies

Table 6.1. Experimental and calculated vibrational frequencies of the phenoxyl radical in aqueous solution from phenol and phenol-OD and their assignments. The calculated frequencies are based on the equilibrium molecular geometry of the microhydrated phenoxyl radical from a previous study.⁸⁴

Phenol		Phenol-OD		Calculated Frequency (cm ⁻¹)	Assignment
FFT Analysis		FFT Analysis			
Frequency (cm ⁻¹)	Power (norm.)	Frequency (cm ⁻¹)	Power (norm.)		
		529	1.8 × 10 ⁻²	541	CCC bend
814	1.5 × 10 ⁻²	808	1.6 × 10 ⁻¹	818	Ring breath / CCC bend
970	3.2 10 ⁻²	984	7.9 × 10 ⁻²	988	CCC trigonal bend
1002	1	1001	1	1006	Ring breath / CH bend
1025	8.2 × 10 ⁻²	1027	8.0 × 10 ⁻²	1007	HCCH torsion
1174	7.2 × 10 ⁻³			1164	CH bend
1268	3.2 × 10 ⁻³	1260	5.0 × 10 ⁻³	1295	CC stretch / CH bend
1228	8.3 × 10 ⁻³	835	1.6 × 10 ⁻²	1197 (870)	OH (OD) wag*
1502	7.0 × 10 ⁻²	1159	6.0 × 10 ⁻³	1414 (1170)	OH (OD) bend*
		2364	6.5 × 10 ⁻²		D ₂ O OD stretch
		2469	4.7 × 10 ⁻³		
1557	1.1 × 10 ⁻²	1553	7.7 × 10 ⁻³		Oxygen
2332	6.7 × 10 ⁻³	2328	6.7 × 10 ⁻³		Nitrogen

* Vibrational mode of PhOH•⁺ or PhOD•⁺.

obtained from DFT calculations. We note that there is no evidence of impulsive stimulated Raman scattering (ISRS) of phenol since the ΔA signal and its associated time-domain oscillations disappear when the ionizing-pump power is attenuated to suppress strong-field photoionization. Furthermore, the static UV absorption spectrum of aqueous phenol does not

overlap with the probe spectral range. This is evident from the absorbance spectrum of 4 mM aqueous phenol, which has a maximum absorption of 270 nm (Fig. 6.8).

Wave packet dynamics could be initiated in PhO• radical, the proton transfer product, *via* two possible pathways. First, ultrafast proton transfer from PhOH•⁺ radical cation could serve as an impulse for generating vibrational coherences in PhO• radical. In this mechanism, non-reactive vibrational modes of PhOH•⁺ radical cation, whose frequencies are lower than the reaction rate, are essentially frozen during an ultrafast chemical reaction. As a result, the vibrational probability densities of PhOH•⁺ radical cation along these modes are projected onto the PES of PhO• radical, thus yielding a nonstationary vibrational state. Such reaction-driven coherences have been observed in photofragments following ultrafast photodissociation.^{78,79} Second, vibrational wave packet motion could be launched in PhOH•⁺ radical cation following the displacive mechanism uncovered in our recent work. Subsequent vibrational coherence transfer to the PhO• product is possible for vibrational modes that are weakly coupled to proton transfer and that exhibit minimal vibrational coordinate transformation between the PhOH•⁺ reactant and the PhO• product. The transfer of vibrational coherence between different electronic states was recently observed in transition metal complexes after ultrafast intersystem crossing.^{80,81} As discussed below, our experimental results favor the mechanism involving vibrational coherence transfer.

To obtain further insight into the vibrational wave packet dynamics, we analyze the $\langle E(t) \rangle$ traces for photoionized aqueous PhOH and PhOD in the time domain by fitting them to a sum of exponentially damped cosine functions

$$\langle E(t) \rangle = \sum_{i=1}^n A_i e^{-t/T_{2,i}} \cos(\omega_i t + \phi_i), \quad (6.5)$$

Figure 6.8. Static UV-visible absorption spectrum of 4 mM aqueous phenol, measured with a 1-mm path length quartz cuvette. The absorption maximum is at 270 nm.

In the above expression, A_i , $T_{2,i}$, ω_i and ϕ_i are the amplitude, dephasing time, vibrational frequency and phase of the vibrational mode Q_i , respectively. The fit parameters are summarized in Tables 6.2 and 6.3. Compared to FFT analysis, time-domain fitting directly furnishes the dephasing times of the vibrational modes.

6.4. Discussion

6.4.1. Transient Absorption of Ionized Aqueous Phenol

The time constants τ_{r1} and τ_{d1} are assigned respectively to the timescales for the formation of the hydrated electron and its subsequent disappearance by geminate recombination. This assignment is supported by the similarity of the corresponding decay associated difference spectrum, $\Delta A_1(\lambda)$, to the absorption spectrum of the hydrated electron. Multiphoton ionization

Table 6.2. Time-domain analysis results and calculated vibrational frequencies of the phenoxyl radical produced from photoionization of phenol (PhOH) in aqueous solution and their assignments. The calculated frequencies are based on the equilibrium geometry of the microhydrated phenoxyl radical. The assignments are based on a previous study.⁸⁴

Experimental				Calc. Freq. (cm ⁻¹)	Assignment
Freq. (cm ⁻¹)	Amplitude (meV)	Damping Time (ps)	Phase (π -rad)		
813 $\pm$ 1	0.23 $\pm$ 0.02	0.44 $\pm$ 0.04	0.67 $\pm$ 0.03	818	Ring breath / CCC bend
974 $\pm$ 2	0.61 $\pm$ 0.09	0.24 $\pm$ 0.02	1.14 $\pm$ 0.06	988	CCC trigonal bend
1001 $\pm$ 1	0.31 $\pm$ 0.01	2.32 $\pm$ 0.12	0.59 $\pm$ 0.01	1006	Ring breath / CH bend
1027 $\pm$ 1	0.14 $\pm$ 0.01	1.39 $\pm$ 0.20	0.41 $\pm$ 0.03	1007	HCCH torsion
1167 $\pm$ 3	0.07 $\pm$ 0.02	1.35 $\pm$ 0.78	1.24 $\pm$ 0.01	1164	CH bend
1262 $\pm$ 3	0.20 $\pm$ 0.11	0.85 $\pm$ 0.50	1.04 $\pm$ 0.16	1275	CH bend
1246 $\pm$ 4	0.52 $\pm$ 0.01	0.18 $\pm$ 0.02	0.73 $\pm$ 0.07	1197	OH wag*
1497 $\pm$ 5	1.09 $\pm$ 0.25	0.16 $\pm$ 0.02	1.89 $\pm$ 0.06	1414	OH bend*
1559 $\pm$ 1	0.02 $\pm$ 0.01	2.33 $\pm$ 0.99	0.40 $\pm$ 0.06		Oxygen
2330 $\pm$ 1	0.04 $\pm$ 0.01	2.21 $\pm$ 0.42	1.97 $\pm$ 0.03		Nitrogen

* Vibrational mode of PhOH•⁺.

of the phenol solute and the liquid water solvent injects electrons into the conduction band of liquid water. Subsequent trapping of the electrons followed by vibrational cooling yields hydrated electrons.⁶²⁻⁶⁸ The hydrated electrons can undergo geminate recombination with the phenoxyl (PhO•) and hydroxyl (OH•) radicals, produced *via* the ionization of phenol and water, respectively. Although geminate recombination is characterized by nonexponential kinetics,^{64,82} it can be approximated as an exponential decay in our analysis due to the narrow range of time delays ($\sim$ 4 ps) sampled in our measurements.^{76,83}

Table 6.3. Time-domain analysis results and calculated vibrational frequencies of the phenoxyl radical produced from photoionization of deuterated phenol (PhOD) in aqueous solution and their assignments. The calculated frequencies are based on the equilibrium geometry of the microhydrated phenoxyl radical. The assignments are based on a previous study.⁸⁴

Experimental				Calc. Freq. (cm ⁻¹)	Assignment
Freq. (cm ⁻¹)	Amplitude (meV)	Damping Time (ps)	Phase (π -rad)		
528 $\pm$ 1	0.21 $\pm$ 0.01	0.79 $\pm$ 0.04	1.51 $\pm$ 0.01	541	CCC bend
809 $\pm$ 1	0.32 $\pm$ 0.01	1.51 $\pm$ 0.07	0.18 $\pm$ 0.01	818	Ring breath / CCC bend
996 $\pm$ 1	0.76 $\pm$ 0.03	0.42 $\pm$ 0.02	0.37 $\pm$ 0.02	988	CCC trigonal bend
1001 $\pm$ 1	0.35 $\pm$ 0.01	3.01 $\pm$ 0.06	0.23 $\pm$ 0.01	1006	Ring breath / CH bend
1026 $\pm$ 1	0.22 $\pm$ 0.12	1.23 $\pm$ 0.11	0.24 $\pm$ 0.02	1007	HCCH torsion
1253 $\pm$ 2	0.18 $\pm$ 0.02	0.48 $\pm$ 0.06	0.41 $\pm$ 0.04	1295	CC str / CH bend
839 $\pm$ 4	0.62 $\pm$ 0.10	0.14 $\pm$ 0.02	1.58 $\pm$ 0.05	870	OD Wag*
1166 $\pm$ 6	2.55 $\pm$ 0.46	0.07 $\pm$ 0.01	0.69 $\pm$ 0.06	1170	OD Bend*
2373 $\pm$ 1	21.87 $\pm$ 0.88	0.07 $\pm$ 0.01	1.86 $\pm$ 0.02		OD Stretch from
2498 $\pm$ 3	19.65 $\pm$ 1.65	0.06 $\pm$ 0.01	1.66 $\pm$ 0.02		D ₂ O
1553 $\pm$ 1	0.15 $\pm$ 0.01	0.54 $\pm$ 0.06	1.10 $\pm$ 0.03		Oxygen
2330 $\pm$ 1	0.07 $\pm$ 0.01	5.52 $\pm$ 0.40	1.30 $\pm$ 0.01		Nitrogen

* Vibrational mode of PhOD^{•+}.

Fitting $\Delta A_1(\lambda)$ to the reported molar extinction coefficients of the hydrated electron yields an initial concentration of 29 ± 4 mM (Fig. 6.9(a)) for hydrated electrons produced from the ionization of aqueous phenol. Repeating the same analysis for the pure liquid water sample, strong-field-ionized under experimental conditions identical to those of aqueous phenol, yields an initial hydrated electron concentration of 26 ± 4 mM (Fig. 6.9(b)). Since hydrated electrons

Figure 6.9. Plot of ΔA_1 as a function of molar extinction coefficient of hydrated electron, and the linear fit function, in red for **(a)** ionized aqueous phenol and **(b)** ionized liquid water. The gradient of the linear fit function corresponds to the product of the concentration of hydrated electron, c_e and the sample path length, L , which are $(2.056 \pm 0.007) \times 10^{-5}$ M cm and $(1.838 \pm 0.006) \times 10^{-5}$ M cm respectively.

can be produced from the aqueous phenol sample by the ionization of both the phenol solute and the water solvent, the difference between the two concentrations corresponds to the concentration of the ionized phenol solute, $c_0(\text{PhOH}^{\bullet+}) \sim 3$ mM. From this concentration, we obtain an ionization fraction of $\sim 2\%$.

Aside from τ_{r1} and τ_{d1} , global fitting also uncovers an additional time constant, τ_{d2} , for ionized aqueous phenol. The corresponding decay associated difference spectrum, $\Delta A_2(\lambda)$, exhibits negative peaks at 384 nm and 399 nm (Fig. 6.5(c)), which coincide with the absorption maxima of the $\text{PhO}^{\bullet}$ radical. The appearance of $\Delta A_2(\lambda)$, with negative peaks superimposed on a broad, positive feature skewed towards the long-wavelength side, implies the growth of the $\text{PhO}^{\bullet}$ radical absorption peaks and the decrease of the long-wavelength ΔA signal with time. Such a spectral evolution is suggestive of vibrational relaxation of the $\text{PhO}^{\bullet}$ radical. As such, we tentatively assign τ_{d2} to the timescale for vibrational relaxation. This timescale is

comparable to $\tau_{d2} = 0.93 \pm 0.02$ ps recently reported for the related tyrosyl radical anion, produced by the sub-6-fs photodetachment of the tyrosinate dianion.⁷⁶

It is interesting to compare the vibrational relaxation timescales, τ_{d2} , for PhO• produced *via* two different routes: (1) the photoionization of phenol and (2) the photodetachment of phenoxide. The latter was the subject of a recent study,⁸⁴ employing the data analysis procedure outlined here gives a vibrational relaxation time of 1.02 ± 0.03 ps. By comparison, the vibrational relaxation of PhO• radical produced *via* the photoionization of aqueous phenol is $\sim 2\times$ slower. The difference in the vibrational relaxation times reflects the different precursors to PhO• formation for the two vibrational relaxation pathways. In the case of pathway 1, the Franck-Condon region corresponds to the equilibrium geometry of aqueous PhOH, whereas the Franck-Condon region for pathway 2 corresponds to the equilibrium geometry of the PhO⁻ anion. *Ab initio* calculations of the equilibrium geometries of PhOH, PhO⁻, and PhO• show that pathway 1 is accompanied by a significantly larger extent of structural rearrangement than pathway 2 (Table 6.4). For example, the contraction of the C–O bond length increases by a factor of $\sim 2\times$ on going from pathway 2 ($\Delta R = -5.8$ pm) to pathway 1 ($\Delta R = -11.0$ pm). It is conceivable that the larger extent of structural rearrangement leads to slower vibrational relaxation dynamics.

Beyond the negative features located at 384 nm and 399 nm, the $\Delta A_2(\lambda)$ spectrum also exhibits dips at 419 nm and 441 nm. The constant energy spacing of 1135 ± 34 cm⁻¹ between the four features suggests that they arise from a single vibronic progression (Fig. 6.10). The energy spacing is consistent with the C–H bend frequency of PhO• (mode 9a in the Wilson notation),^{84,85} previously observed at 1162 cm⁻¹ for aqueous PhO•. The dips at 419 nm and 441 nm appear in an overall positive region of the $\Delta A_2(\lambda)$ spectrum, thus implying a decay, albeit with reduced decay amplitude. It should be noted that the ${}^2B_1 \leftarrow {}^2B_1$ absorption

Table 6.4. Calculated bond lengths of phenol, phenoxide, and the phenoxyl radical. The differences in bond lengths between PhOH and PhO•, and between PhO[−] and PhO•, reflect the extent of structural rearrangement that are associated with pathways 1 and 2, respectively.

Bond	Bond Length / pm			Difference / pm	
	Phenol (A)	Phenoxide Anion (B)	Phenoxyl Radical (C)	(A) – (C)	(B) – (C)
C1 – C2	139.6	141.7	144.8	5.2	3.1
C2 – C3	139.5	139.3	137.2	-2.3	-2.1
C3 – C4	139.5	139.8	141.1	1.6	1.3
C4 – C5	139.6	139.8	141.1	1.5	1.3
C5 – C6	139.3	139.3	137.2	-2.1	-2.1
C1 – C6	139.6	141.7	144.8	5.2	3.1
C1 – O7	137.8	132.6	126.8	-11	-5.8

spectrum of the vibrationally cold PhO• radical does not exhibit peaks at 419 nm and 441 nm; as such, their appearance in the $\Delta A_2(\lambda)$ spectrum suggests that they are vibrationally hot bands, consistent with the PhO• radical being produced in a vibrationally excited state from the PhOH•⁺ precursor.

According to Eq. 6.3, the offset spectrum, $\Delta A_0(\lambda)$, in principle represents the ΔA spectrum recorded at 0 fs time delay. However, since global fitting is performed from 100-fs time delay onwards, it is more appropriate to consider $\Delta A_0(\lambda)$ as the ΔA spectrum recorded in the first ~100 fs. The appearance of spectral signatures of the PhO• radical absorption (at 383 nm and 400 nm) in $\Delta A_0(\lambda)$ therefore suggests that proton transfer from PhOH•⁺ occurs on the ~100-fs timescale. The PhO• radical absorption sits atop an offset absorption that extends beyond the maximum wavelength of 450 nm probed in the experiments. This offset could be due to a combination of the absorption of the vibrationally hot PhO• radical and the PhOH•⁺

Figure 6.10. A linear fit to the transition energies of the vibronic bands reveals a spacing of $1135 \pm 34 \text{ cm}^{-1}$ for the vibronic progression.

radical cation. We note that the absorption at 425 nm that was previously attributed to $\text{PhOH}\cdot^+$ is not observed in $\Delta A_0(\lambda)$.⁷⁰ Aqueous $\text{PhOH}\cdot^+$ is expected to yield a ΔA signal of $\sim 4 \times 10^{-3}$ peaked at 419 nm, based on the concentration of ionized PhOH, $c_0(\text{PhOH}\cdot^+) \sim 3 \text{ mM}$, and both the molar extinction coefficient and the position of the absorption maximum of $\text{PhOH}\cdot^+$ in concentrated aqueous acid ($\epsilon_{\text{max}} = 1800 \pm 150 \text{ M}^{-1} \text{ cm}^{-1}$ at 419 nm). Although the peak at 419 nm in $\Delta A_0(\lambda)$ more likely originates from a hot band transition of the $\text{PhO}\cdot$ radical – it is too narrow to be attributed to the broad differential absorption expected for the $\text{PhOH}\cdot^+$ radical cation – we cannot exclude the possibility of an underlying, broad absorption due to the $\text{PhOH}\cdot^+$ radical cation.

6.4.2. Vibrational Wave Packet Dynamics of Ionized Phenol

The vibrational modes observed from the spectral first-moment time trace would only be observed when, for a given vibrational mode, there is a displacement between the potential energy curves of the phenol and the phenol radical cation. From the time-domain analysis, while most of the vibrational frequencies have dephasing times on the order of a few hundred femtoseconds to a few picoseconds, some vibrational frequencies exhibit dephasing times of ~ 0.1 ps (Tables 6.2 and 6.3). With the aid of *ab initio* calculations (see below), we assign these short-lived vibrational frequencies to the phenolic O–H (O–D) bend and wag modes of the $\text{PhOH}\cdot^+$ ($\text{PhOD}\cdot^+$) radical cation (see below). We note that time-domain fitting does not yield the O–H and O–D stretching modes, predicted to be at 2593 cm^{-1} and 1909 cm^{-1} , respectively. This peculiarity will be addressed below by examining the *ab initio* PES for proton transfer. Nevertheless, the ultrafast dephasing of the O–H and O–D bend and wag modes suggests that the $\text{PhOH}\cdot^+$ and $\text{PhOD}\cdot^+$ radical cations exhibit ultrafast proton transfer, which leads to their rapid depopulation, and hence, the rapid loss of vibrational coherence. The detection of vibrational coherences from transient species whose differential absorption signatures are not apparent is remarkable but not unprecedented – we recently demonstrated the possibility of resolving the vibrational wave packet dynamics of the tryptophan radical, prepared by the strong-field photodetachment of the aqueous tryptophan anion, even though its absorption signature in the visible is obscured by the strong absorption band of the hydrated electron byproduct.⁷⁷

Interestingly, time-domain analysis of ionized PhOD reveals vibrational frequencies at $2373 \pm 1\text{ cm}^{-1}$ and $2498 \pm 2\text{ cm}^{-1}$, with comparable ultrafast dephasing times of 0.07 ± 0.01 ps

and 0.06 ± 0.01 ps, respectively. In the FFT power spectrum (Fig. 6.6(d)), the 2373-cm^{-1} component appears as a peak whereas the 2498-cm^{-1} component appears as a dip, suggesting that these two frequency components do not oscillate in phase. The O–D stretch frequency for aqueous neutral PhOD⁸⁶ of 2670 cm^{-1} exclude the possibility that these frequency components are produced *via* ISRS of aqueous neutral PhOD. Strong-field ionization of pure liquid D₂O, however, yields an O–D stretch frequency of $2370 \pm 2\text{ cm}^{-1}$ in both the time-domain analysis (Fig. 6.11(a)) and the FFT power spectrum (Fig. 6.11(b)). This result suggests that the 2373-cm^{-1} component arises from the bulk D₂O solvent response,⁸⁷ whereas the 2498-cm^{-1} component is associated with the ionization of aqueous PhOD. The origin of the latter frequency requires further investigation. Here, we tentatively assign it to the O–D stretch of the D₂O molecules that are hydrogen-bonded to the oxygen lone pairs of PhOD. Upon ionization to give PhOD^{•+} radical cation, the weakened intermolecular O_{Ph}···D₂O hydrogen bond results in a blueshift of the intramolecular O–D stretch frequency of bulk D₂O. Further support for this assignment comes from the ultrashort dephasing time of the 2498-cm^{-1} component, suggesting that it is associated with the solvent, albeit not in the bulk. Its observation suggests that the primary hydration shell of ionized molecules in aqueous solution also could undergo intramolecular structural rearrangement.

Following our tentative assignment of the vibrational frequencies listed in Tables 6.1, 6.2 and 6.3, we seek to estimate the lifetimes of the transient PhOH^{•+} and PhOD^{•+} radical cations from the measured dephasing times. Within the optical Bloch picture for a quantum beat involving states $|a\rangle$ and $|b\rangle$, the dephasing time T_2 is related to the population lifetimes of the individual states, T_{1a} and T_{1b} , and the pure dephasing time, T_2^* , *via* the relation⁸⁸

$$\frac{1}{T_2} = \frac{1}{2} \left(\frac{1}{T_{1a}} + \frac{1}{T_{1b}} \right) + \frac{1}{T_2^*}. \quad (6.6)$$

Figure 6.11. (a) First-moment, $\langle E(t) \rangle$, time trace of the ionized liquid D₂O. The inset shows the enlarged $\langle E(t) \rangle$ from 0.2 ps to 1 ps. **(b)** FFT power spectrum of the $\langle E(t) \rangle$ trace of ionized liquid D₂O. Note that the oxygen and nitrogen vibrational frequencies are also observed at 1552 cm⁻¹ and 2334 cm⁻¹, respectively.

In the present context, $|a\rangle$ and $|b\rangle$ correspond to the vibrational ground and excited states of the vibrational modes of phenol radical cation with dominant OH or OD character. The vibrational coherent superposition leads to wave packet motion involving the phenolic OH or OD bond. These vibrational states undergo depopulation *via* proton transfer; in addition, the vibrationally excited state can also undergo intramolecular vibrational relaxation (IVR), i.e., $1/T_{1a} = 1/\tau_{PT,a}$ and $1/T_{1b} = 1/\tau_{PT,b} + 1/\tau_{IVR}$, where $\tau_{PT,i}$ is the proton transfer timescale for state $|i\rangle$ and τ_{IVR} is the IVR timescale (Fig. 6.12). Assuming that the proton transfer timescale is independent of vibrational state, i.e., $\tau_{PT,a} \sim \tau_{PT,b} \sim \tau_{PT}$, we obtain

$$\frac{1}{\tau_{PT}} = \frac{1}{T_2} - \frac{1}{T_2^*} - \frac{1}{2\tau_{IVR}}. \quad (6.7)$$

The above expression suggests that the measured damping time T_2 represents a lower bound for τ_{PT} . Based on the T_2 values in Tables 6.2 and 6.3, we infer $\tau_{PT}^{(H)} > 0.18 \pm 0.02$ ps and $\tau_{PT}^{(D)} > 0.14 \pm 0.02$ ps for PhOH^{•+} and PhOD^{•+}, respectively. Given typical timescales for pure

Figure 6.12. Schematic energy level diagram of phenol (PhOH), phenol radical cation ($\text{PhOH}^{\bullet+}$) and phenoxy radical ($\text{PhO}^{\bullet}$). $|a\rangle$ and $|b\rangle$ correspond to the vibrational ground and excited states of the vibrational modes of phenol radical cation with dominant OH or OD character. These vibrational states can undergo depopulation via proton transfer (PT), while the vibrationally excited state can also undergo intramolecular vibrational relaxation (IVR).

dephasing and intramolecular vibrational relaxation of the order of $\gtrsim 1$ ps,^{89,90} we expect the measured dephasing times to closely approach the time constant for proton transfer, i.e., $T_2 \sim \tau_{\text{PT}}$. In this case, the timescale $\tau_{\text{PT}} \sim 0.2$ ps suggests that proton transfer cannot be the impulse for creating vibrational coherences in the $\text{PhO}^{\bullet}$ product because it does not meet the criterion $\tau_{\text{PT}} < 2\pi/\omega_i$, required to excite vibrational modes of frequency ω_i observed in the FFT power spectra (Figs. 6.6(b) and 6.6(d)).

6.4.3. Theoretical Calculations

To compute the vibrational frequencies of the phenol radical cation by *ab initio* simulations, we first construct a model of the phenol radical cation in its non-proton transferred geometry. For this, we employ the microhydrated $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_3$ model, comprising three water molecules directly hydrogen-bonded to the phenolic OH group, with one water molecule

serving as a hydrogen bond acceptor and two water molecules serving as hydrogen bond donors. This microhydrated model is further embedded in a polarizable continuum to mimic the dielectric environment of the water solvent. The optimized geometry of $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_3$ is shown in Fig. 6.13(b). The $\text{O}\cdots\text{O}$ distance between $\text{PhOH}^{\bullet+}$ and the hydrogen bond accepting H_2O , $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$, is 2.533 Å, whereas the $\text{O}\cdots\text{O}$ distance between $\text{PhOH}^{\bullet+}$ and the hydrogen bond donating H_2O molecules, $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{d}}$, is 3.269 Å. These $\text{O}\cdots\text{O}$ distances are consistent with those expected for hydrogen bonded systems.^{91,92} The phenol radical cation O–H bond length, $\text{O}_{\text{Ph}}-\text{H}$, is 1.030 Å, similar to $\text{O}_{\text{Ph}}-\text{H}$ of 0.987 Å for neutral $\text{PhOH}-(\text{H}_2\text{O})_3$ with the same hydrogen bonding configuration, confirming that proton transfer does not occur in $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_3$. This result is supported by the calculated potential energy curve along the $\text{O}_{\text{Ph}}-\text{H}$ coordinate (Fig. 6.14) and is also consistent with previous *ab initio* calculations and experiments performed on the gas-phase $\text{PhOH}^{\bullet+}-\text{H}_2\text{O}$, which does not exhibit proton transfer.

The absence of proton transfer in $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_3$ suggests its possible use as a model for the transient aqueous phenol radical cation. The equilibrium geometries of $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_3$ and $\text{PhOD}^{\bullet+}-(\text{D}_2\text{O})_3$, along with those obtained for isolated $\text{PhOH}^{\bullet+}$ and $\text{PhOD}^{\bullet+}$, in all cases with PCM solvation, are shown in Fig. 6.13 and the vibrational frequencies summarized in Appendix D. Comparison of the vibrational frequencies obtained for the microhydrated and isolated species suggests that explicit solvation can lead to large changes in the calculated vibrational frequencies. The vibrational frequencies that are unique to $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_3$ and $\text{PhOD}^{\bullet+}-(\text{D}_2\text{O})_3$, i.e., those associated with modes that exhibit significant displacement of the phenolic OH and OD bonds, are given in Tables 6.1, 6.2 and 6.3. The O–H (O–D) bend and wag frequencies calculated for $\text{PhOH}^{\bullet+}-(\text{H}_2\text{O})_3$ ($\text{PhOD}^{\bullet+}-(\text{D}_2\text{O})_3$) are in good agreement with the frequencies of the short-lived vibrational modes obtained from time-domain analysis and assigned to the transient phenol radical cation. However, the O–H and O–D stretch frequencies,

Figure 6.13. The equilibrium geometries of (a) the phenol radical cation, (b) the microhydrated phenol radical cation, (c) the phenol-OD radical cation and (d) the microhydrated phenol-OD radical cation, with PCM solvation. The carbon, hydrogen and oxygen atoms are in gray, white and red respectively.

predicted to be at 2593 cm^{-1} and 1909 cm^{-1} , respectively, are notably missing from the experimental data.

The unobserved vibrational coherences of the O–H and O–D stretches of the phenol radical cation is indicative of the coupling of these modes to the reaction coordinate for proton transfer, in analogy with the previously observed quenching of coherences along vibrational modes that drive internal conversion,⁹³ intersystem crossing,⁹⁴ electron transfer,⁹⁵ and ESPT.¹⁹ To explore this possibility, we employ DFT to calculate the reduced PES for proton transfer.

Figure 6.14. Potential energy curve as a function of the phenol O_{ph}-H bond length for the microhydrated phenol radical cation, PhOH•⁺-(H₂O)₃.

Previous experimental and theoretical studies have shown that proton transfer occurs when the water molecule in PhOH•⁺-H₂O is further hydrogen-bonded to two hydrogen bond-accepting water molecules.^{32,33} In our work, we coordinate two water molecules to the hydrogen bond-accepting water molecule in PhOH•⁺-(H₂O)₃, yielding PhOH•⁺-(H₂O)₅. Its optimized geometry clearly reflects proton transfer (Fig. 6.15): the O_{ph}-H distance of 1.551 Å is elongated relative to 1.002 Å in neutral PhOH-(H₂O)₅. Using this model, we performed a 2D constrained geometry optimization along the O_{ph}-H and O_{ph}⋯O_a coordinates to generate a 2D PES. The choice of these coordinates is motivated by earlier studies of excess proton transport in water,^{96,97} which reveal the need to include both the O-H distance and the intermolecular proton donor-acceptor O⋯O distance in models of proton transfer. Moreover, an investigation of proton transfer in PhOH•⁺-NH₃ has shown that the modeling of ultrafast proton transfer must include changes in the intermolecular O⋯N distance.³⁶ The PES obtained from our 2D constrained geometry optimization is shown in Fig. 6.16(a), where the dashed line denotes the

Figure 6.15. The equilibrium geometry of the microhydrated phenol radical cation, $\text{PhOH}\cdot^+-(\text{H}_2\text{O})_5$.

minimum energy path. Fig. 6.16(b) shows the plot of the potential energy as a function of $\text{O}_{\text{Ph}}-\text{H}$ distance along the minimum energy path as well as a section of the PES where the $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ distance is fixed at that of the Franck-Condon region.

Constraining the $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ distance in $\text{PhOH}\cdot^+-(\text{H}_2\text{O})_5$ to that of the Franck-Condon region yields a stabilization energy of 0.265 eV for proton transfer, albeit with a 99-meV-high barrier (Fig. 6.16(b), dashed line). The minimum energy path, on the other hand, is barrierless and energetically downhill by 0.271 eV (Fig. 6.16(b), solid line). The contraction of the $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ distance enroute to proton transfer along the minimum energy path – from 2.617 Å at the Franck-Condon region to 2.367 Å, where the proton transfer is barrierless – is consistent with the results obtained from theoretical studies of excess proton transport in water.^{96,97} The coupling between $\text{O}_{\text{Ph}}-\text{H}$ elongation and the intermolecular $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ hindered translational mode implies an $\text{O}_{\text{Ph}}-\text{H}$ stretch frequency that changes with $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ distance, a condition that favors the ultrafast dephasing of vibrational wave packet motion along the $\text{O}_{\text{Ph}}-\text{H}$ stretch

Figure 6.16. (a) 2D PES for the PT of the microhydrated phenol radical cation, $\text{PhOH}\bullet^+-(\text{H}_2\text{O})_5$, as a function of $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ and $\text{O}_{\text{Ph}}-\text{H}$ distances. The locations of the Franck-Condon region and the proton transfer product are denoted “FC” and “PT”, respectively, and the connecting minimum energy path is represented by the dashed line. Potential energy curves of $\text{PhOH}\bullet^+-(\text{H}_2\text{O})_5$ as a function of $\text{O}_{\text{Ph}}-\text{H}$ distance along the minimum energy path (red solid line) and where the $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ distance is fixed at that of the Franck-Condon region (blue dashed line).

coordinate. Other features of the *ab initio* PES that facilitate the rapid loss of phase coherence include the highly anharmonic nature of the $\text{O}_{\text{Ph}}-\text{H}$ stretching mode itself, evident from comparing the potential energy curves of $\text{PhOH}\bullet^+-(\text{H}_2\text{O})_3$ (Fig. 6.14) and $\text{PhOH}\bullet^+-(\text{H}_2\text{O})_5$, as well as the large possible displacement of the wave packet along the $\text{O}_{\text{Ph}}-\text{H}$ coordinate, which enhances anharmonic coupling of the $\text{O}_{\text{Ph}}-\text{H}$ stretch to other vibrational modes. Taken together, these factors promote the ultrafast dephasing of the $\text{O}_{\text{Ph}}-\text{H}$ and $\text{O}_{\text{Ph}}-\text{D}$ stretch, thus possibly explaining the unobserved vibrational coherence of these modes.

Aside from its role in facilitating ultrafast dephasing, the concomitant contraction of the intermolecular $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ distance during $\text{O}_{\text{Ph}}-\text{H}$ elongation also imposes a lower limit on the time constant for proton transfer. Fig. 6.16(a) shows that the $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ distance oscillates by

approximately one period as the reaction proceeds from the Franck-Condon region (2.617 Å) to the proton-transferred form (2.565 Å) along the minimum-energy path. According to *ab initio* simulations of the $\text{PhOH}\bullet^+(\text{H}_2\text{O})_3$ model, the vibrational frequency of the $\text{O}_{\text{Ph}}\text{--O}_{\text{a}}$ hindered translation mode is 207 cm^{-1} (Fig. 6.17). The corresponding vibrational period of 161 fs is therefore expected to dictate the minimum timescale on which proton transfer occurs, consistent with the experimentally determined $\tau_{\text{PT}} \sim 0.2\text{ ps}$.

We perform wave packet trajectory calculations on the *ab initio* reduced PES to compute the theoretical lifetime of the phenol radical cation. The calculated vibrational probability densities at 0, 100, and 200 fs are shown in Figs. 6.18(a) – 6.18(c) as contour plots. The vibrational probability density at 0 fs (Fig. 6.18(a)) corresponds to the wave packet in the Franck-Condon region, obtained by diagonalizing the 2D reduced Hamiltonian on the $\text{PhOH}(\text{H}_2\text{O})_5$ potential and projecting it onto the $\text{PhOH}\bullet^+(\text{H}_2\text{O})_5$ radical cation surface. A secondary contour feature emerges at 100 fs (Fig. 6.18(b)), with $\text{O}_{\text{Ph}}\text{--H}$ distances increasing to $>1.5\text{ Å}$ and $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ distances decreasing to $<2.4\text{ Å}$, supporting the notion that proton transfer is accompanied by simultaneous contraction of the $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ intermolecular distance. Fig. 6.18(c) shows that the proton transferred form is the dominant species by 200 fs. To quantify the lifetime of the $\text{PhOH}\bullet^+$ radical cation, we determine the population in the Franck-Condon region as a function of time. The resultant time trace shows that the population in the Franck-Condon region decreases rapidly, reaching its $1/e$ value in 193 fs (Fig. 6.19). This suggests a lifetime of 193 fs for the aqueous $\text{PhOH}\bullet^+$ radical cation, consistent with our experimental results. Interestingly, inspection of the time-dependent population reveals a weak modulation with a frequency of 229 cm^{-1} (Fig. 6.19 inset), assigned to the vibrational frequency of the $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ intermolecular hindered translation mode, in good agreement with the frequency of 207 cm^{-1} obtained from *ab initio* vibrational frequency calculations.

Figure 6.17. Theoretical calculation reveals an intermolecular vibrational mode at 207 cm^{-1} , which reveals an $\text{O}_{\text{ph}}\cdots\text{O}_{\text{a}}$ contraction, that promotes the rapid proton transfer process of the phenol radical cation. Note that the blue arrows represent the direction and magnitude of the displacement of the atoms. The carbon, hydrogen and oxygen are represented in gray, white and red respectively.

6.5. Conclusion

We have employed femtosecond optical pump-probe spectroscopy to investigate the ultrafast dynamics of ionized aqueous phenol. Intense laser pulses of sub-6-fs duration are used to drive the strong-field multiphoton ionization of aqueous phenol *via* a four-photon process. Global analysis of the resultant time-resolved ΔA spectra performed from 100 fs onwards does not reveal a clear spectral signature for the $\text{PhOH}^{\bullet+}$ radical cation ionization product. Instead, the absorption signature of $\text{PhO}^{\bullet}$ radical dominates the ΔA spectra, indicating the rapid

Figure 6.18. Ab initio quantum dynamics simulations of proton transfer in aqueous $\text{PhOH}\bullet^+$ radical cation yields the time-dependent vibrational probability density at time delays of **(a)** 0 fs, **(b)** 100 fs, and **(c)** 200 fs

depopulation of the $\text{PhOH}\bullet^+$ radical cation by proton transfer. Time-domain analysis of the vibrational wave packet dynamics, however, reveals distinct vibrational frequencies that can be assigned to the O–H wag and bend modes of $\text{PhOH}\bullet^+$ radical cation. These assignments are supported by *ab initio* vibrational frequency calculations and isotopic substitution experiments performed on ionized aqueous PhOD in D_2O . From the dephasing times of these vibrational frequencies, we obtain an estimated lifetime of ~ 0.2 ps for the $\text{PhOH}\bullet^+$ radical cation. The reduced potential energy surface obtained from *ab initio* calculations suggests that proton

Figure 6.19. Calculated population dynamics of aqueous $\text{PhOH}\bullet^+$ radical cation, obtained from *ab initio* quantum dynamics simulations. The population decays to a $1/e$ value in 193 fs. The inset shows the FFT power spectrum, which reveals an oscillatory frequency of 229 cm^{-1} .

transfer is coupled to the intermolecular $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ hindered translation between $\text{PhOH}\bullet^+$ and the neighboring proton-accepting water molecule. Contraction of the $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$ distance leads to barrierless proton transfer, analogous to excess proton transport in water. As such, the frequency of this intermolecular motion, found to be 207 cm^{-1} according to *ab initio* calculations, dictates the timescale for proton transfer and is consistent with $\tau_{\text{PT}} \sim 0.2\text{ ps}$. This timescale is supported by wave packet trajectory calculations, performed on the *ab initio* reduced potential energy surface, which yield a $\text{PhOH}\bullet^+$ lifetime of 193 fs. Within the context of radiation damage induced by ionizing radiation, ultrafast proton transfer is an effective channel for diminishing the chemical reactivity of $\text{PhOH}\bullet^+$ because the PT product, $\text{PhO}\bullet$, is a weaker oxidant [$E_{\text{red}}^0(\text{PhO}\bullet) = 0.86\text{ V}$ vs. $E_{\text{red}}^0(\text{PhOH}\bullet^+) = 1.6\text{ V}$; both potentials referenced to NHE].⁹⁸

It is instructive to compare the ultrafast proton transfer observed herein with intermolecular ESPTS. Previous extensive investigations of intermolecular ESPTS have correlated the timescale for proton transfer with pK_a^* .²⁴ The strongest photoacids have $pK_a^* < -7$ and exhibit $\tau_{PT} \sim 100$ fs, limited by the frequency of the intermolecular vibration between the proton donor and proton acceptor. Consistent with this classification, time-resolved fluorescence upconversion performed on the recently discovered superphotoacid, quinone cyanine 9 (QCy9), with a pK_a^* of -8.5 , revealed proton transfer occurring on the ~ 100 -fs timescale;²⁵ subsequent measurements with improved time resolution elucidated a biphasic proton transfer process, characterized by time constants of ~ 40 fs and ~ 200 fs.²⁸ In the spirit of correlating the pK_a to the timescale for proton transfer, we note that a recent study of the photodetached tryptophan anion revealed that the indole radical cation sidechain, with a pK_a of 4.2 ,⁹⁹⁻¹⁰¹ is stable with respect to PT on the few-picosecond timescale. In comparison, the phenol radical cation has a significantly lower pK_a of -2.75 .⁷² Our study reveals that $\text{PhOH}^{\bullet+}$ radical cation exhibits the same intermolecular vibration-limited ultrafast proton transfer as QCy9, which in turn suggests the strong-field ionization of weak acids, such as PhOH, as a possible approach to generating strong acids that undergo ultrafast proton transfer.

References

1. Wang, J. H., Facilitated Proton Transfer in Enzyme Catalysis. *Science* **1968**, *161* (3839), 328-334.
2. Schowen, K. B.; Limbach, H. H.; Denisov, G. S.; Schowen, R. L., Hydrogen Bonds and Proton Transfer in General-catalytic Transition-state Stabilization in Enzyme Catalysis. *Biochim. Biophys. Acta Bioenerg.* **2000**, *1458* (1), 43-62.
3. Mitchell, P., Coupling of Phosphorylation to Electron and Hydrogen Transfer by a Chemi-Osmotic type of Mechanism. *Nature* **1961**, *191* (4784), 144-148.
4. Cukier, R. I.; Nocera, D. G., Proton-Coupled Electron Transfer. *Annu. Rev. Phys. Chem.* **1998**, *49* (1), 337-369.
5. Huynh, M. H. V.; Meyer, T. J., Proton-Coupled Electron Transfer. *Chem. Rev.* **2007**, *107* (11), 5004-5064.
6. Reece, S. Y.; Nocera, D. G., Proton-Coupled Electron Transfer in Biology: Results from Synergistic Studies in Natural and Model Systems. *Annu. Rev. Biochem.* **2009**, *78* (1), 673-699.
7. Weinberg, D. R.; Gagliardi, C. J.; Hull, J. F.; Murphy, C. F.; Kent, C. A.; Westlake, B. C.; Paul, A.; Ess, D. H.; McCafferty, D. G.; Meyer, T. J., Proton-Coupled Electron Transfer. *Chem. Rev.* **2012**, *112* (7), 4016-4093.
8. Erez, Y.; Huppert, D., Excited-State Intermolecular Proton Transfer of the Firefly's Chromophore D-Luciferin. *J. Phys. Chem. A* **2010**, *114* (31), 8075-8082.
9. Presiado, I.; Erez, Y.; Simkovitch, R.; Shomer, S.; Gepshtein, R.; Pinto da Silva, L.; Esteves da Silva, J. C. G.; Huppert, D., Excited-State Proton Transfer of Firefly Dehydroluciferin. *J. Phys. Chem. A* **2012**, *116* (44), 10770-10779.

10. Gosset, P.; Taupier, G.; Crégut, O.; Brazard, J.; Mély, Y.; Dorkenoo, K.-D.; Léonard, J.; Didier, P., Excited-State Proton Transfer in Oxyluciferin and Its Analogues. *J. Phys. Chem. Lett.* **2020**, *11* (9), 3653-3659.
11. Stoner-Ma, D.; Jaye, A. A.; Matousek, P.; Towrie, M.; Meech, S. R.; Tonge, P. J., Observation of Excited-State Proton Transfer in Green Fluorescent Protein using Ultrafast Vibrational Spectroscopy. *J. Am. Chem. Soc.* **2005**, *127* (9), 2864-2865.
12. Fang, C.; Frontiera, R. R.; Tran, R.; Mathies, R. A., Mapping GFP Structure Evolution during Proton Transfer with Femtosecond Raman Spectroscopy. *Nature* **2009**, *462* (7270), 200-204.
13. Chen, Y.-H.; Sung, R.; Sung, K., Insights into Excited State Intramolecular Proton Transfer: An Alternative Model for Excited State Proton Transfer of Green Fluorescence Protein. *J. Phys. Chem. A* **2018**, *122* (28), 5931-5944.
14. Tarkka, R. M.; Jenekhe, S. A., Excited-State Intramolecular Proton Transfer in Polymers. *MRS Online Proceedings Library* **1995**, *413* (1), 97-102.
15. Jagadesan, P.; Eder, G.; McGrier, P. L., The Excited-State Intramolecular Proton Transfer Properties of Three Imine-linked Two-Dimensional Porous Organic Polymers. *J. Mater. Chem. C* **2017**, *5* (23), 5676-5679.
16. Yeo, H.; Khan, A., Photoinduced Proton-Transfer Polymerization: A Practical Synthetic Tool for Soft Lithography Applications. *J. Am. Chem. Soc.* **2020**, *142* (7), 3479-3488.
17. Kosower E. M.; Huppert, D., Excited State Electron and Proton Transfers. *Annu. Rev. Phys. Chem.* **1986**, *37* (1), 127-156.
18. Tolbert, L. M.; Solntsev, K. M., Excited-State Proton Transfer: From Constrained Systems to “Super” Photoacids to Superfast Proton Transfer. *Acc. Chem. Res.* **2002**, *35* (1), 19-27.

19. Lee, J.; Kim, C. H.; Joo, T., Active Role of Proton in Excited State Intramolecular Proton Transfer Reaction. *J. Phys. Chem. A* **2013**, *117* (7), 1400-1405.
20. Takeuchi, S.; Tahara, T., Coherent Nuclear Wavepacket Motions in Ultrafast Excited-State Intramolecular Proton Transfer: Sub-30-fs Resolved Pump–Probe Absorption Spectroscopy of 10-Hydroxybenzo[h]quinoline in Solution. *J. Phys. Chem. A* **2005**, *109* (45), 10199-10207.
21. Heo, W.; Uddin, N.; Park, J. W.; Rhee, Y. M.; Choi, C. H.; Joo, T., Coherent Intermolecular Proton Transfer in the Acid–Base Reaction of Excited State Pyranine. *Phys. Chem. Chem. Phys.* **2017**, *19* (28), 18243-18251.
22. Lennox, J. C.; Kurtz, D. A.; Huang, T.; Dempsey, J. L., Excited-State Proton-Coupled Electron Transfer: Different Avenues for Promoting Proton/Electron Movement with Solar Photons. *ACS Energy Letters* **2017**, *2* (5), 1246-1256.
23. Zhou, P.; Han, K., Unraveling the Detailed Mechanism of Excited-State Proton Transfer. *Acc. Chem. Res.* **2018**, *51* (7), 1681-1690.
24. Simkovitch, R.; Shomer, S.; Gepshtein, R.; Huppert, D., How Fast Can a Proton-Transfer Reaction Be beyond the Solvent-Control Limit? *J. Phys. Chem. B* **2015**, *119* (6), 2253-2262.
25. Simkovitch, R.; Karton-Lifshin, N.; Shomer, S.; Shabat, D.; Huppert, D., Ultrafast Excited-State Proton Transfer to the Solvent Occurs on a Hundred-Femtosecond Time-Scale. *J. Phys. Chem. A* **2013**, *117* (16), 3405-3413.
26. Simkovitch, R.; Shomer, S.; Gepshtein, R.; Roth, M. E.; Shabat, D.; Huppert, D., Comparison of the Rate of Excited-State Proton Transfer from Photoacids to Alcohols and Water. *J. Photochem. Photobiol. A* **2014**, *277*, 90-101.

27. Simkovitch, R.; Shomer, S.; Gepshtein, R.; Shabat, D.; Huppert, D., Excited-State Proton Transfer from Quinone-Cyanine 9 to Protic Polar-Solvent Mixtures. *J. Phys. Chem. A* **2014**, *118* (10), 1832-1840.
28. Lee, C.; Chung, S.; Song, H.; Rhee, Y. M.; Lee, E.; Joo, T., Excited State Proton Transfer of Quinone Cyanine 9: Implications on the Origin of Super-Photoacidity. *ChemPhotoChem* **2021**, *5* (3), 245-252.
29. Kim, S. K.; Li, S.; Bernstein, E. R., Excited State Intermolecular Proton Transfer in Isolated Clusters: 1-naphthol/ammonia and Water. *J. Chem. Phys.* **1991**, *95* (5), 3119-3128.
30. Stanley, R. J.; Jr., A. W. C., Cluster Ion Dip Spectroscopy of Hydrogen Bonded Phenol(H₂O)_n clusters, n = 0–4. *J. Chem. Phys.* **1991**, *94* (12), 7744-7756.
31. Sato, S.; Mikami, N., Size Dependence of Intracuster Proton Transfer of Phenol–(H₂O)_n (n = 1–4) Cations. *J. Phys. Chem.* **1996**, *100* (12), 4765-4769.
32. Sawamura, T.; Fujii, A.; Sato, S.; Ebata, T.; Mikami, N., Characterization of the Hydrogen-Bonded Cluster Ions [Phenol–(H₂O)_n]⁺ (n = 1–4), (Phenol)₂⁺, and (Phenol–Methanol)⁺ As Studied by Trapped Ion Infrared Multiphoton Dissociation Spectroscopy of Their OH Stretching Vibrations. *J. Phys. Chem.* **1996**, *100* (20), 8131-8138.
33. Kleinerhmanns, K.; Janzen, C.; Spangenberg, D.; Gerhards, M., Infrared Spectroscopy of Resonantly Ionized (Phenol)(H₂O)_n⁺. *J. Phys. Chem. A* **1999**, *103* (27), 5232-5239.
34. Ishikawa, H.; Kurusu, I.; Yagi, R.; Kato, R.; Kasahara, Y., Quantitative Temperature Dependence of the Microscopic Hydration Structures Investigated by Ultraviolet Photodissociation Spectroscopy of Hydrated Phenol Cations. *J. Phys. Chem. Lett.* **2017**, *8* (11), 2541-2546.

35. Shen, C.-C.; Tsai, T.-T.; Ho, J.-W.; Chen, Y.-W.; Cheng, P.-Y., Communication: Ultrafast Time-Resolved Ion Photofragmentation Spectroscopy of Photoionization-Induced Proton Transfer in Phenol-Ammonia Complex. *J. Chem. Phys.* **2014**, *141* (17), 171103.
36. Shen, C.-C.; Tsai, T.-T.; Wu, J.-Y.; Ho, J.-W.; Chen, Y.-W.; Cheng, P.-Y., Watching Proton Transfer in Real Time: Ultrafast Photoionization-Induced Proton Transfer in Phenol-Ammonia Complex Cation. *J. Chem. Phys.* **2017**, *147* (16), 164302.
37. Loh, Z.-H.; Doumy, G.; Arnold, C.; Kjellsson, L.; Southworth, S. H.; Haddad, A. A.; Kumagai, Y.; Tu, M.-F.; Ho, P. J.; March, A. M.; Schaller, R. D.; Yusof, M. S. B. M.; Debnath, T.; Simon, M.; Welsch, R.; Inhester, L.; Khalili, K.; Nanda, K.; Krylov, A. I.; Moeller, S.; Coslovich, G.; Koralek, J.; Minitti, M. P.; Schlotter, W. F.; Rubensson, J.-E.; Santra, R.; Young, L., Observation of the Fastest Chemical Processes in the Radiolysis of Water. *Science* **2020**, *367* (6474), 179-182.
38. Ambrosio, F.; Pasquarello, A., Reactivity and Energy Level of a Localized Hole in Liquid Water. *Phys. Chem. Chem. Phys.* **2018**, *20* (48), 30281-30289.
39. Koppenol, W. H.; Liebman, J. F., The Oxidizing Nature of the Hydroxyl Radical. A Comparison with the Ferryl Ion (FeO^{2+}). *J. Phys. Chem.* **1984**, *88* (1), 99-101.
40. Bonin, J.; Robert, M., Photoinduced Proton-Coupled Electron Transfers in Biorelevant Phenolic Systems. *Photochem. Photobiol.* **2011**, *87* (6), 1190-1203.
41. Sjödin, M.; Styring, S.; Wolpher, H.; Xu, Y.; Sun, L.; Hammarström, L., Switching the Redox Mechanism: Models for Proton-Coupled Electron Transfer from Tyrosine and Tryptophan. *J. Am. Chem. Soc.* **2005**, *127* (11), 3855-3863.
42. Irebo, T.; Reece, S. Y.; Sjödin, M.; Nocera, D. G.; Hammarström, L., Proton-Coupled Electron Transfer of Tyrosine Oxidation: Buffer Dependence and Parallel Mechanisms. *J. Am. Chem. Soc.* **2007**, *129* (50), 15462-15464.

43. Defelippis, M. R.; Murthy, C. P.; Faraggi, M.; Klapper, M. H., Pulse Radiolytic Measurement of Redox Potentials: The Tyrosine and Tryptophan Radicals. *Biochemistry* **1989**, 28 (11), 4847-4853.
44. Sobolewski, A. L.; Domcke, W., Photoinduced Electron and Proton Transfer in Phenol and Its Clusters with Water and Ammonia. *J. Phys. Chem. A* **2001**, 105 (40), 9275-9283.
45. Schütz, M.; Bürgi, T.; Leutwyler, S.; Fischer, T., Intermolecular Bonding and Vibrations of Phenol·H₂O (D₂O). *J. Chem. Phys.* **1993**, 98 (5), 3763-3776.
46. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. *Gaussian 16 Rev. C.01*, Wallingford, CT, 2016.
47. Becke, A. D., Density-Functional Exchange-Energy Approximation with Correct Asymptotic Behavior. *Phys. Rev. A* **1988**, 38 (6), 3098-3100.
48. Lee, C.; Yang, W.; Parr, R. G., Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density. *Phys. Rev. B* **1988**, 37 (2), 785-789.

49. Tomasi, J.; Mennucci, B.; Cammi, R., Quantum Mechanical Continuum Solvation Models. *Chem. Rev.* **2005**, *105* (8), 2999-3094.
50. Hagan, M. T.; Menhaj, M. B., Training Feedforward Networks with the Marquardt Algorithm. *IEEE Trans. Neural Netw.* **1994**, *5* (6), 989-993.
51. Hu, Q.; Song, H.; Johnson, C. J.; Li, J.; Guo, H.; Continetti, R. E., Imaging a Multidimensional Multichannel Potential Energy surface: Photodetachment of $\text{H}^-(\text{NH}_3)$ and NH_4^- . *J. Chem. Phys.* **2016**, *144* (24), 244311.
52. Feit, M. D.; Fleck, J. A.; Steiger, A., Solution of the Schrödinger Equation by a Spectral Method. *J. Comput. Phys.* **1982**, *47* (3), 412-433.
53. *CRC Handbook of Chemistry and Physics*. 101st edition ed. ed.; Taylor & Francis Ltd.: London, United Kingdom, 2020.
54. Mora-Diez, N.; Egorova, Y.; Plommer, H.; Tremaine, P. R., Theoretical Study of Deuterium Isotope Effects on Acid–Base Equilibria under Ambient and Hydrothermal Conditions. *RSC Adv.* **2015**, *5* (12), 9097-9109.
55. Lozovoy, V. V.; Pastirk, I.; Dantus, M., Multiphoton Intrapulse Interference. IV. Ultrashort Laser Pulse Spectral Phase Characterization and Compensation. *Opt. Lett.* **2004**, *29* (7), 775-777.
56. Pestov, D.; Lozovoy, V. V.; Dantus, M., Multiple Independent Comb Shaping (MICS): Phase-Only Generation of Optical Pulse Sequences. *Opt. Express* **2009**, *17* (16), 14351-14361.
57. Ambrosio, F.; Guo, Z.; Pasquarello, A., Absolute Energy Levels of Liquid Water. *J. Phys. Chem. Lett.* **2018**, *9* (12), 3212-3216.
58. Roberts, G. M.; Chatterley, A. S.; Young, J. D.; Stavros, V. G., Direct Observation of Hydrogen Tunneling Dynamics in Photoexcited Phenol. *J. Phys. Chem. Lett.* **2012**, *3* (3), 348-352.

59. Ayuso, D.; Kimura, M.; Kooser, K.; Patanen, M.; Plésiat, E.; Argenti, L.; Mondal, S.; Travnikova, O.; Sakai, K.; Palacios, A.; Kukk, E.; Decleva, P.; Ueda, K.; Martín, F.; Miron, C., Vibrationally Resolved B 1s Photoionization Cross Section of BF₃. *J. Phys. Chem. A* **2015**, *119* (23), 5971-5978.
60. Ghosh, D.; Roy, A.; Seidel, R.; Winter, B.; Bradforth, S.; Krylov, A. I., First-Principle Protocol for Calculating Ionization Energies and Redox Potentials of Solvated Molecules and Ions: Theory and Application to Aqueous Phenol and Phenolate. *J. Phys. Chem. B* **2012**, *116* (24), 7269-7280.
61. Ambrosio, F.; Miceli, G.; Pasquarello, A., Electronic Levels of Excess Electrons in Liquid Water. *J. Phys. Chem. Lett.* **2017**, *8* (9), 2055-2059.
62. Pépin, C.; Goulet, T.; Houde, D.; Jay-Gerin, J. P., Observation of a Continuous Spectral Shift in the Solvation Kinetics of Electrons in Neat Liquid Deuterated Water. *J. Phys. Chem. A* **1997**, *101* (24), 4351-4360.
63. Mostafavi, M.; Lampre, I., An Overview of Solvated Electrons: Recent Advances. In *Recent Trends in Radiation Chemistry*, pp 21-58.
64. Crowell, R. A.; Bartels, D. M., Multiphoton Ionization of Liquid Water with 3.0–5.0 eV Photons. *J. Phys. Chem.* **1996**, *100* (45), 17940-17949.
65. Silva, C.; Walhout, P. K.; Yokoyama, K.; Barbara, P. F., Femtosecond Solvation Dynamics of the Hydrated Electron. *Phys. Rev. Lett.* **1998**, *80* (5), 1086-1089.
66. Vilchiz, V. H.; Kloepper, J. A.; Germaine, A. C.; Lenchenkov, V. A.; Bradforth, S. E., Map for the Relaxation Dynamics of Hot Photoelectrons Injected into Liquid Water via Anion Threshold Photodetachment and above Threshold Solvent Ionization. *J. Phys. Chem. A* **2001**, *105* (10), 1711-1723.
67. Pshenichnikov, M. S.; Baltuška, A.; Wiersma, D. A., Hydrated-electron Population Dynamics. *Chem. Phys. Lett.* **2004**, *389* (1), 171-175.

68. Elkins, M. H.; Williams, H. L.; Shreve, A. T.; Neumark, D. M., Relaxation Mechanism of the Hydrated Electron. *Science* **2013**, *342* (6165), 1496-1499.
69. Chen, X.; Larsen, D. S.; Bradforth, S. E.; van Stokkum, I. H. M., Broadband Spectral Probing Revealing Ultrafast Photochemical Branching after Ultraviolet Excitation of the Aqueous Phenolate Anion. *J. Phys. Chem. A* **2011**, *115* (16), 3807-3819.
70. Oliver, T. A. A.; Zhang, Y.; Roy, A.; Ashfold, M. N. R.; Bradforth, S. E., Exploring Autoionization and Photoinduced Proton-Coupled Electron Transfer Pathways of Phenol in Aqueous Solution. *J. Phys. Chem. Lett.* **2015**, *6* (20), 4159-4164.
71. Ganapathi, M. R.; Hermann, R.; Naumov, S.; Brede, O., Free Electron Transfer from Several Phenols to Radical Cations of Non-Polar Solvents. *Phys. Chem. Chem. Phys.* **2000**, *2* (21), 4947-4955.
72. Das, T. N., Oxidation of Phenol in Aqueous Acid: Characterization and Reactions of Radical Cations vis-à-vis the Phenoxyl Radical. *J. Phys. Chem. A* **2005**, *109* (15), 3344-3351.
73. Torche, F.; Marignier, J.-L., Direct Evaluation of the Molar Absorption Coefficient of Hydrated Electron by the Isosbestic Point Method. *J. Phys. Chem. B* **2016**, *120* (29), 7201-7206.
74. Wei, Z.; Li, J.; Wang, L.; See, S. T.; Jhon, M. H.; Zhang, Y.; Shi, F.; Yang, M.; Loh, Z.-H., Elucidating the Origins of Multimode Vibrational Coherences of Polyatomic Molecules Induced by Intense Laser Fields. *Nat. Commun.* **2017**, *8* (1), 735.
75. Wei, Z.; Li, J.; Zhang, H.; Lu, Y.; Yang, M.; Loh, Z.-H., Ultrafast Dissociative Ionization and Large-Amplitude Vibrational Wave Packet Dynamics of Strong-Field-Ionized Diiodomethane. *J. Chem. Phys.* **2019**, *151* (21), 214308.

76. Bin Mohd Yusof, M. S.; Lim, Y. L.; Loh, Z.-H., Ultrafast Vibrational Wave Packet Dynamics of the Aqueous Tyrosyl Radical Anion Induced by Photodetachment. *Phys. Chem. Chem. Phys.* **2021**, 23 (34), 18525-18534.
77. Bin Mohd Yusof, M. S.; Debnath, T.; Loh, Z.-H., Observation of Intra- and Intermolecular Vibrational Coherences of the Aqueous Tryptophan Radical Induced by Photodetachment. *J. Chem. Phys.* **2021**, 155 (13), 134306.
78. Zhu, L.; Sage, J.; Champion, P., Observation of Coherent Reaction Dynamics in Heme Proteins. *Science* **1994**, 266 (5185), 629-632.
79. Zhu, L.; Widom, A.; Champion, P. M., A Multidimensional Landau-Zener Description of Chemical Reaction Dynamics and Vibrational Coherence. *J. Chem. Phys.* **1997**, 107 (8), 2859-2871.
80. Monni, R.; Capano, G.; Auböck, G.; Gray, H. B.; Vlček, A.; Tavernelli, I.; Chergui, M., Vibrational Coherence Transfer in the Ultrafast Intersystem Crossing of a Diplatinum Complex in Solution. *Proceedings of the National Academy of Sciences* **2018**, 115 (28), E6396-E6403.
81. Karak, P.; Ruud, K.; Chakrabarti, S., Demystifying the Origin of Vibrational Coherence Transfer Between the S1 and T1 States of the Pt-pop Complex. *J. Phys. Chem. Lett.* **2021**, 12 (40), 9768-9773.
82. Klopfer, J. A.; Vilchiz, V. H.; Lenchenkov, V. A.; Germaine, A. C.; Bradforth, S. E., The Ejection Distribution of Solvated Electrons Generated by the One-Photon Photodetachment of Aqueous I^- and Two-Photon Ionization of the Solvent. *J. Chem. Phys.* **2000**, 113 (15), 6288-6307.
83. Peon, J.; Hess, G. C.; Pecourt, J.-M. L.; Yuzawa, T.; Kohler, B., Ultrafast Photoionization Dynamics of Indole in Water. *J. Phys. Chem. A* **1999**, 103 (14), 2460-2466.

84. Debnath, T.; Mohd Yusof, M. S. B.; Low, P. J.; Loh, Z.-H., Ultrafast Structural Rearrangement Dynamics Induced by the Photodetachment of Phenoxide in Aqueous Solution. *Nat. Commun.* **2019**, *10* (1), 2944.
85. Wilson, E. B., The Normal Modes and Frequencies of Vibration of the Regular Plane Hexagon Model of the Benzene Molecule. *Phys. Rev.* **1934**, *45* (10), 706-714.
86. Evans, J. C., The Vibrational Spectra of Phenol and Phenol-OD. *Spectrochim. Acta* **1960**, *16* (11), 1382-1392.
87. Pattenaude, S. R.; Streacker, L. M.; Ben-Amotz, D., Temperature and Polarization Dependent Raman Spectra of Liquid H₂O and D₂O. *J. Raman Spectrosc.* **2018**, *49* (11), 1860-1866.
88. Demtröder, W., Laser Spectroscopy: Fourth Edition. *Laser Spectroscopy: Fourth Edition* **2008**, *1*, 1-457.
89. Dean, J. C.; Rafiq, S.; Oblinsky, D. G.; Cassette, E.; Jumper, C. C.; Scholes, G. D., Broadband Transient Absorption and Two-Dimensional Electronic Spectroscopy of Methylene Blue. *J. Phys. Chem. A* **2015**, *119* (34), 9098-9108.
90. Rafiq, S.; Scholes, G. D., Slow Intramolecular Vibrational Relaxation Leads to Long-Lived Excited-State Wavepackets. *J. Phys. Chem. A* **2016**, *120* (34), 6792-6799.
91. Jeffrey, G. A., *An Introduction to Hydrogen Bonding*. Oxford University Press: New York, 1997.
92. Gilli, P.; Pretto, L.; Bertolasi, V.; Gilli, G., Predicting Hydrogen-Bond Strengths from Acid–Base Molecular Properties. The pK_a Slide Rule: Toward the Solution of a Long-Lasting Problem. *Acc. Chem. Res.* **2009**, *42* (1), 33-44.
93. Lee, C.; Seo, K.; Kim, M.; Joo, T., Coherent Internal Conversion from High Lying Electronic States to S₁ in Boron-dipyrromethene Derivatives. *Phys. Chem. Chem. Phys.* **2021**, *23* (44), 25200-25209.

94. Kim, J.; Kang, D.-G.; Kim, S. K.; Joo, T., Role of Coherent Nuclear Motion in the Ultrafast Intersystem Crossing of Ruthenium Complexes. *Phys. Chem. Chem. Phys.* **2020**, 22 (44), 25811-25818.
95. Yoneda, Y.; Kudisch, B.; Rafiq, S.; Maiuri, M.; Nagasawa, Y.; Scholes, G. D.; Miyasaka, H., Vibrational Dephasing along the Reaction Coordinate of an Electron Transfer Reaction. *J. Am. Chem. Soc.* **2021**, 143 (36), 14511-14522.
96. Marx, D.; Tuckerman, M. E.; Hutter, J.; Parrinello, M., The Nature of the Hydrated Excess Proton in Water. *Nature* **1999**, 397 (6720), 601-604.
97. Marx, D., Proton Transfer 200 Years after von Grotthuss: Insights from *Ab Initio* Simulations. *ChemPhysChem* **2006**, 7 (9), 1848-1870.
98. Li, C.; Hoffman, M. Z., One-Electron Redox Potentials of Phenols in Aqueous Solution. *J. Phys. Chem. B* **1999**, 103 (32), 6653-6656.
99. Posener, M. L.; Adams, G. E.; Wardman, P.; Cundall, R. B., Mechanism of Tryptophan Oxidation by Some Inorganic Radical-Anions: A Pulse Radiolysis Study. *J. Chem. Soc., Faraday Trans. 1* **1976**, 72, 2231-2239.
100. Baugher, J. F.; Grossweiner, L. I., Photolysis Mechanism of Aqueous Tryptophan. *J. Phys. Chem.* **1977**, 81 (14), 1349-1354.
101. Solar, S.; Getoff, N.; Surdhar, P. S.; Armstrong, D. A.; Singh, A., Oxidation of Tryptophan and N-methylindole by $N_3^\bullet$, $Br_2^{\bullet-}$, and $(SCN)_2^{\bullet-}$ Radicals in Light- and Heavy-Water Solutions: A Pulse Radiolysis Study. *J. Phys. Chem.* **1991**, 95 (9), 3639-3643.

Chapter 7

Summary and Future Work

7.1. Summary

This thesis outlines the use of few-femtosecond transient absorption spectroscopy to resolve the ultrafast dynamics, such as vibrational wave packet dynamics, of ionized or photodetached biomolecules in aqueous solution, and utilizing theoretical calculations to complement the observed experimental results in our studies.

In Chapter 3, we study the vibrational wave packet dynamics of photodetached tyrosinate dianion in aqueous solution by utilizing few-femtosecond near-ultraviolet (UV) probe. The photodetachment process reveals seven unique vibrational modes that are coupled to the photodetachment process, and are assigned with the aid of density functional theory (DFT) calculations. Time-domain analysis is performed to reveal the amplitude, dephasing time and phase of each vibrational mode. Theoretical calculations reveal a few-picometer changes in the C–C and C–O bond lengths of the phenoxide sidechain of the tyrosinate dianion. These changes are comparable to the early study involving the photodetachment of aqueous phenoxide anion;¹ however, we do observe that in the presence of the amino acid backbone in the tyrosine radical anion, it changes the relative fast Fourier-transform (FFT) intensities and decreases the dephasing time of certain vibrational modes. Moreover, the DFT calculations of the microhydrated tyrosyl radical dianion and tyrosyl radical anion suggests that photodetachment is accompanied by the elongation of the hydrogen bonded O···O distances. This study provided the benchmark to study other larger biomolecules in aqueous solution.

In Chapter 4, we explore the vibrational wave packet dynamics of photodetached tryptophan anion in aqueous solution by utilizing both few-femtosecond UV probe and visible probe. With the UV probe, we are able to observe the absorption band at 334 nm, however, with the visible-NIR probe, the absorption band expected at 580 nm (ϵ_{Trp} at 580 nm = $3000 \text{ M}^{-1} \text{ cm}^{-1}$)^{2,11} is not observed as it is obfuscated by the absorption of the ground-state hydrated electron, which is $\sim 4\times$ (ϵ_{e^-} at 580 nm = $11792 \text{ M}^{-1} \text{ cm}^{-1}$)^{12,13} stronger than the absorption of tryptophan radical. Remarkably, we are able to elucidate the vibrational wave packet dynamics of tryptophan radical in the visible-NIR region and resolve eight unique vibrational modes that are coupled to the photodetachment process, which are assigned with the aid of DFT calculations. Moreover, the time-domain analysis is performed to reveal the amplitude, dephasing time and phase of each vibrational modes. Theoretical calculations reveal a few-picometer changes in the C–C, C–N and N–H bond lengths of the indole sidechain of the tryptophan anion.

In Chapter 5, we examine the ultrafast dynamics of photodetached phenylalaninate anion in aqueous solution, in particular the photodissociation behavior and ultrafast vibrational wave packet dynamics of phenylalanine radical. Early studies suggest that the phenylalaninate anion undergoes two pathways – a photodissociation pathway and photodetachment pathway – upon interaction with radiation.¹⁴⁻¹⁶ The time-resolved differential absorption, $\Delta A(\lambda, t)$, spectrum yields the absorption spectrum of the aqueous phenylalanine radical. The absorption spectrum reveals the wavelength-dependent molar extinction coefficient of phenylalanine radical, which has not been observed thus far. Moreover, a vibronic progression is observed, which can be characterized by a spacing of $482 \pm 15 \text{ cm}^{-1}$, consistent to the ν_{16b} vibrational modes of phenylalanine radical. The vibrational wave packet analysis affords nine unique vibrational modes that are coupled to the photodetachment process, with one of the vibrational

is a first overtone of the most intense vibrational mode. Time-domain analysis yields a dephasing time of 0.13 ± 0.07 ps for the overtone, markedly shorter than the dephasing time of 5.5 ± 0.7 ps obtained for the fundamental frequency. Surprisingly, *ab initio* calculations reveal only sub-0.1-pm changes in the bond lengths between the equilibrium geometry of phenylalaninate anion and the phenylalanine radical, even though the vibrational modes observed are consistent with the phenyl vibrational modes. Moreover, *ab initio* calculations of the potential energy curve along the C1–C2 elongation coordinate show that the isolated phenylalanine radical undergoes direct decarboxylation, contrary to the experimental results. However, the addition of explicit water molecules of hydration into the model results in the emergence of a dissociation barrier, where several models of the microhydrated phenylalanine radical are unable to overcome the dissociation barrier. This result explains the absence of ultrafast decarboxylation on the few-picosecond timescale in our experiments and points to the importance of including explicit water molecules of solvation in theoretical models.

In Chapter 6, we investigate the ultrafast photoionization-induced proton transfer process of ionized aqueous phenol with a near-UV probe. The spectral signature of the phenol radical cation, $\text{PhOH}\cdot^+$, is not observed, and global fit analysis performed from 100 fs onwards did not afford an additional time constant that corresponds to the decay of the $\text{PhOH}\cdot^+$ radical cation or the formation of the phenoxyl radical, $\text{PhO}\cdot$. Time domain analysis of the vibrational wave packet dynamics, however, reveals distinct vibrational frequencies that are associated with the O–H wag and bend modes of $\text{PhOH}\cdot^+$ radical cation. These assignments are supported by DFT calculations and isotopic substitution experiments performed on ionized aqueous deuterated phenol (PhOD) in D_2O . From the dephasing times of these vibrational frequencies, we are able to obtain an estimate of the timescale of the proton transfer process of ~ 0.2 ps, which is also the lifetime of the $\text{PhOH}\cdot^+$ radical cation. Theoretical calculations reveal the proton transfer involves an initial contraction of the $\text{PhOH}\cdot^+$ radical cation and one of the

surrounding water molecules through the oxygen – oxygen atoms (i.e., the $\text{O}_{\text{Ph}}\cdots\text{O}_{\text{a}}$), followed by the elongation of the $\text{O}_{\text{Ph}}\text{--H}$ bond of the $\text{PhOH}\bullet^+$ radical cation, which is a barrierless process. This initial contraction is consistent with an intermolecular vibrational mode at 207 cm^{-1} , which dictates the timescale for proton transfer. Wave packet propagation simulation reveals that the population of the $\text{PhOH}\bullet^+$ radical cation disappears within $\sim 0.2\text{ ps}$ to form the proton-transferred product, $\text{PhO}\bullet$ radical, which supports the estimated lifetime of $\text{PhOH}\bullet^+$ radical cation from the time-domain analysis.

7.2. Future Work

In this thesis, it has been shown that utilizing few-femtosecond transient absorption spectroscopy is an excellent tool to elucidate ultrafast dynamics that occurs in various systems. However, as nothing is perfect and there is always place for improvement, the presented studies are not without exceptions.

For the study on the ultrafast vibrational wave packet dynamics of photodetached tyrosinate dianion, further studies and calculations are to be performed to investigate the solvation dynamics driven by photoionization or photodetachment. Solvation dynamics arises when the solvent responds to a sudden change in the charge distribution of a single solute particle due to photoexcitation or photoionization. Understanding the behavior of the solvent due to this change is relevant to understanding chemical reactions that happen in the solution phase such as proton transfer reactions. We suggest investigating the solvation dynamics of such system by utilizing time-dependent Stokes Shift method to obtain the fluorescence emission spectrum of the aqueous biomolecules and observe the spectral shift due to solvation dynamics.^{17,18}

The study on the ultrafast vibrational wave packet dynamics of photodetached tryptophan anion in aqueous solution was unable to elucidate the absorption band of the tryptophan radical in the visible region due to the overlap with the strong absorption signal of the ground-state hydrated electron. Future work can be performed to suppress the absorption signal of the ground-state hydrated electron by performing a detrapping experiment. The detrapping experiment can be performed by introducing a third pulse, which is a long UV pulse at ~ 400 nm. Such detrapping studies have been performed previously.^{19,20} The long UV pulse will suppress the absorption signal of the hydrated electron by exciting the hydrated electron to the conduction band of liquid water. This will result in the depletion of the population of the ground-state hydrated electron that interferes with the absorption of tryptophan radical at 580 nm. Moreover, tryptophan radical does not have significant absorption at ~ 400 nm, thus this will reduce the interference of the detrapping 400 nm pulse with the tryptophan radical dynamics. Therefore, the spectral signature associated with the hydrated electron would become less prominent and tryptophan radical would persist longer and the absorption band at 580 nm associated with tryptophan radical would be revealed.

In the study on the ultrafast dynamics of photodetached phenylalaninate anion in aqueous solution, there is no evidence to suggest decarboxylation occurs when the phenylalaninate anion undergoes photodetachment within the picosecond timescale. To further investigate the discrepancy observed from previous studies, femtosecond stimulated Raman scattering (FSRS) can be utilized to detect the Raman modes of carbon dioxide produced from the decarboxylation pathway. With regard to the theoretical calculations, we do note that the microhydrated models of phenylalanine radical constructed only account for the direct interaction of the phenylalanine radical with the water molecules, and not the interaction of the water molecules that are further away from the phenylalanine radical, thus the microhydrated model is an approximation of the thermodynamics properties of the phenylalanine radical.

Further theoretical calculations can be performed by employing Quantum Mechanics/Molecular Mechanics (QM/MM) calculation where the calculation can treat the solute as point charges for a larger solvation sphere.

References

1. Debnath, T.; Mohd Yusof, M. S. B.; Low, P. J.; Loh, Z.-H., Ultrafast Structural Rearrangement Dynamics Induced by the Photodetachment of Phenoxide in Aqueous Solution. *Nat. Commun.* **2019**, *10* (1), 2944.
2. Bent, D. V.; Hayon, E., Excited State Chemistry of Aromatic Amino Acids and Related Peptides. III. Tryptophan. *J. Am. Chem. Soc.* **1975**, *97* (10), 2612-2619.
3. Bryant, F. D.; Santus, R.; Grossweiner, L. I., Laser Flash Photolysis of Aqueous Tryptophan. *J. Phys. Chem.* **1975**, *79* (25), 2711-2716.
4. Redpath, J. L.; Santus, R.; Ovadia, J.; Grossweiner, L. I., The Oxidation of Tryptophan by Radical Anions. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **1975**, *27* (2), 201-204.
5. Baugher, J. F.; Grossweiner, L. I., Photolysis Mechanism of Aqueous Tryptophan. *J. Phys. Chem.* **1977**, *81* (14), 1349-1354.
6. Solar, S.; Getoff, N.; Surdhar, P. S.; Armstrong, D. A.; Singh, A., Oxidation of Tryptophan and N-methylindole by $\text{N}_3^\bullet$, $\text{Br}_2^{\bullet-}$, and $(\text{SCN})_2^{\bullet-}$ Radicals in Light- and Heavy-Water Solutions: A Pulse Radiolysis Study. *J. Phys. Chem.* **1991**, *95* (9), 3639-3643.
7. Kandori, H.; Borkman, R. F.; Yoshihara, K., Picosecond Transient Absorption of Aqueous Tryptophan. *J. Phys. Chem.* **1993**, *97* (38), 9664-9667.
8. McGimpsey, W. G.; Görner, H., Photoionization of Indole, N-Methylindole and Tryptophan In Aqueous Solution upon Excitation at 193 nm. *Photochem. Photobiol.* **1996**, *64* (3), 501-509.
9. Stevenson, K. L.; Papadantonakis, G. A.; LeBreton, P. R., Nanosecond UV Laser Photoionization of Aqueous Tryptophan: Temperature Dependence of Quantum Yield,

- Mechanism, and Kinetics of Hydrated Electron Decay. *J. Photochem. Photobiol. A* **2000**, *133* (3), 159-167.
10. Tsentalovich, Y. P.; Snytnikova, O. A.; Sagdeev, R. Z., Properties of Excited States of Aqueous Tryptophan. *J. Photochem. Photobiol. A* **2004**, *162* (2), 371-379.
 11. Larson, B. C.; Pomponio, J. R.; Shafaat, H. S.; Kim, R. H.; Leigh, B. S.; Tauber, M. J.; Kim, J. E., Photogeneration and Quenching of Tryptophan Radical in Azurin. *J. Phys. Chem. B* **2015**, *119* (29), 9438-9449.
 12. Hare, P. M.; Price, E. A.; Bartels, D. M., Hydrated Electron Extinction Coefficient Revisited. *J. Phys. Chem. A* **2008**, *112* (30), 6800-6802.
 13. Torche, F.; Marignier, J.-L., Direct Evaluation of the Molar Absorption Coefficient of Hydrated Electron by the Isosbestic Point Method. *J. Phys. Chem. B* **2016**, *120* (29), 7201-7206.
 14. Mittal, L. J.; Mittal, J. P.; Hayon, E., Biphotonic Photodissociation of Phenylalanine in Aqueous Solution at 20°C. *Chem. Phys. Lett.* **1973**, *18* (3), 319-322.
 15. Mittal, L. J.; Mittal, J. P.; Hayon, E., Primary Processes in the Photochemistry of Phenylalanine and Derivatives in Aqueous Solution. Biphotonic Photoionization and Photodissociation Reactions. *J. Am. Chem. Soc.* **1973**, *95* (19), 6203-6210.
 16. Bent, D. V.; Hayon, E., Excited State Chemistry of Aromatic Amino Acids and Related Peptides. II. Phenylalanine. *J. Am. Chem. Soc.* **1975**, *97* (10), 2606-2612.
 17. Jimenez, R.; Fleming, G. R.; Kumar, P. V.; Maroncelli, M., Femtosecond Solvation Dynamics of Water. *Nature* **1994**, *369* (6480), 471-473.
 18. Kumpulainen, T.; Rosspeintner, A.; Vauthey, E., Probe Dependence on Polar Solvation Dynamics from fs Broadband Fluorescence. *Phys. Chem. Chem. Phys.* **2017**, *19* (13), 8815-8825.

19. Hee Son, D.; Kambhampati, P.; Kee, T. W.; Barbara, P. F., One-photon UV Detrapping of the Hydrated Electron. *Chem. Phys. Lett.* **2001**, 342 (5), 571-577.
20. Son, D. H.; Kambhampati, P.; Kee, T. W.; Barbara, P. F., Delocalizing Electrons in Water with Light. *J. Phys. Chem. A* **2001**, 105 (36), 8269-8272.

Appendix

Appendix A

Table A1. Calculated vibrational frequencies of the tyrosyl radical anion and the microhydrated tyrosyl radical with polarizable continuum model (PCM) solvation. The assignment of atoms is based on the equilibrium geometries of the tyrosyl radical anion and the microhydrated tyrosyl radical anion, shown in Figs. 3.4(b) and 3.4(d), respectively in Chapter 3. Numbered assignments denote benzene ring modes in Wilson notation.^{1,2}

Calculated Vibrational Frequency (cm ⁻¹)			Assignment
Tyrosyl Radical Anion	Microhydrated Tyrosyl Radical Anion		
1	38	42	Benzyl Ring Torsion / COO ⁻ Twist
2	52	53	Chain Torsion
3	59	61	Benzyl Ring Torsion
4	80	77	COO ⁻ Twist / Chain CH ₂ Rock
5	157	176	11
6	176	186	Chain Bend
7	241	252	NH ₂ Rock
8	294	295	10b
9	322	337	15
10	351	356	Chain CH ₂ Rock / NH ₂ Twist
11	383	386	16a
12	389	388	Chain Bend
13	458	469	3
14	483	482	6a
15	519	522	16b
16	556	558	16b

17	622	622	6b
18	679	676	4
19	715	715	4
20	763	768	4
21	792	795	10a
22	805	816	12
23	825	832	1
24	841	789	17b
25	862	850	NH ₂ Wag
26	884	886	17b
27	893	876	Benzene Out-of-plane CH Bend
28	968	938	Chain CH ₂ Rock
29	988	992	5
30	990	994	19a
31	996	1000	17a
32	1076	1074	19b
33	1123	1127	14
34	1158	1155	Chain CH ₂ Twist / NH ₂ Wag
35	1175	1185	8a
36	1219	1221	Chain CH ₂ Twist / Chain CH Wag
37	1231	1240	13
38	1271	1264	Chain CH ₂ Wag / Chain CH Wag
39	1275	1293	9b
40	1316	1304	Chain CH Wag / Chain CH ₂ Wag
41	1343	1350	Chain CH ₂ Wag
42	1355	1340	COO ⁻ Bend / Chain CH Wag / Chain CH ₂ Twist
43	1401	1400	Chain CH Wag / NH ₂ Twist
44	1429	1420	20a
45	1452	1462	18b
46	1483	1485	Chain CH ₂ Bend

47	1506	1508	18b
48	1515	1520	20a
49	1580	1586	NH ₂ Bend / COO ⁻ Asymmetric Stretch
50	1605	1626	9a
51	1620	1622	NH ₂ Bend
52	3035	3042	Chain CH ₂ Symmetric Stretch
53	3054	3067	Chain CH Stretch
54	3089	3089	Chain CH ₂ Asymmetric Stretch
55	3166	3173	7b
56	3169	3177	7b
57	3188	3195	20b
58	3190	3197	2
59	3485	3494	NH ₂ Symmetric Stretch
60	3569	3582	NH ₂ Asymmetric Stretch

Appendix B

Table B1. Calculated vibrational frequencies of the tryptophan radical and the microhydrated tryptophan radical with PCM solvation. The assignment of atoms is based on the equilibrium geometries of the tryptophan radical and the microhydrated tryptophan radical, shown in Figs. 4.5(b) and 4.5(d), respectively in Chapter 4.

Calculated Vibrational Frequency (cm ⁻¹)			Assignment
	Tryptophan Radical	Microhydrated Tryptophan Radical	
1	34	31	Indole Torsion
2	58	66	Chain Torsion
3	72	88	Indole Torsion
4	80	75	Chain Deformation
5	156	152	Indole Torsion
6	220	223	Butterfly
7	225	190	NH ₂ Rock
8	246	240	Chain Torsion
9	263	261	Chain CH ₂ Rock
10	324	327	NH ₂ Wag
11	346	343	NH ₂ Twist
12	395	396	Indole Out-of-plane Deformation
13	420	419	Indole Out-of-plane Deformation
14	472	477	Indole In-plane Deformation
15	517	515	Chain Torsion
16	548	549	Indole In-plane Deformation
17	558	866	Indole CH and NH Out-of-plane Bend
18	571	572	Indole Out-of-plane Deformation
19	591	599	Indole NH Out-of-plane Bend / Chain Torsion

20	601	586	Indole In-plane Deformation / Indole NH Out-of-plane Bend
21	651	640	Indole Out-of-plane Deformation
22	722	728	NH ₂ Wag
23	748	750	Indole Out-of-plane Deformation
24	757	759	Indole CH Out-of-plane Bend
25	765	766	Indole CH Out-of-plane Bend
26	779	775	Indole CH Out-of-plane Bend / NH ₂ Wag
27	777	782	Indole CH Out-of-plane Bend
28	806	812	COO ⁻ Bend
29	875	1029	Indole CH Out-of-plane Bend
30	878	882	Indole CH and NH Out-of-plane Bend
31	881	875	Indole CH Out-of-plane Bend
32	941	935	Chain CC (C2 – C3) Stretch
33	958	957	CH ₂ Rock / Indole CH Bend
34	965	965	Indole CH Out-of-plane Bend
35	1000	999	Indole CH Out-of-plane Bend
36	1035	1033	Indole In-plane Deformation
37	1075	1076	Indole In-plane Deformation
38	1075	1113	Indole CH Bend / Chain CH ₂ Twist / Chain CH Wag
39	1124	1122	Indole CH and NH Bend
40	1149	1148	Indole CH and NH Bend
41	1166	1179	Indole CH and NH Bend
42	1181	1183	Indole CH Bend
43	1194	1193	NH ₂ Twist / Chain CH Wag
44	1223	1242	Indole CH and NH Bend
45	1266	1272	Indole CH and NH Bend
46	1290	1289	Indole CH Bend / Chain CH ₂ Wag
47	1310	1313	Indole CH Bend
48	1335	1340	Indole CH Bend
49	1345	1349	Indole CC Stretch

50	1369	1367	Indole CC Stretch
51	1371	1373	Indole CC and CN Stretch
52	1412	1412	NH ₂ Twist/ Chain CH Wag
53	1452	1466	Indole CC Stretch
54	1485	1487	CH ₂ Bend
55	1493	1496	Indole CC Stretch
56	1521	1553	Indole CC Stretch
57	1525	1525	CC (C3 – C4) Stretch
58	1570	1572	COO ⁻ Asymmetric Stretch
59	1611	1610	Indole CC Stretch
60	1634	1628	NH ₂ Bend
61	1645	1644	Indole CC Stretch
62	3043	3039	Chain CH Stretch / Chain CH ₂ Symmetric Stretch
63	3048	3047	Chain CH Stretch
64	3095	3091	Chain CH ₂ Asymmetric Stretch
65	3180	3179	Indole CH Stretch
66	3189	3187	Indole CH Stretch
67	3198	3196	Indole CH Stretch
68	3205	3203	Indole CH Stretch
69	3271	3262	Indole CH Stretch
70	3512	3508	NH ₂ Symmetric Stretch
71	3597	3594	NH ₂ Asymmetric Stretch
72	3619	3046	Indole NH Stretch

Appendix C

Section C1. Time-Dependent Density Functional Theory Calculations

We employ time-dependent density functional theory (TD-DFT), as implemented in the GAUSSIAN 16 program, to calculate the electronically excited states and the vertical excitation energies of the isolated phenylalaninate anion and microhydrated phenylalaninate anion $\text{Phe}(\text{H}_2\text{O})_{10}^-$. Geometry optimization of the isolated and microhydrated phenylalaninate anion is performed by using B3LYP/6-311G++(d,p). The geometry is then used in the TD-DFT calculations of the excited states. For the isolated phenylalaninate anion, the calculations identify two excited states (labelled #13 and #14) that are located 5.8 eV above the ground state and whose one-photon transition from the ground state bears significant oscillator strength. Information on the molecular orbitals that comprise each electronically excited state, along with their configuration interaction expansion coefficients, are given below. Note that the orbitals labelled 43 and 44 are the HOMO-1 and HOMO of the phenylalaninate anion, shown in Fig. 5.14 of the main text.

Excited State 13:	Singlet-A	5.7869 eV	214.25 nm	f=0.0446
43 → 47	0.13847			
43 → 48	0.19898			
43 → 49	0.17094			
43 → 50	0.13377			
43 → 53	-0.21341			
43 → 54	-0.19169			
44 → 49	0.13867			
44 → 50	0.35004			
44 → 51	-0.21800			
44 → 53	0.21867			
44 → 54	0.12298			

Excited State 14:	Singlet-A	5.8302 eV	212.66 nm	f=0.0249
43 → 47	0.28293			
43 → 48	0.40151			
43 → 50	0.14062			
43 → 53	-0.10366			
43 → 54	-0.10163			
44 → 49	-0.13654			

44 → 50	-0.14467
44 → 51	0.24322
44 → 53	-0.22775
44 → 54	-0.13815

For the microhydrated phenylalaninate anion $\text{Phe}(\text{H}_2\text{O})_{10}^-$, the calculations identified four excited states (labelled #8 to #11) that are located 5.8 eV above the ground state, where excited state #9 and #10 bear the largest oscillator strength. Note that the orbitals labelled 93 and 94 are the HOMO-1 and HOMO of the microhydrated phenylalaninate anion.

Excited State 8:	Singlet-A	5.7292 eV	216.41 nm	f=0.0038
91 → 95	-0.10117			
93 → 95	0.66478			
93 → 100	0.10434			

Excited State 9:	Singlet-A	5.8024 eV	213.68 nm	f=0.0199
94 → 100	0.60595			
94 → 101	0.23865			
94 → 102	-0.11948			
94 → 103	0.12781			

Excited State 10:	Singlet-A	5.8701 eV	211.21 nm	f=0.0252
91 → 95	-0.12378			
92 → 95	0.11160			
92 → 96	-0.27994			
92 → 97	-0.16477			
92 → 98	-0.17985			
93 → 96	0.41906			
93 → 97	-0.15469			
93 → 98	-0.33356			

Excited State 11:	Singlet-A	5.8905 eV	210.48 nm	f=0.0095
91 → 95	0.66262			
93 → 96	0.10494			

Table C1. Calculated vibrational frequency of phenylalanine radical and microhydrated phenylalanine radical with PCM solvation. The assignment of atoms and vibrational modes are based on the equilibrium geometries of the phenylalanine radical and the microhydrated phenylalanine radical in Figs. 5.9(a) and 5.9(b) respectively. Numbered assignments denote benzene ring modes in the Wilson notation.^{1,3}

Calculated Vibrational Frequency / cm ⁻¹								Assignment
	Phe•	Phe (H ₂ O) ₃ •	Phe (H ₂ O) ₄ •	Phe (H ₂ O) _{6a} •	Phe (H ₂ O) _{6b} •	Phe (H ₂ O) ₉ •	Phe (H ₂ O) ₁₀ •	
1	8	7	23	15	22	22	18	CO ₂ Twist / Phenylalanine Twist
2	15	44	24	53	42	47	63	CO ₂ Twist / Benzene Twist
3	22	49	67	25	15	57	15	CO ₂ rock / 2- phenylethanamine Rock
4	31	24	32	36	31	32	28	Benzene Twist
5	46	99	44	101	58	53	53	CO ₂ Rock / 2- phenylethanamine Rock
6	62	54	94	287	107	81	49	CO ₂ Twist
7	100	105	173	175	166	103	178	Benzene Rock
8	111	94	167	67	105	171	103	NH ₂ Twist / CH ₂ Twist / CH Wag
9	281	158	319	315	314	299	323	16b
10	326	263	139	187	158	205	652	NH ₂ Rock
11	339	329	344	344	343	343	345	CH ₂ Rock
12	351	466	368	710	440	699	595	NH ₂ Twist / CH ₂ Rock
13	415	415	415	415	415	415	414	16a
14	505	505	512	513	511	515	515	16b
15	594	612	444	438	453	598	624	Ring Deformation

16	617	296	610	610	575	598	611	Ring Deformation
17	634	634	634	634	580	621	634	6b
18	637	636	651	647	658	658	691	CO ₂ Bend / NH ₂ Twist
19	658	653	668	674	670	684	682	CO ₂ Scissor
20	687	507	755	781	727	784	770	NH ₂ Wag
21	712	712	712	713	635	712	712	11
22	762	758	761	761	670	760	759	11
23	833	834	837	838	837	836	836	6a
24	857	858	859	859	859	858	858	10a
25	922	889	902	903	899	905	904	17b
26	929	931	939	940	936	940	940	10b
27	974	954	975	982	969	972	968	NH ₂ Twist / Chain CC Stretch / 17b
28	988	992	993	992	992	992	992	17a
29	1005	1009	1010	1009	1009	1010	1010	5
30	1015	1015	1015	1015	1015	1015	1015	12
31	1047	1048	1048	1048	1048	1048	1048	19a
32	1092	1097	1105	1107	1100	1115	1113	19b
33	1147	1147	1165	1078	1156	1158	1155	NH ₂ Rock / CH ₂ Twist
34	1174	1176	1176	1177	1176	1177	1176	8b
35	1195	1200	1199	1199	1198	1200	1199	14, 8b
36	1203	1224	1234	1236	1230	1230	1231	NH ₂ Twist / CH ₂ Twist
37	1216	1221	1221	1221	1222	1221	1223	CH ₂ Wag
38	1230	1294	1299	1299	1298	1299	1298	1 (19a, 12, 7a, 20a, 8a, 12, 6a)*
39	1334	1330	1332	1332	1331	1330	1330	15
40	1343	1351	1354	1354	1352	1350	1351	CH ₂ Wag / NH ₂ Twist
41	1356	1365	1367	1367	1365	1365	1366	3
42	1371	1254	1267	1273	1261	1278	1276	CO ₂ Symmetric Stretch

43	1432	1457	1458	1456	1458	1447	1448	CH ₂ Rock / CH Rock
44	1473	1464	1467	1468	1466	1467	1468	CH ₂ Symmetric Bend
45	1478	1483	1482	1482	1483	1482	1482	18b
46	1523	1525	1525	1525	1525	1525	1525	18a
47	1617	1620	1619	1619	1620	1620	1620	9b
48	1637	1640	1640	1640	1640	1640	1640	9a / NH ₂ Wag
49	1638	1650	1682	1698	1701	1715	1712	9a / NH ₂ Wag
50	2381	1960	1897	1884	1920	1847	1850	CO ₂ Asymmetric Stretch
51	2920	3007	3017	3020	3013	3026	3026	CH ₂ Symmetric Stretch
52	3000	3072	3088	3089	3080	3089	3090	CH ₂ Asymmetric Stretch
53	3154	3160	3139	3134	3147	3136	3137	CH Stretch
54	3155	3158	3157	3157	3157	3157	3157	13
55	3157	3164	3164	3164	3164	3164	3164	7b
56	3167	3172	3172	3172	3172	3172	3173	20a (7a, 2, 13)*
57	3175	3180	3180	3179	3179	3180	3180	20b
58	3187	3190	3189	3189	3190	3190	3190	2
59	3523	3556	3357	3280	3408	3260	3267	NH ₂ Symmetric Stretch
60	3622	3668	3468	3380	3516	3404	3449	NH ₂ Symmetric Stretch

*Numbers in parentheses refers to minor contributions of phenyl vibrational modes to the main vibrational mode, in descending order of importance.

Table C2. Calculated vibrational frequency of microhydrated 2-phenylethanamine and microhydrated phenylalanine radical with PCM solvation. The assignment of atoms and vibrational modes are based on the equilibrium geometry of microhydrated phenylalanine radical in Fig. 5.9(b). Numbered assignments denote benzene ring modes in the Wilson notation.^{1,3}

Calculated Vibrational Frequency / cm ⁻¹			
	Microhydrated 2-phenylethanamine	Microhydrated Phenylalanine Radical [Phe(H ₂ O) ₄ •]	Assignment
1	59	67	CO ₂ Rock / 2-phenylethanamine Rock
2	124	94	CO ₂ Twist
3	112	173	Benzene Rock
4	286	319	16b
5	339	344	CH ₂ Rock
6	355	368	NH ₂ Twist / CH ₂ Rock
7	339	344	CH ₂ Rock
8	415	415	16a
9	503	512	16b
10	604	651	CO ₂ Bend / NH ₂ Twist
11	647	610	Ring Deformation
12	634	634	6b
13	757	668	CO ₂ Scissor
14	711	712	11
15	762	761	11
16	833	837	6a
17	857	859	10a
18	924	902	17b
19	927	939	10b
20	993	975	NH ₂ Twist / 17b
21	987	993	17a
22	1006	1010	5

23	1015	1015	12
24	1046	1048	19a
25	1092	1105	19b
26	1151	1165	NH ₂ Rock / CH ₂ Twist
27	1173	1176	8b
28	1194	1199	14, 8b
29	1208	1234	NH ₂ Twist / CH ₂ Twist
30	1218	1299	1 (19a, 12, 7a, 20a, 8a, 12, 6a)*
31	1243	1221	CH ₂ Wag
32	1335	1332	15
33	1345	1354	CH ₂ Wag / NH ₂ Twist
34	1355	1367	3
35	1438	1458	CH ₂ Rock / CH Rock
36	1474	1467	CH ₂ Symmetric Bend
37	1478	1482	18b
38	1522	1525	18a
39	1616	1619	9b
40	1636	1640	9a / NH ₂ Wag
41	1669	1682	9a / NH ₂ Wag
42	2908	3017	CH ₂ Symmetric Stretch
43	3002	3088	CH ₂ Asymmetric Stretch
44	3139	3139	CH Stretch
45	3154	3157	13
46	3156	3164	7b
47	3167	3172	20a (7a, 2, 13)*
48	3174	3180	20b
49	3186	3189	2
50	3453	3357	NH ₂ Symmetric Stretch
51	3550	3468	NH ₂ Symmetric Stretch

*Numbers in parentheses refers to minor contributions of phenyl vibrational modes to the main vibrational mode, in descending order of importance.

Appendix D

Table D1. Calculated vibrational frequencies of the phenol radical cation and microhydrated phenol radical cation with PCM solvation. The assignment of atoms and symmetry are based on the equilibrium geometries of the phenol radical cation, the microhydrated phenol radical cation, the phenol-OD radical cation and the microhydrated phenol-OD radical cation in Figs. 6.13(a), 6.13(b), 6.13(c) and 6.13(d) respectively, in Chapter 6. The three vibrational frequencies indicated by asterisk (*) are the unique vibrational modes associated with phenolic OH (OD) of phenol radical cation (phenol-OD radical cation).

Calculated Vibrational Frequency (cm ⁻¹)					Assignment	Symmetry
Phenol Radical Cation		Phenol-OD Radical Cation				
Isolated	Micro-hydrated	Isolated	Micro-hydrated			
1	396	475	374	457	CO Bend	A'
2	524	534	519	528	CCC Bend	
3	572	578	568	576	CCC Bend	
4	818	821	808	813	Ring Breath / CCC Bend	
5	990	995	989	995	CCC Trigonal Bend	
6	995	999	995	999	CH Bend / Ring Breath	
7	1089	1108	1103	1112	CH Bend / CC Stretch	
8	1149	1414	922	1014	OH (OD) Bend*	
9	1186	1201	1187	1202	CH Bend	
10	1195	1275	1166	1334	CH Bend	
11	1356	1379	1331	1384	CH Bend	
12	1384	1396	1378	1395	CC Stretch	
13	1396	1170	1390	1112	CC Stretch	

14	1450	1468	1429	1435	CC Stretch	
15	1491	1508	1486	1501	CO Stretch	
16	1539	1544	1538	1543	CC Stretch	
17	1653	1653	1652	1651	CC Stretch	
18	3188	3213	3188	3213	CH Stretch	
19	3206	3203	3206	3203	CH Stretch	
20	3214	3209	3214	3209	CH Stretch	
21	3220	3219	3220	3219	CH Stretch	
22	3226	3223	3226	3223	CH Stretch	
23	3710	2593	2701	1909	OH (OD) Stretch*	
24	182	192	178	192	Ring Deformation	
25	362	372	356	379	Ring Twist	
26	432	439	425	450	Ring Boat	
27	601	1197	469	870	OH (OD) Wag*	
28	611	634	609	633	CH Wag	A''
29	783	813	780	813	CH Wag / Ring Boat	
30	799	796	791	789	CH Wag	
31	945	958	940	958	CH Wag / Ring Boat	
32	997	1017	995	1017	HCCH Torsion	
33	1007	1012	1006	1012	HCCH Torsion	

References

1. Wilson, E. B., The Normal Modes and Frequencies of Vibration of the Regular Plane Hexagon Model of the Benzene Molecule. *Phys. Rev.* **1934**, *45* (10), 706-714.
2. Grace, L. I.; Cohen, R.; Dunn, T. M.; Lubman, D. M.; de Vries, M. S., The R2PI Spectroscopy of Tyrosine: A Vibronic Analysis. *J. Mol. Spectrosc.* **2002**, *215* (2), 204-219.
3. Gardner, A. M.; Wright, T. G., Consistent Assignment of the Vibrations of Monosubstituted Benzenes. *J. Chem. Phys.* **2011**, *135* (11), 114305.