Engineering neodymium-sensitized nanoconstructs for near-infrared enabled photomedicine

Yu, Zhongzheng

2019

Yu, Z. (2019). Engineering neodymium-sensitized nanoconstructs for near-infrared enabled photomedicine. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/136621>

<https://doi.org/10.32657/10356/136621>

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

Downloaded on 26 Apr 2023 19:29:10 SGT

**ENGINEERING NEODYMIUM-SENSITIZED
NANOCONSTRUCTS FOR NEAR-INFRARED
ENABLED PHOTOMEDICINE**

YU ZHONGZHENG

**SCHOOL OF CHEMICAL AND BIOMEDICAL
ENGINEERING**

2019

**ENGINEERING NEODYMIUM-SENSITIZED
NANOCONSTRUCTS FOR NEAR-INFRARED
ENABLED PHOTOMEDICINE**

YU ZHONGZHENG

**SCHOOL OF CHEMICAL AND BIOMEDICAL
ENGINEERING**

A thesis submitted to the Nanyang Technological University
in partial fulfillment of the requirement for the degree of

Doctor of Philosophy

2019

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

22 July 2019

.....

Date

A handwritten signature in black ink, appearing to be 'Yu Zhongzheng', is written over a light blue rectangular background.

.....

Yu Zhongzheng

Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate 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.

22 July 2019

.....

Date

.....

Prof Timothy T.Y. Tan

Authorship Attribution Statement

This thesis contains material from 3 paper(s) published in the following peer-reviewed journal(s) / from papers accepted at conferences in which I am listed as an author.

Chapter 2 is published as Z. Yu, W. K. Chan and T. T. Y. Tan. Neodymium-Sensitized Nanoconstructs for Near-Infrared Enabled Photomedicine. *Small* (2019). DOI: 10.1002/sml.201905265.

The contributions of the co-authors are as follows:

- I prepared the manuscript drafts.
- The manuscript was revised by Mr. Chan, A/Prof. Tan

Chapter 3 is published as Y. Zhang, [#] Z. Yu, [#] J. Li, Y. Ao, J. Xue, Z. Zeng, X. Yang and T. T. Y. Tan. Ultrasmall-Superbright Neodymium-Upconversion Nanoparticles *via* Energy Migration Manipulation and Lattice Modification: 808 nm-Activated Drug Release. *ACS nano*, 11(3), 2846-2857 (2017). DOI:10.1021/acsnano.6b07958. [#]Co-first Author

The contributions of the co-authors are as follows:

- A/Prof Tan provided the initial project direction and edited the manuscript drafts.
- A/Prof. Zhang and I prepared the manuscript drafts.
- A/Prof Zhang and I co-designed the study with A/Prof Tan. I have done all the synthesis, characterization and analysis work of UCNPs at School of Chemical and Biomedical Engineering.

- Dr. Li, Mr. Ao, Dr. Xue and A/Prof Zhang conducted the *in vitro* and *in vivo* controlled-release work in Hu. Prof. Yang provided funding and place for *in vivo* test.
- Dr. Zeng helped to do the EDX analysis using SEM.

Chapter 5 is published as Z. Yu,[#] W. Hu,[#] H. Zhao, X. Miao, Y. Guan, W. Cai, Z. Zeng, Q. Fan, T. T. Y. Tan. Generating New Cross-Relaxation Pathways by Coating Prussian Blue on NaNdF₄ To Fabricate Enhanced Photothermal Agents. *Angewandte Chemie (International ed. in English)*, 58(25), 8536 (2019). DOI: 10.1002/ange.201904534. [#]Co-first Author

The contributions of the co-authors are as follows:

- I co-designed this study with Dr. Hu.
- I prepared the manuscript drafts.
- The manuscript was revised by Dr. Hu, A/Prof Tan and Prof. Fan.
- I performed all the materials synthesis, characterization and analysis work.
- Dr. Zhao conducted the photothermal comparison work.
- Ms. Miao conducted the cell viability test.
- Dr. Guan assisted to measure the NIR emission lifetime.
- Mr. Cai conducted element mapping and high resolution TEM test.
- Dr. Zeng helped to discuss and add DLS data in revision.

22 July 2019

.....
Date

.....
Yu Zhongzheng

Acknowledgements

I would like to express my most sincere gratitude to my supervisor, Associate Professor Timothy Tan, for his excellent guidance and strong support during my whole Ph.D. career. I really appreciate his way of treating student equally, sharing his personal experience, encouraging me whenever I am faced with barriers. It is such a privilege to work under his supervision, since he always trusts me and gives me the opportunity to express my creativity. His positive and optimistic attitude towards research and life has inspired me and will encourage me all the time.

I am grateful to have Associate Prof. Yan Zhang as my senior, for her guidance at the start of my Ph.D. career and collaboration in my first work. I would like to thank Dr. Wenbo Hu for his strong support and encouragement to convert an idea into a top journal paper by providing photothermal-related test equipment and funding support. Thanks Dr. Yong Yao Chun for his help in the hydrogel-related work. I would like to give my appreciation of having Dr. Ziming He, Dr. Jing Liu, Dr. Jingwen Xue, Dr. Zhiping Zeng, Ms. Liwei Wang as my seniors and colleagues for the care and help I received on my life in Singapore.

I would like to present special thanks to my parents, who give me unconditional love and support. I am grateful to have so many close friends in Nanyang Technological University to share sorrow and happiness in my Ph.D. life and give me numerous good memories.

Table of Contents

STATEMENT OF ORIGINALITY	I
SUPERVISOR DECLARATION STATEMENT	II
AUTHORSHIP ATTRIBUTION STATEMENT	III
ACKNOWLEDGEMENTS.....	V
TABLE OF CONTENTS	VI
LIST OF FIGURES.....	VIII
LIST OF SCHEMES AND TABLES.....	X
LIST OF PUBLICATIONS	XI
ABSTRACT	1
CHAPTER 1 INTRODUCTION	4
1.1 BACKGROUND.....	4
1.2 MOTIVATION AND OBJECTIVES OF THIS THESIS	5
1.3 ORGANIZATION OF THIS THESIS	7
CHAPTER 2 LITERATURE REVIEW	9
2.1 INTRODUCTION	9
2.2 MERITS AND SHORTCOMINGS OF Nd ³⁺ IONS	11
2.3 ENERGY CONVERSION IN Nd ³⁺ -SENSITIZED NANOCONSTRUCTS.....	15
2.3.1 UC.....	15
2.3.2 DC.....	21
2.3.3 Photothermal Conversion.....	25
2.4 NIR-ENABLED PHOTOMEDICINE	26
2.4.1 Biomedical Imaging.....	27
2.4.2 NIR-Triggered Chemotherapy.....	35
2.4.3 Photodynamic Therapy.....	38
2.4.4 Photothermal Therapy.....	41
2.4.5 Optogenetics	43
2.5 CONCLUSIONS, CHALLENGES AND PERSPECTIVES	45
CHAPTER 3 ULTRASMALL-SUPERBRIGHT NEODYMIUM-UPCONVERSION NANOPARTICLES VIA ENERGY MIGRATION MANIPULATION AND LATTICE MODIFICATION: 808 NM-ACTIVATED DRUG RELEASE	48
3.1 INTRODUCTION	48
3.2 MATERIALS AND METHODS	53
3.3 RESULTS AND DISCUSSIONS.....	64
3.3.1 Fabrication and Characterization of Ultrasmall UCNPs.....	64

3.3.2 The Role of Intermediate Layer.....	70
3.3.3 Concentration of Nd^{3+} Ions and Lattice Modification by Ca^{2+} ions.....	74
3.3.4 808-Activated Drug Release in vitro and in vivo	78
3.4 CONCLUSIONS.....	90
CHAPTER 4 THICKNESS TUNING OF SENSITIZING AND INERT SHELLS IN NEODYMIUM-SENSITIZED TETRALAYER NANOCONSTRUCTS FOR OPTIMAL 4-PHOTON ULTRAVIOLET UPCONVERSION AND NEAR-INFRARED CROSS-LINKED HYDROGEL.....	92
4.1 INTRODUCTION	92
4.2 MATERIALS AND METHODS	94
4.3 RESULTS AND DISCUSSION	101
4.3.1 Design, Synthesis and Characterization of Tetralayer UCNPs.....	101
4.3.2 Tuning the Thickness of Sensitizing Layer in CSS UCNPs.....	105
4.3.3 Tuning the Thickness of Inert Layer in CSSS Tetralayer UCNPs.....	107
4.3.4 808 nm-Activated Cross-Linking of Gelatin-Based Hydrogel.....	111
4.4 CONCLUSIONS.....	115
CHAPTER 5 GENERATING NEW CROSS-RELAXATION PATHWAYS BY COATING PRUSSIAN BLUE ON NaNdF_4 TO FABRICATE ENHANCED PHOTOTHERMAL AGENTS.....	117
5.1 INTRODUCTION	117
5.2 MATERIALS AND METHODS	119
5.3 RESULTS AND DISCUSSIONS.....	126
5.3.1 Fabrication and Characterization of NdNP@PB Nanocomplex	126
5.3.2 Comparison of Photothermal Properties and PAI in vitro	132
5.3.3 Proposed Mechanism of Generating New Cross-Relaxation Pathways.....	134
5.3.4 PTT and PAI in vivo.....	140
5.4 CONCLUSIONS.....	147
CHAPTER 6 CONCLUSIONS AND FUTURE WORK.....	149
6.1 CONCLUSIONS.....	149
6.2 FUTURE WORK	151
REFERENCE	153

List of Figures

FIGURE 2.1 ABSORPTIONS AND EMISSIONS OF LANTHANIDE IONS.....	10
FIGURE 2.2 TUNABLE ABSORPTION OF LANTHANIDES BY COMBINING WITH DIFFERENT DYES.	14
FIGURE 2.3 ABSORPTIONS AND EMISSIONS OF LANTHANIDES.	18
FIGURE 2.4 ENERGY TRANSFER AND EVOLUTION OF UCNPs.	21
FIGURE 2.5 SELF-DC MECHANISM.	23
FIGURE 2.6 DC ENERGY TRANSFER WITH OTHER LANTHANIDES.	25
FIGURE 2.7 ENERGY DIAGRAM OF PHOTOTHERMAL CONVERSION.....	26
FIGURE 2.8 RECENT ACHIEVEMENTS IN FUNCTIONALIZED NIR-II IMAGING.	29
FIGURE 2.9 RECENT ACHIEVEMENTS IN LIFETIME-RESOLVED MULTIPLEXING IMAGING. .	32
FIGURE 2.10 RECENT ACHIEVEMENTS IN PHOTOACOUSTIC IMAGING.	34
FIGURE 2.11 RECENT ACHIEVEMENTS IN NIR-CONTROLLED RELEASE FOR CHEMOTHERAPY.	37
FIGURE 2.12 RECENT ACHIEVEMENTS IN PHOTODYNAMIC THERAPY.....	40
FIGURE 2.13 RECENT ACHIEVEMENTS IN PHOTOTHERMAL THERAPY.	42
FIGURE 2.14 RECENT ACHIEVEMENTS IN OPTOGENETICS.	44
FIGURE 3.1 SYNTHESIS OF ULTRASMALL CSS UCNPs.....	52
FIGURE 3.2 HRTEM IMAGES OF AS-SYNTHESIZED CSS UCNP NANOPARTICLES.....	65
FIGURE 3.3 XRD PATTERNS OF AS-SYNTHESIZED CORE, CS AND CSS NANOPARTICLES. .	66
FIGURE 3.4 EDX OF AS-SYNTHESIZED CORE, CS AND CSS NANOPARTICLES.	66
FIGURE 3.5 COMPARISON OF EMISSION SPECTRA OF DIFFERENT CONFIGURATIONS.	67
FIGURE 3.6 TEM IMAGES OF DIFFERENT UCNPs.	70
FIGURE 3. 7 TUNING OF THE INTERMEDIATE LAYER.....	71
FIGURE 3.8 TUNING OF THE CONCENTRATION OF Nd^{3+} IONS AND LATTICE MODIFICATION BY Ca^{2+} IONS.....	75
FIGURE 3.9 TEM IMAGES OF UCNPs WITHOUT Ca^{2+} DOPING.	77
FIGURE 3.10 IN VITRO NIR-CONTROLLED RELEASE OF DOX.....	80
FIGURE 3.11 PORE SIZE TEST BY BET.....	81
FIGURE 3.12 THE COLLOIDAL STABILITY OF $\text{UCNP}@ \text{MSiO}_2\text{-RU}$ NANOPARTICLES IN WATER.	81
FIGURE 3.13 FTIR SPECTRA OF THE $\text{UCNP}@ \text{MSiO}_2$, RU COMPLEX (RU), AND $\text{UCNP}@ \text{MSiO}_2\text{-RU}$	81
FIGURE 3.14 UV/VIS ABSORPTION SPECTRA TO PROVE CONJUGATION AND RELEASE.	82
FIGURE 3.15 FLUORESCENCE SPECTRA ($\lambda_{\text{EX}} = 480 \text{ nm}$) TO SHOW RELEASE.	82
FIGURE 3.16 CELL VIABILITY TEST.....	85
FIGURE 3.17 IN VIVO NIR-CONTROLLED RELEASE.	86
FIGURE 3.18 THE HISTOLOGY ANALYSIS OF THE MAJOR ORGANS.	89

FIGURE 3.19 <i>IN VIVO</i> BIODISTRIBUTION OF NEODYMIUM ELEMENT IN MAJOR ORGANS....	90
FIGURE 4.1 SYNTHESIS PROCESS OF TETRALAYER UCNPs.....	104
FIGURE 4.2 XRD PATTERNS OF CORE, CS, CSS AND CSSS UCNPs.....	105
FIGURE 4.3 SYNTHESIS AND COMPARISON OF CSS UCNPs WITH DIFFERENT SENSITIZING LAYER THICKNESS.....	107
FIGURE 4.4 DIFFERENT TETRALAYER CONFIGURATIONS AND COMPARISON OF THEIR EMISSION SPECTRA.	108
FIGURE 4.5 COMPARISON OF 3-PHOTON AND 4-PHOTON EMISSION AND POSSIBLE MECHANISM.	110
FIGURE 4.6 NIR-ENABLED CROSS-LINKING OF GELMA.	113
FIGURE 4.7 CELL VIABILITY TEST OF UCNPs.	115
FIGURE 5.1 SYNTHESIS AND CHARACTERIZATION OF NdNP@PB NANOCOMPLEX.	128
FIGURE 5.2 HRTEM IMAGES OF NdNP, PB AND NdNP@PB.....	129
FIGURE 5.3 THE ABSORPTION OF NdNPs, PB AND NdNP@PB NANOCOMPLEX.....	129
FIGURE 5.4 DYNAMIC LIGHT SCATTERING CURVES OF NdNPs, PB AND NdNP@PB NANOCOMPLEX.	130
FIGURE 5.5 DIGITAL PICTURES OF NdNP@PB NANOCOMPLEX TO SHOW STABILITY.....	130
FIGURE 5.6 LONG-TERM STABILITY TEST BY MEASURING THE AVERAGE HYDRODYNAMIC SIZE.	131
FIGURE 5.7 COMPARISON OF PHOTOTHERMAL PERFORMANCE.....	133
FIGURE 5.8 PROPOSED NEW CROSS-RELAXATION PATHWAYS AND CELL VIABILITY TEST.	136
FIGURE 5.9 ABSORBANCE OF PB AND NdNPs FROM 900 TO 1100 NM.	137
FIGURE 5.10 CHECK THE PULSE WIDTH AND IRF SITUATION.	138
FIGURE 5. 11 COMPARISON OF <i>IN VITRO</i> PTT.....	139
FIGURE 5.12 <i>IN VIVO</i> PAI AND PTT.	142
FIGURE 5.13 BIODISTRIBUTION OF NdNP@PB NANOCOMPLEX.	143
FIGURE 5.14 COMPARISON OF <i>IN VIVO</i> PTT.	143
FIGURE 5.15 <i>IN VIVO</i> LOCAL TEMPERATURE CHANGE.	144
FIGURE 5.16 COMPARISON OF <i>IN VIVO</i> PTT.	145
FIGURE 5.17 BODY WEIGHT CHANGES OF HELA TUMOR-BEARING MICE IN DIFFERENT GROUPS.	146
FIGURE 5.18 STAINING OF TUMORS IN CONTROL GROUPS.....	146
FIGURE 5.19 THE HISTOLOGY ANALYSIS OF THE MAJOR ORGANS.	147
FIGURE 6.1 ABSORPTION OF WATER AND AUTOFLUORESCENCE OF EX VIVO MOUSE UNDER 808 NM EXCITATION.	151

List of Schemes and Tables

SCHEME 3.1 THE DESIGN OF FROM LARGE TWO-LAYER UCNPs TO ULTRASMALL-SUPERBRIGHT SANDWICH-STRUCTURED UCNPs.	49
SCHEME 5.1 FABRICATION OF NdNDF ₄ @PB CORE/SHELL STRUCTURE TO GENERATE NEW CROSS-RELAXATION PATHWAYS TO ENHANCE PHOTOACOUSTIC IMAGING SIGNAL AND PHOTOTHERMAL THERAPY EFFICACY.	118
TABLE.1 EXPERIMENTAL QUANTUM YIELDS.	69

List of Publications

1. **Yu, Z.**,[#] Hu, W.,[#] Zhao, H., Miao, X., Guan, Y., Cai, W., ... & Tan, T. T. Y.* (2019). Generating New Cross-Relaxation Pathways by Coating Prussian Blue on NaNdF₄ to Fabricate Enhanced Photothermal Agents. *Angewandte Chemie (International ed. in English)*, 58(25), 8536. [#]Co-first Author
2. **Yu, Z.**, Chan, W. K., & Tan, T. T. Y.* (2019). Neodymium-Sensitized Nanoconstructs for Near-Infrared Enabled Photomedicine. *Small* (accepted). DOI: 10.1002/sml.201905265.
3. Zhang, Y.,[#] **Yu, Z.**,[#] Li, J., Ao, Y., Xue, J., Zeng, Z., ... & Tan, T. T. Y.* (2017). Ultrasmall-superbright neodymium-upconversion nanoparticles *via* energy migration manipulation and lattice modification: 808 nm-activated drug release. *ACS nano*, 11(3), 2846-2857. [#]Co-first Author
4. Zeng, Z., Xiao, F. X., Phan, H., Chen, S., **Yu, Z.**, Wang, R., ... & Tan, T. T. Y.* (2018). Unraveling the cooperative synergy of zero-dimensional graphene quantum dots and metal nanocrystals enabled by layer-by-layer assembly. *Journal of Materials Chemistry A*, 6(4), 1700-1713.
5. Ao, Y., Zeng, K., Yu, B., Miao, Y., Hung, W., **Yu, Z.**, ... & Yang, X. (2019). An Upconversion Nanoparticle Enables Near Infrared-Optogenetic Manipulation of the *Caenorhabditis elegans* Motor Circuit. *ACS nano*, 13(3), 3373-3386.
6. Li, J., Huang, J., Ao, Y., Li, S., Miao, Y., **Yu, Z.**, ... & Yang, X. (2018). Synergizing upconversion nanophotosensitizers with hyperbaric oxygen to remodel

the extracellular matrix for enhanced photodynamic cancer therapy. ACS applied materials & interfaces, 10(27), 22985-22996.

7. Chen, Y., Song, J., He, G. S., Hu, W., **Yu, Z.**, Zhou, T., ... & Prasad, P. N. (2018). Polymer-assisted room-temperature synthesis of highly luminescent perovskite nanocrystals with superior water resistance for WLED. Materials Letters, 232, 138-141.

Abstract

Lanthanide-doped nanoconstructs have gained increasing attention in recent decades due to their unique properties, especially optical properties. The design of various lanthanide-based nanosystems are expected to contribute to medical and health applications, due to their advantageous properties such as excellent photostability, non-photobleaching and low cytotoxicity, etc. Among all kinds of lanthanide ions, neodymium (Nd^{3+}) ions are highly favorable since they can absorb near-infrared light, specifically at 808 nm, and upconvert them to higher-energy light or heat. This biological transparent absorption can overcome the heating up effect caused by conventional ytterbium (Yb^{3+}) ions sensitization. However, the low conversion efficiency and potential long-term toxicity of Nd^{3+} -sensitized nanoconstructs are huge obstacles to their clinical translations. This thesis has contributed to this field in the design and fabrication novel Nd^{3+} -sensitized nanoconstructs for near-infrared (NIR) enabled photomedicine, making them potentially safer, more efficient and closer to clinical usage.

First of all, sandwich-structure ultrasmall (<10 nm) Nd^{3+} -sensitized upconversion nanoparticles (UCNPs) have been synthesized for the first time to exploit the benefits of ultrasmall nanoparticles while maintaining a relatively strong blue emission. We adopt a combinatorial strategy of energy migration manipulation and

crystal lattice modification, creating ultrasmall-superbright Nd^{3+} -sensitized nanoparticles with 2 orders of magnitude enhancement in upconversion luminescence. Furthermore, we apply the nanoparticles to 808 nm light-mediated drug release. The results indicate time-dependent cancer cells killing and better antitumor activities. These ultrasmall-superbright dots have unraveled more opportunities in upconversion photomedicine with the promise of potentially safer and more effective therapy.

Furthermore, we have tuned the configuration of tetralayer Nd^{3+} -sensitized UCNPs in a fixed size for the first time. Specifically, we tune the thickness of sensitizing layer containing Nd^{3+} ions and inert layer of gadolinium (Gd^{3+}) ions in a fixed combined thickness of 5 nm in tetralayer UCNPs to exclude size effect and enhance 4-photon conversion (360 nm, UV emission) process. An optimal combination of thickness of sensitizing and inert layer is uncovered to be 3 and 2 nm respectively, showing a new strategy of balancing sensitization and surface passivation to enhance 4-photon emission, while 3-photon emission (475 nm, blue) is mainly influenced by the overall nanoparticle size and its emission intensity remains similar in all the tetralayer UCNPs. In addition, 808-nm cross-linked hydrogel has been demonstrated. Our results have uncovered the structural parameters for optimal UV upconversion emissions and provided design guidance for lanthanide nanostructural configuration.

Taking advantage of the low photon conversion efficiency of UCNPs, the last part of this thesis demonstrates the boosting the nonradiative process by generating new cross-relaxation pathways. Cross-relaxation among sensitizers is commonly regarded as deleterious in fluorescent materials, although favorable in photothermal agents. Herein, we coat Prussian blue (PB) on NaNdF₄ nanoparticles (NdNPs) to fabricate core-shell nanocomplexes with new cross-relaxation pathways between the ladder-like energy levels of Nd³⁺ ions and continuous energy band of PB. The photothermal conversion efficiency was improved significantly and the mechanism of the enhanced photothermal effect was investigated. *In vivo* photoacoustic imaging and photothermal therapy demonstrated the potential of the enhanced photothermal agents. Moreover, the concept of generating new cross-relaxation pathways between different materials is proposed to contribute to the design of all kinds of enhanced photothermal agents.

In summary, this thesis has provided some novel strategies to fabricate Nd³⁺-sensitized nanoconstructs to enhance the upconversion efficiency or photothermal conversion efficiency. We hope the strategies and studies presented in thesis can further enhance the attractiveness of lanthanide-based materials towards clinical translation.

CHAPTER 1 Introduction

1.1 Background

The recent decades have witnessed the rapid development of nanotechnology, along with the emergence of all kinds of nanomaterials. Materials exhibit unique properties at the nanoscale. Researchers are exploring and utilizing these unique properties to design numerous nanoconstructs for technical revolutions. In the biomedical field, enhanced permeability and retention (EPR) effect has been developed to adapt nanoconstructs with certain sizes for more accumulations in tumor sites.[1] Nanomedicines are thus highly favorable in cancer treatments. Human has the dream of controlling the power of light since ancient times. Therefore, the combination of light and nanoconstructs forms a new method for the diagnosis, treatment, understanding of health and disease.[2]

There have been many types of photomedicine, including carbon materials,[3] noble-metal materials,[4] lanthanide materials,[5] quantum dots,[6] organic small molecules and nanoparticles.[7] Different materials show their advantages and disadvantages. While lanthanide materials showed irreplaceable properties, such as large and easy Stokes and anti-Stokes shifts, sharp absorption and emission, inherent coupling of optical and magnetic properties, excellent photo- and chemical stabilities.[8] These properties give enough room for the design and applications of

lanthanide nanoconstructs. Based on these properties, plentiful combinations of different types of lanthanides have been developed. Neodymium (Nd^{3+}) ions featuring 808 nm absorption have deeper penetration depth and negligible heating up effect. Hence, they are popular in NIR-enabled photomedicine.[9]

This thesis will mainly focus on the design, fabrication and application of Nd^{3+} -sensitized nanoconstructs for NIR-enabled photomedicine for chemotherapy, photo-cross-linking and photothermal therapy.

1.2 Motivation and Objectives of This Thesis

Even the Nd^{3+} -sensitized nanoconstructs are attractive in NIR-enabled photomedicine, there are some key challenges for their clinical applications.

The first challenge is the low quantum yield and conversion efficiency of Nd^{3+} -sensitized nanoconstructs when they are applied as light converters. The power density of 808 nm is limited to below 0.33 W cm^{-2} in the clinical treatments. To get a strong large anti-Stokes shift under such a low excitation power density is quite challenging. The second challenge is the controlment of energy migration pathways in Nd^{3+} -sensitized nanoconstructs. Nd^{3+} ions have ladder like energy levels, which will cause cross-relaxation to quench luminescence. Careful and smart design of the configuration is vital, especially for multiphoton conversion processes. The

third challenge is the sharp and weak absorption of Nd^{3+} ions. The narrow and sharp emission of lanthanide ions is an excellent advantage over other materials, while it has been a barrier for absorption. How to obtain a wide and strong absorption during the fabrication of Nd^{3+} constructs is still a conundrum. Last but not least, the potential long-term toxicity issue is always a puzzle at the current stage due to the slow excretion and high accumulation in reticuloendothelial system.[10] Study to have a clear view of the excretion pathways and high tumor accumulation is needed even the lanthanide-doped nanoparticles are reported to be safe at current stage.[11]

The objectives of this thesis are listed as below.

1. Synthesize ultrasmall (<10 nm) sandwich-structure Nd^{3+} -sensitized UCNPs for the first time *via* energy migration and lattice modification approaches to maintain the strong blue emission at ultrasmall range. Apply these ultrasmall-superbright UCNPs in 808 nm-controlled DOX release.
2. Tune the configuration of tetralayer UCNPs to enhance the multiphoton UV emission at 360 nm. Generate a new enhancement method by tuning configuration in the same size to enhance UV emission and demonstrate their applications in NIR-enabled cross-linking.

3. Fabricate core/shell NdNP@PB nanocomplex for the first time to generate new cross-relaxation pathways to enhance the photothermal conversion efficiency. Demonstrate their application in photoacoustic imaging and photothermal therapy.

1.3 Organization of This Thesis

The thesis aims to provide novel Nd³⁺-sensitized nanoconstructs for enhanced therapeutic efficacy as NIR-enabled photomedicine, making Nd³⁺-sensitized nanoconstructs safer, more efficient and closer to clinical applications. The organization of this thesis is listed as below.

Chapter 2 is a literature review to comprehensively interpret the possible energy transfer pathways of Nd³⁺-sensitized nanoconstructs, which may inspire the generation of the design of novel Nd³⁺-sensitized nanoconstructs. Their applications as photomedicine in recent years have been summarized, such as biomedical imaging, NIR-triggered chemotherapy, photodynamic therapy, photothermal therapy and optogenetics.

The following chapters list individual works to achieve the objectives of this thesis. Chapter 3 includes the synthesis of ultrasmall sandwich-structure UCNPs with intense blue emission with control experiments to optimize the synthesis process. Chapter 4 extends the work of Chapter 3 to add an additional inert layer on the

sandwich-structure UCNPs. Meanwhile, the UV emission has been enhanced by tuning the thickness of sensitization and inert layer with a total thickness of 5 nm.

Chapter 5 convert the harmful cross-relaxation in light-to-light conversion to boost the photothermal conversion efficiency by generating new cross-relaxation pathways between different materials

At last, Chapter 6 concludes all these finished works and gives suggestions for future work.

CHAPTER 2 Literature Review

2.1 Introduction

Lanthanide-doped nanoconstructs have gained increasing attention due to their unique magnetic and optical properties. The gradual filling of 4f orbits endows lanthanides abundant metastable energy levels from ultraviolet (UV) to visible (VIS) to near infrared (NIR) range, thus generating numerous narrow absorption and emission bands.[5] Energy transfer between different energy levels in the same type and different types of lanthanide ions can generate a large range of emissions. Tunable large Stokes and anti-Stokes shifts can be uniquely generated in lanthanide-doped nanoconstructs. Moreover, lanthanide ions have superior photo- and chemical stability, no photobleaching, which can overcome the limitations of commonly used organic dyes.[8] Therefore, design of lanthanide-doped nanoconstructs to get strong emissions for various biomedical applications has been a research hotspot.

Different types of lanthanide ions have been applied as sensitizers to absorb energy from excitation light, while NIR-absorbing lanthanide sensitizers are highly favorable in biomedical applications, since NIR light has a deeper penetration depth.[12] The improved penetration depth in biological tissues is due to the reduced absorption and scattering, compared with the conventional visible range

(400-700 nm).[13] The first near-infrared window (NIR-I, 650-950 nm), is define as biological transparency window. NIR-I light can achieve a penetration depth to several millimeters, while disadvantaged by tissue autofluorescence.[13, 14] Second near-infrared window (NIR-II, 1000-1700 nm) has the merits of further reduced scattering and tissue autofluorescence.[15] Typically, ytterbium (Yb^{3+}), erbium (Er^{3+}) and neodymium (Nd^{3+}) ions have been utilized as NIR photosensitizers due to their absorption around 980, 808/1530 and 808 nm, respectively (Figure 2.1a). However, the absorptions of water and biological tissues around 980 and 1530 nm are strong, which will cause a sharp attenuation of excitation light and cause heating up effect (Figure 2.1b, c).[16, 17] Hence, 808 nm excitation is highly favorable to minimize the heating up effect.

Figure 2.1 Absorptions and emissions of lanthanide ions. (a) The absorption spectra of Yb^{3+} , Er^{3+} and Nd^{3+} ions under the same molar concentration. (b) The absorption of water from 700 to 1100 nm (c) Temperature increase induced by 980 and 808 nm laser irradiation in mice. Reproduced with permission.[16] Copyright 2013, American Chemical Society. (d) Typical luminescent emissions of

lanthanides in visible range. Reproduced with permission.[18] Copyright 2014, American Chemical Society. (e) Main luminescent emissions in NIR-II range. Reproduced with permission.[19] Copyright 2016, American Chemical Society.

Unlike the single energy states ($^2F_{5/2}$) of Yb^{3+} ions, Nd^{3+} ions feature ladder-like energy states, which can cause severe cross-relaxation among themselves or with other emitter ions when they have direct interaction. Therefore, careful design of the configuration of Nd^{3+} -sensitized nanoconstructs is vital to enhance the application efficacy.[20] Many efforts have been made in recent studies to apply Nd^{3+} -sensitized nanoconstructs as photomedicine for various biomedical applications. Currently, there is no comprehensive review on the Nd^{3+} -sensitized nanoconstructs to give an overall depiction of the properties of Nd^{3+} ions and their potential applications in the field of health and medical technology.

In this literature review chapter, we will give an introduction of Nd^{3+} -sensitized nanoconstructs including their (I) merits and shortcomings, (II) energy conversion processes and pathways and (III) recent discussions for potential applications as photomedicine. A detailed discussion about the existing problems and challenges of Nd^{3+} -sensitized nanoconstructs, including the corresponding potential solutions is also addressed and emphasized in this literature review.

2.2 Merits and Shortcomings of Nd^{3+} Ions

All lanthanide elements share a similar electronic configuration, which is

[Xe]4fⁿ5d¹6s² with n=0 (La) to 14 (Lu). The gradual filling of 4f orbits and internal f-f transitions make most lanthanide ions luminescent except La³⁺ and Lu³⁺ ions.[5] Transitions between f orbitals of lanthanide ions are parity forbidden and some f-f transitions are even spin-forbidden.[21] Thus, the intra-configuration f-f transitions are weak. The absorption of lanthanide ions is commonly limited to small absorption coefficients (<10 M⁻¹ cm⁻¹) and the corresponding emissions are also narrow and identical.[22] These properties make lanthanides attractive in all kinds of optical and biomedical applications.

The emission of lanthanides can cover almost the entire spectrum from UV to NIR range (300 nm to 2.2 μm, Figure 2.1d, e), thus tuning the type of dopants and ratio of lanthanides have been and still is a research hotspot to achieve new emission properties for various different applications.[18, 19] However, the weak absorbance of lanthanide sensitizers is a vital limitation, since it requires high or expensive excitation power source to achieve certain functions. In the field of photomedicine, NIR-absorbing photosensitizers are highly favorable due to the higher penetration depth of NIR photon and lower attenuation rate compared with higher-energy light (VIS or UV light). Light with a wavelength from 700 to 1100 nm is regarded as optical transparent, which is a great benefit for applications such as bioimaging and phototherapy. Currently, there are only several types of lanthanide sensitizers to be applied as NIR photosensitizers, including Yb³⁺, Nd³⁺ and Er³⁺ ions. The absorption

properties of these photosensitizers under the same molar concentration have been measured and compared (Figure 2.1a). The widely used Yb^{3+} ions have a single absorption around 980 nm, which is unique and attractive. Nevertheless, the strong water absorption band around 980 nm makes Yb^{3+} -sensitized nanoconstructs not ideal for biomedical or health applications, as it will cause strong heating up effect and kill normal cells and tissues by just shining 980 nm laser (Figure 2.1c).[16] 808 nm excitation may provide a good solution to overcome the issues of heating up effect and shallow penetration depth, since the absorption of water around 808 nm is quite weak (Figure 2.1b). Nd^{3+} ions have multiple relatively strong absorption peaks around 730 and 808 nm. Even Er^{3+} ions have been reported to be able to be excited by 808 nm light, their absorbance is much weaker than that of Nd^{3+} ions. In a nutshell, Nd^{3+} ions are most suitable for NIR enabled photomedicine at the current stage among all kinds of lanthanide ions, having attractive traits such as deeper penetration depth, minimal heating up effect, high chemical and photo-stability, unique light conversion ability and ease of multifunctionality.

However, the absorption of Nd^{3+} ions is weak and narrow compared with other dyes or photosensitizers. The absorption cross section of Nd^{3+} ions at 800 nm is just around $2 \times 10^{-20} \text{ cm}^2$. [23] The limitation of power density for safe usage of 808 nm laser in clinical treatments is 0.33 W cm^{-2} . [24] Therefore, strategies to enhance and broaden the absorbance of Nd^{3+} ions have been investigated, one of which is to

combine the lanthanide-doped nanoparticles (NPs) with other organic and inorganic dyes, since these dyes have much higher absorption cross section, for example indocyanine green (ICG) has an absorption cross section of $6 \times 10^{-16} \text{ cm}^2$ at 800 nm.[23] Tunable absorption properties have been achieved by changing the type of dyes due to previous reports, such as IR-783, IR-806, IR-808, IR-820, IR-845, ICG, Prussian blue (Figure 2.2).[25-28]

Figure 2.2 Tunable absorption of lanthanides by combining with different dyes. (a) Absorption and emission spectra of ICG, absorption of Nd³⁺ and Yb³⁺ ions. Reproduced with permission.[25] Copyright 2016, Wiley-VCH. (b) Absorption of Yb³⁺-sensitized UCNP with and without IR-806 dye. Reproduced with permission.[26] Copyright 2016, American Chemical Society. (c) UCNP with tunable excitation wavelength. Reproduced with permission.[27] Copyright 2015, Royal Society of Chemistry. (d) Absorption spectra of NaNdF₄ nanoparticles, Prussian blue and their nanocomplex. Reproduced with permission.[28] Copyright 2019, Wiley-VCH.

Even the combination of dye and lanthanides can enhance and broaden the absorbance of lanthanide ions, while the energy transfer efficiency, photostability and water solubility of dyes, especially for organic dyes, are still serious limitations yet to be solved. The current reported combinations of lanthanides and organic dye cannot remain stable in aqueous environment.[19] Good surface functionalization strategies of the dyes on lanthanide nanocrystals while maintaining efficient energy transfer from dye to lanthanides will be highly favored yet very difficult to achieve.

2.3 Energy Conversion in Nd³⁺-Sensitized Nanoconstructs

Nd³⁺-sensitized nanoconstructs can convert the absorbed energy to luminescence or heat *via* radiative or nonradiative relaxation pathways, respectively. Therefore, the light conversion pathways can be broadly divided into three types: (I) upconversion (UC), (II) downconversion (DC) and (III) photothermal conversion. Among all these conversion methods, UC and DC are radiative conversion processes and photothermal is a nonradiative conversion process. All these processes have great potentials in biomedical applications. This section will give a thorough review, to the best of authors' ability and knowledge, of the state-of-art of energy conversion in Nd³⁺-sensitized nanoconstructs, which hopefully will provide ideas and inspire novel Nd³⁺-sensitized nanoconstructs.

2.3.1 UC

UC is a nonlinear light conversion process, in which the absorbed two or more low-

energy photons can be excited to higher energy states and emit a high energy photon.[8] Among all kinds of UC materials, lanthanide-doped nanoconstructs are most attractive, due to their high photo- and chemical stability, low autofluorescence and large anti-Stokes shift from NIR to VIS or UV range, which cannot be achieved in other UC materials. Triplet-triplet annihilation (TTA) UC is a type of organic UC process involving a triplet sensitizer and a triplet acceptor.[29] TTA allows low power density excitation and higher quantum yield, while the anti-Stokes shifts are not large enough, usually from VIS to VIS.[29] Compared with most currently used direct UV or VIS excitation phototherapy, such as skin phototherapy and cancer photodynamic therapy, NIR enabled UC has a deeper penetration depth and is safer than UV exposure. The key limitation of UC process is the weak conversion efficiency and low quantum yield, since the large anti-Stokes shift usually involves multiphoton conversion process and is hindered by weak 4f-4f transition.

2.3.1.1 Self-UC of Nd³⁺ Ions

Self-UC in Nd³⁺ ions was observed in Nd³⁺-doped yttrium fluoride (Nd³⁺:YLiF₄, also Nd³⁺: YLF) and Nd³⁺-doped yttrium aluminum borate (Nd³⁺:YAl₃(BO₃)₄, also Nd³⁺:YAB) laser crystal under 810 nm excitation to generate the emission at 413, 523, 588, 640, and 661 nm, which were attributed to $^2D_{5/2}/^2P_{1/2} \rightarrow ^4I_{9/2}$, $^4G_{7/2}/^2K_{13/2}/^4G_{9/2} \rightarrow ^4I_{9/2}$, $^2G_{7/2}/^4G_{5/2} \rightarrow ^4I_{9/2}$, $^2H_{11/2} \rightarrow ^4I_{9/2}$, and $^4F_{9/2} \rightarrow ^4I_{9/2}$ transitions (Figure 2.3a, d).[23, 30, 31] With the development of nanotechnology,

many studies have geared towards making Nd^{3+} -sensitized self-UC into nanomaterials and modifying the host matrix from fluoride compositions to sodium fluoride (NaF), resulting in a significant enhancement of upconversion luminescence (UCL) to ~ 400 times.[32] Even though nanomaterials with self UC properties can be easily synthesized, the UCL is not strong enough since most emission will be quenched by the cross-relaxation between Nd^{3+} ions when the nanoparticles are highly Nd^{3+} -doped. Prasad's group has utilized $\text{NaYF}_4:\text{Nd}^{3+}$ nanoparticles to overcome concentration quenching effect by combining the Nd^{3+} -sensitized self-UC nanoparticles with ICG and enhance the emission under a relatively high concentration of Nd^{3+} ions (Figure 2.3b, c).[23] However, the quantum yield and UC fluorescence is still not comparable with other Nd^{3+} co-doped system. In addition, multiple emission peaks from self-UC nanomaterials are usually not suitable for biomedical applications

With the development of lanthanide technology, Nd^{3+} ions are mostly used in combination with other lanthanide ions for more efficient UC emission. Nonetheless, the understanding the self-UC processes will be beneficial for the conceptual design and engineering of all kinds of Nd^{3+} -sensitized UC system.

Figure 2.3 Absorptions and emissions of lanthanides. (a) Energy level diagram and corresponding excited-state absorption (ESA) and energy transfer upconversion (ETU) processes of Nd^{3+} ions. (b) Emission spectra of Nd^{3+} -doped UCNPs with different dopant concentrations. (c) Corresponding digital pictures for luminescence under 808 nm excitation. (d) Proposed self-UC mechanism. Reproduced with permission[23]. Copyright 2016, American Chemical Society.

2.3.1.2 Nd^{3+} Co-Doped UC

Complex energy levels of Nd^{3+} ions provide adequate space for various designs with different functionalities, while the ladder-like energy levels will cause severe cross-relaxation and energy back transfer when they directly interact with the activators. Therefore, Yb^{3+} ions with single energy level and wide applications in 980 nm-activated UC systems have been applied as bridging ions to spatially separate Nd^{3+} ions and emitter ions. Energy transfer pathways of Nd^{3+} - Yb^{3+} -emitters ($\text{Tm}^{3+}/\text{Er}^{3+}$ etc.) so as to minimize the quenching effect between sensitizing Nd^{3+} and the emitter ions have been established (Figure 2.4a).[33] The high quantum efficiency with negligible energy back transfer between Nd^{3+} and Yb^{3+} ions will promise a comparable or stronger UCL intensity compared with Yb^{3+} -sensitized UCNPs.[34, 35] Under 808 nm excitation, Nd^{3+} -sensitized

nanoconstructs can generate different UC emissions depending on the emitters. For example, Tm^{3+} ions emit at 360, 450, 475, 649, and 696 nm that correspond with $^1\text{D}_2 \rightarrow ^3\text{H}_6$, $^1\text{D}_2 \rightarrow ^3\text{F}_4$, $^1\text{G}_4 \rightarrow ^3\text{H}_6$, $^3\text{F}_2 \rightarrow ^3\text{H}_6$, and $^3\text{F}_3 \rightarrow ^3\text{H}_6$, and Er^{3+} ions emit at 520, 550, and 650 nm that correspond with $^2\text{H}_{11/2} \rightarrow ^4\text{I}_{15/2}$, $^4\text{S}_{3/2} \rightarrow ^4\text{I}_{15/2}$ and $^4\text{F}_{9/2} \rightarrow ^4\text{I}_{15/2}$, respectively.

In recent decades, most reports have focused on the tuning of doing ratio of different ions. However, to reduce cross-relaxation and other non-radiative processes, the dopant concentrations of sensitizers are limited to a relatively low level.[36] The reported emitters doping percentage are typically limited below 5% (for example, 1% for Tm^{3+} , 2% for Er^{3+}) to maintain a relatively strong UC emission. Other efforts were directed to tuning the ratio of Yb^{3+} ions to obtain stronger emission, and the typical values for Yb^{3+} -sensitized UCNPs are 20-30% in the core.[37] The ratio of Nd^{3+} ions is definitely a key point in Nd^{3+} -sensitized UCNPs, while this problem is rather complex due to the involvement the configuration of nanoparticles. Recent studies have thus shifted gears to rational design of nanostructure configuration to overcome the limitation of a low doping concentration of sensitizers and emitters.

The evolution of nanostructure configuration design is depicted in Figure 2.4b. Tri-doped core-shell Nd^{3+} -sensitized UCNPs were first introduced by Han's group with an inert shell, while the doping ratio of Nd^{3+} ions is controlled below 1%.[38]

Highly-doped Core-shell Nd^{3+} -sensitized UCNP were achieved by Liu's group by co-doping Nd^{3+} (1%), Yb^{3+} , $\text{Tm}^{3+}/\text{Er}^{3+}/\text{Ho}^{3+}$ in the core and applying a high concentration of Nd^{3+} (~20%) in the shell.[39] Yan et al. have designed a core-shell structure by adding $\text{Yb}^{3+}/\text{Nd}^{3+}$ co-doped shell on the conventional Yb^{3+} -sensitized core to separate Nd^{3+} ions and emitters.[40] While this separation is not throughout, since Nd^{3+} ions can still directly interact with emitters on the core/shell interface. To reduce undesired energy loss, a sandwich-structured UC system was first developed by Yao's group to boost UC emission by spatially separate Nd^{3+} and emitters ($\text{Tm}^{3+}/\text{Er}^{3+}/\text{Ho}^{3+}$) to achieve a high doping concentration up to 90% of Nd^{3+} ions in the outer layer.[41] Surface passivation has also been an effective approach to enhance the UC efficiency since it creates a barrier to avoid direct interaction of surface ions with the surrounding environment to reduce surface quenching. The emerging tetralayer structure by combining the sandwich structure with inert layer will lead to a stronger emission intensity.[42] Rational design of the configuration has contributed to obtain higher UC efficiency and brighter UC emission. The novel design of configuration may contribute to solve the main limitation of low quantum yield for UCNP system and benefit a large amount of new UC-based attractive applications.

Figure 2.4 Energy transfer and evolution of UCNPs. (a) Typical energy transfer diagram of Nd^{3+} - Yb^{3+} - $\text{Tm}^{3+}/\text{Er}^{3+}$ pathway. Reproduced with permission. [37] Copyright 2015, The Royal Society of Chemistry. (b) Evolution of Nd^{3+} -sensitized tri-doped UCNPs with inert shell to tri-doped UCNPs with active shell to sandwich-structure UCNPs separating Nd^{3+} and emitter ions to tetralayer UCNPs.

2.3.2 DC

Compared with Nd^{3+} -sensitized UC process, Nd^{3+} -sensitized DC process has a much higher quantum yield since it doesn't require multi-photon conversion. However, there are only limited types of lanthanides which can be applied in NIR systems, both excitation (808 nm for Nd^{3+} ions, 980 nm for Yb^{3+} ions and 1530 nm for Er^{3+} ions) and emission (1058 nm for Nd^{3+} , 1180 nm for Ho^{3+} , 1475 nm for Tm^{3+} and 1530 nm for Er^{3+} ions). Water has strong absorption bands around 980 and 1530 nm, especially 1530 nm, which will cause heating up effect for excitation and signal attenuation for emission. Therefore, Nd^{3+} -sensitized downconversion nanoparticles (DCNPs) are considered most suitable in the NIR-II range and are garnering more attention recently and demonstrated in various biomedical

applications.

2.3.2.1 Self-DC of Nd^{3+} Ions

Nd^{3+} ions are unique since they can sensitize NIR light and give out DC NIR emission at the same time. Self-DC in Nd^{3+} -ions is an efficient pathway and has attracted greater attention in recent decades. The key limitations for self-DC of Nd^{3+} -ions are concentration quenching and surface quenching. Under the excitation of 730[43]/808/860 nm laser, Nd^{3+} ions can generate emissions at 900, 1058 and 1332 nm ($^4\text{F}_{3/2}$ to $^4\text{I}_{9/2}$, $^4\text{I}_{11/2}$ and $^4\text{I}_{13/2}$, respectively, Figure 2.5a, b) in the NIR range during the DC process. An increase in the concentration of Nd^{3+} ions entails the increase in cross-relaxation process, quenching fluorescence and simultaneously generating heat at higher doping ratio attributed to shorter ionic distance (Figure 2.5c).[44] The lifetime of Nd^{3+} -doped DCNPs can be tuned by changing the dopant concentration of Nd^{3+} ions (Figure 2.5d).[44] Therefore, the optimal doping concentration of Nd^{3+} -doped DCNPs is limited to a relatively low 2%.[45] Another key challenge of Nd^{3+} -doped DCNPs is surface quenching since direct contact of surface ions with surface molecules will generate surface phonons [46], while surface defects of nanoparticles will also quench the emission. In the fabrication of Nd^{3+} -doped DCNPs, a careful design to balance and elimination of concentration and surface quenching is currently essential. Even though the quantum yield (QY) of Nd^{3+} -doped DCNPs is much higher than that of Nd^{3+} -doped UCNPs, while the aim of improving the QY of DCNPs to high enough (>30%) has not been achieved

yet. Therefore, there is plenty of room for the improvement of DC efficiency.

Figure 2.5 Self-DC mechanism. (a) Energy transfer diagram of Nd^{3+} ions to show the self-DC emission. (b) Emission spectrum of NaNdF_4 nanoparticles and digital pictures of NIR-II fluorescence (inset). Reproduced with permission.[28] Figure 2.5a, b, copyright 2019, Wiley-VCH. (c) Emission intensity change influenced by Nd^{3+} concentrations. Reproduced with permission.[45] Copyright 2019, Elsevier. (d) Tuneable lifetime of different Nd^{3+} -doped DCNPs. Reproduced with permission.[44] Copyright 2017, The Royal Society of Chemistry.

2.3.2.2 DC between Different Lanthanides

Recent studies have tried to improve the QY of Nd^{3+} -doped self-DCNPs and expand their applications in all kinds of biomedical applications. While Nd^{3+} ions still possess other possibilities, they can transfer energy to other lanthanides, serving as sensitizers/ bridge ions and give out different NIR-II emissions. The most widely reported DC between different lanthanide ions is $\text{Nd}^{3+}\text{-Yb}^{3+}\text{-X}^{3+}$, X can be Er, Ho,

Tm, or Pr (Figure 2.6a).[19] This process is similar to the Nd^{3+} -sensitized co-doped UC process, since it also involves the bridging Yb^{3+} ions. Thus, it requires careful design and inevitable UC process aiming for higher quantum yield. Reducing the types of lanthanides involved is intended to reduce the energy loss in the DC process. The transfer between Nd^{3+} and Yb^{3+} ions has been reported to possess a high quantum yield of $\sim 20.7\%$ with emission at 980 nm *via* so-called energy transfer highway (Figure 2.6b).[47] Another reported DC between different lanthanide ions is Nd^{3+} - Ho^{3+} , which will generate both emissions of Nd^{3+} ions and a new emission of 1167 nm of Ho^{3+} ions (Figure 2.6c), though relatively weak.[48]

With the development of NIR-II technology, more combination of lanthanides with NIR-II emission and Nd^{3+} ions are expected to be uncovered with more efficient energy transfer to obtain a higher quantum yield in the DC process.

Figure 2.6 DC energy transfer with other lanthanides. (a) $\text{Nd}^{3+}@\text{Yb}^{3+}@\text{Yb}^{3+}/\text{X}^{3+}$ ($\text{X} = \text{Er}, \text{Ho}, \text{Tm}$, or Pr) DC energy transfer pathways with dye sensitization. Reproduced with permission.[19] Copyright 2016, American Chemical Society. (b) Energy transfer highway between Nd^{3+} and Yb^{3+} ions under the protection of CaF_2 inert shell. Reproduced with permission.[47] Copyright 2017, American Chemical Society. (c) Energy transfer diagram between Nd^{3+} and Ho^{3+} ions. Reproduced with permission.[48] Copyright 2017, The Royal Society of Chemistry.

2.3.3 Photothermal Conversion

Nd^{3+} ions have mostly been studied for all kinds of photo-to-photo conversion processes, while their photothermal conversion ability is underrated. Heat generation by Nd^{3+} ions is mainly decided by the cross-relaxation pathways between Nd^{3+} ions, along with other nonradiative processes in energy migration, which usually requires a high dopant concentration (Figure 2.7a-c).[49] Nd^{3+} -doped nanoparticles can be designed to convert light to heat in situ with a high stability

and ease of morphology modification.[50] The key limitation for Nd^{3+} ions as photothermal agents (PTAs) is the weak absorption around 808 nm compared with other widely studied PTAs, especially organic PTAs.[28] Our group has recently fabricated NdNP@PB nanocomplex by coating a thin layer of PB on NaNdF_4 NPs (Figure 2.7d). This combination has generated new and much shorter cross-relaxation pathways between Nd^{3+} ions and PB, significantly boosting the photothermal conversion efficiency to 60.8%.[28] This new concept of uncovering new cross-relaxation pathways between different PTAs should inspire new PTA nanoconstructs with great efficiency.

Figure 2.7 Energy diagram of photothermal conversion. Schematic diagram of radiative process to generate (a) fluorescence and (b, c) nonradiative processes including cross-relaxation and energy migration to generate heat in Nd^{3+} ions under 808 nm excitation and (d) generation of new cross-relaxation pathways by coating PB on NaNdF_4 . Reprinted with permission.[49] Figure 2.7a-c, copyright 2014, AIP Publishing LLC. Reprinted with permission.[28] Figure 2.7d, copyright 2019, Wiley-VCH.

2.4 NIR-Enabled Photomedicine

Based on the above-mentioned energy conversion capability, Nd^{3+} -sensitized

nanoconstructs have been widely demonstrated in NIR-enabled photomedicine, namely biomedical imaging, NIR-triggered chemotherapy, photodynamic therapy (PDT), photothermal therapy (PTT) and optogenetics.

2.4.1 Biomedical Imaging

Biomedical imaging is a helpful tool for visualizing all kinds of tissues and organs and monitoring their structures and functionalities before, during and after medical treatments. Different biomedical imaging modalities have been developed to achieve higher signal/ noise ratio, sensitivity and resolution and faster feedback. In recent years, Nd³⁺-sensitized nanoconstructs have been created and applied in different imaging modalities. In all kinds of imaging modalities, UCL has been well established, summarized and applied in bioimaging and anticounterfeit applications.[5, 51] Meanwhile, the generated UC emission is usually of high energy and short wavelength, the emission is easily scattered or absorbed in biological tissue. This review will mainly focus on some new imaging modalities with recent research progress.

2.4.1.1 NIR-II Imaging

The emergence of near infrared window II (NIR-II, 1000-1700 nm) imaging technology has further extended the penetration depth and resolution compared with NIR-I and visible emission due to the weaker autofluorescence and absorption and scattering of the biological tissues.[15, 17] Among all kinds of NIR-II imaging

probes, Nd^{3+} -sensitized DCNPs are very attractive and have been demonstrated in different kinds of functionalized NIR-II imaging modalities. Li et al. developed a small molecular NIR-II imaging probe based on Nd-diethylene triamine pentaacetate acid (Nd-DTPA) by the inspiration of Gd-DTPA, a magnetic resonance imaging contrast agent approved by USFDA (Figure 2.8a).[52] This NIR-II imaging probes can generate strong 1050 and 1330 nm emissions, which has shown good contrast and fast renal clearance (Figure 2.8b). Tiny tumor detection and X-ray imaging were also demonstrated. Cao et al. applied the energy transfer highway between Nd^{3+} and Yb^{3+} ions to form $\text{NaYF}_4:\text{Yb,Nd}@ \text{CaF}_2$ DCNPs to achieve a *in vivo* blood vessel imaging with a high spatial resolution (~ 0.19 mm, Figure 2.8c).[47] He et al. reported a Nd^{3+} -sensitized $\text{NaYF}_4/\text{Nd } 7\% @ \text{NaYF}_4$ DCNP coated with DSPE-mPEG, which showed a high affinity to skeleton and good potential for bone imaging (Figure 2.8d).[53] Wang et al. modified the surface of $\text{NaGdF}_4: 5\% \text{ Nd} @ \text{NaGdF}_4$ DCNPs with complementary DNA and follicle-stimulating hormone ($\text{FSH}\beta$) peptide to improve the ovarian tumor targeting efficiency.[54] This functionalized DCNP construct showed a high NIR-II imaging contrast and a deep tissue penetration depth (8 mm), high accumulation in tumor by sequence injection strategy to give a consist and clear tumor margin with MRI (Figure 2.8e).

Figure 2.8 Recent achievements in functionalized NIR-II imaging. (a) Scheme of the molecular structure of Nd-DTPA and (b) *in vivo* NIR-II imaging with high accumulation in kidney and fast renal clearance. Reproduced with permission.[52] Copyright 2018, Elsevier. (c) *In vivo* NIR imaging of blood vessel in mice. Reproduced with permission.[47] Copyright 2017, American Chemical Society. (d) *In vivo* NIR-II imaging of mice bone in the 1345 (up) and 1064 (bottom) nm windows. Reproduced with permission. [53] Copyright 2019, American Chemical Society. (e) Comparison of MRI and NIR-II fluorescence imaging of subcutaneous human ovarian adenocarcinoma. Reproduced with permission.[54] Copyright 2018, Springer Nature Publishing Group.

2.4.1.2 Lifetime-Resolved Multiplexed Imaging

The development of NIR-II technology has shown great potential in deep tissue imaging. While in a complicated biological system, different tissues have different optical properties (absorption, scattering etc.), which will influence the quantitative imaging and multiplexed imaging.[13, 55] *In vivo* tunable multiplexed NIR-II imaging is yet challenging due to the limited types of NIR-II emitters and hurdle of

interference of biological tissues.[55] Zhang's group reported a Nd^{3+} -sensitized NIR-II DCNPs with different configuration to obtain easily tunable lifetime at 1525 nm (Figure 2.9a).[56] Lifetime-resolved *in vivo* multiplexing imaging was demonstrated. Three types of DCNPs with different lifetimes were conjugated with three different antibodies and then injected intravenously in mice to check for the expressions of biomarker in tumor (Figure 2.9b). Notably, lifetime-resolved imaging is not affected by the tissue thickness and wavelength-dependent attenuation from different biological substances. By designing brighter DCNPs or changing the excitation source to NIR-II range, the current penetration depth of 8 mm is supposed to boost to several centimeters. Ortgies et al. also reported *in vivo* multiplexed imaging by tuning the lifetime of $\text{NaYF}_4:\text{Yb},\text{Nd}@ \text{CaF}_2$ by adjusting the concentration of Nd^{3+} dopant.[57]

At the same time, Zhang's group presented a new multiplexing concept by combining the UC emission and decayed lifetime to create binary encoding (Figure 2.9c). Similar to the DCNPs system, they changed the thickness of intermediate layer of the sandwich structure to tune the decayed lifetime while the emission peaks of UCNPs remained the same in the tuning process in blue (Tm^{3+}), green (Er^{3+}), red emissions (Er^{3+} and Pr^{3+}), which is consistent with our former report of tuning the intermediate layer will change the fluorescence in the sandwich-structured UCNPs (Figure 2.9e).[35, 58] They tried to build up a time-resolved

multiplexing imaging system to guarantee every single microsphere can be identified by its specific lifetime code to achieve high capacity (Figure 2.9d). They further demonstrated the security inks using UCNPs with different lifetimes to show distinct colors under the lifetime channel, while showing the same color when seeing through the color channel (Figure 2.9f).

In vivo multiplexing showed the potential to track numerous types of drugs, cells or tumors etc. utilizing the same excitation sources with limited types of emitters. This Nd³⁺-sensitized NIR-II lifetime-resolved *in vivo* multiplexing technology may contribute to the development of diagnostic filed.

Figure 2.9 Recent achievements in lifetime-resolved multiplexing imaging. (a) Pseudocolour-mapped lifetime images of the Nd^{3+} -sensitized Er^{3+} -emissive nanoparticles with different configuration acquired by the time-resolved NIR-II imaging system. (b) Lifetime-resolved imaging for *in vivo* multiplexing of tumor biomarkers. Nanoparticles are conjugated to three antibodies (anti-Er, anti-PR and anti-HER2) to quantify the biomarker expressions in tumor *via* lifetime-resolved imaging. Reproduced with permission. [56] Copyright 2018, Springer Nature Publishing Group. (c) Schematic illustration of UC wavelength and lifetime binary encoding. (d) Illustration of time-

resolved imaging system. (e) Emission spectra of blue, green and red emissions of UCNPs and decay curves of UCNPs with different shell thickness. (f) Lifetime populations for each of the three-color bands and demonstration of multiplexing imaging and security inks using the time-resolved imaging scanning system. Reproduced with permission.[58] Copyright 2018, Wiley-VCH.

2.4.1.3 Photoacoustic Imaging

Photoacoustic imaging (PAI) is an imaging modality based on the photothermal expansion of tissue to generate acoustic signal under the excitation of laser, which is known as photoacoustic (PA) effect [59] and has the distinctive advantages of high contrast of optical imaging and high spatial resolution of ultrasound.[60] The contrast of PAI is mainly decided by the photothermal conversion ability of the PTAs.[28]

Nd^{3+} -sensitized nanoconstructs have been demonstrated as a promising PTA. Wang et al. combined the Nd^{3+} -sensitized UCNPs with different kinds of dyes for different purposes (Figure 2.10a).[61] As a two-photon dye, AF-350 was selected to absorb the pulsed laser and DM-Nitro was chosen as a visible-light absorbing dye. Both the combinations of UCNPs+DM-Nitro and AF-350+DM-Nitro showed enhanced photoacoustic signals since the UC-converted light by has been transferred to acoustic signal absorbed by DM-Nitro (Figure 2.10b). Prasad's group has also demonstrated enhanced PAI by loading ICG into the mesoporous silica shell coated on the UCNPs to avoid the rapid hydrolysis of ICG (Figure 2.10c).[62] Our group uncovered a new cross-relaxation pathway in photothermal effect and demonstrated

enhanced PAI by fabricating Nd^{3+} -doped nanoparticles (NdNPs) coated with Prussian blue (PB) (core-shell NdNP@PB nanocomplex) (Figure 2.10d).[28] The enhanced photothermal conversion efficiency was attributed to a significantly shorter cross-relaxation pathway between Nd^{3+} ions and PB, resulting in a much stronger non-radiative pathway. NdNP@PB nanocomplex showed excellent PA contrast to visualize a clear tumor profile with fast clearance (Figure 2.10e, Chapter 5 in this thesis).

Figure 2.10 Recent achievements in photoacoustic imaging. (a) Scheme to show the pulsed-laser-based PAI and intensity-modulated continuous-wave laser-based PAI. (b) PAI obtained with 800 nm excitation of tube phantom imaged through 1.3-cm-thick chicken breast tissue. Reproduced with permission.[61] Copyright 2017, American Chemical Society. (c) Mice brain imaging after injection of $\text{UCNP@mSiO}_2\text{-ICG}$ and pure ICG. Reproduced with permission.[62] Copyright 2017, Springer Nature Publishing Group. (d) *In vitro* PAI of NdNPs, PB and NdNP@PB nanocomplex and PAI of NdNP@PB nanocomplex under different concentrations. (e) *In vivo* PAI using NdNP@PB nanocomplex as contrast agents. Reproduced with permission.[28] Copyright 2019, Wiley-VCH.

2.4.2 NIR-Triggered Chemotherapy

Photo-triggered release of therapeutics can manipulate spatial and temporal release of drugs by simply shining light, achieving better therapeutic efficacy and targeting. Compared with UV or visible light-enabled system, NIR-triggered release system allows deeper penetration depth and alleviate phototoxicity concerns. Nd^{3+} -sensitized UCNP which can convert 808 nm to higher-energy light in situ have been demonstrated in different controlled release system. Our group has synthesized ultrasmall (<10 nm) Nd^{3+} -sensitized UCNP with superbright blue emission for the first time and demonstrated their application in 808-nm-activated doxorubicin (DOX) release (Chapter 3 in this thesis).[35] We coated mesoporous silica shell onto the ultrasmall UCNPs and loaded DOX in the mesoporous silica shell, the pores opening sealed by surface-functionalized ruthenium (Ru)-polymer. 808 nm-upconverted blue emission from the UCNP triggered bond-severing of Ru-polymer from silica shell, after which DOX can be released (Figure 2.11a).[35] Dai et al. reported an 808 nm-to-UV controlled release system by loading DOX in mesoporous silica shell, modifying the surface with the platinum(IV) pro-drug and adamantane, and blocking the DOX by adding β -cyclodextrin through a host-guest interaction.[63] By the activation of 808 nm light, UCNP can emit UV light to activate the platinum(IV) pro-drug to transfer to platinum(II) complexes with higher toxicity and uncover the blocking of DOX at the same time (Figure 2.11b). Besides loading of anticancer drugs, UCNP nanoconstructs can be designed to

release nitric oxide (NO) as demonstrated by Zhang et al. Roussin's black salt (RBS) was loaded on Tween-modified UCNPs to fabricate the 808 nm-enabled NO-release platform.[64] When excited by 808 nm light, UCNPs can give out UV and blue emission resulting in the photolysis of RBS to produce NO. The synergistic effect of NO and DOX can effectively kill cancer stem-like cells in MCF-7 cells (Figure 2.11c).

Loading drug in nanocarriers always requires additional space. Layers with mesopores or channels have been coated on the surface of UCNPs. Building plenty room to store anti-cancer drug outside of UCNPs is challenging but beneficial. Li et al. combined the UCNPs with porphyrin-based metal-organic framework (MOFs) *via* anisotropic growth (Figure 2.11d).[65] The porphyrin-based MOFs have highly porous structure for DOX encapsulation and overlapping absorption with the green emission of UCNPs for simultaneous PDTs as combined therapeutic system. Zhao et al. demonstrated a NIR-induced decomposition of nanocapsules by lay-by-layer assembly of U/DCNPs and azobenzene functionalized polymers for more efficient tumor accumulation (Figure 2.11e).[66] By shining the NIR light, UCNPs can emit UV and blue light to trigger the photoisomerization of azobenzene groups to release DOX and break down of the large capsules to smaller nanoparticles to enable rapid clearance.

Despite many interesting studies and significant advancements in the field of NIR-triggered therapeutics release by Nd^{3+} -sensitized UCNP, some major issues remain: (1) Low loading efficiency, which is limited by the space through coating additional layers on the UCNP. When the space is too large, UCNP may not generate strong enough emission to achieve controlled release. (2) Leakage and irreversible release. Nanosystems with strong sealant and reversible release are highly favorable. (3) Long-term toxicity. The NPs fabricated for controlled release is usually fairly large (>10 nm) and may face the problem of slow clearance.

Figure 2.11 Recent achievements in NIR-controlled release for chemotherapy. (a) Scheme to show the ultrasmall Nd^{3+} -sensitized UCNP with mesoporous silica shell to load and 808 nm-activated release of DOX by coating Ru-polymer. Reproduced with permission.[35] Copyright 2017, American Chemical Society. (b) Scheme to show the fabrication and controlled release of UCNP@mSiO₂-DPP-DOX@CD. Reproduced with permission.[63] Copyright 2017, The Royal Society of Chemistry. (c) Scheme to show the NIR triggered NO release and DOX to kill cancer stem cells. Reproduced with permission. [64] Copyright, 2017 Science China Press. (d) Fabrication of UCMOFs by growing nMOFs onto UCNP. Reproduced with permission.[65] Copyright 2017,

American Chemical Society. (e) Fabrication of UCNPs-doped hollow polymer nanocapsules and NIR induced decomposing process, and *in vivo* study of NIR-induced decomposition process. Reproduced with permission.[66] Copyright 2018, Wiley-VCH.

2.4.3 Photodynamic Therapy

PDT requires the involvement of photosensitizers (PS) and excitation of light to generate reactive oxygen species (ROS) to cause cell death. Light source applied in PDT is usually UV or visible light, which has the shortcoming of shallow penetration depth up to 1-2 mm in tissue.[42, 67] In NIR photomedicine, PS are combined with UCNPs, which act as phototransducers to generate right emission for *in situ* activation of PS. There are many types of PS that have been combined with UCNPs, such as MC540 (merocyanine 540),[68] ce6 (chlorin(e6)),[69] TMPyP4 (tetra-(N-methyl-4-pyridyl)porphyrin tetratosylate),[70] RF (riboflavin),[71] HP (hematoporphyrin),[72] HA (hypocrellin a),[73] RB (rose Bengal),[74] SPCD (silicon phthalocyanine dihydroxide),[75] Zn-PC (zinc phthalocyanine),[76] MB (methylene blue),[77] Ppa (pyropheophorbide a)[78] etc. Their chemical structures have been listed (Figure 2.12e).[79] Some inorganic PS are also applied with UCNPs in PDT including titanium dioxide (TiO₂),[80] graphitic carbon nitride quantum dots (CNQDs, Figure 2.12c),[81] C60 (C60 fullerene)[82] and hydrogenated black TiO₂ (H-TiO₂),[83] SnWO₄[84] etc. Different strategies of combining UCNPs and PS have been reported, including electrostatic interaction, physical absorption by mesopores, π - π stacking and

covalent bonding etc. Figure 2.12a showed a typical conjugation of RB on UCNPs.[74]

Recent research has focused on how to solve the hypoxia problem to generate enough ROS by providing hyperbaric oxygen with UCNPs+RB to remodel the extracellular matrix for deeper penetration depth (Figure 2.12b) .[85] Other studies focus on enhancing UCNPs, PS or UCNPs connection for higher ROS generation and obtain improved PDT efficacy. He et al. tuned the UC emission peaks to match with the red-light-absorbing PS for stronger spectral overlap (Figure 2.12d).[86] Hou et al. reported a modified PS from pristine TiO_2 to H- TiO_2 to get broadened light absorption and improved performance (Figure 2.12f).[83] Yang et al. co-loaded MC540 with ZnPc to fully utilize the green and red emission of UCNPs (Figure 2.12g).[68] The aim of these works is to get enhanced UCNPs, PS or UCNPs connection for enough ROS and obtain improved PDT efficacy.

Figure 2.12 Recent achievements in photodynamic therapy. (a) Formation of UCNP-RB conjugates. Reproduced with permission.[74] Copyright 2016, American Chemical Society. (b) Scheme to show the UCNP-HBO to remodel collagen in ECM for enhanced PDT. Reproduced with permission.[85] Copyright 2018, American Chemical Society. (c) PDT treatment using UCNP-PLL@CNs and the cell apoptosis pathway. Reproduced with permission.[81] Copyright 2017, Wiley-VCH. (d) Spectrally mismatched and matched platforms of photosensitizers and UCNP-PDT to get maximum absorption of photosensitizers. Reproduced with permission.[86] Copyright 2018, American Chemical Society. (e) Chemical structures of partial photosensitizers that have been applied to combine with UCNP. Reproduced with permission.[79] Copyright 2018, The Royal Society of Chemistry. (f) Scheme to show the UCNP@H-TiO₂ nanocomposite for PDT. Reproduced with permission.[83] Copyright 2019, American Chemical Society. (g) Synthesis process of the dual-photosensitizers FP-UCNP-MC540-ZnPc nanoconstructs. Reproduced with permission.[68] Copyright 2017, The Royal Society of Chemistry.

2.4.4 Photothermal Therapy

PTT is a direct cancer treatment method by converting the energy of light to heat to kill cancer cells at temperature above 43°C. Nd³⁺-doped NPs (NdNPs) were applied as PTAs using different host lattice, including LaF₃, [49, 87] V₂O₅ in the form of NdVO₄ [88], NaYF₄ [44] and NaF [28]. NdNPs can be excited under multi-wavelengths, while 808 nm irradiation showed the highest temperature increase (Figure 2.13a). [88] As elucidated in Figure 7, the temperature increase is much higher as the concentration of Nd³⁺ dopant increases (Figure 2.13a). [44] Even 808 nm is shown to be able to penetrate the ~2 mm chicken tissue (Figure 2.13b), the temperature increase under the safety limit of 808 nm laser (0.33 W cm⁻²) is not enough to kill cancer cells (attributed to the weak absorption of Nd³⁺ ions). [88] This is mainly due to the weak absorption of Nd³⁺ ions. To overcome the weak absorption issue, Liu et al. reported ultrasmall pH-responsive Nd³⁺-doped NaDyF₄ NPs for accurate tumor location and enhanced photothermal performance (Figure 2.13e). [50] Modified by gallic acid-Fe (III) complex, NdNPs can aggregate rapidly in low pH condition and get excellent enhanced *in vivo* PTT efficacy via tumor-microenvironment-responsive aggregation (Figure 2.13c, d and f). Other types of PTAs with stronger absorption were combined with NdNPs. Ding et al. decorated Nd³⁺-sensitized UCNPs on the surface of polydopamine (PDA) to get the multifunctional imaging guided PTAs. [89] The PDA@UCNPs showed good photothermal performance and tumor inhibition effect under the excitation of 808

nm (Figure 2.13g). Our work of NdNP@PB nanocomplex have also demonstrated the enhanced photothermal performance *in vitro* and *in vivo* (Figure 2.13h and i, chapter 5 in this thesis).[28]

Figure 2.13 Recent achievements in photothermal therapy. (a) Thermal images of NdVO₄ under the excitation of different wavelengths (up) and thermal images of nanoparticles with different Nd³⁺ dopant concentration (bottom). Reproduced with permission. [44, 88] Copyright 2016, Wiley-VCH. Copyright 2017, The Royal Society of Chemistry. (b) Thermal images of a subcutaneous injection (~2 mm depth) of Nd³⁺-doped NPs in chicken tissue under different 808 nm laser excitation power densities. Reproduced with permission. [88] Copyright 2016, Wiley-VCH. (c) Thermal images of Nd³⁺-doped NaDyF₄ NPs under 808 nm laser excitation with different power densities and different pH conditions. (d) Thermal images of the tumor bearing mice pre- and post-injection. (e) Scheme to show the pH-responsive in situ aggregation of Nd³⁺-doped NaDyF₄ NPs. (f) Tumor growth curves of all groups. Reproduced with permission.[50] Copyright 2017, Ivyspring International Publisher.

(g) Thermal images to show the *in vivo* PTT of PDA@UCNPs under 808 nm excitation. Reproduced with permission.[89] Copyright 2017, Tsinghua University Press and Springer-Verlag Berlin Heidelberg. (h) Temperature change and (i) thermal images of NdNP@PB, PB and NdNPs under the same excitation power density of 808 nm laser. Reproduced with permission.[28] Copyright 2019, Wiley-VCH.

2.4.5 Optogenetics

Optogenetics is a technique to use light to control the activities and functions of neurons that have light-sensitive ion channels.[26] Temporal manipulation of electrical and biochemical signals can be achieved by shining light on the light-activated channels controlled by microbial opsins.[90] The current microbial opsins are responsive to visible light with a shallow penetration depth, such as channelrhodopsin-2 (ChR2)[90] responsive to 470 nm and halorhodopsin[91] responsive to 530 nm. To achieve deeper tissue activation of these light-responsive ion channels, UCNPs are applied as the phototransducers to convert 808 nm to specific emission wavelength. Ai et al. presented a metabolic labelling strategy to covalently bind the dibenzyl cyclooctyne (DBCO)-coated UCNPs on the cell membrane to achieve precise regulation of ChR2 channel (Figure 2.14a).[92] Upon the irradiation of 808 nm laser, UCNPs will emit blue light (470 nm) to activate ChR2 to generate photocurrents for Ca^{2+} ion influx (Figure 2.14b). DBCO-UCNPs lead to significant increase of Ca^{2+} ions fluorescence (Figure 2.14c). *In vivo* fluorescence imaging of zebrafish proved that DBCO-UCNPs successfully enabled the Ca^{2+} response (Figure 2.14d). Ao et al. demonstrated the activation of Chrimson

and change of locomotion behaviors of *C. elegans* by green emissive UCNP (Figure 2.14e).[93] Under the excitation of 808 nm laser, Er^{3+} -doped UCNP will emit green to red light to cause an instantaneous and sustained reduction of action potential frequency, while 808 nm laser will not affect the frequency and amplitude of muscle action potentials (Figure 2.14f). *C. elegans* locomotion was successfully achieved by shining 808 nm laser on UCNP containing, demonstrating an efficient UCNP-based optogenetics approach (Figure 2.14g). UCNP have also been applied as nanocarriers to load antigen to trigger the immune response to dendritic cells to achieve immunotherapy.[94, 95]

Figure 2.14 Recent achievements in optogenetics. (a) Scheme to show the covalent localization of UCNP on the cell membrane to achieve precise regulation of ion channel function. (b) Regulation of cell activities under 808 nm excitation by measuring photocurrents in ChR2-expressing HEK293 cells under 808 nm and 480 nm excitation. (c) Cellular Ca^{2+} imaging before and after 808 nm excitation. (d) *In vivo* fluorescence imaging with and without 808 nm treatment in zebrafish to show the Ca^{2+} response. Reproduced with permission.[92] Copyright 2017, Wiley-VCH. (e) Scheme to show a green emissive UCNP to activate Chrimson and change the locomotion behaviours. (f) Representative muscular action potential firing before, during, and after illumination by NIR light

with or without UCNPs. (g) Example body postures of *C. elegans* that express Chrimson in GABAergic motor neurons. Reproduced with permission. [93] Copyright 2019, American Chemical Society.

2.5 Conclusions, Challenges and Perspectives

This review gives a systematic summary of the merits and shortcomings of Nd^{3+} -sensitized nanoconstructs, discussed the three energy conversion pathways of Nd^{3+} -sensitized nanoconstructs including UC, DC, photothermal conversion, and lists their potential applications as NIR-enabled photomedicine, such as biomedical imaging, NIR-controlled release for chemotherapy, PDT, PTT, and optogenetics in recent years. We hope that this review will give a comprehensive understanding of the conversion mechanism in Nd^{3+} -sensitized nanoconstructs and a guidance for the future design of all types of nanosystems for new functionalities or enhanced performance as photomedicine.

Some hurdles for the translation of 808 nm-triggered Nd^{3+} -sensitized nanosystems towards technology development are listed as follows. (I) Low quantum yield of Nd^{3+} -sensitized nanoconstructs. The laser intensity safety limit for 808 nm laser is only 0.33 W cm^{-2} for biomedical applications. Based on this low excitation intensity, the UC and DC processes may not generate emissions strong enough for the intended applications. Thus, effective strategies to enhance the quantum yield is needed, especially for systems requiring low excitation power density. (II) The

weak absorption of Nd^{3+} ions. The poor absorption of Nd^{3+} ions results in significant wastage of most of the energy from the excitation source. (III) The potential toxicity of nanoconstructs. Most of the studies of Nd^{3+} -sensitized nanoconstructs as photomedicine are carried out by intravenous/ intratumor injection, which involve potential toxicity issues. Though studies have shown that Nd^{3+} -sensitized nanoconstructs were safe and can be excreted, the lack of long-term toxicity studies has hindered the clinical translation of such nanoconstructs.

To resolve these issues, possible solutions are listed as follows: (I) Optimizing the dopant concentration of the involved lanthanide ions could possibly reduce energy loss during the various energy transfer pathways described in this review. (II) A rational design of the nanomaterial configuration could confine the energy transitions to a specific range, reduce energy loss and therefore boost photon conversion efficiency. (III) The combination of lanthanide ions with other stable sensitizers is an effective approach to enhance the emissions for biomedical applications. (IV) The potential toxicity of Nd^{3+} -sensitized nanoconstructs may be resolved by comprehensive long-term studies of excretion pathways using different sizes and with different surface modifications. (V) Boosting their performance at the ultrasmall range with high renal clearance may be a potential solution. (VI) Exploration of the possibilities of Nd^{3+} -sensitized nanoconstructs for *ex vivo* applications, such as wearable devices, may lead to faster and more favorable

translational outcomes.

.

CHAPTER 3 Ultrasmall-Superbright Neodymium-Upconversion

Nanoparticles *via* Energy Migration Manipulation and Lattice

Modification: 808 nm-Activated Drug Release

3.1 Introduction

Lanthanide upconversion nanoparticles (UCNPs) are emerging optical agents attractive in biomedical field, owing to their large anti-Stokes shift, deep penetration ability, high signal-to-noise ratio, excellent photostability, biocompatibility and ease of functionalization.[96-99] Ytterbium (Yb^{3+})-doped UCNPs that can be irradiated at 980 nm laser have been the conventional focus in such application. Yb^{3+} features a single energy level that lies at 980 nm ($^2\text{F}_{5/2}$). Upon irradiation, Yb^{3+} -doped UCNPs absorb 980 nm photons and then transfer the energy to the activators (Er^{3+} , Ho^{3+} or Tm^{3+} in most cases). Through successive absorption of photons and energy transfer, the activator can be promoted to high excited states and give rise to upconverted ultraviolet, visible or near-infrared emission through radiative relaxation. However, the strong absorption of 980 nm light by water in biological samples usually attenuates deep tissue imaging and causes potential thermal damage to cells and tissues.[100] Therefore, recent research has focused on using $\text{Nd}^{3+}/\text{Yb}^{3+}/\text{Ln}^{3+}$ -based UCNPs that can be excited with 808 nm laser, which provide the benefits of minimal water absorption and maximal tissue

transparency.[100-102] In this configuration, Nd^{3+} ion serves as the primary sensitizer to absorb 808 nm photons due to the fact that Nd^{3+} has 10 times higher absorption cross section than that of Yb^{3+} in 808 nm.[100] Moreover, energy transfer between Nd^{3+} and Yb^{3+} can reach up to 70% quantum efficiency with negligible back energy transfer, and thus, greatly enhance the absorption of photons.[34] Yb^{3+} ion is the bridging sensitizer to facilitate energy transfer from Nd^{3+} to the activator ions.

Scheme 3.1 The design of from large two-layer UCNPs to ultrasmall-superbright sandwich-structured UCNPs.

However, Nd^{3+} ion comprises of complex energy transitions compared to a single energy transition in Yb^{3+} and is capable of inducing harsh quenching effect caused by efficient energy back transfer from activator to $^4\text{I}_J$ of Nd^{3+} . A pioneer work reported by Liu's group showed that its concentration can only be up to 1 mol% in $\text{Nd}^{3+}/\text{Yb}^{3+}/\text{Ln}^{3+}$ tridoped system, leading to poor absorption of 808 nm photons and thus weak upconversion luminescence.[102] To suppress the deleterious cross-

relaxation, core-shell structure is preferred to spatially separate activator ions from Nd^{3+} by doping them in the core and shell, respectively.[101, 102] Approximately 7 times enhancement was achieved using core/shell structure, as Nd^{3+} concentration in the shell was increased up to 10-20%.[102] However, it still suffers from a limited harvesting efficiency. Yao *et al.*, on the other hand, developed a quenching-shield core/shell/shell nanostructure ($\text{NaYF}_4:\text{Yb,Er}@ \text{NaYF}_4:\text{Yb}_{0.1}@ \text{NaYb}_{0.1}\text{F}_4:\text{Nd}_{0.9}$) that greatly increased Nd^{3+} ratio in the sensitizing layer up to 90%, which thus was capable of emitting highly intensive upconversion.[101] However, the intermediate layer aimed to reduce the photon quenching effect required to be precisely controlled around 1.5 nm, and forming a uniform sensitizing layer with high Nd^{3+} concentration was difficult due to large ionic radius of Nd^{3+} .[101] Therefore, it remains a challenge to fabricate high-emissive Nd^{3+} -sensitized nanoparticles, where a relatively high concentration of Nd^{3+} content is adopted and quenching effect is largely circumvented.

The lanthanide nanoparticles reported in most existing studies were of relatively large size (> 20 nm).[34, 42, 100-112] Ultrasmall Nd^{3+} -sensitized nanoparticles (< 10 nm) with controlled morphology and intense emission are highly favorable and have not been reported. Ultrasmall nanoparticles enable easy access to intracellular compartment, fast kidney filtration and induce less toxicity.[113-115] Additional benefit includes longer blood half-times due to reduced uptake by the

reticuloendothelial system, which is more beneficial for the particles delivered through intravenous injection to the targeted sites.[116, 117] However, upconversion luminescence is known to be size-dependent and the decrease in the size of UCNPs comes at the expense of weaker emission, due to fewer emitters and more surface defects and vibration energy diffusion.[118-120] To retain excellent optical properties and minimize emission loss induced by surface quenching, non-radiative deactivation and surface effects must be carefully engineered. Therefore, an effective synthetic approach to prepare ultrasmall Nd^{3+} -sensitized UCNPs with uniform size and high luminescence efficiency is highly desirable yet very difficult to achieve.

Herein, a simple synthetic strategy of ultrasmall Nd^{3+} -sensitized nanoparticles in the form of $\text{NaGd}_{0.695}\text{F}_4:\text{Yb}_{0.2},\text{Tm}_{0.005},\text{Ca}_{0.1}@ \text{NaYb}_{0.9}\text{F}_4:\text{Ca}_{0.1}@ \text{NaNd}_{0.6}\text{F}_4:\text{Gd}_{0.3},\text{Ca}_{0.1}$ is reported to obtain the 808 nm excited multilayer UCNPs featuring controlled morphology and intense upconversion luminescence. In our design, NaGdF_4 is chosen as the seed by virtue of its low phonon energy and readiness to form ultrasmall nanoparticles. As a proof-of-concept experiment, Tm^{3+} ion with a ladder like energy levels is selected as the model activator and is set to be 0.5 mol% to maximize blue emission. NaYbF_4 is employed to form the bridging sensitizer-enriched intermediate shell in order to minimize the deleterious cross-relaxation

energy loss and relay energy from Nd^{3+} to Tm^{3+} . Ca^{2+} ion is incorporated into every layer of the nanoparticles to ensure the full-grown of nanoparticles, which subsequently, offers better size-uniformity and more importantly, reduces energy quenching induced by surface defects in the nanoparticles. To this end, the quenching effect from Nd^{3+} ions can be largely eliminated (Figure 3.1a). Their application in light-responsive drug release was also demonstrated.

Figure 3.1 Synthesis of ultrasmall CSS UCNP. (a) Schematic diagram of $\text{NaGdF}_4:\text{Yb,Tm,Ca}@ \text{NaYbF}_4:\text{Ca}@ \text{NaNdF}_4:\text{Gd,Ca}$ nanoparticles showing a primary-sensitizer abundant outer layer, a bridging sensitizer-enriched intermediate layer and crystal defects modified by Ca^{2+} ions to suppress luminescence quenching. (b) Scheme showing the synthetic process for $\text{NaGdF}_4:\text{Yb,Tm,Ca}@ \text{NaYbF}_4:\text{Ca}@ \text{NaNdF}_4:\text{Gd,Ca}$ nanoparticles and TEM images of $\text{NaGdF}_4:\text{Yb,Tm,Ca}$ (c); $\text{NaGdF}_4:\text{Yb,Tm,Ca}@ \text{NaYbF}_4:\text{Ca}$ (d);

NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca@NaNdF₄:Gd,Ca (e) nanoparticles. Scale bar: 50 nm.

3.2 Materials and Methods

Materials and Reagents: gadolinium acetate hydrate, ytterbium acetate hydrate, thulium acetate hydrate, calcium acetate hydrate, neodymium acetate hydrate, 1-octadecene (90%), oleic acid (90%), sodium hydroxide (> 98%), ammonium fluoride (98%), hexadecyltrimethylammonium bromide (CTAB, ≥98%), tetraethyl orthosilicate (TEOS, 98%), hydrochloride acid (37%), doxorubicin hydrochloride (DOX, 98%), 2,2'-bipyridine (98%), LiCl, ruthenium (III) chloride trihydrate (technical), potassium hexafluorophosphate (98%), trimethylphosphine in THF, 3-aminopropyltriethoxysilane (APTES), CCK8 assays were all purchased from Sigma-Aldrich and used as received. Cyclohexane, ethanol, ethyl acetate, acetone and diethyl ether were purchased from Tedia, Singapore. Bovine serum albumin (BSA) was purchased from J&C Chemical CO. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS, Invitrogen), penicillin/streptomycin, trypsin (0.05%) and LIVE/DEAD® Viability/Cytotoxicity Kit were all purchased from Life Technologies. Alamar Blue assays (Invitrogen) was purchased from Invitrogen.

Synthesis of NaGdF₄:Yb,Tm,Ca nanoparticles: a water solution (2 ml) containing Gd (CH₃COO)₃, Yb(CH₃COO)₃, Tm(CH₃COO)₃ and Ca(CH₃COO)₃ with a total lanthanide amount of 0.5 mmol was added to a 50 ml flask containing

oleic acid (4 ml) and 1-octadecene (10 ml). The resulting mixture was heated at 150 °C for 1 h and then cooled down to room temperature. Subsequently, a methanol solution (10 ml) containing NH_4F (1.6 mmol) and NaOH (1 mmol) was added and stirred at 50 °C for 30 min. The reaction temperature was then increased to 110 °C for 15 min to remove the residual methanol from the reaction mixture. Upon removal of methanol, the solution was heated to 300 °C and maintained at this temperature under an argon flow for 30 min. The solution was then cooled down to room temperature naturally. The resulting nanoparticles were precipitated out with an addition of ethanol, collected by centrifugation, and finally dispersed in 4 ml of cyclohexane.

Synthesis of $\text{NaGdF}_4\text{:Yb,Tm,Ca@NaYbF}_4\text{:Ca}$ nanoparticles: a water solution (2 ml) containing $\text{Yb}(\text{CH}_3\text{COO})_3$ and $\text{Ca}(\text{CH}_3\text{COO})_3$ with a total lanthanide amount of 0.5 mmol was added to a 50 ml flask containing oleic acid (4 ml) and 1-octadecene (10 ml). The resulting mixture was heated at 150 °C for 1 h and then cooled down to 50 °C. The as-synthesized $\text{NaGdF}_4\text{:Yb,Tm,Ca}$ core nanoparticles in 4 ml of cyclohexane were added with a methanol solution (10 ml) containing NH_4F (1.6 mmol) and NaOH (1 mmol). The resulting mixture was stirred at 50 °C for 30 min. The reaction temperature was then increased to 110 °C for 15 min to remove the residual methanol from the reaction mixture. Upon removal of methanol, the solution was heated to 300 °C and maintained at this temperature under argon gas

for 30 min. The solution was then cooled down to room temperature naturally. The resulting nanoparticles were precipitated out with an addition of ethanol, collected by centrifugation, washed with ethanol, and finally dispersed in 4 ml of cyclohexane.

Synthesis of NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca@NaNdF₄:Gd,Ca nanoparticles

(UCNPs): a water solution (2 ml) containing Gd(CH₃COO)₃, Nd(CH₃COO)₃ and Ca(CH₃COO)₃ with a total lanthanide amount of 0.5 mmol was added to a 50 ml flask containing oleic acid (4 ml) and 1-octadecene (10 ml). The resulting mixture was heated at 150 °C for 1 h and then cooled down to 50 °C. The as-synthesized NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca in 4 ml of cyclohexane were added with a methanol solution (10 ml) containing NH₄F (1.6 mmol) and NaOH (1 mmol). The resulting mixture was stirred at 50 °C for 30 min. The reaction temperature was then increased to 110 °C for 15 min to remove the methanol from the reaction mixture. Upon removal of methanol, the solution was heated to 300 °C and maintained at this temperature under an argon gas for 1 h. The solution was then cooled down to room temperature naturally. The resulting nanoparticles were precipitated out with an addition of ethanol, collected by centrifugation, washed with ethanol, and finally dispersed in 4 ml of cyclohexane.

Synthesis of UCNP@mSiO₂ nanoparticles: UCNP in cyclohexane (0.1 M, 0.5 ml)

were added in an aqueous CTAB solution (5 ml, 0.05M). The resulting solution was stirred for 1 h to form an oil-in-water emulsion. The mixture was heating up to 80 °C and maintained at the temperature for 30 min to evaporate cyclohexane. The resulting solution was added to a mixture of water (25 ml) and an aqueous solution of NaOH (1.8 ml, 2 M). The mixture was heated to 70 °C under stirring. Then, TEOS (0.12 ml) and ethyl acetate (1.8 ml) were added to the reaction mixture. The reaction was maintained at 70 °C for 6 h. The nanoparticles were isolated *via* centrifugation at 5000 rpm, and washed with ethanol and stored in ethanol (20 ml). To extract CTAB from the nanoparticles, HCl (40 µl) was added to the dispersion to adjust pH of the solution to ~ 1.4. The dispersion was stirred for 3 h at 60 °C. The nanoparticles were isolated *via* centrifugation at 5000 rpm and washed with ethanol. The UCNP@mSiO₂ nanoparticles were dispersed in ethanol.

Loading DOX into UCNP@mSiO₂ nanoparticles: doxorubicin hydrochloride (1 mg) was dissolved in ethanol (25 ml). NaGdF₄:Yb, Tm,Ca@NaYbF₄:Ca@NaGdF₄:Nd,Ca @mSiO₂ (~ 25 mg) were added in the ethanol solution of doxorubicin. The mixture was kept in the dark and stirred at room temperature for 24 h. The DOX-NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca@NaGdF₄:Nd,Ca@mSiO₂ was collected by centrifugation at 7000 rpm.

Synthesis of ruthenium complex Ru[(2,2'-bipyridine)₂(trimethylphosphine)((3-aminopropyl)triethoxysilan)](PF₆)₂: It was synthesized according to a literature method.[121] Three steps were performed to obtain the final ruthenium complex. Briefly, RuCl₃·3H₂O (0.5g), 2,2'-bipyridine (0.6 g), and LiCl (0.46 g) were dissolved in DMF (20 ml) and refluxed for 8 h with magnetic stirring. The reaction mixture was then cooled to room temperature, and acetone (32 ml) was added. The resulting mixture was kept at 0°C overnight. The mixture was then filtered, yielding a red-violet filtrate and a black crystalline substance. The solid was washed three times with 10 ml water and diethyl ether and subsequently dried to yield Ru(bpy)₂Cl₂. Secondly, the obtained Ru(bpy)₂Cl₂ (260 mg) was suspended in 20 ml of a 1:1 mixture of methanol and water, and refluxed under argon flow. 1.2 ml of 1M trimethylphosphine in THF was added by syringe. The reaction was kept for 2h. The resulting aqueous solution was filtered to remove any solids, and the dark orange solid of [Ru(bpy)₂(PMe₃)(Cl)](PF₆)₂ was precipitated out with the addition of KPF₆ at 0 °C. The solid was washed three times with cold water and dried. In the third step, [Ru(bpy)₂(PMe₃)(Cl)](PF₆)₂ (50 mg) was dissolved in 15 ml of a mixture of ethanol/H₂O (1:2). The solution was heated at 80 °C. After 1 h, APTES (64 µl) was added and the solution was reacted for 5h. The final product of Ru[(2,2'-bipyridine)₂(trimethylphosphine)((3-Aminopropyl)triethoxysilane)](PF₆)₂ (Ru) was obtained and used without further purification.

Synthesis of DOX-UCNP@mSiO₂-Ru nanoparticles by capping DOX-

UCNP@mSiO₂ nanoparticles with Ru polymer: aqueous solution of NaOH (10 μ l, 1M) were added to the suspension of NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca@NaNdF₄:Gd,Ca@mSiO₂ nanoparticles (~25 mg) and Ru (2.3 mg) in ethanol (25 ml). The mixture was stirred for 12 h at room temperature. NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca@NaGdF₄:Nd,Ca@mSiO₂-Ru nanoparticles were collected by centrifugation at 7000 rpm and washed by ethanol.

NIR light-triggered controlled drug release:

DOX-UCNP@mSiO₂-Ru nanoparticles were suspended in a 3.5 ml DI water in a quartz cuvette. The mixture was put in an ultrasonic for 30 min to disperse the powder uniformly in the solution. Then the mixture was irradiated with a 808 nm laser for different time at the power density of 0.75 W cm⁻². After every time point, the release doxorubicin was measured using photoluminescence spectra with an excitation wavelength of 485 nm.

Cell lines and cell culture:

4T1 cells were cultured and kept in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 1% penicillin/streptomycin (Life Technologies) in an incubator at 37 C°, 95 % humidity and 5% CO₂. Treatment with trypsin (0.05%) (Life Technologies) for 10 min was used to detach the cells for subculturing.

***In vitro* cell viability assessment of the DOX-UCNP@mSiO₂-Ru nanoparticles**

in the absence of light irradiation: to evaluate the cytotoxicity of the nanoparticles, 4T1 cells were incubated with DOX-UCNP@mSiO₂-Ru nanoparticles as a function of nanoparticles concentration and incubation time. Data are presented as mean $\pm$ standard deviation for three independent experiments. 4T1 cells were plated in 96-well plates with a cell density of 1×10^4 cells per well and allowed to grow into full confluence. Then, medium was replaced by refresh one with nanoparticles of different concentrations and the cells were incubated for further 24 h. CCK8 assays were performed at each time point. Cell viability was calculated based on the absorbance of 450 nm using microplate reader. The cytotoxicity was expressed as the percentage of the cell viability compared to that of untreated control cells.

***In vitro* cell viability assessment of the nanoparticles under light irradiation:**

for laser irradiation experiments, 4T1 cells were seeded in 96-well plates at a density of 1×10^4 per cells and cultured for 24 hours. PBS, DOX-UCNP@mSiO₂, DOX-UCNP@mSiO₂-Ru nanoparticles were added into the well and incubated for another 4 h. A NIR laser (808 nm, 0.75 W cm^{-2}) was used to irradiate cells. Light was irradiated either after 4 hours of incubation with nanoparticles, or without adding the nanoparticles. The irradiation time varies from 0 to 90 minutes. Fresh medium was replaced and the cells were then incubated for another 24 h. For the

cytotoxicity analysis, CCK8 assays was performed and the cultures were incubated for 3h to allow the formation of yellow-color formazan dye. Cell viability was calculated based on the absorbance of 450 nm using microplate reader.

Live/Dead cell viability test: cells were seeded into 24-well plates with a cell density of 5×10^4 cells per well. After adhesion, the medium was replaced with or without serum-free medium containing nanoparticles of different concentrations and the cells were incubated for 1h. Then the medium was replaced with fresh serum-free medium and NIR laser irradiation was applied for 10, 30, 60 and 90 min, respectively. Cell viability was assessed using the LIVE/DEAD[®] Viability/Cytotoxicity Kit (Molecular Probes, Life Technologies) following the manufacturer's instructions. Briefly, the culture medium was poured out and the cells washed with PBS. The working solution containing 2 mM Calcein AM and 4 mM EthD-1 was then added directly to each well. After incubation at room temperature for 30 min, the cells were then observed using a fluorescence microscope (emission at 515 nm and 635 nm) (Axio Observer, Zeiss, Germany) with an attached camera.

Tumor model: Animal experiments were carried out with approval of the Institutional Animal Care and Use Committee of Huazhong University of Science and Technology. Female Balb/c mice (5 ~ 6 weeks old, 18 ~ 20 g) were used in this

work. Xenografted mice tumor model was established by subcutaneous injection of 4T1 cells. Briefly, cells were diluted with PBS and then injected subcutaneously to each mouse at the back with around 3×10^6 cells. After 7 ~ 10 days, tumor grew to be around 70 ~ 100 mm³.

In vivo antitumor assessment: Tumor-bearing Balb/c mice were randomly divided into five groups (n=6, per group). The tumor-bearing Balb/c mice was subcutaneous injected with (a) 50 μ l PBS, (b) 50 μ l PBS + laser, (c) pure DOX, (d) DOX-UCNP@mSiO₂-Ru, (e) DOX-UCNP@mSiO₂-Ru + laser. All the groups were received only one time intratumor injection with 50 μ l PBS or nanoparticles. The tumor site of the mice in the group b and group e received 808 nm laser irradiation (0.75 W cm⁻²) for 60 min after injection. Irradiation was repeated on Day 2. Tumor volume and mice body weight were measured every 2 days, followed by dissection in 14 days post-injection. The tumor volume was measured using the volume of an ellipsoid and it was calculated using the formula: Tumor volume (V) = length*width²/2. Relative tumor volume was calculated as V/V₀ (V₀ was the corresponding tumor volume when the treatment was initiated). At the end of the treatment, mice were sacrificed. The tumors and major organs of mice, such as heart, liver, spleen, lung and kidney, were collected, weighted, imaged and analyzed by H&E staining. The tumors harvested were also imaged and analyzed by TUNEL staining.

***In vivo* biodistribution studies:** Healthy Balb/c female mice weighted 18 ~ 20 g were randomly divided into 6 groups. Mice were intravenously injected with 10 mg/kg nanoparticles (100 μ l for each mouse) separately. At different time point post-injection (3 h, 6 h, 12 h, 1 d, 3 d and 7 d), mice were anesthetized and sacrificed. The major organs of the mice, such as heart, liver, spleen, lung and kidney, were collected, weighted and stored in 4% para-formaldehyde solution. Each organ was treated with HNO₃ and H₂O₂ (v/v = 1:1) at 70 °C till a clear solution was obtained. The Nd element of the samples was measured by ICP. Three repeats were done for each group.

Characterization of as-synthesized nanoparticles: transmission electron microscopy (TEM) images were recorded on a JEOL JEM-2100F microscope operating at 200 kV. Energy-dispersive X-ray (EDX) spectroscopy was done using a JEOL FESEM-7600F field emission scanning electron microscope (system resolution:1.5 nm, electron source: Thermal FEG, X-ray analysis: INCA). Powder X-ray diffraction analysis (XRD) was carried out on a D8 Advance Bruker powder X-ray diffractometer with Cu K α radiation (λ = 1.5406 Å) from 10° to 80° with a counting time of 1s per step. To obtain the upconversion photoluminescence spectra, samples were dispersed in cyclohexane (1 mg ml⁻¹) in a standard quartz cuvette at room temperature, and fluorescence spectra were acquired on a Fluoromax-4,

Horiba Jobin Yvon Spectrofluorometer with a commercial 808 nm NIR laser. Photoluminescence spectra of DOX release were obtained using a Shimadzu RF-5301 PC spectrofluorometer fitted with a 150 W xenon lamp as the excitation source with a resolution of 1 nm. Hydrodynamic size and stability of the nanoparticles were measured using dynamic light scattering (DLS) in a Malvern Nano Zetasizer system by Malvern Instruments. UV-vis spectrum was recorded on a Shimadzu UV-3600 spectrophotometer. Fourier Transform Infrared Spectroscopy (FTIR) was conducted in a Digilab FTS 3100 instrument. Brunauer-Emmett-Teller (BET) was conducted in micromeritics ASAP 2020 V3.01H. The Live/Dead cell viability was analyzed by a Zeiss Observer.Z1 confocal microscopy. Element analysis was measured on a Teledyne Leeman Labs Inc. Prodigy High Dispersion Inductively-Coupled Plasma (ICP).

Measurement of upconversion luminescence quantum yields: UCNPs in cyclohexane (1 mg ml^{-1} , 3ml) were placed in a quartz cuvette, which was inserted into an integrating sphere in the Fluoromax-4 (Horiba Jobin-Yvon Spectrofluorometer). The spectrum of excitation radiation not absorbed by the sample was measured at the wavelength from 790 to 810 nm through two neutral density filter. The spectrum of emission of each sample was measured from 350 to 660 nm without any neutral density filter. Pure cyclohexane (3 ml) was used as the blank background to record the blank excitation and emission spectra. Excitation

and emission spectra were both corrected.

The absolute quantum yield of each sample was then determined according to the equation:

$$\phi = \frac{I_{em,uc} - I_{em,sol}}{I_{ex,sol} - I_{ex,uc}}$$

where $I_{em,uc}$ is the integrated intensity over the wavelength range of the peak of the UCNPs, $I_{em,sol}$ is the integrated intensity over the wavelength range of the peak of pure cyclohexane, $I_{ex,sol}$ is the integrated intensity of the unabsorbed excitation radiation over the given wavelength range of cyclohexane and $I_{ex,uc}$ is the integrated intensity of the unabsorbed excitation radiation over the given wavelength range of UCNPs.

3.3 Results and Discussions

3.3.1 Fabrication and Characterization of Ultrasmall UCNPs

The sandwich-structured $\text{NaGdF}_4:\text{Yb,Tm,Ca}@\text{NaYbF}_4:\text{Ca}@\text{NaNdF}_4:\text{Gd,Ca}$ nanoparticles were synthesized layer-by-layer through a modified thermal decomposition method (Figure 1b).[122, 123] TEM images revealed that core $\text{NaGdF}_4:\text{Yb,Tm,Ca}$, core/shell $\text{NaGdF}_4:\text{Yb,Tm,Ca}@\text{NaYbF}_4:\text{Ca}$, and core/shell/shell $\text{NaGdF}_4:\text{Yb,Tm,Ca}@\text{NaYbF}_4:\text{Ca}@\text{NaNdF}_4:\text{Gd,Ca}$ nanoparticles (denoted hereafter as UCNPs) displayed narrow size distribution and excellent monodispersity with average diameter of 5.5 ± 0.7 nm, 7.5 ± 0.6 nm and 9.3 ± 0.9 nm, respectively (Figure 3.1c-e). The increase particle size along with maintained

structural uniformity primarily suggests the successful growth of the premediated shell. The as-synthesized multilayer nanoparticles were confirmed to be single crystals with a hexagonal phase by high-resolution TEM and XRD analysis (Figure 3.2, 3.3). The XRD patterns of core/shell and core/shell/shell nanoparticles are similar to that of the core nanoparticles, but with an increase in peak signal intensity. The peak position and intensities of the nanoparticles matched with JCPDS 27-0699. The increased intensities are attributed to the increase in size. EDX confirmed the presence of all elements in the core, core/shell and core/shell/shell nanoparticles, implying the shells were covered on core nanoparticles layer by layer (Figure 3.4). All these evidences prove the successful formation of the sandwich-structured nanoparticles.

Figure 3.2 HRTEM images of as-synthesized CSS UCNPs nanoparticles. Scale bar: 5 nm.

Figure 3.3 XRD patterns of as-synthesized core, CS and CSS nanoparticles.

Figure 3.4 EDX of as-synthesized core, CS and CSS nanoparticles. (a) core, (b) CS and (c) CSS, revealing the presence of all elements in the core, core/shell and core/shell/shell nanoparticle.

Figure 3.5 Comparison of emission spectra of different configurations. (a) Room temperature emission spectra of $\text{NaGdF}_4\text{:Yb,Tm,Ca@NaYbF}_4\text{:Ca@NaNdF}_4\text{:Gd,Ca}$; $\text{NaGdF}_4\text{:Yb,Tm,Nd@NaGdF}_4\text{:Nd,Yb}$; $\text{NaGdF}_4\text{:Yb,Tm,Nd@NaGdF}_4\text{:Nd}$ and $\text{NaGdF}_4\text{:Yb,Tm,Nd@NaGdF}_4$ nanoparticles. (b) Photographs of dominate blue emission of (i) $\text{NaGdF}_4\text{:Yb,Tm,Ca@NaYbF}_4\text{:Ca@NaNdF}_4\text{:Gd,Ca}$; (ii) $\text{NaGdF}_4\text{:Yb,Tm,Nd@NaGdF}_4\text{:Nd,Yb}$; (iii) $\text{NaGdF}_4\text{:Yb,Tm,Nd@NaGdF}_4\text{:Nd}$; (iv) $\text{NaGdF}_4\text{:Yb,Tm,Nd@NaGdF}_4$ nanoparticles. (c) Energy transfer mechanism responsible for the upconversion emission of $\text{NaGdF}_4\text{:Yb,Tm,Ca@NaYbF}_4\text{:Ca@NaNdF}_4\text{:Gd,Ca}$; $\text{NaGdF}_4\text{:Yb,Tm,Nd@NaGdF}_4\text{:Nd,Yb}$; $\text{NaGdF}_4\text{:Yb,Tm,Nd@NaGdF}_4\text{:Nd}$ and $\text{NaGdF}_4\text{:Yb,Tm,Nd@NaGdF}_4$ nanoparticles. Vertical and wavy arrows represent non-radiative transitions, curved arrows represent non-radiative energy transfer. Violet, blue and red arrows represent ultraviolet, blue and red emissions. (d) Room temperature emission spectra of the $\text{NaGdF}_4\text{:Yb,Tm,Ca@NaYbF}_4\text{:Ca@NaNdF}_4\text{:Gd,Ca}$ and $\text{NaGdF}_4\text{:Yb,Tm,Ca@NaNdF}_4\text{:Gd,Ca@NaYbF}_4\text{:Ca}$ nanoparticles. (e) Schematic diagram of energy

transfer between (i) $\text{NaGdF}_4:\text{Yb,Tm,Ca}@ \text{NaYbF}_4:\text{Ca}@ \text{NaNdF}_4:\text{Gd,Ca}$ and (ii) $\text{NaGdF}_4:\text{Yb,Tm,Ca}@ \text{NaNdF}_4:\text{Gd,Ca}@ \text{NaYbF}_4:\text{Ca}$ nanoparticles, showing that ytterbium-enriched intermediate layer is vital in bridging the primary sensitizer in the outer shell with the activator in the core. All samples were dispersed in cyclohexane (1 mg ml^{-1}). Spectra were recorded under excitation of 808 nm laser at a power density of 7.5 W cm^{-2} .

Under 808 nm laser excitation, the as-synthesized nanoparticles showed characteristic Tm^{3+} emission peaks at 360 nm, 450 nm, 475 nm, 649 nm and 696 nm that can be assigned to $^1\text{D}_2 \rightarrow ^3\text{H}_6$, $^1\text{D}_2 \rightarrow ^3\text{H}_4$, $^1\text{G}_4 \rightarrow ^3\text{H}_6$, $^3\text{F}_2 \rightarrow ^3\text{H}_6$ and $^3\text{F}_3 \rightarrow ^3\text{H}_6$ transitions, respectively (Figure 3.5 a, c). In order to demonstrate the feasibility of our design in enhancing the upconversion luminescence, we compared the luminescence with that of previously reported nanoparticles. We observed markedly weak emission in the triple-doped system, even with the coating of an inert shell ($\text{NaGd}_{0.79}\text{F}_4:\text{Yb}_{0.2},\text{Tm}_{0.005},\text{Nd}_{0.005}@ \text{NaGdF}_4$) (Figure 3.5a insert). Doping Nd^{3+} ions into the inert shell has improved the luminescence performance of the nanoparticles ($\text{NaGd}_{0.79}\text{F}_4:\text{Yb}_{0.2},\text{Tm}_{0.005},\text{Nd}_{0.005}@ \text{NaGdF}_4:\text{Nd}_{0.2}$), however, the intensity were suppressed by 117 fold compared with our nanoparticles, due to the deleterious cross-relaxation between Tm^{3+} and Nd^{3+} (Figure 3.5a insert). In view of the active role of incorporating appropriate amount of Yb^{3+} ions to form an active shell in enhancing the upconversion emission, $\text{NaGd}_{0.79}\text{F}_4:\text{Yb}_{0.2},\text{Tm}_{0.05},\text{Nd}_{0.005}@ \text{NaGd}_{0.65}\text{F}_4:\text{Nd}_{0.3},\text{Yb}_{0.05}$ nanoparticle was designed and the optical properties were compared.[111] It was found that the intensity of luminescence was decreased by approximately 48 fold (Figure 3.5a

insert). The digital photographs in Figure 3.5b showed visual evidence of comparison of the blue emissions. It should be noted that these nanoparticles were of the similar size, therefore, size effect was ruled out in our study (Figure 3.6). The absolute quantum yield of the as-synthesized upconversion nanoparticles was measured to be 0.464%, which is 25, 58 and 155 fold higher than that of $\text{NaGd}_{0.79}\text{F}_4:\text{Yb}_{0.2},\text{Tm}_{0.05},\text{Nd}_{0.005}@\text{NaGd}_{0.65}\text{F}_4:\text{Nd}_{0.3},\text{Yb}_{0.05}$ (0.018%), $\text{NaGd}_{0.79}\text{F}_4:\text{Yb}_{0.2},\text{Tm}_{0.005},\text{Nd}_{0.005}@\text{NaGdF}_4:\text{Nd}_{0.2}$ (0.008%) and $\text{NaGd}_{0.79}\text{F}_4:\text{Yb}_{0.2},\text{Tm}_{0.005},\text{Nd}_{0.005}@\text{NaGdF}_4$ (0.003%), respectively (excitation density of 7.5 W cm^{-2}) (Table 1). Taken together, these results indicated the success of our nanoparticles in enhancing the luminescence.

Table.1 Experimental quantum yields.

Configuration	Quantum Yield (%) @7.5W/cm ²
$\text{NaGdF}_4:\text{Yb},\text{Tm},\text{Nd}@\text{NaGdF}_4$	0.003
$\text{NaGdF}_4:\text{Yb},\text{Tm},\text{Nd}@\text{NaGdF}_4:\text{Nd}$	0.008
$\text{NaGdF}_4:\text{Yb},\text{Tm},\text{Nd}@\text{NaGdF}_4:\text{Nd},\text{Yb}$	0.018
$\text{NaGdF}_4:\text{Yb},\text{Tm},\text{Ca}@\text{NaYbF}_4:\text{Ca}@\text{NaNdF}_4:\text{Gd},\text{Ca}$	0.464

Quantum yield experimental errors are estimated to be $\pm 50\%$ relative error.

Figure 3.6 TEM images of different UCNPs. (a) $\text{NaGd}_{0.79}\text{F}_4:\text{Yb}_{0.2},\text{Tm}_{0.005},\text{Nd}_{0.005}@\text{NaGdF}_4$, (b) $\text{NaGd}_{0.79}\text{F}_4:\text{Yb}_{0.2},\text{Tm}_{0.005},\text{Nd}_{0.005}@\text{NaGdF}_4:\text{Nd}_{0.2}$, (c) $\text{NaGd}_{0.79}\text{F}_4:\text{Yb}_{0.2},\text{Tm}_{0.05},\text{Nd}_{0.005}@\text{NaGd}_{0.65}\text{F}_4:\text{Nd}_{0.3},\text{Yb}_{0.05}$, scale bar: 100 nm.

3.3.2 The Role of Intermediate Layer

To identify the dominant effect responsible for enhanced emission in our nanoparticles, we conducted a series of control experiments and compared their emission intensities. Firstly, we synthesized core/shell/shell $\text{NaGdF}_4:\text{Yb},\text{Tm},\text{Ca}@\text{NaNdF}_4:\text{Gd},\text{Ca}@\text{NaYbF}_4:\text{Ca}$ nanoparticles, in which the two outer layers were switched. It was found that the emission intensity of the nanoparticles showed 25 times lower than that of $\text{NaGdF}_4:\text{Yb},\text{Tm},\text{Ca}@\text{NaYbF}_4:\text{Ca}@\text{NaNdF}_4:\text{Gd},\text{Ca}$, highlighting the important role of Yb^{3+} -enriched intermediate layer in the energy transfer (Figure 3.5d). Due to Nd^{3+} - Nd^{3+} interactions, the excitation energy migrated along the Nd^{3+} sublattice can readily arrive at the interface of core nanoparticles (Figure 3.5e). Yb^{3+} ions in the core extracted the excitation energy from Nd^{3+} ions through interionic nonradiative relaxation, followed by entrapment by Tm^{3+} in the core. However, the severe quenching caused by back energy transfer from Tm^{3+} to Nd^{3+} occurred when

NaGdF₄:Yb,Tm,Ca@NaNbF₄:Gd,Ca@NaYbF₄:Ca was employed in this study, which is the main disadvantage of using Nd³⁺-sensitized UCNPs. In NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca@NaNbF₄:Gd,Ca system, Yb³⁺-enriched intermediate layer spatially separated Nd³⁺ from Tm³⁺ and refrained from the interfacial quenching interaction between Tm³⁺ and Nd³⁺, and thus, improved the energy transfer efficiency.

Figure 3.7 (a) Room temperature emission spectra of NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca@NaNdF₄:Gd,Ca nanoparticles with different Yb³⁺ concentration. (b) Room temperature emission spectra of the NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca@NaNdF₄:Gd,Ca nanoparticles with different intermediate shell thickness. All samples were dispersed in cyclohexane (1 mg ml⁻¹). Spectra were recorded under excitation of 808 nm laser at a power density of 7.5 W cm⁻². (c-f) Representative TEM images of NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca@NaNdF₄:Gd,Ca nanoparticles with different intermediate shell thickness. The sizes of nanoparticles were measured to be (c) 7.7±0.6 nm, (d) 9.3±0.9 nm; (e) 11.1±0.3 nm and (f) 13.4±0.7 nm, respectively. The intermediate shells were prepared using the same core nanoparticles. Scale bar: 50 nm.

To shed more light on the role of Yb³⁺-enriched intermediate layer in energy transfer, the effect of Yb³⁺ concentration in the intermediate layer on the overall photoluminescence was studied. Gd³⁺ was chosen to replace Yb³⁺ and the concentration of Nd³⁺ in the outer layer was fixed at 60 mol%. As depicted in Figure 3.7a, a steady suppression of emission was observed when Yb³⁺ ion was gradually replaced by Gd³⁺. There was no detectable upconversion emission when Yb³⁺ was completely substituted by Gd³⁺. This result indicated that upconversion intensity of the nanoparticles was strongly affected by Yb³⁺ ions, which formed the Yb³⁺-mediated sub-lattice enabling excitation-energy migration from primary sensitizer to activator. Increasing Yb³⁺ concentration enlarges the absorption cross-section, leading to the enhanced efficiency of energy transfer from Nd³⁺ to Tm³⁺. Moreover, a higher concentration of Yb³⁺ ions resulted in shorter Yb³⁺-Yb³⁺ interionic distance within the confined nanosub-lattice, thus, ensured an efficient energy transfer from

Nd^{3+} in the outer layer to Tm^{3+} . [124]

Modulation of Yb^{3+} - Yb^{3+} interionic distance can also be manipulated by tuning the shell thickness to control the interlayer energy transfer from Nd^{3+} to Tm^{3+} . A detailed investigation was conducted to study the influence of the shell thickness of NaYbF_4 in bridging the energy transfer. Three more control samples were prepared and the shell thickness was precisely control to be 0.4, 1.8, 3.8 and 6.1 nm (Figure 3.7c-e). An enhancement of upconversion emission accompanied by the increase in the intermediate shell thickness until the intensity reached a maximum when the interlayer thickness was controlled at about 1.8 nm; after which, the luminescence decreased. It is reported that the energy transfer rate is proportional to r^{-6} (r is the average donor-acceptor separation) within the dipole-dipole interaction. [125, 126] Thus, narrowing of transition layer confines migration of excitation energy within the nanoshell and prevents the excitation energy from travelling a long distance at a high Yb^{3+} concentration. However, when Yb^{3+} - Yb^{3+} interionic distance exceeds the optimal, back energy transfer from Tm^{3+} to Yb^{3+} takes place according to the following scheme $[\text{Tm} (^1\text{G}_4) + \text{Yb} (^2\text{F}_{7/2}) = \text{Tm} (^3\text{H}_4) + \text{Yb} (^2\text{F}_{5/2})]$, which induces effectively reduction of the upconversion emission intensity. The results confirmed that the intermediate ytterbium-enriched layer played a vital role in limiting the possibility of producing unwanted cross-relaxation pathways, but also affecting the process of bridging efficient energy transfer from the Nd^{3+} in the core layer to the

upconverting ions in the inner shell layer.

3.3.3 Concentration of Nd^{3+} Ions and Lattice Modification by Ca^{2+} ions

We next verified the effect of Nd^{3+} concentration on the overall upconversion luminescence, as Nd^{3+} features as two-edged sword in harvesting the excitation energy. As anticipated, the emission intensity of Tm^{3+} increased with an increase of Nd^{3+} concentration from 20 mol% to 60 mol%, and the intensity declined afterward from 60 mol% to 90 mol% (Figure 3.8a). The gradual increase of Nd^{3+} concentration within the outer shell layer is consistent with overall absorption of excitation energy, leading to elevated photon harvest efficiency and a stronger upconversion emission. However, at higher Nd^{3+} concentration, the enhanced mutual interactions between Nd^{3+} ions consume the excitation energy. Moreover, the good overlap of Tm^{3+} emission ($^3\text{H}_4 \rightarrow ^3\text{F}_4$) with Nd^{3+} absorption ($^4\text{I}_{9/2} \rightarrow ^4\text{I}_{15/2}$) assured an efficient back energy transfer from the metastable Tm^{3+} excite levels to the $^4\text{I}_J$ levels of Nd^{3+} .

Figure 3.8 Tuning of the concentration of Nd³⁺ ions and lattice modification by Ca²⁺ ions (a) Room temperature emission spectra of CSS nanoparticles with different Nd³⁺ concentration in the out shell. (b) Room temperature emission spectra of NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca@NaNdF₄:Gd,Ca and NaGdF₄:Yb,Tm@NaYbF₄@NaNdF₄:Gd nanoparticles. (c) Schematic diagram of energy transfer mechanism between (i) NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca@NaNdF₄:Gd,Ca and (ii) NaGdF₄:Yb,Tm@NaYbF₄@NaNdF₄:Gd nanoparticles, showing that Ca²⁺-dopant modified the lattice defects, and enhance the energy transfer efficiency. All samples were dispersed in cyclohexane (1 mg ml⁻¹). Spectra were recorded under excitation of 808 nm laser at a power density of 7.5 W cm⁻².

Up to now, host lattice manipulation has been proven to be useful in modulating the upconversion luminescence.[127-131] In this work, Ca^{2+} ion was chosen as the dopant. Emission intensity of the nanoparticles with 10 mol% Ca^{2+} showed 3 times enhancement than that without Ca^{2+} (Figure 3.8b). For UCNPs with ultrasmall size, their surface-to-volume ratio is very high and a large portion of dopants is located at the surface. Thus, excitation energy will then be easily quenched by surface defects, impurities, and vibrational deactivation from solvents or surface-bound ligands. Inducing the complete coverage of core and shells by modifying the host lattice is rather a straightforward method to reduce the surface defect density. NaGdF_4 without Ca^{2+} doping as core is hard to realize the complete coverage of core by shells and hence, the obtained nanoparticles usually exhibit a non-uniform size distribution (Figure 3.9). Ca^{2+} has lower valence than that of Gd^{3+} , when Ca^{2+} was introduced to replace Gd^{3+} , the charge imbalance in NaGdF_4 occurred (Figure 3.8c). F^- vacancies were formed in the crystal lattices to meet the requirement of charge compensation. The increasing electron charge density of the crystal can slow down the diffusion of F^- ions to the surface due to increased charge repulsion, and the quantity of the transient electric dipoles on the nanoparticles surface increases, resulting in a diffusion controlled process.[132] Full coverage of nanoparticles can be achieved in this process, leading to a better size-uniformity and fewer surface defects. It is well known that the upconversion emission of lanthanides strongly depends on not only the location of the 4f energy levels of the lanthanide ions, but

also the site symmetry of the lanthanide ions relative to the host, which is significantly affected by their local crystal field environment. A decrease in crystal symmetry proved to be an effective approach to enhance the upconversion luminescence of lanthanide materials in terms of intermixing the f states of lanthanide ions with higher electronic configurations to break the forbidden 4f-4f transitions of lanthanide ions.^{39,40} In this study, the formation of F⁻ vacancies induced by the substitution of Gd³⁺ with Ca²⁺ in the host lowers the local crystal field symmetry around Yb³⁺ and Tm³⁺ ions, which in turn affects the intra 4f-4f transition possibility of lanthanide ions and then enhances the upconversion efficiency.⁴¹ Studies also proved that the perfection in crystallinity of the host materials led to the enhancement of upconversion efficiency.^{41,42} Together, modifying the crystal lattice by doping Ca²⁺ can not only lead to an excellent monodispersity, but also suppress energy loss and enhance the upconversion intensity.

Figure 3.9 TEM images of UCNPs without Ca²⁺ doping. Scale bar: 20 nm.

3.3.4 808-Activated Drug Release *in vitro* and *in vivo*

Encouraged by the high upconversion luminescence of as-synthesized nanoparticles, we demonstrated their use in NIR light-triggered drug release. In our design, mesoporous silica with high surface-to-volume ratio and easy surface modification properties is coated on the surface of the upconversion nanoparticles (UCNP@mSiO₂), which enables not only high drug loading efficiency, but also good water dispersity.⁴³ Doxorubicin (DOX), a typical chemotherapy drug, is loaded into the mesoporous silica *via* diffusion. The blue-light-cleavable ruthenium (Ru), as the molecular valve, is grafted on the surface of UCNP@mSiO₂ nanoparticles to prevent leakage of DOX before reaching the target site (Figure 3.10a). Upon 808 nm light irradiation, the upconverted blue light triggers the cleavage of Ru and uncaps the pores to release DOX and kill the cancer cells. The UCNP@mSiO₂ nanoparticles exhibited relatively uniform morphology with an average diameter of 16 nm (Figure 3.10b). The average pore size and the Brunauer-Emmett-Teller (BET) surface area of UCNP@mSiO₂ were ~2.857 nm and ~289.93 m²/g, respectively (Figure 3.11). To demonstrate the successful conjugation of Ru complex on the surface of nanoparticles, we measured upconversion luminescence spectra of UCNP@mSiO₂ and UCNP@mSiO₂-Ru nanoparticles and it was noted that the intensity of upconversion luminescent at 470 nm decreased significantly in the spectrum of UCNP@mSiO₂-Ru (Figure 3.10c). The hydrodynamic sizes of the UCNP@mSiO₂ and DOX-UCNP@mSiO₂-Ru nanoparticles were determined to be

26.33 nm and 32.42 nm, respectively. The size increase (~ 6 nm) is attributed to the Ru coating and the water molecules associated with Ru. The colloidal stability of the nanoparticles in water was also evaluated using DLS at different time point (Figure 3.12). The data showed that there was no obvious change in the particle size for 3 days, which prove that our particles were stable in DI water in short term. The particles began to aggregate after 3 days, suggesting that further modification may be needed for long-term studies. FTIR and UV/Vis absorption further confirmed that the success of conjugation of Ru complex on the surface of nanoparticles (Figure 3.13). UV/Vis absorption confirmed that upconverted blue light can trigger the cleavage of the Ru complex (Figure 3.14). The drug loading efficiency, measured by fluorescence spectroscopy and UV/vis absorption spectroscopy, was 2.78% and 3.12% (27.8 μg and 31.2 μg DOX in 1 mg nanoparticles), respectively (Figure 3.15). Laser irradiation was carried out on the DOX-UCNP@mSiO₂-Ru to demonstrate the NIR light-controlled drug release. Approximately 42% of DOX was released after 808 nm light irradiation with 0.75 W cm⁻² for 120 min (Figure 3.15). It should be noted that the loaded DOX have not decomposed during 120 min light irradiation with the power density of 0.75 W cm⁻². In addition, control experiment of DOX-UCNP@mSiO₂-Ru without 808 nm light irradiation was measured to monitor whether DOX would premature leak from the nanoparticles. No obvious release was detected up to 4 hours (Figure 3.16b).

Figure 3.10 In vitro NIR-controlled release of DOX. (a) Schematic illustration of functionalization of CSS UCNP with mesoporous silica and DOX for chemo-drug loading and release. (b) TEM images of mesoporous silica coated CSS UCNP. Scale bar: 20 nm. (c) Room temperature spectra of UCNP; UCNP@mSiO₂ and UCNP@mSiO₂-Ru nanoparticles, samples were dispersed in DI water (1 mg ml⁻¹), spectra were recorded under excitation of 808 nm laser at a power density of 7.5 W cm⁻². (d) Cell viability of 4T1 cells with DOX-UCNP @mSiO₂-Ru (concentration: 50 µg ml⁻¹) under different time points. Control: cells with PBS, laser irradiation, UCNP@mSiO₂-Ru, DOX-UCNP@mSiO₂-Ru and DOX-UCNP@mSiO₂ under identical conditions. Detection of photodamage by fluorescence microscopy using fluorescent probes at the DOX-UCNP@mSiO₂-Ru concentration of 50 µg ml⁻¹ at time of (e) 0 min, (f) 10 min, (g) 30 min, (h) 60 min, (i) 90 min, respectively (double-staining with calcein-AM and ethidium homodimer), scale bar: 100 µm.

Figure 3.11 Pore size test by bet. N_2 adsorption-desorption isotherm (a) and pore size distribution (b) of $\text{UCNP}@m\text{SiO}_2$.

Figure 3.12 The colloidal stability of $\text{UCNP}@m\text{SiO}_2\text{-Ru}$ nanoparticles in water. Error bars indicate standard deviations, $n = 3$.

Figure 3.13 FTIR spectra of the $\text{UCNP}@m\text{SiO}_2$, Ru complex (Ru), and $\text{UCNP}@m\text{SiO}_2\text{-Ru}$. In the spectrum of Ru, the band at 1601 cm^{-1} and $1400\text{-}1490\text{ cm}^{-1}$ are assigned to the bipyridine rings and the bands at $730\text{-}790\text{ cm}^{-1}$ belong to C-H. In the spectrum of $\text{UCNP}@m\text{SiO}_2$, the band at $1000\text{-}1100\text{ cm}^{-1}$ and $\sim 795\text{ cm}^{-1}$ are assigned to Si-O-Si asymmetric stretching and Si-O-Si symmetric stretching,

respectively.

Figure 3.14 UV/VIS absorption spectra to prove conjugation and release. UV/Vis absorption spectra of (a) UCNP@mSiO₂ and UCNP@mSiO₂-Ru, (b) DOX-UCNP@mSiO₂-Ru upon 808 nm light irradiation. The absorbance peak decreases, which indicates the cleavage of the Ru complex.

Figure 3.15 Fluorescence spectra ($\lambda_{\text{ex}} = 480 \text{ nm}$) to show release. (a) (black line: the total solution of DOX before irradiation; red line: total solution of DOX subtracted the amount of DOX loading into the nanoparticles) and (b) UV/Vis absorption spectra of Solution 1 (the total solution of DOX before irradiation) and Solution 3 (total solution of DOX subtracted the amount of DOX loading before irradiation).

into the nanoparticles). The drug loading efficiency measured by fluorescence spectroscopy and UV/Vis absorption spectroscopy is 2.78% and 3.12% (27.8 and 31.2 μg doxorubicin in 1 mg nanoparticles), respectively, (c) DOX release profile for DOX-UCNPs@mSiO₂-Ru, (d) Drug release profile of DOX-UCNPs@mSiO₂-Ru at different time interval (30, 60, 90, 120 min).

Prior to evaluate the therapeutic effect of the DOX-UCNP@mSiO₂-Ru, *in vitro* cytotoxicity evaluation of the nanoparticles in 4T1 cells using CCK8 assays showed that the nanoparticles had a cell viability of greater than 90% up to 100 $\mu\text{g}/\text{ml}$ for 24 h, indicating that without laser illumination, DOX-UCNP@mSiO₂-Ru nanoparticles showed no apparent cytotoxic effect in 24 h incubation (Figure 3.16). Subsequently, the chemotherapeutic effect of DOX-UCNP@mSiO₂-Ru was investigated at different time point with 808 nm laser irradiation (Figure 3.10d). The viability of cells was inhibited to 47% at the first 30 min. Irradiation time from 60 min to 90 min further reduced cell viability from 31% to 24%. The fact that the reduction in cell viability increased with irradiation time is because the extended light irradiation triggers more Ru polymer to cleave and more “valves” are opened to diffuse DOX out and kill the cancer cells subsequently. Control experiments under identical condition using laser irradiation only, UCNPs@mSiO₂-Ru, DOX-UCNP@mSiO₂-Ru without laser irradiation and DOX-UCNP@mSiO₂-Ru were carried out for comparison purpose. Treatment with laser irradiation caused no significant effect on the growth of cells, indicating the safe use of 808 nm laser. Negligible cytotoxicity was observed towards cancer cells in the groups of UCNPs@mSiO₂-Ru and DOX-UCNP@mSiO₂-Ru without laser irradiation. In the

group of DOX-UCNP@mSiO₂ with laser irradiation, the overall cell survival rate was comparable to that of experiment group, although the cell cytotoxicity was higher than that of the DOX-UCNP@mSiO₂-Ru nanoparticles with laser irradiation in the initial 30 min. It is because that without the molecular valve, DOX in the DOX-UCNP@mSiO₂ nanoparticles diffuses out easier than that capped inside of the DOX-UCNP@mSiO₂-Ru nanoparticles and causes cytotoxicity effect. As both nanoparticles carried the equal amount of the DOX, the overall cytotoxicity was comparable in the investigation time. It is known that the nanocarriers encounter challenges in the physiological environment in the body, leakage will inevitably take place to some extent on the path to the targeted sites due to a lack of proper protection. The demerit can be overcome by encapsulating the nanocarriers with stimuli-responsive gatekeeper. In our design, light, served as stimuli, triggered the controlled-release of drug when the nanoparticles reach the targeted site, enabled spatial and temporal accurate control. In addition, cell viability was also determined by staining live and dead cells with calcein-AM and ethidium homodimer, respectively. Live and dead cells were visualized as green and red emissions. After 10 min of irradiation, cell death was initiated for the DOX-UCNP@mSiO₂-Ru nanoparticles treated cells. After 30 min of irradiation, cell death was obviously noticeable; significantly reduced cell viability was observed after 90 min NIR irradiation (Figure 3.10e). In comparison, the fluorescence microscopy of the cells after incubated with the UCNP@mSiO₂ and DOX-UCNP@mSiO₂-Ru

nanoparticles was also studied by staining live and dead cells with calcein-AM and ethidium homodimer, respectively. No obvious toxicity toward the cells was detected after incubation with the UCNP@mSiO₂ and DOX-UCNP@mSiO₂-Ru nanoparticles in the investigation time (Figure 3.16). Taken together, these results have clearly demonstrated that upon NIR light irradiation, Nd³⁺-sensitized nanoparticle coated with Ru polymer provides an excellent platform for photo-modulated drug release.

Figure 3.16 Cell viability test. (a) Viability of HeLa cells incubated with different nanoparticles and concentrations for 24 h, (b) Incubation of HeLa cells with pure PBS, UCNP@mSiO₂, and UCNP@mSiO₂-Ru or DOX loaded nanoparticles in the dark did not cause a decrease of cell viability, hereby excluding leakage or non-incorporated DOX, (c) Effects of light exposure (808 nm, 0.75 W/cm²) on the viability of HeLa cells without nanoparticles for different time. Control: cells in the absence of nanoparticles at room temperature.

Figure 3.17 In vivo NIR-controlled release. (a) Tumor growth curves of mice in different groups after various treatments (n=6 per group). All mice received only a single injection of various agents in the experiment. Irradiation was conducted immediately by 808 nm laser at the power density of 0.75 W cm^{-2} for 1 h. Irradiation was repeated once in two days when the experiment was initiated. (b) Average tumor weight after various treatments. (c) Body weight of mice on different groups after various treatments. (d) Images of H&E stained tumor slices collected after 14-day treatment from different groups after various treatments. (e) Images of the tumor slices examined by TUNEL assay after 14-day treatment from different groups after various treatments, scale bar: $100 \mu\text{m}$. * $p < 0.05$.

As a proof-of-concept study, the *in vivo* therapeutic efficacy was evaluated by intratumor injection of the DOX-UCNP@mSiO₂-Ru nanoparticles (DOX: 2.5 g/kg; 50 mg per mice) into the tumor-bearing mice. The tumor-bearing Balb/c mice were randomly divided into five groups (n=6) including group a: PBS, group b: PBS + laser, group c: free DOX, group d: DOX-UCNP@mSiO₂-Ru and group e: DOX-UCNP@mSiO₂-Ru + laser. Tumor sites in the group b: PBS + laser and group e:

DOX-UCNP@mSiO₂-Ru + laser were exposed to 808 nm laser irradiation (0.75 W cm⁻²) for 1 h. Group c treated with free DOX received intratumor injection with the same amount of DOX carried by UCNP@mSiO₂ nanoparticles. Tumor growth was monitored in terms of tumor volume changes and tumor weight (Figure 3.17a, b). As expected, the groups treated with PBS, PBS + laser and DOX-UCNP@mSiO₂-Ru displayed no appreciable effect on tumor growth, which tumor grew 8 times larger on day 14 than it was initial. Mild inhibition of tumor growth was observed in the free DOX group, as tumors grew 6 times larger after 14 days. However, the group of DOX-UCNP@mSiO₂-Ru with laser treatment showed the best tumor growth inhibition efficacy, with 4 times tumor growth. The efficiency of the as-synthesized nanoparticles in the tumor inhibition along with the light controllable release of DOX led us to rationally identify a better design approach for photo-controlled drug release.

The body weight of the mice, an important parameter to evaluate the systemic toxicity of the nanoparticles to the body, was also monitored in the experiment (Figure 3.17c). It was found that there was no apparent decrease during the treatment between the experiment group and control groups, indicating that there was no obvious side effect. At day 14, mice were sacrificed. Tumor and major organs were excised and weighted. Hematoxylin and eosin (H&E) staining of tumor slices and the major organs was conducted for the mice in the five groups (Figure

3.17d, 3.18). In the control groups of PBS, PBS + laser and DOX-UCNP@mSiO₂-Ru without laser irradiation, cancer cells showed activating proliferation with many blue dyeing nucleuses. However, the nucleus of cancer cells became shrinkage and condensation with dark dyeing in the free DOX treatment group. For the DOX-UCNP@mSiO₂-Ru treated group, cancer cells proliferated with more intercellular substance compared with other 4 groups. *In situ* TUNEL staining was conducted on tumor tissue slice excised from the 5 groups of treated mice to study the apoptosis *in vivo*. The nuclei were stained in dark blue, while the apoptotic ones were stained in brown. As shown in Figure 3.17e, DOX-UCNP@mSiO₂-Ru with laser treatment caused a higher percentage of TUNEL-positive apoptotic cells among the five experiment groups. Moreover, H&E staining images of the major organs, such as heart, liver, spleen, kidney and lung in the treatment group and the control groups, showed negligible toxicity. Together, these results demonstrated that the treated group, which resulted in significant apoptosis and necrosis on cancer cells, has a better antitumor effect than other 4 groups. In addition, *in vivo* tissue biodistribution of UCNP@mSiO₂-Ru nanoparticles in mice was measured quantitatively using inductively coupled plasma (ICP) (Figure 3.19). ICP analysis showed that early uptake and accumulation of nanoparticles took place primarily in the liver and spleen, with little nanoparticle accumulation in the heart, kidney or lung. At 1-day post-injection, the signal was greatly reduced in the liver and spleen. The nanoparticles were largely cleared by day 7 post-injection, indicating that they were

not retained for a long period of time. The overall amount of the nanoparticles reduced gradually in the mice's body over time suggesting that they might be excreted through the mice's urine and feces. These data were consistent with the *in vivo* biodistribution studies conducted by Zhang's group.[133] Taken together, we believed that the as-synthesized Nd^{3+} -sensitized nanoparticles had negligible toxicity.

Figure 3.18 The histology analysis of the major organs, including liver, heart, lung, kidney, and spleen, in the mice in the five groups after 14-day treatment.

Figure 3.19 *In vivo* biodistribution of neodymium element in major organs, including heart, liver, spleen, lung and kidney of mice after intravenous injection of UCNPs@mSiO₂-Ru nanoparticles and sacrificed at different time points. Error bars indicate standard deviations, n = 3.

3.4 Conclusions

The current work has overcome a major bottleneck of poor luminescence associated with ultrasmall (< 10 nm) upconversion nanoparticles. By undertaking a combinatorial strategy of energy migration manipulation and crystal lattice modification, we have created ultrasmall-superbright Nd³⁺-sensitized upconversion nanoparticles. We propose that introducing a Yb³⁺-enriched intermediate layer can prevent undesirable cross-relaxation and incorporating Ca²⁺ into the host lattice can reduce luminescence-quenching defects. The improved optical feature of current lanthanide nanoparticles was demonstrated to be effective and promising in 808 nm-mediated chemotherapy. By making the upconversion nanoparticles safer and more efficient, we wish to generate stronger interests amongst clinicians and garner stronger appeals for regulators. We also hope that our approach to nanosystem

engineering could stimulate ideas for the rational design of ultrasmall nanoparticles with enhanced functionalities and/or efficiencies for a wider range of applications, such as waste conversion, energy harvesting and storage.

CHAPTER 4 Thickness Tuning of Sensitizing and Inert Shells in Neodymium-Sensitized Tetralayer Nanoconstructs for Optimal 4-Photon Ultraviolet Upconversion and Near-Infrared Cross-Linked Hydrogel

4.1 Introduction

Upconversion nanoparticles (UCNPs) have drawn increasing attention owing to their unique capability to convert near infrared (NIR) excitation light to ultraviolet (UV)-visible range.[9, 134] This large anti-Stokes shift renders UCNPs designed for photomedicine deeper penetration depth and safer than those activated by direct UV or visible exposure.[135-138] UCNPs also have the advantages of excellent photostability, non-photobleaching, biocompatibility, showing great potential for various clinical applications.[139] However, the low upconversion efficiency, especially for four-photon UV emission process, is a major barrier to the applications of UCNPs.[8, 140] Many strategies have been undertaken to enhance UV upconversion (UC) efficiency, such as particle size increase, core/shell structuring, modification of host lattice, tuning emitter and sensitizer doping ratio, dye sensitization, and high energy excitation irradiation.[5] To obtain strong 4-photon emission in a conventional ytterbium (Yb^{3+})-sensitized system, a high-power 980 nm laser is usually applied, which inevitably causes heating up effect

and rapid cell death.[38] Neodymium (Nd^{3+})-sensitized upconversion nanoparticles absorbing at 808nm have been developed to minimize heating up effect, thus are more suitable for biomedical applications.[16] However, very few works undertake studies to enhance the emission of four-photon process of Nd^{3+} -sensitized UCNPs.

We have demonstrated that a sandwich-like nanostructure, spatially separating emitter ions (Tm^{3+} , Er^{3+} , Ho^{3+}) from sensitizer ions (Nd^{3+}) into different layers could eliminate energy back transfer from emitters to Nd^{3+} and enhance upconversion efficiency.[20, 35] Existing studies mainly focused on tuning the concentration of different ions, especially the concentration of Nd^{3+} as sensitizing ions, while few studies revealed the significance of tuning the thickness of sensitizing layer.[34, 141] Coating an inert shell on the sensitizing layer passivates nanoparticle surface and suppresses non-radiative surface recombination.[142] The generation of tetralayer UCNPs by combining sandwich-like configuration and an inert outer layer provides new opportunities for improving emission intensity.[42, 143] However, coating an extra layer will inevitably create another variable of particle size, a key factor that has not been adequately addressed in previous study.[144]

In this work, we prepare tetralayer core-shell-shell-shell (CSSS) Nd^{3+} -sensitized UCNPs, spatially configured into emitter-insulator-sensitizer-passivator

respectively. We tune the thickness of the 2 outermost shells only but fix the overall particle size to exclude size effect of the tetralayer Nd^{3+} -sensitized UCNPs. We discuss the energy migration mechanism of the 4-photon emission process arisen from balancing different shell thicknesses of the nanoconstructs to optimize the 4-photon emission (360 nm) of Nd^{3+} -sensitized UCNPs. Furthermore, we demonstrate near-infrared triggered cross-linking of a gelatin hydrogel as a potential near-infrared tissue sealant solution for wound closures with minimal heating up effect.

4.2 Materials and Methods

Materials: Gadolinium acetate hydrate ($\text{Gd}(\text{CH}_3\text{COO})_3 \cdot x\text{H}_2\text{O}$), ytterbium acetate hydrate ($\text{Yb}(\text{CH}_3\text{COO})_3 \cdot x\text{H}_2\text{O}$), thulium acetate hydrate ($\text{Tm}(\text{CH}_3\text{COO})_3 \cdot x\text{H}_2\text{O}$), neodymium acetate hydrate ($\text{Nd}(\text{CH}_3\text{COO})_3 \cdot x\text{H}_2\text{O}$), calcium acetate hydrate ($\text{Ca}(\text{CH}_3\text{COO})_2 \cdot x\text{H}_2\text{O}$), 1-octadecene (90%), oleic acid (90%), sodium hydroxide (NaOH , > 98%), ammonium fluoride (NH_4F , 98%), nitrosonium tetrafluoroborate (NOBF_4), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), high glucose Dulbecco's Modified Eagle Medium (DMEM), fluorescein diacetate (FDA) and propidium iodide (PI) were all purchased from Sigma-Aldrich and used as received. Cyclohexane and ethanol

were purchased from Tedia, Singapore. Gelatin powder were purchased from Wako Pure Chemical Industries, Ltd., Japan. Fetal bovine serum (FBS; HyClone™, GE Healthcare Life Sciences, US) and 100 × Antibiotic-Antimycotic (ABAM; Gibco®, Life Technologies, US) were obtained from Thermo Fisher Scientific, US.

Synthesis of NaGd_{0.69}F₄:Yb_{0.2},Tm_{0.01},Ca_{0.1} nanoparticles: Gd(CH₃COO)₃·xH₂O, Yb(CH₃COO)₃·xH₂O, Tm(CH₃COO)₃·xH₂O and Ca(CH₃COO)₂·xH₂O with a total lanthanide amount of 1 mmol was added to a 100 ml flask containing oleic acid (6 ml) and 1-octadecene (14 ml). The resulting mixture was heated at 140 °C for 1 h and then cooled down to room temperature. Subsequently, a methanol solution (6 ml) containing NH₄F (0.96 mmol) and NaOH (0.6 mmol) was added and stirred at 70 °C for 45 min to remove the residual methanol from the reaction mixture. Upon removal of methanol, the solution was heated to 300 °C and maintained at this temperature under an argon flow for 45 min. The solution was then cooled down to room temperature naturally. The resulting nanoparticles were precipitated out with an addition of ethanol, collected by centrifugation, and finally dispersed in 6 ml of cyclohexane.

Synthesis of NaGd_{0.69}F₄:Yb_{0.2},Tm_{0.01},Ca_{0.1}@NaYb_{0.9}F₄:Ca_{0.1} nanoparticles: Yb(CH₃COO)₃·xH₂O and Ca(CH₃COO)₂·xH₂O with a total lanthanide amount of 1 mmol was added to a 100 ml flask containing oleic acid (6 ml) and 1-octadecene

(14 ml). The resulting mixture was heated at 140 °C for 1 h and then cooled down to room temperature. The as-synthesized NaGdF₄:Yb,Tm,Ca core nanoparticles in 3 ml of cyclohexane were added with a methanol solution (3 ml) containing NH₄F (0.48 mmol) and NaOH (0.3 mmol). The resulting mixture was stirred at 70 °C for 45 min to remove the residual methanol from the reaction mixture. Upon removal of methanol, the solution was heated to 300 °C and maintained at this temperature under argon gas for 15 min. The solution was then cooled down to room temperature naturally. The resulting nanoparticles were precipitated out with an addition of ethanol, collected by centrifugation, washed with ethanol, and finally dispersed in 6 ml of cyclohexane.

Synthesis of

NaGd_{0.69}F₄:Yb_{0.2},Tm_{0.01},Ca_{0.1}@NaYb_{0.9}F₄:Ca_{0.1}@NaNd_{0.6}F₄:Gd_{0.3},Ca_{0.1}

nanoparticles: Nd(CH₃COO)₃·xH₂O, Gd(CH₃COO)₃·xH₂O and Ca(CH₃COO)₂·xH₂O with a total lanthanide amount of 1 mmol was added to a 100 ml flask containing oleic acid (6 ml) and 1-octadecene (14 ml). The resulting mixture was heated at 140 °C for 1 h and then cooled down to room temperature. 3 ml of the as-synthesized NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca core-shell nanoparticles in cyclohexane were added with a methanol solution (3 ml) containing NH₄F (0.48 mmol) and NaOH (0.3 mmol). The amount of NH₄F and NaOH can be adjusted for different thickness of NaNdF₄:Gd,Ca layer. The resulting mixture was stirred at 70

°C for 45 min to remove the methanol from the reaction mixture. Upon removal of methanol, the solution was heated to 300 °C and maintained at this temperature under an argon gas for 45 min/1 h. The solution was then cooled down to room temperature naturally. The resulting nanoparticles were precipitated out with an addition of ethanol, collected by centrifugation, washed with ethanol, and finally dispersed in 6 ml of cyclohexane.

Synthesis of

NaGd_{0.69}F₄:Yb_{0.2},Tm_{0.01},Ca_{0.1}@NaYb_{0.9}F₄:Ca_{0.1}@NaNd_{0.6}F₄:Gd_{0.3},Ca_{0.1}@

NaGd_{0.9}F₄:Ca_{0.1} nanoparticles: Gd(CH₃COO)₃·xH₂O and Ca(CH₃COO)₂·xH₂O

with a total lanthanide amount of 1 mmol was added to a 100 ml flask containing oleic acid (6 ml) and 1-octadecene (14 ml). The resulting mixture was heated at 140 °C for 1 h and then cooled down to room temperature. 3 ml of the as-synthesized NaGdF₄:Yb,Tm,Ca@NaYbF₄:Ca@NaNdF₄:Gd,Ca core-shell-shell nanoparticles in cyclohexane were added with a methanol solution (4 ml) containing NH₄F (0.64 mmol) and NaOH (0.4 mmol). The resulting mixture was stirred at 70 °C for 45 min to remove the methanol from the reaction mixture. Upon removal of methanol, the solution was heated to 300 °C and maintained at this temperature under an argon gas for 30 min. The solution was then cooled down to room temperature naturally. The resulting nanoparticles were precipitated out with an addition of ethanol, collected by centrifugation, washed with ethanol, and finally dispersed in 6 ml of

cyclohexane.

Preparation of ligand-free tetralayer UCNPs: The prepared UCNPs in hexane was first precipitated by ethanol. The bottom transparent pilot was treated with 2 ml 0.1M HCl aqueous solution. Then put the mixture into the ultrasonic machine and ultrasonicated for 1 h to remove the OA on the surface. The OA removed tetralayer UCNPs were collected via centrifugation and treated again if the redispersion is not clear. The ligand-free UCNPs were washed by and stored in DI water.

Synthesis of methacrylated gelatin (GelMA): Methacrylated gelatin precursor was synthesized using the carbodiimide cross-linking reaction. Briefly, 2 w/v % gelatin solution was prepared by dissolving gelatin powder in DI water and heating at 60 °C. 0.51 ml methacrylic acid was added into gelatin solution, followed by 0.609 g N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride and 0.189 g N-hydroxysuccinimide. The pH of the mixture was adjusted to 4.7 and allow to react overnight. The pH of the mixture was adjusted to 7 to stop the reaction. Finally, the mixture was dialyzed against DI water for 2 days and lyophilized to get the final dried product.

Synthesis of NIR-activated hydrogel: Lyophilized GelMA was dissolved in 1×PBS to get a 10 w/v% aqueous solution. LAP was added into the 0.5ml hydrogel

precursor solution to get a final concentration of 1% w/v%. 0.5 ml UCNPs aqueous solution with a concentration of 10 mg/ ml was mixed with the GelMA+LAP solution and then poured into 24 well plate and crosslink under the irradiation of NIR laser for 3 min.

Cytotoxicity studies of UCNPs: HaCaT cells were seeded onto 24 well plates with a cell density of 3×10^4 cells per well and top up with high glucose DMEM supplemented with 10% FBS and $1 \times$ ABAM. The cells were then incubated at 37°C and 5% CO₂ for overnight. After adhesion, the culture medium was removed and replaced with culture medium containing different concentrations of UCNPs. The cells were then incubated together with the presence of UCNPs for 24h. Cell viability was assessed using PrestoBlue™ Cell Viability Reagent (Invitrogen™, Thermo Fisher Scientific, US) according to manufacturer's protocol. Cells after the treatment were also stained with 5 mg/ml FDA and 2 mg/ml PI. The Live/dead staining images were obtained using a ZEISS fluorescence microscope.

HDFs proliferation studies using GelMA and GelMA+UCNPs: HDFs were cultured on GelMA and GelMA+UCNPs using a 96 well plate and initial seeding density of 1×10^4 cells per well. Each well was topped up with high glucose DMEM supplemented with 10% FBS and $1 \times$ ABAM. The samples cultured with HDFs were incubated at 37°C and 5% CO₂. The metabolite activity at day 1, 3, 5 and 7

were assessed using PrestoBlue™ Cell Viability Reagent (Invitrogen™, Thermo Fisher Scientific, US) according to manufacturer's protocol. At the same time points, live/dead images were also obtained by first stained the cells with 5 mg/ml FDA and 2 mg/ml PI, follow by imaged using a ZEISS fluorescence microscope.

Characterizations: Transmission electron microscopy (TEM) images were recorded on JEOL 2010 UHR and JEOL JEM-2100F microscope operating at 200 kV. Powder X-ray diffraction analysis (XRD) was carried out on a D8 Advance Bruker powder X-ray diffractometer with Cu K α radiation ($\lambda = 1.5406$ Å) from 10° to 80° with a counting time of 1s per step. To obtain the upconversion photoluminescence spectra, samples were dispersed in cyclohexane (1 mg ml⁻¹) in a standard quartz cuvette at room temperature, and fluorescence spectra were acquired on a Fluoromax-4, Horiba Jobin Yvon Spectrofluorometer with a commercial 808 nm CW laser. Zeta potential of UCNPs, GelMA, UCNPs+GelMA were measured using dynamic light scattering (DLS) in a Malvern Nano Zetasizer system by Malvern Instruments. UV-vis spectrum was recorded on a Shimadzu UV-3600 spectrophotometer. Fourier Transform Infrared Spectroscopy (FTIR) was conducted in a Digilab FTS 3100 instrument. The Live/Dead cell viability was analyzed by a Zeiss Observer Z1 confocal microscopy (ZEISS, Germany).

4.3 Results and Discussion

4.3.1 Design, Synthesis and Characterization of Tetralayer UCNPs

In the current Nd^{3+} -sensitized UC system design, Tm^{3+} ions are doped in the core as UV/ blue light emitters. Nd^{3+} ions serve as primary sensitizing ions, Yb^{3+} ions in core and intermediate layer is designed as bridging ions to transfer energy from Nd^{3+} to Yb^{3+} , separating Nd^{3+} and Tm^{3+} in different layers to minimize cross-relaxation and back energy transfer between Nd^{3+} and Tm^{3+} ions. Adding an inert layer on the sandwich-structure to fabricate tetralayer structure will passivate the surface and enhance UC emission. Core-shell-shell-shell (CSSS) Nd^{3+} -sensitized UCNPs were designed and synthesized, spatially configured into emitter-insulator-sensitizer-passivator respectively (Figure 4.1f).

In detail, NaGdF_4 has been chosen as the host matrix to get easy control the size and shape of all these nanoparticles. Ca^{2+} ions are doped in each layer to enhance the UC conversion efficiency.[35] Previous studies have proven the optimal concentration for UV emission of Tm^{3+} is 1%, and the optimal concentration of Nd^{3+} in outer layer is 60%.[35, 145] As the primary focus of this work is thickness modulation of the outer two layers, the concentration of Tm^{3+} , Yb^{3+} ions in the core is fixed at 1%, 20%, the concentration of Yb^{3+} ions in intermediate (second) layer is fixed at 90%, and the concentration of Nd^{3+} ions in the sensitizing (third) layer is fixed at 60%.

$\text{NaGd}_{0.69}\text{F}_4:\text{Yb}_{0.2},\text{Tm}_{0.01},\text{Ca}_{0.1}@\text{NaYb}_{0.8}\text{F}_4:\text{Gd}_{0.1},\text{Ca}_{0.1}@\text{NaNd}_{0.6}\text{F}_4:\text{Gd}_{0.3},\text{Ca}_{0.1}@\text{NaGd}_{0.9}\text{F}_4:\text{Ca}_{0.1}$ tetralayer nanoparticles were synthesized layer-by-layer *via* a modified thermal decomposition method (Figure 4.1a).[28, 35] Transmission electron microscopy (TEM) images of $\text{NaGdF}_4:\text{Yb},\text{Tm},\text{Ca}$ (core), $\text{NaGdF}_4:\text{Yb},\text{Tm},\text{Ca}@\text{NaYbF}_4:\text{Gd},\text{Ca}$ (core-shell, expressed as CS), $\text{NaGdF}_4:\text{Yb},\text{Tm},\text{Ca}@\text{NaYbF}_4:\text{Gd},\text{Ca}@\text{NaNdF}_4:\text{Gd},\text{Ca}$ (core-shell-shell, expressed as CSS) and $\text{NaGdF}_4:\text{Yb},\text{Tm},\text{Ca}@\text{NaYbF}_4:\text{Gd},\text{Ca}@\text{NaNdF}_4:\text{Gd},\text{Ca}@\text{NaGdF}_4:\text{Ca}$ (core-shell-shell-shell, expressed as CSSS) showed uniform morphology, with an average diameter of 16.2 ± 0.7 , 18.4 ± 0.6 , 24.2 ± 0.6 and 28.2 ± 0.5 nm, respectively (Figure 4.1b-e). The increase of particle size indicated successful epitaxial growth of different shell layers. Scanning TEM was utilized to further confirm the multilayer core-shell structure. Energy-dispersive X-ray (EDX) line scan analysis gave further evidence of tetralayer configuration by measuring the element distribution of a single UCNPs (Figure 4.1g, h). EDX element mapping confirmed the success of doping all kinds of elements (Figure 4.1f). XRD spectra of Core, CS, CSS and CSSS showed good crystallinity and the peak position matched with JCPDS 27-0699 (Figure 4.2).

In this design, we want to exclude the size effect to shed light on how the thickness of sensitizing layer and inert layer can influence the emission property of UCNPs.

Therefore, the total thickness of sensitizing layer and inert layer is fixed as 5 nm (Figure 4.1i). By controlling the growth time for different layers, the thickness of sensitizing layer is precisely controlled at 1,2,3 and 4 nm and correspondingly, the thickness of inert layer is controlled at 4,3,2 and 1 nm. Under 808 nm irradiation, the tetralayer UCNPs displayed typical Tm^{3+} emission peaks at 360, 450, 475, 649, and 696 nm (Figure 4.1j).[146]

Figure 4.1 Synthesis process of tetralayer UCNPs. (a) Scheme showing the synthetic processes and TEM images of the (b) core 16.2 ± 0.7 nm; (c) core-shell 18.4 ± 0.6 nm; (d) core-shell-shell 24.2 ± 0.6 nm; (e) core-shell-shell-shell 28.2 ± 0.5 nm nanoparticles. (f) Dark-field STEM image of a single CSSS UCNP (top left) and its corresponding EDX elemental mapping images. (g) EDX line scan profiles of a single CSSS UCNP along the line shown in the dark-field STEM image and (h) the corresponding element distribution to prove the CSSS structure. (i) Scheme of thickness tuning in tetralayer UCNPs. (j) Energy transfer diagram for emissions involving different numbers of photons.

Figure 4.2 XRD patterns of Core, CS, CSS and CSSS UCNPs.

4.3.2 Tuning the Thickness of Sensitizing Layer in CSS UCNPs

We first investigated the effect of sensitizer layer thickness alone on the emission property of the UCNPs. Sandwich-structure CSS UCNPs with different sensitizing layer thicknesses were prepared with the same CS nanoparticles (~18.2 nm). The thickness of sensitizing layer was precisely tuned from 1 nm to 4 nm by changing the reaction time from 15 min to 60 min (Figure 4.3a), resulting in 20.2, 22.2, 24.2, and 26.3 nm sizes of as-synthesized CSS UCNPs, respectively (Figure 4.3 b-e). With increasing thickness of sensitizing layer, an enhancement of 360 and 475 nm emission was observed (Figure 4.3f). In the traditional sandwich-structure configuration, the 3-photon (475 nm) emission process is more favorable as it is

much stronger than other emission peaks. From the results, it can be observed that the thickness of sensitizing layer plays a vital role in enhancing the upconversion process *via* two main mechanisms. In the first place, thicker sensitizing layer can provide stronger absorption under same excitation power density. In addition, a thicker layer endows a self-passivating property, keeping a greater portion of Nd^{3+} ions in the sensitizing layer from directly interacting with surrounding environment to reduce the quenched luminescence by vibrating surface molecules (Figure 4.3h).[46] When the thickness of sensitizing layer increased from 1 to 4 nm, 360 nm emission increased by 41.3-fold and 475 nm emission increased 29.2-fold (Figure 4.3g), while the enhancement of 360 nm emission is more obvious than 475 nm. Higher photons excitation process is more sensitive to the increase of transferred energy. To obtain a strong 4-photon emission in UC process, rational design of nanoparticle configuration to enable greater energy transferred to higher energy states is a more feasible strategy than applying expensive and high-power laser excitation. Meanwhile, many studies have shown that high Nd^{3+} dopant concentration will cause the decrease in emission intensity due to non-radiative cross-relaxation between Nd^{3+} ions in a shorter ionic distance. Therefore, Nd^{3+} dopant concentration was limited to below 60% as consistent to the current study. Thus, a different strategy must be adopted in order to further enhance upconversion emission in the 360 and 475 nm as the concentration of Nd^{3+} ions and the thickness of sensitizing layer cannot be infinitely increased.

Figure 4.3 Synthesis and comparison of CSS UCNPs with different sensitizing layer thickness. (a) Scheme showing the tuning process of sensitizing layer and TEM images of the (b) CSS 15 min 20.2 ± 0.6 nm; (c) CSS 30 min 22.2 ± 0.7 nm; (d) CSS 45 min 24.2 ± 0.6 nm; (e) CSS 60 min 26.3 ± 0.6 nm nanoparticles. (f) Room temperature emission spectra and (g) comparison of the emission intensity of 360 nm and 450 nm of CSS UCNPs (dispersed in hexane, 1 mg ml^{-1}) with different sensitizing layer thickness (power density $10 \text{ W} \cdot \text{cm}^{-2}$). (h) Schematic diagram of energy transfer mechanism for tuning the thickness of sensitizing layer.

4.3.3 Tuning the Thickness of Inert Layer in CSSSS Tetralayer UCNPs

The coating of an outermost inert layer in fluorescent nanoparticulate system for surface passivation has been widely adopted to enhance UC emission by minimizing surface defects and preventing the direct interaction of sensitizing ions and surrounding solvent environment.[147] Nevertheless, few studies discuss in detail how the thickness of inert layer can influence UC process involving different number of absorbed photons, and most of studies neglected size effect during

comparison. For nano-dimensional materials, size effect is highly crucial especially for biomedical applications. Larger nanoparticle size may lead to accumulation in some organs and difficulty for excretion, hence creating potential nanotoxicity. In the current work, the total size of tetralayer UCNPs was fixed at 28 nm (Figure 4.4a), with the combined thickness of sensitizing and inert layers fixed at 5 nm. We then tuned the thickness of sensitizing and inert layers to maximize energy absorption and reduce nonradiative surface quenching in order to obtain an optimal combination of layer thickness for efficient energy transitions.

Figure 4.4 Different tetralayer configurations and comparison of their emission spectra. (a) Different tetralayer configurations with same size and TEM images of the CSSS 15/60 (b) 28.2 ± 0.6 nm; CSSS 30/45 (c) 28.1 ± 0.5 nm; CSSS 45/30 (d) 28.3 ± 0.6 nm; CSSS 60/15 (e) 28.2 ± 0.5 nm nanoparticles. (f-i) Comparison of emission spectra of different CSSS and their seeding CSS (power density $10 \text{ W} \cdot \text{cm}^{-2}$).

The tetralayer UCNPs were synthesized by epitaxial growth of NaGdF_4 inert layer

with different shell thickness from 4 nm to 1 nm. TEM images of CSSS with different shell thickness show a uniform hexagonal morphology and similar size at 28 nm (Figure 4.4 b-e). The PL intensity of tetralayer UCNPs was tested and compared with the CSS counterparts (Figure 4.4 f-i). By coating an inert layer, all these tetralayer UCNPs show stronger emission intensities than their corresponding CSS UCNPs, which consists with the commonly accepted theory. The emissions at 360 nm (a 4-photon process) and 475 nm (a 3-photon process) were also compared. , The emissions of tetralayer UCNPs at 360 nm increased 72.6, 30.7, 9.4 and 2.9-fold, respectively as the thickness of coated inert layer decreased from 4 nm to 1 nm (Figure 4.5c). At the same time, the emission of 475 nm increased 27, 3.4, 1.3 and 1.1-fold (Figure 4.5d). The thinner the sensitizing layer is, the enhancement effect of surface passivation is more obvious. After coating an inert layer, these tetralayer CSSS UCNPs with different configurations have similar intensities of 475 nm emission, while the emission intensities of 4-photon process of 360 nm vary significantly (Figure 4.5 a, b). Interestingly, the optimal combination of thickness of sensitizing and inert layer is 3 and 2 nm, and the enhancement of 360 nm emission for CSSS UCNPs is much more obvious than that of 475 nm.

Figure 4.5 Comparison of 3-photon and 4-photon emission and possible mechanism. (a) Room temperature emission spectra of tetralayer UCNP with different sensitizing and inert layer thickness (power density 10 W cm^{-2}). (b-d) Comparison of emission intensities at 360 nm and 475 nm of different CSS and CSSS UCNP. (e-f) Schematic diagram of energy transfer mechanism for balancing the thickness of sensitizing layer and inert layer.

A recent report has shown that vibrating surface molecules chelated to exposed surface ions, particularly sensitizing ions (Yb^{3+} or Nd^{3+} ions), can generate surface phonons, and thus quenching the luminescence of UCNP.[46] Coating an additional layer of inert layer can insulate the sensitizing ions from vibrating surface molecules, achieving stronger emission intensity (Figure 4.5e). In the tetralayer CSSS UCNP system, we had demonstrated for the first time that tuning the thickness of sensitizing layer and inert layer rendered the nanoconstructs a balance of absorption of energy and surface energy loss, enabling an optimal UV emission. For energy transfer processes involving different number of photons, the emission intensity generally follows a power dependent relationship $I \propto P^n$, in which P is the

pumping power (expressed as $E_{\text{absorption}} - E_{\text{loss}}$) [145] and n is the number of photons involved in the process.[36, 148] P is proposed be expressed as $E_{\text{absorption}} - E_{\text{loss}}$. [145] The 360 nm emission is a four-photon conversion process and is more sensitive to the change of excitation energy than the three-photon process. Thus, with an adequate thickness of sensitizing layer and inert layer, P is maximized for the generation of a stronger 4-photon emission at a relatively low pumping power (Figure 4.5f). The current work has successfully demonstrated a simple strategy to balance the sensitization and passivation processes *via* nanostructure engineering of the tetralayer nanoparticle configuration, focusing on the use of the intrinsic energy transfer mechanism of UCNPs to enhance the emission intensity of 4-photon emission process instead of employing extrinsic tools such as a high power laser source.

4.3.4 808 nm-Activated Cross-Linking of Gelatin-Based Hydrogel

The current significantly enhanced UV-emission from the tetralayer UCNPs further prompted a proof-of-concept demonstration of an 808 nm-activated hydrogel system. In this system, tetralayer UCNPs serve as nanotransducers converting low energy NIR light to high energy UV light which initiate the crosslinking of methacrylated gelatin with the assistance of a photo-initiator (Lithium phenyl-2,4,6-trimethylbenzoylphosphinate, expressed as LAP).[149, 150] Figure 4.6a, b shows the schematic synthesis and cross-linking process of gelatin-based hydrogel (GelMA). For commonly used photo-initiators, they require light source with high

energy, especially UV light. However, these light sources have limited penetration depth due to their strong interactions with biological tissues. The use of UV-emitting UCNPs in combination with a photo-initiator addresses issues pertaining to safety of direct UV exposure as well as low penetration depth. OA-capped UCNPs were converted to ligand-free UCNPs (verified by FTIR, Figure 4.6c) to render hydrophilic properties such the UCNPs are dispersible in the hydrogel matrix.[151]

Figure 4.6 NIR-enabled cross-linking of GelMA. (a, b) Scheme of the synthesis of GelMA and crosslinking between GelMA. (c) FTIR spectra of OA-capped UCNPs and bare UCNPs. (d) Emission spectra of UCNPs and absorption of LAP photo-initiator. (e) Digital pictures to show the transparency of cross-linked GelMA and GelMA+UCNPs. (f) Digital pictures to show the cross-linking under NIR irradiation. (g) Proliferation curves of cells on GelMA and GelMA+UCNPs. (h) Live/dead staining of cells on tissue culture plate (TCP) as contrast, GelMA and GelMA+UCNPs (green fluorescent represents live cells, red fluorescent represents dead cells and scale bar represents

200 μ m).

Methacrylated gelatin (GelMA) precursor was synthesized *via* a similar reported method using the carbodiimide cross-linking reaction (Figure 4.6 a, b).[152] The zeta potentials of ligand-free UCNPs, GelMA and GelMA+UCNPs were measured to be 33.2 ± 0.27 , -12.1 ± 0.27 , 19.8 ± 0.95 mV. The prepared ligand-free UCNPs and GelMA precursor mixture was then cross-linked upon 808 nm irradiation (Figure 4.6d), since the absorption of LAP overlapped with the UV emission of UCNPs. On the contrary, the mixed precursor without 808 nm laser exposure remained as liquid and fell off the wall when it was turned over (Figure 4.6f). We further tested the cytotoxicity of UCNPs using HaCaT cells and human dermal fibroblasts (HDFs) on GelMA and GelMA+UCNPs (Figure 4.6h, 4.7). The results show a high cell viability of 90% in ligand-free UCNPs even at a high concentration of UCNPs of 400 μ g ml⁻¹. The HDFs can successfully attach and proliferate for 7 days on GelMA and GelMA+UCNPs (Figure 4.6g, h). The inclusion of UCNPs in GelMA showed enhance proliferation of HDFs, probably due to the increase stiffness induced by the presence of UCNPs in the hydrogel.

Figure 4.7 Cell viability test of UCNPs. (a) Cell viability test under different concentrations of UCNPs. Corresponding live/dead staining of cells with different concentrations from (b) 0, (c) 1, (d) 9, (e) 36, (f) 81, (g) 144, (h) 225, (i) 400 µg ml⁻¹.

4.4 Conclusions

In this work, we have studied the configuration design of Nd³⁺-sensitized tetralayer UCNPs by confining the total thickness of sensitizing layer and inert layer in 5 nm for the first time. Utilizing this method, it has been found that the three-photon (470 nm) emission of tetralayer UCNPs was mainly influenced by size and the four-photon (360 nm) emission of tetralayer UCNPs was largely influenced by configuration instead of size. The strongest emission was observed with a thickness

of 3 nm of sensitizing layer and 2 nm inert layer by optimizing the absorption and surface quenching to favor 3 and 4-photon upconversion processes. This work describes a new strategy to exploit the intrinsic energy transition mechanism of lanthanide ions to enhance high-number-photon upconversion processes without the need to use high excitation power sources such as expensive pulse laser dipoles.[145, 153] We further demonstrated NIR-crosslinked hydrogel system by incorporating the current enhanced UV-emission UCNPs with a UV-cross-linkable hydrogel system. This strategy may be adopted to various hydrogel crosslinking, degradation and oxidation processes, enabling NIR-activated drug delivery, photodynamic therapy, tissue glue, biocatalysis and detection which current state-of-art is limited by the shallow penetration depth and potential UV toxicity.

CHAPTER 5 Generating New Cross-Relaxation Pathways by Coating Prussian Blue on NaNdF₄ to Fabricate Enhanced Photothermal Agents

5.1 Introduction

Recent decades have witnessed the development of photothermal agents (PTAs), among which near-infrared (NIR) absorbing PTAs are highly favorable due the higher penetration depth of NIR photons and minimal absorption in biological tissues.[154, 155] Different types of NIR-driven PTAs, such as noble metal nanostructures, carbon materials, semiconducting nanoparticles, rare-earth doped nanoparticles and organic compounds have been developed and demonstrated in photoacoustic imaging (PAI) and photothermal therapy (PTT).[60, 156-161] The intensity of ultrasonic signal in PAI and therapeutic efficacy of PTT is largely decided by the photothermal conversion efficiency of PTAs. How to improve the photothermal conversion efficiency of PTAs, especially inorganic PTAs, has been and still is a research hotspot.

Scheme 5.1 Fabrication of NdNdF₄@PB core/shell structure to generate new cross-relaxation pathways to enhance photoacoustic imaging signal and photothermal therapy efficacy.

Inorganic PTAs have the advantages of excellent photostability, multifunctionality, and ease of synthesis and modification. While the existing popular inorganic PTAs are faced with different shortcomings, such as morphology-dominated unstable absorption of gold nanorods, toxic metal components of semiconducting nanoparticles, complicated synthesis process of carbon nanotubes and weak absorption of rare-earth doped nanoparticles.[162-165] Among all kinds of inorganic PTAs, neodymium (Nd³⁺)-doped nanoparticles (NdNPs) and Prussian blue (PB) have been reported and demonstrated as PTAs. NdNPs, such as LaF₃:Nd³⁺ and NdVO₄, feature good photostability and ease of multifunctionality as NIR fluorescent markers.[44, 49, 87, 88] However, the photothermal performance of

reported NdNPs is yet not satisfying due to their weak absorption around 808 nm. The photothermal effect of NdNPs is mainly decided by the cross-relaxation (CR) between Nd^{3+} ions.[49] CR effect is enhanced when the doping ratio of Nd^{3+} ions is higher. Even for highly Nd^{3+} -doped PTAs, they still need a high excitation power density to achieve the threshold temperature for cancer cells ablation (generally believed $>43^{\circ}\text{C}$). PB, a US Food and Drug Administration approved compound for clinical use, has great potentials in various biomedical applications due to their excellent biocompatibility.[166, 167] While the maximum absorption of PB is located within 690-720 nm and the photothermal conversion efficiency of PB under 808 nm irradiation is just around 20%.[168, 169] To get good photothermal performance, 808 nm laser with high excitation power density or some expensive laser diodes are required (730,785 nm etc.), which largely limits the applications of PB as PTA.[170, 171]

5.2 Materials and Methods

Materials and Reagents: neodymium acetate hydrate, 1-octadecene (90%), oleic acid (90%), sodium hydroxide ($> 98\%$), ammonium fluoride (98%), nitrosonium tetrafluoroborate (95%) were all purchased from Sigma-Aldrich and used as received. Hexane, ethanol, acetone, were purchased from Tedia, Singapore. Iron (II) sulfate hepta-hydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 98%) was purchased from Alfa Aesar.

Synthesis of NaNdF₄ nanoparticles: 1 mmol of Nd (CH₃COO)₃ was added to a 100 ml flask containing oleic acid (6 ml) and 1-octadecene (14 ml). The resulting mixture was heated to 140 °C and maintained for 1 h under the protection of N₂ flow and then cooled down to room temperature. Subsequently, a methanol solution (10 ml) containing NH₄F (0.96 mmol) and NaOH (0.6 mmol) was added and stirred at 70 °C for 45 min to remove the residual methanol from the reaction mixture. Upon the removal of methanol, the solution was heated to 300 °C and maintained at this temperature under a nitrogen flow for 40 min. The solution was then cooled down to room temperature naturally. The resulting nanoparticles were precipitated out with an addition of ethanol, collected by centrifugation, and finally dispersed in 6 ml of cyclohexane.

Surface modification of NaNdF₄ nanoparticles: The as-synthesized NaNdF₄ nanoparticles (NdNPs) were capped with oleic acid ligands. To coat Prussian blue (PB) on the surface of NaNdF₄ nanoparticles, oleic acid was replaced by BF₄⁻ with the treatment of nitrosonium tetrafluoroborate (NOBF₄). 60 µl NOBF₄ (1M) in DMF was mixed with 1 ml oleic acid capped NdNPs dispersed in hexane (5 mg ml⁻¹) at room temperature. Then the mixture was gently shaken for 30 s and put on the vortex and rotated for 2 min. Stopped the shake when there was a clear separation of upper layer and bottom layer to extract NdNPs from hexane to DMF. The mixture was then centrifuged at 13000 rpm for 25 min to get the transparent purple pilot.

The BF_4^- stabilized NdNPs were weighed for further treatment. 0.5 ml saturated aqueous solution of citric acid (CA) was mixed with the BF_4^- capped NdNPs and then ultrasonicated for 30 min at room temperature to get a clear solution. The resultant solution was centrifuged and washed to get rid of the excess of CA. The CA-capped NdNPs were then dispersed in 4ml DI water.

Synthesis of NdNP@PB: The obtained 2 ml CA-capped NdNPs aqueous solution were mixed with 2 ml aqueous solution of FeSO_4 (5 μM) and CA (90 μM). Under the magnetic stirring, 2 ml solution of $\text{K}_3\text{Fe}(\text{CN})_6$ (5 μM) were slowly added at room temperature. After vigorous stirring for 1h, the solution was centrifuged and washed with acetone and DI water, and then re-dispersed in DI water for further characterization and usage.

Synthesis of PB: 5 ml aqueous solution of $\text{K}_3\text{Fe}(\text{CN})_6$ (5 μM) are mixed with 1ml aqueous solution of CA (90 μM). Under vigorous magnetic stirring, 5 ml aqueous of FeSO_4 (5 μM) was added at room temperature. The stirring was kept for 1h, the solution was then mixed with acetone and centrifuged. The obtained blue precipitant was washed with DI water and acetone to remove the excess of CA and re-dispersed in DI water for further usage.

Characterization: transmission electron microscopy (TEM) and high resolution

TEM images were recorded on JEOL 2010 UHR, JEOL JEM-2100F and JEOL JEM 2100PLUS microscope operating at 200 kV. STEM image and EDX elemental mapping images were recorded on a JEOL JEM-2100F scanning TEM. Powder X-ray diffraction analysis (XRD) was carried out on a D8 Advance Bruker powder X-ray diffractometer with Cu K α radiation ($\lambda = 1.5406$ Å) from 10° to 80° with a counting time of 1s per step. To obtain the photoluminescence spectra, samples were dispersed in water (1 mg ml⁻¹) in a standard quartz cuvette at room temperature, and fluorescence spectra and the corresponding fluorescence decay time were acquired on a DeltaFlex (Horiba Scientific, IBH, UK) equipped with a NIR PMT H10330-75 (Hamamatsu, Japan) and a modulated 808 nm laser (Orient KOJ Limited, Tianjin, PRC.) for excitation. Hydrodynamic size and stability of the nanoparticles were measured using dynamic light scattering (DLS) in a Malvern Nano Zetasizer system by Malvern Instruments. UV-vis spectrum was recorded on a Shimadzu UV-3600 spectrophotometer. Fourier Transform Infrared Spectroscopy (FTIR) was conducted in a Digilab FTS 3100 instrument. The temperature change was recorded by an infrared thermal camera (Fotric 225, Shanghai, China). Photoacoustic imaging signal was tested and collected using the Vevo LAZR imaging system software package or OsiriX.

Photothermal performance of NdNP@PB: Photothermal performance of NdNP@PB (0.5 mg mL⁻¹, 100 μ l) was obtained and analyzed by irradiating of an

808 nm NIR laser at power density of 0.6 W cm^{-2} for 5 min. The temperature change was recorded every 10 s by the infrared thermal camera. NdNPs and PB as control groups were measured under the same condition. The calculation of photothermal conversion efficiency was determined according to the previous study of Roper's et al.[172, 173] The detailed calculation formula has been listed as below.

According to the well-established model reported by Roper's et al., the photothermal conversion efficiency was calculated by the following formula:

$$\eta = \frac{hS(T_{Max} - T_{surr}) - Q_{Dis}}{I(1 - 10^{-A_{\lambda}})}$$

where h represents heat-transfer coefficient, S represents the surface area of the container, T_{Max} represents the equilibrium temperature (the highest temperature the sample can reached with a laser irradiation), T_{Surr} represents the surrounding temperature, Q_{Dis} is the baseline energy input of the sample cell and the A_{λ} is the absorbance of nanoparticles at λ of 808nm.

Photothermal stability of NdNP@PB: The solution of NdNP@PB were irradiated by a 808 nm NIR laser (0.6 W cm^{-2}) for 5 min. Then, the laser was closed and NdNP@PB was cooled naturally for 5 min. There are total five heating and cooling cycles. The temperature change was recorded every 10 s by the infrared thermal camera. NdNPs and PB were measured under the same condition.

***In vitro* cell viability assessment:** To evaluate the cell viability of NdNP@PB

nanocomplex, the nanocomplex was dissolved in PBS and 10 μ l DMSO under different concentrations. HELA cells were cultured in 96-well plates for 20 h and incubated with nanocomplex of different concentrations for 8h. The cytotoxicity test was performed in dark (without 808 nm laser irradiation). The cell viability test was tested under different concentrations after 808 nm laser irradiation (1 W cm^{-2}) for 15 min. The cell was co-stained by calcein AM and propidium iodide and their fluorescence images were collected by confocal microscope.

Animals and tumor model: Female HELA tumor-bearing BALB/c nude mice were purchased from Nanjing Mergene Life Science Co.,Ltd. And used in this work according to the guideline of the Laboratory Animal Center of Nanjing Mergen Life Science Co., Ltd. HELA cells (1×10^6) were subcutaneously injected into the wanted region of the nude mice to obtain HELA tumor-bearing mice. After 16 days, tumor grew to 70 mm^3 . Tumor volume is calculated according to the formula $V = LW^2/2$, in which L and W represent the longitudinal and transverse diameter of the tumor, respectively.

***In vivo* photoacoustic imaging and photothermal antitumor assessment:** Tumor bearing BALB/c nude mice were randomly divided into 4 groups and each group contained 5 mice. Each group was injected intravenously with 150 μ l (a) PBS, (b) PBS+laser, (c) NdNP@PB (0.5mg/ml), (d) NdNP@PB (0.5 mg ml^{-1})+laser. Control

groups of (e) PB (0.5mg/ml), (f) PB (0.5mg/ml)+laser, (g) NdNPs (0.5mg/ml) and (h) NdNPs (0.5mg/ml)+laser were added and operated under the same experiment conditions. All these groups were treated once. PA imaging was first performed, and the optimal photo treatment time was decided by the highest accumulation dosage of nanocomplex in tumor. The real-time *in vivo* PAI was performed using Endra Nexus 128 PA tomography system (Endra, Inc., Ann Arbor, MI). The excitation wavelength was fixed at 808 nm. The PAI signal was collected by 128 ultrasonic transducers with 5.8 MHz center frequency in hemispherical fashion in a chamber containing water 37 °C water. The PAI raw data and PAI intensity was analyzed by OsiriX Lite software. The tumor sites of mice in group (b), (d), (f), and (h) were irradiated by 808 nm laser (0.6 W cm^{-2}) for 10 min after 1h of injection. The temperature change in the tumor site induced by PBS+laser and NdNP@PB+laser was measured. The tumor volume and body weight were monitored every 2 days in the following 14 days after injection. At the end of treatment, all these mice were sacrificed and the tumor and major organs of mice were collected, weighed, imaged and stained by H&E staining. The tumor sites were also treated with TUNEL staining.

5.3 Results and Discussions

5.3.1 Fabrication and Characterization of NdNP@PB Nanocomplex

We present a simple strategy to coat a thin layer of PB on NdNPs to create NdNP@PB core/shell nanocomplex with exceptionally enhanced photothermal conversion efficiency of 60.8%. The as-synthesized core/shell nanocomplex combine the advantages of stronger and broader absorption of PB and greater photothermal conversion capability of NdNPs utilizing the absorbed light energy. Moreover, coating of PB layer can generate new CR pathways between NdNPs and PB, which enhance the photothermal conversion efficiency compared with standalone NdNPs and PB.

NdNPs were synthesized by a modified thermal decomposition method.[35] Nd^{3+} was chosen as the only lanthanide ion in the nanocrystal so it will be solely accountable for any possible CR effect. The X-ray diffraction (XRD) pattern of NdNPs (Figure 5.1 c) showed typical diffraction peaks of hexagonal NaNdF_4 crystals, which is also consistent with JCPDS card 27-756. Transmission electron microscopy (TEM) images of NdNPs showed a narrow size distribution and good monodispersity with a size of 17.7 ± 1.2 nm (Figure 5.1 e). PB was coated on the surface of NdNP based on an existing method (Figure 5.1 a).[174] NdNPs were first treated with nitrosonium tetrafluoroborate (NOBF_4) to remove the oleic acid on the surface of the NPs. To stabilize the NdNPs and provide growth sites for PB, citric

acid (CA) was added to form the new surfactant of NdNPs. Fourier transform infrared spectroscopy (FTIR) pattern proves the success transformation of the surfactant from OA to BF_4^- to CA (Figure 5.1 b). CA-coated NdNPs were mixed with a proper amount of $\text{K}_3\text{Fe}(\text{CN})_6$ and FeSO_4 in aqueous solution to form NdNP@PB nanocomplex. XRD pattern of NdNP@PB shows combined peaks of NdNPs and PB, which indicates the successful coating of PB (Figure 5.1c). TEM image of as-synthesized NdNP@PB nanocomplex showed a core/shell structure, with a thin PB shell thickness of ~ 3 nm (Figure 5.1f). High-resolution TEM images confirmed the core/shell-structure nanocomplex was successfully synthesized (Figure 5.1g, 5.2). Scanning TEM images prove the existence of all the elements (Figure 5.1h). The absorbance of NdNP@PB nanocomplex at 808nm was enhanced compared with NdNPs (Figure 5.1d, 5.3), depicted by a color change from pink to blue by coating PB on NdNPs (Figure 5.1i). The average hydrodynamic diameters of NdNPs and NdNP@PB nanocomplex were 19.11 and 28.89 nm, and NdNP@PB nanocomplex was tested to be stable in DI water, phosphate buffer solution (PBS, PH=7.4) and fetal bovine serum (FBS) containing Dulbecco's modified Eagle Medium (DMEM) by dynamic light scattering (Figure 5.4, 5.5 and 5.6).

Figure 5.1 Synthesis and characterization of NdNP@PB nanocomplex. (a) Synthesis route of NdNP@PB ; (b) FTIR of NdNPs with different surfactants; (c) XRD pattern and (d) UV-Vis absorption spectra of NdNPs , PB and NdNP@PB ; (e) TEM image of NdNPs ; (f) TEM image, (g) HRTEM image and (h) Dark-field STEM image (top left) and the corresponding EDX elemental mapping images of NdNP@PB nanocomplex; (i) Digital pictures of NdNP@OA , NdNP@CA and

NdNP@PB.

Figure 5.2 HRTEM images of NdNP, PB and NdNP@PB. HRTEM images of (a) NdNP, (b) PB and (c) fully wrapped NdNP@PB, (d) TEM image of PB.

Figure 5.3 The absorption of NdNPs, PB and NdNP@PB nanocomplex. Under the same concentration ($200 \mu\text{g ml}^{-1}$).

Figure 5.4 Dynamic light scattering curves of NdNPs, PB and NdNP@PB nanocomplex to show the hydrodynamic diameters of NdNPs and NdNP@PB.

Figure 5.5 Digital pictures of NdNP@PB nanocomplex to show stability. Digital pictures of NdNP@PB nanocomplex in (a) PBS and (b) FBS (10%) containing DMEM from day 1 to 3 to 5 ($\sim 200 \mu\text{g ml}^{-1}$). The pictures didn't show any precipitation from day 1 to 5, which further proved that CA-stabilized NdNP@PB nanocomplex can stay stable in physiological environment.

Figure 5.6 Long-term stability test by measuring the average hydrodynamic size of NdNP@PB nanocomplex in (a) DI water, PDI from day 1 to day 5, 0.219 ± 0.021 , 0.275 ± 0.02 , 0.293 ± 0.006 , 0.277 ± 0.037 , 0.361 ± 0.068 , (b) PBS, PDI from day 1 to day 5, 0.437 ± 0.073 , 0.269 ± 0.013 , 0.24 ± 0.024 , 0.527 ± 0.41 , 0.251 ± 0.001 , and (c) FBS (10%) containing DMEM, PDI from day 1 to

day 5, 0.301 ± 0.004 , 0.327 ± 0.052 , 0.286 ± 0.009 , 0.285 ± 0.011 , 0.29 ± 0.003 .

5.3.2 Comparison of Photothermal Properties and PAI *in vitro*

The photothermal properties of as-synthesized NdNPs, PB and NdNP@PB were tested and compared. Under the irradiation of 808 nm laser at 0.6 W cm^{-2} , NdNPs and PB caused temperature increases of 12.1 and 17.4°C, respectively. While NdNP@PB had a much higher temperature change, which is around 65°C (Figure 5.7a, b). The temperature change under different concentrations (0 to 0.4 mg/ml) indicated a concentration-dependent temperature increase (Figure 5.7d). The photothermal conversion efficiency of NdNPs and PB is calculated to be 8.7% and 19.8% according to the model reported by Roper et al.[173] The photothermal conversion efficiency of NdNP@PB has been largely improved to 60.8% , which is higher than most reported PTAs.[159, 175-177] The stability of all these materials was further investigated. NdNPs, PB, and NdNP@PB all showed excellent stability after five cycles of 808 nm laser irradiation at 0.6 W cm^{-2} (Figure 5.7c).

Figure 5.7 Comparison of photothermal performance. Comparison of (a) temperature increases, (b) thermal images, (c) photothermal heating and cooling cycles of NdNPs, PB and NdNP@PB (0.5 mg ml⁻¹) illuminated with 808 nm laser (0.6 W cm⁻²). (d) Temperature change of NdNP@PB under different concentrations. (e) Comparison of PA images of NdNPs, PB and NdNP@PB (0.2 mg ml⁻¹). (f) PA images (g) corresponding plot of PA amplitude of NdNP@PB with increasing concentration of NdNP@PB (0.06 to 1 mg ml⁻¹).

We also demonstrated PAI using all these three types of PTAs. NdNP@PB showed high photoacoustic contrast and while the PA signal of NdNPs and PB was almost not detectable under same test conditions (Figure 5.7e). The PA amplitude of NdNP@PB is also linear to its concentration (Figure 5.7f, g). High photothermal conversion efficiency, strong PA contrast and excellent photostability of NdNP@PB make it a very promising PTA for PTT and PAI.

5.3.3 Proposed Mechanism of Generating New Cross-Relaxation Pathways

It is evident that the combinatorial effect of NdNPs and PB led to an enhancement in photothermal conversion efficiency even the absorbance of NdNP@PB nanocomplex is weaker than that of PB under the same concentration (Figure 5.3). We thus studied the possible interaction between NdNPs and PB and proposed a new mechanism to explain this enhancement. Nd³⁺ ions feature complex ladder-like energy levels (Figure 5.8a). When Nd³⁺ ions are exposed to 808 nm irradiation, photons can be excited to ⁴F_{5/2} state and then non-radiatively drop to ⁴F_{3/2}, at which photons can decay to lower energy states *via* radiative processes to generate 900, 1058, 1332 nm (⁴F_{3/2} to ⁴I_{9/2}, ⁴I_{11/2}, ⁴I_{13/2}, respectively) emissions. CR between ⁴F_{3/2} to ⁴I_{15/2} and ⁴I_{9/2} to ⁴I_{15/2} of different Nd³⁺ ions (Figure 5.8b) and other nonradiative transitions to ground state (Figure 5.8a) generate the photothermal effect. The increase of Nd³⁺ concentration will increase CR effect due to the shorter ionic distance at a higher concentration of Nd³⁺ ions. Even for 100% Nd³⁺-doped NdNPs, a relatively strong NIR emission indicates that the CR1 process is not strong enough.

It's generally believed that CR process happens between same lanthanide ions. In this work, we postulated the generation of new CR2 pathways by coating PB on NdNPs, which inherently do not exist in NdNPs. This brings PB's continuous energy band in close contact with ladder-like energy levels in NdNPs. When photons in NdNPs drop from $^4F_{5/2}$ to $^4F_{3/2}$, photons in PB layer may jump from lower to higher energy states with the same energy difference between $^4F_{5/2}$ and $^4F_{3/2}$ to form a much shorter CR2 pathways, which will generate more heat in NdNPs and bring the absorbed photons closer to the maximum absorption of PB at the same time. By measuring the fluorescence of NdNP@PB nanocomplex, we can observe a sharp decrease of the fluorescence intensities of 900, 1058, 1332 nm emission (Figure 5.8d). These emissions are all generated from radiative transitions from $^4F_{3/2}$ state. The decrease or even elimination of these emissions is direct evidence of the change in energy transfer pathways from radiative to non-radiative since PB doesn't absorb 1058 nm (Figure 5.9), hence converting the energy of light to heat. We further tested the lifetime of 1058 nm emission of NdNPs and NdNP@PB, which is relatively strong. The lifetime of 1058 nm emission has decreased from 1.59 to 1.29 μ s (Figure 5.8e, 5.10), which further evidenced the generation of new CR2 pathways between NdNPs and PB based on $^4F_{3/2}$ states.

Figure 5.8 Proposed new cross-relaxation pathways and cell viability test. Simplified diagrams of (a) radiative and nonradiative process in single Nd^{3+} ion, (b) cross-relaxation between Nd^{3+} ions, (c) generation new cross-relaxation pathways between Nd^{3+} ions and PB. Comparison of (d) NIR emission (e) lifetime decay curves of NdNPs and NdNP@PB at 1058 nm. (1 mg ml^{-1}). Inset: digital pictures of NIR fluorescence of NdNPs (left) and NdNP@PB (right). (f) Cell viability test with and without 808 nm illumination under different concentrations of NdNP@PB. (g) Confocal

fluorescence images of HeLa cancer cells, cells incubated with NdNP@PB (500 $\mu\text{g ml}^{-1}$) under illumination of 808 nm laser (1 W cm^{-2}). Cells were co-stained by calcein-AM and propidium iodide (PI). Scale bar 100 μm .

Figure 5.9 Absorbance of PB and NdNPs from 900 to 1100 nm.

Figure 5.10 Check the pulse width and IRF situation of (a) NdNPs and (b) NdNP@PB to prove the reliability of decay profiles with lifetime. Luminescence decay profiles with average lifetime of (c) NdNPs at 1058 nm is calculated to be 1.59 μ s, and (d) NdNP@PB at 1058 nm is calculated to be 1.29 μ s.

Encouraged by the excellent photothermal performance of nanocomplex, we studied the efficacy of all these three types of PTAs for PTT *in vitro*, along with the cytotoxicity evaluation. HeLa cells were incubated with NdNPs, PB and NdNP@PB at concentrations from 0 and 500 $\mu\text{g ml}^{-1}$. The results showed a high cell viability under a high concentration of 500 $\mu\text{g ml}^{-1}$ in the dark (Figure 5.8f, 5.11). This proved that NdNPs, PB and NdNP@PB had no apparent cytotoxic effect. For the groups treated with NdNP@PB+laser, the cell viability has decreased to 8%, demonstrating that most cancer cells were killed upon 808 nm irradiation, which is more efficient than NdNPs+laser (98.7%) and PB+laser (85.9%) (Figure 5.11). It proved the excellent *in vitro* photothermal efficacy of NdNP@PB nanocomplex (Figure 5.8f, g).

Figure 5.11 (a) Cell viability test with and without 808 nm illumination under different concentrations of NdNPs. (b) Cell viability test with and without 808 nm illumination under different concentrations of PB. Confocal fluorescence images of HeLa cancer cells, cells incubated with (c) NdNPs and (d) PB ($500 \mu\text{g ml}^{-1}$) under illumination of 808 nm laser (1 W cm^{-2}). Cells were co-stained by calcein-AM and propidium iodide (PI). Scale bar $100 \mu\text{m}$.

5.3.4 PTT and PAI *in vivo*

In vivo PA imaging and PTT were further demonstrated in tumor bearing mice. NdNPs, PB and NdNP@PB nanocomplex were injected *via* tail vein with PA images recorded over time. Due to the relatively small size of NdNPs, PB and NdNP@PB nanocomplex, they were rapidly accumulated in the tumor site. After 1h injection, the PA signal showed the maximum accumulation of NdNP@PB (Figure 5.12a). Additionally, bio-distribution of NdNP@PB in major organs and tumor of mice was measured quantitatively by PA imaging (Figure S9). The nanocomplex was mainly distributed in liver, kidney and tumor. The bright NdNP@PB nanocomplex showed good PA imaging contrast and a clear tumor profile, which is much stronger than that of NdNPs and PB (Figure S10). Using the highest accumulation of NdNP@PB at the tumor site which happened at 1-h post-injection, PTT was conducted at the optimal time point. The tumor-bearing mice were treated with 808 nm laser irradiation for 10 min at a power density of 0.6 W cm^{-2} . Upon the irradiation, the temperature of tumor increased to above $43 \text{ }^{\circ}\text{C}$ (Figure S11), which is sufficient for cell ablation. Tumor growth was monitored by measuring tumor volume changes and tumor weight (Figure 5.12b, c). The groups

of 808 nm laser irradiation and NdNP@PB without laser irradiation showed no tumor suppression. For the group treated with NdNP@PB nanocomplex and 808 nm irradiation, the tumor growth was significantly suppressed after one-time injection and treatment (Figure 5.12d). The control groups of NdNPs and PB with laser irradiation showed much weaker PTT efficacies due to the insufficient temperature increase to below 43°C, which is consistent with *in vitro* results (Figure S11,12). Tumor inhibition rate of nanocomplex+laser has been improved to 76.7%, compared with 14.0% of NdNPs+laser and 18.8% of PB+laser.

Figure 5.12 In vivo PAI and PTT. (a) *In vivo* photoacoustic imaging of tumor using NdNP@PB nanocomplex. (b) Tumor growth curves of mice in different groups after various treatments (n=5 per group). (c) Average tumor weight after various treatments. (d) Digital photos of tumor from each group collected after 15-day treatments. Images of (e) H&E stained and (f) TUNEL stained tumor slices collected after 15-day treatments. Scale bar 100 μ m.

Figure 5.13 Biodistribution of NdNP@PB nanocomplex. *Ex vivo* (a) photoacoustic (PA) imaging and PA quantification (b) of NdNP@PB nanocomplex in major organs of mice after 24 h systemic administration. Error bars were based on standard error of mean ($n = 4$). The PA data were acquired at 808 nm.

Figure 5.14 Comparison of in vivo PTT. *In vivo* PA images of tumor using (a) NdNPs, (b) PB.

Figure 5.15 In vivo local temperature change induced by (a) 808 nm laser irradiation, (b) NdNP@PB+ laser, (c) NdNPs+laser and (d) PB+laser in tumor site at the same power density (0.6 W cm^{-2}).

Figure 5.16 Comparison of in vivo PTT. (a) Tumor growth curves of mice in control groups of PB, PB+laser, NdNPs, NdNPs+laser after various treatments (n=5 per group). (b) Average tumor weight of control groups of PB, PB+laser, NdNPs, NdNPs+laser after various treatments. (c) Digital photos of tumor from control groups of (5) PB, (6) PB+laser, (7) NdNPs, and (8) NdNPs+laser collected after 15-day treatments. The tumor inhibition rate of NdNPs@PB nanocomplex+laser has increased to 76.7%, compared with 18.8% of PB+laser with and 14.0% of NdNPs+laser under same experiment conditions.

The mice body weight is also monitored to evaluate the systemic toxicity of the nanocomplex (Figure 5.17). There was no obvious difference during the treatment for all these groups, indicating that NdNPs, PB and NdNP@PB nanocomplex had no apparent side effects. Hematoxylin and eosin (H&E) staining of tumor slices and major organs was conducted for the mice in all groups (Figure 5.12e and Figure 5.18, 5.19). No significant histopathological abnormalities were found in the major organs for all groups, indicating good biosafety of NdNPs, PB and NdNP@PB nanocomplex. For the tumor slices of control groups, no obvious necrosis was observed. While large area of necrosis was found in the group treated with NdNP@PB under laser irradiation after PTT treatment (Figure 5.12e). In situ TdT-mediated DUTP-biotin nick end labeling (TUNEL) staining was also performed on

the tumor slices (Figure 5.12f). No obvious apoptosis can be observed in control groups. For the group of NdNP@PB with 808 nm laser irradiation, TUNEL staining shows a clear apoptosis for tumor cells after PTT treatments. Our data show that NdNP@PB nanocomplex has good PTT efficacy at cellular level and is also consistent with the tumor growth curves in Figure 4b. All these *in vivo* data sets prove that NdNP@PB nanocomplex has great potential as an excellent PTA for PAI and PAI-guided PTT treatments with high efficacy and negligible *in vivo* toxicity.

Figure 5.17 Body weight changes of HELA tumor-bearing mice in different groups.

Figure 5.18 Staining of tumors in control groups. Images of (a) H&E stained and (b) TUNEL stained tumor slices from control groups of PB, PB+laser, NdNPs and NdNPs+laser collected after 15-day treatments. Scale bar 100 μm.

Figure 5.19 The histology analysis of the major organs, including liver, heart, lung, kidney, and spleen, in the mice in the eight groups after 15-day treatment.

5.4 Conclusions

In summary, we have synthesized NdNP@PB core/shell nanocomplex as a potential PTA for PAI and PAI-guided PTT. By coating a thin layer of PB on NdNPs, new cross-relaxation pathways with shorter energy relaxation distance and higher nonradiative energy loss have been generated to enhance the photothermal

performance. The photothermal conversion efficiency of NdNP@PB has been proved to 60.8%, which is higher than most reported PTAs. *In vitro* and *in vivo* PAI and PTT results show excellent imaging contrast and good therapeutic efficacy, along with good biocompatibility. This work has provided a new strategy to enhance the photothermal performance of inorganic PTAs *via* coupling of lanthanide-doped materials featuring ladder-like energy levels with other materials possessing a continuous energy band. We hope the current strategy of generating new cross-relaxation pathways between existing PTAs will contribute to the enhancement of photothermal conversion efficiency and the development of PAI and PAI-guided PTT.

CHAPTER 6 Conclusions and Future Work

6.1 Conclusions

In this thesis, I have successfully developed three novel Nd^{3+} -sensitized nanoconstructs and demonstrated their potential applications as NIR-enabled photomedicines. By engineering the size, configuration, energy transfer pathways, these Nd^{3+} -sensitized nanoconstructs showed enhanced properties and excellent efficiencies.

First all of, I have achieved the nano-manipulation of Nd^{3+} -sensitized nanoconstructs synthesis. By modifying the host lattice and controlling experiment conditions and parameters, the size of sandwich-structure UCNPs can be controlled to below 10 nm with uniform morphology. The thickness of sensitizing and inert layer can be tuned in a fixed size, which is very difficult to achieve and has been rarely reported.

Secondly, I have developed a better understanding of energy migration in Nd^{3+} -sensitized nanoconstructs. The important role of intermediate layer of Yb^{3+} ions to separate Nd^{3+} ions and emitters has been demonstrated to significantly enhance upconversion emission. In addition, I observe that 4-photon upconversion process is more sensitive to the change of thickness of different shells, while 3-photon

conversion process is more sensitive to size change, regardless of the shell configuration. I have proposed that generating new cross-relaxation pathways between lanthanide ions and other PTAs with continuous energy band can boost the thermal conversion efficiency and verified *via* photothermal therapy and photoacoustic imaging. These attempts will enrich the understanding of energy migration in Nd^{3+} -sensitized nanoconstructs.

Last but not least, I have demonstrated enhanced therapeutic efficacy of these Nd^{3+} -sensitized nanoconstructs. The 808nm-controlled release of DOX shows a higher tumor inhibition efficiency than if treated with DOX alone. Tetralayer UCNPs show a strong 360 nm emission and efficient cross-linking of gelatin-based hydrogel. NdNP@PB nanocomplex showed a high photothermal conversion efficiency of 60.8%, which is higher than most reported PTAs.

In summary, the method and mechanism presented in this work in the fabrication novel Nd^{3+} -sensitized nanoconstructs is expected to contribute to the understanding of energy transfer in different configurations and development of novel lanthanide-doped nanosystems. I hope that the efforts presented in this dissertation will drive lanthanide nanoconstructs closer towards medical and health applications.

6.2 Future Work

Through this thesis, I have developed different types of Nd^{3+} -sensitized nanoconstructs for NIR-enabled photomedicine. Based on the completed and published work, there are still some aspects which need further study to be included as follows.

1. DC with other lanthanides with strong NIR-II emission. NIR-II photomedicine is now a research hotspot.[17, 178] However, there are only limited types of NIR-II emitters, even fewer for NIR-II sensitizers. The existing popular NIR-II emitter/sensitizer Er^{3+} ions with emission at 1530 nm face potential pitfalls due to strong water absorption (Figure 6.1a).[14, 179] Autofluorescence of mice organs under 808 nm excitation is quite strong below 1150 nm (Figure 6.1b). [14] Efficient energy transfer from Nd^{3+} ions to new type of NIR-II emitters to achieve suitable NIR-II emission (1150-1400) with a relatively high QY is in urgent need.

Figure 6.1 Absorption of water and autofluorescence of ex vivo mouse under 808 nm excitation.

(a) Absorption of water and (b) autofluorescence of *ex vivo* mouse under 808 nm excitation.

Reproduced with permission.[14] Copyright 2017, Macmillan Publisher Limited.

2. Integration of Nd^{3+} -sensitized nanoconstructs in portable or wearable devices.

Applying Nd^{3+} -sensitized nanoconstructs for *in vivo* photomedicine will always face questions and criticism of potential long-term toxicity. To circumvent this issue, it may be worth putting some effort to exploit the upconversion properties of Nd^{3+} -sensitized nanoconstructs in the treatment of skin disorders by manufacturing portable or wearable devices containing Nd^{3+} -sensitized nanoconstructs.

3. There are abundant opportunities for combining Nd^{3+} -UCNPs with other inorganic sensitizers, such as perovskites, to enhance their absorption properties towards the visible light spectrum, such as ambient lighting and sunlight. Perovskites have been demonstrated as potential photosensitizers,[180] and new strategies to fabricate stable perovskites-lanthanide nanocomposites to overcome the poor stability of perovskites while enhancing efficient energy transfer will be highly beneficial to the state-of-art.

Reference

1. Greish, K., *Enhanced permeability and retention (EPR) effect for anticancer nanomedicine drug targeting*, in *Cancer Nanotechnology*. 2010, Springer. p. 25-37.
2. Regan, J.D., *The science of photomedicine*. 2012: Springer Science & Business Media.
3. Zhang, M., R.R. Naik, and L. Dai, *Carbon nanomaterials for biomedical applications*. Vol. 5. 2015: Springer.
4. Dykman, L. and N. Khlebtsov, *Gold nanoparticles in biomedical applications: recent advances and perspectives*. Chemical Society Reviews, 2012. **41**(6): p. 2256-2282.
5. Zhang, Y., et al., *Engineering lanthanide-based materials for nanomedicine*. Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 2014. **20**: p. 71-96.
6. Klostranec, J.M. and W.C.W. Chan, *Quantum Dots in Biological and Biomedical Research: Recent Progress and Present Challenges*. Advanced Materials, 2006. **18**(15): p. 1953-1964.
7. Elsabahy, M. and K.L. Wooley, *Design of polymeric nanoparticles for biomedical delivery applications*. Chemical Society Reviews, 2012. **41**(7): p. 2545-2561.
8. Li, X., F. Zhang, and D. Zhao, *Lab on upconversion nanoparticles: optical properties and applications engineering via designed nanostructure*. Chem Soc Rev, 2015. **44**(6): p. 1346-78.
9. Liu, B., et al., *808-nm-Light-Excited Lanthanide-Doped Nanoparticles: Rational Design, Luminescence Control and Theranostic Applications*. Adv Mater, 2017. **29**(18).
10. Sharifi, S., et al., *Toxicity of nanomaterials*. Chem Soc Rev, 2012. **41**(6): p. 2323-43.
11. Gnach, A., et al., *Upconverting nanoparticles: assessing the toxicity*. Chem Soc Rev, 2015. **44**(6): p. 1561-84.
12. Yang, Y., J. Aw, and B. Xing, *Nanostructures for NIR light-controlled therapies*. Nanoscale, 2017. **9**(11): p. 3698-3718.
13. Bashkatov, A.N., et al., *Optical properties of human skin, subcutaneous and mucous tissues in the wavelength range from 400 to 2000 nm*. Journal of Physics D: Applied Physics, 2005. **38**(15): p. 2543-2555.
14. Hong, G., A.L. Antaris, and H. Dai, *Near-infrared fluorophores for biomedical imaging*. Nature Biomedical Engineering, 2017. **1**(1).
15. Smith, A.M., M.C. Mancini, and S. Nie, *Bioimaging: second window for in vivo imaging*. Nat Nanotechnol, 2009. **4**(11): p. 710-1.
16. Wang, Y.F., et al., *Nd(3+)-sensitized upconversion nanophosphors: efficient in vivo bioimaging probes with minimized heating effect*. ACS Nano, 2013. **7**(8): p. 7200-6.
17. Li, C. and Q. Wang, *Challenges and Opportunities for Intravital Near-Infrared Fluorescence Imaging Technology in the Second Transparency Window*. ACS Nano, 2018. **12**(10): p. 9654-9659.
18. Wang, F. and X. Liu, *Multicolor tuning of lanthanide-doped nanoparticles by single wavelength excitation*. Acc Chem Res, 2014. **47**(4): p. 1378-85.
19. Shao, W., et al., *Tunable Narrow Band Emissions from Dye-Sensitized Core/Shell/Shell*

- Nanocrystals in the Second Near-Infrared Biological Window*. J Am Chem Soc, 2016. **138**(50): p. 16192-16195.
20. Zhong, Y., et al., *Elimination of photon quenching by a transition layer to fabricate a quenching-shield sandwich structure for 800 nm excited upconversion luminescence of Nd³⁺-sensitized nanoparticles*. Advanced materials, 2014. **26**(18): p. 2831-7.
 21. Vogler, A. and H. Kunkely, *Excited state properties of lanthanide complexes: Beyond ff states*. Inorganica Chimica Acta, 2006. **359**(12): p. 4130-4138.
 22. Bünzli, J.-C.G., *Benefiting from the unique properties of lanthanide ions*. Accounts of chemical research, 2006. **39**(1): p. 53-61.
 23. Wei, W., et al., *Alleviating Luminescence Concentration Quenching in Upconversion Nanoparticles through Organic Dye Sensitization*. J Am Chem Soc, 2016. **138**(46): p. 15130-15133.
 24. Guo, B., et al., *Through Scalp and Skull NIR-II Photothermal Therapy of Deep Orthotopic Brain Tumors with Precise Photoacoustic Imaging Guidance*. Adv Mater, 2018. **30**(35): p. e1802591.
 25. Chen, G., et al., *Efficient Broadband Upconversion of Near-Infrared Light in Dye-Sensitized Core/Shell Nanocrystals*. Advanced Optical Materials, 2016. **4**(11): p. 1760-1766.
 26. Wu, X., et al., *Dye-Sensitized Core/Active Shell Upconversion Nanoparticles for Optogenetics and Bioimaging Applications*. ACS Nano, 2016. **10**(1): p. 1060-6.
 27. Wu, X., et al., *Tailoring dye-sensitized upconversion nanoparticle excitation bands towards excitation wavelength selective imaging*. Nanoscale, 2015. **7**(44): p. 18424-8.
 28. Yu, Z., et al., *Generating New Cross-Relaxation Pathways by Coating Prussian Blue on NaNdF₄ To Fabricate Enhanced Photothermal Agents*. Angew Chem Int Ed Engl, 2019.
 29. Ye, C., et al., *Photon upconversion: from two-photon absorption (TPA) to triplet-triplet annihilation (TTA)*. Phys Chem Chem Phys, 2016. **18**(16): p. 10818-35.
 30. Chuang, T. and H.R. Verdun, *Energy transfer up-conversion and excited state absorption of laser radiation in Nd: YLF laser crystals*. IEEE journal of quantum electronics, 1996. **32**(1): p. 79-91.
 31. Jaque, D., et al., *Up-conversion luminescence in the Nd³⁺: YAB self frequency doubling laser crystal*. Optical Materials, 1998. **10**(3): p. 211-217.
 32. Li, M., et al., *Controllable energy transfer in fluorescence upconversion of NdF₃ and NaNdF₄ nanocrystals*. Optics express, 2010. **18**(4): p. 3364-3369.
 33. Fu, G., et al., *Prussian blue nanoparticles operate as a new generation of photothermal ablation agents for cancer therapy*. Chem Commun (Camb), 2012. **48**(94): p. 11567-9.
 34. Lu, F., et al., *Highly Emissive Nd³⁺-Sensitized Multilayered Upconversion Nanoparticles for Efficient 795 nm Operated Photodynamic Therapy*. Advanced Functional Materials, 2016. **26**(26): p. 4778-4785.
 35. Qin, Q.-S., et al., *Ultralow-power near-infrared excited neodymium-doped nanoparticles for long-term in vivo bioimaging*. Nanoscale, 2017. **9**(14): p. 4660-4664.
 36. Wei, W., et al., *Cross Relaxation Induced Pure Red Upconversion in Activator- and Sensitizer-Rich Lanthanide Nanoparticles*. Chemistry of Materials, 2014. **26**(18): p. 5183-5186.

37. Huang, X. and J. Lin, *Active-core/active-shell nanostructured design: an effective strategy to enhance Nd³⁺/Yb³⁺ cascade sensitized upconversion luminescence in lanthanide-doped nanoparticles*. Journal of Materials Chemistry C, 2015. **3**(29): p. 7652-7657.
38. Shen, J., et al., *Engineering the Upconversion Nanoparticle Excitation Wavelength: Cascade Sensitization of Tri-doped Upconversion Colloidal Nanoparticles at 800 nm*. Advanced Optical Materials, 2013. **1**(9): p. 644-650.
39. Xie, X., et al., *Mechanistic Investigation of Photon Upconversion in Nd³⁺-Sensitized Core-Shell Nanoparticles*. Journal of the American Chemical Society, 2013. **135**(34): p. 12608-12611.
40. Wang, Y.-F., et al., *Nd³⁺-sensitized upconversion nanophosphors: efficient in vivo bioimaging probes with minimized heating effect*. ACS nano, 2013. **7**(8): p. 7200-7206.
41. Zhong, Y., et al., *Elimination of photon quenching by a transition layer to fabricate a quenching-shield sandwich structure for 800 nm excited upconversion luminescence of Nd³⁺-sensitized nanoparticles*. Advanced materials, 2014. **26**(18): p. 2831-2837.
42. Yang, G., et al., *A Single 808 nm Near-Infrared Light-Mediated Multiple Imaging and Photodynamic Therapy Based on Titania Coupled Upconversion Nanoparticles*. Chemistry of Materials, 2015. **27**(23): p. 7957-7968.
43. Yu, X.-F., et al., *Highly Efficient Fluorescence of NdF₃/SiO₂Core/Shell Nanoparticles and the Applications for in vivo NIR Detection*. Advanced Materials, 2008. **20**(21): p. 4118-4123.
44. Marciniak, L., et al., *Heterogeneously Nd(3+) doped single nanoparticles for NIR-induced heat conversion, luminescence, and thermometry*. Nanoscale, 2017. **9**(24): p. 8288-8297.
45. Li, X., et al., *808nm laser-triggered NIR-II emissive rare-earth nanoprobe for small tumor detection and blood vessel imaging*. Mater Sci Eng C Mater Biol Appl, 2019. **100**: p. 260-268.
46. Zhou, J., et al., *Activation of the surface dark-layer to enhance upconversion in a thermal field*. Nature Photonics, 2018. **12**(3): p. 154-158.
47. Cao, C., et al., *Energy Transfer Highway in Nd(3+)-Sensitized Nanoparticles for Efficient near-Infrared Bioimaging*. ACS Appl Mater Interfaces, 2017. **9**(22): p. 18540-18548.
48. Feng, Y., et al., *Neodymium-doped NaHoF₄ nanoparticles as near-infrared luminescent/T₂-weighted MR dual-modal imaging agents in vivo*. Journal of Materials Chemistry B, 2017. **5**(3): p. 504-510.
49. Rocha, U., et al., *Nd³⁺ doped LaF₃ nanoparticles as self-monitored photo-thermal agents*. Applied Physics Letters, 2014. **104**(5): p. 053703.
50. Liu, Y., et al., *Ultra-small pH-responsive Nd-doped NaDyF₄ Nanoagents for Enhanced Cancer Theranostic by in situ Aggregation*. Theranostics, 2017. **7**(17): p. 4217-4228.
51. Lee, J., et al., *Ultra-Wideband Multi-Dye-Sensitized Upconverting Nanoparticles for Information Security Application*. Advanced Materials, 2017. **29**(1).
52. Li, Y., et al., *Second near-infrared emissive lanthanide complex for fast renal-clearable in vivo optical bioimaging and tiny tumor detection*. Biomaterials, 2018. **169**: p. 35-44.
53. He, S., et al., *High Affinity to Skeleton Rare Earth Doped Nanoparticles for Near-Infrared II Imaging*. Nano Lett, 2019. **19**(5): p. 2985-2992.
54. Wang, P., et al., *NIR-II nanoprobe in-vivo assembly to improve image-guided surgery for*

- metastatic ovarian cancer*. Nat Commun, 2018. **9**(1): p. 2898.
55. Fan, Y. and F. Zhang, *A New Generation of NIR-II Probes: Lanthanide-Based Nanocrystals for Bioimaging and Biosensing*. Advanced Optical Materials, 2019: p. 1801417.
 56. Fan, Y., et al., *Lifetime-engineered NIR-II nanoparticles unlock multiplexed in vivo imaging*. Nat Nanotechnol, 2018. **13**(10): p. 941-946.
 57. Ortgies, D.H., et al., *Lifetime-Encoded Infrared-Emitting Nanoparticles for in Vivo Multiplexed Imaging*. ACS Nano, 2018. **12**(5): p. 4362-4368.
 58. Zhou, L., et al., *High-Capacity Upconversion Wavelength and Lifetime Binary Encoding for Multiplexed Biodetection*. Angew Chem Int Ed Engl, 2018. **57**(39): p. 12824-12829.
 59. Wang, L.V. and J. Yao, *A practical guide to photoacoustic tomography in the life sciences*. Nat Methods, 2016. **13**(8): p. 627-38.
 60. Weber, J., P.C. Beard, and S.E. Bohndiek, *Contrast agents for molecular photoacoustic imaging*. Nature methods, 2016. **13**(8): p. 639-50.
 61. Wang, D., et al., *Nonlinear Photoacoustic Imaging by in situ Multiphoton Upconversion and Energy Transfer*. ACS Photonics, 2017. **4**(11): p. 2699-2705.
 62. Lv, R., et al., *Stable ICG-loaded upconversion nanoparticles: silica core/shell theranostic nanoplatfrom for dual-modal upconversion and photoacoustic imaging together with photothermal therapy*. Sci Rep, 2017. **7**(1): p. 15753.
 63. Dai, Y., et al., *808 nm near-infrared light controlled dual-drug release and cancer therapy in vivo by upconversion mesoporous silica nanostructures*. Journal of Materials Chemistry B, 2017. **5**(11): p. 2086-2095.
 64. Zhang, X., et al., *Near infrared light triggered nitric oxide releasing platform based on upconversion nanoparticles for synergistic therapy of cancer stem-like cells*. Science Bulletin, 2017. **62**(14): p. 985-996.
 65. Li, Y., et al., *Heterodimers Made of Upconversion Nanoparticles and Metal-Organic Frameworks*. J Am Chem Soc, 2017. **139**(39): p. 13804-13810.
 66. Zhao, T., et al., *Near-Infrared Triggered Decomposition of Nanocapsules with High Tumor Accumulation and Stimuli Responsive Fast Elimination*. Angew Chem Int Ed Engl, 2018. **57**(10): p. 2611-2615.
 67. Lucky, S.S., K.C. Soo, and Y. Zhang, *Nanoparticles in photodynamic therapy*. Chem Rev, 2015. **115**(4): p. 1990-2042.
 68. Yang, M., et al., *A Nd(3+) sensitized upconversion nanosystem with dual photosensitizers for improving photodynamic therapy efficacy*. Biomater Sci, 2019. **7**(4): p. 1686-1695.
 69. Feng, L., et al., *Multifunctional mesoporous ZrO₂ encapsulated upconversion nanoparticles for mild NIR light activated synergistic cancer therapy*. Biomaterials, 2017. **147**: p. 39-52.
 70. Yuan, Q., et al., *Targeted bioimaging and photodynamic therapy nanoplatfrom using an aptamer-guided G-quadruplex DNA carrier and near-infrared light*. Angew Chem Int Ed Engl, 2013. **52**(52): p. 13965-9.
 71. Khaydukov, E.V., et al., *Riboflavin photoactivation by upconversion nanoparticles for cancer treatment*. Sci Rep, 2016. **6**: p. 35103.
 72. Fan, W., et al., *A smart upconversion-based mesoporous silica nanotheranostic system for synergetic chemo-/radio-/photodynamic therapy and simultaneous MR/UCL imaging*.

- Biomaterials, 2014. **35**(32): p. 8992-9002.
73. Choi, S.Y., et al., *Synthesis of upconversion nanoparticles conjugated with graphene oxide quantum dots and their use against cancer cell imaging and photodynamic therapy*. Biosens Bioelectron, 2017. **93**: p. 267-273.
 74. Li, Y., et al., *A Versatile Imaging and Therapeutic Platform Based on Dual-Band Luminescent Lanthanide Nanoparticles toward Tumor Metastasis Inhibition*. ACS Nano, 2016. **10**(2): p. 2766-73.
 75. Liu, Y., et al., *Hypoxia Induced by Upconversion-Based Photodynamic Therapy: Towards Highly Effective Synergistic Bioreductive Therapy in Tumors*. Angew Chem Int Ed Engl, 2015. **54**(28): p. 8105-9.
 76. Guo, H., et al., *Singlet oxygen-induced apoptosis of cancer cells using upconversion fluorescent nanoparticles as a carrier of photosensitizer*. Nanomedicine: Nanotechnology, Biology and Medicine, 2010. **6**(3): p. 486-495.
 77. Chen, C.W., et al., *Plasmon-Enhanced Photodynamic Cancer Therapy by Upconversion Nanoparticles Conjugated with Au Nanorods*. ACS Appl Mater Interfaces, 2016. **8**(47): p. 32108-32119.
 78. Zhou, A., et al., *Pyropheophorbide A and c(RGDyK) comodified chitosan-wrapped upconversion nanoparticle for targeted near-infrared photodynamic therapy*. Mol Pharm, 2012. **9**(6): p. 1580-9.
 79. Hamblin, M.R., *Upconversion in photodynamic therapy: plumbing the depths*. Dalton Trans, 2018. **47**(26): p. 8571-8580.
 80. Lucky, S.S., et al., *Titania coated upconversion nanoparticles for near-infrared light triggered photodynamic therapy*. ACS nano, 2015. **9**(1): p. 191-205.
 81. Chan, M.H., et al., *Minimizing the Heat Effect of Photodynamic Therapy Based on Inorganic Nanocomposites Mediated by 808 nm Near-Infrared Light*. Small, 2017. **13**(21).
 82. Liu, X., et al., *Separately doped upconversion-C60 nanoplatfrom for NIR imaging-guided photodynamic therapy of cancer cells*. Chem Commun (Camb), 2013. **49**(31): p. 3224-6.
 83. Hou, Z., et al., *Hydrogenated Titanium Oxide Decorated Upconversion Nanoparticles: Facile Laser Modified Synthesis and 808 nm Near-Infrared Light Triggered Phototherapy*. Chemistry of Materials, 2019. **31**(3): p. 774-784.
 84. Zhang, M., et al., *SnWO₄-based nanohybrids with full energy transfer for largely enhanced photodynamic therapy and radiotherapy*. Biomaterials, 2018. **155**: p. 135-144.
 85. Li, J., et al., *Synergizing Upconversion Nanophotosensitizers with Hyperbaric Oxygen to Remodel the Extracellular Matrix for Enhanced Photodynamic Cancer Therapy*. ACS Appl Mater Interfaces, 2018. **10**(27): p. 22985-22996.
 86. He, S., et al., *Leveraging Spectral Matching between Photosensitizers and Upconversion Nanoparticles for 808 nm-Activated Photodynamic Therapy*. Chemistry of Materials, 2018. **30**(12): p. 3991-4000.
 87. Rocha, U., et al., *Subtissue thermal sensing based on neodymium-doped LaF₃ nanoparticles*. Acs Nano, 2013. **7**(2): p. 1188-1199.
 88. del Rosal, B., et al., *Neodymium-Based Stoichiometric Ultrasmall Nanoparticles for Multifunctional Deep-Tissue Photothermal Therapy*. Advanced Optical Materials, 2016. **4**(5): p. 782-789.

89. Ding, X., et al., *Multifunctional core/satellite polydopamine@Nd³⁺-sensitized upconversion nanocomposite: A single 808 nm near-infrared light-triggered theranostic platform for in vivo imaging-guided photothermal therapy*. Nano Research, 2017. **10**(10): p. 3434-3446.
90. Boyden, E.S., et al., *Millisecond-timescale, genetically targeted optical control of neural activity*. Nat Neurosci, 2005. **8**(9): p. 1263-8.
91. Zhang, F., et al., *Multimodal fast optical interrogation of neural circuitry*. Nature, 2007. **446**(7136): p. 633-9.
92. Ai, X., et al., *Remote Regulation of Membrane Channel Activity by Site-Specific Localization of Lanthanide-Doped Upconversion Nanocrystals*. Angew Chem Int Ed Engl, 2017. **56**(11): p. 3031-3035.
93. Ao, Y., et al., *An Upconversion Nanoparticle Enables Near Infrared-Optogenetic Manipulation of the Caenorhabditis elegans Motor Circuit*. ACS Nano, 2019.
94. Xiang, J., et al., *Antigen-loaded upconversion nanoparticles for dendritic cell stimulation, tracking, and vaccination in dendritic cell-based immunotherapy*. ACS nano, 2015. **9**(6): p. 6401-6411.
95. Lee, E.S., et al., *Recent Advances in Polymeric Nanomedicines for Cancer Immunotherapy*. Adv Healthc Mater, 2019. **8**(4): p. e1801320.
96. Dong, H., et al., *Lanthanide Nanoparticles: From Design toward Bioimaging and Therapy*. Chemical Reviews, 2015. **115**(19): p. 10725-10815.
97. Idris, N.M., et al., *Upconversion nanoparticles as versatile light nanotransducers for photoactivation applications*. Chemical Society Reviews, 2015. **44**(6): p. 1449-1478.
98. Prodi, L., et al., *Imaging agents based on lanthanide doped nanoparticles*. Chemical Society Reviews, 2015. **44**(14): p. 4922-4952.
99. Zhang, Y., et al., *Engineering lanthanide-based materials for nanomedicine*. Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 2014. **20**(1): p. 71-96.
100. Wang, Y.F., et al., *Nd³⁺-sensitized upconversion nanophosphors: Efficient in vivo bioimaging probes with minimized heating effect*. ACS Nano, 2013. **7**(8): p. 7200-7206.
101. Zhong, Y., et al., *Elimination of Photon Quenching by a Transition Layer to Fabricate a Quenching-Shield Sandwich Structure for 800 nm Excited Upconversion Luminescence of Nd³⁺-Sensitized Nanoparticles*. Advanced Materials, 2014. **26**(18): p. 2831-2837.
102. Xie, X., et al., *Mechanistic investigation of photon upconversion in Nd(3+)-sensitized core-shell nanoparticles*. Journal of the American Chemical Society, 2013. **135**(34): p. 12608-11.
103. Ma, D., et al., *NaGdF₄:Yb³⁺/Er³⁺@NaGdF₄:Nd³⁺@Sodium-Gluconate: Multifunctional and Biocompatible Ultrasmall Core-Shell Nanohybrids for UCL/MR/CT Multimodal Imaging*. ACS Applied Materials and Interfaces, 2015. **7**(30): p. 16257-16265.
104. Liu, B., et al., *Poly(Acrylic Acid) Modification of Nd³⁺-Sensitized Upconversion Nanophosphors for Highly Efficient UCL Imaging and pH-Responsive Drug Delivery*. Advanced Functional Materials, 2015. **25**(29): p. 4717-4729.
105. Peng, H.Y., et al., *Sequential growth of sandwiched NaYF₄:Yb/Er@NaYF₄:Yb@NaNdF₄:Yb core-*

- shell-shell nanoparticles for photodynamic therapy*. *Applied Surface Science*, 2015. **357**: p. 2408-2414.
106. Hou, Z., et al., *808 nm Light-triggered and hyaluronic acid-targeted dual-photosensitizers nanoplatfrom by fully utilizing Nd³⁺-sensitized upconversion emission with enhanced anti-tumor efficacy*. *Biomaterials*, 2016. **101**: p. 32-46.
 107. Zeng, L., et al., *808 nm-excited upconversion nanoprobles with low heating effect for targeted magnetic resonance imaging and high-efficacy photodynamic therapy in HER2-overexpressed breast cancer*. *Biomaterials*, 2016. **103**: p. 116-127.
 108. Lv, R., et al., *Integration of Upconversion Nanoparticles and Ultrathin Black Phosphorus for Efficient Photodynamic Theranostics under 808 nm Near-Infrared Light Irradiation*. *Chemistry of Materials*, 2016. **28**(13): p. 4724-4734.
 109. He, F., et al., *Optimization of upconversion luminescence of Nd³⁺-sensitized BaGdF₅-based nanostructures and their application in dual-modality imaging and drug delivery*. *Dalton Transactions*, 2016. **45**(4): p. 1708-1716.
 110. Wang, Z., et al., *Nd³⁺-sensitized NaLuF₄ luminescent nanoparticles for multimodal imaging and temperature sensing under 808 nm excitation*. *Nanoscale*, 2015. **7**(42): p. 17861-17870.
 111. Huang, X., *Giant Enhancement of upconversion emission in (NaYF₄:Nd/Yb/Ho)/NaYF₄:Nd, Yb) core/shell nanoparticles excited at 808 nm*. *Optical Letters*, 2015. **40**(15): p. 3599-3602.
 112. Ai, F., et al., *A core-shell-shell nanoplatfrom upconverting near-infrared light at 808 nm for luminescence imaging and photodynamic therapy of cancer*. *Scientific Reports*, 2015. **5**: p. 10785.
 113. Yong, Y., et al., *Tungsten Sulfide Quantum Dots as Multifunctional Nanotheranostics for In Vivo Dual-Modal Image-Guided Photothermal/Radiotherapy Synergistic Therapy*. *ACS Nano*, 2015. **9**(12): p. 12451-12463.
 114. Chen, G., et al., *Ultrasmall monodisperse NaYF₄:Yb³⁺/Tm³⁺ nanocrystals with enhanced near-infrared to near-infrared upconversion photoluminescence*. *ACS Nano*, 2010. **4**(6): p. 3163-3168.
 115. Gargas, D.J., et al., *Engineering bright sub-10-nm upconverting nanocrystals for single-molecule imaging*. *Nat Nano*, 2014. **9**(4): p. 300-305.
 116. Liu, C., et al., *Magnetic/Upconversion Fluorescent NaGdF₄:Yb,Er Nanoparticle-Based Dual-Modal Molecular Probes for Imaging Tiny Tumors in Vivo*. *ACS Nano*, 2013. **7**(8): p. 7227-7240.
 117. Bouzigues, C., T. Gacoin, and A. Alexandrou, *Biological applications of rare-earth based nanoparticles*. *ACS Nano*, 2011. **5**(11): p. 8488-8505.
 118. Wang, F., J. Wang, and X. Liu, *Direct evidence of a surface quenching effect on size-dependent luminescence of upconversion nanoparticles*. *Angewandte Chemie*, 2010. **49**(41): p. 7456-60.
 119. Schietinger, S., et al., *Observation of Size Dependence in Multicolor Upconversion in Single Yb³⁺, Er³⁺ Codoped NaYF₄ Nanocrystals*. *Nano Letters*, 2009. **9**(6): p. 2477-2481.
 120. Shan, J., et al., *Anomalous Raman Scattering of Colloidal Yb³⁺,Er³⁺ Codoped NaYF₄ Nanophosphors and Dynamic Probing of the Upconversion Luminescence*. *Advanced*

- Functional Materials, 2010. **20**(20): p. 3530-3537.
121. He, S., et al., *Ultralow-intensity near-infrared light induces drug delivery by upconverting nanoparticles*. Chemical Communications, 2015. **51**(2): p. 431-434.
 122. Su, Q., et al., *The effect of surface coating on energy migration-mediated upconversion*. Journal of the American Chemical Society, 2012. **134**(51): p. 20849-20857.
 123. Schäfer, H., et al., *Synthesis and Optical Properties of KYF₄/Yb, Er Nanocrystals, and their Surface Modification with Undoped KYF₄*. Advanced Functional Materials, 2008. **18**(19): p. 2913-2918.
 124. Wen, H., et al., *Upconverting near-infrared light through energy management in core-shell-shell nanoparticles*. Angewandte Chemie - International Edition, 2013. **52**(50): p. 13419-13423.
 125. Zhou, B., et al., *Photon Upconversion Through Tb³⁺-Mediated Interfacial Energy Transfer*. Advanced Materials, 2015. **27**(40): p. 6208-6212.
 126. Dexter, D.L., *A THEORY OF SENSITIZED LUMINESCENCE IN SOLIDS*. Journal of Chemical Physics, 1953. **21**(5): p. 836-850.
 127. Xi, Y., et al., *Ultra-small Nd(3+)-doped nanoparticles as near-infrared luminescent biolabels of hemin in bacteria*. Nanoscale, 2016. **8**(3): p. 1288-92.
 128. Yin, W., et al., *Enhanced red emission from GdF₃:Yb³⁺,Er³⁺ upconversion nanocrystals by Li⁺ doping and their application for bioimaging*. Chemistry - A European Journal, 2012. **18**(30): p. 9239-9245.
 129. Lin, H., et al., *Simultaneous size and luminescence control of NaYF₄:Yb³⁺/RE³⁺ (RE = Tm, Ho) microcrystals via Li⁺ doping*. Optical Materials, 2015. **45**: p. 229-234.
 130. Singh, A.K., S.K. Singh, and S.B. Rai, *Role of Li⁺ ion in the luminescence enhancement of lanthanide ions: Favorable modifications in host matrices*. RSC Advances, 2014. **4**(51): p. 27039-27061.
 131. Tian, G., et al., *Mn²⁺ Dopant-Controlled Synthesis of NaYF₄:Yb/Er Upconversion Nanoparticles for in vivo Imaging and Drug Delivery*. Advanced Materials, 2012. **24**(9): p. 1226-1231.
 132. Lei, L., et al., *Modifying the size and uniformity of upconversion Yb/Er:NaGdF₄ nanocrystals through alkaline-earth doping*. Nanoscale, 2013. **5**(22): p. 11298-11305.
 133. Abdul Jalil, R. and Y. Zhang, *Biocompatibility of silica coated NaYF₄ upconversion fluorescent nanocrystals*. Biomaterials, 2008. **29**(30): p. 4122-4128.
 134. Bagheri, A., et al., *Lanthanide-Doped Upconversion Nanoparticles: Emerging Intelligent Light-Activated Drug Delivery Systems*. Adv Sci (Weinh), 2016. **3**(7): p. 1500437.
 135. Han, S., et al., *Upconversion Nanoparticles/Hyaluronate-Rose Bengal Conjugate Complex for Noninvasive Photochemical Tissue Bonding*. ACS Nano, 2017. **11**(10): p. 9979-9988.
 136. Fan, W., W. Bu, and J. Shi, *On The Latest Three-Stage Development of Nanomedicines based on Upconversion Nanoparticles*. Adv Mater, 2016. **28**(21): p. 3987-4011.
 137. Idris, N.M., et al., *Upconversion nanoparticles as versatile light nanotransducers for photoactivation applications*. Chem Soc Rev, 2015. **44**(6): p. 1449-78.
 138. Zhu, X., et al., *Anti-Stokes shift luminescent materials for bio-applications*. Chem Soc Rev,

2017. **46**(4): p. 1025-1039.
139. Chen, G., et al., *Upconversion nanoparticles: design, nanochemistry, and applications in theranostics*. Chem Rev, 2014. **114**(10): p. 5161-214.
140. He, S., et al., *Ultralow-intensity near-infrared light induces drug delivery by upconverting nanoparticles*. Chem Commun (Camb), 2015. **51**(2): p. 431-4.
141. Ma, C., et al., *Optimal Sensitizer Concentration in Single Upconversion Nanocrystals*. Nano Lett, 2017. **17**(5): p. 2858-2864.
142. Johnson, N.J., et al., *Direct Evidence for Coupled Surface and Concentration Quenching Dynamics in Lanthanide-Doped Nanocrystals*. J Am Chem Soc, 2017. **139**(8): p. 3275-3282.
143. He, F., et al., *Optimization of upconversion luminescence of Nd(3+)-sensitized BaGdF5-based nanostructures and their application in dual-modality imaging and drug delivery*. Dalton Trans, 2016. **45**(4): p. 1708-16.
144. Wang, F., J. Wang, and X. Liu, *Direct evidence of a surface quenching effect on size-dependent luminescence of upconversion nanoparticles*. Angew Chem Int Ed Engl, 2010. **49**(41): p. 7456-60.
145. Jin, L.M., et al., *Enhancing Multiphoton Upconversion from NaYF₄:Yb/Tm@NaYF₄ Core-Shell Nanoparticles via the Use of Laser Cavity*. ACS Nano, 2017. **11**(1): p. 843-849.
146. Dong, H., L.D. Sun, and C.H. Yan, *Energy transfer in lanthanide upconversion studies for extended optical applications*. Chem Soc Rev, 2015. **44**(6): p. 1608-34.
147. Bian, W., et al., *Direct Identification of Surface Defects and Their Influence on the Optical Characteristics of Upconversion Nanoparticles*. ACS Nano, 2018.
148. Liu, H., et al., *Balancing power density based quantum yield characterization of upconverting nanoparticles for arbitrary excitation intensities*. Nanoscale, 2013. **5**(11): p. 4770-5.
149. Brown, T.E., et al., *Photopolymerized dynamic hydrogels with tunable viscoelastic properties through thioester exchange*. Biomaterials, 2018. **178**: p. 496-503.
150. Hong, Y., et al., *A strongly adhesive hemostatic hydrogel for the repair of arterial and heart bleeds*. Nat Commun, 2019. **10**(1): p. 2060.
151. Kong, W., et al., *A General Strategy for Ligand Exchange on Upconversion Nanoparticles*. Inorg Chem, 2017. **56**(2): p. 872-877.
152. Eke, G., et al., *Development of a UV crosslinked biodegradable hydrogel containing adipose derived stem cells to promote vascularization for skin wounds and tissue engineering*. Biomaterials, 2017. **129**: p. 188-198.
153. Dawson, P. and M. Romanowski, *Excitation Modulation of Upconversion Nanoparticles for Switch-like Control of Ultraviolet Luminescence*. J Am Chem Soc, 2018.
154. Shanmugam, V., S. Selvakumar, and C.S. Yeh, *Near-infrared light-responsive nanomaterials in cancer therapeutics*. Chemical Society Reviews, 2014. **43**(17): p. 6254-87.
155. Zou, L., et al., *Current Approaches of Photothermal Therapy in Treating Cancer Metastasis with Nanotherapeutics*. Theranostics, 2016. **6**(6): p. 762-72.
156. Li, J.L., D. Day, and M. Gu, *Ultra-low energy threshold for cancer photothermal therapy using transferrin-conjugated gold nanorods*. Advanced Materials, 2008. **20**(20): p. 3866-

- 3871.
157. Chou, S.S., et al., *Chemically Exfoliated MoS₂ as Near-Infrared Photothermal Agents*. *Angewandte Chemie*, 2013. **125**(15): p. 4254-4258.
 158. Lyu, Y., et al., *Intraparticle Molecular Orbital Engineering of Semiconducting Polymer Nanoparticles as Amplified Theranostics for in Vivo Photoacoustic Imaging and Photothermal Therapy*. *ACS Nano*, 2016. **10**(4): p. 4472-81.
 159. Liu, Y., et al., *Dopamine-melanin colloidal nanospheres: an efficient near-infrared photothermal therapeutic agent for in vivo cancer therapy*. *Advanced Materials*, 2013. **25**(9): p. 1353-9.
 160. Maji, S.K., et al., *Upconversion nanoparticles as a contrast agent for photoacoustic imaging in live mice*. *Advanced Materials*, 2014. **26**(32): p. 5633-8.
 161. Yang, K., et al., *Multimodal imaging guided photothermal therapy using functionalized graphene nanosheets anchored with magnetic nanoparticles*. *Advanced Materials*, 2012. **24**(14): p. 1868-72.
 162. Taylor, A.B., A.M. Siddiquee, and J.W. Chon, *Below melting point photothermal reshaping of single gold nanorods driven by surface diffusion*. *Acs Nano*, 2014. **8**(12): p. 12071-12079.
 163. Han, S., et al., *Efficient Self-Assembly Synthesis of Uniform CdS Spherical Nanoparticles-Au Nanoparticles Hybrids with Enhanced Photoactivity*. *Advanced Functional Materials*, 2014. **24**(24): p. 3725-3733.
 164. Wu, M.-C., et al., *Graphene-based photothermal agent for rapid and effective killing of bacteria*. *ACS nano*, 2013. **7**(2): p. 1281-1290.
 165. Sheng, Y., et al., *Rare-earth doped particles as dual-modality contrast agent for minimally-invasive luminescence and dual-wavelength photoacoustic imaging*. *Scientific reports*, 2014. **4**: p. 6562.
 166. Fu, G., et al., *Prussian blue nanoparticles operate as a new generation of photothermal ablation agents for cancer therapy*. *Chemical Communications (Cambridge, United Kingdom)*, 2012. **48**(94): p. 11567-9.
 167. Chen, W., et al., *Cell Membrane Camouflaged Hollow Prussian Blue Nanoparticles for Synergistic Photothermal-/Chemotherapy of Cancer*. *Advanced Functional Materials*, 2017. **27**(11): p. 1605795.
 168. Hoffman, H.A., et al., *Prussian blue nanoparticles for laser-induced photothermal therapy of tumors*. *RSC Advances*, 2014. **4**(56): p. 29729-29734.
 169. Cai, X., et al., *Enabling Prussian Blue with Tunable Localized Surface Plasmon Resonances: Simultaneously Enhanced Dual-Mode Imaging and Tumor Photothermal Therapy*. *ACS Nano*, 2016. **10**(12): p. 11115-11126.
 170. Kim, T., et al., *Photoacoustic Imaging of Human Mesenchymal Stem Cells Labeled with Prussian Blue-Poly(l-lysine) Nanocomplexes*. *ACS Nano*, 2017. **11**(9): p. 9022-9032.
 171. Liu, Y., et al., *Optimization of Prussian Blue Coated NaDyF₄:x%Lu Nanocomposites for Multifunctional Imaging-Guided Photothermal Therapy*. *Advanced Functional Materials*, 2016. **26**(28): p. 5120-5130.
 172. Lin, H., et al., *A Two-Dimensional Biodegradable Niobium Carbide (MXene) for Photothermal Tumor Eradication in NIR-I and NIR-II Biowindows*. *J Am Chem Soc*, 2017. **139**(45): p. 16235-16247.

-
173. Roper, D.K., W. Ahn, and M. Hoepfner, *Microscale heat transfer transduced by surface plasmon resonant gold nanoparticles*. The Journal of Physical Chemistry C, 2007. **111**(9): p. 3636-3641.
174. Jing, L., et al., *Prussian blue coated gold nanoparticles for simultaneous photoacoustic/CT bimodal imaging and photothermal ablation of cancer*. Biomaterials, 2014. **35**(22): p. 5814-21.
175. Sun, L., et al., *Phase-Transition Induced Conversion into a Photothermal Material: Quasi-Metallic WO_{2.9} Nanorods for Solar Water Evaporation and Anticancer Photothermal Therapy*. Angewandte Chemie International Edition, 2018. **57**(33): p. 10666-10671.
176. Lin, H., et al., *A Two-Dimensional Biodegradable Niobium Carbide (MXene) for Photothermal Tumor Eradication in NIR-I and NIR-II Biowindows*. Journal of the American Chemical Society, 2017. **139**(45): p. 16235-16247.
177. Jia, Q., et al., *Biocompatible Iron Phthalocyanine-Albumin Assemblies as Photoacoustic and Thermal Theranostics in Living Mice*. ACS applied materials & interfaces, 2017. **9**(25): p. 21124-21132.
178. Fan, Y. and F. Zhang, *A New Generation of NIR-II Probes: Lanthanide-Based Nanocrystals for Bioimaging and Biosensing*. Advanced Optical Materials, 2019.
179. Liu, L., et al., *Er(3+) Sensitized 1530 nm to 1180 nm Second Near-Infrared Window Upconversion Nanocrystals for In Vivo Biosensing*. Angew Chem Int Ed Engl, 2018. **57**(25): p. 7518-7522.
180. Pan, G., et al., *Doping Lanthanide into Perovskite Nanocrystals: Highly Improved and Expanded Optical Properties*. Nano Lett, 2017. **17**(12): p. 8005-8011.