Development of two-dimensional ensembles of plasmonic nanostructures for surface enhanced raman scattering (SERS)

Duan, Bo

2014

Duan, B. (2014). Development of two-dimensional ensembles of plasmonic nanostructures for surface enhanced raman scattering (SERS). Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/64308>

<https://doi.org/10.32657/10356/64308>

**Development of two-dimensional ensembles of
plasmonic nanostructures for surface enhanced Raman
scattering (SERS)**

Duan Bo

School of Chemical and Biomedical Engineering

2014

**Development of two-dimensional ensembles of
plasmonic nanostructures for surface enhanced Raman
scattering (SERS)**

Duan Bo

School of Chemical and Biomedical Engineering

A thesis submitted to the Nanyang Technological University

in partial fulfilment of the requirement for the degree of

Doctor of Philosophy

2015

Acknowledgements

First and foremost, to Prof Duan Hongwei and Prof Hemond, for their invaluable supervision throughout my graduate study. Their immense knowledge and devotion to research have always inspired me. They have shown great patience in guiding me along the research road, giving me trainings on process skills and soft skills, which are lifelong and go beyond research field alone.

My gratitude also to Dr Wang Xiujuan, who provided generous technical support in Raman instrumentation, electron microscopic instrumentation, and many others.

My sincere appreciation to the exceptional team at Prof Duan's lab, for their open-handed help, the stimulating discussions, as well as all the fun we had working together.

And finally, to my family, who encouraged me to take this road and being with me through both good and difficult times.

Table of Contents

Acknowledgements.....	2
Table of Contents.....	2
List of Abbreviations	4
List of Figures	6
List of Tables	15
Summary.....	16
Chapter 1 Introduction	19
Chapter 2 Literature review	22
2.1. Raman spectroscopy.....	22
2.1.1. Origins of Raman scattering	22
2.1.2. Raman vs. infrared vs. fluorescence	24
2.2. Surface enhanced Raman scattering (SERS)	27
2.2.1. From Raman to SERS.....	27
2.2.2. SERS enhancement mechanisms	28
2.3. Hotspots in plasmonic nanostructures for SERS	30
2.3.1. Hotspots from sharp tips and curvatures.....	30
2.3.2. Hotspots from nanogaps	36
2.4. Two dimensional arrays of plasmonic nanostructures as SERS substrates ...	43
2.5. Applications of SERS in food safety	48
Chapter 3 Chemical processing of interfacial assembly of metal nanowires for SERS detection of food contaminants.....	53
3.1. Introduction	53
3.2. Experimental section.....	56
3.3. Results and discussion.....	58
3.4. Conclusion.....	74
Chapter 4 SERS by graphene-nanosheet-gapped plasmonic nanoparticle arrays for multiplex DNA detection.....	75
4.1. Introduction	75
4.2. Experimental section.....	79
4.3. Results and discussion.....	82
4.4. Conclusion.....	98
Chapter 5 SERS assay based on magnetoplasmonic nanostructures for multiplex detection of bacterial pathogens.....	99

5.1. Introduction	99
5.2. Experimental section	102
5.3. Results and discussion.....	104
5.4. Conclusion.....	118
Chapter 6 Seeded Growth of Gold Nanostar array on Graphene Oxide for Surface Enhanced Raman Scattering and Catalysis.....	120
6.1. Introduction	120
6.2. Experimental section	123
6.3. Results and discussion.....	126
6.4. Conclusion.....	139
Chapter 7 General conclusion and future research	140
7.1. Conclusions	140
7.2. Recommendations for future work.....	142
References.....	144
List of Publications	160

List of Abbreviations

AuNP	Gold Nanoparticle
AuNR	Gold Nanorod
TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
NIR	Near-infrared
SPR	Surface plasmon resonance
LSPR	Localized surface plasmon resonance
SERS	Surface enhanced Raman scattering
CTAB	Cetyltrimethylammonium bromide
PEG	Poly(ethylene glycol)
EM	Electromagnetic enhancement mechanism
CM	Chemical enhancement mechanism
IR	Infrared
FT-IR	Fourier transform infrared spectroscopy
PA	Photoacoustic
FEM	finite element calculations
PVP	Poly-N-vinyl-2-pyrrolidone
LB	Langmuir-Blodgett
DTBE	2,2'-dithiobis[1-(2-bromo-2-methyl-
DCE	1,2-dichloroethane
TBA ⁺ NO ₃ ⁻	Tetrabutylammonium nitrate
MBA	Mercaptobenzoic acid

ELISA	Enzyme-linked immunosorbent assay
PCR	Polymerase chain reaction
HPLC	High performance liquid chromatography
GC	Gas chromatography

List of Figures

Figure 2.1 A diagram shows electron transitions between energy levels during light scattering. The black arrows, which point upward, represent absorption of electrons. The green arrow represents Rayleigh scattering, the blue arrow represents Stokes scattering, and the red arrow represents Anti-Stokes scattering.	23
Figure 2.2 Raman and FT-IR spectra of milk sample. Enlarged picture shows the molecular vibrational states along with a simplified Jablonski diagram. (Reprinted with permission from ref 5. Copyright 2012 The Royal Society of Chemistry)	25
Figure 2.3 Schematic illustration of SERS electromagnetic enhancement based on localized surface plasmon resonance.	29
Figure 2.4 Transmission and scanning (insets) electron microscopy images of the nanostars in order of increasing size (A–F). Their synthesis in high yield is evident. Samples A–F were synthesized by addition of the following volumes of Au seed: (A) 700, (B) 540, (C) 400, (D) 270, (E) 135, and (F) 45 μ L. (Reprinted with permission from ref 24. Copyright 2008 American Chemical Society).....	31
Figure 2.5 (A) TEM image of gold nanorods. (B) SERS spectra of Raman dye molecules collected from gold nanorods. (C) Absorption spectra of gold nanorods. (D) SERS spectra of gold nanorods (left ordinate) and spherical nanoparticle (right ordinate). (Adapted with permission from ref.27. Copyright (2012) American Chemical Society.).....	33
Figure 2.6 (A) Optical image of a single smooth silver nanowire; (B) SERS intensity map of silver nanowire; (C) cross-sectional SERS intensities collected at each micrometer pixel along silver nanowire; and (D) SERS spectra collected at points 1, 2, 3, and 4 in (B). (E) Optical image of single etched silver nanowire with (F) SERS intensity map; (G) cross-sectional SERS intensities collected at each micrometer pixel along etched silver nanowire; and (H) the SERS spectra collected at points 1', 2', 3', and 4' in (F). (Reprinted (adapted) with permission from ref 32. Copyright (2012) American Chemical Society.)	34
Figure 2.7 SERS spectra of (a) monomers, (b) dimers, and (c) trimers of Au@Ag NPs. (Adapted with permission from ref.45. Copyright (2010) American Chemical Society.)	36

Figure 2.8 Scheme of nanogap plasmonic nanoparticle synthesis process and TEM images. (Reprinted with permission from ref. 44 (2014) American Chemical Society)

.....39

Figure 2.9 (a) Scheme of target DNAs detection by gold particle-on-wire system. (b) SERS spectra of Au particle-on-wire system (blue spectrum) and a single Au NW (magenta spectrum). The inset shows plasmonic absorption spectra of Au NPs (green spectrum), Au NWs (magenta spectrum), and Au particle-on-wire systems (blue spectrum). (c) SEM images of gold particle-on-wire structure (top) and a clean NW (bottom). (Reprinted with permission from ref 45. Copyright (2010) American Chemical Society.).....41

Figure 2.10 a) Schematic illustration of how contact angle of a particle with oil-water interface affect its location. Left: contact angle is less than 90°; center: contact angle equal to 90°; right: contact angle is larger than 90°. b) Chemical structures of Au@DTBE (left) and γ -irradiated Au@BMPA nanoparticles (right). (Reprinted with permission from ref 71. Copyright (2004) WileyVCH Verlag GmbH & Co. KGaA.) 45

Figure 2.11 Schematic illustration of optofluidic SERS system for chemical food contaminants (thiram and melamine) detection.....50

Figure 2.12 (a) Scheme of fabrication process of gold nanoparticle-graphene hybrid SERS substrate. (b) Single DNA detection process. (c) Multiplex DNA detection process. (Adapted with permission from ref 83. Copyright (2012) American Chemical Society.)51

Figure 3.1 Scheme of the fabrication process of Ag-Au bimetallic nanowire array. AgNWs are first self-assembled at water/hexane interface and form a monolayer. Upon addition of gold precursor, galvanic reaction occurs between Ag and Au, forming bimetallic Ag-Au nanowire arrays.....56

Figure 3.2 (a) SEM image and (b) TEM image of AgNWs prior to interfacial self-assembly. (c) SEM image of AgNWs after interfacial self-assembly. (d) Optical microscopic pictures of AgNWs before (d1) and after (d2) interfacial self-assembly. 60

Figure 3.3 (a) TEM image of Ag-Au nanowires at Au/Ag ratios of 1:35. (b) and (c) SEM image of Ag-Au nanowire array (Au/Ag ratios of 1:35) at different magnifications. (d) TEM image of Ag-Au nanowires at Au/Ag ratios of 1:18. (e) and

(f) SEM image of Ag-Au nanowire array (Au/Ag ratios of 1:18) at different magnifications.....	62
Figure 3.4 (a) TEM and (b) SEM images of Ag-Au bimetallic nanowire at Ag/Au ratio equals to 9:1.....	62
Figure 3.5 (a) UV-Vis spectra of AgNW colloid (black), AgNW monolayer (red), and Ag-AuNW array (blue). (b) SERS spectra of R6G (100 nM) collected on Ag-AuNW arrays at Ag/Au ratios ranging from 9:1 to 35:1.....	64
Figure 3.6 UV-Vis spectra of Ag-AuNW array at Ag/Au ratios ranging from 9:1 to 35:1.	65
Figure 3.7 (a) XRD spectra of AgNW (red, bottom) and bimetallic Ag-AuNW (blue, top). (b) EDS spectra of bimetallic Ag-AuNW.	65
Figure 3.8 (a) SERS spectra of R6G with concentrations of 10 pM, 50 pM, 100 pM, 1 nM, 10 nM, 50 nM, and 100 nM (from bottom to top) and (b) the plot of the SERS intensity at 1617 cm^{-1} as a function of R6G concentration.....	67
Figure 3.9 SERS spectra of R6G on Ag-Au nanowire array when Au/Ag ratios equals to 1:9.	68
Figure 3.10 (a) SERS spectrum of thiram on Ag-Au bimetallic nanowire array (black trace) and normal Raman spectrum of thiram powder (red trace). (b) SERS spectrum of melamine on Ag-Au bimetallic nanowire array (black trace) and normal Raman spectrum of melamine powder (red trace).	70
Figure 3.11 (a) SERS spectra of thiram in water at different concentrations (from top to bottom : 500, 100, 10, 1, 0.5, and 0.1 nM) acquired in-situ from Ag-AuNW array. (b) Quantitative curves of thiram sensing in water on Ag-AuNW array. The Raman intensity of thiram peak at 1385 cm^{-1} was plotted as a function of thiram concentration. The error bars were obtained from multiple measurements on different array areas. (c) SERS spectra of thiram in mango juice at different concentrations (from top to bottom: 500, 200, 100, 10, and 1 nM) acquired in-situ from Ag-AuNW array. (d) Quantitative curves of thiram sensing in mango juice on Ag-AuNW array. The Raman intensity of thiram peak at 1385 cm^{-1} was plotted as a function of thiram concentration. The error bars were obtained from multiple measurements on different array areas.	71
Figure 3.12 (a) SERS spectra of melamine in water at different concentrations (from top to bottom : 10 μM , 5 μM , 1 μM , 100 nM, 50 nM, 10 nM, 1 nM) acquired in-situ from Ag-AuNW array. (b) Quantitative curves of melamine sensing in water on Ag-	

AuNW array. The Raman intensity of melamine peak at 685 cm^{-1} was plotted as a function of melamine concentration. The error bars were obtained from multiple measurements on different array areas. (c) SERS spectra of melamine in milk at different concentrations (from top to bottom: $10\text{ }\mu\text{M}$, $5\text{ }\mu\text{M}$, $2\text{ }\mu\text{M}$, $1\text{ }\mu\text{M}$, 100 nM , 50 nM , 10 nM) acquired in-situ from Ag-AuNW array. (d) Quantitative curves of melamine sensing in milk on Ag-AuNW array. The Raman intensity of melamine peak at 685 cm^{-1} was plotted as a function of melamine concentration. The error bars were obtained from multiple measurements on different array areas.73

Figure 4.1 Schematic illustration of the preparation of trilayer Au@Ag-tGO-Au@Ag SERS substrate based on alternating loading of interfacially assembled Au@Ag nanoparticles and thiolated graphene oxide, followed by PEGylation of Au@Ag nanoparticles.77

Figure 4.2 Schematic illustration of the SERS detection of DNA based on the trilayer GO-gapped plasmonic arrays of Au@Ag core-shell nanoparticles.....79

Figure 4.3 (a) XPS spectra of graphene oxide (GO) and cysteine functionalized graphene oxide (tGO). (b) The deconvoluted C 1s spectra of GO and tGO. (c) Raman spectra of GO and tGO. (d) AFM image and height profile of tGO.....83

Figure 4.4 (a) UV–Vis spectra of Au nanoparticle dispersion (black line), Au@Ag nanoparticle dispersion (red line), 2D assembly of Au@Ag nanoparticles (blue line), and the Au@Ag-tGO-Au@Ag trilayer construct (purple line). The spectra have been offset vertically for better comparison of absorption maxima. The Insets are photographs of Au@Ag nanoparticle dispersion (left) and the interfacially assembled nanoparticles (right). (b-d) SEM images of 2D assembly of Au@Ag nanoparticles (b), bilayer Au@Ag-tGO structure (c) and Au@Ag-tGO-Au@Ag sandwich structure (d) deposited on glass substrates.84

Figure 4.5 TEM images of (a) Au NPs and (b) Au@Ag NPs.85

Figure 4.6 SERS spectra collected on Au@Ag NP monolayer (red line, bottom), Au@Ag-tGO bilayer structure (black line, middle) and Au@Ag-tGO-Au@Ag sandwich type substrate (blue line, top).....87

Figure 4.7 SEM images of (a) Au@Ag-tGO-Au@Ag sandwich structure with PEGylation and (b) Au@Ag-tGO-Au@Ag sandwich structure without PEGylation. .87

Figure 4.8 SERS spectra of RhB on colloidal Au@Ag nanoparticles with (red line, bottom) and without (black line, top) PEG brushes.....	88
Figure 4.9 (a-b) SERS spectra of RhB (100 nM) on (a) PEGylated and (b) non-PEGylated substrates: Au@Ag NP monolayer (blue line, bottom), Au@Ag-tGO bilayer structure (red line, middle), and Au@Ag-tGO-Au@Ag sandwich type substrate (black line, top). (c) SERS intensity of RhB on Au@Ag-tGO-Au@Ag with (black) and without (red) PEGylation as a function of RhB concentration. The numbers represent the factor of SERS intensity increase after PEGylation. (d) SERS spectra of RhB on PEGylated Au@Ag-tGO-Au@Ag substrate at different concentrations, from bottom to top: 0.05, 0.5, 1, 5, 10, 50, and 100 nM RhB.	88
Figure 4.10 (a) Fluorescence quenching of TAMRA labeled single-stranded probe DNA (10 nM) (black line, top) in the presence of citrate stabilized Au@Ag nanoparticles (red line, bottom) and PEGylated Au@Ag nanoparticles (blue line, middle). (b) Fluorescence quenching of TAMRA labeled double-stranded DNA (5 nM) (black line) in the presence of citrate stabilized Au@Ag nanoparticles (red line) and PEGylated Au@Ag nanoparticles (blue line).....	93
Figure 4.11 (a) The dependence of SERS signal intensity at 1626 cm^{-1} on the concentration of HEX-labeled reporter DNA on PEGylated Au@Ag-fGO-Au@Ag trilayer substrate. (b) SERS spectra of HEX-labeled reporter DNA in the absence and presence of target DNA, and in the presence of a non-complementary sequence. (Blue trace (top): HEX labeled reporter DNA (20 nM), red trace (middle): HEX-labeled reporter DNA incubated in presence of non-complementary DNA sequence, and black trace (bottom): HEX labeled reporter DNA for the detection of 1.0 nM complementary target DNA. (c) SERS spectra of HEX-labeled reporter DNA (20 nM) for the detection of target DNA of different concentrations, from top to bottom: 0, 0.01, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.6 nM. (d) Comparison of SERS intensity of HEX-labeled reporter DNA at 1626 cm^{-1} for the detection of target DNA (black) and non-complementary DNA (red) of various concentrations.....	94
Figure 4.12 (a) SERS spectra of Cy5 labeled reporter DNA (50 nM) for the detection of target DNA of different concentrations (from top to bottom: 0, 0.02, 0.1, 0.2, 1.0, and 2.0 nM). Black line is the background spectra of 50 nM reporter DNA in absence of target DNA. (b) SERS peak intensity of Cy5 labeled reporter DNA (50 nM) at 1592 cm^{-1} for the detection of target DNA of varying concentrations.	95

Figure 4.13 (a) SERS spectra of TAMRA labeled reporter DNA (20 nM) for the detection of target DNA of different concentrations (from top to bottom: 0, 0.01, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 nM). Black line is the background spectra of 20 nM reporter DNA with the absence of target DNA. (b) SERS peak intensity of TAMRA labeled reporter DNA (20 nM) at 1645 cm^{-1} for the detection of target DNA of varying concentrations.96

Figure 4.14 (a) SERS spectra of a mixture of reporter DNAs (20 nM for HEX, 15 nM for TAMRA, and 50 nM for Cy5). (b-d) SERS spectra collected for the detection of target DNAs (1.0 nM) of (b) *E. coli* O157:H7, (c) *Staphylococcus aureus*, and (d) *Listeria monocytogenes*. Triangle, star, and hexagon respectively indicate characteristic Raman peaks of TARMA, HEX, and Cy5, respectively.97

Figure 5.1 Schematic illustration of the detection of target DNAs by the particle-on-array SERS substrate. (A) Preparation of Au nanopikes array and deposition of capture DNA onto the array. (B) Conjugation of reporter DNA with magnetoplasmonic nanoparticles and enrichment of target DNA through DNA hybridization between reporter DNA and target DNA. (C) Formation of particle-on-array sandwiched assay through DNA hybridization between capture DNA and target DNA.101

Figure 5.2 SEM images of Au@Ag@PDA monolayer before (a) and after addition of (b) 50 μL (c) 100 μL (d) 200 μL and (e) 300 of HAuCl_4 solution (1 mM). (f) SEM images of gold nanoflake substrate at a smaller magnification.106

Figure 5.3 EDX of Au@Ag@polydopamine and Au flake substrate Au@Ag@polydopamine.107

Figure 5.4 UV-vis absorption spectra of Au@Ag@polydopamine colloid (black trace), Au@Ag@polydopamine monolayer (red trace) and gold nanospike substrate (blue trace).108

Figure 5.5 (a) UV-vis absorption spectra of gold nanospike substrates at varying amount of gold chloride. (from left to right: red trace: without gold chloride; green trace: 50 μL gold chloride; purple trace: 100 μL gold chloride; black trace: 200 μL gold chloride; blue trace: 300 μL gold chloride). (b) SERS spectra of CV (200 nM) on gold nanospike substrates at varying amount of gold chloride. (from bottom to top: black

trace: without gold chloride; red trace: 50 μ l gold chloride; blue trace: 100 μ l gold chloride; purple trace: 200 μ l gold chloride; green trace: 300 μ l gold chloride).	109
Figure 5.6 TEM images of (a) magnetic beads (MBs), (b) polydopamine coated magnetic beads (MB@PDA), (c) PDA coated magnetic beads with gold nanoseeds (MB@Au seed), and (d) magnetic beads with a gold shell (MB@Au shell).	110
Figure 5.7 UV-vis absorption spectra of magnetic beads colloid (black trace), magnetic beads with PDA layer (red trace), magnetic beads with gold seeds (blue trace), and magnetic beads with a gold layer (purple trace).	112
Figure 5.8 SEM images of DNA conjugated MB@Au shell on gold nanospike substrates at varying target DNA concentrations (a=100 fM, b = 1 pM, c = 100 pM, d = 10 nM).	113
Figure 5.9 (a) SERS spectra of Cy5 at different concentrations of target DNA, from bottom to top: 10 fM, 0.1 pM, 1 pM, 10 pM, 100 pM, 1 nM, and 10 nM. (b) Quantitative plots of SERS intensities at 1598 cm^{-1} versus concentration of target DNA strands. Noncomplementary target DNA strands and single-base mismatch DNA strands were used as controls. The error bars represent standard deviation of three measurements.	115
Figure 5.10 (a) SERS spectra of ROX at different concentrations of target DNA, from top to bottom: 0.1 pM, 1 pM, 10 pM, 100 pM, 1 nM, and 10 nM. (b) Quantitative plots of SERS intensities at 1648 cm^{-1} versus concentration of target DNA strands. Noncomplementary target DNAs were used as controls. The error bars represent standard deviation of three measurements.	117
Figure 5.11 (a) SERS spectra of TAMRA at different concentrations of target DNA, from top to bottom: 0.1 pM, 1 pM, 10 pM, 100 pM, 1 nM, and 10 nM. (b) Quantitative plots of SERS intensities at 1649 cm^{-1} versus concentration of target DNA strands. Noncomplementary target DNAs were used as controls. The error bars represent standard deviation of three measurements.	117
Figure 5.12 Multiplex detection of three different types of pathogen DNA using MB@Au-on-Au nanospike SERS system. (a) SERS spectra obtained when the sample included three types of target DNAs (<i>L. monocytogenes</i> , <i>S. aureus</i> , and <i>B. anthracis</i>). (b) SERS spectra obtained when the sample included two types of target DNAs (<i>L. monocytogenes</i> and <i>B. anthracis</i>). (c) SERS spectra obtained when the sample included two types of target DNAs (<i>S. aureus</i> , and <i>B. anthracis</i>). (d) SERS spectra	

obtained when the sample included two types of target DNAs (*L. monocytogenes* and *S. aureus*). Triangle, star, and hexagon stand for representative Raman peaks of ROX, TARMA, and Cy5 respectively. 118

Figure 6.1 Schematic illustration of gold nanostar-GO hybrid synthesis process. (a) GO nanosheets; (b) gold nanoseed-GO hybrid; (c) gold nanostar-GO hybrid. 123

Figure 6.2 SEM images of (a) Bare GO Sheet, (b) Gold nanoseed-GO hybrid and (c) Gold nanostar-GO hybrid; (d) TEM image of Gold nanostar-GO hybrid. 126

Figure 6.3 FTIR spectra of graphene oxide (GO). 126

Figure 6.4 (a) UV-vis absorption spectra of aqueous dispersion of GO, gold nanoseed-GO hybrid, gold nanostar colloid, as well as gold nanostar-GO hybrid. (b) Raman spectra of GO, gold nanoseed-GO hybrid, and gold nanostar-GO hybrid. 129

Figure 6.5 (a) SERS spectra of CV on different substrates (purple: GO; blue: gold nanoseed-GO hybrid; red: gold nanostars; black: gold nanostar-GO hybrid). (b) SERS spectra of CV at concentrations equal to 5, 20, 200, 1000, 10000, and 20000nM, from bottom to top. (c) Quantitative curves of CV sensing on gold nanostar-GO hybrid. The Raman intensity of CV peak at 1671 cm^{-1} was plotted as a function of CV concentration. The error bars were obtained from measurements collected from three different batches. 131

Figure 6.6 SERS spectra of FePc (a) and RhB (b) on different substrates (purple: GO; blue: gold nanoseed-GO hybrid; red: gold nanostars; black: gold nanostar-GO hybrid), inserts show chemical structures of FePc and RhB, respectively. 134

Figure 6.7 SERS spectra of CV (0.1 μM), RB (3 μM), FePc (10 μM), and mixture solution. 135

Figure 6.8 TEM image of a single gold nanostar. 136

Figure 6.9 (a) Raman spectra of 4-NTP on different substrates (purple: GO; blue: gold nanoseed-GO hybrid; red: gold nanostars; black: gold nanostar-GO hybrid). (b) Time dependent SERS spectra of 4-NTP on gold nanostar-GO hybrid. (c) Time dependent SERS spectra of 4-NTP on gold nanostars. (d) Plot of $\ln(I/I_0)_{1568}$ against time for gold nanostar-GO hybrid and gold nanostars. gold nanostars and gold nanostar-GO hybrid dispersions have approximately identical particle concentrations. 136

List of Tables

Table 1 DNA sequences used in this work	91
Table 2 SERS intensity ratio of GO with and without gold nanoparticle hybrid.	131

Summary

In this thesis, I developed new strategies towards two-dimensional (2D) ensembles of plasmonic nanostructures and explored their uses as Surface Enhanced Raman Scattering (SERS) substrates. SERS is capable of detecting vibrational fingerprints of single molecules, and has stimulated widespread interest in fields that necessitate ultrasensitive detection. The intense electromagnetic field surrounding plasmonic metallic nanostructures, resulting from the excitation of localized surface plasmon resonance (LSPR), plays a major role in the enormous amplification of the Raman signal for molecules in their close proximity. Major challenges exist in the development of SERS substrate with a high density of hotspots and a built-in mechanism for selected analytes, which are of considerable interest for achieving improved fundamental understanding on the structure-SERS activity correlation and practical sensing applications.

To address these challenges, I took advantages of recent advances in wet-chemical synthesis and interfacial assembly of inorganic nanostructures to develop 2D arrays of plasmonic nanostructures with tailored spectroscopic profiles for optimized SERS signal amplification.

Firstly, I developed Ag-Au bimetallic nanowire arrays by virtue of interfacial self-assembly of Ag nanowires, followed by chemical processing through a galvanic replacement reaction. Nanospikes formed on the nanowires during the galvanic reaction led to give rise to a high density of hotspots across the entire nanowire array. We further employed this SERS substrate for the detection of two types of food contaminants: adulteration agent (melamine) and pesticide (thiram). Limits of detection (LOD) of 10 nM and 1 nM were achieved for melamine in milk samples and

thiram in juice samples, which are lower than the LOC imposed by governmental agencies.

Secondly, to endow the substrate with the ability of active enrichment of analytes, I further developed a tri-layer SERS substrate with single layer graphene nanosheets sandwiched between two-dimensional (2D) arrays of closely packed plasmonic nanoparticles. In this design, thiolated graphene oxide (GO) nanosheets served multi-functional roles as a 2D scaffold to anchor plasmonic nanoparticles through metal-sulfur bonding, a nanometer-thick spacer to create SERS-active nanogaps between two layers of nanoparticle arrays, and a molecule harvester for targeted analytes by virtue of π - π interaction. The ability of graphene derivatives to bind DNA molecules, coupled with the powerful multiplexing of SERS spectroscopy, led to multiplexed detection of a group of tumor suppressor genes down to 0.1 nM.

Thirdly, a sandwiched assay was developed based on the combinational use of chemically engineered 2D array of Au@Ag@polydopamine core-shell nanoparticles and magnetoplasmonic nanoparticles that contain a magnetic core and a plasmonic shell. Our results had shown that magnetoplasmonic nanoparticles not only allows for magnetic enrichment of targeted DNA, by forming a sandwiched structure with the 2D array in particle-on-array configuration, their Au shell led to strong plasmonic coupling with the 2D array, which consequently afforded ultrasensitive detection of gene sequences of bacterial pathogens as low as 0.1 nM.

Fourthly, I developed an alternative approach toward 2D ensembles of plasmonic nanostructures by employing GO nanosheets as scaffolds for seeded growth of Au nanostars. The functional groups on chemically derived GO serve as nucleation sites for Au seeds, which enabled the seeded growth of Au nanostars limited on the GO substrates. The branched shape of Au nanostars and their close attachment on the GO

scaffolds collectively led to high SERS activity. Model aromatic molecules such as crystal violet and 4-nitrothiophenol were detected. Moreover, our results have shown that it is possible to monitor the chemical conversion of 4-nitrothiophenol into 4-aminothiophenol, catalyzed by Au nanostars, using SERS spectroscopy.

In summary, my thesis work has led to the development of a number of 2D ensembles of plasmonic nanostructures that possessed abundant SERS hotspots. Their high SERS activity, coupled with the built-in mechanisms for selective enrichment of analyte molecules at SERS hotspots, offered the possibility to detect a broad range of targets, from small chemical molecules to large biomolecules such as nucleic acids that of considerable current interest for water quality management, food safety screening, and medical diagnostics.

Chapter 1 Introduction

Surface enhanced Raman spectroscopy (SERS) is an advanced variation of Raman spectroscopy. It relies on metallic substrates that have nanoscale roughness for amplification of normal Raman signals. Magnitude of amplification of Raman signals can reach approximately 10^{14} , leading to a limit of detection (LOD) at single molecule level. Several reviews have been dedicated to single molecule SERS detections.¹⁻³ Electromagnetic hot spot is one of the most important aspects of SERS. One important category of SERS hotspots is nanoscale gaps (nanogaps) formed among metallic nanostructures that are adjacent to each other. Plasmonic coupling between adjacent metallic nanostructures lead to enormously amplified electromagnetic fields, which are responsible for enhancement of Raman signals. The major limitation with nanogap SERS enhancement is that enhancement factors vary significantly from point to point on a large scale. Because size of gaps is at nanometer scale, enhancement generated from these nanogaps is primarily localized at the nanoscale gap region. In light of the conflicting nature of sensitivity and uniformity, a SERS substrate that has both large-area uniformity and high sensitivity is highly sought-after.

The goal of this thesis is to design and fabricate efficient SERS substrates that have a high density of hotspots uniformly dispersed across a large area. Interfacial self-assembly is a convenient and highly efficient method to prepare two dimensional nanostructure arrays. In this thesis study, stable and uniform nanostructures arrays prepared from interfacial self-assembly technique were used as building blocks for subsequent fabrication of SERS enhancing substrates. These SERS enhancing substrates were subsequently utilized in food safety monitoring and biomedical diagnosis.

The first type of SERS enhancing substrates are single layer metal nanowire arrays assembled at oil/water interfaces. Silver nanowires arrays were first prepared at hexane/water interfaces through interfacial self-assembly method. These nanowire arrays subsequently underwent in situ galvanic replacement reactions with HAuCl_4 and grew into bimetallic Ag-Au nanowire arrays. Galvanic replacement reactions led to formation of nanospikes on Ag-Au nanowires, which generated strong electromagnetic field across Ag-Au nanowires arrays. The interfacial bimetallic nanowire arrays were utilized as efficient SERS substrates for detecting trace chemical contaminations in the food sample matrixes.

The second type of SERS enhancing substrates consists of a monolayer of chemically derived graphene oxide sandwiched between two layers of plasmonic nanoparticles. Metal nanoparticle arrays were prepared by assembling metal nanoparticles at hexane/water interfaces and then transferring to solid substrates. We encapsulated metal nanospheres using polymer poly(ethylene glycol) to direct absorption of analyte onto graphene oxide. The multi-functional role of functional graphene oxide as a 2D scaffold, nanospacer and molecule harvester made it possible to selectively position targeted analytes in the SERS hotspots. This sandwich type SERS substrate was subsequently employed in multiplex detection of three types of tumor suppressor genes.

The third type of SERS substrate based on magneto-plasmonic nanoparticles for detection of pathogen oligonucleotides. A particle-on-array configuration was designed. In this particle-on-array configuration, 'particle' refers to gold coated magnetic beads, and 'array' refers to a gold substrate with a rough surface. Gold coated magnetic beads were conjugated with reporter DNA strands, which then hybridized with half of the target DNA sequence. The other half of the target DNA strand

hybridized with capture DNA, which were immobilized on the gold array substrate. In this way, reporter DNA, target DNA, as well as capture DNA strands were sandwiched between plasmonic nanoparticles and metal platform. Strong plasmonic coupling between magneto-plasmonic nanoparticle and underlying substrate led to amplification of electromagnetic field at their proximity. Raman dye molecules that were attached to the reporter DNA strands thus benefit from strong electromagnetic field and generate large Raman signals. To achieve a further increase in sensitivity, magnetic field was incorporated to enrich target DNA strands and lowered limit of detection by two orders of magnitude.

Lastly, we developed an alternative approach toward 2D ensembles of plasmonic nanostructures by employing graphene oxide nanosheets as scaffolds for seeded growth of gold nanostars. Graphene oxide had a dual functionality: it acted as a nucleation site for gold nanostar growth and a scaffold for nanostar assembly. Star shaped gold nanoparticles offered great enhancement in Raman signal intensities attributable to their branched shape. Gold nanostars formed a close-packed pattern on graphene oxide, generating a high density of hot spots for highly efficient Raman enhancement. The obtained gold star/GO hybrid exhibited superior SERS activity than isolated gold star nanoparticles. The limit of detection for crystal violet was 1.0 nM, which was an order of magnitude lower than gold star colloid. Catalytic conversion of 4-nitrothiolphenol (4-NTP) molecules to 4-aminothiolphenol (4-ATP) was studied in situ by tracking SERS spectra of the reactant. Our results showed that the bifunctional star/GO hybrid material could be applied in both trace detection and pollution treatment.

Chapter 2 Literature review

2.1. Raman spectroscopy

2.1.1. Origins of Raman scattering

Raman spectroscopy is a powerful optical technique to identify and characterize analyte molecules. It provides us with structural information of the analyte molecules on both vibrational and rotational levels of the electronic states. Raman spectroscopy can be used for both identification and quantification of materials: characteristic Raman frequencies are useful in determining the composition of a material, and intensities of Raman bands are often used to provide a measure of concentrations of certain compositions. On top of that, Raman spectroscopy is a highly versatile technique that can be practiced on gas, liquid, as well as solid samples. Raman spectroscopy, as its name suggests, relies on Raman scattering, which is inelastic scattering of an incident light on the analyte molecules. When sample materials are illuminated by a monochromatic radiation, the molecules will absorb the radiation and scatter it in two different ways: (1) elastic scattering (or Rayleigh scattering) - the wavelength of scattered light is the same with incoming light; (2) inelastic scattering - the emission energy is smaller (Stokes scattering) or larger (anti-Stokes scattering) than incident energy. In inelastic scattering, variations of the emission energy are reflected in the changes of emission frequencies. The shift of frequencies of the scattered light as compared with the incident light is called the Raman effect. This frequency shift depends on vibrational, rotational and other low frequency transitions in molecules. We can look at this particular light-matter interaction from both electromagnetic and quantum points of view.

From an electromagnetic point of view, light is a form of electromagnetic (EM) radiation carrying radiant energy. A metal nanoparticle can be viewed as a collective of free electrons around a central positive ion core. When the incoming light encounters sample materials and interacts with the sample molecules, it induces electric dipole moment $P = \alpha E$, where E is electrical field of the incident EM wave and α is molecular polarizability. This dipole moment, which comes from periodic separation of charge within the molecules, will cause the molecules to vibrate in various ways: stretching, rocking, scissoring etc.

Figure 2.1 A diagram shows electron transitions between energy levels during light scattering. The black arrows, which point upward, represent absorption of electrons. The green arrow represents Rayleigh scattering, the blue arrow represents Stokes scattering, and the red arrow represents Anti-Stokes scattering.

Figure 2.1 illustrates light scattering process from the quantum point of view. When the light incidents on sample molecules, it will excite the molecules and cause electrons of the molecules to jump from the ground state to an electronic state. If the electrons return to its original ground state, the molecules will emit light with the same frequency as the incident light. Elastic scattering, or Rayleigh scattering happens. In situations where the electrons return to a state different from its original electronic

state, the molecules will emit light that has a different frequency from the incident light. In this case, inelastic scattering occurs. Inelastic scattering can be further divided into two subcategories: Stokes scattering and Anti-Stokes scattering. In Stokes scattering, the emitted electrons have energy lower than the incident electrons, and the frequency of the emitted electrons shifts to the longer wavelength. The opposite process is called anti-Stokes scattering, where the emitted electrons have energy higher than the incident electrons. Notice that in anti-Stokes scattering, the electrons start from an electronic state higher than the ground state. This characteristic of anti-Stokes scattering makes the anti-Stokes line weaker than the Stokes line, because that extra energy has to be put in to excite the molecules prior to irradiation. As a result, in Raman spectroscopy, the lines we observe are typically Stokes lines.

2.1.2. Raman vs. infrared vs. fluorescence

Raman spectroscopy, infrared spectroscopy, as well as fluorescence spectroscopy is all vibrational spectroscopy. The fundamental differences among them are that Raman spectroscopy measures scattering of incident light, infrared spectroscopy examines absorption of infrared light, and fluorescence spectroscopy measures emission of light from analyte molecules after exposure to incident lights.⁴

Figure 2.2 Raman and FT-IR spectra of milk sample. Enlarged picture shows the molecular vibrational states along with a simplified Jablonski diagram. (Reprinted with permission from ref 5. Copyright 2012 The Royal Society of Chemistry)

Infrared (IR) absorption and Raman scattering are complementary to each other. The differences between Raman spectroscopy and infrared spectroscopy lie in that: (1) Infrared is excited by light in the mid-IR range, while light ranging from ultra-violet to mid-IR can excite Raman. (2) As their names suggest, Raman scattering arises from scattering of light by the molecules while IR absorption is the result of absorption of light by vibrating molecules. (3) IR bands stem from dipole vibrations (i.e. functional groups, O-H, N-H, C=O) while Raman spectra come from polarizable vibrations (i.e. carbon backbones, C=C, aromatics).⁵ **Figure 2.2** shows analysis of milk samples using Fourier transform infrared spectroscopy (FT-IR) and Raman spectroscopy.⁶ Spectra of FT-IR and Raman indicate that strong Raman peaks correspond to weak bands in IR spectra, and vice versa. This observation is in agreement with the report that in certain molecules, especially centrosymmetric molecules, IR and Raman bands appear exclusively.⁷ For example, water molecules have an intense absorption in IR spectroscopy and strongly interfere with IR bands of the analyte molecules. On the

other hand, scattering of water molecules in Raman spectroscopy is very weak. Because of that, Raman spectroscopy can be used in liquid samples without concern of interferences from water solvent. We can also get a perceptive of the differences between Raman and IR from the Jablonski diagram, which shows transitions of electron states that happen during Raman and IR measurements. Infrared radiation is not able to provide enough energy for electrons to transit between different energy states, and IR measurements are limited to rotational and vibrational transitions only.

Fluorescence emission, on the other hand, is a competing phenomenon with Raman scattering. Raman spectroscopy measures scattering of light from materials while fluorescence spectroscopy measures emission of light from analyte molecules after exposure to incident lights.⁴ During fluorescence emissions, electrons are promoted from the ground state to an excited state and then return to ground state. Because electrons relax to a lower electronic state while in the excited state, the photons emitted by the molecules have a lower energy than the absorbed photons. During the relaxation process, energy of the electrons is lost as vibrations or heat without any photo emitted.⁸ Fluorescence spectra are relatively broad (typically 50-100 nm, half-widths), and they can easily overlap when multiple probes are used simultaneously. Raman peaks, on the other hand, have a half-width that is less than one nanometer. Because of its narrow-band characteristic, Raman spectroscopy can detect as many as six types of analytes at the same time.⁹ It is also worth noting that Raman can work with near infrared or even infrared excitations, but fluorescence phenomena starts to disappear when excitations approach the IR spectrum. This is of great importance in biological applications because living tissues have a wide absorption spectrum and the optical absorption in the visible range especially ultraviolet region is quite strong.

2.2. Surface enhanced Raman scattering (SERS)

2.2.1. From Raman to SERS

Raman spectroscopy has gained significant research attention since its discovery in 1928.¹⁰ Two important advantages of this technique are its abilities to provide molecular fingerprints of materials and to carry on simultaneous detection of multiple analytes. Despite these apparent advantages, utilization of Raman spectroscopy is not as popular as other analytical spectroscopy such as Fourier-transform infrared (FTIR) and fluorescence spectroscopy. The biggest limitation of Raman spectroscopy lies in its inherent weak signals, which prevents its wide applications.¹¹ The characteristic of Raman is mainly attributed to two factors: (1) most sample molecules have small Raman scattering cross sections, (2) amount of incoming photons that undergoes Raman scattering is very small. Raman scattering cross section describes the probability for light to be scattered by the molecules, and it is highly species specific. Take benzene molecule as a example, the scattering cross section is only $2.8 \times 10^{-29} \text{ cm}^2 \text{ molecule}^{-1} \text{ sr}^{-1}$, which is 12-14 orders of magnitude smaller than typical fluorescence cross sections.¹² In addition, Raman scattering is a very inefficient process. Of all the incoming photons, only one out of 10^7 undergoes Raman scattering.¹³ Due to the above reasons, sensitivity of normal Raman spectroscopy is extremely low, therefore greatly hindering its application in trace detection.

The discovery of surface enhanced Raman spectroscopy (SERS), which provides signal enhancement of several orders of magnitudes, managed to overcome the limitation of low sensitivity and at the same time preserved other desirable properties of Raman spectroscopy. Nie and Kneipp first reported observation of single-molecule using SERS. In order to achieve single-molecule level detection, SERS enhancement

factors in the range of 10^{14} - 10^{15} are required.^{14, 15} Ever since their pioneering reports on single molecule detection using surface enhanced Raman, more and more studies¹⁶⁻¹⁹ have been carried out on single molecule or near single molecule detection, pushing sensitivity of SERS to be comparable to and sometimes even higher than that of fluorescence.

2.2.2. SERS enhancement mechanisms

SERS enhancement mechanisms have been a matter of debate ever since the first discovery of SERS effect.²⁰ Although the exact mechanisms have not been fully established, two theories have been extensively discussed in the literature: Electromagnetic enhancement (EM), which arises from enhanced electromagnetic field at and near metallic nanostructure due to excitation of surface plasmon resonance; and chemical enhancement (CM), which stems from charge transfer between analyte molecules and nanostructure. Electromagnetic enhancement is independent of the sample molecules, while chemical enhancement is highly dependent on the chemical nature of the analyte molecule.¹² It is worth noting that in electromagnetic enhancement, metallic nanostructures not only enhance the incident laser field but also the Raman scattered field. The enhancement factor of SERS signal intensity will thus be proportional to the fourth power of the enhancement of local electric field (E^4). It had been reported that contribution factor of electromagnetic enhancement (EM) can be as high as 10^{12} ,²¹ while chemical enhancement (CE) accounts for a much smaller increase of 1-2 orders in magnitude.^{22, 23}

Figure 2.3 Schematic illustration of SERS electromagnetic enhancement based on localized surface plasmon resonance.

Electromagnetic enhancement relies greatly on localized surface plasmon resonance (LSPR) property of the enhancing substrates. LSPR refers to collective oscillation of conduction electrons in nanometer-sized metal structures under excitation of an incident light.²⁴ This collective electron resonance leads to enhanced electromagnetic field at (or close to) metal nanostructure surface. As shown in **Figure 2.3**, when two nanostructures are close to each other, their free electrons will interact. This near-field plasmonic coupling between two closely positioned nanostructures will lead to generation of exceptionally strong electromagnetic field at the small gaps among nanostructures. Analyte molecules located in the vicinity of metal nanostructure surface or nanogap junctions will experience dramatically enlarged electromagnetic field, which is the key for amplification of Raman signal intensity. One characteristic of electromagnetic enhancement mechanism is that the enhancement of electromagnetic field is highly surface confined: the magnitude dramatically decays as further away from the substrate surface.

As enlarged electromagnetic field comes from plasmon resonance of the substrate, it is reasonable to assume that this enhancement is radiation wavelength dependent.

Van Duyne et al. studied SERS spectra of benzenethiol on lithographically fabricated silver nanoparticle arrays using wavelength-scanned Raman excitation spectroscopy.²⁵ SERS spectra obtained by excitations from 425 - 505 nm and 610 - 800 nm were found to correlate with LSPR of nanoparticle array: optimum excitation wavelength is blue-shifted to the LSPR maximum absorption peak of nanoparticle array. Another work carried out by this group indicated that excitation wavelengths close to LSPR extinction maximum induced maximum enhancement in SERS signals.²⁶ However, this rule may not apply to complex and disordered plasmonic nanostructures. E. C. Le Ru and co-workers showed that extinction maximum failed to be a good indicator of maximum SERS enhancement in dimers, quadrimers, and aggregates.²⁷

2.3. Hotspots in plasmonic nanostructures for SERS

As electromagnetic enhancement largely relies on localized surface plasmon resonance (LSPR) property of the enhancing substrates, it is expected that electromagnetic enhancement can be easily manipulated by modifying LSPR properties of SERS substrates. Design and fabrication of SERS active substrates that are capable of generating large electric-field are of critical importance in harvesting large amplification of signals, because electromagnetic component of SERS enhancement factor is proportional to the fourth power of electric-field enhancement.²⁸ To achieve high EM enhancement, hotspots where electromagnetic field is greatly enhanced are thus highly thought-after. In general, SERS hotspots are located at sharp tips and/or nanoscale gaps (nanogaps) of metallic nanostructures.

2.3.1. Hotspots from sharp tips and curvatures

Electromagnetic field at the sharp tips and curved features of anisotropic metal nanoparticles is up to a hundredfold higher than that at smooth metal surfaces.²⁹

Concentration of electric field at particle tips is commonly referred to as the 'lightning rod effect', which results from concentration of dipolar fields of the particles at their tips.³⁰

Figure 2.4 Transmission and scanning (insets) electron microscopy images of the nanostars in order of increasing size (A–F). Their synthesis in high yield is evident. Samples A–F were synthesized by addition of the following volumes of Au seed: (A) 700, (B) 540, (C) 400, (D) 270, (E) 135, and (F) 45 μL . (Reprinted with permission from ref 24. Copyright 2008 American Chemical Society).

Star shaped gold nanoparticle is an interesting group of nanostructures for SERS applications. They have a spherical core with several (5-10) sharp tips pointing out. Those sharp tips are responsible for generation of largely enhanced electromagnetic fields.³¹ Studies on single star shaped gold nanoparticle showed that a SERS enhancement factor on the order of 10^7 could be achieved at the tips of the nanoparticles.^{32, 33} Magnitude of SERS enhancement closely correlates with the morphologies of gold nanostars. Because most of the synthesis routes adopt a seeded growth method, morphologies of gold nanostars can be conveniently controlled by varying seed volume in the growth solution. Generally, if all other reaction conditions

are held constant, a smaller seed volume will lead to a larger nanostar size, which was accompanied by larger core size, more branches and larger branch aspect ratio. Optical properties of nanostar structures are very sensitive to changes in their morphologies. As star size grows bigger, both short plasmon band and long plasmon band experience a red shift. Because the enhancement effect of SERS is dependent on both plasmonic properties of metallic nanostructures³⁴ as well as excitation wavelengths, variation of nanostar morphologies will surely lead to a variation in enhancement factor. For example, Tuan et. al carried out solution-based SERS measurements on a series of gold nanostars with size ranging from 45 to 116 nm (**Figure 2.4**), and studied correlation between size and SERS efficiencies of nanostars.³⁵ As large electric field enhancements are located in close proximity of sharp tips, larger branch aspect ratio and larger fraction of Raman dye molecules absorbed to tip area are expected to lead to higher enhancement intensities. However, it is important to note that excitation wavelength also plays a key role in determining the electromagnetic field. The plasmonic peak of gold nanostars shift to longer wavelength as the star size increases. In order to achieve maximum electromagnetic field enhancement, the excitation wavelength has to be adjusted to match the plasmonic peak for better resonance. It is safe to conclude that SERS enhancement factors are dependent on multiple aspects. To obtain an optimum enhancement, all the experiment parameters need to be chosen carefully.

Figure 2.5 (A) TEM image of gold nanorods. (B) SERS spectra of Raman dye molecules collected from gold nanorods. (C) Absorption spectra of gold nanorods. (D) SERS spectra of gold nanorods (left ordinate) and spherical nanoparticle (right ordinate). (Adapted with permission from ref.27. Copyright (2012) American Chemical Society.)

Another important category of anisotropic plasmonic nanostructures is rod shaped nanoparticles. Numerous publications on gold and silver nanorods can be found in fields such as sensing, drug delivery tracking,³⁶ in vivo imaging,³⁷ and photothermal heating treatments³⁸ etc. Large electromagnetic field is concentrated at the ends of nanorods. Gambhir et al. combined photoacoustic (PA) and Raman approaches for ovarian cancer imaging using gold nanorods as molecular imaging agents.³⁷ The rationale for choosing gold nanorods over gold nanospheres is that they have large optical absorption cross sections, which maximize both PA and SERS signal intensities. Their anisotropic shape, as seen in **Figure 2.5**, also offer more efficient

SERS enhancement and better extravasations into tumor space. Intensity of SERS signal from gold nanorods was found to be ten times higher than that from gold nanospheres on a per particle basis. They also found that by varying the aspect ratio of nanorods, plasmonic bands of nanorods could be tuned to better match excitation wavelength: a closer match between plasmonic band and excitation wavelength led to enhancements that are more efficient.

Figure 2.6 (A) Optical image of a single smooth silver nanowire; (B) SERS intensity map of silver nanowire; (C) cross-sectional SERS intensities collected at each micrometer pixel along silver nanowire; and (D) SERS spectra collected at points 1, 2, 3, and 4 in (B). (E) Optical image of single etched silver nanowire with (F) SERS intensity map; (G) cross-sectional SERS intensities collected at each micrometer pixel along etched silver nanowire; and (H) the SERS spectra collected at points 1', 2', 3', and 4' in (F). (Reprinted (adapted) with permission from ref 32. Copyright (2012) American Chemical Society.)

Recently, metallic nanowires have attracted increasing attention in SERS applications because of their large anisotropy. Gold and silver nanowires can be synthesized in colloid through the help of surface ligands such as polyvinylpyrrolidone (PVP),³⁹ hexadecyltrimethylammonium bromide (CTAB),⁴⁰ etc. Or they can be prepared through seeded growth on a solid substrate.⁴¹ They normally have diameters in the nanometer range and lengths in micrometer range. Smooth nanowires are not good candidates as SERS substrate because their smooth longitudinal surfaces are less efficient in light coupling than the two ends.⁴² However, by creating a rough surface with sharp tips on the longitudinal surfaces, large SERS enhancement could be achieved.⁴³⁻⁴⁵ Mixtures of ammonia solution (NH_4OH) and hydrogen peroxide (H_2O_2) at various ratios are commonly used as etchants to oxidize silver. **Figure 2.6** shows optical images of smooth silver nanowire and etched silver nanowire, their corresponding SERS intensity maps, as well as SERS spectra at different spots on the wire.⁴⁶ Optical images show that the nanowires used in this study have an average length of 10 μm and an average diameter around 120 nm. On smooth silver nanowires, large SERS intensities are only observed at the ends of the nanowires, which is similar to that observed on rod shaped nanoparticles. The longitudinal surface, which takes up most of the surface area of nanowire, exhibit little enhancement. On etched silver nanowires, however, we can observe a high density of rough wrinkles distributed across the entire nanowire surface. Creation of a rough nanowire surface is key to generation of large SERS enhancements, because surface plasmon scattering increases at roughened silver nanowire surface. Raman mapping confirmed our observation by showing that strong SERS signals can be obtained from any position on the nanowire.

2.3.2. Hotspots from nanogaps

When multiple plasmonic nanostructures are located in close proximity to each other, localised plasmonic resonances of each nanostructure will interact with each. This will lead to coupled plasmonic oscillations, which give rise to largely enhanced electric field in the gaps among nanostructures.⁴⁷ The strong electromagnetic field at those gap regions (hot-spots) can be utilized to enhance Raman scattering intensity of analyte molecules located at the those hot spots. Earlier SERS substrates rely on colloidal aggregates to generate nanogaps. The Raman signals are generally irreproducible because enhancement factor is very sensitive to surface morphology of the substrate. In addition, random aggregation is time-dependent and non-reversible, the Raman spectra have to be recorded within a limited time duration when the enhancement is highest. In light of the above limitations, a more sophisticated fabrication technique that could offer controlled nanogap generation is needed. Controlled assembly of individual plasmonic nanoparticles offers a promising new route for nanogap generation.

Figure 2.7 SERS spectra of (a) monomers, (b) dimers, and (c) trimers of Au@Ag NPs.

(Adapted with permission from ref.45. Copyright (2010) American Chemical Society.)

Nanoparticle dimer structure is indisputably the simplest form of nanoparticle assembly and had been under intensive research for the past decades. Nanoparticle dimers can be prepared through physical method such as lithographic technique,^{48, 49} or wet chemical method using organic molecules such as protein⁵⁰ and oligonucleotides as linkers, or they can simply be prepared using small molecules as aggregation inducer.⁵¹ Electromagnetic (EM) enhancement greatly depends on inter particle distances. Theoretical studies using finite element calculations (FEM) showed that a 0.5 nm separation is required for EM enhancement bigger than 10^8 , whereas a spacing below 0.5 nm and even slight fusing gave rise to EM enhancement of 10^{10} .⁵² Another interesting finding was that, regardless of spacing and particle diameters, less than 3% of the total analyte molecules accounted for more than 90% of the SERS signal.⁵² This phenomenon indicates that low concentration and even single molecule detection using dimer structures is quite challenging, as less molecules will be able to access highly localized EM enhancement spots at low analyte concentrations. To better utilize enormous field enhancement at the gap region, it is highly advantageous to selectively place analyte molecules in the nanogaps. In Chen's work, Au@Ag core-shell nanoparticles of size 20nm were coated with a monolayer of 2-naphthalenethiol molecules, which acted as Raman dye and nanocluster formation inducer.⁵¹ Instead of fixing dimers and trimers to solid substrates, they encapsulated the nanoclusters with a polymer shell and dispersed them in water. The interparticle distances were observed to be 0.8 nm in each dimer and trimer structure. SERS enhancement resulted from this short distance was significant. Their calculations showed that SERS intensities of 2-naphthalenethiol at dimer structure was greater by a factor of 16 than those at single nanoparticle, whilst the enhancement factor increased to a surprising 87 at trimer structures (**Figure 2.7**).

The challenge of using dimer structures for surface enhanced Raman measurement is precise positioning of analyte molecules in the gap region for enhanced electromagnetic field. The most common approach is to use analyte molecules as aggregation inducers, as shown in the above example. Some groups also reported using DNA strands as linking agents, which provide a controlled assembly approach.⁵³⁻⁵⁵ By attaching nanoparticles to either ends of oligonucleotides, dimer and trimer assemblies in various configurations can be obtained through Watson-Crick base-pairing interactions.⁵⁵ Because target DNA strands were positioned right at the nanogap region, a linear relationship between target concentration and signal intensity could be obtained. However, the approach of using oligonucleotides as dimer structure linkers falls short in precise controlling of inter particle distances. This is because DNA molecules are flexible, they tend to exist in a coil form instead of a stretched form. When the DNA molecules are twisted, the distances among nanostructures become difficult to control.

To summarize, although dimer structures are easy to prepare and at the same time able to provide large Raman signal enhancements, they have several inherent limitations: formation of nanogaps is not reproducible; size of nanogaps is not uniform; large enhancement is confined at the nanogap region while average enhancement of the whole ensemble is moderate.

Figure 2.8 Scheme of nanogap plasmonic nanoparticle synthesis process and TEM images. (Reprinted with permission from ref. 44 (2014) American Chemical Society)

Biological and biomedical applications of Raman require near infrared (NIR) excitation, because fluorescence emission from the analyte can be significantly suppressed in this regime. Tuning plasmon resonance of metal nanostructures by tuning their size and shape is convenient, but the extent is limited. Metal nanoshells, which contain a centre core and an outer shell, offer a wide degree of tunability of the plasmon resonance. In this type of nanostructures, interaction of plasmons comes from inner core and outer shell. Plasmon resonance peaks can be tuned conveniently by varying the gap between core and shell. Lim et al. pioneered the development of metal nanoshells by proposing gold nanobridged nanogap particles (Au-NNPs), which had a uniform narrow gap of 1 nm width between gold core and outer shell.⁵⁶ In their experiment, gold nanoparticles (AuNPs) with a diameter of 20 nm were first modified with thiolated and Raman dye labelled DNA strands through gold-thiol interactions. DNA modified AuNPs were then used as seeds for subsequent growth of gold shells. To stabilize the nanogap particles they used polymer poly-N-vinyl-2-pyrrolidone (PVP) as stabilizer. Positions of Raman dye in Au-NNPs were manipulated by varying dye position on DNA strands. Loading number of Raman dyes in each Au-NNP was controlled by controlling the ratio between non-Raman dye labelled and Raman dye

labelled DNA strands. As gold shell grew from budding structure to half-shell and finally whole-shell, colour of Au-NNPs colloid evolved from pink to blue, to purple, and in the end, to red wine colour. 3D finite-element calculation of Au-NNP revealed that electric field located at the nanogap region was twice higher than that at nanogap particle without bridges, and 30 times stronger than silica-insulated core-shell nanoparticle. By precisely positioning Raman dye right at the hot spot region, enhancement factors in the range of 1.0×10^8 and 5.0×10^9 were accomplished on most of the nanobridged nanogap particles. The most significant achievement of this work was creation of built-in and uniform hotspots in nanoparticles with self-contained Raman dye molecules. This provided better protection of Raman dyes, larger enhancement, as well as a more homogeneous morphology as compared with random aggregated nanoparticle clusters. However, the role of DNA molecules as gold shell growth template was not well explained, and the size of nanogaps were fixed at one nanometer. To develop a more convenient and reproducible fabrication method for nanogap particles, our group worked on an alternative approach to synthesize nanogap plasmonic nanoparticles that had Raman dye pre-deposited in the interior gaps.⁵⁷ Instead of using DNA molecules as gold shell growth template, we used block copolymers to control the growth of gold shells, and more importantly, the gap distance between core and shell. Scheme of the synthesis process of nanogap plasmonic nanoparticle and their TEM images are shown in **Figure 2.8**. Amphiphilic block copolymers labelled with Raman dye were first self-assembled on gold nanoparticle surface; an outer gold shell was then formed by in-situ reduction of Au precursor on hydrophilic polymer brushes. Raman dye molecules, which were attached to hydrophobic brushes, were sandwiched between core and shell. Aside from the advantages mentioned in above work, our polymer mediated nanogap particles have an

important advancement that nanogap size can be tuned from 1.5 nm to 11 nm by controlling the thickness of polymer shell. By analyzing the SERS signal intensities at different gap sizes, we found that by increasing gap size from 1.5 nm to 5 nm and then 11 nm, the enhancement factor could drop by one order of magnitude each time.

Figure 2.9 (a) Scheme of target DNAs detection by gold particle-on-wire system. (b) SERS spectra of Au particle-on-wire system (blue spectrum) and a single Au NW (magenta spectrum). The inset shows plasmonic absorption spectra of Au NPs (green spectrum), Au NWs (magenta spectrum), and Au particle-on-wire systems (blue spectrum). (c) SEM images of gold particle-on-wire structure (top) and a clean NW (bottom). (Reprinted with permission from ref 45. Copyright (2010) American Chemical Society.)

Nanogaps formed between plasmonic nanoparticles and a metallic substrate also provide large electromagnetic field. Micrometer-sized metallic substrates, such as nanowires and planar substrates do not have efficient light-couplings themselves.

However, by placing a nanometer-sized metallic particle adjacent to the substrate, plasmonic couplings efficiency is greatly improved. Take a metallic wire for example, light propagates along the wire in the longitudinal direction. A metallic nanoparticle that is placed near to the wire surface could act as an nanoantenna, which generates efficient light coupling with the propagating plasmons.⁵⁸ The gap between nanoparticle and underlying substrate is considered as the hot spot for SERS. Positioning analyte molecules right at the gap region could lead to generation of enormously enhanced signal intensities. In Taejoon's work, target oligonucleotides are positioned at the gap region through specific hybridizations among target DNA with capture DNA as well as reporter DNA.⁵³ The scheme of their work is shown in **Figure 2.9**, along with optical and spectroscopic results. We can clearly see that, after DNA hybridization, gold nanoparticles are attached smooth gold nanowire surface. The authors claimed a limit of detection of 10 pM using this detection system. As gold nanoparticles are all loosely dispersed on nanowire surface, this SERS enhancement is believed to be generated from plasmonic coupling between nanoparticle and substrate.

So far, one of the biggest challenges in SERS substrate fabrication is that one cannot achieve a high sensitivity and reproducibility at the same time. Rough surfaces of metallic nanostructures, especially those in the colloid form, are a group of excellent SERS substrates. They have a large quantity of hotspots distributed across the entire surface, thus the reproducibility is high. However, they can only provide a moderate SERS enhancement. SERS substrates with nanogaps can provide enormous SERS enhancement in the order of 10^{14} , sensitive enough for single molecule detection. However, because the scale of nanogaps is in the nanometer range, the enhanced SERS regions are highly localized and the reproducibility of SERS signals on the entire substrate is quite low. Two-dimensional arrays of plasmonic nanostructures

shows great potential to be employed as sensitive and reproducible SERS substrates because they contain a large number of hotspots uniformly distributed on a planar array. Examples of two-dimensional arrays of plasmonic nanostructures as SERS substrates will be illustrated in the following sub-chapter.

2.4. Two dimensional arrays of plasmonic nanostructures as SERS substrates

Plasmonic nanostructures have excellent electronic, magnetic, optical, as well as catalytic properties.⁵⁹ Their light-interacting properties, especially LSPR properties, have made them a valuable material in numerous applications such as surface plasmon resonance (SPR), surface enhanced Raman spectroscopy (SERS), and surface-enhanced fluorescence, etc. Organizing plasmonic nanoparticles into two-dimensional (2D) and three-dimensional (3D) arrays could introduce newfangled collective properties to them. Plasmonic properties of nanostructure arrays can be readily controlled by tuning the intrinsic properties (composition, size, shape, etc.) of individual plasmonic nanoparticles, number of nanoparticles, as well as their interactions. Closely packed 2D and 3D arrays of plasmonic nanoparticles are of great importance to SERS because they possess a high density of hotspots homogeneously distributed over a large area.

Plasmonic nanostructure arrays can be fabricated by either top-down approaches or bottom-up approaches. Current top-down approaches for nanostructure array fabrication include electron beam lithography,⁶⁰ electrochemical roughening,⁶¹ and nanosphere lithography,⁶² etc. One of the disadvantages of lithographic techniques is that resolutions are limited to ~10 nm,⁶³ while a sub-10 nm gap distance is required for large signal enhancement in SERS. Other drawbacks such as high cost, lengthy

operating time, complicated operation process, etc. all post a big challenge for high-throughput fabrication and practical applications.

In bottom-up approaches, various nanostructures are first synthesized in colloids and then assembled into two-dimensional ensembles. Assembly of nanoparticles can be achieved through controlled evaporation of solvents⁶⁴ or with the aid of external fields (magnetic field,^{65, 66} electronic field,⁶⁷ and shear forces⁶⁸) and template.⁶⁹ It can also happen in colloid with the aid of molecular-linkers (e.g. DNA strands⁷⁰⁻⁷²). Other methods include convective assembly,⁷³ layer-by-layer assembly,⁷⁴ spin-casting,⁷⁵ the Langmuir-Blodgett (LB) techniques.^{76, 77} Among all these assembly methods, construction of self-assembled nanoparticle films at liquid-liquid interfaces via chemical approaches serve as a promising route and is gaining great momentum, mainly due to its easy and fast fabrication,⁷⁸ low cost, and potential for large scale fabrication of various nanometer-sized building blocks.⁷⁹

An interfacial self-assembled nanoparticle array consists of a monolayer of nanoparticles that reside at the oil-water interfaces. For nanoparticles to stay at oil-water interfaces, the contact angles of nanoparticles with oil-water interface have to be close to 90°. Otherwise, nanoparticles tend to move to the water phase or oil phases, depending on their hydrophobicity. Approaches to bring contact angles to 90° include introducing a hydrophobic coating onto nanoparticles^{80, 81} and reducing surface charges of nanoparticles.^{82, 83}

Figure 2.10 (a) Schematic illustration of how contact angle of a particle with oil-water interface affect its location. Left: contact angle is less than 90° ; center: contact angle equal to 90° ; right: contact angle is larger than 90° . (b) Chemical structures of Au@DTBE (left) and $\gamma\text{-Fe}_2\text{O}_3\text{@BMPA}$ nanoparticles (right). (Reprinted with permission from ref 71. Copyright (2004) WileyVCH Verlag GmbH & Co. KGaA.)

Duan et. al reported that capping AuNPs with a hydrophobic polymer 2,2'-dithiobis[1-(2-bromo-2-methyl-propionyloxy)ethane] (DTBE) could bring contact angle of AuNPs with toluene-water interface to 90° .⁸¹ Similarly, coating $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles with 2-bromo-2-methylpropionic acid (BMPA) could render a contact angle of 90° for $\gamma\text{-Fe}_2\text{O}_3\text{@BMPA}$ nanoparticles. They showed that if the contact angle was less than 90° , particle tended to go to the water phase, and vice versa (**Figure 2.10 a**). Thus bringing contact angle of AuNPs with toluene-water interface to 90° facilitated AuNPs to spontaneously transfer to toluene-water interface and assemble into a metallic thin film. They also found that the terminal functional group 2-bromopropionate played a vital role in determining the degree of the contact angles (**Figure 2.10 b**). Other factors such as the length of ligand hydrophobic chains and

hydrophobicity of original nanoparticles did not affect assembly of nanoparticles at oil-water interface.

Currently, most of the synthetic routes for plasmonic nanoparticles are aqueous phase based.²⁸ Direct assembly of hydrophilic nanoparticles without hydrophobic coating will be more convenient and efficient. Reincke et.al found that adding ethanol into heptane-gold colloid could led to formation of a gold nanoparticle layer at heptane-water interface.⁸² They claimed that addition of ethanol reduced surface charge density of AuNPs, which brought the contact angle closer to 90°, facilitated AuNPs to move into water-heptane interface and form a densely packed monolayer. The organic phase is not limited to heptane, a range of organic solvents such as carbon tetrachloride (CCl₄), chlorobenzene (C₆H₅Cl), chloroform (CHCl₃) etc. can form interfaces with water and provide assembly platforms.⁸³ By selecting the appropriate assembly promoter, various shaped nanoparticles (e.g. nanowires, nanocubes, and nanosheets) can be brought to oil-water interfaces and form closely packed films.⁸³

Important factors that affect the formation and stability of the interfacial particle assembly include particle size, particle shape, property of oil phase, as well as interparticle interactions.⁸⁴ When the size of nanoparticles decreases to nanometer scale, thermal energy needs to be taken into consideration. Both thermal fluctuation and interfacial free energy play important roles in controlling nanoparticle assembly at the liquid-liquid interface.⁸⁵ Particle geometry is also important. Israelachvili et al. found that repulsion forces were smallest for spherical particles among wires, rods, and spherical particles.⁸⁶ Thus self-assembly of spherical particles is most popular among literature. Schulz and co-workers reported self-assembly of gold nanoparticles with anisotropic shapes (gold nanorods (AuNRs) and branched gold nanoparticles) at

water-butanol interface.⁷⁹ Assembly of nanoparticles was achieved by simply adding butanol to AuNP colloid. However, the film formed consist of 3D nanoparticle clusters and void areas, and film formation mechanism was not discussed. Liu et. al investigated a variety of oil-water systems, they found that oil with a polarity closer to that of water favored the assembly process because of synergistic effect of the interfacial energy and polarity of different solvents.⁸³

Two-dimensional arrays prepared at liquid-liquid interfaces through self-assembly are an important class of SERS substrates. A high density of hotspots exists on a relatively large area with satisfactory uniformity. Oil-water interface is a flexible and stable supporting platform itself, SERS measurements can be carried out in-situ without the need of solid supporting substrates. Joshua et al. pioneered the work of in-situ SERS detection using self-assembled nanoparticle arrays at liquid-liquid interface.⁸⁷ Spherical gold nanoparticles were self-assembled into a monolayer at 1,2-dichloroethane (DCE) and water interface through emulsification. Analyte loading onto nanoparticle surface was carried out during the self-assembly process by simply dissolving analytes in the DCE or water phase. All of the analytes, either resonant (MGITC) or non-resonant, water-soluble (MNBI) or DCE-soluble (MATT), showed distinctive peaks at a relatively low concentration. A LOD of several femtomolar was achieved for resonant analyte MGITC and a LOD in the range of 10-400 picomolar was obtained for non-resonant analytes (MNBI and MATT). Other than spherical nanoparticles,⁸⁸ gold nanorods,⁸⁹ silver nanowires,⁹⁰ as well as mixture of nanowires and nanoparticles⁹¹ have also been assembled at liquid-liquid interfaces and used directly for analyte detection in either water or oil phase.

2.5. Applications of SERS in food safety

Food safety is one of the most important priorities in public health sector. Dietary exposure to food contaminants can lead to serious health problems. Because of the frequent outbreaks of foodborne illnesses, food safety is gaining greater public attention and causing bigger consumer concerns. Foodborne pathogens are one of the major concerns in food safety. According to the Center for Disease Control and Prevention (CDC), *Salmonella*, Norovirus, *Campylobacter*, Toxoplasma, *E. Coli* O157, *Listeria* and *Clostridium perfringens* are the pathogens responsible for 90% of the pathogen-caused illnesses.⁹² Toxins emitted by pathogens are another source of food contamination. Exotoxins generated by bacteria and mycotoxins excreted by fungi are able to cause illnesses even without the presence of microbes. Aside from biological threats, chemical such as pesticides and adulterants as well as adulterations also pose significant threat to human health. Dietary exposure to residues of pesticides and adulterants can lead to serious health problems. Although it is not practical to eliminate pesticide residues in foods, governments have established limits of pesticide and fungicide residues to retain them at a safe level. Adulterants are substances that are illegally added into foods for economic benefits. Notable food adulteration scandals include adding cheaper oil during the production of refined and high-value oil,⁶ contaminating animal feed with industrial oil,⁶ adulterating milk powder with melamine to increase the apparent protein content, etc.

Conventional methods for pathogen detection have evolved from culturing to enzyme-linked immunosorbent assay (ELISA) and finally to polymerase chain reaction (PCR). The most traditional detection method for pathogen is the culturing technique, which consists of incubation, enrichment, and a final confirmation test.

Later, enzyme-linked immunosorbent assay (ELISA) technique was developed, which greatly shortened the detection duration and increased the sensitivity of traditional culture-based methods. The disadvantage of ELISA method is that a relatively large amount of antigen is required to generate a readable signal, thus limiting applications of ELISA in situations where trace amount of pathogen is present. Polymerase chain reaction (PCR) shows better sensitivity and specificity than ELISA, and has become the method-of-choice for oligonucleotides analysis recently. During PCR, target nucleic acid sequences can be amplified exponentially, greatly lowering the limit of detection. On the other hand, small molecule detections are usually performed using immunological and chromatography methods, for instance, high performance liquid chromatography (HPLC), gas chromatography (GC), ELISA, etc.⁹³

All of these above methods provide a satisfactory level of both sensitivity and specificity. However, they suffer the disadvantages of being time-consuming and expensive. In addition, most of them require complicated sample pretreatment and handling. All of those drawbacks prevent them from carrying out real time and high throughput applications. Vibrational spectroscopy including infrared spectroscopy and Raman spectroscopy are well known for providing a "fingerprint" of the analyte molecules. Besides being highly specific, they are also simple to operate and do not require complicated sample pretreatment. However, they both have their associated disadvantages. Infrared spectroscopy is limited to detecting solid samples, because water and most of the solvents will interfere with analysis of target molecules. Raman spectroscopy is insensitive to the form of analytes. It can be applied in detection of solid, gaseous, as well as liquid samples. However, the signal output is quite low. A relatively large amount of analytes and high laser power are required to generate signals. Thanks to the rapid development of nanotechnology, new techniques that

utilize optical, electrochemical, as well as catalytic properties of nanomaterials emerge and become attractive alternatives to traditional detection methods. Surface enhanced Raman spectroscopy (SERS) is a technique that combines Raman spectroscopy and plasmonic properties of nanostructures. It is a highly promising assessment tool because of its high sensitivity, molecular specificity, easy sample preparation, and the ability to detect multiple chemicals simultaneously in one matrix. It can detect a wide range of analytes ranging from large organisms such as foodborne pathogen to small molecules such as food contaminants.

Figure 2.11 Schematic illustration of optofluidic SERS system for chemical food contaminants (thiram and melamine) detection.

A common type of contaminates found in fruit juice are small molecules such as pesticides and plasticizer. Current detection methods such as liquid chromatography and mass spectroscopy are time consuming and require complicated sample pretreatment. SERS is an ideal alternative technique because it is highly specific, free from tedious sample preparation, and at the same fast and cheap. By using a proper enhancing substrate, femtomolar detection sensitivity can be achieved.⁹⁴ Combining

SERS technique with microfluidic device can add value to both systems. Soroush et.al designed a portable open channel microfluidic SERS sensing system by integrating both optofluidic and SERS into one system.⁹⁵ The scheme of the detection system is shown in **Figure 2.11**. Analyte solution and silver colloid are introduced into the system at the same time and flow through the microfluidic channel to the end detection point. Efficient analyte-nanoparticle binding could be achieved during the transportation. Silica microspheres placed at the end of the channel stop the flow and concentrate the analyte. This concentration step could greatly enhance sensitivity of the detection system. The authors claimed a detection limit of 63 ppb for melamine and LOD of 50 ppt for thiram.⁹⁵ Other applications of SERS in food safety include detection of color dye Sudan I in chili powder,⁹⁶ detection of melamine in milk samples,⁹⁷ detection of toxins⁹⁸ as well as pesticides such as thiram.⁹⁹

Figure 2.12 (a) Scheme of fabrication process of gold nanoparticle-graphene hybrid SERS substrate. (b) Single DNA detection process. (c) Multiplex DNA detection

process. (Adapted with permission from ref 83. Copyright (2012) American Chemical Society.)

Molecular methods that detect the nucleic acids of foodborne pathogen are gaining an increase of attention because of their high sensitivity, superb specificity, fast and convenient operation, as well as excellent reproducibility. As compared with the standard method polymerase chain reaction (PCR), SERS provides a comparable sensitivity and is much more convenient. DNA detection systems make use of specific hybridizations between DNA strands, either direct hybridization or sandwich type hybridization. In direct hybridization, only two complementary DNA strands are involved, while as in sandwich type hybridization, three DNA strands: capture DNA, target DNA, and reporter DNA participate. Presence of target DNA will lead to assembly of nanoparticles into a controlled pattern, reducing distance between Raman dye molecules and Raman enhancing substrate. A typical example of DNA detection based on sandwich type DNA hybridization is shown in **Figure 2.12**.¹⁰⁰ Capture DNA strands were first conjugated to gold nanoparticles on the substrate through thiol-gold interactions. After that, target DNA strands were introduced into the system and hybridized with capture DNA. In the final step, reporter DNA were brought in and hybridized with the hanging end of target DNA, thus bringing Raman dyes on reporter DNA strands closer to the enhancing substrates. The authors claimed a detection limit of 10 pM.

Chapter 3 Chemical processing of interfacial assembly of metal nanowires for SERS detection of food contaminants

3.1. Introduction

Food safety is one of the most important priorities in public health. Because of the continuous emergences of new organisms and chemicals that pose potential threats to humans, risks related to food safety increase over the years, making food safety issues a global concern.¹⁰¹ Foodborne pathogens and toxins those pathogens produce are major factors leading to microbial foodborne illnesses.^{98, 102} Aside from biological threats, chemical contaminants such as pesticides and adulterants also pose significant risk to human health. Thiram, a sulfur-containing compound that functions as both fungicide and animal repellent,¹⁰³ is an immense concern for food safety. Dietary exposure to thiram can cause skin irritations, eye illnesses, as well as liver damages.¹⁰⁴ The U.S. Environmental Protection Agency (EPA) sets the maximum residue limit of pesticides and fungicides in water to be 1 ppb.⁹⁵ Melamine (2,4,6-triamino-1,3,5-triazine) is a nitrogen-rich chemical commonly used in polymer production as fire-retardant additives. The high nitrogen content (67% by mass) of melamine is one of the major contributors to its flame retardant properties. However, its high nitrogen content has also been illegally exploited: people add melamine into pet and human foods to make the measured protein content appear higher than the actual content.¹⁰⁵ Consumption of melamine-contaminated food can cause formation of kidney stones, acute renal failure and even death.^{95, 106, 107} The U.S. FDA set a maximum permitting concentration of 2.5 ppm melamine in foods, and China established a safety limit of 1 ppm melamine for infant formula.¹⁰⁸

Conventional methods for the detection of low molecular weight food contaminants are mass spectroscopy,¹⁰⁹ immunological methods (ELISA), and chromatography methods (high performance liquid chromatography (HPLC), gas chromatography (GC), etc.).⁹³ Although these analytical methods provide a satisfactory level of both sensitivity and specificity, most of them are time-consuming and costly for screening large quantity samples. Complicated sample pretreatment and handling process also prevent them from carrying out real time and for onsite applications. There is increasing demand for analytical tools allowing for sensitive and selective detection of food contaminants and providing real-time readout with relatively simple instrumental setup for on-site sampling. Nanostructure-based sensing assays that utilize optical, electrochemical, and/or catalytic properties of nanomaterials have become attractive alternatives to traditional detection methods.¹¹⁰ Surface enhanced Raman spectroscopy (SERS) is a highly promising assessment tool due to its high sensitivity, molecular specificity, easy sample preparation, and the ability to detect multiple chemicals simultaneously in one matrix. Here we report a new strategy of using chemically processed metal nanowire arrays for sensitive detection of food contaminants in real samples. Plasmonic nanostructures with concentrated electromagnetic field at their surfaces are capable of amplifying the SERS signal of molecules in their close proximity. Concentrated electromagnetic fields are often located at nanoscale gaps between plasmonic nanostructure surfaces or at sharp tips/edges on rough surfaces. It has been reported that rough silver nanowire exhibit higher SERS enhancement abilities than smooth nanowires,⁴⁶ and several strategies had been proposed to introduce additional enhancement by either co-assembling nanowires with plasmonic nanoparticles in an alternative manner,⁹¹ or simply combining silver nanoparticles and silver nanowires randomly,¹¹¹ or decorating silver

nanowires with silver nanoparticles.¹¹² However, those methods involve synthesis of two types of nanostructures, and the morphology of nanowire-nanoparticle assembly is difficult to control.

In the current study, we developed arrays of metal nanowires with rough surfaces through one-step chemical processing of smooth nanowire arrays. In contrast to the physical co-assembly method, chemical processing of silver nanowires through galvanic replacement reactions can give rise to a high density of nanospikes on the entire nanowire array. The scheme of the fabrication process of Ag-Au bimetallic nanowire array is shown in **Figure 3.1**. Our strategy involves interfacial assembly of silver nanowires as well as chemical processing of silver nanowire arrays. Interfacial assembly of silver nanowires gives rise to a large area of densely packed nanowires at water/hexane interface. Of equal importance is that chemical processing of nanowire arrays leads to formation of a high density of nanospikes on the surfaces of nanowires, which are vital to generation of strong localized electromagnetic field. Collectively, the AgAu bimetallic nanowire arrays showed excellent SERS activity in sensing food contaminants thiram and melamine. In particular, the nanowire arrays represent a promising class of SERS substrates that provide *in situ* sensing of small molecules.

Figure 3. 1 Scheme of the fabrication process of Ag-Au bimetallic nanowire array. AgNWs are first self-assembled at water/hexane interface and form a monolayer. Upon addition of gold precursor, galvanic reaction occurs between Ag and Au, forming bimetallic Ag-Au nanowire arrays.

3.2. Experimental section

3.2.1 Materials. Gold(III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), poly(vinyl pyrrolidone) (PVP, MW = 40 000), thiram, and melamine were bought from Sigma Aldrich. Ethylene glycol (EG) was obtained from Merck Chemical Company and used as received. Silver nitrate (AgNO_3) was purchased from Stream Chemicals. Milk and juice were bought from a local supermarket. Solvents such as ethanol were obtained from Fluka with HPLC purity. All glassware had been cleaned with aqua regia and thoroughly rinsed with DI water.

3.2.2 Synthesis of silver nanowires. Silver nanowires were synthesized following a modified Xia's method.¹¹³ In a typical synthesis, 8.4 mL of AgNO_3 (0.12M, dissolved in EG) and 2.52 mL of PVP (0.6M, dissolved in EG) were first added into 10 mL of EG. 4.0 μmol KBr solution in 0.4 mL of EG was added drop wise to the mixed solution, followed by gradual heating to 200 °C in 16 min. The solution was further heated at boiling for 30 s and then cooled down to room temperature. Ag nanowire suspension

was cleaned with acetone by centrifugation at 1000g for 15 min, followed by ethanol wash.

3.2.3 Preparation of Ag-Au bimetallic nanowire arrays. Silver nanowires were assembled into a monolayer film at water/hexane interface through a interfacial self-assembly technique. Briefly, 1 mL of Ag nanowire colloid (0.1 mg/mL) was mixed with 1 mL hexane in a centrifuge tube. After applying moderate shaking for 30 seconds, a film formed at water/hexane interface. To generate nanospikes, different volumes of H_{Au}Cl₄ solution were added drop wise (~ 1 minute per drop) to Ag nanowire film under gentle shaking. The resultant Ag-Au bimetallic monolayer was subject to vortex for another 5 minutes and used directly.

3.2.4 Preparation of SERS samples. We prepared a stock solution of 10 μ M melamine in milk (full fat content) by adding 12.8 mg of melamine powder into 10 mL milk and sonicating for 5 min to help melamine dissolve. To prepare milk samples containing varying concentrations of melamine, the stock solution was further diluted with pure milk. Milk spiked with melamine was centrifuged at 10,000 rpm for 15 min to remove components that may interfere with melamine detection. Thiram solution was prepared following a similar procedure. A 10 mM thiram solution was prepared in ethanol and then diluted with water to different concentrations. Real sample detection was carried out by spiking thiram solution into mango juice. Centrifugation of thiram spiked juices was also carried out to remove potential molecules that would generate false signals.

3.2.5 Characterization and measurements. UV-visible absorption spectra were recorded using a Shimadzu UV2501 spectrophotometer. SEM images were obtained using a field-emission scanning electron microscope (JSM-6700F). X-ray

photoelectron spectroscopy (XPS) measurement was carried out on a Kratos-Axis spectrometer with monochromatic Al K α (1486.7 eV) X-ray radiation (15 kV and 10 mA) and hemispherical electron energy analyzer. Raman spectra were acquired using a Renishaw Raman microscope. The 785 nm excitation line was used for both normal Raman and SERS measurements. Laser beam was focused on a spot with size of one micrometer using a 50 $\times$ objective.

3.3. Results and discussion

Silver nanowires were synthesized in solution phase via a seeded growth method. (Detailed synthesise procedure can be found in the experimental section.) **Figure 3.2a and b** are representative SEM and TEM images of freshly synthesized silver nanowires (AgNWs). Both images show that AgNWs have a smooth surface and uniform diameter of 40 nm. Self-assembly of nanoparticles at water-oil interfaces is dependent on factors including the size of nanoparticles, surface coating of the nanoparticles, which affects the contact angle of the nanoparticles at the interface, as well as interfacial energy of the biphasic system.⁸¹ Previous studies have shown that adding external solvents such as ethanol into the nanoparticle colloid could effectively reduce the surface charge of the particles and consequently tune their contact angle at the water-oil interface to be close to 90°, which provides the largest free energy change and give rise to stable two-dimensional assemblies of the nanoparticles at the interfaces.^{78, 82} Polyvinylpyrrolidone (PVP), which is a polymer that interacts with Ag nanoparticle surface through carboxyl oxygen atom,¹¹⁴ acts as the stabilizing agent of AgNWs and played an important role in ordered alignment and close packing of AgNWs. Upon the addition of hexane in the water dispersion of AgNWs, a shiny film that resembled the color of bulk silver formed at water-hexane interfaces in less than

10 seconds after vigorous shaking for better mixing. This metal-like color of AgNW film results from the strong coupling of compact packing of individual AgNWs, as evidenced in **Figure 3.2c**. Silver nanowires aligned parallel to each other, side by side forming a closely packed monolayer at water/hexane interface. Dark-field microscopy images of AgNWs as presented in **Figure 3.2d** show that lengths of AgNWs vary from 70 μm to more than 100 μm . It is challenging to control the length of AgNWs precisely using the synthesis method we adopted (reduction of AgNO_3 by ethylene glycol in the presence of PVP), however the width of AgNWs could be readily controlled, which is of greater importance in self-assembly process and subsequent galvanic replacement reactions. Comparing microscopy images of AgNWs before (**Figure 3.2d1**) and after (**Figure 3.2d2**) assembly, we can see that assembly process brings randomly dispersed AgNWs into a well-aligned pattern, forming a shiny film at a large-scale area.

Figure 3. 2 (a) SEM image and (b) TEM image of AgNWs prior to interfacial self-assembly. (c) SEM image of AgNWs after interfacial self-assembly. (d) Optical microscopic pictures of AgNWs before (d1) and after (d2) interfacial self-assembly.

Upon addition of HAuCl_4 , Ag was oxidized to Ag^+ ions by Au^{3+} and eventually released from nanowires. Au^{3+} was reduced to Au at the same time and deposited onto nanowire surfaces. Previous reports have found that Ag^+ ions can react with Cl^- ions, which are produced during Au formation, and formed insoluble AgCl, which promoted directional growth of Au.¹¹⁵ One of the reasons AgCl could direct isotropic growth of Au is because Au and AgCl have quite different lattice (4.080 Å for Au and 5.5491 Å for AgCl),¹¹⁵ unlike Au and Ag which have similar lattice (4.090 Å for Ag and 4.080 Å for Au).^{116, 117} Previous studies have demonstrated growth of Au spikes from Ag nanoparticle seeds¹¹⁸ and growth of Au needles on Ag nanowires¹¹⁹ under similar conditions. It is worth noting that reaction temperature also affects the morphology of

bimetallic Ag-Au nanowire arrays. Higher reaction temperature leads to the generation of smooth Ag-Au bimetallic nanostructures because of higher solubility of AgCl and faster reaction rate.¹¹⁸ TEM and SEM images of Ag-Au bimetallic nanowires (**Figure 3.3a and b**) show that short spike-like knurls start to form on the surface of AgNWs at an Au/Ag ratio of 1:35. We attributed the anisotropic growth of Ag-Au bimetallic nanostructures to galvanic replacement reaction because no other reducing agent was added throughout the reaction process. TEM image (**Figure 3.3a**) reveals that the center portion of nanowires is lighter than the edges, indicating formation of hollow structures. Formation of hollow structures may be attributed to the existence of pinhole structures on Au-Ag nanowire surface, which allows both Au^{3+} and Ag^+ to diffuse across the shell until the inner Ag core is completely reacted.¹²⁰ The SEM image (**Figure 3.3c**) at a lower magnification reveals that nanowire array is well preserved at a large scale. The morphology of Ag-Au nanowires varies with increasing ratios between Au and Ag. As shown in **Figure 3.3d**, further increasing Au/Ag ratio to 1:18 leads to increase of both density and size of nanospikes increase, at the same time AgNW templates were etched to a greater extend. This is expected because as we continue adding in HAuCl_4 , more and more silver were oxidized by Au^{3+} and dissolved away from nanowire while more Au^{3+} were reduced and deposited onto nanowire surface. Formation of a denser layer of nanospikes not only modifies optical properties of Ag-Au nanowire arrays by extending plasmonic absorption to NIR range, but also produces a more efficient SERS enhancement because strong electric field is generated at the tips, edges, as well as gaps among nanospikes. From **Figure 3.3e and 3.3f** it is observed that the wire-like morphology of Ag-Au nanowires is well preserved and nanowire arrays are still intact. When the Au/Ag ratio increased to 1:9, however, silver nanowires become fragmented and the wire shape is almost destroyed

(**Figure 3.4**). The array pattern was severely damaged and we no longer observed any continuous film at the water/hexane interface.

Figure 3. 3 (a) TEM image of Ag-Au nanowires at Au/Ag ratios of 1:35. (b) and (c) SEM image of Ag-Au nanowire array (Au/Ag ratios of 1:35) at different magnifications. (d) TEM image of Ag-Au nanowires at Au/Ag ratios of 1:18. (e) and (f) SEM image of Ag-Au nanowire array (Au/Ag ratios of 1:18) at different magnifications.

Figure 3. 4 (a) TEM and (b) SEM images of Ag-Au bimetallic nanowire at Ag/Au ratio equals to 9:1.

Changes in morphology of Ag-Au nanowire arrays can be conveniently observed by the color changes that happen during the reaction process: the nanowire arrays changed from silvery to yellow and finally brownish black. Changes in optical responses and plasmonic features are also reflected in the UV-Vis spectra, as presented in **Figure 3.5a**. Plasmonic absorptions of the bimetallic nanowire arrays are dependent on both arrangements of nanowires as well as the composition of them. AgNW colloid exhibits two peaks at 354 nm and 370 nm, which correspond to their longitudinal mode and transverse mode of plasmon resonances, respectively.^{113, 121} When the AgNWs assembled into a monolayer film, the transverse plasmon band remained unchanged at 370 nm, while the longitudinal plasmon band broadened and shifted to 480 nm. The red shift and broadening of longitudinal plasmonic peak may be attributed to the plasmonic coupling of neighboring nanowires.¹²²⁻¹²⁴ Absorption spectra of Ag-Au nanowire film exhibits a significantly broadened peak that is further red shifted to the range of 600-800 nm. The red shift of plasmon band confirms replacement of silver by gold. It also indicates that element gold deposit on silver nanowire surface rather than forming isolated nanoparticles.

Figure 3. 5 (a) UV-Vis spectra of AgNW colloid (black), AgNW monolayer (red), and Ag-AuNW array (blue). (b) SERS spectra of R6G (100 nM) collected on Ag-AuNW arrays at Ag/Au ratios ranging from 9:1 to 35:1.

Strong scattering of Ag-Au nanowire arrays is evident even at a relatively low concentration of HAuCl_4 (Ag/Au ratio is 35:1), and remained relatively unchanged as Ag/Au ratio decreases (**Figure 3.6**). This is believed to stem from the growth of spikes on nanowire surfaces and hollow structures formed in nanowire cores.¹²⁵ X-ray Diffraction (XRD) technique was used to determine the composition of as formed composite. XRD patterns of Ag nanowire and Ag-Au bimetallic nanowire array are presented in **Figure 3.7a**. Ag nanowire monolayer exhibits two diffraction peaks, which are assigned to the (111) and (200) planes of fcc metallic silver, respectively.¹¹⁹ The ratio between the intensities of the (200) and (111) diffraction peaks is calculated

to be 0.17. On the spectra representing Ag-Au nanowire array (blue trace), an additional peak is observed, which can be attributed to formation of AgCl.¹²⁶ The peaks of Ag and Au are difficult to differentiate due to their similar lattice structures.¹²⁷ The composition of bimetallic nanowire was also characterized by Energy-dispersive X-ray spectroscopy (EDX). The EDX pattern of Ag-Au nanowire arrays shown in **Figure 3.7b** reveals that both Ag and Au are present on Ag-Au nanowire arrays.

Figure 3. 6 UV-Vis spectra of Ag-AuNW array at Ag/Au ratios ranging from 9:1 to 35:1.

Figure 3. 7 (a) XRD spectra of AgNW (red, bottom) and bimetallic Ag-AuNW (blue, top). (b) EDS spectra of bimetallic Ag-AuNW.

In our work, by etching smooth AgNW surfaces with HAuCl_4 , we are able to create a high density of nanopikes on AgNW surface. Because plasmonic hotspots can be formed at the nanoscale junctions among metal structures with high curvatures, those nanopikes will generate highly localized and strongly enhanced electromagnetic field at their vicinity. Additionally, because bimetallic Ag-AuNWs are closely aligned with each other, hotspots are formed at the junctions among nanopikes, leading to enormously enhanced Raman signals from analyte molecules spatially confined in enhanced fields. In this regard, we used R6G as probe molecules to determine the optimal ratio between Ag and Au for fabrication of Ag-AuNW arrays, which are used subsequently in SERS applications. SERS spectra of R6G at the same concentration on Ag-AuNW arrays with different Ag/Au ratios were collected and presented in Figure 3.5b. All spectra show identical Raman peak positions, which are characteristic Raman peaks of molecule R6G. Signal intensities collected from bare AgNW arrays are the lowest. This is as expected because smooth AgNW arrays possess the least amount of hotspots. Also plasmon absorption peak of AgNW arrays centered at 450 nm is far from being resonant with the excitation wavelength (785 nm). As Ag/Au ratio decreases from 35:1 to 18:1, signal intensities of R6G are observed to amplify by two times. However further reducing Ag/Au ratio to 9:1 leads to a reduction in signal intensity by four times. The highest signal intensity is observed when Ag/Au ratio equals to 18:1. This trend of change in SERS signal intensities could be elucidated by change in nanowire array morphologies with varying Ag/Au ratios: when ratio of Ag/Au reduces from 35:1 to 18:1, more nanopikes begin to form on nanowire surface, leading to dramatically increased electromagnetic field. Continuous addition of HAuCl_4 will lead to severe etching of AgNWs, destroying the wire-like morphology and array stability, leading to a destructed array. To demonstrate substrate-to-substrate

repeatability of the Ag-Au nanowire array, we carried out SERS measurements of R6G on three substrates and calculated the standard deviation. SERS spectra of R6G with concentrations of 10 pM, 50 pM, 100 pM, 1 nM, 10 nM, 50 nM, and 100 nM and the plot of the SERS intensity at 1617 cm^{-1} as a function of R6G concentration are shown in the **Figure 3.8**. The low variation of the measured peak heights demonstrates the high array-to-array repeatability. On the contrary, the intensity of SERS spectra of a fixed concentration of R6G collected on the same substrate varies a lot, as shown in **Figure 3.9**. In the subsequent SERS measurements, we fabricated Ag-AuNW arrays following a Ag/Au ratio of 18:1, and applied Ag-AuNW array substrate in detection of thiram, a sulfur-containing fungicide, and melamine, a nitrogen-rich food contaminant.

Figure 3. 8 (a) SERS spectra of R6G with concentrations of 10 pM, 50 pM, 100 pM, 1 nM, 10 nM, 50 nM, and 100 nM (from bottom to top) and (b) the plot of the SERS intensity at 1617 cm^{-1} as a function of R6G concentration.

Figure 3. 9 SERS spectra of R6G on Ag-Au nanowire array when Au/Ag ratios equals to 1:9.

A thiram molecule contains one S-S bond and two S=C bonds, which account for its high sulfur content. Upon interaction with gold or silver surfaces, the S-S bond cleave and bind to gold or silver surfaces through strong thiol-metal covalent bonds.⁹⁹

Figure 3.10a shows the SERS spectrum of thiram obtained from Ag-Au bimetallic nanowire array (black trace) and normal Raman spectrum of thiram powder (red trace). The peak at 560 cm^{-1} , the dominant peak in the normal Raman spectrum which is assigned to $\nu(\text{S-S})$, becomes much less significant in SERS spectrum. While the peaks at 1385 cm^{-1} ($\rho(\text{CH}_3)$) and 1513 cm^{-1} ($\nu(\text{C-N})$), which are hardly observable in the normal Raman spectrum, are greatly enhanced and become dominant peaks in the SERS spectrum. The differences in normal Raman spectrum and surface enhanced Raman spectrum of thiram are clear evidence that above-mentioned chemical interactions occurred between thiram molecules and Ag-Au bimetallic nanowire surfaces. To evaluate the SERS performance of our substrate in sensing applications, we carried out quantitative SERS measurements of thiram in water solution and juice samples. **Figure 3.11a** shows SERS spectra of thiram in water solution with

concentrations increasing from 0.1 to 500 nM. The chemical structure of thiram is shown in the inset. The most intense peak of thiram is observed at 1385 cm^{-1} , which assigns to $\rho(\text{CH}_3)$.^{99, 128} Intensity of thiram characteristic Raman peak is observed to decrease with reducing thiram concentration. When thiram concentration dropped to below 0.1 nM, signals can no longer be clearly distinguished, indicating a limit of detection of 0.1 nM for thiram, which is lower than maximum acceptable limit of 4 nM (1 ppb) as set by the U.S. Environmental Protection Agency (EPA).⁹⁵ Quantitative relationship between peak intensity and thiram concentration is plotted and presented in **Figure 3.11b**. Intensity of peak at 1385 cm^{-1} increases swiftly with increasing thiram concentration, and gradually reached a plateau at concentrations higher than 100 nM. The above results prove that Ag-AuNW array substrate can be readily employed for quantitative analysis of thiram in water. Next, we carried out detection of thiram spiked in real samples: fresh mango juice that was purchased from a local supermarket. Juice samples spiked with thiram were centrifuged to remove solid residues to avoid interference with SERS detection. Detection parameters are the same as those applied in water solution. **Figure 3.11c** shows concentration dependent Raman spectra of thiram spiked in juice samples, with concentrations of melamine ranges from 1 nM to 500 nM. Characteristic peak of thiram at 1385 cm^{-1} can be clearly observed in all concentrations. Quantitative relationship between peak intensity and thiram concentration is plotted and presented in **Figure 3.11d**. Intensity of peak at 1385 cm^{-1} increases with increasing thiram concentration, and gradually slows down at concentrations higher than 200 nM. Raman peak at 1385 cm^{-1} with thiram concentration as low as 1 nM can be observed in the SERS spectra, indicating a detection limit of 1 nM for thiram in juice samples. Limit of detection for thiram in juice samples is one magnitude higher than that in water solution. We attribute this to

the centrifugation pretreatment process, during which some of the thiram molecules absorbed onto large protein molecules are removed along with impurities.¹²⁹ Nevertheless, this detection limit is still much lower than that set by the EPA regulation, which is 4.0 nM.

Figure 3. 10 (a) SERS spectrum of thiram on Ag-Au bimetallic nanowire array (black trace) and normal Raman spectrum of thiram powder (red trace). (b) SERS spectrum of melamine on Ag-Au bimetallic nanowire array (black trace) and normal Raman spectrum of melamine powder (red trace).

Figure 3. 11 (a) SERS spectra of thiram in water at different concentrations (from top to bottom : 500, 100, 10, 1, 0.5, and 0.1 nM) acquired in-situ from Ag-AuNW array. (b) Quantitative curves of thiram sensing in water on Ag-AuNW array. The Raman intensity of thiram peak at 1385 cm⁻¹ was plotted as a function of thiram concentration. The error bars were obtained from multiple measurements on different array areas. (c) SERS spectra of thiram in mango juice at different concentrations (from top to bottom: 500, 200, 100, 10, and 1 nM) acquired in-situ from Ag-AuNW array. (d) Quantitative curves of thiram sensing in mango juice on Ag-AuNW array. The Raman intensity of thiram peak at 1385 cm⁻¹ was plotted as a function of thiram concentration. The error bars were obtained from multiple measurements on different array areas.

Melamine sensing was carried out following a process similar to the detection of thiram. Melamine is a ring compound containing a triazine ring with three amino

functional groups. Normal Raman spectrum of melamine and SERS spectrum of melamine are presented in **Figure 3.9b**. The dominant Raman peak of melamine, which is assigned to in-plane ring breathing mode,¹³⁰ shifted from 676 cm^{-1} in the normal Raman spectrum to 682 cm^{-1} in SERS spectrum, while other peaks remain relatively unchanged. This finding is in accordance with that reported by Kim et al., whose study suggests that melamine interacts with metal surfaces through the amino groups.¹²⁹ The SERS spectra of melamine with concentrations increasing from 1 nM to 10 μM were collected and shown in **Figure 3.12a**, with the chemical structure of melamine shown in the inset. The results show that the SERS signal of melamine can be easily visualized even at a concentration of 1 nM, revealing a limit of detection of 1 nM for melamine, which is much lower than maximum acceptable limit of 20 μM (2.5 ppm) as set by the U.S. FDA.¹⁰⁸ **Figure 3.12b** shows quantitative relationship between the peak intensity at 685 cm^{-1} and the concentration of melamine. Peak intensity is observed to correlate positively with the concentration of melamine: as the concentration of melamine increases from 10 nM to 10 μM , the intensity of peak at 685 cm^{-1} increases to a maximum of 8000. To further illustrate the practical usage of our SERS substrate, we carried out the detection of melamine spiked in real milk samples. To eliminate interference of proteins and other milk components, milk spiked with melamine was pretreated by centrifugation to remove large molecular weight components, such as proteins and fats. **Figure 3.12c** shows the concentration dependent Raman spectra of melamine spiked in milk, with concentrations of melamine ranging from 10 nM to 10 μM . Characteristic peak of melamine at 685 cm^{-1} can be clearly observed in all concentrations. No interference from proteins or other milk components is observed, indicating successful pretreatment of milk sample by centrifugation. Quantitative relationship between peak intensity and melamine

concentration is plotted and presented in **Figure 3.12d**. Raman peak at 685 cm^{-1} with melamine concentration as low as 10 nM can be observed in the SERS spectra, indicating a detection limit of 10 nM for melamine in milk samples.

Figure 3. 12 (a) SERS spectra of melamine in water at different concentrations (from top to bottom : $10\text{ }\mu\text{M}$, $5\text{ }\mu\text{M}$, $1\text{ }\mu\text{M}$, 100 nM , 50 nM , 10 nM , 1 nM) acquired in-situ from Ag-AuNW array. (b) Quantitative curves of melamine sensing in water on Ag-AuNW array. The Raman intensity of melamine peak at 685 cm^{-1} was plotted as a function of melamine concentration. The error bars were obtained from multiple measurements on different array areas. (c) SERS spectra of melamine in milk at different concentrations (from top to bottom: $10\text{ }\mu\text{M}$, $5\text{ }\mu\text{M}$, $2\text{ }\mu\text{M}$, $1\text{ }\mu\text{M}$, 100 nM , 50 nM , 10 nM) acquired in-situ from Ag-AuNW array. (d) Quantitative curves of melamine sensing in milk on Ag-AuNW array. The Raman intensity of melamine peak

at 685 cm^{-1} was plotted as a function of melamine concentration. The error bars were obtained from multiple measurements on different array areas.

3.4. Conclusion

The work presented here describes fabrication of silver-gold nanowire arrays by virtue of interfacial self-assembly of silver nanowires, followed by galvanic replacement reactions. Nanospikes formed on nanowire surfaces during the galvanic reaction lead to generation of a high density of hotspots across entire nanowire arrays. We further employed this two-dimensional silver-gold nanowire arrays as surface enhancement Raman scattering (SERS) substrates for in situ sensing of two types of food contaminants: adulteration agent (melamine) and pesticide (thiram). We achieved limits of detection (LOD) of 10 nM and 1 nM for melamine in milk sample and thiram in juice sample, respectively. Both results are lower than maximum acceptable concentrations imposed by government agencies.

Chapter 4 SERS by graphene-nanosheet-gapped plasmonic nanoparticle arrays for multiplex DNA detection

4.1. Introduction

Surface enhanced Raman scattering (SERS), capable of detecting vibrational fingerprints of single molecules,^{14, 15} has stimulated widespread interest from diverse backgrounds in which ultrasensitive measurements are of considerable value, ranging from medical diagnostics¹³¹⁻¹³³ to food safety^{94, 134} and environmental regulation.^{135, 136} Raman signals of molecules in close proximity to plasmonic nanostructures are greatly amplified because of confined electromagnetic field arising from the excitation of localized surface plasmon resonance (LSPR).^{137, 138} In particular, tight junctions between strongly coupled plasmonic nanoparticles exhibiting locally enhanced field are highly efficient SERS hotspots. Both top-down lithographic and bottom-up self-assembly methods are being actively explored for fabricating hotspot-rich SERS substrates that can produce sensitive, uniform and consistent signals across entire substrates.^{60, 83, 138-140} Ordered arrays of plasmonic nanoparticles can be readily obtained using lithographic patterning techniques. However, it remains challenging to produce large areas of nanoparticle arrays containing exceedingly small nanogaps below 5 nm, which are most efficient in SERS signal amplification.^{141, 142} In this context, self-assembly of structure-tailored nanoparticles that allows for scalable production of closely packed nanoparticle ensembles has attracted increasing attention.^{87-89, 91, 139, 143, 144} Notably, although considerable successes have been achieved in developing SERS substrates with abundant hotspots,⁵⁶ most SERS substrates reported previously lack a mechanism to selectively position analytes of interest in the SERS hotspots for maximum detection sensitivity.

Here we report a self-assembly approach toward a new class of trilayer SERS substrates with single layer graphene nanosheets sandwiched between two-dimensional (2D) arrays of closely packed plasmonic nanoparticles. Single-atom-thick graphene nanosheets exhibit intriguing optical, electronic, and structural properties.^{145, 146} Hybrid materials of structurally integrated graphene derivatives and metal nanoparticles are under intense research for collective optical^{147, 148} and catalytic¹⁴⁹ properties. In our design, graphene oxide (GO) nanosheets play multifunctional roles as a 2D scaffold to anchor plasmonic nanoparticles through metal-sulfur bond, a nanometer-thick spacer to create SERS-active nanogaps between two layers of nanoparticle arrays, and a molecule harvester for targeted analytes by virtue of π - π interaction. As illustrated in **Figure 4.1**, the trilayer construct is built up through alternative loading of interfacially assembled array of Au@Ag core-shell nanoparticles and thiolated graphene oxide (tGO) nanosheets, followed by coating the nanoparticles with poly(ethylene glycol) (PEG) to minimize adsorption of targeted analytes on their surfaces. The nanogap created between the two layers of nanoparticle arrays and molecule harvesting property of the tGO nanospacer, in conjugation with the stealth PEG coating on plasmonic nanoparticles, collectively leads to precise positioning of selective analytes such as aromatic molecules¹⁵⁰ and single-stranded DNA (ssDNA)¹⁵¹ at the nanogap hotspots for ultrasensitive SERS detection.

Self-assembly of nanoparticles at oil-water interfaces, thermodynamically driven by favorable interfacial energy, has emerged as a promising approach to fabricating large areas of closely attached functional nanocrystals of diverse chemical composition and structures, with interparticle distances tailorable by surface coatings and solvent systems.^{78, 81, 82, 152} Recently, the resulting 2D arrays of functional nanocrystals at oil-water interfaces have found potential uses in SERS,^{87, 89}

electrochemical biosensors,¹⁵³ and chemical catalysis.⁵⁹ Nevertheless, in contrast to our trilayer substrate with a built-in target enrichment mechanism imparted by the tGO nanospacer, existing SERS substrates based on a single layer of interfacially assembled plasmonic nanostructures, either freestanding at oil-water interfaces^{53, 54} or anchored on solid substrates,⁹ rely primarily on random adsorption of molecules on the nanostructures rather than preferential accumulation in the hotspot region.

Figure 4. 1 Schematic illustration of the preparation of trilayer Au@Ag-tGO-Au@Ag SERS substrate based on alternating loading of interfacially assembled Au@Ag nanoparticles and thiolated graphene oxide, followed by PEGylation of Au@Ag nanoparticles.

Ultrasensitive, multiplex detection of DNA sequences is of considerable interest for both fundamental molecular biology research and practical uses in a wide range of fields such as diagnostics, food safety, and forensics. Major efforts have been made to develop DNA SERS sensors based on DNA-functionalized plasmonic nanostructures.^{9, 53, 54} Previous research has demonstrated that exposed nucleobases of flexible ssDNA endow them with strong affinity to GO nanosheets due to π - π interaction.^{154, 155} This affinity allows us to develop a “signal-off” DNA sensor using our trilayer SERS substrate. Two unique oligonucleotide strands containing part of the complementary sequence of target DNA are linked to magnetic beads (MB) and a Raman dye and serve as capture and reporter DNA, respectively. In a sandwich assay (**Figure 4.2**), MB-bound capture DNA first hybridizes with the target DNA to separate and concentrate it from the sample under test, and the overhanging region of the target DNA is then recognized by the Raman-encoded reporter DNA. In absence of the targeted DNA, the reporter DNAs are not removed and all bind to GO nanospacer, leading to the strongest SERS signal. However, when the targeted DNA is present to hybridize with the capture and reporter DNA, Raman tags on the reporter DNA are not able to locate inside the nanogap hotspots after hybridization, which turns off the SERS signal. In addition to the extraordinary signal amplification of SERS spectroscopy, the structure-defined spectral fingerprints of individual Raman tags and narrow bandwidth of Raman peaks, along with single-wavelength excitation of multiple tags, collectively provide unique opportunities for multiplexed detection. Here, using the DNA sequences of three food-borne pathogens as model targets, we have shown that our tGO-gapped plasmonic substrates enable highly efficient multiplexed DNA detection down to a concentration of 10 pM.

Figure 4. 2 Schematic illustration of the SERS detection of DNA based on the trilayer GO-gapped plasmonic arrays of Au@Ag core-shell nanoparticles.

4.2. Experimental section

Materials. Graphite, hydrogen tetrachloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), silver nitrate, trisodium citrate, hexane, methanol, nitric acid, hydrochloric acid, sulphuric acid, hydrogen peroxide, (3-mercaptopropyl) trimethoxysilane (MPTMS), methoxypolyethylene glycol amine (PEG- NH_2 , MW= 750 Da) and L-cysteine were purchased from Sigma-Aldrich and used without purification. Rhodamine B was acquired from Sigma-Aldrich at >90% purity. ssDNAs were purchased from Shanghai Sangon Biotechnology Incorporation (Shanghai, China). Water used in this experiment was deionized water (DI water). Glassware used for all experiments were cleaned using aqua regia (HCl/HNO_3 3:1 (v/v)) and rinsed with copious amounts of DI water prior to use.

Functionalization of Graphene Oxide (GO). Graphene oxide (GO) was synthesized using modified Hummers method^{53, 54} by oxidation of graphite powder.

Synthesized GO nanosheets were purified via multiple centrifugations and then re-dispersed in water to form a well-dispersed suspension. Further functionalization of GO nanosheets with L-cysteine was carried out following our previously reported method.⁴⁴

Au@Ag nanoparticle preparation. Au@Ag nanoparticles with a diameter of 30 nm were synthesized by a seed-mediated method using 15 nm Au nanoparticles as seed. 15 nm Au nanoparticles were prepared by direct reduction of HAuCl₄ using sodium citrate as both reducing agent and capping agent. In a typical synthesis, HAuCl₄ (60 mg) was dissolved in DI water (400 mL) and heated to boiling. Upon boiling, a solution of trisodium citrate tetrahydrate (200 mg, 4.5 mL) was quickly injected. Heating was maintained for another 30 minutes, and the resultant wine-red solution was cooled to room temperature. To synthesize 30 nm core-shell nanoparticles, 2 ml of as-prepared 15 nm Au seed nanoparticles was first diluted with 30 ml of DI water, L-ascorbic acid (10 mg/mL, 10 mL) was added, followed by slow addition of silver nitrate (0.2 mg/mL, 5 ml).

Fabrication of Au@Ag-tGO-Au@Ag sandwich structured substrate. Glass substrates were first cleaned by sonication in ethanol and water for 10 minutes each. To make the surface hydrophilic, cleaned glass substrates were treated with piranha solution (3:1 v/v H₂SO₄ (98%)/H₂O₂ (30%)) at 80 °C for 30 minutes. After rinsing with copious amounts of water and ethanol, the glass substrates were dried thoroughly and then immersed in 5 mM MPTMS solution for 30 minutes for silanization. Silane modified glass substrates were then rinsed thoroughly with chloroform and water to remove excess MPTMS. To prepare self-assembled Au@Ag NP film, hexane (0.5 mL) was added into the aqueous suspension of Au@Ag NPs (1 mL), followed by slow addition of methanol (0.1 mL). Au@Ag nanoparticles spontaneously transferred to the

water/hexane interface and self-assembled into a single layer film. This film formation process could be accelerated by shaking. Self-assembled Au@Ag nanoparticle film was then transferred onto silane-modified glass substrates and dried in ambient air. Subsequent immersion in tGO dispersion for 4 hours successfully yielded a tGO-coated substrate. Lastly, a second layer of Au@Ag nanoparticle film was prepared and transferred onto the glass substrate, resulting in a substrate with tGO nanosheets sandwiched between two monolayers of Au@Ag nanoparticle assemblies. PEGylation was performed by soaking the substrates in PEG-NH₂ solution for 4 hours.

Synthesis of magnetic beads and DNA conjugation. Polydopamine (PDA) coated magnetic beads (MB@PDA) were prepared by in situ growth of polydopamine on magnetic beads in pH 8.5 tris buffer through self-polymerization of dopamine. Thiol-functionalized capture DNA was attached on MB@PDA colloid via Michael addition reaction at salt concentration of 80 mM for 8 h, followed by PEGylation with PEG-NH₂ (MW= 2000 Da). MB-DNA conjugates were purified by an external magnetic field and then mixed with test solutions containing target DNA. After an incubation of 4 h at room temperature, solid was separated by magnetic field and redispersed in buffer (10 mM phosphate, pH=7.4) solution containing 80 mM NaCl. MBs carrying target DNA were then subjected to Raman-dye-tagged reporter DNA and incubated for another 4 h. The solid was again separated by magnetic field and the solution containing free reporter DNA that was left after hybridization was further introduced to Au@Ag-tGO-Au@Ag substrate and incubated for 2 h before measurement.

Characterization and measurements. UV-visible absorption spectra were recorded using a Shimadzu UV2501 spectrophotometer. SEM images were obtained using a field-emission scanning electron microscope (JSM-6700F). Raman spectra were acquired using a Renishaw Raman microscope. The 514 nm excitation line from an

air-cooled Ar(+) laser was used for both normal Raman and SERS measurements. The laser beam has a spot size of 1 μm using a 50 $\times$ objective. A single scan with an integration time of 10 s was performed for each spectra acquisition. X-ray photoelectron spectroscopy (XPS) measurement was carried out on a Kratos-Axis spectrometer with monochromatic Al K α (1486.7 eV) X-ray radiation (15 kV and 10 mA) and hemispherical electron energy analyzer.

4.3. Results and discussion

GO nanosheets prepared by the modified Hummers method carry a high density of oxygen-containing groups, primarily hydroxyl and epoxy groups in the basal plane and carboxylic acid groups at the edges, which have low affinity to metal surfaces. To function as a 2D anchoring scaffold for stable loading of metal nanostructures, new functionalities with stronger binding with metal surfaces are necessitated. Here, we have introduced thiol groups, which can form metal-sulfur bonds, by taking advantage of the ring-opening reaction of epoxy groups on the basal plane of GO and the primary amine group of cysteine.^{156, 157} X-ray photoelectron spectroscopy (XPS) measurements (**Figure 4.3a**) show that a new peak (167.0 eV) associated with S 2p level binding energy appears in the modified tGO, confirming the successful functionalization. In the deconvolved C 1s peaks of GO and tGO (**Figure 4.3b**), the weaker peak (284.6 eV) of sp²-bonded carbon in GO becomes the dominant peak in tGO, in comparison with the epoxy/hydroxyl (286.9 eV) and carbonyl (288.5 eV) peaks, suggesting that cysteine in large excess also leads to partial reduction of GO. Recent reports have shown that reductive thiol-containing molecules such as glutathione can effectively reduce GO.¹⁵⁸ In addition, Raman spectra (**Figure 4.3c**) display an increased intensity ratio of D and G band after the reaction, which is consistent with the partial restoration

of the sp^2 carbon network in tGO. It should be mentioned that tGO maintains excellent colloidal stability in aqueous media and exists as individual nanosheets, which is essential for its use as a nanospacer in the trilayer SERS substrate. Atomic force microscopy (AFM) measurements (**Figure 4.3d**) reveal that the nanosheets have a representative thickness of about 1.2 nm, which is consistent with the thickness of a single layer of chemically derived GO.

Figure 4. 3 (a) XPS spectra of graphene oxide (GO) and cysteine functionalized graphene oxide (tGO). (b) The deconvoluted C 1s spectra of GO and tGO. (c) Raman spectra of GO and tGO. (d) AFM image and height profile of tGO.

Figure 4. 4 (a) UV–Vis spectra of Au nanoparticle dispersion (black line), Au@Ag nanoparticle dispersion (red line), 2D assembly of Au@Ag nanoparticles (blue line), and the Au@Ag-tGO-Au@Ag trilayer construct (purple line). The spectra have been offset vertically for better comparison of absorption maxima. The Insets are photographs of Au@Ag nanoparticle dispersion (left) and the interfacially assembled nanoparticles (right). (b-d) SEM images of 2D assembly of Au@Ag nanoparticles (b), bilayer Au@Ag-tGO structure (c) and Au@Ag-tGO-Au@Ag sandwich structure (d) deposited on glass substrates.

We synthesized core-shell Au@Ag nanoparticles of 30 nm through controlled seeded growth of Ag shell on citrate-stabilized Au nanoparticles of 15 nm in diameter. Addition of AgNO₃ solution into Au nanoparticle dispersion containing L-ascorbic acid quickly led to a color change from red to bright yellow and LSPR of the

nanoparticles shifted from 520 nm to 400 nm (**Figure 4.4a**). Transmission electron microscopy (TEM) observation (**Figure 4.5**) reveals that a 7 nm Ag layer with lower contrast formed on the Au seeds, leading to core-shell Au@Ag nanoparticles of ~ 30 nm in size. Previous reports have shown that Ag shell dominates the optical properties of Au@Ag nanoparticles¹⁵⁹ and Au@Ag nanoparticles exhibit Raman enhancement efficiencies comparable to those of pure Ag nanoparticles.^{128, 160, 161}

Figure 4. 5 TEM images of (a) Au NPs and (b) Au@Ag NPs.

The tendency of nanoparticles to accumulate at oil-water interfaces is closely related to the size of the nanoparticles, interfacial energy of the biphasic system, and contact angle of the nanoparticles at the interface, where a contact angle of 90° provides the largest free energy change.⁸¹ It has been reported that adding alcohol into biphasic mixture of aqueous dispersion of charged particles and organic solvent reduces charge density on the particles and consequently tune their contact angle at the oil-water interface close to 90°, giving rise to 2D-assemblies of the particles at the interface.^{78, 82} Here, methanol was added into the mixture of aqueous dispersion of Au@Ag nanoparticles and hexane to trigger their interfacial assembly. As shown in the inset of **Figure 4.4a**, Au@Ag nanoparticles moved to the interface and formed a

layer exhibiting metallic reflectance upon the addition of methanol followed by vigorous shaking to facilitate the migration of the nanoparticles. The LSPR peak of Au@Ag nanoparticles experienced a drastic red-shift from 400 nm to 700 nm, indicative of a strong interparticle plasmonic coupling. The interfacial assembly was transferred to a glass substrate, which was pretreated with (3-mercaptopropyl)-trimethoxy-silane. The pretreatment was to introduce thiol groups on the substrate for stable anchoring of the nanoparticles. Without it, the loaded nanoparticles were quickly washed off once in contact with aqueous medium. A monolayer of closely packed nanoparticles was observed in SEM image (**Figure 4.4b**). The coated substrate was further immersed in the tGO aqueous dispersion to introduce tGO nanospacer on top of the nanoparticle monolayer (**Figure 4.4c**). The presence of a homogeneous layer of tGO was confirmed by the consistent Raman signal of chemically derived GO across the entire substrate of a few centimeters in lateral size (**Figure 4.6**). In contrast, unmodified GO without thiol groups cannot form stable loading on the nanoparticle array, evidenced by the absence of Raman signal from GO. Afterwards, the second layer of Au@Ag nanoparticles was added by dip-coating to form the sandwiched Au@Ag-tGO-Au@Ag trilayer construct, as shown in **Figure 4.4d**. The binding of thiol functional groups on the tGO basal plane with nanoparticle surfaces aligns the 2D anchoring scaffold parallel to the substrate, which in turn leads to the formation of nanogaps between the bottom and top nanoparticle monolayers. The close attachment of two layers of Au@Ag nanoparticles gives rise to further red shift and broadening of the LSPR peak. PEG chains (molecular weight = 750 Da) with one primary amine end group were coated onto the Au@Ag nanoparticles by ligand exchange. Notably, sandwiching tGO between the two layers of Au@Ag nanoparticle arrays to form the nanogap gave rise to an increase of tGO Raman signal by a factor of 8 (**Figure 4.6**).

SEM observations confirm that the PEGylated nanoparticle arrays did not undergo structural changes and remained stable for at least 3 months (**Figure 4.7a**). In contrast, without PEGylation, the nanoparticles apparently fuse together to form large clusters within 48 h after preparation (**Figure 4.7b**).

Figure 4.6 SERS spectra collected on Au@Ag NP monolayer (red line, bottom), Au@Ag-tGO bilayer structure (black line, middle) and Au@Ag-tGO-Au@Ag sandwich type substrate (blue line, top).

Figure 4.7 SEM images of (a) Au@Ag-tGO-Au@Ag sandwich structure with PEGylation and (b) Au@Ag-tGO-Au@Ag sandwich structure without PEGylation.

Figure 4.8 SERS spectra of RhB on colloidal Au@Ag nanoparticles with (red line, bottom) and without (black line, top) PEG brushes.

Figure 4.9 (a-b) SERS spectra of RhB (100 nM) on (a) PEGylated and (b) non-PEGylated substrates: Au@Ag NP monolayer (blue line, bottom), Au@Ag-tGO

bilayer structure (red line, middle), and Au@Ag-tGO-Au@Ag sandwich type substrate (black line, top). (c) SERS intensity of RhB on Au@Ag-tGO-Au@Ag with (black) and without (red) PEGylation as a function of RhB concentration. The numbers represent the factor of SERS intensity increase after PEGylation. (d) SERS spectra of RhB on PEGylated Au@Ag-tGO-Au@Ag substrate at different concentrations, from bottom to top: 0.05, 0.5, 1, 5, 10, 50, and 100 nM RhB.

We next evaluated the SERS activity of the PEGylated Au@Ag-tGO-Au@Ag substrate using Rhodamine B (RhB) as a model Raman probe. Both the tGO-gapped architecture and PEGylation coating appear to make major contributions to the superior SERS activity of the substrate. RhB is a commonly used Raman dye molecule that adsorbs on citrate-stabilized Au@Ag nanoparticles primarily through electrostatic interaction. Our results demonstrate that PEG coating grafted on colloidal Au@Ag nanoparticles can effectively suppress the non-specific adsorption of RhB molecules on the SERS-active sites of their surfaces (**Figure 4.8**). We reason that a dense layer of PEG chains either forms a physical barrier to block the access of RhB molecules to the nanoparticle surfaces or occupies the SERS-active sites.

When the Au@Ag nanoparticle surfaces are PEGylated, the trilayer Au@Ag-tGO-Au@Ag structure exhibits a dramatic signal enhancement by a factor of 20 relative to that of the bilayer Au@Ag-tGO substrates for RhB concentration of 100 nM, as shown in **Figure 4.9a**. Graphene derivatives with a sp^2 hybridized carbon network have been widely employed for loading aromatic molecules through $\pi-\pi$ interaction. In a PEGylated trilayer substrate, this molecule harvesting property of tGO gives rise to preferential enrichment of RhB molecules in the SERS hotspot between the two layers of Au@Ag NP arrays, thereby resulting in considerably improved

SERS activity with respect to that of the PEGylated Au@Ag-tGO bilayer substrate, in which tGO nanosheets are placed on top of Au@Ag NPs instead of in the nanogap SERS hotspots. We have found that PEGylation of the nanoparticle surfaces in the trilayer construct gives rise to a marked increase in SERS signal (black lines in **Figure 4.9a, b**) and the degree of increase becomes more prominent at low concentrations of analytes (**Figure 4.9c**). In contrast, PEGylation of the nanoparticles in the bilayer Au@Ag-tGO and monolayer Au@Ag substrates both led to obvious signal decrease. To avoid interference caused by nanoparticle fusion, freshly prepared non-PEGylated substrates were used in the SERS measurements for comparison. The closely packed Au@Ag NP array contains highly SERS-active interstitial spaces and less active superficial surfaces. As discussed above, PEGylation of Au@Ag NPs deactivates the nanoparticle surfaces for capturing RhB probes, and consequently leads to reduced SERS activity for PEGylated monolayer and bilayer substrates (**Figure 4.9a and b**). For the trilayer substrate, however, the deactivation of Au@Ag nanoparticle surfaces by PEGylation minimizes the binding of RhB molecules to the less SERS-active superficial surfaces of nanoparticles, which would otherwise occur in a non-PEGylated substrate, giving rise to selective accumulation of RhB molecules on the tGO nanospacer where the SERS hotspots are located. The enhancement induced by PEGylation, defined as the factor of SERS intensity increase after PEGylation, became more pronounced at low RhB concentration and increased from 2.6 at 100 nM to 7.9 at 5 nM (**Figure 4.9c**), demonstrating the importance of selectively positioning molecules in the SERS hotspots for ultrasensitive detection. The trilayer PEGylated Au@Ag-tGO-Au@Ag substrate gives rise to sensitive detection of RhB (**Figure 4.9d**) and a SERS enhancement factor of 2.7×10^8 (see Supporting Information). Although recent studies have demonstrated that graphene has SERS activity, leading to an

enhancement factor of 2 to 17 through a chemical effect, the contribution of electromagnetic enhancement plays a dominant role in the PEGylated Au@Ag-tGO-Au@Ag substrate, considering the enhancement factor it exhibits.

The PEGylated trilayer substrate was further utilized for the detection of gene sequences of bacterial pathogens such as *E. coli* O157:H7, *Staphylococcus aureus*, and *Listeria monocytogenes*, which are food-borne pathogens of significant public health risks. Sequence information of the DNA used in this work is summarized in **Table 1**.

Table 1 DNA sequences used in this work

Oligonucleotide	Sequence
Cy5 labeled <i>Staphylococcus aureus</i>	<p>Target DNA: 5-TGT TAC GAT TGT GTG AAT ACT CGT CTA ATG TCG TCC TTT G -3</p> <p>Probe DNA: 5- GTA TTC ACA CAA TCG TAA CA-SH-3</p> <p>Reporter DNA: 5-Cy5-CAA AGG ACG ACA TTA GAC GA-3</p>
TAMRA labeled <i>Listeria Monocytogenes</i>	<p>Target DNA: 5- CGC GTG TTT CTT TTC GAT TAG GAC TTG CAG GCG GAG -3</p> <p>Probe DNA: 5-ATC GAA AAG AAA CAC GCG-SH-3</p> <p>Reporter DNA: 5-TAMRA-CTC CGC CTG CAA GTC CTA-3</p>
HEX labeled <i>E.Coli</i> O157:H7	<p>Target DNA: 5-AGC GTC CGG GAA TTC ACC CGC AAT GGC TTC CAG CAC ATCC-3</p> <p>Single base mismatch DNA: 5-AGC GTC CCG GAA TTC ACC CGC AAT GGC TTC CAG CAC ATCC-3</p> <p>Probe DNA: 5-CGG GTG AAT TCC</p>

	CGG ACG CT-SH-3
	Reporter DNA: 5-HEX-GGA TGT GCT GGA AGC CAT TG-3

Ultrasensitive detection of DNA sequences relies on the selection enrichment of reporter DNA in the nanogap hotspots. We have found that fluorescence quenching of TAMRA tags on ssDNA by Au@Ag nanoparticles decreased from 84% to 9% (**Figure 4.10**) after PEGylation, confirming that PEG grafts can effectively prevent adsorption of ssDNA onto nanoparticle surfaces.⁵⁰ We further examined SERS detection of HEX-labeled reporter DNA using PEGylated Au@Ag-tGO-Au@Ag substrate. **Figure 4.11a** shows that SERS signal of HEX dye at 1626 cm⁻¹ gradually increased in the DNA concentration range of 2-100 nM and eventually leveled off. A typical sandwich assay involves three steps: First, target DNA of E. coli O157:H7 in 1 mL of sample solution is recognized by the MB-bound capture DNA and magnetically enriched; second, a predetermined amount (20 μ L, 20 nM) of HEX-tagged reporter DNA is then introduced to hybridize with overhanging region of the concentrated target DNA; At last, the sandwich structure formed on MB is magnetically separated and the remaining free reporter DNA is subjected to SERS measurement. **Figure 4.11b** shows that the target DNA with an original concentration of 1.0 nM caused 80% signal drop for reporter DNA of 20 nM. In contrast, scramble non-matching DNA gave rise to minimal signal changes of less than 10%, which could result from non-specific removal of the reporter DNA. This setup allowed for sensitive “signal-off” detection of DNA sequences down to 10 pM (**Figure 4.11c**). Furthermore, the detection system also showed excellent specificity. As demonstrated in **Figure 4.11d**, while the increase of target DNA concentration from 10 pM to 1.6 nM led to continuous signal drop, the

SERS signal remained consistently unchanged in presence of non-complementary DNA of the same concentration.

Figure 4. 10 (a) Fluorescence quenching of TAMRA labeled single-stranded probe DNA (10 nM) (black line, top) in the presence of citrate stabilized Au@Ag nanoparticles (red line, bottom) and PEGylated Au@Ag nanoparticles (blue line, middle). (b) Fluorescence quenching of TAMRA labeled double-stranded DNA (5 nM) (black line) in the presence of citrate stabilized Au@Ag nanoparticles (red line) and PEGylated Au@Ag nanoparticles (blue line).

Figure 4. 11 (a) The dependence of SERS signal intensity at 1626 cm^{-1} on the concentration of HEX-labeled reporter DNA on PEGylated Au@Ag-fGO-Au@Ag trilayer substrate. (b) SERS spectra of HEX-labeled reporter DNA in the absence and presence of target DNA, and in the presence of a non-complementary sequence. (Blue trace (top): HEX labeled reporter DNA (20 nM), red trace (middle): HEX-labeled reporter DNA incubated in presence of non-complementary DNA sequence, and black trace (bottom): HEX labeled reporter DNA for the detection of 1.0 nM complementary target DNA. (c) SERS spectra of HEX-labeled reporter DNA (20 nM) for the detection of target DNA of different concentrations, from top to bottom: 0, 0.01, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.6 nM. (d) Comparison of SERS intensity of HEX-labeled reporter DNA at 1626 cm^{-1} for the detection of target DNA (black) and non-complementary DNA (red) of various concentrations.

Figure 4. 12 (a) SERS spectra of Cy5 labeled reporter DNA (50 nM) for the detection of target DNA of different concentrations (from top to bottom: 0, 0.02, 0.1, 0.2, 1.0, and 2.0 nM). Black line is the background spectra of 50 nM reporter DNA in absence of target DNA. (b) SERS peak intensity of Cy5 labeled reporter DNA (50 nM) at 1592 cm^{-1} for the detection of target DNA of varying concentrations.

Figure 4.13 (a) SERS spectra of TAMRA labeled reporter DNA (20 nM) for the detection of target DNA of different concentrations (from top to bottom: 0, 0.01, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 nM). Black line is the background spectra of 20 nM reporter DNA with the absence of target DNA. (b) SERS peak intensity of TAMRA labeled reporter DNA (20 nM) at 1645 cm⁻¹ for the detection of target DNA of varying concentrations.

Figure 4. 14 (a) SERS spectra of a mixture of reporter DNAs (20 nM for HEX, 15 nM for TAMRA, and 50 nM for Cy5). (b-d) SERS spectra collected for the detection of target DNAs (1.0 nM) of (b) *E. coli* O157:H7, (c) *Staphylococcus aureus*, and (d) *Listeria monocytogenes*. Triangle, star, and hexagon respectively indicate characteristic Raman peaks of TAMRA, HEX, and Cy5, respectively.

Simultaneous detection of multiple DNA sequences is of particular importance in rapid identification of pathogens. A large group of Raman dyes with distinct spectral profiles are readily available for tagging ssDNA, opening the possibility for multiplexed DNA detection. Here, HEX, Cy5, and TAMRA were used to encode reporter DNAs for the detection of gene sequences of *E. coli* O157:H7, *Staphylococcus aureus*, and *Listeria monocytogenes*, respectively. SERS spectra of Cy5 and TAMRA labeled reporter DNA are shown in **Figure 4.12** and **Figure 4.13**. Raman peaks at 1626, 1465/1594, and 1645 cm^{-1} were selected as the characteristic

peaks for HEX,¹⁶² Cy5,⁵³ and TAMRA¹⁶³ tags. Different concentrations of reporter DNAs (20 nM for HEX, 15 nM for TAMRA, and 50 nM for Cy5) were mixed to match their differences in Raman cross-section, leading to a spectrum containing combined Raman peaks of all three dyes (**Figure 4.14a**). In representative measurements, addition of selected target DNA of 1.0 nM into the mixture specifically turned off the corresponding Raman peaks (**Figure 4.14b-d**), demonstrating the multiplexed capability of our SERS sensor.

4.4. Conclusion

In summary, we have developed a modular approach to fabricating efficient SERS substrates based on the use of chemically derived GO and interfacial assembly of plasmonic nanoparticles as building blocks. The multifunctional role of tGO as a 2D scaffold, nanospacer and molecule harvester makes it possible to selectively position targeted analytes in the SERS hotspots. The modular nature of our strategy and recent advances in tailored wet-chemical synthesis of plasmonic nanocrystals collectively provide new opportunities for achieving fundamental insights on the architecture-SERS performance relationship. Furthermore, the ability of graphene derivatives to bind ssDNA, coupled with the powerful multiplexing of SERS spectroscopy, empower this SERS substrate for ultrasensitive multiplexed genetic detection of pathogens.

Chapter 5 SERS assay based on magnetoplasmonic nanostructures for multiplex detection of bacterial pathogens

5.1. Introduction

Bacterial pathogens are a leading cause of foodborne illnesses,¹⁶⁴ infectious diseases,¹⁶⁵ as well as bioterrorism outbreaks.¹⁶⁶ The exposure to bacterial pathogens has become a major risk for public health and environmental safety. The classical methods for detection of bacterial pathogens rely on prolonged culturing and isolation steps¹⁶⁷ as well as expensive polymerase chain reaction (PCR) technique.¹⁶⁸ There is an increasing demand for analytical tools allowing for sensitive and selective detection of multiple pathogens simultaneously and providing real-time readout with relatively simple instrumental setup for on-site sampling. Fluorescence sensors represent an important class of optical DNA sensors that is under intense development to address this critical challenge.^{151, 169, 170} However, fluorescent probes currently available, including organic fluorophores and semiconductor quantum dots, often suffer from fluorescence quenching and interference from background signals in complex milieu. The spectral overlapping between different fluorophores, resulted from the broad emission peaks, also limits their capacity for multiplexed detection. Pathogen DNA sensors based on gold nanoparticles have received increasing attention recently.¹³⁸ The activation of interparticle plasmonic coupling, as a result of DNA hybridization, is accompanied by prominent color changes of gold nanoparticle colloid, giving rise to colorimetric sensors with a wide range of DNA detectable by naked eyes. Despite the considerable progress achieved in the past decade, the colorimetric detection mechanism creates significant challenges to use this type of sensors for quantitative multiplexed detection due to the lack of signal differentiation between multiple targets,

and generating readout in heavily colored environment is the other formidable problem to overcome.

Here we report a new strategy of using surface enhanced Raman scattering (SERS) for quantitative, multiplexed detection of pathogen DNA. Plasmonic nanostructures with concentrated electromagnetic field at their surfaces are capable of amplifying the SERS signal of molecules in their close proximity.⁵⁴ Interparticle plasmonic coupling has been found to enhance the electromagnetic field in the interstices of nanoparticle aggregates, affording dramatically improved SERS effect.^{54, 136} This effect has been utilized to develop SERS sensors for DNA sequences by taking advantage of specific DNA hybridization.^{53, 171, 172} In the current study, we have developed a sandwiched SERS substrate based on the combinational use of chemically engineered two-dimensional (2D) arrays of Au@Ag@polydopamine core-shell nanoparticles and magnetoplasmonic nanoparticles that contain a magnetic core and a plasmonic shell, as depicted in **Figure 5.1**. In our design, 2D arrays of Au@Ag@polydopamine core-shell nanoparticles are chemically engineered into a layer of spike-like gold nanostructures for large enhancement of Raman signals. Magnetoplasmonic nanoparticles allows for magnetic enrichment of targeted DNA. Formation of a particle-on-array configuration with the 2D array through sequence-specific DNA hybridization leads to strong plasmonic coupling and enhancement of the Raman signals, which consequently afforded ultrasensitive detection of gene sequences of bacterial pathogens as low as 0.1 nM. Unlike fluorescence sensors based on organic dyes, the Raman spectra of multiple SERS probes can be captured simultaneously under the same laser excitation, which, in conjugation with the narrow SERS peaks, provide unique advantages for multiplexed detection. Our strategy built upon the well-established DNA hybridization based detection, the ability of magnetoplasmonic

nanoparticles to provide magnetic enrichment and plasmonic coupling, as well as the high multiplex capacity of SERS spectroscopy offers great flexibility to develop a library of pathogens DNA sensors for on-demand screening of complex environmental and biological samples. Our results have shown that SERS-encoded reporter DNA strands collectively give rise to selective and sensitive detection of *Staphylococcus aureus*, *Listeria Monocytogenes*, and *Bacillus anthracis*.

Figure 5. 1 Schematic illustration of the detection of target DNAs by the particle-on-array SERS substrate. (A) Preparation of Au nanospikes array and deposition of capture DNA onto the array. (B) Conjugation of reporter DNA with magnetoplasmonic nanoparticles and enrichment of target DNA through DNA hybridization between reporter DNA and target DNA. (C) Formation of particle-on-array sandwiched assay through DNA hybridization between capture DNA and target DNA.

5.2. Experimental section

Chemicals and materials. Hydrogen tetrachloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), silver nitrate, trisodium citrate, L-hexane, methanol, nitric acid, hydrochloride, sulphuric acid, hydrogen peroxide, (3-mercaptopropyl)trimethoxysilane (MPTMS), sodium dodecyl sulfate (SDS) and phosphate buffer were purchased from Sigma-Aldrich and used without purification. The Rhodamine B Raman dye was acquired from Sigma-Aldrich at >90% purity. DNA strands were purchased from Shanghai Sangon Biotechnology Incorporation (Shanghai, China). The DNA sequences are listed in Supporting information. Water used in this experiment was deionized water (DI water). Glassware used for all experiments was cleaned using aqua regia (HCl/HNO_3 3:1 (v/v)) and rinsed with copious amounts of DI water prior to use.

Gold nanospike array preparation and DNA conjugation. Polydopamine (PDA) coated magnetic beads (MB@PDA) were prepared by in situ growing polydopamine polymer on magnetic beads in pH 8.5 Tris buffer through self-polymerization. Gold shell coated magnetic beads were synthesized via the seed growth method by reducing gold from $\text{HAuCl}_4\text{-K}_2\text{CO}_3$ aqueous solution in the presence of $\text{NH}_2\text{OH}\cdot\text{HCl}$. Thiol-functionalized DNA probes (both reporter and capture DNA, 1 OD) were treated with TCEP in PBS buffer (pH=7.4) for two hours. Deprotected reporter DNA probes were added into MB@Au colloid and incubated for 12 hours. Phosphate buffer was added into the above mixture to reach a final concentration of 10 mM phosphate with pH equals to 7.4. Salt aging was carried out for two days to reach a final salt concentration of 80 mM. The mixture was separated by magnetic field and redispersed in buffer (10 mM phosphate, pH=7.4) solution containing 0.1% SDS and 60 mM NaCl.

Preparation of gold nanospike array and DNA conjugation. Synthesize of Au@Ag@PDA colloids and interfacial self-assembly of Au@Ag@PDAs was carried out following the same method reported in one of our earlier publications.⁵⁹ To fabricate gold nanoflake substrates, aqueous HAuCl₄ (1 mM) was added dropwise onto Au@Ag@PDA monolayer films with the presence of hydroxylamine hydrochloride (NH₂OH·HCl) (30 mM). Deprotected capture DNA probes were drop casted onto gold nanospike substrate and incubated for 12 hours at room temperature in a humid chamber.

Magnetic-plasmonic sandwich assay. For sandwich hybridization, varying amount of target DNA probes were added into MB@Au-reporter probe conjugates and incubated in hybridization buffer for four hours. Target DNA strands were separated from MB@Au conjugates via magnetic separation. MB@Au-reporter/target conjugates were washed with PBS buffer three times and dispersed in hybridization buffer. They were dropped onto gold nanospike substrate conjugated with capture DNA and incubated six hours. The substrate was rinsed with hybridization buffer two times and finally with PBS buffer.

Instruments. UV-visible absorption spectra were recorded using a Shimadzu UV2501 spectrophotometer. Scanning electron microscope images (SEM) were obtained using a field-emission scanning electron microscope (JSM-6700F). Raman spectra were acquired using a Renishaw Raman microscope. The 633 nm excitation line from an air-cooled Ar(+) laser was used for SERS measurements. Laser beam was focused on a spot with size of 1 μm using a 50× objective. A single scan with an integration time of 10 s was performed for each spectra acquisition. X-ray photoelectron spectroscopy (XPS) measurement was carried out on a Kratos-Axis spectrometer with

monochromatic Al K α (1486.7 eV) X-ray radiation (15 kV and 10 mA) and hemispherical electron energy analyzer.

5.3. Results and discussion

Synthesis of Au@Ag@polydopamine colloids and self-assembly of Au@Ag@polydopamine nanoparticles at water/oil interfaces had been reported in our previous work⁵⁹ in which the catalytic property of Au@Ag@polydopamine monolayer films was thoroughly investigated. Au@Ag@polydopamine nanoparticles are a class of gold-silver core-shell nanoparticles coated with a layer of polydopamine. The thickness of polydopamine coating is 4 nm on average. When Au@Ag@polydopamine nanoparticles were assembled into a monolayer, the average distance between neighboring nanoparticles was about 8 nm, as depicted in **Figure 5.2a**. Generation of a largely enhanced electromagnetic field requires the plasmonic nanoparticles to be either closely assembled for activation of strong plasmonic coupling between nanoparticles, or to be highly branched for excitation of plasmon at the tip region¹⁷³ Star shaped gold nanoparticles have been reported with the capability of generating $\sim 10^7$ enhanced Raman signals for analytes located at the tip region.³² To function as an efficient SERS substrate, the Au@Ag@polydopamine nanoparticle arrays need to be chemically modified. Previous literature reported synthesis of rough gold nanocrystals through galvanic replacement reactions between smooth silver nanoparticles and gold chloride.¹⁸⁻²⁰ In our experiment, we added HAuCl₄ solution onto Au@Ag@polydopamine monolayer to initiate galvanic replacement reactions between gold chloride and the Ag shell. Chemical growth of spike-like gold nanostructures happens simultaneously in the presence of reducing agent hydroxylamine hydrochloride (NH₂OH·HCl). The size and morphology of spike-like

gold nanostructures can be controllably tuned by varying the amount of gold chloride introduced into the system. Keeping the concentration of hydroxylamine hydrochloride (30 mM) constant, Au@Ag@polydopamine monolayer gradually evolved from an array of smooth Au@Ag@polydopamine nanoparticles to a dense layer of spike-like nanostructures as the volume of gold chloride was increased solution from 50 to 300 μL . As depicted in **Figure 5.2b**, at the initial stage when 50 μL of gold chloride solution was added, nanostructures with dimensions ranging from 5 to 10 nm began to appear and were randomly scattered on the Au@Ag@polydopamine monolayer. As we gradually increased the amount of gold chloride from 50 μL to 100 μL , nanostructures grew to 25 nm in diameter with the emergence of multiple tips (**Figure 5.2c**). When 200 μL of gold chloride was added, nanostructures appeared as nanostars and their size grew to 100 nm (**Figure 5.2d**). Eventually, a dense layer of spike-like nanocrystals formed on the original Au@Ag@polydopamine monolayer when 300 μL of gold chloride was added (**Figure 5.2e and f**). Continue increasing the amount of gold chloride (400-500 μL) did not lead to any further change in morphology. **Figure 5.2f** shows the SEM image of final nanospike substrates at a lower magnification. The substrate exhibits a high density of nanospikes on a large scale, affording a reproducible SERS enhancement capability across the whole substrate. To study chemical composition of the gold nanospike substrate, we performed EDX analysis on Au@Ag@polydopamine monolayer before and after chemical modification. The EDX pattern of Au@Ag@polydopamine monolayer (**Figure 5.3**) contains both silver and gold diffraction peaks, with an average atomic ratio of Ag/Au equals to 3.8 as calculated from three measurements. However, diffraction peak corresponding to Ag is absent on EDX spectra of gold nanospike substrate, suggesting that all Ag was oxidized by gold chloride and the final

substrate contains gold nanostructures with a high purity.²⁰ It is worth noting that the polydopamine layer plays an important role in nanospikes formation. If we remove the polydopamine layer by plasmon cleaning, the nanospikes will not be formed. Instead, we observe flower-like gold mesostructures with an average diameter of 1.5 μm formed on the substrate surface. We are not sure of the mechanism behind this phenomenon yet, and further study will be carried out.

Figure 5.2 SEM images of Au@Ag@PDA monolayer before (a) and after addition of (b) 50 μL (c) 100 μL (d) 200 μL and (e) 300 of HAuCl_4 solution (1 mM). (f) SEM images of gold nanoflake substrate at a smaller magnification.

Figure 5. 3 EDX of Au@Ag@polydopamine and Au flake substrate Au@Ag@polydopamine.

Figure 5.4 shows UV-vis absorption spectra of Au@Ag@polydopamine colloid, Au@Ag@polydopamine monolayer film, and gold nanospike substrate, respectively. Interfacial assembly of Au@Ag@polydopamine nanoparticles gave rise to a strong interparticle plasmonic coupling, which is evidenced by an 82 nm red-shift of the absorption peak from that (443 nm) of free Au@Ag@polydopamine nanoparticles in colloid to that (525 nm) of Au@Ag@polydopamine monolayer. Chemical modification of Au@Ag@polydopamine monolayer further red-shifted its absorption band to 622 nm, due to replacement of silver by gold as well as elongated tips and edges of the flake-like structures.¹⁷⁴ The extent of red-shift is dependent on the amount of gold chloride added. UV-vis absorption spectra of gold nanospike substrate at varying amount of gold chloride are collected and presented in **Figure 5.5a**. The absorption maximum red-shifts from 525 nm to 558 nm when 50 μ l of gold chloride was added, and further red-shift from 558 nm to 603 nm when the amount of gold chloride increased to 100 μ l. The absorption maximum was observed to be centered at 613 nm when 200 μ l gold chloride was added, and finally reached 632 nm when the

amount of gold chloride increased to 300 μ l and more. To maximize signal output, we chose 633 nm as the Raman laser wavelength for this system because the best SERS excitation is obtained when the laser excitation wavelength is near to the plasmon absorption wavelength.²⁵ Formation of nanospikes with sharp edges are important for large electromagnetic field generation, due to a lightening effect that is also observed in rod shaped nanoparticles and nanocrystals with a high curvature.^{35, 175-177} We measured the SERS spectra of crystal violet (CV) molecules on gold arrays using a 633 nm incident laser. From **Figure 5.5b**, the SERS spectra cannot be observed on the Au@Ag@polydopamine nanoparticle array, which is as expected as the interparticle distance between Au@Ag@polydopamine nanoparticles is around 8 nm and the absorption maximum of Au@Ag@polydopamine nanoparticle array is centered around 525 nm. The SERS spectra however, can be easily identified on gold arrays, and the signal intensity increases with increasing amount of gold chloride, which is highly consistent with the morphologies of gold arrays.

Figure 5.4 UV-vis absorption spectra of Au@Ag@polydopamine colloid (black trace), Au@Ag@polydopamine monolayer (red trace) and gold nanospike substrate (blue trace).

Figure 5.5 (a) UV-vis absorption spectra of gold nanopike substrates at varying amount of gold chloride. (from left to right: red trace: without gold chloride; green trace: 50 μ l gold chloride; purple trace: 100 μ l gold chloride; black trace: 200 μ l gold chloride; blue trace: 300 μ l gold chloride). (b) SERS spectra of CV (200 nM) on gold nanopike substrates at varying amount of gold chloride. (from bottom to top: black trace: without gold chloride; red trace: 50 μ l gold chloride; blue trace: 100 μ l gold chloride; purple trace: 200 μ l gold chloride; green trace: 300 μ l gold chloride).

A core-shell structured magnetoplasmonic nanoparticle has a magnetic core that consists of numerous Fe_3O_4 nanoparticles and a gold shell surrounding the magnetic core. **Figure 5.6** shows how the morphology and structure of magnetoplasmonic nanoparticles evolve at different growth processes of gold shell. The core part of the magnetoplasmonic nanoparticles is a magnetic nanoparticle-embedded sulfonated polystyrene bead, with a diameter of approximately 100 nm (**Figure 5.6 a**). A 10 nm-thick polydopamine (PDA) layer then grew onto magnetic beads in pH 8.5 Tris buffer through self-polymerization, forming PDA coated magnetic beads (MB@PDA) (**Figure 5.6 b**). This PDA layer functionalizes as stabilizer for magnetic beads as well as growth site for subsequent seed development. **Figure 5.6 c** shows TEM image of MB@PDA loaded with gold nanoseeds. The gold nanoseeds are 14 nm diameter gold

nanoparticles that are densely distributed on the surface of MB@PDA. In the presence of gold precursor and reducing agent hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$), gold seeds grew bigger and eventually coalesced to form a continuous metallic shell (**Figure 5.6 d**). The thickness of gold shell amounts to 10 nm, contributing to an increase in the diameter of magnetoplasmonic nanoparticles from 100 nm to 120 nm.

Figure 5.6 TEM images of (a) magnetic beads (MBs), (b) polydopamine coated magnetic beads (MB@PDA), (c) PDA coated magnetic beads with gold nanoseeds (MB@Au seed), and (d) magnetic beads with a gold shell (MB@Au shell).

The change in the morphology of the nanoparticles is reflected in the change of their optical properties. The magnetic bead (MB) colloid exhibited a brown color, which turned into a red color after loading gold nanoseeds on MB surfaces. This red color is a characteristic color of spherical gold nanoparticles. The color of the colloid further changed to dark purple, blue, and finally greenish blue during growth of gold shell. Quantitative analysis of optical properties of magnetoplasmonic nanoparticles was carried out by studying their plasmonic absorption spectra, which are shown in **Figure 5.7**. The spectra feature of magnetic beads loaded with gold nanoseeds is a combination of absorption spectra of magnetic beads and that of 14 nm gold spheres, a well-defined plasmonic resonance band can be observed at 520 nm after loading gold nanoseeds on magnetic beads. This absorption peak centered at the same position with 14 nm gold colloid, indicating negligible plasmonic coupling among gold nanoseeds on magnetic bead surfaces. The absorption peak of MB@Au shell structure is significantly broadened and red-shifted to 700 nm. This plasmonic peak reveals successful formation of a complete gold shell. The elevation of curves is considered part of the absorption spectrum, which is commonly seen in shell-type plasmonic materials.¹⁷⁸

Figure 5.7 UV-vis absorption spectra of magnetic beads colloid (black trace), magnetic beads with PDA layer (red trace), magnetic beads with gold seeds (blue trace), and magnetic beads with a gold layer (purple trace).

Oligonucleotides containing part of the complementary sequence of target DNA were functionalized with thiol groups and Raman dye molecules at each ends. They served as reporter DNA and were conjugated with MB@Au nanoparticles through gold-sulfur covalent bonding to form nanoparticle-DNA conjugates. Oligonucleotides containing the other half of the complementary sequence of target DNA were functionalized with thiol groups and served as capture DNA. They were immobilized on gold nanospike substrates through gold-sulfur covalent bonding. In a sandwich DNA assay, reporter DNA conjugated with MB@Au nanoparticles first hybridized with target DNA strands to separate them from the analyte solution and enrich by magnetic field. The over-hanging end of target DNA strands then hybridized with thiolated capture DNA strands, which were immobilized on gold nanospike substrates. Hybridizations between target DNA and capture DNA can lead to formation of sandwiched structure with a particle-on-array configuration, which can be visualized by SEM images shown in **Figure 5.8**. We observed that the number of MB@Au nanoparticles on gold nanospike substrates increased with increasing concentration of

target DNA strands. At a low target DNA concentration (100 fM), we observed only 4-5 MB@Au nanoparticles on a $2\ \mu\text{m} \times 2\ \mu\text{m}$ nanospike substrate (**Figure 5.8a**). Increasing target DNA concentration by ten-fold (1 pM) led to a slightly higher density of MB@Au nanoparticles on gold nanospike substrates, about 15 MB@Au nanoparticles can be observed on a $2\ \mu\text{m} \times 2\ \mu\text{m}$ substrate (**Figure 5.8b**). Further increasing target DNA concentration by two orders of magnitude (100 pM) led to a higher coverage of MB@Au nanoparticles on gold nanospike substrates (**Figure 5.8c**), and saturation was reached when target DNA concentration increased to 10 nM (**Figure 5.8d**).

Figure 5. 8 SEM images of DNA conjugated MB@Au shell on gold nanospike substrates at varying target DNA concentrations (a=100 fM, b = 1 pM, c = 100 pM , d = 10 nM).

The corresponding concentration-dependent SERS spectra are shown in **Figure 5.9a**. The peak at 1366 cm^{-1} is assigned to the methine chain deformation of Cy5,¹⁷⁹ while the one at 1598 cm^{-1} is assigned to $\nu(\text{C}=\text{N})_{\text{stretch}}$.¹⁸⁰ This particle-on-array setup allowed for sensitive "signal-on" detection of DNA sequences down to 100 fM. Although we can still observe SERS signals of Cy5 when target DNA concentration is as low as 10 fM, the signal intensity is comparable with that from non-complementary DNA strands. So the limit of detection (LOD) is estimated to be 100 fM. The SERS intensity increased gradually and reached a plateau when target DNA concentration reached 10 nM. Quantitative analysis was carried out by plotting the SERS spectra signal intensity of the peak located at 1580 cm^{-1} as a function of concentration of target DNA strands (**Figure 5.9b**). The error bars are standard deviations derived from three measurements at different locations. Note that errors at lower DNA concentrations are higher, which may stem from bigger sampling errors from serial dilution as well as more random distributions of MB@Au nanoparticles on the substrates.

To assess the selectivity of particle-on-array SERS sensing system, the SERS intensity with complementary target DNA, single-base mismatched DNA, and non-complementary DNA were presented in the concentrations from 10 fM to 10 nM. In **Figure 5.9b**, it can be seen that SERS signals obtained in the presence of non-complementary DNA strands are quite weak. The intensities of peak located at 1580 cm^{-1} fluctuate at around 150 regardless of noncomplementary DNA concentrations, suggesting that there exists some weak nonspecific binding between mismatch DNA strands. Comparing SERS signal intensities at the DNA concentration of 10 nM, we observed an intense SERS signal of Cy5 in the presence of perfectly complementary target DNA, while the SERS signal was dramatically decreased in the presence of

single-base mismatch DNA strands. The intensity of the peak at 1580 cm^{-1} is ~ 8000 for perfectly complementary target DNA, and the intensity dramatically reduced to ~ 2000 when single-base mismatch target DNA was used. This big difference indicates that DNA hybridization occurs most efficiently when target DNA is perfectly complementary with the probe and reporter DNA strands. It demonstrated that this particle-on-array SERS system could efficiently discriminate single-base mismatched DNA strands.

Figure 5. 9 (a) SERS spectra of Cy5 at different concentrations of target DNA, from bottom to top: 10 fM, 0.1 pM, 1 pM, 10 pM, 100 pM, 1 nM, and 10 nM. (b) Quantitative plots of SERS intensities at 1598 cm^{-1} versus concentration of target DNA strands. Noncomplementary target DNA strands and single-base mismatch DNA strands were used as controls. The error bars represent standard deviation of three measurements.

Multiplex detection is one of the most attractive features of SERS. When it comes to DNA detection, the ability to detect multiplex DNA strands simultaneously is of critical importance. In our study, we carried out multiplex detection of three different types of pathogen oligonucleotides, namely: *Staphylococcus aureus*, *Listeria*

Monocytogenes, as well as Bacillus anthracis. Staphylococcus aureus and Listeria Monocytogenes are food-borne pathogens that can cause food poisoning when the food is not properly handled.¹⁰² Bacillus anthracis is a bacterium that can cause anthrax disease, which can result in pneumonia, blood infection, and even death.³¹ Reporter DNA strands corresponding to each pathogen were labeled with different Raman dye molecules: Cy5, TAMRA, and ROX. These dye molecules are distinctively structured and have unique spectra with minimum overlap. SERS spectra of TAMRA and ROX labeled DNA are shown in **Figure 5.10** and **Figure 5.11**, respectively. Raman peaks at 1626, 1465/1594, and 1645 cm^{-1} were selected as the characteristic peaks for ROX,^{32, 33} Cy5,¹² and TAMRA³⁴ tags. **Figure 5.12** shows the results of multiplex detection of the target DNA strands. The trace on top (**Figure 5.12 a**) presents the spectra of SERS detection of three types of target DNA strands. Different concentrations of target DNA strands (10 nM for ROX, 10 nM for Cy5, and 30 nM for TARMA) were mixed because different Raman dye molecules have varying Raman cross-sections. Characteristic Raman peaks of all three Raman dyes could be observed simultaneously in the same spectrum, as marked by different symbols. The SERS spectra of combinations of two types of target DNA strands are shown in **Figure 5.12 b-d**. Removing one of the target DNA strands from the mixture specifically turned off corresponding Raman peaks, demonstrating the multiplexed capacity of particle-on-array SERS substrate.

Figure 5. 10 (a) SERS spectra of ROX at different concentrations of target DNA, from top to bottom: 0.1 pM, 1 pM, 10 pM, 100 pM, 1 nM, and 10 nM . (b) Quantitative plots of SERS intensities at 1648 cm⁻¹ versus concentration of target DNA strands. Noncomplementary target DNAs were used as controls. The error bars represent standard deviation of three measurements.

Figure 5. 11 (a) SERS spectra of TAMRA at different concentrations of target DNA, from top to bottom: 0.1 pM, 1 pM, 10 pM, 100 pM, 1 nM, and 10 nM. (b) Quantitative plots of SERS intensities at 1649 cm⁻¹ versus concentration of target DNA strands. Noncomplementary target DNAs were used as controls. The error bars represent standard deviation of three measurements.

Figure 5. 12 Multiplex detection of three different types of pathogen DNA using MB@Au-on-Au nanospike SERS system. (a) SERS spectra obtained when the sample included three types of target DNAs (*L. monocytogenes*, *S. aureus*, and *B. anthracis*). (b) SERS spectra obtained when the sample included two types of target DNAs (*L. monocytogenes* and *B. anthracis*). (c) SERS spectra obtained when the sample included two types of target DNAs (*S. aureus*, and *B. anthracis*). (d) SERS spectra obtained when the sample included two types of target DNAs (*L. monocytogenes* and *S. aureus*). Triangle, star, and hexagon stand for representative Raman peaks of ROX, TARMA, and Cy5 respectively.

5.4. Conclusion

This work focus on the detection of oligonucleotides of pathogens using particle-on-array assays: magnetoplasmonic nanoparticles (gold shell coated magnetic beads) coupled with a gold nanospike array. This detection system employed sandwich type

DNA structure consisting of reporter, target, as well as capture DNA strands. Plasmonic coupling between magneto-plasmonic nanoparticles and the underlying gold nanospike array led to generation of hot spots. DNA strands were precisely positioned at the hotspots through specific hybridization between complementary DNA strands. Using oligonucleotides of *Staphylococcus aureus* as a model system, our SERS system demonstrates a limit of detection of 100 fM. Simultaneous detection of three types pathogen oligonucleotides was also achieved using this platform.

Chapter 6 Seeded Growth of Gold Nanostar array on Graphene Oxide for Surface Enhanced Raman Scattering and Catalysis

6.1. Introduction

Raman scattering spectroscopy is a highly useful technique for molecule fingerprinting. The measured Raman spectrum, which comes from the vibrational behavior of analyte molecules, gives a high degree of information on chemical structures of molecules. Raman peaks usually have very narrow bandwidths ranging from 10 to 20 cm^{-1} , making this technique suitable for simultaneous multiplexed analysis.⁹ However, application of Raman spectroscopy in trace detection has been rare due to its inherent low sensitivity. SERS is an advanced type of Raman scattering spectroscopy that could provide signal enhancement of several orders of magnitudes through properly designed SERS substrates. Trace analyte sensing can be accomplished with SERS, while preserving "fingerprint" specificity and multiplex ability.

Noble metal nanostructures or nanoparticles are commonly used for fabrication of SERS-active substrates due to their unique optical properties. People have been carefully tailoring the shape, size and composition of noble metal nanostructures in order to achieve the desired optimum SERS performance. Star shaped gold nanoparticles, with an inner round core and a few branches protruding out, is an exciting type of plasmonic nanostructures to be employed in SERS applications due to the following aspects: (1) sharp tips of the nanostars give rise to strongly enhanced fields without the need for particle aggregation,³³ (2) hybridization of tip and core plasmons could results in a further increase in the local electric field,¹⁸¹ (3) larger

surface area allows more analyte molecules to be absorbed on their surface.³¹ Analyte molecules must be positioned near or at metallic substrate for significant SERS amplification. However many molecules, especially aromatic molecules, have a low affinity to metal surface. Graphene oxide is a 2-D structure with a monolayer of carbon atoms. Its rich oxygen sites and aromatic domains give it a high absorption ability of aromatic compounds. Other superior properties of GO, including large surface area, high production rate, and low cost, makes it an ideal material in SERS application.

Considerable efforts have been made in synthesizing gold nanostructure-GO hybrid materials in aqueous phase. Two main routes are in situ chemical reduction of gold ions and ex situ adsorption of AuNPs. Pham and co-workers have prepared AuNPs-GO composites by functionalizing both AuNPs and GO and then immobilize AuNPs on GO by covalent bonding through amidation reaction.¹⁸² Zhou et al. prepared AuNPs/GO composites by first preparing AgNP-GO composites and then adding in HAuCl₄ for galvanic reaction.¹⁸³ Zhang et al. used a green route to synthesis Au nanostructures on tannic acid (TA)-functionalized graphene oxide (GO) using TA as a reducing and immobilizing agent.¹⁸⁴ TA is a phenolic hydroxyl-rich compound that contains rich aromatic rings and bind with GO through π - π interaction. Phenolic hydroxyls of TA then reduce Au³⁺ to metallic Au through direct redox reaction between TA and Au³⁺. Choi and co-workers integrated AuNPs to GO through electrostatic self-assembly.¹⁸⁵ GO is prepared using modified Hummers' method, which has a negative surface charge. AuNPs are functionalized with 4-dimethylaminopyridine (DMAP) to give them a positive charge. Positively charged AuNPs and negatively charged GO are then coupled to form a hybrid nanocomposite based on electrostatic self-assembly. Lu et al. synthesized AuNP decorated GO

nanosheets using polyoxyethylene sorbitol anhydride monolaurate (TWEEN 20) as a stabilizing agent for GO as well as a reducing and immobilizing agent for AuNPs.¹⁸⁶ Huang et al. prepared Au-GO and Au-rGO composites by noncovalent attachment of AuNPs pre-modified with 2-mercaptopyridine (2-MPy) to GO and rGO sheets.¹⁸⁷ 2-mercaptopyridine contains aromatic molecules thus could assembly on GO sheets via physisorption through π - π stacking, van der Waals forces etc. Most of the synthesis methods involve functionalization of either gold nanostructure or graphene oxide, which is complicated and time-consuming. Also nanostructures that have been hybridized with GO are limited to spherical gold nanoparticles with size smaller than 50 nm, little study has explored hybridizing GO with more complex structured gold nanoparticles such as gold nanostars. While single isolated gold nanostars are able to offer great enhancement in Raman signal intensities due to the presence of localized and enhanced electromagnetic field at the tips,³³ assembling them on GO nanosheets will lead to generation of more hotspots at the junctions among neighboring nanostars, which will provides additional signal enhancement.

Herein we report in-situ synthesis of gold nanostars on graphene oxide using seeded growth method. Its catalytic properties were studied using reduction of 4-nitrothiophenol as a model system by monitoring the reduction process of 4-nitrothiophenol in the presence of hydrogen gas via Raman spectroscopy. Nanoparticles have unique properties that impart them with a wide range of applications in catalysis and optical sensing. The high catalytic activity of nanoparticles stems from their large active surface area and high surface/volume ratio. Nanoparticles with sharp tips generate largely enhanced Raman signals, this property makes them ideal optical sensing materials. By integrating nanoparticles and graphene oxide, we are able to achieve a bifunctional composite that is both plasmonically and

catalytically active. Applying SERS to monitoring catalytic reactions can thus be monitored in situ using SERS, which is advantageous than other conventional methods because it is fast and highly surface-selective. Synthesis procedure of Gold nanostar-GO hybrid is illustrated in **Figure 6.1**. GO dispersion is prepared using modified Hummers' method from graphite.¹⁸⁸ Spherical AuNPs with diameters of 14nm are loaded onto GO sheets by chemical reduction of AuCl_4^- via sodium citrate. Growth of gold nanostars from 14 nm spherical gold nanoparticles is achieved by template growth with CTAB as capping agent. The resultant gold nanostars exhibit closely packed assembly on graphene oxide nanosheets, providing efficient Raman enhancement. Its performance as SERS substrate was studied using crystal violet (CV), Rhodamine B (RhB), and Iron(II) phthalocyanine (FePc) as probe molecules.

Figure 6.1 Schematic illustration of gold nanostar-GO hybrid synthesis process. (a) GO nanosheets; (b) gold nanoseed-GO hybrid; (c) gold nanostar-GO hybrid.

6.2. Experimental section

Materials. Graphite, Hydrogen tetrachloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), trisodium citrate, Silver nitrate, L-Ascorbic acid, Cetyl trimethylammonium bromide (CTAB), and hydrochloride were purchased from Sigma-Aldrich and used without purification. Raman probe molecules crystal violet (CV), Rhodamine B (RB), and Iron(II) phthalocyanine (FePc) were also acquired from Sigma-Aldrich at >90% purify. Water used in this experiment was deionized water (DI H_2O). Glassware used throughout all

the experiments were cleaned using aqua regia solution (HCl/HNO₃ 3:1 (v/v)) and rinsed with copious amounts of DI water prior to use.

Synthesis of Graphene Oxide (GO). GO was synthesized from graphite powder with modified Hummer's method. We first dissolved 10 gram of K₂S₂O₈ and 10 gram of P₂O₅ in 50 ml concentrated H₂SO₄ solution, the temperature was kept at 80°C. 12 gram of graphite powder was then added to the above solution and was under constant stirring at 80°C. After 4.5 hours, the solution was then cooled to room temperature and diluted with about 2 L DI water. The mixture solution was left standing overnight to separate graphite from supernatant. This pretreated graphite was dried to powder at 100°C (2 g) was put into concentrated H₂SO₄ (46 ml) with ice bath. KMnO₄ (6 g) was added gradually with stirring, the temperature of the solution was kept below 10°C during addition process. After stirring 2 hours, 96 ml of DI water was added into the above mixture. After stirring for another 30 mins, the reaction was terminated by adding 300 ml of DI water and 5 ml of 30% H₂O₂ solution. The mixture was centrifuged and subjected to several cycles of suspension in 5% HCl solution and finally separated by centrifugation. In order to completely remove metal ions and acids, the graphite oxide was subjected to multiple cycles of washing with DI water until the pH value of the supernatant reached 6. The final GO product was suspended in distilled water to give a viscous, brown dispersion (15 mg/ml).

Synthesis of gold nanoseed-GO hybrid. 14nm diameter gold nanoparticles were synthesized by citrate reduction of HAuCl₄ in the presence of GO. HAuCl₄ (4.5mg) was first dissolved in 30ml of DI water, into which 1mg of GO was added. The solution was heated to boiling, and 15mg of sodium citrate as dissolved in 300uL water was injected quickly under vigorous stirring. Boiling was continued for another 15mins. The seed solution was cooled to room temperature before use.

Synthesis of gold nanostars and gold nanostar-GO hybrid. 10ml of DI water, 200ul of HAuCl_4 (25mM), 20ul of HCl (1M), 1ml of seed solution, and 10ul of AgNO_3 (40mM) were added into reaction bottle under vigorous stirring. 100ul of LAA(100mM) was then injected into above solution. The color of the solution turns into purple and then dark blue in a few seconds time. After 2-3 mins of continuous stirring, 500ul of CTAB (10mM) was added to stabilize gold nanostars. Finally, CTAB stabilized gold nanostars were centrifuged to remove excess CTAB. Seed and nanostar solution without GO was synthesized following the same route except no GO was added in the synthesis procedure. Gold stars and AuStar-GO composites were adjusted to contain same weight concentration of Au.

Catalytic activity of gold nanostar on graphene. We studied the catalytic activity of gold nanostar on graphene in borohydride reduction of 4-nitrothiophenol (4-NTP). In a typical experiment, 4-nitrothiophenol was added into 1 ml of gold nanostar on graphene dispersion, resulting in final concentration of $10\mu\text{M}$. 50 μl of 10g/l freshly prepared NaBH_4 was added to the dispersion and time-dependent SERS spectra were recorded at intervals of 10 s at room temperature.

Characterization and measurements. UV-visible absorption spectra were recorded using a Shimadzu UV2501 spectrophotometer. SEM images were obtained using field-emission scanning electron microscope (JSM-6700F). TEM images were taken from JEOL JEM 2010 electron microscope. In preparation of TEM samples, 10 μL of aqueous solution of gold nanostars were drop casted onto a 300-mesh TEM grid and dried in air. Raman spectra were acquired using a RENISHAW Raman microscope. 632.8nm excitation line from an air-cooled He-Ne laser was used for SERS measurements. Laser beam was focused on a spot with size of 2-5 μM using a 50X

objective. Single scan with an integration time of 10s was performed for each spectra acquisition.

6.3. Results and discussion

Figure 6.2 SEM images of (a) Bare GO Sheet, (b) Gold nanoseed-GO hybrid and (c) Gold nanostar-GO hybrid; (d) TEM image of Gold nanostar-GO hybrid.

Figure 6.3 FTIR spectra of graphene oxide (GO).

Morphological changes happened during gold nanostar-GO hybrid synthesis process were monitored by both SEM and TEM. **Figure 6.2a** shows SEM image of bare GO nanosheets. Prior to gold nanoseed loading, GO nanosheets exhibit a silk-like morphology, with plenty of wrinkles and folding on the surfaces as well as at the edges. These intrinsic morphological properties of GO give it a large surface area and thus a high loading ability of gold nanoparticles. Degree of GO reduction was studied by FT-IR. The result is shown in **Fig 6.3**. The peak at 1065 cm^{-1} and 1225 cm^{-1} corresponds to C-O (ν (epoxy or alkoxy)) and C-O-C (ν (epoxy symmetrical ring deformation vibration)) respectively, peaks associated with C-OH (ν (carboxyl)) and C=C are located at 1400 cm^{-1} and 1620 cm^{-1} respectively, and C=O in carboxylic acid and carbonyl moieties (ν (carbonyl)) can be correlated with the peak at 1740 cm^{-1} .^{189, 190} Chemical conversion of graphite into GO is a well established method for large scale synthesis of graphene related materials. GO synthesized through chemical oxidation of graphite contains hydroxyl and epoxy functional groups on its basal plane as well as carboxyl functional groups at its edges.^{148, 191} These oxygen functionalities are believed to be introduced by sulfuric/nitric acid treatment^{192, 193} as well as aqueous sonication.¹⁹⁴ The abundant possession of functional groups makes GO highly hydrophilic and therefore readily dispersed in aqueous solution. A 30 min bath sonication helps exfoliate GO into monolayer nanosheets and maintain a consistent dispersion. Stable dispersion of monolayer GO sheets can be further reduced to graphene or transformed into self-assembled films⁸³ and self-standing papers.¹⁵³

Gold nanoseed-GO hybrid was prepared by reducing HAuCl_4 on GO surface using sodium citrate. Prior to addition of sodium citrate, GO and HAuCl_4 were mixed in a flask and aged for one hour to ensure complete interaction of HAuCl_4 and oxygen

functionalities of GO.¹⁹⁰ The mixed solution was then heated to boiling, followed by quick injection of sodium citrate. **Figure 6.2b** shows the SEM image of gold nanoseed-GO hybrids. We can clearly see a homogenous distribution of discrete gold nanoseeds on GO nanosheet surfaces. The average size of these nanoseeds is 14 nm in diameter. It is also observed that some gold seeds are brighter while some appear darker. This is as expected because gold nanoseeds will grow on both sides of GO nanosheets. Those nanoseeds which appear darker are probably formed on the rear of the GO sheets, and have a reduced surface conductivity because of the shielding effect of GO. In our experiment, graphene oxide nanosheets are not functionalized with thiol groups or any other functional groups to exert a binding force toward gold nanoseeds. Instead, oxygen functionalities that inherently exist on GO surfaces are believed to be responsible for successful formation of gold nanoseeds. Goncalves et al. reported that the presence of oxygen functionalities on GO gives it active sites for nucleation and growth of gold nanoparticles.¹⁹⁰ In their work, they showed that rich oxygen functionalities are responsible for gold nanoparticles attachment to GO surface. Control experiments on reduced GO show that gold nanoparticle agglomerates will grow if density of oxygen functionalities is reduced, and no gold nanoparticles would be able to attach to rGO once oxygen functionalities are removed. The mechanism is proposed as follows: the oxygen functional groups give GO surface a negative charge, and help attract free gold(III) ions in the solution through electrostatic interaction. Subsequent addition of reducing agent reduces gold ions to metallic AuNPs at the GO surface. Degree of GO reduction was studied by Fourier transform infrared (FT-IR) spectroscopy. The result is shown in **Fig 6.3**. The peak at 1065 cm⁻¹ and 1225 cm⁻¹ corresponds to C-O (ν (epoxy or alkoxy)) and C-O-C (ν (epoxy symmetrical ring deformation vibration)) respectively, peaks associated with C-OH (ν (carboxyl)) and

C=C are located at 1400 cm^{-1} and 1620 cm^{-1} respectively, and C=O in carboxylic acid and carbonyl moieties (ν (carbonyl)) can be correlated with the peak at 1740 cm^{-1} .^{189,}
¹⁹⁰ To prepare an assembly of gold nanostars on GO nanosheets, gold nanoseeds-GO hybrid is subject to a growth solution to start in-situ growth of star shaped gold nanoparticles. **Figure 6.2 c** and **Figure 6.2 d** show SEM and TEM images of a GO sheet carrying an assembly of star shaped gold nanoparticles, respectively. Gold nanostars have an average size of 100 nm, with 6-8 sharp tips protruding out from the central core.

Figure 6.4 (a) UV-vis absorption spectra of aqueous dispersion of GO, gold nanoseed-GO hybrid, gold nanostar colloid, as well as gold nanostar-GO hybrid. (b) Raman spectra of GO, gold nanoseed-GO hybrid, and gold nanostar-GO hybrid.

Successful formation of gold seeds and growth of gold nanostars on GO templates were further demonstrated via UV-Vis spectroscopy. UV-visible spectroscopy is a useful approach for monitoring gold nanoparticle growth, the absorption spectra reflect changes in nanoparticle size, shape, inter-particle distance, etc. **Figure 6.4a** shows UV-vis absorption spectra of aqueous dispersion of GO, gold nanoseed-GO hybrid, gold nanostar colloid, as well as gold nanostar-GO hybrid. Bare GO dispersion exhibits two peaks: an absorption peak at around 230 nm, which is associated with

$\pi \rightarrow \pi^*$ transitions of aromatic C-C bonds, and a shoulder at 300 nm, which comes from $n \rightarrow \pi^*$ transitions of C=O bonds.¹⁹⁵ On the curve reflecting gold nanoseed-GO hybrid, we observe an additional characteristic plasmonic peak at 520 nm. This can be attributed to formation of gold nanoseeds on GO, because plasmon resonance peak of 14 nm spherical gold nanoparticle colloid is centered around 520 nm. On the curve representing sample gold nanostar-GO hybrid, absorption peak at 520 nm broadened and red shifted to 700 nm, indicating growth of gold nanostars on GO nanosheets. This change in plasmonic absorption spectra of hybrid material is consistent with that observed during growth of gold nanostars without the presence of GO, as reported by other groups.^{31, 35, 196} Absorption spectrum of gold nanostars in the absence of GO is also measured in our experiment. Comparing the spectra of gold nanostars dispersed in water and gold nanostars assembled on GO nanosheets, we observed a 40 nm red shift in plasmon peaks of gold nanostars when gold nanostars were assembled on graphene oxide nanosheets, which is due to changes in dielectric environment of gold nanostars. Raman enhancement resulted from loading GO with gold nanoparticles was evidenced from **Figure 6.4 b**. Raman intensities of characteristic bands of GO were observed to be largely enhanced after formation and growth of gold nanoseeds and nanostars, respectively. Graphene oxide has two characteristic bands: the band at 1350 cm^{-1} , which is classified as D band, is a disorder-activated Raman mode that indicates extensive oxidation of the graphite; whereas G band, which is centered at 1600 cm^{-1} , comes from the vibration of sp^2 -hybridized carbon atoms in the hexagonal framework. The enhancement effect was quantitatively analyzed by calculating the SERS intensity ratio N before and after gold nanoparticle loading. As seen in **Table 6.1**, loading 14 nm gold nanoparticles on GO amplified the Raman bands by around 5 times, while growth of gold nanostars lead to 19 times of enhancement. This enhancement of signals comes

from enhanced electromagnetic field at gold nanoparticle surfaces. Before loading gold nanoparticles, the spectrum we collected from GO is normal Raman spectrum, which is quite weak. With gold nanoparticles deposited on GO surfaces, large electromagnetic field is generated in close proximity to the surfaces of gold nanoparticles. For the portions of GO that are located within the electromagnetic field, significantly enhanced Raman signals will be generated.

Table 2 SERS intensity ratio of GO with and without gold nanoparticle hybrid.

	D band	$N=I/I_{GO}$	G band	$N=I/I_{GO}$
GO	2037	1	1545	1
AuNP/GO	10173	4.99	8545	5.53
AuStar/GO	33246	16.32	29617	19.17

Figure 6.5 (a) SERS spectra of CV on different substrates (purple: GO; blue: gold nanoseed-GO hybrid; red: gold nanostars; black: gold nanostar-GO hybrid). (b) SERS spectra of CV at concentrations equal to 5, 20, 200, 1000, 10000, and 20000 nM, from bottom to top. (c) Quantitative curves of CV sensing on gold nanostar-GO hybrid. The Raman intensity of CV peak at 1671 cm⁻¹ was plotted as a function of CV concentration. The error bars were obtained from measurements collected from three different batches.

SERS enhancement ability of gold nanostar-GO substrate is originated from two factors: (1) gold nanostars possess several sharp tips that act as hotspots for generation of localized SERS enhancement; (2) gold nanostars could assembly into a close-packed pattern on GO template, the particle-to-particle, especially tip-to-tip distances are largely reduced as compared with free gold nanostars. These as-formed nanogaps will act as tight junctions, where electromagnetic field is greatly enlarged, thus providing additional SERS enhancement. To demonstrate this, SERS efficiencies of GO, gold nanoseed-GO, gold nanostars-GO, and gold nanostars were investigated using crystal violet (CV) as probe molecules. These sharp tips are responsible for generation of highly localized and largely enhanced electromagnetic fields - a lightening effect which is also observed in rod shaped nanoparticles and nanocrystals with a high curvature.^{35, 175-177} A single star shaped gold nanoparticle holds several hotspots located at the tip area, thus a gold nanostar colloid containing stable and well separated nanostars is able to provide satisfactory Raman enhancement without the need of bringing nanoparticles to a aggregated state.^{33, 175} We do not observe any agglomeration of gold nanostars or nanoparticles with other random shapes in all of the samples. It indicates that seeded growth method using gold nanoparticle-GO hybrid as seed material could produce stable gold nanostar-GO hybrid with a high yield.

Electromagnetic enhancement mechanism (EM) is a long-range effect that requires nanoscale roughed metallic substrates, while chemical enhancement mechanism (CM) is a short-range effect that requires the molecule to be in contact with or very close to the substrate. It has been reported that monolayer GO facilitates charge transfer between graphene and analyte molecules, leading to a CM

enhancement of 2-17 times.¹⁹⁷ Electromagnetic enhancement is not likely to happen on bare GO because the surface plasma of graphene is in the terahertz range not visible light range.¹⁹⁷ CV has a molecular structure similar to graphene. When deposited on GO, these molecules should lie parallel to the surface of aromatic graphene owing to the π - π stacking, which means that the distance between them is small enough for CM. However, CV is under non-resonant excitation in our experiment condition (785nm laser excitation) and chemical enhancement from GO is very limited, thus SERS signal from CV is barely observable using bare GO as enhancing substrate, as shown in **Figure 6.5 a**. Weak Raman peaks begin to appear after loading GO with gold nanoseed, which can be attributed to the weak SERS enhancement effect of 14 nm gold nanoparticles. Raman intensities on gold nanostar-GO hybrid are much higher than those on gold nanoseed-GO hybrid, signifying more efficient SERS enhancement from gold star-GO hybrid. Raman signals of probe molecules on gold nanostars without GO template were also measured and found to be weaker than those measured with GO template. The magnitude of enhancement is almost the same for peaks at 1621, 1179, and 915 cm^{-1} , which correspond to ring C-C stretching, ring C-H bending, as well as ring skeletal vibration, indicating a parallel alignment of CV molecules and GO surface. At lower CV concentration (less than 100nM), Raman bands (D band and G band) of GO appear as broad continuous background from 1100 cm^{-1} to 1700 cm^{-1} and are removed during background subtraction. At a higher CV concentration (larger or equal to 100nM), Raman scattering of GO is suppressed and D band or G band was no longer present. This observation is in agreement with previous literature¹⁹⁸ and may be explained by competitive SERS enhancement on GO and analyte molecules. Quantitative evaluation of SERS performance of gold nanostar-GO substrate was carried out by measuring Raman intensity of CV at different concentrations. **Figure**

6.5b shows the representative SERS spectra of CV on gold nanostar-GO substrate at different CV concentrations. SERS intensity of the strongest vibration peak at 1617cm^{-1} was also plotted with respect to CV concentration. The SERS intensity is observed to increase in response to increasing CV concentration, and approach to a saturation level when CV concentration is larger than $1\mu\text{M}$. The limit of detection (LOD), which is defined as the concentration of CV when signal to noise (S/N) ratio is equal to 3, is 1nM . Enhancement factor is calculated to be 4.8×10^5 .

Figure 6.6 SERS spectra of FePc (a) and RhB (b) on different substrates (purple: GO; blue: gold nanoseed-GO hybrid; red: gold nanostars; black: gold nanostar-GO hybrid), inserts show chemical structures of FePc and RhB, respectively.

We further tested SERS enhancement properties of gold nanostar-GO hybrid using Rhodamine B (RhB) and iron phthalocyanine (FePc) as probe molecules. **Figure 6.6** shows Raman signals of RhB and FePc on different substrates. Raman signals of RhB and FePc are barely observable using bare GO as enhancing substrate. Weak SERS signals of RhB begin to appear on gold nanoseed-GO hybrid, while that of FePc cannot be observed. Beside electromagnetic field, inherent properties of analyte molecules such as cross section are also factors that influence intensity of Raman

signals. CV and RhB are dye molecules with cross sections orders of magnitudes larger than that of FePc, thus their characteristic Raman signals could be observed with a moderate enhancement. Similar with that observed from CV, gold nanostars-GO hybrid gives 4-5 times signal enhancement compared with free gold nanostars.

Figure 6.7 SERS spectra of CV (0.1 μ M), RB (3 μ M), FePc (10 μ M), and mixture solution.

The width of Raman peaks for most organic compounds are typically 10-100 times narrower than those of fluorescence, thus minimizing the potential for overlapping in the response from different labels. This unique property of Raman scattering spectroscopy gives it the advantageous application of multiplex detection. To explore this potential, three different Raman probes, namely CV, RhB, and FePc were added into gold nanostar/GO hybrid solution and Raman spectra of the resultant mixture solution was collected. Characteristic peaks of CV (424 cm^{-1} , 442 cm^{-1} , and 1621 cm^{-1}), RhB (622 cm^{-1} and 1650 cm^{-1}), and FePc (684 cm^{-1} , 1342 cm^{-1} , and 1453 cm^{-1}) could be clearly observed in the mixture spectrum (**Figure 6.7**), indicating successful multiplexing.

Figure 6.8 TEM image of a single gold nanostar.

Figure 6.9 (a) Raman spectra of 4-NTP on different substrates (purple: GO; blue: gold nanoseed-GO hybrid; red: gold nanostars; black: gold nanostar-GO hybrid). (b) Time dependent SERS spectra of 4-NTP on gold nanostar-GO hybrid. (c) Time dependent SERS spectra of 4-NTP on gold nanostars. (d) Plot of $\ln(I/I_0)_{1568}$ against time for

gold nanostar-GO hybrid and gold nanostars. gold nanostars and gold nanostar-GO hybrid dispersions have approximately identical particle concentrations.

We studied catalytic properties of gold nanostar-GO hybrid materials using reduction of 4-nitrothiophenol (4-NTP) as a model system by monitoring the reduction process of 4-NTP in the presence of hydrogen gas via Raman spectroscopy. The unique morphology of gold nanostars makes it an ideal substrate for in situ monitoring of catalytic reaction via SERS. It was reported that gold nanocrystals with size larger than 10nm were catalytically inert,¹⁹⁹ thus smaller nanoparticles are more suitable for catalytic applications. However SERS efficiencies increase with size of nanocrystals, and nanocrystals with size smaller than 10 nm will be ineffective for SERS. From TEM image of gold nanostars (**Figure 6.8**), we can see that the radius of curvature at the tips of gold nanostars are significantly less than 10 nm, those tips act as both hot spot for SERS and active site for catalysis. 4-NTP first formed a monolayer on the surface of Au nanostars through chemisorptions, they are subsequently reduced to 4-ATP by gold nanostars upon addition of NaBH₄. **Figure 6.9 a** shows SERS spectra of 4-NTP on GO, gold nanoseed-GO hybrid, gold nanostars, and gold nanostar-GO hybrid. Raman peaks of 4-NTP on GO and gold nanoseed-GO hybrid cannot be observed, indicating limited Raman enhancement of GO and 14 nm spherical AuNPs. Characteristic peaks at 854 cm⁻¹ (C-H wagging), 1076 cm⁻¹ (C-S) stretching, 1334 cm⁻¹ (O-N-O stretching) and 1571 cm⁻¹ (phenyl-ring mode)²⁰⁰ can be clearly observed on gold nanostars, and gold nanostar-GO hybrid. **Figure 6.9 b and c** show the time-dependent SERS spectra of 4-NTP on gold nanostar-GO hybrid and gold nanostar, respectively. Peak at 1334 cm⁻¹ gradually decreases and two peaks of 4-ATP at 1486 cm⁻¹ and 1587 cm⁻¹ begin to emerge, indicating reduction of 4-NTP to

4-NTP, which is catalyzed by gold nanostar-GO hybrid. Peaks at 1140 cm^{-1} , 1380 cm^{-1} , and 1422 cm^{-1} that corresponds to Raman peaks of dimercaptoazobenzene (DMAB) were also observed in the middle of the catalytic reaction. This observation is in agreement with previous reports that 4-NTP could undergo SPR-induced photocatalytic dimerization on gold nanoparticle surfaces.^{199, 201} It is generally assumed that this catalysis reaction process follows pseudo-first-order kinetics. A reaction rate constant of $k = 0.28\text{ min}^{-1}$ were derived from the linearly fitted slope of the $\ln(I/I_0)$ vs. t plot (**Figure 6.9d**). The rate constant of gold nanostars were also determined using the same methods. The results show that stars are able to catalyze hydrogenation of 4-NTP, due to the unique morphology of stars. However gold nanostar-GO has a higher rate constant than gold nanostars. The whole reduction reaction completes in 10 mins in the presence of gold nanostar-GO catalyst, as evidenced by the complete disappearance of the peak at 1568 cm^{-1} . It takes 16 mins for the reduction reaction to complete with gold nanostar as catalyst. This is because of the synergistic catalytic effect of GO. High catalytic activities of gold nanostar-GO hybrid is explained by Zhang et al by strong metal-graphene interactions and electron transfer between gold nanostars and the 2D graphene oxide.¹⁸⁷ Jasuja et al also reported catalytic and SERS performance of Au-GO composites.²⁰² In their experiment, metal NPs are microwave-reduced, and the improved catalytic performance is explained by enhanced density of accessible catalytic sites of the metal NPs. The fact that GO can help adsorb nitrothiophenols also facilitates catalytic reaction. Another factor which greatly affect catalytic activity is amount of available active sites. Gold nanostars have to be stabilized with an excess amount of surfactant such as cetyl trimethylammonium bromide (CTAB), which blocks accessibility of analytes onto star surfaces. On the other hand, GO could effectively stabilize gold nanostars that are

assembled on GO surfaces, eliminating the need to introduce excess stabilizer and free most of the active sites on star surfaces.

6.4. Conclusion

In summary, we developed an alternative approach toward 2D ensembles of plasmonic nanostructures by employing graphene oxide nanosheets as scaffolds for seeded growth of gold nanostars. Graphene oxide had a dual functionality: it acted as a nucleation site for gold nanostar growth and a scaffold for nanostar assembly. Star shaped gold nanoparticles offered great enhancement in Raman signal intensities attributable to their branched shape. Gold nanostars formed a close-packed pattern on graphene oxide, generating a high density of hot spots for highly efficient Raman enhancement. The obtained gold star/GO hybrid exhibited superior SERS activity than isolated gold star nanoparticles. The limit of detection for crystal violet was 1.0 nM, which was an order of magnitude lower than gold star colloid. Catalytic conversion of 4-nitrothiophenol (4-NTP) molecules to 4-aminothiophenol (4-ATP) was studied in situ by tracking SERS spectra of the reactant. Our results showed that the bifunctional star/GO hybrid material could be applied in both trace detection and pollution treatment.

Chapter 7 General conclusion and future research

7.1. Conclusions

In this thesis study, I developed SERS substrates based on two-dimensional ensembles of plasmonic nanostructures. Interfacial self-assembly technique represents a straightforward approach to produce a large area of hotspot-rich SERS substrates. We thrived to obtain a high sensitivity through creation of hotspots and selective enrichment of analytes at hotspots.

In chapter 3, we presented development of a silver-gold nanowire arrays by virtue of interfacial self-assembly of silver nanowires, followed by galvanic replacement reactions. The resultant silver-gold nanowire arrays served as excellent sensing platforms for small molecules detection. We successfully detected two types of food contaminants, adulteration agent (melamine) and pesticide (thiram). Limit of detection (LOD) for melamine in milk sample was 10 nM, and LOD for thiram in juice sample was 1 nM. Both results meet regulation requirements of the governments.

In chapter 4, we presented fabrication of an efficient SERS substrates based on the use of chemically derived GO and interfacial assembly of plasmonic nanoparticles as building blocks. In this design, single layer graphene nanosheets were sandwiched between two-dimensional (2D) arrays of closely packed plasmonic nanoparticles. This sandwich type SERS substrate was subsequently utilized in multiplex detection of three types of tumor suppressor genes.

In chapter 5, we designed the third type of SERS substrate that had a particle-on-array configuration, and applied it in detection of pathogen oligonucleotides. In this particle-on-array configuration, 'particle' refers to magnetoplasmonic nanoparticles, and array refers to a gold substrate. Both magnetoplasmonic nanoparticles and gold

substrate were conjugated with the two single-stranded DNA complimentary to the two ends of target DNA. In this way, target DNA strands were sandwiched between plasmonic nanoparticles and metal platform for enhancement of SERS signals. Multifunctional magnetoplasmonic nanoparticles offer both magnetic enrichment and plasmonic coupling, thus greatly increasing sensitivity of the SERS assay. Using oligonucleotides of *Staphylococcus aureus* as a model system, this SERS system demonstrated a detection limit as low as 100 fM.

In chapter 6, we developed an alternative approach toward 2D ensembles of plasmonic nanostructures by employing graphene oxide nanosheets as scaffolds for seeded growth of gold nanostars. Graphene oxide had a dual functionality: it acted as a nucleation site for gold nanostar growth and a scaffold for nanostar assembly. Star shaped gold nanoparticles offered great enhancement in Raman signal intensities attributable to their branched shape. Gold nanostars formed a close-packed pattern on graphene oxide, generating a high density of hot spots for highly efficient Raman enhancement. The obtained gold star/GO hybrid exhibited superior SERS activity than isolated gold star nanoparticles. The limit of detection for crystal violet was 1.0 nM, which was an order of magnitude lower than gold star colloid. Catalytic conversion of 4-nitrothiophenol (4-NTP) molecules to 4-aminothiophenol (4-ATP) was studied in situ by tracking SERS spectra of the reactant. Our results showed that the bifunctional star/GO hybrid material could be applied in both trace detection and pollution treatment.

7.2. Recommendations for future work

The future research is proposed as follows:

1. Advancements in nanotechnology have opened the possibility of synthesizing plasmonic nanostructures with various morphologies. Because plasmonic properties of nanostructures are closely related to their morphologies, and SERS efficiencies rely largely on plasmonic properties of the enhancing nanostructures, we can further optimize current SERS performance by tuning the morphologies of the nanostructures. In our previous research, we had explored silver nanowires, Au@Ag core-shell nanospheres, magnetoplasmonic nanoparticles, and star shaped gold nanoparticles. Other plasmonic nanostructures such as core-shell nanogap nanoparticles, nanorods, nanoprisms, etc. are also promising SERS substrates. We have seen a growing interest in synthesizing SERS probes with built-in hotspots, which could provide highly uniform and efficient SERS activity as well as overcome the biological barrier of size limitation. The wide LSPR window of gold nanorods, in conjugation with the controlled plasmonic coupling in the ensembles, allows for tuning the LSPR of ensembles into near-infrared (NIR) region, which is beneficial for applying these ensembles in biological and clinical applications. We will continue explore assembly of nanorods and nanogap particles for detection of oligonucleotides, protein molecules, as well as whole cells.

2. SERS as a optical spectroscopy technique has the advantages such as high sensitivity, optical stability, and the ability to multiplex signals from different target analytes. In light of these characteristics, one of the future works is to develop them into real time sensing assays for in-situ chemical and biological detection. The SERS enhancing substrates we developed are very versatile. They can reside at liquid-liquid

interfaces and be used as disposable sensing platforms, or they can be supported by a solid substrate and further developed into re-usable sensing platforms. By incorporating portable Raman spectrometer and sensing substrates together, we can carry on in-situ chemical detection in both natural and biological settings.

SERS imaging is another promising area to explore. The advantages of SERS, such as multiplexing capabilities, photo-stability and high contrast by NIR laser excitation, make SERS imaging a promising novel imaging methodology. By coupling SERS imaging technique with above mentioned SERS probes, we can expand our current application to tissue diagnostics and cancer cell detections.

3. In this thesis work, we utilized our SERS sensing platforms in both small molecule detection as well as oligonucleotides detection. Application of our SERS sensing platforms could be expanded to proteins such as biomarkers on bacteria cells and cancer biomarkers in human blood serum. SERS probes conjugated with secondary antibodies or other functional groups can be utilized in protein sensing through specific antigen-antibody interactions on a sandwich immuno-assay pattern.

Adding magnetic separation overcomes the problem of possible interferences in the sample solution. Magnetic field separation is one promising technique to replace tedious filtration/centrifugation separation. Coupling SERS detection scheme with magnetic bead separation provides new opportunities for detection of a diverse range of targets, including small molecules and proteins, in raw samples. In our future research, we are able to detect samples of DNA extractions from target pathogens.

References

1. Kneipp, K.; Kneipp, H.; Itzkan, I.; Dasari, R. R.; Feld, M. S. *Chemical Reviews* **1999**, 99, (10), 2957-2976.
2. Qian, X. M.; Nie, S. M. *Chemical Society Reviews* **2008**, 37, (5), 912-920.
3. Le Ru, E. C.; Etchegoin, P. G. *Annual Review of Physical Chemistry* **2012**, 63, (1), 65-87.
4. Lakowicz, J. R., *Principles of Fluorescence Spectroscopy*. Springer: **2010**.
5. Reichenbacher, M.; Popp, J., Vibrational Spectroscopy. In *Challenges in Molecular Structure Determination*, Springer Berlin Heidelberg: **2012**; pp 63-143.
6. Ellis, D. I.; Brewster, V. L.; Dunn, W. B.; Allwood, J. W.; Golovanov, A. P.; Goodacre, R. *Chemical Society Reviews* **2012**, 41, (17), 5706-5727.
7. Yang, W. h.; Hulteen, J.; Schatz, G. C.; Van Duyne, R. P. *The Journal of Chemical Physics* **1996**, 104, (11), 4313-4323.
8. So, P. T. C.; Dong, C. Y., Fluorescence Spectrophotometry. In *eLS*, John Wiley & Sons, Ltd: **2001**.
9. Cao, Y. C.; Jin, R.; Mirkin, C. A. *Science* **2002**, 297, (5586), 1536-1540.
10. Masters, B. R. *Optics and photonics* **2009**.
11. Zavaleta, C. L.; Garai, E.; Liu, J. T. C.; Sensarn, S.; Mandella, M. J.; Van de Sompel, D.; Friedland, S.; Van Dam, J.; Contag, C. H.; Gambhir, S. S. *Proceedings of the National Academy of Sciences* **2013**, 110, (25), E2288–E2297.
12. Ko, H.; Singamaneni, S.; Tsukruk, V. V. *Small* **2008**, 4, (10), 1576-1599.
13. Zhu, Z.; Li, Q.; Bai, B.; Fan, S. *Nanoscale Res Lett* **2014**, 9, (1), 1-8.
14. Kneipp, K.; Wang, Y.; Kneipp, H.; Perelman, L. T.; Itzkan, I.; Dasari, R. R.; Feld, M. S. *Physical Review Letters* **1997**, 78, (9), 1667-1670.
15. Shuming Nie, S. R. E. *Science* **1997**, 1102-1106.

16. Moyer, P. J.; Schmidt, J.; Eng, L. M.; Meixner, A. J.; Sandmann, G. W.; Dietz, H.; Plieth, W. *J. Am. Chem. Soc.* **2000**, 122, (22), 5409-5410.
17. Michaels, A. M.; Nirmal, M.; Brus, L. E. *J. Am. Chem. Soc.* **1999**, 121, (43), 9932-9939.
18. Bosnick, K. A.; Jiang; Brus, L. E. *The Journal of Physical Chemistry B* **2002**, 106, (33), 8096-8099.
19. Lee, J.-H.; Nam, J.-M.; Jeon, K.-S.; Lim, D.-K.; Kim, H.; Kwon, S.; Lee, H.; Suh, Y. D. *ACS Nano* **2012**, 6, (11), 9574-9584.
20. Litorja, M.; Haynes, C. L.; Haes, A. J.; Jensen, T. R.; Van Duyne, R. P. *The Journal of Physical Chemistry B* **2001**, 105, (29), 6907-6915.
21. Le Ru, E. C.; Blackie, E.; Meyer, M.; Etchegoin, P. G. *The Journal of Physical Chemistry C* **2007**, 111, (37), 13794-13803.
22. Aroca, R., *Surface-enhanced Vibrational Spectroscopy*. John Wiley & Sons: Chichester, **2006**.
23. Campion, A.; Ivanecky, J. E.; Child, C. M.; Foster, M. *J. Am. Chem. Soc.* **1995**, 117, (47), 11807-11808.
24. Endo, T.; et al. *Science and Technology of Advanced Materials* **2005**, 6, (5), 491.
25. McFarland, A. D.; Young, M. A.; Dieringer, J. A.; Van Duyne, R. P. *The Journal of Physical Chemistry B* **2005**, 109, (22), 11279-11285.
26. Ye, J.; Hutchison, J. A.; Uji-i, H.; Hofkens, J.; Lagae, L.; Maes, G.; Borghs, G.; Van Dorpe, P. *Nanoscale* **2012**, 4, (5), 1606-1611.
27. Le Ru, E. C.; Galloway, C.; Etchegoin, P. G. *Physical Chemistry Chemical Physics* **2006**, 8, (26), 3083-3087.
28. Park, Y.-K.; Yoo, S.-H.; Park, S. *Langmuir* **2007**, 23, (21), 10505-10510.
29. Teng Qiu, W. Z., Paul K. Chu. *Recent Patens on Nanotechnology* **2009**, 3, 10-20.

30. Liao, P. F.; Wokaun, A. *The Journal of Chemical Physics* **1982**, 76, (1), 751-752.
31. Rodríguez-Lorenzo, L.; Álvarez-Puebla, R. n. A.; de Abajo, F. J. G. a.; Liz-Marzán, L. M. *The Journal of Physical Chemistry C* **2009**, 114, (16), 7336-7340.
32. Hrelescu, C.; Sau, T. K.; Rogach, A. L.; Jäckel, F.; Feldmann, J. *Applied Physics Letters* **2009**, 94, (15), -.
33. Hrelescu, C.; Sau, T. K.; Rogach, A. L.; Jäckel, F.; Laurent, G.; Douillard, L.; Charra, F. *Nano Letters* **2011**, 11, (2), 402-407.
34. Kahraman, M.; Daggumati, P.; Kurtulus, O.; Seker, E.; Wachsmann-Hogiu, S. *Sci. Rep.* **2013**, 3.
35. Khoury, C. G.; Vo-Dinh, T. *The Journal of Physical Chemistry C* **2008**, 112, (48), 18849-18859.
36. Zong, S.; Wang, Z.; Chen, H.; Yang, J.; Cui, Y. *Analytical Chemistry* **2013**, 85, (4), 2223-2230.
37. Jokerst, J. V.; Cole, A. J.; Van de Sompel, D.; Gambhir, S. S. *ACS Nano* **2012**, 6, (11), 10366-10377.
38. von Maltzahn, G.; Centrone, A.; Park, J.-H.; Ramanathan, R.; Sailor, M. J.; Hatton, T. A.; Bhatia, S. N. *Advanced Materials* **2009**, 21, (31), 3175-3180.
39. Hu, L.; Kim, H. S.; Lee, J.-Y.; Peumans, P.; Cui, Y. *ACS Nano* **2010**, 4, (5), 2955-2963.
40. Critchley, K.; Khanal, B. P.; Górzny, M. Ł.; Vigderman, L.; Evans, S. D.; Zubarev, E. R.; Kotov, N. A. *Advanced Materials* **2010**, 22, (21), 2338-2342.
41. He, J.; Wang, Y.; Feng, Y.; Qi, X.; Zeng, Z.; Liu, Q.; Teo, W. S.; Gan, C. L.; Zhang, H.; Chen, H. *ACS Nano* **2013**, 7, (3), 2733-2740.
42. Wei, H.; Hao, F.; Huang, Y.; Wang, W.; Nordlander, P.; Xu, H. *Nano Letters* **2008**, 8, (8), 2497-2502.

43. Vigderman, L.; Zubarev, E. R. *Langmuir* **2012**, 28, (24), 9034-9040.
44. Tian, C.; Ding, C.; Liu, S.; Yang, S.; Song, X.; Ding, B.; Li, Z.; Fang, J. *ACS Nano* **2011**, 5, (12), 9442-9449.
45. Lee, S. J.; Baik, J. M.; Moskovits, M. *Nano Letters* **2008**, 8, (10), 3244-3247.
46. Goh, M. S.; Lee, Y. H.; Pedireddy, S.; Phang, I. Y.; Tjiu, W. W.; Tan, J. M. R.; Ling, X. Y. *Langmuir* **2012**, 28, (40), 14441-14449.
47. Romo-Herrera, J. M.; Alvarez-Puebla, R. A.; Liz-Marzan, L. M. *Nanoscale* **2011**, 3, (4), 1304-1315.
48. Chirumamilla, M.; Toma, A.; Gopalakrishnan, A.; Das, G.; Zaccaria, R. P.; Krahne, R.; Rondanina, E.; Leoncini, M.; Liberale, C.; De Angelis, F.; Di Fabrizio, E. *Advanced Materials* **2014**.
49. Osberg, K. D.; Rycenga, M.; Harris, N.; Schmucker, A. L.; Langille, M. R.; Schatz, G. C.; Mirkin, C. A. *Nano Letters* **2012**, 12, (7), 3828-3832.
50. Xu, H.; Käll, M. *ChemPhysChem* **2003**, 4, (9), 1001-1005.
51. Chen, G.; Wang, Y.; Yang, M.; Xu, J.; Goh, S. J.; Pan, M.; Chen, H. *J. Am. Chem. Soc.* **2010**, 132, (11), 3644-3645.
52. McMahon, J.; Henry, A.-I.; Wustholz, K.; Natan, M.; Freeman, R. G.; Duyne, R.; Schatz, G. *Analytical and Bioanalytical Chemistry* **2009**, 394, (7), 1819-1825.
53. Kang, T.; Yoo, S. M.; Yoon, I.; Lee, S. Y.; Kim, B. *Nano Letters* **2010**, 10, (4), 1189-1193.
54. Dong-Kwon Lim, K.-S. J., Hyung Min Kim, Jwa-Min Nam & Yung Doug Suh. *Nature Materials* **2010**, 9, 60-67.
55. Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P.; Schultz, P. G. *Nature* **1996**, 382, (6592), 609-611.

56. Dong-Kwon Lim, K.-S. J., Jae-Ho Hwang, Hyoki Kim, Sunghoon Kwon, Yung Doug Suh & Jwa-Min Nam. *Nature Nanotechnology* **2011**, 6, 452–460.
57. Song, J.; Duan, B.; Wang, C.; Zhou, J.; Pu, L.; Fang, Z.; Wang, P.; Lim, T. T.; Duan, H. *J. Am. Chem. Soc.* **2014**.
58. Knight, M. W.; Grady, N. K.; Bardhan, R.; Hao, F.; Nordlander, P.; Halas, N. J. *Nano Letters* **2007**, 7, (8), 2346-2350.
59. Zhou, J.; Duan, B.; Fang, Z.; Song, J.; Wang, C.; Messersmith, P. B.; Duan, H. *Advanced Materials* **2013**, 701-705.
60. Caldwell, J. D.; Glembocki, O.; Bezares, F. J.; Bassim, N. D.; Rendell, R. W.; Feygelson, M.; Ukaegbu, M.; Kasica, R.; Shirey, L.; Hosten, C. *ACS Nano* **2011**, 5, (5), 4046-4055.
61. Gopinath, A.; Boriskina, S. V.; Premasiri, W. R.; Ziegler, L.; Reinhard, B. r. M.; Dal Negro, L. *Nano Letters* **2009**, 9, (11), 3922-3929.
62. Haynes, C. L.; Van Duyne, R. P. *The Journal of Physical Chemistry B* **2001**, 105, (24), 5599-5611.
63. Cho, W. J.; Kim, Y.; Kim, J. K. *ACS Nano* **2011**, 6, (1), 249-255.
64. Ming, T.; Kou, X.; Chen, H.; Wang, T.; Tam, H.-L.; Cheah, K.-W.; Chen, J.-Y.; Wang, J. *Angewandte Chemie International Edition* **2008**, 47, (50), 9685-9690.
65. Lee, S. H.; Liddell, C. M. *Small* **2009**, 5, (17), 1957-1962.
66. Tang, S. K. Y.; Derda, R.; Mazzeo, A. D.; Whitesides, G. M. *Advanced Materials* **2011**, 23, (21), 2413-2418.
67. Kim, H.-Y.; Lee, M.; Park, K. J.; Kim, S.; Mahadevan, L. *Nano Letters* **2010**, 10, (6), 2138-2140.
68. Murali, S.; Xu, T.; Marshall, B. D.; Kayatin, M. J.; Pizarro, K.; Radhakrishnan, V. K.; Nepal, D.; Davis, V. A. *Langmuir* **2010**, 26, (13), 11176-11183.

69. Zhang, Q.; Gupta, S.; Emrick, T.; Russell, T. P. *J. Am. Chem. Soc.* **2006**, 128, (12), 3898-3899.
70. Prinz, J.; Schreiber, B.; Olejko, L.; Oertel, J.; Rackwitz, J.; Keller, A.; Bald, I. *The Journal of Physical Chemistry Letters* **2013**, 4, (23), 4140-4145.
71. Pilo-Pais, M.; Watson, A.; Demers, S.; LaBean, T. H.; Finkelstein, G. *Nano Letters* **2014**, 14, (4), 2099-2104.
72. Kühler, P.; Roller, E.-M.; Schreiber, R.; Liedl, T.; Lohmüller, T.; Feldmann, J. *Nano Letters* **2014**.
73. Malaquin, L.; Kraus, T.; Schmid, H.; Delamarche, E.; Wolf, H. *Langmuir* **2007**, 23, (23), 11513-11521.
74. Srivastava, S.; Kotov, N. A. *Accounts of Chemical Research* **2008**, 41, (12), 1831-1841.
75. Coe-Sullivan, S.; Steckel, J. S.; Woo, W. K.; Bawendi, M. G.; Bulović, V. *Advanced Functional Materials* **2005**, 15, (7), 1117-1124.
76. Cote, L. J.; Kim, F.; Huang, J. *J. Am. Chem. Soc.* **2008**, 131, (3), 1043-1049.
77. Liu, J.-W.; Zhu, J.-H.; Zhang, C.-L.; Liang, H.-W.; Yu, S.-H. *J. Am. Chem. Soc.* **2010**, 132, (26), 8945-8952.
78. Li, Y.-J.; Huang, W.-J.; Sun, S.-G. *Angewandte Chemie International Edition* **2006**, 45, (16), 2537-2539.
79. Schulz, K. M.; Abb, S.; Fernandes, R.; Abb, M.; Kanaras, A. G.; Muskens, O. L. *Langmuir* **2012**, 28, (24), 8874-8880.
80. Kim, B.; Tripp, S. L.; Wei, A. *J. Am. Chem. Soc.* **2001**, 123, (32), 7955-7956.
81. Duan, H.; Wang, D.; Kurth, D. G.; Möhwald, H. *Angewandte Chemie International Edition* **2004**, 43, (42), 5639-5642.

82. Reincke, F.; Hickey, S. G.; Kegel, W. K.; Vanmaekelbergh, D. *Angewandte Chemie International Edition* **2004**, 43, (4), 458-462.
83. Liu, J.-W.; Zhang, S.-Y.; Qi, H.; Wen, W.-C.; Yu, S.-H. *Small* **2012**, 8, (15), 2412-2420.
84. Zhang, S.-Y.; Regulacio, M. D.; Han, M.-Y. *Chemical Society Reviews* **2014**.
85. Lin, Y.; Skaff, H.; Emrick, T.; Dinsmore, A. D.; Russell, T. P. *Science* **2003**, 299, (5604), 226-229.
86. Younjin Min, M. A., Kai Kristiansen, Yuval Golan & Jacob Israelachvili. *Nature materials* **2008**, 7, 527-538.
87. Michael P. Cecchini, V. A. T., Jack Paget, Alexei A. Kornyshev, Joshua B. Edel. *Nature materials* **2013**, 12, 165-171.
88. Konrad, M. P.; Doherty, A. P.; Bell, S. E. J. *Analytical Chemistry* **2013**, 85(14), 6783-6789.
89. Kyungsuk Yum, Soon Gweon Hong, Kevin E. Healy, Luke P. Lee. Physiologically ...Kihoon Kim, Hye Soo Han, Inhee Choi, Chiwon Lee, SoonGweon Hong, Sang-Hee Suh, Luke P Lee, Taewook Kang. Interfacial Liquid-state Surface-enhanced Raman Spectroscopy, *Nature Communications*, **2013**, 4, 2182
90. Shi, H.-Y.; Hu, B.; Yu, X.-C.; Zhao, R.-L.; Ren, X.-F.; Liu, S.-L.; Liu, J.-W.; Feng, M.; Xu, A.-W.; Yu, S.-H. *Advanced Functional Materials* **2010**, 20, (6), 958-964.
91. Zhang, S.-Y.; Liu, J.-W.; Zhang, C.-L.; Yu, S.-H. *Nanoscale* **2013**, 5, (10), 4223-4229.
92. Scallan, E. H., R. M.; Angulo, F. J.; Tauxe, R. V.; Widdowson, M.-A.; Roy, S.L.; Jones, J. L.; Griffin, P. M. *Emerging Infect. Dis* **2011**, 17, (7).

93. Xiaomeng, W.; Jing, C.; Bosoon, P.; Yao-Wen, H.; Yiping, Z., The Use of Silver Nanorod Array-Based Surface-Enhanced Raman Scattering Sensor for Food Safety Applications. In *Advances in Applied Nanotechnology for Agriculture*, American Chemical Society: **2013**, Vol. 1143, pp 85-108.
94. Peng, B.; Li, G.; Li, D.; Dodson, S.; Zhang, Q.; Zhang, J.; Lee, Y. H.; Demir, H. V.; Yi Ling, X.; Xiong, Q. *ACS Nano* **2013**, 7 (7), 5993-6000.
95. Yazdi, S. H.; White, I. M. *Analytical Chemistry* **2012**, 84, (18), 7992-7998.
96. Cheung, W.; Shadi, I. T.; Xu, Y.; Goodacre, R. *The Journal of Physical Chemistry C* **2010**, 114, (16), 7285-7290.
97. Liu, B.; Lin, M.; Li, H. *Sens. & Instrumen. Food Qual.* **2010**, 4, (1), 13-19.
98. Agata, N.; Ohta, M.; Yokoyama, K. *International Journal of Food Microbiology* **2002**, 73, (1), 23-27.
99. Jae Soo Kang, S. Y. H., Chul Jae Lee, Mu Sang Lee. *Bulletin of the Korean Chemical Society* **2002**, 23, (11), 1604-1610.
100. He, S.; Liu, K.-K.; Su, S.; Yan, J.; Mao, X.; Wang, D.; He, Y.; Li, L.-J.; Song, S.; Fan, C. *Analytical Chemistry* 2012, 84, (10), 4622-4627.
101. Craig, A. P.; Franca, A. S.; Irudayaraj, J. *Annual Review of Food Science and Technology* **2013**, 4, (1), 369-380.
102. Swaminathan, B.; Feng, P. *Annual Review of Microbiology* **1994**, 48, (1), 401-426.
103. Zhang, L.; Jiang, C.; Zhang, Z. *Nanoscale* **2013**, 5, (9), 3773-3779.
104. Krieger, R., A toxicological assessment of sulfur as a pesticide. In *Hayes's handbook of pesticide toxicology*, Academic Press: **2010**; Vol. 1, p 1889.
105. Cheng, Y.; Dong, Y.; Wu, J.; Yang, X.; Bai, H.; Zheng, H.; Ren, D.; Zou, Y.; Li, M. *Journal of Food Composition and Analysis* **2010**, 23, (2), 199-202.

106. Cheng, Y.; Dong, Y. *Food Control* **2011**, 22, (5), 685-689.
107. Zhu, L.; Gamez, G.; Chen, H.; Chingin, K.; Zenobi, R. *Chemical Communications* **2009**, (5), 559-561.
108. Ai, K.; Liu, Y.; Lu, L. *J. Am. Chem. Soc.* **2009**, 131, (27), 9496-9497.
109. Malaj, N.; Ouyang, Z.; Sindona, G.; Cooks, R. G. *Analytical Methods* **2012**, 4, (7), 1913-1919.
110. Baffou, G.; Quidant, R. *Chemical Society Reviews* **2014**, 43 (11), 3898-3907.
111. Preciado-Flores, S.; Wheeler, D. A.; Tran, T. M.; Tanaka, Z.; Jiang, C.; Barboza-Flores, M.; Qian, F.; Li, Y.; Chen, B.; Zhang, J. Z. *Chemical Communications* **2011**, 47, (14), 4129-4131.
112. Chen, L.-M.; Liu, Y.-N. *Journal of Raman Spectroscopy* **2012**, 43, (8), 986-991.
113. Sun, Y.; Yin, Y.; Mayers, B. T.; Herricks, T.; Xia, Y. *Chemistry of Materials* **2002**, 14, (11), 4736-4745.
114. Gao, Y.; Jiang, P.; Liu, D. F.; Yuan, H. J.; Yan, X. Q.; Zhou, Z. P.; Wang, J. X.; Song, L.; Liu, L. F.; Zhou, W. Y.; Wang, G.; Wang, C. Y.; Xie, S. S.; Zhang, J. M.; Shen, D. Y. *The Journal of Physical Chemistry B* **2004**, 108, (34), 12877-12881.
115. Pedireddy, S.; Li, A.; Bosman, M.; Phang, I. Y.; Li, S.; Ling, X. Y. *The Journal of Physical Chemistry C* **2013**, 117, (32), 16640-16649.
116. Qingbo, Z.; Jim Yang, L.; Jun, Y.; Chris, B.; Jixuan, Z. *Nanotechnology* **2007**, 18, (24), 245605.
117. Sun, Y.; Xia, Y. *Nano Letters* **2003**, 3, (11), 1569-1572.
118. Yuan, H.; Ma, W.; Chen, C.; Zhu, H.; Gao, X.; Zhao, J. *The Journal of Physical Chemistry C* **2011**, 115, (47), 23256-23260.
119. Bi, Y.; Hu, H.; Lu, G. *Chemical Communications* **2010**, 46, (4), 598-600.

120. Fu, H.; Yang, X.; Jiang, X.; Yu, A. *Langmuir* **2013**, 29, (23), 7134-7142.
121. Zhang, J.; Li, X.; Sun, X.; Li, Y. *The Journal of Physical Chemistry B* **2005**, 109, (25), 12544-12548.
122. Tao, A.; Kim, F.; Hess, C.; Goldberger, J.; He, R.; Sun, Y.; Xia, Y.; Yang, P. *Nano Letters* **2003**, 3, (9), 1229-1233.
123. Kelly, K. L.; Coronado, E.; Zhao, L. L.; Schatz, G. C. *The Journal of Physical Chemistry B* **2002**, 107, (3), 668-677.
124. Quinten, M.; Leitner, A.; Krenn, J. R.; Aussenegg, F. R. *Opt. Lett.* **1998**, 23, (17), 1331-1333.
125. Mulvihill, M. J.; Ling, X. Y.; Henzie, J.; Yang, P. *J. Am. Chem. Soc.* **2009**, 132, (1), 268-274.
126. Shim, J. H.; Yang, J.; Kim, S.-j.; Lee, C.; Lee, Y. *Journal of Materials Chemistry* **2012**, 22, (30), 15285-15290.
127. Srnová-Šloufová, I.; Lednický, F.; Gemperle, A.; Gemperlová, J. *Langmuir* **2000**, 16, (25), 9928-9935.
128. Liu, B.; Han, G.; Zhang, Z.; Liu, R.; Jiang, C.; Wang, S.; Han, M.-Y. *Analytical Chemistry* **2011**, 84, (1), 255-261.
129. Kim, A.; Barcelo, S. J.; Williams, R. S.; Li, Z. *Analytical Chemistry* **2012**, 84, (21), 9303-9309.
130. Lin, M.; He, L.; Awika, J.; Yang, L.; Ledoux, D. R.; Li, H.; Mustapha, A. *Journal of Food Science* **2008**, 73, (8), T129-T134.
131. Xie, W.; Schlucker, S. *Physical Chemistry Chemical Physics* **2013**, 15, (15), 5329-5344.
132. Song, J.; Zhou, J.; Duan, H. *J. Am. Chem. Soc.* **2012**, 134, (32), 13458-13469.

133. Stuart, D. A.; Yuen, J. M.; Shah, N.; Lyandres, O.; Yonzon, C. R.; Glucksberg, M. R.; Walsh, J. T.; Van Duyne, R. P. *Analytical Chemistry* **2006**, 78, (20), 7211-7215.
134. Lin, M., The Application of Surface-Enhanced Raman Spectroscopy to Identify and Quantify Chemical Adulterants or Contaminants in Foods. In *Handbook of Vibrational Spectroscopy*, John Wiley & Sons, Ltd: **2006**.
135. López-Tocón, I.; Otero, J. C.; Arenas, J. F.; Garcia-Ramos, J. V.; Sanchez-Cortes, S. *Analytical Chemistry* **2011**, 83, (7), 2518-2525.
136. Yin, J.; Wu, T.; Song, J.; Zhang, Q.; Liu, S.; Xu, R.; Duan, H. *Chemistry of Materials* **2011**, 23, (21), 4756-4764.
137. Alvarez-Puebla, R. A.; Liz-Marzán, L. M. *Small* **2010**, 6, (5), 604-610.
138. Camden, J. P.; Dieringer, J. A.; Zhao, J.; Van Duyne, R. P. *Accounts of Chemical Research* **2008**, 41, (12), 1653-1661.
139. Lu, G.; Li, H.; Zhang, H. *Small* **2012**, 8, (9), 1336-1340.
140. Yan, B.; Thubagere, A.; Premasiri, W. R.; Ziegler, L. D.; Dal Negro, L.; Reinhard, B. r. M. *ACS Nano* **2009**, 3, (5), 1190-1202.
141. Etchegoin, E. C. L. R. a. P. G., *Principles of Surface-Enhanced Raman Spectroscopy* **2009**.
142. Zheng, Y.; Thai, T.; Reineck, P.; Qiu, L.; Guo, Y.; Bach, U. *Advanced Functional Materials* **2013**, 23, (12), 1519-1526.
143. Wen, T.; Majetich, S. A. *ACS Nano* **2011**, 5, (11), 8868-8876.
144. Chen, A.; DePrince, A. E.; Demortière, A.; Joshi-Imre, A.; Shevchenko, E. V.; Gray, S. K.; Welp, U.; Vlasko-Vlasov, V. K. *Small* **2011**, 7, (16), 2365-2371.
145. 145. Kian Ping Loh, Q. B., Goki Eda & Manish Chhowalla. *Nature Chemistry* **2010**, 2, 1015-1024.

146. Xie, L.; Ling, X.; Fang, Y.; Zhang, J.; Liu, Z. *J. Am. Chem. Soc.* **2009**, 131, (29), 9890-9891.
147. Huang, J.; Gao, X.; Jia, J.; Kim, J.-K.; Li, Z. *Analytical Chemistry* **2014**, 86 (17), 8506-8513.
148. Liu, X.; Cao, L.; Song, W.; Ai, K.; Lu, L. *ACS Appl. Mater. Interfaces* **2011**, 3, (8), 2944-2952.
149. Zhang, S.; Shao, Y.; Liao, H.-g.; Liu, J.; Aksay, I. A.; Yin, G.; Lin, Y. *Chemistry of Materials* **2011**, 23, (5), 1079-1081.
150. Wang, P.; Liang, O.; Zhang, W.; Schroeder, T.; Xie, Y.-H. *Advanced Materials* **2013**, 25(35): 4918-4924.
151. He, S.; Song, B.; Li, D.; Zhu, C.; Qi, W.; Wen, Y.; Wang, L.; Song, S.; Fang, H.; Fan, C. *Advanced Functional Materials* **2010**, 20, (3), 453-459.
152. Cheng, L.; Liu, A. P.; Peng, S.; Duan, H. W. *Acs Nano* **2010**, 4, (10), 6098-6104.
153. Xiao, F.; Song, J.; Gao, H.; Zan, X.; Xu, R.; Duan, H. *ACS Nano* **2011**, 6, (1), 100-110.
154. Wu, M.; Kempaiah, R.; Huang, P.-J. J.; Maheshwari, V.; Liu, J. *Langmuir* **2011**, 27, (6), 2731-2738.
155. Lu, C.-H.; Yang, H.-H.; Zhu, C.-L.; Chen, X.; Chen, G.-N. *Angewandte Chemie International Edition* **2009**, 48, (26), 4785-4787.
156. Mei, Q.; Zhang, Z. *Angewandte Chemie International Edition* **2012**, 51, (23), 5602-5606.
157. Fang, Z.; Wang, Y.; Song, J.; Sun, Y.; Zhou, J.; Xu, R.; Duan, H. *Nanoscale* **2013**, 5(20), 9830-9838.

158. Gao, H.; Xiao, F.; Ching, C. B.; Duan, H. *ACS Appl. Mater. Interfaces* **2012**, 4, (5), 2801-2810.
159. Sánchez-Iglesias, A.; Carbó-Argibay, E.; Glaria, A.; Rodríguez-González, B.; Pérez-Juste, J.; Pastoriza-Santos, I.; Liz-Marzán, L. M. *Chemistry – A European Journal* **2010**, 16, (19), 5558-5563.
160. Yang, X.; Gu, C.; Qian, F.; Li, Y.; Zhang, J. Z. *Analytical Chemistry* **2011**, 83, (15), 5888-5894.
161. Shen, A.; Guo, J.; Xie, W.; Sun, M.; Richards, R.; Hu, J. *Journal of Raman Spectroscopy* **2011**, 42, (4), 879-884.
162. Faulds, K.; Smith, W. E.; Graham, D. *Analytical Chemistry* **2003**, 76, (2), 412-417.
163. Yazdi, S. H.; Giles, K. L.; White, I. M. *Analytical Chemistry* **2013**, 85, (21), 10605-10611.
164. Batt, C. A. *Science* **2007**, 316, (5831), 1579-1580.
165. Peleg, A. Y.; Hooper, D. C. *New England Journal of Medicine* **2010**, 362, (19), 1804-1813.
166. Gilligan, P. H. *Current Opinion in Microbiology* **2002**, 5, (5), 489-495.
167. Maalouf, R.; Fournier-Wirth, C.; Coste, J.; Chebib, H.; Saïkali, Y.; Vittori, O.; Errachid, A.; Cloarec, J.-P.; Martelet, C.; Jaffrezic-Renault, N. *Analytical Chemistry* **2007**, 79, (13), 4879-4886.
168. Hoshino, T.; Kawaguchi, M.; Shimizu, N.; Hoshino, N.; Ooshima, T.; Fujiwara, T. *Diagnostic Microbiology and Infectious Disease* 48, (3), 195-199.
169. Zuo, X.; Xia, F.; Xiao, Y.; Plaxco, K. W. *J. Am. Chem. Soc.* **2010**, 132, (6), 1816-1818.

170. Sato, K.; Hosokawa, K.; Maeda, M. *J. Am. Chem. Soc.* **2003**, 125, (27), 8102-8103.
171. Zhang, H.; Harpster, M. H.; Park, H. J.; Johnson, P. A.; Wilson, W. C. *Analytical Chemistry* **2010**, 83, (1), 254-260.
172. Qian, X.; Zhou, X.; Nie, S. *J. Am. Chem. Soc.* **2008**, 130, (45), 14934-14935.
173. Osinkina, L.; Lohmüller, T.; Jäckel, F.; Feldmann, J. *The Journal of Physical Chemistry C* **2013**, 117 (43), 22198–22202.
174. Haes, A. J.; Zou, S.; Schatz, G. C.; Van Duyne, R. P. *The Journal of Physical Chemistry B* **2004**, 108, (22), 6961-6968.
175. Dondapati, S. K.; Sau, T. K.; Hrelescu, C.; Klar, T. A.; Stefani, F. D.; Feldmann, J. *Acs Nano* **2010**, 4, (11), 6318-6322.
176. Fales, A. M.; Yuan, H.; Vo-Dinh, T. *Langmuir* **2011**, 27, (19), 12186-12190.
177. Yuan, H.; Liu, Y.; Fales, A. M.; Li, Y. L.; Liu, J.; Vo-Dinh, T. *Analytical Chemistry* **2012**, 85, (1), 208-212.
178. Oldenburg, S. J.; Averitt, R. D.; Westcott, S. L.; Halas, N. J. *Chemical Physics Letters* **1998**, 288, (2–4), 243-247.
179. Mahajan, S.; Richardson, J.; Brown, T.; Bartlett, P. N. *J. Am. Chem. Soc.* **2008**, 130, (46), 15589-15601.
180. Hu, J.; Zhang, C.-y. *Biosensors and Bioelectronics* **2012**, 31, (1), 451-457.
181. Barbosa, S.; Agrawal, A.; Rodríguez-Lorenzo, L.; Pastoriza-Santos, I.; Alvarez-Puebla, R. n. A.; Kornowski, A.; Weller, H.; Liz-Marzán, L. M. *Langmuir* **2010**, 26, (18), 14943-14950.
182. Pham, T. A.; Choi, B. C.; Lim, K. T.; Jeong, Y. T. *Applied Surface Science* **2011**, 257, (8), 3350-3357.

183. Zhou, X.; Huang, X.; Qi, X.; Wu, S.; Xue, C.; Boey, F. Y. C.; Yan, Q.; Chen, P.; Zhang, H. *The Journal of Physical Chemistry C* **2009**, 113, (25), 10842-10846.
184. Zhang, Y.; Liu, S.; Lu, W.; Wang, L.; Tian, J.; Sun, X. *Catalysis Science & Technology* **2011**, 1, (7), 1142-1144.
185. Choi, Y.; Bae, H. S.; Seo, E.; Jang, S.; Park, K. H.; Kim, B.-S. *Journal of Materials Chemistry* **2011**, 21, (39), 15431-15436.
186. Lu, W.; Ning, R.; Qin, X.; Zhang, Y.; Chang, G.; Liu, S.; Luo, Y.; Sun, X. *Journal of Hazardous Materials* **2011**, 197, (0), 320-326.
187. Huang, J.; Zhang, L.; Chen, B.; Ji, N.; Chen, F.; Zhang, Y.; Zhang, Z. *Nanoscale* **2010**, 2, (12), 2733-2738.
188. Hummers, W. S.; Offeman, R. E. *J. Am. Chem. Soc.* **1958**, 80, (6), 1339-1339.
189. Gao, H.; Xiao, F.; Ching, C. B.; Duan, H. *ACS Appl. Mater. Interfaces* **2011**, 3, (8), 3049-3057.
190. Goncalves, G.; Marques, P. A. A. P.; Granadeiro, C. M.; Nogueira, H. I. S.; Singh, M. K.; Grácio, J. *Chemistry of Materials* **2009**, 21, (20), 4796-4802.
191. Lerf, A.; He, H.; Forster, M.; Klinowski, J. *The Journal of Physical Chemistry B* **1998**, 102, (23), 4477-4482.
192. Li, L.; Wu, G.; Xu, B.-Q. *Carbon* **2006**, 44, (14), 2973-2983.
193. Zhang, G.; Sun, S.; Yang, D.; Dodelet, J.-P.; Sacher, E. *Carbon* **2008**, 46, (2), 196-205.
194. Yang, D.-Q.; Rochette, J.-F.; Sacher, E. *The Journal of Physical Chemistry B* **2005**, 109, (16), 7788-7794.
195. Paredes, J. I.; Villar-Rodil, S.; Martínez-Alonso, A.; Tascón, J. M. D. *Langmuir* **2008**, 24, (19), 10560-10564.

196. Pandian Senthil, K.; Isabel, P.-S.; Benito, R.-G.; Abajo, F. J. G. d.; Luis, M. L.-M. *Nanotechnology* **2008**, 19, (1), 015606.
197. Ling, X.; Xie, L.; Fang, Y.; Xu, H.; Zhang, H.; Kong, J.; Dresselhaus, M. S.; Zhang, J.; Liu, Z. *Nano Letters* **2009**, 10, (2), 553-561.
198. Sun, S.; Wu, P. *Physical Chemistry Chemical Physics* **2011**, 13, (47), 21116-21120.
199. Huang, J.; Zhu, Y.; Lin, M.; Wang, Q.; Zhao, L.; Yang, Y.; Yao, K. X.; Han, Y. *J. Am. Chem. Soc.* **2013**, 135, (23), 8552-8561.
200. Yang, X. M.; Tryk, D. A.; Hasimoto, K.; Fujishima, A. *Applied Physics Letters* **1996**, 69, (26), 4020-4022.
201. Huang, Y.-F.; Zhu, H.-P.; Liu, G.-K.; Wu, D.-Y.; Ren, B.; Tian, Z.-Q. *J. Am. Chem. Soc.* **2010**, 132, (27), 9244-9246.
202. Jasuja, K.; Linn, J.; Melton, S.; Berry, V. *The Journal of Physical Chemistry Letters* **2010**, 1, (12), 1853-1860.

List of Publications

1. **Duan B**, Zhou J, Fang Z, Lam Z.Q, Wang X, Ng C-L, Li C,* Hemond, H.F.,* Duan H,* Surface-Enhanced Raman Scattering by Graphene-Nanosheet-Gapped Plasmonic Nanoparticle Arrays for Multiplexed Genetic Detection of Food-Borne Pathogens. (Submitted to *Nanoscale*)
2. **Duan B**, Pu L, Duan H. Raman-Active Plasmonic nanostructures for pathogen detection. (to be submitted to *Future Medicine*)
3. **Duan B**, Zhou J, Hemond, H.F.,* Duan H,* Chemical processing of interfacial assembly of metal nanowires for SERS detection of food contaminants. (to be submitted to *Analytical Chemistry*)
4. **Duan B**, Duan H,* “Particles-on-an-Array” SERS Assay: Combining Magnetoplasmonic Nanoparticles and Engineered Plasmonic Nanoarrays for Multiplexed Genetic Detection of Bacterial Pathogen. (to be submitted to *JACS*)
5. **Duan B**, Duan H,* Seeded Growth of Gold Nanostar array on Graphene Oxide for Surface Enhanced Raman Scattering and Catalysis. (to be submitted to *Langmuir*)
6. Zhou, J. J.; **Duan, B.**; Song, J. B.; Wang, C. X.; Duan, H. W. Interfacial Assembly of Mussel-Inspired Au@Ag@ Polydopamine Core–Shell Nanoparticles for Recyclable Nanocatalysts. *Adv. Mater.* 2014, 26, 701-705
7. Song, J. B; **Duan, B.**; Wang, C.; Duan, H. W. SERS-Encoded Nanogapped Plasmonic Nanoparticles: Growth of Metallic Nanoshell by Templating Redox-Active Polymer Brushes. *J. Am. Chem. Soc.*, 2014, 136, 6838–6841.
8. Song, J. B.; Pu, L.; Zhou, J. J.; **Duan, B.**; Duan, H. W. Biodegradable Theranostic Plasmonic Vesicles of Amphiphilic Gold Nanorods. *ACS Nano*. 2013, 7, 9947-9960.

9. Song, J. B.; Fang, Z.; Wang, C. X.; Zhou, J. J.; **Duan, B.**; Pu, L.; Duan, H. W.
Photolabile plasmonic vesicles assembled from amphiphilic gold nanoparticles for
remote-controlled traceable drug delivery. *Nanoscale*. 2013, 13, 5816–5824.