**Examining the gilded cage:
synthesis, optical properties and plasmonic applications
of gold nanocages**

By

Alyssa Staff

A thesis submitted to the Department of Chemistry
in partial fulfillment of the requirements for the degree of
Master of Science

To

Carleton University
Department of Chemistry
Ottawa, Ontario, Canada

April 2014

© Copyright 2014, Alyssa Staff

The undersigned hereby recommends to the
Department of Chemistry
acceptance of the thesis

**Examining the gilded cage:
synthesis, optical properties and plasmonic applications
of gold nanocages**

Submitted by
Alyssa Staff

in partial fulfillment of the requirements for the degree of
Master of Science

Dr. Anatoli Ianoul
(Supervisor)

Abstract

The methods of synthesis and characterization of the optical properties of gold nanocages produced by templated galvanic exchange of silver nanocubes is reported. The effect of surface modifications and substrate interactions were observed using ultra-violet, visible and near-infrared spectrophotometry and fluorescence spectroscopy. The refractive index sensitivity of the gold nanocage structures was altered using various combinations of conditions including size, shape, surface functionalization, substrate support, and capping with dielectric shells. Atomic force, scanning electron, and transmission electron microscopic methods were used for imaging and characterization of the gold nanocages. The potential applications of gold nanocages with various environmental and surface conditions were investigated based on the ability to tune the localized surface plasmon resonance modes to a series of desired wavelengths and bandwidths. Collaboration with the Albert group (Department of Electronics) was carried out with the successful assembly, as well as limit of detection and sensitivity improvements, of a protein biosensor supported on a titled fibre Bragg grating. Investigations into the fluorescence quenching effects of silica-capped gold nanocages was carried out using a tailored selection of fluorescent dyes to enable potential enhancement and/or quenching using the same nanostructure. The optical properties of gold nanocages allow for many potential uses of the same nanostructure in a wide variety of applications, including biomedical and biosensing applications.

Acknowledgements

My thanks go to Dr. Anatoli Ianoul for direction and encouragement throughout the duration of this program, and to the faculty and staff of the Department of Chemistry for providing an understanding and helpful work environment.

My thanks go to all of the professors with whom I had the privilege to work and with those that gave the opportunity to further my knowledge in this field with classes. I greatly appreciate the opportunity to be continuously learning and applying the effort I have made to reach this point in my education and career.

Perhaps last, but certainly not least, many thanks go out to my friends, family, colleagues and sympathetic graduate students for support during the toiling and late hours of graduate research.

Table of Contents

Abstract	iii
Acknowledgements	iv
Equations, Tables and Figures	vii
Glossary of Abbreviations and Acronyms	ix
1 Introduction	1
1.1 Objectives of This Study	2
1.2 Interactions with Light	3
1.2.1 Localized Surface Plasmon Resonance	3
1.2.2 Applications of LSPR	6
1.2.2a) Refractive Index Sensing	7
1.2.2b) Biosensors	16
1.2.2c) Heating	18
1.2.2d) Fluorescence Enhancement	22
1.2.2e) Fibre Optics and Optical Fibre Sensors	28
1.2.2f) Photovoltaics	29
1.2.2g) Other Potential Applications	31
1.3 Choice of Material	34
1.4 Synthesis Methods	35
1.4.1 Polyol Synthesis for Silver Nanostructures	36
1.4.1a) Metal Precursor	37
1.4.1b) Capping Agent	38
1.4.1c) Trace Materials	39
1.4.2 Galvanic Displacement for Gold Nanostructures	40
1.5 Surface Modifications Made to Gold Nanocages	42
1.5.1 Alkyl-Chain Functionalization (HDT)	42
1.5.2 Core-Shell Modifications (Silica-Coating)	44
1.6 Preparation for Intended Applications	46
1.6.1 Deposition Methods	46
1.6.1a) Langmuir-Blodgett Technique	47
1.6.1b) Self-Assembly Method	50
1.6.2 Fluorescence Investigations	52
1.6.3 Refractive Index Sensitivity Determination	54
1.7 Characterization Methods	55
1.7.1 Ultraviolet-Visible and Near-Infrared Spectroscopy	55
1.7.2 Transmission and Reflection Spectroscopy	57
1.7.2a) Slide-based Substrates	57
1.7.2b) Optical Fibre Substrates	58
1.7.3 Fluorescence Spectroscopy	60
1.7.4 Microscopic Methods	61

1.7.4a) Atomic Force Microscopy	62
1.7.4b) Scanning Electron Microscopy	62
1.7.4c) Transmission Electron Microscopy	63
2 Experimental.....	64
2.1 Synthesis of Silver Nanocubes.....	64
2.2 Synthesis of Gold Nanocages	66
2.3 Surface Modifications to Gold Nanocages	68
2.3.1 Hydrophobic Functionalization	68
2.3.2 Silica-Shell Formation	68
2.4 Monolayer Deposition of Gold Nanocages	71
2.4.1 Langmuir-Blodgett Technique	71
2.4.2 Self-Assembly Method	76
2.5 Preparation for Characterization	77
2.5.1 Absorbance: UV-vis and NIR Spectroscopy.....	77
2.5.2 Transmission and Reflection of Slide-Substrates.....	78
2.5.3 Refractive Index Sensitivity Determination.....	78
2.5.4 Fluorescence Spectroscopy	79
2.5.5 Microscopy: AFM, SEM and TEM	80
2.5.6 Transmission of Optical-Fibre Substrates	81
3 Results and Discussion	82
3.1 Syntheses: Effects of Size, Shape and Composition	82
3.2 Depositions: Substrate and Density Effects	90
3.3 Surface Modifications: Effects of Altering the Local Environment	103
3.3.1 Hydrophobic Functionalization	103
3.3.2 Core-Shell Formation: Silica Shell Formation	105
3.3.3 Silica Shell Etching Variations.....	114
3.4 Refractive Index Sensitivity Determinations	120
3.4.1 Colloidal Suspensions.....	121
3.4.2 Slide Substrate-Supported.....	123
3.5 Fluorescence Enhancement and/or Quenching	127
3.5.1 Proof-of-Concept: Using Available Resources	127
3.5.2 Effect of AuNC@SiO ₂ on Fluorescence Quenching: Tailored for LSPR Overlap.....	136
3.5.3 Potential for Time-Dependent Fluorescence Emission Effects	156
3.6 Application and Publication: Gold Nanocages on a TFBG-Based Biosensor	158
4 Conclusions	167
5 Future Works	171
6 References	173

Equations, Tables and Figures

Equation 1.1 – Lorentz-Lorenz equation	11
Equation 1.2 – Reduction of ethylene glycol	36
Equation 1.3 – Galvanic Exchange Reactions	40
Equation 1.4 – Beer-Lambert Law	56
Equation 1.5 – Beer's Law	58
Equation 1.6 – Quantum Yield Equation	61
Equation 3.1 – Galvanic Displacement Reaction	87
Table 1.1 – RIS and FOM of Gold Nanostructures	11
Table 1.2 – Fluorescent Dye Information	53
Table 1.3 – Quantum Yield of the Fluorescent Dyes	61
Table 2.1 – Modifications to Stöber Method	69
Table 3.1 – Stöber Method Modification Results	111
Table 3.2 – Refractive Indices of Solutions	120
Table 3.3 – RIS of Colloidal AuNC@SiO ₂	123
Table 3.4 – RIS of Substrate-Supported AuNC@SiO ₂	124
Table 3.5 – Quenching Data of Rh6G, Rh640 and LDS-751	135
Table 3.6 – Quenching Data of Rh800, RhB, NBC, Cryptocyanine	144
Figure 1.1 – Lycurgus Cup	2
Figure 1.2 – Schematic of LSPR	5
Figure 1.3 – Schematic of Induced Fields	6
Figure 1.4 – Visualization of Plasmon Modes	9
Figure 1.5 – LSPR by Nanostructure Shape	10
Figure 1.6 – Micrographs of Various Gold Nanostructures	21
Figure 1.7 – Lignum Nepphriticum, <i>pterocarpus indicus</i>	23
Figure 1.8 – Fluorescent Dye and LSPR Overlap	27
Figure 1.9 – Potential drug delivery mechanisms using nanostructures	33
Figure 1.10 – Binding of PVP versus Citrate	39
Figure 1.11 – Schematic of Galvanic Displacement	41
Figure 1.12 – Schematic Representations HDT Monolayers	44
Figure 1.13 – LB Deposition Illustration	47
Figure 1.14 – FDTD Determinations	49
Figure 1.15 – Alkoxysilane Structures	51
Figure 1.16 – TFBG Transmission Spectrum	59
Figure 2.1 – Synthesis Product of Gold Nanocages	67
Figure 2.2 – Clean Water Isotherm	72
Figure 2.3 – Gold Nanocage LB Isotherm	73
Figure 2.4 – Visualization of Gold Nanocage LB Monolayer Pressures	74
Figure 2.5 – Silicon Thin-Film and Glass Slide Substrates	75
Figure 3.1 – LSPR Progress of Silver Nanocube Synthesis	83
Figure 3.2 – LSPR Shift with Respect to Size	84
Figure 3.3 – LSPR Progress of Galvanic Displacement	86
Figure 3.4 – LSPR Changes from Silver Nanocubes to Gold Nanocages	88
Figure 3.5 – Micrographs of AuNC Synthesis Products	89
Figure 3.6 – LSPR Shift with Respect to Substrate Refractive Index	91

Figure 3.7 – LSPR Shift with Respect to Silicon Film Thickness	92
Figure 3.8 – Absorbance, Reflection and Transmission of gold nanocages	95
Figure 3.9 – Reflection Spectra, Low Density Monolayers	97
Figure 3.10 – Reflection Spectra, High Density Monolayers	98
Figure 3.11 – Trends of Reflection Intensity.....	99
Figure 3.12 – Trends of Transmission Intensity	100
Figure 3.13 – Transmission Spectra	101
Figure 3.14 – SEM and AFM Micrographs	102
Figure 3.15 – LSPR shift with Respect to HDT Coating	104
Figure 3.16 – TEM Micrograph of HDT-Capped Gold Nanocages	105
Figure 3.17 – SiO ₂ Coating Attempts	106
Figure 3.18 – TEM Micrographs of SiO ₂ Coating Attempts	106
Figure 3.19 – LSPR Shifts with Stöber Method Modifications	108
Figure 3.20 – TEM Micrograph of Bare Gold Nanocage	108
Figure 3.21 – TEM Micrographs of Further Stöber Method Modifications.....	109
Figure 3.22 – Further Developments of Modified Stöber Methods	112
Figure 3.23 – LSPR Shift During Etching	113
Figure 3.24 – LSPR of Gold Nanocage Products.....	115
Figure 3.25 – LSPR Observations During Continuous Etching Series.....	116
Figure 3.26 – Micrographs of Etching Progress.	118
Figure 3.27 – LSPR Observations During Sequential Etching Series.....	119
Figure 3.28 – RIS Determination of Colloidal Sample	122
Figure 3.29 – Images of HDT-Capped Monolayer.....	124
Figure 3.30 – RIS Determination of Substrate-Supported Sample	125
Figure 3.31 – Spectra Before/After RIS.....	126
Figure 3.32 – Rh6G Spectra versus AuNC LSPR	128
Figure 3.33 – Calibrated Emission Intensities of Rh6G	128
Figure 3.34 – Fluorescence quenching using uncoated gold nanocages	129
Figure 3.35 – Quenching of Rh6G from Modified Stöber Methods	130
Figure 3.36 – Rh640 Spectral Properties	131
Figure 3.37 – LDS-751 Spectral Properties	132
Figure 3.38 – Quenching of Rh640 by [AuNC].....	134
Figure 3.39 – Quenching of LDS-751 by [AuNC]	134
Figure 3.40 – RhB Spectral Properties.....	137
Figure 3.41 – Rh800 Spectral Properties	138
Figure 3.42 – NBC Spectral Properties	139
Figure 3.43 – Cryptocyanine Spectral Properties	140
Figure 3.44 – Quenching by Best Overlap	142
Figure 3.45 – 3D mapping of RhB Emissions.....	147
Figure 3.46 – 3D mapping of Rh800 and AuNC@SiO ₂ (15 nm shells)	148
Figure 3.47 – 3D mapping of Rh800 and AuNC@SiO ₂ (13 nm shells)	149
Figure 3.48 – 3D mapping of Rh800 and AuNC@SiO ₂ (10 nm shells)	150
Figure 3.49 - 3D mapping of Cryptocyanine and AuNC@SiO ₂ (13 nm shells)	151
Figure 3.50 - 3D mapping of Cryptocyanine and AuNC@SiO ₂ (10 nm shells)	152
Figure 3.51 - 3D mapping of NBC and AuNC@SiO ₂ (15 nm shells).....	153
Figure 3.52 - 3D mapping of NBC and AuNC@SiO ₂ (13 nm shells).....	154
Figure 3.53 – 3D mapping of NBC and AuNC@SiO ₂ (10 nm shells)	155
Figure 3.54 – Emission After Prolonged Exposure.....	157

Glossary of Abbreviations and Acronyms

AFM	– atomic force microscopy
AgNC	– silver nanocube
APTES	– (3-aminopropyl)-triethoxysilane
APTMS	– (3-aminopropyl)-trimethoxysilane
AuNC	– gold nanocages
AuNC@SiO ₂	– silica-capped gold nanocages
CTAB	– cetyl trimethylammonium bromide
DBFT	– double-beam fluorescence thermometry
DNA	– deoxyribonucleic acid
DSC	– differential scanning calorimetry
EDC	– N'-ethylcarbodiimide hydrochloride
EG	– ethylene glycol
ELISA	– enzyme-linked immunosorbent assay
FDTD	– finite-difference time-domain
FOM	– figure of merit
HDT	– hexadecanethiol
HMT	– N-hexamethylenetetramine
LB	– Langmuir-Blodgett
LDS-751	– also known as Styryl 8
LoD	– limit of detection
LSPR	– localized surface plasmon resonance
MPTS	– (3-mercaptopropyl)-trimethoxysilane
MUA	– 11-mercaptoundecanoic acid
NBC	– Nile Blue chloride
NHS	– N-hydroxysuccinimide
NIR	– near-infrared
PMAA	– poly(methacrylic acid)
PNIPAM	– poly(N-isopropylacrylamide)
poly-HEMA	– poly(2-hydroxyethyl methacrylate)
PVP	– polyvinylpyrrolidone
Rh640	– Rhodamine 640 (also known as Rhodamine 101, Rh101)
Rh800	– Rhodamine 800
Rh6G	– Rhodamine 6G
RhB	– Rhodamine B
RIS	– refractive index sensitivity
RIU	– refractive index unit
SAM	– self-assembled monolayer
SEM	– scanning electron microscopy
SERS	– surface-enhanced Raman spectroscopy
TEM	– transmission electron microscope
TEOS	– tetraethyl orthosilicate
TFBG	– tilted fibre Bragg grating
UV-vis	– ultraviolet-visible
VOC	– volatile organic compound

1 Introduction

Nanotechnology is a rapidly evolving and incredibly intriguing field of study. The use of nanomaterials in an astounding variety of applications allows for seemingly endless possibilities. Small variations in methods of preparation, use conditions, and type of material can have drastic effects on the properties of a nanomaterial, thus altering the potential range of applications. There are many areas of chemistry that are included under the general terminology of *nanotechnology research*. One of the many intriguing areas of research and application is plasmonic nanotechnology. The investigation of interactions between electromagnetic radiation and particles or materials of much smaller dimensions than the wavelength of the radiation has yielded the observation that these nanomaterials behave drastically different than their macro-sized or bulk counterparts. One of the first known observations of this phenomenon was the Lycurgus Cup. This glass chalice is observed as jade green in ambient light, but placing a light source within the chalice reveals a brilliant red in the glass (as seen in *Figure 1.1*). This is caused by gold and silver nanoparticles embedded in the glass, each approximately 70 nm in diameter.^{1,2}

This drastic change of observable properties is caused by localized surface plasmon resonance (LSPR). LSPR is the optical phenomenon arising from the collective oscillation of surface electrons in a metal nanostructure.³ The resonance of the electron density is directly affected by the size and composition of the nanostructure, as well as by the nature of the immediate environment. This affectation is the primary research interest in working with plasmonic nanomaterials as it defines an inherent tuneability of the range of absorption of

the material, which can be used in turn to optimize a given system of plasmonic nanoparticles for use in a particular application. Applications of particular interest include fluorescence enhancement, improvement of refractive index sensitivity, enhanced absorption for photovoltaic platforms, electromagnetic field-induced heating, and optical fibre-based biosensors.

Figure 1.1 – Lycurgus Cup: Light-induced colour change of Lycurgus Cup caused by gold and silver nanoparticles in the glass. Reprinted with permission from Macmillan Publishers Ltd: Nature Outlook, reference 1, copyright 2013.

1.1 Objectives of This Study

The principle of all LSPR applications is based on the interaction of light with the utilized assembly, thus a system wherein the surface material can be quickly and easily modified to change the range of said interaction could be versatile enough to provide a broad range of applications using the same material. The proposed material to achieve this versatility of applications for this project is cubic gold nanocages. The review of this investigation aims to

demonstrate the reasoning for choosing cubic gold nanocages as the primary structure and material for these potential applications. Each application has specific ranges of light interaction to achieve the desired ranges of results, thus this work is to explain how these ranges can be achieved using cubic gold nanocages. The proposed methods of tuning the LSPR range of the gold nanocages include surface modifications and controlled environmental alterations.

1.2 Interactions with Light

Richard Feynman's 1959 lecture "Plenty of Room at the Bottom" was a key inspirational challenge to scientists of the day to push the limitations of small-scale science beyond what they thought would be possible.⁴ The term "nanotechnology" was coined in 1974 by Dr. Norio Taniguchi in Japan to describe precision machining of materials to within atomic-scale dimensional tolerances.⁵ Since these beginnings, the field of research in nanoscale materials and technology has grown exponentially.

1.2.1 Localized Surface Plasmon Resonance

Plasmonic nanotechnology encompasses most of the research investigating interactions between metallic nanoparticles and light. This interest in plasmonic structures is based on the nanomaterial's ability to capture, concentrate and/or propagate light.² A plasmon is a collection of electron density able to oscillate relative to a fixed position of positive ions. Oscillations with observable amplitude are more commonly found in metals. This resonance is a

series of quantized oscillations that are induced by an electromagnetic field, typically within the range of ultraviolet to visible light. The nature of a metal's plasmon oscillation is the primary influence on a metal's optical properties. When the plasmon is confined to a small surface, however, the optical properties are drastically different from those observed in bulk metal. This is one of the main sources of interest in plasmonic nanomaterials. When metals are confined to the nanoscale in three dimensions, the plasmonic oscillation at the surface of the metal nanostructure is referred to as the *localized* surface plasmon resonance (LSPR)⁶ (see *Figure 1.2*). Plasmon oscillation also occurs in bulk metal, but for the purpose of interactions with electromagnetic fields only the surface electrons are considered. This is to account for the limited depth of interaction an electromagnetic field can have with a material. It has been found that the depth of penetration into bulk gold and silver structures extends to a maximum of 50 nm.⁷ For most applications in plasmonic nanotechnology, this maximum depth of influence is well within the bounds of at least one dimension of the nanostructure being utilized. Plasmonic materials are used to scatter and/or absorb light for a number of applications, as described in *Section 1.2.2*, or to enhance existing emission or absorption of a system into a more desired range of the electromagnetic spectrum. Noble metal nanoparticles have a series of unique properties, among which is their ability to support surface plasmons. Noble metals are those that can form stable solids of pure metal that are not easily oxidized (or at least not beyond a passivation layer of metal oxide at the surface of the solid), as opposed to many metals that are only stable as oxide formations.

Figure 1.2 – Schematic of LSPR: Visual representation of LSPR of metal nanostructure (not to scale) and induced electron cloud oscillation. Reproduced with permission from Elsevier Limited, reference 136, copyright 2010.

The impact of the interacting electromagnetic fields has been extensively studied in the field of computational nanotechnology. The class of problems allowing for analytical solutions to a number of surface plasmon conditions is collectively called “Mie theory”. Gustav Mie, in 1908, analytically solved the first problem⁸, that of plane waves interacting with a uniform sphere of a given relative dielectric constant surrounded by a uniform medium of a different given relative dielectric constant. This solution was the introduction to more complicated problems and conditions, of which all the solutions involve series of complex differential equations. For systems of particles so much smaller than the incident wavelength, simplified computational equations have been utilized and have yielded theoretical results in good accord with experimental results.² The approach most commonly used for theoretical computations of noble metal plasmonics is now finite-difference time-domain (FDTD). This allows for discrete steps of time to enable more easily resolved interpretations of the resonance of

the plasmonic structure being analyzed. It is from these theoretical calculations that the distance of influence from a nanostructure's surface can be known, as well as the corresponding electric field strength, and it is from the knowledge of those factors that near-field plasmonic applications begin. The fine tuning of the nanostructure is different for each intended application, though the theory of interactions with electromagnetic fields spans the wide variety of uses.

Figure 1.3 – Schematic of Induced Fields: (A) Induced electric fields³¹ and (B) its distance-dependent strength² for a 30 nm silver nanosphere. Reproduced with permission from Elsevier Limited, reference 31, copyright 2010. Reprinted with permission from reference 2 (S.Gray, *J. Phys. Chem. C*, vol. 117, no. 5, pp. 1983-1994, 2013). Copyright 2013 American Chemical Society.

1.2.2 Applications of LSPR

There are many, many applications of plasmonic nanomaterials. A large portion of these applications are sensing platforms that utilize the tuneability of the plasmon resonance of the nanomaterials with the intent of increasing specificity. The ability to tune the plasmonic resonance stems from adjusting the

size, shape, composition and surroundings of the plasmonic nanomaterial being used.

The ability of noble metals to support surface plasmons has provoked considerable interest in nanoparticle applications for plasmonics and photonics. The wide range of plasmon wavelengths has made noble metal nanomaterials uniquely suited for many plasmonic applications, among which are catalysis, optics, chemical and biological sensing, and medical therapeutics.⁶ The vast improvements in recent years to synthesis and characterization methods of these noble metal nanoparticles have facilitated the use of these plasmonic nanoparticles. Control over the size, shape and composition of the nanoparticles, as well as the nature of the surrounding environment, has been extensively investigated.^{6,9-10} It is these properties that allow for tunability of frequency, strength and bandwidth of the resulting signals, yielding a very wide range of potential applications.¹¹ These applications encompass theranostics^{7,12-13}, surface plasmon-based sensors¹⁴⁻¹⁵, concentration of light^{7,14}, plasmonic circuitry¹⁴, waveguides¹⁶, and thermal applications¹⁷. Some of the current and potential applications of noble metal nanostructures are described in the following subsections, a number of which were included in the intended applications for the nanostructures prepared in this work.

1.2.2a) Refractive Index Sensing

In previous and on-going work, the plasmon resonances of silver nanostructures have been shown to be highly sensitive to any change of the

refractive index of the surrounding medium.^{14,18-19} The degree of this sensitivity is often controlled by the type of metal, as well as the size and shape of the nanostructure. Refractive index sensitivity (RIS) itself is defined as the shift of the plasmon resonance maximum per refractive index unit (nm/RIU). One of the principles used to compare the RIS of plasmonic nanostructures is the figure of merit (FOM). The FOM is determined as the ratio of the RIS to the full-width-at-half-maximum of the plasmon resonance mode in question. A higher FOM is dependent on two primary factors: the uniformity of size (and shape) of the nanostructures, and the inherent line width of the plasmon mode being observed.¹⁸ The line width of a plasmon resonance mode is affected by the amount of spatial confinement to which the plasmon oscillation is subjected. For example, with a cubic nanostructure, there are three primary resonances observable: dipolar, quadrupolar, and octopolar. (See *Figure 1.4a*) The dipolar mode is the least spatially confined of these three resonances, resulting in the most broad line width. The octopolar is the most spatially confined of these resonances, thus it has the most narrow line width, though the octopolar mode is difficult to observe clearly for most structures. The sharp-edged cubic nanostructures give the clearest demonstration of an octopolar mode while most other shapes, including truncated cubes, have interference from the dipolar or quadrupolar mode that can obscure any observable octopolar mode.

Figure 1.4 – Visualization of Plasmon Modes: Plasmon modes (B) of a large silver nanocube and 3D representation³⁰ of the oscillations of each mode (A). Reproduced with permission from John Wiley and Sons, reference 30, copyright 2013.

The shape of the nanostructure dictates the number of plasmon resonance poles observable for a particular nanostructure. As previously mentioned, a cubic structure has three primary plasmon resonance modes, whereas a spherical nanostructure will only have one observable resonance mode, that of the dipole resonance.¹⁹ Chen *et al* prepared larger, more complex nanostructures that have alternate resonance modes observable by spectroscopy for the purpose of determining their RIS, as seen in *Figure 1.5*.²⁰

Figure 1.5 – LSPR by Nanostructure Shape: A sample of LSPR spectra varying by shape of nanostructure.²⁰

A – nanospheres, line a
 B – nanocubes, line b
 C – nanobranched (spectrum not shown)
 D – nanorods, aspect ratio = 2.4; line c
 E – nanorods, aspect ratio = 3.4; line d
 Line e – nanorods of aspect ratio 4.6

Reprinted with permission from reference 20 (H. Chen, *et al*, *Langmuir*, vol. 24, no. 10, pp. 5233-5237, 2008). Copyright 2008 American Chemical Society.

The value of RIS of a nanostructure is determined by varying the medium surrounding the nanostructure and observing any shift of plasmon resonance peaks. This is typically observed in solution to ensure the same refractive index is on all sides of the nanostructure. The refractive index of the surrounding solution is changed by either changing the primary solvent outright, or by creating a solution of known refractive index using a combination of solvents. The refractive index of a mixed solution can be calculated using the Lorentz-Lorenz equation²⁰, as shown below. The refractive index sensitivity is then determined by observing the shift of one resonance mode peak for each solution of a different refractive index, and computing the shift per refractive index unit, thus the RIS is given units of nm/RIU.

$$\frac{n_{1,2}^2 - 1}{n_{1,2}^2 + 2} = \varphi_1 \frac{n_1^2 - 1}{n_1^2 + 2} + \varphi_2 \frac{n_2^2 - 1}{n_2^2 + 2}$$

Equation 1.1 – Lorentz-Lorenz equation: Lorentz-Lorenz equation for refractive index of a liquid mixture.²⁰

$n_{1,2}$: refractive index of liquid mixture;

n_1, n_2 : refractive indices of pure liquids respectively;

Gold nanoparticles	length (nm)	diameter (nm)	LSPR max. (nm)	RIS (nm/RIU)	FOM
nanospheres		15	527	44	0.6
nanocubes	44		538	83	1.5
nanobranched	80		1141	703	0.8
nanorods	40	17	653	195	2.6

Table 1.1 – RIS and FOM of Gold Nanostructures: Summary of reported RIS and FOM of varying shapes of gold nanostructures (as reported by Chen *et al.*²⁰).

The knowledge of the refractive index sensitivity of numerous nanostructures led to developments in the field of solution-based sensors. Once a particular system has been calibrated to find its RIS, it can be placed in a new solution and the refractive index of that solution can be interpolated. This advantage leads to a simple form of refractive index sensing. It is in this concept that many plasmonic sensors find their basis.

There have been a number of developments made in the use of LSPR sensors, both for bio-sensing and for chemical sensing. Wu *et al* used the concept of RIS-based plasmon sensing to observe the change of refractive index surrounding the plasmonic nanoparticles (which in this case were silver

nanocubes).²¹ The biological sensors using LSPR and RIS-based sensing will be reviewed further in the next section of this work. The basis of all of these biosensors is the plasmon mode shift with respect to the binding of a biomolecule. This rapid and label-free sensor has a similar line of thought in chemical sensors. The ability to determine the relative concentration of a volatile organic compounds (VOCs), the pH of a system, and compounds in aqueous solutions by the shift of the plasmon modes of a particular nanostructure is a very interesting combination of chemical techniques.²²

The Lu research group in Taiwan, using gold and silver nanoparticles, self-assembled onto single and stacked substrates, was able to observe spectral responses to changes in concentration of target gases passing over and through their sensor arrays. Their results showed good sensitivity and specificity to a number of target gases, measured simultaneously using plasmon modes shifts observed with ultraviolet-visible (UV-vis) spectrophotometry, indicative of the RIS of each array.²³ Their main work with LSPR tuning came from the surface modification of the metal nanoparticles after their self-assembled deposition onto the desired substrates. The modifications, using thiolated solutions, were to form another self-assembled monolayer, this one on the metal nanoparticles themselves, again altering the environment surrounding the metal nanoparticles, inducing further RIS changes from that initially observed in colloidal solutions. This modification of the RIS of the metal nanoparticles is what allowed the researchers to observe further changes influenced by the presence of low concentrations of target gases. As the gases adsorbed to the functionalized

surface of a nanoparticle, a change of conformation of the thiolates' alkyl chains induced a new refractive index around the nanoparticle, observable as a plasmon resonance shift by UV-vis spectroscopy. These arrays were determined to have limits of detection as low as 5 ppm.²² A similar concept, using gold island films and layers of polystyrene sulfonic acid (PSS) and polystyrene, was developed by Karakouz *et al.*²⁴ The polymers bound to the gold nano-islands would expand or shrink upon exposure to various target gases. This expansion and contraction altered the refractive index surrounding the gold nano-islands, inducing a shift of the LSPR mode peak.

By the same principle of conformation induced refractive index modification, Nuopponen *et al* utilized aqueous dispersions of gold nanoparticles coated with a stimuli-sensitive diblock copolymer to observe LSPR shifts as a function of pH and temperature.^{22,25} The functionalized gold nanoparticles were also observed to aggregate based on the pH of the solution, inducing a further shift of the plasmon mode. The outer block of the diblock copolymer used was poly(methacrylic acid) (PMAA), a polymer whose solubility in solutions of varying pH has been extensively studied. The trend is such that solubility can be increased or decreased by the respective increase or decrease of the pH of the solution. As the solubility of the PMAA block was decreased, the demixing temperature of the lower block (poly(-N-isopropylacrylamide), PNIPAM) was also lowered. The entire arrangement of the diblock copolymer around the gold nanoparticle was altered with changing pH, thus inducing quantifiable LSPR shifts, observable by optical spectroscopy. In addition to changing the RIS, the

functionalization of the gold nanoparticles with this diblock copolymer also colloiddally stabilized the aggregates of particles. The polymer-coated nanoparticles were subjected to heating experiments, the results of which vary greatly to those without polymer coating. Typical uncoated gold nanoparticles would tend to sinter irreversibly together when subjected to heat, while the diblock copolymer-coated gold nanoparticles remained separated in solution by the polymer itself, allowing for moderate, reversible alterations of particle cluster sizes. An alternative pH sensor array was developed by Mack *et al*, where a gold film deposited over a polymer layer with patterned nanowells used the expansion and contraction of a hydrogel above the gold layer to detect the changing pH.²⁶ The hydrogel was comprised primarily of a pH-sensitive polymer, poly(2-hydroxyethyl methacrylate) (poly-HEMA), which has also been utilized by Jiang *et al* over substrate-bound gold nanocrescents in yet another pH-by-refractive index sensing nanoarray. These sensor arrays use the same principle of expansion and/or contraction to alter the refractive index of the surrounding environment, and both have been found to be very stable with repeatable results after one month of storage.^{22,26-27}

A further example of an application of LSPR sensing includes the detection of aqueous ammonia or copper ions. The detection of aqueous ammonia is based on Le Chatelier's principle of exchanging silver nanoparticles ($\text{Ag}_{(s)}$) to coordinated diamminesilver ions ($\text{Ag}(\text{NH}_3)_2^+_{(aq)}$). This exchange alters the shape and/or size of the colloidal silver nanoparticles, inherently altering the LSPR modes. This plasmon mode alteration may be drastic enough to cause a

distinguishable colour change, allowing for colorimetric sensing as well as LSPR sensing.^{22,28} A method of detection of copper ions by LSPR sensing was developed by Choi *et al* using gold nanoparticles functionalized with ligands typically used to form copper ion complexes. The ligands were chosen such that the absorption of the metal-ligand complex matched the plasmon resonance of the silver nanoparticles, causing quenching of the nanoparticle plasmon scattering, observable with very low concentrations of copper ions.^{22,29}

The limits of detection for LSPR sensors are generally improved by increasing the FOM of a particular sensing array. Changes induced on a narrow LSPR peak will have a greater impact than on a broad LSPR peak, making smaller changes more significant and increasing the limit of detection for the influencing analyte. If the sensing array is intended to be constructed on a substrate, then the shape of that substrate has a direct effect on the LSPR phenomena of the nanocrystals being used. Nanostructures with sharp edges and a high aspect ratio have been seen to have high RIS and the use of substrates to further break the symmetry of the nanoparticle, inducing hybridized plasmon modes, allows for greater control of the LSPR mode of the nanoparticle. This in turn can be utilized in sensing applications to optimize the limit of detection (LoD) and FOM of a sensing array. Comparisons have been made between planar substrate, those with shallow concave facets, and those with high aspect ratio cylindrical pores to observe the induced changes to the LSPR modes of silver nanocubes.³⁰ The highest sensitivity by these substrate designs was observed using the substrate made with cylindrical pores. The increase is

suspected to be caused by inter-particle coupling across a wall between two pores. The dielectric substrate, located between two nanoparticles, amplifies the hybridized resonance modes of the nanocube, and with two nanocubes' plasmons resonating toward the same pore wall, the resonance is further amplified. The inter-particle distance, even without a dielectric medium between the two nanoparticles, also has an effect on the RIS of the sensing array. The El-Sayed research group has investigated the effects of plasmonic coupling in noble metal nanostructures, including distance-dependence.³¹

There are many methods of modifying the refractive index sensitivity of a particular nanostructure, and it is the combinations of these various methods that have induced such a wide variety of LSPR-based refractive index sensors. The nanostructures used in all of the applications mentioned previously were made of silver, gold, or a combination of silver and gold nanoparticles. The reasoning for these choices over other noble metal options is reviewed in *Section 1.3*.

1.2.2b) Biosensors

Similar to LSPR-based sensors for the detection of changing refractive index are LSPR-based biological sensors. The LSPR mode of the sensing array has an induced change of maximum wavelength when a target biomolecule interacts with the array. LSPR-based biological sensors have an advantage over typical bioassays with the fact that they are label-free, allowing for the target molecule to be detected directly and instantaneously. Traditional bioassays, such as an enzyme-linked immunosorbent assay (ELISA), have rigorous rinsing and exposure procedures, eventually allowing a second antibody to be captured by

the target biomolecule, which then generates the signal used to measure the concentration of the target. By removing multiple steps from the sensing platform with LSPR-based sensors, additional information about the target molecule can be inferred (such as the analysis of real-time binding kinetics) while reducing the sensing area required for a signal to be produced, hence improving the potential LoD for the target biomolecule. The sensitivity and specificity of the biosensor is still limited to the affinity of the capture molecule. This capture molecule can be bound to a nanoparticle surface, whence the LSPR mode would be shifted once the target molecule has been captured. The capturing of the target molecule induced a change of local refractive index surrounding the nanoparticle, which translates as an induced change of maximum absorbance of the plasmon mode. The choice of nanoparticle to be used in the biosensing array is influenced by the RIS of that nanoparticle, as well as the biocompatibility of the nanomaterial.²²

LSPR sensing has been used in the analysis of binding kinetics and the detection of many biomolecules. Some of these studies included biotin-streptavidin interaction²², a number of antibody-antigen interactions^{22,32}, DNA hybridization and sequence identification^{22,33}, as well as protein and drug binding for targeted delivery.^{22,34} One main issue with many biomolecule sensors is biocompatibility. The principle may be sound, but if the nanostructure being developed has toxic effects, the platform is less likely to be able to be developed for *in vivo* use and presents limitations of reproducibility dependent on the viability of the target molecule. Chung *et al* presented a method of surface modification of silver nanoparticles to improve the biocompatibility of the

nanoparticles.³⁵ Silver, as well as many other metals, are known to have harmful biological effects, especially when considering sizes in the nanoscale and possible cellular uptake. The enhancement of biocompatibility of the silver nanoparticles used in their study was achieved by synthesizing phospholipid derivatives containing disulfide groups and binding them directly to the silver nanoparticles being formed as silver-thiolate bonds. The external group of the phospholipid (phosphorylcholine) improved the biocompatibility of the metal nanoparticle by improving the cellular uptake without releasing silver ions or particles into the cells. The biocompatibility, cell viability and cellular uptake were all observed using a fluorescent dye also attached to the nanoparticle. These protected nanoparticles allowed for plasmon resonance effects to be observed within cells and tissues. The use of metal nanostructures for the improvement of protein optical fibre biosensors' limit of detection was one of the applications being explored in this project³⁶ (see *Section 3.6*). The use of substrates to immobilize the nanoparticles before modification with capture molecules is a method being explored in order to provide an easy-to-use interface.^{22,36} A number of the biosensors previously mentioned also use fluorescence enhancement and/or quenching as a measure of the binding of a target molecule.^{22,33} This additional factor of LSPR effects will be reviewed in *Section 1.2.2d*).

1.2.2c) Heating

High temperatures are known to cause protein denaturation, and it is this fact that is being used in medical hyperthermia treatments. Protein denaturation

in a cancer cell is a method of reducing the resistivity of the cell to chemotherapy or radiotherapy treatments, as well as a method of inducing nonaggressive cell death. The range of temperature within which protein denaturation occurs is from 43 to 48 °C. This is well above the standard human body temperature of 37 °C. Localized heating is yet another application of noble metal and semiconductor nanostructures.³⁷

The nanostructures being investigated for heating applications can be classed into three generic groups, dependent on their particular role in the thermal treatment: nanoparticles acting as thermometers (real-time thermal sensing of treated cells or tissues); nanoparticles for temperature-activated drug delivery; and nanoparticles for selective local heating. The small size of the nanostructures allows for easy and efficient intracellular incorporation after being transported through the bloodstream. For the third class of nanoparticles, the localized heating is typically induced through an external stimulus, either by an oscillating magnetic field (for magnetic nanoparticles) or by optical radiation (for photoactivated nanoparticles). The depth of penetration of optical radiation into human tissue does pose a limitation on the photoactivated nanoparticles, though nanoparticles tailored to have an optical excitation near 800 nm have shown promise for long-range photothermal treatments by the minimal light absorption through the tissue at this wavelength. The vast majority of nanoparticles being used in biological applications are made of gold, generally chosen for its stability and non-reactivity in biological conditions. The shape-dependent heating efficiency has been investigated by the Fluorescence Imaging Group in Spain,

wherein they determined that between nanorods, nanoshells, nanostars and nanocages, the nanorods and long-edged nanostars had the highest heating efficiency. (See *Figure 1.6* for TEM images of the shapes used in Maestro *et al.*'s investigations.) The heating efficiency of the system is defined as the fraction of laser power reaching the gold nanoparticle that is converted to heat. Gold nanoparticles have very low fluorescence efficiency, so almost all of the optical power is converted to heat. The absorption of the nanoparticles at a given wavelength directly correlates to the heating efficiency, thus the researchers used double-beam fluorescence thermometry (DBFT) with thermosensitive CdSe fluorescent quantum dots to determine the heating efficiency of the nanoparticles in solution. This method allows for the simultaneous excitation of the trace amount of quantum dots (at 488 nm) and of the gold nanoparticles (at 808 nm). The fluorescence emission of the quantum dots is known to shift by 0.1 nm per degree Celsius, allowing for the temperature change to be measured as an average in solution.³⁷

Figure 1.6 – Micrographs of Various Gold Nanostructures: TEM images of different shapes used in heating investigations by Maestro *et al.* (a) gold nanorods; (b) nanoshells; (c, d) “long” and (e, f) “short” gold nanostars; and (g, h) gold nanocages. Reprinted with permission from reference 37 (L.M. Maestro, *et al*, *Langmuir*, vol. 30, no. 6, pp. 1650-1658, 2014). Copyright 2014 American Chemical Society.

Other heating applications involve self-assembled monolayers (SAM) on supported gold nanofilms. These applications are generally used to study the desorption and melting temperatures and the heat capacity of the SAMs. Zhang *et al* used methods of differential scanning calorimetry (DSC) to determine these characteristics with respect to hexadecanethiol monolayers on polycrystalline gold.³⁸ These properties are the basis of investigating surface-modified nanostructures for various thermal treatment applications. If the functionalization molecule cannot withstand temperature changes, or is prone to desorption upon heating in solutions, then that particular molecule-nanoparticle combination would not be a viable option for *in vivo* thermal situations. For the restricted spaces of a living cell, there is no room for the steric hindrance of extra molecules

surrounding the nanoparticle being used, so every molecule present is meant for a purpose, and it would lose that purpose, lessening the efficiency of the entire array if it were to desorb or decompose *in situ*.

1.2.2d) Fluorescence enhancement

The earliest known record of the phenomenon of fluorescence is a cup made of the wood from the narra tree, known as the *lignum nephriticum* cup. The wood from this tree, when in contact with water, would change the colour of the water from clear to opalescent hues (varying shades of blues and oranges depending on light and angle), as in fluorescent shades (see *Figure 1.7*). The written account of this strange wood was recorded by Nicolás Monardes in 1565. The source of the fluorescence has since been determined to be maltaline, the oxidation product of one of the flavonoids found in the wood.³⁹ Developments in the progress and discovery of fluorescence materials and properties in the last 400 years have made our modern use of fluorescent materials possible. The radiative decay of an optically excited compound or structure is very well understood, and this phenomenon has been exploited in many applications, including biomedical dyes and sensors, as well as in combination with other chemical techniques for sensing applications. Fluorescence is a popular area of research now, especially with respect to its enhancement using nanostructures.

Figure 1.7 – Lignum Nepphriticum, *pterocarpus indicus* : Cup of Philippine *Lignum Nepphriticum* (*pterocarpus indicus*), and flask containing its fluorescent infusion.³⁹

The mechanism of fluorescence enhancement stems from effects of electromagnetic interactions between the fluorescence excitation, emission, and the plasmon modes of the nanoparticles. The first of these effects is the polarization of the incident light causing an increase in the incident field acting on the adsorbed molecules, while also causing the surface plasmon to resonate and radiate, thus increasing the intensity of the emitted light. The metal surface itself can also cause a change in the lifetime of the fluorescent molecule bound to or found near the surface by facilitating a conduit for the excess energy, generally leading to a faster emission after the excitation of the fluorophore.⁴⁰⁻⁴¹ The enhancement effects are very sensitive to environmental changes, especially

when those changes also modify the plasmon resonance mode of the nanoparticle incorporated into the system. For example, the range of fluorescence enhancement using a number of silver nanostructures has varied from 10 to 100 times the intensity observed without the use of the nanostructures, while gold nanostructures yielded enhancement factors of 2 to 30.⁴¹⁻⁴³ The variation of these enhancement factors stems from both the electronic structure of the metal, as well as the physical shape and orientation of the nanostructures being used. Silver allows for a much greater enhancement of fluorescence than gold, as is evidenced by the more common use of gold nanostructures to quench fluorescence emission rather than enhancement. The structure of the arrays being utilized also has a significant influence on the enhancement of the emission intensity. One of the most significant alterations of structure is the change from isolated particles to aggregates or clusters of particles. The clusters of particles have different enhancement patterns brought about by the coupling of the plasmonic fields of the particles when they are very close together. The cavities between clustered particles have more surface area with which a fluorophore molecule can interact, thus creating a potentially higher enhancement in those cavities seen as an average signal intensity higher than that seen with isolated particles.⁴⁰⁻⁴¹

Noble metal nanostructures are of particular interest in this area of study, mainly for the potential application of multi-use systems. Ultra-small noble metal nanoclusters have already been found to be fluorescent without the addition of a dye. These very tiny groupings of atoms, either silver or gold, are documented to

fluoresce in the red region of the visible spectrum.⁴⁴ The synthesis of these nanoclusters must be very controlled. Noble metals have a tendency to prefer forming larger, more stable nanostructures. Huang *et al* developed a method involving the formation of dendrimer capsules in order to maintain small sizes during synthesis. Once stable, the fluorescence was easily observed for both the silver and gold nanoclusters. An application for which these nanoclusters might be considered is cellular labelling, though the small size of the nanoclusters may also increase the cytotoxicity with respect to larger particles.⁴⁵ The use of larger nanostructures is thus more desirable for potential biological applications. This poses its own challenges however, with the fact that noble metal nanostructures with width above 5 nm do not have fluorescent properties. The majority of the metal structures quench any nearby fluorescence by the nature of their ability to transfer energy through the conduction bands of the structures and emit that transferred energy in non-radiative decay. Thus, the use of surface modification is a typical method used to develop fluorescence enhancement, as well as variations of the structure of the nanoparticle can be used to control fluorescence quenching.⁴⁶

A popular method of surface modification of nanoparticles for control of fluorescence enhancement or quenching is the use of silica shells. Silica nanoparticles have been used shown to increase the intensity of fluorescence emission, thought to be caused by the high scattering ability of silica as a dielectric medium, allowing for an increased collection efficiency of the emission field.⁴⁷ Silica is usually an insulator, but it can be electronically polarized by an

applied electric field (making it a dielectric), such as that of oscillating conduction electrons at the contact point between a noble metal nanostructure and the silica structure. There are a number of principles that influence the likelihood of a noble metal nanostructure to enhance or quench fluorescence emission respectively, though the primary consideration is the overlap between the excitation or emission of the fluorophore with the LSPR of the nanostructure. If the absorption spectrum of the nanostructure overlaps well with the absorption (excitation) spectrum of the fluorophore, there is likely to be enhancement, whereas if the maximum absorption of the nanostructure overlaps with the emission range of the fluorophore, there is likely to be quenching of emission intensity (see *Figure 1.8*).⁴⁸⁻⁴⁹ There are applications where quenching is the desired effect, and Reineck *et al* determined that the most efficient quenching occurred when the fluorescence emission is expected 30 nm red-shifted of the LSPR of the nanoparticle.⁵⁰ One way to control this placement is to create a system of core-shell nanoparticles, such as gold or silver nanoparticles coated in silica (metal-silica core-shell structures have also been used for attaining enhancement). The plasmon bands of gold nanostructures are always of higher wavelength than those of silver nanostructures, allowing for simpler fluorescence enhancement arrays with silver. The same silica shells have been applied to both gold and silver nanostructures, with almost opposite results. Any thickness of silica shell on a gold nanoparticle was shown to have the same or increased quenching over the use of an uncoated gold nanoparticle, while the same thicknesses of silica shells on a silver nanoparticle had the same or increased enhancement as bare

silver nanoparticles. The extent of the quenching or enhancement with respect to the shell thickness demonstrated a distance-dependence for the fluorescence arrays. Using the silica shells as rigid spacers of controllable distances, the fluorophores closer to the metal nanoparticle showed an increased effect over that seen with thicker silica shells.⁵⁰⁻⁵³ Alternative core-shell structures include DNA shells (crosslinked to increase aggregation)⁵⁴ and Rhodamine B shells (changing thickness of shell modulated fluorescence emission by surface-enhanced fluorescence).⁵⁵

Figure 1.8 – Fluorescent Dye and LSPR Overlap: The absorption (dashed) and emission (dotted) spectra of Cascade Yellow dye show good overlap with the plasmon bands of silver and gold, respectively, for fluorescence enhancement/quenching investigations. Reproduced with permission from John Wiley and Sons, reference 51, copyright 2006.

Other works with fluorescence and nanoparticles includes creating hybrid multifunctional colloidal nanostructures. Bigall *et al* have explored the use of fluorescent, plasmonic, and magnetic properties in a number of combinations. Their aim has been to investigate these combinations for use in complex applications where one property alone would not be sufficient for the intended analysis. An example of such an application is in biomedicine with the goal of both labelling and destroying target tissue. Researchers have used fluorescent nanostructures to label tissues, and are also investigating the use of plasmonic nanoparticles as heat sources for destroying damaged or infected tissue, but the combination of the two has been thus far limited by the general tendency of noble metal plasmonic nanoparticles to quench surrounding fluorescence emission. The addition of an insulating layer, as well as the effects of distance and type of functionalization, are what Bigall *et al*, among many others, have been reviewing. Further progress is expected in the understanding of the combination of magnetic, fluorescent, and plasmonic nanoparticles (including combinations of all three types of nanoparticles) as the desire for hybrid systems increases.⁵⁶

1.2.2e) Fibre Optics and Optical Fibre Sensors

One branch of LSPR-based sensing is with the use of optical fibres as a sensing platform. The optical field of the fibre can be enhanced by coating it with plasmonic films, or depositing plasmonic nanoparticles onto the surface. The enhancement is induced by the coupling between the optical modes of the fibre and the LSPR of the nanostructure. The enhancement of the optical field also increases the sensitivity of the sensing platform by utilizing the nanostructures'

increased sensitivity to changes in their local environment. The plasmonic coupling effects of silver nanoparticles bound to an optical fibre sensor have been studied extensively by the Kundu research group in India. Their studies demonstrated an increased shift in the absorption spectrum of the sensor with the increased adsorbed particle density. More nanoparticles bound to the fibre platform allowed for increased interparticle coupling as well as increased coupling to the fibre's optical field.⁵⁷ Tu *et al* recently reported on the comparison between the use of solid nanoparticles versus hollow nanoparticles for increased optical enhancement and sensitivity.⁵⁸ Their work used solid gold nanoparticles and hollow gold nanocages, similar to a part of the studies to be presented in this work (see *Section 3.6*). Solid gold nanoparticles on optical fibres were shown to have higher sensitivity to surrounding refractive index changes than those coated with silver nanoparticles, and further sensitivity was noted with the use of hollow gold nanostructures (caused by intra-particle coupling, previously unobtainable with filled nanostructures). These sensing platforms are of particular interest by their flexibility and tunability, as well as by being relatively inexpensive and simple to prepare. The quick and sensitive responses of optical fibre-based sensors allow them to be potentially used in a wide variety of applications, including biosensors.

1.2.2f) Photovoltaics

The ongoing research for the development of solar cells stems from our as-yet limited ability to capture the full potential energy from a light source. The typical photovoltaic arrays in production now have an average efficiency of near

3 %, leaving the vast majority of the potential energy wasted in this endeavour. This efficiency is a measure of the photon-to-current ratio of a particular array. The difficulty in achieving a higher efficiency stems from the relatively narrow range of absorption compared to the spectrum of photon energy available to absorb. The use of plasmonic nanoparticles in a photovoltaic array is an area of research intended to improve the absorption efficiency of the array. Silver nanostructures have been moderately explored for this application, with promising results when the plasmon resonance was made to couple with the semiconductor layer of a solar cell.¹⁴ Alternative methods of increasing the solar cell array's efficiency include the assembly of superparticles (diameter between 200 and 400 nm) from smaller nanoparticles (in order to control the absorption range via the interparticle spacing)⁵⁹, or using core-shell or core-shell-shell nanostructures of metal, silica, and titania.⁶⁰ In both of these methods, the attempt is to have a minimum separation, but not direct contact, between the metal nanoparticle and the semiconducting layer, either by functionalized self-assembly, or applying a silica thin film before coating with the conducting titania film. All of these modifications aim at broadening the absorption range of the solar cell array, however one main obstacle is the production cost. Without a drastic increase of efficiency, the cost to produce arrays with silver, gold or other noble metal nanoparticles is extravagant. The research will continue, though mass production of altered solar cells will be some time coming.

1.2.2g) Other Potential Applications

The above sections have briefly reviewed a number of applications for noble metal nanostructures, for which the gold nanocages produced in this study were evaluated. There are many other potential applications, a few of which will be reviewed very briefly in this section.

Surface-enhanced Raman spectroscopy (SERS) and SERS-based sensors, with the continuous advances in nanofabrication and nanotechnology, are very versatile methods of analysis. High sensitivity made possible by the inherently high-intensity signals of Raman-active molecules allowing for a variety of surface modifications to be investigated for improved sensitivity and detection. Research in surface modifications in recent years have altered from roughened surfaces to structured and/or random deposition of pre-synthesized nanoparticles.⁶¹⁻⁶³ The nanoparticles present discrete regions of optical field coupling, altering the sensitivity of the entire sensing array. The shape and material of the nanoparticles being used also alters the SERS signal significantly. Even small changes of shape have significantly different shifts, such as slight truncation of the corners of a cubic structure, as demonstrated by McLellan *et al.*⁶⁴ The process by which the surface is modified can also have an effect on the SERS signals produced. Direct surface modification, such as roughening or lithographic techniques, have shown very different shifts and intensities than deposited or pre-assembled nanostructures.⁶⁵⁻⁶⁶ An interesting case observed by the El-Sayed research group was that hollow gold nanoframes, contrary to previously studied solid gold nanostructures, showed a reduction of the SERS efficiency. This reduction is believed to be caused by the interaction between the

outside and inside of the hollow nanostructure. A solid nanostructure only has external influences on the LSPR and induced electronic fields, whereas a hollow nanostructure has an additional influence of the internal surfaces inducing their own fields and coupling with the external surfaces' fields.⁶⁷ This altered nanostructure added a new level of complication to the investigation. Zoppi *et al* later functionalized the gold nanocages to be able to observe both SERS and fluorescence spectroscopy changes in order to develop a very sensitive sensing probe.⁶⁸ These recent advances have further developed the range of applications for noble metal nanostructures with Raman-active molecules, and methods are being developed for the modification of SERS substrates to allow for tailored enhancement mechanisms. Controlled deposition and selective galvanic displacement of silver nanowires have allowed Netzer *et al* to tailor their SERS substrates⁶⁹, a feature certain to further expand these applications.

A number of other applications of noble metal nanostructures in plasmonic applications include the self-assembly of gold nanoparticles to modify indium tin oxide electrodes⁷⁰; the self-orientation of silver nanocubes for assembly of plasmonic nanojunctions⁷¹; the use of silver films with cadmium sulphide nanowires, or Oregon Green-doped silica-coated gold nanoparticles for the production of plasmon lasers⁷²⁻⁷³; as well as the use of surface-modified hollow silver, gold, or silver/gold alloy nanostructures for the catalytic degradation of 4-nitrophenol and 4-aminophenol⁷⁴⁻⁷⁵, or catalytic glucose oxidation.⁷⁶

Thermotherapy, theranostic, and drug delivery mechanisms (general and targeted) using a variety of gold nanoparticles are also being developed.^{12,21,34,77-}

⁸⁶ The functionalization of the gold nanoparticles with thiolated molecules have allowed for a number of sensing platforms and aptamer-based delivery modes to be developed, including the sensing of a biomarker for Alzheimer's disease⁸⁷ and targeted thermotherapy to cancer cells.⁸⁸⁻⁹⁰ The use of hollow gold nanostructures is a promising method of drug delivery currently being investigated. Targeted cell imaging using modified gold nanoparticles is also being investigated, for *in-vitro* and real-time *in-vivo* imaging.⁹¹⁻⁹² The versatility of external functionalization, along with the strong plasmon resonance and stability in biological conditions, make gold nanostructures a very promising option for a number of biomedical applications.

Figure 1.9 – Potential drug delivery mechanisms using nanostructures:

(A) release mechanism for gold nanocages coated with smart polymers Reprinted with permission from reference 12 [Xia, *et al*, *Acc. Chem. Res.*, vol. 44, no. 10, pp. 914-924, 2011). Copyright 2011 American Chemical Society.

(B) other commonly used loading techniques that may be applied to gold nanocage platforms. Reproduced with permission from Elsevier Limited, reference 34, copyright 2010.

1.3 Choice of Material

The plasmonic properties of gold and silver nanostructures are being extensively studied in current research. Since the shape and size of the nanostructure has a direct impact on these properties, the on-going development of control over the size and shape of the nanoparticles is constantly altering the range of applications for both silver and gold nanostructures. Modified synthesis methods are also becoming increasingly robust and reproducible, allowing for larger scale syntheses of the nanostructures being investigated.

The work presented herein uses gold nanocages with post-synthesis surface modifications made as needed to direct the investigation toward an intended application. Previous work involved the synthesis, characterization and application of silver nanocubes.^{19,93} The intention for this work was to contrast the utilization of gold nanocages in similar characterization settings and applications. The silver nanocubes used previously showed good sensitivity to refractive index, especially when supported on a dielectric substrate. Silver nanostructures, especially those with well-defined and sharp edges, have shown excellent LSPR tunability and sensitivity for plasmon resonance sensing applications.^{22,43,94-95} The characterization and investigative potential for applications of gold nanocages for this project was prompted by the potential of high sensitivity at longer wavelengths and the idea that gold is more favourable for use in biological and biomedical applications.

The potential seen in reported research before this project began demonstrated the size and shape dependence of the RIS of various gold

nanoparticles²⁰ (as seen in *Table 1.1*), as well as the potential for molecular sensing platforms using other gold nanostructures.²² These basic results, as well as the well-known biocompatibility of gold, was the basis of choosing gold as the primary material to be utilized in this project. Large gold nanoframes, varying from mostly spherical to forms of cubic structures, are being investigated for their sensing efficiency at near-infrared (NIR) wavelengths,^{68,96} with promising results based on the high strength surface plasmon fields generated by the interaction between internal and external fields of the hollow nanoparticles. The gold nanocages investigated in this project were smaller than those previously reported, a feature intended to allow for functionalization and/or coatings to control the LSPR in the UV-vis range of the electromagnetic spectrum for various applications.

1.4 Synthesis Methods

Nanostructures in general have two main preparatory routes: “top-down” or “bottom-up”. A top-down approach involves starting with a bulk substance and using lithographic techniques, such as etching, mask-guided deposition, or electron beam lithography to create a desired nanostructure.⁹⁷ This method is typically limited to solid structures and it is difficult to create colloidal dispersions with the resulting nanostructures. A bottom-up approach is more versatile in this manner as it typically involves the formation of nanocrystals from ions in solution. The growth and applications of the resulting nanocrystals may be either substrate-supported or colloidal-solution based, with simple modifications and

functionalizations used to tailor the resulting nanostructures to the desired shapes and sizes.

1.4.1 Polyol Synthesis for Silver Nanostructures

The production of hollow, cubic gold nanocages requires sacrificial silver nanocubes as a starting structure. The synthesis of silver nanostructures has been extensively investigated, with a *polyol* synthesis method becoming the most common synthesis process used in recent years. This method is called a polyol method as it uses an alcohol containing multiple hydroxyl groups as the main solvent. A polyol synthesis method also requires a reducing agent, a capping agent, and a metal precursor. The progression of the reaction and the resulting product can be varied using temperature changes, the presence of trace ions, and the concentration of the reagents being used. The typical choices of solvent for a polyol synthesis of silver nanoparticles are ethylene glycol, 1,2-propylene glycol, or 1,5-pentanediol. For this project, ethylene glycol was used as both the primary solvent and the reducing agent. The hypothesized mechanism of the reduction of silver ions to silver crystals is that ethylene glycol, when heated in the presence of oxygen will form glycoaldehyde, a reductant strong enough to spontaneously reduce silver ions in the same solution.¹⁴

Equation 1.2 – Reduction of ethylene glycol: Reduction of ethylene glycol to glycoaldehyde when heated in the presence of oxygen.

Other syntheses processes have used aqueous solutions using polyelectrolytes⁹⁸⁻¹⁰⁰, citrate (known as the *Turkevich method* when forming gold nanoparticles)^{57,101-102}, copper chloride¹⁰³, chloroplatinic acid (H_2PtCl_6)¹⁰⁴, dimethylformamide¹⁰⁵, hexamethylenetetramine⁷⁶, or hydroxylamine¹⁰⁶ to stabilize and reduce the metal precursor, all of which result in different nanocrystal shapes. Seed-mediated¹⁰⁷⁻¹⁰⁹ and electrochemical¹¹⁰⁻¹¹¹ synthesis methods can also be employed, though they are typically ideal for very large or complex nanostructures. All of the methods listed above are completed in either hydrophilic or aqueous solutions, though hydrophobic solvents are also being investigated. The challenge with hydrophobic solvents is in achieving the same level of control over the product's size and shape, thus alternative etching agents and capping agents are needed to achieve similar results.¹¹²

1.4.1a) Metal Precursor

The metal precursor used in this project for the preparation of silver nanocubes was silver nitrate. This was chosen as the precursor in the polyol synthesis for its ready availability as well as for the fact that the complimentary cation would not interfere with the progression of the synthesis. The rate of addition of the metal precursor also affects the structure being formed.¹¹³ An alternative silver precursor is silver trifluoroacetate (CF_3COOAg), with similar nanocrystal products to those made from silver nitrate when used in a polyol synthesis.¹¹⁴ Other noble metal nanostructures are made in similar fashions to those of silver, including platinum, palladium, gold, ruthenium and rhodium.^{82,98-99,115-117} Plasmonic nanoparticles include those made of cobalt, nickel, iron and

copper (among others), though the synthesis of these particles trends toward oxide nanoparticles, thus the more stable noble metals are used more often.¹¹⁸ The timing of the synthesis must be carefully monitored as overgrowth can occur with extended reaction time, resulting in truncated octahedrons¹¹⁹, extended shapes¹²⁰, or branched nanoparticles.⁹⁹

1.4.1b) Capping Agent

The capping agent of a synthesis is the primary method of dictating the specificity of shapes produced. Without a capping agent to guide the crystal growth, the particles would undergo rearrangement to achieve the lowest possible surface energy, known as the *Wulff shape*. For noble metals, the nanoparticle Wulff shape is a truncated octahedron⁷, as it is a close approximation of a spherical particle while still maintaining a crystal lattice (spherical particles having the lowest surface area allow for the lowest surface energy arrangement). Thus, various capping agents have been investigated and utilized in research to enable the monodisperse production of a number of different shapes of silver nanoparticles.¹⁴ The capping agent used in the synthesis of silver nanocubes for the extent of this work was polyvinylpyrrolidone (PVP). PVP has been shown to selectively bind to the (1 0 0) crystallographic planes of a metal crystal growing in solution, promoting the growth of all other faces, eventually leaving only the (1 0 0) faces of a cubic structure (see *Figure 1.10*).¹²¹ Alternative capping agents for shape control of noble metal nanocrystal growth include polyelectrolytes⁹⁸, facet-specific peptide sequences¹²², citrate^{9,57,123-124}, iso-luminol¹²⁵, cetyltrimethylammonium bromide (CTAB)³, and

surfactants such as stearyltrimethylammonium chloride¹¹⁵ or oleylamine¹¹⁶. Preparation of silver nanoparticles without the use of a capping agent is also possible so long as the desired shape is spheroid nanoparticles.¹²⁶

Figure 1.10 – Binding of PVP versus Citrate: Schematic of preferential binding of (A) a citrate group to the (111) face, and (B) a PVP repeating unit to the (100) face of a silver crystal. Reprinted with permission from reference 121 [Xia, *et al*, *J. Phys. Chem C*, vol. 116, no. 41, pp. 21647-21656, 2012]. Copyright 2012 American Chemical Society.

1.4.1c) Trace Materials

One type of trace ion that is desired for some of the synthesis methods of silver nanocubes is sulfide anions. S^{2-} is known to increase the rate of reduction of silver ions, though this ion must be present only in very small amounts to avoid the precipitation of silver sulfide in the solution, typically causing rapid aggregation of the silver crystals being formed.¹²⁷ Trace amounts of sodium sulfide were added during the synthesis process of the silver nanocubes prepared for this project, with the intention of increasing the rate of reaction. An alternative choice of trace ion is that of a halide.¹²⁸ These anions act as an

oxidative etchant for the silver crystals being formed, promoting the formation of twinned-seeded starting particles and encouraging the growth of larger nanostructures, such as nanowires or very large nanocubes when using chloride ions.^{113,129-130} A source of bromide ions can be added, with resulting structures trending toward right bipyramids and nanobars.^{113,131} The rate of the reaction can also be increased using microwave irradiation^{100,132}, or by bubbling hydrogen gas through the polyol synthesis reaction.¹³³ Etching to desired shapes during or after synthesis can be achieved by adding oxygen^{130,134} or trace amounts of nitric acid, though excess results in acid digestion of the nanoparticles.¹³⁵

1.4.2 Galvanic Displacement for Gold Nanostructures

Half Reactions:

Combined reaction for galvanic exchange:

Equation 1.3 – Galvanic Exchange Reactions: (a, b) Half reactions and reduction potentials of the reduction of silver and gold ions¹⁸¹; (c) complete reaction for galvanic exchange of gold for silver.

Once the silver nanocubes have been prepared, the gold nanocages are synthesized by a simple galvanic displacement using tetrachloroauric acid. This displacement occurs spontaneously because Au^{3+} has a higher reduction potential than Ag^+ , allowing the silver of the template nanostructure to be oxidized as the gold deposits in its place¹³⁶ (as described in *Equation 1.3*). PVP

is added to the solution to preserve the cubic shape during the metal-ion exchange. This stage of the preparation was an aqueous solution, producing easily repeatable results.^{12,137-139} Galvanic exchange has also been utilized in the formation of nanoboxes made of palladium-silver and platinum-silver alloys¹⁴⁰, as well as a number of other hollow gold shapes¹³⁶. A pathway of patterned displacement is suggested by Cobley *et al.* (see *Figure 1.11*), wherein an alloy of gold and silver is first formed at the exposed faces of the nanocube, followed by the progressive oxidation of silver resulting in first an alloyed hollow cubic shape then a gold nanocages with partially open faces.¹³⁶

Figure 1.11 – Schematic of Galvanic Displacement: Schematic illustrating the major morphological and structural changes involved in the galvanic displacement reaction between AgNC and H_{Au}Cl₄. Reproduced with permission from Elsevier Limited, reference 136, copyright 2010.

There are a number of minor alterations seen in literature to the general procedure of galvanic displacement of silver with gold. In some cases, the silver nanocrystals are synthesized in the same vessel to which the gold solution is added, making the synthesis a one-pot procedure.¹⁸ An alternative method involves the formation of gold nanocrystal seeds to begin a four-step protocol to form gold nanocages.⁷⁶ Both of these methods were attempted for this project

(see *Section 2.2*), however a more simple procedure was found to be easily reproduced with good consistency. This simple method used previously prepared silver nanocubes and a low concentration of tetrachloroauric acid (HAuCl_4) to slowly exchange the silver nanocubes to hollow gold nanocages, as per Skrabalak *et al.*¹³⁷, and was the primary method of gold nanocage preparation for this project.

1.5 Surface Modifications Made to Gold Nanocages

To enable the use of the same nanoparticle in a number of different applications, simple surface modifications can be completed. These modifications may be for the intent of self-assembly, RIS adjustment, LSPR shifting, or application design. All modifications to the surface of the nanoparticle, by etching or molecular binding, will inherently change the RIS of the nanoparticle. The extent of this alteration stems from whether the change was intentional or simply a side effect of the surface modification. The two main surface modification methods used for this project were functionalization with an alkyl chain and silica-coating of the gold nanocages. Other surface modifications possible include peptide binding for targeted biomedicine applications⁸⁰, disulfide binding for improved biocompatibility³⁵, and fluorophore or quantum dot binding for emission enhancement and/or quenching applications⁴⁹.

1.5.1 Alkyl-Chain Functionalization (HDT)

Most plasmonic applications have the RIS of a nanostructure tuned to an acceptable working range for the specific application. The functionalization of a

nanostructure alters this sensitivity, a fact which must be considered before investigating an application that requires a molecule to be coated onto the nanostructure. Generally, unmodified nanoparticles have a higher RIS than surface-modified nanostructures. The electromagnetic field of a nanoparticle decays away from the particle surface, thus the strongest sensitivity occurs close to the nanoparticle surface.⁶⁵ A functionalization molecule will typically form a monolayer on the surface of the nanoparticle, the thickness of which corresponds to the decreased sensitivity of the nanoparticle. This can, however, be an effect outweighed by the function of the newly bound monolayer.

One molecule that was used for gold nanocage functionalization in this project was hexadecanethiol (HDT). The intention of this modification was the potential for increased packing order when depositing the nanocages onto substrates. The long alkyl chains create organized packing structures between themselves through van der Waal's interactions. This organized packing allows for a method of self-controlled distance of separation upon deposition.³⁸ The fact that HDT also forms a self-assembled monolayer on gold and silver is another favourable attribute of this functionalization method. A thiolated alkane will spontaneously form a covalent bond with the surface atoms of the nanostructure, which forms a very well ordered monolayer following the crystallographic nature of the nanostructure.

Figure 1.12 – Schematic Representations HDT Monolayers:

Schematic representations of

(A) packing order of HDT on a gold surface. Reprinted with permission from reference 182 (Terrill, *et al*, *Langmuir*, vol. 14, no. 4, pp. 845-854, 1998). Copyright 1998 American Chemical Society.

(B) alkyl-chain structure of HDT¹⁸⁴

(C) SAM formation of HDT on a metal surface. Reprinted with permission from reference 183 (Kim, *et al*, *J. Appl. Phys.*, vol. 106, p. 083518, 2009). Copyright 2009 AIP Publishing LLC.

1.5.2 Core-Shell Modifications (Silica-Coating)

A different method of surface modification is that of creating a core-shell system. Metal core and dielectric shell nanostructures have proven to be particularly interesting for a number of applications. Some of those applications include photothermal drug release⁸⁴ and self-assembly of core-shell films¹⁴¹. There is one synthesis method in particular that is often modified to suit specific situations, being the *Stöber method*. This method was initially developed for the synthesis of monodisperse, micron-sized silica spheres.¹⁴² The general process of this method is the hydrolysis of an alkyl silicate, followed by condensation of

the newly formed silicic acid, using ammonia as a catalyst. The controlled growth of silica shells on metal nanostructures is completed much the same, though the amount of alkyl silicate is typically much lower than originally utilized. The spheres first synthesized by Stöber *et al* were 0.05 to 2 μm in diameter, while the shells desired around the nanostructures are typically between 3 and 100 nm. An alternative to the Stöber method that has been developed involves a microemulsion to create confined spaces within which the core-shell nanostructures are synthesized.¹⁴³ This method uses a surface primer in order to control the silica thickness on the nanoparticle, which has been seen to allow for the use of high concentrations of particles with consistent results. Other methods of creating metal-core silica-shell nanostructures involve the use of water-soluble micelles, though this is typically with the intent of creating very thick shells on very small nanoparticles.¹⁴⁴

The intent of creating core-shell structures with noble metal nanoparticles has a basis in protecting the nanoparticle or nanostructure from external environments. Nanostructures, both made of gold and silver, have tendencies to be altered when exposed to electron beams, heat and pH changes. Using silica as a protective coating is one method often used to stabilize the nanostructures in harsh environments.¹⁴⁵ The thickness of the shell can be determined using a Mie-type model for the shift of a nanoparticle's LSPR mode with respect to the thickness of the shell. Khlebtsov *et al* used a spherical core-shell approximation to determine the approximate thickness of the silica shell being formed on gold nanocages, with good agreement to the experimental results.¹⁴⁶ The structure of

the silica shell being formed is influenced by the length and amount of PVP surrounding the nanostructures, the water content of the solution, the concentration of ammonia added, as well as the type of nanoparticle being used.¹⁴⁷ Once formed, the silica shells can themselves be functionalized externally for other applications.^{68,84,141,148}

1.6 Preparation for Intended Applications

There are a number of steps to be completed between the synthesis of gold nanocages and the induction into a desired application. Many applications incorporate a substrate, thus the nanocages must be deposited somehow, while other applications require the use of varying solvents or the presence of other optically important molecules. The nanocages must therefore be prepared and applied to these changing environments.

1.6.1 Deposition Methods

The deposition of gold nanocages has three primary methods: drop-cast, Langmuir-Blodgett technique, and self-assembly. Of these three, drop-casting is the only method that does not allow for organized assembly, instead resulting in random placement and aggregation upon solvent evaporation. For the use in applications, a more ordered, or at least controlled, deposition is desired, thus the use of Langmuir-Blodgett technique and self-assembly methods.

1.6.1a) Langmuir-Blodgett Technique

The term *Langmuir-Blodgett*, in reference to deposition technique for films, was first coined in 1934. It was then that Irving Langmuir and Katherine Blodgett discovered that it is possible to homogeneously deposit a monolayer of organic molecules onto a solid surface by inserting the solid surface into an aqueous solution containing a monolayer of the organic molecules.¹⁴⁹ Since its initial discovery, this method has been utilized extensively, and expanded to include the deposition of solid particles in combination with organic surfactants onto various forms of substrates. These advances, especially in recent years, made to include complex nanomaterials typically thought too large or heavy to be deposited via Langmuir-Blodgett (LB) technique, have allowed this method to be used to form detailed and complex surface and deposition arrangements, some of which are not easily achieved by other deposition methods.¹⁵⁰⁻¹⁵¹

Figure 1.13 – LB Deposition Illustration: illustrative representation of the transfer of a monolayer of nanoparticles by LB technique via capillary forces (A-water subphase; B-LB trough barrier; C-deposition substrate; D-nanostructure sample). Reproduced with permission from John Wiley and Sons, reference 150, copyright 2009.

The mechanism of the formation of LB films is simple. It begins with a thin layer of water on a specially designed trough. The material to be deposited as a film is first made to form a monolayer at the air-water interface of the water in the LB trough. This material is preferentially amphiphilic to allow for the majority of the material to remain at the surface of the water. If the material is too hydrophobic, there will likely be pockets of concentrated material on the surface of the water, while being too hydrophilic has the material dispersing into the water subphase instead of remaining on the surface. Once an even monolayer of amphiphilic material has been achieved, at the desired density (measured as surface pressure while on the LB trough), a solid surface or substrate can be vertically lifted through the monolayer, whereupon the monolayer of deposition material will quantitatively and qualitatively transfer onto the exiting surface via capillary action and surface tension.

Many different substrates can and have been used to receive LB films, the main criterion being that it is not a hydrophobic surface (one such surface would repel the water and monolayer, thus preventing the transfer of the monolayer). Patterning can also be achieved by using tailored mixes of different amphiphilic molecules, such as two different phospholipids or a polyelectrolyte and surfactant mixture.¹⁵²⁻¹⁵⁸ When depositing nanoparticles by LB technique, an amphiphilic spacer is often used to prevent aggregation and provide more control over the interparticle spacing of the deposited monolayer. The orientation of phospholipid monolayers has been extensively studied, the results of which provide an insight into the mechanism of packing and surface coverage as a function of surface

pressure, for lipids, nanoparticles, and mixtures of the two.¹⁵⁹ The LSPR of plasmonic nanoparticles changes drastically upon deposition, especially onto a dielectric substrate. This change is induced by the alteration of the electronic field interactions on one surface of the nanoparticle (seen clearly with cubic structures) as well as by the permanent change of refractive index near the same surface (see *Figure 1.14*).^{155,160}

Figure 1.14 – FDTD Determinations: FDTD determination of resonance modes influenced by substrate (A=peak#1, B=peak#2); (C) changes to observable LSPR upon silver nanocube approach to substrate.⁷

1.6.1b) Self-Assembly Method

Self-assembly on gold structures has its origin with the discovery of spontaneous, organized binding of disulfides onto a gold surface in 1983. The bond formed between gold and sulfur is a strong, semi-covalent bond, though it is unstable in the presence of water. Self-assembled monolayers developed on gold since then typically involve either a disulfide or an alkanethiol.¹⁶¹⁻¹⁶² These monolayers are highly organized, especially when a long-chained thiolate is used to form the monolayer. The alkane chains will also organize themselves to a state of order dependent on the density of monolayer formed, similar to the organization seen with phospholipids at different densities in an LB film. The density of the monolayer can be controlled by the concentration of the sulfur-containing compound added to the system.

There are a number of intents for the formation of self-assembled monolayers on a gold nanostructure. At high density, the monolayer can form a protective coating, especially with the use of multi-dentate molecules, to enhance resistance against metal corrosion and particle decomposition.¹⁶³ Other applications of self-assembled monolayers on gold nanostructures include surface modification for further functionalization (such as coatings or targeted etching) and the assembly of large areas of organized nanostructures (possibly extending in three dimensions as well).

There are a few popular binding molecules currently being utilized, among which are (3-aminopropyl)trimethoxysilane (APTMS), (3-aminopropyl)triethoxysilane (APTES), and (3-mercaptopropyl)trimethoxysilane

(MPTMS).^{70,164-165} These alkoxy-silanes are of particular interest for their double-ended functionality. The nitrogen or sulfur end favour binding to a gold or silver surface, while the silane group can be adsorbed to another surface or particle. This dual functionality allows for the capture of gold nanoparticles (when the silane-end is fixed first) or for a monolayer formation on a gold surface or on gold nanoparticles (when the sulfur- or nitrogen-end is bound first).

Figure 1.15 – Alkoxy-silane Structures: Structures of APTES (A), APTMS (B), and MPTMS (C).¹⁸⁵

Polyelectrolytes can also be utilized to form self-assembled monolayers on gold or silver nanostructures. Layer-by-layer assemblies can be constructed by using the alternating charges of certain polyelectrolytes. The self-assembly is then driven by electrostatic attraction.¹⁶⁶⁻¹⁶⁷ The use of phospholipids in a colloidal suspension of gold nanoparticles can also yield self-assembled regions upon deposition, much like with LB films.¹⁶⁸ Three-dimensional self-assembly occurs at high pressures with functionalized nanoparticles, but it can be precisely controlled with flat-sided nanostructures (such as nanocubes) by selectively functionalizing faces of the nanostructure.¹⁶⁹

1.6.2 Fluorescence Investigations

Upon coating noble metal nanostructures with silica, fluorescence enhancement and quenching studies are often used as an important characterization method. Thin-film silica over silver nanocubes has been shown to enhance the fluorescence emission by an average factor of 10 (with respect to emission intensity at same concentration without nanoparticles). Thin films of silica over gold nanostructures is typically intended for quenching studies, with quenching increasing with the thickness of the silica shell. The prediction of whether the presence of nanoparticles in solution with a fluorophore will enhance or quench the fluorescence emission is based on the overlap of the excitation and/or emission wavelengths with the LSPR maxima of the nanoparticle. Thus the fluorophores chosen for the study of silica-coated gold nanocages are different for those used in silver nanocube studies. The fluorophores used in this study were Rhodamine 6G (Rh6G), Rhodamine 640 (Rh640), LDS 751, Rhodamine 800 (Rh800), Rhodamine B (RhB), Nile Blue chloride (NBC), and 1,1'-diethyl-4,4'-carbocyanine iodide (cryptocyanine). The overlap of the LSPR absorption of gold nanocages with the excitation and/or emission of the fluorophores used in this work is discussed in *Section 3.5*.

Fluorophore Name	Excitation, Emission Wavelengths (nm)	Structure
Rhodamine 6G	529; 554
Rhodamine 640	545; 588
LDS-751	543; 712
Rhodamine 800	545; 569
Rhodamine B	684; 704
Nile Blue chloride	629; 662
Cryptocyanine	709; 721

Table 1.2 – Fluorescent Dye Information: Basic information and structure of 7 fluorescent dyes used in this project. (Structures obtained from chemBlink database¹⁸⁶)

1.6.3 Refractive Index Sensitivity Determination

The measure of refractive index sensitivity follows a simple procedure with easily reproduced results. Typical analysis involves the use of solutions of ethylene glycol, ethanol and/or water. Varying the ratios of these solvents in a homogenous solution allows the refractive index of the solution to be easily manipulated. The refractive index of the solution can be calculated using the Lorentz-Lorenz equation (*Equation 1.1*). A known concentration of the nanostructure being investigated is then added to the solution and UV-vis spectroscopy (and/or NIR spectroscopy) is used to observe the LSPR peak position with respect to changing refractive index, from which the RIS of the nanostructure is obtained. The slope of this typically linear relation represents the RIS of the nanostructure. Any modifications made to the surface of the nanostructure will alter the RIS, thus this process is repeated after surface modifications.

RIS can also be determined for a nanostructure deposited onto a substrate. The solution is exchanged around the nanostructure, and so long as the nanostructure is firmly bound to the substrate, the RIS measured will be with respect to the unbound faces of the nanostructure. There is a small amount of surface rearrangement that occurs upon solvent exchange, more so as the refractive index of the solution increases. This is observed by a shift of the LSPR when replaced in a previously measured medium. It is for this reason that RIS is carried out as the last characterization method for substrate-supported nanostructures.

1.7 Characterization methods

A number of characterization methods were used throughout this project. There were a few spectroscopic and microscopic methods used at different stages, for varying states of samples being synthesized or used. Some important information regarding these methods is discussed in the sections to follow.

1.7.1 Ultraviolet-Visible and Near-Infrared Spectroscopy

Ultraviolet-visible (UV-vis) and near-infrared (NIR) spectrophotometry were used extensively throughout this project. The LSPR signals are observed as absorption maxima using these spectrophotometric methods, the strongest of which is the dipolar resonance. When the nanostructure has sufficiently sharp edges, the multipolar resonances can also be observed, such as the quadrupolar and octopolar resonances for cubic structures. Gold nanocages and silver nanocubes both have their LSPR well within the range of UV-vis and NIR wavelengths, though the placement within those ranges depends on the composition and size of the nanostructure, as well as the surrounding environment. The range for silver nanocubes in this project was from 300 to 600 nm, while a range of 400 to 2500 nm was needed for gold nanocages.

Glass, quartz and polymethacrylate cuvettes were available to use for the various methods of spectroscopy, the choice of which was made depending on the range of absorption to be observed. Glass is known to absorb light below 380 nm and above 800 nm, thus if to be used for colloidal suspensions or monolayer deposition, a baseline without nanostructures was required to eliminate the interfering absorption. Quartz cuvettes are the prime candidates for absorption

measure from 200 to 2500 nm, thus the range under 400 nm was more clearly characterized (though quartz cuvettes are more fragile than glass, making them more delicate to clean after use with self-adsorbing nanoparticles).

UV-vis and NIR spectroscopy have easily recognizable features that are intrinsic to the samples being characterized. The width of the maximal peak observed is representative of the monodispersity of the sample. Slight variations of size and shape will alter the maximum LSPR peak, though when the variations are not consistent throughout the entire sample, the peak observed is a summation of their absorbances, thus making it broader than a monodisperse sample. The intensity of the absorption observed follows the Beer-Lambert law for concentration of nanoparticles (see *Equation 1.4*). The actual concentration of these (as moles of nanoparticles per litre) is difficult to determine since the extinction coefficient (also known as molar absorptivity) changes for each particular combination of size, shape and nanostructure composition. The concentration of silver nanocubes and gold nanocages cannot be directly correlated to the original amount of silver added to the synthesis unless the amount lost to cleaning, unreacted reagents, and *in-situ* characterization can be quantified. The concentration observed by the absorption intensity is thus a relative concentration between samples.

$$A=\epsilon lc$$

Equation 1.4 – Beer-Lambert Law: Beer-Lambert Law to relate absorbance to concentration. (A =absorbance; ϵ =molar absorptivity ($\text{L mol}^{-1} \text{ cm}^{-1}$); l =path length through cuvette (cm); c =concentration of compound in solution (mol L^{-1}).)

Absorption can also be used to characterize substrate-supported nanocages. The substrate in question is first used to baseline the spectrometer (or collected as absorption against air for later subtraction) in order to accurately capture the particular interference of that substrate. Each substrate used was slightly different (in film thickness, surface features and substrate thickness), thus a more accurate representation of the effect of the substrate on the LSPR of the nanocages is obtained by removing that same interference from the sample's spectra. The intensity of the absorbance observed then represents the relative surface coverage of the substrate with the nanostructures. Angle-dependent absorption and LSPR shifts can also be observed on substrate-supported samples.

1.7.2 Transmission and Reflection Spectroscopy

Substrate-supported nanoparticles are analyzed for transmission and reflection properties between 300 and 900 nm, or above 1500 nm for fibre optic substrates.

1.7.2a) Slide-based Substrates

Transmission and reflection spectra were obtained for monolayers of gold nanocages deposited by LB technique onto thin-film silicon and glass substrates. The range of absorption and transmission spectra obtained was 340 nm to 1025 nm. Transmission spectra had baseline subtractions against the blank substrates (as with UV-vis absorbance). The theory behind correlating transmission minima to absorption maxima is according to Beer's law (see *Equation 1.5*), though there

are often differences observed. These differences are the influences of plasmonic fields and substrate effects, thus the maxima and minima of the same sample's LSPR may not align as expected. Reflection spectra are obtained separately, and are an unusual characterization to be observed. Typically, reflection is counted as part of the absorption spectra along with scattering, but it is possible to obtain reflection separately to better analyze the sample. The intensity of reflection of a sample is compared to that of a clean, unblemished mirror representing 100 % reflection. With metal nanostructures, the reflection measurements obtained can be related to the coverage of the substrate.

$$A = \log_{10} \left(\frac{1}{T} \right) = \log_{10} \left(\frac{100}{\%T} \right)$$

Equation 1.5 – Beer's Law: Beer's Law, relation of intensities of absorbance (A) and transmittance (T).

1.7.2b) Optical Fibre Substrates

Transmission spectra were also obtained for optical fibre-supported nanocages. This particular application was for the intention of developing biosensing platforms on the basis of resonance shifting, much like colloidal or slide-supported nanoparticles. The cladding resonance of the optical fibre is the most sensitive to coupling interactions, thus it was this resonance that was monitored as nanostructures, receptor molecules and target molecules are sequentially bound to the surface of the optical fibre. The addition of plasmonic

nanostructures to the surface of the optical fibre is for the intent of increasing the sensitivity and limit of detection of the biosensing platform. This sensitivity can also be observed as an increase of the RIS of the optical fibre set up. Recent developments include polymers as well as solid or hollow noble metal nanostructures being bound to the fibre.^{58,170} The application investigated in this project added the feature of a tilted fibre Bragg grating to these developing sensing platforms (to be discussed in *Section 3.6*).

Figure 1.16 – TFBG Transmission Spectrum: Transmission spectrum of TFBG, fibre-based platform. Range of interest indicated by red brackets; cladding mode indicated by red arrow.³⁶

1.7.3 Fluorescence Spectroscopy

Fluorescence spectroscopy was used to measure the enhancement and quenching potential of silica-coated gold nanocages. As the project progressed, the choice of fluorophores was altered to better suit the particular samples being analyzed. The fluorescence compounds used are listed below (also see in *Table 1.2*). The process of collecting fluorescence emission spectra of the varying fluorophores was repeated with different silica shell thicknesses for the intent of observing whether intensity was dependant on the thickness of the shell. Relative concentrations of fluorophore to gold nanocages was also considered for observable trends.

The maximum excitation of each of the fluorophores was found first by UV-vis spectroscopy, where the maximum absorption was observed at the same wavelength as the maximum excitation. The maximum emission of each was then observed using fluorescence spectroscopy before adding any nanocages to the solution. Polymethacrylate cuvettes were used exclusively for fluorescence spectroscopy in this project. This choice was to minimize potential interference, as glass has strong absorption signals at much of the desired range of investigation. A quartz cuvette could have been used for fluorescence investigations, but polymethacrylate was chosen first for availability and then for consistency between samples. The intensity of the emission was observed as a function of the concentration of fluorophore. Some of the fluorophores revealed evidence of self-quenching, thus ideal working concentrations were chosen accordingly. The intensity for each fluorophore at the same concentrations was

directly dependent on the quantum yield of each compound, as seen in *Table 1.3* below. With the varying porosity of the silica shells, there was a suspected effect to be observed as the fluorescent compounds diffused into the shells' pores, thus the same samples were repeated 24 hours after initial addition of the gold nanocages.

$$\Phi = \frac{\text{\# photons emitted}}{\text{\# photons absorbed}}$$

Equation 1.6 – Quantum Yield Equation: Quantum yield for a fluorescence event.

Fluorescent Dye	Quantum Yield ¹⁷¹
Rhodamine 6G	0.95
Rhodamine 640	0.01
LDS-751	0.4
Rhodamine 800	0.288
Rhodamine B	0.7
Nile Blue chloride	0.27
Cryptocyanine	0.007

Table 1.3 – Quantum Yield of Fluorescent Dyes: Quantum yields of the fluorescent dyes used in this project.

1.7.4 Microscopic Methods

Optical microscopy, in many forms, was used to image and characterize various stages of this project. An optical microscope could not be used to observe the samples prepared in this work as the nanostructures prepared were

much too small for standard objective magnification. Other work has yielded the use of nanowires, some of which were able to be identified through an optical microscope when they were of sufficient diameter and length, but the small nanocubes and nanocages prepared for this work were much too small to be resolved without alternative methods.

1.7.4a) Atomic Force Microscopy

Atomic force microscopy (AFM) was used to image monolayers of deposited nanocages on slide substrates in an effort to determine the approximate nanostructure size, monodispersity, and the density of the monolayer. The mode of imaging used here was exclusively non-contact mode, dependent on van der Waal's interactions to alter the tip amplitude with respect to changes in surface topography. This method of AFM allows for extended tip usage and repeated sample imaging by the lack of force exerted on the sample. The resolution obtainable by AFM allowed for individual nanostructures to be easily resolved, though the shapes are broadened and slightly rounded by the inherent nature of this imaging method. The tip is repelled by the static forces of the surface of the sample, creating tip convolution and slightly distorting the image of the nanostructure it is approaching.

1.7.4b) Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used during this project for the exclusive purpose of imaging monolayers of gold nanocages deposited by LB technique. This microscopic method allows for much higher resolution than AFM

to provide a more precise and accurate analysis of nanostructure size and density. The resolution of SEM is excellent for relatively broad areas being imaged, though does not provide atomic resolution for more detailed structural analysis. In preparation for SEM, most samples must be sputtered with a thin film of gold to provide a conducting material with which the electrons will easily interact. However, this preparation method did not need to be completed for the gold nanocages imaged in this project, allowing for higher resolution and contrast between the nanocages and the substrate in the resulting images.

1.7.4c) Transmission Electron Microscopy

Transmission electron microscopy (TEM) was the method of characterization with the highest imaging resolution used in this project. Atomic resolution was obtained with careful calibration; the TEM used was capable of up to one million times magnification and resolution as precise as 0.24 nm. TEM was thus used to determine the size of the nanocubes and nanocages prepared, as well as the silica-shell thickness throughout that stage of the project. This method of imaging also allowed for a qualitative analysis of the monodispersity of a sample, and was used in combination with RIS of silica-coated nanocages to approximate the shell thickness of later samples prior to imaging. TEM has the uncomplicated method of sample preparation, with the samples simply drop cast onto TEM grids (designed as a carbon thin-film on a copper grid array). Once the solvent evaporates, the sample is permanently placed on the TEM grid and can be stored indefinitely before imaging and can be imaged repeatedly.

2 Experimental

All glassware used for the synthesis of silver nanocubes and gold nanocages was washed with aqua regia (one-third concentrated $\text{HNO}_{3(\text{aq})}$ (Anachemia) and two-thirds concentrated $\text{HCl}_{(\text{aq})}$ (Anachemia) by volume) for 15 to 20 minutes, followed by extensive water rinsing (52 times with tap water, 38 times with distilled water, and 15 times with Milli-Q distilled, deionized water) and were fully dried to ensure removal of contaminants before use.

2.1 Synthesis of Silver Nanocubes

Silver nanocubes (AgNC) were prepared using a modified polyol synthesis method. Solutions of PVP (0.08 g/mL; MW 40000, Sigma-Aldrich), Na_2S (3mM; Sigma-Aldrich) and AgNO_3 (282 mM; Sigma-Aldrich) were each prepared in anhydrous ethylene glycol, (EG, Sigma-Aldrich). 35 mL of EG was heated at 150 °C for one hour (on a stirring hotplate; Corning Life Sciences) in a round-bottomed flask (Pyrex Vista Glassware), capped with an injection stopper (Chemglass) and submerged in a stirring mineral oil bath (Sigma-Aldrich). 5 mL of the PVP solution, 400 μL of the Na_2S solution, and 2.5 mL of the AgNO_3 solution were added sequentially with ten minute interludes. The progress of the AgNC formation was monitored by periodic UV-vis characterization (Shimadzu UV-2450). The observable properties of the synthesis solution was another indication of the progress of the reaction: it became opaque as nanocubes were formed with the colour of the solution changing from clear to yellow, orange, redish-brown, and finally to green-ochre. The placement and sharpness of the dipolar mode (between 405 and 500 nm) was a key observation, as well as the

sharpness of the octopolar mode (at 350 nm) for determination of the sharpness of the silver nanocubes being formed. Once suitable properties were obtained, the flask was removed from the heated bath and submerged in an ice bath until cool. The product was then centrifuged at 12000 rpm in a micro-centrifuge (Sorval Legend Micro21, Thermo Scientific) to enable the exchange of the EG solvent with ethanol (95 %, Commercial Alcohols). Washing was continued by repeated cycles of redispersion in ethanol (using sonication; Water-bath sonicator, Elmasonic) and centrifugation to remove the supernatant solution until condensed down to a total product volume of 2 to 4 mL. Once washed 3 to 4 times further in ethanol, the silver nanocubes were stable enough to leave refrigerated for up to one week, though further washing was required before further reactions to ensure good monodispersity in the sample.

Variations made to this synthesis method were very few for this project. The average length of a synthesis reaction (from adding AgNO_3 solution to submerging flask in ice bath) was approximately 15 minutes. Trace amounts of water or chloride in the solution had a tendency to slow the reaction (up to approximately 45 minutes) and would interfere with the formation of monodisperse nanocubes. One alternative method for the synthesis of AgNC was attempted during this project with the intent of forming large nanocubes. This alternative method was a 24 hour reaction with an intentional addition of $\text{HCl}_{(\text{aq})}$ to promote slow and even growth of large AgNC as per Im *et al.*¹⁷² The results were not easily reproducible, thus the above polyol method was used to form the products used in all subsequent investigations.

2.2 Synthesis of Gold Nanocages

Hollow gold nanocages (AuNC) used for further analysis and applications in this project were synthesized using a simple galvanic displacement method with pre-made AgNC (as described previously) used as sacrificial templates. A large amount of 0.1 mM $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}_{(\text{aq})}$ (Sigma-Aldrich) in 18.2 M Ω distilled deionized water (MilliQ pure water filtration) was prepared. The AgNC prepared previously were exchanged to deionized water by centrifugation and redispersed in 0.5 mL deionized water. 10 mL of 0.01 g/mL PVP in deionized water was also prepared and added to the AgNC suspension. This solution was heated to boiling before adding the HAuCl_4 solution. The 0.1 mM HAuCl_4 solution was added at a rate of approximately 2 mL per minute until the solution was observably deep blue. The progression of colour change is typically as follows: opaque green-ochre or redish-brown (of AgNC) to redish-purple, purple, violet, blue-violet, grey-blue, and finally to a translucent deep royal blue (solution remains mostly opaque until this point; see *Figure 2.1*). The progress of the galvanic exchange was monitored by UV-vis spectroscopy, as an observation of the changing intensity ratio between the decreasing silver dipole and the increasing gold dipole (further red-shifted than AgNC, between 500 and 700 nm). Once a satisfactory exchange was completed, the reaction was allowed to continue heating and stirring for approximately 10 minutes to ensure no gold ions remained in solution. The galvanic exchange rate of silver to gold is 3 to 1, which is why the resulting structures will remain hollow so long as there is not an extreme excess of gold salt added to the reaction. The AuNC solution was then condensed to 2 mL in

water by centrifugation and redispersion cycles (as with AgNC). Once condensed, the AuNC product was washed with a saturated solution of NaCl (Caledon) in deionized water to remove any remaining silver ions in solution before continuing washing with deionized water for refrigerated storage. Once washed, the AuNC are very stable in solution and can be used in further studies and applications for up to 3 months. This method of AuNC synthesis was modified from the *Nature Protocol* by Skrabalak *et al.*¹³⁷

Figure 2.1 – Synthesis Product of Gold Nanocages: Newly synthesized gold nanocages seen as a translucent, royal blue, colloidal suspension.

Alternative procedures were also attempted for the synthesis of gold nanocages. PVP, AgNO_3 and $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ in 1,5-pentanediol were refluxed together for one hour as per Lee *et al.*¹⁸; and a seed-growth protocol from HAuCl_4 and trisodium citrate with sequential growth solutions of HAuCl_4 , PVP and hexamethylenetetramine (HMT) as per Zhang *et al.*⁷⁶ were attempted.

Neither of these methods yielded easily repeatable and well-formed products, thus the above exchange method was used to produce gold nanocages used in further investigations and applications.

2.3 Surface Modifications to Gold Nanocages

Simple surface modifications, for the intent of specific investigations and applications, were made to the AuNC prepared in this project.

2.3.1 Hydrophobic Functionalization

The gold nanocages prepared previously were exchanged by centrifugation to a 95 % ethanol. 0.5 mL of the 2 mL synthesis product was dispersed in 1mL of ethanol and was added to 9 mL of a prepared solution of 1-hexadecanethiol (HDT, 1 mM; Sigma-Aldrich). This solution was stirred overnight at room temperature (10 to 12 hours). The resulting product was washed at least three times with ethanol by centrifugation-and-redispersion cycles, then redispersed in 1 mL ethanol for storage and use.

2.3.2 Silica-Shell Formation

The processes used to coat the AuNC with silica shells were modified Stöber methods. The original method developed by Werner Stöber in 1968 yielded monodisperse silica spheres¹⁴², thus the modifications were made to allow for shell growth around the nanostructures present. The method developed during this project began according to Khlebtsov *et al*, wherein the synthesized AuNC were redispersed in 4 mL of distilled deionized water and added to 18 mL

isopropanol (99.5 %, Caledon) before adding 0.5 mL of ammonium hydroxide (30 wt% in water; Anachemia) and 0.05 mL of tetraethylorthosilicate (TEOS; 99.999 %, Sigma-Aldrich) while stirring for one hour. The expected thickness of the product silica shells by this method was 45 nm.¹⁷³ This being too thick for any influence of the LSPR beyond the silica shell, the method was modified by varying the volume and concentration of NH₄OH as well as the volume of TEOS in series. The table below lists the first set of method modifications and the predicted thicknesses according to Khlebtsov *et al.*

Trial Number	Volume 28% NH₄OH (mL)	Volume TEOS (μL)	Volume Ratio NH₄OH/TEOS	Predicted SiO₂ Shell Thickness (nm)
1	0.2680	25	10.72	45 ¹⁷³
2	0.2680	12.5	21.44	22.5
3	0.2680	5	53.60	9
4	0.2680	2.5	107.20	4.5
5	0.1340	25	5.36	22.5
6	0.0535	25	2.14	9
7	0.0268	25	1.07	4.5
8	0.1340 (0.268 x 14 %)	25	5.36	22.5
9	0.0536 (0.268 x 5.6 %)	25	2.14	9
10	0.0268 (0.268 x 2.8 %)	25	1.07	4.5

Table 2.1 – Modifications to Stöber Method: Modifications to Stöber method of capping gold nanostructures with silica (Trials 8, 9 and 10 were repeated ratios of NH₄OH:TEOS, with the intention of monitoring for any difference of silica shell with altered rates of addition).

Some of the method modifications listed yielded more promising results than others for the intent of obtaining thin silica shells on the AuNC. These methods were further explored for reproducibility, as well as adding a hardening process in an attempt to reduce the pore size of the silica shells. This hardening process was modified from a method published by Aslan *et al* in 2007¹⁷⁴, and had 2.5 μL of TEOS added to the pre-coated AuNC in ethanol with low heating (60 $^{\circ}\text{C}$) for 16 hours in closed vials, followed by centrifugation and redispersion washing with ethanol. A third method was also added, with easily repeated results, wherein a small fraction of the uncoated AuNC (50 μL of the 2 mL synthesis product) were dispersed in 0.8 mL ethanol with 10 μL deionized water, 10 μL NH_4OH (28 %) and 3 μL of TEOS added in a closed vial, and heated at 60 $^{\circ}\text{C}$ for 16 hours. This small volume of product was again washed with ethanol, and proved to be reproducible, including with larger fractions of AuNC (with the other reagents' volume adjusted to maintain the same ratios). The issue with this final method was that the silica shells were still consistently too thick for the intended applications, while stopping the reaction before 16 hours yielded inconsistently-coated AuNC. Thus, timed etching cycles of the prepared silica-coated AuNC ($\text{AuNC}@\text{SiO}_2$) were completed using 300 μL portions of 6 mM $\text{NaOH}_{(\text{aq})}$ (Caledon) in deionized water while stirring at 1000 rpm. The etching cycles were monitored for etching progress every hour (approximate etching rate observed to be 1 to 2 nm per hour).

The thickness of the resulting silica shells throughout the methods investigated was observed by UV-vis spectroscopy, RIS determination (using

Schimidzu UV-2450 spectrometer), and TEM imaging with the help of Carleton's Nano Imaging Facility (FEI Tecnai G2 TEM).

2.4 Monolayer Deposition of Gold Nanocages

Some of the intended applications required substrate-supported nanostructures, thus there were two methods used in this project to deposit monolayers of AuNC, as described below.

2.4.1 Langmuir-Blodgett Technique

The LB apparatus (NimaTech; NimaTR516 software) was prepared for deposition of AuNC by first thoroughly cleaning the trough and the aspirator nozzle with chloroform (Caledon) and surfactant-free Kimwipes (Fisher Scientific). Once dry, the trough was filled with fresh Milli-Q distilled, deionized water such that a meniscus of approximately 5 mm was visible above the edge of the trough. The surface of the water was evacuated using the aspirator, followed by the full volume of the trough while watching for changes in surface tension (evidence of likely contamination on the trough). Further cleaning with chloroform was completed if required. A Wilhelmy plate (adapted to calibrated pieces of Fisherbrand filter paper) was installed at this point for measuring the surface pressure of the depositing monolayer, and the trough was refilled with fresh deionized water. The water was allowed to equilibrate for 15 to 20 minutes in a closed environment before obtaining an isotherm of the surface of the water (see *Figure 2.1*). If the isotherm was not seen to remain at 0 mN/m throughout the

extent of the trough compression, the trough was again cleaned with chloroform and the process was repeated until a satisfactory isotherm was obtained.

Figure 2.2 – Clean Water Isotherm: Isotherm obtained during preparation stages of deposition by LB technique: clean water on isotherm with very little contamination.

For the deposition of unmodified and functionalized AuNC, the preparation method was the same: the AuNC were exchanged to chloroform and redispersed in 250 μL of chloroform and 10 μL ethanol. The prepared AuNC solution was then carefully added to the surface of the water phase on the LB trough through careful dropwise addition using a microsyringe. The chloroform was allowed to evaporate from the sample while the surface was again reaching equilibrium. The trough compression was adjusted such that an even monolayer of AuNC was seen on the water's surface. Pressure annealing was used to align the functionalized particles without gaps in the monolayer's continuity. Unmodified

AuNC had a tendency to diffuse into the water subphase, thus the monolayer deposition was carried out as quickly as possible to avoid excess sample loss. Isotherms of the samples to be deposited were obtained to discover the pressures needed for monolayer phase-changes (liquid-expanded, liquid-condensed and solid analogue phases; see *Figure 2.3*). These phases defined the ranges of low, medium and high density monolayers to be deposited (see *Figure 2.3*).

Figure 2.3 – Gold Nanocage LB Isotherm: Isotherm of gold nanocages on the LB trough in preparation for deposition. Areas A, B and C correspond to liquid-expanded, liquid-condensed and solid phases, respectively.

Figure 2.4 – Visualization of Gold Nanocage LB Monolayer Pressures: Gold nanocages in monolayer on surface of LB trough's water subphase at (A) low, (B) medium, and (C) high densities, corresponding to liquid-expanded, liquid-compressed, and solid phase analogues, respectively.

The slide substrates were prepared by cleaning with sonication in methanol (Caledon) and water in sequence. The substrate was then submerged below the monolayer on the surface of the LB trough and raised slowly (2 mm/min, controlled by Nima LB program) with automated pressure control. This automatic process allows for an even density monolayer to be deposited. As the substrate was raised out of the solution, it removed the monolayer from the surface of the water and adsorbed it to the slide substrate (through capillary forces as discussed previously), lowering the total AuNC density on the water surface, thus the automated control allowed for the desired pressure and AuNC density to remain constant throughout the deposition. This deposition process

was repeated for each sample, with a varying number of slide-substrates coated with AuNC monolayers depending on the density deposited (lower pressure allowed for more slides to be dipped and coated before reaching insufficient sample remaining on the trough for the next deposition). The slide substrates had a silicon thin-film covering half of one face of the slide (to allow for simultaneous deposition on silicon and on glass), thus this was deemed the primary face of the slide, upon which the even monolayer of AuNC was deposited. The back of the slide had an uneven layer also deposited onto it which was wiped off with a Kimwipe wetted with methanol. The deposited monolayers of AuNC, functionalized or unmodified, were then imaged using AFM (Ntegra NT-MDT) and SEM (Tescan Vega-II XMU VPSEM, Nano Imaging Facility), as well as characterized by UV-vis and NIR (Cary5) spectroscopy for absorption, reflection and/or transmission (Ocean Optics).

Figure 2.5 – Silicon Thin-Film and Glass Slide Substrates: (Left) A sample of clean, unused silicon thin-film and glass substrate typically used with depositions by LB technique. (Right) Three such silicon-glass substrates after deposition with medium-density monolayers of gold nanocages.

2.4.2 Self- Assembly Method

The use of optical fibres for the development of sensing platforms required a more complex, though less involved, method of deposition of gold nanocages. The tilted fibre Bragg grating (TFBG) was first inscribed into the core of a single-mode optical fibre (by pulsed laser etching through a phase mask, at a tilt of 10° from the normal axis of the fibre). A thin gold mirror was also plated by electroless deposition on the end of the Bragg grating for easy of sensing ability. These methods were developed and performed by the Albert lab group in the Department of Engineering. Once the base TFBG sensor had been prepared, four self-assembled coatings were applied, ending with AuNC. Between each layer, the fibre was rinsed with Milli-Q distilled, deionized water. The first layer to be deposited onto the fibre was one of (3-aminopropyl)trimethoxysilane (APTMS; Sigma-Aldrich) using a solution of 10 % in deionized water, self-assembled while the fibre was submerged in the solution for 2 hours. This monolayer was for the intent of silanization of the surface of the fibre. The second self-assembled monolayer was of glutaraldehyde (2.5 % in water; Sigma-Aldrich), assembled over 30 minutes onto the APTMS layer. Cysteamine (0.5 M for 1 hour; Sigma-Aldrich) made up the third self-assembled monolayer on the optical fibre for the intent of providing a thiol-ended compound with which to bind the AuNC. The tri-layered TFBG was then submerged in a solution of AuNC for 2 hours, resulting in an even layer of AuNC covalently bound to the submerged portion of the TFBG platform.

Further layers were required for the investigation of a biosensing platform (with Dr. Sandrine Lepinay). These layers included a self-assembled monolayer of 11-mercaptoundecanoic acid (MUA, 10 mM in ethanol; Sigma-Aldrich). The carboxyl groups of this last layer were activated by an aqueous solution of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 0.2 M; Sigma-Aldrich) and N-hydroxysuccinimide (NHS 0.05 M; Sigma-Aldrich) in preparation for binding of avidin (1 mg/mL; Sigma-Aldrich). This final layer of avidin provided the receptor half of the avidin-biotin biosensor. The AuNC were placed beneath this layer for the intent of increasing the sensitivity and limit of detection of the TFBG biosensor. For the full procedure, refer to the publication in *Biosensors and Bioelectronics* by Lepinay *et al.*³⁶, in Section 3.6.

2.5 Preparation for Characterization

Throughout this project, numerous characterization methods were used to determine the nature and properties of the structures being synthesized and used in investigative applications. There are certain conditions that were followed when using these characterization methods to ensure reproducible results.

2.5.1 Absorbance: UV-vis and NIR Spectroscopy

UV-vis spectroscopy was used extensively throughout this project. The relative concentration of the samples being analyzed was monitored throughout most stages, though due to the time-sensitive nature of the synthesis processes, the results obtained then were purely for qualitative analysis. The spectra of colloidal AgNC, AuNC and AuNC@SiO₂ were primarily in ethanol, though a new

baseline was obtained at the start of each sample set regardless of the solvent change or lack thereof. Once the synthesis products were stable, all spectra obtained had quantifiable relative concentrations determined by the precise addition of a known volume of nanoparticles into a known volume of solvent and homogeneously mixed before analysis. The concentrations determined during analysis and applications were usually relative to the total concentration of AgNC produced during the initial synthesis.

NIR spectroscopy was used for the observing the absorbance of AuNC deposited onto slide-substrates. These spectra were obtained by fixing the slide substrates (blank and coated with both unmodified and HDT-functionalized AuNC) at a distance calibrated for free-standing samples from the light source.

2.5.2 Transmission and Reflection of Slide-Substrates

Transmission and reflection spectra of the AuNC-deposited slides were obtained using an Ocean Optics apparatus (USB 2000 detector; PX-2 pulsed xenon light source; LS-1 tungsten halogen lamp) calibrated against blank slides or a clean mirror, respectively.

2.5.3 Refractive Index Sensitivity Determination

RIS determination was completed using the UV-vis spectrometer for colloidal solutions of AuNC and silica-coated AuNC. Once deposited onto slide substrates, the LSPR of the AuNC was too far red-shifted to be clearly seen using the UV-vis spectrometer. It was then that the NIR spectrometer was used to determine the RIS of deposited unmodified and HDT-functionalized AuNC. In

both cases, solutions of pure deionized water, 1/3 EG in water, 2/3 EG in water, and EG were used to obtain a range of refractive indices. The refractive index of each solution was calculated using the Lorentz-Lorenz equation, resulting in values of 1.3334, 1.3606, 1.3883, and 1.4166 refractive index units (RIU), respectively. An alternative data set was used when desired, using 0 %, 25 %, 50 %, 75 %, and 100 % EG in water (1.3334, 1.3538, 1.3744, 1.3953, and 1.4166 RIU, respectively). Once the absorption maxima of these refractive index ranges were obtained, data processing to obtain the slope of wavelength shift per RIU yielded the RIS of each sample.

2.5.4 Fluorescence Spectroscopy

The impact of AuNC@SiO₂ on the intensity of fluorescence emission was observed using a Cary Eclipse fluorescence spectrometer. The best excitation wavelength of each of the fluorophores being used was found using UV-vis spectroscopy, then the emission wavelength was found using fluorescence spectroscopy before adding AuNC@SiO₂ to the solution. The fluorescence analysis was performed exclusively in ethanol and a blank emission baseline was obtained accordingly. The excitation and emission wavelengths of each fluorophore were listed previously (see *Table 1.2*). The fluorophores being used were at first kept at the same concentration in order to observe any fluorophore-AuNC ratio relations, but the intensity of some of the fluorophores was too low for the same concentrations, thus the concentrations were adjusted in order to provide comparable ranges of intensity. When no fluorescence enhancement was observed, only varying degrees of quenching, the silica shell on the AuNC

was etched further. AuNC with no silica shell were also tested for fluorescence emission affectation (exclusively quenching). Once relatively thin silica shells were obtained, the choice of fluorophores used for each sample was made according to the overlap of the LSPR of the AuNC with either the excitation or emission of the fluorophores. Three-dimensional mapping of excitation versus emission for the most promising fluorophores (with and without AuNC@SiO₂ present) was obtained to enable the identification of plasmon effects where present.

2.5.5 Microscopy: AFM, SEM and TEM

AFM required very little preparation other than calibration of the instrument and the probe tip to obtain clear images. The AuNC samples deposited onto slide substrates required no further modification before imaging by AFM.

SEM imaging of the AuNC monolayers on slide substrates also required no additional preparation. TEM imaging of the colloidal samples prepared required drop-cast deposition onto purchased TEM grids. TEM was used for analysis of AgNC, AuNC, and AuNC@SiO₂ samples. The calibration of the electron microscopes and imaging of the samples using both the SEM and TEM was completed with the help of Dr. Jianqun Wang in the Nano Imaging Facility.

AFM and SEM were also used by Dr. Sandrine Lepinay to observe and determine the density of the AuNC bound to the surface of the TFBG biosensing platform.

2.5.6 Transmission of Optical Fibre-Substrates

The transmission through the TFBG optical fibre sensing platform was observed using a JDS Uniphase OMNI 2 Swept Wavelength System fibre optic component analyzer, measured and analyzed by Dr. Sandrine Lepinay. The sensors were connected to the analyzer using a spliced connector and scanned from 1520 to 1620 nm. Transmission spectra were obtained after each monolayer addition to the surface of the fibre. The main resonance being monitored through sequential additions to the TFBG platform began at 1538 nm (being red-shifted with each modification).

3 Results and Discussion

3.1 Syntheses: Effects of Size, Shape and Composition

The use of silver nanocubes was an essential part of this project as they serve as the templates for the formation of gold nanocages. The plasmonic properties of the silver nanocubes have been extensively investigated in previous work by many members of the Ianoul lab group. Only two key properties of silver nanocubes were noted during the scope of this project, mainly during the synthesis of the silver nanocubes themselves. During a polyol synthesis of silver nanocubes, single-crystal silver seeds are quickly formed, then grown in a controlled manner toward nanocubes using PVP as a capping agent. This progressive growth can be observed as changes to the LSPR spectrum of the product being formed. As seen in *Figure 3.1*, there is a continued red-shift of the LSPR maximum as the reaction progresses, indicative of the increase in size of the silver product in the reaction solution. The shape of the silver nanostructure is monitored by the appearance of the octopolar plasmon mode at 350 nm as the reaction progresses. As the cubic shape becomes well-defined, this small peak is observed more distinctly. With the growth of these nanocubes, the separation increases between the octopolar mode at 350 nm and the dipolar mode (red-shifted maximum past 400 nm). For relatively small silver nanocubes, the reaction is stopped once this separation is well defined.

Figure 3.1 – LSPR Progress of Silver Nanocube Synthesis: Observable general trend of the progress of the synthesis of silver nanocubes starting with small seeds and progressing to cubic shapes (evident by the emergence of a small but well-defined peak at 350 nm (octopolar mode of cubes)).

The other key plasmonic property of silver nanocubes noted during this project is the LSPR shift with respect to the size of the silver nanocubes in solution. As the size of the silver nanocubes directly dictates the gold nanocubes formed from them, size-control is an important factor to note. The majority of the gold nanocubes used for later investigations in this project were between 30 and 50 nm in diameter, though the potential for using larger gold nanocubes is easily achievable. As seen in *Figure 3.2*, the LSPR of silver nanocubes is red-shifted

with increasing size. The dipolar mode is observed as the maximum of the silver nanocube spectrum and is most influenced by the size of the nanocube. The octopolar mode at 350 nm is not greatly affected by the increasing size of the nanocube, only by the overall shape of the silver nanostructure. As the nanocubes increase in size, a third plasmon mode can be observed as a shoulder blue-shifted from the dipolar maximum (near 390 nm in *Figure 3.2*). This shoulder is the emergence of the quadrupolar mode of the silver nanocubes, becoming more distinct as the silver nanocube size continues to increase.

Figure 3.2 – LSPR Shift with Respect to Size: The shift of the LSPR of silver nanocubes is used to predict the diameter of the nanocubes by the inherent red-shift of the dipolar maximum with increasing size.

Similar to the LSPR changes during the synthesis of silver nanocubes are those during the synthesis of gold nanocages, as seen in *Figure 3.3*. The dipolar maximum is drastically red-shifted (as is the nature of gold nanostructures as opposed to those composed solely of silver), and the silver dipolar peak can be seen to become progressively less distinct as more silver is oxidized in the reduction of gold. The LSPR of the gold nanocages continues to red-shift slowly as more gold is added, though this is not with respect to the overall size of the nanocube (since it was the silver nanocubes used as a template). This is instead a shift observed with respect to increasing thickness of gold in the nanocage walls. A part of this same shift is the influence of the oxidation of silver; as more silver is oxidized, there is less to form a gold-silver alloy in the structure of the nanocages, thus the average absorption observed becomes more like that of a pure gold nanostructure. The octopolar mode of the silver nanocubes can also be observed to distort away from 350 nm toward 340 nm and become less distinct. This is likely caused by the corners of the product nanocages becoming slightly rounded as the exchange occurs, causing a less intense resonance.

For every gold atom reduced, there are three silver atoms oxidized (see *Equation 3.1* below). It is by this principle that the product nanostructure can be hollow. The gold will preferentially deposit along the edges of the disappearing silver nanocube, eventually leaving only a cubic nanocage structure.

Figure 3.3 – LSPR Progress of Galvanic Displacement: The progression of the galvanic displacement during the silver nanocube-templated synthesis of gold nanocages can be observed by UV-vis spectroscopy as more gold salt is added to oxidize the silver into solution.

Equation 3.1 – Galvanic Displacement Reaction: The complete equation of the galvanic displacement reaction occurring between silver nanocubes and tetrachloroauric acid is shown, leaving insoluble silver chloride as a product of the reaction to be removed during cleaning.

For most plasmonic nanostructures, the LSPR will red-shift as a function of the size of the structure. This principle stands for gold nanocages, though it is not always a clear relation. The structure of the nanocage itself also influences the LSPR observed. For this reason, the principle of size-dependence of the silver nanocubes used as templates does not directly correlate to the LSPR pattern of the gold nanocages as seen in *Figure 3.4*. It is a more complex correlation for relative LSPR for this particular structure. The porosity of the faces, the thickness of the nanocage walls and edges, and the composition of the alloy making up the nanostructure all add their influence to the LSPR shift, not just the size of the nanocage. This makes it difficult to predict the trend of the size of nanocage produced unless the original size of the templating silver nanocubes is known. The gold nanocages are formed directly on the silver nanocube with the gold depositing into the spaces vacated by the oxidized silver. Thus the final product is typically within 5 nm in edge length of the original silver nanocube templates.

Figure 3.4 – LSPR Changes from Silver Nanocubes to Gold Nanocages: The synthesis of gold nanocages from silver nanocubes does not follow the same size-dependent trend seen during the synthesis of silver nanocubes. The porosity, gold nanocages wall thickness and the percentage of silver-gold alloy remaining also influence the LSPR of the nanocages, not just the size of the nanostructure. (The matching colours of spectral lines correspond to a particular silver nanocubes template producing a particular gold nanocage product.)

The monodispersity of the gold nanocages in a particular sample is primarily dictated by that of the silver nanocubes used to template the exchange reaction. The process of galvanic exchange can also distort the shape of the nanocube as the silver in solution may again reduce onto the structure in the lowest possible energy coordination, pushing the average shape away from cubic into spheroid nanostructures. With minimal contamination in solution, the majority of the nanostructures remain cubic, as seen in *Figure 3.5*.

Figure 3.5 – Micrographs of AuNC Synthesis Products: A few of the AuNC synthesis products; the porosity of the nanocages varied between trials, as did the wall thickness, though the structures within each sample had very similar properties.

3.2 Depositions: Substrate and Density Effects

The plasmonic property that is utilized in almost every application of noble metal plasmonic nanostructures is their inherent sensitivity to changing refractive index. One of the ways to change the refractive index surrounding the nanostructure is to deposit them onto substrates. Langmuir-Blodgett technique was used to deposit unmodified and hydrophobic-functionalized gold nanocages onto slide substrates of glass and varying thicknesses of silicon thin films on glass. As seen in *Figure 3.6*, the initial deposition of gold nanocages from solution onto a glass (or silicon) substrate yields a blue-shifted LSPR spectrum. The nanocages are suddenly exposed to air on all but one face, which is adsorbed onto the slide substrate. The capping agents, when removed from solution, flattened onto the surface of the nanocage with an average thickness less than 1 nm, allowing for the LSPR to be influenced by the next layer of the surrounding environment, that being air in this case. Air, having a lower refractive index than the solution in which they were previously suspended, lowers the wavelength at which the LSRR is observed.

The main consideration for substrate-supported nanostructures is not this initial blue-shift, however, but the subsequent red-shift and broadening of the dipolar mode as the refractive index of the substrate is altered. Adding a thin film of silicon to the glass slide increases the total refractive index of the media with which the nanocages are interacting, inducing an increase of the LSPR wavelength. This property, altering the LSPR of substrate-supported nanostructures by altering the nature of the substrate, can be applied to the

interest of substrate effects (such as in photovoltaic applications). The density of the deposited monolayer also influences the sensitivity of the LSPR to the refractive index of the surrounding environment. With a high-density monolayer, the electron oscillations of the gold nanocages are affected more by the proximity of other nanocages than the nature of the substrate. *Figure 3.7* shows the effect of substrate-silicon thickness on the LSPR of the gold nanocages used in this project with respect to the density of the deposited monolayer.

Figure 3.6 – LSPR Shift with Respect to Substrate Refractive Index: The LSPR shift with respect to deposition onto a slide substrate and subsequent influences of substrate refractive indices. Once deposited, a higher refractive index induced a red-shift of the LSPR.

(A)

(B)

Figure 3.7 – LSPR Shift with Respect to Silicon Film Thickness: (A, previous page) Summary plot of LSPR shift with respect to silicon thin-film thickness demonstrates higher sensitivity with lower density monolayers; (B, previous page, C) sample series of the spectra observed in the analysis of LSPR red-shift with respect to increasing silicon thickness, for small nanocages at high density and larger nanocages at low density, respectively.

Reflection and transmission properties were also observed for the monolayers of gold nanocages prepared by Langmuir-Blodgett technique. The LSPR position was not expected to shift away from that seen by normal absorption (as shown in *Figure 3.8*), thus the property under investigation in this case was the change of intensity. The reflection maxima did red-shift slightly, though the extent of that shift is difficult to determine with current instrument limitations. The shift is likely in part caused by internal reflection in the substrate inducing an interaction with the face of the nanocage in contact with the substrate instead of the only the unbound faces. Beer's law can be used to

calculate an alternate absorbance spectrum from transmission data. This new absorbance does not accurately account for scattering or reflection, thus the maximum does not typically align exactly with the experimentally obtained absorbance maximum, though it was usually seen to be in good agreement. These comparisons were made with the smaller gold nanocages (approximate edge length of 35 nm) for this project. Attempts were made to compare the reflective and transmission properties of the larger nanocages as well (approximate edge length of 50 nm), but only intensity information can be confidently compared as the instrument available for reflection and transmission did not have the near-infrared light source needed to obtain more accurate readings for an LSPR above 900 nm.

Figure 3.9 is a sample of the spectra observed by reflection for gold nanocages deposited at low density on slide substrates made with varying thickness of silicon thin-films. The observable trend is at first unclear until the note of the density of the monolayer is made. At low density, the majority of the surface of the substrate is still open to direct interaction with incident light, thus the increase in reflection observed between the glass substrate and the thinnest silicon film (10 nm) is caused by the nature of the substrate. Silicon thin films are more reflective than bare glass, though it is also observed that the intensity of this reflection decreases with increasing silicon film thickness. The reflection property from the gold nanocages is more easily observed with a high-density monolayer, as in *Figure 3.10*, where it was observed that there is no definitive influence from the substrate on the reflection of light off the gold nanocages.

Figure 3.8 – Absorbance, Reflection and Transmission of gold nanocages: (A), (B) on previous page; comparison of experimentally determined absorbance, reflection and transmission of gold nanocages deposited by LB technique onto a slide-substrate show moderate agreement between the LSPR maxima and minimum observed of the respective spectra. The absorption of the gold nanocages as determined by applying Beer's Law to the transmission spectrum, provides an approximate estimate of the extent of scattering or reflection that is normally only seen as an over absorption. Each series of spectra represent a different combination of conditions, being small nanocubes at high density on a thin-film of silicon of 10 nm (A); small nanocubes at high density on bare glass (B); and small nanocubes at low density on bare glass (C).

Figure 3.9 – Reflection Spectra, Low Density Monolayers: Sample of spectra collected for reflection studies; sample here is small gold nanocages (35 nm in edge-length) at low density of a variety of substrates. (The low density monolayers were achieved by LB technique without any surface modifications made to the

Figure 3.10 – Reflection Spectra, High Density Monolayers: Sample of spectra collected for reflection studies; sample here is small gold nanocages (35 nm in edge-length) at high density of a variety of substrates. (The high density monolayers were achieved by LB technique with HDT-capped gold nanocages.)

The definitive trend observable from the reflection spectra of gold nanocage monolayers was that the density of the monolayer does directly affect the intensity of the reflection observed. The reflection intensity increases with increased monolayer density, as seen in *Figure 3.11*. This is likely as the increased monolayer allows the collection of closely packed gold nanocages to act more like a thin film of gold instead of individual nanostructures, thus having a reflection more inherent to bulk gold than nanoparticles.

Figure 3.11 – Trends of Reflection Intensity: Intensity of reflection spectra seen to increase with increasing AuNC monolayer density. Solid lines are high density monolayers, while dashed lines are low density monolayers. The red line represents the intensity of the mirror used as a blank for this investigation (represents 100 % reflection).

Black lines – 35 nm AuNC on glass;

Green lines – 35 nm AuNC on 10 nm silicon thin-film;

Blue lines – 50 nm AuNC on 30 nm silicon (dashed blue line is a medium density monolayer)

Transmission had the same trend as reflection with respect to monolayer density, albeit reversed. The intensity of transmission was seen to decrease as the density of the gold nanocage monolayer increased, as shown in *Figure 3.12*.

Figure 3.12 – Trends of Transmission Intensity: Intensity of transmittance spectra seen to decrease with increasing AuNC monolayer density. Solid lines are high density monolayers, while dashed lines are low density monolayers. The red line represents the intensity of the clear blank used for this investigation (represents 100 % transmission).

Black lines – 50 nm AuNC on 30 nm silicon thin-film (dashed black line is a medium density monolayer);

Green lines – 35 nm AuNC on 25 nm silicon thin-film;

Blue lines – 35 nm AuNC on 20 nm silicon thin-film.

There was no observable trend of substrate effects on the transmission spectra of the monolayers of gold nanocages. *Figure 3.13* shows the spectra obtained for a sample at low and at high density. There is as yet no trend to be found for the transmission of gold nanocages, though the previously observed effects of porosity and silver-gold alloy composition warrant future investigation into possible hidden correlations.

Figure 3.13 – Transmission Spectra: Sample transmission spectra observed for high (A) and low (B) density monolayers of gold nanocages on various substrates.

SEM and AFM images of some of the deposited monolayers were collected as a method of evaluating the density of the prepared depositions. A few of these images are shown in *Figure 3.14*.

Figure 3.14 – SEM and AFM Micrographs: Sample SEM and AFM images for characterization of low density monolayers (left column) and high density monolayers (right column) prepared by LB film deposition technique.

3.3 Surface Modifications: Effects of Altering the Local Environment

3.3.1 Hydrophobic Functionalization

During the deposition of gold nanocages by Langmuir-Blodgett technique onto slide substrates, it was observed that the as-synthesized gold nanocages were unable to achieve close packing in a monolayer. Hydrophobic surface functionalization was a method used previously to create close ordered arrangements of silver nanocubes, thus this was also attempted with the gold nanocages used in this project. The surface modification achieved by exchanging the PVP capping agent with a self-assembled monolayer of HDT also changed the LSPR observed in solution, as seen in *Figure 3.15*. The shift seen with smaller gold nanocages was much greater than that seen with larger nanocages. This difference may be attributed to the smaller nanocages being more sensitive to changes in the amount of aggregation in the solution. The smaller nanocages, when aggregated together by increased Van der Waals forces between their capping molecules, have an increased number of surrounding nanocages influencing the plasmon modes of their respective neighbours than would the larger nanocages. This is also why the LSPR of the functionalized nanocages is seen to have a significantly broader bandwidth than those left unmodified. The HDT-capped nanocages were used to form high density monolayers on the slide substrates (as seen by SEM and AFM in *Figure 3.14*). TEM images were also used to observe the increased packing order induced by the alkyl-chain interactions. The extent of the packing order observed by TEM imaging was

limited since the samples had been drop-cast instead of precisely deposited by LB technique (see *Figure 3.16*).

Figure 3.15 – LSPR shift with Respect to HDT Coating: LSPR shift induced by surface functionalization of gold nanocages.

Figure 3.16 – TEM Micrograph of HDT-Capped Gold Nanocages: TEM imaging of HDT-capped gold nanocages revealed domains of ordered aggregates after

3.3.2 Core-Shell Formation: Silica Shells

The other surface modification employed during this project was coating the gold nanocages with silica shells. These core-shell structures of varying shell-thicknesses had distinct effects on the LSPR of the suspended nanostructures. Silver nanocubes have previously been coated with thick silica shells, thus the principle of creating a core-shell gold nanocage and silica-shell system was first established according to methods by Khlebtsov *et al.*¹⁷³ and Aslan *et al.*¹⁷⁴, as seen in *Figure 3.17*. The red-shift of the LSPR (and the change of RIS; see *Section 3.4*) was an indication of silica shells being formed, though TEM later revealed that they were not at all well-formed (see *Figure 3.18*).

Figure 3.17 – SiO₂ Coating Attempts: SiO₂ coating attempts following methods by Aslan *et al.* and Khlebtsov *et al.* LSPR shift leads one to assume silica shells are being formed (not the case when further investigations completed).

Figure 3.18 – TEM Micrographs of SiO₂ Coating Attempts: TEM images of “AuNC@SiO₂” products using methods as per Aslan *et al.* (left image: showing some shells, but not well formed around the gold nanocages. Some silica particles formed independent of the gold nanocages.) and Khlebtsov *et al.* (right image: no visible shells on any of the gold nanocage, nor any visible silica spheres formed).

Modified Stöber methods were then attempted to further determine a reliable method of coating the gold nanocages evenly with silica. *Table 2.1* in *Section 2.3* lists the changes made to the method. All of the modified methods yielded a red-shift of the LSPR in solution (as seen in *Figure 3.19*) thus RIS determination was completed on all of the products in an aim to determine the best combinations (see *Section 3.4*). TEM imaging was performed on a number of the products to help correlate potential silica shell thickness with the RIS results. Fluorescence spectroscopy was performed using the products of these ten method modifications (see *Section 3.5*).

Figure 3.19 – LSPR Shifts with Stöber Method Modifications: All of the modifications made to the Stöber method (listed previously) showed some degree of red-shifting of the LSPR shift, indicative of the formation of some sort of silica shell (or silica nanoparticles in contact with the gold nanocages).

Figure 3.20 – TEM Micrograph of Bare Gold Nanocage: Uncoated gold nanocages were also imaged for characterization purposes before attempting to coat with silica shells.

Figure 3.21 - TEM Micrographs of Further Stöber Method Modifications: Trials 1 (top left), 2 (top right) and 3 (middle left) produced poorly formed silica coatings on the gold nanocages. Many parts of the nanocages were still exposed to surrounding environment while other portions of the same nanocages had very thick piles of silica attached to them. Trials 4 (bottom left) and 6 (bottom right) produced very thin, but well-formed silica shells. ($\text{NH}_4\text{OH}:\text{TEOS}$ ratios of 10.72, 21.44, 53.60, 107.2 and 2.14, respectively)

Two of the first ten modifications to the Stöber method provided potentially reproducible results (see *Table 3.1*), thus these particular methods were repeated with slight modifications in an attempt to optimize the method to be used. Two of these repeated methods involved an extended period of time intended to harden the silica shells by having a slight excess of TEOS in the reaction solution, both of which were seen to have even shells forming (by TEM, see *Figure 3.22*). The silica shells were still very thick, thus they were etched with NaOH in an attempt to reduce the overall thickness. *Figure 3.23* demonstrates the etching process as observed by UV-vis spectroscopy. Within the first 30 minutes of etching there is a blue-shift of the LSPR of the coated nanostructures. From the TEM images, it was determined that the initial etching time first dissolves the small spheres of silica that were also formed during the coating process. Once these small pieces were gone, the silica shells themselves were etched, albeit slowly. As the silica shells reduce in thickness, the LSPR is red-shifted as an indication of the reduced distance between the nanostructure and the suspending solvent. The limited distance of penetration of a plasmonic field dictates the need for thin shells in order to retain sensitivity to the surrounding environment (especially being the basis of plasmonic sensors). Fluorescence spectroscopy was also used to analyze these modified coatings, as seen in *Section 3.5*.

Trial Number	Volume Ratio NH ₄ OH/TEOS	SiO ₂ Shell Thickness (TEM imaging, nm)	RIS* (nm/RIU)	Description
0	N/A	0 ± 0	268.7 ± 0.5	Uncoated AuNC
1	10.72	5.00 ± 2.39	196.5 ± 47.0	Incomplete monolayer
2	21.44	3.27 ± 1.81	256.1 ± 16.3	Incomplete monolayer
3	53.60	1.92 ± 1.14	314.9 ± 44.1	Incomplete monolayer
4	107.20	1.54 ± 0.66	297.3 ± 42.9	Mostly even monolayer
5	5.36	3.27 ± 1.81	253.2 ± 21.4	Incomplete monolayer
6	2.14	2.06 ± 1.04	238.5 ± 15.0	Uneven monolayer
7	1.07	1.02 ± 1.71	238.5 ± 15.0	Incomplete monolayer
8	5.36**	2.16 ± 1.50	237.3 ± 35.6	Incomplete monolayer
9	2.14**	0.79 ± 1.10	197.7 ± 23.2	Incomplete monolayer
10	1.07**	1.91 ± 1.02	238.5 ± 15.0	Incomplete monolayer

*Refractive index sensitivity (RIS) determination discussed in *Section 3.4*

**Volume ratios repeated using lower concentration NH₄OH (see *Table 2.1* in *Section 2.3*)

Table 3.1 – Stöber Method Modification Results: Results of initial 10 Stöber method modifications.

Figure 3.22 – Further Developments of Modified Stöber Methods:
Above: (Left) Coating and hardening of silica shell occur simultaneously in 16-hour reaction; (Right) etching for 3 hours* with $\text{NaOH}_{(\text{aq})}$ to reduce shell thickness.
Below: (Left) AuNC with thin coating (from Trial 4 method of first Stöber modifications) hardened with further addition of TEOS over 16 hours, producing very thick shells; (Right) etching for 3 hours* with $\text{NaOH}_{(\text{aq})}$ to reduce shell thickness.

*(approximate etch rate of 1 to 2 nm per hour)

Figure 3.23 – LSPR Shift During Etching: The LSPR shifts observed during the etching of the silica shells from 18 to 16 nm (A), and from 53 to 47 nm (B).

3.3.3 Silica Shell Etching Variations

These last modifications to the Stöber method were used to optimize the process to be used for the remainder of this project. The general process was 16 hours of simultaneous coating and hardening (3:50 TEOS: AuNC by volume, assuming full AuNC synthesis product was first condensed to 2 mL), followed by etching with NaOH (300 μ L, 6 mM). The last variation made to this method was to the etching stage, being either one single addition of NaOH, or sequential etching at predetermined time intervals. The coating process prior to etching formed even silica shells around the gold nanocages with thicknesses varying from 17 to 35 nm, mostly dependent on the size of the nanocages being coated (smaller structures have less surface to cover, thus thicker shells are formed with the same amount of reagents in solution as the thinner shells on larger nanostructures). Three different gold nanocage samples were used for the subsequent investigations, all of approximately the same size nanocages though their respective LSPR spectra differ significantly, as seen in *Figure 3.24*.

The effectiveness of the etching variations was evaluated primarily by RIS determination and fluorescence spectroscopy (see *Sections 3.4* and *3.5*, respectively). TEM imaging was used periodically throughout this stage of the project to confirm predicted thicknesses of silica shells and trends in the etching and fluorescence results.

Figure 3.24 – LSPR of Gold Nanocage Products: Three samples of gold nanocages with the same average edge length. The variations of the LSPR observed are caused by porosity and silver-alloy content remaining in the product nanostructure.

The general trend of LSPR shift observed during the continuous etching series is demonstrated in *Figure 3.25*. One curious notion observed in this series was that the shells seem to redeposit or reassemble between etching hours 10 and 15. This is suspected to be caused by the etched silica remaining in solution, given enough time, will redeposit on the remaining shell. Some of the re-deposited silica filled pores in the surface of the shell, though the average shell thickness increased as this happens. In response to this observation, a sequential etching series was developed in an attempt to continue to reduce the shell thickness surrounding the gold nanocages.

Figure 3.25 – LSPR Observations During Continuous Etching Series: (one addition of NaOH allowed to etch for up to 15 hours) General trend is no real etching occurred. Shells seemed to be slightly thinner at the 5-hour time, but then grew again between 10 and 15 hours. (A) shows one series of sample etching results, with the three trends shown in (B).

The continuous etching series used one single addition of NaOH to the sample and was stirred for the desired time before washing and completing characterization. For the sequential etching series, NaOH was added and allowed to etch for up to 5 hours, followed by washing the sample and exchanging into clean solvent before adding the next addition of NaOH for the next etching time interval. This method allowed for a continually thinning silica shell, though the TEM images collected revealed that the porosity of the silica shells increased as the overall thickness decreased, directly affecting the RIS of these core-shell systems (see *Section 3.4*). *Figure 3.26* summarizes the LSPR movement as etching continued for these three samples of silica-coated gold nanocages.

Figure 3.26 – Micrographs of Etching Progress: TEM images of etching progress via sequential etching series. Each pane is an increased etching time of 5 hours, starting with no etching on the far left panes. The thinnest silica shells were found after 20 hours of etching (with an average near 10nm), though the drastic increase in porosity of the silica shells with each subsequent etching cycle also greatly affects the optical properties of the nanostructure.

Figure 3.27 – LSPR Observations During Sequential Etching Series: (A) shows a sample of the LSPR changes observed for one of the gold nanocage samples; (B) demonstrates the continued etching of the silica shells with each subsequent addition of NaOH_(aq).

3.4 Refractive Index Sensitivity Determinations

RIS is the basis of many of the potential applications of gold nanocages, thus it was one of the largest investigations of this project. The RIS determinations included both colloidal suspension of gold nanocages and silica-capped gold nanocages, and the slide-substrate supported gold nanocages on various thicknesses of silicon thin films.

The refractive index sensitivities of gold nanocages were determined using three solvents of fixed refractive index (95 % ethanol, 1.3658 RIU; deionized water, 1.3334 RIU; and ethylene glycol, 1.4166 RIU) in combination with a varying choice of solutions of intermediate refractive indices. These intermediate ranges were typically solutions of 1/3 and 2/3 ethylene glycol in water, or 25 %, 50 % and 75 % ethylene glycol in water. The refractive indices of these mixed solutions were calculated using the Lorentz-Lorenz equation (*Equation 1.1*), the results of which are shown in *Table 3.2*.

Solution Ratio	RIU
1/3 ethylene glycol in water	1.3606
2/3 ethylene glycol in water	1.3883
25% ethylene glycol in water	1.3538
50% ethylene glycol in water	1.3744
75% ethylene glycol in water	1.3953

Table 3.2 – Refractive Indices of Solutions: Refractive indices of solutions for RIS determination

3.4.1 Colloidal Suspensions

Figure 3.28 displays a sample of the spectra obtained for RIS determination of gold nanocages in solution, both unmodified and capped with silica shells. The RIS of each series was determined as the slope of the linear fit to the relation of LSPR position to the refractive index of the solution (as seen in *Figure 3.28d*).

RIS determination was used throughout the modifications of silica capping and etching methods in attempts to estimate the average silica shell thickness at a given stage. TEM imaging was used to compare the true average shell thickness with these original estimates in order to improve the approximations before TEM of later samples. Preliminary estimates were thought to follow a trend of increasing RIS with decreasing shell thickness, whereupon once the RIS of etched-shell reached the same RIS as uncoated gold nanocages it was thought that the shell was either very thin or had been etched away completely. This was not the case, as revealed by the TEM images of the same etched samples. What was found instead was that the increased porosity of the silica shells drastically increased the RIS of the gold nanocages' LSPR, to the point in some cases that the RIS determined for the etched samples was nearly equal to that of the uncoated nanocages despite still having 10 to 15 nm of silica shell surrounding the nanocage core. *Table 3.3* summarizes the determinations of RIS of gold nanocages, with average edge length of 36 nm (± 6.3 nm), with respect to the silica shell thickness surrounding them.

Figure 3.28 – RIS Determination of Colloidal Sample: (A , B, C) Shown are some of the spectra collected during RIS determination trials, for colloidal suspensions of uncoated AuNC, and AuNC@SiO₂ without and with sequential etching, respectively, (D) The linear plots of the LSPR wavelength with respect to the refractive index of the medium describe the relation to refractive index sensitivity in terms of nm per RIU.

SiO₂ Shell Thickness (nm)	RIS (nm/RIU)	Description
0 ($\pm$ 0 nm)	282.7 ($\pm$ 10.1 nm/RIU)	Uncoated AuNC (36 $\pm$ 6.3 nm)
10.8 ($\pm$ 3.2 nm)	248.7 ($\pm$ 13.3 nm/RIU)	Very porous shell
15.0 ($\pm$ 2.1 nm)	192.3 ($\pm$ 32.6 nm/RIU)	Very porous shell
17.3 ($\pm$ 2.1 nm)	100.2 ($\pm$ 8.5 nm/RIU)	Unmodified SiO ₂
30.8 ($\pm$ 2.5 nm)	61.4 ($\pm$ 88.5 nm/RIU ^{**})	Shell too thick for use

^{**}Shell thickness greater than 20 nm shields LSPR from environmental changes and cannot be used for plasmonic applications involving detection of altered conditions.

Table 3.3 – RIS of Colloidal AuNC@SiO₂: Summary of RIS determinations for colloidal AuNC@SiO₂.

3.4.2 Slide Substrate-Supported

The monolayers of gold nanocages deposited by Langmuir-Blodgett technique were also used to investigate the RIS properties of these nanostructures. The monolayers of low and medium density depositions were submerged in solutions of water, 1/3 and 2/3 ethylene glycol in water, and ethylene glycol to compare their LSPR spectra against that seen in air. The monolayers of high density were achieved by first functionalizing the gold nanocages with hydrophobic HDT, allowing for much closer packing during the deposition onto slide substrates. This hydrophobic functionalization, however, added a complication to further investigations. The adsorption of the functionalized nanocages onto the surface of the glass or silicon thin-film slide did not have a strong enough interaction to withstand submerging the slides into aqueous solutions. The monolayer was immediately and completely pulled away and aggregated at the surface of the solution when the slide was submerged in

any solution prepared for RIS determination (see *Figure 3.29*). Thus the RIS of high-density monolayers of gold nanocages was not determined.

Figure 3.29 – Images of HDT-Capped Monolayer: HDT-functionalized nanocages deposited as a monolayer by LB technique did not withstand being resubmerged into an aqueous solution. (Left = Before; Right = After)

The RIS determination of the low and medium density monolayers of gold nanocages on slide substrates was completed in the same manner as the colloidal suspensions. A sample collection and RIS determination series is shown in *Figure 3.30*, with a summary of RIS by density, silicon thin-film thickness and nanocage size reported in *Table 3.4*.

AuNC Average Edge Length (nm)	Silicon Thin-Film Thickness	RIS (nm/RIU)
35 (low density)	0 nm (bare glass)	151.9 ($\pm$ 10.5 nm/RIU)
	20 nm	508.7 ($\pm$ 102.2 nm/RIU)
50 (low density)	0 nm (bare glass)	273.6 ($\pm$ 9.3 nm/RIU)
	20 nm	91.1 ($\pm$ 1.1 nm/RIU)
50 (medium density)	0 nm (bare glass)	208.2 ($\pm$ 57.4 nm/RIU)
	20 nm	230.1 ($\pm$ 26.5 nm/RIU)

Table 3.4 – RIS of Substrate-Supported AuNC@SiO₂: Summary of RIS determinations for substrate-supported AuNC@SiO₂.

Figure 3.30 – RIS Determination of Substrate-Supported Sample: Shown are some of the spectra collected during RIS determination trials of substrate-supported gold nanocages at low density on glass (A), low density on 20 nm silicon thin-film (B), and medium density on 20 nm silicon thin film (C)

(D) The linear plots of the LSPR wavelength with respect to the refractive index of the medium describe the relation to refractive index sensitivity in terms of nm per RIU.

When evaluating properties of substrate-supported nanostructures, RIS determination must be the last characterization completed because of the surface rearrangement induced by repeated submersion in solutions of increasing refractive index. This rearrangement alters the LSPR observed, as shown in *Figure 3.31*. The alteration affects all sizes and surface functionalizations of gold nanocages to such an extent that the change is immediately visible upon UV-vis characterization. Normally NIR is needed for substrate-supported gold nanocages though the before- and after-RIS LSPR shoulders are also drastically different below 900nm.

Figure 3.31 – Spectra Before/After RIS: Observation of monolayer rearrangement after RIS.

3.5 Fluorescence Enhancement and/or Quenching

Most applications of gold nanoparticles involving fluorescence utilize their inherent quenching for the purpose of monitoring target concentrations or localized aggregation. These applications have so far had their fluorescence dyes chosen with respect to the target molecule as opposed to the gold nanoparticle. The ability to estimate the quenching or enhancement of a fluorophore's emission intensity based on the type of gold nanocage being used (mainly the thickness of a capping agent around it) could be a useful measure in expanding the choice of fluorophores for a variety of applications.

3.5.1 Proof-of-Concept: Using Available Resources

The resources available as this portion of the project began were not very well suited for determining the quenching or enhancement potential of silica-capped gold nanocages. They were, however, essential in determining which fluorescent dyes were to be acquired for further investigations.

Rhodamine 6G (Rh6G) is one of the fluorescent dyes most commonly used in a wide variety of applications including bioassays and SERS. It has a strong excitation and emission signal well within the visible spectrum (529 nm and 554 nm, respectively). Rh6G was the first fluorophore used in this project in combination with gold nanocages. As shown in *Figure 3.32*, neither the excitation nor emission of Rh6G have much overlap with any of the gold nanocages used in this project, thus initial predictions were not expecting enhancement at all, though to what degree the emission would be quenched was then unknown.

Figure 3.32 – Rh6G Spectra versus AuNC LSPR: Rh6G excitation and emission spectra shown to overlap with AuNC LSPR

Many fluorophores are known to self-quench with higher concentrations, thus calibration spectra of each fluorophore to be used were obtained to determine a good working range for each dye. The calibration concentrations and emission spectra of Rh6G are shown in *Figure 3.33*.

Figure 3.33 – Calibrated Emission Intensities of Rh6G: Rh6G calibrated emission intensity with respect to concentration.

Silica spheres, without a metal core, are known to enhance fluorescence emissions, thus a comparative analysis of uncoated gold nanocages was completed, as well as gold nanocages without any fluorescent dye. Tiny gold nanoclusters have characteristic fluorescence properties while under a certain size, but larger nanostructures do not have the same inherent fluorescence, as demonstrated in *Figure 3.34*.

Figure 3.34— Fluorescence quenching using uncoated gold nanocages.

Fluorescence spectroscopy was used during the method development processes of the silica-coated gold nanocages, in combination with RIS and TEM, to determine the thickness of the silica shell formed. Rh6G was the fluorophore utilized for the first round of Stöber-method modifications (see *Figure 3.35*), all of which showed significant quenching. The TEM imaging collected of

the same gold nanostructures revealed that the shells were mostly incomplete, thus any potential fluorescence enhancement on the part of small silica spheroids around the nanocages would have been countered by the quenching from the exposed metal in the solution.

Figure 3.35 – Quenching from Modified Stöber Methods: Rh6G emission quenched by products of the Stöber modification trials.

As the silica-coating method was further developed, two different fluorophores already on hand were chosen to continue the investigations. These two dyes were Rhodamine 640 (Rh640; excitation 545 nm, emission 588 nm; see *Figure 3.36*) and LDS-751 (excitation 543 nm, emission 712 nm; see *Figure 3.37*). Of the immediately available dyes, these two were chosen for their

increased overlap with the LSPR of the gold nanocages. Again, however, neither of these had much excitation overlap with the LSPR spectra.

Figure 3.36a) – Rh640 Spectral Properties: Rh640 excitation and emission spectra shown to overlap with AuNC LSPR

Figure 3.36b) – Rh640 Spectral Properties: Rh640 calibrated emission intensity with respect to concentration

Figure 3.37a) – LDS-751 Spectral Properties: LDS-751 excitation and emission spectra shown to overlap with AuNC LSPR

Figure 3.37b) – LDS-751 Spectral Properties: LDS-751 calibrated emission intensity with respect to concentration

The results observed with the silica-coated gold nanocages and Rh640 or LDS-751 were significantly different from those observed with Rh6G. The silica-capping method had been improved to the point of forming even shells of relatively low porosity (though still thick), and the degree of quenching of the fluorescence emission was lessened with the even silica shells. There was also an easily observable trend of increasing the quenching efficiency of the solution by increasing the concentration of the gold nanocages with respect to the fluorophore being used (see *Figures 3.38 and 3.39*) The fluorescence emission observed for silica-capped gold nanocages with LDS-751 was particularly high. The LSPR of the gold nanocages used at this point had a maximum wavelength of 648 nm, almost directly between the excitation and emission wavelengths of LDS-751. The reasonably large overlap of the higher modes of the silica-capped gold nanocages with the excitation wavelength of the dye likely allowed for a small amount of fluorescence enhancement using the thick silica shells, though it was then quenched by the overlap with the emission wavelength, showing a resulting fluorescence emission near even to that seen without gold nanocages.

At this stage, there was still no measurable enhancement, but the quenching of the dyes' emissions had gained a measure of control based on the approximate thickness of the evenly coated silica shells on the gold nanocages. *Table 3.5* summarizes the quenching of Rh6G, Rh640 and LDS-751 with respect to the thickness of the silica shell on the gold nanocages.

Figure 3.38 – Quenching of Rh640 by [AuNC]: Rh640 emission intensity affected by the concentration of AuNC in solution

Figure 3.39 – Quenching of LDS-751 by [AuNC]: LDS-751 emission intensity affected by the concentration of AuNC in solution

Fluorophore (dye)	LSPR of AuNC (bare) (nm±0.5nm)	LSPR of AuNC@SiO ₂ (nm±0.5nm)	SiO ₂ shell thickness (nm)	Porosity of SiO ₂ shell	Relative [AuNC]/[Dye] (%v/v)	% Fluorescence Emission (relative to emission without AuNC)
Rhodamine 6G (Rh6G) (ex 529nm, em 554nm)	640	N/A	0 ± 0	N/A	4	45.37 ± 0.08
		691	54.70 ± 2.62	Low	4	67.3 ± 0.95
		648	16.00 ± 1.11	Low	4	79.57 ± 0.81
		648	2.06 ± 1.04	High	4	42.92 ± 1.04
		651	1.54 ± 0.66	Low	4	51.63 ± 0.07
Rhodamine 640 (Rh640) (ex 545nm, em 588nm)	640	691	54.70 ± 2.62	Low	5	95.64 ± 0.16
					10	77.11 ± 0.20
					15	76.54 ± 0.20
		648	16.00 ± 2.62	Low	5	89.32 ± 0.17
					10	82.15 ± 0.19
LDS-751 (ex 543nm, em 712nm)	640				15	77.89 ± 0.20
		691	54.70 ± 2.62	Low	5	100.61 ± 0.86
					10	95.50 ± 0.91
					15	93.64 ± 0.93
		648	16.00 ± 2.62	Low	5	97.54 ± 1.57
					10	98.70 ± 0.66
					15	98.27 ± 0.88

Table 3.5 - Quenching Data of Rh6G, Rh640 and LDS-751: Fluorescence quenching with respect to silica shell: Rh6G, Rh640, LDS-751

3.5.2 Effect of AuNC@SiO₂ on Fluorescence Quenching: Tailored for LSPR Overlap

With the investigation of controllable fluorescence quenching using silica-capped gold nanocages well begun, the choice of fluorophores to use was further tailored for excitation and/or emission overlap with the LSPR of the nanocages. The fluorophores then purchased for the remainder of this investigation were rhodamine B (RhB; excitation 545 nm, emission 569 nm), rhodamine 800 (Rh800; excitation 684 nm, emission 704 nm), Nile Blue chloride (NBC; excitation 629 nm, emission 662 nm), and cryptocyanine (excitation 709 nm, emission 721 nm). The excitation, emission and calibration spectra of each of these four fluorophores are seen in *Figures 3.40 to 3.43*, respectively. The choice of these fluorophores allowed for overlaps of the LSPR of the gold nanocages being used, with the excitation, emission, or both wavelengths of the fluorophore.

Figure 3.40a) – RhB Spectral Properties: RhB excitation and emission spectra shown to overlap with AuNC LSPR.

Figure 3.40b) – RhB Spectral Properties: RhB calibrated emission intensity with respect to concentration.

Figure 3.41a) – Rh800 Spectral Properties: Rh800 excitation and emission spectra shown to overlap with AuNC LSPR.

Figure 3.41b) – Rh800 Spectral Properties: Rh800 calibrated emission intensity with respect to concentration.

Figure 3.42a) – NBC Spectral Properties: NBC excitation and emission spectra shown to overlap with AuNC LSPR.

Figure 3.42b) – NBC Spectral Properties: NBC calibrated emission intensity with respect to concentration.

Figure 3.43a) – Cryptocyanine Spectral Properties: Cryptocyanine excitation and emission spectra shown to overlap with AuNC LSPR.

Figure 3.43b) – Cryptocyanine Spectral Properties: Cryptocyanine calibrated emission intensity with respect to concentration.

The silica-capped gold nanocages used during this stage of the investigation all had very well formed and even shells. The relative porosity of the silica shell increased with etching time, though these pores in the surface of the nanostructure had the potential to provide controllable distances between the fluorophore molecule and the gold nanocages without the use of an adsorbing or covalently-bound functionalization molecule. A similar pattern to that seen with LDS-751 was observed for these four fluorophores in that the concentration of the nanocages affected the resulting emission intensity, and so too did the thickness of the silica shells. As shown in *Figure 3.44(a-d)*, each of the fluorophores chosen for specific gold nanocage samples had varying degrees of emission quenching based on the thickness of the silica shell; the extent of the quenching trends are summarized in *Table 3.6*.

Figure 3.44a) – Quenching by Best Overlap: Quenching of fluorescence emission using AuNC@SiO₂ with best LSPR overlap (RhB + AuNC@SiO₂ with 17.5 nm silica shell).

Figure 3.44b) – Quenching by Best Overlap: Quenching of fluorescence emission using AuNC@SiO₂ with best LSPR overlap (Rh800 + AuNC@SiO₂ with 16.6, 17.5 and 29.5 nm of silica shells).

Figure 3.44c) – Quenching by Best Overlap: Quenching of fluorescence emission using AuNC@SiO₂ with best LSPR overlap (NBC + AuNC@SiO₂ with 17 nm silica shell).

Figure 3.44d) – Quenching by Best Overlap: Quenching of fluorescence emission using AuNC@SiO₂ with best LSPR overlap (cryptocyanine + AuNC@SiO₂ with 30 and 18 nm silica shells).

Fluorophore (dye)	LSPR of AuNC (bare) (nm±0.5nm)	LSPR of AuNC@SiO ₂ (nm±0.5nm)	SiO ₂ shell thickness (nm)	Porosity of SiO ₂ shell	Relative [AuNC]/[Dye] (%v/v)	% Fluorescence Emission (relative to emission without AuNC)
Rhodamine B (<i>RhB</i>) (ex 545nm, em 569nm)	642	N/A	0 ± 0	N/A	4	47.28 ± 0.17
		660	18.92 ± 2.33	Low	2	92.58 ± 0.09
					4	85.83 ± 0.09
	641				8	71.30 ± 0.11
		641	15.03 ± 2.07	High	2	96.70 ± 0.13
					4	93.197 ± 0.07
Rhodamine 800 (<i>Rh800</i>) (ex 684nm, em 704nm)	642	N/A	0 ± 0	N/A	4	44.06 ± 3.34
		660	18.92 ± 2.33	Low	2	92.00 ± 1.60
					4	82.53 ± 1.785
	683				8	47.35 ± 3.11
		641	15.03 ± 2.07	High	1.33	63.06 ± 0.77
		N/A	0 ± 0	N/A	2	48.77 ± 1.47
	683				2	45.41 ± 0.18
		698	30.96 ± 2.44	Low	2	96.62 ± 0.082
					4	89.70 ± 0.089
	670				1.33	61.66 ± 0.78
			10.79 ± 3.20	High	2	45.34 ± 1.58

Table 3.6a - Quenching Data of Rh800, RhB, NBC, Cryptocyanine: Fluorescence quenching with respect to silica shell: RhB and Rh800

Fluorophore (dye)	LSPR of AuNC (bare) (nm $\pm$ 0.5nm)	LSPR of AuNC@SiO ₂ (nm $\pm$ 0.5nm)	SiO ₂ shell thickness (nm)	Porosity of SiO ₂ shell	Relative [AuNC]/[Dye] (%v/v)	% Fluorescence Emission (relative to emission without AuNC)
Nile Blue Chloride (NBC) (ex 629nm, 662nm)	642	N/A	0 $\pm$ 0	N/A	4	48.09 $\pm$ 4.65
					2	84.68 $\pm$ 2.64
					4	83.12 $\pm$ 2.69
					8	60.14 $\pm$ 3.72
	683	N/A	15.03 $\pm$ 2.07	High	2	86.90 $\pm$ 0.28
					4	88.22 $\pm$ 0.52
					2	47.23 $\pm$ 4.73
					2	102.55 $\pm$ 2.18
	683	698	30.96 $\pm$ 2.44	Low	4	93.12 $\pm$ 2.40
					8	88.085 $\pm$ 2.54
					2	81.23 $\pm$ 0.30
					4	64.35 $\pm$ 0.71
Cryptocyanine (ex709nm, ex 721nm)	683	670	10.79 $\pm$ 3.20	High	2	93.76 $\pm$ 0.69
					4	92.35 $\pm$ 1.14

Table 3.6b - Quenching Data of Rh800, RhB, NBC, Cryptocyanine: Fluorescence quenching with respect to silica shell: NBC and cryptocyanine

As mentioned above, the fluorophores were chosen specifically for the gold nanocages prepared for this investigation. The effect of the LSPR overlapping with the excitation wavelength was of particular interest, both for the potential for fluorescence enhancement and the control over small amounts of quenching if enhancement was not achieved. Three-dimensional fluorescence spectroscopy was used at this point to observe changes across a range of excitation and emission wavelengths of the fluorescent dyes alone in solution versus with silica-coated gold nanocages (see *Figures 3.45 to 3.48*). All of the solutions containing the gold nanocages exhibited an increased intensity around the maximum excitation wavelength. The excitation and emission wavelengths are very close together for many of the fluorophores, thus a pattern of broadening the excitation peak will directly affect the intensity of the emission peak observed. The broadening of the excitation peak can be observed through the subtraction of the normalized emissions of the fluorophore alone and with the silica-capped gold nanocages in solution.

The subtraction of the two emission mappings, with and without silica-capped gold nanocages, allowed one to better observe the influence of the gold nanocages' LSPR on the bandwidth of the excitation of the fluorophore. *Table 3.6* lists the combinations of fluorophores with AuNC@SiO₂ structures that provided the best potential overlap, thus these silica-capped structures were investigated using the 3D mapping shown below.

Figure 3.45 – 3D mapping of RhB Emissions: 3D mapping of emission spectra of RhB with and without AuNC@SiO₂ structures in solution (top right and top left respectively), and subtraction of the two (bottom middle). The gold nanocages used here had 15 nm silica shells with an LSPR maximum of 641 nm.

RhB excitation and emission did not overlap well with any of the AuNC@SiO₂ LSPR maxima, thus the sample with the nearest LSPR maxima was chosen for this portion of the investigation, still revealing a broadening of the excitation peak evidenced by an increased intensity at the leading edge of the emission range.

Figure 3.46 – 3D mapping of Rh800 and AuNC@SiO₂ (15 nm shells): 3D mapping of emission spectra of Rh800 with and without AuNC@SiO₂ structures in solution (top right and top left respectively), and subtraction of the two (bottom middle). The gold nanocages used here had 15 nm silica shells with an LSPR maximum of 641 nm.

Figure 3.47 – 3D mapping of Rh800 and AuNC@SiO₂ (13 nm shells): 3D mapping of emission spectra of Rh800 with and without AuNC@SiO₂ structures in solution (top right and top left respectively), and subtraction of the two (bottom middle). The gold nanocages used here had 13 nm silica shells with an LSPR maximum of 689 nm.

Figure 3.48 – 3D mapping of Rh800 and AuNC@SiO₂ (10 nm shells): 3D mapping of emission spectra of Rh800 with and without AuNC@SiO₂ structures in solution (top right and top left respectively), and subtraction of the two (bottom middle). The gold nanocages used here had 10.8 nm silica shells with an LSPR maximum of 670 nm.

Figure 3.49 - 3D mapping of Cryptocyanine and AuNC@SiO₂ (13 nm shells): 3D mapping of emission spectra of cryptocyanine with and without AuNC@SiO₂ structures in solution (top right and top left respectively), and subtraction of the two (bottom middle). The gold nanocages used here had 13 nm silica shells with an LSPR maximum of 689 nm.

Figure 3.50 - 3D mapping of Cryptocyanine and AuNC@SiO₂ (10 nm shells): 3D mapping of emission spectra of cryptocyanine with and without AuNC@SiO₂ structures in solution (top right and top left respectively), and subtraction of the two (bottom middle). The gold nanocages used here had 10.8 nm silica shells with an LSPR maximum of 670 nm.

Figure 3.51 - 3D mapping of NBC and AuNC@SiO₂ (15 nm shells): 3D mapping of emission spectra of NBC with and without AuNC@SiO₂ structures in solution (top right and top left respectively), and subtraction of the two (bottom middle). The gold nanocages used here had 15 nm silica shells with an LSPR maximum of 641 nm.

Figure 3.52 - 3D mapping of NBC and AuNC@SiO₂ (13 nm shells): 3D mapping of emission spectra of NBC with and without AuNC@SiO₂ structures in solution (top right and top left respectively), and subtraction of the two (bottom middle). The gold nanocages used here had 13 nm silica shells with an LSPR maximum of 689 nm.

Figure 3.53 – 3D mapping of NBC and AuNC@SiO₂ (10 nm shells): 3D mapping of emission spectra of NBC with and without AuNC@SiO₂ structures in solution (top right and top left respectively), and subtraction of the two (bottom middle). The gold nanocages used here had 10.8 nm silica shells with an LSPR maximum of 670 nm.

3.5.3 Potential for Time-Dependence Fluorescence Emission Effects

The silica shells of core-shell nanostructures are known to be porous, more so after a number of etching procedures. The size of the pores will vary generally depending on the conditions during the reaction for the initial formation of the silica shells. With respect to solid-faced nanostructures, these pores can generally be controlled and used for studies of diffusion kinetics of a particular dye or dye-labeled molecule. For the case of hollow metal nanostructures, such as with gold nanocages, the growth pattern of the silica shell is more erratic as it deposits along the inside of the walls as well, thus the pore sizes may no longer be consistent with previous knowledge. Investigations into time-dependence fluorescence, using variations of emission intensity and the relative diffusion rates of various sized fluorophores would be one way to determine the approximate pore sizes of these the nanocages. This particular characteristic would be useful when considering capture-and-release mechanism for biomedical applications, such as timed-release or targeted delivery. The evidence of the pores in the silica shell can be seen in *Figure 3.54*, where the degree of quenching of the fluorophore was drastically increased after 24hours. This could present an interesting investigation with respect to silica shell thickness relating to the porosity of the same silica shell, though a logical process of investigation could begin with controlling first the shell thickness and the porosity of the shell independently for investigation of those properties separately.

Figure 3.54 – Emission After Prolonged Exposure: Increased quenching observed after 24 hours.

3.6 Application and Publication: Gold Nanocages on a TFBG-Based Biosensor

An application of the gold nanocages investigated in this project was the work of collaboration with Dr. Sandrine Lepinay from the Albert lab group in the Department of Electronics. The intent of this work was to improve the limit of detection of a biosensor made on an optical fibre substrate, for the detection of the binding of biotin to avidin immobilized on the surface of the fibre. Both gold nanoparticles and gold nanocages were used in this investigation, bound to the surface of a tilted fibre Bragg grating via a series of thin-film and monolayer depositions to create the sensing film. The improvement of the limit of detection, and the increase in sensitivity to environmental changes was the result of a number of factors: the reduced non-specific absorption onto the gold nanostructure by external surface functionalization of the gold nanostructures; and an optical field enhancement due to the coupling of the gold nanostructures' LSPR with the photonic modes of the optical fibre.

Improved detection limits of protein optical fiber biosensors coated with gold nanoparticles

Sandrine Lepinay^{a,b,*}, Alyssa Staff^b, Anatoli Ianoul^b, Jacques Albert^b

^a Department of Electronics, Carleton University, 1125 Colonel By Drive, Ottawa, Canada K1S 5B6

^b Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Canada K1S 5B6

ARTICLE INFO

Article history:
Received 24 July 2013
Received in revised form
26 August 2013
Accepted 27 August 2013
Available online 14 September 2013

Keywords:
Avidin-biotin complex
Biosensors
Gold nanocages
Gold nanospheres
Tilted fiber Bragg grating

ABSTRACT

The study presented herein investigates a novel arrangement of fiber-optic biosensors based on a tilted fiber Bragg grating (TFBG) coated with noble metal nanoparticles, either gold nanocages (AuNC) or gold nanospheres (AuNS). The biosensors constructed for this study demonstrated increased specificity and lowered detection limits for the target protein than a reference sensor without gold nanoparticles. The sensing film was fabricated by a series of thin-film and monolayer depositions to attach the gold nanoparticles to the surface of the TFBG using only covalent bonds. Though the gold nanoparticle integration had not yet been optimized for the most efficient coverage with minimum number of nanoparticles, binding AuNS and AuNC to the TFBG biosensor decreased the minimum detected target concentrations from 90 nM for the reference sensor, to 11 pM and 8 pM respectively. This improvement of minimum detection is the result of a reduced non-specific absorption onto the gold nanoparticles (by functionalization of the external surface of the gold nanoparticles), and of an optical field enhancement due to coupling between the photonic modes of the optical fiber and the localized surface plasmon resonances (LSPR) of the gold nanoparticles. This coupling also increased the sensitivity of the TFBG biosensor to changes in its local environment. The dissociation constant (K_d) of the target protein was also characterized with our sensing platform and found to be in good agreement with that of previous studies.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Interest in biosensors extends not only to research but also to industry applications where detection, diagnosis and determination of biomolecules are high priorities. Some such industry fields include food- and water-quality control, security, and health, all of which have a continuously increasing demand for biosensors. The current challenge is to design simple, inexpensive, accurate, sensitive and reliable biosensor platforms. In response to this, many devices and methods have been developed to detect binding events, though optical measurement is the most widely used of the transduction methods. Among optical-based biosensors, optical fibers are new, tiny, flexible platforms that are being used with increasing frequency as biosensor transducers. Optical fibers are able to make quick and sensitive responses, and can be employed as an intrinsic or extrinsic biosensor (Nguyen et al., 2012). The biosensors presented in this work were intrinsic-type, label-free biosensors, utilizing a single-mode tilted fiber Bragg grating (TFBG). This optical fiber biosensor platform was classified as an evanescent wave sensor and combined various technologies to create a simple and robust

platform that has been successfully applied to a wide range of interactions (Maguis et al., 2008). Recently, the Albert group has demonstrated that the efficiency of such TFBG sensors can be further improved by coating the fiber with a thin metallic layer, thus allowing the excitation of surface plasmon polaritons (Shevchenko et al., 2011). By the same principle, the Albert group also showed that TFBG are able to excite localized surface plasmon resonances (LSPR) in nanoscale particles (Bialiyeu et al., 2012). The unique optical property of supporting LSPR have made noble metal nanoparticles the subject of much research interest, and they are also of key importance in various studies of biological applications, including bioactive colloidal crystals (Gosecka et al., 2011), immunodiagnostic assays (Bousalem et al., 2005), chemical sensors (Gehan et al., 2010; Kaewsanaha et al., 2013), biosensors (Bedford et al., 2012; Bemand-Mantel et al., 2010), plasmonic nanostructures (Fang and Zhu, 2013), and hybrid nanocomposites (Rozenberg and Tenne, 2008; Spitalsky et al., 2010; Zou et al., 2008). This popularity and diversity stems from the fact that LSPR, compared to surface plasmon resonances (SPR), are much more localized, allowing for probing processes at the platform interface with spatial sensitivities well within the nanometer scale (Anker et al., 2008; Mayer and Hafner, 2011; Piliarik et al., 2012).

The majority of nanoparticle-enhanced SPR studies reported to-date have been focused on the use of DNA-functionalized gold nanoparticles (typically 10–15 nm in diameter), because of the

* Corresponding author at: Department of Electronics, Carleton University, 1125 Colonel By Drive, Ottawa, Canada K1S 5B6. Tel.: +1 613 520 1636.
E-mail address: sandrine.lepinay@carleton.ca (S. Lepinay).

well-established procedures for the preparation of DNA conjugates on planar gold surfaces (Gosecka et al., 2011; Hayashida et al., 2005). However, for planar detection of SPR, there are currently very few reports concerning larger nanoparticles sizes or non-spherical particles for the detection of proteins or nucleic acids (Sendroui et al., 2009; Sim et al., 2010; Yu and Irudayaraj, 2007). The most complete study of this line of investigation to-date compared different nanoparticle shapes and sizes as part of a sandwich assay in combination with a gold SPR chip for the detection of thrombin to demonstrate that attomolar concentrations can be detected by this technique (Kwon et al., 2012). Branching from the utilization of LSPR with a gold planar surface, other studies using silica nanoparticles have been reported (Lin et al., 2009; Lin et al., 2010; Pamlisky et al., 2006). For instance, Li et al. (2013) showed that transistor biosensors using silicon nanowires for the detection of protein could reach detection limits on the order of femtomolar concentrations. However, the study and work presented herein is the first known report of coupling TFBG and noble metal nanoparticles for the fabrication of biosensors.

These experiments were carried out using an unmodified and inexpensive standard telecommunication single-mode fiber. From the optics point of view, it is well known that a single-mode fiber's transmission power loss is less than 4%/km (Corning, 2010), making optical fibers well suited for remote operation over large distances (up to several kilometers). Moreover, the flexibility of optical fibers allows for a very robust sensor that is also simple to manufacture and to measure, using widely available instrumentation developed by the telecommunications industry. A TFBG transducer also offers very important advantages for sensor platforms in its temperature insensitivity and the preservation of the fiber's structural integrity under stressful environments (Albert et al., 2013; Chan et al., 2007).

Recent work prior to this study had demonstrated that silver nanowires can be used to significantly amplify the sensitivity of the TFBG sensor by introducing large and distinct anisotropy with respect to optical transmission (Bialayeu et al., 2012). With this amplification of sensitivity in mind, it was decided to transfer this technique to a biosensor application. Two biosensors using gold nanoparticles (nanospheres and nanocages respectively) were studied and compared to a reference biosensor without nanoparticles. The amplification performance of the gold nanoparticles with respect to the enhancement of the LSPR was monitored by the detection of a protein. The goal of this study is to determine the influence of the nanoparticles' shape or surface area in contact with the biosensor platform on the LSPR properties, as evidenced by spectral shifts of the TFBG response due to LSPR changes. The gold nanospheres (AuNS) obtained for this study had a maximal absorbance in colloidal solution at a wavelength of 530 nm, whereas the LSPR of the gold nanocages (AuNC) in colloidal solution is in the visible and near-infrared (vis-NIR) range depending on the size and porosity of the nanocages (Skrabalak et al., 2008). The nanocages also provide a larger contact area as binding can occur on the inside surfaces. The TFBG spectral region of interest ranges from 1520 to 1620 nm, because of the high quality factor resonances that can be generated at those wavelengths. Gold nanoparticles were of interest for this range because of their LSPR sensitivity within the near-infrared (NIR) and infrared (IR) ranges of the electromagnetic spectrum when arranged in supported nanostructures, including when deposited on optical fibers, as described in this work. In previous work it was determined that the propagating modes of optical fibers are strongly perturbed within the region of interest by very sparse coatings of metallic nanoparticles (Bialayeu et al., 2012; Shao et al., 2011; Zhou et al., 2013). This perturbation is caused by coupling between the resonance of the fiber cladding-mode and the localized plasmonic resonances of the nanoparticle coatings, including collective resonance modes that occur at longer wavelengths.

The result of such coupling is the increase of the effective refractive index of the fiber's cladding-modes thereby causing measurable wavelength shifts of the grating resonances. The effective refractive index of an optical fiber is typically altered by varying the type and nature of the fiber's cladding (Albert et al., 2013), though in the work presented here, the cladding is instead modified by the interaction of gold nanoparticles.

Molecular recognition is the main criterion of general biosensing, thus the essential component of an effective biosensor is the ability of a specific molecular recognition probe to selectively target the desired analyte. For this study, biotin was chosen as the target molecule. The biotin molecules were to be bound to avidin molecules that had been covalently attached to the gold nanoparticles, which were in turn immobilized on the TFBG surface. Biotin/avidin combination chemistry has been used extensively as a model in protein sensing, primarily because of the strong attraction between the two ($K_d = 10^{-15}$ mol/L). The complex biotin/avidin has been applied successfully as molecular recognition probes for a variety of sensor techniques, including prism-based SPR and surface-enhanced Raman spectroscopy (Fu et al., 2013), as well as nanoscale field-effect transistors (Li et al., 2013). Utilizing the advantages of this well-known complex with its strongly recognizable affinity and signature in combination with the easily-obtained and durable platform of a TFBG, determining the effectiveness of a biosensor comprised of gold nanoparticles on the surface of a single-mode fiber platform was the key interest in this study. The experimental results indicated that the nanoparticle-incorporated biosensors can be used for the real-time monitoring of both the self-assembly of the sensing film on the TFBG surface, as well as for the detection of protein at various concentrations in aqueous solutions. The dissociation constant (K_d) of the target biotin bound to the avidin biosensor surface was also determined and was found to be comparable to that found in other biotin/avidin complex studies.

2. Materials and methods

2.1. The TFBG basic sensor

The optical fiber sensor used was made up of a standard single-mode optical fiber (Corning SMF-28) with a TFBG inscribed in the fiber's core. The TFBG was manufactured using intense ultraviolet light at 193 nm from a pulsed laser incident source on a phase mask, inscribing the grating in the fiber's core. The grating's planes were inscribed with a tilt of 10° relative to the normal axis of the fiber (as seen in Fig. 1). This angle allowed the sensor to operate in aqueous solutions with refractive indices between 1.31 and 1.34 RIU. After the grating was inscribed, a thin gold mirror was formed by electroless deposition on the downstream end of the Bragg grating. This technique is based on the reduction of metallic ions from a solution to a solid surface without electrical potential (Bialayeu et al., 2011; Hrapovic et al., 2003). The interest of depositing a mirror at the end of the fiber is in that it allows a convenient and easy use of the sensor since it can be simply dipped directly into the testing and sampling solutions. In a TFBG sensor, coupling between the forward-propagating core-modes and the counter-propagating cladding-modes occurs, yielding a familiar transmission spectrum (inset in Fig. 1(B)). The molecular recognition event is measured through changes in the spectral transmission response of the TFBG (as described in Section 2.4 below).

2.2. Synthesis of gold nanocages

Gold nanocages were synthesized by a two-step process involving the synthesis of silver nanocubes followed by the galvanic

Fig. 1. (A) TFBG sensor setup with a reflective mirror; (B) inset shows a typical spectrum of the transmitted light after reflection in the gold mirror with the cut-off area indicated by the red bracket. The red arrow shows the wavelength of 1538 nm under observation throughout the study. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

displacement of silver with gold to form the gold nanocages. All non-water reagents and solvents were purchased from Sigma-Aldrich Co.

The silver nanocubes were synthesized using a basic polyol method well known in literature (Mahmoud et al., 2011). 35 mL of ethylene glycol was heated to 150 °C and stirred in a round-bottomed flask submerged in an oil bath for 1 h. Solutions of poly(vinylpyrrolidone) (PVP, 55,000 MW), sodium sulfide (Na_2S), and silver nitrate (AgNO_3) were prepared in ethylene glycol to concentrations of 80 mg mL^{-1} , 3 mM, and 282 mM respectively. 5 mL of the prepared PVP solution was added to the hot ethylene glycol and stirred for 10 min. 400 μL of the Na_2S solution was then added and stirred for 5 min. 2.5 mL of the AgNO_3 solution was then added at a rate of approximately 1 mL min^{-1} , with continued heating and stirring. The color change of the solution from clear and colorless to opaque greenish-grey was a good indication of the progression of the reaction. The shape and size of the synthesized particles was monitored by ultraviolet-visible (UV-vis) spectroscopy by extracting a small aliquot of the reaction solution and diluting it in ethanol. The reaction was quenched by placing the reaction flask into an ice bath when the strongest peak (dipolar plasmon peak for nanocubes) red-shifted to a desired wavelength (this red-shift is known to be size-dependent (Mahmoud et al., 2011)) and a small but distinct peak at 350 nm was visible (indicative of sharp-edged nanocube structures). The product silver nanocubes were then spun in a microcentrifuge to condense them to a smaller volume and exchange their colloidal solvent from ethylene glycol to ethanol. Washing by continued centrifugation and exchange of supernatant solution was carried out further to ensure stable and relatively monodisperse silver nanocubes in the solution.

The gold nanocages were synthesized by galvanic displacement of the previously prepared silver nanocubes (Skrabalak et al., 2008). The colloidal silver nanocubes in ethanol were exchanged

to be suspended in distilled, deionized water, and then redispersed in 10 mL of a previously prepared solution of 1 mg mL^{-1} PVP in deionized water. The solution was heated to boiling in an oil bath for 10 min. A solution of 0.1 mM of tetrachloroaurate trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) was prepared and added to the hot silver solution until the solution became deep, translucent blue in color, and a dipolar resonance above 600 nm was observed by UV-vis spectroscopy (the exact shifting of the resonance peak was dependant on the size of the starting silver nanocubes and the ratio of gold to silver remaining in the nanostructure). A typical galvanic displacement had a gold:silver ratio of 1:5, allowing for the formation of hollow structures. The resulting product of gold nanocages was then condensed and washed with water to remove excess PVP and silver chloride by-product, before exchanging and redispersing in a desirable deposition solvent.

2.3. Fabrication of the TFBG biosensors

(3-aminopropyl)trimethoxysilane (APTMS), glutaraldehyde, cysteamine, gold nanospheres (AuNS, 30 nm), 11-mercaptoundecanoic acid (MUA, 98%), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), avidin, biotin, and phosphate buffer tablets (PBS, 100 mM, pH 7.4) were purchased from Sigma-Aldrich Co. All buffer solutions were prepared using doubly deionized water from a Milli-Q water system (Thermo Fisher Scientific Inc.). After each modification of the fiber's surface, the entire array was rinsed with Milli-Q deionized water.

The effectiveness and sensitivities of the sensors containing nanoparticles were compared against the reference sensor without nanoparticles. The reference sensor consisted of only two layers of thin films. The first thin-film layer was solely APTMS (10% in water), self-assembled over 2 h in order to functionalize the outside wall of the TFBG basic sensor by silanization. The second thin-film layer was glutaraldehyde (2.5% in water), a simple and well-known amine-reactive homobifunctional crosslinker, self-assembled onto the APTMS layer over 30 min. This layer then reacted spontaneously with avidin (1 mg mL^{-1}) over 1 h, covalently bonding the avidin on the surface of the TFBG. Solutions containing different biotin concentrations were then tested on all the prepared biosensor platforms.

The fabrication of the sensors utilizing gold nanoparticles began with the same thin-films of APTMS and glutaraldehyde, as described above. The subsequent binding of the gold nanoparticles required a thiol compound, thus cysteamine was chosen for this purpose. The prepared TFBG platform was dipped into an aqueous solution of cysteamine (0.5 M) for 1 h. Finally, the TFBG platform was dipped into a solution of gold nanoparticles to covalently bind the nanoparticles (either AuNS or AuNC) to the exposed thiol sites on the fiber. In a previous study, Bialayeou demonstrated that the sensitivity of a TFBG increased with the deposition of nanowires (Bialayeou et al., 2012). Using those results, the deposition time of the gold nanospheres (AuNS) was set at 1 h. To achieve approximately the same initial wavelength for the two sensor platforms, the deposition time for the gold nanocages (AuNC) was 2 h. The different deposition times and particle shapes result in a red-shift of the wavelength of interest (1538 nm) by 135 nm. Then to graft the biomolecule to the TFBG, the gold-coated TFBG platform was functionalized with a self-assembled monolayer of MUA (10 mM in ethanol). The carboxyl groups of this latest monolayer were activated by a freshly prepared aqueous solution of EDC (0.2 M) and NHS (0.05 M) for 20 min. The activated sensor was finally immersed in a solution of avidin (1 mg mL^{-1}) for 1 h. The TFBG biosensor was then rinsed overnight in PBS to deactivate any remaining active groups on the surface, and finally tested with different concentrations of biotin in solution.

Fig. 2. (A) Shifting of the 1538 nm peak observed by transmission spectra during fabrication steps of the biosensor. With respect to the minimum of each spectra, from left to right are scans of the TFBG platform with each subsequent layer addition: bare TFBG fiber (black); addition of APTMS thin-film (red); cysteamine functionalization (dark blue); addition of glutaraldehyde layer (pink); immobilization of AuNS (light blue); functionalization of AuNS with MUA (dark purple); grafting of avidin (light purple); and after immersion in a test solution of biotin (green; 10^{-4} M). (B) The average position shifts of the cut-off wavelength during the sensor construction (by each additional layer of functionalization) and a biotin test. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.4. Analysis of the experimental data

A typical transmission spectrum obtained with the TFBG sensor submerged in water is presented in Fig. 1(B). The wavelength denoted by the arrow represents the cladding resonance known to be most sensitive to coupling interactions. This resonance corresponds to the last mode guided by the cladding, before the cut-off point beyond which light escapes into the outer medium. This last guided mode is the one with an evanescent field that has the maximum penetration in the outer medium while still being confined by the cladding, and hence maximum sensitivity to changes occurring on top of the cladding. The analysis presented in this paper is based on tracking the changes to this cut-off wavelength of approximately 1538 nm. Analysis of the transmission signal's changes was carried out by comparing the intensity of this peak as well as the shifting of the wavelength away from its original position. This method can be accurately applied when the change of refractive index is relatively small between trials and platform types.

2.5. Devices

The light source used in scanning the sensors was a JDS Uniphase OMNI 2 Swept Wavelength System fiber optic component analyzer, scanning from 1520 to 1620 nm. All our sensors were simply connected to this instrument via a spliced connector.

A VCA Optima system (AST products Inc.) was used for contact angle analysis in tandem with the associated software, VCA OptimaXE version 1.90.0.15. The size of droplet used in this analysis was 10 μ L of water.

The electron microscopes used for analysis throughout this study were a scanning electron microscope (model Tescan Vega-II XMU VPSEM) and a transmission electron microscope (model FEI Tecnai G2 F20).

3. Results and discussion

3.1. Characterization of nanoparticles

Two different geometries of gold nanoparticles were used in the fabrication of the biosensors used in this study. Ultraviolet-visible (UV-vis) spectroscopy and transmission electron microscopy (TEM) were used to observe and characterize their structure and composition parameters during and after synthesis.

The gold nanoparticles obtained from Sigma-Aldrich Co. had an average diameter of 30 nm. The concentration estimated by the supplier was 1.8×10^{11} nanoparticles/mL. From the UV spectrum taken to confirm their characterization, maximum absorption was observed at 520 nm.

The size of the synthesized cubic gold nanocages was determined by analysis of obtained TEM images, being of an average edge length of 30 nm. The AuNC are hollow with porous cubic faces, allowing for functionalization inside and outside of the nanoparticle structure. This particularity of structure gives the AuNC a higher surface contact area than the AuNS. The synthesized solution of AuNC was diluted to achieve a similar intensity at maximum absorption to that of AuNS. The concentration of the AuNC was then within an order of magnitude to that of the AuNS at 1.8×10^{11} nanoparticles/mL. Similar to previous experiments (Skrabalak et al., 2008), maximum absorption observed by vis-NIR spectroscopy for the AuNC solution was 620 nm.

3.2. Fabrication of the biosensor

Transmission spectra were obtained between the additions of the functionalization layers and thin-films throughout the preparation of the TFBG biosensor. As shown in Fig. 2(A), each subsequent modification of the TFBG's surface induced a further red-shift of the cut-off wavelength near 1538 nm as well as a decrease of the peak's intensity.

Fig. 2(B) shows the position shifts of the resonance of interest in the transmission spectra, as induced by changes to be overall film thickness during the biosensor fabrication and tests. After binding the layers required to immobilize the gold nanoparticles (APTMS, glutaraldehyde, and cysteamine thin-films), the total red-shift observed was 134 pm. As the film thickness increased, the average refractive index of the cladding's local environment (comprised of the thin-film layers and water interactions) also increased. This altered the cladding-mode propagation, which in turn changed the resonance matching conditions between the incident core guided light and this cladding mode. The end result of these changes is a shift of the transmission spectrum notch associated with coupling to this particular cladding mode. Similarly, the addition of the MUA functionalization and avidin binding also produced an increase in the refractive index of the cladding environment. Upon testing the biosensor with biotin at a concentration of 10^{-4} M, a red-shift of approximately 13 pm was observable, indicative of the specific interaction between avidin and biotin.

3.3. Nanoparticles immobilization

A chemical modification of the TFBG surface enabling the covalent binding of the gold nanoparticles was incorporated into the biosensor fabrication process. This modification allowed the nanoparticle layer to be grafted into the biomolecule sensing layer. The film structure extending from the surface of the TFBG resembled a self-assembled multilayer of successive imine bonds, ending with the gold nanoparticles, as described in the schematic in Fig. 3(A).

The surface of the TFBG was first functionalized with APTMS. The silanol ends allowed a covalent binding (Kaas and Kardos, 1971) between the fiber surface and the molecule, creating a very stable chemical structure and a uniform organic layer over the TFBG by the self-assembled orientation of the long carbonyl chain of the APTMS molecule. Cysteamine was used to cap the first set of layers of the sensing film, the thiol group of which then spontaneously reacted with gold (Liu et al., 2001). Used to bind the amino groups of the APTMS layer to those of the cysteamine layer was the amine-reactive cross-linker glutaraldehyde (Habeeb and Hiramoto, 1968). This cross-linker covalently bound cysteamine to the system being fabricated and caused a reticulation of the APTMS monolayer. This reticulation shortened the distance between the base TFBG platform and the unbound solution, increasing the sensitivity of the sensing platform.

AFM imaging, seen in Fig. 3(B), showed the AuNC bound to the TFBG platform. The required absorption and binding time for each type of gold nanoparticle was determined experimentally. The criterion of completion of binding of the gold nanoparticles was to achieve a similar shift of the transmission peak of interest in a series of spectra for both types of nanoparticles. The density of the bound gold nanoparticles corresponding to the same maximal shift in the TFBG platform's spectra was determined by analysis of scanning electron microscopy (SEM) images of each respective platform, and was found to be approximately 30 particles/ μm^2 for AuNC and 34 particles/ μm^2 for AuNS.

3.4. Characterization of the gold nanoparticles bound to the TFBG surface

The same deposition of thin-films and nanoparticles as described previously was performed on a glass slide to enable confirmation of the desired modification and functionalization as carried out on the TFBG platform.

A contact angle goniometer was used to confirm the binding of the gold nanoparticles to the TFBG platform, as well as the

functionalization by MUA of the gold nanoparticles. The contact angle of the TFBG platform without bound gold nanoparticles averaged at 60° , whereas with gold nanoparticles bound to the surface, either AuNS or AuNC, the average contact angle increased to 68° . This increase in contact angle was caused by the reaction between the free thiol ends of the cysteamine molecules and the gold nanoparticles, thus capping the TFBG surface with the hydrophobic noble metal nanostructures. When the gold nanoparticles were subsequently functionalized with MUA, the capping layer then consisted of mostly carboxylic acid groups, making it more hydrophilic, consequently decreasing the contact angle. However, for this functionalization, there was a distinct difference between the two types of gold nanoparticles. The average contact angle for MUA-capped AuNS was 58.5° , whereas that of MUA-capped AuNC was 50.5° . This was explained by the difference of specific surface contact area between the two nanoparticle shapes. AuNS are solid spheres with a specific surface area of approximately 19 nm^2 for binding MUA, while AuNC have porous faces in the cubic structure. The specific surface area for binding MUA was thus much greater as the inner and outer surface of the AuNC can be functionalized by the thiol-ended molecule. The amount of MUA molecules bound to each AuNC was significantly higher than to each AuNS, thus significantly decreasing the hydrophobicity initially caused by the gold nanoparticles at the surface of the TFBG platform. This decrease in hydrophobicity was an important factor used to increase the sensitivity of the biosensor by allowing more unbound solution to come into contact with the protein binding sites of the TFBG platform.

The TEM images, (A) in Fig. 4 display samples of the commercially available AuNS and the AuNC synthesized for this study (C). The average diameter of the AuNS, and the average side length of the AuNC was 30 nm. The functionalization of the gold nanoparticles by MUA was faintly visible by TEM imaging, as seen in Fig. 4 (B and D) as a darker halo around the nanoparticles in contrast to the imaging grid beneath the nanoparticles. The thickness of this layer was estimated to average 11.8 nm as determined by the TEM analysis software. This value was close to the expected thickness of 10 nm as found with High Resolution Electron Energy Loss Spectroscopy by Magnee et al., (2003).

3.5. Sensor parameters

Sensor performance was characterized by analysis of sensitivity, specificity and the dissociation constant, (K_d) with respect to the target biomolecule.

Fig. 3. (A) Schematic representation of the successive chemical additions occurring on the surface of the TFBG platform prior to the binding of gold nanoparticles; (B) atomic force microscopy (AFM) image of the fiber surface coated with AuNC. The circle indicates one of many AuNC identified clearly by parallel orientation relative to the surface of the fiber.

Fig. 4. TEM images of (A) bare AuNS, (B) AuNS functionalized with MUA, (C) synthesized AuNC, and (D) AuNC functionalized with MUA.

Fig. 5. (A) Responses obtained from test solutions of varying biotin concentration. (B) specificity sensorgrams obtained from each biosensing platform. (Step * corresponds to a PBS rinsing).

The first round of experiments involved exposing the TFBG sensor to solutions in a range of biotin concentrations in order to establish response curves of both types of TFBG platforms. Fig. 5 depicts the signal shift ($\Delta\lambda$), versus biotin concentration. $\Delta\lambda$ was defined as $\lambda - \lambda_0$, where λ_0 was the 1538 nm peak position measured after the functionalization with avidin and λ was the position of the same peak in the presence of biotin for each concentration of biotin tested.

The response curves observed for each biosensor platform were pseudo-first order curves demonstrating steep initial signal increases followed by regions of plateau, indicating signal saturation on the biosensor. Points on the sensorgrams are separated by about 2 min, the time it takes for our system to complete a full scan. While there is evidence of biotin-avidin binding kinetics (especially for the NC case), faster sampling would be needed to fully resolve the kinetics. Faster interrogation systems exist for the measurement of fiber grating wavelength shifts (at least to kHz) rates, but there would be trade-offs to explore because higher speeds often translate into increased measurement noise. The linear increase region of the response curves were used to determine the sensitivity of the biosensor arrays. The sensitivity of the reference biosensor, constructed without gold nanoparticles was $14.96 \pm 2.28 \text{ pm L mol}^{-1}$. The biosensor platforms constructed with AuNS and AuNC had sensitivities of $1.41 \pm 0.25 \text{ pm L mol}^{-1}$ and $5.61 \pm 0.20 \text{ pm L mol}^{-1}$ respectively. The uncertainty limits were calculated using statistics from different experiments for each case. It was clear from these results that the reference sensor had the best sensitivity. The avidin layer was bound directly to the glutaraldehyde layer in this arrangement, thus allowing the surface to be fully saturated with protein.

The evaluation of the specificity of the biosensors was carried out using an alternative protein, namely bovine serum albumin (BSA). Fig. 5(B) represents the peak shifting ($\Delta\lambda$) versus time for the

detection first of BSA, then of biotin. On the reference biosensor, BSA and biotin shifted the signal by 10 pm and 3.8 pm respectively. The two proteins were thoroughly bound to the surface, as evidenced by the continuous binding activity of the glutaraldehyde layer even after rinsing the biosensor platform. This continued binding activity was caused by the nucleophilic nature of the proteins allowing for spontaneous reaction with the aldehyde group at the surface of the TFBG platform. The non-specific binding evidenced with the reference sensor also explained the apparent increase of sensitivity in that the platform is readily absorbing any protein in solution at low concentrations, not just the target protein.

By the same consideration, BSA caused a peak shift of less than 2.6 pm for both nanoparticle-containing biosensors, while $\Delta\lambda$ for the target biotin was between three to four times that of BSA. The non-specific absorption for these platforms was not influenced by the global charge of the proteins. The isoelectric points of BSA and biotin are 5 and 3.5 respectively (Ge et al., 1998; Uptima) indicating that biotin is a highly polar molecule. Moreover, at pH 7.4, the MUA monolayer and biotin have the same global charge, thus they are not attracted. Therefore, the non-specific absorption to these biosensors could be a result of the hydrophobic interactions between the proteins and the gold nanoparticles, especially considering that it is well known that proteins stick to hydrophobic surfaces including metal (Hladky and Bujis, 1996). For this reason, it is essential to maintain at least a minimum separation between the protein and the inorganic nanoparticles while minimizing interference with the biosensor's binding sites. The simplest method to achieve this minimum separation is to utilize a self-assembled monolayer (SAM) to act as a barrier on the gold nanoparticles. An MUA SAM on the gold nanoparticles reduced the amount of protein absorbed to the gold structures, though it did not completely stop protein absorption because of the hydrophobicity of MUA inherent to the grouping of the eleven-carbon chains. However, the absorption of proteins onto MUA is significantly less than that onto bare gold, thus

reducing the non-specific absorption as compared to the reference sensor, allowing the biosensors containing gold nanoparticles to better discriminate in favor of the target protein.

The limit of detection (LOD) of biotin was calculated to be the concentration that yields a signal 3 times the average noise level (Vial and Jardy, 1999). The limits of detection for the reference, AuNS, and AuNC biosensors were $9 \times 10^{-8} \text{ mol L}^{-1}$, $1.1 \times 10^{-11} \text{ mol L}^{-1}$, and $8 \times 10^{-12} \text{ mol L}^{-1}$ respectively. Comparable studies included the use of gold nanodots on an optical fiber with a LOD of $6 \times 10^{-12} \text{ mol L}^{-1}$ (Lin et al., 2010). Other techniques for biotin detection, including electrochemical magneto (Linares et al., 2012) and lateral flow immunoassays (Kergaravat et al., 2011), achieved a LOD on the order of $10^{-8} \text{ mol L}^{-1}$. The LOD obtained in this study by the use of gold nanoparticle-incorporated biosensors were at least comparable to the previously determined value by Lin et al. The gold nanoparticles had distinct LSPR, furthering the localization of light in and around the nanoparticles and led to the increase of the sensitivity of the wavelength shifts of the TFBG resonances. Since the gold nanoparticles were incorporated at the outer edge of the biosensor's thin film layers, the sensitivity to environmental changes was also increased, especially with respect to target solution concentrations. LSPR are defined as the oscillations of conduction electrons in the surface of the metallic nanoparticles. These oscillations are very strongly enhanced in the presence of electromagnetic fields, and are sensitive to changes in the local environment of the nanoparticles. The process of detection of proteins at the interface of a biosensor has been shown to be more precise when the spatial sensitivities were on the nanometer scale (Anker et al., 2008; Mayer and Hafner, 2011; Piliarik et al., 2012). Furthermore, Gao et al. (2012) demonstrated that cuboids nanoparticles created nanoscale plasmonic junctions, unique to the flat contact surface of the nanostructure, that allowed the propagation of the LSPR through all like-oriented nanocrystals within the nanostructure. This phenomenon has also been described with respect to the surface of a TFBG (Bialayeu et al., 2012). The dependence of the nanostructure orientation on the chain length of the binding layers aided to explain the lower LOD when using AuNC than when using AuNS. The AuNC had a more distinctive and discernible orientation than the AuNS, allowing for a greater enhancement of the transmission signal when bound to the TFBG biosensor platform.

The determination of the dissociation constant (K_d) is another parameter essential to the development and comparison of efficiency of biosensors. K_d is the parameter used to characterize the binding strength between two interacting molecules, which in this case specifically applied to the target biotin interacting with avidin bound to biosensor platform.

The K_d of the biotin-avidin complex was determined by the least-squares fit of the data for the low biotin concentrations, where the concentration of biotin, was less than or equal to $5 \times 10^{-10} \text{ mol/L}$ according to the Langmuir absorption isotherm model (Chang et al., 2012; Li et al., 2013), as shown in Eq. (1) below.

$$[\text{biotin}]/\Delta\lambda = 1/\Delta\lambda_{\text{max}}[\text{biotin}] + K_d/\Delta\lambda_{\text{max}} \quad (1)$$

As shown in Fig. 6, the least-squares fit of the data obtained for the AuNC biosensor gave K_d for the biotin-avidin complex to be $9 \times 10^{-14} \text{ mol L}^{-1}$. The same least-squares fit analysis applied to the data obtained with the AuNS biosensor gave K_d to be $7 \times 10^{-14} \text{ mol L}^{-1}$. These results are close to the accepted literature value of K_d reported earlier ($10^{-15} \text{ mol L}^{-1}$), as well as close to the K_d of transistors biosensors (Green, 1963; Li et al., 2013). These results demonstrate that TFBG coated with gold nanoparticles can be used as a biosensing platform for the detection of biomolecule targets at small concentrations allowing for the development of a series of portable biosensors that can be applied

Fig. 6. Shifting of the 1538 nm peak with respect to biotin concentration for the AuNC biosensor. The inset shows the least-squares fit (Eq. (1)) of the measured ratio of concentration of biotin to wavelength shift as a function of concentration of biotin.

in situ for the measurement of biomolecular interactions without perturbing the surrounding environment.

4. Conclusion

This study demonstrated how a TFBG sensor, used in reflection mode with a single-mode fiber, can be applied as a biosensor to detect biomolecules. The results indicated that the TFBG can be used as an optical transducer for making biosensors and constitutes an inexpensive alternative to biosensing platforms currently being employed. Specifically, this study showed that the system can be easily modified with chemical and nanostructured thin-films, and can be used for in situ measurements. The simple methods of modification can also be monitored continuously throughout the addition of each functionalization layer, including through the binding of biotin to avidin grafted onto the surface of the TFBG platform. By having a functional sensor throughout the fabrication process, the quality of the surface functionalization can be assessed prior to using the sensor for the target application (and "bad" sensors sorted out). Binding gold nanoparticles to the surface of the TFBG biosensor provided a strong signal for the detection of biotin in a phosphate buffer solution. The gold nanoparticles were used as a magnification layer to enhance the light localization in and around the nanoparticles, thus enhancing the peak shifts in the transmission spectra. The AuNC were found to induce a lower LOD and sensitivity than the AuNS in the biosensor, likely due to their increased contact area (for a similar overall size). The LOD of the TFBG biosensor associated with gold nanoparticles was three orders of magnitude greater than that of the reference biosensor, demonstrating that this sensor platform is a powerful yet sensitive biosensor.

References

- Albert, J., Shao, L.Y., Caucheteur, C., 2013. Laser and Photonics Reviews 7 (1), 83–108.
- Anker, J.N., Hall, W.P., Lyandres, O., Shah, N.C., Zhao, J., Van Duyne, R.P., 2008. Nature Materials 7 (6), 442–453.
- Bedford, E.E., Spadavecchia, J., Pradier, C.M., Gu, F.X., 2012. Macromolecular Bioscience 12 (6), 724–739.
- Bernard-Mante, D., Chehimi, M.M., Millot, M.C., Caribonier, B., 2010. Surface and Interface Analysis 42 (6–7), 1035–1040.
- Bialayeu, A., Bottonley, A., Prezgot, D., Janoul, A., Albert, J., 2012. Nanotechnology 23, 44.
- Bialayeu, A., Caucheteur, C., Ahamad, N., Janoul, A., Albert, J., 2011. Optics Express 19 (20), 18742–18753.

- Bousalem, S., Benabdenahmane, S., Sang, Y.Y.C., Mangeney, C., Chehimi, M.M., 2005. *Journal of Materials Chemistry* 15 (30), 3109–3116.
- Chan, C.F., Chen, G., Jafari, A., Laronche, A., Thomson, D.J., Albert, J., 2007. *Applied Optics* 46 (7), 1142–1149.
- Chang, K.S., Sun, C.J., Chiang, P.L., Chou, A.C., Lin, M.C., Liang, C., Hung, H.H., Yeh, Y. H., Chen, C.D., Pan, C.Y., Chen, Y.T., 2012. *Biosensors and Bioelectronics* 31 (1), 137–143.
- Corning, 2010. http://www.corning.com/opticalfiber/product/SMF-28_Ull_fiber.aspx.
- Fang, Z., Zhu, X., 2013. *Advanced Materials* 25 (28), 3840–3856.
- Fu, C., Hu, C., Liu, Y., Xu, S., Xu, W., 2013. *Analytical Methods* 4 (10), 3107–3110.
- Gao, B., Arya, G., Tao, A.R., 2012. *Nature Nanotechnology* 7 (7), 433–437.
- Ge, S., Kojio, K., Takahara, A., Kujiyama, T., 1998. *Journal of Biomaterials Science, Polymer Edition* 9 (2), 131–150.
- Gehan, H., Hilal, L., Chehimi, M.M., Aubard, J., Hohenau, A., Feldj, N., Mangeney, C., 2010. *ACS Nano* 4 (11), 6491–6500.
- Gosecka, M., Grifette, N., Mangeney, C., Chehimi, M.M., Slomkowski, S., Basinska, T., 2011. *Colloid and Polymer Science* 289 (13), 1511–1518.
- Green, N.M., 1963. *Biochemical Journal* 89 (3), 585–591.
- Habeeb, A.F.S.A., Hiramoto, R., 1968. *Archives of Biochemistry and Biophysics* 126 (1), 16–26.
- Hayashida, M., Yamaguchi, A., Misawa, H., 2005. *Japanese Journal of Applied Physics* 44 (50–52), L1544–L1546.
- Hady, V., Bujis, J., 1996. *Current Opinion in Biotechnology* 7 (1), 72–77.
- Hrapovic, S., Liu, Y.L., Enright, G., Bensebaa, F., Luong, J.H.T., 2003. *Langmuir* 19 (9), 3958–3965.
- Kass, R.L., Kardos, J.L., 1971. *Polymer Engineering and Science* 11 (1), 11–18.
- Kaewwansha, C., Tangboriboonrat, P., Polpanich, D., Eissa, M., Elaisari, A., 2013. *ACS Applied Materials and Interfaces* 5 (6), 1857–1869.
- Kergaravat, S.V., Gómez, G.A., Fabiano, S.N., Laube Chávez, T.I., Plivdori, M.J., Hernández, S.R., 2011. *Talanta* 57 (0), 484–490.
- Kwon, M.J., Lee, J., Waik, A.W., Lee, H.J., 2012. *Analytical Chemistry* 84 (3), 1702–1707.
- Li, B.R., Chen, C.W., Yang, W.L., Lin, T.Y., Pan, C.Y., Chen, Y.T., 2013. *Biosensors and Bioelectronics* 45, 252–259.
- Lin, S.P., Pan, C.Y., Tseng, K.C., Lin, M.C., Chen, C.D., Tai, C.C., Yu, S.H., Sun, Y.C., Lin, T.W., Chen, Y.T., 2009. *Nano Today* 4 (3), 235–241.
- Lin, Y., Zou, Y., Mo, Y., Guo, J., Undquist, R.G., 2010. *Sensors* 10 (10), 9397–9406.
- Linares, E.M., Kubota, L.T., Michaelis, J., Thalhammer, S., 2012. *Journal of Immunological Methods* 375 (1–2), 264–270.
- Liu, Y.C., Wang, C.M., Hsiung, K.P., 2001. *Analytical Biochemistry* 299 (2), 130–135.
- Magnee, R., Maazouz, M., Doneux, C., Bodino, F., Rudolf, P., Teiller-Billy, D., Pireaux, J.J., 2003. *Journal of Physical Chemistry B* 107 (19), 4567–4572.
- Maguis, S., Lafont, G., Ferdinand, P., Carbonnier, B., Kham, K., Mekhalif, T., Millot, M.C., 2008. *Optics Express* 16 (23), 19049–19062.
- Mahmoud, M.A., Qian, W., El-Sayed, M.A., 2011. *Nano Letters* 11 (8), 3285–3289.
- Mayer, K.M., Häfner, J.H., 2011. *Chemical Reviews* 111 (6), 3828–3857.
- Nguyen, T.H., Hardwick, S.A., Sun, T., Grattan, K.T.V., 2012. *IEEE Sensors Journal* 12 (1), 255–260.
- Patolsky, F., Zheng, G., Lieber, C.M., 2006. *Nature Protocols* 1 (4), 1711–1724.
- Pilank, M., Šipová, H., Kvasnicka, P., Gallier, N., Krenn, J.R., Homola, J., 2012. *Optics Express* 20 (1), 672–680.
- Rozenberg, B.A., Tenne, R., 2008. *Progress in Polymer Science* 33 (1), 40–112.
- Sendroiu, I.E., Warner, M.E., Com, R.M., 2009. *Langmuir* 25 (19), 11282–11284.
- Shao, L.-Y., Coyle, J.P., Barry, S.T., Albert, J., 2011. *Optical Materials Express* 1 (2), 128–137.
- Shevchenko, Y., Francis, T.J., Blair, D.A.D., Walsh, R., DeRosa, M.A., Albert, J., 2011. *Analytical Biochemistry* 83, 7027–7034.
- Sim, H.R., Wark, A.W., Lee, H.J., 2010. *Analyst* 135 (10), 2528–2532.
- Skrabalak, S.E., Chen, J.Y., Sun, Y.G., Lu, X.M., Au, L., Cobley, C.M., Xia, Y.N., 2008. *Accounts of Chemical Research* 41 (12), 1587–1595.
- Spitalsky, Z., Tasis, D., Papagelis, K., Galiotis, C., 2010. *Progress in Polymer Science* 35 (3), 357–401.
- Uptima, I., www.interchim.fr/ft/10685A.pdf.
- Vial, J., Jardy, A., 1999. *Analytical Chemistry* 71 (14), 2672–2677.
- Yu, C., Irudayaraj, J., 2007. *Biophysical Journal* 93 (10), 3684–3692.
- Zhou, W., Mandia, D.J., Griffiths, M.B.E., Biallageu, A., Zhang, Y., Gordon, P.G., Barry, S.T., Albert, J., 2013. *Optics Express* 21 (1), 245–255.
- Zou, H., Wu, S., Shen, J., 2008. *Chemical Reviews* 108 (9), 3893–3957.

4 Conclusions

The investigation into the synthesis, optical properties and potential plasmonic applications of gold nanocages, comparing unmodified to surface functionalization and capping in colloidal and substrate-supported environments, presents a variety of information with respect to tuning the localized surface plasmon resonance to desired wavelengths and peak shapes. Unmodified gold nanocages can be tuned during synthesis toward a more or less red-shifted LSPR position relative to their silver nanocube templates depending on the ratio of gold to silver added and the porosity of the faces. The LSPR can be further tuned with the use of substrates of known refractive indices. Once deposited, an increased refractive index on the part of the supporting substrate will red-shift the LSPR peak by a predictable amount related to the refractive index sensitivity of the nanocages. Colloidal nanocages also have LSPR sensitivity to surface functionalization. Whether the nanocages are fully enclosed in a silica shell or simply modified with a monolayer of surfactant or hydrophobic molecules, the environment immediately surrounding the gold nanocages has been modified, inducing a predictable and repeatable shift of the LSPR of the nanocages. The width of the LSPR mode of the gold nanocages was primarily tuneable by the polydispersity of the structures in solution. The more monodisperse samples showed a narrower LSPR band, while those with a variety of shapes and sizes were much broader. The ability to tune the LSPR of the gold nanocages to the desired maximum wavelength and width is definitely a property required for further use in plasmonic applications. Many applications are based on the

interaction within a specific range of the electromagnetic spectrum, thus having an LSPR that can be modified to fall within a desired range (with controllable intensity based on concentration of nanostructure) allows for gold nanocages to be a candidate in the development of these applications.

The reflection and transmission properties of substrate-supported nanocages are inherent to their monolayer density. High density monolayers behave much like solid gold films, revealing bulk reflection properties instead of the plasmonic resonance-related properties seen at low density. This is a property that should be considered if monolayers of medium to high density are to be used in applications involving absorption and transmission of light, for if the intent is to absorb light, having too much lost to reflection will impact the effectiveness of the assembly (such as with photovoltaic application).

The RIS of the gold nanocages can also be tuned. Substrate-supported nanocages have a higher RIS with respect to the substrate when arranged in a low density monolayer. For the case of surface modified nanocages, thinner shells and self-assembled monolayers have a greater impact on the RIS of the nanocages, primarily caused by the limited reach of influence of the plasmon field itself. Modifications close to the surface, or a series of modifications within the induced field just beyond the surface of the nanostructure will have a stronger interaction with the LSPR than if the modification extends beyond the range of the induced electric field (when the LSPR is influenced by an apparent change of suspension medium instead of a surface modification).

The influence of unmodified and silica-capped gold nanocages on the intensity of fluorescence emission is related to the LSPR of the nanostructure being used and its overlap with the excitation or emission wavelengths of a particular fluorophore. The greatest control of the degree of quenching of a fluorophore was achieved as the LSPR maximum of the nanocages overlapped nearly perfectly with the excitation maximum of the fluorophore, allowing the thickness of the silica shell to be the primary influence on the quenching of the emission spectrum. With good LSPR-excitation overlap, the gold nanocages with thinner silica shells were seen to quench less than those with thicker shells, though the only instance of enhancement observed was with the LSPR maximum placed between the maxima of excitation and emission. The greatest degree of quenching was seen with bare gold nanocages, though intensities near the same were achieved with silica shells of low porosity near 20 nm thick as the LSPR overlapped both the excitation and emission spectra of that particular fluorophore. The intrinsic property observed for all solutions of silica-capped gold nanocages in the presence of fluorescent dyes was that the excitation band of the fluorophore was broadened as a result of any overlap with the LSPR of the gold nanostructure. This broadening effect was more noticeable as the overlap with the excitation band increased, but even a small overlap with a shoulder or the tail of the LSPR band induced this observable optical property. All of the gold nanocage assemblies showed evidence of increased quenching with increased concentration relative to that of the dye being used, regardless of the LSPR-excitation or LSPR-emission overlap.

Both the RIS of the gold nanostructure assembly and the potential for fluorescence enhancement or quenching are useful properties for plasmonic sensing applications. RIS and fluorescence quenching can be easily tuned based on the nature of the gold nanocage assembly being used, thus the potential integration into a number of sensing platforms is of particular interest. Sensitivity can also be increased by the limited contact surface of the gold nanocages providing less interference than a solid nanostructure. Many plasmonic sensing platforms use changing refractive indices at the surface of the assembly as the sensing basis, thus the inherently high RIS of gold nanocages provides a useful basis for their integration into these assemblies.

5 Future Works

The work presented herein provided a moderate range of characterization and optical properties of gold nanocages; there is, of course, always more to be investigated. Current work underway by colleagues and collaborators of the lanoul lab include heating experiments of nanostructures (including gold nanocages) bound to the surface of optical fibres for the investigation of the stability of the structures in varying conditions and specific bioapplications involving optical detection. Alternative oxide coatings and surface modifications are also of particular interest in the furtherance of this research, including titanium and vanadium oxide films and coatings for modifying the range of LSPR absorption and applications of the nanostructures.

SERS investigations were not conducted at all with the gold nanostructures used in this project. It is a spectroscopic method that will be used to investigate the enhancement potential the gold nanocages in future work. The potential to increase the sensitivity of the Raman signals is high due to the surface contact of the gold nanocages limited only the edges, where the LSPR is strongest. The lack of solid material, through which the signal would normally diffuse, may provide a stronger enhancement and greater sensitivity than those seen with solid gold nanostructures, thus SERS investigations are definitely to be completed.

Further investigation of aspects of this project would be beneficial for developing specific applications of the gold nanocages. Using a surface modification molecule that would allow for high density monolayers with

increased stability for RIS determination (perhaps functionalizing the surface of the slide substrate as well) would be one investigation useful for continuing to tailor the maximum light absorption ranges on silicon slide for potential photovoltaic applications. In-depth investigations into potentially time-dependent fluorescence emission are another curious property to be researched further. The pore size of the silica-coated nanocages may have an effect on the rate of diffusion of the dye molecules into the shell itself, which may provide further quenching or enhancement trends based on average distance travelled and concentration found within the surface of the silica shell at a given time.

Other hollow plasmonic nanostructures may be able to be synthesized in the same manner as the cubic gold nanocages. The shape and size specificity of silver nanostructures is well understood, thus the potential for other hollow gold structures lies in the stability of structure to be formed (it would be very interesting to investigate the potential for high aspect ratio nanostructures such as nanorods or nanowires).

6 References

- [1] N. Savage, *Nature Outlook*, vol. 495, pp. S8-S9, 2013.
- [2] S. Gray, *J. Phys. Chem. C*, vol. 117, no. 5, pp. 1983-1994, 2013.
- [3] E. Ringe, J. M. McMahon, K. Sohn, C. Cobley, Y. Xia, J. Huang, G. C. Schatz, L. D. Marks and R. P. Van Duyne, *J. Phys. Chem. C*, vol. 114, no. 29, pp. 12511-12516, 2010.
- [4] R. Feynman, "Plenty of Room at the Bottom," 1959.
- [5] N. Taniguchi, "On the basic concept of 'nano-technology'," in *Proc. of ICPE*, Tokyo, 1974.
- [6] S. M. Morton, D. W. Silverstein and L. Jensen, *Chemical Reviews*, vol. 111, no. 6, pp. 3962-3964, 2011.
- [7] X. Lu, M. Rycenga, S. E. Skrabalak, B. Wiley and Y. Xia, *Ann. Rev. Phys. Chem.*, vol. 60, pp. 167-192, 2009.
- [8] G. Mie, *Annalen der physik*, vol. 25, no. 3, pp. 377-445, 1908.
- [9] M. R. Jones, K. D. Osberg, R. J. Macfarlane, M. R. Langille and C. A. Mirkin, *Chemical Reviews*, vol. 111, no. 6, pp. 3736-3827, 2011.
- [10] M. B. Cortie and A. M. McDonagh, *Chemical Reviews*, vol. 111, no. 6, pp. 3713-3735, 2011.
- [11] P. Zijlstra and M. Orrit, *Rep. Prog. Phys.*, vol. 74, no. 10, p. 106401, 2011.
- [12] Y. Xia, W. Li, C. M. Cobley, J. Chen, X. Xia, Q. Zhang, M. Yang, E. C. Cho and P. K. Brown, *Accounts of Chemical Research*, vol. 44, no. 10, pp. 914-924, 2011.
- [13] S. E. Skrabalak, J. Chen, Y. Sun, X. Lu, L. Au, C. M. Cobley and Y. Xia, *Accounts of Chemical Research*, vol. 41, no. 12, pp. 1587-1595, 2008.
- [14] M. Rycenga, C. M. Cobley, J. Zeng, W. Li, C. H. Moran, Q. Zhang, D. Qin and Y. Xia, *Chemical Reviews*, vol. 111, no. 6, pp. 3669-3712, 2011.
- [15] N. C. Lindquist, P. Nagpal, K. M. McPeak, D. J. Norris and S.-H. Oh, *Rep. Prog. Phys.*, vol. 75, no. 3, p. 036501, 2012.
- [16] S. Lal, S. Link and N. J. Halas, *Nature Photonics*, vol. 1, no. 11, pp. 641-648, 2007.
- [17] G. V. Hartland, *Chemical Reviews*, vol. 111, no. 6, pp. 3858-3887, 2011.
- [18] Y. H. Lee, H. Chen, Q.-H. Xu and J. Wang, *J. Phys. Chem. C*, vol. 115, no. 16, pp. 7997-8004, 2011.
- [19] A. Staff, "Investigation of Silicon Substrates' Effects on the Plasmonic Signatures of Silver Nanocubes," Carleton University, Ottawa, 2012.
- [20] H. Chen, X. Kou, Z. Yang, W. Ni and J. Wang, *Langmuir*, vol. 24, no. 10, pp. 5233-5237, 2008.
- [21] H.-J. Wu, J. Henzie, W.-C. Lin, C. Rhodes, Z. Li, E. Sartorel, J. Thorner, P. Yang and J. T. Groves, *Nature Methods*, vol. 9, no. 12, pp. 1189-1191, 2012.
- [22] K. M. Mayer and J. H. Hafner, *Chemical Reviews*, vol. 111, no. 6, pp. 3828-3857, 2011.
- [23] K.-J. Chen and C.-J. Lu, *Talanta*, vol. 81, no. 4-5, pp. 1670-1675, 2010.
- [24] T. Karakouz, A. Vaskevich and I. Rubinstein, *J. Phys. Chem B*, vol. 112, pp. 14530-14538, 2008.

- [25] M. Nuopponen and H. Tenhu, *Langmuir*, vol. 23, no. 10, pp. 5352-5357, 2007.
- [26] N. H. Mack, J. W. Wackerly, V. Malyarchuck, J. A. Rogers, J. S. Moore and R. G. Nuzzo, *Nano Letters*, vol. 7, no. 3, pp. 733-737, 2007.
- [27] H. Jiang, J. Markowski and J. Sabarinathan, *Optics Express*, vol. 17, no. 24, pp. 21802-21807, 2009.
- [28] S. T. Dubas and V. Pimpan, *Talanta*, vol. 76, no. 1, pp. 29-33, 2008.
- [29] Y. Choi, Y. Park, T. Kang and L. P. Lee, *Nature Nanotechnology*, vol. 4, no. 11, pp. 742-746, 2009.
- [30] T. Konig, R. Kodiyath, Z. A. Combs, M. A. Mahmoud, M. A. El-Sayed and V. V. Tsukruk, *Part. Part. Syst. Charact.*, vol. 31, no. 2, pp. 274-283, 2013.
- [31] P. K. Jain and M. A. El-Sayed, *Chemical Physics Letters*, vol. 487, no. 4, pp. 153-164, 2010.
- [32] E. Petryayeva and U. J. Krull, *Analytica Chimica Acta*, vol. 706, no. 1, pp. 8-24, 2011.
- [33] Y.-T. Li, H.-S. Liu, H.-P. Lin and S.-H. Chen, *Electrophoresis*, vol. 26, pp. 4743-4750, 2005.
- [34] J. Xie, S. Lee and X. Chen, *Advanced Drug Delivery Reviews*, vol. 62, no. 11, pp. 1064-1079, 2010.
- [35] Y.-C. Chung, I.-H. Chen and C.-J. Chen, *Biomaterials*, vol. 29, no. 12, pp. 1807-1816, 2008.
- [36] S. Lepinay, A. Staff, A. Ianoul and J. Albert, *Biosensors and Bioelectronics*, vol. 52, pp. 337-344, 2014.
- [37] L. M. Maestro, P. Haro-González, A. Sánchez-Iglesias, L. M. Liz-Marzán, J. G. Solé and D. Jaque, *Langmuir*, vol. 30, no. 6, pp. 1650-1658, 2014.
- [38] Z. S. Zhang, O. M. Wilson, M. Y. Efremov, E. A. Olson, P. Braun, W. Senaratne, C. K. Ober, M. Zhang and L. H. Allen, *Applied Physics Letters*, vol. 84, no. 25, pp. 5198-5200, 2004.
- [39] W. E. Safford, "Lignum Nephriticum - Its History and an Account of the Remarkable Fluorescence of its Infusion," in *Annual Report of the Board of Regents of the Smithsonian Institution*, Washington, Government Printing Office, 1916, pp. 271-298.
- [40] M. Moskovits, *Reviews of Modern Physics*, vol. 57, no. 3, pp. 783-828, 1985.
- [41] S. Gruenbaum, *The Fluorescence Enhancement Effects of Gold Nanoparticles*, Oxford, Ohio: Miami University, 2005.
- [42] J. R. Lakowicz, C. D. Geddes, I. Gryczynski, J. Malicka, Z. Gryczynski, K. Aslan, J. Lukomska, E. Matveeva, J. Zhang, R. Badugu and J. Huang, *Journal of Fluorescence*, vol. 14, no. 4, pp. 425-441, 2004.
- [43] J. Lukomska, J. Malicka, I. Gryczynski and J. R. Lakowicz, *Journal of Fluorescence*, vol. 14, no. 4, pp. 417-423, 2004.
- [44] A. Mooradian, *Physical Review Letters*, vol. 22, no. 5, pp. 185-186, 1969.
- [45] S. Huang, C. Pfeiffer, J. Hollmann, S. Friede, J. J.-C. Chen, A. Beyer, B. Haas, K. Volz, W. Heimbrot, J. M. Montenegro Martos, W. Chang and W. J. Parak, *Langmuir*, vol. 28, no. 24, pp. 8915-8919, 2012.
- [46] E. Fort and S. Grésillon, *Journal of Applied Physics D*, vol. 41, no. 1, p. 013001, 2008.

- [47] B. Krajnik, Gajda-Rączka, D. Piątkowski, P. Nyga, B. Jankiewicz, E. Hofmann and S. Mackowski, *Nanoscale Research Letters*, vol. 8, no. 1, pp. 146-152, 2013.
- [48] Y. Zhao, Y. Jiang and Y. Fang, *Chemical Physics*, vol. 323, no. 2, pp. 169-172, 2006.
- [49] J. Zhang, R. Badugu and J. R. Lakowicz, *Plasmonics*, vol. 3, no. 1, pp. 3-11, 2008.
- [50] P. Reineck, D. Gómez, S. H. Ng, M. Karg, T. Bell, P. Mulvaney and U. Bach, *ACS Nano*, vol. 7, no. 8, pp. 6636-6648, 2013.
- [51] O. G. Tovmachenko, C. Graf, D. J. van den Heuvel, A. van Blaaderen and H. C. Gerritsen, *Advanced Materials*, vol. 18, pp. 91-95, 2006.
- [52] J. Zhu, J.-j. Li, A.-q. Wang, Y. Chen and J.-W. Zhao, *Nanoscale Research Letters*, vol. 5, no. 9, pp. 1496-1501, 2010.
- [53] Y.-F. Huang, K.-H. Ma, K.-B. Kang, M. Zhao, Z.-L. Zhang, Y.-X. Liu, T. Wen, Q. Wang, W.-Y. Qiu and D. Qiu, *Colloids and Surfaces A*, vol. 421, pp. 101-108, 2013.
- [54] P. C. Ray, A. Fortner, J. Griffin, C. K. Kim, J. P. Singh and H. Yu, *Chemical Physics Letters*, vol. 414, pp. 259-264, 2005.
- [55] J. Zhu, K. Zhu and L.-q. Huang, *Physics Letters A*, vol. 372, no. 18, pp. 3283-3288, 2008.
- [56] N. C. Bigall, W. J. Parak and D. Dorfs, *Nano Today*, vol. 7, no. 4, pp. 282-296, 2012.
- [57] R. Dutta, B. P. Singh and T. Kundu, *J. Phys. Chem. C*, vol. 117, no. 33, pp. 17167-17176, 2013.
- [58] M. H. Tu, T. Sun and K. T. V. Grattan, *Sensors and Actuators B*, vol. 191, pp. 37-44, 2014.
- [59] T. Wang, X. Wang, D. LaMontagne, Z. Wang, Z. Wang and Y. C. Cao, *J. Amer. Chem. Soc.*, vol. 134, pp. 18225-18228, 2012.
- [60] S. W. Sheehan, H. Noh, G. W. Brudvig, H. Cao and C. A. Schmuttenmaer, *J. Phys. Chem. C*, vol. 117, no. 2, pp. 927-934, 2013.
- [61] M. J. Banholzer, J. E. Millstone, L. Qin and C. A. Mirkin, *Chem. Soc. Rev.*, vol. 37, no. 5, pp. 885-897, 2008.
- [62] S. E. J. Bell and N. M. S. Sirimuthu, *Chem. Soc. Rev.*, vol. 37, no. 5, pp. 1012-1024, 2008.
- [63] R. J. C. Brown and M. J. T. Milton, *J. Raman Spectrosc.*, vol. 39, pp. 1313-1326, 2008.
- [64] J. M. McLellan, A. Siekkinen, J. Chen and Y. Xia, *Chemical Physics Letters*, vol. 427, pp. 122-126, 2006.
- [65] K. M. Kosuda, J. M. Bingham, K. L. Wustholz and R. P. Van Duyne, "Nanostructures and Surface-Enhanced Raman Spectroscopy," in *Comprehensive Nanoscience and Technology*, Evanston, Oxford: Academic Press, 2011, pp. 263-301.
- [66] P. L. Stiles, J. A. Dieringer, N. C. Shah and R. P. Van Duyne, *Annu. Rev. Anal. Chem.*, vol. 1, pp. 601-626, 2008.
- [67] M. A. Mahmoud and M. A. El-Sayed, *Nano Letters*, vol. 9, no. 8, pp. 3025-3031, 2009.
- [68] A. Zoppi, S. Trigari, E. Giorgetti, M. Muniz-Miranda, M. Alloisio, A. Demartini, G. Dellepiane, S. Thea, G. Dobrikov and I. Timtcheva, *Journal of Colloid and Interface Science*, vol. 407, pp. 89-94, 2013.

- [69] N. L. Netzer, Z. Tanaka, B. Chen and C. Jiang, *J. Phys. Chem. C*, vol. 117, no. 31, pp. 16187-16194, 2013.
- [70] B. Ballarin, M. C. Cassani, E. Scavetta and D. Tonelli, *Electrochimica Acta*, vol. 53, no. 27, pp. 8034-8044, 2008.
- [71] B. Gao, G. Arya and A. R. Tao, *Nature Nanotechnology*, vol. 7, no. 7, pp. 433-437, 2012.
- [72] R. F. Oulton, V. J. Sorger, T. Zentgraf, R.-M. Ma, C. Gladden, L. Dai, G. Bartal and X. Zhang, *Nature*, vol. 561, no. 7264, pp. 629-632, 2009.
- [73] M. A. Noginov, G. Zhu, A. M. Belgrave, R. Bakker, V. M. Shalaev, E. E. Narimanov, S. Stout, E. Herz, T. Suteewong and U. Weisner, *Nature*, vol. 460, no. 7259, pp. 1110-1112, 2009.
- [74] J. Lee, K. Han and D.-J. Jan, *Applied Catalysis A*, vol. 469, pp. 380-386, 2014.
- [75] M. A. Mahmoud and M. A. El-Sayed, *Nano Letters*, vol. 11, no. 3, pp. 946-953, 2011.
- [76] Y. Zhang, F. Xu, Y. Sun, C. Guo, K. Cui, Y. Shi, Z. Wen and Z. Li, *Chem. Eur. J.*, vol. 16, no. 30, pp. 9248-9256, 2010.
- [77] W. Cai, T. Gao, H. Hong and J. Sun, *Nanotechnology, Science and Applications*, vol. 1, pp. 17-32, 2008.
- [78] J. Chen, M. Yang, Q. Zhang, E. C. Cho, C. M. Cobley, C. Kim, C. Glaus, L. V. Wang, M. J. Welch and Y. Xia, *Adv. Funct. Mater.*, vol. 20, no. 21, pp. 3684-3694, 2010.
- [79] X. Huang, P. K. Jain, I. H. El-Sayed and M. A. El-Sayed, *Nanomedicine*, vol. 2, no. 5, pp. 681-693, 2007.
- [80] R. Singh and J. W. J. Lillard, *Experimental and Molecular Pathology*, vol. 86, no. 3, pp. 215-223, 2009.
- [81] S. E. Skrabalak, J. Chen, L. Au, X. Lu, X. Li and Y. Xia, *Advanced Materials*, vol. 19, no. 20, pp. 3177-3184, 2007.
- [82] T.-H. Tran and T.-D. Nguyen, *Colloids and Surfaces B*, vol. 88, no. 1, pp. 1-22, 2011.
- [83] B. M. Wohl and J. F. J. Engbersen, *Journal of Controlled Release*, vol. 158, no. 1, pp. 2-14, 2012.
- [84] J. Yang, D. Shen, L. Zhou, W. Li, X. Li, C. Yao, R. Wang, A. M. El-Toni, F. Zhang and D. Zhao, *Chemistry of Materials*, vol. 25, pp. 3030-3037, 2013.
- [85] M. S. Yavuz, Y. Cheng, J. Chen, C. M. Cobley, Q. Zhang, M. Rycenga, J. Xie, C. Kim, K. H. Song, A. G. Schwartz, L. V. Wang and Y. Xia, *Nature Materials*, vol. 8, no. 12, pp. 935-939, 2009.
- [86] B. Zheng, N. Zettsu, M. Fukuta, M. Uenuma, T. Hashimoto, K. Gamo, Y. Uraoka, I. Yamashita and H. Watanabe, *Chemical Physics Letters*, vol. 506, no. 1, pp. 76-80, 2011.
- [87] A. J. Haes, L. Chang, W. L. Klein and R. P. Van Duyne, *J. Amer. Chem. Soc.*, vol. 127, pp. 2264-2271, 2005.
- [88] B. N. Khlebstov, E. V. Panfilova, G. S. Terentyuk, I. L. Maksimova, A. V. Ivanov and N. G. Khlebstov, *Langmuir*, vol. 28, no. 24, pp. 8994-9002, 2012.

- [89] M. A. Mackey, F. Saira, M. A. Mahmoud and M. A. El-Sayed, *Bioconjugate Chemistry*, vol. 24, pp. 897-906, 2013.
- [90] W. Li, X. Cai, C. Kim, G. Sun, Y. Zhang, R. Deng, M. Yang, J. Chen, S. Achilefu, L. V. Wang and Y. Xia, *Nanoscale*, vol. 3, no. 4, pp. 1724-1730, 2011.
- [91] J. Gao, X. Huang, H. Liu, F. Zan and J. Ren, *Langmuir*, vol. 28, no. 9, pp. 4464-4471, 2012.
- [92] Y. Wang, Y. Liu, H. Luehmann, X. Xia, D. Wan, C. Cutler and Y. Xia, *Nano Letters*, vol. 13, no. 2, pp. 581-585, 2013.
- [93] A. Bottomley, D. Prezgot, A. Staff and A. Ianoul, *Nanoscale*, vol. 4, pp. 6374-6382, 2012.
- [94] D. E. Charles, D. Aherne, M. Gara, D. M. Ledwith, Y. K. Gun'ko, J. M. Kelly, W. J. Blau and M. E. Brennan-Fournet, *ACS Nano*, vol. 4, no. 1, pp. 55-64, 2010.
- [95] N. J. Halas, S. Lal, W.-S. Chang, S. Link and P. Nordlander, *Chemical Reviews*, vol. 111, no. 6, pp. 3913-3961, 2011.
- [96] M. A. Mahmoud and M. A. El-Sayed, *J. Amer. Chem. Soc.*, vol. 132, no. 36, pp. 12704-12710, 2010.
- [97] E. M. Goldys, N. Calander and K. Drozdowicz-Tomsia, *J. Phys. Chem. C*, vol. 115, no. 3, pp. 676-682, 2011.
- [98] H. Chen, Y. Wang and S. Dong, *Inorg. Chem.*, vol. 46, pp. 10587-10593, 2007.
- [99] J. Chen, B. Lim, E. P. Lee and Y. Xia, *Nano Today*, vol. 4, no. 1, pp. 81-95, 2009.
- [100] S. Kundu, V. Maheshwari, S. Niu and R. F. Saraf, *Nanotechnology*, vol. 19, no. 6, p. 065604, 2008.
- [101] T. Huang and X.-H. N. Xu, *J. Mater. Chem.*, vol. 20, pp. 9867-9876, 2010.
- [102] P. Zhao, L. Na and D. Astruc, *Coordination Chemistry Reviews*, vol. 257, no. 3, pp. 638-665, 2013.
- [103] X. Hong, D. Wang, S. Cai, H. Rong and Y. Li, *J. Amer. Chem. Soc.*, vol. 134, pp. 18165-18168, 2012.
- [104] S. J. Ku, S. A. Woo, D. Seo, H. Soon and J.-B. Kim, *European Polymer Journal*, vol. 47, no. 3, pp. 305-310, 2011.
- [105] K.-J. Lee, Y.-I. Lee, J. Lee, N. V. Myung and Y.-H. Choa, *Current Applied Physics*, vol. 12, pp. S53-S58, 2012.
- [106] L. Minati, F. Benetti, A. Chiappini and G. Speranza, *Colloids and Surfaces A*, vol. 441, pp. 623-628, 2014.
- [107] F.-R. Fan, D.-Y. Liu, Y.-F. Wu, S. Duan, Z.-X. Xie, Z.-Y. Jiang and Z.-Q. Tian, *J. Amer. Chem. Soc.*, vol. 130, no. 22, pp. 6949-6951, 2008.
- [108] L. Vigderman and E. R. Zubarev, *Langmuir*, vol. 28, no. 24, pp. 9034-9040, 2012.
- [109] Q. Zhang, W. Li, C. Moran, J. Zeng, J. Chen, L.-P. Wen and Y. Xia, *J. Amer. Chem. Soc.*, vol. 132, pp. 11372-11378, 2010.
- [110] C.-J. Huang, P.-H. Chiu, Y.-H. Wang, W. R. Chen and T. H. Meen, *J. Electrochem. Soc.*, vol. 153, no. 8, pp. D129-D133, 2006.

- [111] C.-J. Huang, Y.-H. Wang, P.-H. Chiu, M.-C. Shih and T.-H. Meen, *Materials Letters*, vol. 60, pp. 1896-1900, 2006.
- [112] Y. Ma, W. Li, J. Zeng, M. McKiernan, Z. Xie and Y. Xia, *J. Mater. Chem.*, vol. 20, no. 18, p. 3586, 2010.
- [113] C. M. Cobley, S. E. Skrabalak, D. J. Campbell and Y. Xia, *Plasmonics*, vol. 4, no. 2, pp. 171-179, 2009.
- [114] Q. Zhang, W. Li, L.-P. Wen, J. Chen and Y. Xia, *Chemistry*, vol. 16, no. 33, pp. 10234-10239, 2010.
- [115] Y. Nakao and K. Kaeriyama, *Journal of Colloid and Interface Science*, vol. 110, no. 1, pp. 82-87, 1986.
- [116] J. Ren and R. D. Tilley, *J. Amer. Chem. Soc.*, vol. 129, no. 11, pp. 3287-3291, 2007.
- [117] Y. Xiong, J. Chen, B. Wiley, Y. Xia, Y. Yin and Z.-Y. Li, *Nano Letters*, vol. 5, no. 7, pp. 1237-1242, 2005.
- [118] O. Masala and R. Seshadri, *Annual Review of Materials Research*, vol. 34, pp. 41-81, 2004.
- [119] C. M. Cobley, M. Rycenga, F. Zhou and Z.-Y. X. Y. Li, *Angewandte Chemie*, vol. 48, no. 26, pp. 4824-4827, 2009.
- [120] X. Xia and Y. Xia, *Nano Letters*, vol. 12, no. 11, pp. 6038-6042, 2012.
- [121] X. Xia, J. Zeng, Q. Zhang, C. H. Moran and Y. Xia, *J. Phys. Chem. C*, vol. 116, no. 41, pp. 21647-21656, 2012.
- [122] C.-Y. Chiu, Y. Li, L. Ruan, X. Ye, C. B. Murray and Y. Huang, *Nature Chemistry*, vol. 3, no. 5, pp. 393-399, 2011.
- [123] D. T. Nguyen, D.-J. Kim and K.-S. Kim, *Micron*, vol. 42, no. 3, pp. 207-227, 2011.
- [124] B. Pietrobon, M. McEachran and V. Kitaev, *ACS Nano*, vol. 3, no. 1, pp. 21-26, 2009.
- [125] F. Li, D. Tian and H. Cui, *Luminescence*, vol. 28, no. 1, pp. 7-15, 2013.
- [126] J. A. Jacob, S. Kapoor, N. Biswas and T. Mukherjee, *Colloids and Surfaces A*, vol. 301, no. 1, pp. 329-334, 2007.
- [127] A. R. Siekkinen, J. M. McLellan, J. Chen and Y. Xia, *Chemical Physics Letters*, vol. 432, no. 4, pp. 491-496, 2006.
- [128] M. R. Langille, M. L. Personick, J. Zhang and C. A. Mirkin, *J. Amer. Chem. Soc.*, vol. 134, pp. 14542-14554, 2012.
- [129] S. H. Im, Y. T. Lee, B. Wiley and Y. Xia, *Angewandte Chemie*, vol. 44, pp. 2154-2157, 2005.
- [130] B. Wiley, T. Herricks, Y. Sun and Y. Xia, *Nano Letters*, vol. 4, no. 9, pp. 1733-1739, 2004.
- [131] B. J. Wiley, Y. Xiong, Z.-Y. Li, Y. Yin and Y. Xia, *Nano Letters*, vol. 6, no. 4, pp. 765-768, 2006.
- [132] T. Zhao, J.-B. Fan, J. Cui, J.-H. Liu, X.-B. Xu and M.-Q. Zhu, *Chemical Physics Letters*, vol. 501, no. 4, pp. 414-418, 2011.
- [133] Y. T. Lee, S. H. Im, B. Wiley and Y. Xia, *Chem. Phys. Lett.*, vol. 411, pp. 479-483, 2005.
- [134] A. Taguchi, S. Fujii, T. Ichimura, P. Verma, Y. Inouye and S. Kawata, *Chemical Physics Letters*, vol. 462, no. 1, pp. 92-95, 2008.

- [135] R. Ma, C. Levard, M. S. M., Y. Cheng, J. Liu, F. M. Michel, G. E. J. Brown and G. V. Lowry, *Environ. Sci. & Tech.*, vol. 46, no. 2, pp. 752-759, 2012.
- [136] C. M. Copley and Y. Xia, *Mater. Sci. Eng. R*, vol. 70, no. 3, pp. 44-62, 2010.
- [137] S. E. Skrabalak, L. Au, X. Li and Y. Xia, *Nature Protocols*, vol. 2, no. 9, pp. 2182-2190, 2007.
- [138] J. Chen, J. M. McLellan, A. Siekkinen, Y. Xiong, Z.-Y. Li and Y. Xia, *J. Amer. Chem. Soc.*, vol. 128, no. 46, pp. 14776-14777, 2006.
- [139] Y. Sun and Y. Xia, *J. Amer. Chem. Soc.*, vol. 126, pp. 3892-3901, 2004.
- [140] J. Chen, B. Wiley, J. McLellan, Y. Xiong, Z.-Y. Li and Y. Xia, *Nano Letters*, vol. 5, no. 10, pp. 2058-2062, 2005.
- [141] J. Ye, B. Van de Broek, R. De Palma, W. Libaers, K. Clays, W. Van Roy, G. Borghs and G. Maes, *Colloids and Surfaces A*, vol. 322, no. 1, pp. 225-233, 2008.
- [142] W. Stöber, A. Fink and E. Bohn, *Journal of Colloid and Interface Science*, vol. 26, pp. 62-69, 1968.
- [143] Y. Han, J. Jiang, S. S. Lee and J. Y. Ying, *Langmuir*, vol. 24, no. 11, pp. 5842-5848, 2008.
- [144] S.-G. Kim, N. Hagura, F. Iskandar and K. Okuyama, *Advanced Power Technology*, vol. 20, no. 1, pp. 94-100, 2009.
- [145] Y. Imura, S. Hojo, C. Morita and T. Kawai, *Langmuir*, vol. 30, no. 7, pp. 1888-1892, 2014.
- [146] B. N. Khlebtsov, V. A. Khanadeev, E. V. Panfilova, O. A. Inozemtseva, A. M. Burov and N. G. Khlebtsov, *Journal of Quantitative Spectroscopy & Radiative Transfer*, vol. 121, pp. 23-29, 2013.
- [147] J.-G. Oh and H. Kim, *Current Applied Physics*, vol. 13, no. 1, pp. 130-136, 2013.
- [148] S.-R. Yang and B. O. Kolbesen, *Applied Surface Science*, vol. 255, no. 5, pp. 1726-1735, 2008.
- [149] G. L. J. Gaines, *Thin Solid Films*, vol. 99, no. 1, pp. 9-13, 1983.
- [150] S. Acharya, J. P. Hill and K. Ariga, *Advanced Materials*, vol. 21, no. 29, pp. 2959-2981, 2009.
- [151] K. Ariga, Y. Yamauchi, T. Mori and J. P. Hill, *Advanced Materials*, vol. 25, no. 45, pp. 6477-6512, 2013.
- [152] X. Chen, N. Lu, H. Zhang, M. Hirtz, L. Wu, H. Fuchs and L. Chi, *J. Phys. Chem. B*, vol. 110, no. 15, pp. 8039-8046, 2006.
- [153] E. S. Erickson, P. W. Livanec, J. F. Frisz and R. C. Dunn, *Langmuir*, vol. 25, no. 9, pp. 5098-5102, 2009.
- [154] F.-W. Hsiao, Y.-L. Lee and C.-H. Chang, *Colloids and Surfaces B*, vol. 73, no. 1, pp. 110-115, 2009.
- [155] F.-W. Hsiao, Y.-L. Lee and C.-H. Chang, *Colloids and Surfaces A*, vol. 351, no. 1, pp. 18-25, 2009.
- [156] P. Moraille and A. Badia, *Langmuir*, vol. 18, pp. 4414-4419, 2002.
- [157] P. Moraille and A. Badia, *Langmuir*, vol. 19, pp. 8041-8049, 2003.
- [158] B. Pignataro, L. Sardone and G. Marletta, *Mater. Sci. Eng. C*, vol. 22, no. 2, pp. 177-181, 2002.

- [159] M. R. Rodríguez Niño, A. Lucero and J. M. R. Patino, *Colloids and Surfaces A*, vol. 320, no. 1, pp. 260-270, 2008.
- [160] N. Y.-W. Tang and A. Badia, *Langmuir*, vol. 26, no. 22, pp. 17058-17067, 2010.
- [161] R. G. Nuzzo and D. L. Allara, *J. Am. Chem. Soc.*, vol. 105, no. 13, pp. 4481-4483, 1983.
- [162] E. Delamarche, B. Michel, H. A. Biebuyck and C. Gerber, *Advanced Materials*, vol. 8, no. 9, pp. 719-729, 1996.
- [163] L. Srisombat, A. C. Jamison and T. R. Lee, *Colloids and Surfaces A*, vol. 390, no. 1, pp. 1-19, 2011.
- [164] S. Begum, I. P. Jones, D. E. Lynch and J. A. Preece, *Journal of Experimental Nanoscience*, vol. 7, no. 1, pp. 1-16, 2012.
- [165] M. Hu, S. Noda, T. Okubo, Y. Yamaguchi and H. Komiyama, "Structure and morphology of self-assembled 3-mercaptopropyltrimethoxysilane layers on silicon oxide," University of Tokyo, Tokyo, 2012.
- [166] M. M. de Villiers, D. P. Otto, S. J. Strydom and Y. M. Lvov, *Advanced Drug Delivery Reviews*, vol. 63, no. 9, pp. 701-715, 2011.
- [167] M.-H. Lin, H.-Y. Chen and S. Gwo, *J. Am. Chem. Soc.*, vol. 132, no. 32, pp. 11259-11263, 2010.
- [168] A. Mogilevsky, R. Volinsky, Y. Dayagi, N. Markovich and R. Jelinek, *Langmuir*, vol. 26, no. 11, pp. 7893-7898, 2010.
- [169] M. Rycenga, J. M. McLellan and Y. Xia, *Advanced Materials*, vol. 20, no. 12, pp. 2416-2420, 2008.
- [170] T. H. Nguyen, S. A. Hardwick, T. Sun and K. T. V. Grattan, *IEEE Sensors Journal*, vol. 12, no. 1, pp. 255-260, 2012.
- [171] S. Prahl, "PhotochemCAD," Oregon Medical Laser Center, 5 March 2012. [Online]. Available: omlc.ogi.edu/spectra/PhotochemCAD/html/. [Accessed 10 April 2014].
- [172] S. H. Im, Y. T. Lee, B. Wiley and Y. Xia, *Angewandte Chemie*, vol. 117, no. 14, pp. 2192-2195, 2005.
- [173] B. Khlebtsov, E. Panfilova, V. Khanadeev, O. Bibikova, G. Terentyuk, A. Ivanov, V. Rumyantseva, I. Shilov, A. Ryabova, V. Loshchenov and N. G. Khlebtsov, *ACS Nano*, vol. 5, no. 9, pp. 7077-7089, 2011.
- [174] K. Aslan, M. Wu, J. R. Lakowicz and C. D. Geddes, *J. Fluoresc.*, vol. 17, no. 2, pp. 127-131, 2007.
- [175] B. J. Wiley, S. H. Im, Z.-Y. Li, J. McLellan, A. Siekkinen and Y. Xia, *J. Phys. Chem. B*, vol. 110, pp. 15666-15675, 2006.
- [176] Y. Sun and Y. Xia, *Science*, vol. 298, no. 5601, pp. 2176-2179, 2002.
- [177] D. S. Kilin, O. V. Prezhdo and Y. Xia, *Chem. Phys. Lett.*, vol. 458, pp. 113-116, 2008.
- [178] H.-J. Wu, J. Henzie, W.-C. Lin, C. Rhodes, Z. Li, E. Sartorel, J. Thorner, P. Yang and J. T. Groves, *Nature Methods*, vol. 9, no. 12, pp. 1189-1191, 2012.

- [179] M. Lisunova, J. Norman, W. Xingfei, S. Jenkins, J. Chen and D. K. Roper, *Materials Letters*, vol. 117, pp. 241-243, 2014.
- [180] M. A. Mahmoud and M. A. El-Sayed, *J. Phys. Chem. C*, vol. 112, pp. 14618-14625, 2008.
- [181] P. Vanýsek, "Electrochemical Series," in *Handbook of Chemistry and Physics, 92nd Edition*, Cleveland, Ohio, CRC Press, 2011, pp. 5-80 - 5-89.
- [182] R. Terrill, T. Tanzer and P. Bohn, *Langmuir*, vol. 14, no. 4, pp. 845-854, 1998.
- [183] C. K. Kim, G. M. Marshall, M. Martin, M. Bisson-Viens, Z. Wasilewski, F. Bensebaa and J. J. Dubowski, *Journal of Applied Physics*, vol. 106, p. 083518, 2009.
- [184] Royal Society of Chemistry, "ChemSpider," Royal Society of Chemistry, 2014. [Online]. Available: <http://www.chemspider.com/Chemical-Structure.17019.html> . [Accessed 10 April 2014].
- [185] Sigma-Aldrich Co., "Sigma-Aldrich Catalog," Sigma-Aldrich Co., 2014. [Online]. Available: www.sigmaaldrich.com. [Accessed 10 April 2014].
- [186] chemBlink, "Online database of chemicals from around the world," chemBlink, 2014. [Online]. Available: www.chemblink.com/index.htm. [Accessed 10 April 2014].