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UNIVERSITY**

**SYNTHESIS AND SELF-ASSEMBLY OF
PLASMONIC METAL NANOPARTICLES FOR
SURFACE-ENHANCED RAMAN SCATTERING SPECTROSCOPY**

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SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

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ABSTRACT

Plasmonic metal nanoparticles (MNPs) exhibit localized surface plasmon resonance, which enables surface-enhanced Raman scattering (SERS) spectroscopy and photothermal effect. SERS is a sensitive vibrational spectroscopic technique capable of providing molecular fingerprint of analytes for qualitative and quantitative studies, whereas photothermal effect allows the conversion of photons into heat. Although SERS is a powerful analytical tool, it can be limited to certain factors such as fluorescence emission, highly dilute samples, and unstable target analytes. In this thesis, we will be discussing the synthesis and self-assembly of MNPs to improve the applications of SERS. For synthesis, we develop a new cubic MNP, known as nanoporous gold nanoframes (NGNs), produced from a seed-mediated method. The NGN synergizes nanoscale porosity and hollow nanostructure in which lower porosity generates greater SERS capability. We also demonstrate the multifunctionality of NGN, which is able to combine photothermal effect and SERS for in-situ heating and SERS analysis. We successfully apply this for SERS monitoring of bovine serum albumin's denaturation. For self-assembly, we demonstrate the fabrication of a superhydrophobic NIR-SERS platform (S-nIR-NGN) from electrostatic self-assembly of NGNs, in which an optimum static contact angle of $169 \pm 1^\circ$ is obtained. S-nIR-NGN enables the SERS detection of a fluorescent dye, Nile blue A, up to 10^{-12} M which improves the limit of detection by 50-fold compared to gold nanostars. Finally, we demonstrate the self-assembly of Ag nanocubes on liquid droplet in an organic phase for the fabrication of plasmonic liquid marbles (PLM). The merging of PLMs is performed to produce an isolated environment which protects its encapsulating analytes from degradation, enabling accurate SERS analysis. Using azo dye as a model reaction, we are able to detect bisphenol A up to 1×10^{-15} mole, improving its detection limit by 4 orders of magnitude compared to a SERS-based

aptasensor platform. From the works done, we envision the diversification of hollow MNPs, benefiting from the generic synthesis method introduced, which can lead to new discoveries of optical or physical properties for tailored applications. Additionally, the synergy of superhydrophobicity, photothermal effect, and PLM with SERS shows the importance of tailored platform for specific target analytes to refine the field of analytical chemistry.

CHAPTER 1

Introduction

“Nanotechnology” is a term coined by Professor Norio Taniguchi in 1974 to describe nanoscale semiconductor processes.¹ Today, it is used to describe the science and engineering research conducted at the nanoscale. Nanotechnology has garnered tremendous interest due to unique properties only obtainable or greatly enhanced at the nanoscale and has been studied across many scientific fields, such as chemistry,² physics,³ biology,⁴ and engineering.⁵ The importance of nanotechnology is apparent in the increase of global scientific and academic publications, up to 1500% in 15 years since the end of the 20th century and is projected to grow throughout the 21st century.⁶

A forefront of nanotechnology is nanoparticles (NPs), which are particles in the nanoscale and the bridge between bulk solids and atoms. The uniqueness of NPs arises from its very small size, leading to high surface-area-to-volume ratio, surface layer with large amount of atoms, and the capability to exhibit quantum effects. Such characteristics have made its way into valuable applications like catalysis^{7,8} and drug delivery⁹ which utilizes the high surface-area-to-volume ratio and surface modification capabilities respectively. NPs can exist in many forms such as metals,¹⁰ metal oxides,¹¹ polymers,¹² semiconductors,¹³ and carbon materials;¹⁴ and also exist in different morphologies such as cubes,¹⁵ rods,¹⁶ spheres,¹⁷ cylinders,¹⁸ and hollow-like structures.¹⁹ To determine which form and morphology of NPs to study, we need to know the function and objective we want to achieve. For example, carbon NPs are useful for energy storage and energy conversion,²⁰ and hollow-like structures are useful for transport of molecules within their core for biomedical purposes.²¹ In this thesis, we will be focusing on metal NPs (MNPs) and its optical properties.

1.1 Plasmonic Metal Nanoparticles

MNPs have been a fascination since ancient time, where they were devised and exploited for decorative purposes as seen in the famous 4th century Roman Lycurgus cup which changes color from green to red according to the direction of light shined.²² Medieval stained glasses commonly found in churches across Europe from the 10th century obtained their vibrant colors by incorporating MNPs such as Ag MNPs which produces yellow color.²³ Although MNPs were successfully utilized at that time, the understanding behind their exclusive optical properties was unknown. This drives researchers to study MNPs and scientific studies could be dated back to Michael Faraday's study on colloidal Au MNPs in 1857 which is still optically active today.²⁴ To understand the unique optical properties of MNPs we need to look into the field of plasmonics.

Plasmonics is a field that deals with the interaction of electromagnetic field and free electrons in a metal which covers the generation, transmission, manipulation, and control of this interaction. MNPs that are able to interact with an electromagnetic field are known as plasmonic MNPs and they have been a major component in the field of optics for the past decade. The emergence of extensive studies on plasmonic MNPs is contributed by three major factors, which include the development of methods to produce an array of MNPs, advancement in characterization methods, and the expansion of modeling and simulation of complex MNPs' optical responses.²⁵

The interaction of electromagnetic field and free electrons in a metal leads to coherent oscillations of conduction-band electrons, recognized as plasmons, which controls the optical properties of a metal.²⁶ Surface plasmon resonance (SPR) is the coherent oscillations of surface conduction-band electrons excited by electromagnetic radiation which occur at the interface of two opposite dielectric constant media.²⁷ SPR can be in two different forms, propagating or localized as illustrated in Figure 1.1.

Figure 1.1. Schematic diagram of (A) propagating surface plasmon resonance and (B) localized surface plasmon resonance when electromagnetic radiation interacts with metal. Propagating surface plasmon resonance involves the propagation of plasmons over a metal-dielectric interface and decays overtime. For localized surface plasmon resonance, plasmons are confined within a metal nanoparticle leading to the ability to absorb or scatter light in the nanoscale scale. Reproduced from Reference 28.

For propagating SPR (Figure 1.1A), plasmon propagates along a planar metal-dielectric interface in the x -, y -direction and gradually decay. For localized SPR (LSPR), light interacts with MNPs smaller than the incident wavelength (Figure 1.1B), leading to locally confined plasmons within the MNPs.²⁸ LSPR allows MNPs to strongly absorb or scatter light and opens the pathway to the ability of compressing light into nanoscale dimensions, resulting in enhancement of electromagnetic field locally.²⁵

Importantly, LSPR is affected by many parameters such as the size, shape, composition and dielectric environment of the MNPs.²⁹ For example, the LSPR of Au NPs undergoes red-shift when the size of the Au NPs increases due to retardation effect.^{30,31} In terms of shape, the optical responses of MNPs are uniquely dependent on it, in which anisotropic MNPs display multiple scattering peaks in the visible region but highly symmetric spherical particles usually display a single scattering peak.³² A model example is the difference in spherical Au NPs and Au nanorods, in which Au nanorods have both longitudinal and transverse plasmon modes but spherical Au NPs only have a singular dipole plasmon mode.³³ Similarly, as the aspect ratio (ratio of length to

thickness) of Au nanorods differ, i.e. their size, the LSPR extinction would also change.³⁴ In addition, single MNPs and assembly of MNPs would generate different LSPR, in which coupling of LSPR occurs when MNPs comes together.³⁵ This is shown in the assembly of Ag nanowires, where parallel and vertically stacked Ag nanowires create electromagnetic coupling at the intersections and is applied as a 3D sensing platform³⁶. Another example can be observed in the assembly of Ag octahedra, where the orientation of the assembled MNPs produced three distinct plasmonic superlattices, in which standing octahedra generates greater electromagnetic coupling compared to two more densely packed configuration.³⁷ This proves that having more MNPs is not necessarily better and structural design is significant in a microscopic structure of assembled MNPs for plasmonics. These parameters allow researchers to tune the LSPR of MNPs and apply it for specific applications, namely sensing (surface-enhanced Raman scattering spectroscopy) and photothermal heating.

1.1.1 Surface-enhanced Raman Scattering Spectroscopy

Raman spectroscopy is a qualitative vibrational spectroscopy technique which is highly sensitive and non-destructive. In 1928, Raman and Krishnan experimentally proved inelastic scattering of light, known today as Raman scattering. Raman spectroscopy utilizes the interaction of photons with a molecule. When a laser interacts with a molecule, two types of scattering take place. The first is Rayleigh (elastic) scattering, in which the photons do not shift in wavelength. The second is Raman (inelastic) scattering, where scattered photons either have a lower energy (Stokes) or higher energy (anti-Stokes). The change in wavelength of scattered light (Raman shift) is determined by the chemical composition of the interacting molecules and is very specific, allowing Raman spectroscopy to be a highly sensitive sensing technique.³⁸ In addition, Raman spectroscopy is non-destructive, fast, does not need sample preparation, and is

able to test sample in different phases; making it a valuable technique in analytical chemistry. However, Raman scattering cross sections are usually 14 orders of magnitudes smaller than that of fluorescence, resulting in significantly weaker Raman signal than fluorescence emission.³⁹ This major drawback coupled with inefficient detectors and excitation source limits the use of Raman spectroscopy for decades.

A turning point occurred in 1974 when Fleischmann et al. discovered the enhancement of pyridine's Raman signal on roughened Ag electrode and claimed it is due to large number of adsorption sites on the roughened surface.⁴⁰ In 1977, two groups, Jeanmaire et al. and Albrecht et al., studied the Raman signal enhancement independently and came up with two mechanisms, electromagnetic enhancement (EME) and chemical enhancement (CE), which laid the foundation of what we know today as surface-enhanced Raman scattering (SERS) spectroscopy.⁴¹

Figure 1.2. Schematic diagram of SERS (A) chemical enhancement and (B) electromagnetic enhancement mechanism. Reproduced (adapted) from Reference 42 with permission of The Royal Society of Chemistry. EME is dependent on the surface of MPNs and contributes greatly to SERS effect more than CE, therefore the study of MNPs' LSPR is of great interest (C) Electromagnetic field enhancement location, also known as hot-spots, of different MNPs showing the dependence of LSPR on the shape of the MNP; the Raman scattering of molecules in these hot-spots are enhanced leading to SERS effect. Reprinted (adapted) with permission from Reference 39. Copyright 2005 American Chemical Society.

CE involves the changes to molecules' electronic states attributed to chemisorptions or chemical bond formation between the molecule and metals (Figure 1.2A). A charge-transfer state is formed which increases the probability of a Raman transition by supplying a pathway for resonant excitation.³⁹ Since this mechanism relates to the interaction of molecules and the metal surface, it is site specific and analyte dependent, in which molecules need to be directly adsorbed on the metal surface to experience CE. It is considered to contribute an average enhancement factor of 10^2 .³⁹

Conversely, EME involves the enhancement of MNPs' electromagnetic field and the confinement of molecules within the enhanced electromagnetic field (hot-spots (Figure 1.2C)). As mentioned, the optical properties of MNPs are affected by different parameters, which determine the resonant frequency of its conduction electrons. When MNPs interacts with electromagnetic radiation of similar frequency, it causes the collective oscillation of conduction electrons (LSPR). When this occurs, LSPR will absorb and scatter the resonant electromagnetic radiation, and generate hot-spots. Enhancement of the local incident field and enhancement of the re-emitted Raman scattering from molecules that are in the region of these hot-spots leads to SERS effect with average enhancement factor of $\geq 10^4$ (Figure 1.2B).^{39,42,43} Therefore, EME is considered the main contributor for SERS and is related to the properties of the MNPs instead of the chemical interaction between the molecules and the metal surface. Consequently, the research field encompassing MNPs is ever expanding to optimize its LSPR for specific SERS. Regardless of mechanisms, MNPs is proven to be key for SERS and have been applied for practical uses such as detection of trace toxins^{44,45} and anti-counterfeiting security labels.⁴⁶

Additionally, besides being a qualitative analytical method, SERS spectroscopy can also be applied for quantitative analysis. This is possible when a linear relationship between Raman intensities with their corresponding concentration of target analyte is

established, usually through the plot of a semi-log⁴⁷⁻⁴⁹ or a log-log graph.^{50,51} Semi-log and log-log plots are statistical methods using logarithmic scale to visualize and represent exponential data clearer for the better understanding of the relationship between the two variables plotted.⁵² Therefore, besides being a powerful qualitative analytical tool, the viability of SERS spectroscopy for quantitative analysis further validates its importance in the field of analytical chemistry.

1.1.2 Photothermal Effect

Photothermal effect is used to describe the conversion of photons to heat. The relation of heat and metal is well known, in which metal conducts heat easily through free electrons. However, for MNPs its interaction with photons is much more complicated, enabling it to exhibit photothermal effect, highlighting another benefit of MNPs compared to its bulk material. As mentioned, MNPs exhibit LSPR when interacting with electromagnetic radiation of similar frequency leading to enhancement of electromagnetic field. Besides this, a series of nonradiative processes allows the conversion of the absorbed light into heat. Initially after laser irradiation, fast phase loss of coherently excited electrons through femtosecond electron-electron collisions produce hot electrons, with temperatures of these hot electrons up to 720 °C. This is followed by energy transfer from electrons to phonons within ~1 picosecond, in which the electron-phonon interactions produce a hot lattice with a few tens of degrees temperature increment.⁵³

Three outcomes can occur from this photothermal effect, firstly phonon-phonon relaxation within ~100 picoseconds causes the lattice to cool down and pass its heat to the surrounding medium. Secondly, the ablation of MNPs or desorption of surface-capping molecules can occur when lattice heat content is adequate. Thirdly, MNPs undergo physical changes due to sufficiently high lattice heat. Heating and cooling of the lattice are opposing processes and when the rate of heating is much more rapid than the rate of

cooling, accumulation of tremendous heat at the lattice is capable of altering MNPs structure such as melting or disintegration in nanoseconds.⁵⁴ These three outcomes determine the application of MNPs with its photothermal effect. For example, photothermal treatment with MNPs exploits the heating of its surrounding medium to kill cancer cells,⁵⁵ and laser induced thermal annealing of nanoporous Au NPs uses photothermal effect to modify the porosity of the NPs to enable the tunability of its plasmonic properties.⁵⁶

1.2 Synthesis of Metal Nanoparticles

As mentioned, the emergence of extensive studies on MNPs is contributed by development of methods to produce an array of MNPs. The synthesis of MNPs is divided into two approaches, “top-down” or “bottom-up”. The basis of top-down approach is using a bulk material to obtain MNPs through mechanical or physical process. Conversely, the basis of bottom-up approach is the formation of seeds through atomic nucleation followed by growth of the seeds to form MNPs. The general technique for top-down and bottom-up synthesis of plasmonic MNPs is lithography and wet chemical synthesis, respectively.

Lithography is a technique that builds on pattern transfer. Although there are many different types of lithography techniques, they follow a similar strategy whereby a default pattern is produced on a flat substrate’s surface followed by the transfer of the pattern to a metal film. The transfer of pattern can be either additive or subtractive. In the additive method, metals are deposited into cavities of a patterned template followed by the removal of the template. Conversely, in the subtractive method, patterned template is positioned on a bulk metal surface and unprotected metal parts are removed.⁵⁷ The oldest and most common technique of lithography is optical lithography, in which light reacts with a photoresist to produce desired patterned template.⁵⁸ However, it is limited to the

wavelength of light used, and nanoscale features are not favorable. This is overcome by electron-beam lithography which is the generally used technique for the top-down fabrication of MNPs.²⁵ It utilizes the reaction of electron-beam resist and a focused electron-beam which enables nanostructured patterns to be drawn from the resist. Once the nanostructures are drawn, metal is deposited on it and the resist is dissolved in a solvent. Metal deposited on unpatterned resist will be removed leaving the desired MNPs. Although the top-down approach manages to produce MNPs, there are major drawbacks. Firstly, the shape and size of synthesized MNPs are limited to the template, which are relatively flat. Therefore shape-controlled MNPs with distinct sharp corners are inconceivable, which is crucial in plasmonics.⁵⁹ Secondly, MNPs synthesized are generally polycrystalline which causes plasmon damping and weakens the LSPR, making it disadvantageous for sensing purposes.⁶⁰ Lastly, it is not time and cost effective because it involves two processes and unwanted metal are disposed and wasted.

Wet chemical syntheses are primarily the main method of bottom-up approach due to it being thermodynamically and kinetically dependent, enabling the control of nucleation and growth. There are three distinct stages in a wet chemical synthesis, which are nucleation, evolution of nuclei into seeds, and growth of seeds into MNPs.^{61,62} Nucleation is the first stage of any crystallization process to form nuclei which are minuscule clusters consisting of very few atoms. Wet chemical syntheses typically start with the reduction of ionic metal precursors to form atoms. Based on LaMer et al. mechanistic study,⁶³ the concentration of metal atoms rises over time until a point of supersaturation where atoms start to aggregate into nuclei. The nuclei would then grow as the concentration of metal atoms decreases and nucleation stops when the concentration of metal atoms drops below the level of minimum supersaturation. A continuous supply of atoms from the precursor allows the growth of nuclei. Structural fluctuations become energetically costly when a cluster of nuclei grow past a critical size consequently locking

them into a well-defined structure, known as seeds. The seeds will gradually grow into MNPs until an equilibrium state is achieved between the atoms in the solution and the atoms on the MNPs' surfaces.

Shape-controlled MNPs are abundantly produced as a result of development in wet chemical syntheses. Controlling the nucleation or growth of seeds enables researchers to synthesize MNPs with different shapes and sizes which is crucial for plasmonics. The control of nucleation can be done homogeneously or heterogeneously and the control of seed growth can be achieved by regulating surface energies.

Homogeneous nucleation is when the formation of nuclei to seeds to MNPs occurs in a same solution. For shape-controlled MNPs it is important to control the rate of nucleation for homogeneous nucleation because uneven growth rate will yield polydisperse MNPs. This could be done by sequential addition of metal precursors or modifying the reactivity of the metal precursors.⁶¹ Conversely, in heterogeneous nucleation, the formation of seeds is first carried out in a separate synthesis step followed by the growth of MNPs in another step. Since seeds are preformed, there are less stringent and wider ranges of growth conditions for shape controlled MNPs. Murphy et al. have shown that separating nucleation and growth by adding preformed seeds in solutions of metal precursor and its reducing agent allows the control of shape and size of the synthesized MNPs, producing Au nanorods with different aspect ratios.^{10,16}

Subsequently, surface energy is most important in determining the growth of shape-controlled MNPs and it is controlled thermodynamically or kinetically.⁶⁴ Thermodynamic control dictates the growth to form the most stable product, therefore predicted product will have the lowest total surface energy. For example, a face-centered cubic structure with facets, arranged in an energetic sequence, of $\gamma_{[111]} < \gamma_{[100]} < \gamma_{[110]}$ would grow in a way to maximize the [111] facets. Kinetic control dictates the product by the rate of growth from the primary nuclei. For example, if the growth of high energy

surfaces occurs rapidly, it could lead to the expansion of the lower energy surface through elongation, producing single crystal facets.

A common practice to produce shape-controlled MNPs is the addition of capping agents during synthesis.⁶² Capping agents primarily acts to prevent the aggregation of synthesized MNPs; however it is also able to affect the surface energy of MNPs through chemisorption, allowing the control of growth on certain facets. This is exemplified in the synthesis of polyhedral Ag nanocrystals using polyol reaction where Ag salt is reduced by a diol at near reflux temperature in the presence of a capping agent, polyvinylpyrrolidone (PVP).⁶⁵ The synthesis successfully produced well-defined Ag nanocube and upon further reaction the evolution of the cubes into truncated cubes, cuboctahedra, truncated octahedral, and octahedra took place.

Certainly, the synthesis of shape-controlled MNPs is not just governed by a single change or addition; it is a mix of different contributing factors allowing researchers to continuously discover new types of MNPs which could be classified as spherical, rod-like, 2D polygonal, 3D polyhedral, branched, complex, and hollow structures.⁶⁶

1.2.1 Hollow Metal Nanoparticles

Among the many classes of MNPs, hollow nanostructures are particularly interesting because they have noticeable different physical and chemical properties compared solid nanostructures of similar dimensions. Firstly, hollow MNPs are nanostructures devoid of non-functional metallic cores, such as nanoframes (Figure 1.3A)⁶⁷ and nanoboxes.⁶⁸ Several methods are available to synthesize hollow MNPs, including galvanic replacement,⁶⁸⁻⁷² selective deposition,^{73,74} electrochemical deposition,⁷⁵ and thermal decomposition.⁷⁶

Figure 1.3. (A) Cubic Au nanoframes, an example of hollow MNPs, nanostructures devoid of non-functional metallic cores. Reproduced from Reference 67. (B) DDA simulation of a cubic Au nanoframe showing electromagnetic field coupling between the internal and external surface due to an empty core. Reprinted (adapted) with permission from Reference 78. Copyright 2010 American Chemical Society. (C) UV-Vis spectra of Au nanoframes showing the tunability of its LSPR from the visible to NIR region by increasing the amount of AuCl₂⁻ precursor in a galvanic replacement synthesis. (D) DDA calculate scattering spectra of Au nanoframes with different wall length (l) to wall thickness (t) ratio. Reproduced from Reference 77.

The optical properties of hollow MNPs are distinctly unique because their LSPR can be extended into the near infrared region (NIR). This is shown with cubic Au nanoframes, in which tailoring the ratio of wall thickness to wall length produces LSPR extinctions and scattering efficiency from the visible to NIR range (Figure 1.3C, D).⁷⁷ Additionally, coupling of LSPR is possible in a single cubic Au nanoframe between the internal and external surface due to an empty core (Figure 1.3B).⁷⁸ This results in a greater enhancement of local electromagnetic fields than a solid cube. Besides the unique optical properties, hollow MNPs also exhibit a larger surface area than solid MNPs, making them highly efficient catalyst.^{74,79} Last but not least, the hollow interior allows the use of hollow MNPs to transport analytes and has been shown to be effective for drug delivery.⁸⁰

1.3 Self-assembly of Metal Nanoparticles

Assembly of MNPs is an expanded field following the synthesis of MPNs, driven by the synergy of individual building blocks to form an entity with enhanced or new properties. Self-assembly of MNPs is the spontaneous organization of individual building blocks to form ordered macroscopic structures caused by external forces and/or direct interactions between MNPs.⁸¹ In plasmonics, where the shape and size of an overall nanostructure influences its optical properties and functionality, it is important to have a control over the self-assembly of MNPs to prevent randomly assembled product. In order to do so, the internal interactions and external forces responsible for self-assembly have to be modulated. This has been successfully achieved either directly through interactions of MNPs or indirectly through templates or external field.⁸²

One of the strategies for self-assembly is the balance of attractive forces and repulsive forces of MNPs in solutions.⁸³ Examples of the attractive forces are covalent or hydrogen bonding, dipole-dipole interactions, electrostatic attraction between oppositely charged ligands, van der Waals forces and depletion forces; examples of the repulsive forces are steric forces and electrostatic repulsion between similarly charged ligands. An example which exploits the site-specific interactions of chemically heterogeneous MNPs is the assembly of Au nanorods into metal-polymers; which could be assembled side-by-side or end-to-end depending on the distinct ligands on the short and long facets of the nanorods.⁸⁴ The Au nanorods carried cetyl trimethyl ammonium bromide (CTAB) on the long side and polystyrene at the ends. When a poor polystyrene solvent was added to the solution of nanorods, end-to-end assembly was produced; whereas side-by-side assembly was produced by the addition of poor CTAB solvent.

Another strategy of self-assembly is the use of templates to organize the MNPs, and a broad range of materials such as carbon nanotubes, proteins, block-copolymer, or even viruses could be used as templates.⁸² The interaction between MNPs and the

template causes the MNPs to be arranged in structures defined by the shape of the template. There are two types of templates, which are hard templates and soft templates. Hard templates lack control of the deposited MNPs spacing but provide well-defined shapes whereas soft templates, particularly soft biological templates, provide the hierarchical structure of organized MNPs by exploiting their well-defined binding sites and their natural actions.⁸³ An example of hard template self-assembly is the deposition of Au nanorods on the surface of carbon nanotubes.⁸⁵ The Au nanorods were functionalized with negatively charged PVP whereas the carbon nanotubes were coated with positively charged poly(diallyldimethyl) ammonium chloride resulting in end-to-end organization of the MNPs on the surface of the coated carbon nanotubes due to the lower surface potential of the nanorod ends. On the other hand, an example of soft template self-assembly is the use of tobacco mosaic virus to organize MNPs on its inner and outer surfaces.⁸⁶ At an acidic pH, AuCl_4^- and PtCl_6^- were electrostatically driven to the outer, positively charged, surface of the virus and at neutral pH the Ag^+ ions were driven to the inner, negatively charged, surface. The metallization was achieved by controlling the surface charges of the virus.

Lastly, self-assembly could be assisted by external forces such as light and electrical or magnetic field; which could increase speed, precision, and the ability to manipulate the assembly of MNPs.⁸³ An example is the formation of superparamagnetic maghemite ($\gamma\text{-Fe}_2\text{O}_3$) nanocubes into 1D assemblies (chain) or 3D superlattices when placed in a magnetic field.⁸⁷ Electric fields, which include alternating current (a.c.) and direct current (d.c.), induce polarization of MNPs, resulting in dipole-dipole interactions between adjacent MNPs forming chains aligned parallel to the electric field lines; the chain length grows with the electric field strength, MNPs concentration, and dielectric permittivity of the media. For example, under the action of d.c. field, CdS was organized into centimeter-long chains and when the d.c. field was removed, the chains retained but

mild shaking breaks the arrangement and converts the chain into randomly aligned configuration. Alternatively, the switching of the d.c. field direction causes the chains to relax to randomly oriented cluster followed by reordering into opposite direction of the original direction, forming chains again.⁸⁸ On the other hand, a.c. field allows the assembly of MNPs without the interference of electrolysis or eletro-osmosis and the interaction of a.c. field with MNPs is commonly referred as dielectrophoresis.⁸⁹ The assembly of Au nanowires occurred spanning from one end of an electrode to another when a.c. field was applied in a Au NP suspension and the assembly of different types of MNPs was also possible. Last but not least, light-assisted assembly depends on irradiation-induced changes of photoactive ligands, such as molecule with azobenzene functional group which undergo trans and cis isomerization under UV and visible light respectively. UV radiation caused trans-cis isomerization of azobenzene dithiol ligand, inducing molecular dipole on the azobenzene unit and the dipolar interactions between the ligand containing MNPs result in ordered 3D arrays assembly.⁹⁰

The above-mentioned are fundamental strategies for self-assembly of MNPs and a few examples of their implementations. It is important to determine the desired outcome of a self-assembly process to choose a suitable strategy. For SERS, the assembly of MNPs is generally carried out to create a sensing platform with greater enhancement capabilities and reproducibility. When MNPs are assembled, the coupling of LSPR between adjacent MNPs happen, which generate greater hot-spots.⁹¹ This is exemplified in the fabrication of large dense SERS-active Au nanoprism monolayer films.⁹² Similar to the effect of a MNP's size and shape on its optical properties, the form of assembled MNPs would also affect the LSPR coupling. It has been shown that the orientation of Ag nanocubes⁹³ and Ag octahedra^{94,95} in two-dimensional metasurfaces generate different hot-spots for the optimization of surface-enhanced Raman scattering. Besides generating more hot-spots, assembly of MNPs could lead to new mechanical properties for extended

applications. Two particular properties are superhydrophobicity and the ability to trap a liquid in what is called a liquid marble.

1.3.1 Superhydrophobic Plasmonic Platform

Superhydrophobicity is one of many wetting states in which the contact angle of a water droplet with a platform is more than 150° .⁹⁶ Superhydrophobicity can be observed in nature such as a lotus leaf giving them self-cleaning capabilities, and even in living organisms like the legs of a water strider allowing it to rest on water surfaces.⁹⁷ Microscopic images of these organisms show microstructures arranged in an orderly and dense manner, producing a microscale rough surface. This inspiration from nature leads to engineering of superhydrophobic platforms with many applications such as membranes for distillation⁹⁸ and self-cleaning surfaces.⁹⁹ To produce a superhydrophobic surface, two important factors have to be considered which are structure and chemical composition. For structure, according to Young's equation which describes the interaction of gas, liquid, and solid surface tensions, to obtain a high contact angle of water, a platform has to have a low surface tension.¹⁰⁰ However, the equation is only applicable for a smooth surface. In 1936, an additional study was done to relate surface roughness with Young's equation in which an increase in surface roughness enhances the anti-wetting capability of a platform.¹⁰¹ This study leads to one of two superhydrophobic states, known as Wenzel state. Another wetting state was studied in 1944, known as Cassie and Baxter state,¹⁰² which also describes the interaction of water with a rough surface. The difference between both states is, for Wenzel state, water droplet permeates into the gaps of the rough structure while for Cassie and Baxter state, water droplet sits on top of the rough structure with sustained air gaps. This leads to a difference in contact angle hysteresis.¹⁰³ Contact angle hysteresis (CAH) is defined as the angle in which a water droplet slips off a surface and can be determined by subtracting the advancing contact angle and receding

contact angle. Since water permeates into gaps for Wenzel state, it will be pinned down and does not slip off easily, leading to high CAH of $>90^\circ$. Conversely, for Cassie and Baxter state, water sits on top of the rough surface and air gaps are present making it easy to slip off; leading to a very low CAH of $<10^\circ$.¹⁰⁴ Although rough surfaces have been proven to provide anti-wetting capabilities, they typically only exhibit hydrophobic properties, which is when the contact angle merely exceeds 90° . Enhancing a hydrophobic platform to a superhydrophobic platform can be achieved by modifying its surface chemistry with hydrophobic molecules.⁹⁷ Therefore, to produce a superhydrophobic platform with MNPs, we need to assemble them in a way that produces microscopic roughness followed by functionalization with hydrophobic ligands. Additionally, depending on desired functionality, a superhydrophobic surface could be either one of the two mentioned states. For example, a self-cleaning surface would be required to follow Cassie and Baxter state⁹⁹ or a surface could be a Wenzel state for the ease of in-situ detection.¹⁰⁵

Figure 1.4. (A) A water droplet on superhydrophobic platform dries overtime, showing the shrinkage of the water droplet into a small spot. Reproduced (adapted) from Reference 106 with permission of The Royal Society of Chemistry. (B-C) A $1\mu\text{L}$ water droplet on a superhydrophobic platform and its subsequent drying spot compared to a (D-E) hydrophilic platform shows the analyte concentrating effect. Reprinted (adapted) with permission from Reference 44. Copyright 2014 American Chemical Society.

For SERS, superhydrophobicity has been incorporated by exploiting an outcome called analyte concentrating effect (Figure 1.4).¹⁰⁶ When an analyte containing water droplet is dropped onto a hydrophilic platform, it would spread out and after it dries it would leave a large area of analytes. However if a similar water droplet is dropped onto a superhydrophobic platform, the high contact angle would lead to minimum contact between the water droplet and the platform. As the water dries, analytes will be concentrated into a small spot, resulting in the analyte concentrating effect. This is particularly useful for low concentration analytical solution because instead of spreading the scarce analytes over a large area, the analytes are brought together into a small concentrated spot. Therefore, by assembling a superhydrophobic SERS platform, analyte concentrating effect allows ultratrace analysis. This is shown in the assembly of Ag nanocubes^{107,108} and Ag nanowire⁴⁴ into superhydrophobic platform with the ability to detect trace analytes up to 10^{-16} M.

1.3.2 Plasmonic Liquid Marble

Similar to a superhydrophobic surface, liquid marble takes inspiration from nature. Aphids manage waste through coating of their liquid excretion with a layer of wax, forming a spherical structure which will be rolled off from their nest. This is crucial for the survival of young or immobile aphids that could drown and die in the sticky waste.¹⁰⁹

Liquid marble is a term used to describe a droplet of liquid encapsulated with micro- or nano-scaled solids, coined and pioneered by Quéré et al. in 2001 (Figure 1.5A).¹¹⁰ In that work, a small amount of liquid was rolled on a very hydrophobic powder bed, in which the grains instinctively coat the droplet. Such a simple experiment yields an astonishing outcome, which is the seclusion of the water droplet from its surrounding. This leads to the ability to contain and transport a liquid droplet and draws similarity to a

superhydrophobic platform. From this simple and thoughtful discovery, in merely one and a half decade, liquid marble has been studied intensively and has found its way into various applications such as a detector of surface water pollution¹¹¹ and microorganism cultivator.¹¹² Ultimately, liquid marble has been commonly used as miniaturized micro-reactors due to its small dimension, the use of chemical reagents and solvents are reduced, reaction conditions can be accurately controlled, and reaction time is shortened.¹¹³

It may seem intuitive that a hydrophobic solid repels water and is not compatible with one another. However, through theoretical calculations, replacing part of a droplet's liquid-air interface with a solid-liquid interface through the attachment of a particle lowers the surface free energy, which is energetically favored (Figure 1.5B).¹¹⁴ Therefore, formation of liquid marble is not limited to hydrophobic solids, allowing the use of other functional solids. Examples of solids used to produce liquid marbles are magnetic/magnetizable particle and shaped particle.¹¹⁵ The type of particle used as the shell of a liquid marble will determine its functionality, such as the use of magnetic particles allow the control of a liquid marbles's movement in a magnetic field¹¹⁶ or the use of silver nanowires allow a liquid marble to be a catalytic reactor.¹¹⁷

Figure 1.5. (A) Schematic of the formation of liquid marble. (B) Surface free energy is reduced when a solid particle attach to a water droplet, replacing part of the droplet's liquid-air interface with a solid-liquid interface. Reproduced (adapted) from Reference

114 with permission of The Royal Society of Chemistry. (C) Schematic and picture of a plasmonic liquid marble produced with Ag nanocubes. (D) Application of the Ag nanocube plasmonic liquid marble for multiplex sensing of coumarin and methylene blue. Reproduced from Reference 118.

Plasmonic liquid marble (PLM) uses MNPs as encapsulating solid, therefore allowing the detection of analytes in the water droplet with SERS (Figure 1.5C).¹¹⁸ Conventional SERS platform involves the assembly of MNPs on a substrate, which typically requires multiple fabrication steps. For example, Ag NPs were incorporated with a graphene foam to produce a SERS substrate for the detection of methylene blue.¹¹⁹ PLM negates this by its simple assembly of adding a drop of sample solution on a MNP powder bed, producing a substrate-less sensing platform. Additionally, the formation of a uniform core-shell causes the aggregation of MNPs on the surface of the liquid droplet, producing more hot-spots for greater LSPR scattering. Besides the simplicity of fabrication, submerging a PLM into a different phase solution allows multiplex sensing, as shown in the detection of coumarin (in the outer hexane solution of PLM) and methylene blue (in encapsulated water of PLM) of a Ag nanocube PLM (Figure 1.5D).¹¹⁸ Last but not least, the isolation of a solution from external interferences coupled with the ease of manipulation makes PLM a suitable microreactor for in-situ mechanistic studies. This is shown in the mechanism and kinetic study of aryl-diazonium surface grafting reaction with a Ag nanocube PLM.¹²⁰

1.4 Outlook & Motivation

MNPs have proven to play a crucial role in the field of analytical chemistry by enhancing Raman spectroscopy sensitivity, making an exceptional sensing technique even better through the use of plasmonics. As discussed, this enhancement is known as SERS. A subgroup to SERS is NIR-SERS, which uses laser wavelength in the NIR region to

excite plasmonic MNPs. NIR-SERS is useful for preventing common sources of SERS signal loss such as plasmonic heating and photobleaching, in addition to a beneficial decrease in fluorescence.¹²¹ To utilize NIR-SERS, plasmonic MNPs need to exhibit LSPR in the NIR region. This is achievable by hollow MNPs, which have also proven useful in the field of catalysis and biomedicine. Therefore, it is a motivation to contribute to the field of nanomaterials by developing synthesis method for new novel hollow MNPs. In addition to sensing, plasmonics generates potential photothermal effect allowing localized heating. Therefore we are motivated to study the photothermal capabilities of our newly synthesized hollow MNPs and its potential application.

In addition to synthesis, self-assembly enables researches to tune the LSPR of MNPs through plasmonic coupling. Besides that, the synergy from bringing together individual building blocks to form ordered macroscopic structure can produce new physical properties, such as the discussed superhydrophobicity and PLM. Therefore, it is a motivation to use these mechanical properties to assist sensing of analytes. We notice that current superhydrophobic SERS platform do not exploit the NIR laser wavelength. This gives us the motivation to fabricate a superhydrophobic NIR-SERS platform and this may be achieved by utilizing hollow MNPs since their skeletal shape imparts surface roughness which is important for superhydrophobicity. Conversely, one of the unique properties of liquid marble is its ability to merge with one another to form a larger liquid marble. Although studies have been carried out on PLM, only singular PLM are used. This gives us the motivation to study the feasibility of PLMs merging and by having two separate encapsulated reactants, we anticipate the protection of products as soon as it is formed. This will be beneficial for products that are sensitive to external interferences, such as dyes that undergo photobleaching when exposed to light, since the MNPs forms a protective core shell in PLMs.

1.5 References

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CHAPTER 2

Nanoporous Gold Nanoframes with Minimalistic Architectures: Lower Porosity Generates Stronger Surface-Enhanced Raman Scattering Capabilities*

Abstract

Current synthesis of gold nanoframes has only demonstrated morphological control over wall thickness and wall length. Here, we demonstrate the ability to control the nanoscale porosity of these nanoframes using a templated seed-mediated approach. The porosity on these nanoporous gold nanoframes (NGN) is tuned by controlling the crystallite size of Au nanoparticles deposited on the AgCl templates. The yield of the NGNs approaches 100 %. Despite its minimalist architectural construction, the NGNs are mechanically robust, retaining its morphology even after multiple centrifugation and sonication rounds. We further highlight that decreasing porosity on the NGN leads to improved surface-enhanced Raman scattering (SERS) enhancement. Increasing the constituent Au crystallite size decreases the porosity, but increases the surface roughness of NGN, hence leading to greater SERS enhancement. The introduction of porosity in a gold nanoframe structure through our synthesis method is novel and generic, suggesting the extendibility of our method to other types of templates.

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2.1 Introduction

Gold (Au) nanoframes are skeletal nanostructures devoid of non-functional metallic cores. Their minimalist architecture provides exclusive optical,¹⁻³ catalytic,⁴ and electrical⁵ properties distinct from solid nanostructures of similar dimensions. In particular, the hollow cores of Au nanoframes provide additional internal surfaces to promote coupling between the internal and external surface plasmon resonances. The resulting increase in local electromagnetic fields in turn enhances the surface-enhanced Raman scattering (SERS) capabilities of such nanoframes.^{3,5,6} Additionally, the associated LSPRs of Au nanoframes are in the near-infrared region,^{3,7} ideal for the trace detection of molecules with reduced or low fluorescence background.^{8,9} Current synthetic methods to synthesize Au nanoframes include galvanic replacement,^{3,7,10-12} selective deposition,^{4,13} electrochemical deposition,¹⁴ and thermal decomposition.¹⁵ Even though these studies have demonstrated morphological control over frame wall thickness and wall length, the synthesis of Au nanoframes with different porosity has not yet been demonstrated.

Nanoscale porosity arises from crystallites interconnecting to form nano-sized ligaments with pores between them, where porosity is affected by the packing density of different crystallite sizes.^{16,17} It is a distinctive form of surface structure, where the ligaments are comprised of numerous kinks and step edges.¹⁸ The presence of such ligaments imparts unique optical,¹⁹ sensing,^{20,21} and catalytic properties^{22,23} to the nanoporous Au structures in comparison to their non-porous counterparts. For instance, nanoporous Au nanoparticles exhibit enhanced surface plasmon resonance tunability compared with a smooth-surfaced Au nanoparticle.¹⁹ In particular, two-dimensional nanoporous Au thin film supports localized surface plasmon resonance (LSPR).²⁴ The enhanced localized electromagnetic fields associated with the interconnected ligaments combined with the large surface area makes nanoporous Au structures excellent SERS platforms.^{20,25,26} The SERS capability of nanoporous Au structures is dependent on

geometric parameters such as ligament size, pore size, and surface roughness, making it a highly active, tunable SERS platform worth studying.^{27,28} Thus, tunable porosity is crucial to enable the tuning of LSPRs and electromagnetic field of nanoporous Au structures. In addition, nanoscale porosity enhances the permeability for even more efficient capturing of analyte molecules.

Here, we introduce a simple and high-yield seed-mediated method to synthesize cubic nanoporous Au nanoframes (NGNs) with tunable wall porosity for SERS sensing. We aim to introduce nanoscale porosity to further increase surface roughness and bring about higher hot spot density for greater SERS capability. Tunable porosity is imparted by the control over the Au crystallite sizes deposited during synthesis. Our NGNs are mechanically robust, retaining their structural integrity even after repeated sonications and various morphological, surface, and chemical characterizations. Characterization of our NGN's morphology at different stages of the reactions is conducted to provide insights on the mechanism of the NGN formation. We systematically investigate the effect of the porosity on the SERS capability of the NGNs, elucidating the effect of Au crystallite size and surface roughness on the SERS behaviors. Notably, SERS signals from the NGNs are nearly 9000-fold stronger than typical SERS substrates such as Au nanorods of equal size.

2.2 Experimental Section

2.2.1 Materials

Silver nitrate (99+ %, AgNO₃), gold (III) chloride trihydrate ($\geq 99.9\%$), ethylene glycol anhydrous (99.8%, EG), hydroquinone (98%), polyvinylpyrrolidone (PVP, $M_w = 1,300,000$) were purchased from Sigma-Aldrich; ammonium hydroxide (25% in water) was purchased from Sinopharm Chemical Reagent Co., Ltd. Ethanol (absolute) was purchased from Fisher Scientific. Acetone was purchased from P.P. Chemical. Hydrochloric acid (37%, HCl) was purchased from Schedelco. All chemicals were used without further purification. Milli-Q water ($> 18.0 \text{ M}\Omega\cdot\text{cm}$) was purified with Sartorius Arium 611 UV ultrapure water system.

2.2.2 Synthesis of silver chloride (AgCl) nanocubes. The synthesis method was adapted from Woong Kim *et al.*²⁹ 0.4 g of PVP was dissolved in 30 mL of EG and 0.4 g of AgNO₃ was dissolved separately in 20 mL of EG. The dissolved PVP and AgNO₃ were added into a 100 mL round bottom flask and mixed well at 1500 rpm. 1.23 mL of concentrated HCl (37%) was added to the solution dropwise. The mixture was then heated at 150 °C for 20 minutes. The final product was milky white in color and the mixture was then left to cool to room temperature before purification. The AgCl nanocubes were purified by washing with acetone once and ultrapure water twice using centrifugation. The purified AgCl nanocubes were stored away from light source and the weight/volume ratio was determined.

2.2.3 Determination of the AgCl nanocubes weight/volume ratio. To determine the weight/volume ratio of the purified AgCl nanocubes, 3 empty Eppendorf vials were weighed. 1 mL of AgCl nanocubes was pipetted into the empty Eppendorf vials, and were centrifuged at 10,000 rpm/10 minutes. The supernatant was removed and the residue was further dried by placing the vials in a drying oven. The Eppendorf vials with the dried samples were weighed once again and the weight/volume ratio was determined by

subtracting the weight of the Eppendorf vials with samples, with the empty Eppendorf vials; and by taking the average out of the 3 different vials.

2.2.4 Synthesis of NGNs. PVP was dissolved in ultrapure water to prepare a PVP solution with concentration of 7 mg/mL. Aqueous HAuCl_4 and hydroquinone solutions were separately prepared with concentrations of 40.4 mM and 28.2 mM respectively. 4.5 mL of PVP solution was pipetted into a 20 mL glass vial and stirred at 650 rpm. Variable amounts of AgCl stock solution (x , given in μL) were added into the PVP solution. Different amount of hydroquinone (y , given in mL) was then added, during which the solution changed from white to faint red. Finally, HAuCl_4 (z , given in μL) was added into the solution. The solution was continuously stirred for 1 minute at room temperature to complete the process. For the NGNs synthesized in Figure 2.1, the amount of AgCl used was 33 μL (84 mM), using 55 μL of HAuCl_4 (40.4 mM), and 130 μL , 110 μL , 100 μL of hydroquinone (28.2 mM) for NGN1, NGN2, NGN3 respectively. This was followed by the etching off of the AgCl nanocube template to produce the gold nanostructure.

To etch away the AgCl nanocube template, ammonium hydroxide was added in excess (around 1 mL of concentrated solution per reaction) and the solution was sonicated and vortexed. The etched Au nanostructures were purified via consecutive centrifugation at 500 rpm with water and ethanol.

2.2.5 Surface-enhanced Raman Scattering (SERS) Measurement. SERS spectra and xy hyperspectral imaging were obtained using Ramantouch microspectrometer (Nanophoton, Osaka, Japan). NGNs were drop-cast on patterned Si substrates, which were then immersed in 10 mM of 4-MBT overnight before measuring SERS. The substrates were rinsed with ethanol to remove excess 4-MBT prior to SERS measurements. An excitation laser wavelength of 785 nm was used, with an excitation power of 0.05 mW at an exposure time of 10 seconds/line with a 100 $\times$ / 0.90 NA objective lens. 10 particles were measured for each NGN and only particles which are

approx. 500 nm in wall length and 150 nm in wall thickness were analysed by indexing the Si substrates. Normal Raman spectra of 4-MBT was obtained using 0.5 M 4-MBT solution with a 100× / 0.90 NA objective lens using the same hyperspectral imaging mode with an exposure time of 3600 s/line.

2.2.6 Characterization. NGNs dispersed in ethanol were drop-cast on a clean silicon substrate and dried with nitrogen prior to scanning electron microscopy (SEM) characterization using JEOL JSM 7600F SEM operating at an accelerating voltage 5 kV in the back-scattered mode. 100 NGNs from the SEM images were taken into consideration for the wall length and wall thickness distribution. Energy-dispersive X-ray spectroscopy (EDS) was carried out with JEOL JSM 7600F SEM at 30 kV. X-ray photoelectron spectroscopy XPS spectra were measured using a Phoibos 100 spectrometer with a monochromatic Mg X-ray radiation source. XPS Peak 4.1 (freeware available at <http://www.phy.cuhk.edu.hk/~surface>) was used to fit all the XPS spectra. High-resolution transmission electron microscopy (HR-TEM) images were acquired using a JOEL-JEM-2100 electron microscope at an accelerating voltage of 200 kV. 50 crystallites from HR-TEM images were taken into consideration for the measurement of Au crystallite sizes of the NGNs. UV-Vis measurements were obtained with Agilent Technologies Cary 60 spectrophotometer. Electrochemical active surface area (ECSA) analysis was carried out with electrochemical gold oxide stripping cyclic voltammetry, reaction in 0.5 M H₂SO₄ aqueous solution at a scan rate of 5 mVs⁻¹ at room temperature.

2.3 Results and Discussion:

2.3.1 Synthesis of NGN

NGNs are synthesized using a seed-mediated approach, starting with the selective deposition of Au on silver chloride (AgCl) sacrificial templates, followed by template removal (Figure 2.1A). Cubic AgCl templates with average wall length of 425 ± 96 nm are synthesized by ion-exchanging silver nitrate with hydrochloric acid in ethylene glycol, using polyvinylpyrrolidone (PVP) as capping agent (Figure 2.1B).²⁹ HAuCl₄ is then added to the purified AgCl nanocube solution together with hydroquinone as reducing agent in the presence of PVP. The dispersion changes from white (Figure 2.1E) to grayish (Figure 2.1F), and the SEM image indicates that Au nanoparticles are selectively deposited on the edges and corners of the AgCl templates (Figure 2.1C). This selective Au deposition is attributed to the higher reactivity of the edges and vertices, brought about by the lower coordination number of the surface atoms along these regions.³⁰ Etching off the AgCl nanocube templates using concentrated ammonium hydroxide (NH₄OH) gives rise to a bluish-gray dispersion (Figure 2.1D, G). SEM characterization shows the ligaments of the NGN consist of Au crystallites integrating along the edges and corners of the etched-off AgCl template, exhibiting undulating roughness to form the NGNs. The NGNs have rigid, cubic framework, with edge-to-edge wall lengths of 464 ± 93 nm and wall thicknesses of 128 ± 13 nm. The longer edge-to-edge wall length of NGN than AgCl templates indicates that the growth of Au nanoparticles occurs on top of the sacrificial templates.

Figure 2.1. (A) Schematic and (B, C, D) SEM images illustrating the formation of NGN. (B) (inset: size distribution of AgCl template) AgCl nanocube templates are selectively deposited with reduced Au^{3+} (Au^0) on the (C) edges and corners, followed by etching off of the AgCl nanocube templates to yield the desired (D) porous nanoframes. (E-G) Corresponding changes in solution colour at each stage of the reaction (B-D).

Next, we demonstrate the ability to manipulate the porosity of the NGNs by systematically controlling the sizes of the Au crystallites formed on the NGNs. It has been shown that surface texture and particle sizes can be tuned by controlling the amount of precursor added during synthesis.³¹ Thus, by tuning the molar ratio of hydroquinone to HAuCl_4 during the synthesis from 1.65, 1.40, to 1.27, three types of NGNs are obtained, with the constituent Au crystallite sizes ranging from 7 ± 2 nm, 22 ± 4 nm, to 30 ± 5 nm, respectively (Figures 2.2A-C, 7ii). These NGNs are correspondingly denoted as NGN1, NGN2, and NGN3.

Figure 2.2. (i-ii) SEM images of the respective NGN; (iii) color of the NGNs solution. The amount of precursors used for all samples are similar except for the amount of hydroquinone (HQ) added, with the molar ratio of HQ:HAuCl₄ being (A) 1.65, (B) 1.40, and (C) 1.27.

The overall wall lengths for NGN1, NGN2, and NGN3 are in the range of 400 - 600 nm (Figure 2.3), indicating a similar growth of Au crystallites on top of the AgCl templates.

Figure 2.3. The wall length and wall thickness of the NGNs are 464 ± 93 nm and 128 ± 13 nm, 530 ± 99 nm and 125 ± 10 nm, and 489 ± 90 nm and 123 ± 14 nm for (A) NGN1, (B) NGN2, and (C) NGN3 respectively.

Notably, the yields of the synthesized NGNs are nearly 100 % with relatively good monodispersity (Figure 2.4). The color of their respective solutions changes from bluish-gray to reddish-blue with the increase in Au crystallite size (Figure 2.2iii and side-by-side comparison in Figure 2.5). In addition, the NGNs are mechanically robust as they do not break apart after sonication and throughout the characterizations performed. This makes the NGNs useful for practical applications such as molecular detection in unknown solutions without the worry of them losing their structural integrity.

Figure 2.4. (A-C) SEM images of NGN1, NGN2, and NGN3, respectively, indicating yield of close to 100%.

Figure 2.5. (A-C) Solution colour of NGN1, NGN2, and NGN3, respectively. As the pore size increases from left to right, the colour changes from bluish-grey to reddish-blue.

At lower molar ratio (<1.2), incomplete and broken NGNs are formed due to lesser reduced Au^+ that are not able to connect well (Figure 2.6A). Conversely, nanoboxes are formed at higher molar ratios (> 1.65) due to the excess reducing agent present in the reaction which can also reduce Ag^+ (Figure 2.6B).

Figure 2.6. Control experiment to determine the effect of the molar ratio of hydroquinone to HAuCl_4 in which at lower molar ratio of 1.0 (A) the reducing agent is not enough to reduce the Au^+ causing the growth of incomplete NGN as observed with strands of Au nanoparticles and incomplete NGN; and at higher molar ratio of 2.0 (B) the excess amount of reducing agent reacts with the Ag^+ from the AgCl template leading to the formation of nanoboxes instead of NGN.

2.3.2 Characterizations of NGN

We perform high-resolution transmission electron microscopy (HR-TEM) characterization to gain insight on the surface structure and atomic arrangements of the NGNs (Figure 2.7).

Figure 2.7. (i) TEM images of individual (A) NGN1, (B) NGN2, and (C) NGN3 which clearly exhibit the porosity and high polycrystallinity of the NGNs. (ii) Zoom-in images for the estimated calculation of the crystallite size of the Au particles. The crystallite sizes for NGN1, NGN2, and NGN3 7 ± 2 nm, 22 ± 4 nm, to 30 ± 5 nm respectively. The increase in crystallite size in the order of NGN3 > NGN2 > NGN1 is similar with the calculated grain size using Scherrer equation from the XRD analysis. (iii) HR-TEM of the NGNs shows distinct lattice fringes. The d -spacing is measured to be $2.4 \text{ \AA}$, corresponding to Au (111) crystal.

The frame nature of our nanoparticles and the various roughness (hence the porosity) of the frame structures are clearly distinguished in the TEM images (Figure 2.7i). The NGN

ligaments consist of interconnecting Au crystallites along the edges and vertices of the cubic template which lead to pores being formed in the interstitial region. Under similar frame dimensions, increasing the size of interconnecting Au crystallites in order of NGN1 < NGN2 < NGN3 decreases the packing density of the Au particles which would affect the porosity of the NGN (Figure 2.7ii). The crystallinity of the Au particles on the NGNs are also evident, where characteristic lattice fringes of face-centered cubic (fcc) Au (111) facets with d-spacing of 2.4 Å are observed (Figure 2.7iii).³²

The crystallinity and grain sizes of the NGNs are also studied using X-ray diffraction (XRD, Figure 2.8). The XRD spectra of NGN1, NGN2, and NGN3 are similar, showing five diffraction peaks at 38.3°, 44.5°, 64.8°, 77.7°, and 81.8° in the 2θ range of 30-85° (Figure 2.8A).

Figure 2.8. (A) XRD spectra for NGN1, NGN2, and NGN3; (B) Full-width half maximum (FWHM) at 38.3° diffraction peak and calculated crystallite size using Scherrer equation.

These peaks correspond to the fcc phase of metallic Au (JCPDS, card No. 04-0784), and can be indexed to the (111), (200), (220), (311), and (222) reflections, respectively.²² The diffraction peak at 38.3°, which corresponds to the (111) plane, is the most intense for all three NGNs due to large exposed area of the (111) facet. This observation corroborates well with the HR-TEM characterization (Figure 2.7iii). In addition, the diffraction peaks for NGN1 are broader than those of NGN2 and NGN3, arising from the much smaller crystallite size of NGN1. The peak broadening also implies lattice defects.³³ Using the Scherrer equation and the full-width half maximum (FWHM) of the diffraction peak at 38.3° to determine the crystallite size (as shown below), we find the crystallite sizes for NGN1, NGN2, and NGN3 increase from 4.7 nm, to 10.1 nm, and 13.0 nm, respectively (Figure 2.8B).

Calculation of crystallite grain size

Scherrer equation:

$$\text{Crystallite grain size} = \frac{K\lambda}{\beta \cos\theta}$$

K = shape factor (0.94)

λ = X-ray wavelength (1.5406 Å = 0.15406 nm)

β = Full-width half maximum (FWHM)

θ = Bragg angle ($2\theta = 38.3$; $\theta = 19.15$)

For NGN1

FWHM = 1.88° = 0.03288 radian

$$\begin{aligned}\text{Crystallite grain size} &= (0.94 \times 0.15406) \div (0.03288 \times \cos 19.15) \\ &= 4.7 \text{ nm}\end{aligned}$$

For NGN2

$$\text{FWHM} = 0.87^\circ = 0.01513 \text{ radian}$$

$$\begin{aligned} \text{Crystallite grain size} &= (0.94 \times 0.15406) \div (0.01513 \times \cos 19.15) \\ &= 10.1 \text{ nm} \end{aligned}$$

For NGN3

$$\text{FWHM} = 0.68^\circ = 0.01178 \text{ radian}$$

$$\begin{aligned} \text{Crystallite grain size} &= (0.94 \times 0.15406) \div (0.01178 \times \cos 19.15) \\ &= 13.0 \text{ nm} \end{aligned}$$

This slight difference between the crystallite size measured from HR-TEM and XRD likely arises from the fact that the measured HR-TEM particle size could be an overlap of crystallites, thus giving a larger value. Nonetheless, both characterization techniques point to an increasing Au crystallite size and reduced crystal lattice imperfection from NGN1 to NGN3. These would certainly affect plasmonic scattering and SERS capabilities of the NGNs.

The combination of various characterization techniques to elucidate the crystallite sizes in the three samples allows us to understand the effect of hydroquinone in tuning the porosity of the various NGNs. Larger amounts of hydroquinone accelerates the reduction rate of Au^{3+} to Au^0 , forming smaller Au particle islands with higher packing density along the edges and vertices of the AgCl templates. This results in NGNs with higher surface area as shown by electrochemically active surface area (ESCA) analysis (Figure 2.9A). ECSA is performed using electrochemical Au oxide stripping cyclic voltammetry, where the Au oxide reduction curve area at 0.9 V are compared among different NGNs. Based on the specific charge of $450 \mu\text{Ccm}^{-2}$ for Au oxide reduction,²² the mass-normalized

surface areas of NGN1, NGN2, and NGN3 are estimated at 35.3, 10.2, and 3.4 m²g⁻¹, respectively (Figure 2.9B). Conversely, as the amount of hydroquinone decreases, the rate of Au³⁺ reduction is slower. Larger Au particles are formed with lower packing density, eventually leading to lower surface area. Since pores are formed at the interstitial region of the interconnected Au crystallites, the surface area of the NGNs reflects the porosity of the NGN, in which larger surface area indicates greater porosity. Thus, the porosity of the NGNs increases in the order of NGN1 > NGN2 > NGN3. The decrease in porosity coincides with the increase in Au crystallite size which leads to profound effect on the NGNs' SERS capabilities.

Figure 2.9. (A) Cyclic voltammogram of NGN1, NGN2, and NGN3. (B) Calculated electrochemically active surface area (ECSA) of the NGNs showing the decrease of surface area as the porosity increases from NGN1 to NGN2 to NGN3.

We further characterize the surface elemental composition of the NGNs using X-ray photoelectron spectroscopy (XPS, Figure 2.10). The high-resolution Au 4f scan shows

two peaks separated by 3.7 eV at 87.0 eV and 83.3 eV, corresponding well to metallic Au (Figure 2.10A).³⁴ In addition, metallic Ag is also present, where Ag 3d scan shows a primary doublet at 373 and 367 eV with an intensity ratio of 2:3. This is attributed to the spin-orbital splitting of Ag 3d_{3/2} and Ag 3d_{5/2} respectively.²² A much smaller doublet at 373 eV and 368 eV is also observed in the Ag 3d scan, arising from the trace amount of AgCl leftover from the AgCl nanocube templates.³²

Figure 2.10. (A, B) XPS spectra of the NGNs in region of Au 4f and Ag 3d, respectively. (C-E) shows the EDS mapping of the NGN1, NGN2, and NGN3 respectively, with SEM images of the particle being mapped, individual mapping of Au & Ag elements and the combine mapping of both the elements.

Energy-dispersive X-ray spectroscopy (EDS) mapping is also carried out to determine the elemental distribution of Au and Ag in the NGNs (Figure 2.10C-E). The elemental maps show that Au is homogeneously distributed throughout the NGN surfaces with trace amount of Ag present. The atomic percentage of Au/Ag for NGN1, NGN2, NGN3 is 77/23, 84/16, 92/8 respectively, confirming that the NGNs are predominantly Au (Figure 2.10C, D, E). The presence and difference of Ag atomic percentage can be explained by the additional amount of hydroquinone added during synthesis, which can be used to reduce both Au^{3+} and Ag^+ to their respective metallic states upon the completion of the Au reduction reaction. Under our experimental conditions, Au^{3+} is preferentially reduced due to its higher reduction potential (1.50 V vs. SHE) than Ag^+ (0.22 V vs. SHE). A larger amount of hydroquinone is used in the synthesis of NGN1, and this implies that the additional reducing agent can also reduce Ag^+ from the AgCl template after the reduction of Au^{3+} is complete. This leads to a higher relative atomic percentage of Ag^0 in the system, whereas NGN3 has the lowest amount of Ag.

The extinction spectra of the three NGNs exhibit a general broad peak across the visible to the near-IR region (Figure 2.11), consistent with the unique characteristic of Au nanoframes.^{3,7}

Figure 2.11. UV-Vis spectra of the NGNs with the vertical dashed line representing the excitation wavelength at 785 nm. They all exhibit broad peak across the visible to the

near-IR region which is consistent with the unique characteristic of GNFs whereby their LSPR are extended to the near-IR region. Dark field images shows the change in colour from reddish to orangey as the porosity changes.

In addition, the darkfield scattering images show NGNs' scattering color changing from dark red (NGN1) to bright reddish-orange (NGN3) with decreasing porosity under similar illumination intensity (Figure 2.11). This suggests an increase in scattering capability from NGN1 to NGN3 in relation to the increase of the collective constituent Au nanoparticle size, as it has been reported that larger Au nanoparticle size has higher scattering efficiency.³⁵ The localized surface plasmon resonances of Au nanoframes are usually tuned by controlling the ratio of wall thickness to wall length.^{1,3,7} Here, we demonstrate that nanoscale porosity can also be used to tune the scattering behaviors of plasmonic NGNs without changing the wall length and wall thickness.

2.3.3 NGN Surface-enhance Raman Scattering capability

The effect of porosity on the resulting SERS capability of our NGNs is investigated using 4-methylbenzenethiol (4-MBT) as the probe molecule at an excitation wavelength of 785 nm. Using 4-MBT allows us to more accurately quantify the subsequent SERS enhancement factor due to its ability to form a self-assembled monolayer on the Au surfaces. 4-MBT exhibits two major characteristic peaks at 1080 cm^{-1} and 1578 cm^{-1} (Figure 2.12G). The peak at 1578 cm^{-1} is assigned to a phenyl stretching motion and the peak at 1080 cm^{-1} is due to the combination of phenyl ring-breathing mode, CH in-plane bending, and CS stretching.³⁶ The average intensity of 10 individual particles is taken into account to determine the SERS capability of the NGNs (Figure 2.12). The SERS map generated using the 1080 cm^{-1} vibrational mode is overlaid with the SEM image of the same particles to directly elucidate the SERS behaviors of the NGNs (Figure 2.12A-F). The NGNs chosen for measurements have identical wall lengths

of ~ 500 nm and wall thicknesses ~ 150 nm and are well spaced apart. This enables us to investigate the effects of porosity on SERS efficiency and to ensure a fair comparison between all the NGNs. The difference in porosity significantly impacts the resulting SERS intensity of the NGNs. Even though the overall dimensions are similar, NGN3 exhibits the highest SERS signal at 257 ± 63 counts, followed by NGN2 (154 ± 36 counts), and NGN1 (30 ± 16 counts).

Figure 2.12. Overlaid SERS and SEM images, and SEM images of single (A,B) NGN1, (C,D) NGN2, (E,F) NGN3. The colour scheme is assigned by the relative intensity at 1080 cm^{-1} due to phenyl stretching motion and combination of phenyl ring-breathing mode, CH in-plane bending of 4-MBT. (G) Single particle SERS spectra of 4-MBT, 10 single particles were taken into consideration for each spectrum. (H) SERS enhancement factor of the NGNs.

In geometry, a cube consist of 12 edges, therefore a NGN can be compared to 12 Au nanorods arranged in a cubic structure. Using Au nanorods (average width and length of ~ 100 nm and 150 nm, respectively) with a similar LSPR peak with the NGNs as a comparison, the SERS intensity of NGN1, NGN2, and NGN3 are stronger than the Au nanorods by 1054-fold, 5500-fold, and 9179-fold, respectively for the detection of 4-

MBT (Figure 2.13). The NGNs outperform the Au nanorod due to its rougher surface which increases hot spots for SERS detection.³⁷

Figure 2.13. (A) UV-Vis spectrum of Au nanorods (AuNRs) used for comparison with the vertical dashed line showing a similar peak with the corresponding UV-Vis spectra of NGNs. (B) Overlaid SERS and SEM images of gold nanorods with excitation laser wavelength of 785 nm and an excitation power of 0.05 mW, similar to NGNs, but with an exposure time of 6 minutes/line, 36 times longer than the exposure time used for the NGNs. (C) Zoom-in SEM image of the red border area shows 4 single gold nanorods, which shows no SERS enhancement, with one gold nanorod trimer showing SERS enhancement. (D-E) Zoom-in SEM image of the green border area shows a single gold nanorod with its SERS spectrum. Assuming the value of the gold nanorods spectra as 1 counts, the counts normalized to the exposure time of NGNs (10 seconds/line) would be 1/36 counts, which is 0.028 counts. Thus NGN1 (30 ± 16 counts), NGN2 (154 ± 36 counts), and NGN3 (257 ± 63 counts) are better than gold nanorods for SERS detection of 4-MBT by 1054-fold, 5500-fold, and 9179-fold respectively.

The SERS enhancement factor based on the 1080 cm^{-1} vibrational mode is determined to be 1.0×10^3 , 1.8×10^4 , and 8.9×10^4 for NGN1, NGN2, and NGN3, respectively (detailed calculations shown below). The SERS enhancement factor

increases with increase in crystallite size, even after adjusting for the difference in surface area (and hence number of probe molecules present) for the different NGNs.

Figure 2.14. Estimation of the dimension of a NGN using TEM image for the calculation of enhancement factor. The darker outline is assumed to be the overlay of the wall thickness.

Figure 2.15. (A) Cross section dimension of the NGNs from the TEM image. (B) A wall of the NGN which consist of 4 long walls and 8 short walls.

Calculation of enhancement factor (EF)

The enhancement factor is calculated using the equation below:

$$EF = \frac{I_{SERS}}{I_{NORMAL}} \times \frac{N_{NORMAL}}{N_{SERS}}$$

We chose the peak at 1080 cm^{-1} to calculate the enhancement factor.

$$\begin{aligned} I_{NORMAL} &= 167.2/3600 \text{ s} \\ &= 0.46 \text{ (based on } 0.5 \text{ M } 4\text{-MBT solution, } 100 \times \text{ objective lens, line scan, } \\ &\quad 3600 \text{ s/line)} \end{aligned}$$

$$\begin{aligned} N_{NORMAL} &= \text{number of molecules within the measured laser spot} \\ &= V_{\text{solution}} \times \text{concentration of molecules} \times \text{Avogadro's number} \end{aligned}$$

$$\begin{aligned} V_{\text{solution}} &= \pi \times x \times y \times z \text{ (} x=1.20/2=0.60 \mu\text{m, } y=1.18/2=0.59 \mu\text{m, } z=14.76 \mu\text{m)} \\ &= 3.142 \times 600 \text{ nm} \times 590 \text{ nm} \times 14760 \text{ nm} \\ &= 1.64 \times 10^{10} \text{ nm}^3 \end{aligned}$$

$$\begin{aligned} N_{NORMAL} &= 1.64 \times 10^{10} \text{ nm}^3 (=1.64 \times 10^{-17} \text{ m}^3) \times 0.5\text{M} (=500 \text{ mol/m}^3) \times 6.022 \times 10^{23} \\ &= 4.94 \times 10^9 \end{aligned}$$

$$\begin{aligned} N_{SERS} &= \text{number of molecules on the surface of the nanoparticles within the} \\ &\quad \text{measured laser spot} \end{aligned}$$

Laser spot diameter = 610 nm ($100 \times$ objective lens) > diameter of the nanoparticles

$$N_{SERS} = \text{surface area of single nanoparticle} \div \text{surface area of } 4\text{-MBT}$$

$$\text{Surface area of } 4\text{-MBT} = 2.3 \times 2.3 \text{ \AA}^2 / \text{molecule} = 0.0529 \text{ nm}^2/\text{molecule}$$

Assuming the Au nanoparticle seeds making up the NGN are uniform in size with simple cubic packing throughout the NGN, the amount of Au particle seed in a NGN could be calculated. Thus the volume of a NGN could be estimated through the following reaction using the dimension in Figure 2.14 and 2.15:

$$\text{Number of Au seeds in a plane } (\chi) = \left(\frac{127}{\text{Au seed diameter}}\right)^2 - \left(\frac{64}{\text{Au seed diameter}}\right)^2$$

$$\text{Number of Au seeds in 4 long walls } (\alpha) = 4 \left(\frac{500}{\text{Au seed diameter}}\right) \times \chi$$

$$\text{Number of Au seeds in 8 short walls } (\beta) = 8 \left(\frac{246}{\text{Au seed diameter}}\right) \times \chi$$

$$\text{Volume of NGN} = (\alpha + \beta) \times \text{Au seed volume}$$

By estimating the volume of a NGN we could calculate the mass of a single NGN and thus the surface area of the NGN.

The calculation carried out on NGN1, NGN2, and NGN3 using the Au seed diameter of 4.7 nm, 10.1 nm, and 13.0 nm respectively yield a similar volume of $2.5 \times 10^7 \text{ nm}^3$

Thus, the estimated mass of a NGN would be:

$$\begin{aligned} \text{Mass} &= \text{Volume} \times \text{Density} \\ &= (2.5 \times 10^{-14} \text{ cm}^3) \times (19.30 \text{ g/cm}^3) \\ &= 4.825 \times 10^{-13} \text{ g} \end{aligned}$$

Therefore, the surface area of a single nanoparticle would be the ECSA value multiplied with the estimated mass above.

For NGN1

$$\mathbf{I_{SERS}} = 30$$

$$\text{Surface area} = 35.3 \text{ m}^2\text{g}^{-1}$$

$$\begin{aligned} \text{Surface area of single nanoparticle} &= 35.3 \text{ m}^2\text{g}^{-1} \times 4.825 \times 10^{-13} \text{ g} \\ &= 1.70 \times 10^7 \text{ nm}^2 \end{aligned}$$

$$\begin{aligned} \mathbf{N_{SERS}} &= 1.70 \times 10^7 \text{ nm}^2 \div 0.0529 \text{ nm}^2/\text{molecule} \\ &= 3.22 \times 10^8 \text{ molecule} \end{aligned}$$

$$\begin{aligned} \mathbf{EF} &= [30/0.46] \times [4.94 \times 10^9 / 3.22 \times 10^8] \\ &= 1.00 \times 10^3 \end{aligned}$$

For NGN2

$$\mathbf{I_{SERS}} = 154$$

$$\text{Surface area} = 10.2 \text{ m}^2\text{g}^{-1}$$

$$\begin{aligned}\text{Surface area of single nanoparticle} &= 10.2 \text{ m}^2\text{g}^{-1} \times 4.825 \times 10^{-13} \text{ g} \\ &= 4.92 \times 10^6 \text{ nm}^2\end{aligned}$$

$$\begin{aligned}\mathbf{N_{SERS}} &= 4.92 \times 10^6 \text{ nm}^2 \div 0.0529 \text{ nm}^2/\text{molecule} \\ &= 9.30 \times 10^7 \text{ molecule}\end{aligned}$$

$$\begin{aligned}\mathbf{EF} &= [154/0.46] \times [4.94 \times 10^9/9.30 \times 10^7] \\ &= 1.78 \times 10^4\end{aligned}$$

For NGN3

$$\mathbf{I_{SERS}} = 257$$

$$\text{Surface area} = 3.4 \text{ m}^2\text{g}^{-1}$$

$$\begin{aligned}\text{Surface area of single nanoparticle} &= 3.4 \text{ m}^2\text{g}^{-1} \times 4.825 \times 10^{-13} \text{ g} \\ &= 1.64 \times 10^6 \text{ nm}^2\end{aligned}$$

$$\begin{aligned}\mathbf{N_{SERS}} &= 1.64 \times 10^6 \text{ nm}^2 \div 0.0529 \text{ nm}^2/\text{molecule} \\ &= 3.10 \times 10^7 \text{ molecule}\end{aligned}$$

$$\begin{aligned}\mathbf{EF} &= [257/0.46] \times [4.94 \times 10^9/3.10 \times 10^7] \\ &= 8.90 \times 10^4\end{aligned}$$

We ascribe the increase in SERS efficiency from NGN1 to NGN3 to the increase in the electromagnetic field as the Au crystallite increase in size.³⁷ This is because the intensity of electromagnetic field produced upon excitation with incident photons is highly dependent of the amount of electrons excited, and such, a higher volume generates a greater electromagnetic field.³⁸ In addition, increase of the constituting Au crystallite size leads to the increase of surface roughness.³⁹ This is evident in the SEM and HR-TEM

images (Figures 2.2 and 2.7), in which the surface roughness becomes more prominent from NGN1 to NGN3 with the increase of the Au crystallite size constituting the NGNs. Findings on nanoporous Au and surface roughness shows that rough surfaces on nanoporous Au gives rise to greater SERS enhancement.^{25,40} Consequently, the presence of larger Au crystallite and high surface roughness in NGN3 implies that the SERS signals are more effectively scattered as compared to the other two NGNs.

2.4 Conclusions

In conclusion, we have developed a seed-mediated approach to synthesize mechanically robust nanoporous Au nanoframes in high yields (nearly 100%) through the selective deposition of Au on a cubic template. The Au crystallite size of the NGNs can be easily controlled by the amount of hydroquinone added, thus affecting their porosity. Our NGNs shows admirable SERS capability, with the NGN3 as the best SERS substrate due to its large Au crystallite size and surface roughness. To our knowledge, this is the first reported synthetic method to produce porous nanostructure of such shape (frame), which offers itself as another interesting prospect in the field of material science, particularly molecular sensing. In addition, our synthesis strategy suggest the possibility of being applied to other template shapes, as long as there is a difference in relative surface energy, to obtain different shaped hollow and porous nanostructures. Through this work, we have introduced a method to synthesize porous hollow nanostructures which can be adopted to produce nanostructures with enhanced properties, primarily in the field of SERS as demonstrated and potentially in the field of catalysis due to their superior surface area and network that aids in the mass transfer of reactants and increase in catalytic active sites.

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CHAPTER 3

Electrostatic Self-assembly of Nanoporous Gold Nanoframes for the Production of Superhydrophobic Near Infrared Surface-Enhanced Raman Scattering Platform

Abstract

In surface-enhanced Raman scattering (SERS) spectroscopy, fluorescence emission has been known to suppress vibrational signals, causing a significant problem in detecting fluorescent analytes. Additionally, highly dilute analytical samples inhibit SERS analysis due to sparse analytes, producing weak or indistinguishable Raman peaks. These can be overcome by near infrared (NIR) laser excitation source which prevents fluorescence of fluorophores with excitation wavelength in the visible to ultraviolet region and by a superhydrophobic platform which concentrates sparse analytes into a localized area. Here, we synergize NIR-SERS with superhydrophobicity through the electrostatic self-assembly of nanoporous gold nanoframes (NGN), producing a superhydrophobic NIR-SERS platform (S-nIR-NGN). We systematically study the impact of surface roughness, either from self-assembly time or coating thicknesses, on the S-nIR-NGN antiwetting properties. We demonstrate the self-assembly time of 40 minutes and ALD coating thickness of 1 nm of alumina produce the best superhydrophobic performance in which static contact angle of $169 \pm 1^\circ$ and contact angle hysteresis of $17 \pm 1^\circ$ is obtained. Finally, we apply the S-nIR-NGN for the detection of Nile blue A (NBA), a common histology stain, and successfully improve its limit of detection by 50-fold compared to gold nanostars while suppressing fluorescence emission. The ability of our S-nIR-NGN to suppress fluorescence emission and detect highly dilute analytes proves to be applicable and beneficial to the field of analytical chemistry.

3.1 Introduction

Fluorescence emission is a possible outcome when photon interacts with a molecule which can suppress Raman signal by producing a broad background. Therefore, analytes that fluoresce strongly such as large biological molecules¹ and dyes² are a challenge for surface-enhanced Raman scattering (SERS) spectroscopy. Near infrared SERS (NIR-SERS), in which laser wavelength in the NIR region is used as the excitation source, can circumvent fluorescence emission.³ This is because most fluorophores have an excitation wavelength in the visible and ultraviolet (UV) region,^{4,5} therefore NIR laser wavelength can prevent light absorption, leading to no fluorescence emission. Additionally, NIR-SERS can prevent the loss of SERS signal from photobleaching which can be caused by visible region laser. It has been successfully applied for the detection of Eosin Y, an important historical dye and is determined to be a suitable detection method for art applications.⁶

Besides fluorescence emission, detection of highly dilute analytical samples can be challenging, especially when spread over a hydrophilic SERS platform. This can be overcome by the fabrication of superhydrophobic SERS platform.⁷⁻⁹ Superhydrophobicity can be observed in nature such as a rose petal or lotus leaf¹⁰ and has found its way into chemical sensing due to a phenomenon known as “analyte concentrating effect”.¹¹ It refers to the concentration of analytes into a small spot after the drying of an aqueous droplet due to minimal contact between the droplet with the superhydrophobic surface. The analyte concentrating effect allows the detection of trace analytes, such as the SERS detection of adenine up to 3.7×10^{-14} M,¹² because it prevents random dispersion and it concentrates to a definitive localized spot. However, current superhydrophobic SERS platform is observed to use laser excitation wavelength in the visible region, therefore the detection of trace analytes that fluoresce can be a challenge.

Here, we fabricate a superhydrophobic NIR-SERS platform using our previously synthesized nanoporous gold nanoframes (NGN) as the building block. NGN is chosen because it is a hollow nanostructure with distinguishable roughness throughout the surface which is an important parameter to obtain superhydrophobicity. Additionally, the LSPR of NGN is in the NIR region which is crucial for the application of NIR-SERS, because it enables the interaction of electromagnetic field and free electrons when a NIR laser source is used. We use electrostatic self-assembly to fabricate our superhydrophobic NIR-SERS (S-nIR-NGN) platform and systematically study the effect of self-assembly time on surface roughness and superhydrophobicity. We also study the effect of coating thicknesses on the anti-wetting properties of the platform and chose the best parameter, determined from static contact angle, contact angle hysteresis, and analyte concentration effect, to produce the most efficient superhydrophobic surface. Finally, we apply the superhydrophobic NIR-SERS platform for the detection of Nile blue A (NBA), a common histology stain for biological imaging which fluoresces under visible excitation wavelength, to demonstrate the capability of our platform in suppressing fluorescence background and detecting trace analytes.

3.2 Experimental section

3.2.1 Materials

Silver nitrate (99+ %, AgNO_3), gold (III) chloride trihydrate ($\geq 99.9\%$), ethylene glycol anhydrous (99.8%, EG), hydroquinone (98%), polyvinylpyrrolidone (PVP, $M_w = 1,300,000$), 11-mercaptoundecanoic acid (95%), 3-aminopropyltriethoxysilane (99%), 1H,1H,2H,2H-perfluorodecanethiol (97%), 1H,1H,2H,2H-perfluorooctyltriethoxysilane (98%), trimethylaluminum (electronic grade, $\geq 99.9999\%$) were purchased from Sigma-Aldrich; ammonium hydroxide (25% in water) was purchased from Sinopharm Chemical Reagent Co., Ltd. Ethanol (absolute) and methanol (absolute) were purchased from Fisher Scientific. Acetone was purchased from P.P. Chemical. Hydrochloric acid (37%, HCl) was purchased from Schedelco. All chemicals were used without further purification. Milli-Q water ($> 18.0 \text{ M}\Omega\cdot\text{cm}$) was purified with Sartorius Arium 611 UV ultrapure water system.

3.2.2 Synthesis of NGN. NGN was synthesized using a method previously developed by our group.¹³ Briefly, sacrificial AgCl nanocube template was first synthesized through ion exchange of AgNO_3 and HCl in the presence of PVP as the capping agent.¹⁴ Addition of aqueous HAuCl_4 and hydroquinone into the AgCl nanocube solution resulted in the selective deposition of gold on the corners and edges of the cubic template. Etching off the AgCl template with NH_4OH produced the NGN. In this study, we chose the least porous NGN, synthesized with the molar ratio of hydroquinone to HAuCl_4 of 1.27, because it produced the roughest surface with strongest SERS capability.

3.2.3 Fabrication of S-nIR-NGN platform. 20 μL of 11-mercaptoundecanoic acid dissolved in ethanol (1 mM) was added into 5 mL of NGN (0.5 mg/mL in 1:5 ratio of hexane:ethanol solution). The functionalization was left overnight and functionalized NGN was redispersed in ethanol to remove excess ligands. To prevent aggregation of NGN, the purification process was carried out by letting the NGN precipitate from a

solution over time followed by the removal of the liquid phase, instead of using centrifugal force. $1 \times 1 \text{ cm}^2$ of oxygen plasma-treated silicon substrate was submerged in an anhydrous toluene solution with 2% v/v of 3-aminopropyltriethoxysilane for 60 minutes and rinsed with anhydrous toluene, methanol, and water accordingly to remove unfunctionalized ligands and deprotonate the substrate surface. Electrostatic self-assembly was performed by submerging the positively-charged silicon substrate into 2 mL of negatively-charged NGN solution and leaving it for a pre-determined amount of time. The silicon substrate containing the self-assembled NGN was then removed, rinsed with ethanol, and blow dried with N_2 gas twice. Removal of charged ligands on the NGN was performed to ensure exclusion of false Raman shifts by placing the silicon substrate with self-assembled NGN in a solution of 1H,1H,2H,2H-perfluorodecanethiol (5 mM). The self-assembled NGN was then stabilized by atomic layer deposition (ALD) of alumina in which the coating thicknesses was varied from 0.5 nm to 5 nm. Finally, the alumina coated self-assembled NGN was placed in a vacuum chamber with 20 μL of stock 1H,1H,2H,2H-perfluorooctyltriethoxysilane to impart chemical-induced hydrophobicity, producing the S-nIR-NGN platform.

3.2.4 SERS Measurement. SERS spectra and x-y hyperspectral imaging were obtained using Ramantouch microspectrometer (Nanophoton, Osaka, Japan). 2 μL of analyte solution were used throughout the SERS study, in which it was drop-casted and left to dry at ambient condition on the S-nIR-NGN platform. An excitation laser wavelength of 785 nm was used, with an excitation power of 0.4 mW at an exposure time of 60 seconds/line with a 20 $\times$ / 0.45A objective lens, for all sample analysis including normal Raman and control studies. 10 populations, approximately $1.0 \mu\text{m}^2$, were taken into account for each analysis.

3.2.5 Characterization. Imaging of the S-nIR-NGN platform was performed by scanning electron microscopy (SEM) characterization using JEOL 7600F JSM operating at an

accelerating voltage of 5kV in the back-scattered mode. Energy-dispersive X-ray spectroscopy (EDS) was performed with JEOL 7600F JSM at 30 kV. Surface roughness was analyzed by atomic force microscopy (AFM) using Bruker Dimension ICON with NanoScope V controller from Bruker. Silicon probes (Tap300Al-G with 30-nm aluminium reflex coating) from BudgetSensor was used for a non-contact mode analysis. Contact angle analyses were performed with Theta Lite tensiometer equipped with a Firewire digital camera; static contact angle measurements were carried out with a 4 μ L sessile water droplet. Advancing and receding contact angles for contact angle hysteresis were obtained using drop shape analysis routine of a growing and shrinking drop of water, respectively. Each contact angle analysis was performed at least five times at different spots across every substrate. UV-Vis measurement was obtained with Agilent Technologies Cary 60 spectrophotometer.

3.3 Results and discussions

3.3.1 Fabrication of S-nIR-NGN platform

We fabricate the S-nIR-NGN platform using electrostatic self-assembly of NGN (Figure 3.1). Firstly, we functionalize NGN and silicon substrate with oppositely charged ligands, 11-mercaptopundecanoic acid and 3-aminopropyltriethoxysilane respectively. The positively-charged silicon substrate is then immersed face up in the negatively-charged NGN solution for a certain amount of time. Electrostatic forces drive the NGN to self-assemble onto the silicon substrate and by controlling the immersion time, we can control the amount of assembled NGN (Figure 3.2). Following the electrostatic self-assembly of NGN, we perform functionalization with 1H,1H,2H,2H-perfluorodecanethiol to remove the charged ligands. We choose 1H,1H,2H,2H-perfluorodecanethiol because it has been shown to display a featureless SERS spectrum.⁷ We then perform atomic layer deposition (ALD) of alumina for stability by preventing the redispersion of assembled NGN while maintaining their pores through conformal coating. Lastly, we functionalize the assembled NGN with 1H,1H,2H,2H-perfluorooctyltriethoxysilane to impart chemical-induced hydrophobicity, producing the S-nIR-NGN platform.

Figure 3.1. Schematic of the fabrication of S-nIR-NGN platform. Using electrostatic guided self- assembly of NGNs, we are able to obtain uniform monolayer of assembled NGN in which its rough surface coupled with chemical-induced hydrophobicity produces a superhydrophobic NIR-SERS platform. Digital image shows the uniformity of our assembled NGN in which it exhibits a reddish blue color.

3.3.2 Surface roughness of S-nIR-NGN and its antiwetting capabilities

We choose NGN as the building block because of its hollow and rough surface morphology which is crucial for achieving superhydrophobicity, where rougher surfaces produces higher static contact angle with liquid droplets.¹⁵ As the immersion times increases from 10 to 80 minutes, more NGN is assembled on the silicon substrate and after 40 minutes it reaches maximum packing density (Figure 3.2). From a $5 \times 5 \mu\text{m}^2$ area, and taking the average of 50 areas, the amount of assembled NGN increases from 10 ± 2 to 15 ± 1 to 18 ± 1 and finally 20 ± 2 with the increase in immersion time from 10 to 40 minutes respectively. Assembled NGN from 10 to 40 minutes are correspondingly denoted as S-nIR-NGN10, S-nIR-NGN20, S-nIR-NGN30, and S-nIR-NGN40.

Figure 3.2. (A-H) SEM images of assembled NGN from 10-80 minutes respectively with time interval of 10 minutes. Low magnification images shows the large uniform monolayer of NGN assembled through electrostatic self-assembly. (I) Amount of NGN in a $5 \times 5 \mu\text{m}^2$ area showing the maximum amount obtained at 40 minutes, in which 50 areas were taken into account for statistical calculations.

We carry out a control experiment to prove the importance of electrostatic attraction, in which an unfunctionalized silicon substrate is immersed in a functionalized NGN solution. This results in inhomogeneous assembly of NGN (Figure 3.3), showing the crucial role of charged driven self-assembly.

Figure 3.3. (A-C) SEM images of different area on an assembled NGN platform with unfunctionalized Si substrate produced non-uniform assembly. (D) Image of the Si substrate shows inhomogeneous assembly. This shows the importance of electrostatic guided assembly to produce homogeneous assembly of NGN.

We perform atomic-force microscopy (AFM) analysis on assembled NGN to quantify the root-mean-square (rms) roughness to determine its surface asperities (Figure 3.4E). Initial cross-sectional analysis (Figure 3.4F), performed by plotting a line through as many NGNs possible within 10 μm , shows the decrease in exposed substrate with the increase in amount of NGNs from S-nIR-NGN10 to S-nIR-NGN40. Following rms roughness analysis shows the increase in average roughness from S-nIR-NGN10 (169 ± 38 nm) to S-nIR-NGN20 (184 ± 44 nm) to S-nIR-NGN30 (235 ± 16 nm) and S-nIR-NGN40 (275 ± 5 nm) (Figure 3.4G). The increase in surface roughness can be related to the increase in assembled NGN with the increase in immersion time, which also leads to the decrease in empty gaps as seen in SEM images in Figure 3.4A-D. The decrease in standard deviations from S-nIR-NGN10 to S-nIR-NGN40 further prove the decrease in empty gaps that are being filled up with NGN, producing a more uniform assembled NGN platform.

Figure 3.4. (A-D) SEM images of self-assembled NGN at different immersion time. (E) AFM images with subsequent (F) cross-sectional analysis of corresponding immersion time. (G) Resulting average root-mean-square roughness obtained from AFM, static contact angle, and contact angle hysteresis shows the increase in surface roughness produces better S-nIR-NGN platform.

Antiwetting properties of the S-nIR-NGNs coated with 1 nm of alumina and functionalized with 1H,1H,2H,2H-perfluorooctyltriethoxysilane are determined with 2 μ L of water droplet. S-nIR-NGN10 with the lowest surface roughness exhibit hydrophobic properties ($90^\circ < \theta < 150^\circ$)¹⁶ with the static contact angle of $136 \pm 1^\circ$ (Figure 3.4G). Conversely, S-nIR-NGN20 to S-nIR-NGN40 exhibit superhydrophobic properties ($\theta > 150^\circ$)¹⁶ with increasing static contact angles from $159 \pm 2^\circ$ to $169 \pm 1^\circ$ respectively. This corroborate well with the increase of surface roughness from S-nIR-NGN10 to S-nIR-NGN40, in which increase in surface roughness enhances hydrophobic capabilities.¹⁷

We also measure contact angle hysteresis, the difference in advancing and receding contact angles, which is crucial to understand the water adhesion behavior on our assembled NGN. Water adhesion behavior directly affects the analyte concentrating effect which consequently affects SERS measurement. S-nIR-NGN10 demonstrates lotus-like characteristics (contact angle hysteresis $> 90^\circ$)¹⁸ indicating high liquid-solid adhesion (Figure 3.5E). From S-nIR-NGN20 to S-nIR-NGN40, intermediate adhesion characteristics between lotus-like and rose-like (contact angle hysteresis $< 10^\circ$)¹⁸ are exhibited. Additionally, we observe a decrease in contact angle hysteresis from S-nIR-NGN20 to S-nIR-NGN40 with S-nIR-NGN40 almost conforming to a rose-like surface, indicating the decrease in liquid-solid adhesion as the immersion time increases. We attribute the decrease in contact angle hysteresis to the increase in number of assembled NGN, in which more air pockets are present, preventing the water droplet to permeate and adhere on the substrate as opposed to less assembled NGN with more empty spaces.

Therefore, S-nIR-NGN40 which exhibit greater contact angle and smaller contact angle hysteresis than S-nIR-NGN10 to S-nIR-NGN30 is chosen to be used as our superhydrophobic NIR-SERS platform.

3.3.3 Coating thickness of S-nIR-NGN40 and its antiwetting capabilities

Redispersion of assembled NGN when in contact with water is a recurring problem first noticeable in our groups previous work of assembling silver nanocube into a superhydrophobic platform.⁷ We use the similar method of stabilizing the metal nanoparticles through thermal evaporation of a silver film. However, it is unable to prevent redispersion (Figure 3.5A) due to lesser contact area of the NGN with the silicon substrate caused by its hollow core, not allowing the silver film to form uniformly. In addition, the silver film covers up the pores of NGN (Figure 3.5B) which will affect its SERS scattering capabilities in addition to decrease surface roughness, making it unsuitable to stabilize the assembled NGN.

Figure 3.5. (A-B) Redispersion of assembled NGN after water contact is not prevented from thermal deposition coating of 2nm Cr and 50 nm Ag, as seen by the dark spots on the SEM image where NGNs were initially at but no longer present. Additionally, the pores of NGN are covered up, making it an unsuitable method to stabilize the assembled NGN. (C-D) Atomic layer deposition of 0.5 nm alumina is able to prevent the redispersion of the assembled NGN, as no dark spots are present to show the lost of NGN. The darker region in the image is a mark left by an evaporated water droplet to prove the

image taken is where the water interacted with the assembled NGN. Furthermore, the pores of NGN are retained, making it a suitable method to stabilize the assembled NGN.

Using ALD of alumina, we can prevent the redispersion of the assembled NGN while maintaining the pores through conformal coating (Figure 3.5C, D). Coating thickness down to 0.5 nm is shown to sufficiently provide stability making ALD a suitable method to stabilize the assembled NGN. In addition to the assembly of NGN, ALD coating thicknesses produce different antiwetting capabilities. ALD coating thicknesses of 0.5 nm, 1 nm, 2 nm, and 5 nm are coated on S-nIR-NGN40, and are denoted as S-nIR-NGN40-0.5, S-nIR-NGN40-1, S-nIR-NGN40-2, and S-nIR-NGN40-5 respectively (Figure 3.6A-D). Similarly, we perform AFM analysis to determine the rms roughness of the ALD coated NGN (Figure 3.6E). As the coating thickness increases from 0 nm to 5 nm, we found the rms roughness to decrease from 26 ± 3 nm to 23 ± 2 nm to 21 ± 1 nm to 16 ± 3 nm and 13 ± 2 nm respectively (Figure 3.6G). This is attributed to the smoothening of the NGN ligaments as the coating thickness increases. Initial cross-sectional analysis (Figure 3.6F, inset) also shows the decrease in peak valleys and smoothening of peaks as the coating thickness increases, further proving the decrease in surface roughness.

Figure 3.6. (A-D) SEM images of NGN coated with alumina thickness of 0.5 nm, 1 nm, 2 nm, and 3 nm respectively. Porosity of the NGN is still visible and not covered up as the coating thicknesses increase, which is crucial for SERS analysis. Inset SEM images shows the NGN tilted, in which it retains the empty core from its frame-like nanostructure. Therefore, the current coating thicknesses are suitable to stabilize the assembled NGN (E) AFM images with subsequent (F) cross-sectional analysis of corresponding coating thickness. (G) Resulting average root-mean-square roughness obtained from AFM, static contact angle, and contact angle hysteresis shows the increase in superhydrophobicity from 0.5 nm to 1 nm coating followed by the decrease in superhydrophobicity from 1 nm to 2 nm and 5 nm coating. The decrease in superhydrophobicity is attributed by the decrease in surface roughness whereas the

increase in superhydrophobicity is attributed to a more uniform coating allowing efficient functionalization.

Contact angle analysis with a 2 μ L water droplet shows an increase of contact angle from S-nIR-NGN40-0.5 ($160 \pm 1^\circ$) to S-nIR-NGN40-1 ($169 \pm 1^\circ$), followed by a gradual decrease from S-nIR-NGN40-1 to S-nIR-NGN40-2 ($166 \pm 1^\circ$) and S-nIR-NGN40-5 ($163 \pm 1^\circ$) (Figure 3.6G). The decrease in contact angle from S-nIR-NGN40-1 to S-nIR-NGN40-5 corroborates well with the general decrease in surface roughness. However, this is counterintuitive for S-nIR-NGN40-0.5 which has the roughest surface but exhibit the smallest contact angle.

Energy-dispersive X-ray spectroscopy (EDS) analysis explains this observation (Figure 3.7). Outline of Al mapping on S-nIR-NGN40-0.5 (Figure 3.7A) does not show clear Al within the boundary, while it becomes more apparent from S-nIR-NGN40-1 to S-nIR-NGN40-5 (Figure 3.7B-D). This implies incomplete coating of alumina for S-nIR-NGN40-0.5, leaving pinholes of Au. The atomic percentage of Au/Al for S-nIR-NGN40-0.5 further proves this by it having the highest atomic percentage of Au (80%) compared to S-nIR-NGN40-1 to S-nIR-NGN40-5 which decreases gradually. This corroborate well with a study on sub-nm ALD coating of alumina which yields pinholes at sub-nm coating thicknesses.¹⁹ Therefore, the functionalization of 1H,1H,2H,2H-perfluorooctyltriethoxysilane on S-nIR-NGN40-0.5 is not efficient to impart chemical-induced hydrophobicity, causing it to exhibit the smallest contact angle.

Figure 3.7. (A-D) EDS mapping of S-nIR-NGN with different alumina coating thickness of 0.5 nm, 1 nm, 2 nm, and 5 nm respectively. Hand drawn outlines of NGN for Al mapping (red color map) show little visible Al for the 0.5 nm coating, but is visible for 1 nm and progressively noticeable for 2 nm and 5 nm coating. This shows the incomplete coating of NGN with 0.5 nm Alumina causing improper functionalization with 1H,1H,2H,2H-perfluorooctyltriethoxysilane which leads to poor superhydrophobicity. Conversely, the increase of Al coating from 1 nm to 2 nm decreases the surface roughness therefore, 1 nm is the optimum coating thickness.

We also investigate the contact angle hysteresis analysis and it corroborates with the contact angle and surface roughness analysis (Figure 3.6G). As the surface roughness decreases from S-nIR-NGN40-1 to S-nIR-NGN40-5, the contact angle hysteresis increases as the liquid-solid adhesion increases. Conversely, the contact angle hysteresis

is highest for S-nIR-NGN40-0.5 as explained by the improper functionalization for chemical-induced hydrophobicity. To affirm our analysis, we tested the analyte concentrating effect using silica beads as a marker.

When 2 μL of aqueous silica beads is dropped on a hydrophilic silicon substrate, it spreads out and dries with an average area of 36 mm^2 (Figure 3.8). However, when it is dropped on our superhydrophobic S-nIR-NGN platform, it is confined into a water droplet and subsequent drying spot size is shown in Figure 3.9A-D. Analyte concentrating effect is obtained by dividing the average area of drying on the hydrophilic substrate with the average area of drying on our S-nIR-NGN platform (Figure 3.9D). S-nIR-NGN40-1 exhibits the best analyte concentrating effect with 82-fold, followed by S-nIR-NGN40-2 with 55-fold and S-nIR-NGN40-0.5 and S-nIR-NGN40-5 with comparable values of 28-fold and 27-fold respectively. This corresponds well with our contact angle hysteresis analysis, in which lower liquid-solid adhesion enables the water droplet to contract to a smaller spot size as it dries.

Figure 3.8. 2 μL of SiO_2 beads dried on a hydrophilic surface with average area of 36 mm^2 .

Figure 3.9. (A-D) Analyte concentrating effect of SiO₂ beads (2μL) on S-nIR-NGN platform with 0.5 nm, 1 nm, 2 nm, and 5 nm coating thickness respectively. (E) Corresponding average area and analyte concentrating effect further proves 1 nm coating thickness produces the best S-nIR-NGN platform.

Therefore, we can conclude that S-nIR-NGN40-1 is the best S-nIR-NGN platform and we use it for further SERS study.

3.3.4 Superhydrophobic NIR-SERS platform

We choose Nile blue A (NBA) as a target molecule for SERS detection because it has an absorption peak at 600 nm which spread across much of the visible spectrum (Figure 3.10A). Therefore, using an excitation laser wavelength of 532 nm yields a SERS spectrum with high fluorescence background for the SERS detection of 10⁻⁶ M NBA (Figure 3.10B). NBA exhibits a characteristic peak at 592 cm⁻¹, assigned to a ring

deformation from incorporating nitrogen of the phenoxazine ring,^{20,21} which will be monitored across different analyte concentrations.

Figure 3.10. (A) UV-Vis spectrum of Nile Blue A (10^{-6} M). (B) SERS spectra of NB, at excitation wavelength of 532 nm and excitation power of 0.46 mW, with high fluorescence background that suppresses its characteristic Raman peak.

To show our S-nIR-NGN40-1 suppresses fluorescence background, the SERS spectra obtained and plotted in Figure 3.11, using the excitation wavelength of 785 nm, are unprocessed. Comparing the SERS spectra in Figure 3.10B and Figure 3.11, it is clear that NIR-SERS suppresses the fluorescence background, producing a flat spectrum with the distinguishable peak of 592 cm^{-1} . A control analysis on S-nIR-NGN40-1 without the target analyte yields a clean, undistinguishable SERS spectrum from 550 cm^{-1} to 1600 cm^{-1} (Figure 3.12A). Therefore, detection of trace analytes sensing is accurate without any non-analyte interferences.

Figure 3.11. (A) SERS spectra of Nile blue A (NBA) with characteristic peaks at 592 cm⁻¹ and 665 cm⁻¹ obtained from a 2μL droplet on the S-nIR-NGN platform. (B) Detection of NBA at different concentrations showing the detection limit of 10⁻¹² M. (C) SERS intensity (counts per second) of 592 cm⁻¹ for NBA concentration of 10⁻⁴ M to 10⁻¹² M plotted as semi-log graph. Linear relationship of SERS intensity and analyte concentration from 10⁻⁴ M to 10⁻¹⁰ M indicates the quantitative ability of the S-nIR-NGN platform within this range.

The average SERS intensity of 10 populations is taken into account for the detection of NBA across different concentrations. Figure 3.11B shows the magnification near the 592 cm⁻¹ characteristic peak for 10⁻⁴ to 10⁻¹⁴ M of NBA. At 10⁻⁴ M, the SERS intensity is 1658 ± 164 counts. Decreasing the concentration of NBA decreases the SERS intensity from 1241 ± 259 counts to 872 ± 311 counts to 408 ± 115 counts and finally 254 ± 67 counts for 10⁻⁶ M, 10⁻⁸ M, 10⁻¹⁰ M, and 10⁻¹² M respectively. At 10⁻¹⁴ M, the peak at

592 cm^{-1} is no longer present and the resulting SERS spectrum is featureless. Therefore the detection limit of our S-nIR-NGN platform for NBA is 10^{-12} M.

Comparing our detection limit with a study using gold nanostars, we successfully lower the detection limit of NBA by 50-fold.²² A linear SERS response is also observed as a function of NBA concentration from 10^{-4} M to 10^{-10} M when a semi-log graph is plotted (Figure 3.11C). Semi-log plot is a statistical method using logarithmic scale to visualize and represent exponential data clearer for the better understanding of the relationship between the two variables plotted.²³ For quantitative SERS study, a linear relationship obtained from the semi-log plot between SERS intensity and its corresponding analyte concentration is commonly used to prove its feasibility within the range of the linearity. For example, the use of silver nanocluster films for quantitative SERS analysis of glucose in aqueous solution,²⁴ quantitative analysis of diaquat dibromide monohydrate in water with SERS-based microdroplet sensor,²⁵ and silver nanocubes assembled on polyethylene film for quantitative study of thiram,²⁶ used the semi-log plot to prove their capabilities. Therefore, the linear relationship, with the equation $y = 2494.53 + 208.23x$ ($R^2 = 0.9990$), indicates our S-nIR-NGN platform is applicable for quantitative analysis of analytes within the concentration range of 10^{-4} M to 10^{-10} M.

We obtain the average of 10 normal Raman of NBA with the concentration of 10^{-4} M (410 ± 153 counts) (Figure 3.12B) to calculate the analytical enhancement factor (AEF) and quantify the SERS enhancement of our S-nIR-NGN platform. AEF is defined as $[(I_{\text{SERS}})/(I_{\text{Raman}})] \times [(C_{\text{Raman}})/(C_{\text{SERS}})]$, where I_{SERS} and I_{Raman} are the intensity of SERS and normal Raman at lowest concentration respectively, and C_{SERS} and C_{Raman} are the corresponding concentration used to obtained the intensity.²⁷ The AEF is therefore calculated, as shown below, to be 6.2×10^7 , demonstrating that our S-nIR-NGN platform enhances SERS signal by the factor of 10^7 compared to a hydrophilic substrate.

Figure 3.12. (A) SERS background of S-nIR-NGN platform. (B) Normal Raman of NBA (10^{-4} M). (C) SERS spectra of NBA on 30 nm gold film, which could only detect up to 10^{-4} M of NBA.

Calculation of analytical enhancement factor (AEF)

The AEF is calculated using the equation below:

$$AEF = \frac{I_{SERS}}{I_{Raman}} \times \frac{C_{Raman}}{C_{SERS}}$$

I_{SERS} = intensity of SERS at lowest concentration

I_{Raman} = normal Raman at lowest concentration respectively

C_{SERS} = corresponding concentration used to obtained I_{SERS}

C_{Raman} = corresponding concentration used to obtained I_{Raman}

For this study:

I_{SERS} = 254 counts

I_{Raman} = 410 counts

C_{SERS} = 10^{-12} M

C_{Raman} = 10^{-4} M

$$\begin{aligned} \text{Therefore, } AEF &= \frac{254}{410} \times \frac{10^{-4}}{10^{-12}} \\ &= 6.2 \times 10^7 \end{aligned}$$

Using a 30 nm Au film for comparison, the SERS detection of 10^{-4} M NBA at similar acquisition conditions as the S-nIR-NGN platform yields 440 ± 240 counts (Figure 3.12C). Decreasing the NBA concentration to 10^{-6} M produces a featureless SERS spectrum. Comparing the intensity of 10^{-4} M NBA, the S-nIR-NGN platform is stronger than the gold film by 7-fold. The inability of the gold film to detect 10^{-6} M NBA further proves that having NGN and superhydrophobicity on a substrate leads to better SERS capability.

3.4 Conclusions

We have successfully fabricated a superhydrophobic NIR-SERS platform (S-nIR-NGN) through the electrostatic self-assembly of NGN, in which studies were performed to achieve optimum superhydrophobicity. The immersion time during electrostatic self-assembly of 40 minutes and ALD coating thickness of 1 nm of alumina was found to produce the best S-nIR-NGN platform, which exhibit static contact angle of $169 \pm 1^\circ$, contact angle hysteresis of $17 \pm 1^\circ$, and a 82-fold analyte concentrating effect. The superhydrophobicity is proven functional with the SERS detection of NBA in which a detection limit of 10^{-12} M was achieved. This is an improvement by 50-fold, compared to gold nanostar. More importantly, the use of NIR laser excitation source is proven beneficial for the suppression of fluorescence background, an interference which hinders efficient SERS analysis. Therefore, the synergy of superhydrophobicity and NIR-SERS in S-nIR-NGN platform benefits the field of analysis by allowing the detection of trace analytes which fluoresce strongly, such as large biological molecules and dyes.

3.5 References

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CHAPTER 4

Multifunctional Nanoporous Gold Nanoframes: Combining Photothermal Heating and Surface-enhanced Raman Scattering (SERS) for In-situ Heating and Sensing

Abstract

Multifunctional plasmonic metal nanoparticles exhibiting combined photothermal effect and surface-enhanced Raman scattering (SERS) enable in-situ studies of heating effect on analytes. Current methods to obtain such multifunctionality are limited to multistep fabrication, whereas as-synthesized plasmonic metal nanoparticles are limited to analytes which facilitate surface-enhanced resonance Raman scattering (SERRS) and reproducibility due to random morphology of the metal nanoparticles. Here, we demonstrate the multifunctionality of nanoporous gold nanoframes (NGN), capable of photothermal effect and SERS at different laser excitation wavelengths. The NGN allows regular SERS analysis while improving reproducibility with its well defined nanostructure. We demonstrate the heating capabilities of the NGN in which water is heated to approximately 70 °C with 532 nm laser excitation wavelength. We also demonstrate a good degree of control over the temperature of our NGN in relation to time with the tunable step size of ± 10 °C. Using 785 nm laser for SERS analysis, we monitor the heating changes of bovine serum albumin, a protein which denatures at a temperature > 50 °C, demonstrating the multifunctionality of NGN. Combined photothermal effect and SERS benefit studies of temperature-sensitive analytes with increased accuracy and time efficiency due to in-situ heating which eliminates the removal of sample from the acquisition chamber.

4.1 Introduction

Multifunctional plasmonic nanoparticles exhibit more than one functionality, which enables more advanced applications from the synergy of properties. For example, the synergy of magnetism and photothermal effect in a plasmonic shell–magnetic core nanoparticle enables the isolation and photothermal destruction of cancer cells.¹ Photothermal effect, in which plasmonic metal nanoparticles convert absorbed light into heat, is an outcome of localized surface plasmon resonance (LSPR).^{2,3} Additionally, LSPR can produce electromagnetic field enhancement which is utilized in surface-enhanced Raman scattering (SERS). The ability of localized heating has primarily been applied for bionanotechnology, commonly for cancer cell therapy.⁴⁻⁷ This is achieved through the heating of plasmonic metal nanoparticles' surrounding environment which destroys target analytes. Although extensive researches have been carried out on photothermal effect for biological applications, the study of it coupled with SERS sensing is not as widespread. SERS is a highly sensitive vibrational spectroscopic technique which has been successfully applied for chemical and biological analyses such as molecular detections⁸⁻¹⁰ and bioimaging.^{11,12} Therefore, the coupling of photothermal effect with SERS can be applied for the monitoring of temperature-sensitive analytes, such as proteins¹³ and DNA.¹⁴ An example of successful coupling is the SERS monitoring of photothermal ablation of *S. aureus* and *E. coli*, bacteria that are harmful to humans, which can be useful for measuring in-situ antibacterial response during a photothermal treatment.¹⁵

Currently, the study of combined photothermal effect and SERS is not extensive. Existing methods to obtain this multifunctionality is surface modification of gold nanorods with photosensitizer¹⁶ and the formation of a hybrid nanostructure, in which gold nanoparticles are wrapped with graphene oxide.^{15,17} In both methods, the photosensitizer and graphene oxide provide the photothermal effect and gold

nanoparticles provide the SERS capability. Although functional, these strategies require multistep fabrication and a simpler method is desired. As-synthesized plasmonic metal nanoparticles to exhibit combined photothermal effect and SERS capability have been produced in the form of gold nanorods¹⁸ and “ill-defined gold nanoparticles”.¹⁹ However, the gold nanorods are limited to analytes which enables surface-enhanced resonance Raman scattering (SERRS), in which its electronic transition have to match the laser excitation wavelength, and the “ill-defined gold nanoparticles” faces the problem of reproducibility due to its random morphology. A common characteristic of these multifunctional gold nanoparticles is the use of two excitation laser wavelength for photothermal effect and SERS sensing separately. This eliminates the complication of heating while sensing with the use of a single laser wavelength, allowing controlled heating and controlled sensing.

Here, we introduce an as-synthesized multifunctional plasmonic metal nanoparticle capable of exhibiting photothermal effect and SERS sensing with different laser excitation wavelength. The plasmonic metal nanoparticle is known as nanoporous gold nanoparticles (NGN), which was introduced in our previous work. The NGN has been shown to outperform gold nanorods for SERS sensing and also enables the reproducibility of heating and SERS analysis with its well defined porous frame-like nanostructure. We demonstrate its photothermal effect when irradiated with 532 nm laser and determine its temperature profile as irradiation time increases. Switching the excitation laser wavelength to 785 nm enables SERS detection and we analyze the heating of bovine serum albumin (BSA) to demonstrate the capability of in-situ heating and analysis.

4.2 Experimental Section

4.2.1 Materials

Silver nitrate (99+ %, AgNO_3), gold (III) chloride trihydrate ($\geq 99.9\%$), ethylene glycol anhydrous (99.8%, EG), hydroquinone (98%), polyvinylpyrrolidone (PVP, $M_w = 1,300,000$), 11-mercaptoundecanoic acid (95%), 3-aminopropyltriethoxysilane (99%), 1H,1H,2H,2H-perfluorodecanethiol (97%), 1H,1H,2H,2H-perfluorooctyltriethoxysilane (98%), trimethylaluminum (electronic grade, $\geq 99.9999\%$) were purchased from Sigma-Aldrich; ammonium hydroxide (25% in water) was purchased from Sinopharm Chemical Reagent Co., Ltd. Ethanol (absolute) and methanol (absolute) were purchased from Fisher Scientific. Acetone was purchased from P.P. Chemical. Hydrochloric acid (37%, HCl) was purchased from Schedelco. Bovine serum albumin (BSA) Lyophilised pH ~ 7 was purchased from BioWest. All chemicals were used without further purification. Milli-Q water ($> 18.0 \text{ M}\Omega\cdot\text{cm}$) was purified with Sartorius Arium 611 UV ultrapure water system.

4.2.2 Synthesis and self-assembly of NGN. Synthesis of NGNs was according to the seed-mediated approach developed by our group.²⁰ Briefly, sacrificial AgCl nanocube template was synthesized through ion exchange between AgNO_3 and HCl in the presence of PVP as the capping agent.²¹ Selective deposition of gold on the corners and edges of the cubic AgCl nanocube template was achieved through the addition of aqueous HAuCl_4 and hydroquinone (molar ratio of hydroquinone to HAuCl_4 of 1.27). NGNs were finally obtained after the etching off of the AgCl template with NH_4OH .

Self-assembly of NGNs was performed according to the methods used for the fabrication of superhydrophobic NGN platform. Briefly, synthesized NGNs and silicon substrate were functionalized with 11-mercaptoundecanoic acid and 3-aminopropyltriethoxysilane respectively to induce electrostatic charges. The positively-charged silicon substrate was submerged into 2 mL of negatively-charged NGN solution for 40 minutes allowing electrostatic self-assembly. The self-assembled NGNs were then

functionalized with 1H,1H,2H,2H-perfluorodecanethiol to ensure the removal of interferences in Raman analyses followed by atomic layer deposition of 1 nm alumina to stabilize the NGNs and prevent redispersion. Finally, the self-assembled NGNs were functionalized with 1H,1H,2H,2H-perfluorooctyltriethoxysilane to impart chemical-induced hydrophobicity.

4.2.3 Determination of NGN photothermal capability and temperature profile. Water was used as a medium to determine the temperature profile of NGN. Firstly, the calibration curve of heated water was obtained by heating water to a desired temperature with a hot plate and electronic contact thermometer, followed by point mode Raman analysis with 532 nm excitation wavelength, 135 mW excitation power, and 1 second acquisition time. Ratio of Raman peak intensities at 3250 cm^{-1} (I_1) and 3410 cm^{-1} (I_2) assigned to four and three hydrogen-bonded water molecules respectively²² was plotted against the temperature it was acquired which produced the calibration curve with a linear relationship of $y = 0.95 + (-0.003x)$.

Self-assembled NGN, coated with 1 nm alumina, was immersed in water by placing it in a cylindrical container with the diameter of 2 cm and adding 500 μL of water. Using the radius of the container and volume of the water added, we approximated the water film to be 1.6 mm thick. The NGNs were then irradiated with 532 nm excitation wavelength, 135 mW excitation power, and x minute(s) acquisition time. The average of 3 spectra was obtained at each acquisition time and I_1/I_2 was plotted against the acquisition time which was then translated into temperature with the water calibration curve.

4.2.4 Determination of multifunctional capability of NGNs. Using a superhydrophobic NGN platform, we drop-casted 2 μL of 10^{-4} M of BSA and allowed it to dry. We then acquired the SERS spectrum of BSA with 785 nm excitation wavelength, 0.1 mW excitation power, and 30 second acquisition time. Without moving the superhydrophobic

NGN platform, we switched the laser excitation wavelength to 532 nm and irradiated the similar spot with 135 mW excitation power for 9 minutes. We then switched the laser excitation wavelength back to 785 nm and acquired the resulting SERS spectrum with similar excitation power and acquisition time as before.

4.2.5 Characterization. SERS spectra were obtained using Ramantouch microspectrometer (Nanophoton, Osaka, Japan) with a 20× / 0.45A objective lens. Heating and temperature monitoring were performed with IKA RCT Basic Stirrer/Hotplate and ETS-D5 Electronic Contact Thermometer respectively. Imaging of the superhydrophobic NGN platform was performed by scanning electron microscopy (SEM) characterization using JEOL 7600F JSM operating at an accelerating voltage 5kV in the back-scattered mode.

4.3 Results and Discussions

4.3.1 Photothermal Capability and Temperature Profile of NGN

NGNs are synthesized using the seed-mediated approach, in which selective deposition of Au on the corners and edges of AgCl nanocube templates followed by the etching of the template with NH_4OH produces the plasmonic metal nanoparticles.²⁰ In chapter 2, we have demonstrated the SERS capabilities of NGN with 785 nm laser excitation wavelength, in which lower porosity exhibit greater SERS performance. Additionally, UV-Vis spectroscopy analysis on the NGN demonstrated an extinction spectrum with a broad peak from 500 nm to 1000 nm (Figure 4.1). The extinction spectrum of plasmonic metal nanoparticles consist of its scattering and absorption wavelengths, therefore the utilization of 532 nm laser excitation wavelength for NGN's photothermal effect is feasible. Figure 4.1 shows the schematic of the multifunctionality of NGN, in which we are able to obtain photothermal effect and SERS sensing capabilities by employing different laser excitation wavelengths. Similarly, the self-assembly of NGNs is performed using electrostatic approach to ensure a uniform monolayer of NGN is obtained for reproducible analysis. This also gives rise to superhydrophobicity which will assist the SERS analysis because it enables us to pinpoint target analytes when a sample droplet dries due to analyte concentrating effect. Therefore the self-assembly of NGN to obtain the best static contact angle ($169 \pm 1^\circ$) and contact angle hysteresis ($17 \pm 1^\circ$) is performed.

Figure 4.1. Schematic of the multifunctionality of NGN. The extinction spectrum of NGN (pink box) shows the feasibility of using both 532 nm and 785 nm laser excitation wavelengths for different purposes. Using 785 nm laser excitation wavelength for SERS sensing, a switch of laser excitation wavelength to 532 nm enables photothermal effect. This allows simple and precise in-situ heating of analytical samples to monitor changes with SERS sensing.

We utilize Raman fingerprint of water at laser excitation of 532 nm to quantify the heating capabilities of our NGN. Water is used as the heating medium because it has high heat capacity (4.186 joule/gram °C) and a high boiling point (100 °C), therefore temperature fluctuations are minimal for accurate quantification. Importantly, the hydrogen bonds of water, which is affected by temperature, produce characteristic Raman shifts. This enables the use of SERS for temperature determination of the NGN, which

allows straightforward and uncomplicated photothermal and SERS utilization in a similar system.

Figure 4.2A shows the Raman spectra of water obtained at three different temperatures which exhibits characteristic Raman shifts at 3250 cm^{-1} (I_1) and 3410 cm^{-1} (I_2), assigned to four and two hydrogen-bonded water molecules respectively.²² As temperature increases, the kinetic energy of water increases. This leads to the disruption of stronger hydrogen-bonded water molecules (four hydrogen bonds), resulting in the reduction of Raman intensity at 3250 cm^{-1} .²³ This is clearly observed in Figure 4.2A in which higher water temperature produce lower Raman intensity at 3250 cm^{-1} . Therefore, water at different temperature produces different ratio of $I_1:I_2$, in which I_1/I_2 decreases with the increase in temperature. Performing Raman analyses on water that is heated to known temperatures enable the plot of a calibration curve as seen in Figure 4.2B. The relation of I_1/I_2 and temperature from $20\text{ }^{\circ}\text{C}$ to $80\text{ }^{\circ}\text{C}$ shows satisfying linearity with the linear equation of $y = 0.95 + (-0.003x)$ and R^2 value of 0.95.

Figure 4.2. (A) Raman spectra of water at different temperature, in which an increase in temperature weakens the stronger hydrogen-bonded water leading to the decrease in intensity at 3250 cm^{-1} (I_1). (B) Calibration curve of water with the ratio of $I_1: I_2$ against temperature showing a linear relationship (obtained from the average of 3 readings).

To determine the photothermal capability of NGN, the self-assembled NGN is first submerged in 500 μL of water, with a thin film of approximately 1.6 mm of water above it. Water film is preferable than a water droplet because it allows proper focusing of the laser source through its level surface, as opposed to the convex curvature of a water droplet. Additionally, a larger volume of water such as in our NGN sample ensures minimal mass changes after heating for more precise measurement. For example, a small water droplet may shrink in size if a high temperature is achieved, causing disparity in laser focusing before and after heating. But a bulk water film in our NGN sample undergoes minimal changes due to its larger volume.

Using 532 nm laser wavelength and a constant excitation power of 135 mW, we then vary the acquisition time from 1 minute to 9 minutes and irradiate the NGNs. Figure 4.3A shows Raman spectra of heated water at different irradiation times. We observe the decrease in I_1/I_2 as we increase the irradiation time, as seen in Figure 4.3B. This shows the increase in temperature over time, which corroborates well with the increase in excitation time. We are able to determine the temperature profile of our NGN using the linear equation from calibration curve of water (Figure 4.3B). From the average of 3 measurements each, as the time increases from 1 minute to 9 minutes, the localized temperature of our NGN increases from 21 ± 4 °C to 26 ± 5 °C to 32 ± 8 °C to 35 ± 5 °C to 45 ± 5 °C to 47 ± 7 °C to 58 ± 8 °C to 65 ± 2 °C and 68 ± 3 °C. This temperature profile shows that we have a good degree of control over the temperature of our NGNs in relation to time with the tunable step size of ± 10 °C, enabling manipulation of its photothermal effect for specific heating.

Figure 4.3. (A) Resulting Raman spectra of water film on self-assembled NGNs irradiated with 532 nm excitation wavelength at different times. (B) Decrease in I_1/I_2 as irradiation time increases which reflects the rise of water temperature as photothermal effect become stronger with longer irradiation time. The control of temperature with the stepsize of ~ 10 °C is possible by manipulating time.

We estimate the laser spot diameter to be $1.44 \mu\text{m}$, using the formula, $d = 1.22 \lambda / \text{NA}$, derived from Rayleigh criterion,²⁴ in which d is the laser spot diameter, λ is the laser excitation wavelength, and NA is the numerical aperture of the microscope objective used. Using this laser spot size, we estimate an average of 4 ± 1 NGN is able to be irradiated in a scan (Figure 4.4A). Such little quantity of NGN capable of reaching ~ 70 °C shows great potential of its photothermal effect. However, for future studies, to precisely determine the amount of NGN involve in the photothermal heating, knife-edge measurement²⁵ should be carried out to accurately determine the laser spot size. Figure 4.4B and C show the NGN before and after heating respectively, in which it retains its shape and porosity. Therefore, in the current temperature range, the NGNs are stable and do not undergo morphological changes which is important for further SERS sensing.

Figure 4.4. (A) SEM image of self-assembled NGN with red circles approximated as laser spot size of $1.44\ \mu\text{m}$. A maximum average amount of 4 ± 1 NGN is determined within this spot size which shows the exceptional potential of our NGNs photothermal capability. (B-C) SEM images of NGNs before and after photothermal heating in which they retain their shape and surface morphology.

To ensure the photothermal effect is produced by the NGN, we did a control experiment in which an empty spot with no NGN was irradiated with laser and similar condition was used in the presence of NGN (Figure 4.5). There is an obvious difference in Raman intensity of I_1 (Figure 4.5A) and further analysis shows the I_1/I_2 decreases when NGN is present (Figure 4.5B). The corresponding temperatures shows the increase in $7\ ^\circ\text{C}$ when the laser source irradiates NGN, therefore validating the photothermal capabilities of our NGN.

Figure 4.5. (A) Raman spectra of water without the presence of NGN (black) and with the presence of NGN, obtained at similar irradiation conditions (laser excitation power of 135 mW and acquisition time of 2 minutes). (B) Resulting I_1/I_2 of each spectra and temperature shows the increase in water temperature when NGN is present. Therefore, photothermal effect of NGN is validated.

4.3.2 Photothermal effect coupled with SERS sensing

To demonstrate the multifunctional capability of our NGN to exhibit photothermal and SERS effect, we exploit the denaturation of protein, namely bovine serum albumin (BSA), in which SERS sensing before and after heating can provide validation of the photothermal effect through the changes in the protein's structure. Denaturation studies of proteins are commonly performed to determine protein stability, which is important in biological science, such as its potential use to comprehend the effects of point mutations involved in many genetic diseases.²⁶ BSA is a protein which undergoes denaturation at the temperature of > 50 °C,²⁷ therefore it is well suited for our study in which a change in temperature can be observed with the change of the SERS spectrum.

We begin the multifunctional analysis by obtaining the SERS spectrum of BSA with 785 nm laser, which is denoted as 0 minute. After the first scan, we performed a

rescan as a control to determine the inability of 785 nm laser to induce photothermal heating. Without removing the sample from the acquisition chamber, a switch of laser excitation wavelength to 532 nm is performed for the photothermal effect. The similar spot is irradiated for 9 minutes to ensure the temperature of $> 50\text{ }^{\circ}\text{C}$ is reached. Finally, the laser excitation wavelength is switched back to 785 nm to acquire the resulting SERS spectrum after the photothermal effect, which is denoted as 9 minutes.

Figure 4.6 shows these three SERS spectra with one spectrum exhibiting drastic changes in its Raman shifts. At 0 minutes, four characteristic peaks of BSA can be observed at 990 cm^{-1} , 1210 cm^{-1} , 1460 cm^{-1} , and 1540 cm^{-1} which are denoted as Phe ring breathing, Tyr + Phe, CH_2 and CH_3 scissoring, and Amide II respectively.²⁸ A rescan is performed, in which the four characteristic peaks remain; indicating the 785 nm laser did not induced photothermal heating. At 9 minutes, these four peaks drastically reduce in intensity and are practically indistinguishable. The decrease in peak intensity is commonly observed as proteins denature,^{12,29} therefore the photothermal effect of our NGN have denatured the BSA. This observation proves the operational photothermal effect coupled with SERS sensing of our NGN and shows the potential application for analysis of temperature sensitive analytes such as proteins and DNA.

Figure 4.6. SERS spectra of BSA before (0 min) and after photothermal heating (9 min). A rescan is performed after 9 minutes from the first analysis with 785 nm to ensure the

change of peaks is caused by photothermal heating. 4 characteristics peaks at 990 cm^{-1} , 1210 cm^{-1} , 1460 cm^{-1} , and 1540 cm^{-1} are observed to diminish after the application photothermal effect which produced temperature higher than denaturation temperature of BSA.

4.4 Conclusions

In conclusion, we have produced a capable multifunctional metal nanoparticle with NGN, which can exhibit photothermal effect and SERS scattering with just the switch of laser excitation wavelength. The control of temperature can be accomplished by managing the irradiation time in which a maximum temperature of approximately $70\text{ }^{\circ}\text{C}$ is achieved in the current study. Due to instrumental limitations, in which the CCD undergoes saturation at acquisition time above 9 minutes, the steady state temperature of the photothermal heating of the NGN could not be determined. Therefore, further analysis with different instruments such as thermocouple or IR camera should be performed to enhance the understating of the photothermal capabilities of the NGN. With just 4 ± 1 NGNs approximated to contribute to current temperature, we anticipate a greater increase in heating capabilities with a larger amount of NGNs. This multifunctional capability enables in-situ heating with laser which is more time effective than ex-situ heating by eliminating the need of substrate removal from the acquisition chamber. Eliminating the removal of substrate from the acquisition chamber also allows a more accurate study of the changes in target analytes since the resulting spectral changes are obtained from the same analytes in the constant laser spot. Therefore, as-synthesized multifunctional NGNs are beneficial for in-situ study of heating effects with SERS sensing to monitor changes.

4.5 References

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CHAPTER 5

Plasmonic Liquid Marble Merging: An Isolated Environment for Accurate Ultrasensitive Surface-Enhanced Resonance Raman Scattering (SERRS) Detection

Abstract

Plasmonic liquid marbles (PLM) are proficient substrate-less SERS platform, known for its ease of fabrication, ability to isolate analytes, and effortless multiplex sensing. However, current studies of PLMs are limited to single PLM and the exploit of the merging capability of liquid marbles is yet to be studied. Here, we demonstrate the merging of PLMs by utilizing the mirror-like, soft shell of Ag nanocubes formed at the organic phase. Using azo coupling as our model reaction, we demonstrate the advantages of PLMs merging. With one PLM constituting our target analyte (bisphenol A (BPA)) and another PLM constituting our reagent (nitrobenzene diazonium (dNB)), upon merging, the resulting PLM provides protection to the azo dye (BPA+dNB), as soon as it is formed. This is highly important as the exposure of BPA+dNB at ambient conditions leads to photobleaching, producing substandard surface-enhanced resonance Raman scattering (SERRS) results. Therefore, the merging of PLM enables accurate analytical studies of trace analytes and additionally providing an isolated environment which increases the stability of the encapsulant. Furthermore, we improve the detection limit of BPA by 4 orders of magnitude with a substantial decrease in detection time.

5.1 Introduction

Liquid marbles are exceptional micro reactors due to its ease of fabrication and manipulation,^{1,2} ability to reduce chemical reagents and solvents for precise control of reactions,^{3,4} isolation of reactions from external interferences,^{5,6} and elimination of platform instability.⁷ One of the unique mechanical properties of liquid marbles is its ability to merge with another liquid marble to form a singular, larger unit.⁸ This merging behavior enables liquid marbles to be a micro mixer and it has been applied to mix reactants in the encapsulating liquid⁹ or mix the encapsulant to produce Janus liquid marble.¹⁰ Plasmonic liquid marble (PLMs) are innovated by encapsulation with plasmonic metal nanoparticles, generating the capability of detecting analytes in the constituent liquid droplet through the use of surface-enhanced Raman scattering (SERS) spectroscopy.¹¹ PLMs proves to be advantageous than conventional substrate-based SERS sensors due to the elimination of multistep fabrication, ease of multiplex detection, and isolation for in-situ studies.¹² Investigation on PLMs merging for triggering and monitoring chemical reaction as well as identification of the product in-situ is therefore significant for further development of PLM as real microfactory platform.

Azo coupling is an organic reaction that produces an azo compound from a diazonium compound and an aromatic compound.¹³ In recent years, azo coupling reaction has been exploited to increase SERS sensitivity through the formation of azo dyes which enables surface-enhanced resonance Raman scattering (SERRS).¹⁴ SERRS occurs when the excitation laser wavelength matches the electronic transition of analytes, increasing the sensitivity of SERS.¹⁵ Therefore, the formation of azo dyes enables ultrasensitive detection of phenol molecules with weak affinity to plasmonic metal nanoparticles such as thyrotropin-releasing hormone,¹⁶ phenolic estrogens,¹⁷ histidine, and tyrosine.¹⁸ However, conventional SERS/SERRS sensing in which dyes interact with plasmonic metal nanoparticles in an ambient condition face the potential complication of

photobleaching,¹⁹ causing inaccurate detection. Additionally, production of diazonium compounds, a precursor for azo coupling, is potentially hazardous because they are explosive.^{20,21}

Here, we exploit PLMs for azo coupling reaction through the merging of two PLMs with precursors of the reaction, enabling the protection of the azo dye product from external influences, such as photobleaching, as soon as it is produced, leading to accurate and fast ultrasensitive in-situ SERRS detections. Furthermore, PLMs as micro reactors allow safe handling of diazonium compounds due to the small amount used and the protection with a core-shell. We demonstrate conditions suitable for the formation and merging of PLMs, to ensure uniform encapsulation with plasmonic metal nanoparticles. Using azo coupling as the model reaction, we are able to detect trace amount of bisphenol A (BPA), an essential component in industrial production of plastic, which is harmful to the environment and potentially to humans.^{22,23}

5.2 Experimental Section

5.2.1 Materials

Silver nitrate (99+%, AgNO_3), anhydrous 1,5-pentanediol ($\geq 97\%$), polyvinylpyrrolidone (PVP, average $M_w = 55,000$), hydrochloric acid (HCl, 37 %), 4-nitroaniline ($\geq 99\%$), sodium nitrite (NaNO_2 , $\geq 97\%$), decane (anhydrous, $\geq 99\%$), bisphenol A (BPA, $\geq 99\%$) were purchased from Sigma Aldrich. Copper (II) chloride (CuCl_2 , $\geq 98\%$) was purchased from Alfa Assar. Sodium carbonate (Na_2CO_3 , AR grade) was purchased from Sinopharm Chemical Reagent co., Ltd. Ethanol (absolute) was purchased from Fisher Scientific. All chemicals were used without further purification. Milli-Q water ($> 18.0 \text{ M}\Omega\cdot\text{cm}$) was purified with Sartorius Arium 611 UV ultrapure water system.

5.2.2 Synthesis and purification of Ag nanocubes. Ag nanocubes were prepared from a polyol method described in literature.²⁴ Briefly, AgNO_3 (20 mg/mL), PVP (20 mg/mL), and CuCl_2 (8 mg/mL) were dissolved separately in 10 mL 1,5-pentanediol. 35 μL of CuCl_2 solution was then added to the AgNO_3 solution. 20 mL of 1,5-pentanediol was added into a 100-mL round bottomed flask and heated to 190°C for 10 minutes. 250 μL of PVP precursor was added to the flask dropwise every 30 seconds while 500 μL of AgNO_3 precursor was injected every min. The addition process proceeded until tea-like color was observed.

Synthesized Ag nanocubes were purified by removal of 1,5-pentanediol from the mixture through centrifugation. The Ag nanocubes were subsequently redispersed in 10 mL of ethanol and 100 mL of aqueous PVP solution (0.2 g/L). The solution was vacuum filtered with Durapore® polyvinylidene fluoride filter membranes (Millipore) with pore sizes of 5000, 650, 450, and 220 nm, repeated numerous times for each pore size in descending order. The Ag nanocubes were finally redispersed in ethanol (10 mg/mL) and stored in the dark, below room temperature for further use.

5.2.3 HCl treatment of Ag nanocubes. Surfactant removal from the surface of Ag nanocubes was referred to method in literature.²⁵ 100 μL of purified Ag nanocubes was added to 5 mL of HCl (0.1 M) in water while stirring. After 3 hours, the Ag nanocubes colloidal suspension was purified from excess HCl with abundant amount of pure water and ethanol. Finally, the HCl-treated Ag nanocubes were redispersed in ethanol (10 mg/mL).

5.2.4 Preparation of bisphenol A encapsulated PLM. Bisphenol A solution with varying concentrations was prepared by dissolving solid BPA in 2% Na_2CO_3 aqueous solution followed by serial dilutions. 4 μL of HCl-treated Ag nanocubes was transferred into a 2 mL centrifuge tube and dried with N_2 gas. 250 μL of decane was subsequently added to the dried HCl-treated Ag nanocubes. The solution was sonicated to uniformly redisperse the HCl-treated Ag nanocubes in decane. A 2 μL droplet of BPA solution was added into Ag nanocube-containing decane suspension and rolled to form PLM.

5.2.5 Preparation of nitrobenzene diazonium encapsulated PLM. Diazotization of 4-nitroaniline (1.25 mM) was carried out by dissolving it in 0.1 M HCl aqueous solution. 1 μL of aqueous 4-nitroaniline solution was subsequently mixed with 1 μL of 5% NaNO_2 aqueous solution producing nitrobenzene diazonium. Similar to the preparation of bisphenol A encapsulated plasmonic liquid marble, 4 μL of HCl-treated Ag nanocubes was transferred into a 2 mL centrifuge tube and dried with N_2 gas. 250 μL of decane was subsequently added to the dried HCl-treated Ag nanocubes. The solution was sonicated to uniformly redisperse the HCl-treated Ag nanocubes in decane. The 2 μL droplet of nitrobenzene diazonium solution was added into Ag nanocube-containing decane suspension and rolled to form PLM.

5.2.6 Merging of PLMs. Nitrobenzene diazonium encapsulated PLM was transferred to the centrifuge tube containing bisphenol A encapsulated PLM using a pipette with a sufficient tip opening which ensured the transferred PLM retains its form. Once

transferred, the centrifuge tube was lightly shaken to bring the PLMs together. This led to the merging of PLMs. The merged PLM was left to react for a minimum time of 1 minute before further analysis.

5.2.7 Characterization. Imaging of the PLM was performed by scanning electron microscopy (SEM) characterization using JEOL 7600F JSM operating at an accelerating voltage of 5kV in the back-scattered mode. UV-Vis measurement was obtained with Agilent Technologies Cary 60 spectrophotometer. SERS/SERSS measurements were performed using x-y imaging mode of the Raman touch microspectrometer (Nanophoton Inc., Osaka, Japan) with a 50× objective lens (N.A. = 0.55), an excitation laser wavelength of 532 nm, excitation power of 100 μ W, and acquisition time of 1 second. SERS/SERSS imaging experiments were carried out on at least three PLMs to ensure the reproducibility of SERS/SERSS signal.

5.3 Results and Discussions

5.3.1 Demonstration and characterization of PLM merging

Single-crystalline Ag nanocubes, synthesized in high yield from the polyol method,²⁶ is chosen as the building block of our PLMs due to well-defined edges and tips which generate strong localized electromagnetic fields for SERS enhancement. The Ag nanocubes are treated with HCl for the removal of surfactants to ensure no interference in Raman signal during SERS analysis and to facilitate the approach of target molecules to the Ag nanocube surface for signal enhancement. We form PLM by introducing a sessile aqueous droplet into Ag nanocubes suspension in decane followed by rolling the droplet to ensure the Ag nanocubes uniformly adsorb on the droplet surface (Figure 5.1A). The formed Ag nanocubes shell assembled on the surface of individual aqueous droplet functions as a protection layer from outer environment and as an optically active plasmonic layer for Raman signal amplification of the encapsulated substance. Additionally, the formation of PLM in an organic phase is beneficial to prevent the evaporation of encapsulated liquid which leads to the collapse of the PLM.²⁷

Figure 5.1. (A) Formation of plasmonic liquid marble through the self-assembly of Ag nanocubes on an aqueous droplet in organic phase. (B) Schematic of plasmonic liquid marble merging. (C) Digital image of the plasmonic liquid marble with SEM image showing the adsorbed Ag nanocubes forming a mirror-like soft shell. (D) Digital images of plasmonic liquid marble merging in which two plasmonic liquid marbles fuse together, producing a larger singular unit.

Scanning electron microscopy (SEM) imaging shows the closely packed Ag nanocubes shell on the aqueous droplet-oil interface (Figure 5.1C). Previous study in our group using Ag nanocubes for the formation of PLM produced an analytical SERS enhancement factor of approximately 10^8 for methylene blue detection in liquid phase.¹¹ This is attributed to the large amount of hot spot produced from the close packing of Ag nanocubes, which validates the use of Ag nanocubes in this study.

Importantly, the shell of the PLM is quite soft, which allows the easy merging between marbles to form an enclosed new marble with larger size. We apply such merging process between marbles that contain different reactants to trigger chemical reactions and read out the information on the product inside PLM by non-invasive SERS technique (Figure 5.1B). Merging of PLMs is performed by adding two formed PLMs in a similar decane solution. The soft Ag shell enables immediate fusion of the PLMs, forming a larger singular PLM, bringing reactants together for resulting reaction (Figure 5.1D). We do not observe any leakage of encapsulated liquid as the surrounding decane solution remains colorless after the formation of azo dye. Additionally, the merged PLM displays Ag mirror appearance with no exposure of the encapsulated droplet. Therefore, a uniform and stable PLM is fabricated from the merging of smaller PLMs, enabling the isolation of resulting product from external interferences.

5.3.2 Performing Azo coupling model reaction through PLM merging

Azo coupling produces azo compound from an organic reaction between a diazonium compound and an aromatic compound, which will be the constituent liquids in the pre-merged PLMs. In this study, the aromatic compound used is BPA, which is our target analyte. SERS detection of BPA, a harmful compound to the environment and potentially to humans,²⁸ faces the issue of weak affinity of phenolic molecules to plasmonic metal nanoparticles surfaces.²⁹ Consequently, complex and time-consuming methods to increase the affinity of BPA to the surface of plasmonic metal nanoparticles surfaces have been studied, such as surface modifications of the metal nanoparticles²⁹ and the use of aptamers.^{30,31} On the contrary, the azo coupling reaction of BPA allows a simpler and time efficient ultrasensitive SERS detection through the formation of azo dyes, which possess larger Raman cross section.

The diazonium compound is used as the reagent to assist the detection of BPA; hence excess amount is always utilized to ensure complete formation of the azo dye. To produce a diazonium compound, diazotization of aromatic amines with nitrous acid is commonly performed. It is a spontaneous reaction which yields reactive and unstable product, in which the diazonio group ($-N_2^+$) can be lost as N_2 gas at ambient condition.³² Therefore, in situ formation of the diazonium compound is crucial for every analysis to ensure proper precursors are used for the azo coupling reaction. We employ nitrobenzene diazonium (dNB) as the diazonium compound in this study because it has a strong electron withdrawing group which enhances stability and allows azo coupling reaction at room temperature.³³ Importantly, it forms azo dye after coupling with BPA which exhibits a bright red color. The reaction between the dNB and BPA is shown in Figure 5.2A below.

Figure 5.2. (A) Reaction steps to produce nitrobenzene diazonium and azo dye. Digital images show the solution color of corresponding compounds. (B) UV-Vis spectra of BPA solution and BPA+dNB solution showing the emergence of an absorption peak after azo coupling reaction.

Diazotization of 4-nitroaniline is performed with HCl and NaNO₂ to produce dNB (Figure 5.2A). Both the dNB and BPA solution are colorless. Mixing of these reactants led to azo coupling, in which dNB attacks bisphenol A in the ortho position to the hydroxyl group, and the reaction completes in a few minutes.³³ 1 minute of reaction time is sufficient for the azo coupling reaction in PLM due to the microliter amount of reactants used. The delocalization of π electron enables the azo compound to exhibit color. This is observed in the bright red solution color produced (BPA+dNB) after the reaction of the two colorless dNB and BPA solutions. UV-Vis spectroscopy measurements on BPA+dNB and BPA solutions (Figure 5.2B) demonstrate the emergence of absorption peak (λ_{max} : 480 nm) from 400 to 600 nm for BPA+dNB, whereas BPA exhibit no absorption within this range. Therefore, the use of 532 nm laser

excitation wavelength, which overlaps with the absorption spectrum of formed azo dye, during SERS analysis enables increase in analyte sensitivity with SERRS.

5.3.3 SERS/SERRS analysis of PLMs

We perform SERS analysis (Figure 5.3A) on the two individual PLMs, constituting our target analyte (BPA, 2×10^{-9} mole) and our reagent (dNB, 1.25 mM), and SERRS analysis on the merged PLMs, constituting azo dye after reaction (BPA+dNB). dNB produces Raman shifts at 1350 cm^{-1} , 1470 cm^{-1} and 1600 cm^{-1} which corresponds to NO_2 stretching, phenyl-N-N stretching and ring deformation, and phenyl C=C stretching respectively.³⁴ However, BPA produces an indistinguishable Raman spectrum. This shows the inability of SERS to detect BPA without complex and time-consuming methods to increase the affinity of BPA to the plasmonic metal nanoparticles. Upon merging of PLMs to produce azo dye, we observe the emergence of two new Raman shifts at 1400 cm^{-1} and 1440 cm^{-1} . These peaks correspond to N=N stretching,³⁴ indicating the presence of azo compound. Therefore, the detection of BPA is possible after the merging of PLMs with constituent reactants. Since the Raman shift at 1400 cm^{-1} exhibits stronger intensity, it is chosen as the characteristic peak for the quantitative detection and analysis of BPA.

Figure 5.3. (A) SERS spectra of individual PLMs with BPA and dNB, and SERS spectrum of the final merged PLM with BPA+dNB. (B) SERS spectra of BPA+dNB with different mole amount of BPA, showing the detection limit of our PLMs merging (1×10^{-15} mole). (C) SERS intensity of BPA+dNB ranging from 1×10^{-9} mole to 1×10^{-15} mole, with a linear relationship established between 1×10^{-11} mole to 1×10^{-15} mole.

To ensure the emergence of Raman peak at the 1400 cm^{-1} due to azo coupling reaction, we perform control SERS analysis on PLM with dNB and merged PLMs with 0 mole BPA and 1.25 mM dNB. Firstly, SERS analysis on PLM with dNB (Figure 5.4A) over the time period of 5, 10, and 20 minutes shows minimal changes. This demonstrates the stability of the encapsulated reactant. Importantly, there is no peak at the 1400 cm^{-1} Raman shift, which will not interfere with the detection of BPA. Subsequently, SERS

analysis of merged PLMs with 0 mole BPA (Figure 5.4B) and dNB yielded comparable spectra to pure dNB. Similarly, there is no peak noticeable at 1400 cm^{-1} . Therefore, the emergence of such peak after the merging of PLMs with BPA and dNB demonstrate the formation of azo dye, enabling the detection of BPA.

Figure 5.4. (A) SERS spectra of PLM with dNB from 5 to 20 minutes showing the stability of the encapsulated reactant which do not interfere with the detection of BPA. (B) SERS spectrum of merged PLM with 0 mole of BPA with dNB. The absence of 1400 cm^{-1} Raman shift shows the peak correspond to the azo compound formed with the presence of BPA.

Following the confirmation of our SERRS analytical method, a detection limit of BPA in our PLM is determined up to 1×10^{-15} mole (Figure 5.3B). This improves the detection limit of BPA by 4 orders of magnitude from previously reported SERRS-based aptasensor platform, which requires detection time of up to 40 minutes.³⁰ Therefore, in addition to the improved detection limit, our PLM merging system drastically reduces the detection time. The decrease in SERRS intensity with the decrease in mole of BPA can be observed clearly in Figure 5.3C. We observe a linear relationship in SERRS intensity

from 1×10^{-11} to 1×10^{-15} mole of BPA with the equation of $y = 426.88 + 26.56x$ ($R^2 = 0.99445$). Therefore, quantitative analysis of BPA within this mole range is possible through our PLM merging.

Figure 5.5. UV-Vis measurement of BPA+dNB at different mole amount of BPA.

Furthermore, we highlight the advantage of our PLM-based SERRS detection of BPA over colorimetric detection using UV-Vis spectroscopy (Figure 5.5), in which its detection limit is 1×10^{-8} mole. Our SERRS measurement improves the detection limit by 7 orders of magnitude. Additionally, our SERRS-based detection offer richer molecular vibration information, require small amount of sample, and allow non-invasive in-situ analysis.

5.3.4 Advantages of PLM merging

To study the importance of forming PLMs to isolate reactants prior to reaction, we perform control experiments with different PLM fabrication methods. Firstly, we have our current PLM merging method in which PLMs of BPA and dNB are first formed followed by their merging in decane (method A, Figure 5.6A). Secondly, we mix BPA and dNB in ambient condition followed by the formation of PLM with the BPA+dNB

solution in decane (method B, Figure 5.6B). Lastly, we mix BPA and dNB in decane to form the azo dye followed by fabrication of the PLM in decane (method C, Figure 5.6C). The concentrations (1×10^{-10} mole BPA, 1.25 mM dNB) and amount of reactants (2 μ L each) used in all the methods are similar to ensure a fair comparison. For method B and method C, the formation of PLM was performed after 1 minute of exposed reaction.

Figure 5.6. Fabrication of plasmonic liquid marble with different methods and their corresponding SERRS spectrum. (A) PLM merging, in which PLMs is formed with BPA and dNB in decane followed by their merging to yield BPA+dNB. (B) Azo coupling reaction in air between BPA and dNB followed by the formation of PLM in decane. (C) Azo coupling reaction in decane followed by the formation of PLM in decane. (D) Raman intensity of BPA+dNB for the different PLM fabrication method.

The merging of PLMs, method A, produced the highest Raman intensity at 1400 cm^{-1} (380 ± 26 cps). Although similar amount of reactants are used, the formed azo dye

by method B and method C exhibit lower Raman intensity than that in method A by approximately 37.4 % and 93.2 % respectively (Figure 5.6D). The decrease in Raman intensity can be attributed to photobleaching of azo dye because the product solution in method B and C was exposed to visible light irradiation before encapsulation to form PLM. Photobleaching is a process in which a dye loses its fluorescence capability due to permanent depletion of ground state molecules when molecules are excited to the singlet or the triplet excited state.^{35,36}

To verify the photobleaching effect, two similar droplets of BPA+dNB solution were prepared at the same time and one of the droplets was covered from exposure of light whereas another was left exposed (Figure 5.7A). At different time intervals, a digital image of both droplets was taken and we observe a noticeable decrease in color intensity for the exposed droplet as time increases. We also notice the slight difference in color intensity of the closed droplet as time increases. This shows the sensitivity of the BPA+dNB solution to degrade at ambient conditions and even greater susceptibility to photobleaching when constantly exposed to light. Additionally, the drastic decrease in Raman intensity for method C could be attributed to the possible diffusion of exposed BPA+dNB into the organic phase (decane). As seen in Figure 5.7B, at the beginning of BPA and dNB addition to decane, the decane solution was clear with the formation of BPA+dNB at the base of the sample bottle. Upon stirring, the decane solution becomes slightly orange in color. This demonstrates the diffusion of the azo dye into the organic phase.

Figure 5.7. (A) Photobleaching of BPA+dNB is shown by two similar solution that was prepared at the same time with one of the droplet covered from exposure of light (left droplet) and another left exposed (right droplet). At different time interval, a digital image was taken and the exposed droplet undergoes drastic change in color in which the color intensity decrease as time increases. (B) Diffusion of BPA+dNB into the organic phase (decane) is shown. At the beginning of addition to form BPA+dNB, the decane was clear. Upon stirring, the decane solution became orange in color.

Method B and method C shows the importance of PLM merging. A 1 minute interval in producing PLM after obtaining the BPA+dNB solutions can drastically influence the SERS intensity. This is highly critical for the detection of trace analytes as photobleaching may cause false negative results. Therefore, the protection of products from external interferences, as soon as it forms, through the merging of the PLMs, enables us to address this issue and provide a pathway for accurate SERRS analysis.

Additionally, we examine the stability of BPA+dNB solution in our merged PLMs. Using the PLM formed with method A, we perform SERRS analysis on it after 2 days (Figure 5.8A). The SERRS spectrum after 2 days (Figure 5.8A ii) retains characteristic peaks same as the SERRS spectrum obtained after 1 minute of reaction through PLM merging (Figure 5.8A i). Importantly, the Raman shift at 1400 cm^{-1} remains comparable with only 3.5 % difference in Raman intensity between the time span of 1 minute and 2 days. Conversely, when a BPA+dNB solution was exposed in air for 30

minutes before the formation of PLM (method B), the resulting SERRS spectrum (Figure 5.8B ii) exhibits drastic changes compared to the exposure of 1 minutes before PLM formation (Figure 5.8B i). The crucial Raman shift intensity of 1400 cm^{-1} decrease by 93.3 % as the BPA+dNB solution was exposed for a longer time. This further demonstrates photobleaching of the azo compound. Therefore, the advantage of PLM merging is its ability to protect products, as soon as it is formed, for accurate in-situ analytical studies and to retain the product for prolong period of time (up to 2 days in current study), enabling further ex-situ studies.

Figure 5.8. (A) SERRS spectra of BPA+dNB solution in merged PLM after (i) 1 minute and (ii) 2 days show comparable results. (B) SERRS spectra of PLM formed after the exposure of BPA+dNB solution in air for (i) 1 minute and (ii) 30 minutes show drastic changes in Raman intensity.

5.4 Conclusions

In conclusion, we have developed a method for the merging of PLMs. This is achieved with the Ag mirror-like soft shell composed of Ag nanocubes formed on a spherical aqueous droplet/oil interface. We demonstrated the advantages of PLM merging with azo coupling reaction, in which the product is protected as soon as it is formed from external interferences. This is particularly crucial for detection of trace and photo-sensitive analytes because external interferences such as photobleaching may induce false negative on analytical results. We are able to improve the detection limit of BPA, a pollutant from modern industrial production, with SERRS in addition to being less time consuming. Finally, the instantaneous formation of products together with the metallic shell (SERS platform) from merging of PLMs also suggests the capability for in-situ analysis of reaction kinetics.

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CHAPTER 6

Conclusions & Outlook

6.1 Conclusions

In this thesis, we have introduced a novel plasmonic metal nanoparticle (MNP), known as nanoporous gold nanoframes (NGNs), synthesized through a seed-mediated approach, and demonstrated the effect of its porosity on surface-enhanced Raman scattering (SERS). Electrostatic self-assembly of NGNs was then performed to produce a superhydrophobic platform, which was coupled with near infrared SERS (NIR-SERS) for sensing of trace analytes that fluoresce strongly. Additionally, we demonstrated the multifunctional capability of NGN which exhibits photothermal effect and SERS at different laser excitation wavelengths. Finally, we introduced the merging of plasmonic liquid marbles (PLMs) as isolated micro reactors for the protection of products from external interferences with the encapsulating Ag nanocube core shell. We believe the work done provide substantial contributions to the field of analytical chemistry through the development of SERS sensing, by fabricating novel plasmonic MNPs and the use of photothermal effect, superhydrophobicity, and PLM to support SERS sensing. Nevertheless, there are many opportunities to expand the work of this thesis.

6.2 Outlook

Firstly, the seed-mediated synthesis of NGN provides a foundation for the synthesis of other shape-controlled nanoporous hollow nanoparticles. The cubic shape of the NGN is dictated by the AgCl cubic template; therefore changing the shape of the sacrificial template will ideally change the shape of the NGN. However, it is crucial for the sacrificial template to exhibit difference in relative surface energy for the selective

deposition of reduced metal precursors. An example will be the use of octahedral AgCl nanocrystal¹ which will ideally yield octahedral NGN. Since localized surface plasmon resonance is affected by shape,² the change of a NGN's shape will produce different optical properties. Additionally, physical properties of a hollow nanostructure will be affected by its shape. For example, although octahedral NGN and cubic NGN have 12 connecting edges, octahedral NGN will have 8 empty faces whereas a cubic NGN will have 6 empty faces. Therefore, the syntheses of other novel shape-controlled nanoporous hollow nanoparticles is worth studying as different optical and physical properties can possibly lead to enhanced or new applications.

Secondly, current self-assembly of NGNs using electrostatic method produced a monolayer of NGN, which exhibited surface roughness that was exploited for superhydrophobicity. The self-assembly of NGN into other macroscopic structures are yet to be studied. Assembly of gold nanoframes into dimers has been shown to increase SERS capability due to the increase in "hot spots" from plasmon coupling between two monomers.³ Similarly, assembly of Ag nanowires into three-dimensional (3D) woodpile-like structures has been shown to increase the amount of "hot spots" from cross linking of the wires, but is limited to three layers due to impenetrable laser source at higher amount of layers.⁴ Therefore, the assembly of NGN into a 3D fishnet-like structure can be worth investigating due to the increase in "hot spots" from the coupling between NGNs and the hollow core with porous frames can possibly allow deeper penetration depth of a laser beam, therefore allowing a greater amount of nanoparticles stacking for greater SERS capability.

Thirdly, we have demonstrated the multifunctional capability of NGN in combining photothermal heating with SERS. However, additional analysis can be performed to further understand the photothermal effect for a better optimization in its application. Photothermal conversion efficiency,⁵ in which how efficient the NGN

converts photons into heat is yet to be studied. This is important because there are NGNs with different porosity and the effect of the porosity on its photothermal conversion efficiency is unknown, which restrict the optimization of our NGNs' photothermal effect. Additionally, the current high temperature (approximately 70 °C) achieved by a small amount of NGNs (~ 4 NGNs) suggest the greater extendibility of heating capabilities with a larger array of NGN. Therefore, the study of NGNs' photothermal effect can be extended from the promising results of current studies.

Lastly, the study of reaction kinetics can be achieved with SERS by monitoring the change in Raman shifts overtime.^{6,7} Consequently, PLM merging provides the capability of real-time and in-situ study of reaction kinetics from the merging of reactants encapsulated in the PLMs. Importantly, the MNP provides a protection to encapsulated products as demonstrated in our PLM merging work. Therefore, the development of PLM merging can be beneficial to fundamental study of reaction kinetics, especially for products that are prone to photobleaching. Additionally, the use of NGN as the core shell of a PLM to conceive a 3D substrate-less photothermal-SERS micro reactor can be interesting. A photothermal miniature reactor, in the form of graphene liquid marble, has demonstrated the capability of modulating reaction kinetics with its heating capability.⁸ Therefore, a NGN PLM can potentially assist the study of reaction kinetics with its multifunctional heat modulation and SERS detecting capabilities. Finally, the merging studies of PLM is currently limited to two individual PLMs, the merging of three or more PLMs, such as feasibility, experimental conditions, and applications, can be worth studying

6.3 References

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CHAPTER 7

List of Publications

1. **Chew, W. S.**; Pedireddy, S.; Lee, Y. H.; Tjiu, W. W.; Liu, Y.; Yang, Z.; Ling, X. Y., Nanoporous Gold Nanoframes with Minimalistic Architectures: Lower Porosity Generates Stronger Surface-Enhanced Raman Scattering Capabilities. *Chemistry of Materials* **2015**, 27 (22), 7827-7834.
2. Miao, Y.-E.; Lee, H. K.; **Chew, W. S.**; Phang, I. Y.; Liu, T.; Ling, X. Y., Catalytic liquid marbles: Ag nanowire-based miniature reactors for highly efficient degradation of methylene blue. *Chemical Communications* **2014**, 50 (44), 5923-5926.
3. **Chew, W.S.** 2nd Workshop on the Chemistry of Energy Conversion, Singapore, **2016**, Poster (Honorable Mention).
4. **Chew, W.S.** 9th Asian Photochemistry Conference, Singapore, **2016**, Contributed Speaker.