Plasmonic colloidosomes: dynamic three-dimensional platform for biphasic, microfluidic and airborne surface-enhanced raman scattering applications

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**NANYANG
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SINGAPORE

**PLASMONIC COLLOIDOSOMES: DYNAMIC THREE-
DIMENSIONAL PLATFORM FOR BIPHASIC,
MICROFLUIDIC AND AIRBORNE SURFACE-ENHANCED
RAMAN SCATTERING APPLICATIONS.**

PHAN-QUANG GIA CHUONG

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

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PHAN-QUANG GIA CHUONG

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

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

2019

Statement of Originality

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

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Authorship Attribution Statement

This thesis contains material from 4 paper(s) published in the following peer-reviewed journal(s) in which I am listed as an author.

Chapter 2 is published as G.C. Phan-Quang, H.K. Lee, I.Y. Phang and X.Y. Ling. Plasmonic Colloidosomes as Three - Dimensional SERS Platforms with Enhanced Surface Area for Multiphase Sub - Microliter Toxin Sensing. *Angewandte Chemie International Edition* **54**, 9691 –9695 (2015). DOI: 10.1002/anie.201504027

The contributions of the co-authors are as follows:

- A/Prof Ling and Dr Phang provided the initial research direction and edited the manuscript drafts.
- I prepared the manuscript drafts. The drafts were revised by Dr Lee.
- I performed the synthesis of Ag particles, plasmonic colloidosomes and their characterization. I collected ultraviolet spectra of Ag colloidal particles, scanning electron microscope (SEM) images of Ag and colloidosomes.
- I performed the testing of surface-enhanced Raman scattering (SERS) performance of plasmonic colloidosomes and their sensing ability. I collected, evaluated and processed all Raman data.

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The contributions of the co-authors are as follows:

- A/Prof Ling provided the initial research direction and edited the manuscript drafts.
- I prepared the manuscript drafts. The drafts were revised by Dr Lee.
- I performed the synthesis of Ag particles, plasmonic colloidosomes and their characterization. I collected ultraviolet spectra of Ag colloidal particles, scanning electron microscope (SEM) images of Ag and colloidosomes.

- I performed the reaction of dimethyl yellow protonation on plasmonic colloidosomes and conducted real-time SERS monitoring.
- I collected, evaluated and processed all Raman data.
- I analyzed and performed calculations to determine apparent reaction kinetics.

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- I prepared the manuscript drafts. The drafts were revised by Dr Lee.
- I performed the synthesis of Ag particles, plasmonic colloidosomes and their characterization. I collected ultraviolet spectra of Ag colloidal particles, scanning electron microscope (SEM) images of Ag and colloidosomes.
- Wee, Yang and Feng designed the fluidic chips and performed the initial trials on flowing plasmonic colloidosomes and conducted real-time SERS monitoring using Nanophoton device.
- I optimized and performed online sensing with uRaman device. I collected, evaluated and processed all Raman data.

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- A/Prof Ling, Dr Phang and I provided the initial research direction. A/Prof Ling and I edited the manuscript drafts.
- I prepared the manuscript drafts. The drafts were revised by Dr Lee and Koh.

- I performed the synthesis of Ag particles, plasmonic colloidosomes and their characterization. I collected ultraviolet spectra of Ag colloidal particles, scanning electron microscope (SEM) images of Ag and colloidosomes.
- Teng and Yim examined the nebulizers to introduce Ag particles/plasmonic colloidosomes into the air. Teng performed the initial trials collecting in-air SERS signals.
- Koh assisted in the SEM analysis.
- Dr Tan, Dr Phang and Tok assisted and controlled stand-off Raman systems and in-air SERS measurements.
- I optimized and performed in-air sensing with uRaman and stand-off device. I collected, evaluated and processed all Raman data.

Appendix contains the SERS peak assignment tables in the above published particles.

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With love,

Phan-Quang Gia Chuong

Nanyang Technological University

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Summary

The sensing of toxic pollutants in solution and hazardous vapors in air is highly important for early recognition and prevention of natural disasters, diseases, and terrorism activities. Current commercial sensing methods such as fluorescence, UV-vis and chromatography do not provide sufficient molecular fingerprints of target analytes to prevent false positives from similar molecules. As a temporary solution, surface-enhanced Raman scattering (SERS) has enabled ultratrace detection with highly specific molecular information, yet suffers the need for stringent laser alignment while only capable of static measurements due to the use of rigid 2D substrates. This thesis introduces ‘plasmonic colloidosomes’ – micron-sized water droplets coated with Ag nanoparticles – as 3D substrate-less platforms to tackle the above problems in sensing and SERS spectroscopy. These droplets possess a robust, spherical and highly SERS active plasmonic shells comprising of Ag nanoparticle clusters, allowing their establishment as the first “dual-phase tri-analyte” detection system, as demonstrated in Chapter 2. Such breakthrough in biphasic molecular sensing across liquid-liquid interface allows us to directly investigate ultras-small interfacial reactions. In particular, Chapter 3 discusses the seamless SERS monitoring of dimethyl yellow interfacial protonation performed on plasmonic colloidosomes, which reveals the presence of two highly similar products. Chapter 4 presents the incorporation of plasmonic colloidosomes with online sensing device, realizing the rapid high through-put analysis of multiple samples. A highlight of our work, as described in Chapter 5, is the preparation of the world’s first macroscale 3D SERS ‘in-air sensing’ platform, by incorporating the colloidosomes within an liquid aerosol. These achievements also represent a giant leap towards the potential development of stand-off and substrate-less spectroscopic methodology to detect gas toxins/airborne weapons remotely.

Chapter 1

Introduction: Colloidosomes as 3D SERS platforms

Abstract. Surface-enhanced Raman scattering (SERS) is an attractive detection technique that offers more than 10^{10} -fold amplification in Raman fingerprints of target analytes. Current SERS platforms are predominantly two-dimensional (2D) substrates which suffer drawbacks in terms of low hotspot volume in the z-direction, poor mobility and flexibility to incorporate in other media and having only one SERS active surface. We recognize the immense potential of colloidosomes, nanoparticle-coated emulsion droplets, as highly dynamic and substrate-less 3D SERS platforms upon their fabrication with plasmonic particle building-blocks. Owing to their robust 3D spherical shells, these micron-sized emulsion droplets can address the aforementioned challenges of 2D nanometer-thick SERS platforms. In this chapter, we introduce SERS and the pressing issues with current 2D SERS platforms, followed by the concept of colloidosomes, their fabrication technique, physical properties and applications. We also discuss current works on using colloidosomes as sensors and reactors, which ultimately leads to our motivation and objectives in this thesis – building the world’s first 3D SERS-active colloidosomes for a vast range of SERS-based applications across multiple media.

1.1 Surface-enhanced Raman Scattering (SERS)

Surface-enhanced Raman scattering (SERS) is a non-destructive detection method that enhances Raman vibrational fingerprints of target analytes by up to 10^{10} -fold, using plasmonic nanostructures of Ag, Au, Cu and Al.¹ SERS enhances normally weak Raman signals via two main mechanisms, namely electromagnetic enhancement and chemical enhancement.² Electromagnetic enhancement, affording up to 10^8 -fold enhancement and also the major contributor of SERS, arises from the interaction of light with the collective oscillation of the metal's conduction band electrons, which form localized surface plasmon resonances (LSPRs). This optical phenomenon concentrates strong electromagnetic field at the metal surfaces, which amplifies both excitation light and scattered Raman signals of any molecules that experience this field within a distance of 10 nm (*Figure 1.1*).³ On the other hand, chemical enhancement, contributing up to 10^3 -fold enhancement, results from the increased polarizability of the typically chemisorbed molecule on the plasmonic metal surface due the formation of analyte-metal charge-transfer states.³

Figure 1.1. Electromagnetic enhancement mechanism of SERS. Scheme illustrating the mechanism of the electromagnetic enhancement effect of SERS which amplifies the Raman

molecular vibrational fingerprints of molecules near the plasmonic surface. Adapted from Ref 2 with permission from The Royal Society of Chemistry.

Recent years have witnessed a fast-paced shift away from using conventional methods such as fluorescence and chromatography toward SERS for analytical research and applications. For instance, the past decade birthed 20000 SERS-related research works, while only 13000 works on fluorescence and 2500 works on chromatography techniques in the field of sensing (*data retrieved from Web of Science, from 2011-2018*). Such paradigm shift arises due to SERS's key advantage of affording molecule-specific information via the elucidation of vibrational fingerprints characteristic to each molecule. This important feature allows the differentiation of molecules with similar structures and physical properties, which is a longstanding challenge that plagues conventional analytical methods.¹ Thus, SERS emerges as a highly reliable next generation sensing technique, providing various opportunities in the development of applicable SERS platforms for real-life purposes.

1.2 Development and Challenges of Current Two-dimensional (2D) SERS Platforms

In a bid to greatly improve the applicability of SERS in the last four decades, a great deal of effort has been devoted to the engineering of novel SERS platforms that function in both static and dynamic (flowing liquid or on-going reaction) measurements.⁴ Single 0D nanoparticles (NPs) confine EM field at the nanometer length scale and act as highly localized hotspots for fundamental investigation and sensing in the solution phase using colloidal nanoparticles.⁴ However, due to their small size and limited enhancement as a single particle, these 0D particles are not practical for large-scale detection purposes. On the other hand, better SERS signals arose from aggregates of nanoparticles due to the plasmonic coupling effect,

whereby the electromagnetic fields between the tips and/or edges of neighboring particles are immensely enhanced.³ However, due to the restriction of fabrication methods, most of the aggregated SERS substrates were formed by physical methods directly from colloidal solutions by increasing the ionic bonding among particles.⁴ These aggregates allow ultrasensitive detection down to the single molecule detection, but offer very poor reproducibility in the point to point SERS intensity due to their random aggregation, thus inhibiting quantitative applications.⁵ As a consequence, during the last 15 years, researchers have imparted both sensitivity and reproducibility together within the so-called 2D plasmonic platforms.⁶ Such 2D plasmonic platforms are organized assembly of nanoparticles on solid substrates (typically glass or silicon wafers) and commonly fabricated by lithography colloidal imprinting,⁷ self-assembly,⁸ or Langmuir-Blodgett/Schaefer deposition techniques.^{9,10}

The creation of 2D SERS platforms initiated an emerging trend in utilizing SERS as a rapid detection tool in lieu of conventional analytical methods such as fluorescence due to the much higher sensitivity and molecular-specificity. With up to 10^{14} fold enhancement created by a nanogap hotspot with only a single layer 2D substrate, SERS has found its stance in various sensing scenarios in multiple media, namely the detection of toxins in food samples,^{9,10} the detection of biomarkers in blood,¹¹ or the detection of pollutants in gases.^{12,13} In particular, a superhydrophobic-oleophobic 2D substrate comprising of Ag nanowires has enabled femtomole-level detection of melamine and Sudan I in liquid food samples (*Figure 1.2A*).¹⁰ Besides, SERS-based gas sensing substrates, while less prominent, have laid important milestones in the highly specific recognition of small airborne molecules such as CO, N₂, toluene or even explosives which remain challenging to detect with other sensing techniques (*Figure 1.2B*).¹²⁻¹⁴ These substrates typically comprise of Ag or Au spheres modified with a probe molecule that exhibit spectral changes upon interaction with the small gas molecules. Thus, the detection is often indirect, due to the low Raman cross-section and also the poor

affinity to metal surface of the aforementioned gas molecules which raise difficulties in obtaining direct Raman fingerprints from them.¹⁵

Figure 1.2. 2D SERS substrates. (A) Superhydrophobic-oleophobic 2D substrate comprising of Ag nanowires for femtomole-level detection of melamine in liquid food samples. Adapted with permission from Ref 10. Copyright 2014 American Chemical Society. (B) Ag nanoparticles on a paper allowing the detection of airborne trinitrotoluene. Adapted with permission from Ref 13. Copyright 2014 American Chemical Society.

However, 2D SERS substrates have several drawbacks that hamper their commercial applications. Firstly, the Raman excitation laser confocal volume is a three-dimensional (3D) space that has large depths ranging from sub-micrometers to several millimeters,¹⁶ and this laser volume is severely underutilized by 2D planar SERS substrates that only possess SERS-active hotspots in a single Cartesian plane. Furthermore, background light and airborne

particles are collected in the unutilized laser illumination volume in the z-direction which contributes to signal interferences and results in suppressed analyte SERS intensities and poor signal-to-noise ratio.⁴ Secondly, most 2D planar substrates have only one active plasmonic surface where analytes need to be deposited on that specific surface to be detected and strong signals are achieved only when the excitation laser is perpendicular to the substrate. Hence, these 2D platforms require strict laser focus during measurements whereby any minor misalignment of laser path or its focal plane from the substrate surface can result in significant decrease in signal intensity. Such restrictions raise various challenges in on-site applications using portable Raman spectrometers, where stationary detection set-up and stringent laser alignment are challenging to achieve. In addition, this inflexibility is particularly limiting in the case of gas-phase detection, where the analyte can approach the platform from all directions. Furthermore, substrate-based SERS platforms are bound to solid supports, and thus have poor mobility and unable to be incorporated within non-solid media such as liquid-liquid or liquid-air interfaces,¹⁷ flowing solution,¹⁸ or air,¹⁹ for the detection of highly dynamic/volatile analytes. Therefore, the construction of 3D SERS platforms with extended plasmonic hotspots in all x-, y- and z- directions can effectively address the above issues in laser misfocus tolerance, underutilization of the laser focal depth and having only one active surface.

1.3 Colloidosomes: Formation and Properties

Colloidosomes are 3D spherical micron-sized emulsion droplets coated with nano/sub-micron particles (*Figure 1.3*).²⁰ Possessing the Pickering-emulsion motif of liquid droplets coated with a vast choice of encapsulating solid which grant them corresponding properties, colloidosomes are commonly referred to as miniaturized liquid marbles.⁴ One extraordinary feature of the colloidosome system is the presence of two phases that accompany each other: the inner phase (dispersed phase) and the outer phase (continuous phase).²¹ This equips

colloidosomes with unique characteristics of an emulsion system such as enlarged surface area and interactive dual-phase nature.²²⁻²⁴ The principle of colloidosome formation lies within the self-assembly of particles at the fluid-fluid interfaces to minimize the contact between the two immiscible phases during emulsification that increases the interfacial area.²⁰ Such assembly lowers the total energy of the emulsion system and stabilizes the emulsion.²⁵⁻²⁷ Conventional colloidosomes are typically fabricated in liquid-liquid system,²⁸ yet there are classes of colloidosomes that exist in liquid-air system,^{29,30} or even air-air system (hollow colloidosomes).³¹⁻³³ *Figure 1.3B* displays the first liquid-based colloidosome synthesized with silica particles,²⁸ igniting an era of intensive research on colloidosome fabrication and engineering.

Figure 1.3. Colloidosomes (A) Scheme illustrating the basic formation of colloidosomes as particle-stabilized emulsion droplets. (B) The first colloidosome synthesized with silica particles. From Ref 28. Reprinted with permission from AAAS.

The simplest method of colloidosome preparation is via emulsification of a three-component system including two immiscible phases and encapsulating particles (*Figure 1.3A*) using stirrer, sonicator or homogenizer. The emulsification breaks the original immiscible liquids into emulsion droplets while the particles adsorb and self-assemble at the emulsion interface.²³ Notably, if the particles remain in colloidal state without aggregation during the emulsification, the resulting colloidosome shells are covered with a single layer of unlocked particles.³⁴ Otherwise, if the particles aggregate in the continuous phase during emulsification, the shell will comprise of a disordered and multilayered of particles in form clusters.³⁵ Only if the particles aggregate in the dispersed phase, a rigid shell with ordered particles will be formed.³⁵ However, colloidosomes formed with this simple method possess the least robust shell as the only driving force for the self-assembly of particles is the stabilization of interfacial energy, thus the particles are prone to disintegrate from the shell with time.²⁰ One solution to reinforce the shells formed after emulsification is sintering the colloidosomes with absorbing polymer, adding an extra layer of inter-particle connections besides the non-covalent interactions such as electrostatic and Vander Waals force.^{3,35} Concurrently, click-chemistry is a powerful technique to enhance shell robustness by linking the particles via strong covalent bonds such as amide,³⁶ or azide-alkyne,³⁷ efficiently sealing the shells and allowing colloidosomes to be applied in the field of microencapsulation for flavor and drugs preservation. On the other hand, there are also several fabrication techniques in the preparation of colloidosomes in different templates. The afore-mentioned emulsification method yields colloidosomes in liquid-liquid template, typically water-in-oil or oil-in-water colloidosomes. More unique templates such as water-in-oil-in-water,³⁸ or water-in-water colloidosomes,³⁹ are prepared using multiple-channel microfluidic devices, or aqueous gel-core method.⁴⁰ Furthermore, decanting the continuous phase followed by drying affords liquid-in-air

colloidosomes.⁴¹ Also, a series of hollow air-in-air colloidosomes have been prepared with air-flown method for efficient evaporation of both dispersed and continuous liquids.⁴² Especially, colloidosomes can also be formed with more than one type of encapsulating particles by fusing colloidosomes made from different particles via simple emulsification,⁴³ or electrohydrodynamic coalescence,⁴⁴ resulting in Janus and patchy colloidosomes that possess multifunctional shells.

1.4 Current Applications of Colloidosomes

Colloidosomes sketch various horizons in the field of microcapsules, miniaturized sensing devices and microreactors for their collective advantages such as micron-size, high surface area and most importantly, dual-phase nature. The most common and emerging application of colloidosomes is their use as capsules for encapsulation and controlled release, highly favorable for the preservation and delivery of bioactive molecules in pharmaceuticals, food, cosmetics and agriculture.⁴⁵⁻⁴⁷ Their easy and straight-forward fabrication allows a time- and cost-effective method to mass produce microcapsules in thousand-scale quantity, highlighting the powerful emulsion template of colloidosomes. The robust and tightly sealed colloidosomes shell can become (semi)permeable or fully open to release the encapsulated components based on a variety of external or internal triggering sources (Figure 1E), most common of which are pH,⁴⁶ temperature,³⁰ and light.⁴⁸ For instance, pH responsive colloidosomes have been engineered for the specific pH-controlled encapsulation and release of dextran (*Figure 1.4A*).⁴⁶

Figure 1.4. Current applications of colloidosomes (A) Scheme illustrating the pH-controlled encapsulation and release of dextran with colloidosomes. Republished with permission of The Royal Society of Chemistry, from Ref 46; permission conveyed through Copyright Clearance Center. (B) Microscopic images and fluorescence spectra monitoring the absorption and recognition of fructose with colloidosomes. Republished with permission of The Royal Society of Chemistry, from Ref 49; permission conveyed through Copyright Clearance Center. (C) Scheme and microscopic images of catalytic colloidosomes for the cleavage of a chlorophenol-based molecule and the reaction progress. Adapted with permission from Ref 52 Copyright 2009 Wiley.

Besides, we would also like to highlight the attractive application of colloidosomes as micro-sensors. Its expanded surface area offers an ultrasensitive technique for liquid phase sensing with amplified collective signals,⁴ allowing strong signal enhancement from low

volume sample. Moreover, the encapsulating particles can also be modified with probe molecules that can directly or indirectly interact with target analytes to elude signals.³ While these collective advantageous grant colloidosomes immense potential in the field of sensing, colloidosomes have not been widely utilized as chemical sensors due to the limitation of common readout techniques (UV-Vis and fluorescence) that have poor detection limit and are not adaptable with low volume samples. There has been one report on utilizing colloidosomes for fluorescence-based fructose recognition with embedded host molecules on the colloidosome shells and in the dispersed phase (*Figure 1.4B*).⁴⁹ This methodology sketches new horizons in using microcapsules as novel micron-sized platforms for chemical analysis and detection with the concurrent development of readout techniques.

Colloidosome's ultralow volume of down to only few picoliters has also enabled its use as microreactors to perform small volume chemistry in the last decade.⁵⁰ Researchers exploit the high surface area and small confinement volume of colloidosomes as micro-emulsions to boost reaction efficiency, while imparting them with functional particles to manipulate the reaction conditions. In particular, a family of catalytic colloidosomes offers a miniaturized yet highly efficient platform to perform heterogeneous catalytic reactions.⁵¹ Colloidosomes made from polymer@Au particles are highly thermo responsive and able to be heated up to desired temperatures, allowing reactions with high activation energy to be conducted within the ultrasmall vessels.⁵⁰ Remarkably, the dual-phase template of colloidosomes is immensely helpful in the investigation of interfacial reactions, commonly applied in enzymatic biochemical reactions (*Figure 1.4C*).⁵²

1.5 Motivation and Objectives

The sensing of toxic pollutants in solution and hazardous vapors in air is highly important for early recognition and prevention of natural disasters, diseases, and terrorism attacks.

Current sensing and detection technology typically relies on methods such as chromatography, UV-vis/fluorescence absorption, and mechanical detectors. However, these conventional methods are unable to elucidate the molecular structural information of target analytes, which allows the differentiation of molecules with closely-resembling structures. SERS has enabled ultratrace detection with highly specific molecular information, yet suffers the need for stringent laser alignment while only capable of static measurements due to the use of rigid 2D substrates. As mentioned in section 1.2, while 2D SERS substrates can yield excellent SERS enhancement and sensitivity, there are several other challenges that limit their application as practical on-site sensors, namely poor hotspot density in all spatial directions and poor mobility. Furthermore, substrate-based platforms are unable to be applied in interfacial sensing or in-air sensing, which also hampers its utilization in interfacial or gas-phase reaction investigation.

We recognize the immense potential of colloidosomes as highly functional 3D and substrate-less SERS platform when coated with plasmonic nanoparticles, due to their ensemble of benefits in size, morphology, interfacial area, emulsion nature and robustness. In particular, colloidosomes possess 3D spherical structure and exhibit high surface area. Upon coating with plasmonic particles, the proposed ‘plasmonic colloidosomes’ offer immense 3D SERS-active surface for the sensing of target analytes on both sides of the interface. Such 3D surface is omnidirectional and abundant in all spatial directions and thus does not require stringent laser alignment and focusing. Moreover, colloidosomes inherit the nature of an emulsion and exist as immiscible liquids. Thus, SERS-active particles are embedded right at the interface between two liquids and able to afford SERS fingerprints of analytes present in both sides of the colloidosome shell. This emulsion nature further allows potential applications in reaction investigation at the liquid-liquid interface. Furthermore, colloidosomes are highly robust and can sustain in both liquid and air without any solid substrate support, featuring various

opportunities in their incorporation with detection platforms such as microfluidic or even aerosol for high through-put and gas detection.

Figure 1.5. The proposed venture of SERS from 2D substrates into 3D substrate-less colloidosomes.

In overall, we envision the incorporation of plasmonic particles within the colloidosome shell to form the world’s first 3D SERS-active plasmonic colloidosomes – for molecular sensing in multiple media and conditions (*Figure 1.5*). As elaborated in the previous discussion, such substrate-less 3D plasmonic droplets can solve the aforementioned pressing problems of 2D SERS substrates such as poor spatial hotspot volume, poor mobility, low tolerance to misalignment and the inability to be utilized in dynamic detection platforms. This venture from 2D substrates to 3D substrate-less platforms boosts the efficiency and practicality of SERS as an analytical tool for modern world applications. Herein, the objective of this thesis is to develop a novel class of colloidosomes with plasmonic building blocks and apply them in various sensing tasks. In particular, Chapter 2 introduces the design and optimize of plasmonic colloidosome fabrication technique, followed by the characterization of plasmonic colloidosomes in terms of physical properties and plasmonic properties. We also showcase the first “dual-phase tri-analyte” SERS detection of multiple analytes across the liquid-liquid interface with plasmonic colloidosomes. Chapter 3 discusses the use of plasmonic colloidosomes in the *in situ* SERS monitoring of a biphasic protonation reaction at the water-

decane interface, demonstrating an attractive application of plasmonic colloidosomes as microreactors that allow direct reaction monitoring. Subsequently, Chapter 4 describes the incorporation the plasmonic colloidosomes within a microfluidic channel which realizes on-line high through-put analysis of multiple samples. The colloidosomes are able to fully encapsulate the analyte samples and prevent channel and inter-sample contamination. We also achieve highly accurate quantification of multiple analytes in sequence. Chapter 5 highlights the preparation of the world's first macroscale 3D SERS 'in-air sensing' platform, by incorporating the colloidosomes within a liquid aerosol. The resulting 'aerosolized plasmonic colloidosomes' is an omnidirectional plasmonic aerosol cloud that exhibits intense and uniform SERS response in all x, y and z directions over centimeter lengthscale. We also conclude the thesis with an overview outlook in Chapter 6. Ultimately, our achievements represent a giant leap towards the development of 3D SERS-active structures as next-generation molecular sensors that can adapt to various detection media and conditions.

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Chapter 2

Plasmonic Colloidosomes as Three-dimensional SERS Platforms with Enhanced Surface Area for Multiphase Sub-microliter Toxin Sensing**

Abstract. Colloidosomes are robust microcapsules attractive for molecular sensing owing to their characteristic micron-size, large specific surface area, and dual-phase stability. However, current colloidosome sensors are limited to qualitative fluorogenic receptor-based detection, which restrict their applicability to a narrow range of molecules. Here, we introduce plasmonic colloidosome constructed from Ag nanocubes as an emulsion-based 3D SERS platform. The colloidosomes exhibit excellent mechanical robustness, flexible size tunability, versatility to merge, and ultrasensitivity in SERS quantitation of food/industrial toxins down to sub-femtomole levels. Using just 0.5 μL of sample volumes, our plasmonic colloidosomes exhibit > 3000-fold higher SERS sensitivity over conventional suspension platform. Notably, we demonstrate the first high-throughput multiplex molecular sensing across multiple liquid phases simultaneously.

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2.1 Introduction

Colloidosomes are three-dimensional (3D) spherical microcapsules formed by the self-assembly of colloidal particles at an emulsion interface, whereby a dispersed liquid is encapsulated within a continuous liquid medium.¹⁻⁷ Typically, a microliter volume of dispersed liquid is emulsified into tens of thousands of micrometer-sized liquid core-solid shell structures with a concurrent hundred-fold enhancement in effective surface area.^{1,3-7} The versatility choice of their building blocks also allows easy incorporation of unique properties into colloidosomes,⁸⁻¹⁰ which enables the wide applicability of colloidosomes in controlled delivery and release,¹¹ and small-volume biphasic catalysis.¹²

One less explored yet greatly attractive application of colloidosomes is their use as miniaturized molecular sensing platforms,¹³ owing to their microliter volume, large surface area, and excellent interfacial stability.^{14,15} Previously, fluorescence-based colloidosome sensor has been used to qualitatively detect fructose by embedding colloidosomes with fluorogenic receptors.¹³ However, such method provides limited molecular information and also narrows the choice of detectable analyte. These limitations can be addressed using “plasmonic colloidosomes” constructed from noble metal (Ag or Au) nanoparticles which utilize the surface-enhanced Raman scattering (SERS) effect,¹⁶ arising from the intense electromagnetic field upon incident excitation, to provide highly specific vibrational fingerprint of molecules even at ultratrace concentration.^{17,18}

In addition, the 3D morphology of the plasmonic colloidosomes enables efficient laser interaction due to their high SERS hot spot density across all spatial planes within the laser illumination volume, and also high tolerance toward laser focus misalignment.¹⁹ Colloidosomes also enable direct and simultaneous SERS detection of multiple molecules present in two immiscible phases owing to their robust emulsion-based template. Hence, plasmonic colloidosomes can overcome the general drawbacks of current interfacial SERS

platforms based on liquid-liquid assembly of two-dimensional (2D) nanoparticle monolayer,²⁰⁻²³ such as the latter's under-utilization of laser confocal volume and potential susceptibility to signal fluctuations on thermal/physical agitation.²⁴ The application of plasmonic colloidosomes in interfacial SERS sensing therefore provides an ideal generic molecular sensor that can greatly facilitate on-site detection of multi-phase samples in food and environmental analysis.

Herein, we demonstrate the fabrication of plasmonic colloidosomes and their application as an emulsion-based 3D SERS platform for the multiplex toxin detection in sub-microliter volumes of both water and organic phases. Our fabrication strategy involves the emulsification of immiscible liquids in the presence of plasmonic Ag nanocubes. We first examine their physical and mechanical properties such as shell thickness, durability, ability to merge and size-tunability. We then determine the 3D SERS-active areas of our colloidosomes and subsequently study the single-phase ultratrace and quantitative SERS detection of toxin down to sub-femtomole levels. Our plasmonic colloidosomes are further evaluated for multiplex detection of analytes in the same phase, and also across two immiscible liquid phases.

2.2 Results and Discussion

2.2.1 Preparation and physical properties of plasmonic colloidosomes

We employ Ag nanocubes as the building blocks for the construction of plasmonic colloidosomes because of their strong localized surface plasmons upon excitation by visible light,¹⁷ and also their well-defined tips and edges that produce intense electric field for SERS enhancement.²⁵ Ag nanocubes with excellent monodispersity of ~120 nm (*Figure 2.1*), are functionalized with perfluorodecanethiol (PFDT) to impart omniphobicity,^{24,25} essential for stabilizing our water-in-oil colloidosomes.⁶

Figure 2.1 Characterization of Ag nanocubes. (A) SEM image of as-synthesized Ag nanocubes and (B) its size distribution. (C) Extinction spectrum of colloidal Ag nanocubes. The peaks at 348, 412, 525 and 674 nm can be assigned to octupole (348 nm), quadrupole (412 nm and 525 nm), and dipole resonances (674 nm), respectively.²⁶

Plasmonic colloidosomes are prepared by simply shaking a mixture of 0.5 μL dispersed water in 200 μL continuous decane in the presence of 0.20 mg PFDT-functionalized Ag nanocubes (*Figure 2.2*). Decane is employed as the continuous phase due to their high immiscibility with water (only 10^{-8} g/1 ml) thus creating high interfacial energy which drives the self-assembly of Ag nanocubes at the emulsion surface. Decane also has lighter density than water (0.73 g/mL versus 1 g/mL) and the formed colloidosomes can be sustained within the continuous phase rather than floating on top, making them easier to characterize and conduct measurement on. Moreover, due to its high boiling point (174°C), decane does not evaporate quickly and acts as a protection layer for the colloidosomes under potential heating from laser during SERS measurement. Most importantly, decane exhibits weak Raman signals that are invisible under our experimental laser condition (0.06 mW and 1-10 seconds collection time) The resulting spherical water-in-decane colloidosomes have average diameter of (40 ± 10) μm and possess rough shells composed of Ag nanocube clusters (*Figure 2.3*).

Figure 2.2. Schematic illustrating the formation of plasmonic colloidosomes

Figure 2.3. (A) SEM image of a colloidosome in its native environment (water-in-decane) and (B) its magnified section. (C) SEM image of a dried colloidosome and (D) its magnified section. (E) SERS spectra of colloidosomes formed by Ag nanocubes functionalized with different thiol ligands and poly(vinylpyrrolidone).

We also compare the SERS responses of colloidosomes formed from Ag nanocubes of different surface ligands in terms of background signals, which is crucial for latter application in ultratrace and multiplex detection. Generally, we observe that colloidosomes are readily formed from Ag nanocubes grafted with PFDT, poly(vinylpyrrolidone) and also alkylthiols such as dodecanethiol and hexadecanethiol (*Figure 2.3E*). Colloidosomes formed with perfluorodecanethiol-functionalized Ag nanocubes demonstrate featureless SERS spectrum with weak signals only at 741, 767 and 811 cm^{-1} (< 10 counts/s), possibly due to the weak

Raman activity of C-F bonds. In contrast, alkylthiol- and poly(vinylpyrrolidone)-grafted colloidosomes exhibit strong background signals (> 50 counts/s) across a wide Raman shift window of $500 - 1600\text{ cm}^{-1}$, where molecular vibrational fingerprints typically occur. The strong background interferences are due to the coupling of the metal-sulfur bond stretch with the longitudinal acoustic vibrations of the molecular alkane chain. Hence, it is evident that perfluorodecanethiol is ideal for colloidosomes formation and their subsequent application as highly sensitive and multiplex sensing platforms due to their minimal SERS interferences.

Plasmonic colloidosomes also demonstrate wide size-tunability via the manipulation of the water/Ag nanocubes (shortened as water/Ag) ratio during emulsification. As the water/Ag ratio is increased from 2.5 to 12.5 $\mu\text{L}/\text{mg}$, the colloidosomes' diameter increases linearly from the initial $\sim 40\text{ }\mu\text{m}$ to $\sim 200\text{ }\mu\text{m}$ (*Figure 2.4*). In contrast, the colloidosomes' size is not influenced by decane volume (*Figure 2.5*). The linear relationship between colloidosomes' diameter and water/Ag ratio indicates the presence of an optimal Ag nanocube shell thickness to stabilize the water/decane interface, which we further affirm the consistency of colloidosomes' shell thickness using SEM imaging (*Figure 2.6*). Based on the hollow frames of dried colloidosomes, we approximate the shell thickness at $(1560 \pm 360)\text{ nm}$, which is equivalent to (13 ± 3) layers of Ag nanocube. These closely-packed clusters of Ag nanocubes are essential in generating intense electromagnetic field for Raman enhancement due to plasmonic coupling across all spatial directions (x, y and z).¹⁷ Hence, it is clearly evident that our plasmonic colloidosomes are highly versatile in accommodating a diverse range of microliter volumes, while maintain consistent shell property. Hereupon, we use 40- μm plasmonic colloidosomes fabricated from 2.5 $\mu\text{L}/\text{mg}$ water-Ag ratio for latter discussion, unless otherwise stated, to emphasize the ultralow sample volume required for colloidosome preparation.

Figure 2.4. (A - E) Microscopic images (left) of colloidosomes formed from different water/Ag ratio and their respective size distribution.

Figure 2.5. Effect of decane volume on the formation of plasmonic colloidosomes and their SERS activities. Mass of Ag, volume of water and concentration of methylene blue are fixed at 0.20 mg, 0.5 μL and 10^{-6} M, respectively. SERS intensity is quantified using methylene blue's 1633 cm^{-1} band.

(A) 2.5 $\mu\text{L}/\text{mg}$ water/Ag ratio

(B) 5.0 $\mu\text{L}/\text{mg}$ water/Ag ratio

(C) 7.5 $\mu\text{L}/\text{mg}$ water/Ag ratio

Figure 2.6. SEM images of dried colloidosomes formed using (A) 2.5, (B) 5.0 and (C) 7.5 $\mu\text{L}/\text{mg}$ water/Ag ratio. (i) An overview of the entire colloidosome, and (ii, iii) their magnified sections of the shell. The green dotted lines outline the colloidosomes' shells, which are subsequently used to determine both their thickness and number of Ag nanocube layers present. Calculation of the number of Ag nanocube layers, which is estimated by dividing the mean thickness by the mean Ag cube edge length, assuming the cubes are stacked layer-by-layer: Ag nanocube layers : $1560 \text{ nm}/120 \text{ nm} = \sim 13$ layers

Together with the formation of $> 10^4$ colloidosomes and > 26 -fold enhancement in surface area compared to the original water droplet (calculation below), plasmonic

colloidosomes provide an ultrasensitive and large-area SERS platform for latter ultratrace toxin detection.

Calculation 2.1: Estimation of number of colloidosomes and surface area.

Assuming the original 0.5 μL of water is divided equally to form colloidosomes with average diameter of 40 μm and the Ag shell thickness (1560 ± 360) nm is negligible compared to the droplet size, we estimate the number of colloidosomes formed per fabrication as followed:

Total number of colloidosomes per fabrication

$$\begin{aligned} N_{\text{colloidosome}} &= V_{\text{water}} / V_{\text{colloidosome}} \\ &= V_{\text{water}} / [4(d / 2)^3 \pi / 3] \\ &= 0.5 \mu\text{L} / (4(38 \mu\text{m} / 2)^3 \pi / 3) \\ &= 17402 \end{aligned}$$

where N, V, S and r refer to number of colloidosomes, volume, surface area and radius, respectively.

Total surface area of colloidosomes

$$\begin{aligned} S_{\text{colloidosome, total}} &= N_{\text{colloidosome}} \times S_{\text{colloidosome, individual}} \\ &= 17402 \times [4\pi(38 \mu\text{m} / 2)^2] \\ &= 7.9 \times 10^{-3} \text{ dm}^2 \end{aligned}$$

Total surface area of original water droplet prior to colloidosome formation

$$\begin{aligned} r_{\text{droplet}} &= [V_{\text{water}} / (4 \times \pi / 3)]^{1/3} \\ &= [0.5 \mu\text{L} / (4 \times \pi / 3)]^{1/3} \\ &= 4.9 \times 10^{-3} \text{ dm} \\ S_{\text{water droplet}} &= 4\pi(r_{\text{droplet}})^2 \\ &= 4\pi(4.9 \times 10^{-3} \text{ dm})^2 \end{aligned}$$

$$= 3.0 \times 10^{-4} \text{ dm}^2$$

Overall increase of surface area on colloidosome formation

$$S_{\text{colloidosome}} / S_{\text{water droplet}} = (7.9 \times 10^{-3}) \text{ dm}^2 / (3.0 \times 10^{-4}) \text{ dm}^2 = 26 \text{ times}$$

(end of Calculation 2.1)

2.2.2 Plasmonic activity of plasmonic colloidosomes

We perform x-y and x-z hyperspectral SERS imaging of individual colloidosomes containing methylene blue dye (1 μM) to identify their SERS active area. From the x-y SERS imaging, we observe that the SERS-active regions (brightly-lit area in *Figure 2.7A–C*) of plasmonic colloidosomes exhibit characteristic vibrational modes of methylene blue (*Figure 2.8, Table 1 in Appendix*), which resembles the normal Raman spectrum of the same molecule without Ag nanoparticles (*Figure 2.9*). The featureless SERS spectrum obtained in the absence of the dye affirms that the observed signals originate from the target analyte and not from the surface ligands or solvent.

Figure 2.7. SERS imaging of plasmonic colloidosomes. Schematics (top) and corresponding x-y SERS images (bottom) when laser is focused (A) on top, and (B) at the mid-

plane of the colloidosome. (C) Schematics (top) and the corresponding x-z SERS image (bottom) image of colloidosome. (D) SERS intensity-distance profile along the white dotted line of (B), from (1) to (2). (A–D) uses 20× objective lens. (E) SERS image of colloidosomes using 4× objective lens. All the colloidosomes contain methylene blue 10^{-6} M and are submerged in decane.

Using the most intense C=C stretching mode of methylene blue at 1633 cm^{-1} (*Table S1 in Appendix*), we observe a strong dependency of the SERS-active areas on the confocal plane of excitation laser when using a 20× objective with $\sim 3.6\text{ }\mu\text{m}$ resolvable resolution. For instance, focusing the laser confocal plane at the top and mid-plane of plasmonic colloidosome result in strong SERS responses ($\sim 4900\text{ counts/s}$) mainly confined to the top and edge of colloidosome (Figure 2.7A-B, D), respectively. Subsequent x-z SERS imaging of the plasmonic colloidosomes further exemplifies the localization of strong SERS-activity ($\sim 4720\text{ counts/s}$) to the Ag nanocube shell (Figure 2.7C).¹⁷ Collectively, both the x-y and x-z SERS images highlight the applicability of entire colloidosome's shell for SERS detection. This is again illustrated using a wide-field and higher depth field 4× objective lens where the whole 3D plasmonic shell of colloidosomes exhibits strong SERS activity ($\sim 4000\text{ counts/s}$), even at attomole level of methylene blue (Figure 2.7E, calculation below). This features an attractive analytical enhancement factor (AEF) of $> 10^6$ arising from the plasmonic colloidosome shell.

Figure 2.8. (A) SERS spectra and (B) SERS intensity of the 1633 cm^{-1} signal methylene blue encapsulated in plasmonic colloidosomes with concentration ranging from 5 fmol to 5 pmol. Control refers to blank colloidosomes in the absence of methylene blue.

Figure 2.9. Normal Raman spectrum of aqueous methylene blue solution (10^{-2} M), with an exposure time of 100s.

Calculation 2.2: Estimation of average number of molecules in one colloidosome and analytical enhancement factor (AEF)

Basis: $0.5 \text{ }\mu\text{L}$ of 10^{-6} M methylene blue solution to form colloidosomes.

Mole of methylene blue present = $5 \times 10^{-13} \text{ mol}$

Total number of colloidosomes = 17402.

Estimated number of molecules in one colloidosome = $[(5 \times 10^{-13}) \times (6.022 \times 10^{23})] / 17402$

With reference to the 1633 cm^{-1} SERS band, we calculate the analytical enhancement factor of methylene blue detection as followed:

$$\begin{aligned}\text{Analytical EF} &= [(I_{\text{SERS}}) / (I_{\text{Raman}})] \times [(C_{\text{Raman}}) / (C_{\text{SERS}})] \\ &= [7 / (831 / 100)] \times (10^{-2} / 10^{-9}) \\ &= 8 \times 10^6\end{aligned}$$

where N_{SERS} and N_{Raman} are the corresponding concentrations measured using plasmonic colloidosomes (10^{-9} M ; $5 \times 10^{-16} \text{ mol}$ over $0.5 \text{ }\mu\text{L}$) and normal Raman of methylene blue (10^{-2} M , $5 \times 10^{-9} \text{ mol}$ over $0.5 \text{ }\mu\text{L}$), respectively. I_{SERS} and I_{Raman} are the time-normalized intensities measured using SERS and normal Raman, respectively, at their corresponding concentration.

(end of calculation 2.2)

Furthermore, consistent SERS activities are observed from colloidosomes of varying sizes (*Figure 2.10*) and also over an extended storage duration of > 340 hours (*Figure 2.11*), highlighting that plasmonic colloidosomes are durable and produce reproducible signals for various sample volumes.

Figure 2.10. Top-plane SERS images of colloidosomes of different sizes, produced from (A) 2.5 (B) 5.0 and (C) 7.5 $\mu\text{L}/\text{mg}$ of water/Ag ratio. All the colloidosomes contain 10^{-6} M aqueous methylene blue solution. (D) Comparison of SERS intensities of methylene blue across different colloidosomes' sizes.

Figure 2.11. (A) SERS image of colloidosomes encapsulating methylene blue (10^{-6} M) (i) after 340 hours and (ii) freshly prepared, with representative pixels indicating the intensity of 1633 cm^{-1} signal in counts/s. Scale-bar is 25 μm . (B) SERS spectra of methylene blue (10^{-6} M) encapsulated within plasmonic colloidosomes when freshly prepared (bottom) and after storage in decane for 340 hours (top).

2.2.3 Mixing of plasmonic colloidosomes for multiplex detection

The mechanically robust plasmonic colloidosomes also demonstrate excellent versatility via their ability to merge upon re-emulsification to homogenize their encapsulated aqueous content (*Figure 2.12A*). When two plasmonic colloidosomes are placed together, each separately containing methylene blue ($1\ \mu\text{M}$) and rhodamine 6G ($1\ \mu\text{M}$), SERS bands unique to methylene blue ($456\ \text{cm}^{-1}$ and $503\ \text{cm}^{-1}$) and rhodamine 6G ($620\ \text{cm}^{-1}$, *Table S3 in Appendix*) are observed distinctly in the respective colloidosomes (*Figure 2.12C*) with no apparent cross-transfer of analytes. Upon re-emulsification (*Figure 2.12D*), the colloidosomes merge and equally homogenize their encapsulated contents into newly merged colloidosomes (*Figure 2.13*), which exhibit a multiplex SERS spectrum, consisting of signals characteristic to both methylene blue and rhodamine 6G at $456\ \text{cm}^{-1}$ and $620\ \text{cm}^{-1}$, respectively (*Figure 2.12B, D*)

Figure 2.12. (A) Schematic illustrating the merging of colloidosomes. (B) SERS spectra for the multiplex detection of methylene blue and rhodamine 6G in a single colloidosome (top),

and also individual detection of methylene blue (middle) and rhodamine 6G (bottom) in separate colloidosomes. (C) SERS images overlaid with corresponding optical image of two distinct colloidosomes before mixing. (D) SERS image overlaid with optical image of a colloidosome containing both analytes after mixing. SERS images are color-indexed using blue and red for methylene blue (i; 456 cm^{-1}) and rhodamine 6G (ii, 620 cm^{-1}), respectively. Magenta color-indexed (iii) denotes the presence of both methylene blue and rhodamine 6G in a single colloidosome.

Figure 2.13. Histogram representing the SERS intensities of methylene blue (blue; 456 cm^{-1} SERS band) and rhodamine 6G (red; 620 cm^{-1} SERS band) for different detection schemes, before and after merging.

Using the characteristic SERS bands at 456 cm^{-1} and 620 cm^{-1} , the individual (non-merged) colloidosomes exhibit SERS intensity of $\sim 980\text{ counts/s}$ for methylene blue ($1\text{ }\mu\text{M}$), and $\sim 1130\text{ counts/s}$ for rhodamine 6G ($1\text{ }\mu\text{M}$), respectively (*Figure 2.12*). Whereas the merged colloidosome demonstrates the SERS intensities of both methylene blue and rhodamine 6G at $\sim 790\text{ counts/s}$ and $\sim 750\text{ counts/s}$ (*Figure 2.12*). These decrease of SERS intensities are due to a two-fold reduction of analyte concentrations upon merging, which are also quantitatively

tallied to a control colloidosome containing both 0.5 μM of methylene blue and 0.5 μM of rhodamine 6G (*Figure 2.13*). Hence, it is clearly evident that the merging the plasmonic colloidosomes leads to homogeneous distributions of analytes in the aqueous phase. Such ability to merge and homogenize aqueous content via re-emulsification is crucial in the field of sensing and micro-reaction, where additional species such as reactants and spectroscopic reference can be easily added into colloidosomes on demand.

2.2.4 Dual-phase tri-analyte sensing with plasmonic colloidosomes

The plasmonic colloidosomes also excel in multiplex detection and quantification of toxins simultaneously across a liquid-liquid interface. To demonstrate a triplex interfacial detection, we separately encapsulate malachite green (5 pmol, 9% mol) and rhodamine 6G (0.5 pmol, 1% mol) in plasmonic colloidosomes and mix them in a decane solution containing dimethyl yellow (50 pmol, 90% mol; *Figure 2.14B*, see *Calculation 2.3*). The overall multiplex SERS spectrum in *Figure 2.14C* exhibits distinguishable fingerprint signals that can be indexed to individual analytes; 808 and 929 cm^{-1} for malachite green, 620 cm^{-1} for rhodamine 6G and 483, 739 and 905 cm^{-1} for dimethyl yellow (*Table S2-S5 in Appendix*). When the characteristic SERS band of malachite green at 808 cm^{-1} is selected, only a portion of the colloidosomes (cyan) demonstrate SERS activity (*Figure 2.14D*). Likewise, when the SERS band of rhodamine 6G 620 cm^{-1} is highlighted, high SERS activities are observed on the remaining portion of colloidosomes (red; *Figure 2.14D*). On the other hand, the selection of SERS band at 905 cm^{-1} , which is unique only to dimethyl yellow in the exterior phase, reveals that all the colloidosomes (yellow) demonstrate SERS responses to the common organic-soluble analyte, (*Figure 2.14E*). Most importantly, the intensities of the selected SERS bands of different analytes in the multiplex detection can be quantitatively matched within the standard of deviation of those observed in the individual detection scheme (*Figure 2.15*). This highlights

the ability to quantify both absolute amount and relative percentage of each toxins in a mixture. To the best of our knowledge, this is the first interfacial SERS sensing using a miniaturized “dual-phase tri-analyte” multiplex detection set-up. This is an advancement to existing interfacial SERS methods, which are typically restricted to the determination of relative concentrations between two analytes only, possibly due to signal fluctuations and also relatively larger SERS platform size that is unsuitable for the analysis of additional analyte compartment.²⁴ It is therefore evident that the excellent mechanical robustness and interfacial stability, large specific 3D SERS-active area, and micron-sized of colloidosomes are all crucial factors in achieving highly-sensitive, miniature SERS platform capable of complex sensing and quantification of multiple analytes and/or liquid phases.

Figure 2.14. (A) SERS intensity at various mole numbers of malachite green, rhodamine 6G and dimethyl yellow in their individual detection. (B) Schematic depicting the “dual-phase tri-analyte” multiplex detection using plasmonic colloidosomes. (C) SERS spectra of multiplex detection (black) and also individual detections of malachite green (green), rhodamine 6G (red) and dimethyl yellow (yellow). SERS images of multiplex detection using plasmonic

colloidosomes when characteristic bands of (D) malachite green (cyan) and rhodamine 6G (red), and also (E) dimethyl yellow (yellow) are selected.

Calculation 2.3: Calculation of relative percentage of each toxin and accuracy of the multiplex detection.

Our multiplex detection in Figure 2.14C is performed with 5 pmol malachite green, 0.5 pmol rhodamine 6G and 50 pmol dimethyl yellow. The quantification of each toxin is performed as follow:

- **Malachite green** : %Malachite green = $5/(5 + 0.5 + 50) = 9\%$ mol

In the individual detection, 5 pmol malachite green afford (89 ± 23) counts/s for its 808 cm^{-1} signal. In the multiplex detection, 5 pmol malachite green afford (101 ± 9) counts/s, which is within the standard of deviation of the individual sensing.

- **Rhodamine 6G** : %Rhodamine 6G = $0.5/(5 + 0.5 + 50) = 1\%$ mol

In the individual detection, 0.5 pmol rhodamine 6G afford (75 ± 18) counts/s for its 620 cm^{-1} signal. In the multiplex detection, 0.5 pmol rhodamine 6G afford (80 ± 20) counts/s, which is within the standard of deviation of the individual sensing.

- **Dimethyl yellow** : %Dimethyl yellow = $50/(5 + 0.5 + 50) = 90\%$ mol

In the individual detection, 50 pmol dimethyl yellow afford (27 ± 9) counts/s for its 905 cm^{-1} signal. In the multiplex detection, 50 pmol dimethyl yellow afford (29 ± 15) counts/s, which is within the standard of deviation of the individual sensing.

(end of Calculation 2.3)

Figure 2.15. Comparison of SERS intensities of malachite green (green; 808 cm^{-1}), rhodamine 6G (red; 620 cm^{-1}) and dimethyl yellow (yellow; 905 cm^{-1}) in both the individual and multiplex detection.

2.3 Conclusion

In conclusion, we have constructed size-tunable plasmonic colloidosomes using Ag nanocube as emulsion-based 3D multiplex SERS sensing platforms for ultralow sample volumes. Our plasmonic colloidosomes are capable of ultratrace detection of both aqueous- and organic-soluble toxins down to sub-femtomole levels, and also quantitative analysis over 5-order of magnitudes, all using just sub-microliter sample volumes. By exploiting the micron-sized plasmonic colloidosomes and their large and reproducible SERS enhancement of $> 10^6$ -fold, we demonstrate the first “dual-phase tri-analyte” interfacial detection scheme for the high through-put detection and quantification of three analytes across multiple liquid phases simultaneously. The ensemble of benefits of plasmonic colloidosomes enables them as an immensely attractive, miniaturized SERS analytical platform crucial for on-site detection in the field of forensic and also industrial, food and environment safety.

2.4 Materials and Methods

Materials. Silver nitrate ($\geq 99\%$), anhydrous 1,5-pentanediol (PD, $\geq 97\%$), poly(vinyl pyrrolidone) (PVP, average MW = 55,000); 1H,1H,2H,2H-perfluorodecanethiol (PFDT, $\geq 97\%$), rhodamine 6G (R6G, dye content $\sim 95\%$), decane (anhydrous, $> 99\%$), methylene blue (MB, $\geq 82\%$), coumarin 30 (99%; simplified as coumarin for discussion), malachite green (MG, oxalate salt, dye content $\geq 90\%$) and dimethyl yellow (DY, analytical standard, $\geq 98\%$) were purchased from Sigma Aldrich; copper (II) chloride ($\geq 98\%$) was from Alfa Aesar; ethanol (ACS, ISO, Reag. Ph Eur) was from EMSURE®; toluene (BAKER ANALYZED® A.C.S. Reagent) was from Avantor; propan-2-ol (HPLC grade) was from Fisher Scientific. All chemicals were applied without further purification. Milli-Q water ($> 18.0\text{ M}\Omega\cdot\text{cm}$) was purified with a Sartorius Arium® 611 UV ultrapure water system.

Synthesis and purification of silver nanocubes. The preparation of silver (Ag) nanocubes was carried out based on the polyol method described in literature.²⁷ 10 mL PD solutions of CuCl_2 (8 mg/mL), PVP (20 mg/mL) and AgNO_3 (20 mg/mL) were prepared separately by sonication and vortex. 35 μL CuCl_2 solution was added to the AgNO_3 solution. 250 μL PVP precursor was added dropwise every 30 s while 500 μL AgNO_3 precursor was injected every min using a quick addition to a 10 minute-preheated 20 mL PD solution. The addition was continued until the mixture turned orange brown. For the purification, PD was first removed by washing the mixture with acetone followed by ethanol. The suspension was then dispersed in 10 mL ethanol and 100 mL aqueous PVP solution (0.2 g/L) and filtered using Durapore®polyvinylidene fluoride filter membranes (Millipore) with pore sizes ranging from 5000 nm, 650 nm, 450 nm and 220 nm, several times for each pore size. SEM imaging was performed, from which the edge lengths of 250 Ag nanocubes were measured and analyzed using ImageJ software. The as-synthesized nanocubes were found to be obtained in high yield of approximately 40 mg/synthesis.

Functionalization of Ag nanocubes with perfluorodecanethiol. 20 mg of purified Ag nanocubes were immersed in 10 mL of 1:1 propan-2-ol/hexane solution containing 0.1 mM of 1H,1H,2H,2H-perfluorodecanethiol (PFDT) for 6 h at room temperature. The colloidal suspension was then washed with copious amounts of ethanol and decane, and subsequently dispersed in 1.0 mL of decane.

Preparation of colloidosomes. 0.5 – 5.0 μL ultrapure water was added to 200 μL decane suspension containing 0.10 mg – 0.2 mg perfluorodecanethiol-functionalized Ag nanocubes or 0.75 mg perfluorodecanethiol-functionalized Ag octahedra. Colloidosomes were formed by emulsification via vigorous shaking.

Mixing of colloidosomes. Colloidosomes containing rhodamine 6G and colloidosomes containing methylene blue were all transferred into 200 μL of decane. The colloidosomes were subsequently merged via re-emulsification on vigorous shaking.

SERS analysis of analytes using colloidosomes. Methylene blue (3.20 mg/mL, 10^{-2} M), rhodamine 6G (4.79 mg/mL, 10^{-2} M), malachite green (4.63 mg/mL, 10^{-2} M) were prepared in aqueous solution using ultrapure water. Coumarin (0.34 mg/mL, 10^{-3} M) was prepared in 10 % (v/v) toluene/decane solution, dimethyl yellow (2.25 mg/mL, 10^{-2} M) was prepared in decane solution. Serial dilutions were performed to give a series of concentrations, ranging from 10^{-3} M to 10^{-12} M.

For SERS detection involving 20 $\times$ (N.A. 0.45) objective lens: The as-prepared plasmonic colloidosomes were dispensed onto a 3 $\times$ 3 mm silicon substrate and submerged in 5.0 μL of decane. The laser was focused at the top and edge of the colloidosome. Both the colloidosome formation and SERS imaging for different analytes and concentrations were performed at least thrice to ensure the reproducibility of the SERS signal. All SERS intensities and spectra were obtained by averaging an area at least 20 individual spectrum in the hyperspectral map. *Under our data collection mode which collectively averages large areas

multiple pixels in the hyperspectral image, we are obtaining signals from the total number of analyte molecules within a wide area of multifold hotspots – instead of measuring the number of analyte molecules within a single laser spot. Therefore, due to the measurement of total analyte molecule numbers, we are able to obtain linear relationship over a wide concentration range as the total intensity increases as the total number of analytes increases. This is different from the data collection method whereby single data points (featuring single laser spots) are taken, which reflects the density of analyte molecules within the laser spot and results in a non-linear range at low concentration, because the molecule density per laser spot might remain the same while total number of molecules increases*

For SERS detection involving 4× (N.A. 0.13) objective lens: All the colloidosomes were dispensed onto a 3×3 mm silicon substrate and submerged in 5.0 μL of decane. Both the colloidosome formation and SERS imaging for different analytes and concentrations were performed at least thrice to ensure the reproducibility of the SERS signal. All scan area were pre-defined at $1850 \times 1350 \mu\text{m}$ and completely filled with colloidosomes for comparison across various analyte concentrations. All SERS intensities and spectra were obtained by averaging all the spectra (400×300 spectra) in the scanned area.

For control SERS experiment using suspension method: A suspension prepared from 2.0 mg of perfluorodecanethiol-functionalized Ag nanocubes in 50 μL of coumarin solution was evenly sonicated. Raman spectra from 5.0 μL of the suspension were collected at least thrice using the 4× (N.A. 0.13) objective lens. The mass of Ag and volume of coumarin solution used in this control were identical to the detection using colloidosome to ensure fair comparison. SERS intensities and spectra were obtained by averaging all the spectra in the scanned area ($1850 \times 1350 \mu\text{m}$).

For normal Raman evaluation of various analytes: Raman spectra were collected using the 4× (N.A. 0.13) objective lens from 0.5 μL of the respective analyte solution;

methylene blue, rhodamine 6G, malachite green in aqueous solution; dimethyl yellow and coumarin in decane and toluene/decane solution (1:9 v/v), respectively. Raman intensities and spectra were obtained by averaging all the spectra in the scanned area ($1850 \times 1350 \mu\text{m}$).

Material characterization. SEM imaging was performed with JEOL-JSM-7600F microscope and QX-102 capsules.²⁸ UV-vis spectroscopic measurements were conducted with a Cary 60 UV-Vis spectrometer. Microscopic images were taken with Olympus BX51 microscope under $10\times$ (0.30 BD) objective lens. SERS measurements were performed using both x-y and x-z imaging mode of the Ramantouch microspectrometer (Nanophoton Inc, Osaka, Japan) with an excitation wavelength of 532 nm. When using $20\times$ (N.A. 0.45) objective lens, laser power was set at $64.4 \mu\text{W}$ with 1 s accumulation time (unless otherwise stated) for data collection between 200 cm^{-1} to 1800 cm^{-1} . All SERS spectra and intensities were obtained by averaging at least 20 individual spectra from each SERS image. When using $4\times$ objective lens (N.A. 0.13), laser power was set at $133.3 \mu\text{W}$ with 1 s accumulation time (unless otherwise stated) for data collection between 200 cm^{-1} to 1800 cm^{-1} . All SERS spectra and intensities were obtained by averaging at least 3 average spectra of the entire scanned area of $1850 \times 1350 \mu\text{m}$ (equivalent to 400×300 spectra).

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Chapter 3

Isolating Chemical Reactions at the Picoliter-scale: Parallel Control of Reaction Kinetics at the Liquid-liquid Interface**

Abstract. Miniaturized liquid-liquid interfacial reactors offer enhanced surface area and rapid confinement of compounds of opposite solubility, yet unable to provide *in situ* molecular-level reaction monitoring at the interface. Here we utilize plasmonic colloidosomes with Ag octahedra strategically assembled at the water-in-decane emulsion surface as a pico-reactor to perform reactions at the liquid-liquid interface. Our plasmonic colloidosomes can isolate ultrasmall amount of <200 pL solutions, for parallel monitoring of multiple reactions simultaneously. We are also able to *in situ* monitor the interfacial protonation of dimethyl yellow on multiple colloidosomes containing different reaction environments via hyperspectral SERS imaging. Furthermore, the obtained SERS spectra allows us to resolve the presence of structural-isomeric products of similar physical properties, which is otherwise indiscernible by other analytical method.

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3.1 Introduction

Colloidosomes inherit the characteristics of an emulsion system, including dual-phase nature, large surface area and remarkable interfacial stability.^{1,2} They can be imparted with additional functionality via the choice of nanoparticles used.³ Notably, an attractive application of colloidosomes is their use as liquid-liquid interfacial picoreactors,^{4,5} due to their unique confinement of multiple reagents of opposite solubility within close proximity at their permeable shells.⁶ Furthermore, the enhanced interfacial area of such picoreactors facilitates diffusion-limited phase transfer of molecules and heat across an interface,⁵ and improves interfacial reaction efficiency by tens of order of magnitude compared to macroscopic systems.^{2,7,8} For instance, several Diels-Alder reactions are performed in ultra-small capsules without the need to alter the temperature of the bulk solvent due to the capsule's ability to confine temperature and trigger reactions.⁷ However, a major drawback of current colloidosome reactors is their reliance on *ex-situ* monitoring techniques which could not offer vital molecular information.⁹ Consequently, it is not possible to study the intrinsic reaction dynamic and differentiation of physically-similar isomeric species in the reaction's native biphasic environment.

Plasmonic colloidosomes constructed from noble metal nanoparticles are excellent three-dimensional (3D) surface-enhanced Raman spectroscopy (SERS) platforms that tackle the aforementioned limitations.¹⁰ Equipped with high density of intense SERS hotspots, ultrasensitive plasmonic colloidosomes provide specific molecular vibrational fingerprints simultaneously across immiscible liquids.¹⁰ Their ability to compartmentalize various liquids creates immense potential as a pico-scale reactor for parallel reaction screening.¹¹ Most importantly, they enable *in-situ* tracking of molecular events directly at the native liquid-liquid interface, which is crucial for elucidating reaction kinetics and mechanism,^{12,13} and ultimately allows the discovery and optimization of new reaction pathways in synthetic chemistry.¹⁴

Herein, we fabricate Ag octahedra-stabilized plasmonic colloidosome to confine picoliter of liquids for parallel monitoring of multiple liquid-liquid interfacial reactions. Our model reaction is the interfacial protonation of dimethyl across the decane-water interface. Together with computational density functional theory (DFT) simulation, our *in-situ* SERS highlights the successful differentiation and quantification of two unprecedented isomeric products, exemplifying the superiority of plasmonic colloidosome over conventional high performance liquid chromatography (HPLC) technique in resolving physically-similar isomers. Correspondingly, the reaction order and its kinetics are determined based on the consumption of H⁺ protons over trials with multiple pH values, which exemplifies the excellent quantitative SERS capability of plasmonic colloidosome. The high through-put monitoring of interfacial reaction is also demonstrated on multiple colloidosomes placed in a single organic phase.

3.2 Results and Discussion

3.2.1 Preparation and properties of plasmonic colloidosomes with Ag octahedra

Ag octahedra nanoparticles of (350 ± 40) nm (*Figure 3.1*) are used as encapsulating solids for the fabrication of plasmonic colloidosomes due to their highly efficient light scattering effect and high adaptability to 785 nm laser (which avoids the oversensitive surface-enhance resonance-Raman scattering (SERRS) effect of protonated dimethyl yellow under 532 nm laser).¹⁵ Colloidosomes are prepared by intense emulsification of micro-liter aqueous droplet in colloidal decane suspension of hydrophobic perfluorodecanethiol-grafted Ag octahedra (*Figure 3.2*). The as-formed spherical colloidosomes exhibit an average diameter of (72 ± 20) μ m (*Figure 3.2B*) with closely-packed plasmonic shells comprising of (5 ± 1) layers of Ag octahedra (*Figure 3.2C, D*). Furthermore, plasmonic colloidosomes demonstrate the strongest SERS activities at their Ag shell which is capable of sensing methylene blue down to ~ 20 attomole level (*Figure 3.2E-G, 3.3*), corresponding to an analytical enhancement factor

of 10^5 (Figure 3.3, see Calculation 3.1). This is attributed to the high density of intense SERS hot spot arising from closely-packed Ag octahedra clusters across the colloidosome's three-dimensional surface. Due to their size-independent SERS sensitivity, from hereon, we use plasmonic colloidosomes of diameter $(72 \pm 20) \mu\text{m}$, corresponding to $\sim 195 \text{ pL}$, for our latter reaction control and study.¹⁰

Figure 3.1. Characterization of Ag octahedra. (A) SEM image of as-synthesized Ag octahedra and (B) its size distribution. (C) Extinction spectrum of colloidal Ag octahedra. The peaks in the 400 - 600 nm window are assigned as hexapole and higher order resonances and the broad peak at $\sim 810 \text{ nm}$ is assigned as quadrupole resonance.¹⁶

Figure 3.2. Fabrication and characterization of plasmonic colloidosomes (A) Schematic illustration of the formation of plasmonic colloidosomes with PFDT-grafted Ag octahedral and their (B) microscopic images. SEM images of (C) a hollow colloidosome shell and (D) the magnified segment of the dash yellow box in (C). x-y SERS images of a colloidosome encapsulating methylene blue 10^{-6} M when laser is focused on its (E) top and (F) mid-plane. (G) SERS intensity-distance profile along the white dotted line of (F), from (1) to (2).

Figure 3.3. (A) SERS spectra and (B) normal Raman spectrum (200s exposure time) of methylene blue obtained using colloidosomes, and water droplet respectively. (C) SERS intensity of the 1633 cm^{-1} signal methylene blue encapsulated in plasmonic colloidosomes with concentration ranging from 20 amol to 0.2 pmol. Control refers to blank colloidosomes in the absence of methylene blue.

Calculation 3.1. SERS enhancement factor of Ag octahedra colloidosomes.

SERS measurements are conducted on single colloidosomes with diameter of $(72 \pm 20) \text{ nm}$, or volume of $\sim 195 \text{ pL}$. For example, a single colloidosome encapsulating 10^{-7} M methylene blue solution contains $\sim 2 \times 10^{-17}$ mole methylene blue. We report the methylene blue concentration in its actual mole number to emphasize on the ultratrace amount of analyte that can be detected with plasmonic colloidosome platform.

With reference to the 1633 cm^{-1} SERS band, we calculate the analytical enhancement factor of methylene blue detection as followed:

$$\begin{aligned}
 \text{Analytical EF} &= [(I_{\text{SERS}}) / (I_{\text{Raman}})] \times [(C_{\text{Raman}}) / (C_{\text{SERS}})] \\
 &= [(7/10) / (122 / 200)] \times (10^{-2} / 10^{-7}) \\
 &= 10^5
 \end{aligned}$$

where N_{SERS} and N_{Raman} are the corresponding concentrations measured using plasmonic colloidosomes (10^{-7} M; 2×10^{-17} mol encapsulated as per colloidosome) and normal Raman of methylene blue (10^{-2} M), respectively. I_{SERS} and I_{Raman} are the time-normalized intensities measured using SERS and normal Raman, respectively, at their corresponding concentration.

3.2.2 Performing miniaturized interfacial protonation of dimethyl yellow in plasmonic colloidosomes.

We use plasmonic colloidosome to conduct a miniaturized interfacial protonation of dimethyl yellow (DY) at the decane-water interface (*Figure 3.4*), to demonstrate its dual-functionality as a permeable picoreactor for liquid-liquid interfacial reaction and also an *in-situ* SERS monitoring platform. The reaction generally involves the protonation of organic-soluble DY (yellow; in decane) into aqueous-soluble protonated dimethyl yellow (red; HDY^+) at the interface, followed by the diffusion of HDY^+ into the encapsulated aqueous phase (*Figure 3.5*). It is also noteworthy that the interfacial protonation of DY happens only at pH values lower than their pKa value of 3.3.¹⁷ Such interfacial protonation reactions are important and widely applied as a separation technique to extract organic molecules from organic phase into aqueous phase for purification or latter characterization.¹⁸ However, there has been no report on the quantitative examination of the kinetics and molecular events occurring at the native interfacial reaction site.

Figure 3.4. Interfacial protonation of dimethyl yellow across the colloidosome shell. (A) Schematic illustration of the interfacial protonation of dimethyl yellow (DY - yellow) to form protonated dimethyl yellow (HDY⁺ - red) on colloidosomes encapsulating pH 1 solution. (B) Molecular scheme of the interfacial protonation of dimethyl yellow across the interface. (C) SERS spectrum obtained on colloidosomes encapsulating pH 1 submerged in DY 10⁻² M solution in decane with time. Blank control refers to colloidosome encapsulating the same pH solution in pure decane. HDY⁺ SERS spectrum was obtained with colloidal Ag solution. (D) SERS images of a colloidosome encapsulating pH 1 in dimethyl yellow solution at (i) 1 min and (ii) 15 min, with both DY's 1150 cm⁻¹ (yellow-indexed) and HDY's 1283 cm⁻¹ (red-indexed) signals are specified.

Figure 3.5. (A) HDY⁺ 10⁻² M solution in aqueous pH 1 solution and DY 10⁻² M solution in decane. Neutral DY form is only soluble in organic decane solvent and while charged HDY⁺ form is soluble in acidic aqueous solution. (B) Protonation and diffusion of DY across the decane-water interface performed in conventional two-phase system: (i) formation of HDY⁺ in the aqueous phase when pH 1 is used and (ii) no observable reaction when pH 7 is used. The two forms of dimethyl yellow are characterized by distinct optical characteristics, namely the different maximum absorption wavelengths at 435 nm and 515 nm, for DY and HDY⁺, respectively.¹⁹

Our *in-situ* SERS measurements are performed with time-dependent x-y SERS imaging with laser focused on top of the colloidosome over 25 min (*Figure 3.4C*; *Figure 3.6*), aiming to investigate the reaction kinetics and molecular information. Briefly, colloidosomes encapsulating aqueous pH 1 or pH 7 solutions are immersed in a decane solution containing excess DY of 10⁻² M. Control experiment using colloidosome in pH 7 exhibits only DY's C-N stretching mode at 1150 cm⁻¹ over the entire reaction duration,¹⁹ indicating no observable formation of HDY⁺ (*Figure 3.4C*). Upon changing to pH 1 colloidosome, the SERS spectrum clearly exhibits the evolution of new vibrational features at 1283 and 1633 cm⁻¹, which are attributed to HDY⁺'s N=N and C=C stretching modes, respectively.¹⁹ The SERS image of a pH 1 colloidosome prior to the reaction only shows DY's 1150 cm⁻¹ signal (yellow-indexed), whereas the SERS profiling of the same colloidosome after 15 minutes of reaction is

overwhelmed by HDY⁺ signature peak (red-indexed) at 1283 cm⁻¹ (Figure 3.4D). This is attributed to the protonation of DY by H⁺ at liquid-liquid interface to generate protonated HDY⁺ ions which gradually penetrate from the interface into the encapsulated aqueous phase (pH 1; Figure 3.5). This also agrees with our control set-up involving bulk biphasic protonation of DY. Hence, we affirm the shell permeability of plasmonic colloidosome and exemplify its immense capability for compartmentalization of reactants to perform interfacial reactions involving the dynamic diffusion of molecules across the interface.

Figure 3.6. Schematic of experimental set-up for the *in situ* SERS monitoring of interfacial protonation of dimethyl yellow (in external decane phase) with plasmonic colloidosomes (encapsulating aqueous H⁺ solution).

3.2.3 Resolving the isomers of protonated dimethyl yellow

Plasmonic colloidosome also excels in the *in-situ* resolution of isomer structures in the reaction. We observe evident asymmetry in all the HDY⁺ SERS features in pH 1 colloidosomes (Figure 3.7A, B). Generally, each asymmetric peak can be attributed to the presence of two contributing peaks (Figure 3.7A). In particular, the C-H wagging mode at 1180 cm⁻¹ region comprises of 1178 and 1190 cm⁻¹ peaks, the C-N stretching mode at 1220 cm⁻¹ region

comprises of 1220 and 1229 cm^{-1} , and the N=N stretching mode at 1283 cm^{-1} region comprises of 1281 and 1305 cm^{-1} signals (*Figure 3.7A*). These results reveal the presence of two isomeric products in DY protonation reaction with similar vibrational profiles that overlaps with each other. In combination with our density function theory (DFT) simulation results, we attribute this observation to the two major potential protonation sites in DY molecule, each of which situates at one of the N=N azo nitrogen atoms (red circles in *Figure 3.7C*), labelled as HDY⁺(N1) and HDY⁺(N2) accordingly, owing to their large coefficients in the two highest occupied molecular orbitals (HOMO; *Figure 3.8A*), also the most electron-rich atoms (*Figure 3.8B*), and possess strongest affinity to H⁺ protons according to Pearson hard-soft acid-base theory.²⁰

Figure 3.7. Interpretation of SERS spectrum of DY and HDY⁺, with likely molecular structures and interactions with Ag. (A) Magnified spectrum of dotted region in the ‘HDY⁺ on pH 1 colloidosomes’ spectrum in (B) with multiply-fitted peaks of two protonated isomers

of HDY⁺. (B) SERS spectra of HDY⁺ observed on colloidosomes encapsulating pH 1 solution submerged in DY solution for 15 minutes, in comparison the DFT-simulated SERS spectra of both isomers of HDY⁺. (C) Molecular structures of both isomers of HDY⁺ with Ag-6 cluster (for SERS simulation) optimized by DFT-simulation. (D) Relative contribution ratio of fitted C-N stretching mode at 1220 cm⁻¹ experimental peak in HDY⁺ spectrum, with respect to time.

Figure 3.8. (A) Molecular representation of the two highest energy HOMOs of DY molecule, where the azo group contributes high coefficients in. (B) Electrostatic potential map of DY molecule. The red dash-box highlights the N=N azo group.

In addition, DFT simulation also affirms the protonation of DY's azo nitrogens to form isomeric HDY⁺(N1) and HDY⁺(N2) (Figure 3.7C), respectively. The simulated SERS spectra

for both HDY⁺ isomers demonstrate same characteristic vibrational modes of HDY⁺ with slight differences in the vibrational energies: C-H wagging modes at 1204 and 1197 cm⁻¹, C-N stretching modes at 1249 and 1232 cm⁻¹, N=N stretching mode at 1320 and 1324 cm⁻¹ for HDY⁺(N1) and HDY⁺(N2), respectively (*Figure 3.7B, Table S2 in Appendix*). This agreement with the experimental spectrum clearly exemplifies the presence of both protonation isomeric products HDY⁺(N1) and HDY⁺(N2), which have not been resolved in literature SERS studies of this molecule.¹⁹ Importantly, the normal Raman spectrum of HDY⁺ (without the presence of any Ag particles) also exhibit identical peak asymmetry (*Figure 3.9*), which affirms that the asymmetry indeed arises from the presence of the two isomers of HDY⁺, rather than from its different absorption configurations on Ag surface. The isomeric ratio analysis is performed using C-N stretching mode of HDY⁺ in the 1220 cm⁻¹ region, which comprises of two signals at 1220 and 1229 cm⁻¹ which are assigned HDY⁺(N2) and HDY⁺(N1) isomer (*Figure 3.10, Table S2*), respectively. This feature is also located in a clear window free from DY SERS interference. Notably, we observe a constant relative contribution ratio of HDY⁺(N2) / HDY⁺(N1) at (1.5 ± 0.2) throughout the reaction time (*Figure 3.11C*). Such relative contribution ratio is also observed to be generally constant for other characteristic vibrational mode of HDY⁺ (*Figure 3.11*). In fact, relative SERS contribution ratio is an excellent quantity to monitor the reaction progress.²¹ The constant SERS contribution ratio indicates that the isomeric products of HDY⁺ are directly formed in a thermodynamically-determined ratio from the start of reaction.²² This can be vital in asymmetric synthesis where mixtures of isomeric products are common but remain indiscernible by chromatography techniques.²³ We also highlight the superiority of plasmonic colloidosome over HPLC technique, which is unable to resolve the above isomers with highly similar structures and physical properties (*Figure 3.12*).

Figure 3.9. (A) Comparison of HDY⁺ SERS spectrum and normal Raman spectrum, with DFT-simulated spectra. (B) DFT-optimized molecular structures of the isomers. (C) Zoom-in segment of the dotted-box region in (A) and the peak fit analysis of the 1180, 1220 and 1283 cm⁻¹ vibrational bands observed in both SERS and normal Raman.

Figure 3.10. (A) Fitted HDY⁺(N2) and HDY⁺(N1) C-N stretching modes within experimental 1220 cm⁻¹ peak in time-resolved HDY⁺ spectra. (B) DFT-Simulated C-N stretching modes of

HDY⁺(N2) and HDY⁺(N1). (C) Relative SERS contribution ratio of fitted C-N stretching peaks for HDY⁺(N2)/HDY⁺(N1) in 1220 cm⁻¹ experimental peak in HDY⁺ spectrum, with respect to time.

Figure 3.11. Relative SERS contribution ratio for HDY⁺(N2)/HDY⁺(N1) analyzed in 1180, 1220 and 1283 cm⁻¹ experimental peaks in HDY⁺ spectrum, with respect to time. The difference in the apparent ratio values is attributed to the difference in the Raman activity and Raman cross section of different vibrational modes.²⁴

Figure 3.12. High performance liquid chromatography (HPLC) elution profile and the zoom-in signal of HDY⁺ observed at 20.80 min (MeOH/ACN gradient elution, detection wavelength

435 nm). It is impossible to resolve the two isomers in the peak due to their highly similar polarity.

3.2.4 Calculation of reaction kinetics

The capability of plasmonic colloidosome for the elucidation of critical reaction dynamic is further demonstrated by quantitatively investigating the temporal evolution of product HDY⁺ *in-situ* at the liquid-liquid interface. We would like to emphasize that SERS intensity obtained at the shell is accurate for the kinetic modelling of the chemical species in both phases due to their fast diffusion rate over the ultrasmall droplet volume (see Calculation 3.2). Briefly, the overall reaction equation of DY's interfacial protonation are denoted in equation (1), respectively.

where k is the rate constant of our model interfacial reaction.

We treat [DY] as a constant since there is a 10⁶-fold excess of DY amount (2.5×10^{-5} mol) in the external organic phase compared to H⁺ in the encapsulated aqueous phase (2.0×10^{-11} mol), as exemplified by the constant intensity of DY's C-N stretching SERS band (1150 cm⁻¹) of (1510 ± 160) counts (*Figure 3.13, 3.14*). We also note that H⁺ proton is Raman non-active, hence we use the SERS responses of HDY⁺ for time-dependent profiling of H⁺ consumption. The formation of HDY⁺ product is tracked based on the relative intensity (R_t) of HDY⁺ SERS signal (at 1283 cm⁻¹) compared to DY's constant SERS intensity (at 1150 cm⁻¹) at time t (equation 2). This is to minimize errors arising from experimental fluctuations.

$$R_t = \frac{I_{1283 \text{ cm}^{-1},t}}{I_{1150 \text{ cm}^{-1},t}} = \frac{a_{HDY}[HDY^+]_t}{a_{DY}[DY]} = c[HDY^+]_t, \text{ where } c = \frac{a_{HDY}}{a_{DY}[DY]} \quad (2)$$

where α_{HDY} and α_{DY} (counts.L.mol⁻¹) are specific activity constants of HDY⁺ and DY, respectively (see Calculation 3.2). To obtain [H⁺] necessary for the determination of k_{app} , we assume that all [H⁺] are consumed for the conversion of DY into HDY⁺. Subsequently, we use the infinity quantity method to indirectly extract the consumption profile of H⁺ concentration based on the production of HDY⁺ (equation 3).²²

$$c[H^+]_t = R_\infty - R_t = 4.6 - R_t \quad (3)$$

where c denotes the proportionality constant in this relationship, which is proven to correlate with the aforementioned c in equation 2. R_∞ and R_t are the relative SERS intensity of HDY⁺/DY at infinity time and time, t , respectively. R_∞ remains constant at (4.6 ± 0.5) beyond 15 min. Our derivation leads to the following relationship between $(4.6 - R_t)$ in equation (4) of the reaction obeys first-order (reaction order is discussed in Calculation 3.2)

$$\ln c[H^+] = \ln(4.6 - R_t) = -k_{\text{app}}t + A \quad (4)$$

where $A = \ln [H^+]_0 + \ln c$

Using equation (4), we indeed observe an evident linear relationship in the plot of $\ln(4.6 - R_t)$ against reaction time, t (*Figure 3.15*, Calculation 3.2), which verifies that the formation of HDY⁺ obeys the 1st order kinetic with respect to H⁺. Through the determination of its gradient in the plot for equation (4), the apparent rate constant (k_{app}) of our model interfacial reaction is determined to be 0.09 min⁻¹, which agrees with the value obtained at pH 2 (*Figure 3.16*; Calculation 3.2). We also affirm the similar reaction kinetic using other vibrational modes of HDY⁺ for reaction monitoring, such as the C=C stretching mode at 1633 cm⁻¹ (*Figure 3.17*). Notably, we also observe a consistent kinetic profile by measuring the SERS spectra at half-plane of the colloidosome (*Figure 3.18*), which exemplifies the advantage of plasmonic colloidosome as a 3D SERS platform.

Figure 3.13. Area intensity of DY's 1150 cm^{-1} signal with time, on colloidosomes pH 1 (red) and pH 7 (black). This also highlights the advantage of our stable SERS platform over existing colloidal systems in SERS monitoring, where the latters are prone to large signal fluctuations due to aggregation effect over time.^{21,25} In fact, our plasmonic colloidosome has demonstrated its signal stability and reproducibility owing to the precedently assembled Ag shell skeleton.²⁶

Calculation 3.2 Reaction kinetics

Use of DY as the reference.

Our reaction is performed with plasmonic colloidosomes submerged in the bulk organic phase containing DY 10^{-2} M solution (*Figure 3.6*). We observe a constant SERS intensity (1510 ± 160 counts) of DY signals, namely the 1150 cm^{-1} peak when colloidosomes of both pH 1 and pH 7 are used in the reaction. Hence, we use the above signal as a reference to standardize the evolution of the HDY⁺ signals (I_{1283}/I_{1150}) in our kinetic calculation. The observation may be due to the excess amount of DY molecules as compared to the H⁺ ions in the plasmonic colloidosomes, and the diffusion of excess DY to replenish the reacted DY at the interface is fast enough to maintain signal stability within our exposure time of 10s.²⁷

- Mole number of DY in the external phase :

$$n_{DY} = [DY] \times V_{org} = 10^{-2} M \times 2.5 \text{ mL} = 2.5 \times 10^{-5} \text{ mol}$$

- Mole number of H^+ in a plasmonic colloidosome :

$$n_{H^+} = [H^+] \times V_{colldosome} = 10^{-1} M \times 195 \text{ pL} = 1.95 \times 10^{-11} \text{ mol}$$

Therefore, DY is in 10^6 -fold excess in comparison to H^+ amount, and hence its concentration in the organic phase is insignificantly affected during the course of reaction.

HDY^+ in the aqueous phase can also diffuse over the entire encapsulated capsule fast enough, such that the SERS signals observed at the Ag shell to be representative of its amount in the whole vessel. More importantly, HDY^+ in the internal aqueous phase can diffuse at a much greater rate due to the ultrasmall volume of $< 200 \text{ pL}$ of our vessel. In particular, small picoliter droplets allow up to 8 orders of magnitude faster rate of diffusion and homogenization as compared to bulk vial-based solution, according to the following formula of diffusion rate in liquid system:^{28,29}

$$t \approx r^2 / D$$

(t is diffusion time required for droplet homogenization, r is droplet diameter, D is diffusion coefficient of the analyte)

Hence, we perform the kinetic calculation based on the SERS intensity observed at the colloidosome shell which can accurately reflect the amount of reactants and products in both phases. Briefly, the overall reaction equation and the rate equation of DY's interfacial protonation are denoted in equation (1) and (2), respectively.

$$\text{rate of equation (1)} = -\frac{d[H^+]}{dt} = k[H^+]^n [DY]^m \quad (2)$$

where k is the rate constant of our model interfacial reaction, $[H^+]$ and $[DY]$ are the concentrations of H^+ and DY, respectively, while n and m are integers representing the rate order of H^+ and DY, respectively.

We did attempt to quantify the concentration HDY^+ in the water phase, by isolating HDY^+ in plasmonic colloidosomes to obtain an intensity-concentration calibration curve similar to the methylene blue case. However, our colloidosome preparation procedure involves an intense emulsification step which facilitates the diffusion of the encapsulated HDY^+ to the external decane phase as DY molecules, according to the equilibrium $DY_{(org)} + H^+_{(aq)} \rightleftharpoons HDY^+_{(aq)}$. Even though the inner aqueous phase contains pH 1 solution which minimize the formation of DY, we still observe a great amount of DY diffused to the decane phase (both physically and via SERS measurement in *Figure 3.13*), which we attribute to the concentration gradient difference. Hence, we are unable to estimate the actual concentration of the HDY^+ remained within the colloidosomes for quantification. Nevertheless, we are able to retrieve the kinetics based on the monitoring of the SERS intensity of HDY^+ signals, with correlation to the stable DY signal from the excess DY in the organic phase. Such methodology of reaction monitoring based on solely SERS intensity without correlation to the actual concentration is indeed proven to be an accurate and reliable technique to investigate reaction kinetics.²¹ Hence, we are confident that our analysis based on SERS intensity monitoring genuinely reflects the kinetics of this reaction.

Figure 3.14. Diffusion of DY to the external phase as shown by (A) digital images of the vials before and after emulsification and formation of colloidosomes. Even though HDY⁺(red) is originally dissolved in water, the intense emulsification step facilitates the formation of DY (yellow) and its diffusion to the decane phase. (B) SERS spectra measured on colloidosomes sample prepared with HDY⁺ 10⁻³ M and 10⁻⁴ M, with the control of DY spectrum. The DY's 1150 cm⁻¹ mode is always present, which proves the difficulties in the isolation of HDY⁺ in the colloidosomes.

We treat [DY] as a constant since there is a 10⁶-fold excess of DY amount (2.5×10^{-5} mol) in the external organic phase compared to H⁺ in the encapsulated aqueous phase (2.0×10^{-11} mol), as exemplified by the constant intensity of DY's C-N stretching SERS band (1150 cm⁻¹) of (1510 ± 160) counts (Figure S9). Together with the assumption of $n = 1$ (to be verified later), we can therefore rewrite equation (2) into a pseudo 1st order reaction as simplified in equation (3).

$$-\frac{d[H^+]}{dt} = k[H^+]^n[DY]^m$$

$$-\frac{d[H^+]}{dt} = k_{app}[H^+]^n, \text{ where } k_{app} = k[DY]^m \quad (3)$$

where k and k_{app} represents the intrinsic and apparent rate constant, respectively, t is the time from the start of reaction and $[DY]$ is a constant.

By integrating the pseudo 1st order rate law (equation 3), we obtain the 1st order integrated rate law as described in equation (4)

$$[H^+] = [H^+]_o e^{-k_{app}t}$$

$$\ln[H^+] = -k_{app}t + \ln[H^+]_o \quad (4)$$

where $[H^+]_o$ is the initial concentration of H^+ at $t = 0$ min.

We note that H^+ proton is Raman non-active and uses the SERS responses of HDY^+ instead for latter time-dependent profiling of H^+ consumption. $[HDY^+]$ is directly proportional to the SERS intensity of its characteristic SERS band at 1283 cm^{-1} according to the relationship in equation 5. We track the formation of HDY^+ product based on the relative intensity of its SERS signal compared to DY 's constant SERS intensity at 1150 cm^{-1} (equation 6). This is to eliminate the possibility of any intensity change arising from random fluctuation of our experimental set-up instead.

$$I_{1283 \text{ cm}^{-1},t} = a_{HDY}[HDY^+]_t \quad (5)$$

$$R_t = \frac{I_{1283 \text{ cm}^{-1},t}}{I_{1150 \text{ cm}^{-1},t}} = \frac{a_{HDY}[HDY^+]_t}{a_{DY}[DY]} = c[HDY^+]_t, \text{ where } c = \frac{a_{HDY}}{a_{DY}[DY]} \quad (6)$$

where α_{HDY} and α_{DY} (counts.L.mol^{-1}) are specific activity constants of HDY^+ and DY , respectively. Such activity constant is commonly used to relate the SERS intensity of chemical species with their concentration; it is a comprehensive term comprising of contribution from

laser intensity, the effective molecular SERS cross-section of the corresponding vibrational mode, and experimental conditions including the sample temperature and the solvent diffraction constant.²⁴ These aforementioned factors, and therefore α_{HDY} and α_{DY} , are essentially constant as all the experimental conditions for our SERS measurements are precisely controlled to ensure their consistency.

To obtain $[H^+]$ necessary for the determination of k_{app} , we assume that all $[H^+]$ are consumed for the conversion of DY into HDY^+ . Subsequently, we use the infinity quantity method to indirectly extract the consumption profile of H^+ concentration.^{22,30} Infinity quantity method is a powerful technique to relate product formation profile to the reactant consumption, which state that the difference in a physical quantity of the product at time infinity (∞) and a specific time (t) from the onset of reaction is directly proportional to the reactant concentration (equation 7).

$$R_{\infty} - R_t = c[HDY^+]_{\infty} - c[HDY^+]_t = c[H^+]_t \quad (7)$$

(derivation below)

(1) Concentration of reactants and product at different time :

(2) Prove of $R = k[HDY^+]$:

$$\frac{I_{1283}}{I_{1150}} = \frac{\alpha_{HDY}[HDY^+]}{\alpha_{DY}[DY]} \longrightarrow R = I_{1283}/I_{1150} = \alpha_{HDY}[HDY^+]/\alpha_{DY}[DY] = c[HDY^+]$$

(α is specific activity; $[DY]$ = constant concentration in excess;
 $c = \alpha_{HDY}/\alpha_{DY}[DY]$)

(3) Derivation of $R_{\infty} - R_t = c([H^+]_t)$:

$$\begin{aligned} R_{\infty} - R_t &= c[HDY^+]_{\infty} - c[HDY^+]_t && \text{(from (2))} \\ &= c([HDY^+]_{\infty} - [HDY^+]_t) \\ &= c([H^+]_0 - [H^+]_{\infty} - [H^+]_0 + [H^+]_t) && \text{(from (1))} \\ &= c([H^+]_t - [H^+]_{\infty}) \\ &= c([H^+]_t) && ([H^+]_t \gg [H^+]_{\infty} \text{ at initial stage}) \end{aligned}$$

where c denotes the proportionality constant in this relationship, which is proven to correlate with the aforementioned c . R_{∞} can be obtained from the plateau value of the relative SERS intensity (R)-time plot, which exhibits an initial incremental growth of SERS intensity that

eventually remained constant at (4.6 ± 0.5) beyond 15 min (Figure 4A, B). Consequently, $R_\infty = (4.6 \pm 0.5)$ and equation (7) can be simplified as followed:

$$c[H^+]_t = 4.6 - R_t \quad (8)$$

By substituting equation (8) into equation (4),

$$\begin{aligned} \ln[H^+] &= -k_{app}t + \ln[H^+]_0 \\ \ln[H^+] + \ln c &= -k_{app}t + \ln[H^+]_0 + \ln c \\ \ln(c[H^+]) &= -k_{app}t + A, \text{ where } A = \ln[H^+]_0 + \ln c \\ \ln(4.6 - R_t) &= -k_{app}t + A \end{aligned} \quad (9)$$

Using equation (9), we note an evident linear relationship in the plot of $\ln(4.6 - R_t)$ against reaction time, t (Figure S10A). The presence of such linear relationship therefore verify that the interfacial protonation of DY obeys the 1st order kinetic, where the formation of HDY^+ is only dependent on the concentration of H^+ . Through the determination of its gradient in the plot for equation (9), the apparent rate constant (k_{app}) of our model interfacial reaction is determined to be 0.09 min^{-1} . Notably, the apparent rate constant derived at pH 1 is also in agreement with the value obtained at pH 2 (0.09 min^{-1} ; Figure S11).

To confirm the order of the reaction, we also perform the profiling of $1/c[H^+]$ vs. time (equation 10) according to the integrated rate law of second-order reaction :

$$\begin{aligned} 1/[H^+] &= k_{app}t + 1/[H^+]_0 \\ 1/c[H^+] &= k_{app}t/c + 1/c[H^+]_0 \\ 1/(4.6 - R_t) &= k_{app}t/c + A' \quad \text{where } A' = 1/c[H^+]_0 \end{aligned} \quad (10)$$

We do observe that the plot of $1/c[H^+]$ or $1/(4.6 - R_t)$ vs. time does not produce a linear plot (Figure S10B). Hence, it affirms that the reaction indeed obeys the first-order kinetics (as determined above) and not second-order.

Figure 3.15. Plot of (A) $\ln(c[H^+])$ vs time and (B) $1/c[H^+]$ vs time in the reaction on pH 1 colloidosome.

Figure 3.16. Plot of $\ln(c[H^+])$ vs time in the reaction on pH 2 colloidosome.

Figure 3.17. Reaction monitoring using HDY⁺ 1633 cm⁻¹ peak. (A) Area ratio of HDY's 1633 cm⁻¹ peak and DY's 1150 cm⁻¹ peak on the pH 1 colloidosomes submerged in decane over time. (B) Plot of $\ln(c[H^+])$ vs time in the reaction on pH 1 colloidosome.

Figure 3.18. Similar growth profile of HDY⁺ formation based on I_{1283}/I_{1150} ratio obtained on colloidosome with laser focused on top- and mid-plane.

(end of Calculation 3.2)

3.2.5 Parallel isolated reactions with plasmonic colloidosomes.

Additionally, plasmonic colloidosomes enable high throughput SERS monitoring of multiple interfacial reactions on colloidosomes. Colloidosomes encapsulating pH 1, pH 2 and pH 7 solutions are monitored as isolated picoreactors in the same DY solution simultaneously within the same imaging screen but independently (*Figure 3.19A, C*). All colloidosomes response similarly to DY in the external phase (labelled yellow) and exhibit uniform DY's 1150 cm^{-1} SERS intensities throughout the reaction (*Figure 3.19B, C*). Notably, pH 1 colloidosomes exhibit the steepest growth profile of HDY⁺ signals (*Figure 3.19B*), which is also illustrated in our color-indexed SERS image where the pH 1 colloidosome is brightly lit when HDY⁺ 1283 cm^{-1} signal is selected (labelled red, *Figure 3.19C(ii)*). Concurrently, pH 2 colloidosomes display a ~ 5 -fold weaker red color intensity as observed in the same SERS image. We observe a ~ 10 -fold slower initial formation rate of HDY⁺ in pH 2 colloidosomes relative to pH 1 colloidosomes (initial gradients of R_I vs time plot of 0.224 min^{-1} and 0.029 min^{-1} respectively),²² which obeys the previously determined first order kinetic of our model interfacial reaction ($\text{rate} = k_{\text{app}}[\text{H}^+]$; *Figure 3.19A, 3.20*). On the contrary, control pH 7 colloidosomes do not exhibit observable HDY⁺ signals in the SERS spectrum and remain invisible in the SERS image of HDY⁺ (*Figure 3.19C(ii)*). We also exclude errors resulting from crosstalk among closely-spaced plasmonic colloidosomes,¹⁰ and also pH-induced effect on its Ag shell and SERS activities (*Figure 3.21*). Our results therefore emphasize on the importance of colloidosome's robustness to efficiently isolate reactions for high through-put reaction monitoring.

Figure 3.19. The formation of HDY⁺ at different pH. (A) Area ratio of I_{1283}/I_{1150} peak and (B) time-resolved maps of the SERS spectra on the colloidosomes pH 1, pH 2 and pH 7 submerged in dimethyl yellow 10^{-2} M solution over time. (C) SERS images of a mixture of pH 1, pH 2 and pH 7 colloidosomes submerged in DY solution for after 15 minutes with (i) 1150 cm^{-1} (yellow-indexed) chosen and (ii) 1283 cm^{-1} (red-indexed) chosen. In the case of 1283 cm^{-1} shift, only the pH 1 colloidosomes exhibit strong signals, while pH 2 colloidosomes shows weak signals and pH 7 colloidosomes do not response to the shift.

Figure 3.20. Initial rate analysis of the HDY⁺ formation observed on colloidosome pH 1 and pH 2.

Figure 3.21. SERS background of blank colloidosomes encapsulating pH 1 to pH 14.

3.3 Conclusion

In conclusion, we have fabricated plasmonic colloidosomes from highly SERS active Ag octahedra, as a dual-phase picoreactor for simultaneous in-situ reaction monitoring and molecular analysis of liquid-liquid interfacial reaction. Applied in the decane-water interfacial

protonation of dimethyl yellow on a < 200 pL droplet, our platform excels exemplifying the protonated isomers of HDY⁺ in a constant thermodynamic ratio and the pseudo-first-order kinetic with the apparent rate constant of 0.09 min^{-1} of the reaction, as retrieved from in-situ SERS monitoring of the reaction progress. We also perform the parallel reaction monitoring with plasmonic colloidosomes, demonstrating their benefits as potential picoreactors for high through-put screening of reactions involving the multiple components soluble in both a single or two different phases. Therefore, plasmonic colloidosomes feature multiple advantages as a SERS microplatform for small-scale study of interfacial phenomena commonly encounter in the areas of food chemistry, clinical analysis and drug treatment.

3.4 Materials and Methods

Chemicals. Silver nitrate ($\geq 99 \%$), anhydrous 1,5-pentanediol (PD, $\geq 97 \%$), poly(vinyl pyrrolidone) (PVP, average MW = 55,000); 1H,1H,2H,2H-perfluorodecanethiol (PFDT, $\geq 97 \%$), 4-methylbenzenethiol (4-MBT, $\geq 98\%$), decane (anhydrous, $> 99\%$), methylene blue (MB, $\geq 82 \%$), and dimethyl yellow (DY, analytical standard, $\geq 98\%$) were purchased from Sigma Aldrich; copper (II) chloride ($\geq 98 \%$) was from Alfa Aesar; ethanol (ACS, ISO, Reag. Ph Eur) was from EMSURE®; toluene (BAKER ANALYZED® A.C.S. Reagent) was from Avantor; propan-2-ol (HPLC grade) was from Fisher Scientific. All chemicals were applied without further purification. Milli-Q water ($> 18.0 \text{ M}\Omega \cdot \text{cm}$) was purified with a Sartorius Arium® 611 UV ultrapure water system.

Synthesis and purification of silver octahedra. The preparation of silver (Ag) octahedra was carried out based on the polyol method described in literature, first starting with the intermediate Ag nanocubes.^{16,31} 10 mL PD solutions of CuCl_2 (8 mg/mL), PVP (20 mg/mL) and AgNO_3 (20 mg/mL) were prepared separately by sonication and vortex. 35 μL CuCl_2 solution was added to the AgNO_3 solution. 250 μL PVP precursor was added dropwise every

30 s while 500 μL AgNO_3 precursor was injected every min using a quick addition to a 10 minute-preheated 20 mL PD solution. The addition was continued until the mixture turned orange brown, followed by the further addition of 30 ml of PVP (20 mg/mL) and AgNO_3 (40 mg/mL with 120 μL CuCl_2) (both precursors were separately prepared in PD). The reaction was allowed to proceed until the precursors were used up. For the purification, PD was first removed by washing the mixture with acetone followed by ethanol. The suspension was then dispersed in 10 mL ethanol and 100 mL aqueous PVP solution (0.2 g/L) and filtered using Durapore® polyvinylidene fluoride filter membranes (Millipore) with pore sizes ranging from 5000 nm, 650 nm, 450 nm, several times for each pore size. SEM imaging was performed, from which the edge lengths of 250 Ag octahedra were measured and analyzed using ImageJ software.

Functionalization of Ag octahedra with perfluorodecanethiol. 50 mg of purified Ag octahedra were immersed in 10 mL of 1:1 propan-2-ol (IPA)/ethanol solution containing 0.1 mM of 1H,1H,2H,2H-perfluorodecanethiol (PFDT) for 6 h at room temperature. The colloidal suspension was then washed with copious amounts of ethanol, and subsequently dispersed in 1.0 mL of 1:1 IPA/ethanol.

Preparation of colloidosomes. 5 μL ultrapure water was added to 200 μL decane suspension containing 0.75 mg perfluorodecanethiol-functionalized Ag octahedra (referred to as Ag from this point onwards). Colloidosomes were formed by emulsification via vigorous shaking.

SERS characterization of colloidosomes. Methylene blue (3.2 mg/mL, 10^{-2} M) was prepared in aqueous solution using ultrapure water. Serial dilutions were performed to give a series of concentrations, ranging from 10^{-3} M to 10^{-12} M. For SERS detection involving 20 $\times$ (N.A. 0.45) objective lens, the as-prepared plasmonic colloidosomes were dispensed onto a 3 $\times$ 3 mm silicon substrate and submerged in 2.5 mL decane. The laser was focused at the top

and edge of single colloidosomes. Both the colloidosome formation and SERS imaging for different analytes and concentrations were performed at least thrice to ensure the reproducibility of the SERS signal. All SERS intensities and spectra were obtained by averaging at least 20 individual spectrum. For normal Raman evaluation of various analytes, Raman spectra were collected using the 20× (N.A. 0.45) objective lens from 0.5 μ L of the respective analyte solution, with 100 s exposure time. Raman intensities and spectra were obtained by averaging at least 20 individual spectrum.

SERS analysis of Dimethyl yellow protonation and diffusion. The plasmonic colloidosomes prepared with aqueous solution with varying pH were dispensed onto a 3 $\times$ 3 mm silicon substrate and submerged in 2.5 mL decane solution of 10^{-2} M dimethyl yellow (DY). The laser was focused at the top of single colloidosomes. SERS imaging was performed at 2.5 minute interval over a period of 30 minutes. Both the colloidosome formation and SERS imaging were performed at least thrice to ensure the reproducibility of the SERS signal. All SERS intensities and spectra were obtained by averaging at least 20 individual spectrum.

SERS spectrum of protonated dimethyl yellow. The SERS spectrum using was done using 0.01 mg of perfluorodecanethiol-grafted Ag octahedra dispersed in 0.5 mL of HDY⁺ 10^{-3} M. The SERS imaging were performed at least thrice to ensure the reproducibility of the SERS signal. All SERS intensities and spectra were obtained by averaging at least 20 individual spectrum.

Density functional theory (DFT) simulation. The calculation of DY and HDY⁺ on Ag surface were carried out using the unrestricted B3LYP exchange-correlation functional, as implemented in the Gaussian 09 computational chemistry package. The 6-31g (d) basis set was used for all atoms except Ag, for which the LANL2DZ basis set was employed. The Ag surface was modeled using an Ag-6 cluster for optimum simulated results within minimal computational time.

Material characterization. SEM imaging was performed with JEOL-JSM-7600F microscope and QX-102 capsules.³² UV-vis spectroscopic measurements were conducted with a Cary 60 UV-Vis spectrometer. Microscopic images were taken with Olympus BX51 microscope under 10× (0.30 BD) objective lens. SERS measurements were performed using both x-y and x-z imaging mode of the Ramantouch microspectrometer (Nanophoton Inc, Osaka, Japan) with an excitation wavelength of 785 nm. When using 20× (N.A. 0.45) objective lens, laser power was set at ≤ 0.06 mW with 10 s accumulation time (unless otherwise stated) for data collection between 200 cm^{-1} to 1800 cm^{-1} . Low laser power is used so decane signals are not shown in our measurement. All SERS spectra and intensities were obtained by averaging at least 20 individual spectra from each SERS image. High performance liquid chromatography (HPLC) was performed with Shimadzu SDP-20A, with MeOH/Acetonitrile gradient dilution over 40 minutes, and dual detection wavelengths at 435 nm.

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Chapter 4

On-line Flowing Colloidosomes for Sequential Multi-Analyte and High Throughput SERS Analysis **

Abstract. 3D plasmonic colloidosomes are superior SERS sensors due to their high sensitivity and excellent laser misalignment tolerance. Here, we incorporate plasmonic colloidosomes in a microfluidic channel for on-line SERS detection. Our method resolves the poor signal reproducibility and inter-sample contamination in the existing on-line SERS platforms. Our flow system offers rapid and continuous on-line detection of 20 samples in < 5 minutes with excellent signal reproducibility. The isolated colloidosomes prevents cross-sample and channel contamination, allowing accurate quantification of samples with six orders of magnitude concentration range. Our system demonstrates high resolution multiplex detection with fully preserved signal and Raman features of individual analytes in a mixture. High throughput multi-assay analysis is performed, which highlight our system is capable of rapid identification and quantification of a sequence of samples containing various analytes and concentrations.

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4.1 Introduction

Thus far in the previous chapters, current sensing applications of plasmonic colloidosomes are performed with static state measurements that under-utilize their advantages as a dynamic substrate-less sensing platform.¹⁻⁶ Also, static measurements greatly limit the detection throughput and generally require accurate laser alignment prior to every measurement.⁷ We recognize the immense potential of the incorporation of plasmonic colloidosomes with on-line SERS detection system to significantly boost the throughput quantity and detection time, enabling automation of SERS multi-assay sensing.⁸⁻¹⁰ This reinforces their use as a miniaturized yet powerful platform for the identification of toxins or active biological compounds,⁵ especially applied in medical diagnosis, genetic and pharmaceutical modulations where sample input quantity is large and sample volume is resource-constrained.⁸⁻¹²

On-line SERS is an emerging microscale detection technique due to its time-efficiency and the ability to afford highly specific vibrational fingerprints of analytes, which remains impossible using conventional UV-vis detectors or liquid/gas chromatography.¹¹ It typically involves passing of an analyte solution containing plasmonic colloidal particles through a flow channel, where SERS measurement is performed at a fixed position.¹³ Such method offers rapid SERS detection, however it suffers from poor signal reproducibility and stability. This is due to the random particle distribution in a flow channel that leads to laser misalignment, and/or particle aggregation along the flow channel during the detection.¹⁴ Alternatively, substrate-based on-line SERS platforms with particles embedded along a flow channel can eliminate particle aggregation issue and provide stable signals during detection.¹⁵ Firstly, plasmonic nanoparticles are already assembled onto a rigid substrate and thus do not experience gradual settling down or aggregation over time. Secondly, the bed provides a defined and fixed target for laser focal alignment, which results in consistent SERS monitoring of analytes in the flow

channel. However, these substrates require time-consuming pre-assembly of nanoparticles and are easily contaminated as analyte molecules are gradually adsorbed onto the channel,^{14,16} which make them impractical for prolonged usage. In fact, they are most suitable for 1 time usage as a flow of analytes can contaminate the particles, which might cause the appearance of previous analytes' signals in subsequent measurements. Hence, our proposed plasmonic colloidosome system is an ideal substrate-less platform for on-line SERS detection that can tackle the above limitations. Their robust 3D shell skeletons consisting of assembled Ag clusters provide high and stable hotspot density within laser illumination volume, affording reproducible SERS signal.^{17,18} Moreover, target analytes encapsulated within colloidosomes remain isolated,¹ thus prevent analyte cross-talking and channel contamination for sequential detection, providing a practical solution to rapid on-line analysis.

Here, we introduce plasmonic colloidosomes for on-line sequential SERS detections of multiple samples through a microfluidic channel, as a prototype on-line Raman analyzer for practical multi-assay analysis. Plasmonic colloidosomes encapsulating different toxic dyes and at various concentrations are flowed through a channel where SERS signals are obtained from a fixed laser exposure spot. Our online Raman analyzer demonstrates highly reproducible detection of >20 batches of sample in <5 minutes using a single flow channel, and is able to analyze samples of alternating concentrations without any inter-sample contamination. This is a critical advancement from conventional colloidal online SERS channels, which suffer excessive contamination and poor signal consistency as presented in our control experiments. This real-time analytical platform also excels in resolving individual analytes within short accumulation time of 500 ms in a multiplex detection of a flow containing a mixture of toxins. We illustrate the sequential on-line detection with specific identification and quantification of up to four toxic dyes with closely similar SERS features, exhibiting the strength of SERS

technique and plasmonic colloidosomes in high throughput assay, which is especially crucial in the field of analytical chemistry and biology.¹⁰

4.2 Results and Discussion

4.2.1 Designing online colloidosome-based detection system

Plasmonic colloidosomes are typically prepared using hydrophobic perfluorodecanethiol (PFDT)-functionalized Ag nanocubes of ~ 121 nm edge length,¹ and exist as robust water-in-decane droplets in the diameter range of (90 ± 20) μm (*Figure 4.1*). The colloidosomes are stable and can flow smoothly in the decane continuous phase in the flow channel (500 μm inner diameter) (see Materials and Methods, *Figure 4.2*).

Figure 4.1. (A) SEM image of as-synthesized Ag nanocubes and (B) microscopic image of the plasmonic colloidosomes.

Figure 4.2. Microfluidic glass flow channel on a glass cover slip. The glass flow channel is connected to a Teflon inlet and outlet.

To perform the on-line sensing of single-analyte samples in the flow channel (*Figure 4.3A*), plasmonic colloidosomes are first prepared using 0.5 μL of methylene blue (MB, 10^{-4} M), and stored in 500 μL decane to prevent solvent evaporation.¹ Subsequently, multiple 1 μL samples of colloidosomes containing 10^{-4} M MB are subjected into the flow channel at intervals of 10 s, flow rate at 0.10 mL/min, where time-lapsed spectra are obtained at every 500 ms. Decane is used to flush the channel in between colloidosome injections. In the flow channel, plasmonic colloidosomes move as a cluster. It is noteworthy that the colloidosomes are prepared off-chip, the on-line measurement refers to the in-situ and time-lapsed collection of spectra under a fixed laser spot. Our results demonstrate highly consistent SERS intensity of ($18.1 \text{ k} \pm 1.3 \text{ k}$) counts, using the 1630 cm^{-1} signal of MB as benchmark from here onwards (*Table S1 in Appendix*). All SERS spectra indicate identical features and similar intensities within 8.6% relative standard deviation (RSD, *Figure 4.3C*, see Calculation 4.1). We also note that the 3D colloidosome clusters occupy the entire channel, giving rise to 26-fold amplified SERS-active surface area. This ensures our laser collection volume is always occupied with the 3D assembled plasmonic colloidosomes, and yield highly reproducible and uniform intensity.^{1,18} The excellent reproducibility of our system allows reliable high throughput analysis of more than 20 analyte samples in less than 5 minutes. To our knowledge, this is the first online SERS platform that elucidates time-lapsed spectroscopic profiles of multiple sample inputs in a sequential detection, in comparison with existing platforms that are typically demonstrated with only one-time sample input due to substrate contamination.^{14,16,19,20}

Figure 4.3. Plasmonic colloidosomes in microfluidic channel for on-line SERS detection

(A) 3D scheme of the sequential on-line SERS detection of analyte samples encapsulated within plasmonic colloidosomes. (B) Time-lapsed waterfall plot of output SERS spectra obtained within 4 minutes of flowing various samples containing methylene blue 10^{-4} M. (C) Time-lapsed intensity profile of methylene blue 1630 cm^{-1} peak demonstrating reproducibility of the on-line detection platforms: using plasmonic colloidosome flow (top) and colloidal Ag flow (control – bottom).

On a different note, *Figure 4.4* shows the overlay Raman image over microscopic image of colloidosomes in the channel, whereby the brightly lit area display an average of up to ~ 4000 counts/s for methylene blue 1630 cm^{-1} signal, which is comparable to the intensity observed when measured without the channel using the same instrument and setup. This indicates the high laser transparency of the thin glass wall ($140\text{ }\mu\text{m}$) of the TLC channel that allows maximum laser penetration and thus high SERS intensity captured from the inner colloidosomes, highlighting the suitability of using cost-effective TLC tube as a flow channel for on-line SERS detection.

Figure 4.4. Static SERS image of colloidosomes encapsulating methylene blue 10^{-6} M in flow channel.

Calculation 4.1: Laser spot size and channel size

The laser spot diameter used for our on-line system can be theoretically determined according to the following equation,²¹ where D is spot diameter, λ is laser wavelength, N.A is lens numerical aperture:

$$\begin{aligned}
 D &= 1.22 \lambda / \text{N.A} \\
 &= 1.22 \times 532\text{ nm} / 0.4 \\
 &= 1622.6\text{ nm} \\
 &\sim 1.62\text{ }\mu\text{m}
 \end{aligned}$$

The laser collection volume can be calculated as followed²², in which we take into account the enlargement of the laser spot size under our detection condition involving the glass tube and decane solvent :

$$V_{\text{collection}} = \pi^{3/2} \kappa D^3$$

(κ is a geometric factor, $\kappa = 2.33n/N.A$ where n is the refraction index, in this case $n_{\text{decane}} = 1.44$ and $n_{\text{glass}} = 1.5$, thus overall $n = 1.44 \times 1.5 = 2.16$)

$$\begin{aligned} &= \pi^{3/2} (2.16)(1.62 \mu\text{m})^3 \\ &= 51.15 \mu\text{m}^3 \end{aligned}$$

We also compare the relative experimental laser spot size and the colloidosome cluster volume per detection in *Figure 4.5*. We also estimate the number of colloidosomes per detection to illustrate the consistent SERS active area within the laser collection volume that gives rise to the reproducible intensity as followed:

Our colloidosome's mean diameter is determined at $90 \mu\text{m}$. Thus, the volume of each colloidosomes is:

$$V_{\text{colloidosome}} = 4/3\pi.r^3 = 4/3\pi (90/2 \mu\text{m})^3 = 3.82 \times 10^5 \mu\text{m}^3$$

The volume of the colloidosome cluster flowing in the channel :

$$V_{\text{cluster}} = \pi \times r_{\text{channel}}^2 \times h_{\text{cluster}} = \pi \times (500/2 \mu\text{m})^2 \times 650 \mu\text{m} = 1.27 \times 10^8 \mu\text{m}^3$$

The estimated number of colloidosomes per detection (assumed closely-packed in the cluster) :

$$N_{\text{colloidosome}} = V_{\text{cluster}} / V_{\text{colloidosome}} = 1.27 \times 10^8 \mu\text{m}^3 / 3.82 \times 10^5 \mu\text{m}^3 = \sim 300 \text{ colloidosomes}$$

Figure 4.5. (A) Microscopic image of the colloidosome cluster in the flow channel (when the flow is stopped temporarily) under 4× objective lens. (B) Image of the laser spot focused at the collection point, under 20× lens.

Our calculations show that the estimated laser collection volume is smaller than a colloidosome cluster, which is also evident in Figure S4B where the laser spot diameter is only $\sim 7 \mu\text{m}$. Hence, it is both theoretically and experimentally evident that the laser collection volume is always occupied with consistent total SERS active area, which gives rise to the reproducible intensity.

(end of Calculation 4.1)

In addition, the detection system is highly sensitive to minor change of analyte in the laser illumination volume. This is indicated by the instantaneous on/off signal response between the change of MB to decane baseline background solution with intensity difference of ~18k counts (*Figure 4.3C*). Such rapid on/off response reduces the overall detection time while provides real-time feedback of the molecular events occurred within the laser illumination volume,^{7,19} which is vital for in situ detection and monitoring.^{3,4} The system also resolves the unstable SERS signal issue encountered in conventional on-line SERS platforms using colloidal particle flow.¹⁴ This is demonstrated in our control experiment involving the detection of multiple MB samples, with conventional single-phase Ag colloidal flows (*Figure 4.3C*). Such system exhibits poor signal reproducibility (RSD =28.3%) and significant channel contamination after 7 rounds of samples (~ 90 s onwards) whereby MB signal is consistently observed throughout the detection. This is due to the direct exposure of analyte to the channel. The high intensity fluctuation is attributed to the uneven distribution of particles and their gradual aggregation, which makes it impractical for subsequent analysis. On the other hand, plasmonic colloidosomes encapsulate the analyte molecules within its interior volume which prevents channel contamination. More importantly, the colloidosomes are always submerged in an immiscible outer decane phase in the flow channel, which ensures a smooth flow while preventing direct contact between the colloidosomes and the channel wall. Hence, the experiments highlight the advantage of using plasmonic emulsion droplets for on-line SERS because analytes are encapsulated within robust micro-droplets submerged in a second solvent layer, and thus tackle the contamination issue.

4.2.2 Online high through-put quantification of multiple samples

Our on-line SERS platform also offer highly accurate quantitative detection of samples of different concentrations without any inter-sample contamination, especially when switching from concentrated sample to the diluted ones. Seven samples containing of MB with concentration difference in the range of 5 orders of magnitude (10^{-2} M to 10^{-7} M, *Figure 4.6*, labelled sample 1 to 7, respectively) are flowed through the channel in random order. A time-intensity profile is constructed, where distinct intensity can be quantitatively assigned to MB of specific concentrations using a double log scale calibration curve (*Figure 4.6B*, *Figure 4.7*). Specifically, sample 1 and 6 exhibit (2300 ± 502) counts, which match with the intensity obtained from the reference 10^{-7} M sample (*Figure 4.6*) ; sample 2, 4, 5 and 7 exhibit $(18.1\text{k} \pm 1.3\text{k})$ counts which corresponds to the 10^{-4} M sample; and the most intense signal from sample 3 exhibits up to $(38\text{k} \pm 1.8\text{k})$ counts which corresponds to the most concentrated sample of 10^{-2} M. Importantly, the detection of highly concentrated 10^{-2} M solution (sample 3) does not contaminate the channel and the subsequent samples of 100-fold lower concentrations (10^{-4} M, sample 4 and 5). Our contamination-free system is also affirmed by the zero intensity of MB signals at every decane flush intervals. Similarly, the trace concentration 10^{-7} M sample (sample 6) exhibits its corresponding intensity after the detection of 1000-fold higher concentration samples. The ability tackles the issue of inter-sample contamination in conventional on-line detection platforms using single phase colloidal solution as illustrated in our control experiment (*Figure 4.8*). The direct exposure of colloidal nanoparticles to the channel leads to its contamination and thus affect the intensity readout of the subsequent sample with 1000-fold lower concentration. For instance, the 10^{-5} M samples suffer strong inter-sample contamination and display inaccurate intensity of up to $\sim 23\text{k}$ counts which is comparable to the $\sim 25\text{k}$ counts observed in 10^{-2} M samples. Such cross-talking is unfavorable for sequential sensing of samples of different concentrations, which is resolved using our

plasmonic colloidosomes with high encapsulation efficiency. Therefore, this ability grants our online colloidosome-based SERS platform immense practical value for the development of reliable Raman analyzer.

Figure 4.6. Contamination-free, quantitative and high throughput on-line SERS detection of samples containing different concentrations. (A) 3D scheme of the on-line SERS detection of samples containing methylene blue 10^{-7} M, 10^{-4} M and 10^{-2} M in random order. (B) Time-lapsed 2D contour plot of output SERS spectra obtained from on-line detection, with the time-lapsed intensity profile of 1630 cm^{-1} signal (yellow-dotted box). The blue rectangular bars indicate the known average intensity at each respective concentration, obtained from methylene blue calibration curve.

Figure 4.7. (A) Methylene blue SERS spectrum and (B) intensity-concentration (double log scale) calibration curve constructed based on 1630 cm⁻¹ signal (highlighted in (A)). (C) SERS spectra of malachite green detected in plasmonic colloidosomes with concentration ranging from 10⁻² M to 10⁻⁹ M. Control refers to blank colloidosomes in the absence of malachite green.

Figure 4.8. Time-lapsed intensity profile of methylene blue 1630 cm⁻¹ peak demonstrating inter-sample contamination on the on-line detection platform using multiple Ag colloidal flows of methylene blue 10⁻² M and 10⁻⁵ M.

4.2.3 Multiplex quantification

The ability to resolve multiple analytes in the same sample is crucial because real-life specimens are typically complex systems containing multiple species.¹³ A sample containing 10^{-4} M MB and 10^{-3} M crystal violet (CV) gives rise to a multiplex spectrum containing the fingerprint signals of both analytes (*Figure 4.9A*), namely 1039, 885 and 667 cm^{-1} for MB (labelled green), and 1590, 1540 and 725 cm^{-1} for CV (labelled magenta, *Table S5 in Appendix*). Our investigation on the analytes' distinct signals in the 550 – 800 cm^{-1} window reveals that the intensity of the signals in the multiplex detection match well with that measured in the individual detection (*Figure 4.9B, C*). In particular, MB's 667 cm^{-1} signal achieves similar intensities (within one standard deviation error) of (910 ± 70) and (880 ± 100) counts; and CV's 725 cm^{-1} signal achieves (2030 ± 250) and (1960 ± 300) counts, in the individual and multiplex detection respectively. The hydrophobic self-assembled monolayer of thiol ligands (PFDT) on our Ag surface serves to prevent the adsorption competition among analyte molecules with different affinity to metal surface, thus ensure fair SERS enhancement for all analytes in a multiplex detection. Additionally, our further experiments involving the multiplex detection of analytes with large concentration difference by 2-3 magnitude orders (*Figure 4.10, 4.11*) also exemplifies the excellent ability of our on-line platform in simultaneous qualitative and quantitative multiplex analysis, which is highly adaptable for potential applications in high throughput in vivo investigation of multi-component complex live systems.^{23,24} In addition, the ability to fully resolve Raman fingerprints of multiplex analyte paves the way for rapid sensing without tedious sample treatment and analyte isolation process commonly performed prior to conventional analysis methods.

Figure 4.9. Multiplex on-line detection of sample containing two analytes. (A) Experimental multiplex SERS spectrum exhibiting all vibrational fingerprints of both MB and CV, with comparison to the reference SERS spectra of the dyes. (B) Magnified window of the green-dotted box in (B), showing MB (667 cm⁻¹) and CV (725 cm⁻¹) fingerprints, which are used to calculate the correlation between multiplex and single detection, as show in (C).

Figure 4.10. (A) Experimental multiplex SERS spectra of mixture samples containing different analyte ratio/concentration, with comparison to the reference SERS spectra of the individual

MB and CV dye. (B) Magnified window of the red-dotted box in (B), showing MB (667 cm^{-1}) and CV (725 cm^{-1}) fingerprints, whose intensity is presented in (C).

Figure 4.11. Comparison between the peak intensity observed in single and multiplex detection, based on (A) MB 667 cm^{-1} peak and (B) CV 725 cm^{-1} .

4.2.4 Online identification and quantification of multiple samples of different analytes.

Multiple samples of different analytes and concentrations can also be resolved, validating its practical value and potential for sequential analysis with high throughput quantity (*Figure 4.12A*). Four unidentified samples containing MB, CV, malachite green (MG) and rhodamine 6G (R6G) of different concentrations are flowed through the online system in random order. We obtain a well-resolved waterfall plot of SERS spectra with four distinct signals (labelled sample 1 to 4 respectively; *Figure 4.12B,C*). We are able to identify these unknown dyes by

investigating the percentage of spectral correlation with a library of reference SERS spectra. For instance, only the SERS profile of sample 1 achieves absolute correlation, 1.0 with R6G reference spectrum (labelled red in *Figure 4.12C*, *Table S3 in Appendix*), indicating that sample 1 contains R6G dye. Sample 2 has the highest correlation degree of 1.0 with MG reference spectrum among the 4 samples, affirming the presence of MG in sample 2 (labelled green, *Table S4 in Appendix*). We do observe positive correlation of 0.5 for sample 3 and 0.25 for sample 4 with MG reference spectrum, which could be due to the shared SERS features of MG, CV and MB that leads to the partial correlations. Hence, we set the benchmark that only samples that yield absolute correlation of 1.0 can be correlated to the reference analyte, as it indicates the agreement of all SERS fingerprints. This highlights that our system can differentiate molecules with similar molecular structures and physical properties with SERS fingerprint comparison,¹⁷ featuring a powerful analysis technique. Similarly, sample 3, and 4 are identified as CV and MB (labelled purple and blue in *Figure 4.12C* respectively). The high intensity also arises from the additional surface-enhanced resonance Raman scattering (SERRS) effect due to the overlapping of the excitation laser line (532 nm) and the absorption maxima of the dyes used. These intensity values obtained from the SERS spectra also reveals the concentration of the dyes, as they are quantitatively correlated with the intensity-concentration calibration curves (double log scale, *Figure 4.7, 4.13, 4.14, 4.15*). That is, 10^{-6} M R6G in sample 1, 10^{-2} M MG in sample 2, 10^{-2} M CV in sample 3 and 10^{-4} M MB in sample 4. The above results demonstrate the superiority of plasmonic colloidosomes in providing accurate information about the sample contents via SERS technique, and the immense potential of our on-line detection platform for on-site high through-put analysis of large sample quantity.

Figure 4.12. Sequential and quantitative multi-analyte on-line SERS detection. (A) 3D scheme of the on-line SERS detection of samples containing rhodamine 6G (R6G), malachite green (MG), crystal violet (CV) and methylene blue (MB). (B) Time-lapsed 2D contour plot of output SERS spectra obtained within 2 minutes exhibiting specific SERS fingerprints of each analyte, with respective concentrations obtained from tallying the peak intensities with calibration curves. (C) Time-lapsed correlation degree to specific reference spectra for differentiation of compounds with closely-resembling SERS features.

Figure 4.13. (A) Rhodamine 6G SERS spectrum and (B) intensity-concentration (double log scale) calibration curve based on 620 cm⁻¹ signal (highlighted in (A)). (C) SERS spectra of rhodamine 6G detected in plasmonic colloidosomes with concentration ranging from 10⁻³ M to 10⁻⁷ M. Control refers to blank colloidosomes in the absence of rhodamine 6G.

Figure 4.14. (A) Malachite green SERS spectrum and (B) intensity-concentration (double log scale) calibration curve based on 1628 cm⁻¹ signal (highlighted in (A)). (C) SERS spectra of malachite green detected in plasmonic colloidosomes with concentration ranging from 10⁻² M to 10⁻⁹ M. Control refers to blank colloidosomes in the absence of malachite green.

Figure 4.15. (A) Crystal violet SERS spectrum and (B) intensity-concentration (double log scale) calibration curve based on 1628 cm^{-1} signal (highlighted in (A)). (C) SERS spectra of crystal violet detected in plasmonic colloidosomes with concentration ranging from 10^{-2} M to 10^{-6} M . Control refers to blank colloidosomes in the absence of crystal violet.

4.2.5 Online identification and quantification of cytosine

To demonstrate the applicability of our system for real-life applications, we have added an additional on-line detection of non-dye cytosine molecule spiked within tap water (at alternating concentrations of 8 mg/mL and 0.8 mg/mL) to demonstrate the potential of our plasmonic colloidosome-based on-line channel as a universal SERS platform for real-life applications (*Figure 4.16*). The detection of cytosine indicates that our colloidosomes are not limited to dye molecules, yet can be further developed and applied in biomedical diagnosis/genome monitoring based on micro-DNA detection. Further examinations on the SERS detection of biomolecules within highly complex matrices such as urine and other body fluids are undergoing with plasmonic particles functionalized with specific capturing agents to selectively extract and detect the target analytes within the matrices.

Figure 4.16. (A) Time-lapsed waterfall spectra of the on-line detection of cytosine samples with alternating concentrations (8mg/mL, 0.8 mg/mL and 8 mg/mL). (B) SERS intensity (collected at 800 cm^{-1}) versus time profile of various colloidosomes of alternating concentrations passing through the microfluidic tube, highlighted in the blue-dotted box in (A).

4.3 Conclusion

In conclusion, we design the first on-line microfluidic platform compatible with highly sensitive plasmonic colloidosomes for multi-assay SERS detection. Our platform prevents inter-sample cross-talking and channel contamination, allowing the reproducible detection and accurate quantification of samples with largely different concentrations. We also achieve high resolution multiplex detection with fully preserved Raman features and signal intensity of a mixture of analytes within one sample. Our platform is also capable of multi-assay style analysis that provides rapid identification and quantification of a sequence of samples

containing different analytes and concentrations. Ultimately, the collective advantages of fusing plasmonic colloidosomes with on-line SERS detection sketch new horizons in the development of superior portable sensors for on-site high throughput detection, widely applicable in medical diagnosis, food sampling, forensics analysis and environmental monitoring.

4.4 Materials and Methods

Chemicals. Silver nitrate ($\geq 99\%$), anhydrous 1,5-pentanediol (PD, $\geq 97\%$), poly(vinyl pyrrolidone) (PVP, average MW = 55,000); 1H,1H,2H,2H-perfluorodecanethiol (PFDT, $\geq 97\%$), 1H,1H,2H,2H-perfluorooctyltriethoxysilane (98%), rhodamine 6G (R6G, dye content $\sim 95\%$), decane (anhydrous, $> 99\%$), methylene blue (MB, $\geq 82\%$), malachite green (MG, oxalate salt, dye content $\geq 90\%$) and crystal violet (CV, dye content $\geq 90\%$) were purchased from Sigma Aldrich; copper (II) chloride ($\geq 98\%$), cytosine ($\geq 98\%$) was from Alfa Aesar; ethanol (ACS, ISO, Reag. Ph Eur) was from EMSURE®; toluene (BAKER ANALYZED® A.C.S. Reagent) was from Avantor; propan-2-ol (HPLC grade) was from Fisher Scientific. All chemicals were applied without further purification. Milli-Q water ($> 18.0\text{ M}\Omega\cdot\text{cm}$) was purified with a Sartorius Arium® 611 UV ultrapure water system.

Synthesis and purification of silver nanocubes. The preparation of silver (Ag) nanocubes was carried out based on the polyol method described in literature.²⁵ 10 mL PD solutions of CuCl_2 (8 mg/mL), PVP (20 mg/mL) and AgNO_3 (20 mg/mL) were prepared separately by sonication and vortex. 35 μL CuCl_2 solution was added to the AgNO_3 solution. 250 μL PVP precursor was added dropwise every 30 s while 500 μL AgNO_3 precursor was injected every min using a quick addition to a 10 minute-preheated 20 mL PD solution. The addition was continued until the mixture turned orange brown. For the purification, PD was first removed by washing the mixture with acetone followed by ethanol. The suspension was then dispersed in 10 mL ethanol and 100 mL aqueous PVP solution (0.2 g/L) and filtered using

Durapore® polyvinylidene fluoride filter membranes (Millipore) with pore sizes ranging from 5000 nm, 650 nm, 450 nm and 220 nm, several times for each pore size. SEM imaging was performed, from which the edge lengths of 250 Ag nanocubes were measured and analyzed using ImageJ software. The as-synthesized nanocubes were found to be obtained in high yield of approximately 40 mg/synthesis.

Functionalization of Ag nanocubes with perfluorodecanethiol. 20 mg of purified Ag nanocubes were immersed in 10 mL of 1:1 propan-2-ol/hexane solution containing 0.1 mM of 1H,1H,2H,2H-perfluorodecanethiol (PFDT) for 6 h at room temperature. The colloidal suspension was then washed with copious amounts of ethanol and decane, and subsequently dispersed in 1.0 mL of decane.

Preparation of colloidosomes. Methylene blue (3.20 mg/mL, 10^{-2} M), rhodamine 6G (4.79 mg/mL, 10^{-2} M), malachite green (4.63 mg/mL, 10^{-2} M), crystal violet (4.08 mg/mL, 10^{-2} M) were prepared in aqueous solution using ultrapure water. Serial dilutions were performed to give a series of concentrations, ranging from 10^{-3} M to 10^{-8} M. 0.5 μ L of the aqueous solution of the above dyes was added to 500 μ L decane suspension containing 0.10 mg perfluorodecanethiol-functionalized Ag nanocubes (referred to as Ag from this point onwards). Colloidosomes were formed by emulsification via vigorous shaking.

Preparation of microfluidic flow channel. 0.5 mm inner diameter (ID) thin-layer-chromatography (TLC) tubes are soaked in 1H,1H,2H,2H-perfluorooctyltriethoxysilane 2% solution in water/ethanol (4:1 v/v) for 5 minutes, and flushed with the same silane solution for 3 times, to impart hydrophobicity. Before flushed with ethanol and let dry. The hydrophobic TLC tubes are then connected to 0.7 mm ID Teflon tubes via air-tight tape. The system is then fixed on a glass slide with duct tape. The TLC tube is used as main flow channel for online detection, and Teflon tubes are used as inlet (connected to syringe pump) and outlet.

Static SERS imaging of colloidosomes. Colloidosomes containing methylene blue of 10^{-6} M are transferred to the flow channel via pipette. There is no external flow and the colloidosomes remain static in the tube. The segment with the most colloidosomes is imaged with Ramantouch microspectrometer (see Material characterization).

Online SERS analysis of analytes using colloidosomes. 1 μ L of the plasmonic colloidosomes in decane (one sample) were transferred into the inlet via pipette, which is then connected to a syringe containing decane being pumped at 0.10 mL/min, controlled with a NE-1000 syringe pump. After time intervals of minimum 10 s (for flushing and injection of new samples, time interval can be extended with lower through-put quantity), the flow is stopped temporarily and the inlet is disconnected from the decane source for the addition of the next sample batch. The flow is continued again and the process repeats for several sequential online analysis.

SERS intensity analysis: Each signal of analyte sample typically comprises of 4-5 spectra, the average intensity of which is used as the intensity for quantitative analysis.

Calibration curve construction: The samples containing the dyes of concentrations from 10^{-2} to 10^{-9} M are injected and flowed through the channel in similar methods as described above. The intensity corresponding to each of the concentrations is obtained for the construction of the double log scale calibration plot. For each of the concentrations, 3 experiments are performed to obtain the average intensity. The lowest concentration shown in the calibration plot is the lowest concentration that can display signals (limit of detection), that are distinguishable from the blank control (colloidosomes encapsulating pure water without the presence of any analyte).

For online SERS experiment with plasmonic colloidosome samples of different MB concentration: The samples are injected and flowed through the channel in similar methods as described above. The time intervals are extended to 15 s accordingly to the sample through-put

quantity (7 samples). The samples are eluted at 12.5, 28, 43, 57, 65, 85 and 93 s; labelled sample 1 to 7 respectively.

For online SERS experiment with plasmonic colloidosome sample containing dye mixture: MB and CV are mixed in one aqueous solution with the mentioned final concentrations. The mixed solution is made into plasmonic colloidosome samples, which are injected and flowed through the channel in similar methods as described above.

For online SERS experiment with plasmonic colloidosome samples of different analytes and concentrations: The samples are injected and flowed through the channel in similar methods as described above. The time intervals are extended to 25 s accordingly to the sample through-put quantity (4 samples). The samples are eluted at 14, 40, 65 and 98 s, labelled sample 1 to 4 respectively.

For control online SERS experiment using colloidal suspension method: A suspension prepared from 100 mg of perfluorodecanethiol-functionalized Ag nanocubes in 500 μ L of the dye solution (same Ag/analyte solution volume to prepare colloidosome samples) was sonicated and homogenized. The colloidal suspension is pumped from a syringe with the same flow rate through the flow channel. Subsequent samples are pumped in sequence, right after the previous sample finished.

Data processing of time-lapsed SERS spectra. Time-lapsed SERS spectra are obtained with u-Raman controlling software uSoft (Technospex Pte. Ltd, Singapore). The time-lapsed spectra are then subjected to baseline subtract treatment with OriginPro software.

Material characterization. SEM imaging was performed with JEOL-JSM-7600F microscope. UV-vis spectroscopic measurements were conducted with a Cary 60 UV-Vis spectrometer. Static SERS measurements were performed using both x-y imaging mode of the Ramantouch microspectrometer (Nanophoton Inc, Osaka, Japan) with an excitation wavelength of 532 nm. When using 4 $\times$ objective lens (N.A. 0.13), laser power was set at 133.3

μW with 1 s accumulation time (unless otherwise stated) for data collection between 200 cm^{-1} to 1800 cm^{-1} . All on-line SERS measurements were performed using time-lapsed mode of uRaman-532 (Technospex Pte. Ltd, Singapore) with laser power 50 mW with 500 ms accumulation time for data collection between 200 cm^{-1} to 1800 cm^{-1} , under $20\times$ objective lens (N.A. 0.4).

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Chapter 5

Plasmonic Hotspots in the Air: Omnidirectional and Three-dimensional Platform for Stand-off In-air SERS Sensing of Airborne Species **

Abstract. Molecular-level airborne sensing is critical for early prevention of disasters, diseases and terrorism. Currently, most 2D surface-enhanced Raman spectroscopy (SERS) substrates used for air sensing have only one functional surface and exhibit poor SERS active depth. Here, we introduce ‘aerosolized plasmonic colloidosomes’ (APC) as airborne plasmonic hotspots for direct in-air SERS measurements. Our APC functions as a macro-scale 3D and omnidirectional plasmonic cloud that receives laser irradiation and emits signals in all directions. Importantly, it brings about an effective plasmonic hotspot in ~ 2.3 cm lengthscale, which affords 100-fold higher tolerance to laser misalignment along the z axis comparing with 2D SERS substrates. Our APC exhibits extraordinary omnidirectional property and demonstrates consistent SERS performance independent of laser and analyte introductory pathway. Furthermore, we showcase the first in-air SERS detection in a stand-off condition at 200 cm distance, highlighting the immense applicability of our 3D omnidirectional plasmonic cloud for remote airborne sensing in threatening/inaccessible areas.

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5.1 Introduction

Airborne sensing of explosive vapors, chemical hazards and pathogens is critical for early recognition and prevention of disasters, diseases and terrorism.¹⁻³ Generally, airborne sensing is performed using methods such as gas chromatography,⁴ photoelectric/ionization detectors,⁵ and nanomechanical sensors.^{6,7} Despite their widespread usage, these conventional methods do not provide direct and specific molecular fingerprints of target analytes.⁸ The identification and differentiation of gas molecules with similar physical properties remain challenging yet crucial to prevent potential false signals from interferences.⁹ As a solution, surface-enhanced Raman spectroscopy (SERS) has been employed as a general detection technique that provides instantaneous molecular fingerprints read-out,¹⁰ for the trace detection of various species present in the air using ultrasensitive substrates.¹¹⁻¹⁴ However, SERS sensing of airborne species often require additional molecular collection systems such as electrodynamic precipitation or fluidic stream to accumulate airborne molecules to the SERS substrates.^{2,15} These substrate-based platforms are further limited by the need for stringent laser focal and directional alignment during measurements – any slight deviation of laser path or its focal point can result in dramatic decrease in signal intensity.^{16,17}

To overcome these pressing problems, an omnidirectional and three-dimensional (3D) SERS platform is ideal for airborne detection within an extended volume due to their ability to receive laser irradiation and transmit signals in all directions. We hypothesize that incorporating plasmonic nanoparticles within an isotropic aerosol to form a 3D plasmonic ‘cloud’ can function as a macro-scale omnidirectional SERS platform to tackle the aforementioned issues of 2D substrates.^{18,19} Such plasmonic clouds are independent to laser angle alignment and have advantageous flexibility in dynamic gas phase environment, permitting the detection of analyte molecules introduced from any direction. On the other hand, 2D substrates have merely one functional surface where only analytes deposited on the specific

surface can be detected. Moreover, aerosols extending up to centimeter-scale can be easily produced using a commercial spray device, allowing the formation of macroscale 3D plasmonic volume with high tolerance to laser misfocus. Hence, aerosols of plasmonic nanoparticles have immense potential to couple with stand-off Raman devices for remote sensing in dangerous/inaccessible areas where accurate laser positioning from meter-range distances remains non-trivial.

Here, we introduce ‘aerosolized plasmonic colloidosomes’ (APC) as an omnidirectional 3D platform that serves as in-air SERS hotspots for the identification of airborne molecules in their native environment. An aerosol containing plasmonic colloidosomes is first introduced into the air using an aerosolizer, where its physical properties such as spatial dimensions and stability are evaluated. We further examine the SERS characteristic of our APC clouds, exemplifying them as a macro-scale 3D SERS active volumes that extends over several centimeters required for omnidirectional SERS measurement. Comparison with conventional 2D substrates further highlights that our APC exhibits >100-fold larger SERS-active volume crucial to circumvent laser misalignment issue typical in in-air detection. When coupled with stand-off Raman system, our APC notably represents the world’s first proof-of-concept in-air SERS sensor for remote detection of airborne species up to 200 cm away. Our work lays the foundation for the development of remote air sensing technology, which is especially critical for defense and security sectors.

5.2 Results and Discussion

5.2.1 Preparation and characterization of aerosolized plasmonic colloidosomes

To prepare omnidirectional 3D plasmonic clouds for in-air SERS, we first fabricate plasmonic colloidosomes and subsequently aerosolize them using a commercial nanosprayer

device (*Figure 5.1A, B*). Briefly, plasmonic colloidosomes of an average diameter of (7 ± 3) μm are fabricated by emulsifying a water/hexane system using single-crystalline Ag nanocubes (edge length ~ 120 nm) as building blocks (*Figure 5.2, 5.32A*).²⁰ We hereby employ hexane as the continuous solvent due to its low boiling point (68°C) that allows quick evaporation in the further aerosolization, thus exposing our plasmonic colloidosome shells to interact with airborne analytes. Our plasmonic colloidosomes are spherical and possess multilayer shells composed of (10 ± 3) Ag nanocube layers, giving rise to a high density of SERS hotspots (*Figure 5.3B, C*). Subsequently, we transfer the as-synthesized colloidosomes to an aerosolizer disk that uses high-rate vibration at a regularity of 1 GHz to eject aerosols containing colloidosomes. APC is produced instantly in the form of a mist, and can spread up to ~ 8 cm away from the nozzle within ~ 1 second upon activation of the device (*Figure 5.1B; Figure 5.3*). Upon collection of the aerosolized contents, a large portion of colloidosomes have retained their original shape and size (7 ± 3) μm (*Figure 5.1C, D; Figure 5.5*). This indicates that they can withstand the physical impacts of the expulsion process through the aerosolizer nozzle (*Figure 5.6*). We estimate that a concentration of 68000 colloidosomes/ cm^3 (equivalent to 10^9 Ag nanocubes/ cm^3) is introduced within the APC volume per second (*Calculation 5.1*), providing substantial quantity of SERS hotspots in the air for the subsequent in-air detection. In addition, we obtain circular coverage areas of APCs with diameters of 0.8, 1.5 and 3.0 cm by depositing the aerosol on Si substrates at 1, 3 and 5 cm away from the aerosolizer nozzle (*Figure 5.1E, 5.5*). Notably, the circular cross-sections of APCs also suggest their omnidirectional property, where the three-dimensional cloud of APCs indicates that the plasmonic hotspots are available in all directions for in-air analyte detection. Such property is highly advantageous for in-air sensing of airborne species that are typically randomly dispersed.

Figure 5.1. Aerosolized plasmonic colloidosomes (APC). (A) Schematic illustrating plasmonic colloidosomes as a 3D plasmonic cloud for stand-off SERS measurement of airborne species. (B) Digital image of the plasmonic mist dispensed from the aerosolizer. (C) Microscopic image of aerosolized colloidosomes collected in a hexane solution after spray. (D) SEM image of a colloidosome collected on a Si substrate after spray. (E) Digital images showing APC's cross-sectional coverage area (yellow-dash circle in second image) obtained by depositing the aerosol on Si substrates at 1, 3 and 5 cm away from the aerosolizer nozzle (as illustrated in the 3D scheme).

Figure 5.2. SEM image of as-synthesized Ag nanocubes.

Figure 5.3. (A) Microscopic images of as-prepared plasmonic colloidosomes (in hexane). (B) SEM image of a full plasmonic colloidosomes. (C) SEM image of a partially opened colloidosome showing a shell thickness of around (10 ± 3) Ag nanocube layers.

Figure 5.4. Aerosolizer device. (A) Digital image of an aerosol mist formed using a commercial humidifier. (B) Aerosolizer chip and membrane extracted from the humidifier.

Figure 5.5. (A) Overall SEM images of colloidosomes collected from the spray on Si substrate at 1, 5 and 7 cm away from the spray nozzle. (B) Colloidosome density per mm² of the collected Si substrates. (C) Magnified SEM images of colloidosomes collected on Si substrate after spray. (D) Size distribution of colloidosomes collected after spray. The colloidosome density on the Si substrate maintains in the range of ~5500/mm² to ~7000/mm² when the substrate is ≤ 5 cm away, but drops to ~2000/mm² when the separation is > 5 cm (Figure S4). This indicates that the aerosol has the highest density of colloidosomes extending up to 5 cm from the nozzle, which can be utilized for subsequent SERS detection.

Figure 5.6. Microscopic images of the aerosolizer membrane inlet and outlet holes. The device inlet hole size is around 25 μm while the outlet holes are 10 μm in width. Only colloidosomes smaller than 10 μm are able to pass through and the bigger ones are otherwise reemulsified into

smaller ones, resulting in the loss of Ag particles. Thus, we intentionally prepare small colloidosomes with diameter $< 10 \mu\text{m}$.

Calculation 5.1. Calculation of colloidosome concentration in aerosol

We start with $3.0 \mu\text{L}$ of water and produce colloidosomes (droplets) of $7 \mu\text{m}$ diameter. Hence, the total number of colloidosomes formed is:

$$N_{\text{colloidosomes}} = V_{\text{water}} / V_{\text{colloidosomes}} = 3 \mu\text{L} / \left(\frac{4}{3} \pi \left(\frac{7}{2} \mu\text{m} \right)^3 \right) = 1.7 \times 10^7 \text{ colloidosomes}$$

Typically, one whole batch of colloidosomes is transferred to the aerosolizer reservoir. We assume all colloidosomes are transferred. A whole batch of colloidosome emulsion of $500 \mu\text{L}$ can be sprayed continuously over 10 seconds. Due to gravity, the colloidosomes always settle down and accumulate at the nozzle, causing APC to be dispensed in its highest concentration possible. Hence, we are unable to tune the concentration of colloidosomes per volume that can be sprayed out. This calculation estimates the amount of colloidosomes within the APC:

We observe that the APC can extend up to 8 cm length and 2 cm width, hence the cylindrical volume that the colloidosomes are introduced into is estimated as :

$$V_{\text{air}} = \pi(1\text{cm})^2 \times 8\text{cm} = 25 \text{ cm}^3$$

The concentration of colloidosomes sprayed out in air per 1 second :

$$\begin{aligned} C_{colloidosome} &= 1.7 \times 10^7 \text{ colloidosomes} / 10 \text{ s} \times 25 \text{ cm}^3 \\ &= 68000 \text{ colloidosomes cm}^{-3} \text{ s}^{-1} \end{aligned}$$

We are also able to calculate the concentration of Ag nanocubes being sprayed out as followed:

Ag nanocube edge length is 121 nm.

Ag nanocube volume is : $V_{Ag \text{ cube}} = (121 \text{ nm})^3 = 1.77 \times 10^6 \text{ nm}^3 = 1.77 \times 10^{-15} \text{ cm}^3$

Ag density is 10.49 g/cm^3 , hence the weight of one Ag cube is :

$$m_{Ag \text{ cube}} = 10.49 \text{ g.cm}^{-3} \times 1.77 \times 10^{-15} \text{ cm}^3 = 1.86 \times 10^{-14} \text{ g}$$

We use 4.80 mg of Ag cubes in total for one batch of colloidosomes, thus the number of Ag cubes is:

$$N_{Ag \text{ cubes}} = 4.80 \text{ mg} / 1.86 \times 10^{-14} \text{ g} = 2.58 \times 10^{11}$$

The concentration of cubes sprayed out in air per 1 second :

$$C_{Ag \text{ cubes}} = 2.58 \times 10^{11} / 10 \text{ s} \times 25 \text{ cm}^3 = 10^9 \text{ cm}^{-3} \text{ s}^{-1}$$

(end of Calculation 5.1)

5.2.2 Large three-dimensional SERS-active volume of aerosolized plasmonic colloidosomes.

The major advantage of APCs over 2D SERS substrates lies within its 3D effective plasmonic volume over centimeter-scale. To demonstrate and characterize the SERS active

volume and sensitivity along the spatial axes of APC, we simultaneously record the 1077 cm^{-1} fingerprint of 4-MBT-functionalized APCs across the corresponding x, y, and z axes at $500\text{ }\mu\text{m}$ step intervals as they are sprayed into a detection chamber (4-MBT acts as signalling molecule, *Figure 5.7A,B*, *5.8*, *Calculation 5.2*). Upon projection of the intensity profiles along the axes, we obtain a large 3D SERS active volume with $\sim 2.3\text{ cm}$ in the vertical spray direction (y-axis, main axis of the spray) and $\sim 1.0\text{ cm}$ and $\sim 0.98\text{ cm}$ in x- and z-axes, respectively (full-width half-maximum (FWHM) of intensity profiles, *Figure 5.7B*, *Figure 5.9*). While this cuboid shape is modelled after the spray in the chamber and does not fully represent the standalone plasmonic cloud, it still affirms that the SERS-active volume of our APC clouds is three-dimensional and extends over centimeter-scale regions, a significant leap from conventional 2D SERS substrates with micro-scale SERS active depth. It is also critical to design a SERS platform that possesses higher tolerance to laser misfocus, and can produce consistent signal readout over larger region in z-axis. Notably, the FWHM of the intensity profile along the z-axis for APC is calculated at up to 9.8 mm (0.98 cm , *Figure 5.7C*). In our control experiments with 2D SERS substrate, the SERS active volume along the z-axis is measured at only $\sim 200\text{ }\mu\text{m}$ in FWHM of the intensity profile. Based on the above results, it is clear that the effective 3D SERS active layer of APC is 100-fold superior to that of conventional 2D substrates. This unprecedented centimeter-scale 3D focal volume is a breakthrough from the conventional focal plane, and provides immense flexibility in laser focal alignment, which is especially crucial for in-air SERS measurements. On a side note, we observe that even after the nozzle is turned off, 4-MBT signals are still detectable after $\sim 2\text{ s}$ (*Figure 5.10*), indicating the airborne suspension time of APC last up to 2 s . Nevertheless, we choose to constantly introduce APC into the detection chamber to maximize the in-air hotspot density.

Figure 5.7. 3D characteristic of APC. (A) Schematic illustration on the spread of APC (with 4-MBT) in the x, y, and z directions. (B – D) Comparison of SERS detection capabilities between (i) APC and (ii) 2D substrate based on different parameters. (B) SERS active volume along x, y, z axes, (C) SERS intensity of 4-MBT signal observed when laser focal point is moved along z-axis (D) SERS intensity recorded when each platform is rotated at different angles from the original position with respect to laser beam.

Figure 5.8. The detection chamber (length (z) $\times$ width (x) $\times$ height (y) = 12 cm $\times$ 4 cm $\times$ 4 cm) and the defined axis used to measure the x, y and z dimension of the APC with respect to the fixed laser spot. In actual detections, analytes are introduced from the opposite end of the laser.

Figure 5.9. Projection of 3D SERS active volume of APC via x,y,z intensity profiles.

Figure 5.10. 4-MBT intensity profile observed from 4-MBT APC showing aerosol suspension time after APC spray is stopped.

Calculation 5.2 Theoretical calculation of laser confocal volume

Our laser is irradiated via a L-shape horizontal lens with 0.08 N.A and 4 μm beam radius.

The confocal volume is calculated as:²¹

$$V_c = \pi^{3/2} \kappa \omega^3 \quad (\omega \text{ is beam radius})$$

Where $\kappa = 2.33 \frac{n}{N.A} = 2.33 \frac{1.00}{0.08} = 29.125$ (we perform in-air measurement, refractive index of air $n = 1$)

Hence, the theoretical confocal volume can be estimated as:

$$V_c = \pi^{3/2} \kappa \omega^3 = \pi^{3/2} 29.125 \times (4 \mu\text{m})^3 = 10379 \mu\text{m}^3$$

Our system is coupled with a horizontal laser source with a large focal volume of $10^4 \mu\text{m}^3$ so that large volume of in-air signals can be collected.

(end of Calculation 5.2)

The omnidirectional property of APC gives it uniformity and reproducibility in SERS performance in all directions, rendering it independent on the angle of laser irradiation. To examine this unique property, we measure the SERS signals by rotating the injection angle of APCs along the y-axis from 0 to 180° with respect the laser beam (*Figure 5.7A; Figure 5.8*), thus exposing different sides of the APC to the laser pathway. We obtain consistent 4-MBT signals from APC clouds with a relative standard deviation of 5% when we systematically rotate the APC injection angles at 0°, 45°, 90°, 135° and 180° along the y-axis (*Figure 5.7D*). In contrast, 2D substrates exhibits highest intensity only when the substrate surface is directly facing the laser beam (original position, 0°) and a 45° rotation leads to a 90% decrease in signal intensity (*Figure 5.7D(ii)*). These observations highlight the importance of APC's 3D SERS volume to operate uniformly in all directions, offering significantly higher tolerance to laser directional misalignment compared to a 2D substrate.

5.2.3 In-air SERS detection with aerosolized plasmonic colloidosomes

The in-air SERS performance of our APCs is further evaluated by detecting various aerosolized liquid analytes, an important class of airborne species that is commonly encountered in medical diagnosis (saliva),²² environmental monitoring (haze and mist),²³ and defence (sprayed nerve and biological agents).²⁴ Our detection model uses aerosolized methylene blue (MB) as probe molecules, where blank aerosolized plasmonic colloidosomes are the substrate-less SERS platforms. Typically, 5 μmol MB aerosols (in ethanol) and a 500 μL suspension of 4.8 mg APCs (in hexane) are introduced simultaneously into the detection chamber from two separate inlets; one from side and one from top (*Figure 5.11A*). The SERS signals are collected instantaneously in mid-air where the aerosolized colloidosomes and MB come into direct contact and mixing (yellow dash-circle in *Figure 5.11A*). We obtain SERS spectra with distinct fingerprints of MB including C-N-C deformation modes at 456 and 503

cm^{-1} , and aromatic C-C stretching at 1630 cm^{-1} , respectively (*Table S1 in Appendix*). When $5\text{ }\mu\text{mol}$ aerosolized MB is introduced into the detection chamber, the vibrational mode centered at 1630 cm^{-1} exhibits a SERS intensity of $\sim 5900\text{ counts/s}$. (*Figure 5.12*). The featureless spectra obtained just by spraying APC (without MB) or MB (without Ag) clearly affirms that the aforementioned signals arise from the successful in-air SERS detection of MB molecules using APCs (*Figure 5.11B*). This SERS response also demonstrates that our in-air APCs are able to interact effectively with aerosolized liquid analytes. On the other hand, aerosolized Ag nanocubes only give rise to 1500 counts of signal when MB aerosols of the same concentration is used (*Figure 3B, Figure S9*). Furthermore, aerosolized plasmonic colloidosomes are capable of detecting MB down to 0.5 nmol ($\sim 450\text{ counts}$, *Figure 5.12*), giving rise to an analytical enhancement factor (AEF) of 1.8×10^5 (*Figure 5.11C*). In contrast, single Ag particle spray can only achieve a detection limit of $5\text{ }\mu\text{mol}$ MB, with AEF value of only 36 (*Figure 5.13, Calculation 5.3*). These results demonstrate that APC outperforms single Ag particles, and is a superior platform for in-air SERS sensing owing to their unique multilayered Ag shells, to effect manifold SERS hotspots due to plasmonic couplings and thus increasing hotspot-analyte interaction in a highly dynamic in-air environment.

Figure 5.11. In-air detection of airborne species. (A) Experimental setup for SERS detection of methylene blue aerosol using APC (yellow dashed-circle indicates laser illumination spot). (B) Comparison of SERS performance of our 3D colloidosome with control experiment using single Ag nanocubes. (C) SERS intensity of MB (0.5 μmol) when MB is introduced from the back and side of (i) APC and (ii) substrate. The intensity is based on the characteristic fingerprint at 1630 cm^{-1} . (E) SERS spectra of other airborne species detected using APC ('control' refers to blank APC without any analyte introduced)

Figure 5.12. (A) Time dependent-SERS intensity of aerosolized MB (5 μmol) using APC (pink) and single particle spray (purple). Intensities were taken at characteristic 1630 cm^{-1} peak. (B) SERS spectra of different amounts of aerosolized MB using APC (“Control” experiment refers to APC in the absence of analyte). (C) Double-log scale intensity-concentration calibration curve of methylene blue aerosol in the in-air SERS detection with aerial colloidosomes.

Figure 5.13. SEM image of collected single Ag nanoparticle spray on Si substrate.

Calculation 5.3. Calculation of APC’s analytical enhancement factor in MB detection.

With reference to the 1630 cm^{-1} SERS band, we calculate the analytical enhancement factor of methylene blue detection as followed:

$$\begin{aligned}
 \text{Analytical EF} &= [(I_{\text{SERS}}) / (I_{\text{Raman}})] \times [(C_{\text{Raman}}) / (C_{\text{SERS}})] \\
 &= [403 / (2231 / 10)] \times (10^{-1} / 10^{-6}) \\
 &= 1.8 \times 10^5
 \end{aligned}$$

where N_{SERS} and N_{Raman} are the corresponding concentrations measured using plasmonic colloidosomes (10^{-6} M; 0.5 nmol / 500 μ L) and normal Raman of methylene blue (10^{-1} M, 10 μ mol over 500 μ L), respectively. I_{SERS} and I_{Raman} are the time-normalized intensities measured using SERS and normal Raman, respectively, at their corresponding concentration.

Figure 5.14. Normal Raman spectrum of methylene blue obtained in air.

Similarly, the analytical enhancement factor of methylene blue detection for single Ag nanoparticles can be calculated as followed:

$$\begin{aligned} \text{Analytical EF} &= [(I_{\text{SERS}}) / (I_{\text{Raman}})] \times [(C_{\text{Raman}}) / (C_{\text{SERS}})] \\ &= [821 / (2231 / 10)] \times (10^{-1} / 10^{-2}) \\ &= 36 \end{aligned}$$

where N_{SERS} and N_{Raman} are the corresponding concentrations measured using Ag nanocubes (10^{-2} M; 5 μ mol over 500 μ L) and normal Raman of methylene blue (10^{-1} M, 10 μ mol over 500 μ L), respectively. I_{SERS} and I_{Raman} are the time-normalized intensities measured using SERS and normal Raman, respectively, at their corresponding concentration.

(end of Calculation 5.3)

Airborne species are usually non-static and could be highly dynamic due to environmental conditions such as wind and air disturbance. Hence, an isotropic SERS platform increases the flexibility and feasibility in detection, eradicating the need for substrate alignment or positioning. To demonstrate the isotropic property of our APCs, we systematically vary the

direction from which MB aerosol is introduced into the APC spray. In our set-up, MB aerosol (0.5 μmol) is sprayed from the side and the back of SERS chamber. The APC affords highly reproducible and consistent SERS signals (~ 2000 counts) regardless of the analyte direction (Figure 5.11C, 5.14). In contrast, control 2D Ag substrate has only one active side and thus displays a dramatically poorer SERS performance. For instance, the 2D SERS substrate is only able to detect MB when the analyte is introduced from the side (~ 3000 counts), but not able to efficiently interact with the analyte (MB intensity < 50 counts) when it is introduced from the back. This result again exemplifies the importance of APC's 3D nature to achieve omnidirectional SERS detection of airborne species. Moreover, we are also able to perform the in-air identification of several volatile molecules such as 4-MBT vapors (vapor pressure, $P_{\text{MBT}} = 0.807$ mmHg), toluene and naphthalene aerosols (Figure 5.11D, Table S6-8 in Appendix for peak assignments). Among the analytes, we notice that the colloidosomes generally produce better intensity with large conjugated analyte (methylene blue) and absorbing thiol 4-MBT. Nevertheless, we are able to differentiate each airborne molecule by their characteristic SERS fingerprints, thus highlighting the strength of SERS in providing molecular-level identification to circumvent false positive commonly encountered in commercial air sensors.

Figure 5.15. Two-way detection chamber allowing the introduction of aerosolized MB from two different directions. We observe that APC is independent of the directions of analyte aerosol, while substrate is strongly dependent.

5.2.4 Stand-off in-air SERS detection with aerosolized plasmonic colloidosomes

Stand-off Raman spectroscopy is an analytical tool that is often used in operational scenarios whereby the spectrometer is separated from dangerous (explosion/disaster) or inaccessible (waterfall/mountainous) sample sites. Using a Raman spectrometer equipped with long distance objective lens (*Figure 5.16A-C, 5.17*), we successfully perform an in-air stand-off SERS detection of aerosolized MB upon its interaction with APC at a distance of 200 cm (~ 600 counts and ~ 200 counts for 50 nmol and 5 nmol respectively, *Figure 5.16D*). On the other hand, a featureless spectrum is obtained when signals are collected from MB aerosol in the absence of APC, indicating stand-off Raman alone does not yield sufficient signals for trace analyte detection. Moreover, quantitative multiplex spectra exhibiting characteristic vibrational features of MB (1630 cm^{-1} peak) and R6G (620 cm^{-1} peak, *Table S3 in Appendix*) are obtained when a mixture of 50 nmol of MB and 5 nmol of rhodamine 6G are sprayed with APC (*Figure 5.16E*). This demonstrates the immense potential in the development and application of APC for real-life sensing of airborne samples, which often contain more than one chemical species. To our knowledge, this is the first in-air stand-off SERS sensing demonstration in a substrate-less manner. While the overall sensitivity and detection limit of the aerosol platform is poorer in comparison to static substrates due to the vigorous spraying condition, the ensemble benefits enabled by APC effectively eliminates the need of tedious laser focusing processes. Collectively, these insights are important for the further development of stand-off, substrate-less SERS technology in identifying explosives/ hazardous compounds in inaccessible sites.

Figure 5.16. Stand-off SERS detection. (A, B) Scheme and digital image depicting the set-up of stand-off SERS for detection of methylene blue aerosol using APC. (C) Digital image of telescope lens used for stand-off detection. (D) SERS spectra of different methylene blue amount introduced into air per second, detected with APC using our set-up shown in (B). (E) Scheme and SERS spectra of in-air multiplex detection of both methylene blue (MB) and rhodamine 6G (R6G) by APC. The characteristic peaks of MB (456, 1630 cm⁻¹) and R6G (620 cm⁻¹) are highlighted in blue and yellow respectively.

Figure 5.17. Stand-off Raman detection set-up (background and irrelevant objects have been blurred to allow clearer focus on set-up)

5.2.5 Comparison between our method and conventional gas-phase analyzers.

The current gold standard for gas analysis is gas chromatography – mass spectroscopy (GC-MS), which separates the components in the air sample and provides elution time and molecular weight information of the components. While this method is able to provide rough identification and quantification through the peak position, molecular weight and peak intensity, the obtained information are not absolute and accurate at the molecular level due to the lack of molecular fingerprints. Similar molecular isomers with same molecule weight and physical properties appear as one peak in the GC-MS chromatogram, thus raising a serious issue in the high possibility of false-positives.¹⁰ Furthermore, GC-MS requires collection of air samples and submission to instrumental lab for analysis, followed by chromatography-based separation, which takes days to obtain results.

Electrochemical (EC) sensors and metal-oxide semiconductor (MOS) sensors are specifically designed as portable devices for on-site sensing.^{2,3} Both techniques measure the current generated when target gases are present at the electrode, through electrochemical current and conductivity measurements, respectively. As such, the amount of gas present is proportional/ inversely proportional to the types and concentration of gas present. It should be

noted that the current measurements from these sensors, similar to GC-MS, are also indirect signals. These current measurements do not contain any specific molecular information, and therefore not specific to a particular gas. These indirect current signals need to be converted to a target gas concentration through calibration database. Hence, the major limitation of electrochemical or metal-oxide semiconductor sensors is the effect of interfering gases. For instance, it is known that SO_2 will incur -165% signal interference on NO_2 electrochemical sensors.²⁶ This means that the presence of SO_2 will cancel out the NO_2 signals in an EC sensor reading. On the other hand, non-dispersive infrared (NDIR) sensors, similar to Raman, provides molecular information on the gas present. Hence it is able to measure a wide range of gases. However, its sensitivity is only at part-per-million levels. Moreover, the IR signals can be severely interfered by the presence of water and water vapor, and once again giving rise to false positive/negative signals. Hence, the growth of the NDIR sensors are hampered by its limitations.

Hence, our proposed SERS-based solution is ideal for the open-air and on-site analysis of air composition. We have strategically built our platform to meet the unresolved issues with current commercial EC, MOS and NDIR gas sensors in the market. Firstly, SERS provides high molecular specificity by measuring the intrinsic Raman vibrational fingerprints of target molecules, thus preventing the possibility of false signals. Secondly, SERS is water interference-free as water does not exhibit Raman signals in our targeted spectral window of detection. This indicates our SERS technique is feasible in highly humid outdoor environment. Notably, we have also designed our substrates with ultrahigh density of plasmonic hotspots that potentially achieve down to ppb detection limit.

5.3 Conclusion

In conclusion, we demonstrate aerosolized plasmonic colloidosomes (APC) as a 3D plasmonic cloud containing $\sim 10^9$ Ag nanocubes/cm³ for in-air SERS detection of airborne species. The APC serves as an omnidirectional plasmonic active cloud that extends over centimeter-lengthscale. Our platform directly tackles the poor SERS-active depth and laser focal misalignment tolerance issues of conventional 2D SERS substrates. Its omnidirectionality property also brings about consistent SERS signals independently of laser and analyte introductory pathways. Importantly, we demonstrate the substrate-less multiplex detection of airborne liquid aerosols in a stand-off detection from a distance of 200 cm. These collective advantages of APC as an in-air SERS platform open up new horizons in fundamental SERS research, as well as detection technology in general for on-site and remote applications in environmental analysis and terrorism control.

5.4 Materials and Methods

Chemicals. Silver nitrate ($\geq 99\%$), anhydrous 1,5-pentanediol (PD, $\geq 97\%$), poly(vinyl pyrrolidone) (PVP, average MW = 55,000); 1H,1H,2H,2H-perfluorodecanethiol (PFDT, $\geq 97\%$), 4-Methylbenzenethiol (4-MBT, 98%), naphthalene (99%), methylene blue (MB, $\geq 82\%$) were purchased from Sigma Aldrich; acetone (HPLC grade) was purchased from TEDIA, copper (II) chloride ($\geq 98\%$); ethanol (ACS, ISO, Reag. Ph Eur) was from EMSURE®; toluene and hexanes (BAKER ANALYZED® A.C.S. Reagent) was from Avantor; propan-2-ol and chloroform (HPLC grade) was from Fisher Scientific. All chemicals were applied without further purification. Milli-Q water ($> 18.0\text{ M}\Omega\cdot\text{cm}$) was purified with a Sartorius Arium® 611 UV ultrapure water system.

Synthesis and purification of silver nanocubes. The preparation of silver (Ag) nanocubes was carried out based on the polyol method described in literature.²⁵ 10 mL PD solutions of CuCl₂ (8 mg/mL), PVP (20 mg/mL) and AgNO₃ (20 mg/mL) were prepared separately by sonication and vortex. 35 μ L CuCl₂ solution was added to the AgNO₃ solution. 250 μ L PVP precursor was added dropwise every 30 s while 500 μ L AgNO₃ precursor was injected every min using a quick addition to a 10 minute-preheated 20 mL PD solution. The addition was continued until the mixture turned orange brown. For the purification, PD was first removed by washing the mixture with acetone followed by ethanol. The suspension was then dispersed in 10 mL ethanol and 100 mL aqueous PVP solution (0.2 g/L) and filtered using Durapore® polyvinylidene fluoride filter membranes (Millipore) with pore sizes ranging from 5000 nm, 650 nm, 450 nm and 220 nm, several times for each pore size. SEM imaging was performed, from which the edge lengths of 250 Ag nanocubes were measured and analyzed using ImageJ software. The as-synthesized nanocubes were found to be obtained in high yield of approximately 40 mg/synthesis.

Functionalization of Ag nanocubes with perfluorodecanethiol. 20 mg of purified Ag nanocubes were immersed in 10 mL of 1:1 propan-2-ol/hexane solution containing 0.1 mM of 1H,1H,2H,2H-perfluorodecanethiol (PFDT) for 6 h at room temperature. The colloidal suspension was then washed with copious amounts of ethanol and hexane, and subsequently dispersed in 1.0 mL of hexane.

Preparation of colloidosomes. 3.0 μ L water was added to 500 μ L hexane suspension containing 4.80 mg perfluorodecanethiol-functionalized Ag nanocubes (referred to as Ag from this point onwards). Colloidosomes were formed by emulsification via vigorous shaking. The colloidosomes were settled down and collected for further aerosolizing.

Extraction of aerosolizer disk. Commercially available ‘Nano Handy Mist Spray’ purchased from SKG was opened and the aerosolizer disk (attached with battery and

controlling chip) was extracted for further use. A 1-cm conical portion of a pipette tip was attached on the aerosolizer disk (via UV-cured gel) to be used as a colloidosome reservoir. The disk was activated putting a magnet near the chip, which closed the circuit upon magnetic induction.

Preparation of 3D Printed chamber. 3D models were designed in AutoDesk 3Ds Max 2016, and exported to and printed with FormLabs 1+ 3D printer using clear resins FL02. The chamber was designed such that the colloidosome can be sprayed into from top down, and the analyte can be introduced from the side. The chamber interior and the laser source (objective lens) is separated with a quartz slide to protect the lens. The distance from the analyte inlet to the laser source is kept at 12 cm to prevent analyte aerosol from reaching and condensing onto the quartz slide.

In-air SERS characterization of aerosolized plasmonic colloidosomes (APC) and data collection. ~500 μ L colloidosomes (in 10 mM 4-MBT solution in hexane) was loaded into the reservoir on the aerosolizer disk. Colloidosomes (now functionalized with 4-MBT) were then sprayed into the 3D printed chamber and signal collection were obtained in mid-air by illuminating the target spot with a horizontal laser beam. (We are required to perform the characterization in a chamber (because spraying plasmonic particles and solvents directly into air would severely contaminate and damage our spectrophotometer system, especially the objective lens.) Time-lapsed SERS spectra were then obtained with u-Raman controlling software uSoft (Technospex Pte. Ltd, Singapore) while the colloidosomes were continuously being dispensed. With accumulation time of 1000 ms, we obtained 10-60 spectra per detection depending on consistency of signals. The time-lapsed spectra were then subjected to baseline subtract treatment using OriginPro software. X, y, z-dimension characterization of the colloidosome mist were performed by obtaining signals of 4-MBT adsorbed to APCs while moving the chamber in x,y and z direction through the center point of the quartz slide with a

controllable stage, at 500 μm step size. Upon obtaining the x,y,z intensity profiles, a 3D projection of the profiles was constructed using Matlab software. For control experiment with substrate, the substrate is moved at 50 μm step size instead, due to its high sensitivity to the change in laser focal plane. The obtained intensity profile is then represented by a mathematically fitted peak. The 3D volume was constructed likewise. Aerosol stability experiment was performed by collecting the Raman signals continuously from when the APCs were being sprayed and up to 10 seconds after the inlet shutter is closed (spraying stopped). Before and between experiment trials, the quartz slide is cleaned with IPA/ethanol to remove all possible chemicals that might have been deposited onto its surface, and Raman measurements are performed when the chamber is empty (with quartz glass) to ensure no interfering signals from any adsorbed chemicals on the quartz are observed. (Note: Our detection method includes the initial record of all the background light, with the spectrophotometer aperture opened and the laser shutter closed, before every measurement. In this way, we obtain a “Dark” spectrum including all the background interference. Only after the “Dark” is obtained, we proceed to record the spectra with the laser shutter opened. All the SERS spectra (with laser exposed) undergo auto-subtraction from the “Dark” spectrum obtained previously. Hence, we can eliminate the background interference and enhance the SERS signals that come from our analytes themselves).

Control experiment – 2D Ag substrate. 4.80 mg Ag nanocube was dropcasted onto a Si substrate and let dry. The substrate was then submerged in 10 mM 4-MBT solution in hexane to allow adsorption of 4-MBT molecules onto the Ag nanocubes. The substrate was erected perpendicular to the horizontal laser. 4-MBT signals were monitored while the substrate was moved along the z-axis at 50 μm step size (parallel with respect to the laser beam).

In-air SERS sensing with aerial colloidosomes and data collection. ~500 μL of colloidosomes was loaded into the reservoir on the aerosolizer disk. Ethanolic methylene blue

(MB) aerosol was sprayed into the chamber using another ‘Nano handy mist’ device. For 4-MBT vapor detection, 100 mg of solid 4-MBT was put in the chamber, which was then sealed with parafilm (4-MBT will vaporize and saturate the chamber). For toluene, 1 mL of toluene is introduced into the chamber as aerosol using the ‘Nano handy mist’. For naphthalene, a 0.89 M (saturated) solution of naphthalene in hexane is introduced into the chamber as aerosol. For the multiplex sensing of MB and rhodamine 6G (R6G), MB and R6G were prepared in ethanol where by concentration of MB and R6G are 10^{-3} M and 10^{-4} M respectively (corresponding to a mole number introduced into air per second of 50 nmol and 5 nmol). Colloidosomes were then sprayed into the chamber once the analytes (aerosol/vapors) are dispensed and signal collection were obtained in mid-air by focusing the laser within the colloidosome cloud. Time-lapsed SERS spectra were obtained with u-Raman controlling software uSoft (Technospex Pte. Ltd, Singapore) for ~ 10 - 60 seconds depending on signal consistency, while the colloidosomes were continuously being sprayed out. The acquisition time was 1 s and we obtained 10-60 spectra per trial. The time-lapsed spectra were then subjected to baseline subtract treatment with OriginPro software. Only spectra with analyte’s SERS fingerprints were selected and averaged. Typically, we observed ~1 to 3/10 spectra in which the analyte peaks could be observed. This can be explained based on the probability whereby the analyte molecules and Ag hotspot contact and give rise to SERS signals that can be captured within the laser illumination volume, given the highly disruptive spraying condition. All measurements were performed 3-5 times for each analyte and each concentration, or until sufficient spectra are obtained. Each reported spectrum and intensity was an average of at least 10 spectra in which analyte fingerprints were observed.

Methylene blue (MB) calibration curve. The samples containing the dyes of concentrations from 10^{-2} to 10^{-7} M are introduced in similar methods as described above. Typically, we introduce 50 μ L of aerosolized MB per second into the detection chamber. Thus,

the concentration of MB is reported under the mole number (5 μmol to 0.5 nmol) introduced per second, as the SERS acquisition time is 1 second. This truly reflects the actual MB amount being detected in-air. The average intensity corresponding to each concentration is obtained and the double log scale calibration of average intensity against concentration was plotted. The lowest concentration shown in the calibration plot is the lowest concentration that can display signals (limit of detection), that are distinguishable from the blank control (colloidosome spray without the presence of any analyte).

Control experiment with single particle spraying. The control experiment was performed with similar in-air SERS detection protocol as described above. In the reservoir, colloidosome solution was replaced with a 300 μL solution containing 4.8 mg colloidal Ag nanocubes in ethanol. The amount of Ag used in colloidal solution and in colloidosome solution remains the same.

Material characterization. SEM imaging was performed with JEOL-JSM-7600F microscope. UV-vis spectroscopic measurements were conducted with a Cary 60 UV-Vis spectrometer. SERS measurements were performed using time-lapsed mode of uRaman-532 (Technospex Pte. Ltd, Singapore) with laser power 100 mW with 1000 ms accumulation time for data collection between 200 cm^{-1} to 1800 cm^{-1} , fitted an L-shaped horizontal lens (0.08 N.A, 4 μm radius beam) whose working distance was measured to be $\sim 2.0\text{ cm}$. For stand-off Raman detection, a 200 mm Nikon lens whose working distance can be adjusted and can be extended up to $> 200\text{ cm}$, was mounted on the uRaman-532 module.

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Chapter 6

Summary and Outlook

6.1 Summary

SERS has emerged as a reliable analytical tool that offers highly molecular-specific Raman fingerprints of target analytes, and is commonly employed in the sensing of toxic pollutants in solution and hazardous vapors in air. Such sensing is highly important for the early recognition and prevention of natural disasters, diseases and terrorism activities yet conventional methods such as fluorescence, UV-Vis, gas chromatography, photoelectric/ionization and nanomechanical sensors fail to provide molecular information of target analytes, and differentiate closely-resembling molecules. SERS tackles this issue by enabling ultratrace detection with highly specific Raman molecular fingerprints that differentiate closely resembling molecules. However, current SERS technique relies on rigid 2D substrates which require stringent laser alignment while only capable of static measurements. Moreover, such 2D substrates have critical drawbacks in terms of low hotspot volume in the z-direction, poor mobility and flexibility to incorporate in other media and having only one SERS active surface.

This thesis introduces ‘plasmonic colloidosomes’ – micron-sized water droplets coated with Ag nanoparticles – as 3D substrate-less SERS platforms to tackle the above problems in sensing and SERS spectroscopy. In Chapter 2, we report the fabrication of these droplets via emulsification method, which grants them robust, spherical and highly SERS active plasmonic shells comprising of Ag nanoparticle clusters. The shells give rise to $>10^6$ -fold more enhanced vibrational Raman fingerprints of target analytes and allow the ultrasensitive femtomole-level detection of analytes presence on both sides of the plasmonic shell. We achieve the world’s first “dual-phase tri-analyte” detection, in which three food toxins existing in two immiscible solvents are simultaneously detected. Such breakthrough ability in biphasic detection of

chemicals across liquid-liquid interface allows us to *in situ* monitor multiple picoliter-small reaction compartments, as demonstrated in Chapter 3. In particular, we perform the SERS-based visualization of the reaction progress of dimethyl yellow interfacial protonation, and extract the Raman fingerprints of two isomeric products formed upon the protonation.

Following up in Chapter 4, we describe the incorporation the plasmonic colloidosomes within a microfluidic channel which realizes on-line high through-put analysis of multiple samples. The colloidosomes are able to fully encapsulate the analyte samples and prevent channel and inter-sample contamination. We also achieve highly accurate quantification of multiple analytes in sequence.

A highlight of our work is the design of the world's first macroscale 3D SERS 'in-air sensing' platform, by incorporating the colloidosomes within a liquid aerosol. Chapter 5 introduces 'aerosolized plasmonic colloidosomes', an omnidirectional plasmonic aerosol cloud that exhibits intense and uniform SERS response in all x, y and z directions over centimeter lengthscale. Such isotropic SERS performance of our APCs is critical for in-air sensing especially when it is impossible to control analyte impingent direction and accurately position a detection laser for optimal performance under actual dynamic air conditions. These achievements also represent a giant leap towards the potential development of stand-off and substrate-less spectroscopic methodology to detect gas toxins/airborne weapons remotely.

6.2 Outlook

While plasmonic colloidosomes feature immense SERS performance and flexibility to be incorporated with other multi-media detection platforms such as microfluidic and in-air detection, we recognize certain limitations in our current colloidosome research. Firstly, the current library of detectable target analytes achieved by our platform is still limited to common chemicals used in proof-of-concept SERS demonstrations such as dyes, for e.g. methylene blue

and rhodamine 6G, or thiols, for e.g 4-methylbenzenethiol, which have high Raman cross-sections and/or affinity to plasmonic surface. However, plasmonic colloidosomes have not been applied for the sensing of other molecules with poor Raman cross-sections and low affinity to metal such as gas molecules (H_2 , N_2 and $CO...$), nitrotoluene explosives or biomolecules. Such detection is necessary in real-life sensing tasks for air monitoring, product quality control, forensics and medical diagnosis.^{1,2} It is currently challenging to detect such molecules with plasmonic colloidosomes due to the presence of the organic continuous phase which diffuses and suppresses signals while hampering target molecules to be concentrated at the plasmonic hotspots. Hence, the further development of plasmonic colloidosomes as a next-generation 3D SERS sensor requires the upgrade of its core hotspot quality in terms of sensitivity towards small molecules. Currently, current colloidosome shells only consist of plasmonic particles without any functionality and it therefore raises the need to equip these particles with molecular-trapping or molecular-probing moiety. The former can be achieved by modifying plasmonic nanoparticles with metal-organic-framework (MOF) such as zeolite-imidazole-framework-8 (ZIF-8) or ZIF-71, which exhibits outstanding sorption ability to concentrate a vast range of molecules as large as bicyclic aromatic compounds close to the plasmonic surface.³ In particular, our preliminary research has achieved a handful of 2D MOF-SERS platforms with Ag@ZIF-8 moiety,^{3,4} which display excellent sorption of CO_2 , aniline, toluene and naphthalene within the ZIF pores and enable their SERS detection due to the increased local concentration at the Ag surface. On the other hand, plasmonic particles can also be modified with probe molecules that exhibit spectral changes upon interaction with target analytes.¹ When coupled with chemometrics method to extract the differences in the SERS spectra of probe molecule, this allows the indirect detection of single or even multiple target analytes. Interaction probe molecules can be freely tailored from a vast library of boronic acids (for diol detection), crown ligands (for metal detection), or even DNA strains (for biomolecule

detection).^{5,6} In overall, such modification of the plasmonic building blocks can bring upon several advantages to the plasmonic colloidosomes, granting them upgrading abilities to sense more molecules and ultimately creating opportunities in their commercialization as a universal 3D SERS sensor that adapts to multiple real-life sensing tasks. Furthermore, the expanded library of detectable molecules also allows plasmonic colloidosomes to be applied in the investigation of various interfacial reactions at the ultrasmall scale.

Secondly, our platform engineering methodology still relies on make-shift techniques, which are sufficient for proof-of-concept demonstrations but not applicable for the mass production of plasmonic colloidosomes for potential commercial applications. The emulsification step to form colloidosomes from the immiscible bulk solutions is based on hand shaking, which induces relatively inhomogeneous droplet sizes. This process can be automated with robotic arms for better outcome. Moreover, our current method to aerosolize plasmonic colloidosomes utilizes handheld mist sprayers, which result in imperfect aerosols with limited reproducibility and irregular particle density. This raises difficulty in in-air measuring due to inconsistent signals. Hence, it requires a large number of measurements to achieve notable fingerprints of airborne molecules with appropriate signal-to-noise ratio. This protocol can be further optimized with the use of scientific particle aerosolizers or nebulizers to produce homogeneous and high quality plasmonic aerosols for accurate quantitative sensing.⁷ Additionally, online flowing colloidosomes in fluidic systems can potentially be formed in situ with the aid of dual-channel microfluidic device with accurately controlled flow rates. Such development direction can significantly improve the mass formation of plasmonic colloidosomes and plasmonic colloidosome-based platforms for industrial applications.

Importantly, current plasmonic colloidosome shells are formed based on the sole self-assembly of plasmonic particles at the liquid-liquid interface without any further procedures to enhance shell strength. This raises problems, although rarely, in particle detachment over time

or shell disintegration when the colloidosomes are subjected to measurable external forces, as demonstrated in their aerosolization. Thus, we propose further strengthening of the plasmonic shells using click-chemistry to integrate the shell particles, or external coating to protect the original plasmonic layers. Ultimately, the collective upgrading properties in hotspot sensitivity, production reproducibility and shell robustness will sketch several new horizons in the development of plasmonic colloidosomes as a multi-purpose platform for SERS-based application in both detection and non-detection areas.

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Appendix

Assignment of SERS vibrational fingerprints.

Table S1. SERS band assignments of methylene blue.

SERS band (cm ⁻¹)	Band assignment
456	C-N-C deformation
503	C-N-C deformation
1406	C-H in plane ring deformation
1446	C-N stretching
1633	Ring C-C stretching

Table S2(a). SERS band assignments of dimethyl yellow (DY)

Experimental SERS band (cm ⁻¹)	DFT-Simulated SERS band (cm ⁻¹)	Band assignment.
1150	1183	C-N _{azo} stretching
1202	1233	C-N _{azo} stretching
1415	1477	N=N stretching
1604	1643	Ring C-C stretching

Table S2 (b). SERS band assignments of protonated dimethyl yellow (HDY⁺)

Experimental SERS band (cm ⁻¹)	DFT-Simulated SERS band of HDY ⁺ (N1) (cm ⁻¹)	DFT-Simulated SERS band of HDY ⁺ (N2) (cm ⁻¹)	Band assignment.
1180	1204	1197	C-H wagging
1220	1249	1237	C-N _{azo} stretching
1283	1320	1324	N=N stretching
1410	1423	1440	N=N stretching
1604	1634	1644	Ring C-C stretching
1633	1671	1680	Ring C-C stretching

Table S3. SERS band assignments of rhodamine 6G

SERS band (cm ⁻¹)	Band assignment
620	In-plane Xanthene ring deformation
783	Out-of-plane C-H bend
1194	In-plane C-H bend
1305	C-O-C stretch
1372	Ring C-C stretching
1513	Ring C-C stretching
1585	Ring C-C stretching
1662	Ring C-C stretching

Table S4. SERS band assignments of malachite green

SERS band (cm ⁻¹)	Band assignment
808	Out-of-plane ring C-H vibration
929	Ring skeletal vibration
1377	N-phenyl stretching
1408	N-phenyl stretching
1605	Ring C-C stretching
1633	Ring C-C stretching

Table S5. SERS band assignment of crystal violet.

SERS band (cm ⁻¹)	Band assignment
417	C-C-C and C-N-C bending
437	C-N-C bending
725	C-N stretching
800, 917	C-C-C bending
1172	C-C-C asymmetric stretching
1398	C-C and C-H bending, C-C-C stretching
1587	Ring C-C stretching
1628	Ring C-C stretching + N-H bending

Table S6. SERS band assignments of 4-methylbenzenethiol (4-MBT).

SERS band (cm ⁻¹)	Band assignment
816	C-H ring torsion
1077	Phenyl ring-breathing, C-H in-plane bending, and C-S stretching
1582	Phenyl ring stretching motion

Table S7. SERS band assignments of toluene.

SERS band (cm ⁻¹)	Band assignment
787	C-N-C deformation
1005	C-C stretching
1084	C-H bend
1212	C-CH ₃ stretching
1603	C-C ring stretching

Table S8. SERS band assignments of naphthalene.

SERS band (cm ⁻¹)	Band assignment
815	Ring breathing
1054	C-C stretch
1302	C-C stretch, C-H bend
1684	C-C ring stretch, C-H bend

- END OF THESIS -