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Constructing liquid marble as particle-assembled microdroplet for multiplex sensing and reaction modulation/monitoring

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Abstract

Liquid marbles are particle-assembled microdroplet platforms that have garnered paramount importance as both microsensor and microreactor owing to their excellent robustness, highly customizable properties as well as need of minute sample/reaction volumes. However, three main challenges relevant to the field have been identified; (1) limitation of molecular sensing to a single fluid phase, (2) invasive and ex-situ reaction monitoring techniques and (3) lack of heating mechanism and active mass transportation system for reaction control. The objectives of my thesis therefore aim to address these limitations, empowering liquid marbles as efficient and multifunctional micro-sensors/reactors applicable in broad scientific/technological fields such as nanotechnology, green processes, and synthetic chemistry. In chapter 2, we demonstrate the fabrication of plasmonic liquid marble prepared using Ag particles as a multiplex SERS sensor capable of identifying and quantifying analytes present across an immiscible liquid-liquid interface. Such plasmonic liquid marble is further exploited as a microreactor-sensor hybrid in chapter 3 for the rapid and on-site read-out of reaction events within the microreactor at the molecular-level. Chapter 4 and 5 mainly focus on the enhancing and tuning of reaction kinetics using liquid marble-based microreactors. For chapter 4, we apply graphene liquid marble as photothermal-active miniature reactor to remotely control its temperature for direct kinetic modulation on the encapsulated reaction. In chapter 5, we demonstrate the spinning of a magnetically-active liquid marble to induce an active mass transportation system within the enclosed 3D microdroplet. Such spinning phenomenon imparts a spiral acceleration of enclosed molecules towards the exterior encapsulating shell for improved catalytic performance and controllable reaction dynamics. Lastly, I conclude my thesis with a summary of my four-year research works and provide an outlook for continuous and significant progress in this emerging field.

Contents

Acknowledgements.....	I
Abstract.....	III
Chapter 1: Introduction.....	1
1.1 Liquid marbles and their formation.....	2
1.2 Properties of liquid marbles	4
1.3 Incorporation of additional functionalities	8
1.4 Stimuli-responsive liquid marbles.....	11
1.5 Applications of liquid marbles	16
1.5.1 Liquid marble as micro-sensor	17
1.5.2 Liquid marble as miniature reactor	22
1.6 Motivations and objectives.....	27
1.7 References	29
Chapter 2: Plasmonic liquid marbles: A miniature substrate-less surface-enhanced Raman spectroscopy (SERS) platform for quantitative and multiplex ultratrace molecular detection.....	35
2.1 Introduction	36
2.2 Results and discussion.....	38
2.3 Conclusions	56
2.4 Materials and methods	57
2.5 References	60
Chapter 3: Identifying enclosed chemical reaction and dynamics at the molecular level using shell-isolated miniaturized plasmonic liquid marble	64
3.1 Introduction	65
3.2 Results and discussion.....	66
3.3 Conclusions	92

3.4	Materials and methods	93
3.5	References	97
Chapter 4: Graphene liquid marbles as photothermal miniature reactors for reaction kinetics modulation		101
4.1	Introduction	102
4.2	Results and discussion.....	103
4.3	Conclusions	126
4.4	Materials and methods	127
4.5	References	130
Chapter 5: Dynamic rotating liquid marble for directional and enhanced mass transportation in three-dimensional microliter droplets		133
5.1	Introduction	134
5.2	Results and discussion.....	136
5.3	Conclusions	155
5.4	Materials and methods	155
5.5	References	160
Chapter 6: Summary and outlook		164
6.1.	Summary	164
6.2.	Outlook.....	167
6.3	References	169
List of publications and technology disclosures.....		170

Chapter 1

Introduction

Abstract. Liquid marbles are particle-assembled microdroplets formed when nano/micro-sized particles spontaneously adsorb on the surface of the liquid droplets. An encapsulating shell is consequently formed which effectively confines and isolates such small-volume liquid droplet from the external environment while still preserving its fluid-like properties. Owing to their unique liquid core-solid shell configurations, liquid marbles have been demonstrated as a robust and versatile microplatform with extensive unique physical/mechanical properties. More importantly, liquid marbles can be easily incorporated with endless functionalities, making them immensely attractive for a wide range of targeted applications. In this chapter, we introduce the concept of liquid marbles, with emphasis placed on their formation, physical/mechanical properties, as well as the ease to tailor such microplatform with additional functionalities. This will be followed by a review on the applications of such particle-assembled microdroplet as microsensor and microreactor. At the end of this chapter, we identify three challenges associated with current liquid marble progress and subsequently describe the main objectives of this thesis.

1.1 Liquid marbles and their formation

Liquid marbles are three-dimensional (3D) spherical entities comprising of a micro-/millimeter-sized liquid droplet core enclosed within a solid nano/microparticle shell (Figure 1A).¹⁻⁵ These particle-assembled microdroplets have dimensions in the millimeter to centimeter ranges, and can be prepared easily by rolling a liquid droplet on a bed of pulverized solid particles (Figure 1B). Driven by the large surface tension of liquid microdroplet (equation 1),³ particles are spontaneously assembled and locked at the liquid-gas interfaces upon contact to minimize the overall system energy through the creation of a liquid-particle-air system (Figure 1C).

$$\Delta E_1 = -\pi b^2 \gamma (1 + \cos \theta_Y)^2 \quad (1)$$

where ΔE is the surface energy of liquid-particle-air system, b is the radius of the particle, γ is the surface tension at the liquid-vapor interface, and θ_Y is the Young angle of the particle which describes its hydrophobicity/hydrophilicity; the Young angle is the contact angle of a sessile water droplet when placed on top of these particles.

Figure 1.1. (A) Schematics of the spontaneous self-assembly of a rolling liquid droplet on a bed of pulverized particles. (B) Digital image of the as-fabricated quasi-spherical liquid marble. (C) Illustration of the minimization of surface free energy upon the attachment of a small particle onto the surface of a liquid droplet. Reproduced with permission from ref 4. Copyright 2011 The Royal Society of Chemistry.

Through such formation mechanism, the liquid microdroplet is effectively isolated in a loosely packed particle shell to produce discrete liquid marbles which retain both the soft and fluid-like characteristic of liquids. The preparation of liquid marbles is therefore analogous to the natural process of aphids to transform liquids into “soft solids”,⁶ whereby they encapsulate their liquid wastes with a thin layer of wax to impart enhanced mobility for rapid disposal. Furthermore, the encapsulating particle shell also doubles as a protective layer to overcome the rapid evaporation issues typical to small-volume liquids by effectively reducing the liquid-air surface area.⁷⁻⁹ For instance, the formation of graphite liquid marbles decreases the evaporation of the enclosed droplet by two-fold as compared to an exposed droplet.⁷ Liquid marbles are therefore emerging substrate-less microdroplet platforms due to their one-step preparation which overcomes the need of complex fabrication techniques

commonly encountered in substrate-based platforms and/or microfluidic devices.¹⁰⁻¹³ While liquid marbles are mainly prepared using high surface-tension liquid such as water, these microplatforms can easily be extended to organic solvents with relatively lower surface tension such as toluene, ethanol, octane and dimethylsulfoxide (DMSO).^{14, 15} It is also noteworthy that the formation of liquid marble is independent of the surface properties of the encapsulating solids (equation 1).³ Consequently, liquid marbles can be prepared using a diverse selection of hydrophobic and hydrophilic particles.³ For instance, aqueous-based liquid marbles coated with hydrophobic ($\theta_Y > 90^\circ$) particles such as polytetrafluoroethylene and hydrophilic ($\theta_Y < 90^\circ$) building blocks like graphite and carbon black have been reported.^{7, 16, 17} Together with the facile manipulation of liquid marbles owing to their macroscopic dimension, such versatility of preparation enables liquid marbles as attractive platforms with high customizability towards their target applications.

1.2 Properties of liquid marbles

Liquid marbles demonstrate versatile volume attained simply by pre-defining the volume of liquid droplet used for their preparations.^{2, 18-21} Consequently, these droplet-based platforms can be miniaturized from centimeter size down to the micrometric regime by using milliliter and microliter liquid droplets, respectively. The resulting shape of liquid marble is dependent on the volume of the enclosed liquid droplet as well as the effective surface tension of the entire liquid-particle-air ensemble (typically 40 mJ m^{-2} to 70 mJ m^{-2}).²²⁻²⁶ Generally, at a specific surface tension, liquid marble exists as spherical structure at low liquid volume and eventually demonstrate “puddle-like” behavior as volume increases.^{2-4, 20} Gravity dominates in the latter scenario such that the height of the liquid marble remains constant even with further increase of liquid volume. The maximal height which liquid marble can attain is also dictated by the effective surface tension of liquid marble; a

larger effective surface tension of liquid marble increases the maximal height achievable by the liquid marble. Such dependency of the shape of a liquid marble on the interplay of surface tension and gravity can be described using equation 2,³ which is also the basis of the “puddle height method” commonly employed to estimate the effective surface tension of liquid marble. The collective ability of liquid marbles to effectively tailor their geometrical shapes and sizes empower them to easily access narrow fluidic channels. This feature facilitates their integration with current microtechnologies such as lab-on-a-chip system,²⁷⁻³¹ which remains a challenge using rigid substrate-supported platforms.^{11, 32-34} Synergizing liquid marble with modern microdevices is imperative to align with increasing efforts to miniaturize (bio)chemical processes for high throughput screening and greener operation by minimizing chemical/solvent consumptions.^{14, 18, 35-37}

$$\gamma_{\text{eff}} = \rho g H^2 / 4 \quad (2)$$

where γ_{eff} is the effective surface tension of the liquid marble, ρ is the density of the liquid, g is gravitational constant and H is the maximal marble height.

Liquid marbles also exhibit non-stick character which allows their facile maneuver on both solid and liquid surfaces without any leakage or disruption to their structural integrity, remarkably even when placed on external hydrophilic surfaces.^{1, 38-42} This is achieved due to the Cassie-Baxter-type anti-wetting properties of the hydrophobic shell which generates air pockets between adjacent particles and the enclosed liquid surface,⁴³⁻⁴⁶ analogues to the “lotus” effect of superhydrophobic platforms. In this way, direct contact of the enclosed aqueous droplet with external surfaces can therefore be eliminated. Such isolation of liquid microdroplets within the protective particle shell also confers liquid marbles with excellent mechanical robustness; they are

able to bounce at elevated height,^{1, 2, 41} roll at slight inclination of underlying platform,^{1, 47} as well as rapid and on-demand merging or splitting of liquid marbles (Figure 1.2A, 1.2B).⁴⁸ It is also worth mentioning that the robustness of liquid marbles during merging and splitting operations renders them ideal for the homogenization of multiple enclosed contents to trigger chemical reactions at the microliter-scale, notably without needing engineered microfluidic devices with designed hydrodynamic and/or designated mixing microcompartments.⁴⁹⁻⁵³

Figure 1.2. (A, B) Digital images of liquid marbles merging and splitting, respectively. Reproduced with permission from ref 48. Copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA. (C) An aqueous-based liquid marble floating on the water surface. Reproduced with permission from ref 47. Copyright 2008 Elsevier. (D) Schematic depicting the liquid-liquid separation enabled by the encapsulating shell of liquid marble that is resting on a pool of liquid. Reproduced with permission from ref 4. Copyright 2011 The Royal Society of Chemistry.

In addition to its intrinsic mechanical robustness, liquid marbles are also capable of interacting stably with its surrounding liquid environment which further creates additional avenues to enhance their properties along with the creation of a new window of opportunities for their latter applications. For instance, liquid marble prepared using hydrophobic particles can be suspended atop of underlying liquid with high surface tension such as water (Figure 1.2C, 1.2D), and also submerged stably in low surface tension organic liquids (including hexane and decane) without affecting its particle-assembled soft interfaces.^{4, 5, 15, 47} Such interaction of liquid marbles with an external liquid environment is vital to enhance the stability of enclosed microdroplet against evaporations. The presence of a pool of water underneath an aqueous-based liquid marble enhances the longevity of liquid marble by 4-fold due to the increased humidity surrounding the liquid marble which retards the evaporation of enclosed aqueous droplet.⁵⁴⁻⁵⁶ Likewise, the immersion of liquid marble in organic liquids virtually extends its lifetime indefinitely as long as the exterior continuous phase is still present.^{15, 57} Achieving the long-term stability of liquid marbles is crucial for their potential applications in the prolonged tracking of temporal reaction events to elucidate the associated mechanism and kinetics.

More importantly, the direct contact of liquid marbles with an external liquid phase potentially extends them into a variety of particle-assembled soft interfaces including water-particle-water, water-particle-oil and water-particle-air configurations. Liquid marbles can consequently be extended to broad interfacial studies/applications targeting gas- and/or liquid-related processes at fluid-particle-fluid boundary.⁵⁸⁻⁶⁰ One possible example of these processes includes the scrubbing of gas whereby gas molecules dissolve in liquid absorbent upon their physical contact. Novel multiphasic applications utilizing liquid marbles are therefore made possible because of the direct access of such particle-assembled soft interfaces to both the immiscible liquids situated within

and outside the encapsulating shell. Potential multiphasic applications could involve the simultaneous molecular sensing across a liquid-liquid interface, as well as innovative reaction design using an immiscible two-solvent system whereby reaction between molecules of contrasting solubility characteristics solvents are confined at the ultrathin liquid-liquid boundary.

1.3 Incorporation of additional functionalities

The most remarkable feature of liquid marbles lies in the facile incorporation of additional functionalities into these microplatforms simply via the choice of encapsulating materials (Figure 1.3) and/or modifying the surface moieties of the particles.^{14, 25, 30, 31, 37, 39, 61-65} These attributes range from the improved chemical resistivity of the liquid marble's shell, to more sophisticated characteristics such as particle-induced plasmonic and catalytic properties. For instance, acid and base-resistant liquid marbles constructed using Teflon powder ensure their stabilities in respective gaseous NH_3 and HCl environments by eliminating potential chemical reactions of gas/vapor with the liquid marble shell.^{60, 66} This ability is vital for their subsequent application as acidic/basic gas sensors when further combined with fluorescent and colorimetric reaction indicators that are encapsulated within the liquid marbles.

Figure 1.3. Liquid marbles demonstrate high versatility and can be formed from a variety of encapsulating solid materials such as lycopodium, Teflon and carbon black (left to right). Reproduced with permission from ref 5. Copyright 2012 The Royal Society of Chemistry.

Likewise, catalytically-active liquid marble can be achieved via the deposition of noble metal particle (Ag or Au) on the surface of liquid microdroplet. By encapsulating a reaction microdroplet with Ag nanowires, the concept of catalytic liquid marble has been proposed as a novel strategy for heterogeneous catalysis involving a reaction core-catalyst shell template.¹⁸ Using the degradation of methylene blue with sodium borohydride reductant as a model reaction, the support-free and rate-controllable reduction of such environment toxin with near 100 % efficiency has been demonstrated in < 15 min. This rapid and efficient catalytic degradation of methylene blue is attributed to the presence of the noble metallic shell which functions as electron relay to facilitate electron transfer from the reductant to methylene blue. Building on this concept, Wei et al. further improve the catalytically-active liquid marble by decorating the Ag nanoparticles onto the surface of hydrophobic cyclomatrix polyphosphazene particles (PZAF).⁶⁷ PZAF is selected as the backbone of the encapsulating shell owing to its adsorption behavior towards acidic dyes such as methylene blue via an acid-base interaction. Their strategy hence effectively synergizes the catalytic functionality of Ag nanoparticles with a strong methylene blue-sorbing material to accelerate the diffusion and

concentration of the reactant molecules near Ag catalyst. Using this approach, they also achieve near complete toxin degradation with rate constants reaching 0.179 min^{-1} , which is ~ 4 -fold higher compared to the liquid marble (0.41 min^{-1}) formed solely from Ag nanowire.

Plasmonic-active liquid marble can also be realized using noble metal (such as Ag or Au) nanoparticles as the encapsulating material to support their latter application as a surface-enhanced Raman spectroscopy (SERS)-based molecular sensor.⁶⁸ Briefly, plasmonic nanoparticles are able to interact with light to cause localized surface plasmon resonance (LSPR), which is the coherent oscillation of its free conduction electrons.⁶⁹⁻⁷² Such collective oscillation of the metal's electrons creates a secondary electric field that can constructively interfere with oncoming electromagnetic radiation to generate an intense electromagnetic field that is confined to the surface of the plasmonic nanoparticles. When coupled with Raman spectroscopy, a vibrational-based technique which offers molecule-specific and quantitative information based on inelastic light scattering from molecules, these intense electromagnetic fields can be exploited to dramatically boost the Raman scattering responses from probe molecules that are adsorbed on or near the surface of plasmonic nanoparticles. This phenomenon is known as the surface-enhanced Raman scattering (SERS) which provides a 10^6 enhancement of Raman signal even using a single and isolated plasmonic nanoparticle.^{69, 71-73} It is also noteworthy that molecules directly attached to the metallic surface can further contribute to the overall SERS effect by undergoing a chemical enhancement (up to 10^2). This phenomenon arises from the increase in electronic polarizability by means of charge transfer between the noble metal and the target molecule. While both electromagnetic and chemical enhancements are essential processes to boost Raman responses, it is often difficult to index their overall contributions and therefore will not be discussed extensively in this thesis.⁷³ Utilizing Ag nanoparticles adsorbed on the oleic acid-water interface,⁶⁸ previous work has reported the formation of plasmonic-active

oil-in-water liquid marble to strongly confine light at the plasmonic shell for subsequent exploitation for SERS-based molecular detection. With the use of sodium 1-phenyl-1H-terazole-5-thiolate (PPT) as SERS probe, the authors demonstrated plasmonic-active liquid marble as a soft microsensor to elucidate distinct molecular vibrational fingerprint of targeted PPT molecules.

1.4 Stimuli-responsive liquid marbles

Liquid marbles also function as stimuli-responsive microdevices capable of on-demand manipulation and actuation by incorporating an active particle shell that is responsive to external stimulants such as pH,^{74, 75} light irradiation,⁷⁶⁻⁷⁸ electric field and magnetic field.^{14, 30, 31, 39, 62-65} pH-responsive liquid marble has been prepared by encapsulating aqueous microdroplet with poly[2-(diethylamino)ethyl methacrylate]-terminated polystyrene beads (PDEA-PS) with a pKa of 7.3.^{60, 66} At basic pH $\gg$ 7.3, pH-responsive amine moieties in PDEA-PS are largely deprotonated which causes the particle to turn hydrophobic for effective stabilization of the entire liquid marble structure. Upon the decrease of pH below its pKa, these amine functionalities rapidly protonate to form cationic ammonium ions which are hydrophilic. As a result, such hydrophilic latex particles readily rupture liquid marble even within < 1 min upon contact with underlying acidic aqueous medium.

Photothermal-active liquid marbles constructed from light-absorbing materials such as polypyrrole and carbon black have also demonstrated instantaneous light-to-heat conversion upon laser irradiation, achieving a shell's surface temperature of > 100 °C.⁷⁶ Such on-demand and remote heating stems from the excellent photothermal properties of these encapsulating materials. Strong photoabsorption over a broad spectrum of irradiation wavelengths by these materials allows the rapid and highly localized heating at the irradiated zone upon non-radiative vibrational relaxation of

photoexcited electrons.^{79, 80} Such localized heating of liquid marble is critical to prolong the lifetime of small-volume liquid whereby sufficient heat energy is supplied to trigger target physical/chemical processes while effectively minimizing the evaporation rate of the entire enclosed microdroplet. Localized heating using laser irradiation is therefore distinct from conventional conduction/convective heating, where the latter usually affects the entire liquid system and causes rapid evaporation issues.⁸¹ When suspended atop of water surface, asymmetric heat generation upon laser irradiation on a single face of photothermal liquid marble creates a surface tension gradient on the underlying water surface (Figure 1.4A, 1.4B).⁷⁶ This spatial variation of exterior water surface tension consequently drives a Marangoni-type movement of liquid marble that can be controlled through precise laser irradiation on such photothermal-active encapsulating shell. Marangoni-based actuation of liquid marble can be further coupled with miniaturized “ship-like” structures to trigger precise movement and even accurate steering of these “ship” to designated locations (Figure 1.4C).

Figure 1.4. (A) Illustration and (B) experimental demonstration on the Marangoni-type maneuver of liquid marble when the laser is irradiated on a single face of photothermal-active liquid marble. (C) Using multiple liquid marbles to phototaxis a boat under sunlight. Inset is a close-up image on the experimental set-up, involving two liquid marble anchored to a boat-like structure carrying two $60\ \mu\text{L}$ water droplet. Reproduced with permission from ref 76. Copyright 2016 Wiley-VCH Verlag GmbH & Co. KGaA.

Similarly, liquid marbles can also be modified to render them responsive to an externally applied electric field.^{62-65, 82} Encapsulating water microdroplet with polytetrafluoroethylene (PTFE) particles, Shum et al. reported the electro-coalescence of two contacting liquid marbles when they are charged by an applied voltage (Figure 1.5).⁶⁵ The threshold voltage of the DC electric field to

effect electro-coalescence of the liquid marbles depends on two factors, namely the type of stabilizing particles used and the surface tension of the enclosed water phase. At the threshold voltage, the authors first observed the formation of a connecting bridge through the build-up of electric stress. The liquid bridge subsequently broadens and eventually leads to the merging of the two liquid marbles. Such controlled electro-coalescence of adjacent liquid marbles highlights their potential use as small-volume reaction vessels to trigger (bio)chemical reactions involving multiple reactants upon their electro-coalescence.

Figure 1.5. The coalescence of two liquid marbles using electric field and the evolution of current during the coalescence process. Reproduced with permission from ref 65. Copyright 2016 The Royal Society of Chemistry.

Notably, magnetic-responsive liquid marbles represent an emerging class of stimuli-responsive liquid marbles as they offer a simple-yet-powerful approach for precise and swift manipulation of liquid microdroplets using an external magnet.^{14, 30, 31, 39} Superparamagnetic and

hydrophobic Fe_3O_4 particles are typically employed for the fabrication of such emerging magnetic-responsive liquid marble. The applied magnetic field conveyed by an external permanent magnetic can be utilized to actuate the entire liquid marble (Figure 1.6A, 1.6B) and/or manipulate its encapsulating shell. For instance, a permanent magnet placed directly underneath the liquid marble leads to the on-demand opening of the magnetic shell to expose the encapsulated microdroplet (Figure 1.6C).¹⁴ When the magnet is removed, Fe_3O_4 building blocks reversibly and quickly spread across the microdroplet surfaces to re-encapsulate it. The applied magnetic field can also force the controlled coalescence of adjacent magnetically-active liquid marbles almost instantaneously within < 100 ms (Figure 1.6 C).³⁹ Moreover, these magnetic liquid marbles exhibit swift actuation on both flat and curved solid substrate and can even overcome gravity forces by using a sufficiently strong magnet. A remarkable velocity reaching 0.32 m.s^{-1} has been achieved using a magnetic liquid marble,³⁹ whereby such maximum velocity is easily modulated by their droplet volume, along with the strength of magnetic fields of both the external magnet and the encapsulating particles. These unique features of magnetic liquid marble could potentially expedite the development of magnetically-actuated microdroplet system with on-demand positioning and merging capabilities.³¹ With a virtually endless library of functional solids available for microdroplet encapsulation together with its ability to form Janus (liquid marble with two different encapsulating materials at either ends) or even more complex multi-layered shell, liquid marble is truly a versatile particle-assembled microdroplet platform adept for a vast range of targeted applications.

Figure 1.6. (A, B) Magnetic-actuated liquid marble movement such as rolling on a solid surface and gliding across a liquid surface, respectively. (C) Demonstration of the on-demand opening of shell using a permanent magnetic followed by the merging of two adjacent liquid marbles. A chemiluminescence reaction is used to signal the successful merging of the two liquid marbles. Reproduced with permission from ref 14. Copyright 2010 Wiley-VCH Verlag GmbH & Co. KGaA.

1.5 Applications of liquid marbles

So far, we have described liquid marble as a particle-assembled microdroplet platform and identified the key factors on its formation mechanisms. After which, we discussed the intrinsic properties of liquid marble and subsequently highlighted its ability to incorporate addition functionalities simply via the choice of encapsulating solids and their surface moieties. This ability

empowers liquid marbles as an immensely attractive microplatform for precise customization towards their intended applications. Examples of liquid marble-based applications include controllable transportation in fluidic system,^{30, 31} micro-pumps,⁸³ micro-sensors,^{60, 66} and even as isolated and miniature reactors.^{14, 35, 37, 67} In this section, we will mainly discuss on the recent advent of liquid marble as microsensor and miniature reactor to establish extensive understanding within the context of this thesis.

1.5.1 Liquid marble as micro-sensor

Liquid marbles are excellent candidates for molecular sensing owing to their miniature sizes and ability to exist as discrete and isolated compartments which allow high throughput detection. These micro-sensors do not require large sample volume, hence making them ideal as detection platform where samples are scarce or highly toxic, especially in the fields of forensic, biological profiling, as well as industrial and environmental safety. Being clearly visible to the naked eye, liquid marbles can also serve as a visual molecule sensor without the need of specialized instrumental aid. Here, we review the achievements of liquid marbles for molecular sensing, ranging from non-molecular-specific colorimetric and optical absorption techniques, and eventually to molecular identification and quantification at the molecular level using SERS.

Colorimetric sensing is a commonly employed detection technique that provides a “yes” or “no” evaluation to the presence of target chemical species through drastic color changes associated with dye-based indicator solution. On exposure to external gas or liquid analytes, an alteration to the indicator’s chromophore triggers a distinct color change that is easily recognized by the naked eyes.⁶⁶ Liquid marbles are attractive microplatforms for colorimetric-based sensing because they can efficiently isolate micro/milliliter-sized indicator microdroplet within a chemical-resistant particle

shell for high throughput analyte detections. Furthermore, liquid marble's gas-permeable shell enables gas analytes to easily enter and interact with the enclosed indicator solution for efficient molecular sensing. Such loosely-packed particle shell also allows operators to directly recognize the color changes associated with the enclosed indicator solution, thus eliminating the need to disrupt the particle shell for monitoring. For instance, Shen et al. exploited the porosity of liquid marbles for parallel colorimetric detection of acidic/basic gas by enclosing aqueous pH indicator solutions within chemical-resistant Teflon particle shell (Figure 1.7).⁶⁶ Their strategy generally utilizes different colorimetric-based pH indicator and pH-sensitive fluorescence molecules for the sensing of both acidic and basic gases. Liquid marbles containing pH-indicator phenolphthalein undergo a change to its color from colorless to pink upon exposure to basic ammonia vapor. Similarly, HCl vapor was detected by liquid marbles containing 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS); HPTS is a fluorescent dye ($pK_a \sim 7.3$) that exhibits pH dependent fluorescent color change from blue (405 nm) at pH 6 to green (450 nm) at pH 8. On exposure to the acidic gas, HPTS-containing liquid marble gives a distinct blue fluorescent color under UV light even when its appearance remains unchanged under ambient light. Their findings therefore highlight the possibility of simultaneous gas/vapors detection using chemically-stable liquid marbles that are equipped with multiple detection mechanisms (e.g. colorimetry and change in fluorescent color).

Figure 1.7. (i) Liquid marbles for gas sensing containing HPTS indicator under ambient lighting before and after exposure to HCl vapour. Liquid marbles containing the HPTS indicator under UV light (ii) before and (iii) after exposure to HCl vapour. Reproduced with permission from ref 66. Copyright 2010 The Royal Society of Chemistry.

Another colorimetric-based detection approach using liquid marbles was proposed by Fujii et al. and involves the encapsulation of an aqueous dye solution within pH-responsive poly[2-(diethylamino)ethyl methacrylate]-terminated polystyrene beads (PDEA-PS; $pK_a = 7.3$).^{74, 75} Upon the lowering of pH below its pK_a , the liquid marble rapidly ruptured in < 1 min due to the protonation of the PDEA-PS's amine functional groups into hydrophilic cationic ammonium ions. This in turn releases the encapsulated dye as visual affirmation of the acidic nature of surrounding aqueous environment. While colorimetric sensing offers rapid preliminary results easily recognized

by naked eyes, such method usually provides limited molecular-specific and quantitative information.

To overcome the limitations of colorimetric sensing, common analytical tools such as electrochemistry and optical absorption spectroscopy are coupled to liquid marbles to enrich the information of analytes present.³¹ On-line electrochemical detection has been integrated with liquid marbles by immersing a three-electrode set-up into the enclosed droplet of an opened magnetic liquid marble (Figure 1.8). Using square-wave voltammetric analysis, Lin et al. systematically varied the applied voltage and measured the electrochemical current responses. These electrochemical responses are related to the reduction/oxidation reaction of the analyte, and the peak current and voltage position of these signals are indicative of analyte concentration and its identity, respectively. The authors indexed the well-defined electrochemical peaks to the oxidation of dopamine which can be clearly measured up to an analyte concentration of 1 μ M, with a limit of detection reaching $\sim$ 40 nM. They also reported the linear relationship between peak currents with dopamine concentration, hence indicating the capabilities of their electrochemical approach to quantify the concentrations of target analytes. Moreover, facile shell opening of magnetic liquid marbles further facilitates the use of optical transmission spectroscopy for fast and non-destructive molecular detection. To realize such transmission-mode detection, they flattened the exposed microdroplet with two hydrophobic glass slides to minimize the reflection and refraction issues associated with curved air-water interface. They successfully detected 8 mM of aqueous acid blue 25 solution encapsulated within the liquid marble using its characteristic absorption peak at 615 nm. In addition to qualitative analysis, optical spectroscopy-based quantitative measurement was also highlighted using a glucose assay. Such assay was performed using a colorimetric enzyme-kinetic method, where glucose is converted into quinoneimine (absorbance peak at 545 nm) by the enzyme. An excellent linear relationship

between the absorbance-glucose concentrations was obtained which allows quantitative measurement of glucose in the range of up to 6 mM. Although representing facile strategies for sensitive and quantitative molecule detection, these electrochemical and optical absorption strategies narrow the choices of suitable analyte; these molecules need to be electro-active and able to absorb in the UV-Vis region, respectively. More importantly, both methods only provide partial information on the actual molecular structure required for accurate identification.

Figure 1.8. Electrochemical detection using liquid marble. (A) Transporting (1 – 3) a magnetic liquid marble on glass substrate and subsequent opening of its magnetic shell using a magnet (4). (B) Immersing three-electrode probe into the enclosed microdroplet (5 – 6), recording of electrochemical responses (7), and removing electrode probe out of the droplet (8). (C) Closing of liquid marble (9 – 10) followed its removal after detection (11 – 12). Reproduced with permission from ref 31. Copyright 2014 Wiley-VCH Verlag GmbH & Co. KGaA.

Recent advent in liquid marble-based sensing involves the incorporation of vibrational-based surface-enhanced Raman spectroscopy (SERS). This spectroscopic technique allows the elucidation of molecular vibrational fingerprints for accurate identification of analytes present in the liquid microdroplet. Moreover, SERS is also capable of ultra-trace and quantitative detection even down to the attomolar level, thus making it an ideal characterization technique for molecular sensing using liquid marble as a micro-sensor. For instance, plasmonic-active liquid marble can be prepared by encapsulating oleic acid microdroplet with Ag nanoparticles (diameter < 70 nm).⁶⁸ When subjected to laser irradiation, sodium 1-phenyl-1H-terazole-5-thiolate (PPT) analyte molecules near/at the SERS-active shell undergo Raman scattering to yield their vibrational fingerprints. These vibrational spectra contain information on PPT's unique molecular bonds, thus highlighting the enormous potential of SERS and liquid marble-based microsensory ensemble to achieve accurate identification of analytes, even with microliter amounts of sample.

1.5.2 Liquid marble as miniature reactor

Miniaturizing chemical/biological processes in 3D liquid marble-based reactors offers numerous benefits such as high throughput reaction screening and enhanced reaction kinetics. These advantages arise from the minimized use of chemical/solvents, enlarged surface area-to-volume ratio, as well as the reduced reactants' diffusion paths (down to milli/micrometric length scale) to access the entire reaction volume. For instance, aqueous methylene blue solution enclosed within catalytic Ag liquid marble exhibits strong dependency of degradation kinetics on the size/volume of the microreactor.¹⁸ A 5-fold enhanced degradation kinetic was demonstrated simply by reducing the reaction droplet's volume from 80 to 5 μL , attaining an optimal rate constant of 0.41 min^{-1} with an almost 100 % degradation efficiency in < 10 min. Liquid marbles are therefore appealing as robust

and green miniature reactors which have been extensively applied for (bio)chemical reactions including nanoparticle synthesis,³⁶ blood typing,³⁵ and even cell cultivation.⁸⁴ Here, the recent progress of liquid marble microreactors are highlighted with a focus mainly placed on the precise control of reaction kinetics using inert and/or functional encapsulating solid particles. This will be continued by a brief review on current reaction monitoring techniques employed on such particle-assembled microdroplet reactors.

The initial applications of liquid marbles as microreactors mainly utilize an inert particle shell with the main purpose of providing an isolated and confined environment for the reacting microdroplet. Polytetrafluoroethylene (PTFE)-encapsulated liquid marble has been demonstrated as a micro-bioreactor for tumor growth owing to the non-adhesive and chemical-resistant nature of its shell which disfavor cell-PTFE interactions.⁸⁴ The confined volume of liquid marble enhances the intimate interaction between neighboring cells and results in their rapid growth and aggregations. Such micro-bioreactor does not require specialized instrumentation to induce cell aggregations and requires only minimal human intervention. This liquid marble-based micro-bioreactor therefore allows the study of cell aggregations typical in cancer cells, and can possibly be extended to other cell types including embryonic cells. In addition to tumor growing, liquid marble can also function as a biosensor for rapid blood typing.³⁵ This is achieved by enclosing blood samples within a precipitated calcium carbonate (PCC) shell (Figure 1.9). The antibody solution was subsequently injected and any bioreaction within the liquid marble was monitored using a digital camera. In this bioreaction, the presence of corresponding antigen causes red blood cells to agglutinate, resulting in the separation of the enclosed microdroplet into two distinct shades of red; bright red on top and dark red at the bottom. Absence of the corresponding antigen does not cause any visible separation. Collectively in the two examples discussed, bio-samples are isolated within microliter-sized liquid

marbles and therefore significantly reduces potential hazards/risks associated with the contamination to the surroundings. These micro-biosensors can also be easily burned to completely eliminate any post-process bio-contamination. Collectively, the advancement in droplet-based micro-biosensor creates new opportunities in tissue engineering and biomedical sciences.

Figure 1.9. Application of liquid marbles as microbiosensors for blood typing. These images are the summary of the results after the injection of corresponding antibodies into the marble microreactors. Green ticks denote the separation of agglutinated red blood cell. Cross signs represent the conditions at which color separation arising from agglutination were not observed. Reproduced with permission from ref 35. Copyright 2011 Wiley-VCH Verlag GmbH & Co. KGaA.

One major advantage of liquid marble-based microreactors lie in their ability to enhance the kinetics of enclosed reaction simply by varying the types of encapsulating particle used; additional functions such as catalytic and photocatalytic properties can be incorporated.^{18, 85} These properties directly impact the frequency and efficiency of intermolecular reactant collisions which essentially form the basis of high-performance bio(chemical) reactions. For instance, silica-based liquid marble provides a reactive encapsulating shell for the regulations of the silver mirror reaction.³⁷ This is achieved by varying the amount of deprotonated silanol groups (Si-O-) present on these silica particles. Negatively-charged silanol groups on silica beads promote their electrostatic interaction with positively-charged Ag ion (Ag^+), thus forming an initial Ag^+ layer directly bonded to the silica surfaces. Exposure of this Ag^+ layer to reducing agent resulted in the formation of Ag metal which subsequently acts as a nucleating site. Ag metals were further deposited onto the particles and eventually formed a Ag mirror coating throughout the silica particles.

On-demand triggering of enclosed reaction has also been realized using liquid marble enclosed within magnetic lanthanide-doped upconversion nanoparticles (UCNPs).⁸⁵ Upon irradiation with near-infrared light, these UCNP particles are able to convert low-energy photons into high-energy visible light. Consequently, the high-energy photons were introduced into the enclosed microdroplet to initiate a photocatalytic reaction. Such on-demand triggering of a chemical reaction allows their potential applications in photodynamic therapy, and also facilitates the remote control of multi-step (bio)chemical reactions.

The ability to assess or monitor chemical/physical processes within the liquid marble reactor at real-time is another vital aspect for the elucidation of reaction mechanism and dynamics. These information are crucial to achieve efficient and selective reaction via rational reaction design. Current reaction monitoring techniques on liquid marbles mainly revolve around ex situ methods,

whereby the particle shells are often disrupted at pre-determined stages to extract the reacting microdroplet for further elemental/molecular analysis (such as UV-Vis absorption spectroscopy).^{14, 18, 67} As an example, Lin et al. has demonstrated the exploitation of magnetically-active liquid marble as a “lab-in-a-droplet” system where reaction monitoring is conducted on-line by opening/closing the shell using an external magnetic (Figure 1.10).³¹ By partially opening the particle shell, electrochemical measurements can be performed using a miniaturized three-electrode probe. Similarly, reaction species can also be tracked using transmission-based optical techniques on a fully-opened magnetic liquid marble. These monitoring techniques have thus far offered adequate qualitative and quantitative insights for the study of a reaction’s mechanism and kinetics.

Figure 1.10. Schematic demonstrating the concept of liquid marble-based magnetically-actuated reacting system for “on-line” detection. Typical processes of this system include (1, 2) preparation of liquid marble, (3) opening of magnetic shell, (4) reflection-mode optical measurement, (5) electrochemical sensing of molecule, (6) full opening of magnetic shell, (7) transmission-mode optical detection, (8, 9) encapsulation of liquid marble driven by IR heating and (10) exit for the removal of liquid marble. Reproduced with permission from ref 31. Copyright 2014 Wiley-VCH Verlag GmbH & Co. KGaA.

1.6 Motivations and objectives

Liquid marbles are emerging robust platforms that have attracted paramount interest owing to their ensemble of benefits intrinsic to particle-assembled microdroplet systems, as well as excellent versatility in terms of the choice of active encapsulating materials and types of liquid microdroplets.¹⁻⁵ These versatilities enable the facile incorporation of a virtually endless combination of functionalities into liquid marbles specially tailored for their intended applications. Liquid marble-based applications include microdroplet transportation system, micro-pumps, micro-sensors, and miniature reactors, which are especially important in the field of nanotechnology, synthetic chemistry, and lab-on-a-chip fluidic devices.

Despite the immense potential offered by liquid marbles, there are still three main challenges present in the application of liquid marbles as micro-sensor and micro-reactor. (1) Current detection mode does not fully exploit the dual phase behavior of liquid marble whereby molecular sensing is limited to the aqueous-soluble analytes enclosed within the microplatform.^{66, 68} Sensing at the liquid-particle-liquid interface is important for concurrent detection in both aqueous and organic phases without needing prior phase separation. Thus, such multiplex detection scheme is advantageous over conventional protocols involving gas- or high-performance liquid chromatography. (2) Reaction monitoring techniques are restricted to ex-situ invasive protocols which often require the quenching of the entire reaction using non-reaction-related chemical species and/or deliberate disruption of the liquid marble shell.^{18, 67} These manipulations of the reaction systems may interfere with the reaction itself, hence inhibiting the accurate elucidation of reaction mechanism and kinetics. An in situ and real-time monitoring method capable of molecular-level read-out should be incorporated into liquid marble for representative reaction investigations, especially for multi-step reactions. (3) Current liquid marble miniature reactors are restricted to

reactions that occur in ambient conditions, and do not include an active mass transportation mechanism to facilitate mixing for homogeneous and rapid reaction.^{14, 35} Integrating a heating mechanism and active mass transportation system into liquid marble are anticipated to overcome high energy barrier of inert reaction and enhance reaction performance.

The objectives of my thesis aim to overcome the aforementioned limitations of liquid marble-based platforms, empowering them as efficient and multifunctional micro-sensor and miniature reactor applicable in broad scientific/technological fields such as nanotechnology, green chemistry, and synthetic chemistry. In chapter 2, we will first demonstrate the fabrication of a plasmonic liquid marble prepared using Ag particles as a multiplex SERS sensor capable of identifying and quantifying analytes present in both the encapsulated and exterior liquid phases simultaneously. Subsequently in chapter 3, we further exploit plasmonic liquid marble as a reactor-sensor hybrid for the rapid and on-site read-out of reaction events within the microreactor at the molecular-level. Using aryl-diazonium surface grafting as a model reaction, we will highlight the importance of such real-time and non-invasive reaction monitoring technique to elucidate the mechanism of a two-step tandem reactions and their associated distinct reaction kinetic models. For chapter 4 and 5, we mainly focus on the incorporation of two novel strategies to dramatically enhance and modulate reaction kinetics using liquid marble microreactor. Chapter 4 involves the fabrication of graphene liquid marbles as photothermal-active miniature reactors to introduce a remote heating mechanism capable of precise temperature control of the reactors simply via laser irradiation. Such temperature control can therefore be employed for direct kinetic modulation of the encapsulated reaction microdroplet, as well as potentially activating inert reactions with high energy barrier. In chapter 5, we demonstrate the spinning of a magnetically-active liquid marble to induce an active mass transportation system within the enclosed 3D microdroplet. Such spinning

phenomena impart a spiral acceleration of enclosed molecules towards the exterior encapsulating shell. Using a shell-catalyzed model reaction involving the multi-step degradation of malachite green, we exemplify the importance of such active and unique mass transfer system to enhance and regulate reaction kinetics, which is remarkably ~ 3 -fold faster compared to conventional stirbar-based convective flow. In chapter 6, I will conclude the thesis and provide an outlook that discusses current drawbacks of liquid marbles that limit their advancement towards real-world applications, as well as identifies potential strategies to overcome these bottlenecks.

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Chapter 2

Plasmonic liquid marbles: A miniature substrate-less surface-enhanced Raman spectroscopy (SERS) platform for quantitative and multiplex ultratrace molecular detection**

Abstract. Inspired by aphids, liquid marbles have been studied extensively and have found application as isolated micro-reactor, as micro-pumps, and in sensing. However, current liquid marble-based sensing methodologies are limited to qualitative colorimetric-based detection. Herein, we describe the fabrication of a plasmonic liquid marble as a substrate-less analytical platform which, when coupled with ultrasensitive SERS, enables simultaneous multiplex quantification and the identification of ultratrace analytes across separate phases. Our plasmonic liquid marble demonstrates excellent mechanical stability and is suitable for the quantitative examination of ultratrace analytes, with detection limits as low as 0.3 fmol, which corresponds to an analytical enhancement factor of 5×10^8 . The results of our simultaneous detection scheme based on plasmonic liquid marbles and an aqueous-solid-organic interface quantitatively tally with those found for the individual detection of methylene blue and coumarin.

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2.1 Introduction

Aphids encapsulate their excreted liquid waste within a thin layer of wax to form sphere-like structures, known as “liquid marbles”, to prevent the flooding of their colony.² Inspired by the natural process of transforming liquids into “soft” solids with enhanced mobility, extensive research on liquid marbles fabricated by the spontaneous encapsulation of water droplets with pulverized hydrophobic micro- or nanoparticles have been studied for the past decade.³⁻⁵ The encapsulating hydrophobic particles impart strong water-repelling properties on the liquid marbles to prevent any interaction of the water droplet with the underlying platform, thus giving liquid marbles a unique non-stick behavior.^{6, 7} Given the flexibility in the choice of solid particles to form liquid marbles and their excellent mechanical robustness, it is no surprise that liquid marbles are fast emerging as an alternative substrate-less platform.⁸⁻¹³ Various applications exploiting the superhydrophobicity (contact angle $\geq 150^\circ$) of liquid marbles and their ability to isolate small-volume liquids¹⁴ have been reported, including controlled transportation in microfluidic system,^{15-17 15-17 15-17} their use as isolated micro-reactors,^{18, 19} micro-pumps,²⁰ and in sensing.²¹⁻²³

Liquid marbles offer tremendous potential as miniaturized and substrate-less sensing platforms for trace-analyte detection. Although there have been reports of the application of liquid marbles for qualitative colorimetry-based detection,²¹⁻²³ no study on the quantitative spectroscopic sensing of trace analyte has been conducted. We envisage the use of a combination of plasmonic liquid marble, formed by using metallic (Ag or Au) nanoparticles, with surface-enhanced Raman spectroscopy (SERS) as an attractive ensemble to produce a substrate-less analytical platform to enable the simultaneous quantification and identification of target analytes.

Typically, conventional SERS sensors are fabricated by the assembly of Ag/Au nanoparticles on solid substrates.²⁴⁻²⁷ These substrate-based platforms have demonstrated 10^4 to 10^{12} -fold

enhancement in Raman signals,²⁸⁻³⁰ providing molecular fingerprints even for femto-/attomolar detection.³¹⁻³³ This behavior can be attributed to the intense electromagnetic fields generated by the incident excitation of localized surface plasmon resonances (LSPRs) of the plasmonic nanoparticles.^{34, 35} However, substrate-based SERS sensors are limited by their need for multistep fabrication, and sequential protocols are required for multiplex analysis involving different phases. Even though there have been reports on the use of nanoparticle monolayers assembled at liquid-liquid or liquid-air interfaces for simultaneous substrate-less detection of multiple analytes across interfaces,^{36, 37} this approach generally lacks robustness as the nanoparticle assembly is prone to disruption from thermal/physical agitation which ultimately affects SERS signal. Hence, the synergy of miniaturized, mechanically stable, readily prepared plasmonic liquid marbles with ultrasensitive SERS is immensely promising in terms of both the quantification and identification of trace analyte and also the multiplex simultaneous detection of analytes in separate phases. The application of plasmonic liquid marbles as an analytical platform therefore offers great versatility and time-efficiency for the detection of samples involving multiple analytes and/or phases, as are commonly observed in industrial discharge and polluted water.

Herein, we demonstrate the fabrication of silver-nanoparticle-based plasmonic liquid marbles and their application as a substrate-less SERS platform for multiplex and quantitative ultratrace molecular detection.³⁸ We use single-crystalline Ag nanocubes as the encapsulating hydrophobic powder for liquid-marble formation. The physical properties of the plasmonic liquid marbles, especially their mechanical stability and robustness as mobile, superhydrophobic platforms are demonstrated. Using SERS as the detection technique, we find that these plasmonic liquid marbles are capable of ultratrace molecular sensing down to the femtomole level. The ability of the plasmonic liquid marbles to sense ultratrace analytes in a single phase (aqueous or organic) and also

simultaneously across the oil/water interface is examined.

2.2 Results and discussion

Single crystalline Ag nanocubes are chosen as the building blocks for the formation of plasmonic liquid marbles, as their well-defined edges/tips can generate intense localized electromagnetic fields for SERS enhancement upon excitation by a laser in the visible region (Figure 2.1A). Furthermore, Ag nanocubes can be synthesized in high yield by the polyol route.³⁹ Approximately 40 mg of dried Ag nanocube powder can be produced per synthesis, which enables the formation of multiple plasmonic liquid marbles. The Ag nanocubes exhibit excellent monodispersity, with an average edge length of (125 ± 5) nm (Figure 2.1B). Characteristic localized surface plasmon resonances of Ag nanocubes are also observed at 348 nm, 412 nm, 525 nm and 674 nm (Figure 2.1C) of the extinction spectrum, which can be indexed to octupole (348 nm), quadrupole (412 nm and 525 nm), and dipole resonances (674 nm), respectively.⁴⁰

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.

To confer hydrophobicity to the Ag nanocubes, the as-synthesized poly(vinylpyrrolidone)-capped Ag nanocubes are first functionalized with 1*H*,1*H*,2*H*,2*H*-perfluorodecanethiol (PFDT) and subsequently dried and pulverized to yield the hydrophobic Ag nanocube powder (Figure 2.2A). The perfluorodecanethiol-grafted Ag nanocubes exhibit excellent anti-wetting property (water contact angle: $(153 \pm 5)^\circ$; Figure 2.2B); in contrast, as a powder, poly(vinylpyrrolidone)-capped Ag nanocubes undergo rapid wetting by water (Figure 2.2C). This comparison clearly emphasizes the need to graft hydrophobic moiety onto the Ag nanocubes to impart hydrophobicity, which is essential for subsequent liquid-marble formation.

Figure 2.2. Characterization of different Ag nanocube powders. (A) SEM image of the pulverized Ag nanocube powder. (B) and (C) are digital images of a 3 μ L water droplet deposited on hydrophobic perfluorodecanethiol-grafted Ag nanocube powder and poly(vinylpyrrolidone)-grafted Ag nanocube powder, respectively. (D) SERS spectra of perfluorodecanethiol-grafted and poly(vinylpyrrolidone)-grafted Ag nanocube powder.

To further demonstrate the necessity of perfluorodecanethiol grafting, we compare the SERS responses of perfluorodecanethiol-grafted Ag nanocubes with poly(vinylpyrrolidone)-capped Ag nanocubes. In Figure 2.2D, poly(vinylpyrrolidone)-grafted Ag nanocube powders are observed to exhibit strong and broad SERS background across Raman shift region between 400 cm^{-1} to 1800 cm^{-1} , where most molecular vibrational fingerprints are typically present.⁴¹ On the other hand, perfluorodecanethiol-grafted Ag nanocube powder generally gives a relatively flat and featureless SERS spectrum across the Raman shift region of interest, with only weak SERS bands at 741 cm^{-1} , 767 cm^{-1} and 811 cm^{-1} .⁴¹ Hence, potential SERS signal interferences caused by perfluorodecanethiol will not affect our latter application of liquid marble for ultratrace molecular detection using SERS.

Plasmonic liquid marbles are formed by rolling a $3\text{ }\mu\text{L}$ water droplet over a bed of the hydrophobic Ag nanocube powder (Figure 2.3A); the resulting plasmonic liquid marble appears dull grayish-green due to the diffuse scattering of light from the powder (Figure 2.3B). The static contact angle of the plasmonic liquid marble as measured on a hydrophilic glass coverslip is $(164 \pm 3)^\circ$ (Figure 2.3C) and indicates minimal interaction between the plasmonic liquid marble and the solid surface. The amount of Ag nanocube powder required to form a single plasmonic liquid marble is estimated to be approximately 0.18 mg via gravimetric analysis and corresponds to a powder thickness of around $1.7\text{ }\mu\text{m}$ or 13 layers of Ag nanocubes (refer to Calculation 2.1).

Figure 2.3. Fabrication and characterization of plasmonic liquid marble. (A) A plasmonic liquid marble is formed by placing a 3 μL water droplet on perfluorodecanethiol-functionalized Ag nanocubes. (B) Digital photographic image of a plasmonic liquid marble. (C) Contact angle of the same plasmonic liquid marble. (D) Rolling and (E) bouncing of a plasmonic liquid marble (from left to right). (F) Submersion and floating of a plasmonic liquid marble in hexane and on water (yellow layer), respectively.

Calculation 2.1. Estimation of the Ag nanocube shell thickness and the number of Ag nanocube layers of plasmonic liquid marble

Basis: One liquid marble

Mass of Ag powder used to form one marble = $1.75 \times 10^{-4} \text{ g}$

Density of Ag = 10.49 g/cm^3

Volume of Ag powder used = Mass of Ag powder / Density of Ag

$$= (1.75 \times 10^{-4}) / 10.49$$

$$= 1.6682 \times 10^{-5} \text{ cm}^3$$

Assuming that Ag nanocubes are entirely suspended on water surface:

Volume of marble = volume of Ag powder used + volume of sessile water droplet used

$$= (1.6682 \times 10^{-5}) + (3 \times 10^{-3}) \text{ cm}^3$$

$$\frac{4}{3} \times \pi \times r_{\text{marble}}^3 = 3.016682 \times 10^{-3} \text{ cm}^3$$

$$r_{\text{marble}} = 8.963555 \times 10^{-2} \text{ cm}$$

Assume that sessile water droplet within liquid marble is spherical:

$$\text{Volume of sessile water droplet used} = \frac{4}{3} \times \pi \times r_{\text{water}}^3 = 3 \times 10^{-3} \text{ cm}^3$$

$$r_{\text{water}} = 8.947002 \times 10^{-2} \text{ cm}$$

$$r_{\text{powder}} = r_{\text{marble}} - r_{\text{water}}$$

$$= 8.963555 \times 10^{-2} \text{ cm} - 8.947002 \times 10^{-2} \text{ cm}$$

$$= 1.6553 \times 10^{-4} \text{ cm}$$

$$\approx 1655 \text{ nm}$$

Based on average Ag nanocube length = $125 \pm 5 \text{ nm}$:

Estimated layers of Ag nanocubes ≥ 13

The plasmonic liquid marble is then subjected to various mechanical tests, including rolling and bouncing, to evaluate its suitability as a substrate-less analytical platform for interfacial applications. The plasmonic liquid marble demonstrates excellent non-stick properties with lotus-like behavior, where a slip-off angle of $(4 \pm 1)^\circ$ (Figure 2.3D) is observed. The plasmonic liquid marble is also mechanically stable and does not break even when dropped from a height of 1 cm (Figure 2.3E). Furthermore, the plasmonic liquid marble can be submerged in some liquids and

float on others, thus highlighting its suitability for interfacial applications (Figure 2.3F). The excellent mechanical properties of our plasmonic liquid marble can be largely attributed to the Cassie-Baxter-like (lotus-like) anti-wetting behavior of water on the perfluorodecanethiol-grafted Ag nanocubes, which creates “air-gaps” that prevent the direct interaction of encapsulated water with underlying platform and also cushioning from mechanical impact.^{6, 9} The mechanical characterization studies collectively demonstrate the mechanical robustness of our plasmonic liquid marble as a substrate-less system and also its suitability for interfacial applications.

To identify the SERS-active areas on the plasmonic liquid marbles, x-y and x-z hyperspectral SERS imaging are performed on the 3 μ L plasmonic liquid marbles encapsulating methylene blue (0.3 nmol) submerged in hexane (Figure 2.4A). The SERS spectrum obtained exhibits characteristic vibrational modes of methylene blue (Figure 2.4C; assignment in Table 2.1).⁴² The x-y SERS image overlaid with its optical microscopic image (Figure 2.4B) shows that the edges of the plasmonic liquid marbles are brightly lit when the most intense C=C ring stretching mode of methylene blue at 1634 cm^{-1} is selected. A waterfall plots of SERS spectra in the x-y plane (Figure 2.4C) from the interior (i) to the exterior (ii) of the plasmonic liquid marble further indicates that the strongest signals ((4347 ± 453) counts/s) are recorded along the edges of the liquid marble, where the Ag nanocube powder is located. In areas away from the Ag nanocube powder, SERS intensities average around (505 ± 29) counts/s in the interior of the plasmonic liquid marble, whereas negligible signals of (115 ± 25) counts/s are recorded at the exterior of the plasmonic liquid marble (Figure 2.4D). Similarly, maximum SERS intensity is also observed at the edge in x-z SERS image (Figure 2.5A – C), where a maximum intensity of (5257 ± 681) counts/s is observed (Figure 2.5D). The strong SERS intensities at the edge of the liquid marble is expected, as SERS is a surface analytical method in which the electromagnetic field enhancement decays exponentially with increasing separation

between the analyte and the surface of Ag nanocubes.^{43, 44} The SERS intensity line profiles for both x-y and x-z imaging therefore hint at the suitability of the entire plasmonic liquid marble edge for molecular sensing. The confinement of SERS activities on the Ag nanocube shell is again demonstrated by measuring the full-width half-maximum (FWHM) of the SERS intensity profiles of the x-y (FWHM = 7.8 μm) and x-z (FWHM = 16.3 μm) SERS images, which agree well with estimated values of 5.2 μm and 10.2 μm , respectively, by summing the optical resolution of our Raman system (Figure 2.6) and the estimated thickness of the Ag nanocube powder. The slight differences are mainly ascribed to ambient vibration as well as slight movement of the plasmonic liquid marble in a fluid environment. The larger discrepancy along the x-z plane is also attributed to the poorer resolution of the system along this plane as well as greater vibrational movement of the motorized stage in the z-direction. From this point onwards, we always use x-y SERS imaging, as there is less interference than that observed for x-z imaging, and only SERS spectra at the edge of plasmonic liquid marble are discussed.

Figure 2.4. Characterization of the SERS-active areas by using x-y SERS imaging of plasmonic liquid marble containing methylene blue. (A) Illustration of (i) SERS detection of the encapsulated aqueous methylene blue and (ii) x-y SERS imaging on plasmonic liquid marble. (B) Overlay of the x-y SERS image of plasmonic liquid marble containing methylene blue (0.3 nmol) with its optical image. The black dotted line denotes the location at which the SERS intensity line profile is obtained.

(C) SERS spectra and (D) SERS intensities profiling along the black dotted line in (B). Negative and positive distances correspond to the interior and exterior of liquid marble, respectively, with the edge defined as distance = 0 μm .

Table 2.1. SERS vibrational modes of methylene blue and their band assignments

On plasmonic liquid marble (cm^{-1})	Band assignment ⁴²
457	C-N-C skeletal deformation
508	C-N-C skeletal deformation
1407	C-H in plane ring deformation
1447	asymmetry C-N stretching
1634	asymmetry C=C ring stretching

Figure 2.5. Characterization of the SERS-active areas using x-z SERS imaging of plasmonic liquid marble containing methylene blue. (A) Illustration of (i) SERS detection of the encapsulated aqueous methylene blue and (ii) x-z SERS imaging on plasmonic liquid marble. (B) X-z SERS image of plasmonic liquid marble containing methylene blue (0.3 nmol). The white dotted line denotes the location at which the SERS intensity line profile is obtained. Scale bar refers to 50 μm (C) SERS spectra and (D) SERS intensities profiling along the white dotted line in (B). Negative and positive distances correspond to the interior and exterior of liquid marble, respectively, with the edge defined as distance = 0 μm .

Figure 2.6. (A – C) Determination of optical resolution for x-, y- and z-axis respectively. Briefly, line-mode SERS imaging of 0.1 μm fluorescent microspheres under the same experimental conditions are performed to obtain intensity line profile. Optical resolutions are determined as $(3.5 \pm 0.2) \mu\text{m}$, $(2.3 \pm 0.3) \mu\text{m}$ and $(8.5 \pm 1.5) \mu\text{m}$ for x-, y-, and z-axis, respectively

We characterize the SERS responses of our plasmonic liquid marbles in three scenarios, namely (1) an analyte within the encapsulated aqueous phase, (2) an analyte outside of the liquid marble, in the organic phase and (3) analytes in both the interior aqueous phase and exterior organic phase. Methylene blue and coumarin are used as the probe analytes for the aqueous and organic phase, respectively. Quantitative SERS detection of methylene blue encapsulated within the 3 μL plasmonic liquid marble is conducted by loading various concentrations of the analyte molecules into the marble (Figure 2.7A). The magnified SERS spectra in the Raman shift window between 1550 cm^{-1} and 1750 cm^{-1} for methylene blue (0.3 fmol – 0.3 nmol) show that the detection limit of methylene blue is 0.3 fmol, for which a SERS intensity of (57 ± 13) counts/s with a signal-to-noise ratio higher than 3:1 is observed (Figure 2.7B, 2.7C). A plasmonic liquid marble in the absence of methylene blue gives a featureless SERS spectrum (“control” in Figure 2.7C), demonstrating that perfluorodecanethiol adsorbed on Ag nanocubes does not interfere with ultratrace sensing owing to the weak/moderate Raman response associated with C-F group.⁴¹ The correlation of SERS intensities with analyte concentration indicates a linear range between 0.3 pmol to 0.3 nmol

(orange-dotted line, Figure 2.7D). At lower concentrations between 0.3 fmol to 0.3 pmol, the SERS intensities remain relatively constant at approximately 50 counts/s. This behavior is attributed to the sub-monolayer Langmuir adsorption of analytes onto the Ag nanocube surfaces, where analyte aggregates are sparingly distributed such that SERS intensity typically arise from only a single aggregate within the laser excitation volume, as illustrated in Figure 2.8.^{31, 33} Furthermore, the analytical enhancement factor (AEF) of the plasmonic liquid marble using methylene blue concentrations of 0.3 fmol is calculated to be 5×10^8 (refer to Figure 2.9 for normal Raman spectrum of methylene blue; refer to Calculation 2.2). This value indicates that our system is able to provide an approximately 10^8 -fold enhancement of Raman intensity relative to that observed in the absence of the encapsulating plasmonic Ag nanocube powder. This high AEF is mainly attributed to the presence of high SERS hotspot densities in the Ag nanocube powder where Ag nanocubes are closely packed together.^{45, 46}

Figure 2.7. SERS sensing of methylene blue in the aqueous phase encapsulated within the plasmonic liquid marble. (A) Illustration of SERS detection of the encapsulated aqueous methylene blue. (B) Typical SERS spectrum of methylene blue. The black dotted box indicates the Raman shift window of interest. (C) SERS spectra and (D) SERS intensities of methylene blue at concentrations ranging from 0.3 fmol to 0.3 nmol. “Control” refers to SERS analysis on plasmonic liquid marble without methylene blue.

Figure 2.8. Adsorption model of methylene blue on plasmonic liquid marble. (A) Langmuir adsorption isotherm fitting of SERS intensity-concentrations plot using encapsulated aqueous methylene blue of concentrations between 0.3 fmol and 0.3 nmol. (B) Illustration of the effect of Langmuir adsorption on SERS intensity, using a substrate for an example.

Figure 2.9. Raman spectrum of methylene blue obtained from the normal Raman analysis of aqueous methylene blue solution (10^{-3} M) submerged in hexane. The acquisition time is 200 s.

Calculation 2.2. Analytical enhancement factor (AEF) using methylene blue

$$\begin{aligned}\text{Analytical EF} &= [(I_{\text{SERS}}) / (I_{\text{Raman}})] \times [(C_{\text{Raman}}) / (C_{\text{SERS}})] \\ &= [57 / (209 / 200)] \times (10^{-3} / 10^{-10}) \\ &= 5 \times 10^8\end{aligned}$$

where C_{SERS} and C_{Raman} are the corresponding concentration measured using plasmonic liquid marble (10^{-10} M; 3×10^{-16} mol over 3 μL) and normal Raman (10^{-3} M), respectively. I_{SERS} and I_{Raman} are the signals recorded on SERS and normal Raman at their respective concentration per unit time.

Further to the quantitative ultratrace detection of an aqueous analyte encapsulated within the plasmonic liquid marble, the exterior of the plasmonic liquid marble can also be used for analyte detection in the organic phase (Figure 2.10A). Figure 2.10B shows a typical SERS spectrum of coumarin, an organic-soluble compound, and its SERS bands assignment are detailed in Table 2.^{47, 48} The variation in the SERS intensity of the most intense ring stretching band at 780 cm^{-1} in the concentration range between 0.1 nmol and 1 μmol is shown in Figure 2.10C. The detection limit for coumarin in the organic phase is 0.1 nmol, with an average SERS intensity of (24 ± 11) counts/s.

The linearity range is determined to be between 0.1 nmol and 1 μ mol from the correlation plot of SERS intensity against the concentration of coumarin (orange-dotted line, Figure 2.10D). The AEF based on coumarin is estimated to be 10^7 (refer to Figure 2.11 for normal Raman spectrum of coumarin; refer to Calculation 2.3), which is comparable to the AEF calculated by using methylene blue. The slight decrease in the analytical enhancement factor (by about one order of magnitude) for detection in organic phase is probably due to the relative increase in background interference contributed by Raman-active organic solvent molecules (Figure 2.11) at the highly diluted analyte concentrations (< 0.1 nmol). Nevertheless, the generally high AEF ($> 10^7$) is a clear demonstration that the plasmonic liquid marble is able to provide strong signal enhancement on its Ag nanocube shell, both internally and externally, for sensing applications.

Figure 2.10. SERS sensing of coumarin in organic phase outside the plasmonic liquid marble. (A) Illustration of SERS detection of coumarin in the organic phase. (B) Typical SERS spectrum of coumarin. The black dotted box indicates the Raman shift window of interest. (C) SERS spectra and

(D) SERS intensities for coumarin at concentrations ranging from 0.1 nmol to 1 μmol . “Control” refers to SERS analysis on a plasmonic liquid marble in the absence of coumarin.

Table 2.2. SERS vibrational modes of coumarin and their band assignments

On plasmonic liquid marble (cm^{-1})	Band assignment ^{47, 48}
622	out of plane ring deformation
655	C-H wag
747	ring stretching
780	ring stretching
1119	C-H scissoring
1549	asymmetric C=C stretching
1592	asymmetric C=C stretching
1635	asymmetric C=C stretching

Figure 2.11. Normal Raman analysis of coumarin in ethanol. Qualitative comparison of normal Raman spectra of (i) pure ethanol and (ii) 10^{-3} M coumarin in ethanol, respectively. The acquisition time is 200 s. We will like to emphasize that coumarin in the initial 10 volume% toluene/hexane solution, which demonstrate limited solubility of 10^{-4} M, does not yield a Raman band unique to coumarin in the 780 cm^{-1} region. Hence, we measure the normal Raman of coumarin in ethanol,

which shows better solubility of coumarin (10^{-3} M). Unique Raman band of coumarin can be identified at 780 cm^{-1} , with intensity of approximately 36 counts. Hence, we use Raman band at 780 cm^{-1} of coumarin in ethanol to estimate the AEF of plasmonic liquid marble.

Calculation 2.3. Analytical enhancement factor (AEF) using coumarin

$$\begin{aligned}\text{Analytical EF} &= [(I_{\text{SERS}}) / (I_{\text{Raman}})] \times [(C_{\text{Raman}}) / (C_{\text{SERS}})] \\ &= [24 / (36 / 200)] \times (10^{-3} / 10^{-8}) \\ &= 1 \times 10^7\end{aligned}$$

where C_{SERS} and C_{Raman} are the corresponding concentration measured using plasmonic liquid marble (10^{-8} M; 10^{-10} mol over 10 mL) and normal Raman (10^{-3} M), respectively. I_{SERS} and I_{Raman} are the signals recorded on SERS and normal Raman at their respective concentration per unit time.

The capability of our plasmonic liquid marble for simultaneous ultratrace detection of both analytes across the liquid-solid-liquid interface is demonstrated in Figure 2.12. In this setup, methylene blue (3 pmol) is encapsulated within the marble, and coumarin (10 nmol) is present in the organic phase (Figure 2.12A). The SERS spectrum of the interfacial detection of methylene blue and coumarin shows fingerprint peaks corresponding to both probe molecules (Figure 2.12B). A comparison of this SERS spectrum with those for single-analyte detection shows that the blue peaks (457 cm^{-1} and 508 cm^{-1}) can be explicitly indexed to methylene blue while the orange peaks (622 cm^{-1} , 655 cm^{-1} , 747 cm^{-1} and 780 cm^{-1}) can be assigned to coumarin. Importantly, the SERS intensities of the selected bands ((48 ± 7) counts/s and (42 ± 22) counts/s for 457 cm^{-1} and 655 cm^{-1} , respectively) in the simultaneous detection scheme can be quantitatively tallied to the individual detection of both methylene blue and coumarin (Figure 2.12C), with accuracy up to one standard

deviation. To the best of our knowledge, this interfacial detection technique is the first for which the SERS intensities of different analytes can be directly related for both simultaneous and individual detection. In contrast, with other methods based on the interfacial assembly of nanoparticles, only relative concentrations of the analytes can be resolved.^{36, 37} Hence, it is evident that the mechanical stability of our plasmonic liquid marble provides an analytical platform less prone to environmental interferences with SERS signals. Thus, a reproducible SERS responses across different plasmonic liquid marbles is possible, as well as the concurrent determination of multiple analytes with different concentrations in separate phases.

Figure 2.12. Multiplex interfacial SERS analysis of methylene blue (aqueous phase) and coumarin (organic phase) with plasmonic liquid marble. (A) Illustration of the SERS detection scheme. (B) SERS spectrum for the multiplex interfacial detection of methylene blue and coumarin (top). Individual SERS spectra of methylene blue in the aqueous phase (middle) and coumarin in the organic phase (bottom) at the same respective concentrations are included for comparison. (C)

Comparison of SERS intensity of methylene blue (3 pmol) and coumarin (10 nmol) from individual and simultaneous detection using plasmonic liquid marble. SERS intensities of methylene blue and coumarin are based on SERS band at 457 cm^{-1} and 655 cm^{-1} respectively.

Over the course of SERS measurements, the potential complications of the plasmonic liquid marble drying and the organic phase vaporizing do not affect our analyses and SERS reproducibility. The drying of the plasmonic liquid marble in air takes slightly over an hour to complete (Figure 2.13A), and at least thirty minutes for the volume of the water droplet to be halved. This process will certainly be longer when the plasmonic liquid marble is submerged in the organic phase. In relation to the vaporizing of the organic phase during SERS measurements, four consecutive SERS measurements on the same plasmonic liquid marble using 10 nmol coumarin over a period of 30 min, with four-minute intervals between each measurement, shows that the SERS signals of coumarin remain stable throughout the experimental duration (Figure 2.13B). SERS signals of the 780 cm^{-1} mode averaged around 60 counts and are all within experimental errors of each other. On the other hand, every SERS measurement for aforementioned molecular sensing using plasmonic liquid marble only require at most ten minutes to be completed due to rapid readout of SERS signals. Consequently, the SERS data collected are in no way affected by the drying of the plasmonic liquid marble or the organic phase, thus demonstrating that our detection methodology is dynamically stable which enables high signal reproducibility over the course of the data collection.

Figure 2.13. Effect of evaporation of encapsulated water and exterior organic phase on the plasmonic liquid marble and concentration of coumarin, respectively. (A) Drying of plasmonic liquid marble under ambient conditions, from a complete liquid marble (0 s) to approximately half of the initial volume (1800 s) and eventually to complete dryness (3960 s). (B) Variation of the SERS intensities (780 cm^{-1} band) of 10 nmol of coumarin with time. Each bar represents one x-y imaging experiment lasting 4 min and the interval between each measurement is 4 min.

2.3 Conclusions

In summary, we have introduced plasmonic liquid marbles as a substrate-less analytical platform for the simultaneous interfacial detection of multiple analytes. This process is facilitated by the mechanical robustness of the plasmonic liquid marbles, which are capable of rolling, bouncing, and remain stable in various solvents. The quantitative detection of individual analytes reaches femtomole levels for the aqueous-based detection of methylene blue and nanomole levels for the organic-phase detection of coumarin. Multiplex detection of these two analytes is also demonstrated

at ultratrace levels. The protocol for the fabrication of plasmonic liquid marble is also generic and can be extended to other nanoparticles or a combination of nanoparticles to tailor the plasmonic response and mechanical properties of the liquid marbles. The multiple advantages of plasmonic liquid marbles will enable them to find applications in lab-on-a-chip systems for on-site ultratrace and/or quantitative sensing in various fields such as industrial/environment safety, criminology and anti-terrorism.

2.4 Materials and methods

Chemicals. Silver nitrate ($\geq 99\%$), anhydrous 1,5-pentanediol ($\geq 97\%$), poly(vinylpyrrolidone) (average MW = 55,000), 1H,1H,2H,2H-perfluorodecanethiol ($\geq 97\%$), methylene blue ($\geq 82\%$) and coumarin 30 (99%; herein denoted as coumarin) were purchased from Sigma Aldrich; copper (II) chloride ($\geq 98\%$) was from Alfa Aesar; ethanol (ACS, ISO, Reag. Ph Eur) was from EMSURE®; n-hexane (AR, $\geq 95\%$) was obtained from RCI Labscan; toluene (BAKER ANALYZED® A.C.S. Reagent) was from Avantor; propan-2-ol (HPLC grade) was from Fisher Scientific. All chemicals were used 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 Ag nanocubes. The preparation of Ag nanocubes was carried out following the method described in literature.³⁹ Briefly, 10 mL of copper (II) chloride (8 mg/mL), poly(vinylpyrrolidone) (20 mg/mL) and silver nitrate (20 mg/mL) were separately dissolved in 1,5-pentanediol. The chemicals were sonicated and vortexed repeatedly to dissolve them. 35 μL copper (II) chloride solution was then added to the silver nitrate solution. Then, 20 mL 1,5-pentanediol in a 100 mL round bottomed flask was heated to 190 °C for 10 min. 250 μL poly(vinylpyrrolidone) precursor was added to flask dropwise every 30 s while 500 μL silver

nitrate precursor was injected every min using a quick addition. The addition process continued until the greenish coloration of the reaction mixture faded off.

For the purification of Ag nanocubes, 1,5-pentanediol was first removed from the mixture through centrifugation. The Ag nanocubes solution was then dispersed in 10 mL ethanol and 100 mL aqueous poly(vinylpyrrolidone) solution (0.2 g/L). The resulting solution was vacuum filtered using Durapore® polyvinylidene fluoride filter membranes (Millipore) with pore sizes ranging from 5000 nm, 650 nm, 450 nm and 220 nm, repeated several times for each pore size. SEM imaging was performed, from which the edge lengths of 100 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.

Preparation of plasmonic liquid marble. Purified Ag nanocubes were then functionalized by immersion into a 5 mM 1H,1H,2H,2H-perfluorodecanethiol in 1:1 propan-2-ol/hexane solution for at least 15 h. The colloidal suspension was then washed with copious amounts of hexane and ethanol and dried to obtain the hydrophobic Ag nanocube powders. The coarse Ag nanocube powders were pulverized to achieve a finer texture. A 3 μ L sessile water droplet was then dispensed onto a bed of fine Ag nanocube powders and rolled to form the plasmonic liquid marble.

Analysis of methylene blue and coumarin on plasmonic liquid marble. Methylene blue (3.20 mg/mL, 10^{-2} M) and coumarin 30 (34.7 μ g/mL, 10^{-4} M) were prepared in solution using ultrapure water and 10 % toluene/hexane mixture respectively. Serial dilutions were performed to give a series of concentrations ranging from 10^{-3} M to 10^{-10} M and 10^{-5} M to 10^{-8} M for methylene blue and coumarin, respectively. For SERS analysis using the interior aqueous solution of liquid marble, a 3 μ L methylene-blue-containing water droplet of each concentration was used for the formation of liquid marble and later transferred into hexane (approximately 10 mL). For SERS

analysis using the exterior of liquid marble, a 3 μL ultrapure water droplet was used instead and transferred into the coumarin solution of each concentrations (approximately 10 mL). For SERS analysis on both the interior and exterior of liquid marble, a 3 μL aqueous solution of methylene blue (10^{-6} M) was used for the formation of liquid marble and transferred into a 10^{-6} M coumarin solution (approximately 10 mL). Each set-up was then subjected to SERS characterization.

Material characterizations. SEM imaging was performed using a JEOL-JSM-7600F microscope. Dark-field imaging and spectroscopy were performed on an inverted microscope (Nikon, Ti-U) using reflective mode. The excitation source was the unpolarized output of a tungsten-halogen lamp. Scattered light was collected with a 100 $\times$ (N.A. 0.90) objective and coupled into a dispersive imaging spectrometer (iDus 401, Andor). Contact angles, rolling, bouncing and drying of liquid marble were captured on a Theta Lite tensiometer equipped with a Firewire digital camera. Static contact angle was measured with a 3 μL ultrapure water droplet. Rolling of liquid marble was performed on a flat stage and the inclination angle was adjusted slowly till the liquid marble started to roll. Bouncing of liquid marble was performed by dropping it at a height of approximately 1 cm. Both rolling and bouncing were captured using a frame rate of 60 fps. Drying of liquid marble was carried out under ambient conditions. 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 (power = 64.4 μW). A 20 $\times$ (N.A. 0.45) objective lens with 1 s accumulation time (unless otherwise stated) was used for data collection between 200 cm^{-1} to 1800 cm^{-1} . At least three x-y SERS imaging experiments (each x-y SERS image is 400 $\mu\text{m} \times 490 \mu\text{m}$) were carried out on different areas of at least two plasmonic liquid marbles to ensure the reproducibility of the SERS signal. All SERS spectra were obtained by averaging at least 20 individual spectra from various plasmonic liquid marble(s), except for x-y and x-z SERS intensity

line profiling, where 9 spectra were used instead. All SERS characterizations were typically completed within the first 10 minutes upon the transfer of liquid marble into hexane or coumarin solution.

Authors contributions. H.K.L. and X.Y.L. designed research. H.K.L. and J.W. performed research. H.K.L., Y.H.L., I.Y.P. and X.Y.L. analyzed the results and wrote the manuscript. All authors discussed the project. X.Y.L. supervised the project.

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Chapter 3

Identifying enclosed chemical reaction and dynamics at the molecular level using shell-isolated miniaturized plasmonic liquid marble**

Abstract. Current microscale tracking of chemical kinetics is limited to destructive ex situ methods. Here we utilize Ag nanocube-based plasmonic liquid marble (PLM) microreactor for in situ molecular-level identification of reaction dynamics. We exploit the ultrasensitive surface-enhanced Raman scattering (SERS) capability imparted by the plasmonic shell to unravel the mechanism and kinetics of aryl-diazonium surface grafting reaction in situ, using just a 2 μL reaction droplet. This reaction is a robust approach to generate covalently functionalized metallic surfaces, yet its kinetics remain unknown to date. Experiments and simulations jointly uncover a two-step sequential grafting process. An initial Langmuir chemisorption of sulfonicbenzene diazonium (dSB) salt onto Ag surfaces forms an intermediate sulfonicbenzene monolayer (Ag–SB), followed by subsequent autocatalytic multilayer growth of Ag–SB₃. Kinetic rate constants reveal 19-fold faster chemisorption than multilayer growth. Our ability to precisely decipher molecular-level reaction dynamics creates opportunities to develop more efficient processes in synthetic chemistry and nanotechnology.

**This chapter has been published in “Identifying Enclosed Chemical Reaction and Dynamics at the Molecular Level Using Shell-Isolated Miniaturized Plasmonic Liquid Marble”, J. Phys. Chem. Lett., 2016, 7, 1501.¹ Reproduced with permission from ref 1. Copyright 2016 American Chemical Society.

3.1 Introduction

Liquid marbles are robust particle-encapsulated microreactors widely applied for the isolation of microlitersized (bio)chemical reactions arising from their ease of fabrication and manipulation.²⁻⁴ In particular, synergizing plasmonic liquid marbles (PLMs) assembled using metallic nanoparticles with ultrasensitive surface-enhanced Raman scattering (SERS) spectroscopy creates excellent microtestbeds to investigate the kinetics and pathways of confined reactions in their native environments without external interference, especially for hazardous or explosive reactions.^{5,6} Unraveling these molecular-level events, particularly for aqueous-based reactions, will enable us to rationally design and control synthetic approaches for the ultimate goal of improving reaction efficiency.⁷⁻⁹ In comparison with other reaction tracking platforms, PLM can potentially overcome current limitations that require large reaction volumes or sophisticated characterization tools without introducing platform instability.¹⁰⁻¹²

Here we combine PLM microreactor with vibrational spectroscopy-based SERS to elucidate molecular-level kinetic pathway of aryl-diazonium grafting reaction onto silver (Ag) surfaces using just 2 μ L of reaction solution, and quantify its intrinsic kinetics without disturbing the reaction progress. While aryl-diazonium grafting is a powerful approach to form covalently functionalized metallic surfaces for important industrial applications,¹³⁻¹⁵ the dynamics of this highly reactive grafting currently remain unresolved.¹⁶⁻¹⁹ From our in situ SERS reaction tracking, we unveil a hitherto undocumented two-step grafting reaction and their respective kinetic rate constants. Experimental and simulation evidence reveal an initial Langmuir chemisorption of aryl-diazonium onto Ag to form a surface intermediate, followed by a subsequent autocatalytic multilayer growth.

3.2 Results and discussion

PLM functions as a confined microreactor for the in situ SERS monitoring of dynamic molecular events occurring during sulfonicbenzene diazonium (dSB) grafting of sulfonicbenzene species onto Ag surfaces (Figure 3.1A). Prior to PLM formation, we subject the as-synthesized, PVP-capped Ag nanocubes (nanocube edge length = (122 ± 6) nm; Figure 3.1B, 3.1C) to aqueous HCl treatment to reduce both Ag-bound PVP and their strong SERS interference, which is important for subsequent accurate SERS read-out during in situ monitoring of surface grafting reaction. The HCl-treated Ag nanocubes (edge length = (115 ± 6) nm; Figure 3.1D, 3.1E) exhibit a relatively flat and featureless SERS spectrum with weak SERS bands (~ 5 counts/s; Figure 3.1F, 3.1G) at 1143 cm^{-1} , 1269 cm^{-1} , 1432 cm^{-1} and 1451 cm^{-1} , which are characteristic vibration mode involving the backbone carbon, hydrogen groups and pyrrolidone groups of polyvinylpyrrolidone.²⁰ On the other hand, PVP-capped Ag nanocubes demonstrate strong (~ 60 counts/s) and broad SERS background signal extending between Raman shift region of $400\text{ cm}^{-1} - 1800\text{ cm}^{-1}$, with distinct SERS bands that can be indexed to polyvinylpyrrolidone at 1143 cm^{-1} , 1269 cm^{-1} , 1351 cm^{-1} , 1377 cm^{-1} , 1432 cm^{-1} , 1451 cm^{-1} and 1500 cm^{-1} . Hence, it is evident that the HCl treatment of Ag nanocube is efficient in reducing the interfering signal from polyvinylpyrrolidone by $\sim 85\%$ (based on SERS band at 1500 cm^{-1}), and provide a clean background vital for the accurate identification and SERS monitoring of reaction species. Hereon, all the plasmonic liquid marbles are prepared using HCl-treated Ag nanocubes unless otherwise stated.

Figure 3.1. (A) Assembling a PLM microreactor by using Ag nanocube building blocks to encapsulate a 2 μL aqueous reaction droplet. The reaction droplet contains sulfanilic acid (SA), NaNO_2 , and HCl for the generation of sulfonicbenzene diazonium (dSB) salt in situ. (B) SEM image of as-synthesized, PVP-coated Ag nanocubes and (C) their size distribution, average edge length of (122 ± 6) nm. (D) SEM image of HCl -treated Ag nanocubes and (E) their size distribution, average length of (115 ± 6) nm. The slight decrease of Ag nanocubes edges is due to the rounding of edge and vertices by acid etching. (F) Digital image of the as-fabricated PLM. (G) Comparison of SERS background between plasmonic liquid marble formed using PVP-capped (black) and HCl -treated Ag nanocubes (red). Both the liquid marbles are prepared by encapsulating pure water.

PLM is easily formed by using Ag nanocube building blocks to encapsulate a 2 μL aqueous reaction precursor droplet. We systematically tune the ratio of Ag nanocube mass to droplet volume ratio during plasmonic liquid marble fabrication to optimize its SERS activities for rapid signal read-out crucial for real-time monitoring of reaction. Generally, we observe that the increase of Ag mass-to-droplet volume from 4 $\mu\text{g}/\mu\text{L}$ to 20 $\mu\text{g}/\mu\text{L}$ leads to the formation of plasmonic liquid marble with reddish brown Ag nanocube shell to eventually a shiny silver-like appearance (Figure 3.2A), respectively. Such phenomenon can be attributed to the intense plasmonic coupling of adjacent Ag nanocube arising from the increasing compactness and uniformity of the Ag nanocube shell (Figure 3.2B) with higher concentration of Ag nanocube incorporated,²¹ concentration ≤ 10 $\mu\text{g}/\mu\text{L}$ leads to formation of discontinuous Ag nanocube islands while concentration ≥ 20 $\mu\text{g}/\mu\text{L}$ is able to form a continuous and compact Ag nanocube shell. Consequently, the reddish brown coloration of Ag nanocube shell at low particle concentration (4 $\mu\text{g}/\mu\text{L}$) resemble the appearance of Ag nanocube colloidal solution, while high nanocube concentration of Ag nanocube (> 20 $\mu\text{g}/\mu\text{L}$) give rise to a very compact assembly that approximate bulk Ag. We further estimate that the plasmonic shell comprises of $\sim 1, 2, 3, 4$ and 5 layers of Ag nanocube (refer to Calculation 3.1) at a Ag nanocube mass-to-droplet volume ratio of 5 $\mu\text{g}/\mu\text{L}$, 10 $\mu\text{g}/\mu\text{L}$, 15 $\mu\text{g}/\mu\text{L}$, 20 $\mu\text{g}/\mu\text{L}$ and 25 $\mu\text{g}/\mu\text{L}$, respectively.

Figure 3.2. (A) Digital images of plasmonic liquid marble encapsulating different volume of water. The mass of Ag nanocubes used is constant at 40 μg . Scale bar, 10 mm. (B) Microscopic images of plasmonic liquid marble's Ag nanocube shells using various Ag mass-to-droplet volume ratio. The region marked by white contour indicates the exposed water droplet's surface. The site marked by orange circle refer to Ag nanocube island suspended on water droplet surface. (C) Schematic depicting the cross section of an idealized plasmonic liquid marble. r_1 and r_2 refer to radius of core water droplet and radius of plasmonic liquid marble, respectively. (D) Comparison of SERS intensities of 0.1 mM aqueous methylene blue droplet, using characteristic SERS band at 1623 cm^{-1} , encapsulated by plasmonic liquid marble formed using various Ag mass-to-droplet volume ratio. Laser power = 100 μW .

Calculation 3.1. Determining the shell thickness and corresponding layers of Ag nanocube at various Ag-to-water ratio

Assuming all of Ag nanocubes are entirely suspended on water/decane interface, the thickness of plasmonic liquid marble shell can be estimated with the following equation which idealized the formation of Ag nanocube shell (Figure 3.2C):

$$\text{Thickness of Ag nanocube shell} = r_2 - r_1 = \sqrt[3]{\frac{3V_M}{4\pi}} - \sqrt[3]{\frac{3V_d}{4\pi}} \quad (1)$$

where r_1 = radius of core water droplet, r_2 = radius of plasmonic liquid marble, V_M = Volume of the entire liquid marble, V_d = Volume of the core water droplet = 2 μL .

$$V_M = \text{Volume of Ag nanocubes} + V_d$$

$$= (\text{Mass of Ag nanocubes} / \text{Density of Ag}) + V_d$$

Since density of Ag = 10.49 g/cm^3

$$V_M = \frac{m_{\text{Ag}}}{10.49} + (2 \times 10^{-3}) \text{ cm}^3 \quad (2)$$

By substituting equation (2) into (1), the thickness of Ag nanocubes shell can be obtained as 123 nm, 248 nm, 372 nm, 500 nm, and 620 nm for Ag nanocube mass-to-droplet volume ratio of 5 $\mu\text{g}/\mu\text{L}$, 10 $\mu\text{g}/\mu\text{L}$, 15 $\mu\text{g}/\mu\text{L}$, 20 $\mu\text{g}/\mu\text{L}$, 25 $\mu\text{g}/\mu\text{L}$, respectively. Using average HCl-treated Ag nanocube edge length of 115 nm, the layers of Ag nanocube encapsulating the water droplet can therefore be estimated at ~ 1, 2, 3, 4 and 5, respectively.

We subsequently investigate the effect of Ag nanocube layers on the SERS-enhancing capability of plasmonic liquid marble. Briefly, various plasmonic liquid marble are prepared by encapsulating 2 μL aqueous methylene blue droplet (0.1 mM) using different Ag mass-to-droplet volume ratio and then subjected for SERS evaluation. The SERS spectra obtained demonstrate characteristic vibrational modes of methylene blue.²² As shown in Figure 3.2D, the intensity of

SERS band at 1623 cm^{-1} is enhanced by $> 80\%$, from 1166 counts/s to 2105 counts/s, as the concentration of Ag nanocube increases from $5\text{ }\mu\text{g}/\mu\text{L}$ to $15\text{ }\mu\text{g}/\mu\text{L}$, respectively. This is due to the intense plasmonic coupling between adjacent particles in the Ag nanocube shell. However, further increases of Ag nanocube concentration from $20\text{ }\mu\text{g}/\mu\text{L}$ to $25\text{ }\mu\text{g}/\mu\text{L}$ leads to a decrease of SERS intensity from 2047 counts/s to 1480 counts/s instead. We attribute such decrease of SERS intensity to the reduced accessibility of excitation laser to the Ag nanocube/droplet interface caused by the potential light shielding effect from thick outer Ag nanocube layers.^{23,24} Characterized by the high uniformity of Ag nanocube shell and also its intense SERS-enhancing capability, the Ag mass-to-droplet volume ratio for the fabrication of plasmonic liquid marble is therefore optimized at $20\text{ }\mu\text{g}/\mu\text{L}$ for subsequent in situ SERS study. Correspondingly, we determine the analytical enhancement factor of our plasmonic liquid marble for methylene blue to be 4.5×10^8 (Figure 3.3; refer to Calculation 3.2).²⁵

Figure 3.3. (A) SERS spectra and (B) SERS intensity of 1623 cm^{-1} from $2\text{ }\mu\text{L}$ aqueous methylene blue solution encapsulated in plasmonic liquid marble with concentration ranging from 2 fmol to 0.2 nmol . The control denote plasmonic liquid marble in the absence of methylene blue. (C) Normal Raman spectrum of 10^{-2} M aqueous methylene blue solution using an acquisition time of 50 s .

Calculation 3.2. Analytical enhancement factor of plasmonic liquid marble using methylene blue as probe molecule

Analytical enhancement factor of plasmonic liquid marble is determined by comparing the SERS and Raman intensity of methylene blue's 1623 cm^{-1} band at its lowest detectable concentration when in the presence and absence of plasmonic liquid marble, respectively.

$$\begin{aligned}\text{Analytical EF} &= [I_{\text{SERS}} / I_{\text{Raman}}] \times [C_{\text{Raman}} / C_{\text{SERS}}] \\ &= [76.45 / (84.63 / 50)] \times [10^{-2} / 10^{-9}] \\ &= 4.5 \times 10^8\end{aligned}$$

where C_{SERS} and C_{Raman} are the corresponding concentration measured using plasmonic liquid marble (10^{-9} M ; $2 \times 10^{-15}\text{ mol}$ over $2\text{ }\mu\text{L}$) and normal Raman (10^{-2} M ; $2 \times 10^{-8}\text{ mol}$ over $2\text{ }\mu\text{L}$), respectively. I_{SERS} and I_{Raman} are the signals recorded using SERS and normal Raman at their respective concentration per unit time.

We demonstrate the excellent SERS reproducibility of by evaluating the temporal SERS responses of encapsulated and non-reacting aqueous methylene blue solution, which functions as an ex-situ internal reference. The characteristic SERS band of methylene blue at 1623 cm^{-1} demonstrate a highly consistent average SERS intensity (at least 500 random SERS spectra) with $< 1\%$ signal fluctuation (Figure 3.4), even for an extended duration of 1 h. Such consistent SERS intensities highlight both the stability and homogeneity of our plasmonic liquid marble in prolonged experimental duration, which is important to provide both strong and reproducible SERS activities for reliable temporal molecular read-out. Collectively, the analytical SERS enhancement factor of $> 10^8$ with high signal reproducibility are collectively attributed to the extensive 3D plasmon coupling among the Ag nanocubes. These characteristics enable us to utilize PLM as an efficient SERS

platform to monitor the kinetics of short-lived chemical species formation and decomposition in our model reaction.

Figure 3.4. SERS intensities of characteristic SERS band of aqueous methylene blue solution (0.1 mM) encapsulated in plasmonic liquid marble at various time intervals. At each time point, the SERS intensity value is obtained by averaging at least 500 random spectra.

Real-time and noninterfering SERS monitoring of the surface grafting reaction is performed directly in its native environment using 2.5 mM dSB salt as the reactant (Figure 3.5A). Owing to its high reactivity, dSB salt is generated freshly in situ within the PLM by premixing sulfanilic acid (SA), sodium nitrite (NaNO_2), and hydrochloric acid (HCl) in the precursor droplet (Figure 3.5B, 3.5C).²⁶ A clear evolution of various vibrational fingerprints is evident in the time-dependent SERS spectra over the course of 1 h (Figure 3.5D. 3.5E). The initially featureless spectrum in the absence of dSB (background) gives rise to two distinct SERS bands at 983 cm^{-1} and 1555 cm^{-1} at 150 s. Both SERS bands continue to increase in intensity until 360 s, followed by a steady signal reduction to below the detection limit of our system at 2400 s. The onset of signal reduction coincides with a

concurrent emergence of two new distinct SERS bands at 1072 cm^{-1} and 1572 cm^{-1} at $t > 360\text{ s}$. These new vibrational modes exhibit a gradual intensity increase before ultimately dominating the SERS spectrum at $t > 2400\text{ s}$. The SERS spectral changes are indicative of a multistep surface reaction. This is supported by the stable environment provided by our PLM with consistent SERS enhancement ($<1\%$ signal fluctuation) over a similar time frame. Consequently, peak intensity changes observed over time can be directly correlated with concentration changes of a particular chemical species on the surfaces of Ag nanocubes. The initial appearance and diminishing of the vibrational modes at 983 cm^{-1} and 1555 cm^{-1} signify the formation and subsequent consumption of a reaction intermediate as the grafting reaction progresses. The formation of the thermodynamically stable product is characterized with the formation of new vibrational modes at 1072 cm^{-1} and 1572 cm^{-1} .

Figure 3.5. (A) Schematic depicting in situ SERS monitoring of surface grafting reaction using PLM with 2.5 mM dSB. (B) Characteristic UV-vis spectra of sulfanilic acid and sulfonicbenzene diazonium salt produced by in-situ generation method. Extinction peak at 249 nm can be attributed

to sulfanilic acid, and the peak at 268 nm represent the formation of sulfonicbenzene diazonium salt.²⁶ (C) UV-vis spectra of sulfonicbenzene diazonium salt recorded at pre-defined timings of 1 min, 6 min, 10 min, 30 min, and 60 min upon the addition of NaNO₂. All the reactions are performed in ice bath. (D, E) Spectral evolution over the course of surface grafting. Time-resolved color-coded intensity map (D) and corresponding SERS spectra (E) show distinct appearance and diminishing of the 983 cm⁻¹ and 1555 cm⁻¹ bands (blue) up to 2400 s. As these bands disappear, new bands at 1072 cm⁻¹ and 1572 cm⁻¹ begin to appear after 360 s (orange).

To identify the reaction species involved in the reaction, we perform density function theory (DFT) simulations of probable surface species that could be involved in the dSB grafting reaction. These species include Ag-bound sulfonicbenzene monomer (Ag-SB), dimer (Ag-SB₂), trimer (Ag-SB₃), and also azo-coupled SB dimer (Ag-SB-N=N-SB) (Figure 3.6A).^{17, 27} In the first step, dSB is covalently grafted onto the Ag surfaces to form a Ag-SB monolayer. First, the experimental SERS spectrum at 360 s is in close agreement with simulated Ag-SB's SERS spectrum, exhibiting characteristic vibrational modes of sulfonicbenzene backbone (Figure 3.6B). The most intense SERS bands at 983 cm⁻¹ and 1555 cm⁻¹ are assigned to in-plane deformation and stretching mode of benzene ring in SB moiety, respectively (Table 3.1). While qualitative comparison of SERS spectra with PLM containing pure SA affirms the sulfonicbenzene backbone of dSB intermediate, we note the SERS intensities in the control spectrum in the absence of other grating reactants are ~ 20 times weaker and remain almost constant over 3000 s (Figure 3.7). This further supports that SB is chemically grafted onto Ag instead of mere physisorption. The absence of a characteristic diazonium (-N₂⁺) vibrational mode also excludes the possibility of dSB physisorption on Ag nanocubes (Figure 3.8).¹⁷

Figure 3.6. (A) Optimized molecular configurations for various chemical species including Ag-SB, Ag-SB₂, Ag-SB₃, Ag-SB-N=N-SB. These chemical species are attached to an Ag pyramidal cluster, constructed using 10 Ag atoms, to represent their grafting onto Ag surfaces and also for latter SERS spectra DFT calculations. (B) DFT simulated Raman spectra of Ag-SB, Ag-SB₂, Ag-SB₃, and Ag-SB-N=N-SB compared to the experimental SERS spectrum of dSB grafted on Ag nanocubes at 360 s and 3000 s. The blue and orange SERS bands highlight the close resemblance of experimental SERS spectra with the simulated SERS spectra of Ag-SB and Ag-SB₃, respectively.

Table 3.1. Assignment of both experimental and simulated SERS spectra of various molecular species, including Ag-SB, Ag-SB₂, Ag-SB-N=N-SB and Ag-SB₃.^{17, 27-29}

Band position (cm ⁻¹)						
SERS (360 s)	SERS (3000 s)	Ag-SB	Ag-SB ₂	Ag-SB-N=N-SB	Ag-SB ₃	Assignment
1555	1572	1556	1551	1519	1564	v(1 st layer C=C)
			1594	1556	1652	v(2 nd layer C=C)
1459		1459	1448	1449		v(C=C) + β(C-H)
		1330, 1316	1332		1333	v _{as} (SO ₃)
1188		1184, 1092		1195, 1096	1182	β(C-H)
1124	1124	1133	1139		1134	v _s (SO ₃)
983	1072	984	1006, 987	1016, 993	1043	δ(ring)
707		691	690	722	719	v _s (C-S) + δ(ring)
498		502	557			γ(ring)
		422	418	433	416	γ(ring)
350		343				ρ(ring)
333		337			337	v(Ag-C)

v, stretching; δ, in-plane deformation; β, in-plane bending; γ, out-of-plane deformation;

ρ, rocking; as, asymmetric; s, symmetric; ring, benzene ring

Figure 3.7. Time-resolved spectral evolution of PLM containing pure SA (10 mM). Almost consistent SERS responses of ~ 8 counts/s are observed during duration of 3000 s under identical measurement conditions.

Figure 3.8. SERS spectra of aqueous dSB droplet encapsulated in plasmonic liquid marble for 150 s and 2400 s. The absence of SERS bands at ~ 2300 cm^{-1} , which is indexed to diazonium group ($-\text{N}_2^+$),¹⁷ for both 150 s and 2400 s suggests the cleavage of dinitrogen during the surface grafting reaction of sulfonicbenzene diazonium salt onto Ag surfaces.

Second, we observe a characteristic Ag–C vibration mode in the 300 cm^{-1} to 400 cm^{-1} region experimentally and in silico (Figure 3.9A). In contrast, this band is not found in both the physisorbed SA and the background signals of PLM (Figure 3.9A). The broad Ag–C band can be further deconvoluted into two peaks centered at 333 cm^{-1} and 350 cm^{-1} (Figure 3.9B). The 333 cm^{-1} band is indexed to the Ag–C bond stretching mode in Ag–SB,^{30,31} while the 350 cm^{-1} band is assigned to benzene-ring rocking vibration (Figure 3.9C).

Figure 3.9. (A) Experimental SERS spectra of dSB reaction on Ag surface at 360 s (pink line), sulfanilic acid (green line), and pure water PLM background (black line) as well as DFT-calculated SERS spectrum for Ag–SB in the 300 cm^{-1} to 600 cm^{-1} region (blue line). Agreement between experimental and calculated SERS spectra further confirms Ag–SB formation at 360 s. Molecular model highlighting Ag–C stretching is shown on the right. Deconvolution of (B) experimental and (C) DFT-simulated Ag–C band. For (B), the 333 cm^{-1} and 350 cm^{-1} bands correspond to Ag–C stretching and benzene-ring rocking, respectively. For (C), Ag–C stretching is centered at a simulated Raman shift of 337 cm^{-1} , while the rocking vibration of benzyl ring is positioned at 343 cm^{-1} .

Third, the chemical grafting reaction of dSB on Ag nanocubes can be further affirmed via XPS spectral analysis of the Ag nanocubes surface. Briefly, we first deposit a constant mass of Ag nanocubes onto silicon wafer. After drying with nitrogen gas, 20 μ L dSB reactants (5 mM) or sulfanilic acid (5 mM) are deposited on the Ag nanocube and allowed to react for at least 12 h under 1 $^{\circ}$ C to 2 $^{\circ}$ C. The samples are subsequently washed with copious amount of water and dried with nitrogen before XPS measurement. A control HCl-treated Ag nanocube experiment is also subjected to the same preparation procedures but in the absence of reactants. For Ag nanocubes exposed to dSB solution, we observe a strong decrease of Ag 3d peaks intensity and the upshifting of peaks position by ~ 0.16 eV (Figure 3.10A), relative to the case of control HCl-treated Ag nanocube. Conversely, there are no obvious changes of peak intensity for Ag nanocubes exposed to SA compared to Ag 3d peaks of the control sample, while only a slight upshifting of ~ 0.09 eV is observed. The decrease of Ag 3d peaks intensity for Ag nanocube exposed to dSB can be attributed to the inhibition of Ag 3d signal by Ag surface-grafted SB multilayer,³² which is also in agreement with the SERS data. Likewise, the increase in the binding energy of Ag 3d generally correlates with the chemical environment changes of Ag atom by electron-withdrawing group, also in line with our experiment that Ag surface is grafted with sulfonic benzene groups. Moreover, the S 2p core level XPS spectra (S atom of sulfonic group) of Ag nanocubes derived with dSB for 120 and 3600 s reveal a gradual increase of signals (Figure 3.10B), while the N 1s signal (N atom from PVP residue even after acid treatment) concurrently decreases (Figure 3.10C). Such information on the S 2p and N 1s XPS spectra affirm the existence of SB groups on Ag surface, and also PVP are removed during the surface grafting reaction, respectively. High-resolution C1s spectra also identify the chemical interaction of grafted sulfonic benzene with the Ag surface. In the control HCl-treated Ag nanocubes, characteristic C 1s Gaussian-fitted peaks of polyvinylpyrrolidone (PVP; 284.0 eV, 284.6 eV, 285.3

eV, and 287.3 eV) are mainly observed due to the presence of residual PVP on Ag nanocubes surface after acid treatment (Figure 3.10D).³³ Upon the exposure of Ag nanocube to dSB, two additional XPS peaks are identified at 283.0 and 286.5 eV (Figure 3.10F), which can be indexed to Ag-C and C-S, respectively.³⁴⁻³⁶ On the other hand, Ag-C and C-S bonds are not located on Ag nanocube exposed to sulfanilic acid instead (Figure 3.10E), which is in good agreement with our expectation that no grafting reaction will occur. Consequently, the identification of both the presence of Ag-C and C-S bonds upon the exposure of Ag nanocube to dSB is therefore a clear evidence that sulfonic benzene is chemically grafted onto the Ag nanocube's surface via the formation of Ag-C bond.

Figure 3.10. (A) Ag 3d core level XPS spectra of blank, HCl-treated Ag nanocubes, and also Ag nanocubes exposed to 5 mM sulfanilic acid (SA) solution or 5 mM dSB solution for 3600 s. (B) S 2p and (C) N 1s core level XPS spectra of blank, HCl-treated Ag nanocubes, and also Ag nanocubes exposed to dSB solution for 120s and 3600 s, respectively. C1s core level XPS spectra of (D) blank, HCl-treated Ag nanocubes, and (E, F) Ag nanocubes exposed to sulfanilic acid solution and dSB

solution for 1 h, respectively. All the Ag nanocubes samples are washed with copious amount of water and dried with nitrogen gas before XPS measurement.

The second step of the grafting reaction involves the reaction between free dSB with Ag-SB to form Ag-SB₃ multilayer structure. The major experimental vibration mode at 1572 cm⁻¹ observed at 3000 s matches well with the simulated Ag-SB₃ bands at 1564 cm⁻¹ (Figure 3.6B). We also note that another major experimental vibrational mode at 1072 cm⁻¹ exhibits moderate deviation from the simulated SERS band at 1043 cm⁻¹ (Figure 3.6B). This is possibly due to the differences in experimental structure/orientation of the multilayer structure on Ag nanocube as compared with the simulated Ag-SB₃ model. In addition, our single-molecule simulation does not account for the coverage effects of multiple-molecule surface grafting that could influence the experimental SERS bands.¹⁷ Nevertheless, the former vibration is assigned to in-plane benzene ring deformation, and the latter is indexed to first benzene ring stretching of Ag-SB₃ (Table 3.1). We consider only the formation of multilayer SB structures at the ortho position in our simulations due to its reduced steric hindrance compared with the meta position,³⁷ which is situated near Ag surface. In addition, the formation of Ag-SB₃ is energetically favored over other possible multilayer SB structures, including Ag-SB₂ and Ag-SB-N=N-SB. The energy change upon adsorption (Figure 3.11) of the dSB molecule on the SB monomer-bonded vertex of pyramidal Ag₁₀ cluster to form various SB multilayer-bonded Ag are calculated as:

$$\Delta E = E(\text{Ag}_{10}\text{vSB}_3) + 2E(\text{N}_2) + E(\text{H}_2) - E(\text{Ag}_{10}\text{vSB}) - 2E(\text{dSB})$$

$$\Delta E = E(\text{Ag}_{10}\text{vSB}_2) + E(\text{N}_2) + 1/2E(\text{H}_2) - E(\text{Ag}_{10}\text{vSB}) - E(\text{dSB})$$

$$\Delta E = E(\text{Ag}_{10}\text{vSB-N=N-SB}) + 1/2E(\text{H}_2) - E(\text{Ag}_{10}\text{vSB}) - E(\text{dSB})$$

where the $E(\text{Ag}_{10}\text{vSB})$, $E(\text{dSB})$, $E(\text{N}_2)$ and $E(\text{H}_2)$ are calculated total energies of Ag_{10} cluster with one SB molecule adsorbed on its vertex, dSB molecule, nitrogen and hydrogen gas in vacuum, respectively.

Figure 3.11. Calculated energy changes of adsorption of dSB molecules onto Ag-SB to form Ag-SB₃, Ag-SB₂, and Ag-SB-N=N-SB, respectively.

The total molecular energy reduces by more than 2-fold upon Ag-SB₃ generation from Ag-SB intermediate as compared with both Ag-SB₂ and Ag-SB-N=N-SB. Furthermore, huge spectral mismatches and the absence of strong azo (N=N) stretching preclude the existence of these two species (Figures 3.6B, 3.12, Table 3.1). All of these results jointly suggest the formation of Ag-SB₃ in the second step. Hence, our in situ SERS reaction monitoring using PLM evidently demonstrates the first molecular-level confirmation of a two-step mechanism for the surface grafting reaction between Ag and the aryldiazonium salt (Figure 3.13). Such reaction mechanism is

especially crucial for the derivation of representative kinetic information, especially the rate constants. Compared with other in situ methods or quantitative characterization techniques such as electrochemistry¹⁶ and quartz crystal microbalance,^{18, 19} our PLM-based in situ SERS monitoring can clearly distinguish the multistep reaction by identifying intrinsic intermediates/products and therefore elucidate the reaction mechanism.

Figure 3.12. Comparison of experimental SERS spectrum recorded at 2400 s and the calculated SERS spectrum for Ag-SB-N=N-SB model in the range of 1800 - 2200 cm⁻¹.

Figure 3.13. (A) Two-step mechanism of diazonium-based surface grafting reaction. (B) Graphical representation of (A).

To elucidate the dynamics of the grafting reaction, we study the temporal SERS spectral evolution when the dSB concentration is varied between 0.5 mM and 5 mM. We base our analysis on the 983 cm^{-1} peak for step 1 and 1072 cm^{-1} peak for step 2, as they are sufficiently distinct to provide accurate spectral evaluation (Figure 3.14A - C).

Figure 3.14. (A) Time-resolved color-coded intensity map and (B) corresponding SERS spectra recorded in situ from 2 μL of PLM containing 0.5 mM dSB. (C) Relative time-dependent intensity changes of 983 cm^{-1} (denoting Ag–SB formation and consumption) and 1072 cm^{-1} (denoting Ag–SB₃ formation) SERS bands using 0.5 mM dSB. Time-dependent plot of relative SERS intensity changes of (D) 983 cm^{-1} and (E) 1072 cm^{-1} bands for initial dSB concentrations between 0.5 mM and 5 mM. The intensity changes fit well with (D) the Langmuir chemisorption isotherm (dotted lines) and (E) the autocatalytic logistic growth model (solid lines), respectively. (F) Plot of apparent reaction rate constants, k_{app} , of steps 1 (blue dots) and 2 (orange dots) at various dSB concentrations. The gradients of the best-fit lines correspond to the respective intrinsic rate constants of the surface

grafting reactions. Error bars in panels C – E correspond to signal variations collected from at least three separate experiments and in panel F correspond to uncertainty arising from curve fittings in panels D and E.

Step 1 of the grafting reaction is characterized by an initial rapid intensity increase before gradually reaching a plateau with the formation of Ag–SB monolayer. Increasing dSB concentrations leads to a shorter time to reach plateau (Figure 3.14D). This intensity growth trend agrees well with the derived Langmuir chemisorption kinetic isotherm (refer to Calculation 3.3).^{18, 38} The apparent chemisorption rate constant of step 1 ($k_{app,step1}$) ranges from 3.2 ms^{-1} to 60 ms^{-1} at 0.5 mM to 5 mM dSB (Figure 3.14F), respectively. Quantitative agreement between our experimental results with Langmuir chemisorption model further validates the covalent grafting of dSB onto Ag surface to form an Ag–SB monolayer, in line with our observation of Ag–C vibration.

Calculation 3.3. Derivation of modified Langmuir chemisorption isotherm

$$\begin{aligned}\frac{d\theta_t}{dt} &= k_1 (1 - \theta) c - k_d \theta \\ \theta_t &= 1 - \exp(-k_{app,step1} t)\end{aligned}\tag{3}$$

where θ is the monomer SB coverage fraction on Ag surface, t is the reaction time, k_1 is the chemisorption rate constant of sulfonicbenzene diazonium on Ag, which is independent of sulfonicbenzene diazonium salt concentration (dSB), k_d is the desorption rate constant, c denotes the concentration of dSB, $k_{app, step1} = k_1 c + k_d$.

Since the surface coverage of SB monomer bonded on Ag is proportional to SERS intensity I , the surface coverage fraction of SB monomer, θ can be expressed as

$$\theta_t = \frac{I_t}{I_{max}}$$

where I_t is the integration intensity of SERS mode at 983 cm^{-1} at reaction time t , I_{max} is the maximum value of the integration intensity of this mode.

Equation 3 can therefore be expressed as

$$I_t = I_{max} [1 - A \exp(-k_{app,step1}t)] \quad (4)$$

where I_t is SERS intensity at time t , I_{max} is maximum SERS intensity, A is proportionality constant, $k_{app, step 1}$ is the apparent chemisorption rate constant of step 1, t is time.

The temporal SERS intensity evolution in step 2 ($t \geq 600\text{ s}$) of the grafting reaction exhibits a logistic type growth curve (Figure 3.14E). This growth is characterized by an initial slow growth, which subsequently accelerates as reaction progress before slowing down to an eventual plateau.³⁹ After 600 s, we observe a simultaneous onset of the 1072 cm^{-1} SERS band, indicating the formation of Ag-SB₃, and a diminishing intensity for the SERS band at 983 cm^{-1} correlated with a decrease in Ag-SB population (Figure 3.14A – C, 3.14E). The multilayer formation of Ag-SB₃ resulting from the reaction between dSB and Ag-SB is an autocatalytic process that can be described using a Prout–Tompkins model (refer to Calculation 3.4).⁴⁰ Our experimental observations comply well with the proposed autocatalytic reaction kinetic model, with apparent formation rate constants ($k_{app,step2}$) ranging from 2.1 ms^{-1} to 5.4 ms^{-1} at 0.5 mM to 5 mM dSB (Figure 3.14F).

Calculation 3.4. Derivation of modified autocatalytic Slogistic model

By assuming a first-order autocatalytic reaction, the rate equation can be written as

$$\frac{d\alpha}{dt} = k_2\alpha(1 - \alpha)c \quad (5)$$

where, α is the conversion fraction of multilayer, t is the time of reaction, c denote the dSB concentration, and k_b is the multilayer formation rate constant that is

conversion-fraction-independent.

Integrating equation (5) and rearranging

$$\alpha = \left(1 + \exp^{-k_{app,step2}(t-t_{1/2})}\right)^{-1} \quad (6)$$

where $k_{app,step2} = k_b c$, $t_{1/2}$ is an integration constant, representing the characteristic time of reaction when the conversion approach to 50%, t is reaction time

Correspondingly, the conversion fraction of multilayer can be expressed as

$$\alpha = \frac{I_t}{I_{max}} \quad (7)$$

where I_t is the integrated intensity of SERS mode at 1072 cm^{-1} at reaction time t , I_{max} is the maximum value of integrated intensity of such SERS mode.

Combining (6) and (7), we can get

$$I_t = I_{max} \left(1 + \exp^{-k_{app,step2}(t-t_{1/2})}\right)^{-1} \quad (8)$$

where $k_{app,step2}$ is the concentration-dependent apparent rate constant of step 2 and $t_{1/2}$ is the time to reach half of maximum intensity.

A linear k_{app} -dSB concentration relationship exists for both reaction steps, where increasing dSB concentration enhances the apparent rate constant (Figure 3.14F). We make use of the following relationship $k_{app} = kC + A$ to determine the actual reaction rate constants (k), where k_{app} is the apparent rate constant, C is concentration of dSB reactant, and A is a constant. The actual reaction rate constants for steps 1 (k_1) and 2 (k_2) are determined as $13.0 \text{ M}^{-1} \text{ s}^{-1}$ and $0.7 \text{ M}^{-1} \text{ s}^{-1}$, respectively. k_1 is ~ 19 times higher than k_2 , further supporting our proposed sequential two-step reaction mechanism and not a concurrent single-step reaction. The lower k_2 is likely due to an induction time needed to kickstart autocatalysis. Ag-SB₃ autocatalyzes step 2 by stabilizing incoming dSB to facilitate the growth of new Ag-SB₃, which is in accordance to the nucleation site buildup model.³⁹

Such stabilization could be originated from dipole-ionic ($-\text{SO}_3\text{H}$ with $-\text{N}_2^+$) or pi interaction (between aromatic backbones). Consequently, initial Ag-SB₃ formation is slow due to the lack of Ag-SB₃ species. As the reaction progresses, the initially generated Ag-SB₃ then catalyzes and accelerates the subsequent grafting of dSB onto adjacent Ag-SB, consistent with the nucleation site buildup model. The difference in rate constants also implies that we can potentially control the extent of mono- or multilayer formation on the Ag surfaces and even extend our system to form supramolecular networks.

We further demonstrate the PLM as an isolated microreactor for in situ reaction kinetics investigation by comparing with ex situ techniques and with other SERS microplatforms. Control plasmonic liquid marble encapsulating 2 μL of 2.5 mM dSB droplet are placed under ice bath conditions and ruptured at pre-defined timings for UV-vis measurements. By utilizing ex situ UV-vis characterization and an extinction-concentration calibration curve (Figure 3.15A, 3.15B), we can only observe a decrease in dSB's characteristic absorption peak at 268 nm with reaction time (Figure 3.15C). C/C_0 exhibits a single exponential decrease from 1.0 to ~ 0.3 from 0 s to 3600 s (Figure 3.15D), respectively, which could not exemplify the two-step process of diazonium-based surface grafting reaction. Normalizing the absorption changes against the initial dSB concentration results in a single exponential decrease with an apparent rate constant of $1.8 \times 10^{-3} \text{ s}^{-1}$ (refer to Calculation 3.5). This rate constant reflects only on the speed at which dSB is consumed in the surface grafting process but does not provide any valuable information on the complex dynamics occurring in the two-step reaction.

Figure 3.15. (A) UV-vis spectra of in-situ generated dSB at different concentration. The dSB solutions are prepared by direct mixing of sulfanilic acid in HCl with excess NaNO_2 . The in-situ generated dSB concentration generally approximate the corresponding concentration of sulfanilic acid used. (B) Extinction of dSB's characteristic 268 nm peak versus their respective concentration. (C) Ex situ absorption spectra collected from breaking 2 μL of aqueous PLM droplet containing 2.5 mM of dSB at predefined time intervals. The absorption band at 268 nm corresponds to that dSB absorption. (D) Time-dependent plot of normalized dSB concentrations (C/C_0). Error bars correspond to intensity fluctuations from at least three independent experiments.

Calculation 3.5. Determination of Apparent Rate Constant using UV-vis characterization

The experimental ex-situ UV-vis results can be described using equation 9, and has an apparent resultant rate constant of $1.8 \times 10^{-3} \text{ s}^{-1}$.

$$\frac{C_t}{C_0} = 1 - (\Delta \frac{C}{C_0})_{\max} + (\Delta \frac{C}{C_0})_{\max} \exp(-kt) \quad (9)$$

where C_0 is concentration of dSB droplet at $t = 0 \text{ s}$, $(\Delta \frac{C}{C_0})_{\max}$ is the maximum reduction of dSB concentration after grafting reaction, k is the grafting reaction rate constant, and t is reaction time.

We further compare our PLM microreactor with other control SERS microplatforms, including Ag nanocube colloidal solution, Ag nanocube pre-deposited on Si, and thermal-evaporated Ag film (Figure 3.16, 3.17). All reaction set-up employ 2 μL of 5 mM aqueous dSB solutions, 40 μg of Ag nanocubes and are also subsequently immersed in decane to reduce evaporation of the reacting solution for fair evaluation with plasmonic liquid marble. The time-dependent SERS signal are collected for each systems. Using a pre-defined timing at 240 s for comparison, plasmonic liquid marble clearly provides strong and distinct SERS spectrum (signal-to-noise ratio > 3 ; Figure 3.17) of Ag-SB formation with an intensity of ~ 200 counts/s for the characteristic SERS band at 983 cm^{-1} . The SERS intensity provided by plasmonic liquid marble is > 40 -fold higher compared to the control SERS micro-platforms, which the latter generally provides < 5 counts/s of SERS intensity or an absence of distinguishable SERS bands is observed (Figure 3.16). The strong and reproducible SERS signals offered by PLM are critical in enabling temporal investigation of rapid reactions or reaction species, especially small molecules with low SERS cross sections.

Figure 3.16. Time-dependent SERS spectra of the surface grafting reaction of sulfonicbenzene diazonium onto Ag recorded from various SERS platforms including (A) Ag nanocube colloidal solution, (B) Ag nanocube cluster pre-deposited on Si wafer and (C) thermal-evaporated Ag film.

Figure 3.17. PLM (green line) clearly shows much stronger SERS signals than Ag nanocube colloidal suspension (purple line), Ag nanocube cluster predeposited on Si wafer (orange), and thermal-evaporated Ag film on Si wafer (black line). All SERS spectra are recorded at 240 s using identical mass of Ag nanocubes for fair comparison.

3.3 Conclusions

We quantitatively uncover a two-step pathway for the covalent grafting of aryl-diazonium species onto metallic surfaces through the use of PLM coupled with ultrasensitive and real-time SERS reaction tracking. The spectral evolution can be accurately modeled using Langmuir chemisorption and logistic growth models for the first and second steps, respectively. By solving the respective intrinsic rate constants experimentally, we find a 19-fold faster Langmuir chemisorption than the subsequent multilayer growth. This kinetic difference enables us to control the formation of functional mono- or multilayers using only 2 μL of reactants. The comprehensive and representative mechanistic and kinetic information offered by our platform certainly opens up opportunities for the precise regulation of reaction efficiency, which is especially crucial in the field of synthetic chemistry and nanotechnology.

3.4 Materials and methods

Chemicals. Silver nitrate ($\geq 99\%$), anhydrous 1,5-pentanediol ($\geq 97\%$), poly(vinylpyrrolidone) (PVP; average MW = 55000), hydrochloric acid (HCl; 37 %), methylene blue ($\geq 82\%$), sulfanilic acid (SA; $\geq 99\%$), sodium nitrite ($\geq 97\%$), decane (anhydrous, $\geq 99\%$) were purchased from Sigma Aldrich; copper (II) chloride ($\geq 98\%$) was from Alfa Assar; ethanol (ACS, ISO, Reag. Ph Eur) was from EMSURE®; n-hexane (AR, $\geq 95\%$) was obtained from RCI Labscan; All chemicals were used 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 Ag nanocubes. Ag nanocubes were prepared following the method in literature.⁴¹ Briefly, copper (II) chloride (8 mg/mL), poly(vinylpyrrolidone) (20 mg/mL) and silver nitrate (20 mg/mL) were separately dissolved in 10 mL 1,5-pentanediol after repeated sonication and vortex mixing. 35 μL copper (II) chloride solution was subsequently added to the silver nitrate solution. 20 mL of 1,5-pentanediol was added into a 100-mL round bottomed flask and heated to 190 °C for 10 min. 250 μL poly(vinylpyrrolidone) precursor was added to flask dropwise every 30 s while 500 μL silver nitrate precursor was injected every min using a quick addition. The addition process continued until the greenish coloration of the mixture faded off.

For the purification of Ag nanocubes, 1,5-pentanediol was first removed from the mixture through centrifugation. The Ag nanocubes were then dispersed in 10 mL ethanol and 100 mL aqueous poly(vinylpyrrolidone) solution (0.2 g/L). The resulting solution was vacuum filtered using Durapore® polyvinylidene fluoride filter membranes (Millipore) with pore sizes ranging from 5000 nm, 650 nm, 450 nm, and 220 nm, repeated several times for each pore size. The Ag nanocubes were then re dispersed in ethanol (10 mg/mL) and stored in fridge for further use.

HCl treatment of Ag nanocubes. The removal of surfactant from Ag nanocubes surface was referred to method in literature.⁴² 100 μ L of purified Ag nanocubes in ethanol suspension (10 mg/mL) was added to 5 mL hydrochloric acid (0.1 M) in water under stirring condition. After 3 h, the Ag nanocubes colloidal suspension was then washed with copious amount of pure water and ethanol to remove excess HCl. The HCl-treated Ag nanocubes were subsequently redispersed in ethanol (10 mg/mL) for further use.

In-situ generation of sulfonichbenzene diazonium salt. Various concentrations of sulfanilic acid (10 mM, 5 mM, 2.5 mM and 1 mM) were first prepared in 0.1 M HCl aqueous solution. The aqueous sulfanilic acid solutions were then mixed with equi-volume of aqueous sodium nitrite solution (1.6 M) and placed in an ice bath to generate dSB in-situ.^{26, 43} Consequently, the efficient concentrations of sulfanilic acid, HCl and sodium nitrite were (5 mM, 2.5 mM, 1.25 mM, 0.5 mM), 0.05 mM and 0.8 M, respectively. Since a rapid and complete generation of dSB from sulfanilic acid is observed in Figure 3.5, the concentrations of dSB generated were the same as respective concentration of sulfanilic acid used.

Preparation of plasmonic liquid marble (PLM). Typically, 4 μ L ethanolic solution of HCl-treated Ag nanocubes (10 mg/mL) was added into a 2 mL centrifuge tube and dried with nitrogen gas. 300 μ L decane was then added to the centrifuge tube and sonicated to uniformly disperse the Ag nanocubes in decane. A 2 μ L water droplet, either in the presence or absence of reactants, was dispensed into Ag nanocube-containing decane suspension and rolled to form plasmonic liquid marble.

SERS measurement on plasmonic liquid marble. The as-prepared plasmonic liquid marbles (2 μ L) encapsulating various chemical species were transferred onto the Raman samplestage for SERS evaluations. The SERS characterization for both plasmonic liquid marbles encapsulating

ultrapure water and aqueous methylene blue solution were performed at room temperature ($\sim 22\text{ }^{\circ}\text{C}$), while the in-situ SERS monitoring of surface grafting reaction involving sulfonicbenzene diazonium salt was conducted under ice bath condition. We would like to emphasize that the first SERS read-out for each in-situ SERS monitoring was typically after 100 s, due to the time required to transfer plasmonic liquid marble on the sample stage and subsequent focusing on its top surface. The as-prepared plasmonic liquid marbles ($2\text{ }\mu\text{L}$) encapsulating various chemical species were transferred onto the Raman sample stage for SERS evaluations. The SERS characterization for both plasmonic liquid marbles encapsulating ultrapure water and aqueous methylene blue solution were performed at room temperature ($\sim 22\text{ }^{\circ}\text{C}$), while the in-situ SERS monitoring of surface grafting reaction involving sulfonicbenzene diazonium salt was conducted under ice bath condition. We would like to emphasize that the first SERS read-out for each in-situ SERS monitoring was typically after 100 s, due to the time required to transfer plasmonic liquid marble on the sample stage and subsequent focusing on its top surface. For surface grafting reaction monitoring, the sample is not illuminated over the entire duration of the stipulated timings. Instead, the stipulated timings refer to the time, relative to the initial encapsulation of dSB reactant into PLM, at which respective SERS measurements are performed. SERS measurement at each time point require an overall duration of only $\sim 15\text{ s}$ and the actual SERS measurement is performed using a hyperspectral x-y imaging mode with an acquisition time of 1 s/line and a laser power of 0.1 mW . At least 100 individual spectra were obtained by averaging from various PLMs over an area of $\sim (6 \times 160)\text{ }\mu\text{m}^2$. The laser-induced heating and its effect on the reaction process is neglected owing to the low laser power (0.1 mW) used, short laser exposure time ($< 20\text{ s}$), and also reaction performed under ice bath condition.

Density functional theory (DFT) calculation. The calculation of grafted sulfonicbenzene on Ag surface were carried out using the unrestricted BVP86 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 a pyramid consisting of 10 Ag atoms. After geometry optimization of the pyramidal Ag cluster, the sulfonicbenzene monomer, dimer, dimer connected with azo group, and trimer molecule were placed on its vertex. The whole system was then relaxed with all the Ag atoms fixed.

Ex-situ UV-vis characterization of plasmonic liquid marble. Plasmonic liquid marble was prepared via the encapsulation of 2 μL aqueous dSB (2.5 mM) solution under ice bath conditions. The plasmonic liquid marbles were subsequently ruptured at pre-defined timings of 0 s, 120 s, 360 s, 600 s, 1200 s, 1800 s and 2400 s for UV-vis measurements to quantify the concentration of unreacted dSB precursor.

Preparation and SERS evaluation of control SERS micro-platforms. For fair comparison with plasmonic liquid marble, we generally prepared control SERS micro-platforms using the same mass of Ag nanocube (40 μg), reaction volume (2 μL) and dSB concentrations (5 mM), which were similarly immersed in decane to minimize evaporation of reacting solution. Ag colloidal solution was prepared by suspending 40 μg of Ag nanocube in a 2 μL water droplet, which also contain precursors for dSB in-situ generation. For Ag nanocube clusters pre-deposited on Si, 40 μg of HCl-treated Ag nanocube was dispensed on a $\sim 10\text{ mm}^2$ Si wafer. Upon the evaporation of ethanol, the SERS platform was further dried using a stream of N_2 gas. For Ag film, it was prepared using a home-built thermal evaporator deposition system, whereby 25 nm of Ag was deposited on a Si wafer at a rate of 0.5 $\text{\AA}/\text{s}$. 2 μL aqueous dSB (5 mM) droplets were then deposited on the latter two SERS micro-platforms for subsequent SERS evaluation.

Characterization. SEM imaging was performed using JEOL-JSM-7600F microscope. UV-vis spectra was measured with Cary 60 UV-vis spectrometer. XPS spectra were measured using

a Phoibos 100 spectrometer with a monochromatic Mg X-ray radiation source. All XPS spectra were fit using XPS Peak 4.1 (free software accessible at <http://www.phy.cuhk.edu.hk/Bsurface>). SERS measurements were performed using both x-y and x-z imaging mode of the Raman touch microspectrometer (Nanophoton Inc., Osaka, Japan) with an excitation wavelength of 532 nm and laser power of 100 μ w (unless otherwise stated). A 50 $\times$ objective lens (N.A. = 0.55) with 1 s acquisition time (unless otherwise stated) was used for data collection. X-y SERS imaging experiments were carried out on at least three PLMs to ensure the reproducibility of SERS signal for each reaction time. All SERS spectra were obtained by averaging at least 100 individual spectra from various PLMs. SERS intensities were represented using the integrated peak area of corresponding SERS bands.

Authors contributions. X.H., H.K.L. and X.Y.L. designed research. X.H., H.K.L. and Y.L. performed experimental research. W.H. and S.L. conducted simulation studies. X.H., H.K.L., Y.H.L. and X.Y.L. analyzed the results and wrote the manuscript. All authors discussed the project. X.Y.L. supervised the project.

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Graphene liquid marbles as photothermal miniature reactors for reaction kinetics modulation**

Abstract. We demonstrate the fabrication of graphene liquid marbles as photothermal miniature reactors with precise temperature control for reaction kinetics modulation. Graphene liquid marbles show rapid and highly reproducible photothermal behavior while maintaining their excellent mechanical robustness. By tuning the applied laser power, swift regulation of graphene liquid marble's surface temperature between 21 °C and 135 °C and its encapsulated water temperature between 21 °C and 74 °C are demonstrated. The temperature regulation modulates the reaction kinetics in our graphene liquid marble, achieving a 12-fold superior reaction rate constant for methylene blue degradation than at room temperature.

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4.1 Introduction

Liquid marbles are formed by the spontaneous encapsulation of liquid droplets by pulverized solid particles.²⁻⁵ They are promising miniature reactors owing to their ability to isolate microliter liquid, ease of fabrication, and excellent mechanical robustness.^{6, 7} Such miniature reactor is ideal for reactions involving costly and hazardous reagents/processes, and useful in preliminary reaction screening. Liquid marble reactors have been applied for blood typing,⁸ nanocomposite synthesis,⁹ photochemical polymerization,¹⁰ and heterogeneous catalysis.¹¹ However, current applications of liquid marble reactors are restricted to reactions of low activation energy or at room temperature due to the lack of a heating mechanism. It is essential to incorporate a heating mechanism into liquid marble to broaden its application for reactions requiring precise control and elevated temperature.

Graphene is a promising candidate for heatable liquid marble miniature reactor due to their excellent photothermal properties.¹² Their strong photoabsorption over a wide range of wavelengths allows rapid and localized heating upon vibrational relaxation of photoexcited electrons.¹³ Currently, the incorporation of graphene are mainly in the form of suspensions or films,¹⁴⁻¹⁶ which require tedious reactant/product recovery procedures, and/or film fabrication protocols. Hence, the combination of graphene and liquid marble allows easy-to-prepare heatable miniature reactor, and permits a broad range of processes with precise temperature and reaction kinetic control. Notably, the localized photothermal heating is crucial as thermal energy is supplied to the small volume reaction on-demand without changing the bulk liquid medium temperature.¹⁷ This reduces the evaporation issues of miniature reactors and minimizes energy waste, making it superior to conduction/convection heating.¹⁷

Herein, we demonstrate the fabrication of graphene liquid marbles (GLM) and their application as remotely heatable miniature reactors for reaction kinetic modulation. The physical and mechanical properties of GLM as isolated and robust miniature reactors are characterized. We then

demonstrate the instant heating of GLM with surface temperatures tunable between 21 °C to 135 °C by controlling the laser power. The actual encapsulated water temperature is probed using temperature-dependent Raman scattering spectroscopy. The application of GLM for reaction kinetics modulation is subsequently evaluated by performing a temperature-dependent degradation of methylene blue. We also highlight the easy post-reaction recovery of encapsulated liquid and emphasize the superior photothermal properties of GLM over other materials.

4.2 Results and discussion

GLM are fabricated by rolling microliter water droplets on a bed of pulverized perfluorooctylsilane-grafted graphene nanoplatelets (thickness $\sim$ 12 nm, lateral size of a few microns; Figure 4.1A, 4.1B). To evaluate the hydrophobicity of graphene nanoplatelets upon perfluorooctylsilane functionalization, 3 μ L water droplets are placed on respective beds of un-functionalized and functionalized graphene nanoplatelets (Figure 4.1C). Water is observed to spread readily across a bed of un-functionalized graphene, resulting in a small contact angle of $< 5^\circ$. On the other hand, perfluorooctylsilane-functionalized graphene exhibits a contact angle of $(108 \pm 2)^\circ$, indicating that hydrophobicity can be rendered to graphene via perfluorooctylsilane functionalization.

Figure 4.1. (A) Preparation of graphene liquid marble (GLM). (B) SEM image of perfluorooctylsilane-grafted graphene nanoplatelets. (B) Contact angle (CA) of a 3 μL water droplet placed on graphene flakes, before (top) and after (bottom) functionalization.

We then prepare GLM by rolling different volume of water droplets on a bed of perfluorooctylsilane-functionalized graphene nanoplatelets (Figure 4.2A). Graphene nanoplatelets are observed to wrap around the surface of the water droplet into a closely-packed and assembled into a rough shell (Figure 4.2B) with a thickness estimated at 220 μm (refer to Calculation 4.1). For small-volume graphene liquid marbles ($< 20 \mu\text{L}$), the shape adopted are mainly spherical (Figure 4.1C) which can be attributed to the dominance of surface tension over the effect of gravity.⁷ As the droplet volume increases to $> 20 \mu\text{L}$, a flatten puddle-like shape is observed which is due to the gravity-induced distortion of the graphene liquid marble when gravity becomes the dominant force. Nevertheless, the successful formation of GLM with between 5 mL to 80 mL clearly demonstrating the versatile reaction capacity of such miniature reactors. Our GLM also demonstrates excellent non-stick properties, exhibiting high static contact angle of $\sim 152^\circ$ and low slip-off angle of $\sim 20^\circ$ (Figure 4.2C, 4.2D) even on a hydrophilic glass platform. Hence, the wetting state of the graphene liquid marble can be approximated to the Cassie-Baxter model (lotus-behavior),^{18, 19} where the

closely-packed graphene nanoplatelets isolate the water droplet and inhibit its direct interaction with the underlying hydrophilic platform. Otherwise, the liquid marble will rupture due to the rapid outflow of encapsulated water and subsequent spreading across the hydrophilic platform.

Figure 4.2. (A) Digital image of a 40 mL GLM. (B) Bright field image of graphene liquid marble. (C) Contact angle images of graphene liquid marbles fabricated with volumes ranging from 5 μL to 80 μL. (E) Rolling of graphene liquid marble on a platform with inclination angle of 20°.

Calculation 4.1. Estimation of layer thickness of graphene liquid marble

Basis: One 40 μL graphene liquid marble, which is assumed as a sphere with core-shell structure.

Mass of graphene powder used to form one marble = 7.2×10^{-4} g

Volume of graphene power used = mass of graphene / density of graphene nanoplatelet

$$= (7.2 \times 10^{-4} \text{ g}) / 0.053 \text{ g.cm}^{-3}$$

$$= 1.36 \times 10^{-2} \text{ cm}^3$$

Volume of marble = volume of graphene powder used + volume of sessile water droplet used

$$= (1.36 + 4) \times 10^{-2} \text{ cm}^3$$

$$\frac{4}{3} \times \pi \times r_{\text{marble}}^3 = 5.36 \times 10^{-2} \text{ cm}^3$$

$$r_{\text{marble}} = 0.234 \text{ cm}$$

For water droplet:

$$\frac{4}{3} \times \pi \times r_{\text{water}}^3 = 4.0 \times 10^{-2} \text{ cm}^3$$

$$r_{\text{water}} = 0.212 \text{ cm}$$

The shell thickness is therefore calculated as:

$$r_{\text{shell}} = r_{\text{marble}} - r_{\text{water}}$$

$$= (0.234 - 0.212) \text{ cm}$$

$$= 0.022 \text{ cm}$$

We determine the effective surface tension of graphene liquid marble using the “puddle height method”.²⁰ Briefly, the side-profile digital images of graphene liquid marbles are collected as their volumes increase systematically between 5 μL to 300 μL . Consequently, the height-volume profile of graphene liquid marbles can be attained and the effective surface tension (γ) of graphene liquid marble is determined. The height-volume profile exhibits an initial rapid increases of height from 2.41 mm to 4.67 mm as volume increases from 10 μL to 120 μL (Figure 4.3), respectively. Subsequently, the height remained at a maximal of ~ 4.77 mm even when the volume of graphene liquid marble increases to 300 μL . The two-phase increases of height is typical and is attributed to the increasing gravitational distortion to the liquid marble as its volume increases,⁷ resulting in a

transformation from a spherical structure to a puddle-shape structure. The effective surface tension of graphene liquid marble is therefore determined to be $\sim 59 \text{ mJ/m}^2$ (refer to Calculation 4.2), which agrees well with the typical reported effective surface tension between 40 mJ/m^2 to 75 mJ/m^2 as obtained from a variety of liquid marbles.³

Figure 4.3. Height-volume co-relationship of graphene liquid marble.

Calculation 4.2. Determination of the surface tension of graphene liquid marble

With the maximal height determined to be 4.77 mm (Figure 4.3), the effective surface tension could be calculated as:

$$\gamma = \rho g H^2 / 4 \sin^2(\theta/2)$$

where γ is the surface tension on the marble–air interface, $\rho = 1.0 \times 10^3 \text{ kg/m}^3$ is the density of water; $g = 9.80665 \text{ m/s}^2$ is the Newton's gravitational constant; H is the maximum height of a puddle in large volumes, and an average contact angle of 152° is observed.

$$\gamma = [1.0 \times 10^3 \text{ kg/m}^3 \times 9.80665 \text{ m/s}^2 \times (4.77 \times 10^{-3} \text{ m})^2] / 4 \sin^2 76^\circ$$

$$= 59.30 \text{ mJ/m}^2 \approx 59 \text{ mJ/m}^2 \text{ (2.s.f.)}$$

The photothermal behavior of GLM is investigated upon laser irradiation (532 nm, laser power = 112 mW), and its spatially resolved, front-temperature profiles are monitored using an infrared (IR) camera (Figures 4.4A). Prior to laser irradiation, a 40 mL GLM exhibits a homogeneous surface temperature of 21 °C (Figure 4.4B, 0 s). Within 1 s of irradiation, the surface temperature of the central irradiated zone (cross-sectional distance = 0 mm) instantaneously increases to about 90 °C (Figure 4.4C, 4.4D), with a temperature change rate $(dT/dt)_{\max}$ of ca. 600 °C.s⁻¹ (Figure 4.4E). The temperature at the central irradiated zone remains plateau at 90 °C throughout 60 s of laser irradiation (Figure 4.4D). The spatial-temperature plots exhibit Gaussian-like profiles, indicating localized photothermal heating due to the use of a Gaussian laser beam.²¹ We also note a slower heat conduction process from the central irradiated zone to the non-irradiated zone (cross-sectional distance $\geq \pm 1$ mm) through the thermally-conductive GLM. Such heat transfer results in a gradual temperature increase on the non-irradiated surface from 21 °C to 30 °C after 60 s of laser irradiation (Figure 4.4C, 4.4D), during which $(dT/dt)_{\max} \approx 0.5$ °C.s⁻¹ (Figure 4.4F).

Figure 4.4. (A) Schematic representation of the photothermal set-up. (B) Thermograms, (C) spatial temperature profiles and (D) temperature-time profiles of graphene liquid marble when the irradiation laser is switched on. 1st derivative temperature-time profiles of the heating process representing temperature changing rates (dT/dt) of (E) central irradiated zone and (F) non-irradiated zone.

Generally, the photothermal behavior of GLM can be categorized as a two-step heating process. Firstly, the drastic temperature increment is attributed to the instantaneous photothermalization of light (< 10 ps) by graphene.¹³ A subsequent temperature plateau indicates that a thermal equilibrium is reached, in which the photothermal heat generation is balanced by heat dissipation through thermal conduction to the entire graphene shell, the encapsulated water, and the environment. In contrast, no temperature change is observed on an irradiated 40 mL water droplet (to

be discussed later), affirming that the photothermal responses of our GLM originate from their graphene shells. The photothermal efficiency of GLM (η), which is the ratio of the thermal energy to the total irradiation energy,²² is estimated to be 15 % (refer to Calculation 4.3) and is comparable to most graphene-based photothermal agents.^{14, 15, 23}

Calculation 4.3. Calculation for the photothermal efficiency of GLM

A theoretical model based on the energy balance of graphene shell during the laser heating is employed to analyze the photothermal conversion process and determine its conversion efficiency (η). In the initial stage ($t < 1$ s), the heat generated greatly surpassed the heat dissipation via thermal conduction, allowing the efficiency of photothermal conversion upon laser irradiation of graphene liquid marble to be estimated.¹⁴ At the initial stage, the converted thermal energy can be approximated as the temperature distribution in the purple ring highlighted in Figure 4.5A, assuming the effect from thermal conduction is negligible. Due to the nature of Gaussian laser beam applied, 95 % of energy conversion occurred within this circle area with a radius of $1.224 \times w_0 = 0.1$ cm,²⁴ where $w_0 = 0.81$ cm is the waist size obtained with the Gaussian equation (as highlighted in Figure 4.5B). Hence, graphene shell within this area is selected as overall irradiated zone for analysis, where temperature increment are observed.

In order to determine photothermal efficiency, we combine equation 1 - 3 to derive equation 4:

$$Q_{\text{output}} = C_{\text{graphene}} \times m_{\text{graphene}} \times \Delta T \quad (1)$$

$$Q_{\text{input}} = I \times t \quad (2)$$

$$\eta = Q_{\text{output}}/Q_{\text{input}} \quad (3)$$

$$\begin{aligned} &= (C_{\text{graphene}} \times m_{\text{graphene}} \times \Delta T) / (I \times t) \\ &= [(C_{\text{graphene}} \times m_{\text{graphene}}) / (I)] \times (\Delta T/t) \end{aligned} \quad (4)$$

where Q_{output} is the converted thermal energy from laser energy; C_{graphene} is the specific heat capacity of graphene shell, assumed to be similar to that of graphite ($0.7 \text{ J} \cdot \text{g}^{-1} \cdot \text{K}^{-1}$);²⁵ m_{graphene} is the mass of graphene within overall irradiated zone; $\Delta T = T - T_0$, the temperature difference between temperature at time t and starting temperature, T_0 . Temperature at time t is obtained by averaging that of overall irradiated zone; Q_{input} is the laser energy absorbed by graphene; I is the incident laser power; η is the photothermal conversion efficiency.

For mass of graphene (m_{graphene})

Mass of graphene within the circle ring, m_{graphene} , can be estimated from the corresponding proportions of total shell mass. Assuming the section of front face to be an ellipse, as exemplified from the thermogram, its major and minor axis is measured to be 0.23 cm and 0.17 cm, respectively. The radius of irradiation zone is previously estimated to be 0.1 cm. The proportion of overall irradiation zone to area of front-half of graphene shell can be simply calculated as:

$$(\pi \times 0.1 \times 0.1) / (\pi \times 0.23 \times 0.14) = 31 \%$$

Thus, m_{graphene} can be estimated to be:

$$m_{\text{graphene}} = (1/2) \times (31 \%) \times m_{\text{total,shell}} = 1.12 \times 10^{-4} \text{ g}$$

For rate of temperature change ($\Delta T/t$):

Rate of temperature change, $\Delta T/t$, can be estimated from the dT/dt at the moment of triggering the laser (Figure 4.5C, 4.5D) since a linear temperature-time relationship is evident for $t < 1 \text{ s}$.

$$(\Delta T/t) = (dT/dt) = 200 \text{ }^\circ\text{C/s}$$

For photothermal conversion efficiency (η):

Using equation (4):

$$\begin{aligned} \eta &= [(C_{\text{graphene}} \times m_{\text{graphene}}) / (I)] \times (\Delta T / t) \\ &= [(0.7 \times 1.12 \times 10^{-4}) / (112)] \times 200 \approx 15 \% \end{aligned}$$

Figure 4.5. (A) Thermogram of graphene liquid marble at 1 s after switching on the laser at a power of 112 mW and (B) corresponding Gaussian-fitted spatial temperature distribution on the horizontal line across central irradiated zone. (C) Temperature-time profiles of overall irradiated zone (within the purple ring) during photothermal heating and subsequent cooling. (D) 1st derivative curve of (C), representing temperature changing rates (dT/dt) within the overall irradiated zone (interior of purple ring) for the heating/cooling processes. Temperature for (C) and (D) refer to the average temperature achieved within the overall irradiated zone.

Next, the cooling process of the GLM is studied by switching off the laser (Figure 4.6A). Similarly, the cooling process at the central irradiation zone occurs in two stages (Figure 4.6B, 4.6C); an initial rapid temperature decrease from about 90 °C to 46 °C within 1 s ($(dT/dt)_{\max} \approx -600 \text{ }^{\circ}\text{C.s}^{-1}$; Figure 4.6D), followed by a gradual temperature decrease of the entire graphene surface to the preheated state of 21 °C within 60 s ($(dT/dt)_{\max} \approx -0.5 \text{ }^{\circ}\text{C.s}^{-1}$; 4.6E). The longer duration of the cooling process to achieve thermal equilibrium ($\approx 30 \text{ s}$) is possibly due to a slower heat dissipation

from the graphene shell to air. Overall, the results highlight the instantaneous photothermal properties of GLM, and also its high thermal conductivity to achieve rapid thermal modulation.^{21, 26}

Figure 4.6. (A) Thermograms, (B) spatial temperature profiles and (C) temperature-time profiles of graphene liquid marble when the irradiation laser is switched off. 1st derivative temperature-time profiles of the cooling process representing temperature changing rates (dT/dt) of (D) central irradiated zone and (E) non-irradiated zone.

The heat conduction on GLM is further evidenced by its lateral-profile photothermal imaging (Figure 4.7), in which the temperature elevates progressively across the entire graphene shell. In a typical experimental set-up (Figure 4.7A), a 532 nm laser (laser power = 112 mW) is applied to irradiate our 40 μ L graphene liquid marble while a perpendicularly-placed infrared camera monitors

the spatially-resolved, side temperature profile of the liquid marble. Similar to the front profile imaging, we observe an initial instantaneous increase and subsequent plateauing of temperature in the laser irradiated region on graphene liquid marble (Figure 4.7B, 4.7C). Furthermore, a relatively slower heat transfer process is found to propagate from the central irradiated zone to the non-irradiated zone of lower temperature, achieved via conduction in the thermal-conductive graphene shell. Such heat transfer eventually spread across and increases the temperature of the entire graphene liquid marble. We also note that the heat transfer from the central irradiated zone generally prefers a pathway along the top of graphene liquid marble. This is possibly due to a relatively faster heat dissipation from the lower region of liquid marble to the underlying glass substrate, which has a higher thermal conductivity than air.²⁶ As the laser is switched off, a rapid drop in temperature of central irradiated zone is also observed (Figure 4.7D, 4.7E). The entire bulk of graphene liquid marble eventually cools to the pre-heated state of 21 °C via heat dissipation in the vicinity of underlying glass substrate first and subsequently from the top of liquid marble. Hence, the ability for the entire liquid marble to heat, via photothermal heating and subsequent thermal conduction, and later cooled to pre-heated state collectively demonstrate the importance of having an encapsulating layer of high thermal conductivity and intense photothermal properties. Hereafter, for the ease of discussion, only the maximum surface temperature (central irradiated zone) of GLM will be discussed.

Figure 4.7. (A) Schematic illustration of the side profile thermal imaging of graphene liquid marble irradiated by 532 nm laser. (B) Digital image (top) and thermogram (bottom) of 40 μL graphene liquid marble, without laser irradiation, in a typical photothermal experiment set-up. (C, E) Thermograms and (D, F) spatial temperature distribution profiles of graphene liquid marble when irradiation laser is switched on (heating) and subsequently switched off, respectively. The orange dotted lines denote the locations at which the temperature-distance profiles are obtained. Laser power = 112 mW.

The surface temperature of our GLM can also be modulated by the irradiation laser power. Equilibrium temperature (at $t = 60$ s) from 33 $^{\circ}\text{C}$ to 135 $^{\circ}\text{C}$ are achieved by tuning the laser power from 11 mW to 214 mW, respectively (Figure 4.8A). Similar Gaussian-like spatial temperature profiles and a two-stage heating process are observed (Figure 4.8A,B). The thermal energy generated

at the graphene shell can subsequently be conducted to heat the encapsulated water (to be discussed later). Upon turning off the laser, GLM rapidly cools to about 21 °C in 60 s (Figure 4.8C). Both heating and cooling processes demonstrate linear temperature responses to the applied laser power (Figure 4.8D), highlighting the tunable, wide temperature range on GLM at the instance of laser irradiation.

Figure 4.8. (A) Gaussian-fitted spatial temperature profiles of GLM at different laser power ($t = 60$ s). (B) Temperature–time profiles on GLM when the irradiation laser is switched (B) on and (C) off. (D) Temperature changes of GLM with laser power. (E) Heat/cool cycling test on GLM (laser power=112 mW). (F) Temperature changes as a function of graphene liquid marble volume, upon laser irradiation at 112 mW for 1 min. (B–F) indicate maximum surface temperature. All volumes are 40 μL for (A–E).

The rapid responsiveness and high reproducibility of photothermal heating are further illustrated by subjecting a 40 μ L GLM to heating/cooling cycles on laser irradiation (112 mW). We observe the precise cycling of GLM surface temperature between 21 $^{\circ}$ C and 90 $^{\circ}$ C over six successive cycles (Figure 4.8E). The extent of photothermal heating is also observed to be highly reproducible across different volumes of graphene liquid marble (Figure 4.8F). Using a laser power of 112 mW, graphene liquid marbles with volume ranging from 10 μ L to 60 μ L all exhibit consistent temperature increment of approximately 80 $^{\circ}$ C, attributed to the localization of photothermal heating. Hence, it is evident that our graphene liquid marble can potentially accommodate an extensive range of microliter-sized reaction volumes while enabling timely and precise thermal control essential for temperature-sensitive reactions.

GLM possesses more superior photothermal heating over liquid marbles formed using other materials. Upon laser irradiation (214 mW), GLM exhibits the highest surface temperature of about 135 $^{\circ}$ C (Figure 4.9), which is $\geq$ 2-fold higher than liquid marbles of other materials ($\leq$ 65 $^{\circ}$ C). We affirm the temperature rise originates from photothermal heating on the encapsulating materials, as evident from the negligible temperature response from water ($T = 23$ $^{\circ}$ C). The lower temperature increment of these controls is possibly due to weaker photoabsorption and photothermal efficiency at 532 nm.

Figure 4.9. Temperature–time profiles of liquid marbles prepared from various encapsulating materials (laser power= 214 mW). Maximum surface temperature is used for comparison.

Thus far, only the surface temperature of GLM is examined and the determination of actual encapsulated water temperature is also crucial for a latter application as remotely heatable miniature reactor. Here, the encapsulated water temperature of 40 mL GLM is quantified using the Raman fingerprint of water (Figure 4.10A). All spectra exhibit water bimodal OH stretching, with characteristic temperature-dependent Raman bands at 3250 cm^{-1} (I_1) and 3450 cm^{-1} (I_2 ; Figure 4.10B), assigned to strongly and weakly hydrogen-bonded water molecules,^{27, 28} respectively. Generally, elevated temperature disrupts the hydrogen bonding between water molecules and reduces the proportion of strongly hydrogen-bonded water molecules. To correlate the water temperature in our graphene liquid marble under irradiation, we construct a calibration graph that is achieved by thermally heating bulk water (in the absence of graphene) to a pre-defined temperature and measurement of their I_1/I_2 ratio. Our calibration graph (Figure 10C) yields a linear (I_1/I_2 ratio)-temperature relationship of $[I_1/I_2 = (-0.004 \times T) + 0.995]$, where T denotes the temperature of the water in °C. Our observation agrees well with the literature; the ratio of I_1/I_2 decreases from 0.84 to 0.68 as laser power increases from 11 mW to 112 mW (Figure 10D), respectively. Hence, the temperature of water encapsulated in GLM is determined to range between 35 °C to 74 °C (Figure

10D).

Figure 4.10. A) Illustration of the differences in interaction between water molecules during photothermal heating. B) Raman spectra of encapsulated water in GLM, at different irradiation laser powers. (C) Calibration curve relating the intensity ratio of two temperature-dependent Raman bands of water (I_1/I_2 ; I_1 : 3250 cm^{-1} , I_2 : 3450 cm^{-1}) with the corresponding temperatures. (D) I_1/I_2 ratio and corresponding temperature of encapsulated water in GLM, at different irradiation laser powers.

To determine the origin of changes in Raman spectra for laser-irradiated graphene liquid

marble, we perform similar Raman evaluation on non-photothermal Al_2O_3 -based liquid marble (Figure 4.11). Under a bright field microscope, Al_2O_3 grains closely packed on the water droplet surface to form the liquid marble (Figure 4.11A). Unlike the case of graphene liquid marble, the I_1/I_2 ratio of encapsulated water in Al_2O_3 -based liquid marble are observed to remain invariant with laser power which ranges from 11 mW to 112 mW (Figure 4.11B). Thus, we exclude the effect of laser-induced changes on the I_1/I_2 ratio of water; that is, the I_1/I_2 changes as observed in graphene liquid marble (Figure 4.10) is due to the rise of encapsulated water temperature resulting from photothermal heating. The negligible changes to I_1/I_2 is also consistent with our findings that there is no significant photothermal heating on Al_2O_3 liquid marble, as quantified by infrared camera (Figure 4.9). Hence, the photothermal heat generated on the thermally conductive graphene shell can clearly modulate the temperature of encapsulated water.

Figure 4.11. (A) Bright field image of Al_2O_3 liquid marble. (B) Raman spectra of water encapsulated in Al_2O_3 -liquid marble, measured under different laser power.

GLM as a remotely heatable miniature reactor is evaluated by studying the temperature-dependent degradation of methylene blue (Figure 4.12A). Typically, 40 mL GLM, containing 1 mM methylene blue and 0.4 M NaBH_4 , are irradiated using a laser power of 112 mW.

At predefined time intervals, we extracted the encapsulated aqueous solution and observed a steady decrease of the characteristic 665 nm extinction peak intensity of methylene blue (Figure 4.12B),¹¹ indicating its degradation over laser irradiation time. Using an extinction-concentration calibration curve (Figure 4.12C, 4.12D), a degradation efficiency of > 70 % is achieved ($C/C_0 = 0.3$; C_0 and C denote the initial concentration and concentration at time t , respectively) after 10 min of photothermal heating (Figures 4.12E). In contrast, only ≤ 10 % of the degradation efficiency is achieved in the absence of photothermal heating or NaBH_4 , probably due to contributions from photobleaching, heat-induced molecular transformation, and/or adsorption of methylene blue (Figures 4.12E, 4.12G). The methylene blue reduction by NaBH_4 is assumed to be a pseudo-first-order reaction; $\ln(C / C_0) = -k_{\text{app}} \times t$, in which k_{app} and t denote the degradation rate constant and time (Figure 4.12F), respectively.¹¹ Consequently, k_{app} is calculated at 0.01 min^{-1} and 0.12 min^{-1} for a laser power of 0 mW and 112 mW (Figure 4.12F), respectively. Hence, remotely heatable GLM provide tremendous enhancement in both the degradation efficiency (> 7-fold), and reaction kinetics (> 12-fold) of the methylene blue degradation reaction compared to the absence of photothermal heating. The temperature-dependent reaction kinetics of methylene blue degradation can also be modulated by the laser power. By tuning the laser power to 44 mW and 82 mW, k_{app} is easily modulated to 0.03 min^{-1} and 0.06 min^{-1} (Figure 4.12F), respectively. Such dependency of reaction kinetics on the temperature clearly follows the Arrhenius equation (equation 5),¹⁷ as demonstrated in the linear relationship for the plot of $(\ln K)$ against $(1 / T)$ (Figure 4.12H).

$$\text{Arrhenius equation: } \ln K = \frac{E_a}{R} \frac{1}{T} + \ln A \quad (5)$$

where K is rate constant; E_a is activation energy; R is gas constant; T is temperature of the encapsulated water in kelvin; A is the pre-exponential factor.

Figure 4.12. (A) Schematic representation of GLM as remotely heatable miniature reactor. (B) Extinction spectra of methylene blue solution extracted from GLM at different time intervals (laser power = 112 mW). (C) UV-vis spectra of aqueous methylene blue solution at various pre-defined concentrations and (D) the corresponding calibration curve relating absorbance, at characteristic 665 nm, with the concentrations. (E) Plot of normalized methylene blue concentrations (C/C_0) as a function of irradiation duration for different laser power applied. (F) Plot of $-\ln(C/C_0)$ against irradiation duration for different laser power applied. (G) UV-vis spectra of 40 μL aqueous methylene blue solution, in the absence of NaBH_4 , extracted from graphene liquid marble before (black) and after (red) laser irradiation for 10 min. Laser power applied at 112 mW. (H) Plot of $\ln K$ against $(1/T)$ for the rate constant (K) obtained at different encapsulated water temperature (T).

To investigate the potential effect of water evaporation on the determination of 1st order rate constant, we examine the drying process of graphene liquid marble on laser irradiation. The power of laser is selected at 112 mW, which is in accordance to the power used for methylene blue degradation. We observe that the graphene liquid marble require at least 12 min of laser irradiation

for the encapsulated water volume to be halved (Figure 4.13A, 4.13B), which is ~ 4 -fold faster compared to the absence of laser irradiation. The graphene liquid marble also remained intact and does not rupture throughout the irradiation duration, emphasizing its stability even on photothermal heating. The accelerated evaporation of water is due to the increase of its temperature upon photothermal heating on the graphene shell.

Figure 4.13. (A) Drying of a 40 μL graphene liquid marble under atmospheric condition. (B) Thermograms recorded at 1 min, 4 min, 8 min, 12 min and 16 min of laser irradiation during the thermal-accelerated drying process of 40 μL graphene liquid marble. Laser power = 112 mW. (C) Drying of a 40 μL graphene liquid marble when heated at 90 $^{\circ}\text{C}$ using a hot-plate.

Notably, our graphene liquid marble is expected to be minimally affected by the accelerated water evaporation since a methylene blue degradation experiment under laser irradiation typical last for 10 min. This hypothesis is further supported by the close-fitting of the experimental observations to the linear co-relation line (Figure 4.12F), whereby the degradation rate constant can be determined from its slope. We also note that the experimental data at $t = 10$ min experiences a slight positive deviation from the linear co-relation line, possibly due to a relatively greater extent of water

evaporation which consequently increases methylene blue concentration. Such deviation is generally reduced or unobservable at lower irradiation power of ≤ 82 mW, due to the slower water evaporation rate over the course of analysis. Nevertheless, the well-defined linear co-relationship between $-\ln(C/C_0)$ and time enables representative degradation rate constants to be derived at various laser power applied, and also demonstrate the ability of our graphene liquid marble to modulate reaction kinetic simply by controlling the extent of photothermal heating.

To exemplify the advantage of localized photothermal heating over conventional hot-plate bulk heating, we monitor the structural changes to 40 μ L graphene liquid marbles upon laser irradiation and when placed on a hot-plate. Both the localized photothermal heating and hot-plate heating are set at a surface temperature of ~ 90 °C for fair comparison. The hot-plate heating of graphene liquid marble leads to a completely dried state within just 3 min (Figure 4.13C). Hence, localized photothermal heating exhibit at least 5-fold reduced evaporation rate compared to conventional hot-plate bulk heating. Such a thermal modulation of reaction kinetics by localized heating therefore makes GLM the first demonstration of a remotely heatable and isolated miniature reactor, with > 5 -fold prolonged lifetime compared to conventional bulk heating (Figure 4.13C). This is especially important for small-volume reaction or miniature reactor.

The importance of GLM as a confined microreactor/heater hybrid is again highlighted by comparing control platforms comprising of bare reaction solution droplets and graphene solution mixtures (Figure 4.14). For bare droplet (Figure 4.14A), the 665 nm peak intensity, and thus the concentration of methylene blue, remain rather constant even under laser irradiation (power = 112 mW) over an extended duration of 10 min. Hence, a poor methylene blue degradation efficiency ($\eta \sim 26$ %; $C / C_0 = 0.74$) and slow rate constant (0.04 min^{-1}) are achieved (Figure 4.14C, 4.14D). This is mainly attributed to the absence of graphene encapsulating layer, which therefore do not allow the

conversion of light energy into thermal energy that is required to accelerate the reduction of methylene blue by NaBH_4 . In addition, this control experiment also supplement our previous observation that photo-bleaching contributes minimally to the methylene blue degradation (Figure 4.12G), even on direct exposure of reaction mixture to irradiation laser. The extent of photo-bleaching is expected to be further reduced especially when the graphene shell of liquid marble is present to prevent/minimize direct laser exposure to the encapsulated aqueous solution.

Figure 4.14. UV-vis spectra of aqueous methylene blue solution extracted from (A) bare aqueous droplet and (B) graphene-solution mixture at different time interval, upon irradiation with laser at a power of 112 mW. Graphene-solution mixture is obtained by rupturing and subsequent gentle stirring of graphene liquid marble. (C) Plot of normalized methylene blue concentrations (C/C_0) as a function of irradiation duration for different types of miniature reactor. (D) Plot of $-\ln(C/C_0)$ against irradiation duration for different types of miniature reactor. All volumes are 40 μL .

For the graphene-solution mixture (Figure 4.14B), perfluorooctylsilane-functionalized graphene are mainly suspended on the surface of water droplet due to their hydrophobicity. Upon laser irradiation, the methylene blue concentration is observed to decrease readily, giving a lower degradation efficiency ($\eta \sim 50\%$; $C/C_0 = 0.50$) and slow rate constant (0.07 min^{-1} ; Figure 4.14C, 4.14D). Hence, such graphene-solution mixture generally exhibits ~ 1.7 -fold lower degradation rate constant than graphene liquid marble (Figure 4.14D). We attribute the lower efficiency of such suspension system, despite the presence of photothermal heating, to (1) the inefficient heat transfer throughout the entire liquid marble due to shell discontinuity, and also (2) greater rate of heat dissipation resulting from increased exposure area and direct contact of reaction mixture with underlying glass substrate.

4.3 Conclusions

In summary, graphene liquid marbles have been fabricated as robust, isolated, and remotely heatable miniature reactors for the enhancement of methylene blue reaction kinetics by more than twelve times, achieving $> 70\%$ degradation efficiency. GLM also demonstrates rapid, reproducible, and strong photothermal heating to achieve encapsulated water temperatures ranging between $21\text{ }^{\circ}\text{C}$ to $74\text{ }^{\circ}\text{C}$, simply by varying the laser power. The ensemble of benefits enables GLM as an attractive miniature reactor for a vast library of reactions, including heat-activated processes and reaction kinetics modulation. This is crucial in fields involving costly and hazardous conditions, in which small-scale preliminary reactions are preferred. The inhibition of encapsulated water evaporation and studies of thermal flow within GLM are currently under investigation.

4.4 Materials and methods

Materials. Graphene (average thickness ~ 12 nm) and MoS₂ ($\geq 99\%$, particle size: ~ 90 nm) were purchased from Graphene Supermarket; silver nitrate ($\geq 99\%$), anhydrous 1,5-pentanediol ($\geq 97\%$), poly(vinylpyrrolidone) (average MW = 55,000), 1H,1H,2H,2H-perfluorooctyltriethoxysilane ($\geq 97\%$), 1H,1H,2H,2H-perfluorodecanethiol ($\geq 97\%$), Al₂O₃ (CG-20) and spiropyran (98 %) were from Sigma Aldrich; dimethylformamide (HPLC grade) was obtained from Fisher Scientific; copper (II) chloride ($\geq 98\%$) was from Alfa Aesar; ethanol (ACS, ISO, Reag. Ph Eur) was from EMSURE®. All chemicals were used 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.

Functionalization of graphene and fabrication of graphene liquid marble.

Perfluorooctylsilane-grafted graphene nanoplatelets were obtained by dispersing 100 mg of graphene nanoplatelets in 25 mM of perfluorooctylsilane in DMF solution. The mixture was ultrasonicated for several hours and then stirred overnight. After that, it was washed with copious amount of ethanol to remove the excess perfluorooctylsilane. The perfluorooctylsilane-grafted graphene were collected by centrifugation, dried overnight in the oven at 60 °C and subsequently pulverized to obtain fine powders. Graphene liquid marbles were then formed by rolling water droplets, ranging from 5 μL to 80 μL , on a bed of pulverized perfluorooctylsilane-grafted graphene.

Synthesis and purification of Ag nanocubes. The preparation of Ag nanocubes was carried out following the method described in literature.²⁹ Briefly, 10 mL of copper (II) chloride (8 mg/mL), poly(vinylpyrrolidone) (20 mg/mL) and silver nitrate (20 mg/mL) were separately dissolved in 1,5-pentanediol. The chemicals were sonicated and vortexed repeatedly to dissolve them. 35 μL copper (II) chloride solution was then added to the silver nitrate solution. Then, 20 mL 1,5-pentanediol in a 100 mL round bottomed flask was heated to 190 °C for 10 min. 250 μL

poly(vinylpyrrolidone) precursor was added to flask dropwise every 30 s while 500 μ L silver nitrate precursor was injected every min using a quick addition. The addition process continued until the greenish coloration of the reaction mixture faded off.

For the purification of Ag nanocubes, 1,5-pentanediol was first removed from the mixture through centrifugation. The Ag nanocubes solution was then dispersed in 10 mL ethanol and 100 mL aqueous poly(vinylpyrrolidone) solution (0.2 g/L). The resulting solution was vacuum filtered using polyvinylidene fluoride filter membranes (Sigma Alrich) with pore sizes ranging from 5000 nm, 650 nm, 450 nm and 220 nm, repeated several times for each pore size. SEM imaging was performed, from which the edge length of 100 Ag nanocubes were measured and analyzed using ImageJ software.

Functionalization and fabrication of liquid marble controls using various materials.

MoS₂ and Al₂O₃ solid particles were functionalized using the same protocol as graphene. Ag nanocubes were functionalized by immersion in an ethanolic solution of perfluorodecanethiol (5 mM) for at least 15 hr. Spiropyran was used directly without additional modification. Liquid marbles of various materials were then formed by rolling water droplets on their respective hydrophobic-grafted solids.

Photothermal tests on graphene liquid marble. 40 μ L graphene liquid marble was formed, immediately transferred to a stage and irradiated with a 532 nm laser which was directed at the center of the liquid marble. For front-profile thermal imaging, the infrared camera was placed adjacent to the laser and directed to the front of graphene liquid marble. For side profile thermal imaging, the infrared camera was placed at 90° and directed to the side of graphene liquid marble. Thermograms of graphene liquid marbles were then recorded (typically 60 s) during both the photothermal heating and subsequent cooling as laser was switched on and off, respectively. Cycle

test was performed at a heating-cooling interval of 10 s; 5 s for heating and 5 s for cooling. The laser powers for investigation were selected at 11 mW, 44 mW, 82 mW, 112 mW, and 214 mW.

Using Raman scattering spectroscopy to determine the actual water temperature inside graphene liquid marble. 40 μL graphene liquid marbles were formed using milli-Q water. A 532 nm laser (laser power = 112 mW) was applied as both the Raman excitation and heating source simultaneously. The laser powers investigated were selected at 11 mW, 44 mW, 82 mW, and 112 mW, respectively, which were in accordance with those applied for photothermal tests. All Raman spectra are normalized over the Raman intensity at 3450 cm^{-1} .

Graphene liquid marble as remote-heatable miniature reactor for modulation of methylene blue degradation kinetics. 40 μL of water droplets, containing 0.4 M NaBH_4 and 1 mM methylene blue, were rolled on perfluorooctylsilane-functionalized graphene to form graphene liquid marble. The graphene liquid marbles were then immediately irradiated with the laser (laser power = 112 mW). At pre-defined duration of 0 min, 3 min, 6 min and 10 min, the encapsulated aqueous methylene blue solutions were extracted out, diluted 20-fold using water and subsequently quantified with UV-vis spectroscopy. The extinction peak at 665 nm was selected for the determination of methylene blue concentrations for reaction kinetic study. For the intensity-concentration calibration curve, the methylene blue concentrations were systematically varied between 0 mM to 0.1 mM and co-related to their respective 665 nm peak intensity. The concentration of methylene blue at selected time intervals were then derived from the calibration plot. Laser powers investigated were selected at 44 mW, 82 mW, and 112 mW, respectively. The dye degradation ability of both bare aqueous methylene blue solution droplet (40 μL) and a suspension of methylene blue and perfluorooctylsilane-functionalized graphene, formed by rupturing a 40 μL graphene liquid

marble, were also investigated for comparison.

Characterization. SEM imaging was performed using a JEOL-JSM-7600F microscope. Static contact angle, rolling, and drying of graphene liquid marble were captured on a Theta Lite tensiometer equipped with a Firewire digital camera. Bright-field images were captured on an Olympus BX51BD microscope. FLIR SC7000 IR thermal imaging camera was used to capture the thermograms of graphene liquid marble. Laser used for irradiating graphene liquid marble has an excitation wavelength of 532 nm and its power was tuned by using ND filters. Raman measurements were performed using the point mode of the Ramantouch microspectrometer (Nanophoton Inc., Osaka, Japan) with an excitation wavelength of 532 nm (power was tuned to be 11 mW, 44 mW, 82 mW and 112 mW). A 20× objective len (N. A. 0.45) with 20 s accumulation time was used for data collection between 2440 cm⁻¹ and 4420 cm⁻¹. UV-vis spectroscopy measurements were carried out using Carry-60 UV-vis spectrometer.

Authors contributions. W.G., H.K.L. and X.Y.L. designed research. W.G., H.K.L., and I.Y.P. performed research. W.G., H.K.L. and X.Y.L. analyzed the results and wrote the manuscript. All authors discussed the project. T.L., I.Y.P. and X.Y.L. supervised the project.

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Chapter 5

Dynamic rotating liquid marble for directional and enhanced mass transportation in three-dimensional microliter droplets**

Abstract. The ability of an artificial microdroplet to mimic the rotational behavior of living systems is crucial for dynamic mass transportation but remains challenging to date. Herein, we report dynamic microdroplet rotation using a liquid marble (RLM) and achieve precise control over mass transportation and distribution in a three-dimensional (3D) microdroplet. RLM rotates synchronously with an external magnetic field, creating circular hydrodynamic flow and an outward centrifugal force. Such spin-induced phenomena direct a spiral movement of entrapped molecules and accelerate their diffusion and homogenization in the entire liquid. Moreover, we demonstrate the rotation rate-controlled (between 0 rpm and 1300 rpm) modulation of shell-catalyzed reaction kinetics from 0.13 min^{-1} to 0.62 min^{-1} . The directed acceleration of reactants toward a catalytically active shell surface is 3-fold faster than conventional stir bar-based convective flow. RLM as an efficient magnetohydrodynamics transducer will be valuable for dynamical control over mass transportation in microdroplet-based chemical, biological, and biomedical studies.

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5.1 Introduction

Rotation is a ubiquitous natural phenomenon that remains challenging to mimic in artificial microscale systems. Biological systems in particular actively utilize rotation to achieve three-dimensional (3D) cellular organization and morphogenesis that is otherwise unachievable by spatial cellular migration.^{2,3} This is evident in the collective rotational locomotion of epithelial cells in both humans and a *Drosophila* egg chamber.^{4, 5} For example, healthy Michigan Cancer Foundation (MCF)-10A mammary cells rotate to assemble laminins and collagen onto their functional surfaces.⁴ In chemistry, consistent rotation of a liquid medium is necessary to homogenize the reactant distribution, to direct mass transportation, and to modulate reaction kinetics.⁶⁻⁸ In this aspect, artificial droplet-based systems are appealing as stimuli-responsive microdevices to attain remote droplet taxis and on-demand modulation of droplet composition via fusion and fission.⁹⁻¹¹ However, current droplet-based studies predominantly involve microfluidic systems and are limited by sophisticated fabrication/actuation protocols to achieve control over droplet composition.¹² Furthermore, these naked droplets lack active functional surfaces which greatly restricts their potential applications such as in core-shell heterogeneous catalysis.

Liquid marbles are emerging platforms for facile droplet manipulation and are also capable of overcoming the aforementioned limitations of microfluidic approach.^{13, 14} Formed via the simple entrapment of micro/nanosized particles onto liquid surfaces, liquid marbles (LMs) can effectively isolate a microdroplet within a potentially functional encapsulating shell.¹⁵⁻¹⁸ More importantly, LMs incorporated with magnetic particles enable magnetic field-induced spatiotemporal actuation and on-demand release of encapsulated microdroplets.¹⁹⁻²¹ However, current studies have not demonstrated the ability to achieve dynamic rotation on these LMs, with most works limited to linear droplet translation, which narrows their application to mass delivery and release.²²⁻²⁴

Achieving rotational motion will transform these LMs into platforms capable of transducing mechanical energy input into hydrodynamic forces for guided advection of encapsulated contents toward the functional solid shell, analogous to the rotational function for morphogenesis in living systems.⁵ Such guided advection can boost the efficiency of small-volume reactions, providing valuable insight on process optimization and targeted mass transportation crucial in the fields of nanotechnology, synthetic chemistry, and bioinspired technologies.^{17, 25-30}

Herein, we demonstrate the dynamic rotation of LMs and utilize this rotation for active manipulation of the transportation of molecular/particle cargo within the rotating liquid marble (RLM). Fabricated using magnetic Fe₃O₄ nanoparticles, we demonstrate that RLM and its enclosed aqueous droplet can rotate synchronously along its center of mass in the presence of an external magnetic field. Such mechanical rotation effectively transduces magnetic energy input to create hydrodynamic flow and centrifugal forces within the RLM. We harness these rotational dynamics to gain precise spatiotemporal control over the interior fluid composition of the RLM, enhancing microscale reaction homogeneity and achieving effective particle separation. More importantly, by using the rotational dynamics to accelerate molecular transportation toward the active shell surface, we demonstrate the ability to linearly and quantitatively modulate the kinetics of a cascade chemical reaction involving shell-catalyzed decomposition of H₂O₂ to generate ·OH radical for the oxidative degradation of malachite green (MG) toxin. Such a reaction-diffusion process is further highlighted to overcome the conventional reaction limit imposed by typical vortex flow based on commercial micro-stirbars.

5.2 Results and discussion

The collective magnetotaxis of magnetic nanoparticles on the shell endows LMs with a superior rotating capability. We form the RLM by rolling a microdroplet of water on a bed of hydrophobic Fe_3O_4 nanoparticles (diameter ~ 12 nm; Figure 5.1A, 5.1B). The magnetic nanoparticles are spontaneously trapped at the air/water interface to isolate the droplet for the minimization of the overall system's free energy.^{19, 31} Such uniform coverage of nanoparticles on a water droplet surface is also crucial to impart superior mechanical robustness to LMs, as compared to those created using micro-sized solids.³² The as-prepared RLM is nonwetting and can float on a water surface (Figure 5.2A).³¹ It can also be maneuvered by a permanent magnet. In the presence of a rotating magnetic field, the RLM rotates along its mass center axis (Figure 5.2A). By tracking the white Al_2O_3 tracer deposited atop the black RLM using a high-speed camera, we observe precise and linear modulation of the RLM rotational rate over a wide range of 100 rpm to 1300 rpm (Figure 5.2B). The rotation is synchronous with the external rotating field, with only $< 7\%$ deviation ($\Delta\omega/\omega$) from the applied rotational rates (Figure 5.2C). Furthermore, the rotation of the RLM is reproducible and can be sustained for at least 30 min while preserving its structural integrity, whereby Fe_3O_4 particles remain largely attached to the droplet surface.

Figure 5.1. (A) TEM image of as-synthesized Fe₃O₄ nanoparticles with average size of (12 ± 2) nm.

(B) Digital images of a 12 μ L water droplet placed on hydrophobic Fe₃O₄ nanoparticles powder bed and the formed LM after rolling the water droplet.

Figure 5.2. (A) Schematic illustration of an aqueous microdroplet encapsulated within a hydrophobic Fe₃O₄ nanoparticle shell (left) to form a magnetically-actuated rotating liquid marble (RLM) on a water pool surface (middle). Such rotation creates a rotational hydrodynamic flow and centrifugal force for directed particle/molecular transportation within the microdroplet. The blue arrow indicates the centrifugal force created by RLM. (B) Linear correlation of a 12 μ L LM spinning dynamics (ω) with the applied rotating magnetic field conveyed by a commercial stirrer. Inset is a

digital image of a 12 μL liquid marble rotating on water surface. White alumina powder is used to track its motion. (C) Plot depicting the relative deviation of LM's rotational speeds from the actual rotational rate of externally applied magnetic field for an experimental duration of 30 mins.

To verify the actual rotation of the encapsulated microdroplet under an applied rotating magnetic field, we deposit a 12 μL aqueous droplet on the hydrophobic Fe_3O_4 powder bed to form a partial magnetic shell at the bottom of the liquid. Such partial encapsulation is crucial for clear visual examination of interior microdroplet motion. Under an applied rotating magnetic field (300 rpm), the adhered Fe_3O_4 nanoparticles are collectively actuated to drive rotation of the droplet. This is evident from the repetitive spinning motion of a hydrophobic and non-magnetically active Sudan I powder tracer (orange) along the periphery of the microdroplet surface (Figure 5.3A, 5.3B). The observation is also in accordance with previous reports.³³ Because the Sudan I tracer is suspended on the top hemisphere of the exposed microdroplet and in the absence of a magnetic shell, we therefore evidently demonstrate that the rotation of the magnetic shell drives the spinning of the enclosed droplet rather than the mere gliding of the Fe_3O_4 building blocks across the surface of a stationary microdroplet. The liquid rotation is important to create an interior hydrodynamic flow to facilitate microdroplet homogenization and also direct mass transportation of encapsulated contents.

Figure 5.3. (A) Schematic illustration and (B) digital images of the magnetically-actuated rotation of a 12 μL water droplet deposited on a bed of hydrophobic Fe_3O_4 nanoparticles powder. Red Sudan I powders are suspended on the microdroplet surface to track its motion. (C) Scheme depicting the experimental setup to investigate the mass transport mechanism of dye molecules in a rotating water pool (100 μL). The controlled rotation of water is achieved by attaching the liquid containment atop of stirbar, where external magnetic field is subsequently applied. (D) Digital images demonstrating the mass flow patterns of aqueous methylene blue solution when the dye is deposited at (i) the center and (ii) edge of the liquid media, respectively. All scale bars, 1 mm. (E) Schematic of (i) outward and (ii) inward spiral mass transportation trajectories dominated by rotational hydrodynamic flow, centrifugal force, and also molecular diffusion arising from the concentration gradient.

We subsequently evaluate the mass transport dynamics of encapsulated molecules to elucidate critical information on the hydrodynamic flow and potential centrifugal force in a rotating liquid system. Noting the intrinsic difficulties in the accurate tracking of molecular flow in a

spherical microdroplet, we reenact the rotation of the LM at a larger volume to investigate the mass transfer dynamics unique to a rotating liquid medium (Figure 5.3C). This is achieved experimentally via visual monitoring of the motion of concentrated methylene blue solutions (1 μ L, 10 mM) when added to a 100 μ L rotating aqueous medium attached atop a magnetic stirbar. When the dye solution is deposited at the center of an aqueous medium rotating at 300 rpm, the resultant flow pattern exhibits a spiral shape that extends to the peripheral of the solution within just 2 s (Figure 5.3D-i). Subsequently, the dye solution is mainly confined adjacent to the liquid containment and eventually distributes across the entire volume in ≤ 10 s. When a dye solution is placed at the edge of the rotating liquid instead, a similar spiral flow pattern is also clearly demonstrated (Figure 5.3D-ii). We also observe the mass transportation to be much slower where the spiral patterns largely situate near the edge of the liquid for a longer period of time (60 s) and gradually spread over the entire volume after only 100 s. Moreover, the dye solutions for both cases exhibit slow and passive molecular diffusion under static conditions, which could not spread uniformly across the entire liquid (Figure 5.4), even after an extended period of 120 s. These controls therefore affirm the origin of the spiral molecular flow as a consequence of the liquid rotation.

Figure 5.4. Slow and non-uniform diffusion of aqueous methylene blue solution in static water media. The dye solutions are deposited at the center (top) and edge (bottom) of liquid media.

The differences in mass transport phenomena for the two scenarios (Figure 5.3D) evidently highlight the presences of three major contributions arising from spin-induced hydrodynamic flow and centrifugal force and also intrinsic molecular diffusion (Figure 5.3E). First, there is a circular hydrodynamic flow across the entire 3D microdroplet owing to the rotational behavior of the enclosed droplet. This is also supported experimentally from the observation of curved flow patterns (Figure 5.3D). Second, the circular hydrodynamic flow in turn imparts a centrifugal force on the microdroplet, which always drives particles/molecules in an outward direction, as affirmed by the sedimentation of nanoparticles at high RLM rotational speed (Figure 5.5) and also in our previous report.³¹ The centrifugal force is proportional to the mass of the object (m) and square of the rotation speed (ω^2), as well as the distance of the object to the rotating axis. Because the masses of our dye molecules are non-zero, the methylene blue molecules are expected to be influenced by the centrifugal forces, whereby they are actuated from the center to the peripheral of the entire 3D droplet. Notably, both the circular dynamic flow and centrifugal forces are intrinsic to the rotating entity and are independent of the concentration of the encapsulated particles/molecules. Third, molecular diffusion arising from a concentration gradient could also cause molecules to migrate from regions of high molecular concentration to low concentration, regardless of the spin-induced forces. The distinct flow patterns from the two contrasting cases therefore result from the differences in the overall interaction between these three factors. For instance, concentrated dye solution added to the center of the rotating liquid experiences a large outward-driving force from the cumulative effect from both the centrifugal force and molecular diffusion. When coupled with the radial hydrodynamic flow, a spiral flow is observed that rapidly travels toward the edge of the aqueous medium. On the contrary, dye molecules first deposited at the edge of the aqueous solution experience an opposing inward-flowing molecular diffusion and outward flowing centrifugal force.

This correspondingly leads to a spiral flow pattern that travels relatively slower toward the interior and, hence, requires longer duration for microdroplet homogenization. Nevertheless, these results jointly demonstrate the directed mass transportation and rapid homogenization enabled by rotation of the liquid. Hence, the RLM is clearly an efficient magnetohydrodynamic transducer, functioning as a microvessel for the dynamic spatiotemporal advection of mass in three-dimensional space. Our microdroplet-based platform is analogous to the *Drosophila* flies' egg chamber,⁵ whereby constituting epithelial cells actuate collectively to rotate the entire organ-like structure for spatial positioning of its actin bundles on the surface during morphogenesis.

Figure 5.5. Digital image of the top-view of an exposed Ag nanocube-containing microdroplet inside a static liquid marble (SLM; left) and a rotating liquid marble (RLM; right) after spinning at 1300 rpm for 5 mins. Scale bars, 1 mm.

Our precise control over the hydrodynamic flow and the consequential centrifugal force in a RLM renders it an attractive platform to achieve enhanced molecular transfer for improved reaction kinetics and homogeneity. We demonstrate this capability using Au-catalyzed reduction of blue resazurin to pink resorufin using NaBH_4 .³⁴ Au nanoparticles (3 nm; localized surface plasmon resonance at 510 nm; Figure 5.6) and NaBH_4 are sequentially added to an exposed Fe_3O_4 -stabilized resazurin droplet, followed by rotating the RLM at 300 rpm for 2 min (Figure 5.7A, 5.7B).

Quantitative analysis on the representative images at different reaction stages, via their transformation into a RGB color histogram (area encompassing > 1800 pixels), reveals the initial dominance of reactant resazurin represented by the blue pixels (Figure 5.7C).³⁵ The original blue resazurin droplet subsequently transforms entirely into the characteristic pink coloration of resorufin over the entire droplet after rotation. On the contrary, the reaction droplet kept under static conditions exhibits an inhomogeneous spatial distribution of red and blue colorations that contribute approximately equally to the RGB evaluation of the microdroplet, indicating the incompleteness of the reaction. Absorption measurements further affirm ~ 100 % reduction of the resazurin extinction peak (600 nm; Figure 5.7D) within 2 min in a RLM rotating at 300 rpm and a < 50 % conversion in a static reaction droplet, possibly due to slow and random molecular diffusion (Figure 5.8). The results clearly demonstrate the importance of the RLM's dynamic rotation to drive circular hydrodynamic flow in the microreactor for overcoming the intrinsically slow and random molecular diffusion in a 3D droplet. This is crucial to achieve enhanced micromixing to yield a homogeneous reaction environment for representative reaction investigations.

Figure 5.6. The extinction spectra of as-synthesized Au nanoparticles colloidal solution. The peak at 510 nm is assigned to its localized surface plasmon resonance.¹⁶ The inset digital image depicts the as-synthesized Au nanoparticles colloidal solution.

Figure 5.7. (A) Reaction scheme depicting Au nanoparticle-catalyzed reduction of blue resazurin to pink resorufin using NaBH_4 . (B) Digital image of adding 1 μL Au nanoparticles and 1 μL NaBH_4 into an opened 10 μL resazurin LM using a pipette. The shell opening is manipulated by the permanent magnet. Scale bar, 1 mm. (C) (i) Side view photographic images and (ii) histograms displaying the 2D spatial RGB analysis on the reacting microdroplet encapsulated within the initially-formed RLM (left, “before”), a RLM after remaining static for 2 min (middle, “static”), and a RLM after rotation at 300 rpm for 2 min (right, “after rotation”). The white solid frames in (ii) indicate the selected area for RGB analysis. All scale bars, 1 mm. (D) Extinction spectra of reaction microdroplet encapsulated in RLM under the three respective conditions in (C).

Figure 5.8. Extinction spectra of the reaction droplet at different reaction times. The insets show digital images of the reaction progress in the reaction droplet at different reaction stages, all in the absence of Fe_3O_4 nanoparticles.

We further combine the dynamic mass transportation capability of a RLM with its functional shell to perform an enhanced heterogeneous shell-catalyzed reaction. We choose a cascade chemical reaction involving Fe_3O_4 shell-catalyzed decomposition of H_2O_2 to generate $\cdot\text{OH}$ radical, followed by subsequent oxidative degradation of the environmental toxin MG (Figure 5.9A).³⁶ We first form a RLM using premixed aqueous MG and H_2O_2 , followed by spinning at 500 rpm. At predefined timings, we extract the entire microdroplet and use absorption measurements to quantify the non-degraded MG using an extinction calibration curve (Figure 5.9B, 5.9C). MG's absorption at 616 nm decreases over the course of our temporal studies, signifying its progressive degradation (Figure 5.9D). The RLM achieves a degradation efficiency of $\sim 90\%$ (Figure 5.9E) after spinning at 500 rpm for 8 min ($C/C_0 = 0.1$; C_0 and C denote the concentration of MG at 0 min and t min, respectively). In contrast, a static LM and naked MG reaction droplet can only achieve much lower degradation efficiencies of 66 % and 3 %, respectively (Figure 5.10A - C).

Figure 5.9. (A) Shell-catalyzed oxidative degradation of malachite green (MG) in RLM. The pink arrow indicates the accelerated and outward transportation of MG by the circular hydrodynamic flow and centrifugal force inside RLM. (B) Extinction spectra of various pre-defined malachite green concentrations and (C) their corresponding calibration curve relating absorbance (616 nm) with the concentration of malachite green. (D) Time-dependent absorption spectra of MG solution extracted from 12 μL RLM rotating at 500 rpm. (E) Time-dependent normalized MG concentrations (C/C_0) for static LM (0 rpm) and RLM rotating between 100 rpm to 1300 rpm. (F) Time-dependent plots of $-\ln(C/C_0)$ for RLMs at various rotating speeds. Eye-guided solid lines show the dependence of MG degradation efficiency on rotation speed. Arrow indicates the direction of increasing rotational speed. (G) A linear relationship is observed between k_{app} (apparent rate constants) and RLM's rotational rate.

Figure 5.10. Extinction spectra of malachite green solution extracted from different reaction assays at various reaction times from (A) static liquid marble and (B) static naked droplet. The inset image depicts the control microplatforms. (C) Plots of normalized malachite green concentrations (C/C_0) as a function of reaction time in static liquid marble and static reaction droplet.

Using the reaction efficiency of the naked droplet as a benchmark, we deduce that the catalytic nature of Fe_3O_4 nanoparticles accounts for $\sim 63\%$ enhanced degradation efficiency. More importantly, the RLM's dynamic mass transportation capability at 500 rpm boosts the MG degradation efficiency by an additional $\sim 24\%$ as compared to the static LM (Figure 5.10). In addition to the enhanced degradation efficiency enabled by our RLM, the kinetics of MG degradation can also be precisely modulated by simply tuning RLM's speed between 0 rpm and 1300 rpm (Figure 5.9E - G). As demonstrated by the static LM (0 rpm), the base degradation efficiency is 66 % due to the passive transport of reactants toward the catalytic shell. Increasing the rotation speed to 700 rpm leads to a linear increase in degradation efficiency by $\sim 30\%$, reaching an overall degradation efficiency of $\sim 95\%$ within 8 min (Figure 5.9E; Table 5.1). Further increasing the rotational rate to 1300 rpm drives the reaction efficiency to $\sim 100\%$ in just 5 min. Because the degradation of MG follows a pseudo-first-order reaction (Figure 5.9F), we approximate its kinetics using the integrated rate law, $\ln(C/C_0) = -k_{\text{app}}t$, where k_{app} and t denote the degradation rate constant and reaction time, respectively.³⁷ We observe a linear relationship between the RLM's speed and k_{app} ,

confirming the pseudo-first-order kinetics (Figure 5.9G). k_{app} increases by ~ 5 -fold from 0.13 min^{-1} to 0.62 min^{-1} as the RLM's speed increases from 0 rpm to 1300 rpm, respectively, directly demonstrating the ability of the RLM to modulate the kinetics of MG degradation. More importantly, the molecular transfer coefficient of MG molecules from the bulk microdroplet to the RLM's catalytic shell spinning at 1300 rpm is $\sim 4 \text{ }\mu\text{m/s}$ (refer to Calculation 5.1). This transfer rate is ~ 2 -fold higher than that of kinesin-based biomolecular motors ($1 \text{ }\mu\text{m/s}$ to $2 \text{ }\mu\text{m/s}$), which are widely known for their rapid mobilization of vital molecules to targeted cellular sites.³⁸ It is also worth noting that as a microplatform, the RLM also demonstrates 2-fold enhanced degradation kinetics relative to a reaction microdroplet suspended with an identical mass of Fe_3O_4 nanoparticles (Figure 5.11).

Table 5.1. The degradation efficiency of malachite green using rotating liquid marble, naked reaction droplet, and reaction droplet covered with iron oxide powder after 8 mins of reaction time under various rotation speeds.

Spinning/Stirring speed (rpm)	Degradation efficiency of malachite green		
	12 μL rotational liquid marble	12 μL naked droplet	140 μL reaction solution covered with a layer of Fe_3O_4 (%)*
	(%)	(%)	
0	66	3	8
100	74	8	11
300	83	13	17
500	90	16	20
700	95	19	26
1300	99	-	-

* Note that we are unable to incorporate a magnetic micro-stirbar into a 12 μL micro droplet without interfering the catalytic Fe_3O_4 nanoparticles; the magnetic nanoparticles are attracted and adhered to

the stirbar instead of suspending on top of the liquid surface. To ensure fair comparison of the effect of pure stirring on the degradation efficiency of MG, we therefore increase the reaction droplet volume to 140 μL . This ensures a complete immersion of micro stir bar without interfering the catalytic layers suspended on the liquid surface.

Calculation 5.1. Derivation of integrated pseudo-first-order rate law and mass transfer coefficient.

The oxidative degradation of malachite green follows a pseudo-first-order reaction kinetics (Figure 5.9G), where its kinetics can be approximated using equation 1.³⁹

$$\frac{dC}{dt} = -kC \quad (1)$$

where k refers to the reaction rate constant contributed from catalytic shell in static LM.

Under rotation, the centrifugal force can drive molecular transfer towards the active shell surface for reaction. Such centrifugal force-driven mass transfer can be expressed using equation 2.^{40, 41}

$$\frac{dC}{dt} = -\frac{k_c}{V} AC \quad (2)$$

where k_c denotes the centrifugal force-driven molecule transfer coefficient to the active shell surface in RLM; A and V are the surface area and the drop volume of LM; C and t denote the MG concentration and the reaction time, respectively.

Hence, combining equation (1) and (2), the degradation rate of malachite green can be further expressed as equation 3.

$$\frac{dC}{dt} = -\left(k + \frac{k_c A}{V}\right) C \quad (3)$$

Equation 3 can be integrated to obtain the following rate law in equation 4:

$$\ln(C/C_0) = -k_{app} t \quad (4)$$

where $k_{app} = k + \frac{k_c A}{V}$, and k_{app} is the apparent rate constant.

The rate constants exhibit a good linear relationship with the rotation velocity. Hence, we determine

the intrinsic catalytic reaction rate constant in a static liquid marble (0 rpm) at 0.11 min^{-1} . Hence, the spin-induced reaction rate increment can be expressed as

$$\frac{k_c A}{V} = 0.0004 \omega \quad (5)$$

where ω is the spinning speed of LM in rpm.

Correspondingly, we can deduce the relationship between mass transfer coefficient and the spinning speed follows equation 6.

$$k_c = \frac{0.0004 V}{A} \times \omega \quad (6)$$

In our case of 12 μL liquid marble, the ratio of volume to surface area,

$$V/A = (4/3 \times \pi \times R_{\text{droplet}}^3) / (4 \times \pi \times R_{\text{droplet}}^2) = 473 \mu\text{m}$$

$$R_{\text{droplet}} = 1420 \mu\text{m}$$

Hence, in RLM, the equation 6 can also be expressed as:

$$k_c = 0.2 \times \omega \quad (7)$$

The unit of k_c is $\mu\text{m/s}$.

For one 12 μL liquid marble spinning at 1300 rpm, the mass transfer coefficient,

$$k_c = 0.2 \times (1300 / 60) = 4.33 \mu\text{m/s}$$

Figure 5.11. Comparison of the degradation reaction kinetics of malachite green in RLM with two control microsystems involving naked reaction droplet and the same suspension mixture of reaction droplet with Fe_3O_4 nanoparticles.

We also compare the degradation efficiency of our RLM against commercial micro-stirbars that are conventionally used to homogenize laboratory reactions. Two control setups are chosen: a 12 μL naked microdroplet and a 140 μL reaction microdroplet covered atop with equivalent catalyst mass, both stirred using a micro-stirbar (size 5×2 mm; Figure 5.12). Both setups demonstrate similar linear stir rate-dependent degradation efficiency (Figure 5.12A; Table 5.1). However, micromixing in both control setups enhances degradation efficiency by only $\sim 15\%$ between 0 and 700 rpm (Figure 5.12A, 5.12B). In contrast, our RLM provides a ~ 2 -fold increment of enhanced degradation efficiency and > 3 -fold boost of mass transfer coefficient relative to these controls (Figure 5.13; refer to Calculation 5.2).

Figure 5.12. (A) Benchmarking degradation efficiency of (i) 12 μL RLM against conventional laboratory micro-stir bar setups. Setup (ii) comprises reaction solution (140 μL) covered atop with equivalent mass of catalytic nanoparticles and setup (iii) consists of a naked 12 μL reaction droplet. Both control setups are stirred using micro-stirbars. (B) Plot of the rotating/stirring induced increment of degradation efficiency as rotational rates increase from 0 to 700 rpm using different type of platforms (i) RLM, (ii) mixture, and (iii) droplet, as depicted in (A).

Figure 5.13. (A) Plots of $-\ln(C/C_0)$ versus reaction time for control that consists of reaction solution (140 μL) covered atop with magnetic nanoparticles, which also contains a fully-immersed micro stir bar at various stirring speeds. The inset is a scheme depicting the control set-up. (B) Plot of apparent reaction rate constants, k_{app} , of malachite green degradation reaction in the control experiment at different stirring speeds. The fitted curve shows a linear relationship between k_{app} and the stirring rate.

Calculation 5.2. Determining mass transfer coefficient for convective stirring in reaction solution covered atop with magnetic nanoparticles.

Utilizing the same method as Calculation 5.1, we can also deduce the relationship between mass transfer coefficient and stirring rate for convective stirring in reaction solution (140 μL) covered atop with magnetic nanoparticles, and also contains a fully-immersed micro-stirbar, follows equation 8.

$$k_c = \frac{0.00003V}{A} \times \omega \quad (8)$$

The reaction droplet volume, $V = 140 \text{ mm}^3$.

The surface area of magnetic nanoparticles cover layer, $A = \pi r^2$

where r is the radius of the cylindar reaction solution container.

$$V/A = 140 / (3.14 \times 4.5^2) = 2200 \text{ } \mu\text{m}$$

$$r = 4500 \text{ } \mu\text{m}$$

Hence, the relationship between the mass transfer coefficient and the spinning rate can be also expressed as:

$$k_c = 0.06 \times \omega \quad (9)$$

Compared with equation 7 for RLM with radial mass transfer mechanism, the mass transfer coefficient in stirring convective mass transfer therefore exhibits ~ 3.3 times lower than RLM at the same rotation speeds.

Such improvements are attributed to the differences in mass transport phenomena arising from the circular hydrodynamic flow and outward-driving centrifugal force in a RLM compared to the typical convective flow using a stirbar (Figure 5.14). The stirbar utilizes vortex flow to achieve convective mass transportation, drawing reactants from the bulk microdroplet toward the agitation origin instead of toward the catalyst.⁴² Our results therefore collectively exemplify the superiority of

rotating hydrodynamic flow in a RLM to drive reaction performance beyond the conventional convective mass transportation limit in a stir bar system. Such controlled advection of reactants toward the functional shell in the RLM is important for heterogeneous catalysis using a LM microreactor and other reacting core/catalytic shell systems. Our RLM is also easily recyclable and capable of sustaining an excellent catalytic degradation efficiency of > 90 % over multiple degradation cycles, highlighting its suitability for practical applications (Figure 5.15).

Figure 5.14. Schematic illustration of different hydrodynamic flow using (i) RLM and (ii) commercial micro-stirbar.

Figure 5.15. Evaluation on the catalytic efficiency and reusability of Fe_3O_4 nanoparticles over four consecutive degradation cycles. Spin rate and reaction time are at 700 rpm and 8 min, respectively.

5.3 Conclusions

In conclusion, we achieve unique dynamic rotation using the RLM, creating an active rotational hydrodynamic flow to direct and improve molecular/particle transport within an enclosed microdroplet. The rotational dynamics of the RLM can be precisely controlled by tuning the rotational speeds of the applied magnetic field from 0 rpm to 1300 rpm. The circular hydrodynamic flow induced by the RLM also generates a centrifugal force on the microdroplet, enabling 3-fold faster molecular transport than traditional stirbar-based convective mass transfer. In addition, our dynamic active mass transportation renders the RLM a stimuli-responsive microreactor for the linear and 5-fold kinetic modulation of MG degradation without increasing catalyst amount. Together with its ease of preparation and robustness as well as reversible opening/closing, a RLM is immensely attractive for dynamic manipulation of microdroplet-based chemical/biological reaction and a rotary 3D microsystem for efficient cell cultivation, which is otherwise unachievable by simple spatial migrations. Such on-demand modulation and guiding of particle/molecular transport in three-dimensional space are also crucial in catalytic and surface-sensitive sensory microdevices. Moreover, our RLM provides new opportunities for 3D artificial droplet-based microsystems to mimic the complex and dynamic processes in living systems.

5.4 Materials and methods

Materials. Iron (II) chloride, iron (III) chloride, 1H,1H,2H,2H-perfluorooctyltriethoxysilane ($\geq 97\%$), Al_2O_3 (CG-20), silver nitrate ($\geq 99\%$), copper (II) chloride ($\geq 98\%$), poly(vinylpyrrolidone) (average MW = 55,000), 1,5-pentaediol ($\geq 97\%$), resazurin sodium salt, malachite green oxalate, methylene blue ($\geq 82\%$), Sudan I, gold (III) chloride hydrate and hydrogen peroxide (30%) were purchased from Sigma-Aldrich. Ammonia solution (25 %) was from Merck.

Ethanol (ACS, ISO, Reag. Ph Eur) was from EMSURE®. Sodium borohydride (97 %) was from Alfa Aesar. All chemicals were used without further purification. Mili-Q water (> 18.0 MΩ.cm) was purified with a Sartorius Arium 611® UV ultrapure water system.

Synthesis of Fe₃O₄ nanoparticles. The preparation of Fe₃O₄ nanoparticles was carried out following the method described in literature.¹⁹ Briefly, aqueous NH₄OH solution (1.5 M) was added dropwise to 200 mL water/ethanol (4:1 v/v) containing FeCl₃ (0.509 g, 3.14 mmol), FeCl₂ (0.191 g, 1.51 mmol), and 1H,1H,2H,2H-perfluorooctyltriethoxysilane (0.20 mL) under nitrogen protection and vigorous stirring until pH 8. After stirring for 24 h, the resulting precipitate was isolated from the solution with a bar magnet, washed with copious amount of water and ethanol, and dried for 2 hours – 3 hours in the oven at 65 °C. The Fe₃O₄ nanoparticles were subsequently pulverized to obtain fine powders.

Fabrication of liquid marble (LM). Liquid marbles were formed by rolling 12 µL water droplets on a bed of pulverized perfluorooctyltriethoxysilane–modified Fe₃O₄ nanoparticles.

Evaluation of rotation speed of liquid marble under applied rotating magnetic field. Prior to the spinning test, the performance of magnetic stirrer was calibrated with a commercial ellipse stirrer bar (size of 15 mm × 6 mm). For spinning evaluation, 12 µL liquid marble was first formed and transferred onto a water pool in a petri dish placed on top of magnetic stirrer plate, using a previously optimized magnetic field-to-RLM distance of 8 mm.³¹ Trace amount of white alumina powder was added on top of rotational liquid marble for the tracking of its motion. The rotating magnetic field conveyed by commercial stirrer was then activated and pre-set at 100 rpm – 1300 rpm. A maximum duration of 20 s was allowed for the marble to equilibrate with the applied magnetic field. The equilibrium response of our liquid marble to this applied field were subsequently captured using a slow-motion video (400 fps) for 3 s. All experimental were repeated at least thrice to ensure

the reproducibility of the results.

Spinning durability of rotating liquid marble (RLM): The rotational motion durability test was performed by spinning magnetic liquid marble at 100 rpm, 300 rpm, 500 rpm, 700 rpm, and 1300 rpm for 30 mins. At least three slow motion videos were taken at each pre-defined timings of 2 min, 5 min, 8 min, 10 min, 15 min, 20 min, 25 min, and 30 min.

Characterization of the hydrodynamic flow inside RLM. A 12 μL water droplet was placed on hydrophobic Fe_3O_4 powder bed to form a partial magnetic shell at the bottom of the droplet. A rotational magnetic field (300 rpm) was applied. Sudan I powder were added on top of exposed liquid droplet as droplet motion tracer.

Characterization of the flow pattern in circular hydrodynamic flow. 1 μL 10 mM methylene blue droplet was added to the center and edge of 100 μL water contained in a cap of centrifuge tube, respectively. Under an applied rotational magnetic field (300 rpm), the trajectory of the dye molecule in two scenarios were recorded by Camera. Similarly, the dye diffusion in static liquid phase were also recorded as control.

Synthesis and purification of Ag nanocubes. Ag nanocubes were prepared following the method in literature.¹⁶ Briefly, copper (II) chloride (8 mg/mL), poly(vinylpyrrolidone) (20 mg/mL) and silver nitrate (20 mg/mL) were separately dissolved in 10 mL 1,5-pentanediol after repeated sonication and vortex mixing. 35 μL copper (II) chloride solution was subsequently added to the silver nitrate solution. 20 mL of 1,5-pentanediol was added into a 100 mL round bottomed flask and heated to 190 $^{\circ}\text{C}$ for 10 min. 250 μL poly(vinylpyrrolidone) precursor was added to flask dropwise every 30 s while 500 μL silver nitrate precursor was injected every min using a quick addition. The addition process continued until the greenish coloration of the mixture faded off.

For the purification of Ag nanocubes, 1,5-pentanediol was first removed from the mixture

through centrifugation. The Ag nanocubes were then dispersed in 10 mL ethanol and 100 mL aqueous poly(vinylpyrrolidone) solution (0.2 g/L). The resulting solution was vacuum filtered using Durapore® polyvinylidene fluoride filter membranes (Millipore) with pore sizes ranging from 5000 nm, 650 nm, 450 nm, and 220 nm, repeated several times for each pore size. The Ag nanocubes were then re-dispersed in ethanol (10 mg/mL) and stored in fridge for further use.

Separation of Ag nanocubes from aqueous suspension droplet in spinning liquid marble.

Liquid marble encapsulating 12 μ L Ag nanocubes aqueous suspension (0.01 mg/mL) was prepared and subsequently transferred onto water surface. After spinning at 1300 rpm for 5 mins, the liquid marble was transferred out from water surface to the glass slide and the encapsulated droplet was fully exposed using external permanent magnet. The encapsulated droplet was extracted using pipette and further diluted into 2 mL pure water for UV-vis spectroscopy measurement. The same aqueous suspension of Ag nanocubes was encapsulated in static liquid marble as control.

Reduction of resazurin to resorufin in rotational liquid marble: Liquid marble encapsulating 10 μ L resazurin (0.1 mM) was formed and subsequently transferred onto glass slide surface. The marble shell was then opened using a permanent magnet to reveal its encapsulated contents. Subsequently, 1 μ L NaBH₄ (0.01M, reducing reagent) and 1 μ L Au nanoparticles colloidal solution (catalytic reagent) were added in sequence into the resazurin droplet. The marble shell was closed by removing the permanent magnet and the marble was immediately transferred onto surface of water pool in petri dish. After rotation at 300 rpm for 2 mins, the marble was transferred back to the glass slide and the encapsulated reaction droplet was exposed and isolated from the marble shell using external permanent magnet. The encapsulated aqueous content was further diluted into 2 mL pure water for UV-vis spectroscopy measurement. The same reaction was also conducted in static liquid marble, and naked droplet as controls.

Rotational liquid marble as catalytic microreactor for oxidative degradation of malachite green. The degradation of malachite green was carried out in the catalytic rotational liquid marble. Firstly, equal volume of H_2O_2 (1 M) was added to aqueous malachite green (1 mM) solution to obtain the mixture solution of H_2O_2 (0.5 M) and malachite green (0.5 mM). Then, 12 μL of the as-obtained solution were dropped on the hydrophobic Fe_3O_4 powder bed to form liquid marbles and was immediately transferred onto water surface and allowed to rotate at 500 rpm. At pre-defined duration of 0 min, 2 min, 5 min, and 8 min, the encapsulated malachite green solution was extracted out and diluted into 2 mL pure water for quantified UV-Vis spectroscopy measurement. The degradation of malachite green by H_2O_2 in static liquid marble was also conducted. In addition, 12 μL reaction solution droplets in the absence of Fe_3O_4 nanoparticles and 140 μL reaction solution with surface covered by Fe_3O_4 layers under static condition or stirred using micro-stirbar (5 mm $\times$ 2 mm), respectively, were also carried out as controls. Control microreactor involving a suspension mixture of reaction droplet with Fe_3O_4 nanoparticles was formed by the deliberate rupturing of a 12 μL liquid marble upon its formation. This is to ensure that a consistent amount of Fe_3O_4 nanoparticles was dispersed in an equal volume of malachite green solution.

Modulation of degradation kinetic of malachite green by tuning the rotational rate of liquid marble microreactor. The rotational effect of magnetic liquid marble on the modulation of encapsulated reactions was further carried out at selected rotating speed of 100 rpm, 300 rpm, 500 rpm, and 700 rpm. Naked mixture solution containing malachite green and H_2O_2 and 140 μL reaction solution with surface covered by Fe_3O_4 layers were also studied under stirring condition using micro stir bar at the selected speed of 100 rpm, 300 rpm, 500 rpm, and 700 rpm.

Recyclability of RLM. The recyclability of our catalytic magnetic liquid marbles were obtained by opening the marble using magnet to withdraw the interior solution droplet, followed by

the re-encapsulation of new reaction droplet on the bed of recycled Fe_3O_4 powder. To evaluate the degradation efficiency, the encapsulated aqueous droplet was extracted after spinning at 700 rpm for 8 mins by opening the marble shell with magnet and diluted into 2 mL pure water for UV-vis spectroscopy measurement

Characterization. Transmission electron microscopy (TEM) images were collected on a JEM-1400 (JEOL) operated at 100 kV. IKA color squid magnetic stirrer plate was used for liquid marble spinning test. Nikon 1 V2 digital camera was used to capture the slow-motion video (400 fps) of rotational liquid marble. UV-vis spectra was measured with Cary 60 UV-vis spectrometer. The digital images were analyzed with Image J.

Authors contributions. X.H., H.K.L. and X.Y.L. designed research. X.H., and H.K.L. performed research. X.H., H.K.L., Y.H.L. and X.Y.L. analyzed the results and wrote the manuscript. All authors discussed the project. X.Y.L. supervised the project.

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Summary and outlook

6.1. Summary

Liquid marbles are particle-assembled microdroplet platforms that have garnered paramount importance as both microsensor and microreactor owing to their excellent mechanical robustness and highly customizable properties. However, we identified three main challenges which could potentially impede their subsequent progress. These challenges include (1) molecular sensing of analytes present only in the encapsulated liquid phase, (2) invasive and ex-situ reaction monitoring techniques and (3) lack of heating mechanism and active mass transportation system to enhance and modulate reaction kinetics. In this thesis, we overcome the challenges systematically to showcase liquid marble as a simple-yet-powerful multifunctional microdevice built on the concept of particle-assembled microdroplet.

In chapter 2, we introduced the fabrication of plasmonic liquid marble using Ag nanocube as encapsulating material and their subsequent application as a substrate-less SERS platform for detection of analytes concurrently across two immiscible aqueous and organic phases. Plasmonic liquid marble demonstrates quantitative and ultratrace detections of methylene blue (aqueous soluble) and coumarin (organic solvent soluble) analytes down to femtomole level, with an analytical enhancement factor of $> 10^8$. Notably, the results from the multiplex detection scheme are quantitatively tallied with those measured during the individual detection of respective analytes. The ensemble of benefits offered by plasmonic liquid marble potentially allow them as an efficient

sensor in fields such as industrial and environmental safety where multiphasic samples are prevalent.

We further exploit plasmonic liquid marble as a reactor-sensor hybrid for in situ reaction monitoring in chapter 3. Utilizing the ultrasensitive SERS activities of the plasmonic shell, we demonstrate the in situ read-out of the reaction mechanism and kinetics of aryl-diazonium surface grafting process, with the use of a mere 2 μL of reaction droplet. Corroborating experimental SERS evolution with simulations, we uncover our model reaction as a two-step tandem process. Reaction first proceeds with the chemisorption of sulfonicbenzene diazonium (dSB) salt onto metallic surface to form an intermediate sulfonicbenzene monolayer (Ag-SB). After which, the intermediate Ag-SB undergoes an autocatalytic multilayer growth to form Ag-SB₃ via the addition of two SB moieties. Distinct kinetic rate constants between the first and second steps are also determined using SERS, whereby the initial chemisorption exhibits a 19-fold faster kinetic compared to the later multilayer growth. Plasmonic liquid marbles' abilities as a reactor-sensor hybrid therefore render them ideal for the unravelling of molecular-level reaction dynamics with the ultimate aim of optimizing a reaction design especially relevant in nanotechnology and synthetic chemistry.

In chapter 4, graphene liquid marble is fabricated as a robust and photothermal-active miniature reactor capable of remote heating simply upon laser irradiation. By modulating the power of irradiation laser, the surface temperature of the graphene shell can be swiftly regulated between 21 °C to 135 °C. Photothermal-generated heat can subsequently be conducted through the graphene shell to heat the encapsulated aqueous reaction microdroplet, reaching a water temperature of 21 °C to 74 °C determined using Raman spectroscopy. This heat generated is employed to enhance and systematically tune the reaction kinetics of enclosed microdroplet. Using the degradation of methylene blue as our model reaction, such regulation of microreactor's temperature allows graphene liquid marble to achieve a 12-fold better reaction rate constant as compared to those

performed under room temperature. This study therefore provides valuable insights on the use of graphene liquid marble for a wide range of (bio)chemical reactions, including heat-activated processes and precise control over reaction kinetics.

Lastly in chapter 5, we realize the unprecedented spinning of a magnetically-active liquid marble for precise control over mass transportation and distribution within the enclosed 3D microdroplet. Rotating liquid marble is able to spin synchronously (0 rpm to 1300 rpm) with an external applied magnetic field which imparts a centrifugal force acting on the enclosed droplet. Together with the circular hydrodynamic flow, the outward pushing centrifugal force directs a spiral motion of entrapped molecules towards the entire 3D volume. This phenomenon is essential to accelerate diffusion and rapid homogenization across the whole liquid microdroplet. Utilizing a shell-catalyzed degradation of malachite green as a model, the control over liquid marble's rotating rate allows the linear modulation and enhancement of reaction kinetics by > 5-fold. Furthermore, we determine that our rotating liquid marble is able to achieve > 3-fold faster advection of molecules towards the catalytic shell as compared to traditional stirbar-based convective flow. Rotating liquid marble is therefore an efficient magneto-to-hydrodynamic transducer which offer great opportunities for precise and dynamical control over mass transportation in microdroplet-based (bio)chemical reactions. Rotating liquid marble can also potentially double as a 3D artificial cell to imitate cellular processes for detailed investigations.

6.2. Outlook

In this thesis, we have offered several novel insights to significantly boost the appeal of liquid marble as a microsensor and microreactor. Nevertheless, continuous research efforts should be maintained to further explore and drive liquid marble as a universal microplatform suitable for real life sensing applications, as well as high-performance microreactor for high throughput biomedical screening and optimization of synthetic protocols. For instance, current plasmonic liquid marble-based micro-sensor could be coupled with statistical tools such as principal component analysis (PCA) to accurately index a highly sophisticated vibrational fingerprint into respective constituting spectra of individual analytes that are present.¹ Plasmonic liquid marble, which is also electrically active, could also be applied as a spectroelectrochemical microsensor for dual-mode detection based on spectroscopy and electrochemistry. Furthermore, the surface of plasmonic building blocks could be chemically functionalized to “fish out” targeted molecules from a pool of molecular interferences for selective detection within a complex sample. Collectively, these strategies aim to further our studies beyond controlled laboratory settings, whereby real samples typically comprises of complex matrixes with a variety of analytes and potential molecular interferences that could complicate detailed spectroscopic/electrochemical studies. Such complex samples are ubiquitous in numerous aspects such as forensic, biosensing and even water pollutions.²⁻⁴

While liquid marble functions as an excellent microreactor capable of isolating the reaction microdroplet, these reactions are mainly confined to the enclosed liquid phase and do not fully utilize the dual phase stability of liquid marble. Hence, liquid marble microreactor can be further extended for conducting interfacial application. This direction is aligned with growing efforts on the development of biphasic reaction involving an immiscible binary solvent system, which have

recently been demonstrated as an advanced synthetic strategy involving multiple analytes of contrasting polarities that are soluble only in either of the solvents.^{5, 6} By confining reaction only at the liquid-particle-liquid boundary, these interfacial microreactor could potentially be applied for the synthesis of wide and ultrathin plate-like structures/polymer.

Another avenue of research could be directed towards the fabrication of non-spherical liquid marble. One apparent advantage of such geometrical transformation is the enlarged surface area-to-volume ratio which has direct impact on heterogeneous catalytic performance, surface-sensitive molecular detection, and even discovering of novel collective photonics and magnetic properties. Although the fabrication of non-spherical “armoured” bubbles have been reported,⁷ we note that these structures can only be formed individually through applied mechanical force such as compression which is non-ideal for future application. Hence, additional research works could focus on different liquid marble formation techniques together with the use of stimuli-responsive particles to effect high throughput distortion to the conventional spherical liquid marble.

Multifunctional Janus liquid marble can also be potentially fabricated by merging plasmonic liquid and graphene liquid marble to imbue the micro-entity with both in-situ SERS reaction monitoring and laser-induced remote-heating capabilities.⁸⁻¹⁰ Such synergism will provide an attractive universal, multi-functional miniature reactor that can essentially be extended to a wide variety of chemical and/or biological reactions, including the activation of inert processes, acceleration of reaction rate at elevated temperature, and/or for the in-situ studies of reaction mechanism, intermediates and kinetics. These are especially important for the optimization of reaction designs in numerous crucial field such as nanotechnology, synthetic chemistry, as well as biochemical/medical investigations. Possible extension to such Janus microplatform could also

include the multiple combinations involving plasmonic, photothermal, and rotating liquid marbles.

Liquid marble-in-liquid marble system is also a potential area of great interest where such sophisticated and multi-layered designs are promising for the pre-programming of physical and/or chemical processes. For instance, these hierarchical liquid marble platforms could enable diffusion-regulated multi-step reaction and/or automated reaction-purification sequence as molecules transit across adjacent layers. Furthermore, such design of multi-layered liquid marble platforms can also render multiple functionalities to a single liquid marble ensemble, whereby the properties of this ensemble are expected to be highly symmetrical and uniform throughout the 3D space. This contrasts with current reported Janus or “watermelon-like” configurations,¹⁰ where the additional unique properties are mainly confined to only one-face of the liquid marble or on regions where the active particles are present, respectively.

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