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NANOMATERIALS FOR SENSING, HEATING AND APPLICATIONS IN COMPOSITES AND EMULSION BIOFOULING

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NANOMATERIALS FOR SENSING, HEATING AND APPLICATIONS IN
COMPOSITES AND EMULSION BIOFOULING

BY

AKRAM ABBASI

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

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IN

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ABSTRACT

Nanoscale materials often exhibit fascinating physical, chemical, and biological properties which are considerably different from those of their macro-and/or micro-scale counterparts. These unique characteristics have led to considerable growth over the last decade in nanomaterial-enabled applications in areas such as biomedicine, environmental sensing and remediation, and energy production and storage. In this dissertation, techniques are presented to create new nanomaterials relevant to these areas.

Gold nanostructures are an example of a nanomaterial with great interest in biomedical therapy and sensing applications due to their high chemical and physical stability, ease of synthesis and surface functionalization and their unique optical properties. High photothermal heat generation efficiency and their biocompatibility have made them an effective tool for thermal destruction of cancer cells. Magnified electromagnetic field on their surfaces allows gold nanostructures to be used as ultrasensitive nano-sensors for analyte detection using surface-enhanced Raman scattering (SERS). There remains significant interest in further improving the synthesis and design of gold nanostructures to maximize their potential applications. In biomedicine, for instance, the absorbance spectra of gold nanostructures need to be stable and tuned to the near-infrared (NIR) window (650–1350 nm) where biological tissues have minimal light absorption, while yet it is also desirable for the nanostructures to degrade to avoid accumulation in the body after treatment. Likewise in SERS applications, it is important that the nanostructures are stable and resist surface fouling,

while yet analytes with low affinity for the metal surface must be enriched at the surface to overcome poor detection limits.

A versatile templating strategy was developed to create gold nanoshells on different dielectric cores for photothermal heating and sensing applications. The strategy allows for tunable NIR absorbance and high degradation capabilities upon laser irradiation using soft templates. When carbon nanomaterials are used as the template, the carbon improves the affinity of different analytes to the surface of the hybrid nanostructures, yielding sensitive SERS-active materials for monitoring aqueous pollutants.

In the area of environmental remediation, nanoparticles with mixed wettability properties have been recently explored as alternative oil spill dispersants to help avoid the negative effects of surfactants on marine species. Adequate dispersing qualities and low toxicity are generally considered as the criteria for a good oil dispersant. However, their impact on the oil biodegradation process is still poorly understood. The attachment of bacteria to the oil/water interface, as the first step in oil biodegradation, was investigated to study the potential inhibitory effect of two commercially available dispersants, Corexit-9500 and Tween 20, and also carboxyl-terminated carbon black nanoparticles on biodegradation process.

Finally, conductive nanoparticles were used to enhance the electrical conductivity of polymer nanocomposites. While graphene, with a high aspect ratio and excellent conductivity, seems a promising filler to induce conductivity into a polymer, dispersing it uniformly within a polymer network remains a challenge due to their strong attractive forces. To assist dispersion, carbon black nanoparticles were used as a secondary filler to prevent restacking the graphene sheets within the polymer matrix. As a result, the

electrical conductivity of the composite was significantly increased and sustained even at high nanoparticle loading.

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PREFACE

This dissertation is written in manuscript format. The first chapter is an introduction covering the main topics of the dissertation. The second chapter entitled “Near-Infrared Responsive Gold-Layersome Nanoshells” was published in *Langmuir* (*Langmuir* 33 (2017): 5321-5327) in May 2017. The third chapter entitled “Carbon Black Templated Gold Nanoparticles for Analyte Detection by Surface Enhanced Raman Spectroscopy” is currently under review at ACS Applied Nanomaterials. The fourth chapter entitled “Attachment of *Alcanivorax borkumensis* to Hexadecane-In-Artificial Sea Water Emulsion Droplets” was published in *Langmuir* (*Langmuir* 34 (2018): 5352-5357) in April 2018. The fifth chapter entitled “Massive and Sustained Enhancement of Electrical Conductivity of Polystyrene Using Multilayer Graphene and Carbon Black as Primary and Secondary Fillers” is in preparation for submission in ACS Applied Polymer Materials. Supporting information for “Attachment of *Alcanivorax borkumensis* to Hexadecane-In-Artificial Sea Water Emulsion Droplets” manuscript is provided in Appendix S1.

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CHAPTER 1

INTRODUCTION

1.1 Gold nanoparticles

Gold nanoparticles (Au NPs) have attracted significant interest in different fields of nanoscience and nanotechnology including cancer therapy¹, cellular imaging², drug delivery³, heating⁴, sensing⁵, electronics⁶, and photocatalysis⁷. Their success in this broad range of applications is mainly due to their high chemical and physical stability as well as biocompatibility, facile surface conjugation to a variety of organic and biological ligands, and more importantly unique and tunable optical properties associated to their surface plasmons, a function for Au NPs which makes them distinct from bulk metal.⁸

Figure 1. 1: Schematic of collective oscillation of surface plasmon of a spherical gold nanoparticle, in resonance with electric field of incident light

When the size of a metallic particle decreases to smaller than the wavelength of a photon, the oscillating electric field of incident light polarizes the conduction electrons over the whole volume of the nanoparticle. The displacement of electron cloud from the positively charged lattice generates a restoring Coulombic force to pull back the polarized electrons.⁹ This collective oscillation of free electrons in resonance with

incident light is called localized surface plasmon resonance (LSPR)¹⁰, which is the origin of the intense color of Au NPs dispersion as well as many other unique optical properties. Localized surface plasmons can only be excited by the frequency of light in resonance with their oscillation. Tuning plasmonic nanoparticles LSPR wavelength to the desired wavelength has been the subject of numerous studies.¹¹⁻¹⁴ Plasmon resonance depends on the charge density distribution over the plasmonic particles;¹⁵ thus any change in size and shape of the particles can alter their surface plasmon resonance peak location. While varying the diameter of Au NPs changes the optical properties only slightly, engineering anisotropic nanoparticles tune the surface plasmons oscillation dramatically.¹⁶

Figure 1. 2: In photothermal therapy, gold nanoparticles first accumulate within tumors via the enhanced permeability and retention (EPR) effect, then with NIR laser irradiation, LSPR induced heat is generated to kill the surrounding cancer cells.¹⁷

The incident light which excites the plasmons is absorbed by the gold nanoparticles and causes vibrations of the metal lattice that is converted to heat. The high photothermal conversion efficiency of gold nanoparticles, as well as their tunability to near-infrared (NIR) wavelengths, has made them attractive for photothermal therapy.¹⁸ Biological tissue and blood show maximum optical transmissivity in the 650-1350 nm wavelength range (NIR window).¹⁹ Plasmonic nanoparticles can be accumulated into tumors via the enhanced permeability and retention (EPR) effect²⁰ and then illuminated

with NIR laser light. The heat generated from the absorption of the NIR light leads to a temperature increase above body temperature (37°C), causing irreversible photothermal destruction of tumor tissue while avoiding damage to healthy cells (see Figure 1.2).²¹ Recently, gold nanoparticle-mediated photothermal therapy in combination with other therapeutic approaches such as chemotherapy, immunotherapy and gene regulation has been explored as an enhanced multimodal approach for cancer treatment.²²⁻²⁴ Optimal size and structure of nanoparticles play critical roles in their performance. Small particles(<20nm) are rapidly cleared through the renal system while larger particles accumulate into the body after treatments since they are nonbiodegradable.²⁵ Gold nanoparticle shape can also be engineered to transport drugs which enable them to be used in the targeted photo-mediated drug delivery.²⁶ While loading drugs onto the exterior surface of particles is an easy approach through Au-thiol bonding, the drugs are susceptible to immune recognition and degradation.²⁷ The first use of gold nanoparticles in targeted photothermal therapy was conducted using NIR responsive gold nanoshells formed on silica templates.²⁸ LSPR peak of nanoshells is tuned from visible to NIR wavelengths by the adjustment of the shell thickness which was first shown by Neeves et al.²⁹ A hybridization model by Prodan et al.³⁰ describes the redshifts in the LSPR wavelength of nanoshells, as the results of the interaction of the plasmons of the outer and inner shell-surfaces due to the finite thickness of the shell.

LSPR excitation also results in an amplification of local electric field at the surface of gold nanoparticles that can enhance the inherently weak Raman signal from molecules in close proximity to the surface.³¹ This phenomenon, called surface-enhanced Raman scattering (SERS) was first observed in the spectra of pyridine on a

roughened silver surface in 1974.³² After the enhancement mechanism was discovered in 1977, the interest in SERS has grown exponentially. It is becoming one of the most popular ultrasensitive spectroscopic techniques in medicine, biology and environmental monitoring.³³ Raman scattering is an extremely weak process in which only one in every 10^8 photons scatters inelastically.³⁴ The intensity of the scattered radiation is proportional to the square of the magnitude of the electromagnetic field of light incident on the analyte ($I \propto E_0^2$).³⁵ In SERS due to the evanescent oscillation of surface plasmons, the electromagnetic field is the field amplified by the LSPR. Thus, the SERS intensity is enhanced compared to the Raman intensity of the analyte by an enhancement factor (EF) as:

$$EF = \frac{|E_{out}|^2 |E'_{out}|^2}{|E_0|^4} \quad (1)$$

where, E_{out} and E'_{out} are the magnified local fields generated by the incident light and the scattered light, respectively.³⁵

The maximum enhancement occurs in narrow gaps between gold nanoparticles and at sharp nanoscale tips and crevices within individual anisotropic nanoparticles due to the associated high confinement of the electrons.⁷ Various anisotropic nanoparticles such as nanorods, nanocubes, nanostar, nanoplates, and nanoflowers have been synthesized with regions of high curvature to intensify local electromagnetic field, termed as “hot spots”.³⁶ In addition to the number of hot spots per particle, the wavelength that nanoparticles are excited can affect the magnitude of enhancement. The best spectral location of the LSPR for maximum enhancement is in the close proximity of the laser excitation wavelength.³⁵ In this regard, gold nanoshells can be an ideal candidate as reproducible SERS substrates because of the ease of their LSPR peak

adjustment to the desired wavelength.³⁷ Wang et al.³⁸ showed that individual gold nanoshells unlike gold nanospheres, are SERS active due to the interaction of inner and outer shell surfaces and can be used for sensing applications.

However, surface plasmons have evanescent wave character and the enhanced electromagnetic field penetrates just a short distance from the metal-dielectric interface (up to 5nm).³⁹ So the presence of probe or analyte molecules in close proximity to the metal surface is a critical factor in Raman enhancement that makes the detection of those molecules with low affinity for metal surfaces such as polycyclic aromatic hydrocarbons (PAHs) a big challenge. Some current techniques introduce various metal surface functionalization to capture such analytes for the surface of nanoparticles.⁴⁰ However, the inherent complication of this approach, as well as the possibility for the surface passivation by surface ligands, restrict the broad applicability of this method. Combination of gold nanoparticles with different carbon nanomaterials (such as graphene, carbon nanotubes and graphene oxide) have also been investigated as an effective alternative approach in selective trapping aromatic molecules.⁴¹

A focus of this dissertation is to develop a novel templating strategy for the synthesis of gold nanoparticles with different functionalities. Gold nanostructures are formed on different dielectric cores and optimized for photothermal heating and sensing applications. Photothermal efficiencies for hollow gold -liposome nanostructures and also the SERS detection of a variety of analytes in aqueous solutions using gold-carbon nanoparticles, are studied.

1.2 Bacterial Oil Biodegradation

Oil biodegradation is the major natural process for the treatment of oil pollution in seawater. Due to the existence of naturally occurring hydrocarbons in all marine environments, numerous microorganisms have evolved to utilize hydrocarbons as a major source of energy for growth.⁴² These microorganisms are ubiquitous in nature and dominate microbial communities after an oil spill.⁴³ *Alcanivorax spp.*, for example, were shown to compose 70–90% of the prokaryotic cells within 1–2 weeks of entering oil to seawater.⁴⁴ The initial step in the biodegradation process is to transform the terminal carbon into a primary alcohol through membrane-bound oxygenase⁴⁵, so direct contact with the hydrocarbon substrates is an essential step for bacterial oil degradation. However, the low solubility of many oils in water has made the oil bioavailability a limiting factor in bacterial oil degradation.⁴³ One biological strategy to enhance the bioavailability of water-insoluble hydrocarbons is emulsification of the hydrocarbon by microbially-synthesized surfactants.⁴⁶ All strains growing on oil as the sole source of carbon and energy produce a broad range of biosurfactants ranging from low molecular weight lipopeptides and glycolipids to high molecular weight compounds such as polysaccharides, lipopolysaccharides, lipoproteins.⁴⁷ However, in an oil spill where large volumes of petroleum hydrocarbon enter into an open system, bio-emulsification never reaches a high enough value to effectively emulsify oil.⁴²

Dispersants are routinely applied to oil-contaminated waters during a response to marine oil spills. In the Deepwater Horizon oil spill in 2010, which resulted in the release of about 5 million barrels of crude oil, nearly 1.8 million gallons of Corexit 9500 were used to emulsify oil emanating from the seafloor, as well as break up surface

slicks.⁴⁸ Toxicity effects such as disruption of cell membranes, interference with cell membrane surface receptors, reactions with cellular components or irreversible blockage of enzyme active sites resulting from the uptake of dispersants by the bacteria have been reported for different dispersants.⁴⁹ However, their impact on the oil biodegradation process is still poorly understood. At first, the dispersants were believed to promote biodegradation rate based on their ability to generate micron-sized droplets and enhancing the oil-water interface area. However, many studies have shown that dispersants practically either make no difference or even inhibit biodegradation.^{50, 51} Efforts to develop safer and more effective dispersant are ongoing. In this regard, colloidal particles such as carbon-based nanoparticles, silica, and clay nanoparticles have recently been examined as effective alternative marine oil spill dispersants.⁵²⁻⁵⁴

Adequate dispersing qualities and low toxicity to marine species have generally been assumed as the criteria for a good oil dispersant. While attachment is a vital step for bacteria to degrade the oil, potential inhibitory effects of a dispersant is a critical factor that should be considered when developing alternative dispersants. Therefore, a focus of this dissertation is to explore the way in which an oil-degrading bacterium interacts with an oil-water interface populated with different dispersants, by using fluorescence microscopy and cryogenic scanning electron microscopy (cryo-SEM) to image cells at the oil-water interfaces.

1.3 Electrically Conductive Composites

Polymer-based composites have attracted enormous interest over the last few decades because of their lightweight, chemical and corrosion resistance and ease of manufacturing.⁵⁵ Different filler materials can be embedded into the polymer matrix to

impart specific functionalities to the composites. Electrical conductivity is induced into a polymer matrix by incorporating a conductive filler beyond a critical volume loading known as percolation threshold.⁵⁶ At this concentration, conductive particles contact one another, forming a continuous conductive network which results in a jump in the electrical conductivity. Today, electrically conductive polymer composites exhibit enormous applications in a wide range of fields including antistatic plastic materials, electrodes for batteries, sensors, electromagnetic interference (EMI) shielding materials.^{57, 58}

Figure 1. 3: TEM images of a (a) Carbon black nanoparticle with fractal structure (scale bar = 50nm) and (b) Multi-layer graphene with two-dimensional structure, comprising sp^2 hybridized carbon atoms (scale bar = $2\mu m$).

Various conductive fillers such as carbon blacks, graphite, carbon fibers and nanotubes, metal particles have been used to impart conduction to polymers.^{59, 60} The selection of appropriate filler materials is essential to achieve the desired properties of the composites. Metal particles have high intrinsic electrical conductivity but show a low tendency to form a conductive network. Fractal structured carbon black nanoparticles at high volume loading are extensively used in rubber composites as a filler to improve the mechanical performance and to impart electrical conductivity to prevent static charge buildup.⁶¹ Providing a low loading at percolation is a critical aspect

for easier processing, lowering the final cost and also for mechanical properties, as fillers can act as nucleation sites for crack growth.⁶² Fillers with high surface area and easier distribution into a non-conductive matrix provide high conductivity enhancement at lower loading levels.⁶³

Graphene is a one atom-thick sheet of hexagonally arrayed sp^2 -bonded carbon atoms, first fabricated in 2004.⁶⁴ Since then, graphene has attracted considerable attention as an ideal nanofiller to modify different polymers owing to its outstanding properties; their high aspect ratio and exceptional in-plane electrical conductivity has enabled them to provide conductivity to the polymer with minimum loading. If graphene sheets are modeled as ideally dispersed and randomly rotated disks of aspect ratio AR (AR = disk diameter/thickness), the percolation threshold ϕ_c is given by $\phi_c = 1.5(\phi_{\text{sphere}}/\text{AR})$.⁶⁵ Here, ϕ_{sphere} is the percolation threshold for monodispersed spheres, i.e., $\phi_{\text{sphere}} = 0.29$. Considering AR value of around 10^4 for graphene, the percolation threshold ϕ_c can be theoretically lowered as low as 0.001; but their high surface area also promotes their agglomeration by van der Waals interactions.⁶⁶ Their uniform dispersion in a host polymer is still a challenge.

The key goal of this project is to fabricate conductive graphene-polymer composite by developing a method to enhance the dispersion of graphene sheets. A third component was used as a secondary filler which is dispersed throughout the polymer network during processing to prevent graphene sheets restacking. The result of this study should be useful in lowering the graphene loading in conductive composites and consequently lowering the final cost.

1.4 References

1. Huang, X.; Qian, W.; El-Sayed, I. H.; El-Sayed, M. A., The potential use of the enhanced nonlinear properties of gold nanospheres in photothermal cancer therapy. *Lasers Surg Med* **2007**, *39* (9), 747-53.
2. Huang, X.; El-Sayed, I. H.; Qian, W.; El-Sayed, M. A., Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods. *J. Am. Chem. Soc.* **2006**, *128* (6), 2115.
3. Ghosh, P.; Han, G.; De, M.; Kim, C. K.; Rotello, V. M., Gold nanoparticles in delivery applications. *Advanced Drug Delivery Reviews* **2008**, *60* (11), 1307-1315.
4. Zielinski, M. S.; Choi, J. W.; La Grange, T.; Modestino, M.; Hashemi, S. M. H.; Pu, Y.; Birkhold, S.; Hubbell, J. A.; Psaltis, D., Hollow Mesoporous Plasmonic Nanoshells for Enhanced Solar Vapor Generation. *Nano Lett.* **2016**, *16* (4), 2159-2167.
5. Saha, K.; Agasti, S. S.; Kim, C.; Li, X.; Rotello, V. M., Gold Nanoparticles in Chemical and Biological Sensing. *Chemical reviews.* **2012**, *112* (5), 2739-2779.
6. Barsotti, R. J.; Vahey, M. D.; Wartena, R.; Chiang, Y. M.; Voldman, J.; Stellacci, F., Assembly of Metal Nanoparticles into Nanogaps. *Small* **2007**, *3* (3), 488-499.
7. Daniel, M. C.; Astruc, D., Gold nanoparticles: Assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chem. Rev.* **2004**, *104* (1), 293-346.
8. Amendola, V.; Pilot, R.; Frascioni, M.; Marago, O. M.; Iati, M. A., Surface plasmon resonance in gold nanoparticles: a review. *J Phys Condens Matter* **2017**, *29* (20), 203002.
9. Kelly, K. L.; Coronado, E.; Zhao, L. L.; Schatz, G. C., The Optical Properties of Metal Nanoparticles: The Influence of Size, Shape, and Dielectric Environment. *The Journal of Physical Chemistry B* **2003**, *107* (3), 668-677.
10. Mayer, K. M.; Hafner, J. H., Localized surface plasmon resonance sensors. *Chem Rev* **2011**, *111* (6), 3828-57.

11. Casu, A.; Cabrini, E.; Dona, A.; Falqui, A.; Diaz-Fernandez, Y.; Milanese, C.; Taglietti, A.; Pallavicini, P., Controlled synthesis of gold nanostars by using a zwitterionic surfactant. *Chemistry* **2012**, *18* (30), 9381-90.
12. Hu, M.; Chen, J. Y.; Li, Z. Y.; Au, L.; Hartland, G. V.; Li, X. D.; Marquez, M.; Xia, Y. N., Gold nanostructures: engineering their plasmonic properties for biomedical applications. *Chem. Soc. Rev.* **2006**, *35* (11), 1084-1094.
13. Cathcart, N.; Chen, J. I. L.; Kitaev, V., LSPR tuning from 470 to 800 nm and Improved Stability of Au-Ag Nanoparticles Formed by Gold Deposition and Rebuilding in the Presence of Poly(styrene sulfonate). *Langmuir : the ACS journal of surfaces and colloids* **2017**, *34* (2).
14. Wolf, A.; Kodanek, T.; Dorfs, D., Tuning the LSPR in copper chalcogenide nanoparticles by cation intercalation, cation exchange and metal growth. *Nanoscale* **2015**, *7* (46), 19519-19527.
15. Eustis, S.; El-sayed, M. A., Why gold nanoparticles are more precious than pretty gold: Noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes. *Chem. Soc. Rev.* **2006**, *35* (3), 209-217.
16. Li, N.; Zhao, P.; Astruc, D., Anisotropic Gold Nanoparticles: Synthesis, Properties, Applications, and Toxicity. *Angewandte Chemie International Edition* **2014**, *53*, 1756-1789.
17. Riley, R. S.; Day, E. S., Gold nanoparticle-mediated photothermal therapy: applications and opportunities for multimodal cancer treatment. Hoboken, USA, 2017; Vol. 9, pp n/a-n/a.
18. Wang, X.; Wang, C.; Cheng, L.; Lee, S. T.; Liu, Z., Noble metal coated single-walled carbon nanotubes for applications in surface enhanced Raman scattering imaging and photothermal therapy. *J Am Chem Soc* **2012**, *134* (17), 7414-22.
19. Jain, P. K.; El-sayed, M. a., Universal Scaling of Plasmon Coupling in Metal Nanostructures : Extension from Particle Pairs to Nanoshells Universal Scaling of Plasmon Coupling in Metal Nanostructures : Extension from Particle Pairs to Nanoshells. **2007**.

20. Maeda, H.; Wu, J.; Sawa, T.; Matsumura, Y.; Hori, K., Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *Journal of Controlled Release* **2000**, *65* (1), 271-284.
21. Rengan, A. K.; Bukhari, A. B.; Pradhan, A.; Malhotra, R.; Banerjee, R.; Srivastava, R.; De, A., In vivo analysis of biodegradable liposome gold nanoparticles as efficient agents for photothermal therapy of cancer. *Nano Lett* **2015**, *15* (2), 842-8.
22. Issels, R. D., Hyperthermia adds to chemotherapy. *European Journal of Cancer* **2008**, *44* (17), 2546-2554.
23. Park, H.; Yang, J.; Lee, J.; Haam, S.; Choi, I.-H.; Yoo, K.-H., Multifunctional nanoparticles for combined doxorubicin and photothermal treatments. *ACS nano* **2009**, *3* (10), 2919.
24. Nair, R.; Christie, C.; Ju, D.; Shin, D.; Pomeroy, A.; Berg, K.; Peng, Q.; Hirschberg, H., Enhancing the effects of chemotherapy by combined macrophage-mediated photothermal therapy (PTT) and photochemical internalization (PCI). *Lasers in Medical Science* **2018**, *33* (8), 1747-1755.
25. Perrault, S. D.; Walkey, C.; Jennings, T.; Fischer, H. C.; Chan, W. C. W., Mediating tumor targeting efficiency of nanoparticles through design. *Nano Lett.* **2009**, *9* (5), 1909.
26. Wu, G. H.; Milkhailevsky, A.; Khant, H. A.; Fu, C.; Chiu, W.; Zasadzinski, J. A., Remotely triggered liposome release by near-infrared light absorption via hollow gold nanoshells. *J. Am. Chem. Soc.* **2008**, *130* (26), 8175-+.
27. Skrabalak, S. E.; Chen, J.; Au, L.; Lu, X.; Li, X.; Xia, Y., Gold Nanocages for Biomedical Applications. *Advanced Materials* **2007**, *19* (20), 3177-3184.
28. Hirsch, L. R.; Stafford, R. J.; Bankson, J. a.; Sershen, S. R.; Rivera, B.; Price, R. E.; Hazle, J. D.; Halas, N. J.; West, J. L., Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 13549-54.
29. Neeves, A. E.; Birnboim, M. H., Composite structures for the enhancement of nonlinear-optical susceptibility. *Journal of the Optical Society of America B* **1989**, *6*, 787.

30. Prodan, E.; Radloff, C.; Halas, N. J.; Nordlander, P., A hybridization model for the plasmon response of complex nanostructures. *Science (New York, N.Y.)* **2003**, *302* (5644), 419.
31. Jackson, J. B.; Halas, N. J., Surface-enhanced Raman scattering on tunable plasmonic nanoparticle substrates. *Proc Natl Acad Sci U S A* **2004**, *101* (52), 17930-5.
32. Fleischmann, M.; Hendra, P. J.; McQuillan, A. J., Raman spectra of pyridine adsorbed at a silver electrode. *Chemical Physics Letters* **1974**, *26* (2), 163-166.
33. Sharma, B.; Frontiera, R. R.; Henry, A.-I.; Ringe, E.; Van Duyne, R. P., SERS: Materials, applications, and the future. *Materials Today* **2012**, *15* (1-2), 16-25.
34. McNay, G.; Eustace, D.; Smith, W. E.; Faulds, K.; Graham, D., Surface-enhanced Raman scattering (SERS) and surface-enhanced resonance Raman scattering (SERRS): a review of applications. *Appl Spectrosc* **2011**, *65* (8), 825-37.
35. Alvarez-Puebla, R. A., Effects of the Excitation Wavelength on the SERS Spectrum. *J Phys Chem Lett* **2012**, *3* (7), 857-66.
36. Alvarez-Puebla, R.; Liz-Marzán, L. M.; García de Abajo, F. J., Light Concentration at the Nanometer Scale. *The Journal of Physical Chemistry Letters* **2010**, *1* (16), 2428-2434.
37. Xie, Y.; Chen, T.; Guo, Y.; Cheng, Y.; Qian, H.; Yao, W., Rapid SERS detection of acid orange II and brilliant blue in food by using Fe₃O₄@Au core-shell substrate. *Food Chem* **2019**, *270*, 173-180.
38. Wang, H.; Kundu, J.; Halas, N. J., Plasmonic nanoshell arrays combine surface-enhanced vibrational spectroscopies on a single substrate. *Angew Chem Int Ed Engl* **2007**, *46* (47), 9040-4.
39. Stiles, P. L.; Dieringer, J. A.; Shah, N. C.; Van Duyne, R. P., Surface-enhanced Raman spectroscopy. *Annu Rev Anal Chem (Palo Alto Calif)* **2008**, *1*, 601-26.
40. Kubackova, J.; Fabriciova, G.; Miskovsky, P.; Jancura, D.; Sanchez-Cortes, S., Sensitive surface-enhanced Raman spectroscopy (SERS) detection of organochlorine pesticides by alkyl dithiol-functionalized metal nanoparticles-induced plasmonic hot spots. *Anal Chem* **2015**, *87* (1), 663-9.

41. Zhang, K.; Yao, S.; Li, G.; Hu, Y., One-step sonoelectrochemical fabrication of gold nanoparticle/carbon nanosheet hybrids for efficient surface-enhanced Raman scattering. *Nanoscale* **2015**, *7* (6), 2659-66.
42. Ron, E. Z.; Rosenberg, E., Biosurfactants and oil bioremediation. *Current Opinion in Biotechnology* **2002**, *13* (3), 249-252.
43. Grant, C.; Deszcz, D.; Wei, Y. C.; Martinez-Torres, R. J.; Morris, P.; Folliard, T.; Sreenivasan, R.; Ward, J.; Dalby, P.; Woodley, J. M.; Baganz, F., Identification and use of an alkane transporter plug-in for applications in biocatalysis and whole-cell biosensing of alkanes. *Sci Rep* **2014**, *4*, 5844.
44. Head, I. M.; Jones, D. M.; Roling, W. F., Marine microorganisms make a meal of oil. *Nat Rev Microbiol* **2006**, *4* (3), 173-82.
45. Rojo, F., Specificity at the end of the tunnel: understanding substrate length discrimination by the AlkB alkane hydroxylase. *J Bacteriol* **2005**, *187* (1), 19-22.
46. Cameotra, S. S.; Makkar, R. S., Biosurfactant-enhanced bioremediation of hydrophobic pollutants. *Pure and Applied Chemistry* **2010**, *82* (1).
47. Overholt, W. A.; Marks, K. P.; Romero, I. C.; Hollander, D. J.; Snell, T. W.; Kostka, J. E., Hydrocarbon-Degrading Bacteria Exhibit a Species-Specific Response to Dispersed Oil while Moderating Ecotoxicity (vol 82, pg 518, 2016). *Appl. Environ. Microbiol.* **2016**, *82* (17), 5477-5477.
48. John, V.; Arnosti, C.; Field, J.; Kujawinski, E.; MacCormick, A., The Role of Dispersants in Oil Spill Remediation: Fundamental Concepts, Rationale for Use, Fate, and Transport Issues. *Oceanography* **2016**, *29* (3), 108-117.
49. Kleindienst, S.; Paul, J. H.; Joye, S. B., Using dispersants after oil spills: impacts on the composition and activity of microbial communities. *Nat. Rev. Microbiol.* **2015**, *13* (6), 388-396.
50. Kleindienst, S.; Seidel, M.; Ziervogel, K.; Grim, S.; Loftis, K.; Harrison, S.; Malkin, S. Y.; Perkins, M. J.; Field, J.; Sogin, M. L.; Dittmar, T.; Passow, U.; Medeiros, P. M.; Joye, S. B., Chemical dispersants can suppress the activity of natural oil-degrading microorganisms. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112* (48), 14900-14905.

51. Grimberg, S. J.; Stringfellow, W. T.; Aitken, M. D., Quantifying the biodegradation of phenanthrene by *Pseudomonas stutzeri* P16 in the presence of a nonionic surfactant. *Appl. Environ. Microbiol.* **1996**, 62 (7), 2387-2392.
52. Creighton, M. A.; Ohata, Y.; Miyawaki, J.; Bose, A.; Hurt, R. H., Two-dimensional materials as emulsion stabilizers: interfacial thermodynamics and molecular barrier properties. *Langmuir* **2014**, 30 (13), 3687-96.
53. Saha, A.; Nikova, A.; Venkataraman, P.; John, V. T.; Bose, A., Oil emulsification using surface-tunable carbon black particles. *ACS Appl Mater Interfaces* **2013**, 5 (8), 3094-100.
54. Omarova, M.; Swientoniewski, L. T.; Tsengam, I. K. M.; Panchal, A.; Yu, T.; Blake, D. A.; Lvov, Y. M.; Zhang, D.; John, V., Engineered Clays as Sustainable Oil Dispersants in the Presence of Model Hydrocarbon Degrading Bacteria: The Role of Bacterial Sequestration and Biofilm Formation. *ACS Sustainable Chemistry & Engineering* **2018**, 6 (11), 14143-14153.
55. Thomas, S.; Shanks, B.; Chandrasekharakurup, S.; Adegbola, T. A., *Design and applications of nanostructured polymer blends and nanocomposite systems*. Amsterdam, Netherlands : William Andrew: Amsterdam, [Netherlands], 2016.
56. Nan, C. W.; Shen, Y.; Ma, J., Physical Properties of Composites Near Percolation. *Annual Review of Materials Research* **2010**, 40 (1), 131-151.
57. Zhang, W.; Dehghani-Sani, A. A.; Blackburn, R. S., Carbon based conductive polymer composites. *Journal of Materials Science* **2007**, 42 (10), 3408-3418.
58. Seenaa, V.; Fernandes, A.; Pant, P.; Mukherji, S.; Ramgopal Rao, V., Polymer nanocomposite nanomechanical cantilever sensors: material characterization, device development and application in explosive vapour detection. *Nanotechnology* **2011**, 22 (29), 295501.
59. Sih, B. C.; Wolf, M. O., Metal nanoparticle—conjugated polymer nanocomposites. *Chemical Communications* **2005**, (27), 3375-3384.
60. Balberg, I., A comprehensive picture of the electrical phenomena in carbon black–polymer composites. *Carbon* **2002**, 40, 139-143.

61. Huang, J.-C., Carbon black filled conducting polymers and polymer blends. *Advances in Polymer Technology* **2002**, *21* (4), 299-313.
62. Evora, V. M. F.; Shukla, A., Fabrication, characterization, and dynamic behavior of polyester/TiO₂ nanocomposites. *Materials Science & Engineering A* **2003**, *361* (1), 358-366.
63. Kyrylyuk, A. V.; Hermant, M. C.; Schilling, T.; Klumperman, B.; Koning, C. E.; van der Schoot, P., Controlling electrical percolation in multicomponent carbon nanotube dispersions. *Nat Nanotechnol* **2011**, *6* (6), 364-9.
64. K., G. A., Graphene: Status and Prospects. *Science* **2009**, *324*, 1530-1534.
65. Kim, H.; Abdala, A. A.; Macosko, C. W., Graphene/Polymer Nanocomposites. *Macromolecules* **2010**, *43* (16), 6515-6530.
66. Chakraborty, I.; Bodurtha, K. J.; Heeder, N. J.; Godfrin, M. P.; Tripathi, A.; Hurt, R. H.; Shukla, A.; Bose, A., Massive electrical conductivity enhancement of multilayer graphene/polystyrene composites using a nonconductive filler. *ACS Appl Mater Interfaces* **2014**, *6* (19), 16472-5.

CHAPTER 2

Near Infrared Responsive Gold-Layersome Nanoshells

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2.1 Abstract

Anionic liposomes coated with the cationic polyelectrolyte poly-L-lysine (PLL), or layersomes, were used as soft, self-assembled templates for synthesizing gold nanoshells that absorb near infrared radiation. The gold-nanoshells were formed using two techniques (a) direct reduction of tetrachloroauric acid on the layersomes, and (b) reduction of a tetrachloroauric acid/potassium carbonate ‘growth’ solution on nanosized gold seeds bound to the surface of layersomes. The resulting structures were characterized by transmission and scanning electron microscopy, and visible-near infrared spectroscopy. Direct reduction produced discrete gold nanoparticles on the layersomes. The slower reduction from the growth solution on the gold seeds resulted in more complete shells. The absorption spectra of these suspensions were sensitive to the synthesis method. The morphology of the gold shells was tuned for absorption at the biologically safe and tissue-penetrating NIR wavelengths, and laser irradiation at 810 nm produced significant heat. These gold-layersome nanoshells have the potential to be used for photothermal therapy, photothermally mediated drug delivery, and biomedical imaging.

2.2 Introduction

Gold nanostructures have attracted significant interest in medicine because of their biocompatibility, localized surface plasmon resonance (LSPR), and facile conjugation to a variety of biomolecular ligands.¹⁻⁵ Their LSPR can be tuned to near infrared (NIR) wavelengths by engineering their morphologies.⁶⁻⁸ NIR radiation can penetrate into body tissue and blood to depths of several millimeters,⁹ making these particles useful for biomedical imaging and photothermal therapy.¹⁰⁻¹²

Averitt *et al.*¹³ synthesized spherical nanoparticles consisting of a gold shell and a dielectric core. Reducing the shell thickness led to increased interactions between the inner and outer surface plasmons that produced a significant red shift in the absorption spectrum.^{9, 14, 15} Moving the SPR peak from ultraviolet-visible (UV-Vis) to NIR wavelengths by adjustment of the shell thickness was also shown by Neeves *et al.*¹⁶ By irradiating gold–silica nanoshells with a NIR laser, Hirsch *et al.*¹⁷ demonstrated photothermal destruction of targeted cancer cells.¹⁸ Although the formation of shells over hard cores leads to uniform shell thicknesses, the key disadvantages of these structures for therapy are that they are nonbiodegradable and not easily cleared through the renal system.²

One strategy for making plasmon resonant nanostructures with good biocompatibility and degradation capabilities is to use liposomes as templates, rather than hard inorganic cores. Troutman *et al.*¹⁹ developed biodegradable plasmon resonant structures that had a 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposome core and a gold shell. Upon NIR irradiation, the lipid membranes were disrupted and the gold shells degraded into 5–8 nm nanoparticles.^{2, 19, 20} Thus, hydrophilic and/or hydrophobic drugs can be encapsulated within these gold-coated liposomes, and these drugs can be released by NIR triggering.^{21–24} The gold nanoparticles produced after NIR irradiation can be easily cleared through the renal system.²⁵ Reduction of gold ions on liposome templates is a facile one step process. To avoid salt induced aggregation of the liposomes, the gold ion concentration in the surrounding solution must be limited. Rather than a continuous gold shell, this leads to arrays of gold nanoparticles on the liposome surface.²⁶

Figure 2. 1: Procedures for synthesis of gold-layersome nanoshells. (a) Direct reduction from a tetrachloroauric acid solution on the surface of layersomes. (b) Formation of nanoshells on gold-seeded layersomes by reduction from a growth solution consisting of a mixture of tetrachloroauric acid and potassium carbonate

We propose that a cationic polyelectrolyte overlaid on a liposome surface can significantly enrich that surface either with anionic gold precursor ions, or with anionic gold nanoparticles that act as heterogeneous nucleation sites for further growth. The intermediate polyelectrolyte also removes direct interaction of gold nanoparticles with lipids, stabilizing the liposome structure upon absorption of the charged gold nanoparticles.²⁷⁻³⁰ We used poly-L-lysine (PLL)-coated liposomes, referred to as layersomes, as templates to prepare gold-layersome nanoshells. The shells were synthesized using two techniques, outlined in Figure 2.1 - (a) direct reduction, where tetrachloroauric acid (HAuCl_4) bound to the layersome surfaces was reduced by ascorbic acid (AA) to metallic gold, and (b) seeded growth, where ~ 3 nm diameter anionic gold nanoparticles were first attached to the surface of the layersomes. A

growth solution consisting of a mixture of HAuCl_4 and potassium carbonate (K_2CO_3) was then added. The Au seeds act as heterogeneous nucleation sites, and the slow formation of metallic gold upon addition of AA to this growth solution allowed a shell to be formed on the uncoated parts of the surface. Although this two-step process has been used previously to create nanoshells on hard templates,^{16-18,31} this is the first report of the seeded growth method being used to obtain gold nanoshells on a polyelectrolyte-coated liposome as a soft, biocompatible template. The nanoshell morphologies created by these two preparation methods are distinct, leading to different absorption characteristics. NIR-triggered (at 810 nm) heating of the gold-layersome nanoshell suspensions is demonstrated. A simple lumped parameter model is used to extract the fraction of the incident NIR laser power that is absorbed and converted to heat, or the photothermal conversion coefficients, for each of these nanoshell suspensions. This coefficient shows large differences for the suspensions prepared by the two methods.

2.3 Experiments

2.3.1 Materials

1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) (DOPG) were purchased from Avanti Polar Lipids. Poly-L-Lysine hydrobromide (PLL, MW=15,000-30,000), tetrakis(hydroxymethyl)phosphonium chloride (THPC, 80 wt% solution in water), tetrachloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), potassium carbonate (K_2CO_3), sodium hydroxide (NaOH) and ascorbic acid (AA) were purchased from Sigma-Aldrich. All materials were used as received.

2.3.2 Preparation of layersomes

A 10 mM solution DOPC:DOPG at a molar ratio of 1:1 was formed in chloroform. The solvent was removed in a rotary evaporator, and a thin lipid film formed on the sides of a round-bottom flask. The dry film was hydrated with deionized water and then vigorously vortexed at 50°C, well above the gel-fluid transition temperature for either lipid. 1 ml aliquots of this suspension were extruded 11 times at room temperature in an Avanti Polar Lipids mini-extruder with 100 nm pore polycarbonate membranes to produce unilamellar liposomes with a narrow size distribution. The liposome suspension was diluted to 5 mM total lipid concentration. A 1 mg/ml PLL solution was then titrated into the liposome suspension. PLL was added in sufficient quantities to quickly go past the point of zero charge for the layersomes, thus avoiding aggregation.²⁷

2.3.3 Preparation of gold-layersome nanoshells

Direct reduction. Aqueous solutions of HAuCl_4 and AA were prepared at concentrations of 50 mM and 200 mM respectively. HAuCl_4 solutions were added to the layersome suspensions at $[\text{HAuCl}_4]:[\text{lipid}]$ molar ratios of 0.5:1, 1.5:1, and 3:1. The translucent white color of these layersome suspensions abruptly changed to blue when AA was added. This color change is indicative of gold shells forming on the layersome surfaces. At molar ratios of $[\text{HAuCl}_4]:[\text{lipid}]$ of 5:1 or 7:1, the ‘free’ gold particles formed in the bulk solution after addition of AA, and these particles aggregated quickly. The aggregates dragged gold-layersome structures out of the suspension. Therefore we limited our experiments to a maximum $[\text{HAuCl}_4]:[\text{lipid}]$ ratio of 3:1. As a control, gold nanoparticles (AuNPs) were synthesized by adding freshly made AA solution to a 50

mM HAuCl₄ solution in the absence of layersomes. All experiments were performed at a molar ratio of AA to HAuCl₄ of 4:1, well above the stoichiometric ratio of 1.5:1.

Seeded growth. A basic solution was prepared by adding 100 μ l of a THPC solution (1.2 vol% in water) to 50 μ l of 1 M NaOH in 4.5 ml of water. THPC is a strong reducing agent. Au seed suspensions with $\sim$ 3nm gold particles were formed by adding 100 μ L of 50 mM HAuCl₄ to the THPC containing basic solution.³² Assuming that all the HAuCl₄ was reduced to 3 nm diameter gold nanoparticles, the Au particle concentration in this suspension was calculated to be 7.6×10^{17} particles/mL. Gold seeds were targeted to cover about 25% of the layersome surfaces. This coverage would allow the layersome surfaces to remain cationic, as well as strike a balance between having enough seeds to provide an adequate number of heterogeneous nucleation sites and sufficient space between the seeds to allow for gold growth between the seed particles. To achieve this coverage approximately, 0.05 ml of the Au seed suspension and 1 ml of the layersome suspension (the layersome number concentration was estimated to be $\sim 3.8 \times 10^{13}$ layersomes/ml) were mixed and stirred for 30 min. The resulting suspension was centrifuged three times at 8000g for 10 min. At this acceleration, any gold particles less than a diameter of 60nm would remain suspended by Brownian motion. The supernatant containing any unattached Au seeds was removed each time, and the light brown precipitate was redispersed in deionized water.

The gold ‘growth’ solution was prepared by mixing 150 μ l of 50 mM HAuCl₄ with a solution containing 5 mg of K₂CO₃ dissolved in 10 ml water. The solution was aged in the dark for 24 h before use. The solution appeared transparent and yellow initially, but became colorless after mixing for 30 min. The loss of color is caused by the

hydrolysis of HAuCl_4 to $[\text{AuCl}_x(\text{OH})_{4-x}]^-$ ($x=0-4$). The reduction rate of these anions to metallic gold upon addition of AA is slower than that for HAuCl_4 .³³ This attenuated reduction rate promotes the formation of gold shells on the layersome surface rather than nucleation of nanoparticles in the bulk solution.

The Au seed-decorated layersome suspension was mixed with 0.75 mM gold growth solution at $[\text{HAuCl}_4]:[\text{lipid}]$ molar ratios varying from 0.1:1, 0.5:1, 1.5:1 and 3:1. AA was then added to the mixture at a $[\text{AA}]:[\text{HAuCl}_4]$ molar ratio of 4:1, resulting in gold nanoshells on the layersomes.

2.3.4 Characterization

Samples were imaged using transmission electron microscopy (JEOL JEM-2100), at an accelerating voltage of 200 KV, and scanning electron microscopy (Zeiss Sigma VP FESEM). Cryo-TEM samples were prepared in a Vitrobot Mark III unit, and transferred to a Gatan 626 DH cold stage for imaging. Cryo-SEM samples were prepared in a Gatan Alto 2500 device. Elemental maps were created using Energy Dispersive X-Ray (EDX) analysis with an Oxford Inca system. A Malvern Zetasizer Nano ZS was used to determine the hydrodynamic diameter of the liposomes and layersomes, and the zeta potentials of the liposomes, layersomes, and Au seeds. Absorption spectra were collected using a Perkin–Elmer Lambda 1050 UV-Vis-NIR scanning spectrophotometer over wavelengths from 500 nm to 1200 nm. All samples were diluted four times in water for absorption measurements. Absorption from deionized water was used as the reference.

2.3.5 Photothermal behavior

The photothermal behavior of the nanoshell suspension was probed using an 810 nm NIR laser with a power rating of 20 W/cm². The experimental setup is shown in Figure 2.2. In a typical experiment, 600 µl of the suspension was placed in a glass vial at room temperature. The sample was irradiated 5 times for 10 min each from the top through a glass cover. The temperature rise during irradiation was monitored using a thermocouple, embedded through the side of the vial. Between each irradiation, the samples were allowed to cool back to room temperature. To assess the robustness of the gold nanoshells, some of the samples were examined after the fifth irradiation cycle using transmission electron microscopy.

Figure 2. 2: The NIR laser heating experiment. The sample is in a glass vial and is irradiated from the top through a glass slide. A thermocouple embedded through the side of the vial is used to monitor the transient sample temperature.

2.4 Results and discussion

2.4.1 Layersome formation

Hydrodynamic diameters and zeta potentials of the liposomes and layersomes are shown in Figures 2.3a,b respectively. The surface charge was dominated by the DOPG for the liposomes, giving a zeta potential of -63 mV. PLL deposited spontaneously on

the anionic liposomes, due to polycation binding. As a consequence, the hydrodynamic diameter increased as shown in Figure 2.3a. The zeta potential of the layersomes was +50 mV, shown in Figure 2.3b. Cryo-TEM images of the liposomes and layersomes are shown in Figures 2.3c,d respectively. The increase in membrane thickness of layersomes is a result of the deposition of cationic PLL on the anionic liposome surface. We note that the sizes of the layersomes shown in Figure 2.3d are not representative of the overall distribution.

Figure 2. 3: (a) Number weighted hydrodynamic diameters for liposomes and layersomes. The average diameters for liposomes and layersomes are 106 nm and 122 nm. (b) Zeta potentials for liposomes and layersomes. The liposomes have a zeta potential of -63 mV, while the zeta potential of the layersomes is +50 mV. Error bars are shown. Cryo-TEM images of (c) liposomes and (d) layersomes. The thickness of the layersome membrane is noticeably higher than that of the liposomes, because of deposition of the PLL on the liposome surfaces.

Figure 2. 4: (a) Images of vials containing AuNPs prepared by reduction of 50 mM HAuCl_4 solution by a 200 mM ascorbic acid solution, and gold-layered nanoshell suspensions prepared by direct reduction of 50 mM HAuCl_4 on the layersomes for $[\text{HAuCl}_4]:[\text{lipid}]$ molar ratios of 0.5:1, 1.5:1 and 3:1. (b) Vis-NIR absorbance spectra of the samples shown in (a). (c) SEM image of the AuNPs formed in the solution without layersomes. SEM images of gold nanoshells with $[\text{HAuCl}_4]:[\text{lipid}]$ molar ratios of (d) 0.5:1 (e) 1.5:1 and (f) 3:1. The insets show a magnified image of one of the particles. The molar ratio of AA: HAuCl_4 was 4:1 for all samples.

2.4.2 Gold-layered nanoshells-direct reduction

Figure 2.4a shows vials containing AuNPs only (control) as well as those containing samples prepared by the addition of ascorbic acid to the layersomes in the HAuCl_4 solution. The AuNPs suspension was red and displayed a narrow absorption spectrum with a peak at 530 nm. The gold-layered nanoshell suspension was blue, and this color did not change significantly for different HAuCl_4 to lipid molar ratios. All of the gold-layered nanoshell suspensions showed similar broad absorption spectra between 500 and 1200 nm (Figure 2.4b). Figure 2.4c shows that the AuNPs from the suspension without layersomes were approximately 40-50 nm in diameter. In this direct

reduction method, gold nanoparticles precipitated on a layersome core at all $[\text{HAuCl}_4]:[\text{lipid}]$ ratios, as shown in Figures 2.4d,f. The red shift in the absorption spectrum compared to the absorption with just gold nanoparticles in suspension suggests that the gold nanoparticles absorbed on the layersome surfaces with interparticle distances short enough to give plasmon coupling.³⁴

2.4.3 Gold-layersome nanoshells - seeded growth

Au seeds prepared by reduction of a HAuCl_4 solution by a strong reducing agent, THPC, are shown in Figure 2.5a. The seeds have an average diameter of ~ 3 nm, and zeta potential of -30 mV. When a suspension containing these anionic Au seeds was added to the cationic layersomes in the suspension, the particles were bound to the surface of the layersomes, as shown in Figure 2.5b. The precipitation of gold on these seeds from the growth solution resulted in the growth of Au seeds on the layersome surface, as shown in Figure 2.5c. Further reduction from the gold growth solution led to the formation of a more complete gold layer.

Figure 2. 5: TEM images of (a) Au seeds with diameter of ~ 3 nm (b) Au seeded layersomes (c) Gold islands form on the layersome surface when gold growth solution, $[\text{Au}(\text{OH})_4]^-$, is reduced over the gold seeds.

Figure 2. 6: (a) Effect of $[\text{HAuCl}_4]:[\text{lipid}]$ molar ratio on UV-Vis-NIR absorbance spectra of gold-layersome nanoshells prepared by the seeded growth method.(b) Cryo-SEM of gold nanoshell with $[\text{HAuCl}_4]:[\text{lipid}]$ molar ratio of 0.1:1. SEM images of gold nanoshells with $[\text{HAuCl}_4]:[\text{lipid}]$ molar ratios of (c) 0.5:1. The elemental map shows the colocalization of Au and P, indicating that the gold shell forms on the lipid/layersome template. (d) 1:1 and (e) 3:1.

Figure 2.6a shows absorption spectra of gold-layersome nanoshells prepared through seeded growth at different HAuCl_4 to lipid molar ratios. For a $[\text{HAuCl}_4]:[\text{lipid}]$ molar ratio of 0.1:1, the spectrum is similar to that from a gold nanoparticle suspension. As gold deposits on the seeds, they form islands on the layersome surfaces, as shown in Figure 2.6b, and their interparticle distance is too large to produce plasmon coupling. As the $[\text{HAuCl}_4]:[\text{lipid}]$ molar ratio increases to 1.5:1, more gold is deposited, the interparticle distance decreases, and the absorbance peak red shifts until the shell is complete (Figures 2.6c,d). Energy-dispersive X-ray spectroscopy (EDS) displays colocalization of phosphorous, coming from the liposome, and gold, confirming the

presence of gold on the surface of the layersomes (Figure 2.6c). Beyond a $[\text{HAuCl}_4]:[\text{lipid}]$ molar ratio of 1.5:1, additional gold formation increases the shell thickness (Figure 2.6e), and the absorbance spectrum blue-shifts, because of decreased coupling between surface plasmons in the inner and outer surfaces of the shell.^{9, 35}

We followed standard procedures for sample preparation for cryo-SEM to further examine these particles. These structures, shown in Figure 2.7, are created when the cold diamond knife slices through a vitrified gold nanoshell suspension. Some of the gold shells get sectioned in this process and are seen in the images. The particles are hollow, and the gold shell thickness, as estimated from these images is between 10-20nm. The Vis-IR absorption spectrum of the sample is also characteristic of hollow shells, and not of solid particles of these dimensions.

Figure 2. 7: Cryo-SEM images of gold nanoshells prepared by (a) direct synthesis, $[\text{HAuCl}_4]:[\text{lipid}]$ molar ratio of 0.5, and (b) the seeded growth technique, $[\text{HAuCl}_4]:[\text{lipid}]$ molar ratio of 3:1. The particles are hollow, and the shell thickness is between 10-20nm.

2.4.4 NIR heating

The suspensions of gold–layersome nanoshells with a $[\text{HAuCl}_4]:[\text{lipid}]$ molar ratio of 1.5:1, prepared through the direct reduction and seeded growth techniques, were subjected to NIR laser radiation at 810 nm. The gold shells absorb at this wavelength

and act as local heat sources that raise the bulk temperature of the solution. The transient temperature profiles measured by the thermocouple are shown in Figure 2.8a. After 10 min of irradiation, the temperature increased by 40–50 °C from ambient temperature. In contrast, the temperature of water without the gold-layered nanoshells rose by only ~7 °C, reflecting partial absorption at NIR wavelengths by the water, the vial material, and the thermocouple.

At the length scales corresponding to the vial dimensions, these suspensions can be treated as a sample with a volumetrically homogeneous heat source. A lumped parameter analysis that ignores spatial temperature variations was used to model the transient heating behavior of the samples. The resulting energy balance gives

$$mC_p \frac{dT(t)}{dt} = \dot{Q} - hA(T(t) - T_\infty) \quad (1)$$

where $T(t)$ is the transient sample temperature, m and C_p are the sample mass and the specific heat capacity, respectively (because the gold-layered nanoshell concentration is low, we assumed the heat capacity of the suspension to be the same as that of water), $\dot{Q}$ is the absorbed laser power/volume of sample, h is the convective heat transfer coefficient, T_∞ is the ambient temperature, and A is the surface area for heat transfer, based on the vial dimensions shown in Figure 2.2.

If the fraction of the incoming laser power absorbed by the suspension is K_{abs} , then $\dot{Q} = P \times K_{abs}$, and the transient temperature profile resulting from the integration of Eq. (1) is

$$T(t) = \frac{P \times K_{abs}}{hA} [1 - \exp\left(-\frac{hA}{mC_p} t\right)] + T_\infty \quad (2)$$

K_{abs} and h were obtained first by doing a two-parameter fit for the data from the sample prepared through the seeded growth method, giving $K_{abs} = 0.37$ and $h = 34$ W/m²°K. Keeping h fixed, K_{abs} for the direct reduction sample was determined by fitting that experimental data to Eq. (2). Using these fitted values for K_{abs} , 37% and 29% of the laser power is being converted to heat in the gold-layersome nanoshell suspensions prepared by the seeded growth and direct reduction methods respectively. Figure 2.8b shows greater absorption at 810 nm for the seeded growth sample, in agreement with the trend in the transient temperature. We note that about 5% of the laser power is absorbed by just the water, the vial and thermocouple. This is a small effect compared to absorption in the presence of the gold-coated layersomes.

Figure 2. 8: (a) Transient temperature measurements and heat transfer model predictions (shown by the dashed lines) during irradiation at 810nm for gold-layersomes nanoshells prepared through direct reduction and seeded growth. The [HAuCl₄]:[lipid] molar ratios for

both samples was 1.5:1. The control sample had only water. (b) Absorption spectra of samples prepared by direct reduction and seeded growth methods. The gray dashed line is at 810 nm. (c) Transient temperature measurements at the 5th cycle of irradiation for the two samples and that of gold nanoparticles in a suspension. The fit from the heat transfer model is shown by the dashed line. (d) TEM image of gold nanoparticles prepared by reducing HAuCl₄ by addition of an ascorbic acid solution. TEM images of gold nanoshells after five cycles of heating prepared by (e) direct reduction and (f) seeded growth.

The suspensions prepared by the two methods were then subjected to four additional cycles of 810 nm irradiation. Each cycle consisted of 10 min of irradiation followed by no irradiation until the sample cooled back to room temperature. The temperatures of samples for the fifth irradiation cycle are shown in Figure 2.8c. Interestingly, this temperature transient is similar for samples prepared by either method. When a suspension containing only gold nanoparticles prepared by adding AA to a HAuCl₄ solution at an [AA]:[HAuCl₄] molar ratio of 4:1 was allowed to age for several hours, the particles aggregated. TEM images of those particles are shown in Figure 2.8d. This suspension was irradiated using the same laser. The transient temperature profile mimics that for the gold-layered nanoshell suspensions at the fifth cycle. The absorption coefficient was also similar to that from the first heating cycle of the gold-layered nanoshell suspension prepared by direct reduction, where individual gold particles were the primary constituents of the shell on the surface of the layersomes. Thus, the transient temperature profiles indicate that repeated irradiation of the gold-layered nanoshell suspensions resulted in a breakup of the gold shell into nanoparticles. This degradation was confirmed by TEM images of samples after NIR irradiation, shown in Figure 2.8e,f.

2.5 Conclusion

Two methods have been used for the synthesis of gold nanoshells on layersome surfaces. In the direct reduction approach, reducing tetrachloroauric acid with ascorbic acid leads to the covering of the layersome surface with gold nanoparticles. Absorbance spectra exhibit red shifts as the tetrachloroauric acid to lipid ratios are increased as a result of the greater proximity of gold nanoparticles formed on the surface. When gold nanoparticles were used as heterogeneous nucleation sites, the absorption spectra were broad and red-shifted at low gold growth solution concentrations as complete shells formed. At higher gold growth solution concentrations, the spectrum showed a blue shift because the increased shell thickness resulted in less coupling between plasmons at the inner and outer surfaces. NIR illumination at 810 nm for the nanostructure suspensions showed a distinct temperature rise, indicating that the gold–layersome nanoshells absorb strongly at that wavelength and dissipate that energy as heat. Repeated NIR irradiation degraded the gold coating on the surfaces of the layersomes into 5–10 nm gold nanoparticles that can be cleared through the renal system. Thus, the NIR-triggered gold–layersome nanoshell suspension has the potential to be used in photothermal therapy and photothermally targeted drug delivery as well as in imaging.

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2.7 References

1. Abadeer, N. S.; Murphy, C. J., Recent Progress in Cancer Thermal Therapy Using Gold Nanoparticles. *The Journal of Physical Chemistry C* **2016**, *120* (9), 4691-4716.
2. Rengan, A. K.; Jagtap, M.; De, A.; Banerjee, R.; Srivastava, R., Multifunctional gold coated thermo-sensitive liposomes for multimodal imaging and photo-thermal therapy of breast cancer cells. *Nanoscale* **2014**, *6* (2), 916-23.
3. Daniel, M. C.; Astruc, D., Gold nanoparticles: Assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chem. Rev.* **2004**, *104* (1), 293-346.
4. Hu, M.; Chen, J. Y.; Li, Z. Y.; Au, L.; Hartland, G. V.; Li, X. D.; Marquez, M.; Xia, Y. N., Gold nanostructures: engineering their plasmonic properties for biomedical applications. *Chem. Soc. Rev.* **2006**, *35* (11), 1084-1094.
5. Huang, X.; El-Sayed, M. A., Gold nanoparticles: Optical properties and implementations in cancer diagnosis and photothermal therapy. *Journal of Advanced Research* **2010**, *1*, 13-28.
6. Li, N.; Zhao, P.; Astruc, D., Anisotropic Gold Nanoparticles: Synthesis, Properties, Applications, and Toxicity. *Angewandte Chemie International Edition* **2014**, *53*, 1756-1789.
7. Kedia, A.; Kumar, P. S., Controlled reshaping and plasmon tuning mechanism of gold nanostars. *J. Mater. Chem. C* **2013**, *1* (30), 4540-4549.
8. Sanchez-Gaytan, B. L.; Swanglap, P.; Lamkin, T. J.; Hickey, R. J.; Fakhraai, Z.; Link, S.; Park, S.-J., Spiky Gold Nanoshells: Synthesis and Enhanced Scattering Properties. *The Journal of Physical Chemistry C* **2012**, *116* (18), 10318-10324.
9. Jain, P. K.; El-sayed, M. a., Universal Scaling of Plasmon Coupling in Metal Nanostructures : Extension from Particle Pairs to Nanoshells Universal Scaling of Plasmon Coupling in Metal Nanostructures : Extension from Particle Pairs to Nanoshells. **2007**.
10. Link, S.; El-Sayed, M. A., Size and Temperature Dependence of the Plasmon Absorption of Colloidal Gold Nanoparticles. *J. Phys. Chem.* **1999**, *103*, 4212.

11. Jain, P. K.; Lee, K. S.; El-Sayed, I. H.; El-Sayed, M. A., Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: Applications in biological imaging and biomedicine. *J. Phys. Chem. B* **2006**, *110*, 7238-7248.
12. Bardhan, R.; Lal, S.; Joshi, A.; Halas, N. J., Theranostic Nanoshells: From Probe Design to Imaging and Treatment of Cancer. *Accounts of Chemical Research* **2011**, *44*, 936-946.
13. Averitt, R.; Sarkar, D.; Halas, N., Plasmon Resonance Shifts of Au-Coated Au₂S Nanoshells: Insight into Multicomponent Nanoparticle Growth. *Physical Review Letters* **1997**, *78*, 4217-4220.
14. Prodan, E.; Radloff, C.; Halas, N. J.; Nordlander, P., A hybridization model for the plasmon response of complex nanostructures. *Science (New York, N.Y.)* **2003**, *302*, 419-422.
15. Lal, S.; Link, S.; Halas, N. J., Nano-optics from sensing to waveguiding. *Nat. Photonics* **2007**, *1*, 641-648.
16. Neeves, A. E.; Birnboim, M. H., Composite structures for the enhancement of nonlinear-optical susceptibility. *Journal of the Optical Society of America B* **1989**, *6*, 787.
17. Hirsch, L. R.; Stafford, R. J.; Bankson, J. a.; Sershen, S. R.; Rivera, B.; Price, R. E.; Hazle, J. D.; Halas, N. J.; West, J. L., Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 13549-54.
18. Rengan, A. K.; Bukhari, A. B.; Pradhan, A.; Malhotra, R.; Banerjee, R.; Srivastava, R.; De, A., In Vivo Analysis of Biodegradable Liposome Gold Nanoparticles as Efficient Agents for Photothermal Therapy of Cancer. *Nano Lett.* **2015**, *15*, 842-848.
19. Troutman, T. S.; Barton, J. K.; Romanowski, M., Biodegradable plasmon resonant nanoshells. *Adv Mater* **2008**, *20* (13), 2604-+.
20. Jin, Y., Multifunctional compact hybrid Au nanoshells: A new generation of nanoplasmonic probes for biosensing, imaging, and controlled release. *Accounts of Chemical Research* **2014**, *47*, 138-148.

21. Leung, S. J.; Romanowski, M., NIR-activated content release from plasmon resonant liposomes for probing single-cell responses. *ACS Nano* **2012**, *6*, 9383-9391.
22. Brewer, E.; Coleman, J.; Lowman, A., Emerging Technologies of Polymeric Nanoparticles in Cancer Drug Delivery. *Journal of Nanomaterials* **2011**, *2011*, 1-10.
23. Wu, G. H.; Milkhailovsky, A.; Khant, H. A.; Fu, C.; Chiu, W.; Zasadzinski, J. A., Remotely triggered liposome release by near-infrared light absorption via hollow gold nanoshells. *J. Am. Chem. Soc.* **2008**, *130* (26), 8175-+.
24. Erickson, T. a.; Tunnell, J. W., Gold Nanoshells in Biomedical Applications. *Nanomaterials for the life Science Vol. 3: Mixed Metal Nanomaterials* **2009**, *3*, 1-44.
25. Choi, H. S.; Liu, W.; Misra, P.; Tanaka, E.; Zimmer, J. P.; Itty Ipe, B.; Bawendi, M. G.; Frangioni, J. V., Renal clearance of quantum dots. *Nat. Biotechnol.* **2007**, *25*, 1165.
26. Troutman, T. S.; Barton, J. K.; Romanowski, M., Biodegradable Plasmon Resonant Nanoshells. *Adv Mater* **2008**, *20* (13), 2604-2608.
27. Volodkin, D.; Ball, V.; Schaaf, P.; Voegel, J. C.; Mohwald, H., Complexation of phosphocholine liposomes with polylysine. Stabilization by surface coverage versus aggregation. *Biochimica et Biophysica Acta - Biomembranes* **2007**, *1768*, 280-290.
28. Wang, F.; Liu, J., Self-healable and reversible liposome leakage by citrate-capped gold nanoparticles: probing the initial adsorption/desorption induced lipid phase transition. *Nanoscale* **2015**, *7*, 15599-15604.
29. Jin, Y. D.; Gao, X. H., Spectrally Tunable Leakage-Free Gold Nanocontainers. *J. Am. Chem. Soc.* **2009**, *131* (49), 17774-17776.
30. Wang, M.; Liu, Y.; Zhang, X.; Luo, L.; Li, L.; Xing, S.; He, Y.; Cao, W.; Zhu, R.; Gao, D., Gold nanoshell coated thermo-pH dual responsive liposomes for resveratrol delivery and chemo-photothermal synergistic cancer therapy. *J. Mater. Chem. B* **2017**, *5* (11), 2161-2171.
31. Troutman, T. S.; Barton, J. K.; Romanowski, M., Biodegradable plasmon resonant nanoshells. *Advanced Materials* **2008**, *20*, 2604-2608.

32. Duff, D. G.; Baiker, A.; Edwards, P. P., A New Hydrosol of Gold Clusters. 1. Formation and Particle Size Variation. *Langmuir* **1993**, *9*, 2301-2309.
33. Young, J. K.; Lewinski, N. A.; Langsner, R. J.; Kennedy, L. C.; Satyanarayan, A.; Nammalvar, V.; Lin, A. Y.; Drezek, R. A., Size-controlled synthesis of monodispersed gold nanoparticles via carbon monoxide gas reduction. *Nanoscale Research Letters* **2011**, *6*, 428.
34. Rechberger, W.; Hohenau, A.; Leitner, A.; Krenn, J. R.; Lamprecht, B.; Aussenegg, F. R., Optical properties of two interacting gold nanoparticles. *Optics Communications* **2003**, *220* (1-3), 137-141.
35. Oldenburg, S. J.; Averitt, R. D.; Westcott, S. L.; Halas, N. J., Nanoengineering of optical resonances. *Chemical Physics Letters* **1998**, *288*, 243-247.

CHAPTER 3

Carbon Black Templated Gold Nanoparticles for Analyte Detection by Surface Enhanced Raman Spectroscopy

(under review in ACS Applied Nanomaterials)

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3.1 Abstract

A novel approach is reported to create hybrid gold nanostructures for surface enhanced Raman Scattering (SERS) using polycation-layered fractal carboxyl-terminated carbon black nanoparticles (CB) as templates. Using this approach, the dependence of nanostructure surface topography and absorbance characteristics on SERS detection was examined for a number of distinct probe molecules. Anionic CB nanoparticles were first coated with the cationic polyelectrolyte poly-L-lysine (PLL), and gold-carbon black (Au-PLLCB) particles were formed by the reduction of tetrachloroauric acid that had accumulated on the surface of the PLL-coated CB templates. The Au-PLLCB particles produced strong SERS signals for 4-nitrobenzene thiol (4-NBT) in ethanol, and for Congo red, crystal violet, and nitrate ions in water. The fractal morphology of the underlying carbon black template and the presence of PLL promote the formation of sharp gold spikes on the surface, yielding hot spots for Raman enhancement. The carbon also acts as an absorbent for organic molecules, allowing analytes even with poor affinity for the gold surface to concentrate in regions close enough to the particle surfaces to enable SERS. The morphology and chemical nature of the underlying template make the Au-PLLCB a broadly applicable substrate for detecting of a wide range of analytes in solution.

3.2 Introduction

Engineering the shape and surface topology of gold nanostructures allows tuning of their localized surface plasmon resonance (LSPR) wavelength, making them attractive for sensing, photothermal applications and imaging.¹⁻⁴ Upon excitation at appropriate wavelengths, the collective oscillation of surface plasmons can result in strong

absorption of the incident energy.^{5, 6} If the surface topology of these particles has nanoscale features, such as sharp tips and edges, the electric field near these features, termed hot spots, is enhanced significantly due to the high confinement of electrons.⁷ The amplified electric field enhances the inherently weak Raman signal from molecules in close proximity to the surface, a phenomenon called Surface Enhanced Raman Scattering (SERS).⁸ As a result, it is possible to detect organic, inorganic and biological molecules at low concentrations with SERS.⁹⁻¹¹

Different size and shaped particles, such as nanorods, nanotriangles, nanocages and nanostars have been synthesized for SERS applications.¹²⁻¹⁴ The maximum Raman signal enhancement can be expected when the excitation wavelength matches the peak LSPR wavelength for the particle.¹⁵ Core-shell structures are promising as SERS substrates as their LSPR peak can be adjusted to a desired wavelength by varying the size and/or the shell thickness.¹⁶⁻¹⁸ The development of robust SERS substrates with high enhancement factors for the detection of a wide range of molecules and ions is an active area of research,¹⁹⁻²² and is the focus of this work.

The evanescent wave character of surface plasmons in metal nanoparticles implies that the local electric field decays exponentially away from the surface, with a typical decay length of approximately 5 nm.^{23, 24} Probe molecules must therefore be in close proximity to the metal surface to provide detectable Raman signals. Many types of molecules such as polycyclic aromatic hydrocarbons (PAHs) cannot be analyzed using conventional SERS substrates due to their poor affinity for metal surfaces.²⁵ To address the issue, various surface functionalization strategies have been introduced that promote capture of specific analytes on the metal surface.²⁶⁻²⁸ Usually, each ligand is specific

only to a small subset of analytes restricting the broad applicability of this method.²⁹ A combination of gold nanoparticles with carbon nanomaterials (*e.g.*, graphene, graphene oxide and carbon nanotubes) is an alternative approach for physisorption of diverse molecules for higher SERS enhancement.³⁰⁻³³ The high specific surface area of several carbon nanomaterials and their affinity for a range of molecules have made them effective adsorbents.³⁴ This adsorbing property of underlying carbon can be exploited for bringing molecules in close proximity to metal surfaces.³⁵

We describe a simple strategy to produce gold-carbon nanoparticles that are effective SERS substrates for the detection of a wide range of analytes in aqueous solution. We show the potential of these gold-carbon nanoparticles for the detection of two groups of important anthropogenic water contaminants - organic dyes and nitrate ions. The persistence of dyes in the environment has potential carcinogenic and mutagenic effects.³⁶ Excess amounts of nitrates (for example, coming from fertilizer runoff) in water bodies can cause large increases in aquatic plant growth, leading to local hypoxia that negatively affects aquatic life.³⁷

3.3 Experiments

3.3.1 Materials

A para amino benzoic acid-terminated carbon black suspension in water at pH 7.5 was obtained from Cabot Corporation. Poly-L-lysine hydrochloride (PLL, MW = 15,000–30,000), tetrachloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), 4- nitrobenzenethiol (4-NBT), crystal violet (CV), congo red (CR), sodium nitrate and ascorbic acid (AA) were purchased from Sigma-Aldrich. Deionized water (DIW), with a resistance of 18 M Ω ,

was obtained from a Millipore Direct-3Q purification system. All materials were used as received.

Figure 3. 1: Synthesis of gold-coated carbon black (CB) nanoparticles. CB NPs were first coated with the cationic polyelectrolyte poly-L-lysine (PLL). PLL concentrates anionic gold tetrachloride (AuCl_4^-) near the particle surfaces. Metallic gold is precipitated on the particles by reduction of the gold tetrachloride ions with ascorbic acid.

3.3.2 Preparation of PLL-coated CB NP suspensions

In a typical synthesis of PLL coated CB nanoparticles, 1 mL of a 0.015wt% CB suspension was added dropwise to 4cc of a 0.019wt% aqueous solution of PLL, and stirred for 30 min. The suspension was then centrifuged at 17,000g for 60 min. The supernatant was removed, and the pellet was redispersed in 5 mL of DIW using a vortex mixer. Centrifuging removed unbound PLL from the suspension. Suspensions prepared with PLL:CB weight ratios ranging from 0.5:1 to 10:1 were used to monitor the adsorption of PLL on the CB surfaces by zeta potential measurements. Two suspensions were prepared with a PLL:CB weight ratio of 5:1 (PLLCB5 NPs) for subsequent precipitation of gold. One was centrifuged to remove unbound PLL and the other was

used without centrifugation. The presence of unbound PLL has a strong impact on the morphology of the gold forming on the CB particles.

3.3.3 Preparation of structured gold nanoparticles

Aqueous solutions of 50 mM HAuCl_4 and 75 mM ascorbic acid were used for the synthesis of structured gold nanoparticles. An ascorbic acid to HAuCl_4 molar ratio of 1.5:1 was used for all experiments.

Gold-coated CB nanoparticles were prepared using the steps outlined in Figure 3.1. First, 1 ml of PLLCB5 NPs from the centrifuged suspension was mixed with 80 μl of the HAuCl_4 solution, and the gold tetrachloride ions were reduced using 80 μl of ascorbic acid. These nanoparticles are labeled $\text{Au-PLLCB}_{\text{CF}}$. To incorporate the potential shape directing effect of PLL for maximizing roughness of gold on the carbon black template, 80 μl of HAuCl_4 solution were also reduced in a 1 ml suspension of PLLCB5 NPs that was not centrifuged. The gold tetrachloride ions, bound to PLLCB5 NP surfaces, were reduced by addition of 80 μl ascorbic acid. These nanoparticles are called Au-PLLCB NPs. Gold nanoparticles were also prepared without CB and are labeled Au-PLL NPs. For these samples, 80 μl of HAuCl_4 .

3.3.4 Preparation of SERS-active substrates and detection experiments

Dispersions of $\text{Au-PLLCB}_{\text{CF}}$, Au-PLLCB and Au-PLL NPs were pelleted by centrifugation at 3000g for 10 min and resuspended in 400 μl deionized water (DIW) using vortex mixing to reduce the colloidal suspension to $\sim 35\%$ of the initial volume. Silicon wafers (5 mm $\times$ 5 mm) were plasma cleaned, and 30 μl of $\text{Au-PLLCB}_{\text{CF}}$, Au-PLLCB and Au-PLL suspensions were deposited onto wafers and allowed to dry under

ambient conditions to form a continuous layer of nanoparticles. The particle loading on the silicon wafers was estimated to be $\sim 2.4 \mu\text{g}/\text{mm}^2$. 4-NBT in 15 μl of ethanol was deposited on each wafer and dried by vacuum evaporation. The SERS activity of Au-PLLCB_{CF}, Au-PLLCB and Au-PLL was compared. For the detection of other analytes, the particle-loaded wafers were immersed in 0.5 ml of 1 μM aqueous solutions of CR, CV and different concentrations of sodium nitrate solution for 30 min. The wafers were washed and dried by vacuum evaporation.

UV–vis absorption spectroscopy was conducted to determine the concentrations of CR and CV solutions before and after contact with the wafers. The amounts adsorbed on each wafer was estimated by subtracting the concentration of analyte in solution after exposure from the initial concentration of the analyte and multiplying this concentration difference by the withdrawn volume.

3.3.5 Characterization

Nanoparticles were imaged using transmission electron microscopy (JEOL JEM-2100) at an accelerating voltage of 200 kV, and by scanning electron microscopy (Zeiss Sigma VP FESEM). Particles were examined on a Rigaku Ultima IV X-Ray diffractometer. An Oxford Inca energy dispersive X-ray (EDX) system was used for composition analysis. A Malvern Zetasizer Nano ZS was used to determine the zeta potentials of the CB and PLLCB NPs. Three different samples were analyzed, and three measurements were made for each sample; the average zeta potentials and standard deviations from these measurements were reported. Absorption spectra were collected using a UV–vis–NIR scanning spectrophotometer (Jasco, Tokyo, Japan) over wavelengths from 400 to 1300 nm. Absorption from deionized water was used as the

reference. Raman spectra were recorded by using a Sierra portable system (Snowy Range Instrument) that has a 785 nm laser operating at 100 mW. The operational wavenumber range is 200–2000 cm^{-1} with a resolution of 8 cm^{-1} . The spot size on the sample was approximately 30 μm in diameter. By rastering the focused laser beam over the sample, a 20 mm^2 area was interrogated without a loss in resolution.³⁸ Each SERS spectrum was accumulated for 10 s.

Figure 3. 2: (a) TEM image of CB NPs shows the fractal structure of CB (scale bar = 200 nm). The inset shows magnified image of a CB NP consisting of 10-30 nm primary particles that fused together (scale bar = 50 nm). (b) Zeta potential of PLLCB NPs as a function of the PLL/CB weight ratio. The carboxylated CB NPs had an initial zeta potential of -44.7 ± 0.5 .

3.4 Results and discussion

3.4.1 Particle characterization

Figure 3.2a, shows CB NPs, consisting of primary particles with a diameter between 10 nm and 30 nm, fused together to form a fractal structure. These CB NPs have a zeta potential of -44.7 ± 0.5 mV because of deprotonation of carboxylate groups on the surface at pH 7. Poly-L-lysine (PLL) is a cationic polyelectrolyte with lysine as a repeat unit. When the CB NPs are exposed to a PLL solution, PLL binds to the particles and

reverses the surface charge. The zeta potential increases, and then starts to plateau beyond a PLL:CB weight ratio of $\sim 5:1$ at +60 mV. The cationic groups in PLL serve as adsorption sites for gold chloride anions.

Figure 3. 3: (a) SEM image of Au-PLLCB_{CF} NPs. The bright regions are gold precipitated on the carbon black surface (scale bar = 200 nm). The inset shows a magnified image of a particle (scale bar = 50 nm). (b) XPS spectra of the Au-PLLCB_{CF} NPs and PLLCB5 NPs and (c) XRD pattern of Au-PLLCB_{CF} NPs. (d) UV-vis-NIR absorbance spectra of the Au-PLLCB_{CF} shows a peak maximum at 633 nm. No peaks are observed for PLLCB5 NPs.

Addition of ascorbic acid reduces the gold chloride ions to gold on the surface of the PLLCB particles, forming clusters as shown in Figure 3.3a. The PLL coating promotes the adsorption of gold tetrachloride anions on the PLLCB surface. The bulk concentration of gold tetrachloride reduces to below the supersaturation limit. No gold is nucleated in the bulk solution. Figure 3.3b shows a XPS spectrum of both PLLCB5 and Au-PLLCB_{CF} NPs. The PLLCB5 XPS spectrum contains peaks at 285 eV for C-C

bonds, 400 eV for the $\text{NH}_2\text{-C}$ bonds and 533.6 eV for the O=C bonds, confirming PLL coverage on the carbon black surface. For Au-PLLCB, the presence of Au 4f7/2 and 4f5/2 doublets with binding energies of 84.0 eV and 87.6 eV, which are typical values for Au^0 , demonstrate that gold has been deposited onto the surface of PLLCB.

Figure 3. 4: SEM images of (a) Au-PLLCB formed by reduction of HAuCl_4 in a dispersion of PLLCB with unbound PLL (b) Au-PLL NPs formed by the addition of HAuCl_4 to a solution of PLL and reduction of that solution using ascorbic acid (scale bar = 200 nm). (c) UV-vis-NIR absorbance spectra of Au-PLLCB and Au-PLL, showing peaks maxima at 770 nm and 1179 nm, respectively. (d) Raman spectra of Au-PLL and Au-PLLCB NPs. The peaks at 1324 cm^{-1} and 1595 cm^{-1} are characteristic for carbon nanomaterials (D-band and G band, respectively). The weak peak at 1446 cm^{-1} corresponds to the bending mode of CH_2 in the PLL lysine side chain.

The nanoparticles were examined by X-ray diffraction (XRD), and the results are shown in Figure 3.3c. Peaks at 38.2°, 44.4°, 64.6°, 77.6° and 81.7°, correspond to the

(111), (200), (220), (311), and (222) facets of the FCC phase of Au. The underlying CB does not exhibit any peaks implying the carbon was amorphous. Vis-NIR absorbance spectra of aqueous dispersion of Au-PLLCB_{CF} NPs show a peak at 633 nm, whereas no peak is appeared for the PLLCB5 in the range of 500 to 1200 nm. The location of the LSPR peak of these nanoparticles depends on their size and surface morphology.

Figure 3.4a shows Au-PLLCB NPs with tip-like protrusions formed in the suspension that had unbound PLL. In this sample, the gold nanostructure first forms on the CB surface. PLL from the solution then binds to specific crystal facets of gold through the NH_3^+ groups on their lysine side chains.³⁹ This adsorbed PLL then promotes further formation and growth of sharp gold tips.^{40, 41}

Gold tetrachloride anions were also reduced in PLL solution only (no CB; Figure 3.4b). PLL adsorbs on nucleated gold particles, modulating the shape of the growing particles, and led to the formation of flower-like nanoparticles.⁴² These results are consistent with those from other groups that have studied the structure directing roles of surfactants and polymers by selective binding to crystal facets.^{43, 44} This morphological variation, with and without CB present, shifts the LSPR peak from 770 nm for Au-PLLCB to 1179 nm for Au-PLL NP (Figure 3.4c).

The Raman spectra of Au-PLLCB show three distinct peaks; the D-band (1324 cm^{-1}), which is a disorder-activated Raman mode, and the G-band (1595 cm^{-1}), which is characteristic of sp^2 -hybridized carbon atoms (Figure 3.4d). The sp^2 – hybridized carbon is important for the adsorption of aromatic molecules through π - π interactions.⁴⁵ The low intensity peak at 1446 cm^{-1} for both Au-PLLCB and Au-PLL NPs is due to bending mode of CH_2 in the lysine side chain of adsorbed PLL on the surface ⁴⁶. Both

particles are positively charged with zeta potential values of $48.5 \pm 1.75 \text{ mV}$ and $61.5 \pm 0.95 \text{ mV}$ for Au-PLLCB and Au-PLL NPs, respectively; further confirming the presence of PLL presence on the surface of both particles. However, low lysine signal intensity from Au-PLL NPs can be the result of the low enhancement effect from those particles.

3.4.2 Evaluation of the SERS signals from the particles

The assignments of the Raman bands for 4-NBT, congo red, crystal violet and nitrate ion are shown in Table 1.

Table 3. 1: Raman shifts of four probe molecules

Probe molecules	Raman shift (cm^{-1})	Band assignment ⁴⁷⁻⁵¹
4-NBT	1079	C-S stretch
	1110	In plane C-H bending
	1331	NO_2 symmetric stretch
	1568	Ring C-C stretch
CR	1154	Phenyl-N stretch
	1286	Phenyl-phenyl stretch
	1373	Naphthyl ring C-C stretch
	1593	Phenyl ring C-C stretch
CV	913	Ring skeletal vibration of radial orientation
	1175	Ring C-H in-plane bending
	1374	N-phenyl stretch
	1586 & 1621	Ring C-C stretch
NO_3^-	1048	N-O stretch

To evaluate the effect of nanoparticle surface topography on Raman enhancement, the SERS activity of Au-PLLCB_{CF}, Au-PLLCB and Au-PLL substrates was compared after evaporation of 10 μ M 4-NBT in ethanol on each substrate. Several peaks are observed in the SERS spectrum for CB templated gold substrates (Au-PLLCB_{CF}, Au-PLLCB) and the Au-PLL substrate (Figure 3.5a). The more ‘rounded’ morphology of the Au-PLLCB_{CF} particles gave a much weaker signal than from the Au-PLLCB and was not pursued further. Au-PLLCB and Au-PLL nanoparticles have sharp spikes and edges on their surface that act as hot spots for Raman enhancement. The strongest signals came from the Au-PLLCB substrate.

Figure 3. 5: (a) Raman signals from Au-PLLCB_{CF}, Au-PLLCB and Au-PLL substrates for 10 μ M 4-NBT. SEM images show corresponding particles (scale bars = 100 nm). The Au-PLLCB_{CF} particles show a smoother surface topology than the Au-PLL CB particles; this lack of sharp features results in weakest Raman signals for 4-NBT. (b) The intensity of the SERS peaks of 4-NBT (1331 cm^{-1}) at different concentration of 4-NBT from Au-PLLCB and Au-PLL substrates. The value “n” in (b) represents $n_{1331} = I_{\text{Au-PLLCB}} / I_{\text{Au-PLL}}$ which is the SERS intensity ratio of the 1331 cm^{-1} peak from two substrates at each 4-NBT concentration.

Figure 3. 6: Raman spectra of (a) congo red and (b) crystal violet on Au-PLLCB and Au-PLL substrates from 1 μM aqueous solution. The excitation wavelength used was 785 nm. Reduction of analyte concentration due to adsorption after 30 min of immersing of substrate in 1 μM solution of (d) congo red and (e) crystal violet, calculated based on UV-vis absorption intensity of the analytes

The 4-NBT peak located at 1331cm^{-1} was chosen to demonstrate the increase in SERS intensity from Au-PLLCB compared to Au-PLL. As shown in Figure 3.5b, the Raman signal intensity for both substrates increases with as the 4-NBT concentration goes from 1 μM to 1 mM. We analyzed the intensity ratio of the 1331 cm^{-1} peak between the Au-PLLCB and Au-PLL substrates ($n_{1331}=I_{\text{Au-PLLCB}}/I_{\text{Au-PLL}}$). For 1 μM 4-NBT, the intensity ratio $n_{1331} = 100$), represents two order of magnitude stronger signals from Au-PLLCB compared to that of Au-PLL particles. This is remarkably high and can be attributed to the numerous hot spots distributed on the anisotropic surfaces of the Au-PLLCB NPs, as well as the effective LSPR excitation of nanoparticles at 785 nm. Au-PLL NPs have a flower-like structure comprising of 2D anisotropic nanocrystals or

nanoplates with deep crevices. The sharp corners and edges in the Au nanoplates exhibit excellent Raman enhancement.⁵² However, the flat surfaces of the nanoplates show little SERS activity.⁴³ So the SERS signals in this case, are dominated by just molecules adsorbed at the corners and edges of nanoplates which result in the weak Raman signals for low concentration of 4-NBT. However, as the 4-NBT concentration increases, corners and edges adsorb more molecules, resulting in higher signals. The Raman intensity ratio reduces to $I_{1331} = 20$, at 1 mM 4-NBT. This result signifies the importance of the distribution of hot spots on the gold since the presence of analyte molecules in their proximity plays an important role in the degree of enhancement.

To investigate the role of the underlying carbon on the Raman enhancement due to analyte adsorption on the nanoparticle surface, two organic dyes with different surface charges were used as Raman probes. Congo red (CR) is an anionic azo dye with one central biphenyl group and two symmetric naphthalenic groups and crystal violet (CV) is a positively charged triphenylmethane dye.⁵⁰ Raman peaks of CR and CV are shown in Figures 3.6a,b. Considering 1154 cm^{-1} and 1174 cm^{-1} as representative peaks for CR and CV, respectively, Au-PLLCB particles result in 16- (CR) and 65-fold (CV) higher Raman signals than those from Au-PLL particles. This result can be rationalized by the synergistic effect of analyte affinity towards the substrates and their SERS activity. The positively charged Au-CBPLL and Au-PLL substrates show the same affinity to the negatively charged CR as confirmed by surface adsorption (Figure 3.6c). The 16-fold higher signal from Au-PLLCB is due to the higher SERS activity of Au-PLLCB compared to Au-PLL. On the other hand, the greater enhancement effect of the Au-PLLCB for CV (65 times higher than from Au-PLL) can be attributed to the

combination of its higher SERS activity and higher adsorption affinity to the surface by π - π interactions, which concentrate more probe molecules on the surface of the nanoparticles. Polycyclic aromatic compounds with short alkyl chains can be selectively adsorbed by carbon black nanoparticles.⁵³ Our results suggest that π - π interactions are more significant than electrostatic repulsion for CV.

Figure 3. 7: (a) Raman spectra of sodium nitrate (100 μ M) on Au-PLLCB versus Au-PLLCB substrate. The N-O stretch from the nitrate ions appears at 1048 cm^{-1} . (b) SERS intensity at 1048 cm^{-1} at different concentrations of sodium nitrate. Each data point represents the average intensity at 1048 cm^{-1} for each concentration of sodium nitrate from three different experiments, with the standard deviation shown by the error bars. The inset shows the linear region of the SERS intensity – sodium nitrate concentration curve. R^2 value of 0.9970 obtained for the linear region.

3.4.3 Detection of nitrate ions

Given the effectiveness of Au-PLLCB for detection of CR and CV, we explored its potential to detect nitrate ions in water. As shown in Figure 3.7a, a 100 μ M sodium nitrate a pronounced Raman band at 1048 cm^{-1} , coming from the N-O stretching mode of nitrate ion. Intensity of the peak at 1048 cm^{-1} was then used for quantitative detection of sodium nitrate (Figure 3.7b). The SERS signal intensity increases with increasing

nitrate concentration up to 1 mM, after which the signal remains almost constant as the hot spots on the surface become saturated by nitrate ions. As shown in the inset of Figure 3.7b, the intensity- concentration curve is linear up to 500 μM with a linear regression coefficient value (R^2) of 0.9970. The limit of detection ($\text{LOD} = 3 \frac{\sigma}{\text{slope}}$) is estimated using slope of the regression line and uncertainty in the line intercept (σ),⁵⁴ to be 15 μM . We note that for environmental purposes, 10 ppm or $\sim 117 \mu\text{M}$ concentration has been reported to be the desired detection limit of nitrate ions.⁵¹

3.5 Conclusion

A novel template precipitation approach has been used for the hybridization of gold with carbon nanomaterials to generate highly active SERS particles for the detection of a wide variety of analytes. The adsorbed cationic polyelectrolyte, PLL, on carbon black concentrates the AuCl_4^- anions on the surface, providing an enriched interface for reducing the anions to gold nanostructures. Equally important was the ability to further tailor the surface topography of the gold nanoparticles using PLL as a shape-directing agent, producing sharp tips and edges that enhance incident electric fields. As a result, the Au-PLLCB substrate was capable of detecting of both cationic and anionic aromatic dyes due to the dual adsorbing role of the underlying carbon template via π - π interactions and the PLL coating via charge attraction. Furthermore, the substrate was able to provide quantitative detection of nitrate ions at and below environmentally relevant concentrations. These results suggest that Au-PLLCB hybrid particles have great potential as sensitive SERS-active materials for detection of a wide range of analytes in aqueous solutions.

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3.7 References

1. Jain, P. K.; Lee, K. S.; El-Sayed, I. H.; El-Sayed, M. A., Calculated Absorption and Scattering Properties of Gold Nanoparticles of Different Size, Shape, and Composition: Applications in Biological Imaging and Biomedicine. *J. Phys. Chem.* **2006**, *110* (14), 7238-7248.
2. Xia, Y.; Xiong, Y.; Lim, B.; Skrabalak, S. E., Shape-controlled synthesis of metal nanocrystals: simple chemistry meets complex physics? *Angew Chem Int Ed Engl* **2009**, *48* (1), 60-103.
3. Huang, X.; El-Sayed, I. H.; Qian, W.; El-Sayed, M. A., Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods. *J. Am. Chem. Soc.* **2006**, *128* (6), 2115.
4. Saha, K.; Agasti, S. S.; Kim, C.; Li, X.; Rotello, V. M., Gold Nanoparticles in Chemical and Biological Sensing. *Chemical reviews.* **2012**, *112* (5), 2739-2779.
5. Mayer, K. M.; Hafner, J. H., Localized surface plasmon resonance sensors. *Chem Rev* **2011**, *111* (6), 3828-57.
6. Abbasi, A.; Park, K.; Bose, A.; Bothun, G. D., Near-Infrared Responsive Gold-Layersome Nanoshells. *Langmuir* **2017**, *33* (21), 5321-5327.
7. Daniel, M. C.; Astruc, D., Gold-Nanoparticles-Assembly-Supramolecular-Chemistry-QuantumSizeRelated-Properties-and-Applications-Toward-Biology-Catalysis-and-Nanotechnology. *Chem. Rev.* **2004**, *104*, 293-346.
8. McNay, G.; Eustace, D.; Smith, W. E.; Faulds, K.; Graham, D., Surface-enhanced Raman scattering (SERS) and surface-enhanced resonance Raman scattering (SERRS): a review of applications. *Appl Spectrosc* **2011**, *65* (8), 825-37.
9. Ding, S.-Y.; Yi, J.; Li, J.-F.; Ren, B.; Wu, D.-Y.; Panneerselvam, R.; Tian, Z.-Q., Nanostructure-based plasmon-enhanced Raman spectroscopy for surface analysis of materials. *Nature Reviews Materials* **2016**, *1* (6).
10. Park, M.; Hwang, C. S. H.; Jeong, K.-H., Nanoplasmonic Alloy of Au/Ag Nanocomposites on Paper Substrate for Biosensing Applications. *ACS applied materials & interfaces.* **2018**, *10* (1), 290-295.

11. Lu, P.; Lang, J.; Weng, Z.; Rahimi-Iman, A.; Wu, H., Hybrid Structure of 2D Layered GaTe with Au Nanoparticles for Ultrasensitive Detection of Aromatic Molecules. *ACS applied materials & interfaces*. **2018**, *10* (1), 1356-1362.
12. Alvarez-Puebla, R.; Liz-Marzán, L. M.; García de Abajo, F. J., Light Concentration at the Nanometer Scale. *The Journal of Physical Chemistry Letters* **2010**, *1* (16), 2428-2434.
13. Reguera, J.; Langer, J.; Jimenez de Aberasturi, D.; Liz-Marzan, L. M., Anisotropic metal nanoparticles for surface enhanced Raman scattering. *Chem Soc Rev* **2017**, *46* (13), 3866-3885.
14. Khoury, C. G.; Vo-Dinh, T., Gold Nanostars For Surface-Enhanced Raman Scattering: Synthesis, Characterization and Optimization. *Journal of physical chemistry*. **2008**, *112* (48), 18849-18859.
15. Alvarez-Puebla, R. A., Effects of the Excitation Wavelength on the SERS Spectrum. *J Phys Chem Lett* **2012**, *3* (7), 857-66.
16. Xie, Y.; Chen, T.; Guo, Y.; Cheng, Y.; Qian, H.; Yao, W., Rapid SERS detection of acid orange II and brilliant blue in food by using Fe₃O₄@Au core-shell substrate. *Food Chem* **2019**, *270*, 173-180.
17. Wang, H.; Kundu, J.; Halas, N. J., Plasmonic nanoshell arrays combine surface-enhanced vibrational spectroscopies on a single substrate. *Angew Chem Int Ed Engl* **2007**, *46* (47), 9040-4.
18. Jackson, J. B.; Halas, N. J., Surface-enhanced Raman scattering on tunable plasmonic nanoparticle substrates. *Proc Natl Acad Sci U S A* **2004**, *101* (52), 17930-5.
19. Sharma, B.; Frontiera, R. R.; Henry, A.-I.; Ringe, E.; Van Duyne, R. P., SERS: Materials, applications, and the future. *Materials Today* **2012**, *15* (1-2), 16-25.
20. Joseph, D.; Huh, Y. S.; Han, Y.-K., A top-down chemical approach to tuning the morphology and plasmon resonance of spiky nanostars for enriched SERS-based chemical sensing. *Sensors and actuators*. **2019**, *288*, 120-126.
21. Yang, Y.; Zhu, J.; Zhao, J.; Weng, G.-J.; Li, J.-J.; Zhao, J.-W., Growth of Spherical Gold Satellites on the Surface of Au@Ag@SiO₂ Core-Shell Nanostructures

Used for an Ultrasensitive SERS Immunoassay of Alpha-Fetoprotein. *ACS applied materials & interfaces*. **2019**, *11* (3), 3617-3626.

22. Lee, T.; Wi, J.-S.; Oh, A.; Na, H.-K.; Lee, J.; Lee, K.; Lee, T. G.; Haam, S., Highly robust, uniform and ultra-sensitive surface-enhanced Raman scattering substrates for microRNA detection fabricated by using silver nanostructures grown in gold nanobowls. *Nanoscale*. **2018**, *10* (8), 3680-3687.

23. Stiles, P. L.; Dieringer, J. A.; Shah, N. C.; Van Duyne, R. P., Surface-enhanced Raman spectroscopy. *Annu Rev Anal Chem (Palo Alto Calif)* **2008**, *1*, 601-26.

24. Amendola, V.; Pilot, R.; Frascioni, M.; Marago, O. M.; Iati, M. A., Surface plasmon resonance in gold nanoparticles: a review. *J Phys Condens Matter* **2017**, *29* (20), 203002.

25. Zhang, K.; Yao, S.; Li, G.; Hu, Y., One-step sonoelectrochemical fabrication of gold nanoparticle/carbon nanosheet hybrids for efficient surface-enhanced Raman scattering. *Nanoscale* **2015**, *7* (6), 2659-66.

26. Kubackova, J.; Fabriciova, G.; Miskovsky, P.; Jancura, D.; Sanchez-Cortes, S., Sensitive surface-enhanced Raman spectroscopy (SERS) detection of organochlorine pesticides by alkyl dithiol-functionalized metal nanoparticles-induced plasmonic hot spots. *Anal Chem* **2015**, *87* (1), 663-9.

27. Guerrini, L.; Garcia-Ramos, J. V.; Domingo, C.; Sanchez-Cortes, S., Functionalization of Ag nanoparticles with dithiocarbamate calix4arene as an effective supramolecular host for the surface-enhanced Raman scattering detection of polycyclic aromatic hydrocarbons. *Langmuir : the ACS journal of surfaces and colloids* **2006**, *22* (26), 10924.

28. Dejesus, J. F.; Trujillo, M. J.; Camden, J. P.; Jenkins, D. M., N-Heterocyclic Carbenes as a Robust Platform for Surface-Enhanced Raman Spectroscopy. *Journal of the American Chemical Society* **2018**, *140* (4), 1247.

29. Oliverio, M.; Perotto, S.; Messina, G. C.; Lovato, L.; De Angelis, F., Chemical Functionalization of Plasmonic Surface Biosensors: A Tutorial Review on Issues, Strategies, and Costs. *ACS applied materials & interfaces* **2017**, *9* (35), 29394.

30. Zeng, F.; Xu, D.; Zhan, C.; Liang, C.; Zhao, W.; Zhang, J.; Feng, H.; Ma, X., Surfactant-Free Synthesis of Graphene Oxide Coated Silver Nanoparticles for SERS

Biosensing and Intracellular Drug Delivery. *ACS Applied Nano Materials* **2018**, *1* (6), 2748-2753.

31. Abraham, S.; König, M.; Srivastava, S. K.; Kumar, V.; Walkenfort, B.; Srivastava, A., Carbon nanostructure (0-3 dimensional) supported isolated gold nanoparticles as an effective SERS substrate. *Sensors & Actuators: B. Chemical* **2018**, *273*, 455-465.

32. Liu, H.; Li, Y.; Dykes, J.; Gilliam, T.; Burnham, K.; Chopra, N., Manipulating the functionalization surface of graphene-encapsulated gold nanoparticles with single-walled carbon nanotubes for SERS sensing. *Carbon* **2018**, *140*, 306-313.

33. Xin, W.; De Rosa, I. M.; Ye, P.; Severino, J.; Li, C.; Yin, X.; Goorsky, M. S.; Carlson, L.; Yang, J.-M., Graphene template-induced growth of single-crystalline gold nanobelts with high structural tunability. *Nanoscale*. **2018**, *10* (6), 2764-2773.

34. Dichiaro, A. B.; Benton-Smith, J.; Rogers, R. E., Enhanced adsorption of carbon nanocomposites exhausted with 2,4-dichlorophenoxyacetic acid after regeneration by thermal oxidation and microwave irradiation. *Environ. Sci.: Nano* **2014**, *1* (2), 113-116.

35. Baik, S. Y.; Cho, Y. J.; Lim, Y. R.; Im, H. S.; Jang, D. M.; Myung, Y.; Park, J.; Kang, H. S., Charge-selective surface-enhanced Raman scattering using silver and gold nanoparticles deposited on silicon-carbon core-shell nanowires. *ACS nano* **2012**, *6* (3), 2459.

36. Sultan, M., Polyurethane for removal of organic dyes from textile wastewater. *Environmental Chemistry Letters* **2017**, *15* (2), 347-366.

37. Tran, C. T. K.; Tran, H. T. T.; Bui, H. T. T.; Dang, T. Q.; Nguyen, L. Q., Determination of low level nitrate/nitrite contamination using SERS-active Ag/ITO substrates coupled to a self-designed Raman spectroscopy system. *Journal of Science: Advanced Materials and Devices* **2017**, *2* (2), 172-177.

38. Mosier-Boss, P. A.; Putnam, M. D., The evaluation of two commercially available, portable Raman systems. *Anal Chem Insights* **2013**, *8*, 83-97.

39. Ma, L. L.; Feldman, M. D.; Tam, J. M.; Paranjape, A. S.; Cheruku, K. K.; Larson, T. A.; Tam, J. O.; Ingram, D. R.; Paramita, V.; Villard, J. W.; Jenkins, J. T.; Wang, T.; Clarke, G. D.; Asmis, R.; Sokolov, K.; Chandrasekar, B.; Milner, T. E.;

Johnston, K. P., Small multifunctional nanoclusters (nanoroses) for targeted cellular imaging and therapy. *ACS nano* **2009**, 3 (9), 2686.

40. Xu, L.; Guo, Y.; Xie, Y.; Zhuang, J.; Yang, W.; Li, T., Three-dimensional assembly of Au nanoparticles using dipeptides. *Nanotechnology* **2002**, 13, 725 - 728.

41. Tam, J. M.; Tam, J. O.; Murthy, A.; Ingram, D. R.; Ma, L. L.; Travis, K.; Johnston, K. P.; Sokolov, K. V., Controlled Assembly of Biodegradable Plasmonic Nanoclusters for Near-Infrared Imaging and Therapeutic Applications. *ACS Nano* **2010**, 4 (4), 2178-2184.

42. Borwankar, A. U.; Willsey, B. W.; Twu, A.; Hung, J. J.; Stover, R. J.; Wang, T. W.; Feldman, M. D.; Milner, T. E.; Truskett, T. M.; Johnston, K. P., Gold nanoparticles with high densities of small protuberances on nanocluster cores with strong NIR extinction. *RSC Adv.* **2015**, 5 (127), 104674-104687.

43. Song, C. Y.; Zhou, N.; Yang, B. Y.; Yang, Y. J.; Wang, L. H., Facile synthesis of hydrangea flower-like hierarchical gold nanostructures with tunable surface topographies for single-particle surface-enhanced Raman scattering. *Nanoscale* **2015**, 7 (40), 17004-11.

44. Topete, A.; Alatorre-Meda, M.; Villar-Alvarez, E. M.; Cambon, A.; Barbosa, S.; Taboada, P.; Mosquera, V., Simple control of surface topography of gold nanoshells by a surfactant-less seeded-growth method. *ACS Appl Mater Interfaces* **2014**, 6 (14), 11142-57.

45. Björk, J.; Hanke, F.; Palma, C.-A.; Samori, P.; Cecchini, M.; Persson, M., Adsorption of Aromatic and Anti-Aromatic Systems on Graphene through π - π Stacking. *The Journal of Physical Chemistry Letters* **2010**, 1 (23), 3407-3412.

46. Hernandez, B. P., F. Derbel, N. Coninck, J. Ghomi, M, Vibrational Analysis of Amino Acids and Short Peptides in Hydrated Media. VI. Amino Acids with Positively Charged Side Chains: L-Lysine and L-Arginine. *J. Phys. Chem. B* **2010**, 114, 1077-1088.

47. Dong, B.; Fang, Y.; Xia, L.; Xu, H.; Sun, M., Is 4-nitrobenzenethiol converted to p,p'-dimercaptoazobenzene or 4-aminothiophenol by surface photochemistry reaction? *Journal of Raman Spectroscopy* **2011**, 42 (6), 1205-1206.

48. Abdelsalam, M., Surface enhanced raman scattering of aromatic thiols adsorbed on nanostructured gold surfaces. *Open Chemistry* **2009**, *7* (3).
49. Liu, F.; Gu, H.; Yuan, X.; Dong, X., Semi-Quantitative Analysis of Gentian Violet by Surface-Enhanced Raman Spectroscopy Using Silver Colloids. *Applied Spectroscopy* **2010**, *64* (11), 1301-1307.
50. Bonancêa, C. E.; do Nascimento, G. M.; de Souza, M. L.; Temperini, M. L. A.; Corio, P., Substrate development for surface-enhanced Raman study of photocatalytic degradation processes: Congo red over silver modified titanium dioxide films. *Applied Catalysis B: Environmental* **2006**, *69* (1-2), 34-42.
51. Mosier-Boss, P. A.; Lieberman, S. H., Detection of Nitrate and Sulfate Anions by Normal Raman Spectroscopy and SERS of Cationic-Coated, Silver Substrates. *Applied Spectroscopy* **2000**, *54* (8), 1126-1135.
52. Lin, W. H.; Lu, Y. H.; Hsu, Y. J., Au nanoplates as robust, recyclable SERS substrates for ultrasensitive chemical sensing. *J Colloid Interface Sci* **2014**, *418*, 87-94.
53. Waarden, M. v. d., Adsorption of aromatic hydrocarbons in nonaromatic media on carbon black. *Journal of Colloid Science* **1951**, *6* (5), 443-449.
54. Mocak, J.; Bond, A. M.; Mitchell, S.; Scollary, G., A statistical overview of standard (IUPAC and ACS) and new procedures for determining the limits of detection and quantification: Application to voltammetric and stripping techniques (Technical Report). *Pure and Applied Chemistry* **1997**, *69* (2).

CHAPTER 4

Attachment of *Alcanivorax borkumensis* to Hexadecane-in-Artificial Sea Water Emulsion Droplets

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4.1 Abstract

Alcanivorax borkumensis (AB) is a marine bacterium that dominates bacterial communities around many oil spills because it enzymatically degrades the oil while using it as a nutrient source. Several dispersants have been used to produce oil-in-water emulsions following a spill. Compared to surface slicks, the additional oil–water surface area produced by emulsification provides greater access to the oil and accelerates its degradation. We deliberately cultured AB cells using hexadecane as the only nutrient source. We then examined the first critical step of the biodegradation process, the attachment of these AB cells to hexadecane–water interfaces, using fluorescence microscopy and cryogenic scanning electron microscopy. The hexadecane-in-artificial sea water (ASW) emulsions were produced by gentle shaking and were stabilized either by AB alone, by Corexit 9500, by Tween 20, or by carbon black particles. When no dispersants were used, AB stabilizes the emulsion, and bacterial cells attach to the hexadecane droplets within the first 3 days. When Corexit 9500 was used as the dispersant, AB did not attach to the hexadecane droplets over 3 days, and many AB cells in the aqueous phase appeared dead. Only limited attachment was observed after 7 days. No AB attachment was observed over 3 days when Tween 20 was used as the dispersant. However, the bacteria used Tween 20 in the ASW as a nutrient. Large amounts of AB attached to carbon black stabilized hexadecane droplets within 3 days. An analysis that accounts for van der Waals and electrostatic interactions is unable to predict all of these observations, indicating that the attachment of AB to the hexadecane is a complex phenomenon that goes beyond simple physiochemical effects. While these experiments do not mimic conditions in the open ocean where the large amount of water dilutes any

emulsion stabilizer, they provide important insights on bacteria adhesion to oil, a critical step in the oil degradation process following a marine spill.

4.2 Introduction

Bacteria play an essential role in the degradation of crude oil following marine spills.¹ *Alcanivorax borkumensis* (*AB*) is a marine bacterium that dominates bacterial communities around many oil spills because it uses the oil as a nutrient source, and enzymatically degrades a wide range of alkanes, including linear and branched aliphatics, and isoprenoid hydrocarbons. They comprise up to 80–90% of the oil-degrading microbial community within 1–2 weeks of oil entering sea water.² Because of the pivotal role of *AB* in oil-spill remediation it serves as a model organism to understand the alkane biodegradation process.³⁻⁵

Dispersants have been widely applied to oil-contaminated waters during a response to marine oil spills. These dispersants help emulsify the oil, typically into 1-100 μm diameter droplets. The enhanced oil-water interface area compared to having a surface slick promotes the availability of the oil to *AB*, thus enhancing degradation.⁶ In the Deepwater Horizon oil spill in 2010, nearly 1.8 million gallons of Corexit 9500 were used to emulsify oil emanating from the sea floor, as well as break up surface slicks. Corexit 9500 is a mixture of Span 80 (4.4 wt.%), Tween 80 (18 wt.%), Tween 85 (4.6 wt.%), and AOT (18 wt.%), all dissolved in propylene glycol and petroleum distillates.⁷

Mixed wettability particles have also been examined as dispersants to help avoid some of the potential negative effects of surfactants on microbial species.^{8, 9} High energy, usually through vortexing or homogenization is typically required for these mixed wettability particles to breach oil-water interfaces. However, unlike surfactants,

these particles remain at the oil-water interfaces even after extreme dilution, due to the high energy required for their desorption.¹⁰⁻¹² Particle-stabilized emulsions are therefore typically more stable than emulsions stabilized by surfactants, making particles an attractive alternative to surfactants for this application.

The presence of dispersants at oil-water interfaces can affect the bacterial degradation process.⁸ Dispersants can disrupt bacterial cell membranes, interfere with cell surface receptors, react with cellular components, or block enzyme active sites.¹³ Their presence at oil-water interfaces can affect bacteria attachment and thus the degradation process.^{8, 14, 15} Some studies suggest that dispersants enhance biodegradation,^{16, 17} while others conclude that dispersants either make no difference or even inhibit biodegradation.¹⁸⁻²⁰ These conflicting results require a fuller understanding of the degradation process.

Due to the low solubility of many oils in water, and because bacteria are present in the water, bacteria must attach to the oil-water interfaces before degradation gets initiated.⁵ During aerobic degradation, the terminal hydrocarbon is transformed into a primary alcohol through membrane-bound oxygenase.³ The direct contact of *AB* with the oil surface can significantly increase the rate of hydrocarbon uptake into the cells, thereby accelerating degradation as well as enhancing bacteria growth.²¹ Bacterial attachment to the interface precedes biofilm formation.²² The biofilm allows bacterial cohesion and acts as a nutrient sink which is useful when the *AB* responds to stressors such as shear or changes in pH.²³

In this work we seek to understand the first critical step in the degradation process – the attachment of *AB* to oil-water interfaces. We use fluorescence microscopy and

cryogenic scanning electron microscopy (cryo-SEM) to image *AB* cells at the oil-water interfaces and in the aqueous phase. We chose hexadecane as the oil because it is degraded by *AB* and has a low solubility (9×10^{-8} M at 25°C) in water.

4.3 Materials

A. borkumensis (ATCC® 700651™) was obtained from the American Type Culture Collection. Corexit 9500 (called Corexit in the rest of the paper) was obtained from Nalco. Tween 20, hexadecane, NaCl, MgCl₂, MgSO₄, CaCl₂, KCl, KNO₃, HK₂O₄P and hexamethyldisilazane (HMDS) were purchased from Fisher Scientific. Para-amino benzoic acid (PABA)-terminated, and thus hydrophilic, carbon black (CB) particles at a 15 wt %, pH 7.5 suspension in water, was obtained from Cabot Corporation.¹⁰ A Molecular Probes LIVE/DEAD® *BacLight*™ Bacterial Viability Kit (L7007) was purchased from Thermofisher. Artificial seawater (ASW) was used as the aqueous phase. Its composition was 19.45 g/L NaCl, 5.9 g/L MgCl₂, 3.24 g/L MgSO₄, 1.8 g/L CaCl₂, 0.55 g/L KCl, 2 g/L KNO₃ and 0.126 g/L HK₂O₄P in deionized water (DIW).

4.4 Sample preparation

To train the bacteria to respond specifically to hexadecane, *AB* was deliberately cultured in 10 mL of ASW supplemented with 0.5 mL of hexadecane. The culture was incubated at 30 °C on an orbital shaker (SCILOGEX SK-O180-E) rotating at 100 rpm, for 72 h, by which time the *AB* was in an exponential growth phase. The suspension was then centrifuged for 10 min at 4000g. The supernatant was decanted, and the pellet containing the cells was resuspended in ASW. The centrifugation and resuspension

steps were repeated twice to remove any traces of the hexadecane. The remaining pellet was dispersed in ASW, and this suspension was used in the subsequent experiments.

Figure 4. 1: Experimental setup to study the attachment of AB to hexadecane-in-ASW emulsions. (a) Hexadecane deposited on ASW containing AB and shaken to make emulsion, (b) hexadecane with Corexit (volumetric ratio of Corexit/hexadecane is 1:20) deposited on ASW containing AB and shaken to make emulsion, and (c) Tween 20- or CB-stabilized emulsions deposited on ASW containing AB and shaken. The concentration of Tween 20 in ASW was 0.06 mM, and the CB loading in ASW was 0.015 wt %.

Samples were prepared using the procedures illustrated in Figure 4.1a, Hexadecane was deposited on the ASW containing AB, and then shaken. (b) Hexadecane containing Corexit (volumetric ratio of 1:20 Corexit:hexadecane) was deposited on the ASW containing AB and then shaken. (c) 0.5 mL of hexadecane was added to ASW containing 0.06 mM Tween 20 and then vortexed at 3000 rpm for 30 s, forming an emulsion. This emulsion was added to the AB-containing ASW and shaken. Or a 0.015 wt % CB suspension was formed in ASW, followed by vortexing at 3000 rpm for 2 min. The cations in the ASW salt out many of the carboxylate groups on the surface of the

CB, making the particles partially hydrophobic. Hexadecane was then added to the suspension, and the mixture was vortexed at 3000 rpm for 2 min., emulsifying the oil. During emulsion formation, most of the CB particles transferred from the aqueous phase to the hexadecane–ASW interfaces. This emulsion was added to the ASW containing AB and then shaken. The hexadecane to ASW volume ratio was 0.05:1 in all the experiments. An orbital shaker (SCILOGEX SK-O180-E), rotating at 100 rpm, was used to mix all the samples for up to 14 days. The sample temperature was maintained at 30 °C.

4.5 Sample visualization

To image *AB* cells, 100 µL of the bacteria dispersion was diluted by a factor of 50 with ASW and passed through a 0.08 µm pore filter. The cells on the filter were fixed with 4% glutaraldehyde and then stained with a 2% osmium tetroxide solution. The specimen was dehydrated through a graded ethanol series (35, 50, 75, 95, 95, 100, 100% ethanol). After each step, the sample was washed twice with ASW. *AB* cells were then air dried using HMDS as a drying agent.^{24,25} The dried *AB* cells were sputtered with gold, then imaged in a Zeiss Sigma VP field emission scanning electron microscope.

At various time points during the experiment, 50 µL of sample was removed and stained with a dye containing equal parts of a SYTO9 green-fluorescent nucleic acid and a propidium iodide red-fluorescent nucleic acid mixed in 1 ml of deionized water, then incubated in the dark for 15 min. A Cytoviva microscope equipped with a Dual Mode Fluorescent Module was used to obtain fluorescence and darkfield images of the samples. Bacteria with intact cell membranes emit green, whereas bacteria with

damaged membranes emit red, easily allowing us to distinguish between live and dead *AB* cells.

A Gatan Alto 2500 cryo system was used to prepare samples for cryogenic scanning electron microscopy (cryo-SEM). Approximately 5 μL of the sample was placed on a holder, and then plunged into liquid nitrogen. The rapidly frozen specimen was fractured using a cold flat-edge knife in a chamber cooled to -130°C . The sample temperature was then raised to -95°C for 5min, to enhance surface topological details by differential sublimation of the hexadecane and water. The sample was cooled back to -130°C , sputtered with gold, and moved from the preparation chamber to the imaging stage. Samples were imaged on a Zeiss Sigma VP field emission scanning electron microscope.

Figure 4. 2: SEM image showing *AB*. The bacteria were incubated at 30°C in ASW containing 0.5 vol % hexadecane. After fixation and air drying, a gold-sputtered sample was imaged. The bacteria are rod shaped. The red arrows show aggregates. Scale bar = 2 μm . The inset shows strands of EPS, marked by blue arrows, connecting cells to form aggregates. Scale bar = 0.2 μm

4.6 Result and discussion

SEM images of individual bacteria as well as some aggregates are shown in Figure 4.2. These rod-shaped bacteria are approximately 1.0–1.5 μm long and have a diameter of $\sim 0.5 \mu\text{m}$.^{26, 27} The cells in aggregates are connected by strands of exopolymeric substances (EPS), identified by blue arrows in the inset.^{28, 29}

During alkane metabolism *AB* excretes anionic glucolipids that act as surfactants.³⁰ Thus, even though no dispersants were added, emulsions formed after following the procedure shown in Figure 4.1a. The droplet diameters range from 2 μm -100 μm . Because these are hexadecane-trained *AB* cells, the bacteria attach to the hexadecane-ASW interfaces within 3 days (Figure 4.3a), as expected.^{5, 31}

Figure 4. 3: Images of droplets formed after 3 days following the process shown in Figure 4a. (a) Fluorescence image of oil droplets. The green borders indicate live *AB* attached to the hexadecane-ASW interfaces. Scale bar = 10 μm . (b) Cryo-SEM image of a hexadecane droplet. The red arrow points to the biofilm formed around the oil droplet. The ribbon-like structures are artifacts arising from solidification of a salt solution. (c) Cryo-SEM image of another oil droplet, showing *AB* attached to the hexadecane droplet. Scale bar = 5 μm . The region in the red box is

magnified and shown in the inset. The strands seen in the inset, marked by the blue arrow, are exopolymers that keep the biofilm intact.

Cryo-SEM images of these droplets, shown in Figures 4.3b,c, reveal details that are not visible by fluorescence microscopy. Figure 4.3b shows a hexadecane droplet with a biofilm, identified with a red arrow as the thick layer at the oil-water interface. Biofilms are known to contain of adherent cells and exopolymers.³² The red arrow in the inset of Figure 4.3c shows an individual bacterium at the interface. Also visible (blue arrow) is a network of exopolymers that keep the biofilm intact.

Figure 4. 4: Images of droplets after 3 days of *AB* incubation in ASW, supplemented with a hexadecane layer containing Corexit (volume ratio of Corexit:hexadecane is 1:20) (a) Fluorescence image overlaid on a darkfield image of the same region. No bacteria are seen on the droplet surfaces, and many of the *AB* in the ASW are dead. The white circles are the droplets. Scale bar = 10 μ m. (b) Cryo-SEM image. No bacteria are visible at the oil water interface. The ribbon-like structures are artifacts arising from solidification of the ASW solution. Scale bar = 5 μ m.

When Corexit was present in the hexadecane and samples were prepared using the procedure outlined in Figure 4.1b, no bacteria were found to attach to the hexadecane droplets after 3 days (Figure 4.4a). In addition, most bacteria are dead by day 3. No

bacteria can be seen in the cryo-SEM image of the droplets shown in Figure 4.4b, including in the magnified region shown in the inset.

Figure 4. 5: Fluorescence images of Corexit stabilized droplets after 7, 10 and 14 days of incubation with *AB* in ASW. For samples from days 7 and 10, fluorescence images were overlaid on dark-field images of the same region. (a) The image from day 7 shows some attachment of *AB* to the hexadecane. (b) At day 10 droplet sizes are small, and very few *AB* are attached to the droplets. (c) At day 14, many dead bacteria are seen. The red arrow in the inset points to the floc that develops after two weeks. Scale bars in (a)-(c) = 10 μm (d) A cryo-SEM image of the floc shows the droplets are aggregated and connected by strands of the exopolymer excreted by the *AB*. Scale bar = 2 μm .

By day 7, some bacteria did attach to the hexadecane-ASW interfaces, indicating that the hexadecane-trained *AB* cells were able to find the oil eventually. These are shown in Figure 4.5a. In addition, the hydrocarbon components of Corexit can get oxidized and act as a nutrient source for *AB* growth,¹⁷ allowing some ‘Corexit-trained’ bacteria to bind to the hexadecane-ASW interfaces. After 10 days in the shaker, many small hexadecane droplets had formed, but most had no *AB* cells on their surfaces

(Figure 4.5b). After 2 weeks, many dead bacteria are seen and a large fluffy floc was observed floating on the surface of the vial, shown in the inset of Figure 4.5c. These flocs contain hexadecane, and their formation is a physiological conservation mechanism.^{17, 33, 34} The debris from lysed cells remains in the floc, and is used as a nutrient by other live cells.³⁵ Exopolymers released into the water provide the glue for the aggregates. Figure 4.5d is a cryo-SEM image of the floc, showing oil droplets connected by strands of exopolymer. We emphasize that laboratory conditions with a finite amount of ASW do not mimic the large dilution experienced by the surfactants in an actual marine spill.

Figure 4. 6: (a) Fluorescence microscopy (scale bar = 10 μ m) and (b) Cryo-SEM images (scale bar = 5 μ m) of Tween 20 stabilized hexadecane droplets 3 days after incubation with *AB* in ASW. The fluorescence image was overlaid on the dark-field image of the same region to visualize the droplets (white circles) as well as *AB*. The magnified area in the inset of image 6(b) shows no bacteria attachment on the hexadecane droplet.

Following the procedure outlined in Figure 4.1c, we exposed a Tween 20-stabilized hexadecane-in-ASW emulsion to *AB* in ASW. Figure 4.6a shows that *AB* did not attach to the hexadecane droplets after 3 days. However, the bacteria are alive and dispersed in the aqueous phase, suggesting that *AB* can metabolize free Tween 20 and use it as a

nutrient.^{7,36} Figure 4.6b shows a cryo-SEM image of a Tween 20 stabilized hexadecane droplet taken after 3 days. No bacteria are seen at the interface. Previous studies have shown that Tween can absorb strongly and create a stable monolayer at oil-water interfaces.³⁷ The monolayer can persist even under dilution,³⁷ and the adsorption of other surfactants to oil–water interfaces containing pre-adsorbed Tween surfactants is significantly inhibited.³⁸ The absence of *AB* cells at the hexadecane-water interfaces is suggestive of a similar inhibition mechanism, compounded by the presence of Tween as a nutrient within the ASW.^{39, 40}

Figure 4. 7: Fluorescence image of bacteria incubated for 3 days in ASW containing (a) Tween 20. Live bacteria are visible. (b) No added Tween 20. No live bacteria are seen. Scale bars =10 μ m. (c) Evolution of optical density of AB suspension in ASW, with and without Tween 20.

To further confirm that *AB* can indeed use Tween 20 as a nutrient, we added this surfactant to an *AB* suspension in ASW. Figure 4.7a shows several live bacteria after 3 days of incubation. No live *AB* cells were detected after 3 days of incubation in ASW alone, as seen in Figure 4.7b. The transient optical densities of these suspensions, shown in Figure 4.7c, confirm that *AB* grows in the presence of Tween 20, but does not in only ASW.

Carbon black particles have recently been studied as potential dispersants for marine oil spills.^{10, 41} We followed the procedure outlined in Figure 4.1c and incubated *AB* with emulsions stabilized by CB. Figure 4.8a shows that a compact layer of live cells was formed around the oil droplets in 3 days. CB particles are also seen at the interfaces. The presence of CB does not prevent the gram-negative bacteria from attaching to the oil droplets. Cryo-SEM images, shown in Figure 4.8b, provide a more detailed picture of the bacteria and carbon black particles at the hexadecane-ASW interfaces. The interfacial layer contains bacterial cells and carbon black aggregates, as seen in the insets of Figure 4.8b.

Figure 4. 8: Images of CB stabilized hexadecane-in-ASW emulsion droplets after 3 days of incubation with *AB* in ASW. (a) Fluorescence images show the co-presence of *AB* and CB

particles (black spots) at the hexadecane-ASW interfaces. Scale bar = 10 μ m (b) Cryo-SEM images. The red arrows point to the CB particles. Scale bar = 5 μ m.

We conducted additional experiments and did an analysis to provide further insight into these experimental observations. The details are shown in the Supporting Information file. We observed that AB cells get lysed in DIW, and there is essentially no absorption of AB to the CB particles in that medium (Figure S.1a). Therefore, we focus our discussion on the interactions of AB and CB in ASW, where we observed some attachment (Figure S.1b). We measured the zeta potentials of CB and AB in ASW. We then used a Derjaguin–Landau–Verwey–Overbeek (DLVO) analysis along with the Derjaguin approximation to estimate the force between an AB cell and a CB particle, idealized as spheres of diameters 1600 and 120 nm, respectively. The interaction between the AB and CB is weakly attractive through ASW, as shown in Figure S.2. The weak attraction predicted by this analysis is consistent with the observation of small amounts of binding of AB cells with CB particles.

A model that only has electrostatic and van der Waals contributions to the interaction energy (DLVO analysis) between AB cells and surfactant- or particle-covered hexadecane would predict that AB cells would attach to Tween 20-covered hexadecane droplets as the attractive van der Waals forces would dominate. Because of the low zeta potential of cells in ASW (−7.2 mV) even consideration of image charge repulsion⁴² of the bacteria at the interface cannot completely explain the hindrance to adsorption of AB to the Tween-decorated interface. Thus, the lack of attachment implies that AB cells are unable to displace Tween 20 from the interface,^{37, 38} or a biochemical element, that has not been explored in this work.

We note that the AB cells in ASW are partially hydrophobic. They could potentially act like rod-shaped Pickering particles, penetrate the hexadecane–ASW interfaces, and stabilize emulsions when no other emulsifiers are added. However, the gentle shaking in all our experiments makes it unlikely that enough energy is being imparted to these cells to allow them to breach the hexadecane–ASW interfaces.⁴³ Additionally, we see no direct evidence of AB cells spanning across hexadecane–ASW interfaces. Thus, unlike “Pickering” particles, the wettability of the AB cells and their morphology are unlikely to be playing a very direct role in emulsion formation.

If bacterial cells are thought of as passive colloidal particles, their attachment would only be affected by physicochemical factors that would include van der Waals and electrostatic interactions, as well as cell morphology. However, bacteria generate a biological response as they adapt to different conditions.⁴⁴ These are reflected in modifications to cell surface structures, such as flagella and fimbriae, and excretion of exopolysaccharides that make the AB cells surface-active, as well as promote attachment to the oil–water interfaces.⁴⁴

4.7 Conclusions

We have examined the interaction of hexadecane-trained AB cells with surfactant- or particle-decorated hexadecane droplets. Corexit and Tween 20 were used as chemical dispersants, while surface-modified CB was used as a particle-based “Pickering” emulsifier.

Without any added dispersant, the hexadecane droplets are covered by a large number of AB cells, forming a dense bacterial film at the oil surface. When Corexit was used as the emulsion stabilizer, no AB attachment was observed after 3 days of

incubation, and most AB cells in the ASW were dead. After 7 days, some AB cells moved to the oil water interface. The presence of some live bacteria in the ASW indicates that AB cells metabolizes one of the components of Corexit. Tween 20 forms a stable and packed layer at the oil–water interface that makes it difficult for the bacteria to contact the oil. However, AB cells survive in ASW by using free Tween 20 in the aqueous phase as a nutrient. CB did not interfere with AB attachment to the oil droplets. AB cells and CB aggregates were observed at the interfaces. A model that considers only electrostatic and van der Waals interactions between the AB cells at the surfactant or particle-covered oil water interfaces would not predict all of our observations. The attachment of these bacterial cells to hexadecane droplets is a complex interplay between physicochemical interactions and biochemical processes.

4.8 Acknowledgments

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4.9 References

1. Ron, E. Z.; Rosenberg, E., Biosurfactants and oil bioremediation. *Current Opinion in Biotechnology* **2002**, *13* (3), 249-252.
2. Head, I. M.; Jones, D. M.; Roling, W. F., Marine microorganisms make a meal of oil. *Nat Rev Microbiol* **2006**, *4* (3), 173-82.
3. Rojo, F., Specificity at the end of the tunnel: understanding substrate length discrimination by the AlkB alkane hydroxylase. *J Bacteriol* **2005**, *187* (1), 19-22.
4. Sabirova, J. S.; Becker, A.; Lunsdorf, H.; Nicaud, J. M.; Timmis, K. N.; Golyshin, P. N., Transcriptional profiling of the marine oil-degrading bacterium *Alcanivorax borkumensis* during growth on n-alkanes. *FEMS Microbiol. Lett.* **2011**, *319* (2), 160-168.
5. Schneiker, S.; Martins dos Santos, V. A.; Bartels, D.; Bekel, T.; Brecht, M.; Buhrmester, J.; Chernikova, T. N.; Denaro, R.; Ferrer, M.; Gertler, C.; Goesmann, A.; Golyshina, O. V.; Kaminski, F.; Khachane, A. N.; Lang, S.; Linke, B.; McHardy, A. C.; Meyer, F.; Nechitaylo, T.; Puhler, A.; Regenhardt, D.; Rupp, O.; Sabirova, J. S.; Selbitschka, W.; Yakimov, M. M.; Timmis, K. N.; Vorholter, F. J.; Weidner, S.; Kaiser, O.; Golyshin, P. N., Genome sequence of the ubiquitous hydrocarbon-degrading marine bacterium *Alcanivorax borkumensis*. *Nat Biotechnol* **2006**, *24* (8), 997-1004.
6. Joye, S. B.; Kleindienst, S.; Gilbert, J. A.; Handley, K. M.; Weisenhorn, P.; Overholt, W. A.; Kostka, J. E., Responses of Microbial Communities to Hydrocarbon Exposures. *Oceanography* **2016**, *29* (3), 136-149.
7. Place, B. J.; Perkins, M. J.; Sinclair, E.; Barsamian, A. L.; Blakemore, P. R.; Field, J. A., Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. *Deep-Sea Res. Part II-Top. Stud. Oceanogr.* **2016**, *129*, 273-281.
8. John, V.; Arnosti, C.; Field, J.; Kujawinski, E.; MacCormick, A., The Role of Dispersants in Oil Spill Remediation: Fundamental Concepts, Rationale for Use, Fate, and Transport Issues. *Oceanography* **2016**, *29* (3), 108-117.
9. Rodd, A. L.; Creighton, M. A.; Vaslet, C. A.; Rangel-Mendez, J. R.; Hurt, R. H.; Kane, A. B., Effects of surface-engineered nanoparticle-based dispersants for marine oil spills on the model organism *Artemia franciscana*. *Environ Sci Technol* **2014**, *48* (11), 6419-27.

10. Saha, A.; Nikova, A.; Venkataraman, P.; John, V. T.; Bose, A., Oil emulsification using surface-tunable carbon black particles. *ACS Appl Mater Interfaces* **2013**, 5 (8), 3094-100.
11. Creighton, M. A.; Ohata, Y.; Miyawaki, J.; Bose, A.; Hurt, R. H., Two-dimensional materials as emulsion stabilizers: interfacial thermodynamics and molecular barrier properties. *Langmuir* **2014**, 30 (13), 3687-96.
12. Cremaldi, J.; Ejaz, M.; Oak, S.; Holleran, M. K.; Roberts, K.; Cheng, G.; Wang, Y.; Grayson, S. M.; John, V.; Pesika, N. S., Polymer grafted hard carbon microspheres at an oil/water interface. *J Colloid Interface Sci* **2016**, 470, 31-38.
13. Kleindienst, S.; Paul, J. H.; Joye, S. B., Using dispersants after oil spills: impacts on the composition and activity of microbial communities. *Nat. Rev. Microbiol.* **2015**, 13 (6), 388-396.
14. Efroymsen, R. A.; Alexander, M., Biodegradation by an *Arthrobacter* Species of Hydrocarbons Partitioned into an Organic Solvent. *Appl. Environ. Microbiol.* **1991**, 57 (5), 1441-1447.
15. Peziak, D.; Piotrowska, A.; Marecik, R.; Lisiecki, P.; Wozniak, M.; Szulc, A.; Lawniczak, L.; Chrzanowski, L., Bioavailability of hydrocarbons to bacterial consortia during Triton X-100 mediated biodegradation in aqueous media. *Acta Biochim. Pol.* **2013**, 60 (4), 789-793.
16. Grimberg, S. J.; Stringfellow, W. T.; Aitken, M. D., Quantifying the biodegradation of phenanthrene by *Pseudomonas stutzeri* P16 in the presence of a nonionic surfactant. *Appl. Environ. Microbiol.* **1996**, 62 (7), 2387-2392.
17. Baelum, J.; Borglin, S.; Chakraborty, R.; Fortney, J. L.; Lamendella, R.; Mason, O. U.; Auer, M.; Zemla, M.; Bill, M.; Conrad, M. E.; Malfatti, S. A.; Tringe, S. G.; Holman, H. Y.; Hazen, T. C.; Jansson, J. K., Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. *Environ Microbiol* **2012**, 14 (9), 2405-16.
18. Bookstaver, M.; Bose, A.; Tripathi, A., Interaction of *Alcanivorax borkumensis* with a Surfactant Decorated Oil-Water Interface. *Langmuir* **2015**, 31 (21), 5875-81.
19. Kleindienst, S.; Seidel, M.; Ziervogel, K.; Grim, S.; Loftis, K.; Harrison, S.; Malkin, S. Y.; Perkins, M. J.; Field, J.; Sogin, M. L.; Dittmar, T.; Passow, U.;

Medeiros, P. M.; Joye, S. B., Chemical dispersants can suppress the activity of natural oil-degrading microorganisms. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112* (48), 14900-14905.

20. Overholt, W. A.; Marks, K. P.; Romero, I. C.; Hollander, D. J.; Snell, T. W.; Kostka, J. E., Hydrocarbon-Degrading Bacteria Exhibit a Species-Specific Response to Dispersed Oil while Moderating Ecotoxicity (vol 82, pg 518, 2016). *Appl. Environ. Microbiol.* **2016**, *82* (17), 5477-5477.

21. Stelmack, P. L.; Gray, M. R.; Pickard, M. A., Bacterial adhesion to soil contaminants in the presence of surfactants. *Appl. Environ. Microbiol.* **1999**, *65* (1), 163-168.

22. Rendueles, O.; Ghigo, J. M., Multi-species biofilms: how to avoid unfriendly neighbors. *FEMS Microbiol Rev* **2012**, *36* (5), 972-89.

23. Jefferson, K. K., What drives bacteria to produce a biofilm? *FEMS Microbiol Lett* **2004**, *236* (2), 163-73.

24. Hazrin-Chong, N. H.; Manefield, M., An alternative SEM drying method using hexamethyldisilazane (HMDS) for microbial cell attachment studies on sub-bituminous coal. *J. Microbiol. Methods* **2012**, *90* (2), 96-99.

25. Braet, F.; deZanger, R.; Wisse, E., Drying cells for SEM, AFM and TEM by hexamethyldisilazane: A study on hepatic endothelial cells. *Journal of Microscopy-Oxford* **1997**, *186*, 84-87.

26. Yakimov, M. M.; Golyshin, P. N.; Lang, S.; Moore, E. R. B.; Abraham, W. R.; Lunsdorf, H.; Timmis, K. N., *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *Int. J. Syst. Bacteriol.* **1998**, *48*, 339-348.

27. Dorobantu, L. S.; Yeung, A. K. C.; Foght, J. M.; Gray, M. R., Stabilization of oil-water emulsions by hydrophobic bacteria. *Appl. Environ. Microbiol.* **2004**, *70* (10), 6333-6336.

28. Liang, X.; Liao, C.; Thompson, M. L.; Soupir, M. L.; Jarboe, L. R.; Dixon, P. M., *E. coli* Surface Properties Differ between Stream Water and Sediment Environments. *Front Microbiol* **2016**, *7*, 1732.

29. Hori, K.; Matsumoto, S., Bacterial adhesion: From mechanism to control. *Biochemical Engineering Journal* **2010**, 48 (3), 424-434.
30. Barbato, M.; Scoma, A.; Mapelli, F.; De Smet, R.; Banat, I. M.; Daffonchio, D.; Boon, N.; Borin, S., Hydrocarbonoclastic *Alcanivorax* Isolates Exhibit Different Physiological and Expression Responses to n-dodecane. *Front Microbiol* **2016**, 7, 2056.
31. Abraham, W. R.; Meyer, H.; Yakimov, M., Novel glycine containing glucolipids from the alkane using bacterium *Alcanivorax borkumensis*. *Biochim. Biophys. Acta-Lipids Lipid Metab.* **1998**, 1393 (1), 57-62.
32. Gong, H.; Bao, M.; Pi, G.; Li, Y.; Wang, A.; Wang, Z., Dodecanol-Modified Petroleum Hydrocarbon Degrading Bacteria for Oil Spill Remediation: Double Effect on Dispersion and Degradation. *ACS Sustainable Chemistry & Engineering* **2016**, 4 (1), 169-176.
33. Passow, U.; Ziervogel, K.; Asper, V.; Diercks, A., Marine snow formation in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. *Environmental Research Letters* **2012**, 7 (3), 035301.
34. Kragh, K. N.; Hutchison, J. B.; Melaugh, G.; Rodesney, C.; Roberts, A. E.; Irie, Y.; Jensen, P. O.; Diggle, S. P.; Allen, R. J.; Gordon, V.; Bjarnsholt, T., Role of Multicellular Aggregates in Biofilm Formation. *MBio* **2016**, 7 (2), e00237.
35. Flemming, H. C.; Wingender, J.; Szewzyk, U.; Steinberg, P.; Rice, S. A.; Kjelleberg, S., Biofilms: an emergent form of bacterial life. *Nat. Rev. Microbiol.* **2016**, 14 (9), 563-575.
36. Kostka, J. E.; Prakash, O.; Overholt, W. A.; Green, S. J.; Freyer, G.; Canion, A.; Delgardio, J.; Norton, N.; Hazen, T. C.; Huettel, M., Hydrocarbon-degrading bacteria and the bacterial community response in gulf of Mexico beach sands impacted by the deepwater horizon oil spill. *Appl Environ Microbiol* **2011**, 77 (22), 7962-74.
37. Reichert, M. D.; Walker, L. M., Interfacial tension dynamics, interfacial mechanics, and response to rapid dilution of bulk surfactant of a model oil-water-dispersant system. *Langmuir* **2013**, 29 (6), 1857-67.
38. Kirby, S. M.; Anna, S. L.; Walker, L. M., Sequential adsorption of an irreversibly adsorbed nonionic surfactant and an anionic surfactant at an oil/aqueous interface. *Langmuir* **2015**, 31 (14), 4063-71.

39. Li, J.-L.; Chen, B.-H., Surfactant-mediated Biodegradation of Polycyclic Aromatic Hydrocarbons. *Materials* **2009**, *2* (1), 76-94.
40. Jin, D. Y.; Jiang, X.; Jing, X.; Ou, Z. Q., Effects of concentration, head group, and structure of surfactants on the degradation of phenanthrene. *J. Hazard. Mater.* **2007**, *144* (1-2), 215-221.
41. Powell, K. C.; Chauhan, A., Interfacial tension and surface elasticity of carbon black (CB) covered oil-water interface. *Langmuir* **2014**, *30* (41), 12287-96.
42. Wang, H.; Singh, V.; Behrens, S. H., Image Charge Effects on the Formation of Pickering Emulsions. *J Phys Chem Lett* **2012**, *3* (20), 2986-90.
43. Kaz, D. M.; McGorty, R.; Mani, M.; Brenner, M. P.; Manoharan, V. N., Physical ageing of the contact line on colloidal particles at liquid interfaces. *Nature materials* **2011**, *11* (2), 138.
44. Goulter, R. M.; Gentle, I. R.; Dykes, G. A., Issues in determining factors influencing bacterial attachment: a review using the attachment of *Escherichia coli* to abiotic surfaces as an example. *Lett Appl Microbiol* **2009**, *49* (1), 1-7.

CHAPTER 5

Massive and Sustained Enhancement of Electrical Conductivity of Polystyrene Using Multilayer Graphene and Carbon Black as Primary and Secondary Fillers

(in preparation for ACS Applied Polymer Materials)

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5.1 Abstract

The high electrical conductivity of multilayer graphene and their sheet-like morphology with a low percolation threshold makes them an attractive filler to induce electrical conductivity in an insulating polymer. However, strong van der Waals force between the flat faces of graphene causes agglomeration and makes them difficult to disperse in a polymer. The volume loading required for forming a percolating conductive network of these MLGs then increases dramatically, and the advantage of their sheet-like morphology is lost. To enhance the dispersion of MLGs, carbon black nanoparticles (CB) were added to a polystyrene (PS) matrix containing 2.5 vol% MLG. The electrical conductivity of the composite increased by several orders of magnitude. CB particles at low loadings act as dispersion aids and prevent MLG restacking; excess CB at high loadings cause no conductivity drop and allow for a sustained electrical conductivity for loadings of up to 40 vol %. Using Raman spectroscopic imaging and wide-angle X-ray diffraction, we confirmed that the CB reduces the agglomeration and enhances dispersion of the MLG sheets in PS, leading to a significant increase in its electrical conductivity

5.2 Introduction

Electrically conductive polymer composites consisting of conductive fillers dispersed in a polymer matrix, have attracted interest over the last few decades because of their lightweight, chemical and corrosion resistance and ease of manufacturing.^{1,2} As the electrical conductivity of the filler and the matrix are different by many orders of magnitude, percolation of a conducting filler is required to achieve electrical conductivity in the otherwise insulating matrix. The volume loading corresponding to

a sharp increase in electrical conductivity is known as the percolation threshold.^{3, 4} Filler content is a critical aspect in the production of conductive polymer composites. Providing a low loading at percolation has a significant benefit for mechanical properties, as filler materials can act as nucleation sites for crack growth.⁵

The geometric features of conductive fillers and also their distribution in a matrix have significant roles in lowering the critical limit for making conductive composites.^{3,} ⁶ Graphene, due to its remarkable electrical, thermal, stiffness and strength properties, is a promising nanofiller in composite materials.^{7, 8} If graphene sheets are modeled as ideally dispersed and randomly rotated disks of aspect ratio AR (AR = disk diameter/thickness), the percolation threshold ϕ_c is given by $\phi_c = 1.5(\phi_{\text{sphere}}/\text{AR})$.⁹ Here, ϕ_{sphere} is the percolation threshold for spheres, i.e., $\phi_{\text{sphere}} = 0.29$ ($\phi_{\text{sphere}} = 0.29$ is for monodispersed spheres; that number is lower if there is polydispersity, but remains of the same order of magnitude). Graphene nanosheets have large surface areas that promote agglomeration by van der Waals interactions. Their dispersion in a host polymer remains a challenge.^{10, 11} One way to prevent restacking of MLG is to incorporate a second filler that acts as a spacer between the sheets.¹² Using non-conducting silica nanoparticles as a second filler resulted in an enhanced dispersion of graphene sheets, leading to a remarkable increase of the electrical conductivity of the composite as the loading of the non-conducting filler was increased.¹³

In this work, multilayer graphene (MLG) was used as a primary filler. MLGs are several microns in lateral dimensions and are of 8-10 carbon layers thick, providing a lateral dimension to thickness ratio of the order of 10^3 . We utilized carbon black nanoparticles (CB) as a secondary filler. CB is largely used in rubber composites as a

filler not only to improve the mechanical performance but also to impart electrical conductivity to prevent static charge buildup.¹⁴ We investigated the effect of the incorporation of CB as second filler for enhancement of MLG dispersion in a polymer, and its corresponding synergistic effect on electrical conductivity. We used Raman spectroscopy and wide-angle X-ray diffraction to show enhanced dispersion of the MLG sheets in a PS network, at different loadings of CB.

5.3 Materials and methods

MLG with lateral dimensions of 25 μ m are purchased from XG Sciences, USA. Polystyrene (MW 121,000) pellets are purchased from Styrolution, USA. Carbon black BP2000 with an approximate particle diameter of 100-300 nm and DBP around 3.3 cc/g is obtained from Cabot. N, N-dimethylformamide (DMF) and methanol are purchased from Fisher Scientific, USA.

Following our previous procedure for preparing MLG/polystyrene composite materials,¹³ 5g of the polystyrene pellets are added to 30ml of DMF and the solution is stirred magnetically for 12 h. CB is dispersed in DMF and sonicated for 1.5hrs. The MLG is then added to CB dispersion and then the mixture is sonicated for 1 hr. The loading of MLG in DMF is 0.001gm/ml. The suspension is then mixed with the PS solution and magnetically stirred for 2 h. This mixed suspension is then poured into methanol, an antisolvent for PS. The PS precipitates and creates the composite and engulfing all the particles into the solidified matrix. The composite is then dried in an oven for 18 h at 90 °C. Following hot pressing at 120 °C to get rid of all entrapped air bubbles, a disk-like shape is prepared for electrical conductivity measurements. All reported loadings are based upon the volume percent in the final composite.

A standard two-point probe using a constant current source (Keithley Instruments model 6221) is used to obtain bulk electrical conductivity. The surfaces of specimens are coated with silver paint to reduce contact resistance in these samples. The voltage drops across the specimen is measured to calculate the resistance of the sample. This is normalized with the dimensions of the sample to produce the electrical conductivity.

Raman spectroscopy was performed in a backscattering configuration using a WITec confocal microscope. A 100X/0.9NA microscope objective was used to focus 532 nm wavelength light from a frequency-doubled Nd:YAG laser, resulting in a lateral resolution of approximately 0.36 μm . The scattered light was then collected using the same objective. The light was dispersed by a monochromator using a 600 g/mm grating and was detected by a thermoelectrically-cooled charge coupled device (CCD) camera. A total of 2400 individual spectra were collected over a $30 \times 20 \mu\text{m}^2$ area of each composite. The integration time was 2.5 s.

Figure 5. 1: Electrical conductivity of PS-MLG2.5-CB and PS-CB composites. PS-MLG2.5-CB composites contain 2.5 vol% MLG. Incorporating CB as a second filler results in a remarkable increase of the electrical conductivity of the composite of several orders of magnitude beyond samples without CB (at the same MLG loading).

5.4 Result ad discussion

The electrical conductivity versus the CB content (vol %) for the composites filled with the carbon black (PS-CB) and for the ternary composites containing 2.5 vol% of MLG and CB (PS-MLG2.5-CB) are shown in Figure 5.1.

The percolation threshold for the CB, which can be estimated according to the volume loading where the slope became considerably steeper, is about 8 vol%. At this concentration, CB particles contact one another, forming a continuous conductive network which result in a jump in the electrical conductivity. As the CB content increases beyond this critical content, more conductive pathways are established in the polymer matrix, allowing the gradual increase in the electrical conductivity until it reaches to the intrinsic conductivity of CB.¹⁵

While PS composite having 2.5 vol% MLG does not exhibit electrical conductivity, introducing 1.5 vol% loading of CB, improves the conductivity of PS-MLG2.5 composite remarkably by six orders of magnitude. Since this volume loading of CB is well below its percolation threshold, the observed conductivity enhancement reveals that the CB particles acted as spacers between MLG sheets, instead of forming their own network. The conductivity increases further as the loading of the carbon black is increased up to 12 vol%, and then starts to plateau.

The conductivity values for PS-MLG2.5-CB composites with different loading of CB are compared with previously reported data for PS composites with the same loading of MLG but using silica nanoparticles as the secondary fillers (PS-MLG2.5-silica).¹³ Interestingly, at volume loadings of the secondary fillers up to 8 vol %, both conducting and non-conducting nanoparticles resulted in similar conductivity enhancement of

composite. Above 8 vol% content, CB nanoparticles enhance the conductivity one order of magnitude beyond that of the silica nanoparticles at equal volume loadings. Conducting CB connects the MLG particles.¹⁶ However, it is not until 12% by volume loading of secondary filler that there is significant difference in the magnitude of electrical conductivity between PS-MLG2.5-CB and PS-MLG2.5-silica composites. Beyond a loading of 12%, , excessive silica particles start to break the connectivity of the MLG network, leading to a significant drop in the electrical conductivity to approximately 10^{-3} S/m.¹³ In contrast, using CB, the enhancement of electrical conductivity of polystyrene is sustained to a higher value of 442 S/m.

Figure 5. 2: Raman spectra from multilayer graphene (MLG) and carbon black (CB) showing the characteristic D, G and 2D bands.

We used Raman spectroscopic images and wide-angle X-ray diffraction from the PS-MLG-CB composites to show the degree of MLG dispersion at different CB loadings. Typical Raman spectra obtained from the MLG and CB are shown in Figure 5.2. They both have the G-band, appearing at 1580 cm^{-1} originating from a normal first order Raman scattering of sp^2 carbons and another peak at 1350 cm^{-1} which is a disorder induced D-band.¹⁷ MLG also exhibits a two-phonon band at 2700 cm^{-1} (2D-band) which

is a second order Raman mode of graphene with no disorder.¹⁸ The dispersion of MLG in PS with different loading of CB was obtained by Raman mapping taken in an area of each sample, by focusing on the 2D-band of multilayer graphene at 2700 cm^{-1} .

Figure 5.3: Optical image and corresponding Raman mapping images showing the distribution of MLG (all samples contain 2.5% volume MLG) in the polystyrene matrix with (a) No CB, (b) 1.5 vol % CB, (c) 12 vol. % CB and (d) 25 vol.% CB. Intensity distribution of the Raman signal at the peak wavenumber 2700 cm^{-1} . Raman intensities greater than 60 are depicted in red. The dispersion of MLG improves after introducing CB. (e) X-ray diffraction plots around the graphite (0 0 2) peaks. (f) Average crystallite size of MLG agglomerates obtained using Scherrer's equation. The crystal size decreases as CB is added, up to 12 vol%, indicating less restacking and improved dispersion of MLG.

Figure 5.3 shows optical microscope images and MLG distribution map for composites with different volume loadings of CB from 0 to 25 vol%. Without CB, the MLG are agglomerated rather than well dispersed in the PS as seen in Figure 5.3a. With 1.5 vol% CB, dispersion of the MLG is enhanced, and the conductivity of the composite rises dramatically to 10^{-4} S/m. As the CB loading is increased to 12 vol %, the MLG are dispersed more uniformly throughout the composite (Figure 5.3c) and the dispersion is maintained for higher CB loading at 25 vol%.

Figure 5.3e shows the graphite (0 0 2) diffraction peaks from the PS-MLG-CB composites. The average crystallite dimensions of MLG crystallites for each CB loading can be calculated using the measured full width at half maximum of graphite peak using Scherrer equation.¹² Figure 5.3f shows that the MLG crystallite size decreases as CB loading increases, indicating improved dispersion of the MLG with increasing loading of CB.

5.5 Conclusion

The electrical conductivity of PS-MLG2.5-CB composites was studied for 2.5 vol% MLG and different loading of CB. Loading of CB filler below its percolation threshold significantly increases electrical conductivity of the composite by acting as spacers to separate the MLG sheets. At higher loadings of CB, establishment of additional conductive pathways by CB results in sustained and higher conductivity values compared to using non-conductive secondary filler. The enhanced dispersion of MLG sheets throughout polymer network at different loadings of CB was also confirmed by Raman spectroscopic images and wide-angle X-ray diffraction.

5.6 Acknowledgements

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5.7 References

1. Zhang, W.; Dehghani-Sani, A. A.; Blackburn, R. S., Carbon based conductive polymer composites. *Journal of Materials Science* **2007**, *42* (10), 3408-3418.
2. Yang, Y. N.; Jin, Z.; Dan, W.; Yongsheng, C.; Lei, J., Two-Dimensional Graphene Bridges Enhanced Photoinduced Charge Transport in Dye-Sensitized Solar Cells. *ACS Nano* **2010**, *4* (2), 887-897.
3. Nan, C. W.; Shen, Y.; Ma, J., Physical Properties of Composites Near Percolation. *Annual Review of Materials Research* **2010**, *40* (1), 131-151.
4. Balberg, I., A comprehensive picture of the electrical phenomena in carbon black-polymer composites. *Carbon* **2002**, *40*, 139-143.
5. A., R. M.; Javad, R.; Zhou, W.; Huaihe, S.; Zhong-Zhen, Y.; Nikhil, K., Enhanced Mechanical Properties of Nanocomposites at Low Graphene Content. *ACS Nano* **2009**, *3* (12), 3884-3890.
6. Kyrylyuk, A. V.; Hermant, M. C.; Schilling, T.; Klumperman, B.; Koning, C. E.; van der Schoot, P., Controlling electrical percolation in multicomponent carbon nanotube dispersions. *Nat Nanotechnol* **2011**, *6* (6), 364-9.
7. Stankovich, S.; Dikin, D. A.; Dommett, G. H.; Kohlhaas, K. M.; Zimney, E. J.; Stach, E. A.; Piner, R. D.; Nguyen, S. T.; Ruoff, R. S., Graphene-based composite materials. *Nature* **2006**, *442* (7100), 282-6.
8. K., G. A., Graphene: Status and Prospects. *Science* **2009**, *324*, 1530-1534.
9. Kim, H.; Abdala, A. A.; Macosko, C. W., Graphene/Polymer Nanocomposites. *Macromolecules* **2010**, *43* (16), 6515-6530.
10. Shojaei, S. A.; Zandiatashbar, A.; Koratkar, N.; Lucca, D. A., Raman spectroscopic imaging of graphene dispersion in polymer composites. *Carbon* **2013**, *62*, 510-513.
11. Zandiatashbar, A.; Picu, C. R.; Koratkar, N., Control of Epoxy Creep Using Graphene. *Small* **2012**, *8* (11), 1676-1682.

12. Chakraborty, I.; Shukla, A.; Bose, A., Core-shell rubbery fillers for massive electrical conductivity enhancement and toughening of polystyrene-graphene nanoplatelet composites. *Journal of Materials Science* **2016**, *51* (23), 10555-10560.
13. Chakraborty, I.; Bodurtha, K. J.; Heeder, N. J.; Godfrin, M. P.; Tripathi, A.; Hurt, R. H.; Shukla, A.; Bose, A., Massive electrical conductivity enhancement of multilayer graphene/polystyrene composites using a nonconductive filler. *ACS Appl Mater Interfaces* **2014**, *6* (19), 16472-5.
14. Huang, J.-C., Carbon black filled conducting polymers and polymer blends. *Advances in Polymer Technology* **2002**, *21* (4), 299-313.
15. Pang, H.; Xu, L.; Yan, D.-X.; Li, Z.-M., Conductive polymer composites with segregated structures. *Progress in Polymer Science* **2014**, *39* (11), 1908-1933.
16. Wu, D.; Lv, Q.; Feng, S.; Chen, J.; Chen, Y.; Qiu, Y.; Yao, X., Polylactide composite foams containing carbon nanotubes and carbon black: Synergistic effect of filler on electrical conductivity. *Carbon* **2015**, *95*, 380-387.
17. Bîru, E. I.; Iovu, H., Graphene Nanocomposites Studied by Raman Spectroscopy. In *Raman Spectroscopy*, 2018.
18. Malard, L. M.; Pimenta, M. A.; Dresselhaus, G.; Dresselhaus, M. S., Raman spectroscopy in graphene. *Physics Reports* **2009**, *473* (5-6), 51-87.

APPENDICES

Supplementary file for

“Attachment of *Alcanivorax borkumensis* to Hexadecane-in-Artificial Sea Water Emulsion Droplets”

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S1-Physicochemical (DLVO) model for the interaction of *AB* with CB particles

The para-aminobenzoic acid (PABA) -terminated CB particles in DI-water carry a negative surface charge at neutral pH and in deionized water. However, in ASW many carboxylate groups on the surface of the CB are salted out by cations. *AB* are gram-negative bacteria with an outer membrane consisting of a lipid bilayer containing lipopolysaccharides.¹ The dissociation of carboxyl and phosphate groups in the peptidoglycan and lipopolysaccharides on the cell membrane surface imparts a negative charge to the surfac,² in deionized water (DIW). In ASW, the zeta potential is reduced. Measured zeta potentials of *AB* and CB in DIW and ASW are reported in Table S1. We caution that there is some aggregation of the CB in ASW so the zeta potential numbers may not be accurate but are qualitatively correct.

Table S.1: Zeta potentials of *AB* and CB in DIW, ASW

	Zeta potential in deionized water (mV)	Zeta potential in artificial sea water (mV)
<i>AB</i>	-32.2 ±0.40	-7.2±0.27
Carbon black	-44.7±0.50	-15.9±1.42

The We calculated the interaction between the CB and the *AB* through the ASW solution as well as DIW using a DLVO model. We assumed that the Hamaker constant for *AB* was $50 \times 10^{-21}J$ (value for a typical oil). The Hamaker constants for CB and water were taken to be $275 \times 10^{-21}J$ and $37 \times 10^{-21}J$ respectively.³ Zeta potentials of *AB* and CB were taken from Table S1.

The total energy of interaction per unit area between the two semi-infinite plates of *AB* and CB with either DIW or ASW in between is given by:

$$G^{Total} = G^{VW} + G^{EL}$$

The van der Waals term G^{VW} is:⁴

$$G^{VW} = \frac{-A_{123}}{12\pi D^2},$$

where D is the separation distance and A_{123} is the composite Hamaker constant calculated using the following combining relation: $A_{123} \approx (\sqrt{A_{11} - A_{33}})(\sqrt{A_{22} - A_{33}})$. Here A_{11} is the Hamaker constant for AB , A_{22} that for CB and A_{33} that for the medium in between.

The Gouy-Chapman model for electrostatic interaction for two surfaces with unequal potentials, is given by:

$$G^{EL} = \frac{64n_0kT}{\kappa} \frac{\gamma_1\gamma_2 \exp(-\kappa D)}{\kappa},$$

Where,

$$\gamma_1 = \tanh\left(\frac{Ze\psi_1}{4kT}\right), \quad \gamma_2 = \tanh\left(\frac{Ze\psi_2}{4kT}\right)$$

For an ionic solution, the inverse Debye length $\kappa = 2.32 \times 10^9 \sum z_i^2 C_i$, where C_i is molar concentration of ion i , z_i is the valence of that ion, and the sum is taken over all ions in the solution. ψ_1, ψ_2 are surface potentials of AB and CB and we take those to be the zeta potentials. Figure S.1 shows the interaction energy versus separation distance for AB and CB interacting through DIW and ASW.

Figure S. 1: Interaction energy versus separation distance for two semi-infinite flat plates made of AB and CB separated by either DIW or ASW.

The Derjaguin approximation^{5, 6} was then used to get the force as a function of separation distance (D) between an AB sphere of radius $R_1 = 800\text{nm}$ and a CB sphere of radius $R_2 = 100\text{ nm}$, and is shown in Figure S.2.

$$F(D) = 2\pi\left(\frac{R_1 R_2}{R_1 + R_2}\right)G(D)$$

The prefactor multiplying the energy-separation distance expression is a positive number that depends only on the dimensions of the two spheres, so the sign of the energy-separation distance curve determines if the force between the CB and AB is attractive (negative values of energy) or repulsive (positive values energy).

Figure S. 2: Force versus separation distance between AB and CB, each modeled as spheres.

The force-distance curve remains positive for the *AB*/DIW/CB interaction and negative for *AB*/ASW/CB interaction.

Figure S. 3: Fluorescence images of *AB* and *CB* dispersion in (a) DIW and (b) ASW. The fluorescence images were overlaid on the dark-field images of the same region to visualize the *CB* particles as well as *AB*. Fluorescence images (a) show no attachment of *AB* to *CB* in DIW, and (b) some attachments of *AB* and *CB* particles (black spots) in ASW. Scale bars = 10 μ m

We show experimental results of mixing *CB* with *AB* in DIW and in ASW in Figure S.3. There is little binding of the *AB* and *CB* in DIW, and only some in ASW. The lack of salt in DIW also causes the *AB* to get lysed, and further inhibits any binding.

S1-References

1. Hori, K.; Matsumoto, S., Bacterial adhesion: From mechanism to control. *Biochemical Engineering Journal* **2010**, 48 (3), 424-434.
2. Liang, X.; Liao, C.; Thompson, M. L.; Soupir, M. L.; Jarboe, L. R.; Dixon, P. M., E. coli Surface Properties Differ between Stream Water and Sediment Environments. *Front Microbiol* **2016**, 7, 1732.
3. Jr., R. A. F., *Nanomedicine, Volume I: Basic Capabilities*. Landes Bioscience: Georgetown, TX, 1999; Vol. 1.
4. Gregory, J., Approximate expressions for retarded van der waals interaction. *J. Colloid Interface Sci.* **1981**, 83 (1), 138–145.
5. Rentsch, S.; Pericet-Camara, R.; Papastavrou, G.; Borkovec, M., Probing the validity of the Derjaguin approximation for heterogeneous colloidal particles. *Phys Chem Chem Phys* **2006**, 8 (21), 2531-8.
6. Israelachvili, J. N., *Intermolecular and Surface Forces*. Elsevier: 2011.