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Mitchell Truong for the degree of Honors Baccalaureate of Science in Chemical Engineering presented on May 31, 2013. Title: Synthesis of gold nanoparticle surfactants and their self-assembly at oil-water interfaces.

Abstract Approved: _____

Dr. Joseph McGuire

Amphiphilic gold nanoparticle surfactants can self-assemble at oil-water interfaces to form stable Pickering emulsions. These nanoparticle surfactants have previously been synthesized by functionalizing gold nanoparticles with thiol terminated polyethylene glycol (PEG-thiol), and subsequently with an alkane-thiol. It is necessary to improve the bio-compatibility of the nanoparticles if they are to be used in medical applications. The amino acids L-Cysteine and L-Methionine are already present in the human body and are therefore expected to improve the bio-compatibility of the nanoparticle surfactants when used instead of an alkane-thiol. Similar to the ligands used in previous studies, these amino acids have thiol functional groups that can potentially covalently bond to the gold nanoparticles. Studies were done to develop a method to functionalize these gold nanoparticles with thiol containing amino acids instead of alkane-thiols. Dynamic light scattering (DLS) and UV-Vis experiments were conducted to measure hydrodynamic size and peak absorbance of perfluorohexane (PFH) in water emulsions stabilized by nanoparticle surfactants. Emulsions of PFH in water were successfully stabilized by gold nanoparticle surfactants functionalized with PEG-thiol and L-cysteine. These particle-stabilized emulsions have a tunable plasmon resonance that makes them useful as contrasting agents for medical imaging and for targeted cancer therapy.

Key Words: Nanoparticle, Surfactant, Self-Assembly

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Synthesis of gold nanoparticle surfactants and their self-assembly at oil-water interfaces

by

Mitchell Truong

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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Mitchell Truong, Author

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I. INTRODUCTION

According to the National Cancer Institute, it is estimated that over 1.6 million people will be diagnosed with cancer in 2013 [1]. Current treatments such as chemotherapy are systemic; they harm both cancerous and non-cancerous cells throughout the entire patient's body. Therefore, there is an appeal in developing targeted cancer therapies. One potential way to destroy a cancerous tumor is to cause localized heating near the cancerous tissue only. If heated sufficiently, the cells local to the heating site will die and create scar tissue that the body can remove on its own. Other researchers have shown that gold nanoshells can provide this desired localized heating effect when exposed to a focused-beam light source [2]. These nanoshells are designed to be about 140 nm in diameter, causing them to be photoactive in the near-infrared-window (approximately 650 to 900 nm), where biological tissues are semi-transparent [3]. However, these solid nanoshells are relatively large and have the potential to be retained in the body after treatment is complete. It is unknown if these nanoshells have any adverse, long-term health effects in the body. To address this, the Pozzo group has developed nanostructures which are around the same size as the nanoshells mentioned earlier [4]. One of these nanostructures consists of an oil droplet core encased in a layer of tightly packed gold nanoparticle surfactants that are 12 nm in diameter, effectively mimicking the shape of the aforementioned nanoshell. This creates a structure that can be heated in a similar fashion, yet has the potential to be deconstructed later where the individual constituents could be small enough to passively exit the body post treatment.

Just like the solid nanoshells, these gold "armored" oil droplets are photoactive in the NIR-window due to their plasmon resonance [5]. An IR laser can be used on the

nanostructures to cause localized heating. Targeted cancer therapy is but one application of these nanostructures. This heating also causes reversible expansion of the oil droplets. A sound wave is emitted during expansion, which can be used to increase contrast in ultrasound imaging as shown in Figure 1 [6]. This has advantages over traditional fluorocarbon contrasting agents because the nanostructures are smaller and can more easily navigate through vasculature to the site of interest. In addition, the nanostructures expand only under localized irradiation potentially providing higher resolution because divergence of lasers in biological tissue is expected to be less so than for traditional ultrasounds waves.

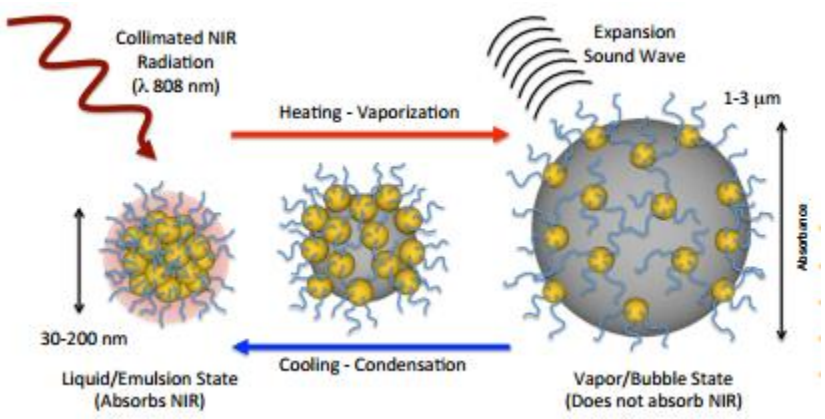

Figure 1. An illustration representing the expansion of a gold "armored" oil droplet for ultrasound imaging [6].

By the same virtue, this expanding oil droplet can be used to mechanically break up fibrin structures, as a potential means to remove unwanted blood clots. This allows for individual clots to be removed without the use of thrombolytic drugs in a patient, where the drugs could cause unexpected bleeding elsewhere. Because of the usefulness of these nanostructures, it is essential to understand how to reproducibly fabricate them.

II. BACKGROUND

Previously, the Pozzo group developed a simple one-pot method for synthesis of amphiphilic gold nanoparticle surfactants [7]. These amphiphilic gold nanoparticle surfactants can self-assemble to form clusters, similar to how detergents form micelles, but more importantly these functionalized nanoparticles can assemble at oil-water interfaces to form stable Pickering emulsions that have tunable optical properties. These nanoparticle surfactants have previously been synthesized by functionalizing gold nanoparticles with a hydrophilic ligand: thiol terminated polyethylene glycol (PEG-thiol), and subsequently with a hydrophobic ligand: an alkane-thiol, rendering the nanoparticle amphiphilic. During functionalization, the alkane-thiol can displace an already bound PEG chain because of competitive binding as illustrated in Figure 2 [8].

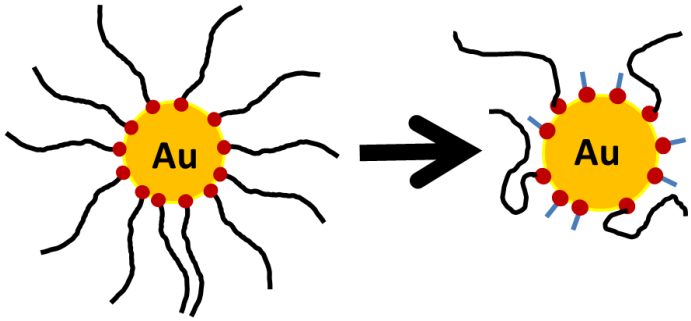

Figure 2. Demonstration of competitive binding of the long PEG and short alkane-thiol to an Au particle. As the small thiol binds to the gold, it can displace the longer PEG chains [8].

After the particle is rendered amphiphilic, it will begin to assemble to form clusters like shown in Figure 3. Cluster size (number of particles aggregating in a single structure) and geometry are controlled by the concentration of PEG chains that remain bound to the

particle surface after functionalization with the small alkane-thiol [7]. The higher the PEG to small thiol ratio is, the smaller the clusters will be.

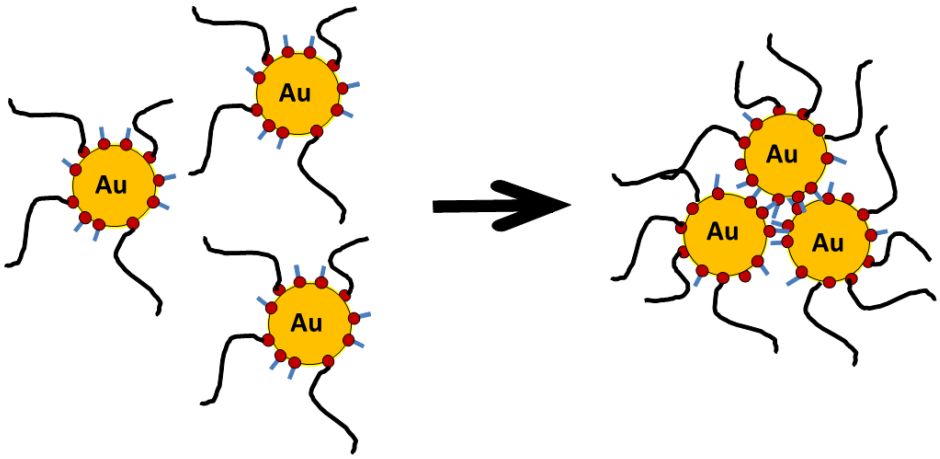

Figure 3. An illustration showing the controlled self-assembly of functionalized gold nanoparticle surfactants. PEG to short thiol ratio determines self-assembly behavior, such as size and geometry of the clusters [7].

Subsequently, these particles can assemble at the oil-water interface of an oil droplet in an emulsion [5]. Figure 4 summarizes the different nanostructures that can be formed. The wavelength of light at which this oil and gold nanostructure has the highest absorbance can be controlled by the overall size of the nanostructure, as well as the tightness of the packing of the gold nanoparticles on the surface of the oil droplet.

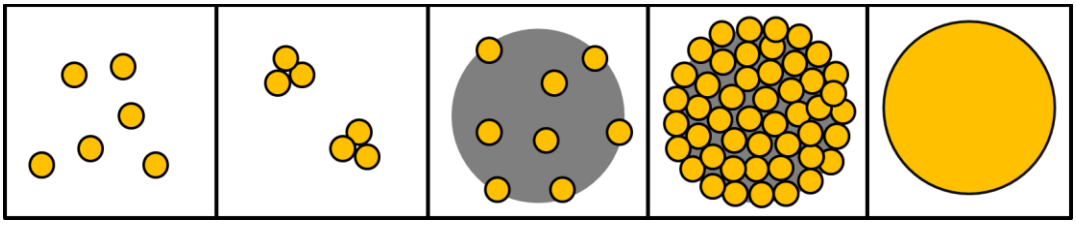

Figure 4. An illustration of the various nanostructures that can be formed: (a) Individual Au nanoparticles, (b) Clusters of functionalized particles, (c) Oil droplet stabilized by functionalized Au, (d) Oil with closely packed Au, (e) "traditional" solid gold nanoshell.

Because biological tissue is semi-transparent in the NIR-window, it is desired to maximize nanostructure light absorbance at a wavelength within that range. Figure 5 shows the absorbance of light in biological tissue for a wavelength sweep.

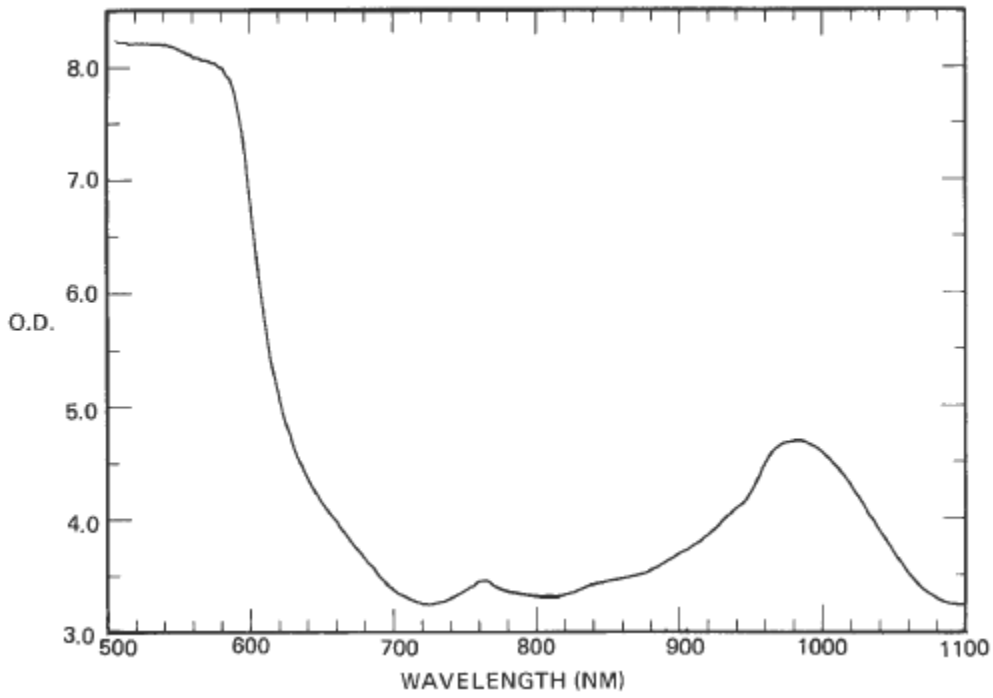

Figure 5. The optical density (absorbance) of light in human tissue based on the wavelength. Notice that the absorbance is lowest in the 650 to 900 nm window. The goal for this study was to maximize nanostructure absorbance for a laser at 800 nm [9].

As stated earlier, stable Pickering emulsions for this purpose were created using oil in conjunction with these gold nanoparticle surfactants. Those gold nanoparticles were functionalized with PEG and alkane-thiol. It is commonly understood that PEG is a bio-friendly compound, however alkane-thiols can pose some harm to the human body. An alkane-thiol, such as octane thiol or butanethiol, can cause convulsions and central nervous system depression when a person is exposed at sufficient concentrations [10]. It is desired to replace the alkane-thiol with a more bio-friendly ligand. In contrast, the

thiol containing amino acids L-cysteine and L-methionine are already present in the human body and are therefore expected to improve the bio-compatibility of the nanoparticle surfactants if used instead of an alkane-thiol. The thiol functional groups in these amino acids can potentially covalently bond to the gold nanoparticles. Studies were done to develop a method to functionalize these gold nanoparticles with thiol containing amino acids instead of alkane-thiols, and to use the particles to create stable emulsions.

III. MATERIALS AND METHODS

Cleaning glassware

Glassware was cleaned in preparation for gold nanoparticle synthesis. The glassware must be cleaned as contamination provides unwanted nucleation sites for particles to form. A 120 g sample of potassium hydroxide was dissolved in 120 mL of water in a 1 L Erlenmeyer flask. This solution was raised to a final volume of 1 L by gradually adding 200-proof ethanol. This cleaning solution can be reused to clean more than one piece of glassware, until the solution is dark brown in color. The solution was left in the flask for 12 hours. The flask was emptied, thoroughly rinsed with 5 volumes of deionized water, and left to dry in an oven for 1 hour, after which the 1 L flask was stored, covered for nanoparticle synthesis later.

Gold nanoparticle synthesis

A 1 g sample of gold chloride trihydrate (purchased from Sigma Aldrich) was combined with 2.5 mL of DI water to create a 1 M stock solution. A 0.5 mL sample was extracted and diluted with 500 mL of DI water, and set aside for later. A 38.8 mM solution of sodium citrate in water was created by mixing 0.57 g of sodium citrate with 50 mL of DI water. The gold diluted gold solution was put on a hotplate and brought to a rolling boil. The sodium citrate was added to the gold solution. The color of the mixture went from yellow to clear (gold was reduced) to black (gold nanorods formed) to dark red (rods broke into 12 nm-diameter gold nanoparticles). The mixture was left to boil for an additional 20 minutes at which point the heat was turned off and stirring continued for an another 15 minutes. The 12 nm-diameter gold nanoparticle solution was stored covered, away from light [7, 11].

Functionalization of gold nanoparticles with PEG-thiol

A solution of 5 mM polyethylene glycol (PEG) was made by mixing 135 µL of 150 mM dithiothreitol (DTT) in water with 0.22 g of PEG and 3.9 mL of water. This solution was left to rest for 12 hours to allow the DTT to prepare the PEG to bind to gold. This PEG solution was added to 400 mL of 0.024 wt% 12 nm gold nanoparticle dispersion and left to react for 4 days to functionalize the gold at a ratio of 5.0 PEG chains/nm^2 exposed Au surface area.

Functionalization of gold nanoparticles using small thiols

The PEG-functionalized nanoparticle dispersion was then concentrated to 0.13 wt% and functionalized with a small thiol: octane thiol, L-methionine, or L-cysteine. It was necessary to concentrate the gold solution because it would increase the reaction rate of the gold nanoparticles with the small thiol, and also there needed to be enough gold (relative to the amount of oil) to create stable emulsions later. The gold dispersion was combined with and vortexed with the various small thiols at concentrations from 1 to 1000 thiols/nm^2 Au and stored to react for one to four days. Functionalization of one of the samples was brought to a stop by removing the L-cysteine in a 1000 L-cysteine/nm^2 sample by dialyzing for five days after the sample was left to functionalize for one day.

Creating nanostructures in an emulsion using perfluorohexane oil (PFH)

To attempt to create a stable emulsion, 0.0086 g of perfluorohexane oil was added per 1 mL of gold dispersion. This oil, water, and gold mixture was then sonicated for 1 second on and 2 seconds off for a total of 45 seconds, to allow for the oil to break up into droplets and for the functionalized gold to break apart from their clusters into individual nanoparticles and assemble at the oil-water interfaces.

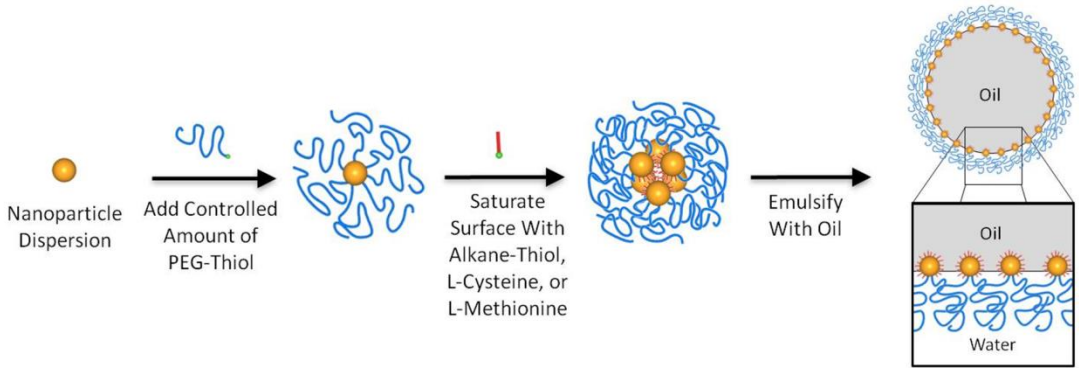

Figure 6. An illustration demonstrating the overall process to functionalize the gold nanoparticles and assemble them into gold-oil nanostructures in an emulsion [7].

Characterization using UV-Vis, and Dynamic Light Scattering (DLS)

Samples of gold dispersion or of emulsion 0.2 mL in volume were diluted with 4 mL of water to run in UV-Vis and DLS. The UV-Vis was setup to do a wavelength sweep between 300 and 1100 nm to determine which wavelength peak absorbance occurred, and the DLS was setup to determine the Z-average radius of the nanostructures to understand assembly behavior.

IV. RESULTS AND DISCUSSION

Controlled functionalization using L-Cysteine

Figure 7 show a summary of the functionalization experiments using PEG and L-cysteine. It was expected that if the nanoparticles had been successfully functionalized such that they could form clusters, that those same nanoparticles would be sufficiently amphiphilic so that they could later be used to assemble at an oil-water interface to make stable Pickering emulsions [5]. All samples were first functionalized with 5 PEG/nm^2, then functionalized with varying amounts of L-cysteine, and the size of the nanostructures were monitored over four days using DLS. The solid black line (about 25 nm-radius) therefore represents the hydrodynamic radius of 12 nm-diameter gold nanoparticles with only PEG attached to their surfaces.

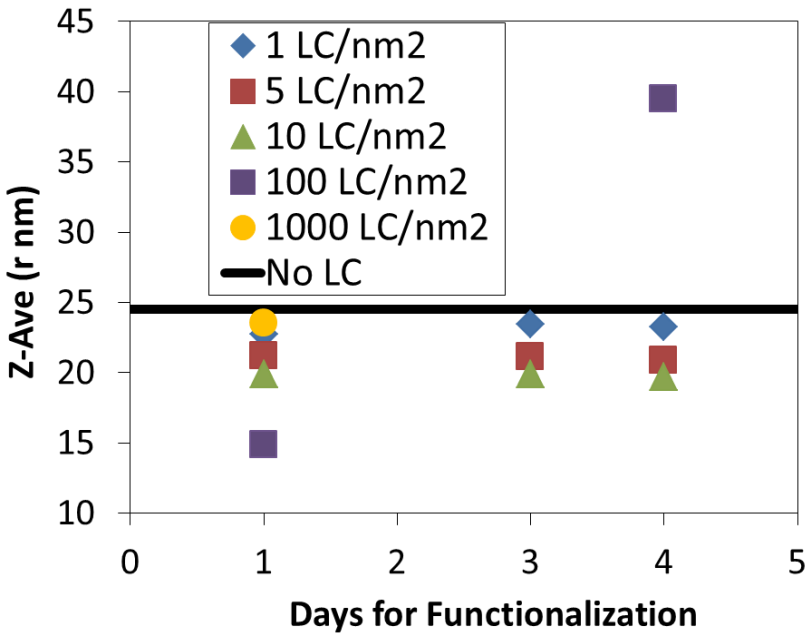

Figure 7. Average hydrodynamic radius of Au assemblies functionalized first with 5 PEG/nm^2 Au and subsequently with L-cysteine. Time zero represents when the samples were functionalized with L-cysteine. Concentrations of 10 LC/nm^2 and less did not exhibit clustering. The 1000 LC/nm^2 Au sample uncontrollably aggregated and crashed out of solution after day 1.

It was expected that as the relatively short L-cysteine bonded to the surface it would displace some of the relatively long PEG already attached to it, thus the nanoparticles would exhibit a reduction in hydrodynamic radius. Once enough L-cysteine binds to the gold to render the nanoparticle sufficiently amphiphilic, it was expected that the particles would begin to form clusters. Therefore, there would be an increase in the average size of the structures [7]. All samples showed a decrease in size after one day of functionalization with L-cysteine, indicating that L-cysteine was bonding with the gold. Samples of 1, 5, and 10 LC/nm^2 Au did not show a change in size after day one which suggests that although L-cysteine was at the gold surface there was not enough to render the particles sufficiently amphiphilic for subsequent clustering. In contrast, the 100 LC/nm^2 sample showed an increase in radius from 15 nm to 40 nm between the first and fourth days showing that the particles were self-assembling. Between days one and two the 1000 LC/nm^2 nanoparticles aggressively aggregated into large, millimeter sized clusters and crashed out of solution. This may have been because too much of the L-cysteine bound to the surface, displacing the PEG that would have been preventing the particles from aggregating into large structures. Therefore concentrations between 100 and 1000 LC/nm^2 were used in further experiments to create nanoparticles for stabilizing oil droplets in an emulsion.

L-cysteine functionalized gold for emulsion stabilization

Another set of gold nanoparticles were functionalized with 1000 LC/nm^2 for one day and then dialyzed for five days to remove the L-cysteine and prevent it from replacing all of the PEG on the gold's surface. These particles were then used with perfluorohexane (PFH) oil to attempt to make a stable emulsion. Figure 8 compares the size of individual nanoparticles functionalized with only PEG, with the sizes of oil and gold nanostructures made with PFH and nanoparticles functionalized with PEG and either octane thiol or L-cysteine. Octane thiol was the base case used to compare against L-cysteine.

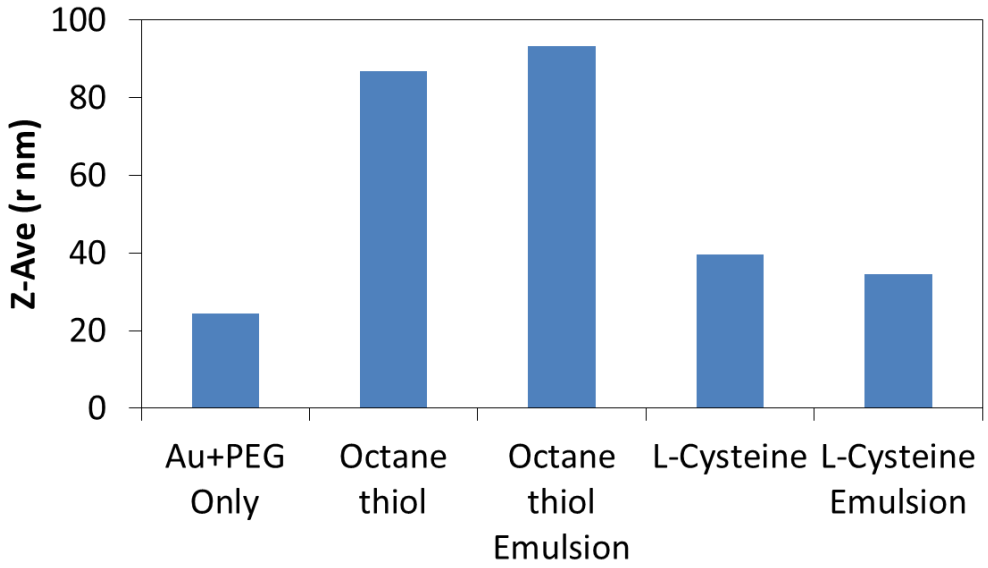

Figure 8. Average hydrodynamic radius of Au assemblies measured using DLS. All are first functionalized with PEG, and are then functionalized with either 1500 octane thiol/nm^2 Au, or dialyzed 1000 L-cysteine/nm^2 Au. Stable emulsion droplets of PFH were expected to be about 100 nm in radius.

Average radius increased from about 25 nm to about 85 nm after the nanoparticles were functionalized in the traditional fashion using octane thiol. This showed that after functionalization the particles began aggregating into structures about 85 nm in radius. When an attempt was made to make an emulsion the average nanostructure radius

increased to about 93 nm. A PFH oil droplet stabilized by gold nanoparticles was expected to be about 100 nm in radius because of the relative amounts of oil and gold added to the samples. Therefore, it looked like nanoparticles functionalized with octane thiol were able to stabilize oil droplets. Nanoparticles showed an increase in size from 25 nm to 40 nm after functionalization with L-cysteine and dialysis. Again, this increase in size suggested that the nanoparticles were aggregating. An attempt to make an emulsion with the L-cysteine particles was made, but seemed unsuccessful. The average size of the structures in the emulsion were only about 38 nm, nowhere near the expected 100 nm radius for stable PFH oil droplets.

Despite the lack of change in the size measured using DLS, there was an obvious visual difference between the solutions containing particles functionalized with PEG only, particles that were also functionalized with L-cysteine, and particles that were used to try to create an emulsion by sonification with PFH, as shown in Figure 9.

Figure 9. Au+PEG only (Left), L-Cysteine functionalized (Center), L-Cysteine Emulsion with PFH (Right)

Under analysis using a wavelength sweep in UV-Vis shown in Figure 10, it was apparent that the samples functionalized with L-cysteine, with or without the addition of oil, absorbed light considerably differently than individual nanoparticles functionalized with PEG only.

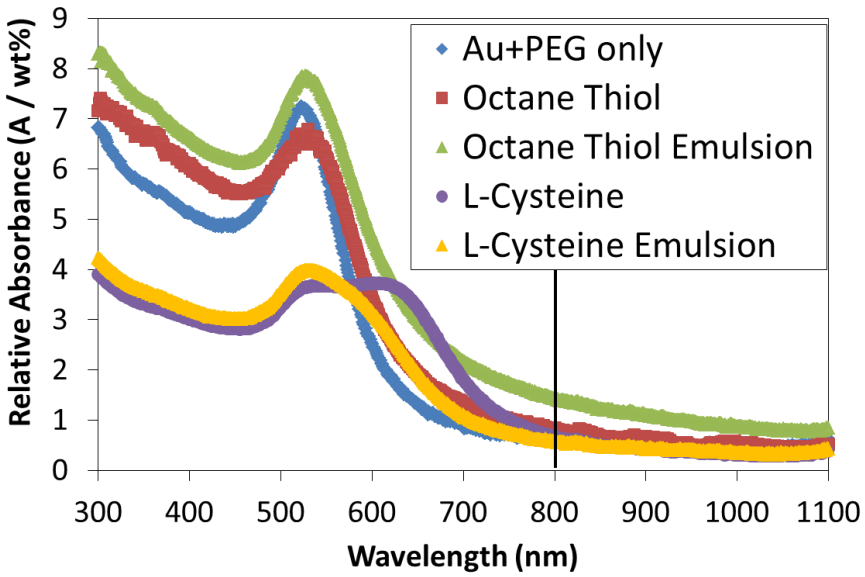

Figure 10. UV-Vis spectrum. Relative absorbance is measured absorbance divided by Au wt% of the sample. It is desired to maximize the abs at 800 nm.

From a qualitative standpoint, the shapes of the curves looked drastically different. However, the peak absorbance of individual gold nanoparticles and of the L-cysteine emulsion structures were both about 525 nm, suggesting that perhaps the particles in the emulsion were not actually on the oil droplet, but were actually individual particles in solution like in Figure 4a. However, if the nanoparticles were in solution as individual particles, and were not stabilizing any interfaces, the oil would have separated from the water and collected at the bottom of the sample vial within minutes. As seen in Figure 9 (right), the solution is homogenous and there are not two distinct liquid phases, which means that the oil droplets must be stabilized somehow. Indeed, there were stable

micron-sized droplets visible in the solution when viewed under a 40x microscope (even days after fabrication) as shown in Figure 11. This indicates that the gold nanoparticle surfactants were functionalized properly to successfully assemble at the oil-water interface. The gold-oil structures that formed were much larger than expected, and consequently too large to have given a size signal when size was measured using DLS (only the smaller remaining clusters would have been detected). This explains why the size of the L-cysteine functionalized particles (about 40 nm) apparently did not change much after the addition of oil and sonification as seen in Figure 8.

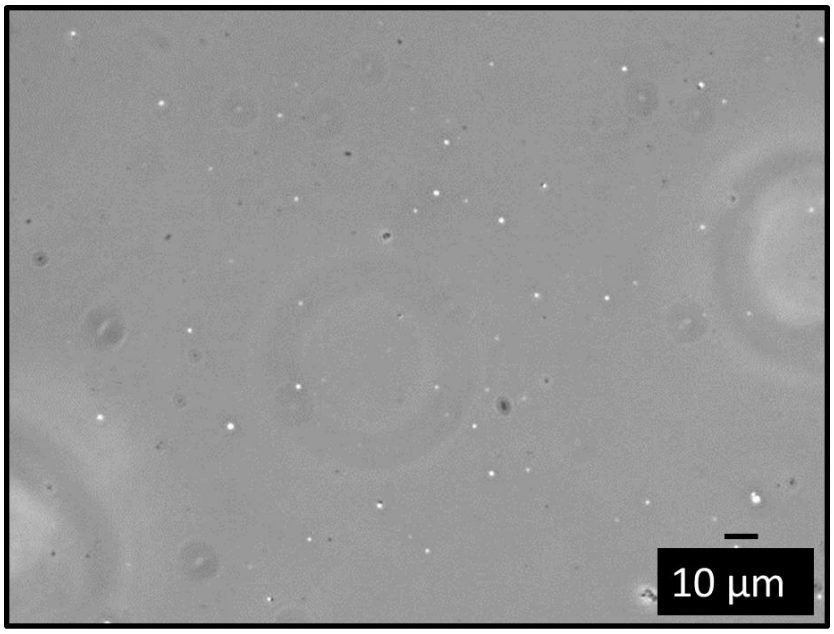

Figure 11. 40x microscope image of perfluorohexane droplets stabilized by L-cysteine functionalized Au in water. Droplets are about 1 micron in diameter.

A reason why the nanoparticles in the emulsion showed similar absorbance to individual particles even though they were assembled at the oil-water interface is because the particles could have been very spaced out while on the oil droplet, like in Figure 4c. The

arrangement of the gold in Figure 4c more closely resembles the individual gold particles, like in Figure 4a, than it does the tightly packed gold-oil structure in Figure 4d.

Conclusions

In summary, L-cysteine can be used in lieu of alkane-thiols as means of functionalizing gold nanoparticles to make them amphiphilic and surface active. Gold nanoparticles functionalized with PEG and L-cysteine can be used to stabilize perfluorohexane oil droplets in water. These stable oil droplets were larger than expected at 1000 nm instead of 200 nm in diameter.

Similar tests to those performed with L-cysteine were run with L-methionine as well. The Z-average radius of the L-methionine functionalized nanoparticles were typically around 25 nm; they did not differ greatly in size from nanoparticles functionalized with PEG only, suggesting that L-methionine did not bind to gold well, and those particles were not amphiphilic. In addition, there were no signs of stable oil droplet formation after the addition of PFH and subsequent sonification of the nanoparticles. UV-Vis and DLS showed no noticeable change in peak absorbance or size, nor were any droplets visible under the microscope. For these reasons L-methionine data were omitted from this report.

Future Work

Future work would include reducing the size of the stable oil droplets by increasing the ratio of gold nanoparticle surfactants to the volume of oil added. Also, it would be useful to try to create stable emulsions using oils with different boiling points than perfluorohexane, such as hexane or hexadecane.

BIBLIOGRAPHY

1. Howlader, N., Noone, A. M., Krapcho, M., Garshell, J., Neyman, N., Altekruse, S. F., Kosary, C. L., Yu, M., Ruhl J, Tatalovich, Z., Cho, H., Mariotto, A., Lewis, D. R., Chen, H. S., Feuer, E. J., & Cronin, K. A. (2012, November). SEER Stat Fact Sheets: All Sites. *SEER Cancer Statistics Review*. Retrieved May 4, 2013, from http://seer.cancer.gov/statfacts/html/all.html.
2. Loo, C., Lowery, A., Halas, N. J., West, J. & Drezek, R. (2005). Immunotargeted nanoshells for integrated cancer imaging and therapy. *Nano Letters, 5*(4), 709-711.
3. Delpy, D. T., & Cope, M. (1997). Quantification in tissue near-infrared spectroscopy. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences, 352*(1354), 649-659.
4. Larson-Smith, K., Jackson, A., & Pozzo, D. C. (2012). SANS and SAXS analysis of charged nanoparticle adsorption at oil-water interfaces. *Langmuir, 28*(5), 2493-2501.
5. Larson-Smith, K., & Pozzo, D. C. (2012). Pickering emulsions stabilized by nanoparticle surfactants. *Langmuir, 28*(32), 11725-11732.
6. Pozzo, D. C. (2012) Multifunctional nanostructures based on nanoparticle surfactants for ultrasound imaging and photothermal therapy. Unpublished, Department of Chemical Engineering at the University of Washington, Seattle WA.
7. Larson-Smith, K., & Pozzo, D. C. (2011). Scalable synthesis of self-assembling nanoparticle clusters based on controlled steric interactions. *Soft Matter,* (7), 5339-5347.
8. Larson-Smith, K., & Pozzo, D. C. (2012). Competitive adsorption of thiolated polyethylene glycol and alkane-thiols on gold nanoparticles. *Langmuir, 28*(37), 13157-13165.
9. Smith, K. C. (2013, May 16). *Photobiological Sciences Online.* Retrieved May 26, 2013, from http://www.photobiosci.info/PhotobioInArt_files/hand1.png.
10. (April 2013). 1-Octanethiol. *Haz-Map.* Retrieved May 27, 2013, from http://hazmap.nlm.nih.gov/category-details?id=194&table=copytblagents.
11. Frens, G. (1973). Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nature-Physical Science,* (241), 20-22.