CHAPTER I

NANOPARTICLES FOR BIOLOGICAL APPLICATIONS AND MIMICRY

Introduction

Since the development of water-soluble, ligand capped nanoparticles almost 15 years ago,[1] the use of nanoparticles in biological systems has increased dramatically. This is due in part to the fact that they can be chemically modified to mimic an antigen or biological marker, can be used for imaging in vivo, or even in catalysis. The chemistry to conjugate functional ligands and macromolecules to these monolayer protected clusters (MPCs) s has been well developed, especially place-exchange[2] and amide linkage[3], and can be adapted to fit a myriad of systems, such as, antigen/antibody interaction, *via* different synthetic routes. Through these reactions nanoparticles can be multi-functionalized to create a broad spectrum of utility, whether presenting multiple epitopes off the same antigen or two different reactive species from a catalyst.

This dissertation explores the unique properties of nanoparticles in biological applications. In chapter 4, nanoparticles are conjugated with cytochrome-c to help facilitate in the heme group transition from the Fe^{+2} to Fe^{+3} charge state. Nanoparticles work well in this application because they can charge, hold, and release electrons from the gold core. Later, in chapter 5, *in vivo* aspects of nanoparticles are explored as they are injected into mice and their effects monitored in respect to clearance, retention, and immunological response. More aspects of nanoparticles are seen in the appendices with

the re-formulation of particles for in vivo research and the study for their formation from a precursor to the particle.

This chapter will discuss the creation, both historical and synthetic, of nanoparticles and their uses in an ever expanding niche within the literature. It will highlight key techniques used to characterize nanoparticles and methods for the validation of functionalized nanoparticles. While the quartz crystal microbalance is quickly discussed later in this introduction, it will be covered in depth during chapters 2 and 3 in relation to virus and protein detection. It is acknowledged that a broad spectrum of nanometer sized materials; virus-like particles, quantum dots, polymeric nanoparticles, colloids, nanorods, is present in the literature. However, the focus of this chapter is stable, water-soluble gold core MPCs and their targeted use in and with biological systems.

Transition of Colloids to Monolayer Protection

The scientific study of colloidal gold particles dates back to Faraday in the mid 19th century.[4] The synthesis and characterization of water soluble gold colloids as small as 18 nm by electron microscope was completed by Turkevich and co-workers in 1951.[5] Schiffrin and Brust, 43 years later, reported gold particles stabilized by a monolayer of alkanethiols. Murray and co-workers termed these monolayer protected clusters (MPCs) and defined them as differing from gold colloids because they can be repeatedly dried, isolated from, and redissolved in common solvents without decomposing or aggregating.[6] MPCs are synthesized using a bottom-up approach, suggesting that a wide variety of nanomaterials is possible from a small number of building block materials.[7]

Nanoparticles are created with a variety of core types and capping ligands to create water or organic soluble products with desired functions. Both metallic and non-metallic starting materials are used in the creation of nanoparticles, such as: MPCs,[1,6,8-12] organic polymers,[13-16] virus like particles (VLPs),[17-22] protein particles,[23] colloidal particles,[5,24,25] and semiconductor quantum dots.[26] Thiol capped MPCs have received more focus because of their ease of creation, water and air stability, electrochemical and optical properties, and their ability to be surface functionalized by the addition of biologically relevant ligands, e.g., peptide sequences of epitopes. Gold MPCs can range in size from 1-10 nm, containing approximately 55-1000 gold atoms with molecular weights between 20 – 200+ kDa.[27]

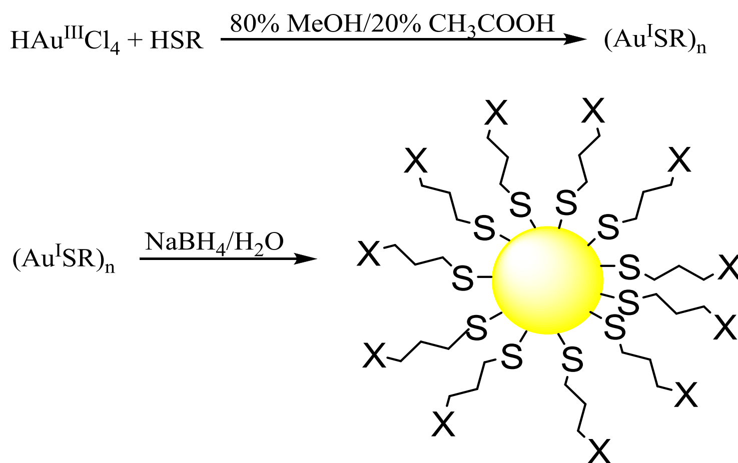

Figure 1. The modified one-phase Brust reaction for the synthesis of gold MPCs. HSR represents any generic thiol ligand.

Nanoparticle Synthesis

Water solubility of MPCs is best accomplished by using a thiolated, polar protecting ligand in a modified Brust reaction as seen in Fig. 1.[1,9] In the Brust reaction, tetrachloroauric acid is reduced from Au^{3+} to Au^{1+} in the presence of the thiol capping

ligand, yielding a ruby red gold-thiol solution. This is either composed of a gold-thiol polymer[6,28] or discrete tetramers[29], depending on the type of ligand and solubility. Following the initial reduction, the gold is further reduced to Au^0 in the presence of sodium borohydride ($NaBH_4$), yielding a purple to dark brown solution. Other potent reducing agents, such as lithium aluminum hydride ($LiAlH_4$) or lithium triethylborohydride, have been used to reduce metal cores, like palladium and platinum.[11,12]

Key examples of thiolate ligands that have been used to produce water soluble and long term (months) air and water stable clusters are tiopronin,[9] glutathione,[30] 4-mercaptobenzoic acid,[31] 1-thio-β-D-glucose,[32] and N,N,N-trimethyl(mercaptoundecyl)ammonium (TMA)[33] as depicted in Fig. 2. These ligands can be selected based on the desired applications of the nanoparticle, namely charge and

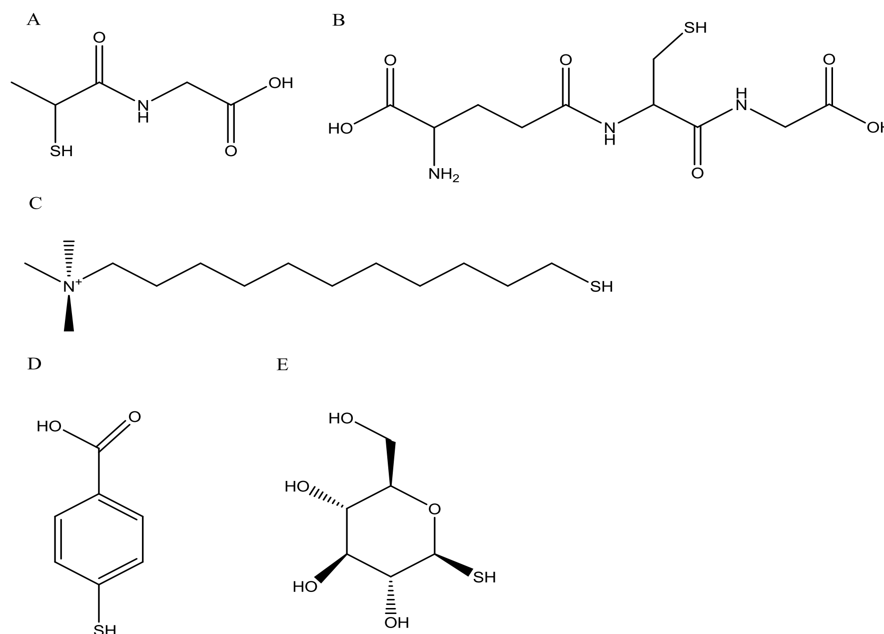

Figure 2. Examples of thiolate ligands used for creating water-soluble gold nanoparticles. The ligands shown are (A) tiopronin, (B) glutathione, (C) TMA, (D) para-mercaptobenzoic acid, and (E) 1-thio-β-D-glucose.

functionality. For charge purposes, the TMA coated particles make for a highly charged positive surface, while tiopronin yields a surface with an overall net negative charge, and as a third option glutathione tends to yield a zwitterionic particle. Another aspect is the functionality or ability to carry out chemistry on the nanoparticle surface. A carboxylic acid termination not only gives a negative charge, but also a means to amide couple the particle to an amine in solution using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC coupling). Using the right mix of functional groups, particles can be coupled to form dimers, trimers, and even networks[34] using simply chemistry. These new particles can then be applied to imaging (dimers, trimers) and thin films (networks).

Nanoparticle Functionalization

Transformation of water soluble MPCs into biological mimics has been accomplished using a variety of synthetic functionalization strategies. However, the most widely used, straight-forward method is the thiol place-exchange reaction seen in Fig. 3.

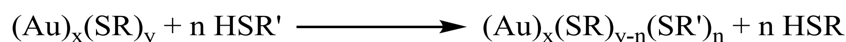

Figure 3. Example of a generic place-exchange reaction. The incoming ligand HSR' will exchange with the current monolayer in a S_n2 type reaction.

In the place-exchange reaction, an incoming ligand, such as a thiol containing molecule, peptide, or biomacromolecule, replaces one of the original capping ligands in a 1:1 ratio. Place-exchange on nanoparticles was first described by Murray and co-workers who used alkanethiolate-clusters with ω-functionalized thiols in toluene.[2] This reaction has since been expanded to aqueous solutions and can also be carried out in aqueous buffer solutions.[10] Multiple research groups have studied the dynamics by which place-

exchange occurs in ligand solutions. According to Murray's work, the rate of ligand exchange depends upon both the concentration of incoming and exiting ligands, implying an associative (S_n2-like) mechanism.[35] Lennox and co-workers, on the other hand, report the reaction is zero-order with respect to the incoming ligand.[36] Zerbetto's lab found that the associative mechanism is accurate, but that the newly introduced ligand interacts with multiple existing ligands on the cluster.[37] These interactions cause the kinetics to change as the reaction proceeds. Nevertheless, while the exact mechanism for place-exchange reactions may be complicated, the utility of place-exchange for functionalizing MPCs results from the simplicity and ease in carrying out this reaction.

Reaction rates also play an important role in the place-exchange dynamics. The reaction rate depends on the chain length and steric bulk of the initial monolayer.[35] Consequently, it is thermodynamically favorable to place-exchange a large biomolecule, such as a peptide or protein fragment, onto a MPC with a small protecting ligand like tiopronin. Further, it is important to consider that subtle differences in the structure of the incoming ligand, such as branching, can have a significant effect on both the rate of

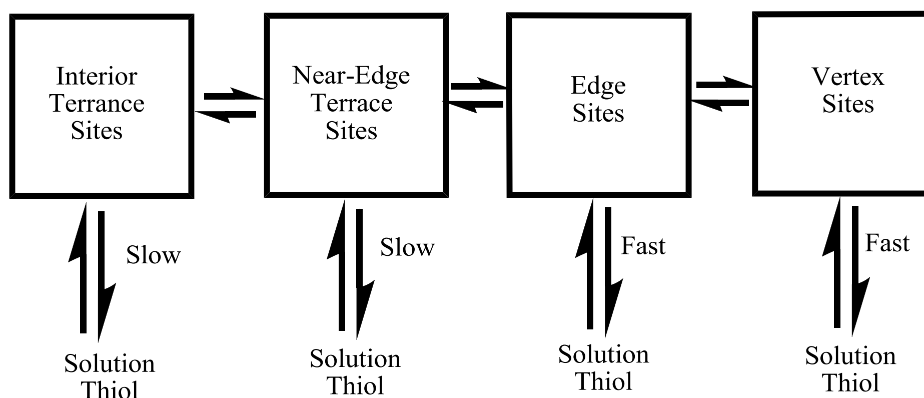

Figure 4. Diagram of the place-exchange rates based on the nanoparticle sites. Exchanges occur fastest at the vertex and edge sites followed by the slower exchange at near-edge and interior sites.[35]

place-exchange and the stability of the monolayer.[38] It should be noted that the reaction is thought to proceed more favorably at different sites on the core: vertex sites > edge sites > near-edge sites > terrace sites[35] as depicted in Fig. 4.

The variations in reactivity due to thermodynamics and kinetics originate from the differences in electron density[39] and steric accessibility[40] of these sites. This property of nanoparticles leads to some degree of predictability, and therefore control, in where place-exchanged functional groups will anchor on the core. The rate of exchange is also increased by oxidative electronic charging of the core by electrochemical means[41] or in the presence of dioxygen.[42] The extent of reaction can be enhanced by increasing the incoming ligand concentration, but it should be noted that the extent of exchange rarely approaches 100%, due to the difficulty of exchange at terrace sites.[35]

It is also important to realize that the rate of ligand place-exchange on MPCs becomes slower as the particles age, probably due to a slow rearrangement of the ligands on the surface to create a more stable layer.[43] Unfortunately, no kinetic or mechanistic study of place-exchange has considered new findings about the presence of gold thiolate tetramer rings on the surface of MPCs as reported by the Cliffel group using mass spectrometry[29] and Häkkinen and co-workers in a theoretical paper.[44] Most recently, Kornberg *et al.* determined the specific crystal structure of a *p*-mercaptobenzoic acid MPC.[45] The X-ray structure, in figure 5, shows surface bridging interactions between gold atoms and the thiol groups of the protecting ligands. Also, the structure contains conformational features specific to the phenyl ligands, *e.g.* phenyl stacking, T-stacking, and sulfur-phenyl interactions. All the recent findings show nuances in surface structure that could help to better explain the complexities of the place-exchange mechanism.

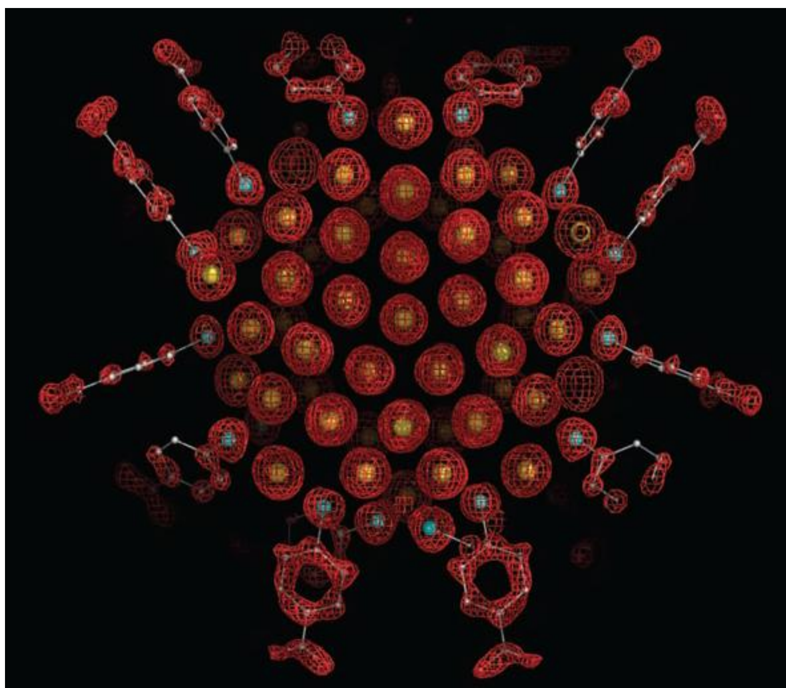

Figure 5. Crystal structure of a PMBA nanocluster showing the various bridging and steeples. The new structure changes the classical perception of a gold core simply capped by a thiol-anchor.[45]

As an alternative to the solution phase place-exchange discussed above, Huo and co-workers have studied solid phase place-exchange reactions. In their original report of this reaction, they employ a polystyrene Wang resin with acetyl protected 6-mercaptohexanoic acid attached via an ester bond.[46] The thiol groups are deprotected and allowed to undergo place-exchange with butanethiolate-protected gold nanoparticles, followed by washing away of unexchanged product, and cleaving of the exchanged particles. Their results show they could place exchange one ligand on to a particle surface. This was proven using coupling chemistry to make dimer nanoparticle complexes, rather than trimers or larger aggregates that would result from multiple exchanged ligands.

The same group has compared this solid phase approach to the solution phase approach and found the solid phase to be advantageous in terms of controlling the number of ligands attached per cluster and preserving the order of ligands on the surface.[47] Recently, the same group has reported a solid phase approach using a non-covalent interaction of the incoming ligand with silica gel,[48] which is depicted in Fig. 6. This strategy employs milder reaction conditions, thus making it amenable to a wider class of molecules, such as large biologically relevant functional groups.

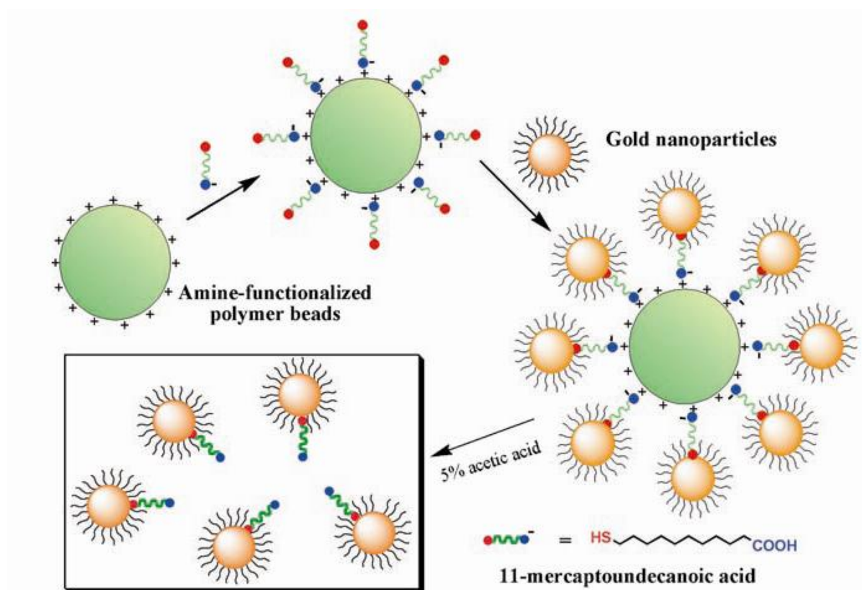

Figure 6. Figure showing the mild conditions of the solid-phase place-exchange.[48]

Macromolecules often contain thiols, for example, cysteine residues in proteins or adenosyl phosphothioate residues in DNA oligonucleotides.[49] These groups make biomolecules readily amenable to the place-exchange reaction. Strategies to introduce thiol groups into macromolecules include, but are certainly not limited to, the use of Traut's reagent (2-iminothiolane)[50] in the case of proteins or primary amine containing

molecules, the inclusion of terminal cysteine residues during the synthesis of peptides, and the conversion of phosphates to phosphorothioates using 3H-1,2-benzodithiole-3-one 1,1-dioxide.[51] It is also possible to introduce ligands into the MPC monolayer which undergo electrostatic interactions with biomolecules, for example, the use of biotin-streptavidin interaction or biotin-anti-biotin interaction.[52] All of these routes provide straightforward methods to ready a group for place-exchange and create functional nanoparticles.

There are other strategies to functionalize MPCs. An important class of these strategies, which is gaining popularity, is the use of simple organic reactions on ligands already bound to the MPC. Examples include triazole cycloaddition to a bromine functionality,[53] direct functionalization of a hydroxyl group,[54] amide coupling, and ester coupling.[3] All of these methods enable post-exchange reaction chemistry to occur, allowing a surface to be modified in a controlled fashion.

Characterizatoin of Monolayer Protected Clusters

A thorough characterization of MPCs and post-functionalized MPCs is critical before applying them in biological uses. Determination of core size is easily accomplished *via* transmission electron microscopy (TEM).[1] Smaller core sizes have also been determined by mass spectrometric methods.[55,56] Thermogravimetric analysis (TGA)[57] provides a facile method to determine the ratio of organic ligand to inorganic core material. Combining the core size and organic to inorganic ratio data yields an approximate average molecular formula for homofunctionalized MPCs.[56] Nuclear magnetic resonance spectroscopy (NMR) is useful for determining the structure and

composition of the protecting monolayer. It can also be used in real-time to monitor place exchange reaction rates.[35] Protecting ligands have broadened peaks in both ^{1}H and ^{13}C spectra due to spin-spin relaxational (T_2) broadening, heterogeneity in binding sites,[58] and dipolar broadening due to packing density gradients.[59,60]

It is critical to characterize MPCs functionalized as a biomolecular mimics for two attributes. The first is the quantity of biologically relevant functional groups attached per cluster, generally reported as an average of all the clusters in a sample. The second factor is the secondary structure of the biomolecules post-conjugation. ^{1}H NMR is a simple way to semi-quantitatively determine the number of biomolecules, for example antigen peptides per cluster, *via* integration of known protecting ligand peak area versus new broadened biomolecule peak area. The accuracy of this method can be enhanced through the use of I_2-induced MPC decomposition (termed the "death reaction") which leads to sharper peaks with less overlap.[61]

Secondary structure determination has proven to be more challenging. Drobny and co-workers describe the use of novel solid-state NMR techniques to investigate the secondary structure of peptides immobilized on gold MPCs via amide coupling.[62] For their experiments, they used cross-polarization magic angle spinning (CPMAS) and double-quantum dipolar recoupling with a windowless sequence (DQDRAWS). They showed a peptide maintained a helical structure upon conjugation, but with a slight change in backbone torsion angle. Mandal and Kraatz recently described similar characterizations of peptides place-exchanged onto MPCs using Fourier transform infrared spectroscopy (FT-IR) and Fourier transform reflection absorption infrared spectroscopy (FT-RAIRS).[63] Using amide I bands, they observed that the secondary

structure of a leucine-rich peptide bound to gold transitions from α-helical to β-sheet with greater surface curvature. Results showed that free peptides, 2-D SAMs on gold, and peptides on 20 nm gold MPCs showed α-helical structure because of less surface curvature. However, 10 nm and particularly 5 nm gold MPCs showed increasing amounts of β-sheet conformation due to the increased surface curvature. Understanding the nature of primary and secondary structure becomes critical as functionalized nanoparticles are used for practical applications.

Antigenic Validation using Immunoassays

One of the most effective ways to validate functionalized nanoparticles' ability to mimic a biological antigen is to look for recognition from a specific antibody. Since monoclonal antibodies are generally targeted for one epitope of interest, they allow for specificity and serve as the keystone in many bioanalytical techniques. This section will quickly highlight some selected analytical tools used to detect antigen mimics in a sensitive and specific fashion.

Enzyme-linked immunosorbent assays (ELISA) describes a family of techniques used for the validation of antigen-antibody interaction through the detection of antigen or antibody. This technique was first described by Engvall and Perlmann in 1971.[64] ELISA generally involves the adsorption of an antigen onto a plastic substrate, followed by recognition with a primary antibody. Then detectable secondary antibody, specific to the primary, is incubated. Secondary antibodies use tags: for example, horseradish peroxidase or alkaline phosphatase that give a detectable signal upon activation with a specific substrate. The results can be made quantitative by using techniques like UV-Vis

to measure binding. An advantage of ELISA is the amplification of the signal over longer time periods.

A powerful tool used by our lab and others to detect antigens against the specific antibodies is the quartz crystal microbalance (QCM).[49,65-71] This technique is based on a piezoelectric oscillator that changes frequency with the addition of a mass load, i.e., the

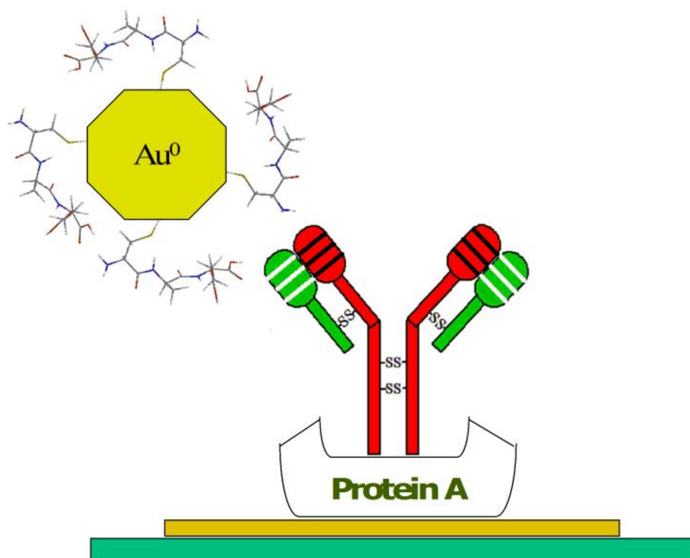

Figure 7. QCM biosensor showing the binding of a biofunctionalized MPC to an antibody. The antibody is held to the sensor and properly aligned using Protein A.[66]

antigen. This frequency shift is then converted to a mass load, so the instrument acts as a highly sensitive mass balance. Depicted in Fig. 7 is a cartoon representation of a QCM biosensor, with a generic antibody-antigen system. The detection limit of QCM technology is continuously improving as higher frequency crystals are developed, reaching easily to the nanogram level and down to the hundreds of picograms. A convenient reason to use QCM for measuring biomimic binding is the built-in amplification of using nanoparticles. Since QCM is essentially a mass detection method,

the large molecular weight of the gold nanoparticle improves the sensitivity for biomimic studies.

Another popular tool for bioanalytical measurements is the optical technique, surface plasmon resonance (SPR).[25,70-72] This technique detects the refractive change of an incident laser source, which then translates to the on- and off-rates of the antigens. SPR utilizes commercially available gold surfaces with pre-fabricated substrates. These pre-fabricated substrates allow for easier surface functionalization to create the biosensor. With sub-nanogram detection limits, SPR is another powerful tool for bioassays.

Nanoparticle Based Mimetics

Nanoparticles are capable of being functionalized with whole proteins, while still undergoing the same biomolecular recognition events as the free proteins. Recently, Rosenzweig and Thanh demonstrated the viability of biomolecular recognition of whole-protein coated gold nanoparticles in the development of an aggregation based assay.[73] They were able to detect anti-protein A in serum by aggregating protein A coated gold nanoparticles and observing an absorbance change at 620 nm.

Kornberg and co-workers described single chain Fv (scFv) antibody fragments conjugated to glutathione gold MPCs.[74] The scFv were rigidly coupled and exhibited specificity in binding to antigen protein. By eliminating the flexible regions present in the whole antibody, rigidity was achieved. Conjugation was accomplished by attaching a cysteine-terminated C-terminal affinity tag (FLAG) to the scFv. To assist the place-exchange of the glutathione with scFv, they used oxidative charging of the metal core. Using cryo-electron microscopy, they were able to verify the antibody activity by

observing the attachment of four Au_{71}-scFV-glutathione units to single *tetrameric* influenza N9 neuraminidase units. In both of these cases, the nanoparticle was used to aid in detection of antibody-antigen binding, without actually using it as a biomimetic building block.

Nanoparticles can be surface functionalized by particle assembly and stabilization with a peptide or by place-exchange with the ligand after particle assembly. The first example of biologically relevant particles is the synthesis of nanoparticles with a protecting peptide from the histidine-rich protein II (HRP-II) of *Plasmodium falciparum*.[75] Using standard fmoc procedures, Wright and co-workers recreated this peptide from HRP II and used it as a stabilizing ligand on different metal core particles: ZnS, Au^0, Ag^0, TiO_2, and AgS. The biological significance comes from the recognition of the particle by a monoclonal antibody specific for *Plasmodium falciparum*. They were able to detect the peptide-encapsulated particles as they would the whole protein. This

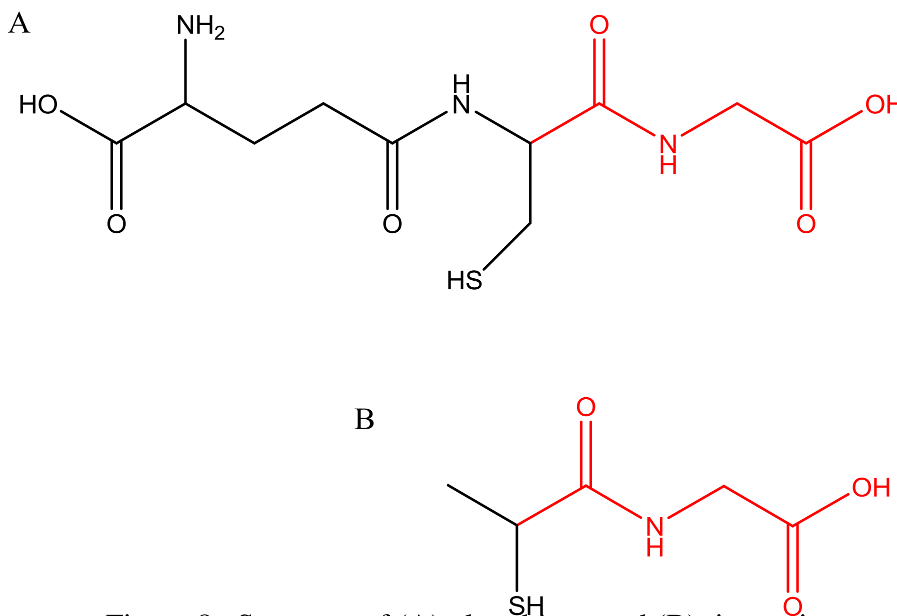

Figure 8. Structure of (A) glutathione and (B) tiopronin. The tiopronin molecule has one less amino acid but is not recognized by a glutathione specific antibody.[66]

antibody-nanoparticle recognition shows that their particle mimics the native epitope.

Recently, Cliffel and co-workers developed several MPCs that mimic antigens of interest. The first was a glutathione (GSH)-passivated gold cluster (GSH-MPC) that was then detected with a polyclonal anti-GSH antibody.[66] The antibody very specifically recognized the GSH-MPC vs. a standard tiopronin-passivated nanoparticle, even though both surface ligands only differ by two amino acids, seen in Fig. 8. While glutathione is not a traditional antigen, it serves as a proof of concept that an MPC can be functionalized with a surface peptide, and then specifically recognized *via* its antibody.

Another MPC this group synthesized contains an epitope from the hemagglutinin (HA) protein of influenza,[67] termed an HA-MPC. The 10-amino acid peptide was again synthesized with standard fmoc procedures with a terminating cysteine residue to promote place-exchange chemistry. This peptide was selected because it is a neutralizing site for influenza and there was a commercially available monoclonal antibody specific for this epitope on HA. Also, this experiment compared 2-D SAMs to 3-D nanoparticles as depicted in Fig. 9. It was shown that the HA-MPC was more efficient in presenting

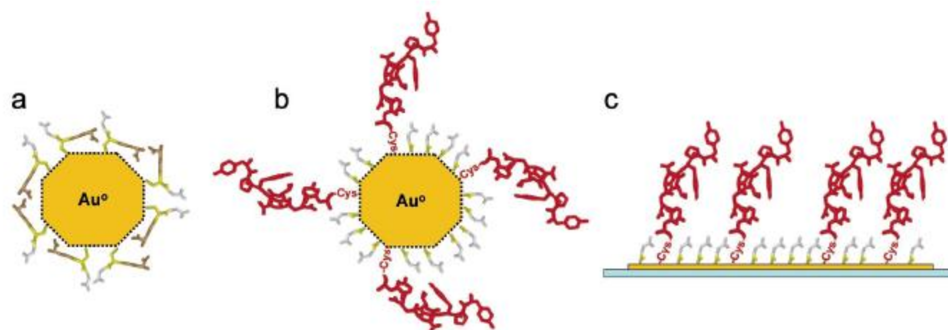

Figure 9. This figure shows MPCs that are place-exchanged with HA epitopes. For contrast, the starting cluster (a) is shown next to the functionalized cluster (b). Also, the increased area of the 3D particle can be seen when compared to the 2D surface (c).[67]

the peptide to the antibody resulting in a higher ratio of antibody to peptide binding when compared to the 2-D surface.

Another novel feature of epitope-presenting MPCs is that they can be separated by size. Using a specific sized particle, the peptide is forced into adopting a conformation closer to the native structure. Previous work by Murray and co-workers had shown that ligands are dynamically attached to the surface and will thus migrate across the MPC to find the most stable conformation possible.[3,35,76] Cliffel's research group applied this concept to their work on the protective antigen (PA) of *B. anthracis*.[69] The PA protein is one of 3 precursors of the anthrax toxin. PA was selected because it precedes the other two proteins (edema factor and lethal factor) in their transport for infection, which makes it an ideal target for neutralizing antibodies. Specifically, the C-terminus and two loops of the PA protein were identified as cell-receptor sites, making them the best candidate for their work.

Again, tiopronin MPCs were used and place-exchanged with the relevant peptide for the regions on PA. Since some of the PA epitopes selected were loop regions, the peptide was designed so that it could mimic its native conformation by putting cysteine

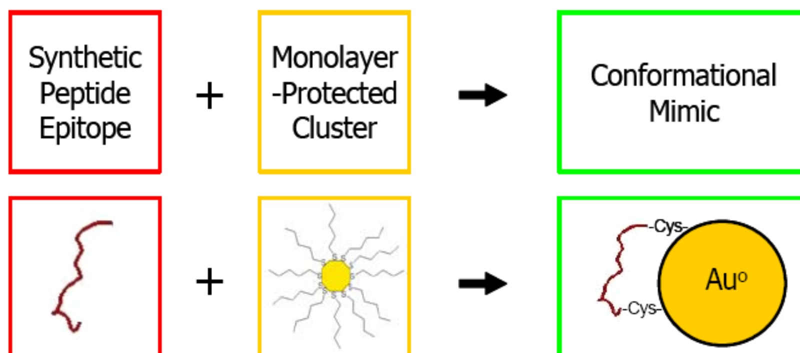

Figure 10. Step-wise creation of a bidentate attached ligand. A peptide is synthesized with a cysteine termination on both sides and then place-exchanged onto a tiopronin MPC.[69]

residues on both the N and C-terminus. This allowed bidentate attachment across the nanoparticle surface to reconstruct the natural loop. Shown in Fig. 10 is the step-wise process in the creation of the conformational mimic.

For comparison a second cluster was created that only had a cysteine on the C-terminus for monodentate attachment. This creates two types of clusters, both with the proper primary structure, but only one with the secondary structure closer to the native conformation, as illustrated in Fig. 11.

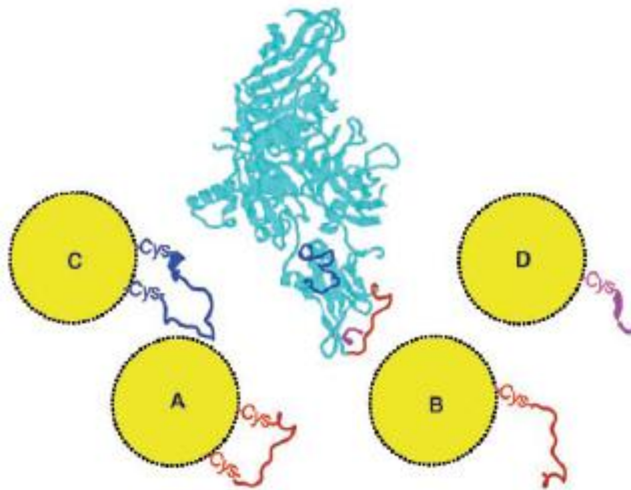

Figure 11. The various MPCs that could be synthesized with either bidentate or monodentate ligands.[69]

A QCM based antibody-antigen binding study revealed that the loop-presenting cluster was more strongly recognized than the linear epitope cluster. More specifically, the loop epitope had a higher affinity constant (K_a) for this particular antibody than the linear epitope, especially at physiological saline concentrations. This data shows that the bidentate structure was better recognized and bound more tightly. This suggests that the

commercial antibody may have a conformational paratope. The quartz crystal microbalance was used to detect the antibody binding to the MPC, which was electrostatically held to the sensor.

Peptides are not only limited to use as the functional group. Naik and co-workers used peptides in a novel and sophisticated way. Multifunctional peptides were used as the reducing agent, gold-protecting ligand, and presenting epitope.[77] Peptide A3 was selected from a phage peptide display library and found to both bind to gold and reduce it. Flag, a peptide commonly used in tagging proteins with a biomolecular recognition domain, was also found to reduce gold. They were able to produce Flag-A3 and A3-Flag gold nanoparticles in a one pot synthesis with good monodispersity that were capable of binding to anti-Flag IgG on glass slides.

As an extension of peptide epitope protected gold MPCs, a collaboration of the Cliffel and Wright research groups synthesized tiopronin MPCs containing a monolayer of either the flag epitope (flag-MPC), HA epitope (HA-MPC), flag and HA epitope (flag/HA-MPC), or no epitope.[78] The peptide epitopes were attached to the cluster *via* a cysteine-terminated polyethylene glycol (PEG) hexamer using place-exchange. The PEG linker provides enhanced accessibility by moving the epitope away from the particle's surface. QCM immunosensors, as previously described, using either anti-flag or anti-HA IgG were used to evaluate the immunological activity of the mimics synthesized. They were able to detect the HA-MPC and HA/flag-MPC using the anti-HA immunosensor, and the flag-MPC and HA/flag-MPC using the anti-flag immunosensor. Neither one detected the tiopronin MPCs without peptide epitopes. In all these trials, biological

recognition serves as a quick means to validate peptide nanoparticles and to determine binding constants.

Cell Targeting

Biomimetic nanoparticles have shown promise as a tool for targeted cell entry. Targeted entry is complex, but the small size of gold nanoparticles, and the functionality available from synthetic peptides, make this delicate task a possibility. Inspired by viruses, Feldheim and co-workers conjugated peptides to BSA via an ester linker, and then conjugated the BSA to gold nanoparticles.[79] The four peptides they used were from viral cell entry/targeting proteins, and they were able to achieve targeted entry of the gold nanoparticles into the nucleus of HepG2 cells. Furthermore, it should be noted that the cells were still viable after entry of the gold nanoparticles.[79]

Gold nanoparticles, as previously mentioned, can be functionalized with many different ligands. Results from Rotello's group show that the charge of the capping ligand can effect how the particle binds to cell surfaces[80]. Positively charge ligands, like TMA, cause an attraction between the particle and the negatively charged cell wall. The increased binding leads to higher toxicity and cell lysis. Conversely, the same negatively charged cell wall has little attraction to a carboxylate-terminated nanoparticle, leading to less cell lysis. Cell walls with no overall charge, however, lysed slightly more with negatively charged particles. These findings present an interesting consideration when conducting studies on the cellular level.

Further work by Schmid and co-workers has shown that very small gold nanoparticles coated with a phosphine ligand (Au_{55} cores, 1.4 nm, $Ph_2PC_6H_4SO_3H$

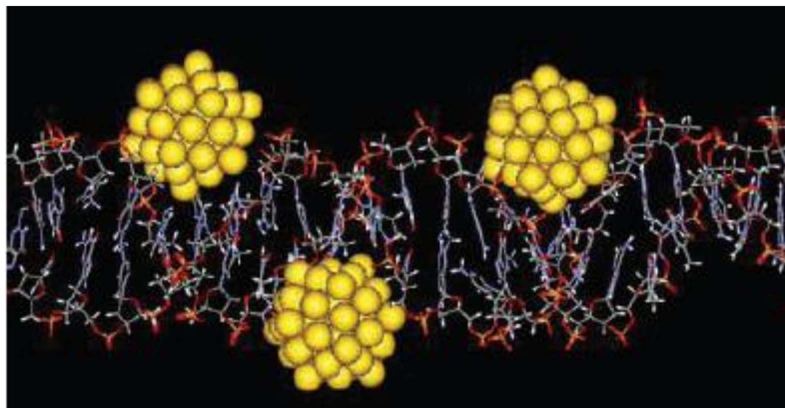

Figure 12: Au_{55} clusters entrenched in the backbone of DNA based on electrostatic attraction.[81]

ligand) can actually bind to DNA in the cell. This is due to gold's preference for the negatively charged backbone of DNA, which partially removes the protecting ligand group.[81] Au_{55} clusters entrenched in the DNA grooves are depicted in Fig. 12. Cell entry and specific targeting can serve as tools to either mark cells for imaging or cause controlled cell death.

The first report of using multi-functionalized gold MPCs for catalysis was by Frigeri and co-workers earlier this decade.[82] Using N-methylimidazole functionalized gold nanoparticles, they were able to catalyze the hydrolysis of an activated ester. Scrimin and co-workers have created water soluble gold MPCs place-exchanged with histidine-phenylalanine dipeptides that are capable of mimicking hydrolytic enzymes.[83] These two examples represent steps toward gold nanoparticle based enzyme mimics that inspired Scimrin and co-workers to term them "nanozymes." More recently, Morse and co-workers were able to use gold nanoparticles to mimic the catalytic activity of an

enzyme in the sponge *Tethya aurantia* responsible for producing silica needles by simply conjugating organic molecules to the protecting monolayer of gold nanoparticles.[84] The catalytic site of the aforementioned enzyme in *Tethya aurantia* uses a nucleophilic –OH group interacting with a hydrogen-bonding imidazole group to accomplish hydrolysis of a silicon alkoxide precursor and subsequent polycondensation to silica. Hydroxy-terminated nanoparticles were afforded simply by using 11-mercaptoundecanol as the protecting ligand in a Brust synthesis. The imidazole-terminated nanoparticles were obtained by using amide coupling of the imidazole functionality to 11-mercaptoundecanoic protected gold nanoparticles.

Many important processes in biology rely on carbohydrate-protein interactions, and it may become convenient to functionalize gold nanoparticles with carbohydrates instead of proteins or peptides. This was first accomplished by Penadés and co-workers when they used carbohydrate functionalized gold nanoparticles to mimic glycocalyx, the sticky film found on the outside of many different cells.[85]

As a further example of non-protein related gold nanoparticle biomimetics, Chen and co-workers observed the high affinity and specificity binding of carbohydrate-encapsulated nanoparticles to concanavalin A.[86] Carbohydrates were attached to the gold core using a thiol linker in a place-exchange reaction. Interaction with concanavalin A was monitored using SPR.

Conclusions

The role of nanoparticles in biotechnology will continue to grow in exciting directions. Many examples of the utility of nanoparticles exist already, and the routes to

create and modify them continue to expand. Specifically, the monolayer protected cluster has many virtues based on its stability and ease of functionalization. As more developments in the understanding of structure and composition are uncovered, the particles can be more synthetically controlled and fine-tuned to applications. They will continue to fill a necessary role in nanotechnology whether as a scaffold for viral mimics, catalyst, imaging agent, or cell entry. Also, newer techniques are using the particles for biosensor signal amplification or in optical methods exploiting their intrinsic fluorescence.

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