Biomacromolecule Surface Recognition Using Nanoparticles

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Abstract: Monolayer-protected nanoparticles represent a new class of receptors, capable of high affinity, multivalent binding with biomolecules. Networks of self-optimizing bioactive substituents can be introduced via facile place-exchange of functionalized thiols, approximating the diversified topology of biological surfaces. Extension of these particles to model systems and in catalysis is described.

Keywords: noncovalent interactions, nanoparticle, monolayer-protected clusters, biomolecular recognition.

I. INTRODUCTION

The use of gold nanoparticles in biological systems dates back to their first application in immunological staining for electron microscopy [1]. These dense particles are available in a range of sizes, making them perfectly suited to act as contrast agents for identification of cellular compartments [2], determination of protein localization [3], or in answering similar biological questions. In these initial studies, the gold clusters were not typically functionalized beyond simple covalent attachment or physisorption of an antibody or enzyme.

Recently, synthesis, materials and nanotechnology approaches have been integrated to apply functionalized gold nanoparticles as elements in assembly and device formation [4, 5]. The application of gold particles has surged in recent years due to an improvement in fabrication methods for both monolayer-stabilized clusters (MPCs) [6] and mixed monolayer protected gold clusters (MMPCs) [7]. These inorganic-organic hybrid materials feature a core-shell structure comprised of a metallic or semiconductor core surrounded by an organic layer; most reports utilizing gold as the metallic component have focused on thiol-functionalized chains to promote adhesion of the monolayer. The reactivity and solubility of the particles is dominated by the properties of the exterior monolayer substituents. In particular, the introduction of pendant groups capable of further intermolecular interactions into the monolayer confers activity to the particle as a whole. Nanoparticles assembled by these monolayer components have resulted in the formation of thin films [8], spherical assemblies [9], and multilayer aggregates [5,10], as well as additional topologies [11].

The applicability of the MMPCs is not limited to the fabrication of nanoscale materials. The availability of water-soluble nanoparticles [12] has allowed the extension of these functionalized particles into the biological realm (Figure 1).

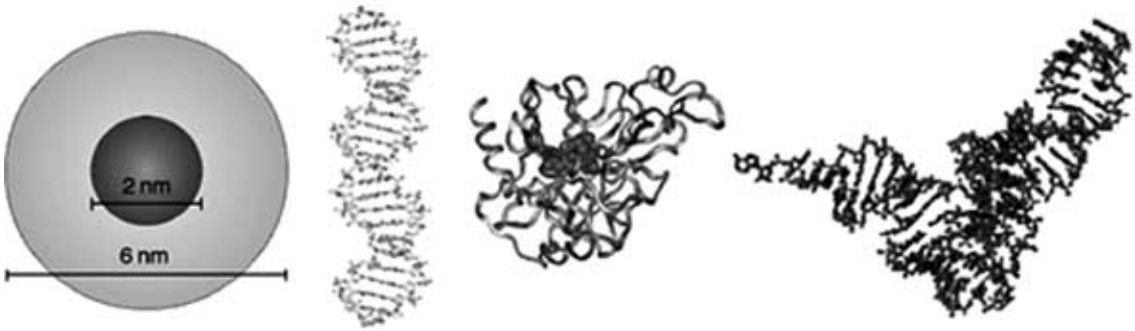

Fig. (1). To-scale schematic of 2 nm gold nanoparticles with an octanethiol monolayer and several potential biological targets: shown are a 20mer DNA duplex, α-chymotrypsin, and phenylalanine tRNA. The similarity in size of the MPC with the biomolecules provides additional surface area in binding, useful in generating high affinity scaffolds.

In addition to immunological staining, gold particles have been applied as passive spectroscopic probes in a multitude of studies [13]. Covalent attachment of biomolecules in these diagnostic applications has provided an initial demonstration that gold nanoparticles are biocompatible scaffolds. Nanoparticles prepared with weakly bound monolayers intended for subsequent displacement, such as via the sodium citrate reduction, reveal that these substituted hosts are also capable of binding biomolecular targets [14]. In this review, however, we will focus on studies that utilize monolayer-protected nanoparticles in the multivalent binding

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of biomolecular targets. These particles can be synthesized in diverse fashions, and are stable under *in vivo* conditions. This stability is essential, since exposed particles have a higher potential to form aggregates upon coming into contact with one another [15]. While other protection schemes are available to prevent unintended place-exchange of the gold surface and guard against aggregation [16], but in general these routes require redesign of the monolayer when introduction of an additional substituent is desired, in contrast to the facile place-exchange reactions permitted by alkanethiol monolayers.

The second focal point of this review is multivalency as a design element for particle-target recognition. As previously mentioned, pendant substituents can confer activity to the particle as a whole. In the cases where a single functional group is used in this fashion, the nanoparticle is primarily being used as a soluble "solid-phase" support or to enhance a spectroscopic response. Examples of studies based on monovalent interactions include binding of streptavidin and biotin-labeled particles [17] or formation of a DNA duplex on a DNA-functionalized gold core [18], utilizing the nanoparticles simply as structural building blocks or visualization aids exploits aspects of nanoparticle capabilities, but does not take full advantage of the unique properties of these scaffolds in biomolecular targeting.

The fabrication and use of multivalent nanoparticles additionally allows comparison to more traditional biological complex formation: protein-protein interactions, for example, must be specified based only on the orientation of a few repeated functional groups. Nevertheless, these few amino acid side chains are sufficient to dictate the myriad of intermolecular interactions *in vivo*. By creating nanoparticles constructed using similar principles of functional group heterogeneity, we can hope to create scaffolds suited for binding to a variety of significant targets.

II. FEATURES OF BIOCOMPATIBLE NANOPARTICLES

Gold nanoparticle hosts are particularly well suited for application to biomolecular systems due to their unique characteristics. MMPCs can be formed in a range of core sizes, from 1-5 nm and beyond, with the majority of research focused on 1.5-2 nm particles (Figure 2) [6, 7]. The contribution of the monolayer to the particle diameter is determined primarily by the length and bulk of the thiol chains [19]. These features are easily manipulated, meaning that the nanoparticles can be adjusted for binding of any size of biomacromolecule. The protective alkanethiol monolayer can be replaced with new thiols [7, 20]; this facile place-exchange allows the incorporation of synthetic substituents. In addition to direct exchange of thiols to introduce new functionality, reactive groups at the nanoparticle surface can be substituted via synthetic methodologies [21]. These reactions are made even easier, in fact, because the gold-sulfur bond effectively acts as a thiol protecting group, preventing cross reactivity. The monolayer is tightly packed at the particle surface, consisting of approximately 100 thiol chains on the surface of a 2 nm gold core [7].

The monolayer flexibility is affected by the composition and bulkiness of the chains. For example, introduction of a hydrogen bonding amide into the chain as compared to a non-bonding ester derivative results in an increased rate of cyanide decomposition of the MMPC due to interchain interactions. In this case, the close association of the chains on the gold facets due to interchain hydrogen bonding leaves the gold surface more exposed at the vertices (Figure 3a) [22]. While hydrogen bonding within the monolayer is one potential way to constrain the chains relative to other monolayer components, the decrease in stability would prevent these particles from application to biomolecular systems. This 'vertex effect' can be compensated for, however, through the introduction of sterically large groups. The place-exchange of these units into the monolayer is less favorable than their smaller counterparts, but can be added in sufficient quantities by controlling the ratio of thiols added during synthesis [23]. Comparison of aromatic substituents with alkane and branched chains reveal that the best hydrogen bonding within the monolayer, and thus protection to decomposition, is offered by the π-stacking of the phenyl groups (Figure 3b) [22]. Presumably, this protection by aromatic components occurs as the flexible chains bend across the vertex gaps to π-stack with partners of the intermolecular interaction. The straight chain thiols provided an intermediate level of hydrogen bonding and protection, while the bulky tertiary carbon chains studied offered the least resistance to decomposition, probably due to a decreased surface coverage of the substituted chains.

In addition to governing access to the gold core and the density of the monolayer, the exterior functional groups are responsible for the solubility and activity of the particle: the inclusion of groups known to bind to a selected guest

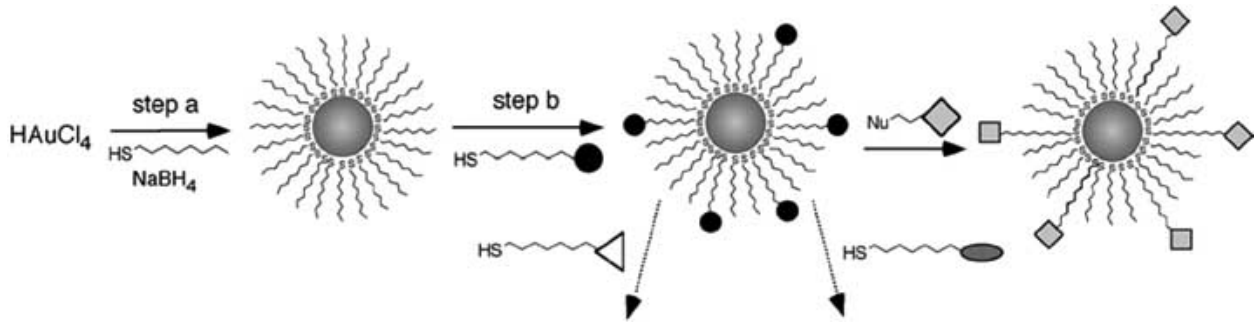

Fig. (2). Fabrication of the nanoparticles and subsequent introduction of functionality *via* the Brust reduction and Murray place-exchange reaction, (steps a and b, respectively). The further introduction of substituents can proceed via equilibrium exchange (downward dashed arrows) or by the use of more traditional reactions on the surface of the particle (square shapes are introduced by nucleophilic attack in this case.

molecule confer this same activity to the MMPC. Fluorophores represent one group of substituents with markedly different behavior on the surface of the particles then when in solution. The gold core of the particles in this case strongly quenches the intrinsic fluorescence, although the degree of quenching is dependent on the fluorophore-particle distance [24].

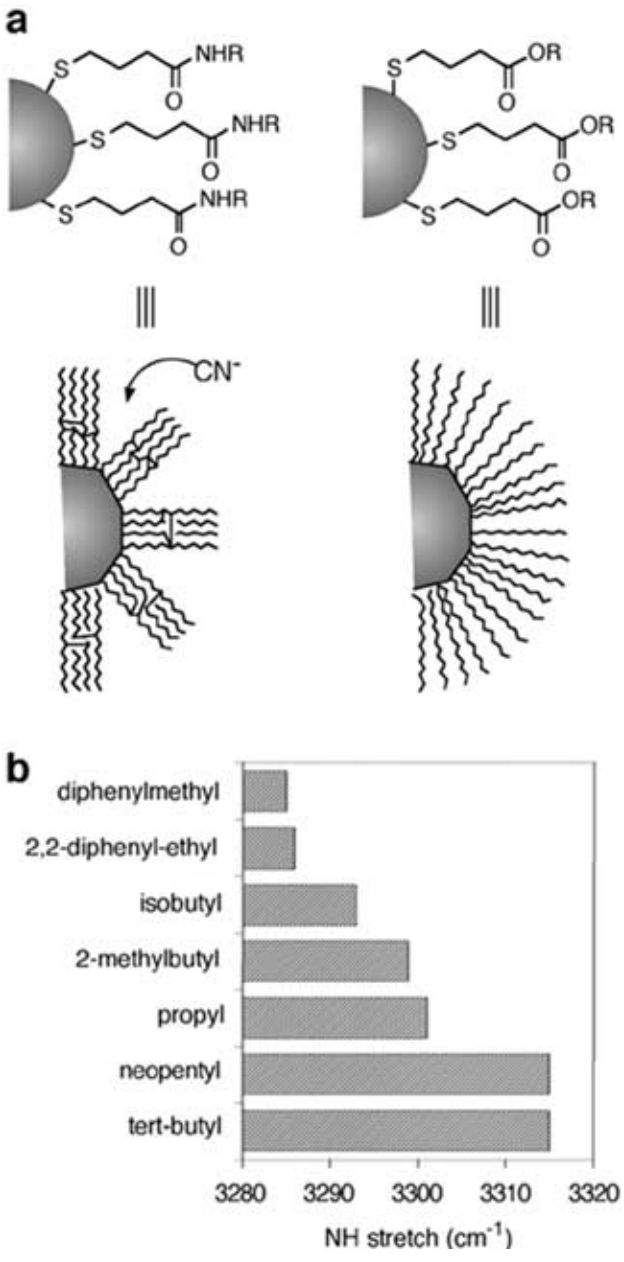

Fig. (3). a) Introduction of hydrogen bonding amides draws the flexible chains toward the facets of the gold surface, leaving the vertices unprotected. The corresponding ester derivative displays no interchain interactions, leaving the monolayer fully extended. b) Interchain interactions within the monolayer components (R groups listed) are monitored by IR as changes in the amide N-H stretch. Decreasing values indicate increased hydrogen bonding. The strongest networks formed also provided the strongest protection from NaCN-induced decomposition.

The capacity of the particles to adopt the recognition characteristics of functional groups at the monolayer exterior has facilitated the application of nanoparticles in biological systems. Initial studies, focused on particularly well-known examples of biological partners [17], indicated that biocompatible substituents act similarly on the nanoparticle surface when compared to their native state. Model systems of biomolecular interactions based on small molecule binding give a demonstration of how multivalency, an important design feature in protein and carbohydrate systems, comes into play. As an example, the flavin cofactor has been demonstrated to interact with its protein partner *in vivo* using hydrogen bonding and π-stacking interactions, as well as other noncovalent forces [25]. Incorporation of a trivalent hydrogen bond host onto a gold particle (MMPC **1**) yields a binding constant with flavin of 193±8 M^{-1} [26]. Introduction of a second functional group appropriate for flavin binding should then increase the binding affinity if the two substituted chains are close enough for flavin to contact them simultaneously [27]. The hydrogen bonding diaminopyridine of MMPC **1** was place-exchanged onto a nanoparticle bearing pyrene-substituted chains to yield MMPC **2** (Figure 4) [28]. This bivalent host displays a 65% increased binding affinity of 323±20 M^{-1} for flavin as compared to the monovalent MMPC **1**. Thus, introduction of the second functionality created an enhanced binding site for the cofactor.

Similarly, Pasquato *et al.* have shown that nanoparticles functionalized with N-methylimidazole binds bis- and tris-Zn-porphyrins in a multivalent fashion [29]. Comparisons of imidazole-functionalized MMPC **3** with a monovalent counterpart showed an increase in binding affinity by up to three orders of magnitude for the tris-porphyrin (Figure 5a). The gain in affinity can be traced to the decreased entropy required for the multiply-functionalized particles to bind the extra porphyrin systems: for the monovalent host, a second or third host molecule must be added to the supramolecular complex to saturate the porphyrin sites. Interestingly, as anticipated, a deviation in binding affinity was seen if the affinity of MMPC **3** for the second and third porphyrins was identical and additive to the initial binding event (Figure 5b). The authors speculate that the difference observed is due to the inability of the porphyrins to bind effectively to the curved surface of the particle, demonstrating the importance of matching the particle surface to its target molecule in addition to the display of multiple substituents for binding. Worth noting is the potential strength of this multivalent interaction: if the tris-porphyrin linker was slightly more adaptable to the pseudo-spherical particle surface, and binding had been additive as the second and third porphyrins were attached, the increase in affinity as compared to the small molecule host would have been larger than five orders of magnitude. This substantial difference is reminiscent of the increased ability of enzymes to process their substrates when compared to uncatalyzed rates.

The previous examples highlight the importance of the tether structure of the recognition element at the particle periphery. Generation of multivalent interactions is simplified because thiol chains are flexible at the monolayer exterior due to the radial nature of the gold core. As the exterior substituents are free to move in space, the relative

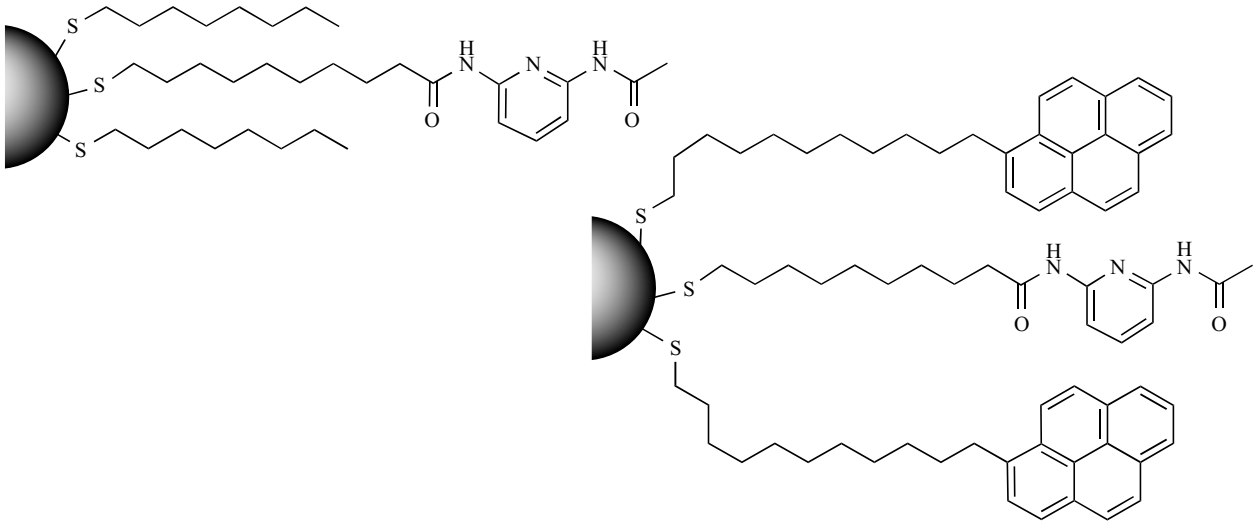

Fig. (4). Structure of hydrogen bonding MMPC **1** and the divalent MMPC **2**. Placement of the diaminopyridine thiol within the aromatic stacking pyrene units increases the flavin-nanoparticle binding affinity by 65%.

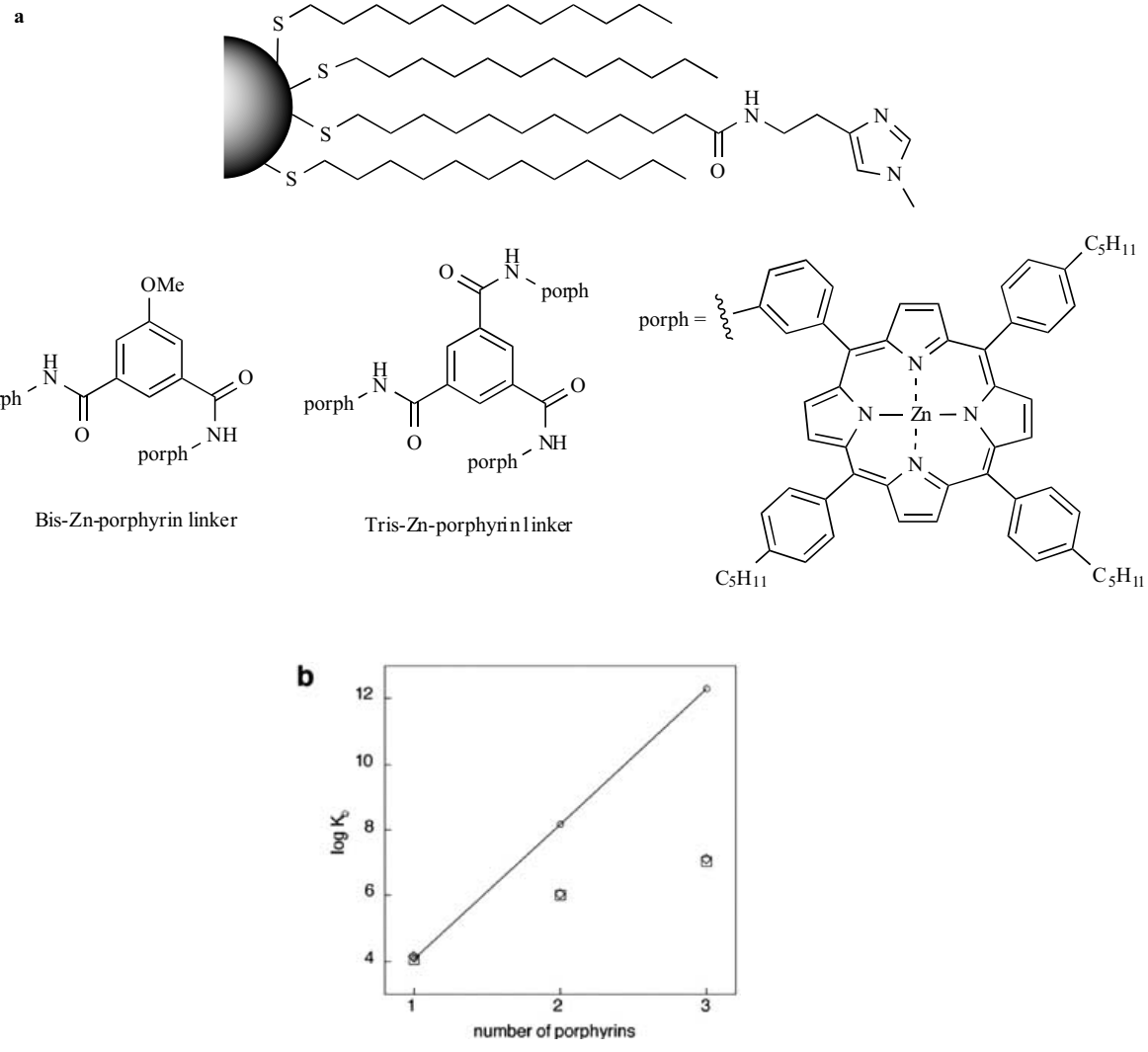

Fig. (5). a) Structure of MMPC **3**, and the bis- and tris-porphyrins. The monovalent porphyrin is unsubstituted. b) Binding affinity observed for MMPC **3** with the three porphyrin derivatives (points) in comparison with that expected if additional porphyrins contributed in an additive fashion to the overall binding (line).

location of the thiols is not fixed. While this introduces uncertainty in the overall topology of the nanoparticle surface, the flexible nature of the thiols also improves the capacity to match the functionality displayed by a guest molecule to optimize binding. Importantly, the optimized conformations available can be further templated due to the mobility of the thiols on the surface of the particle (Figure 6a) [19]. This self-optimization was demonstrated using MMPC **4**, a trifunctionalized nanoparticle consisting of the pyrene and diaminopyridine units of MMPC **2** for flavin binding as well as an octanethiol monolayer to serve as a diluent for the substituted chains [28]. On MMPC **4**, the ω-substituted groups are present only in very low concentrations, making it unlikely for the flavin nucleus to encounter the two moieties simultaneously. Yet, as previously described, flavin is stabilized by both hydrogen bonding groups and aromatic stacking substituents, providing a driving force for rearrangement of the thiols to a configuration allowing the dual binding (Figure 6b). When flavin was added to a solution of MMPC **4**, substantial rearrangement was observed over a 73 hour time period as shown by shifts in flavin NMR resonances. Quantification of these NMR peak shifts showed a 71% increase in the binding constant over the course of the templation process. The slow rearrangement suggests that it is not simply the thiol chains moving in space to maximize interactions, but actual movement of the bound chains on the particle surface to maximize the free energy of the system [19]. The environmentally responsive MMPC **4** bodes well for application to systems with complex surface features, such

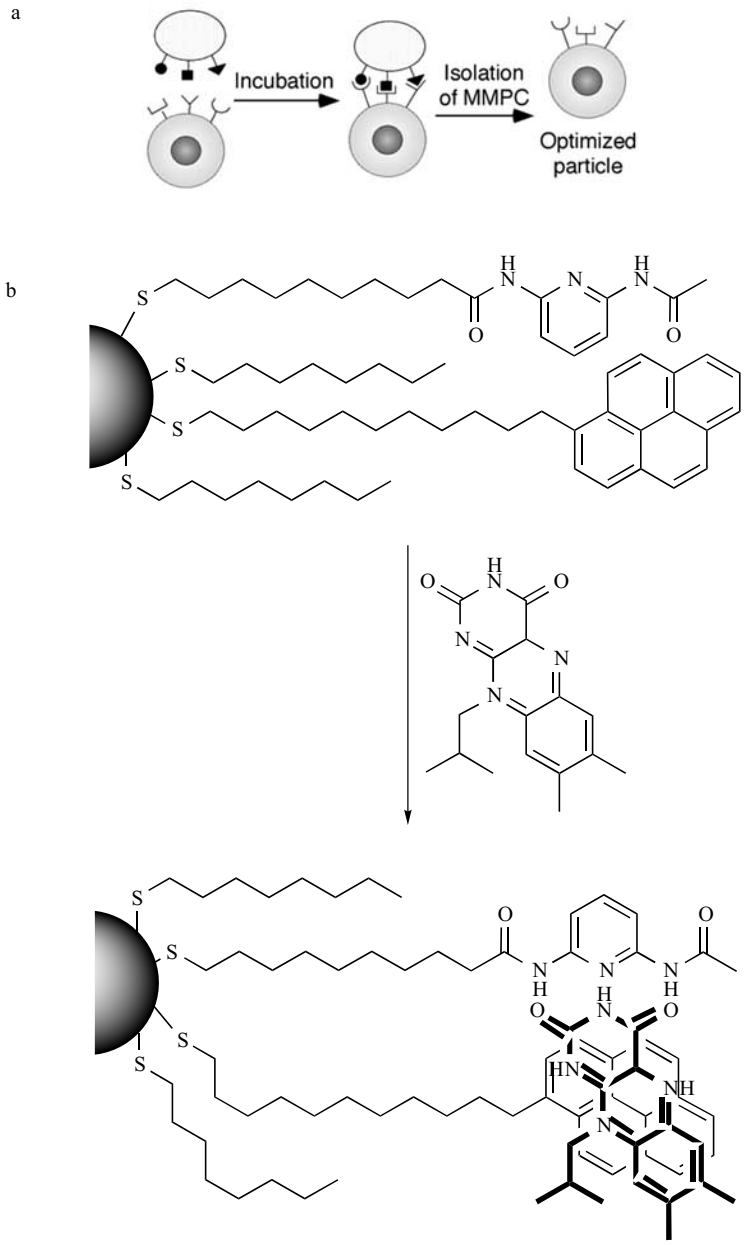

Fig. (6). a) Schematic of self-optimization of functionalized thiols against a biomolecular target. b) Structure of MMPC **4** before and after introduction of the flavin guest. The binding affinity of the trivalent nanoparticle scaffold with the flavin cofactor increased 71% during 73 hours of incubation.

as proteins, nucleic acids, and polysaccharides. Multisubstituted particles can be likened to an immune response: many antibodies with slight variations on sequence, and thus, antigen-binding sites, are generated to find a high affinity partner for the intruding molecule. In the case of the nanoparticles, however, all potential 'sequences' are already present on the particle, and substituents need only to be optimized against any guest molecule to form a tight, selective bond. This templation of the structured, yet flexible monolayer, combined with the ability to incorporate many and varied functionalized thiols indicates that the nanoparticles are excellent candidates for further extension to recognition of complex biomolecules.

III. DNA BINDING

The successful development of synthetic DNA-binding molecules offers the opportunity to directly regulate and control cellular activity. Small molecules have been demonstrated to bind specific DNA sequences [30], or inhibit [31] or promote [32] DNA transcription based on both intercalative and major/minor groove binding. Extension of these principles to nanoparticle systems allows the expedient incorporation of a variety of DNA-binding moieties, as well as the introduction of peptide tags capable of targeting these potential drugs to a desired location. DNA-functionalized nanoparticles have been developed for diagnostic techniques that show high selectivity for a complementary strand, demonstrating their ability to bind DNA with excellent sequence specificity [18]. As previously mentioned, however, we chose to focus in this review on those particles that do not make use of this biologically-active pendant morphology, but instead utilize a network of noncovalent interactions to promote high affinity interactions.

In our laboratory, initial experiments in DNA binding probed the interaction of cationic nanoparticles (MMPC **5**) with a 37mer DNA duplex [33]. Gold cores protected by a mixed monolayer of N,N,N-trimethylammoniumundecane thiol and octane thiol were binding this DNA strand in a 4:1 nanoparticle:duplex ratio (Figure 7a). DNA binding was monitored using a UV centrifugation assay, which requires the DNA change to a 'bound' conformation in order to precipitate from solution; the structure of the DNA in this new conformation was not characterized. While our results and others [34] have shown that extended aggregates of nanoparticles can be assembled using DNA templates in the solid phase, these DNA:nanoparticle complexes in solution form discrete clusters approximately 20 nm in diameter. The ability of a cationic host to simply bind a DNA template is not surprising; the affinity of the electrostatic interaction mediated by the multivalent surface, however, proved to fall within the range of biological complexes. We demonstrated that the nanoparticles, when preincubated with the DNA,

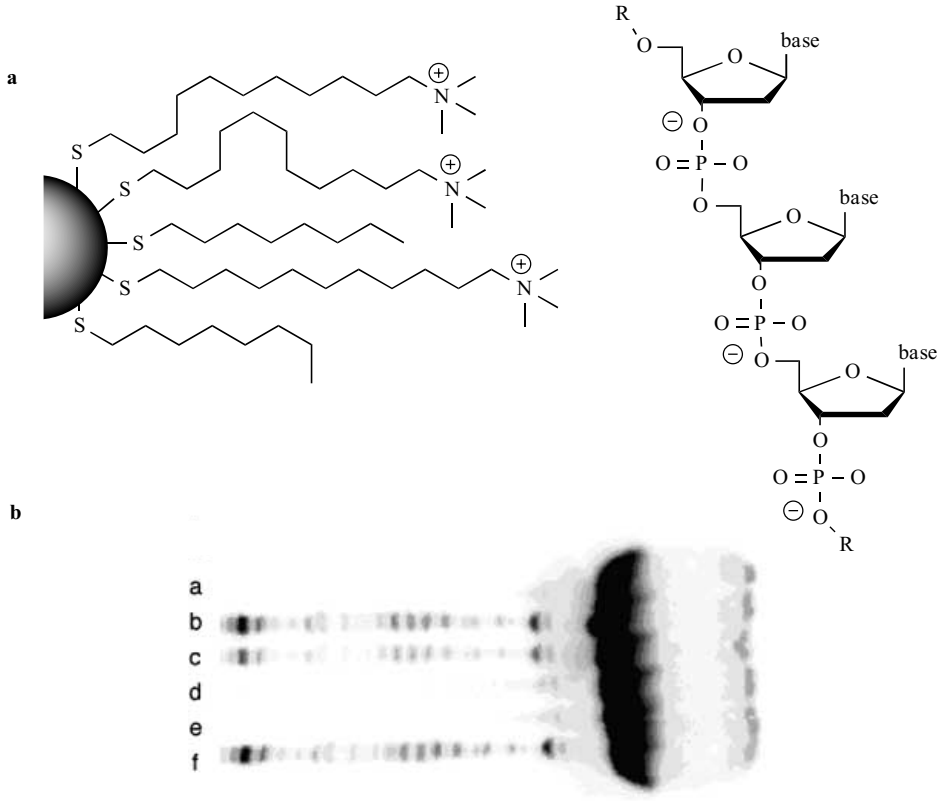

Fig. (7). a) The cationic quaternary ammonium salts of MMPC **5** bind to the phosphate backbone of the DNA. b) Incubation of the nanoparticle and DNA prevents transcription at a 4:1 duplex:particle ratio. Lane a: Control experiment with no DNA shows an impurity in the radioactive GTP. Lanes b-e: DNA and T7 RNA polymerase with increasing concentrations of MMPC **5** (1.8, 3.6, 5.4, and 7.2 eq., respectively). Lane f: DNA and polymerase in the absence of nanoparticles. The large band to the right is free GTP.

could interrupt DNA transcription by T7 RNA polymerase (Figure 7b). The DNA:polymerase complex is estimated to have a K_d of approximately 5 nM [35], suggesting that either MMPC **5** binds with higher affinity than the T7 RNA polymerase or that the altered conformation of DNA when bound to the nanoparticles prevents recognition as the polymerase substrate.

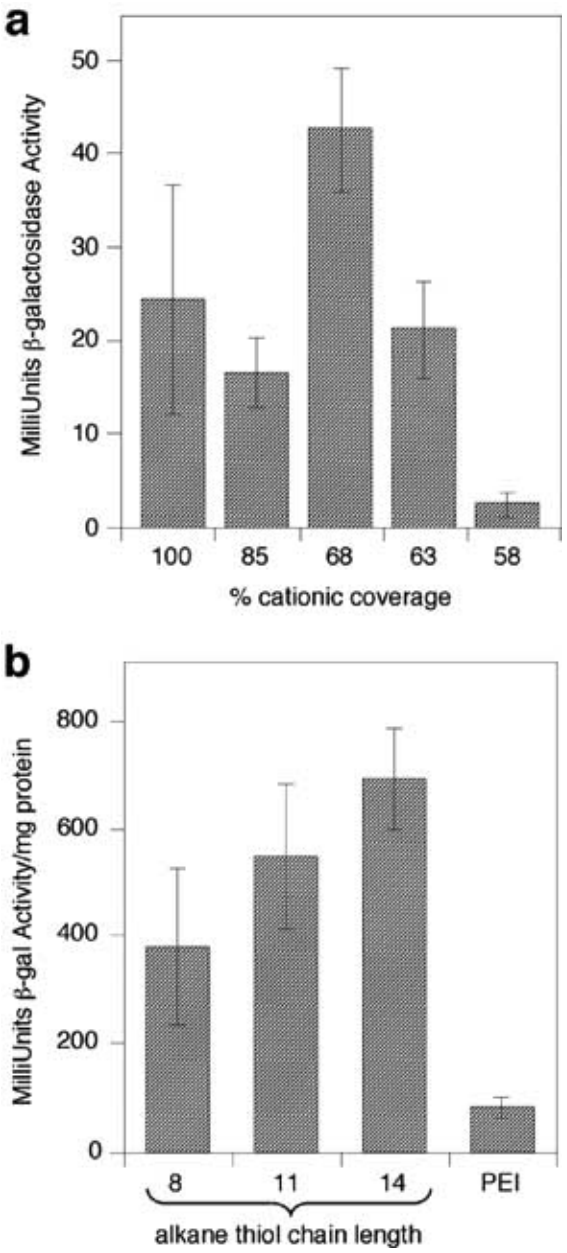

Fig. (8). a) Optimal transfection of the nanoparticle:plasmid complex was seen with 70% cationic coverage, confirming the importance of amphiphilicity in cellular uptake. b) Increasing lengths of the unsubstituted monolayer component promotes additional internalization of the DNA plasmid. The nanoparticles represent a viable vector system, as seen by comparison with polyethyleneimine (PEI).

The ability of the nanoparticles to interrupt a biological process *in vitro* indicates that eventual extension to *in vivo* regulation of DNA expression is possible. In recent studies, we have shown that the nanoparticle:DNA complex can be successfully employed as a transfection vector [36], demonstrating the ability of the particles to function in a biological process in live cells. A series of octanethiol-protected nanoparticles substituted with increasing amounts of quaternary ammonium thiol were briefly incubated with a DNA plasmid encoding β-galactosidase, then introduced to a culture of human embryonic kidney cells. The ratio of nanoparticles to DNA necessary to completely retard the plasmid in an agarose gel and calculated to neutralize all phosphate backbone charges was not sufficient for maximal cellular uptake. This ratio was increased by three-fold for optimal transfection, demonstrating the importance of an overall positive charge on the DNA complex [37]. A comparison of trimethyl ammonium particles showed a varied response with increasing surface charge (Figure 8a). Nanoparticle-mediated internalization of the plasmid complex was most successful with approximately 70% substitution of the cationic thiol, indicating the importance of balance of charged elements for initial interaction with the cell membrane and hydrophobic alkane thiols for subsequent release from the endosomal vesicle membrane [38]. To further examine the effect of hydrophobicity in DNA transfection, we prepared nanoparticles with increasing lengths of the unfunctionalized alkane thiol. Addition of 3 or 6 methylene units increased transfection by approximately 50 or 85%, respectively (Figure 8b). Further increases in length could not be evaluated due to decreased solubility of the nanoparticle: DNA complex. The importance of both the number of cationic substituents as well as variations in the hydrophobicity of the particle in the context of the same cationic surroundings demonstrates the role that multivalency, i.e. consideration of multiple noncovalent interactions, must play in any nanoparticle design.

In contrast to the design of nanoparticles that feature electrostatically complementary substituents, fabrication of DNA-binding gold particles can also proceed via the introduction of DNA-binding elements into the monolayer. Murray *et al.* have utilized the known intercalation of ethidium bromide (EtBr) within the DNA duplex to facilitate binding of cationic and anionic nanoparticles to DNA (MMPC **6** and **7**, respectively) (Figure 9) [31b]. These nanoparticles were functionalized with an average of only one EtBr thiol. MMPC **6** behaved similarly to MMPC **5**: complete and immediate binding of the nanoparticles was observed as monitored by the increased fluorescence of the intercalated ethidium bromide. Surprisingly, intercalation of the EtBr substituent on MMPC **7** was also observed, but only when the tiopronin carboxylates were shielded by salt (at concentrations greater than 0.1 M). Since the intracellular salt concentration ranges from 0.2 to 0.5 M, these carboxylate-functionalized or similar nanoparticles can in fact be applied in the development of DNA-binding scaffolds. The weaker binding of the neutral nanoparticle **7** resulted in slower kinetics, allowing discrimination of two competing binding modes: These two processes are characterized as the interaction of the EtBr with the DNA, either in the major or minor groove, and with the particle itself, due to pairing of the cationic EtBr with the anionic tiopronin. This dual mode of binding raises intriguing possibilities in the design of self-regulating systems.

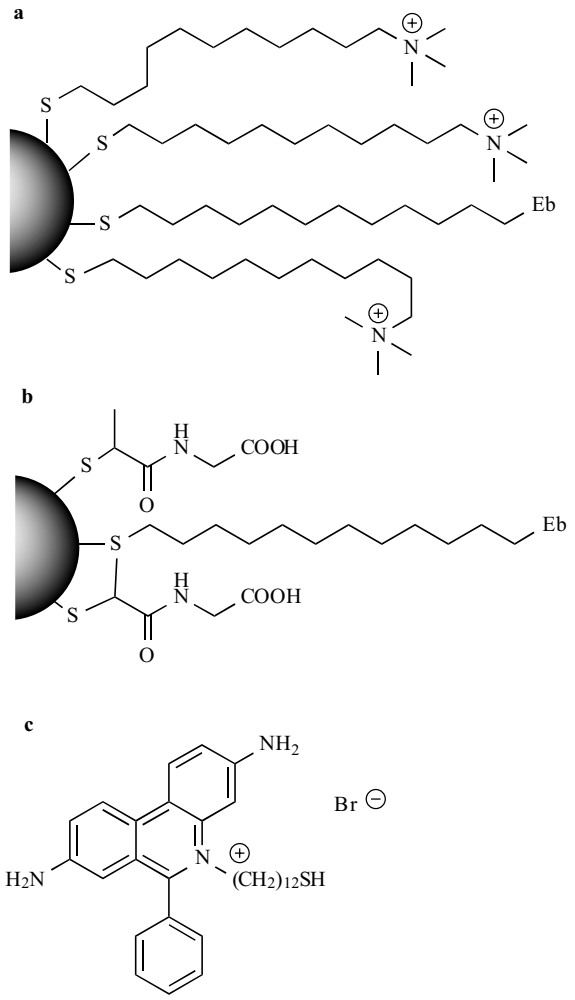

Fig. (9). Structures of MMPCs **6** and **7** and the ethidium thiol (eb). a) MMPC **6** utilizes cationic amines to mediate initial binding to DNA, monitored by increases in ethidium bromide fluorescence upon intercalation. b) MMPC **7** features a tiopronin monolayer, with DNA binding governed by the solvophobic intercalator. c) The ethidium bromide thiol intercalates into the DNA duplex.

IV. PROTEIN BINDING AND THE CONSEQUENCES OF HYDROPHOBIC INTERACTIONS

The ability to bind DNA with cationically functionalized or specifically tagged nanoparticles represents an initial step toward gene therapy techniques. Development of probes for DNA binding, however, can be simplified by approximating the helix as a negative rod, disregarding the fine details of the structural characteristics of DNA as well as specific interactions necessary to facilitate selective binding. To extend the nanoparticles to act as inhibitors in a case where the surface topology was more varied between potential targets, we have applied the nanoparticle as enzyme inhibitors. The creation of protein inhibitors that operate via surface binding allows the extension of protein binding beyond traditional targets [39], such as enzymes with a defined active site. Inhibitors designed for a protein surface may also provide specificity in a family of enzymes that catalyze a similar reaction, preventing a small molecule inhibitor from selectively interacting with only a single family member. Gold nanoparticles are particularly suitable for protein surface binding: the diversity of functional groups that may be incorporated into the monolayer can provide a network of weak intermolecular interactions to strengthen binding between the particle and the protein. In addition, the flexibility of the monolayer at the periphery allows the nanoparticle to more closely map the protein surface, increasing affinity.

In initial studies, we have examined the interaction of carboxylate-functionalized MMPC **8** with α-chymotrypsin (ChT) [40]. ChT has a ring of cationic residues surrounding its active site (Figure 10a). The anionic particles were anticipated to bind at this location, thereby preventing access to the active site and halting hydrolysis of a synthetic ester substrate. The inhibition observed was time- and concentration-dependent, with increasing inhibition observed for nanoparticles with a higher proportion of anionic functionality. No inhibition was seen for the cationic MMPC **5**. All enzymatic activity was interrupted at a nanoparticle:ChT ratio as low as 1:5. The surface area of a 6 nm monolayer-protected nanoparticle is ~110 nm^2, while the ChT footprint is approximately 22 nm^2. The relative size of the two molecules suggests that the nanoparticle surface is fully saturated by the protein at this ratio. Kinetic and CD analysis of binding revealed a two step mechanism of initial electrostatic-mediated binding with an apparent K_i of 10.4 ± 1.3 nM, followed by kinetically irreversible denaturation of the protein on the nanoparticle surface (Figure 10b). While the initial design of the protein inhibitor was simple, incorporating only two monolayer components, the electrostatic complementarity to ChT was sufficient to provide some level of selectivity to this process: elastase, β-galactosidase, and cellular retinoic acid-binding protein displayed minimal interaction with the particles.

Recent reports from the literature [41, 42] show that insertion of an ethylene glycol spacer into the monolayer chains at or near the surface of the particle causes a decrease in nonspecific adsorption and denaturation similar to that observed in 2D systems [43]. In addition, the hydrophilic layer allows the particles to remain dispersed in solutions with high salt concentrations, important for application to *in vivo* conditions [43]. An example of multivalent binding of proteins, in which polyethyleneglycol (PEG) groups are used to maintain protein structure comes from Kataoka *et al.*, in which MMPC **9**, functionalized with PEG-lactose chains, was bound to agglutinin, a bivalent lectin (Figure 11) [44]. The design of the particles was such that the sugar moieties would not extend far enough from the nanoparticle to reach both binding sites on the lectin, resulting in network formation. The PEG interior prevented protein denaturation/adsorption on the surface. Indeed, the particles could be removed and reused via addition of an excess of galactose, the natural substrate for agglutinin. It seems likely that extension of the chains tethering the sugar ring to the nanoparticles would provide a system capable of binding proteins to only one nanoparticle (i.e. no network formation). Accordingly, the length of tether could provide a mechanism for discrimination between lectins with similar substrates based on the orientation of the binding sites. These initial studies of binding protein targets reveal the diversity of systems available for adaptation of nanoparticle hosts.

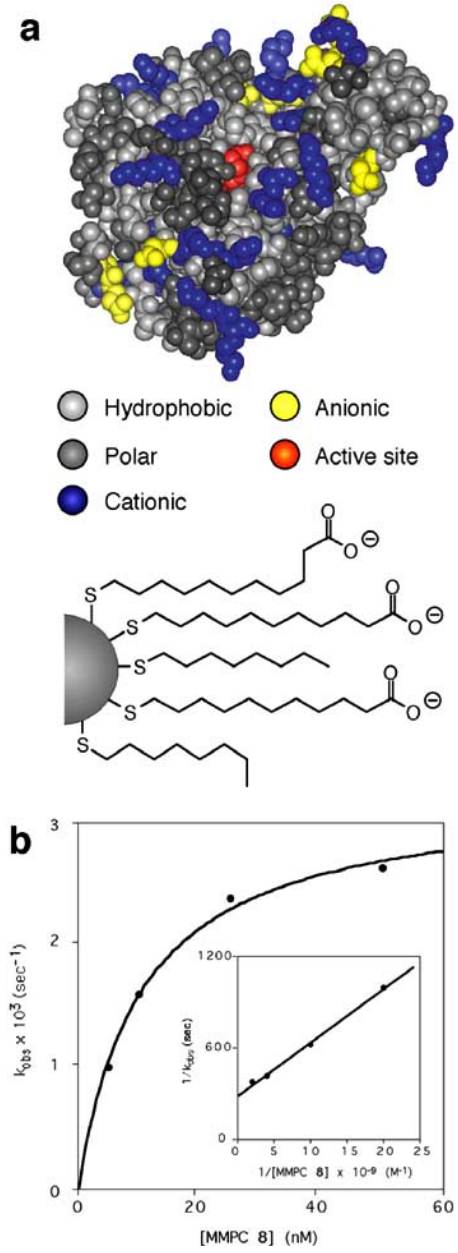

Fig. (10). a) The active site of chymotrypsin is surrounded by a cationic ring of residues, anticipated to promote binding of the anionic MMPC **8**, thus preventing catalysis. b) Kinetic analysis of chymotrypsin activity indicating a two step mechanism of initial electrostatic pairing (K_i = 10.4±1.3 nM) followed by irreversible protein denaturation (inset; denoted by non-zero intercept).

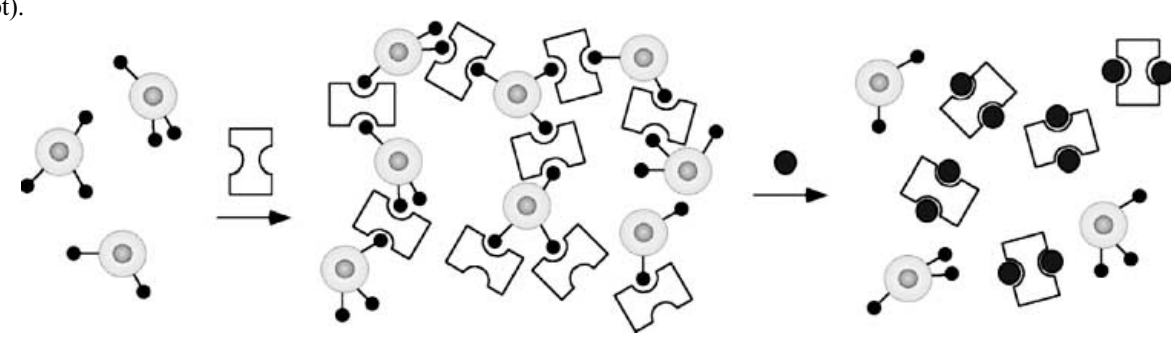

Fig. (11). Lactose-functionalized nanoparticles bind the bivalent lectin (rectangular shape) to form an interconnected network, which can be released by the addition of excess galactose (large circles). The particles, due to their PEG interior, do not adhere to the protein and can be recycled.

V. FURTHER EXTENSIONS OF FUNCTIONALIZED NANOPARTICLES

The first steps described above in applying the nanoparticles as biomolecular hosts provide great promise in mediating disease states or regulating cellular conditions. Yet the unique architecture of the particles also lends these scaffolds to further functions. One significant field of exploration is the use of the nanoparticles in membrane studies, as the flexible nature of the chains, combined with the hydrophobic interior and hydrophilic surface, are highly reminiscent of the fluidity and structure of lipid membranes. The Lewis^x antigen, a trisaccharide attached to membrane lipids, is believed to undergo self-association in the initial steps of cell recognition and adhesion. Because each interaction is weak, cells utilize a polyvalent display of these substituents to provide tight binding. To test this self-assembly mechanism, Penades *et al.* created glyconanoparticles with either PEG- or alkyl-lactose monolayers or with Lewis^x-functionalized chains (MMPCs **10**-**12**, respectively) (Figure 12a) [45]. The Lewis^x association is further mediated by divalent cations. Addition of $CaCl_2$ to all three particles led to the association of MMPC **12**, with no aggregation observed for either MMPC **10** or **11** (Figure 12b). Removal of the cations *via* addition of EDTA reversed the assembly of the trisaccharide-substituted surface, demonstrating the specific role of the sugar in assembly. By varying the nanoparticle properties or diluting the concentration of Lewis^x sugars on the particle surface, we can hope to learn more about the role of these trisaccharides in cellular adhesion [46]. Indeed, these early results demonstrate that further studies of these and other membrane components can be successfully completed within the background of a nanoparticle scaffold.

Beyond the use of the MMPCs in biomolecular binding and model systems, these nanoparticle systems can be used to create of novel materials. The simplest biological systems to deconvolute and recreate at this point are enzymes. Substantial effort has gone into alteration [47] and invention [48] of proteins capable of performing new or modified catalytic transformations. The main principle behind these efforts comes from the realization that active sites are often nothing more than several substituents held in close proximity with proton donors and acceptors nearby. Accordingly, nanoparticles are excellent systems for the development of catalysts because the three-dimensional arrangement of the functional groups on the monolayer surface can be controlled to some extent by varying substituent concentration, introducing hydrogen bonding or

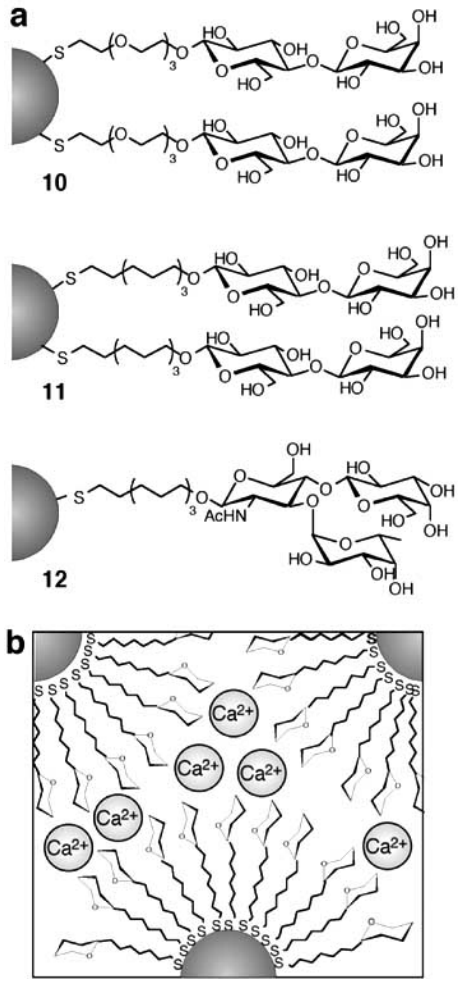

Fig. (12). a) Structure of the glycosylated thiols of MMPCs **10** and **11** (lactose chain) and MMPC **12** (functionalized with the Lewis^x antigen) b) Lewis^x association, mediated by divalent calcium ions, drives particle aggregation. This assembly can be reversed by addition of EDTA to sequester the cations.

similar tethering groups, or via templation against a transition state analog. Pasquato *et al.* have probed the ability of the N-methylimidazole-functionalized MMPC **3** to act as a catalyst in the hydrolysis of a carboxylic acid ester [49]. The cooperativity of the substituted chains increased hydrolysis rates over a small molecule analog by approximately 30 times at the optimal pH for each reaction. For the single imidazole, the pH profile showed only one transition centered at pH 6, with increasing rates of catalysis in increasingly basic conditions. For the multivalent nanoparticle system, however, two transitions were observed: the first was a sharp increase in the rate as the pH increased to 6.5, presumably due to deprotonation of some of the imidazoles and therefore, increased concentrations of general base sites for catalysis. The second transition was a decrease in activity as more of the substituents were deprotonated, probably due to the decrease in available sites for general acid catalysis. This activity profile is reminiscent of many enzymes, which are frequently optimized to operate in a narrow pH range. To test the nanoparticle as a scaffold beyond its ability to simply assemble multiple imidazoles, the authors formed a comicelle of surfactants and a long chain variant of the monovalent host. While a direct comparison was complicated due to changes in concentration of the imidazole hosts, the closest comparison possible indicated that the reaction rate as catalyzed by the comicelles was approximately 65% of that observed with the nanoparticles. While this result at first suggests that the particles do not substantially improve on readily available systems, there is an important distinction between the micelle system and the particles: the formation of the micelles is governed by their critical aggregate concentration, whereas the nanoparticles are concentration-independent.

VI. CONCLUSION

The properties of monolayer-protected gold nanoparticles are specially suited for biomolecular recognition. The gold core can be formed in a range of sizes, and functionalized with diverse substituents through place exchange reactions. Perhaps more important, however, is the flexibility inherent to the monolayer coupled with the ability of the substituents to self-optimize against a given target. These properties have been exploited in binding a range of biomacromolecules, with almost unlimited opportunities for further study. Finally, the nanoparticles have been applied in the development of advanced model systems and catalysts, demonstrating that the use of gold particles in biological studies is constrained only by our imagination.

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REFERENCES

[1] Faulk, W. P.; Taylor, G. M. *Immunochemistry* **1971**, *8*, 1081.

[2] a) Lea, P.; Lee, L. M. Y.; Shi, Q. W.; Takahashi, M.; Youn, W.; Jackowski, G. *Scanning* **1996**, *18*, 259. b) Roth, J.; Zuber, C.; Komminoth, P.; Sata, T.; Li, W. P.; Heitz, P. U. *Histochem. Cell Biol.* **1996**, *106*, 131.

[3] a) Schoning, P. R. *J. Histotechnol.* **1997**, *20*, 31. b) Delaigue, O.; Arbeille, B.; Rossazza, C.; Lemesle, M.; Roingeard, P. *Graefes Arch. Clin. Exp. Ophthalmol.* **1995**, *233*, 331. c) Meeker, R. B.; Swanson, D. J.; Greenwood, R. S.; Hayward, J. N. *Brain Res.* **1993**, *600*, 112. d) Poole, C. A.; Ayad, S.; Gilbert, R. T. *J. Cell Sci.* **1992**, *103*, 1101.

[4] Shenhar, R.; Rotello, V. M. *Acc. Chem. Res.* **2003**, *36*, 549.

[5] Sastry, M.; Rao, M.; Ganesh, K. N. *Acc. Chem. Res.* **2002**, *35*, 847.

[6] Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D. J.; Whyman, R. *J. Chem. Soc., Chem. Commun.* **1994**, 801.

[7] a) Chen, S. W.; Templeton, A. C.; Murray, R. W. *Langmuir* **2000**, *16*, 3543. b) Hostetler, M. J.; Wingate, J. E.; Zhong, C. J.; Harris, J. E.; Vachet, R. W.; Clark, M. R.; Londono, J. D.; Green, S. J.; Stokes, J. J.; Wignall, G. D.; Glish, G. L.; Porter, M. D.; Evans, N. D.; Murray, R. W. *Langmuir* **1998**, *14*, 17.

[8] Sarathy, K. V.; Narayan, K. S.; Kim, J.; White, J. O. *Chem. Phys. Lett.* **2000**, *318*, 543.

[9] a) Boal, A. K.; Gray, M.; Ilhan, F.; Clavier, G. M.; Kapitzky, L.; Rotello, V. M. *Tetrahedron* **2002**, *58*, 765. b) Frankamp, B. L.; Boal, A. K.; Rotello, V. M. *J. Am. Chem. Soc.* **2002**, *124*, 15146. c) Boal, A. K.; Ilhan, F.; DeRouchey, J. E.; Thurn-Albrecht, T.; Russell, T. P.; Rotello, V. M. *Nature* **2000**, *404*, 746.

[10] a) Willner, I.; Shipway, A. N.; Willner, B. In *Molecules as Components of Electronic Devices*; *Amer. Chem. Soc.*: Washington, DC, **2003**; Vol. *844*, p 88. b) Hicks, J. F.; Seok-Shon, Y.; Murray, R. W. *Langmuir* **2002**, *18*, 2288. c) Hao, E. C.; Lian, T. Q. *Chem. Mat.* **2000**, *12*, 3392.

[11] Cole, D. H.; Shull, K. R.; Baldo, P.; Rehn, L. *Macromolecules* **1999**, *32*, 771.

[12] a) Simard, J.; Briggs, C.; Boal, A. K.; Rotello, V. M. *Chem. Commun.* **2000**, 1943. b) Templeton, A. C.; Cliffel, D. E.; Murray, R. W. *J. Am. Chem. Soc.* **1999**, *121*, 7081. c) Templeton, A. C.; Hostetler, M. J.; Warmoth, E. K.; Chen, S. W.; Hartshorn, C. M.; Krishnamurthy, V. M.; Forbes, M. D. E.; Murray, R. W. *J. Am. Chem. Soc.* **1998**, *120*, 4845.

[13] a) Dragnea, B.; Chen, C.; Kwak, E. S.; Stein, B.; Kao, C. C. *J. Am. Chem. Soc.* **2003**, *125*, 6374. b) Souza, G. R.; Miller, J. H. *J. Am. Chem. Soc.* **2001**, *123*, 6734. c) Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757.

[14] Wilson, R. *Chem. Commun.* **2003**, 108. b) Lin, S. Y.; Liu, S. W.; Lin, C. M.; Chen, C. H. *Anal. Chem.* **2002**, *74*, 330. Nath, N.; Chilkoti, A. *J. Am. Chem. Soc.* **2001**, *123*, 8197.

[15] Zheng, M.; Davidson, F.; Huang, X. Y. *J. Am. Chem. Soc.* **2003**, *125*, 7790.

[16] a) Mayya, K. S.; Schoeler, B.; Caruso, F. *Adv. Funct. Mater.* **2003**, *13*, 183. b) Mandal, T. K.; Fleming, M. S.; Walt, D. R. *Nano Lett.* **2002**, *2*, 3. c) Corbierre, M. K.; Cameron, N. S.; Sutton, M.; Mochrie, S. G. J.; Lurio, L. B.; Ruhm, A.; Lennox, R. B. *J. Am. Chem. Soc.* **2001**, *123*, 10411. d) Gittins, D. I.; Caruso, F. *J. Phys. Chem. B* **2001**, *105*, 6846. e) Nuss, S.; Bottcher, H.; Wurm, H.; Hallensleben, M. L. *Angew. Chem. Int. Edit.* **2001**, *40*, 4016. f) Chechik, V.; Crooks, R. M. *Langmuir* **1999**, *15*, 6364.

[17] a) Raschke, G.; Kowarik, S.; Franzl, T.; Sonnichsen, C.; Klar, T. A.; Feldmann, J.; Nichtl, A.; Kurzinger, K. *Nano Lett.* **2003**, *3*, 935. b) Haes, A. J.; Van Duyne, R. P. *J. Am. Chem. Soc.* **2002**, *124*, 10596. c) Riepl, M.; Enander, K.; Liedberg, B.; Schaferling, M.; Kruschina, M.; Ortigao, F. *Langmuir* **2002**, *18*, 7016.

[18] a) Cao, Y. W. C.; Jin, R. C.; Mirkin, C. A. *Science* **2002**, *297*, 1536. b) Park, S. J.; Taton, T. A.; Mirkin, C. A. *Science* **2002**, *295*, 1503. c) Yun, C. S.; Khitrov, G. A.; Vergona, D. E.; Reich, N. O.; Strouse, G. F. *J. Am. Chem. Soc.* **2002**, *124*, 7644. d) Cao, Y. W.; Jin, R.; Mirkin, C. A. *J. Am. Chem. Soc.* **2001**, *123*, 7961.

[19] Hostetler, M. J.; Templeton, A. C.; Murray, R. W. *Langmuir* **1999**, *15*, 3782.

[20] Templeton, A. C., Wuelfing, W. P., Murray, R. W. *Acc. Chem. Res.* **2000**, *33*, 27-36.

[21] Templeton, A. C.; Hostetler, M. J.; Kraft, C. T.; Murray, R. W. *J. Am. Chem. Soc.* **1998**, *120*, 1906.

[22] Paulini, R.; Frankamp, B. L.; Rotello, V. M. *Langmuir* **2002**, *18*, 2368. Boal, A. K.; Rotello, V. M. *Langmuir* **2000**, *16*, 9527.

[23] Ingram, R. S.; Hostetler, M. J.; Murray, R. W. *J. Am. Chem. Soc.* **1997**, *119*, 9175.

[24] a) Gu, T.; Ye, T.; Simon, J. D.; Whitesell, J. K.; Fox, M. A. *J. Phys. Chem. B* **2003**, *107*, 1765. b) Ipe, B. I.; Thomas, K. G.;

Barazzouk, S.; Hotchandani, S.; Kamat, P. V. *J. Phys. Chem. B* **2002**, *106*, 18. c) Wang, T. X.; Zhang, D. Q.; Xu, W.; Yang, J. L.; Han, R.; Zhu, D. B. *Langmuir* **2002**, *18*, 1840.

[25] For a recent review, see: *Flavins and Flavoproteins, 1996,* Stevenson, K.; Massey, V.; Williams, C. Eds.; University of Calgary: Calgary, Canada, **1997**, Vol. *1-3*.

[26] Boal, A. K.; Rotello, V. M. *J. Am. Chem. Soc.* **1999**, *121*, 4914.

[27] Niemz, A.; Rotello, V. M. *Accounts Chem. Res.* **1999**, *32*, 44. b) Rotello, V. M. *Curr. Opin. Chem. Biol.* **1999**, *3*, 747.

[28] Boal, A. K.; Rotello, V. M. *J. Am. Chem. Soc.* **2000**, *122*, 734.

[29] Fantuzzi, G.; Pengo, P.; Gomila, R.; Ballester, P.; Hunter, C. A.; Pasquato, L.; Scrimin, P. *Chem. Commun.* **2003**, 1004.

[30] a) Dervan, P. B.; Edelson, B. S. *Curr. Opin. Struct. Biol.* **2003**, *13*, 284. b)Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2215. Crooke, S. T. *Faseb J.* **1993**, *7*, 533.

[31] a) Gottesfeld, J. M.; Belitsky, J. M.; Melander, C.; Dervan, P. B.; Luger, K. *J. Mol. Biol.* **2002**, *321*, 249. b) Wang, G. L.; Zhang, J.; Murray, R. W. *Anal. Chem.* **2002**, *74*, 4320.

[32] a) Coull, J. J.; He, G. C.; Melander, C.; Rucker, V. C.; Dervan, P. B.; Margolis, D. M. *J. Virol.* **2002**, *76*, 12349. b) Mapp, A. K.; Ansari, A. Z.; Ptashne, M.; Dervan, P. B. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 3930.

[33] McIntosh, C. M.; Esposito, E. A.; Boal, A. K.; Simard, J. M.; Martin, C. T.; Rotello, V. M. *J. Am. Chem. Soc.* **2001**, *123*, 7626.

[34] a) Iacopino, D.; Ongaro, A.; Nagle, L.; Eritja, R.; Fitzmaurice, D. *Nanotechnology* **2003**, *14*, 447. b) Warner, M. G.; Hutchison, J. E. *Nat. Mater.* **2003**, *2*, 272. c) Sauthier, M. L.; Carroll, R. L.; Gorman, C. B.; Franzen, S. *Langmuir* **2002**, *18*, 1825. d) Kumar, A.; Pattarkine, M.; Bhadbhade, M.; Mandale, A. B.; Ganesh, K. N.; Datar, S. S.; Dharmadhikari, C. V.; Sastry, M. *Adv. Mater.* **2001**, *13*, 341. e) Han, S.; Lin, J.; Satjapipat, M.; Baca, A. J.; Zhou, F. *Chem. Commun.* **2001**, 609. f) Richter, J.; Seidel, R.; Kirsch, R.; Mertig, M.; Pompe, W.; Plaschke, J.; Schackert, H. K. *Adv. Mater.* **2000**, *12*, 507.

[35] Kuzmine, I.; Martin, C. T. *J. Mol. Biol.* **2001**, *305*, 559.

[36] Sandhu, K. K.; McIntosh, C. M.; Simard, J. M.; Smith, S. W.; Rotello, V. M. *Bioconjugate Chem.* **2002**, *13*, 3.

[37] a) Kneuer, C.; Sameti, M.; Bakowsky, U.; Schiestel, T.; Schirra, H.; Schmidt, H.; Lehr, C. M. *Bioconjugate Chem.* **2000**, *11*, 926. b) Truong-Le, V. L.; Walsh, S. M.; Schweibert, E.; Mao, H. Q.; Guggino, W. B.; August, J. T.; Leong, K. W. *Arch. Biochem. Biophys*. **1999**, *361*, 47. c) Wolfert, M. A.; Schacht, E. H.; Toncheva, V.; Ulbrich, K.; Nazarova, O.; Seymour, L. W. *Hum. Gene Ther.* **1996**, *7*, 2123.

[38] Sandhu, K. K. Masters Thesis. University of Massachusetts at Amherst, May 2002.

[39] a) Park, H. S.; Lin, Q.; Hamilton, A. D. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 5105. b)Burgess, K. *Acc. Chem. Res.* **2001**, *34*, 826. c) Jain, R. K.; Hamilton, A. D. *Org. Lett.* **2000**, *2*, 1721. d) Lin, Q.; Park, H. S.; Hamuro, Y.; Lee, C. S.; Hamilton, A. D. *Biopolymers* **1998**, *47*, 285.

[40] Fischer, N. O.; McIntosh, C. M.; Simard, J. M.; Rotello, V. M. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 5018.

[41] a) Foos, E. E.; Snow, A. W.; Twigg, M. E.; Ancona, M. G. *Chem. Mat.* **2002**, *14*, 2401. b)Wuelfing, W. P.; Gross, S. M.; Miles, D. T.; Murray, R. W. *J. Am. Chem. Soc.* **1998**, *120*, 12696. c) Zheng, M.; Davidson, F.; Huang X. *J. Am. Chem. Soc.*, **2003**, *125*, 7790.

[42] Weisbecker, C. S.; Merritt, M. V.; Whitesides, G. M. *Langmuir* **1996**, *12*, 3763.

[43] a) Mrksich, M.; Whitesides, G. M. *Trends Biotechnol.* **1995**, *13*, 228. b) Lopez, G. P.; Albers, M. W.; Schreiber, S. L.; Carroll, R.; Peralta, E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 5877.

[44] Otsuka, H.; Akiyama, Y.; Nagasaki, Y.; Kataoka, K. *J. Am. Chem. Soc.* **2001**, *123*, 8226.

[45] de la Fuente, J. M.; Barrientos, A. G.; Rojas, T. C.; Rojo, J.; Canada, J.; Fernandez, A.; Penades, S. *Angew. Chem., Int. Ed.* **2001**, *40*, 2258.

[46] Barrientos, A. G.; de la Fuente, J. M.; Rojas, T. C.; Fernandez, A.; Penades, S. *Chem.-Eur. J.* **2003**, *9*, 1909.

[47] a) Lutz, S.; Benkovic, S. J. *Curr. Opin. Biotechnol.* **2000**, *11*, 319. b) Nixon, A. E.; Ostermeier, M.; Benkovic, S. J. *Trends Biotechnol.* **1998**, *16*, 258.

[48] a) Maglio, O.; Nastri, F.; Pavone, V.; Lombardi, A.; DeGrado, W. F. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3772. b) Marsh, E. N. G.; DeGrado, W. F. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 5150. c) DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3025.

[49] Pasquato, L.; Rancan, F.; Scrimin, P.; Mancin, F.; Frigeri, C. *Chem. Commun.* **2000**, 2253.