Surface recognition of biomacromolecules using nanoparticle receptors

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Nanoparticles present a versatile scaffold to target biomacromolecule surfaces *via* complementary interactions. This review highlights some unique features of nanoparticles that make them particularly attractive resources for biomacromolecular recognition, and displays their use in modulation of structure and function of biomacromolecules.

Introduction

Interactions between biomacromolecules form the basis for a number of cellular processes such as protein–protein interactions, protein–nucleic acid interactions, enzyme activity, and cell surface recognition. Modulation of these interactions through creation of efficient receptors designed to recognize biomacromolecules such as proteins and nucleic acids paves the way for alternative approaches to therapeutic agents, as well as diagnostic biosensors for rapid monitoring of imbalances and illnesses.

Even though recognition of biomolecular surfaces relies on the same fundamental interactions involved in small molecule host–guest systems, regulation of biomacromolecules presents a considerable challenge. This challenge can be attributed to two basic requirements for an effective protein or nucleic acid surface recognition. The first requirement is a large receptor contact area. As an example, large surface areas are required for effective binding of protein surfaces which are both convex and solvent exposed. Insight into this requirement comes from examination of protein–protein interactions, which reveal that surface areas of more than 6 nm^2 per protein are typically buried in such interactions.[1]

The second challenge stems from the complexities of the surfaces involved[2] in terms of their multiple electrostatic,[3] hydrophobic[4] and topological features. A number of "small molecule" systems[5] and macromolecular scaffolds have been used to address this challenge for protein surface recognition. "Small molecule" systems include receptors on calixarene and porphyrin scaffolds,[6] cyclodextrin dimers[7] and transition metal complexes targeted against surface exposed histidines.[8] Polymer scaffolds include multivalent libraries of receptors possessing partially constrained backbones.[9] Such systems demonstrate a certain level of success in modulation of biomolecular function. As discussed in this review, however, nanoparticle-based receptors offer a unique and advantageous platform for biomacromolecular surface recognition.

Core–shell nanoparticle systems such as monolayer protected clusters (MPCs) and mixed monolayer protected clusters (MMPCs) possess four important attributes that make them promising scaffolds for creation of receptors targeted to biomolecular surfaces:

(1) The size of the nanoparticle core can be tuned from 1.5 to 8 nm with overall diameters of 2.5 to 11 nm.[10] This control of core size allows particles to be fabricated on comparable size scales as their biomacromolecular targets (Fig. 1).

(2) Nanoparticles can be fabricated with a wide range of surface functionality in divergent fashion, providing a flexible route to the creation of surface-specific receptors.

(3) MMPCs can be generated with a range of metal and semiconductor cores featuring useful electronic, fluorescence, and magnetic properties. This versatility makes these systems excellent materials for probes and/or diagnostic agents.

(4) MMPCs can self-template to complementary surfaces, which allows increase in the affinity and selectivity of the recognition process on incubation with the guest (*vide infra*).[11,12] This provides a definite advantage over the conventionally used synthetic receptors.

Recent studies have focused on the use of nanoparticles as a "solid phase" support, where they have been used as structural building blocks or as a visualization aid for sensor studies. These studies use pre-formed recognition elements, and include examples such as interactions between streptavidin and biotin-labeled particles[13] or hybridization of complementary DNA strands conjugated to nanoparticles.[14] However, the focus of this review will be the properties and utilization of monolayer

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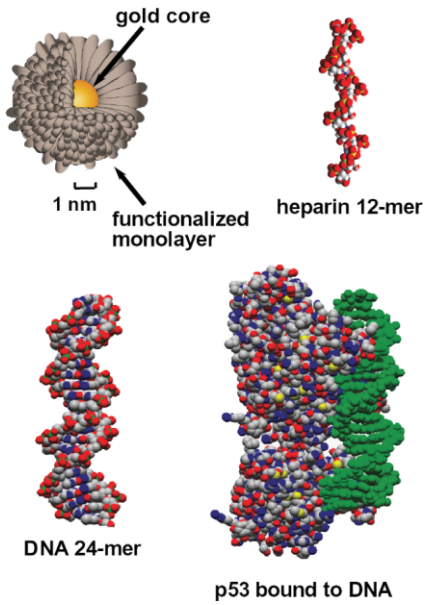

Fig. 1 Relative sizes of a nanoparticle with a 2 nm core and an octanethiol functionalized monolayer and possible biological targets.

and mixed monolayer-protected nanoparticles using the functional organic groups on the nanoparticle surface as multivalent recognition elements targeted at biomolecular surfaces. The use of MPCs and MMPCs as synthetic receptors for biomolecular recognition allows for modulation of activities of proteins and nucleic acids, not possible through the traditional use of nanoparticles as support elements. Further, MPCs and MMPCs can allow comparisons of biological complex formation such as protein–protein interactions based on surface complementarity.

Fabrication and properties of monolayer and mixed monolayer protected clusters

The ease of fabrication and characterization of MPCs and MMPCs renders them a useful tool for biomolecular surface recognition. Core-shell nanoparticles comprise of a cluster of metal atoms forming a polyhedral shape, immediately surrounded by a self-assembled monolayer (SAM).[15] The monolayer serves two functions: it shields the metal core preventing aggregation of the nanoparticles, and provides the opportunity to tailor the surface functionality of the particle.

An important advance in the fabrication of MPCs was introduced by Brust *et al.*[16] featuring reduction of metal salts in the presence of capping ligands such as thiols (Fig. 2a). The reduction process is performed under mild conditions enabling

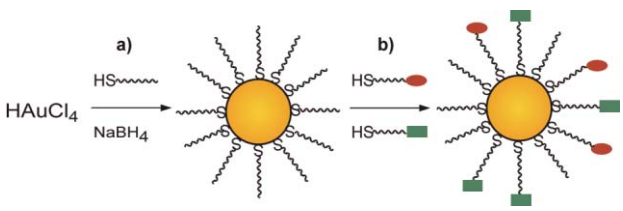

Fig. 2 Synthesis of gold MPCs using (a) the Brust–Schiffrin reaction and MMPCs *via* (b) the Murray place-exchange method.

the use of a wide range of ligand functionality. A range of noble metal cores (Pd, Au, Ag, Pt) can be synthesized and the sizes of the metal cores can be tuned by varying the ratio of the metal salt to the capping ligand, providing a range of sizes. As a point of reference, an MMPC featuring a 2 nm gold core diameter is covered by approximately 100 ligand chains[15] furnishing a surface area of $\sim$110 nm^2 for biomolecular surface interaction.

The monolayers of MPCs can be further elaborated through the Murray place displacement reaction,[17] where ligands are displaced on the MPC, resulting in MMPCs (Fig. 2b). This technique provides for a rapid generation of a wide variety of MMPCs featuring diverse functionalities. In addition to the generation of functionalities at the surface *via* place displacement method, synthetic approaches can be applied to provide reactive groups at the surface.[18]

The reactive groups on the nanoparticles assume a primary importance in mediation of biomacromolecular surface recognition. The end-groups dictate the water solubility of the MMPCs, a prerequisite for most biological studies. Additionally, the end groups dictate the ability of nanoparticles to interact with biomacromolecules. An important aspect of the inclusion of interacting groups on the nanoparticle surface is that covalent attachment of multiple ligands can greatly enhance the strength of biomolecular interactions. For example, carbohydrate–protein interactions are usually characterized by very low affinity. However, as polyvalent interactions between the ligands and receptors are collectively stronger than corresponding monovalent interactions, the low affinity of interactions can be increased by the presentation of multiple groups on the nanoparticles.[19] Another important property of end-groups on the nanoparticle surface is observed in the case of fluorophores. The gold core strongly quenches the intrinsic fluorescence; the extent of quenching depending on the distance of the fluorophore from the core.[20]

Templation of MMPCs

Given the importance of the nanoparticle monolayer end-groups, a versatile receptor can be generated on the MMPC surface for both monotropic as well as multivalent interactions. Significantly, the mobility of the thiols on the self-assembled monolayer (SAM) surface presents the possibility of creating environmentally responsive systems. The mobility of thiols on MMPCs provides a potential method for imprinting/templation through maximization of binding enthalpy (Fig. 3a). This templation was demonstrated by Rotello *et al.* through the time-dependent recognition of flavin by MMPC **1** (Fig. 3b).[11] The nanoparticle featured pyrene as an aromatic stacking element and diamidopyridine as a hydrogen-bonding moiety, both diluted into an octanethiol monolayer. Flavin, which can interact by both hydrogen bonding and aromatic π-stacking was then incubated with MMPC **1**. On addition of flavin to MMPC **1**, substantial rearrangement was observed over a 73 h incubation period, as demonstrated by time-course NMR experiments. Quantification of the results revealed a 71% increase in the binding constant over the course of the templation process. This distinctive feature of MMPCs can be extended to the surface recognition of systems with complex

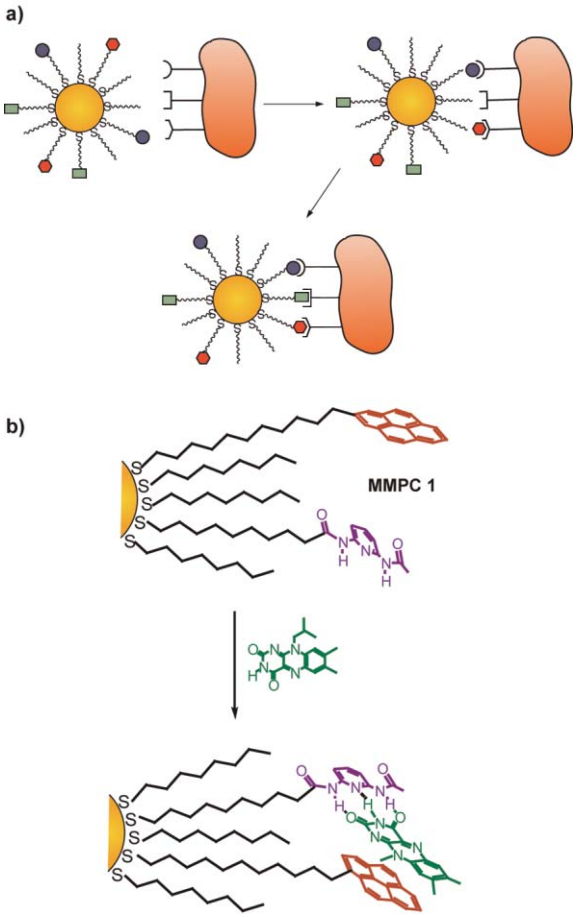

Fig. 3 (a) Schematic representation of an MMPC optimizing to a biomolecule surface. (b) Flavin mediated templation of MMPC **1**.

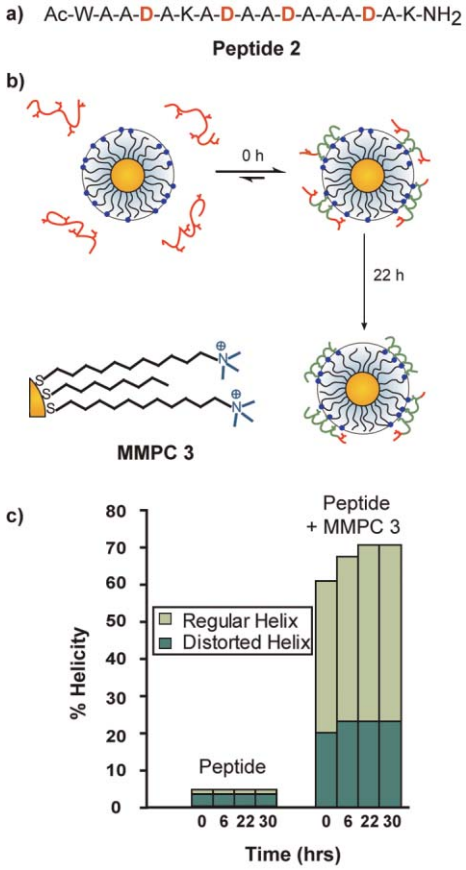

Fig. 4 (a) Peptide **2** sequence. (b) Schematic representation of the peptide binding to MMPC **3** surface. (c) Increase in helicity over time on incubation of the peptide with the nanoparticles demonstrating receptor templation. Distorted helix refers to fraying of the helices at either or both ends.

surface features, such as proteins, nucleic acids and polysaccharides. MMPCs functionalized with multiple recognition groups, can be thus optimized against the target guest molecule with high selectivity and binding affinity.

The ability of MMPCs to template to larger surface area targets was demonstrated through the interaction of nanoparticles with a complementary α-helical peptide. Rotello *et al.* used MMPC **3**, with a trimethylammonium functionalized monolayer, to target a tetraaspartate peptide (Peptide **2**) featuring the aspartate residues in alternating i, i + 3 and i, i + 4 positions (Fig. 4a).[12] This positioning of aspartates provided a cofacial presentation of the carboxylates suitable for recognition by the nanoparticle surface. Addition of the MMPC **3** to the peptide resulted in a significant increase in peptide helicity, from ~4% to ~60%, as determined through circular dichroism (CD) studies. Significantly, the helicity was found to increase over time (by ~20%) demonstrating the ability of the MMPC to template to the peptide surface, further stabilizing the peptide helix (Fig. 4c).

DNA surface recognition

Among biomacromolecules, DNA presents a relatively simple surface for biomolecular surface recognition. Based on intercalation and major/minor groove binding, small molecules have been utilized to bind specific DNA sequences,[21] and to inhibit,[22] or promote[23] DNA transcription. The principles that allow receptor–DNA binding can be extended to nanoparticle systems by incorporation of DNA-binding moieties on the nanoparticle surface. Nanoparticles functionalized with single-stranded DNA have demonstrated high selectivity for complementary sequences.[14] However, as indicated earlier, the focus of this review will be on the utilization of a network of non-covalent interactions to promote high affinity nanoparticle–DNA binding rather than the use of nanoparticles as support elements for direct attachment of biomacromolecules such as proteins and nucleic acids.

To test the ability of MMPCs in modulation of DNA activity through non-covalent interactions, Rotello *et al.* used MMPC **3** to bind to DNA.[24] The trimethylammonium end-groups on the MMPC **3** surface can bind to the negatively charged phosphate backbone of 37mer duplex DNA through electrostatic complementarity (Fig. 5a). The binding of the DNA to the positively charged nanoparticles was monitored through a UV centrifugation assay, which relies on the change of the DNA conformation on binding to the MMPC surface and its subsequent precipitation from the solution. The stoichiometry of association was found to be 4 : 1 nanoparticle to DNA duplex. In solid phase studies,[25] extended aggregates

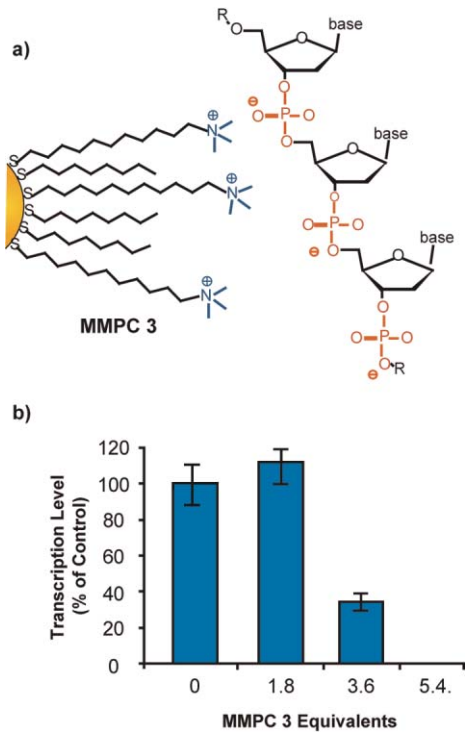

Fig. 5 (a) Structure of MMPC **3** scaffold and the DNA backbone. (b) Percent transcription level decreases with the increase in the amount of MMPC **3**.

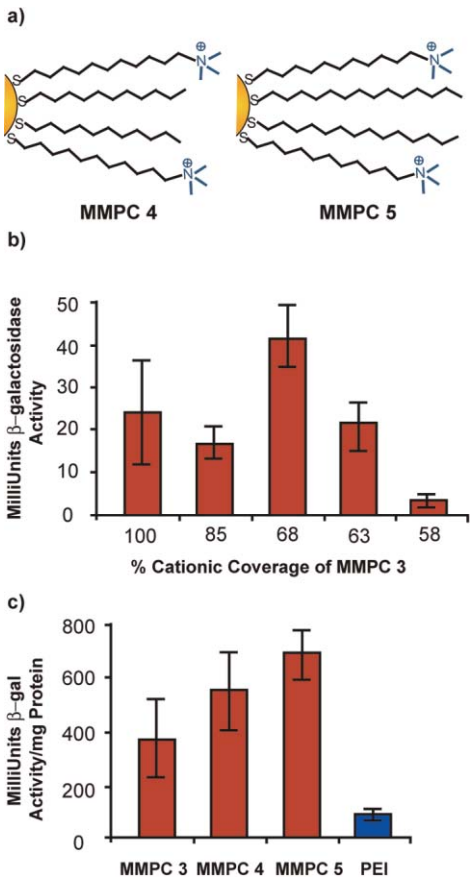

Fig. 6 (a) Structures of MMPCs used to study the effect of hydrophobicity on the transfection efficiency. (b) Optimal transfection is observed with ~68% functionalized MMPC **3** displaying the importance of amphiphilic nanoparticles for delivery. (c) Greater hydrophobic character of MMPCs results in a higher transfection.

of nanoparticles have been assembled using DNA templates. In the present case, however, discrete DNA–MMPC clusters of 20 nm in diameter were obtained in solution, as characterized by dynamic light scattering (DLS), displaying the presence of non-aggregated structures. The strength of the binding was further tested by the ability of the nanoparticles to inhibit DNA transcription *in vitro* (Fig. 5b). The authors demonstrated that on incubation with DNA, the MMPC effectively inhibited DNA transcription by T7 RNA polymerase. The DNA : polymerase complex is estimated to have a K_d of approximately 5 nM,[26] indicating that either MMPC **3** binds with higher affinity than the T7 RNA polymerase or that the altered conformation of the nanoparticle-bound DNA interrupts the recognition process.

The ability of MMPCs to bind DNA suggested the possibility of using nanoparticles for gene delivery into cells. In this study, MMPC **3** with various amounts of the positively charged functional group were synthesized by Rotello *et al.*[27] The MMPCs were briefly incubated with DNA plasmid encoding β-galactosidase before being introduced into human embryonic kidney cells. For optimal transfection, excess of MMPCs were required, demonstrating the importance of an overall positive charge of the DNA–nanoparticle complex for cellular uptake.[28] Studies elucidating the effect of charge and hydrophobicity of the MMPC on the transfection process were performed. Interestingly, the most efficient nanoparticle-mediated internalization of the plasmid was observed with a ~68% coverage of the cationic charge on MMPC **3** (Fig. 6b). This suggests the importance of amphiphilic particles for interaction with the cell membrane for subsequent release from the endosomal vesicle. Further investigations on the effect of hydrophobicity for DNA transfection was studied with increasing lengths of unfunctionalized alkane thiols (Fig. 6a). Significantly, the most efficient vector (MMPC **5**) was ~8 fold more effective than 60 kDa polyethylenimine, a widely used transfection agent. It was also observed that increasing the chain length of the alkane thiol by six methylene units resulted in an increase in plasmid transfection by ~85% with MMPC **5** (Fig. 6c). This study demonstrates that MMPCs can be successfully employed as transfection vectors, displaying the utility of nanoparticles in modulation of an important biological process.

The use of gold nanoparticles for transfection has also been recently studied by Klibanov *et al.*, who in an effort to improve low efficiencies of polycations as gene delivery vectors, have covalently attached branched 2 kDa polyethylenimine (PEI2) to gold nanoparticles (Fig. 7a). These particles were then used to investigate the delivery efficiency into monkey kidney cells *in vitro*.[29] The underlying premise of this study was that conjugating PEI2 to the nanoparticles would increase its effective molecular weight, consequently enhancing DNA binding and condensation resulting in improved transfection. Their studies revealed that the transfection efficiency varied with the PEI : gold molar ratio in the conjugates, with the best

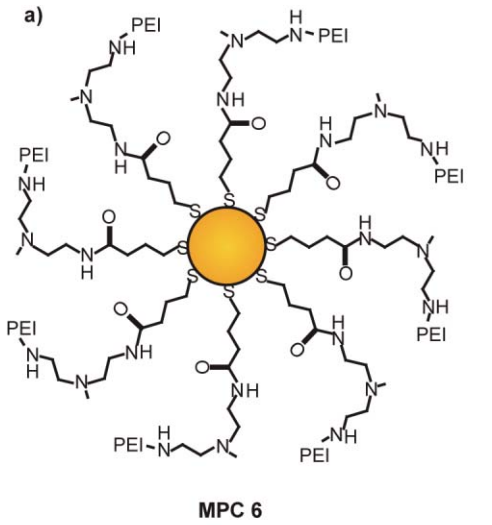

Fig. 7 (a) MPC **6** scaffold featuring branched 2 kDa polyethylenimine (PEI2) conjugated to a gold core. (b) Addition of dodecyl-PEI2 to MPC **6** increases the transfection efficiency. The numbers in the parentheses indicate the ratio of PEI nitrogen to DNA phosphate.

conjugate being 12 times more potent than the unmodified polycation. Further examination of the addition of the *N*-dodecyl-PEI2 to the conjugate during complex formation revealed that the efficiency of the delivery could be doubled (Fig. 7b). Consistent with our studies, the study indicated that the hydrophobicity of the transfection agent could have a beneficial effect on cellular uptake. Importantly, although unmodified PEI2 transfects just 4% of the cells, the PEI2–gold nanoparticle complex transfects 25% and further addition of dodecyl-PEI2 shows transfection into 50% of the cells, as assessed by histochemical staining. The intracellular trafficking of the polyplexes was monitored by transmission electron microscopy (TEM), which revealed the entry of the complexes into the nucleus <1 h after transfection.

In the MMPC-mediated transfection studies described above, DNA binds to the nanoparticles *via* complementary electrostatic interactions. An alternate route to the design of nanoparticles featuring complementary elements can be achieved by incorporation of DNA base pair intercalating moieties into the nanoparticle monolayer. Murray *et al.* have used ethidium bromide (EtBr) (Fig. 8c) as a means of binding cationic (Fig. 8b) and anionic (Fig. 8a) gold nanoparticles to DNA.[22a] In their study, each nanoparticle contains only one or two ethidium thiolate ligands. The binding of the nanoparticles

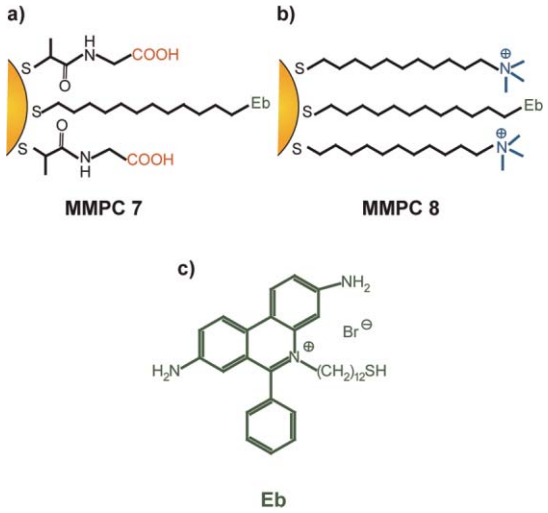

Fig. 8 Structures of mixed monolayers of (a) tiopronin and ethidium side chains and (b) trimethylammonium and ethidium thiolate. (c) Ethidium bromide structure.

was monitored by an increase in EtBr fluorescence on binding to DNA. Binding of the cationic trimethylammonium functionalized nanoparticles to the DNA was efficient and rapid. However, the binding of the tiopronin carboxylates did not occur until NaCl concentrations were greater than 0.1 M. The slower binding of tiopronin–ethidium MPC allowed analysis of two competing binding interactions: first, the binding of EtBr with DNA and second, pairing of cationic EtBr with the anionic tiopronin. This dual mode of binding raises other interesting possibilities in the design of MMPC systems.

Surface recognition of proteins and peptides using nanoparticles

The ability of the MMPCs to bind and regulate DNA provides the possibility of extending the utility of nanoparticles in recognition of more complex systems such as proteins and peptides. Synthetic receptors targeted at protein surfaces allow regulation of enzyme activity as an alternative approach to the traditional active site inhibition.[30] Significantly, such an approach can be exploited for modulation of proteins without a well-defined active site such as proteins involved in signal transduction[31] and dimerization.[32] Additionally, such receptors could provide a potent tool to control protein–protein and protein–nucleic acid interactions that are central to cellular processes.

As an initial step towards recognition of protein surfaces using MMPCs, Rotello *et al.* investigated the interaction between carboxylate-functionalized gold MMPC **9** (Fig. 9b) (6 nm overall diameter) with a model protein, α-chymotrypsin (ChT).[33] ChT has a ring of cationic residues around its active site (Fig. 9a),[34] which provides a suitable target for negatively charged receptors. The MMPC bound to the ChT is anticipated to sterically block the active site to substrate access resulting in complete enzyme inhibition. On incubation with MMPC **9**, the enzyme was completely inhibited; the extent of inhibition being dependent on time and concentration of the anionic nanoparticles. The inhibition displayed a two-step process featuring a fast reversible step due to

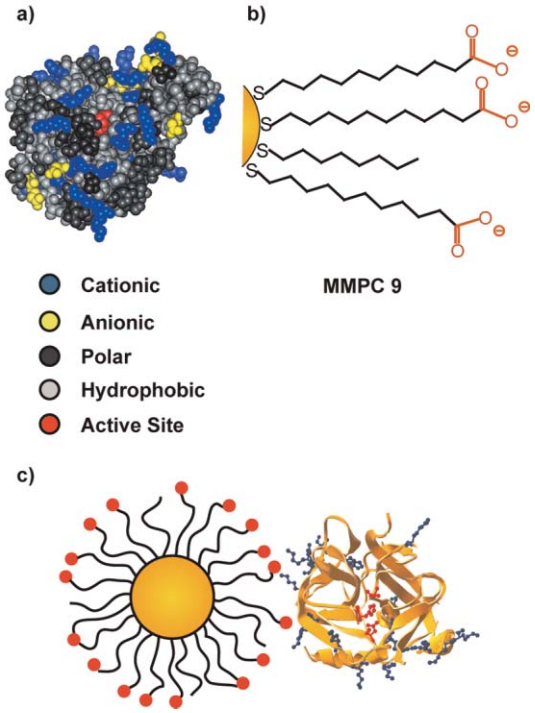

Fig. 9 (a) Chymotrypsin (ChT): the active site is surrounded by cationic residues, (b) structure of MMPC **9** and (c) relative sizes of MMPC **9** (2 nm core diameter) and ChT.

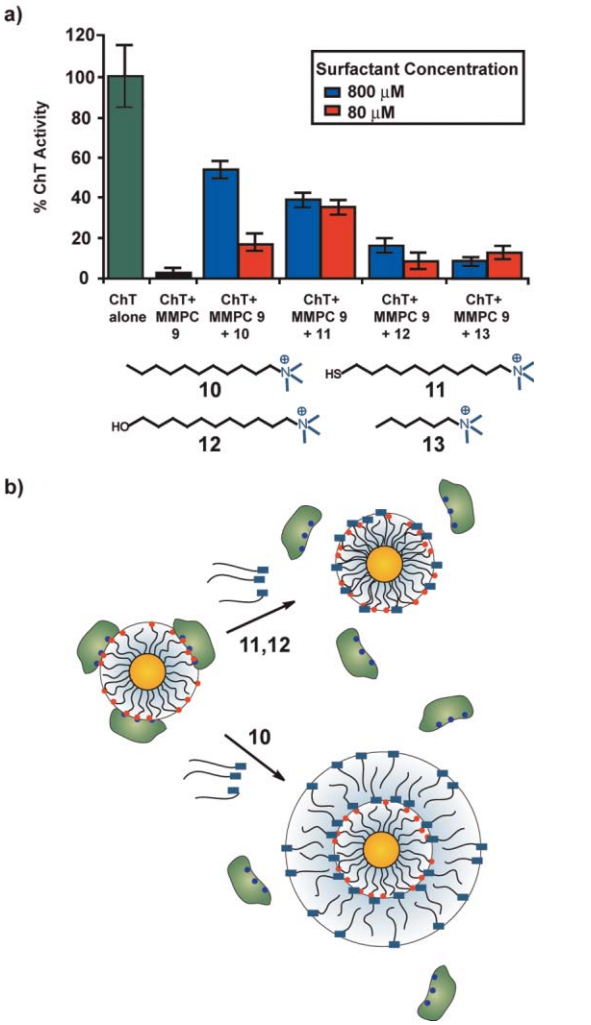

Fig. 10 (a) Surfactant mediated restoration of enzymatic activity of ChT bound to nanoparticle surface. (b) Schematic representation of monolayer modification on surfactant addition resulting in disassociation of ChT from MMPC.

complementary electrostatic binding followed by a slower irreversible process resulting in ChT denaturation on the nanoparticle surface. Kinetic analysis revealed that the inhibition was very effective with a K_d of 10.4 $\pm$ 1.3 nM. The binding ratio of the nanoparticle with the enzyme was found to be 1 : 5, which indicated a complete saturation of the MMPC surface with the protein, given their relative surface areas. This study revealed a certain level of selectivity as elastase, β-galactosidase and cellular retinoic acid-binding protein displayed no significant interaction with MMPC **9**. Additionally, positively charged MMPC **3** displayed no inhibition of ChT activity. Significantly, the initial binding of MMPC **9** to ChT was found in subsequent studies to be strongly dependent on the ionic strength of the solution.[35]

The non-covalent nature of the inhibition of ChT coupled with the unique nature of the MMPC scaffold suggested that attenuation of the complementary charges driving the binding of the MMPC with the enzyme could provide a means of rescuing the enzyme activity. To explore this possibility, derivatives of trimethylammonium-functionalized surfactants (Fig. 10a) were added to the preincubated MMPC–ChT complex.[36] Up to 50% of the native enzyme activity was restored with surfactant **10** (Fig. 10a). Dynamic light scattering (DLS) experiments confirmed the release of ChT from the nanoparticle surface. The conformation of the released ChT was characterized by fluorescence and fluorescence anisotropy, indicating that ChT attained a high degree of native structure upon release. Based on DLS data of MMPC–protein assemblies, two mechanisms were developed to explain the enzymatic reactivation of ChT upon addition of surfactants (Fig. 10b). In the first mechanism, the surfactants **11** and **12** directly modify the monolayer by intercalation and/or chain displacement resulting in attenuation of monolayer charge mediating protein release and subsequent restoration of activity. With alkane surfactant **10**, a larger radius was observed for the MMPC–protein assembly, indicating that a bilayer structure is formed, resulting in protein release.

The aforementioned studies displayed MMPCs to be an effective tool for the modulation of enzyme activity, however, the inhibition proceeded with enzyme denaturation. In applications such as *in vivo* protein delivery and *in vitro* enzyme stabilization, it is essential to retain the native enzyme structure upon binding. Additionally, templation of a nanoparticle to a protein surface requires the retention of native protein conformation. To segregate protein inhibition from change in protein conformation, thioalkyl and thioalkylated oligo(ethylene glycol) (OEG) ligands with chain-end functionality: (i) OEG terminated with hydroxyl group (Fig. 11b), (ii) carboxylate-terminated thioalkyl ligand (Fig. 11a) and (iii) carboxylate terminated OEG (Fig. 11c) were used by Rotello, Emrick *et al.* to fabricate water-soluble CdSe nanoparticle

scaffolds.[37] As ethylene glycol units have been shown to resist non-specific interactions with biomacromolecules,[38] shielding of the hydrophobic monolayer from the protein surface by inserting an ethylene glycol spacer between the functional end-group and the alkane monolayer was anticipated to diminish non-specific interactions of the MMPC-bound protein. Moreover, CdSe nanoparticles can be used as fluorescent tags for bioimaging[39] and this system provides better understanding of the interaction between CdSe nanoparticles and proteins. On incubation with ChT, three levels of control of enzyme activity and structure were observed. No interaction was observed with MPC **15**, which terminated in the hydroxyl end-group. Nanoparticles containing the carboxylate-terminated thioalkyl ligand (MPC **14**) demonstrated binding and denaturation of ChT, as observed in our earlier studies with gold MMPC **9**.[33] However, while the enzyme bound to nanoparticles displaying carboxylate terminated OEG thiols (MPC **16**) showed substantial loss of enzymatic activity, no significant loss in the native structure of the bound enzyme was seen as investigated through circular dichroism (CD) and fluorescence experiments. The binding in the latter case arose primarily from complementary electrostatic interactions of the enzyme and the nanoparticle, which was confirmed through ionic strength studies. This study demonstrates that MMPCs can be used to modulate protein activity and structure, which can form the basis of a number of pragmatic biological applications.

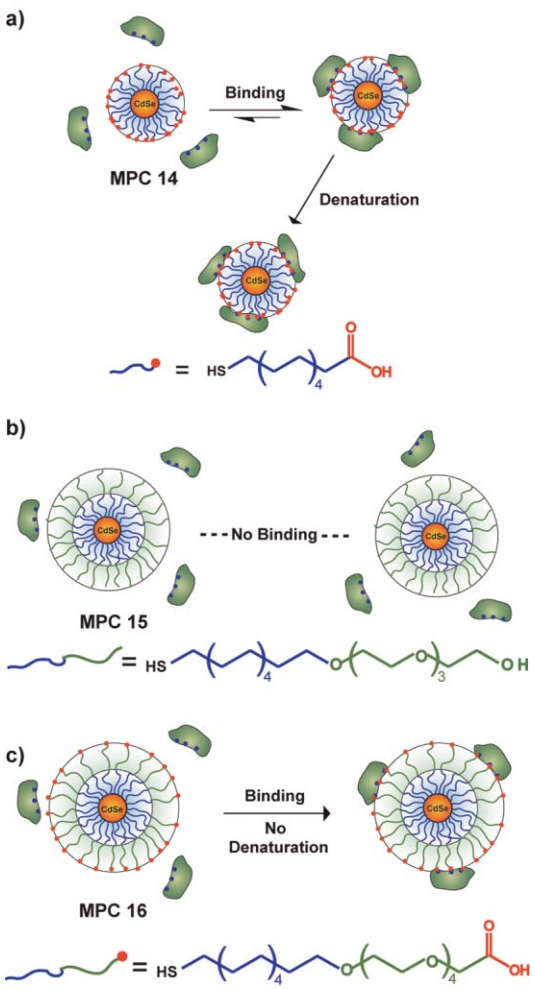

Fig. 11 Three levels of control over ChT structure and function by CdSe based MPCs. (a) ChT binds to MPC **14** and denatures on the nanoparticle surface. (b) No binding of ChT to MPC **15** surface is observed. (c) ChT binds to MPC **16** but retains native conformation.

In addition to recognition of protein for modulation of structure and function through the use of functional end-groups present on MMPC surfaces, nanoparticles can also be utilized to enhance low affinity interaction such as carbohydrate–protein interactions due to their ability to present multiple ligands for such interactions. This has been observed by interactions of mannose-encapsulated nanoparticles with type 1 pili in *E. coli*, as examined by Wu *et al.*[19] Type 1 pili are filamentous proteinaceous appendages that extend from the surface of many Gram-negative organisms and are composed of FimA, FimF, FimG, and FimH proteins. The nanoparticles were found to be stable in various media, of high ionic strength and pH values ranging from 1.5 to 12 indicating high stability and applicability in various biological conditions. TEM examination displayed specific interactions of the nanoparticles with the *E. coli* stain ORN178 containing the mannose-binding site even in the presence of competing free mannose. Other studies investigating carbohydrate–protein interactions have targeted lectins with sugar-coated nanoparticles. Kataoka *et al.* have used ethylene glycol containing lactose-conjugated gold nanoparticles to bind to agglutinin, a bivalent lectin.[40] Exploiting optical properties of gold nanoparticles, they have demonstrated that the nanoparticles exhibited selected aggregation when exposed to the lectin; the aggregation being reversible upon addition of excess galactose (Fig. 12). Importantly, as the degree of aggregation was proportional to the lectin concentration, the target molecule could be quantified with high sensitivity. Protein recognition using carbohydrates has also been examined successfully by mannose, glucose and galactose-encapsulated gold nanoparticles.[41] In another study by Gervay-Hague *et al.*, gold nanoparticles featuring galactosyl and glucosyl headgroups were used to display their relative ability to displace HIV-associated glycoprotein gp120 bound to cellular receptor GalCer, displaying the disruption of one of the molecular events involved in HIV recognition of mucosal membranes.[42] Collectively, these studies demonstrate the applicability of carbohydrate functionalized gold nanoparticles as effective inhibitors of protein–carbohydrate interactions that could be extended to *in vivo* biological systems.

While gold nanoparticles have been used as optical sensors in biological applications, few reports in the literature have revealed the unique benefit of magnetic nanoparticles in recognizing biological surfaces with great efficiency compared to their magnetic bead counterparts. The obvious advantage of magnetic nanoparticles compared to beads results from a higher surface to volume ratio as the beads are 1–5 μm in size as compared to <10 nm for the nanoparticles. Additionally, the smaller size of nanoparticles allows faster movement and easier entry into cells making the magnetic nanoparticles better suited for *in vivo* applications. Xu *et al.* have used magnetic FePt nanoparticles to capture and detect vancomycin-resistant enterococci (VME) and other Gram-positive bacteria at

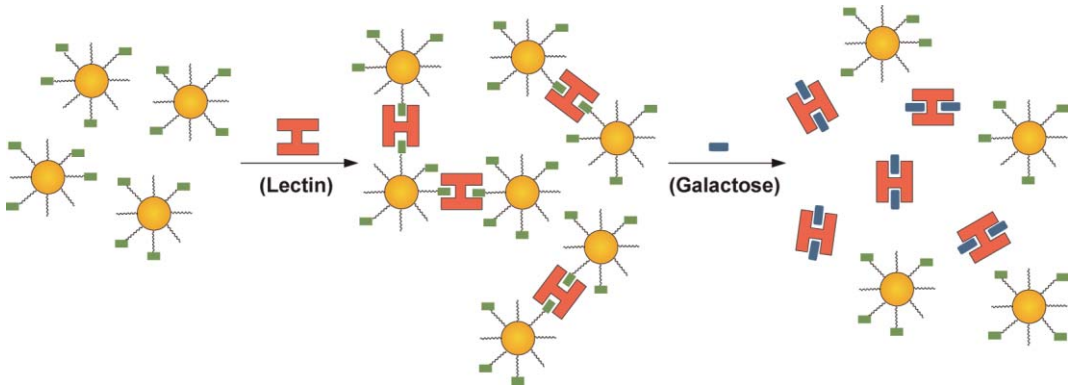

Fig. 12 Lactose-conjugated gold nanoparticles aggregate on addition of lectin, however, addition of excess galactose reverses this association.

concentrations of ~10 cfu (colony forming units) per mL within an hour (Fig. 13).[43] Having functionalized the nanoparticles with the broad spectrum antibiotic, vancomycin (Van), their premise is based upon Van binding to a terminal peptide D-Ala–D-Ala on the cell wall of a Gram-positive bacterium *via* hydrogen bonding. Upon binding of the nanoparticles, the "magnetized" bacteria (with the magnetic nanoparticles attached to the cell surface) are separated from the solution and the binding is confirmed through microscopic analysis and scanning electron micrographs (SEM). In another pragmatic application of FePt magnetic nanoparticles, the authors have synthesized nitrilotriacetic acid (NTA)-modified magnetic nanoparticles which are capable of separating, transporting and anchoring recombinant proteins that are engineered to have six consecutive histidine residues.[44] Such proteins are usually separated *via* metal–chelate affinity chromatography (MCAC), which employs NTA-attached resin to immobilize nickel ions (Ni^{2+}). The study demonstrates the ability of the magnetic nanoparticles to obtain pure proteins directly from lysed cell mixtures through magnetic separation within 10 min. The work also indicates the superiority of the nanoparticles to MCAC columns as they do not exhibit non-specific binding.

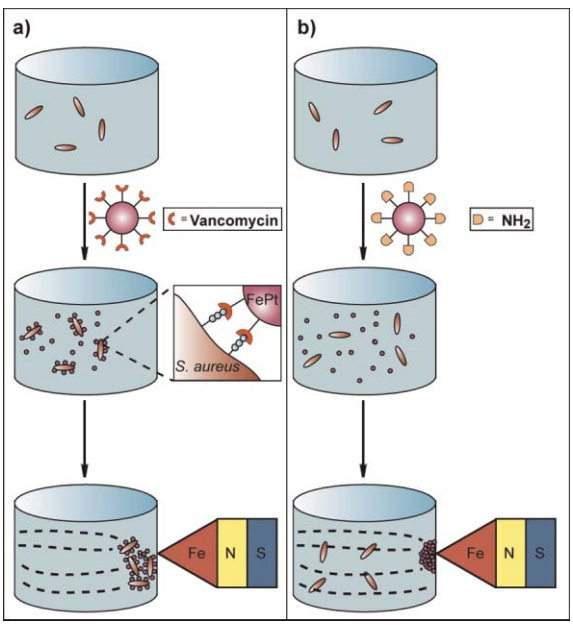

Fig. 13 Schematic illustration of (a) capturing bacteria with vancomycin functionalized magnetic nanoparticles through recognition of terminal peptides on the bacterial cell wall and (b) a control experiment with amine-functionalized magnetic nanoparticles displaying no interaction with the bacteria.

Further applications of functionalized nanoparticles

While numerous versatile properties of core-shelled nanoparticles have been used to exhibit control of structure and activity of proteins and modulation of DNA function, the unique architecture of MPCs and MMPCs also lends them to be utilized for mimicking and elucidating other biological processes. The flexible nature of the alkane chains combined with the hydrophobic interior and hydrophilic surface can be of high semblance with the fluidity and structure of lipid membranes. Taking advantage of this structural aspect of nanoparticles, Penadés *et al.* functionalized gold nanoparticles with neoglycoconjugates of biologically significant oligosaccharides: disaccharide lactose (MPCs **17** and **18**) and trisaccharide Lewis^x (MPC **19**) and demonstrated the selective self-recognition of the Lewis^x functionalized nanoparticles by carbohydrate–carbohydrate interactions.[45] The Lewis^x antigen, a trisaccharide attached to membrane lipids, is believed to undergo self-association in the initial steps of cell adhesion and recognition. This interaction is characterized with very low affinity in nature and is strongly dependent on divalent cations, which makes the study of the adhesion process a challenging one. Their study reveals that while nanoparticles functionalized with lactose do not associate even in the presence of Ca^{2+} ions, Lewis^x MPCs displayed aggregation; this being reversible on addition of EDTA (Fig. 14b). In a further extension of the study, the authors were able to quantify the kinetics of the self-interaction for the antigen.[46] This indicates the importance of the nanoparticle scaffold in mimicking cell membrane structures and elucidating roles of trisaccharides in the cellular adhesion process.

Summary and outlook

Nanoparticles present a versatile scaffold for recognition of biomolecular surfaces. The nanoparticle size can be tuned for optimally scaled systems for biomacromolecules, their surfaces

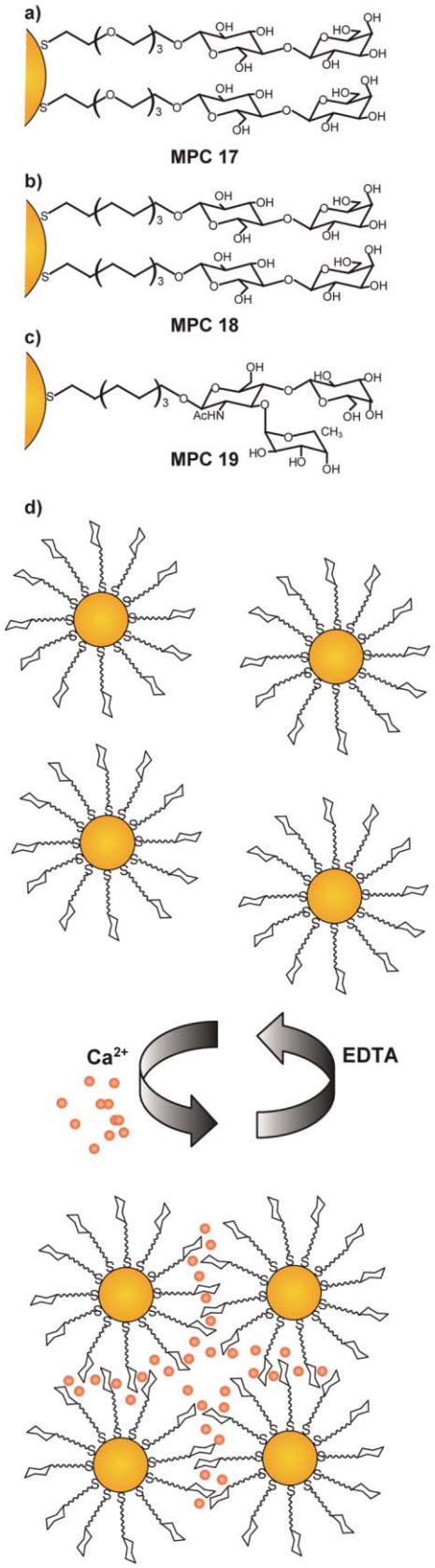

Fig. 14 Structures of (a) MPC **17** and (b) MPC **18** featuring lactose units and (c) MPC **19** functionalized with Lewis^x antigen. (d) MPC **19** undergoes association in the presence of Ca^{2+} ions, however, the aggregation can be reversed on addition of EDTA.

can undergo divergent functionalization and importantly, the nanoparticle surface can be templated to the guest surface providing an environmentally responsive receptor for biomacromolecules. These properties of nanoparticles have been harnessed by a number of groups to provide effective binding of proteins and DNA along with a control over structure and function. The current studies featuring the interactions of nanoparticles with biological molecules can provide a stepping stone to a host of biomedical applications.

The ability of functionalized nanoparticles in mediating biomacromolecular recognition can be extended to development of novel hybrid materials composed of biomacromolecules and nanoparticles. These nanocomposites could feature attributes such as sensing, catalysis, transport or other applications in medicinal and engineering science. The properties of such nanocomposites could encompass tunable features of nanoparticles such as size, surface functionality and core properties[10] with the unique functional properties of biological molecules.[47] Significantly, these two classes of compounds are suitable to address the gap in size that currently exists in the engineering of small-scale devices by the "bottom up" and the "top down" strategies of constructing materials. Proteins and nucleic acids have been utilized as programmable recognition units for assembling nanoparticles into nanostructured and mesostructured supramolecular hybrid architectures in the literature.[48] However, we are still in the process of realizing numerous potential benefits in merging biomacromolecules and nanoparticles at the nanoscale level.

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