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Biomacromolecule Surface Recognition using Nanoparticle Receptors

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Biomacromolecule surface recognition is an important factor in regulating cellular processes. Nanometer-scale mixed monolayer-protected clusters (MMPCs) provide scaffolds for creating receptors targeting biomacromolecular surfaces. Unique features of nanoparticles that make them particularly attractive resources for biomacromolecular recognition and their use in modification of structure and function of biomacromolecules are illustrated in this review.

Keywords: Nanoparticles; Chymotrypsin; Mixed monolayer-protected clusters; Self-assembled monolayers; Surface recognition

INTRODUCTION

Cellular processes featuring inter-biomacromolecule interactions such as protein–protein interactions, protein–nucleic acid interactions, enzyme activity and cell surface recognition are central to the function of biological systems. These interactions also provide attractive targets for synthetic receptors, if we can replicate the specificity of the biological prototypes. Synthetic receptors designed to target biomacromolecules such as proteins and nucleic acids would pave the way for alternative approaches to diagnostic biosensors for rapid monitoring of imbalances and illnesses, as well as therapeutic agents.

While recognition of biomolecular surfaces relies on the same noncovalent interactions involved in small molecule host–guest systems, binding of biomacromolecules presents a substantial challenge. This challenge arises from two basic requirements for effective biomacromolecule surface recognition. A large receptor contact area is essential. For instance, large surface areas are required for effective binding of biomacromolecules that feature solvent-exposed surfaces; examination of protein–protein interactions revealed a buried surface area of 6 nm^2 per protein [1]. The topological, electrostatic [2] and hydrophobic [3] complexity of biomacromolecular surfaces present further complications for the design of receptors [4].

Researchers have undertaken the challenge of protein surface recognition using a number of "small molecule" systems [5] and macromolecular scaffolds. These systems comprise receptors on calixarene and porphyrin scaffolds [6–10], cyclodextrin dimers [11] and transition metal complexes aimed against surface-exposed histidines [12]. Partially constrained backbones [13–15] possessing multivalent libraries of receptors have also been developed using polymer scaffolds. The systems mentioned above establish a certain level of success in the modulation of biomolecular function, but the question of protein surface recognition still remains open.

Monolayer-protected cluster (MPC) and mixed monolayer-protected cluster (MMPC) nanoparticles provide highly useful biomolecular platforms for surface recognition, with four distinctive and significant features. First, we have demonstrated that MMPCs are self-templating: the affinity and specificity of the recognition process is amplified via incubation of these particles with target molecules [16]. Second, various sizes of nanoparticles can be synthesized ranging from 2 to >10 nm, analogous to that of proteins and other biomacromolecules, providing sufficient surface area for effective protein and DNA binding [17] (Fig. 1). Third, these particles

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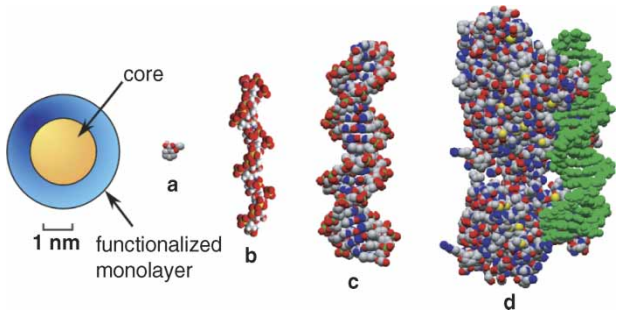

FIGURE 1 Relative sizes of a nanoparticle with a 2 nm core and C_8 monolayer and (a) aspirin, (b) heparin sulfate 12mer, (c) DNA 24-mer, and (d) DNA binding domain of p53 complexed to DNA 20mer.

can be synthesized with a wide variety of metal and semiconductor core materials that incorporate useful fluorescence and magnetic properties. This diversity of materials makes them useful as probes and diagnostic reagents. Finally, nanoparticles can be synthesized with a wide range of surface functionality, providing adaptability in the formation of surface-specific receptors [16–18]. This provides a unique tool for achieving efficient and specific recognition of protein surfaces.

Nanoparticles have been used extensively for the immobilization of biomacromolecules. Such studies include interactions between streptavidin and biotin-labeled particles [19,20] or hybridization of complementary DNA strands conjugated to nanoparticles [17]. However, this review primarily discusses our research, which focuses on the properties and utilization of organic groups functionalized on monolayer- and mixed monolayer-protected nanoparticles. In these investigations, the nanoparticles are used as multivalent recognition elements to target the surfaces of biomacromolecules.

FABRICATION AND PROPERTIES OF MPCs AND MMPCs: SYNTHESIS AND CHARACTERISTICS

The high surface area and stability of MPC and MMPC nanoparticles are ideal for studying the interactions between biomacromolecules and surfaces. The MPC nanoparticles are easily synthesized via the Brust-Schiffrin reduction (Fig. 2a) [21]. The selection of thiols used during synthesis controls the functionality of the monolayer surface, while the stoichiometry controls the particle size. The polyhedral-shaped gold core is instantly enclosed by a self-assembled monolayer (SAM) [22], thus preventing aggregation of the nanoparticles and allowing the surface functionality to be customized. Using the Murray place displacement reaction [23], MMPCs can be readily synthesized using a mixture of functional thiols and MPCs (Fig. 2b). This method offers the ability synthesize a diverse variety of MMPCs with unique recognition groups on the surface [24].

The ability to control the functionality of the end-groups on MMPCs is the key tool for biomacromolecular recognition. These side-chains determine the solubility of the nanoparticles in water and the affinity and specificity of interactions with biomacromolecule targets. The efficiency of interactions of MMPCs with biomacromolecules can be enhanced through functionalization with multiple ligands. In this way, low-affinity interactions can be used to generate polyvalent interactions between the ligands and receptors that are collectively stronger than corresponding monovalent interactions [25]. The gold core also strongly quenches fluorophores, providing a potential method for sensor design [26–28].

TEMPLATION OF MMPCs

The ability to controllably functionalize MMPCs makes them promising receptors for both monotropic and multivalent interactions. Presentation of functionality, however, is crucial to the creation of effective multivalent receptors. The thiols on the nanoparticle are mobile, thus creating an environmentally responsive system on the SAM that can be used to address this issue. Through maximization of binding enthalpy, the mobility of the thiols on the MMPCs provides a potential method for

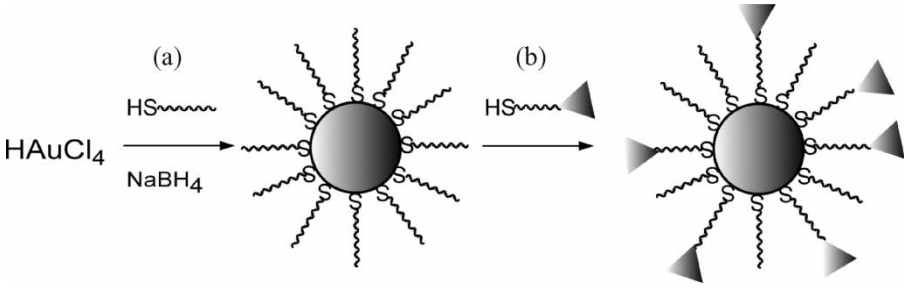

FIGURE 2 Formation of (a) MPCs using the Brust-Schiffrin reduction and (b) MMPCs using the Murray place-exchange.

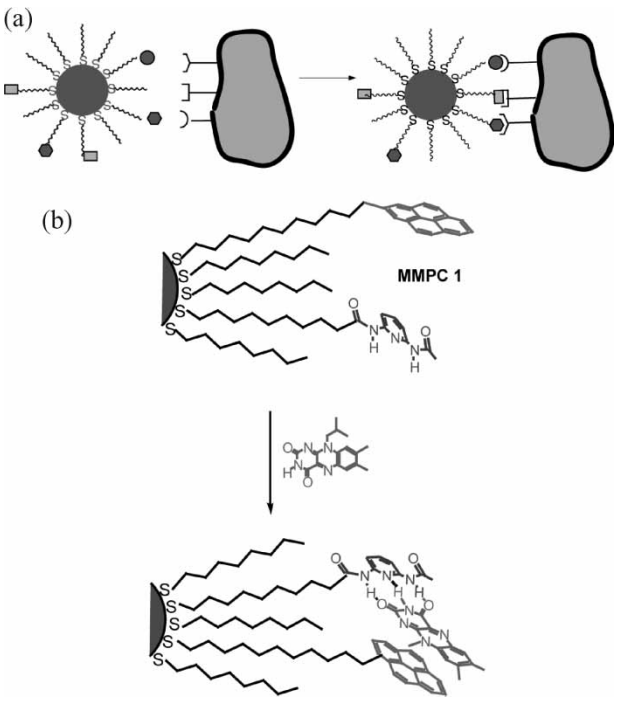

FIGURE 3 (a) Optimization of MMPCs to a biomacromolecule surface. (b) Templation of flavin onto MMPC **1**.

imprinting/templation (Fig. 3a). We have demonstrated this templation with the aid of time-dependent recognition of flavin by MMPC **1** (Fig. 3b). In this MMPC, the pyrene and diamidopyridine functionalized side-chains that were dispersed in an octanethiol monolayer provided aromatic stacking and hydrogen-bonding moieties, respectively. Flavin was incubated with MMPC **1**, enabling hydrogen bonding and aromatic π-stacking interactions. Substantial rearrangement was observed over a 73-h incubation on addition of flavin to MMPC **1**. The results from time-course NMR experiments showed that the binding constant increased by 71% during the templation process. These properties allow for further optimization, such as selectivity and binding affinity, with more complex biomacromolecules (Fig. 3a).

The interaction of nanoparticles with a complementary peptide demonstrated the ability of MMPCs to template biomacromolecules with larger surface areas. A tetraaspartate peptide (Peptide **2**) featuring the aspartate residues in alternating i, i + 3 and i, i + 4 positions (Fig. 4a) was used as a target for MMPC **3** (Fig. 4b) [24]. A cofacial presentation of the carboxylates on peptide **2** in the α-helical conformation allowed recognition of the nanoparticle surface by this arrangement of the aspartates. Circular dichroism (CD) studies showed a substantial increase in peptide helicity from ~4% to ~60% upon addition of MMPC **3** to the peptide. Time-course experiments were used once again to demonstrate MMPC templation, with a substantial increase in helicity (~20%) over 30 h (Fig. 4b).

SURFACE RECOGNITION OF DNA

A relatively simple surface for biomolecular surface recognition is presented by DNA. In previous studies, small molecules have been used to bind specific DNA sequences [29,30], and to inhibit [31,32] or promote [33,34] DNA transcription based on intercalation and major/minor groove binding. High selectivity of nanoparticles with complementary sequences functionalized with single-stranded DNA has been demonstrated [35]. Taking an alternative tack, we have focused on the use of a network of noncovalent interactions to promote high affinity of nanoparticle–DNA binding.

In initial studies, we used cationic MMPC **3** to bind to DNA through noncovalent interactions [35]. Complementary electrostatic charges allow MMPC

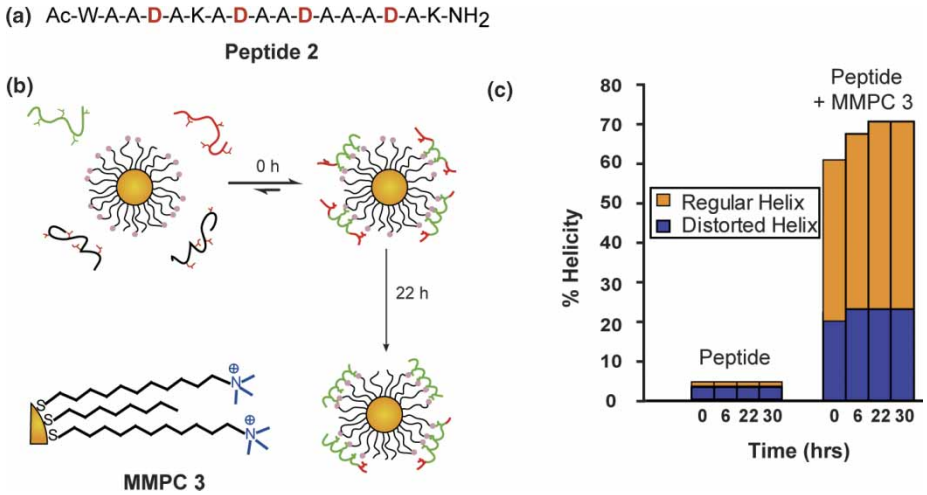

FIGURE 4 (a) Sequence of Peptide **2**. (b) Schematic representation of the peptide binding to the MMPC **3** surface. (c) Incubation of the peptide over time increase its helicity. Fraying of the helices at either ends is referred to as a distorted helix.

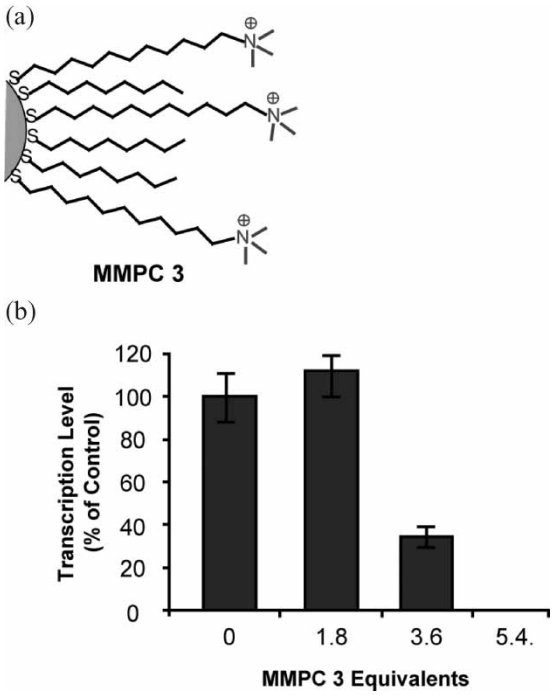

FIGURE 5 (a) Structure of the MMPC **3** scaffold. (b) The increase in the amount of MMPC **3** decreases the level of transcription.

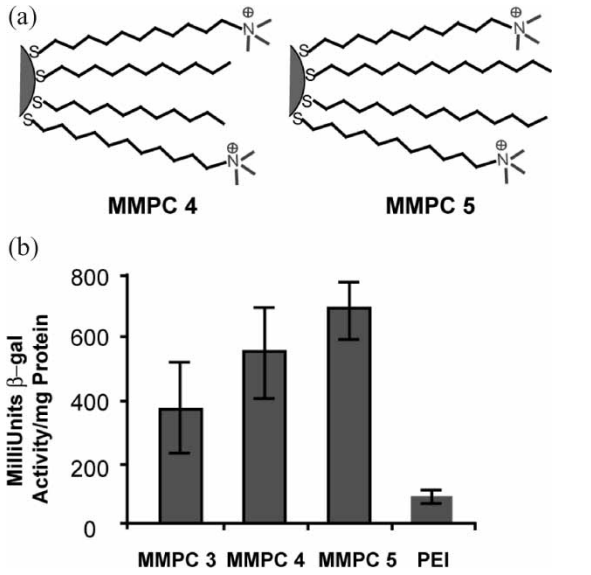

FIGURE 6 (a) Structures of MMPCs used to study the effect of hydrophobicity on the transfection efficiency. (b) A higher transfection is achieved via a greater hydrophobic character of MMPCs.

3 to bind to the negatively charged phosphate backbone of a 37mer duplex DNA (Fig. 5a). This interaction was monitored through a UV centrifugation assay, with a 4:1 stoichiometry of association observed. Further characterization by dynamic light scattering (DLS) indicated the formation of discrete DNA–MMPC clusters of 20 nm in diameter. The ability of the nanoparticles to inhibit DNA transcription *in vitro* was used as a functional test for the efficiency of MMPC–DNA interaction (Fig. 5b). Upon incubation with DNA, MMPC effectively inhibited DNA transcription by T7 RNA polymerase. It is possible that MMPC **3** binds with higher affinity than the T7 RNA polymerase from an estimated K_d of approximately 5 nM [36] for the DNA:polymerase complex. However the K_d could also suggest that the recognition process is interrupted as the nanoparticle-bound DNA conformation is altered, an issue we are currently exploring.

Given the efficiency of MMPC–DNA interaction, subsequent studies using nanoparticles for gene delivery into cells were conducted (Fig. 6) [37]. Before being introduced into human embryonic kidney cells, DNA plasmid encoding β-galactosidase was incubated briefly with the MMPCs. Further studies showed a cationic charge of ~70% coverage to be the most effective for transfection into a plasmid (Fig. 6b), with MMPC **5** (Fig 6a) providing enhanced efficiency relative to MMPC **3** (Fig 5a) and MMPC **4** (Fig 6a). It was also found that MMPC **5** is ~8-fold more effective than 60 kDa polyethyleneimine (PEI).

NANOPARTICLES AS RECEPTORS FOR SURFACE RECOGNITION OF PROTEINS

Control of enzyme inhibition can be regulated by synthetic receptors targeted at the protein's active site [38,39]. Proteins without a well-defined active site can be modulated [40] and dimerized [41,42] by nanoparticles. As in interactions that are central to cellular processes, such receptors could provide a potent tool to control protein–protein and protein–nucleic acid interactions. Because of their diversity in functionalization and the ability to maximize interaction over time through templation, MMPCs provide a suitable platform for examining nanoparticle–protein interactions. As an example, we used α-chymotrypsin (ChT) to investigate the interaction with carboxylate-functionalized gold MMPC **6** (Fig. 7a) (6 nm overall diameter) as an initial step towards recognition of protein surfaces using MMPCs [43]. A suitable target for negatively charged receptors was provided by a ring of cationic residues around the active site of ChT (Fig. 7b). Strong interactions between ChT and MMPC **6** were demonstrated by the complete inhibition of ChT activity [44], presumably from steric blocking of the ChT active site by the bound MMPC. A 1:5 binding ratio of the nanoparticle with the enzyme was found. Given their relative surface areas, this indicated a complete saturation of the MMPC surface with the protein. The inhibition of ChT on the surface of the nanoparticle exhibited a two-step process: a fast reversible step due to complementary electrostatic binding followed by a slower irreversible process that was determined through CD to arise from denaturation of the protein on the MMPC surface.

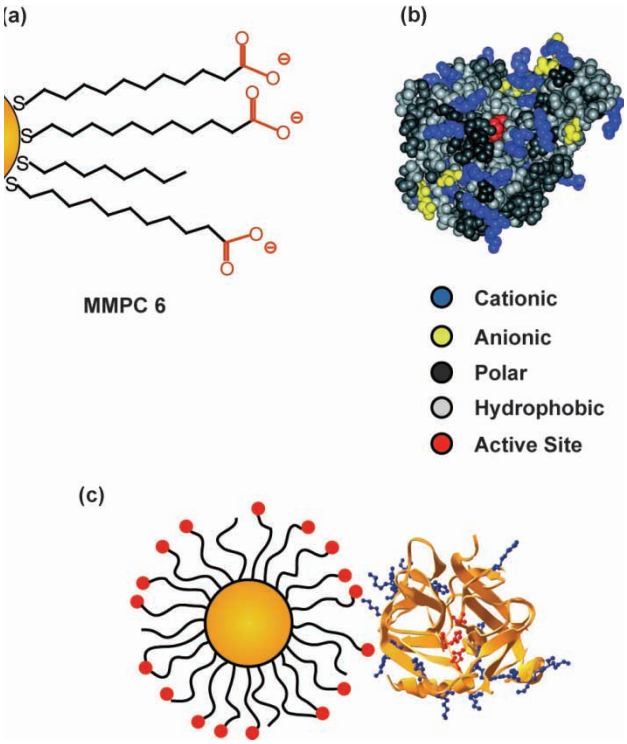

FIGURE 7 (a) Structure of MMPC **6**. (b) The active site of ChT is surrounded by cationic residues. (c) Relative sizes of MMPC **6** (2 nm core diameter) and ChT.

A certain level of selectivity was discovered as no significant interaction with MMPC **6** was displayed with elastase, β-galactosidase and cellular retinoic acid-binding protein. Furthermore, inhibition of ChT activity was not affected by positively charged MMPC **3**. In subsequent studies the initial binding of MMPC **6** to ChT was found to be strongly dependent on the ionic strength of the solution [45].

The unique nature of the MMPC scaffold suggested a possible means of restoring the activity of ChT via electrostatically mediated release and refolding of the protein. Derivatives of trimethylammonium-functionalized surfactants (Fig. 8) were added to the preincubated MMPC–ChT complex to investigate this possibility [46]. With the addition of surfactant **7** (Fig. 8), the activity of ChT was almost immediately restored to up to 50% of native ChT. The release of ChT from the nanoparticle surface was confirmed by DLS experiments, fluorescence and fluorescence anisotropy studies. This study signified that, once released, the native structure of ChT is attained. To explain the enzymatic reactivation of ChT upon addition of surfactants, two principal mechanisms were developed based on DLS data for MMPC–protein assemblies (Fig. 8). Surfactants **8** and **9** directly modify the monolayer by intercalation and/or chain displacement in the first mechanism. The resulting attenuation of the monolayer charge mediates the release of the protein and ensuing activity. A larger radius was observed for

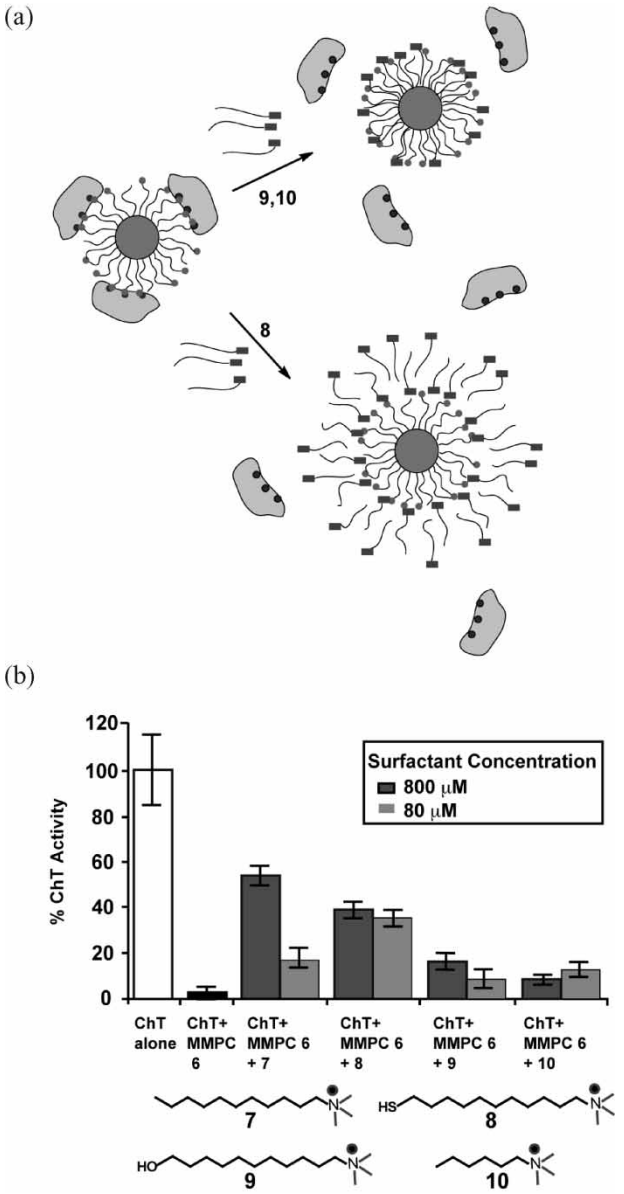

FIGURE 8 (a) Addition of surfactant modifies the monolayer allowing ChT to dissociate from the MMPC. (b) Enzymatic activity of ChT is restored by addition of surfactants.

the MMPC–protein assembly with alkane surfactant **7**, presumably due to bilayer formation upon releasing the protein.

It is essential to retain the native enzyme structure upon binding for applications such as *in vivo* protein delivery and *in vitro* enzyme stabilization. The utility of nanoparticle receptors will be greatly enhanced by the ability to control protein structure during the binding process. Moreover, the retention of native protein conformation is required for templation of nanoparticles to a protein surface. Thioalkyl and thioalkylated oligo(ethylene glycol) (OEG) ligands with chain-end functionality were used to fabricate water-soluble CdSe nanoparticle scaffolds [47] to allow for protein inhibition without a change in protein conformation. The researchers used: (i) OEG

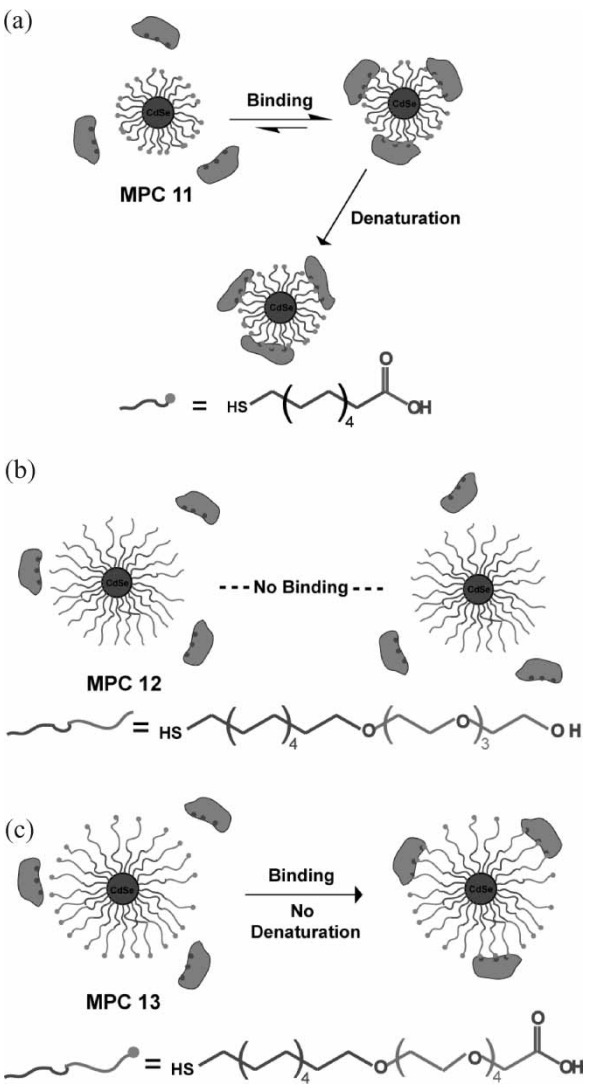

FIGURE 9 Three levels of control over ChT structure and function by CdSe-based MPCs. (a) ChT binds to MPC **11** and denatures on the nanoparticle surface. (b) No observable binding of ChT to the MPC **12** surface. (c) ChT retains native conformation although bound to MPC **13**.

terminated with hydroxyl groups (Fig. 9), (ii) a carboxylate-terminated thiolalkyl ligand (Fig. 9) and (iii) carboxylate-terminated OEG (Fig. 9c), as ethylene glycol units have been shown to resist nonspecific interactions with biomacromolecules [48]. It was anticipated that the OEG spacer between the functional end-group and the alkane monolayer would diminish nonspecific interactions of the MMPC-bound protein by shielding the hydrophobic monolayer from the protein surface. This system provides better understanding of the interaction between CdSe nanoparticles and proteins, an issue that arises when CdSe nanoparticles are used as fluorescent tags for bioimaging [49,50]. Three levels of control of enzyme activity and structure were observed upon incubation with ChT. The hydroxyl end-group-terminated MPC **12** did not interact. However, the carboxylate-terminated thiolalkyl ligand (MPC **11**) bound and denatured ChT. This was observed in earlier studies with gold MMPC **6**. The carboxylate-terminated OEG MMPC showed a substantial loss of enzymatic activity. In this case, no significant loss in the native structure of the bound enzyme was seen as investigated by CD and fluorescence experiments. Ionic strength studies confirmed that the binding in the latter case arose primarily from complementary electrostatic interactions of the enzyme and the nanoparticle.

SUMMARY

The distinct properties of nanoparticles offer a scaffold that can be varied and templated for recognition of biomolecular surfaces. Furthermore, the capability to optimally tune the nanoparticle size and divergent functionalization of the surface provides an environmentally responsive receptor for guest biomacromolecules. A number of groups have used these properties of nanoparticles to effectively bind proteins and DNA, controlling structure and function. A stepping-stone to a host of biomedical applications has been provided with these current studies, highlighting the interactions of nanoparticles with biomacromolecules.

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References

[1] Lo Conte, L.; Chothia, C.; Janin, J. *J. Mol. Biol.* **1999**, *285*, 2177.
[2] Golumbfskie, A. J.; Pande, V. S.; Chakraborty, A. K. *Proc. Natl Acad. Sci. USA* **1999**, *96*, 11707.
[3] Lijnzaad, P.; Argos, P. *Proteins* **1997**, *28*, 333.
[4] de Rinaldis, M.; Ausiello, G.; Cesareni, G.; Helmer-Citterich, M. *J. Mol. Biol.* **1998**, *284*, 1211.
[5] Park, H. S.; Lin, Q.; Hamilton, A. D. *Proc. Natl Acad. Sci. USA* **2002**, *99*, 5105.
[6] Hamuro, Y.; Calama, M. C.; Park, H. S.; Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2680.
[7] Lin, Q.; Park, H. S.; Hamuro, Y.; Lee, C. S.; Hamilton, A. D. *Biopolymers* **1998**, *47*, 285.
[8] Park, H. S.; Lin, Q.; Hamilton, A. D. *J. Am. Chem. Soc.* **1999**, *121*, 8.
[9] Ernst, J. T.; Becerril, J.; Park, H. S.; Yin, H.; Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 535.
[10] Wilson, A. J.; Groves, K.; Jain, R. K.; Park, H. S.; Hamilton, A. D. *J. Am. Chem. Soc.* **2003**, *125*, 4420.
[11] Leung, D. K.; Yang, Z. W.; Breslow, R. *Proc. Natl Acad. Sci. USA* **2000**, *97*, 5050.
[12] Fazal, M. A.; Roy, B. C.; Sun, S. G.; Mallik, S.; Rodgers, K. R. *J. Am. Chem. Soc.* **2001**, *123*, 6283.
[13] Gordon, E. J.; Sanders, W. J.; Kiessling, L. L. *Nature* **1998**, *392*, 30.
[14] Strong, L. E.; Kiessling, L. L. *J. Am. Chem. Soc.* **1999**, *121*, 6193.
[15] Gestwicki, J. E.; Cairo, C. W.; Strong, L. E.; Oetjen, K. A.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 14922.
[16] Boal, A. K.; Rotello, V. M. *J. Am. Chem. Soc.* **2000**, *122*, 734.

[17] 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.
[18] Park, S.; Taton, T. A.; Mirkin, C. A. *Science* **2002**, *295*, 1503.
[19] Niemeyer, C. M. *Agnew. Chem., Int. Ed. Engl.* **2001**, *40*, 4128.
[20] Mann, S.; Shenton, W.; Li, M.; Connolly, S.; Fitzmaurice, D. *Adv. Mater.* **2000**, *12*, 147.
[21] Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D. J.; Whyman, R. *J. Chem. Soc. Chem. Commun.* **1994**, 801.
[22] Templeton, A. C.; Wuelfing, M. P.; Murray, R. W. *Acc. Chem. Res.* **2000**, *33*, 27, **s18**.
[23] 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.
[24] Templeton, A. C.; Hostetler, M. J.; Kraft, C. T.; Murray, R. W. *J. Am. Chem. Soc.* **1998**, *120*, 1906.
[25] Lin, C. C.; Yeh, Y. C.; Yang, C. Y.; Chen, C. L.; Chen, G. F.; Chen, C. C.; Wu, Y. C. *J. Am. Chem. Soc.* **2002**, *124*, 3508.
[26] Ipe, B. I.; Thomas, K. G.; Barazzouk, S.; Hotchandani, S.; Kamat, P. V. *J. Phys. Chem. B* **2002**, *106*, 18.
[27] Wang, T. X.; Zhang, D. Q.; Xu, W.; Yang, J. L.; Han, R.; Zhu, D. B. *Langmuir* **2002**, *18*, 1840.
[28] Gu, T.; Ye, T.; Simon, J. D.; Whitesell, J. K.; Fox, M. A. *J. Phys. Chem. B* **2003**, *107*, 1765.
[29] Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2215.
[30] Dervan, P. B.; Edelson, B. S. *Curr. Opin. Struct. Biol.* **2003**, *13*, 284.
[31] Wang, G. L.; Zhang, J.; Murray, R. W. *Anal. Chem.* **2002**, *74*, 4320.
[32] Gottesfeld, J. M.; Belitsky, J. M.; Melander, C.; Dervan, P. B.; Luger, K. *J. Mol. Biol.* **2002**, *321*, 249.
[33] Mapp, A. K.; Ansari, A. Z.; Ptashne, M.; Dervan, P. B. *Proc. Natl Acad. Sci. USA* **2000**, *97*, 3930.
[34] Coull, J. J.; He, G. C.; Melander, C.; Rucker, V. C.; Dervan, P. B.; Margolis, D. M. *J. Virol.* **2002**, *76*, 12349.
[35] 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.
[36] Kuzmine, I.; Martin, C. T. *J. Mol. Biol.* **2001**, *305*, 559.
[37] Sandhu, K. K.; McIntosh, C. M.; Simard, J. M.; Smith, S. W.; Rotello, V. M. *Bioconjugate Chem.* **2002**, *13*, 3.
[38] Toogood, P. L. *J. Med. Chem.* **2002**, *45*, 1543.
[39] Gadek, T. R.; Nicholas, J. B. *Biochem. Pharmacol.* **2003**, *65*, 1.
[40] Chrunyk, B. A.; Rosner, M. H.; Cong, Y.; McColl, A. S.; Otterness, I. G.; Daumy, G. O. *Biochemistry* **2000**, *39*, 7092.
[41] Zhang, Z. Y.; Poorman, R. A.; Maggiora, L. L.; Heinrikson, R. L.; Kezdy, F. J. *J. Biol. Chem.* **1991**, *266*, 15591.
[42] Cochran, A. G. *Chem. Biol.* **2000**, *7*, R85.
[43] Fischer, N. O.; McIntosh, C. M.; Simard, J. M.; Rotello, V. M. *Proc. Natl Acad. Sci. USA* **2002**, *99*, 5018.
[44] Capasso, C.; Rizzi, M.; Menegatti, E.; Ascenzi, P.; Bolognesi, M. *J. Mol. Recog.* **1997**, *10*, 26.
[45] Verma, A.; Simard, J. M.; Rotello, V. M. *Langmuir* **2004**, *20*, 4178.
[46] Fischer, N. O.; Verma, A.; Goodman, C. M.; Simard, J. M.; Rotello, V. M. *J. Am. Chem. Soc.* **2003**, *125*, 13387.
[47] Hong, R.; Fischer, N. O.; Verma, A.; Goodman, C. M.; Emrick, T.; Rotello, V. M. *J. Am. Chem. Soc.* **2004**, *126*, 739.
[48] Kane, R. S.; Deschatelets, P.; Whitesides, G. M. *Langmuir* **2003**, *19*, 2388.
[49] Chan, W. C. W.; Nie, S. M. *Science* **1998**, *281*, 2016.
[50] Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013.