Binding and templation of nanoparticle receptors to peptide α-helices through surface recognition†

Partha S. Ghosh, Ayush Verma and Vincent M. Rotello*

Received (in Austin, TX, USA) 12th April 2007, Accepted 21st May 2007
First published as an Advance Article on the web 7th June 2007
DOI: 10.1039/b705554d

Nanoparticles featuring highly flexible sidechains template to peptides, demonstrating substantial pre-organization of the particle monolayer.

Protein–protein interactions are dictated through both non-covalent interactions and shape complementarity.[1] In several cases, the exposed face of a helical segment interacts with another helical surface or a shallow cleft of the binding partner.[2] For example, the hydrophobic residues on the helical surface of p53, a tumour suppressor, bind to a deep hydrophobic cleft of MDM2, an oncoprotein, *via* steric complementarity.[3] Therefore, the targeting of α-helices provides a promising tool for biomedical applications.[4] Several synthetic strategies have been successfully developed to stabilize short peptides into α-helices. The strategies include the use of a metal ion,[5] cyclodextrin dimers[6] and tetraguanidinium-based receptors.[7] However, significant stabilization of peptide α-helices by synthetic scaffolds in completely aqueous media is a challenging task.[8]

Nanoparticles offer a unique scaffold for biomacromolecule recognition by proper surface functionalization.[9] In preliminary studies, we used trimethylammonium functionalized gold nanoparticles to stabilize a tetraaspartate peptide (**TAP**) into an α-helix in completely aqueous media.[10] These particles, however, are not biocompatible. Incorporation of a tetra(ethylene glycol) (TEG) unit renders access to a biocompatible nanoparticle through: (a) increasing the water solubility,[11] (b) prevention of non specific interactions with biomolecules,[12] and (c) controlled surface recognition.[13] Here we demonstrate stabilization of an α-helical peptide by TEG-functionalized nanoparticles. Surprisingly, despite the highly flexible structure of the sidechains, these particles display templation to their peptide guests.

Cationic gold mixed monolayer protected clusters (MMPCs) with ~2 nm core diameter were used to induce folding of negatively charged peptide **TAP** into an α-helix (Fig. 1). A series of nanoparticles bearing different charge densities on the surface were fabricated (Table 1). MMPCs **1–3** were synthesized *via* the Murray place exchange method[14] by adding different ratios of two ligands, cationic TEG-trimethylammonium (TTMA) and neutral TEG-hydroxy (TOH) (see ESI). Water-soluble MMPC **4** was prepared according to the literature procedures.[15]

The 17-amino acid peptide **TAP** was engineered such that four aspartate residues were placed at alternating *i*, *i* + 3, and *i* + 4 positions in order to provide a more linear peptide surface suitable for recognition by the colloid (Fig. 1). The ends of the peptide were capped by acetylation of the amino terminus and amidation of the carboxy terminus to decrease the helix macrodipole effect. Moreover, a tryptophan (trp) residue was integrated at the N-terminal as a fluorescent probe.

The ability of MMPC **1** to induce helicity was monitored in aqueous solution (pH 11) using circular dichroism (CD). Varying concentrations (0–10 μM) of nanoparticle were mixed with 15 μM peptide solution, and CD spectra were collected after incubation for 5 min. CD spectra showed a significant increase in helicity (maximum at 192 nm, minima at 208 nm and 222 nm) of the

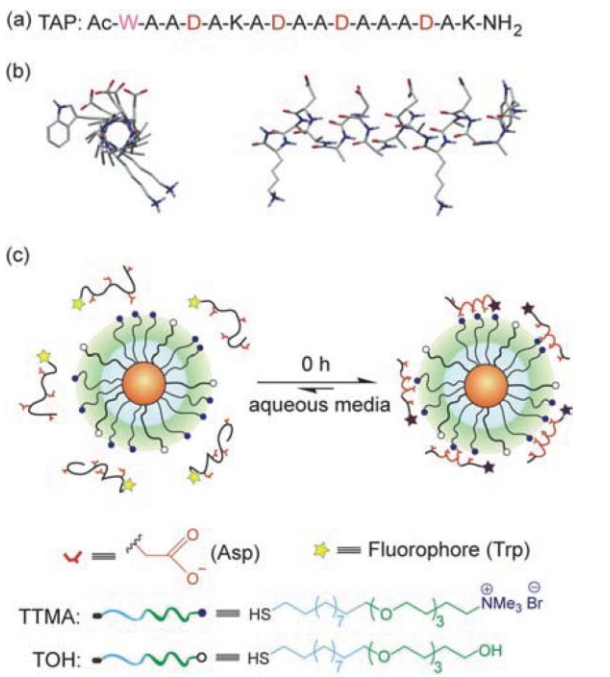

Fig. 1 (a) Amino acid sequence of the target peptide. (b) End and side views of the tetraaspartate peptide. (c) Schematic depiction of the peptide binding in helical conformation on MMPC surface (shown in relative sizes).

Table 1 The series of nanoparticles with different charge density

Nanoparticles	TTMA : TOH	% of cationic charge
MMPC **1**	1 : 0	~100
MMPC **2**	1 : 0.43	~70
MMPC **3**	1 : 0.78	~55
MMPC **4**	0 : 1	0

Department of Chemistry, University of Massachusetts, Amherst, Massachusetts, 01003, USA. E-mail: rotello@chem.umass.edu; Tel: (+1) 413-545-2058

† Electronic supplementary information (ESI) available: Synthesis of particles, DLS study and plots of helicity *versus* time for particles **1** and **3**. See DOI: 10.1039/b705554d

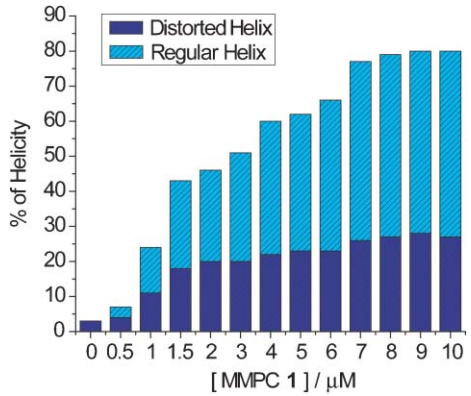

Fig. 2 CD titration of 15 μM peptide with MMPC **1** in pH 11 water: increase in overall helicity (regular and distorted) with increase in nanoparticle concentration.

peptide in presence of the nanoparticle (see ESI). The percent of helicity in each incubation was calculated by curve fitting using DICHROWEB. The overall helicity was obtained by combining the regular helix and the distorted helix. In the absence of nanoparticle, the peptide is in a random coil conformation showing only ~3% of helicity. Upon addition of cationic MMPC **1** to the peptide solution, there is a substantial degree of stabilization in the helical conformation with maximum overall helicity up to ~80% (Fig. 2). By contrast, neutral particle **4** was unable to induce helicity in the peptide, which verifies that the recognition of the peptide by the nanoparticle receptor is due to charge pairing. Likewise, addition of salt to the mixture of MMPC

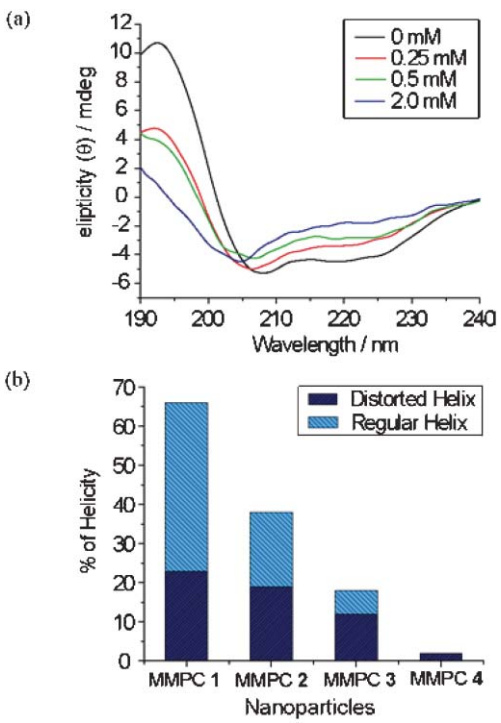

Fig. 3 (a) CD spectra of a mixture of 15 μM peptide and 6 μM of MMPC **1** at different salt concentrations. (b) Calculated overall helicity (regular and distorted) of 15 μM peptide in presence of 6 μM MMPCs in water of pH 11.

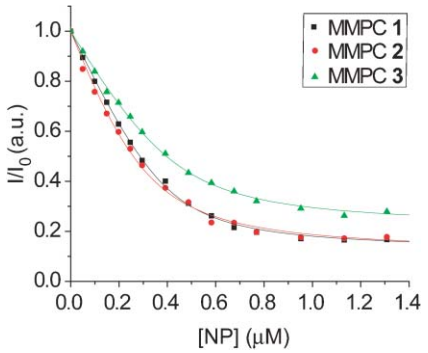

Fig. 4 Binding curves of 2 μM peptide with different MMPCs from fluorescence titration in pH 11 water. Absorbance of nanoparticles was corrected using neutral MMPC **4**.

1 and peptide disrupted their interaction resulting in a sharp decrease in helicity (Fig. 3a), validating electrostatic self-assembly. Furthermore, dynamic light scattering (DLS) studies indicate formation of discrete complexes upon association of peptides with nanoparticles (see ESI).

The electrostatic interaction between particle and peptide was regulated by using MMPCs with different cationic charge density on the surface. CD experiments were performed to observe the effect of charge density on helicity induction. The spectra were obtained after incubation of each of these particles (6 μM) with 15 μM of peptide solution for 5 min and a pronounced change in helicity was observed (Fig. 3b). As expected, helicity decreases with decrease in charge density on the particle surface.

The binding affinity of the peptide with MMPCs **1–4** was further investigated by fluorescence. Trp fluorescence was monitored by adding an increasing amount of nanoparticle to the 2 μM peptide solution. Upon binding with the peptide, MMPCs quenched the fluorescence due to the gold core. The corrected fluorescence intensities (I/I_0) were plotted against nanoparticle concentration and fitted with the binding equation (Fig. 4). The calculated macroscopic binding constants (K_s) are similar for all of the cationic particles (Table 2) with ~5–6 peptides binding per particle. The results reflect that the energy required for conformational change is balanced by the favorable enthalpy change due to ionic interaction.

Gold colloids have the ability to template through maximization of binding enthalpy as the thiols are mobile on the self assembled monolayer (SAM) surface.[10,16] To determine if this was occurring with our particles, the peptide was incubated in the presence of MMPCs over a period of 24 h. As expected, no templation was observed in the case of MMPC **1** (see ESI) due to the complete coverage by the functional group. Significantly, MMPC **2** showed

Table 2 Calculated macroscopic binding constant (K_s), Gibbs free energy change ($-\Delta G$), and binding ratio (n) for the complexation between peptide and cationic nanoparticles in pH 11 water, as determined *via* fluorescence. (The errors in binding constants and binding ratios were around 25% and 5%, respectively)

Nanoparticles	$K_s/10^6$ M^{-1}	$-\Delta G$/kJ mol^{-1}	n
MMPC **1**	4.1	38.4	5.1
MMPC **2**	2.1	36.7	6.7
MMPC **3**	2.9	37.5	4.7

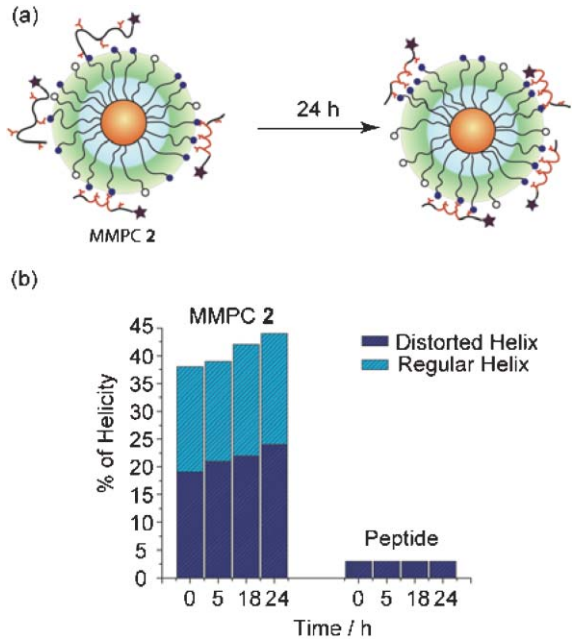

Fig. 5 Particle-assisted templation of peptide: increase in helicity of **TAP** with time on incubation with MMPC **2**.

a 16% increase in helicity within a period of 24 h due to the mobility of the ligands on the nanoparticle surface (Fig. 5). Further decrease in the density of the recognition unit for MMPC **3** resulted in no templation (see ESI). This lack of templation presumably arises from the low concentration of ligands on the surface, increasing the entropic cost of templation.

In addition to providing enhanced binding and helicity for target peptides, the observed templation highlights the preorganized nature of the nanoparticle monolayer. Clearly, structural information is transmitted *via* the highly flexible ligands from the particle surface to the terminus, a distance of $\sim$3 nm.[17] The nature of this monolayer organization is an open and intriguing question that a number of groups are exploring.[18]

In summary, we have demonstrated stabilization of α-helices using nanoparticles featuring flexible TEG-terminated sidechains. The electrostatic interaction and hence the extent of helicity induction can be tuned by tailoring the monolayer of MMPCs. Finally, despite the high flexibility of the ligands, MMPC **2** shows templation of the particle surface to the peptide, demonstrating the broad scope of nanoparticle templation to targets.

The authors are grateful to the NIH (GM077173) for financial support and the Center for Hierarchical Manufacturing ((DMI-0531171) for facilities. We thank Dr C.-C. You (UMass-Amherst), Mr G. Han (UMass-Amherst) and Mr M. Pollier (UMass-Amherst) for their assistance.

Notes and references

1 S. Jones and J. M. Thornton, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 13.
2 D. P. Fairlie, M. L. West and A. K. Wong, *Curr. Med. Chem.*, 1998, **5**, 29.
3 P. H. Kussie, S. Gorina, V. Marechal, B. Elenbaas, J. Moreau, A. J. Levine and N. P. Pavletich, *Science*, 1996, **274**, 948.
4 L. D. D'Andrea, G. Iaccarino, R. Fattorusso, D. Sorriento, C. Carannante, D. Capasso, B. Trimarco and C. Pedone, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 14215.
5 S. Futaki, T. Kiwada and Y. Sugiura, *J. Am. Chem. Soc.*, 2004, **126**, 15762.
6 D. Wilson, L. Perlson and R. Breslow, *Bioorg. Med. Chem.*, 2003, **11**, 2649.
7 (*a*) M. W. Peczuh, A. D. Hamilton, J. Sanchez-Quesada, J. de Mendoza, T. Haack and E. Giralt, *J. Am. Chem. Soc.*, 1997, **119**, 9327; (*b*) T. Haack, M. W. Peczuh, X. Salvatella, J. Sanchez-Quesada, J. de Mendoza, A. D. Hamilton and E. Giralt, *J. Am. Chem. Soc.*, 1999, **121**, 11813.
8 (*a*) B. P. Orner, X. Salvatella, J. S. Quesada, J. de Mendoza, E. Giralt and A. D. Hamilton, *Angew. Chem., Int. Ed.*, 2002, **41**, 117; (*b*) L. K. Tsou, C. D. Tatko and M. L. Waters, *J. Am. Chem. Soc.*, 2002, **124**, 14917; (*c*) Y. Mito-Oka, S. Tsukiji, T. Hiraoka, N. Kasagi, S. Shinkai and I. Hamachi, *Tetrahedron Lett.*, 2001, **42**, 7059.
9 (*a*) A. Verma and V. M. Rotello, *Chem. Commun.*, 2005, 303; (*b*) P. Hazarika, F. Kukolka and C. M. Niemeyer, *Angew. Chem., Int. Ed.*, 2006, **45**, 6827; (*c*) P. Hazarika, B. Ceyhan and C. M. Niemeyer, *Small*, 2005, **1**, 844; (*d*) L. Pasquato, P. Pengo and P. Scrimin, *J. Mater. Chem.*, 2004, **14**, 3481; (*e*) U. Drechsler, B. Erdogan and V. M. Rotello, *Chem.–Eur. J.*, 2004, **10**, 5570.
10 A. Verma, H. Nakade, J. M. Simard and V. M. Rotello, *J. Am. Chem. Soc.*, 2004, **126**, 10806.
11 A. G. Kanaras, F. S. Kamounah, K. Schaumburg, C. J. Kiely and M. Brust, *Chem. Commun.*, 2002, 2294.
12 M. Zheng, F. Davidson and X. Y. Huang, *J. Am. Chem. Soc.*, 2003, **125**, 7790.
13 R. Hong, N. O. Fischer, A. Verma, C. M. Goodman, T. Emrick and V. M. Rotello, *J. Am. Chem. Soc.*, 2004, **126**, 739.
14 A. C. Templeton, M. P. Wuelfing and R. W. Murray, *Acc. Chem. Res.*, 2000, **33**, 27.
15 H. Bayraktar, P. S. Ghosh, V. M. Rotello and M. J. Knapp, *Chem. Commun.*, 2006, 1390.
16 A. K. Boal and V. M. Rotello, *J. Am. Chem. Soc.*, 2000, **122**, 734.
17 The length of the TTMA ligand was calculated by using a 3-21G* basis set, using B3LYP. The calculated length was similar to the DLS data (see ESI).
18 (*a*) W. D. Luedtke and U. Landman, *J. Phys. Chem. B*, 1998, **102**, 6566; (*b*) A. M. Jackson, J. W. Myerson and F. Stellacci, *Nat. Mater.*, 2004, **3**, 330; (*c*) G. A. DeVries, M. Brunnbauer, Y. Hu, A. M. Jackson, B. Long, B. T. Neltner, O. Uzun, B. H. Wunsch and F. Stellacci, *Science*, 2007, **315**, 358.

