Sunlight mediated disruption of peptide-based soft structures decorated with gold nanoparticles†

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This communication reports morphological studies of a novel C_3-symmetric thiolated, tren-based ditryptophan conjugate. The exposed thiol groups on the soft structures interacted with gold nanoparticles and mediated the release of encapsulated fluorescent dye, when exposed to natural sunlight.

The diversity of peptide-based design of soft supramolecular ensembles emanates from the properties engendered in the side-chains of constituent amino acids that range from simple alkyl groups to a host of acidic, basic, hydrophilic, aromatic and heterocyclic groups.[1] These functionalities confer self-assembling and physicochemical properties through a variety of noncovalent interactions such as hydrogen bonds, hydrophobic interactions, salt bridges and a number of other specific interactions such as π–π stacking, cation–π and charge–dipole interactions.[2] Peptide-based soft structures respond to environmental stimuli and offer exciting opportunities in material synthesis, drug delivery, tissue engineering and sensing applications.[3]

One of the key interests in these soft structures concerns the possibility of encapsulation, covalent attachment, or chemiadsorption of bioactive agents for intracellular transport, where the size scale may offer certain advantages during administration *via* intravenous and subcutaneous routes and circumvent issues related to solubility and dispersability. The delivery of biologics could eventually be achieved by applying suitable external stimuli, such as alteration of pH change, exposure to reducing agents, or heat.[4]

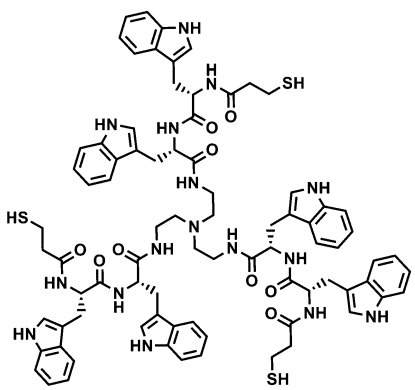
Recent excitement in metal nanoparticle research deals with the fundamental concepts and possible applications of plasmonic photothermal heating of diseased tissues by using visible radiation or laser excitation at resonant frequencies of plasmonic metal nanoparticles.[5] It was also shown that the size of the nanoparticles plays a significant role in determining the thermal transduction efficiencies.[6] Thus, it could be envisaged that thermal transduction *via* plasmonic heating could serve as an external stimulus for soft biological structures.

We have described the design and self-assembly of peptide-based soft structures to study prion octarepeats, as delivery vehicles and in ion-beam milling experiments.[3d,7] In one of the studies, a synthetic triskelion self-assembled to form spherical objects which responded to pH stimuli.[4g] We decided to explore the possibility of using a redox environment as a stimulus to exert pressure on the morphology of peptide soft structures. A C_3-symmetric ditryptophan-tren derivative was conjugated to 3-mercaptopropionic acid and the product was characterized by spectroscopic methods (Scheme 1).

A freshly prepared solution of **1** (1 mM; 9 : 1 methanol : water) revealed a spherical morphology in SEM micrographs (Fig. 1a), which was confirmed by AFM experiments on a mica surface (Fig. 1b). The emergence of self-assembled structures could be attributed to favorable interdigitation of Trp indole rings supported by π–π stacking.[4g] Interestingly, the 10-day incubation of this solution leads to the coalescence of the soft structures (Fig. 1c). It is possible that exposed thiol groups on surface of soft structures form disulfide cross-links upon slow aerial oxidation due to prolonged incubation, thus bringing the spherical structures into close proximity followed by deformation. The latter process was reversed by dithiothreitol treatment giving back the original spherical morphology perhaps due to reduction of the disulfide cross-links (Fig. 1d).

We next tried to exploit thiol–gold nanoparticle interaction to decorate the surface of peptide soft structures. Gold nanoparticles (AuNP) of ~15 nm in size were added to a solution of **1** and UV-visible spectra were recorded. A slight red shift and decrease in the peak height was observed for the plasmon absorbance of AuNPs, but more importantly, there was no colour change, and precipitation of AuNPs was not observed in the presence of the peptide conjugate.[8]

Microscopy analysis of the pre-formed **1**–AuNP interaction was conducted to investigate the morphological details of the hybrid structures. It was observed that a 1 h or longer co-incubation resulted in the attachment of AuNP on to the surface of spherical soft structures (Fig. 2a,b). The AuNP decorated structures were able to retain their integrity for as



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Scheme 1 Structure of the conjugate (Mpa-Trp-Trp)$_3$ tren (**1**). Mpa = 3-mercaptopropionic acid and Trp = tryptophan

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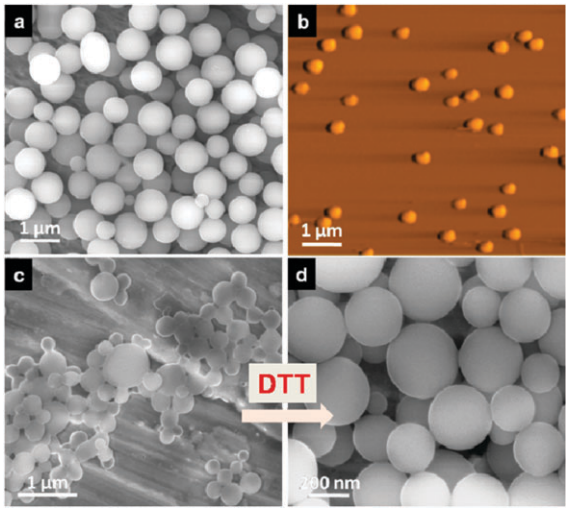

Fig. 1 (a) and (b) SEM and AFM micrographs of **1**, respectively; SEM micrographs of (c) 10 days aged sample, and (d) 10 days old sample treated with a reducing agent, DTT.

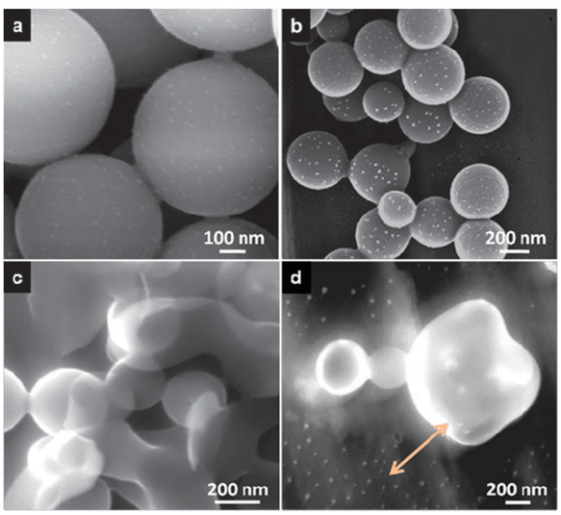

Fig. 2 SEM micrographs: (a) and (b) pre-formed spheres of **1** co-incubated with AuNPs (~15 nm) for 1 h and 3 days, respectively; (c) and (d) **1** co-incubated with AuNPs for 1 h and then exposed to sunlight for 2 h and 6 h, respectively (arrow shows swelling of vesicles and release of AuNPs due to SPR heating).

long as 16 days of incubation. EDAX analysis of these spheres further confirmed the presence of gold on the surface.[8]

Gold nanoparticles and nanorods are known to intensely absorb visible light due to the surface plasmon resonance (SPR) effect. The exposure of metal nanoparticles to electromagnetic radiation facilitates plasmonic heat generation, with a distinct correlation to the shape, number and organisation of the metal nanoparticles.[6] Besides the biological therapeutic uses suggested earlier,[5] plasmonic heating of gold nanoparticles has been used to control the size, shape, and phases of the nanoparticles,[9] for optothermal imaging,[10] and catalysis.[11] Such studies are mostly conducted with the help of lasers corresponding to the SPR maxima, but the use of sunlight and simulated sunlight for plasmonic heating of gold nanoparticles is also reported.[11a,12]

We decided to investigate if plasmonic heating will have an effect on the morphology of peptide-based soft self-assembled structures, as we have an interest in discovering newer stimuli-responsive structures for containment, release and delivery. AuNP immobilized spherical structures from Fig. 2a were directly exposed to sunlight for a period of 6 h in eppendorf tubes. Subsequently, the sample was analyzed by scanning electron microscopy which revealed some deformation in the overall morphology and in a few instances, the spherical objects coalesced resulting in irregular shapes (Fig. 2c). Interestingly, AuNPs were detached and release in the bulk solution, as a result of exposure to sunlight (Fig. 2d). In a control experiment, the size and overall shape of spherical objects from **1** remained unperturbed when exposed to sunlight in the absence of gold nanoparticles under similar conditions.[8]

Based on literature precedent, it could be proposed that a direct exposure to sunlight results in the plasmonic heating of AuNP-decorated peptide soft structures. This thermal energy gets most likely transferred to the self-assembled peptide scaffold thus modifying integrity of their structure. Although, laser beams or near-IR (NIR) sources have the advantage of tunable power for plasmonic heating experiments, a 2 h exposure to sunlight was also sufficient to achieve photothermal heating in the present example.

Morphological changes of the nanospheres as a result of SPR heating encouraged us to investigate whether they can possibly be used for delivery related applications. For latter studies, fluorescent dyes were used for encapsulation and release, with the aid of an external stimulus.[13] We performed a simple experiment to follow the interaction of a fluorescent dye with self-assembled structures of **1**, followed by the fate of dye labeled, AuNP conjugated soft structures upon plasmonic heating.

1 was dissolved in a rhodamine solution (1 μM) and the resulting solution was incubated for 2 h. As the dye interacted with the spherical structures, punctated bright red coloured objects were visualized in the fluorescence microscope (Fig. 3a). These rhodamine encapsulated spheres were then co-incubated with AuNP for 1 h as mentioned earlier. Fluorescence microscopy of rhodamine encapsulated AuNP immobilised spheres, after exposing to sunlight for 6 h, revealed that the intensity of the dye decreased and it was confined to the periphery of the spherical objects (Fig. 3c). Similar observations have also been reported for laser and NIR-mediated plasmonic heating of metal nanoparticle modified polyelectrolyte polymer capsules and synthetic liposomes.[13]

For control studies, dye encapsulated spheres in the absence of AuNP were exposed to sun light[8] and dye encapsulated AuNP immobilised spheres were kept in dark, for 6 h. In both cases dyes were uniformly distributed in the spheres (Fig. 3b), confirming that plasmonic heating could be used as a stimulus for releasing encapsulated material from these hybrid spherical self-assembled structures.

In summary, a new triskelion peptide conjugate was synthesised and its self-assembly process was studied in detail with various microscopy techniques. Time-dependent studies of this conjugate revealed agglomeration of these self-assembled spheres due to thiol oxidation, which was reversed when

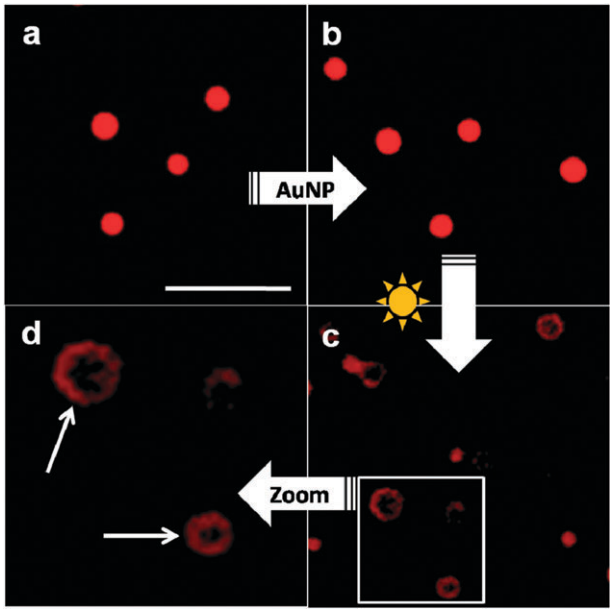

Fig. 3 Fluorescence microscopy images: (a) **1** co-incubated with rhodamine B for 2 h (scale bar: 3 μm); (b) dye encapsulated spheres co-incubated with AuNPs; (c) dye encapsulated AuNP immobilized spheres exposed to sunlight for 6 h; (d) zoomed-in image of structures with rhodamine fluorescence at the periphery.

treated with DTT. Further, we showed that thiol-containing spheres serve as a scaffold for AuNP interaction. Interestingly, AuNP decorated soft structures responded to sunlight-mediated plasmonic heating and as an application, remote release of encapsulated rhodamine B from the hybrid spherical objects was demonstrated.

We realize that considering the smaller size of the AuNPs used in this study, less thermal effect is expected. As a follow-up, we would like to investigate if low thermal effects are sufficient in inducing morphological changes of soft structures. Finally, it is surmised that the tuning of such hybrid structures may open possibilities for discovering biocompatible drug delivery compartments for targeted release of drugs on exposure to sunlight as a release mechanism.

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Notes and references

1 (*a*) X. Zhao and S. Zhang, *Chem. Soc. Rev*., 2006, **35**, 1105; (*b*) H. Rapaport, *Supramol. Chem*., 2006, **18**, 445; (*c*) M. Reches and E. Gazit, *Curr. Nanosci*., 2006, **2**, 105.

2 (*a*) R. S. Prajapati, M. Sirajuddin, V. Durani, S. Sreeramulu and R. Varadarajan, *Biochemistry*, 2006, **45**, 15000; (*b*) J. S. Nowick, *Org. Biomol. Chem*., 2006, **4**, 3869; (*c*) E. Gazit, *FEBS J*., 2005, **272**, 5971; (*d*) M. L. Waters, *Biopolymers*, 2004, **76**, 435; (*e*) C. G. Claessens and J. F. Stoddart, *J. Phys. Org. Chem*., 1997, **10**, 254; (*f*) S. K. Burley and G. A. Petsko, *Science*, 1985, **229**, 23.

3 (*a*) C. L. Chen and N. L. Rosi, *Angew. Chem., Int. Ed*., 2010, **49**, 1924; (*b*) J. P. Jung, J. Z. Gasiorowski and J. H. Collier, *Biopolymers*, 2010, **94**, 49; (*c*) S. Aluri, S. M. Janib and J. A. Mackay, *Adv. Drug Delivery Rev*., 2009, **61**, 940; (*d*) K. B. Joshi, S. Ghosh and S. Verma, *Med. Chem*., 2007, **3**, 605; (*e*) R. J. Mart, R. D. Osborne, M. M. Stevens and R. V. Ulijn, *Soft Matter*, 2006, **2**, 822.

4 (*a*) D. M. Lynn, M. M. Amiji and R. Lager, *Angew. Chem., Int. Ed*., 2001, **40**, 1707; (*b*) A. N. Zelikin, J. F. Quinn and F. Caruso, *Biomacromolecules*, 2006, **7**, 27; (*c*) Y. Li, B. S. Lokitz, S. P. Armes and C. L. McCormick, *Macromolecules*, 2006, **39**, 2726; (*d*) J.-K. Kim, E. Lee, Y.-b. Lim and M. Lee, *Angew. Chem., Int. Ed*., 2008, **47**, 4662; (*e*) M. Gingras, J.-M. Raimundo and Y. M. Chabre, *Angew. Chem., Int. Ed*., 2007, **46**, 1010; (*f*) S. Lee, S. C. Yang, M. J. Heffernan, W. R. Taylor and N. Murthy, *Bioconjugate Chem*., 2007, **18**, 4; (*g*) S. Ghosh, M. Reches, E. Gazit and S. Verma, *Angew. Chem., Int. Ed*., 2007, **46**, 2002; (*h*) E. Kim, D. Kim, H. Jung, J. Lee, S. Paul, N. Selvapalam, Y. Yang, N. Lim, C. G. Park and K. Kim, *Angew. Chem., Int. Ed*., 2010, **49**, 4405.

5 Selected references: (*a*) B. Kang, M. A. Mackey and M. A. El-Sayed, *J. Am. Chem. Soc*., 2010, **132**, 1517; (*b*) J. H. Park, G. von Maltzahn G, M. J. Xu, V. Fogal, V. R. Kotamraju, E. Ruoslahti, S. N. Bhatia and M. J. Sailor, *Proc. Natl. Acad. Sci. U. S. A*., 2010, **107**, 981.

6 (*a*) D. K. Roper, W. Ah and M. Hoepfner, *J. Phys. Chem. C*, 2007, **111**, 3636; (*b*) J. R. Cole, *J. Phys. Chem. C*, 2009, **113**, 12090; (*c*) R. A. Sperling, P. gil Rivera, F. Zhang, M. Zanella and W. J. Parak, *Chem.Soc. Rev*., 2008, **37**, 1896.

7 (*a*) K. B. Joshi and S. Verma, *Angew. Chem., Int. Ed*., 2008, **47**, 2860; (*b*) S. Ghosh, S. K. Singh and S. Verma, *Chem. Commun*., 2007, 2296; (*c*) S. Ghosh and S. Verma, *Chem.–Eur. J*., 2008, **14**, 1415; (*d*) S. Mondal, S. Ghosh and S. Verma, *Tetrahedron Lett*., 2010, **51**, 856; (*e*) N. Gour, C. S. Purohit, R. Puri b, S. Ganesh and S. Verma, *Biochem. Biophys. Res. Commun*., 2009, **378**, 503; (*f*) N. Gour and S. Verma, *Soft Matter*, 2009, **5**, 1789.

8 See Supporting Information.

9 F. Hubenthal, *Eur. J. Phys*., 2009, **30**, S49 and references cited therein.

10 (*a*) D. Boyer, P. Tamarat, A. Maali, B. Lounis and M. Orrit, *Science*, 2002, **297**, 1160; (*b*) V. P. Zharov, K. E. Mercer, E. N. Galitovskaya and M. S. Smeltzer, *Biophys. J*., 2006, **90**, 619; (*c*) X. H. Huang, S. Neretina and M. A. El-Sayed, *Adv. Mater*., 2009, **21**, 4880.

11 Selected recent references: (*a*) X. Chen, H.-Y. Zhu, J.-C. Zhao, Z.-F. Zheng and X.-P. Gao, *Angew. Chem., Int. Ed*., 2008, **47**, 5353; (*b*) C. C. Jia, H. M. Yin, H. Y. Ma, R. Y. Wang, X. B. Ge, A. Q. Zhou AQ, X. H. Xu and Y. Ding, *J. Phys. Chem. C*, 2009, **113**, 16138; (*c*) E. Kowalska, O. O. P. Mahaney, R. Abe and B. Ohtani, *Phys. Chem. Chem. Phys*., 2010, **12**, 2344; (*d*) H. Y. Zhu, X. Chen, Z. F. Zheng, X. B. Ke, E. Jaatinen, J. C. Zhao, C. Guo, T. F. Xie and D. J. Wang, *Chem. Commun*., 2009, 7524.

12 (*a*) N. Harris, M. J. Ford and M. B. Cortie, *J. Phys. Chem. B*, 2006, **110**, 10701; (*b*) Q. Xiang, G. F. Meng, H. B. Zhao, Y. Zhang, H. Li, W. J. Ma and J. Q. Xu, *J. Phys. Chem. C*, 2010, **114**, 2049.

13 (*a*) A. G. Skirtach, C. Dejugnat, D. Braun, A. S. Susha, A. L. Rogach, W. J. Parak, H. Möhwald and G. B. Sukhorukov, *Nano Lett*., 2005, **5**, 1371; (*b*) D. V. Volodkin, A. G. Skirtach and H. Möhwald, *Angew. Chem., Int. Ed*., 2009, **48**, 1807; (*c*) A. G. Skirtach, D. V. Volodkin and H. Möhwald, *ChemPhysChem*, 2010, **11**, 822; (*d*) D. V. Volodkin, M. Delcea, H. Möhwald and A. G. Skirtach, *ACS Appl. Mater. Interfaces*, 2009, **1**, 1705.

