

Cite this: *Chem. Commun.*, 2011, **47**, 9846–9848

www.rsc.org/chemcomm

Polymer control of ligand display on gold nanoparticles for multimodal switchable cell targeting†

Francesca Mastrotto,^a Paolo Caliceti,^a Vincenzo Amendola,^b Sara Bersani,^a Johannes Pall Magnusson,^c Moreno Meneghetti,^b Giuseppe Mantovani,^c Cameron Alexander^c and Stefano Salmaso*^a

Received 5th May 2011, Accepted 15th July 2011

DOI: 10.1039/c1cc12654g

The function of cell-specific ligands on gold nanoparticles can be selectively gated by the action of co-grafted thermosensitive polymers. Below the LCST the responsive chain-extended polymers prevent cell-surface receptors from accessing the affinity ligands while above the LCST, the polymers collapse exposing the ligands and allowing binding to receptors, which in turn promotes cell internalisation.

The spatial and temporal display of chemical functionality is the key to many important natural and synthetic functions, such as cell–cell recognition and communication,¹ reaction engineering² and information processing.³ Control of when and how a ligand or a surface becomes active can endow systems with specific bio-recognition features, surface properties and/or micro-environmental sensitivity.⁴ A wide array of synthetic polymers, self-assembling supramolecular structures and nanoparticles have been prepared that can perform sequential time/site “logic” events in response to stimuli.⁵ Recently, metal nanoparticles have shown potential as combined diagnostics and therapeutics, primarily for *in situ* thermal ablation therapy, drug delivery, radiotherapy enhancement and X-ray Computed Tomography based imaging.⁶

Here we demonstrate control of ligand display at responsive polymer-functionalised gold nanoparticles (AuNPs) and the use of this feature for selective tumour cell targeting. Gold nanoparticles ($d = 18 \pm 11$ nm) were synthesized by Laser Ablation Synthesis in Solution⁷ (LASiS, Fig. S1, ESI†), then decorated with thiolated biotin or folic acid as representative biorecognition and tumour targeting agents.⁸ A thermoresponsive co-polymer, poly(*N*-isopropylacrylamide-*co*-acrylamide) (pNIPAm-*co*-Am), with a cysteine terminus and a Lower Critical Solution Temperature (LCST) of 37 °C^{4b} was subsequently attached to the nanoparticles to bestow ‘stealth’

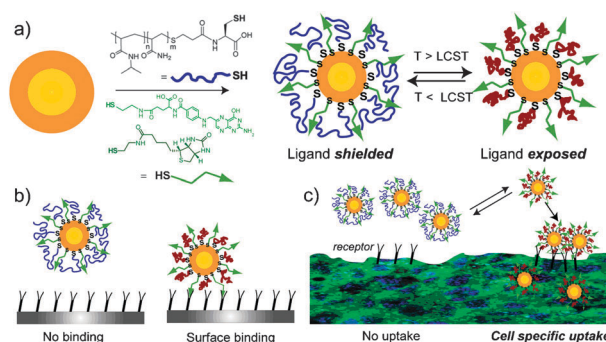


Fig. 1 Schematic of AuNP decoration with targeting agents and thermoresponsive polymers (a), and temperature controlled biorecognition (b and c).

properties at temperature close to normal physiological temperatures. Accordingly, the particles were expected to display chain-extended polymer brushes at their surfaces at 37 °C ($T < \text{LCST}$), thus hiding the targeting molecules on the particle surface, but to expose the ligands to the outer environment at temperatures above 37 °C ($T > \text{LCST}$) when the polymer collapsed into poorly-solvated globules (Fig. 1).

AuNP decoration studies were carried out using biotin-SH/AuNP in the range of 50:1–4000:1 molar ratio and pNIPAm-*co*-Am-SH/AuNP in the range of 350:1–2800:1 molar ratio. Functionalised AuNPs were incubated with avidin coated microtitre plates prior to ELISA assays. As apparent in Fig. 2, at 40 °C ($T > \text{LCST}$) biotin/pNIPAm-*co*-Am coated AuNPs bearing 100 biotin molecules and 700 polymer chains displayed specific recognition properties, with 79% binding efficiency as compared to nanoparticles decorated with biotin only with the same biotin/AuNP molar ratio (polymer free particles). At 34 °C ($T < \text{LCST}$), the same biotin/pNIPAm-*co*-Am coated AuNP exhibited only 9% comparative binding, while biotin free pNIPAm-*co*-Am decorated AuNP did not undergo binding to avidin irrespective of assay temperature. These data indicated that presentation of the nanoparticle-bound biotin ligand to surfaces was dependent on whether the co-attached pNIPAm-*co*-Am chain-collapsed (at 40 °C), or chain-extended (at 34 °C).

The relative extents of surface coverage by both a responsive polymer and a targeting moiety were found to be critical to

^a Department of Pharmaceutical Sciences, University of Padua, via F. Marzolo 5, 35131, Padova, Italy.

E-mail: stefano.salmaso@unipd.it; Fax: +39 0498275366; Tel: +39 0498271602

^b Department of Chemical Sciences, University of Padua, via F. Marzolo 1, 35131, Padova, Italy

^c School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, UK

† Electronic supplementary information (ESI) available: Experimental procedures and additional materials. See DOI: 10.1039/c1cc12654g

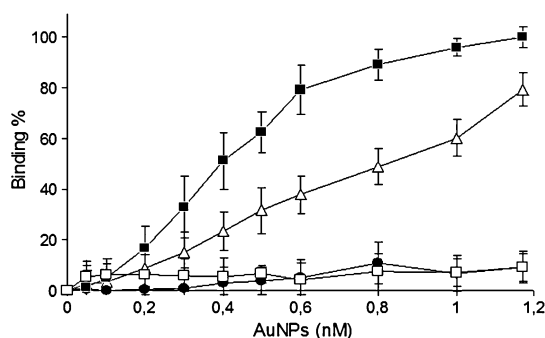


Fig. 2 Binding profile of AuNPs to avidin coated microtitre plates: (■) biotinylated AuNP incubated at 34 and 40 °C (overlapped); (◇) biotin/pNIPAm-co-Am coated AuNP incubated at 40 °C; (●) biotin/pNIPAm-co-Am coated AuNP incubated at 34 °C; (□) pNIPAm-co-Am coated AuNP incubated at 40 °C.

tune the binding selectivity of the polymer/targeting agent decorated AuNP. In fact, nanoparticles bearing 50 biotin molecules and 350 polymer chains showed more limited bio-recognition properties, although the biotin/polymer molar ratio was very similar to AuNPs coated with 100 biotin molecules and 700 polymer chains described above. Therefore, a minimum amount of targeting molecules is required as they can be cooperatively engaged in avidin recognition. On the other hand, nanoparticles bearing 100 biotin molecules and 2680 polymer chains did not bind to the avidin surfaces, indicating that the high polymer density on the particle surface masks the biotin exposition thus impairing the AuNP/avidin interaction (Fig. S6, ESI†).

Particles decorated with 100:700:1 folate-SH/polymer/AuNP molar ratio for folate receptor (FR) overexpressing cancer cell targeting^{9,10} were used on the basis of the best performing targeting agent/polymer composition emerged with the biotinylated gold nanoparticle study.

The UV-Vis spectra and dynamic light scattering (DLS) measurements indicated that naked AuNPs aggregated in buffer irrespective of temperature. By contrast, both pNIPAm-co-Am and folate/pNIPAm-co-Am functionalized AuNP formed stable dispersions in saline buffer and in foetal bovine serum (FBS) at 34 °C while slightly self-associated at 40 °C (Fig. S4 and S5, Table S2, ESI†). Particle de-aggregation was obtained by decreasing the temperature below the LCST. This behavior was ascribed to the reversible changes in colloidal stability as the polymer coatings collapsed or expanded above and below the LCST.

Cell targeting properties of the folate/pNIPAm-co-Am decorated AuNP were evaluated by atomic absorption spectroscopy and by confocal microscopy using MCF7 cells that do not overexpress folate receptor (FR-negative) and KB cells that overexpress the folate receptor (FR-positive).¹¹

In the absence of FBS (Fig. 3a) at 34 °C, very low pNIPAm-co-Am and folate/pNIPAm-co-Am decorated AuNP uptake was obtained either with KB or MCF7 cells and internalization into cells was not affected by the presence of folic acid on the particle surface. This behavior was likely due to the hydrophilic steric shielding conferred by the intermediate molar mass (~8 kDa) pNIPAm-co-Am chains in the extended state below the LCST that prevents protein adsorption, particle aggregation

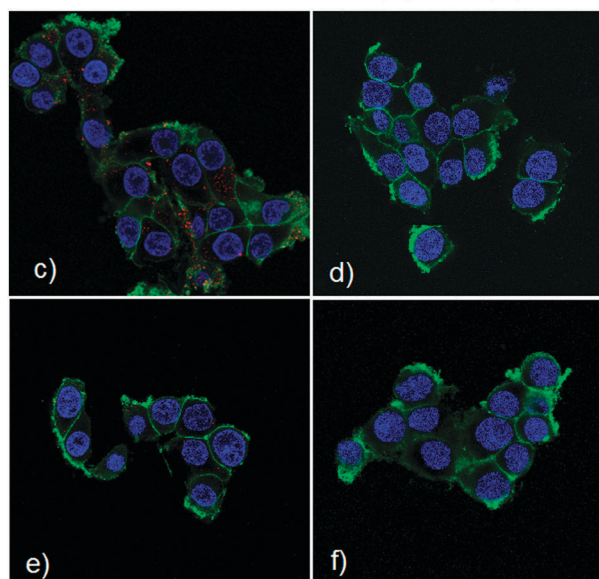
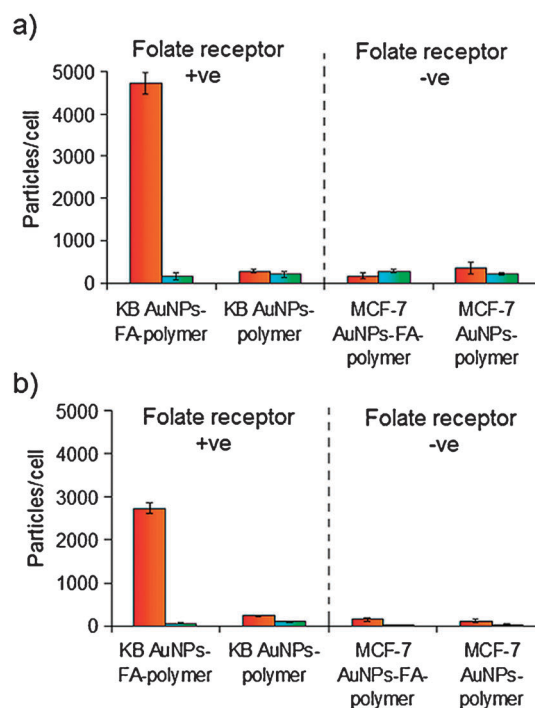


Fig. 3 Cell uptake of thermoresponsive untargeted and targeted AuNPs by KB and MCF7 cells at 34 °C (blue bars) and 40 °C (red bars) in DMEM (a) and in DMEM supplemented with serum (b). Confocal microscopic examination of KB cells treated with AuNPs functionalized with folic acid/pNIPAm-co-Am (c and d) and with pNIPAm-co-Am only (e and f). Cells were incubated at 40 °C (c and e) and 34 °C (d and f). Blue channel = nuclei detection (DAPI); green channel = cell membrane detection (fluorescein-DHPE); red channel = scattered light by AuNP.

as well as folate exposure. Low cell uptake also occurred at 40 °C when pNIPAm-co-Am coated AuNPs were incubated with KB and MCF7 cells and when folic/pNIPAm-co-Am coated decorated AuNPs were incubated with MCF7 cells.

Interestingly, the KB cell incubation with folate/pNIPAm-co-Am decorated AuNPs at 40 °C yielded efficient internalization as about 4700 particles/cell were found. This value was 17, 30 and 28 times higher than that obtained with folic acid free

thermoresponsive AuNP and KB cells at 40 °C, with folate/pNIPAm-co-Am decorated AuNP and KB cells at 34 °C, and MCF7 cells at 40 °C, respectively. Similar behaviour was obtained when cell incubation was performed in the presence of 10% FBS (Fig. 3b), although the overall uptake of folate targeted thermoresponsive AuNPs at 40 °C in KB cells was about 2 times lower. Lower particle uptake was obtained in all experiments carried out in the presence of FBS that may be ascribed to a combination of events, which include either protein adsorption on the nanoparticles or the competition with free folic acid present in the FBS medium. The number of particles per cell detected with folate/pNIPAm-co-Am decorated AuNP at 40 °C in the presence of 10% FBS was close to the value obtained with folate decorated AuNP used as the positive control. Competition studies performed by incubation of KB cells with folate/pNIPAm-co-Am decorated particles in the presence of free folic acid confirmed the receptor mediated cell uptake of the targeted AuNP.

Confocal microscopy studies were carried out to visualize AuNPs by their scattering.¹² This analysis confirmed the data obtained by atomic absorption spectroscopy quantification and indicated that the AuNP uptake was observed *only* for the folate/pNIPAm-co-Am decorated particles at temperatures above the polymer LCST and in the FR-positive cell line (Fig. 3c). The confocal images show that in the case of folate/pNIPAm-co-Am decorated AuNPs most of nanoparticles are located in the cytoplasmic compartment while only few particles are distributed in the peripheral region of the cells. The results confirmed the temperature tunable targeting properties conferred by folic acid and pNIPAm-co-Am decoration (Fig. 3d–f). The data further supported the role of surface-displayed folic acid in cell internalization by ligand–receptor interaction and uptake by potocytosis.¹³ As reported above, the limited nonspecific internalization obtained with the pNIPAm-co-Am decorated AuNP was most probably a consequence of the low particle aggregation under the experimental conditions.

Overall, the results demonstrate that the coil-to-globule transition of thermoresponsive polymers can be used to hide and reveal active functionalities, and that this in turn can be used to direct molecular and cell recognition phenomena. Although there have been numerous prior examples of polymer-directed protein activity¹⁴ and cell attachment,¹⁵ we believe this is the first example wherein a polymer switch has been used to mask and display bioactive ligands and cell internalization in this way. Importantly, the switching behaviour was designed and demonstrated to take place at temperatures just above those in normal physiological tissues, potentially allowing for site-specific activation by medical external procedures¹⁶ or *in situ* activation by local hyperthermia in tumour tissue.¹⁷ This suggests possible *in vitro* or *in vivo* applications in diagnostics and therapeutics, which would be particularly beneficial where dual-triggered polymer-switched display of active functionality could be used to effect external-mediated localisation in a tumour site and/or disease-activated cellular entry in specific tumour cells.

For tumor targeting, polymers with LCST in the range of physiopathological temperatures (37–39 °C) must be selected

while a wider range of LCST can be used for *in vitro* and *ex vivo* diagnostic purposes. Additionally, a wide variety of other stimuli sensitive materials could readily be incorporated to enable the control of ligand display and activation of chemical functionality.

This work was supported by the Eng. Phys. Sciences Res. Council (Grants EP/H006915/1, EP/H005625/1).

Notes and references

- (a) M. Mammen, S. K. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2755–2794; (b) O. J. Barrett, J. L. Childs and M. D. Disney, *ChemBioChem*, 2006, **7**, 1882–1885.
- S. F. M. van Dongen, M. Nallani, J. J. L. M. Cornelissen, R. J. M. Nolte and J. C. M. van Hest, *Chem.–Eur. J.*, 2009, **15**, 1107–1114.
- N. Wagner and G. Ashkenasy, *Chem.–Eur. J.*, 2009, **15**, 1765–1775.
- (a) S. Lee, E. J. Cha, K. Park, S. Y. Lee, J. K. Hong, I. C. Sun, S. Y. Kim, K. Choi, I. C. Kwon, K. Kim and C. H. Ahn, *Angew. Chem., Int. Ed.*, 2008, **47**, 2804–2807; (b) S. Salmaso, P. Caliceti, V. Amendola, M. Meneghetti, J. P. Magnusson, G. Pasparakis and C. Alexander, *J. Mater. Chem.*, 2009, **19**, 1608–1615.
- (a) H. Lomas, I. Canton, S. MacNeil, J. Du, S. P. Armes, A. J. Ryan, A. L. Lewis and G. Battaglia, *Adv. Mater.*, 2007, **19**, 4238–4243; (b) A. Kishimura, A. Koide, K. Osada, Y. Yamasaki and K. Kataoka, *Angew. Chem., Int. Ed.*, 2007, **46**, 6085–6088; (c) G. Pasparakis, M. Vamvakaki, N. Krasnogor and C. Alexander, *Soft Matter*, 2009, **5**, 3839–3841; (d) J. P. Magnusson, A. Khan, G. Pasparakis, A. O. Saeed, W. Wang and C. Alexander, *J. Am. Chem. Soc.*, 2008, **130**, 10852–10853; (e) M. P. Xiong, Y. Bae, S. Fukushima, M. L. Forrest, N. Nishiyama, K. Kataoka and G. S. Kwon, *ChemMedChem*, 2007, **2**, 1321–1327; (f) Y. Z. You, Q. H. Zhou, D. S. Manickam, L. Wan, G. Z. Mao and D. Oupicky, *Macromolecules*, 2007, **40**, 8617–8624; (g) P. M. George, D. A. LaVan, J. A. Burdick, C. Y. Chen, E. Liang and R. Langer, *Adv. Mater.*, 2006, **18**, 577–581.
- (a) P. Diagaradjane, A. Shetty, J. C. Wang, A. M. Elliott, J. Schwartz, S. Shentu, H. C. Park, A. Deorukhkar, R. J. Stafford, S. H. Cho, J. W. Tunnell, J. D. Hazle and S. Krishnan, *Nano Lett.*, 2008, **8**, 1492–1500; (b) W. Eck, A. I. Nicholson, H. Zentgraf, W. Semmler and S. Bartling, *Nano Lett.*, 2010, **10**, 2318–2322.
- V. Amendola, S. Polizzi and M. Meneghetti, *J. Phys. Chem. B*, 2006, **110**, 7232–7237.
- L. C. Hartmann, G. L. Keeney, W. L. Lingle, T. J. H. Christianson, B. Varghese, D. Hillman, A. L. Oberg and P. S. Low, *Int. J. Cancer*, 2007, **121**, 938–942.
- S. F. Atkinson, T. Bettinger, L. W. Seymour, J. P. Behr and C. M. Ward, *J. Biol. Chem.*, 2001, **276**, 27930–27935.
- P. S. Low, W. A. Henne and D. D. Doorneweerd, *Acc. Chem. Res.*, 2008, **41**, 120–129.
- (a) M. McHugh and Y. C. Cheng, *J. Biol. Chem.*, 1979, **254**, 1312–1318; (b) F. Sonvico, C. Dubernet, V. Marsaud, M. Appel, H. Chacun, B. Stella, M. Renoir, P. Colombo and R. Couvreur, *J. Drug Delivery. Sci. Technol.*, 2005, **15**, 407–410.
- S. Klein, S. Petersen, U. Taylor, D. Rath and S. Barcikowski, *J. Biomed. Opt.*, 2010, **15**, 036015.
- R. G. W. Anderson, B. A. Kamen, K. G. Rothberg and S. W. Lacey, *Science*, 1992, **255**, 410–411.
- A. S. Hoffman and P. S. Stayton, *Prog. Polym. Sci.*, 2007, **32**, 922–932.
- (a) E. Wischerhoff, K. Uhlig, A. Lankenau, H. G. Borner, A. Laschewsky, C. Duschl and J. F. Lutz, *Angew. Chem., Int. Ed.*, 2008, **47**, 5666–5668; (b) N. Matsuda, T. Shimizu, M. Yamato and T. Okano, *Adv. Mater.*, 2007, **19**, 3089–3099.
- Z. P. Shen, A. A. Brayman, L. Chen and C. H. Miao, *Gene Ther.*, 2008, **15**, 1147–1155.
- M. R. Dreher, W. G. Liu, C. R. Michelich, M. W. Dewhirst and A. Chilkoti, *Cancer Res.*, 2007, **67**, 4418–4424.