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DNA-mediated Anisotropic Silica Coating of Upconversion Nanoparticles

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We report a facile approach of using DNA molecules as switches to selectively activate silica coating onto specific facets of upconversion nanoparticles. Being simple and reproducible, this method improves the understanding of silica coating mechanism and opens up new opportunities for nanomedicine delivery.

Nanoparticles, as the building blocks in nanobiotechnology, are broadly used as molecular-specific tags[1], responsive sensors[2, 3], nanoscale carriers[4-6], and stimuli-reactive triggers in nanomedicine delivery systems[7, 8]. Among a variety of surface modification strategies for nanoparticles, silica coating attracts substantial attention because a silica capping layer can introduce a stabilized interface for molecular functionalization[9, 10]. When upconversion nanoparticles (UCNPs) become a new family of luminescent nanomaterials, with unique properties in converting near infrared light into visible and UV emissions, silica coating has been commonly used for the surface modification and phase transformation of UCNPs for chemical sensing[11, 12], bioimaging[13-15], intracellular sensing[16, 17], NIR light triggered drug therapy[14, 17, 18] and optogenetics[19].

As the stability, specificity, selectivity, and biocompatibility of nanoparticles rely largely on their surface properties, nowadays exploration of the anisotropic surface properties of nanoparticles becomes a trend for creating novel functional nanomaterials or optimizing the performance of the particles for emerging applications[20-22]. According to previous reports[20, 21, 23], the site-specific silica coating can be achieved by the surface curvature of nanoparticles, but the fundamental mechanism thereof remains unclear. Wang et al.[20] suggest that the prior modification of thiol-terminated methoxypoly(ethylene glycol) (PEG-thiol) could prevent silica deposition onto the ends of gold nanorods (AuNRs). Murphy et al.[23] believe that the oxidization state of capping ligands could be the key to anisotropic silica deposition. For instance, PEG-disulfide can selectively bond onto the ends of AuNRs, facilitating silica deposition on the side facets; while PEG-thoil modification deters silica deposition onto AuNRs as it shows no site preference but covers complete surface of AuNRs. Nevertheless, current publications are mainly focused on AuNPs, the site-specific silica coating onto UCNPs has seldom been reported.

DNA molecules are rich in phosphodiester bonds on their backbones, which enables their direct conjugation onto the trivalent lanthanide ions of UCNPs[24-26]. Moreover, the surfactant molecules of UCNPs are featured with different binding strengths on end (001) and lateral (100)/(010) facets[27]. In our recent study, we have found that DNA molecules exhibit selectivity with regard to the different facets of UCNPs[28]. The intriguing property of DNA molecules has enabled us to obtain both hydrophobic and hydrophilic facets on UCNPs. Such a facet-selective DNA binding might offer a promising route for the anisotropic silica coating on UCNPs by using DNA molecules as capping ligands.

To clearly observe the difference on (001) and (100)/(010) facets, rod-shape UCNPs are utilized. DNA modification is initiated by a ligand exchange method[25, 26]. By shaking the mixture of chloroform containing UCNPs and the aqueous solution that contains synthetic DNA molecules for two hours, the initial surfactants of UCNPs would be replaced, transferring UCNPs from chloroform to the aqueous phase. A micro-emulsion method with minor adjustment is followed to coat silica onto the DNA capped UCNPs[15]. Briefly, the pre-treated UCNPs are dispersed in cyclohexane with a surfactant (IGEPAL® CO-520) and ammonium hydroxide with the help of ultrasonication for 10 minutes. Then orthosilicate (TEOS) cyclohexane solution is added dropwise into the UCNPs suspension under magnetic stir. After 10 hours, pure ethanol is added to the suspension to stop the reaction. The UCNPs are then purified by ethanol and water for 3 times respectively and finally stored in water for further analysis. It should be noted that only after adding CO-520 and the use of ultrasonication can the DNA-modified UCNPs be well-suspended in cyclohexane.

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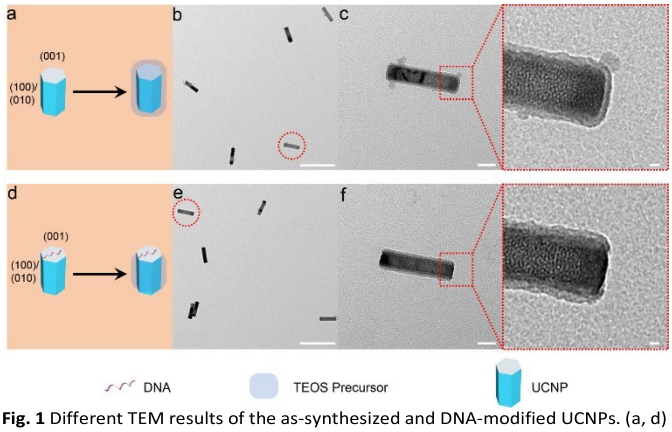

Fig. 1 Different TEM results of the as-synthesized and DNA-modified UCNPs. (a, d) Carton diagram of silica coating onto as-synthesized UCNPs and DNA modified UCNPs. (b) TEM image of the as-synthesized UCNPs after silica coating. (c) High-resolution TEM image of the single UCNP marked by a circle in (b). The squared area is zoomed in for a clear vision. (e) Silica coating of UCNPs modified with DNA molecules. (f)High-resolution TEM image of the single UCNP marked by a circle in (e) and the enlarged tip area. Scale bar: 100 nm.

Characterisation by transmission electron microscopy (TEM) in Figure 1 shows that a uniform silica shell forms all through the surface of as-synthesized UCNPs whereas a silica shell only forms on the (100)/(010) facets of DNA modified UCNPs.

Our recent work[28] proves that in the ligand exchange process, the phosphodiester bonds on the backbone of DNA can only replace the surfactant molecules on the (001) facets of UCNPs, leaving the (100)/(010) facets covered by initial organic surfactants to generate anisotropic surface; nevertheless, phosphate groups on the terminus of DNA show the strongest affinity which replace all the surfactant molecules on the (001) and (100)/(010) facets, rendering UCNPs fully hydrophilic.

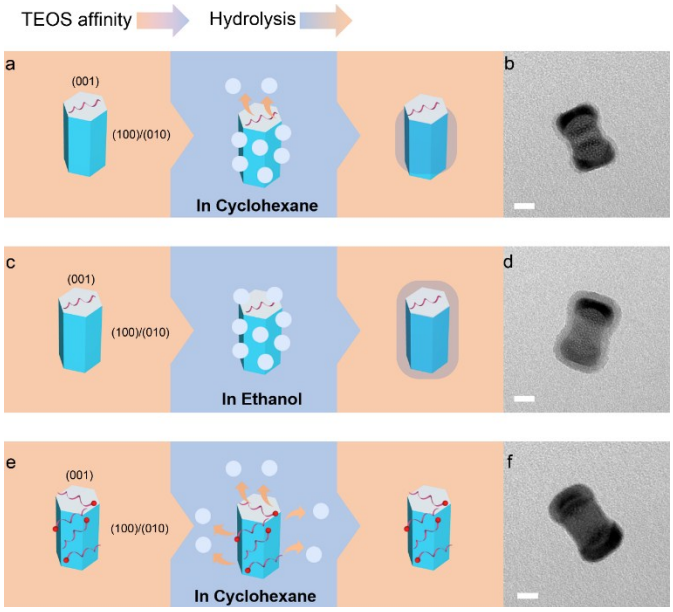

Fig. 2 Investigation on the site-specific silica coating mechanism. (a) Mechanism of selective silica coating: DNA on the (001) facets prevents deposition of TEOS precursor, leading to anisotropic silica coating. (b) TEM image of a dumbbell-shape UCNP with silica coated on (100)/(010) facets. (c) Schematic illustration of silica coating onto UCNPs with DNA modification on (001) and (100)/(010) facets in ethanol. (d) TEM result shows no selectivity in amphiphilic solvent ethanol. (e, f) In cyclohexane, neither (001) nor (100)/(010) facets of UCNPs can be coated with silica if covered by DNA molecules. bar: 20 nm.

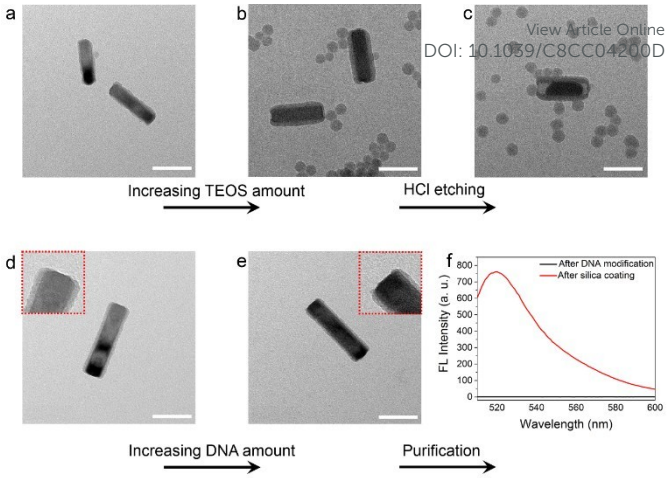

Fig. 3 (a, b) TEM images of UCNPs after silica coating using increased amounts of TEOS. (c) Acid etching of silica coated UCNPs in (b). The etching process initiates from the two ends. (d, e) TEM images of UCNPs after silica coating using an increased amount of DNA by decreasing the pH value. (f) Comparison of fluorescence spectra for supernatants used for UCNPs purification after DNA modification at low pH value (black curve) and after silica coating (red curve). Scale bar: 100 nm.

Therefore, we assume that in organic solvent (e. g. cyclohexane), only the hydrophobic facets of UCNPs exhibit affinity to the silica precursor TEOS to form solid silica layers (Figure 2a and b). To verify this hypothesis, Stöber method that applies ethanol as the reaction medium for silica coating onto UCNPs with hydrophilic surfaces[14, 15, 29] is conducted on the DNA modified UCNPs. Just as we assumed, in amphiphilic solvent ethanol, both hydrophilic (001) facets and hydrophobic (100)/(010) facets of DNA modified UCNPs are affined to TEOS. Thus, silica shells form all through the surface of UCNPs (Figure 2c and d). We further use DNA molecules with phosphate groups modified on the 5' terminus (P-DNA) to replace the organic surfactant molecules on both (001) and (100)/(010) facets and proceed micro emulsion method for silica coating on the totally hydrophilic UCNPs. As shown in Figure 2e and f, the hydrophilic DNA molecules on the surface of UCNPs prevent the deposition of TEOS precursors, therefore no silica layers are formed on the P-DNA modified UCNPs. In conclusion, in the organic solvent cyclohexane, silica layers cannot form onto the UCNPs' facets that are covered by DNA molecules, leading to anisotropic silica structures. Notably, in this work, dumbbell-like UCNPs with round edges are applied for the investigation of site-specific silica coating mechanism. This highlights the feasibility of the selective silica coating technique.

As the source of silica, the amount of TEOS plays a vital role in the anisotropic coating. We increase the concentration of TEOS for 5 times (from 0.5‰ to 2.5‰) to coat silica onto the rod-shape UCNPs. TEM results show that compared with the control group (Figure 3a), a high concentration of TEOS generally leads to thicker silica layers on the (100)/(010) facets of UCNPs; meanwhile, many free silica spheres appear in the system (Figure 3b). However, the concentration of TEOS does not alter the result of anisotropic coating: the (001) facets are still uncoated. To confirm the selective silica coating, we add HCl into the above aqueous suspension of UCNPs, and the (001)

facets of UCNPs are etched due to the lack of silica protection (Figure 3c).

It has been proved that with the decreasing of pH, there is a dramatic increased amount of DNA molecules which can be physically inserted in between the organic surfactant molecules on the (100)/(010) facets of UCNPs[28]. To identify if the pH-incurred DNA molecules on the side facets would influence the site-specific silica coating, we proceed silica coating onto UCNPs which are pre-treated by dye (FITC) labelled DNA molecules under pH 5.5. After reaction, silica shells are found only on the (100)/(010) facets of UCNPs; meanwhile, a strong fluorescence signal is detected in the supernatant used for final product purification, suggesting that TEOS is still only affine to the (100)/(010) facets and further hydrolyses to form silica layers. This indicates that the DNA molecules physically absorbed on the (100)/(010) facets caused by low pH value would not influence the site-specific silica coating. Therefore, there is no need for the delicate control of the concentration of DNA, medium pH and the amount of TEOS to achieve UCNPs with anisotropic silica shells, guaranteeing the reproducibility of this approach.

Toward the bio-applications of UCNPs such as intracellular imaging, we test the fluorescence properties of the UCNPs before and after silica coating/site-specific silica coating at single nanoparticle level by using our home-made confocal microscope (Figure S2). As shown in Figure 4c and g, the fluorescence intensity of UCNPs after silica coating does not change significantly compared with that of the as-synthesized ones. Meanwhile, after DNA modification and facet-selective silica coating, the fluorescence intensity of UCNPs decreases slightly. Similarly, there is no significant change in the intensity ratio of the two main peaks at 560 nm and 660 nm. The slight change of the fluorescence intensity and band ratio could be attributed to the surface quenching effect[30, 31] on the un-coated (001) facets.

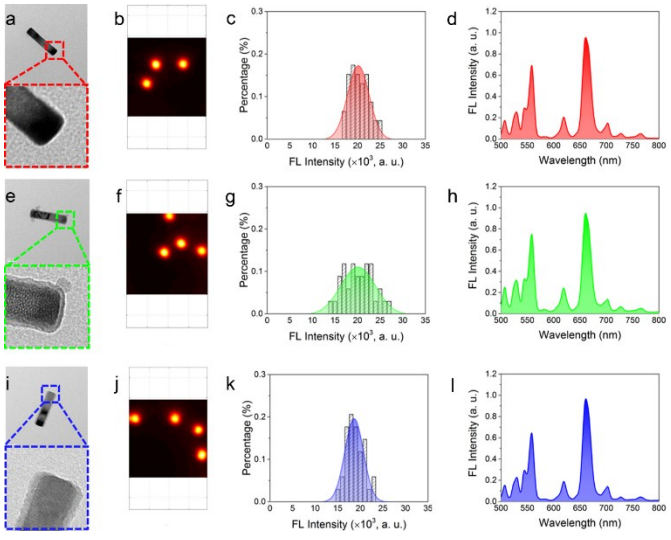

Fig. 4 (a, e and i) TEM images of three kinds of UCNPs: as-synthesized, silica coated, and site-specific silica coated. (b, f and j) Confocal microscopy fluorescence intensity measurement of the three kinds of UCNPs on single nanoparticle level. (c, g and K) Fluorescence intensity of UCNPs. (d, h and l) Calibrated fluorescence spectrum of the three samples to show the change of ratio for each emission bands.

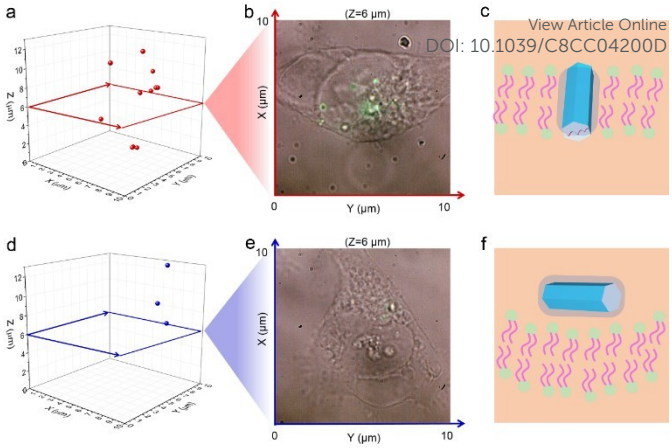

Fig. 5 3D scanning by our home-made microscope reveals the distribution of UCNPs of anisotropic silica shell (a) and whole silica shell (d) inside of two randomly selected cells. (b) and (e) are images of cells with UCNPs (green color dots) inside at Z=6 μm. (c) and (f) show the proposed mechanisms of cell uptake process of two kinds of UCNPs respectively.

The size[32], shape[33-35], and surface properties[36, 37] of nanoparticles would largely influence their cell uptake efficiencies. We find here that the anisotropic silica shells tailored by DNA molecules can also impact the UCNPs' delivery into cells. Figure 5 shows two typical cells treated with rod-shape UCNPs with silica shell and anisotropic silica shell respectively (more images can be found in supporting information). Calibrated by fluorescence intensity, equal amounts (0.03 μmol) of UCNPs with isotropic and anisotropic silica coating are added to cells (MDA-MB-468) and cultured for 3 hours. The UCNPs with anisotropic silica coating exhibit a high cell-uptake efficiency (Figure 5a) whereas the isotropic silica coated UCNPs can hardly penetrate inside cells within 3 hours (Figure 5d). According to our result, it is supposed that the anisotropic surface functionalization of DNA on the tips and silica shell on the lateral facets of rod-shape UCNPs, could lead to the orientation-selective uptake of UCNPs by the cells. Owing to the bio-compatible DNA molecules, cell uptake the rods by orienting the tips toward cell membrane. The silica coated lateral facets of UCNPs facilitate the cell uptake more smoothly towards the intercellular plasma environment.

Conclusions

In all, we suggest a new route in tailored silica coating onto nanoparticles. We have achieved selective silica coating by using DNA molecules which avoids complex chemical synthesis. The technique does not rely on the special morphologies of nanoparticles. Reproducible silica coating could be guaranteed by its facile manipulation that free from delicate control of reagent concentration, which promises this approach for scaling up synthesis. The increased cell uptake efficiency demonstrated in this work would also contribute to nanomedicine development and cell-nanomaterial interaction studies.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

1 X. Qian, X.-H. Peng, D. O. Ansari, Q. Yin-Goen, G. Z. Chen, D. M. Shin, L. Yang, A. N. Young, M. D. Wang and S. Nie, *Nature biotechnology*, 2008, **26**, 83.
2 J. Liu and Y. Lu, *Journal of the American Chemical Society*, 2003, **125**, 6642-6643.
3 P. D. Howes, R. Chandrawati and M. M. Stevens, *Science*, 2014, **346**, 1247390.
4 C. Liu, P. Zhang, X. Zhai, F. Tian, W. Li, J. Yang, Y. Liu, H. Wang, W. Wang and W. Liu, *Biomaterials*, 2012, **33**, 3604-3613.
5 D. Peer, J. M. Karp, S. Hong, O. C. Farokhzad, R. Margalit and R. Langer, *Nature nanotechnology*, 2007, **2**, 751-760.
6 C. Wang, L. Cheng and Z. Liu, *Biomaterials*, 2011, **32**, 1110-1120.
7 B. Yan, J.-C. Boyer, N. R. Branda and Y. Zhao, *Journal of the American Chemical Society*, 2011, **133**, 19714-19717.
8 S. Mura, J. Nicolas and P. Couvreur, *Nature materials*, 2013, **12**, 991.
9 S. Liu and M. Y. Han, *Chemistry–An Asian Journal*, 2010, **5**, 36-45.
10 J.-N. Liu, W.-B. Bu and J.-L. Shi, *Accounts of chemical research*, 2015, **48**, 1797-1805.
11 N. Wang, X. Yu, K. Zhang, C. A. Mirkin and J. Li, *J Am Chem Soc*, 2017.
12 B. Gu, M. Ye, L. Nie, Y. Fang, Z. Wang, X. Zhang, H. Zhang, Y. Zhou and Q. Zhang, *ACS Applied Materials & Interfaces*, 2018, **10**, 1028-1032.
13 Y. Cen, W.-J. Deng, Y. Yang, R.-Q. Yu and X. Chu, *Analytical Chemistry*, 2017, **89**, 10321-10328.
14 S. Han, A. Samanta, X. Xie, L. Huang, J. Peng, S. J. Park, D. B. L. Teh, Y. Choi, Y. T. Chang and Y. Yang, *Advanced Materials*, 2017, **29**.
15 Z. Li, Y. Zhang and S. Jiang, *Advanced Materials*, 2008, **20**, 4765-4769.
16 S. K. Pramanik, S. Sreedharan, H. Singh, C. Smythe, J. Thomas and A. Das, *Chemical Communications*, 2017.
17 J. Xu, F. He, Z. Cheng, R. Lv, Y. Dai, A. Gulzar, B. Liu, H. Bi, D. Yang and S. Gai, *Chemistry of Materials*, 2017.
18 L. Huang, Y. Zhao, H. Zhang, K. Huang, J. Yang and G. Han, *Angewandte Chemie International Edition*, 2017, **56**, 14400-14404.
19 S. Chen, A. Z. Weitemier, X. Zeng, L. He, X. Wang, Y. Tao, A. J. Y. Huang, Y. Hashimotodani, M. Kano, H. Iwasaki, L. K. Parajuli, S. Okabe, D. B. L. Teh, A. H. All, I. Tsutsui-Kimura, K. F. Tanaka, X. Liu and T. J. McHugh, *Science*, 2018, **359**, 679.
20 F. Wang, S. Cheng, Z. Bao and J. Wang, *Angew Chem Int Ed Engl*, 2013, **52**, 10344-10348.
21 X. Zhu, H. Jia, X. M. Zhu, S. Cheng, X. Zhuo, F. Qin, Z. Yang and J. Wang, *Advanced Functional Materials*, 2017, **27**.
22 S. F. Tan, U. Anand and U. Mirsaidov, *ACS nano*, 2017, **11**, 1633-1640.
23 J. G. Hinman, J. R. Eller, W. Lin, J. Li, J. Li and C. J. Murphy, *J Am Chem Soc*, 2017, **139**, 9851-9854. View Article Online DOI: 10.1039/C8CC04200D
24 D. Costa, H. D. Burrows and M. da Graça Miguel, *Langmuir*, 2005, **21**, 10492-10496.
25 L.-L. Li, P. Wu, K. Hwang and Y. Lu, *J Am Chem Soc*, 2013, **135**, 2411-2414.
26 J. Lu, Y. Chen, D. Liu, W. Ren, Y. Lu, Y. Shi, J. Piper, I. Paulsen and D. Jin, *Analytical chemistry*, 2015, **87**, 10406-10413.
27 D. Liu, X. Xu, Y. Du, X. Qin, Y. Zhang, C. Ma, S. Wen, W. Ren, E. M. Goldys and J. A. Piper, *Nature communications*, 2016, **7**, 10254.
28 W. Ren, S. Wen, S. A. Tawfik, Q. P. Su, G. Lin, L. A. Ju, M. J. Ford, H. Ghodke, A. M. van Oijen and D. Jin, *Chemical Science*, 2018, **9**, 4352-4358.
29 N. J. Johnson, N. M. Sangeetha, J.-C. Boyer and F. C. van Veggel, *Nanoscale*, 2010, **2**, 771-777.
30 F. Wang, J. Wang and X. Liu, *Angewandte Chemie*, 2010, **122**, 7618-7622.
31 N. Bogdan, F. Vetrone, G. A. Ozin and J. A. Capobianco, *Nano letters*, 2011, **11**, 835-840.
32 Y. Guo, E. Terazzi, R. Seemann, J. B. Fleury and V. A. Baulin, *Science Advances*, 2016, **2**.
33 C. Graf, D. Nordmeyer, C. Sengstock, S. Ahlberg, J. Diendorf, J. Raabe, M. Epple, M. Köller, J. Lademann, A. Vogt, F. Rancan and E. Rühl, *Langmuir*, 2018, **34**, 1506-1519.
34 Y. Li, M. Kroger and W. K. Liu, *Nanoscale*, 2015, **7**, 16631-16646.
35 E. Hinde, K. Thammasiraphop, H. T. Duong, J. Yeow, B. Karagoz, C. Boyer, J. J. Gooding and K. Gaus, *Nature nanotechnology*, 2017, **12**, 81-89.
36 D. Van Haute, A. T. Liu and J. M. Berlin, *ACS Nano*, 2018, **12**, 117-127.
37 X.-M. Zhu, C. Fang, H. Jia, Y. Huang, C. H. Cheng, C.-H. Ko, Z. Chen, J. Wang and Y.-X. J. Wang, *Nanoscale*, 2014, **6**, 11462-11472.

