DNA-controlled dynamic colloidal nanoparticle systems for mediating cellular interaction

Seiichi Ohta, Dylan Glancy, and Warren C. W. Chan

Version Post-Print/Accepted Manuscript

Citation (published version) S. Ohta, D. Glancy, "DNA-controlled Dynamic Colloidal Nanoparticle Systems for Mediating Cellular Interaction," *Science*, 2016 (Feb), 351, 841-845. JIF = 33.6

Publisher's Statement This is the author's version of the work. It is posted here by permission of the AAAS for personal use, not for redistribution. The definitive version was published in *Science* on 19 Feb 2016: Vol. 351, <http://dx.doi.org/10.1126/science.aad4925>.

How to cite TSpace items

Always cite the published version, so the author(s) will receive recognition through services that track citation counts, e.g. Scopus. If you need to cite the page number of the TSpace version (original manuscript or accepted manuscript) because you cannot access the published version, then cite the TSpace version **in addition to** the published version using the permanent URI (handle) found on the record page.

DNA-controlled dynamic colloidal nanoparticle systems for mediating cellular interaction

Authors: Seichi Ohta,^{1,2} Dylan Glancy,^{1,3} and Warren C. W. Chan^{1,3,4,5*}

Affiliations:

¹ Institute of Biomaterials and Biomedical Engineering, Donnelly Center for Cellular and Biomolecular Research, University of Toronto, 164 College St, Toronto, ON, M5S 3G9, Canada.

² Center for Disease Biology and Integrative Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan.

³ Department of Chemistry, University of Toronto, 80 St George Street, Toronto, Ontario M5S 3H6, Canada.

⁴ Department of Chemical Engineering, University of Toronto, 200 College Street, Toronto, Ontario M5S 3E5, Canada.

⁵ Department of Material Science and Engineering, University of Toronto, 160 College Street, Room 450, University of Toronto, Toronto M5S 3E1, Canada.

*Correspondence to: warren.chan@utoronto.ca

Abstract:

Precise control of biosystems requires developments of materials that can dynamically change physicochemical properties. Inspired by the ability of proteins to alter their conformation to mediate function, we explore the use of DNA as molecular keys to assemble and transform colloidal nanoparticle systems. The systems consist of a core nanoparticle surrounded by small satellites, the conformation of which can be transformed in response to DNA via a toe-hold displacement mechanism. The conformational changes can alter the optical properties and biological interactions of the assembled nanosystem. Photoluminescent signal is altered by changes in fluorophore-modified particle distance, whereas cellular targeting efficiency is increased 2.5 times by changing surface display of targeting ligands. These concepts provide strategies for engineering dynamic nanotechnology systems for navigating complex biological environments.

One Sentence Summary: The optical and biological properties of assembled nanosystems can morph and function dynamically like a protein by using DNA keys.

Main Text:

Fundamental studies on the interactions of nanoparticle designs with biomolecules, cells, tissues, and organs are providing guiding principles to build nanosystems for imaging diagnosing, and treating diseases. An optimal nanoparticle physicochemical property (e.g., sizes, shapes, and surface chemistries) for biological use varies with time and place within the living body and current nanoparticle designs do not have the engineering range to meet these design requirements (1-3). For example, rod-shaped particles are reported to be preferable for tumor penetration (4), while spherical nanoparticles are better for subsequent cellular uptake by cancer cells (5); nanoparticle PEGylation can increase blood circulation time by reducing serum protein adsorption and macrophage uptake (6), but can impede surface-coated antibodies from cell targeting (7). These variations have inspired the development of interactive nanoparticle systems that can alter their properties in response to biological stimuli (8-10). However, dynamic control over the physicochemical properties of nanoparticles, especially particle shape, remains a challenge. DNA provides exquisite control and flexibility in engineering the physicochemical, morphological, optical, and electrical properties of 3-D nanosystems (11-14). But these DNA-assembled structures have not been fully exploited for cellular and biological interaction. Here, we explore the use of DNA to assemble shape-shifting nanostructures with controlled biological function.

We used single-stranded DNA-functionalized gold nanoparticles (AuNPs) of different sizes (denoted as large, medium, and small) as building blocks to assemble shape-changing nanostructures (Fig. 1A). Each AuNP was functionalized with two DNA sequences - one for nanostructure assembly and the other for shape-changing (15). Valency of these nanoparticles were 114, 25, and 6 DNA strands on 13, 6, and 3 nm AuNPs, respectively (Figs. S1-2). The nanoparticle building blocks were assembled into ‘core-satellite’ structures (16, 17) by a linker DNA strand (L1 and L2) whose ends were complementary to two different nanoparticle types. The assembled nanostructure consists of a large core with surrounding satellites of medium and small size (assembly morphology 1 in Fig. 1). An important design feature here is that the linkage between the core and the small satellites contain a single-stranded spacer sequence (17 bases, shown in black in Fig. 1A), which, if precisely designed, can allow nanoparticles to change into different shapes via a ‘toehold strand displacement’ mechanism (18-20) to reconfigure the DNA assembly (Fig. 1A). An attaching strand, A1, was first added in order to anchor small satellites to the medium-sized nanoparticles under hybridization and purification conditions. No significant change in shape was observed by TEM at this step (denoted as “intermediate” structure hereafter). Then, L1_{comp}, a fully complementary detaching strand to the linker strand between the core and small satellites (L1), was added. Because the duplex formation with L1_{comp} is energetically favorable, L1 was displaced from the superstructure, resulting in the detachment of small satellites from the core. Through these two steps, small satellites were relocated from the core to the medium satellite, thereby transforming the nanoassemblies into a new shape whereby medium particles became the new core surrounded by small satellites (assembly morphology 2). Representative TEM images in Fig. 1B show the successful shape change of nanoassemblies mediated by DNA.

TEM images in Fig. 2A show the reversibility of the transforming nano-assemblies. The nanoassemblies first changed their assembled shape from morphology 1 to morphology 2 by the addition of A1 and L1_{comp} strands. Similarly, addition of L1 and A1_{comp} allowed the

nanoassemblies to revert back to their original shape. The low magnification TEM images (Fig. S3) and UV-vis absorbance (Fig. S4) showed the nanoassemblies are monodisperse and DLS measurements show hydrodynamic sizes of 94 and 74 nm for morphology 1 and 2, respectively (Fig. S5). These characterizations indicate that the shape-changing nanoassemblies are colloidally stable in PBS. We analyzed a larger population of nanoassemblies by TEM to determine the efficiency of the shape shift (Fig. 2A). We defined assembly morphology 1 and 2 by particle distance as illustrated in Fig. S6. The proximity of 3 nm (small) particles to either 6 nm (medium) or 13 nm (large) particles was measured for each nanoassembly to determine its conformation. Structures where satellites were concentrated near 3 nm particles were categorized as assembly morphology 1 while assemblies with 3 nm particles more closely associated with 6 nm cores were classified as morphology 2. Nanoassemblies that met neither of the above criteria were categorized as ‘other’. The analysis showed that conversion of morphology 1 to morphology 2 occurred at an efficiency of 89% and was capable of reverting back to morphology 1 at an efficiency of 88%. During these shape-changes, some of the satellites detach from the assembly due to imperfect anchoring; the average number of 6 nm particles on a nanoassembly of morphology 1, morphology 2, and the reversed morphology 1 was 1.8, 1.6, and 1.5, while that of 3 nm was 9.7, 6.6, and 5.6, respectively (Fig. S7). The shape change was further evaluated by two dimensional radial distribution function (RDF) analysis (21). RDF of satellites from a core particle was calculated from the TEM images (Fig. 2B). In morphology 1, a single peak around 16 nm was observed, indicating the isotropic position of 3 and 6 nm satellites with respect to the 13 nm core. On the other hand, broadened multiple peaks were observed in morphology 2, which reflected the new position of 3 nm satellites. This RDF reverted back to the single peak after adding L1 and A1_{comp} strands. These differences confirmed the reversible shape change of nanoassemblies. The shape change was not induced by the addition of DNA that has a non-complementary sequence (Fig. S8), suggesting specificity of this system. This shape-shifting property was not limited to a single design. By changing the linker stoichiometry, we successfully generated core-satellite structures with various numbers of 6 nm secondary cores (two to four) (Fig. S9). Using the same procedures, each of the base-structures could successfully change their shape (Fig. 2c).

We next determined whether the optical properties of the nanoassemblies could be controlled through morphological changes. The optical properties of a nanoparticle, such as fluorescence and surface plasmon resonance are sensitive to the location of neighboring particles. For example, changes in the distance of neighboring fluorescent nanoparticles can alter their Förster resonance energy transfer (FRET) signals(22, 23). Figure 3A describes the overall principle of our experiment. DNA strands on 13, 6, and 3 nm building block particles were end-labeled with FAM, Cy5, or Cy3, respectively, then coated onto the nanoparticle, and assembled. Given that the 3 nm particles are anchored to the 13 nm core in morphology 1, FAM and Cy3 are close enough to induce FRET. When we excite FAM, fluorescence from Cy3 can be observed. However, no FRET is observed by the excitation of Cy3 because the 3 and 6 nm particles are farther from one another. After shifting to the intermediate structure, FRET can be observed by the excitation of either FAM or Cy3 as 3 nm particles are anchored to both 13 and 6 nm particles. When the shape is finally shifted to morphology 2, FRET can be detected only by Cy3 excitation because 3 nm particles are now detached from the 13 nm core. Experimentally, DNA strands were fluorescently labeled according to supplier, coated onto the AuNPs, purified, and the photoluminescent spectra measured to verify successful coating (Fig. S10). Since NP1-NP6 strands were composed of 25 bases, which roughly corresponds to 5-10 kDa of linear polymer

chain, they are remote enough to prevent fluorescent quenching by AuNPs (24). Figs. 3B-C show photoluminescent spectra of the assembled nanostructures at each step of the shape-changing process. Following excitation of FAM, a prominent signal from Cy3 was observed at 561 nm for assembly morphology 1 and the intermediate structure (Fig. 3B). This signal was decreased after shifting to morphology 2. Following Cy3 excitation, the shift from morphology 1 to the intermediate shape was accompanied by the emergence of the signal from Cy5 at 665 nm, but the subsequent shift to morphology 2 did not affect the signal (Fig. 3C). These changes in FRET signals are consistent with the expected position of fluorophores (Fig. 3A), validating our proposed mechanism. Real time monitoring of the shape shift was also carried out by FRET measurement (Fig. 3D). A1 and L1_{comp} strands were sequentially added to the nanoassemblies in a hybridization buffer, during which the changes in fluorescent signals from Cy3 and Cy5, obtained by the excitation of FAM and Cy3 respectively, were measured in real-time at room temperature. The signals from Cy3 and Cy5 respond specifically to the addition of the attaching and detaching strands, illustrating the fidelity of the shape change. We found that each step of the transformation is predominantly complete within 10 min. Assuming first order reaction, the reaction rate constant for the first and the second step of the shape change was estimated to be 1.9×10^{-3} and $1.7 \times 10^{-3} \text{ s}^{-1}$, respectively (Fig. S11). These results not only confirm successful shape-shifting of the nanoassemblies, but also suggest possible applications as imaging probes that are responsive to specific DNA sequences.

Finally, we determined whether we could alter the cellular interaction of our nanoassemblies by changing their shape and thus, indirectly, changing the presentation of the surface chemistry. This is an important feature, as the optimal physicochemical properties to mediate transport in cells, tissues, and whole animals necessitate particles of different sizes, shapes, and surface chemistries (4-7). It is unlikely that current single-particle design systems will be able to fulfill these engineering requirements. Here, we control the presentation of targeting ligands on the surface of the nanoassembly system by changing its shape via DNA. We functionalized the core particle with folic acid (FA) as a targeting ligand for over-expressed folate receptor in many types of cancer cells (Fig. 4A) (25). In assembly morphology 1, FAs are surrounded by 3 and 6 nm satellites, which sterically hinder their targeting ability (OFF state). After transformation to morphology 2, “hidden” FAs are exposed to the outer environment via the relocation of 3 nm satellites, thereby activating the targeting property of the nanoassemblies (ON state).

The FA functionalization was achieved by concurrently conjugating FA-functionalized PEG (Mw = 5 kDa) terminated with thiol and DNA strands to 13 nm particles. The resulting grafting density was 40.0 ± 5.2 FA molecules per particle. The cellular uptake of the FA-functionalized nanoassemblies was examined using folate receptor-expressing U87-MG human glioblastoma-astrocytoma cells (25, 26). The cells were incubated with the nanoassemblies of morphology 1 or 2 in serum free media, as described in the procedures in the Supplementary Materials. Serum was not added to the media to avoid the interaction of non-specifically adsorbed serum proteins with nanoparticles and influencing cellular interaction (27). Fig. 4B shows TEM images of the nanoassemblies internalized by U87-MG cells. Nanoparticles were found within vesicles inside the cells, suggesting that they were internalized via endocytosis. Moreover, as we reported (28), the nanostructures were disassembled into their respective building blocks in the vesicles, whereas fully assembled nanostructures were still found in the culture media.

We quantified the amount of cellular uptake by ICP-MS. Nanoassemblies conjugated with the same amount of PEG but without FA functionalization were used as a control. ICP-MS

revealed that the cellular uptake of FA-conjugated nanoassemblies of morphology 2 was 2.5 times higher than that of morphology 1, indicating the ON/OFF switching of the targeting property. It was also found that the ON/OFF switching was moderately effective even without FA functionalization; the cellular uptake of morphology 2 was 1.5 fold higher than that of morphology 1 (Fig. 4D). We deduced that both shape and surface chemistry (i.e., type of DNA sequences exposed on the outer surface) of the non-targeted nanoassembly is responsible for this difference (Figs. S14-15). This effect is amplified by the FA conjugation. A competition experiment was conducted to confirm the contribution of FA functionalization in cellular uptake. Free FA was added to the culture media with the nanoassemblies (Fig. 4E). The ON/OFF ratio of the control nanoassemblies exhibited little change in the presence of free FA. Conversely, for FA-functionalized assemblies, the ON/OFF ratio significantly decreased with increasing free FA concentration toward control level, suggesting that the cellular uptake was indeed mediated by FA-folate receptor interaction. The results herein demonstrate that DNA-mediated shape change of the nanoassembly can alter the surface display of targeting ligands and thus control its cellular uptake property.

Some natural biomolecules, such as proteins, switch their function by changing their three-dimensional conformation in response to biological signals. This quality allows them to display varying conformations to activate functions or stay dormant until needed. Like these biomolecules, the nanoassemblies presented here alter their fluorescent and cellular uptake properties by transforming their structure in response to specific DNA sequences. A next step is to design the linker sequences to respond to circulating DNA or intracellular RNA sequences, to design the chemistry so that all building blocks reside within proximity to each other during the shape-shifting process, and to determine the impact of payload on shape-shifting property. Although further development is needed for their practical use, the concept presented here can be expanded to develop more complex systems that can mimic the diverse functions of a protein to selectively control the biological functions of the engineered nanosystem. Our proposed design strategy provides a new opportunity and general method to develop future dynamic nanomaterials that can travel through the blood stream and into diseased sites, transforming into the required shapes to overcome diverse biological barriers and, finally, activating their function to diagnose or treat targeted sites.

References and Notes:

1. W. Jiang, B. Y. Kim, J. T. Rutka, W. C. Chan, Nanoparticle-mediated cellular response is size-dependent. *Nat. Nanotechnol.* **3**, 145-150 (2008).
2. R. A. Petros, J. M. DeSimone, Strategies in the design of nanoparticles for therapeutic applications. *Nat. Rev. Drug Discov.* **9**, 615-627 (2010).
3. H. Cabral *et al.*, Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. *Nat. Nanotechnol.* **6**, 815-823 (2011).
4. V. P. Chauhan *et al.*, Fluorescent nanorods and nanospheres for real-time in vivo probing of nanoparticle shape-dependent tumor penetration. *Angew. Chem. Int. Ed.* **50**, 11417-11420 (2011).
5. B. D. Chithrani, A. A. Ghazani, W. C. Chan, Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Lett.* **6**, 662-668 (2006).
6. K. Knop, R. Hoogenboom, D. Fischer, U. S. Schubert, Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives. *Angew. Chem. Int. Ed.* **49**, 6288-6308 (2010).
7. Q. Dai, C. Walkey, W. C. Chan, Polyethylene glycol backfilling mitigates the negative impact of the protein corona on nanoparticle cell targeting. *Angew. Chem. Int. Ed.* **53**, 5093-5096 (2014).
8. C. Wong *et al.*, Multistage nanoparticle delivery system for deep penetration into tumor tissue. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 2426-2431 (2011).
9. G. von Maltzahn *et al.*, Nanoparticles that communicate in vivo to amplify tumour targeting. *Nat. Mater.* **10**, 545-552 (2011).
10. J. W. Yoo, S. Mitragotri, Polymer particles that switch shape in response to a stimulus. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 11205-11210 (2010).
11. M. R. Jones, N. C. Seeman, C. A. Mirkin, Nanomaterials. Programmable materials and the nature of the DNA bond. *Science* **347**, 1260901 (2015).
12. A. Kuzyk *et al.*, DNA-based self-assembly of chiral plasmonic nanostructures with tailored optical response. *Nature* **483**, 311-314 (2012).
13. Y. Zhang *et al.*, Selective transformations between nanoparticle superlattices via the reprogramming of DNA-mediated interactions. *Nat. Mater.* **14**, 840-847 (2015).
14. S. M. Douglas, I. Bachelet, G. M. Church, A logic-gated nanorobot for targeted transport of molecular payloads. *Science* **335**, 831-834 (2012).
15. X. Zhang, M. R. Servos, J. Liu, Instantaneous and quantitative functionalization of gold nanoparticles with thiolated DNA using a pH-assisted and surfactant-free route. *J. Am. Chem. Soc.* **134**, 7266-7269 (2012).
16. X. Xu, N. L. Rosi, Y. Wang, F. Huo, C. A. Mirkin, Asymmetric functionalization of gold nanoparticles with oligonucleotides. *J. Am. Chem. Soc.* **128**, 9286-9287 (2006).
17. J. H. Yoon, J. Lim, S. Yoon, Controlled assembly and plasmonic properties of asymmetric core-satellite nanoassemblies. *ACS Nano* **6**, 7199-7208 (2012).

18. D. Y. Zhang, G. Seelig, Dynamic DNA nanotechnology using strand-displacement reactions. *Nature Chem.* **3**, 103-113 (2011).
19. B. Yurke, A. J. Turberfield, A. P. Mills, Jr., F. C. Simmel, J. L. Neumann, A DNA-fuelled molecular machine made of DNA. *Nature* **406**, 605-608 (2000).
20. Y. Tian, C. Mao, Molecular gears: a pair of DNA circles continuously rolls against each other. *J. Am. Chem. Soc.* **126**, 11410-11411 (2004).
21. X. Yu *et al.*, Distance control in-between plasmonic nanoparticles via biological and polymeric spacers. *Nano Today* **8**, 480-493 (2013).
22. C. S. Yun *et al.*, Nanometal surface energy transfer in optical rulers, breaking the FRET barrier. *J. Am. Chem. Soc.* **127**, 3115-3119 (2005).
23. P. D. Howes, R. Chandrawati, M. M. Stevens, Bionanotechnology. Colloidal nanoparticles as advanced biological sensors. *Science* **346**, 1247390 (2014).
24. L. Y. Chou, W. C. Chan, Fluorescence-tagged gold nanoparticles for rapidly characterizing the size-dependent biodistribution in tumor models. *Adv. Healthc. Mater.* **1**, 714-721 (2012).
25. Z. Xu *et al.*, Folic acid conjugated mPEG-PEI600 as an efficient non-viral vector for targeted nucleic acid delivery. *Int. J. Pharm.* **426**, 182-192 (2012).
26. J. Zhu *et al.*, Targeted cancer theranostics using alpha-tocopheryl succinate-conjugated multifunctional dendrimer-entrapped gold nanoparticles. *Biomaterials* **35**, 7635-7646 (2014).
27. C. D. Walkey *et al.*, Protein corona fingerprinting predicts the cellular interaction of gold and silver nanoparticles. *ACS Nano* **8**, 2439-2455 (2014).
28. L. Y. Chou, K. Zagorovsky, W. C. Chan, DNA assembly of nanoparticle superstructures for controlled biological delivery and elimination. *Nat. Nanotechnol.* **9**, 148-155 (2014).
29. S. D. Perrault, W. C. Chan, Synthesis and surface modification of highly monodispersed, spherical gold nanoparticles of 50-200 nm. *J. Am. Chem. Soc.* **131**, 17042-17043 (2009).
30. K. Zhang *et al.*, Folate-mediated Cell Uptake of Shell-crosslinked Spheres and Cylinders. *J. Polym. Sci. Part A: Polym. Chem.* **46**, 7578-7583 (2008).
31. A. Albanese, K. M. Tsoi, W. C. Chan, Simultaneous quantification of cells and nanomaterials by inductive-coupled plasma techniques. *J. Lab. Autom.* **18**, 99-104 (2013).

Acknowledgments:

WCWC acknowledges the Natural Sciences and Engineering Research Council (NSERC) for support (2014-06397), Canadian Institute of Health Research (MOP-130143), and Prostate Cancer Canada (D2014-12). SO acknowledges the Japan Society for the Promotion of Science (JSPS) for a Research Fellowship (PD, No. 5621) and the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan for a Grant-in-Aid for Young Scientists (B) (No. 26820356). DG acknowledges NSERC for graduate fellowship. The authors thank the Advanced Bioimaging Centre at Mt Sinai Hospital for the use of the TEM and Toronto Nanofabrication

Center at University of Toronto for the use of ICP-MS. We also thank Dr. Leo Chou for interesting discussions.

Figure captions

Fig. 1 Schematic illustration and corresponding TEM images of the shape change of nanoparticle assemblies mediated by DNA. (A) Individual nanoparticles (large, medium, and small) were functionalized with strands of two unique single-stranded DNA sequences (NP1-NP6). By using linker DNA strands containing complementary sequence regions (L1 and L2), they were then assembled into the ‘core-satellite’ structure (assembly morphology 1). To change the shape of the nanoassembly, attaching strands (A1) were added to anchor small satellites to the medium particle (intermediate). After that, the detaching strand (L1_{comp}) was added to dislocate L1, resulting in the relocation of small satellites from the large core to the medium satellites (assembly morphology 2). This shape change can be reversed by adding extra attaching and detaching strands, L1 and A1_{comp}. (B) Representative TEM images of the nanoassemblies of morphology 1, intermediate, and morphology 2. They consist of 13, 6, and 3 nm gold nanoparticles. Orange and red circle indicates 13 and 6 nm particle, respectively. Scale bar is 20 nm.

Fig. 2 Reversibility and design versatility of the nanoassembly shape change. (A) TEM images and histograms of the nanoparticle assemblies at each shape-shifting step. Scale bar is 20 nm. The shape of the nanoassembly was categorized as assembly morphology 1, 2, and others by particle distance (see Fig. S6 for the definition) and then the proportion of each shape was analyzed from TEM images. > 100 assemblies were analyzed for each condition. (B) RDF analysis of nanoassemblies at each shape-shifting step. RDF of satellites from a core particle was analyzed from TEM images. For more details on the analytical procedure, see Supplementary Materials. (C) Shape shift of nanoassemblies with different number of 6 nm particles (two to four). Scale bar is 20 nm.

Fig. 3 Changes in FRET signals from the nanoparticle assemblies induced by shape change. (A) Schematic illustration of the FRET occurring in the nanoassemblies. In assembly morphology 1, FRET between FAM and Cy3 (FRET 1) can be observed by excitation of FAM, whereas FRET between Cy3 and Cy5 (FRET 2) does not occur by excitation of Cy3. After the shift to intermediate structure, FRET is observed between FAM and Cy3 and between Cy3 and Cy5. Then, in morphology 2, FRET can be detected only between Cy3 and Cy5. (B-C) Photoluminescent spectra from nanoassemblies (morphology 1, intermediate, and morphology 2). They are excited at (B) 480 nm for FAM or (C) 520 nm for Cy3. Each spectrum is normalized to the peak from FAM at 519 nm (B) or that from Cy3 at 561 nm (C), respectively. (D) Real time monitoring of FRET signals during the shape change. A1 and L1_{comp} DNA strands were added at 5 min and 60 min to the nanostructure solution, while FRET signals from Cy3 (FRET 1, excitation at 480 nm) and Cy5 (FRET 2, excitation at 520 nm) were monitored in real time.

Fig. 4 Altering cellular uptake properties of the nanoparticle assemblies by shape change. (A) In assembly morphology 1, FA is surrounded by satellite nanoparticles, which impedes its targeting property (“OFF” state). Then after the shift to morphology 2, the “hidden” FA is exposed to the outer environment, which activates the cellular uptake property of the assemblies (“ON” state).

(B-C) TEM images of the FA-functionalized nanoassemblies incubated with U87-MG cells. (B) and (C) represent the result of the nanoassembly internalization for morphology 1 and 2, respectively. In each panel, the left image shows the overall picture of a cell that internalized the assemblies. The right image (i) shows the enlarged images of its corresponding part of the overall picture. The nanoassemblies found in the culture media after the incubation were also shown. The scale bar is 2.0 μm in the low magnification images and 100 nm in enlarged images. For more enlarged images inside cells, see Fig. S12. For nanoassemblies without FA-functionalization, see Fig. S13. (D) Comparison of the amount of cellular uptake of the FA-functionalized nanoassemblies between morphology 1 and morphology 2 measured by ICP-MS. Nanoassemblies without FA functionalization were also used as control. Data are averages $\pm$ standard deviations ($n > 3$). (E) Effect of free FA on the ON/OFF switching ratio of the cellular uptake. The ON/OFF ratio was defined as the ratio of the amount of cellular uptake of the assemblies between morphology 2 and morphology 1 (Raw data is shown in Fig. S16). Data are averages $\pm$ standard deviations ($n > 3$). Statistical comparisons are shown as follows: ** $p < 0.01$, * $p < 0.05$, N.S: not significant.

Figure 1

Figure 2

Figure 3

Figure 4

Supplementary Materials:

Materials and Methods

Figures S1-S16

References (29-31)