Supplementary Material (ESI) for Chemical Communications

Supporting Information

Materials and Methods

(1) 6-nm TiO_2 nanoparticle preparation

The 6-nm TiO_2 particles (Figure S1) were prepared using a high-temperature pyrolysis reaction [1]. Triocytlphosphine oxide (TOPO, 5.0 g, 13.1 mmol)) was degassed at 150 °C for 10 min under vacuum to remove trace water and oxygen, followed by additional heating at 200 °C for 10 min under argon atmosphere. Titanium (IV) tetrabutoxide (0.681 mL, 2.0 mmol) were then added and heated at 200 °C for another 10 min. The temperature was increased to 320 °C with subsequent rapid injection of 0.275 mL (2.5 mmol) $TiCl_4$. This solution then underwent a condensation reaction at this temperature for 2 h with vigorous stirring. Finally, the solution was cooled down to 60°C and addition of excess acetone precipitated the TiO_2 nanocrystals. The precipitate was harvested by centrifugation and washed with acetone several times to remove excess TOPO. The TiO_2 nanocrystals were made water-soluble through ligand exchange of surface TOPO with polyacrylic acid (PAA) [2]. A solution of 0.1 g PAA in 8.0 ml diethylene glycol (DEG) was heated to 110 °C with vigorous stirring under a nitrogen atmosphere. TiO_2 nanocrystals (10.0 mg) in 1.0 ml toluene were injected into the PAA solution and heated to 240 °C for ~5 minutes and the milky solution turned clear. The solution was kept at 240 °C for another 6 hours, then cooled down to room temperature with subsequent addition of dilute HCl to precipitate the nanoparticles. The white TiO_2 powder was then washed three times with water, and finally dispersed in aqueous solution at pH = 6-8.

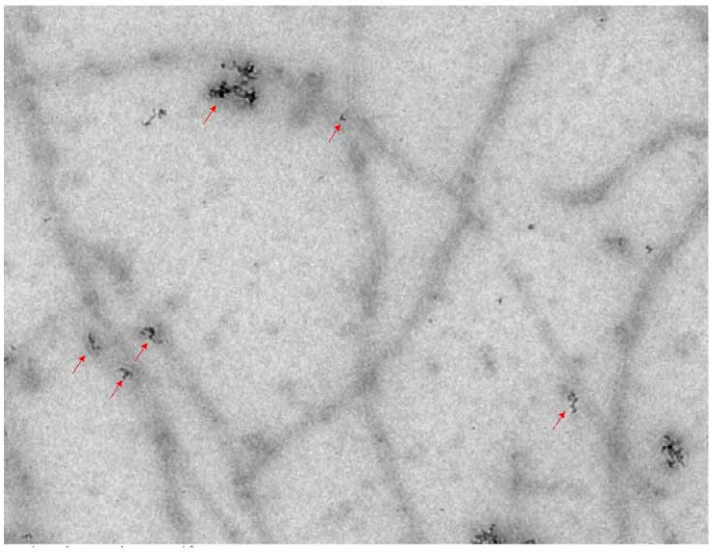
(2) 6-nm TiO_2-streptavidin conjugate preparation

The TiO_2-streptavidin conjugation was accomplished using a protocol which allows for sequential coupling without exposing the streptavidin to EDC, in essence negating the effect of EDC on the carboxyl groups of streptavidin. First, 10 µl of 0.1 M EDC and 15 µl of 0.1 M *N*-hydroxysulfosuccinimide (sulfo-NHS) were added to 1 mL of 5% PAA coated TiO_2 in water. The pH was then adjusted to 6.0 and the reaction stirred at room temperature for 30 minutes. The reaction solution was then eluted down a nap-5 column using a borate buffer at pH 8.0 to remove the derivatized TiO_2 from excess EDC and sulfo-NHS. 30 µl of streptavidin-Alexa Fluor 488 purchased from Invitrogen (2mg/ml) were then added to the derivatized TiO_2 and stirred for 2 hours at room temperature after which the reaction solution was centrifuged for 5 min in order to remove any streptavidin precipitate.

(3) TiO_2 labeling of microtubules

Microtubules (MTs) were polymerized by incubating 5mg/ml tubulin tagged with biotin in G-PEM buffer (80 mM PIPES, pH 6.8, 1 mM $MgCl_2$, 1 mM EGTA, 1 mM GTP), containing 30% glycerol, at 37 °C for 40 minutes. Paclitaxel was then added to a final concentration of 10 □M to stabilize the MT. TEM grids were incubated in 1% poly-L-lysine solution and allowed to sit for 15 minutes at room temperature, after which excess poly-L-lysine was removed with filter paper. The MT solution was diluted with G-PEM/paclitaxel solution to 1mg/ml and incubated with an equal amount of solution of

TiO_2-streptavidin (prepared as above) for 45 min. Approximately 2-4 µl of MT solution was spread over the entire surface of the coated TEM grids and incubated at room temperature for 10 minutes, after which excess solution was removed using filter paper. For light microscopy, the grids were viewed right away. For TEM and x-ray microscopy, the MT were fixed with 2% glutaraldehyde for 10 min, then washed with distilled water three times and dried overnight. The TEM image below shows several TiO_2 streptavidin conjugates labeled within the microtubulin structure.



(4) Microscopy measurement

Fluorescent light microscopy was done using a Zeiss AxioVert 200M fluorescence inverted microscope with a 40X 1.2NA water immersion lens and an AxioCam MRm CCD camera. TEM micrographs were obtained using a Tecnai G2 20 microscope operating at 200 kV with a LaB6 filament and S-TWIN objective lens. Selected area electron diffraction (SAED) was obtained on a JEOL 2010 TEM operating at 200 keV. Scanning transmission X-ray microscopy (STXM) was performed at beamline 5.3.2 at Advanced Light Source, Lawrence Berkeley National Laboratory.[3] A Fresnel zone plate with an outermost zone width of 25nm was used to focus the x-ray onto the sample.

References:

[1] J. J. Urban, W. S. Yun, Q. Gu, H. Park, *J. Am. Chem. Soc.* **2002**, *124*, 1186-1187.
[2] T. Zhang, J. Ge, Y. Hu, Y. Yin, *Nano Lett.* **2007**, *In press*.
[3] A. L. D. Kilcoyne, T. Tyliszczak, W. F. Steele, S. Fakra, P. Hitchcock, K. Franck, E. Anderson, B. Harteneck, E. G. Rightor, G. E. Mitchell, A. P. Hitchcock, L. Yang, T. Warwick, H. Ade, *Journal of Synchrotron Radiation* **2003**, *10*, 125-136.