

Programmed assembly of multi-layered protein/nanoparticle-carbon nanotube conjugates

Mei Li,^a Erik Dujardin^b and Stephen Mann^{a*}

^a Centre for Organized Matter Chemistry, School of Chemistry, University of Bristol, Bristol, UK BS8 1TS

*E-mail: s.mann@bristol.ac.uk

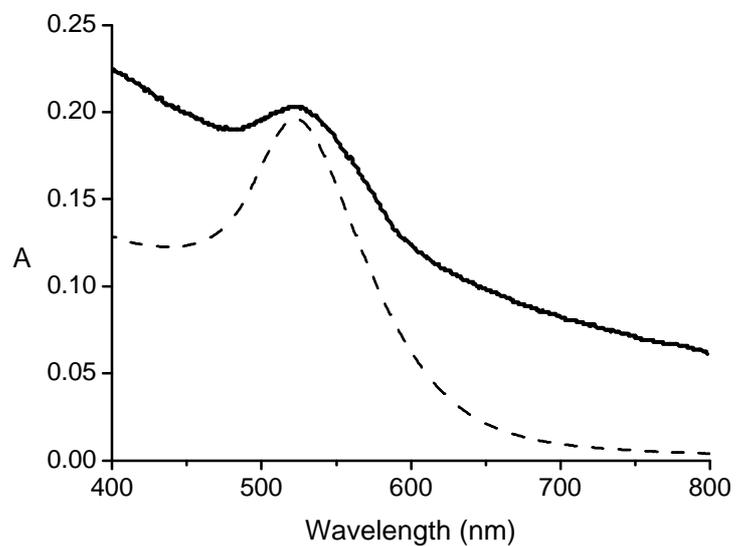
^b NanoSciences group, CEMES-CNRS UPR 8011, 29 rue J. Marvig, 31055 Toulouse Cedex 4, France

Preparation methods for *bFn*

Samples of multi-walled CNTs were prepared by chemical vapor deposition (CVD) and kindly provided by Dr D. Su (Fritz Haber Institute of the Max Planck Society, Berlin, Germany). The dry samples were dispersed in diluted HNO₃ overnight, washed three times with Milli-Q water, and then stored in water at room temperature. Biotinylated horse spleen ferritin (*bFn*) was synthesized by nucleophilic coupling of a water soluble succinimidyl derivative of biotin (sulfosuccinimidyl-6-biotinamido hexanoate, Vector Laboratories, UK) to exposed lysine residues on the surface of ferritin (Mr~500 kDa, 11.4 wt% iron, Sigma, UK) according to standard procedures.

Approximately 60 to 70 lysine residues per molecule were available for biotin coupling. 20 µL of a CNT suspension (~0.5 mg/mL) was added to 500 µL HEPES buffer (100 mM, [*N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid)], pH 8.5) and sonicated for 5 minutes. 50 µL of *bFn* (1.5 mg/mL in HEPES buffer) was added quickly and vortexed. The mixture was placed in a water bath at 70 °C for 5 minutes and then put on ice immediately, and stored in the fridge at 4 °C overnight. Non-CNT-bound *bFn* was removed by three washing cycles, involving centrifugation at 13.2 krpm for 20 minutes and redispersion in 500 µL HEPES buffer to produce a suspension of *bFn*-[CNT] nanoconjugates.

Samples for transmission electron microscopy (TEM) were air-dried at room temperature onto carbon-coated, 3 mm-diameter, copper electron microscope grids, and analysed in bright field mode. Optical spectra were recorded on dispersed samples using a PerkinElmer Lambda 25 UV/VIS spectrometer.



Supplementary data, S1: UV-vis absorption spectra of Au nanoparticle sol (control, dashed line), and conjugated Au nanoparticles in [Au]-DNAb-SA-bFn-[CNT] nanostructure (full line).

M. Li *et al*

Supplementary data, S2. TEM image showing disassembly of Au nanoparticles after addition of excess biotin to a dispersion of [Au]-DNA b -SA- b Fn-[CNT] conjugates. Note that the CNT-attached ferritin molecules remain strongly adsorbed. Scale bar = 100 nm

