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ARTICLE TYPE

Surface Functionalized Silver Nanoparticle Conjugate: Demonstration of Uptake and Release of a Phototherapeutic Porphyrin Dye

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Materials and Methods

Cucurbit[7]uril, CB7, was synthesized by following reported procedure.¹ From the mixture of CB homologues, CB7 was separated out by repeated precipitation using acetone/water (8:2 v/v) mixture and the purity of the sample was tested by NMR and other optical spectroscopic methods. AgNO₃ and NaBH₄ were obtained from Chemco Fine Chemicals, India and Sisco Research Laboratory (SRL), India and were of pure grades. Nanopure water (conductivity of 0.06 μS cm⁻¹), obtained from a Millipore Gradient Elix-3/A10 system was used to prepare the sample solutions.

Synthetic procedure for silver nanoparticles and the CB7-AgNP conjugates:

²⁰ **Type I particles:** Aqueous solutions of silver nitrate (AgNO₃) (200 μl, 2 mM) and sodium borohydride (NaBH₄) (200 μl, 12 mM) was added drop wise simultaneously to water (1 ml) with stirring at room temperature.² The resultant golden yellow solution was stirred for 10 minutes and diluted with water to a total volume of 2 ml.

²⁵ **Type II particles:** Silver nanoparticle solution was prepared by the above procedure. This was added to ~600 μl, 2 mM CB7 solution with stirring and made up to a total volume of 2 ml. Reverse addition of CB7 to the Type I solution was also attempted, however, the solution immediately turned dark pink and precipitated, probably due to the higher nanoparticle concentration as compared to the host CB7.

³⁰ **Type III particles:** An aqueous solution of silver nitrate (200 μl, 2 mM) was added to CB7 solution (600 μl, 2 mM) in water and the mixture was stirred for 15 minutes. Aqueous sodium borohydride (200 μl, 12mM) was added drop wise to the above mixture with stirring at room temperature. Stirring was continued for another 15 minutes and the resultant brownish yellow solution was diluted with water to total volume of 2 ml.

³⁵ Note that after the preparation, the presence of uncomplexed CB7 in solution was removed by repeated centrifugation, precipitation of NP and re-dispersion in case of Type II & Type III particles. In these cases, the initially obtained solution was centrifuged at 10000 rpm for 10 minutes. The precipitate was re-dispersed in 2 ml de-ionized water and again centrifuged at 10000 rpm for 5 minutes and the nanoparticles were redispersed in water for further

measurements. The presence of CB7 moieties on the AgNP surface was verified from the changes in the zeta potential values (see Table S1), which also indicate the increased number of CB7 on the Type III surface. Care has been taken to make sure that during NaBH₄ reduction, the characteristics of the CB7 moieties are not affected as verified from the FT-IR measurements (Fig. S5, ESI).

Absorption spectra were recorded with a Shimadzu 160 A UV-vis spectrophotometer (Kyoto, Japan). Appromate concentrations of these nanoparticles were estimated by using the molar extinction coefficient (ϵ) at 380 nm as $\sim 2 \times 10^4$ M⁻¹cm⁻¹.³ Steady-state fluorescence spectra were recorded using a Hitachi F-4500 spectrofluorimeter (Tokyo, Japan). The time-resolved (TR) fluorescence measurements were carried out using a time-correlated-single-photon-counting (TCSPC) spectrometer (Horiba Jobin Yvon IBH, UK). In the present work, a 374 nm diode laser (~100 ps, 1 MHz repetition rate) was used for sample excitation and an MCP-PMT was used for fluorescence detection. Transmission electron microscopic (TEM) measurements, both bright field low magnification and lattice imaging, were performed using 200 keV electrons in JEOL 2010 UHR TEM microscope. Atomic force microscopic (AFM) images were recorded in semi-contact mode using a NT-MDT solver model instrument with 50 μm scanner head and silicon nitride tip. FTIR spectra were recorded using a Bomem MB102 FTIR machine. A drop of the dispersed sample solution was dried over CaF₂ plate and mounted inside the sample chamber of the FTIR instrument, prior to the recording of FTIR spectrum. Zeta potentials were determined with a Nanosizer Z (Malvern Instruments, Malvern, UK) by phase analysis light scattering. The light source was He-Ne laser operated at 633 nm operating at 4 mW. The zetapotential (ζ) values were calculated from the electrophoretic mobility data using Smoluchowsky approximation. The experiment was carried out using a quartz cuvette (universal 'dip' cell) with 10 mm light pathway.

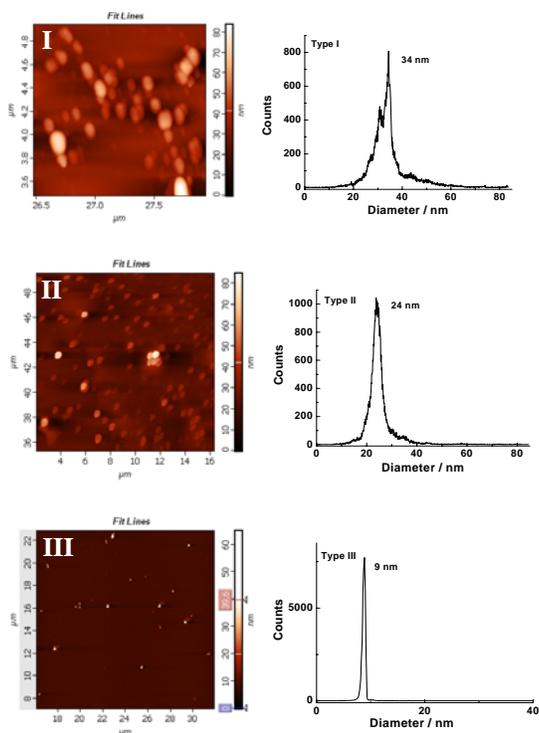


Figure S1: The AFM images of the Type I, Type II and Type III silver nanoparticles. The corresponding particle size distributions are shown along the side panels.

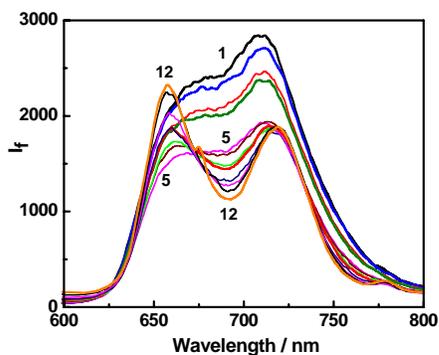


Figure S2: Fluorescence spectral changes recorded for the TMPyP (~0.5 μM) on gradual addition of Type II nanoconjugate. Spectrum 1 corresponds to only TMPyP and spectrum 12 corresponds to the addition of ~10 μM of Type II nanoparticles.

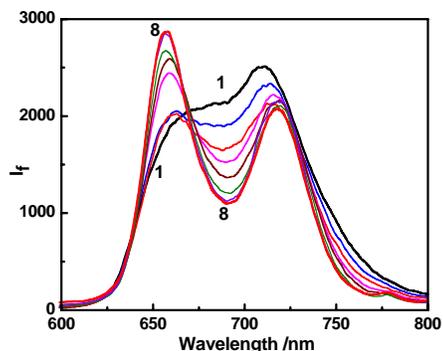


Figure S3: Fluorescence spectral changes recorded for the TMPyP (~0.5 μM) on gradual addition of Type III nanoconjugate. Spectrum 1 corresponds to only TMPyP and spectrum 8 corresponds to the addition of ~2 μM of Type III nanoparticles

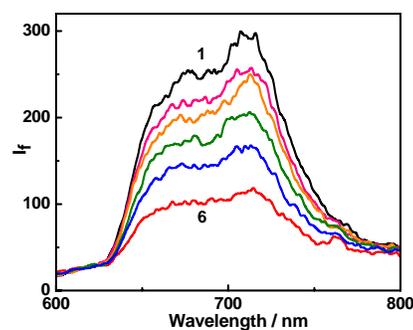


Figure S4: Fluorescence spectral changes recorded for the TMPyP (~0.5 μM) on gradual addition of Type I nanoconjugate. Spectrum 1 corresponds to only TMPyP and spectrum 6 corresponds to the addition of ~10 μM of Type I nanoparticles.

Table S1: Zeta potential values measured for Type I-III particles

System	Zeta potential (mV)
Type I	-36.5 ± 1.5
Type II	-3.0 ± 1.2
Type III	+22.2 ± 1.0

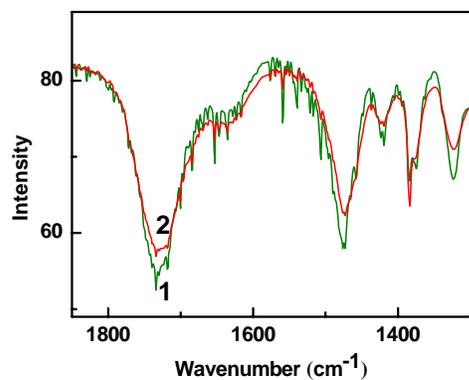


Figure S5: The IR spectra recorded for CB7; in free (1) and as attached to silver nanoparticles (2).

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