Electronic Supplementary Material (ESI) for RSC Advances

SUPPLEMENTARY SUPPORTING INFORMATION

Photomorphogenesis of γ-Globulin: Effect on Sequential Ordering and Knock Out of Gold Nanoparticles Array

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General experimental details and Figures S1-S7 showing TEM and SEM images, DLS and FTIR spectral data of the γ-globulin AuNPs synthesised in the presence and absence of irradiation and under different conditions.

EXPERIMENTAL DETAILS

Materials and methods. Chloroauric acid ($HAuCl_4$), and γ-globulin were purchased from Sigma Aldrich and were used as such without further purification. Doubly distilled water was used for all the studies. UV–Vis and fluorescence spectra were recorded in 1×1 cm quartz cuvettes. The electronic absorption spectra were recorded on a Shimadzu UV–VIS–NIR spectrophotometer.[1] Fluorescence spectra were recorded on a SPEX–Fluorolog F112X spectrofluorimeter.[2] CD spectra were obtained on a JASCO-J-810 Circular Dichroism Spectropolarimeter. The irradiation experiments were carried out in a Rayonet Photochemical Reactor (RPR 350 nm) containing four tubes. Solutions were irradiated in quartz tube and the temperature during the irradiation was maintained at 28 ± 5 °C.

Microscopic analyses of γ-Globulin-AuNPs. The samples for atomic force microscopic (AFM) image were prepared by drop casting solution on a freshly cleaved mica sheets at the required concentrations at ambient conditions. The AFM images were recorded under ambient conditions using a NTEGRA (NT-MDT) system and tapping mode regime. Micro-fabricated TiN cantilever tips (NSG10) with a resonance frequency of 299 kHz and a spring constant of 20-80 Nm^{-1} were used. The conductive atomic force studies have been done by using tapping mode to image protein-nano particle conjugate with a resonance frequency of 299 kHz and a spring constant of 20-80 Nm^{-1}. Micro-fabricated diamond coating was doped with nitrogen cantilever tips (DSP11) and employed an operator-activated external circuit to switch to contact mode for point contact electrical characterization of the selected positions. After completion of the measurement, we have deactivated the external circuit to prevent the damage of the protein fibres. Samples for imaging and electrical properties were prepared by drop casting the sample on HOPG at required concentrations at ambient conditions. For high resolution transmission electron microscope (HRTEM) studies, a drop of nanoparticle solution was placed on a carbon coated Cu grid and the solvent was allowed to evaporate at room temperature. Specimens were imaged on a JEOL 300 kV HRTEM instrument. For scanning electron microscope images (SEM) samples were drop casted and air dried on flat surface of cylindrical brass stubs and subjected to thin gold coating using JOEL JFC-1200 fine coater. The probe was inserted into Zeiss EVO 18 Cryo scanning electron

microscope for taking photographs. OPM images were observed using a Nikon HFX 35A Optiphot-2 polarized light optical microscope, equipped with a Linkam THMS 600.

Synthesis of gold nanoparticles. *Thermal synthesis of gold nanoparticles in presence of γ-globulin.* Aqueous solution of hydrogen tetrachloroaurate hydrate (2 mM) was mixed with different concentrations of γ-globulin (w/v = 0.1, 0.2 and 0.5%) and was heated at 60^0 C for 4 h (GLAu). The reaction was cooled to room temperature, filtered and ultracentrifuged to remove the unbound proteins. The nanoparticles synthesised were further characterized through AFM, SEM, TEM and DLS analyses.

Irradiation of $HAuCl_4$ in presence of γ-globulin. Aqueous solution of hydrogen tetrachloroaurate hydrate (2 mM) was mixed with different concentrations of γ-globulin (w/v = 0.1, 0.2 and 0.5%) and subjected to irradiation in quartz tubes at 350nm using a Rayonet photochemical reactor. The resulting colloidal solutions were characterized using AFM, SEM, TEM and DLS analyses.

Synthesis of gold nanoparticles on self assembled protein fibre by adjusting pH (GLAN). In a typical experiment, 2 mM aqueous solution of hydrogen tetrachloroaurate hydrate (99.999%) was mixed with 0.5% (w/v) of γ-globulin. NaOH solution (0.5 mL, 1 M) was added dropwise to the above mixture after 2 min with vigorous stirring and the mixture was irradiated using Rayonet instrument by using lamp at wavelength of 350 nm. The colour of the solution changed from light yellow to brown. The nanoparticles on the protein fibres synthesised were further characterized through AFM, SEM, TEM and OPM analyses at different time intervals.

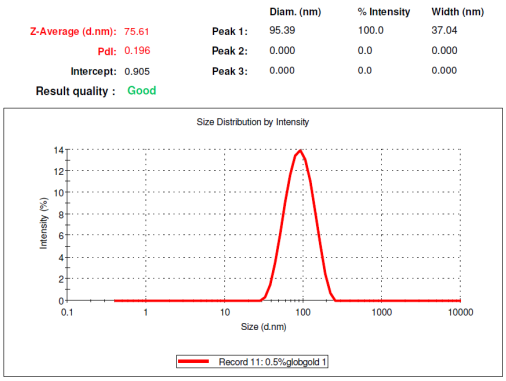

Figure S1. Dynamic light scattering data of 0.5% γ–globulin Au nanoparticles synthesised at 60^oC.

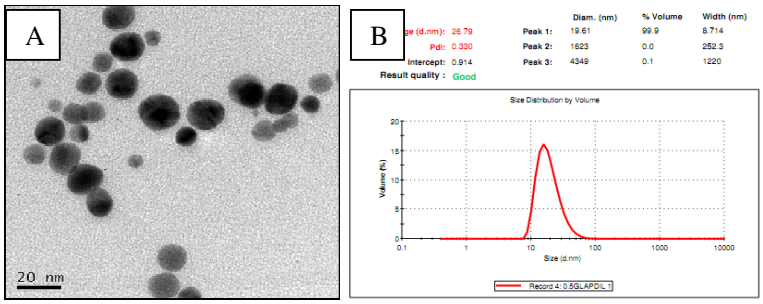

Figure S2. A) TEM images of 0.5% γ-globulin capped gold nanoparticle by irradiation at pH 7 with a light source of 350 nm B) DLS data of 0.5% γ–Globulin capped gold nanoparticles

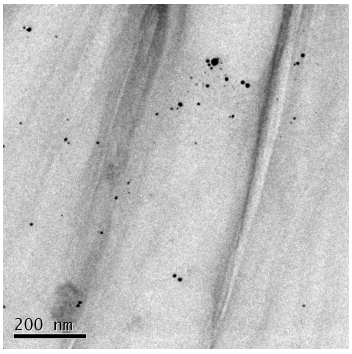

Figure S3. TEM image showing the growth of the protein fibres and the knocking out of the nanoparticles from the protein fibres when 0.5 GLAN was irradiated for 24 h with 350 nm light source.

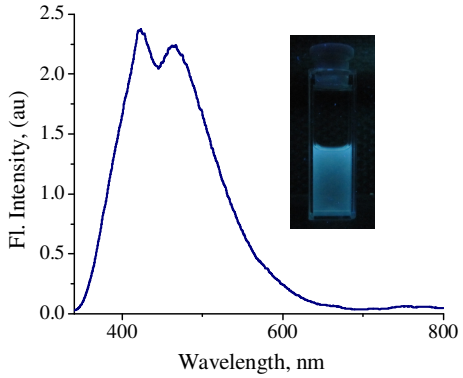

Figure S4. Fluorescence spectra of the γ-globulin protein fibres after the irradiation for 24 h using 350 nm light source in the absence of AuNPs. Inset shows the visual fluorescence of the protein alone.

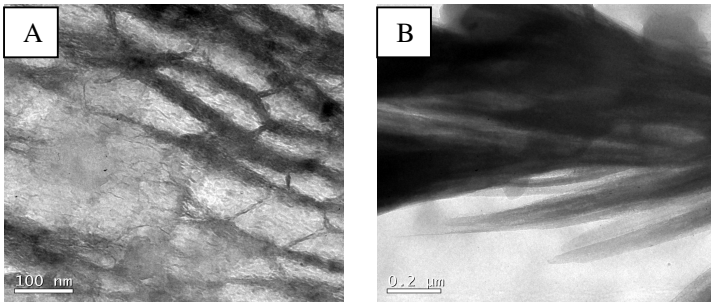

Figure S5. TEM images of the protein fibres in the absence of AuNPs, after A) 6 h and B) 24 h irradiation with 350 nm light source.

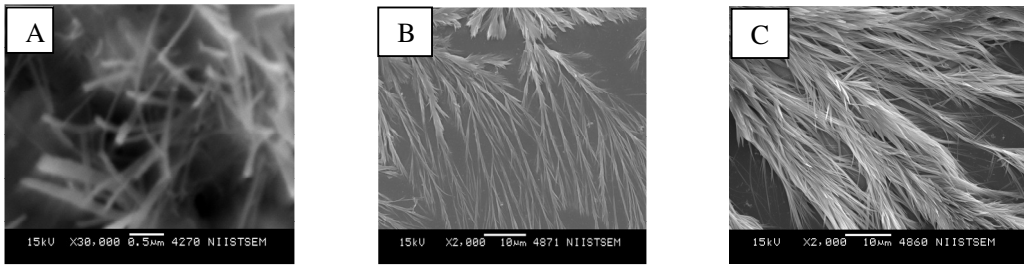

Figure S6. Time dependent SEM images showing the different stages of the growth of protein fibres after A) 3 B) 12 and C) 24 h of irradiation with 350 nm light source.

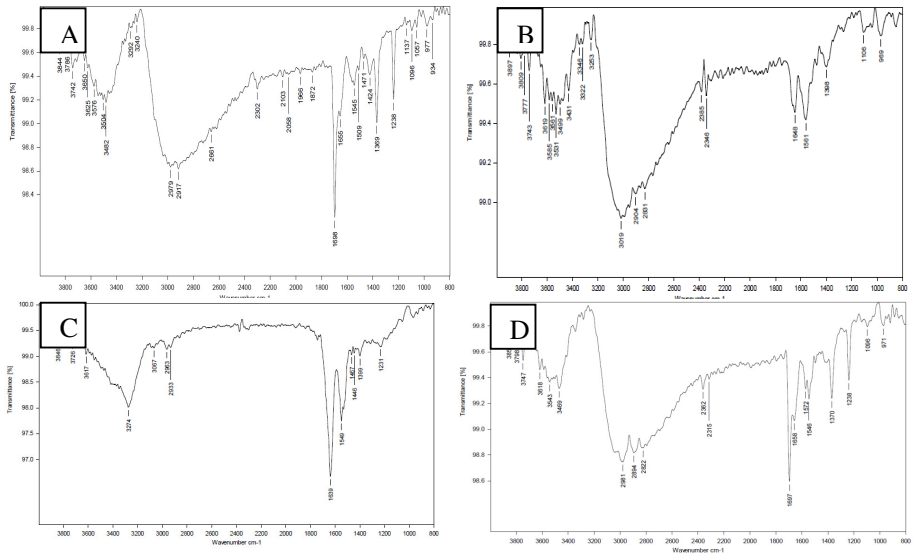

Figure S7. FTIR spectra of 0.5 % γ-globulin gold nanoparticle conjugate (0.5 GLAN) after irradiation A) 3 h, B) 24 h, C) protein alone and D) protein at pH 12 after 24 h irradiation with 350 nm light source.

Table S1. Data summarizing FTIR spectroscopic changes during the protein fibrillar growth.

Protein irradiation conditions*	**Amide I**	**Amide II**
Protein alone at pH 12	1639 cm^{-1}	1549 cm^{-1}
Protein alone at pH 12 after 24 h irradiation	1697 cm^{-1}	1546 cm^{-1}
0.5 GLAN* after 3 h irradiation	1698 cm^{-1}	1545 cm^{-1}
0.5 GLAN* after 24 h irradiation	1648 cm^{-1}	1561 cm^{-1}

*0.5 GLAN = 0.5 % γ-globulin gold nanoparticle conjugate, λ_{ex} = 350 nm.

References

[1] a) P. P. Neelakandan, M. Hariharan and D. Ramaiah, *J. Am. Chem. Soc.* 2006, **128,** 11334; b) P. P. Neelakandan and D. Ramaiah, *Angew. Chem. Int. Ed.* 2008, **47**, 8407.

[2] a) V. S. Jisha, K. T. Arun, M. Hariharan, and D. Ramaiah, *J. Phys. Chem. B* 2010, **114**, 5912; b) V. S. Jisha, K. T. Arun, M. Hariharan and D. Ramaiah, *J. Am. Chem. Soc.* 2006, **128**, 6024.