Supporting Information

Inhibition of glycation induced aggregation of human serum albumin by organic-inorganic hybrid nanocomposite of iron oxide functionalized nanocellulose

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Methods

Isolation of cellulose nanocrystals (CNCs). In brief, properly washed and dried crushed leaves of SC and needles of PR were bleached using 3% (w/v) acidified NaClO₂ solution for 3 h at 80 °C with continuous stirring. The material was then washed and treated again with acidified NaClO₂ solution for overnight at room temperature. Alkali treatment with each of 3% KOH and 5% KOH solution was given for 2 h at 80 °C under stirring. Again, the already treated fibers were dipped in NaClO₂ solution for 1 h at 80 °C under stirring. The fibers obtained were further acid hydrolyzed using 65% (v/v) H₂SO₄ for 1 h at 45 °C under constant stirring. The suspension was then diluted with DDW, centrifuged and dialyzed until complete removal of acidic contents were achieved. Thereafter, mechanical treatment was given for 5 min by high intensity ultrasound processor (Sonics, USA). The aqueous suspension of nanocellulose prepared from SC leaves and PR needles were abbreviated as SC-CNCs and PR-CNCs, respectively. The suspension was further characterized and then freeze dried to obtain dried form of CNCs.

Preparation of ED. In brief, EDTA (3 g) was added to round bottom flask containing 8.3 mL pyridine and 4.16 mL acetic anhydride. The mixture was heated at 65 °C under reflux and kept on stirring for 24 h under nitrogen (N₂) atmosphere. After the completion of reaction, the mixture was cooled to room temperature and vacuum filtered to obtain a solid product. The collected solid was washed repeatedly with diethyl ether and dried further in hot air oven at 60 °C to obtain white solid powder known as ED, which was characterized by ¹H-NMR, ¹³C-NMR and FTIR spectroscopy.

Chemical modification of CNCs with ED to form CNC-ED. For surface modifications of CNCs with ED, 100 mg of SC-CNCs and PR-CNCs were dispersed individually in 10 mL of dimethylformamide (DMF) and then 100 mg of ED was added to both the suspensions. The reaction mixture was kept under stirring at 75 °C for 24 h under reflux conditions. After that, the reaction mixture was filtered under reduced pressure. The collected product was washed several times with DMF, DDW and saturated solution of sodium bicarbonate followed by ethanol wash. After washing, the product was dried in hot air oven at 50 °C. The products were abbreviated SC-CNC-ED and PR-CNC-ED, respectively.

Characterization of CNCs and developed nanocomposites. The surface topography of CNCs and the developed nanocomposites were observed by scanning electron microscopy (SEM; Hitachi S-3400 N, Japan) at different steps during their formation. Transmission electron
microscopy (TEM; Tecnai, Twin 200 KV, FEI, Netherlands) was used to analyze the morphology and particle size of all the samples. TEM-EDX was also performed to know the elemental composition of nanocomposites. The particle size distribution histograms were prepared by using ImageJ software via calculating the size of a large number of NPs by analyzing TEM images. Water uptake (%) capacity of both the SC-CNCs and PR-CNCs was measured as a function of immersing time according to the method detailed in our previous publication. SEM was also used to depict the porous nature of CNCs and nanocomposite films.

The aqueous suspensions of CNCs and NCs were characterized by zeta potential (Zetasizer Nano ZS, Malvern Instruments Ltd.) for surface charge estimations. The presence of particular functional groups was detected by Fourier transform infrared spectroscopy (FTIR, Shimadzu) carried out in the range between 4000-400 cm\(^{-1}\). The amount of Fe present in nanocomposites was determined by flame atomic absorption spectrometry (AAS; Shimadzu AA-6300).

**Thioflavin T (ThT) fluorescence assay to monitor inhibition and/or dissociation of protein aggregation.** To monitor ThT fluorescence of nanocomposites treated or untreated HSA aggregates, aliquots were drawn from each set and mixed with ThT stock solution (2 mM in Tris-HCl, pH-7.0). In the final set, the concentrations of protein and dye were kept at 4 μM and 20 μM, respectively by diluting with Tris-HCl. After ThT addition, samples were incubated for 1 h at 37 °C and then scanned using Varian Carry Eclipse Fluorescence Spectrophotometer. The fluorescence intensity was measured at excitation wavelength of 420 nm, keeping both the slit widths at 10 nm and the emission spectra were recorded between 440-600 nm (\(\lambda_{\text{max}} \sim 480 \text{ nm}\)).

The inhibition and dissociation experiments were further studied at five different time intervals viz., 0, 6, 12, 18 and 24 h using SC-NC-500 and PR-NC-500 along with other controls.

**Congo Red binding assay.** To study the potential of the developed nanocomposites to act as effective inhibiting or dissociating agents against HSA fibril formation, Congo Red binding assay was performed. The incubated set of each sample, with and without the nanocomposites was diluted using Tris-HCl (pH-7.0) and then mixed with Congo Red (2 mM stock prepared in Tris-HCl). The concentration of protein and dye in the final working solution was kept at 4 μM and 10 μM, respectively. The optical density of the samples was measured in the wavelength range of 200-800 nm using Nanodrop (UV-vis spectrophotometer; ND-2000). The Congo Red binding assay of inhibition and dissociation experiments were further studied at five different
time intervals viz., 0, 6, 12, 18 and 24 h using SC-NC-500 and PR-NC-500 along with other controls.

**Transmission electron microscopy (TEM).** For TEM analysis of protein fibrils, the protein in each set of incubated sample was diluted to a concentration of 4 μM and ~ 5 μl sample was placed on 300 mesh copper grid and allowed to dry for 5 min. Then, the grid was negatively stained with 2% aqueous uranyl acetate solution. After that, extra amount of stain was blotted and the grid was washed properly with DDW. The grids were examined under TEM at an accelerating voltage of 200 kV to acquire images at desired magnification.

**Fluorescence microscopy imaging of aggregated proteins.** For imaging, 10 μl of incubated HSA solutions were mixed with 5 μl of ThT. The sample was placed on the glass slide and covered with coverslip. The images were captured using Axio-Zeiss Imager M1 microscope equipped with a fluorescence attachment using a GFP filter.

**Protein intrinsic or tryptophan (Trp) fluorescence.** The intrinsic fluorescence of HSA originates of tryptophan (Trp) show changes in intrinsic fluorescence property resulting from unfolding of the native protein structure. To measure intrinsic fluorescence, incubated HSA protein samples were diluted with Tris-HCl (pH-7.0) to maintain the final concentrations of proteins at 4 μM. The samples were then excited at 290 nm keeping both the slit widths at 5.0 nm (λ max ~ 350 nm). The fluorescence emission spectra were acquired in the wavelength range of 300-400 nm. The protein intrinsic or tryptophan fluorescence of inhibition and dissociation experiments were further studied at five different time intervals viz., 0, 6, 12, 18 and 24 h using SC-NC-500 and PR-NC-500 along with others controls.

**8-Anilinonaphthalene-1-sulfonic acid (ANS) fluorescence.** Aliquots of HSA aggregates with or without the presence of nanocomposites were mixed with ANS stock solution (2 mM in Tris-HCl, pH-7.0), by maintaining the final concentration of protein and dye at 4 μM and 20 μM, respectively. The samples were incubated for 1 h at 37 °C. The fluorescence intensity of ANS was measured at an excitation wavelength of 370 nm and the data were collected between 380-600 nm (λmax ~ 485 nm). The ANS fluorescence studies of inhibition and dissociation experiments were further studied at five different time intervals viz., 0, 6, 12, 18 and 24 h using SC-NC-500 and PR-NC-500 along with respective controls.

**Circular dichroism spectroscopy.** To observe the changes in HSA secondary structure after aggregation experiment, in the presence or absence of nanocomposites, each set of incubated
proteins was diluted with Tris-HCl (pH-7.0). Far-UV (190-260 nm) CD measurements were carried out using J-810 spectropolarimeter (JASCO, Japan) in quartz cuvette with a path length of 0.1 cm at room temperature. CD measurements were expressed in terms of mean residue ellipticity (MRE) in deg.cm².dm mol⁻¹ using equation 1.

\[
MRE = \frac{\text{Observed CD}}{C_p \cdot n \cdot l \times 10}
\]

where \(C_p\) is the molar concentration of proteins, \(n\) is the number of amino acid residues (585) present in HSA and \(l\) is the path length of cell (cm). The CD studies of inhibition and dissociation experiments were further studied at five different time intervals viz., 0, 6, 12, 18 and 24 h using SC-NC-500 and PR-NC-500 along with respective controls.

**Native polyacrylamide gel electrophoresis (Native-PAGE) of nanocomposite treated protein aggregates.** Bradford assay was performed on nanocomposite treated protein samples to quantify the amount of protein present in each sample. For quantification, 5 µl of protein solution from each set of incubated sample was incubated with 250 µl of Bradford reagent for 5 min in dark. Then absorbance was noted at 595 nm using microplate reader. The absorbance of protein was compared with standard curve prepared from pure HSA (0.0625-2.0 mg/mL), to calculate the amount of protein present in each sample. The concentration of proteins was kept same for all the cases (5 µg/µl). The aqueous dispersion of each set of incubated protein sample was electrophoresed using a Bio-Rad electrophoresis system. Each sample (20 µL) was mixed with 10 µl of sample loading buffer (50% glycerol, 0.1% bromophenol blue, and Tris-HCl at pH-6.8). A total of 25 µl of sample and 10 µl of protein marker were loaded individually into the wells for electrophoretic separation using 6% polyacrylamide. The gel was run at a constant voltage of 70 V for 5 h. After a suitable electrophoresis front was achieved, the gel was carefully removed from the plates and then stained with Coomassie Brilliant Blue dye for 2 h. The gel was then destained overnight and finally the gel was imaged using a gel documentation system.

**Native-PAGE of HSA present in the supernatant after magnetic separation of protein-nanocomposite complexes.** In another study, after the incubation of the nanocomposites with HSA, the samples were separated using a magnet. Then, the amount of proteins present in the supernatant was quantified by Bradford assay and loaded for Native-PAGE analysis (using 10% polyacrylamide) following same protocol as described above.
**Matrix Assisted Laser Desorption/Ionization–Time of flight (MALDI-TOF) studies.** For MALDI-TOF analysis, protein bands were isolated from the Native-PAGE using a fresh scalpel and transferred in 2 ml microcentrifuge tubes. The gel pieces were then washed with Milli-Q water (thrice, each time for 10 min). After that, these were destained using 100 mM NH₄HCO₃ in 50 % ACN and rehydrated in 10 mM NH₄HCO₃. Each gel band was then digested using 20 μl of sequencing grade modified trypsin and incubated overnight at 37 °C. To stop the reaction, 1 % TFA (in 80 % ACN) was added to each of the samples. After that, the samples were lyophilized for 24 h. The samples were then re-dissolved in 20-30 μl of 0.1 % TFA in 50 % ACN. Finally, 0.5 μl of each of the sample was taken in CHCA matrix and MALDI-TOF analysis was carried out (UltraflexTreme MALDI-TOF/TOF Mass Spectrometer, Bruker Corporation). A Mass standard starter kit (Bruker) and a standard tryptic BSA digest (Bruker) were used for MS and MS/MS calibrations and fine-tuning the resolution and sensitivity of the system. A combined search (MS and LIFT-MS/MS) was analyzed using MASCOT (Version 2.1, Matrix Science, London, UK) and searched against all entries in the SwissProt database, which contained accessible public protein sequences. All peptide masses were assumed monoisotopic and [M+H]⁺. The other parameters used for search were as follows: taxonomy, Homo sapiens, enzyme, trypsin, the fixed modification, carbamidomethyl (C), the variable modification, Glu-> pyro-Glu (N-term Q) and oxidation (M), parent ion mass tolerance at 50 ppm and MS/MS mass tolerance of 100 ppm and 1.2 Da, one missed cleavage allowed. The identified proteins had to meet the following criteria: (1) be among the top hits on the searching report, (2) individual ions scores indicate identity or extensive homology (p<0.05) and (3) more than two peptides should match. Also, to evaluate protein identification, the percentage of sequence coverage was considered.

**Esterase bio-activity assay.** To evaluate the retention of biological activity of nanocomposite treated albumin proteins, esterase activity was carried out. For this, the protein bands obtained from the supernatant solution of the magnetically separated samples were taken. After obtaining the Native-PAGE, the gel containing protein bands were cut using a sterile scalpel and put in 2ml centrifuge tubes. Similar procedure, as previously mentioned in MALDI studies was followed (washing, destaining and rehydration) for this assay. After overnight incubation, the samples were centrifuged at 12000 rpm for 10 min and the supernatant was removed carefully. The samples were again washed using 200 μl of Milli-Q water, centrifuged at 12000 rpm (twice for 5
min) and the supernatant was removed. A minimum volume (~150-200 µl) of tris-HCl (1.5 M, pH-8.8) was added to all the samples and incubated for 30 min (37 °C, 100 rpm). The sample gel pieces were sonicated for 2 min in an ice bath and kept for overnight incubation at 4 °C. All the samples were then centrifuged (14000 rpm, 20 min, 4 °C) and supernatants were collected in fresh tubes and finally Bradford assay was carried out for determination of the protein concentration of the samples and the esterase activity assay was performed. The assay procedure involved the incubation of extracted protein (~7.2 µM) from all the samples with p-nitrophenyl acetate (50 µM) in PBS (100 mM, pH-7.4) at 37°C for 1 h. The formation of p-nitrophenol was monitored via absorbance at 405 nm using UV-Vis spectrophotometer. The esterase activity of the HSA Native solution was taken as 100%, and activity of nanocomposite treated protein aggregates was compared accordingly.

**DPPH assay:** DPPH scavenging activity was performed according to previous reports. In brief, 100 µl of SC-NC and PR-NC of defined concentrations (100, 200, 500 and 800 µg/ml), IONP, SC-CNC, PR-CNC and HSA+G-Fibril treated SC-NC-500 and PR-NC-500 with respective control were added to 100 µl of 0.2 mM of DPPH prepared in methanol. After incubation for 1 h, absorbance was measured at 520 nm using a multiplate reader (Synergy Microplate Reader, Biotek). Different concentrations of ascorbic acid were taken as positive control. Scavenging activity percentage was calculated by equation (2);

\[
\text{Scavenging activity (\%) = } \frac{\text{Absorbance}_{\text{sample}} \cdot \text{Absorbance}_{\text{control}}}{\text{Absorbance}_{\text{control}}} \times 100
\]  

(2)

**Hemocompatibility Assay:** The isolation of the human blood was done according to the standard protocol approved by Institutional Ethics Committee (IHBT Project No.-IEC/IHBTP-5/Dec-2015). The human blood samples were collected from a healthy volunteer and preserved in trisodium citrate containing vials. The isolation was done by following standard procedure and 500 µl RBCs were used against each test sample. The hemolysis study for bare cellulose and iron oxide nanoparticles along with NCs was studied spectrophotometrically by measuring free hemoglobin (Hb) released from red blood cells (RBCs). First, 2mg of PR-CNC and SC-CNC and 0.4 mg of iron oxide nanoparticles (IONP) along with 1mg, 2mg, 5mg and 8mg of PR-NC and SC-NC were incubated in 500 µl PBS at 37 °C for 1 h. Next, 500 µl of these test samples were added to 500 µl of RBCs solution under aseptic condition. The RBCs treated samples were incubated in an incubator for 3 h under shaking condition (60 rpm) at 37 °C. After incubation,
intact RBCs were pelleted down by centrifugation (4000 rpm, 10 min) and 100 μl of the supernatant was used for recording the absorbance at 540 nm using plate reader. RBCs treated with phosphate buffer saline (0% lysis) and 1% Triton-X (100% lysis) were used as negative and positive control, respectively. Hemolysis percentage was calculated by equation (3);

\[
\text{Hemolysis (\%)} = \frac{(\text{Absorbance of test sample} - \text{Absorbance of negative control})}{(\text{Absorbance of positive control} - \text{Absorbance of negative control})}
\]  \hspace{1cm} (3)

The biconcave shape and morphology of erythrocytes after treatment with test samples were observed under SEM. For this, centrifuged RBCs were re-dispersed into 1 mL of PBS containing 500 μl 1% glutaraldehyde solution in PBS (pH-7.4). The fixed RBC samples were then centrifuged, and the pellets were re-dispersed in 1 mL of water. Finally, these were drop-casted on a double-sided carbon tape and coated with gold and imaged using SEM.
Figure S1. $^1$H-NMR spectra of EDTA (top) and ED (bottom).
ED spectra: $^1$H NMR (300 MHz, DMSO): $\delta$ 3.691 (s, 8H), 2.657 (s, 4H), 3.080 (s, DMSO), 2.496-2.488 (m, DMSO), which is in accordance with the characteristic peaks of H in ED.

Figure S2. $^{13}$C-NMR spectra of EDTA (top) and ED (bottom).
Figure S3. SEM images of SC and PR during different stages of chemical treatment for nanocellulose isolation.

Figure S4. SEM images of SC-CNC, PR-CNC, SC-CNC-ED, PR-CNC-ED, SC-NC and PR-NC.
Figure S5. SEM-EDS spectra of (a) SC-NCs and (b) PR-NCs; TEM-EDX spectra of (c) SC-NCs and (d) PR-NCs showing the peak of elemental Fe in nanocomposites.

Figure S6. Characterization of IONPs showing morphology and surface charge by (a) SEM image, (b) TEM image, (c) SEM-EDS, and (d) zeta potential studies.
Figure S7. SEM images showing presence of pores in SC-CNCs, SC-NCs, PR-CNCs and PR-NCs films.

Figure S8. Zeta potential measurements depicting the surface charge of (a) SC-CNCs, (b) PR-CNCs, (c) SC-CNC-ED, (d) PR-CNC-ED, (e) SC-NCs and (f) PR-NCs.
Figure S9. Digital camera photographs showing the magnetic nature of SC-NCs and PR-NCs in powder and suspension form.
**Figure S10.** FTIR spectra of (a) PR-CNC, (b) SC-CNC, (c) IONP, (d) EDTA, (e) ED, (f) PR-CNC-ED, (g) SC-CNC-ED, (h) PR-NC and (i) SC-NC. The peaks in the high frequency region split at 1813, 1759 and 1689 cm\(^{-1}\) in ED. The low frequency groups split at 1139, 1074, and 991 cm\(^{-1}\). Additionally, the bands at 1245 and 1400 cm\(^{-1}\), related to C–O and C–N stretching, respectively, were also evidence of the ED structure.
Figure S11. Comparison of fibril formation of HSA for native HSA (abbreviated as HSA fibril) and in presence of 0.1 M D-glucose (abbreviated as HSA+G-Fibril) by (a) ThT, (b) Congo Red, (c) ANS, (d) intrinsic fluorescence assay and (e) CD spectra. TEM images of (e) HSA +G-Fibril and (f) HSA fibril. The corresponding fluorescence microscopy images were represented by (h) and (i), respectively.
Figure S12. TEM micrographs of (a) HSA Fibril, (b) HSA+G+IONP, (c) HSA+G+SC-CNC for inhibition and the corresponding fluorescence images were given in (d), (e) and (f). Dissociation TEM micrographs of HSA+G+IONP (g), HSA+G+SC-CNC (h), HSA+G+PR-NC-500 (i), HSA+G+SC-NC-500 (j) and the corresponding fluorescence images were given in (k), (l), (m), and (n). The scale bar for TEM was 200 nm for all the cases whereas the scale was 20 μm for fluorescence images.
Table S1: Monitoring inhibition effect of nanocomposites on protein aggregation by CD studies (change in % α–helix and β–sheet content of protein at different concentrations).

| Sample          | % α–helix | % β–sheet |
|-----------------|-----------|-----------|
| HSA Native      | 28        | 4         |
| HSA Fibril      | 5         | 74        |
| HSA+G-Fibril    | 3         | 80        |
| HSA+G+IONP      | 6         | 62        |
| HSA+G+PR-CNC    | 12        | 58        |
| HSA+ G+SC-CNC   | 15        | 54        |
| HSA+ G+PR-NC-100| 17        | 49        |
| HSA+ G+PR-NC-200| 23        | 27        |
| HSA+ G+PR-NC-500| 25        | 8         |
| HSA+ G+PR-NC-800| 19        | 37        |
| HSA+ G+SC-NC-100| 14        | 45        |
| HSA+ G+SC-NC-200| 27        | 20        |
| HSA+ G+SC-NC-500| 24        | 5         |
| HSA+ G+SC-NC-800| 24        | 9         |

Table S2: Monitoring dissociating effect of nanocomposites on protein aggregation by CD studies (change in % α-helix and β-sheet content of protein at different concentrations).

| Sample          | % α–helix | % β–sheet |
|-----------------|-----------|-----------|
| HSA Native      | 34        | 4         |
| HSA Fibril      | 15        | 70        |
| HSA+G-Fibril    | 12        | 81        |
| HSA+G+IONP      | 6         | 63        |
| HSA+G+PR-CNC    | 12        | 58        |
| HSA+ G+SC-CNC   | 15        | 26        |
| HSA+ G+PR-NC-100| 17        | 27        |
| HSA+ G+PR-NC-200| 19        | 18        |
| HSA+ G+PR-NC-500| 24        | 9         |
| HSA+ G+PR-NC-800| 24        | 16        |
| HSA+ G+SC-NC-100| 21        | 17        |
| HSA+ G+SC-NC-200| 26        | 15        |
| HSA+ G+SC-NC-500| 27        | 6         |
| HSA+ G+SC-NC-800| 23        | 12        |
Figure S14. ThT fluorescence assay of native HSA, HSA+G-Fibril and SC-NC-500 and PR-NC-500 treated nanocomposite for inhibition at (a) 0, (b) 6, (c) 12, (d) 18 and (e) 24 h, respectively. Corresponding relative fluorescence intensity plots were given in (f) – (j), respectively.
Figure S15. ThT fluorescence assay of native HSA, HSA+G-Fibril and SC-NC-500 and PR-NC-500 treated nanocomposite for dissociation at (a) 0, (b) 6, (c) 12, (d) 18 and (e) 24 h, respectively. Corresponding relative fluorescence intensity plots were given in (f) – (j), respectively.
Figure S16. Congo Red assay of native HSA, HSA+G-Fibril and SC-NC-500 and PR-NC-500 treated nanocomposite for inhibition at (a) 0, (b) 6, (c) 12, (d) 18 and (e) 24 h, respectively.
Figure S17. Congo Red assay of native HSA, HSA+G-Fibril and SC-NC-500 and PR-NC-500 treated nanocomposite for dissociation at (a) 0, (b) 6, (c) 12, (d) 18 and (e) 24 h, respectively.
Figure S18. ANS assay of native HSA, HSA+G-Fibril and SC-NC-500 and PR-NC-500 treated nanocomposite for inhibition at (a) 0, (b) 6, (c) 12, (d) 18 and (e) 24 h, respectively. Corresponding relative fluorescence intensity plots were given in (f) – (j), respectively.
Figure S19. ANS assay of native HSA, HSA+G-Fibril and SC-NC-500 and PR-NC-500 treated nanocomposite for dissociation at (a) 0, (b) 6, (c) 12, (d) 18 and (e) 24 h, respectively. Corresponding relative fluorescence intensity plots were given in (f) – (j), respectively.
Figure S20. Protein intrinsic assay of native HSA, HSA+G-Fibril and SC-NC-500 and PR-NC-500 treated nanocomposite for inhibition at (a) 0, (b) 6, (c) 12, (d) 18 and (e) 24 h, respectively. Corresponding relative fluorescence intensity plots were given in (f) – (j), respectively.
Figure S21. Protein intrinsic assay of native HSA, HSA+G-Fibril and SC-NC-500 and PR-NC-500 treated nanocomposite for dissociation at (a) 0, (b) 6, (c) 12, (d) 18 and (e) 24 h, respectively. Corresponding relative fluorescence intensity plots were given in (f) – (j), respectively.
Figure S22. CD studies of native HSA, HSA+G-Fibril and SC-NC-500 and PR-NC-500 treated nanocomposite for inhibition at (a) 0, (b) 6, (c) 12, (d) 18 and (e) 24 h, respectively.
Table S3: Monitoring inhibition effect of nanocomposites on protein aggregation by CD studies (change in % α-helix and β-sheet content of protein at different time intervals).

| Sample (0 h)                  | % α-helix | % β-sheet |
|-------------------------------|-----------|-----------|
| HSA Native                    | 65        | 9         |
| HSA Fibril                    | 59        | 11        |
| HSA+G-Fibril                  | 51        | 13        |
| HSA+G+IONP                    | 56        | 12        |
| HSA+G+PR-CNC                  | 59        | 16        |
| HSA+G+SC-CNC                  | 61        | 13        |
| HSA+G+PR-NC-500               | 64        | 9         |
| HSA+G+SC-NC-500               | 62        | 7         |

| Sample (6 h)                  | % α-helix | % β-sheet |
|-------------------------------|-----------|-----------|
| HSA Native                    | 28        | 4         |
| HSA Fibril                    | 10        | 61        |
| HSA+G-Fibril                  | 2         | 69        |
| HSA+G+IONP                    | 3         | 58        |
| HSA+G+PR-CNC                  | 6         | 46        |
| HSA+G+SC-CNC                  | 3         | 45        |
| HSA+G+PR-NC-500               | 26        | 18        |
| HSA+G+SC-NC-500               | 26        | 14        |

| Sample (12 h)                 | % α-helix | % β-sheet |
|-------------------------------|-----------|-----------|
| HSA Native                    | 26        | 3         |
| HSA Fibril                    | 8         | 70        |
| HSA+G-Fibril                  | 6         | 80        |
| HSA+G+IONP                    | 30        | 70        |
| HSA+G+PR-CNC                  | 13        | 67        |
| HSA+G+SC-CNC                  | 14        | 55        |
| HSA+G+PR-NC-500               | 22        | 8         |
| HSA+G+SC-NC-500               | 24        | 7         |

| Sample (18 h)                 | % α-helix | % β-sheet |
|-------------------------------|-----------|-----------|
| HSA Native                    | 24        | 3         |
| HSA Fibril                    | 40        | 60        |
| HSA+G-Fibril                  | 7         | 74        |
| HSA+G+IONP                    | 5         | 75        |
| HSA+G+PR-CNC                  | 16        | 49        |
| HSA+G+SC-CNC                  | 22        | 30        |
| HSA+G+PR-NC-500               | 23        | 12        |
| HSA+G+SC-NC-500               | 24        | 10        |

| Sample (24 h)                 | % α-helix | % β-sheet |
|-------------------------------|-----------|-----------|
| HSA Native                    | 32        | 4         |
| HSA Fibril                    | 5         | 74        |
| HSA+G-Fibril                  | 3         | 80        |
| HSA+G+IONP                    | 6         | 62        |
| HSA+G+PR-CNC                  | 12        | 58        |
| HSA+G+SC-CNC                  | 15        | 54        |
| HSA+G+PR-NC-500               | 25        | 8         |
| HSA+G+SC-NC-500               | 24        | 5         |
Figure S23. CD studies of native HSA, HSA+G-Fibril and SC-NC-500 and PR-NC-500 treated nanocomposite for dissociation at (a) 0, (b) 6, (c) 12, (d) 18 and (e) 24 h, respectively.
Table S4: Monitoring dissociating effect of nanocomposites on protein aggregation by CD studies (change in % α-helix and β-sheet content of protein at different time intervals).

| Sample (0 h)          | % α-helix | % β-sheet |
|-----------------------|-----------|-----------|
| HSA Native            | 60        | 10        |
| HSA Fibril            | 0         | 95        |
| HSA+G-Fibril          | 0         | 100       |
| HSA+G+IONP            | 0         | 92        |
| HSA+G+PR-CNC          | 0         | 75        |
| HSA+G+SC-CNC          | 0         | 60        |
| HSA+G+PR-NC-500       | 2         | 52        |
| HSA+G+SC-NC-500       | 3         | 51        |
| Sample (6 h)          | % α-helix | % β-sheet |
| HSA Native            | 32        | 9         |
| HSA Fibril            | 0         | 77        |
| HSA+G-Fibril          | 0         | 80        |
| HSA+G+IONP            | 30        | 60        |
| HSA+G+PR-CNC          | 6         | 51        |
| HSA+G+SC-CNC          | 10        | 45        |
| HSA+G+PR-NC-500       | 25        | 10        |
| HSA+G+SC-NC-500       | 28        | 5         |
| Sample (12 h)         | % α-helix | % β-sheet |
| HSA Native            | 38        | 11        |
| HSA Fibril            | 17        | 67        |
| HSA+G-Fibril          | 12        | 93        |
| HSA+G+IONP            | 0         | 35        |
| HSA+G+PR-CNC          | 11        | 45        |
| HSA+G+SC-CNC          | 10        | 49        |
| HSA+G+PR-NC-500       | 35        | 18        |
| HSA+G+SC-NC-500       | 38        | 9         |
| Sample (18 h)         | % α-helix | % β-sheet |
| HSA Native            | 30        | 7         |
| HSA Fibril            | 27        | 36        |
| HSA+G-Fibril          | 20        | 43        |
| HSA+G+IONP            | 5         | 35        |
| HSA+G+PR-CNC          | 8         | 26        |
| HSA+G+SC-CNC          | 6         | 29        |
| HSA+G+PR-NC-500       | 23        | 12        |
| HSA+G+SC-NC-500       | 28        | 9         |
| Sample (24 h)         | % α-helix | % β-sheet |
| HSA Native            | 34        | 4         |
| HSA Fibril            | 15        | 70        |
| HSA+G-Fibril          | 12        | 81        |
| HSA+G+IONP            | 6         | 63        |
| HSA+G+PR-CNC          | 12        | 58        |
| HSA+G+SC-CNC          | 15        | 26        |
| HSA+G+PR-NC-500       | 24        | 9         |
| HSA+G+SC-NC-500       | 27        | 6         |
Table S5: Inhibition effect nanocomposite on different mass of protein aggregates identified using MALDI ToF/ToF mass spectrometry.

| Sample Name         | Score | Protein sequence coverage (%) | M. Mass (kDa) | No. of peptides |
|---------------------|-------|-------------------------------|---------------|-----------------|
| HSA +Native         | 38    | 43                            | 71.3          | 30              |
| HSA Fibril          | 35    | 26                            | 223.6         | 50              |
|                     | 34    | 19                            | 129.7         | 23              |
|                     | 34    | 26                            | 109.1         | 23              |
|                     | 42    | 23                            | 82.3          | 16              |
| HSA+G+Fibril        | 33    | 4                             | 205.8         | 13              |
|                     | 38    | 7                             | 193.3         | 20              |
|                     | 32    | 29                            | 117.2         | 31              |
|                     | 42    | 14                            | 97.4          | 18              |
| HSA+G+IONP          | 43    | 27                            | 181.5         | 32              |
|                     | 32    | 13                            | 123.7         | 25              |
|                     | 38    | 7                             | 153.3         | 20              |
|                     | 34    | 17                            | 70.1          | 15              |
| HSA+G+PR-CNC        | 36    | 14                            | 237.1         | 45              |
|                     | 37    | 32                            | 123.2         | 27              |
|                     | 37    | 31                            | 103.2         | 37              |
|                     | 39    | 21                            | 90.5          | 26              |
| HSA+G+SC-CNC        | 37    | 31                            | 206.1         | 52              |
|                     | 41    | 36                            | 121.7         | 34              |
|                     | 43    | 14                            | 103.5         | 22              |
|                     | 38    | 13                            | 90.1          | 20              |
| HSA+G+PR-NC-500     | 34    | 10                            | 176.6         | 18              |
|                     | 42    | 17                            | 115.4         | 21              |
|                     | 40    | 11                            | 109.1         | 10              |
|                     | 36    | 13                            | 106.9         | 19              |
| HSA+G+SC-NC-500     | 40    | 18                            | 115.4         | 26              |
|                     | 36    | 13                            | 106.9         | 19              |
Table S6: Dissociation effect nanocomposite on different mass of protein aggregates identified using MALDI ToF/ToF mass spectrometry.

| Sample Name         | Score | Protein sequence Coverage (%) | M. Mass (kDa) | No. of peptides |
|---------------------|-------|--------------------------------|---------------|-----------------|
| HSA +Native         | 42    | 9                              | 61.4          | 8               |
| HSA Fibril          | 43    | 22                             | 152.1         | 36              |
|                     | 38    | 24                             | 97.2          | 23              |
|                     | 35    | 11                             | 90.7          | 11              |
|                     | 32    | 19                             | 71.3          | 14              |
| HSA+G-Fibril        | 35    | 6                              | 185.1         | 17              |
|                     | 35    | 23                             | 103.2         | 30              |
|                     | 27    | 27                             | 110.1         | 27              |
|                     | 34    | 14                             | 70.1          | 13              |
| HSA+G+IONP          | 42    | 11                             | 137.1         | 22              |
|                     | 38    | 20                             | 117.1         | 22              |
|                     | 25    | 19                             | 98.3          | 17              |
|                     | 41    | 16                             | 71.3          | 12              |
| HSA+G+PR-CNC        | 35    | 8                              | 124.3         | 11              |
|                     | 32    | 17                             | 129.7         | 22              |
|                     | 35    | 11                             | 95.6          | 14              |
|                     | 30    | 26                             | 71.4          | 15              |
| HSA+G+SC-CNC        | 26    | 5                              | 127.6         | 9               |
|                     | 36    | 16                             | 102.3         | 14              |
|                     | 28    | 10                             | 78.4          | 11              |
|                     | 30    | 26                             | 71.4          | 15              |
| HSA+G+PR-NC-500     | 34    | 9                              | 124.3         | 15              |
|                     | 38    | 27                             | 110.1         | 27              |
|                     | 43    | 30                             | 74.9          | 23              |
|                     | 45    | 22                             | 71.3          | 14              |
| HSA+G+SC-NC-500     | 31    | 15                             | 73.1          | 9               |
|                     | 40    | 19                             | 67.9          | 19              |
Table S7: Different mass of protein aggregates after magnetic separation identified using MALDI ToF/ToF mass spectrometry.

| Sample                        | Score | Protein sequence coverage (%) | M. Mass (kDa) | No. of peptides |
|-------------------------------|-------|-------------------------------|---------------|-----------------|
| Protein samples separated from inhibition samples |       |                               |               |                 |
| HSA Native                    | 93    | 47                            | 66.5          | 38              |
| HSA Fibril                    | 123   | 46                            | 66.5          | 34              |
| HSA+G-Fibril                  | 114   | 47                            | 66.5          | 39              |
| HSA+G+ IONP                   | 101   | 47                            | 66.5          | 37              |
| HSA+G+ PR-CNC                 | 101   | 45                            | 66.5          | 36              |
| HSA+G+ SC-CNC                 | 115   | 45                            | 66.5          | 34              |
| HSA+G+ PR-NC 500              | 94    | 45                            | 66.5          | 37              |
| HSA+G+ SC-NC 500              | 104   | 49                            | 66.5          | 39              |
| Protein samples separated from dissociation samples |       |                               |               |                 |
| HSA Native                    | 101   | 47                            | 66.5          | 37              |
| HSA Fibril                    | 111   | 53                            | 66.5          | 37              |
| HSA+G-Fibril                  | 113   | 44                            | 66.5          | 33              |
| HSA+G+ IONP                   | 99    | 49                            | 66.5          | 38              |
| HSA+G+ PR-CNC                 | 110   | 47                            | 66.5          | 39              |
| HSA+G+ SC-CNC                 | 119   | 47                            | 66.5          | 38              |
| HSA+G+ PR-NC 500              | 87    | 43                            | 66.5          | 35              |
| HSA+G+ SC-NC 500              | 109   | 47                            | 66.5          | 39              |
**Figure S25.** (a) Histogram showing DPPH scavenging activity of the different nanocomposites along with other controls. The corresponding scavenging activity under protein aggregation condition was given in (b). Ascorbic acid has been taken as positive control.

**Figure S26.** Bright field and fluorescence micrograph images of (e) IONP, (f) PR-CNC, (g) SC-CNC and (h) PR-NC 500 treated HEK-293 cells to monitor inhibition of invitro protein aggregation.
Figure S27. Confocal microscopy images of different nanocomposite treated samples to maintain intracellular Ca^{2+} dysregulation for HEK-293 cells.
Figure S28. (a) Histogram showing percentage hemolysis, (b) absorbance study of hemolysis and (c) photographic image of the effect of nanocomposites and its precursors on RBCs.
Figure S29. SEM images of RBCs in the presence of (a) 1X PBS, (b) Triton X-100, (c) IONP,(d)PR-CNC,(e) SC-CNC,(f) PR-NC-100,(g) PR-NC-200,(h) PR-NC-500,(i) PR-NC-800,(j) SC-NC-100,(k) SC-NC-200,(l) SC-NC-500 and (m) SC-NC-800.