

Electronic Supporting Information

Systematic spectroscopic investigation of structural changes and corona formation of bovine serum albumin over magneto-fluorescent nanoparticles

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Materials

Ferrocene, hydrogen peroxide, phosphate buffer solution (PBS), bovine serum albumin (BSA) and sodium dodecyl benzene sulphonate (SDBS) were purchased from Sigma Aldrich. All the solvents used in synthesis and others studies were of analytical grade. All chemicals were used without further purification.

Synthesis of magneto-fluorescent nanoparticles (MFNPs)

In a typical synthesis of MFNPs, ferrocene (0.20 g) was dissolved in acetone solution (60 mL). After intense sonication for 30 min, 5 mL of hydrogen peroxide solution (30%) was slowly added in the above solution, which was then vigorously stirred for another 30 min with magnetic stirring apparatus. The precursor solution was transferred to a 100 ml Teflon-lined stainless steel autoclave. After sealing, the autoclave was heated to and maintained at 200°C for 48 h. The autoclave was then cooled naturally to room temperature. Further after intense sonication for 20 min, the product from the Teflon lined stainless steel autoclave was magnetized for 15 min by a magnet. The supernatant was carefully discarded using a magnet. Finally, the black precipitate was dried at 50°C in a vacuum oven. The dried MFNPs were transferred to 2 μ M aqueous solution of sodium dodecyl benzene sulphonate (SDBS) to prepare amphiphilic MFNPs. The prepared nanoparticles were used for further characterizations and studies.

Crystalline structure studies (X-ray diffraction (XRD) spectroscopy)

Powder X-ray diffraction (PXRD) spectroscopy was recorded on a Rigaku Smart Lab diffractometer, using Cu ($K\alpha$) radiation source from 5 to 80 degree 2θ with scanning rate of 2 degree/min. Samples were mounted on the glass side prior to the scanning.

Transmission electron microscopy (TEM) studies

Particle size and morphology of MFNPs sample were obtained by transmission electron microscopy (TEM) using FEI Tecnai TEM equipped with a LaB₆ source operating at 200 kV. MFNPs and MFNPs-BSA samples were first drop casted on the carbon coated copper grid and dried before subjected to the imaging.

Field emission scanning electron microscopy-energy dispersive x-ray spectroscopic analysis (FESEM-EDAX) studies

Surface morphology of MFNPs was observed using a field-emission scanning electron microscopy FESEM (NOVA NanoSEM 450) at an accelerating voltage of 10 kV. Field emission scanning electron microscopy-energy dispersive x-ray spectroscopic analysis (FESEM-EDAX) was also performed on the same microscope using proper scan area in the specific region of interest in the sample. The samples were drop casted on the silicon wafer and dried before imaging.

Fluorescence quenching studies (Steady state Fluorescence spectroscopy)

In a typical experiment, stock solution of 4.0×10^{-6} mol/L of BSA and 4.319×10^{-5} mol/L of MFNPs were prepared in phosphate buffer solution (PBS) (pH=7.4). An appropriate concentration of amphiphilic MFNPs from 4.319×10^{-5} to 4.319×10^{-4} mol/L were transferred into BSA solution in a 5 mL comparison tubes in a sequence containing 2 mL of phosphate buffer solution (pH 7.4) and shaken carefully. Based on our pre-experiments, BSA concentration was fixed at 4.0×10^{-6} mol/L and MFNPs concentration was varied from 4.319×10^{-5} to 4.319×10^{-4} mol/L. Fluorescence spectra were recorded at three different temperatures (291, 298 and 306 K) in the range of 290–450 nm upon excitation wavelength of 280 nm.

Fluorescence lifetime and anisotropy measurements

Fluorescence anisotropy and lifetime measurements of BSA and BSA-MFNPs were recorded by fixing 280 nm as the excitation wavelength and 340 nm as the emission wavelength. The BSA concentration was 4.0×10^{-6} mol/L while the MFNPs concentration was 4.319×10^{-4} mol/L in phosphate buffer at room temperature.

Fourier transform infrared spectroscopy (FTIR) studies

FTIR spectra of BSA, MFNPs and BSA-MFNPs were recorded in the range of frequency from 4000 to 400 wavenumber. BSA concentration was fixed at 4.0×10^{-6} mol/L while MFNPs concentration was 4.319×10^{-4} mol/L in the phosphate buffer solution.

UV-Vis spectroscopic studies

UV-Vis measurements of BSA, MFNPs and BSA-MFNPs were carried out from the wavelength range from 200 to 800 nm. BSA concentration was fixed at 4.0×10^{-6} mol/L while MFNPs concentration was 4.319×10^{-4} mol/L in phosphate buffer solution.

Circular dichroic (CD) measurement studies

CD spectra of BSA and BSA-MFNPs at 298 K were recorded in the range of 190-280 nm for five concentrations of MFNPs (4.319×10^{-5} to 4.319×10^{-4} mol/L) with fixed BSA concentration at 4.0×10^{-6} mol/L in phosphate buffer solution (PBS).

Raman spectroscopic studies

Raman spectroscopy was performed with the BSA and BSA-MFNPs at room temperature. The spectra was recorded from 200 to 2000 cm^{-1} at 10s acquisition time with 532 nm excitation laser. The concentration of MFNPs and BSA were 4.0×10^{-6} mol/L and 4.319×10^{-4} mol/L in phosphate

buffer solution (PBS) respectively. The samples were drop casted on glass slide prior to Raman measurements.

Dynamic light scattering (DLS) and zeta potential measurement studies

Dynamic light scattering and zeta potential measurements were carried out to examine the hydrodynamic diameter and surface charge of MFNPs in the absence and presence of BSA at room temperature (25°C) using a Malvern Zetasizer Nano ZS with 633 nm He–Ne laser, equipped with an MPT-2 Autotitrator.

Thermogravimetric analysis (TGA) measurement studies

Thermogravimetric analysis (TGA) was performed on a PerkinElmer Thermal Analyzer. The measured weight loss of MFNPs, BSA and BSA-MFNPs samples was recorded from room temperature to 900°C at a heating rate of 10 K/min under a constant nitrogen gas flow of 20 mL/min.

X-Ray photoelectron spectroscopy (XPS) studies

X-ray photoelectron spectra (XPS) was recorded on a VG (ESCALAB250) electron photo spectrometer with a monochromatic Al K α (1486.6 eV) at 15 kV and 10 mA, and all binding energies were referenced to the C1s peak (284.6 eV).

Vibrating sample magnetometer (SQUID-VSM) studies

Magnetic properties of the MFNPs were analyzed using vibrating sample magnetometer-superconducting quantum interference device (VSM-SQUID) (Quantum design Model: Quantum Design MPMS 3) under the vibrating magnetic field. The samples were prepared using the prescribed procedure. VSM is a very sensitive magnetometer due to the utilization of a liquid

Helium cooled SQUID (Super conducting quantum interference device) to measure changes in the magnetic flux which is converted to and measured as current, as sample moves through the superconducting detection coil. The instrument is used to study the magnetic properties at temperature from 1.8 to 400 K under magnetic field up to 7 Tesla. It is possible to measure materials in powder, single crystal or pellet form. Three measurement modes AC susceptibility mode, DC scan mode and VSM mode can be performed.

Supporting Figures

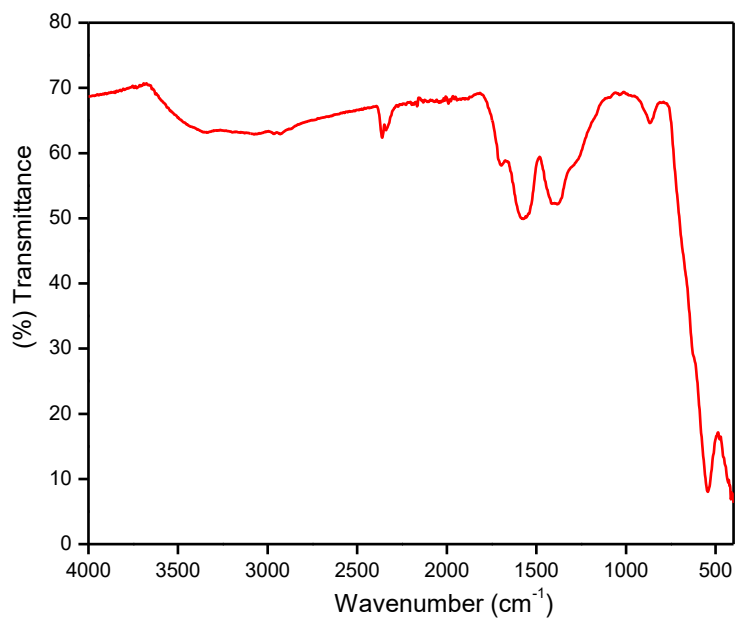


Figure S1. FTIR spectra of MFNPs.

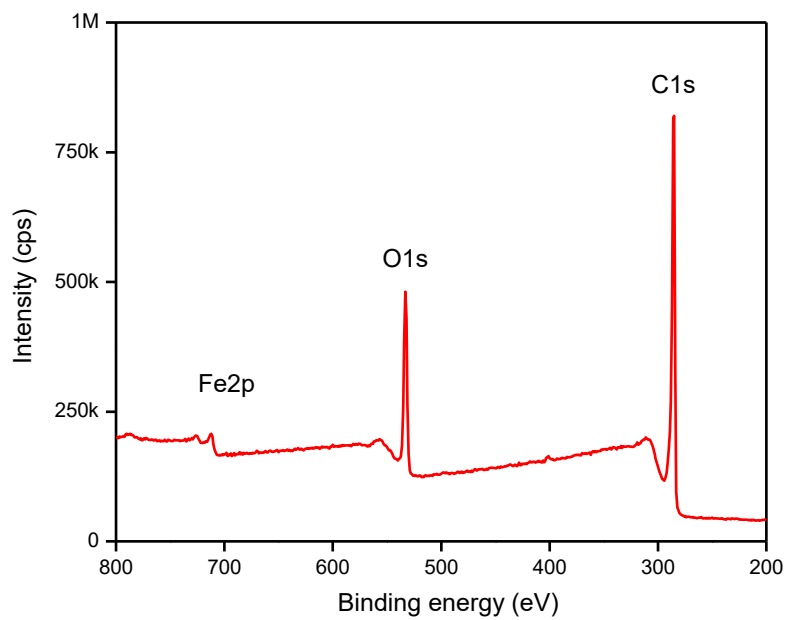


Figure S2. Full survey XPS spectra of MFNPs.

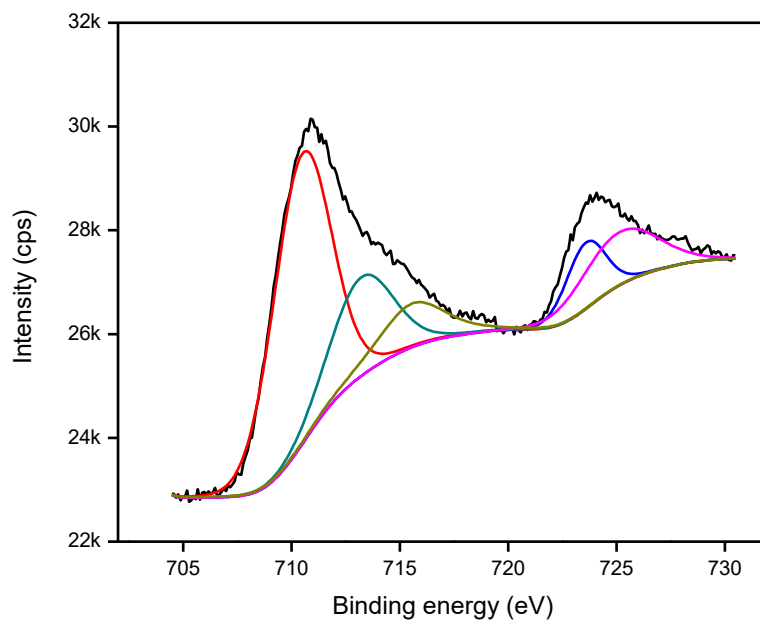


Figure S3. Fe2p XPS spectra of MFNPs.

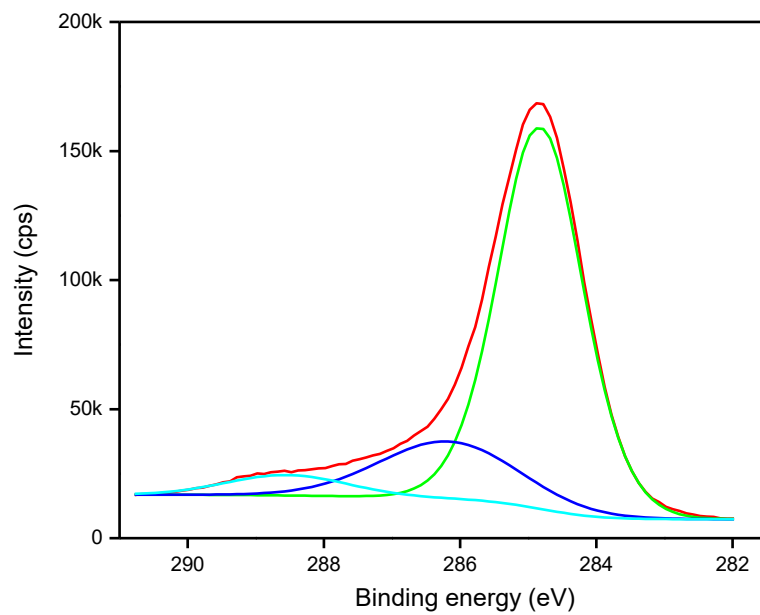


Figure S4. C1s XPS spectra of MFNPs.

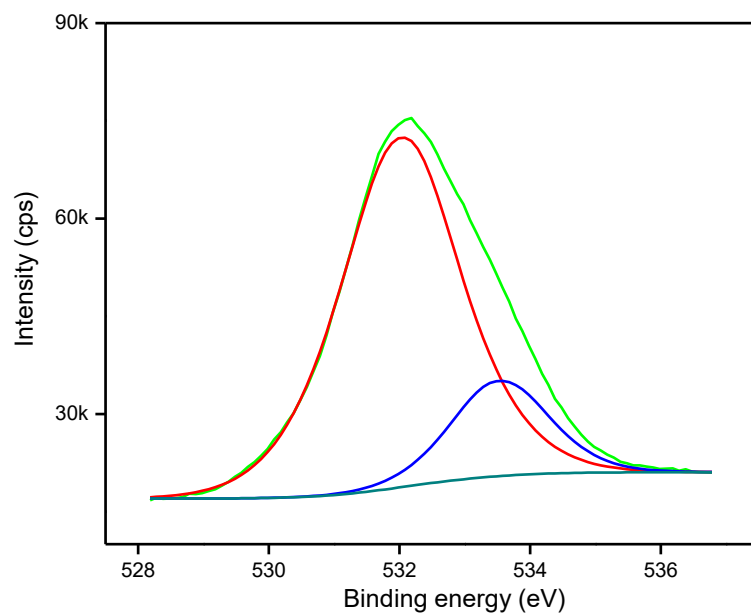


Figure S5. O1s XPS spectra of MFNPs.

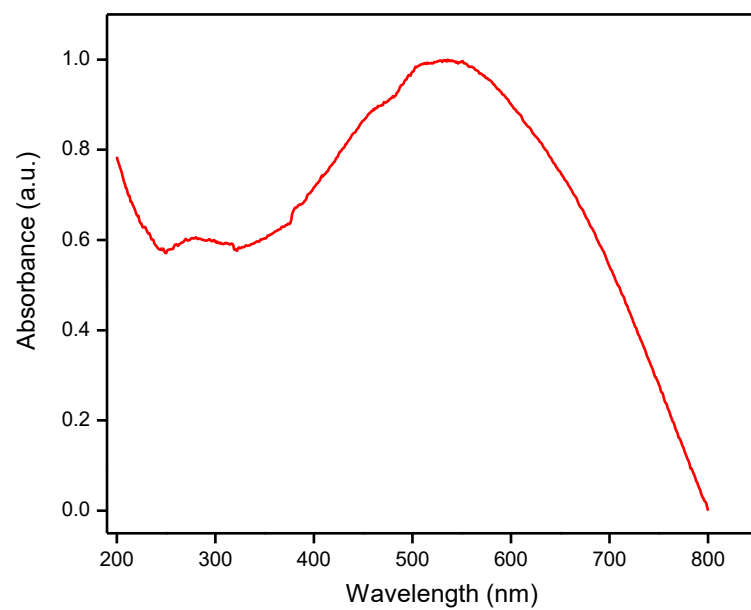


Figure S6. UV-Vis spectra of MFNPs.

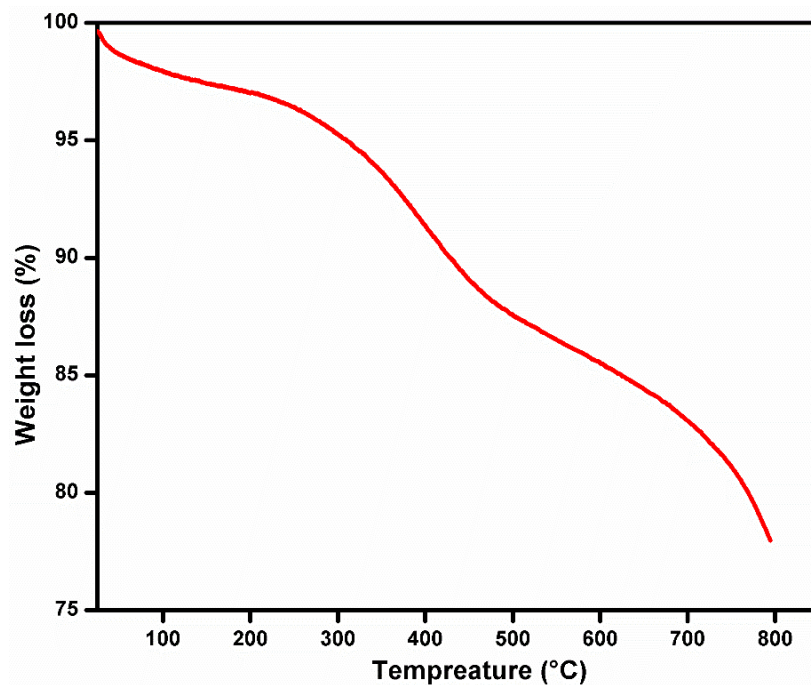


Figure S7. TGA graph of MFNPs.

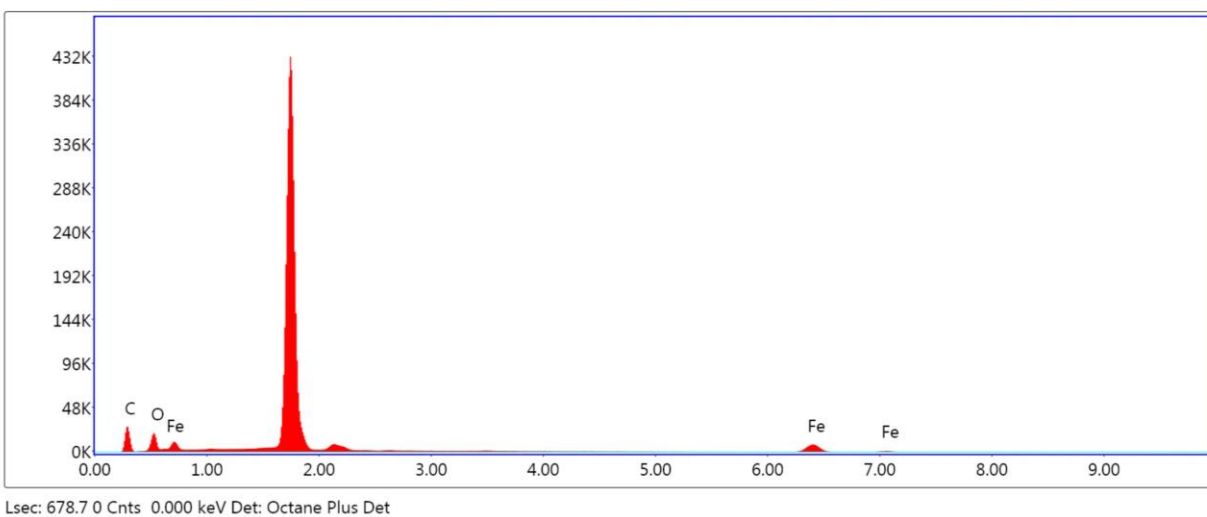


Figure S8. FESEM-EDAX spectroscopic elemental analysis of MFNPs.

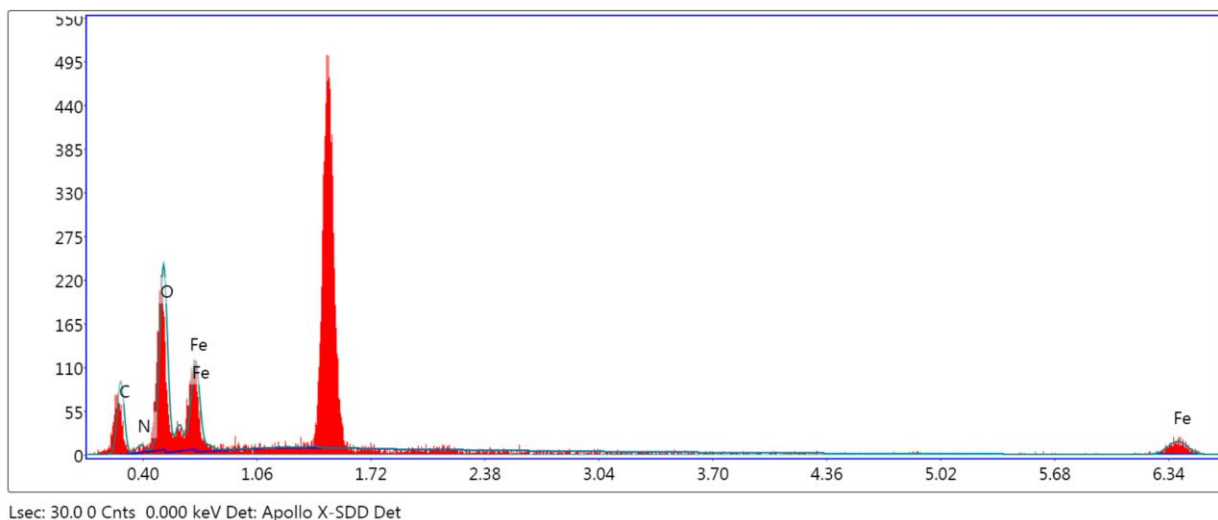


Figure S9. FESEM-EDAX spectroscopic elemental analysis of MFNPs-BSA system.

Table ST1. FESEM-EDAX spectroscopic elemental composition analysis of MFNPs-BSA system.

Element	Weight %	Atomic %
C K	21.28	36.78
N K	3.14	4.66
O K	32.91	42.70
FeL	42.67	15.86

Table ST2: Change in the hydrodynamic diameter and surface zeta potential on increasing concentration of BSA into MFNPs.

Samples	Hydrodynamic diameter (nm)	Zeta potential (mV)
MFNPs	545±10	-10.4
MFNPs-BSA (1µM)	644±10	-13.9
MFNPs-BSA (2µM)	962±10	-12.0
MFNPs-BSA (3µM)	974±10	-13.2
MFNPs-BSA (4µM)	994±10	-13.8
MFNPs-BSA (5µM)	1153±10	-13.9