

Supporting Information

Superparamagnetic Fluorescent Nickel-Enzyme Nanobioconjugates: Synthesis and Characterization of a Novel Multifunctional Biological Probe

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1. Details of Steady-state and Time-resolved Measurements: Steady-state UV-vis absorption and photoluminescence (PL) were done using a Shimadzu UV-2450 spectrophotometer and a Jobin Yvon Fluoromax-3 fluorimeter, respectively. A JEOL JEM-2100 high-resolution transmission electron microscopy (HRTEM) equipped with an energy dispersive X-ray (EDAX) spectrometer was used to characterize the internal structures of samples and to analyze their elemental composition. Samples for TEM were prepared by placing a drop of the colloidal solution on a carbon-coated copper grid and allowing the film to evaporate overnight at room temperature. To obtain the clear TEM images of nanoparticles, the aqueous solution of Ni-CHT sample was dialyzed against double distilled water exhaustively using a dialysis membrane (Spectrum Laboratories, Inc, USA) with a molecular weight cut off (MWCO) of 12000-14000. The dialyzed solution was then placed on a separate carbon-coated copper grid and dried overnight at room temperature. Magnetic measurements, including the zero-field-cooled (ZFC), field-cooled (FC) magnetization, and hysteresis loops, were carried out in a Quantum Design MPMS XL7 SQUID magnetometer on 20 days old sample. The circular dichroism (CD) study was done using Jasco 815 spectropolarimeter using a quartz cell of path-length 10 mm. All of the photoluminescence transients were taken using the picosecond-resolved timecorrelated single photon counting (TCSPC) technique. We used a commercially available picosecond diode laser-pumped (LifeSpec-ps) time-resolved fluorescence spectrophotometer from Edinburgh Instruments, U.K. The picosecond excitation pulse from the picoquant diode laser was used at 375 nm with instrument response function (IRF) of 80 ps. A microchannel-plate-photomultiplier tube (MCP-PMT, Hamamatsu) was used to detect the photoluminescence from the sample after dispersion through a monochromator. For all transients the polarizer on the emission side was adjusted to be at 55° (Magic angle) with respect to the polarization axis of the excitation beam. The observed fluorescence transients were fitted by using a nonlinear least square

fitting procedure to a function $(X(t) = \int_0^t E(t')R(t-t')dt')$ comprising of convolution of the

IRF ($E(t)$) with a sum of exponentials ($R(t) = A + \sum_{i=1}^N B_i e^{-t/\tau_i}$) with pre-exponential factors

(B_i), characteristic lifetimes (τ_i) and a background (A). Relative concentration in a multi-

exponential decay was finally expressed as; $c_n = \frac{B_n}{\sum_{i=1}^N B_i} \times 100$.

To estimate the FRET efficiency of the donor and hence to determine the distance of the donor–acceptor pair, we followed the methodology described in chapter 13 of reference. The Förster distance (R_0) is given by,

$$R_0 = 0.211 [\kappa^2 n^{-4} Q_D J(\lambda)]^{1/6} \quad (\text{in } \text{\AA}) \quad (\text{S1})$$

where κ^2 is a factor describing the relative orientation in space of the transition dipoles of the donor and acceptor. For donor and acceptors that randomize by rotational diffusion prior to energy transfer, the magnitude of κ^2 is assumed to be 2/3. In the present study the same assumption has been made. The refractive index (n) of the medium was assumed to be 1.4. Q_D , the quantum yield of the donor QD in the absence of acceptor, is measured to be 0.51. $J(\lambda)$, the overlap integral, which expresses the degree of spectral overlap between the donor emission and the acceptor absorption is given by,

$$J(\lambda) = \frac{\int_0^\infty F_D(\lambda) \epsilon(\lambda) \lambda^4 d\lambda}{\int_0^\infty F_D(\lambda) d\lambda} \quad (\text{S2})$$

where $F_D(\lambda)$ is the fluorescence intensity of the donor in the wavelength range of λ to $\lambda+d\lambda$ and is dimensionless. $\epsilon(\lambda)$ is the extinction coefficient (in $\text{M}^{-1} \text{cm}^{-1}$) of the acceptor at λ . If λ

is in nm, then $J(\lambda)$ is in units of $M^{-1} \text{ cm}^{-1} \text{ nm}^4$. Once the value of R_0 is known, the donor–acceptor distance (r) can easily be calculated using the formula,

$$r^6 = \frac{[R_0^6(1-E)]}{E} \quad (\text{S3})$$

Here E is FRET efficiency. The transfer efficiency is measured by using the relative fluorescence intensity of the donor in the absence (F_D) and presence (F_{DA}) of the acceptor. The efficiency E is also calculated from the lifetime under these respective conditions (τ_D and τ_{DA}).

$$E = 1 - \left(\frac{F_{DA}}{F_D} \right), \quad (\text{S4})$$

$$E = 1 - \left(\frac{\tau_{DA}}{\tau_D} \right) \quad (\text{S5})$$

The distance measured with eqs. S4 and S5 are revealed as R^S (steady-state measurement) and R^{TR} (time-resolved measurement), respectively.

It has to be noted that equation (S5) holds rigorously only for a homogeneous system (i.e. identical donor-acceptor complexes) in which the donor and the donor-acceptor complex have single exponential decays. However, for donor-acceptor, systems are decaying with multiexponential lifetimes. FRET efficiency (E) is calculated from the amplitude weighted lifetimes¹ $\langle \tau \rangle = \sum_i \alpha_i \tau_i$ where α_i is the relative amplitude contribution to the lifetime τ_i . We have used the amplitude weighted time constants for τ_D and τ_{DA} to evaluate E using equation (S5).

Catalytic activity measurements in our experiment were made using the substrate AAF-AMC. The extinction coefficient used for determining the concentration of AAF-AMC

in buffer (10 mM, pH = 7.0) is $16 \text{ mM}^{-1}\text{cm}^{-1}$ at 325 nm^2 . For the kinetics experiment, the concentration of CHT and Ni-CHT was maintained at $10 \text{ }\mu\text{M}$, whereas that of substrate was maintained at $100 \text{ }\mu\text{M}$. The rate of formation of product was monitored by observing the change in the absorbance at 370 nm of the product with time. The product formed in the catalytic measurement was 7-amido-4methyl coumarin (AMC) and its extinction coefficient at 370 nm is reported to be $7.6 \text{ mM}^{-1}\text{cm}^{-1}$.

2. Calculation of molar extinction coefficient of Ni NPs solution (Ni-CHT) from theoretical considerations: Firstly, the molar concentration of the nanoparticles solutions were calculated by dividing the total number of nickel atoms (N_{total} , equivalent to the initial amount of nickel salt added to the reaction solution) over the average number of nickel atoms per nanosphere (N) determined from TEM, according to the equation³:

$$C = \frac{N_{\text{total}}}{NVN_A} \quad (\text{S6})$$

where V is the volume of the reaction solution in liter and N_A is the Avogadro's constant. It is assumed that the reduction from nickel ions to nickel atoms was 100 % complete. The concentration of the diluted sample was then calculated from this initial concentration. The extinction coefficient of the sample was then determined according to Lambert-Beer law, Eq. (S7) by measuring the absorbance at 417 nm (SPR peak of Ni NPs solution).

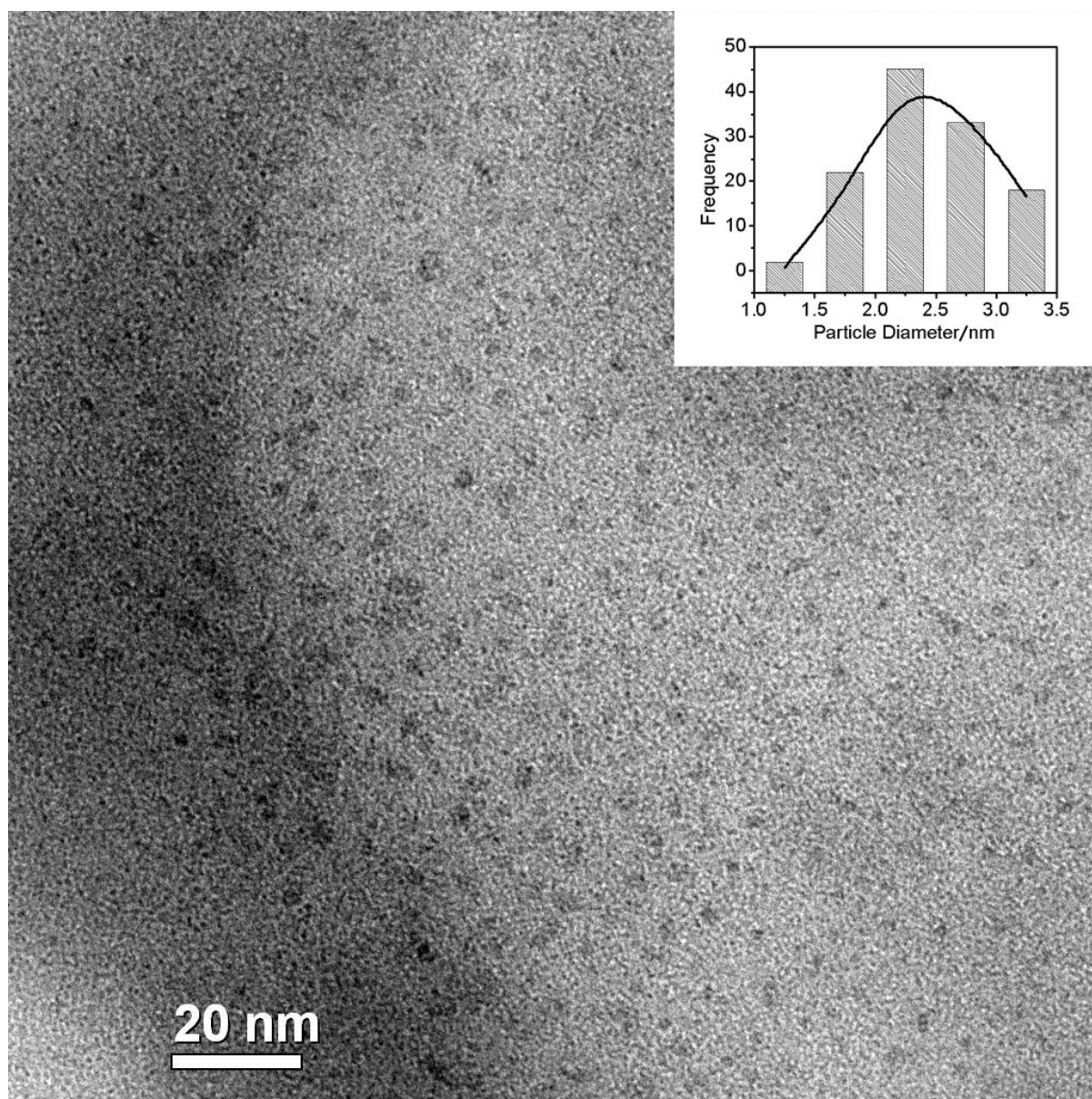
$$A = \epsilon Cl \quad (\text{S7})$$

The molar extinction value obtained at SPR peak is $3347.6 \text{ M}^{-1}\text{cm}^{-1}$.

Figure caption:

Figure S1. (a) TEM image of as-prepared of dialyzed Ni-CHT nanobiocomjugates. The size distributions of the samples are shown in the inset.

Figure S1.



References:

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