

Supporting Information

Metal assisted self-assembled rod like nanostructures for effective cellular internalization

Sourav Bhattacharya¹, Saikat Mukherjee¹, Jayasri Das Sarma² and

Raja Shunmugam^{1*}

Polymer Research Centre, Department of Chemical Sciences,
Indian Institute of Science Education and Research, Kolkata, India.

Email sraja@iiserkol.ac.in

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Experimental Section:

Materials: Exo-oxabicyclo- [2.2.1] hept-5-ene-2, 3 - dicarboxylic anhydride¹, P-amino benzoic acid, acetic anhydride, sodium acetate, 3-amino-1,2,4-triazole-5-thiol, second generation Grubbs' catalyst, were purchased from Sigma Aldrich. dicyclohexyl carbodiimide (DCC), dimethylsulfoxide (DMSO), dichloromethane (DCM), methanol, CDCl₃ were purchased as reagent grade from Aldrich, Acros, Merck and used as received. Dichloromethane (DCM) was distilled over calcium hydride and used for reaction.

Characterization:

Gel Permeation Chromatography (GPC). Molecular weights and PDIs are measured by Waters gel permeation chromatography in THF relative to polymethylmethacrylate (PMMA) as well as polystyrene (PS) standards on systems equipped with Waters Model 515 HPLC pump and Waters Model 2414 Refractive Index Detector at 35 °C with a flow rate of 1 mL/min. HRMS analyses are performed with Q-TOF YA263 high resolution (Waters Corporation) instruments by +ve mode electrospray ionization. *Fluoremetry.* Fluorescence emission spectra are recorded on a Fluorescence spectrometer (Horiba Jobin Yvon, Fluoromax-3, Xe-150 W, 250-900 nm).

Nuclear Magnetic Resonance (NMR). The ¹H NMR spectroscopy is carried out on a Bruker 500 MHz spectrometer using DMSO-d₆, and CDCl₃ as a solvent. ¹H NMR spectra of solutions in DMSO-d₆, and CDCl₃ are calibrated to tetramethylsilane as internal standard (δ H 0.00).

Fourier Transform Infra Red (FT-IR). FT-IR spectra are obtained on FT-IR Perkin-Elmer spectrometer at a nominal resolution of 2 cm⁻¹.

Ultra Violet (UV) Spectroscopy. UV-visible absorption measurements are carried out on U-4100 spectrophotometer HITACHI UV-vis spectrometer, with a scan rate of 500 nm/min.

Dynamic Light Scattering (DLS). Particle size of polymer is measured by dynamic light scattering (DLS), using a Malvern Zetasizer Nano equipped with a 4.0 mW He-Ne laser operating at $\lambda = 633$ nm. All samples are measured in aqueous as well as methanol at room

temperature and a scattering angle of 173° .

Transmission Electron Microscopy (TEM). Low resolution transmission electron microscopy (TEM) is performed on a JEOL 200 CX microscope. TEM grids are purchased from Ted Pella, Inc. and consisted of 3-4 nm amorphous carbon film supported on a 400-mesh coppergrid.

Scanning Electron Microscopy (SEM). High resolution SEM is performed on a Zeiss microscope; SUPRA 55VP-Field Emission Scanning Electron Microscope. High performance variable pressure FE-SEM with patented GEMINI column technology. Schottky type field emitter system, single condenser with crossover-free beam path. Resolution: 1.0 nm at 15 kV; 1.6 nm at 1 kV high vacuum mode. 2.0 nm at 30 kV at variable pressure mode.

Synthesis of **1**:

10 g of maleic anhydride (0.1 mol) was taken in round bottomed flask containing 100 mL of toluene. The mixture was stirred for 30 mins at room temperature and was filtered to get a clear solution.³⁶ The filtered solution was purged with nitrogen to maintain inert atmosphere. 8.2 mL of furan (0.11 mol) was added to the filtrate and the reaction mixture was stirred at room temperature for 48 hours. After completion of the reaction, the product was collected as white precipitate after washing with cold toluene and hexane for several times. The product was dried for overnight under vacuum. Yield: 6.64 g (0.04 mol, 80%). ^1H NMR (DMSO- D_6 , 500 MHz): 6.6 (s, 2H), 5.2 (s, 1H), 3.1 (s, 2H) (**Figure S1**). ^{13}C NMR (DMSO- D_6 , 500 MHz): 171, 136.65, 81, 47.5 (**Figure S2**).

Synthesis of **2**:

Exo - oxabicyclo- [2.2.1] hept-5-ene-2, 3 - dicarboxylic anhydride, 1.914 g (11.5 mmol) is taken in 4 neck reaction flask followed by 35 ml of acetone is added to it and heated until it became clear solution. After that para-amino benzoic acid 1.605 g (11.5 mmol) is added with stirring.

After fifteen minutes, heating is stopped and reaction mixture is allowed to stir for about 30 minutes. The solid is filtered and dried under oven at 55 °C under vacuum. The dried intermediate is then dissolved in 30 ml of dimethyl formamide and heated to 50 °C. Acetic anhydride 15 ml (158.97 mmol) and sodium acetate 0.635 g (7.743 mmol) are added under stirring. The reaction mixture is allowed to stir for three hours at 55 °C. After 3 h the reaction mixture is poured into 500 ml of water acidified by addition of 5 ml concentrated HCl. White color solid is precipitated immediately and filtered the solid and washed with water and dried at 90 °C, under vacuum (80 % yield). ¹H NMR (DMSO-D₆, 500 MHz): δ 13.1 (bs, 1H), 8.0 - 8.2 (m, 2H), 7.4 - 7.5 (m, 2H), 6.6 (s, 2H), 3.1 (s, 2H) (**Figure S3**). ¹³C NMR (DMSO-D₆, 500 MHz): δ 175.43, 166.59, 136.65, 135.78, 130.0, 126.79, 80.86, 47.58 (**Figure S4**).

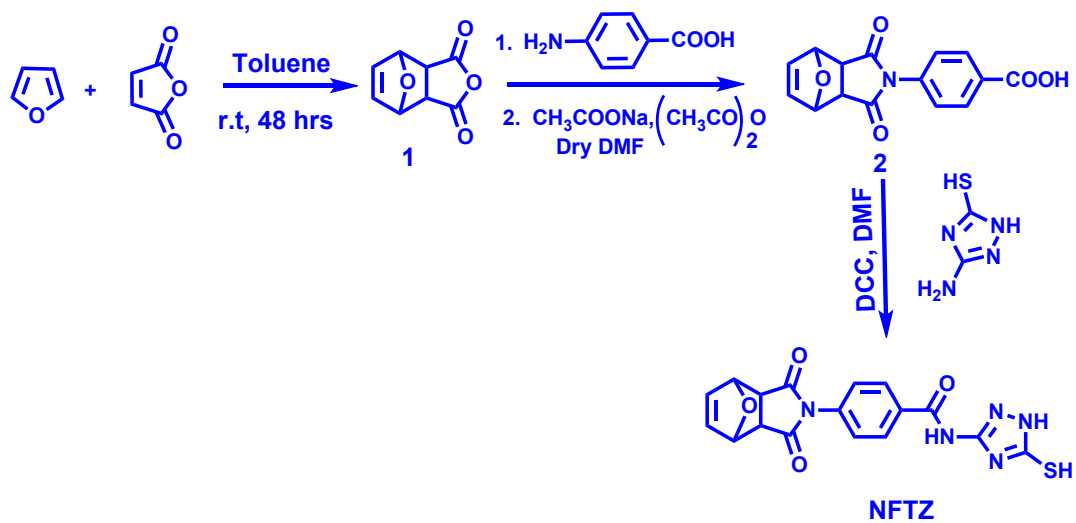
Synthesis of **NFTZ**:

1 g (3.5 mmol) of compound **2** is taken into 6 ml of dimethyl formamide. 0.86 g (4.17 mmol) of Dicyclohexylcarbodiimide (DCC) is added to the reaction mixture. Reaction mixture allowed to stir for 15 min to form the white precipitate at room temperature. Reaction mixture is cooled to 0-5 °C. 3-amino-1,2,4-triazole-5-thiol 0.45 g (3.85 mmol) dissolved in dimethyl formamide and this solution is added to the reaction mixture and continued the reaction mixture overnight. After completion of the reaction monitor by TLC, 15mL EtOAc followed by water is added to the reaction mixture. Organic layer is washed with 2 x 10 ml of water followed by sodium bicarbonate wash. Finally, organic layer is washed with brine solution. Organic layer concentrated under vacuum to yield off-white colour solid which is purified which is purified through column chromatography using 25% DCM, MeOH mixture as mobile phase and silica as stationary phase. (700 mg, 70% yield). ¹H NMR (DMSO-D₆, 500 MHz): δ 7.82 (s, 1H), 7.52 (s,

2H), 7.23 (s, 2H), 6.5 (s, 2H), 5.22 (s, 2H), 3.11 (s, 2H) (**Figure S5**) ^{13}C NMR (CDCl_3 , 400 MHz): δ 175.48, 167.39, 139.42, 138.64, 132.23, 128.32, 126.67, 80.85, 45.27 (**Figure S6**).

Homopolymerization of norbornene derived triazole monomer (NFTZH):

10 mg (0.0347 mmol) of Monomer **NFTZ** is weighed into a separate Schlenk flask, placed under an atmosphere of nitrogen, and dissolved in 1 ml of dry DCM. Into another Schlenk flask, a desired amount of second generation Grubbs' catalyst 1.4 mg (G2, 20 mol %) is added, flushed with nitrogen, dissolved in minimum (0.5 ml) anhydrous dichloromethane. All two flasks are degassed three times by freeze-pump-thaw cycles. The **NFTZ** is transferred to the flask containing the catalyst via a cannula (**Scheme 1**). The reaction is allowed to stir at room temperature until the polymerization is complete (1 h) after that it was quenched with vinyl ethyl ether (0.5 mL). An aliquot is taken for GPC analysis, and the remaining product is precipitated with diethyl ether, dissolved it again THF, passed it through neutral alumina to remove the catalyst and precipitated again in diethyl ether to get pure polymer. Gel permeation chromatography (GPC) is done in tetrahydrofuran (flow rate = 1mL/1min). The molecular weight of homopolymer (**NFTZH**) was measured by using polystyrene standards. $M_n = 9,000$ and PDI = 1.09 (**Figure S8**).



Scheme S1: Synthesis of Norbornene functionalized Triazole monomer (NFTZ).

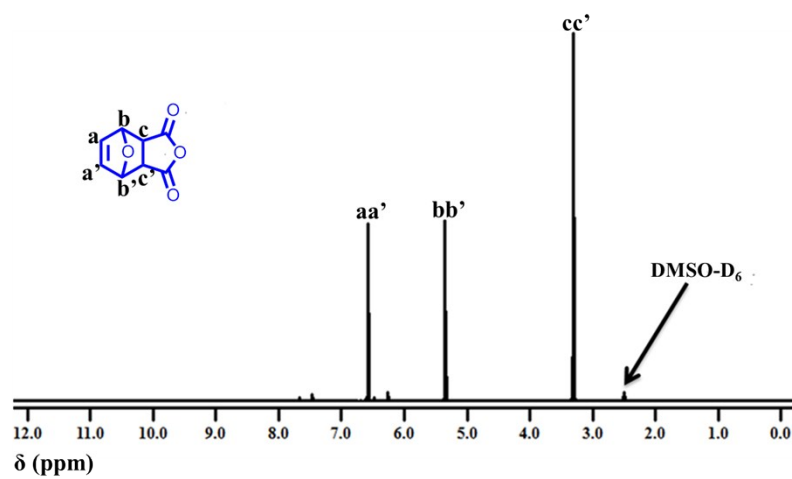


Figure S1: ¹H NMR spectrum of **1** in DMSO-D₆

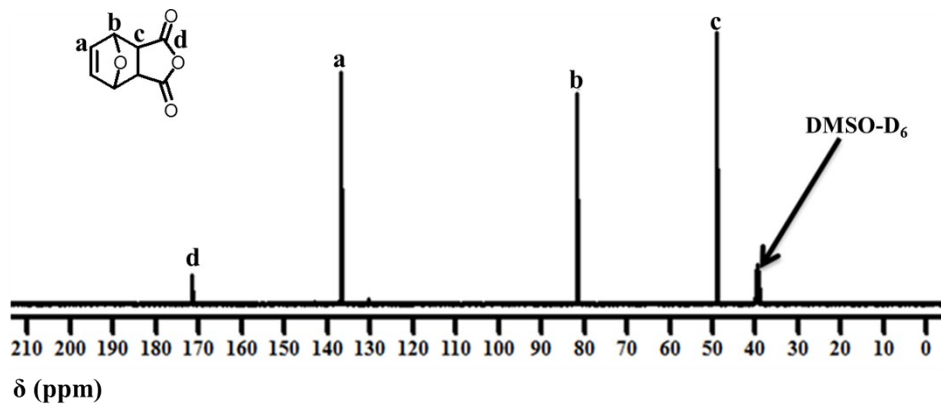


Figure S2: ^{13}C NMR spectrum of **1** in DMSO-D_6

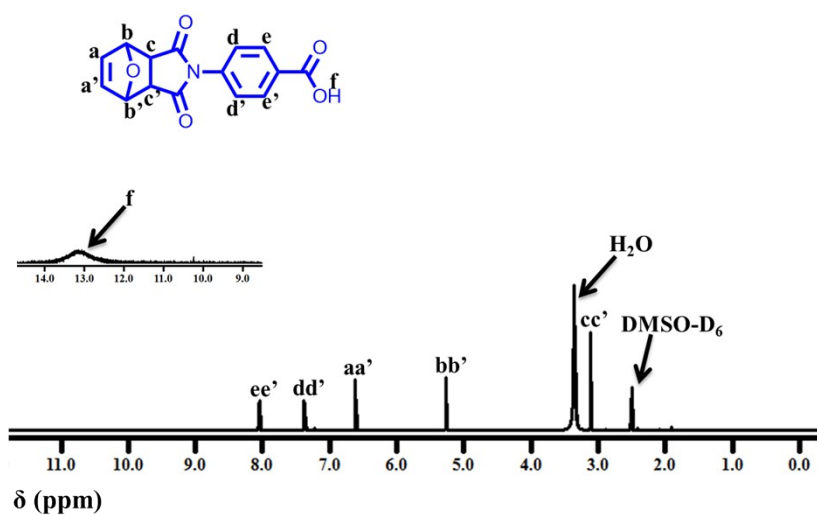


Figure S3: ^1H NMR spectrum of **2** in DMSO-D_6

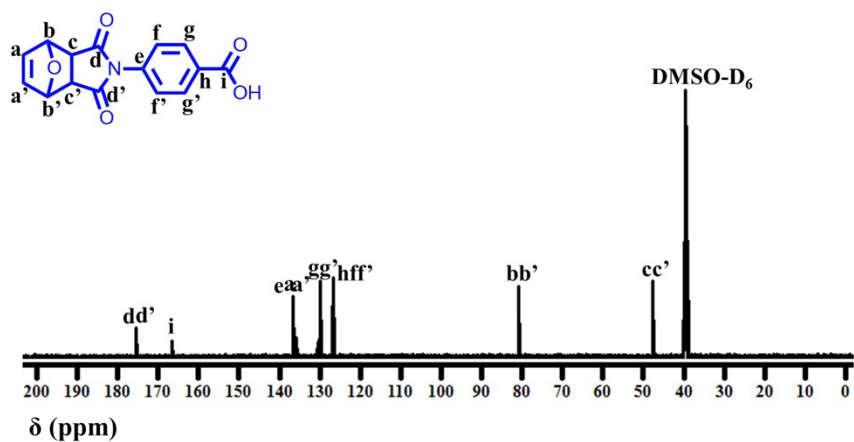


Figure S4: ^{13}C NMR spectrum of **2** in DMSO-D_6

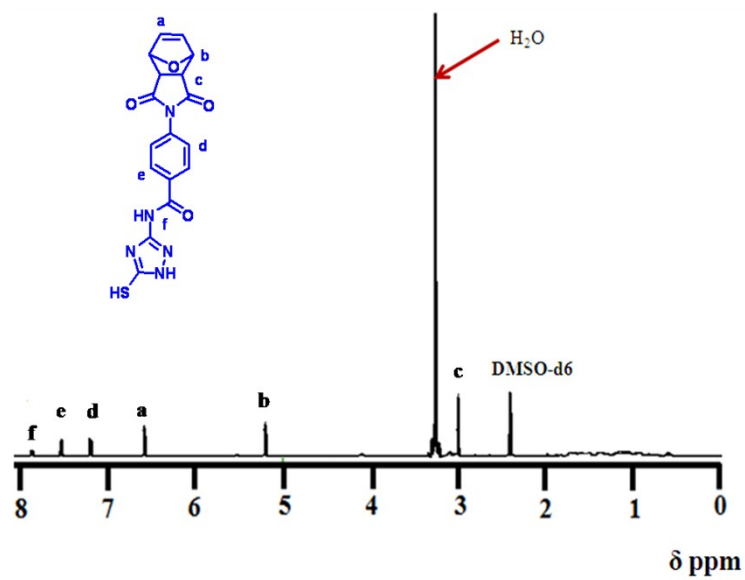


Figure S5: ^1H NMR spectrum of NFTZ in DMSO-d_6

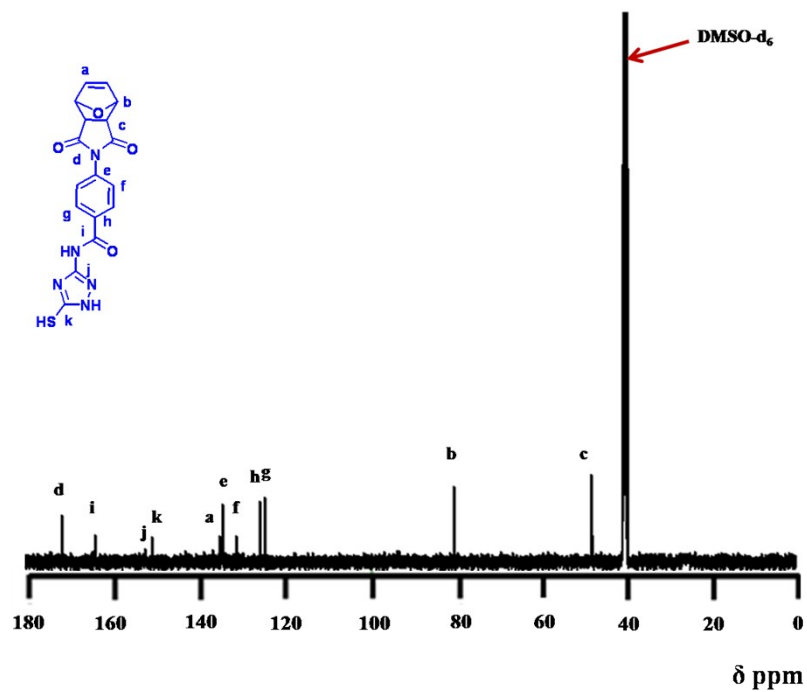


Figure S6: ^{13}C NMR spectrum of NFTZ in DMSO-d_6

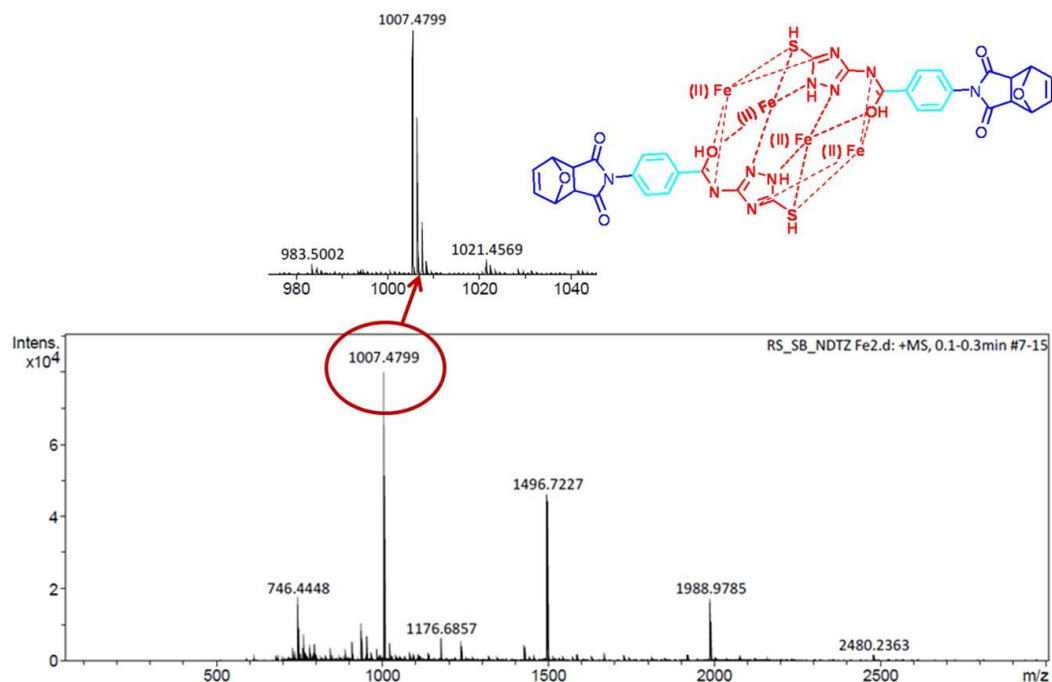


Figure S7: ESI-MS of NFTZ-Fe(II).

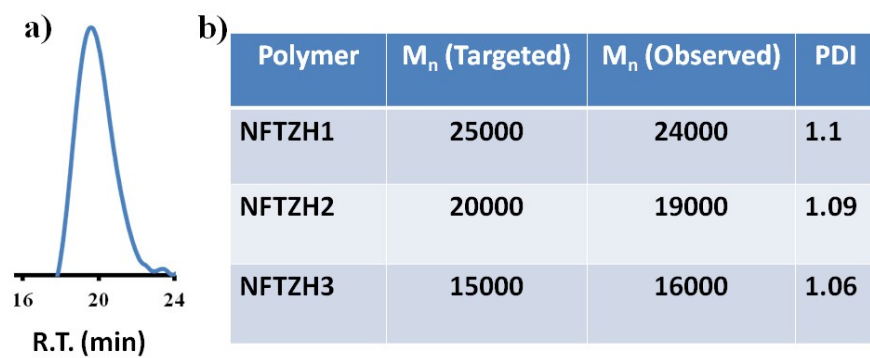


Figure S8: a) GPC chromatogram of triazole based norbornene polymer (NFTZH)

M_n = 9000 Da PDI= 1.09. **b)** Table comprising molecular weight and PDI of NFTZH.

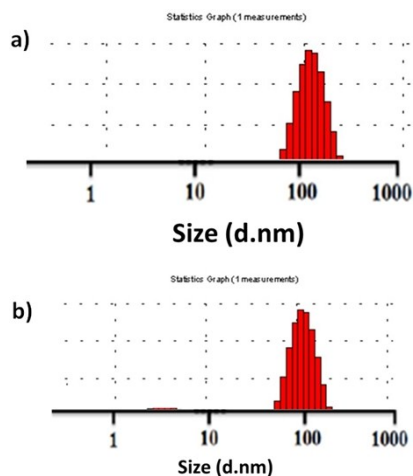


Figure S9: Dynamic light scattering data of **a)** NFTZH in THF-MeOH (1:1 mixture) $Z_{av} = 206$ nm, PDI=0.375 **b)** NFTZH -Fe(II) in THF-Hexane (1:1 mixture) $Z_{av} = 180$ nm, PDI= 0.285 (concentration = 1mg/ml).

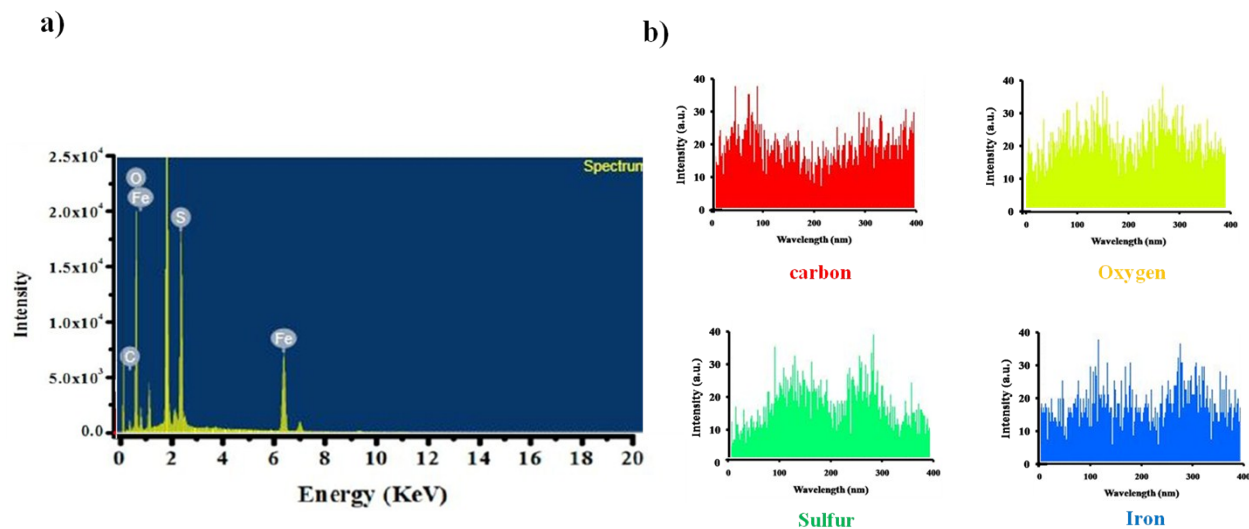


Figure S10: a) and b) Representative EDX analysis of NFTZH-Fe(II) complex

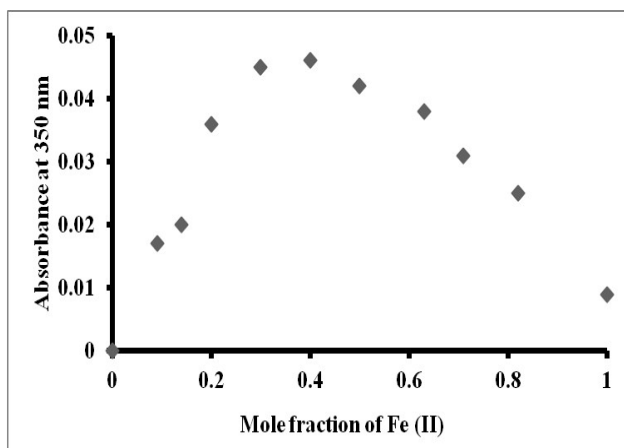


Figure S11: Job's plot of the **NFTZ** monomer and **Fe(II)**. The absorption at 350 nm was plotted against the mole fraction of **Fe(II)** at a constant total concentration [**Fe(II)** + **NFTZ** monomer] of 80 M showing the 1 : 2 binding stoichiometry in 1 : 1 MeOH : H₂O

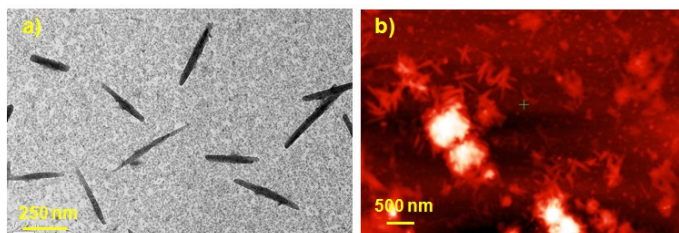


Figure S12: Rod-like aggregates in a solution of 1 wt % **NFTZH-Fe(II)** in THF–MeOH (1:1 mixture) (a) TEM and (b) AFM images.

36. Rao, V. N.; Mane, S.; Kishore, A.; Das Sarma, J.; Shunmugam, R. Norbornene derived doxorubicin copolymers as drug carriers with ph responsive hydrazone linker *Biomacromolecules* **2012**, *13* (1), 221-230.