

Electronic Supplementary Information

Design and Synthesis of a Highly Sensitive “Turn–On” Fluorescent Organic Nanoprobe for Iron(III) Detection and Imaging

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Experimental Details

Reagents and apparatus

All the chemicals in the experiment were of AR grade and used as received from Sinopharm Chemical Reagent Co. Ltd. Water used throughout was obtained from a Mill-Q system (MilliporeCorp., Bedford, USA).

¹H NMR spectra were recorded on a Varian Mercury 600 spectrometer and resonances are reported relative to TMS. Elementary analyses were taken on a Vario EL III elementary analysis instrument. Electrospray ionization mass spectrum was carried out on a Finnigan Trace MS 2000 organic mass spectrometer. The UV–vis spectroscopy measurements were performed on a T6 series UV–vis spectrophotometer (Puxi Co., Beijing, China). Fluorescence measurements were

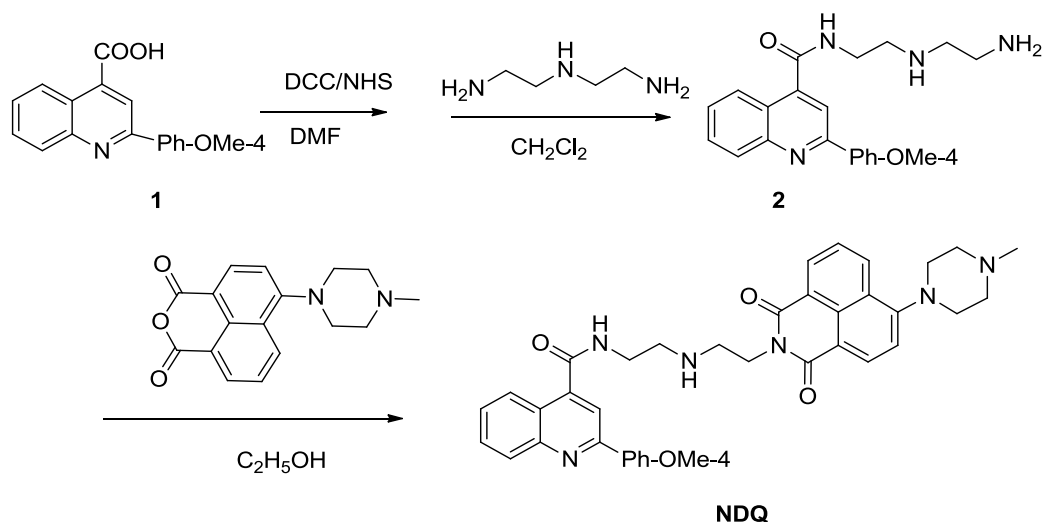
carried out using a LS-45/55 Fluorescence/Phosphorescence Spectrometer (PerkinElmer, USA). The particle size of FONs was determined with dynamic light scattering (DLS) using Nicomp380ZLS Particle Size Analyzer (PSS Co., USA). The morphologies of the FONs were observed on an FEI Tecnai G2 Spirit Bio TWIN electron microscope (FEI Company, Eindhoven, The Netherlands).

Synthesis of NDQ

Synthesis of NDQ is shown in Scheme S1. A mixture of **1** (2.79g, 10 mmol) in 30 mL DMF cooled in an ice bath and N-hydroxysuccinimide (1.44 g, 12.5 mmol) was added followed by DCC (4.12 g, 20 mmol). After approximately 30 min the ice bath was removed and the solution stirred overnight. The mixture was poured into water (150 mL) and extracted with CH₂Cl₂ (3 × 30 mL). The organic phase was washed with water (20 mL), 5% NaHCO₃ (2 × 20 mL), and brine (20 mL) and dried (Na₂SO₄). Concentration in vacuum gave 1-(2-(4-methoxyphenyl)quinoline-4-carbonyl)pyrrolidine-2,5-dione. The product was not purified and used directly in the next step. The residue was dissolved in 30 mL CH₂Cl₂ and 1.03 g (100 mmol) was added dropwise at room temperature. After stirring for 10 h, the reaction mixture was washed sequentially with brine, and dried over anhydrous MgSO₄. Concentration in vacuum gave a brown solid which was purified by flash column chromatography give the product **2**.

To a stirred solution of 4-(4-methylpiperazin-1-yl)-1,8-naphthalic anhydride (0.73 g, 2 mmol) in ethanol (20 mL) at room temperature were added **2** (0.59 g, 2 mmol). After the mixture was stirred and refluxed for 2 h (TLC monitoring), ethanol was removed under reduced pressure. The solid product was purified by flash column chromatography give the target NDQ in 76% yield. ¹H NMR (600 MHz in CDCl₃): 2.43 (s, 3H, NCH₃), 2.69 (t, 4H, *J*=5.4Hz, (CH₂)₂NCH₃), 3.13 (t, 8H, *J*=5.2Hz, CH₂NHCH₂, O=CNHCH₂, CH₂N(C=O)₂), 3.63 (t, 2H, *J*=5.6Hz), 3.85 (s, 3H, OCH₃), 4.26 (t, 2H, *J*=6.0Hz), 6.78 (d, 1H, *J*=7.8Hz, ArH), 7.00 (d, 2H, *J*=8.4Hz, ArH), 7.32 (s, 1H, ArH), 7.36-7.39 (m, 1H, ArH), 7.64-7.66 (m, 1H, ArH), 7.80 (d, 1H, *J*=8.4Hz, ArH), 7.94 (s, 1H, O=CNH), 8.05 (d, 1H, *J*=7.2Hz, ArH), 8.14 (d, 4H, *J*=8.4Hz, ArH),

8.17 (d, 1H, $J=8.4\text{Hz}$, ArH); LC-MS(4.85e5, m/z): 642.4, 322.0. Anal. Calc. $\text{C}_{38}\text{H}_{38}\text{N}_6\text{O}_4$: C, 71.01; H, 5.96; N, 13.08. Found: C, 70.62; H, 6.29; N, 12.61%.



Scheme S1. Synthetic procedure employed for the syntheses of compound NDQ.

Preparation of NDQ FONs

In a typical preparation, a stock solution of NDQ in CH_3CN (5×10^{-3} M) was prepared. To the 9.9 mL of $\text{H}_2\text{O}/\text{MeCN}$ mixed solution ($v/v = 7:3$), 100 μL of the stock solution of NDQ was rapidly injected under vigorous stirring ensured the rapid mixing of both the solutions. The above solution was then stirred for 10 min at room temperature. Finally, 50 μM FONs of NDQ was obtained.

Determination of quantum yields

The quantum yields of FONs were calculated by using quinine sulfate (0.1M H_2SO_4 , $\text{QY}=54\%$) solution as reference together with the following formula (J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd Ed., 1999, Kluwer Academic/Plenum Publishers, New York):

$$\text{QY} = \text{QY}_{\text{ref}} \times [I \times A_{\text{ref}} \times \eta^2] / [I_{\text{ref}} \times A \times \eta_{\text{ref}}^2]$$

Where QY is the quantum yield of unknown; QY_{ref} is the quantum yield of the reference compound; η is the refractive index of the solvent, I is the integrated fluorescence intensity and A is the absorbance at the excitation wavelength. The absorbances at the wavelength of excitation is optimally kept in between $A = 0.02-0.05$ in order to avoid inner filter effects and ensure linear response on the

intensity.

Assay procedure

The NDQ FONs (prepared in 70% H₂O/CH₃CN) solution (10 mL) was initially diluted with a 10 mM phosphate buffer solution (40 mL, pH 7.4) for metal ions detection. In a typical assay, 0.1 mL of sample containing certain amount of metal ion was added to the NDQ FONs (1.9 mL). The mixture was shaken thoroughly and equilibrated for 10 min. Then, the fluorescence spectrum of the solution was measured.

Calculation of binding constant between Fe³⁺ and FONs

The stability constant of the complex was calculated by the linear Benesi–Hildebrand expression (H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703–2707):

$$\frac{I_0}{I-I_0} = \frac{I_0}{[F]} + \frac{I_0}{[F] K_s} \frac{1}{[Fe]}$$

Where I is the change in the fluorescence intensity at 545 nm, K_s is the stability constant, and [F] and [Fe] are the concentrations of FONs and Fe³⁺, respectively. I₀ is the fluorescence intensity of FONs in the absence of Fe³⁺. On the basis of the plot of 1/(I-I₀) versus 1/[Fe], the stability constant can be obtained, which is 7.14×10⁵ M⁻¹.

Figures

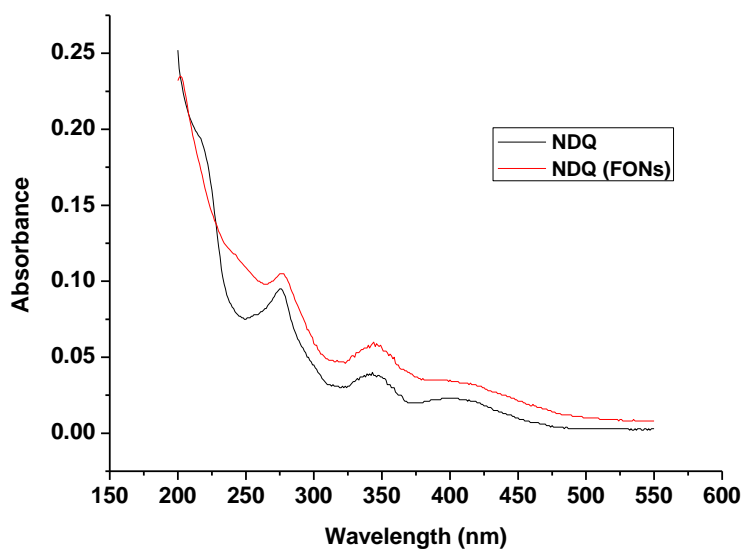


Fig. S1 UV-Vis absorption spectra of compound NDQ and NDQ FONs (50 μM).

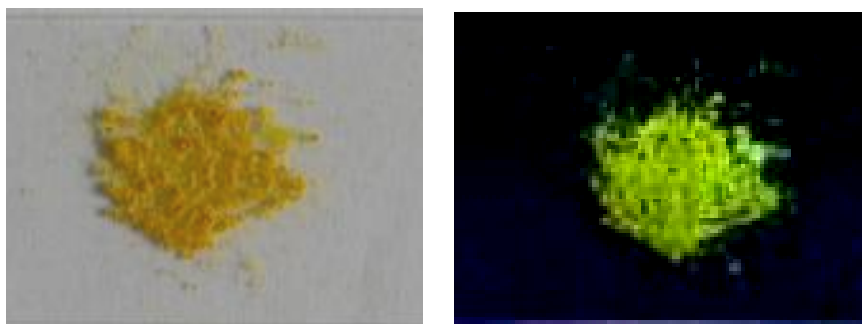


Fig. S2 Photographs of the solid powders of NDQ (from left to right) under ambient (left) and UV (right) illumination.

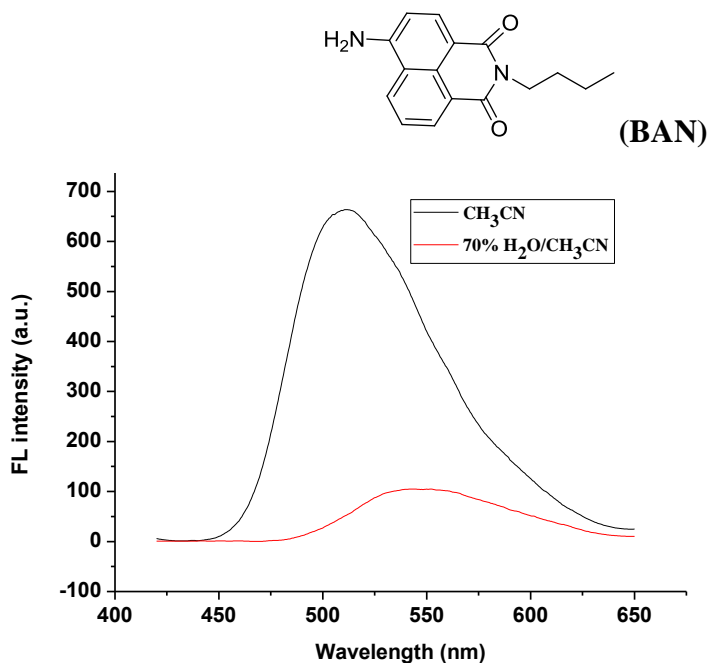


Fig. S3 The structure of N'-butyl-4-amino-1,8-naphthalimide (BAN), and fluorescence spectra of BAN in CH₃CN and in 70% H₂O/CH₃CN solvent (50 μM).

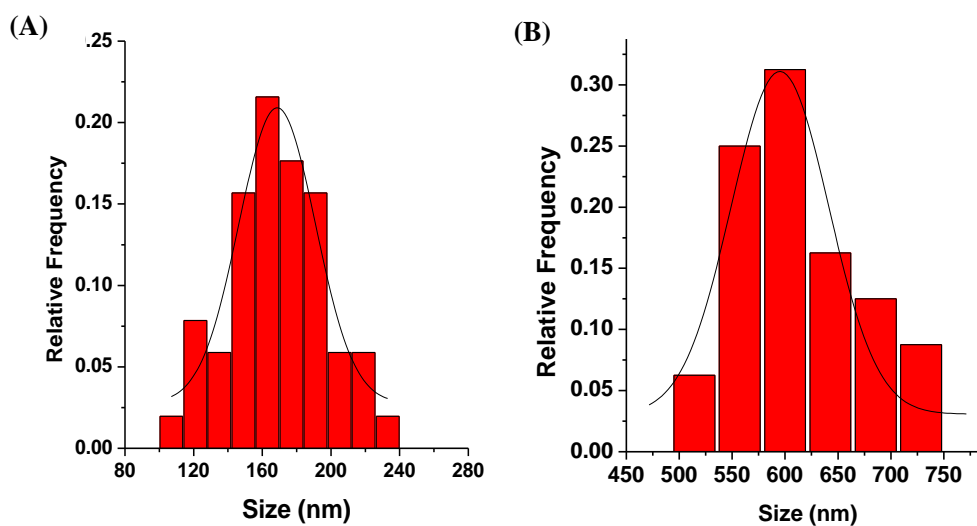


Fig. S4 TEM size distribution of NDQ FONs prepared in (A) 70% H₂O/CH₃CN and (B) in 90% H₂O/CH₃CN (v/v). 150 particles are measured to get the size distribution.

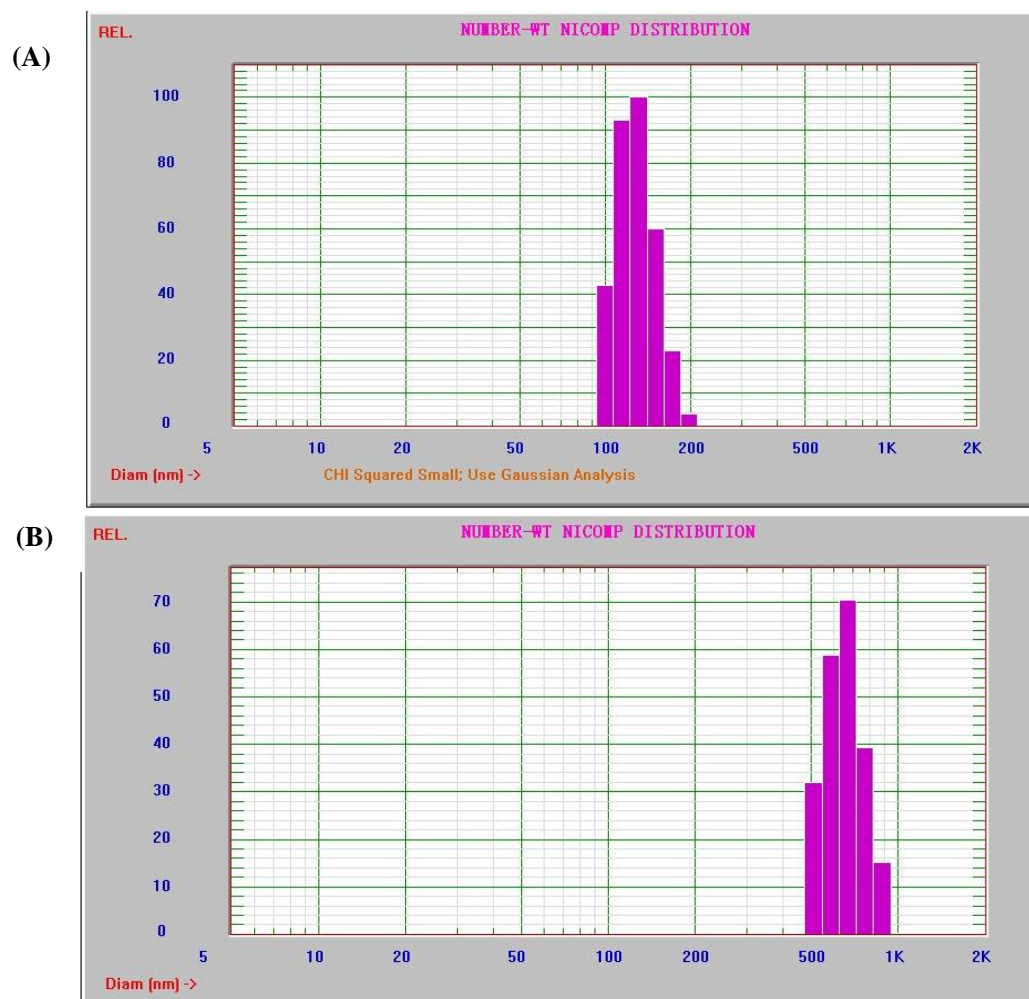


Fig. S5 Dynamic light scattering size distribution graphs of NDQ FONs prepared in (A) 70% H₂O/CH₃CN and (B) in 90% H₂O/CH₃CN (v/v).

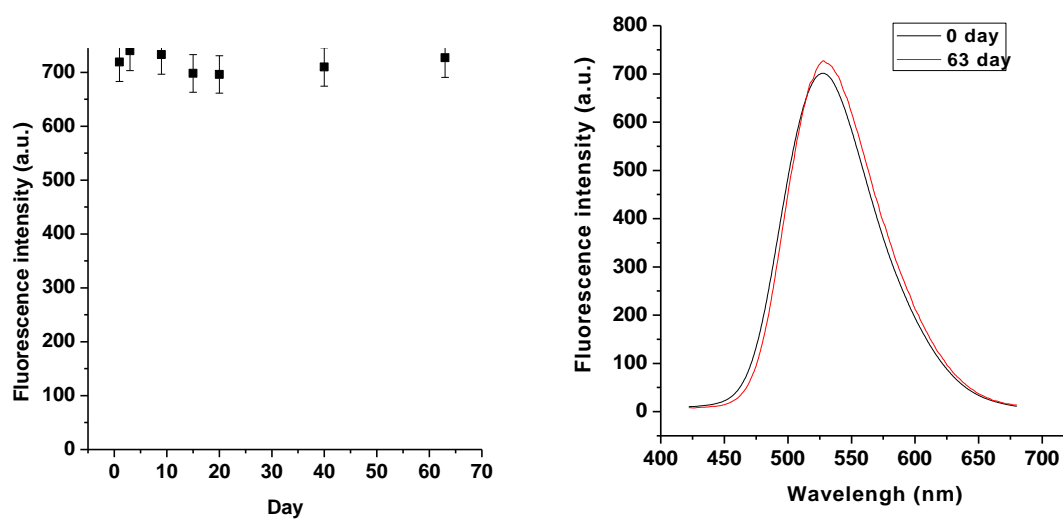


Fig. S6 The fluorescence intensity and fluorescence spectra of NDQ FONs as a function of storing time at room temperature. The results showed that the prepared

FONs are quite stable in water as no significant changes in emission intensity and wavelength were observed for more than two months.

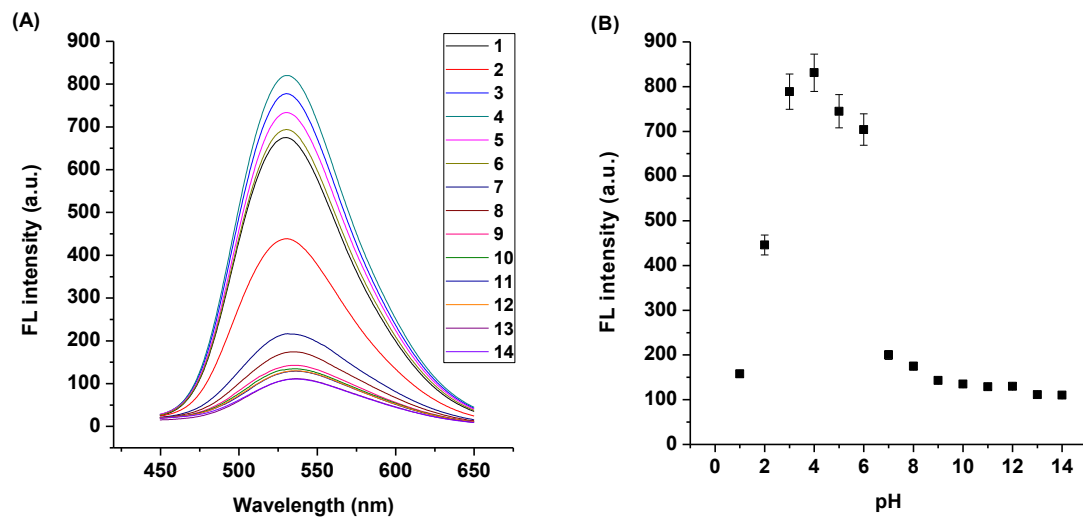


Fig. S7 (A) Fluorescent spectra of NDQ FONs at various pH values (from 1 to 14), (B) fluorescent intensity at 534 nm at different pH from 1 to 14.

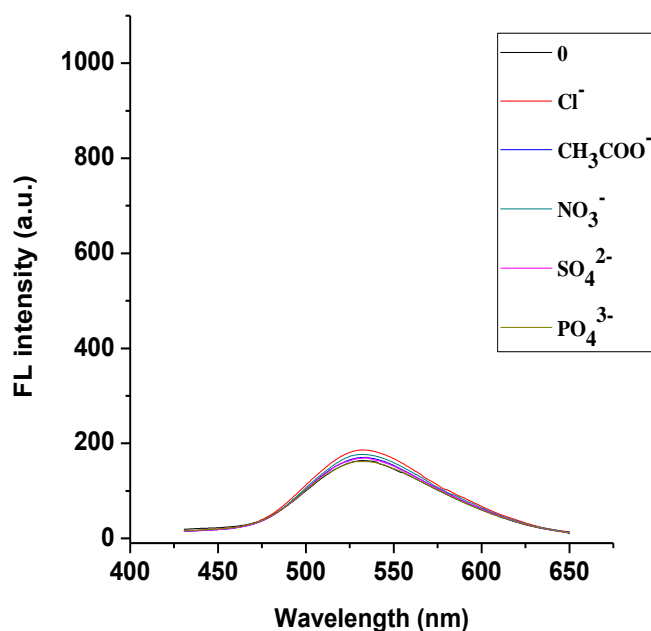


Fig. S8 Fluorescence spectra of NDQ FONs (10 μM) upon addition of a particular anions (1 mM).

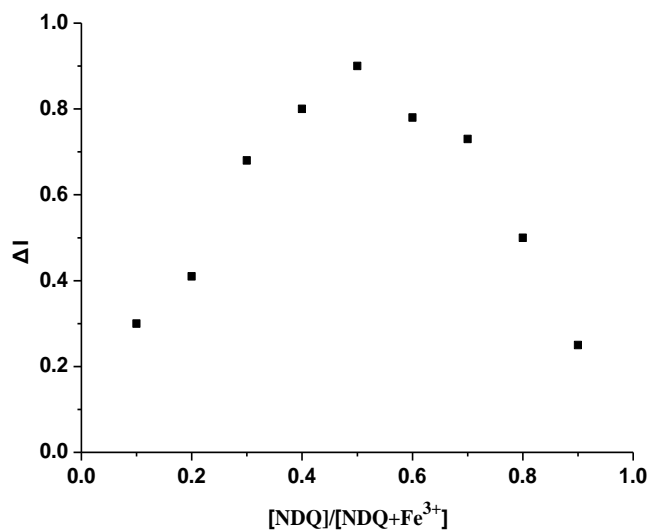


Fig. S9 Job's plot of Fe³⁺/NDQ binding according to the emission at 513 nm. The total molar concentration of 3 and Fe³⁺ is 50 μM.

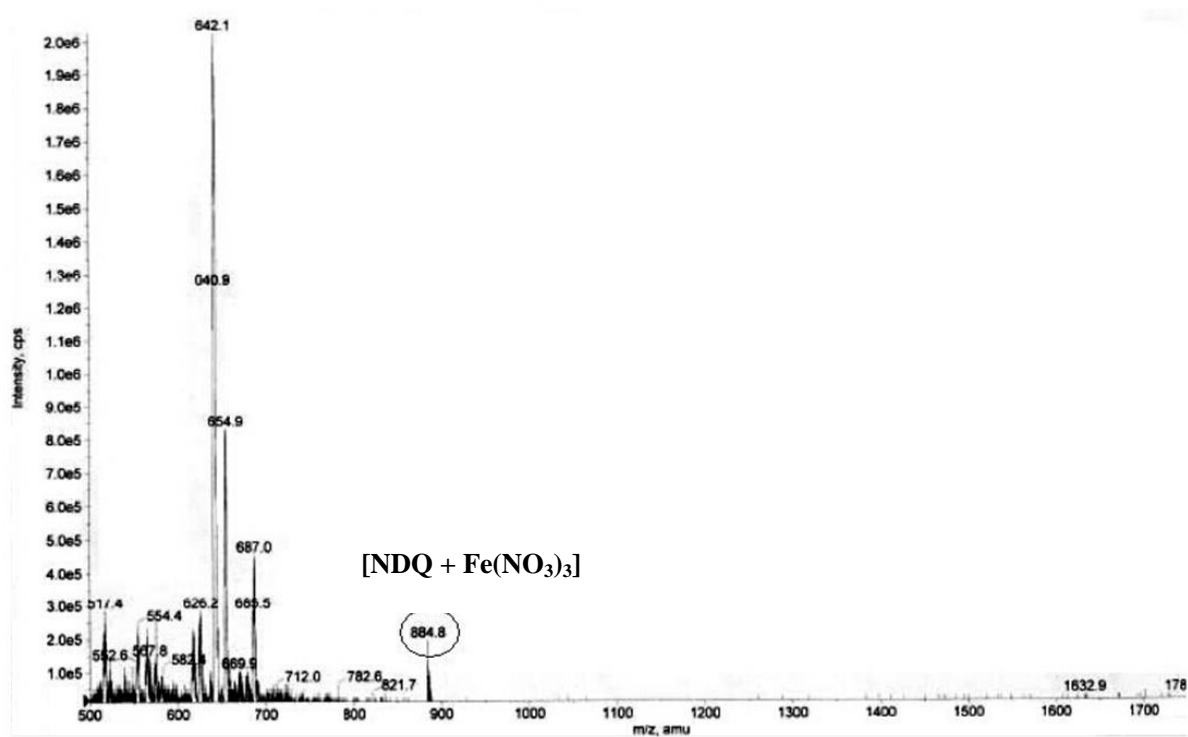


Fig. S10 Mass spectrum of complex NDQ/ Fe(NO₃)₃.

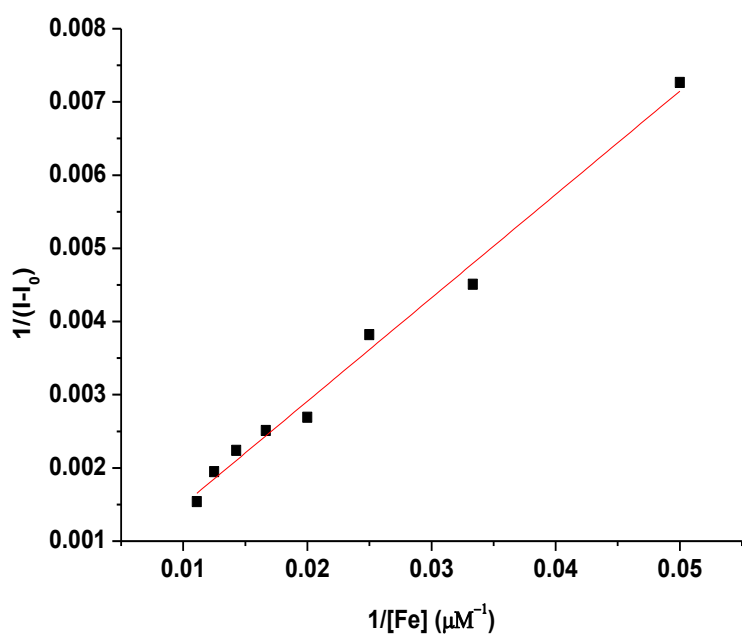


Fig. S11 Benesi-Hildebrand plot of NDQ FONs and Fe^{3+} .

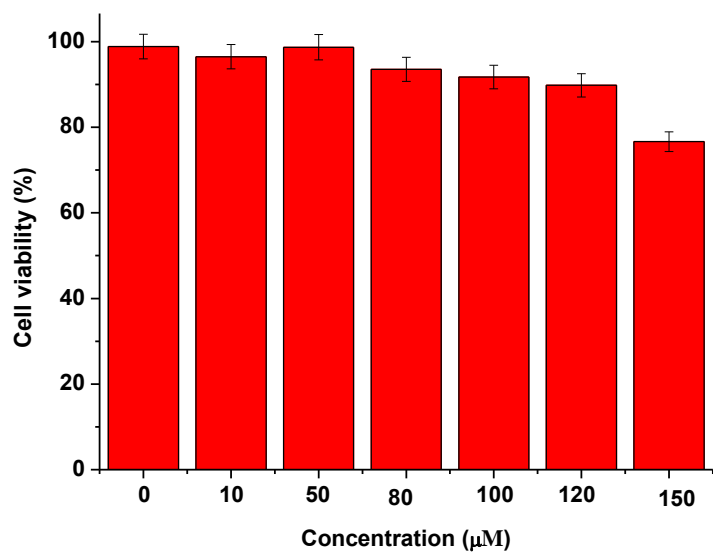


Fig. S12 Effect of NDQ FONs on Hela cells viability.