Supporting Information

Cytochrome C Encapsulated Metal Organic Framework as Bio-material for Sulfate ion Recognition

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Experimental:

Materials and instrumentation:

Bovine heart Cytochrome c (Cyt c), 2, 6-napthlene dicarboxylic acid (H_2NDC), $Mn(NO_3)_2\ 4H_2O$, DMF, Na_2CO_3 (CO_3^{2-}), $NaClO_4$ (ClO_4^-), Na_2HPO_4 (HPO_4^{2-}) and tetrabutyl ammonium salts were purchased from Sigma Aldrich. HEPES purchased from Alfa Aesar. The FTIR spectral studies were performed on Perkin Elmer FT-IR spectrometer using KBr pellets. Fluorescence studies were carried on Agilent Technologies cary eclipse fluorescence spectrophotometer. U.V studies were carried on SHIMADZU UV-2450 spectrophotometer using quartz cuvettes. The fluorescence life time was measured time correlated single photon counting (TCSPC) setup using LASER diode (405 nm) from ISS, USA (ehrones BH fluorescence life time spectrometer). Single crystal X-ray diffraction (SXRD) data was collected on dual core Agilent Technologies super nova CCD system. Powder X-ray diffraction (PXRD) data was collected on Rigaku SmartLab 9 KW rotating anode Powder X-ray diffractometer at room temperature. TEM images collected on SEI TECNAI F20 HRTEM (high resolution transmission electron microscope). The thermogravimetric analysis was carried out using Mettler Toledo thermal analyzer on N_2 atmosphere and heating speed 10 °K/ min.

X-ray crystallography details:

Single crystal X-ray structural studies of **3** were performed on a CCD Agilent Technologies (Oxford Diffraction) SUPER NOVA diffractometer. Data were collected at 293(2) K using graphite-monochromoated Mo Kα radiation (λ_α = 0.71073 Å). The strategy for the Data collection was evaluated by using the CrysAlisPro CCD software. The data were collected by the standard 'phi-omega scan techniques, and were scaled and reduced using CrysAlisPro RED software. The structures were solved by direct methods using SHELXS-97 and refined by full matrix least-squares with SHELXL-97, refining on F^2.[1]

The positions of all the atoms were obtained by direct methods. All non-hydrogen atoms were refined anisotropically. The remaining hydrogen atoms were placed in geometrically constrained positions and refined with isotropic temperature factors, generally $1.2U_{eq}$ of their parent atoms.

(1) Sheldrick, G. M. *Acta Crystallogr., Sect. A* **2008**, *A64*, 112-122. *Program for Crystal Structure Solution and Refinement;* University of Goettingen: Goettingen, Germany, 1997.

Synthesis of 3 and desolvation of 3 (4):

We synthesized Mn-MOF ($Mn_3(NDC)_3(DMF)_4$) **(3)** according to slightly modified previously reported procedure.[RS1a] 2, 6-napthlene dicarboxylic acid (H_2NDC) (0.5 mmol), $Mn(NO_3)_2$ $4H_2O$ (1 mmol) and 8 ml of DMF was placed in parr's acid digestion bomb and then heated in oven at 120 °C for 24 h. The mixture was allowed to cool at room temperature for 12 h to obtain yellow color crystals (0.128 g, yield 69.9%) which were washed with DMF (3×1 ml) and dried under vacuum at room temperature. Compound **3** was characterized by single crystal XRD. Crystal structure of **3** is given below (with and without DMF molecule).

The coordinated DMF molecules **3** were removed by reported procedure.[RS1a] Compound **3** was heated up to 200 °C (at rate of 5 K/min) under vacuum for 24 h. The color of desolvated compound **3** (named as **4**) changes from yellow to gray.

Encapsulation of cyt c in 4 (preparation of ensemble 5):

Compound **4** (100 mg) was immersed in Cyt c (10 mg in 5 ml HEPES buffer, 7.4 pH) solution and incubated at 37 °C for 72 h.[RS1b] After incubation upper orange-red color (probably Cyt c) solution converted into color less. It indicates that Cyt c entered into **4** (desolvated Mn-MOF). After the removal of upper solution the obtained solid was washed with fresh buffer (2×5 ml) solution and was dried under vacuum at room temperature. This product obtained was of orange color. We named it compound **5.**

Sample preparation for spectroscopic and microscopic studies:

2 mg of ensemble **5** was stirred in 3 ml of HEPES buffer (7.4 pH) and stirred for 10 min and allowed to stand for 5 min. The resulting dispersion was used for different spectroscopic and microscopic studies. Tetra butyl ammonium salts of F^-, Cl^-, Br^-, OAC^-, NO_3^-, HSO_4^-, SO_4^{2-}, $H_2PO_4^-$, CN^-, SCN^- and OH^- solutions were prepared in distilled water. For CO_3^{2-}, HPO_4^{2-} and ClO_4^- ions its sodium salt was used. 2.5 mg of bovine heart cytochrome c was dissolved in 3 ml of HEPES buffer, (7.4 pH).

*RS1. (a) B. Liu, R. Zou, R. Zhong, S. Han, H. Shioyama, T. Yamada, G. Maruta, S. Takeda, Q. Xu, *Micropor. Mesopor. Mat.*., 2008, **111**, 470. (b) Y. Chen, V. Lykourinou, C.Vetromile, T. Hoang, L. Ming, R. W. Larsen, S. Ma, *J. Am. Chem. Soc.*, 2012, **134**, 13188.

Computational methods

The three dimensional coordinates of cytochrome c (pdb 2B4Z)[RS2a] were obtained from the Internet at the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank. Cytochrome c is one of the most studied proteins probably due to its electron-transfer properties in aerobic and anaerobic respiration. Particularly, cytochrome c from bovine heart is a small protein, M(r) 12,230 Da, globular (hydrodynamic diameter of 3.4 nm), soluble in different buffer solutions, and commercially available. It is a well studied protein and relatively easy to manipulate from the biochemical and electrochemical viewpoint.

Docking of sulfate ion in the active site of cytochrome c was performed using Argus Lab 4.0.1. The docking program implements an efficient grid-based docking algorithm that approximates an exhaustive search within the free volume of the binding site cavity. The conformational space was explored by the geometry optimization of the sulfate ion. Thus, docking occurs between the flexible sulfate ion and enzymes. The sulfate ion orientation is determined by a shape scoring function based on Ascore and the final positions are ranked by lowest interaction energy values.

Calculations for quantum yield :

Fluorescence quantum yield was determined using optically matching solutions of pyrene (φ_R = 0.65 in ethanol) as standard at an excitation wavelength of 342 nm and quantum yield is calculated using the equation:

$$\Phi_S = \Phi_R \times (A_R / A_S) \times (D_S / D_R) \times [n_S / n_R]^2$$

φ_S and φ_R are the radiative quantum yields of sample and the reference respectively, A_S and A_R are the absorbance of the sample and the reference respectively, D_S and D_R the respective areas of emission for sample and reference, n_S and n_R are the refractive indices of the sample and reference solutions.

*RS2 (a) N. Mirkin, J. Jaconic, V. Stojanoff, A. Moreno, *Proteins*, 2008, **70**, 83.

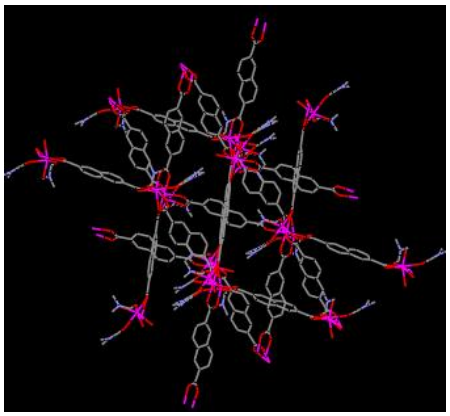

Figure S5a: Synthesized Metal organic framework **3**.

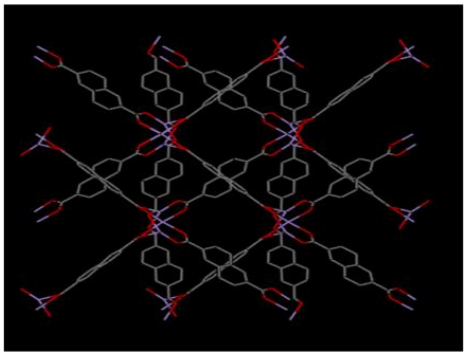

Figure S5b: Synthesized Metal organic framework **3** (along a-axis, H and DMF molecules was removed).

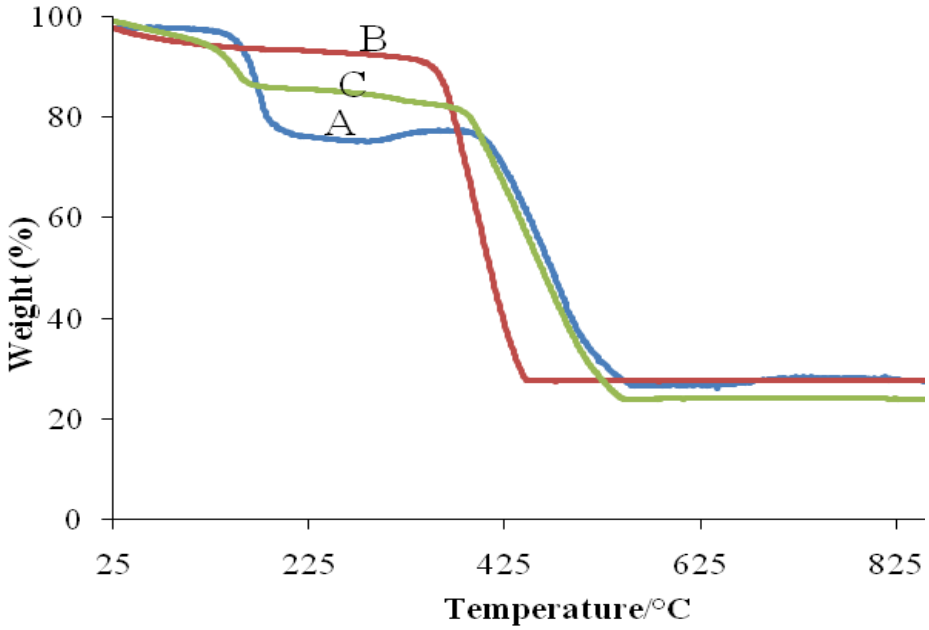

Figure S6: TGA curves of (A) **3** (B) **4** (C) **5**.

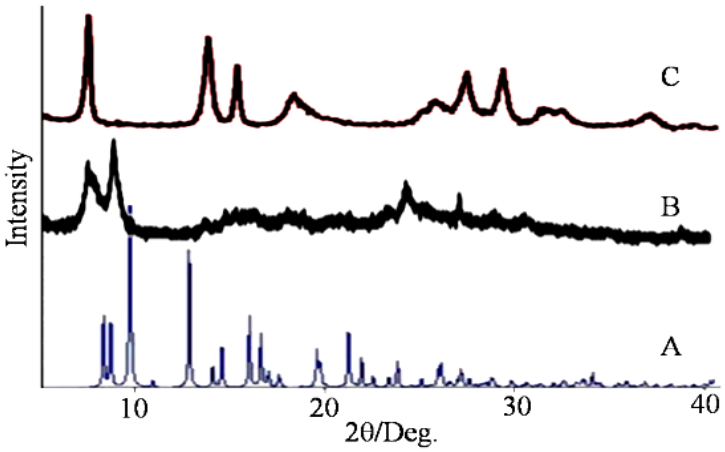

Figure S7. P-XRD of (A) **3** stimulated from single XRD data(B) **4** (C) **5**.

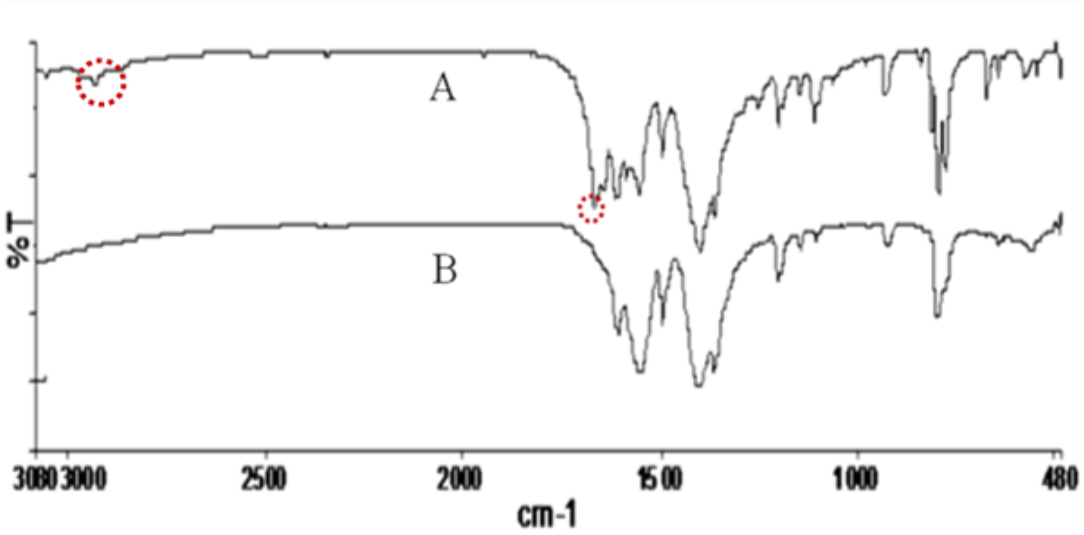

Figure S8. FT-IR spectra of (A) **3** (B) **4**.

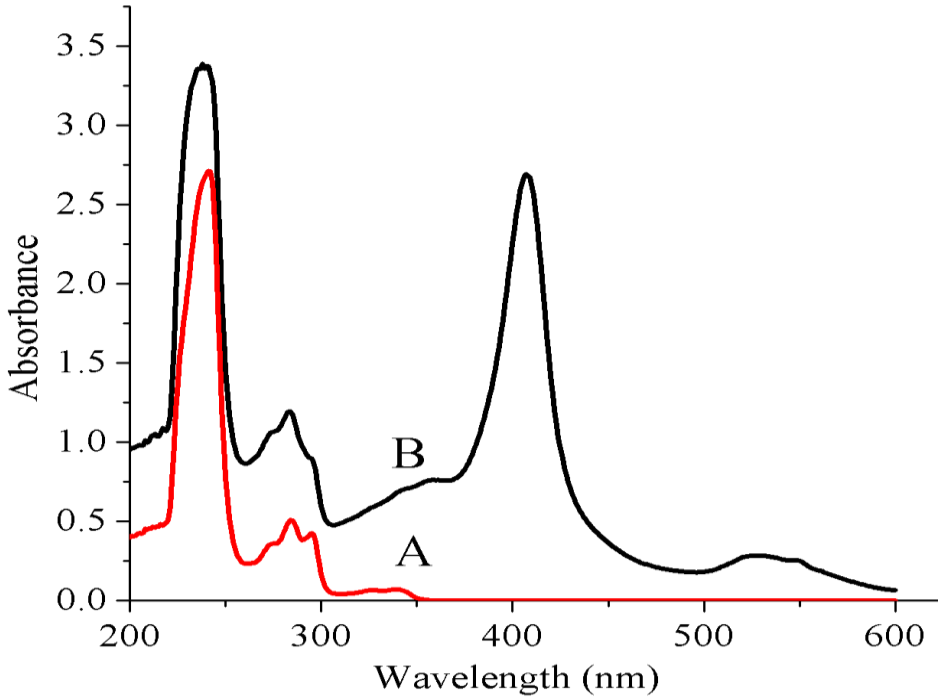

Figure S9. UV-vis spectra of (A) **4** (B) **5**.

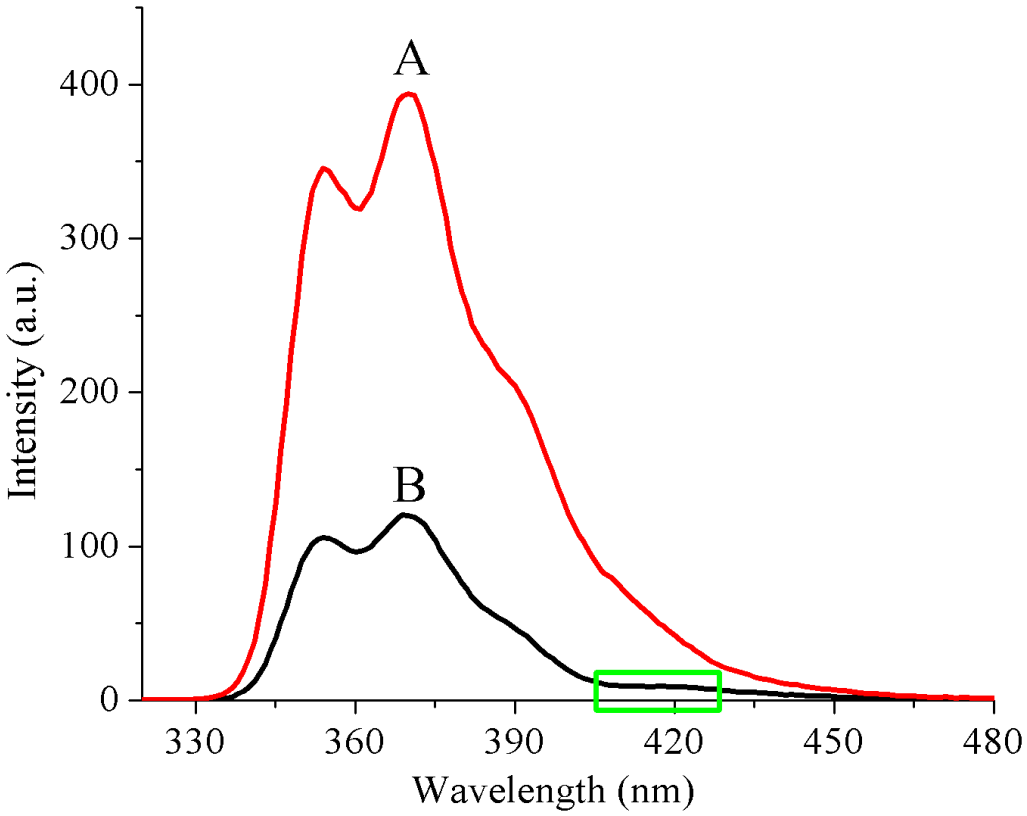

Figure S10. Fluorescence emission spectra of (A) **4** (B) **5**, λ_{ex} at 240 nm.

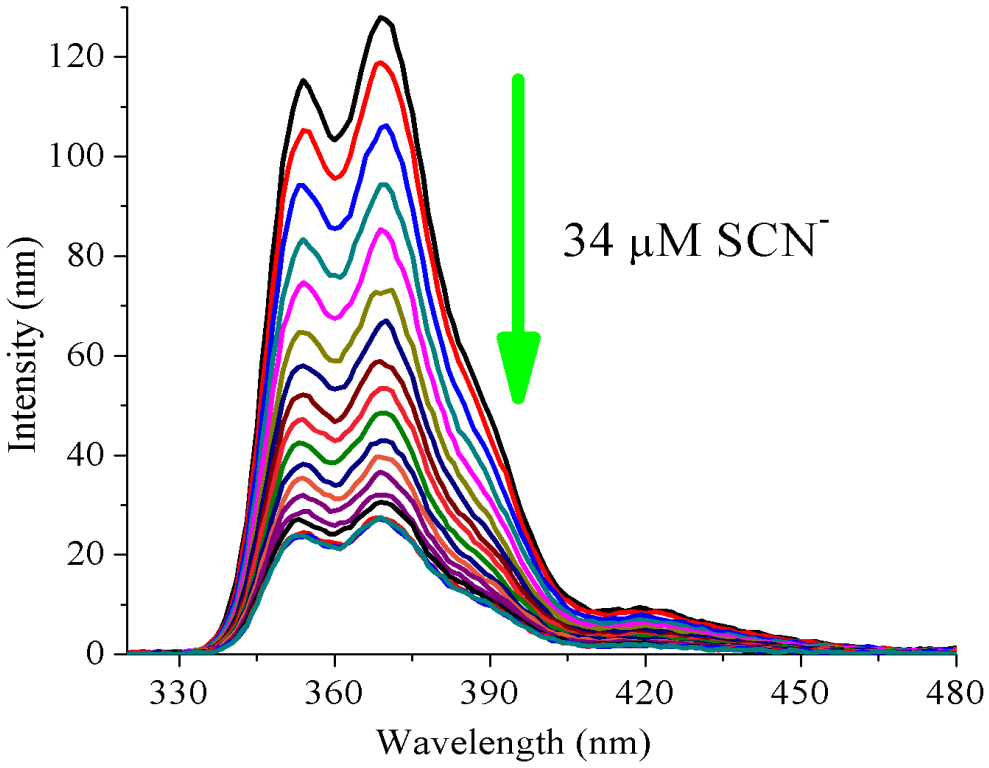

Figure S11: Fluorescence emission spectrum of **5** on addition of 34 μM of SCN^- ions (λ_{ex}= 240 nm).

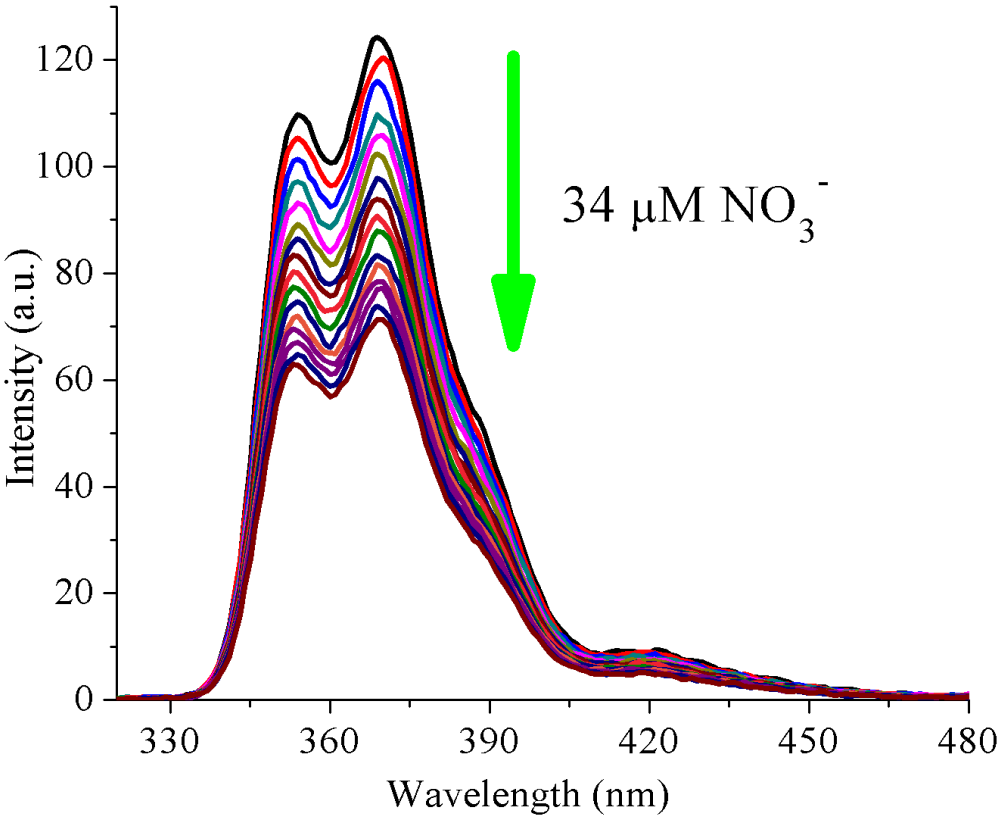

Figure S12. Fluorescence quenching emission spectra of **5** on addition of 34 μM NO_3^- (λ_{ex}= 240 nm).

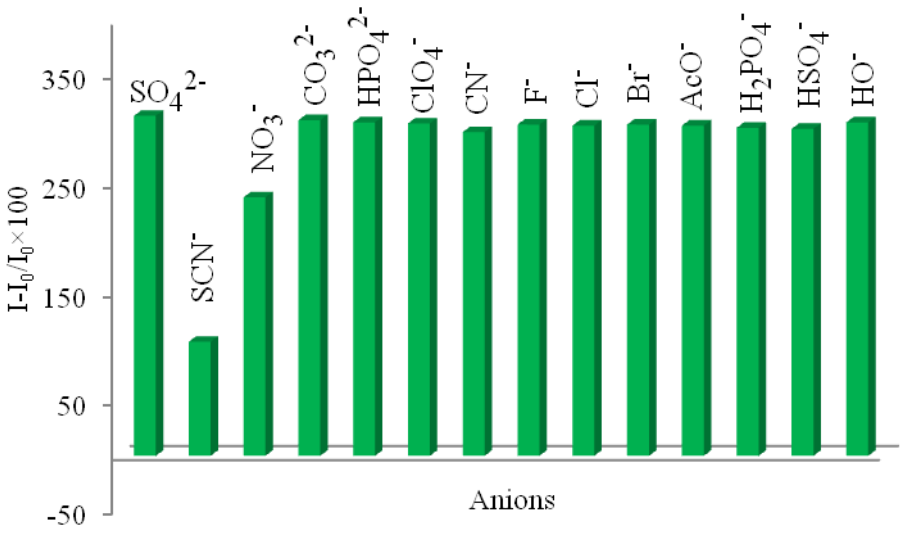

Figure S13: Fluorescence emission changes in **5** on addition of SO_4^- ions alone and SO_4^- ions in presence of other anions. I = the final fluorescence intensity on addition of anions and I_0 is the initial fluorescence intensity of **5**.

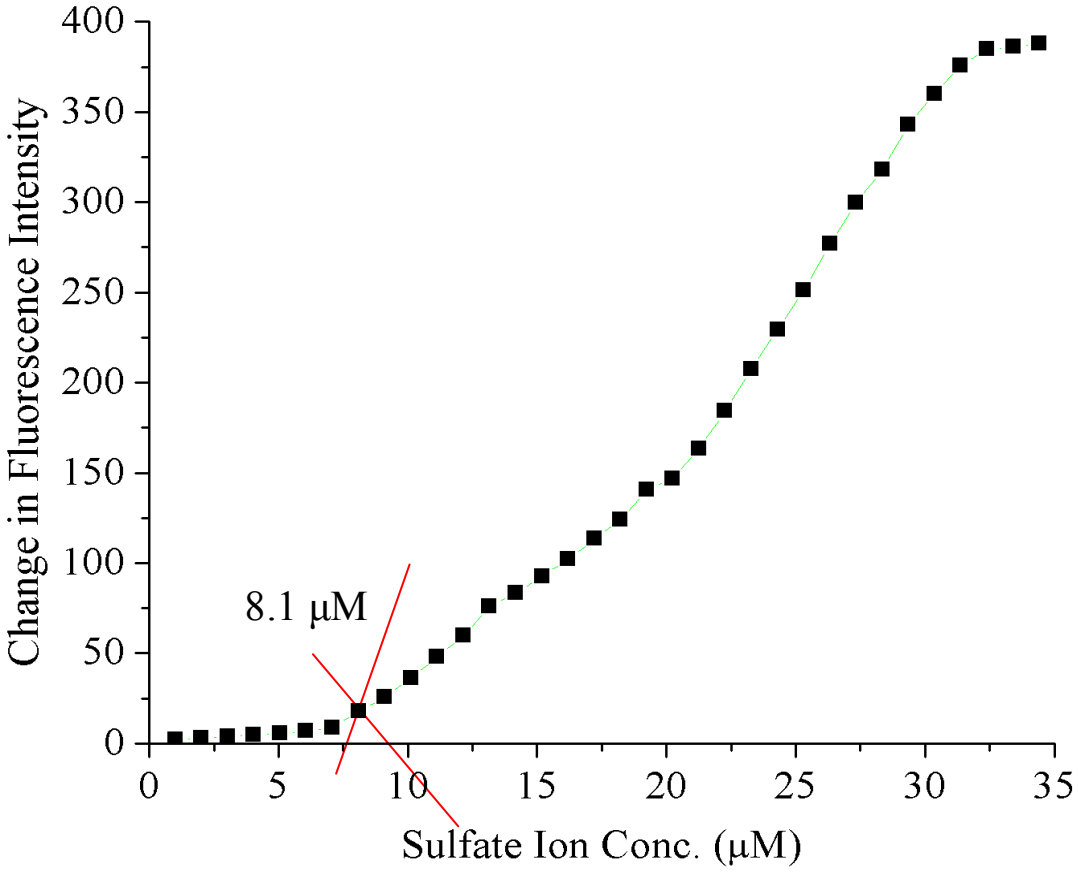

Figure S14: Variation of Fluorescence intensity with sulfate ion concentration and detection limit of sulfate ion is 8.1 µM.

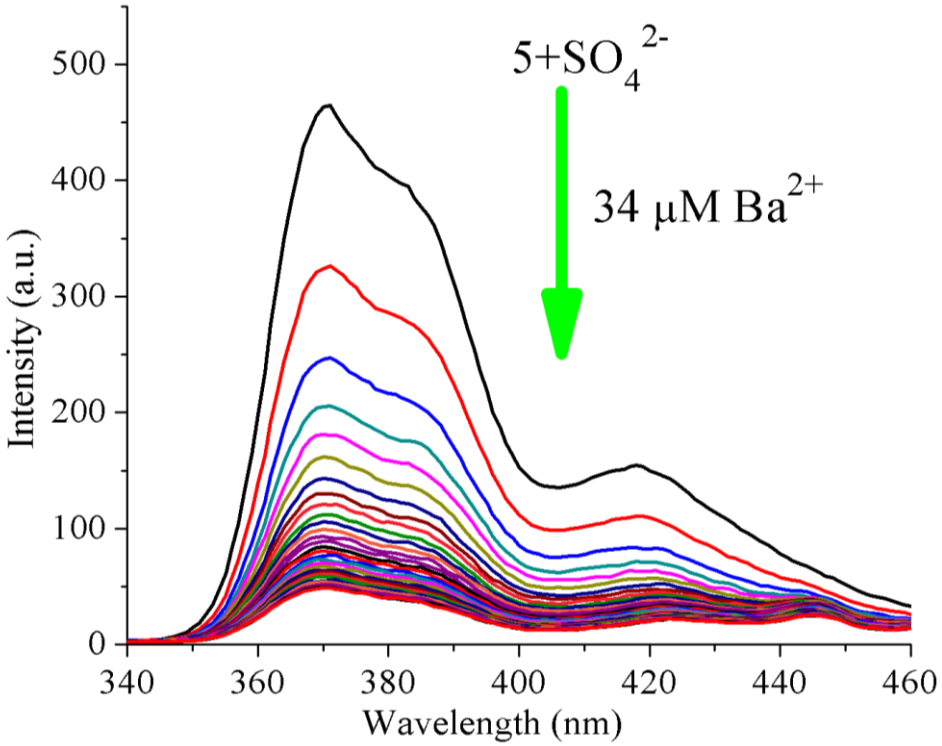

Figure S15: The fluorescence back titration of the **5**+ SO_4^{2-} on the addition of 34 μM Ba^{2+} ($BaCl_2$ $2H_2O$ solution with same concentration of SO_4^{2-}).

Table S1: Selectivity from reported sensors/receptors for sulfate ion

S.NO.	Limit of detection for sulfate ion	Selectivity over other anions	Solvent used	Technique used	Reference
1	Less than 30 μM	Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, AcO^-, $H_2PO_4^-$	DMSO/H_2O	NMR & UV	RS3
2	μM level	Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, CN^-, N_3^-	CH_3OH/CH_3CN	Fluorescence & NMR	RS4
3	15 μM (Approx)	Selective based on sulfate encapsulation	DMF	Crystallization	RS5
4	10 μM	Redox-driven sulfate transfer between two receptors	DMSO	NMR, Uv & Fluorescence	RS6
5	----------	NO_3^-, ClO_4^-, AcO^-, $H_2PO_4^-$	CH_3CN/H_2O	NMR & Uv	RS7
6	----------	Cl^-	CD_3OH/DMSO-D_6	NMR	RS8
7	Between 0.1 to 0.5 mM)	F^-, Cl^-, Br^-,HSO_4^-, $H_2PO_4^-$,NO_3^-, ClO_4^-, AcO^-.	$CHCl_3$	NMR, Uv & CV	RS9
8	----------	$H_2PO_4^-$,NO_3^-, Cl^-, AcO^-.	DMSO-D_6	NMR	RS10
9	----------	Cl^-, Br^-, AcO^-.	$CDCl_3$/CD_3OD	NMR	RS11
10	----------	NO_3^-, HSO_4^-.	DMSO-D_6	NMR	RS12
11	----------	Cl^-, Br^-, I^-, $H_2PO_4^-$.	DMSO-D_6	NMR	RS13
12	----------	Cl-, HSO_4^-, AcO^-, HCO3-, $H_2PO_4^-$, BzO^-.	DMSO-D_6/H_2O	NMR	RS14
13	10 μM (approx)	Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, AcO^-, H_2PO_4.	DMSO	NMR & Uv	RS15
14	2 mM	Cl^-, Br^-, I^-, NO_3^-, ClO_4^-.	D_2O	NMR	RS16
15	----------	Cl^-, I^-, NO_3^-, ClO_4^-, AcO^-, HPO_4^{2-}, SeO_4^{2-}, $S_2O_3^{2-}$.	D_2O	NMR	RS17
16	----------	PO_4^{3-}	THF/Water	Potentiometry	RS18
17	0.5 μM	F^-, Cl^-, Br^-, I^-, HSO_4^-, CN^-, SCN^-, OH^-, $H_2PO_4^-$,NO_3^-, ClO_4^-, AcO^-.	DMSO/ H_2O (pH 7.4)	Fluorescence & Uv	RS19
18	0.1-50 μM	F^-, Cl^-, Br^-, I^-, CN^-, PO_4^{3-}, ClO_4^-, AcO^-.	DMSO/H_2O	Fluorescence & Uv	RS20
This work					
19	8.1 μM	CO_3^{2-}, HPO_4^{2-}, F^-, Cl^-, Br^-, OAC^-, NO_3^-, HSO_4^-, $H_2PO_4^-$, CN^-, SCN^-, ClO_4^- and OH^- (selective from 13 other anions)	100% Water (HEPES buffer, pH 7.4)	Fluorescence & Uv	

RS3. C. D. Jia, B. A. Wu, S. G. Li, X. J. Huang, Q. L. Zhao, Q. S. Li and X. J. Yang, *Angew. Chem., Int. Ed.*, 2011, **50,** 486.
RS4. J.-i. Kim, H. Juwarker, X. Liu, M. S. Lah and K.-S. Jeong, *Chem. Commun.*, 2010, **46,** 764.
RS5. Z. W. Yang, B. A. Wu, X. J. Huang, Y. Y. Liu, S. G. Li, Y. N. Xia, C. D. Jia and X. J. Yang, *Chem. Commun.*, 2011, **47,** 2880–2882.
RS6. M. Li, Y. Hao, B. Wu, C. Jia, X. Huang and X.-J. Yang, *Org. Biomol. Chem.*, 2011, **9,** 5637.
RS7. D. A. Jose, D. K. Kumar, B. Ganguly and A. Das, *Inorg. Chem.*, 2007, **46,** 5817.
RS8. M. J. Kim, H. W. Lee, D. Moon and K. S. Jeong, *Org. Lett.*, 2012, **14,** 5042.
RS9. M. Li, B. Wu, C. Jia, X. Huang, Q. Zhao, S. Shao and X.-J. Yang, *Chem.–Eur. J.*, 2011, **17,** 2272.
RS10. I. Ravikumar and P. Ghosh, *Chem. Commun.*, 2010, **46,** 6741.
RS11. B. Huang, S. M. Santos, V. Felix and P. D. Beer, *Chem. Commun.*, 2008, 4610.
RS12. M. C. Das, S. K. Ghosh and P. K. Bharadwaj, *CrystEngComm*, 2010, **12,** 413.
RS13. M. G. Fisher, P. A. Gale, M. E. Light and S. J. Loeb, *Chem. Commun.*, 2008, 5695.
RS14. P. A. Gale, J. R. Hiscock, C. Z. Jie, M. B. Hursthouse and M. E. Light, *Chem. Sci.*, 2010, **1,** 215.
RS15. C. Jia, B. Wu, S. Li, Z. Yang, Q. Zhao, J. Liang, Q.-S. Li and X.-J. Yang, *Chem. Commun.*, 2010, **46,** 5376.
RS16. J. S. Mendy, M. L. Pilate, T. Horne, V. W. Day and Md. A. Hossain, *Chem. Commun.*, 2010, **46,** 6084.
RS17. P. Mateus, R. Delgado, P. Brandao, S. Carvalho and V. Felix, *Org. Biomol. Chem.*, 2009, **7,** 4661.
RS18. P. D. Beer, J. Cadman, J. M. Lloris, R. Martinez-Manez, M. E. Padilla, T. Pardo, D. K. Smith and J. Soto, *J. Chem. Soc., Dalton Trans.*, 1999, 127.
RS19. R. Saini and S. Kumar, *RSC Adv.*, 2013, **3,** 21856.
RS20. S. Kaur, A. Kaur, N. Kaur, *Materials Letters*, 2013, **100,** 19.

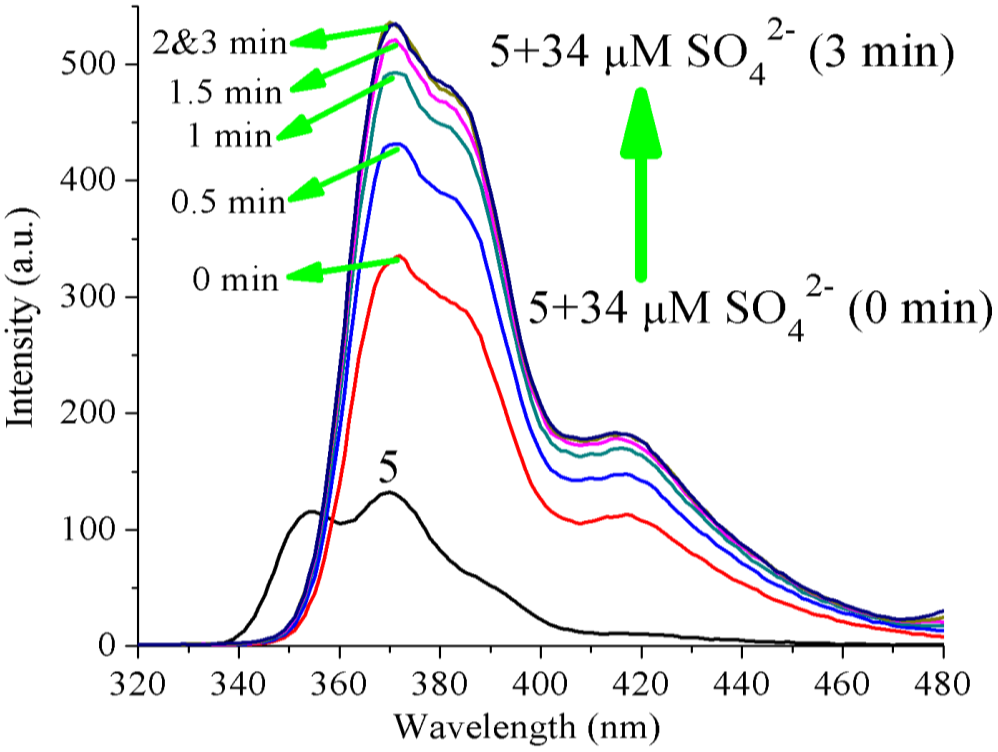

Figure S16: Time scale fluorescence response of the sulfate ion with **5**.

Table **S2**: Fluorescence life time correlation measurement table of **5** & **5**+SO_4^{2-} ions

	5	**5** + SO_4^{2-}
τ_1	4.54 ns, 85.75 %	0,95 ns, 32.40 %
τ_2	0.97ns, 14.25 %	0.13 ns, 50.05 %
τ_3	------No-----	5.07 ns, 17.55 %
τ_{avg}	4.03 ns	1.27 ns
χ^2	0.962	1.3

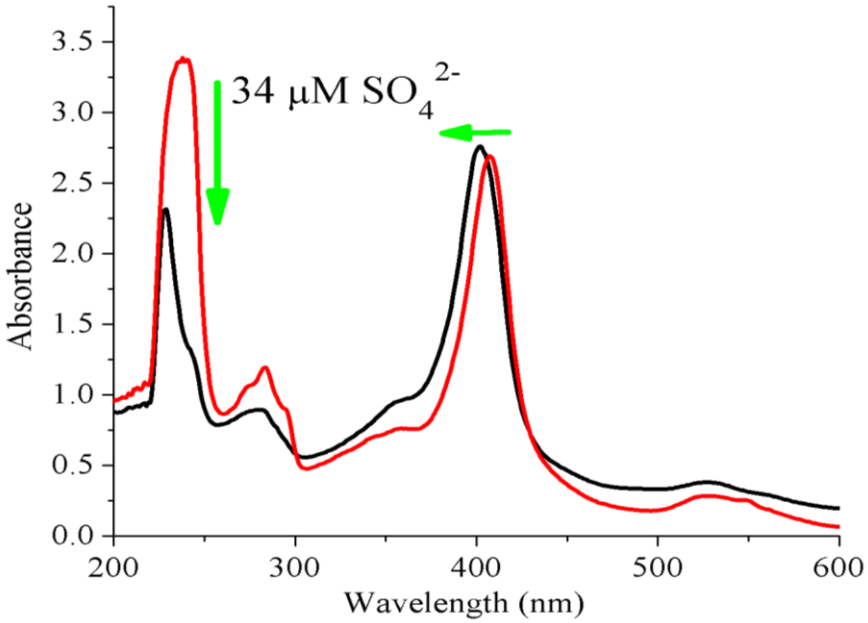

Figure. S17. Uv-vis spectra of **5** upon addition of SO_4^{2-} ions.

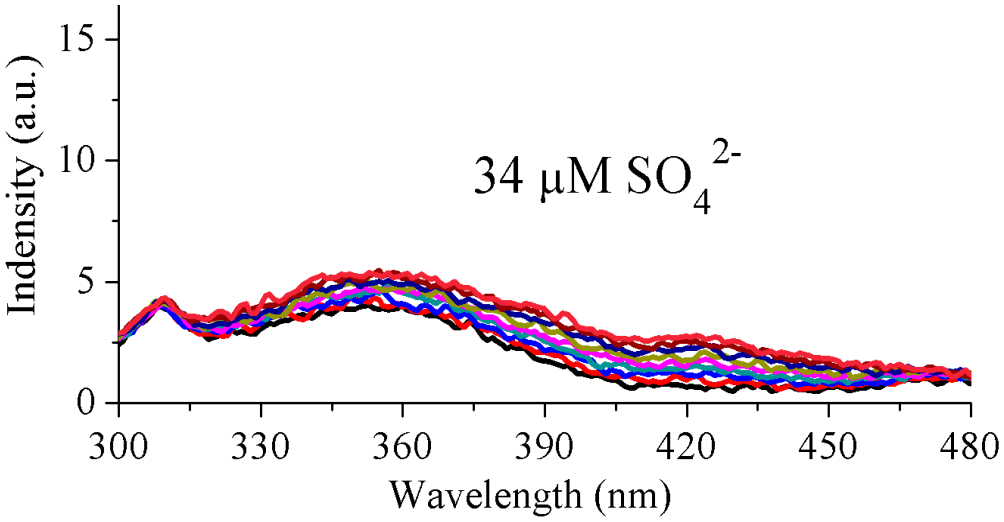

Figure S18. Fluorescence emission spectra of Cytochrome *c* on addition of 34 μM SO_4^- (λ_{ex}= 280 nm).

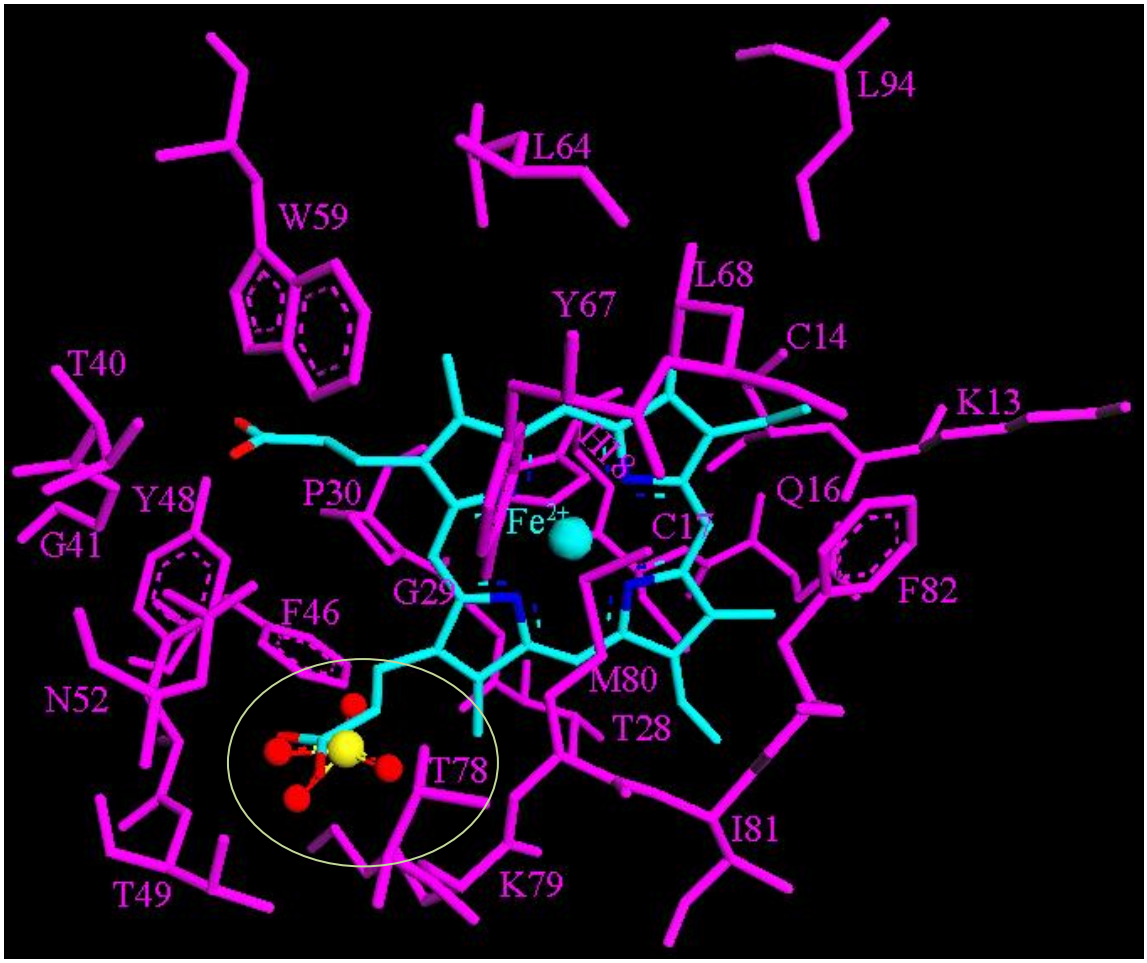

Figure. S19. Docking studies performed with the help of Argus. Sulfate ions docked in the active site of cyt c. Sulfate ion (encircled) is in close proximity to amino acids T49, K79 & M80. The location of sulfate ions is far away from Fe^{2+} ions.