Supporting Information

Cd(II) triggered excimer-monomer conversion of a pyrene derivative: Time dependent red-shift of monomer emission with cell staining application

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Experimental

Materials and methods

High purity HEPES, 2-hydroxy naphthaldehyde and diethylenetriamine were purchased from Sigma Aldrich (India). $Cd(NO_3)_2.4H_2O$ and $CdCl_2$. H_2O were purchased from Merck (India). Solvents used were of spectroscopic grade. Other chemicals were of analytical reagent grade and had been used without further purification except when specified. Mili-Q Milipore® 18.2 MΩ cm^{-1} water was used throughout all the experiments. A JASCO (model V-570) UV–Vis spectrophotometer was used for recording UV-Vis spectra. FTIR spectra were recorded on a JASCO FTIR spectrophotometer (model: FTIR-H20). Mass spectra were performed on a QTOF Micro YA 263 mass spectrometer in ES positive mode. ^1H NMR spectra were recorded using Bruker Avance 300 (300MHz) in DMSO-d_6. Elemental analysis was performed using Perkin Elmer CHN-Analyzer with first 2000-Analysis kit. The steady-state fluorescence emission and excitation spectra were recorded with a Hitachi F-4500 spectrofluorimeter. All pH measurements were performed with Systronics digital pH meter (model 335). All spectra were recorded at room temperature except for fluorescence microscope images.

Imaging system

The imaging system was comprised of an inverted fluorescence microscope (Leica DM 1000 LED), digital compact camera (Leica DFC 420C), and an image processor (Leica Application Suite v3.3.0). The microscope was equipped with a mercury 50 W lamp.

Synthesis of (0E)-N1-((E)-2-((pyren-7-yl)methyleneamino)ethyl)-N2-((pyren-7 yl)methylene)ethane-1,2-diamine ***(L)***

An ethanol solution of pyrene aldehyde (1g, 4.34 mmol) was added drop wise to a solution (30 mL) of diethylenetriamine (0.234 mL, 2.17 mmol) in ethanol under stirring condition at room temperature (Scheme 1). Stirring was continued for further 30 minutes followed by reflux for 10 h. Upon removal of the solvent, a yellow colored residue was obtained. The residue was recrystallized from ethanol. Yield 86 %; M. P. 153^{o}C (± 4^{o}C); QTOF – MS ES^{+} (ESI, Fig. S8): $[M + H]^{+}$ = 528.03 ; elemental analysis data as calculated for $C_{38}H_{29}N_3$ (%): C, 86.50; H, 5.54; N, 7.96. Found (%): C, 85.92; H, 5.43; N, 7.62. FTIR (cm^{-1}) (ESI, Fig. S10): υ(NH) 3376.83, υ(C=N) 1635.8; ^{1}H NMR (300MHz, DMSO-d_6) (ESI, Fig. S14): 3.07 (4H, m); 3.912 (4H, m); 5.15 (1H, s) 7.92-8.49 (10H, m); 8.54 (2H, d, J = 7.5 Hz); 8.60 (2H, d, J = 9.3 Hz); 8.93 (2H, d, J = 9.3 Hz); 9.02 (2H, d, J = 9.9 Hz); 9.33 (2H, d, J = 15 Hz).

*Synthesis of [**L**+Cd^{2+}+DMSO+NO_3^-+H_2O]*

A 10 mL DMSO: water (1:1, v/v) solution of $Cd(NO_3)_2.4H_2O$ (0.116 g, 0.379 mmol) was added slowly to a magnetically stirred solution (10 mL) of the ligand (**L**) (0.2 g, 0.379 mmol) in DMSO. Stirring was continued for 6 h. On slow evaporation of the solvent, a yellow color compound was obtained. The compound was collected from methanol.

QTOF –MS ES^+ (ESI, Fig. S9): $[M+H]^+$ = 800.09. FTIR (cm^{-1}) (ESI, Fig. S11): υ(NH) 3423.98, υ(OH), 3423.98; υ($-NO_3^-$), 1384.16.

Preparation and imaging of cells

Candida albicans cells (IMTECH No. 3018) from exponentially growing culture in yeast extract glucose broth medium (pH 6.0, incubation temperature, 37°C) were centrifuged at 3000 rpm for 10 minutes washed twice with 0.1 M HEPES buffer (pH 7.4). Then cells were treated with Cd^{2+} (10 μM) for 30 minutes in 0.1 M HEPES buffer (pH 7.4) containing 0.01 % Triton X100 as permeability enhancing agent. After incubation, the cells were washed with HEPES buffer and incubated with **L** (10 μM) for 15 minutes. Cells thus obtained were mounted on grease free glass slide and observed under the fluorescence microscope having UV filter. Cells incubated with **L** but without Cd^{2+} were used as control.

Pollen grains were obtained from freshly collected mature buds of *Allamanda puberula* (Aapocynaceae), a common ornamental plant with bell shaped bright yellow flower by crashing stamens on a sterile petriplate and suspending them in normal saline. After crashing the stamina debrishes are removed by filtering through a thin layer of non absorbent cotton and the suspended pollens are collected by centrifugation at 5000 rpm for five minutes. The pollen pellet was then washed twice in normal saline and then incubated in a solution of chromium nitrate (1 mg mL^{-1}) for one hour at ambient temperature. After incubation they are again washed in normal saline as mentioned above and then photographed under various objectives using UV filter in a LEICA Fluorescence microscope in presence and absence of the ligand.

Both Cd^{2+} treated and untreated cells were stained with **L** and observed under fluorescence microscope.

General method of UV-Vis and fluorescence titration

Path length of the cells used for absorption and emission studies was 1 cm. For UV-Vis and fluorescence titrations, 10 μM stock solution of **L** was prepared in DMSO/water (4/1, v/v) HEPES (0.1M) buffer. Working solutions of **L** and Cd^{2+} were prepared from their respective stock solutions. Fluorescence measurements were performed using 5 nm x 5 nm slit width. All the emission and absorbance spectra were recorded after 30 minutes of mixing **L** and Cd^{2+}.

Calculation of Quantum Yield

Fluorescence quantum yields (Φ) were estimated by integrating the area under the fluorescence curves using the equation,

$$\phi_{sample} = \phi_{ref} \times \frac{OD_{ref} \times A_{sample} \times \eta^2_{sample}}{OD_{sample} \times A_{ref} \times \eta^2_{ref}}$$

where A was the area under the fluorescence spectral curve, OD was optical density of the compound at the excitation wavelength and η was the refractive indices of the solvent. Anthracene was used as quantum yield standard (quantum yield is 0.27 in ethanol)[2] for measuring the quantum yields of **L** and [**L**-Cd^{2+}].

Job's plot from fluorescence experiments

A series of solutions containing **L** and Cd^{2+} were prepared such that the total concentration of Cd^{2+} and **L** remain constant (10 μM) in all the sets. The mole fraction (X) of **L** was varied from 0.1 to 0.9. The fluorescence intensity at 450 nm was plotted against the mole fraction of **L** in solution.

Determination of Binding Constant

Tsein group[3] proposed an equation, modified by Li *et al.* which has been used to determine the stoichiometry of the [**L** – Cd^{2+}] complex.

$$[M^{n+}]^m = \frac{1}{n \cdot K} \cdot \frac{1}{[L]_T^{n-1}} \cdot \frac{1-\alpha}{\alpha^n}$$

Where K is complex equilibrium constant, M_mL_n is the complex species, L is ligand and M is the metal ion. Third bracket is used to denote their concentration. α is the ratio between free ligand concentration [L], and its initial concentration $[L]_T$. In our case, the stoichiometric ratio of the Cd^{2+}: **L** was 1:1. So, this equation can be written as $[Cd^{2+}]$ = 1/K(1-α)/α. The curve fitting of the experimental data points using this equation yielded, $K_a = (2.11 \pm 0.05) \times 10^5\ M^{-1}$.

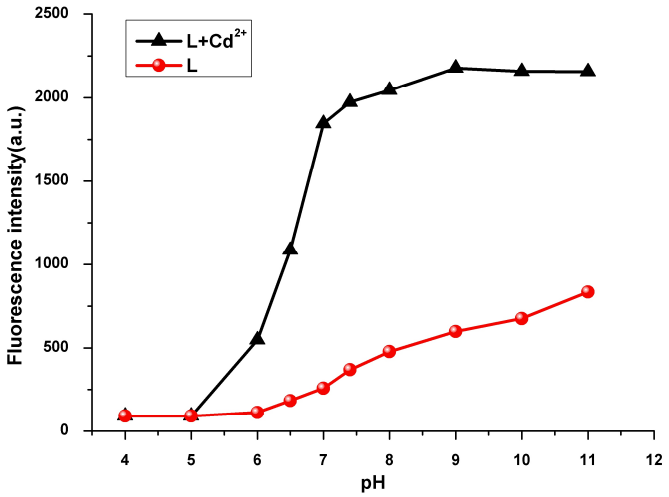

Fig.S1 Effect of pH on the binding efficiency of **L** (10 μM) towards Cd^{2+} (30 equivalents) in HEPES buffer (0.1 M), (DMSO/water = 4/1, v/v) (λ_{em}: 450 nm, λ_{ex}: 375 nm).

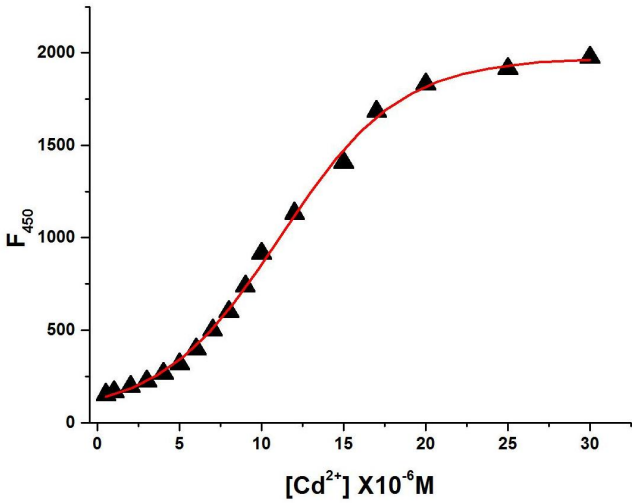

Fig.S2 Plot of fluorescence intensity of **L** (10μM) as a function of externally added $[Cd^{2+}]$ = 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 25, 30 μM [in 0.1 M HEPES buffered, DMSO: water (4:1, v/v), λ_{em}: 450 nm, λ_{ex}: 375 nm].

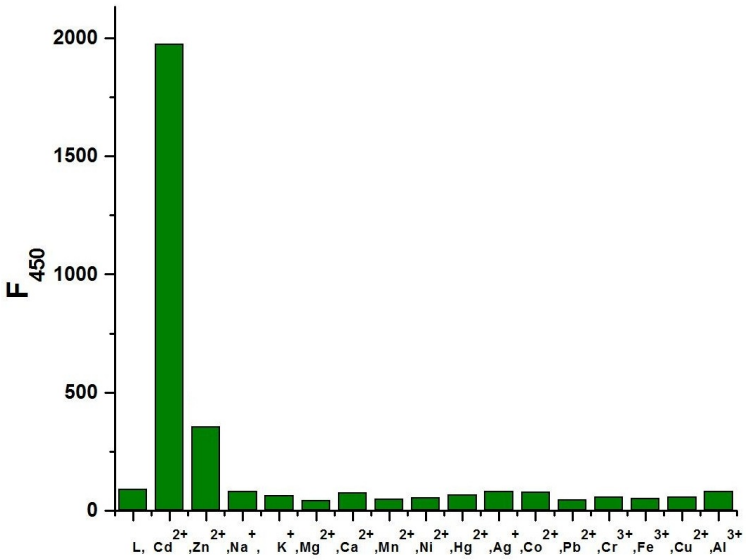

Fig.S3 Effect of different metal ions (M^{n+}) on the fluorescence intensity of **L** (10 μM); (M^{n+} = Cd^{2+}, Zn^{2+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Ni^{2+}, Hg^{2+}, Ag^{+}, Co^{2+}, Pb^{2+}, Cr^{3+}, Fe^{3+}, Cu^{2+}, Al^{3+} (30 μM) (λ_{em}: 450 nm, λ_{ex}: 375 nm, HEPES buffered DMSO: water (4:1, v/v).

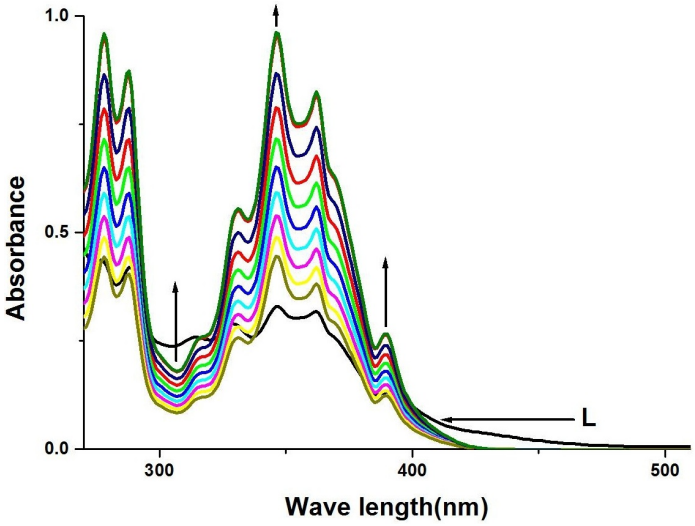

Fig.S4 Absorption spectra of **L** (10 μM) as a function of externally added [Cd^{2+}]. From bottom to top: [Cd^{2+}] = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μM [solvent = 0.1 M HEPES buffered, DMSO: water (4:1, v/v), λ_{em}: 450 nm, λ_{ex}: 375 nm].

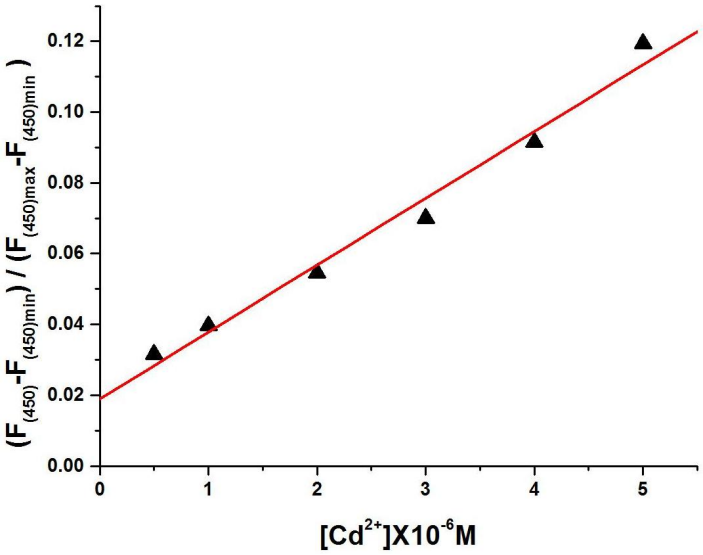

Fig.S5 Emission intensities of **L** (10 μM) as a function of externally added [Cd^{2+}] in HEPES buffer (0.1 M in DMSO/water = 4/1, v/v, pH 7.4, λ_{em}: 450 nm, λ_{ex}: 375 nm). The detection limit is 1.8×10^{-8}.

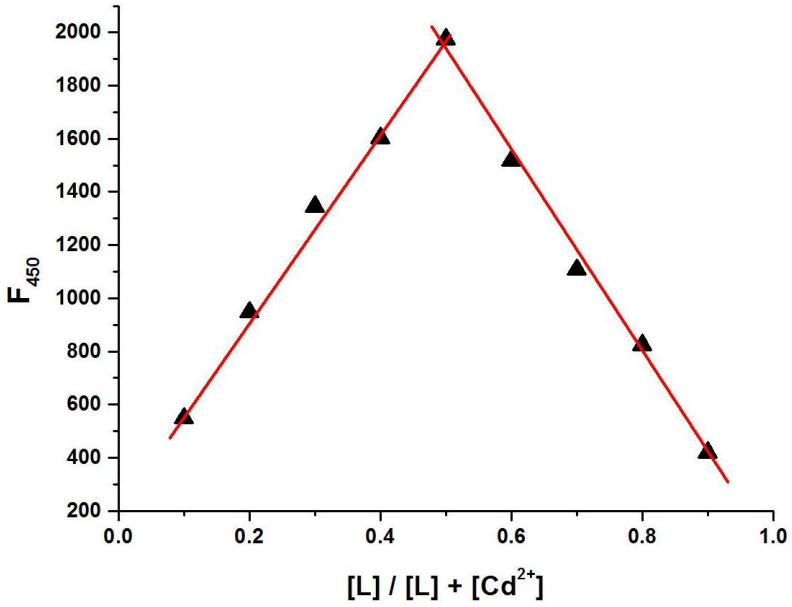

Fig.S6 Job's plot of the complexation reaction between **L** and Cd^{2+} in HEPES buffer (0.1 M) solution (DMSO/water = 4/1, v/v, pH 7.4).

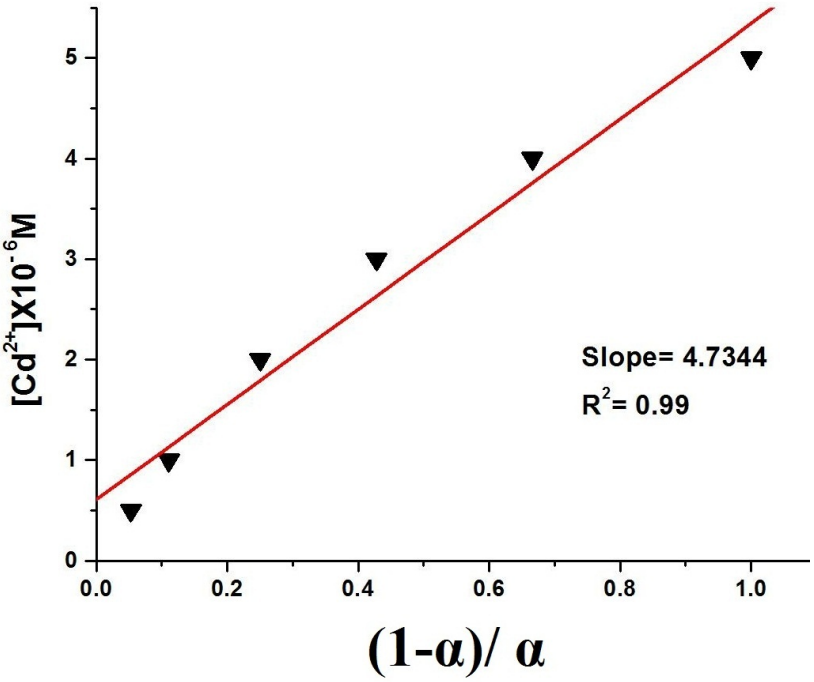

Fig.S7 Determination of binding constant (K_a) of **L** for Cd^{2+} using Li's equation

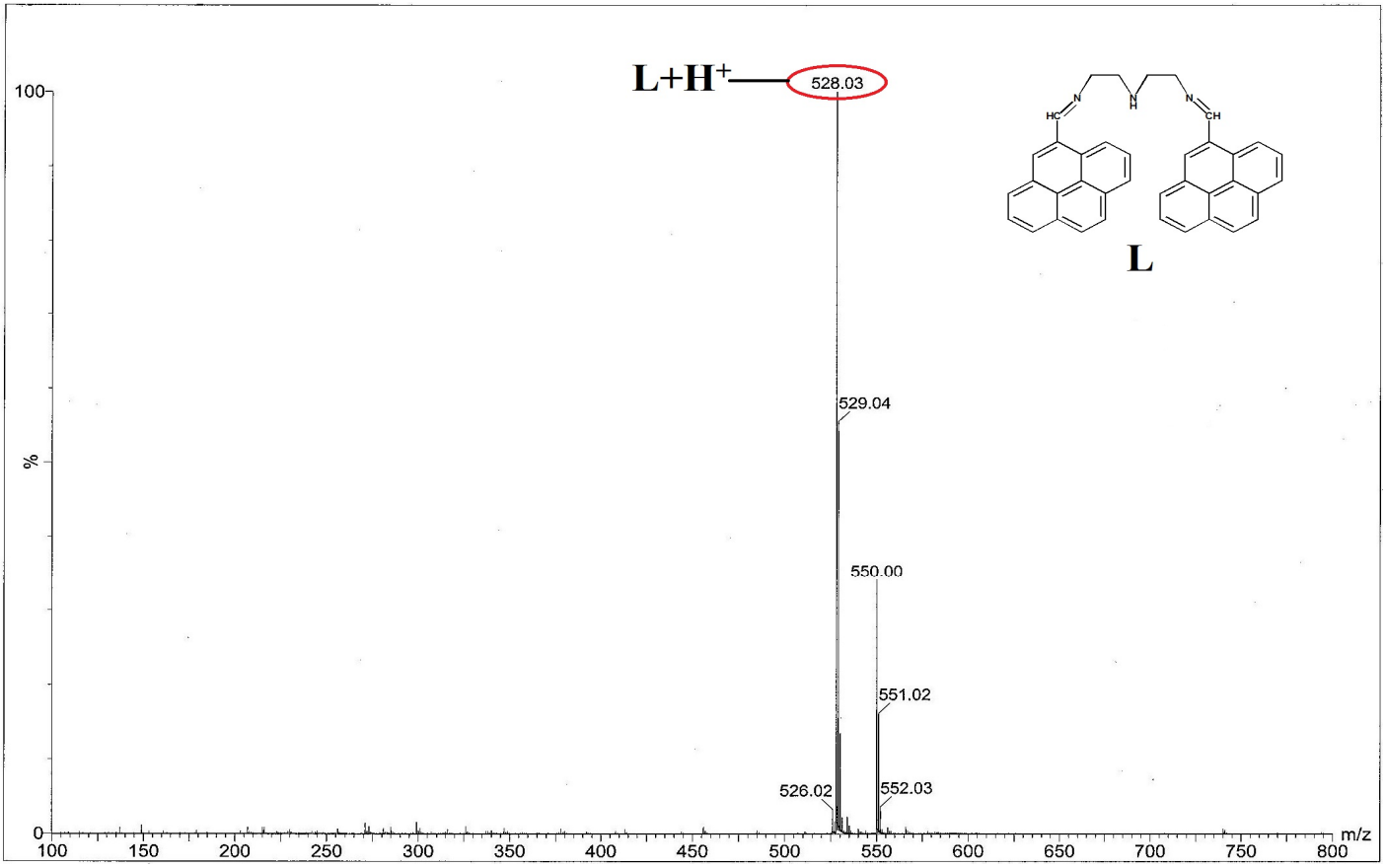

Fig.S8 QTOF-MS spectrum of **L**

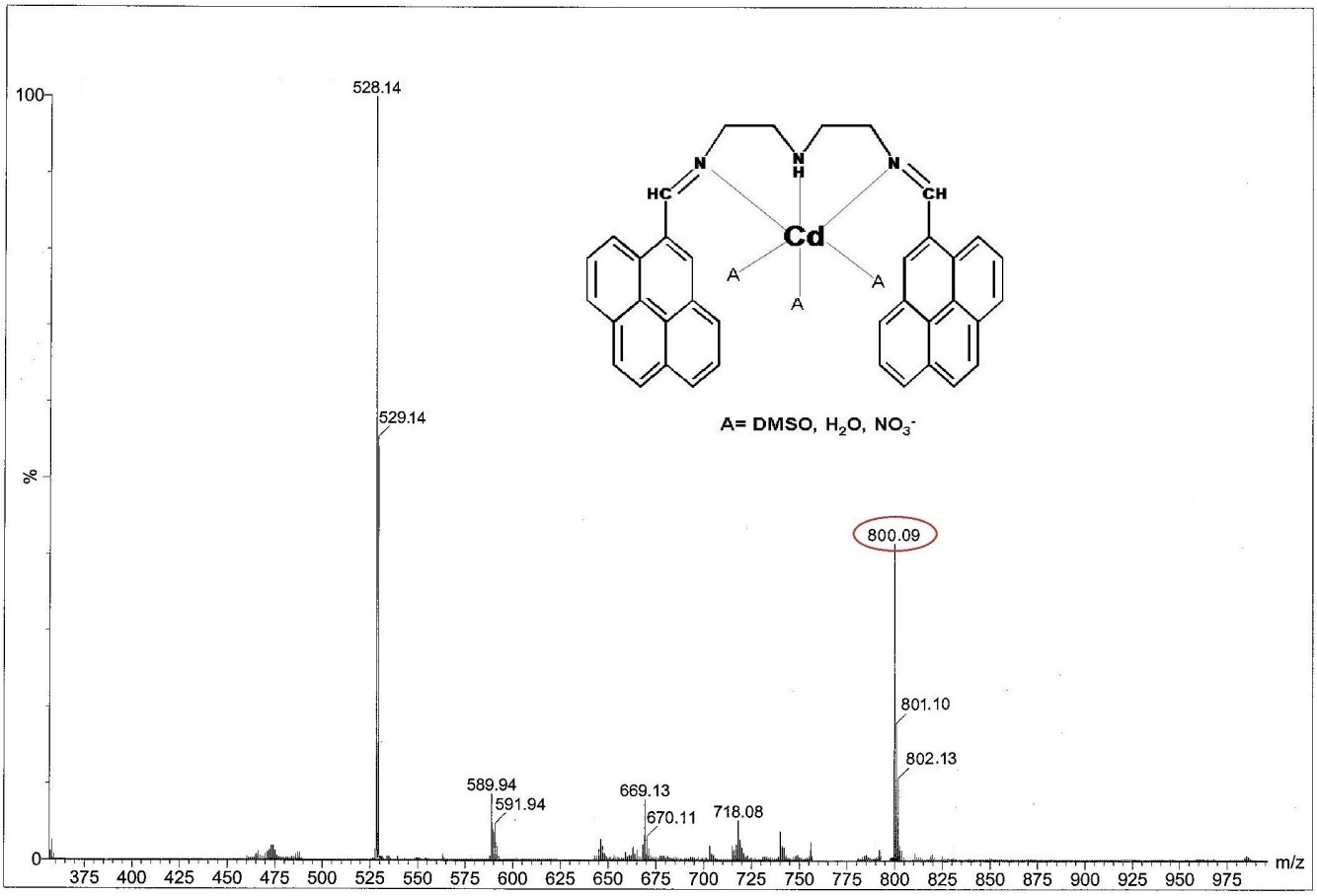

Fig.S9 QTOF-MS spectrum of **L**+Cd^{2+}+DMSO+NO_3^-+H_2O+H^+

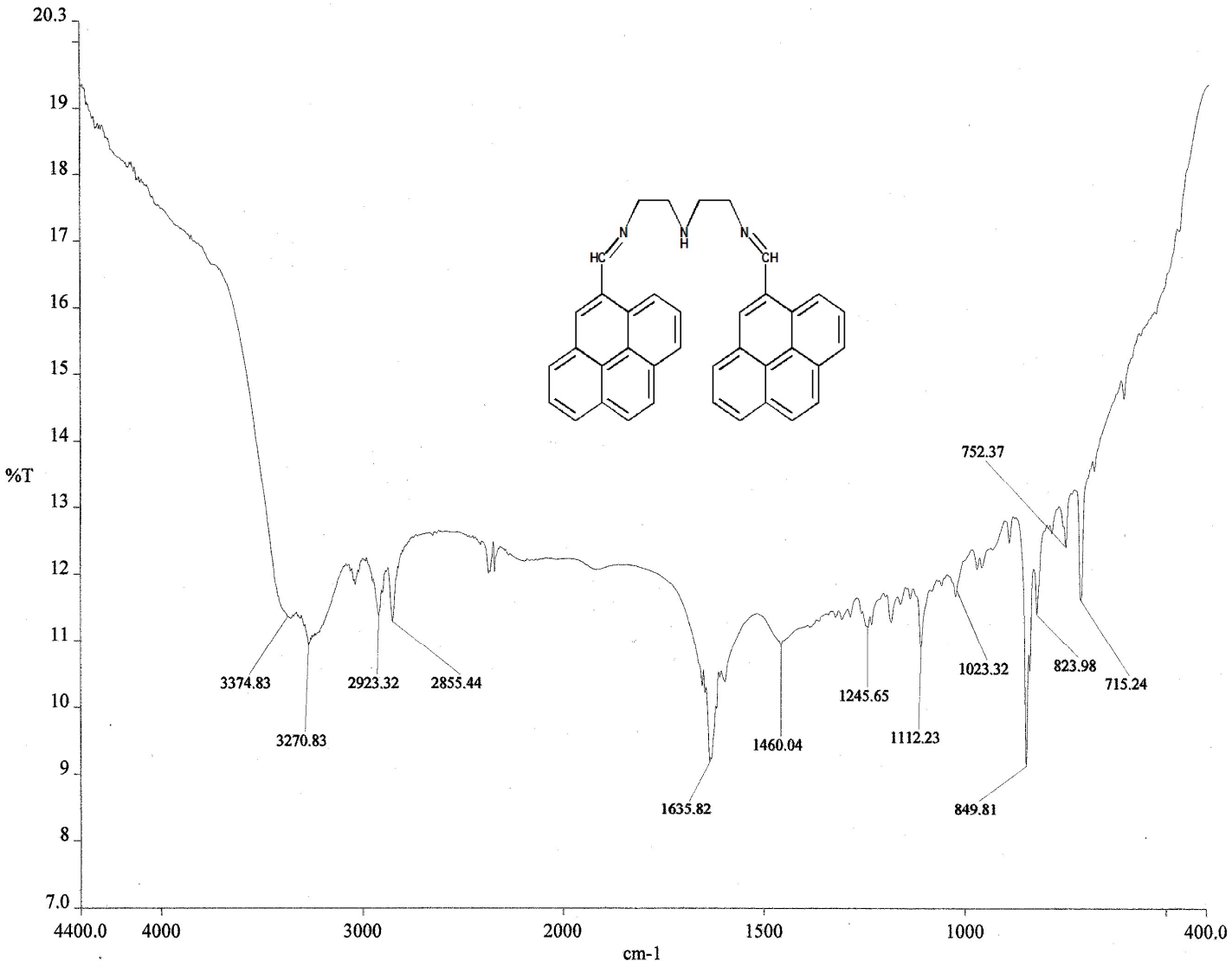

Fig.S10 FTIR spectrum of **L**

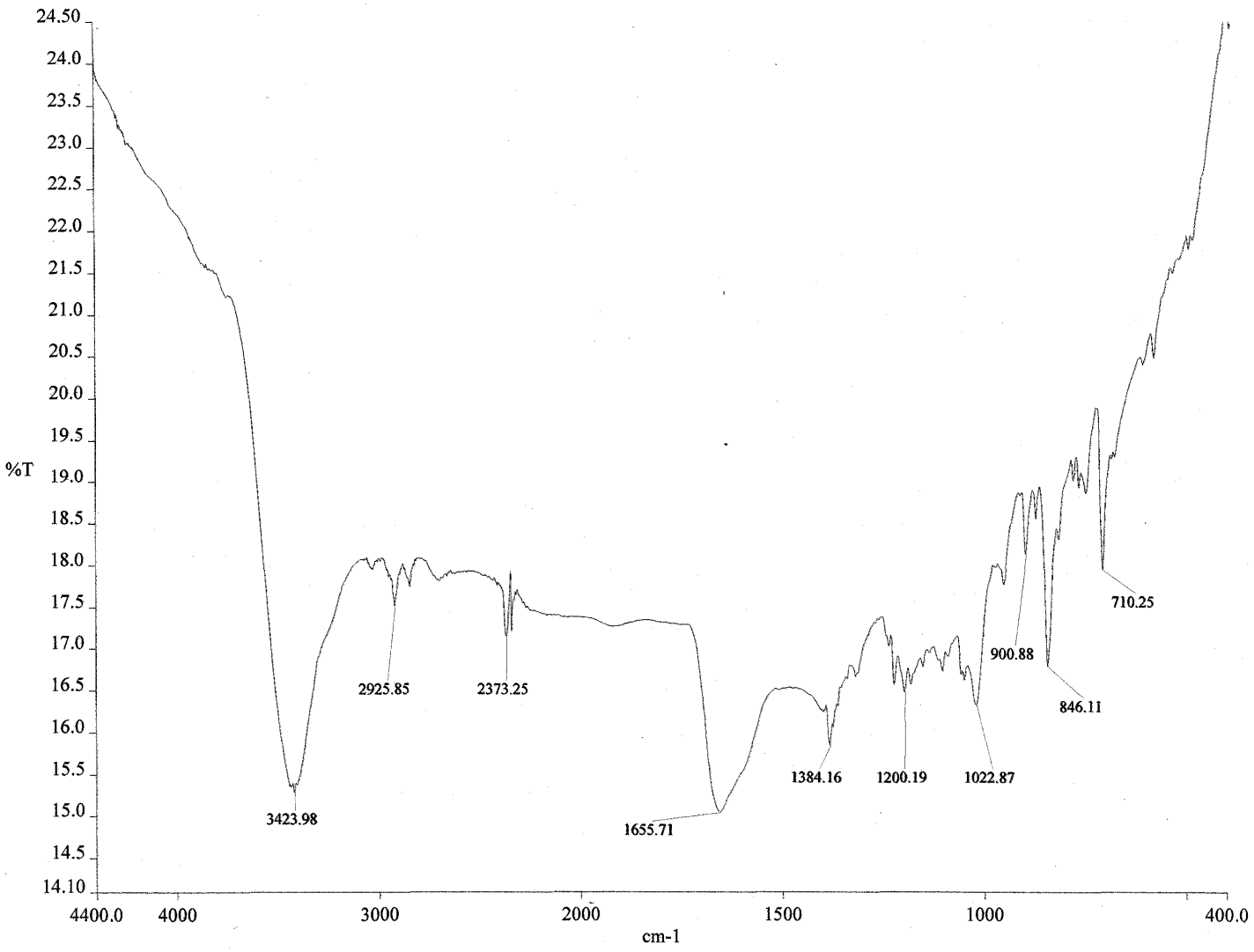

Fig.S11 FTIR spectrum of **[L-**Cd^{2+}] complex

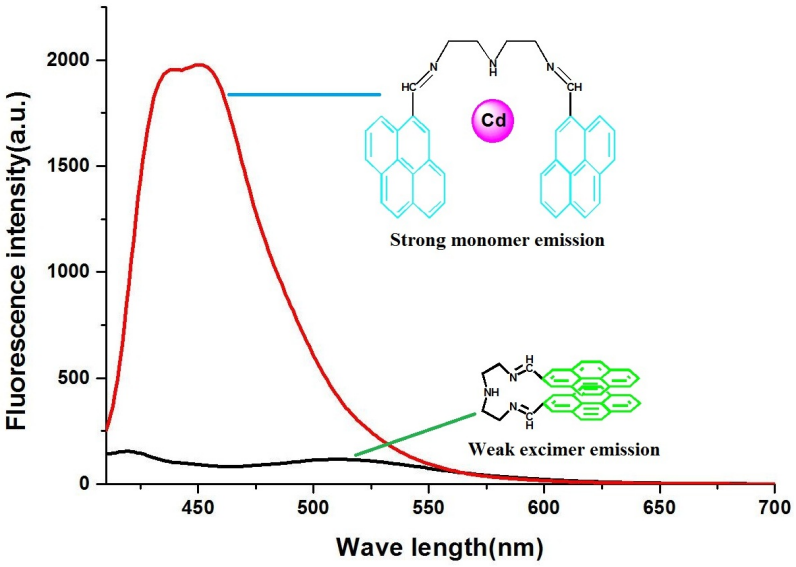

Fig.S12 The changes of fluorescence intensities of pyrene excimer and monomer measured at 525 and 450 nm respectively.

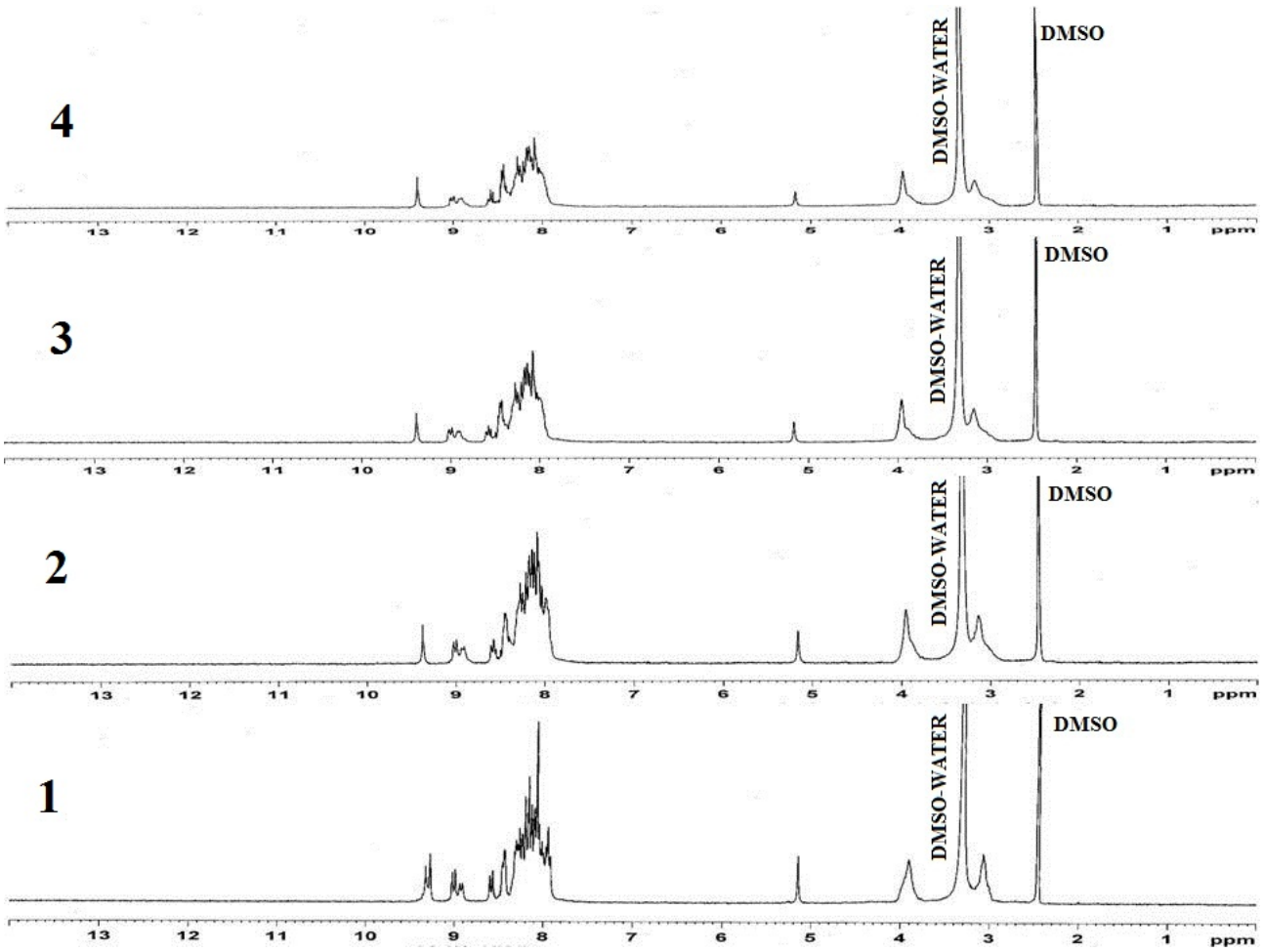

Fig.S13 ^{1}H NMR spectra of **L** in presence of $Cd(NO_3)_2.4H_2O$ in DMSO-*d*6: (1) **L**; (2) **L** with 1.0 equivalent of Cd^{2+}; (3) **L** with 2.0 equivalent of Cd^{2+}; (4) **L** with 3.0 equiv. of Cd^{2+}.

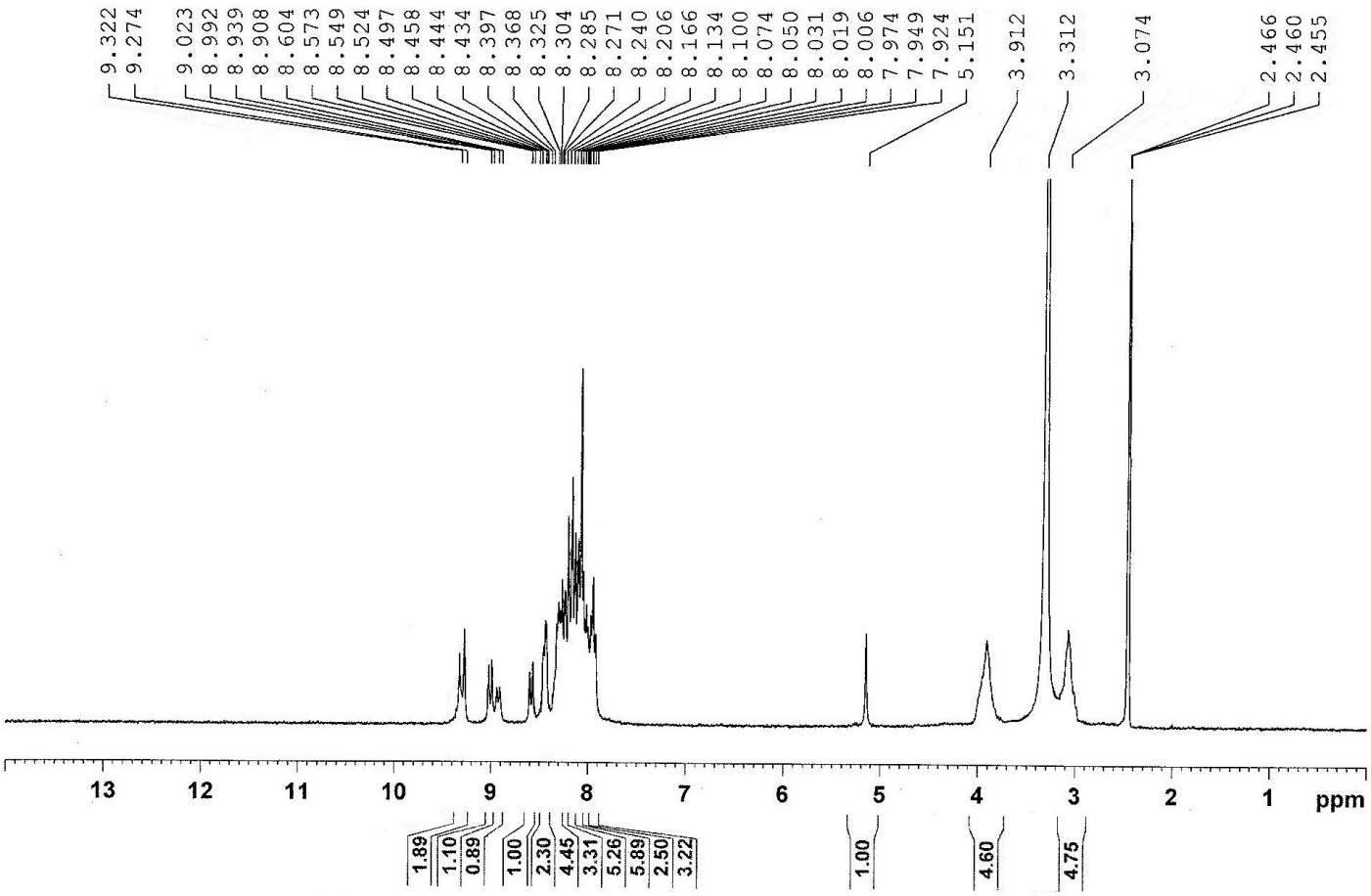

Fig.S14 ^{1}H NMR spectrum of **L**

Table S1. Determination of Cd^{2+} in the spiked drinking water samples

Drinking water source	Cd^{2+} added (μM)	Cd^{2+} found (μM)	Errors (%)
Tap water (Burdwan town)	1.00	0.98 ± 0.3	0.02
Tap water (Durgapur city)	2.00	2.07 ± 0.4	0.07
Tap water (Kolkata)	4.00	3.9 ± 0.3	0.1
Tap water, Kanchrapara, 24 Parganas (N)	7.00	6.8 ± 0.2	0.2
Tap water (Bankura town)	12.0	12.3 ± 0.5	0.3

References

1. E. Austin, M. Gouterman, *Bioinorg. Chem.*, 1978, **9**, 281.

2. W. H. Melhuish, *J. Phys. Chem.*, 1961, **65**, 229.

3. G. Grynkiewcz, M. Poenie, R. Y. Tsein, *J. Biol.Chem.* 1985, **260**, 3440.