Nanomolar Pyrophosphate Detection in Water and in a Self-Assembled Hydrogel of a Simple Terpyridine-Zn^{2+} Complex

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EXPERIMENTAL SECTION

General information and Materials: All reagents and solvents were purchased from commercial sources and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance III HD 300 NMR spectrometer. Chemical shifts were expressed in ppm. Mass spectra were measured on a Micromas (ESI-TOF) spectrometer. UV-Vis absorption spectra were recorded on a Cary 100 Conc. UV-Vis spectrophotometer, whereas fluorescence spectra were obtained on a Varian Cary Eclipse Fluorescence spectrophotometer. SEM image was obtained on Bruker Quantax400 EDS (Zeiss EVO-50XVP), while TEM image was recorded on JEOL JEM-1400HC at 80 kV. Confocal fluorescence microscopy images were recorded in Olympus Fluoview 1000 setup, equipped with argon (488 nm) and He/Ne lasers (546 nm and 633 nm).

Synthesis of 4′-(4-N,N′-dimethylaminophenyl)-2,2′:6′,2′′-terpyridine L: The ligand 4′-(4-N,N′-dimethylaminophenyl)-2,2′:6′,2′′-terpyridine **L** was synthesized according to the literature method.[1] 2-Acetylpyridine (4.84 g, 40 mmol) was added to a solution of 4-dimethylaminobenzaldehyde (3 g, 20 mmol) in ethanol (100 mL). KOH pellets (3.4 g, 85%, 51 mmol) and aq. NH$_3$ (58 mL) were added to the stirred solution and the resulting mixture was stirred at room temperature for 30 hours. The precipitate was then filtered, washed several times with ethanol. The precipitate was then dissolved in chloroform (10 mL) and excess n-hexane added (50 mL). The greenish solid was filtered, washed with n-hexane and dried in air. Yield 2.2 g (6.2 mmol, 31%). ^{1}H NMR (300 MHz, CDCl$_3$) δ/ppm: 8.73-8.75 (m, 2H), 8.72 (s, 2H), 8.66 (dt, 2H, *J* 8.0 Hz, 1.1 Hz), 7.84-7.90 (m, 4H), 7.31-7.35 (m, 2H), 6.82

(dt, 2H, J 9.0 Hz, 2.0 Hz), 3.04 (s, 6H). ^{13}C NMR (75 MHz, CDCl$_3$) δ/ppm: 156.7, 155.7, 151.1, 150.0, 149.0, 136.8, 128.1, 125.6, 123.6, 121.4, 117.6, 112.3, 40.4. HRMS (ESI-TOF) m/z calcd for [M+H]$^+$ (C23H21N4)$^+$: 353.17607; found: 353.1774.

Synthesis of Zn(II) complex ZnCl₂L: To a solution of ZnCl$_2$ (0.02 g, 0.15 mmol) in methanol (5 mL), was added solution of **L** (0.045 g, 0.13 mmol) in dichloromethane (6 mL) and the resulting solution stirred at 50 ^{o}C for 3 hour. The resultant yellow-orange precipitate was filtered, washed with water, methanol, and diethyl ether and dried under vacuum. Yield 0.043 g (60%). Slow evaporation of its acetonitrile solution afforded yellow needles of ZnCl₂L. ^{1}H NMR (300 MHz, DMSO-d$_6$) δ/ppm: 8.81-8.85 (m, 6H), 8.25 (td, 2H, J 7.8 Hz, 1.8 Hz), 8.15 (d, 2H, J 8.9 Hz), 7.82 (m, 2H), 6.82 (d, 2H, J 9.0 Hz), 3.05 (s, 6H). HRMS (ESI-TOF) m/z calcd for [M-Cl]$^+$ (C23H20N4ClZn)$^+$: 451.0662; found: 451.0635.

Fluorescence Study: The receptor stock solution was prepared by adding 100 µL of ZnCl$_2$ (25 mM) solution to 1 mL of **L** (2.5 mM) and diluting the resulting mixture with 50 mL of 0.01 M HEPES buffer (pH 7.4). All solutions for fluorescence measurements were sonicated for 5 minutes after mixing of receptor and analyte to maintain homogeneity.
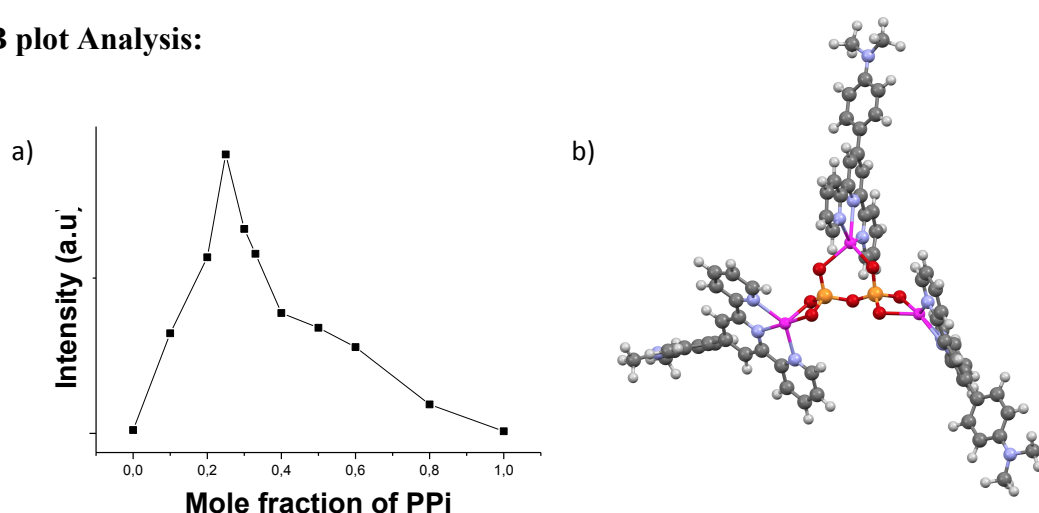
JOB plot Analysis:

Figure S1. a) Job plot analysis for PPi binding to **ZnCl₂L** (50 µM) in 0.01M HEPES buffer (pH 7.4), b) Energy minimized structure of PPi-**ZnCl₂L** (1:3) complex (DFT B3LYP 6-31G* in SPARTAN2014[2]).

Gelation studies: To a stock solution of **L** (200 μL, 12 mM in 0.1 N HCl) taken in a test tube (0.5 cm diameter), 200 μL of $ZnCl_2$ (12 mM in H_2O) was added from top and shaken gently to instantaneously afford a translucent, thermo-irreversible, thixotropic hydrogel.

The gel was spread uniformly over disposable paper strips and then dried thoroughly to prepare PPi sensing strips for fluorescence studies.

X-ray crystallographic study for $ZnCl_2L$: Single crystal X-ray diffraction analysis of **$ZnCl_2L$** was performed at 173 K on an Agilent Super-Nova diffractometer using mirror-monochromatized Mo-$K\alpha$ (λ = 0.71073 Å) radiation. *CrysAlisPro*[3] program was used for the data collection and processing. The intensities were corrected for absorption using the Analytical face index absorption correction method.[4] The structure was solved by charge flipping method with *SUPERFLIP*[5] and refined by full-matrix least-squares methods using the *WinGX*-software,[6] which utilizes the *SHELXL-97* module.[7] All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were introduced in proper positions with isotropic thermal parameters using the 'riding model'. *ORTEP* figure was plotted using *Ortep-3 for Windows*,[8] and structure was analysed with *Mercury* v 3.3.[9]

Table S1. Crystallographic data and structure refinement parameters for **ZnCl₂L**

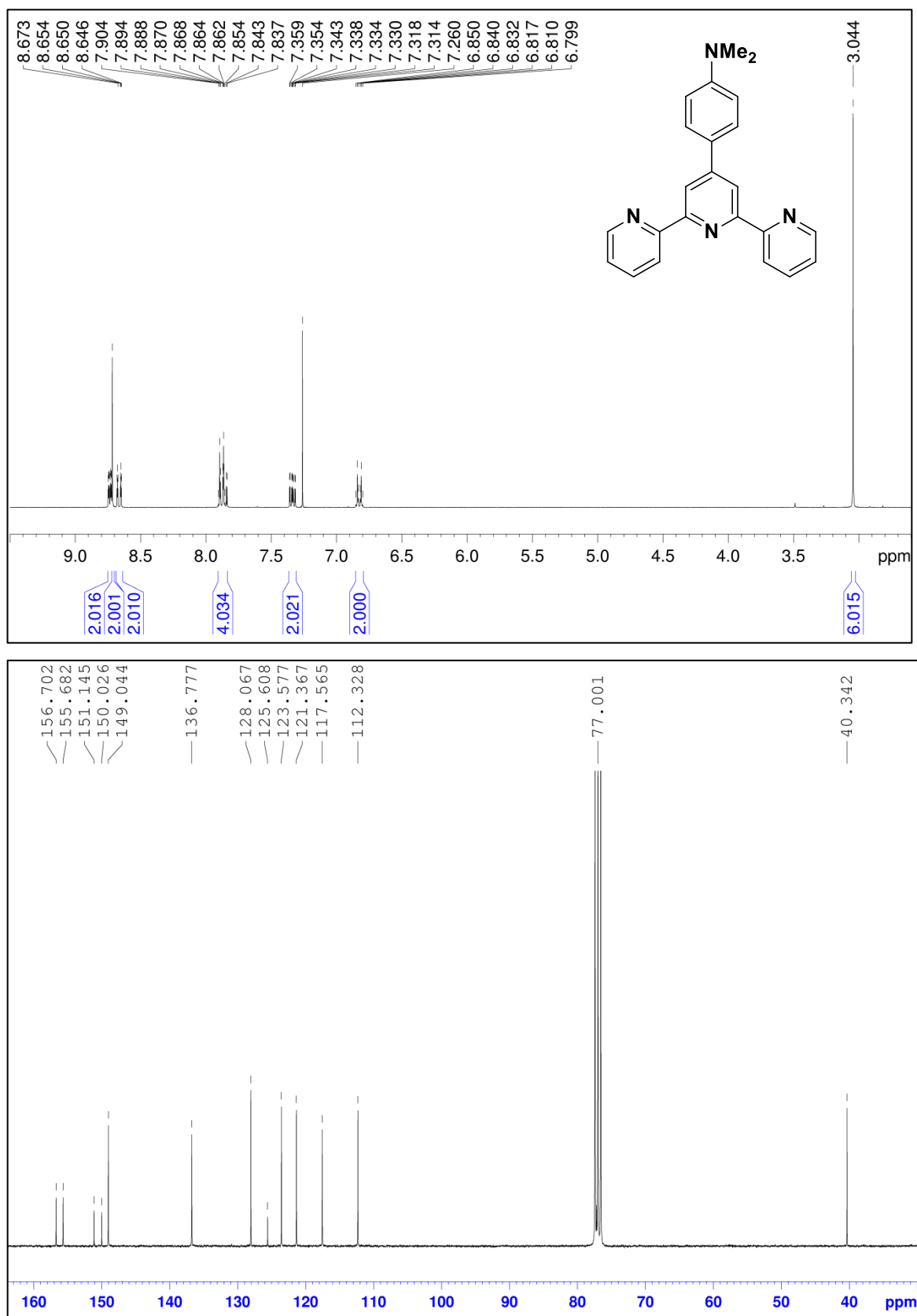|	
CCDC No.	977466
Empirical formula	$C_{23}H_{20}N_4Cl_2Zn_1$
Formula weight	488.70
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal color and shape	yellow, needle
Crystal size	0.58 x 0.06 x 0.05 mm^3
Crystal system	orthorhombic
Space group	Pbcn
Unit cell dimensions	a = 11.6964(3) Å
	b = 15.8328(4) Å
	c = 11.5220(3) Å
	α = 90°
	β = 90°
	γ = 90°
V	2133.72(9) Å^3
Z	4
Density (calculated)	1.521 Mg/m^3
Absorption coefficient	1.419 mm^{-1}
F(000)	1000
Theta range for data collection	2.16 to 25.00°
Index ranges	-13 ≤ h ≤ 9; -18 ≤ k ≤ 15; -12 ≤ l ≤ 13
Completeness to theta = 25.00°	99.8 %
Reflections collected	4734
Independent reflections	1882 [R(int) = 0.0230]
Absorption correction	analytical
Max. and min. transmission	0.948 and 0.689
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1882 / 0 / 140
Goodness-of-fit on F^2	1.062
Final R indices [I>2σ(I)]	R1 = 0.0281, wR2 = 0.0690
R indices (all data)	R1 = 0.0355, wR2 = 0.0727
Largest diff. peak and hole	0.291 and -0.347 e.Å^{-3}

Figure S2. ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **L** in CDCl$_3$.
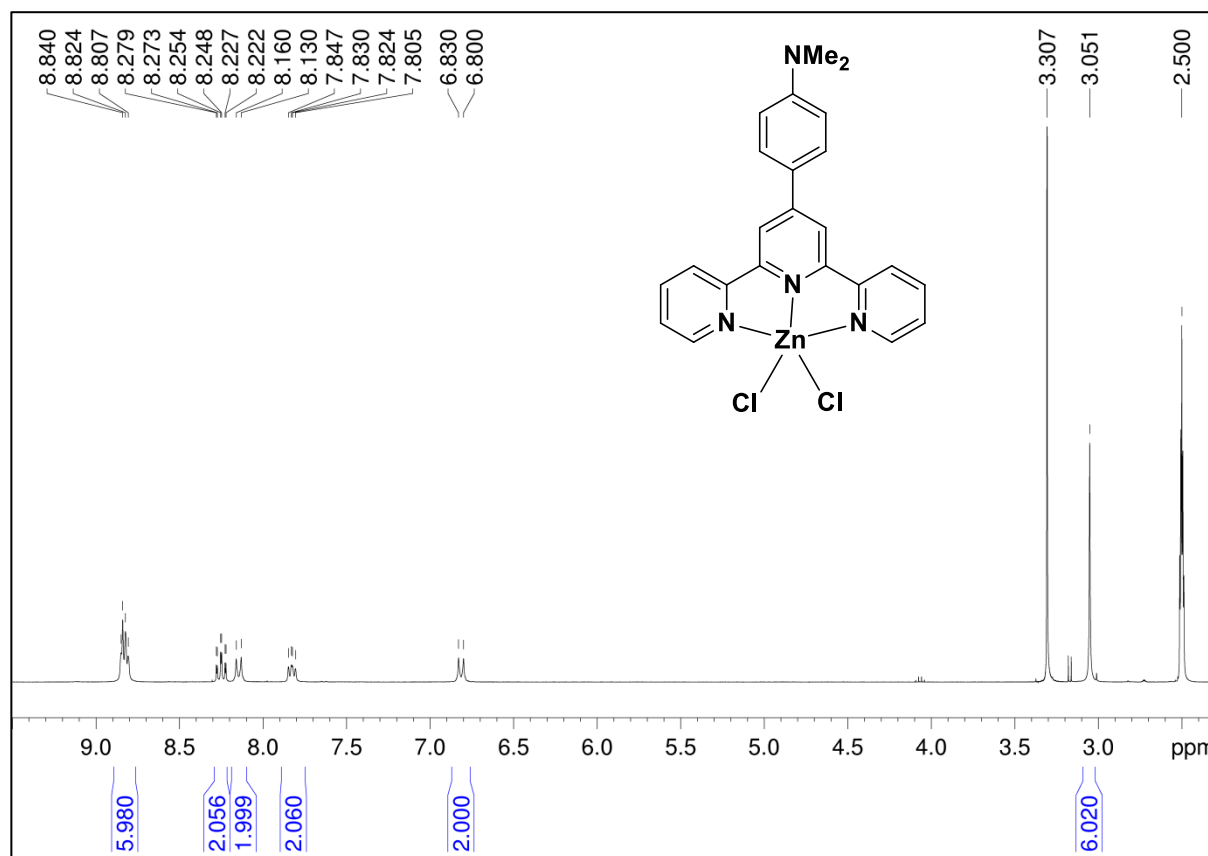
Figure S3. ^{1}H NMR spectrum of **ZnCl$_2$L** in DMSO-d$_6$.

References

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