Selective binding and fluorescence sensing of diphosphate in H_2O *via* Zn^{2+}-induced allosteric regulation of the receptor structure

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Electronic supportino Information

Chemicals.

$Zn(ClO_4)_2 \cdot 6H_2O$ and the sodium salts Na_2HPO_4, $Na_4P_2O_7 \cdot 10H_2O$ and $Na_5P_3O_{10} \cdot 6H_2O$ (in all cases purity ≥ 99.5 %) were purchased form Aldrich and used in all potentiometric, spectrophotometric and potentiometric titrations.

Synthesis of ligand L

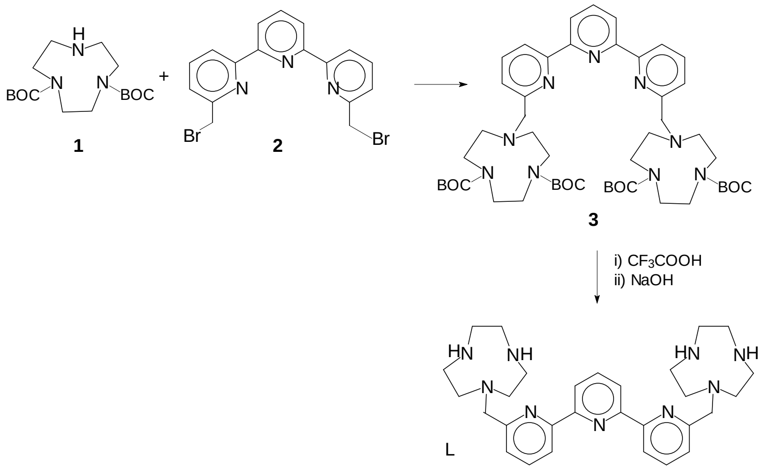
Receptor L was prepared as sketched in Scheme S1. Reaction of 1,4,7-triazacyclononane-1,4-dicarboxylic acid di-*tert*-butyl ester[1] **1** with 6,6”-bis(bromomethyl)-2,2’:6’,2”-terpyridine[2] **2** affords the BOC-protected L derivative **3**, which was subsequently deprotected with CF_3CO_2H.

Synthesis of 3. To a solution of 1,4,7-triazacyclononane-1,4-dicarboxylic acid di-*tert*-butyl ester [$(BOC)_2$-[9]aneN_3] (0.47 g, 1.43 mmol) and KOH (0.12 g, 2.14 mmol) in dry toluene (20 mL) under nitrogen, was added 6,6”-bis(bromomethyl)-2,2’:6’,2”-terpyridine (0.3 g, 0.72 mmol) in dry toluene (30 mL) within 30 min. The mixture was stirred at 70 °C for 6 h and at room temperature for 24 h, filtered, dried over Na_2SO_4, and concentrated under reduced pressure to give a light yellow solid (0.32 g, 48.5% yield). Mp 115 °C. Elem. Anal. Found. (calcd for $C_{49}H_{73}N_9O_8$): C, 64.03 (64.21); H, 8.11 (7.98); N, 13.51 (13.77)%. ^{1}H-NMR (400 MHz, $CDCl_3$), δ 1.36 (s, 36H, C(CH_3)$_3$), 2.71-3.55 (m, 24 H, [9]aneN_3 ring), 3.91 (s, 4H, ArCH_2N), 7.29 (d, 2H, J = 10.4 Hz), 7.81 (t, 1H, 10.0 Hz), 7.93-8.02 (m, 3H), 8.44 (m, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 28.50, 28.56 (C(CH_3)$_3$), 48.94, 49.33, 49.86, 50.49, 50.93, 51.68, 53.40, 54.04, 54.26 ([9]aneN_3 ring), 62.23, 62.30 (ArCH_2N), 79.48, 79.58 (C(CH_3)$_3$), 119.18, 120.76, 123.17, 125.23, 128.16, 128.97, 136.87, 137.64, 155.47, 155.72 (C=O), 159.73.

Synthesis of L·9HBr·3H$_2$O. To a solution of $(Boc)_4$-L (0.91 g, 0.99 mmol) in CH_2Cl_2 (10 mL) was added CF_3CO_2H (10 mL), and the resulting solution was stirred at room temperature under nitrogen for 2 h. The solvent was removed under vacuum and the residue was taken up in water, the pH value was adjusted to 9-10 by adding 1 M NaOH, and the product extracted into $CHCl_3$ (4 x 20 mL). The organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a gummy yellow solid of L. ^{1}H-NMR (400 MHz, $CDCl_3$), δ 2.86-2.95 (m, 24 H, [9]aneN_3 ring), 4.01 (s, 4H, Ar*CH₂*N), 7.28 (d, 2H, J = 10.4 Hz), 7.79 (t, 1H, 10 Hz), 7.92-7.99 (m, 3H), 8.41 (m, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 44.22, 44.92, 50.66 ([9]aneN_3 ring), 60.76 (ArCH_2N), 120.06, 121.22, 123.07, 137.57, 138.16, 155.38, 155.96, 157.91.

The HBr salt of the ligand can be prepared by dissolving the crude product in EtOH (20 mL) and adding HBr 48% resulting in the formation of a yellow precipitate of $L \cdot 9HBr \cdot 3H_2O$ (0.43 g, 33.4% yield). Mp 180 °C with decomposition. Elem. Anal. Found. (calcd for $C_{29}H_{56}N_9Br_9O_3$): C, 26.52 (26.78); H, 4.48 (4.35); N, 9.54 (9.70) %.

Synthesis of the complex $[ZnLH_2](ClO_4)_4 \cdot 2H_2O$. A solution of $Zn(ClO_4)_2 \cdot 6H_2O$ (7.4 mg, 0.02 mmol) in 5 ml of H_2O was slowly added, under stirring, to a solution of L (10 mg, 0.02 mmol) in 5 ml of H_2O. The pH of the solution was adjusted at 7 with NaOH 0.1 M. $NaClO_4$ (100 mg) were than added. The complex was obtained as a colourless solid by slow evaporation of the resulting solution at room temperature (17.3 mg, 85% yield). Elem. Anal. Found. (calcd for $C_{29}H_{47}N_9ZnCl_4O_{18}$): C, 34.32 (34.25); H, 4.69 (4.66); N, 12.53 (12.40) %.



Scheme S1

Potentiometric measurements

Equilibrium constants for protonation and complexation reactions were determined by means of potentiometric measurements (pH = -log [H^+]), carried out in 0.1 M NMe_4Cl at 298.1 ± 0.1 K, in the pH range 2.5-10.5, by using the equipment that has been already described.[3] The reference electrode was an Ag/AgCl electrode in saturated KCl solution. The glass electrode was calibrated as a hydrogen concentration probe by titrating known amounts of HCl with CO_2-free NaOH solutions and determining the equivalent point by the Gran's method.[4] This allow one to determine the standard potential E^o, and the ionic product of water ($pK_w = 13.83 \pm 0.01$). 1×10^{-3} M ligand concentrations was generally employed in the potentiometric measurements. In the study on metal complexation, the metal to ligand molar ratio was varied from 0.5:1 to 3:1, while in the measurements with anions, the anion to ligand molar ratio was varied from 1 to 3:1. The computer program HYPERQUAD[5] was used to calculate the stability constants of the complexes from e.m.f. data. The analysis of the binding ability of the mononuclear Zn^{II} complex towards phosphate anions was carried out either by performing potentiometric titrations on solutions containing the preformed $[ZnLH_2](ClO_4)_4 \cdot 2H_2O$ complex (1×10^{-3} M) and each anion, varying the anion to complex molar ratio from 1 to 3:1 or on solution containing Zn^{II} and L in 1 x 10^{-3} M concentrations and varying the anion to Zn^{II} molar ratio from 1 to 3:1. Data treatment with the HYPERQUAD[5] program lead to equal values of the formation constants of the ternary complexes by using the two methods. Precipitation of the complexes prevents measurements with Zn^{II} to L molar ratios greater 1 in the presence of phosphate anions. At least three measurements (about 100 experimental points each one) were performed for each system. The titration curves for each system were treated either as a single set or as separated entities without significant variations in the values of the protonation or complexation constants.

Spectrophotometric and Spectrofluorimetric Measurements

Absorption spectra were recorded on a Perkin-Elmer Lambda 25 spectrophotometer. Fluorescence emission spectra were collected on a Perkin Elmer LS55 spectrofluorimeter. In the measurements carried out at different pH values, HNO_3 and NMe_4OH were used to adjust the pH values which were measured on a Metrohm 713 pH meter. TRIS buffer (5 mM) was used in the experiments performed at pH 7. In the fluorescence emission measurements, the terpyridine unit of L was excited at 290 nm.

^{1}H NMR measurements.

^{1}H NMR spectra in D_2O solution at different pH values were recorded at 298 K on a Varian 300 MHz instrument. Peak positions are reported relative to HOD at 4.75 ppm. The pH was calculated from the measured pD value by using the relationship pH = pD – 0.40.[6]

Molecular dynamics calculations.

Molecular modelling calculations were carried out on the complex formed by $[ZnLH_2(H_2O)_2]^{2+}$ and the $P_2O_7^{4-}$ anion. The input coordinates of the $[ZnLH_2(H_2O)_2]^{2+}$ complex were built considering that the Zn^{II} ion is normally 5-coordinate in its 1:1 complexes with tpy, the metal ion being coordinated by the 3 nitrogens of tpy and 2 donor atoms of exogenous ligand(s), as pointed out by a search on the Cambridge Structural Database[7] (CSD) on crystallographically characterized 1:1 Zn^{II} complexes with tpy. In particular, the starting coordinates for the metal coordination environment were taken from the crystal structure of bis(O,O'-di-isopropyl-dithiophosphato)-(2,2'-6',2''-terpyridine)-Zn^{II},[8] replacing the two phosphate groups with water molecules. The aliphatic moiety of L and the anion were built using the builder module of the program Maestro.[9] The obtained structure was then optimized at the molecular mechanics level of theory using the OPLS2005 forcefield.[10]

The resulting conformer was then minimized at the DFT/B3LYP[11] level of theory using the LAV3P**+ basis set, which uses an effective core potential for metal atoms, as implemented in the software Jaguar,[12] and an implicit simulation of the aqueous environment by means of the Poisson-Boltzmann equation.[13] The nature of the stationary point as a true minimum was checked by frequency calculation.

References.

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Table S1. Protonation constants of L (NMe_4Cl 0.1 M, 298 K)

Reaction	Log K
$L + H^+ = (HL)^+$	11.07(4)
$(HL)^+ + H^+ = (H_2L)^{2+}$	10.67(4)
$(H_2L)^{2+} + H^+ = (H_3L)^{3+}$	7.03(4)
$(H_3L) + H = (H_4L)^{4+}$	5.57(6)
$(H_4L)^{4+} + H^+ = (H_5L)^+$	3.0(1)

Table S2. Formation constants of the adducts of L with di- and triphosphate (NMe_4Cl 0.1 M, 298 K)

Reaction		$A = P_3O_{10}^{5-}$
	Log K	
$(H_2L)^{2+} + A^{n-} = [H_2LA]^{(2-n)+\ a}$	2.90(9)	2.70(9)
$(H_2L)^{2+} + HA^{(1-n)+} = [H_3LA]^{(3-n)+}$	2.89(7)	2.81(7)
$(H_3L)^{3+} + HA^{(1-n)+} = [H_4LA]^{(4-n)+}$	3.34(9)	3.17(9)
$(H_3L)^{3+} + H_2A^{(2-n)+} = [H_5LA]^{(5-n)+}$	3.14(9)	3.01(9)
$(H_4L)^{4+} + H_2A^{(2-n)+} = H_6LA^{(6-n)+}$	3.5(1)	3.3(1)

[a]: n = 4 for $A = P_2O_7^{4-}$, n = 5 for $A = P_3O_{10}^{5-}$

Table S3. Formation constants of the Zn^{II} complexes with L (NMe_4Cl 0.1 M, 298 K)

Reaction	Log K
$Zn^{2+} + L = [LZn]^{2+}$	16.81(2)
$[ZnL]^{2+} + H^+ = [ZnLH]^{3+}$	10.12(4)
$[ZnLH]^{3+} + H^+ = [ZnLH_2]^{4+}$	8.41(6)
$[ZnLH_2]^{4+} + H^+ = [ZnLH_3]^{5+}$	2.18(5)
$[ZnL]^{2+} + M^{2+} = [Zn_2L]^{4+}$	9.39(6)
$[Zn_2L]^{4+} + 2OH^- = [Zn_2L(OH)_2]^{2+}$	4.13(3)
$[Zn_2L]^{4+} + Zn^{2+} = [Zn_3L]^{6+}$	5.78(2)
$[Zn_3L]^{4+} + OH^- = [Zn_3L(OH)]^{5+}$	2.7(1)

Table S4. Addition constants of the phosphate anions to the mononuclear Zn^{II} complexes with L (NMe_4Cl 0.1 M, 298 K).

Reaction	$A = PO_4^{3-}$	$A = P_2O_7^{4-}$	$A = P_3O_{10}^{5-}$
		log K	
$[ZnL]^{2+}+[A]^{n-} = [ZnLA]^{(2-n)+}$		4.51(7)	3.88(7)
$[ZnLH]^{3+} + [A]^{n-} = [ZnLHA]^{(3-n)+}$	3.5(1)	5.80(7)	4.10(7)
$Zn[LH_2]^{4+} + [A]^{n-} = [ZnLH_2A]^{(4-n)+}$	4.2(1)	6.95(6)	4.69(6)
$[ZnLH_2]^{4+} + [HA]^{(1-n)+} = [ZnLH_3A]^{(5-n)+}$	3.6(1)	5.6(1)	4.3(1)

[a]: n = 3 PO_4^{3-}, for n = 4 for $A = P_2O_7^{4-}$, n = 5 for $A = P_3O_{10}^{5-}$

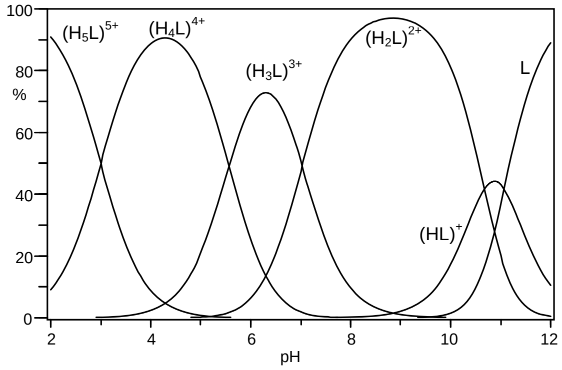

Figure S1. Distribution diagrams of the protonated species of L (L = 1.0×10^{-3} M, NMe_4Cl 0.1 M, 298 K)

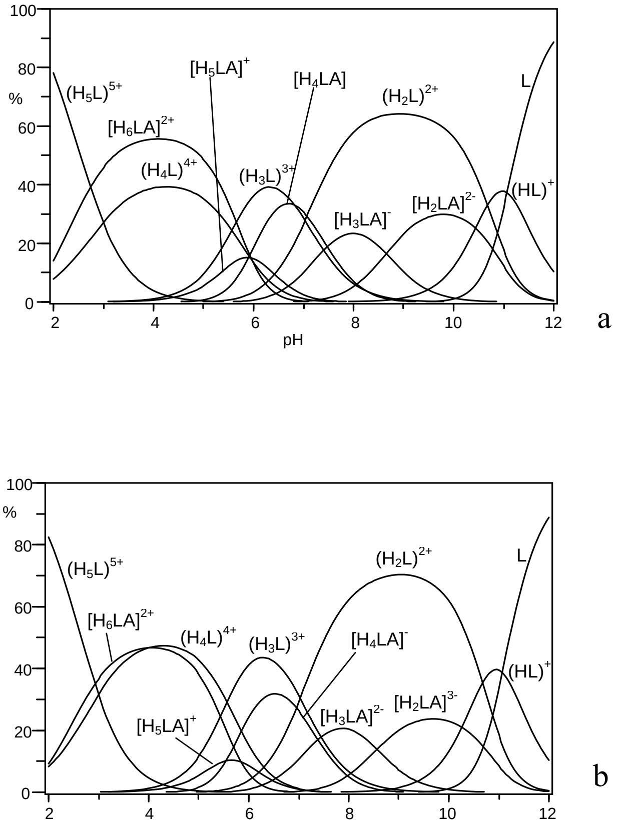

Figure S2. Distribution diagrams of the adducts formed by L with diphosphate (a) and triphosphate (b) ($[L] = [P_2O_7^{4-}] = [P_3O_{10}^{5-}] = 1.0 \times 10^{-3}$ M, NMe_4Cl 0.1 M, 298 K)

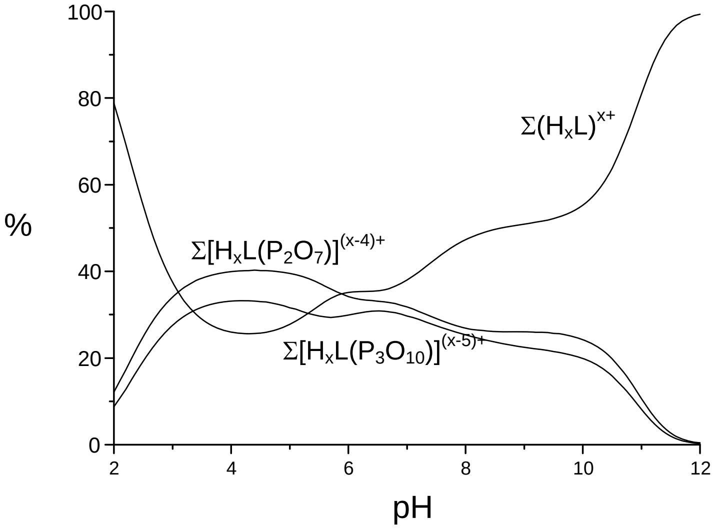

Figure S3. Overall percentages of di- and triphosphate complexed by L *vs* pH ([L] = [$P_2O_7^{4-}$] = [$P_3O_{10}^{5-}$] = 1.0 x10^{-3} M, NMe$_4$Cl 0.1 M, 298 K).

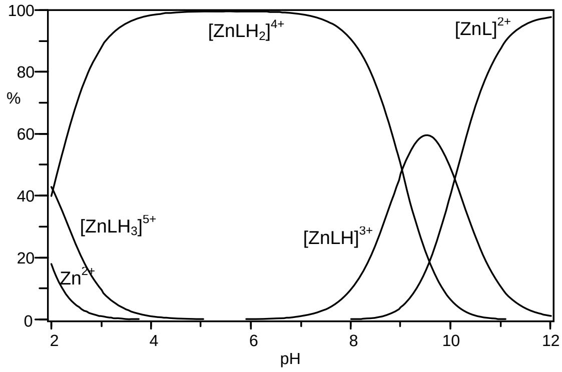

Figure S4. Distribution diagrams of the complexes formed by L with Zn^{II} ([L] = [Zn^{2+}] = 1.0 x 10^{-3} M, NMe$_4$Cl 0.1 M, 298 K)

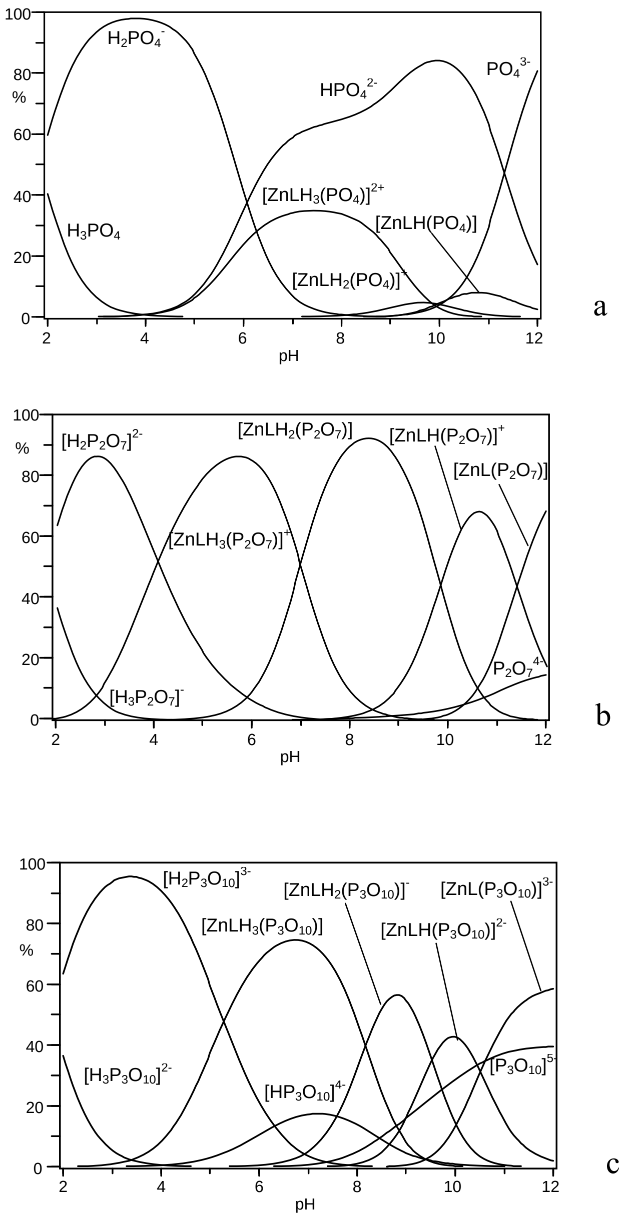

Figure S5. Distribution diagrams of the adducts formed by L with mono- (a), di- (b) and triphosphate (c) ([L] = $[PO_4^{3-}]$ = $[P_2O_7^{4-}]$ = $[P_3O_{10}^{5-}]$ = 1.0 x 10^{-3} M, NMe_4Cl 0.1 M, 298 K)

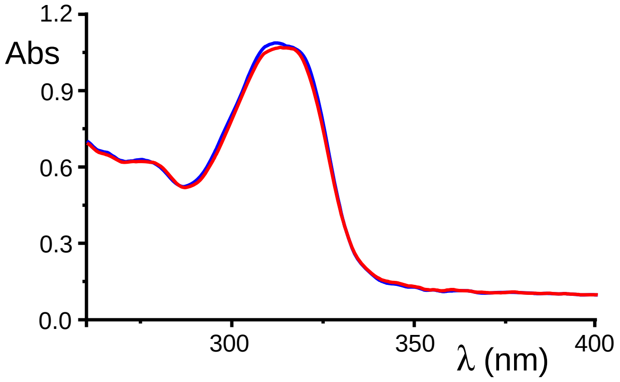

Figure S6. Absorption spectra of complex **I** in the absence (blue line) and in the presence (red line) of diphosphate in aqueous solution at pH 7 ([**I**] = 1.4·10^{-4} M**)**

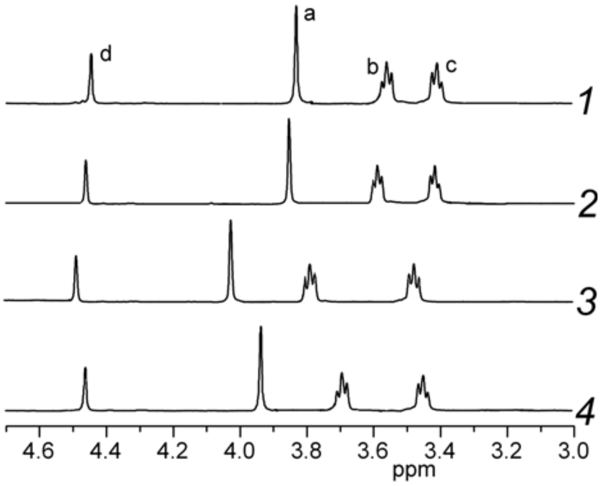

Figure S7. Aliphatic part of the ^{1}H NMR spectra of **I** alone in D_2O (*1*) and in the presence of 1 eq. of PO_4^{3-} (*2*), $P_2O_7^{4-}$ (*3*) and $P_3O_{10}^{5-}$ (*4*).

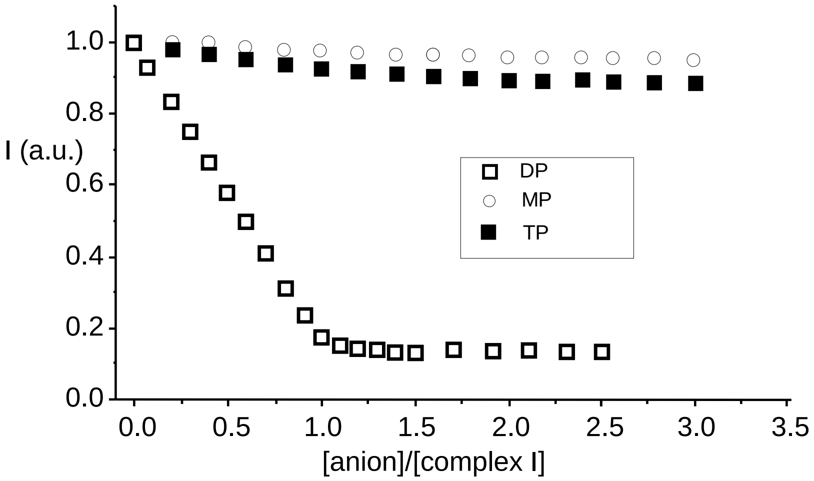

Figure S7. Fluorescence emission intensity of complex **I** at 355 nm in the presence of increasing amount of monophosphate (○), diphosphate (□) and triphosphate (■) in aqueous solution at pH 7.