Phosphate Derivative-Induced Supramolecular Assembly and NIR-Emissive Behaviour of Alkynylplatinum(II) Terpyridine Complexes For Real-Time Monitoring of Enzymatic Activities

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Supporting Information

Instrumentation. UV-Vis absorption spectra were recorded on a Cary 50 (Varian) spectrophotometer equipped with a Xenon flash lamp. Steady state emission spectra were recorded using a Spex Fluorolog-3 Model FL3-211 fluorescence spectrofluorometer equipped with a R2658P PMT detector. CD measurements were recorded using a Jasco (Tokyo, Japan) J-815 CD spectropolarimeter. Temperature-dependent UV-vis, emission and CD spectra were obtained using the respective instruments described with a single cell peltier thermostat equipped to control the working temperatures at 25 and 37 ^{o}C. Microquartz cuvettes with 10-mm path length and 2-mm window width were used for UV-vis, emission and CD measurements.

Chemicals and Materials. *v*-Src kinase,[1] expressed as full-length human Src with *N*-terminal GST-fusion protein using baculovirus expression system, was purchased from Carna Biosciences, Inc. Peptide sequence **P1**[1,2] and its tyrosine-phosphorylated derivative, ***p*P1**, were supplied by GL Biochem (Shanghai) Ltd. Sodium pyrophosphate was purchased from Alfa Aesar. Alkaline phosphatase from bovine kidney, adenosine 5'-triphosphatase from porcine cerebral cortex, adenosine 5'-triphosphate disodium salt, adenosine 5'-diphosphate sodium salt, adenosine 5'-monophosphate disodium salt and other chemicals used were purchased from Sigma-Aldrich. Deionized water (18.2 MΩ) used for measurements was purified by Elga Purelab UHQ system. Complexes **1** and **2** were synthesized according to the previously reported procedures.[3]

Assay Procedure for Measurement of ATP. Mixtures of buffer solution (50 µl; 50 mM Tris-HCl, pH 7.8), platinum(II) complex (100 µl; 1.2 mM), ATP and water of appropriate amount were added to reach a final volume of 500 µl. The resultant mixture was incubated at ambient temperature for 30 min before measurement. All concentrations were calculated as the final concentration in the assay solution mixture, with buffer solution of Tris-HCl (5 mM), pH 7.8 and platinum(II) complex (240 µM). Both UV-vis and emission spectra were recorded at ambient temperature. The emission spectra were recorded with an excitation wavelength of 360 nm and were corrected for PMT response.

Assay Procedure for Mg^{2+} Ion Titration. The assay procedure was similar to that for the measurement of ATP except that the mixture of buffer solution, **1**, ATP and water were incubated at ambient temperature for 30 min, followed by addition of appropriate amount of $MgCl_2$ to the

mixture and incubation for another 15 min prior to the measurement. All concentrations were calculated as the final concentration in the assay solution mixture, with buffer solution of Tris-HCl (5 mM), pH 7.8, **1** (240 μM) and ATP (30 μM).

Assay Procedure for EDTA Titration. The assay procedure was similar to that for the measurement of Mg^{2+} ion titration except that the mixture of buffer solution, **1**, ATP, $MgCl_2$ and water were incubated at ambient temperature for 15 min, followed by addition of appropriate amount of EDTA to the mixture and incubation for another 15 min prior to the measurement. All concentrations were calculated as the final concentration in the assay solution mixture, with buffer solution of Tris-HCl (5 mM), pH 7.8, **1** (240 μM), ATP (30 μM) and $MgCl_2$ (30 μM).

Assay Procedure for Selectivity Analysis. The assay procedure was similar to that for the measurement of ATP except that ATP was replaced by same concentration of interfering substrate in the respective buffer conditions.

Assay Procedure for Specificity Analysis. The assay procedure was similar to that for the selectivity analysis except that ATP was added to the resultant mixture followed by incubation for another 30 min prior to the measurement.

Assay Procedure for Monitoring ATPase Activity. Stock solution of ATPase was prepared in buffer solution (20 mM Tris-HCl, 133 mM NaCl, 3 mM KCl, pH 7.8).[4] Mixture of **1** (100 μl; 1.2 mM), ATP, buffer solution and water of appropriate amount were added to reach a final volume of 500 μl. The resultant mixture was incubated at ambient temperature for 30 min. ATPase of appropriate amount was added to the mixture with both UV-vis and emission spectra recorded at 25 ^{o}C as a function of time. All concentrations were calculated as the final concentration in the assay solution mixture, with buffer solution of Tris-HCl (2 mM), NaCl (13.3 mM), KCl (0.3 mM), pH 7.8, **1** (240 μM) and ATPase (0.4 unit L^{-1}). Both UV-vis and emission spectra were recorded with an excitation at the wavelength of 360 nm and were corrected for PMT response.

Assay Procedure for Measurement of P1 and *p*P1 for *v*-Src Kinase Activity Study. Mixture of buffer solution (50 µl; 50 mM HEPES, 10 mM $MnCl_2$, pH 7.4),[1] ATP (10 µl; 1 mM), platinum(II) complex (37.5 µl; 1.2 mM), **P1** or ***p*P1** and water of appropriate amount were added to reach a final volume of 500 µl. The resultant mixture was incubated at ambient temperature for 15 min before measurement. All concentrations were calculated as the final concentration in the assay solution mixture, with buffer solution of HEPES (5 mM), $MnCl_2$ (1 mM), pH 7.4, ATP (20 µM) and platinum(II) complex (90 µM). Both UV-vis and emission spectra were recorded at 25 ^oC with an excitation at the wavelength of 345 nm and were corrected for PMT response.

Assay Procedure for Monitoring *v*-Src Kinase Activity. Stock solution of *v*-Src kinase was prepared in buffer solution (50 mM HEPES, 10 mM $MnCl_2$, pH 7.4)[1] and incubated at 25 ^oC for 30 min.[5] Mixtures of ATP (10 µl; 1 mM), **2** (37.5 µl; 1.2 mM), **P1**, buffer solution and water of appropriate amount were added to reach a final volume of 500 µl. The resultant mixture was incubated at ambient temperature for 15 min. *v*-Src kinase of appropriate amount was added to the mixture with both UV-vis and emission spectra recorded at 25 ^oC as a function of time with an excitation at the wavelength of 345 nm. The emission spectra obtained were corrected for PMT response. All concentrations were calculated as the final concentration in the assay solution mixture, with buffer solution of HEPES (5 mM), $MnCl_2$ (1 mM), pH 7.4, ATP (20 µM) and **2** (90 µM).

Assay Procedure for Measurement of *p*P1 for ALP Activity Study. Mixture of buffer solution (50 µl; 100 mM Tris, 20 mM $MgCl_2$, pH 7.5 – 9.2),[6,7] **2** (37.5 µl; 1.2 mM), ***p*P1** and water of appropriate amount were added to reach a final volume of 500 µl. The resultant mixture was incubated at 37 ^oC for 15 min before measurement. All concentrations were calculated as the final concentration in the assay solution mixture, with buffer solution of Tris-HCl (10 mM), $MgCl_2$ (2 mM), pH 7.5 – 9.2. Both UV-vis and emission spectra were recorded at 37 ^oC with an excitation at the wavelength of 345 nm and were corrected for PMT response.

Assay Procedure for Monitoring ALP Activity. Stock solution of ALP was prepared in buffer solution (100 mM Tris-HCl, 20 mM $MgCl_2$, pH 7.5 – 9.2)[6,7] and incubated at 37 ^oC for 30 min. Mixtures of **2** (37.5 µl; 1.2 mM), ***p*P1**, buffer solution and water of appropriate amount were

added to reach a final volume of 500 µl. The resultant mixture was incubated at 37 ^{o}C for 15 min. ALP of appropriate amount was added to the mixture with both UV-vis and emission spectra recorded at 37 ^{o}C as a function of time with an excitation at the wavelength of 345 nm. The emission spectra obtained were corrected for PMT response. All concentrations were calculated as the final concentration in the assay solution mixture, with buffer solution of Tris-HCl (10 mM), $MgCl_2$ (2 mM) pH 7.5 – 9.2, and **2** (90 µM).

Determination of Kinetic Parameters from Emission Spectral Measurement on Enzymatic Assays. With the use of the titration curve of emission intensity of the mixture of metal complex, M, and substrate, S, against the concentration of S added, the concentration of S at time *t*, $[S]_t$, under enzyme-catalyzed condition could be determined by correlating with the emission intensity obtained for the mixture of M and S at time *t* after enzyme addition, I_t. A plot of $[S]_t$ against time could thus be obtained and the initial rate of the catalytic reaction by a particular enzyme, V_0, could be determined from the slope of the initial region of this plot.[7] Michaelis-Menten model has been commonly employed to determine the enzyme kinetic characteristic with the use of Michaelis-Menten equation[7,8] [Eq. (1)], which is expressed as

$$V_0 = \frac{V_{max}[S]_0}{K_M + [S]_0} \quad (1)$$

where $[S]_0$ is the initial substrate concentration, V_{max} is the maximum rate of enzyme-catalyzed reaction which is attained at saturated substrate concentration and K_M, the Michaelis-Menten constant, is equivalent to the substrate concentration at which the enzyme-catalyzed reaction rate is half of V_{max}. Also, V_{max} could be defined as

$$V_{max} = k_{cat}/[E]_0 \quad (2)$$

where k_{cat} is the turnover number of an enzyme, indicating the number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated

with the substrate, and $[E]_0$ is the initial concentration of enzyme.[7,8] When the substrate concentration employed in the assay condition is far below the reported K_M value of the particular enzyme, then Eq. (1) could be expressed as

$$V_0 = \frac{V_{max}[S]_0}{K_M} \quad (3)$$

Also, under this condition, the concentration of free enzyme [E] is nearly equal to $[E]_0$, and the Michaelis-Menten equation could be reduced as

$$V_0 = \left(\frac{k_{cat}}{K_M}\right)[S]_0[E]_0 \quad (4)$$

with k_{cat}/K_M, the specificity constant, corresponding to the reaction rate at low substrate concentration, denoted the catalytic efficiency of an enzyme.[7,8] By combining Eq. (3) and (4), the following equation [Eq. (5)] could be obtained,

$$\frac{V_{max}}{K_M} = \left(\frac{k_{cat}}{K_M}\right)[E]_0 \quad (5)$$

With the use of Eq. 5, a linear plot of V_{max}/K_M against $[E]_0$ could be used to determine k_{cat}/K_M from the slope.[7]

References:

[1] H. Akiba, J. Sumaoka and M. Komiyama, *Chem. Eur.–J.*, 2010, **16**, 5018.

[2] Z. Songyang, K. L. Carraway III, M. J. Eck, S. C. Harrison, R. A. Feldman, M. Mohammadi, J. Schlessinger, S. R. Hubbard, D. P. Smith, C. Eng, M. J. Lorenzo, B. A. J. Ponder, B. J. Mayer and L. C. Cantley, *Nature*, 1995, **373**, 536.

[3] a) M. C. L. Yeung, K. M. C. Wong, Y. K. T. Tsang and V. W. W. Yam, *Chem. Commun*., 2010, **46**, 7709; b) C. Yu, K. H. Y. Chan, K. M. C. Wong and V. W. W. Yam, *Chem.–Eur. J.*, 2008, **14**, 4577.

[4] Modified enzymatic assay condition from Sigma Quality Control Test Procedures on ATPase (Sigma A7510)

[5] A. M. Edison, S. C. Barker, D. B. Kassel, M. A. Luther and W. B. Knight, *J. Biol. Chem.*, 1995, **270**, 27112.

[6] Y. Choi, N. H. Ho and C. H. Tung, *Angew. Chem.*, 2007, **119**, 721; *Angew. Chem. Int. Ed.*, 2007, **46**, 707.

[7] Y. Liu and K. S. Schanze, *Anal. Chem.*, 2008, **80**, 8605.

[8] *Biochemistry*, 5th Ed. (Ed.: J. M. Berg, J. L. Tymoczko and L. Stryer), W. H. Freeman, New York, 2002.

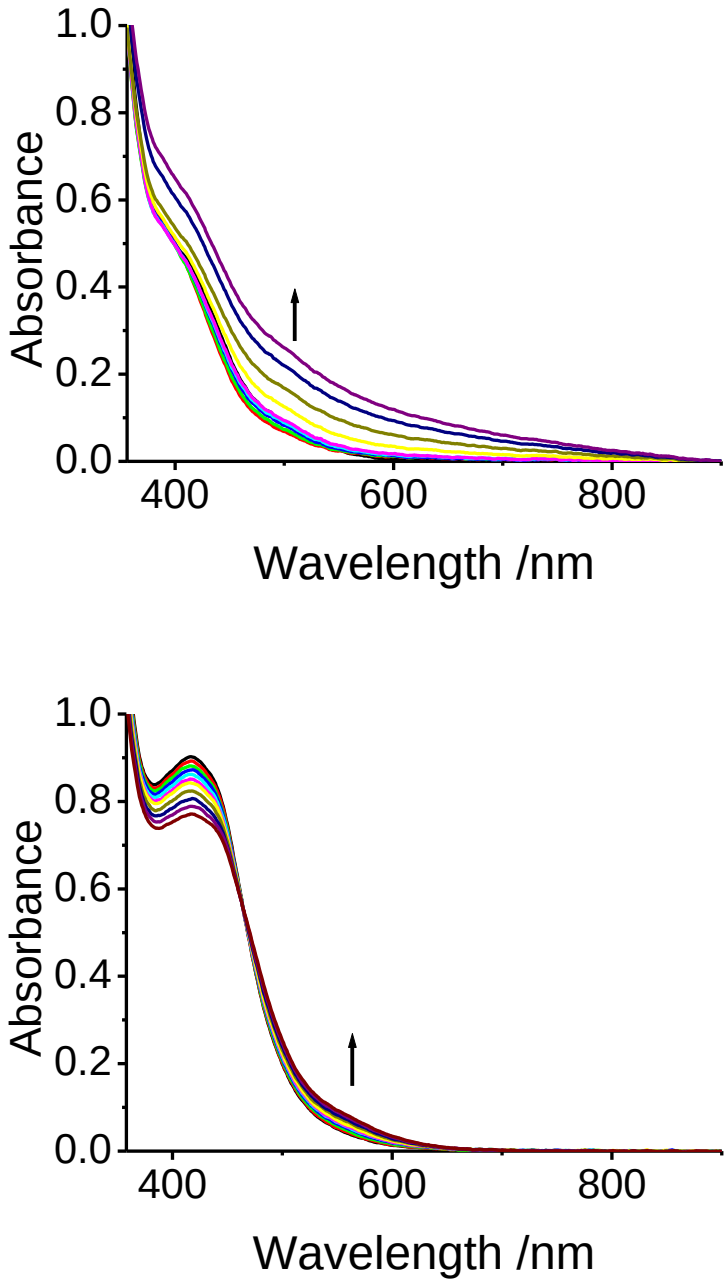

Fig. S1 UV-Vis absorption spectral changes of **1** (240 μM) (top) and **2** (240 μM) (bottom) with increasing concentration of ATP (up to 42 μM). Medium: 5 mM Tris-HCl, pH 7.8.

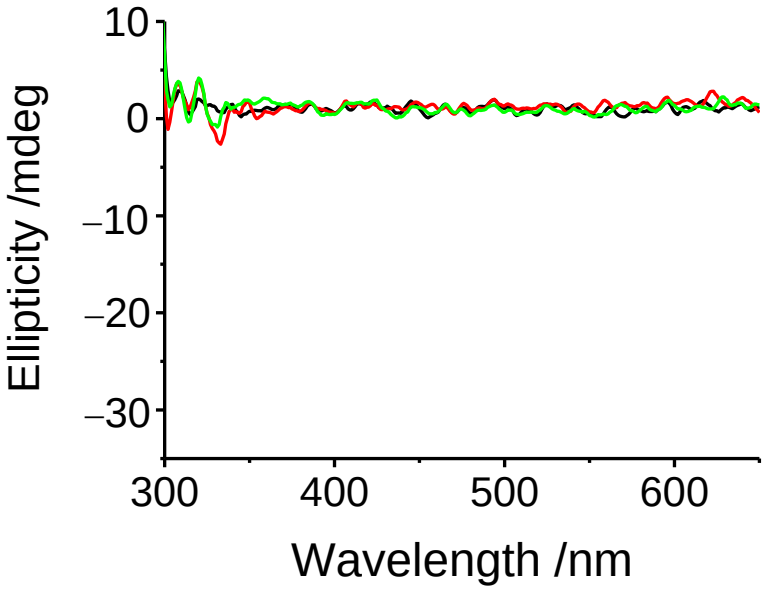

Fig. S2 CD spectral changes of **2** (240 μM) (black) in the presence of ATP (12 μM in red and 30 μM in green). Medium: 5 mM Tris-HCl, pH 7.8.

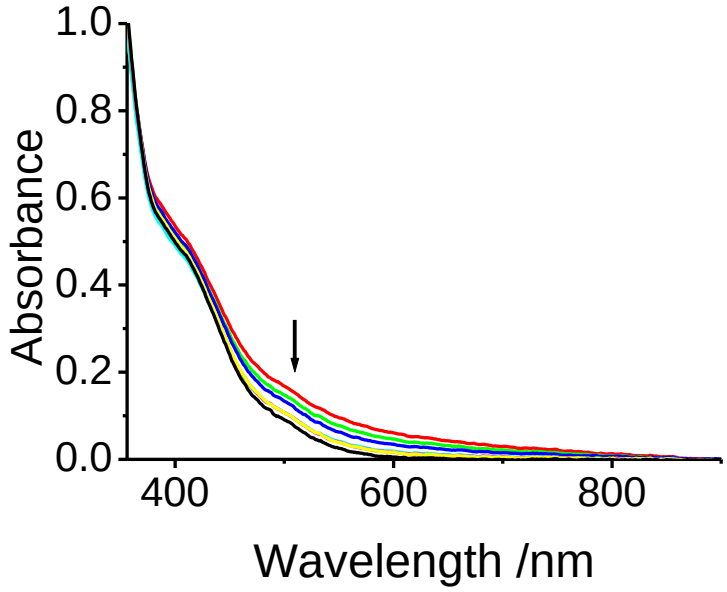

Fig. S3 UV-Vis absorption spectral changes of **1** (240 μM) (—) with ATP (30 μM) (—), and the mixtures upon titration with Mg^{2+} ion (up to 90 μM). Medium: 5 mM Tris-HCl, pH 7.8.

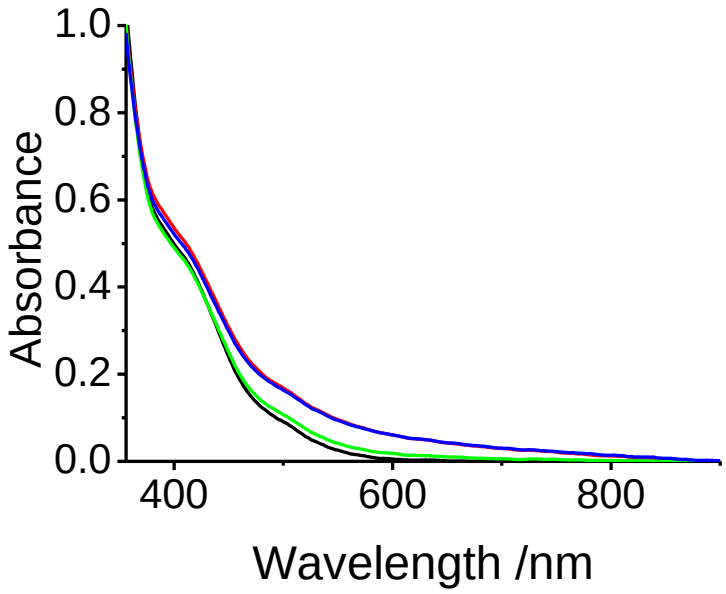

Fig. S4 UV-Vis absorption spectral changes of **1** (240 μM) (—) in the presence of ATP (30 μM) (—), in the presence of ATP (30 μM) and Mg^{2+} ion (30 μM) (—), and in the presence of ATP (30 μM), Mg^{2+} ion (30 μM) and EDTA (60 μM) (—). Medium: 5 mM Tris-HCl, pH 7.8.

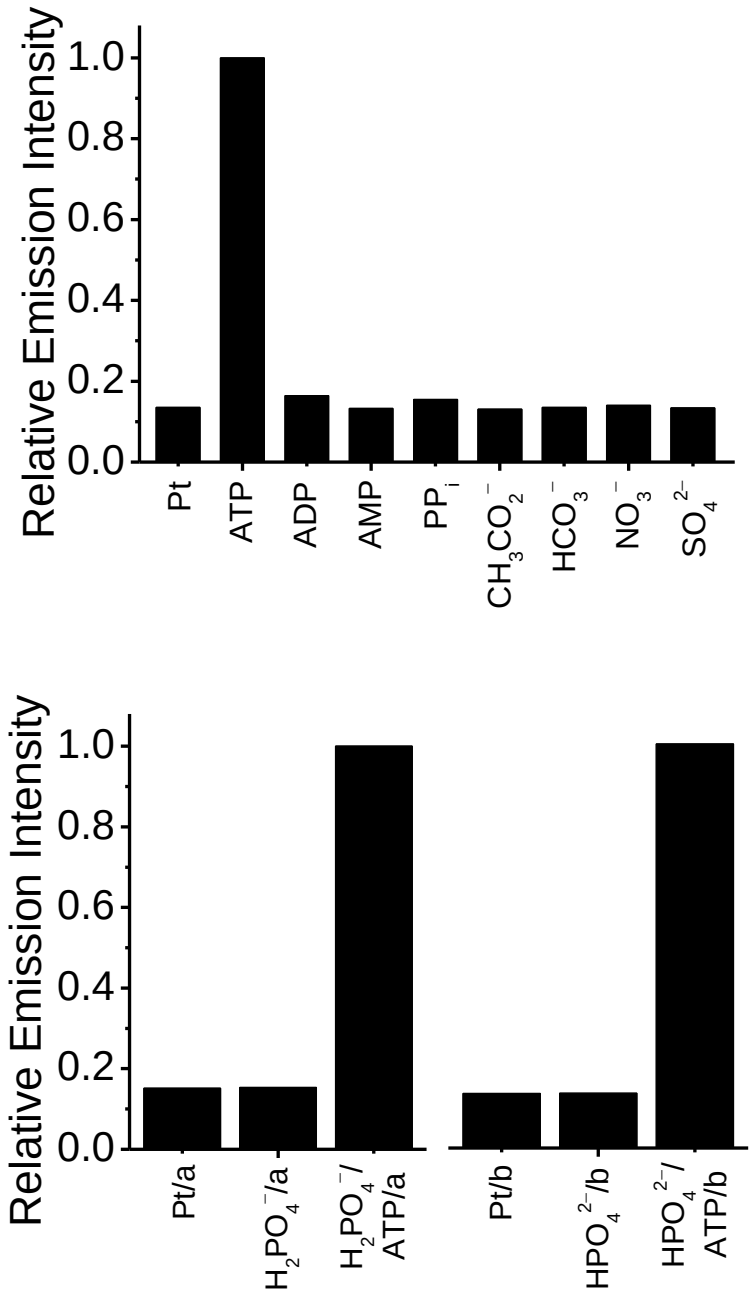

Fig. S5 (Top) Selectivity analysis with various possible anionic interfering substrates in the presence of **1** (240 μM) (Pt) in buffer. Concentrations of anionic substrates for selectivity analysis were tested at 30 μM. Medium: 5 mM Tris-HCl, pH 7.8. (Bottom) Selectivity and specificity analysis with $H_2PO_4^-$ (30 μM) and HPO_4^{2-} (30 μM) in the presence of **1** (240 μM) (Pt) in (a) 5 mM HEPES, pH 6.5, and (b) 5 mM Tris-HCl, pH 7.8.

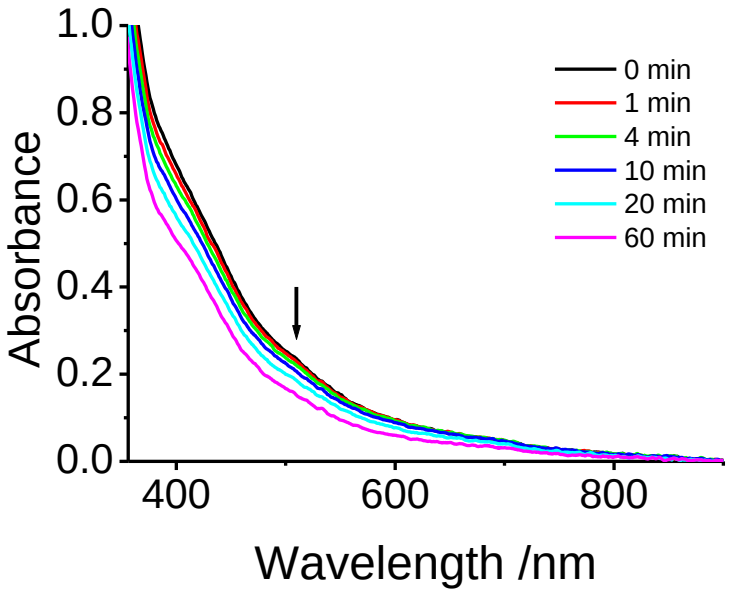

Fig. S6 UV-Vis absorption spectral changes upon addition of ATPase to the mixtures of **1** (240 μM) and ATP (42 μM) at different time intervals. Medium: 2 mM Tris-HCl, 13.3. mM NaCl, 0.3 mM KCl, pH 7.8 at 25 ^{o}C.

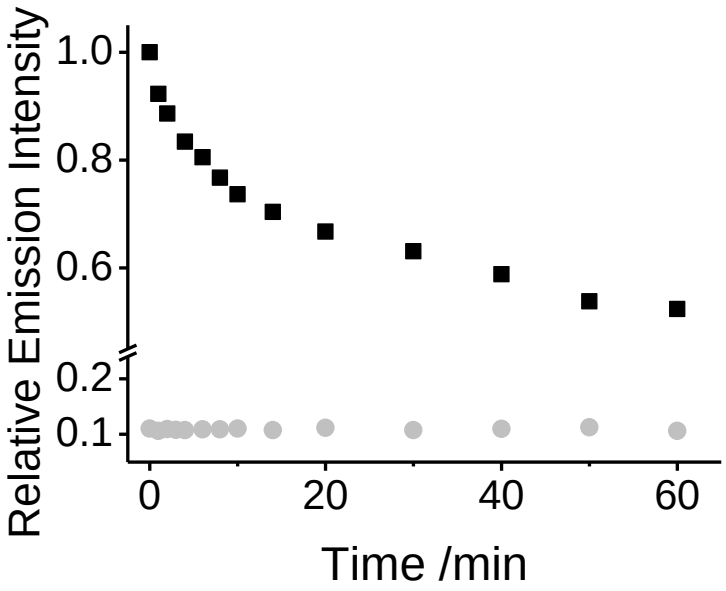

Fig. S7 Plots of relative emission intensity at 768 nm upon addition of ATPase to the mixtures of **1** (240 μM) and ATP (42 μM) (■) or without ATP (●) at different time intervals. Medium: 2 mM Tris-HCl, 13.3. mM NaCl, 0.3 mM KCl, pH 7.8 at 25 ^{o}C.

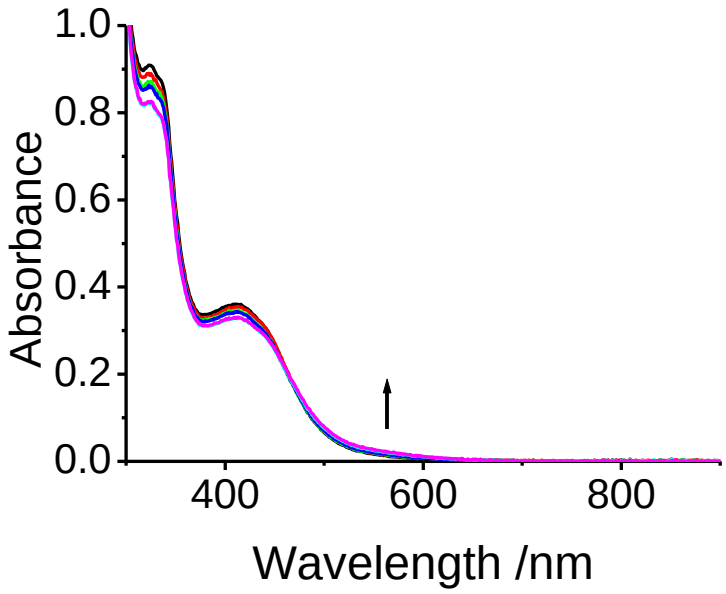

Fig. S8 UV-Vis absorption spectral changes of **2** (90 μM) with increasing concentration of **P1** (up to 7 μM). Medium: 5 mM HEPES, 1 mM $MnCl_2$, 20 μM ATP, pH 7.4.

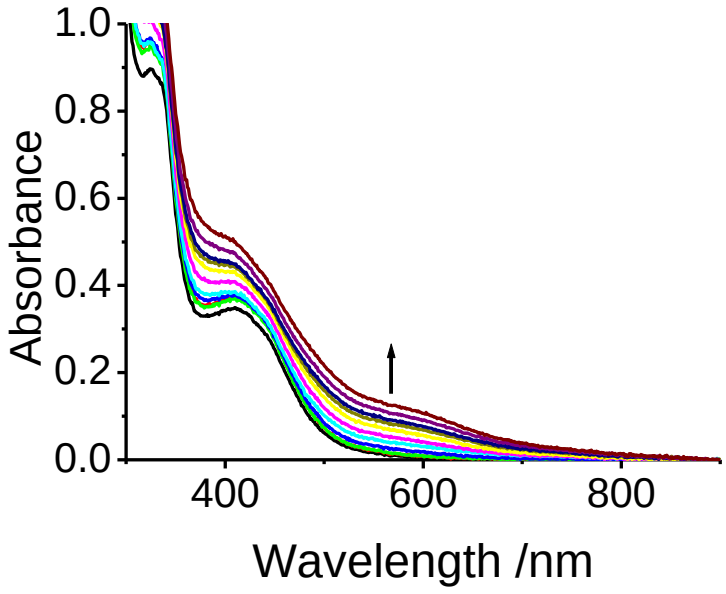

Fig. S9 UV-Vis absorption spectral changes of **2** (90 μM) with increasing concentration of ***p*P1** (up to 8 μM). Medium: 5 mM HEPES, 1 mM $MnCl_2$, 20 μM ATP, pH 7.4.

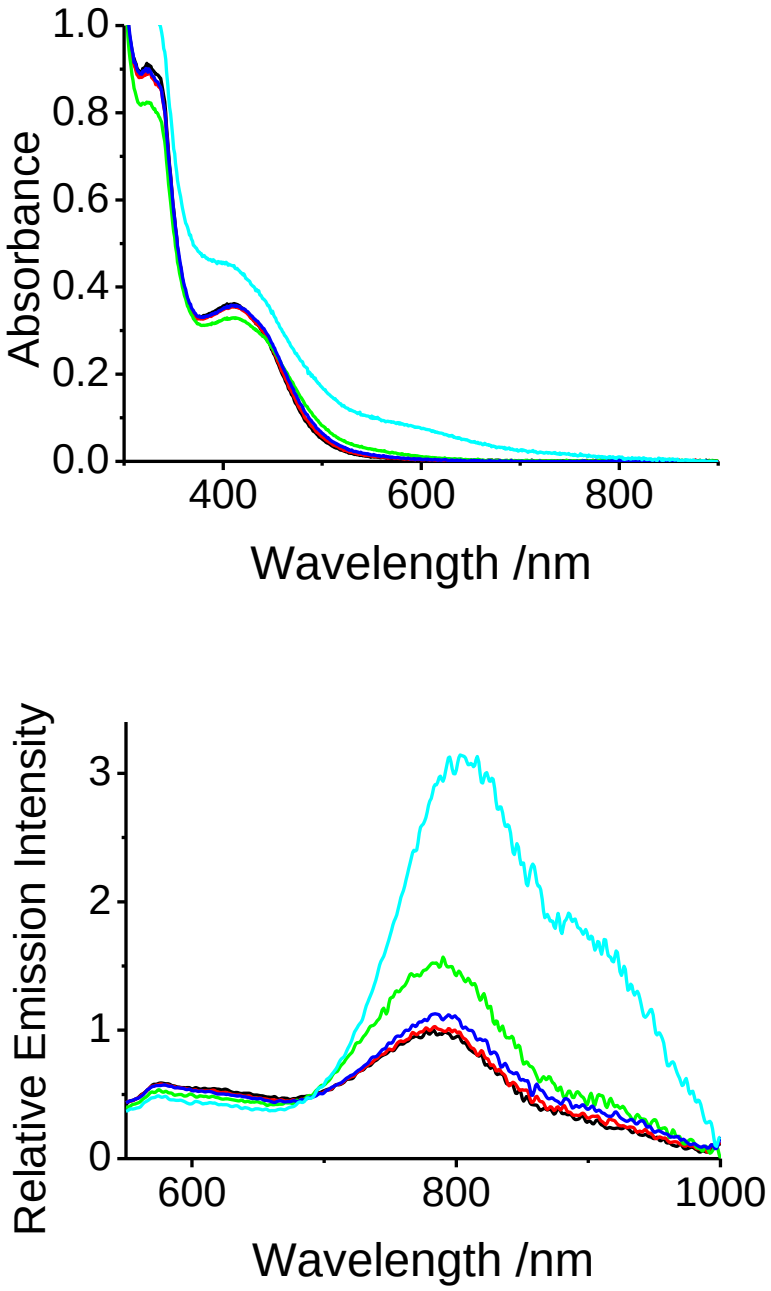

Fig. S10 UV-Vis absorption (top) and emission (bottom) spectral changes of **2** (90 μM) (—), in the presence of $MnCl_2$ (1.0 mM) (—), in the presence of ATP (20 μM) (—), in the presence of $MnCl_2$ (1.0 mM) and ATP (20 μM) (—), and in the presence of $MnCl_2$ (1.0 mM), ATP (20 μM) and ***p*P1** (6 μM) (—). Medium: 5 mM HEPES, pH 7.4.

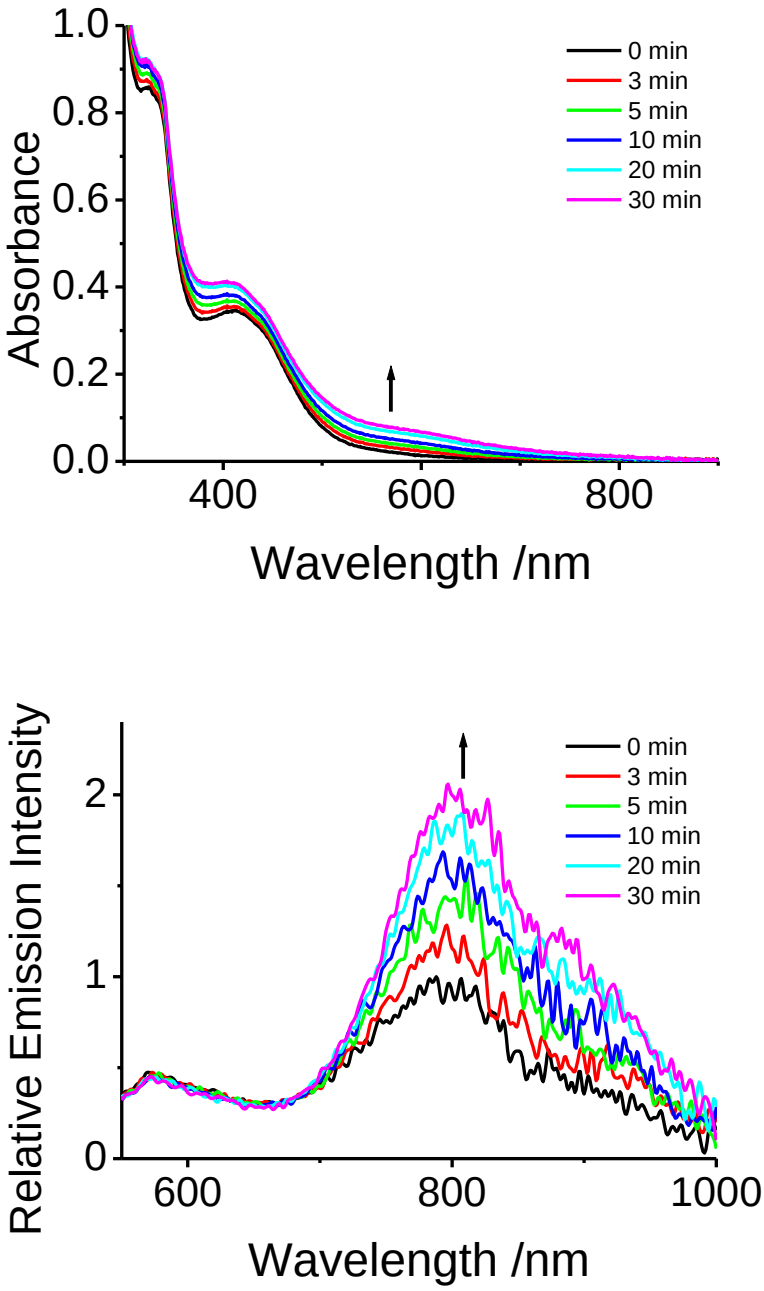

Fig. S11 UV-Vis absorption (top) and emission (bottom) spectral changes upon addition of *v*-Src kinase (0.75 μg ml^{-1}) to the mixtures of **2** (90 μM) and **P1** (4.5 μM) at different time intervals. Medium: 5 mM HEPES, 1 mM $MnCl_2$, 20 μM ATP, pH 7.4 at 25 ^oC.

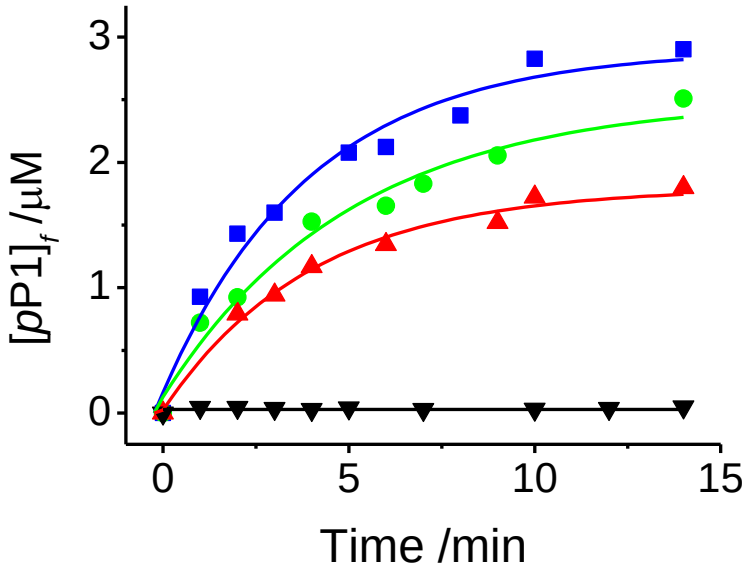

Fig. S12 Plots of [***p*P1**]$_f$ against time with 0 (▼), 2 (▲), 3 (●) and 4.5 (■) μM of initial **P1** concentration upon addition of *v*-Src kinase (0.75 μg ml^{-1}) to the mixtures containing **2** (90 μM). Medium: 5.0 mM HEPES, 1.0 mM $MnCl_2$, 20 μM ATP, pH 7.4 at 25 ^{o}C.

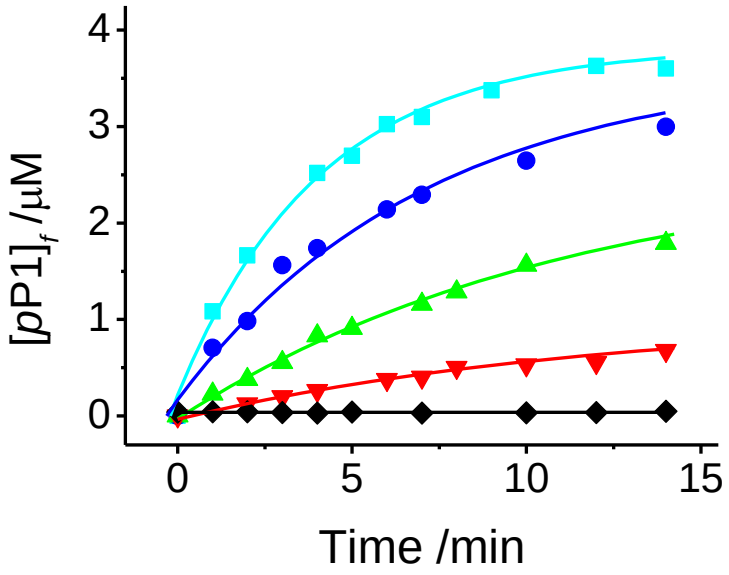

Fig. S13 Plots of [***p*P1**]$_f$ against time upon addition of 1.25 (■), 0.75 (●), 0.3 (▲), 0.1 (▼) and 0 (◆) μg ml^{-1} *v*-Src kinase to the mixtures of **2** (90 μM) with **P1** (6 μM) at different time intervals. Medium: 5 mM HEPES, 1 mM $MnCl_2$, 20 μM ATP, pH 7.4 at 25 ^{o}C.

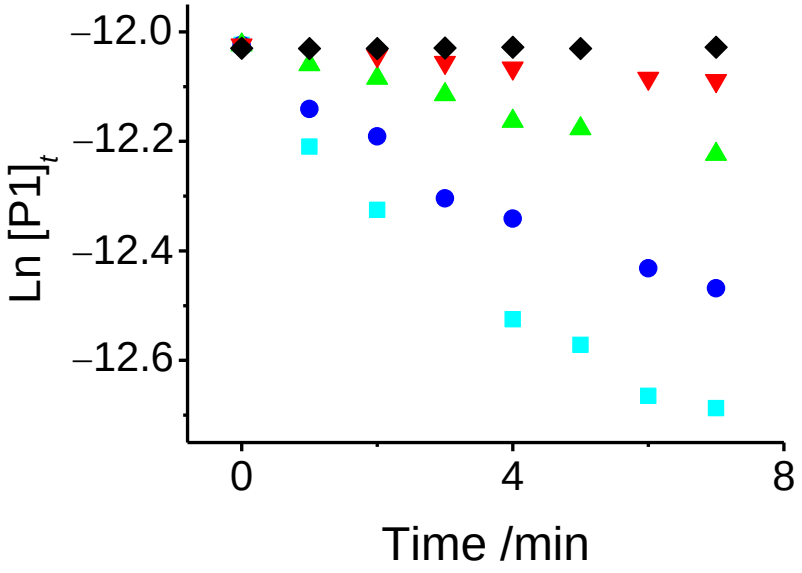

Fig. S14 Plots of natural logarithm of concentration of [**P1**]$_t$ against time upon addition of 1.25 (■), 0.75 (●), 0.3 (▲), 0.1 (▼) and 0 (◆) μg ml^{-1} *v*-Src kinase to the mixtures of **2** (90 μM) with **P1** (6 μM) at different time intervals. Medium: 5 mM HEPES, 1 mM $MnCl_2$, 20 μM ATP, pH 7.4 at 25 ^{o}C.

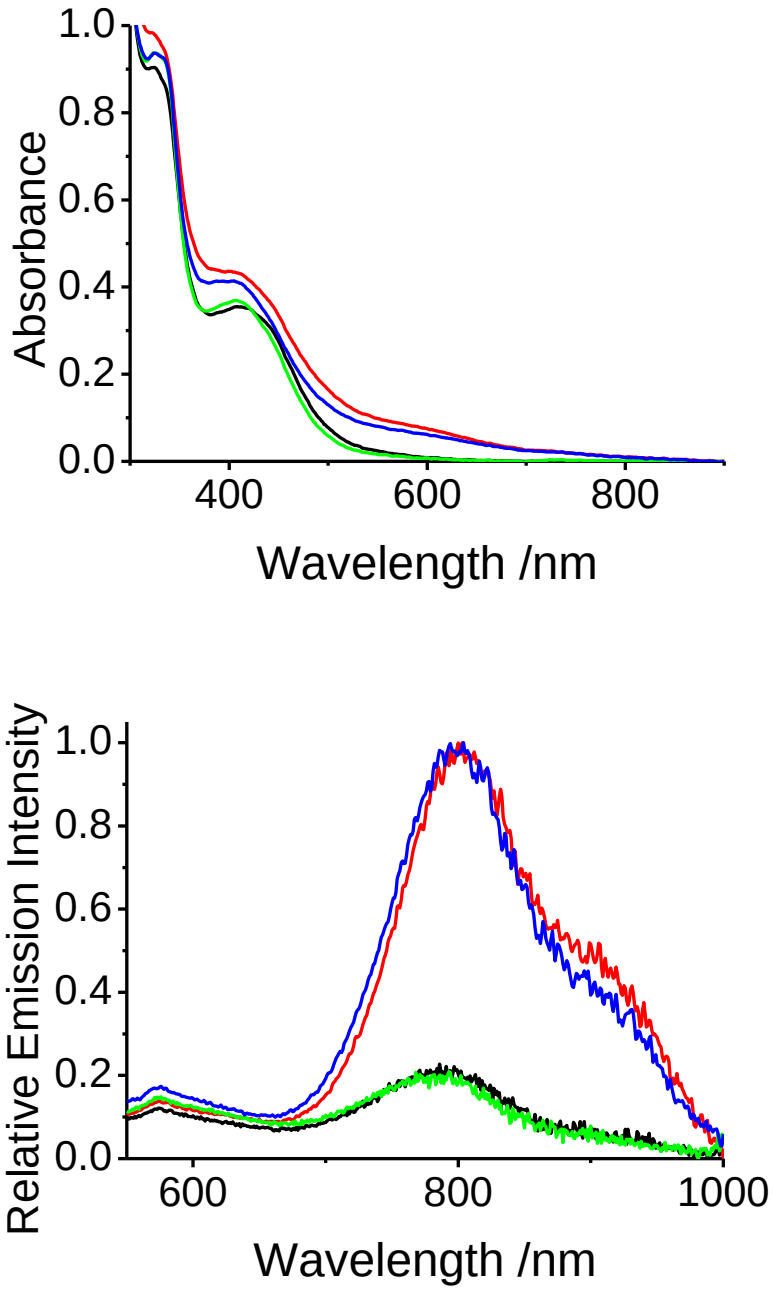

Fig. S15 UV-Vis absorption (top) and emission (bottom) spectral changes of **2** (90 μM) at 25 ^{o}C (—) and 37 ^{o}C (—), and in the presence of ***p*P1** (8 μM) at 25 ^{o}C (—) and 37 ^{o}C (—). Medium: 10 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.7.

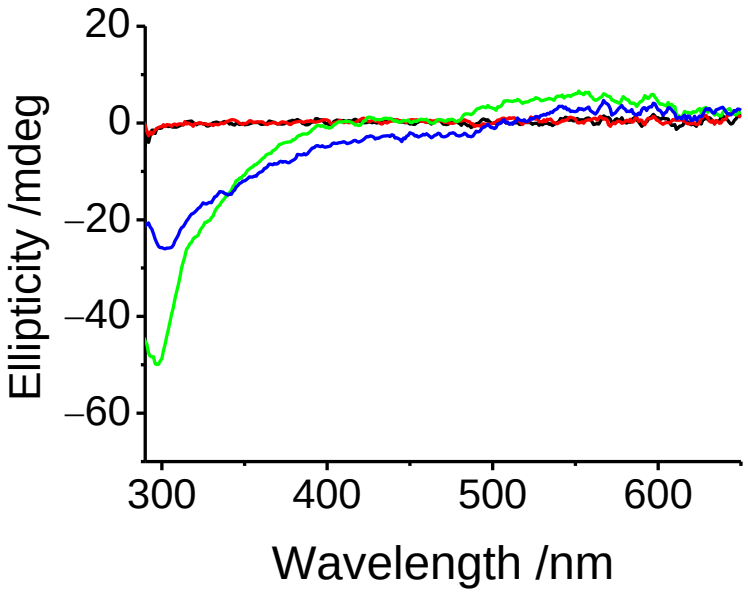

Fig. S16 CD spectra of **2** (90 μM) (—), ***p*P1** (8 μM) (—), and **2** (90 μM) in the presence of ***p*P1** (8 μM) at 25 ^{o}C (—) and 37 ^{o}C (—) respectively. Medium: 10 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.7.

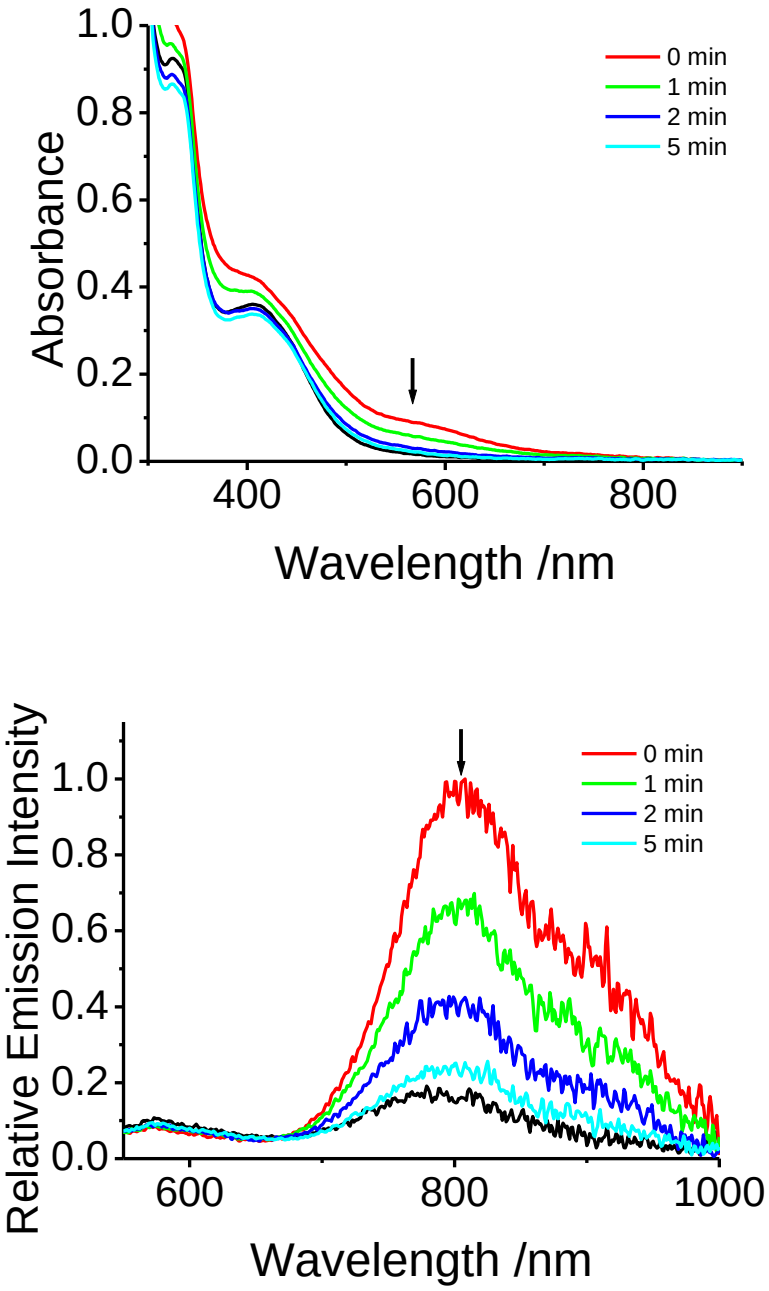

Fig. S17 UV-Vis absorption (top) and emission (bottom) spectral changes of **2** (90 μM) (—), and in the presence of ***p*P1** (8 μM) upon addition of ALP (1 unit ml^{-1}) at different time intervals. Medium: 10 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.7 at 37 ^{o}C.

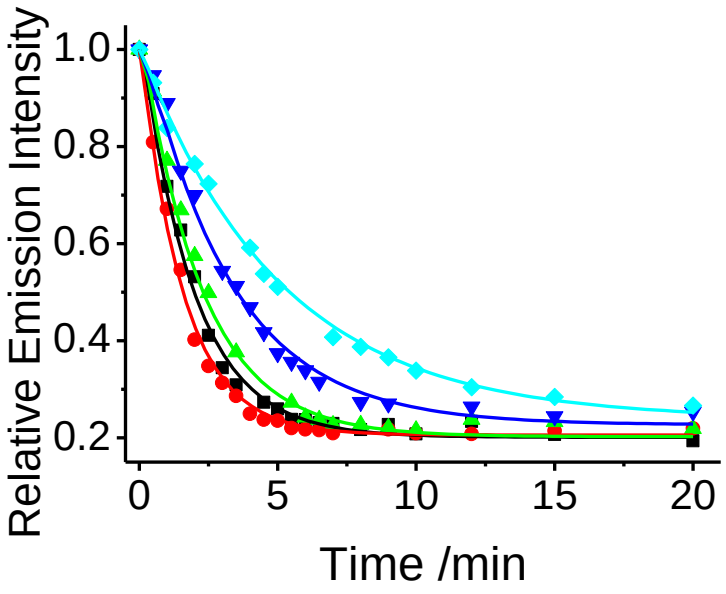

Fig. S18 Plots of relative emission intensity at 805 nm upon addition of ALP (1 unit ml^{-1}) to the mixtures of **2** (90 μM) and ***p*P1** (8 μM) at different time intervals at pH 7.5 (■), 7.7 (●), 8.2 (▲), 8.7 (▼) and 9.2 (◆). Medium: 10 mM Tris-HCl, 2 mM $MgCl_2$ at 37 ^{o}C.

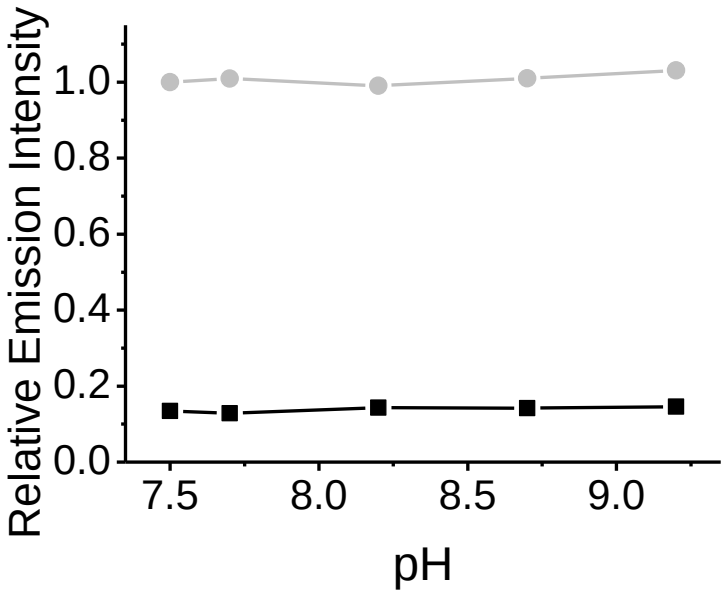

Fig. S19 Plots of relative emission intensity at 805 nm of **2** (90 μM) (■) and in the presence of ***p*P1** (8 μM) (●) at different pH. Medium: 10 mM Tris-HCl, 2 mM $MgCl_2$ at 37 ^{o}C.

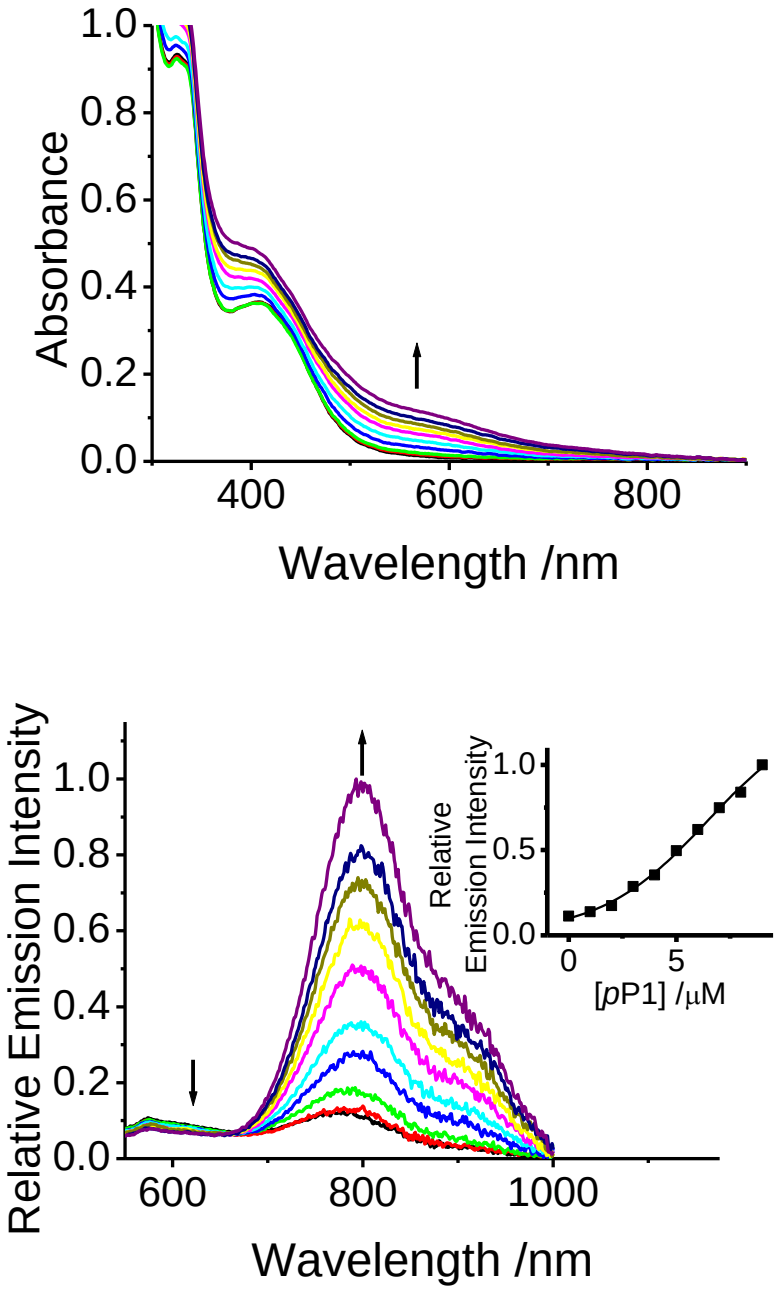

Fig. S20 UV-Vis absorption (top) and emission (bottom) spectral changes of **2** (90 μM) with increasing concentration of ***p*P1**. Inset: plot of relative emission intensity at 805 nm of **2** (90 μM) with various concentrations of ***p*P1**. Medium: 10 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.7 at 37 ^{o}C.

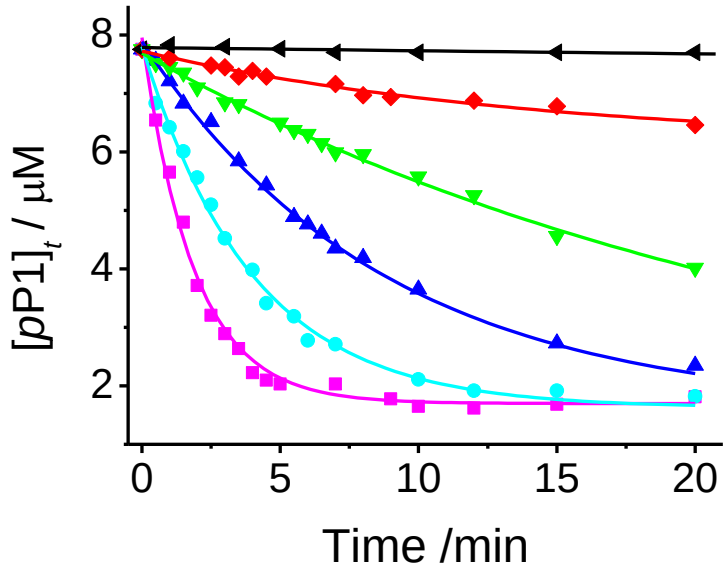

Fig. S21 Plots of [***p*P1**]$_t$ against time upon addition of 1 (■), 0.4 (●), 0.2 (▲), 0.1 (▼), 0.02 (◆) and 0 (◀) unit ml^{-1} of ALP to the mixtures of **2** (90 μM) with ***p*P1** (8 μM) at different time intervals. Medium: 10 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.7 at 37 ^{o}C.

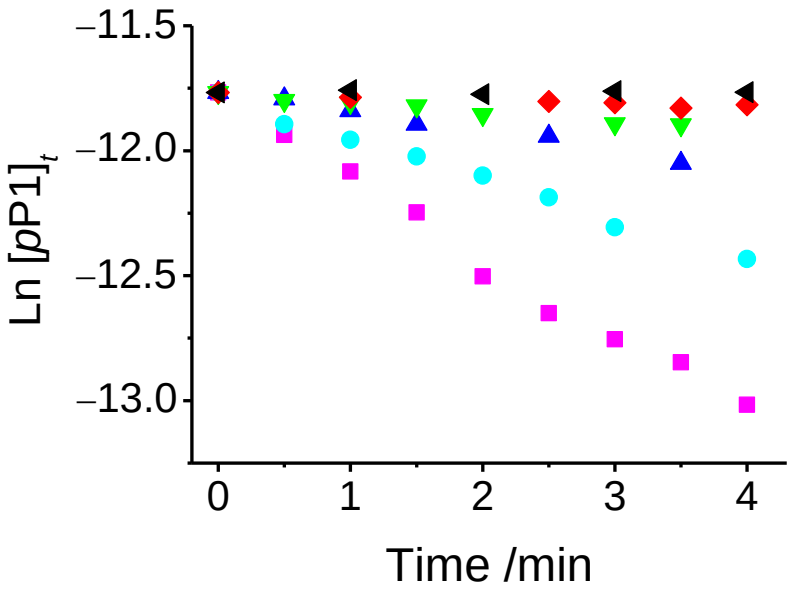

Fig. S22 Plots of natural logarithm of concentration of [***p*P1**]$_t$ against time upon addition of 1 (■), 0.4 (●), 0.2 (▲), 0.1 (▼), 0.02 (◆) and 0 (◀) unit ml^{-1} of ALP to the mixtures of **2** (90 μM) with ***p*P1** (8 μM) at different time intervals. Medium: 10 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.7 at 37 ^{o}C.

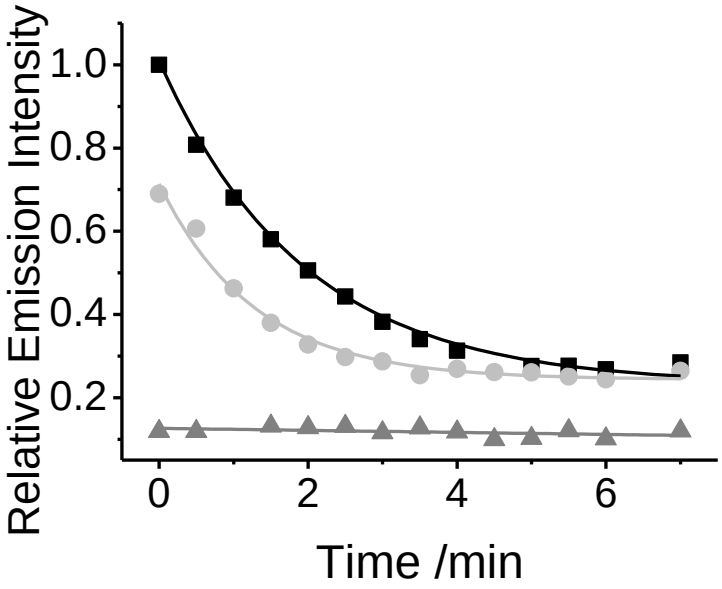

Fig. S23 Plots of relative emission intensity at 805 nm upon addition of ALP (1 unit ml^{-1}) to the mixtures of **2** (90 μM) with 9 (■), 6.5 (●) and 0 (▲) μM of ***p*P1** at different time intervals. Medium: 10 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.7 at 37 ^oC.

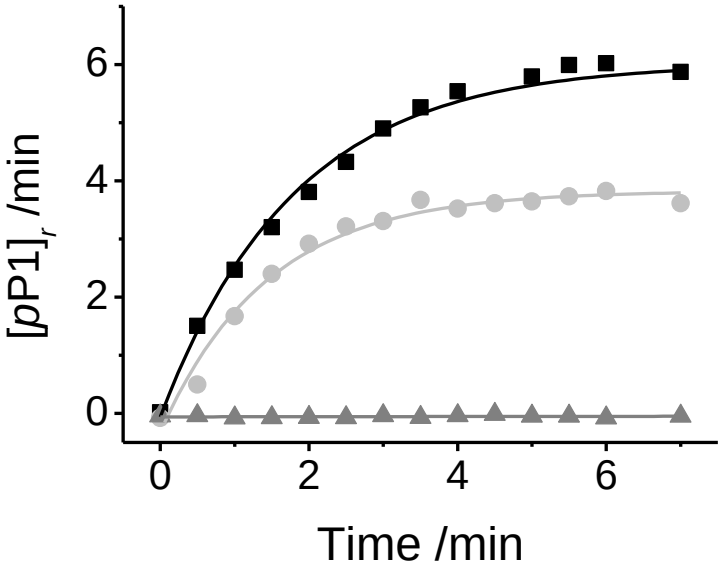

Fig. S24 Plots of [***p*P1**]$_r$ against time upon addition of ALP (1 unit ml^{-1}) to the mixtures of **2** (90 μM) with 9 (■), 6.5 (●) and 0 (▲) μM of ***p*P1** at different time intervals. Medium: 10 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.7 at 37 ^oC.