

## Supplementary Information

### Quantitative analysis of modeled ATP hydrolysis in water by a colorimetric sensor array

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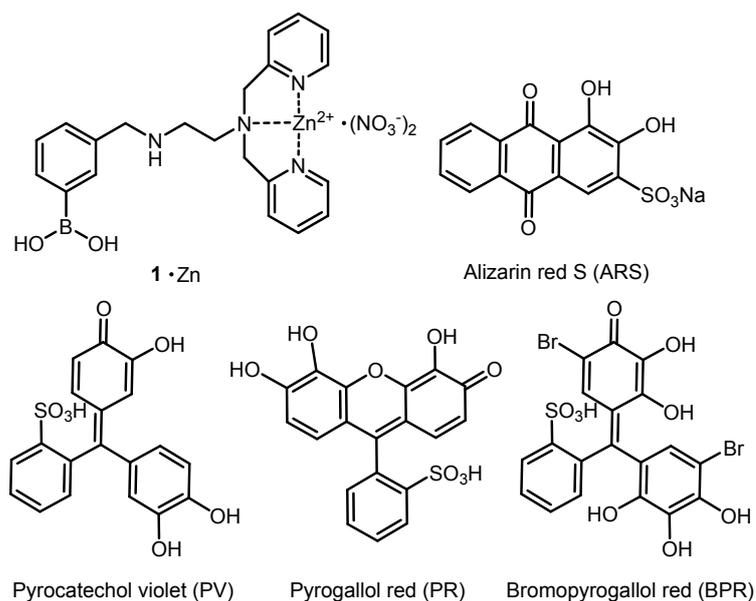
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## 1. General

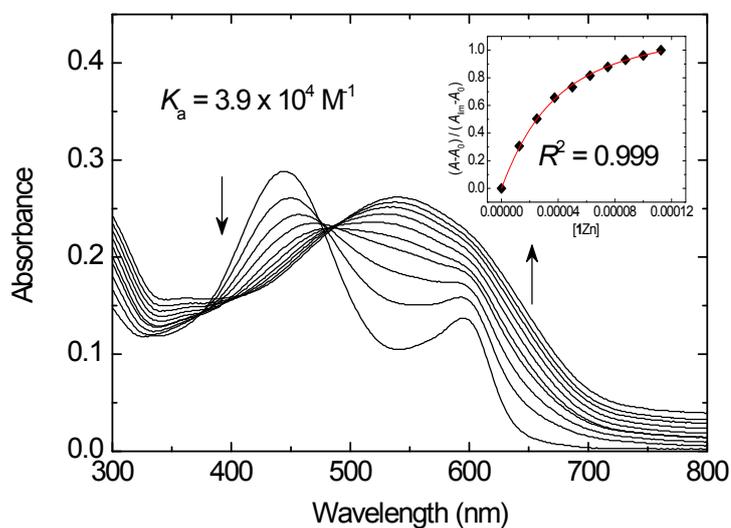
Compound **1**•Zn was prepared according to the literature procedure.<sup>1</sup> Alizarin red S (ARS) was recrystallized from ethanol. The nanopure water was used for all photophysical experiments. The phosphate guests were purchased from Sigma-Aldrich.

UV–vis spectra were measured using a HITACHI U-3010 spectrophotometer. UV–vis spectra were recorded from 300 nm to 800 nm. Titration isotherms were constructed from changes in the absorption maximum. The data analysis for binding constants was performed according to published methods.<sup>2</sup>

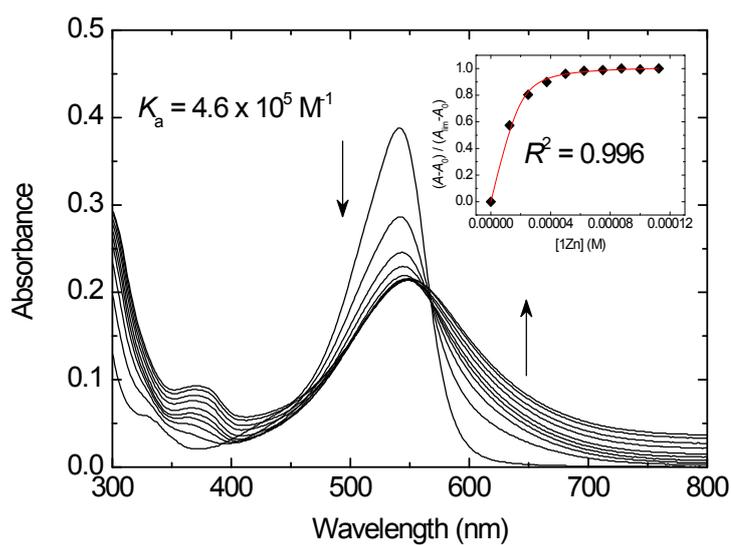
The array experiments for quantitative analysis of the mixtures of PPi, ATP and AMP were performed in 384-well plates using a Beckman BioRAPTR microfluidic robotic dispenser. The fluids (HEPES buffer (10 mM) with KCl (10 mM) at pH 7.5), **1**•Zn (60  $\mu$ M), PV, PG, BPG, ARS (20  $\mu$ M) and analyte solutions) were contact-free dispensed at 200 nL/s as follows. Each experiment was performed in 4 repetitions. Each well received 18  $\mu$ L of buffer, followed by 18  $\mu$ L of **1**•Zn, and 18  $\mu$ L of dye solution, after which the mixture was equilibrated for 2 min to establish the **1**•Zn-dye complex. Subsequently, 18  $\mu$ L of analyte solutions were added. After the analyte was dispensed, the plate was centrifuged (2 min, 2500 rpm,  $T = 22$  °C). Absorption spectra were measured by a BMG FluoStar OMEGA microplate reader. The spectra were recorded from 300 nm to 700 nm. Thus obtained data were then analyzed by Artificial Neural Network-Radial Basis Function (ANN-RBF) to predict the concentrations of PPi, ATP and AMP. ANN-RBF was achieved with PCA as a preprocessing method. The values of the parameters (PCs, hidden nodes and spread values) were optimized to obtain relative standard error for estimation of concentration in the validation set (RSEV%).



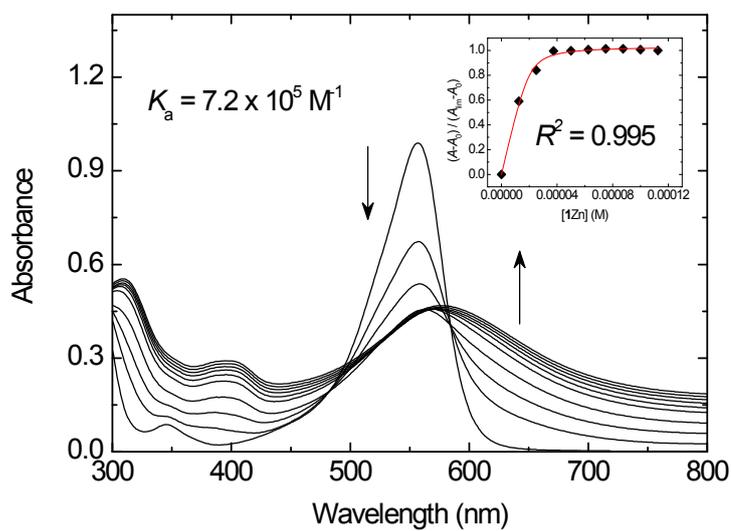
## 2. UV-vis Titration



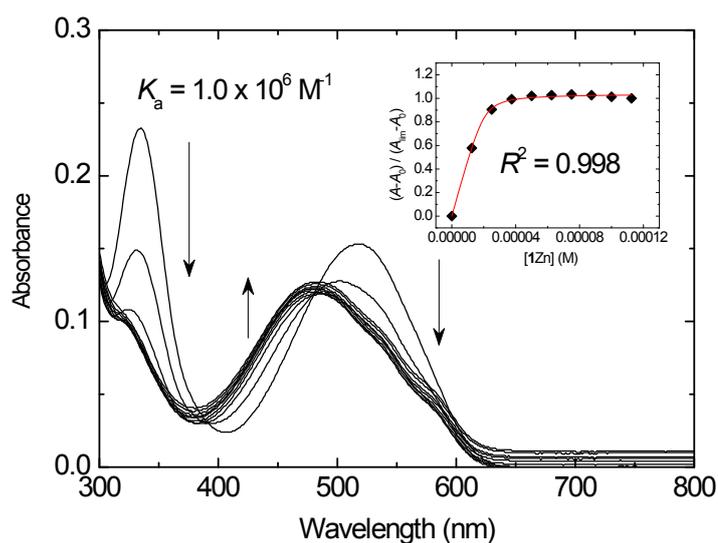
**Fig. S1** UV-vis spectra of PV ( $2.0 \times 10^{-5} \text{ M}$ ) upon the addition of **1·Zn** in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5. [**1·Zn**] = 0–0.11 mM.



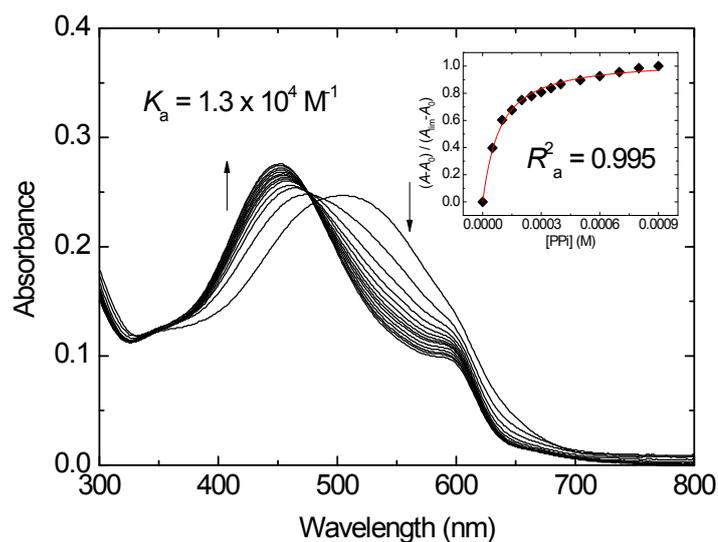
**Fig. S2** UV-vis spectra of PR ( $2.0 \times 10^{-5}$  M) upon the addition of **1•Zn** in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5.  $[1\bullet\text{Zn}] = 0 - 0.11$  mM.



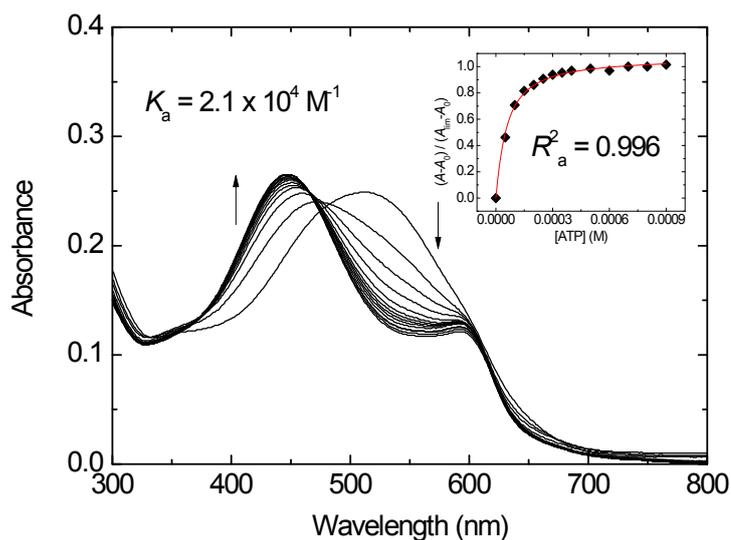
**Fig. S3** UV-vis spectra of BPR ( $2.0 \times 10^{-5}$  M) upon the addition of **1•Zn** in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5.  $[1\bullet\text{Zn}] = 0 - 0.11$  mM.



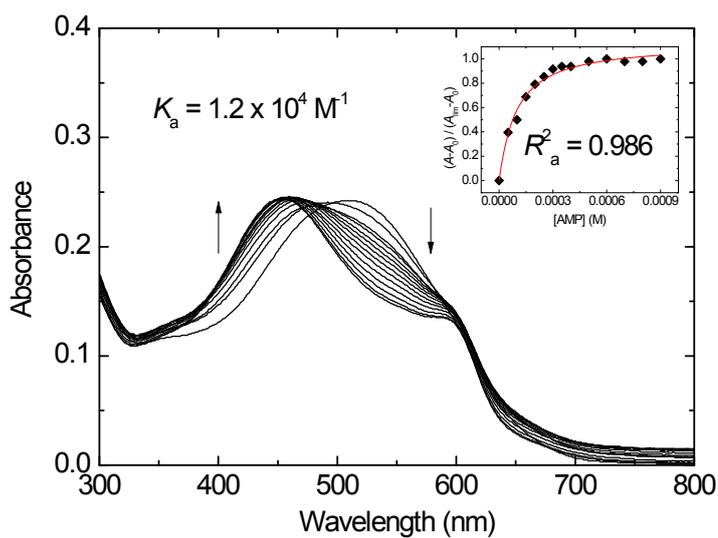
**Fig. S4** UV-vis spectra of ARS ( $2.0 \times 10^{-5}$  M) upon the addition of **1•Zn** in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5.  $[1\bullet\text{Zn}] = 0 - 0.11$  mM.



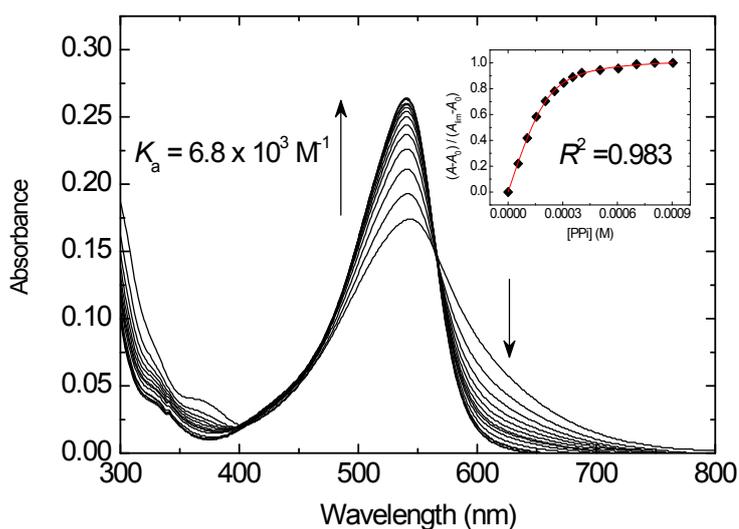
**Fig. S5** UV-vis spectra of **1•Zn** ( $6.0 \times 10^{-5}$  M)-PV ( $2.0 \times 10^{-5}$  M) upon the addition of PPI in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5.  $[\text{PPI}] = 0 - 0.90$  mM.



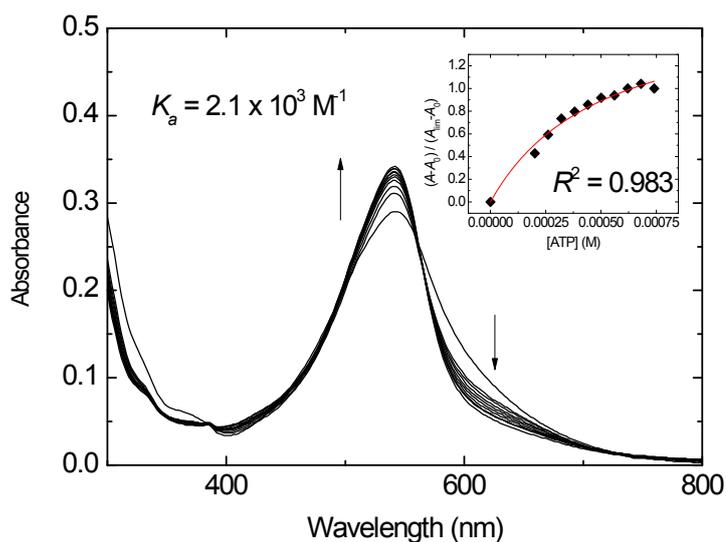
**Fig. S6** UV-vis spectra of  $1 \cdot \text{Zn}$  ( $6.0 \times 10^{-5} \text{ M}$ )-PV ( $2.0 \times 10^{-5} \text{ M}$ ) upon the addition of ATP in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5. [ATP] = 0 – 0.90 mM.



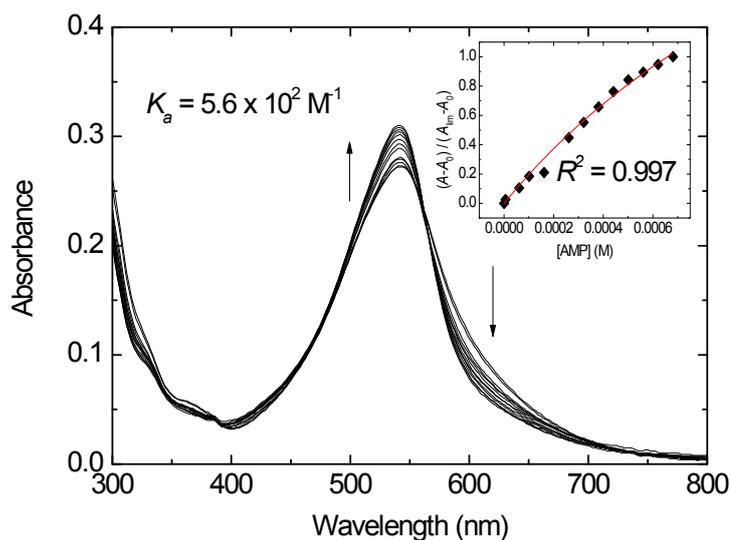
**Fig. S7** UV-vis spectra of  $1 \cdot \text{Zn}$  ( $6.0 \times 10^{-5} \text{ M}$ )-PV ( $2.0 \times 10^{-5} \text{ M}$ ) upon the addition of AMP in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5. [AMP] = 0 – 0.90 mM.



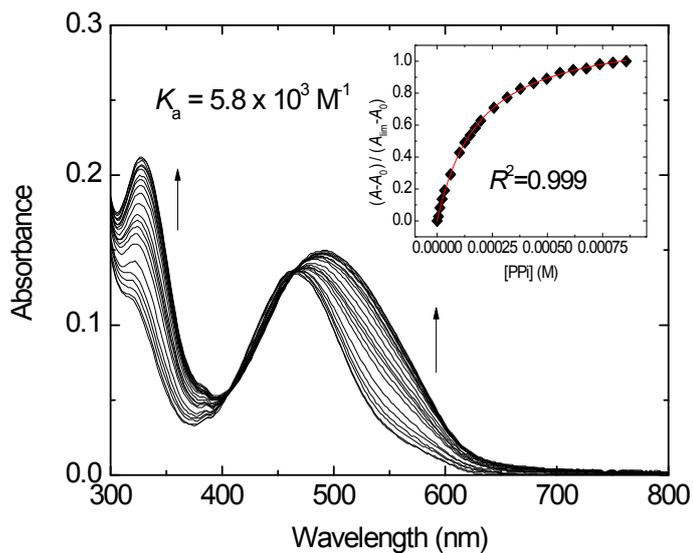
**Fig. S8** UV-vis spectra of  $1 \cdot \text{Zn}$  ( $6.0 \times 10^{-5} \text{ M}$ )-PR ( $2.0 \times 10^{-5} \text{ M}$ ) upon the addition of PPI in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5. [PPI] = 0 – 0.91 mM.



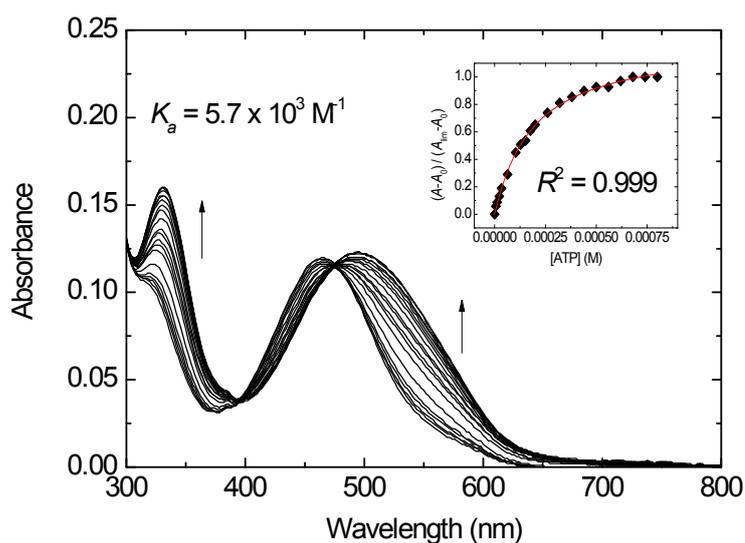
**Fig. S9** UV-vis spectra of  $1 \cdot \text{Zn}$  ( $6.0 \times 10^{-5} \text{ M}$ )-PR ( $2.0 \times 10^{-5} \text{ M}$ ) upon the addition of ATP in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5. [ATP] = 0 – 0.74 mM.



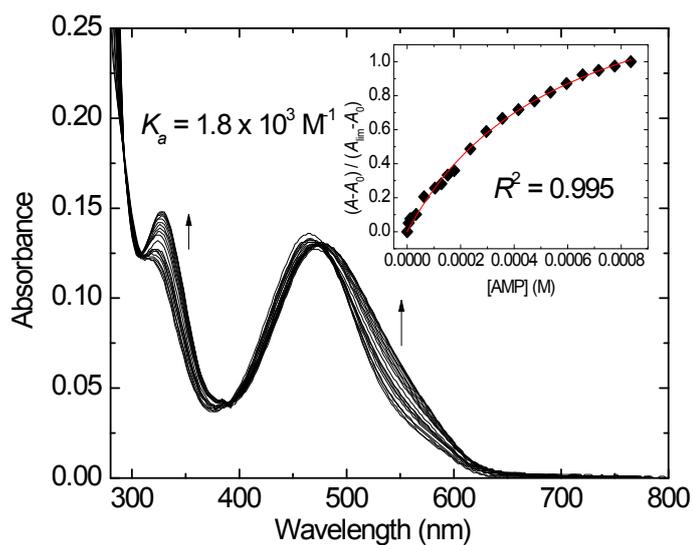
**Fig. S10** UV-vis spectra of **1•Zn** ( $6.0 \times 10^{-5}$  M)-PR ( $2.0 \times 10^{-5}$  M) upon the addition of AMP in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5. [AMP] = 0 – 0.68 mM.



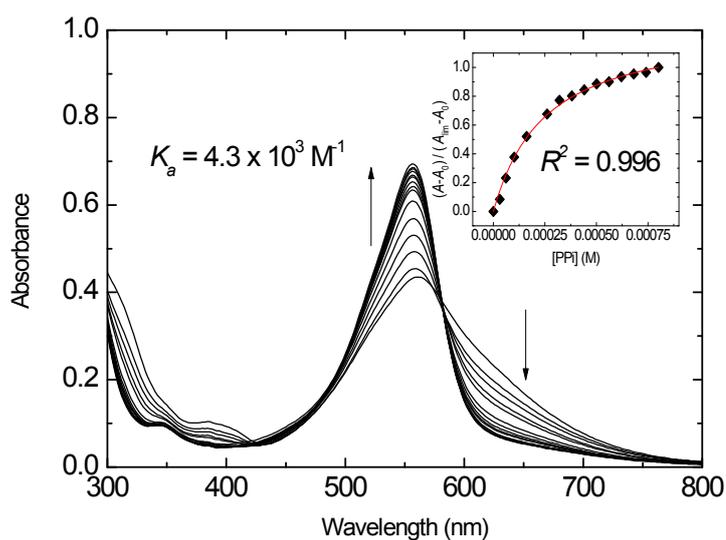
**Fig. S11** UV-vis spectra of **1•Zn** ( $6.0 \times 10^{-5}$  M)-ARS ( $2.0 \times 10^{-5}$  M) upon the addition of PPI in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5. [PPI] = 0 – 0.86 mM.



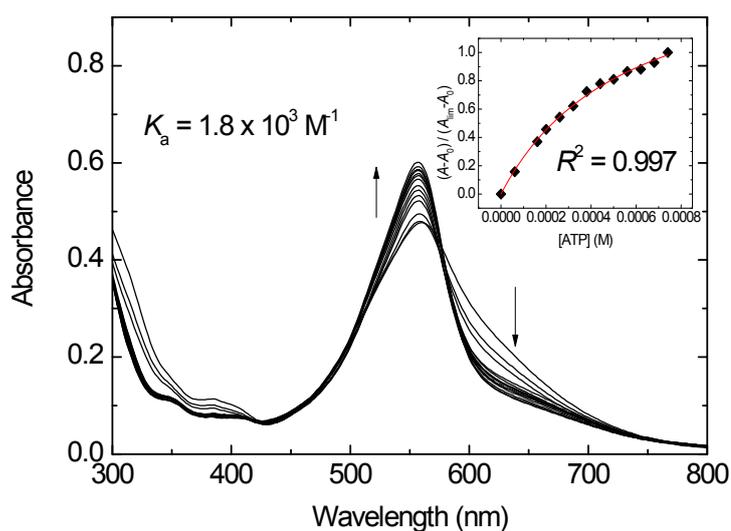
**Fig. S12** UV-vis spectra of  $1 \bullet \text{Zn}$  ( $6.0 \times 10^{-5} \text{ M}$ )-ARS ( $2.0 \times 10^{-5} \text{ M}$ ) upon the addition of ATP in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5.  $[\text{ATP}] = 0 - 0.80 \text{ mM}$ .



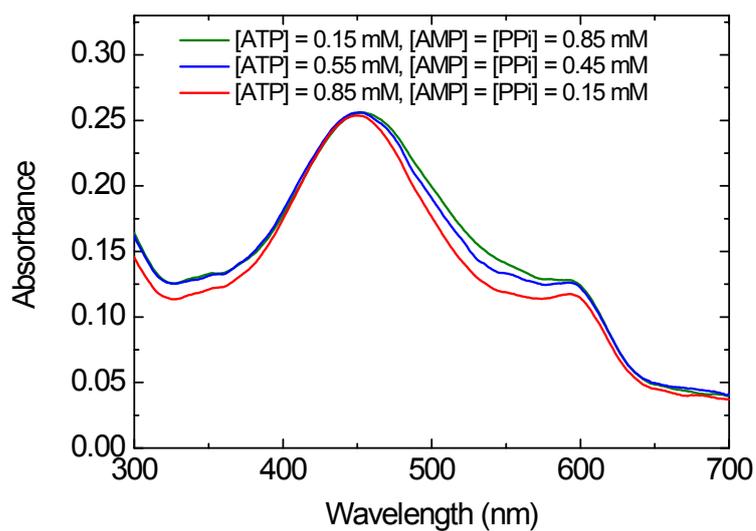
**Fig. S13** UV-vis spectra of  $1 \bullet \text{Zn}$  ( $6.0 \times 10^{-5} \text{ M}$ )-ARS ( $2.0 \times 10^{-5} \text{ M}$ ) upon the addition of AMP in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5.  $[\text{AMP}] = 0 - 0.84 \text{ mM}$ .



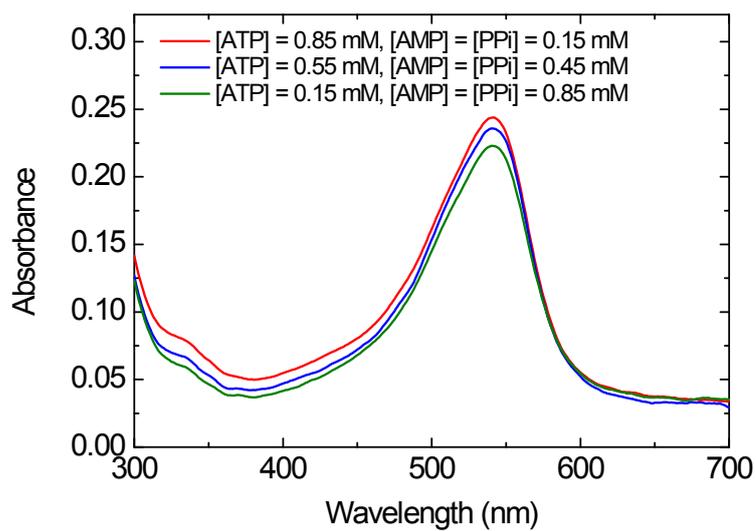
**Fig. S14** UV-vis spectra of  $1 \cdot \text{Zn}$  ( $6.0 \times 10^{-5} \text{ M}$ )-BPR ( $2.0 \times 10^{-5} \text{ M}$ ) upon the addition of PPI in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5. [PPI] = 0 – 0.80 mM.



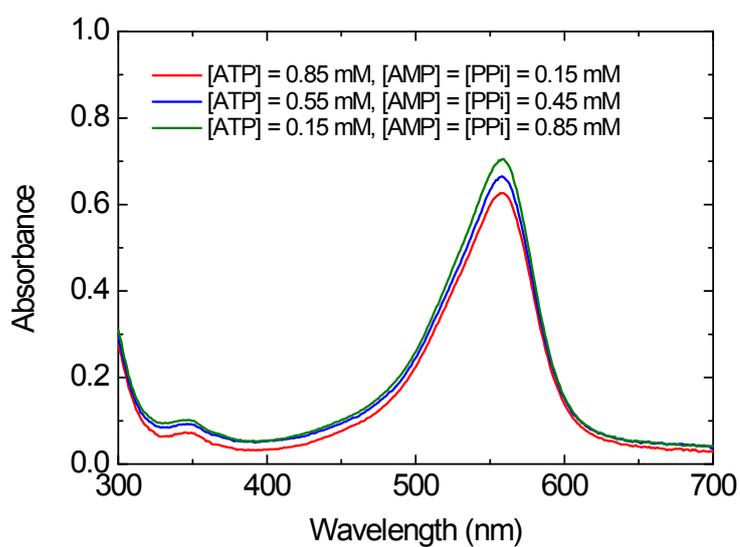
**Fig. S15** UV-vis spectra of  $1 \cdot \text{Zn}$  ( $6.0 \times 10^{-5} \text{ M}$ )-BPR ( $2.0 \times 10^{-5} \text{ M}$ ) upon the addition of ATP in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5. [ATP] = 0 – 0.74 mM.



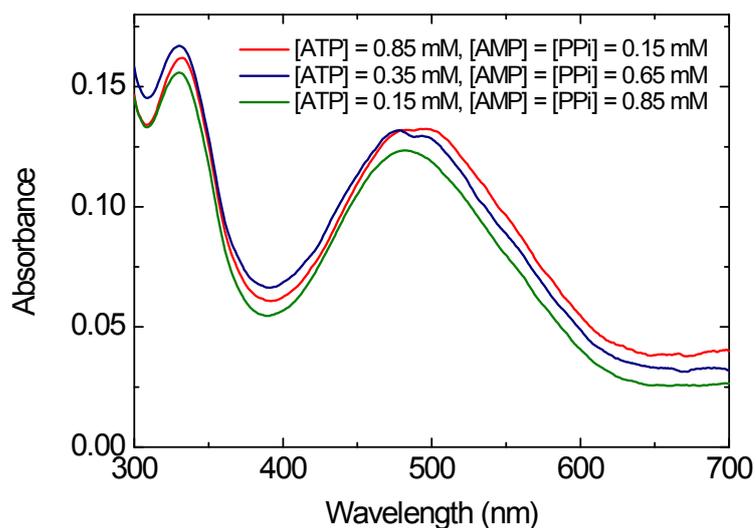
**Fig. S16** UV-vis spectra of  $1 \cdot \text{Zn}$  ( $6.0 \times 10^{-5} \text{ M}$ )-PV ( $2.0 \times 10^{-5} \text{ M}$ ) upon the addition of phosphate mixtures in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5.



**Fig. S17** UV-vis spectra of  $1 \cdot \text{Zn}$  ( $6.0 \times 10^{-5} \text{ M}$ )-PR ( $2.0 \times 10^{-5} \text{ M}$ ) upon the addition of phosphate mixtures in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5.

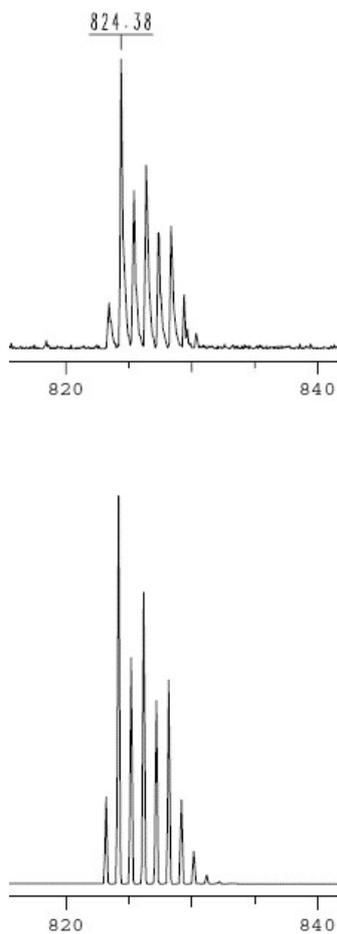


**Fig. S18** UV-vis spectra of  $1\cdot\text{Zn}$  ( $6.0\times 10^{-5}$  M)-BPR ( $2.0\times 10^{-5}$  M) upon the addition of phosphate mixtures in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5.

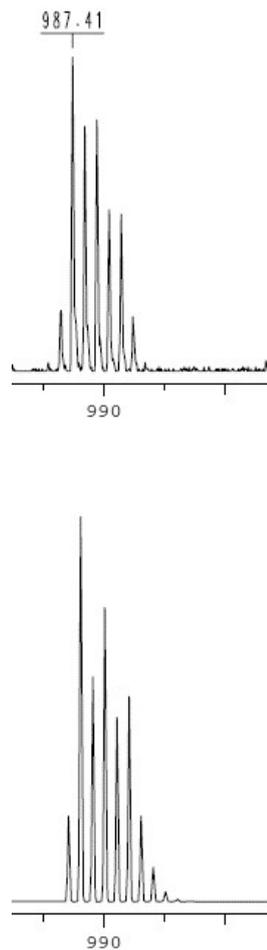


**Fig. S19** UV-vis spectra of  $1\cdot\text{Zn}$  ( $6.0\times 10^{-5}$  M)-ARS ( $2.0\times 10^{-5}$  M) upon the addition of phosphate mixtures in HEPES buffer solution (10 mM) with sodium chloride (10 mM) at pH 7.5.

### 3. MALDI-TOF MS Analysis

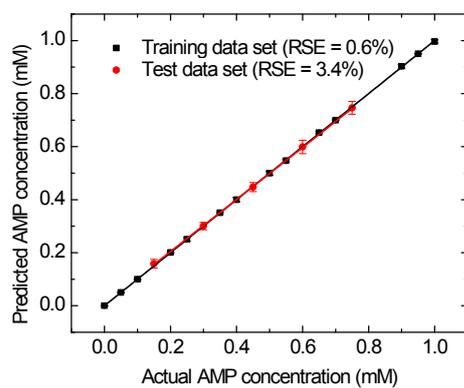
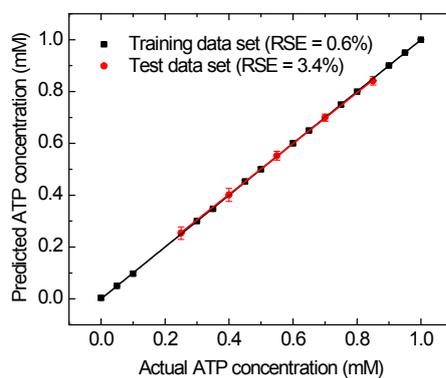
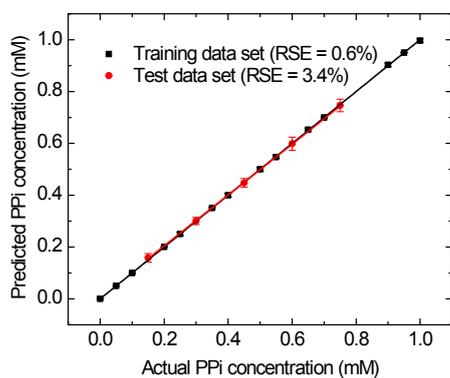
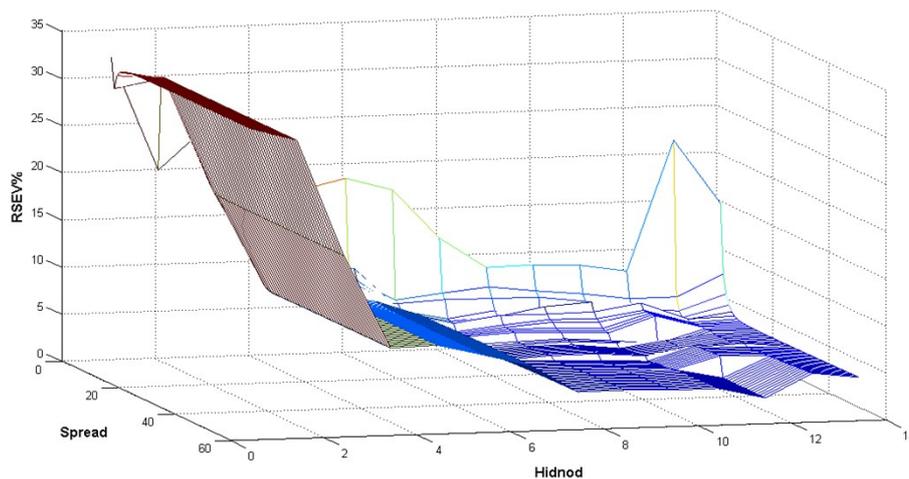


**Fig. S20 Top:** MALDI TOF MS (positive mode) spectra of **1•Zn** and PV, **Bottom:** isotope pattern for  $C_{40}H_{37}BN_4O_9SZn^+$ .



**Fig. S21 Top:** MALDI TOF MS (positive mode) spectra of the complex of **1**•Zn, PPI and PV. **Bottom:** isotope pattern for  $C_{40}H_{36}BN_4NaO_{14}P_2SZn^+$ .

#### **4. Regression Analysis of Phosphate Mixture in Water**



**Fig. S22. Top:** Mesh Plots of %RSEV as a function of number of nodes in the hidden layer and spread for PPI, ATP and AMP mixtures. The lowest RSEV was 3.4%. **Bottom:** The regression analysis of PPI, ATP, and AMP in the mixtures by ANN-RBF.

## References

1. A. Nonaka, S. Horie, T. D. James and Y. Kubo, *Org. Biomol. Chem.*, 2008, **6**, 3621.
2. J. R. Lakowicz, *Principles of Fluorescence Spectroscopy 3rd Ed.*, 2006, Springer, New York, USA.