

Supporting Information

**Minimalist Second-Sphere Engineering with Polyvinyl Alcohol Drives  
Cooperative Hydrolysis in Zn(II) System**

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## Materials

Zinc acetate dihydrate ( $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ ), zinc nitrate hexahydrate ( $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ), zinc chloride ( $\text{ZnCl}_2$ ), ammonium cerium(IV) nitrate ( $\text{Ce}(\text{NH}_4)_2(\text{NO}_3)_6$ ), polyvinyl alcohol (PVA,  $M_w$  9000-10000, 80% hydrolyzed), polyethylene glycol (PEG,  $M_w$  6000), ethylene glycol (EG), bis(4-nitrophenyl) phosphate (BNPP), *p*-nitrophenyl phosphate (*p*-NPP), and 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich. All reagents were of analytical grade and used as received without further purification.

## Instrumentation

UV-vis absorption spectra and kinetic assays were recorded using a Microplate Reader (Thermo Fisher Scientific Co., Ltd., Sweden) equipped with temperature control. Fourier transform infrared (FT-IR) spectra were collected on a Nicolet iS5 spectrometer (Thermo Fisher Scientific Inc., Waltham, USA) to characterize the chemical composition of the polymer matrix and the coordination environment of the metal centers. Fluorescence emission spectra for Nile Red assays were measured using an Agilent Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, USA). Dynamic light scattering (DLS) instrument (Zetasizer Nano ZS, Malvern Instruments, UK) was used to determine the hydrodynamic diameter of the Zn(II) catalytic systems.

## Preparation of PVA with Varying Degrees of Hydrolysis

Commercial PVA80 ( $M_w$  9000-10000, 80% hydrolysis) was used as the starting material. PVA80 (200 mg) was dissolved in a mixed solvent of methanol and water (3 mL, volume ratio 2/1). NaOH aqueous solution (10 M) was added to the mixture in amounts of 4  $\mu\text{L}$  or 28  $\mu\text{L}$  respectively. The reaction mixture was stirred at 70°C for 40 min. The resulting PVA products were precipitated using an acetone and ethyl acetate mixture. The precipitate was dried under vacuum at room temperature overnight. The degree of hydrolysis (DH) was verified by FTIR spectroscopy. To quantitatively determine the DH, the relative peak intensity ratio method was employed based on the Beer-Lambert law. The transmittance spectra were first converted to absorbance ( $A = 2 - \log T\%$ ). The characteristic peak centered at approximately  $1735 \text{ cm}^{-1}$ , corresponding to the C=O stretching vibration of the residual acetate groups, was selected as the indicator of acetylation. The peak at

2940  $\text{cm}^{-1}$ , attributed to the C-H stretching vibration of the polymer backbone, served as the invariant internal reference. The integrated areas of these peaks were calculated after baseline correction. The area ratio ( $R$ ) was defined as  $R = A_{1735}/A_{2940}$ . Using the starting material PVA80 (DH=80%) as the internal standard, the residual acetyl content (Degree of Acetylation,  $DA$ ) and the DH of the modified PVA samples were calculated using the following equations:

$$DA_{\text{sample}} = DA_{\text{std}} \times \frac{R_{\text{sample}}}{R_{\text{std}}}$$

$$DH_{\text{sample}} = 100\% - DA_{\text{sample}}$$

where  $DA_{\text{std}}$  is the known acetyl content of PVA80 (20%), and  $R_{\text{sample}}$  and  $R_{\text{std}}$  represent the integrated area ratios ( $A_{1735}/A_{2940}$ ) of the modified sample and the PVA80 standard, respectively.

### Hydrolysis Activity Assays

For the Zn(II) system, the reaction mixture contained Zn(II) (2 mM) and the additive (PVA, EG, or PEG, 1  $\text{mg mL}^{-1}$ ) and BNPP (2 mM) in HEPES buffer (50 mM, pH 8.0). The hydrolysis process was monitored by measuring the absorbance change at 400 nm using a microplate reader.

For the Ce(IV) system, the concentration of Ce(IV) was 0.2 mM while other conditions remained identical to the Zn(II) system.

For the *p*-NPP hydrolysis experiments, the substrate concentration was set to 4 mM with all other conditions kept the same as the BNPP system.

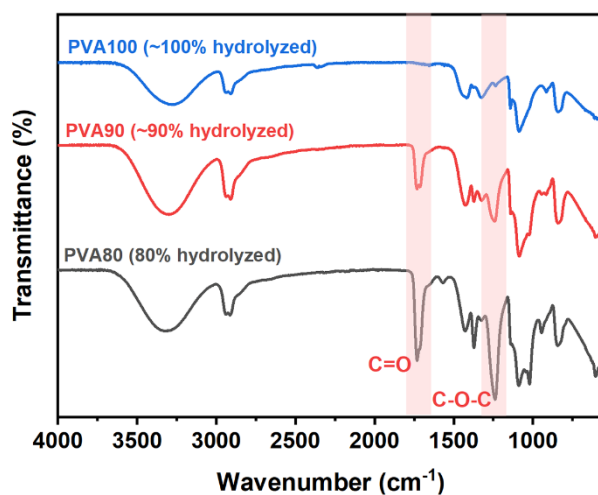
### Kinetic Studies

Kinetic experiments were conducted with either Zn(II) (2 mM) or Ce(IV) (0.2 mM) in the presence of PVA80 (1  $\text{mg mL}^{-1}$ ). The concentration of BNPP was varied across a range of 0, 0.125, 0.25, 0.5, 0.75, 1, 1.5, and 2 mM. The reaction was performed in HEPES buffer (50 mM, pH 8.0). The initial reaction rates were determined by monitoring the absorbance at 400 nm via a microplate reader. The molar extinction coefficient of *p*-nitrophenol at 400 nm in HEPES buffer (pH 8.0) was determined to be 18000  $\text{M}^{-1} \text{cm}^{-1}$ .

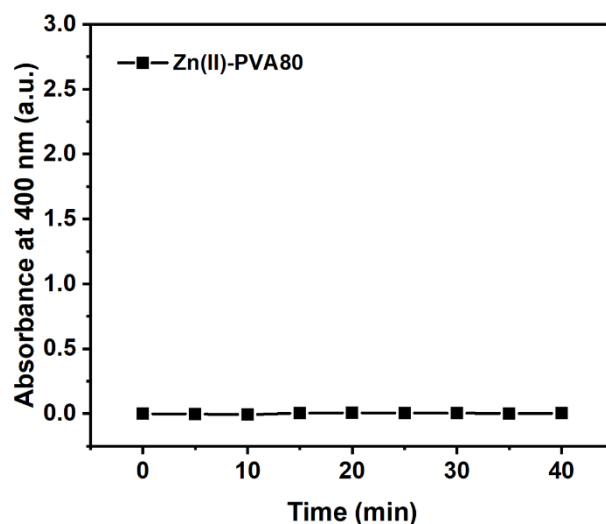
### Nile Red Fluorescence Assays

A stock solution of Nile Red was prepared 2 mM in DMSO. The detection system

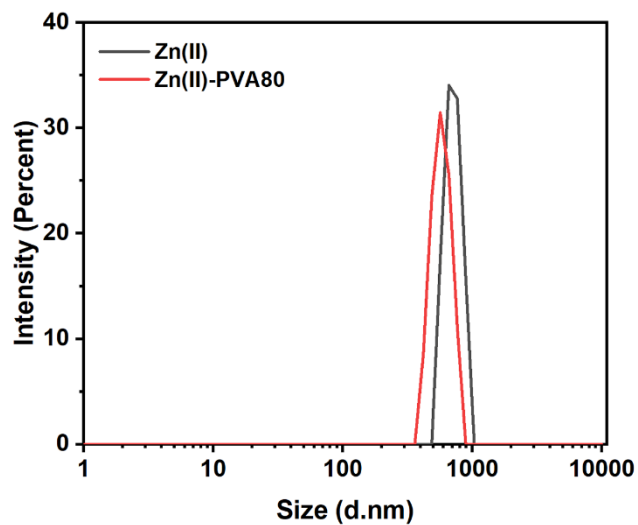
contained Nile Red (1  $\mu\text{M}$ ) and PVA (1  $\text{mg mL}^{-1}$ ) in HEPES buffer (50 mM, pH 8.0). The concentration of the substrate (BNPP or *p*-NPP) in the system was 2 mM. Fluorescence spectra were recorded with an excitation wavelength of 490 nm and the emission was collected from 500 nm to 800 nm. Both excitation and emission slit widths were set to 5 nm.



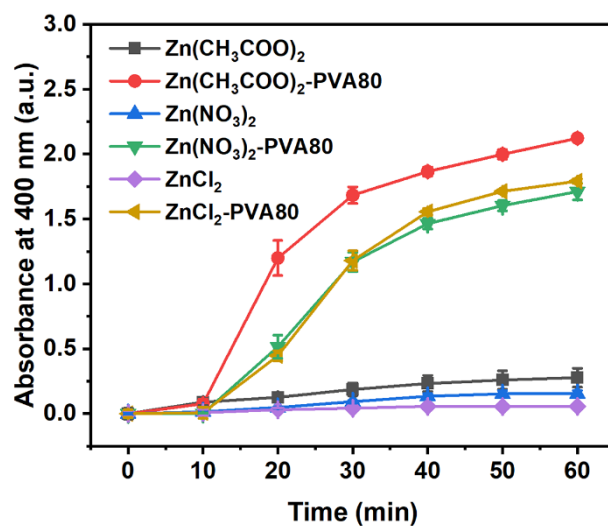
**Figure S1.** FTIR spectra of PVA80, PVA90 and PVA100.



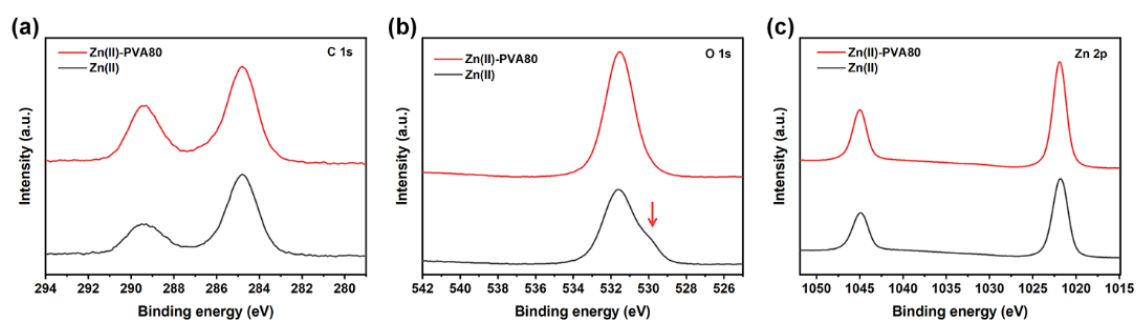
**Figure S2.** Time-dependent absorbance at 400 nm for the Zn(II)-PVA80 system in the absence of BNPP. Conditions:  $[\text{Zn(II)}]=2 \text{ mM}$ ,  $[\text{PVA80}]=1 \text{ mg mL}^{-1}$ , HEPES buffer (50 mM, pH 8.0) at  $37^\circ\text{C}$ .



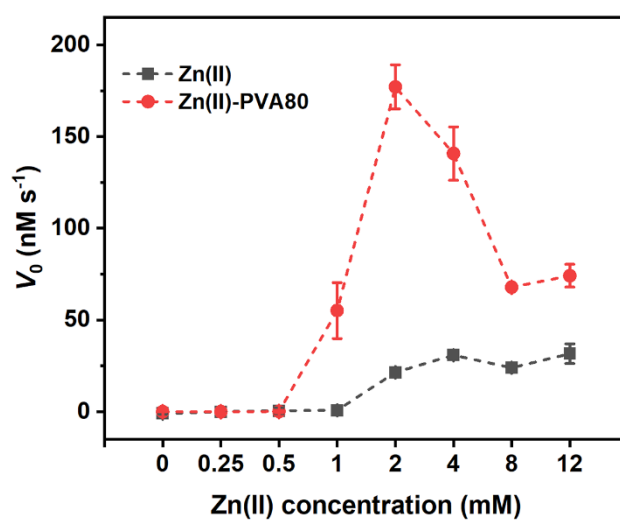
**Figure S3.** Dynamic light scattering (DLS) analysis of Zn(II) and Zn(II)-PVA80 systems in 50 mM HEPES buffer (pH 8.0).



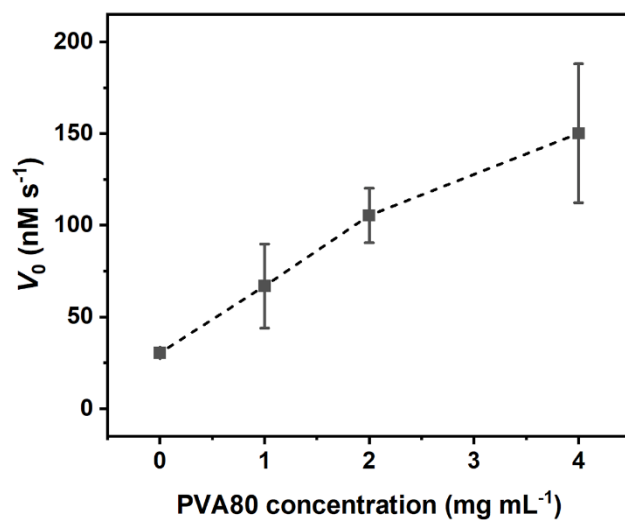
**Figure S4.** Time-dependent absorbance changes at 400 nm for the hydrolysis of BNPP catalyzed by different zinc salts in the presence and absence of PVA80. Conditions: [Zn(II)]=2 mM, [PVA80]=1 mg mL<sup>-1</sup>, [BNPP]=2 mM, HEPES buffer (50 mM, pH 8.0) at 37°C.



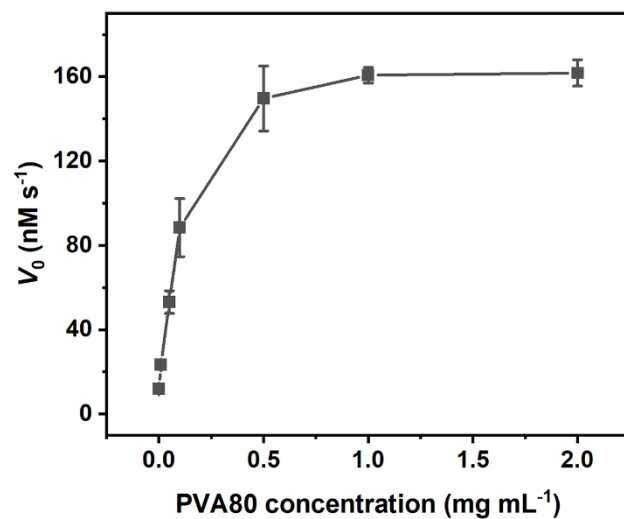
**Figure S5.** High-resolution C 1s (a), O 1s (b) and Zn 2p (c) XPS spectra of the solid precipitate isolated from the Zn(II) or Zn(II)-PVA80 mixture.



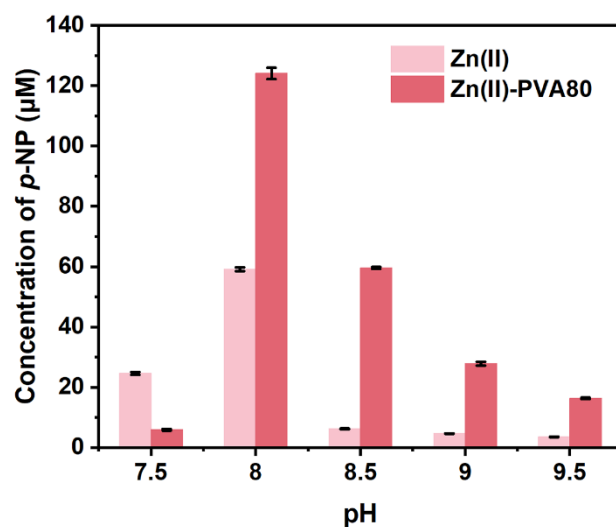
**Figure S6.** Initial hydrolysis rates of the Zn(II) and Zn(II)-PVA80 systems as a function of Zn(II) concentration. PVA80 1 mg mL<sup>-1</sup>, BNPP 2 mM, 50 mM HEPES pH 8 at 37 °C.



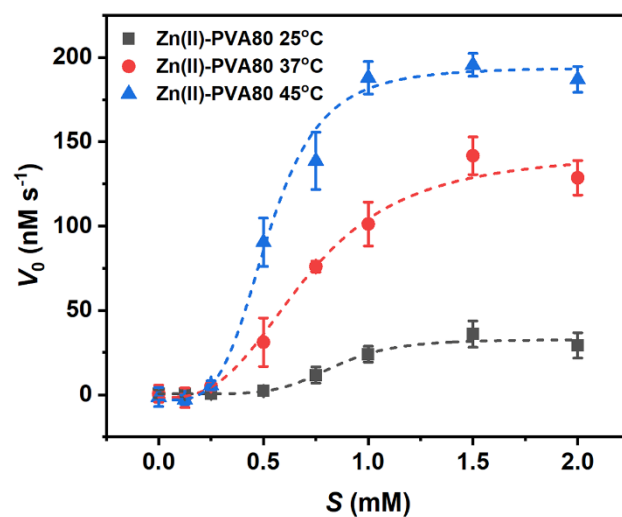
**Figure S7.** Dependence of the initial hydrolysis rate on different PVA80 concentrations (1, 2, 4 mg mL<sup>-1</sup>) at a fixed Zn(II) concentration of 8 mM. BNPP 2 mM, 50 mM HEPES pH 8 at 37 °C.



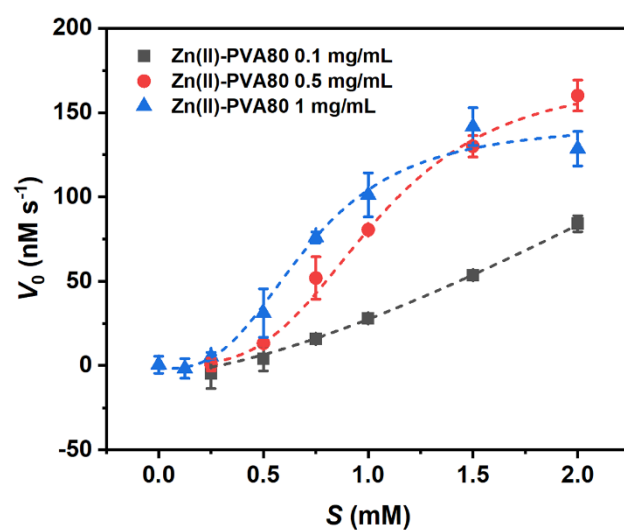
**Figure S8.** Initial hydrolysis rates measured at varying PVA80 concentrations ranging from 0 to 2 mg mL<sup>-1</sup>. Zn(II) 2 mM, BNPP 2 mM, 50 mM HEPES pH 8 at 37 °C.



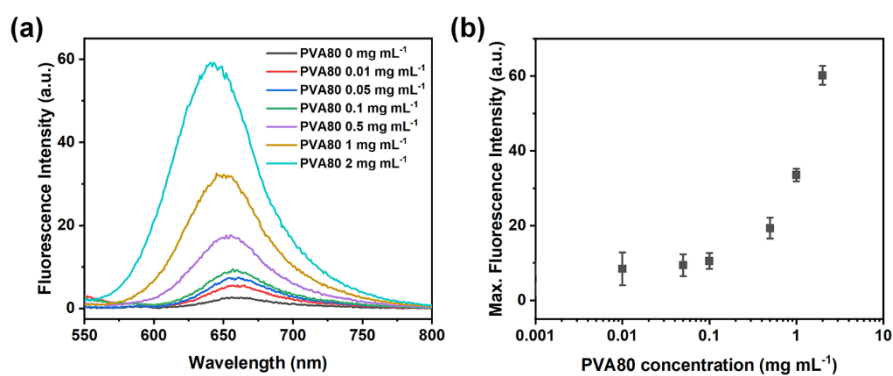
**Figure S9.** Concentration of *p*-nitrophenol (*p*-NP) produced after 1.5 h in Zn(II) and Zn(II)-PVA80 systems under different pH conditions. Zn(II) 2 mM, PVA80 1 mg mL<sup>-1</sup>, BNPP 2 mM, 50 mM HEPES at 37 °C.



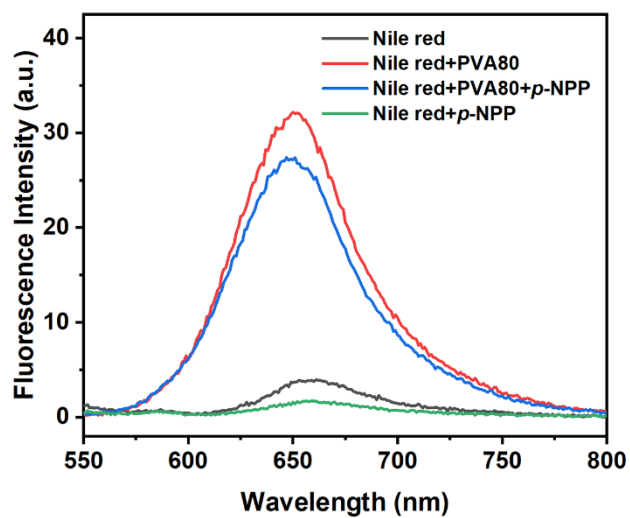
**Figure S10.** Initial hydrolysis rates of the Zn(II)-PVA80 system at different temperatures and varying BNPP concentrations. Zn(II) 2 mM, PVA80 1 mg mL<sup>-1</sup>, 50 mM HEPES pH 8 at 37 °C.



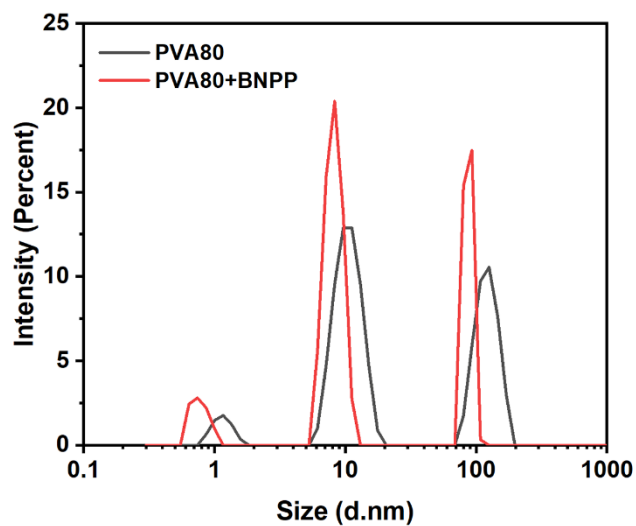
**Figure S11.** Initial hydrolysis rates of the Zn(II)-PVA80 system at different PVA80 concentrations (0.1, 0.5 and 1 mg mL<sup>-1</sup>) and varying BNPP concentrations. Zn(II) 2 mM, 50 mM HEPES pH 8 at 37 °C.



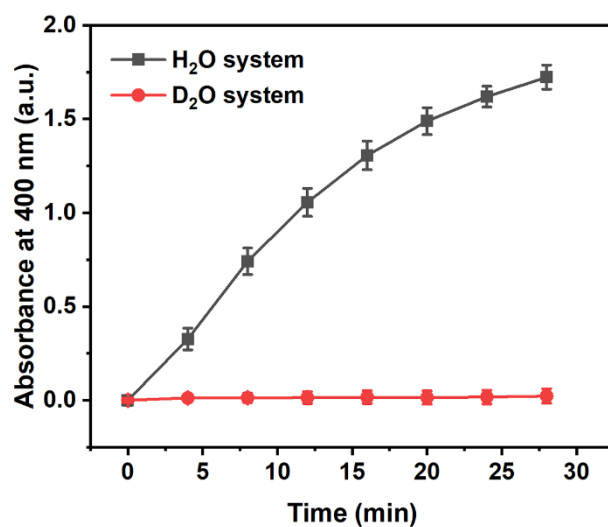
**Figure S12.** (a) Fluorescence emission spectra of the solvatochromic probe Nile Red (0.5 μM) recorded in the presence of increasing concentrations of PVA80 ranging from 0 to 2 mg mL<sup>-1</sup>. (b) Plot of the maximum fluorescence emission intensity as a function of PVA80 concentration.



**Figure S13.** Fluorescence emission spectra of Nile Red in the PVA80 system in the presence of the hydrophilic substrate *p*-NPP.



**Figure S14.** Dynamic light scattering (DLS) analysis of PVA80 in the absence and presence of BNPP in 50 mM HEPES buffer (pH 8.0).



**Figure S15.** Hydrolysis time courses of BNPP (2 mM) in H<sub>2</sub>O and D<sub>2</sub>O for the Zn(II)-PVA80 system, monitored by the absorbance change at 400 nm. Zn(II) 2 mM, PVA80 1 mg mL<sup>-1</sup>, 50 mM HEPES pH 8 at 37 °C.

**Table S1.** X-ray photoelectron spectroscopy (XPS) analysis of the elemental compositions of the solid precipitate isolated from the Zn(II) or Zn(II)-PVA80 mixture.

	C (at%)	O (at%)	Zn (at%)
Zn(II)	24.08	46.39	29.53
Zn(II)-PVA80	23.85	49.27	26.89

**Table S2.** Comparison of kinetic parameters ( $V_{max}$ ,  $K_m$ ,  $k_{cat}$ , and  $k_{cat}/K_m$ ) of different phosphodiesterase mimics for the hydrolysis reactions.

Substrate	Temperature		Hill		$V_{max}$ (nM s <sup>-1</sup> )	$K_m$ (mM)	$k_{cat}$ (s <sup>-1</sup> )	$k_{cat}/K$ (M <sup>-1</sup> s <sup>-1</sup> )	Ref
	(°C)	pH	coefficient	t (n)					
[Zn <sub>4</sub> (Hcpdp) <sub>2</sub> (suc)]Br <sub>2</sub> ·12 H <sub>2</sub> O	BNPP	30	9.5		0.76	7.6	3.05×10 <sup>-6</sup>	4.02×10 <sup>-4</sup>	1
[Zn <sub>2</sub> (L-2H)(AcO)(H <sub>2</sub> O)](PF <sub>6</sub> )·2H <sub>2</sub> O (Zn <sub>2</sub> L <sup>+</sup> )	BNPP	50	7.2			6.15×10 <sup>-2</sup>	4.60×10 <sup>-6</sup>	4.49×10 <sup>-5</sup>	2
[Zn <sub>2</sub> (HL1)(μ-CH <sub>3</sub> COO)](PF <sub>6</sub> )·H <sub>2</sub> O	BNPP	50	9		437	1.96×10 <sup>-3</sup>	1.26×10 <sup>-6</sup>	0.64×10 <sup>-3</sup>	3
[Zn <sub>2</sub> (H <sub>2</sub> L <sup>2</sup> )(OAc) <sub>2</sub> ] <sup>+</sup>	BDNPP	25	9.5			9	3.20×10 <sup>-3</sup>	0.34×10 <sup>-3</sup>	4
[Zn <sub>4</sub> (TPPNOL) <sub>2</sub> (OAc) <sub>3</sub> ](ClO <sub>4</sub> ) <sub>3</sub>	BDNPP		8.5			4.4	1.28×10 <sup>-4</sup>	2.90×10 <sup>-3</sup>	5
C <sub>18</sub> TACN Zn <sup>2+</sup>	HPNPP	40	7		78	0.53	3.00×10 <sup>-3</sup>	5.7	6
Zn(II)2/γ-CD	HPNPP	40	8			3.3	1.20×10 <sup>-2</sup>	3.6	7
Zn(II)-PVA80 (PVA80 1 mg mL <sup>-1</sup> )	BNPP	25	8	5.82	32.75	0.84	1.64×10 <sup>-5</sup>	1.95×10 <sup>-3</sup>	This work
Zn(II)-PVA80 (PVA80 1 mg mL <sup>-1</sup> )	BNPP	37	8	2.94	143.40	0.71	7.17×10 <sup>-5</sup>	1.01×10 <sup>-2</sup>	This work
Zn(II)-PVA80 (PVA80 1 mg mL <sup>-1</sup> )	BNPP	45	8	4.15	194.07	0.52	9.70×10 <sup>-5</sup>	1.86×10 <sup>-2</sup>	This work
Zn(II)-PVA80 (PVA80 0.1 mg mL <sup>-1</sup> )	BNPP	37	8	1.55	-	7.98	-	-	This work
Zn(II)-PVA80 (PVA80 0.5 mg mL <sup>-1</sup> )	BNPP	37	8	3.42	171.73	1.04	8.59×10 <sup>-5</sup>	8.26×10 <sup>-3</sup>	This work
Ce(IV)	BNPP	37	8		57.47	1.75	2.87×10 <sup>-4</sup>	0.16	This work
Ce(IV)-PVA80	BNPP	37	8		84.03	0.92	4.20×10 <sup>-4</sup>	0.45	This work

## References

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