[Supporting Information (SI) to accompany:]

Assembly of the active center of organophosphorus hydrolase in metal–organic frameworks via rational combination of functional ligands

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Section S1. General methods

Paraoxon (diethyl 4-nitrophenyl phosphate) was purchased from Sigma-Aldrich. 2-(cyclohexylamino)ethanesulfonic acid (CHES) was obtained from Shanghai Sangon Biotechnology Co. Ltd. Biphenyl-3,3′,5,5′-tetracarboxylic acid (BPTC) was purchased from J&K Scientific LTD. THF was purchased from Sinopharm Chemical Reagent Co. Ltd and purified by distillation from Na–benzophenone. All other chemicals were of analytical grade purity and were used without further purification. Absorption spectra were obtained with a PerkinElmer Lambda 35 UV−vis spectrometer. The FT-IR spectra (KBr pellets) were recorded on a Nicolet Avatar 360 FT-IR Spectrometer in the range of 4000–400 cm^{-1}. Thermal stability studies were carried out on a SDT Q600 V20.9 Build 20 under nitrogen atmosphere (40–800 ℃ range) at a heating rate of 10 ℃ min^{-1}.
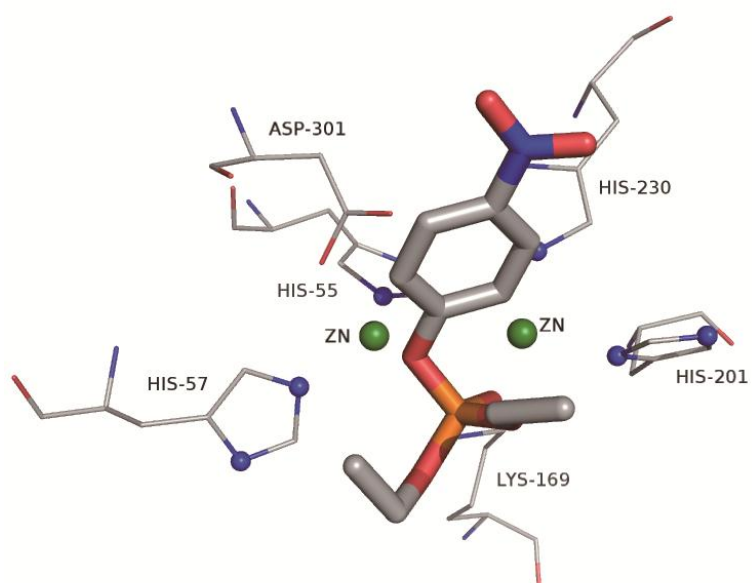
Section S2. Molecular docking of paraoxon with OPH

The OPH and paraoxon required for the docking experiments with Autodock 4.2. [1]were prepared as follows. The OPH (PDB code 1HZY) structure was obtained from the Protein Data Bank (PDB) (http://www.rcsb.org/) in .pdb format. Paraoxon structure was first obtained from the ZINC (http://zinc.docking.org/) in .mol$_2$ format, and then the format was changed to .pdb by UCSF Chimera. Water molecules and heteroatoms were removed from the surface of the OPH, and all hydrogen atoms were restored for ADT calculations. The Lamarckian genetic algorithm was used as the search engine. The active site was defined using AutoGrid. The grid size was set to 60 Å × 60 Å × 60 Å points with grid spacing of 0.375 Å centered on the ligand center of mass. The best conformation was selected for the analysis of protein–ligand interactions, including hydrogen bonds and bond lengths. The 3D structure of organophosphorus hydrolase of docking is shown in Fig. S1.
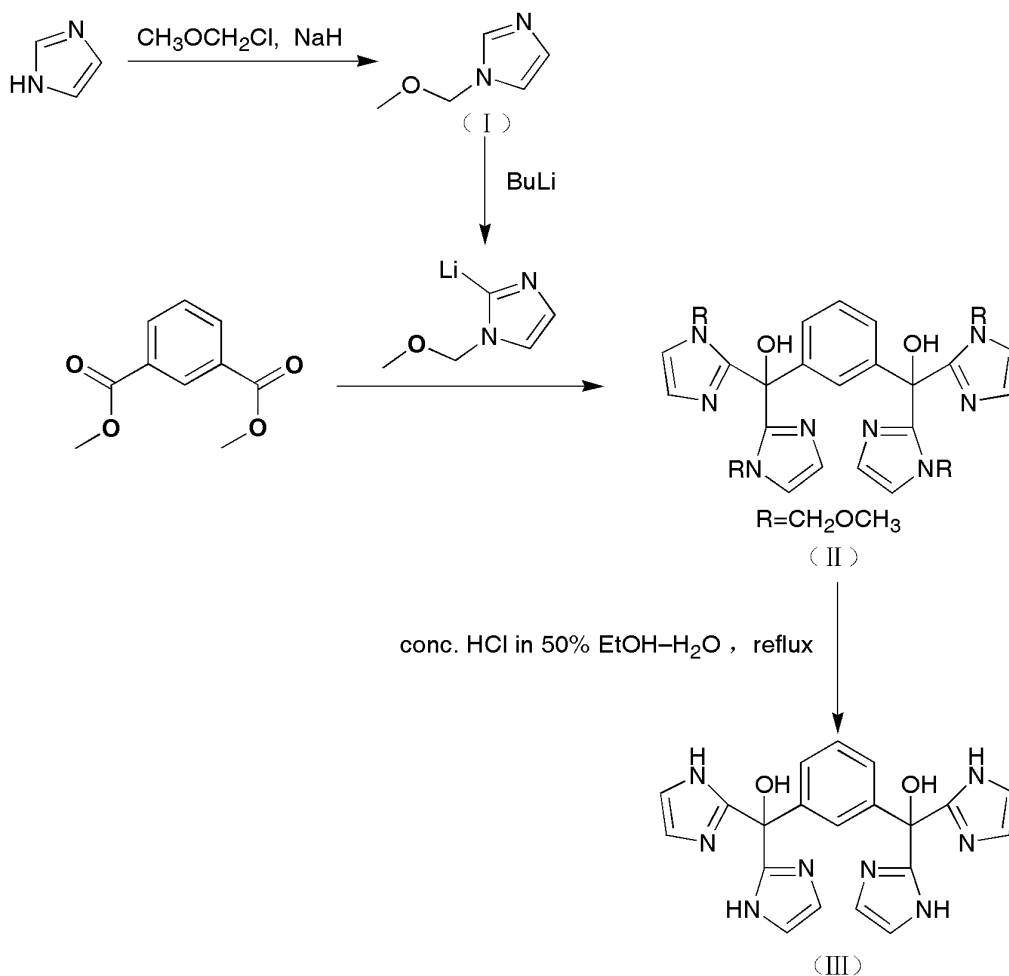
(a)
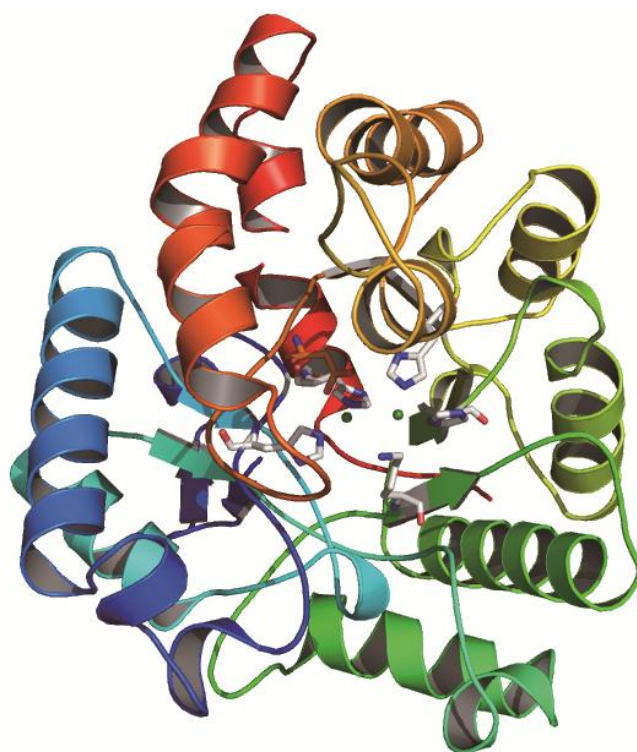
(b)

Fig .S1 (a). 3D structure of OPH.[2] (b) Molecular docking of paraoxon with OPH.

Section S3. Synthesis of 1,3-bis(diimidazol-2-ylhydroxymethyl)benzene (BDIB)

Scheme S1. Synthetic route of 1,3-bis(diimidazol-2-ylhydroxymethyl)benzene(BDIB)

3.1 Synthesis of 1-(methoxymethyl)imidazole (I)[3]

First, NaH (7.10 g, 60% dispersed in mineral oil) was added to a stirred solution of imidazole (3.00 g, 0.0441 mol) in THF (40 mL) at 0 ℃. After stirring for 30 min, chloromethyl methyl ether (4.00 mL, 0.0529 mol) was added dropwise. The reaction mixture was stirred for 3 h at room temperature. After addition of NH_4Cl solution (50 mL), the reaction mixture was extracted with $CHCl_3$ (50 mL × 3). After washing with saturated saltwater and drying over Na_2SO_4, the product was filtrated and spin steamed. The oily product was purified by column chromatography (MeOH : $CHCl_3$ = 1:9; yield, 4.59 g; 99%).

3.2 Synthesis of

**1,3-bis{bis[1-(methoxymethyl)imidazol-2-yl]hydroxymethyl}-benzene (II)**

n-Butyllithium (1.64 mol dm^{-3} in hexane, 13 mL, 21.3 mmol) was added to a stirred solution of 1-(methoxymethyl)imidazole (2.25 g, 20 mmol) in THF (50 mL) at −78 °C over 10 min under N_2. After stirring for 30 min at −78 °C, diethyl isophthalate (0.5826 g, 3 mmol) in THF (10 mL) was added dropwise. The reaction mixture was stirred for 30 min at −78 °C and 4 h at room temperature. After addition of deionized water, the reaction mixture was extracted with EtOAc (100 mL × 3). After drying over Na_2SO_4, the solvent was evaporated in vacuo. The oily product was purified by column chromatography (CH_2Cl_2:MeOH = 10:1) and recrystallized by EtOAc; the yield was 1.29 g (45%).

**3.3 Synthesis of 1,3-bis(diimidazol-2-ylhydroxymethyl)benzene (III, BDIB)**

A solution containing (II) (0.5 g, 0.86 mmol) and HCl concentration (6 mL) in 50% EtOH–H_2O (30 mL) was refluxed overnight. The reaction mixture was evaporated in vacuo, and the residue was recrystallized from MeOH–diethyl ether. Yield is at 0.37 g (78%).^{1}HNMR (300 MHz, CD_3OD) δ 8.21 (s, 1H), 7.77–7.90 (m, 3H), 7.53–7.76 (br, 10H); ^{13}C NMR (100.6 MHz, CD_3OD): δ 71.65, 124.50, 128.04, 131.14, 138.44, 121.00, 145.05. IR: ν = 3437, 3051, 2848, 2754, 2666, 2531, 1714, 1602, 1484, 1419, 1383, 1284, 1161, 1125, 1074, 908, 767, 725, 701, and 560 cm^{-1}; MS (ESI+): m/z = 403.1622[M+H]$^+$. HRMS: Calculated: 402.1553, Found (ESI$^+$): m/z=403.1622[M+H]$^+$.
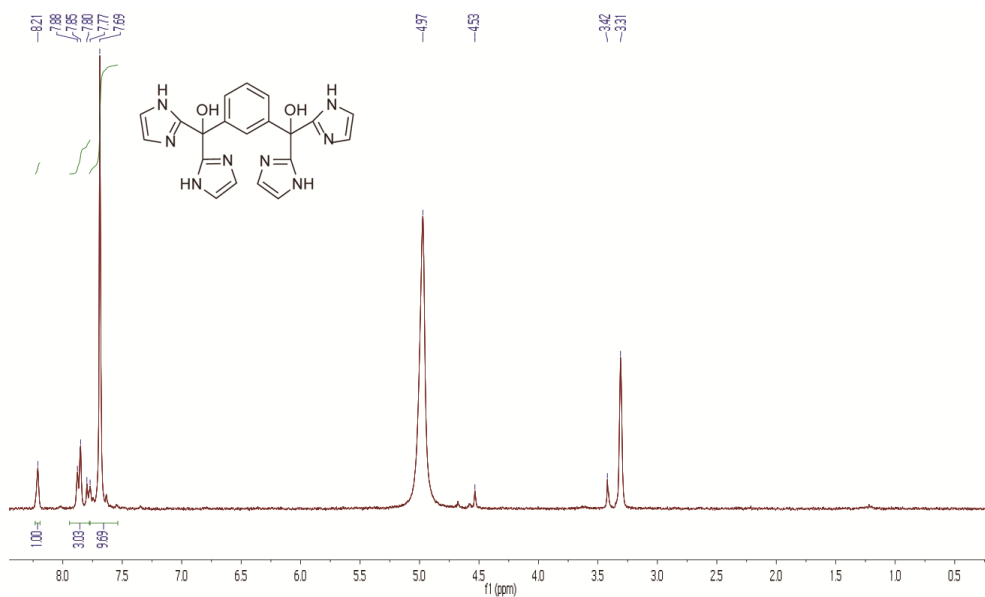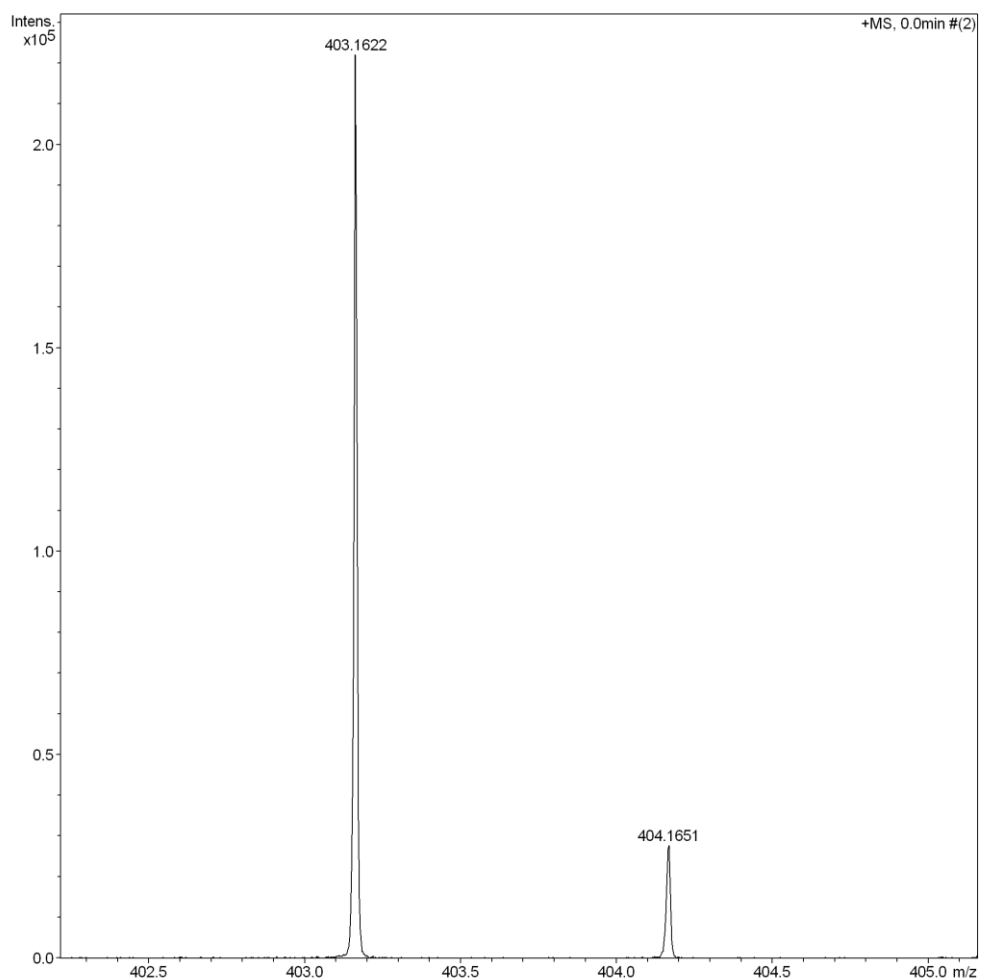
Fig .S2 ^{1}HNMR spectrum of BDIB
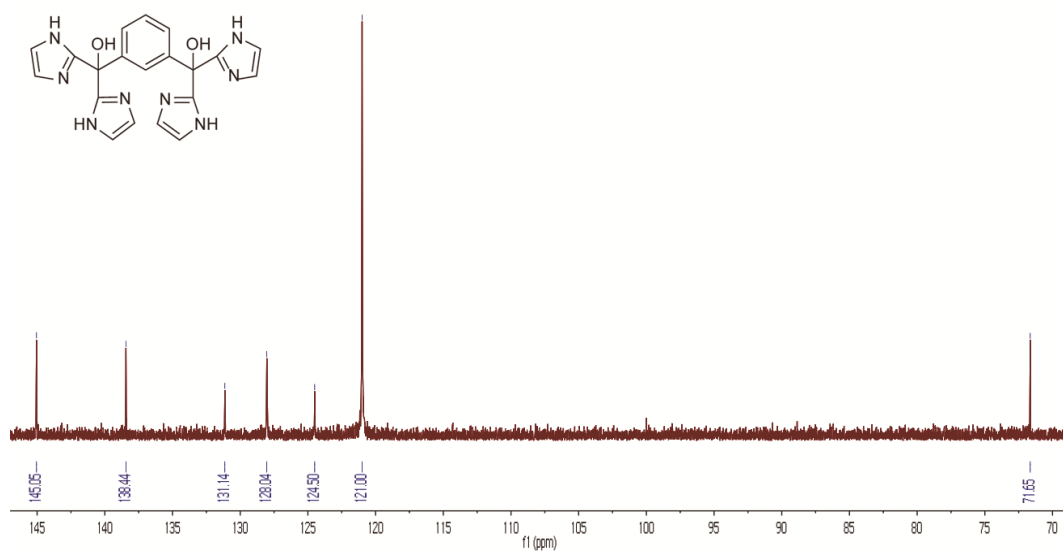
Fig. S3 ^{13}C NMR spectrum of BDIB
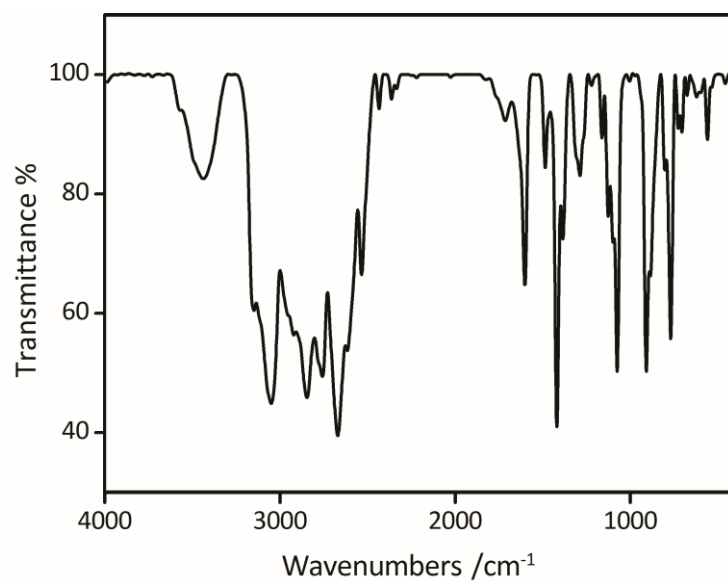
Fig .S4 IR spectrum of BDIB

Fig .S5 Mass spectrum of BDIB

Section S4. Synthesis of SNNU-101

A solution of $ZnSO_4 \cdot 7H_2O$ (17.2 mg, 0.06 mmol), BPTC (9.9 mg, 0.03 mmol), NaOH (65 μL, 0.5 M), BDIB (12.1 mg) in DMF (4 Ml) and deionized water (1.5 mL)was heated at 65 ℃ for three days, and the mixture was then cooled to room temperature at a speed of 3.5 ℃/h. After washing with fresh DMF (3×10 mL) and drying, yellow crystals were obtained (yield, approximately 50% based on Zn). FT-IR for SNNU-101 (solid KBr pellet/cm^{-1}): 3401 (s), 3150 (s), 1660 (s), 1617 (s), 1565 (s), 1490 (w), 1416 (w), 1364 (s), 1138 (w), 1082 (m), 889 (w), 779 (m), 741 (m), and 660 (m).

Section S5. Synthesis of $Zn_2(SO_4)_2 \cdot (BDIB)_2 \cdot 6H_2O$ (Crystal 1)

A 20-mL vial was loaded with $ZnSO_4 \cdot 7H_2O$ (14.37 mg, 0.05 mmol), $C_{20}H_{22}Cl_4N_8O_2 \cdot H_2O$ (20.1 mg), and 6 mL of DMF/H_2O (1:1, v/v). The final mixture was stirred for 1.5 h at room temperature, then increasing to 80 ℃ for days. The resulting block crystals, with a yield of 36.1%, were washed with water and acetone several times. The product was finally dried under vacuum at 45 ℃. Anal. Calcd (%) for $Zn_2(SO_4)_2 \cdot (BDIB)_2 \cdot 6H_2O$ (Mr = 1235.82): C, 38.88; H, 3.91; N, 18.13. Found: C, 39.03; H, 4.22; N, 17.81. IR = (KBr, cm^{-1}): 3654 (w), 3240 (m), 3000 (w), 2802 (w), 2725 (w), 2551 (w), 2294 (m), 1876 (m), 1651 (m), 1556 (s), 1448 (s), 1340 (s), 1112 (vs), 1046 (s), 949 (s), 830 (s), 743 (s), 678 (m).

Section S6.The structure of SNNU-101

6.1 Single crystal X-ray crystallography of SNNU-101

Crystallographic data were carried out on Oxford Gemini E Diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) at T = 293(2) K. The structure was solved by direct methods and refined on F^2 by full-matrix least-squares analysis with SHELXTL-97 software.[4] CIF data have been deposited with the Cambridge Crystallographic Data Centre as numbers CCDC 1560464 (SNNU-101).
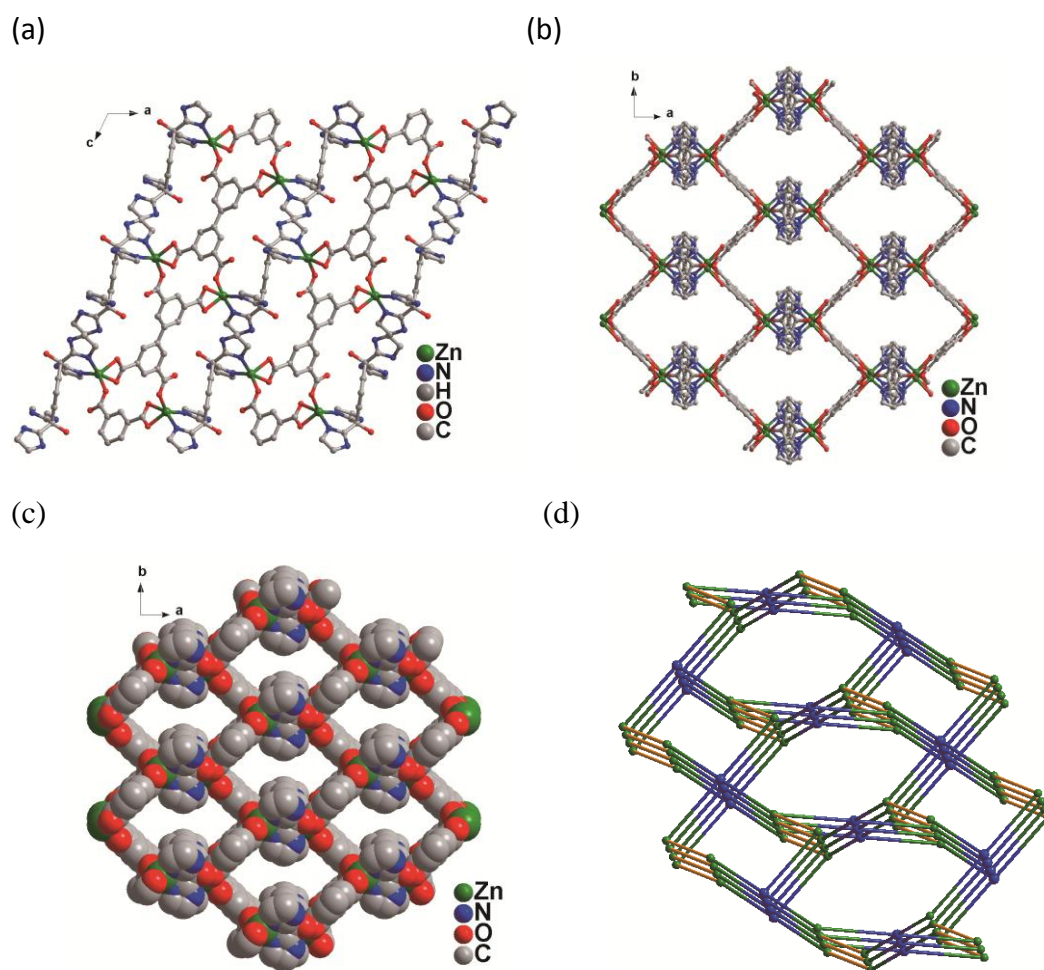
Fig .S6 (a) 2D sheet of SNNU-101 in ac plane. (b) 2D sheet of SNNU-101 in ab plane. (c) Space-filling model of SNNU-101. (d) Schematic view of the (3,4)-connected network of SNNU-101 (green spheres, Zn^{II} atoms; blue spheres, BPTC). (Hydrogen atoms and solvents are removed for clarity).

Table S1. Summary of the crystal data and structure refinement parameters of SNNU-101

Complex	SNNU-101
Empirical formula	$C_{27}H_{33}N_7O_8Zn$
Formula weight	648.97
T / K	123(4)
λ / Å	1.54178
Crystal system	Monoclinic
Space group	$C2/c$
a / Å	25.469(3)
b / Å	12.0512(10)
c / Å	21.5230(17)
α /°	90
β /°	116.159(3)
γ /°	90
V / Å^3	5929.5(9)
Z	8
D_{calcd} / Mg m^{-3}	1.454
μ/mm^{-1}	1.671
F (000)	2704
θ for data collection (deg)	3.87 to 66.04
Reflections collected /unique	11914 / 5174
GOF on F^2	1.047
R(int)	0.0462
Parameters	481
$R_1{}^a$, w$R_2{}^b$ [I>2σ(I)]	0.0664, 0.1769
$R_1{}^a$, w$R_2{}^b$ (all data)	0.0944, 0.1988
ρ_{fin}(max/min) (e ·Å^{-3})	0.410 / -0.607

[a] $R_1 = \Sigma\|F_o\| - |F_c\|/\Sigma |F_o|$. [b] w$R_2 = [\Sigma[w(F_o{}^2 - F_c{}^2)^2]/\Sigma[w(F_o{}^2)^2]]^{1/2}$.

Table S2. Selected Bond Lengths (Å) and Angles ( ) of SNNU-101

|			
Zn(1)-O(1)	1.933(3)	Zn(1)-N(1)	2.004(4)
Zn(1)-O(3)	2.050(3)	Zn(1)-N(3)	2.064(4)
Zn(1)-O(4)	2.377(4)		
O(1)-Zn(1)-N(1)	130.17(14)	O(1)-Zn(1)-O(3)	112.56(14)
N(1)-Zn(1)-O(3)	111.70(15)	O(1)-Zn(1)-N(3)	105.11(17)
N(1)-Zn(1)-N(3)	92.45(15)	O(3)-Zn(1)-N(3)	95.24(13)
O(1)-Zn(1)-O(4)	92.65(15)	N(1)-Zn(1)-O(4)	91.10(15)
O(3)-Zn(1)-O(4)	58.95(12)	N(3)-Zn(1)-O(4)	153.20(13)

symmetry codes: #1 -x+1, y, -z+3/2; #2 -x+3/2, y+1/2, -z+3/2; #3 -x+3/2, y-1/2, -z+3/2; #4 -x+3/2, -y+1/2, -z+2

Table S3. Hydrogen-bonding geometry parameters (Å,) of SNNU-101

D- H⋯A	D-H / Å	H⋯A / Å	D⋯A / Å	D-H⋯A / °
N(2)-H(2)⋯O(7)	0.86	1.92	2.739(8)	157
N(4)-H(4)⋯O(3) [i]	0.86	2.27	2.928(5)	134
O(5)-H(5)⋯O(2) [ii]	0.82	1.88	2.701(5)	173
C(2)-H(2A)⋯O(8) [iii]	0.93	2.43	3.29(2)	154
C(23)-H(23)⋯O(2) [ii]	0.93	2.49	3.215(9)	135
C(24)-H(24B)⋯O(7) [iv]	0.96	2.47	3.408(10)	166
C(24)-H(24C)⋯O(7)	0.96	2.47	2.825(12)	101
C(25)-H(25A)⋯O(6) [v]	0.96	2.54	3.01(3)	110
C(27)-H(27C)⋯O(8)	0.97	2.15	2.58(8)	105

Equivalent positions: (i) 1-x, 1-y, 1-z, (ii) 1-x, y, 3/2-z, (iii) x, 1+y, z, (iv) 1/2-x, 3/2-y, 1-z, (v) -1/2+x, 1/2+y, z

6. 2 Infrared Spectroscopy of SNNU-101
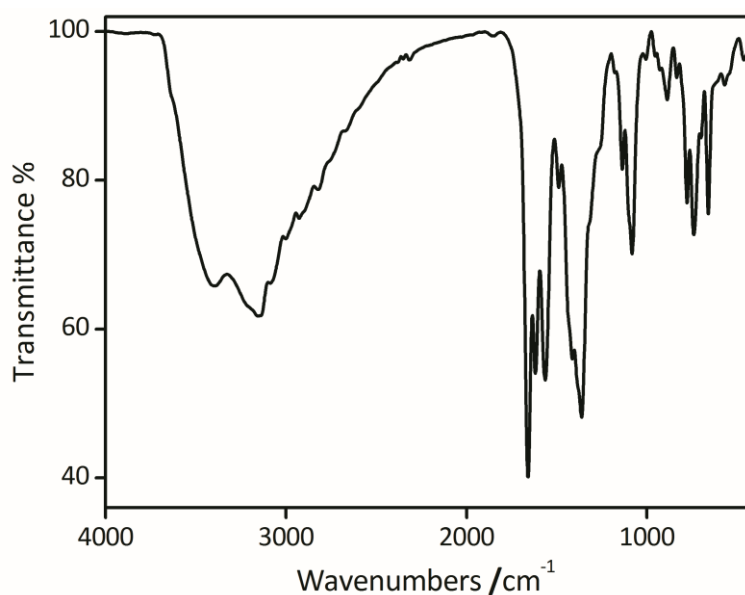
Fig .S7 FT-IR spectrum of SNNU-101.

The FT-IR spectrum of SNNU-101 shows broad bands at 3100 cm^{-1} corresponding to the stretching vibration of O–H and N–H. Broad bands in the range of 3100 and 3000 cm^{-1} are due to the stretching vibrations of =C–H and C–H of the benzene. The band at 1660 cm^{-1} corresponds to the stretching vibration of C=O. The peaks at 1617, 1565, and 1490 cm^{-1} corresponds to the stretching vibration of C=N and C=C of the benzene. The peak at 1416 and 1364 cm^{-1} is due to the stretching vibration of C–N of the coordinate DMF and imidazole, respectively. The peak at 1138 and 1082 cm^{-1} is due to the stretching vibration of C–O.

6.3 Thermal gravimetric analysis (TGA) of SNNU-101
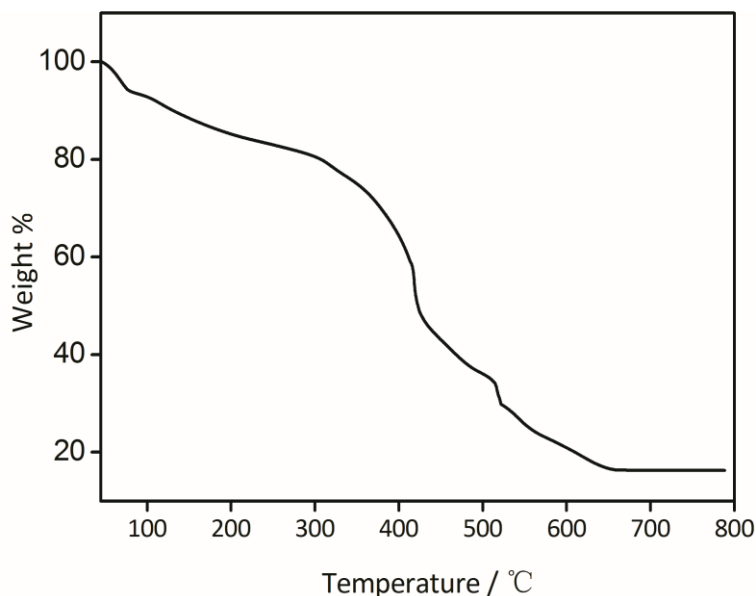
Fig .S8 TGA of SNNU-101

Section S7.Catalysis Experiments of SNNU-101
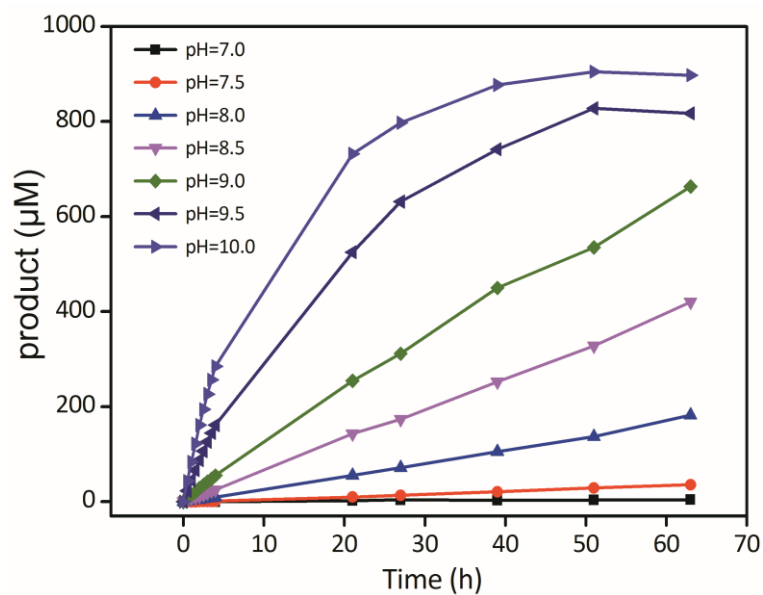
7.1. Paraoxon hydrolysis

The resulting crystals were crushed and ground finely in an agate mortar. A stock solution of 20 mM buffer solution (CHES for pH 9.0) and a solution of 50 mM paraoxon in acetonitrile were prepared. Hydrolysis experiments were carried out at 25 ℃. The crystal powder (1 mg) was suspended in 980 μL (20 mM) of CHES buffer solution (pH = 9.0). The reaction was initiated by injection of 20 μL of 50 mM paraoxon in acetonitrile. A total of 50 μL of the reaction solution was sampled at certain time intervals (i.e., 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 17.0, and 29.0 h), diluted with deionized water, and centrifuged at 10,000 rpm for 1 min. Finally, the supernatant was collected and the absorbance at 400 nm was measured using a UV–vis spectrophotometer to determine the concentration of p-nitrophenol. In controlled experiments, the reaction is performed without 1 mg of crystal powder under identical reaction conditions.
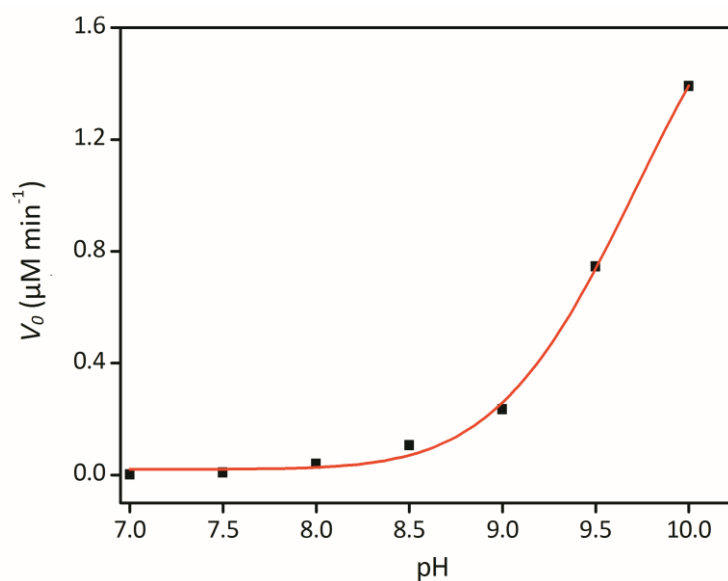
7.2. Kinetics of paraoxon hydrolysis

The kinetics of paraoxon hydrolysis with or without catalyst was monitored by UV−vis. A stock solution of 20 mM buffer solution (CHES for pH 9.0) and a solution of 50 mM paraoxon in acetonitrile were prepared. The crystal powder (1 mg) was suspended in a different volume (20 mM) of CHES buffer solution (pH = 9.0) with a different volume of 50 mM paraoxon in acetonitrile, whereas the total volume of the solution reaches 1 mL and the concentration of paraoxon is 1.00, 1.25, 1.65, 2.50, 5.00, and7.50 mM. The reaction solution (50 μL) was sampled at certain time intervals (0, 15, 30, 45, 60, 75, and 90 min), diluted with deionized water, and centrifuged at 10,000 rpm for 1 min. Finally, the supernatant was collected, and the absorbance at 400 nm was measured using a UV−vis spectrophotometer to determine the concentration of p-nitrophenol.

## 7.3. Effect of pH value on paraoxon hydrolysis

A stock solution of 20 mM buffer solution in deionized water (HEPES for pH 7.0 and 7.5; CHES for pH 8.0, 8.5, and 9.0; and CHES for pH 9.5 and 10.0) were prepared. Crystal powder (1 mg) was suspended in 980 μL (20 mM) at a different buffer solution. The reaction was initiated by injection of 20 μL of 50 mM paraoxon in acetonitrile. The reaction solution (50 μL) was sampled at certain time intervals (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 21, 27, 39, 51, and 63 h), diluted with deionized water, and centrifuged at 10,000 rpm for 1 min. Finally, the supernatant was collected, and the absorbance at 400 nm was measured using a UV−vis spectrophotometer to determine the concentration of p-nitrophenol.

Fig .S9 Hydrolysis of paraoxon over time in the presence of SNNU-101 at different pH value. 1 mM paraoxon, 1.0 mg/mL SNNU-101, pH = 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0, 25 ℃

**Fig .S10** Effect of pH on the paraoxon hydrolysis rate catalyzed by SNNU-101.
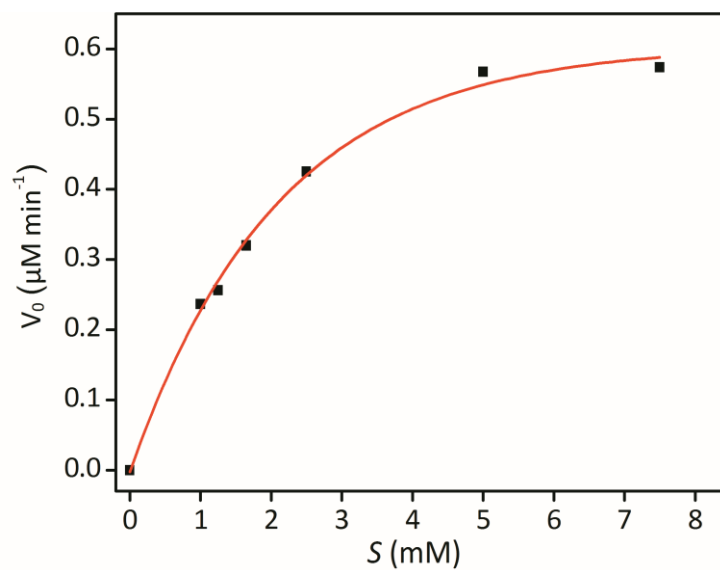
Fig .S11 Michaelis–Menten plot of paraoxon hydrolysis catalyzed by SNNU-101.

Section S8.The structure of Crystal 1

8. 1 Single crystal X-ray crystallography of Crystal 1

CIF data have been deposited with the Cambridge Crystallographic Data Centre as numbers CCDC 1062340 ($Zn_2(SO_4)_2$ $(BDIB)_2$ $6H_2O$,Crystal 1).
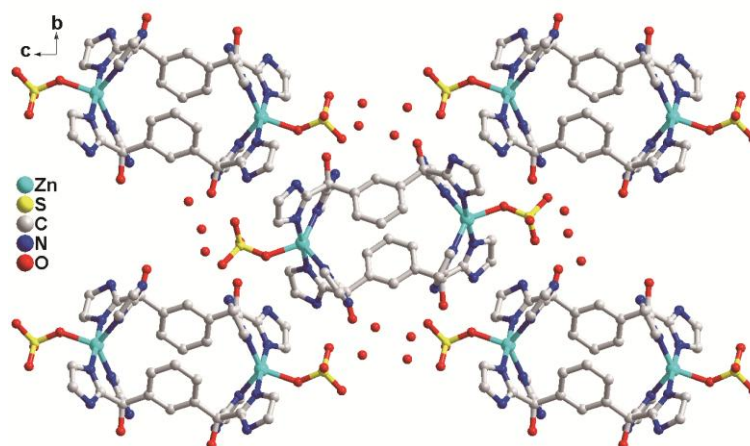
Fig .S12 Packing diagram of Crystal 1 in bc plane.   Hydrogen atoms are removed for clarity.

Table S4. Summary of the crystal data and structure refinement parameters of Crystal 1.

Complex	Crystal 1
Empirical formula	$C_{40}H_{48}N_{16}O_{18}S_2Zn_2$
Formula weight	1235.82
T / K	293(2)
λ/ Å	0.71073
Crystal system	Monoclinic
Space group	$P2_1/n$
a / Å	8.6739(4)
b / Å	13.1233(6)
c / Å	21.5232(8)
α /°	90
β /°	94.249(4)
γ /°	90
V / Å^3	2443.26(18)
Z	2
D_{calcd} / Mg m^{-3}	1.652
μ/mm^{-1}	1.159
F (000)	1232
θ for data collection (°)	3.03 to 26.37
Reflections collected /unique	20574 / 4995
GOF on F^2	1.064
R(int)	0.0414
Parameters	347
$R_1{}^a$, w$R_2{}^b$ [I>2σ(I)]	0.0625, 0.1591
$R_1{}^a$, w$R_2{}^b$ (all data)	0.0873, 0.1764
ρ_{fin}(max/min) (e ·Å^{-3})	0.664 / -0.539

[a] $R_1 = \Sigma \|F_o \| - | F_c \|/\Sigma | F_o |$, [b] w$R_2 = [\Sigma[w(F_o{}^2 - F_c{}^2)^2]/\Sigma[w(F_o{}^2)^2]]^{1/2}$.

Table S5. Selected Bond Lengths (Å) and Angles (ͦ) of Crystal 1.

Zn(1)-O(3)	2.025(3)	Zn(1)-N(6)#1	2.035(4)
Zn(1)-N(2)	2.041(4)	Zn(1)-N(8)#1	2.165(4)
Zn(1)-N(4)	2.188(4)	N(6)-Zn(1)#1	2.035(4)
N(8)-Zn(1)#1	2.165(4)		
O(3)-Zn(1)-N(6)#1	130.27(16)	O(3)-Zn(1)-N(2)	106.50(1)
N(6)#1-Zn(1)-N(2)	123.23(16)	O(3)-Zn(1)-N(8)#1	90.42(15)
N(6)#1-Zn(1)-N(8)#1	86.00(16)	N(2)-Zn(1)-N(8)#1	95.19(16)
O(3)-Zn(1)-N(4)	87.77(15)	N(6)#1-Zn(1)-N(4)	94.59(16)
N(2)-Zn(1)-N(4)	86.06(16)	N(8)#1-Zn(1)-N(4)	178.03(1)

Table S6. Hydrogen-bonding geometry parameters (Å, ͦ) of Crystal 1.

D-H⋯A	D-H / Å	H⋯A / Å	D⋯A / Å	D-H⋯A / ͦ
O(1)-H(1B)...Ow(2)	0.82	1.91	2.732(6)	178
O(2)-H(2B)...O(4) [i]	0.82	2.13	2.947(8)	179
C(2)-H(2A)...N(4)	0.93	2.58	3.229(8)	127
C(4)-H(4A)...O(6)	0.93	2.48	3.209(9)	135
C(10)-H(10A)...O(4) [ii]	0.93	2.55	3.389(8)	150
C(13)-H(13A)...N(8)	0.93	2.58	3.242(8)	128

Equivalent positions: (i) -1/2+x, 1/2-y, -1/2+z, (ii) 1/2+x, 1/2-y, -1/2+z

8.2 Powder X-ray diffraction of Crystal 1

PXRD pattern was measured on a Rigaku D/Max2550VB+/PC diffractometer at 40 kV and 100 mA. The simulated powder pattern was calculated using single-crystal XRD data and processed by the free Mercury 2.3 program provided by the Cambridge Crystallographic Data Centre.
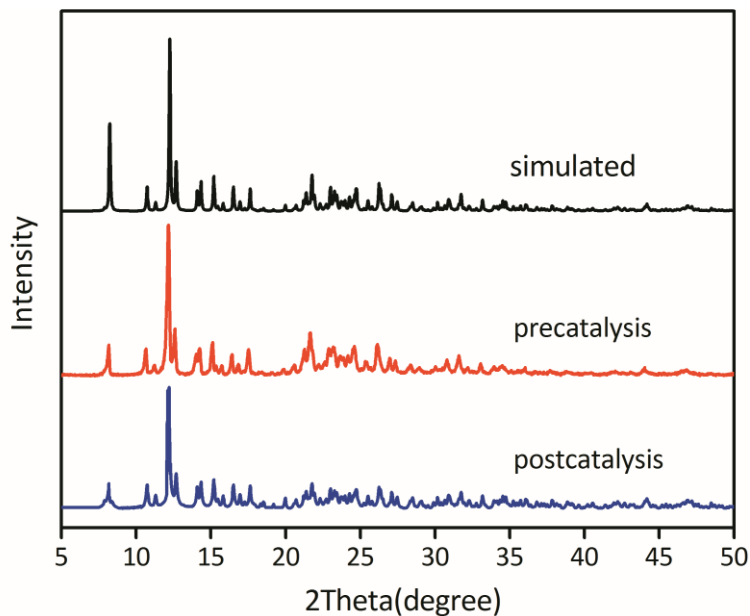

Fig .S13 PXRD of Crystal 1.

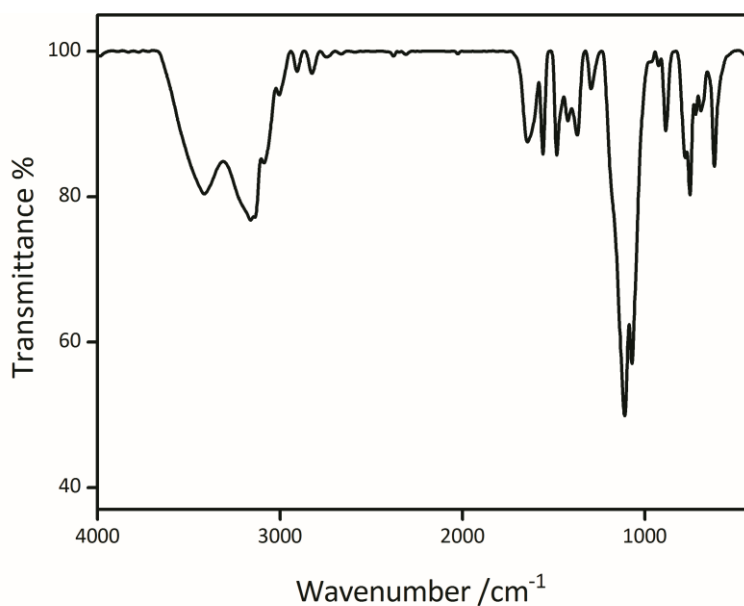## 8. 3 Infrared Spectroscopy of Crystal 1

Fig .S14 FT-IR spectrum of Crystal 1.

Broad bands in the range of 3600 and 3000 cm^{-1} result from stretching vibrations of O–H and N–H. Peaks at 3150 cm^{-1} correspond to stretching vibration of O–H of H$_2$O. Broad bands in the range of 3100 and 3010 cm^{-1} are caused by stretching vibrations of =C–H and C–H of benzene. Peaks in the range of 1670 and 1460 cm^{-1} represent stretching vibrations of C=N of imidazole and C=C of benzene. Peaks at 1292 and 1107 cm^{-1} correspond to stretching vibration of C–N and C–O.

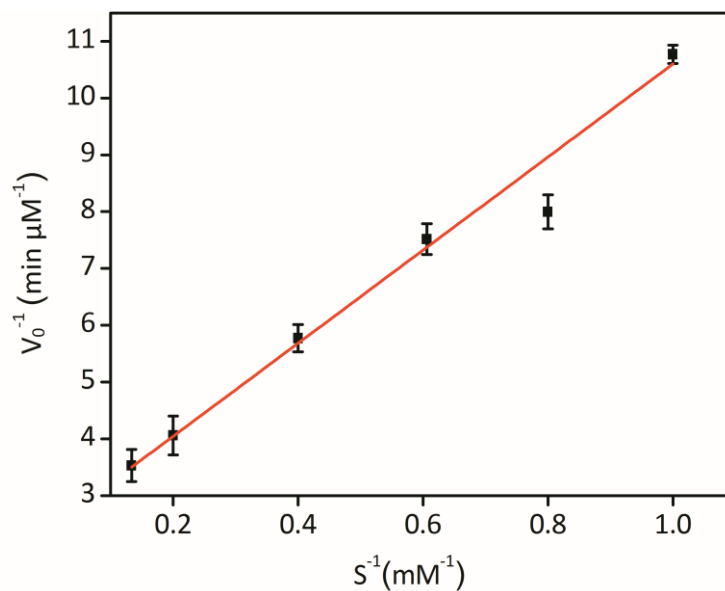**Section S9.Catalysis Experiments of Crystal 1**
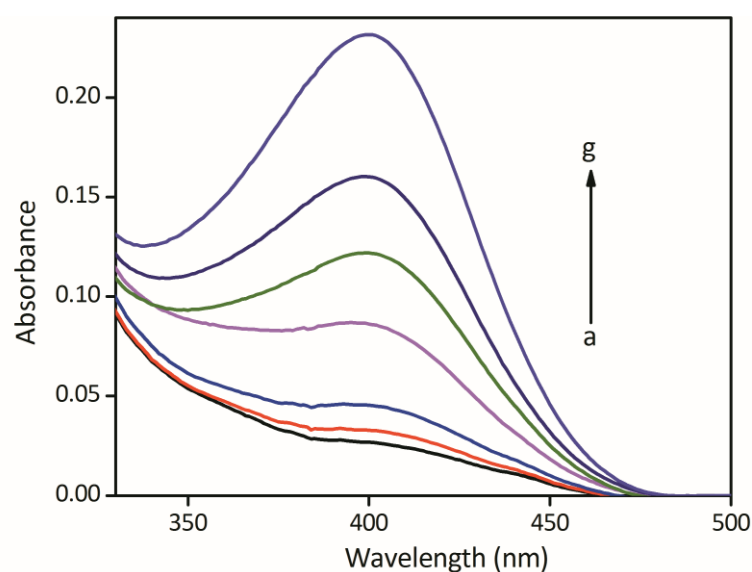
**Fig .S15** UV–vis spectra of the product *p*-nitrophenolate.

(The hydrolysis of 1 mM paraoxon was catalyzed by 1.0 mg/mL Crystal 1 in 20 mM CHES, pH = 9.0 buffer solution at 25 ℃ and $t_{(a-g)}$ = 0.5,1.5, 3.5, 14, 26, 38, and 62 h.)
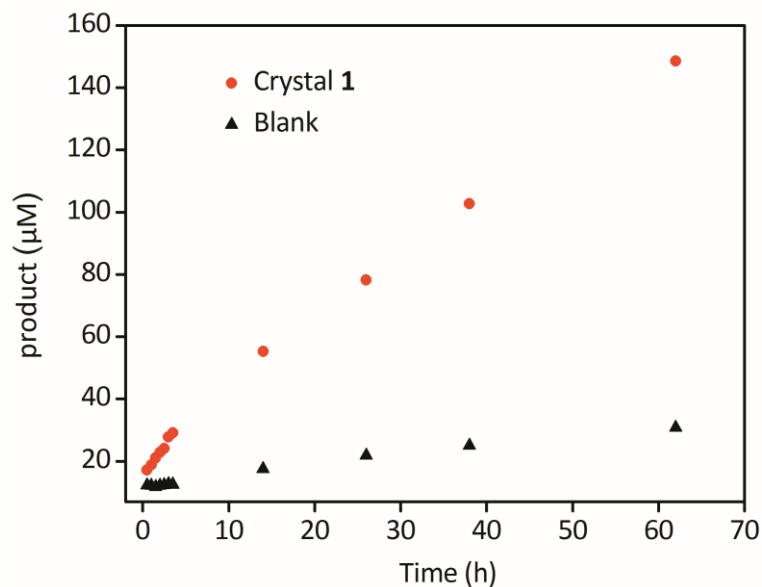
Fig .S16 Catalytic hydrolysis of paraoxon by Crystal 1

Fig .S17   Lineweaver-Burk plot of the catalytic hydrolysis of paraoxon by Crystal 1. (Different paraoxon concentrations at 1.0, 1.25, 1.65, 2.5, 5.0, and 7.5 mM were hydrolyzed by 1.0 mg/mL complex 1 in 20 mM CHES, pH = 9.0 buffer solution at 25 ℃)
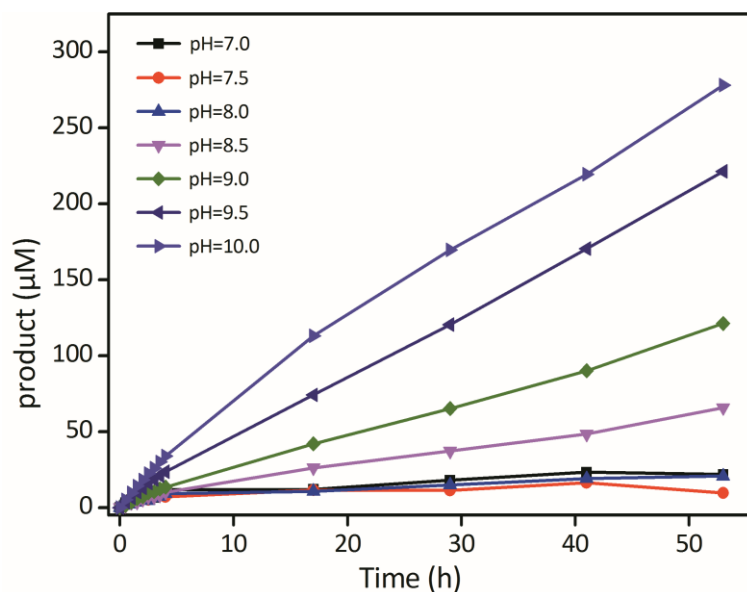
Fig .S18 Catalytic hydrolysis of paraoxon by Crystal 1 in different pH buffer solution (1 mM paraoxon was hydrolyzed by 1.0 mg/mL $Zn_2(SO_4)_2$ (BDIB)$_2$ $6H_2O$ in pH = 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0 buffer solutions at 25 ℃)

Section S10. References

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