

1

## 2

3

4

6

7

8

9

10

11

12

13

14

## Table of Contents

15 **Figure S1.** DNA sequencing of the *E. coli* WT-*ppa* gene and mutant *ppa* gene

16 **Figure S2.** CD spectra of the PP and PPC.

17 **Figure S3.** <sup>1</sup>H NMR spectrum and GPC trace of the pyridyl disulfide-functionalized  
18 pHEMA.

19 **Figure S4.** The influence of 8 mM cysteine on the PPi hydrolysis activity of the PP  
20 and PPC.

21 **Figure S5.** The influence of 8 mM DTT on the PPi hydrolysis activity of the PP and  
22 PPC.

23 **Table S1.** Metal ion selectivity of the PP and PPC.

24 **Table S2.** The effect of pHEMA with different molecular weights on PPC activity.

25 **Table S3.** Michaelis-Menten parameters of sodium pyrophosphate hydrolysis  
26 catalyzed by PPC, the conjugated products and reduced products.

27

WT-*ppa* : ATGAGAGGATCGCATCACCATCACCATCACGGATCCAGCTTACTCAACGTCCCTGCGGGTAAAGATCTGCCGGAAGACAT  
 K148C-*ppa* : ATGAGAGGATCGCATCACCATCACCATCACGGATCCAGCTTACTCAACGTCCCTGCGGGTAAAGATCTGCCGGAAGACAT  
  
 WT-*ppa* : CTACGTTGTTATTGAGATCCCGGCTAACGCAGATCCGATCAAATACGAAATCGACAAAGAGAGCGGCGCACTGTTCTGTTG  
 K148C-*ppa* : CTACGTTGTTATTGAGATCCCGGCTAACGCAGATCCGATCAAATACGAAATCGACAAAGAGAGCGGCGCACTGTTCTGTTG  
  
 WT-*ppa* : ACCGCTTCATGTCCACCGCGATGTTCTATCCATGCAACTACGGTTACATCAACCACACCTGTCTCTGGACGGTGACCCG  
 K148C-*ppa* : ACCGCTTCATGTCCACCGCGATGTTCTATCCATGCAACTACGGTTACATCAACCACACCTGTCTCTGGACGGTGACCCG  
  
 WT-*ppa* : GTTGACGTACTGGTCCCAACTCCGTACCCGCTGCAGCCGGGTTCTGTGATCCGTTGCCGTCGGTTGGCGTTCTGAAAAAT  
 K148C-*ppa* : GTTGACGTACTGGTCCCAACTCCGTACCCGCTGCAGCCGGGTTCTGTGATCCGTTGCCGTCGGTTGGCGTTCTGAAAAAT  
  
 WT-*ppa* : GACCGACGAAGCCGGTGAAGATGCGAACTGGTTGCGGTTCCGCACAGCAAGCTGAGCAAAGAATACGATCACATTAAAG  
 K148C-*ppa* : GACCGACGAAGCCGGTGAAGATGCGAACTGGTTGCGGTTCCGCACAGCAAGCTGAGCAAAGAATACGATCACATTAAAG  
  
 WT-*ppa* : ACGTTAACGATCTGCCTGAAGTGTGAAAGCGCAAAATCGCTCACTTCTTCGAGCACTACAAAGACCTCGAAAAAGGTAAG  
 K148C-*ppa* : ACGTTAACGATCTGCCTGAAGTGTGAAAGCGCAAAATCGCTCACTTCTTCGAGCACTACAAAGACCTCGAAAAAGGTTGC  
  
 WT-*ppa* : TGGGTGAAAGTTGAAGGTTGGGAAAAACGAGAAGCCGCTAAAGCTGAAATCGTTGCCTCCTTCGAGCGCGCAAAGAATAA  
 K148C-*ppa* : TGGGTGAAAGTTGAAGGTTGGGAAAAACGAGAAGCCGCTAAAGCTGAAATCGTTGCCTCCTTCGAGCGCGCAAAGAATAA  
  
 WT-*ppa* : ATAA  
 K148C-*ppa* : ATAA

28

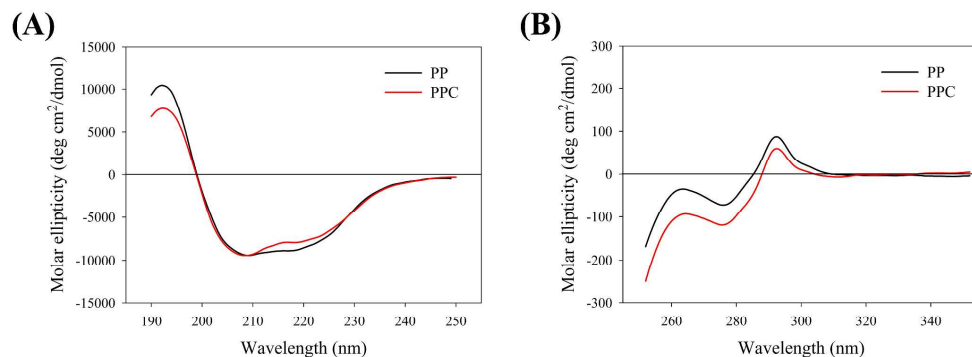
29 **Figure S1.** DNA sequencing results of the *E. coli* WT-*ppa* gene and mutant *ppa* gene.  
 30 (nucleotides in the frame represent mutations).

31 **Table S1.** Metal ion selectivity of the PP and PPC.

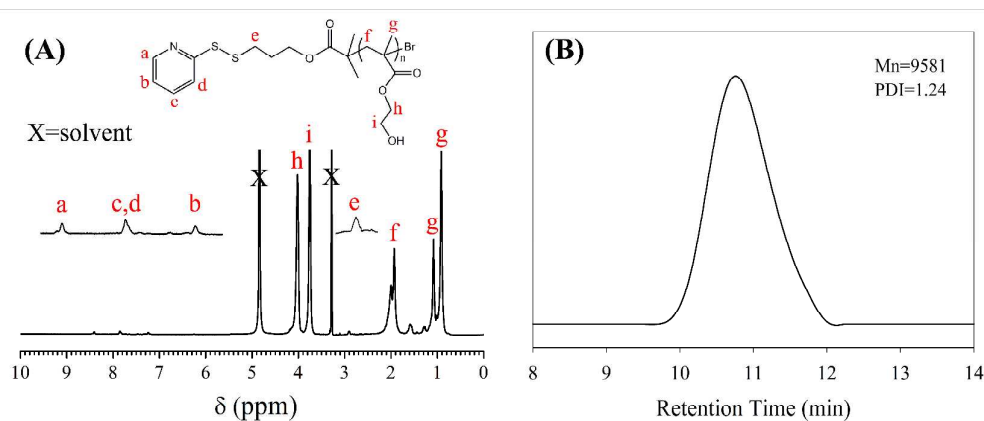
Metal ions	Relative activity (%)	
	PP	PPC
Mg <sup>2+</sup>	100.00 ± 2.17	100.00 ± 3.28
Mn <sup>2+</sup>	4.75 ± 1.55	0.47 ± 1.16
Co <sup>2+</sup>	0.76 ± 1.63	0.87 ± 0.64
Zn <sup>2+</sup>	0.39 ± 1.12	0.86 ± 1.04
Ca <sup>2+</sup>	2.91 ± 2.06	2.78 ± 1.86
Ni <sup>2+</sup>	0.63 ± 0.98	0.09 ± 0.77
Cu <sup>2+</sup>	1.00 ± 1.26	0.06 ± 0.75

32

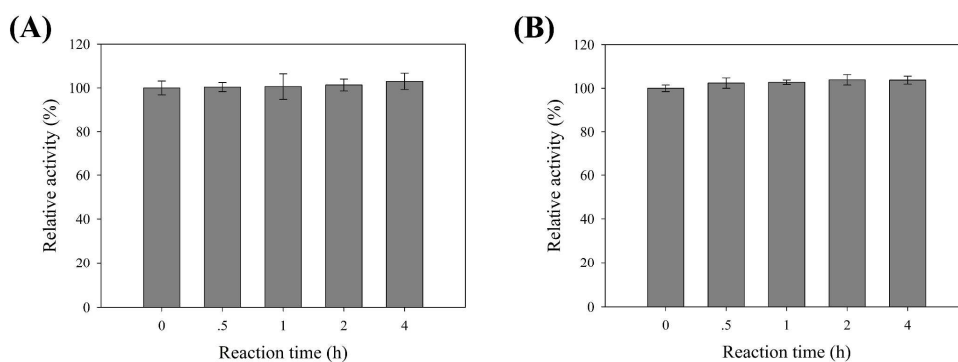
33



**Figure S2.** CD spectra in the near ultraviolet region (A) and far ultraviolet region (B) of the PP and PPC.

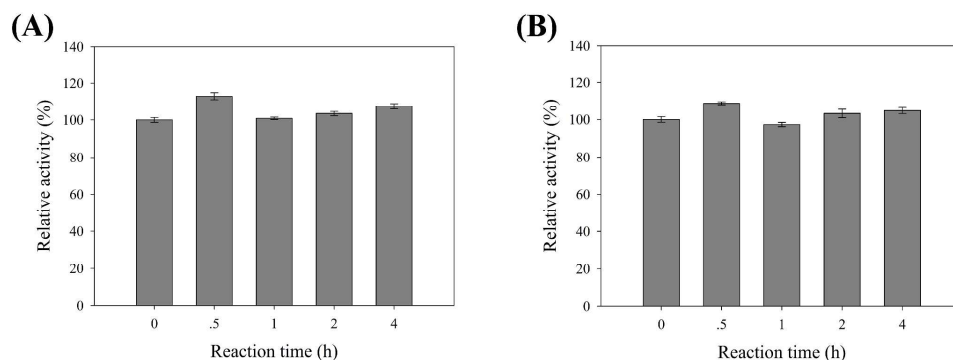


**Figure S3.**  $^1\text{H}$  NMR spectrum (A) (in  $\text{CD}_3\text{OD}$ ) and GPC trace (B) of the pyridyl disulfide-functionalized pHEMA.



**Figure S4.** The influence of 8 mM cysteine on the PPi hydrolysis activity of the PP (A) and PPC (B). Here, a relative activity of 100% corresponds to the activity of PP and PPC without treatment respectively. Each column represents the mean of separate

44 experiments with bar as SE (n = 3).



45

46 **Figure S5.** The influence of 8 mM DTT on the PPi hydrolysis activity of the PP (A)  
 47 and PPC (B). Here, a relative activity of 100% corresponds to the activity of PP and  
 48 PPC without treatment respectively. Each column represents the mean of separate  
 49 experiments with bar as SE (n = 3).

50 **Table S2.** The effect of pHEMA with different molecular weights on PPC activity.  
 51 The relative activity of 100 corresponds to the activity of the PPC without treatment.

Relative activity (%)	PPC	PPC-pHEMA	Reduced PPC-pHEMA
PPC-pHEMA1 <sup>a</sup>	100.00±1.31	0.37±0.01	102.64±1.85
PPC-pHEMA2 <sup>b</sup>	100.00±1.09	0.33±0.02	101.08±0.64

52 <sup>a</sup> pHEMA1:  $M_{n,HNMR}=8019$ , PDI=1.24; <sup>b</sup> pHEMA2:  $M_{n,HNMR}=25049$ , PDI=1.31.

53 **Table S3.** Michaelis-Menten parameters of sodium pyrophosphate hydrolysis  
 54 catalyzed by PPC, the conjugated products and reduced products.

Michaelis-Menten parameters	PPC	PPC-PCMB	Reduced PPC-PCMB	PPC-pHEMA	Reduced PPC-pHEMA
$K_M$ (mM)	1.08	1.10	1.12	0.90	1.06
$k_{cat}$ (s <sup>-1</sup> )	377.7	68.3	335.4	10.4	329.4
$k_{cat}/K_M$ (mM <sup>-1</sup> ·s <sup>-1</sup> )	351.1	62.3	300.0	11.6	312.1

55