Supporting Information

Gold Nanoparticle-Protein Conjugate Dually-Responsive to pH and Temperature for Modulation of Enzyme Activity

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Construction and Purification of Inorganic Pyrophosphatase (PPase)

Mutant-type PPases with additional thiol group far from the active center were generated as previously described.[1] Briefly, the mutants were performed for cloning the mutant *ppa* gene according to the megaprimer PCR method. The forward flanking primer for megaprimer PCR was 5'- CGCAAGCTTTTATTTATTCTTTGCGCGCTC-3', and the reverse one was 5'-CGCGGATCCAGCTTACTCAACGTCCCT-3'. The primer 5'- CACATTAAAGACGTTTGCGATCTGCCTGAACTGC-3' (nucleotides that represent mutations are underlined) was for the mutant N124C-PPase. The BamHI-HindIII fragment of the PCR products were ligated to pQE30 vector. Then the plasmid was transformed into *E. coli* XL1-Blue for protein expression. After IPTG incubation, the cells were pelleted by centrifugation, and disrupted with lysozyme under sonication. The obtain proteins were purified by Ni-NTA Sepharose resin (Shanghai Sangon Biotech Co., Ltd., China) and concentrated using centrifuge filters (Amicon Ultra). The purity of the proteins was verified by sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE, 4% stacking gel, 12% separating gel).

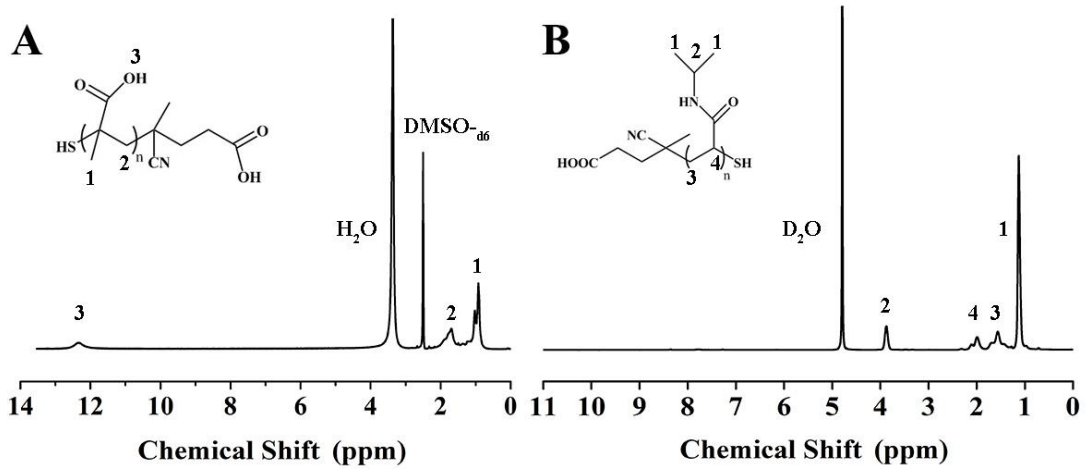

Fig. S1 ^{1}H NMR spectra of pMAA-SH and pNIPAM-SH.

Table S1 The GPC data for pNIPAM-SH.

Sample	$[M]_0/[CTA]_0/[I]_0$	MW (by GPC)	PDI
1	50.0/1/0.2	4700	1.11
2	100.0/1/0.2	9300	1.13
3	250.0/1/0.2	21000	1.17
4	500.0/1/0.2	42000	1.19

Table S2 The GPC data for pMAA-SH.

Sample	$[M]_0/[CTA]_0/[I]_0$	MW (by GPC)	PDI
1	100.0/1/0.2	5500	1.10
2	200.0/1/0.2	9600	1.14
3	300.0/1/0.2	14200	1.15
4	400.0/1/0.2	27600	1.17

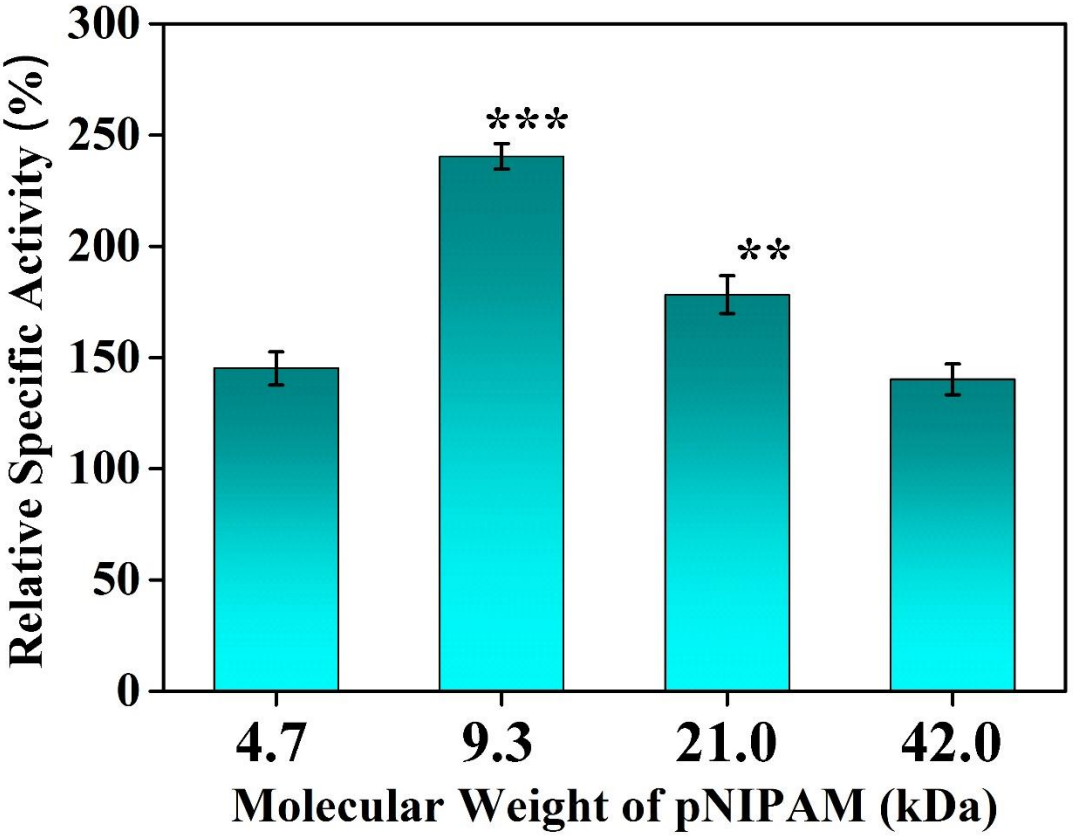

Fig. S2 The relative activity of AuNP-PPase-N-M at 45 ºC/pH 8.0 with different molecular weights of pNIPAM in the conjugates (compared to the activity at 25 ºC) (± SD, n = 3); **p<0.01, ***p<0.001 (4.7 kDa is the control group for the analysis of significant differences).

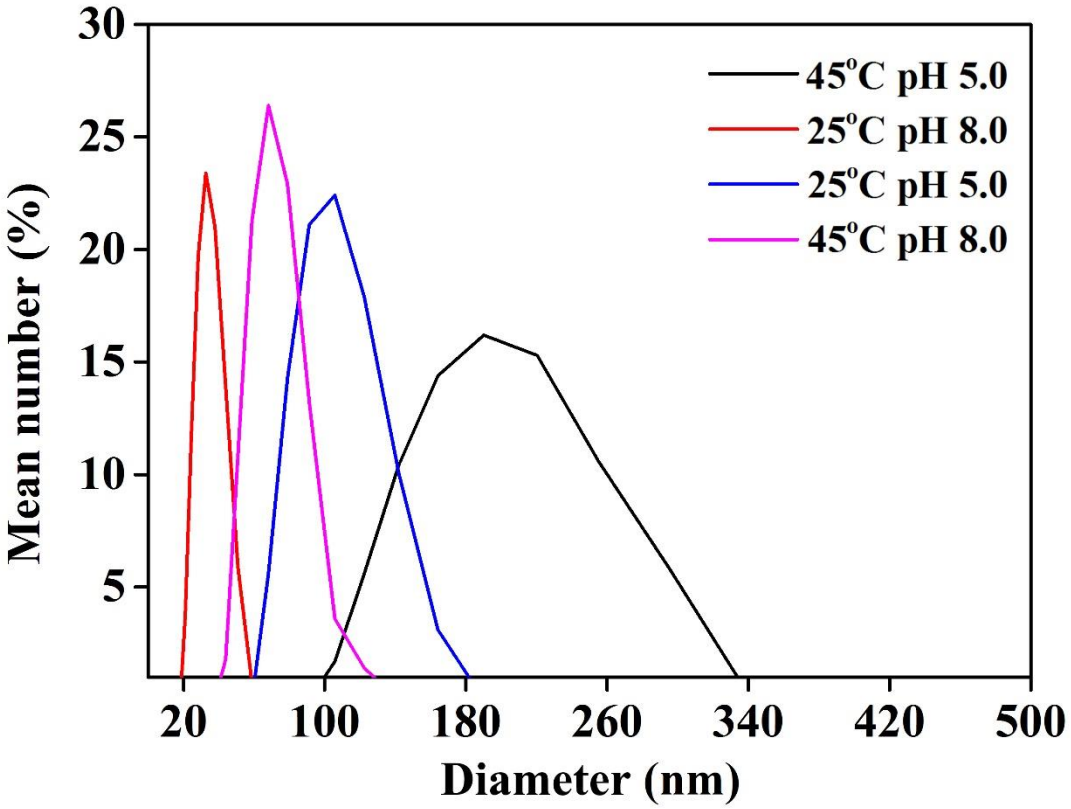

Fig. S3 Hydrodynamic diameter of AuNP-PPase-N-M conjugate under different conditions of temperature and pH determined by DLS.

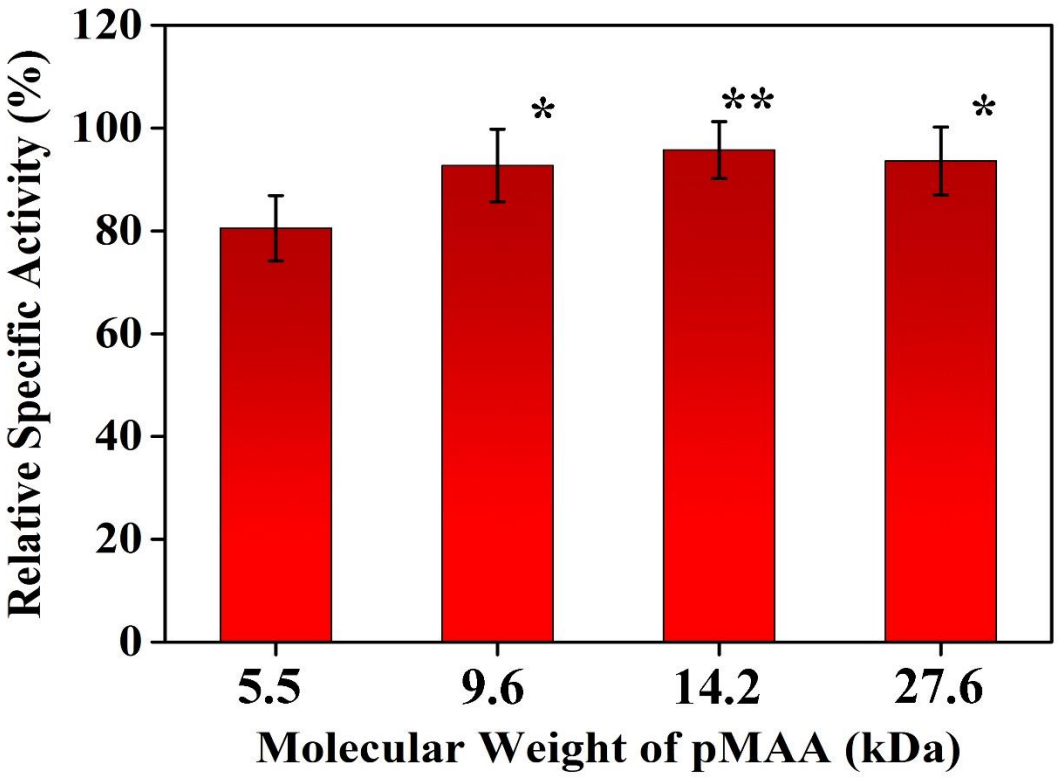

Fig. S4 The relative activity of AuNP-PPase-N-M conjugate at 45 °C/ pH 5.0 compared to 45 °C/ pH 8.0 as a function of pMAA molecular weight (± SD, n = 3); * $p<0.05$, ** $p<0.01$ (5.5 kDa is the control group for the analysis of significant differences). The data show that pMAA of molecular weight in the range of 9.6-27.6 kDa should be equally effective for regulation of PPase activity in AuNP-PPase-N-M conjugate.

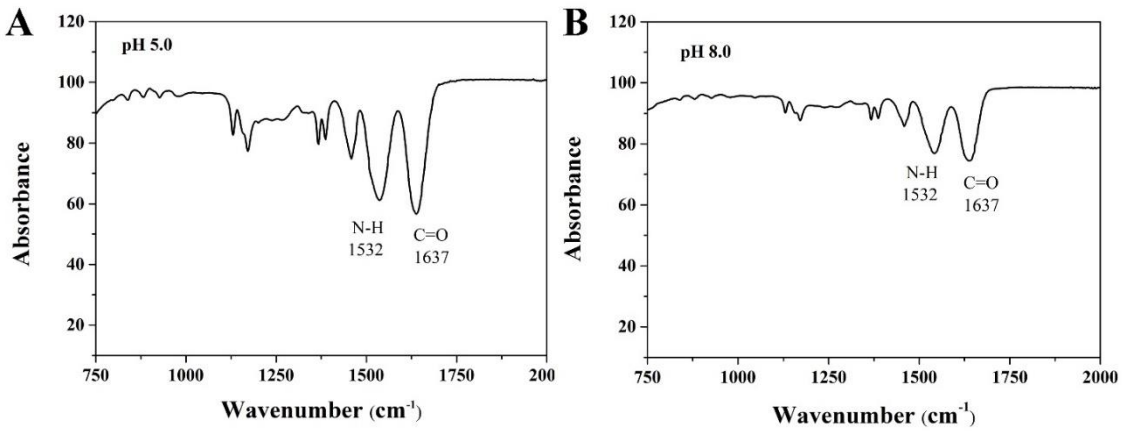

Fig. S5 FTIR spectra of pure pNIPAM at pH 5.0 (A) and pH 8.0 (B).

Table S3 Michaelis-Menten parameters of PPase and AuNP-PPase-N-M.

Parameters	PPase (25 °C /pH 5.0)	AuNP-PPase-N-M (25 °C /pH 5.0)	PPase (45 °C /pH 8.0)	AuNP-PPase-N-M (45 °C /pH 8.0)
Km (mM)	9.3	16.97	0.66	0.59
kcat (S^{-1})	6.04	6.18	731	636
kcat/ Km ($mM^{-1} \cdot S^{-1}$)	0.65	0.36	1107.6	1077.9

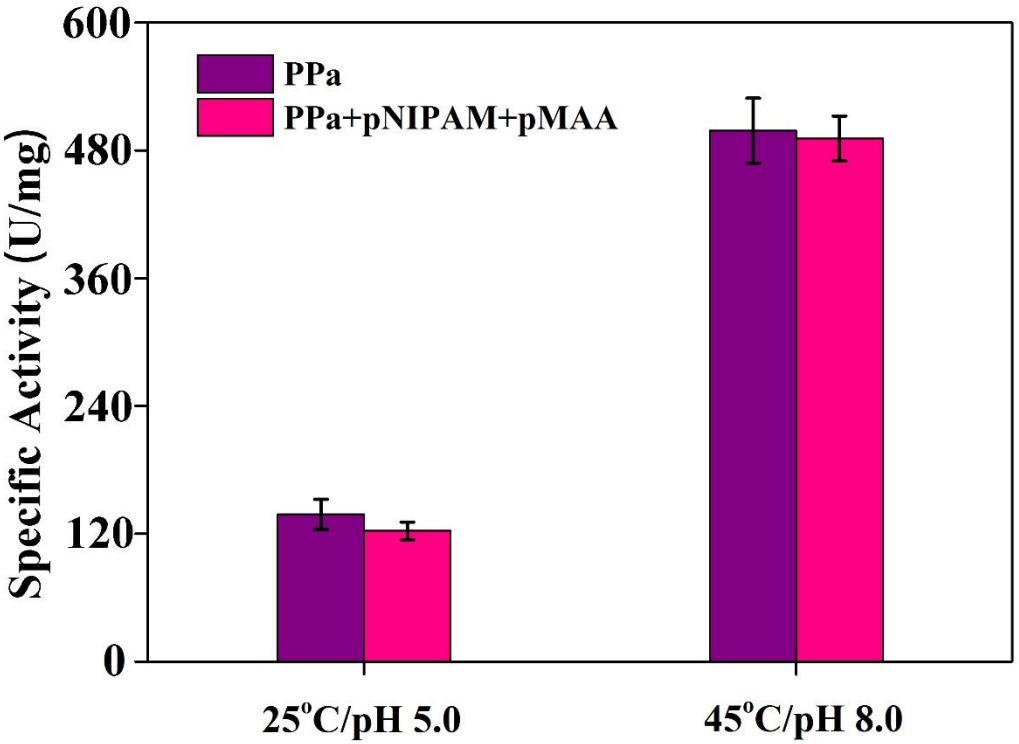

Fig. S6 The effect of polymers on the activity of PPase. Both the protein and polymer are dissolved in the solution. Under the conditions of 25 °C /pH 5.0 and 45 °C /pH 8.0, the enzyme activity has no significant difference before and after polymer treatment.

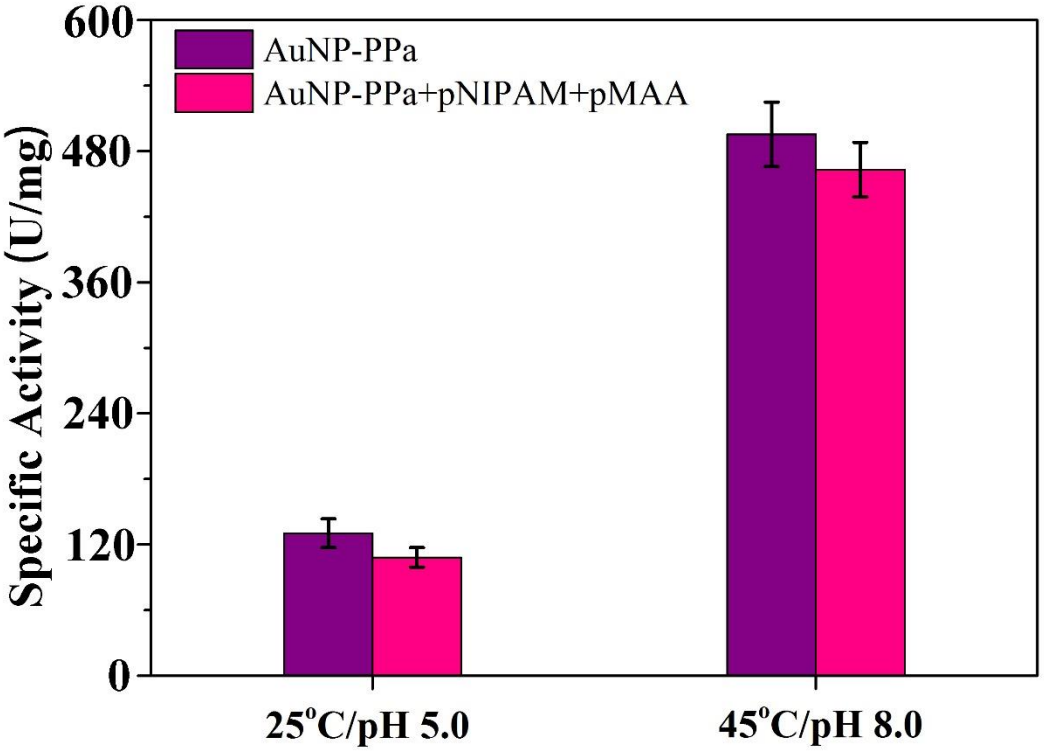

Fig. S7 The effect of polymers on the activity of PPase bound to AuNPs. The polymer are dissolved in the solution and AuNP-PPase is dispersed in the solution. Under the conditions of 25 ºC /pH 5.0 and 45 ºC /pH 8.0, the activity of PPase bound to AuNPs has no significant difference before and after polymer treatment.

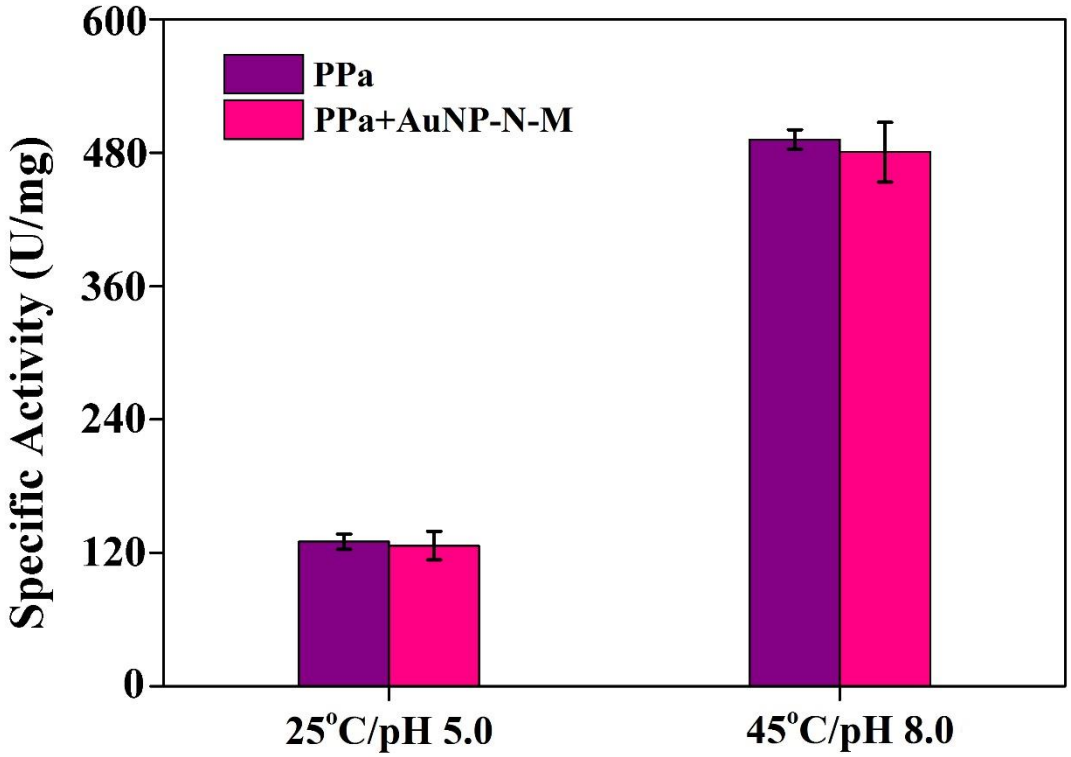

Fig. S8 The effect of polymers bound to AuNPs on the activity of PPase. The protein is dissolved in the solution and AuNP-N-M is dispersed in the solution. Under the conditions of 25 ºC /pH 5.0 and 45 ºC /pH 8.0, the activity of PPase has no significant difference before and after AuNP-N-M treatment.

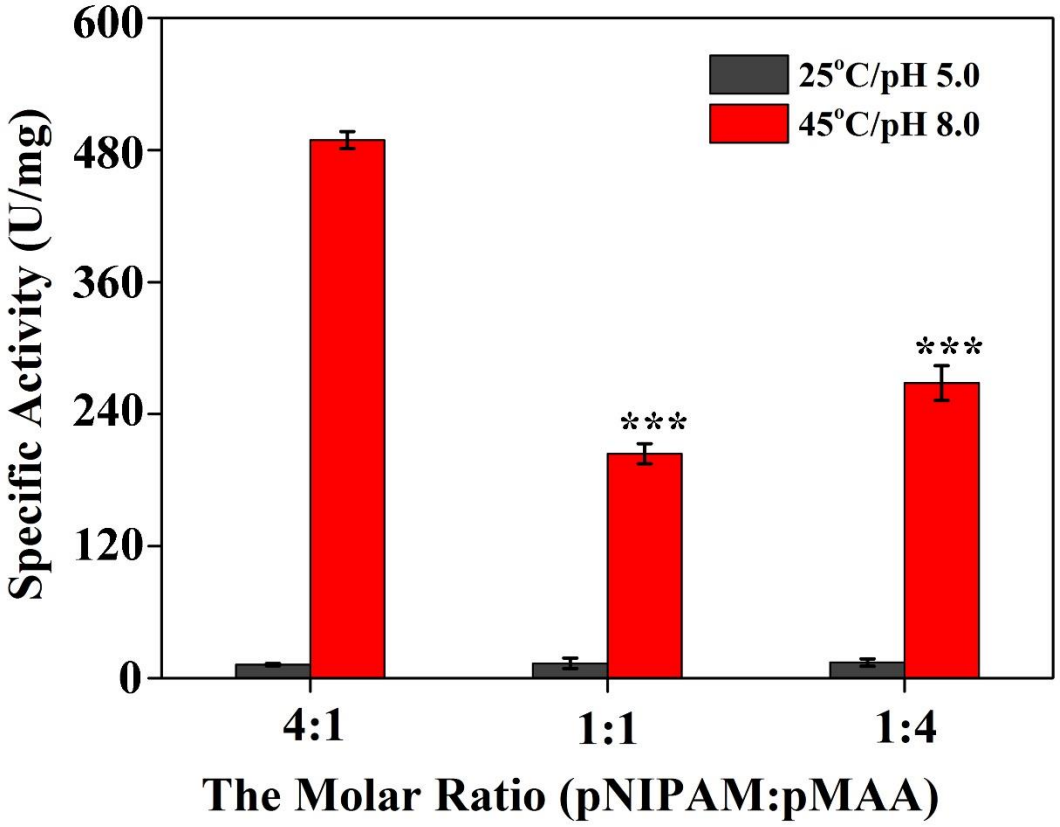

Fig. S9 The effect of the molar ratio of pNIPAM to pMAA on the activity of AuNP-PPase-N-M (± SD, n = 3); ***p<0.001 (4:1 under the same condition is the control group for the analysis of significant differences).

Reference

1. F. Liu, Y. Cui, L. Wang, H. Wang, Y. Yuan, J. Pan, H. Chen and L. Yuan, *ACS Appl. Mater. Interfaces*, 2015, **7**, 11547-11554.