Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

One-step synthesis of glycoprotein mimics *in vitro*: improvement of protein activity, stability and application in CPP hydrolysis

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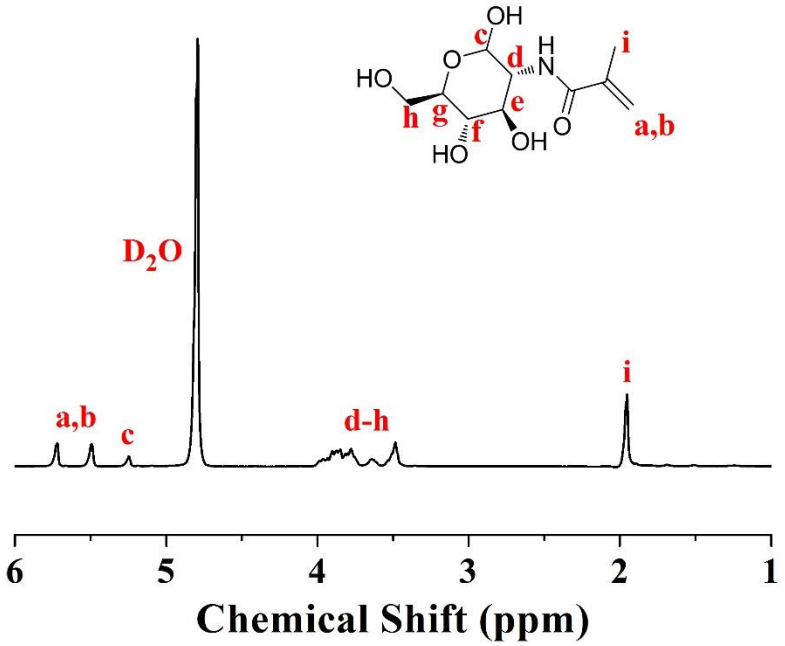

Fig. S1 ^{1}H NMR spectrum of MAG. Solvent: D_2O.

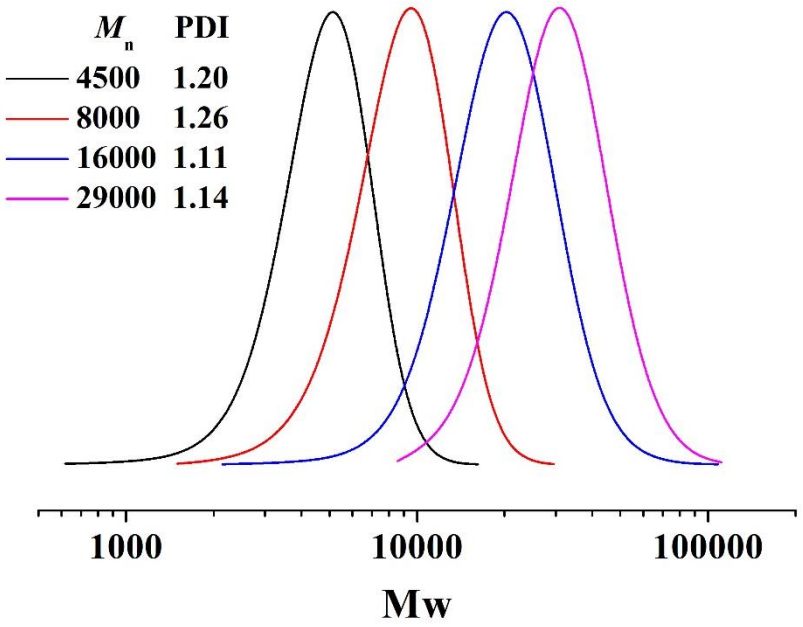

Fig. S2 Aqueous GPC of PMAG with increasing molecular weight.

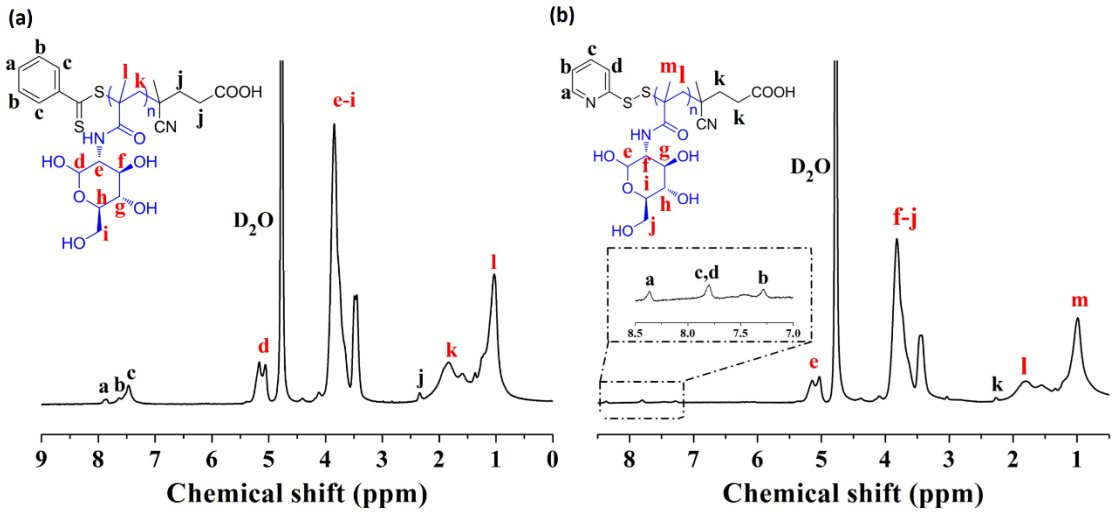

Fig. S3 ^{1}H NMR of PMAG (a) and the corresponding pyridyl-PMAG (b). Solvent: D_2O. The conversion of pyridyl-PMAG was calculated as below[1, 2]:

$$\text{Conversion (\%)}=\frac{\delta_{8.4\text{ppm}}}{\delta_{2.5\text{ppm}}/2}\times 100\%=95.28\%$$

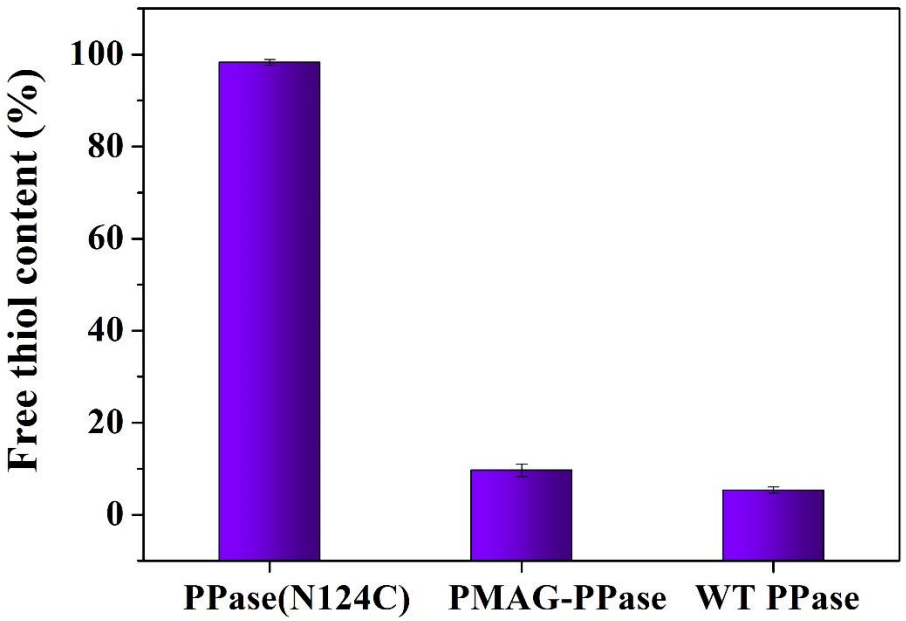

Fig. S4 Free thiol content of PPase(N124C), PMAG-PPase conjugates (after purification) and WT (wild type) PPase (Mean ± SD, n = 3). The conversion of conjugate was calculated as below:

$$\text{Conversion (\%)}=\frac{A_{\text{N124C}}-A_{\text{PMAG-PPase}}}{A_{\text{N124C}}-A_{\text{WT-PPase}}}\times 100\%=\frac{98.26-9.68}{98.26-5.36}\times 100\%=95.35\%$$

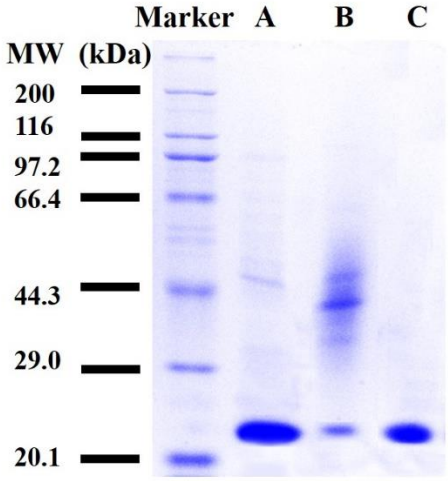

Fig. S5 SDS-PAGE result. Lane M: molecular weight markers; lane A: PPase; lane B: PMAG-PPase conjugate (PM_2-PPase); lane C: conjugate reduced by DTT.

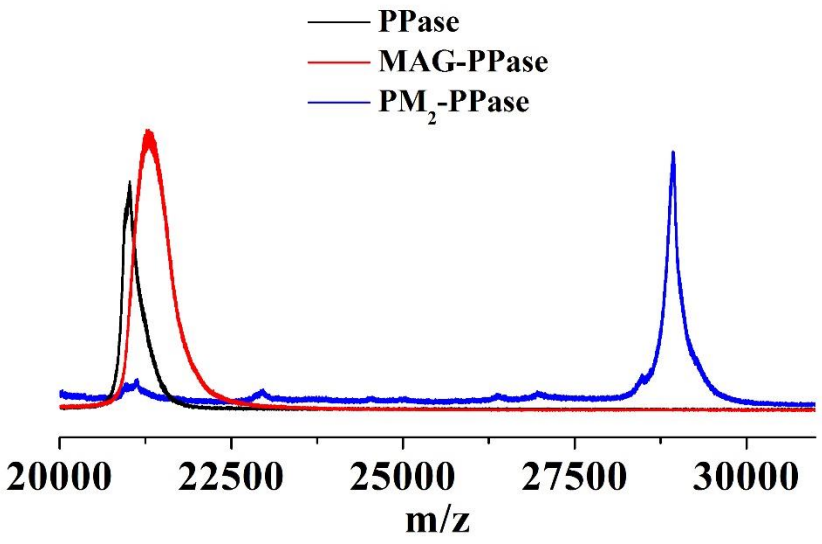

Fig. S6 MALDI-TOF mass of PPase, MAG-PPase and PM_2-PPase. MAG-PPase conjugate was synthesized via thiol-ene click chemistry according to Cobo *et al.*[3] The process was similar with the synthesis of PMAG-conjugates, which the feed molar ratio of MAG/PPase was 50/1, reacted in 10 mM Tris-HCl buffer (pH 8.0) and measured via MALDI-TOF mass.

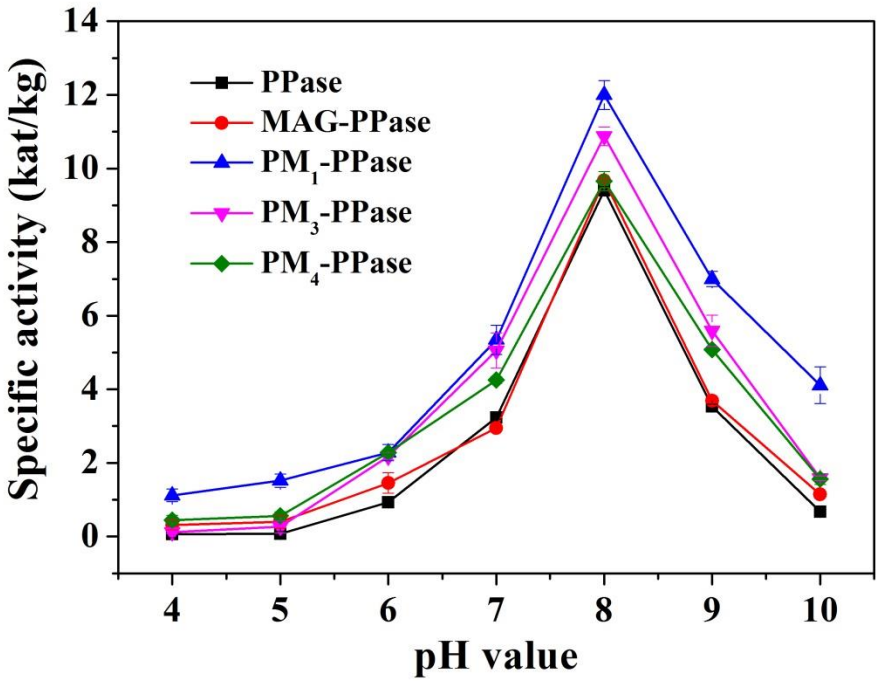

Fig. S7 Specific activities vs. pH value of free PPase, MAG-PPase and PMAG-PPase conjugates of different PMAG molecular weight ($\boldsymbol{M}_n$(PMAG) = 4.5, 16.0, and 29.0 kDa for PM_1, PM_3, and PM_4 respectively) as a function of pH value. Mean ± SD, n = 3.

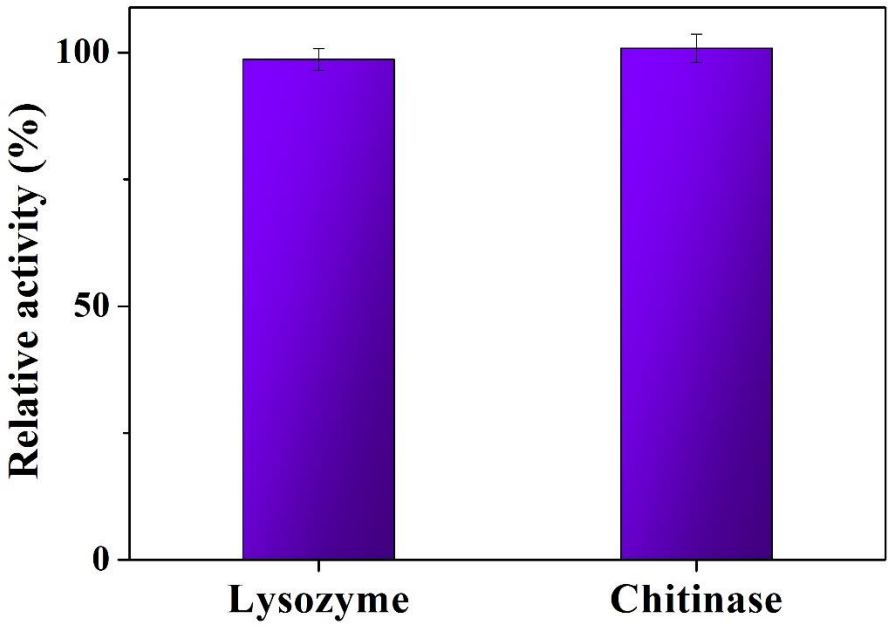

Fig. S8 Tolerance of PM_2-PPase conjugate to lysozyme and chitinase digestion (relative to the specific activity of PM_2-PPase without the incubation of glycosidases, Mean ± SD, n = 3).

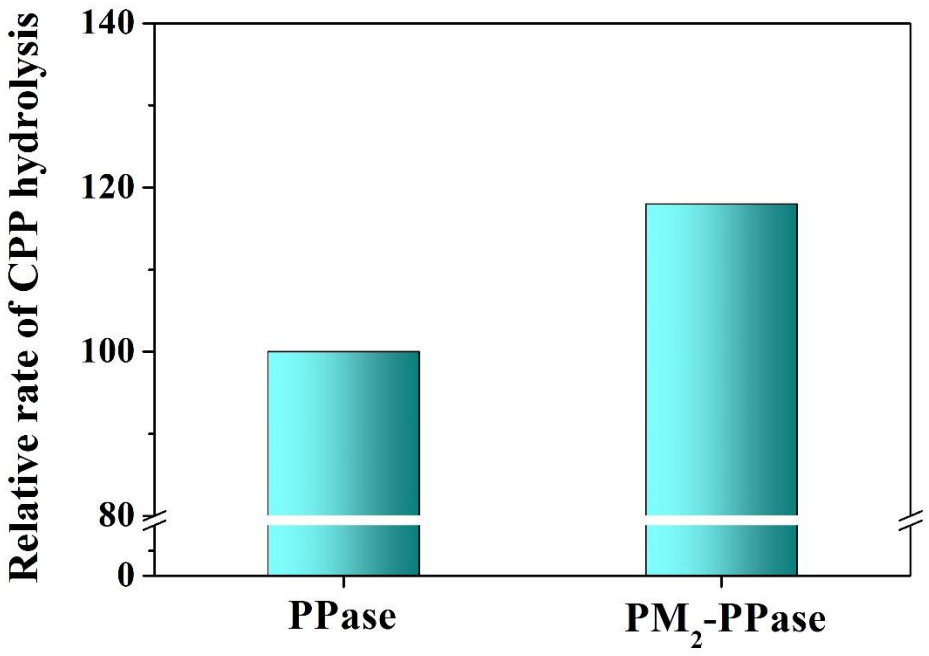

Fig. S9 Relative rate of CPP hydrolysis using PPase and PM_2-PPase, respectively (relative to the hydrolysis rate of unconjugated PPase).

References

1 C. Boyer, V. Bulmus and T. P. Davis, *Macromol. Rapid Commun.*, 2009, **30**, 493-497.

2 C. Boyer, A. Granville, T. P. Davis and V. Bulmus, *J. Polym. Sci. Part A: Polym. Chem.*, 2009, **47**, 3773-3794.

3 I. Cobo, M. Li, B. S. Sumerlin and S. Perrier, *Nat. Mater*. 2015, **14**, 143-159.