Electronic Supplementary Information

for

Improving protein activity and stability in acidic conditions via site-specific conjugation of a pH-responsive polyelectrolyte

Lei Wang,[a] Xin Li,[a] Lin Yuan,*[a] Hongwei Wang,[a] Hong Chen*[a] and John L. Brash[b]

[a] The Key Lab of Health Chemistry and Molecular Diagnosis of Suzhou, Department of Polymer Science and Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, 199 Ren'ai Road, Suzhou, 215123, P. R. China.

[b] School of Biomedical Engineering, Department of Chemical Engineering, McMaster University, Hamilton, Ontario, Canada.

E-mail: yuanl@suda.edu.cn (L. Yuan); chenh@suda.edu.cn (H. Chen)

Table of Contents

Table S1. Specific activities of native PPase and mutants for the hydrolysis of sodium pyrophosphate at pH 8.0. Mean +/- SD, n=3.

PPase samples	Specific activity (kat/kg)
Native PPase	8.57±0.19
K148C mutant	8.77±0.10
N124C mutant	7.20±0.09

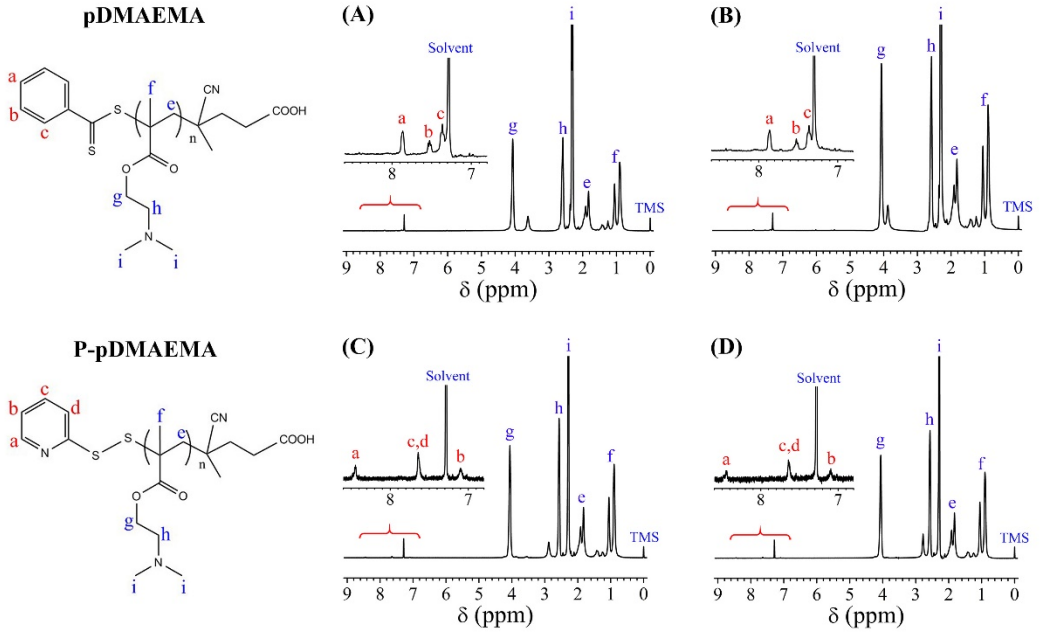
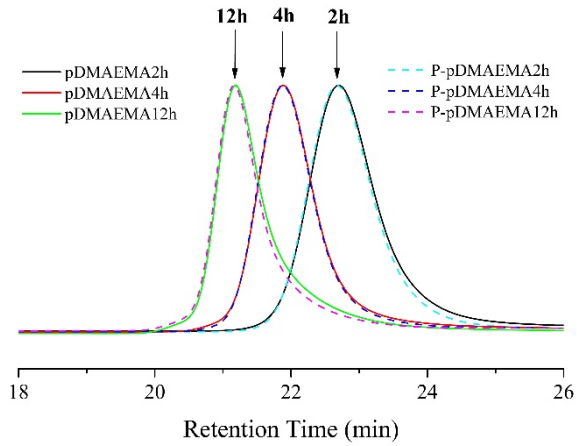


Figure S1. ^{1}H NMR of pDMAEMA (A and B) and the corresponding pyridyl disulfide-functionalized pDMAEMA (C and D). Solvent: $CDCl_3$. A and C: P_2 (Polymerization time: 4 h); B and D: P_3 (Polymerization time: 12 h)

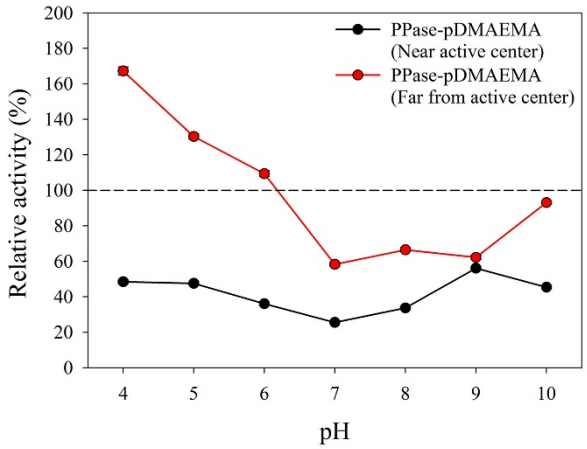
Table S2. ^{1}H NMR results of pDMAEMA with increasing polymerization time.

pDMAEMA	Polymerization time	Monomer conversion	Theoretical M_n	Polymerization degree	M_n, by HNMR
P_1	2 h	34.7%	11 200	72	11 600
P_2	4 h	65.6%	20 900	162	25 700
P_3	12 h	92.9%	29 500	190	30 100



35

36 **Figure S2.** GPC traces of pDMAEMA with increasing molecular weight before (solid lines)
37 and after (dashed lines) pyridyl disulfide functionalization.



38

39 **Figure S3.** Relative activity of PPase-pDMAEMA conjugates compared to that of
40 unconjugated PPase at different pH. Black line: conjugates with polymer near the protein
41 active center. Red line: conjugates with polymer far from the protein active center. Mean +/-
42 SD, n=3.

43 **Table S3.** Hydrodynamic diameter of PPase and PPase-pDMAEMA conjugates at different
44 pH.

Samples	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
PPase	3531.0	3420.3	2682.0	8.3	6.8	6.6	5.3
PPase-P_1	6.7	6.9	7.0	162.7	361.8	15.0	6.1
PPase-P_2	11.0	11.8	10.6	144.7	865.8	12.6	8.0
PPase-P_3	14.9	10.8	10.8	9.9	939.9	7.5	13.1

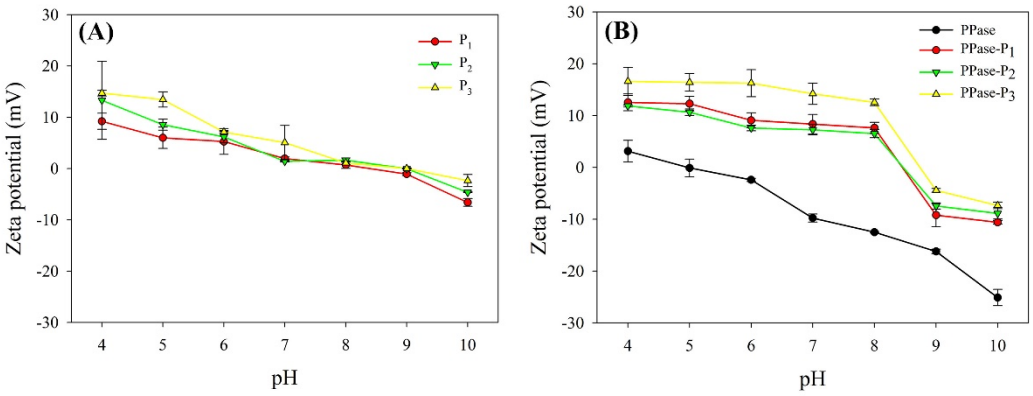


Figure S4. Zeta potential of pDMAEMA with increasing molecular weight (A) and PPase and PPase-pDMAEMA conjugates (B) determined by DLS at different pH. Mean +/- SD, n=3.