Electronic Supplementary Information (ESI)

Covalently Crosslinked Coacervate: Immobilization and Stabilization of Proteins with Enhanced Enzymatic Activity

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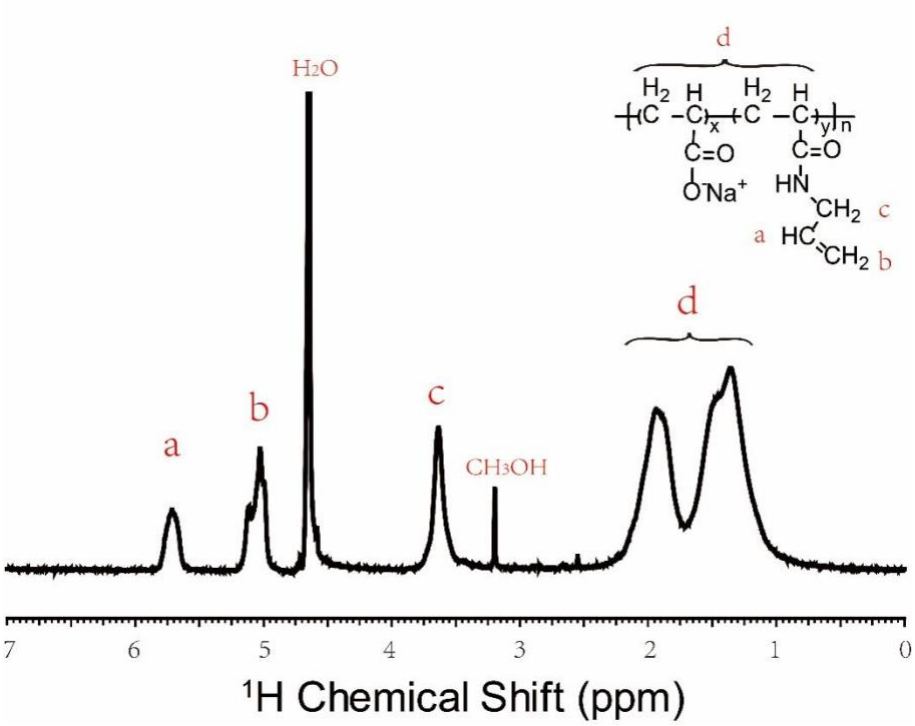

Figure S1. ^{1}H NMR of allyl-PAA using D_2O as the solvent.

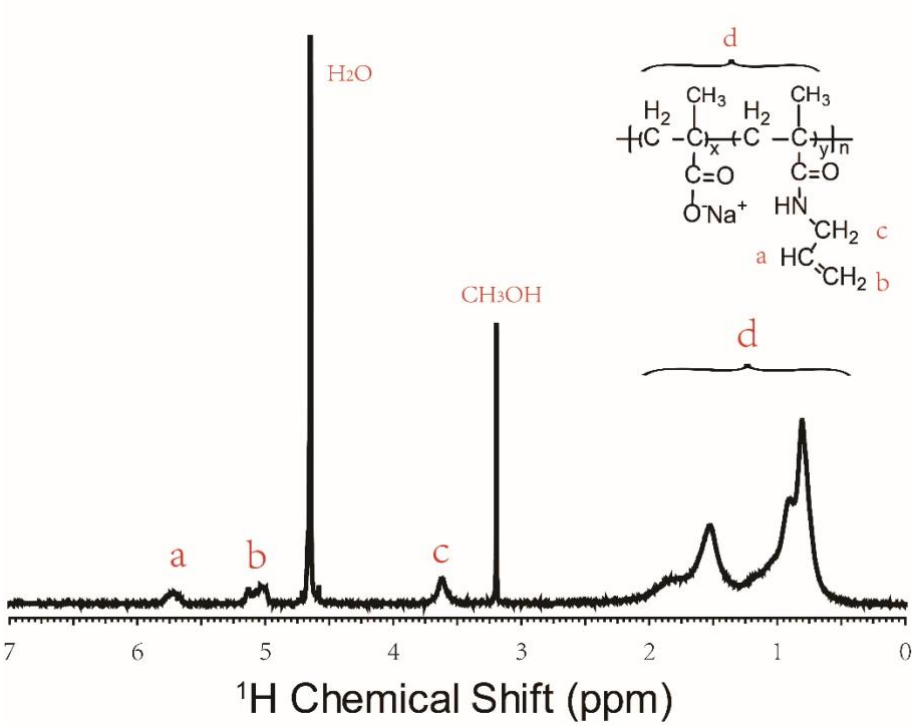

Figure S2. ^{1}H NMR of allyl-PMAA using D_2O as the solvent.

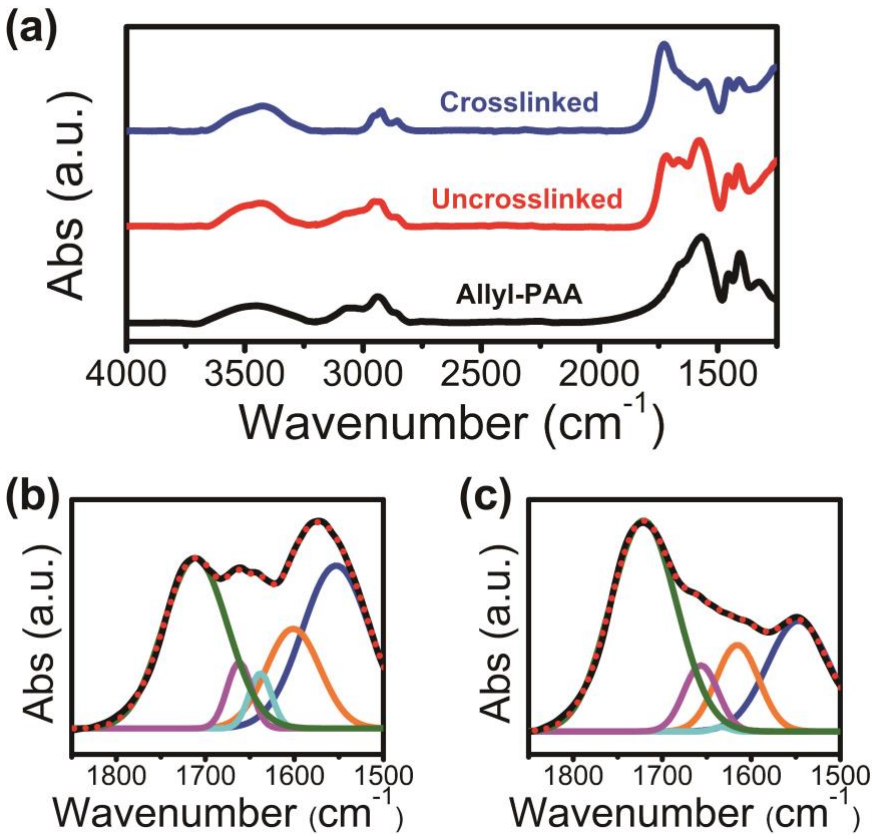

Figure S3. (a) FTIR of dried uncrosslinked and crosslinked BPEI-allyl-PAA coacervate, and deconvolution of FTIR peaks of (b) uncrosslinked BPEI-allyl-PAA coacervate and (c) crosslinked BPEI-allyl-PAA coacervate between 1500 – 1850 cm^{-1} using Gaussian functions. The BPEI-allyl-PAA coacervate was prepared using 10 mM BPEI and 10 mM PAA at pH 6.5. Origin was used to fit multiple Gaussian functions to separate the peaks between 1500 – 1850 cm^{-1}. Absorption bands at ~ 1554 cm^{-1} and 1710 cm^{-1} were attributed to asymmetric stretching of carboxylate group and C=O stretching of carboxyl group, respectively. The absorption peaks at ~1660 cm^{-1} and 1600 cm^{-1} were ascribed to the C=O stretching of amide and $-NH_3^+$ of BPEI, respectively.

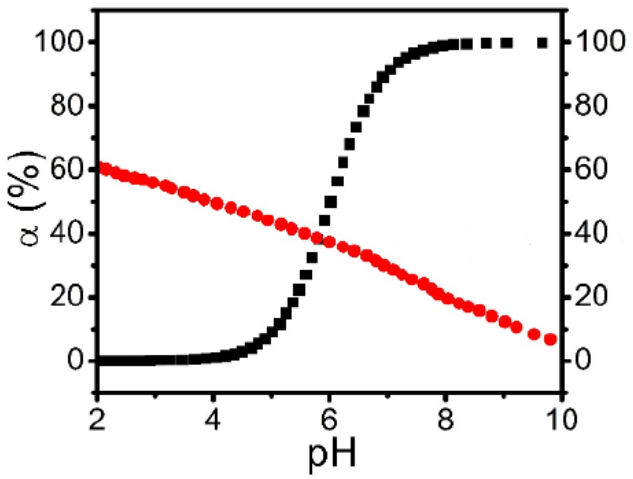

Figure S4. Ionization of allyl-PAA (■) and BPEI (●) as a function of pH. The degree of ionization is reported as α for both the weak acid, allyl-PAA, and weak base, BPEI.

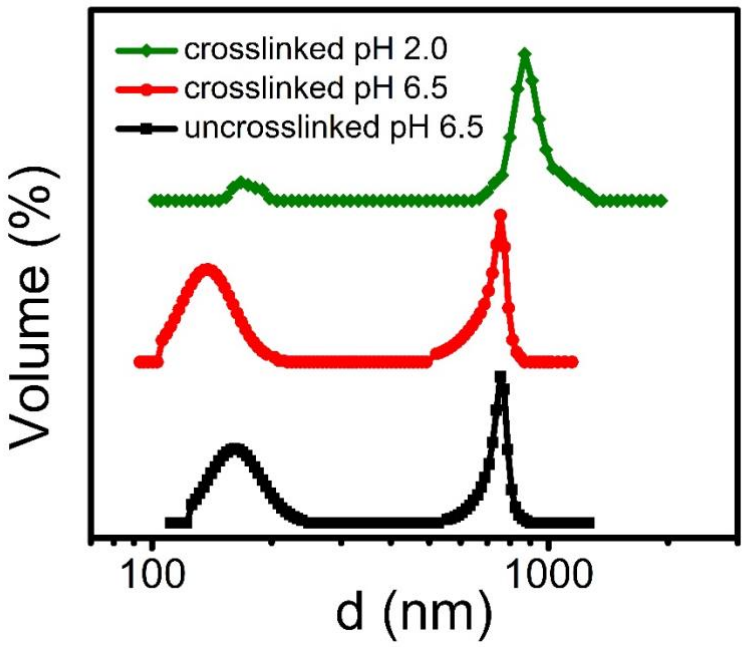

Figure S5. Volume-averaged hydrodynamic diameters of crosslinked (pH 2.0 and 6.5) and uncrosslinked (pH 6.5) BPEI-allyl-PAA coacervate samples determined by dynamic light scattering. For each coacervate sample, the peak with smaller size was attributed to the soluble complexes, while the peak with larger size was assigned to the coacervate.

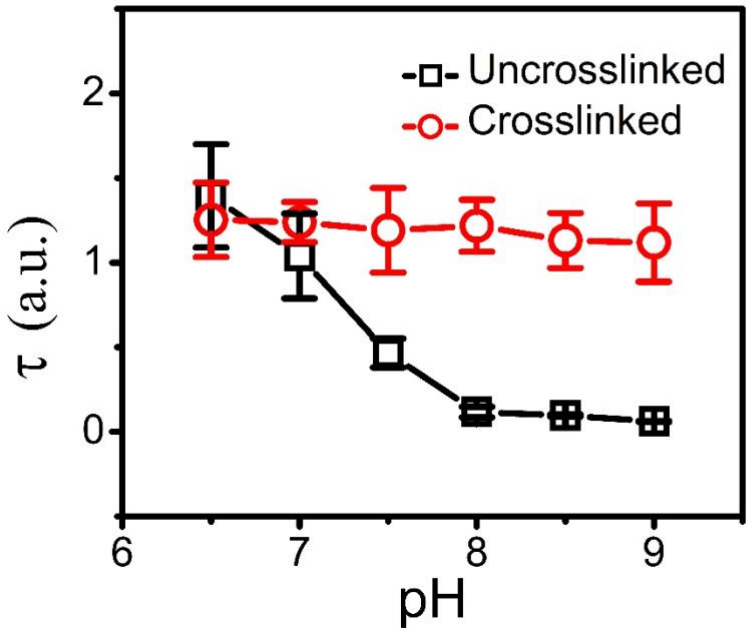

Figure S6. Turbidity of uncrosslinked and crosslinked BPEI-allyl-PMAA coacervate droplets as a function of increasing pH from 6.5 to 9.0. The error bars represent the standard deviation of 3 measurements

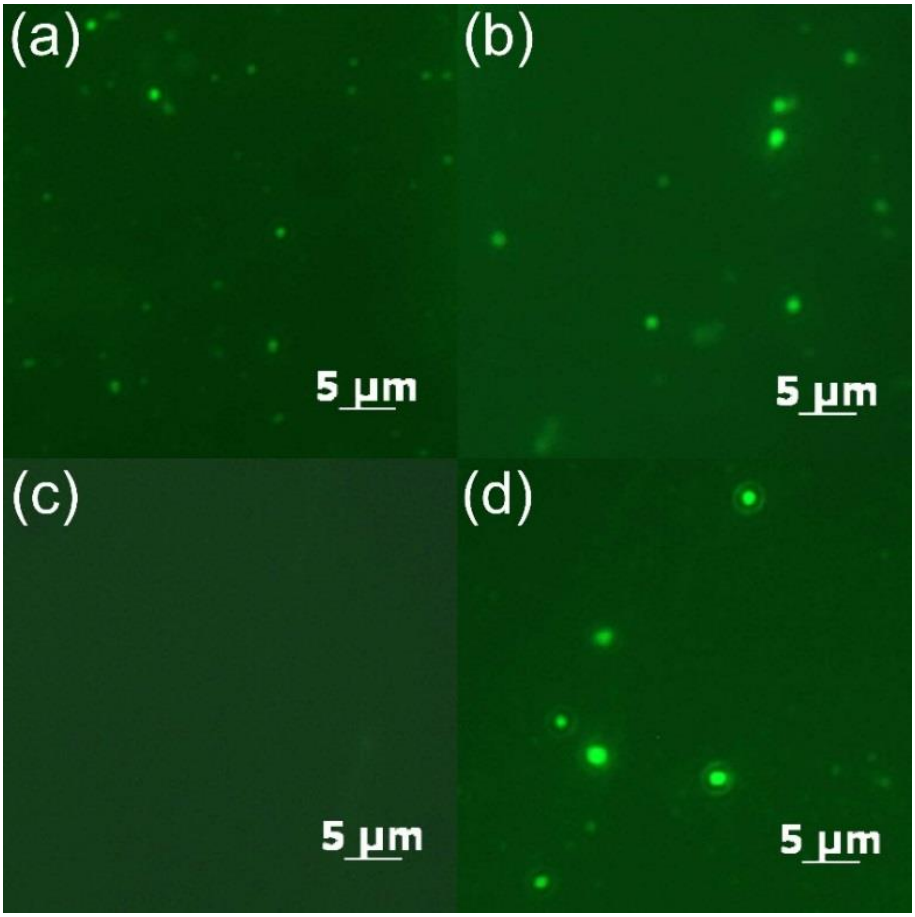

Figure S7. Optical micrographs from fluorescence microscopy of FITC-BSA encapsulated within (a) uncrosslinked coacervates at pH 6.5, (b) crosslinked coacervates at pH 6.5, (c) uncrosslinked coacervates at pH 2.0 and (d) crosslinked coacervates at pH 2.0.

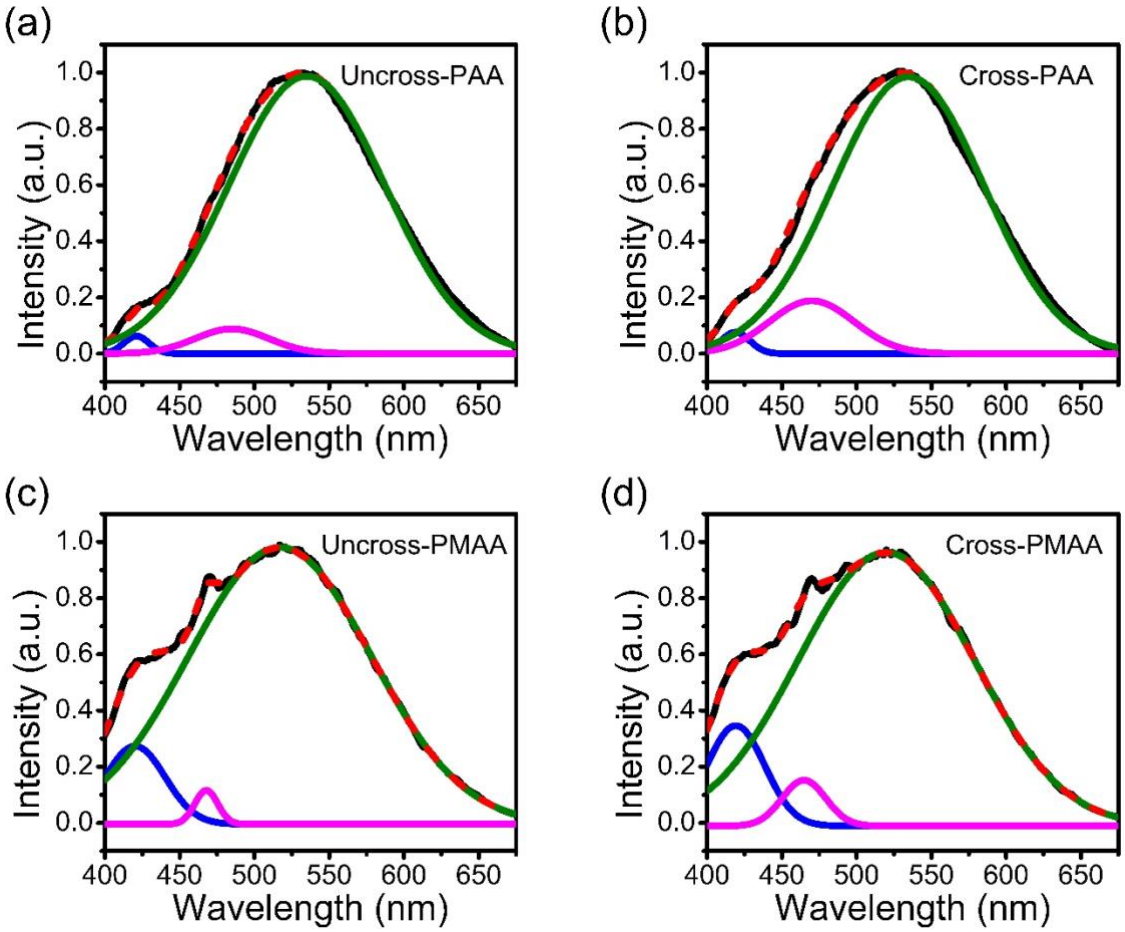

Figure S8. Fluorescence emission spectra of ANS within (a) uncrosslinked BPEI-allyl-PAA, (b) crosslinked BPEI-allyl-PAA, (c) uncrosslinked BPEI-allyl-PMAA and (d) crosslinked BPEI-allyl-PMAA coacervate droplet dispersions recorded at room temperature. The dispersions were excited at 350 nm and emission measured from 400 to 675 nm. Multi-Gaussian fits to fluorescence spectra for ANS within uncrosslinked and crosslinked coacervate. The plots show peak maxima at approximately 420, 480 and 530 nm corresponding to distributions of polar and non-polar environment. The strong emission peak at 530 nm was consistent with emission from the charge transfer state, while the low intensity peaks at 420 and 470 nm was assigned to the non-polar excited state localized on the naphthalene ring of ANS. The large relative intensity of the peak at 530 nm indicates that the sequestered ANS molecules were predominantly in a polar environment rather than a non-polar excited state. Crosslinked coacervate showed an increase in the relative fluorescence intensity of ANS at shorter wavelengths (420 and 470 nm) compared to the uncrosslinked coacervate, indicating that the sequestered ANS experienced a more hydrophobic environment in crosslinked coacervate.

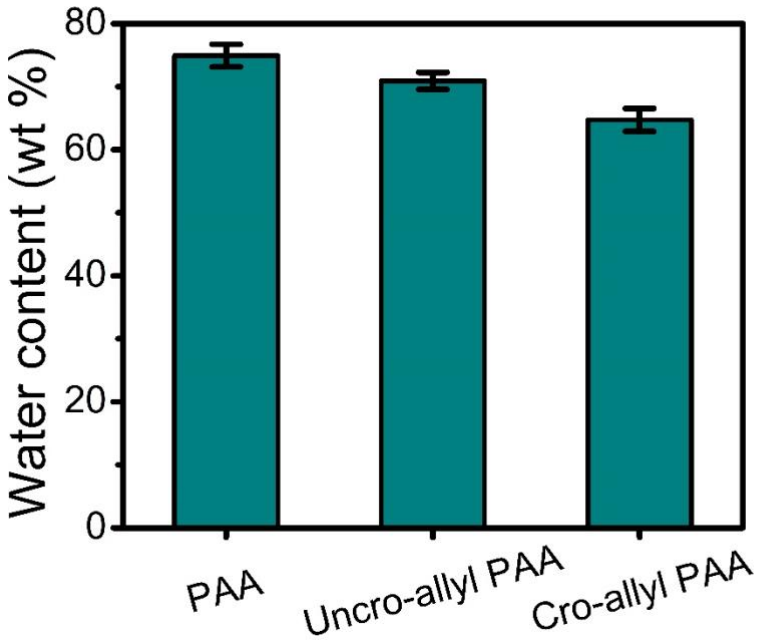

Figure S9. Water content (wt%) of BPEI-PAA coacervate, uncrosslinked BPEI-allyl-PAA coacervate and crosslinked BPEI-allyl-PAA coacervate. The coacervate samples were prepared with an initial pH of 6.5 and a 1:1 molar ratio of BPEI to PAA or allyl-PAA. The error bars represent the standard deviation of 3-5 measurements

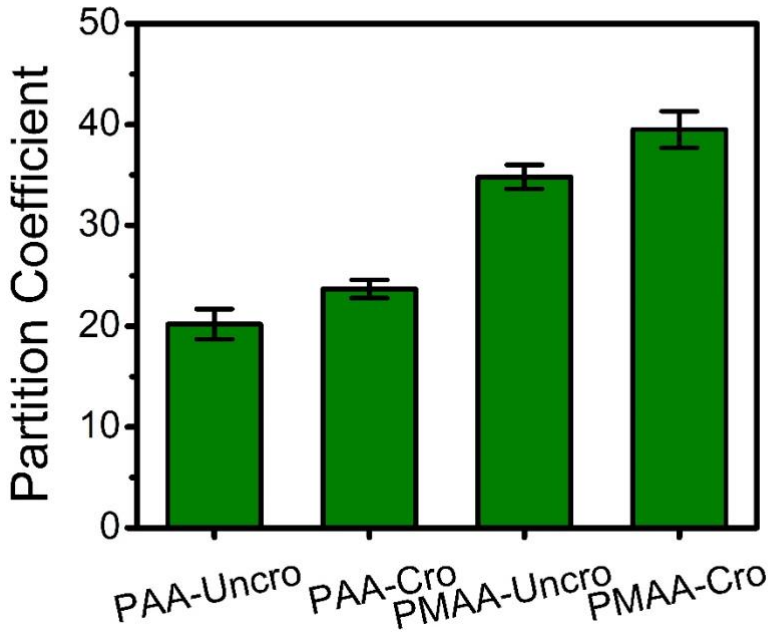

Figure S10. Partition coefficient of substrate 4-NPP into uncrosslinked and crosslinked BPEI-allyl-PAA coacervate as well as into uncrosslinked and crosslinked BPEI-allyl-PMAA coacervate at pH 6.5. The error bars represent the standard deviation of 3-5 measurements

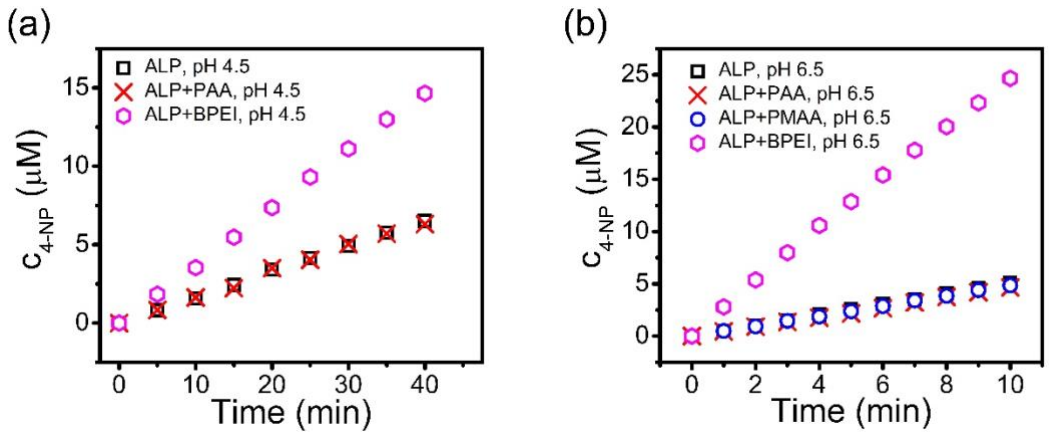

Figure S11. Enzymatic activity of ALP at (a) pH 4.5 and (b) pH 6.5 in aqueous solution without polyelectrolytes (□), with PAA (×), with PMAA (○) or with BPEI (⬡) respectively.