Enzymatic Hydrogelation to Immobilize an Enzyme for High Activity and Stability

Qigang Wang, Weiwei Ge, Zhimou Yang, Ling Wang, Yuan Gao, Bing Xu*

Electronic Supplementary Information

The CD analysis: To get the control hydrogel for CD test, the pH value of the suspension of **3** (38 mg) in 1 mL water was adjusted to about 2.0. As shown in Figure S-1, acid phosphatase in the hydrogel displays same CD peak in 210 nm as that of free acid phosphatase, indicating that this enzymatic hydrogelation process preserves the secondary structure of the enzyme. The Cotton effect at about 220 nm and 230 in Gel and control Gel indicates the superhelical arrangements of the amino acid residues (nπ*transition). The induced helical orientation of the fluorenyl groups in **4** and **3** are the cause of the Cotton effect at 250– 320 nm (ππ* transition) in $AP_{(gel)}$ and the control hydrogel of **3**, respectively.

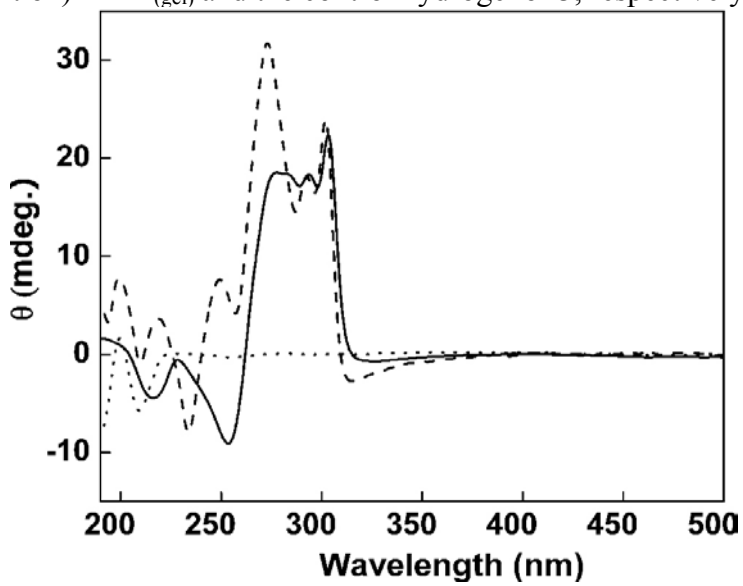

Figure S-1. The CD spectra of $AP_{(gel)}$ (Dash line), $AP_{(free)}$ (Dot line), and the control gel of **3** without AP (Solid line).

Test leakage of $AP_{(gel)}$ in solvents: At first, the insolubility of AP in the selected organic media can be easily confirmed by the observation during enzymatic reaction. The leach of enzyme from hydrogel to water could be measured by incubating 0.5 g of $AP_{(gel)}$ (containing 50 μg AP) in 5 mL of water buffer (100 mM phosphate; pH 6.0). Then the released amount of AP from molecular hydrogel was measured by the Bradford methods. The absorbance of the complex of protein and coomassie brilliant G-250 is measured at 595 nm. After 24h incubation, the 100 μL release solution was added to 2 mL the G-250 solution and measured the absorbance at 595 nm. At the same time, the 100μL free AP standard solution (with 10 μg/mL concentration) was treated as the previous protocol. By comparing their absorbance at 595 nm, the leached amount of AP is (0.0039/0.0302)*100%=12.9 %.

Test of AP absorption by gel in solvents: In one typical test, 10 μL AP (500U) was diluted in 1 mL water buffer, then 0.5g of the gel formed by compound **4** was immersed in the solution of AP. After 24h, 100 μL solution was mixed with the 2 mL of G-250 solution and measured for its absorption at 595 nm. The initial enzyme concentration was also measured using the same method. Using the gel formed by compound **4** in the water buffer as the control, we obtained the absorption ratio of AP by the gel is 8.8%.

The activity test: We first tested the activity of the self-immobilized acid phosphatase by the hydrolysis of p-nitrophenylphosphate (**1**) in different solvents at 298K. After adding the various form AP with 0.2 μg AP to 10ml chloroform, toluene, and n-octane with 10 mM (**1**), the activity of various forms AP was measured by monitoring the absorbance of p-nitrophenol (**2**) at 300 nm with the molar coefficient about

1000 $M^{-1}cm^{-1}$.The whole catalytic reaction is executed at room temperature and with slightly shaking. The activity assay of the various form AP in water at same concentration (20μg/L) followed the Sigma procedure (Acid phosphatase Assay Kit, CS0740) at 298K. The concentration 10 mM of the substrate (**1)** is much larger than K_m value (about 0.5 mM) of the AP in literature. Therefore, the initial reaction rate in the first 5 minutes in the reaction condition is defined as the activity of various forms AP.