Supporting Information

Evaluation of an Immobilized Artificial Carbonic Anhydrase Model for CO_2 Sequestration

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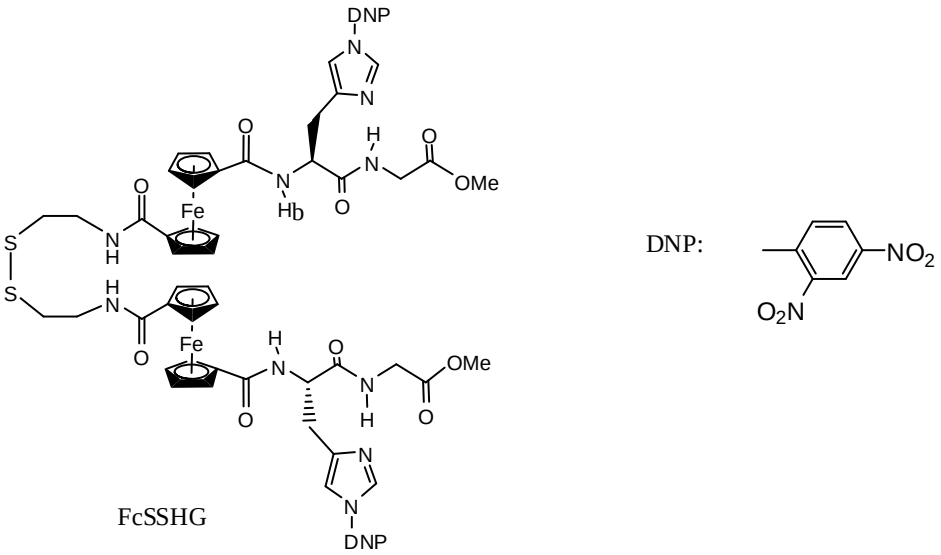
Structure and Characterization of Objective Compound (FcSSHG)

The conjugate FcSSHG was prepared as the structure in Scheme S1, and characterized by ^{1}H NMR, ^{13}C NMR, and high resolution Mass spectrometry as Figure S1-3.



FcSSHG

Scheme S1. The structure of **FcSSHG**.

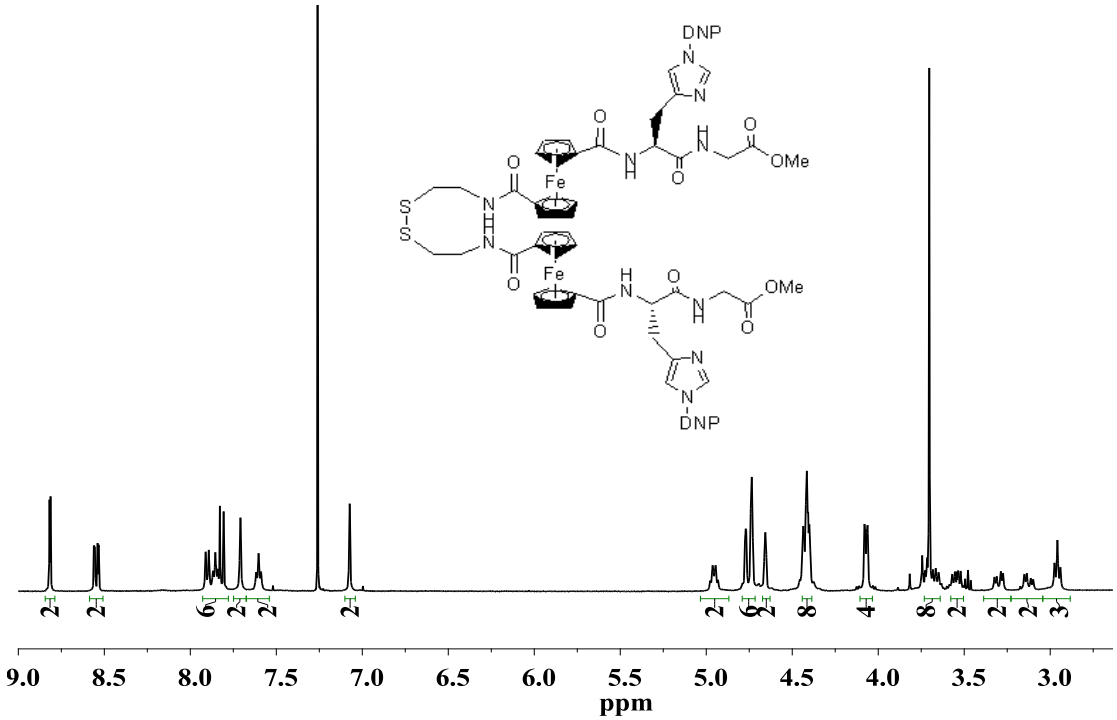


Figure S1. ^{1}H NMR spectrum of **FcSSHG** in $CDCl_3$.

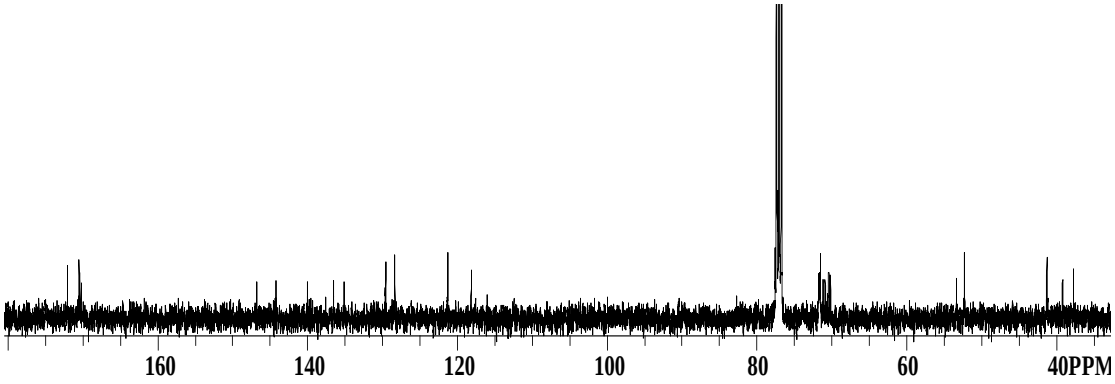


Figure S2. ^{13}C NMR spectrum of **FcSSHG** in $CDCl_3$.

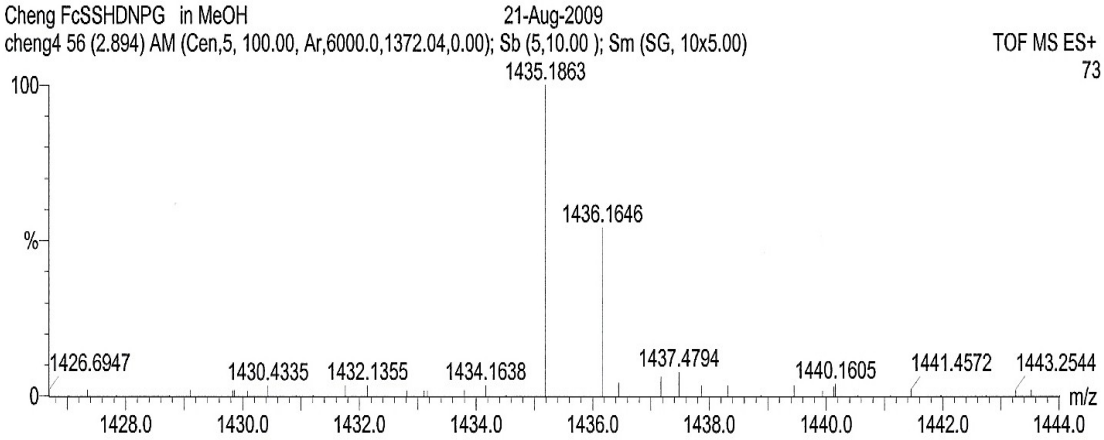


Figure S3. High resolution mass spectrum for the molecular ion cluster of **FcSSHG**.

Preparation of SAM-Fc, SAM2-Fc, SAM3-Fc and Corresponding Zn-AM, Zn-AM2 and Zn-AM3

The SAM-Fc film was prepared by immersing a gold substrate/electrode in 1mM FcSSHG alcoholic solution for 72 h at 4 ºC. 1 mM ethanethiol alcoholic solution was prepared for the following operations. The SAM2-Fc film was prepared by immersing a gold electrode in the mixed solution of 1mM FcSSHG and 1mM ethanethiol (V:V=1:1). The SAM3-Fc film was prepared by immersing a gold electrode in the mixed solution of 1mM FcSSHG and 1mM ethanethiol (V:V=1:5). After the SAM-Fc, SAM2-Fc and SAM3-Fc films were obtained, the gold substrate/electrodes were thoroughly rinsed by alcohol and Millipore water, respectively, then immersed into a 10mM aqueous $Zn(ClO_4)_2$ solution over 1 h at 25 ºC to further achieve the corresponding films of Zn-AM, Zn-AM2 and Zn-AM3. The achieved Zn-AM film was rinsed by Millipore water to remove the physical adsorbed Zn^{2+} ions.

Electrochemical Characterization of SAM-Fc, SAM2-Fc, SAM3-Fc and Zn-AM

All the electrochemical experiments were accomplished on a CHI 1660 instrument with a three electrodes system, gold electrode was used as working electrode, SCE. was used as reference electrode, and the Pt wire was used as counter electrode. 0.73 M $NaClO_4$/0.09 M Tris buffer was the electrolyte solution, and the pH value was adapted by conc. HCl according to the experimental demand.

The electrochemical property of SAM-Fc film on gold electrode was studied in 0.73 M $NaClO_4$/0.09 M Tris buffer solution at pH 7.51. In Figure S4, the CV plots of SAM-Fc were displayed ranging from 0.2V to 0.9V at different scan rates (0.03, 0.05, 0.07, 0.1 and 0.15 $V \cdot s^{-1}$). A good linear relationship between the anodic current i_p and the scan rate was displayed in Figure S5. The Zn-AM film was scanned by the same electrochemical method. The comparison of CV plots between SAM-Fc and Zn-AM was exhibited as Figure S6. Sharp reduction of peak current can be observed in the CV plot of Zn-AM.

The CV plots for SAM-Fc, SAM2-Fc and SAM3-Fc are displayed in Figure S7. The film SAM2-Fc incubated in the 1:1 (FcSSHG : Ethanethiol) mixed solution, show much weaker redox peaks than the film SAM-Fc. This is ascribed to lower concentration of FcSSHG on the gold surface resulted from the lower concentration of FcSSHG in the incubating solution. Contrast to the normal regularity, the film SAM3-Fc incubated in the 1:5 (FcSSHG : Ethanethiol) mixed solution exhibited the strongest CV signal instead of the weakest signal, which is ascribed to the formation of a multilayer film. This can be understood that the bigger space among the peptide sequences in monolayer makes the intermolecular H-bond interactions between free FcSSHG molecules and the immobilized monolayer increase, and results in adsorbed multilayer.

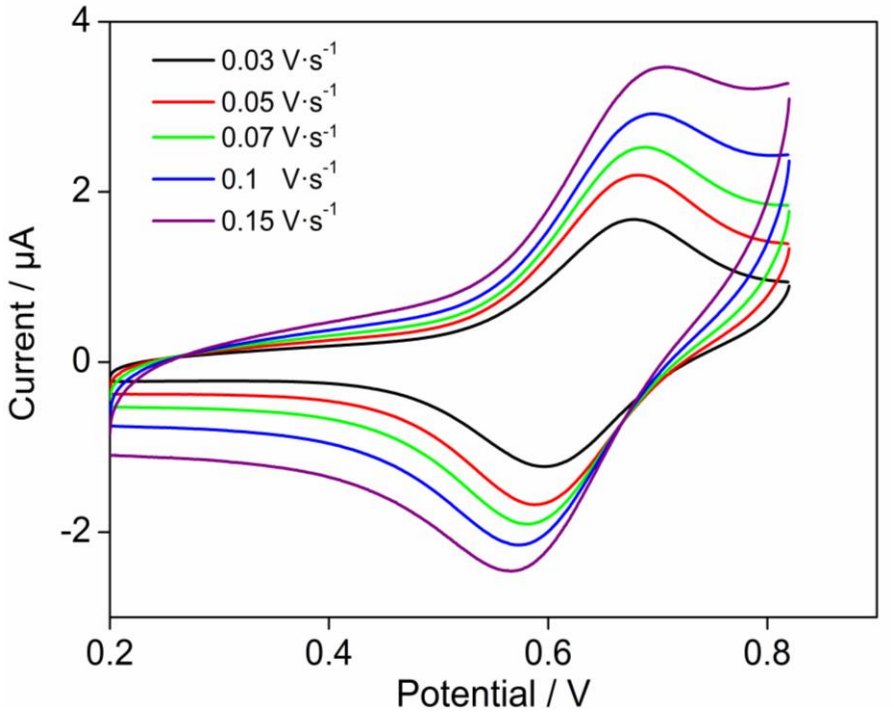


Figure S4. CV plots of SAM-Fc film under different scan rates in 0.73 M $NaClO_4$/0.09 M Tris (pH=7.51).

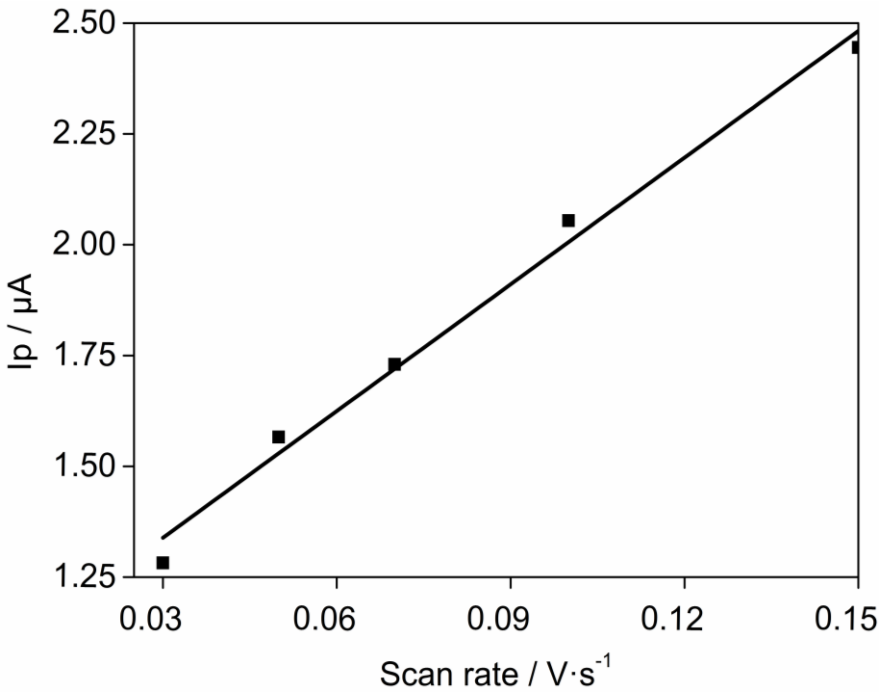


Figure S5. I_p-scan rate plot of SAM-Fc.

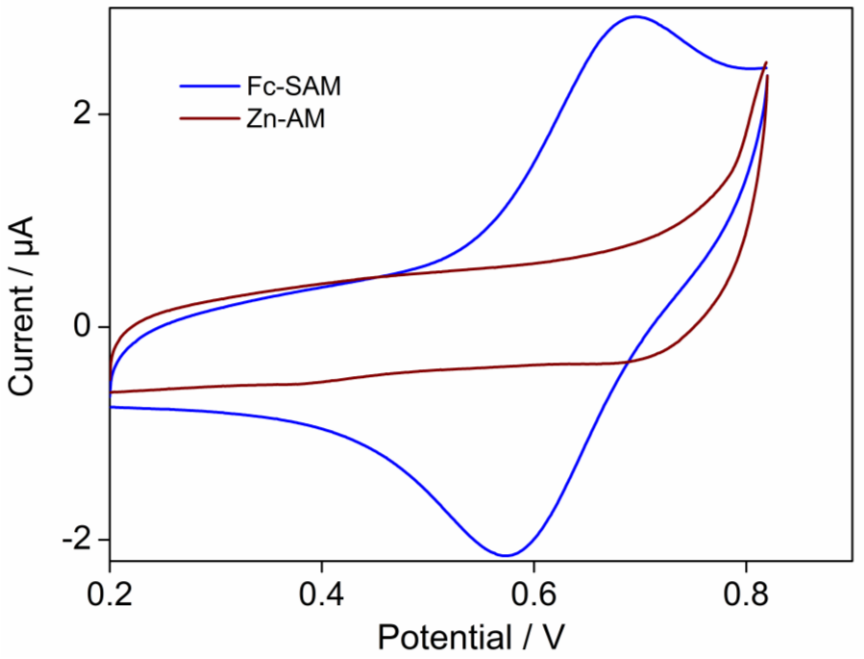


Figure S6. CV plots of SAM-Fc (blue) and Zn-AM (brown) in 0.73 M $NaClO_4$/0.09 M Tris (PH=7.51) under the scan rate 0.1 $V \cdot s^{-1}$.

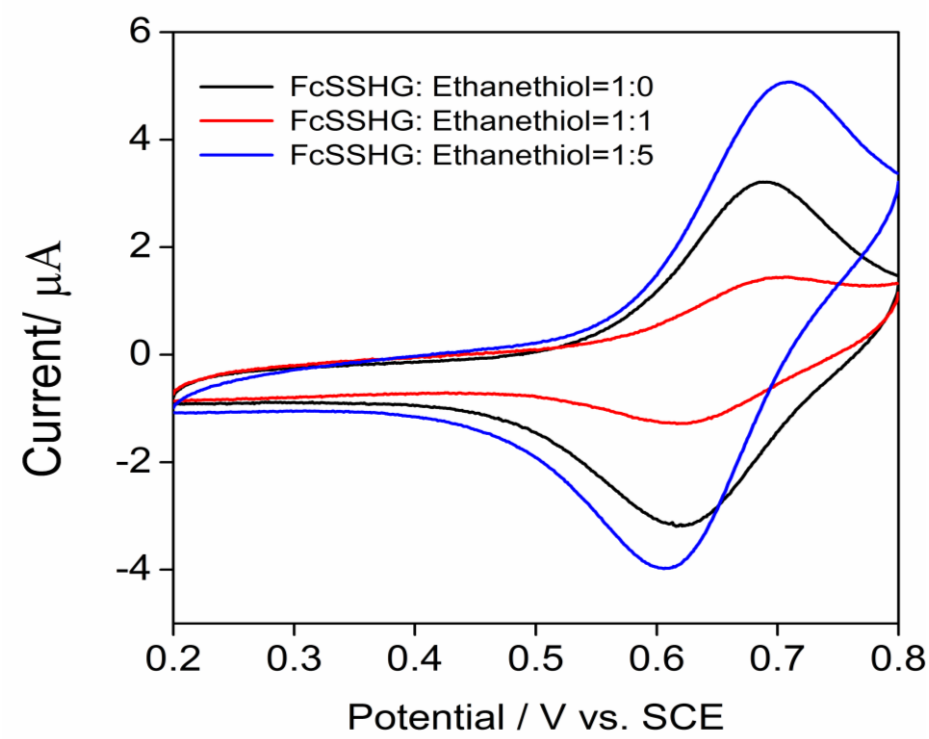


Figure S7. The representative CV plots for SAM-Fc, SAM2-Fc and SAM3-Fc are obtained in 0.73 M $NaClO_4$/0.09 M Tris (PH=7.51) under the scan rate 0.1 $V \cdot s^{-1}$. The film SAM2-Fc incubated in the 1:1 (FcSSHG:Ethanethiol) mixed solution and the film SAM3-Fc incubated in the 1:5 (FcSSHG:Ethanethiol) mixed solution.

XPS studies of SAM-Fc and Zn-AM

The immobilized film of SAM-Fc and its Zn^{2+} complex Zn-AM were further examined by XPS

with the photon energy 1486.7 eV. In the N1s spectra of SAM-Fc (Figure 1(b)), the peaks at 399.9 eV and 406.3 eV are assigned to the nitrogen atoms in the amide and nitro groups respectively. The peaks at 398.9 eV and 401.4 eV are assigned to the C=N-C and C-N-C nitrogen of the imidazole group on the side chain of histidine residue. In the N1s spectra of Zn-AM, the assigned N atom for amide, nitro, C=N-C and C-N-C appeared at 400.1, 406.4, 399.0 and 401.8 eV, respectively.

To obtain more coordination information about the active center in the Zn-AM, we intensively investigated the XPS spectra of the Zn-AM and quantitatively analyzed the XPS spectrums of Fe2p and Zn2p for Zn-AM (Figure S8). The areas of the doublets for Fe2p and Zn2p and their corresponding sensitivity factors are utilized in the quantitative analysis. The atomic sensitivity factors for Fe2p and Zn2p are 2.957 and 5.589, respectively. The surface concentration ratio of Fe:Zn is obtained as $I_{Fe}/S_{Fe}:I_{Zn}/S_{Zn}$ = 2.5:1 (I_{Zn} is the total area of the $Zn2p_{3/2}$ and $Zn2p_{1/2}$, I_{Fe} is the total area of the $Fe2p_{3/2}$ and $Fe2p_{1/2}$). Because the atomic ratio of Fe:N_{imi} in FcSSHG is 1:1, the matching ratio between the N_{imi} and the Zn atoms in the active center, would be equal to the surface concentration ratio between Fe and Zn atoms in the Zn-AM if all the N_{imi} atoms involve in the construction of active centers. The obtained ratio is close to the ideal ratio of N_{imi} : Zn at 3:1 in the active center. The minor difference is probably ascribed to the presence of imperfect matching, e.g. N_{imi}:Zn =1:1, or 2:1.

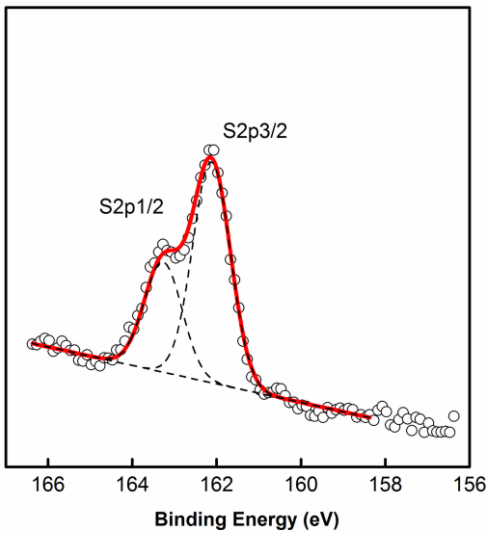


Figure S8a. The detailed S2p spectra of SAM-Fc.

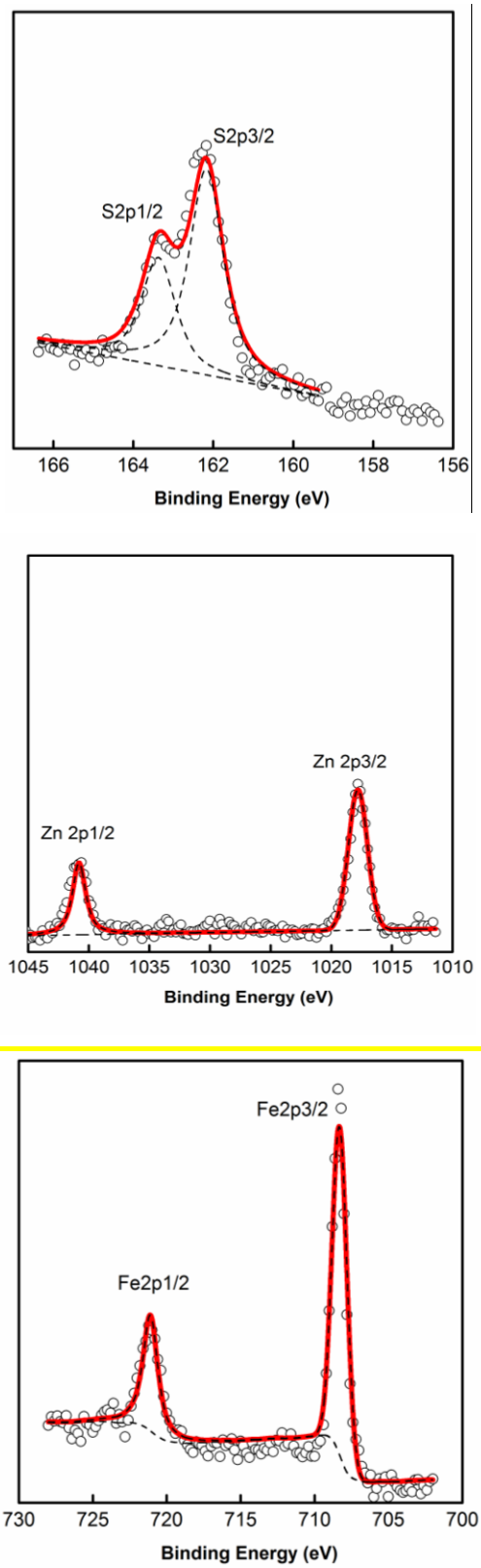


Figure S8b. The detailed S2p/Zn2p/Fe2p spectra of Zn-AM.

The *p*-NPA Assay on the Activity of Zn-AM, Zn-AM2 and Zn-AM3

The same three electrodes system and solution was used, and the same potential applied in the *i*-t experiments was also employed during the period of *p*-NPA assay. The p-NPA solutions with different concentration were prepared in acetonitrile to prevent self-hydrolysis. A 9% (V/V) *p*-NPA acetonitrile solution was injected into electrochemical vial and the solution was sufficiently mixed by a magnetic stirrer during the experimental process. The absorbance of the hydrolysis product, *p*-NP, at 400 nm was observed using the UV kinetics method on a VRIAN Cary 100 UV-Visible spectrophotometer. Each time, around 1 mL sample was taken out to a cuvette by a syringe, and immediately tested on the UV spectrometry, then given back to the vial again. In order to transfer the absorbance intensity to the quantity of *p*-NP, the calibration plot of *p*-NP was worked out as displayed in Figure S9, and the fitting equation is y=11.71536x. As contrast, another system without Zn-AM, which displayed the self hydrolysis situation of *p*-NPA, was performed exactly as that with Zn-AM. The absorbance intensity subtracting the effect of self-hydrolysis can give the quantity of *p*-NP produced by Zn-AM catalysis according to the calibration equation. Next, the catalytic rate υ can be described as the productive rate of *p*-NP in unit catalytic time on unit Zn-AM area.

The catalytic activities of films Zn-AM, Zn-AM2 and Zn-AM3 on the *p*-NPA hydrolysis are studied, respectively, using the same method described in manuscript under the optimum condition. The UV kinetics plots of *p*-NPA hydrolysis are directly shown as Figure S12. The film of Zn-AM displayed outstanding catalysis activity on *p*-NPA hydrolysis, while neither of Zn-AM2 and Zn-AM3 exhibited obvious catalytic activity. These suggest that the peptide sequences environment in the monolayer strongly effect the formation of active center.

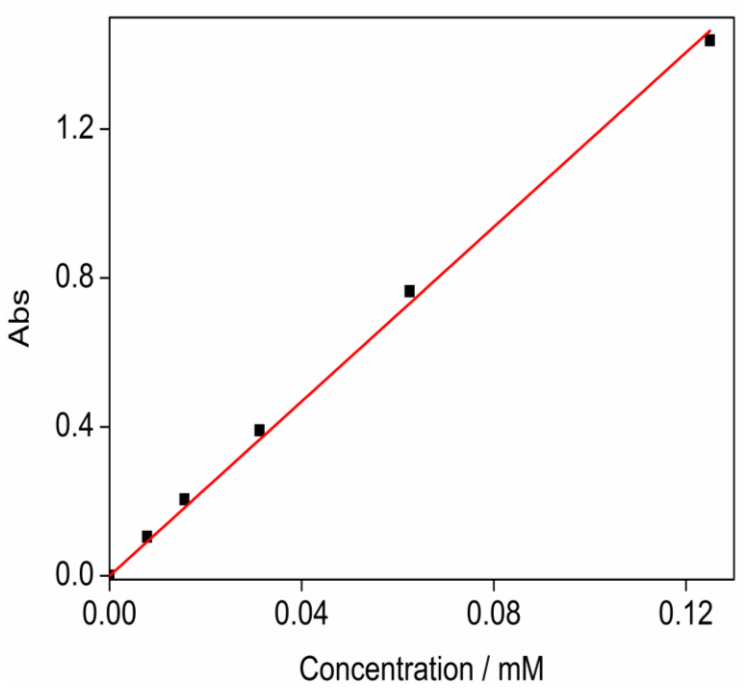


Figure *S9*. Calibration plot of *p*-NP in 0.73 M $NaClO_4$/0.09 M Tris buffer solution (pH=7.51) in presence of 9% (V/V) CH_3CN. The black spots represented the absorbance of *p*-NP at 400 nm recorded at 0, 0.0078, 0.0156, 0.0313, 0.0625 and 0.125 mM, respectively. The red trace was the fitting line, corresponding to the equation y=11.71536x.

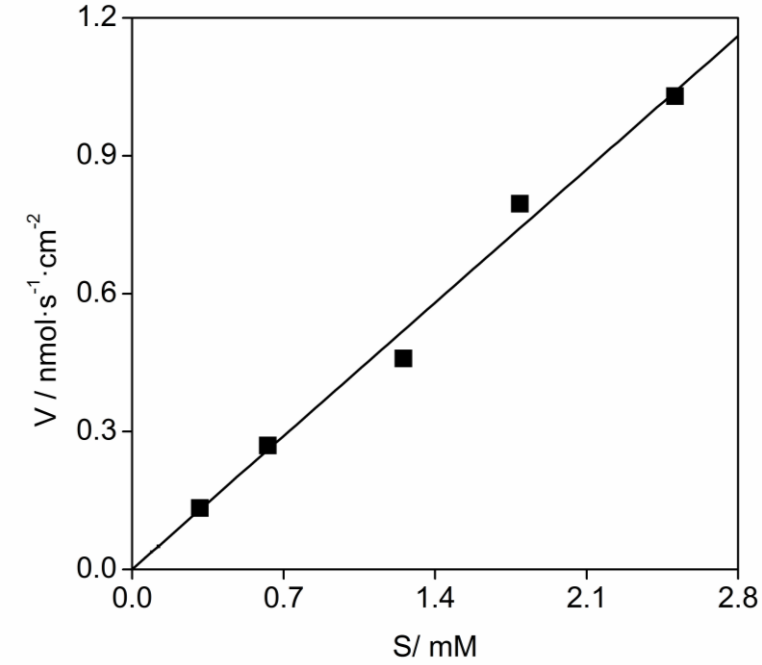


Figure S10. Effect of the *p*-NPA concentration on the activity of Zn-AM. The horizontal axis represents the original *p*-NPA concentration, and the vertical axis represents the catalytic rate over 180 s catalysis.

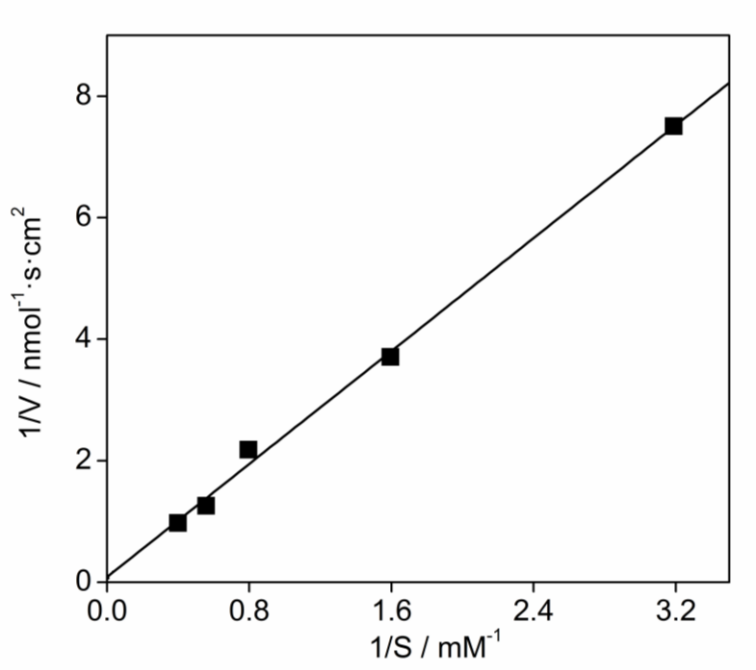


Figure S11. The Lineweaver-Burk plot of Zn-AM in *p*-NPA assay. 1/V represents the reciprocal of catalytic rate V, while 1/S represents the reciprocal of the *p*-NPA concentration. The fitting equation is (1/V) = 0.08554 + 2.32426 (1/S).

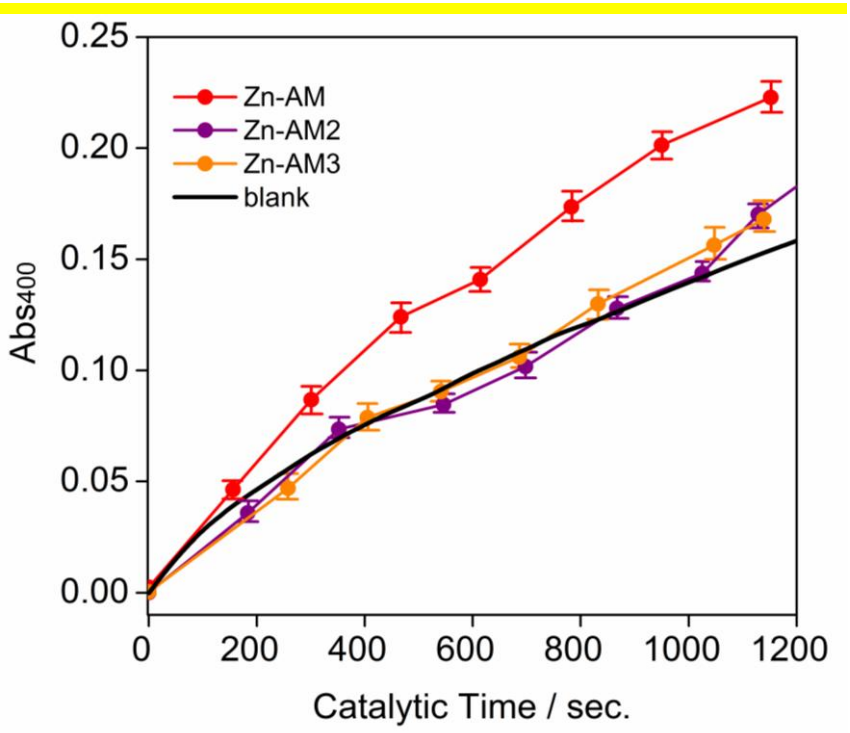


Figure S12. The UV kinetics plots of *p*-NPA hydrolysis at the presence of Zn-AM, Zn-AM2 and Zn-AM3, observed through the absorbance at 400 nm in 0.73 M $NaClO_4$/0.09 M Tris (pH =7.51) containing 9% (V/V) acetonitrile, 25 ºC. The orange line represents the blank affected by the self-hydrolysis of *p*-NPA.

Evaluation of the Catalytic Parameters

The surface concentration of Fc, Γ_{Fc}, was determined as 6.92×10^{-11} mol·cm^{-2} by eq. S1. *Q* is the charge associated with the Fc redox peaks, obtained by integrating the area under the anodic peak of the CV for SAM-Fc. The 'n' is the electron number, for the redox couple Fc/Fc^{+}, n=1. *F* is the faraday constant. 'A' is the area of gold electrode.

In eq. S2 and eq.S3, υ is the generated rate of *p*-NP catalyzed by Zn-AM, υ'_{max} is the maximum apparent rate when substrate concentration is infinite, [*S*] is the concentration of *p*-NPA, $[E]_T$ is the total enzyme concentration, supposed as one third of Γ_{Fc} (eq.S5). K'_m is the apparent Michaelis constant. The values of K'_m and υ'_{max} were calculated out according to the fitting equation (1/V) = 0.08554 + 2.32426 (1/S).

Consequently, the catalytic rate constant κ_{cat}, was obtained according to eq. S4.

$$\Gamma_{Fc} = \frac{Q}{nFA} \qquad \text{(eq. S1)}$$

$$\upsilon = \frac{\upsilon'_{max}[S]}{K'_m + [S]} \qquad \text{(eq. S2)}$$

$$\frac{1}{\upsilon} = \frac{1}{\upsilon'_{max}} + \frac{K'_m}{\upsilon'_{max}} \cdot \frac{1}{[S]} \qquad \text{(eq. S3)}$$

$$k_{cat} = \upsilon'_{max}/[E]_T \qquad \text{(eq. S4)}$$

$$[E]_T = \Gamma_{Fc}/3 \qquad \text{(eq. S5)}$$