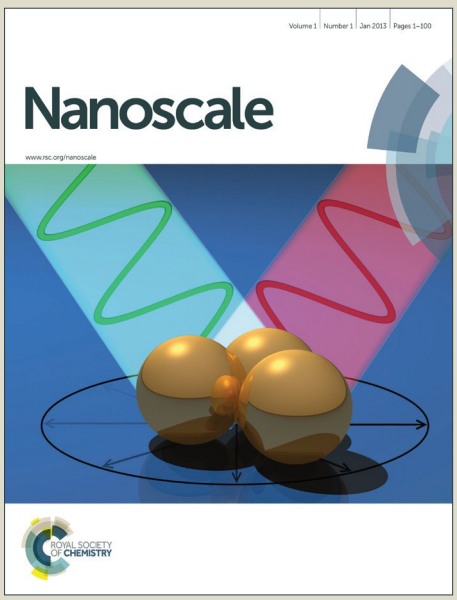
Nanoscale

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/nanoscale

Co-immobilization of multiple enzymes by metal coordinated nucleotide hydrogel nanofibers: improved stability and an enzyme cascade for glucose detection

Hao Liang,*[a] Shuhui Jiang,[a] Qipeng Yuan,[a] Guofeng Li,[a] Feng Wang,[b] Zijie Zhang,[b] and Juewen Liu*[b]

a. State key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing, P.R. China.
E-mail: lianghao@mail.buct.edu.cn

b. Department of Chemistry and Waterloo, Institute for Nanotechnology, University of Waterloo, Waterloo, Canada.
E-mail: liujw@uwaterloo.ca

Fax: +1 519 7460435; Tel: +1 519 8884567 ext. 38919

Abstract

Preserving enzyme activity and promoting synergistic activity via co-localization of multiple enzymes are key topics in bionanotechnology, materials science, and analytical chemistry. This study reports a facile method for co-immobilizing multiple enzymes in metal coordinated hydrogel nanofibers. Specifically, four types of protein enzymes, including glucose oxidase, *Candida rugosa* lipase, α-amylase, and horseradish peroxidase were respectively encapsulated in a gel nanofiber made of Zn^{2+} and adenosine monophosphate (AMP) with a simple mixing step. Most enzymes achieved quantitatively loading and retained full activity. At the same time, the entrapped enzymes were more stable against temperature variation (by 7.5 °C), protease attack, extreme pH (by 2-fold), and organic solvents. After storage for 15 days, the entrapped enzyme still retained 70% activity while the free enzyme nearly completely lost activity. Compared to nanoparticles formed with AMP and lanthanide ions, the nanofiber gels allowed much higher enzyme activity. Finally, a highly sensitive and selective biosensor for glucose was prepared using the gel nanofiber to co-immobilize glucose oxidase and horseradish peroxidase for an enzyme cascade system. A detection limit of 0.3 μM glucose with excellent selectivity was achieved. This work indicates that metal coordinated materials using nucleotides are highly useful for interfacing with biomolecules.

Introduction

Enzymes are powerful biocatalysts, playing important roles in industrial processes, biosensors, and biofuel cells. A key requirement for these applications is good enzyme stability.[1-3] Inspired by cellular processes, in which multiple enzymes often work together, enzymatic cascade reactions have been developed in vitro.[4] Co-immobilization of multiple enzymes could enhance the overall reaction efficiency and specificity, and omit the isolation of reaction intermediates. Recently, co-immobilized enzymes have been demonstrated on a few substrates,[5,6] such as sol–gel derived materials,[7,8] silica particles,[9,10] polymers,[11] DNA,[12,13,14] and proteins.[15,16] We reason that ideal immobilization materials should be cost-effective, trap enzymes under mild conditions, allow easy substrate accessibility, prevent enzyme leaching, and protect the enzymes. Most of the above materials, however, do not satisfy all these requirements.

Coordination polymers (CPs) are organic-inorganic hybrid materials formed by metal ion connectors and organic bridging ligands. Many biomolecules have multiple metal binding sites and offer excellent biocompatibility as a CP component.[17] Nucleotides,[18-20] amino acids,[21,22] peptides,[23,24] and proteins[6,25,26] have all been used to construct CPs. We are interested in using DNA and nucleotides for metal coordination.[27-29] Metal coordination is a mild reaction that does not involve toxic radicals, organic solvents, or other extreme conditions, rendering it attractive for enzyme entrapment. In most cases, nanoparticles are formed by mixing nucleotides and metal ions. However, when encapsulated in nanoparticles, enzymes might have poor substrate accessibility due to the rigid and less porous nanoparticle structures. On the other hand, we reason that hydrogels might be more suitable for enzyme encapsulation due to easy substrate accessibility. Hydrogels are crosslinked porous polymer networks retaining a large amount of water (often >90% water).

Recently, we reported a hydrogel nanofiber self-assembled in water using adenosine monophosphate (AMP) and Zn^{2+}.[30] It is interesting to note that other nucleotides such as GMP and CMP formed nanoparticles instead of hydrogels under the same condition. This gel has a prominent molecular encapsulation property, and it can form reversibly. The nanoscale fiber structures are ideal for substrate diffusion. Herein, we explore this material for immobilizing multiple enzymes and related analytical applications.

Materials and Methods

Chemicals.

Glucose oxidase (GOx) from *Aspergillus*, and horseradish peroxidase (HRP) were purchased from Aladdin. *Candida rugosa* lipase (CRL) was purchased from Sigma-Aldrich, and α-amylase was from Beijing Aoboxing Bio-Tech Co., Ltd. Adenosine 5′-monophosphate (AMP) disodium salt, N-2-hydroxyethyl piperazine-N'-2-ethyl sulfonic acid (HEPES), fluorescein 5(6)- isothiocyanate (FITC), rhodamine B isothiocyanate (RhB), 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and *p*-nitrophenylpalmitate (*p*-NPP) were purchased from Aladdin. Zinc chloride, ethanol, and isopropanol were from Beijing Chemical Works. Gadolinium chloride hexahydrate, europium chloride hexahydrate, and samarium chloride hexahydrate were purchased from Sigma-Aldrich. Glucose, xylose, fructose, mannose, galactose, and bovine serum albumin (BSA) were from Sinopharm Chemical Reagent Co., Ltd. Milli-Q water was used to prepare all the buffers and solution. All other reagents and solvents were of analytical grade and used as received.

Enzyme immobilization.

The aqueous solution of GOx, HRP, CRL and α-amylase (1mg/mL) were separately prepared and stored at 4 °C. Immobilization of the enzymes within the Zn^{2+}/AMP hydrogel was performed by firstly mixing 200 μL of 25 mM AMP dissolved in HEPES (10 mM, pH 7.4), 600 μL of HEPES buffer (10 mM, pH 7.4), and 100 μL of enzymes. Then, 100 μL of $ZnCl_2$ (50 mM) in water was quickly added and mixed. After 1 h, the immobilized enzymes were collected by centrifuging at 8,000 rpm for 10 min. The amounts of protein incorporated into the Zn^{2+}/AMP complexes were measured by the Bradford assay from the absorption intensities of the supernatant solutions. The absorption of the untreated proteins in HEPES buffer was used as reference.

Activity assay of immobilized enzymes.

For the GOx activity assay, 1 mL of glucose (45 mM) solution and 180 μL of ABTS (0.4 mM) were mixed with 200 μL of free GOx (2 μg/mL) or 200 μL of the suspension of the immobilized GOx (containing 2 μg/mL GOx), and 1.8 mL of pH 7.4 HEPES buffer. Then, 200 μL of HRP (10 μg/mL) were added. The mixed samples were incubated at room

temperature for 5 min. The reaction was monitored with a UV/vis spectrometer at 415 nm.

For the HRP activity assay, 200 μL of free HRP (50 μg/mL) or 200μL of the suspension of immobilized HRP (containing 50 μg/mL HRP) was added into 1.9 mL of pH 7.4 HEPES buffer containing 0.08 mM ABTS. Then, 1.9 mL of H_2O_2 (0.4 mM) was added, and the sample was incubated at room temperature for 5 min. The reaction was also monitored a with UV/vis spectrometer at 415 nm. Note that the free and encapsulated enzymes were at the same concentration during enzyme activity assays.

The activity assay of CRL used *p*-NPP as the substrate. First, 1 mL p-NPP (500 μg/mL) was added into 2 mL phosphate buffer solution (50 mM, pH 7.4), then 100 μL of free CRL (100 μg/mL) or 100 μL of the suspension of immobilized CRL (containing 100 μg/mL CRL) was added. The mixed samples were incubated at 37 °C for 5 min. The reaction was monitored with a UV/vis spectrometer at 410 nm.

The activities of both the free and the immobilized α-amylase preparations were determined by measuring the amount of reducing ends formed after enzymatic hydrolysis of starch in the medium according to a reported method.[31]

Co-immobilization of GOx and HRP.

Co-immobilization of enzymes within the Zn^{2+}/AMP hydrogel was performed by mixing 200 μL of 25 mM AMP in HEPES (10 mM, pH 7.4), 600 μL of buffer. Then, 50 μL of GOx and HRP (2 mg/mL each) were added by vortex mixing. At last, 100 μL of $ZnCl_2$ (50 mM) was quickly added and mixed. After 1 h, the immobilized enzymes were collected by centrifuging at 8,000 rpm for 10 min. In co-immobilization, the total immobilized protein ratio (i.e. percentage of immobilized protein) was measured by the Bradford assay, while immobilized HRP was determined by measuring the absorbance at 403 nm.

Enzyme stability test.

For stability test at different pH, the suspension of GOx&HRP@Zn/AMP nanofibers was added into 1 mL of various pH solutions for 4h. Then the residual overall enzymatic activity was measured by recording the absorbance at 415 nm. To test stability at different temperatures, the suspensions of the GOx&HRP@Zn/AMP nanofibers in HEPES buffer (10 mM, pH7.4) were incubated at 50-90 °C for 30 min; or at 55, 60 and 65 °C for 80 min with 10 min intervals for free and co-immobilized enzymes. The half-life ($t_{1/2}$) of the enzyme with

k_d as the decay constant was calculated as $t_{1/2} = \ln 2/k_d$. To test stability against protease degradation, the suspension of the nanofibers in HEPES buffer (10 mM, pH7.4) was incubated with 1 mg/mL of trypsin at 37 °C for 2 h. For solvent stability, the suspensions of the nanofibers in HEPES buffer (10 mM, pH 7.4) were incubated with 50 wt% ethanol or isopropanol at room temperature for 2h. To test its long-term stability, the nanofibers were stored in HEPES buffer (10 mM, pH 7.4) at room temperature. For all these assays, free GOx and HRP enzymes at the same protein concentrations were also treated and analyzed using the same procedure.

Detection of glucose.

Different concentrations of glucose were added into 1.25 mL of 10 mM HEPES buffer (pH 7.4) containing 500 μL of the suspension of GOx&HRP@Zn/AMP nanofibers (containing 84 μg/mL GOx and 75 μg/mL HRP) and 1.6 mM ABTS. The samples were then incubated at room temperature for 10 min. The reaction solution was centrifuged at 8,000 rpm for 10 min, and the absorbance of the supernatants at 415 nm was measured by a UV-1100 spectrophotometer. The selectivity was determined by the absorbance of the supernatants using 100 μM glucose as the substrate, compared to that with 1.0 mM of xylose, fructose, mannose, or galactose, or 1 mg/mL of BSA.

Results and Discussion

Immobilization of single enzymes. Zn^{2+} and AMP self-assemble to form a hydrogel nanofiber upon simple mixing.[30] This property is unique to Zn^{2+} since other divalent or trivalent metals either have no reaction or produce rigid nanoparticle precipitants.[18,30] Similarly, replacing AMP by other nucleotides also failed to produce hydrogel. We reason that hydrogels are ideal for enzyme function to allow good substrate accessibility. To test the enzyme immobilization property of the Zn^{2+}/AMP gels, we employed four types of common enzymes as guest molecules, including glucose oxidase (GOx, pI=4.2), *Candida rugosa* lipase (CRL, pI=5.0), α-amylase (pI=5.2), and horseradish peroxidase (HRP, pI=9.0). These enzymes (100 μg/mL) were respectively mixed with AMP and $ZnCl_2$ (5 mM each), and gels were formed in each case. The amount of incorporated proteins by the Zn^{2+}/AMP gels was calculated from the remaining proteins in the supernatant solutions using the Bradford assay

(Figure 1a). In the inset of Figure 1b, the picture on the left is dispersed gel nanofibers encapsulating rhodamine B (RhB)-labeled HRP before centrifugation. After centrifugation, most of the proteins are in the bottom gel phase. At neutral pH, GOx, CRL and α-amylase are negatively charged, and they all showed nearly 100% loading efficiency. On the other hand, the cationic HRP had a lower efficiency of ~55%. To increase HRP immobilization, we next studied the effect of pH (Figure 1b). The loading improved when pH was higher than the pI of HRP, and at pH 10.5 more than 80% HRP was in the gel (Figure 1b). Therefore, a high loading efficiency is favored with negative charged proteins. The zeta-potential of the Zn^{2+}/AMP gel is close to zero, and the net electrostatic interaction might be low. We reason that the negatively charged enzymes interact with Zn^{2+} in the CP. Recently, Zare and co-workers reported Cu^{2+}-mediated enzyme immobilization in a phosphate buffer,[25] and similar metal/protein interactions may take place here. Overall, this CP is very efficient in immobilizing a broad range of enzymes.

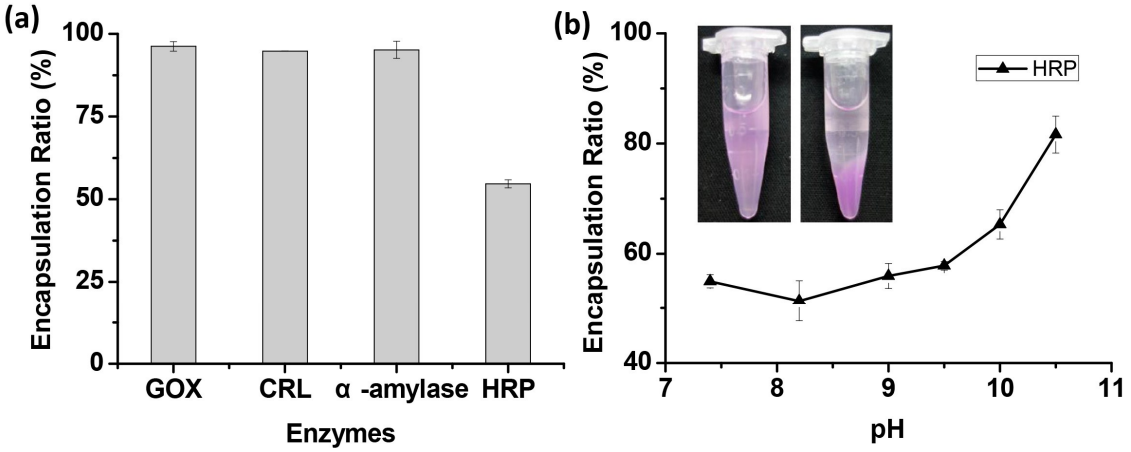


Figure 1. (a) Ratio of encapsulated GOx, CRL, α-amylase, and HRP by the Zn^{2+}/AMP gel nanofiber in a pH 7.4 HEPES buffer. (b) Encapsulation ratio of HRP by the gel in buffers of different pH (from pH 7.4 to 8.2 using HEPES; and from pH 9 to 10.5 using NaOH). Inset: photographs of entrapping RhB-labeled HRP by the Zn^{2+}/AMP gel before (left) and after (right) centrifugation.

To further understand the immobilization mechanism, IR spectra were collected for free AMP, and the Zn^{2+}/AMP hydrogel with or without GOx (Figure S1 and Table S1, ESI). The

phosphate and the C-N stretching vibrations of AMP shifted to higher wavenumbers after coordination with Zn^{2+}, suggesting that both the phosphate and the adenine base in AMP are involved in metal coordination. For the Zn^{2+}/AMP hydrogel with GOx, two IR absorption bands centered at 1660 and 1544 cm^{-1} were observed, attributable to the typical amide I and II absorption bands in protein, respectively.[32-34] This also confirms that GOx was successfully encapsulated into the Zn^{2+}/AMP gel. We next measured the immobilization capacity of the Zn^{2+}/AMP gel by using GOx as the guest protein. Various concentrations of GOx were added, and the maximum immobilization capacity in Zn^{2+}/AMP gel (5 mM) reached ~0.25 mg GOx (Figure S2), which is ~12% of the gel matrix weight.

To characterize the morphology of the gels, we performed transmission electron microscopy (TEM, Figure 2a). The composite containing the enzyme displayed the same morphology as the pure Zn^{2+}/AMP complexes (inset), and the fibril structure was retained. In addition, the dried hydrogels, with or without immobilized enzymes, were amorphous from the X-ray diffraction analysis (Figure S3). For comparison, we also mixed AMP with a lanthanide, Eu^{3+}. Instead of hydrogel, this sample produced aggregated nanoparticles (Figure 2b).

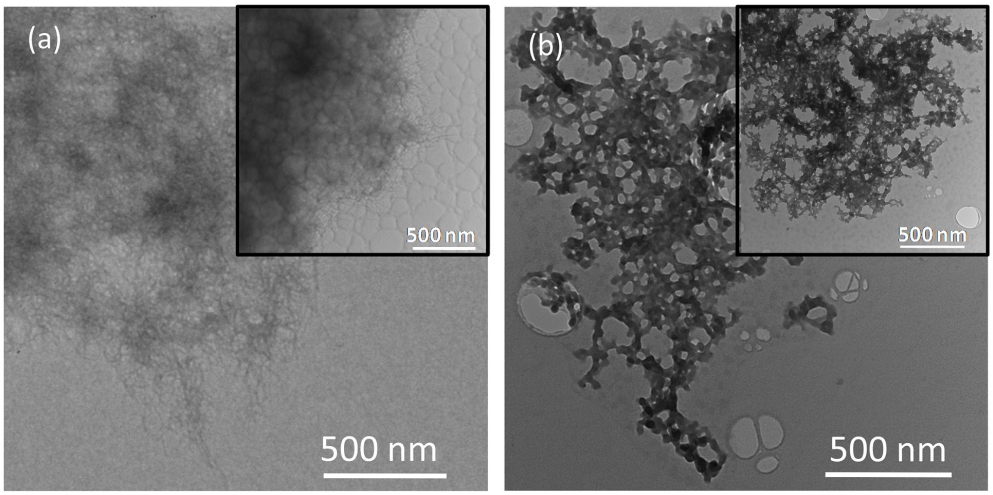


Figure 2. TEM micrographs of (a) the Zn^{2+}/AMP hydrogel with entrapped GOx and without GOx (inset); and (b) the Eu^{3+}/AMP nanoparticles with entrapped GOx and without GOx (inset).

Enzyme activity. Immobilized enzymes usually exhibit lower activity compared with the free ones, mainly due to damages during immobilization, and mass transfer limitations inside solid supports.[25, 35,36] In our system, the activities of GOx, HRP, and α-amylase are almost the same after encapsulation in the gel compared to those of the free enzymes (Figure 3). This is attributable to the high porosity of the gel nanofibers allowing both short diffusion distance and high diffusion coefficient inside the gel. The mild polymerization condition and the overall neutral charge of the gel matrix also help retain the native structure and activity of the enzymes. The slightly decreased hydrolytic activity of the lipase might be due to the retarded diffusion of the hydrophobic substrate into the gel. For comparison, lipase immobilized on Sepharose 6B retained just 50% activity compared to that of the free enzyme, and pullulanase immobilized on activated agar gels was only about 30% of its initial activity.[37, 38] Therefore, our gels are excellent matrix due to its simplicity and nanoscale structures.

To further evaluate the immobilized enzymes, the kinetics of the free and immobilized GOx and HRP were studied. Compared with the free enzymes, the immobilized enzymes have similar k_{cat} and K_m values (differ within 30%, see Table S2). Therefore, such immobilization does not change the enzyme performance, further supporting the data in Figure 3.

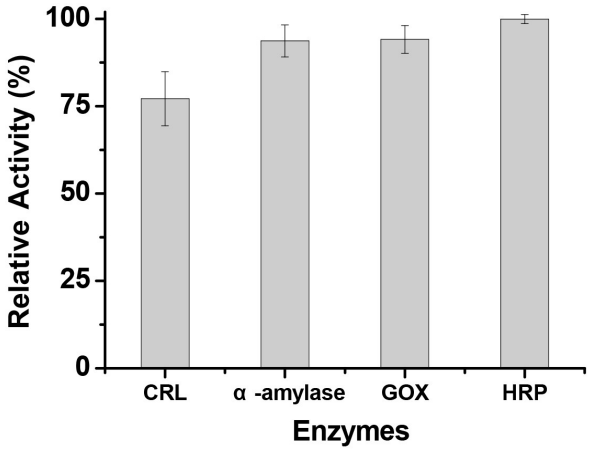


Figure 3. Relative activity of the four enzymes in the Zn^{2+}/AMP hydrogel compared to that of the free enzymes in a pH 7.4 HEPES buffer. The same concentration of the free enzyme and encapsulated enzyme was used for each assay.

Immobilization of multiple enzymes. After establishing the feasibility of using the Zn^{2+}/AMP gel for single enzyme immobilization, co-immobilization of multiple enzymes was further performed. Based on the above studies, GOx and HRP were chosen for a cascade reaction. Co-immobilization was first tested at different pH conditions (pH 7.4, 9.0, and 10.0). The total immobilized proteins was measured by the Bradford assay, while the immobilized HRP was determined by its absorbance at 403 nm.[26] As shown in Figure 4a, higher protein immobilization was achieved at higher pH, consistent with the single enzyme results. The catalytic activities of the co-localized GOx and HRP in the Zn^{2+}/AMP gel were evaluated by reacting with glucose using ABTS as a chromogenic substrate, and they were compared with the concentration of free GOx and HRP (Figure 4b). In the co-immobilization system, the reaction intermediates could rapidly reach the next active site,[39,5] so that the overall activity was improved. Other methods, such as dual-functionalized sequential colocalization,[39] and metal-organic framework nanocrystals,[40] were also used to co-localize GOx and HRP, enhancing the overall product conversion by two-fold compared to the controls. At pH 9 and 10, however, we did not observe much enhancement. This is attributed to the denaturation of GOx at the basic environment.[41] The activity loss of GOx was confirmed by testing the activity of free and singly immobilized GOx (Figure S4). A cartoon showing immobilization of the two enzymes is shown in Figure 4e, and this composite is named GOx&HRP@Zn/AMP nanofibers.

It was previously reported that lanthanide ions can form nanoparticles with AMP.[17] To highlight the advantage of our nanogels, we also co-immobilized GOx and HRP by mixing AMP with Gd^{3+}, Eu^{3+}, and Sm^{3+}. As shown in Figure 4c, the Zn^{2+}/AMP gel exhibited the best encapsulation property for both enzymes, while the lanthanide/AMP nanoparticles only encapsulated GOx efficiently. Furthermore, the catalytic activities of the co-localized GOx and HRP in the lanthanide/AMP nanoparticles were only ~30% of the initial activity of the equivalent free enzymes (Figure 4d). This comparison strengthens the importance of using nanogels instead of nanoparticles for enzyme entrapment.

To further characterize this material in the solution phase, confocal laser scanning microscopy (CLSM) was used. For this purpose, FITC and RhB were used to label GOx and HRP, respectively. Then, the fluorescent-labeled GOx and HRP were co-immobilized by the

same procedure. Both GOx and HRP were present in each Zn^{2+}/AMP hydrogel and they tended to distribute randomly (Figure 4f, left column, merged picture).

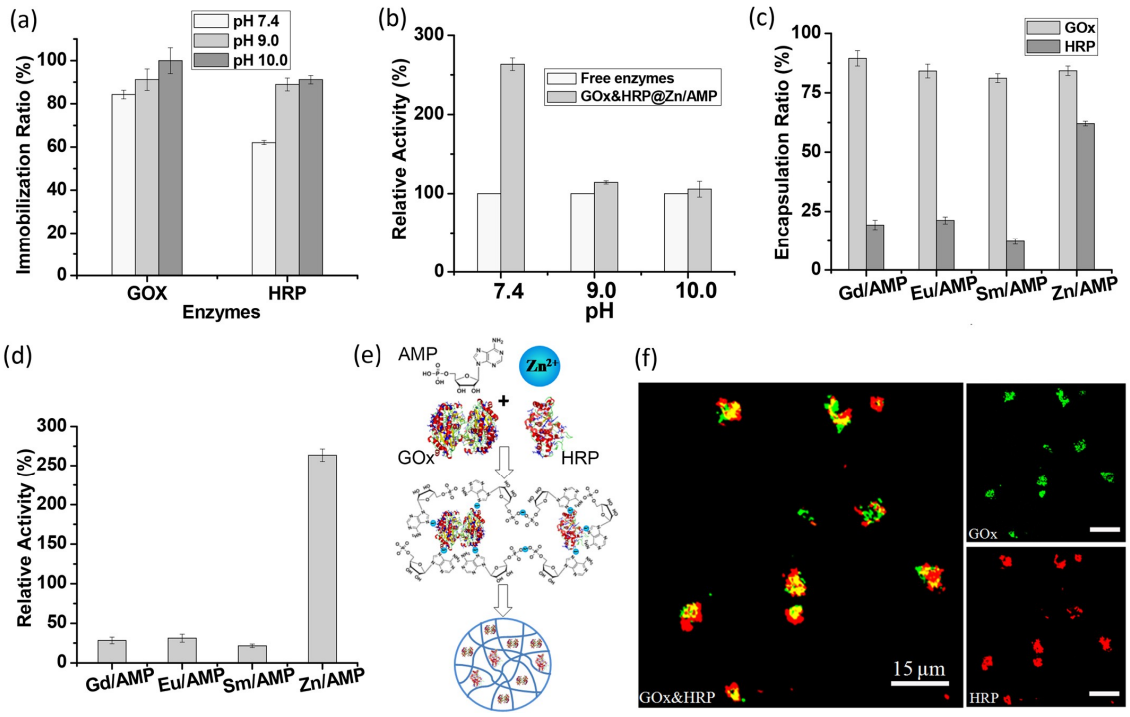


Figure 4. (a) Immobilization ratio of GOx and HRP at different pH. (b) Relative activity of the GOx&HRP@Zn/AMP nanofibers compared to the free enzyme mixtures at different pH. (c) Immobilization ratio of GOx and HRP with the Zn^{2+}/AMP gel or lanthanide/AMP nanoparticles. (d) Relative activity of GOx and HRP co-immobilized by the Zn^{2+}/AMP gel or lanthanide/AMP nanoparticles. (e) A cartoon of enzyme co-immobilization by *in situ* self-assembly of the Zn^{2+}/AMP gel. (f) A CLSM micrograph of the GOx&HRP@Zn/AMP nanofibers (left column). GOx = green, and HRP = red.

Stability test. High enzyme stability is important for applications.[3] Deactivation of enzymes by high temperature and extreme pH are the major reasons for enzyme deactivation. The stability of the GOx&HRP@Zn/AMP nanofibers was examined in at different pH (from 7.4 to 10, Figure 5a) and temperature (from 25 to 90 °C, Figure 5b) and compared to that of the free GOx and HRP in solution. The activity of the GOx&HRP@Zn/AMP nanofibers was

2-fold more stable compared to that of the free enzymes by pH. While the activity of both systems quickly dropped at high temperature, the transition of the immobilized system was 7.5 °C higher. Thus, the Zn^{2+}/AMP nanofibers could protect GOx and HRP from deactivation by heat and base conditions. We also measured the half-life ($t_{1/2}$) of the enzymes by exposing them at elevated temperatures for up to 80 min. The data at 60 °C is shown in Figure S5, where the free enzyme has a $t_{1/2}$ of 38 min, and the immobilized 70 min (Table S3).

Then, the stability of co-immobilized GOx and HRP against protease and organic solvents was also examined. Compared with free GOx and HRP, the activity of GOx&HRP@Zn/AMP nanofibers did not significantly decreased in the presence of trypsin, isopropanol, or ethanol (Figure 5c). Thus, the Zn^{2+}/AMP nanofibers could also protect immobilized enzymes from degradation or deactivation by protease and organic solvents. The long-term storage stability of co-immobilized enzymes is also important for biotechnological application. The GOx&HRP@Zn/AMP nanofibers preserved more than 70% of initial overall activity for 15 days (Figure 5d). The loss of activity in this period might be attributed to protein denaturation and degradation during long-term storage. Under the same condition, however, the free GOx and HRP system lost more than 50% of the overall activity after 5 days. However, the GOx&HRP@Zn/AMP nanofibers can be lyophilized, retaining ~100% activity after storing at 4 °C for 12 days (Figure S6). Taken together, the confinement of the proteins within the Zn^{2+}/AMP nanofibers improved the enzymes' stability against various environmental factors.

Finally, the GOx&HRP@Zn/AMP nanofibers were tested for recycle. The fibers can be easily collected by centrifugation after the reaction, and can be re-dispersed to nanoscale size by vortex mixing. The relative activity decreased by ~20% each to and reached ~20% of the original activity after 8 cycles (Figure S7). This might be due to gradual release of the enzyme from the gel fibers during these operations. Since the enzymes are only physically entrapped, each washing may release a fraction of the enzyme.

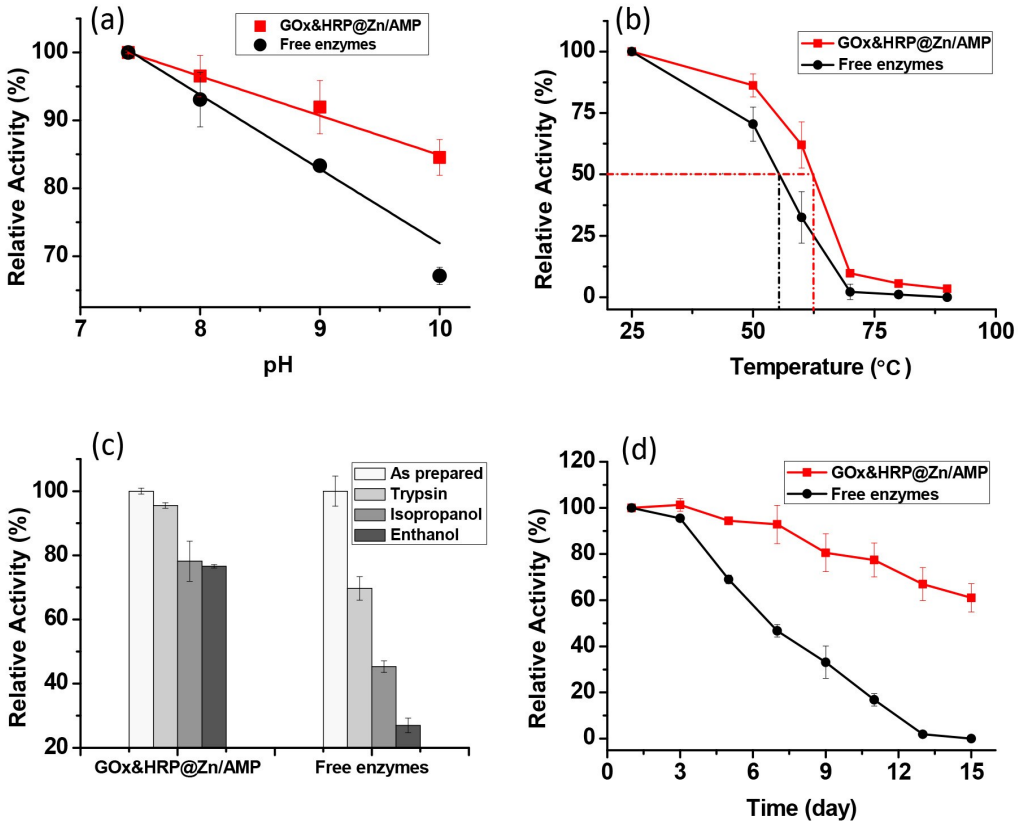


Figure 5. Stability of the GOx&HRP@Zn/AMP nanofibers compared with the equivalent free enzymes (a) at different pH at 25 °C; (b) at different temperatures; and (c) in the presence of 1 mg/mL trypsin or 50 wt% ethanol or isopropanol in pH 7.4 HEPES buffer. (d) Long-term stability of GOx&HRP@Zn/AMP nanofibers compared with the free enzymes at room temperature.

Glucose detection. Given the ease of preparation, high activity, and improved stability, this immobilized composite provides an ideal material for developing biosensors. GOx specifically reacts with glucose to produce H_2O_2, which is a co-substrate for HRP. Next, different concentrations of glucose (0-100 μM) were used to study the response of GOx&HRP@Zn/AMP nanofibers in pH 7.4 HEPES buffer. Figure 6a illustrates a good linearity between the absorbance and the concentration of glucose up to 100 μM (R^2=0.998). The detection limit was calculated to be 0.3 μM glucose based on three times of background standard deviation divided by the slope of the calibration curve. For comparison, the free

enzyme mixture has a smaller slope (~50% of the co-immobilized system), and a detection limit of 0.6 μM glucose. The selectivity of GOx&HRP@Zn/AMP nanofibers was evaluated by monitoring the absorbance at 415 nm in the presence of various competing compounds, including xylose, fructose, mannose, galactose, maltose, and albumin. Although the concentrations of these tested compounds were 10-times higher compared to that of glucose, no visible changes were observed (Figure 6b inset), and the absorbance at 415 nm was much lower. Thus, this composite retains an excellent selectivity towards glucose.[42]

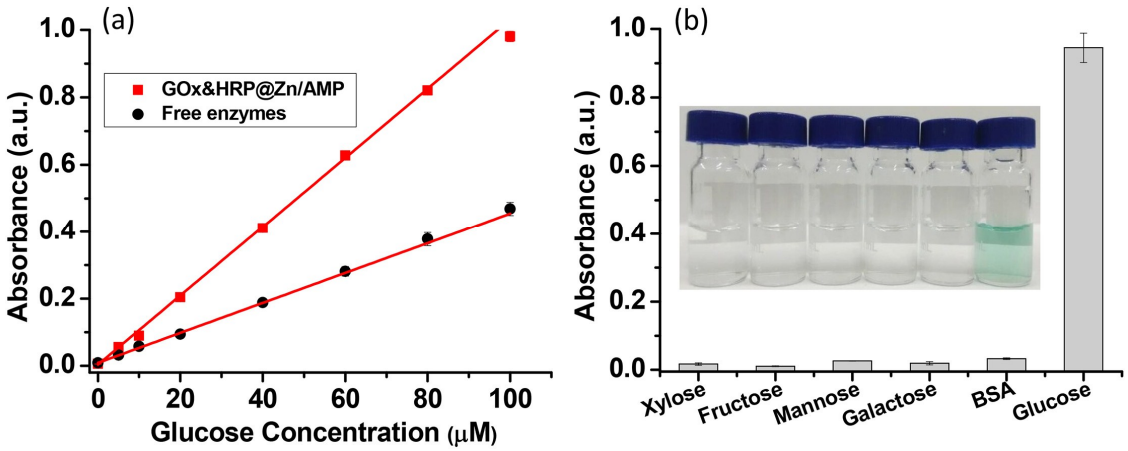


Figure 6. (a) Detection of glucose in solutions with glucose concentrations of 0-100 μM (absorbance at 415 nm was measured after incubation in solution for 10 min at room temperature). Both free enzymes and the co-immobilized enzymes were tested. (b) The response of the GOx&HRP@Zn/AMP nanofibers to 100 μM glucose, or 1.0 mM of xylose, fructose, mannose, or galactose, or 1 mg/mL of BSA.

Conclusions

In summary, we presented a simple, mild, yet highly efficient enzyme immobilization strategy using *in situ* self-assembled Zn^{2+}/AMP gel nanofibers as a supramolecular platform. The binding of proteins to the gel provided a convenient approach for enzyme immobilization and most of enzymes retained high catalytic activities similar to free enzymes. For an enzyme cascade reaction catalyzed by multiple enzymes, the composite enhanced the overall reaction efficiency, since the intermediate can easily reach the next active site for the reaction to

proceed. Moreover, the Zn^{2+}/AMP nanofiber structure made the co-immobilized enzymes with better pH stability, thermal stability, protease and organic solvent tolerance, and long-term stability. Consequently, this facile method will likely find applications in biotechnology, industrial catalysis, biosensing, and biomedical engineering.

Acknowledgement

The authors acknowledge financial support from the Beijing Higher Education Young Elite Teacher Project (YETP0520), the Fundamental Research Funds for the Central Universities (YS1407), the Beijing Natural Science Foundation (2162030), China Scholarship Council, and the Natural Sciences and Engineering Research Council of Canada (NSERC).

Electronic supplementary information (ESI) available. See DOI:

Additional methods, IR and XRD spectroscopy, enzyme loading capacity, enzyme kinetic parameters, and enzyme stability data.

References

1. U. T. Bornscheuer, G. W. Huisman, R. J. Kazlauskas, S. Lutz, J. C. Moore and K. Robins, *Nature*, 2012, **485**, 185-194.
2. R. C. Rodrigues, C. Ortiz, A. Berenguer-Murcia, R. Torres and R. Fernandez-Lafuente, *Chem. Soc. Rev.*, 2013, **42**, 6290-6307.
3. Y. Zhang, J. Ge and Z. Liu, *ACS Catal.*, 2015, **5**, 4503-4513.
4. S. Schoffelen and J. C. M. van Hest, *Curr. Opin. Struct. Biol.*, 2013, **23**, 613-621.
5. S. Schoffelen and J. C. M. van Hest, *Soft Matter*, 2012, **8**, 1736-1746.
6. J. Sun, J. Ge, W. Liu, M. Lan, H. Zhang, P. Wang, Y. Wang and Z. Niu, *Nanoscale*, 2014, **6**, 255-262.

7. A. C. Pierre, *Biocatal. Biotransform.*, 2004, **22**, 145-170.

8. L. Betancor and H. R. Luckarift, *Trends Biotechnol.*, 2008, **26**, 566-572.

9. L. Betancor, C. Berne, H. R. Luckarift and J. C. Spain, *Chem. Commun.*, 2006, 3640-3642.

10. C. Lu, X. Liu, Y. Li, F. Yu, L. Tang, Y. Hu and Y. Ying, *ACS Appl. Mater. Interfaces*, 2015, **7**, 15395-15402.

11. S. M. Kuiper, M. Nallani, D. M. Vriezema, J. J. L. M. Cornelissen, J. C. M. van Hest, R. J. M. Nolte and A. E. Rowan, *Org. Biomol. Chem.*, 2008, **6**, 4315-4318.

12. O. I. Wilner, Y. Weizmann, R. Gill, O. Lioubashevski, R. Freeman and I. Willner, *Nat. Nanotechnol.*, 2009, **4**, 249-254.

13. C. J. Delebecque, A. B. Lindner, P. A. Silver and F. A. Aldaye, *Science*, 2011, **333**, 470-474.

14. Y. Liu, J. Du, M. Yan, M. Y. Lau, J. Hu, H. Han, O. O. Yang, S. Liang, W. Wei, H. Wang, J. Li, X. Zhu, L. Shi, W. Chen, C. Ji and Y. Lu, *Nat. Nanotechnol.*, 2013, **8**, 187-192.

15. J. E. Dueber, G. C. Wu, G. R. Malmirchegini, T. S. Moon, C. J. Petzold, A. V. Ullal, K. L. J. Prather and J. D. Keasling, *Nat. Biotechnol.*, 2009, **27**, 753-759.

16. M. C. Good, J. G. Zalatan and W. A. Lim, *Science*, 2011, **332**, 680-686.

17. Y. Liu and Z. Tang, *Chem.-Eur. J.*, 2012, **18**, 1030-1037.

18. R. Nishiyabu, N. Hashimoto, T. Cho, K. Watanabe, T. Yasunaga, A. Endo, K. Kaneko, T. Niidome, M. Murata, C. Adachi, Y. Katayama, M. Hashizume and N. Kimizuka, *J. Am. Chem. Soc.*, 2009, **131**, 2151-2158.

19. H.-C. Zhou, J. R. Long and O. M. Yaghi, *Chem. Rev.*, 2012, **112**, 673-674.

20. H. Wei, B. Li, Y. Du, S. Dong and E. Wang, *Chem. Mater.*, 2007, **19**, 2987-2993.

21. I. Imaz, M. Rubio-Martínez, W. J. Saletra, D. B. Amabilino and D. Maspoch, *J. Am. Chem. Soc.*, 2009, **131**, 18222-18223.

22. C. Li, K. Deng, Z. Tang and L. Jiang, *J. Am. Chem. Soc.*, 2010, **132**, 8202-8209.

23. A. Mantion, L. Massüger, P. Rabu, C. Palivan, L. B. McCusker and A. Taubert, *J. Am. Chem. Soc.*, 2008, **130**, 2517-2526.

24. M. M. Pires, D. E. Przybyla and J. Chmielewski, *Angew. Chem. Int. Ed.*, 2009, **121**, 7953-7957.

25. J. Ge, J. Lei and R. N. Zare, *Nat. Nanotechnol.*, 2012, **7**, 428-432.

26. Z. Li, Y. Zhang, Y. Su, P. Ouyang, J. Ge and Z. Liu, *Chem. Commun.*, 2014, **50**, 12465-12468.

27. F. Wang, B. Liu, P. J. Huang and J. Liu, *Anal. Chem.*, 2013, **85**, 12144-12151.

28. F. Wang and J. Liu, *Nanoscale*, 2014, **6**, 7079-7084.

29. A. Lopez and J. Liu, *J. Phys. Chem. C*, 2013, **117**, 3653-3661.

30. L. Hao, Z. Zhang, Q. Yuan and J. Liu, *Chem. Commun.*, 2015, 51, 15196-15199.

31. G. Bayramoğlu, M. Yilmaz and M. Y. Arica, *Food Chem.*, 2004, **84**, 591-599.

32. F. Miao, X. Lu, B. Tao, R. Li and P. K. Chu, *Microelectron. Eng.*, 2016, **149**, 153-158.

33. N. Q. Dung, D. Patil, T. T. Duong, H. Jung, D. Kim and S.-G. Yoon, *Sens. Actuators B*, 2012, **166–167**, 103-109.

34. P. Villalba, M. K. Ram, H. Gomez, A. Kumar, V. Bhethanabotla and A. Kumar,

Mater. Sci. Eng. C, 2011, **31**, 1115-1120.

35. J. Kim, J. W. Grate and P. Wang, *Trends Biotechnol.*, 2008, **26**, 639-646.

36. J. Ge, D. Lu, Z. Liu and Z. Liu, *Biochem. Eng. J.*, 2009, **44**, 53-59.

37. C. Mateo, J. M. Palomo, G. Fernandez-Lorente, J. M. Guisan and R. Fernandez-Lafuente, *Enzyme Microb. Technol.*, 2007, **40**, 1451-1463.

38. T. Kuroiwa, H. Shoda, S. Ichikawa, S. Sato and S. Mukataka, *Process Biochem.*, 2005, **40**, 2637-2642.

39. F. Jia, B. Narasimhan and S. K. Mallapragada, *AIChE J.*, 2013, **59**, 355-360.

40. X. Wu, J. Ge, C. Yang, M. Hou and Z. Liu, *Chem. Commun.*, 2015, **51**, 13408-13411.

41. S. B. Bankar, M. V. Bule, R. S. Singhal and L. Ananthanarayan, *Biotechnol. Adv.*, 2009, **27**, 489-501.

42. J. A. Foster, R. M. Parker, A. M. Belenguer, N. Kishi, S. Sutton, C. Abell and J. R. Nitschke, *J. Am. Chem. Soc.*, 2015, **137**, 9722-9729.