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Supporting Information

Thermo-responsive polymer-modified metal-organic frameworks as soft-rigid enzyme-reactors for enhancement of enzymolysis efficiency using a controllable embedding protocol

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Contents

Experimetal section

Chemicals

N-isopropyl acrylamide (NIPAM), glucose oxidase (GOx), *N*-benzoyl-L-arginine-ethyl ester (BAEE) *N*-benzoyl-L-arginine (BA) and 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were supplied by Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Dimethylvinyloxazolinone (VDMA) was gotten from Beijing Institute of Cool Light Fine Chemicals (Beijing, China). UIO-66-NH_2 was bought from Beijing Krre Technology Co., Ltd. (Beijing, China). 1,4-Dioxane, ethyl ether and *N,N*-dimethylformamide (DMF) were obtained from Concord Technology Co., Ltd. (Tianjin, China). Horseradish peroxidase (HRP) was supplied from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Trypsin (TRY) and D-glucose were purchased from Xinjingke Biotechnologies Co., Ltd. (Beijing, China). Glutaminase (Glnase) was brought from MegazymeLtd. D,L-Glutamine (D,L-Gln), D,L-glutamic acid (D,L-Glu) and dansyl chloride (Dns-Cl) were purchased from TCI Chemical Industrial Development Co., Ltd (Shanghai, China). Hydrogen peroxide (H_2O_2, 30.0% w/w), 3,3',5,5'-tetramethylbenzidine (TMB) and andazodiisobutyronitrile (AIBN) were supplied from Beijing Innochem Technology Co., Ltd. (Beijing, China). Cytochrome c (Cyt-c) and fluorescein isothiocyanate isomer (FITC) were bought from Sigma-Aldrich (USA). Acetic acid (HAc) and sodium hydroxide (NaOH) were provided by Beijing Chemical Works (Beijing, China). Tris-(hydroxymethyl)-aminomethane (Tris) was gotten from J&K Scientific Ltd. (Beijing, China). Phosphate buffered saline (PBS) was purchased from ThermoFisher Biochemical Products Co., Ltd. (Beijing, China). Dipeptide L-arginine-L-arginine (L-Arg-L-Arg) was obtained from Nanjing Peptide Biotechnology Co. Ltd. (Nanjing, China). Brilliant Blue G (G-250) was obtained from Alfa Aesar Chemicals Co., Ltd. (Shanghai, China). Phosphoric acid (H_3PO_4), sodium acetate (NaAc) and other chemicals were supplied by Beijing Yili Fine Chemicals Co., Ltd. (Beijing, China). The aqueous solutions were prepared with Milli-Q water (Millipore, Bedford, MA, USA).

Apparatus

The ultraviolet-visible (UV-*vis*) absorption spectra were recorded using a TU-1900 UV-*vis* double-beam spectrometer (Purkinje General, China). The dynamic light scattering (DLS) measurements were carried out with a Zetasizer laser particle analyser (Zetasizer Nano ZS ZEN3600, British). Transmission electron microscopy (TEM) images were obtained using a transmission electron microscope (JEM-2010, Japan electron optics laboratory, Japan) at a voltage of 200 kV. Circular dichroism (CD) spectra were recorded on a Circular dichroism spectrometer (J-1700, JASCO, Japan). The ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR) spectra were recorded on a Bruker Avance III 400 spectrometer (Bruker Corpration, Billerica, MA, USA) using deuterium DMSO as the solvent at room temperature. Confocal Laser Scanning Microscopy (FV1000-IX81, Olympus, Japan) was used to determine the presence of FITC labeled enzymes@MOFVN. Thermogravimetric analyses (TGA) were performed in air with temperature increasing at 10 °C min^{-1} and a temperature range from 40 ^{o}C to 930 ^{o}C using a synchronous thermal analyzer (STA 449 F3 Jupiter, Nestal, Germany). The samples were dried in vacuum at 50 °C for 12 h before TGA analysis. Powder X-ray diffraction (PXRD) patterns were collected on a PANalytical Empyrean diffractometer (Empyrean, PANalytical B.V., Netherlands) at room temperature

Enzymatic activities of GOx@MOFVN

The enzymatic activity of GOx@MOFVNat 25 ^{o}C and 37 ^{o}C were evaluated through tracing the production of H_2O_2 (Equation 1) based on 3,3',5,5'-tetramethylbenzidine (TMB)-H_2O_2-HRP (Equation 2) enzymatic assay.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{Gluconic acid} + H_2O_2 \quad (1)$$

$$TMB + H_2O_2 \xrightarrow{\text{HRP}} \text{oxTMB} + H_2O \quad (2)$$

20.0 μL as-prepared GOx@MOFVN was dispersed into 0.5 mL HAc-NaAc buffer (pH 5.5, 50 mM), followed by adding a series concentration of D-glucose (0.5 mL) solution. The mixture was incubated for 20.0 min at 25 ^{o}C. And then, the mixture was added to 1.0 mL HAc-NaAc buffer (pH 5.5, 50 mM) which contained 0.2 μg/mL HRP and 0.2 mM TMB. The reaction solution was incubated at room temperature for 5.0 min. After incubation, 200.0 μL H_2SO_4 (2.0 M) was added to the reaction solution to terminate reaction. The TMB was oxidized to oxTMB by produced H_2O_2 quantitatively, which was measured at 450 nm by UV-vis spectrophotometer. For the enzymatic activity of GOx@MOFVNat 37 ^{o}C, 25 ^{o}C was replaced by 37 ^{o}C, other steps were same as the above operation processes.

Enzymatic activities of HRP@MOFVN

The enzymatic activity of HRP@MOFVNat 25 ^{o}C and 37 ^{o}C were evaluated through tracing the production of oxTMB using TMB as a peroxidase substrate (Equation 3).

$$TMB + H_2O_2 \xrightarrow{\text{HRP}} \text{oxTMB} + H_2O \quad (3)$$

60.0 μL as-prepared HRP@MOFVN was dispersed into 1.0 mL HAc-NaAc buffer (pH 5.5, 50 mM) containing 0.2 mM H_2O_2, followed by adding a series concentration of TMB (1.0 mL) solution. The mixture was incubated for 20.0 s at 25 ^{o}C. After reaction, 200.0 μL H_2SO_4 (2.0 M) was added to the solution to terminate reaction. The produced oxTMB was measured at 450 nm by UV-vis spectrophotometer. For the enzymatic activity of HRP@MOFVNat 37 ^{o}C, 25 ^{o}C was replaced by 37 ^{o}C, other steps were same as the above operation processes.

Enzymatic activities of TRY@MOFVN

The enzymatic activity of TRY@MOFVNat 25 ^{o}C and 37 ^{o}C were evaluated through tracing the production of *N*-benzoyl-L-arginine (BA) using *N*-benzoyl-L-arginine-ethyl ester (BAEE) as a substrate (Equation 4).

$$\text{BAEE} \xrightarrow{\text{Trypsin}} \text{BA} + CH_3CH_2OH \quad (4)$$

50.0 μL as-prepared TRY@MOFVN was dispersed into 0.5 mL Tris-HCl (pH 8.0, 50.0 mM), followed by adding a series concentration of substrate BAEE (0.5 mL) solution. The mixture was incubated for 10.0 min at 37 ^{o}C. The enzymatic kinetics activity of TRY@MOFVN at 37 ^{o}C was determined by measuring the amount of product BA with capillary electrophoresis (CE) technique and quantitively analyzed by a standard calibration curve. The enzymatic activity of TRY@MOFVN at 25 ^{o}C was tested using the same steps as the above operation processes.

CE performance conditions: A capillary with 75.0 μm inner diameter and 60.0 cm length (45.0 cm effective length, Yongnian Optical Fiber Factory, Hebei, China) was used for CE performance. Buffer solution: 50.0 mM Tris-HCl, pH 8.0; applied voltage, +20.0 KV; sampling, siphoning for 20 s in 15.0 cm height; UV detection at 254 nm.

Enzymatic activities of Cyt-c@MOFVN

The enzymatic activity of Cyt-c@MOFVNat 25 ^{o}C and 37 ^{o}C were evaluated through tracing the production of oxABTS using 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as a substrate (Equation 5).

$$ABTS + H_2O_2 \xrightarrow{Cyt\text{-}c} oxABTS + H_2O \quad (5)$$

50.0 μL as-prepared Cyt-c@MOFVN was dispersed into 2.0 mL PBS (pH 7.2, 6.7 mM) which contained 10.0 mM H_2O_2, followed by adding a series concentration of substrate ABTS (1.0 mL) solution. The mixture was incubated for 5.0 min at 25 ^{o}C. The product oxABTS was measured by UV-vis spectrophotometer at 420 nm. The enzymatic activity of Cyt-c@MOFVN at 37 ^{o}C was tested using the same steps as the above operation processes.

Enzymatic activities of Glnase@MOFVN

The enzymatic activity of Glnase@MOFVNat 25 ^{o}C and 37 ^{o}C were evaluated through tracing the decrease of L-Gln using D,L-glutamine (D,L-Gln) as a substrate (Equation 6).

$$L\text{-}Gln \xrightarrow{Glnase} L\text{-}Glu + NH_3 \quad (6)$$

30.0 μL as-prepared Glnase@MOFVN was dispersed into 30.0 μL 50.0 mM HAc-NaAc (pH 4.9), followed by adding a series concentration of substrate L-Gln (60.0 μL) solution. The mixture was incubated for 10.0 min at 25 ^{o}C. The substrate L-Gln was measured by CE technique and quantitively analyzed by a standard calibration curve. Before CE analysis, the obtained supernatant was boiled for 20.0 min and derivatized with dansyl chloride (Dns-Cl). The enzymatic activity of Glnase@MOFVN at 37 ^{o}C was tested using the same steps as the above operation processes.

CE performance conditions for chiral separation of Dns-D,L-Gln and enzymatic kinetics study: A capillary with 75.0 μm inner diameter and 60.0 cm length (45.0 cm effective length, Yongnian Optical Fiber Factory, Hebei, China) was used for CE performance. Buffer solution: 100.0 mM boracic acid, 10.0 mM ammonium acetate, 3.0 mM zinc acetate and 6.0 mM L-Arg-L-Arg, pH 8.0; applied voltage, -18.0 KV; sampling, siphoning for 10 s in 15.0 cm height; UV detection at 254 nm.

Calibration of BA

The BA in the enzymatic activities test for TRY@MOFVN was quantified based on the standard calibration curve. The corresponding calibration curve of the assay was described as follow. Briefly, a series concentration of BA solution in PBS was prepared. The determination of BA was performed by CE technique. The concentration of BA was proportion to its peak area and the standard calibration curve was obtained.

Calibration of Dns-L-Gln

The substrate L-Gln in the enzymatic activities test for Glnase@MOFVN was quantified based on the standard calibration curve. The corresponding calibration curve of the assay was described as follow. Briefly, a series of L-Gln solutions were prepared and derived with Dns-Cl for CE analysis. The determination of Dns-L-Gln was performed by CE technique. The concentration of Dns-L-Gln was proportion to its peak area and the standard calibration curve was obtained.

Enzymatic kinetic study

Enzymatic kinetic parameters can be calculated based on the Michaelis-Menten equation (Equation 7):

$$V_0 = V_{max}\ (S) / (K_m + (S)) \quad (7)$$

Where, V_0 is the initial catalytic rate; V_{max} is the maximum rate. [*S*] is the initial substrate concentration, and K_m is the Michaelis-Menten constant. The initial catalytic rates V_0 is determined by making the slope of the kinetic curve in the initial phase, and the initial substrate concentration [*S*] is determined at t = 0 s. The kinetic parameters K_m and V_{max} are fitted using Michaelis-Menten equation with the calculated V_0 and [*S*].

Measurement of the enzyme-loading amount

The loading amount of the enzymes within MOFVN was measured by examining the concentration decrease of enzymes in the supernatant before and after immobilization *via* Bradford proteins assay. Typically, 0.5 mL enzyme solution was added into a tube with 1.5 mL deionized water, followed by introducing 1.0 mL Coomassie Brilliant Blue G-250 reagent. After 10.0 min incubation, the solution was collected and detected by a UV-*vis* spectrophotometer. The concentration of the enzymes was proportional to its UV-*vis* absorption intensity at 595 nm.

The enzymes (GOx, HRP, TRY, Cyt-c, Glnase) loading amount was quantified based on the standard calibration curve. The corresponding calibration curve of the assay was described as follow. Briefly, a series of enzymes (GOx, HRP, TRY, Cyt-c, Glnase) solutions were prepared in PBS. Then, 0.5 mL enzyme solution was added into a tube with 1.5 mL deionized water, followed by introducing 1.0 mL Coomassie Brilliant Blue G-250 reagent. After 10.0 min incubation, the solution was collected and detected by a UV-vis spectrophotometer. The concentrations of enzymes were proportion to the UV-*vis* absorption intensity at 595 nm and the standard calibration curve was obtained.

D-glucose detection in rat serum

Three male-Sprague-Dawley-rats (about 250.0 g) were gotten from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). The rat serum samples were pre-treated to eliminate the interferences-proteins. Simply, 0.1 mL of the fresh rat serum samples was diluted by 0.1 mL of ethanol, which was incubated at 25 ^{o}C for 10.0 min. Consequently, the samples were centrifuged at 10,000 rpm for 10.0 min and the supernatant was collected and stored at 4 ^{o}C for further analysis. All experiments using rat samples were performed in accordance with the institutional animal care and used the guidelines of China (GB/T 27416-2014).

The proposed colorimetric GOx@MOFVN-HRP-TMB system was applied to determination of D-glucose concentration in the rat serum samples. 30.0 μL rat serums, GOx@MOFVN (40.0 μL), HRP (10.0 μL, 1.0 mg/mL), TMB (40.0 μL, 1.0 mM), and acetate buffer (2.88 mL, pH 5.5) were mixed. After the mixture was mixed and incubated at 37 °C for 15.0 min. Then, H_2SO_4 (200.0 μL, 2.0 M) was added into the reaction solution to stop reaction. Finally, the UV-*vis* absorption at 450 nm was conducted.

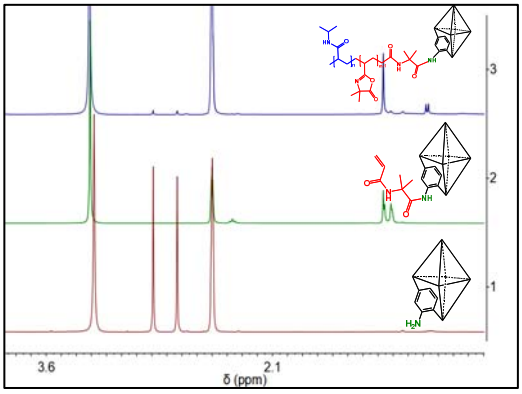

Fig. S1. ^{1}H NMR spectra of MOF, MOFV, and MOFVN.

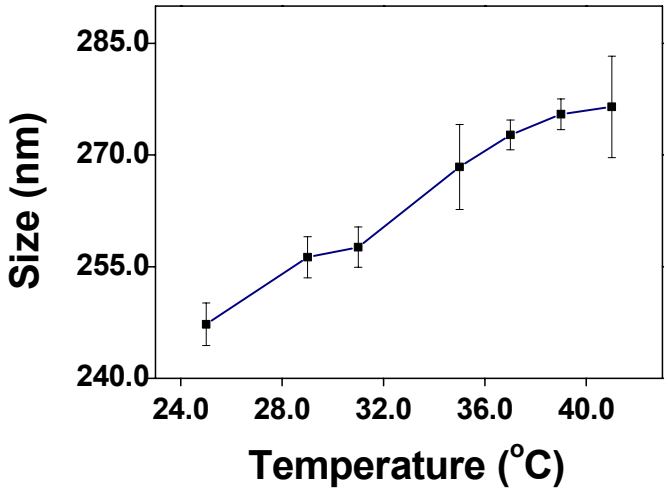

Fig. S2. Size of MOFVN nanoparticles changed with temperature increase.

Table S1 Molecule weight of different enzymes

Enzymes	Molecule weight (KD)
GOx	150.0
Glnase	138.0
HRP	43.0
TRY	23.0
Cyt-c	13.0

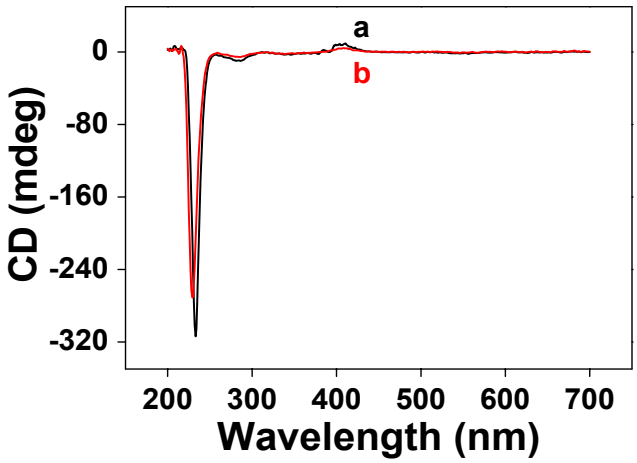

Fig. S3. CD spectra of (a) free HRP and (b) immobilized HRP.

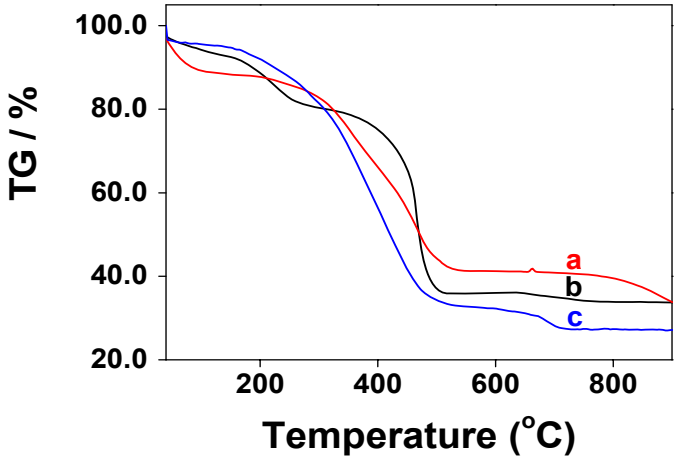

Fig. S4. Thermal gravity analysis of (a) MOF (b) MOFVN and (c) HRP@MOFVN.

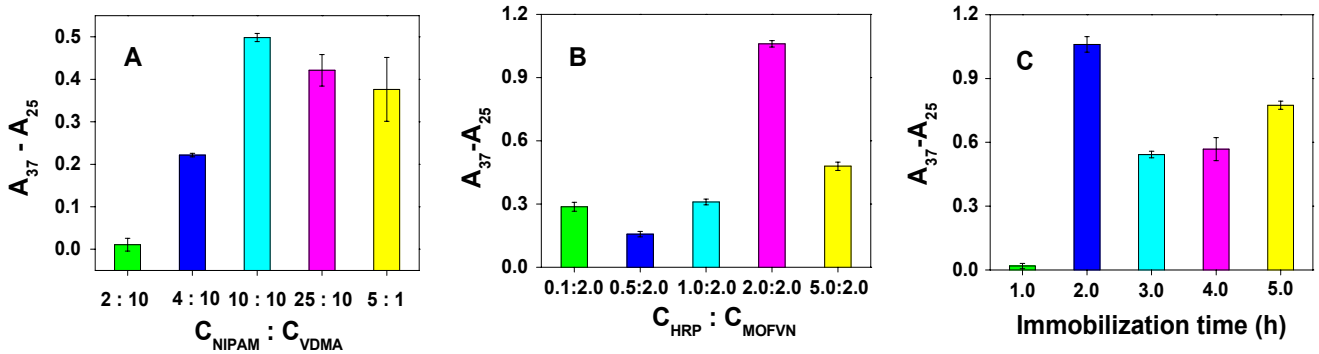

Fig. S5. Optimization of (A) concentration ratio of NIPAM to VDMA; (B) concentration ratio of HRP to MOFVN and (C) immobilization time. A_{25} and A_{37} referred to the UV-*vis* absorption of oxTMB at 450 nm in HRP@MOFVN-TMB-H_2O_2 system at 25 °C and 37 °C, respectively.

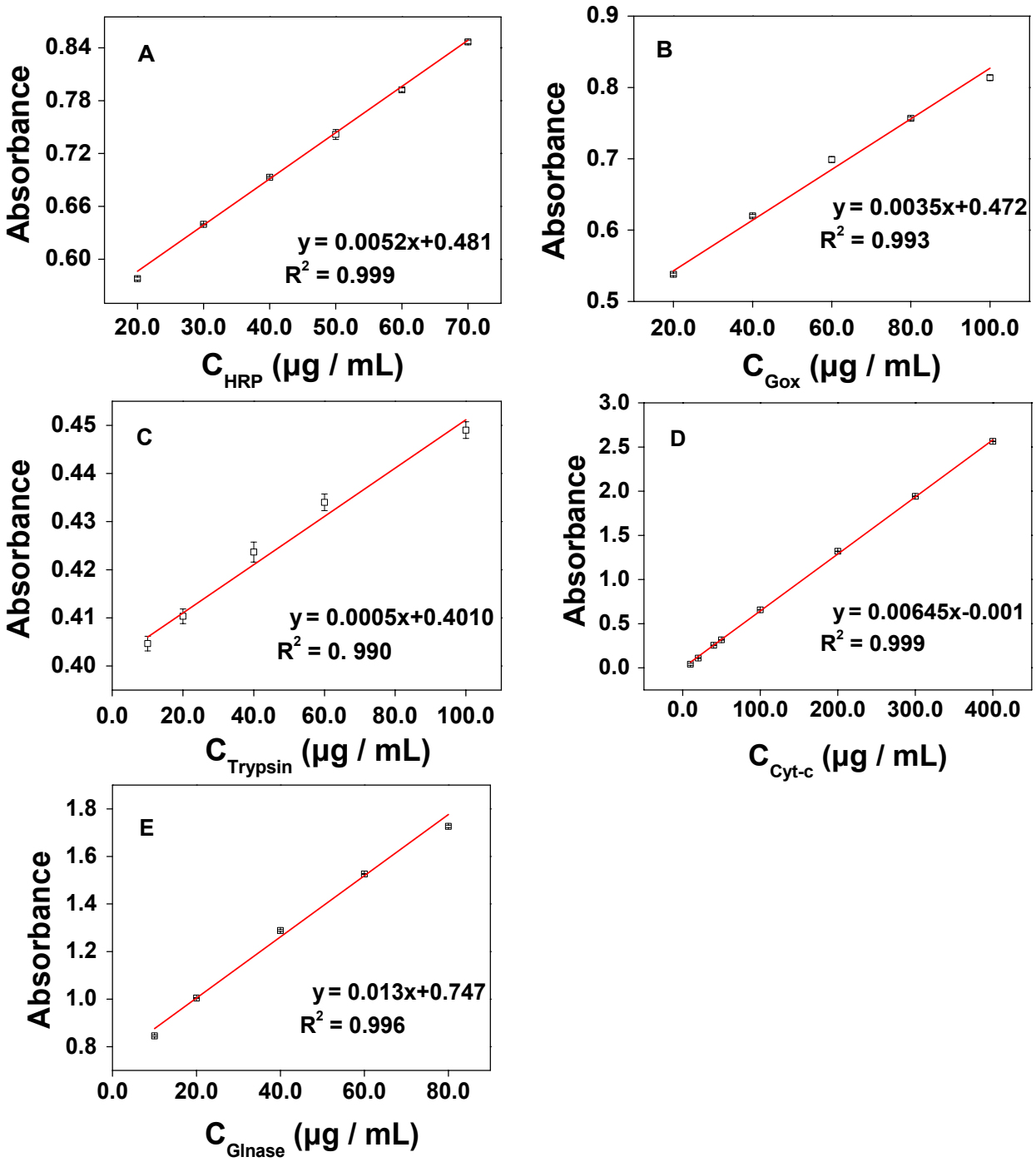

Fig. S6. Linear relationship of (A) HRP, (B) GOx, (C) TRY and (D) Cyt-c concentrations with their UV-vis intensities obtained by Coomassie bright blue assay.

Table S2 The calculated enzyme loading amount in enzymes@MOFVN

Enzymes@MOFVN	GOx@MOFVN	Glnase@MOFVN	HRP@MOFVN	TRY@MOFVN	Cyt-c@MOFVN
Enzyme loading amount (μg / mg)	41.0	43.5	45.9	63.3	148.5

Table S3 Comparison of enzymes loading content in different nano-reactors

Nano-reactors	Immobilized enzymes	Loading content (wt %)*	Ref.
Cyt-c@ZIF-8	Cyt-c	2.7	[1]
HRP@ZIF-8	HRP	0.7	[1]
CAT@ZIF-8	CAT	1.8	[1]
UOx@ZIF-8	UOx	3.3	[1]
ADH@ZIF-8	ADH	4.2	[1]
HRP@MOFs	HRP	0.45	[2]
Cyt-c@MOFs	Cyt-c	1.3	[2]
GOx@MOFs	GOx	4.2	[2]
MB@ZIF-8	MB	18.2	[3]
HRP@ZIF-8	HRP	14.4	[3]
Cyt-c@ZIF-8	Cyt-c	8.0	[4]
CAT@ZIF-90	CAT	5.0	[5]
OVA@ZIF-8	OVA	7.0	[6]
Cyt-c@Tb-mesoMOF	Cyt-c	10.0	[7]
Glnase@MOFVN	Glnase	17.4	
HRP@MOFVN	HRP	18.4	
TRY@MOFVN	TRY	25.3	**This work**
Cyt-c@MOFVN	Cyt-c	59.4	
GOx@MOFVN	GOx	16.4	

* Loading content (wt %) = loading amount in nano-reactors measured / total amount of enzymes used for immobilization x 100%.

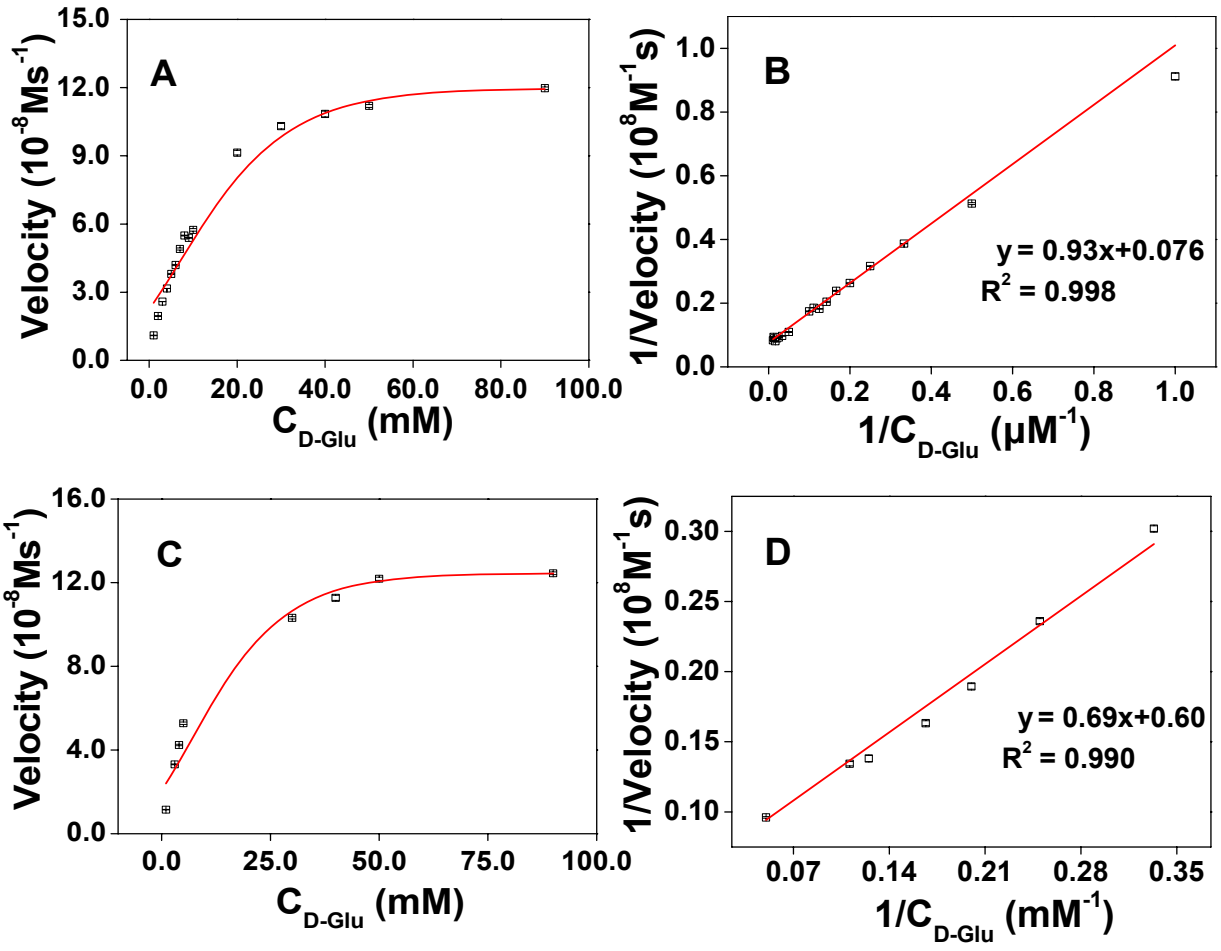

Fig. S7. Kinetic parameters of GOx@MOFVN using D-glucose (D-Glu) as the substrate at 25 ^{o}C (A, B) and 37 ^{o}C (C, D), respectively.

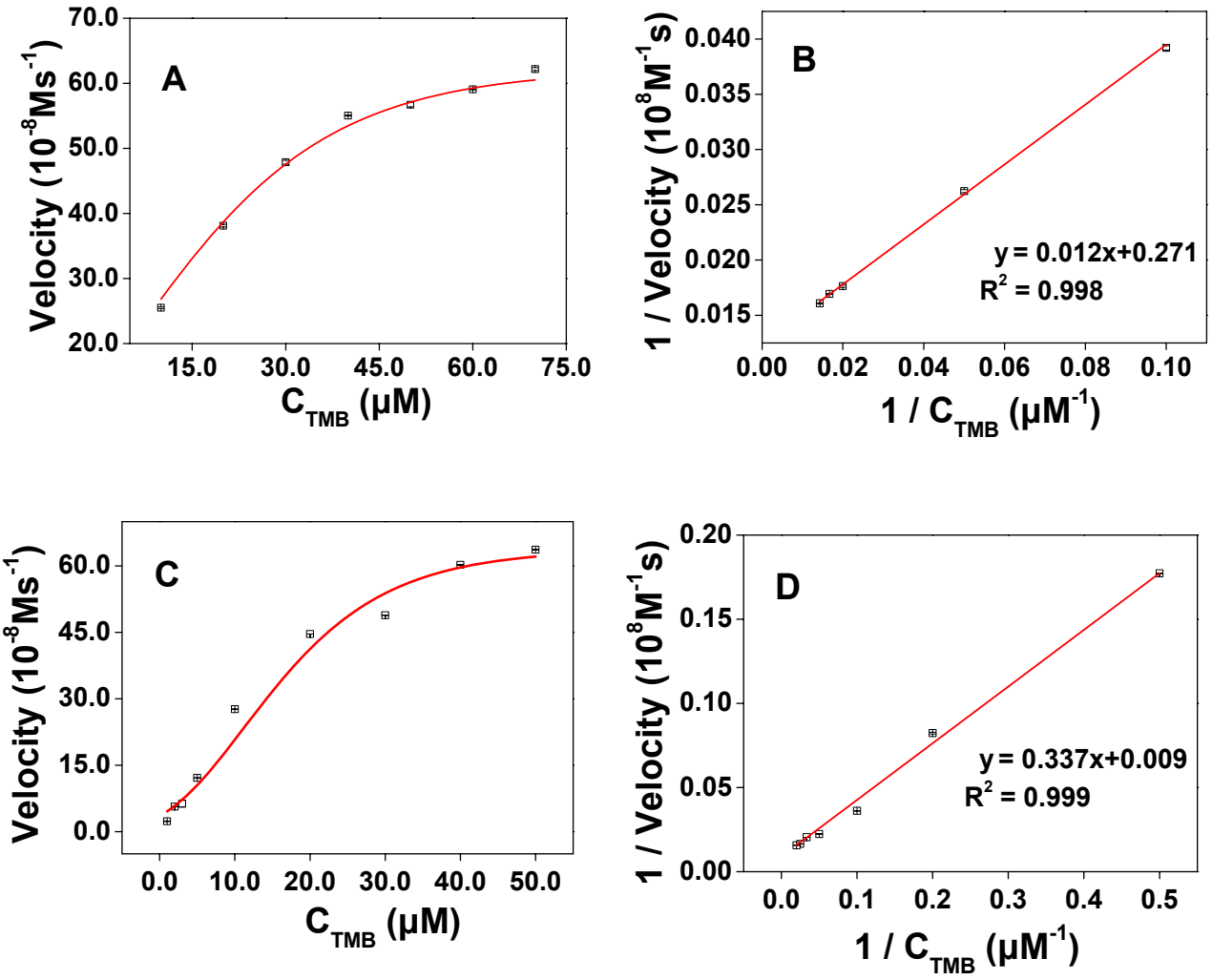

Fig. S8. Kinetic parameters of HRP@MOFVN with TMB as the substrate at 25 ^{o}C (A, B) and 37 ^{o}C (C, D), respectively.

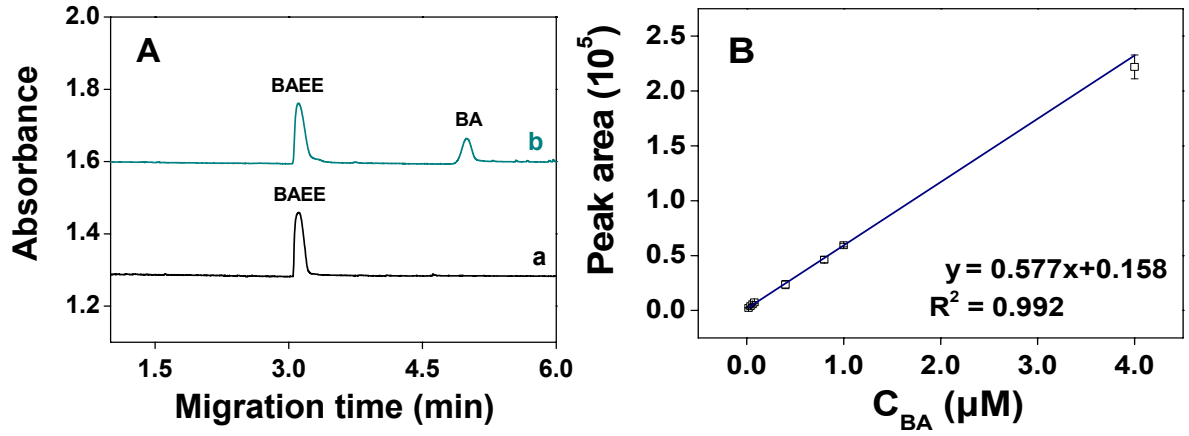

Fig. S9. (A) Electropherograms of BAEE (a) before and (b) after incubation with TRY@MOFVN. (B) Standard calibration curve of BA.

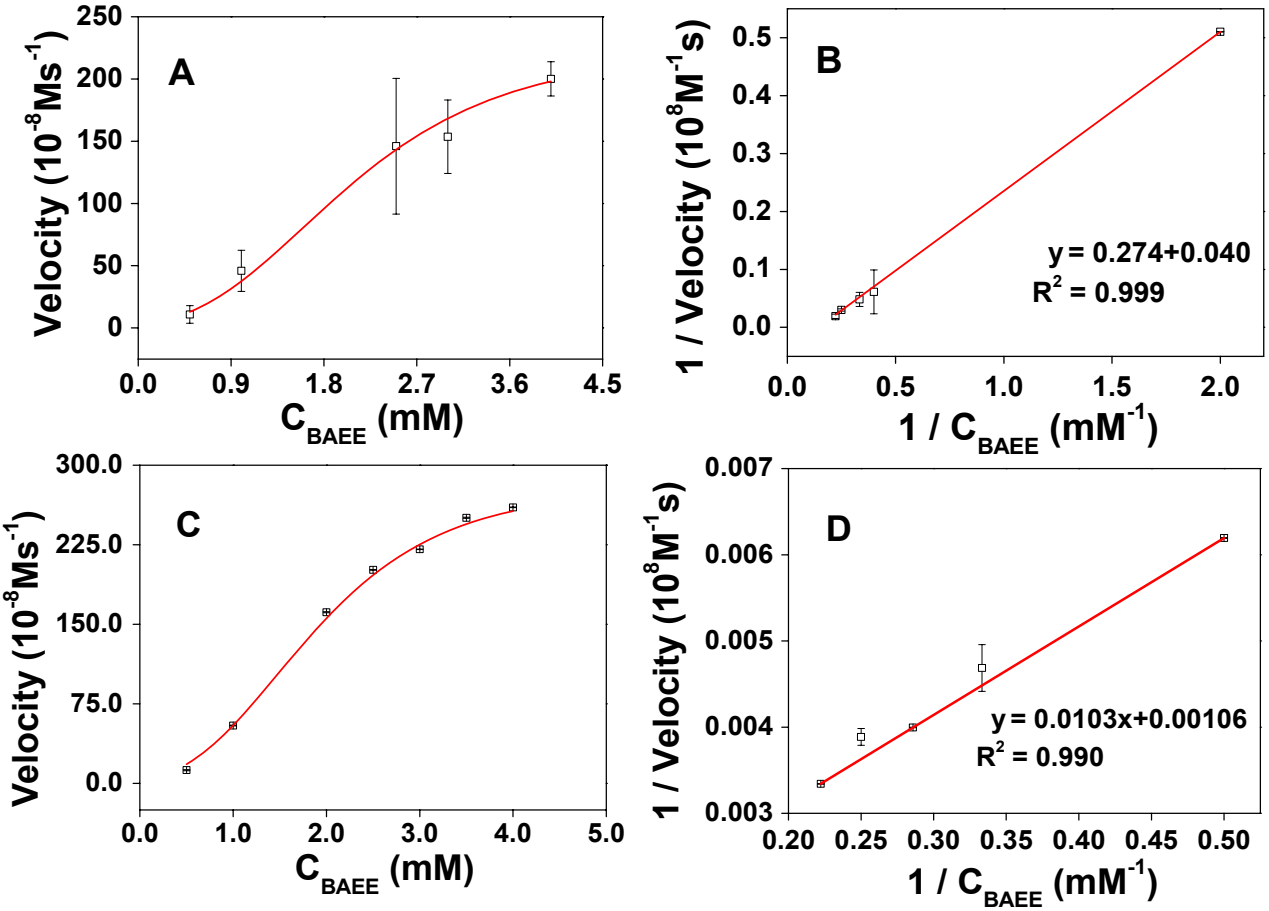

Fig. S10. Kinetic parameters of TRY@MOFVN with BAEE as substrate at 25 ^{o}C (A, B) and 37 ^{o}C (C, D), respectively.

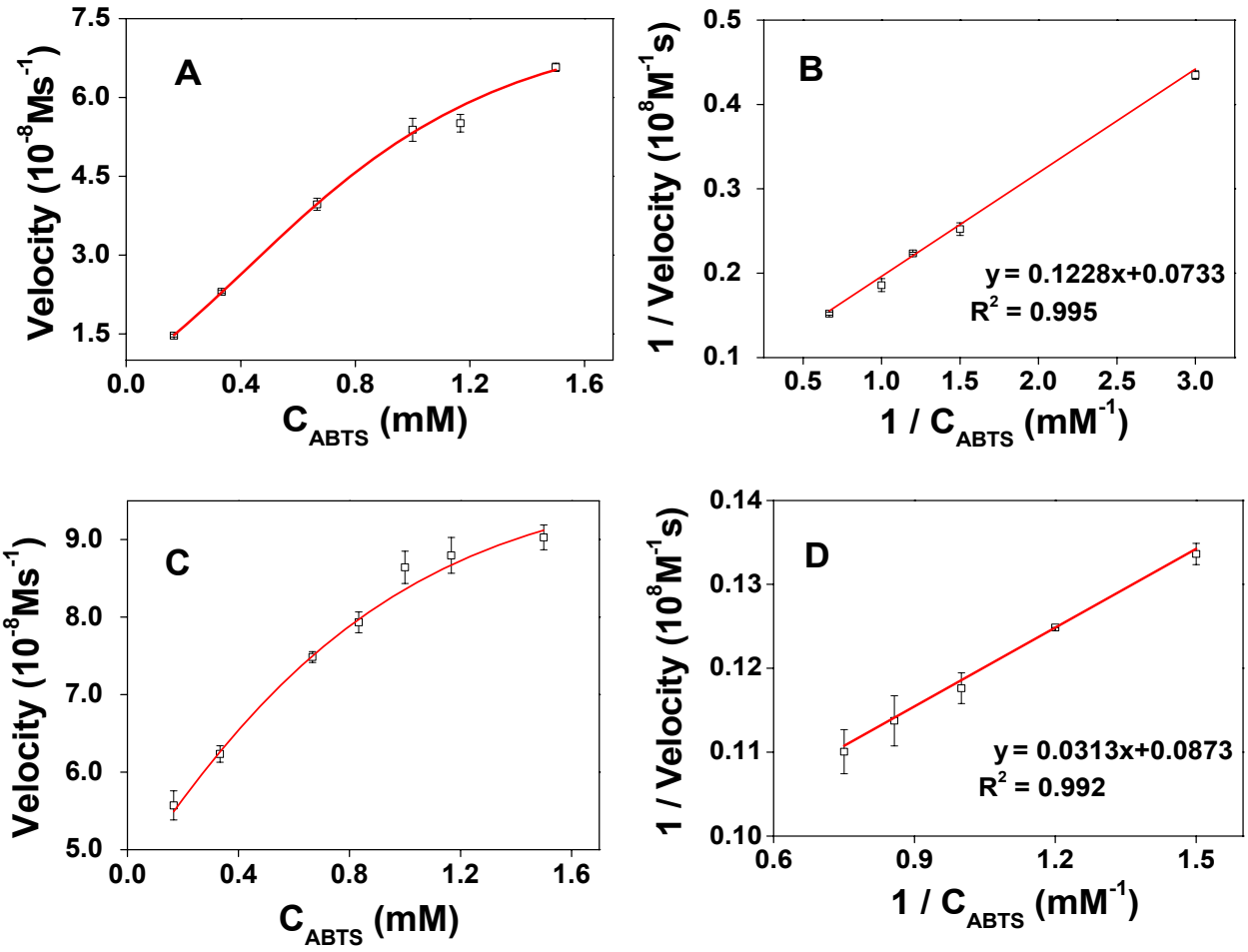

Fig. S11. Kinetic parameters of Cyt-c@MOFVN using ABTS as the substrate at 25 ^{o}C (A, B) and 37 ^{o}C (C, D), respectively.

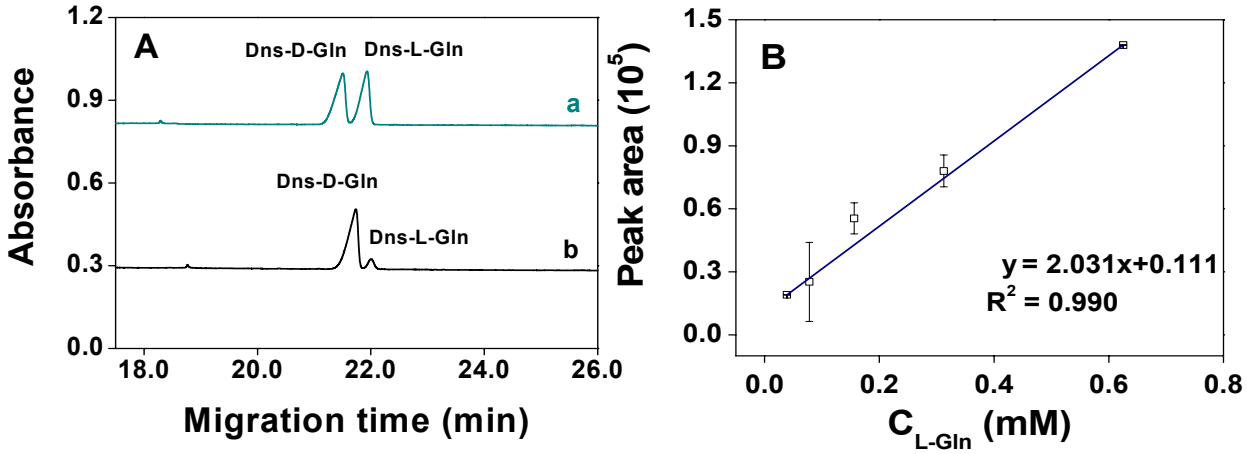

Fig. S12. (A) Electropherograms of D,L-Gln (a) before and (b) after incubation with Glnase@MOFVN. (B) Standard calibration curve of Dns-L-Gln.

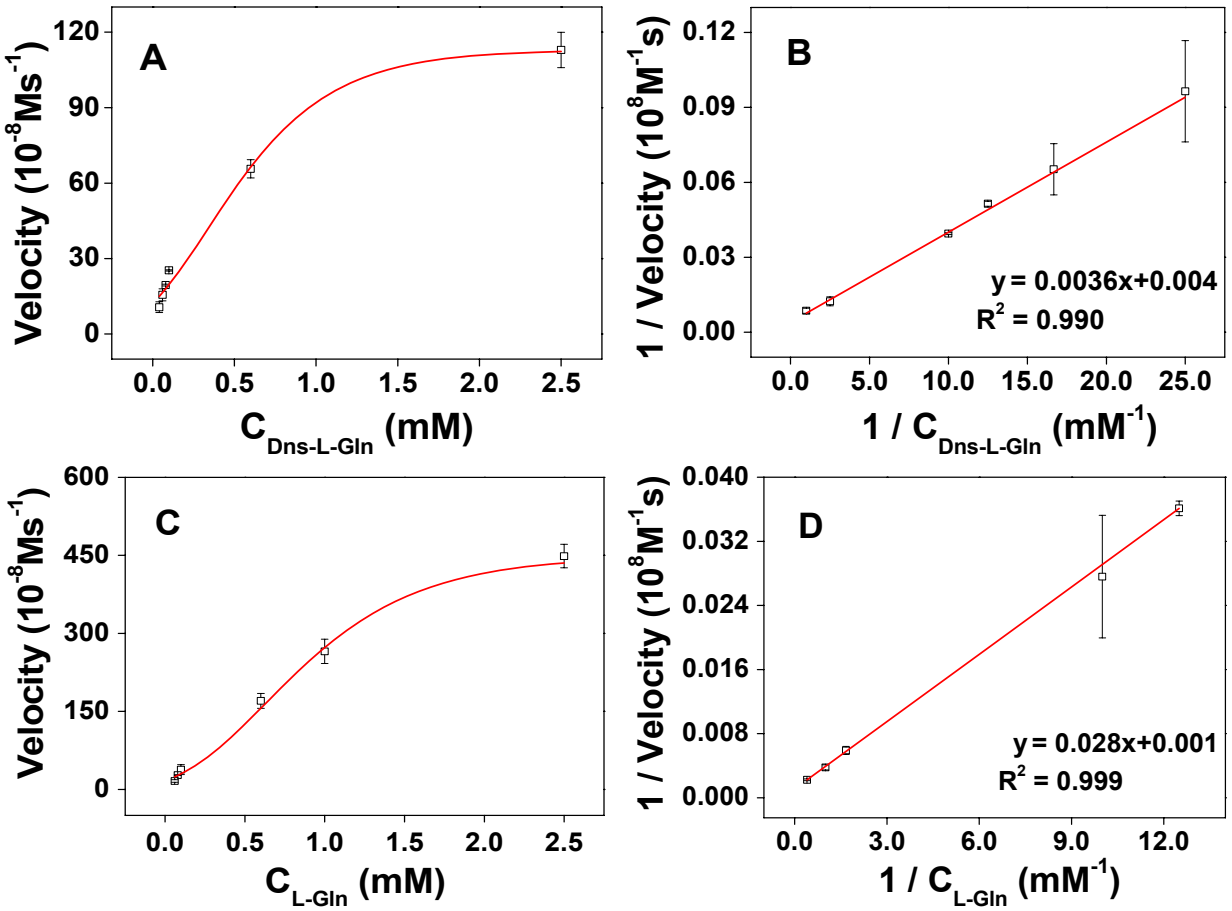

Fig. S13. Kinetic parameters of Glnase@MOFVN at 25 °C (A and B) and 37 °C (C and D), respectively.

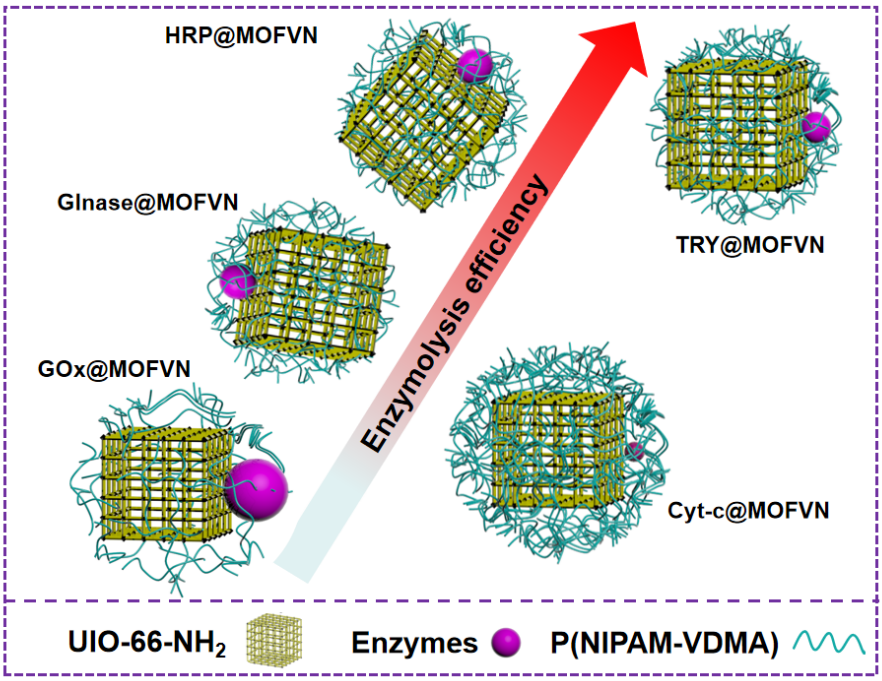

Fig. S14. Illustration of the nano-reactors enzymolysis efficiency affected by molecular weight of the immobilized enzymes.

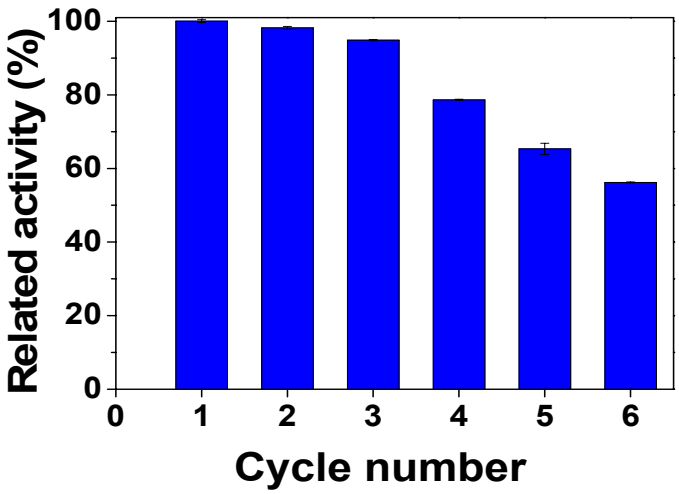

Fig. S15. Recyclability of HRP@MOFVN.

Table S4 Recovery of the proposed method*

Serums	Detected (mM)	Added (mM)	Found (mM)	Recovery (%)	RSD (%)
1	6.19	30.0	39.20	110.01	2.35
		60.0	64.07	96.46	0.99
		90.0	99.54	103.72	1.64
2	6.16	30.0	39.58	111.41	1.18
		60.0	63.38	95.38	1.59
		90.0	110.22	115.63	1.68
3	6.64	30.0	39.92	110.96	0.47
		60.0	65.91	98.78	0.50
		90.0	101.93	105.88	1.67

*Blank controlled rat serums were used for recovery study (n=3).

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