Electronic Supplementary Information

Molecular Recognition of Enzymes and Modulation of Enzymatic Activity by Nanoparticle Conformational Sensors

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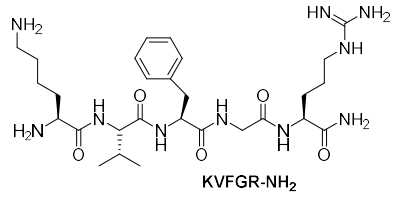
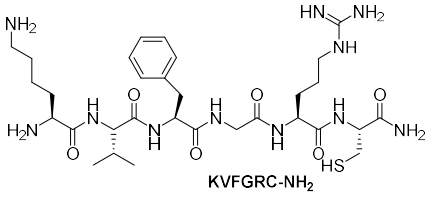
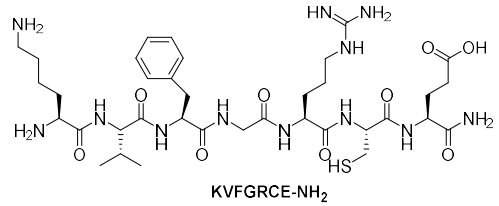
General Method

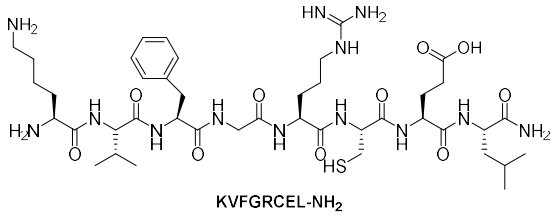
All peptides were purchased from Shanghai Bootech BioScience & Technology Co. Ltd., and reconstituted according to the instructions. All organic solvents and reagents were of ACS-certified grade or higher grade, and were purchased from commercial suppliers. Routine ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400, on a Bruker AV III 600, or on a Varian VXR-400 spectrometer. For chemical analysis, ESI-MS mass was recorded on a Shimadzu LCMS-2010 mass spectrometer. For protein analysis, MALDI−TOF spectra were recorded on a Shimadzu AXIMA Confidence MALDI TOF Mass Spectrometer. Dynamic light scattering (DLS) data were recorded at 25 °C using PDDLS/CoolBatch 90T with PD2000DLS+ instrument. Isothermal titration calorimetry (ITC) was performed using a MicroCal VP-ITC Microcalorimeter with Origin 7 software and VPViewer2000 (GE Healthcare, Northampton, MA). UV-vis spectra were recorded on a Cary 100 Bio UV-visible spectrophotometer. CD spectra were measured on a Jasco J-715 CD Spectrometer.

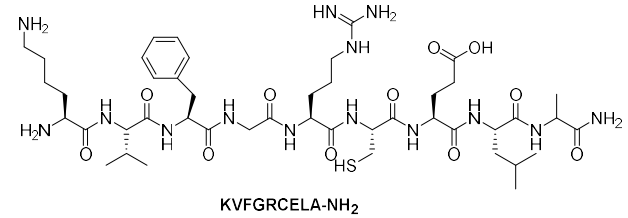
Chart S1. Structures of peptides used in study

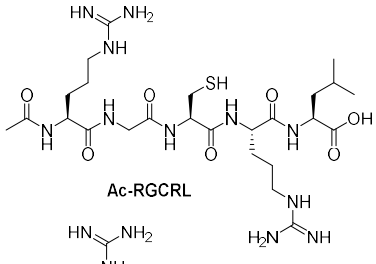


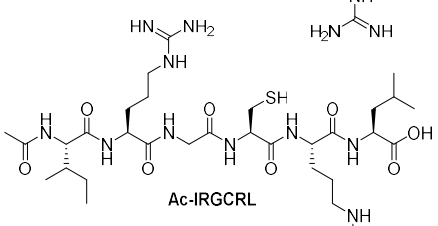


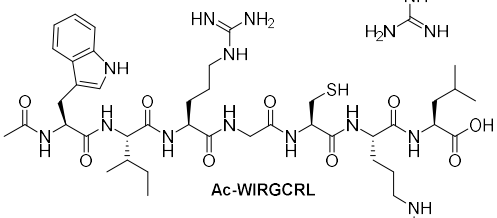


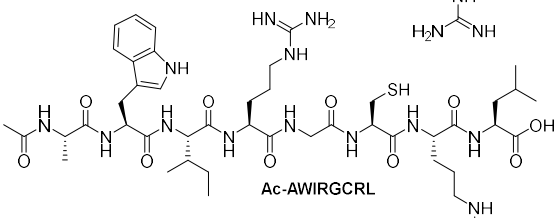


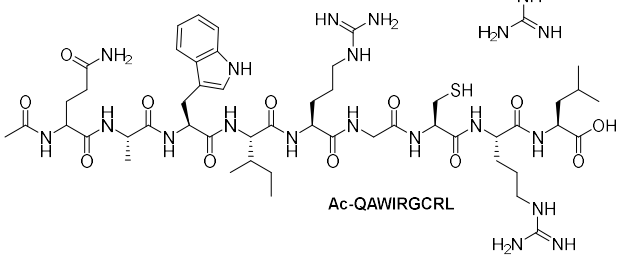




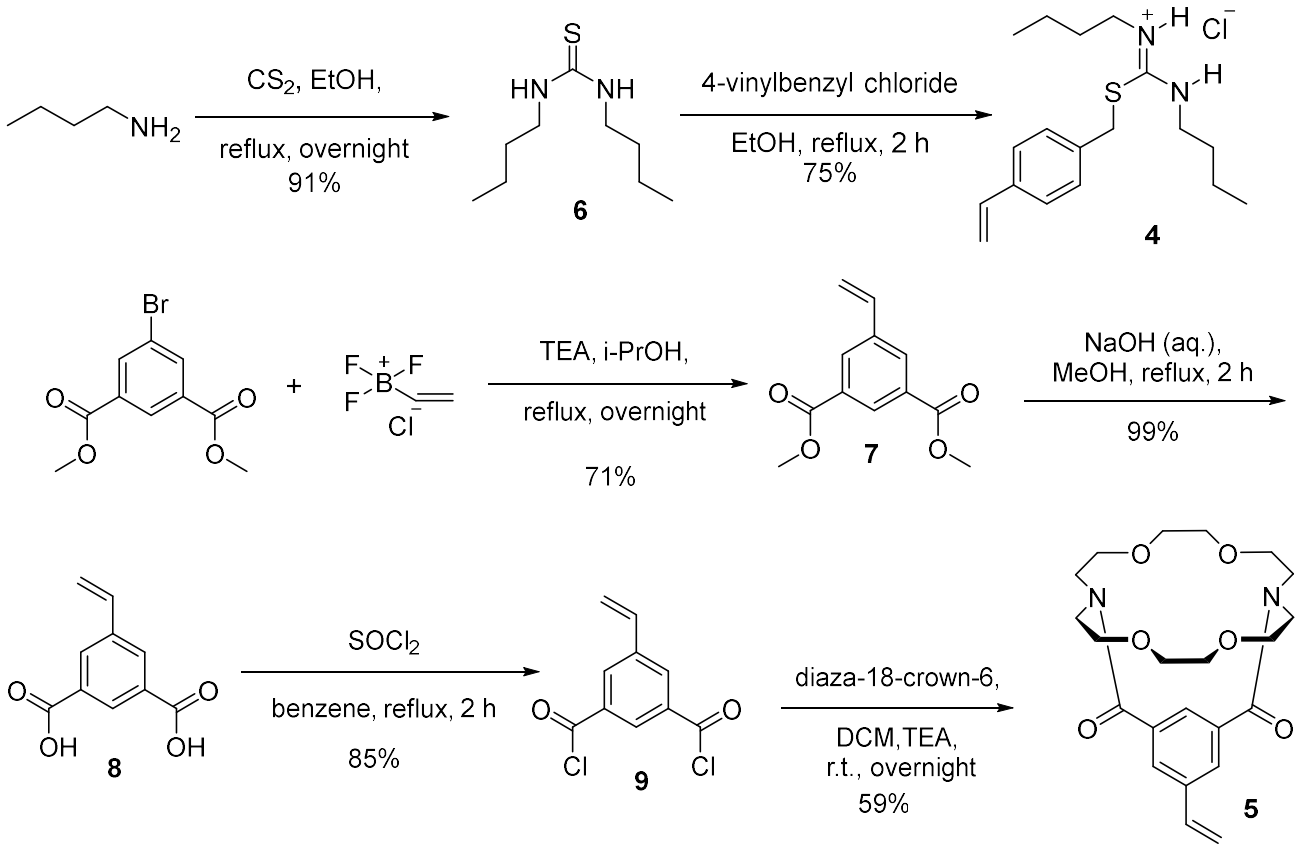




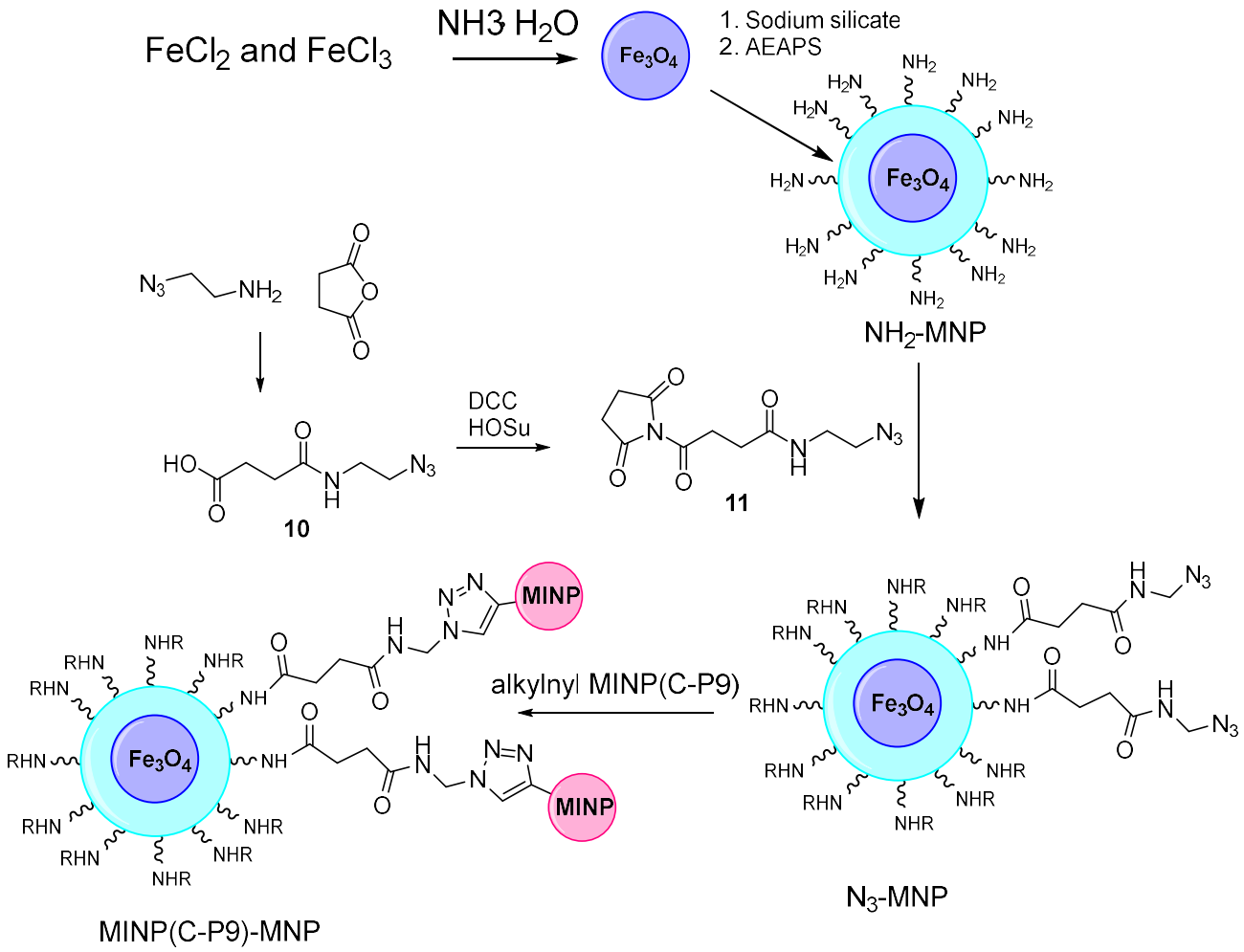




Scheme S1. Preparation of Functional Monomers



Scheme S2. Preparation of MINP-MNP



Syntheses

Syntheses of compounds **1**,[1] **2**,[2] **3**,[3] **4**–**9**,[4] and **10**–**11**[5] were previously reported.

[1] Awino, J. K.; Zhao, Y. *J. Am. Chem. Soc.* **2013**, *135*, 12552−12555.
[2] Zhang, S.; Zhao, Y. *Macromolecules* **2010**, *43*, 4020-4022.
[3] Ryu, E.-H.; Zhao, Y. *Org. Lett.* **2005**, *7*, 1035-1037.
[4] Fa, S.; Zhao, Y. *Chem. Mater.* **2019**, 31, 13, 4889-4896.
[5] Duan, L.; Zangiabadia, M.; Zhao, Y. *Chem. Commun.*, **2020**, 56, 10199-10202

Typical procedure for the synthesis of Functionalized MINPs

Functional monomer (FM) **4** in methanol (75 μL of a 10.9 mg/2 mL solution, 0.0012 mmol**)**, **5** in methanol (25 μL of a 6.7 mg/ mL solution, 0.0004 mmol), and template **N-P5** in methanol (25 μL of an 8.1 mg/mL solution, 0.0004 mmol) were added to a small vial with 100 μL of methylene chloride. The mixture was stirred for 2 h before the organic solvent was removed *in vacuo*. A micellar solution of surfactant **1** (9.3 mg, 0.02 mmol) in H_2O (2.0 mL) was added to the above FM–template complex. Divinylbenzene (DVB, 2.8 μL, 0.02 mmol) and 2,2-dimethoxy-2-phenylacetophenone in DMSO (DMPA,10 μL, 0.0005 mmol) were then added to the solution. The mixture was subjected to ultrasonication for 10 min, followed by the addition of cross-linker **2** in H_2O (10 μL of 410 mg/mL, solution, 0.024 mmol), $CuCl_2$ in H_2O (10 μL of 6.7 mg/mL solution, 0.0005 mmol), and sodium ascorbate in H_2O (10 μL of 99 mg/mL solution, 0.005 mmol). The reaction mixture is stirred slowly at room temperature for 12 h. Surface ligand **3** (10.6 mg, 0.04 mmol), $CuCl_2$ in H_2O (10 μL of 6.7 mg/mL solution, 0.0005 mmol), and sodium ascorbate in H_2O (10 μL of 99 mg/mL solution, 0.005 mmol) were then added. The reaction mixture was stirred at room temperature for another 8 h, purged with nitrogen for 15 min, sealed with a rubber stopper, and irradiated in a Rayonet reactor for 12 h. The reaction mixture was poured into acetone (8 mL). The precipitate collected by centrifugation was washed with a mixture of acetone/water (5 mL/1 mL) three times, followed by methanol/acetic acid (5 mL/0.1 mL) three times. The MINP precipitate was rinsed with methanol (5 mL) and finally with acetone (5 mL) before being dried in air to afford the final MINP as an off-white solid. Typical yields were >80%. The progress of reaction could be monitored by ^{1}H NMR spectroscopy and dynamic light scattering (DLS).

Preparation of MINP-MNP (Scheme S2)

Preparation of the Fe_3O_4 MNPs followed a reported procedure.[6] $FeCl_3 \cdot 6H_2O$ (1.17 g) and $FeCl_2 \cdot 4H_2O$ (0.43 g) were dissolved in 20 mL of deionized water under nitrogen with vigorous stirring by a mechanical stirrer at 85 ºC. 25% $NH_3 \cdot H_2O$ (1.5 mL) was added to the solution and the reaction mixture was stirred for another 15 min. The precipitate formed was collected by filtration and washed water (3 × 5 mL) and ethanol (3 × 5 mL) to afford the Fe_3O_4 nanoparticles as a black powder. The Fe_3O_4 nanoparticles were combined with a solution of sodium silicate (4.75 g) in water and sonicated for 30 min before being heated to 80 °C. Hydrochloric acid was added dropwise to

[6] Liu, X.; Ma, Z.; Xing, J.; Liu, H. *J. Magn. Magn. Mater*. **2004**, 270, 1-6.

adjust the pH value to 6–7. The precipitate formed was collected by filtration and washed with water (3 × 5 mL) and ethanol (3 × 5 mL) to give a black powder.

The silica-coated Fe_3O_4 nanoparticles were dispersed in 15 mL of methanol. The solution was mixed with 15 mL of glycerol and sonicated for 30 min. 3-Aminopropyltriethoxysilane (AEAPS, 1 mL) was added and the reaction mixture was heated to 80–90 °C with rapid stirring for 3 h. The insoluble material was collected by filtration, washed with water (3 × 5 mL) and ethanol (3 × 5 mL), and dried under vacuum. A portion of the NH_2-MNP obtained (10.0 mg) was dispersed in DMSO (0.5 mL) and sonicated for 30 min, followed by the addition of compound **11** (17 mg, 0.06 mmol) and triethylamine (6 μL, 0.08 mmol). The mixture was stirred at room temperature for 1 h. The insoluble material was washed with DMSO (3 × 0.1 mL) to give N_3-MNP as a black powder.

The MINP(P-C9) prepared above, without surface-functionalization with **3**, was added to N_3-MNP (2.0 mg), followed by the addition of $CuCl_2$ (10 μL of a 6.7 mg/mL solution in H_2O, 0.0005 mmol), and sodium ascorbate (10 μL of a 99 mg/mL solution in H_2O, 0.005 mmol). After the mixture was stirred at room temperature for 6 h, the insoluble material was collected with a magnet, washed with distilled water (3 × 5 mL) and ethanol (3 × 5 mL) to give MINP(P-C9)-MNP as a black powder (1.8 mg).

Dynamic Light Scattering (DLS)

Particle size of MINP was determined on a Malvern Zetasizer Nano ZS using the Zetasizer software according to the Stokes-Einstein equation (1).[7] The volume of a spherical nanoparticle (V_{D_h}) was calculated from equation (2). Assuming a density of 1.37 g/cm^3 (the density of protein), the molecular weight of the particle can be calculated using equation (3). A nanoparticle with hydrodynamic diameter of 4.87 nm has a calculated molecular weight of 50 kDa.

$$D_h = \frac{k_B T}{6\pi\eta D_t} \qquad (1)$$

in which D_h is the hydrodynamic diameter, D_t the translational diffusion coefficient measured by dynamic light scattering, T the temperature, k_B the Boltzmann's constant, and η is dynamic viscosity of water (0.890 cP at 298 K).

[7] Erickson, H. P. *Biol. Proced. Online* **2009**, *11*, 32-51.

$$V_{D_h} = \frac{4\pi}{3}\left(\frac{D_h}{2}\right)^3 \quad (2)$$

$$\text{Mw in dalton} = \left(\frac{D_h}{0.132}\right)^3 \quad (3)$$

in which D_h is the hydrodynamic diameter in nm.

ITC Titration

ITC was performed using a MicroCal VP-ITC Microcalorimeter with Origin 7 software and VPViewer2000 (GE Healthcare, Northampton, MA). An average M.W. of 50 KDa was used to prepare all MINP solutions. The determination of binding constants by ITC followed standard procedures.[8,9,10] In general, a solution of an appropriate guest in Millipore water was injected in equal steps into 1.43 mL of the corresponding MINP in the same solution. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of the MINP to the guest. The smooth solid line is the best fit of the experimental data to the sequential binding of N binding site on the MINP. The heat of dilution for the guest, obtained by titration carried out beyond the saturation point, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

General method of enzyme activity measurement.

Lysozyme activity was measured by a modified procedure from the literature. [11] Stock solutions of glycol chitin (5 mg/mL) and lysozyme (1.5 μM) were prepared in 0.1 M of acetate buffer (pH 4.5). Both solutions were incubated at 40 °C for 1 h before 1.0 mL of the glycol chitin solution and 0.5 mL of the lysozyme solution were added to a vial. After the enzyme reaction was allowed to proceed at 40 °C for 3 h, a 750 μL of the reaction mixture was combined with 1.0 mL of potassium ferricyanide (1.5 mM) in 0.5 M Na_2CO_3 solution. The mixture was heated in a boiling water bath for 15 min. After the solution was cooled to room temperature, 1750 μL of water was added and the absorbance at 420 nm was measured with a UV-visible spectrophotometer. The amount of hydrolysis

[8] Wiseman, T.; Williston, S.; Brandts, J. F.; Lin, L. N. *Anal. Biochem.* **1989**, *179*, 131-137.
[9] Jelesarov, I.; Bosshard, H. R. *J. Mol. Recognit.* **1999**, *12*, 3-18.
[10] Velazquez-Campoy, A.; Leavitt, S. A.; Freire, E. *Methods Mol. Biol.* **2004**, *261*, 35-54.
[11] T. Imoto and K. Yagishita, *Agr. Biol. Chem.*, **1971**, 35, 1154-1156.

was determined via a calibration curve (Figure S13) generated from authentic samples of N-acetylglucosamine (NAG).

Extraction of lysozyme from a protein mixture

A stock solution of each protein (0.15 mg/mL for lysozyme, 1.5 mg/mL for other proteins) was prepared with an ammonium bicarbonate buffer (50 mM with 0.5 M NaCl). The protein mixture was prepared by combining 100 μL of the six protein stock solutions. MINP(P-C9)-MNP (2.0 mg) was added to the protein mixture and the mixture was gently shaking at room temperature for 2 h. The solution was removed while a magnet was applied side of the small vial. A 200 μL of ammonium bicarbonate buffer (50 mM with 0.5 M NaCl) was added and the mixture was shaking gently at room temperature for 5 min before the buffer was removed, and the washing step was repeated another two times. To release the bound lysozyme, the MINP(P-C9)-MNP composite was washed with 50 μL of 100 mM acetic acid solution and shaking gently for 1 h. The solution was collected and dried under a flow of nitrogen. The residue was redissolved in 20 μL of ammonium bicarbonate buffer (50 mM with 0.5 M NaCl) and analyzed by MALDI-TOF MS.

MALDI-TOF MS Analysis

The above sample was diluted 1:5 with H_2O before it was analyzed on a Shimadzu AXIMA Confidence MALDI TOF Mass Spectrometer with the following conditions.
Matrix: sinapinic acid (SA) 10 mg/mL in 50% ACH/H_2O, 0.1% TFA
Spotting method: 0.5 uL of the diluted sample and 1.0 uL of the matrix was spotted on the plate, mixed by pipetting, and allowed to air dry.
Ion gate: off
Pulsed Extration: off
Mode: linear positive
Laser power: 130
Calibrant: BSA
Range: 10000-100000 Da

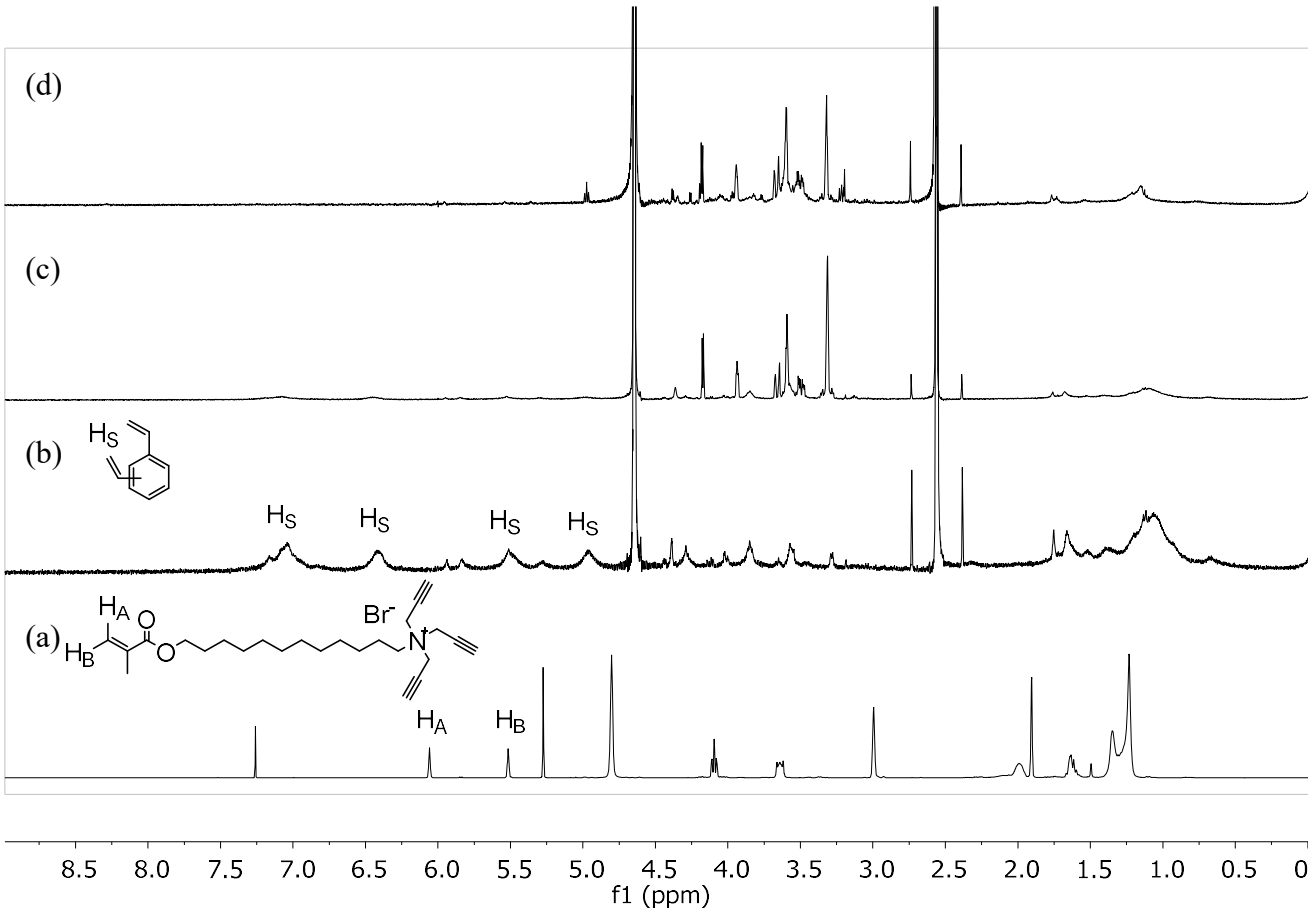


Figure S1. Typical ^{1}H NMR spectra of (a) surfactant **1** in $CDCl_3$, (b) alkynyl-SCM in D_2O, (c) surface-functionalized SCM in D_2O, (d) MINP in D_2O.

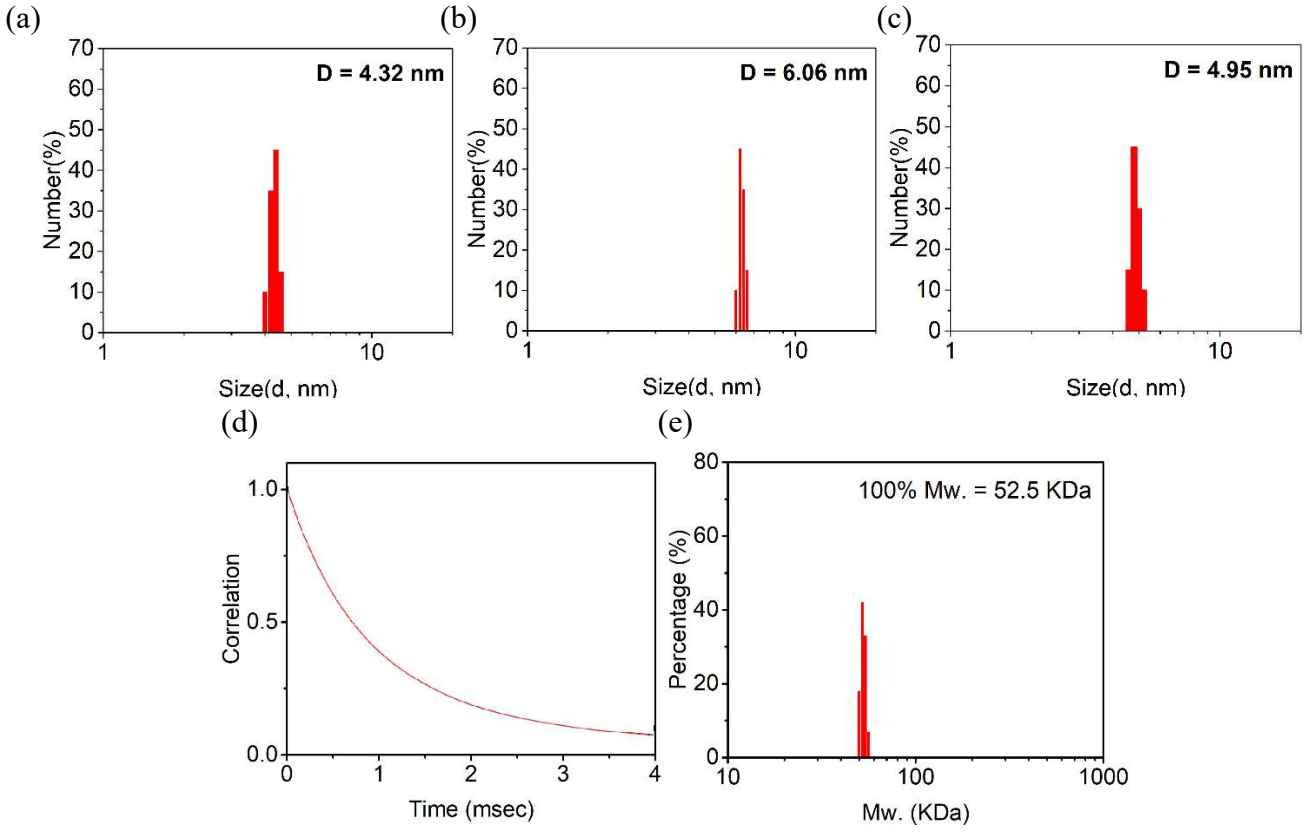


Figure S2. Typical distribution of the hydrodynamic diameters of the nanoparticles in water as determined by DLS for (a) alkynyl-SCM, (b) surface-functionalized SCM, (c) MINP in water. (d) The correlation curve and (e) the corresponding molecular weight of the MINP based on the DLS size.

Table S1. Binding data obtained from ITC titrations. [a]

Entry	Template	Guest	FM **4**	FM **5**	K_a ($\times 10^5$ M^{-1})	N	ΔG (kcal·mol^{-1})	ΔH (kcal·mol^{-1})	ΔS (cal·mol^{-1} deg^{-1})
1	N-P5	N-P5	none	none	1.59 ± 0.49	1.13 ± 0.08	-7.10	-1.83	17.7
2	N-P6	N-P6	none	none	2.20 ± 0.38	1.56 ± 0.03	-7.27	-4.74	8.5
3	N-P7	N-P7	none	none	3.68 ± 0.33	0.72 ± 0.02	-7.60	-24.23	-55.8
4	N-P8	N-P8	none	none	6.25 ± 0.43	1.00 ± 0.01	-7.92	-16.59	-29.1
5	N-P9	N-P9	none	none	8.57 ± 1.24	1.49 ± 0.04	-8.10	-14.03	-19.9
6	C-P5	C-P5	none	none	3.29 ± 0.68	0.98 ± 0.03	-7.10	-1.83	17.7
7	C-P6	C-P6	none	none	3.37 ± 0.59	0.47 ± 0.10	-7.27	-4.74	8.5
8	C-P7	C-P7	none	none	7.97 ± 1.01	0.69 ± 0.01	-7.60	-24.23	-55.8
9	C-P8	C-P8	none	none	10.50 ± 2.09	1.04 ± 0.05	-7.92	-16.59	-29.1
10	C-P9	C-P9	none	none	24.40 ± 6.99	1.29 ± 0.04	-8.64	-31.29	-76.0
11	N-P5	Lysozyme	none	none	1.31 ± 0.20	0.97 ± 0.04	-6.99	-12.65	-19.0
12	N-P6	Lysozyme	none	none	1.61 ± 0.16	0.86 ± 0.03	-7.12	-12.22	-17.1
13	N-P7	Lysozyme	none	none	2.35 ± 0.45	0.75 ± 0.04	-7.33	-6.48	2.9
14	N-P8	Lysozyme	none	none	2.83 ± 0.59	0.87 ± 0.04	-7.44	-6.37	3.6
15	N-P9	Lysozyme	none	none	< 0.001	-	-	-	-
16	C-P5	Lysozyme	none	none	0.49 ± 0.14	1.46 ± 0.09	-6.40	-0.74	19.0
17	C-P6	Lysozyme	none	none	0.61 ± 0.21	1.42 ± 0.11	-6.52	-0.65	19.7
18	C-P7	Lysozyme	none	none	1.56 ± 0.30	0.88 ± 0.05	-7.08	-13.13	-20.3
19	C-P8	Lysozyme	none	none	2.21 ± 0.48	0.76 ± 0.05	-7.30	-10.97	-12.3
20	C-P9	Lysozyme	none	none	3.58 ± 0.52	1.12 ± 0.03	-7.58	-9.61	-6.8

[a] The titrations were performed in 10 mM HEPES buffer (pH 7.0) at 298 K, and the MINPs were prepared without any FM. The K_a values in the parentheses are those determined by ITC titration.

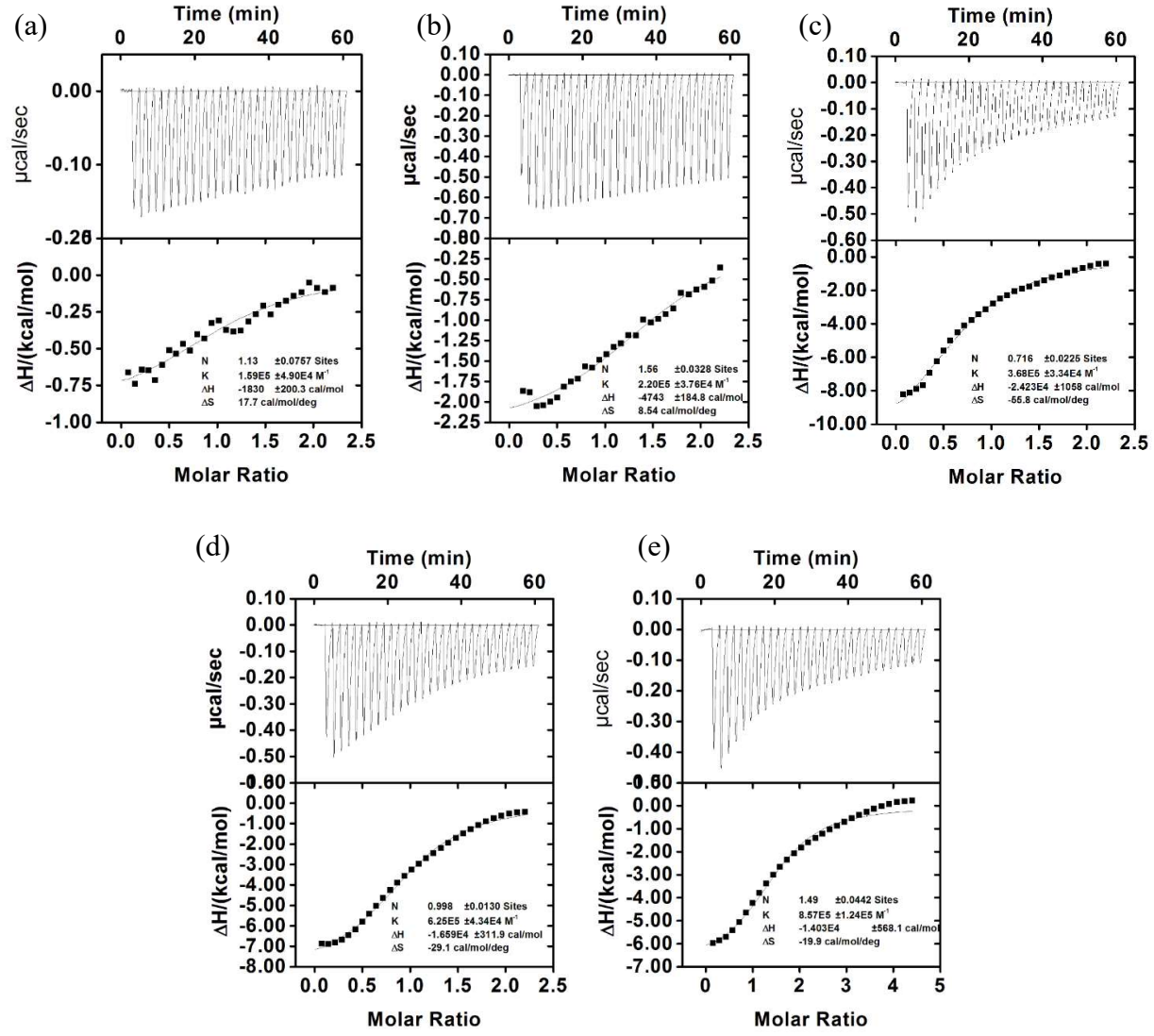


Figure S4. ITC titration curves obtained at 298 K for the titration of (a) MINP(**N-P5**) without functional monomer by peptide **N-P5**, (b) MINP(**N-P6**) without functional monomer by peptide **N-P6**, (c) MINP(**N-P7**) without functional monomer by peptide **N-P7**, (d) MINP(**N-P8**) without functional monomer by peptide **N-P8**, (e) MINP(**N-P9**) without functional monomer by peptide **N-P9**. The MINP concentration in the ITC titration was (a) 20 µM, (b) 20 µM, (c) 10 µM, (d) 10 µM, and (e) 5 µM, respectively. The concentration of peptide in the ITC titration was (a) 200 µM, (b) 200 µM, (c) 100 µM, (d) 100 µM, and (e) 100 µM, respectively. All titrations were performed in 10 mM HEPES buffer (pH 7.0). The data correspond to entries 1, 2, 3, 4, and 5, respectively, in Table S1.

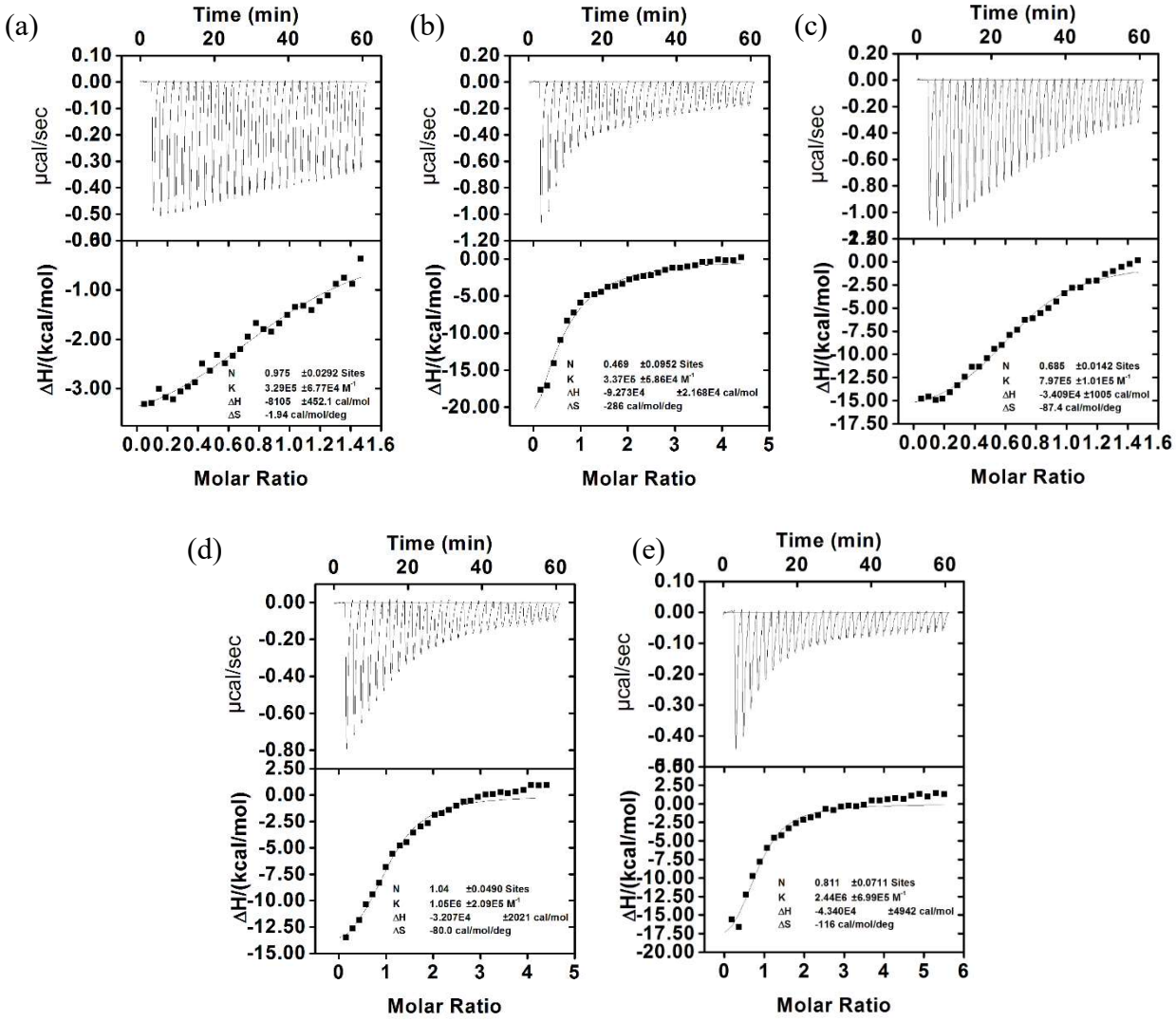


Figure S5. ITC titration curves obtained at 298 K for the titration of (a) MINP(**C-P5**) without functional monomer by peptide **C-P5**, (b) MINP(**C-P6**) without functional monomer by peptide **C-P6**, (c) MINP(**C-P7**) without functional monomer by peptide **C-P7**, (d) MINP(**C-P8**) without functional monomer by peptide **C-P8**, (e) MINP(**C-P9**) without functional monomer by peptide **C-P9**. The MINP concentration in the ITC titration was (a) 15 µM, (b) 5 µM, (c) 15 µM, (d) 5 µM, and (e) 2 µM, respectively. The concentration of peptide in the ITC titration was (a) 100 µM, (b) 100 µM, (c) 100 µM, (d) 100 µM, and (e) 50 µM, respectively. All titrations were performed in 10 mM HEPES buffer (pH 7.0). The data correspond to entries 6, 7, 8, 9, and 10, respectively, in Table S1.

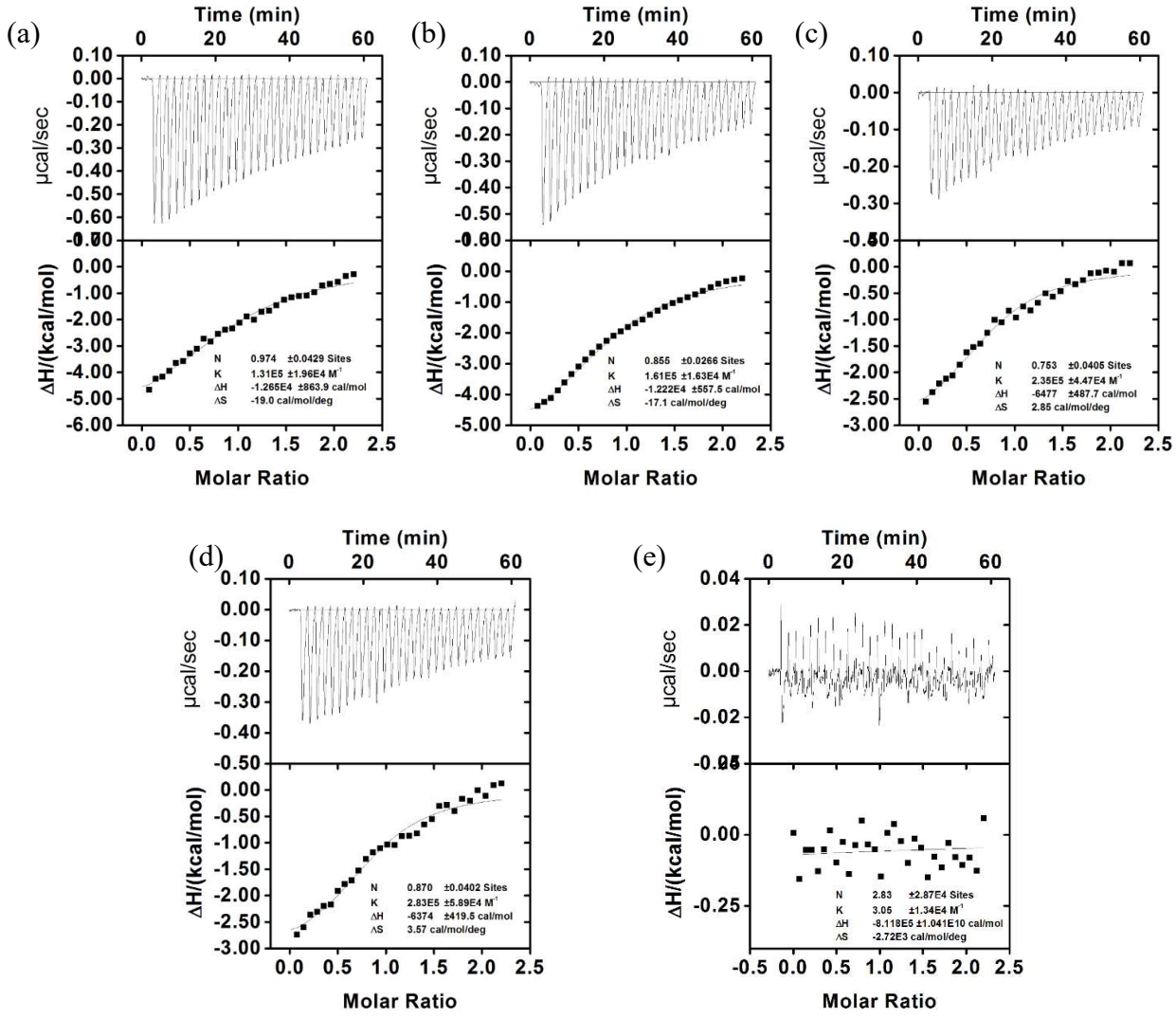


Figure S6. ITC titration curves obtained at 298 K for the titration of (a) MINP(**N-P5**) without functional monomer by lysozyme, (b) MINP(**N-P6**) without functional monomer by lysozyme, (c) MINP(**N-P7**) without functional monomer by lysozyme, (d) MINP(**N-P8**) without functional monomer by lysozyme, (e) MINP(**N-P9**) without functional monomer by lysozyme. The MINP concentration in the ITC titration was (a) 20 µM, (b) 20 µM, (c) 20 µM, (d) 20 µM, and (e) 20 µM, respectively. The concentration of lysozyme in the ITC titration was (a) 200 µM, (b) 200 µM, (c) 200 µM, (d) 200 µM, and (e) 200µ M, respectively. All titrations were performed in 10 mM HEPES buffer (pH 7.0). The data correspond to entries 11, 12, 13, 14, and 15, respectively, in Table S1.

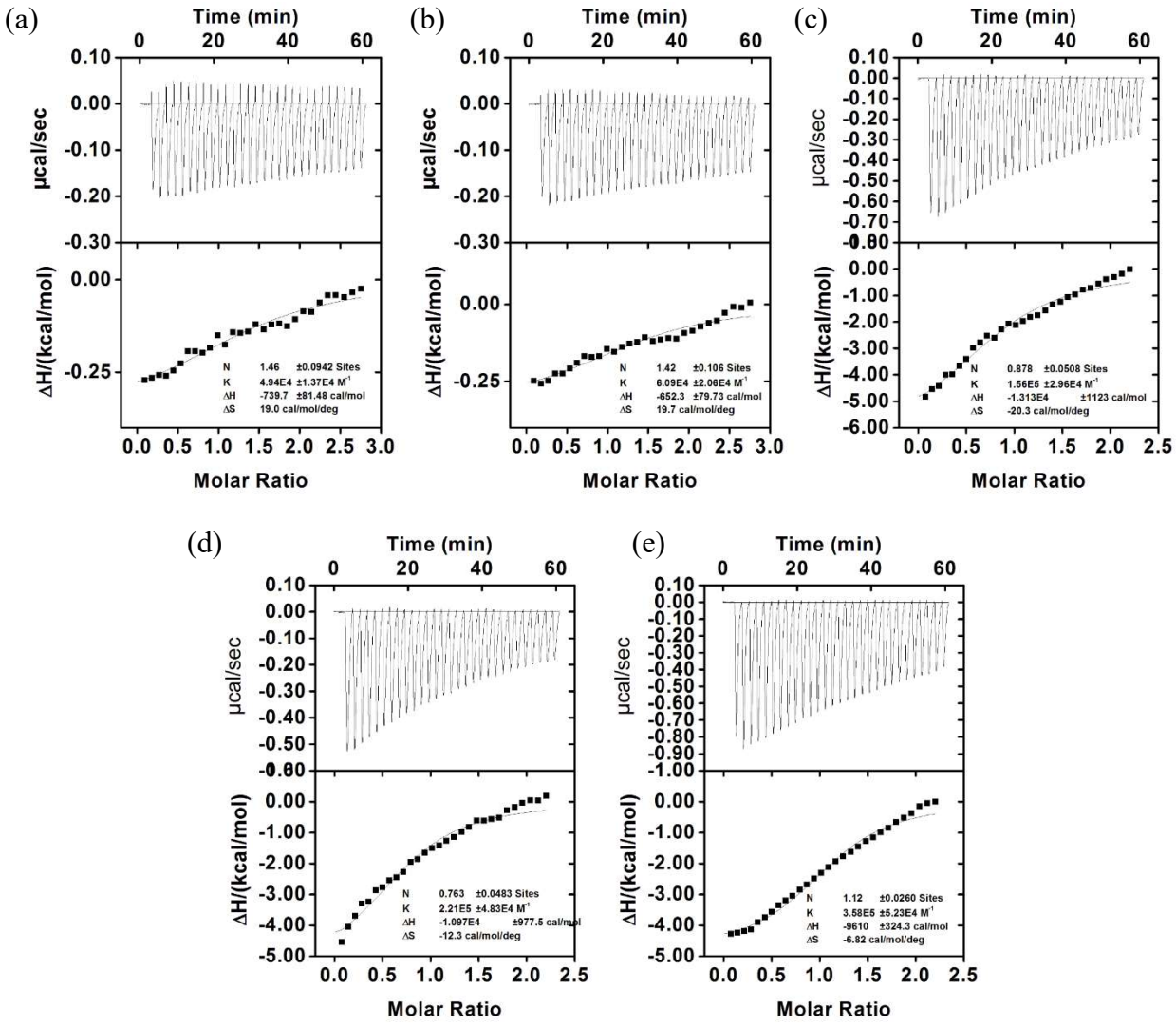


Figure S7. ITC titration curves obtained at 298 K for the titration of (a) MINP(**C-P5**) without functional monomer by lysozyme, (b) MINP(**C-P6**) without functional monomer by lysozyme, (c) MINP(**C-P7**) without functional monomer by lysozyme, (d) MINP(**C-P8**) without functional monomer by lysozyme, (e) MINP(**C-P9**) without functional monomer by lysozyme. The MINP concentration in the ITC titration was (a) 5 µM, (b) 2 µM, (c) 2 µM, (d) 2 µM, and (e) 2 µM, respectively. The concentration of lysozyme in the ITC titration was (a) 50 µM, (b) 50 µM, (c) 50 µM, (d) 50 µM, and (e) 50 µM, respectively. All titrations were performed in 10 mM HEPES buffer (pH 7.0). The data correspond to entries 16, 17, 18, 19, and 20, respectively, in Table S1.

Table S2. Binding data obtained from ITC titrations. [a]

Entry	Template	Guest	FM **4**	FM **5**	K_a ($\times 10^5$ M^{-1})	N	ΔG (kcal·mol^{-1})	ΔH (kcal·mol^{-1})	ΔS (cal·mol^{-1} deg^{-1})
1	N-P5	N-P5	-	3eq.	5.01 ± 0.52	1.27 ± 0.04	-7.77	-9.71	-6.5
2	N-P6	N-P6	-	3eq.	6.17 ± 0.48	1.04 ± 0.03	-8.04	-57.21	-165
3	N-P7	N-P7	1.5eq.	3eq.	7.13 ± 0.71	0.72 ± 0.03	-8.01	-64.63	-190
4	N-P8	N-P8	1.5eq.	3eq.	8.68 ± 1.01	1.14 ± 0.03	-8.13	-34.29	-87.8
5	N-P9	N-P9	1.5eq.	3eq.	11.2 ± 1.14	1.30 ± 0.03	-8.29	-44.35	-121
6	C-P5	C-P5	1.5eq.	2eq.	6.90 ± 0.69	0.86 ± 0.03	-8.12	-83.22	-252
7	C-P6	C-P6	1.5eq.	2eq.	9.46 ± 0.97	1.46 ± 0.05	-8.24	-134.00	-422
8	C-P7	C-P7	1.5eq.	2eq.	10.10 ± 1.42	0.77 ± 0.07	-8.23	-117.60	-367
9	C-P8	C-P8	1.5eq.	2eq.	18.80 ± 2.32	1.45 ± 0.04	-8.62	-101.00	-310
10	C-P9	C-P9	1.5eq.	2eq.	32.50 ± 7.67	1.57 ± 0.06	-9.03	-80.55	-240
11	N-P5	Lysozyme	-	3eq.	1.61 ± 0.31	0.92 ± 0.05	-7.10	-4.25	9.55
12	N-P6	Lysozyme	-	3eq.	2.13 ± 0.16	1.07 ± 0.02	-7.27	-5.35	6.43
13	N-P7	Lysozyme	1.5eq.	3eq.	2.96 ± 0.27	0.91 ± 0.02	-7.46	-4.67	9.37
14	N-P8	Lysozyme	1.5eq.	3eq.	3.3 ± 0.48	1.16 ± 0.03	-7.53	-3.98	11.9
15	N-P9	Lysozyme	1.5eq.	3eq.	1.83 ± 0.57	0.70 ± 0.09	-7.18	-4.73	8.21
16	C-P5	Lysozyme	1.5eq.	2eq.	1.86 ± 0.16	0.73 ± 0.02	-7.19	-9.87	-8.99
17	C-P6	Lysozyme	1.5eq.	2eq.	2.14 ± 0.16	0.73 ± 0.02	-7.27	-24.52	-57.9
18	C-P7	Lysozyme	1.5eq.	2eq.	2.88 ± 0.32	1.03 ± 0.02	-7.44	-5.60	6.19
19	C-P8	Lysozyme	1.5eq.	2eq.	3.23 ± 0.47	0.95 ± 0.03	-7.52	-3.62	13.1
20	C-P9	Lysozyme	1.5eq.	2eq.	4.26 ± 0.47	1.26 ± 0.02	-7.66	-3.52	13.9

[a] The titrations were performed in 10 mM HEPES buffer (pH 7.0) at 298 K, and the MINPs were prepared with FM. The K_a values in the parentheses are those determined by ITC titration. The FM/carboxylate ratio was kept 1.5:1 in the cases with FM **4**. The FM/amine or FM/guanidyl ratio was kept 1:1 in the cases with FM **5**.

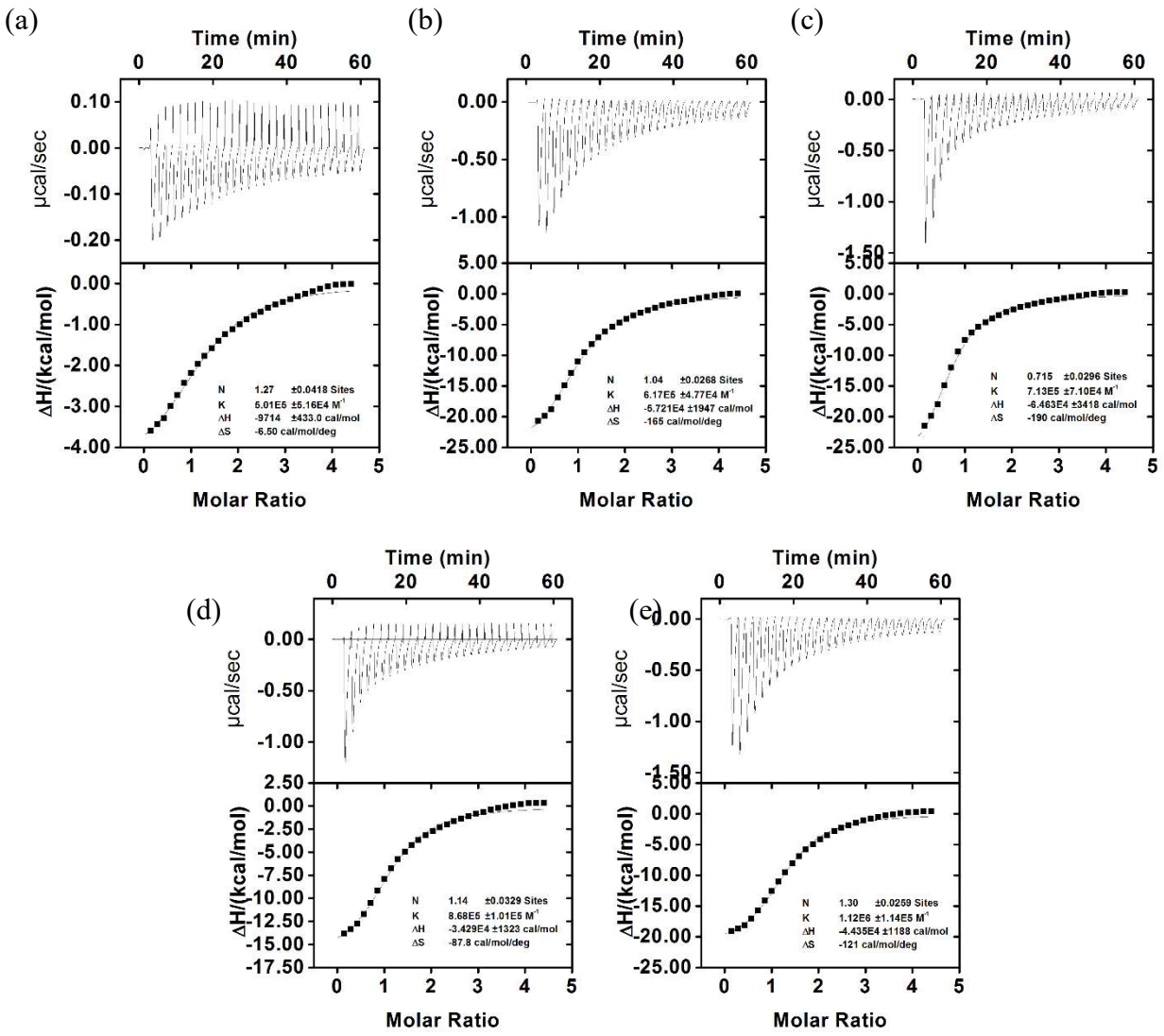


Figure S8. ITC titration curves obtained at 298 K for the titration of (a) MINP(**N-P5**) with FM **5** (1:3) by peptide **N-P5**, (b) MINP(**N-P6**) with FM **5** (1:3) by peptide **N-P6**, (c) MINP(**N-P7**) with FM **4** (1:1.5) and FM **5** (1:3) by peptide **N-P7**, (d) MINP(**N-P8**) with FM **4** (1:1.5) and FM **5** (1:3) by peptide **N-P8**, (e) MINP(**N-P9**) with FM **4** (1:1.5) and FM **5** (1:3) by peptide **N-P9**. The concentration of MINP was 5 µM in all the ITC titrations. The concentration of lysozyme was 100 µM in all the ITC titrations. All titrations were performed in 10 mM HEPES buffer (pH 7.0). The data correspond to entries 1, 2, 3, 4, and 5, respectively, in Table S2.

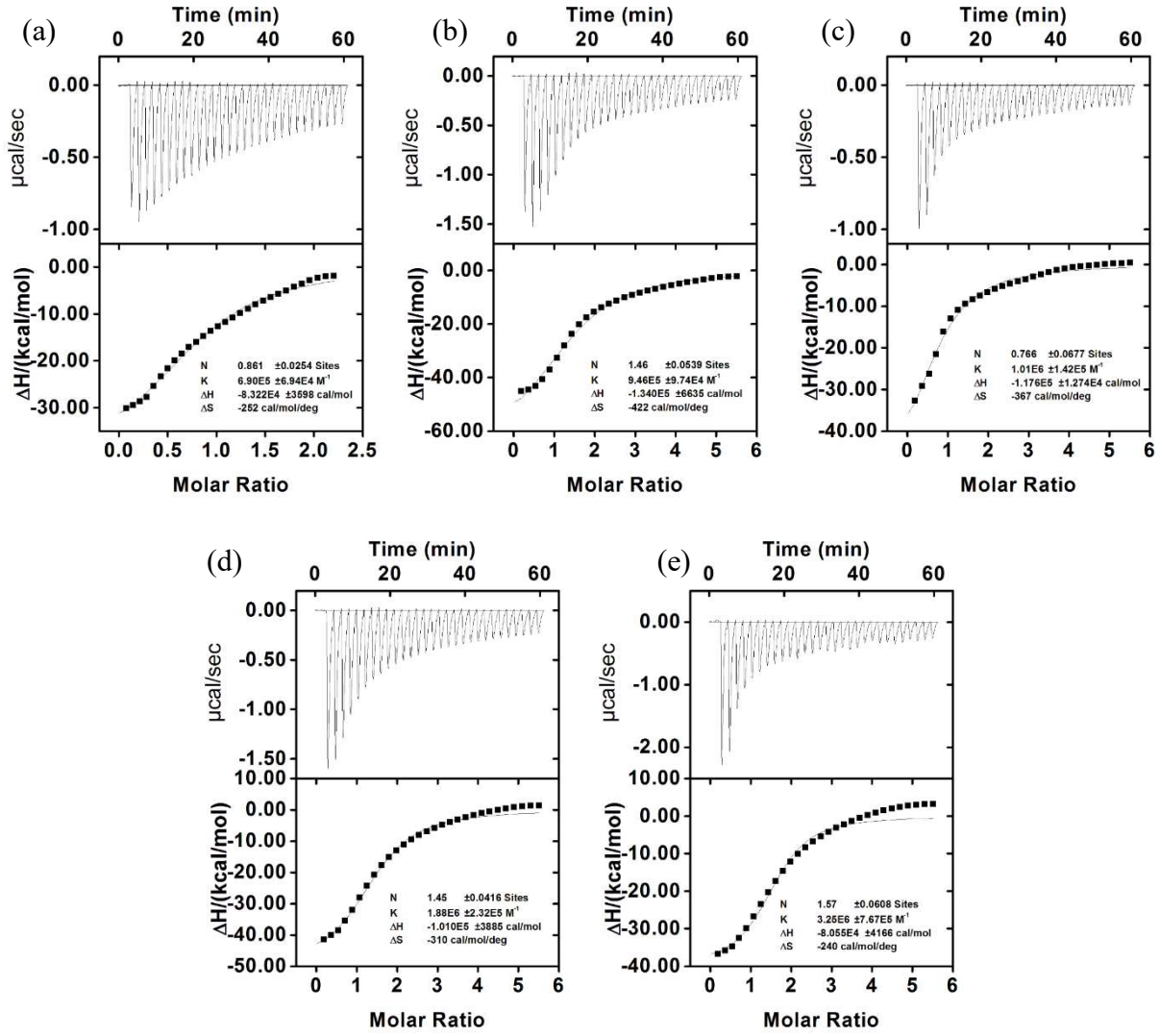


Figure S10. ITC titration curves obtained at 298 K for the titration of (a) MINP(**C-P5**) with FM **4** (1:1.5) and FM **5** (1:2) by peptide **C-P5**, (b) MINP(**C-P6**) with FM **4** (1:1.5) and FM **5** (1:2) by peptide **C-P6**, (c) MINP(**C-P7**) with FM **4** (1:1.5) and FM **5** (1:2) by peptide **C-P7**, (d) MINP(**C-P8**) with FM **4** (1:1.5) and FM **5** (1:2) by peptide **C-P8**, (e) MINP(**C-P9**) with FM **4** (1:1.5) and FM **5** (1:2) by peptide **C-P9**. The concentration of MINP in the ITC titration was (a) 40 µM, (b) 40 µM, (c) 20 µM, (d) 20 µM, and (e) 20 µM, respectively. The concentration of the peptide was (a) 500 µM, (b) 500 µM, (c) 200 µM, (d) 200 µM, and (e) 200 µM, respectively. All titrations were performed in 10 mM HEPES buffer (pH 7.0). The data correspond to entries 6, 7, 8, 9, and 10, respectively, in Table S2.

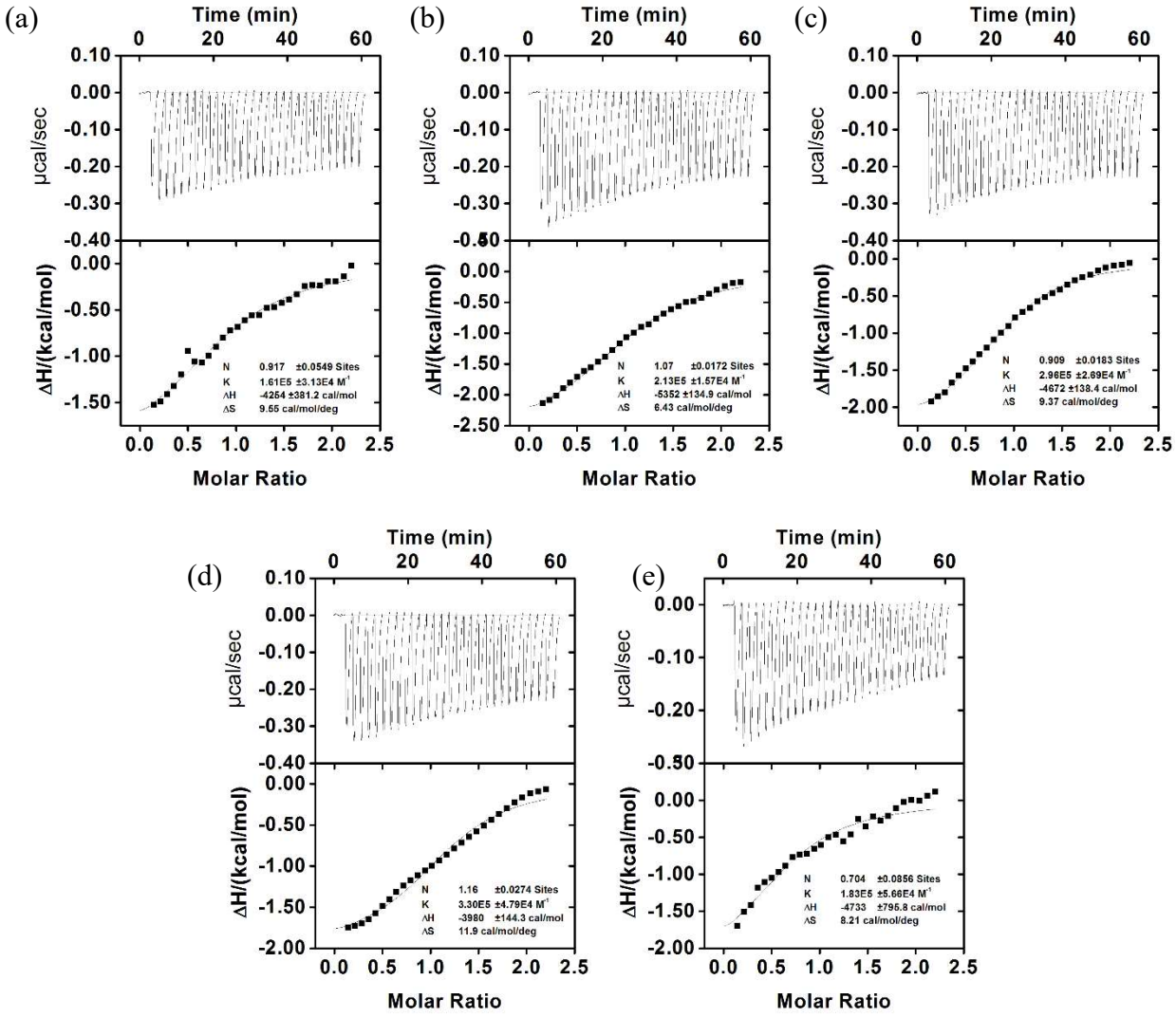


Figure S9. ITC titration curves obtained at 298 K for the titration of (a) MINP(**N-P5**) with FM **5** (1:3) by lysozyme, (b) MINP(**N-P6**) with FM **5** (1:3) by lysozyme, (c) MINP(**N-P7**) with FM **4** (1:1.5) and FM **5** (1:3) by lysozyme, (d) MINP(**N-P8**) with FM **4** (1:1.5) and FM **5** (1:3) by lysozyme, (e) MINP(**N-P9**) with FM **4** (1:1.5) and FM **5** (1:3) by lysozyme. The concentration of MINP was 20 µM in all the ITC titrations. The concentration of lysozyme was 200 µM in all the ITC titrations. All titrations were performed in 10 mM HEPES buffer (pH 7.0). The data correspond to entries 11, 12, 13, 14, and 15, respectively, in Table S2.

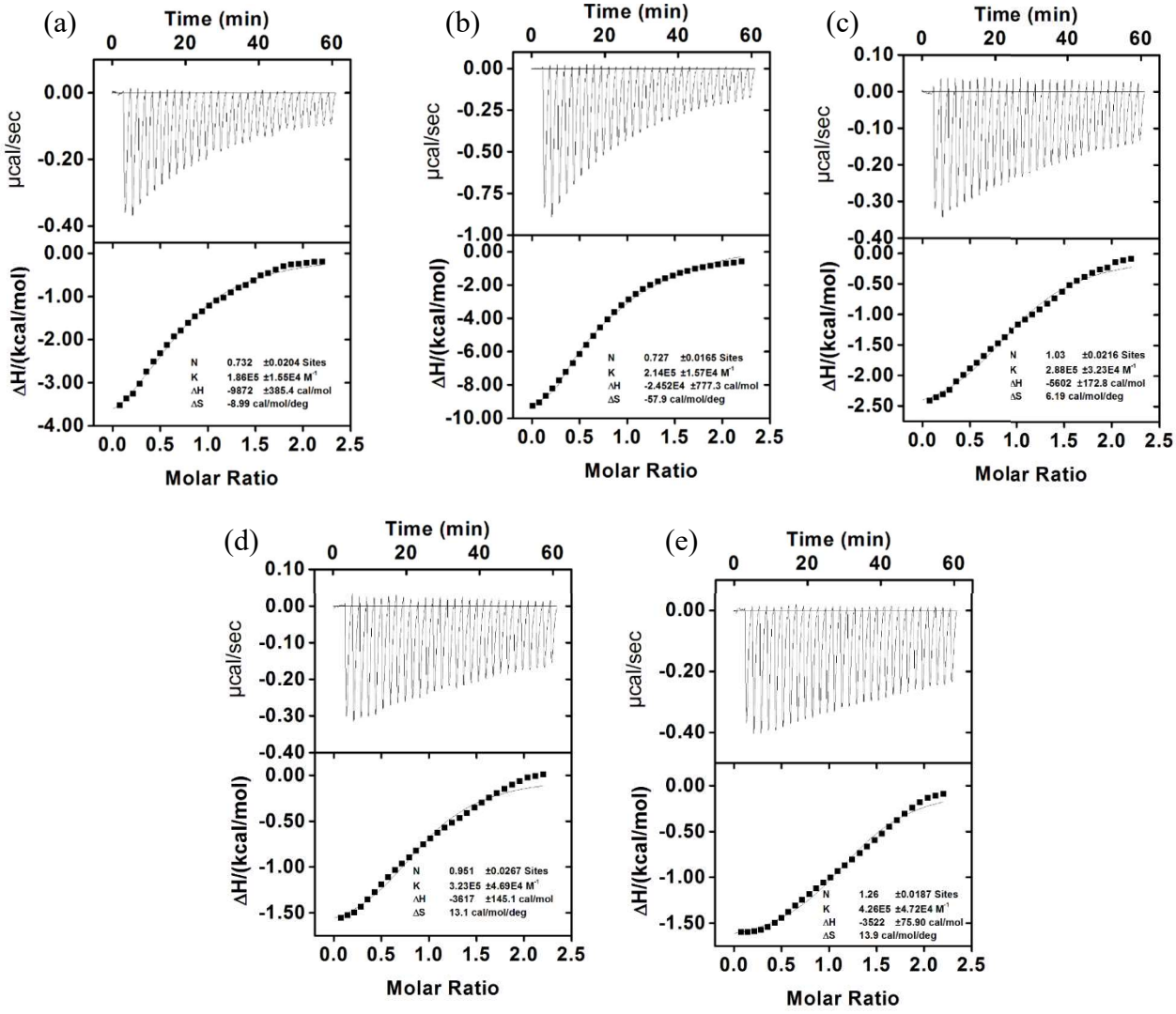


Figure S11. ITC titration curves obtained at 298 K for the titration of (a) MINP(**C-P5**) with FM **4** (1:1.5) and FM **5** (1:2) by lysozyme, (b) MINP(**C-P6**) with FM **4** (1:1.5) and FM **5** (1:2) by lysozyme, (c) MINP(**C-P7**) with FM **4** (1:1.5) and FM **5** (1:2) by lysozyme, (d) MINP(**C-P8**) with FM **4** (1:1.5) and FM **5** (1:2) by lysozyme, (e) MINP(**C-P9**) with FM **4** (1:1.5) and FM **5** (1:2) by lysozyme. The concentration of MINP was 20 µM in all the ITC titrations. The concentration of lysozyme was 200 µM in all the ITC titrations. All titrations were performed in 10 mM HEPES buffer (pH 7.0). The data correspond to entries 16, 17, 18, 19, and 20, respectively, in Table S2.

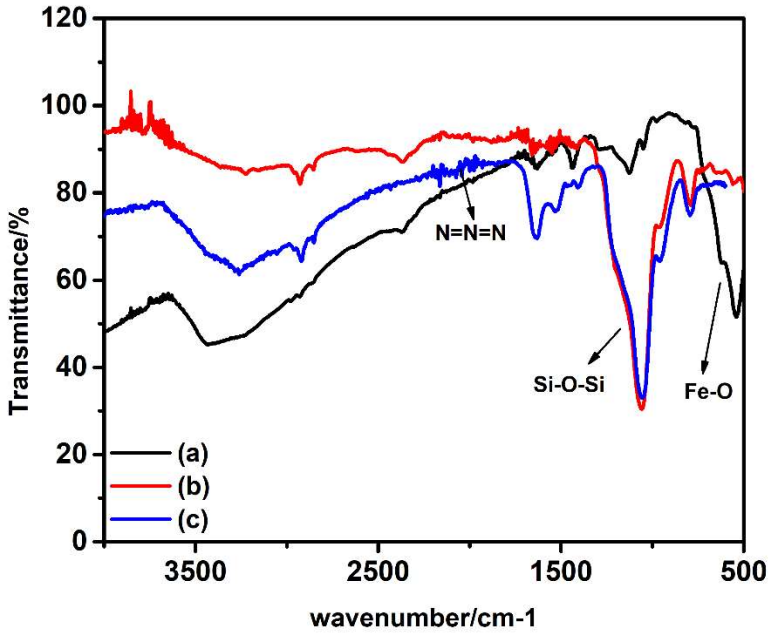


Figure S12. FT-IR of (a) crude magnetic nanoparticles; (b) amine-functionalized magnetic nanoparticles; (c) azido-functionalized magnetic nanoparticles.

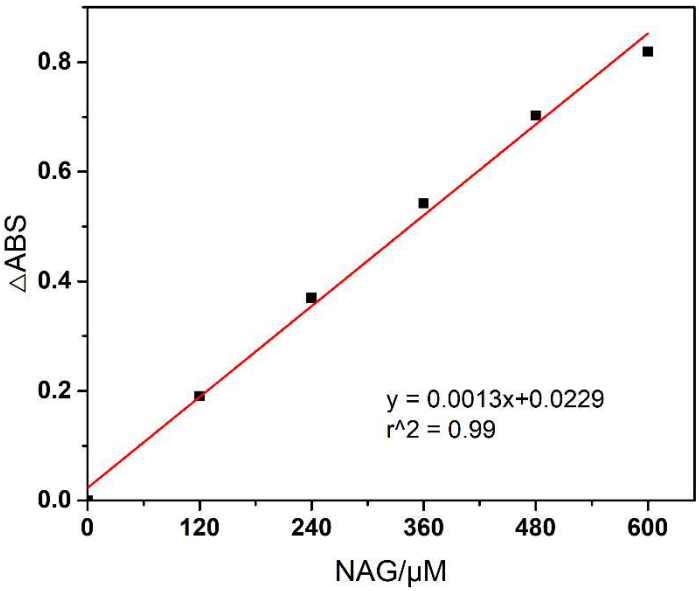


Figure S13. Calibration curve of NAG measured by UV-Vis spectra.

Table S3. Sequences of N- and C-terminal peptides for proteins used in extraction. [a]

Entry	Protein	UniProt	10 AA sequence from the N-terminal	10 AA sequence from the C-terminal
1	lysozyme	P00698	KVFGRCELAA	VQAWIRGCRL
2	cytochrome c	P62894	MGDVEKGKKI	IAYLKKATNE
3	BSA	P02769	MKWVTFISLL	LVVSTQTALA
4	transferrin	P02787	MRLAVGALLV	LLEACTFRRP
5	horseradish peroxidase	P00433	MHFSSSSTLF	VEVVDFVSSM
6	α-chymotrypsin	P00767	CGVPAIQPVL	PWVQETLAAN

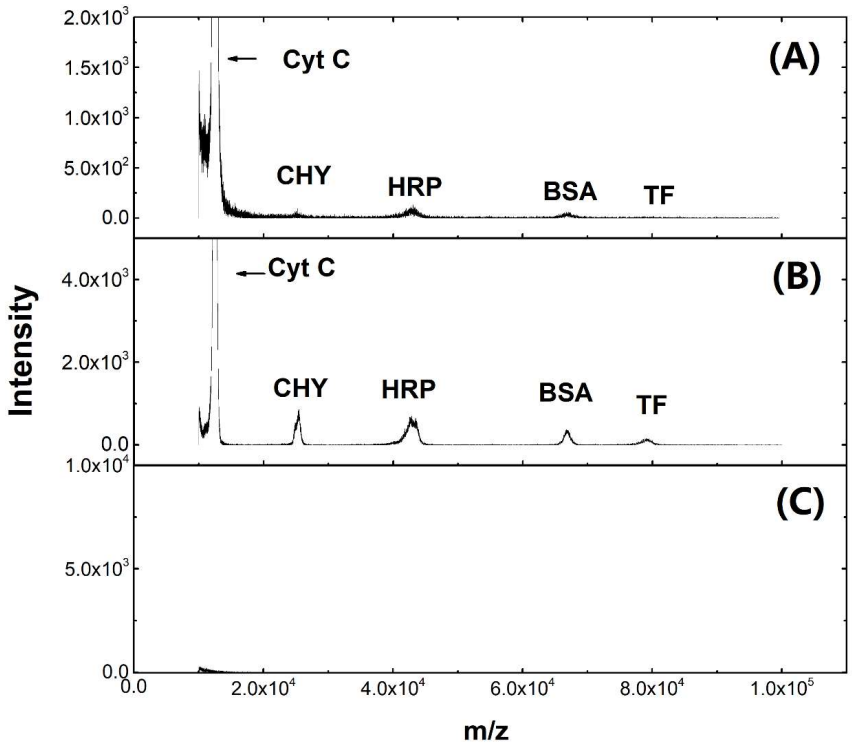


Figure S14. MALDI-TOF MS spectra of (a) a protein mixture, (b) the remaining solution after MINP-MNP extraction, and (c) the released proteins from MINP-MNP. Cyt C = cytochrome complex; CHY = α-chymotrypsin; HRP = horse radish peroxidase; BSA = bovine serum albumin; TF = transferrin. [Proteins] = 1.5 mg/mL. This was the control extraction experiment performed without lysozyme in the mixture showing the nonimprinted proteins were not extracted.