Electronic Supplementary Information

Synthetic Lectins for Selective Binding of Glycoproteins in Water

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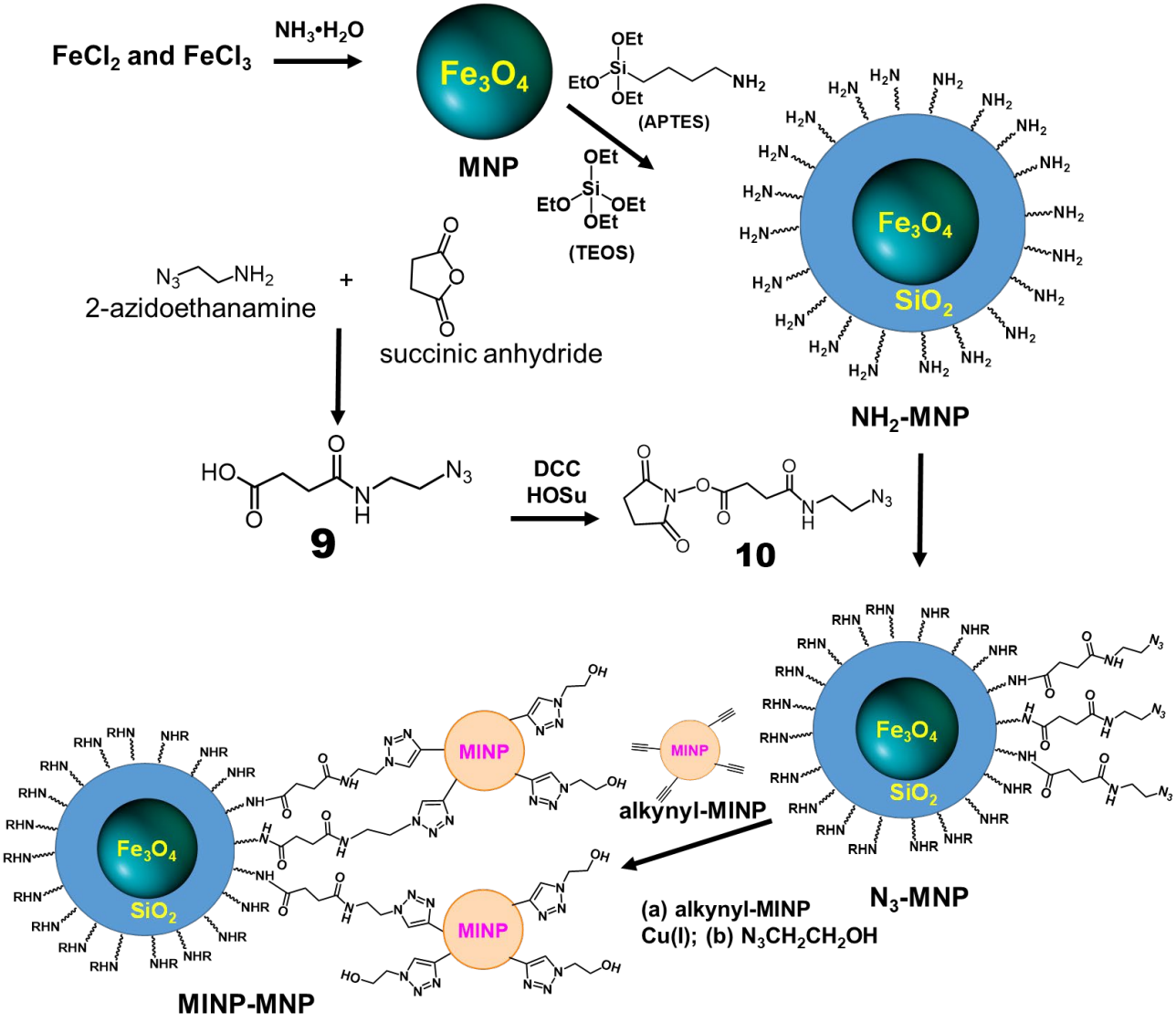
General Method

Routine ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400, Bruker AV II 600, or Varian VXR-400 spectrometer. ESI-MS mass spectra were recorded on Shimadzu LCMS-2010 mass spectrometer. Dynamic light scattering (DLS) data were recorded at 25 °C on a PDDLS/ CoolBatch 90T with PD2000DLS instrument. Isothermal titration calorimetry (ITC) was performed on a MicroCal VP-ITC Microcalorimeter with Origin 7 software and VPViewer2000 (GE Healthcare, Northampton, MA). α1-2,3,6 Mannosidase was purchased from New England BioLabs Inc. Quantification of mannose was performed using an NADP-dependent coupled enzyme assay using the D-mannose/D-fructose/D-glucose assay kit (K-MANGL) from Megazyme (Wicklow, Ireland).

List of Abbreviations

AGP =	alpha-1-acid glycoprotein
APTES =	(3-aminopropyl)triethoxysilane
BSA =	bovine serum albumin
CH2Cl2 =	dichloromethane
Con A =	Concanavalin A
Cyt C =	cytochrome complex
DCC =	N,N'-dicyclohexylcarbodiimide
DLS =	dynamic light scattering
DMPA =	2,2-dimethoxy-2-phenylacetophenone
DVB =	divinylbenzene
FM =	functional monomer
Fuc =	L-fucose
GlcNAc =	N-acetyl-D-glucosamine
HEPES =	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HOSu =	N-hydroxysuccinimide
HRP =	horseradish peroxidase
IF =	imprinting factor
IgG =	Immunoglobulin G
ITC =	isothermal titration calorimetry
MALDI =	matrix-assisted laser desorption/ionization
Man =	D-mannose
MINP =	molecularly imprinted nanoparticle
MNP =	magnetic nanoparticle
NaClO =	sodium hypochlorite
NINP =	nonimprinted nanoparticle
OVA =	ovalbumin
TEOS =	tetraethoxysilane
TF =	transferrin
TFH =	tetrahydrofuran
Xyl =	D-xylose

Scheme S1. ***Preparation of MINP-MNP.***



$FeCl_2$ = iron(II) chloride; $FeCl_3$ = iron(III) chloride; Fe_3O_4 = iron(II,III) oxide; MNP = magnetic nanoparticle; APTES = (3-aminopropyl)triethoxysilane; TEOS = tetraethoxysilane; SiO_2 = silicon oxide; DCC = N,N′-dicyclohexylcarbodiimide; HOSu = N-hydroxysuccinimide; alkylnyl-MINP = alkyne-terminated MINP; $N_3CH_2CH_2OH$ = 2-azidoethanol.

Syntheses

Compound 9. 2-Azidoethanamine (86 mg, 1 mmol) and succinic anhydride (50 mg, 0.5 mmol) were dissolved in dry THF (5 mL). After being stirred at room temperature for 24 h, the reaction mixture

was diluted with CH_2Cl_2 (10 mL). The organic layer was washed with 1 M HCl (10 mL) and water (2 × 10 mL), dried over magnesium sulfate, filtered, and concentrated by rotary evaporation to give a yellowish oil (52 mg, 56 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.45 (s, 4H), 2.74 – 2.68 (m, 2H), 2.57 – 2.49 (m, 2H). ^{13}C NMR (151 MHz, CD_3OD) δ 174.8, 174.7, 50.1, 38.6, 28.8, 28.4. $[M-H]^-$ calcd for $C_6H_9N_4O_3$, 185.0675; found, 185.0680.

Compound 10. N,N'-dicyclohexylcarbodiimide (DCC) (247 mg, 1.2 mmol) was added to a solution of **9** (186 mg, 1 mmol) and N-hydroxysuccinimide (HOSu) (138 mg, 1.2 mmol) in dry THF (10 mL). After the reaction mixture was stirred at room temperature for 24 h, the solid formed was removed by filtration. The filtrate was concentrated by rotary evaporation and the residue was purified by column chromatography over silica gel using 4:1 hexane/ethyl acetate as the eluent to afford a yellowish oil (180 mg, 63 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.45 (s, 4H), 2.74 – 2.68 (m, 2H), 2.57 – 2.49 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.3, 169.1, 168.1, 50.8, 39.0, 30.7, 26.9, 25.6. $[M + H]^+$ calcd for $C_{10}H_{14}N_5O_5$, 284.0995; found, 284.0986.

Oxidative Cleavage of Glycans from Glycoprotein

Preparation of OVA Glycan.[1] A 6% NaClO aqueous solution (2 mL) was added to a stirred solution of ovalbumin (200 mg) in water (10 mL) at room temperature. After 15 min, formic acid (0.1 mL) was added to the reaction mixture slowly. After another 5 min, the precipitate was removed by centrifugation. The supernatant was concentrated by rotary evaporation and the residue was combined with water (1.0 mL). The insoluble material was removed by centrifugation. The supernatant was purified over a Sephadex G-25 column (0.5× 10 cm), and the resulting solution was lyophilized to give

[1] Song, X. Z.; Ju, H.; Lasanajak, Y.; Kudelka, M. R.; Smith, D. F.; Cummings, R. D. Oxidative Release of Natural Glycans for Functional Glycomics. *Nat. Methods* **2016**, *13*, 528-534.

a white powder (3.3 mg). The OVA glycan obtained was analyzed with MALDI-TOF mass spectrometry (Figure S7).

Preparation of HRP Glycan.[1] A 6% NaClO aqueous solution (1 mL) was added to a stirred solution of ovalbumin (100 mg) in water (5 mL) at room temperature. After 15 min, formic acid (0.1 mL) was added slowly to the reaction mixture. After another 5 min, the precipitate was removed by centrifugation. The supernatant was concentrated by rotary evaporation and the residue was combined with water (1.0 mL). The insoluble material was removed by centrifugation. The supernatant was purified over a Sephadex G-25 column (0.5× 10 cm), and the resulting solution was lyophilized to give a white powder (2.9 mg). The HRP glycan obtained was analyzed with MALDI-TOF mass spectrometry (Figure S8).

Preparation and Analysis of Materials

Preparation of MINP.[2] A typical procedure is as follows. A solution of 6-vinylbenzoxaborole (**4**) in methanol (10 μL of a 6.4 mg/mL, 0.0004 mmol) was added to the OVA glycan (**3**) in methanol (10 μL of 56 mg/mL, 0.0004 mmol) in a vial containing methanol (5 mL). After the mixture was stirred for 6 h at room temperature, methanol was removed in vacuo. A micellar solution of compound **6** (5.6 mg, 0.012 mmol), compound **7** (4.5 mg, 0.008 mmol), divinylbenzene (DVB, 2.8 μL, 0.02 mmol), and 2,2-dimethoxy-2-phenylacetophenone (DMPA,10 μL of a 12.8 mg/mL solution in DMSO, 0.0005 mmol) in H_2O (2.0 mL) was added to the glycan–boroxole complex. The mixture was subjected to ultrasonication for 10 min before $CuCl_2$ (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) were added. After the reaction mixture was stirred slowly at room temperature for 12 h, the reaction mixture was transferred into a

[2] Gunasekara, R. W.; Zhao, Y., A General Method for Selective Recognition of Monosaccharides and Oligosaccharides in Water. *J. Am. Chem. Soc.* **2017,** *139*, 829-835.

glass vial, purged with nitrogen for 15 min, sealed with a rubber stopper, and irradiated in a Rayonet reactor for 8 h. Compound **8** (10.6 mg, 0.04 mmol), $CuCl_2$ (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) were added. After being stirred for another 6 h at room temperature, 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), and methanol/acetic acid (5 mL/0.1 mL) for three times and finally with acetone (1×5 mL) to neutral before being dried in air to afford the final MINPs (14 mg).

ITC Titration of OVA with Mannose Enzymatically Cleaved. GlycoBuffer 4 (1.0 μL) and zinc buffer (1.0 μL) were added to a solution of OVA in water (8.0 μL of a 1.3 mg/mL). A 1.0 μL aliquot of α1-2,3,6 mannosidase in water (2 units) was added and the mixture was incubated at 37 °C for 24 h. The mixture was diluted with 10 mM HEPES buffer (pH 7.4) to reach a concentration of 6.0 μM for OVA. The sample obtained was titrated with MINP(OVA) in ITC and reported in Figure 1.

Preparation of MINP-MNP. Preparation of the Fe_3O_4 MNPs was adapted from a reported procedure.[3] A solution of $FeCl_3 \cdot 6H_2O$ (0.28 g, 1 mmol) and $FeCl_2 \cdot 4H_2O$ (0.10 g, 0.5 mmol) in 40 mL 0.1 M HCl (1.5 mL) was added slowly to 12 mL of 1.5 M NH_3 solution until pH 11. The mixture was swirled at room temperature for 2 h. The precipitate was collected by centrifugation, washed with water (3 × 5 mL) and ethanol (3 × 5 mL), and dried at 60 °C to afford the Fe_3O_4 MNP as a black powder (0.17 g). A portion of the MNP (40 mg) was suspended in ethanol (4.0 mL), followed by the addition of tetraethyl orthosilicate (TEOS, 0.5 mL) in water (10 mL) and ethanol (20 mL). The mixture was vortexed at room temperature for 12 h. The precipitate was washed with water (3 × 5 mL) and ethanol (3 × 5 mL) to give a black powder (37 mg). The material obtained (30 mg) was dispersed in 10 mL of water, followed by the addition of 3-aminopropyltriethoxysilane (APTES,

[3] Liu, X.; Ma, Z.; Xing, J.; Liu, H., Preparation and characterization of amino–silane modified superparamagnetic silica nanospheres. *J. Magn. Magn. Mater.* **2004,** *270*, 1-6.

0.10 mL) and CH_3COOH (0.15 mL). The reaction mixture was vortexed at room temperature for 5 h. The precipitate was washed with DMF (3 × 5 mL) and ethanol (3 × 5 mL) to give NH_2-MNP as a black powder (25 mg). A portion of the NH_2-MNP (5.0 mg) was dispersed in DMSO (0.25 mL) and sonicated for 30 min, followed by the addition of compound **10** (8.5 mg, 0.03 mmol) and triethylamine (3 μL, 0.04 mmol). The mixture was vortexed gently at room temperature for 1 h. The precipitate was washed with DMSO (3 × 0.1 mL) to give N_3-MNP as a black powder (3.0 mg). MINP(OVA) prepared above, without surface-functionalization with **8**, 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 vortexed gently at room temperature for 6 h, the precipitate was collected with a magnet, washed with distilled water (3 × 5 mL) and ethanol (3 × 5 mL) to give MINP-MNP as a black powder (1.8 mg).

Extraction of OVA from a OVA/HRP/TF/IgG Mixture. A stock solution of each protein (1.5 mg/mL) was prepared with an ammonium bicarbonate buffer (50 mM with 0.5 M NaCl). The OVA/HRP/TF/IgG protein mixture was obtained by combining 100 μL of the four protein stock solutions. The MINP-MNP composite (2.0 mg) was added to the protein mixture and the resulting mixture was vortexed gently at room temperature for 2 h. The supernatant was removed while a magnet was applied to the MINP-MNP composite. A 200 μL aliquot of the ammonium bicarbonate buffer (50 mM with 0.5 M NaCl) was added and the mixture was vortexed gently at room temperature for 5 min before the buffer was removed. The washing step was repeated two more times. After washing, the MINP-MNP composite was suspended in 50 μL of 100 mM acetic acid solution and vortexed gently for 1 h. The supernatant was collected and dried under a flow of nitrogen. The residue was dissolved in 20 μL of ammonium bicarbonate buffer (50 mM with 0.5 M NaCl) and analyzed by MALDI-TOF MS, with the results shown in Figure 4.

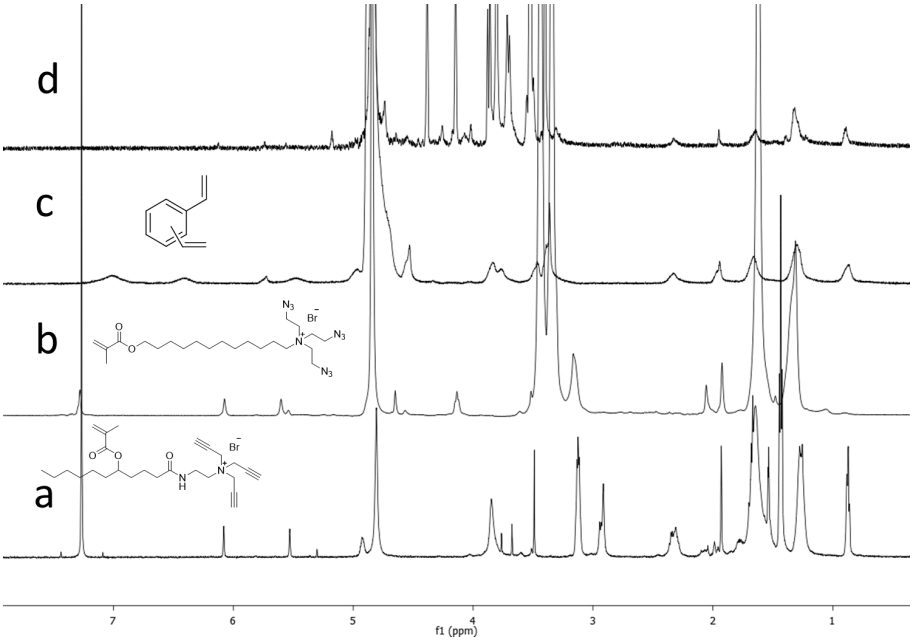


Figure S1. ^{1}H NMR for MINP(OVA). ^{1}H NMR spectra of (a) **6** in $CDCl_3$, (b) **7** in CD_3OD, (c) alkynyl-SCM in D_2O and (d) MINP in D_2O.

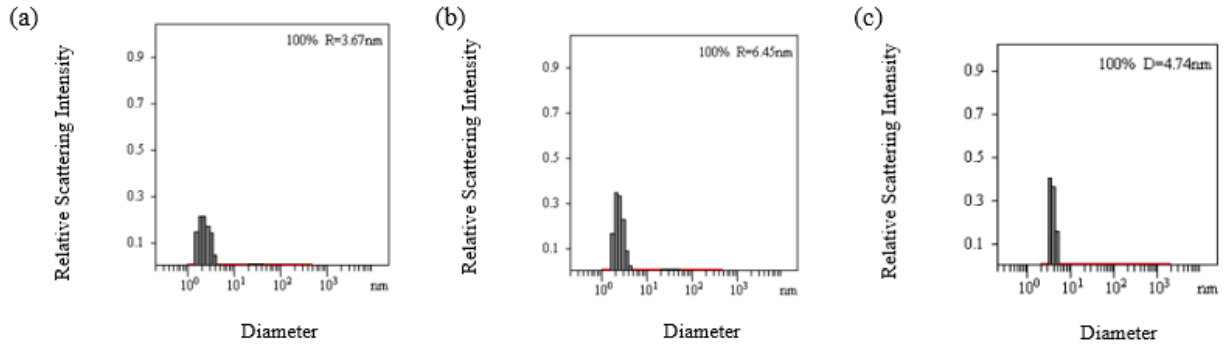


Figure S2. DLS for MINP(OVA). Distribution of the hydrodynamic diameters of the nanoparticles in water as determined by DLS for (a) alkynyl-SCM, (b) surface-functionalized SCM, and (c) MINP after purification.

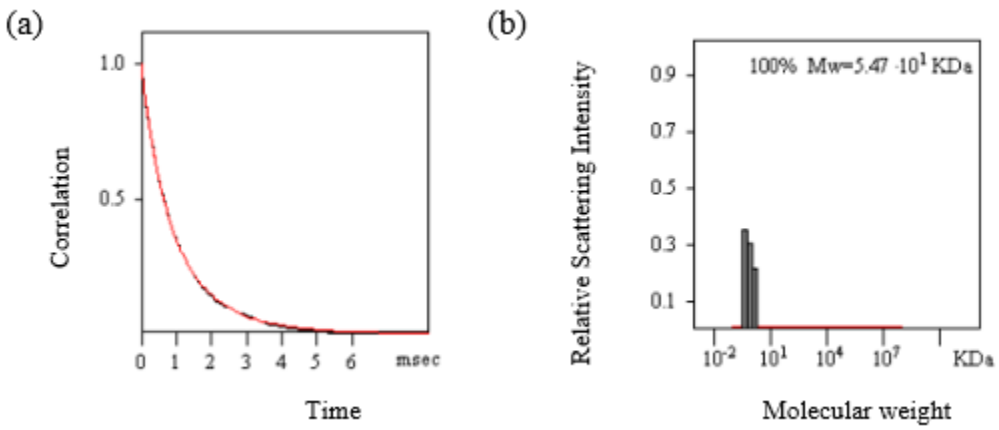


Figure S3. DLS for MINP(OVA). The correlation curve and the distribution of the molecular weight for MINP(OVA) from the DLS. The PRECISION DECONVOLVE program assumes the intensity of scattering is proportional to the mass of the particle squared. If each unit of building block for the MINP(OVA) is assumed to contain 0.6 molecule of compound **6** (MW = 465 g/mol), 0.4 molecule of compound **7** (MW = 558 g/mol), 1 molecule of DVB (MW = 130 g/mol), and 0.6 molecules of compound **8** (MW = 264 g/mol), and 0.16 molecules of compound 4 (MW = 160 g/mol), the molecular weight of MINP(OVA) translates to 66 [= 54700 / (0.6×465 + 0.4×558 + 0.8×264 + 130 + 0.16×264)] of such units.

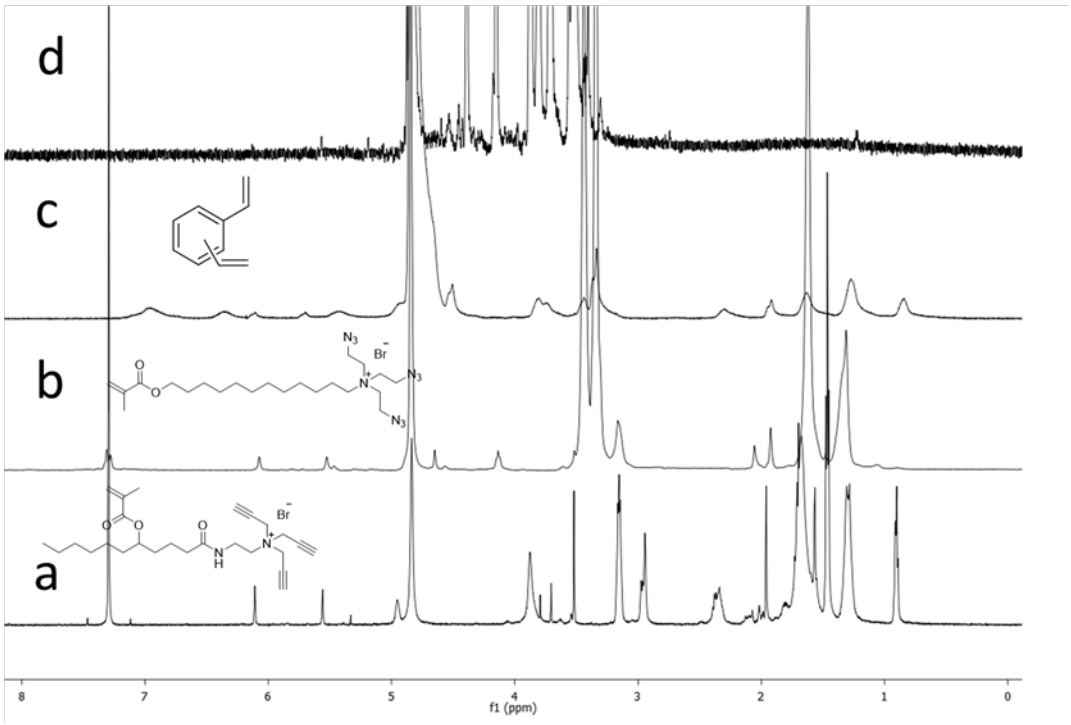


Figure S4. ^{1}H NMR for MINP(HRP). ^{1}H NMR spectra of (a) **6** in $CDCl_3$, (b) **7** in CD_3OD, (c) alkynyl-SCM in D_2O and (d) MINP in D_2O.

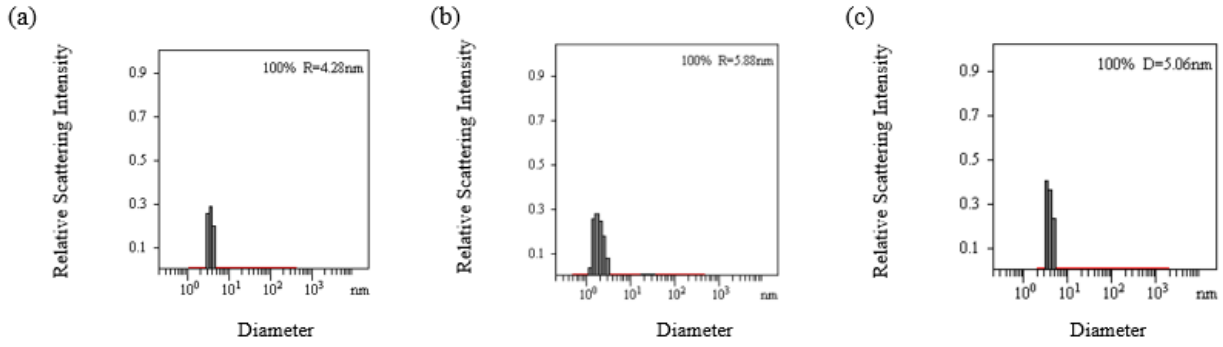


Figure S5. DLS for MINP(HRP). Distribution of the hydrodynamic diameters of the nanoparticles in water as determined by DLS for (a) alkynyl-SCM, (b) surface-functionalized SCM, and (c) MINP after purification.

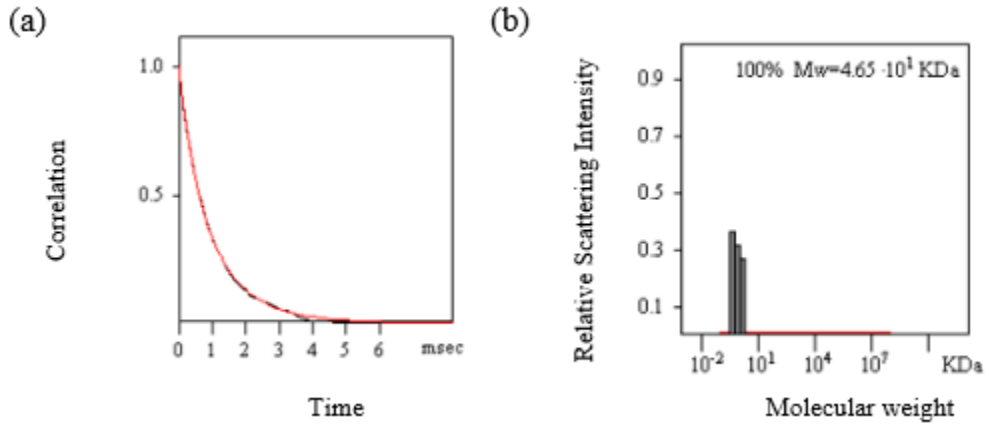


Figure S6. DLS for MINP(HRP). The correlation curve and the distribution of the molecular weight for MINP(HRP) from the DLS. The PRECISION DECONVOLVE program assumes the intensity of scattering is proportional to the mass of the particle squared. If each unit of building block for the MINP(HRP) is assumed to contain 0.6 molecule of compound **6** (MW = 465 g/mol), 0.4 molecule of compound **7** (MW = 558 g/mol), 1 molecule of DVB (MW = 130 g/mol), and 0.6 molecules of compound **8** (MW = 264 g/mol), and 0.08 molecules of compound 4 (MW = 160 g/mol), the molecular weight of MINP(HRP) translates to 57 [= 46500 / (0.6×465 + 0.4×558 + 0.8×264 + 130 + 0.08×264)] of such units.

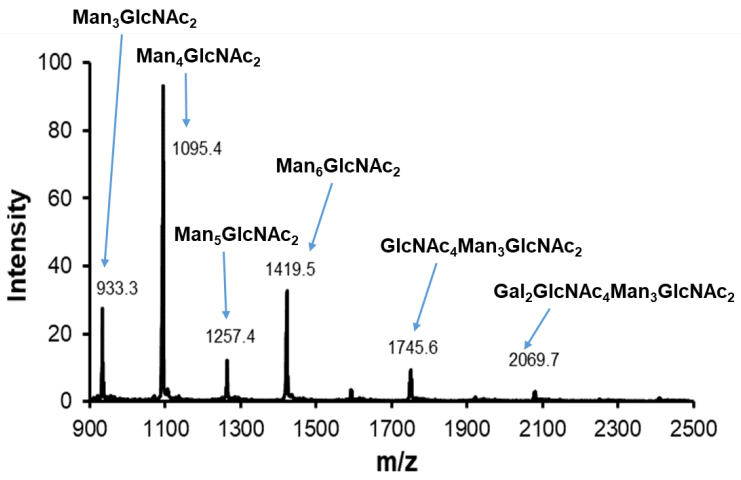


Figure S7. MALDI-TOF MS spectrum of the cleaved OVA glycan. MALDI-TOF MS spectra for the analysis of the glycan cleavage from OVA. A Shimadzu AXIMA Confidence MALDI TOF Mass Spectrometer and an anchor-chip target plate were used for MS analysis. Reflective positive mode was used for the cleaved glycan. 2,5-dihydroxybenzoic acid (DHB) (5 mg/mL in 50% acetonitrile with 0.1% trifluoroacetic acid) was used as matrix.

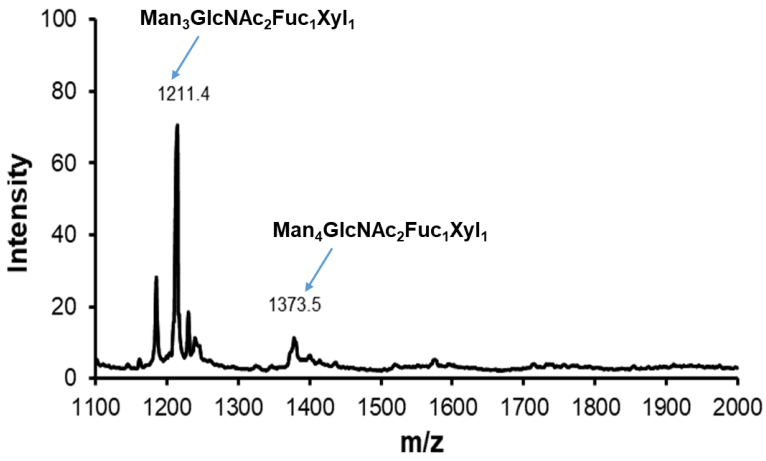


Figure S8. MALDI-TOF MS spectrum of the cleaved HRP glycan. MALDI-TOF MS spectra for the analysis of the glycan cleavage from HRP. A Shimadzu AXIMA Confidence MALDI TOF Mass Spectrometer and an anchor-chip target plate were used for MS analysis. Reflective positive

mode was used for the cleaved glycan. 2,5-dihydroxybenzoic acid (DHB) (5 mg/mL in 50% acetonitrile with 0.1% trifluoroacetic acid) was used as matrix.

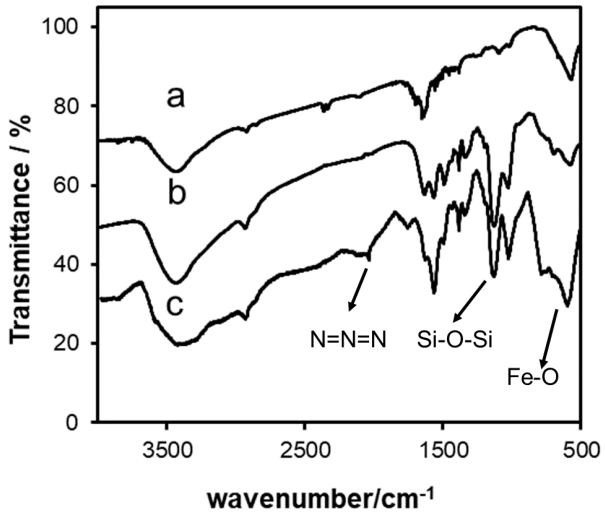


Figure S9. FT-IR. FT-IR of (a) crude magnetic nanoparticles; (b) amine-functionalized magnetic nanoparticles; (c) azido-functionalized magnetic nanoparticles. The peak at 1127 cm^{-1} corresponds to the SiO-H stretching. The peak at 2095 cm^{-1} corresponds to N=N=N antisymmetric stretching. The peak at 2095 cm^{-1} corresponds to the azide antisymmetric stretching. The peak at 580 cm^{-1} corresponds to Fe-O stretching.

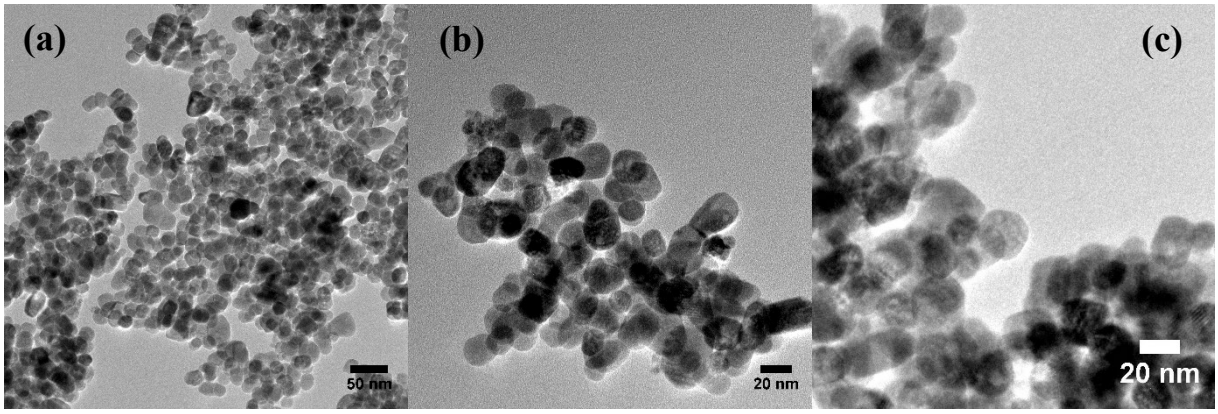


Figure S10. TEM of MINP-MNP. TEM images of MINP-MNP. Transmission electron microscopy was carried out using a TECNAI G2 F20 operated at 200 kV.

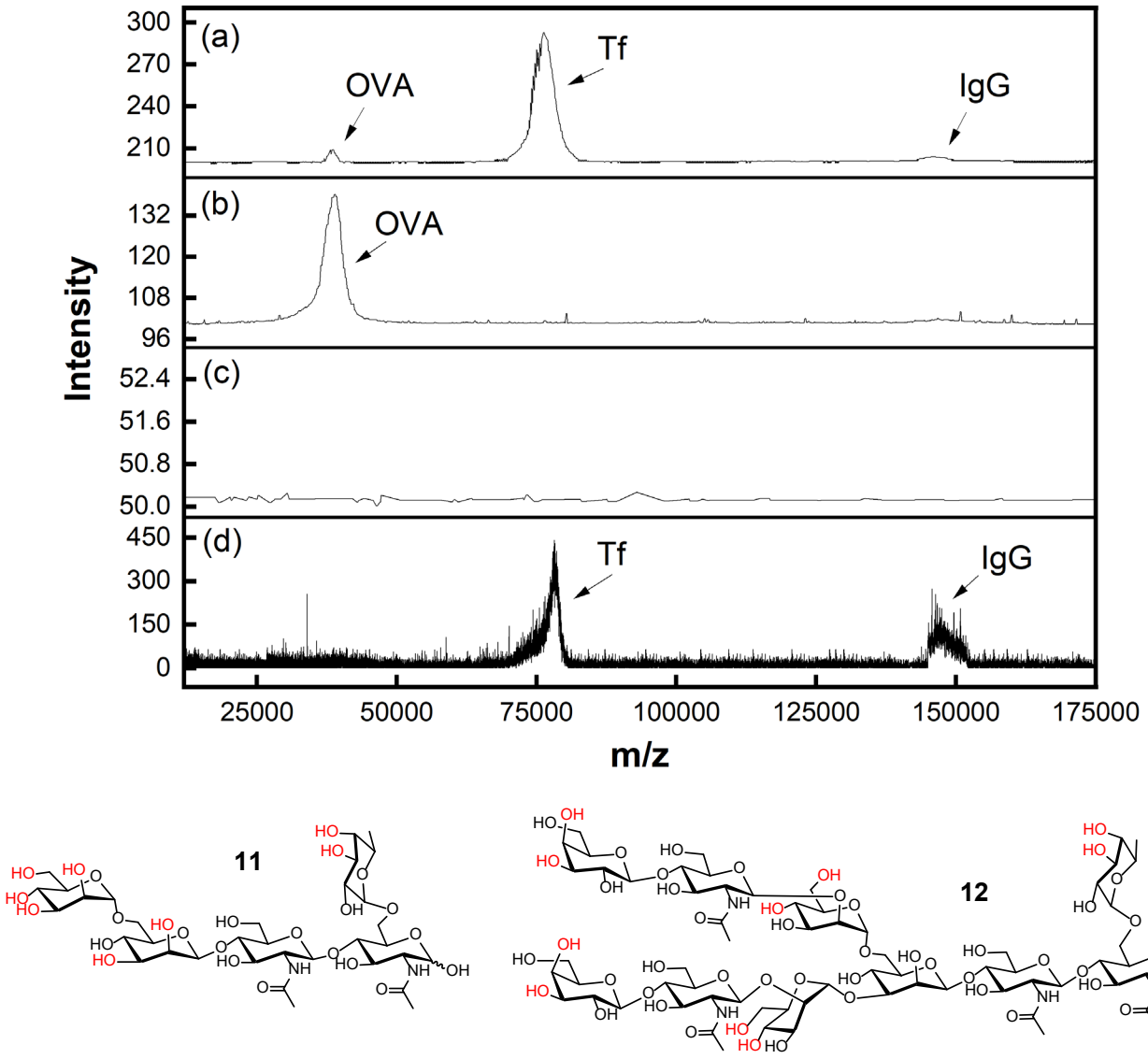


Figure S11. MALDI-TOF MS spectra for the analysis of mixture. MALDI-TOF MS spectra for the analysis of the extraction of ovalbumin in a mixture of three proteins. (a) OVA/TF/IgG mixture. (b) Extracted proteins released from MINP(OVA)-MNP. (c) Extracted proteins released from NINP-MNP. (d) Remaining solution after extraction with MINP(OVA)-MNP. Compound **11** is a representative glycan on transferrin[4] and **12** a glycan on IgG.[5]

[4] Choi, O.; Tomiya, N.; Kim, J. H.; Slavicek, J. M.; Betenbaugh, M. J.; Lee, Y. C. N-Glycan Structures of Human Transferrin Produced by Lymantria Dispar (Gypsy Moth) Cells Using the Ldmnpv Expression System. *Glycobiology* **2003**, *13*, 539-548.

[5] Huhn, C.; Selman, M. H. J.; Ruhaak, L. R.; Deelder, A. M.; Wuhrer, M. Igg Glycosylation Analysis. *Proteomics* **2009**, *9*, 882-913.

Table S1. Binding constants of MINP(OVA) and MINP(HRP) obtained by isothermal titration calorimetry (ITC).[a]

entry	templating glycan source	FM/template ratio	guest[b]	K_a ($\times 10^4$ M^{-1})	-ΔG (kcal/mol)
1	OVA	8:1	OVA	9.41 ± 0.21	6.78
2	OVA	8:1	HRP	0.73 ± 0.02	5.27
3	OVA	8:1	IgG	0.92 ± 0.03	5.40
4	OVA	8:1	AGP	0.051 ± 0.004	3.70
5	OVA	8:1	TF	0.071 ± 0.003	3.89
6	OVA	8:1	BSA	0.01[c]	2.78
7	OVA	8:1	Lysozyme	0.02[c]	3.34
8	OVA	8:1	Trypsin	0.01[c]	2.78
9	OVA	8:1	Cyt C	0.04[c]	3.58
10	HRP	4:1	OVA	0.12 ± 0.02	4.20
11	HRP	4:1	HRP	1.09 ± 0.06	5.50
12	HRP	4:1	IgG	0.41 ± 0.02	4.93
13	HRP	4:1	AGP	0.04[c]	3.58
14	HRP	4:1	TF	0.05[c]	3.67
15	HRP	4:1	BSA	0.02[c]	3.25
16	HRP	4:1	Lysozyme	0.01[c]	2.83
17	HRP	4:1	Trypsin	0.02[c]	2.93
18	HRP	4:1	Cyt C	0.02[c]	3.13

[a] The titrations were performed in duplicates with the indicated errors in HEPES buffer (10 mM, pH 7.4) at 298 K. [b]Glycan indicates the guest was the same templating glycan mixture from the corresponding glycoprotein. [c] Binding was very weak and the binding constant was estimated from ITC titration.

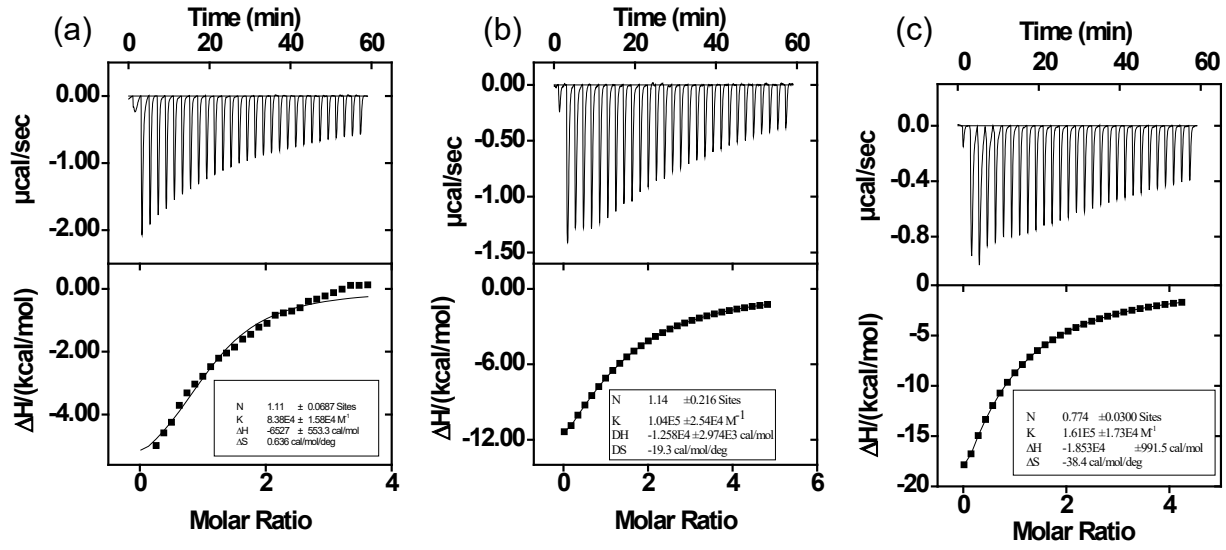


Figure S12. ITC titration of glycan by MINP (OVA) ITC titration curves obtained at 298 K for the titration of glycan with MINP(OVA) of various FM **4**/template ratio (a) 4:1, (b) 6:1, and (c) 8:1 in 10 mM HEPES buffer (pH = 7.4). [glycan] = 5 µM. [MINP(OVA)] = 60 µM. The data correspond to entry 1-3, respectively, in Table 1. 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 MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

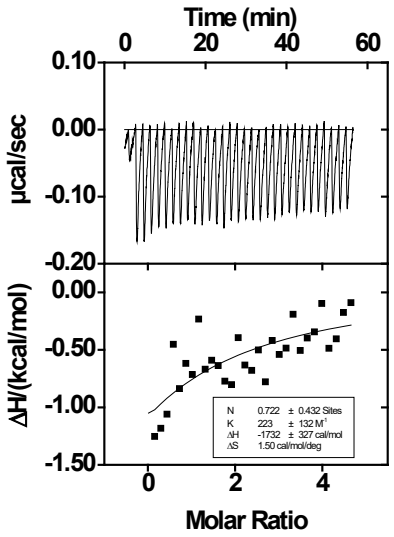


Figure S13. ITC titration of glycan by NINP ITC titration curves obtained at 298 K for the titration of glycan with NINP of FM **4**/template ratio 8:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 4 in Table 1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

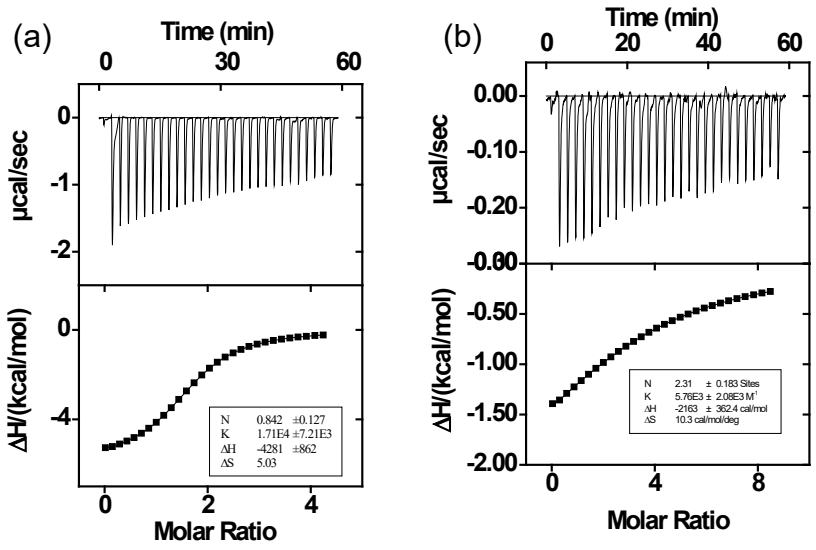


Figure S14. ITC titration of glycoprotein by MINP (OVA) ITC titration curves obtained at 298 K for the titration of OVA(a) or HRP(b) with MINP(OVA) of FM **4**/template ratio 4:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 5-6 in Table 1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

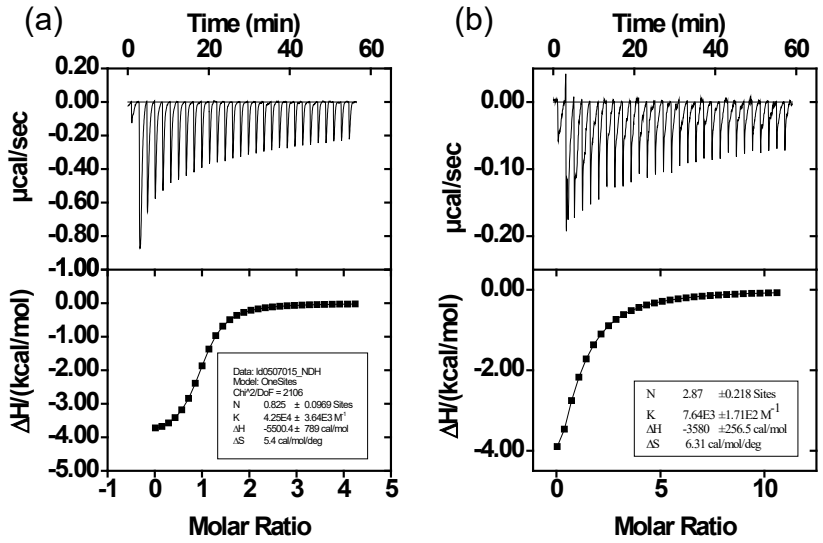


Figure S15. ITC titration of glycoprotein by MINP (OVA) ITC titration curves obtained at 298 K for the titration of OVA or HRP with MINP(OVA) of FM **4**/template ratio 6:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 7-8 in Table 1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

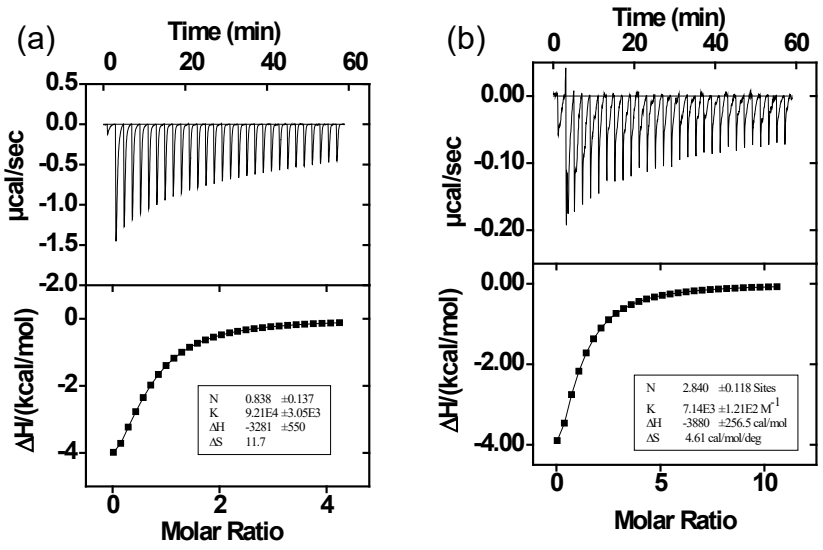


Figure S16. ITC titration of glycoprotein by MINP (OVA) ITC titration curves obtained at 298 K for the titration of OVA or HRP with MINP(OVA) of FM **4**/template ratio 8:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 9-10 in Table 1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

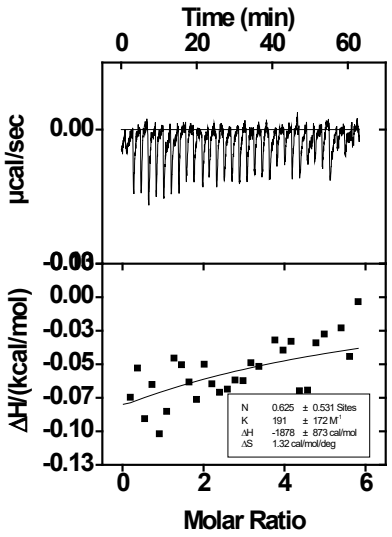


Figure S17. ITC titration of glycoprotein by NINP ITC titration curves obtained at 298 K for the titration of OVA or HRP with NINP of FM **4**/template ratio 8:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 11 in Table 1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

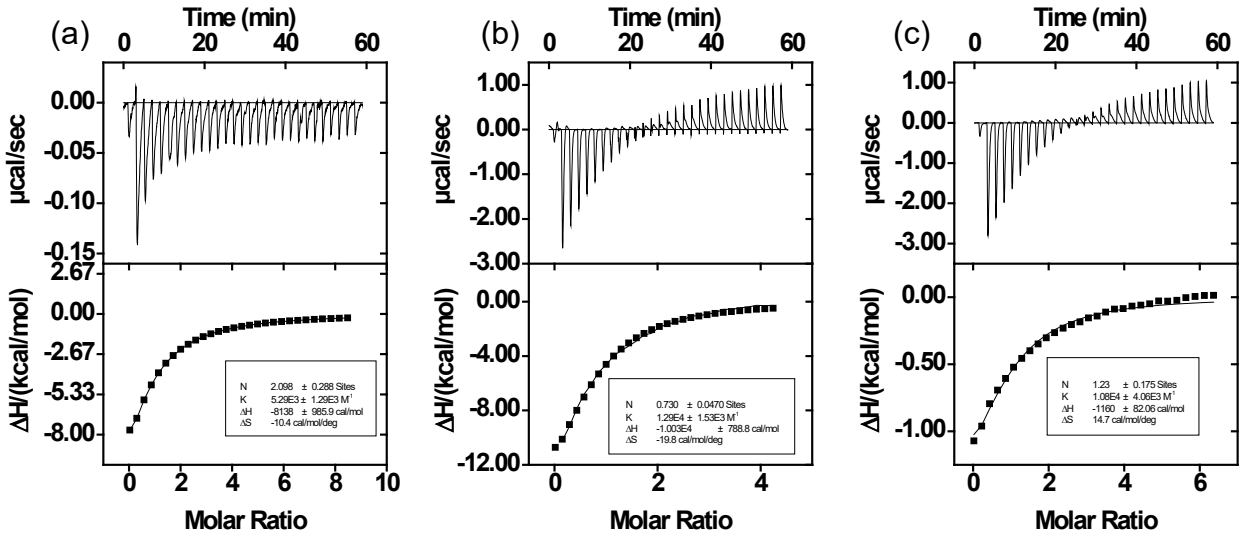


Figure S18. ITC titration of glycan by MINP (HRP) ITC titration curves obtained at 298 K for the titration of glycan with MINP(HRP) of various FM **4**/template ratio (2:1, 4:1, and 6:1) in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 12-14 in Table 1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

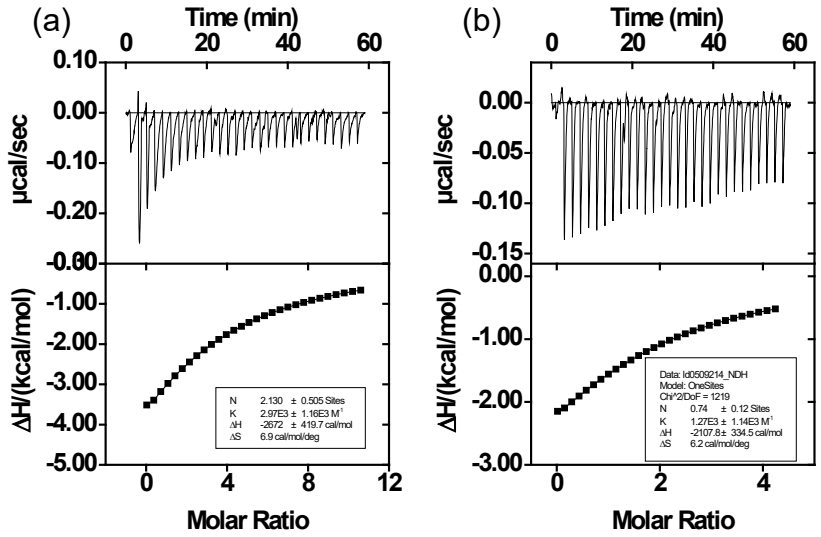


Figure S19. ITC titration of glycoprotein by MINP (HRP) ITC titration curves obtained at 298 K for the titration of glycoprotein with MINP(HRP) of FM **4**/template ratio 2:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 15-16 in Table 1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

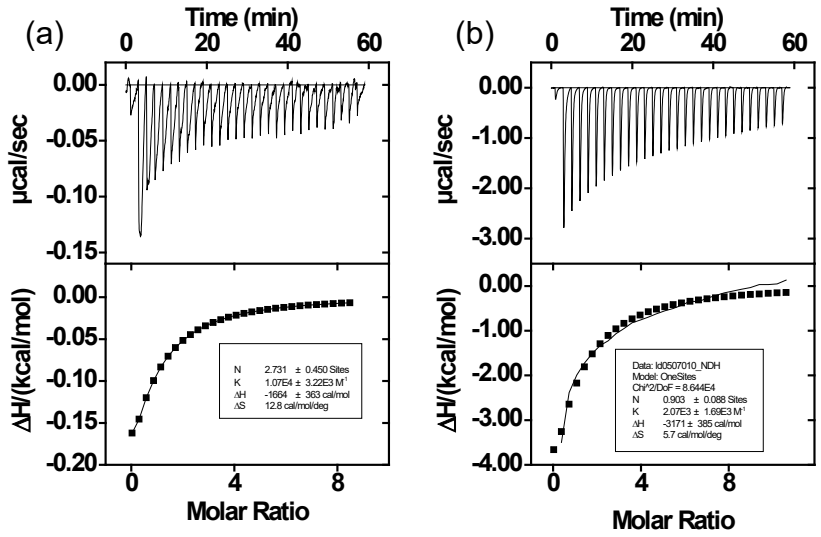


Figure S20. ITC titration of glycoprotein by MINP (HRP) ITC titration curves obtained at 298 K for the titration of glycoprotein with MINP(HRP) of FM **4**/template ratio 4:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 17-18 in Table 1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

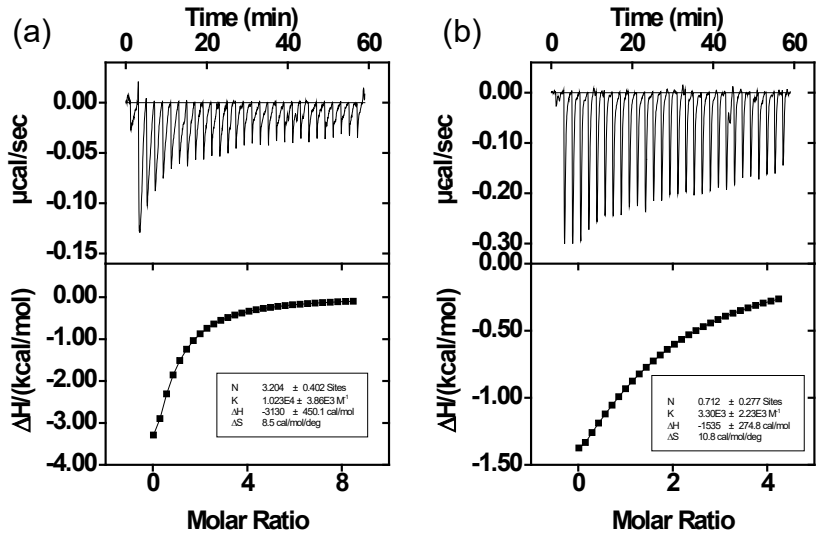


Figure S21. ITC titration of glycoprotein by MINP (HRP) ITC titration curves obtained at 298 K for the titration of glycoprotein with MINP(HRP) of FM **4**/template ratio 6:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 19-20 in Table 1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

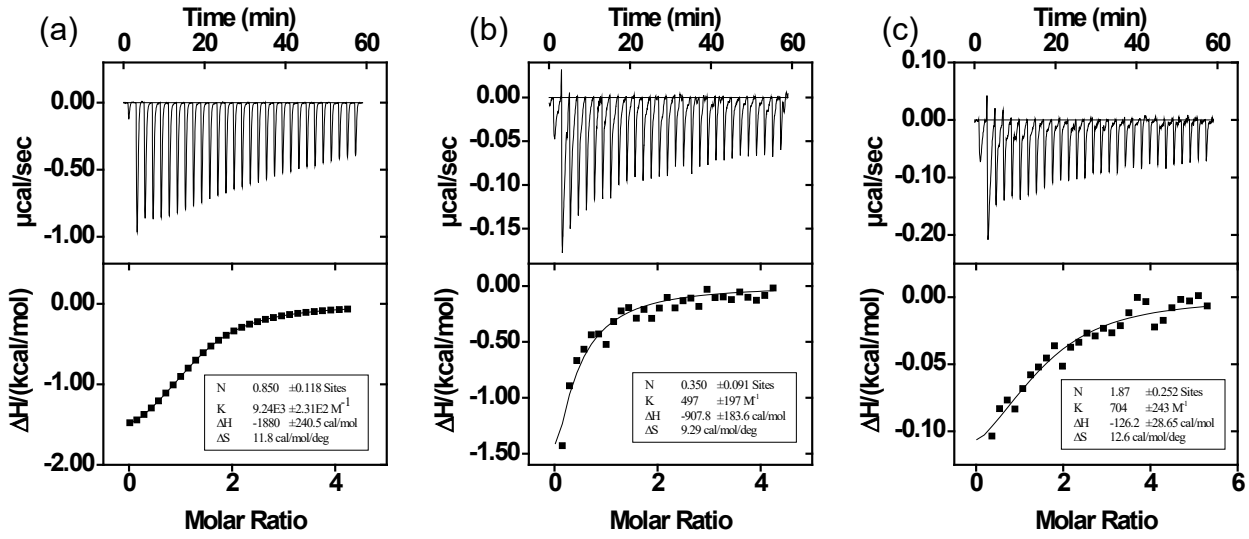


Figure S22. ITC titration of glycoprotein by MINP (OVA) ITC titration curves obtained at 298 K for the titration of IgG(a), AGP(b), or TF(c) with MINP(OVA) of FM **4**/template ratio 8:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 3-5 in Table S1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

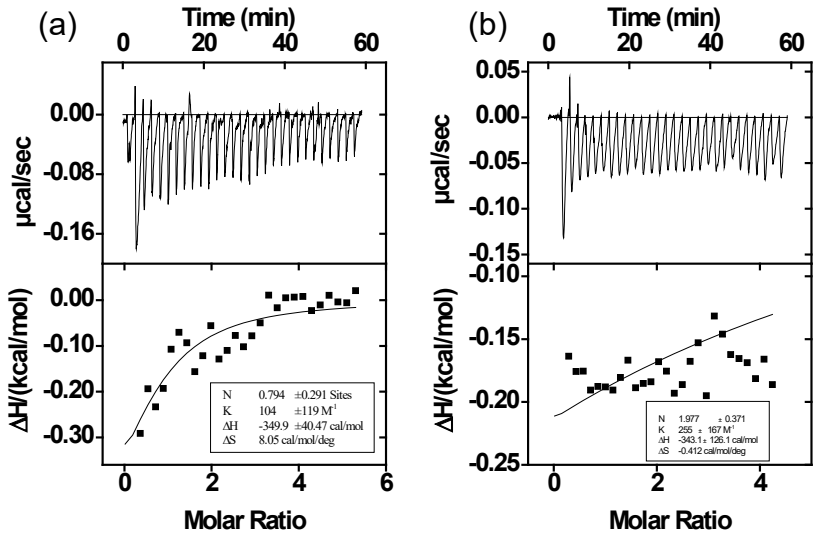


Figure S23. ITC titration of non-glycoprotein by MINP (OVA) ITC titration curves obtained at 298 K for the titration of BSA(a) or Lysozyme (b) with MINP(OVA) of FM **4**/template ratio 8:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 6-7 in Table S1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

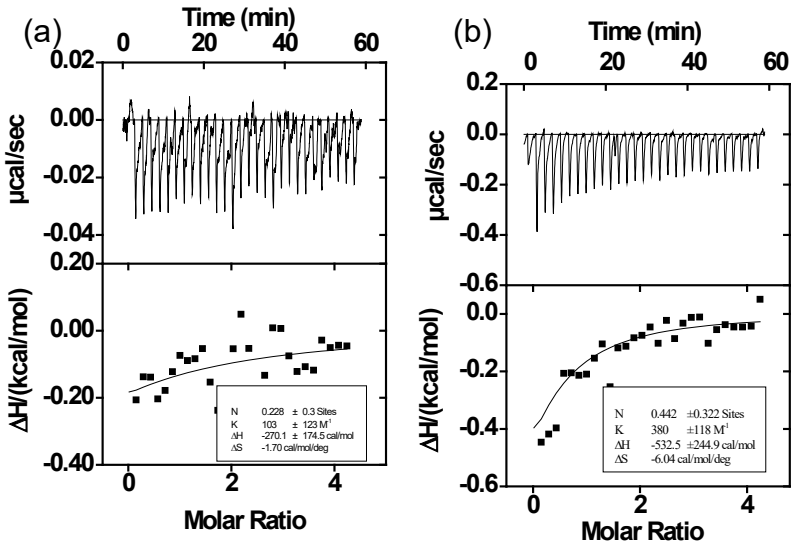


Figure S24. ITC titration of non-glycoprotein by MINP (OVA) ITC titration curves obtained at 298 K for the titration of Trypsin(a) or Cyt C(b) with MINP(OVA) of FM **4**/template ratio 8:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 8-9 in Table S1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

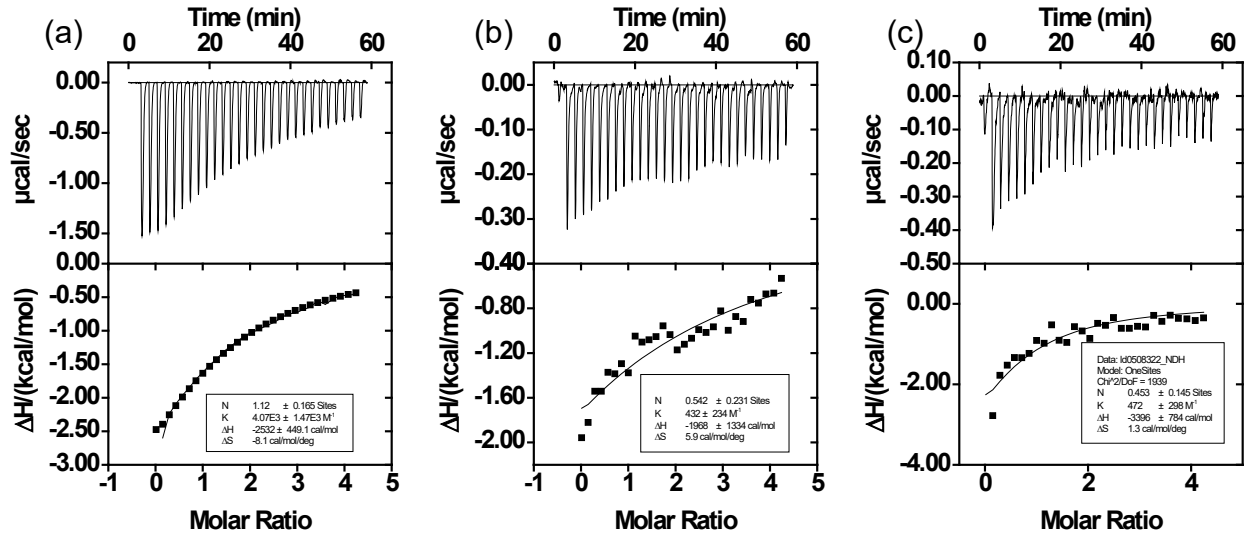


Figure S25. ITC titration of glycoprotein by MINP (HRP) ITC titration curves obtained at 298 K for the titration of IgG(a), AGP(b), or TF(c) with MINP(HRP) of FM **4**/template ratio 4:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 12-14 in Table S1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

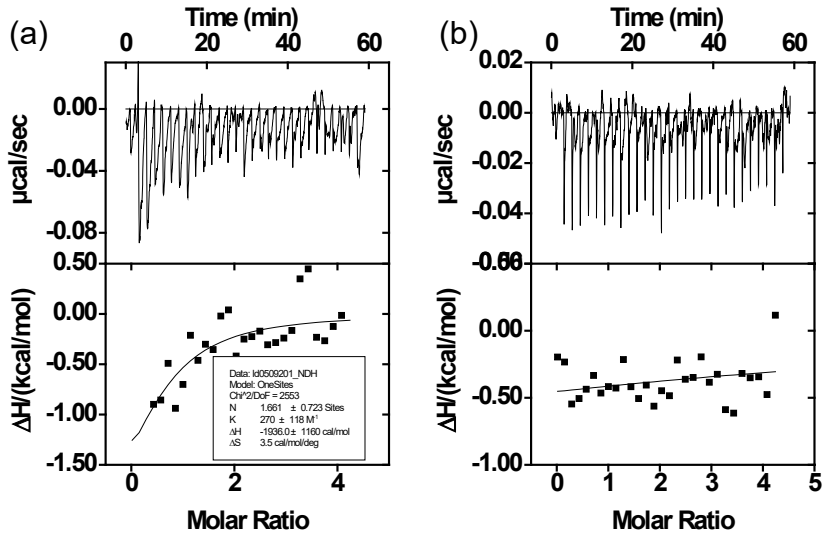


Figure S26. ITC titration of non-glycoprotein by MINP (HRP) ITC titration curves obtained at 298 K for the titration of BSA(a) or Lysozyme (b) with MINP(HRP) of FM **4**/template ratio 4:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 15-16 in Table S1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

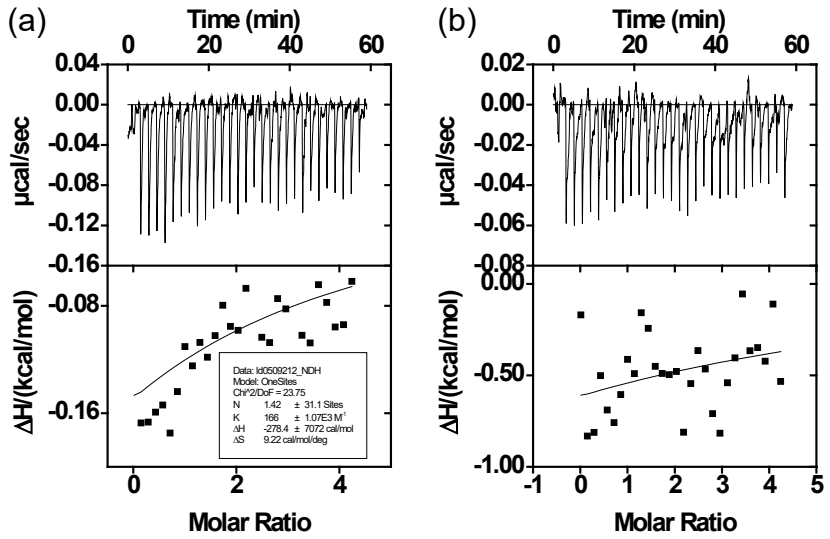


Figure S27. ITC titration of non-glycoprotein by MINP (HRP) ITC titration curves obtained at 298 K for the titration of Trypsin(a) or Cyt C(b) with MINP(HRP) of FM **4**/template ratio 4:1 in 10 mM HEPES buffer (pH = 7.4). The data correspond to entry 17-18 in Table S1. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of MINP to the substrate. The solid line is the best fit of the experimental data to the sequential binding of N equal and independent binding sites on the MINP. The heat of dilution for the substrate, obtained by adding the substrate to the buffer, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

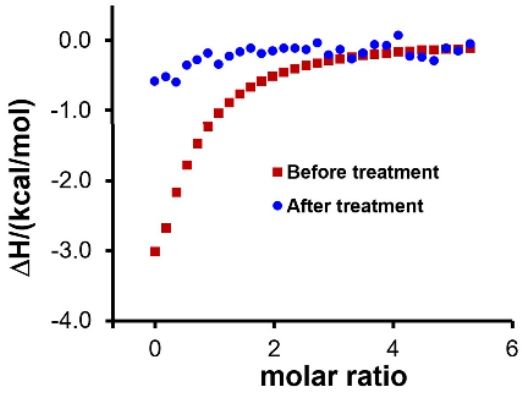
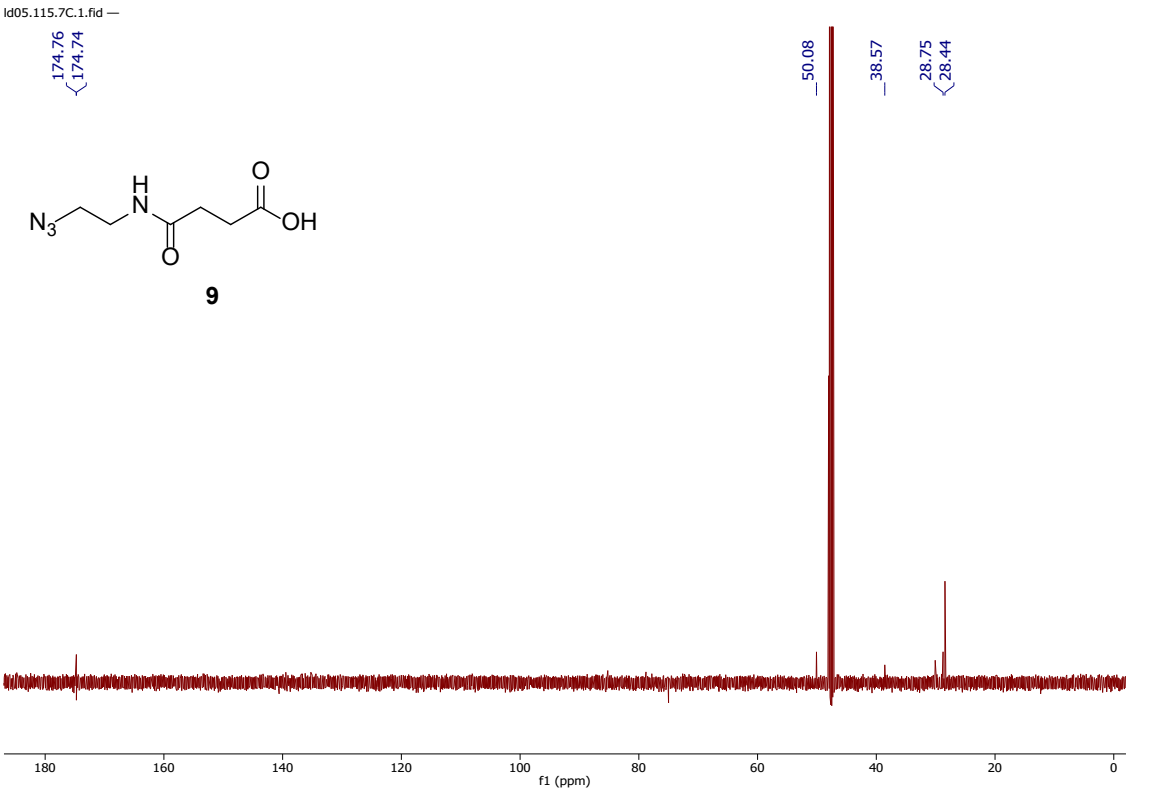
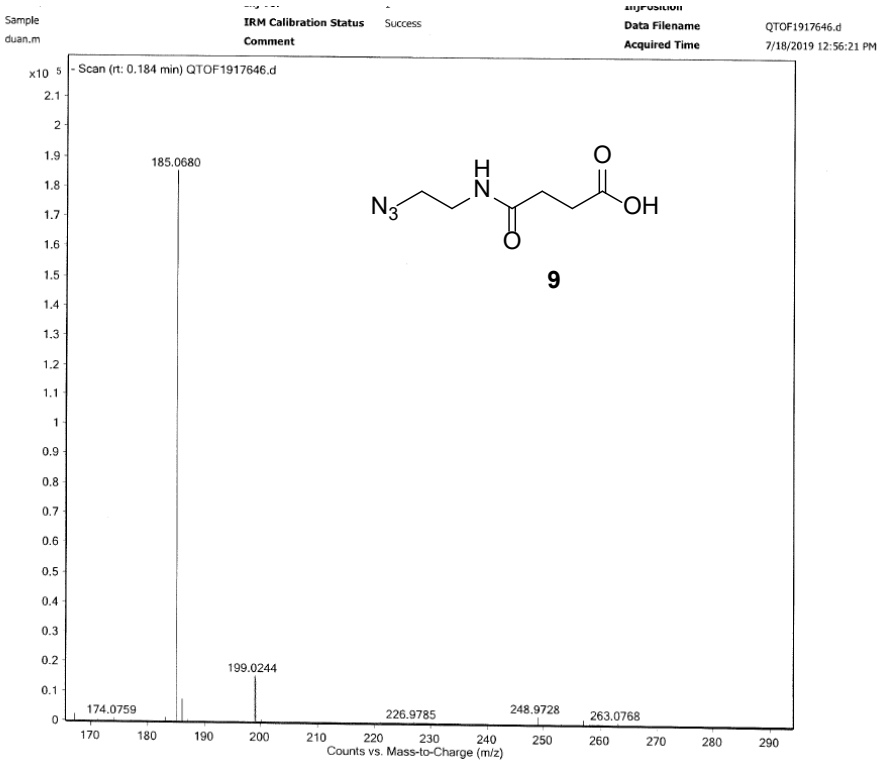


Figure S28. ITC titration curves of OVA. ITC titration curves of OVA by MINP(OVA) prepared with 8:1 FM/template ratio (a) before and (b) after treatment with α1-2,3,6 mannosidase for 24 h in 10 mM HEPES buffer (pH 7.4). [OVA] = 6.0 μM. [mannosidase] = 6 units.

^{1}H NMR, ^{13}C NMR, and MS Spectra

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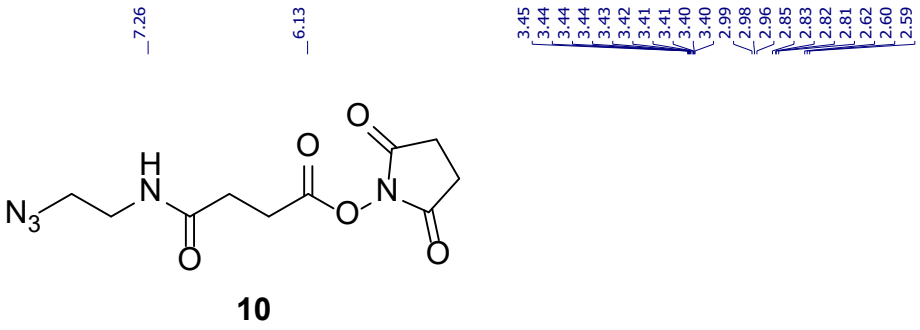
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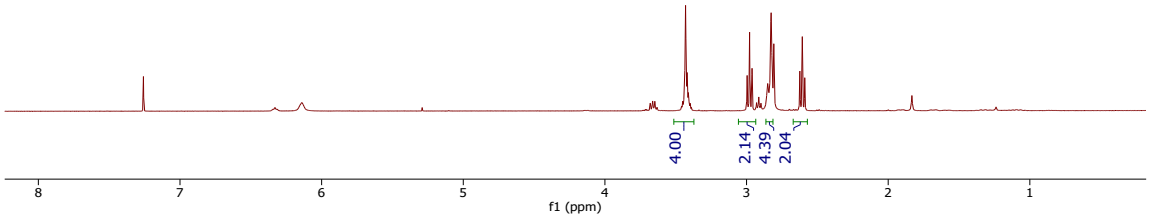
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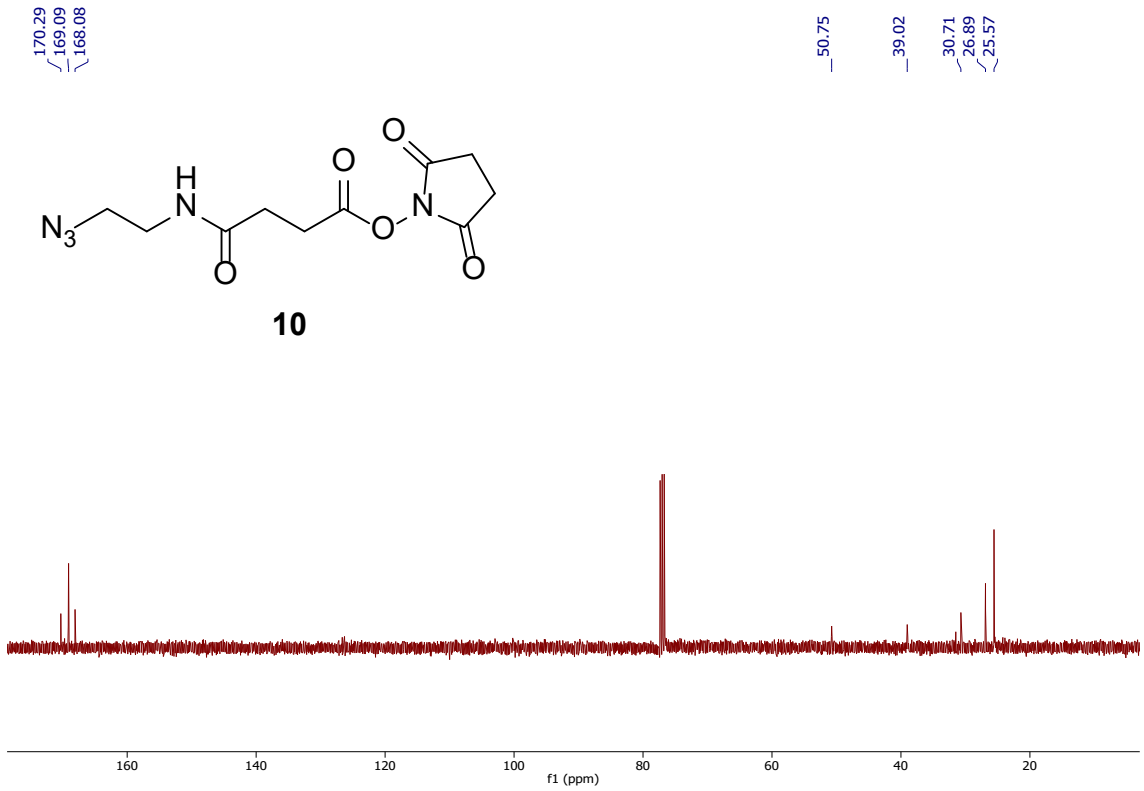
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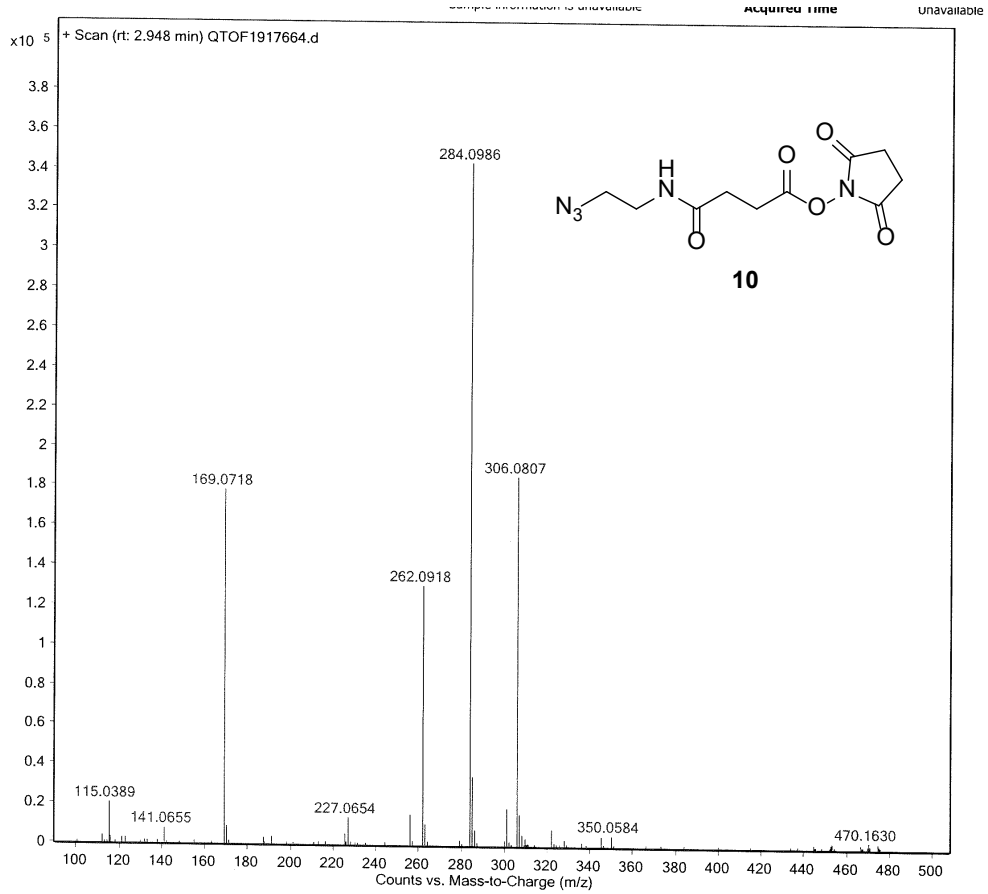
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