Electronic Supplementary Information

An arginine functionalized stationary phase for hydrophilic interaction liquid chromatography

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EXPERIMENTAL SECTION

Chemicals and equipment

Spherical silica particles were provided by Fuji Corp. (Acchrom Corp., 5μm particle size; 300 m^2/g surface area). Chromatographic grade acetonitrile, formic acid and ammonium formate were purchased from Tedia (USA). Ammonium formate and copper iodide were from Acros (USA). L-Arginine was obtained from Aladding Corp. (Shanghai, China). Proteins, ZIC-HILIC materials and chemical reagents, if otherwise mentioned, were ordered from Sigma-Aldrich (St. Louis, MO, USA). GELoader tips were obtained from Eppendorf (Madison, WI). Other reagents were used without further purification. Unless otherwise stated, ultrapure water from a Mill-R04 purification system (Millipore, Germany) was used for solution preparation.

The solid phase nuclear magnetic resonance (NMR) was characterized on an AVANCE DRX-500 (Bruker, Germany). The chromatographic separation was performed on a Waters Alliance HPLC system equipped with a UV absorbance detector and an evaporative light-scattering detector (ELSD) (Waters Corp., USA). The column temperature was 35 °C and the absorbance wavelength was 254 nm.

Synthesis of Arginine-based stationary phase

To click arginine onto silica gel, it is necessary to transfer amino group of arginine into azido needed in the cycloaddition reaction with alkyne-silica gel to form a triazole ring. The synthesis route was similar to that of previous report with slight modification. Briefly, a mixture of arginine (5.226 g, 30 mmol), K_2CO_3 (18 g, 130 mmol), $CuSO_4 \cdot 5H_2O$ (0.075 g, 0.3 mmol) and ISAH (8.5 g, 40.6 mmol, its synthesis route was in detail described previously[1]) in 100 mL MeOH was stirred at room temperature for 9 h. The solution was then treated by rotary evaporation and vacuum filtration to yield brown-yellowish solid. The solid was resolved in water, then the solution was extracted three times with ethyl acetate to get aqueous phase, followed by treatment with rotary evaporation filtration to yield brown-yellowish solid for

further use.

The solid was resolved in 100 mL MeOH, then the solution was adjusted pH to 6 by acetic acid, followed by the addition of terminal alkyne silica gel (3 g, its synthesis route was in detail described previously[2]) and Cu (I) catalyst which was newly prepared by mixing copper (II) acetate (0.6 g, 2 mmol) and sodium ascorbate (1.2g, 4 mmol) in 7.5 mL water. This suspension was stirred slowly at room temperature for 24 h. After filtration, the filter cake was in turn washed with water (300 mL), 10% EDTA (300 mL), warm water (600 mL, 50 ^{o}C), acetone (300 mL), and tetrahydrofuran (50 mL). The product was then obtained and dried in vacuum before packing. The synthesis route was provided in Fig.1.

For comparison, arginine was immobilized on silica with common γ-glycidoxypropyl trimethoxysilane) via common epoxy-addition reaction. The synthesis route was given in the support information of SI-Fig. 1.

The obtained stationary phase was slurry-packed into stainless-steel column (4.6 mm i.d. × 150 mm length) with methanol as slurry solvent and propulsion solvent.

Enrichment of glycopeptides

Click-Arginine-SP material (1 mg) was packed into a GELoader tip after suspending in CH_3CN. The tips were washed and equilibrated with 40 μL of 50% CH_3CN/1% TFA (trifluoroacetic acid) and 80 μL of 80% CH_3CN/1% FA (formic acid), Tryptic fetuin digests were redissolved in 20 μL of 80% CH_3CN/1% FA and loaded into the microcolumns. After subsequently wash with 40 μL of 80% CH_3CN/1% 0.1FA and 40 μL of 65% CH_3CN/1% FA, glycopeptides were eluted with 20 μL 50% CH_3CN/1% FA. When more complex samples were investigated, the amount of Arg-ZIC-SP was increased accordingly.

Adsorption capacity of Click-Arginine-SP toward glycopeptides

In order to determine the adsorption capacity of Click-Arginine-SP for sialylated glycopeptides, fixed volume of purified fetuin glycopeptides enriched with TiO_2 were loaded into Click-Arginine-SP packed microcolumns. The flowthrough fractions were collected and identified with mass spectrometer. The adsorption capacity is calculated

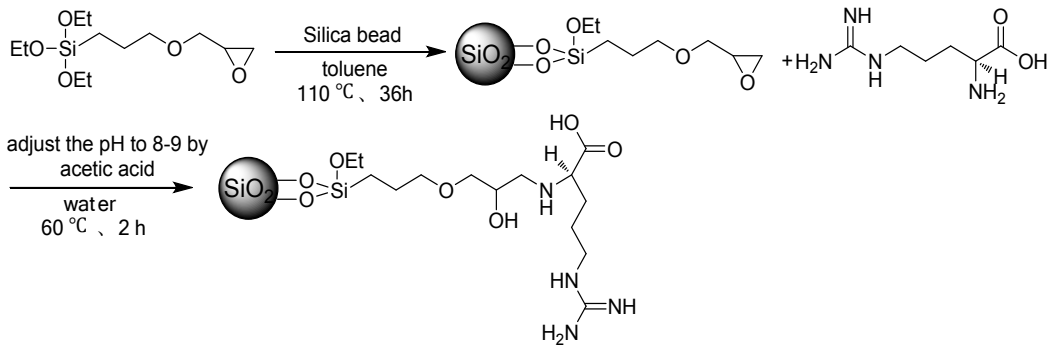
unitl the glycopeptide signals were observed.

Mass spectrum analysis

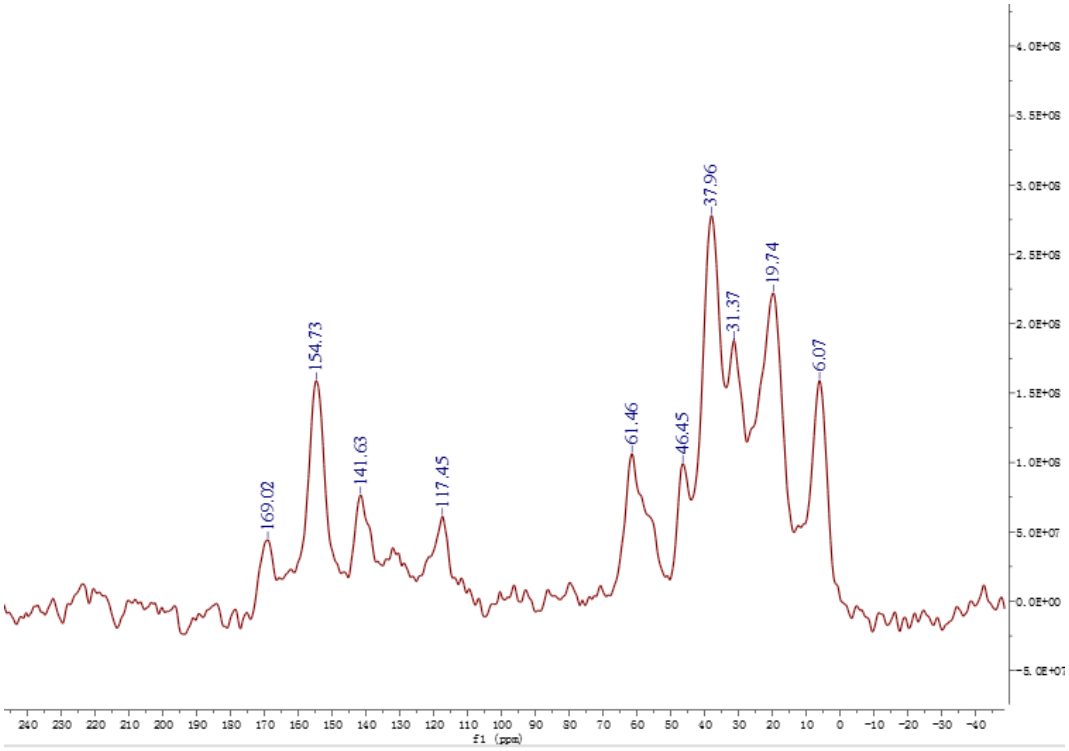
A nano-electrospray ionization-quadrupole time-of-flight mass spectrometer (ESI Q-TOF MS, Waters, Milford, MA, USA) was used to analyze peptide samples. The peptides were pumped into ESI source with Nano Acquity UPLC (Waters, Milford, MA, USA). The MS analysis was carried out under positive ion mode. Full scan MS data were obtained at m/z 600-2000.

Quantum chemistry calculation

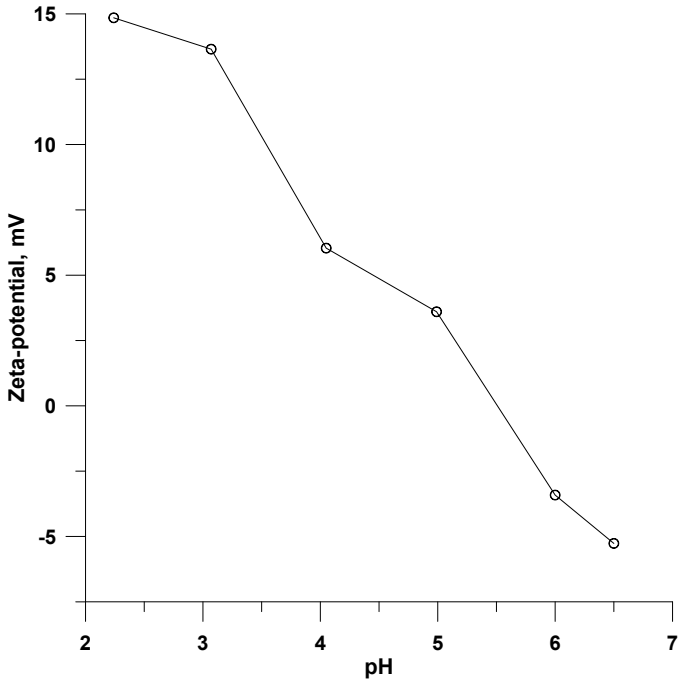
To explore the possible adsorption mechanism of Click-Arginine-SP towards glycopeptides, a much similar simple molecule, sialic acid was chosen to be model. Calculation of inter-molecular interaction was performed with Gaussian 2003W software in two Intel Xeon (R) workstations. The interaction model was acquired by reparative conformational optimum until reaching the minimum energy.



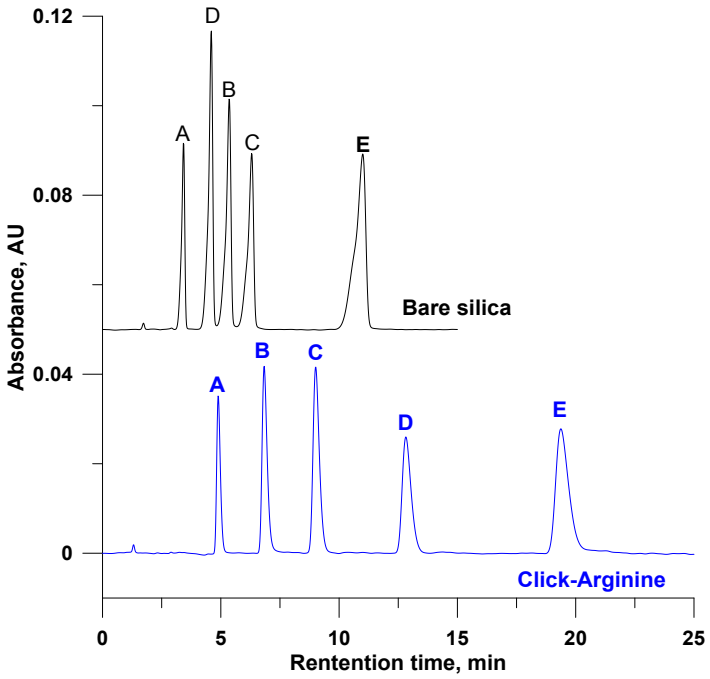
SI-Fig.1 Synthesis route of Arginine-based ZIC-SP via common way



SI-Fig. 2 Solid phase ^{13}C-CP/MAS NMR spectrum of Click-Arginine-SP

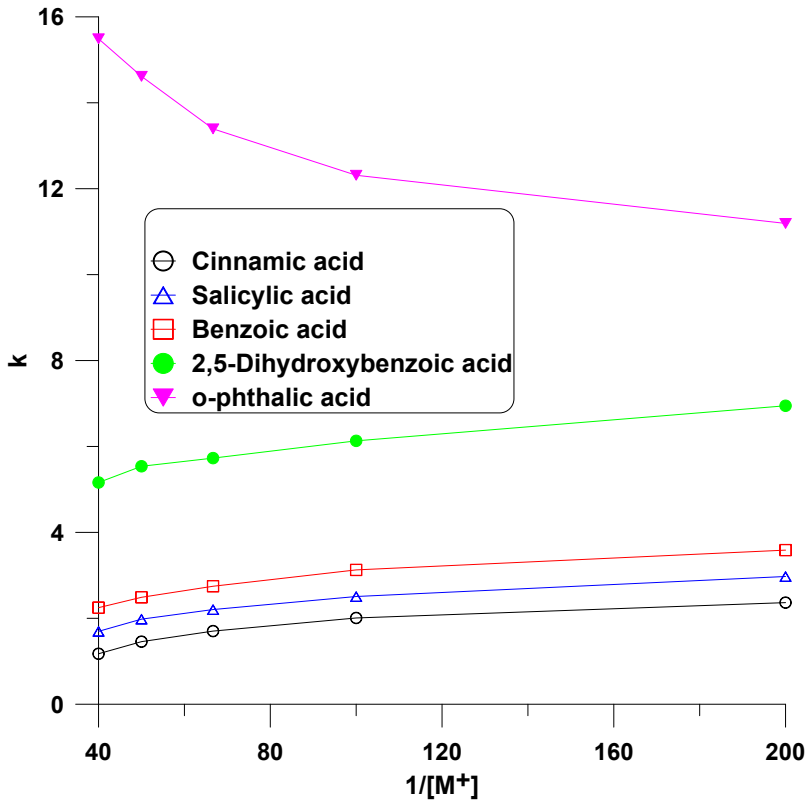


SI-Fig. 3 Mean Zeta-potential of Click-Arginine-SP at different pH



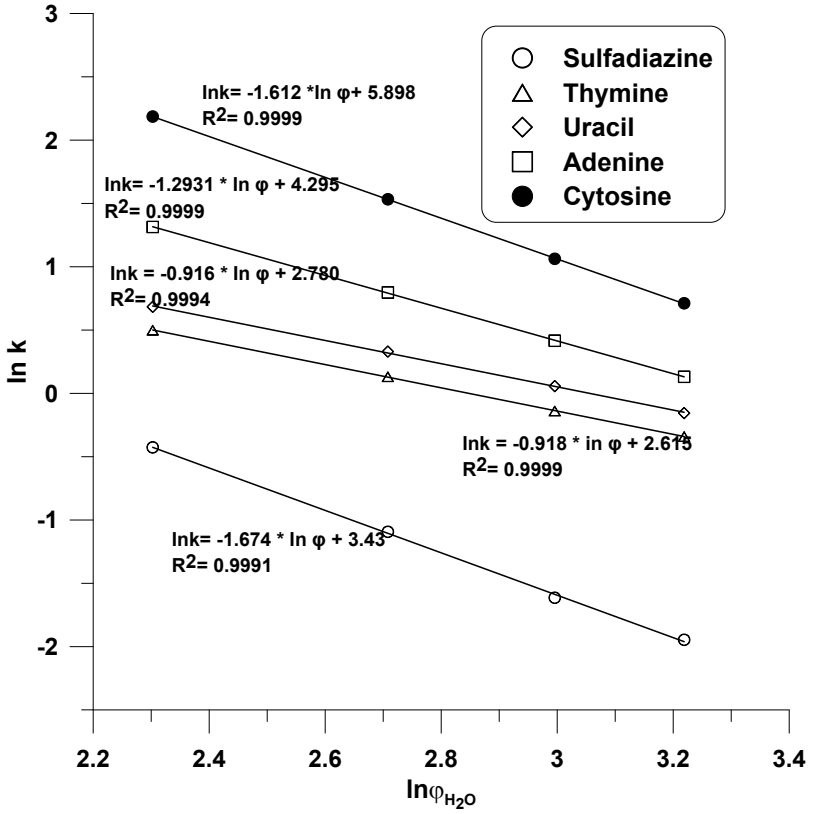
SI-Fig.4 Separation of nucleosides and nucleotides bases on bare silica and Click-Arginine-SP.

Conditions: mobile phase: B: ACN; C: NH_4FA (250 mM NH_4FA, pH, 3.05).90%B/10% C; detection wavelength, 254 nm; flow rate, 1.0 mL/min; column temperature, 30 ^oC**.** Peak identification: A: Uracil, B: Adenine, C: 5 - methyl uridine, D: Uridine, E: Cytosine.



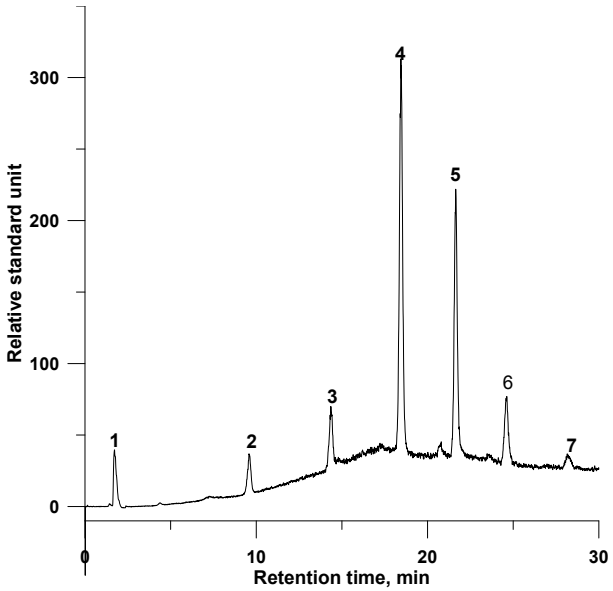
SI-Fig. 5 Effect of electrolyte concentration on the retention

Condition: mobile phase:A:H_2O ;B:ACN;C: NH_4FA (250mM NH_4FA,pH=4).(0%/2%/4%/6%/8%)A/90%B/(10%/8%/6%/4%/2%)C; other conditions same as SI-Fig. 4.



SI-Fig. 6 Effect of water content in the mobile phase on retention.

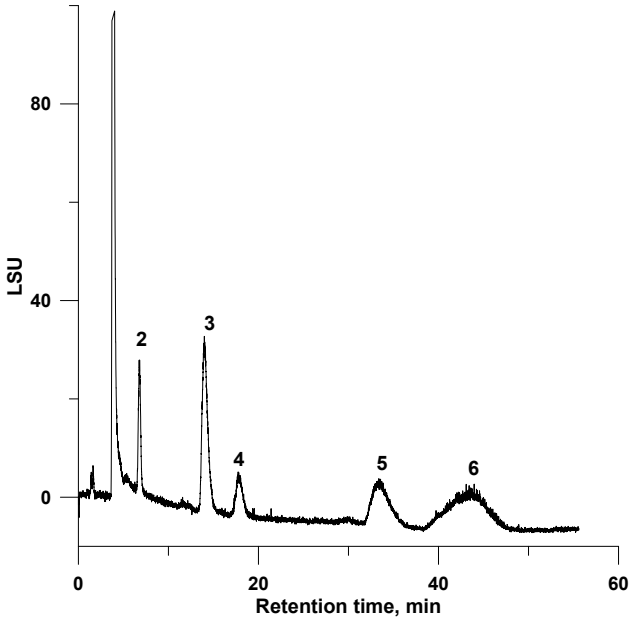
Conditions: mobile phase: A/B=H_2O/ACN; other conditions same as SI-Fig. 4.



SI-Fig. 7 Separation of oligosaccharides bases by Click-Arginine-SP.

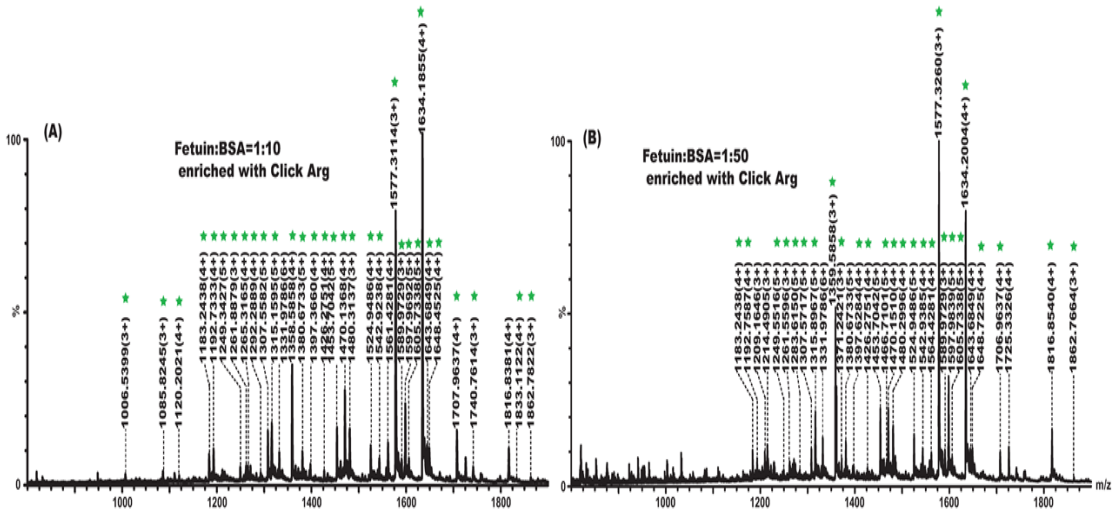
Condition: mobile phase A: H_2O; B: ACN. Gradient 0-20min 20%-40%A; ESD: nitrogen

nebulizer gas 30 psi; tube temperature 50° C; gain 50. Numbers on peaks are the degree of polymerization of oligosaccharide.

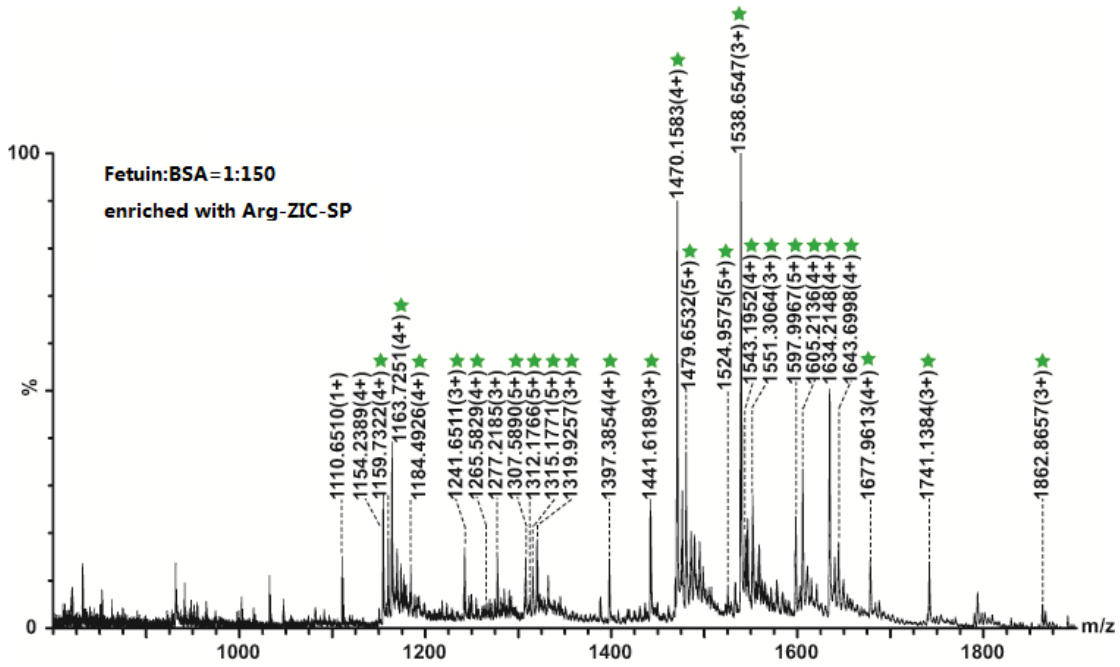


SI-Fig. 8 Separation of sodium alginate by Click-Arginine-SP.

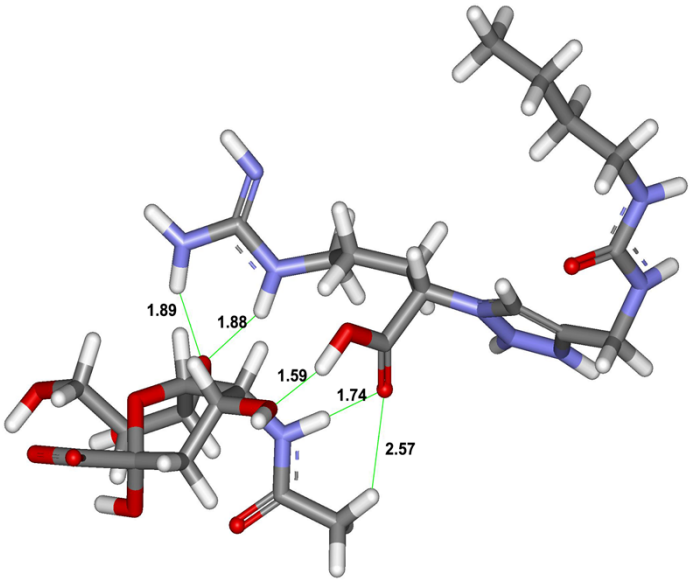
Condition: mobile phase A: H_2O; B: ACN; C: NH_4FA (100mM, pH=6.57). 20%A/60%/20%C. Other conditions same as SI-Fig. 7. Numbers on peaks are the degree of polymerization of sodium alginate.



SI-Fig. 9 Mass spectra of tryptic digests of bovine fetuin and bovine serum albumin (BSA) with ratio of 1:10 AND 1:50 after enrichment with Click-Arginine-SP.



SI-Fig. 10 Mass spectra of tryptic digests of bovine fetuin and bovine serum albumin (BSA) with ratio of 1:150 after enrichment with Click-Arginine-SP.



SI-Fig. 11 Possible interaction model of sialic acid with Click-Arginine-SP functional groups, obtained from quantum chemistry calculation (Gaussian 2003, density

function theory (DFT), at 3-21G level). In this model, the hydrogen bond interactions between sialic acid and guanidyl or carboxyl groups in Click-Arginine-SP are indicated by green dotted lines. The lengths of the bonds are 1.89, 1.88, 1.59, 1.74 Å and 2.57Å, respectively.

References

1. Li, Y.; Liao, J. L.; Nakazato, K.; Mohammad, J.; Terenius, L.; Hjertén, S. *Anal. Biochem.* **1994**, *223*, 153-158
2. Y. Liu, Q. Du, B. Yang, F. Zhang, C. Chu and X. Liang, *Analys*t, 2012, 137, 1624-1628.