SUPPORTING INFORMATION

Hypercrosslinked porous polymer monoliths for hydrophilic interaction liquid chromatography of small molecules featuring zwitterionic functionalities attached to gold nanoparticles hold in layered structure

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Chemicals and Materials. 4-Methylstyrene (99%), vinylbenzyl chloride (mixture of 3- and 4-isomers, 97%), and divinylbenzene (80%, technical grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and purified by passing them through an aluminum oxide column for removal of inhibitor. Azobisisobutyronitrile (AIBN), 3-(trimethoxysilyl)propyl methacrylate, 1-dodecanol, iron (III) chloride, 1,2-dichloroethane, molecular bromine (Br_2), carbon tetrachloride, cystamine dihydrochloride, cysteine, cysteamine, 3-mercaptopropionic acid, ethanolamine, tris(2-carboxylethyl)phosphine hydrochloride (TCEP) solution (0.5 mol/L, pH=7 adjusted with ammonium hydroxide), formic acid, triethylamine, phosphoric acid, ammonium formate, uracil, benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, amylbenzene, Phe-Gly-Phe-Gly, Val-Try-Val, Gly-Phe, Gly-Leu, Gly-Try, Lys-Val, Gly-Gly-Gly, thymine, adenosine, cytidine, cytosine, guanosine, and HPLC-grade solvents (acetonitrile, ethanol, *N,N*-dimethylformamide, tetrahydrofuran) were purchased from Sigma-Aldrich and used as received. Polyethyleneimine (branched, M.W. 70,000, 30% w/v aqueous solution) and polyethyleneimine (M.W. 2,000, 50% w/v aqueous solution) were obtained from Alfa Aesar (Ward Hill, MA, USA). Gold colloids (GNP) with particles sizes of 5, 10, and 15 nm were obtained from Ted Pella, Inc. (Redding, CA, USA). Polyimide coated 100 μm i.d. fused silica capillaries were purchased from Polymicro Technologies (Phoenix,

AZ, USA).

Instrumentation. A syringe pump (Kd Scientific, New Hope, PA) was utilized for pumping reagents through the column during the hypercrosslinking, free radical bromination, and modifications of monolithic columns with cystamine and TCEP. Gold colloids, polyethyleneimine (PEI), and cysteine were pumped through the monolithic capillary columns using a high pressure 260D syringe pump (ISCO, Lincoln, NE, USA) provided with a Rheodyne 7725 manual six-port sample injection valve (Rohnert Park, CA) and a 2 mL loop to avoid contamination of the pump with GNP. A Dionex Ultimate 3000 HPLC system (Sunnyvale, CA, USA) equipped with a 3 nL UV detection cell and an external micro-valve injector with a 4 nL inner sampling loop (Valco, Houston, USA) was used for the chromatographic evaluation. Scanning electron micrographs and energy dispersive X-ray spectra of monoliths were obtained using a Zeiss Gemini Ultra Field-Emission Scanning Electron Microscope (Peabody, MA, USA) integrated with an energy dispersive X-ray spectrometer (Thermo Electron, USA). Nitrogen adsorption/desorption isotherms were measured using a Micromeritics ASAP 2020 surface area and porosimetry analyzer (Norcross, GA) and used for the calculation of surface areas.

Preparation of generic monolithic capillary columns. The inner wall of the 100 μm i.d. fused-silica capillary was vinylized with 3-(trimethoxysilyl)propyl methacrylate to enable covalent attachment of the monolith. A polymerization mixture comprised 21% 4-methylstyrene, 7% vinylbenzene chloride, 12% divinylbenzene, 13% toluene, 47% 1-dodecanol, and AIBN initiator (1% with respect to monomers) (all wt.%) and was homogenized by sonication for 10 min and degassed by purging with nitrogen for 5 min. The solution was then introduced into the vinylized capillary. The capillary was sealed at both ends with a rubber septum and immersed in a thermostated water bath at 70°C for 20 h. After the polymerization reaction was completed, a few centimeters from both ends of the capillary were cut to liberate the virgin structure, and the monolith was flushed with acetonitrile to remove porogens and unreacted components.

Hypercrosslinking. The hypercrosslinking reaction in bulk was carried out using 1.50 g monolithic material pre-swollen in 20 mL of 1,2-dichloroethane for 2 h. The Lewis acid catalyst $FeCl_3$ (1 g) was then added to the slurry cooled in an ice bath. Once the catalyst was homogeneously dispersed, the mixture was allowed to come to the room temperature. The hypercrosslinking reaction was then carried out at 90°C for 4 h. The resulting polymer was separated and washed with methanol, 0.5 mol/L HCl in acetone, and then with methanol again followed by drying in vacuum oven. Hypercrosslinking of generic monolithic columns was performed by flushing the columns with 1,2-dichloroethane at a flow rate of 0.25 μL/min for 2 h. The filtered solution of 50 mg of $FeCl_3$ in 1 mL of 1,2-dichloroethane was pumped through the columns at a flow rate of 0.25 μL/min for 2 h and the columns were held in an ice bath for 1 h. The reaction was carried out at 90 °C for 4 h. The hypercrosslinking columns were then washed completely with the mixture of acetonitrile and water.

Free radical bromination. Free radical bromination of hypercrosslinked poly(4-methylstyrene-co-vinylbenzyl chloride-co-divinylbenzene) monolith was carried out by keeping the column in a column heater at 80ºC and continuously pumping the fresh Br_2 (33 μL)/AIBN (4.2 mg)/CCl_4 (250 μL) solution through the heated column at a flow rate of 30 μL/h for 4 h. The brominated columns were then washed with acetonitrile/water (50/50, v/v).

Reaction with cystamine and tris(2-carboxylethyl)phosphine. Cystamine dihydrochloride was first neutralized with sodium hydroxide. A 50% (v/v) cystamine solution in *N,N*-dimethylformamide (DMF) was pumped through the brominated monolith at room temperature at a flow rate of 0.25 μL/min for 2 h. The column was then sealed with rubber septa at both ends, and the reaction was either heated in an oven or irradiated in a microwave oven. Some modifications were repeated several times to achieve a higher conversion. The capillary column was then flushed with water until the pH of the eluent was neutral, followed by capping unreacted epoxy groups with 1.0 mol/L ethanolamine using the same conditions described above. TCEP solution (0.25 mol/L) was pumped through the monolith at room temperature at a flow rate of 0.25 μL/min for 6 h to achieve the cleavage of disulfide bonds. The

column was then washed with water.

Attachment with gold nanoparticles. A high pressure 260D ISCO syringe pump was used for the modification of monoliths with gold nanoparticles. Colloidal dispersion of GNP was filled in the 2 mL loop attached to a six-port injection valve. By switching the valve, the dispersion contained in the loop was pumped through the functionalized monolithic column at a flow rate of 5 μL/min until the entire column length turned deep red and a pink solution was observed coming out from the capillary outlet. The column was then rinsed thoroughly with water.

Functionalization with polyethyleneimine and cysteine. A solution of polyethyleneimine with a concentration of 10.0 wt% in water was pumped through the monolayer GNP functionalized monolithic columns using the high pressure 260D ISCO syringe pump at a flow rate of 5 μL/min for 2 mL, and was followed by washing with water. A solution of cysteine (1 mol/L) in water was pumped through the dual-layer GNP functionalized monolithic columns using the high pressure 260D ISCO syringe pump at a flow rate of 5 μL/min for 2 mL, and was followed by washing with water.

Preparation of hydrophilic monolith with gold nanoparticle layered architecture. A high pressure 260D ISCO syringe pump was used for the modifications of monolithic columns at a flow rate of 5 μL/min. Monolith with single-layer GNP was obtained by pumping gold colloidal dispersion through the thiol-containing hypercrosslinked monolithic column until complete saturation, visually confirmed by the deep red color of entire column length and a pink solution coming out from the capillary outlet. A solution of polyethyleneimine (PEI) with a concentration of 10.0 wt% in water was pumped through the monolayer GNP monolithic column for 2 mL, and was followed by washing with water. Using PEI as spacer, monolith with dual-layer GNP was prepared by pumping gold colloidal dispersion through PEI-functionalized monolith until achieving complete saturation. Finally, a solution of cysteine (1 mol/L) in water was pumped through the dual-layer GNP monolithic column for 2 mL, followed by washing with water.

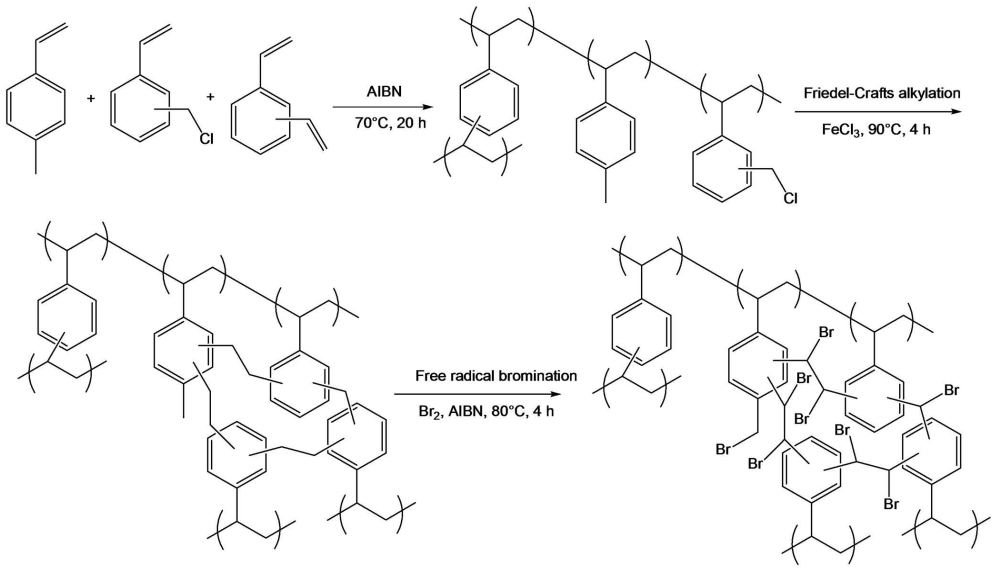

Figure S-1. Scheme of preparation of poly(4-methylstyrene-co-vinylbenzene chloride -co-divinylbenzene) monolith, its hypercrosslinking, and free radical bromination with molecular bromine.

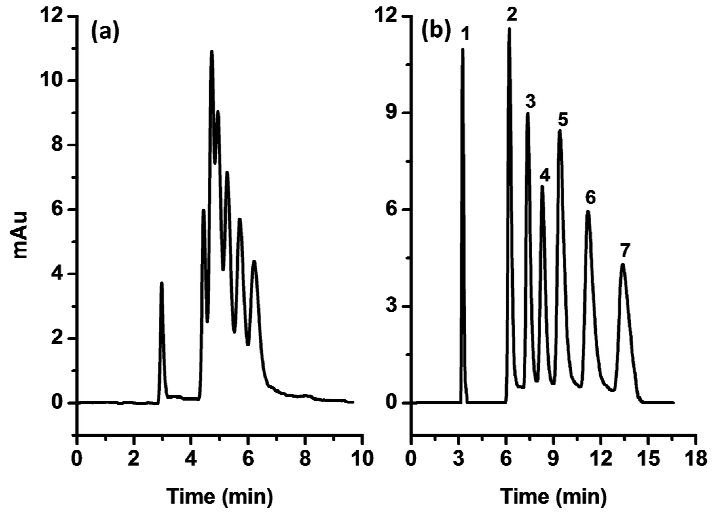

Figure S-2. Separation of uracil and alkylbenzenes using generic poly(4-methylstyrene -co-vinylbenzyl chloride-co-divinylbenzene) monolithic column (a), and its hypercrosslinked counterpart (b). Conditions: Columns: generic 229 mm × 100 μm i.d., hypercrosslinked 203 mm × 100 μm i.d. Mobile phase: 60:20:20 vol% acetonitrile-water-tetrahydrofuran, UV detection 254 nm, flow rate 0.5 μL/ min. Peaks: uracil (1), benzene (2), toluene (3), ethylbenzene (4), propylbenzene (5), butylbenzene (6), and amylbenzene (7).

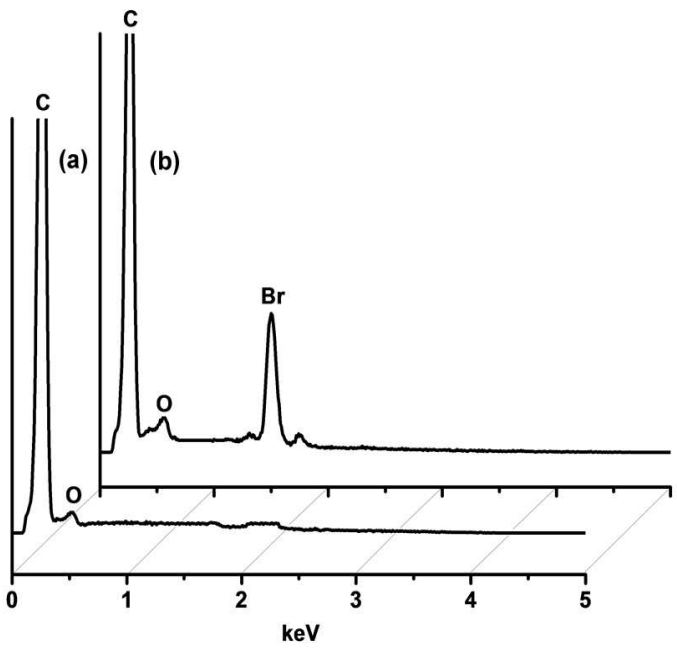

Figure S-3. Energy dispersive X-ray spectra for hypercrosslinked poly(4-methylstyrene-co-vinylbenzene chloride-co-divinylbenzene) monolith (a), and its counterpart after free radical bromination with molecular bromine (b).

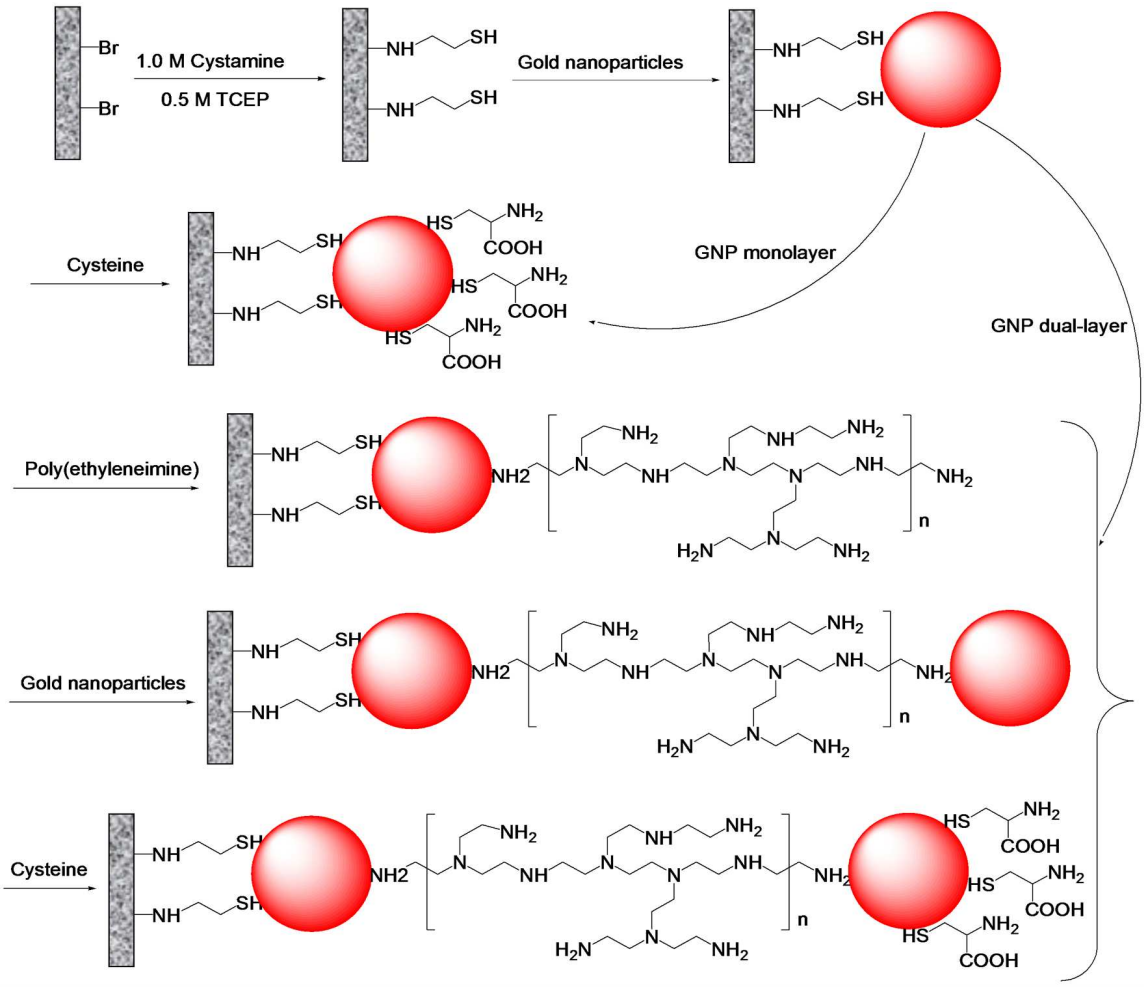

Figure S-4. Scheme of modifications of brominated hypercrosslinked poly(4-methylstyrene-co-vinylbenzene chloride-co-divinylbenzene) monolith with cystamine, TCEP, gold nanoparticles, polyethyleneimine, and cysteine.

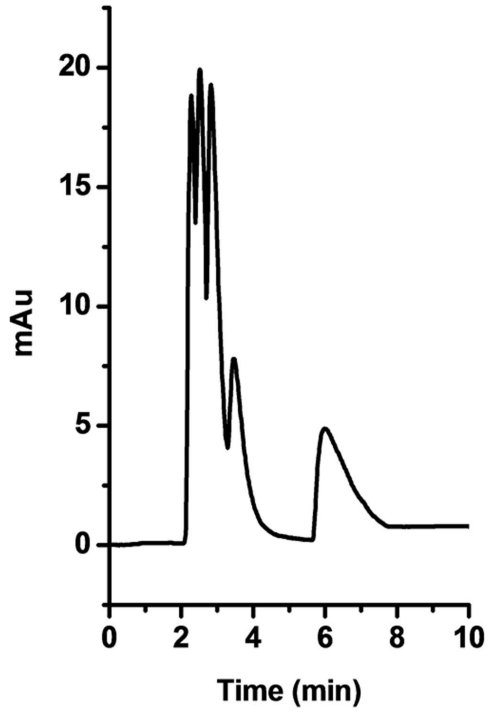

Figure S-5. Isocratic HILIC separation of five nucleosides using monolithic column including 5 nm GNP in dual-layer and functionalized with cysteine. Column: 172 mm × 100 μm i.d. Conditions: mobile phase, 25 mmol/L ammonium formate (pH 3.2) in 90:10 vol% acetonitrile-water; flow rate, 0.5 μL/min; UV detection 254 nm; temperature 25°C.

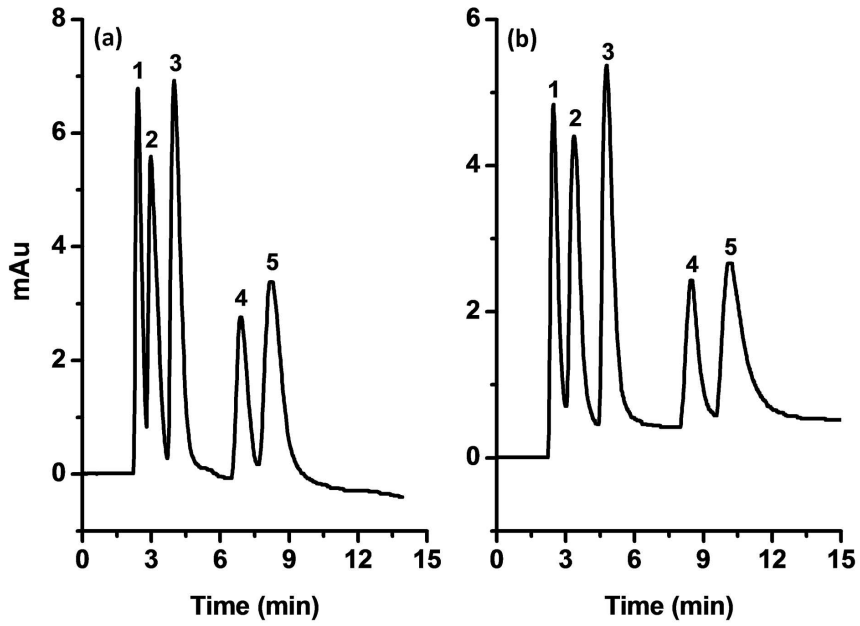

Figure S-6. Isocratic HILIC separation of nucleosides using non-hypercrosslinked monolithic column including 10 nm GNP in (a) monolayer, and (b) dual layer functionalized with cysteine. Conditions: Columns: 154 mm × 100 μm i.d. (a), 161 mm × 100 μm i.d. (b). mobile phase, 25 mmol/L ammonium formate (pH 3.2) in 90:10 vol% acetonitrile-water; flow rate, 0.5 μL/min; UV detection 254 nm; temperature 25°C.Peaks: thymine (1), adenosine (2), cytidine (3), cytosine (4), guanosine (5).

Table S-1 Elemental analysis of brominated monoliths functionalized with cystamine under different reaction conditions

Monolith	Treatment	Reaction time, h	C [a]	Element content, at.%			
				C	N	S	Br
M_I	80 °C	16	1	93.7	1.2	1.2	3.9
M_{II}	120 °C	16	1	93.1	1.8	2.5	2.6
M_{III}	120 °C	16	4	92.7	1.9	3.9	1.5
M_{IV}	Microwave	0.5	1	92.5	2.1	3.8	1.6

[a] Number of reaction cycles.

Table S-2 Gold content in monoliths coated with single and dual layer of gold nanoparticles varying in size

Monolith [a]	Gold nanoparticles		Au, wt%	
	Size, nm	Particles/mL	Monolayer	Dual-layer
M_{10}	10	5.7×10^{12}	4.1	9.5
M_5	5	5.0×10^{13}	1.2	2.7
M_{10} [b]	10	5.7×10^{12}	7.3	17.2
M_{15} [c]	15	1.4×10^{12}	28.1	—

[a] The number in subscript denotes size of the gold nanoparticles used for modification.

[b] Generic poly(4-methylstyrene-vinylbenzene chloride-divinylbenzene) monolith.

[c] Hypercrosslinked poly(4-methylstyrene-vinylbenzene chloride-divinylbenzene) monolith.