Electronic Supporting Information

Photoinduced thiol-ene polymerization reaction for fast preparation of macroporous hybrid monoliths and their application in capillary liquid chromatography

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Experimental section

Materials

Vinyltrimethoxysilane (VTMS) and 1,6-hexanedithiol (2SH) were purchased from J&K Scientic Ltd.. Pentaerythriol tetrakis(3-mercaptopropionate) (4SH), tetravinylsilane (TVS), diethylene glycol diethyl ether (DEGDE), 2,4,6,8-tetramethyl-2,4,6,8-tetravinylcyclotetrasiloxane (TMTVS), trifluoroacetic acid (TFA), formic acid (FA), dithiothreitol (DTT), iodoacetamide (IAA), poly(ethylene glycol) (PEG, Mn=200), insulin (bovine), lysozyme (chicken egg white), bovine serum albumin (BSA), myoglobin (horse heart) and ribonuclease B (bovine pancreas) were obtained from Sigma-Aldrich (St Louis, Mo, USA). Cytochrome c (bovine heart) was obtained from Aladdin (Shanghai, China). 2,2-Dimethoxy-2-phenylacetopheone (DMPA) was purchased from Acros Organics (New Jersey, USA). Tetrahydrofuran (THF) and 1-propanol were gotten from Tianjin Kemiou Chemical Reagent Co. Ltd. (Tianjin, China). Arg-Gly, Trp-Phe, Trp-Tyr and Trp-Trp were purchased from SERVA (Germany). The flexible fused silica capillary tubing (UV transparent coating) with inner dimension of 75 μm was purchased from Polymicro Technologies (Phoenix, AZ, USA). HPLC-grade acetonitrile (ACN) was obtained from Yuwang Group (Shandong, China) and used for preparation of mobile phases. The water used in all experiments was doubly distilled and purified by Milli-Q system (Millipore Inc., Milford, MA, USA).

Instrumentation

The thiol-ene reaction was irradiated in UV crosslinkers (XL-1500A, λ=365 nm, Spectronics Corporation, New York, USA). The microscopic morphology of monolith material was obtained by scanning electron microscopy (SEM, JEOL JSM-5600, Tokyo, Japan). Fourier-transformed infrared spectroscopy (FT-IR) characterization was carried out on Thermo Nicolet 380 spectrometer using KBr pellets (Nicolet, Wisconsin, USA). Water contact angle was characterized on a JC2000C machine with 5 μL water drop (Powereach, Shanghai, China). Thermogravimetry (TG) data were collected on Pyris 1 TGA (Perkin Elmer, USA). Nitrogen adsorption/desorption measurements of dried monoliths were performed on a Quadrasorb SI surface area analyzer and pore size analyzer (Quantachrome Boynton Beach, USA). Elemental analyses were performed on Vario EL III (Elementar, Hanau, Germany).

The cLC (capillary liquid chromatography) experiments were performed on LC system coupled with an Agilent 1100 micropump, a 7725i injector with a 20 μL sample loop and a K-2501 UV detector (Knauer, Berlin, Germany). A T-union connector was used as a splitter, with one end connected to the monolithic column and the other connected to a blank capillary (200 cm×50 μm i.d.). The detection window was made by removing the polyimide coating of fused-silica capillary tubing. All chromatographic data were collected and analyzed using the software program HW-2000 from Qianpu Software (Shanghai, China).

Preparation of bulk monoliths via thiol-ene reaction

As an example for preparing monolith **I**, TMTVS (25.0 mg, 0.072 mmol), 2SH (21.3 mg, 0.142 mmol), DEGDE (80 μL) and 1-propanol (100 μL) were added to a small transparent glass vial. The mixture was under sonication for 2 min, and then 1 μL DMPA/DEGDE solution (0.4 mol L^{-1}) was added. The obtained prepolymerization mixture was further sonication for 2 min and irradiated in UV light (λ=365 nm, 120 mJ cm^{-2}) for 10 min. The cured bulk monoliths were extracted with ethanol to remove residuals for three times.

Similarly, the bulk monoliths (**II-IV**) were prepared according to the composition of prepolymerization mixtures as listed in Table 1. For the following characterization, the bulk monoliths were cut into small pieces and grinded using mortar and pestle. Then the grinding powders were dried in a vacuum at 50 ^{o}C for two days.

Preparation of monolithic columns via thiol-ene reaction

Before preparing monolithic columns, the inner wall of fused-silica capillary was pretreated and modified with VTMS for anchoring monolith matrix. Briefly, the capillary was rinsed using 1.0 mol L^{-1} NaOH, water, 1.0 mol L^{-1} HCl and water for 2 h, successively. After being dried under nitrogen stream, the capillary was filled with VTMS solution in methanol (50%, v/v), sealed with rubbers at both ends and submerged in water bath at 50 ^{o}C for 12 h. Finally, the capillary was rinsed with methanol to flush out the residual reagent and dried under nitrogen flow. The pretreated capillary was cut into a certain length, into which the above-mentioned prepolymerization mixture was introduced with a syringe. After sealing both ends with rubbers, the capillary was irradiated by UV light (λ=365 nm, 120 mJ cm^{-2}) for 10 min. The obtained monolithic columns were then flushed with

methanol to remove residuals.

Separation of proteins on nano-HPLC system

Standard proteins were separated on an Eksigent one dimensional Plus Nano-HPLC system (Eksigent, Dublin, CA) equipped with a UV detector K-2520 from Knaur (Berlin, Germany). 1μL of the standard protein mixture (about 5 μg mL^{-1} of each protein in water) was directly loaded to the analytical column using 100% water (containing 0.1% TFA) before gradient elution. The detection was using a 5 nL flow cell with the detection wavelength set at 214 nm. All the chromatography data were collected and analyzed by Eksigent Control Software.

Preparation of BSA tryptic digest and analysis on cLC-MS/MS

To a 10 mL centrifuge tube were added 2 mg BSA, and 1 mL of denaturing buffer containing 8 mol L^{-1} urea and 0.1 mol L^{-1} ammonium bicarbonate. After the addition of 20 μL of DTT (20 mmol L^{-1} in water) solution, the mixture was incubated at 60 ^{o}C for 1 h. And then, 7.4 mg IAA was added and the mixture was incubated at room temperature in the dark for 40 min. The mixture was diluted 8-fold with 0.1 mol L^{-1} ammonium bicarbonate buffer and digested for 16 h in the presence of trypsin (trypsin/BSA=1/25, w/w). After digesting, the pH of mixture was adjusted to 2-3 by 10% TFA aqueous solution. Solid-phase extraction (SPE) was performed with a homemade C18 cartridge. The collected peptides were dried under vacuum and dissolved in a 0.1% formic acid aqueous solution (2 mL), and then stored in a -20 ^{o}C freezer before cLC-MS/MS analysis.

The cLC-MS/MS experiment was carried out by interfacing a surveyor MS pump to a Finnigan LTQ ion trap mass spectrometer (Finnigan MAT, ThermoFinnigan, San Jose, CA). Mobile phase A was water (containing 0.1% FA), and mobile phase B was ACN (0.1% FA). Tryptic digest was automatically injected onto the column with 100% mobile phase A for 5min. And then the trapped peptides were separated on monolithic column (25 cm×75 μm i.d.) with gradient elution from 5% to 35% mobile phase B in 90 min. The LTQ linear ion trap mass spectrometer equipped with a nanospray ion source. The temperature of the ion transfer capillary was set at 200 ^{o}C. The spray voltage was set at 1.8 kV, and the normalized collision energy was set at 35.0%. One microscan was set for each MS and MS/MS scan. All MS and MS/MS spectra were acquired in the data dependent mode. The mass spectrometer was set that one full MS scan was followed by six MS/MS scans on

the six most intense ions. The dynamic exclusion function was set as follows: repeat count 2, repeat duration 30 s, and exclusion duration 90 s. System control and data collection were done by Xcalibur software version 1.4 (Thermo, USA). The scan range was set from m/z 400 to m/z 1600.

Supplementary Figures

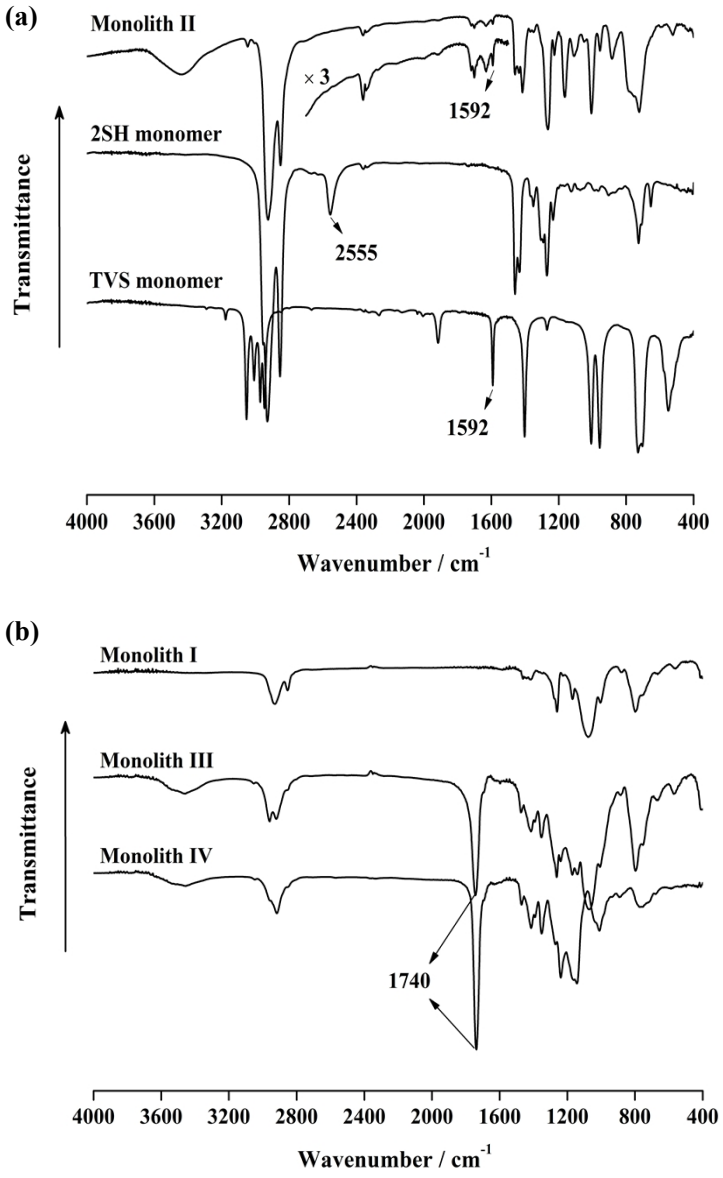

Fig. S1 FT-IR spectra of (a) the TVS and 2SH monomers, and monolith **II**; (b) monoliths **I**, **III** and **IV**.

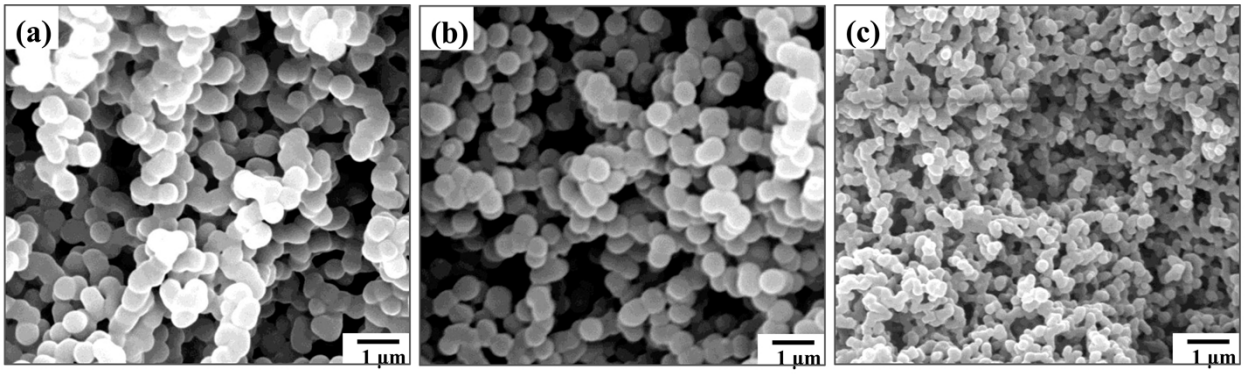

Fig. S2 SEM micrographs of hybrid monoliths using different porogenic solvents (a) TVS/2SH (15.5/26.8, mg/mg) with DEGDE/1-propanol (170/10, v/v); (b) and (c) TVS/4SH (17/45, mg/mg) with DEGDE/1-propanol (103/137, v/v) and DEGDE/PEG200 (140/80, v/v), respectively.

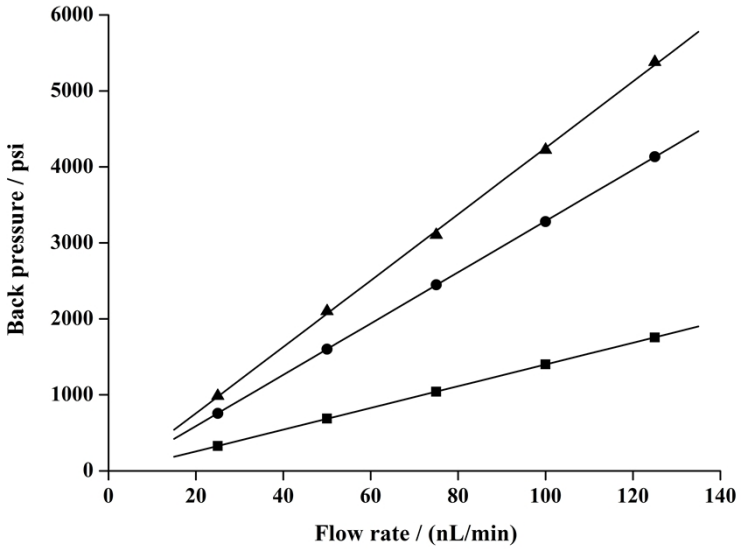

Fig. S3 The relationship between flow rate and back pressure drop on hybrid monolith **II** prepared with different ratio of THF/DEGDE (30/150, (■); 35/145, (●) and 40/140, (▲), v/v). Experimental conditions: column length, 22.8 cm×75 μm i.d.; mobile phase, ACN/water (40/60, v/v).

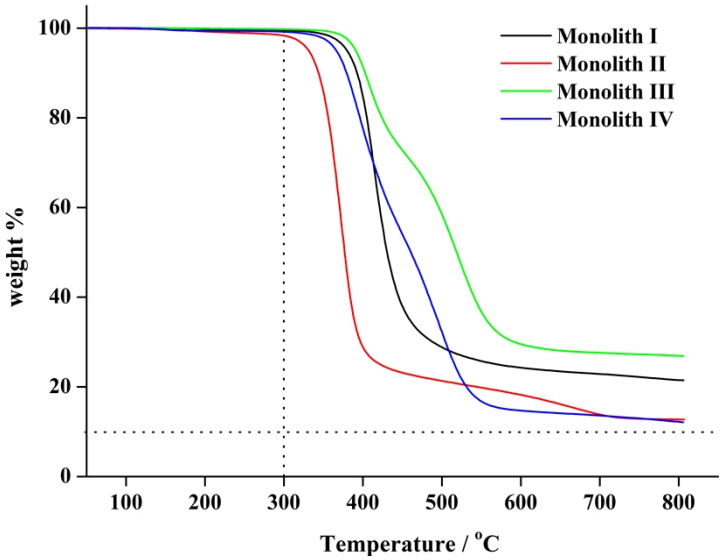

Fig. S4 TG analysis of monoliths **I-IV** at a heating rate of 10 °C min^{-1} under air atmosphere, indicating that pyrolysis begins from about 300 °C.

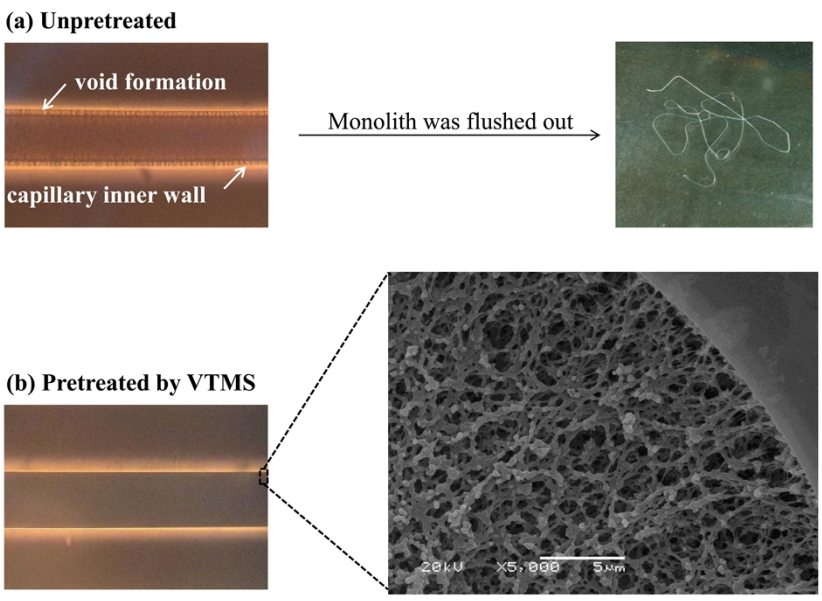

Fig. S5 (a) void formation between monolith and inner wall in the unpretreated capillary. (b) SEM micrograph of hybrid monolith **II** being anchored to the inner wall of UV-transparent capillary pretreated with VTMS.

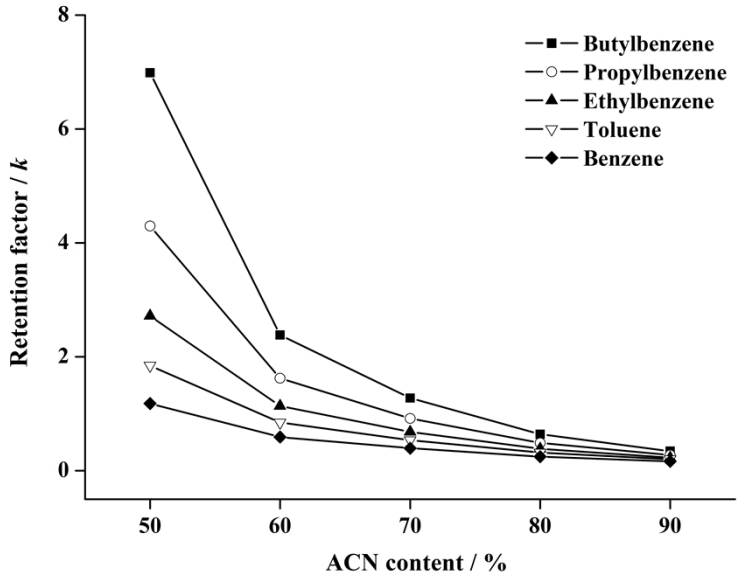

Fig. S6 The effect of ACN content in mobile phases on retention factors of alkylbenzenes on monolith **II**. Experimental conditions: effective length, 25 cm×75 μm i.d.; flow rate, 100 μL min^{-1} (before split); detection wavelength, 214 nm.

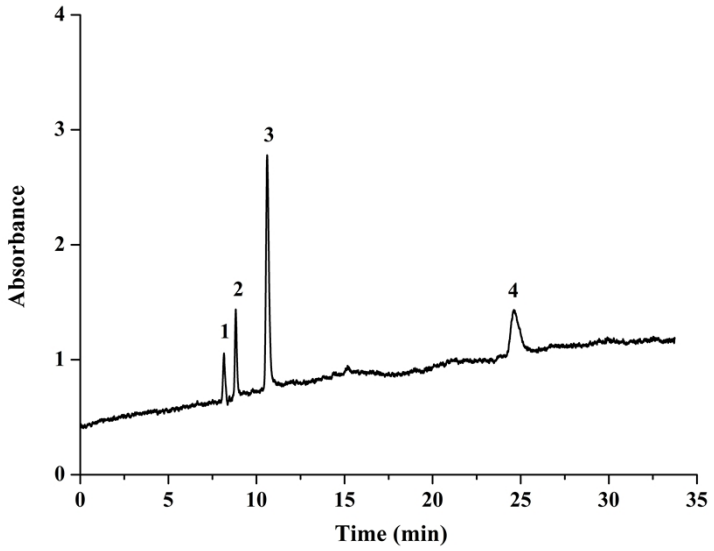

Fig. S7 Separation of peptides on hybrid monolith **II** by cLC under isocratic condition. Analytes: (1) Arg-Gly, (2) Trp-Phe, (3) Trp-Tyr and (4) Trp-Trp. Experimental conditions: effective length, 25 cm×75 μm i.d.; mobile phase, ACN/triethylammonium acetate solution (pH=4.2), (10/90, v/v); flow rate, 80 μL min^{-1} (before split); detection wavelength, 214 nm.

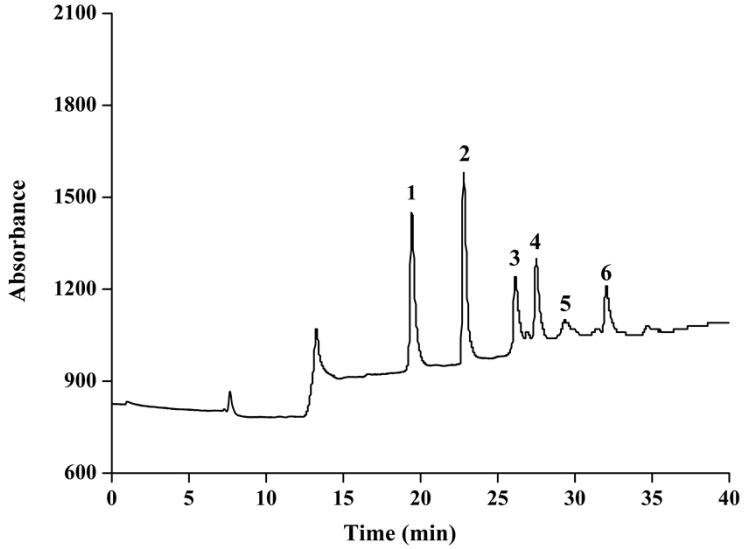

Fig. S8 Separation of proteins on hybrid monolith **II** by nano-HPLC. Solute of standard protein mixture: (1) ribonuclease B, (2) cytochrome c, (3) insulin, (4) lysozyme, (5) BSA and (6) myoglobin; Experimental conditions: effective length, 28 cm×75 μm i.d.; mobile phase A, water with 0.1% TFA, mobile phase B, ACN with 0.1% TFA; gradient, 5% B to 35% B in 30 min; flow rate, 300 nL min^{-1}; detection wavelength, 214 nm.

Supplementary Tables

Table S1 The theoretical and calculated silicon content in monoliths

Monolith	**I**	**II**	**III**	**IV**
Theoretical (%)	17.5	7.5	15.6	5.6
Calculated (%)	10.5	6.0	12.9	5.6

Table S2 Carbon and hydrogen contents in monoliths

Monolith	**I**	**II**	**III**	**IV**
Carbon (%)	44.42	55.50	41.47	48.10
Hydrogen (%)	8.04	9.23	6.18	6.42