



Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: [www.elsevier.com/locate/chroma](http://www.elsevier.com/locate/chroma)



Full length article

## Facile preparation of multi-functionalized hybrid monoliths via two-step photo-initiated reactions for two-dimensional liquid chromatography–mass spectrometry

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### ARTICLE INFO

#### Article history:

Received 3 July 2017

Received in revised form 25 August 2017

Accepted 25 September 2017

Available online xxx

#### Keywords:

Hybrid monolith

Polyhedral oligomeric silsesquioxane

Click

reaction

Two-dimensional separation

### ABSTRACT

A facile approach was developed to prepare hybrid monoliths with different functions via two-step photo-initiated reactions. Firstly, acrylopropyl polyhedral oligomeric silsesquioxane (acryl-POSS) and propargyl acrylate (PA) were used as precursors to synthesize alkyne-functionalized hybrid monoliths via photo-initiated free radical polymerization. Secondly, the hybrid monoliths were modified with 1-octadecanethiol (ODT) and sodium 3-mercaptopropanesulfonate (SMPS) via photo-initiated thiol-yne click reaction to prepare reversed-phase (RP) and strong cation-exchange (SCX) hybrid monoliths, respectively. The results of chromatographic characterization indicated that the column efficiencies for alkylbenzenes on ODT-modified hybrid monolith reached 84,000–87,700 plates per meter at the velocity of 0.58 mm/s, and also revealed a retention-independent efficient performance of small molecules in isocratic elution. The SMPS-modified hybrid monolith exhibited both hydrophobicity and ion-exchange mechanisms, and the dynamic binding capacity was calculated to be  $1.4 \times 10^{-4} \mu\text{mol}/\text{cm}$ . Human Hela cells tryptic digest was well separated on ODT-modified hybrid monolith in one-dimensional RPLC–MS/MS, and 2786 unique peptides and 685 proteins were identified. Furthermore, the SMPS-modified monolith coupled with ODT-modified monolith was used for two-dimensional separation of human Hela cells tryptic digest in SCX–RPLC–MS/MS, and the results showed that 9744 unique peptides and 2749 proteins were identified. Compared to those identified in one-dimensional RP system, the total numbers of unique peptides and proteins identified in SCX–RP system increased by 249.7% and 301.3%, respectively.

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### 1. Introduction

Since the early 1990s, the emergence of monoliths used for nanoHPLC has gained extensive attention on the separation of small molecules and biomacromolecules because of their excellent properties, such as ease of preparation, low amount of mobile phases and samples to accomplish detection, and compatibility with mass spectrometry (MS) [1–5]. Among them, organic-inorganic hybrid monolith was supposed to possess some merits, including wide pH tolerance, good mechanical properties and high permeability [6,7]. Meanwhile, the versatility of hybrid monolith could be simply

regulated by polymerization mixture composition, polymerization conditions and post-modification. Metal-organic framework (MOF) [8,9], covalent-organic framework (COF) [10] or inorganic oxide [11,12] was introduced into monoliths to provide large specific surface areas, sufficient mesopores and high permeability, which could improve the separation efficiency and resolution in small molecules. However, the preparation process of these additives was tedious and time-consuming in most cases. To solve this problem, the introduction of commercial polyhedral oligomeric silsesquioxanes (POSS) could be a facile alternative. Many researches [13–18] reported to prepare various hybrid monoliths based on different POSS monomers, which exhibited excellent physical properties. Especially, the POSS-based hybrid monolithic columns exhibited satisfactory column efficiencies for small molecules in capillary liquid chromatography (cLC) [6,19].

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**Table 1**  
Detailed composition of prepolymerization mixtures and permeability of hybrid monoliths.

Hybrid monolith <sup>a</sup>	PA (w/w, %)	Precursors content (w/v, %)	Porogenic solvents (v/v, %) <sup>b</sup>	Permeability (10 <sup>-14</sup> m <sup>2</sup> )
1	0	21.4	8.6/91.4	1.34
2	25	28.6	7.1/92.9	1.99
3	25	28.6	8.6/91.4	1.46
4	25	28.6	10.7/89.3	1.22
5	40	35.7	7.1/92.9	2.10
6	40	35.7	8.6/91.4	1.66
7	40	35.7	10.7/89.3	1.39

<sup>a</sup> The prepolymerization solution contained 100 mg acryl-POSS and 5  $\mu$ L 10% DMPA (w/w, dissolved in 1-butanol). The capillary was irradiated in UV light (wavelength 365 nm, 120 mJ/cm<sup>2</sup>) for 10 min.

<sup>b</sup> The porogenic solvents were the mixture of THF and 1-butanol (v/v).

Multidimensional liquid chromatography (LC) as a powerful separation technology could enhance the separation performance. Particularly, online multidimensional separation exhibits many advantages such as high sensitivity, minimal loss of sample, and avoidance of sample dilution effect and contamination [20–22]. Our group [23–25] prepared a strong cation-exchange (SCX) monolith in capillary and coupled it as trap column with a reversed-phase (RP) monolithic column for online multidimensional separation in shotgun proteome analysis. Excellent separation performance of this biphasic monolithic column was obtained, owing to the good orthogonality of SCX and RPLC. These results greatly inspired us to further search for different functional hybrid monolithic columns for multidimensional separation.

“Click” chemistry, due to its high yield and simple reaction conditions, has been applied in newly emerged technologies, such as new compounds synthesis, polymer preparation, surface modification and hydrogel formation [26–29]. Many works have been done to prepare beads or monoliths as LC stationary phases via “click” chemistry [14,30–34]. However, photo-initiated thiol-yne click reaction was not adopted to modify POSS-based hybrid monoliths for different chromatographic modes in cLC.

In this study, we prepared alkynyl-functionalized POSS-based hybrid monoliths via photo-initiated free radical polymerization with acrylopropyl polyhedral oligomeric silsesquioxane (arcyl-POSS) and propargyl acrylate (PA) as monomers. Due to the existence of alkynyl group in hybrid monolith, 1-octadecanethiol (ODT) and sodium 3-mercapto-1-propanesulfonate (SMPS) could be successfully bonded on the surfaces of hybrid monoliths via photo-initiated thiol-yne click reaction. The physical characterization of these hybrid monoliths was carried out, and their applications in separation of complicated biological samples in two-dimensional cLC-MS/MS were also performed.

## 2. Materials and methods

### 2.1. Chemicals and reagents

The monomer of acryl-POSS, a hybrid molecule with an inorganic silsesquioxane at the core and organic acrylopropyl groups attached at the corners of the cage, was obtained from Hybrid Plastics, Inc (Hattiesburg, MS, USA). SMPS, ODT, di(ethylene glycol) diethyl ether (DEGDE, 98%), 3-(trimethoxysilyl) propyl methacrylate ( $\gamma$ -MAPS,  $\geq 98\%$ ), formic acid (FA), bovine serum albumin (BSA), protease inhibitor Cocktail (for use with mammalian cell and tissue extracts), phosphatase inhibitor (1 mmol/L NaF and 1 mmol/L Na<sub>3</sub>VO<sub>4</sub>), TPCK treated trypsin, urea, iodoacetamide (IAA), EPA610, 1,4-dithiothreitol (DTT) and polystyrenes (Mw = 800, 4 000, 13 200, 35 000, 50 000, 90 000, 280 000 and 900 000) were purchased from Sigma (St Louis, MO, USA) and used directly without further purification. 2,2-Dimethoxy-2-phenylacetophenone (DMPA, 99%) and PA were purchased from Acros Organics (New Jersey, USA). Thiourea, benzene, toluene, ethylbenzene, propylbenzene,

butylbenzene, ammonium acetate, ethanol, tetrahydrofuran (THF) and 1-butanol were of analytical grade, and obtained from Tianjin Kermel Chemical Plant (Tianjin, China). Deionized water was prepared with a Milli-Q system (Milli-pore, MA, USA). Methanol and acetonitrile (ACN) were HPLC-grade and acquired from Yuwang Group (Shandong, China). Both polyimide-coated fused-silica capillary with 50  $\mu$ m i.d. and UV-transparent fused-silica capillary with 150  $\mu$ m i.d. were the products of Reafine Chromatography Ltd. (Hebei, China). UV-transparent fused-silica capillary with 75  $\mu$ m i.d. was the product of Polymicro Technologies (Phoenix, AZ, USA).

### 2.2. Preparation of alkynyl-functionalized hybrid monoliths

Prior to preparation, the inner wall of UV-transparent capillary has to be covered with a layer of methacrylate groups. In brief, capillary was treated with 0.1 mol/L NaOH (1 h), H<sub>2</sub>O (0.5 h), 0.1 mol/L HCl (4 h), H<sub>2</sub>O (0.5 h) and methanol (0.5 h) in sequence. Then a  $\gamma$ -MAPS solution (50% in methanol, v/v) was injected into the capillary with a syringe. After both ends of capillary were sealed with silicon septa, the capillary was submerged into water at 50 °C overnight. Finally, the capillary was rinsed with methanol to flush out the residual  $\gamma$ -MAPS from the capillary, and was dried under nitrogen flow for 5 h.

Alkynyl-functionalized hybrid monolith (assigned as monolith I) was manufactured in the  $\gamma$ -MAPS-pretreated UV-transparent capillary. A prepolymerization solution consisting of crosslinker (acryl-POSS), functional monomer (PA), photo-initiator (DMPA) and binary porogenic solvents (THF and 1-butanol) was prepared. The detailed composition of prepolymerization solution was shown in Table 1. After ultrasonication for 5 min to degas, the prepolymerization solution was immediately introduced into the  $\gamma$ -MAPS-pretreated capillary. After both ends were sealed with silicon septa, the capillary was settled under UV light for 10 min. The obtained hybrid monolithic column was flushed with methanol to remove the residual. The rest of prepolymerization solution in the vial was also cured under UV light to form bulk monolithic material at the same conditions. After the material was rinsed with ethanol three times, it was cut into small pieces, ground using a mortar and pestle, and then dried in a vacuum at 50 °C for 24 h.

### 2.3. Modification of hybrid monolith I with monothiol-containing monomers

A DEGDE solution containing 15.0% (w/v) ODT and 0.3% (w/v) DMPA was flushed through hybrid monolith I. Then the reaction was performed by UV light irradiation for 15 min. Finally, the ODT-modified hybrid monolith (assigned as monolith II) was obtained after washing with methanol to remove the residual. Another thiol-containing monomer (SMPS) was used to modify hybrid monolith I with the similar method. The SMPS-modified hybrid monolith was named as monolith III.

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