Electronic Supplementary information （ESI）

Aptamer@AuNPs modified POSS-polyethylenimine hybrid affinity monolith with high coverage density of aptamer for sensitive and selective recognition of ochratoxin A†

Xia Yu [a], Hongliang Song [a], Jing Huang [a], Yongxuan Chen [b], Ming Dai [b], Xucong Lin [a,*], Zenghong Xie [a]

[a.] Institute of Food Safety and Environment Monitoring, Fuzhou University, Fuzhou, 350108, China

[b.] Fujian Inspection and Research Institute for product quality, Fuzhou, 350002, China

†Corresponding author: Prof. Xucong Lin

Fax: +86-591-22866131

E-mail address: xulin@fzu.edu.cn

1. Experimental

1.1. Preparation of Apt@AuNPs and POSS-PEI parent matrixes

AuNPs were prepared according to the method reported previously.[1] AuNPs were modified with thiolated aptamers as follows. A 60 μL of TCEP solution (5 mM)was added into a 40 μL of thiolated aptamer (100 μmol/L)solution, and the mixture was incubated at room temperature for 1 h to reduce the disulfide formation of aptamer and form free thiol groups. Then, a 2.0 mL of AuNPs solution (10 nmol/L) was added into this solution. The obtained mixture was vortexed for 2 min and kept in the dark at 4 ℃ overnight. The Apt@AuNPs samples were finally salt-aged with 120 μL 1.0 mol/L NaCl solution for 24 h at 4 ℃.

The fused-silica capillary (100 μm i.d. × 365 μm o.d.) was pretreated according to the previous report.[1] POSS-based hybrid monolithic column was prepared according to the process and feed recipes shown in Fig.1 and Table S1, respectively. The mixtures of monomers were weighed, and then dissolved in porogenic solvents consisting of 1-propanol, 1,4-butanediol and PEG (Mn = 10000) in different ratios (as shown in Table S1). The polymerization mixture was vortexed and mixed under ultrasonication to form a homogeneous solution prior filling the capillary. Then, with both ends sealed by silicon, the capillary columns were immersed in a 50 ℃ water bath for 16 h and rinsed successively with methanol to flush out the residual reagents.

1.2. Calculation of EOF

The electroosmotic flow (EOF of monolithic columns was investigated with capillary electrochromatography. The EOF was calculated as following formula:

$$\mu_{eo} = \frac{L}{t_{02}} - \frac{L}{t_{01}} \qquad \text{（Equation S1）}$$

L (mm) is effective length of capillary monolithic column, t_{01} (s) is the retention time by pressure flow as the driving force, t_{02} (s) is the retention time by pressure flow and electroosmotic flow as the driving force.

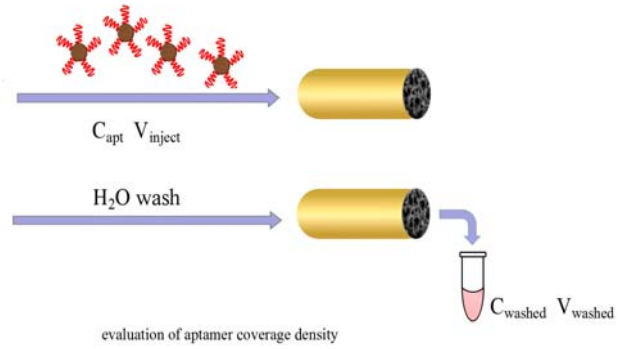
1.3. Calculation of SP

The swelling propensity (SP) is a criterion for the swelling behavior of organic material which might lead to problems such as poor stability of capillary columns, rapidly resulting in reduced efficiencies, loss of resolution and poor reproducibility. The swelling propensity factor (SP) EOF was calculated as following formula:

$$SP = \frac{p(ACN) - p(H_2O)}{p(H_2O)}$$ （Equation S2）

where p is the pressure relative to the viscosity, p =P/η. P was the pressure and η was the viscosity of ACN and H_2O. In general, the SP value of a non-swelling material was zero.[3]

1.4. Calculation of Aptamer coverage density



Scheme S1. The manipulation process of to calculate aptamer coverage density in monolith

As shown in the above shceme, the amount of Apt@AuNPs immobilized on POSS-PEI monolith was evaluated by calculating the content decrease of Apt@AuNPs before and after the mobilization. At first, the Apt@AuNPs solution was pumped into the POSS-PEI monolith until the monolith has been saturated with Apt@AuNPs solution. The total volume of Apt@AuNPs aqueous solution injected into POSS-PEI monolith was calculated. Then the monolithic column was rinsed with water to remove the excess or unbound Apt@AuNPs until no obvious Apt@AuNPs was detected by a UV-Vis spectrometer (UV-2450, Japan) at 285 nm. All the volume of washing solution to remove the unbound Apt@AuNPs from the resultant Apt@AuNPs@POSS-PEI monolith was defined as V_{washed} and calculated.

So the aptamer coverage density could be calculated as below:

$$\rho = \frac{n_{injected} - n_{washed}}{V_{monolith}} = \frac{C_{apt} \times V_{injected} - C_{washed} \times V_{washed}}{V_{monolith}}$$

Where ρ was coverage density of aptamers, $n_{injected}$ and n_{washed} were the amount of Apt@AuNPs injected into POSS-PEI monolith and washed from the column, respectively. $V_{injected}$ was the total inject volume of Apt@AuNPs solution. V_{washed} was the total volume of washing solution to remove the unbound Apt@AuNPs from the monolith. $V_{monolith}$ was the volume of affinity monolith. The concentration C_{washed} of the Apt@AuNPs elution solution was calculated based on the absorbance measured at 520 nm via Beer's Law.

References:

[1] Grabar K C, Freeman R G, Hommer M B, et al., *Analytical Chemistry*, 1995, **67**(4):735-743.

[2] Lin H, Ou J, Tang S, et al., *Journal of Chromatography A*, 2013, **1301**(15):131-138.

[3] Nevejans F, Verzele M. Journal of Chromatography A, 1985, **350**(1):145-150.

2. Supplementary data

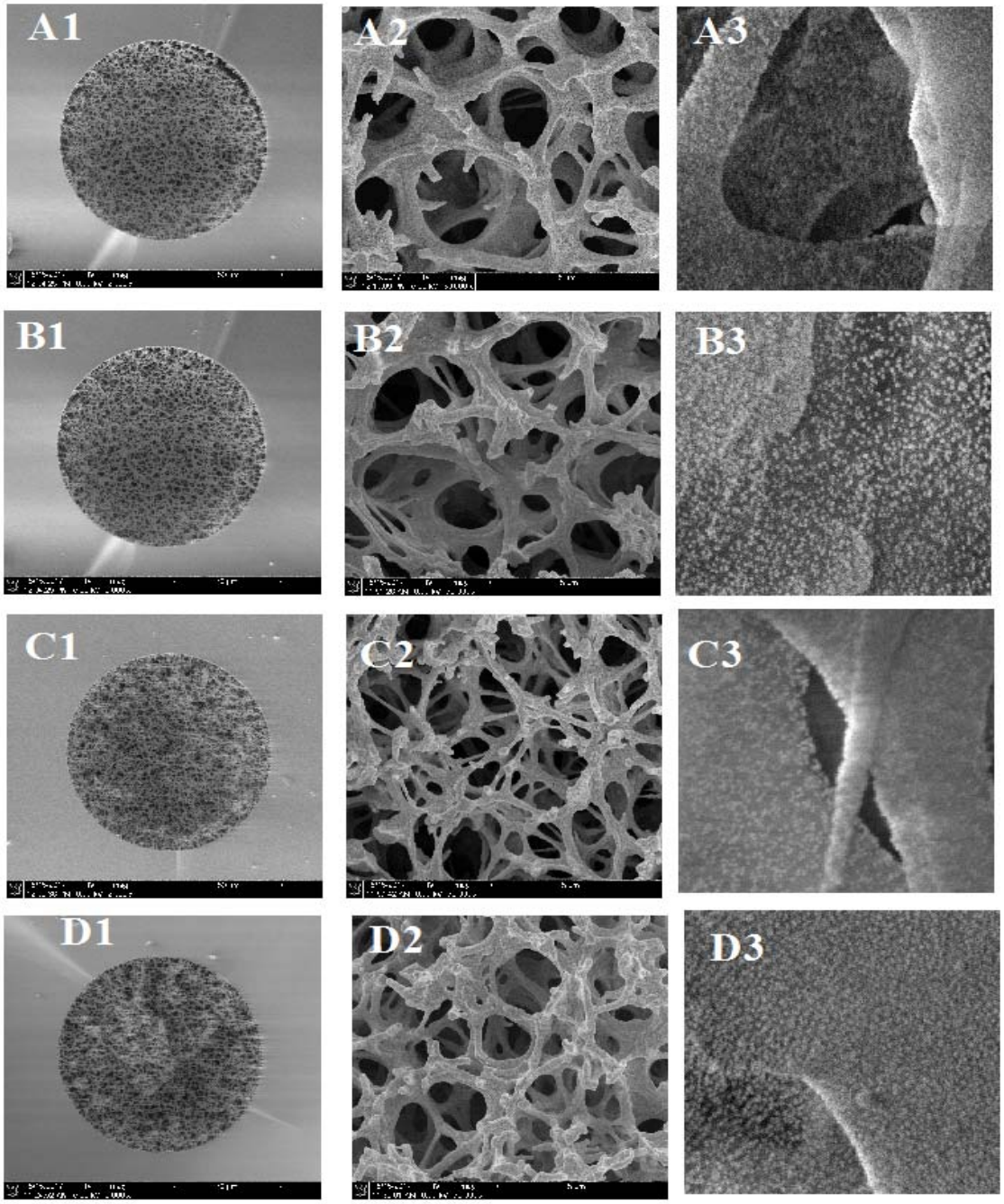


Fig.S1 SEM images of the structure of Apt@AuNPs@POSS-PEI hybrid affinity monoliths with different permeability.

The permeability of A-D series was shown as A_1-A_3: 3.70 × 10^{-14} m^2, B_1-B_3: 1.88 × 10^{-14} m^2, C_1-C_3: 1.481 × 10^{-14} m^2, and D_1-D_3: 1.11 × 10^{-14} m^2 respectively. Each column (A-D) was measured with three SEM magnification times. A_1-A_3, were measured with the SEM magnification times as × 2 000, × 30 000, × 100 000, as well as B_1-B_3, C_1-C_3 and D_1-D_3.

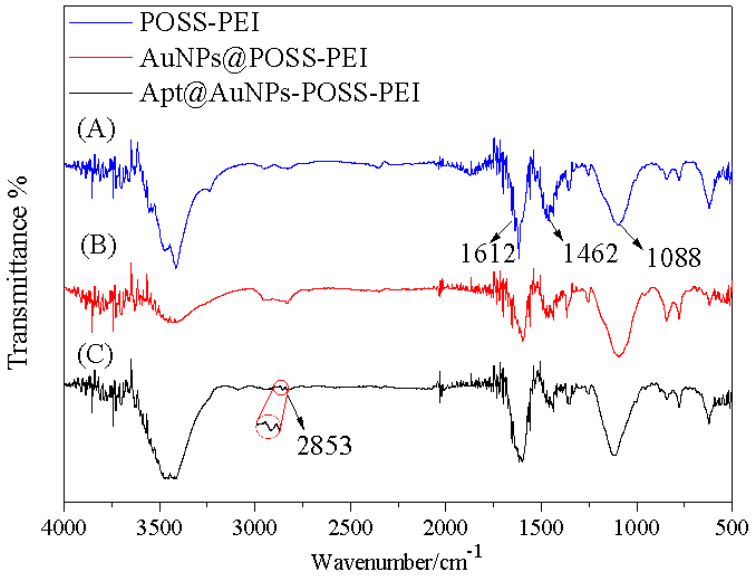


Fig.S2 FT-IR spectra of monolithic columns.

(a) POSS-PEI hybrid monolithic column, (b) AuNPs@POSS-PEI monolithic column,(c) Apt@AuNPs@POSS-PEI monolithic column.

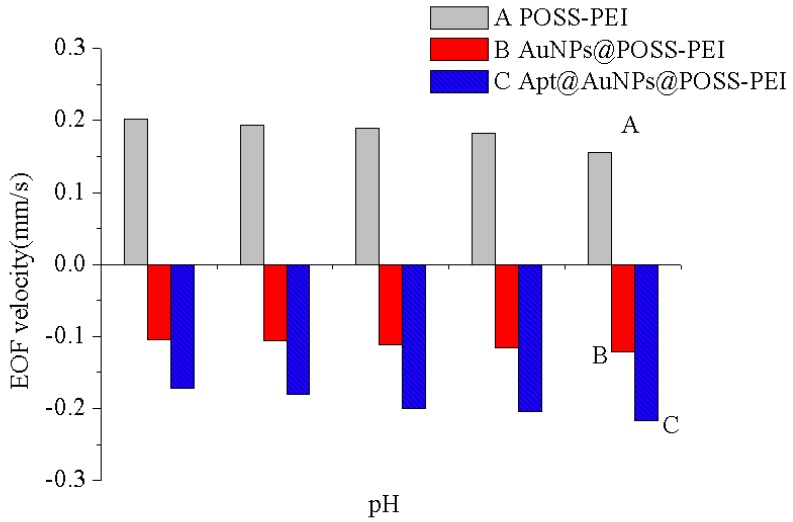


Fig.S3 EOF values of three monoliths under different pH conditions

(a) POSS-PEI, (b) AuNPs@POSS-PEI, (c) Apt@AuNPs@POSS-PEI

Effective length of monolithic column was 20 cm (total length was 40 cm), mobile phase: ACN/ phosphate buffer (5 mmol/L)=70/30 (v/v), pump flow was 0.1 mL/min, applied pressure was 4.0 MPa, detection wavelength was 214 nm, applied voltage was +10 kv. Toluene was used as the EOF marker, and the EOF from anode to cathode was denoted as positive.

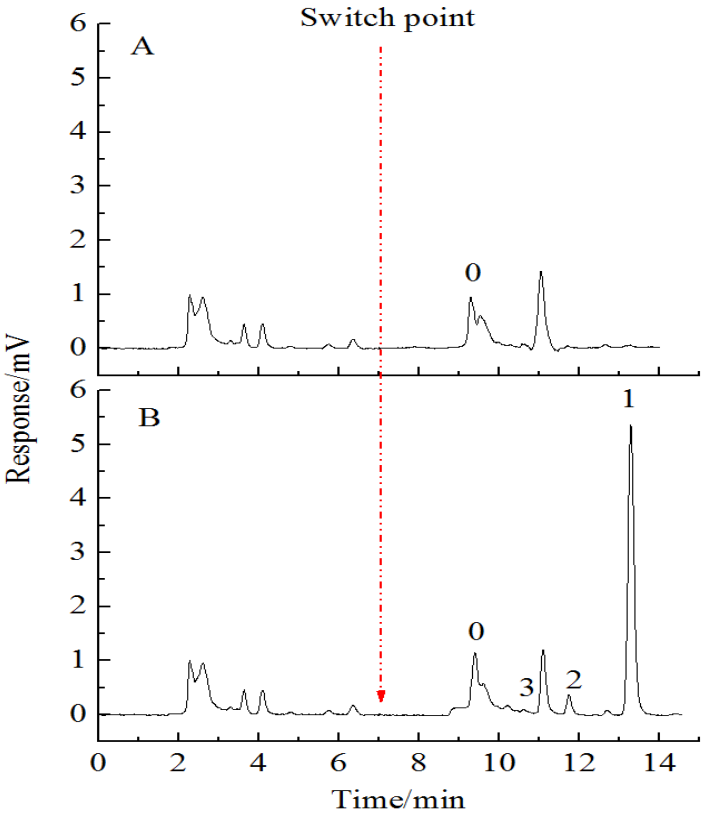


Fig.S4 Affinity recognition of Apt@AuNPs@POSS-PEI monolith to blank sample (A) and mixture solution (B)

A: Chromatogram of blank sample, solute: 0: background peak. B: Chromatogram of the mixture composed of OTA, OTB and AFB_1 with the concentration of 10 ng/mL, solute: 0: background peak, 1: OTA, 2: OTB, 3: AFB_1.

Before the switching point, binding buffer solution (10 mM Tris-HCl, 120 mM NaCl, 5 mM KCl and 20 mM $CaCl_2$, pH 8.50) was used to wash the monolith firstly, and after the switching point, the elution solution composed of TE buffer solution (Tris-HCl 10 mM, EDTA 2.5 mM, pH 8.00) and ACN with the ratio of 30:70 (v/v) was used to elute the OTA.

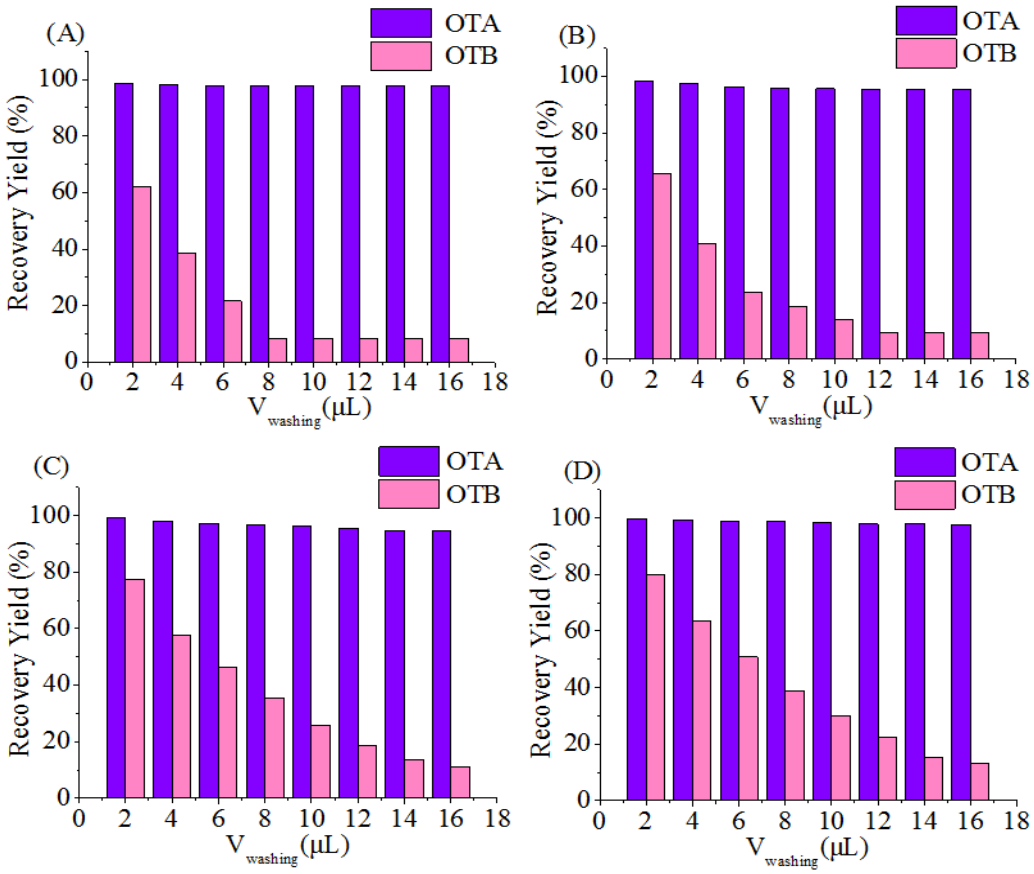


Fig. S5 Recovery yields of OTA and OTB in Apt@AuNPs@POSS-PEI affinity monolithic column under different volumes of binding buffer solution.

Binding buffer solution (10 mM Tris-HCl, 120 mM NaCl, 5 mM KCl and 20 mM $CaCl_2$, pH 8.50), concentration of OTA and OTB: A: 10 ng/mL, B: 25 ng/mL, C: 50 ng/mL, D: 100 ng/mL. Injection volumn was 20 μL, Effective length of Apt@AuNPs@POSS-PEI column was 10 cm.

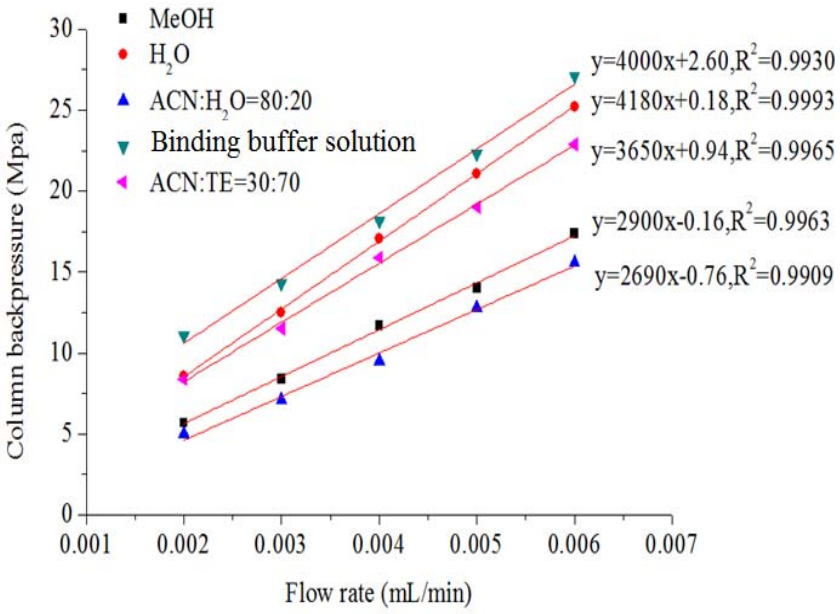


Fig. S6 Effect of mobile-phase flow rate on hybrid affinity monolithic column back-pressure. Experimental conditions: 5.0 cm length × 100 μm i.d. monolithic column, binding buffer solution (pH 8.50) was composed of 10 mM Tris-HCl, 120 mM NaCl, 5 mM KCl and 20 mM $CaCl_2$, the elution solution was ACN: TE = 30:70 (v:v) and TE solution (pH 8.00) was 10 mM Tris-HCl and 2.5 mM EDTA solution.

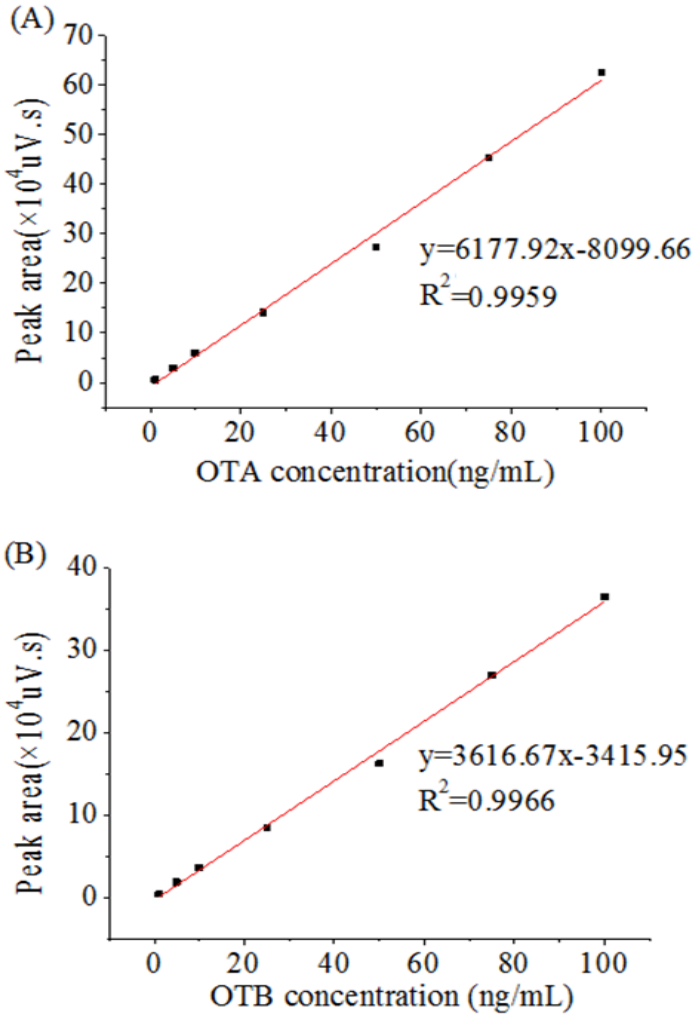


Fig. S7 The calibration curves of OTA (A) and OTB (B) with HPLC.

Experimental conditions: the separation column in HPLC was C18 column (Alltech, USA, 5μm,4.6 mm i.d. × 250 mm) and the injection volume was 20 μL. Isocratic elution was used with a mix of acetonitrile/ 2.0% aqueous acetic acid (62%:38%, v/v) at a flow rate of 1.0 mL/min. The fluorescence excitation and emission wavelengths were 333 nm and 460 nm, respectively.

Table S1 Feed recipes for the preparation of POSS-based monolithic columns

Monolithic column	Monomer-to-solvent Ratio[a] (w:w)	Poss-epoxy: PEI or BDA (w:w)	1,4-Butanediol[b] (w_t%)	1-Propanol[c] (w_t%)	PEG[d] (w_t%)	Permeability[e] K ($\times 10^{-14}$ m^2)
POSS-PEI1200 [f]	25:75	55:45	14%	78.0%	8.0%	1.85
POSS-PEI600 [f]	25:75	55:45	14%	77.5%	8.5%	1.94
POSS-BDA [f]	25:75	65:35	14%	78.0%	8.0%	1.90

a) POSS-epoxy as crosslink and PEI or BDA as monomer, 1,4-Butanediol, 1-Propanol and PEG (Mn = 10000) were as solvent.

b-d) Percentage of 1,4-Butanediol/ 1-Propanol/ PEG in the porogen solvent mixture.

e) the permeability was measured by using methanol. the viscosity of methanol was 0.544 mPa•s. flow rate 0.005 mL/min, 5.0 cm length×100 μm i.d..

f) PEI 1200 (Mn = 1200, 50% in water), PEI 600 and BDA were prepared by ultrapure water to form 50% aqueous solution, respectively.

Table S2 The coverage density of aptamer in Apt@AuNPs@POSS-PEI affinity monolith [a]

n_{apt} (nmol)	$V_{solution}$ (μL)	C_{apt} (nmol/L)	$V_{injected}$ (μL)	C_{eluted} (nmol/L)	V_{eluted} (μL)	$V_{monolith}$ (μL)	ρ (pmol/L)
1.889	2220	851	1400	411	200	0.785	1413

a) POSS-PEI1200 monolith parent matrix, effective length was 10 cm in capillary column (100 μm i.d. × 360 μm o.d.)

Table S3 The reproducibility of hybrid affinity monolithic column

Column	RSD intra-day (n = 3)[a]	RSD inter-day (n = 3)[b]
Apt@AuNPs@POSS-PEI	1.9	2.8

a. RSD intra-day was determined based on three replicates in the same day.

b. RSD inter-day was determined by analyzing the marker in three consecutive days.