Electronic Supplementary Information

A facile AuNPs@aptamer modified mercaptosiloxane-based hybrid affinity monolith with an unusually high coverage density of aptamer for on-column selective extraction of ochratoxin A

Jinxin Chi [a,b], Maolin Chen [a], Lijun Deng [a], Xucong Lin [a,*] and Zenghong Xie [a,*]

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

b. Xiamen huaxia university, Xiamne 361024, China.

*Corresponding author: Prof. Xucong Lin

Fax: +86-591-22866131

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

1. Methods

1.1 Synthesis of AuNPs

(1) AuNPs of 5.0 nm diameter were prepared as below [JACS, 127(15) (2005) 5312-5313]:

In the presence of 1 mL of sodium citrate (10 mM) and 36 mL of fresh pure water (18.25 MΩ), 1 mL of HAuCl4 (10 mM) was reduced by 1 mL $NaBH_4$ (100 mM) with stirring vigorously. Upon addition of the $NaBH_4$, the solution turned a reddish orange color and was allowed to continue stirring for two minute. The resulting mixture was aged for 2-4 hours to finish the hydrolysis of unreacted $NaBH_4$. The gold nanoparticle seeds exhibited a plasmon resonance peak at 513 nm, and had an average diameter of 5.0 nm.

(2) AuNPs of 15-35 nm diameters were prepared as below [Chem. Comm, 19 (19) (2008) 2242-2244]:

A 50mL of $HAuCl_4$ (1 mM) was brought to a vigorous boil with stirring in a round-bottom flask fitted with a reflux condenser. Then, trisodium citrate (38.8 mM) was added rapidly. The mixture was heated under reflux for another 10 min, during which its color changed from pale yellow to deep red. The solution was cooled to room temperature with stirring continuously. Gold nanoparticles exhibited a plasmon resonance peak at 520, 523 and 526 nm respectively, and had the corresponding average diameter of 15, 25 and 35 nm.

(3) According to the reference [Anal. Chem. 2007, 79, 4215-4221], the size of gold nanoparticles can be determined directly by UV-vis spectra. The particle diameter of AuNPs was calculated by the following equation:

$$\mathrm{d} = \exp(B_1 \frac{A_{spr}}{A_{450}} - B_2) \qquad \text{..... (1)}$$

Where A_{spr} is the absorbance of the surface plasma resonance peak and A_{450} is the absorbance at 450nm; B_1=3.00; B_2=2.20.

1.2 Aptamer coverage density

Aptamer coverage density was evaluated according to the method reported previously [Talanta, 154 (2016) 555-559. Analyst, 141(16) (2016) 4961-4967]. By using a UV-Vis spectrometer (UV-2450, Japan) at 285 nm, The aptamer coverage density was calculating by dividing the amount of aptamer by the volume of obtained aptamer-modified monolith.

The aptamer coverage density was calculated as below:

$$\rho = \frac{n_{injected} - n_{eluated}}{V_{monolith}} = \frac{C_{apt} \times V_{injected} - C_{eluated} \times V_{eluated}}{V_{monolith}} \quad \text{..... (2)}$$

Where ρ was coverage density of aptamers on the resultant monolith, $n_{injected}$ and $n_{eluated}$ were the amount of aptamer injected into AuNPs modified poly(TMOS-co-MPTMS) monolith and eluted from the monolith, respectively. $C_{eluated}$ was the concentration of aptamer solution injected into AuNPs modified poly(TMOS-co-MPTMS) monolith, $V_{injected}$ was the volume of aptamer solution injected into AuNPs modified poly(TMOS-co-MPTMS) monolith. $C_{eluated}$ was the concentration of unbound aptamer in the total eluate solution, $V_{eluated}$ was the total volume of unbound aptamer which consists of the eluate solution and the washing solution. $V_{monolith}$ was the volume of obtained aptamer-modified monolith.

1.2 Dynamic binding capacity

Binding capacity of OTA on affinity monolith was measured by dynamic frontal analysis. The calculation equation was shown as below:

$Q_{max}=C\ (V_R-V_0)$

Where Q_{max} is the maximum binding capacity (ng), C is the analyte concentration (ng/mL), V_R is the retention volume (μL), V_0 is the void volume (μL). V_R could be determined from the diagram of breakthrough curve, and corresponded to 1/2 of the value of maximum analyte concentration in the effluent, which was according to the previous reference [*Talanta*, 80 (2009) 614-621]. A 100-ng/mL of OTA solution was used to saturate the aptamer binding sites. The breakthrough curve was constructed by plotting the peak response of OTA in effluent solution versus the volume of OTA solution.

V_0 of the affinity monolith was calculated as $V_0=V_{total}-V_{capillary}$, where $V_{total} = \mu \times t_{total}$, where μ is the flow rate (μL/min) of mobile phase in the capillary, t is the breakthrough time of mobile phase (min). DMF was employed as unretained compound to calculate the void volume.

2. Supplementary data

Fig.S1. SEM of poly(TMOS-co-MPTMS) monolith (a), poly(TMOS-co-MPTMS)@AuNPs monolith (b) and poly(TMOS-co-MPTMS)@AuNPs@Apt affinity monolith (c).

Fig.S2 FTIR spectra of silica-based parent column and aptamer-modified affinity monoliths

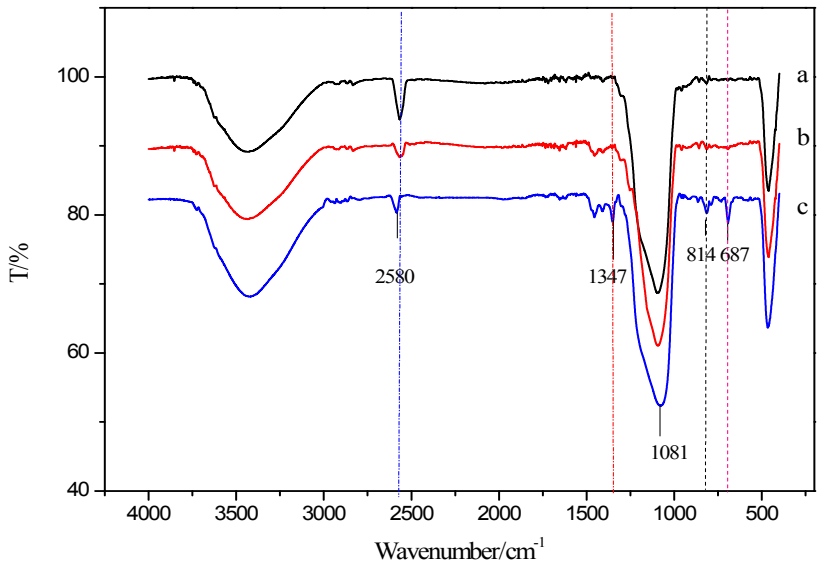
a. poly(TMOS-co-MPTMS) monoliths;

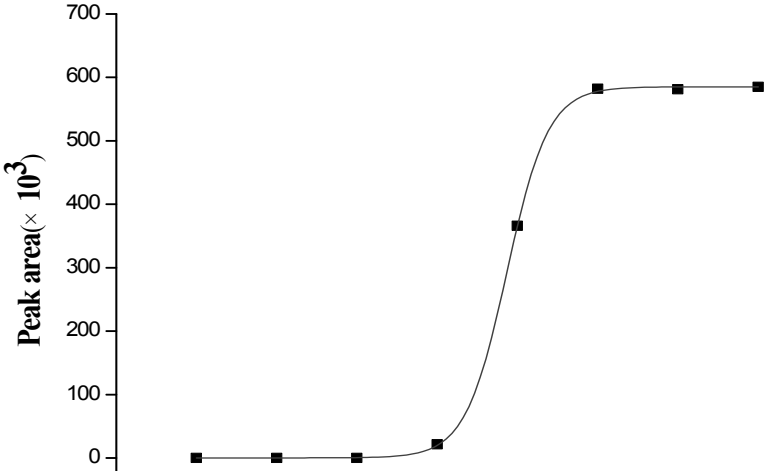
b. poly(TMOS-co-MPTMS)@AuNPs monolith

c. poly(TMOS-co-MPTMS)@AuNPs@Apt monolith

Fig.S3 Breakthrough curve of OTA in poly(TMOS-co-MPTMS)@AuNPs@Apt monolith

Affinity monolith: 10 cm-long, 75 μm i.d. × 365 μm o.d.; flow rate, 0.05 mL/min; OTA: 100ng/mL; DMF was used to estimate the void time.





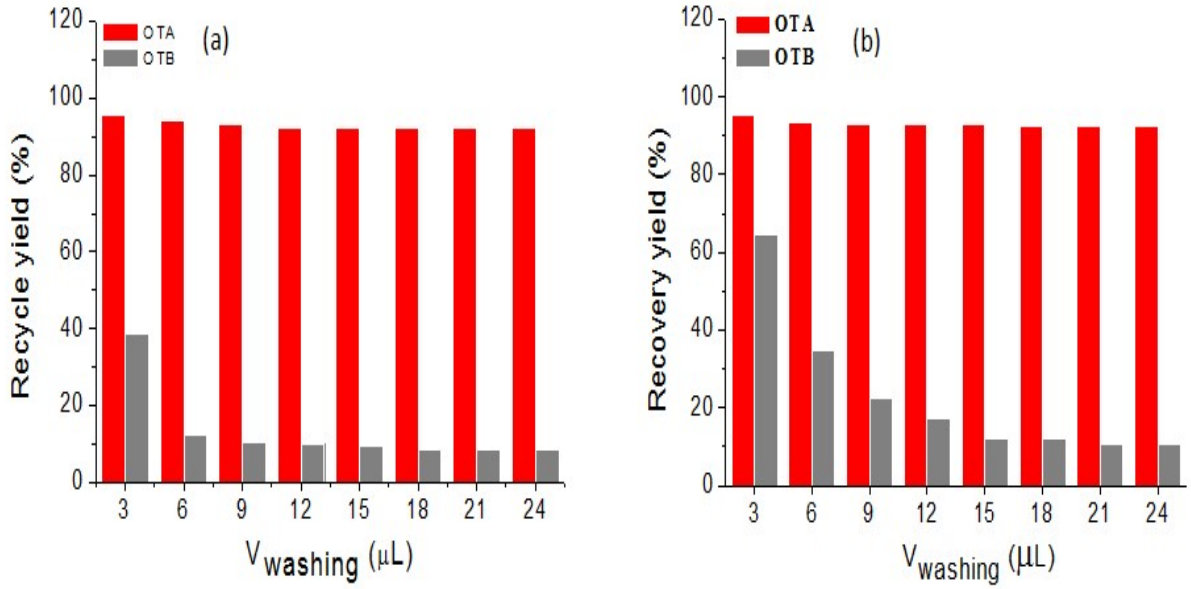


Fig.S4 Recoveries of OTA in the mixture containing high concentration of OTB with the affinity monolith after being washed with different volume of BB solution

Column: poly(TMOS-co-MPTMS)@AuNPs@Apt affinity monolith.

Concentrations of OTA and OTB: a: 25 ng/mL, b: 50 ng/mL.

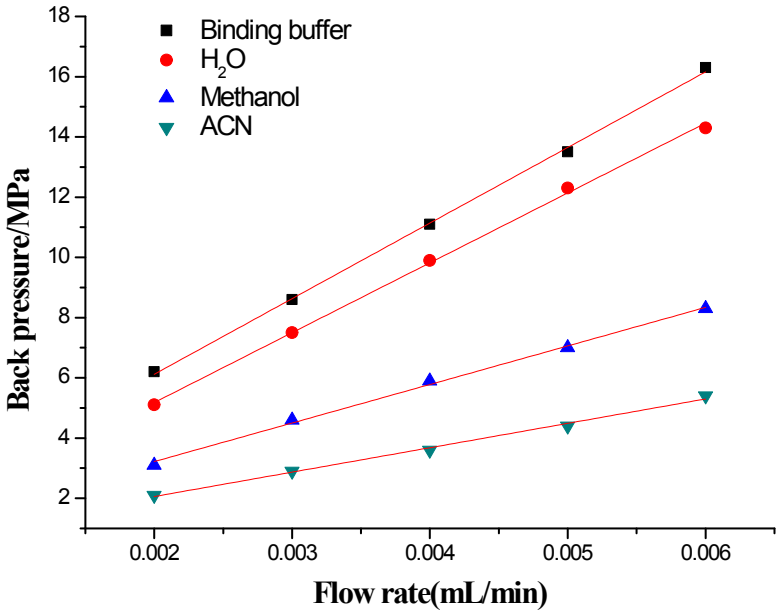


Fig. S5 Linear relationship of the mobile-phase flow rate on back-pressure of poly(TMOS-co-MPTMS)@AuNPs@Apt affinity monolith.

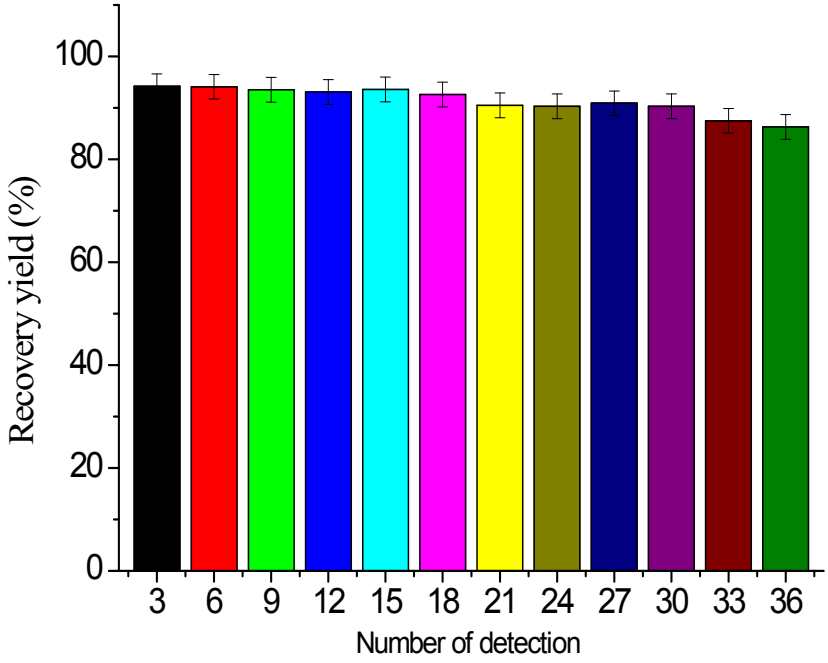


Fig. S6 The lifetime of poly(TMOS-co-MPTMS)@AuNPs@Apt affinity monolithic column.

Table S1 The recipes for fabricating poly(TMOS-co-MPTMS) parent column*

Column	TMOS (uL)	MPTMS (uL)	PEG10000 (mg)	Urea (mg)	HAc (mL)	Permeability K ($\times 10^{-13}$)
A	400	200	135	175	1.22	4.21
B	435	165	135	175	1.22	0.63
C	450	150	135	175	1.22	--
D	435	165	125	175	1.22	2.57
E	435	165	150	175	1.22	1.42
F	435	165	135	160	1.22	--
G	435	165	135	190	1.22	--
H	435	165	135	175	1.12	5.13
I	435	165	135	175	1.32	3.66

* Experimental conditions for measuring permeability: mobile phase, methanol, flow rate, 0.005uL/min. column length, 5cm.

Table S2 Relationships between the diameter of AuNPs and the plasmon resonance peak

No.	Reductant	Wavelength(nm)/Aspr	A450nm	Size/nm
1	sodium citrate + $NaBH_4$	513/0.612	0.484	5
2	sodium citrate	520/0.785	0.479	15
3	sodium citrate	523/0.775	0.432	25
4	sodium citrate	526/0.769	0.402	35

Table S3 Coverage density of poly(TMOS-MPTMS)@ AuNPs@Apt affinity monolith

Columm	n_1(nmol)	n_2(nmol)	ρ (pmol/μL)	Average (n=3) (pmol/μL)
Column1	2	0.440	3531	3494
Column2	2	0.507	3379	
Column3	2	0.210	3592	

a) effective length was 10 cm in capillary column (75 μm i.d. × 365 μm o.d.)

b) ρ was coverage density of aptamer on the monolithic column, n1 and n2 were the amount of aptamer injected into MPTMS-silica hybrid monolithic column and eluted from the column, respectively.

Table S4 Reproducibility of poly(TMOS-MPTMS)@AuNPs@Apt hybrid affinity monolith

	Added(ng/mL)	Found(ng/mL)	Recovery yield	RSD (%)
Run-to-run (n=5)[a)]	10	9.056	90.56	2.0
		9.308	93.08	
		9.420	94.20	
		9.419	94.19	
		9.030	90.30	
Day-to-day (n=5)[b)]	10	9.424	94.24	2.4
		9.002	90.02	
		9.255	92.55	
		9.419	94.19	
		8.959	89.59	
Column-to-column (n=3)[c)]	10	9.350	93.50	2.6
		8.967	89.67	
		9.420	94.20	

a. The intra-day RSDs were determined based on five replicates in the same day.

b. The inter-day RSDs were determined by analyzing the marker based on three replicates in three consecutive days.

c. The column-to-column RSDs were determined based on three columns in the same batch.