Selective Separation of Heavy Metals Based on Metal-Biomass Interactions

金属–生物质相互作用与选择性分离

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东北大学 理学院 分析科学研究中心

The National Symposium on Metallomics (NSM2019), 2019.1.11-13, 北京

Outline

Background

Metal-biomass interactions for heavy metals separation

- ***Bacillus subtilis*-hydrous ferric oxide for As(V) adsorption**
  枯草芽孢杆菌-水合氧化铁复合物：选择性吸附砷(V)
- ***Metallothionein* in selective adsorption of Cd**
  金属硫蛋白用于镉的选择性吸附分离
- **Cr(III) binding phage screening for Cr(III) adsorption**
  噬菌体文库筛选Cr(III)结合肽：选择性吸附Cr(III)
- **Growing AuNPs on Hg^{2+}-binding phage for Hg sensing**
  亲汞噬菌体原位生长纳米金: Hg^{2+}传感

Conclusions

Microbes, plants and animals
To survive at harsh conditions (high level toxic substances)
Resistance to toxic heavy metals

Microbes

Metalloregulatory proteins

MerR, PbrR, ArsR

- monitor specific metal
- regulate intracellular metal levels

Higher organisms

Metal-binding proteins

- metallothioneins
- metallothionein-like protein
- phytochelatins
- metal-binding peptide

Metal-binding proteins

- cysteine-rich, histidine-rich
- selective binding metals
- prevent heavy metal binding with enzyme active sites
- high sorption capacity

Metal-cell interactions

Selective adsorption of specific heavy metals (Cd, Cr, Hg)

Solid Phase Extraction

BIO-SORPTION
Adsorbent

Cell-sorption

Cells
zooblast, alga, yeast, epiphyte, bacteria

细胞，藻类，酵母，真菌，细菌

Biomaterials
chitosan, cellulose, collagen, silk fibroin

壳聚糖，纤维素，胶原质，蚕丝蛋白

N-acetyl, amide, phosphate, hydroxyl, ether, carboxylate, thiol

酰胺基，氨基，磷酸基，羟基，醚，羧基，硫醇

Selective adsorption of metal species

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Conclusions

Environ. Sci. Technol, 2012, 46, 2251-2256
Anal. Chem, 2009, 81,1291-1296

Bacillus subtilis decoration by hydrous ferric oxide for arsenic(V) adsorption

※ Arsenic pollution and toxicity

Organic species { MMA, DMA, TMAO…… / AsB, AsC……

Toxicity: As(III)>As(V)

As(III)/As(V)>Organic arsenic

Inorganic species { As(III) / As(V) ⇒ **Removal and speciation**

※ Arsenic: highly correlated with iron

Iron has a high affinity to arsenic.

Arsenic in iron containing matrix: arsenopyrite, biotite, clays……

※ Aims at…

A selective sorbent for arsenic:

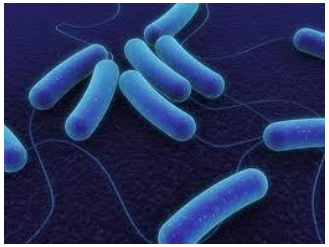
high iron-arsenic affinity + potential selectivity of biological cells.

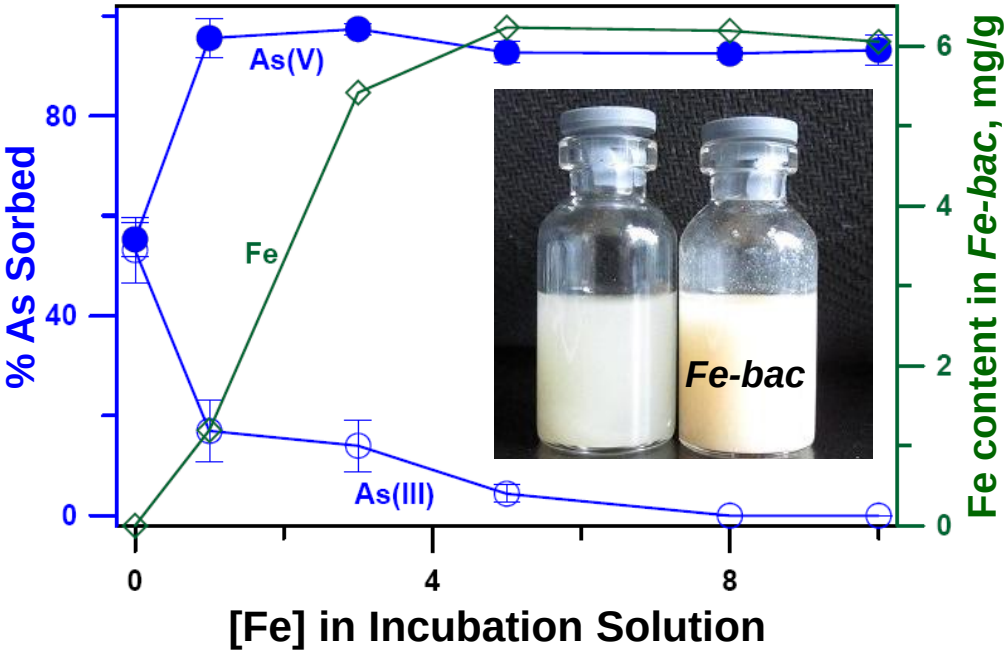
※ Fe(III) loading on *Bacillus subtilis* provides unusual selectivity and sorption capacity to As(V)

Bacillus subtilis **(枯草芽孢杆菌) is treated in Fe^{3+}.**

Fe sorption at non-growth conditions (no nutrient).

The ferrated bacteria: *Fe-bac*.



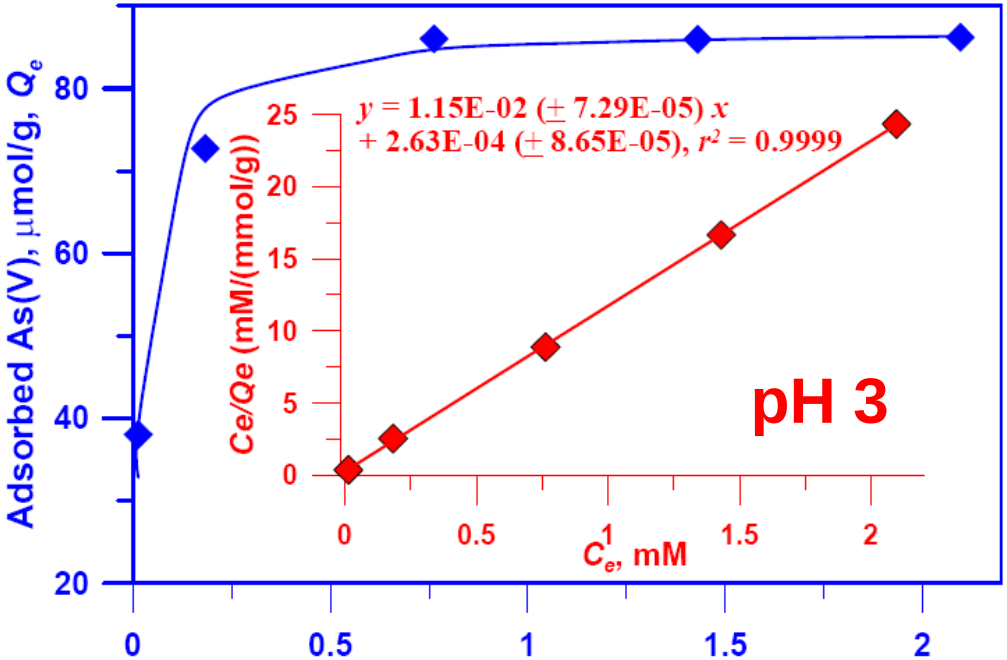


Fe loading on *bacteria* as a function of [Fe^{3+}]

B. subtilis **sorbs As(V) and As(III) at same level;**

Fe loading results in discrimination between As(V) and As(III);

Increase of Fe content improves selectivity to As(V)



Blue: *Langmuir* behavior for As(V) binding by *Fe-bac.*

Red: Linear plot derives sorption capacity (87.6 μmol/g, pH 3).

Fast sorption: 20 ppb iAs in 2 min (>90%).

pH	Native *B. subtilis*		Fe-loaded *B. subtilis* (0.6% w/w)	
	As(III)	As(V)	As(III)	As(V)
	Q_{max} μmol/g	Q_{max} μmol/g	Q_{max} μmol/g (mol As/mol Fe)	Q_{max} μmol/g (mol As/mol Fe)
3	36.9±2.1	22.8±0.1	~0	87.6±1.0 (0.78±0.01)
8	68.6±2.2	31.4±1.2	127±1 (1.1±0.01)	172±4 (1.6±0.03)
10	15.3±0.7	11.9±0.1	110±5.4 (1.0±0.04)	137±3.3 (1.2±0.03)

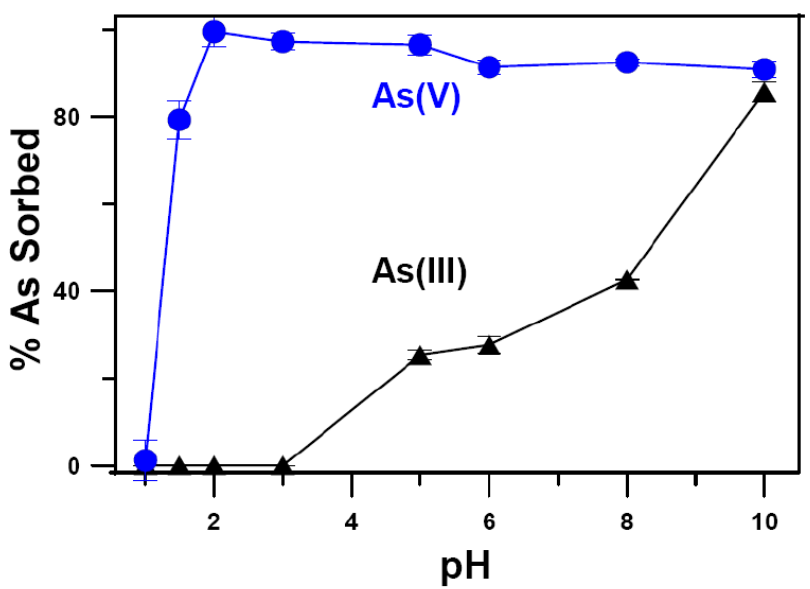
At pH 3, As(V) sorption greatly improved: high selectivity for As(V).

At pH 10, As(V) capacity >11 times higher than native *bac*.

Small amount Fe greatly changes the selectivity and sorption capacity

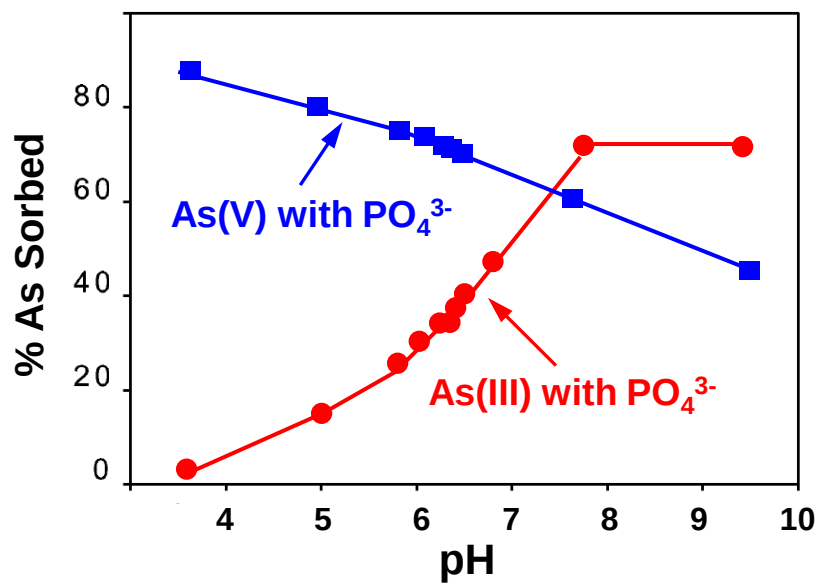
※ The nature of As binding by *Fe-B. subtilis (Fe-bac)*

pH-dependent selectivity to As(V) by *Fe-bac*



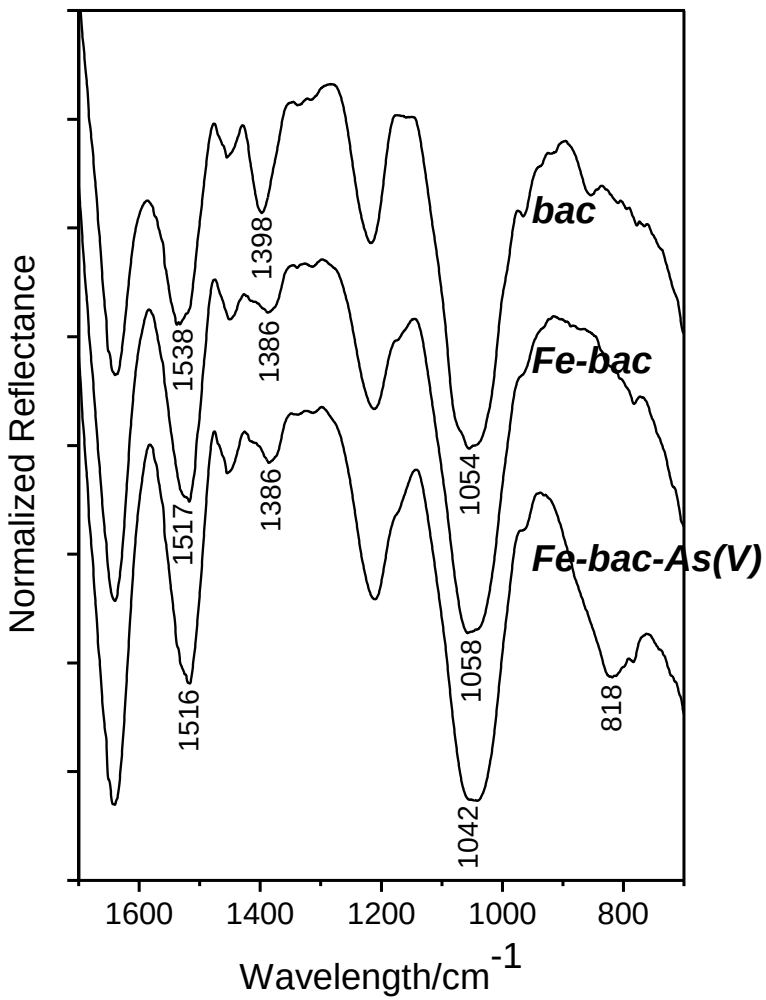
Similar sorption behaviors by *Fe-bac* with HFO (with PO_4^{3-}):
HFO on *B.subtilis*?
Fe-O-As?

pH dependent sorption of iAs by HFO *Environ.Sci.Technol.* **2003,***37*,4182



Sorption competition from the PO_4^{3-} group

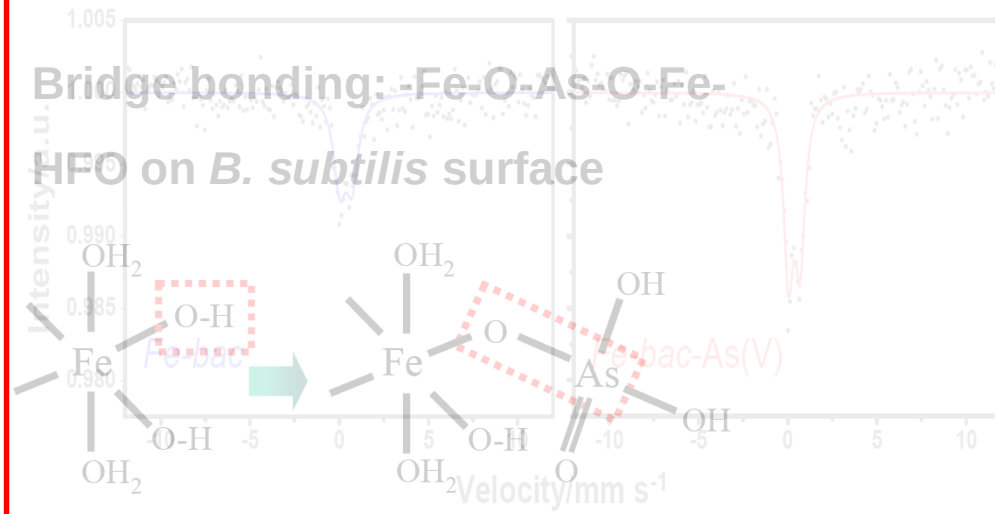
ATR-FTIR



^{57}Fe Mössbauer Spectroscopy

Bridge bonding: -Fe-O-As-O-Fe-

HFO on *B. subtilis* surface



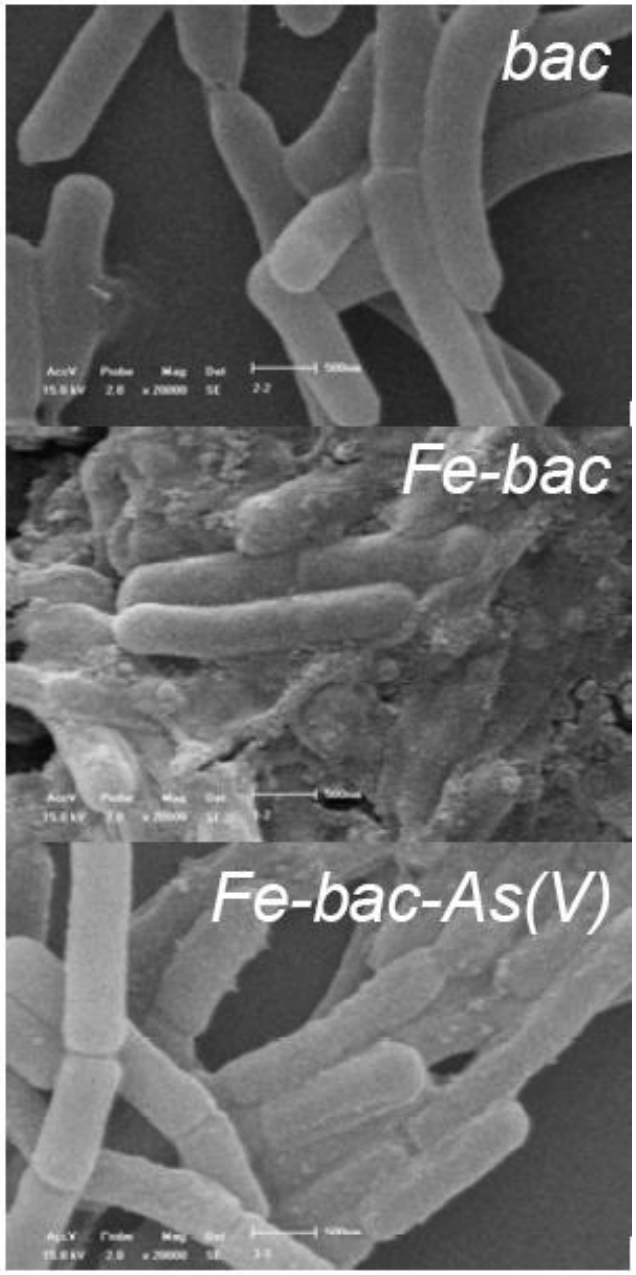
	I.S./mm/s	Q.S./mm/s
Fe-Bac	0.3885	0.6913
Fe-Bac-As(V)	0.3708	0.6598

Fe-O-H to Fe-O-As:

- **Electronegativity H>As**
- **s-electron density;**
- **Isomer Shift (IS)**

Better electron density symmetry

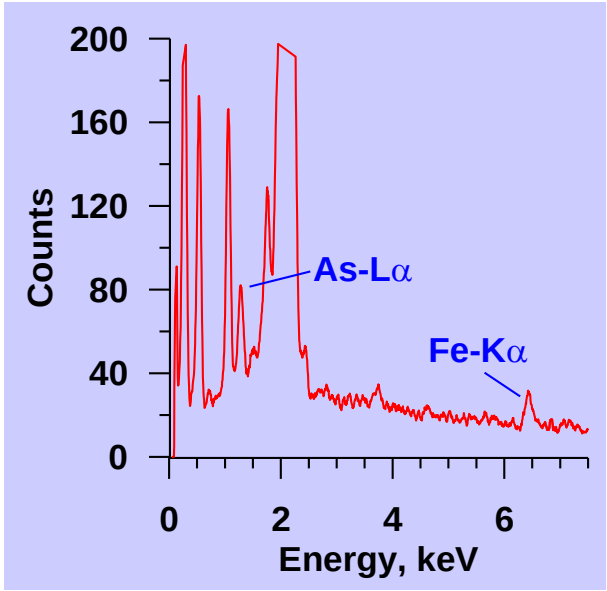
- **Quadrupole Split (QS)**



***Fe-bac*: amorphous nanomaterial, HFO as separate phase.**

Fe(III) sorption and HFO precipitation on cell membrane is a continuum.

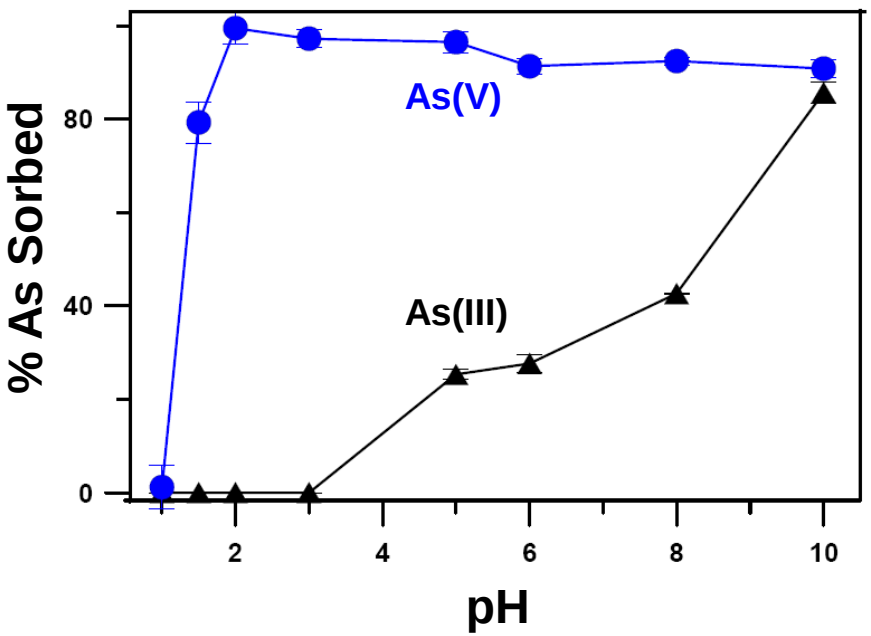
Upon As(V) exposure, *bac* cemented together, via -Fe-O-As-O-Fe- bridges.



※ *Fe-bac* selectivity to As(V): iAs speciation

pH-dependent selectivity to As(V) against As(III) by *Fe-bac*

Selectivity
As(V)/As(III):: ~300



Selective sorption of As(V), pH 3
0.8M HNO_3 elution
ETAAS quantification

Convert As(III) to As(V)
selective sorption at pH 3
0.8M HNO_3 elution
ETAAS

iAs speciation by difference

※ Performance

Sample volume	1 mL
Linear range	0.30-2.00 μg L^{-1}
Detection limit (3σ, n=11)	0.08 μg L^{-1}
RSD (1 μg L^{-1}, n=9)	4%
Enrichment factor	9.2
Retention efficiency	95.6%

※ Validation

Sample	As(III) (μg L^{-1})	As(V) (μg L^{-1})	Spiked As(V)	Recov. (%)
GuanMen Mountain Spring (BenXi)	0.186 ± 0.091	0.383 ± 0.104	0.5	93±3
QiPan Mountain Spring (ShenYang)	0.262 ± 0.074	0.849 ± 0.046	0.5	95±4
GBW 09101 Human hair	Certified total 0.58±0.05*	Found total 0.54±0.12*	*μg g^{-1}	

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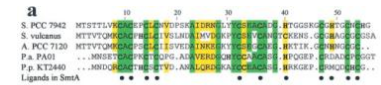
J. Mater. Chem, 2012, 22, 21909-21916
TrAC-Trends Anal Chem, 2015, 66, 90-102
J. Anal. At. Spectrom, 2015, 30, 929-935

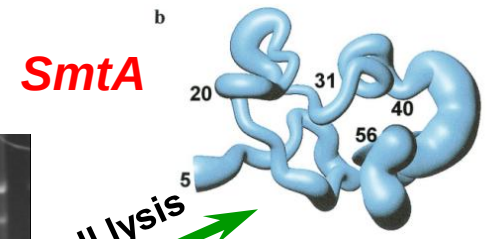
Metallothionein in selective adsorption of Cd
金属硫蛋白用于镉的选择性吸附分离

Metallothionein decorating graphene oxide (GO)

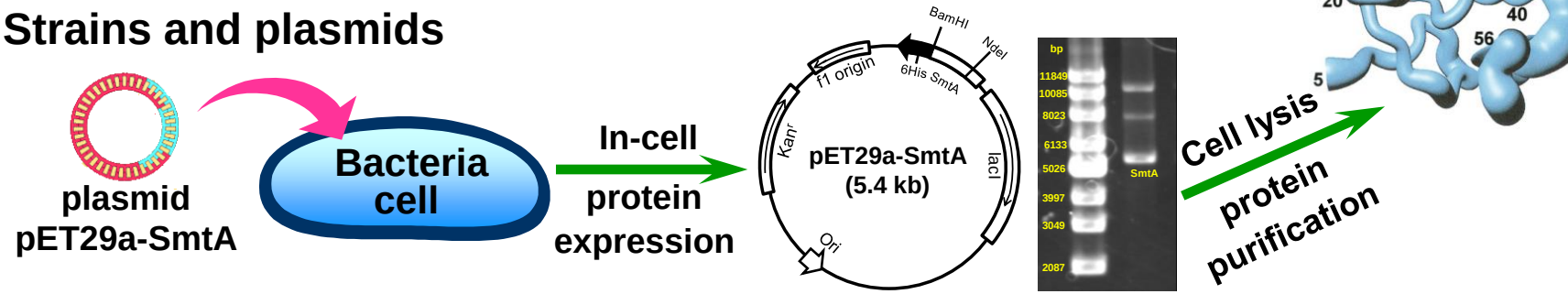
Cyanobacterium metallothionein (SmtA)

Binding capability: Cd>Pb>>Zn

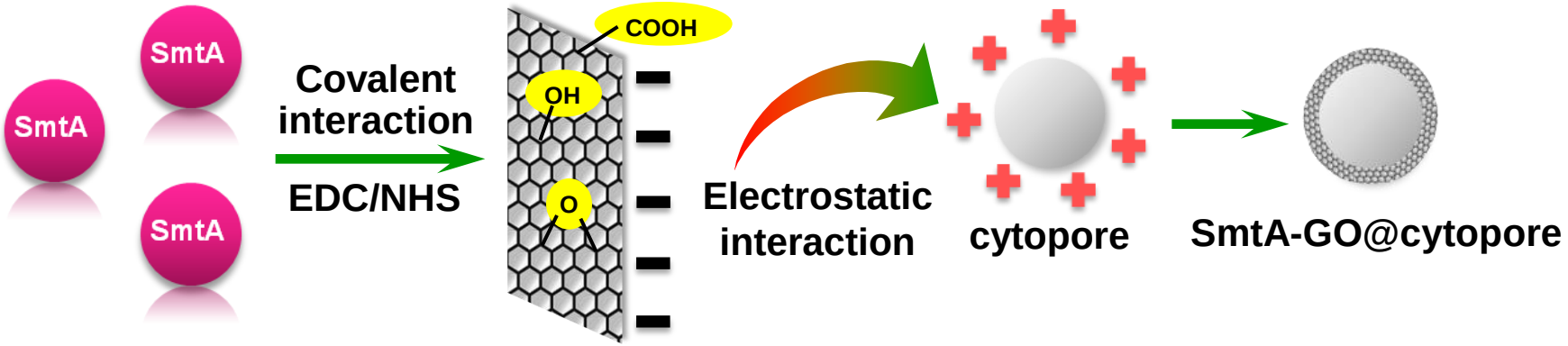




- **Strains and plasmids**

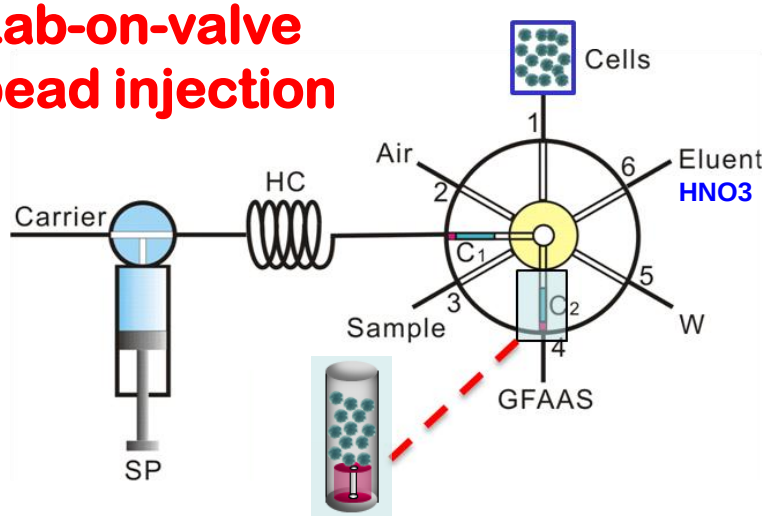


- **Immobilization of SmtA on graphene oxide (GO)-cytopore**



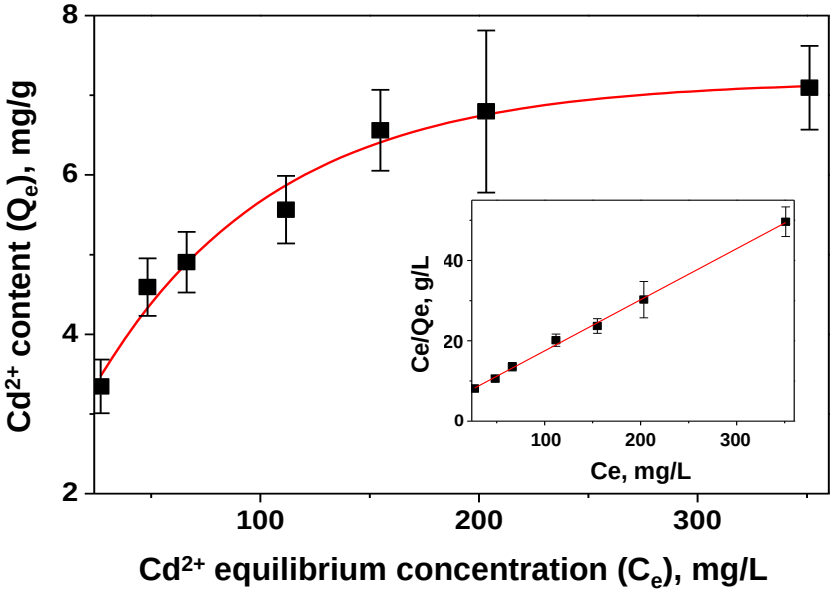
Cadmium adsorption by SmtA-GO/cytopore

Lab-on-valve bead injection

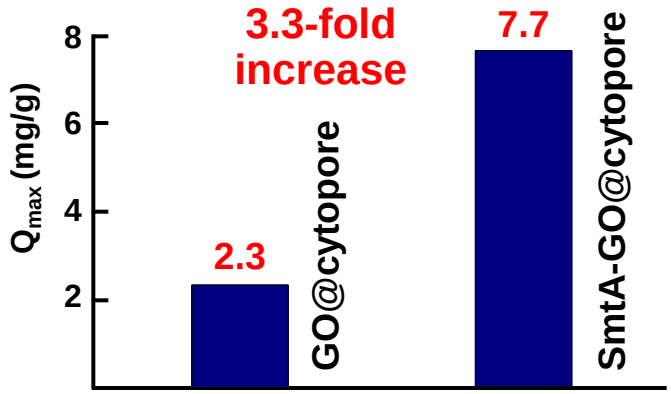


SmtA-GO/cytopore

Sorption isotherm



Sorption capacity



Fitting *Langmuir* adsorption model

Improvement on sorption capacity with respect to GO/cytopore

Selectivity

Cations	Tolerance SmtA-GO/cyto (μg/L)	Tolerance GO/cyto (μg/L)	Selectivity improvement (-fold)
K^+	3,000,000	100	30,000
Na^+	1,500,000	50	30,000
Ca^{2+}	50,000	1	50,000
Mg^{2+}	40,000	0.05	800,000
Co^{2+}	100	0.5	200
Al^{3+}	50	1	50
Cu^{2+}	50	0.05	1000
Ni^{2+}	40	1	40
Zn^{2+}	20	1	20

20-800,000 fold improvement on the tolerance of metal cations

HSAB principle

Hard acid

H^+, Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}

Hard base

OH^-, O^{2-}, ROH, NO_3^-, PO_4^{3-}, SO_4^{2-}

Soft acid

Ag^+, Cd^{2+}, Pd^{2+}, Hg^{2+}

Soft base

RSH, CN^-, $S_2O_3^{2-}$, SCN^-

Borer acid

Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Pb^2

SmtA: 9 cysteines, -SH rich soft base

Cd^{2+}: soft acid

↓

Highly selective retention of cadmium

Analytical performance

Sample volume	1000 μL
Eluent volume	50 μL
LOD (3σ, n=7)	1.2 ng/L
Linear range	5-100 ng/L
Enrichment factor	14.6

Sample analysis

CRMs	Certified (μg/g)	Found (μg/g)
GBW 07404 (soil)	0.35±0.06	0.35±0.07
GBW 08608 (nature water)	11.57±0.72	11.09±0.67

Sample	Found (μg/L)	Spike (μg/L)	Rec. (%)
Snow water	0.069±0.007	0.240	96
Rain water	0.139±0.001	0.120	101
Spring water	0.024±0.005	0.040	99
Sea water	0.676±0.039	0.600	104

Metallothionein isoforms in the adsorption of Cd

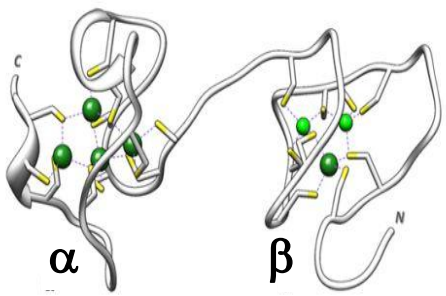
Mammalian MT (rMT)

- 20 cys out of 61 amino acids (33%)
- No aromatic amino acids/His
- α domain: Me4Cys11 cluster
  β domain: Me3Cys9 cluster

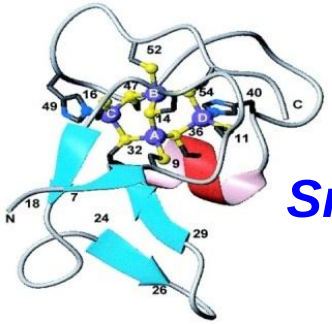
Cyanobacterium MT (*SmtA*)

- 9 cys out of 56 amino acids (16%)
- Contains His residue
- One domain
  Form $Me_4Cys_9His_2$ with Me^{2+}

rMT from rabbit liver

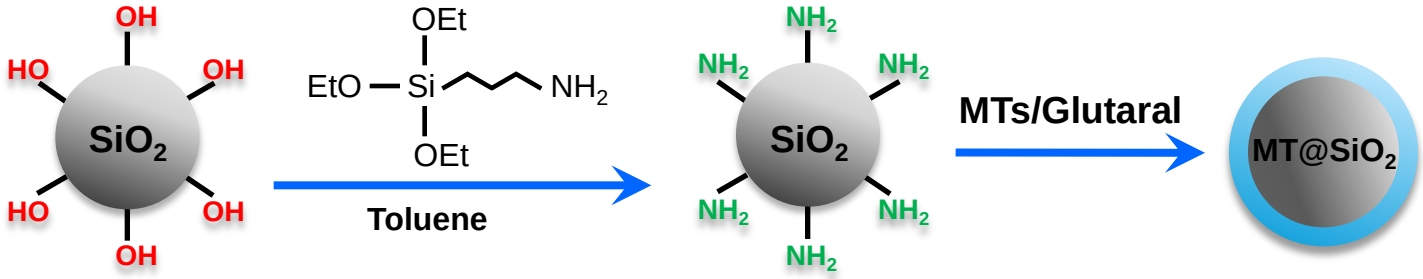


Difference ?

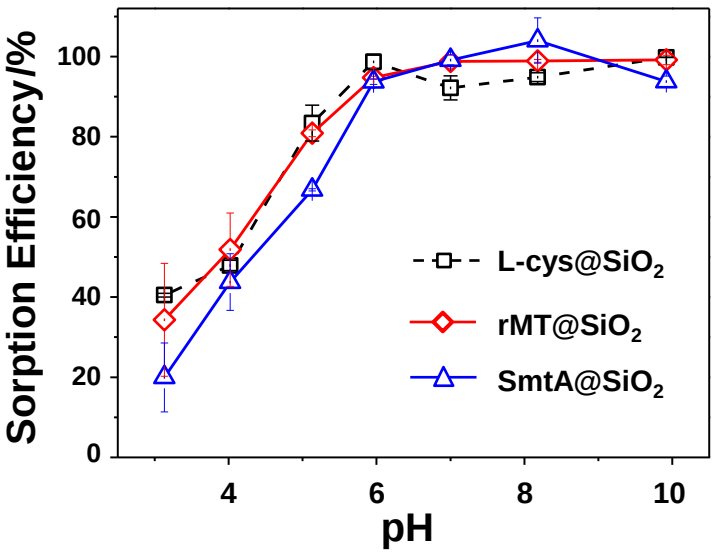


SmtA

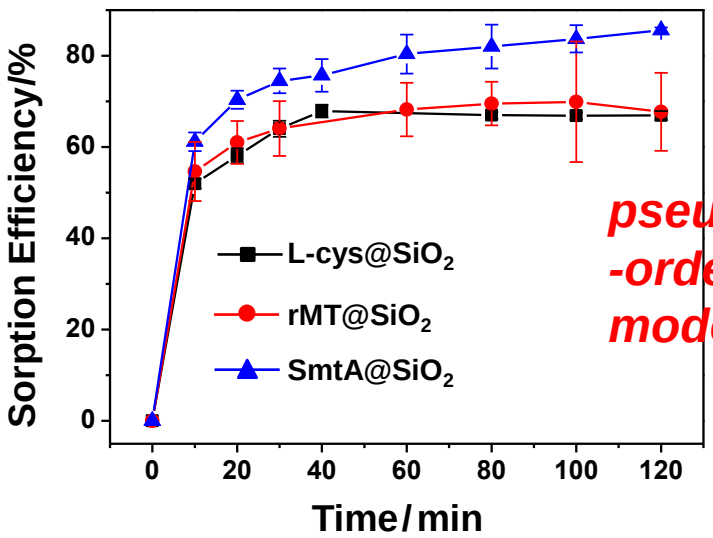
MTs immobilization on SiO_2 (Mammalian MT, *SmtA*, L-cysteine)



pH dependent sorption



Adsorption kinetics



pseudo-second -order kinetics model

pH ↓, sorption decrease

Proton competition

SmtA

more pH dependent

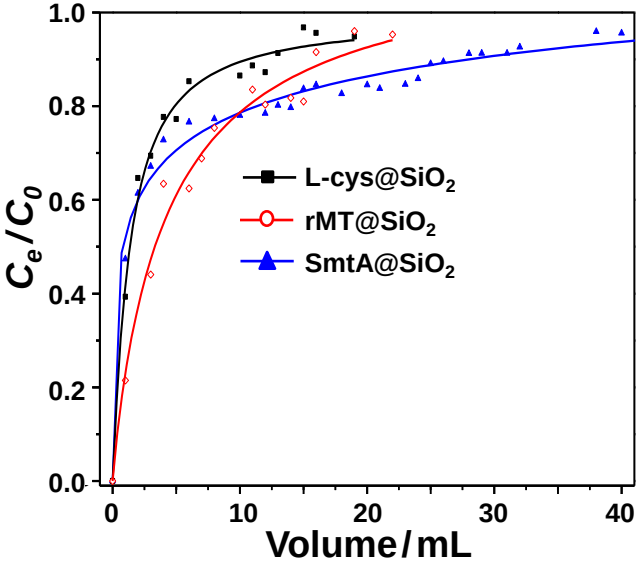
low cys content *vs* rMT

Adsorbent	k_2 10^5 g mg^{-1} h^{1}	q_e 10^{-4} mg g^{-1}	r^2
L-cys@SiO_2	1.34	1.83	0.9994
rMT@SiO_2	1.22	1.88	0.9989
SmtA@SiO_2	0.45	2.36	0.9995

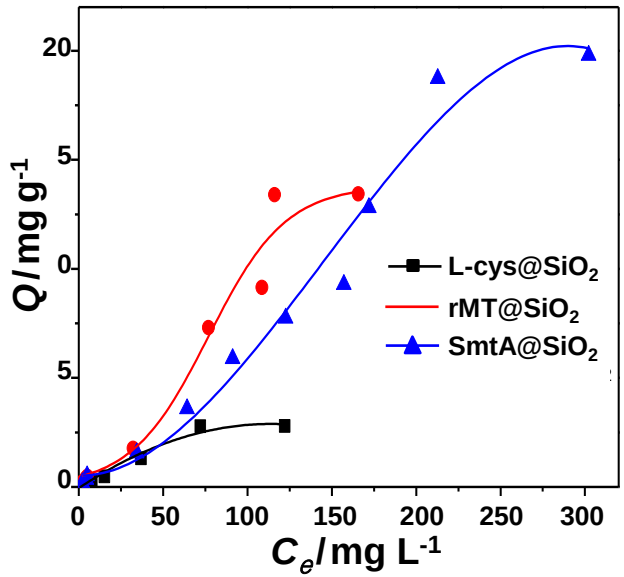
pseudo-second-order kinetics model

Adsorption efficiency: *SmtA* > rMT

Breakthrough



Sorption isotherms



Capacity expected

rMT > *SmtA*.
rMT: binds 7 bivalent metals, *SmtA*: binds 4.

Surface binding & Cd accommodation in *SmtA* framework.

rMT: steric structure is disrupted.
SmtA: intact, 6-His tags co-expression. Remains chelating cavity

Dynamic adsorption rate

SmtA **< rMT < L-cys**

Dynamic sorption capacity

L-cys@SiO_2	0.72 μg g^{-1}
rMT@SiO_2	1.31 μg g^{-1}
SmtA@SiO_2	**1.65 μg g^{-1}**

L-cys < rMT < *SmtA*

Adsorbent	Q_{max}/mg g^{-1}	K_d/mg L^{-1}	r^2
L-cys@SiO_2	3.09	64.11	0.9889
rMT@SiO_2	13.70	135.08	0.9964
SmtA@SiO_2	18.94	219.96	0.9891

Adsorption capacity

L-cys@SiO_2<rMT@SiO_2<SmtA@SiO_2

rMT: Cd^{2+} chelating "cavity" disrupted.

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Appl. Spectrosc. Rev, 2016, 51, 148-161
ACS Appl. Mater. Interfaces, 2015, 7, 21287-21294

Cr(III) binding phage screening for Cr(III) adsorption
噬菌体文库筛选Cr(III)结合肽：选择性吸附Cr(III)

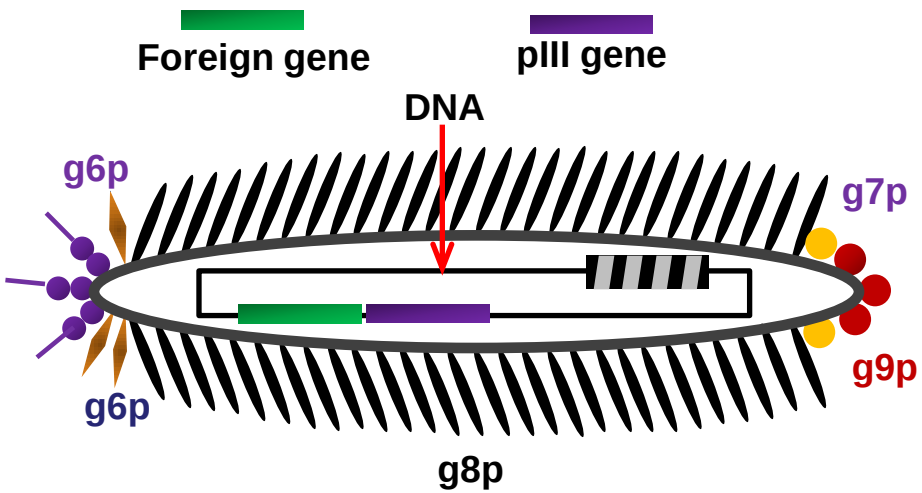
Natural metalloprotein

Limited species

Phage display library screening

Any targets

Phage display peptide library



Peptide display on phage surface

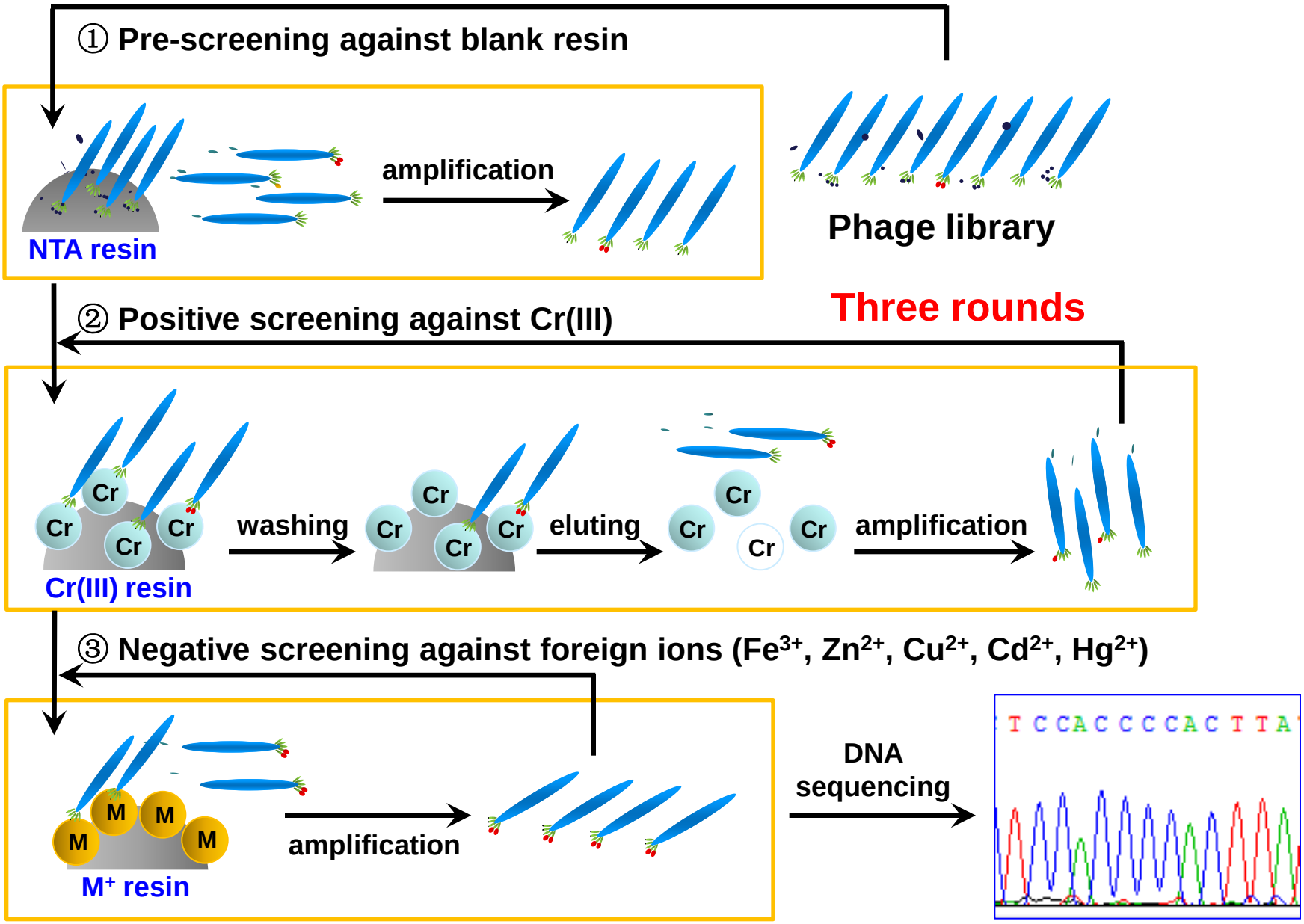
A foreign gene incorporating into a phage to display a peptide on surface. Various peptides make up a phage library.

Phage library

By repeated biopanning with target, phages with peptide ligands for target is selected

A universal tool for metal binding peptide selection

Peptide screening procedure - Cr(III) as model target



Peptide sequence

No	Sequence	Property	-OH
1	ATNKITK	nopbnob	2
2	SKVGYPT	obnnono	3
3	YKASLIT	obnonno	3
9	IQMTDIA	npnoann	1
10	SRHLHEW	obbnban	1
11	APVTSMK	nnnoonb	2

Non-polar amino acid--peptide folding by hydrophobic interaction:

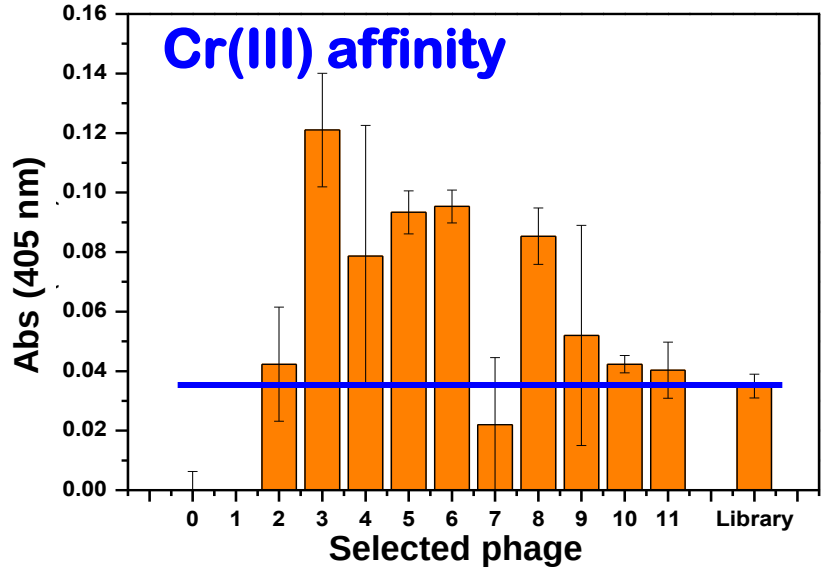
Formation of favorable spatial structure for Cr(III) binding.

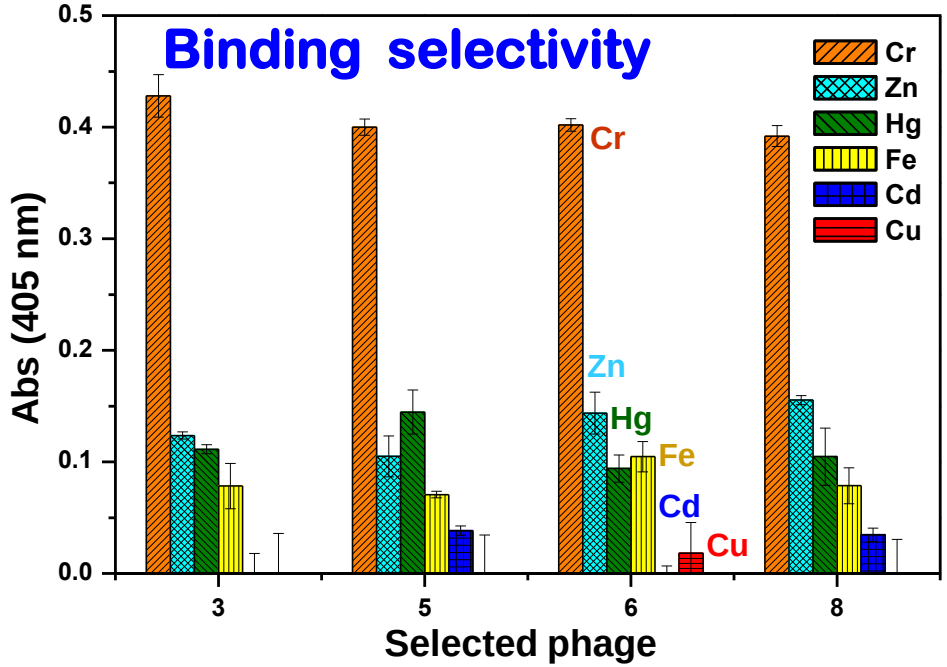
n: non-polar amino acid;
o: -OH containing amino acid;
p: polar and uncharged amino acid;
a: acidic/polar amino acid;
b: basic/polar amino acid

HSAB principle

Cr(III): Hard acid, -OH: Hard base

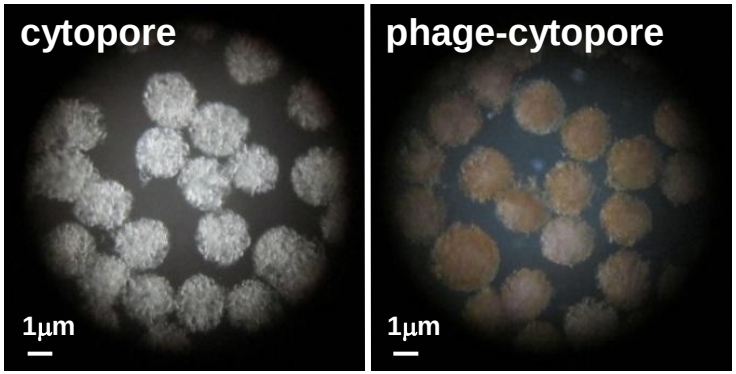
ELISA results



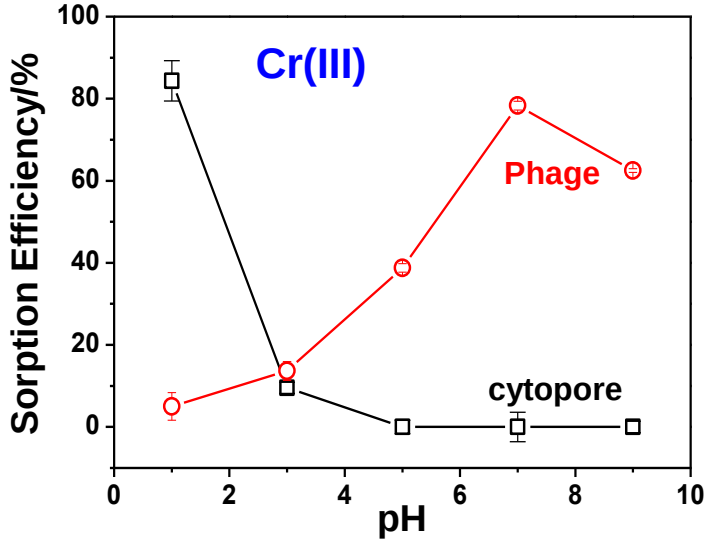


Cr binding phage - Cr(III) adsorption

Phage immobilization



pH dependent sorption



pH ↓, sorption ↓

Cr(VI) pre-removed by cytopore

Selectivity

Co-existing ions	Tolerance ratio
K^+	2,000,000
Na^+	400,000
Ca^{2+}	200,000
Mg^{2+}	40,000
Al^{3+}	320
Fe^{3+}	400
Cu^{2+}	400
Zn^{2+}	200
Pb^{2+}	4,000
Ni^{2+}	2,000
Cd^{2+}	200
Cl^-	400,000
NO_3^-	3,180,000
HCO_3^-	400,000
$H_2PO_4^-$	200,000

Cr(III) binding phage - Cr(III) adsorption

Parameters

Phage	No. 3
Peptide sequence	YKASLIT
pH	7.0
Sorption time	2 h
Eluent	0.1 M HNO_3
Detector	ICPMS

Analytical performance

Sample volume	4000 μL
Eluent volume	400 μL
RSD (250 ng/L, n=7)	3.6%
Linear range	50-500 ng/L
Enrichment factor	7.1

Validation

Sample	Found / μg L^{-1}		Spiked / μg L^{-1}		Found / μg L^{-1}		Recov. / %	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
Tap water	0.06±0.04	0.13±0.03	0.10	0.10	0.14±0.05	0.26±0.04	98	103
Snow water	0.12±0.05	0.08±0.03	0.10	0.10	0.22±0.02	0.19±0.02	100	105
GBW08608	Certified 31.4±2.0	Found 31.9±5.0						

Outline

Background

Metal-biomass interactions for heavy metals separation

***Bacillus subtilis*-hydrous ferric oxide for As(V) adsorption**
枯草芽孢杆菌-水合氧化铁复合物：选择性吸附砷(V)

***Metallothionein* in selective adsorption of Cd**
金属硫蛋白用于镉的选择性吸附分离

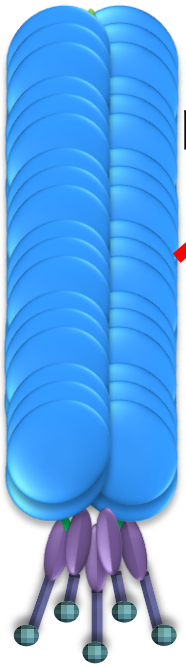
Cr(III) binding phage screening for Cr(III) adsorption
噬菌体文库筛选Cr(III)结合肽：选择性吸附Cr(III)

Growing AuNPs on Hg^{2+}-binding phage for Hg sensing
亲汞噬菌体原位生长纳米金: Hg^{2+}传感

Conclusions

Nanoscale, 2017, 9, 16728-16734

Growing AuNPs on Hg^{2+}-binding phage for Hg sensing
亲汞噬菌体原位生长纳米金: Hg^{2+}传感



金属结合肽
为识别单元

- 还原位点有利于原位生长AuNPs

M13噬菌体表面PVIII 蛋白富含还原性氨基酸：酪氨酸、色氨酸、半胱氨酸、天冬氨酸……

- AuNPs作为信号转换单元

距离/尺寸/形状依赖的表面等离子体共振吸收（SPR）

↓

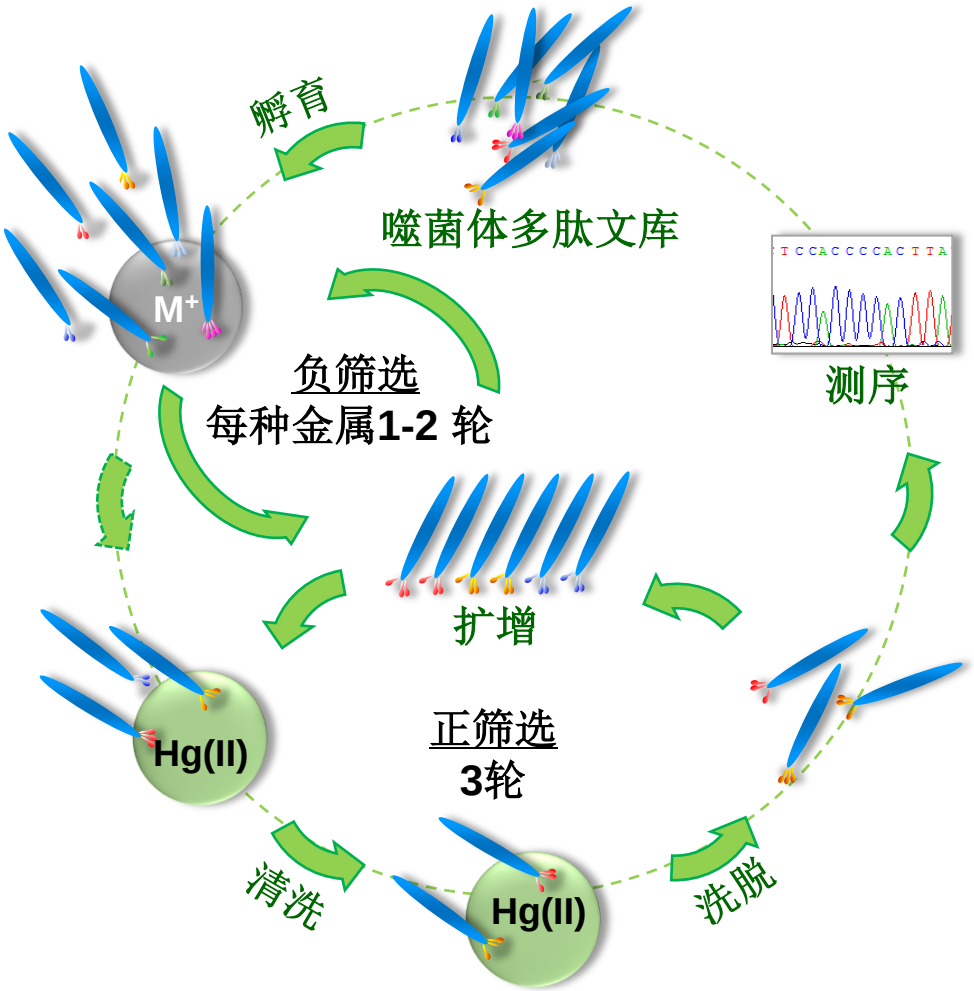
Hg^{2+}

↓

M13 噬菌体为比色传感平台

亲汞噬菌体筛选

生物淘选



1) 金属离子固定化

螯合于磁珠IDA-γ-Fe_2O_3

Zn(II), Cu(II), Fe(III), Cd(II)

2) 负筛选

保留上清液中的噬菌体

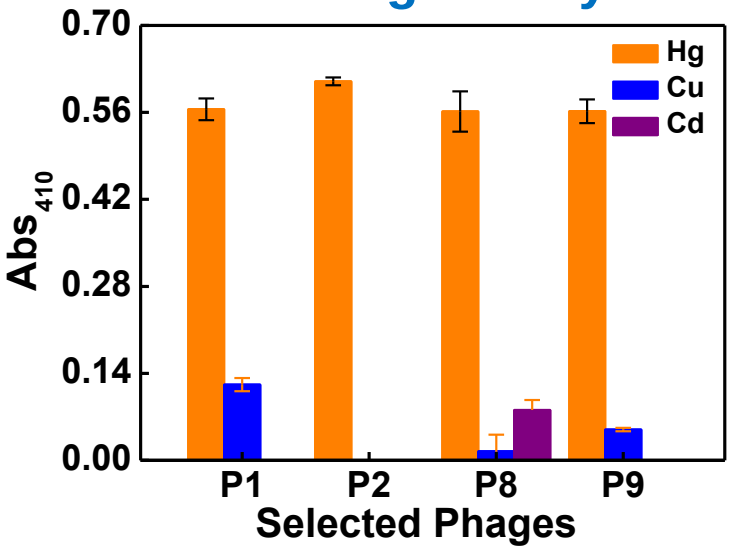
3) 正筛选

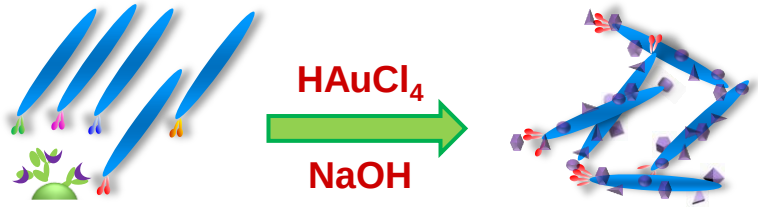
保留与磁珠结合的噬菌体，洗脱、扩增

4) 测序

纳米金原位生长

Binding affinity



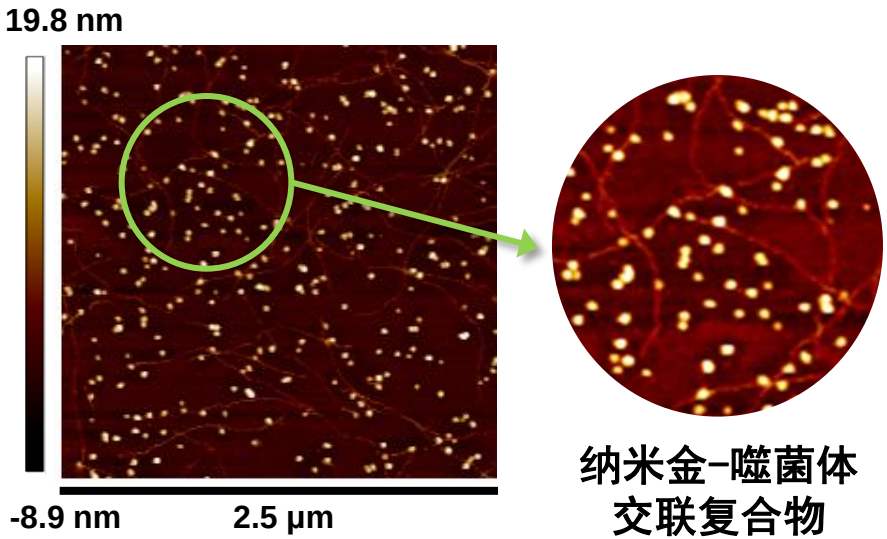


亲汞M13
噬菌体

纳米金-噬菌体
交联复合物

汞结合肽序列

P1	N	L	S	F	N	Y	L
P2	H	H	T	F	Q	S	T
P8	N	V	S	T	L	V	H
P9	T	S	Y	G	R	S	S

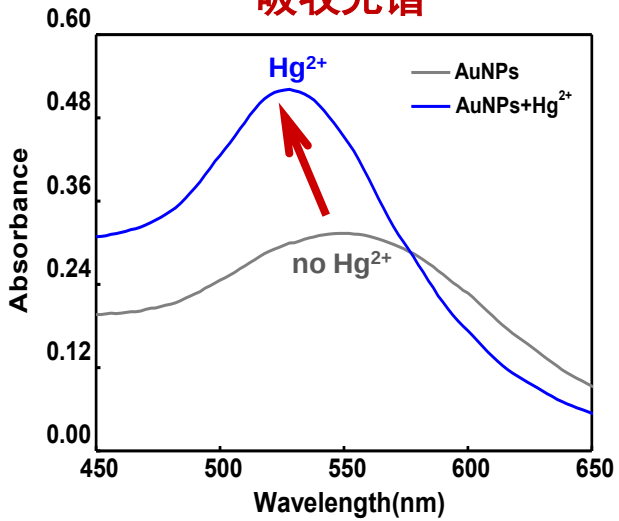


纳米金-噬菌体
交联复合物

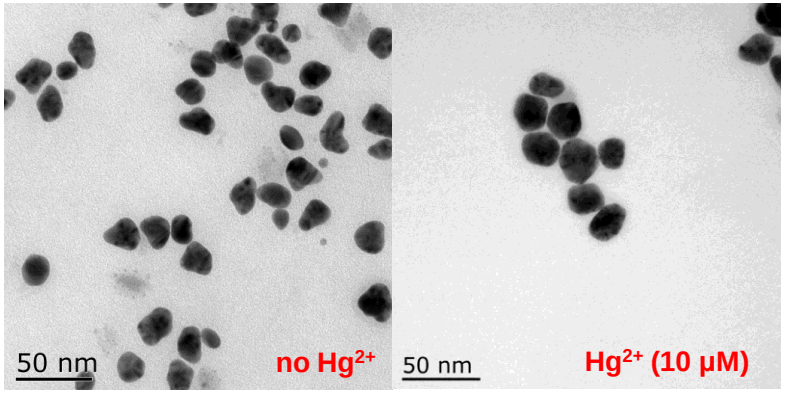
汞 传 感

传感原理

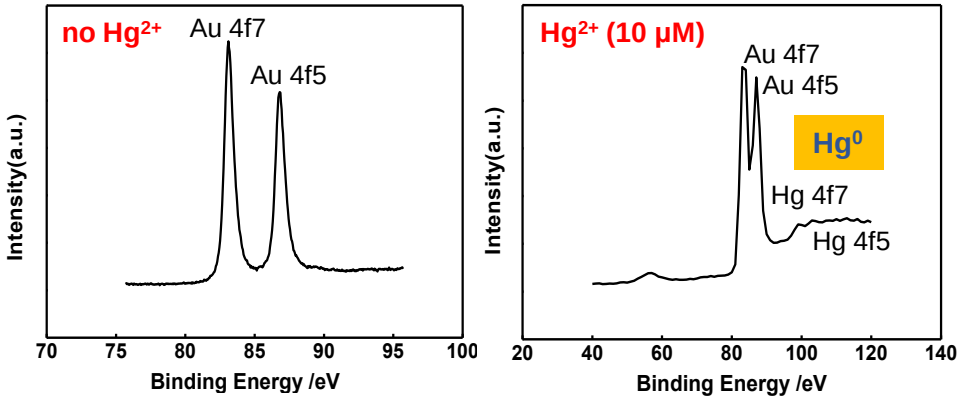
吸收光谱

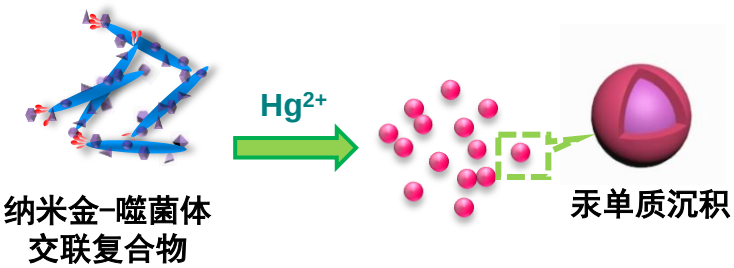


TEM



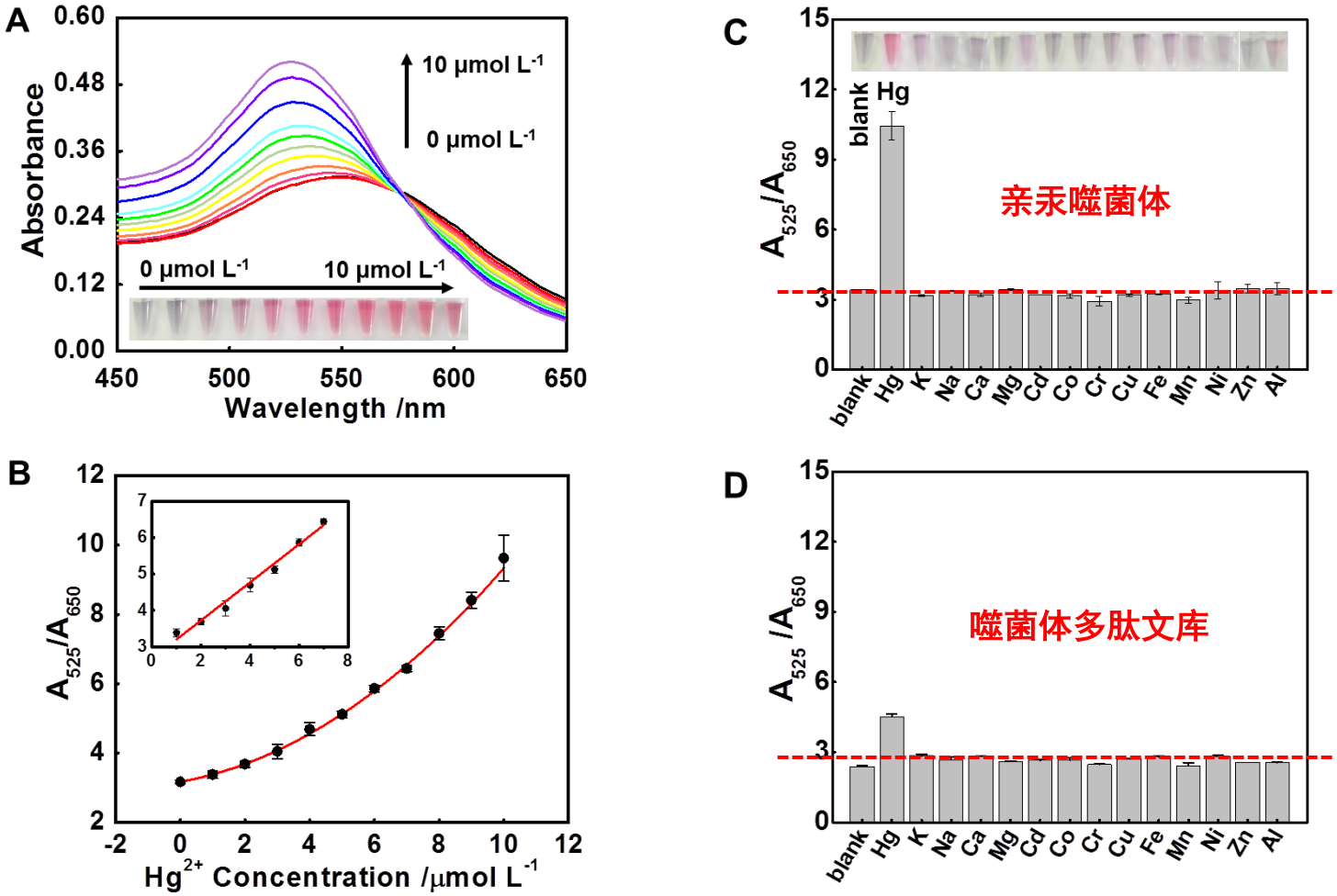
XPS





汞 传 感

灵敏度与选择性



LOD 80 nmol/L (3σ/k, n=5)

汞 传 感

干扰耐受能力

干扰离子	Tolerance level /μM					
	噬菌体 - AuNPs	Bismuthiol II - AuNPs	Dithioerythritol - AuNPs	Tween 20 - AuNPs	T-Hg^{2+}-T - AuNPs	MPA - AuNPs
K^+	100,000	-	-	100	-	-
Na^+	150,000	120	-	100	-	-
Mg^{2+}	30,000	120	600	100	100	100
Ca^{2+}	1,000	120	600	100	100	100*
Al^{3+}	30,000	120	-	100	-	100
Fe^{3+}	300	120	600	100	-	100
Cu^{2+}	500	120	600	100	100	100
Ni^{2+}	1,000	120	600	100	100	100
Co^{2+}	500	120	600	100	100	100
Cr^{3+-}	6,000	4.8	-	100	-	100*
Cd^{2+}	1,000	48	600	-	100	100*
Zn^{2+}	2,000	120	600	100	100	100
Ref.	本研究	[1]	[2]	[3]	[4]	[5]

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2. Y.-R. Kim, R. K. Mahajan, J. S. Kim, H. Kim, *ACS Appl. Mater. Interfaces* 2010, *2*, 292-295.
3. C.-Y. Lin, C.-J. Yu, Y.-H. Lin, W.-L. Tseng, *Anal. Chem.* 2010, *82*, 6830-6837.
4. X. Xu, J. Wang, K. Jiao, X. Yang, *Biosens. Bioelectron.* 2009, *24*, 3153-3158.
5. C. C. Huang, H. T. Chang, *Chem Commun* 2007, 1215-1217.

样品测定

环境水样中汞离子含量分析

Sample	Spiked (μM)	Found (μM)	Recov.(%)	RSD% (n=3)
Snow	2.0	2.46	123.0	4.9
	3.0	2.90	96.5	4.4
	4.0	3.51	87.8	3.6
	5.0	4.55	91.1	7.7
	6.0	6.01	100.2	10.5
	7.0	7.47	106.7	1.5
River water	1.0	1.07	107.0	0.9
	2.0	1.89	94.6	6.3
	3.0	2.87	95.8	8.9
	4.0	4.24	106.0	3.4
	5.0	5.48	110.0	0.4
	6.0	6.40	106.6	1.7
	7.0	6.70	95.7	0.7

Snow LOD 83 nmol/L Linear: 2.0-7.0 μmol/L

River water LOD 80 nmol/L Linear: 1.0-7.0 μmol/L

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***Bacillus subtilis* decorated by hydrous ferric oxide for arsenic(V) adsorption**

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Conclusions

Conclusions

Biological cells: alternative for selective sorption of heavy metals

The wide choice of functional groups on cell surface provides favorable selectivity to metal species.

Metal binding phage screening:
Universal approach for developing highly selective adsorbents for metals.

Acknowledgements

National Natural Science Foundation, China

Northeastern University, China

Thank you for your attention