

Supplementary Information

Poly- γ -glutamic acid coupled *Pseudomonas putida* cells surface-displaying metallothioneins: Composited copper(II) biosorption and inducible flocculation in aqueous solution

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Supplementary Tables

Table S1. Two-way ANOVA analysis of the Cu²⁺ removal efficiency of MB546 and MB546-PGA at 0.05 significance level

	<i>df</i>	Sum of squares	Mean square	<i>F</i> value	<i>P</i> value
Factor A	1	1627.500	1627.500	65.310**	3.082×10^{-10}
Factor B	8	55637.825	6954.728	279.086**	0
Model	9	57265.324	6362.814	255.333**	0
Error	44	1096.466	24.920	n/a	n/a
Corrected total	53	58361.790	n/a	n/a	n/a

Table S2. Fitting parameters for the pseudo-first-order kinetics

<i>C</i> ₀ (mg L ⁻¹)	<i>k</i> ₁ (min ⁻¹)	<i>q</i> _e (mg g ⁻¹)	Standard error of <i>lnq</i> _e	Standard error of <i>-k</i> ₁	<i>F</i> value	Prob> <i>F</i>	<i>R</i> ²
6.4	0.5849	9.3410	0.1360	0.1241	22.2363	0.00920	0.8094
12.8	0.5126	19.3918	0.0647	0.1089	22.1667	0.00925	0.8089
32	0.6078	47.3577	0.0913	0.0992	37.5516	0.00359	0.8797
64	0.5566	87.1369	0.1257	0.1082	26.4550	0.00678	0.8358
128	0.7135	178.1646	0.1682	0.1444	24.4285	0.00780	0.8241

Table S3. Fitting parameters for the pseudo-second-order kinetics

<i>C</i> ₀ (mg L ⁻¹)	<i>k</i> ₂ (g mg ⁻¹ min ⁻¹)	<i>q</i> _e (mg g ⁻¹)	Standard error of <i>k</i> ₂ ⁻¹ <i>q</i> _e ⁻²	Standard error of <i>q</i> _e ⁻¹	<i>F</i> value	Prob> <i>F</i>	<i>R</i> ²
6.4	0.1541	10.4037	0.0188	0.00606	251.96	9.21×10^{-5}	0.9805
12.8	0.0749	19.8886	0.0074	5.3171×10^{-4}	8943.74	7.50×10^{-8}	0.9994
32	0.0396	49.1400	0.0034	5.3237×10^{-4}	1460.61	2.80×10^{-6}	0.9966
64	0.0144	97.4659	0.0015	3.3157×10^{-4}	957.06	6.51×10^{-6}	0.9948
128	0.0089	194.1748	8.8×10^{-4}	1.5708×10^{-4}	1073.41	5.18×10^{-6}	0.9954

Table S4. Fitting parameters of the Freundlich isotherm model for Cu²⁺ biosorption by MB546-PGA

K_f	n	Standard error of $\ln K_f$	Standard error of n^{-1}	F value	Prob> F	R^2
23.16	1.305	0.05242	0.02412	1009.71	6.85×10^{-5}	0.9961

Table S5. Fitting parameters of the Langmuir isotherm model for Cu²⁺ biosorption by MB546-PGA

K_s (mg L ⁻¹)	Q_{max} (mg g ⁻¹)	Standard error of Q_{max}^{-1}	Standard error of $K_s^{-1} Q_{max}^{-1}$	F value	Prob> F	R^2
2.012×10^{-4}	145.99	0.002	0.00183	257.72	5.26×10^{-4}	0.9847

Table S6. Plasmids and oligonucleotide primers used in this study

Plasmids or primers	Phenotypes or sequences ^a	Sources or references
Plasmids		
pTrcHis B	Amp ^r , <i>E. coli</i> expression vector, 4404 bp	Invitrogen
pYNP	Cb ^r , <i>Pseudomonas</i> expression vector harboring the promoter <i>P_{oprL}</i> , 6154 bp	[1]
pYN2S	Cb ^r , <i>Pseudomonas</i> expression vector harboring the <i>P_{oprL}-inaK-N-smtA-smtA</i> fusion gene, 5763 bp	[2]
pMB109	Amp ^r , an <i>E. coli</i> plasmid expressing the fusion gene (<i>inaQ-N</i>) ₂ / <i>gfp</i> ; 6071 bp	[3]
pMB541	Amp ^r , a pMB109 derivative harboring the fusion gene (<i>inaQ-N</i>) ₂ - <i>glnBP</i> , 6162 bp	This study
pMB542	Amp ^r , a pTrcHis B derivative harboring the fusion gene (<i>inaQ-N</i>) ₂ - <i>glnBP</i> , 6185 bp	This study
pMB544	Amp ^r , a pMB542 derivative harboring the fusion gene (<i>inaQ-N</i>) ₂ - <i>glnBP</i> -(<i>smtA</i>) ₂ , 6525 bp	This study
pMB545	Cb ^r , a pYNP-derived <i>Pseudomonas</i> plasmid expressing the fusion gene (<i>inaQ-N</i>) ₂ - <i>glnBP</i> -(<i>smtA</i>) ₂ , 7124 bp	This study
Oligonucleotide primers ^b		
<i>glnBP</i> -F	5'-GGAAGATCTATGCTTAAAATGAAAAAG-3' (<i>Bgl</i> II)	
<i>glnBP</i> 1-R	5'-GTCGGAATTCCTTCTCTTGTGTTTTGCC-3' (<i>Eco</i> RI)	
<i>inaQ-N</i> -F	5'-AGTCCATGGATCTCGACAAGGCGTT-3' (<i>Nco</i> I)	
<i>glnBP</i> 2-R	5'-CTACTGCAGCTTCTCTTGTGTTTTGC-3' (<i>Pst</i> I)	
<i>smtA</i> -F	5'-AGTTGGTACCATGACCTCAACAACG-3' (<i>Kpn</i> I)	
<i>smtA</i> -R	5'-GAAAGAATTCATTAGCCGTGGCAGT-3' (<i>Eco</i> RI)	

Note: ^a Amp^r, ampicillin resistance; Cb^r, carbenicillin resistance; *P_{oprL}*, the constitutive promoter of pYNP in *Pseudomonas* strains; *inaK-N*, N-terminal domain of ice-nucleating gene *inaK*; *glnBP*, glutamine-binding protein gene; *inaQ-N*, N-terminal domain of ice-nucleating gene *inaQ*; *smtA*, cyanobacterial metallothionein protein gene.

^b The underlined sequences indicates the restriction enzyme sites.

References

- [1] T. Ye, C. Yao, Q. Li, H. Zhang and L. Li, Construction and characterization of *Pseudomonas* shuttle expression vector pYMB03, *Chin. J. Appl. Environ. Biol.* 14 (2008) 528–533.
- [2] H. Ni, Z. Xiong, T. Ye, Z. Zhang, X. Ma and L. Li, Biosorption of copper(II) from aqueous solutions using volcanic rock matrix-immobilized *Pseudomonas putida* cells with surface-displayed cyanobacterial metallothioneins, *Chem. Eng. J.* 204–206 (2012) 264–271.
- [3] Q. Li, Q. Yan, J. Chen, Y. He, J. Wang, H. Zhang, Z. Yu and L. Li, Molecular characterization of an ice nucleation protein variant (InaQ) from *Pseudomonas syringae* and the analysis of its transmembrane transport activity in *Escherichia coli*, *Int. J. Biol. Sci.* 8 (2012) 1097–1108.

Supplementary Figures

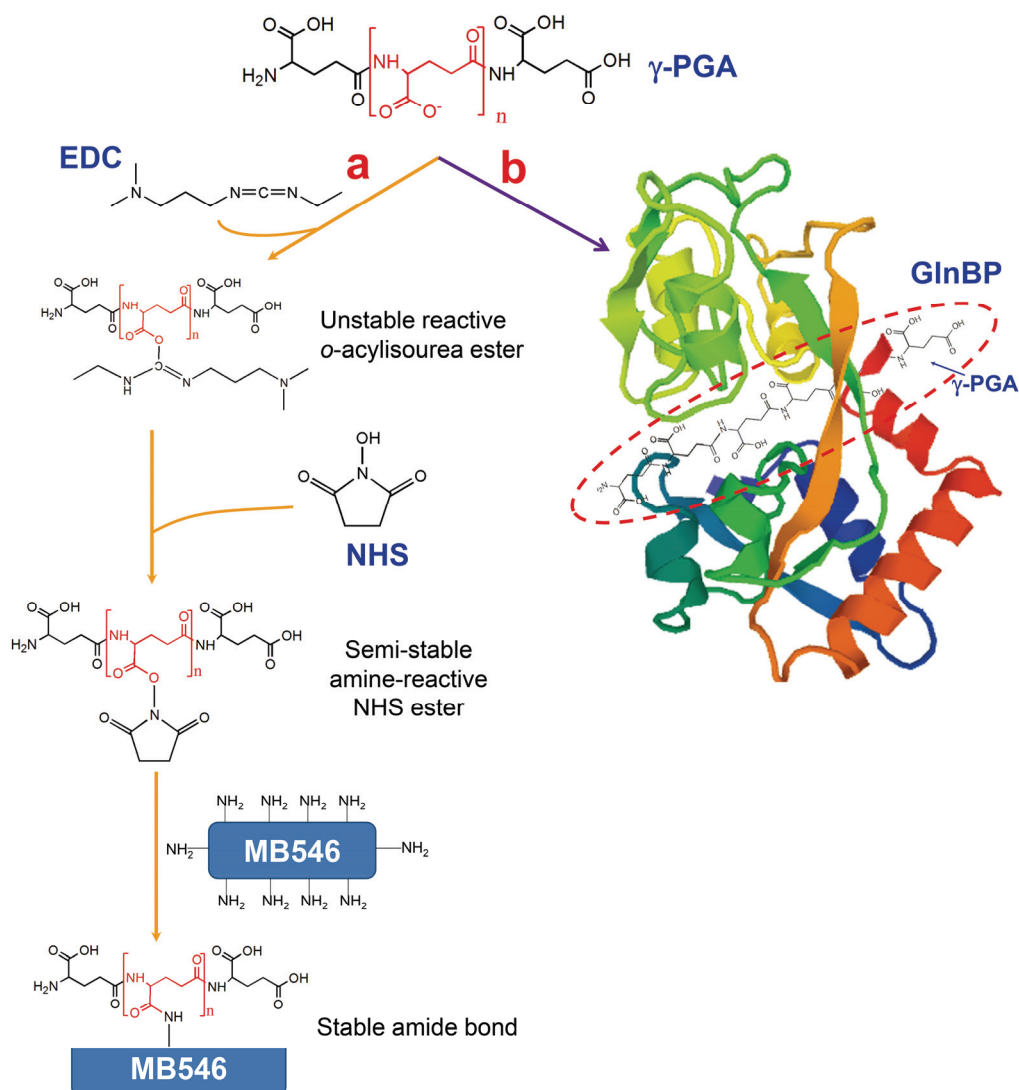


Figure S1. Strategies for the binding of γ -PGA and GlnBP. (a), Formation of amide bonds by activated γ -PGA and the amino groups of the amino acids of GlnBP; (b), Binding of the Gln ligand to the cleft between the two domains of GlnBP. Abbreviations: γ -PGA, γ -polyglutamic acid; GlnBP, glutamine-binding protein; EDC, 1-(3-Dimethylaminopropyl)-3-Ethylcarbodiimide; NHS, *N*-Hydroxysuccinimide.

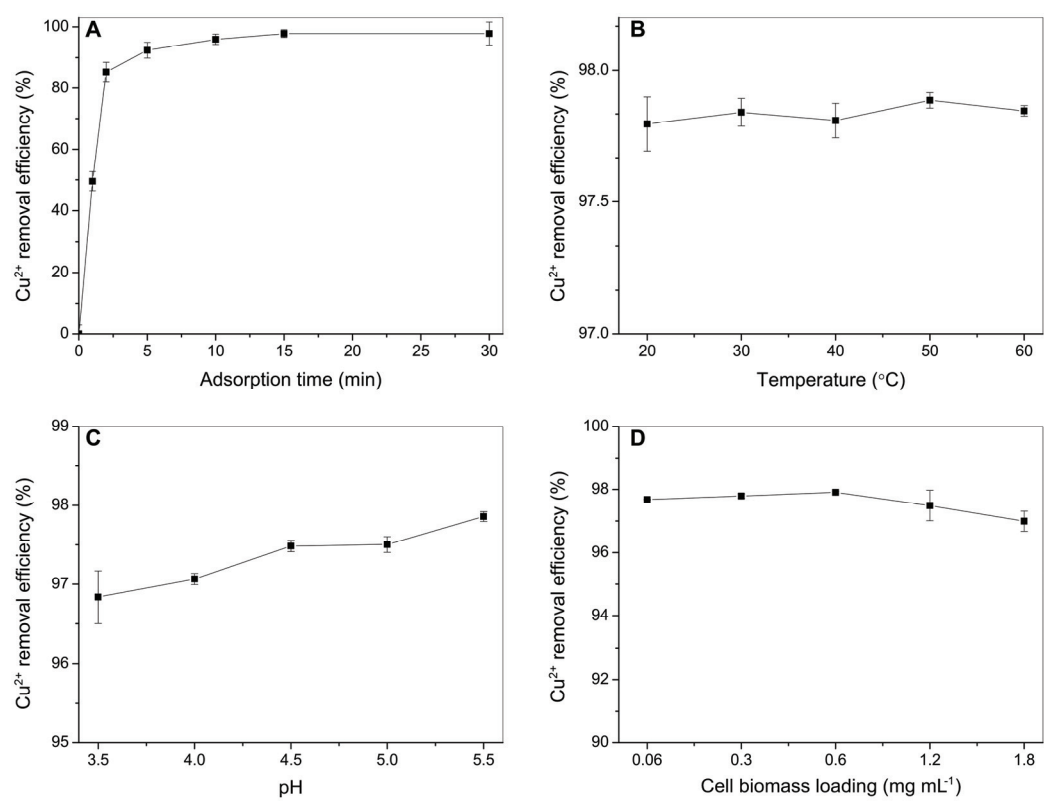


Figure S2. Effects of the adsorption time, pH, temperature and cell biomass loading on the biosorption capacity of Cu^{2+} by MB546-PGA.

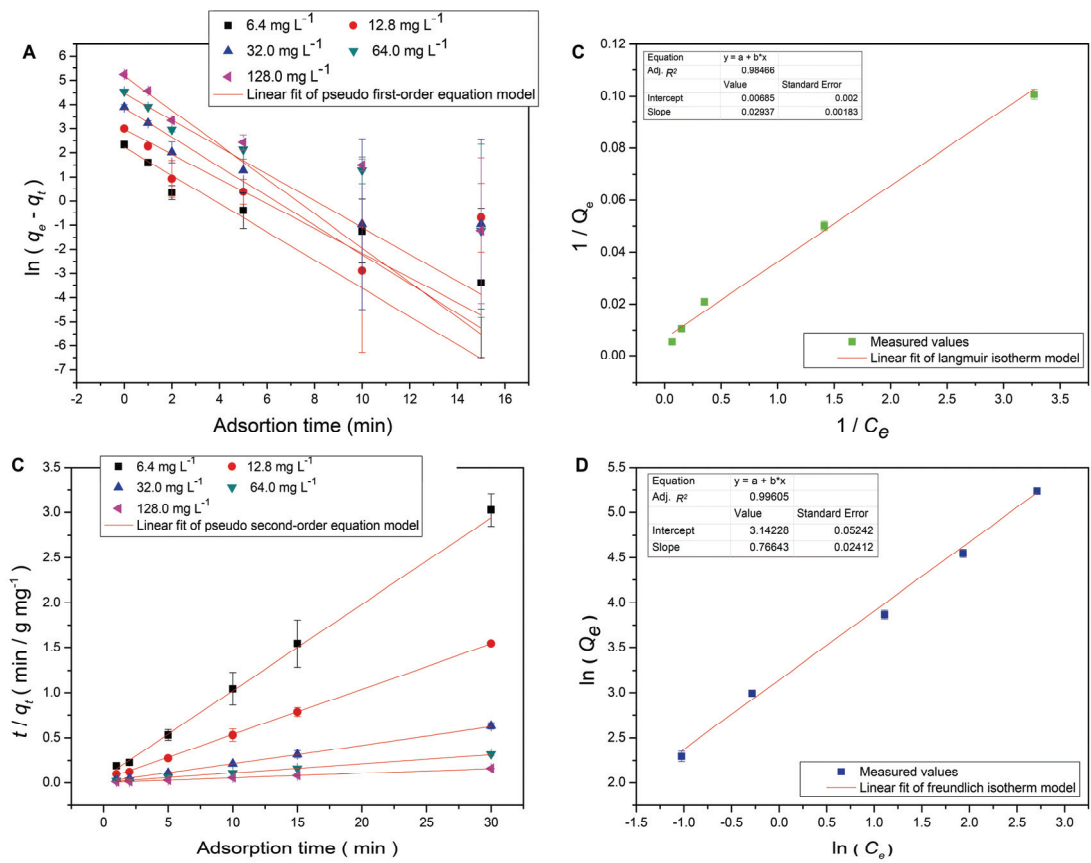


Figure S3. The pseudo-first-order equation (A) and the pseudo-second-order equation (B) modeled sorption data over a time course, and Freundlich isotherm (C) and Langmuir isotherm (D) plots of the MB546-PGA biocomposite.

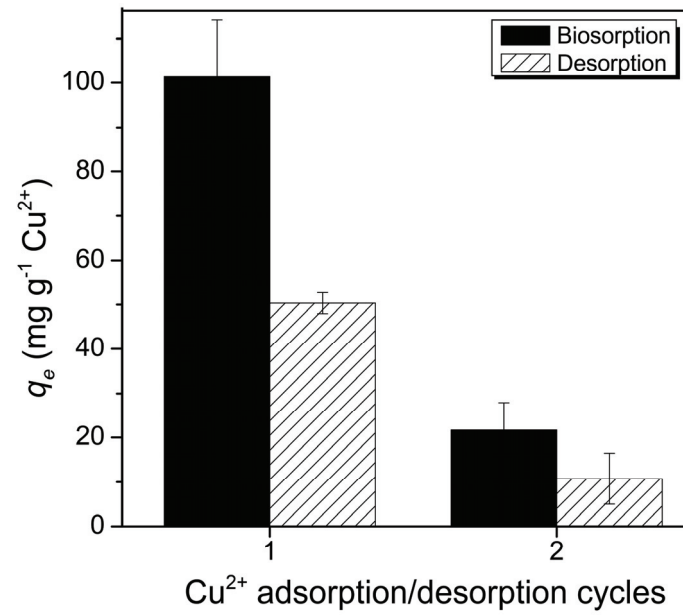


Figure S4. Cu²⁺ adsorption capacity and Cu²⁺ desorption capacity after continuously repeated adsorption and desorption cycles.

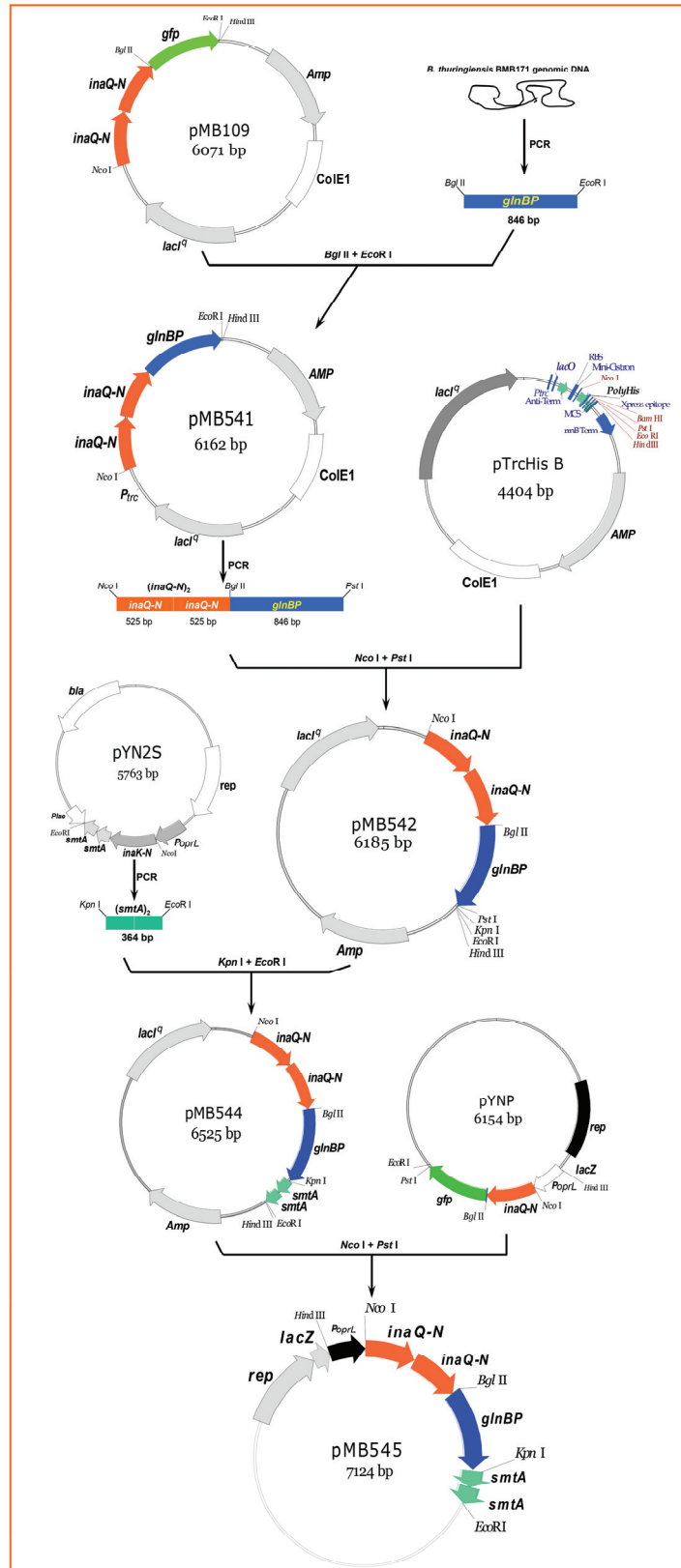


Fig. S5. Schematic illustration of the construction of the recombinant pMB541, pMB542, pMB544, and pMB545 plasmids.