Supporting information

Preparation of boronic acid-functionalized cryogels using modular and clickable building blocks for bacteria separation

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International Collaboration

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Figure S1. FT IR spectra of (a) poly(NIPAm-co-GMA) and (b) poly(NIPAm-co-GMA)@N$_3$. 
Figure S2. GPC trace of poly(NIPAm-co-GMA)@N₃.

$M_n = 21000; \frac{M_w}{M_n} = 1.9$
Figure S3. SEM images of AG-N₃ cryogel (A, B), AG-alkyne cryogel (C, D), AG-alkyne@polymer-N₃ cryogel (E, F). The scale bars are 10 μm in (A), (C), (E), and 1 μm in (B), (D), (F).
Figure S4. Calibration plots for quantification of (A) *E. coli* and (B) *S. epidermidis*. 
Figure S5. SEM images of AG-alkyne@polymer-pBA cryogel loaded with S. epidermidis, after elution using 0.5 M fructose (in 20 mM PBS, pH 9.0, containing 0.5 M NaCl). The scale bars are 10 μm in (A) and 1 μm in (B).
Figure S6. Eluted *E. coli* and *S. epidermidis* before (A) and after (B) cultivation in LB medium at 37 °C for 16 h. The bacteria suspension (0.5 mL) eluted by 0.5 M fructose-PBS buffer was transferred into 8 mL of fresh LB medium for cultivation.
Figure S7. Chromatograms of bacteria separation tested with (A) *E. coli* and (B) *S. epidermidis* on different cryogels: (—) AG-alkyne@polymer-pBA, (--) AG-B-A. The arrows indicate: (a) loading of 10 mM PBS (pH 8.0), (b) loading of *E. coli* or *S. epidermidis* suspensions (OD$_{600} \approx 1$) in 10 mM PBS (pH 8.0), (c) elution with 0.5 M fructose in 20 mM phosphate buffer (pH 9.0, containing 0.5 M NaCl), (d) washing with 100 mM HAC.
Table S1. Physical properties of AG and AG-alkyne@polymer-pBA cryogel

| Temperature | Cryogel samples               | Swelling Degree (g H$_2$O/g Cryogel) | Macroporosity (Volume %) |
|-------------|-------------------------------|-------------------------------------|--------------------------|
| 25°C        | AG cryogel                    | 15.13 ± 0.07                        | 72.42 ± 0.01             |
|             | AG-alkyne@polymer-pBA cryogel | 13.38 ± 0.06                        | 69.05 ± 0.01             |
| 40°C        | AG cryogel                    | 14.84 ± 0.06                        | -                        |
|             | AG-alkyne@polymer-pBA cryogel | 11.95 ± 0.11                        | -                        |
| Adsorbent | Ligands | Targets | Sample | Binding Capacity | Ref. |
|-----------|---------|---------|--------|------------------|-----|
| AG@epoxy@PEI-DFFPBA | boronic acid | *Salmonella* spp., *S. aureus* | 25% cow milk; water | *Salmonella* spp.: (906.60 ± 15.73) × 10^7 CFU/g | [1] |
| | | | | *S. aureus*: (582.59 ± 13.19) × 10^7 CFU/g | |
| N-methylimidazolium functionalized magnetic particles | N-methylimidazolium | *Listeria monocytogenes* | mineral water and tap water | 6.22 × 10^8 CFU/mg | [2] |
| AGe-Si@brush-pBA | boronic acid | yeast cell | - | 6 mg/g | [3] |
| PDA@Fe_3O_4 nanoparticles | ion-exchange | *S. aureus* | tap water | 1.2 × 10^8 CFU/mg | [4] |
| Si@poly(NIPA-co-GMA)@PCAPBA | boronic acid | *E. coli*, *S. epidermids* | water, 25% milk | *E. coli*: 13.4 × 10^7 CFU/mg | [5] |
| | | | | *S. epidermids*: 3.36×10^9 CFU/g | |
| Vancomycin modified PEGylated-magnetic nanoparticles | vancomycin | *Listeria monocytogenes* | lettuce | ~ 2.4 × 10^7 CFU/mg | [6] |
| AG-alkyne@polymer-pBA cryogel | boronic acid | *E. coli*, *S. epidermids* | water, 25% milk | *E. coli*: 2.15×10^9 CFU/g | This work |
Table S3. Comparison of different composite cryogel for chromatographic separation

| Cryogels                              | Ligands            | Targets               | Advantages / Disadvantages                                   | Ref. |
|---------------------------------------|--------------------|-----------------------|--------------------------------------------------------------|------|
| Organic-inorganic cryogel composite   | boronic acid       | nucleosides           | Synthesis under mild conditions; high surface area; simple synthesis conditions | [7]  |
| Tyrosine-imprinted cryogel            | molecularly imprinted polymer | tyrosine             | High selectivity and good reusability                        | [8]  |
| Poly(Hydroxyethyl Methacrylate) cryogel | antibody          | human immunoglobulin M | High specificity and biocompatibility. High-cost             | [9]  |
| PolyAdenine                           | adenine methacrylate | RNA                   | Sing-step synthesis; high RNA binding capacities; simple operation procedures | [10] |
| AGe-Si@brush-pBA                      | boronic acid       | yeast cell; haemoglobin | High surface area and multiple affinity ligands. Complicated synthesis procedures | [3]  |
| poly(HEMA-co-MAAc) cryogel           | ion-exchange       | cisplatin             | High hydrophilicity and binding capacities. Selectivity limited | [11] |
| AG-alkyne@polymer-pBA cryogel         | boronic acid       | *E. coli*, *S. epidermidis* | High ligand density, high binding capacity for bacteria, simple and modular synthesis | This work |
Reference

[1] Zheng, H.; Han, F.; Lin, H.; Cao, L.; Pavase, T.; Sui, J. Preparation of a novel polyethyleneimine functionalized sepharose-boronate affinity material and its application in selective enrichment of food borne pathogenic bacteria. Food Chem. 2019, 294, 468-476.

[2] Wang, Y.; Deng, M.; Jia, L. N-methylimidazolium functionalized magnetic particles as adsorbents for rapid and efficient capture of bacteria. Microchim Acta 2014, 181, 1275–1283.

[3] Hajizadeh, S.; Ye, L. Hierarchical macroporous material with dual responsive copolymer brushes and phenylboronic acid ligands for bioseparation of proteins and living cells. Sep. Purif. Technol. 2019, 224, 95-105.

[4] Gao, X.; Yao, X.; Zhong, Z.; Jia, L. Rapid and sensitive detection of Staphylococcus aureus assisted by polydopamine modified magnetic nanoparticles. Talanta, 2018, 186, 147-153.

[5] Zheng, H.; Gong, H.; Cao, L.; Lin, H.; Ye, L. Photoconjugation of Temperature-and pH-Responsive Polymer with Silica Nanoparticles for Separation and Enrichment of Bacteria. Colloid. Surface. B 2020, 111433.

[6] Meng, X.; Li, F.; Li, F.; Xiong, Y.; Xu, H. Vancomycin modified PEGylated-magnetic nanoparticles combined with PCR for efficient enrichment and detection of Listeria monocytogenes. Sensor. Actuat. B-Chem. 2017, 247, 546-555.

[7] Zhao, S.; Zou, Y.; Wang, Y.; Zhang, H.; Liu, X. Organized cryogel composites with 3D hierarchical porosity as an extraction adsorbent for nucleosides. J. Sep. Sci. 2019, 42, 2140-2147.
[8] Bakhshpour, M.; Göktürk, I.; Bereli, N.; Denizli, A. Molecularly imprinted cryogel cartridges for the selective recognition of tyrosine. Biotechnol. Progr. 2020, 36, e3006.

[9] Bakhshpour, M.; Topcu, A.; Bereli, N.; Alkan, H.; Denizli, A. Poly(Hydroxyethyl Methacrylate) Immunoaffinity Cryogel Column for the Purification of Human Immunoglobulin M. Gels, 2020, 6, 4.

[10] Köse, K.; Erol, K.; Özgür, E.; Uzun, L.; Denizli, A. PolyAdenine cryogels for fast and effective RNA purification. Colloid. Surface. B 2016, 146, 678-686.

[11] Farías, T.; Hajizadeh, S.; Ye, L. Cryogels with high cisplatin adsorption capacity: Towards removal of cytotoxic drugs from wastewater. Sep. Purif. Technol. 2020, 235, 116203.