Supplementary information

for

Self-assembled molecular team of boronic acids at gold surface for specific capture of cis-diol biomolecules at neutral pH

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EXPERIMENTAL SECTION

Materials. Water used for the experiments was prepared using a Milli-Q system (Millipore, Bedford, MA, USA). Acetonitrile and formic acid were purchased from Aldrich (Milwaukee, WI, USA). RNase B (product numbers, R1153 and R7884), RNase A, myoglobin and α-cyano-4-hydroxycinnamic acid (CHCA) were obtained from Sigma (St. Louis, MO, USA). Thiophene-3-boronic acid, 2-mercaptopethamine hydrochloride, adenosine, adenosine monophosphate were from Alfa Aesar (Ward Hill, MA, USA).

Preparation of Fe$_3$O$_4$/Au MNPs. The Au nanoparticles were prepared by the citrate reduction of HAuCl$_4$ according to a literature.\textsuperscript{1} The amino-functionalized Fe$_3$O$_4$ MNPs were synthesized according to another literature.\textsuperscript{2} The Fe$_3$O$_4$/Au core/satellite nanoparticles were prepared according to a literature protocol.\textsuperscript{3,4} Briefly, an aliquot of amino functionalized magnetic nanoparticles dispersed in ethanol (4 mL, ~ 4×10$^{12}$ particles/mL) was placed in a flask along with an excess of gold nanoparticles (40 mL, ~ 7×10$^{12}$ particles/mL), and the mixture was left to react at room temperature for 2 h.

Self-assembly processing. A literature approach,\textsuperscript{5,6} with slight modifications, was used for self-assembly processing. For self-assembling of TBA-molecular teams, the processing solution was a mixture of 12.8 mg thiophene-3-boronic acid and 11.7 mg 2-mercaptopethamine hydrochloride (molar ratio, 1:1) dissolved in H$_2$O / methanol (9:1) solution. To prepare the TBA-MNPs, 30 mg Fe$_3$O$_4$/Au nanoparticles was added to a 40-mL processing solution and reacted at room temperature for 12 h. To prepare the CBA-MNPs, no 2-mercaptopethamine was present in the processing solution and the procedure was otherwise identical. The resulting immobilized Fe$_3$O$_4$/Au nanoparticles were collected by a magnet at the wall and washed with water and ethanol for 3 times each and dried at 50 °C.

Preparation of TBA-modified MALDI target plate. A thin gold layer was deposited onto a mica strip by a Bal-Tec SCD 500 sputter coating system (Liechtenstein, UK). The sputter coating system was operated under 6×10$^{-4}$ mbar and 25 mA for 200 s. The TBA-modified mica strip was prepared by immersing freshly cleaned mica strips into the processing solution containing both thiophene-3-boronic acid and 2-mercaptopethamine and then reacting for 12 h. The resulting mica
strip modified was washed with water and ethanol for 3 times each and dried at 50 °C. It was adhered to a MALDI target plate by conductive adhesive.

**Extraction.** For the extraction by MNPs, 1 mg TBA-MNPs or CBA-MNPs or bare Fe₃O₄/Au MNPs were added to a 200-μL plastic microcentrifugal tube containing analytes dissolved in 20 μL solution of appropriate pH. The tube was shaken on a rotator for 1 h. The MNPs were then collected at the tube wall by applying a magnet to the tube wall and rinsed with 100 μL 100 mM ammonium acetate buffer containing 600 mM NaCl (pH 7.4) and 100 μL 100 mM ammonium acetate buffer (pH 7.4) for 3 times each. After washing, the TBA-MNPs or CBA-MNPs were resuspended in 20 μL 10 mM acetic acid solution for 1 h on a rotator. Finally, the TBA-MNPs or CBA-MNPs were trapped to the tube wall again and the elution buffer was collected by pipetting carefully. The extraction and desorption experiments were carried out under ambient temperature. For the capture by the TBA-modified MALDI target plate, sample drops (2 μL) were added to the plate and removed after 1 h, and rinsed with 100 μL 100 mM ammonium acetate buffer containing 600 mM NaCl (pH 7.4) and 100 μL 100 mM ammonium acetate buffer (pH 7.4) for each 3 times, before acetic acid was added.

**Instrumentation.** ¹¹B NMR spectra were recorded on an Avance DMX 500 MHz instrument (Bruker, Germany) operated at 160 MHz and 25 °C. A solution of BF₃OEt₂ was used as an external reference (δ= -18.2 ppm). Thiophene-3-boronic (6.4 mg) was dissolved in 0.5 mL D₂O with or without the addition of 2-mercaptopropanoic acid (5.8 mg) for measurement. The pH for the mixture was adjusted to 7.0 with ammonia. Zeta potential measurements were carried out on the Malvern Nano Z Zetasizer (Malvern, Worcestershire, UK). The MNPs were dispersed in water when measuring.

CE analysis was performed on a P/ACE MDQ system (Beckman Coulter, Fullerton, CA, USA). A bare fused-silica capillary (Yongnian Optical Fibre Factory, Hebei, China) of 75 μm i.d. × 60 cm (50 cm effective length) was used as the separation column. At the beginning of each day, the capillary was rinsed with 1 M NaOH, water and running buffer for 20 min each. Before each run, the capillary was conditioned with 0.1 M NaOH, water, running buffer for 2 min each. The capillary was thermostated at 25 °C. Samples were injection by pressure at 0.5 psi for 3 or 5 s. The separation voltage was 12 or 20 kV. The UV absorbance was recorded at 214 or 254 nm.
MALDI-TOF MS experiments were implemented on an Autoflex mass spectrometer (Brucker Daltonics, Germany). Positive ion spectra were recorded using stainless steel target plate with standard parameter: a nitrogen laser ($\lambda = 337$ nm), 250 ns pulse duration and 20 kV for accelerating voltage. Saturated CHCA in 0.1% trifluoroacetic acid: acetonitrile (70: 30, v/v) was used as the matrix. After a 1.0-$\mu$L matrix solution was added, the sample spots were allowed to dry. Each spectrum was typically summed with 100 laser shots.

**Reference**


Supporting Figures and Tables

Fig. S1 SEM photograph of the TBA-MNPs (magnification, 300,000 ×).

Fig. S2 CE analysis of adenosine extracted by bare Fe₃O₄/Au MNPs from 1 mg/mL adenosine dissolved in 100 mM sodium phosphate solution at different pH.
**Fig. S3** Dependence of the amount of adenosine monophosphate (represented by the corrected peak area) extracted by per mg of TBA-MNPs and CBA-MNPs on the salt concentration present in the sample. Sample: 1 mg/mL adenosine monophosphate in 10 mM phosphate buffer containing 0, 100, 200, and 400 mM NaCl, pH 7.4 or 8.5, each extracted by TBA-MNPs (pH 7.4) or CBA-MNPs (pH 8.5) for 1 h, desorbed with 10 mM acetic acid for 1 h.

**Fig. S4** Mass spectra for i) direct analysis of human serum spiked with RNase B (final RNase B concentration, 0.05 mg/mL); ii) analytes extracted by TBA-MNPs from the RNase
B-spiked human serum. The different spectrum for RNase B was due to different reagent batch.

**Fig. S5** Photograph of the TBA-modified MALDI plate.

**Fig. S6** Specific capture of glycoprotein by the TBA modified target plate. MALDI-TOF MS spectra for i) direct analysis of the mixture of RNase B and myoglobin with molar ratio of 1:1; ii) analysis of glycoprotein captured by TBA-MNPs from the 1:1 mixture of RNase B and myoglobin. Concentration of RNase B and myoglobin: $7 \times 10^{-6}$ M.
**Table S1.** Measured zeta potential of the MNPs at different stages

<table>
<thead>
<tr>
<th>Magnetic nanoparticles</th>
<th>Zeta potential / mV</th>
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<tbody>
<tr>
<td>amino-functionalized Fe$_3$O$_4$ MNPs</td>
<td>$+26.1 \pm 1.2$</td>
</tr>
<tr>
<td>Fe$_3$O$_4$/Au MNPs</td>
<td>$-23.2 \pm 0.8$</td>
</tr>
<tr>
<td>CBA-MNPs</td>
<td>$-20.1 \pm 0.8$</td>
</tr>
<tr>
<td>TBA-MNPs</td>
<td>$-8.7 \pm 0.8$</td>
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