Supplementary Information

Rational design of Hg\textsuperscript{2+} controlled streptavidin-binding aptamer

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Experimental section

Materials

Unlabeled oligonucleotides were synthesized and purified by Sunbiotech Co. Ltd. (Beijing, China). Carboxyfluorescein (FAM) labeled ssDNAs were synthesized and purified with HPLC by Shanghai Sangon Biological Engineering Technology&Services Co. Ltd. (Shanghai, China). Streptavidin coated sepharose beads (Streptavidin Sepharose High Performance) were obtained from GE Healthcare Bio-Sciences AB (GE Healthcare, Sweden). Streptavidin-coated magnetic beads (Dynabeads M-280 Streptavidin) were purchased from Invitrogen Dynal AS (Oslo, Norway). Hemin was obtained from Beijing XinjingKe Biotechnology (Beijing, China). A hemin stock solution (20 mM) was prepared in DMSO and stored in the dark at -20 °C. 2,2’-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was purchased from Amresco (Solon, USA). All used metal salts (HgCl\textsubscript{2}, PbCl\textsubscript{2}, CdCl\textsubscript{2}, FeCl\textsubscript{2}, MgCl\textsubscript{2}, CaCl\textsubscript{2}, CoCl\textsubscript{2}, ZnCl\textsubscript{2}, MgCl\textsubscript{2}, MnCl\textsubscript{2}, BaCl\textsubscript{2}, NiCl\textsubscript{2} and CuCl\textsubscript{2}) and H\textsubscript{2}O\textsubscript{2} (30%) was obtained from Beijing Chemical Plant (Beijing, China). Working solutions of hemin, ABTS, and H\textsubscript{2}O\textsubscript{2} were freshly prepared with Tris-HCl buffer (25 mM Tris-HCl, 150 mM NaCl, 5 mM KCl, 2 mM MgCl\textsubscript{2}, 0.05% Tween-20, pH=7.4) before used. All reagents were used as received without further purification. The deionized water was prepared on a UPHW-III-90T UP water purification system (Chengdu, China).
**Table S1** all ssDNA sequences used in this paper

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequences (From 5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>St-2-1</td>
<td>FAM-ATTGACCGCTGTGTGACGCAACACTCAAT</td>
</tr>
<tr>
<td>SVHg1</td>
<td>FAM-TTTTACCGCTTTTTTGACGCATTTTTTTTT</td>
</tr>
<tr>
<td>SVHg2</td>
<td>FAM-ATTGACCGCTGTGTGACGCATTCTCAAT</td>
</tr>
<tr>
<td>SVHg3</td>
<td>FAM-TTTTACCGCTGTGTGACGCAACACTTTTT</td>
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<tr>
<td>SVHg4</td>
<td>FAM-ATTGACCGCTGTGTGACGCAACTCTCAAT</td>
</tr>
<tr>
<td>SVHg5</td>
<td>FAM-ATATACCGCTGTGTGACGCAACTCTTTAT</td>
</tr>
<tr>
<td>SVHg5-G4</td>
<td>ATATACCGCTGTGTGACGCAACTCTTTATCATCGCTGGGAGGGAGGGAGGGA</td>
</tr>
</tbody>
</table>

**Influence of Hg^{2+} on the fluorescence intensity of FAM-labeled DNA sequences**

200 μL of 200 nM FAM-labeled DNA sequences (St-2-1, SVHg1, SVHg2, SVHg3, SVHg4, SVHg5) in Tris-HCl buffer (25 mM Tris-HCl, 150 mM NaCl, 5 mM KCl, 2 mM MgCl₂, 0.05% Tween-20, pH=7.4) with or without 10 μM HgCl₂ were denatured at 95°C for 5 min, cooled on ice for 10 min, and then kept at 25°C for 30 min. The fluorescence intensity of the solution was measured with SpectraMax M5 (Molecular Devices, USA) at 538 nm with excitation at 485 nm.

Normalized fluorescence intensity = \( \frac{F_b}{F_0} \times 100\% \),

Wherein, \( F_b \) is the fluorescence intensity of FAM-labeled DNA sequences (St-2-1, SVHg1, SVHg2, SVHg3, SVHg4, or SVHg5) in 25 mM Tris-HCl buffer with 10 μM HgCl₂, \( F_0 \) is the average value of three measured fluorescence intensities of the corresponding FAM-labeled DNA sequences in 25 mM Tris-HCl buffer without 10 μM HgCl₂. Each experiment was repeated three times.

**Binding abilities of different DNA sequences in the presence or absence of Hg^{2+}**

200 μL of 200 nM FAM-labeled DNA sequences (St-2-1, SVHg1, SVHg2, SVHg3, SVHg4, SVHg5) in 25 mM Tris-HCl buffer with or without 10 μM HgCl₂ were denatured at 95°C for 5 min, cooled on ice for 10 min, and then kept at 25°C for 30 min. 10 μL of streptavidin-coated sepharose beads, washed with 200 μL of 25 mM Tris-HCl buffer, was added to the DNA solution and incubated at 25°C for 30 min with shaking. After centrifuging, the fluorescence intensities of the supernatants were measured on SpectraMax M5 at 538 nm with excitation at 485 nm. The amount of bound FAM-labeled DNA sequences were calculated by subtracting the amount of the free ones from the total amount of FAM-labeled DNA sequences.

Bound percentage = \( (1-\frac{F_b}{F_0}) \times 100\% \),
Wherein, \( F_0 \) is the fluorescence intensity of the free FAM-labeled DNA sequences in the supernatant, and \( F_b \) is the fluorescence intensity of original FAM-labeled DNA sequences before incubation. Each experiment was repeated three times.

**Specificity investigation of SVHg5 to Hg\(^{2+}\)**

200 \( \mu \)L of 200 nM FAM-labeled SVHg5 in 25 mM Tris-HCl buffer containing 10 \( \mu \)M different metal salts (HgCl\(_2\), PbCl\(_2\), CdCl\(_2\), FeCl\(_2\), MgCl\(_2\), CaCl\(_2\), CoCl\(_2\), ZnCl\(_2\), MgCl\(_2\), MnCl\(_2\), BaCl\(_2\), NiCl\(_2\), and CuCl\(_2\)) was denatured at 95\(^\circ\)C for 5 min, cooled on ice for 10 min, and then kept at 25 \(^\circ\)C for 30 min. 10 \( \mu \)L of streptavidin coated sepharose beads, washed with 200 \( \mu \)L of 25 mM Tris-HCl buffer, were added to the DNA solution and incubated at 25 \(^\circ\)C for 30 min. After centrifuging, the fluorescence intensities of supernatants were measured on a SpectraMax M5 at 538 nm with excitation at 485 nm. The amount of bound FAM-labeled SVHg5 was calculated by subtracting the amount of the free FAM-labeled SVHg5 from the total amount of FAM-labeled SVHg5.

\[
\text{Bound percentage} = \left(1 - \frac{F_b}{F_0}\right) \times 100%.
\]

Wherein, \( F_0 \) is the fluorescence intensity of the free FAM-labeled SVHg5 in the supernatant, and \( F_b \) is the fluorescence intensity of original FAM-labeled SVHg5 before incubation. Each experiment was repeated three times.

**Flow Cytometric Analysis**

200 \( \mu \)L of 500 nM FAM-labeled SVHg5 in 25 mM Tris-HCl buffer containing different concentration of HgCl\(_2\) (0 \( \mu \)M, 0.05 \( \mu \)M, 0.3 \( \mu \)M, 0.8 \( \mu \)M, 1.5 \( \mu \)M, 3.0 \( \mu \)M, 6.0 \( \mu \)M, 10.0 \( \mu \)M, 20.0 \( \mu \)M) were denatured at 95\(^\circ\)C for 5 min, cooled on ice for 10 min, and then kept at 25 \(^\circ\)C for 30 min. 5 \( \mu \)L of streptavidin-coated sepharose beads, washed with 200 \( \mu \)L of 25 mM Tris-HCl buffer, were added to the solution and incubated at 25 \(^\circ\)C for 30 min. After filtered through a pipette tip (200 \( \mu \)L) containing a frit, the beads were washed with 50 \( \mu \)L of 25 mM Tris-HCl buffer. The fluorescence intensity of the beads was collected on a Beckman flow cytometer (Beckman Coulter celllab Quanta SC) by counting 10,000 events for each sample.

**Peroxidase activity of bound SVHg5-G on Streptavidin-coated magnetic beads**

200 \( \mu \)L of 500 nM SVHg5-G in 25 mM Tris-HCl buffer containing 10 \( \mu \)M different metal salts (HgCl\(_2\), PbCl\(_2\), CdCl\(_2\), FeCl\(_2\), MgCl\(_2\), CaCl\(_2\), CoCl\(_2\), ZnCl\(_2\), MgCl\(_2\), MnCl\(_2\), BaCl\(_2\), NiCl\(_2\), and CuCl\(_2\)) was denatured at 95\(^\circ\)C for 5 min respectively, cooled on ice for 10 min, and then kept at 25 \(^\circ\)C for 30 min. 10 \( \mu \)L of Streptavidin coated magnetic beads, washed with 200 \( \mu \)L of Tris-HCl buffer, were added to the DNA solution and incubated at 25 \(^\circ\)C for 30 min. After separated by
magnet, the magnetic beads were washed by 50 μL of 25 mM Tris-HCl buffer, and then incubated with 10 μL of 5 μM hemin Tris-HCl solution at 25 °C for 1.5 h. Followed by adding 2μL of 50 mM ABTS Tris-HCl solution and 2μL of 25 mM H₂O₂ Tris-HCl solution, incubated at 25 °C for 15 min, a color change could be observed by naked-eye.

**Fig. S1** Influence of Hg²⁺ on the fluorescence intensity of FAM-labeled DNA sequences. Normalized fluorescence intensity=F/F₀×100%, F is the fluorescence intensity of FAM-labeled DNA sequences (St-2-1, SVHg1, SVHg2, SVHg3, SVHg4, or SVHg5) in 25 mM Tris-HCl buffer containing 10 μM HgCl₂. F₀ is the average value of three measured fluorescence intensities of the corresponding FAM-labeled DNA sequence in 25 mM Tris-HCl buffer without 10 μM HgCl₂. Each experiment was repeated three times.