Supporting Information

A fuel-initiated DNA molecular machine for microRNA detection in serum via poly-adenine-mediated spherical nucleic acid

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Chemicals and Reagents

Hydrogen tetrachloroaurate (III) trihydrate (HAuCl\(_4\)·3H\(_2\)O), sodium citrate trihydrate (Na\(_3\)C\(_6\)H\(_5\)O\(_7\)·3H\(_2\)O), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), Tetramethylethylenediamine (TEMED) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.) and all were analytical grade. TBE buffer, 40% acrylamide, Loading buffer, gel-red and fetal bovine serum (FBS) were purchased from Shanghai Sangon (Shanghai, China). Phosphate-buffered saline (PBS) was prepared using 0.1 M NaH\(_2\)PO\(_4\), 0.1 M Na\(_2\)HPO\(_4\), 0.1 M KCl, and 0.9 wt% NaCl. Other reagents were obtained from Sinopharm Chemical Reagents, Co., Ltd. (Shanghai, China) and used without further purification. All oligonucleotides were synthesized and purified by Shanghai Sangon (Shanghai, China) and used without further purification. The sequence and name of the above oligonucleotides are shown in Table S1. All solutions were prepared with Milli-Q water with a resistivity of 18.2 M\(\Omega\)/cm. DEPC water was used for dilution in microRNA experiments.

Preparation and Characterization of Gold Nanoparticles (AuNPs)

AuNPs were synthesized through the method of sodium citrate reduction of HAuCl\(_4\) as previous reported\(^1\). In brief, 3.5 ml 1% Na\(_3\)C\(_6\)H\(_5\)O\(_7\)·3H\(_2\)O was added into the boiling 100 ml aqueous solution containing 1 ml HAuCl\(_4\) (24.28 nM). The solution was kept boiling and stirred for 20 min, and then cooled to room temperature with stirring. The obtained AuNPs were stored at 4\(^\circ\)C for further use. The size and morphology of AuNPs were assessed by TEM. The AuNPs were diluted to 1 nM by water for measuring the
hydration radius and zeta potential of SNA were measured by DLS. The concentration of AuNPs was obtained by UV-Vis spectrophotometer.

**Instrumentation**

Ultraviolet-visible (UV-Vis) spectrophotometer (UV2600, Shimadzu, Japan) was used to measure the concentration of AuNPs and DNA solution. Fluorescence spectrophotometer (FS5, Edinburgh Instruments, UK) was used to measure fluorescence spectra. The TEM images of gold nanoparticles were obtained on a transmission electron microscope (Talos F200x, Thermo Fisher Scientific, USA). Dynamic light scattering (DLS) instrument (Nano-ZS90, Malvern, UK) was used to characterize the hydration diameter and zeta potential of AuNPs and SNAs.
Table S1. The name and sequence of oligonucleotides used in this article.

<table>
<thead>
<tr>
<th>DNA name</th>
<th>Sequence (5’-3’)</th>
</tr>
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<tr>
<td>polyA20-TP</td>
<td>AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGAGTGGTGGAAGGGAGGGACAGTTCTTCAACTGGCAGCTT</td>
</tr>
<tr>
<td>FP</td>
<td>CCTTTCCACACTCT-FAM</td>
</tr>
<tr>
<td>AP</td>
<td>CCAGTTGAAGAACTGTCCCTATATCC</td>
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<tr>
<td>FS</td>
<td>CCAGTTGAAGAACTGTCCCTCTCTTTCCACCACCTCT</td>
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<td>polyA5-TP</td>
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<td>polyA50-TP</td>
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<tr>
<td></td>
<td>GCAGCTT</td>
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<td>Target DNA</td>
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<tr>
<td>Mismatch-10</td>
<td>AAGCTGCCATTTGAAGAACTGT</td>
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<tr>
<td>Mismatch-16</td>
<td>AAGCTGCCAGTTGAATAACTGT</td>
</tr>
<tr>
<td>MicroRNA-22</td>
<td>AAGCUUGCUGUUGAAGAACUGU</td>
</tr>
</tbody>
</table>
**Fig. S1** The average particle size of the prepared AuNPs was obtained by statistical analysis of TEM images.

**Fig. S2** The reaction process of (left) the byproduct (polyA-TP + FP) with FS, producing high leakage; and the reaction process of (right) the diblock DNA (polyA-TP + AP+FP) with FS and target DNA, producing high signal ratio.
Fig. S3 By increasing the AP ratio (A) and salt ion concentration (B) during probe synthesis, the stability of the diblock probe can be enhanced, resulting in the reduction of leakage and increasing of F/F₀. Error bars represent standard deviations from at least three independent tests.
The reaction rate was calculated by fitting the quasi-first order reaction rate and the reaction rate constant $k'$ is 0.05 min$^{-1}$.

**Fig. S4**
Fig. S5 Target DNA detection in serum. (A) As the serum content in the reaction system increases, the signal-to-noise ratio decreases. (B) The fluorescence curve of $F_{\text{polyA20-SNAs}}$ and $F_0$ in 100% FBS and PB buffer. (C) The leakage signal ($F_0/F_{\text{polyA20-SNAs}}$) in FBS and PB buffer. (D) The fluorescence curve of $F_{\text{polyA20-SNAs}}$ in 100% FBS and 50% FBS. Error bars represent standard deviations from at least three independent tests.
Fig. S6 MiR-22 detection in serum. (A) The fluorescence signal increased with the increase in target concentration. (B) The fitting standard curve ($R^2 = 0.9728$) and the LOD is 10 pM. Error bars represent standard deviations from at least three independent tests.

Reference