Supporting Information for

DNA Aptamer Folding on Gold Nanoparticles: from Colloid Chemistry to Biosensors

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1. Experimental Section

1.1 Materials. Trisodium citrate, hydrogen tetrachloroaurate(III) ($HAuCl_4$), 6-mercaptohexan-1-ol (MCH), adenosine, adenosine 5'-triphosphate (ATP), potassium chloride (KCl), sodium chloride (NaCl), lithium chloride (LiCl), inosine, *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA), ethyl acetate and adenosine deaminase (ADA) were purchased from Sigma and used as received. [-^{32}P]-ATP was obtained from Amersham Biosciences. DNA molecules were obtained from Integrated DNA Technologies (IDT).

1.2 Preparation of aptamer-modified AuNPs. AuNPs (~13.5 nm in diameter based on transmission electron microscopy (TEM) measurement) were prepared according to previously described protocols.[1] The concentration was estimated using UV/vis spectroscopy to be ~13 nM, based on an extinction coefficient of 2.7×10^8 M^{-1} cm^{-1} at λ=520 nm.[1a] Aptamer-modified AuNPs were then prepared using the standard surface modification protocol based on Au-S chemistry.[1] Briefly, an AuNP solution (600 μL, ~13 nM) was mixed with thiol-modified oligonucleotides (280 μL, 6.6 μM) (see Table 1 for the sequences of adenosine, K^+ aptamers and their mutants). The solution was incubated at room temperature for 12 h. Tris-HCl buffer (10 μL, 1 M, pH 7.5) and aqueous NaCl (90 μL, 1M) were added, and the mixture was incubated for another 12 h. After that, Tris-HCl buffer (5 μL, 1 M, pH 7.5) and aqueous NaCl (50 μL, 5M) were added, and the mixture was further incubated for 18 h at room temperature. The solution was then separated by a centrifuge at 22000 *g* for 15 min. The precipitated aptamer-modified AuNPs were washed with double-deionized (dd) H_2O (600 μL) and isolated using centrifugation. Finally, adenosine aptamer-modified AuNPs (referred to as Au-Ado and Au-T10Ado for the aptamer without and with T10 linker, respectively) and K^+ aptamer-modified AuNPs (referred to as Au-K) were redispersed in 600 μL adenosine buffer (Tris-HCl (20 mM), pH = 7.5, NaCl (100 mM)) and ddH_2O, respectively. For the synthesis of radioactive oligonucleotide, [γ-^{32}P] ATP (20 μCi) was incorporated using the manufacturer-supplied protocol.

1.3 MCH treatment. The aptamer-modified AuNPs were treated with MCH as described elsewhere.[2] Briefly, Au-Ado (or Au-T10Ado) solution prepared above was diluted with an equal volume of adenosine buffer, MCH was then added to give a final MCH concentration of ~ 4 μM. The MCH treatment was performed at room temperature for 30 min. The reaction was quenched by three washes with equal volumes of ethyl acetate, which removed excess MCH from the aqueous solution. A similar procedure was used for Au-K with an exception that ddH_2O was used instead of adenosine buffer. The aptamer-modified AuNPs were spun down using centrifugation at 22 000 *g* for 15 min. The final Au-Ado (or Au-T10Ado) and Au-K were redispersed in 600 μL adenosine buffer and ddH_2O, respectively.

1.4 Au-Ado (or Au-T10Ado) stability with and without adenosine. Adenosine aptamer folding on Au-Ado (or Au-T10Ado) was conducted in a solution that contained Au-Ado (or Au-T10Ado) (~ 3 nM), adenosine (1 mM), Tris-HCl (20 mM), pH = 7.5, 4 mM $MgCl_2$ and NaCl (100 mM). At this salt concentration (4 mM $MgCl_2$ and100 mM NaCl), both Au-Ado (or Au-T10Ado) and Au-Ado with folded aptamer/adenosine complex (referred to as Au-Ado-Target) (or Au-T10Ado-Target) were stable and the solutions appeared red. To determine their stability, $MgCl_2$ (1 M) solution was gradually added into AuNP solutions until a red-to-purple color change was observed over a short period of time (~ 1 min).

1.5 Au-K stability with and without K^+. K^+ aptamer folding on Au-K was performed in an aqueous solution containing Au-K (~ 3 nM) and KCl (10 mM). Using a strategy that is similar to the adenosine system, the stability of Au-K and Au-K with the folded aptamer/K^+ complex (referred to as Au-K-Target) was studied by gradually adding $MgCl_2$ (1 M) until a red-to-purple color change was observed over a short period of time (~ 1 min).

1.6. Adenosine and K^+ sensing assays. In the case of adenosine, assay solutions (250 μL) contained various amounts of adenosine, Au-T10Ado (~ 3 nM), Tris-HCl (20 mM), pH = 7.5, NaCl (100 mM) and $MgCl_2$ (60 mM). Specifically, a mixture (180 μL) of Au-T10Ado (~ 4.14 nM), Tris-HCl (28 mM) and NaCl (138 mM) was first prepared. A solution (70 μL) of $MgCl_2$ (214 mM) and various amounts of adenosine were then added, after which UV-visible spectra were recorded continuously on a Cary300 UV/vis spectrophotometer for 10 min at room temperature (21 °C) or 35 °C.

A similar assay was performed for the detection of K^+. Briefly, assay solutions (250 L) contained various amounts of K^+, Au-K (~ 3 nM), and $MgCl_2$ (20 mM). UV-visible spectra were recorded continuously on a Cary300 UV/vis spectrophotometer for 10 min at room temperature immediately after the addition of KCl and $MgCl_2$.

1.7. Adenosine deaminase (ADA) sensing assay. Au-T10Ado (~ 3 nM) was mixed with adenosine (1 mM) solution in a buffer containing Tris-HCl (20 mM), pH = 7.5, $MgCl_2$ (60 mM) and NaCl (100 mM). Various amounts of ADA were then added, after which UV-visible spectra were recorded on Cary300 UV/vis spectrophotometer at room temperature. To study the inhibition, the ADA inhibitor, EHNA (400 μM), was incorporated in above Au-T10Ado solution before the addition of ADA.

1.8. Characterizations. TEM samples were prepared by dropping AuNP solutions (4 μL) onto a carbon-coated copper grid. The solution was wicked from the edge of the grid with a piece of filter paper after 1 min. TEM images were taken with a JEOL 1200 EX. UV-visible spectra were recorded on a Cary300 UV/vis spectrophotometer. Dynamic light scattering (DLS) measurements were performed at 25 °C and a scattering angle of 90° using a Brookhaven (Holtsville, NY) 256 channel BI-APD 8590 correlator and a 35 mW 632.8 nm laser. The particle size (diameter) was calculated by cumulative intensity distribution using the program CONTIN. Each reported particle size was the average of 10 measurements.

2. Supporting Figures

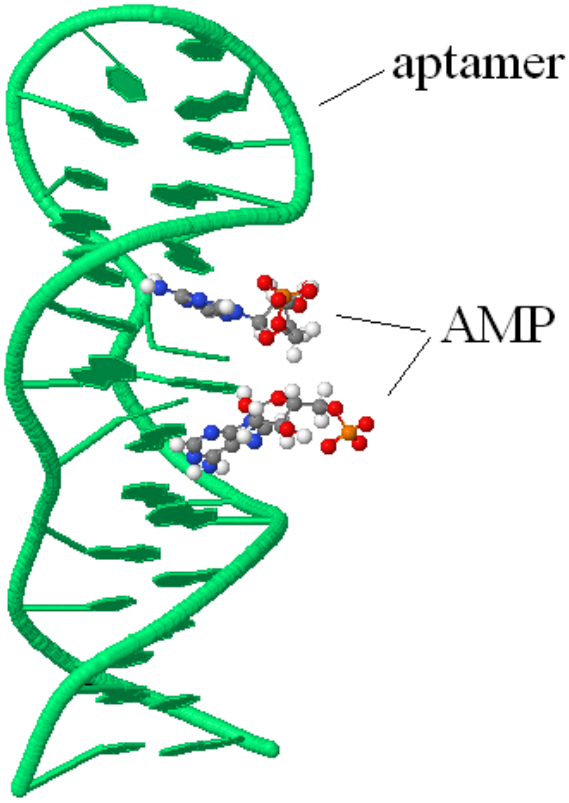

Figure S1. Three-dimensional model for folded DNA aptamer/adenosine monophosphate (AMP) complex. This structure, obtained based on a previous NMR study,[3] is adopted from the RCSB protein data bank. Aptamer backbone together with nucleobases are shown in green. In AMP model structure, C, N, O, P and H are shown in grey, blue, red, orange and white, respectively. One aptamer/AMP complex contains two adjacently bound AMP molecules. Detailed structural information can be found in reference 3.

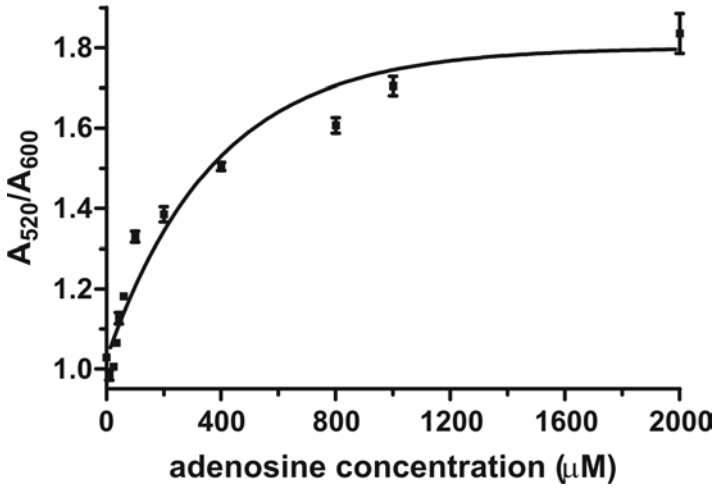

Figure S2. Determination of the dissociation constant (K_d) for adenosine-binding DNA aptamer. The A_{520}/A_{600} (abbreviated as A) vs. adenosine concentration data were fit into a modified one phase exponential association equation, $A = A_{min} + (A_{max} - A_{min}) \times (1\text{-}e^{-k[adenosine]})$, where the best-fit A_{min} = 1.026 with the A_{max} constraint of ≥1.8, with $R^2 = 0.95$. K_d was approximated from the mid-point of the equation, –ln(0.5/k), where k (an arbitrary constant) has a value of 0.002632. By this method, a K_d of 263 μM was obtained.

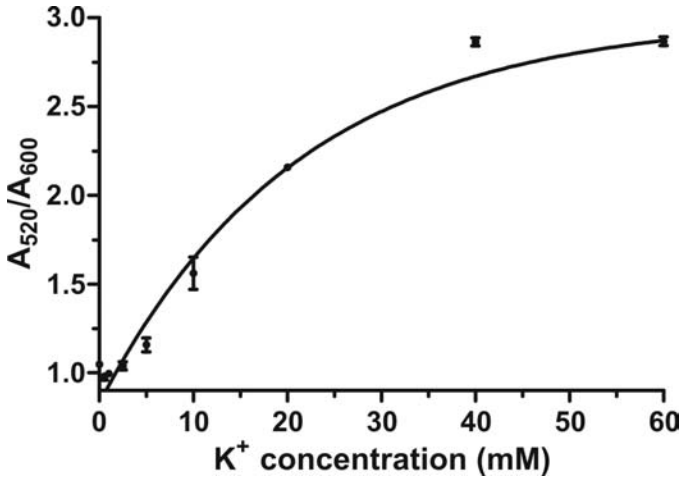

Figure 3. Determination of K_d for potassium ion-binding DNA aptamer. The A_{520}/A_{600} vs. potassium concentration data were fit into a modified one phase exponential association equation, $A = A_{min} + (A_{max} - A_{min}) \times (1\text{-}e^{-k[potassium]})$, where the best-fit A_{min} = 0.83 with the A_{max} constraint = 3, with R^2 = 0.98. K_d was approximated from the mid-point of the equation, –ln(0.5/k), where k has a value of 0.047. By this method, a K_d of 15 mM was obtained.

References

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(2) (a) Park, S.; Brown, K. A.; Hamad-Schifferli, K. *Nano Lett*. **2004**, *4*, 1925-1929. (b) Huang, E.; Satjapipat, M.; Han, S.; Zhou, F. *Langmuir* **2001**, *17*, 1215-1224. (c) Zhao, W.; Chiuman, W.; Brook, M. A.; Li, Y. *ChemBioChem*. **2007**, *8*, 727-731.
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