An Ultra-sensitive Colorimetric Detection of Tetracyclines using the Shortest Aptamer with Highly Enhanced Affinity

Young Seop Kwon‡, Nurul Hanun Ahmad Raston‡, and Man Bock Gu*.  

School of Life Sciences and Biotechnology, Korea University, Anam-dong, Seongbuk-Gu, Seoul 136-713, Rep. of Korea.

* To whom correspondence should be addressed. Tel: +82-2-3290-3417; Fax: +82-2-9286050; Email: mbgu@korea.ac.kr

‡These authors contributed equally to this work.
Supplementary Results

Supplementary Figures

Supplementary Figure 1

Supplementary Figure 1. Specific interaction of all truncated aptamers from groups A, B and C, with different molecules including TCs by using the colorimetric assays, which is represented by the absorbance ratio of AuNPs at 520 and 650 nm wavelengths (A650/A520).
Supplementary Figure 2

Supplementary Figure 2. Molecular interactions of the truncated (a) A1 and (b) A2 aptamers with TET measured by Isothermal Titration Calorimetry (ITC) analysis. TET (50 μM) was titrated into 0.5 μM of A1 and A2 aptamers at 25 °C. The Kd value of A1 aptamer to TET was found to be 3.413 nM, compared to A2 aptamer which is 1.067 nM.
Supplementary Figure 3

Supplementary Figure 3. Molecular interactions of the (a) A2 and (b) original longer (76 mer) aptamers measured by Isothermal Titration Calorimetry (ITC) analysis with 50 μM OTC titrated into 0.5 μM of each aptamers at 25 °C.
Supplementary Figure 4. Molecular interaction of A2 aptamer with TCs. (a) 3-D CPK molecular interaction model showing A2 aptamer-TCs (Skyblue for Tetracycline, Yellow for Oxytetracycline, and Green for Doxycycline) complex obtained from molecular dynamic simulations. (b) Molecular interaction between A2-aptamer and TCs represented by 3-D stick model, showing π-π stacking interaction between 5’-CG binding pocket with TET (left side) and hydrogen bonds formation (right side), in A2 aptamer-TCs interaction complex.
Supplementary Figure 5

Supplementary Figure 5. Specific interaction of various aptamers. A2 aptamer, original 76-mer aptamer and variously truncated A2 aptamers such as scrambled 8-mer, front 4-mer of A2 aptamer, scrambled 4-mer plus GGTG-3’, non-conserved arm-bases and 7-mer to tetracycline and oxytetracycline as the targets by using the colorimetric assays, which is represented by the absorbance ratio of AuNPs at 520 and 650 nm wavelength (A650/A520). Buffer was used as control.
Supplementary Figure 6

Supplementary Figure 6. Molecular interactions of the variously truncated A2 aptamers, such as (a) non-conserved arm-bases, (b) front 4-mer of A2 aptamer, (c) scrambled 4-mers plus GGTG-3’ and (d) 7-mer (5’-CGGTGGT-3’) measured by Isothermal Titration Calorimetry (ITC) analysis with 50 µM TET titrated into 0.5 µM of each truncated aptamers at 25 °C.
## Supplementary Table

### Supplementary Table 1

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.2</td>
<td>5'-CGACCCGCAGGTGCACTGGGCGACGTCTCTGGGTGTGGTGT-3'</td>
</tr>
<tr>
<td>No. 4</td>
<td>5'-CGACGCACGCGTTGGTGGTGATGGTGTGTGTTACACGTGTGTG-3'</td>
</tr>
<tr>
<td>No. 5</td>
<td>5'-ACGTTGACGCTGGTCCGTTTGGTGTCGAGCTGTGTGTGT-3'</td>
</tr>
<tr>
<td>No. 20</td>
<td>5'-CGAGTTGAGCGGGGGGGCGGTACGGGTACTGTATGTGTGGG-3'</td>
</tr>
<tr>
<td>No. 14</td>
<td>5'-ACGTTGACGCTGGTCCGTTTGGTGTCGAGCTGTGTGTGT-3'</td>
</tr>
</tbody>
</table>

Supplementary Table 1. Aptamers that bind to oxytetracycline selected by Systematic Evolution of Ligands by Exponential enrichment (SELEX) method by Javed et al., (2008), which were used for truncation analysis in this study.
Supplementary Table 2

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>5’-CGCTGGTG-3’</td>
</tr>
<tr>
<td>A2</td>
<td>5’-CGGTGGTG-3’</td>
</tr>
<tr>
<td>Scrambled 8-mer</td>
<td>5’-GGCTTCGG-3’</td>
</tr>
<tr>
<td>Front 4-mer of A2 aptamer</td>
<td>5’-CGGT-3’</td>
</tr>
<tr>
<td>Scrambled 4-mer plus GGTG-3’</td>
<td>5’-GCGTGGTG-3’</td>
</tr>
<tr>
<td>Non-conserved arm-bases</td>
<td>5’-CGTCGGTG-3’</td>
</tr>
<tr>
<td>7-mer</td>
<td>5’-CGGTGGT-3’</td>
</tr>
<tr>
<td>Original 76-mer (Aptamer No. 5)</td>
<td>5’-ACGTTGACGCTGGTGCCCGGTGTGCGAGTGTTGTGTTGT-3’</td>
</tr>
</tbody>
</table>

Supplementary Table 2. Variation of truncated and original 76-mer (Aptamer No. 5) aptamers used in this study.
Materials and methods

Materials and ssDNA aptamers

All the chemicals; Tetracycline (TET), oxytetracycline (OTC), doxycycline (DOX), diclofenac (DIC), chlortetracycline (CHLOR), glyphosate (GLY), naproxen (NPX), and carbamazepine (CBZ) were purchased from Sigma-Aldrich, unless specified and used without further purification. AuNPs were synthesized according to previously reported protocol by citrate reduction of HAuCl$_4$. The size of mono-dispersed AuNPs was confirmed to be around 13 nm by performing transmission electron microscopy (TEM, Tecnai 20). UV/vis spectrophotometer (Ultraspec 6300 pro, Amersham Biosciences) was used to characterize the absorption of AuNPs. The concentration of AuNPs was estimated by Beer-Lambert law using extinction coefficient, $2.43 \times 10^8 \text{ M}^{-1}\text{cm}^{-1}$ at 520nm. Five strands of OTC binding ssDNA aptamer (OBA) and all truncated aptamers as mentioned in Supplementary Table 2, were obtained from Genotech Inc. (Korea, Republic of) and sorted after dissolving in distilled water.

Heuristic Approach on Truncation

Three conserved regions with high homogeneity amongst five original aptamer sequences selected from our previous study were observed and truncated to have three different groups of truncated aptamer candidates, which are named as group A (8-mer from 26-33 base from 5’ to 3’ end of the original aptamers), group B (11-mer from 47-58) and group C (18-mer from 41-58). Then, these three groups of truncated candidates were tested for their specificity to TCs by using gold-nanoparticle based colorimetric assay. From this pre-screening experiment, as can be seen in the supplementary figure 1, we found that truncated candidates from group A and some of group C showed good selectivity to TCs. Furthermore, since the truncated
candidates from group A are 8-mer, which is the shortest candidate, yet still retaining high sensitivity to TCs, we selected group A aptamers, instead of group C, for further analysis.

**Gold nanoparticles (AuNPs) Colorimetric Assay**

Specificity Test: A mixture of 360 ul of 2.2 nM AuNPs with 20 ul of 10 uM of truncated aptamer was shaken mildly for 30 min at room temperature. Then, 20 ul of 100 uM of each target (TET, OTC, DOX, DIC, CHLOR, GLY, NPX and CBZ) in binding buffer (100 mM NaCl, 20 mM Tric-HCl, 2 mM MgCl₂, 5 mM KCl, and 1 mM CaCl₂) was added and further incubated for 30 min under the same condition. After adding 44 ul NaCl (1M) gradually into incubated samples, color and spectra changes were observed by naked eye and UV/vis spectrophotometer (Ultraspec 6300 pro, Amersham Biosciences). Buffer was used as control.

AuNPs colorimetric assay for all aptamers (including A2 aptamer, variations of A2 aptamer and original 76-mer aptamer) to both OTC and TET: A mixture of 360 ul of 2.2 nM AuNPs with 20 ul of each aptamer was shaken mildly for 30 min at RT. Then, 20 ul of 100 nM of target (TET or OTC), in binding buffer (100 mM NaCl, 20 mM Tric-HCl, 2 mM MgCl₂, 5 mM KCl, and 1 mM CaCl₂) was added and further incubated for 30 min under the same condition. After adding 44 ul NaCl (1M) gradually into incubated samples, color and spectra changes were observed by naked eye or UV/vis spectrophotometer (Ultraspec 6300 pro, Amersham Biosciences). The aptamer molar ratio to AuNPs was optimized for different length of ssDNA strands. Buffer was used as control.

Dose-dependent experiment of A2 aptamer for TET: A mixture of 360 ul of 2.2 nM AuNPs with 20 ul of 10 uM of A2 aptamer was shaken mildly for 30 min at RT. Then, 20 ul of 0, 0.1, 0.5, 1, 10, 50 and 100 nM of target TET in binding buffer (100 mM NaCl, 20 mM Tric-HCl, 2 mM MgCl₂, 5 mM KCl, and 1 mM CaCl₂) was added and further incubated for 30 min under the same condition. After adding 44 ul NaCl (1M) gradually into incubated samples, color and
spectra changes were observed by naked eye or UV/vis spectrophotometer (Ultraspec 6300 pro, Amersham Biosciences). Buffer was used as control.

Dose-dependent experiment of A2 aptamer and original 76-mer aptamer for OTC: A mixture of 360 ul of 2.2 nM AuNPs with 20 ul of 10 uM of each aptamer (A2 aptamer or original 76-mer aptamer) was shaken mildly for 30 min at RT. Then, 20 ul of 0, 0.1, 0.5, 1, 10, 5, 100, 500 and 1000 nM of OTC in binding buffer (100 mM NaCl, 20 mM Tric-HCl, 2 mM MgCl$_2$, 5 mM KCl, and 1 mM CaCl$_2$) was added and further incubated for 30 min under the same condition. After adding 44 ul NaCl (1M) gradually into incubated samples, color and spectra changes were observed by naked eye or UV/vis spectrophotometer (Ultraspec 6300 pro, Amersham Biosciences). Buffer was used as control.

**Study on Aptamer Affinity by Isothermal Titration Colorimetry (ITC)**

The binding affinities of the aptamer relevant to tetracycline were measured by ITC. ITC experiments were conducted in a VP-ITC machine (MicroCal). In the ITC experiment, 0.5 μM aptamer was loaded into the cell with 50 μM target in the titrating syringe, depending on the binding affinities of the compounds. All chemicals and aptamer were dissolved into the binding buffer (100 mM NaCl, 20 mM Tric-HCl, 2 mM MgCl$_2$, 5 mM KCl, and 1 mM CaCl$_2$). The titration experiments were performed at 25 °C with twenty nine 10 μl injections. The stirring speed during the titration was 290 rpm. Data were analyzed using MicroCal Origin software by fitting to a single-site binding model. Correction for the enthalpy of ligand dilution was carried out by subtracting reference data points of the only target titration without aptamer in the cell.

**Study on Molecular Interactions of Aptamer to Target by Molecular Dynamic (MD)**
simulations.

To discover whether a DNA aptamer’s nucleotide sequence can bind selectively to its target ligands (TET, OTC, and DOX), we first generated aptamer structure based on the sequence 5’-CGGTGGTG-3’ using Maestro programme. Molecular dynamics simulations 5 ns in length with explicit water solvent were performed on the 8-mer using the Desmond software, and the trajectories predicted diverse structural conformations of aptamer. Structures from the every 48ps of the MD trajectory were used to dock the ligands using Glide programme. For each structure, a grid box of 30×30×30 Å³ with a default inner box (10×10×10 Å³) was centred on the structure. Default parameters were used, and no constraints were included. The docking was performed using standard-precision mode with expanded sampling options. In case of expanded sampling, the number of poses per ligand for the initial phase of docking was increased to 20,000 poses, a wider scoring window of 500.0 Kcal/mol was used to keep initial poses, and the best 1000 poses per ligand were kept for minimization. The best docked pose obtained from docking simulations was analyzed.