Supporting information for

Real-time FO-SPR monitoring of solid-phase DNAzyme cleavage activity for cutting-edge biosensing

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1. Material and Methods

1.1. FO-SPR experimental setup

A picture of the in-house developed FO-SPR experimental setup is presented in Figure S1 and was based on Lu et al. All optics are integrated on a computer-controlled robot system to automate the movement of two FO-SPR sensors. Two tungsten halogen light sources (HL-2000, Ocean optics, Dunedin, USA) are used and sent light through a bifurcated fiber (Ocean optics, Dunedin, USA) into the FO-SPR sensor, which is in contact with the bifurcated fiber using a connector. Upon reaching the FO-SPR sensor’s sensing tip, the SPR effect is created and reflected into the second part of the bifurcated fiber to a spectrometer.

### Table S1: Table with DNA sequences

<table>
<thead>
<tr>
<th>Sequence name</th>
<th>Length (nt)</th>
<th>Sequence 5' -&gt; 3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>FO-SPR oligo</td>
<td>33</td>
<td>HS-TTTTTTTTTTTGAGGGATTATAGTAT<strong>CAGCACAA</strong></td>
</tr>
<tr>
<td>AuNP oligo</td>
<td>23</td>
<td>Phos-<strong>CCrGrUCACCAACC</strong>TTTTTTTTT-TSH</td>
</tr>
<tr>
<td>Template</td>
<td>24</td>
<td>TTGGTGACCGGGTTGCTGATACTA</td>
</tr>
<tr>
<td>DNAzyme</td>
<td>35</td>
<td>CGGTTGGTGAGGCTAGCTACAACGAGGTTGCTG</td>
</tr>
<tr>
<td>Inhibitor</td>
<td>52</td>
<td><strong>CAGCGCAACCTCGTTG</strong>ATCAACGCTCGTCTCCCTCAGTA<strong>GCCTACCAACCC</strong></td>
</tr>
<tr>
<td>Target</td>
<td>43</td>
<td>GGTAAGGGCTACTGAGGAGGAGCGAGCGATCAACGAGGTTG</td>
</tr>
<tr>
<td>Non-matching ssDNA</td>
<td>44</td>
<td>CATTCTTGCCCATCTACATTACCTCCTCGCACCACCCCCAGGT</td>
</tr>
</tbody>
</table>

**Note**
1. Bold region is bound by the DNAzyme
2. Underlined region forms the catalytic core. Regions left and right from the catalytic core are called the substrate arms.
3. DNAzyme and substrate sequences are based on Bone et al. (2014).
(USB4000, Ocean Optics, Dunedin, USA). The resulting SPR signal is monitored by a LabVIEW program and the reaction temperatures were controlled by a T1 thermocycler (Biometra, Goettingen, Germany).

1.2. FO-SPR high resolution melting (HRM)

The HRM was performed by incubation of the functionalized FO-SPR sensor in a ligation mix containing 1× Ampligase buffer, 100 nM of ligation template, 2.32 nM functionalized AuNPs in a final volume of 100 µL, but without any Ampligase. Prior to melting, the ligation template, FO-SPR- and AuNP-probe were hybridized for 20 min at 50 °C. Thereafter, the temperature was increased up to 95 °C (ramp speed = 0.1 °C/s) and the melting temperature \(T_m\) was determined as the maximal value of the temperature.
derivative of the FO-SPR sensogram. The melting peaks were fitted with a 1-term Gaussian function using Matlab 2015b.

1.3. Gel electrophoresis

All experiments were performed with a NovexTM XCell SureLock® Mini-Cell The Thermo Fisher Scientific group – Life Technologies (Merelbeke, Belgium), using precasted non-denaturating PAGE gels of 10%. While the Mini Cell was put on ice, the gels were run in 1x TBE buffer with 10 mM MgCl2 for 100 min at 180 V. A first experiment studied different DNAzyme-inhibitor strand ratios, therefore containing a single stranded DNAzyme and inhibitor control (300 nM), followed by a 1:1, 1.2:1, 1.4:1, 1.6:1, 1.8:1 and 2:1 ratio of inhibitor-DNAzyme strand with a fixed 150 nM DNAzyme concentration. In a second experiment, potential target detection was considered based on a single stranded target, inhibitor and DNAzyme control (300 nM), followed by 0, 50, 100, 200 and 400nM ssDNA target concentrations and an inhibitor-target complex control (150-150 nM). Samples were prepared as described in the sections ‘DNAzyme inhibition’ and ‘Target detection’ of the article in 100 µl of which 15 µl was loaded onto the gels with 3 µl of loading dye.

2. Results

2.1. Determination lower melting temperature ligation complex

Since ligation is a temperature dependent process, this parameter was considered during the optimization of the ligation efficiency. The Ampligase requires a third DNA strand or template strand to line up the FO-SPR- and AuNP-probe next to each other, enabling ligation by removing the nick and creating a phosphodiester bond. This alignment relies on simple hybridization with both the FO-SPR- and AuNP-probe, which have a complementarity with the ligation template of 14 and 10 nucleotides respectively.
The optimal ligation temperature is therefore restricted by the lower $T_m$ of the AuNP-probe and was determined by HRM on the FO-SPR sensor. This resulted in an average $T_m$ of $65.05 \pm 0.25$ °C (Figure S2).

![Graph showing FO-SPR high resolution melting. The lower $T_m$ of the ligation complex was determined based on 4 experiments.](image-url)
2.2. SEM images and AuNP count

Figure S3: High resolution SEM images of 4 different FO-SPR sensors after ligation with 0, 10, 100 and 1000 nM ligation template.
Figure S4: AuNP density determined for 0, 10, 100 and 1000 nM template concentrations. For every concentration, three SEM images were obtained on the same optical fiber. Error bars represent the intrafiber variability of one standard deviation (n = 3).

Figure S5: High resolution SEM image of a FO-SPR sensors after 30 min of incubation with the negative control: 0 nM DNAzyme.
2.3. FO-SPR characterization of DNAzyme activity

Cleavage of the AuNP-labelled substrate strands by the DNAzyme releases AuNPs into solution, causing the FO-SPR signal to shift towards lower wavelengths. This FO-SPR shift was monitored in real-time and determined as the difference between the baselines acquired before and after incubation of the FO-SPR sensor with different concentrations of DNAzyme (Figure S7A). In addition, DNAzyme efficiencies were expressed as the ratio of DNAzyme and ligation shifts, obtained for every DNAzyme concentration that
was studied (Figure S7B). Taking advantage of the intrinsic real-time monitoring feature of the described FO-SPR sensor, half-life values were determined as kinetic parameter to evaluate the different DNAzyme concentrations (Figure S8).

Figure S7: A) DNAzyme shifts determined for 0, 0.1, 1, 3.125, 6.25, 12.5, 25, 50 and 100 nM DNAzyme.

B) DNAzyme efficiencies determined as the ratio of DNAzyme and ligation shifts. Error bars represent one standard deviation (n = 3).
Figure S8: Half-life values for 0, 1, 3.125, 6.25, 12.5, 25, 50 and 100 nM DNAzyme. Values were determined based on the DNAzyme incubation step. Error bars represent one standard deviation (n = 3).

2.4. Inhibitor-DNAzyme complex illustration

Figure S9: Illustration of the inhibitor-DNAzyme complex. Top: inhibitor with internal loop and bottom: DNAzyme with the underlined core and the G-T wobble structure in red.
2.5. Visualization of blocked DNAzyme activity by gel electrophoresis

Blocking of the DNAzyme was qualitatively confirmed by gel electrophoresis, comparing different ratios of inhibitor and DNAzyme with two controls. In Figure S10 the first two lanes of the gel contained single stranded control sequences of the DNAzyme and inhibitor respectively. In the following six lanes, different ratios of inhibitor and DNAzyme were loaded after a 30 min hybridization step at 55 °C with a fixed DNAzyme concentration of 150 nM. For the lower inhibitor-DNAzyme ratios 1:1 and 1.2:1, no bands were observed at the level of both controls, but a new band at higher position was observed. A higher band indicates a longer sequence or complex of sequences, which in this case could only be the inhibitor-DNAzyme complex. As the inhibitor-DNAzyme ratio was increased, a second band was observed at the level of the single stranded inhibitor control. This was expected, since the DNAzyme concentration was kept constant, creating an excess of the inhibitor from a 1.2:1 ratio onwards and reaching a visual concentration for all ratios ≥ 1.4.

![Figure S10: Gel electrophoresis of different inhibitor and DNAzyme ratios. From left to right: a DNAzyme control (300 nM); an inhibitor control (300 nM); and increasing ratios of inhibitor and DNAzyme from 1:1 – 2:1 with fixed 150 nM DNAzyme concentration.](image)
2.6. Visualization of target recognition and DNAzyme release by gel electrophoresis

Once the DNAzyme could be blocked, target specific release was also evaluated qualitatively with gel electrophoresis. Figure S11 presents the obtained results, starting with three lanes containing single stranded controls of the target, inhibitor and DNAzyme respectively. Lane 4-9 contained a negative control followed by four different target concentrations and a positive control. As expected, the highest single stranded band was observed for the inhibitor (52 nt), followed by the target (43 nt) and the DNAzyme (35 nt). The band of the negative control was observed at a higher position than the single stranded controls as earlier, however also above the positive control, which has a higher total complementarity of 10 nt compared to the negative control. It was hypothesized that the internal loop of 22 nt in the DNAzyme-inhibitor complex caused slower migration through the gel than the linear hybridization between target and inhibitor. Once target was present (lane 5-8), a band appeared at the level of the positive control, illustrating the preferential binding of the target to the inhibitor and release of the blocked DNAzyme.

Figure S11: Gel electrophoresis of different target concentrations. From left to right: a target control (300 nM); an inhibitor control (300 nM); a DNAzyme control (300 nM); increasing concentrations of target (nM), incubated with a 1.4:1 ratio of inhibitor (180 nM) and DNAzyme (150 nM); and a 1:1 inhibitor-target complex control (150 nM).
This was confirmed by the fact that no bands appeared at the level of the single stranded inhibitor control. Furthermore, for the higher target concentrations the majority of blocked DNAzymes were released and a band was observed at the level of the single stranded DNAzyme control. For the highest target concentration of 400 nM a third band was observed at the position of the single stranded target control as there was a big excess of target compared to inhibitor strand.

2.7. Proof-of-concept detection of a synthetic DNA strand

Target detection with the FO-SPR sensor and specificity were established by first incubating the DNAzyme-inhibitor complex with the target or non-matching ssDNA for 30 min, releasing blocked DNAzyme molecules. Next, the FO-SPR sensor was used to detect the released DNAzymes, corresponding to a certain target concentration. FO-SPR shifts, DNAzyme efficiencies and half-life values were determined as

![Graph](image)

Figure S12: Top panel: DNAzyme shifts determined for corresponding target concentrations of 0, 10, 12.5, 15, 20, 25, 30 nM and a blank containing 30 nM scrambled ssDNA (red); bottom panel: DNAzyme efficiencies determined as the ratio of DNAzyme and ligation shifts. Error bars represent one standard deviation (n = 3).
described earlier but presented in function of the target concentration: 0, 10, 12.5, 20, 25 and 30 nM (Figure S12 and S13).

Figure S13: Half-life values for corresponding target concentrations of 0, 10, 12.5, 20, 25 and 30 nM. Values were determined based on the DNAzyme incubation step. Error bars represent one standard deviation (n = 3).

REFERENCES
