# A new biosensor-based assay for aptamer-protein interaction analysis

Keywords: Aptamers | G-quadruplex | Thrombin | DNA-protein interaction

**Here we show how aptamer-protein interactions can be analyzed with DNA-based biosensors. To this end, we study the salt-dependence and sequence and structure specificity of the well-characterized interaction of thrombin with its aptamer (TBA) using a simple and quick switchSENSE® assay.**

**The automated workflow with standard consumables can be used to study an extensive range of aptamer-target interactions in a DRX² analyzer.**

## Background

Nucleic acid aptamers are single-stranded oligonucleotides engineered to specifically recognize a wide range of targets, including small molecules, ions, proteins, and even viruses and bacteria (Banerjee and Nilsen-Hamilton, 2013). Notably, many aptamers that recognize biologically relevant protein targets, can fold into G-quadruplex structures. G-quadruplexes are four-stranded DNA or RNA structures rich in guanine residues. The thrombin binding aptamer (TBA) is a DNA aptamer with high specificity and affinity for thrombin (Bock *et al.*, 1992). The folding into a G-quadruplex and its binding to thrombin are known to be strongly dependent on the type and concentration of ions present in solution (Bhattacharyya D. *et al.*, 2016). Here, we employ a simple switchSENSE® assay for the determination of kinetic rates and affinities for the TBA-thrombin interaction in the presence of different ions. The switchSENSE® technology reveals very fast kinetics and affinities in the pM range with minimal sample consumption.

## Methods

All experiments were performed on a DRX² instrument on standard multipurpose switchSENSE® chips (order MPC2-48-G1R1-S). The experimental workflow was set up using the switchBUILD software (v. 1.11.0.1, Dynamic Biosensors), as visualized in Figure 1. The aptamer sequence elongated by the cNLB sequence was immobilized on the chip by hybridization to the complementary ssDNA (NLB) on the sensor surface ①. Table 1 lists the DNA sequences used here. After chip functionalization, human-alpha-thrombin (HTI) was injected for 30 s at 6 different concentrations between 0 and 10 nM, followed by dissociation phases of 600 s consisting of injections of running buffer. The measurements were performed with a flow rate of 2000 µL/min at 25°C in TE140 buffer (10 mM Tris, pH 7.4, 140 mM XCl, 0.05% Tween20, 50 µM EDTA, 50 µM EGTA, with XCl representing different salt species: KCl, NaCl, $NH_4Cl$, LiCl). The kinetic data was analyzed with the switchANALYSIS software (v. 1.7.0.34, Dynamic Biosensors) by fitting the fluorescence of all association and dissociation curves of one data set simultaneously (global fit) with a single-exponential fit model.

**Table 1** | DNA sequences.

| | | |
|---|---|---|
| TBA† | 5′ | GGT TGG TGT GGT TGG TTT-cNLB 3′ |
| deadTBA† | 5′ | GTG TGG TGT GTG TGG TTT-cNLB 3′ |
| HTA† | 5′ | TTT GGG TTA GGG TTA GGG TTA GGG-cNLB 3′ |
| cNLB | 5′ | ATC AGC GTT CGA TGC TTC CGA CTA ATC AGC CAT ATC AGC TTA CGA CTA 3′ |

† ligand sequences are flanked by $A_3$ or $A_5$ to minimize "end effects" (stacking interactions with bound analyte)

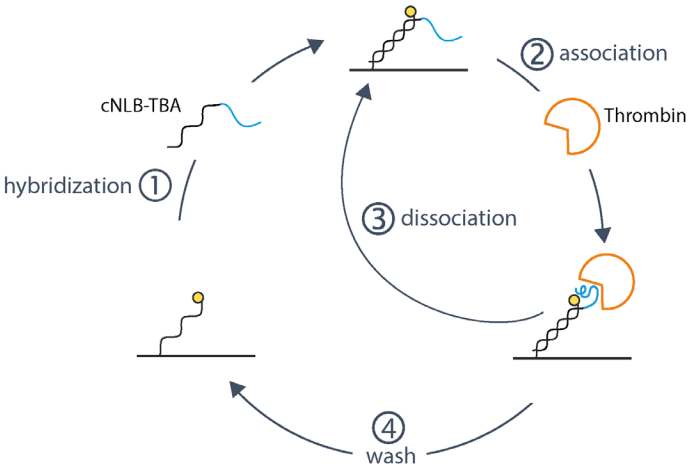


**Figure 1** | Measurement workflow. ① Sensor functionalization with TBA: covalently attached single-stranded DNAs on the measurement electrode are functionalized by hybridizing complementary DNA with a TBA overhang (cNLB-TBA) to the surface. ② Association of thrombin. ③ Dissociation of thrombin. ④ Regeneration: the cNLB-TBA is removed from the sensor surface by denaturing the DNA nanolevers at basic pH. The sensor is now ready for the next measurement cycle. To resolve kinetic rates, the cycle is repeated several times at increasing concentrations of analyte.

## Results

First, we determined the kinetic rates of thrombin binding to TBA and compared the influence of four different ions on the interaction. The workflow shown in Figure 1 was used for all the measurements. The association of thrombin to TBA was observed by a concentration-dependent fluorescence decrease. Correspondingly, dissociation of thrombin resulted in a fluorescence increase back to starting levels. Figure 2 shows the real-time concentration dependent association and dissociation curves of the interaction in the presence of $Li^+$, $Na^+$, $K^+$ and $NH_4^+$.

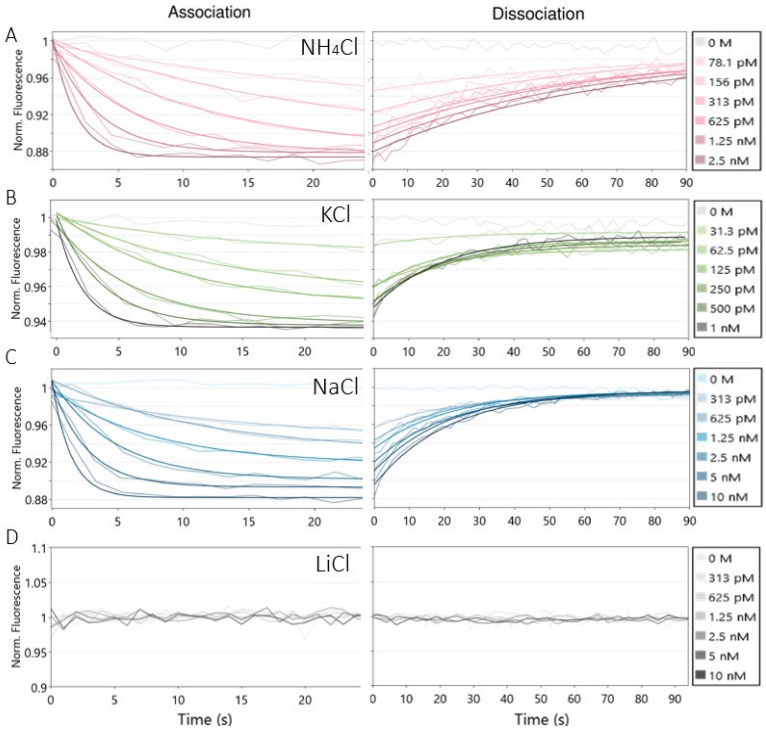


**Figure 2** | Fluorescence signal and fits during association and dissociation phases of thrombin to immobilized TBA carried out in: A) TE140-$NH_4Cl$; B) TE140-KCl; C) TE140-NaCl; D) TE140-LiCl.

The effect of the presence of $Li^+$ ions is controversial (Bhattacharyya *et al.*, 2016), and in the present study no interaction was observed in the presence of up to a concentration of 10 nM. In the presence of KCl, NaCl and $NH_4Cl$, single-exponential binding behavior was observed as expected for a one-to-one-interaction. Global fitting of the curves yielded very fast on-rates ($10^8$ $M^{-1}s^{-1}$) and $K_D$s in the picomolar range, as listed in Table 2 and visualized in the rate plot in Figure 3. The resolution of the extremely fast kinetics without artifacts from rebinding or mass-transport limitation were facilitated by the high flow rate (2000 µl/min) and a low surface density of approximately 400 ligands/µm² (≈ 20 RU), unique features of the **switch**SENSE® technology.

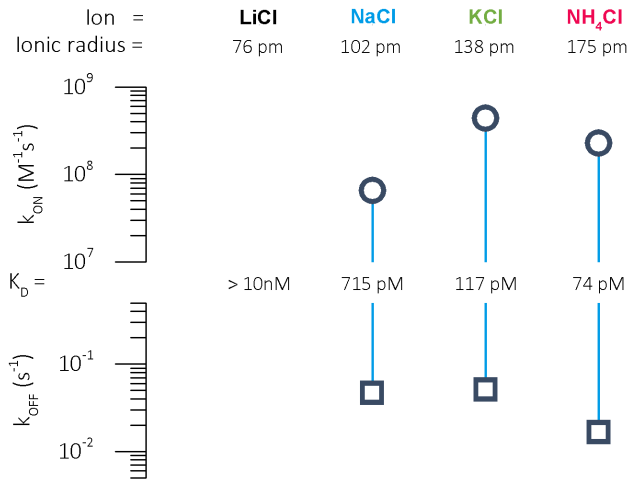


**Figure 3** | Rate plot. Note the increase of affinity (lower $K_D$) correlating with ion size.

For the two physiologically relevant ions $K^+$ and $Na^+$, we found that the affinity in the presence of $Na^+$ is lower than in the presence of $K^+$. This is in good agreement with literature. As shown by Joachimi *et al.*, $K^+$ ions stabilize the folding of TBA into a G-quadruplex and thus facilitate thrombin binding (Joachimi *et al.*, 2009). In contrast, high NaCl concentrations are known to decrease the affinity of the interaction, since $Na^+$ shields the thrombin binding site (Lin *et al.*, 2011). Here, we showed that the affinity

**Table 2** | Summary of thrombin-TBA kinetic parameters in indicated buffers.

| Buffer | $k_{ON}$ ( $10^7$ $M^{-1}s^{-1}$) | $k_{OFF}$ ($10^{-2}$ $s^{-1}$) | $K_D$ (pM) |
|---|---|---|---|
| TE140-$NH_4Cl$ | 22.3 ± 1.2 | 1.66 ± 0.03 | 74 ± 4 |
| TE140-KCl | 4.40 ± 0.24 | 5.13 ± 0.19 | 117 ± 7 |
| TE140-NaCl | 6.54 ± 0.27 | 4.68 ± 0.05 | 715 ± 31 |
| TE140-LiCl | - | - | >> 10 000 |

decrease is mainly caused by a slower on-rate in the presence of $Na^+$. The increase of affinity from $Li^+$ (no binding), over $Na^+$ ($K_D$= 715 pM) to $K^+$ ($K_D$ = 117 pM) seems to be correlated with ion size. To test whether thrombin binding is also facilitated by even bigger cations, we also analyzed the kinetics in the presence of ammonium ions. Interestingly, we found that the affinity is in the same range as in presence of $K^+$, but with slightly lower on- and off-rates.

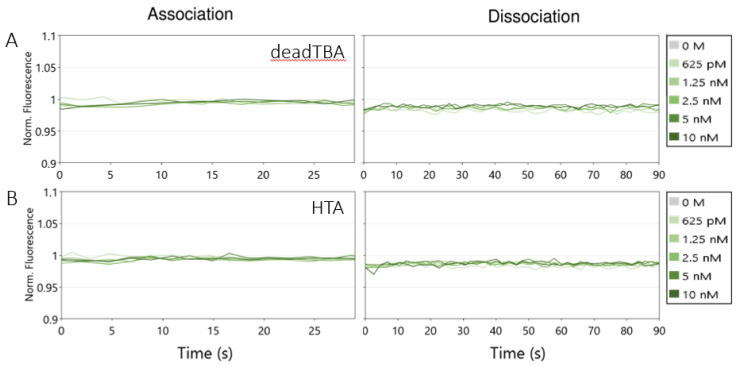


**Figure 4** | Association and dissociation of thrombin in TE140-KCl to/from: A) immobilized deadTBA. B) immobilized HTA. In these conditions, no folding and/or binding was observed.

To study the sequence and structure specificity of the thrombin-aptamer interaction, we used two control sequences: a second G-quadruplex-forming aptamer (Human Telomeric repeat Aptamer, "HTA") and a sequence that does not fold into a G-quadruplex structure ("deadTBA"). For the latter, the TBA sequence was modified by shuffling the bases to disrupt multiple guanines in a row, thereby preventing the formation of the G-quadruplex structure. As shown in Fig. 4, we could not observe any association of deadTBA with thrombin, indicating that folding of the G-quadruplex structure is important for thrombin binding. However, we neither observed thrombin binding to HTA, which is known to form a G-quadruplex structure. This finding underlines not only the structure- but also sequence-specificity of the thrombin-binding aptamer.

## Conclusions

In conclusion, we have developed a functional G-quadruplex-DNA biochip for aptamer-based assays. With this assay the interaction between thrombin and TBA was characterized, determining their binding affinity and kinetics in different buffer conditions. We showed that it is possible to easily modulate the affinity between TBA and thrombin by changing the salts in the running buffer, and that this interaction is highly sequence and structure specific. This **switch**SENSE® assay can serve as a versatile tool to characterize protein-DNA interaction with high sensitivity and specificity.

## References


Banerjee J. and Nilsen-Hamilton M. (2013). Aptamers: multifunctional molecules for biomedical research. J. Mol. Med. *91*:1333-1342.

Bhattacharyya D. *et al.* (2016). Metal cations in G-quadruplex folding and stability. Front Chem *4*:38.

Bock L.C. *et al.* (1992). Selection of single-stranded DNA molecules that bind and inhibit human thrombin. Nature *355*:564–566.

Lin P. *et al.* (2011). Studies of the binding mechanism between aptamers and thrombin by circular dichroism, surface plasmon resonance and isothermal calorimetry. Elsevier, Colloids and Surfaces B: Biointerfaces *88*:552-558.

Joachimi A. *et al.* (2009). A comparison of DNA and RNA quadruplex structures and stabilities. Elsevier, Bioorganic and Medicinal Chemistry *17*:6811-6815.



Authors: Irene Ponzo, Friederike Möller, Nena Matscheko

Contact: info@dynamic-biosensors.com


February 2019


**Dynamic Biosensors GmbH**
Lochhamer Str. 15
82152 Martinsried / Planegg
Germany