Supporting Material

Nanopore force spectroscopy of aptamer-ligand complexes

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Note S1: Universality of results obtained at different loading rates

In Figure S 1, the voltage - dependent lifetime of ATP aptamer – ligand complexes determined from 13 individual experiments is shown. The data obtained at loading rates from 0.5 V/s to 15 V/s all collapse on a single master curve, verifying that the measurements were performed in quasi-equilibrium conditions (1).

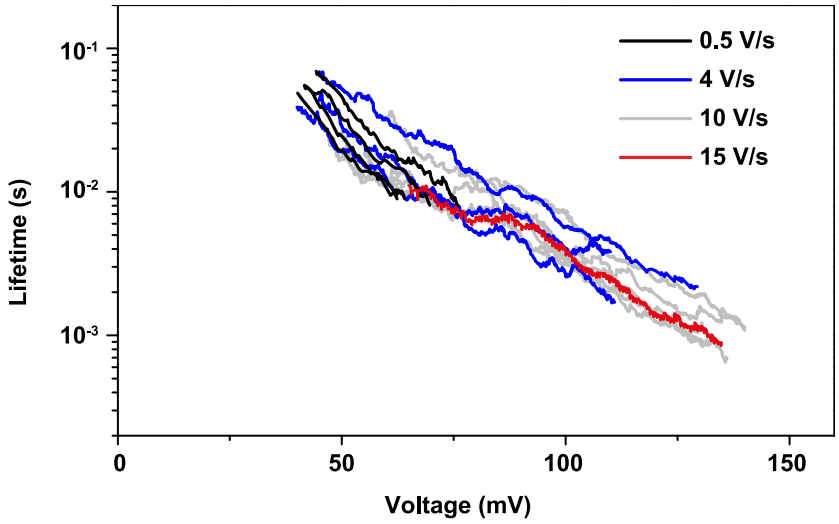

Figure S 1 Voltage dependent lifetime of ATP aptamer - ligand complexes obtained at different loading rates. Each line represents one individual measurement. An ATP concentration of 1 mM was used.

Note S2: Aptamer binding to CTP

We performed control experiments in presence of 1 mM CTP. The results are shown in Figure S 2. As expected, no bound population is observed.

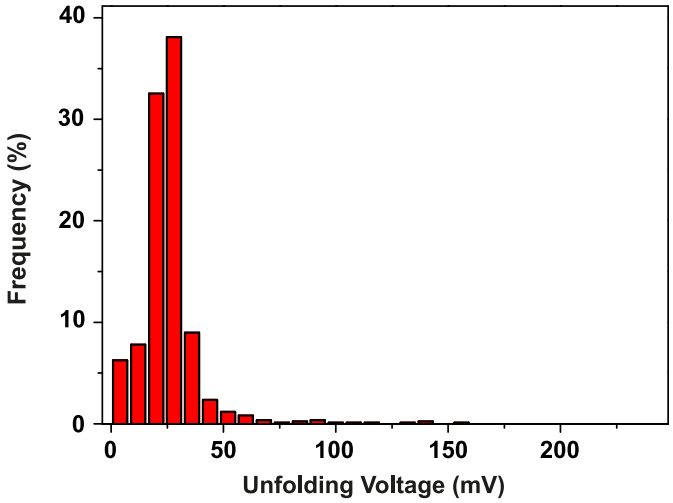

Figure S 2 ATP aptamer unfolding in presence of 1 mM CTP, obtained at a loading rate of 10 V/s.

Note S3: Dissociation constant of the stabilized aptamer structure ATPapt-mod

In Figure S 3, the binding curve of the stabilized ATP aptamer structure is shown. We obtained a dissociation constant of 116 ± 16 µM, which is virtually identical to the value obtained for the conventional aptamer structure ATPapt.

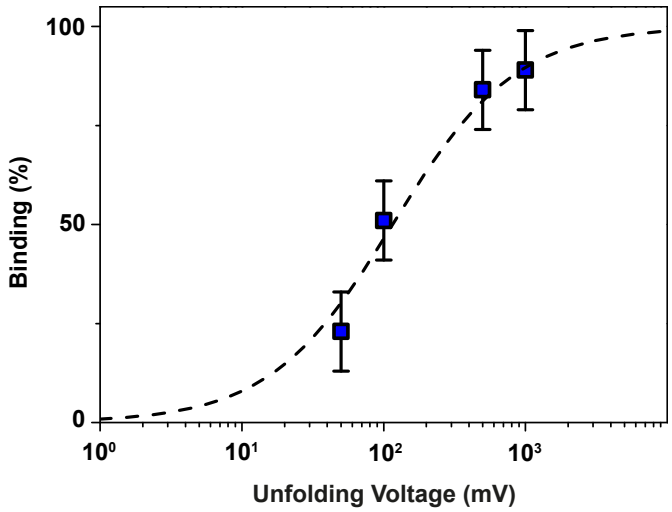

Figure S 3 Fraction of bound aptamer structures vs. ATP concentration for the stabilized aptamer structure ATPapt-mod. The dashed line represents a fit to the hill equation. The fit yields a dissociation constant of 116 ± 16 µM.

Note S4: Thrombin Aptamer Unfolding – Measurement Principle

For the thrombin aptamer was studied using a conventional cis -> trans translocation approach (see Figure S 4). It is captured into the pore at a bias potential of 120 mV by a $(dT)_{50}$ thread on its 5' end. The sudden decrease in trans-pore current upon entry of DNA is detected by an analog circuit, which triggers the patch-clamp amplifier to start a voltage ramp from 50mV to 250mV. The unfolding of the aptamer structure is detected by a sudden increase in current. The corresponding unfolding voltage is recorded.

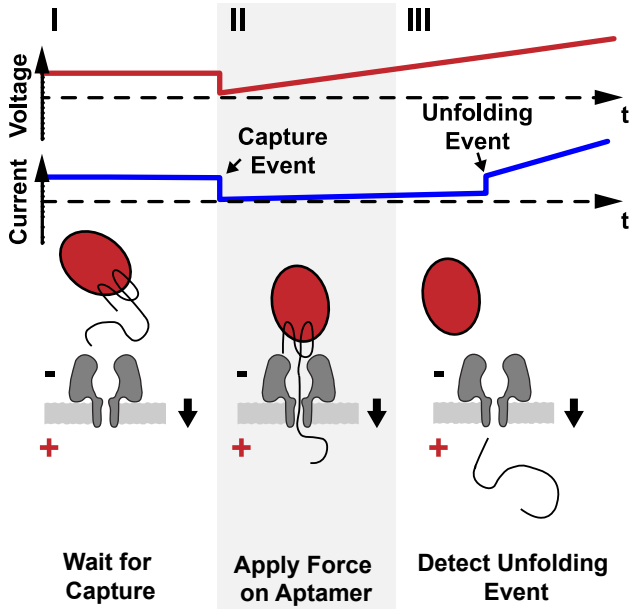

Figure S 4 Measurement Principle. I) A potential of 120 mV is applied to capture an aptamer - ligand complex. Upon detection of a capture event, the potential immediately is switched to 50mV to hold the aptamer structure inside the pore and II) a voltage ramp to 250 mV is started to exert an increasing force on the complex. III) When the aptamer structure unfolds, the DNA molecule escapes from the pore and a sudden increase in the current signal is observed.

Note S5: Stability of the thrombin aptamer structure as a function of potassium concentration

The thrombin binding aptamer structure is known to form a G-quadruplex in solution, which is stabilized by monovalent cations localized between the two G-quartets of the structure (2). The quadruplex stability was found to be strongly dependent on the cation type (3). In the presence of 100 mM KCl and NaCl, melting temperatures of 51 ± 1 °C and 23 ± 1 °C were found, respectively. Thus, only the K^+ binding form may be observed in our present studies which where carried out at 37° C. In another study, the quadruplex stability was characterized as a function of overall ionic strength in KCl solution using AFM force spectroscopy and a strong dependence was found (4). Here, we characterized the stability of the G-quadruplex forming thrombin aptamer structure as a function of $[K^+]$, maintaining the overall ionic strength at 1M by adding NaCl. We performed NFS experiments in presence of 1 M, 100 mM and 10 mM KCl. The obtained lifetime (τ) vs. voltage (V) curves are shown in Figure S 5. The data was fit to an exponential function of the form $\tau = \tau_0 e^{V/V_\beta}$ (5). All three curves share the same value for the global fit parameter V_β (48 mV), while the extrapolated lifetime at zero voltage τ_0

was fitted for every curve individually. We observed a strong decrease in quadruplex stability towards lower potassium concentrations. The extrapolated lifetime at zero voltage decreased from 3.5 ± 1.1 s at 1M KCl to 0.5 ± 1.1 s at 10 mM KCl concentration. As the debye length was maintained at ≈ 0.3 nm in our measurements, this effect may not be explained by a weaker electrostatic shielding at low KCl concentrations.

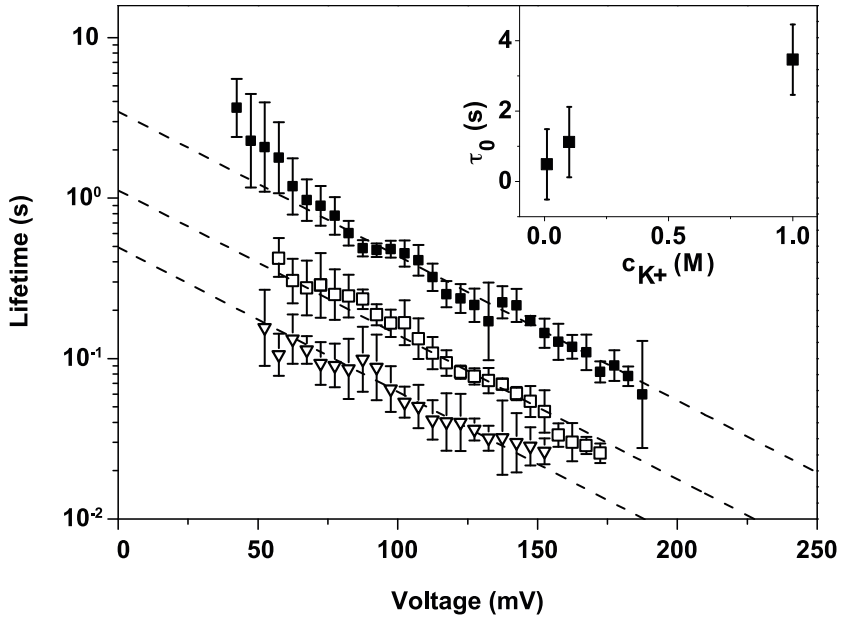

Figure S 5 Thrombin aptamer stability as function of potassium concentration. Voltage dependent lifetime of structures at 1M (full squares), 100 mM (open squares) and 10 mM KCl concentration (open triangles). The overall ionic strength was maintained at 1 M by adding NaCl. Error bars are standard deviations from 5, 3 and 4 independent measurements, respectively. Dashed lines represent mono-exponential fits to the data. Inset: Extrapolated lifetime at zero voltage τ_0 determined from fits as a function of potassium concentration. Error bars are standard deviations obtained from fits.

Note S6: Thrombin detection at elevated KCl concentration.

In addition to the thrombin aptamer unfolding experiments shown in Fig. 6 in the main paper, we performed experiments where we increased the KCl concentration from 10 mM to 100 mM KCl (see Figure S 6). In the presence of thrombin, the observed stability is comparable to the 10 mM case, which is in contrast to the increasing stability of the unbound aptamer structure. Therefore, the stability difference of the bound and unbound case diminishes towards higher KCl concentrations.

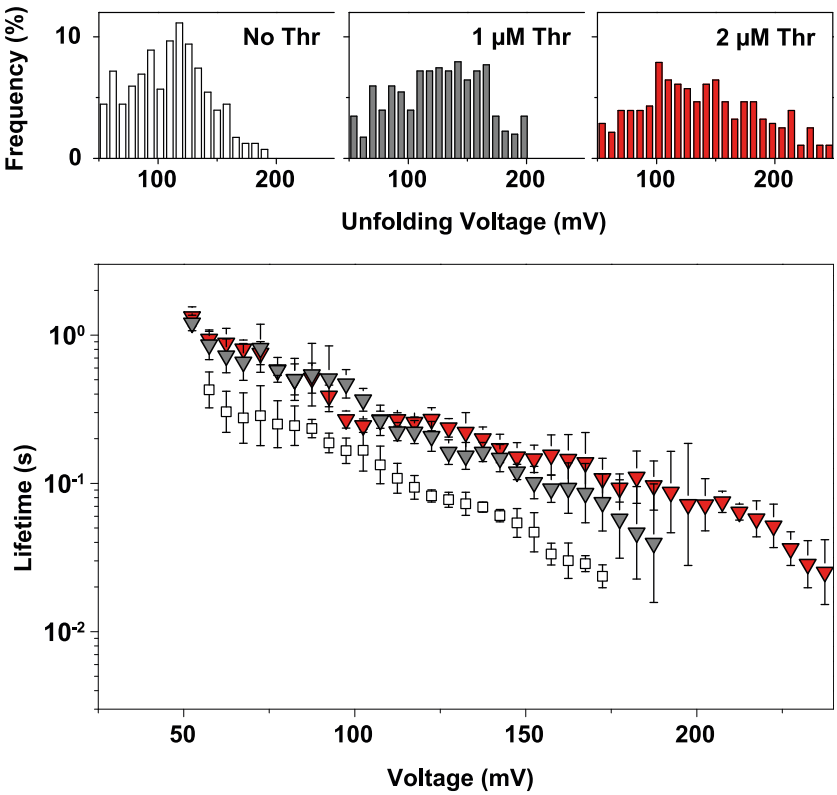

Figure S 6 Thrombin aptamer - ligand complex unfolding at 100 mM K^+ concentration. Top panel: Typical unfolding voltage distributions without (open bars) and with (full bars) addition of thrombin at different concentrations (grey: 1 µM, red: 2 µM). Experiments were performed at a loading rate of 0.2 V/s. Bottom Panel: Voltage dependent lifetime of aptamer structures (open squares) and aptamer target complexes (full triangles, grey: 1 µM, red: 3 µM thrombin) at a potassium concentration of 100 mM. Error bars are standard deviations from 3 independent measurements.

Note S7: Separability of bound/unbound populations for the thrombin aptamer

We characterized the bound population of the thrombin aptamer – ligand complex at high thrombin concentrations, were virtually all aptamers should be bound (cf. Fig. 6). To check whether bound and unbound populations can be separated at intermediate thrombin concentrations, we used Eq. 2 (see main paper) to calculate the unfolding voltage distribution for a bound fraction of 50% (see Figure S 7). The parameters $V_{\beta i}$ and τ_{0i} were determined from single exponential fits to the lifetime vs. voltage curves in presence and absence of thrombin, respectively. The obtained curve shows, that bound and unbound populations overlap strongly and may not be difficult to separate from experimental distributions.

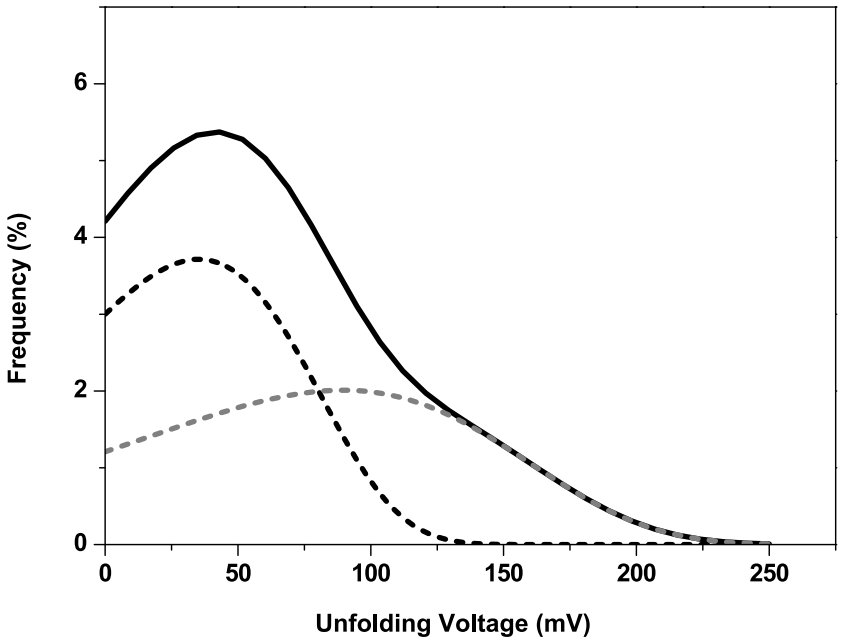

Figure S 7 Simulated unfolding voltage distribution for the thrombin aptamer - ligand complex at a ligand-bound fraction of 50% (solid line). Individual subpopulations are shown as dashed lines (black: unbound, grey: ligand-bound). Curves were calculated from Eq. 2 in the main paper, using V_β and t_0 values that were determined from single exponential fits to the voltage-dependent lifetime in absence and presence of thrombin, respectively (see Fig. 6).

Supporting References

1. Dudko, O. K., J. Mathé, A. Szabo, A. Meller, and G. Hummer. 2007. Extracting Kinetics from Single-Molecule Force Spectroscopy: Nanopore Unzipping of DNA Hairpins. Biophysical Journal 92:4188-4195.
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4. Lynch, S., H. Baker, S. G. Byker, D. Zhou, and K. Sinniah. 2009. Single molecule force spectroscopy on G-quadruplex DNA. Chemistry 15:8113-8116.
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