Ligand-modulated folding of the full-length adenine riboswitch probed by NMR and single-molecule FRET spectroscopy

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Supplementary Tables

Supplementary Table S1. PCR amplified DNA templates used for *in vitro* transcription (written in 5' to 3' direction) [a]

^{112}Asw DNA wt	TAATACGACTCACTATAGGGAGA-TGAAGCCTGATGAGAGCGAAAGC TCGAAACAGCTGTGAAGCTGTC-GCTTCATATAATCCTAATGATATGG TTTGGGAGTTTCTACCAAGAGCCTTAAACTCTTGATTATGAAGTCTGT CGCTTTATCCGAAATTTTATAAAGAGAAGACTCATGAAT
^{112}Asw DNA apoB$_{STAB}$	TAATACGACTCACTATAGGGAGA-ATGGCGCCTGATGAGAGCGAAAG CTCGAAACAGCTGTGAAGCTGTC-GCGCCATATAATCCTAATGATATG GTTTGGGCGTTTCTACCAAGAGCCTTAAACTCTTGATTATGAAGTCT GTCGCTTTATCCGAAATTTTATAAAGAGAAGACTCATGAAT
Forward Primer	TAATACGACTCACTATAGG
Reverse Primer	ATTCATGAGTCTTCTCTTTAT

[a] T7 promoter and hammerhead ribozyme sequences are depicted in grey; mutated residues are underlined

Supplementary Table S2. Oligonucleotide sequences used for DNA splinted enzymatic RNA ligation (written in 5' to 3' direction) [a]

^{112}Asw RNA Fragment 1, wt	GCUUCAUAUAAUCCUAAUGAUA(5-N-U)GGUUUGGGAGUUUCUAC
^{112}Asw RNA Fragment 1, apoB$_{STAB}$	GCGCCAUAUAAUCCUAAUGAUA(5-N-U)GGUUUGGGCGUUUCUAC
^{112}Asw RNA Fragment 1, apoA$_{STAB}$	GCUUCAUAUAAUCCUCGUGAUA(5-N-U)GGUCGGGGAGUUUCUAC
^{112}Asw RNA Fragment 2	p-CAAGAGCC(5-N-U)UAAACUCUUGAUUAUGAAGUCUGUC
^{112}Asw RNA Fragment 3	p-GCUU(5-N-U)AUCCGAAAUUUUAUAAAGAGAAGACUCAUGAAU-bi
DNA Splint	ATTCATGAGTCTTCTCTTTATAAAATTTCGGATAAAGCGACAGACTTC ATAATCAAGAGTTTAAGGCTCTTGGTAGAAACTCCCAAACCATATCAT TAGGATTATATGAAGC

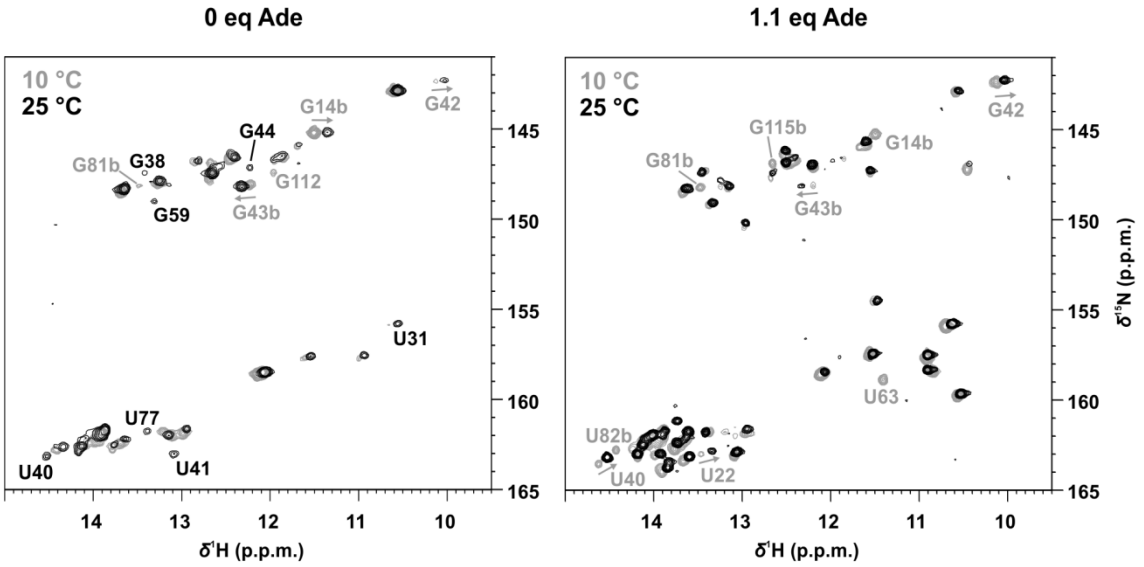
[a] Mutated residues are underlined; abbreviations: 5-amino allyl modified uridine (5-N-U), 5'-phosphate (p), 3'-biotin linker (bi)

Supplementary Table S3. Bulk fluorescence anisotropies of Cy3 (Ex/Em = 525 nm / 565 nm) and Cy5 (Ex/Em = 625 nm / 665 nm) in the free form and in the RNA-coupled form at the selected labelling sites of the full-length 112-nucleotide *add* Asw [a]

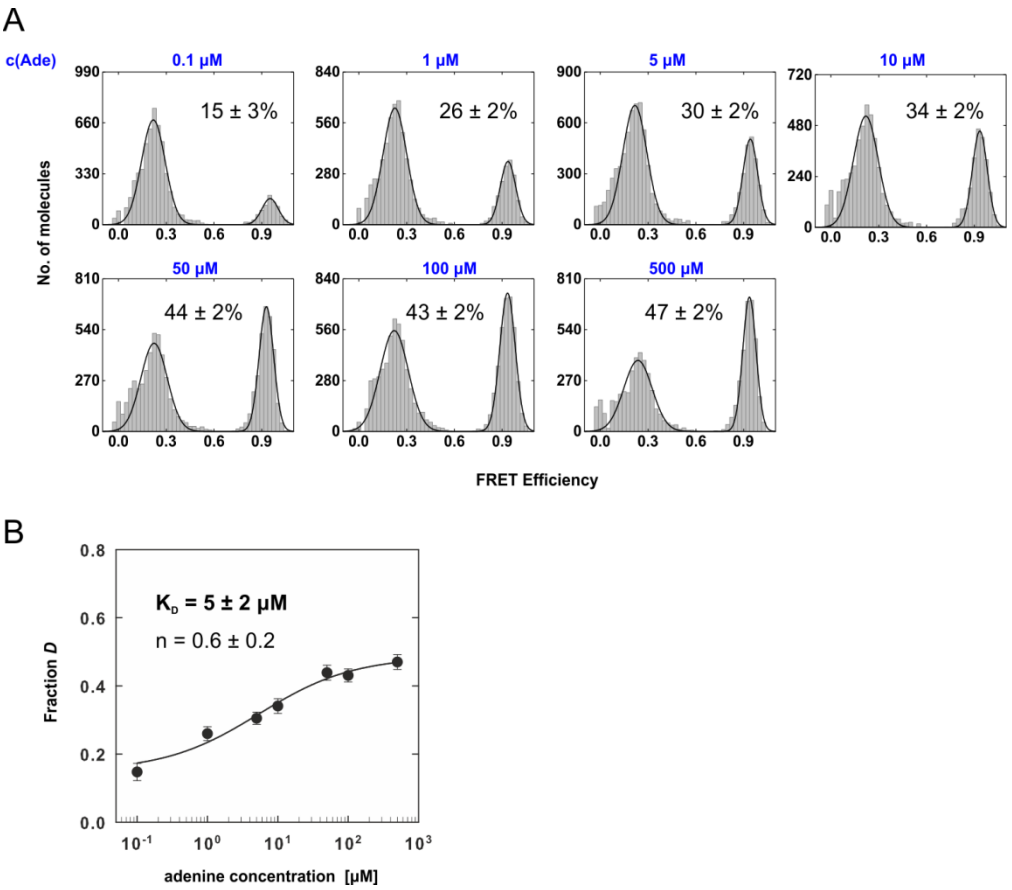
Construct	r
Cy3 free	0.244 ± 0.006
^{112}Asw L2(Cy3)	0.285 ± 0.004
^{112}Asw P5(Cy3)	0.296 ± 0.004
Cy5 free	0.127 ± 0.004
^{112}Asw L3(Cy5)	0.238 ± 0.009
^{112}Asw P5(Cy5)	0.281 ± 0.008

[a] The fluorescence anisotropy *r* was measured at a concentration of ~4 nM single-fluorophore-labelled riboswitch in smFRET immobilization buffer (25 mM K_2HPO_4/KH_2PO_4, 50 mM KCl, pH 7.0) with 2 mM Mg^{2+} at 20 °C. The errors are standard errors obtained over 20 replicates.

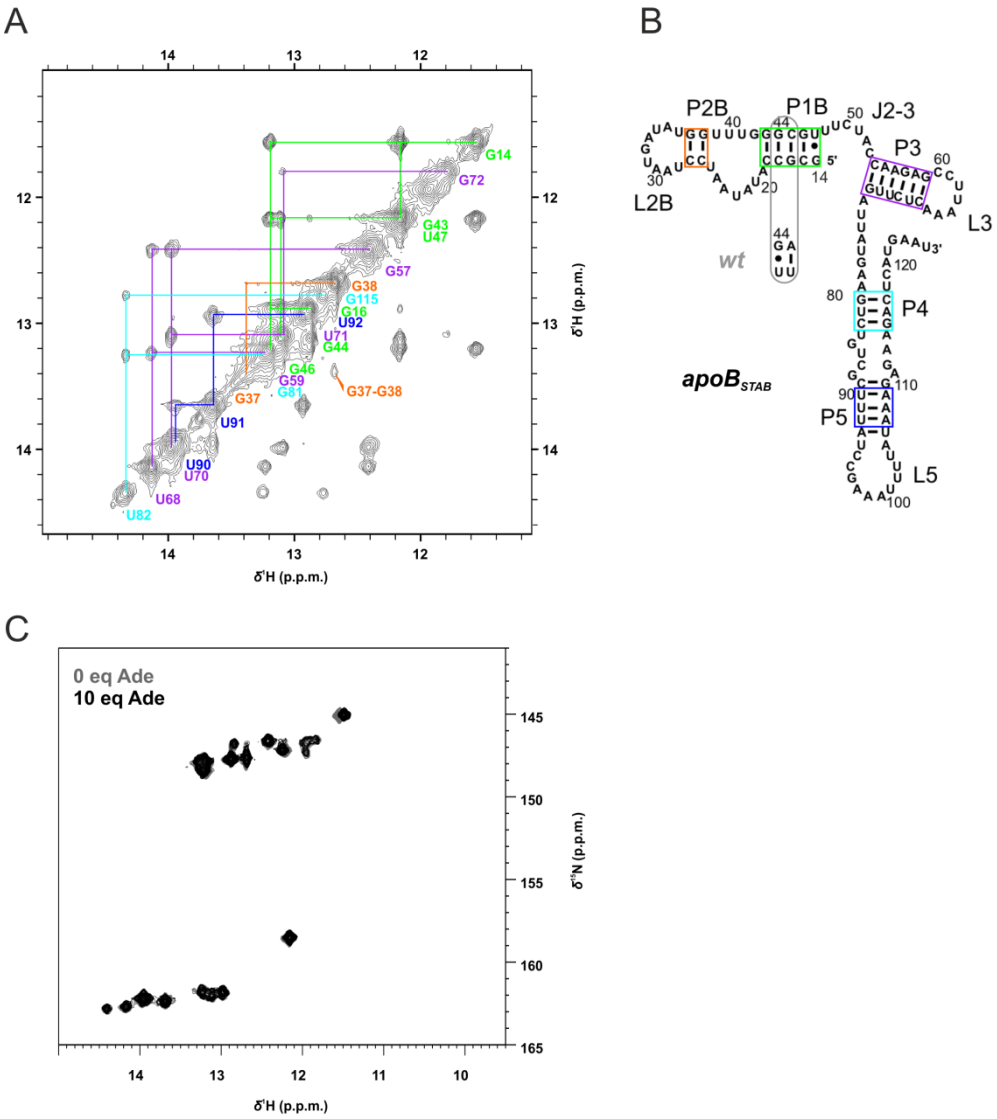
Supplementary Figures



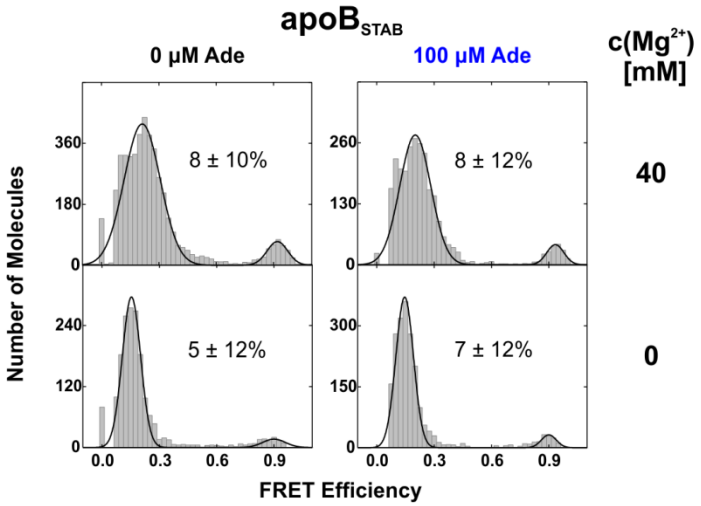
Supplementary Figure S1. Overlay of the imino region of the ^{1}H,^{15}N-BEST-TROSY spectrum of the *add* Asw measured at 25 °C (black) with the assigned ^{1}H,^{15}N-HSQC spectrum measured at 10 °C (grey) without and with adenine in presence of 5 mM Mg^{2+}. Assigned signals that are missing or shifted beyond linewidth at 25 °C are annotated in grey. The missing signals G81b, U82b, G115b and G112 indicate a destabilization of base pairing in the P4 helix. Assigned signals that appear at 25 °C are annotated in black. U31, G38, U40, U41, G44, G59 and U77 indicate pre-folding of the apoA aptamer. The ^{1}H,^{15}N-HSQC spectra and their assignment at 10 °C were adapted from Reining et al. (2)



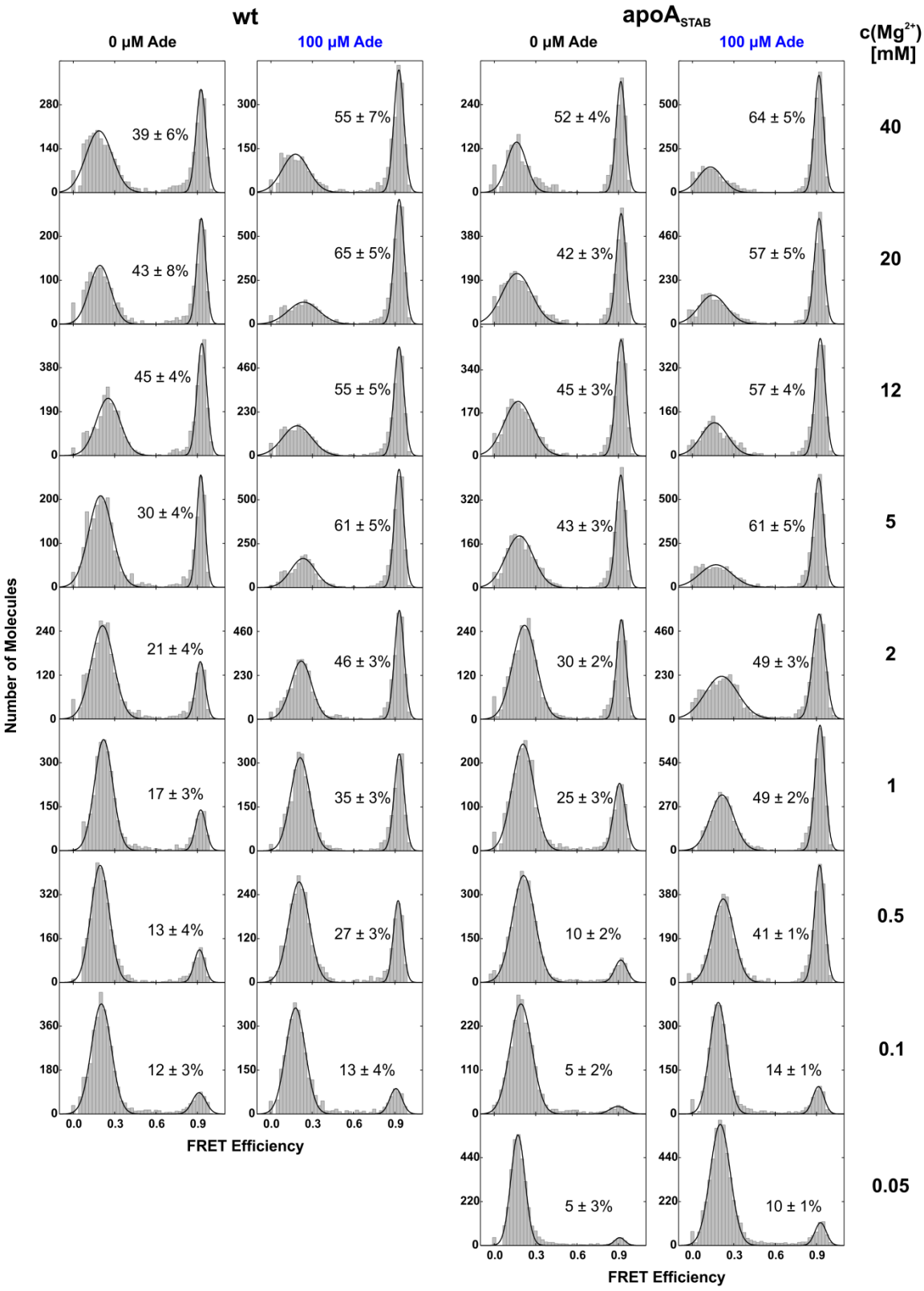
Supplementary Figure S2. (**A**) smFRET histogram analysis of adenine-dependent aptamer docking of the L2,L3-labeled full-length *add* Asw at 2 mM Mg^{2+}. The fractional population of the docked (high-FRET) state is indicated in percent. (**B**) Plot of the fractional population of the docked state determined from the FRET histograms shown in (A) as a function of the adenine concentration. The data have been fitted using the Hill equation to obtain the half-saturating adenine concentration for aptamer docking (K_D) and the Hill coefficient (n).



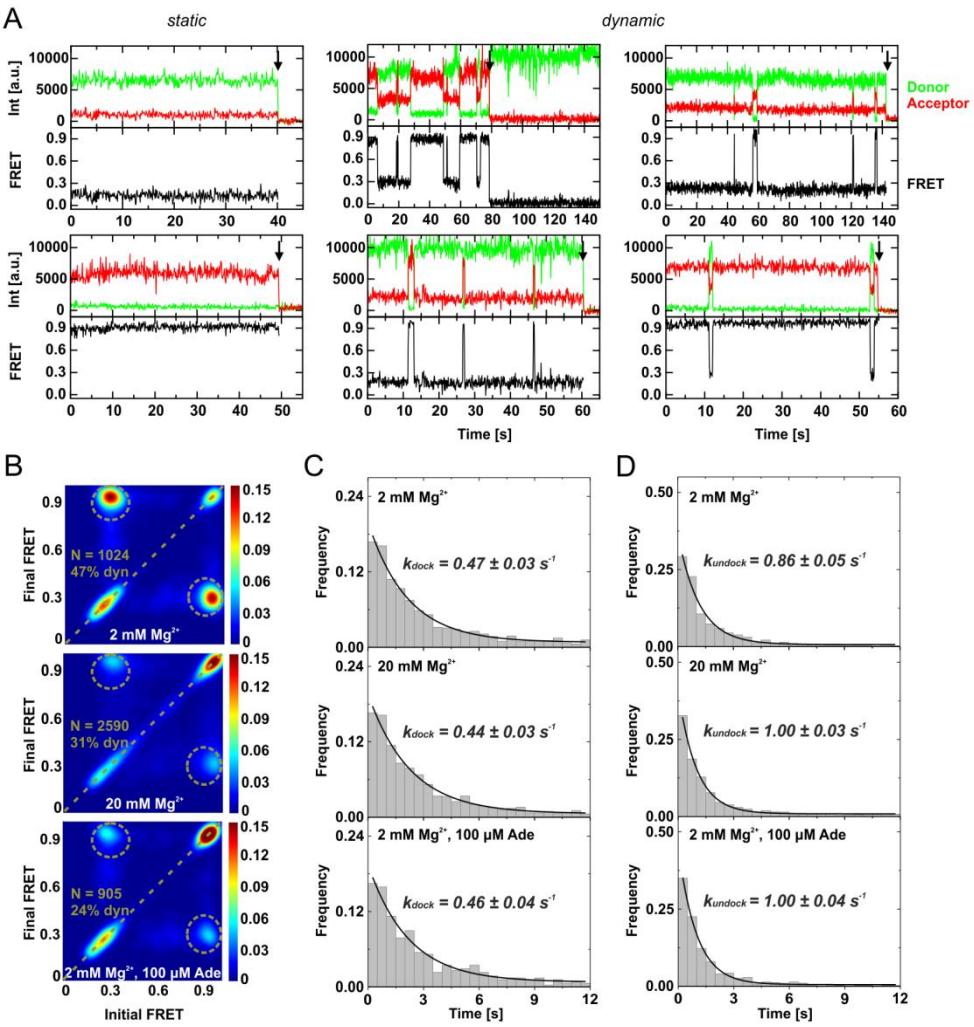
Supplementary Figure S3. (**A**) Imino region of the ^{1}H,^{1}H-NOESY spectrum of the apoB stabilized mutant apoB$_{STAB}$ of the *add* Asw (0.5 mM) without adenine and Mg^{2+} acquired at 900 MHz and 10 °C. Helices P1b, P2b, P3, P4 and P5 are indicated in green, orange, purple, cyan and blue, respectively. (**B**) Secondary structure model of apoB$_{STAB}$. Mutated base pairs are highlighted in comparison to the wildtype. (**C**) Overlay of the imino region of the ^{1}H,^{15}N-HSQC spectra of apoB$_{STAB}$ (0.3 mM) without (grey) and with 10 eq adenine (black) in presence of 5 mM Mg^{2+} measured at 600 MHz and 10 °C. The spectra are superimposable and show that apoB$_{STAB}$ exhibits no adenine dependent base pairing structure.



Supplementary Figure S4. smFRET histogram analysis of Mg^{2+}-dependent aptamer docking of L2,L3-labeled apoB_{STAB} in absence and presence of 100 µM adenine. The fractional population of the docked (high-FRET) state is indicated in percent.



Supplementary Figure S5. smFRET histogram analysis of Mg^{2+}-dependent aptamer docking of L2,L3-labeled wt and apoA_{STAB} in absence and presence of 100 µM adenine. The fractional population of the docked (high-FRET) state is indicated in percent.



Supplementary Figure S6. Mg^{2+}- and adenine-dependent aptamer docking dynamics of the apoA-stabilized-mutant $apoA_{STAB}$ of the full-length *add* Asw analyzed by smFRET. (**A**) Representative smFRET time traces of L2/L3-labeled $apoA_{STAB}$ collected at 2 mM Mg^{2+} without adenine. The aptamer domain of single riboswitches either statically remained in a long-lived undocked or docked state (left panel), or exhibited dynamics between short-lived and long-lived undocked and docked states (middle and right panel). Photobleaching events are indicated by a black arrow. (**B**) Transition occupancy density plots (TODPs) for smFRET traces collected at 2 mM Mg^{2+}, at 20 mM Mg^{2+} and at 2 mM Mg^{2+} with 100 µM adenine. The fraction of molecules that exhibited dynamics (*dyn*) is indicated in percent. N indicates the number of traces included in each TODP. (**C**) Dwelltime histograms of the undocked state created from the dynamic smFRET traces designated in the corresponding TODP in (B). The data were fitted using single-exponential decay functions to extract the indicated docking rate constants. (**D**) Dwelltime histograms of the docked state created from the dynamic smFRET traces designated in the corresponding TODP in (B). The data were fitted using single-exponential decay functions to extract the indicated undocking rate constants.