Supporting Information for:

Mg^{2+} Shifts Ligand-Mediated Folding of a Riboswitch from Induced-Fit to Conformational Selection**

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Supporting Note 1

Assessing the accuracy of our Φ-values. Since Φ-values are sensitive to the free energies $\Delta\Delta G^{\ddagger}_{dock}$ and $\Delta\Delta G^{0}_{dock}$, which are in turn calculated from a ratio of the individual rate constants, it is important to make accurate kinetic measurements for use in smTSA analysis. Therefore, we performed each experiment in three independent replicates to minimize the errors in the rate constants obtained by exponential fitting of docking and undocking dwell times. A minimum of 70 smFRET traces showing multiple transitions were derived for each replicate experiment so that more than 250 dynamic traces were used to obtain the mean and standard deviation (S.D.) of each rate constant. As a result, the errors on the values of k^{∞}_{dock} and k^{∞}_{undock}obtained from non-linear curve fitting are typically ≤ 15 %. We performed error propagation analysis using the uncertainties of the rate constants k^{∞}_{undock} and k^{∞}_{dock} to derive the standard deviations reported for our Φ-values; we found that our modest rate constant errors only slightly influence the Φ-values and do not affect the conclusions of our analysis. Of note, due to the only subtle chemical differences between our chemical ligands, the changes of $\Delta\Delta G^{0}_{dock}$ are small (Table 1) compared to traditional (ensemble) TSA studies where residues are drastically mutated. Caution has thus to be exercised in applying smTSA to study riboswitch folding when the $\Delta\Delta G^{0}_{dock}$values are small (≤ 0.6 kcal/mol), and care should be taken to minimize the measurement errors, as done here. Notably, TSA is still applicable when done carefully, as shown for studying the coupled folding and binding of proteins, even with values of $\Delta\Delta G^{0}_{fold}$ < 0.2 kcal/mol.[1]

Supporting Tables

Table S1

Ligand	$K_{1/2}$ (nM)		k^0_{dock} (s^{-1})		k^∞_{dock} (s^{-1})	
	- Mg^{2+}	**+ Mg^{2+}**	**-Mg^{2+}**	**+ Mg^{2+}**	**- Mg^{2+}**	**+ Mg^{2+}**
$preQ_1$	280±60	44.4±28.3	0	1.20±0.13	3.46±0.36	3.44±0.14
$preQ_0$	3138±25	446±253	0	0.54±0.07	3.28±0.01	2.83±0.49
Guanine	3796±1791	3956±1157	0	0.43±0.04	1.72±0.50	2.10±0.18

Table S1. Parameters obtained from fitting the k_{dock} versus [L] data in the absence and presence of Mg^{2+} with eq. (1).

Table S2

Ligand	$K_{1/2}$ (nM)		k^∞_{undock} (s^{-1})	
	- Mg^{2+}	**+ Mg^{2+}**	**- Mg^{2+} [i]**	**+ Mg^{2+}**
$preQ_1$	NA	0.53±0.06	0.50±0.11	0.41±0.01
$preQ_0$	NA	1.26±0.27	1.06±0.17	0.42±0.04
Guanine	NA	5.01±0.38	2.38±0.13	0.47±0.02

Table S2. Parameters obtained from fitting the k_{undock} versus [L] data in the absence and presence of Mg^{2+} with eq. (2). NA, not applicable. [i]Represents the average value of k_{undock} for all [L] values.

Supporting Figures

Figure S1

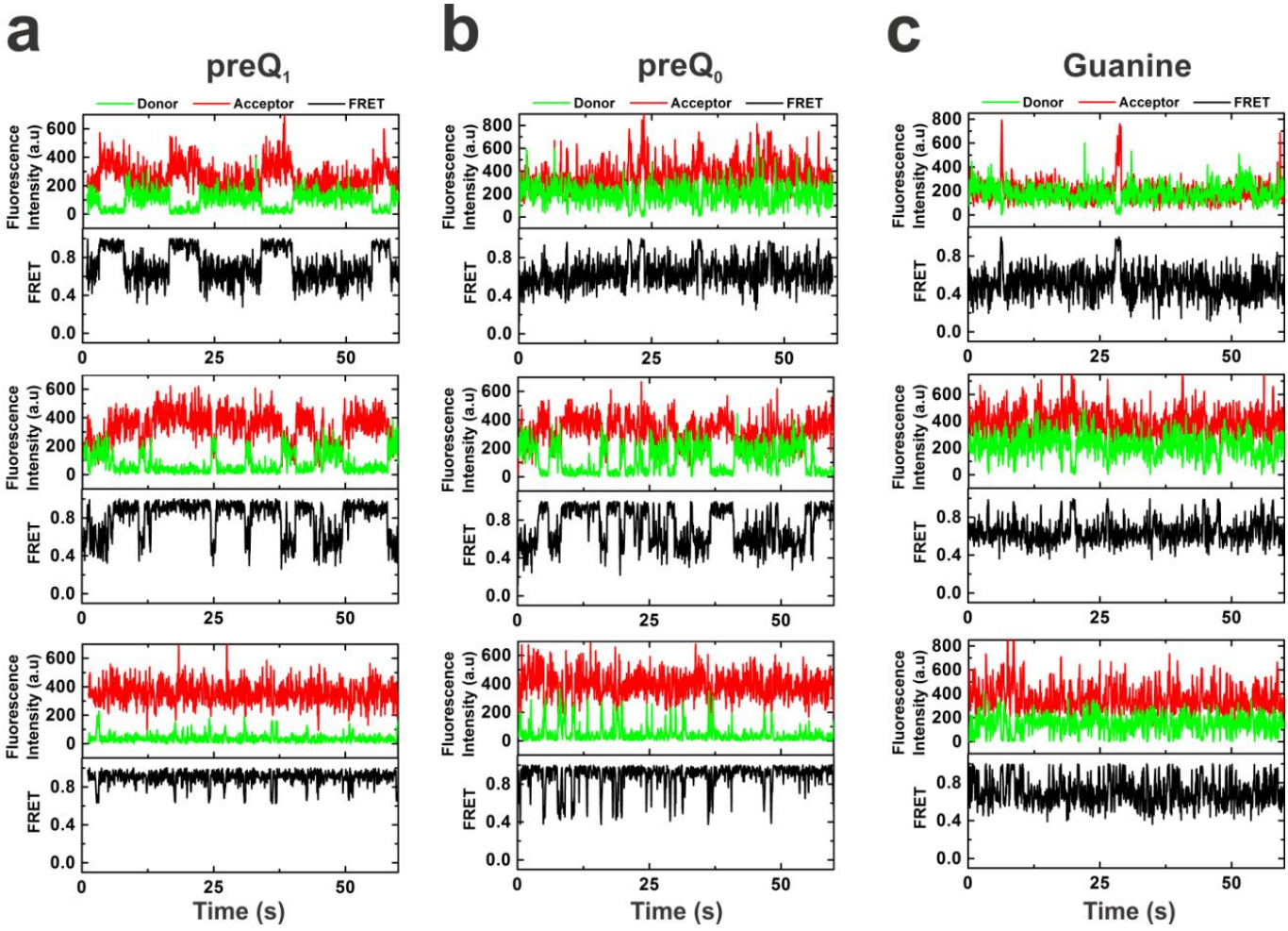

Figure S1. Representative raw smFRET traces for the *Bsu* aptamer in the absence of Mg^{2+}
Exemplary time traces showing anti-correlation between the donor (green) and acceptor (red) fluorescence intensities and the transitions in the FRET ratio (black) at three different concentrations for the ligands (a) $preQ_1$ (top to bottom: 50 nM, 250 nM, 1 µM) (b) $preQ_0$ (top to bottom: 250 nM, 1 µM, 10 µM) and (c) guanine (top to bottom: 500 nM, 1 µM, 10 µM) in 1× smFRET buffer without Mg^{2+}. The corresponding HMM-idealized smFRET traces are shown in Figure 2.

Figure S2

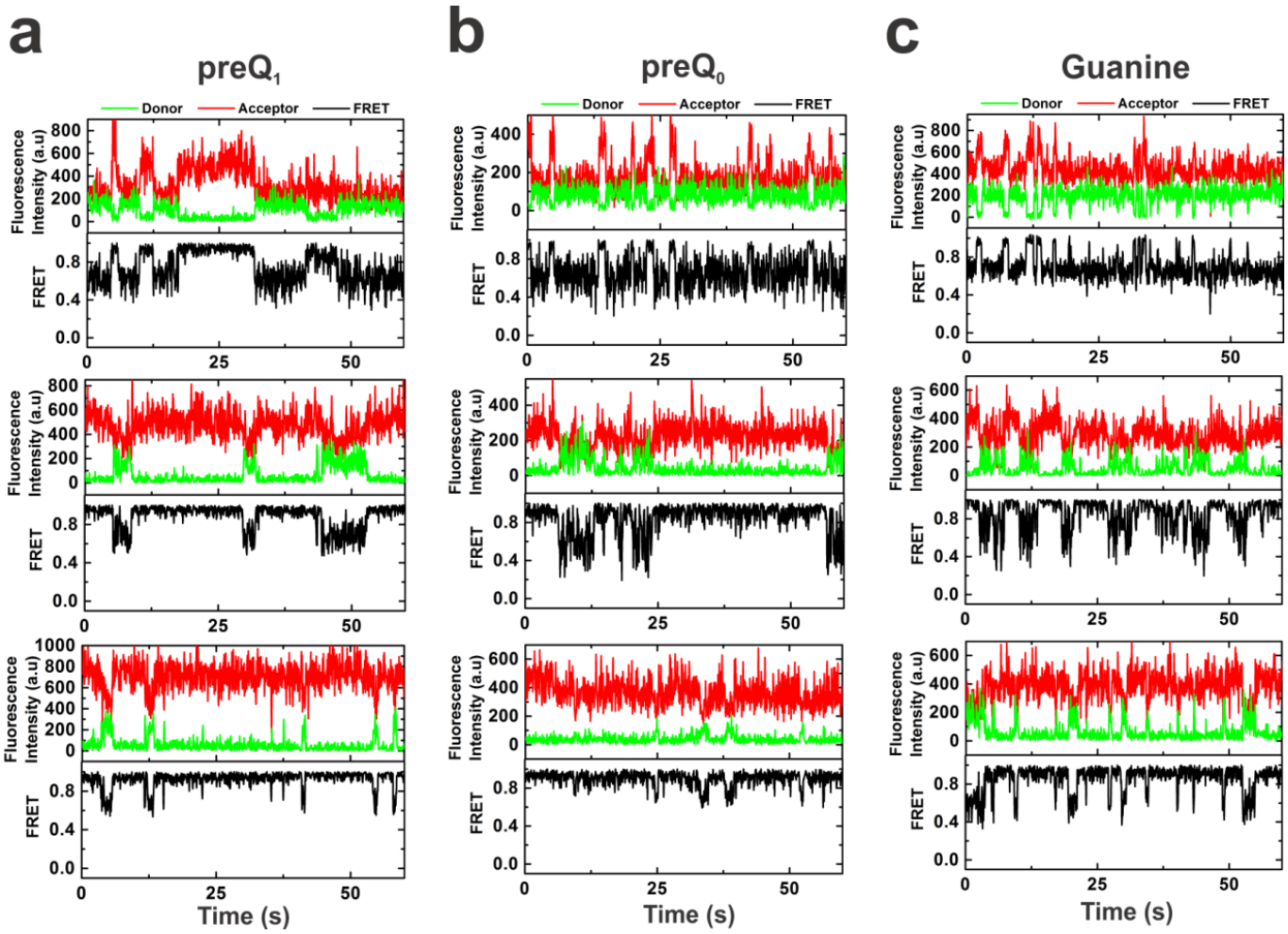

Figure S2. Representative raw smFRET traces for the *Bsu* aptamer in the presence of Mg^{2+}
Exemplary time traces showing anti-correlation between the donor (green) and acceptor (red) fluorescence intensities and the transitions in the FRET ratio (black) are shown at three different concentrations for the ligands (a) $preQ_1$ (top to bottom: 10 nM, 100 nM, 1 µM) (b) $preQ_0$ (top to bottom: 25 nM, 250 nM, 1 µM) and (c) guanine (top to bottom: 50 nM, 500 nM, 10 µM) in 1x smFRET buffer with 1 mM Mg^{2+}. The corresponding HMM idealized smFRET traces are shown in Figure 3.

Figure S3

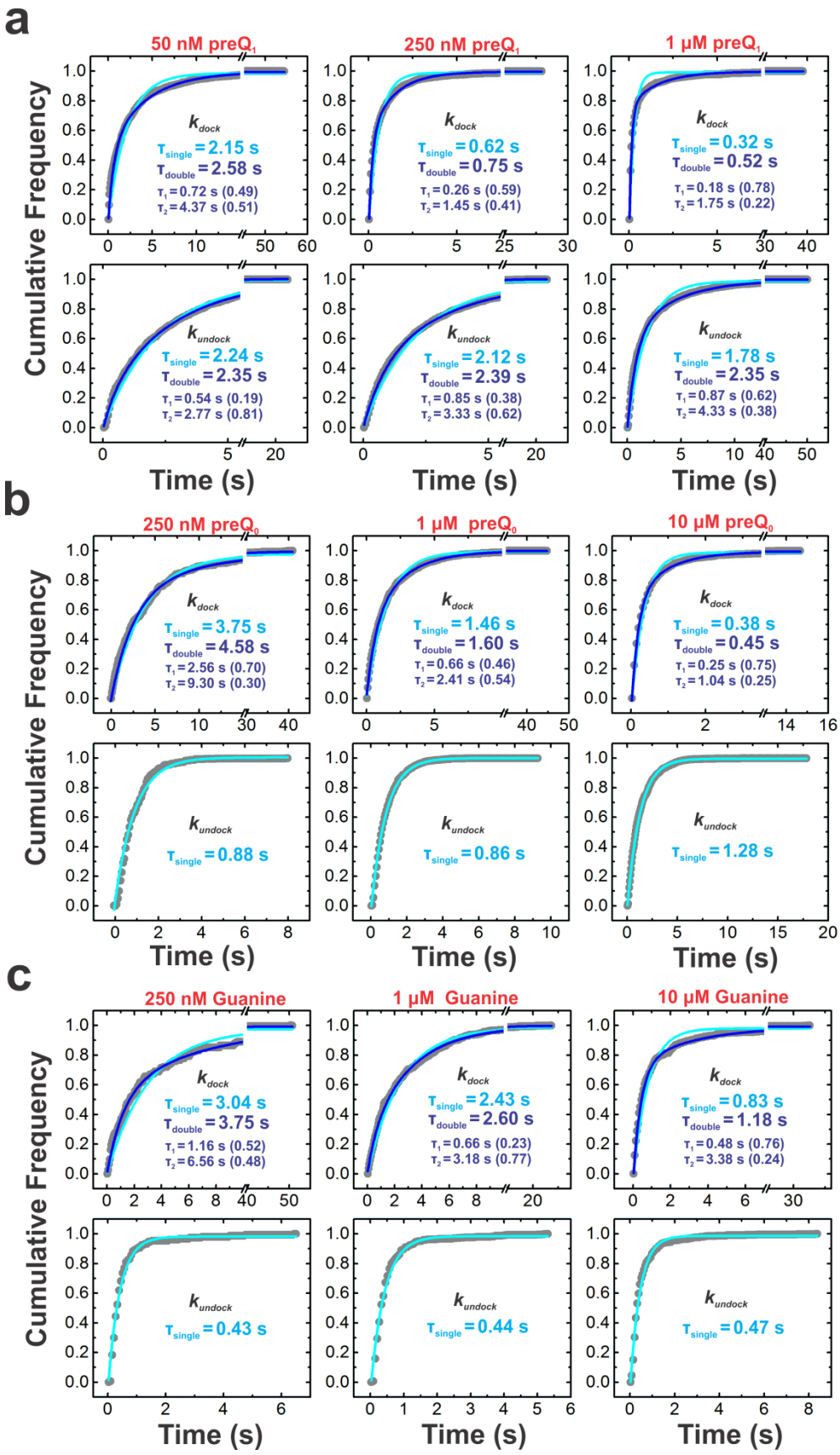
a
50 nM preQ1
250 nM preQ1
1 μM preQ1
k dock
T single = 2.15 s
T double = 2.58 s
τ1 = 0.72 s (0.49)
τ2 = 4.37 s (0.51)
T single = 0.62 s
T double = 0.75 s
τ1 = 0.26 s (0.59)
τ2 = 1.45 s (0.41)
T single = 0.32 s
T double = 0.52 s
τ1 = 0.18 s (0.78)
τ2 = 1.75 s (0.22)
k undock
T single = 2.24 s
T double = 2.35 s
τ1 = 0.54 s (0.19)
τ2 = 2.77 s (0.81)
T single = 2.12 s
T double = 2.39 s
τ1 = 0.85 s (0.38)
τ2 = 3.33 s (0.62)
T single = 1.78 s
T double = 2.35 s
τ1 = 0.87 s (0.62)
τ2 = 4.33 s (0.38)
Cumulative Frequency
Time (s)
b
250 nM preQ0
1 μM preQ0
10 μM preQ0
T single = 3.75 s
T double = 4.58 s
τ1 = 2.56 s (0.70)
τ2 = 9.30 s (0.30)
T single = 1.46 s
T double = 1.60 s
τ1 = 0.66 s (0.46)
τ2 = 2.41 s (0.54)
T single = 0.38 s
T double = 0.45 s
τ1 = 0.25 s (0.75)
τ2 = 1.04 s (0.25)
T single = 0.88 s
T single = 0.86 s
T single = 1.28 s
c
250 nM Guanine
1 μM Guanine
10 μM Guanine
T single = 3.04 s
T double = 3.75 s
τ1 = 1.16 s (0.52)
τ2 = 6.56 s (0.48)
T single = 2.43 s
T double = 2.60 s
τ1 = 0.66 s (0.23)
τ2 = 3.18 s (0.77)
T single = 0.83 s
T double = 1.18 s
τ1 = 0.48 s (0.76)
τ2 = 3.38 s (0.24)
T single = 0.43 s
T single = 0.44 s
T single = 0.47 s

Figure S3. *Bsu* aptamer kinetics in the absence of Mg^{2+}

Representative cumulative dwell time distributions for three different concentrations of (a) $preQ_1$ (50 nM, 250 nM, 1 µM) (b) $preQ_0$ (250 nM, 1 µM, 10 µM) and (c) guanine (250 nM, 1 µM, 10 µM) in the absence of Mg^{2+}. The cumulative dwell-time distributions in the undocked and docked states were fit with single (cyan) and double (blue) exponential functions to obtain τ_{single} and τ_{double}, which were used to estimate the rate constants k_{dock} and k_{undock}, respectively. τ_{single} is the time constant for the single-exponential fit, whereas τ_{double} is the average time constant of the double-exponential fit calculated from the individual short (τ_1) and long (τ_2) time constants and their respective amplitudes as $A_1\tau_1 + A_2\tau_2$. Heterogeneity in the kinetic behavior, mainly in k_{dock}, was observed for all ligands whereas k_{undock} showed some heterogeneity only for $preQ_1$. Therefore, for guanine and $preQ_0$, cumulative distributions of dwell-times in the docked state had to be fit with single-exponential functions, as shown in the bottom panels of b and c.

Figure S4

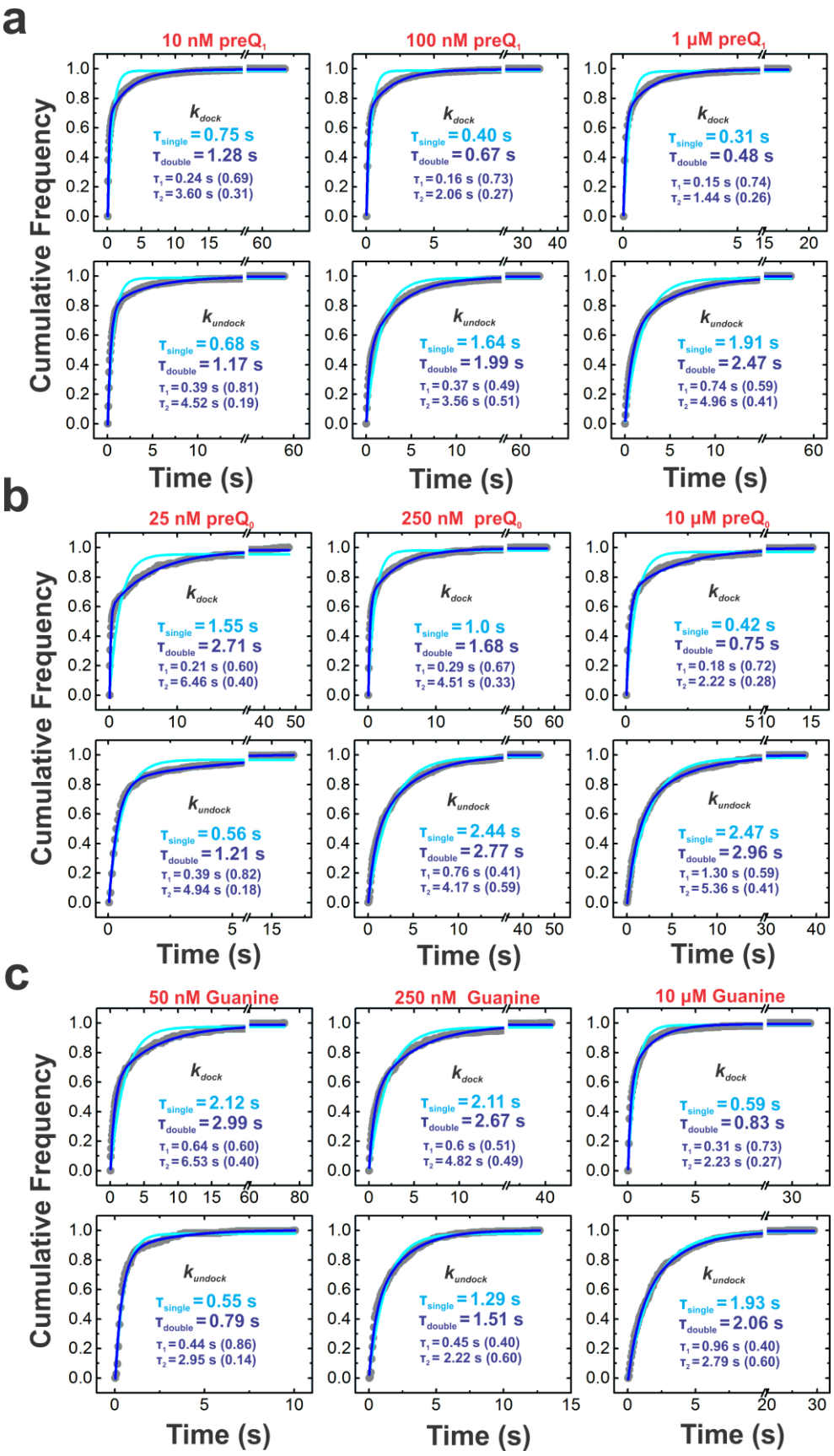
a
10 nM preQ1
100 nM preQ1
1 μM preQ1
Cumulative Frequency
k dock
τ single = 0.75 s
τ double = 1.28 s
τ1 = 0.24 s (0.69)
τ2 = 3.60 s (0.31)
τ single = 0.40 s
τ double = 0.67 s
τ1 = 0.16 s (0.73)
τ2 = 2.06 s (0.27)
τ single = 0.31 s
τ double = 0.48 s
τ1 = 0.15 s (0.74)
τ2 = 1.44 s (0.26)
k undock
τ single = 0.68 s
τ double = 1.17 s
τ1 = 0.39 s (0.81)
τ2 = 4.52 s (0.19)
τ single = 1.64 s
τ double = 1.99 s
τ1 = 0.37 s (0.49)
τ2 = 3.56 s (0.51)
τ single = 1.91 s
τ double = 2.47 s
τ1 = 0.74 s (0.59)
τ2 = 4.96 s (0.41)
Time (s)
b
25 nM preQ0
250 nM preQ0
10 μM preQ0
τ single = 1.55 s
τ double = 2.71 s
τ1 = 0.21 s (0.60)
τ2 = 6.46 s (0.40)
τ single = 1.0 s
τ double = 1.68 s
τ1 = 0.29 s (0.67)
τ2 = 4.51 s (0.33)
τ single = 0.42 s
τ double = 0.75 s
τ1 = 0.18 s (0.72)
τ2 = 2.22 s (0.28)
τ single = 0.56 s
τ double = 1.21 s
τ1 = 0.39 s (0.82)
τ2 = 4.94 s (0.18)
τ single = 2.44 s
τ double = 2.77 s
τ1 = 0.76 s (0.41)
τ2 = 4.17 s (0.59)
τ single = 2.47 s
τ double = 2.96 s
τ1 = 1.30 s (0.59)
τ2 = 5.36 s (0.41)
c
50 nM Guanine
250 nM Guanine
10 μM Guanine
τ single = 2.12 s
τ double = 2.99 s
τ1 = 0.64 s (0.60)
τ2 = 6.53 s (0.40)
τ single = 2.11 s
τ double = 2.67 s
τ1 = 0.6 s (0.51)
τ2 = 4.82 s (0.49)
τ single = 0.59 s
τ double = 0.83 s
τ1 = 0.31 s (0.73)
τ2 = 2.23 s (0.27)
τ single = 0.55 s
τ double = 0.79 s
τ1 = 0.44 s (0.86)
τ2 = 2.95 s (0.14)
τ single = 1.29 s
τ double = 1.51 s
τ1 = 0.45 s (0.40)
τ2 = 2.22 s (0.60)
τ single = 1.93 s
τ double = 2.06 s
τ1 = 0.96 s (0.40)
τ2 = 2.79 s (0.60)

Figure S4. *Bsu* aptamer kinetics in the presence of Mg^{2+}

Representative cumulative dwell time distributions for three different concentrations of (a) $preQ_1$(10 nM, 100 nM, 1 µM) (b) $preQ_0$ (25 nM, 250 nM, 10 µM) and (c) guanine (50 nM, 250 nM, 10 µM) in the presence of Mg^{2+}. The cumulative dwell-time distributions in the undocked and docked states were fit with single (cyan) and double (blue)exponential functions to obtain τ_{single} and τ_{double}, which were used to estimate the rate constants k_{dock} and k_{undock}, respectively. τ_{single} is the time constant for the single-exponential fit and τ_{double} is the average time constant of the double-exponential fit calculated from the individual short (τ_1) and long (τ_2) time constants and their respective amplitudes as $A_1\tau_1 + A_2\tau_2$. In the presence of Mg^{2+}, both k_{dock} and k_{undock} showed heterogeneous double-exponential behavior for all three ligands.

Figure S5

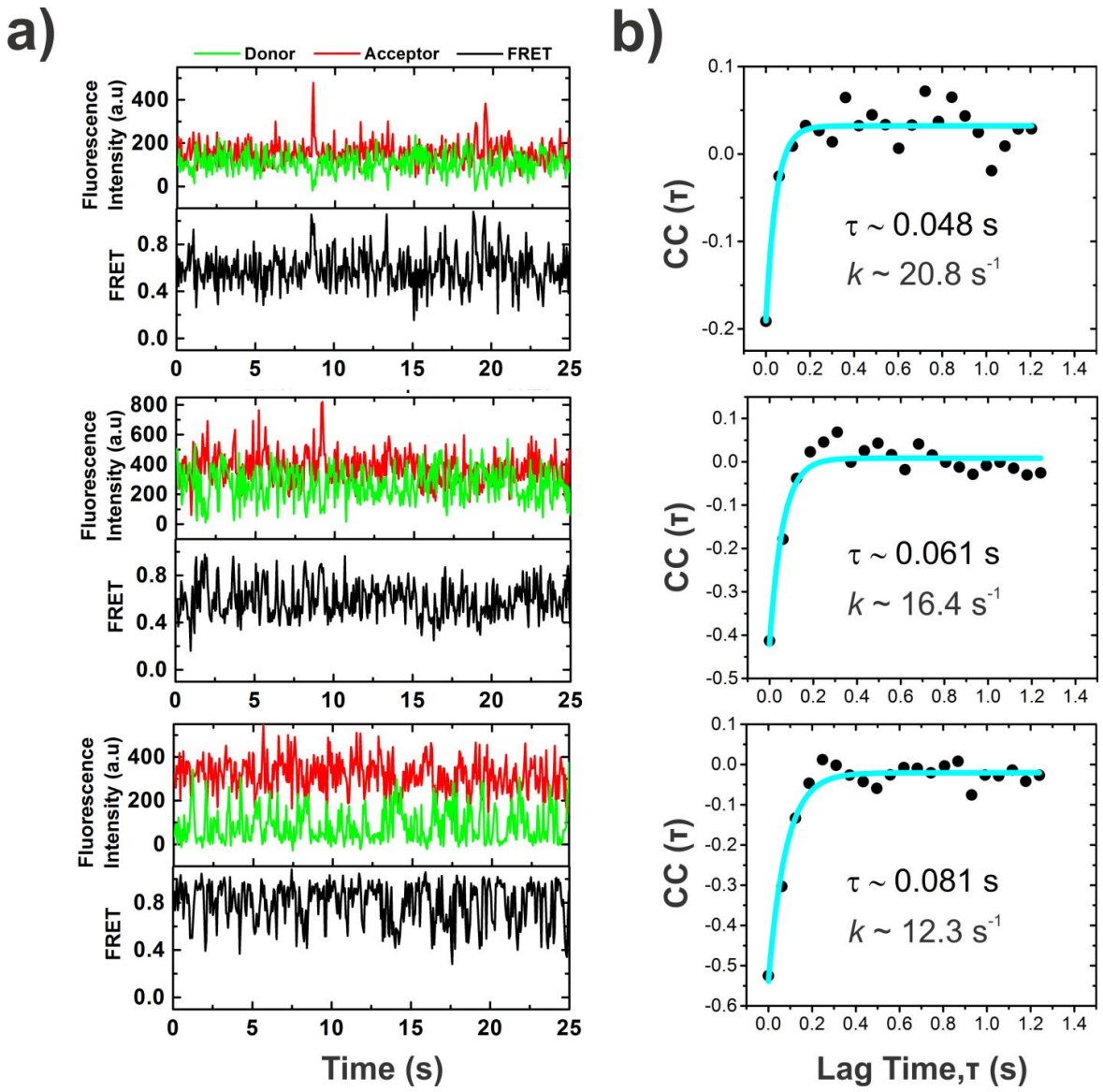

Figure S5. Cross-correlation analysis of the smFRET traces
(a) Exemplary raw smFRET traces showing fast (close to time resolution, ~60 ms) fluctuations in the intensities of donor and acceptor fluorophores in 1× smFRET buffer containing 1 mM Mg^{2+} in the absence of ligand. (b) Cross-correlation functions, CC(τ) of the raw traces shown in (a) were fit with single-exponential functions (cyan) to estimate the lifetimes (τ), the inverse of which equals the combined k_{dock} + k_{undock} rate constant (k_{obs}) of conformational transitions.

Supporting References

(1) Dogan, J.; Mu, X.; Engstrom, A.; Jemth, P. *Sci. Rep.* **2013**, *3*, 2076.