

SUPPORTING INFORMATION

Single-Molecule Fluorescence Reveals Commonalities and Distinctions among Natural and *In Vitro*-Selected RNA Tertiary Motifs in a Multi-Step Folding Pathway

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SUPPORTING METHODS

Single-photon counting measurements. Molecules were immobilized on a streptavidin coated coverslip at a density of ~ 1 molecule / $5 \mu\text{m}^2$ and imaged in a custom-built confocal microscope. To localize single molecules on the surface, the axial focus of the microscope was manually adjusted to locate the surface of the coverslip and a XY piezo stage (Nano-PDQ, Mad City Labs) was used to automatically raster-scan a $60 \mu\text{m} \times 60 \mu\text{m}$ area on the surface of the coverslip. The raster-scan was automatically stopped when a molecule was found, and single-photon counting data were collected until the donor fluorescent dye photo-bleached, at which point the raster-scan was resumed. For imaging, a 561 nm laser (OBIS, Coherent Inc.) was focused on the sample with a 40x microscope objective lens (Zeiss) to form a near-diffraction limited illumination volume (~ 700 nm beam waist at the microscope focus). The emission signal was separated from the laser by a dichroic mirror (Chroma ZT405/488/561/640rpc), spatially filtered through a pinhole, and split into donor and acceptor signals by a second dichroic mirror (Chroma 625DCX). The donor and acceptor signals were band-pass filtered (Chroma ET595/50m and Chroma ET705/72m for the donor and acceptor channels, respectively) and each measured with a dedicated avalanche photodiode (APD; Perkin-Elmer). The APDs produced electronic pulses corresponding to the detection of individual photons, which were recorded by a dual-channel time-interval analyzer board (GT653, GuideTech) operating in time-tagging mode. The resulting data consisted of two vectors with single-photon arrival times from the donor and acceptor channels. The single-photon arrival times were binned to estimate the donor and acceptor fluorescence intensities which were fit to two-state Hidden Markov models using the SMART¹ software package as described in the main text.

Choice of EMCCD vs. single-photon counting. A conventional TIRF setup equipped with an Andor Ultra iXon 897 EMCCD camera allowed data collection at up to 5 ms exposure time. We determined that binning at 5 ms resulted in an underestimation of the combined rate constants $k_{\text{comb}} = k_{\text{dock}} + k_{\text{undock}}$ of $\sim 15\%$ when k_{comb} increases to $\sim 70 \text{ s}^{-1}$ as Mg^{2+} concentration was increased, and this underestimation was expected to worsen at faster k_{comb} (e.g. at higher $[\text{Mg}^{2+}]$). To increase the dynamic range of our measurements, we used single-photon counting with 1 ms binning for conditions at which k_{comb} was expected to be $> 70 \text{ s}^{-1}$. The agreement between data collected with the camera and by single-photon counting (see for example Figure 4 in main text), indicates that there were no significant binning effects in the analyzed data.

Table S1. Sequence of the 515 11ntR-like tetraloop-receptors identified in the Group I Intron Sequence and Structure Database (GISSD).² Sequences were found in locations predicted to form TL/TLR interactions—*i.e.*, tertiary contacts L5b-P6, L9-P5, and L2-P8.³ The total number of tetraloop-receptors found was broken down by group I intron type². Sequences of variants investigated by smFRET in this study are colored: canonical 11ntR (cyan), 11ntR_AC (green), and 11ntR_A/CC (orange).

Sequence residues 1-6	Sequence residues 7-11	Dinucleotide platform	total number found	IA1	IA3	IC1	IC3	IE	IE1	IE2
CCUAAG	UAUGG	AA	96	-	2	76	3	3	4	8
CCUAAA	CAUGG	AA	60	-	4	19	37	-	-	-
GCUAAG	UAUGC	AA	35	5	-	8	-	7	13	2
CCUAAG	UACGG	AA	27	-	-	25	-	-	-	2
UCUAAG	UAUGA	AA	20	-	-	20	-	-	-	-
CCUAAC	UAUGG	AA	14	-	-	14	-	-	-	-
UCUAAG	UACGA	AA	14	-	-	14	-	-	-	-
GCUAAA	CAUGC	AA	14	-	-	13	-	1	-	-
CCUAAA	CACGG	AA	10	-	-	6	4	-	-	-
CCUAAA	CAAGG	AA	10	-	-	-	10	-	-	-
GCUAAG	UACGC	AA	8	-	-	-	-	-	8	-
GCUAAG	UAGGC	AA	7	-	-	6	-	-	1	-
CCUAAC	CACGG	AA	5	-	-	5	-	-	-	-
GCUAAG	UACGC	AA	5	-	-	1	-	4	-	-
CCUAAA	UAUGG	AA	5	-	-	-	5	-	-	-
CCUAAC	CAUGG	AA	4	-	-	4	-	-	-	-
UCUAAG	UAUGG	AA	4	-	-	4	-	-	-	-
CCUAAG	UAGGG	AA	2	-	-	2	-	-	-	-
CCUAAU	GAUGG	AA	2	-	-	2	-	-	-	-
UCUAAA	CAUGA	AA	2	-	-	2	-	-	-	-
GCUAAG	UACGC	AA	2	2	-	-	-	-	-	-
GCUAAA	UAUGC	AA	2	2	-	-	-	-	-	-
CCUAAA	UAUGG	AA	2	-	2	-	-	-	-	-
UCUAAA	CAUGA	AA	2	-	2	-	-	-	-	-
CCUAAC	CAAGG	AA	2	-	-	-	2	-	-	-
CCUAAA	UAAGG	AA	1	-	-	1	-	-	-	-
CCUAAC	AACGG	AA	1	-	-	1	-	-	-	-
CCUAAC	AAUGG'	AA	1	-	-	1	-	-	-	-
CCUAAC	CAGGG	AA	1	-	-	1	-	-	-	-
CCUAAC	UACGG	AA	1	-	-	1	-	-	-	-
CCUAAC	UAGGG	AA	1	-	-	1	-	-	-	-
CCUAAG	CACGG	AA	1	-	-	1	-	-	-	-
CCUAAU	UACGG	AA	1	-	-	1	-	-	-	-
GCUAAG	CAUGC	AA	1	-	-	1	-	-	-	-
UCUAAA	CAUGC	AA	1	-	-	1	-	-	-	-
UCUAAC	UAUGG	AA	1	-	-	1	-	-	-	-

Table S1 continues on next page.

Table S1 continued

Sequence residues 1-6	Sequence residues 7-11	Dinucleotide platform	total number found	IA1	IA3	IC1	IC3	IE	IE1	IE2
UCUAAG	UAGGA	AA	1	-	-	1	-	-	-	-
GCUAAC	UAUGC	AA	1	-	-	-	-	-	1	-
CCUAAA	UAUGG	AA	1	1	-	-	-	-	-	-
GCUAAG	UAUGU	AA	1	1	-	-	-	-	-	-
GCUAAA	UACGC	AA	1	1	-	-	-	-	-	-
CCUAAA	CAAGG	AA	1	-	1	-	-	-	-	-
UCUAAC	UAUGA	AA	1	-	1	-	-	-	-	-
CCUAAA	UACGG	AA	1	-	-	-	1	-	-	-
CCUAAU	UAUGG	AA	1	-	-	-	1	-	-	-
CCUACG	UACGG	AC	41	-	-	39	-	1	-	1
CCUACG	UAUGG	AC	22	-	-	22	-	-	-	-
CCUACC	CACGG	AC	11	-	-	11	-	-	-	-
CCUACC	CAUGG	AC	9	-	-	9	-	-	-	-
CCUACG	CACGG	AC	6	-	-	6	-	-	-	-
CCUACA	CACGG	AC	5	-	-	5	-	-	-	-
CCUACC	UAUGG	AC	3	-	-	3	-	-	-	-
CCUACA	CAGGG	AC	2	-	-	2	-	-	-	-
CCUACC	CAGGG	AC	2	-	-	2	-	-	-	-
CCUACG	UAAGG	AC	2	-	-	2	-	-	-	-
GCUACC	CAUGC	AC	2	-	-	2	-	-	-	-
GCUACG	UACGC	AC	2	-	-	2	-	-	-	-
CCUACA	CAGGA	AC	1	-	-	1	-	-	-	-
CCUACA	CAUGG	AC	1	-	-	1	-	-	-	-
CCUACC	UACGG	AC	1	-	-	1	-	-	-	-
CCUACC	UAGGG	AC	1	-	-	1	-	-	-	-
CCUACG	CAAGG	AC	1	-	-	1	-	-	-	-
CCUACG	UAGGG	AC	1	-	-	1	-	-	-	-
GCUACC	UACGC	AC	1	-	-	1	-	-	-	-
UCUACG	UACGA	AC	1	-	-	1	-	-	-	-
UCUACG	UACGG	AC	1	-	-	1	-	-	-	-
UCUACG	UAGGA	AC	1	-	-	1	-	-	-	-
UCUACG	UAUGG	AC	1	-	-	1	-	-	-	-
CCUAAU	UACGG	AC	1	-	-	1	-	-	-	-
GCUACG	UAUGC	AC	1	-	-	-	-	-	1	-
CCUACG	UAUGG	AC	2	-	-	-	-	-	-	2
CCUGUC	CAUGG	GU	2	-	-	2	-	-	-	-
GCUGUA	AAUGC	GU	2	-	-	2	-	-	-	-
CCUUCA	UAUGG	UC	8	-	-	8	-	-	-	-
GCUUCG	CAUGC	UC	3	-	-	3	-	-	-	-
CCUUCA	AAUGG	UC	1	-	-	1	-	-	-	-
CCUUCG	CACGG	UC	1	-	-	1	-	-	-	-
CCUUCG	UAUGG	UC	1	-	-	1	-	-	-	-

Table S2. Sequences of U₇ and A₇-tethered smFRET constructs. Constructs were assembled from the annealing of three oligonucleotides as previously described.⁴⁻⁶ Oligos 1 and 2 contain the GAAA tetraloop (green) and tetraloop-receptor (red). Oligo 3 is used for surface immobilization via biotin-streptavidin linkage and is complementary to sequence inside parentheses. Bold residues are deoxyribonucleotides. 5' Amino-C6 modifications (5AmMC6) allowed for labeling with Cy3B and Cy5 fluorophores.⁷

Construct	Oligo #	Sequence
11ntR (U ₇)	1	/5AmMC6/GGC GAAA GCCUUUUUUUCGUGUCGU CCUAAG UCGGCGC
	2	/5AmMC6/GCGCCGA UAUGG ACGACACGCC(ACCAAAATCAACCTAAAACCTTACACA)
11ntR (A ₇)	1	/5AmMC6/GGC GAAA GCCAAAAAACGUGUCGU CCUAAG UCGGCGC
	2	/5AmMC6/GCGCCGA UAUGG ACGACACGCC(ACCAAAATCAACCTAAAACCTTACACA)
11ntR_AC (U ₇)	1	/5AmMC6/GGC GAAA GCCUUUUUUUCGUGUCGU CCUACG UCGGCGC
	2	/5AmMC6/GCGCCGA UAUGG ACGACACGCC(ACCAAAATCAACCTAAAACCTTACACA)
11ntR_AC (A ₇)	1	/5AmMC6/GGC GAAA GCCAAAAAACGUGUCGU CCUACG UCGGCGC
	2	/5AmMC6/GCGCCGA UAUGG ACGACACGCC(ACCAAAATCAACCTAAAACCTTACACA)
11ntR_A/CC (U ₇)	1	/5AmMC6/GGC GAAA GCCUUUUUUUCGUGUCGU CCUAAA UCGGCGC
	2	/5AmMC6/GCGCCGA CACGG ACGACACGCC(ACCAAAATCAACCTAAAACCTTACACA)
C7.10 (U ₇)	1	/5AmMC6/GGC GAAA GCCUUUUUUUCGUGUCGU CCUGUG UCGGCGC
	2	/5AmMC6/GCGCCGA UAUGG ACGACACGCC(ACCAAAATCAACCTAAAACCTTACACA)
C7.10 (A ₇)	1	/5AmMC6/GGC GAAA GCCAAAAAACGUGUCGU CCUGUG UCGGCGC
	2	/5AmMC6/GCGCCGA UAUGG ACGACACGCC(ACCAAAATCAACCTAAAACCTTACACA)
C7.2 (U ₇)	1	/5AmMC6/GGC GAAA GCCUUUUUUUCGUGUCGU CCUGUAC UCGGCGC
	2	/5AmMC6/GCGCCGA GAUGG ACGACACGCC(ACCAAAATCAACCTAAAACCTTACACA)
C7.2 (A ₇)	1	/5AmMC6/GGC GAAA GCCAAAAAACGUGUCGU CCUGUAC UCGGCGC
	2	/5AmMC6/GCGCCGA GAUGG ACGACACGCC(ACCAAAATCAACCTAAAACCTTACACA)
C7.9 (U ₇)	1	/5AmMC6/GGC GAAA GCCUUUUUUUCGUGUCGU CCUGAAG UCGGCGC
	2	/5AmMC6/GCGCCGA UAUGG ACGACACGCC(ACCAAAATCAACCTAAAACCTTACACA)
Common to all constructs	3	5'Biotin-TGTGTAAGTTTTAGGTTGATTTTGGT 3'

Table S3. Bayesian analysis of smFRET traces for TL/TLR variants in U₇-tether minimal construct at a range of Mg²⁺ and K⁺ concentrations. The optimum number of FRET states was evaluated by vbFRET which uses evidence maximization and Bayesian statistics to infer the minimal number of FRET states that best describes the data.⁸ Rare frames with aberrant FRET values—*i.e.*, outside the range of 0 to 1—were corrected prior to vbFRET analysis as described by Blanco & Walter.⁹ Representative FRET traces and vbFRET fits are shown in Figure S6. Additionally, the Bayesian information criterion (BIC) of two and three-state models were calculated. The model with the lowest BIC has the optimal fit. Both vbFRET and BIC analysis indicate that the traces are better described by two FRET states. Data shown here were collected by EMCCD camera at a 5 ms exposure time. For analysis of single-photon counting (SPC; 1 ms binning) traces see Figure S7.

Construct	Ionic conditions	Total # traces	% two-state vbFRET	% two-state BIC
11ntR (U ₇)	0.15 mM Mg ²⁺	176	95	87
11ntR (U ₇)	0.25 mM Mg ²⁺	159	96	92
11ntR (U ₇)	0.35 mM Mg ²⁺	230	93	84
11ntR (U ₇)	0.50 mM Mg ²⁺	156	96	91
11ntR (U ₇)	0.65 mM Mg ²⁺	239	89	83
11ntR (U ₇)	0.75 mM Mg ²⁺	127	98	90
11ntR (U ₇)	0.85 mM Mg ²⁺	246	92	84
11ntR (U ₇)	90 mM K ⁺	118	92	96
11ntR (U ₇)	240 mM K ⁺	140	97	92
11ntR_AC (U ₇)	0.15 mM Mg ²⁺	160	100	89
11ntR_AC (U ₇)	0.25 mM Mg ²⁺	123	93	79
11ntR_AC (U ₇)	0.35 mM Mg ²⁺	235	92	95
11ntR_AC (U ₇)	0.50 mM Mg ²⁺	119	94	81
11ntR_AC (U ₇)	0.65 mM Mg ²⁺	226	96	94
11ntR_AC (U ₇)	0.75 mM Mg ²⁺	101	91	84
11ntR_AC (U ₇)	0.85 mM Mg ²⁺	204	96	93
11ntR_AC (U ₇)	1.0 mM Mg ²⁺	119	94	80
11ntR_AC (U ₇)	1.5 mM Mg ²⁺	156	97	78
11ntR_AC (U ₇)	2.0 mM Mg ²⁺	110	94	79
11ntR_AC (U ₇)	2.5 mM Mg ²⁺	116	96	79
11ntR_AC (U ₇)	190 mM K ⁺	107	95	86
11ntR_AC (U ₇)	490 mM K ⁺	79	91	84
11ntR_A/CC (U ₇)	0.15 mM Mg ²⁺	181	98	93
11ntR_A/CC (U ₇)	0.35 mM Mg ²⁺	191	97	95
11ntR_A/CC (U ₇)	0.65 mM Mg ²⁺	171	98	97
11ntR_A/CC (U ₇)	0.85 mM Mg ²⁺	183	98	93
11ntR_A/CC (U ₇)	140 mM K ⁺	132	95	92
11ntR_A/CC (U ₇)	340 mM K ⁺	116	95	96
C7.10 (U ₇)	0.25 mM Mg ²⁺	138	94	78
C7.10 (U ₇)	0.50 mM Mg ²⁺	134	90	91
C7.10 (U ₇)	0.75 mM Mg ²⁺	111	93	90
C7.10 (U ₇)	1.0 mM Mg ²⁺	115	90	91
C7.10 (U ₇)	3.0 mM Mg ²⁺	128	99	92
C7.10 (U ₇)	340 mM K ⁺	116	97	94
C7.10 (U ₇)	1240 mM K ⁺	81	99	94
C7.2 (U ₇)	0.25 mM Mg ²⁺	106	96	85
C7.2 (U ₇)	0.50 mM Mg ²⁺	243	98	92
C7.2 (U ₇)	0.75 mM Mg ²⁺	213	96	86
C7.2 (U ₇)	1.0 mM Mg ²⁺	115	94	93
C7.2 (U ₇)	4.0 mM Mg ²⁺	46	93	89
C7.2 (U ₇)	440 mM K ⁺	119	93	98
C7.2 (U ₇)	1040 mM K ⁺	95	95	91

Table S4. Data for TL/TLR variants in the U₇-tether minimal construct at a range of Mg²⁺ concentrations. All measurements were carried out in with 140 mM K⁺ background. k_{dock} and k_{undock} values were determined from a two-state HMM model using the SMART software package¹ as described previously^{6,10} and equilibrium constants (K_{obs}) were determined from the ratio $k_{\text{dock}}/k_{\text{undock}}$. Standard errors were determined from bootstrapping. Apparent equilibrium constants from cumulative data ($K_{\text{eq,bulk}}$) were determined from integration of the areas under the cumulative FRET distributions shown in supporting summary figures (Figure S9-S82). Raw data are available to download.¹¹ Repeated measurements are from independent experiments performed on different days. Data were collected with either an EMCCD camera (C) or a single-photon counting (SPC) setup as described in Materials and Methods.

Construct	[Mg ²⁺] (mM)	k_{dock} (s ⁻¹)	k_{undock} (s ⁻¹)	K_{obs}	$K_{\text{eq,bulk}}$	Method	Summary Figure ¹¹
11ntR (U ₇)	0.15	16.3 ± 0.5	6.1 ± 0.2	2.7 ± 0.1	3.2	C	S9
11ntR (U ₇)	0.25	23.4 ± 0.6	6.5 ± 0.2	3.6 ± 0.1	4.0	C	S10
11ntR (U ₇)	0.25	25.0 ± 0.8	7.3 ± 0.2	3.4 ± 0.1	3.8	SPC	S11
11ntR (U ₇)	0.35	28.2 ± 0.7	6.0 ± 0.2	4.7 ± 0.2	5.5	C	S12
11ntR (U ₇)	0.50	43 ± 1	6.7 ± 0.2	6.4 ± 0.3	8.3	C	S13
11ntR (U ₇)	0.65	51 ± 2	5.9 ± 0.3	8.5 ± 0.4	9.6	C	S14
11ntR (U ₇)	0.65	60 ± 4	7.3 ± 0.4	8.2 ± 0.7	4.9	SPC	S15
11ntR (U ₇)	0.75	63 ± 3	6.6 ± 0.3	9.5 ± 0.6	10.7	C	S16
11ntR (U ₇)	0.75	72 ± 2	6.5 ± 0.3	11.0 ± 0.6	13.0*	SPC	S17
11ntR (U ₇)	0.85	66 ± 2	5.7 ± 0.2	11.5 ± 0.5	10.1*	C	S18
11ntR (U ₇)	1.0	93 ± 3	7.0 ± 0.3	13.2 ± 0.8	13.9*	SPC	S19
11ntR (U ₇)	1.5	136 ± 3	7.0 ± 0.2	19.4 ± 0.7	15.9*	SPC	S20
11ntR (U ₇)	2.0	176 ± 8	6.5 ± 0.4	27 ± 2	27.6*	SPC	S21
11ntR (U ₇)	3.0	287 ± 13	7.2 ± 0.3	40 ± 2	43.0*	SPC	S22
11ntR (U ₇)	3.5	272 ± 12	7.4 ± 0.6	37 ± 3	22.2*	SPC	S23
11ntR (U ₇)	4.0	262 ± 20	7 ± 1	36 ± 7	15.3*	SPC	S24
11ntR AC (U ₇)	0.15	3.3 ± 0.1	15.8 ± 0.5	0.21 ± 0.01	0.3	C	S25
11ntR AC (U ₇)	0.15	3.6 ± 0.2	14.2 ± 0.6	0.26 ± 0.02	0.4	C	S26
11ntR AC (U ₇)	0.15	3.1 ± 0.2	19.7 ± 0.8	0.16 ± 0.01	0.3	SPC	S27
11ntR AC (U ₇)	0.25	5.6 ± 0.2	15.7 ± 0.4	0.36 ± 0.01	0.5	C	S28
11ntR AC (U ₇)	0.25	5.9 ± 0.2	13.4 ± 0.4	0.44 ± 0.02	0.6	C	S29
11ntR AC (U ₇)	0.35	7.6 ± 0.2	15.6 ± 0.4	0.49 ± 0.02	0.7	C	S30
11ntR AC (U ₇)	0.50	12.4 ± 0.3	14.5 ± 0.4	0.86 ± 0.03	1.0	C	S31
11ntR AC (U ₇)	0.65	14.7 ± 0.3	14.2 ± 0.3	1.03 ± 0.03	1.4	C	S32
11ntR AC (U ₇)	0.75	20 ± 1	11.8 ± 0.4	1.7 ± 0.1	1.9	C	S33
11ntR AC (U ₇)	0.75	19.6 ± 0.8	17.0 ± 0.5	1.15 ± 0.06	1.1	SPC	S34
11ntR AC (U ₇)	0.85	20.8 ± 0.4	13.4 ± 0.2	1.56 ± 0.04	1.9	C	S35
11ntR AC (U ₇)	1.0	28 ± 1	12.5 ± 0.3	2.2 ± 0.1	2.3	C	S36
11ntR AC (U ₇)	1.5	48 ± 1	12.0 ± 0.3	4.0 ± 0.1	5.7	C	S37
11ntR AC (U ₇)	2.0	68 ± 3	12.2 ± 0.6	5.6 ± 0.3	7.4	C	S38
11ntR AC (U ₇)	2.0	82 ± 4	13.2 ± 0.5	6.2 ± 0.4	4.5	SPC	S39
11ntR AC (U ₇)	2.5	75 ± 2	9.2 ± 0.6	8.2 ± 0.6	7.5	C	S40
11ntR AC (U ₇)	3.0	120 ± 6	11.3 ± 0.3	10.6 ± 0.6	7.2*	SPC	S41
11ntR AC (U ₇)	4.0	149 ± 4	11.0 ± 0.4	13.5 ± 0.7	13.1*	SPC	S42
11ntR A/CC (U ₇)	0.15	12.2 ± 0.3	20.3 ± 0.4	0.60 ± 0.02	0.7	C	S43
11ntR A/CC (U ₇)	0.35	21.5 ± 0.5	18.9 ± 0.4	1.13 ± 0.04	1.2	C	S44
11ntR A/CC (U ₇)	0.65	37 ± 1	16.9 ± 0.2	2.17 ± 0.07	2.4	C	S45
11ntR A/CC (U ₇)	0.75	49 ± 1	20.5 ± 0.6	2.38 ± 0.09	2.2	SPC	S46
11ntR A/CC (U ₇)	0.85	44 ± 1	16.5 ± 0.3	2.69 ± 0.08	2.9	C	S47
11ntR A/CC (U ₇)	2.0	105 ± 2	15.3 ± 0.3	6.9 ± 0.2	5.4	SPC	S48
11ntR A/CC (U ₇)	3.0	163 ± 5	13.6 ± 0.4	12.0 ± 0.5	13.9*	SPC	S49
11ntR A/CC (U ₇)	4.5	191 ± 6	12.1 ± 0.3	15.7 ± 0.6	17.7*	SPC	S50
C7.10 (U ₇)	0.25	2.8 ± 0.2	28 ± 1	0.10 ± 0.01	0.3	C	S51
C7.10 (U ₇)	0.50	5.6 ± 0.2	18.4 ± 0.4	0.31 ± 0.01	0.4	C	S52
C7.10 (U ₇)	0.75	9.3 ± 0.2	14.9 ± 0.3	0.63 ± 0.02	0.8	C	S53
C7.10 (U ₇)	1.0	13.2 ± 0.3	13.4 ± 0.3	0.98 ± 0.03	0.9	C	S54

Table S4 continues on next page

Table S4 continued

Construct	[Mg ²⁺] (mM)	k_{dock} (s ⁻¹)	k_{undock} (s ⁻¹)	K_{obs}	$K_{\text{eq,bulk}}$	Method	Summary Figure ¹¹
C7.10 (U ₇)	1.5	21.2 ± 0.5	11.4 ± 0.2	1.85 ± 0.06	2.3	C	S55
C7.10 (U ₇)	1.5	21.6 ± 0.5	10.5 ± 0.2	2.05 ± 0.06	2.8	C	S56
C7.10 (U ₇)	1.5	22.2 ± 0.5	10.6 ± 0.4	2.09 ± 0.09	2.6	C	S57
C7.10 (U ₇)	2.0	28.6 ± 0.9	10.2 ± 0.3	2.8 ± 0.1	3.8	C	S58
C7.10 (U ₇)	3.0	43 ± 1	8.4 ± 0.2	5.2 ± 0.2	5.3	C	S59
C7.2 (U ₇)	0.25	4.2 ± 0.3	65 ± 7	0.06 ± 0.01	0.2	C	S60
C7.2 (U ₇)	0.25	5.1 ± 0.2	103 ± 5	0.05 ± 0.01	0.1	SPC	S61
C7.2 (U ₇)	0.35	5.2 ± 0.1	48 ± 1	0.11 ± 0.01	0.2	C	S62
C7.2 (U ₇)	0.50	6.6 ± 0.1	34.8 ± 0.5	0.19 ± 0.01	0.2	C	S63
C7.2 (U ₇)	0.65	9.5 ± 0.2	28.7 ± 0.7	0.33 ± 0.01	0.4	C	S64
C7.2 (U ₇)	0.75	9.9 ± 0.2	27.2 ± 0.4	0.36 ± 0.01	0.5	C	S65
C7.2 (U ₇)	0.85	11.5 ± 0.3	22.6 ± 0.4	0.51 ± 0.02	0.6	C	S66
C7.2 (U ₇)	1.0	14.7 ± 0.5	18.7 ± 0.6	0.79 ± 0.04	0.5	C	S67
C7.2 (U ₇)	1.0	13.3 ± 0.4	20.3 ± 0.5	0.66 ± 0.02	0.9	C	S68
C7.2 (U ₇)	1.5	21.0 ± 0.4	15.1 ± 0.2	1.40 ± 0.03	1.6	C	S69
C7.2 (U ₇)	2.0	28.4 ± 0.8	12.3 ± 0.4	2.31 ± 0.08	2.5	C	S70
C7.2 (U ₇)	2.5	32 ± 1	9.1 ± 0.3	3.6 ± 0.17	2.1	C	S71
C7.2 (U ₇)	3.0	39 ± 1	9.5 ± 0.3	4.1 ± 0.2	4.4	C	S72
C7.2 (U ₇)	3.0	49 ± 3	11 ± 2	4.3 ± 0.8	2.7	SPC	S73
C7.2 (U ₇)	3.5	44 ± 2	7.8 ± 0.2	5.6 ± 0.2	1.8	C	S74
C7.2 (U ₇)	4.0	54 ± 2	8.9 ± 0.4	6.1 ± 0.4	6.3	C	S75
C7.9 (U ₇)	0.75	28.5 ± 0.5	407 ± 6	0.070 ± 0.002	0.08	SPC	S76
C7.9 (U ₇)	0.85	31.9 ± 0.8	370 ± 4	0.086 ± 0.002	0.1	SPC	S77
C7.9 (U ₇)	1.0	38 ± 1	335 ± 5	0.114 ± 0.004	0.1	SPC	S78
C7.9 (U ₇)	1.5	60 ± 2	263 ± 10	0.23 ± 0.01	0.4	SPC	S79
C7.9 (U ₇)	2.5	96 ± 1	189 ± 4	0.51 ± 0.01	0.5	SPC	S80
C7.9 (U ₇)	3.5	135 ± 2	153 ± 2	0.88 ± 0.02	0.9	SPC	S81
C7.9 (U ₇)	4.5	168 ± 4	131 ± 2	1.28 ± 0.04	1.2	SPC	S82

*The fitting parameters of the cumulative FRET distribution—i.e. mean, variance, and mixing proportion of each of the two FRET components—become difficult to estimate when the mixing proportion of one of the components approaches unity, resulting in inaccurate estimates of $K_{\text{eq,bulk}}$ under these conditions. To obtain a better estimate of $K_{\text{eq,bulk}}$ at conditions when the folded state is greatly favored over the unfolded state (chosen as $K_{\text{obs}} > 10$), the mean and variance of the low FRET component were kept constant during fitting under these conditions.

Table S5. Data for TL/TLR variants in the U₇-tether minimal construct at a range of Ba²⁺ concentrations. All measurements were carried out with a 140 mM K⁺ background. k_{dock} and k_{undock} values were determined from a two-state HMM model using the SMART software package¹ as described previously^{10,12} and equilibrium constants (K_{obs}) were determined from the ratio $k_{\text{dock}}/k_{\text{undock}}$. Standard errors were determined from bootstrapping. Apparent equilibrium constants from cumulative data ($K_{\text{eq,bulk}}$) were determined from integration of the areas under cumulative FRET distributions shown in supporting summary figures (Figure S83-S125). Raw data are available to download.¹¹ Repeated measurements are from independent experiments performed on different days. Data were collected with either an EMCCD camera (C) or a single-photon counting (SPC) setup as described in Materials and Methods.

Construct	[Ba ²⁺] (mM)	k_{dock} (s ⁻¹)	k_{undock} (s ⁻¹)	K_{obs}	$K_{\text{eq,bulk}}$	Method	Summary Figure ¹¹
11ntR (U ₇)	0.125	14.0 ± 0.6	5.1 ± 0.2	2.8 ± 0.2	2.7	C	S83
11ntR (U ₇)	0.25	22.2 ± 0.7	4.3 ± 0.2	5.2 ± 0.3	5.0	C	S84
11ntR (U ₇)	0.25	23 ± 1	7 ± 1	3.4 ± 0.7	1.5	SPC	S85
11ntR (U ₇)	0.50	37 ± 1	3.3 ± 0.1	10.3 ± 0.5	11.7	C	S86
11ntR (U ₇)	0.75	52 ± 1	3.2 ± 0.1	16.1 ± 0.4	16.5	C	S87
11ntR (U ₇)	1.0	79 ± 5	3.9 ± 0.3	20 ± 2	10.3	SPC	S88
11ntR (U ₇)	2.0	190 ± 9	3.4 ± 0.1	56 ± 3	34.3	SPC	S89
11ntR (U ₇)	3.0	237 ± 14	3.6 ± 0.2	65 ± 6	73.3	SPC	S90
11ntR AC (U ₇)	0.125	2.4 ± 0.1	13.9 ± 0.7	0.17 ± 0.01	0.21	SPC	S91
11ntR AC (U ₇)	0.25	3.8 ± 0.2	10.6 ± 0.6	0.36 ± 0.03	0.41	C	S92
11ntR AC (U ₇)	0.25	2.2 ± 0.1	15.1 ± 0.8	0.15 ± 0.01	0.15	SPC	S93
11ntR AC (U ₇)	0.50	11.1 ± 0.3	8 ± 1	1.4 ± 0.2	1.2	SPC	S94
11ntR AC (U ₇)	0.75	8.8 ± 0.3	6.1 ± 0.2	1.45 ± 0.07	1.4	C	S95
11ntR AC (U ₇)	1.0	12.3 ± 0.3	5.7 ± 0.1	2.15 ± 0.08	2.2	C	S96
11ntR AC (U ₇)	2.0	25.8 ± 0.8	4.2 ± 0.2	6.2 ± 0.3	6.5	C	S97
11ntR AC (U ₇)	2.0	31 ± 2	5.1 ± 0.4	6.1 ± 0.5	4.8	SPC	S98
11ntR AC (U ₇)	3.0	52 ± 4	4.1 ± 0.3	13 ± 1	5.8	SPC	S99
11ntR A/CC (U ₇)	0.125	10.7 ± 0.2	17.5 ± 0.4	0.61 ± 0.02	0.89	C	S100
11ntR A/CC (U ₇)	0.25	14.9 ± 0.3	13.3 ± 0.3	1.12 ± 0.03	1.3	C	S101
11ntR A/CC (U ₇)	0.25	19.9 ± 0.4	18.3 ± 0.3	1.08 ± 0.03	1.1	SPC	S102
11ntR A/CC (U ₇)	0.50	24.5 ± 0.4	11.0 ± 0.2	2.24 ± 0.06	2.7	C	S103
11ntR A/CC (U ₇)	0.75	33.9 ± 0.8	8.9 ± 0.2	3.8 ± 0.1	4.4	C	S104
11ntR A/CC (U ₇)	1.0	50 ± 2	10.3 ± 0.2	4.8 ± 0.2	4.2	SPC	S105
11ntR A/CC (U ₇)	2.0	105 ± 3	7.2 ± 0.2	14.6 ± 0.6	13.8*	SPC	S106
11ntR A/CC (U ₇)	3.0	141 ± 9	6.4 ± 0.3	22 ± 2	22.6*	SPC	S107
C7.10 (U ₇)	0.50	3.5 ± 0.2	37 ± 2	0.096 ± 0.01	0.25	C	S108
C7.10 (U ₇)	0.75	5.1 ± 0.2	28.4 ± 0.9	0.18 ± 0.01	0.32	C	S109
C7.10 (U ₇)	1.0	5.8 ± 0.2	24.8 ± 0.6	0.24 ± 0.01	0.37	C	S110
C7.10 (U ₇)	1.2	9.2 ± 0.3	20.4 ± 0.6	0.45 ± 0.02	0.55	C	S111
C7.10 (U ₇)	2.0	11.4 ± 0.2	17.8 ± 0.3	0.64 ± 0.02	0.75	C	S112
C7.10 (U ₇)	3.0	15.1 ± 0.3	16.4 ± 0.3	0.92 ± 0.02	1.0	C	S113
C7.2 (U ₇)	0.25	5.8 ± 0.2	134 ± 4	0.043 ± 0.002	0.05	SPC	S114
C7.2 (U ₇)	0.75	16.4 ± 0.8	52 ± 2	0.31 ± 0.02	0.37	SPC	S115
C7.2 (U ₇)	1.0	25 ± 1	41 ± 2	0.60 ± 0.04	0.62	SPC	S116
C7.2 (U ₇)	1.5	37 ± 2	32 ± 2	1.2 ± 0.1	1.1	SPC	S117
C7.2 (U ₇)	2.0	33 ± 0.5	15.6 ± 0.2	2.13 ± 0.04	2.7	C	S118
C7.2 (U ₇)	3.0	76 ± 2	20 ± 1	3.8 ± 0.2	3.2	SPC	S119
C7.2 (U ₇)	5.0	105 ± 7	15 ± 1	7.2 ± 0.8	4.4	SPC	S120
C7.9 (U ₇)	0.75	43 ± 1	383 ± 5	0.111 ± 0.004	0.15	SPC	S121
C7.9 (U ₇)	1.0	52.4 ± 2	315 ± 7	0.17 ± 0.01	0.23	SPC	S122
C7.9 (U ₇)	1.5	80 ± 2	243 ± 3	0.33 ± 0.01	0.42	SPC	S123
C7.9 (U ₇)	3.0	160 ± 4	157 ± 2	1.02 ± 0.03	1.1	SPC	S124
C7.9 (U ₇)	5.0	211 ± 5	122 ± 3	1.74 ± 0.07	1.8	SPC	S125

*The fitting parameters of the cumulative FRET distribution—i.e. mean, variance, and mixing proportion of each of the two FRET components—become difficult to estimate when the mixing proportion of one of the components approaches unity, resulting in inaccurate estimates of $K_{\text{eq,bulk}}$ under these conditions. To obtain a better estimate of $K_{\text{eq,bulk}}$ at conditions when the folded state is greatly favored over the unfolded state (chosen as $K_{\text{obs}} > 10$), the mean and variance of the low FRET component were kept constant during fitting under these conditions.

Table S6. Data for TL/TLR variants in the A₇-tether minimal construct at a range of Mg²⁺ concentrations. All measurements were carried out with a 140 mM K⁺ background. k_{dock} and k_{undock} values were determined from a two-state HMM model using the SMART software package¹ as described previously^{10,12} and equilibrium constants (K_{obs}) were determined from the ratio $k_{\text{dock}}/k_{\text{undock}}$. Standard errors were determined from bootstrapping. Apparent equilibrium constants from cumulative data ($K_{\text{eq,bulk}}$) were determined from integration of the areas under the cumulative FRET distributions shown in supporting summary figures (Figure S126-S155). Raw data are available to download.¹¹ Repeated measurements are from independent experiments performed on different days. Data were collected with either an EMCCD camera (C) or a single-photon counting (SPC) setup as described in Materials and Methods.

Construct	[Mg ²⁺] (mM)	k_{dock} (s ⁻¹)	k_{undock} (s ⁻¹)	K_{obs}	$K_{\text{eq,bulk}}$	Method	Summary Figure ¹¹
11ntR (A ₇)	0.15	8.9 ± 0.5	7.4 ± 0.3	1.20 ± 0.08	1.5	C	S126
11ntR (A ₇)	0.25	10.9 ± 0.4	7.4 ± 0.2	1.47 ± 0.06	1.6	C	S127
11ntR (A ₇)	0.25	11.8 ± 0.4	9.2 ± 0.6	1.30 ± 0.1	1.1	SPC	S128
11ntR (A ₇)	0.75	19.1 ± 0.6	5.8 ± 0.2	3.3 ± 0.2	3.3	C	S129
11ntR (A ₇)	1.25	24 ± 1	5.8 ± 0.2	4.2 ± 0.2	4.1	C	S130
11ntR (A ₇)	2.0	27 ± 1	6 ± 1	4.5 ± 1	1.7	SPC	S131
11ntR (A ₇)	2.5	25 ± 1	4.7 ± 0.3	5.4 ± 0.4	5.0	C	S132
11ntR AC (A ₇)	0.15	1.4 ± 0.1	19 ± 1	0.07 ± 0.01	0.2	C	S133
11ntR AC (A ₇)	0.25	1.9 ± 0.1	16.8 ± 0.9	0.11 ± 0.01	0.2	C	S134
11ntR AC (A ₇)	0.25	2.0 ± 0.1	18.7 ± 0.4	0.107 ± 0.004	0.2	C	S135
11ntR AC (A ₇)	0.50	3.8 ± 0.1	18.2 ± 0.4	0.21 ± 0.01	0.3	C	S136
11ntR AC (A ₇)	0.75	5.6 ± 0.3	13.6 ± 0.6	0.41 ± 0.03	0.5	C	S137
11ntR AC (A ₇)	1.0	6.7 ± 0.4	12.5 ± 0.5	0.53 ± 0.04	0.8	C	S138
11ntR AC (A ₇)	1.0	7.0 ± 0.3	13.7 ± 0.3	0.51 ± 0.02	0.5	C	S139
11ntR AC (A ₇)	2.0	10.7 ± 0.2	11.1 ± 0.2	0.97 ± 0.03	1.0	C	S140
11ntR AC (A ₇)	2.5	11.5 ± 0.7	9.2 ± 0.4	1.26 ± 0.09	1.4	C	S141
11ntR AC (A ₇)	3.0	13.4 ± 0.6	9.6 ± 0.3	1.39 ± 0.08	1.5	C	S142
C7.10 (A ₇)	0.5	1.7 ± 0.1	19.3 ± 0.9	0.089 ± 0.01	0.2	C	S143
C7.10 (A ₇)	1.0	3.2 ± 0.2	14.6 ± 0.4	0.22 ± 0.01	0.3	C	S144
C7.10 (A ₇)	2.0	4.8 ± 0.1	9.7 ± 0.2	0.49 ± 0.01	0.6	C	S145
C7.10 (A ₇)	3.0	5.5 ± 0.2	8.3 ± 0.4	0.66 ± 0.04	0.8	C	S146
C7.2 (A ₇)	0.50	1.9 ± 0.2	32 ± 2	0.06 ± 0.01	0.2	C	S147
C7.2 (A ₇)	1.0	2.4 ± 0.1	16.5 ± 0.7	0.15 ± 0.01	0.2	C	S148
C7.2 (A ₇)	1.0	2.5 ± 0.1	15.0 ± 0.5	0.17 ± 0.01	0.2	C	S149
C7.2 (A ₇)	1.5	3.0 ± 0.1	11.2 ± 0.4	0.27 ± 0.02	0.3	C	S150
C7.2 (A ₇)	2.0	3.5 ± 0.1	9.4 ± 0.2	0.38 ± 0.02	0.4	C	S151
C7.2 (A ₇)	2.5	3.6 ± 0.1	7.1 ± 0.3	0.50 ± 0.03	0.6	C	S152
C7.2 (A ₇)	3.0	5.0 ± 0.3	6.1 ± 0.2	0.81 ± 0.06	0.8	C	S153
C7.2 (A ₇)	3.5	4.1 ± 0.1	5.5 ± 0.1	0.75 ± 0.03	0.7	C	S154
C7.2 (A ₇)	4.0	4.4 ± 0.2	4.6 ± 0.2	0.94 ± 0.07	0.8	C	S155

Table S7. Data for TL/TLR variants in the U₇-tether minimal construct at a range of K⁺ concentrations. Total concentration of K⁺ contains 40 mM K⁺ from K-MOPS buffer. k_{dock} and k_{undock} values were determined from a two-state HMM model using the SMART software package¹ and equilibrium constants (K_{obs}) were determined from the ratio $k_{\text{dock}}/k_{\text{undock}}$. Standard errors were determined from bootstrapping. Apparent equilibrium constants from cumulative data ($K_{\text{eq,bulk}}$) were determined from integration of the areas under cumulative FRET distributions shown in supporting summary figures (Figure S156-S202). Raw data are available.¹¹ Repeated measurements are from independent experiments performed on different days. Data were collected with EMCCD camera (C) or by single-photon counting (SPC).

Construct	[K ⁺] (mM)	a _z (mM)	k_{dock} (s ⁻¹)	k_{undock} (s ⁻¹)	K_{obs}	$K_{\text{eq,bulk}}$	Method	Summary Figure ¹¹
11ntR (U ₇)	90	70	3.3 ± 0.1	8.2 ± 0.2	0.40 ± 0.02	0.4	C	S156
11ntR (U ₇)	90	70	5.9 ± 0.3	10.1 ± 0.5	0.58 ± 0.04	0.6	SPC	S157
11ntR (U ₇)	140	103	8.0 ± 0.3	6.5 ± 0.2	1.22 ± 0.06	1.5	C	S158
11ntR (U ₇)	140	103	17 ± 1	7.2 ± 0.4	2.4 ± 0.2	2.1	SPC	S159
11ntR (U ₇)	190	134	18.6 ± 0.5	5.2 ± 0.1	3.6 ± 0.1	3.5	C	S160
11ntR (U ₇)	190	134	24.2 ± 0.9	7.1 ± 0.5	3.4 ± 0.3	2.7	SPC	S161
11ntR (U ₇)	240	165	30.3 ± 0.9	4.7 ± 0.2	6.4 ± 0.3	5.6	C	S162
11ntR (U ₇)	240	165	39 ± 3	6.7 ± 0.6	5.8 ± 0.7	3.8	SPC	S163
11ntR (U ₇)	340	224	105 ± 5	4.4 ± 0.7	24 ± 4	10.1*	SPC	S164
11ntR AC (U ₇)	190	134	3.2 ± 0.1	13.1 ± 0.3	0.24 ± 0.01	0.3	C	S165
11ntR AC (U ₇)	240	165	5.2 ± 0.2	10.9 ± 0.3	0.48 ± 0.02	0.6	C	S166
11ntR AC (U ₇)	290	195	7.6 ± 0.2	9.4 ± 0.2	0.81 ± 0.03	1.0	C	S167
11ntR AC (U ₇)	340	224	11.3 ± 0.3	8.4 ± 0.1	1.35 ± 0.04	1.4	C	S168
11ntR AC (U ₇)	390	252	14.3 ± 0.4	7.2 ± 0.2	2.00 ± 0.08	2.2	C	S169
11ntR AC (U ₇)	440	280	19.5 ± 0.6	6.3 ± 0.2	3.1 ± 0.1	3.2	C	S170
11ntR AC (U ₇)	490	308	25.7 ± 0.9	6.3 ± 0.2	4.1 ± 0.2	4.1	C	S171
11ntR A/CC (U ₇)	90	70	5.7 ± 0.2	30.8 ± 0.7	0.19 ± 0.01	0.2	SPC	S172
11ntR A/CC (U ₇)	140	103	8.4 ± 0.2	25.4 ± 0.5	0.33 ± 0.01	0.4	C	S173
11ntR A/CC (U ₇)	190	134	15.0 ± 0.4	21.6 ± 0.4	0.69 ± 0.02	0.7	C	S174
11ntR A/CC (U ₇)	190	134	20.4 ± 0.5	26.1 ± 0.6	0.78 ± 0.03	0.8	SPC	S175
11ntR A/CC (U ₇)	190	134	22 ± 1	25 ± 2	0.87 ± 0.08	0.7	SPC	S176
11ntR A/CC (U ₇)	240	165	23.4 ± 0.5	20.9 ± 0.4	1.12 ± 0.03	1.2	C	S177
11ntR A/CC (U ₇)	290	195	36.2 ± 0.7	18.1 ± 0.1	2.00 ± 0.05	2.7	C	S178
11ntR A/CC (U ₇)	290	195	62 ± 3	20 ± 2	3.1 ± 0.03	2.2	SPC	S179
11ntR A/CC (U ₇)	340	224	49 ± 0.8	17.4 ± 0.3	2.82 ± 0.06	3.1	C	S180
11ntR A/CC (U ₇)	340	224	72 ± 2	19 ± 1	3.81 ± 0.02	2.7	SPC	S181
C7.10 (U ₇)	340	224	4.1 ± 0.2	44 ± 2	0.09 ± 0.01	0.1	C	S182
C7.10 (U ₇)	440	280	6.1 ± 0.3	37 ± 1	0.16 ± 0.01	0.3	C	S183
C7.10 (U ₇)	640	389	11.3 ± 0.3	30 ± 1	0.38 ± 0.02	0.5	C	S184
C7.10 (U ₇)	840	493	17.0 ± 0.6	22.9 ± 0.5	0.74 ± 0.03	0.8	C	S185
C7.10 (U ₇)	1040	595	24.0 ± 0.8	19.2 ± 0.5	1.25 ± 0.05	1.5	C	S186
C7.10 (U ₇)	1240	694	28.7 ± 0.8	16.9 ± 0.6	1.70 ± 0.07	1.8	C	S187
C7.2 (U ₇)	440	280	17.1 ± 0.4	105 ± 2	0.16 ± 0.01	0.3	C	S188
C7.2 (U ₇)	440	280	23.4 ± 0.6	131 ± 3	0.18 ± 0.01	0.2	SPC	S189
C7.2 (U ₇)	540	335	24.1 ± 0.5	85 ± 2	0.29 ± 0.01	0.5	C	S190
C7.2 (U ₇)	640	389	32 ± 1	71 ± 1	0.45 ± 0.02	0.6	C	S191
C7.2 (U ₇)	740	441	40 ± 0.8	60 ± 1	0.67 ± 0.02	1.0	C	S192
C7.2 (U ₇)	740	441	56 ± 2	78 ± 3	0.72 ± 0.03	0.8	SPC	S193
C7.2 (U ₇)	840	493	47 ± 2	52 ± 1	0.91 ± 0.05	1.1	C	S194
C7.2 (U ₇)	1040	595	62 ± 1	41 ± 0.8	1.51 ± 0.04	2.0	C	S195
C7.2 (U ₇)	1040	595	91 ± 2	52 ± 2	1.73 ± 0.08	1.7	SPC	S196
C7.9 (U ₇)	440	280	69 ± 2	518 ± 5	0.13 ± 0.03	0.2	SPC	S197
C7.9 (U ₇)	490	308	90 ± 3	457 ± 13	0.20 ± 0.01	0.3	SPC	S198
C7.9 (U ₇)	540	335	119 ± 3	394 ± 5	0.30 ± 0.01	0.4	SPC	S199
C7.9 (U ₇)	740	441	177 ± 3	322 ± 4	0.55 ± 0.01	0.7	SPC	S200
C7.9 (U ₇)	840	493	248 ± 7	241 ± 8	1.03 ± 0.04	1.0	SPC	S201
C7.9 (U ₇)	1040	595	277 ± 8	210 ± 6	1.32 ± 0.05	1.1	SPC	S202

*The fitting parameters of the cumulative FRET distribution—i.e. mean, variance, and mixing proportion of each of the two FRET components—become difficult to estimate when the mixing proportion of one of the components approaches unity, resulting in inaccurate estimates of $K_{\text{eq,bulk}}$ under these conditions. To obtain a better estimate of $K_{\text{eq,bulk}}$ at conditions when the folded state is greatly favored over the unfolded state (chosen as $K_{\text{obs}} > 10$), the mean and variance of the low FRET component were kept constant during fitting under these conditions.

Table S8. Data for TL/TLR variants in the U₇-tether minimal construct at a range of Na⁺ concentrations. Total concentration of Na⁺ contains 40 mM Na⁺ from Na-MOPS buffer. k_{dock} and k_{undock} values were determined from a two-state HMM model using the SMART software package¹ as described previously^{10,12} and equilibrium constants (K_{obs}) were determined from the ratio $k_{\text{dock}}/k_{\text{undock}}$. Standard errors were determined from bootstrapping. Apparent equilibrium constants from cumulative data ($K_{\text{eq,bulk}}$) were determined from integration of the areas under cumulative FRET distributions shown in supporting summary figures (Figure S203-S241). Raw data are available to download.¹¹ Repeated measurements are from independent experiments performed on different days. Data were collected with either an EMCCD camera (C) or a single-photon counting (SPC) setup as described in Materials and Methods.

Construct	[Na ⁺] (mM)	a _± (mM)	k_{dock} (s ⁻¹)	k_{undock} (s ⁻¹)	K_{obs}	$K_{\text{eq,bulk}}$	Method	Summary Fig. ¹¹
11ntR (U ₇)	90	71	3.2 ± 0.2	12.8 ± 0.5	0.25 ± 0.02	0.3	SPC	S203
11ntR (U ₇)	140	106	10.1 ± 0.5	9.4 ± 0.7	1.1 ± 0.1	1.0	SPC	S204
11ntR (U ₇)	190	138	21 ± 1	7.8 ± 0.6	2.7 ± 0.3	2.0	SPC	S205
11ntR (U ₇)	240	170	36 ± 3	7.7 ± 0.9	4.6 ± 0.6	3.3	SPC	S206
11ntR (U ₇)	290	202	47 ± 1	6.0 ± 0.2	7.8 ± 0.4	9.1	C	S207
11ntR (U ₇)	340	233	80 ± 8	5.5 ± 0.7	15 ± 2	5.3*	SPC	S208
11ntR (U ₇)	340	233	60 ± 2	5.5 ± 0.2	11.1 ± 0.6	8.3*	C	S209
11ntR_AC (U ₇)	290	202	5.6 ± 0.2	17.6 ± 0.6	0.32 ± 0.02	0.5	C	S210
11ntR_AC (U ₇)	390	264	10.0 ± 0.4	14.3 ± 0.5	0.70 ± 0.03	0.8	C	S211
11ntR_AC (U ₇)	440	294	13.3 ± 0.3	13.6 ± 0.3	0.98 ± 0.03	1.1	C	S212
11ntR_AC (U ₇)	490	324	17.8 ± 0.5	12.0 ± 0.4	1.49 ± 0.06	1.6	C	S213
11ntR_AC (U ₇)	540	354	20.5 ± 0.5	11.0 ± 0.4	1.85 ± 0.08	2.0	C	S214
11ntR_AC (U ₇)	590	384	25.2 ± 0.8	10.0 ± 0.3	2.5 ± 0.1	2.5	C	S215
11ntR_AC (U ₇)	840	534	50 ± 1	6.9 ± 0.4	7.2 ± 0.5	7.8	C	S216
11ntR_A/CC (U ₇)	140	106	11.0 ± 0.3	19.3 ± 0.5	0.57 ± 0.02	0.6	SPC	S217
11ntR_A/CC (U ₇)	190	138	20.0 ± 0.8	17.2 ± 0.7	1.16 ± 0.07	1.0	SPC	S218
11ntR_A/CC (U ₇)	240	170	30 ± 1	16 ± 1	1.9 ± 0.1	1.6	SPC	S219
11ntR_A/CC (U ₇)	290	202	42 ± 1	13.8 ± 0.3	3.0 ± 0.1	2.9	SPC	S220
11ntR_A/CC (U ₇)	340	233	55 ± 4	13 ± 2	4.3 ± 0.6	2.5	SPC	S221
11ntR_A/CC (U ₇)	440	294	107 ± 5	10.1 ± 0.7	10.5 ± 0.9	7.8	SPC	S222
C7.10 (U ₇)	340	233	4.6 ± 0.3	44 ± 7	0.11 ± 0.02	0.2	C	S223
C7.10 (U ₇)	440	294	6.4 ± 0.2	38.9 ± 0.8	0.17 ± 0.01	0.3	C	S224
C7.10 (U ₇)	640	414	12.0 ± 0.3	27.8 ± 0.6	0.43 ± 0.02	0.6	C	S225
C7.10 (U ₇)	840	534	16.8 ± 0.5	23.2 ± 0.4	0.73 ± 0.03	0.9	C	S226
C7.10 (U ₇)	1040	653	21.9 ± 0.8	19.5 ± 0.4	1.12 ± 0.01	1.3	C	S227
C7.10 (U ₇)	1240	773	28 ± 1	16.7 ± 0.9	1.7 ± 0.1	2.0	C	S228
C7.2 (U ₇)	390	264	19.8 ± 0.6	56 ± 1	0.36 ± 0.01	0.5	C	S229
C7.2 (U ₇)	440	294	23.7 ± 0.4	53 ± 1	0.45 ± 0.01	0.5	C	S230
C7.2 (U ₇)	490	324	28.6 ± 0.6	43.7 ± 0.5	0.65 ± 0.02	0.9	C	S231
C7.2 (U ₇)	540	354	33.6 ± 0.8	40.5 ± 0.9	0.83 ± 0.03	1.0	C	S232
C7.2 (U ₇)	590	384	36.9 ± 0.9	35.5 ± 0.5	1.04 ± 0.03	1.3	C	S233
C7.2 (U ₇)	640	414	40 ± 2	33 ± 2	1.21 ± 0.08	1.2	C	S234
C7.2 (U ₇)	740	474	52 ± 1	26.3 ± 0.4	1.96 ± 0.06	2.5	C	S235
C7.2 (U ₇)	840	534	59 ± 2	22.1 ± 0.6	2.6 ± 0.1	2.9	C	S236
C7.2 (U ₇)	1040	653	70 ± 3	16.8 ± 0.6	4.2 ± 0.2	3.3	C	S237
C7.9 (U ₇)	540	354	101 ± 5	365 ± 20	0.28 ± 0.02	0.5	SPC	S238
C7.9 (U ₇)	640	414	167 ± 9	269 ± 16	0.62 ± 0.05	0.7	SPC	S239
C7.9 (U ₇)	740	774	192 ± 3	234 ± 3	0.82 ± 0.02	0.8	SPC	S240
C7.9 (U ₇)	1040	653	300 ± 11	141 ± 5	2.1 ± 0.1	2.0	SPC	S241

*The fitting parameters of the cumulative FRET distribution—i.e. mean, variance, and mixing proportion of each of the two FRET components—become difficult to estimate when the mixing proportion of one of the components approaches unity, resulting in inaccurate estimates of $K_{\text{eq,bulk}}$ under these conditions. To obtain a better estimate of $K_{\text{eq,bulk}}$ at conditions when the folded state is greatly favored over the unfolded state (chosen as $K_{\text{obs}} > 10$), the mean and variance of the low FRET component were kept constant during fitting under these conditions.

Table S9. Data for TL/TLR variants in the U₇-tether minimal construct at a range of Rb⁺ concentrations. Total concentration of Rb⁺ contains 40 mM Rb⁺ from Rb-MOPS buffer. k_{dock} and k_{undock} values were determined from a two-state HMM using the SMART software package¹ as described previously^{10,12} model and equilibrium constants (K_{obs}) were determined from the ratio $k_{\text{dock}}/k_{\text{undock}}$. Standard errors were determined from bootstrapping. Apparent equilibrium constants from cumulative data ($K_{\text{eq,bulk}}$) were determined from integration of the areas under cumulative FRET distributions shown in summary figures.¹¹ Repeated measurements are from independent experiments performed on different days. Data were collected with either an EMCCD camera (C) or a single-photon counting (SPC) setup as described in Materials and Methods.

Construct	[Rb ⁺] (mM)	a _± (mM)	k_{dock} (s ⁻¹)	k_{undock} (s ⁻¹)	K_{obs}	$K_{\text{eq,bulk}}$	Method	Summary Figure ¹¹
11ntR (U ₇)	90	74	1.3 ± 0.1	11.5 ± 0.5	0.12 ± 0.01	0.2	C	S242
11ntR (U ₇)	90	74	2.2 ± 0.1	13.7 ± 0.8	0.16 ± 0.01	0.2	SPC	S243
11ntR (U ₇)	140	108	3.5 ± 0.2	9.4 ± 0.3	0.37 ± 0.03	0.5	C	S244
11ntR (U ₇)	140	108	9.8 ± 0.8	9.2 ± 0.5	1.1 ± 0.1	1.0	SPC	S245
11ntR (U ₇)	190	141	9.9 ± 0.5	10.1 ± 0.5	0.99 ± 0.07	1.0	SPC	S246
11ntR (U ₇)	240	172	12.3 ± 0.5	7.6 ± 0.2	1.63 ± 0.08	1.8	C	S247
11ntR (U ₇)	240	172	16.8 ± 0.8	8.2 ± 0.4	2.0 ± 0.1	1.9	SPC	S248
11ntR (U ₇)	340	232	25.0 ± 0.8	5.5 ± 0.3	4.6 ± 0.3	4.4	C	S249
11ntR (U ₇)	340	232	71 ± 4	5.2 ± 0.2	13.6 ± 0.9	10.2	SPC	S250
11ntR AC (U ₇)	290	203	4.7 ± 0.1	12.9 ± 0.3	0.36 ± 0.01	0.5	C	S251
11ntR AC (U ₇)	340	232	5.9 ± 0.2	11.0 ± 0.4	0.54 ± 0.02	0.6	C	S252
11ntR AC (U ₇)	390	261	7.9 ± 0.3	10.3 ± 0.4	0.77 ± 0.04	0.8	C	S253
11ntR AC (U ₇)	440	290	10.8 ± 0.3	9.7 ± 0.3	1.11 ± 0.04	1.3	C	S254
11ntR AC (U ₇)	540	346	15.1 ± 0.4	7.7 ± 0.3	1.95 ± 0.08	1.9	C	S255
11ntR AC (U ₇)	640	400	21.8 ± 0.5	6.9 ± 0.2	3.1 ± 0.1	3.1	C	S256
11ntR A/CC (U ₇)	90	74	6.4 ± 0.3	32 ± 1	0.20 ± 0.01	0.2	SPC	S257
11ntR A/CC (U ₇)	140	108	20.6 ± 0.6	28.5 ± 0.8	0.72 ± 0.03	0.7	SPC	S258
11ntR A/CC (U ₇)	240	172	37 ± 1	25.2 ± 0.7	1.45 ± 0.06	1.3	SPC	S259
11ntR A/CC (U ₇)	340	232	123 ± 8	19.0 ± 0.9	6.5 ± 0.5	4.0	SPC	S260
C7.10 (U ₇)	440	290	4.8 ± 0.3	49 ± 2	0.10 ± 0.01	0.2	C	S261
C7.10 (U ₇)	640	400	8.3 ± 0.3	40.4 ± 0.9	0.21 ± 0.01	0.3	C	S262
C7.10 (U ₇)	840	504	10.7 ± 0.4	33.1 ± 0.7	0.32 ± 0.01	0.4	C	S263
C7.10 (U ₇)	1040	606	14.4 ± 0.6	29.3 ± 0.8	0.49 ± 0.02	0.7	C	S264
C7.10 (U ₇)	1240	707	16.5 ± 0.4	25.6 ± 0.5	0.65 ± 0.02	0.8	C	S265
C7.2 (U ₇)	640	400	29.7 ± 0.8	90.3 ± 2	0.33 ± 0.01	0.5	C	S266
C7.2 (U ₇)	740	453	34 ± 1	75 ± 2	0.46 ± 0.02	0.7	C	S267
C7.2 (U ₇)	840	504	40.3 ± 0.8	69 ± 2	0.59 ± 0.02	0.9	C	S268
C7.2 (U ₇)	1040	606	50.8 ± 0.9	54 ± 1	0.94 ± 0.03	1.5	C	S269
C7.2 (U ₇)	1240	707	61 ± 1	46 ± 1	1.33 ± 0.04	1.8	C	S270
C7.9 (U ₇)	540	346	125 ± 5	431 ± 7	0.29 ± 0.01	0.4	SPC	S271
C7.9 (U ₇)	640	400	194 ± 12	335 ± 24	0.58 ± 0.05	0.7	SPC	S272
C7.9 (U ₇)	740	453	213 ± 3	326 ± 3	0.66 ± 0.01	0.7	SPC	S273
C7.9 (U ₇)	840	504	190 ± 2	356 ± 16	0.53 ± 0.03	0.6	SPC	S274
C7.9 (U ₇)	1040	606	298 ± 8	234 ± 9	1.27 ± 0.06	1.1	SPC	S275

*The fitting parameters of the cumulative FRET distribution—i.e. mean, variance, and mixing proportion of each of the two FRET components—become difficult to estimate when the mixing proportion of one of the components approaches unity, resulting in inaccurate estimates of $K_{\text{eq,bulk}}$ under these conditions. To obtain a better estimate of $K_{\text{eq,bulk}}$ at conditions when the folded state is greatly favored over the unfolded state (chosen as $K_{\text{obs}} > 10$), the mean and variance of the low FRET component were kept constant during fitting under these conditions.

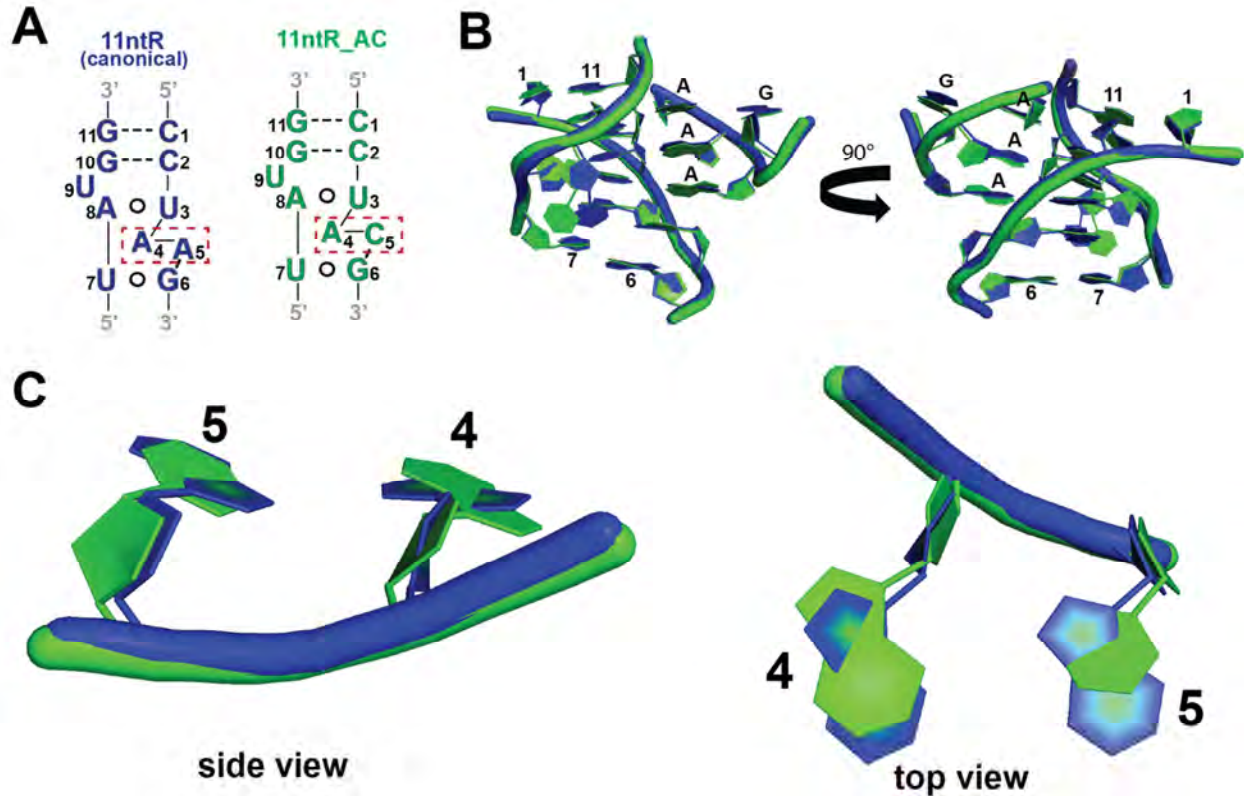


Figure S1. Comparison of tetraloop-receptor variants 11ntR and 11ntR_AC in their docked conformation as observed in crystal structures. (A) Sequence and secondary structures of tetraloop-receptors 11ntR (blue) and 11ntR_AC (green). Residues 4 and 5 (red dashed box) comprise the dinucleotide platform that differs between these two variants. (B) Structures of GAAA/11ntR (blue) and GAAA/11ntR_AC (green) were aligned in Pymol with a total RMSD of 0.58 Å. GAAA/11ntR was extracted from the crystal structure of the P4-P6 domain (PDB 1GID¹³) and GAAA/11ntR_AC was extracted from crystal structure of RNase P (PDB 1NBS¹⁴). (C) Enlarged view of the AA-platform (blue) and the AC-platform (green) in variants 11ntR and 11ntR_AC, respectively.

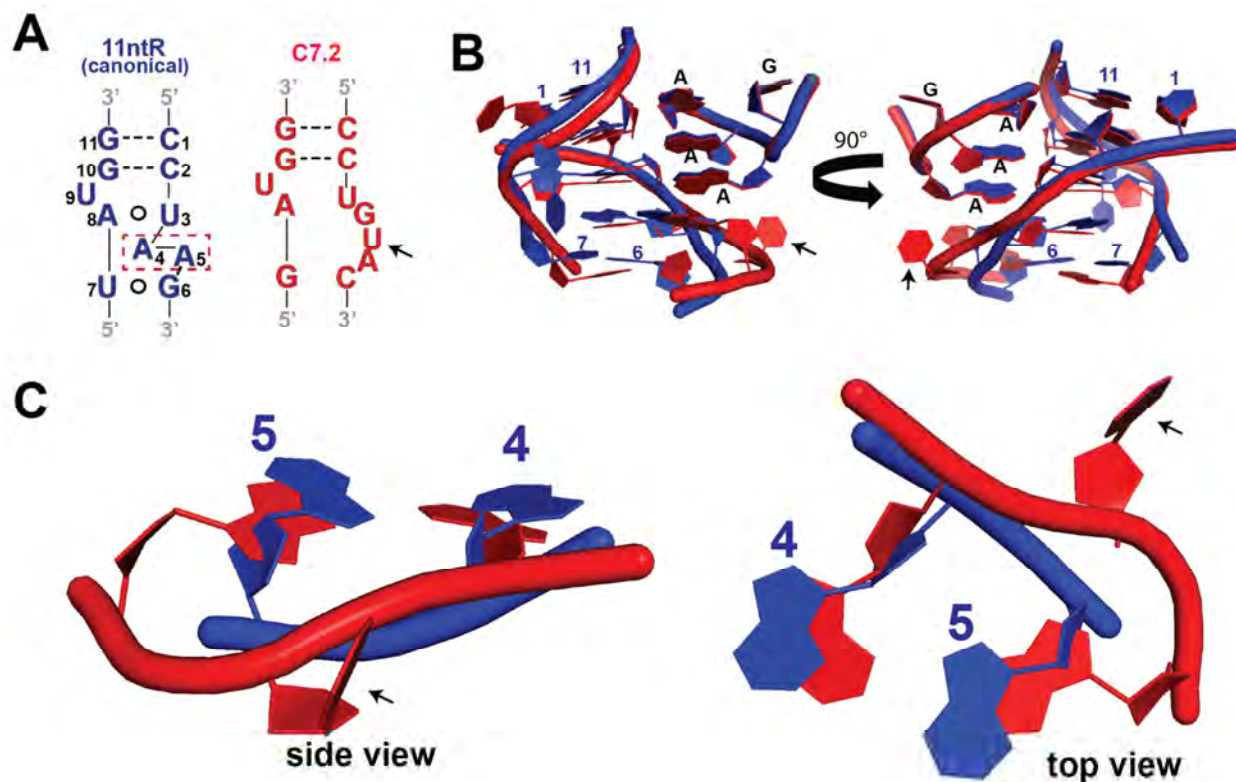


Figure S2. Comparison of experimentally determined structure of TL/TLR GAAA/11ntR and computationally predicted structure of GAAA/C7.2. Structure of GAAA/C7.2 was predicted by stepwise ansatz modeling and tested by single-nucleotide-resolution chemical probing.¹⁵ (A) Sequence of tetraloop-receptors 11ntR (blue) and C7.2 (red). Residues 4 and 5 in 11ntR (red box) comprise the AA-platform. Arrow points to U residue computationally predicted to bulge out in the docked conformation of C7.2. (B) Structure alignment of GAAA/11ntR (PDB 1GID¹³) and computationally predicted structure of GAAA/C7.2. (C) Enlarged view of the AA-platform (blue) and the GUA platform (red) in variants 11ntR and C7.2 respectively. The U residue in the GUA platform is predicted to bulge out.

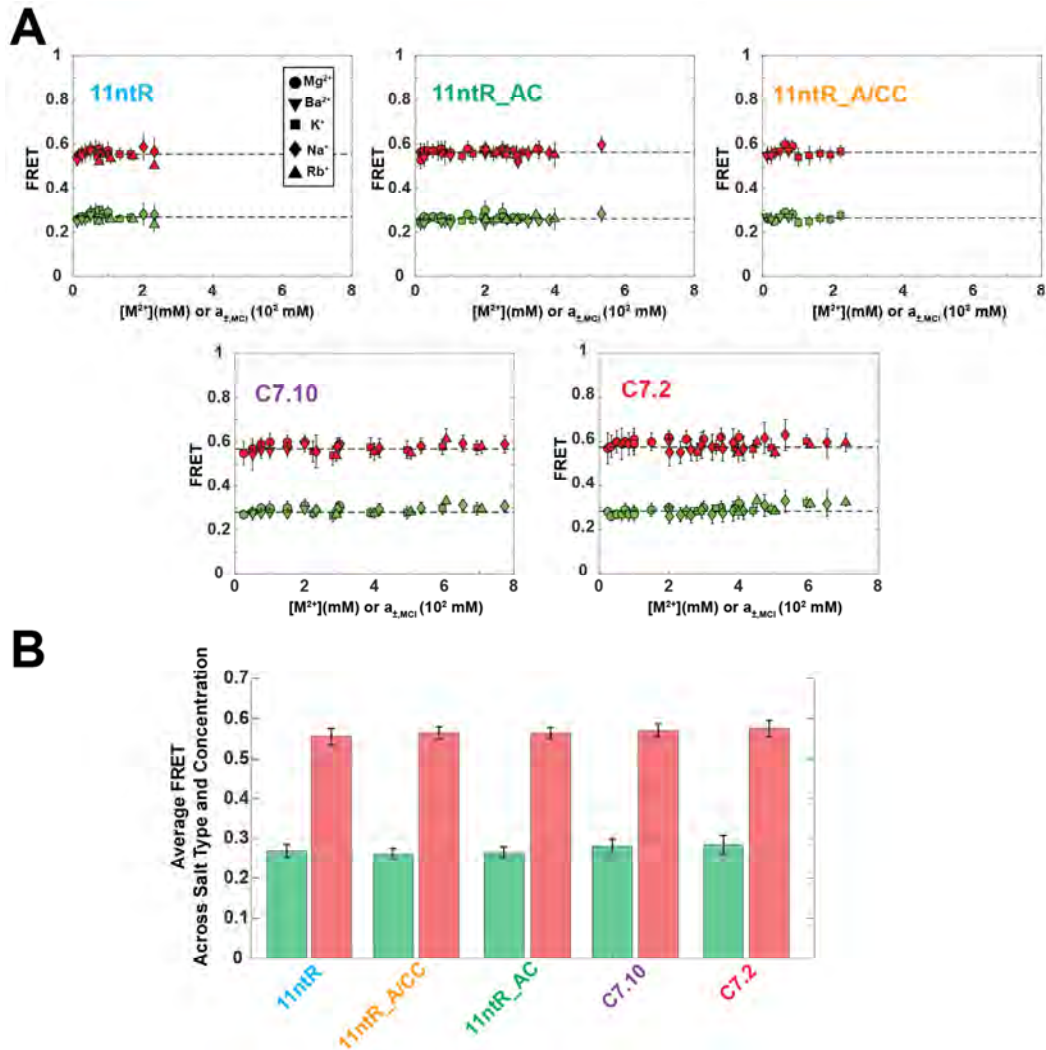
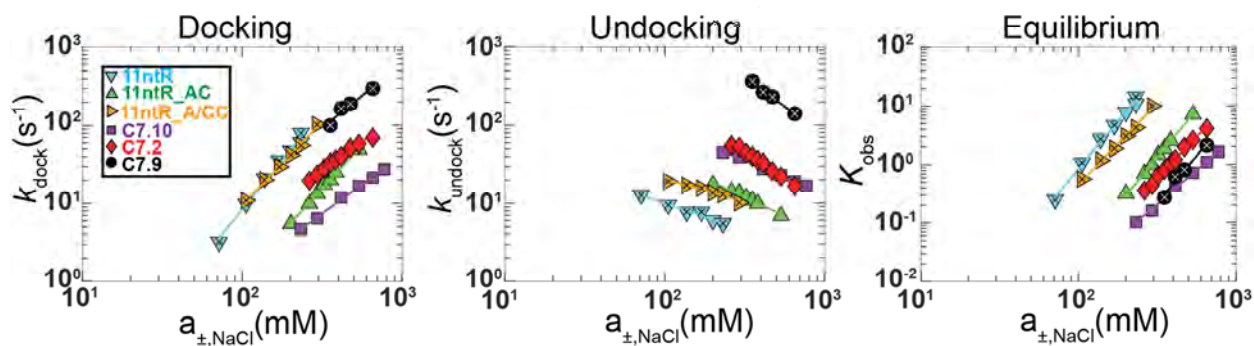


Figure S3. Average FRET states across salt conditions for TL/TLR variants embedded in the U₇-tether smFRET minimal construct. (A) Average low (green) and high (red) FRET states obtained from two-state HMM fits across different concentrations of Mg²⁺ (circles), Ba²⁺ (inverted triangles) and mean activities of KCl (squares), NaCl (diamonds), and RbCl (triangles). Each data point represents the average of a population of single molecules (typically >100 molecules) and error bars are standard deviations. Dashed line is the average across all salt conditions. Data for Mg²⁺ and Ba²⁺ were collected in a background of 140 mM K⁺ (B) Average low (green) and high (red) FRET states across all salt conditions (dashed line in (A)) for each of the TL/TLR variants. Error bars are standard deviations of the averages. Variant C7.9 was not included as no data were collected for this variant with EMCCD camera due to its fast dynamics across all salt conditions tested. Variant C7.9 was characterized by single-photon counting only.

A NaCl



B RbCl

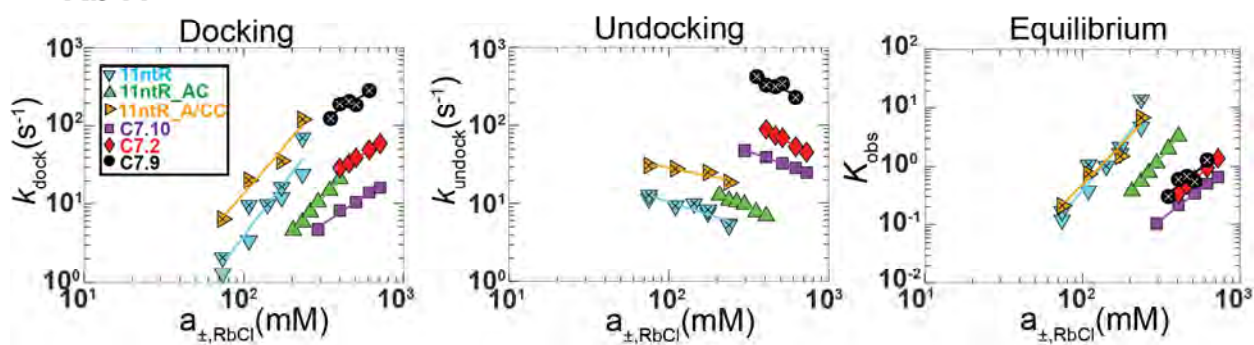


Figure S4. Docking kinetics and thermodynamics of TL/TLR variants in NaCl (A) and RbCl (B). To account for non-ideal electrolyte interactions the data are plotted as a function of the mean activity (a_{\pm}) of the salt solution. Average k_{dock} (left), k_{undock} (middle), and K_{obs} (right) values for each of the TL/TLR variants over a range of NaCl (A) and RbCl (B) concentrations. Data and errors are summarized in Tables S8 and S9 for NaCl and RbCl, respectively. Standard errors determined from bootstrapping were smaller than the size of the symbols. Data marked with an internal “X” were determined by single-photon counting. Linear fits were determined by least squares.

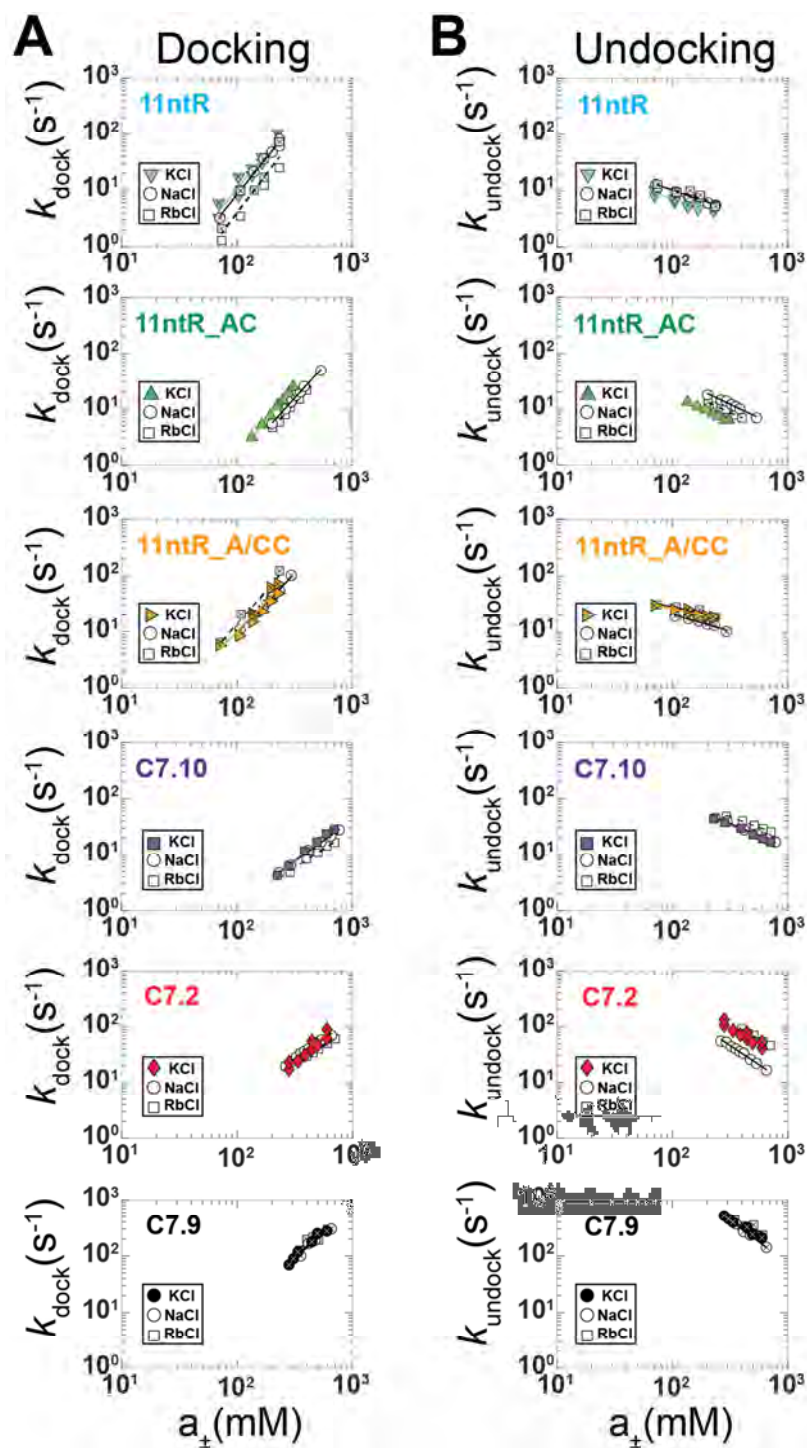


Figure S5. Docking kinetics of variants in different monovalent salts. To account for non-ideal electrolyte interactions the data are plotted as a function of the mean activity (a_{\pm}) of the salt solution. Average k_{dock} (A) and k_{undock} (B) as a function of the activity of KCl, NaCl and RbCl. Data and errors are summarized in Tables S7-S9. Standard errors determined from bootstrapping were smaller than the size of the symbols. Data marked with an internal “X” were determined by single-photon counting. Linear fits were obtained by least-squares.

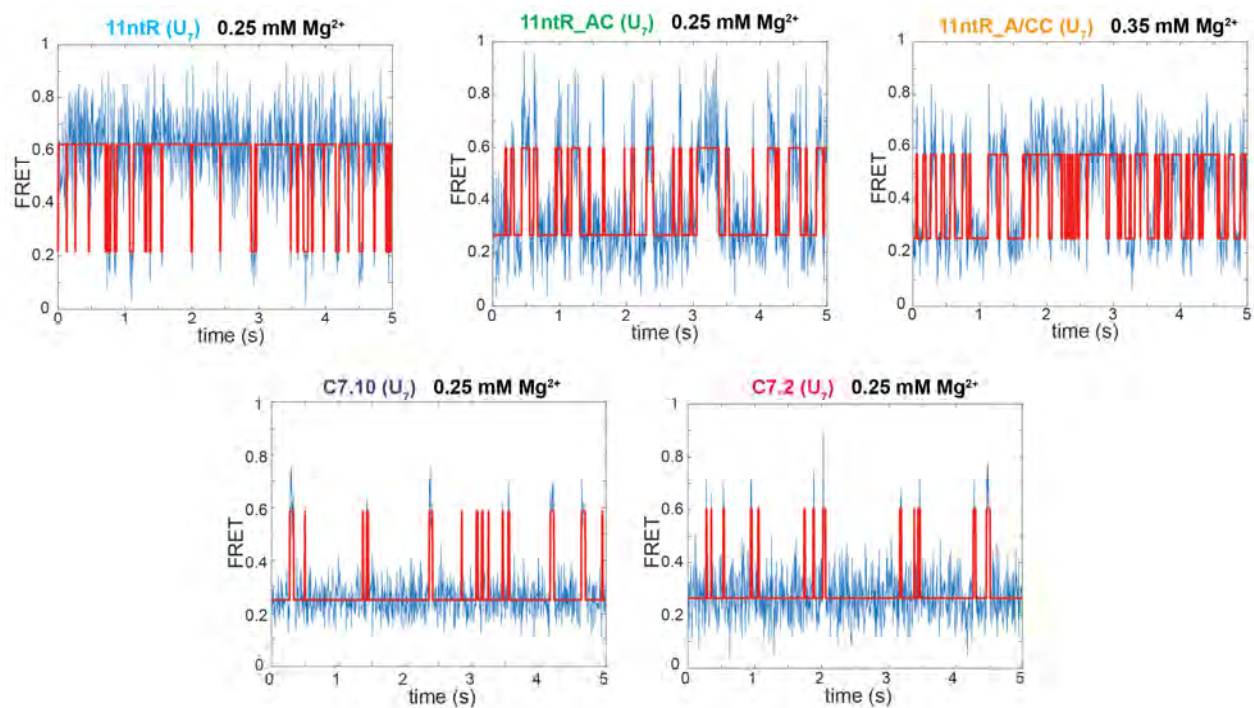


Figure S6. Sample FRET traces and vbFRET fits. vbFRET was one of the tools used to evaluate the optimum number of states. Shown above are traces collected by EMCCD camera at 5 ms. Blue trace is the FRET efficiency calculated as $I_A/(I_A + I_D)$ where I_A and I_D are the intensities of the acceptor and donor channels respectively and the red trace is a two-state state fit performed by vbFRET.

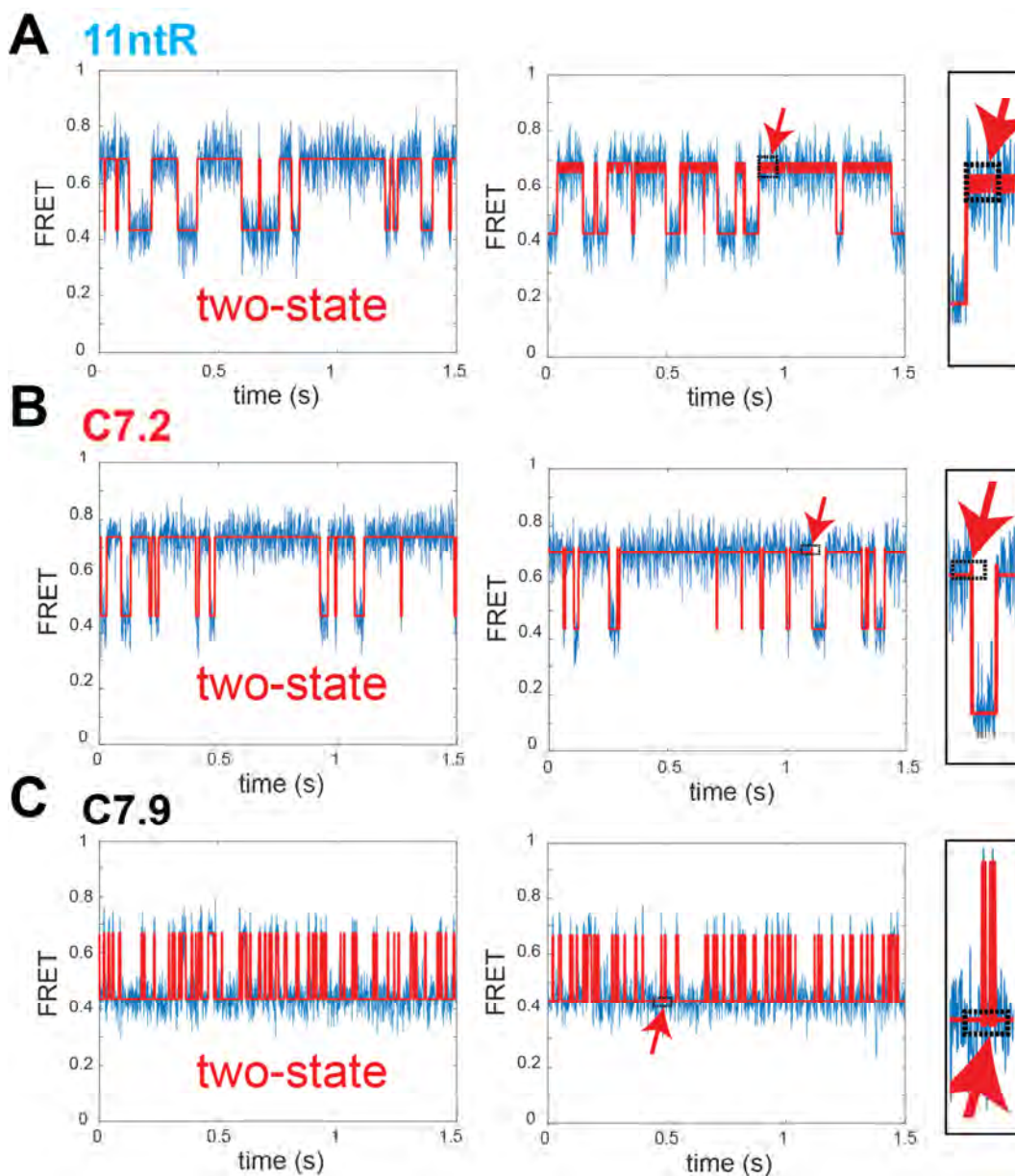


Figure S7. vbFRET analysis of single-photon counting (SPC; 1 ms binning) traces. vbFRET uses Bayesian statistics and evidence maximization to infer the optimum number of states in each trace. Sample vbFRET fits are shown for 11ntR at 0.25 mM Mg^{2+} (A), C7.2 at 3.0 mM Mg^{2+} (B), and C7.9 at 1.5 mM Mg^{2+} (C). Raw data (blue) and vbFRET fit (red) are shown. Examples of 1 ms traces that showed two-state behavior according to vbFRET analysis are shown on the left. A fraction of 1 ms traces showed three-state behavior according to vbFRET. Close-up views of apparent third state (red arrow) are shown on the far right. We concluded that the third FRET state is an artifact or, minimally, could not be reliably established. This conclusion is based on: (1) the third FRET state was short-lived (1 frame) and its FRET value was very similar to one of the other FRET states ($\Delta\text{FRET} < 0.04$), (2) the occurrence and value of the third FRET state was inconsistent between traces within the same dataset. The evidence outlined above suggests that two FRET states are sufficient to describe the data.

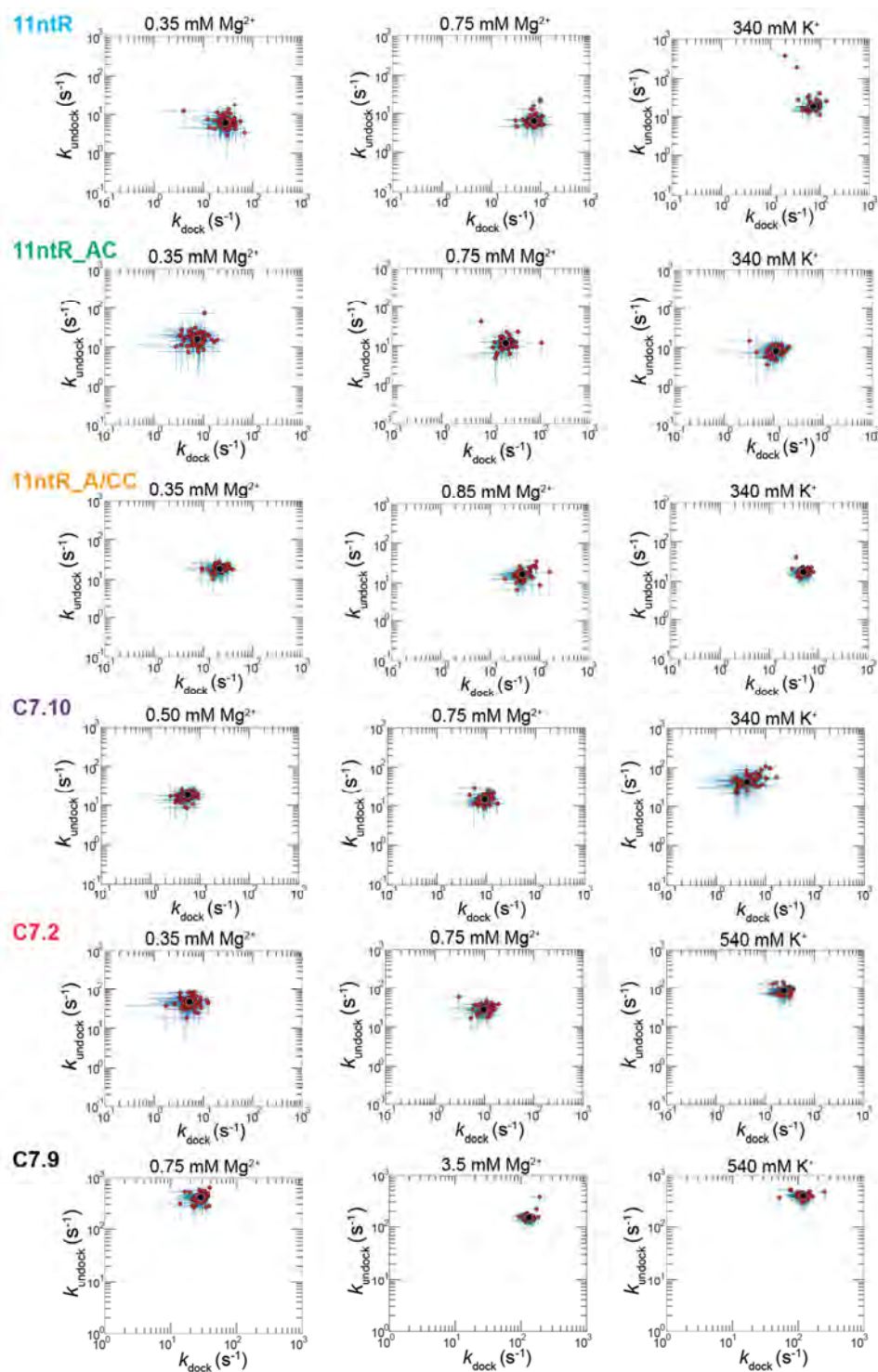


Figure S8. Error on individual HMM fits calculated by SMART for TL/TLR variants in the presence of Mg^{2+} and K^+ . The error on an individual HMM fit depends on the length of the trace, the signal to noise ratio (SNR), and the number of transitions in the trace.¹ Red circles are individual molecules, black larger circle is the median of the population, and the blue bars are 95% confidence intervals calculated using SMART. For clarity, only 70 randomly-selected molecules are shown.

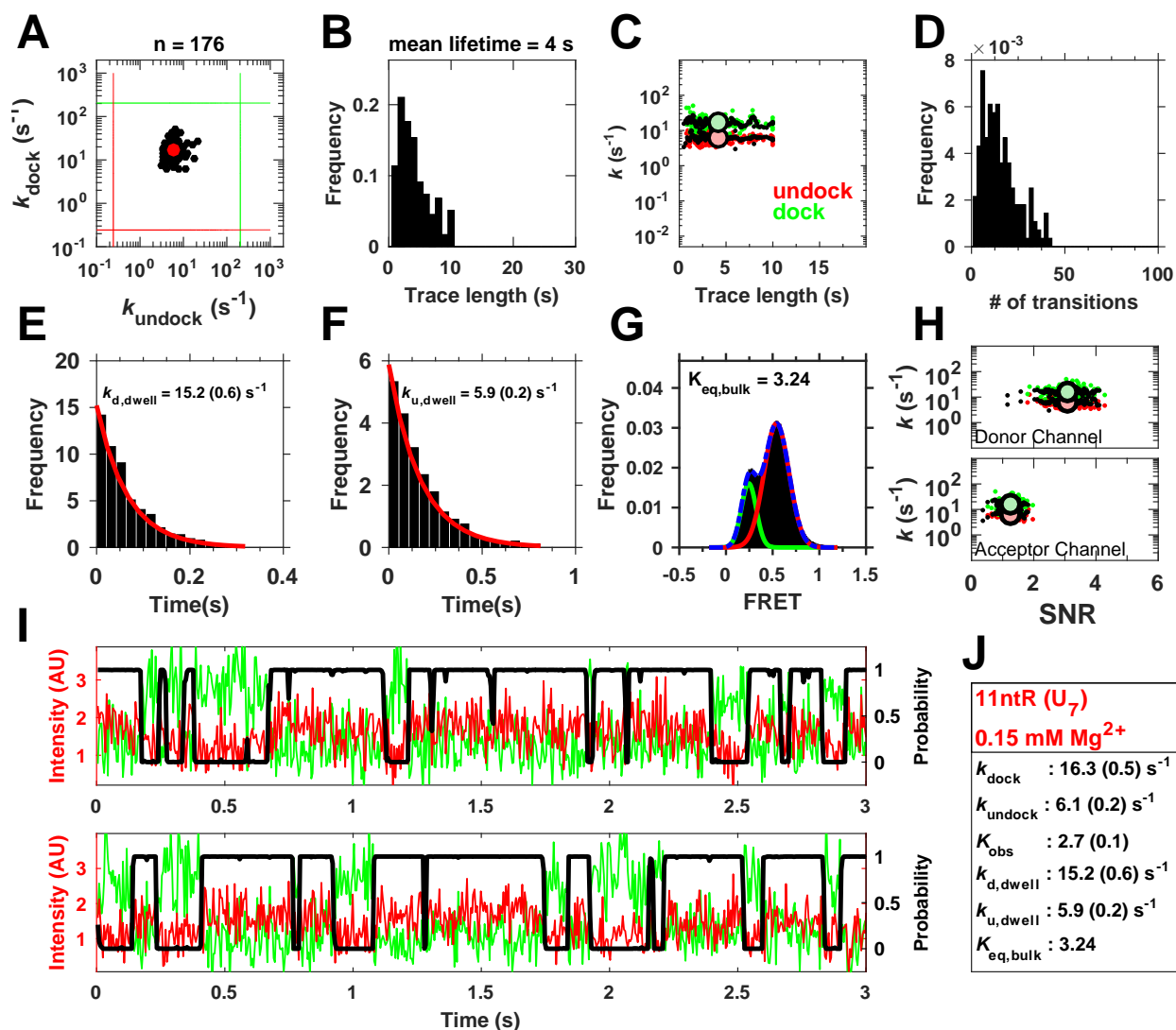


Figure S9. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

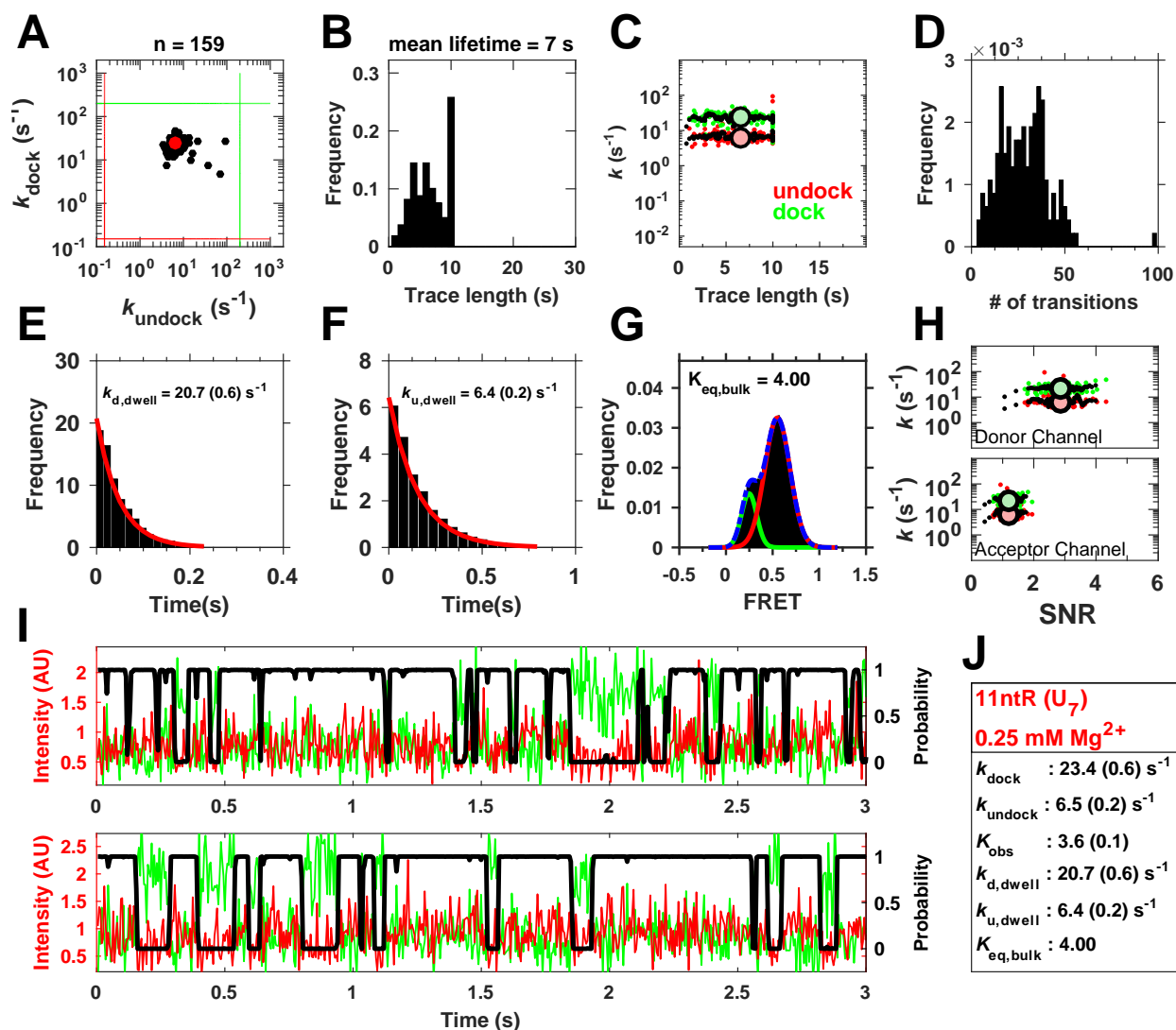


Figure S10. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

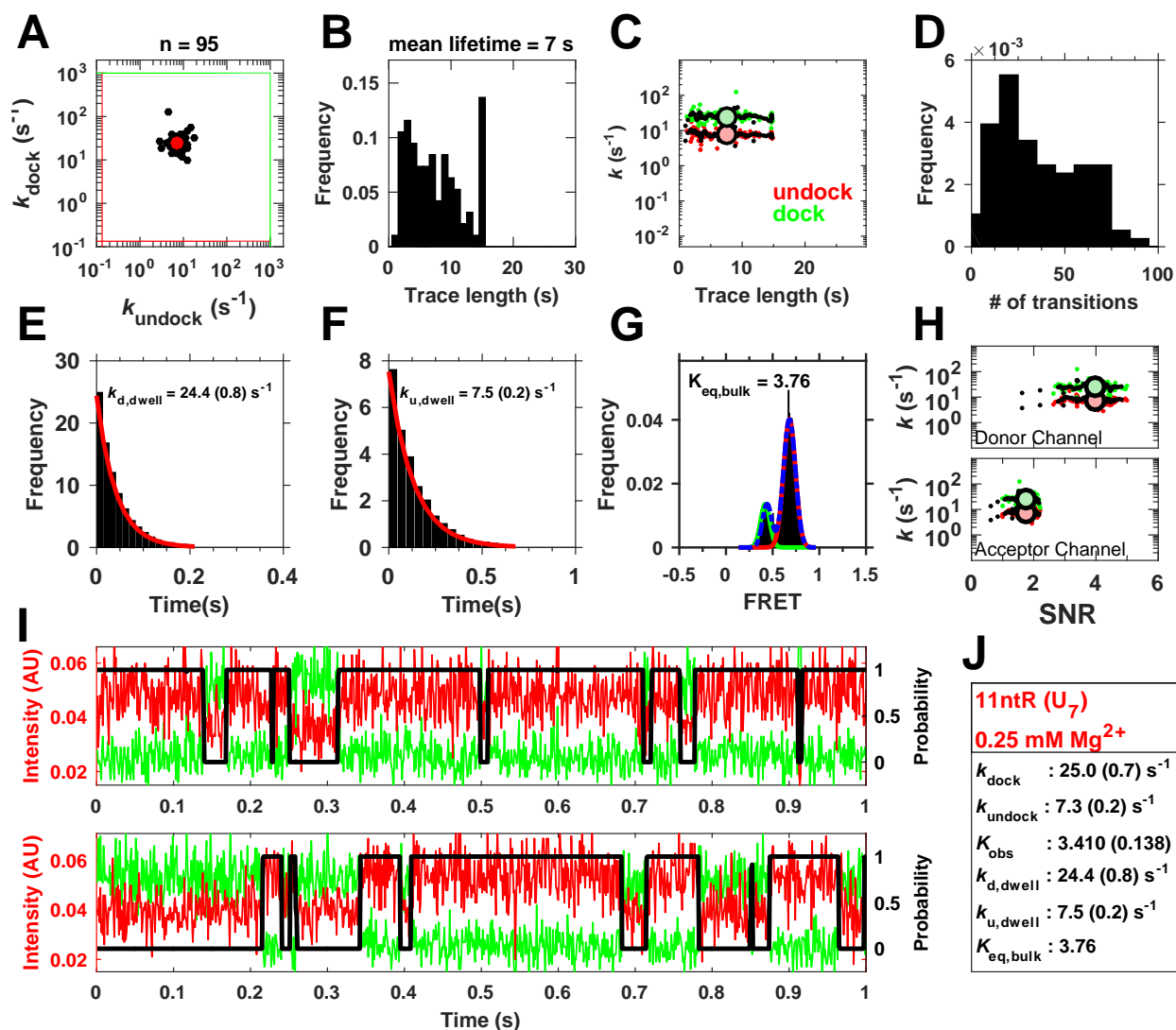


Figure S11. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

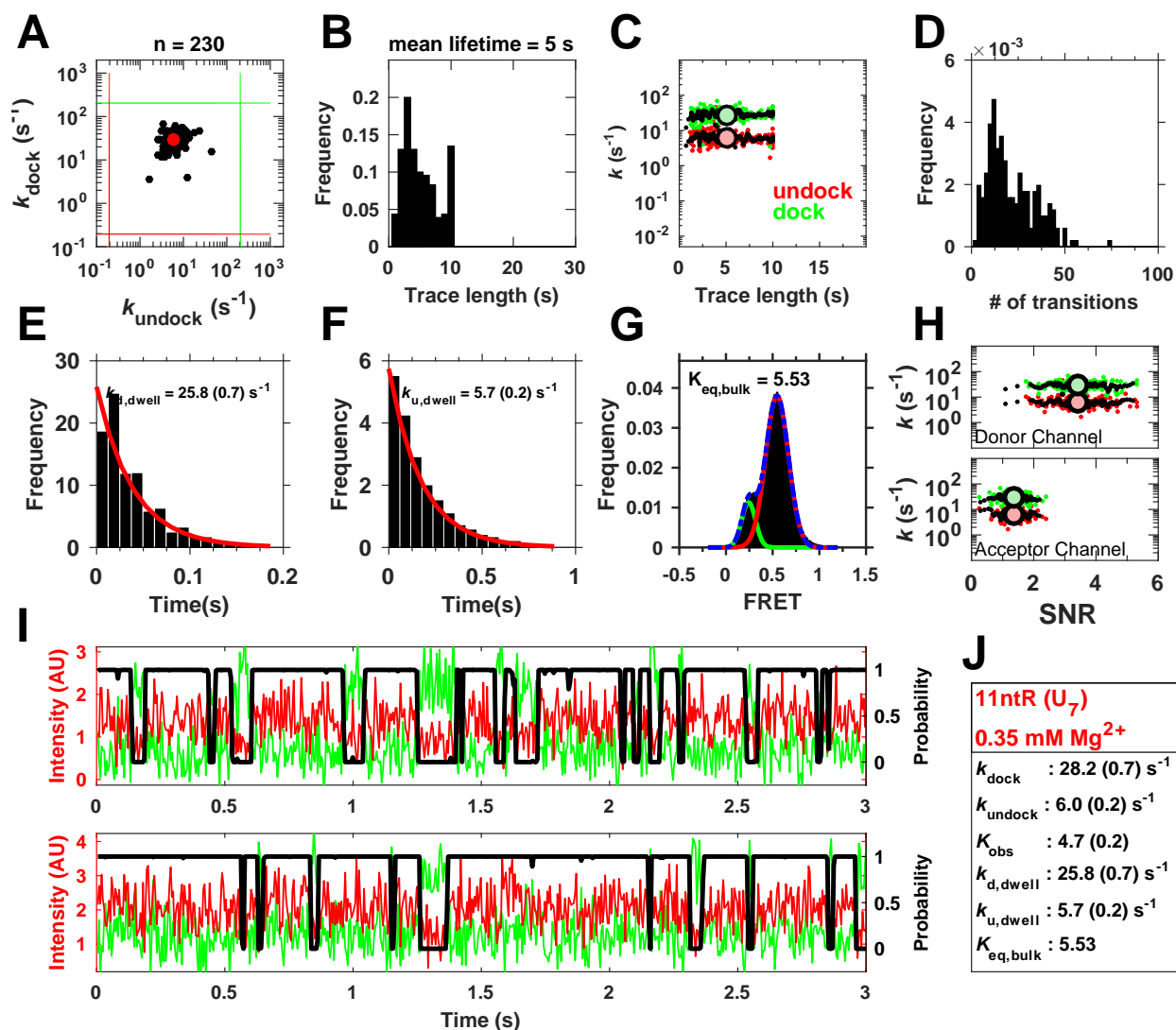


Figure S12. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

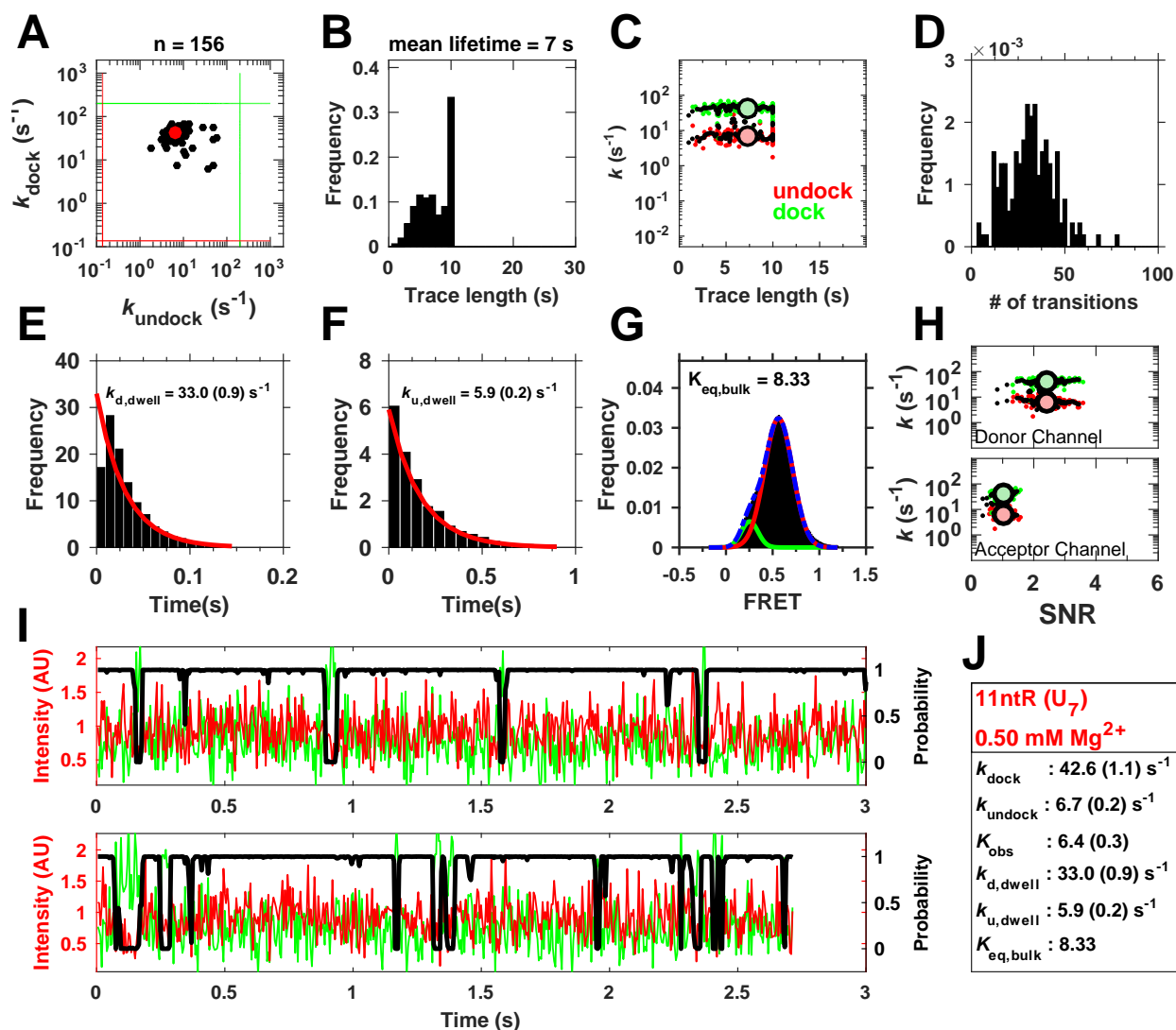


Figure S13. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

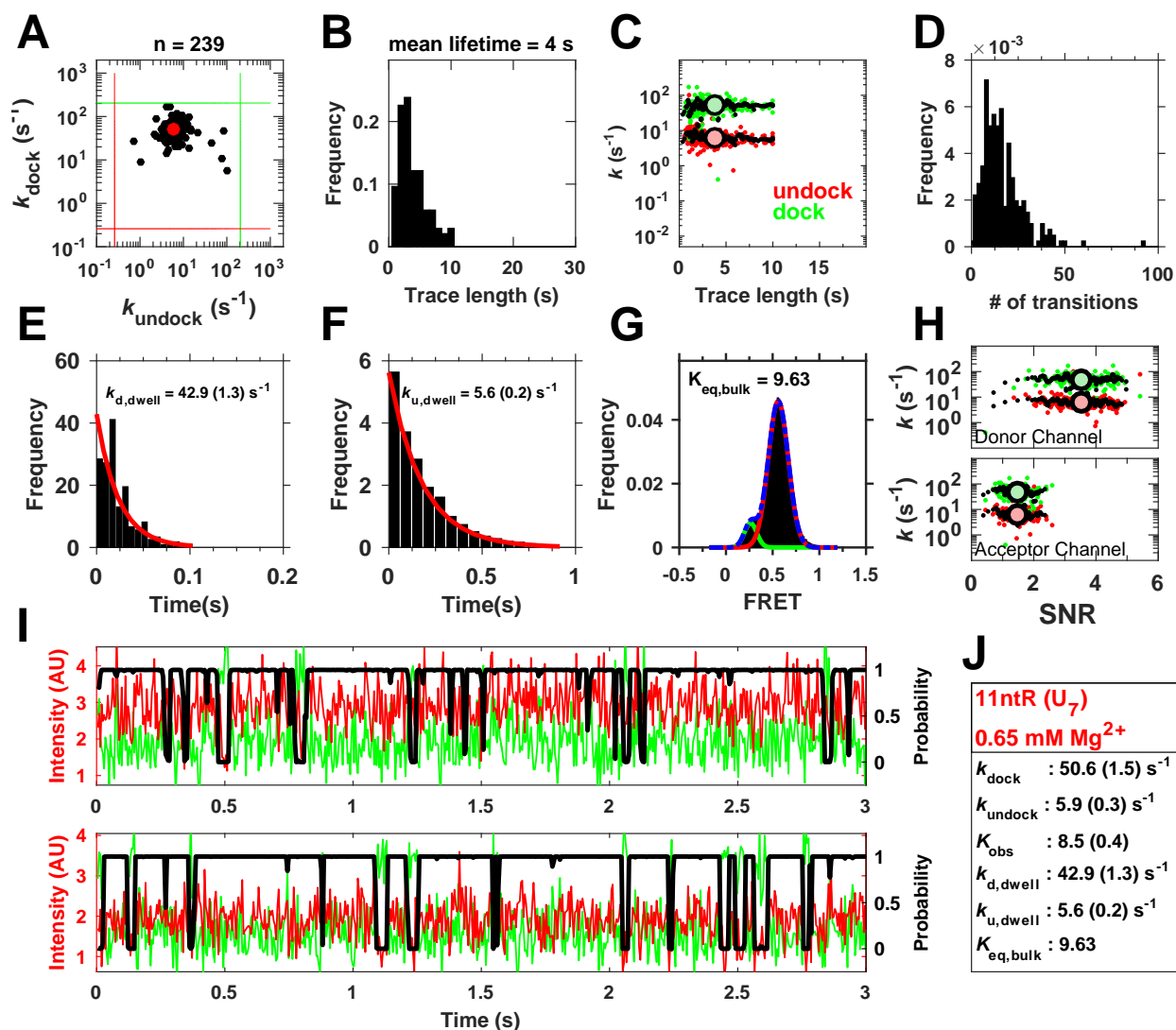


Figure S14. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

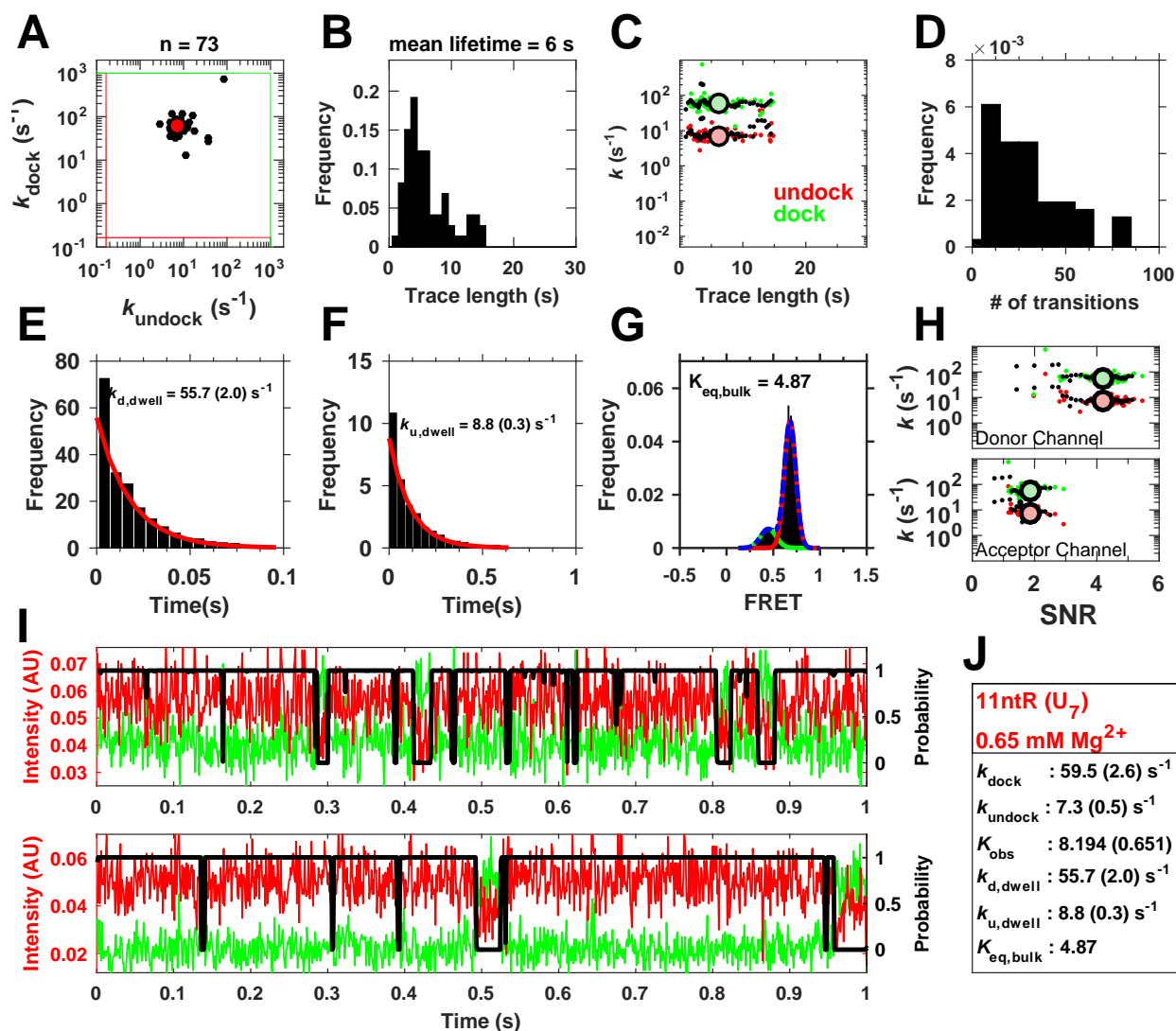


Figure S15. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

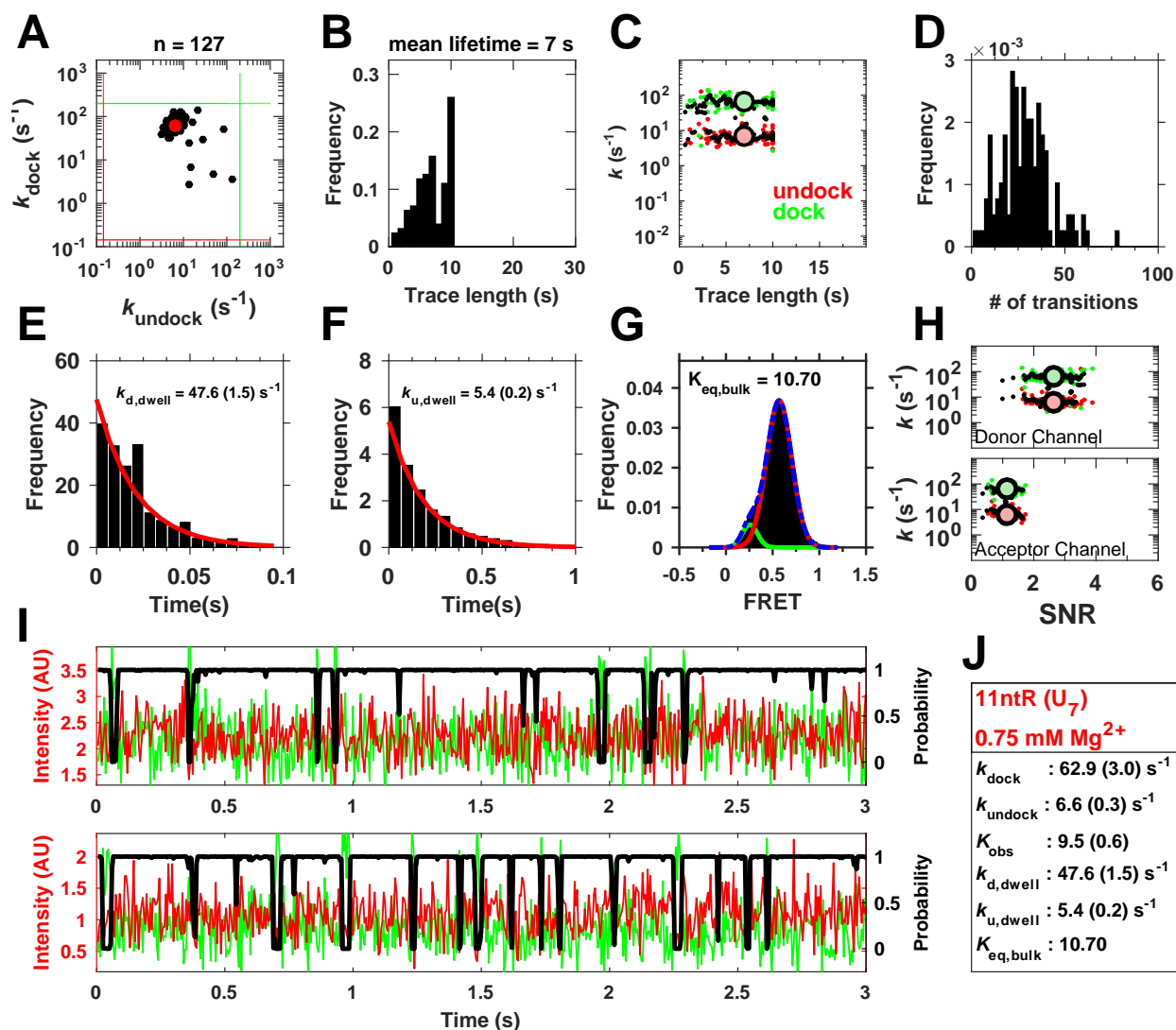


Figure S16. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

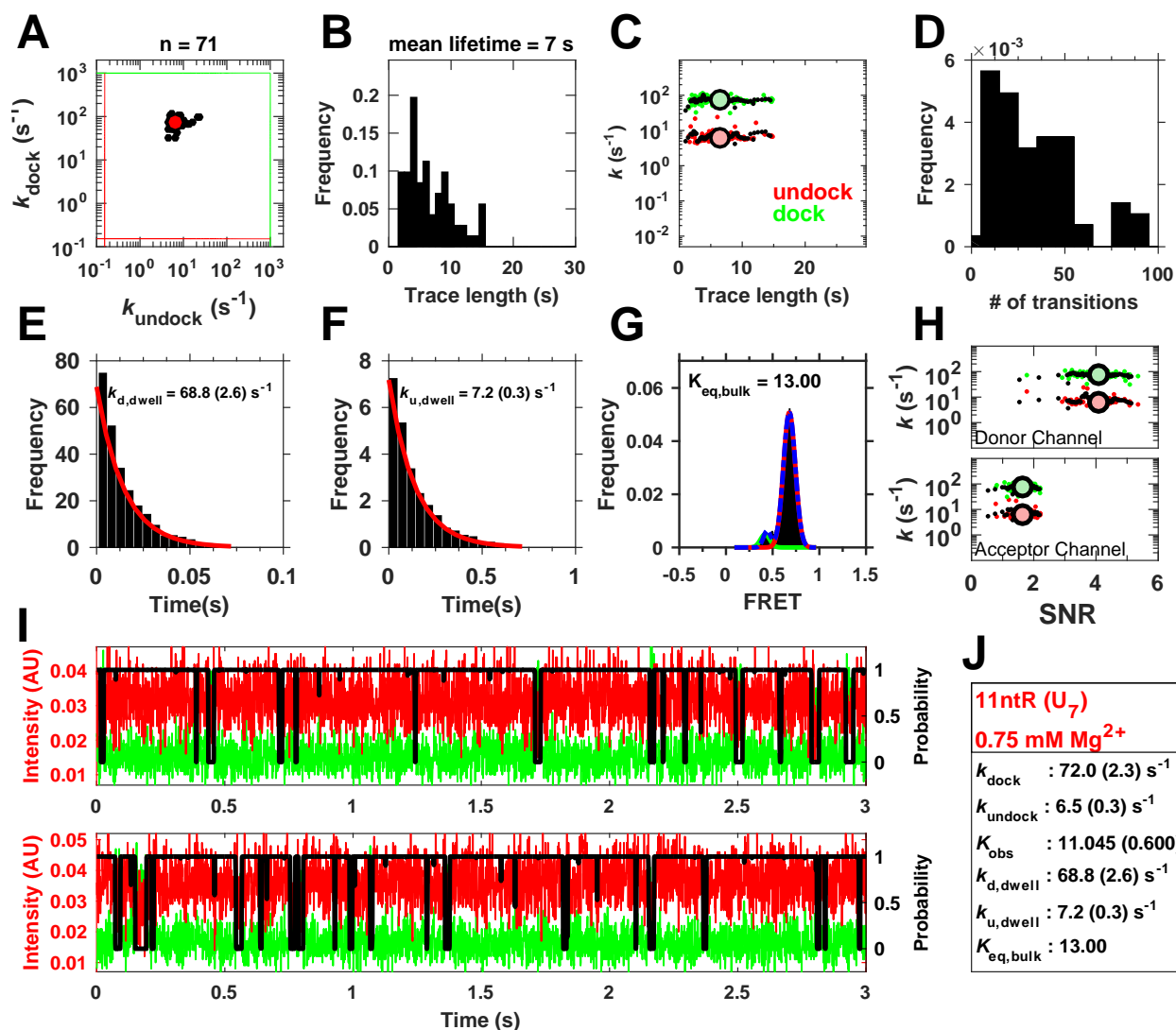


Figure S17. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

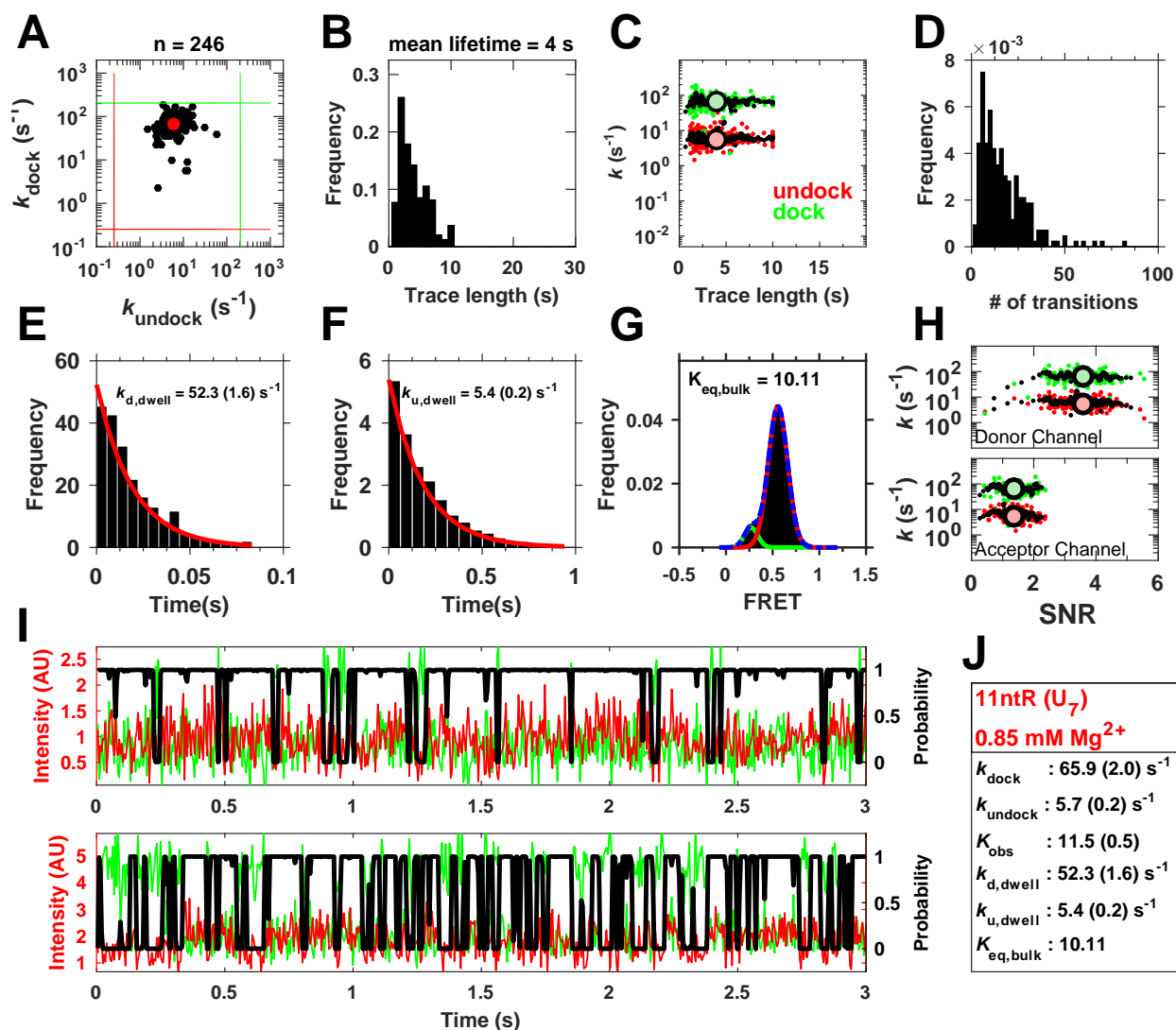


Figure S18. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

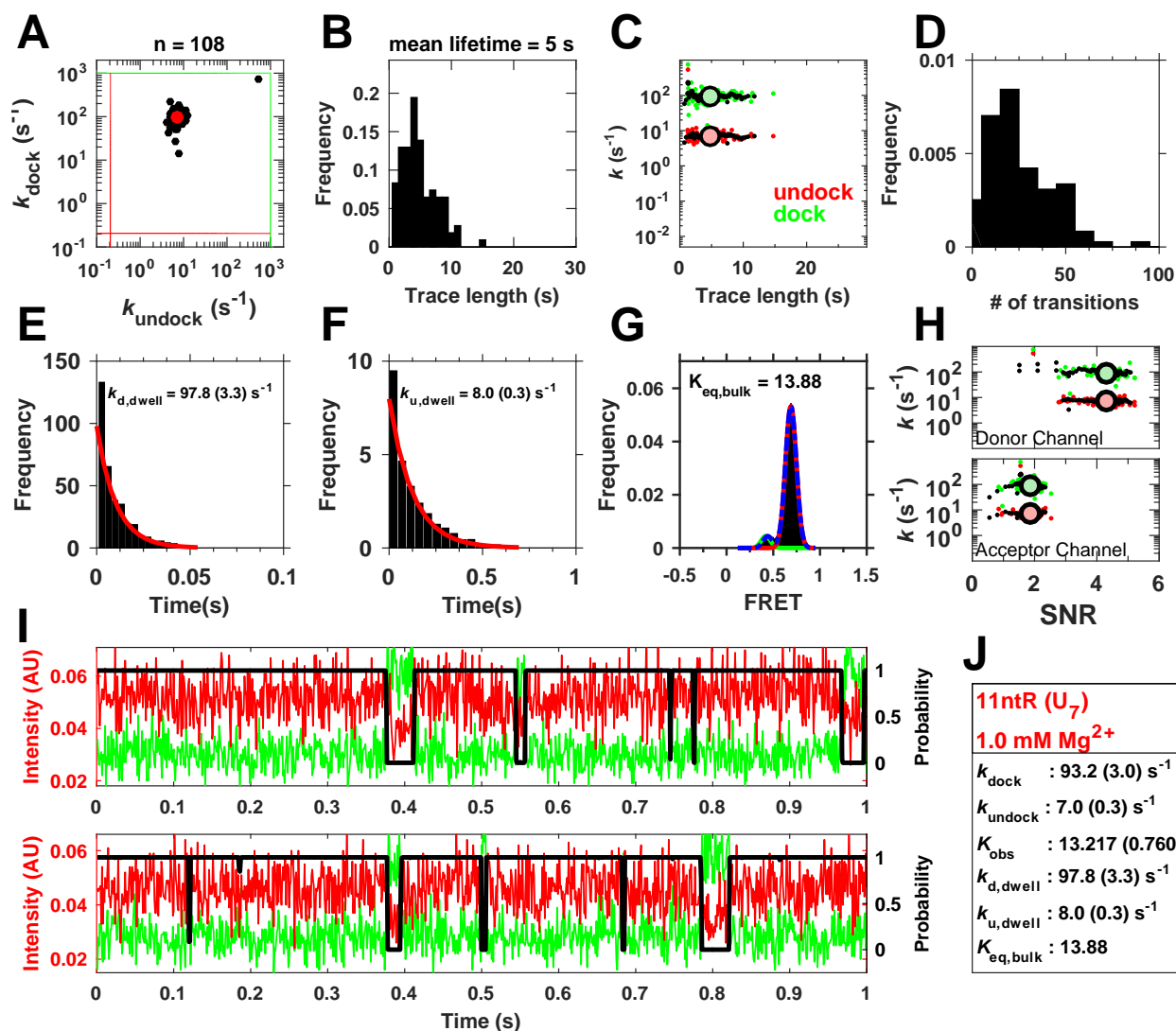


Figure S19. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

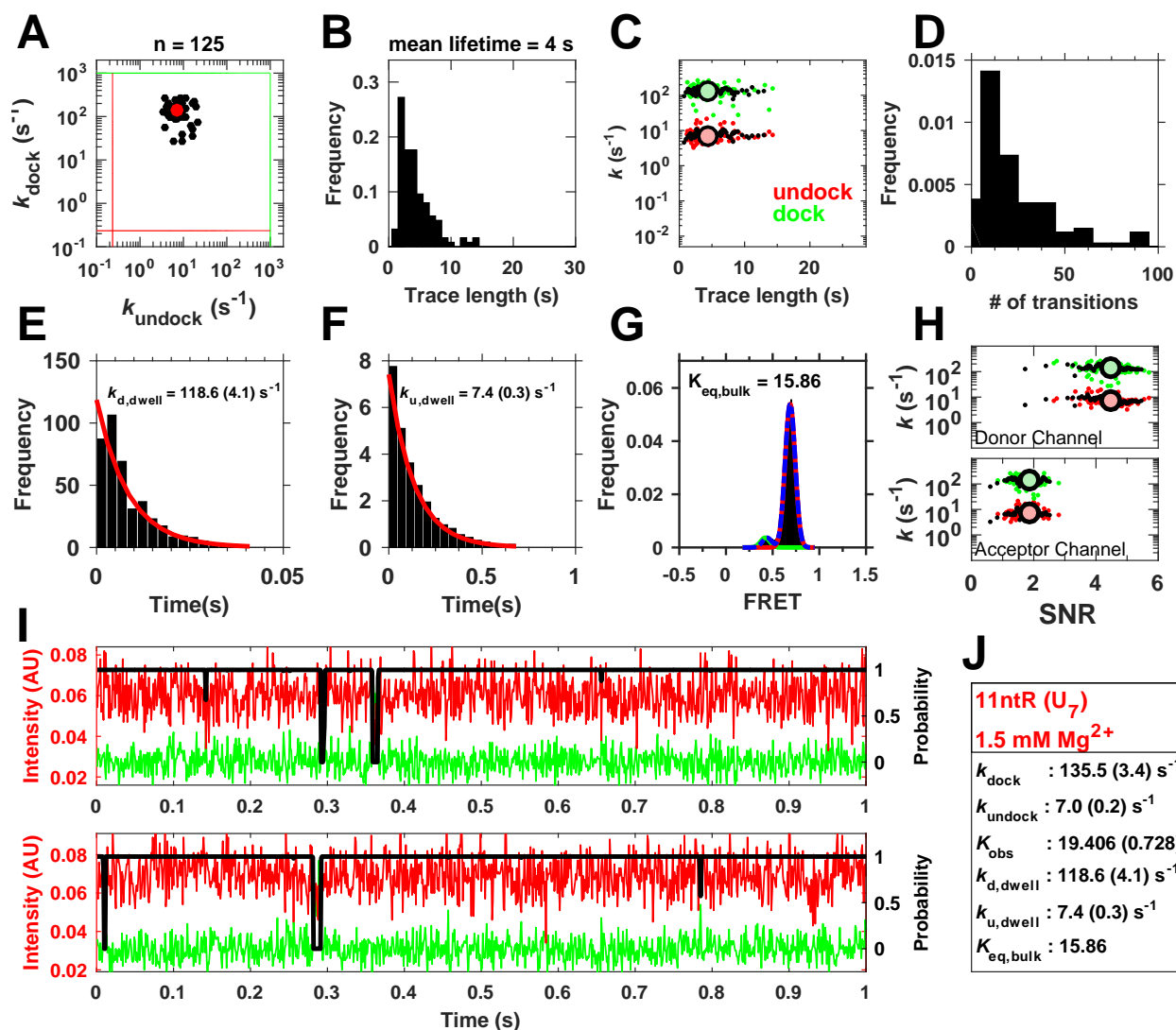


Figure S20. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

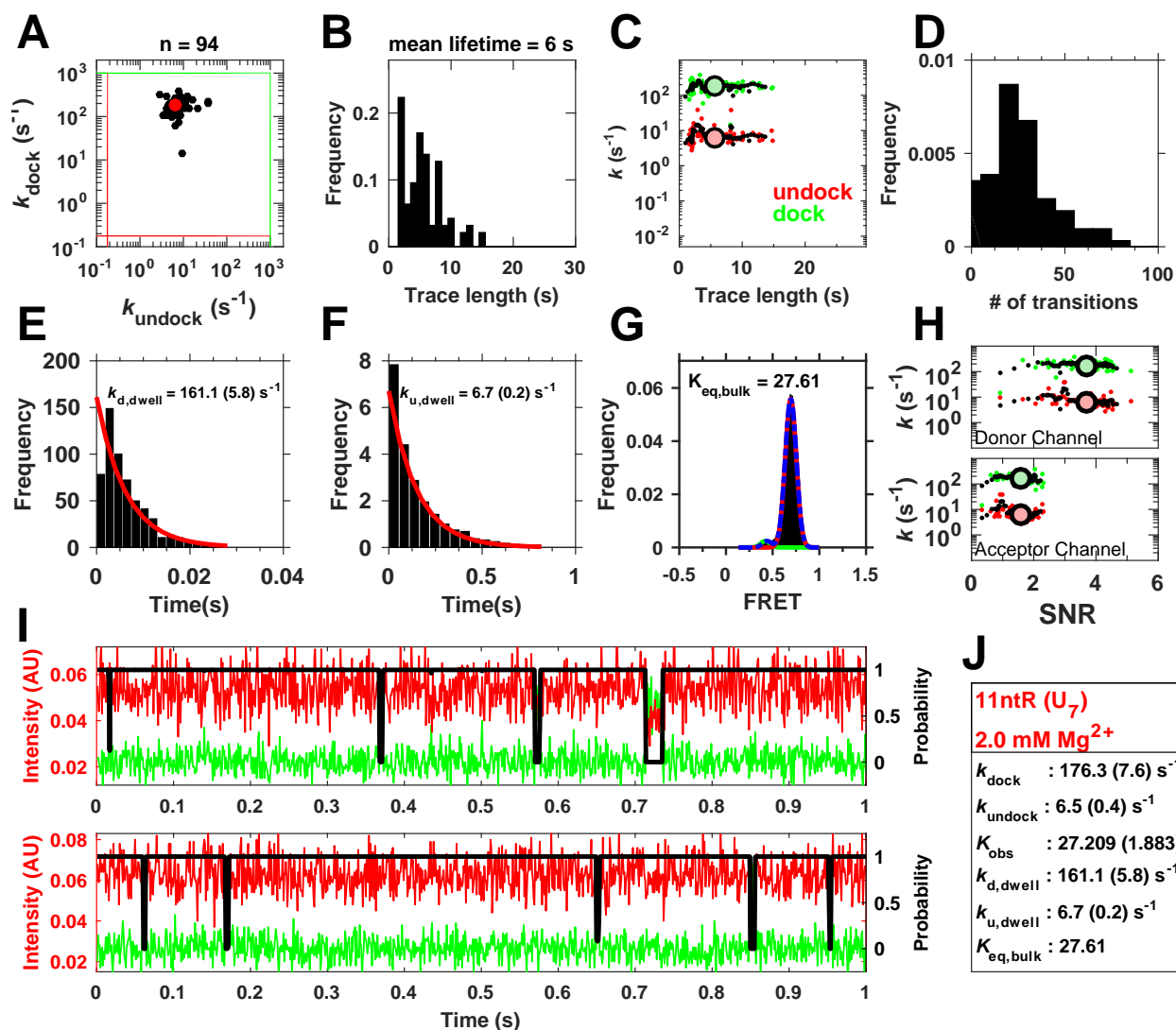


Figure S21. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

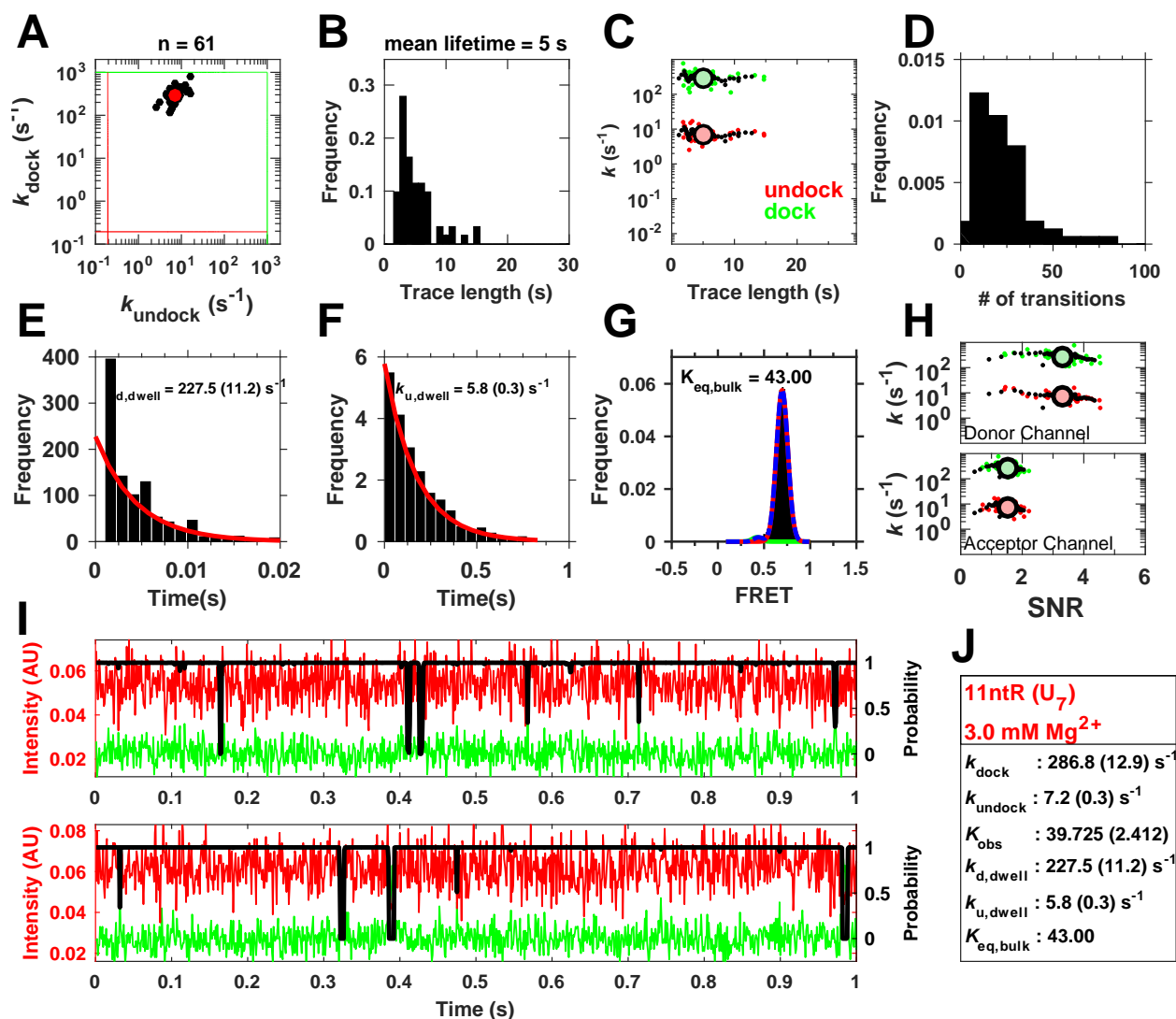


Figure S22. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

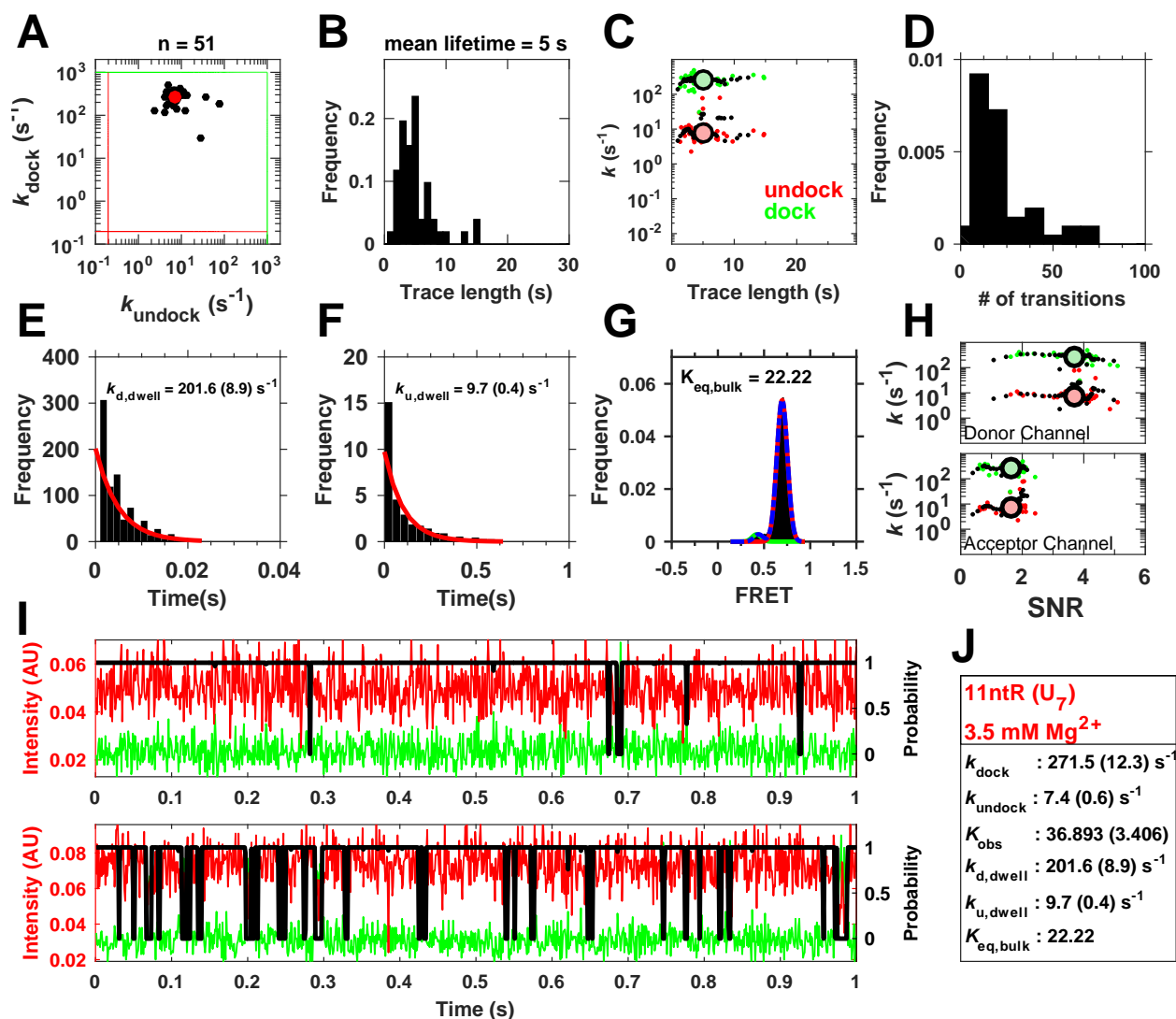


Figure S23. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

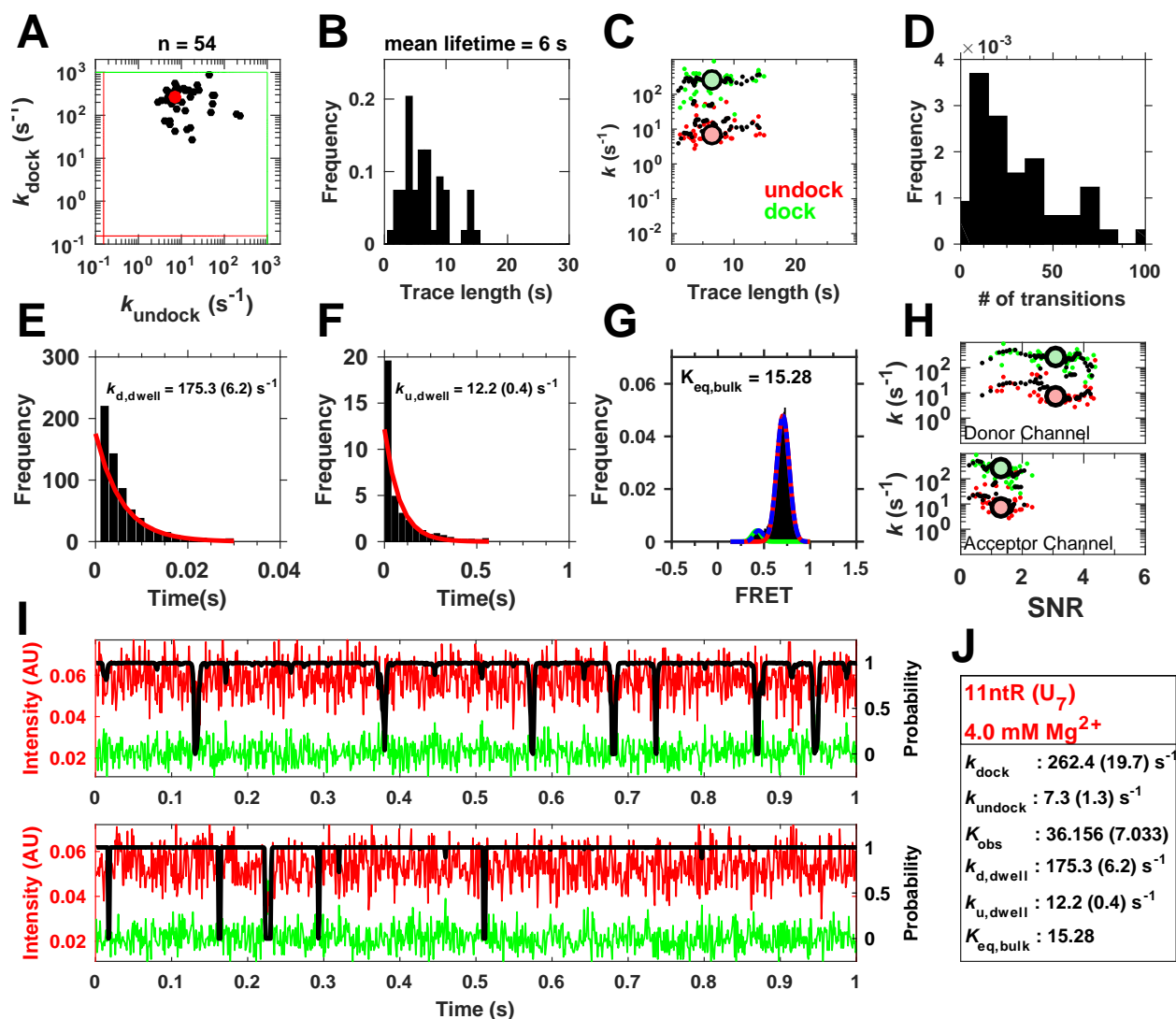


Figure S24. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

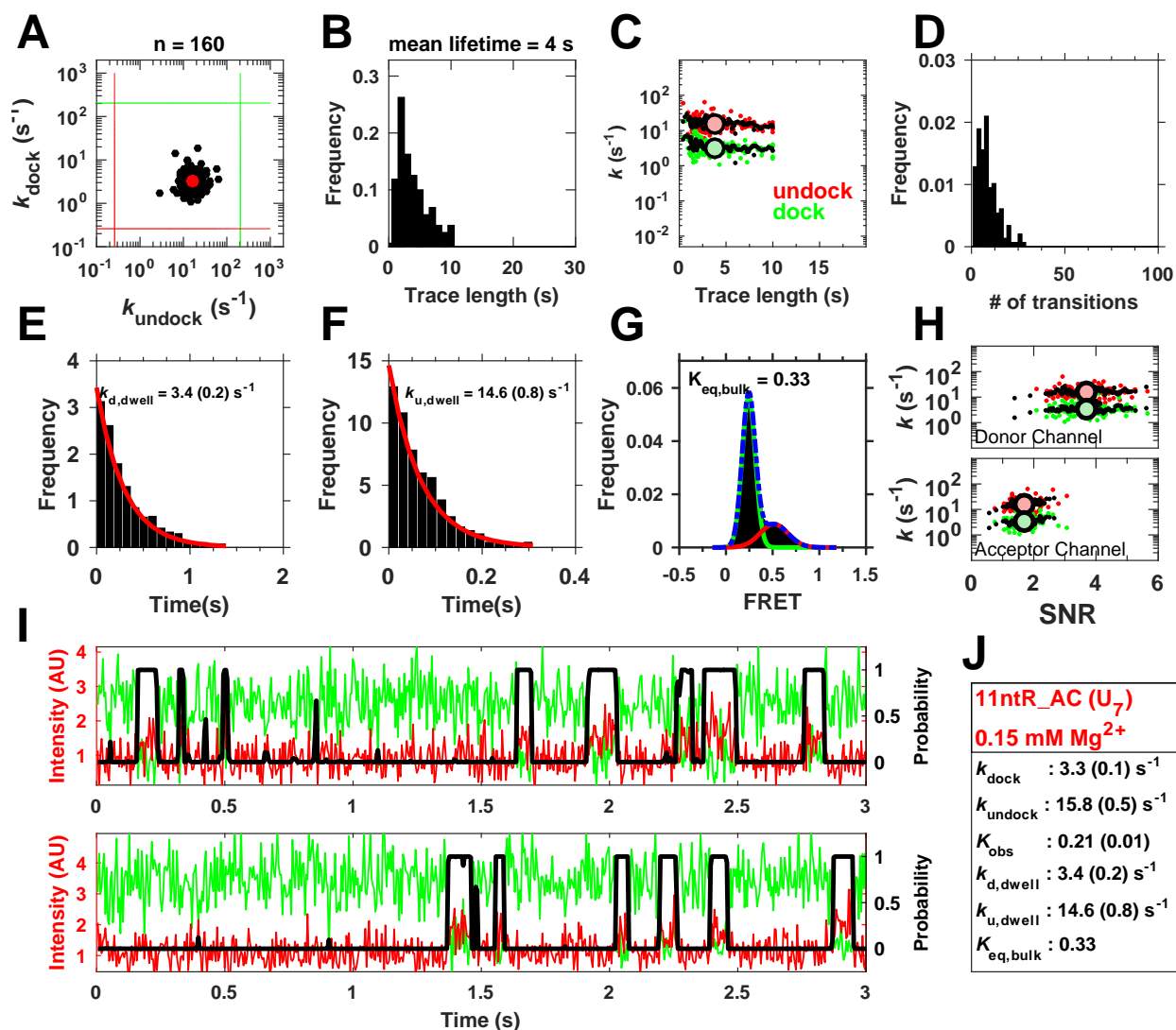


Figure S25. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

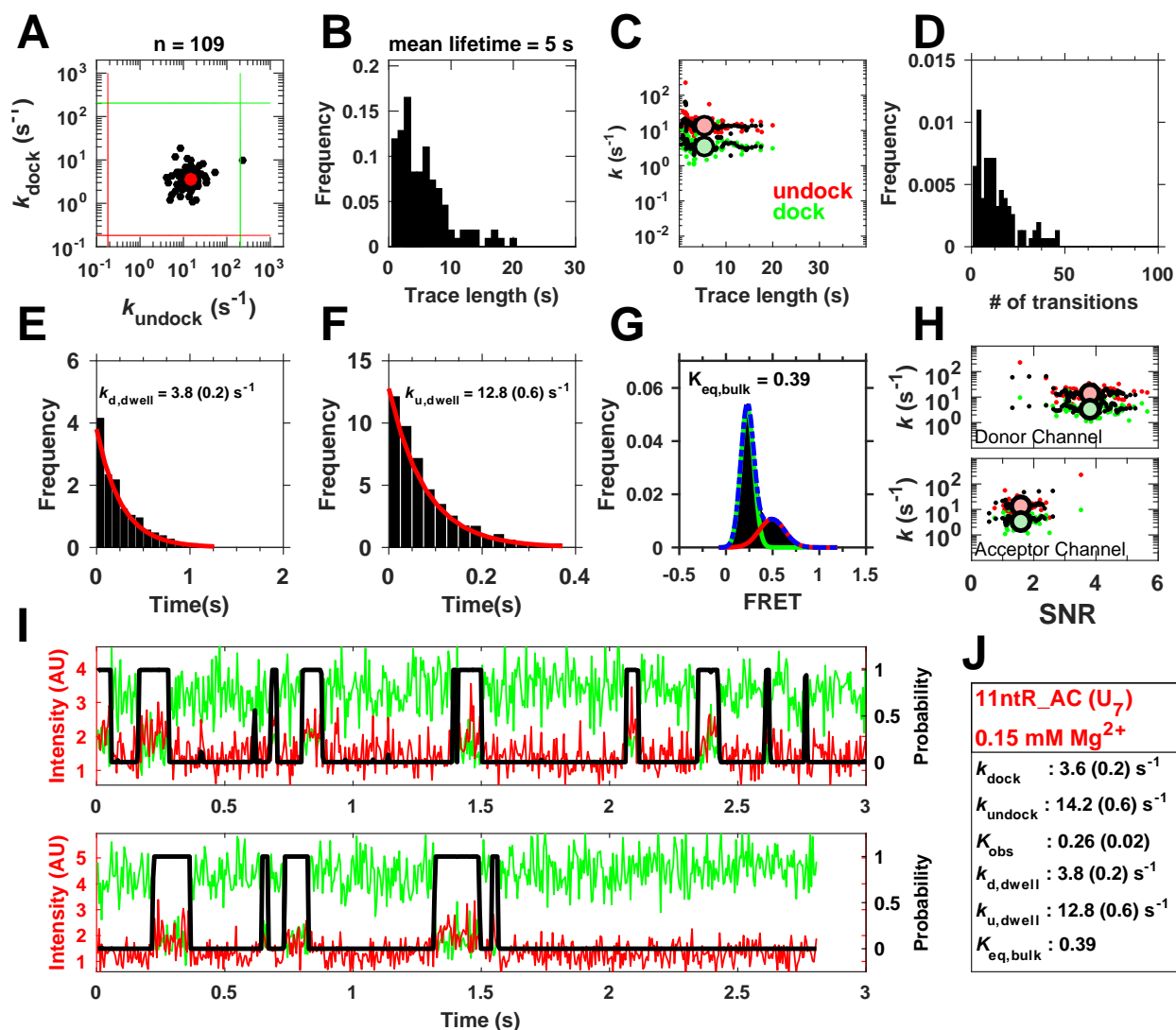


Figure S26. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

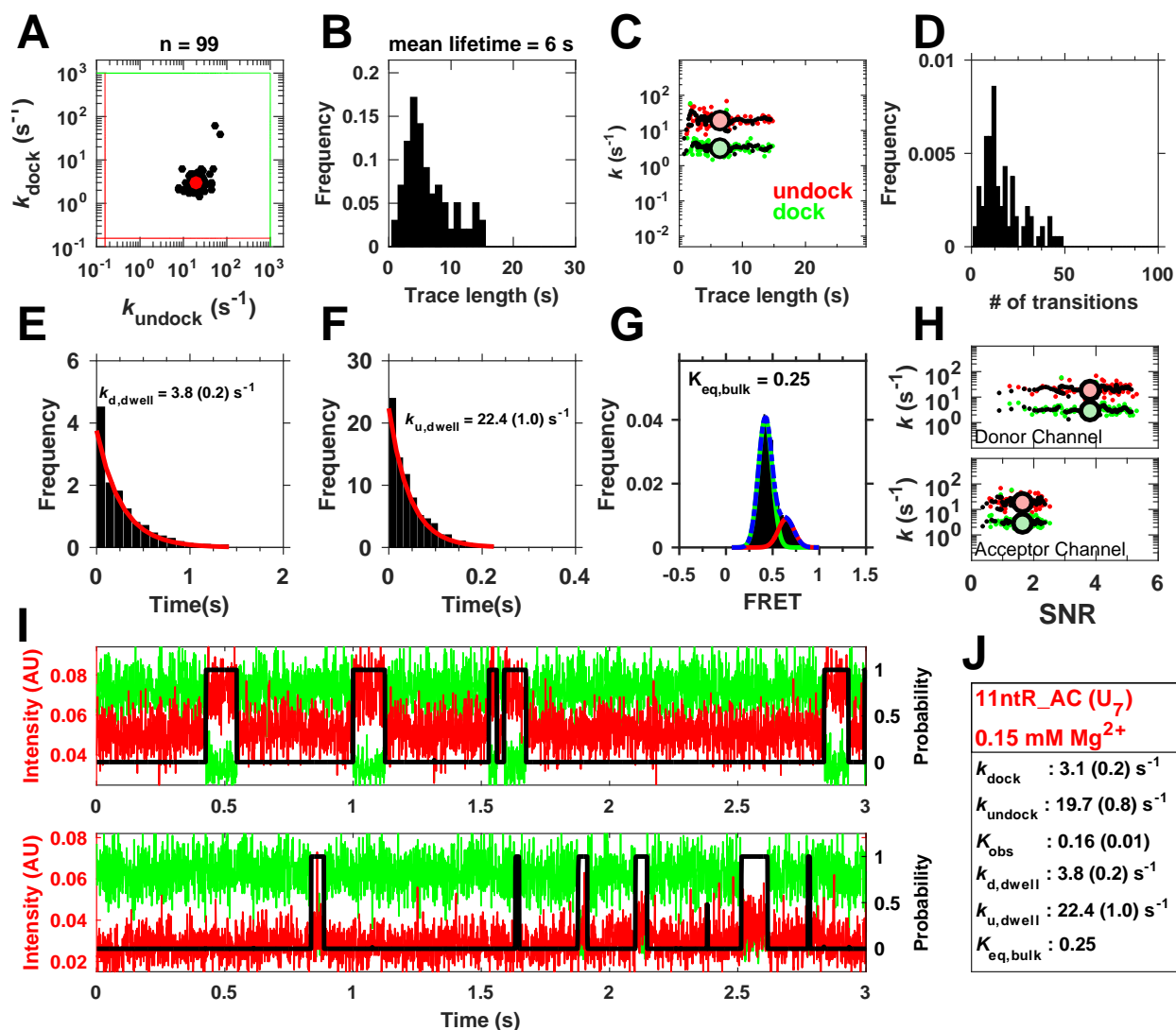


Figure S27. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

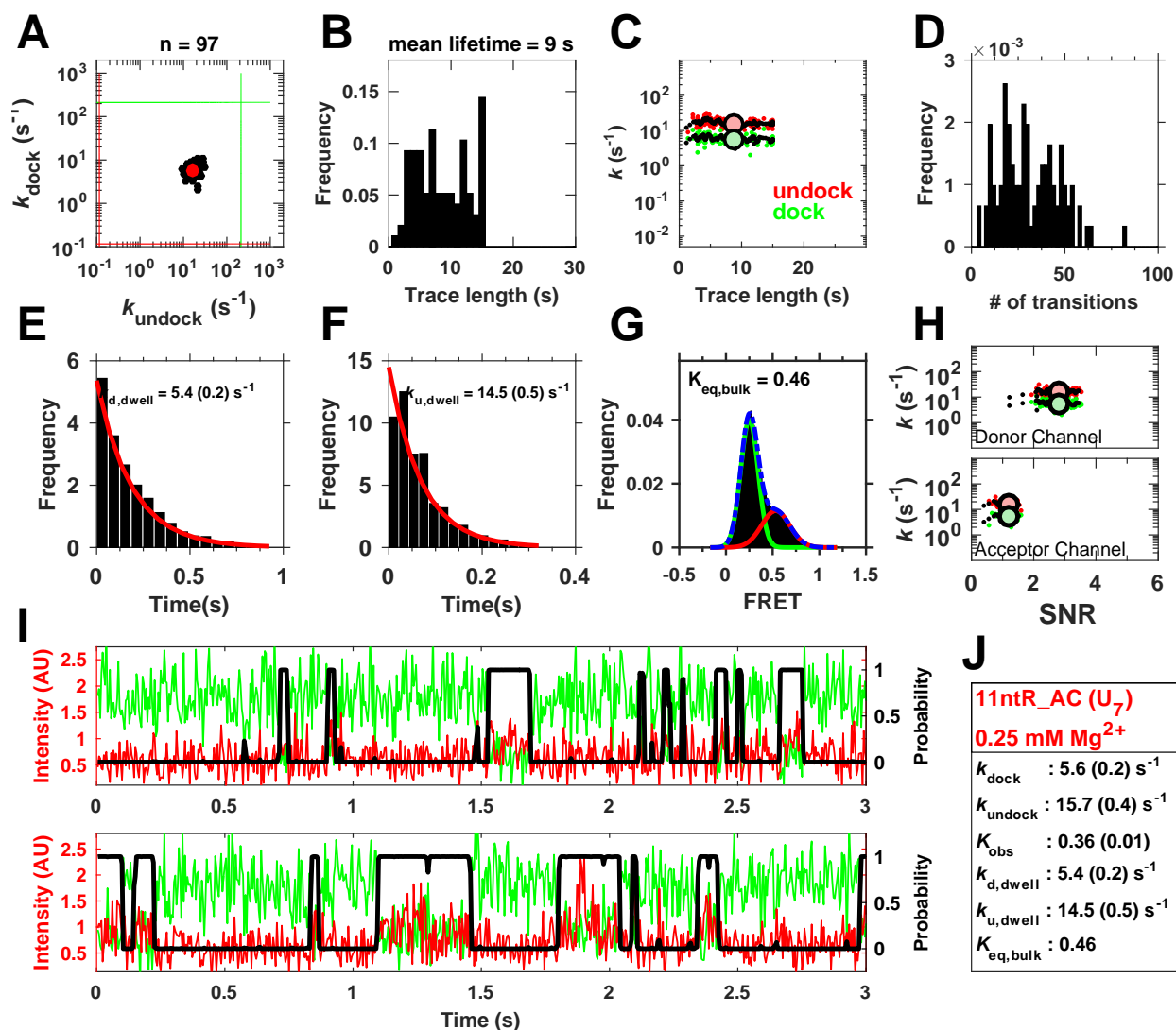


Figure S28. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

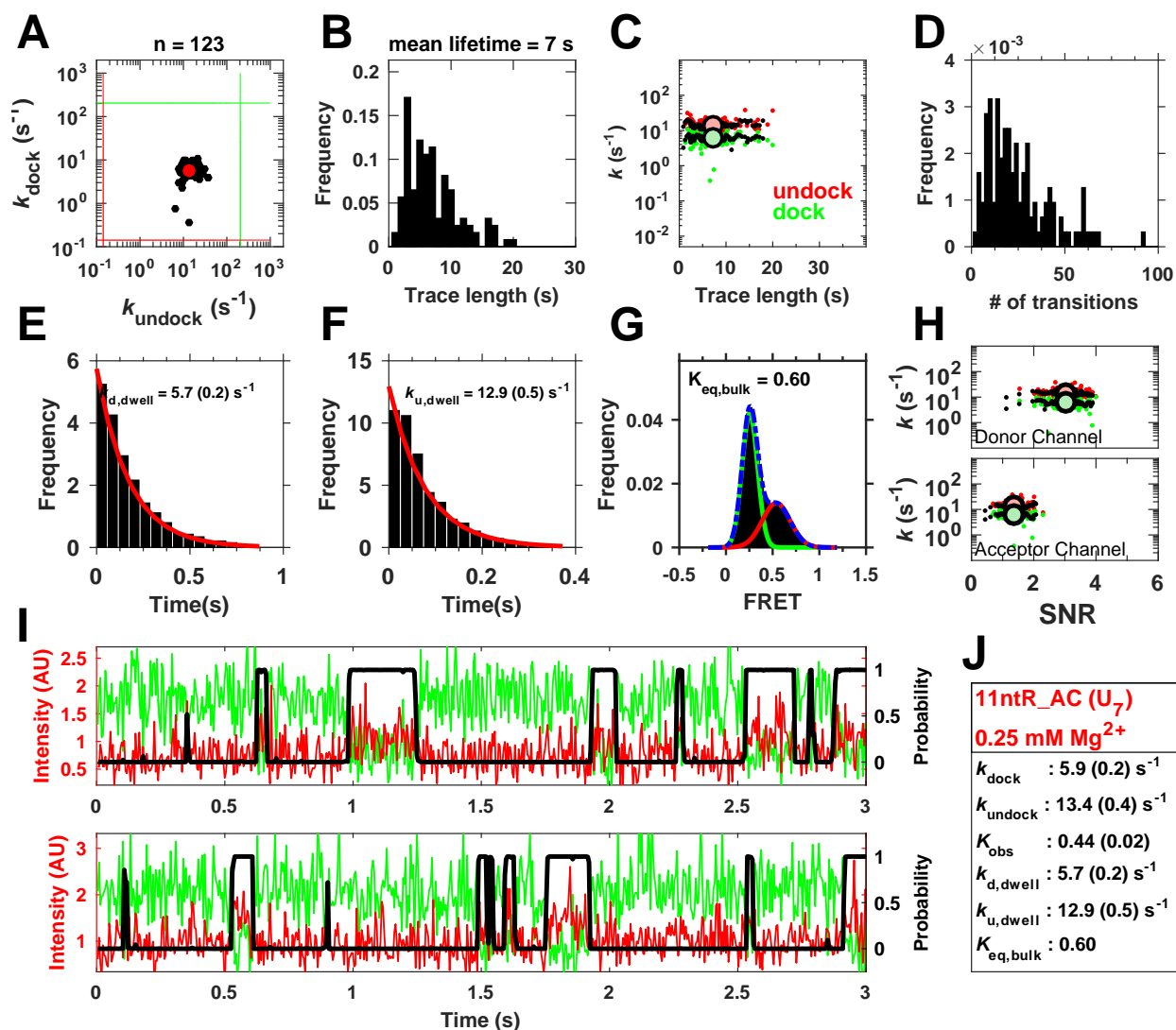


Figure S29. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

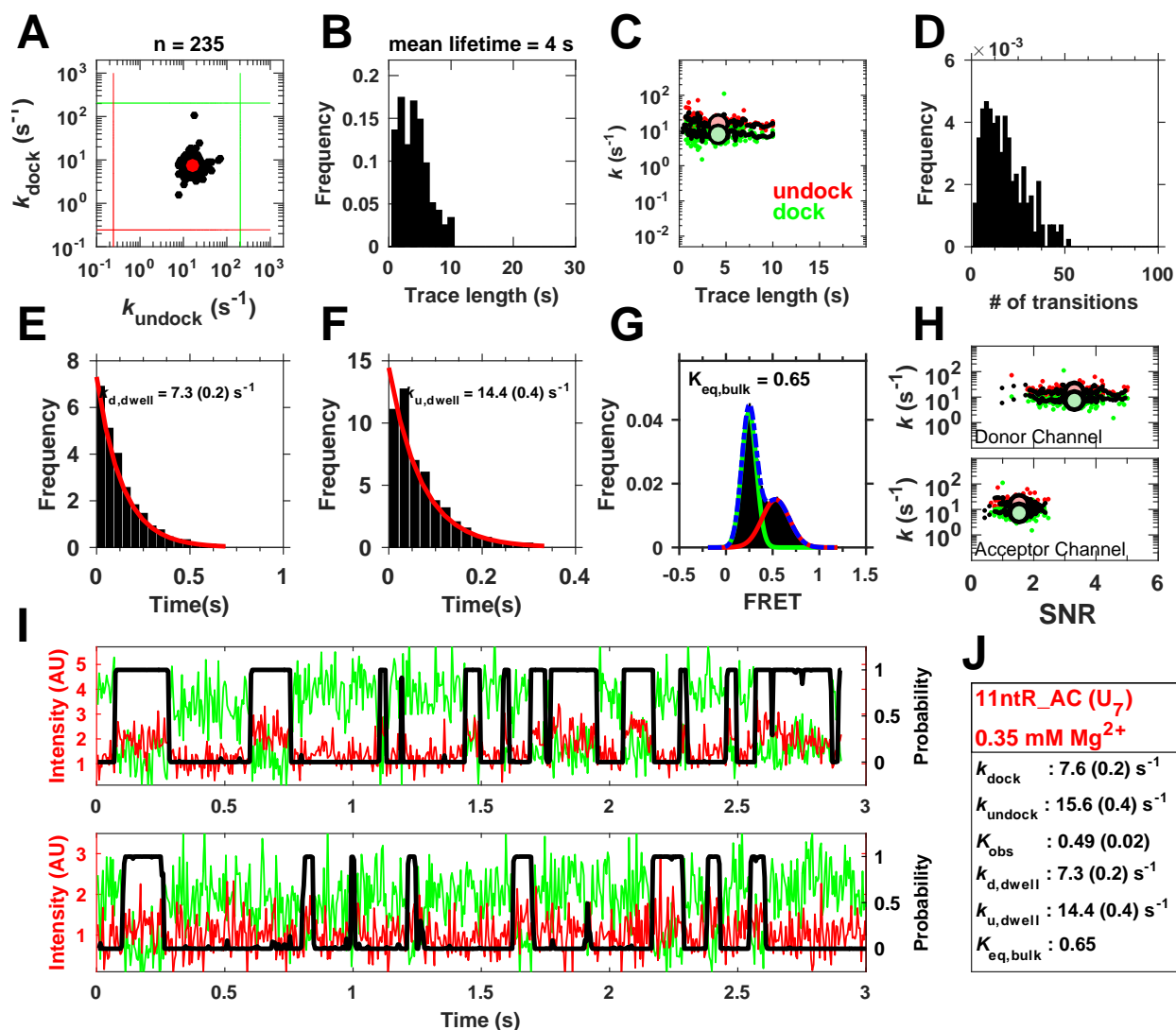


Figure S30. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

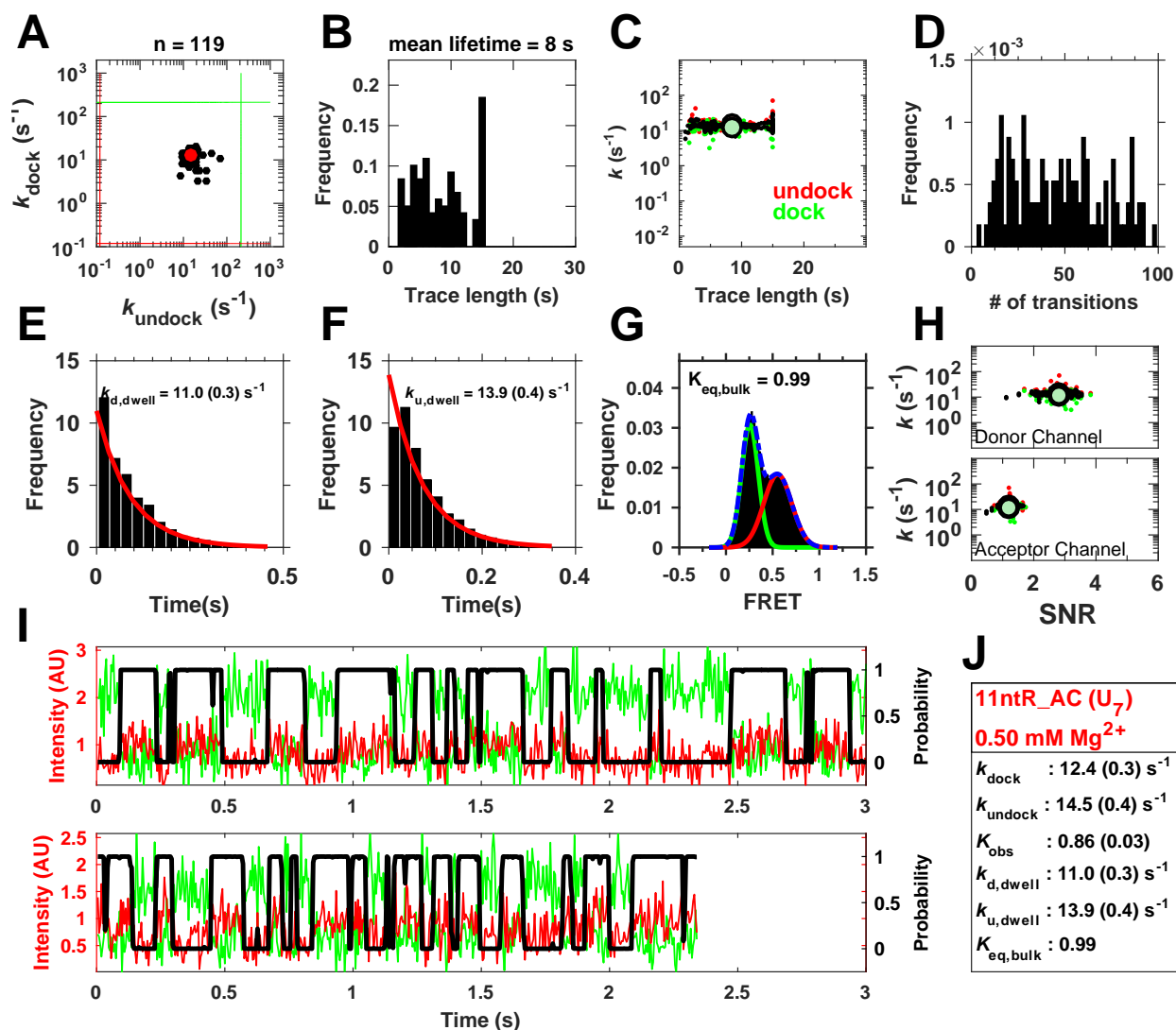


Figure S31. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

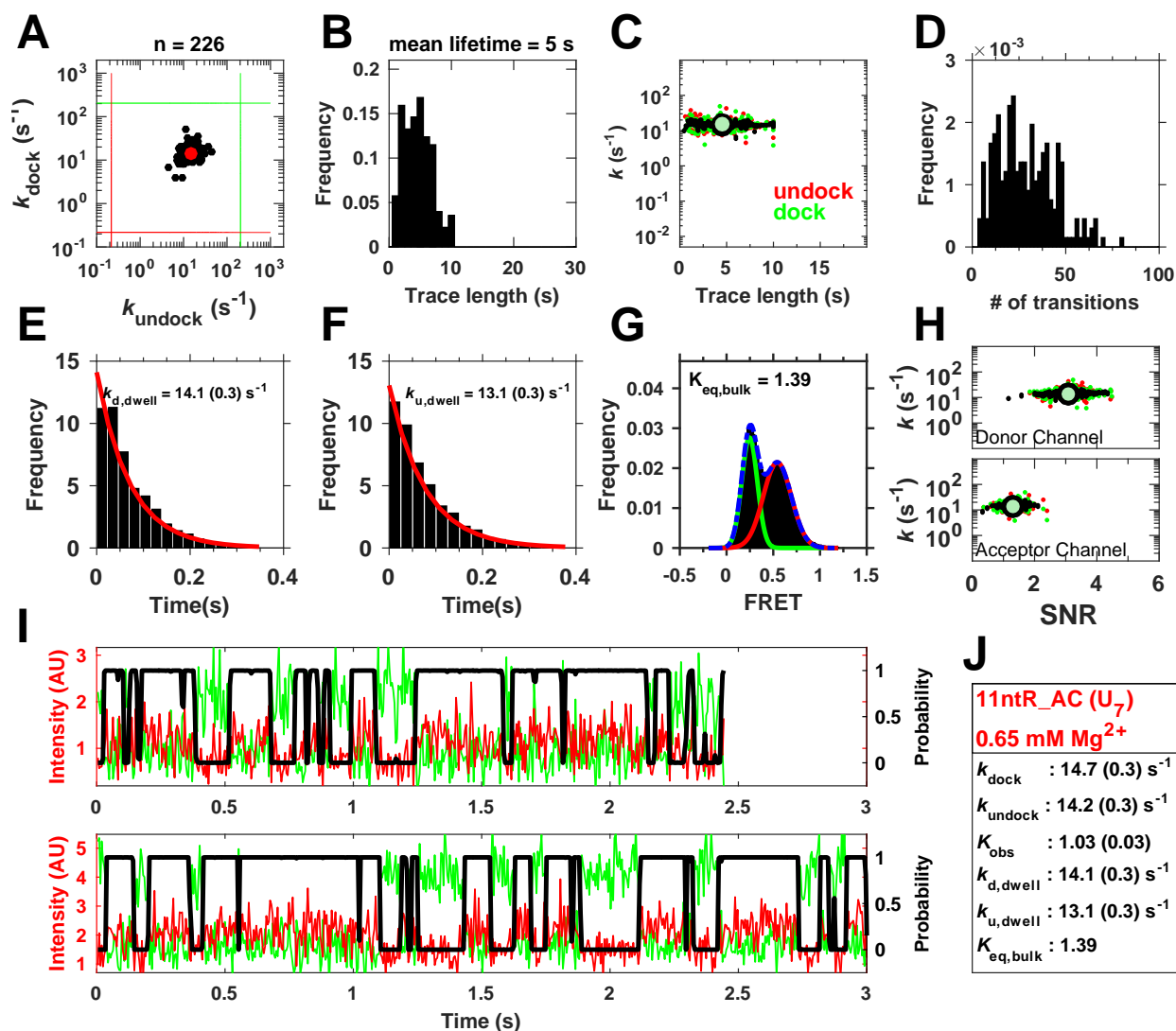


Figure S32. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

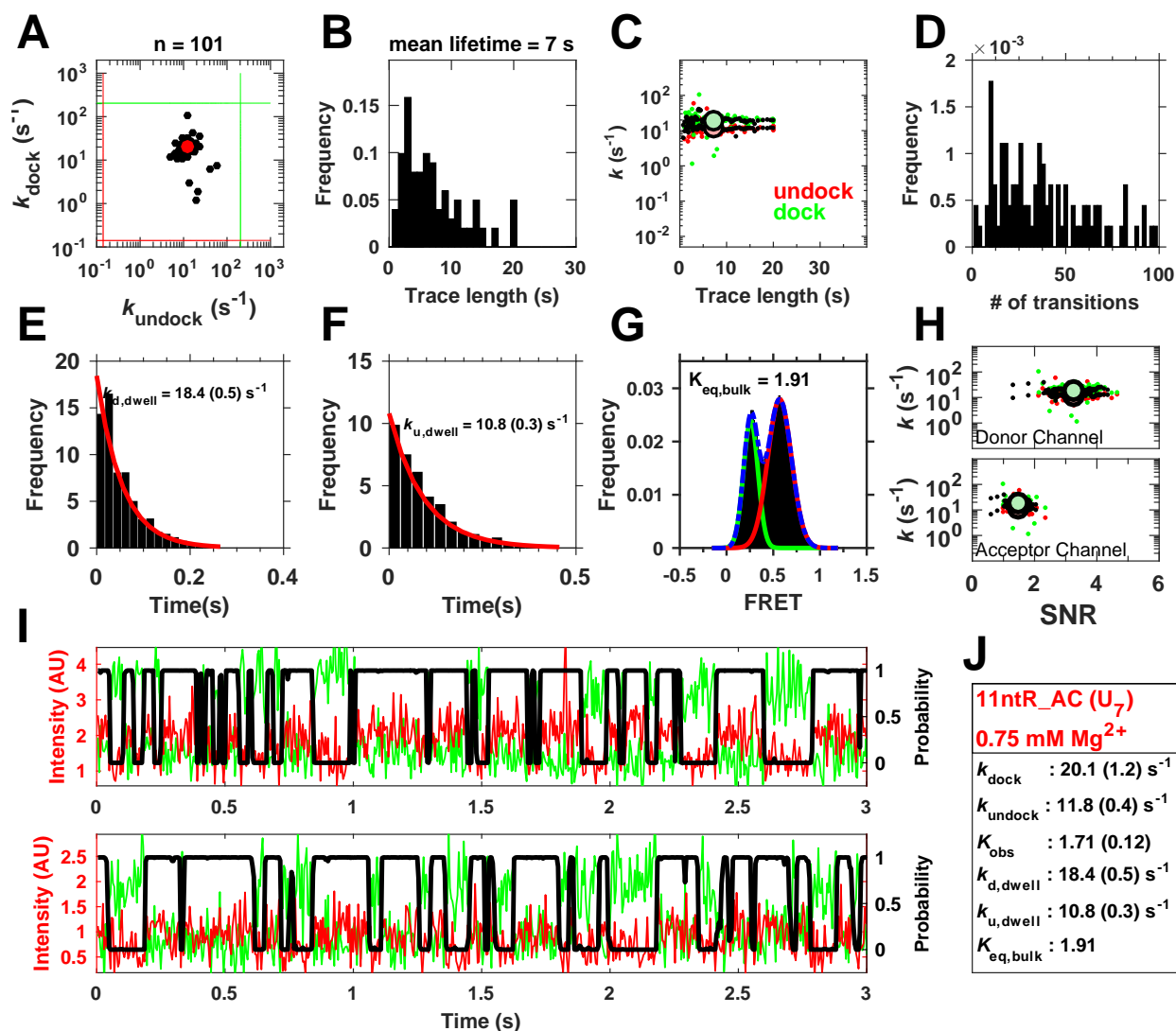


Figure S33. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

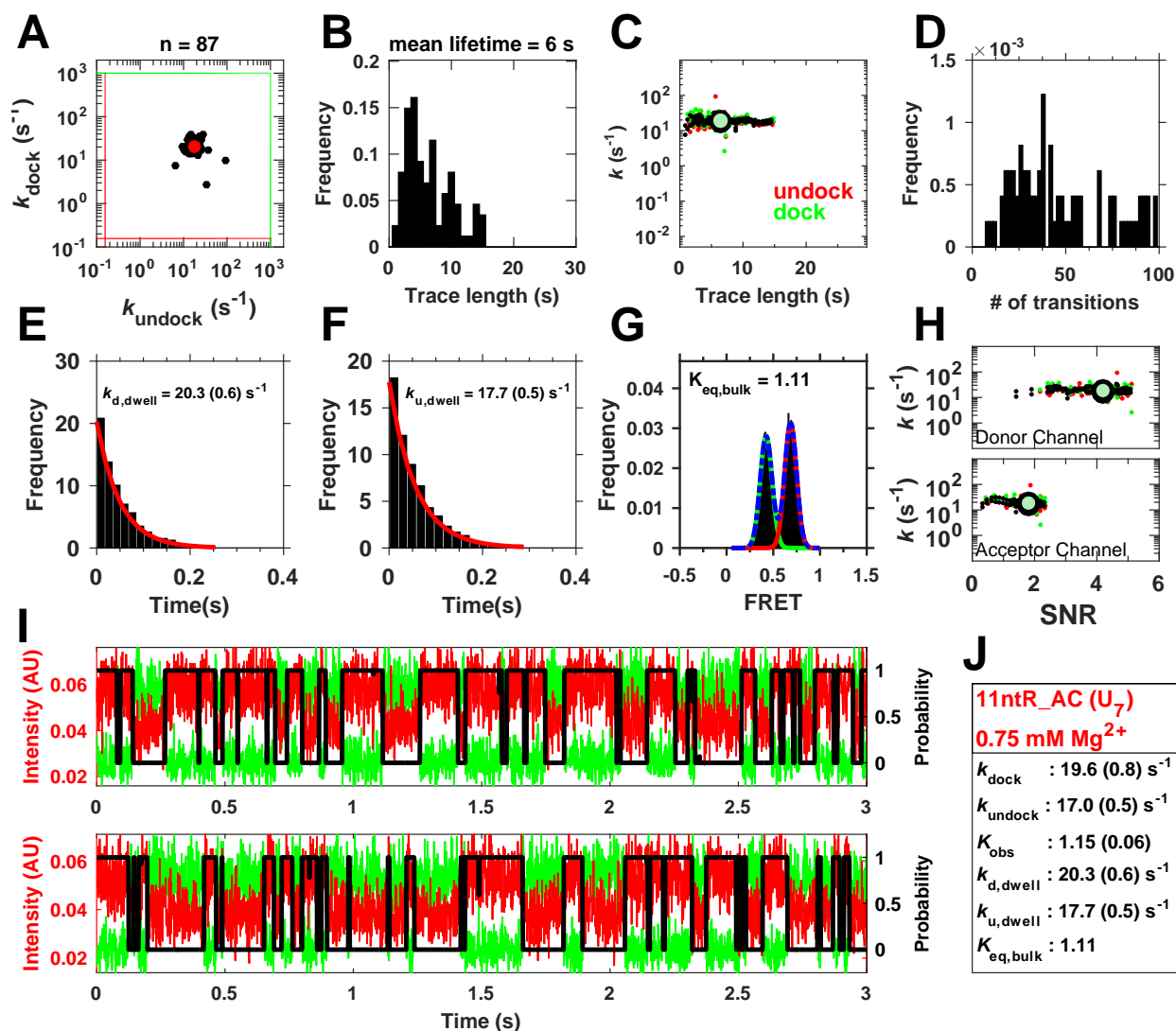


Figure S34. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

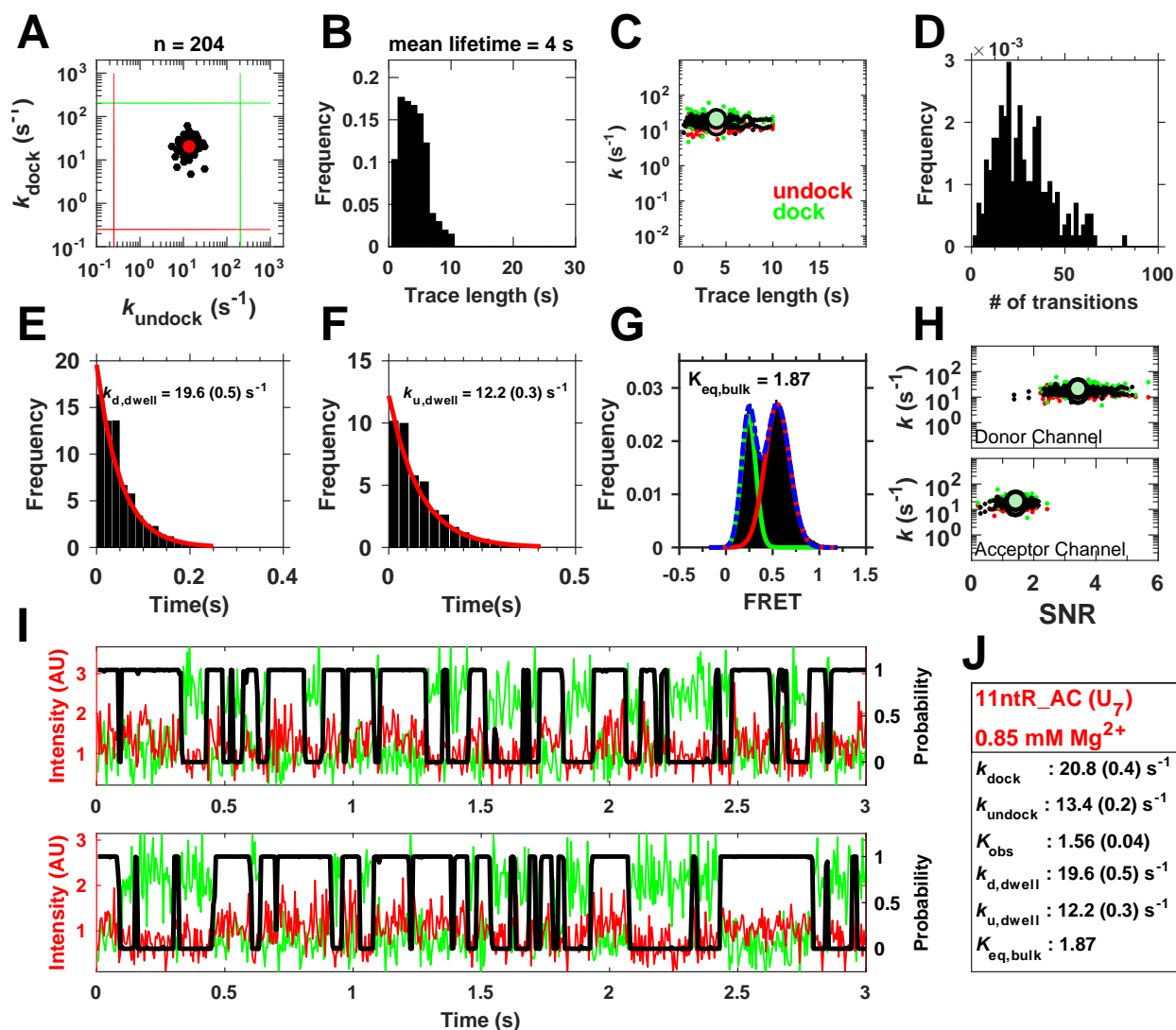


Figure S35. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

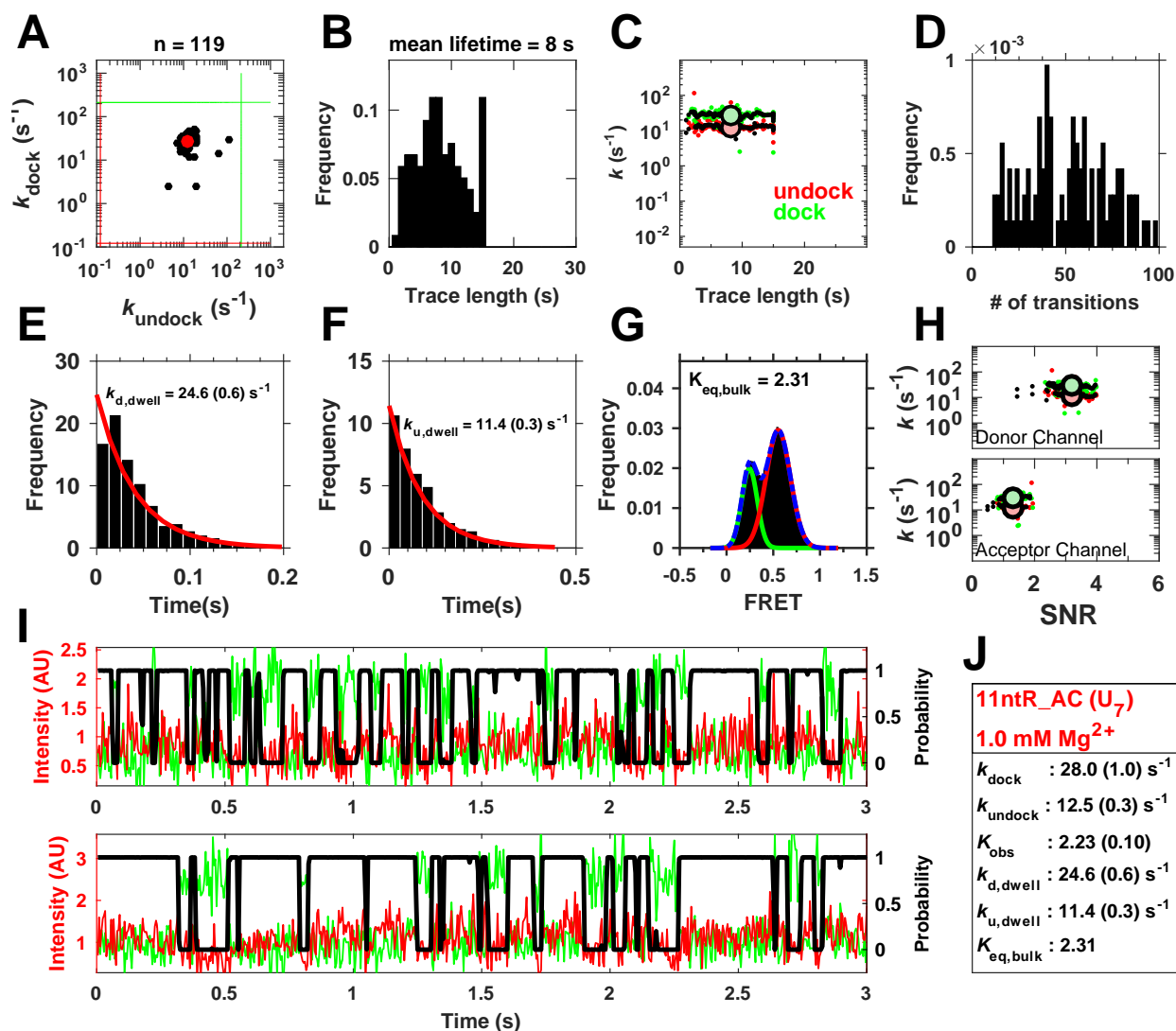


Figure S36. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

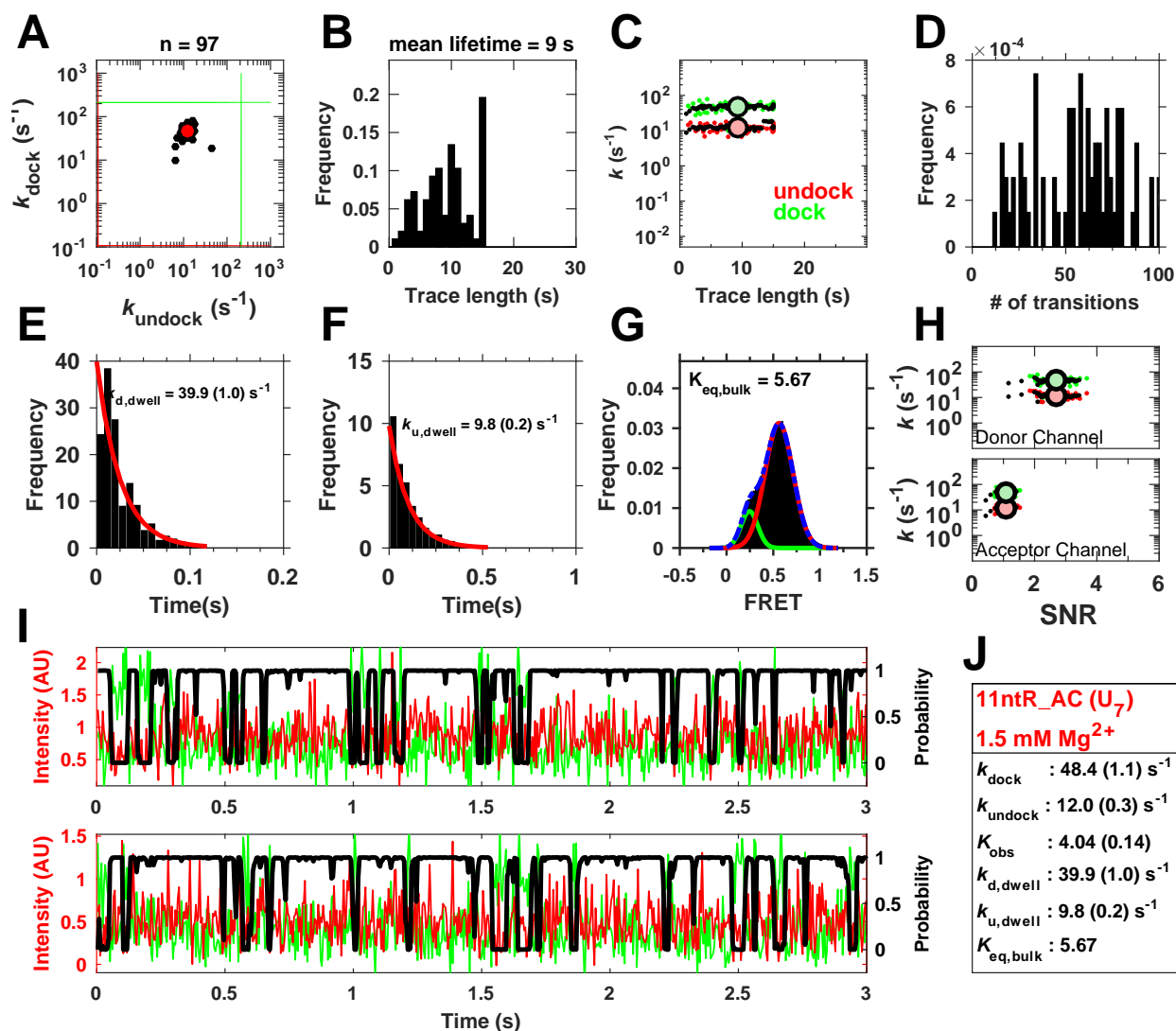


Figure S37. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

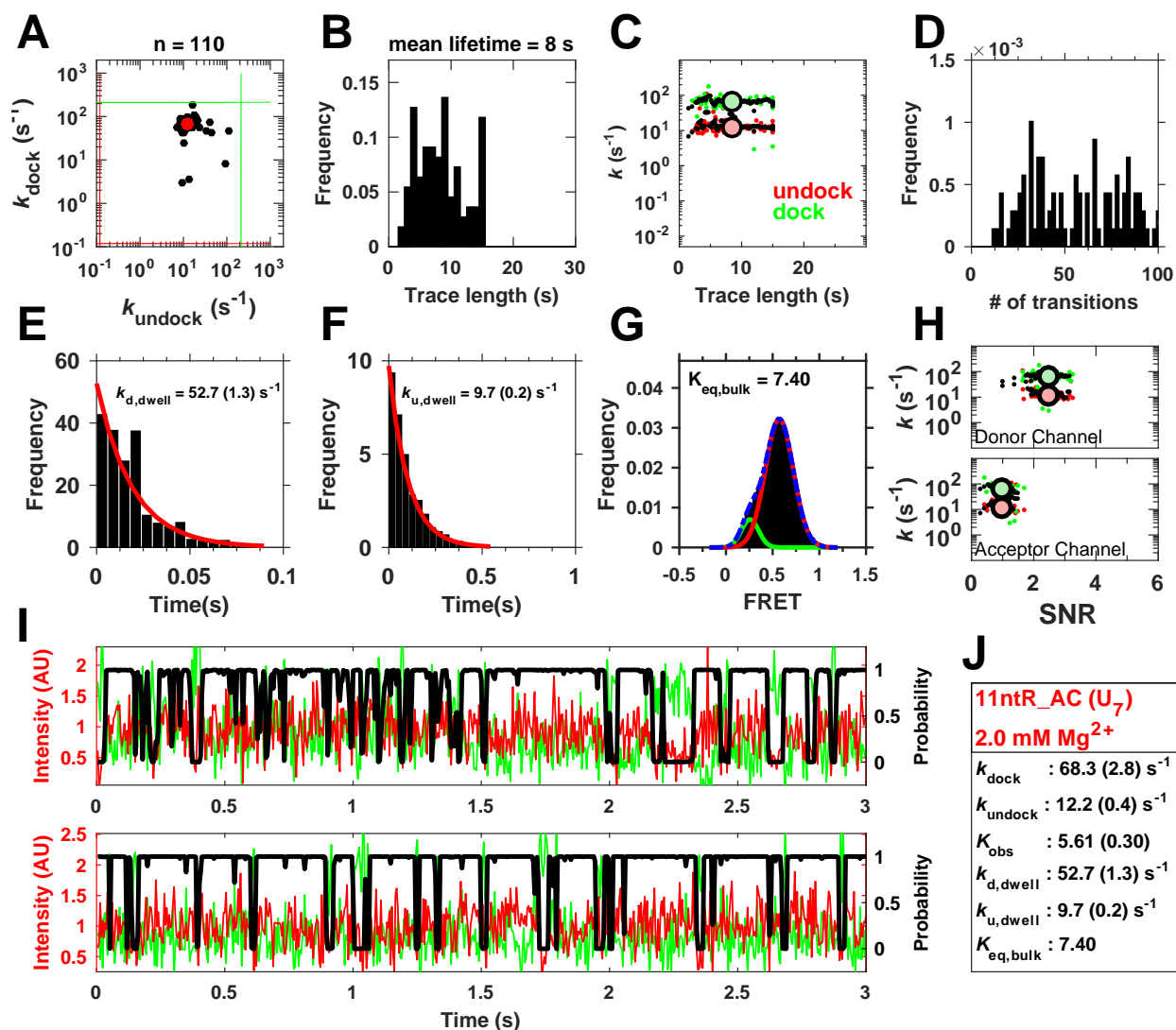


Figure S38. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

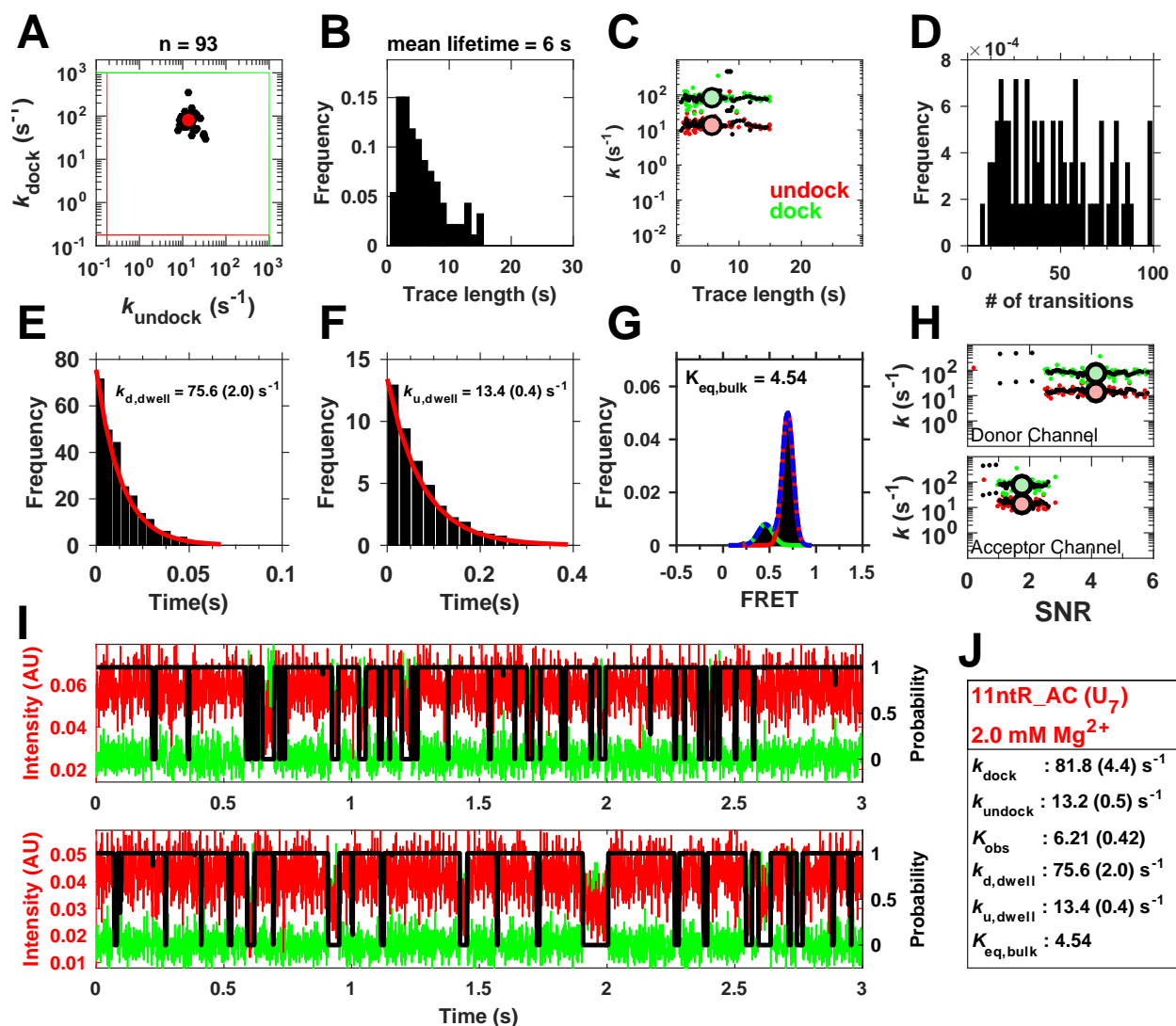


Figure S39. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

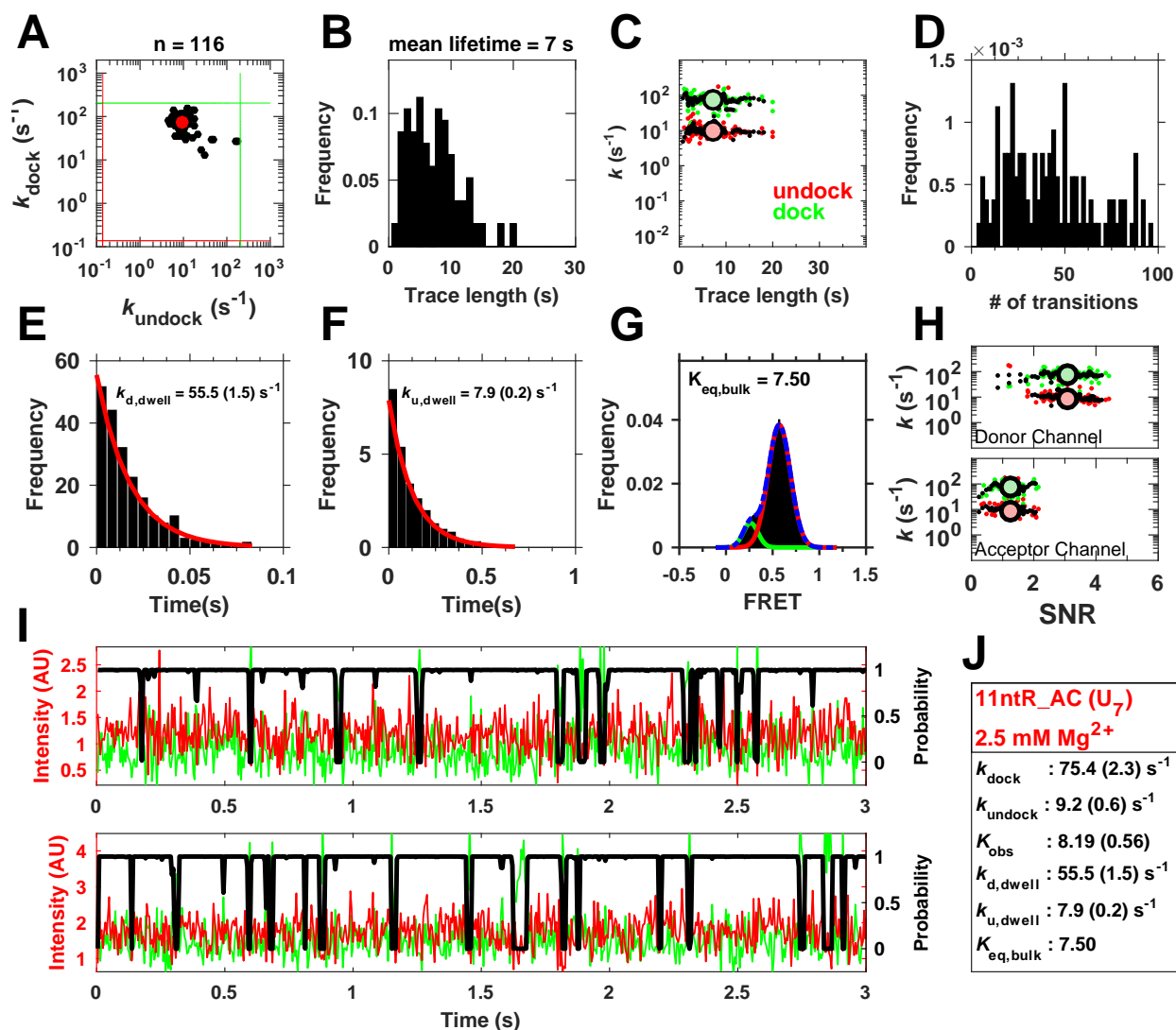


Figure S40. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

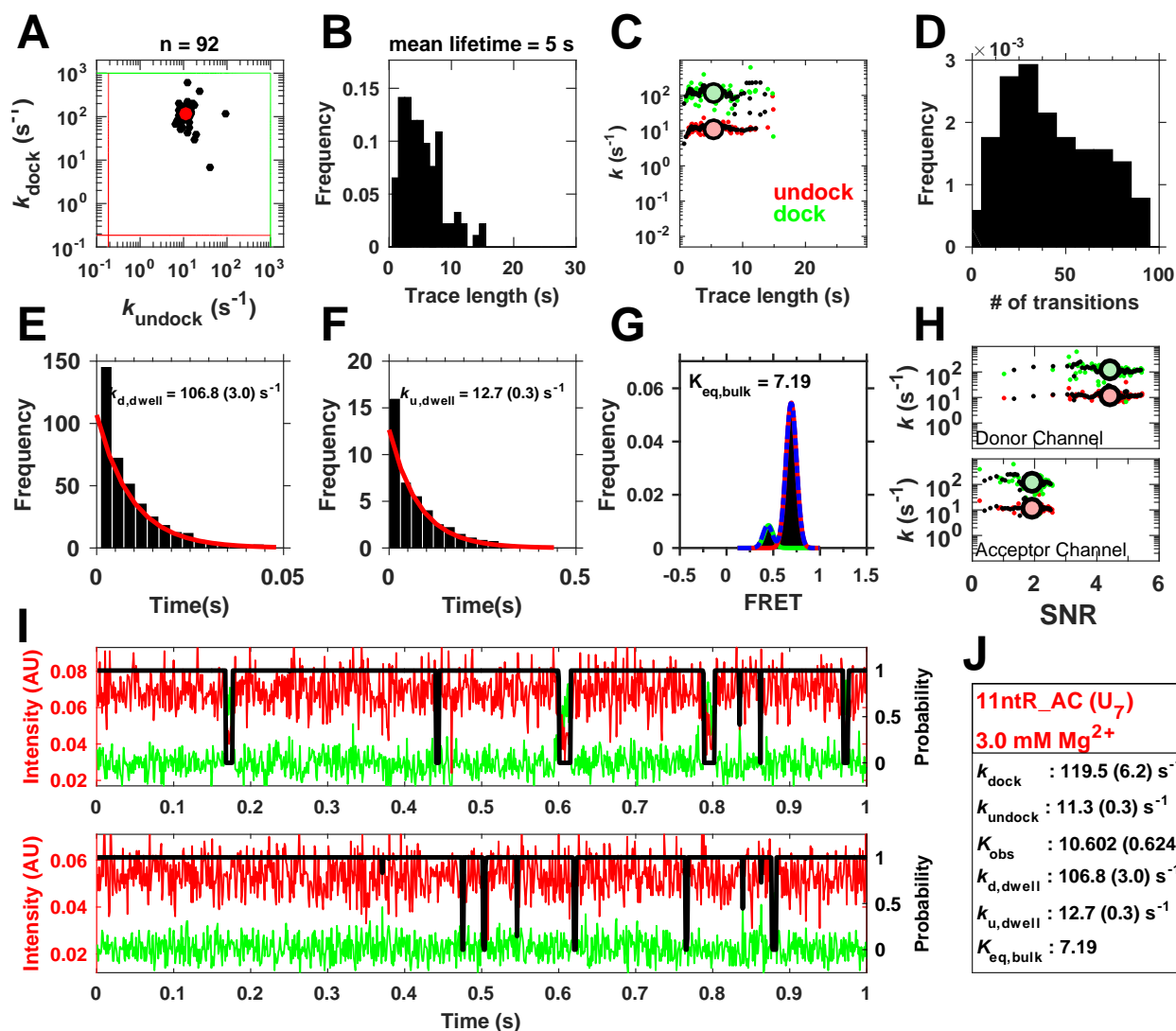


Figure S41. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

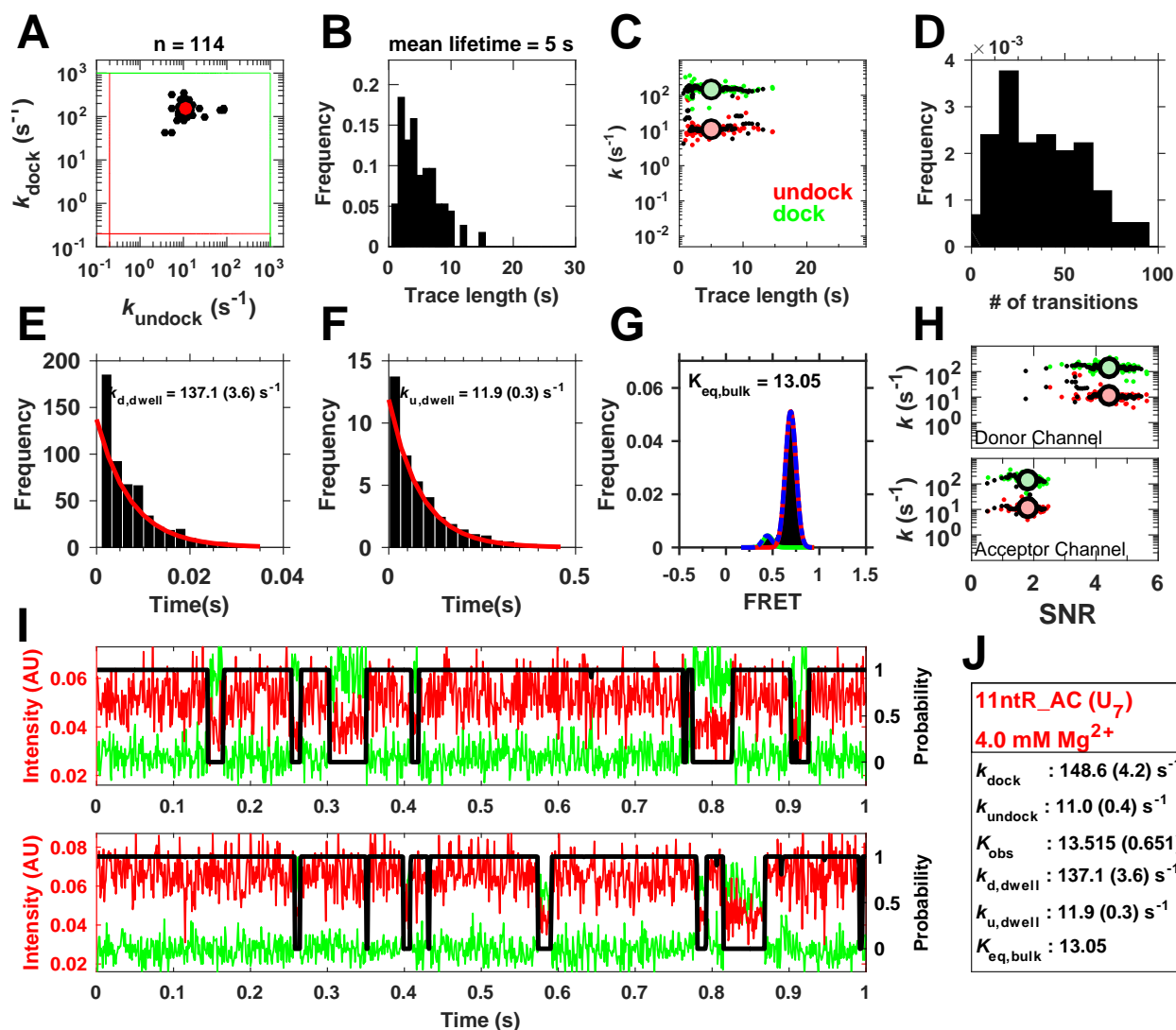


Figure S42. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

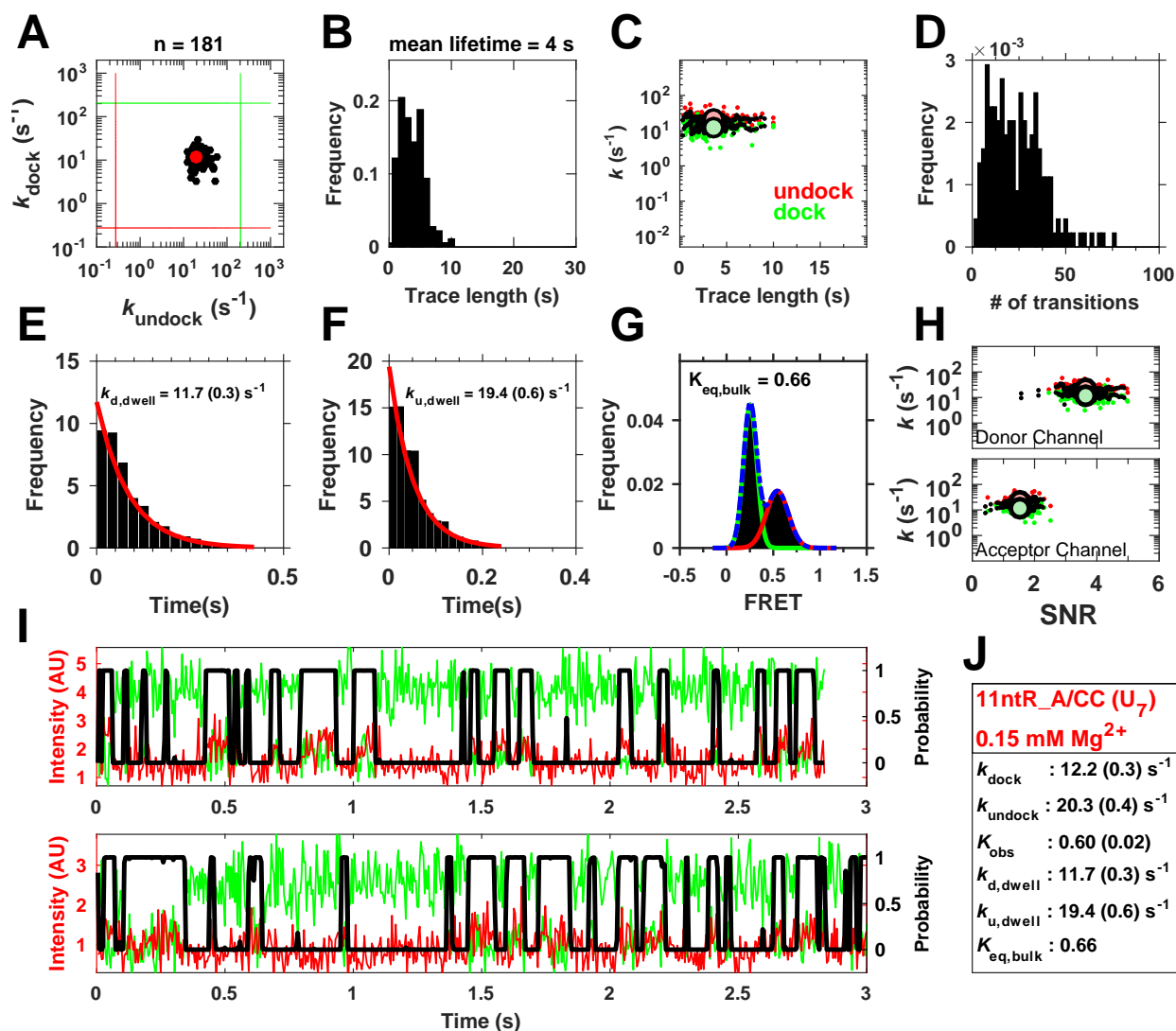


Figure S43. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

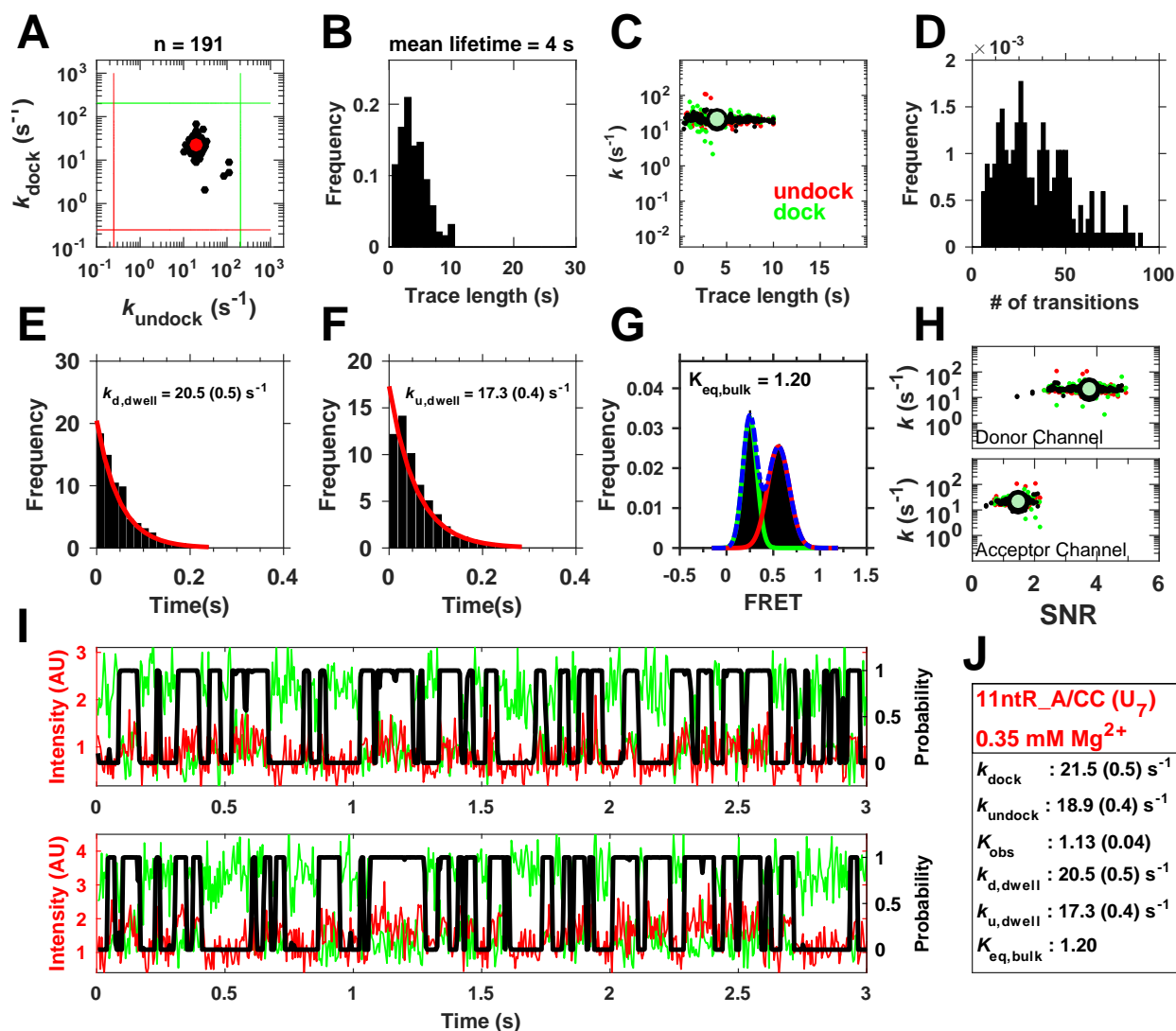


Figure S44. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

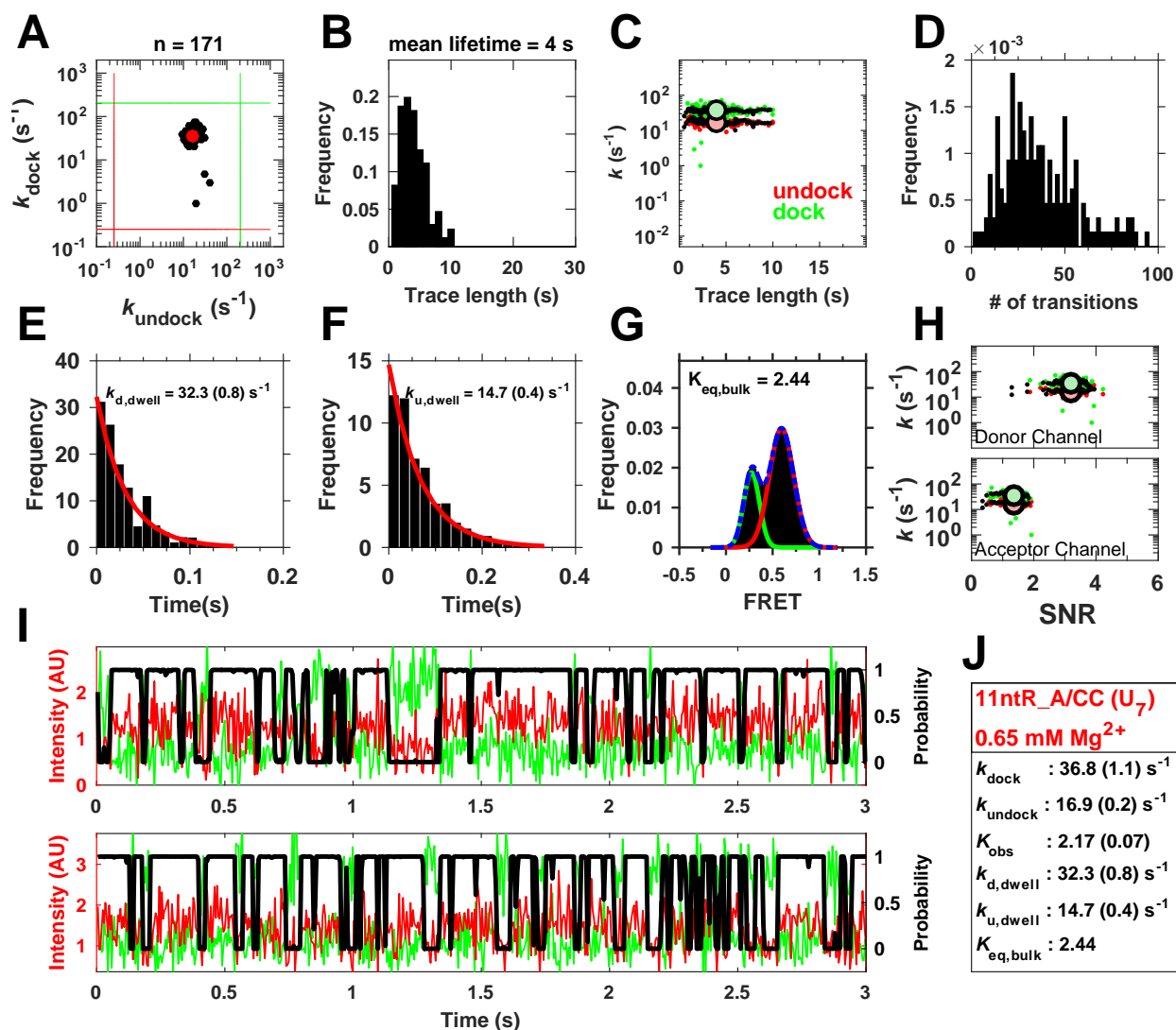


Figure S45. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

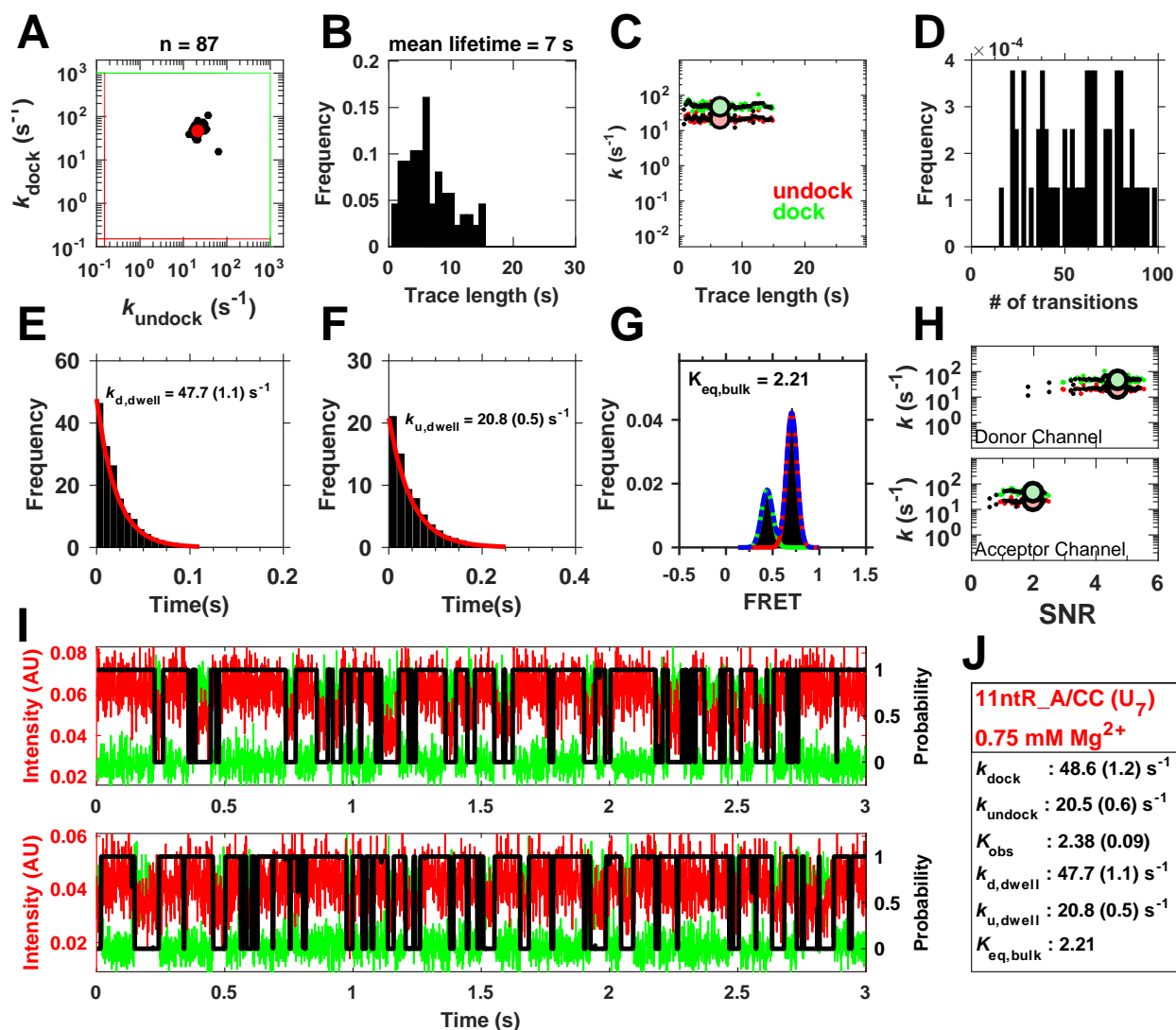


Figure S46. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

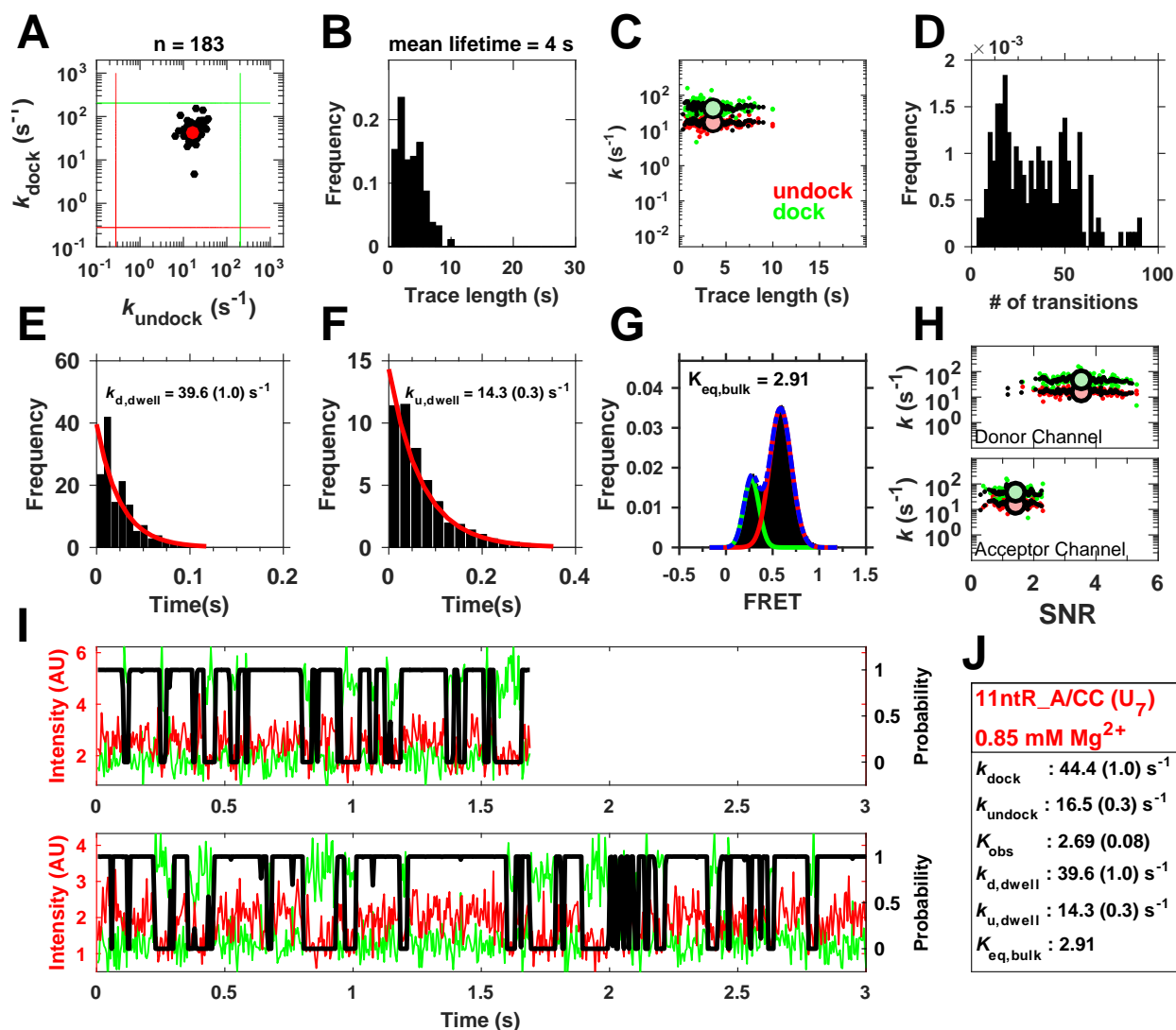


Figure S47. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

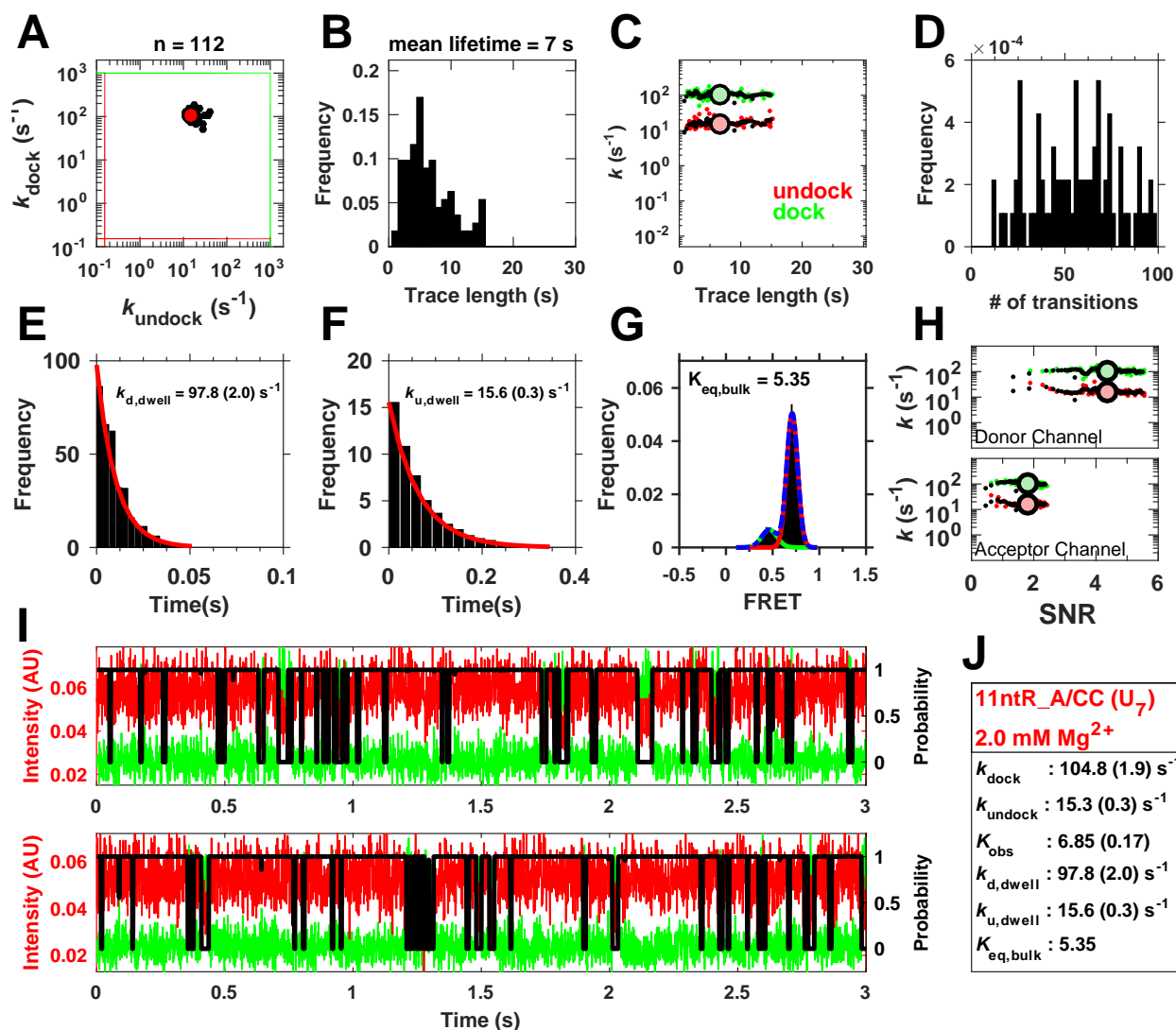


Figure S48. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

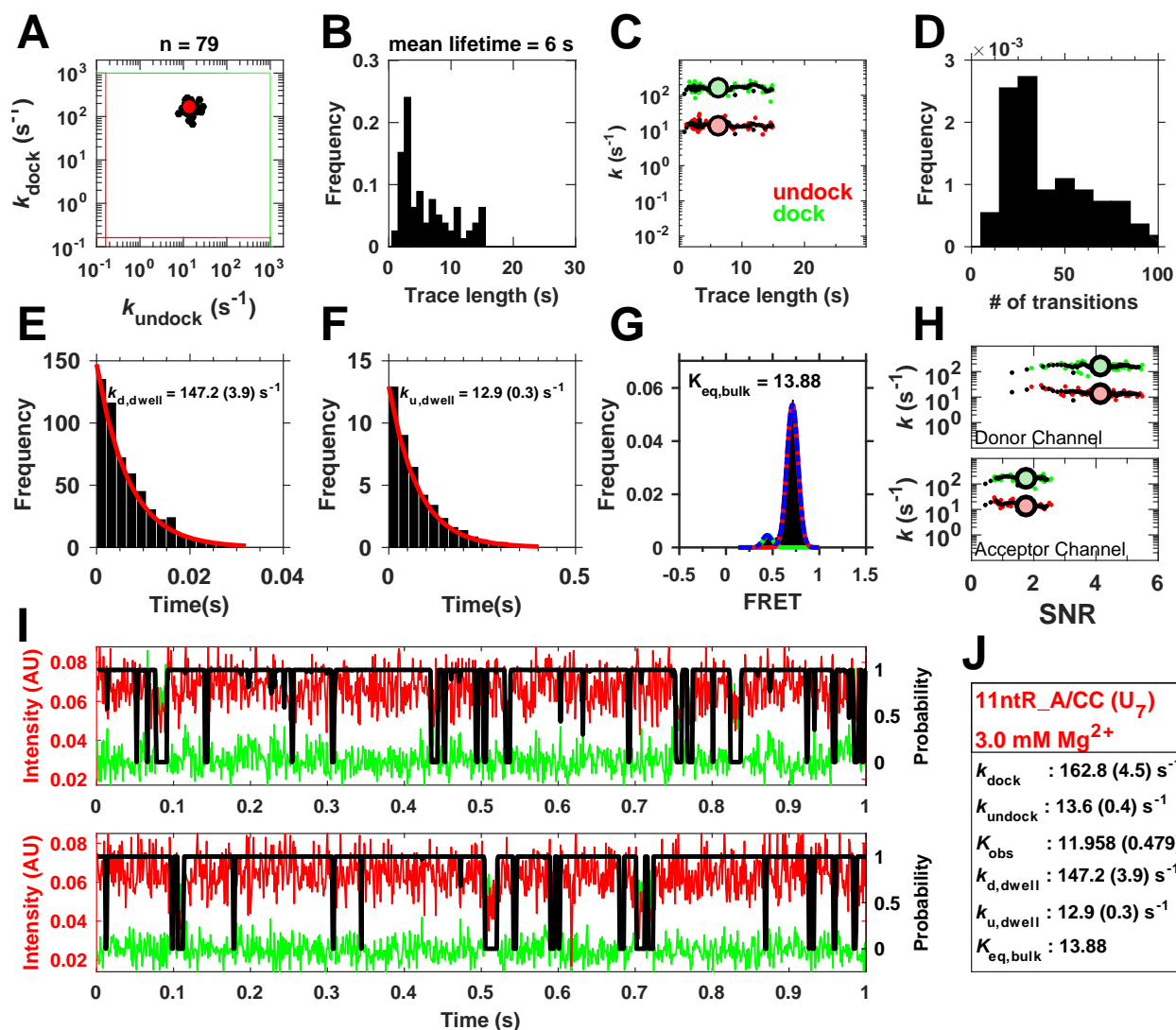


Figure S49. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

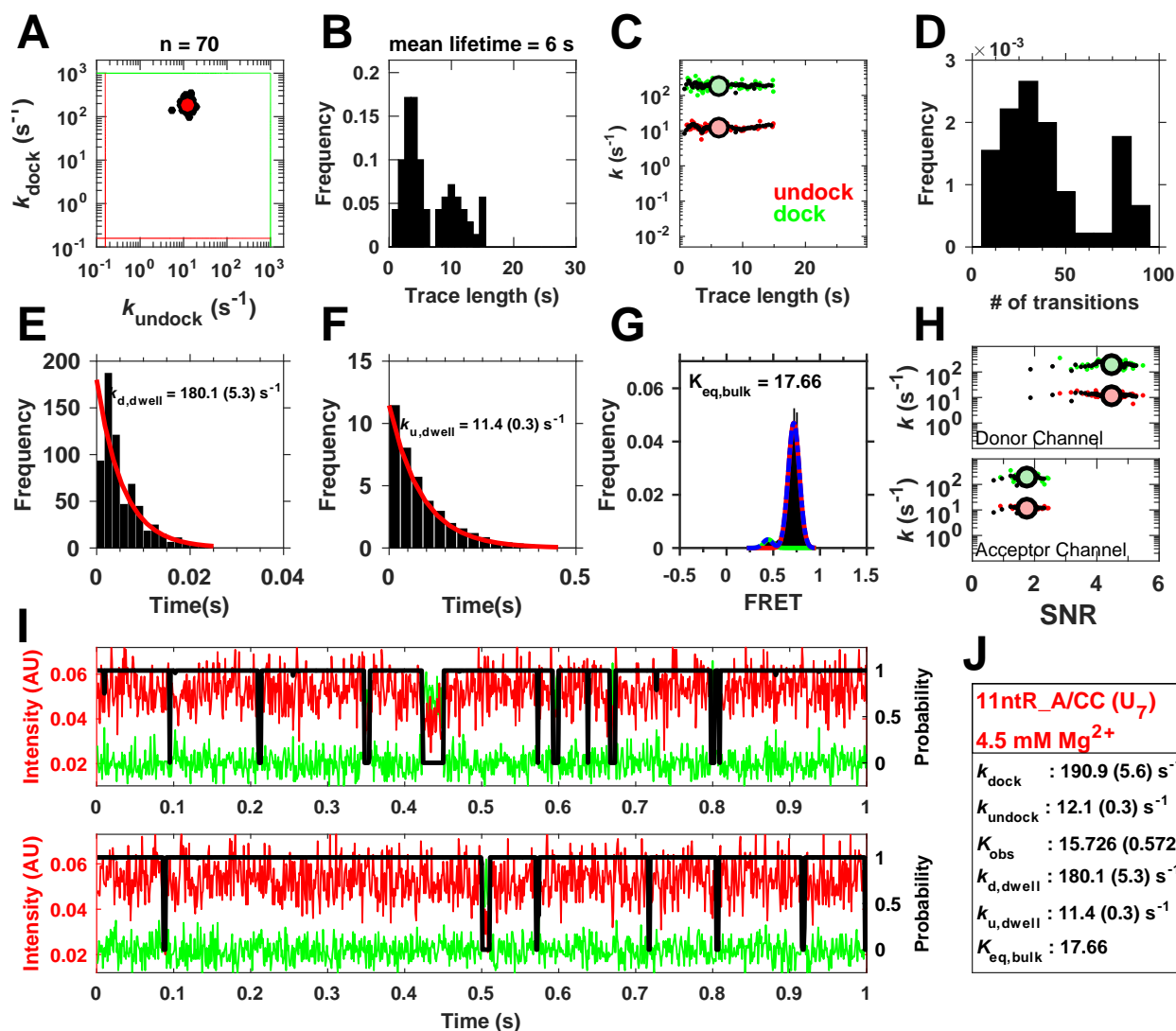


Figure S50. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

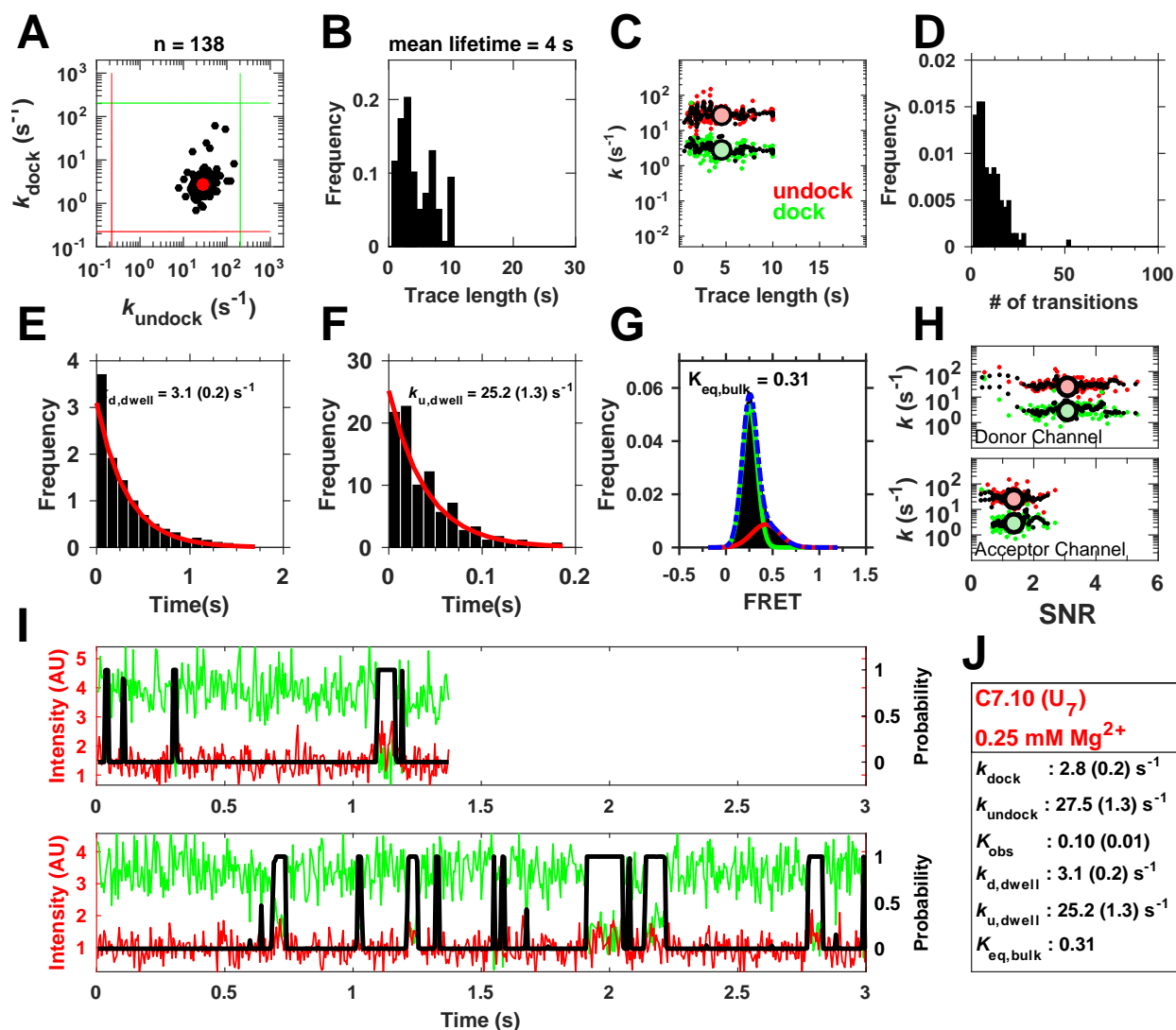


Figure S51. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

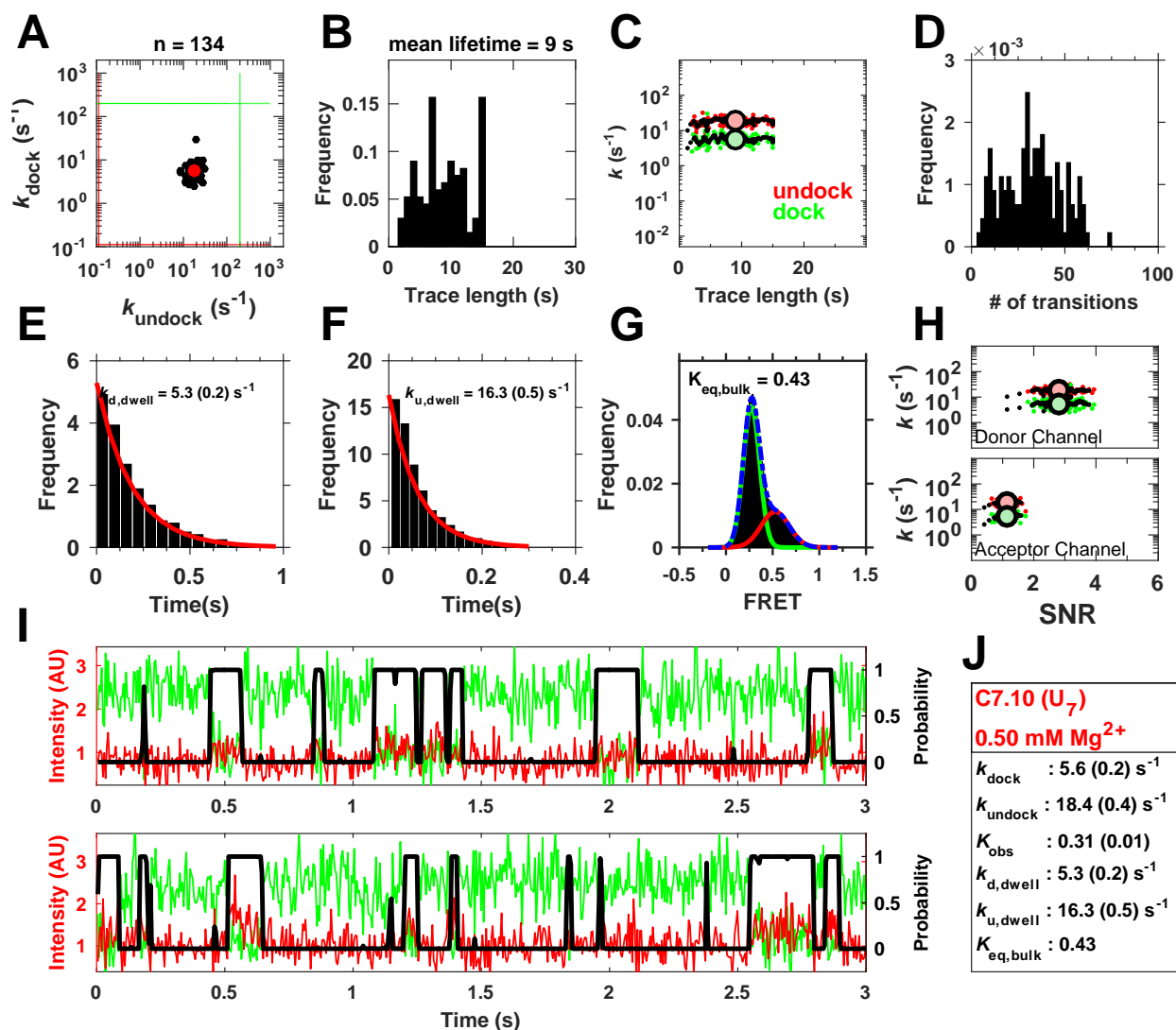


Figure S52. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

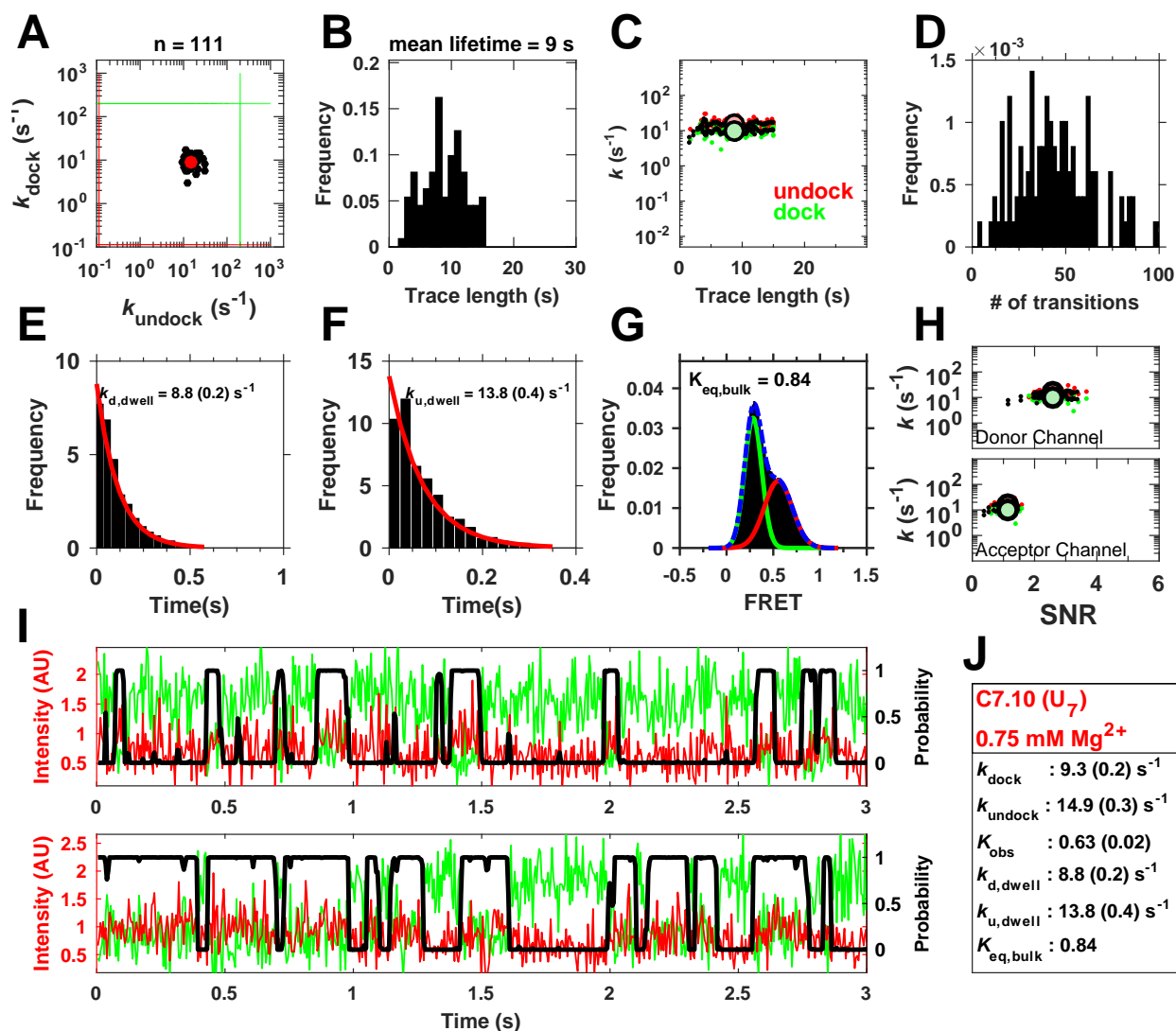


Figure S53. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.



Figure S54. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

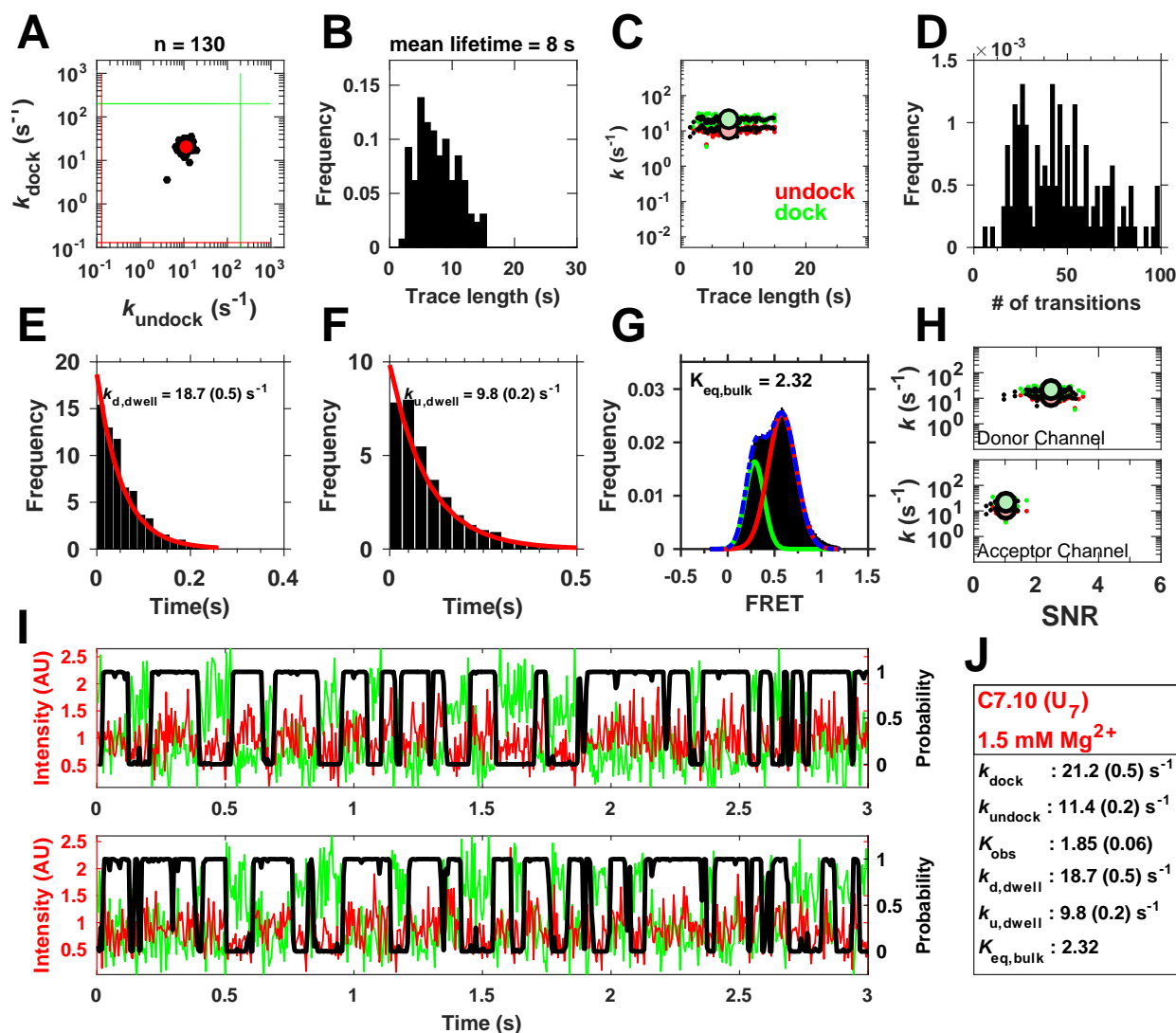


Figure S55. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

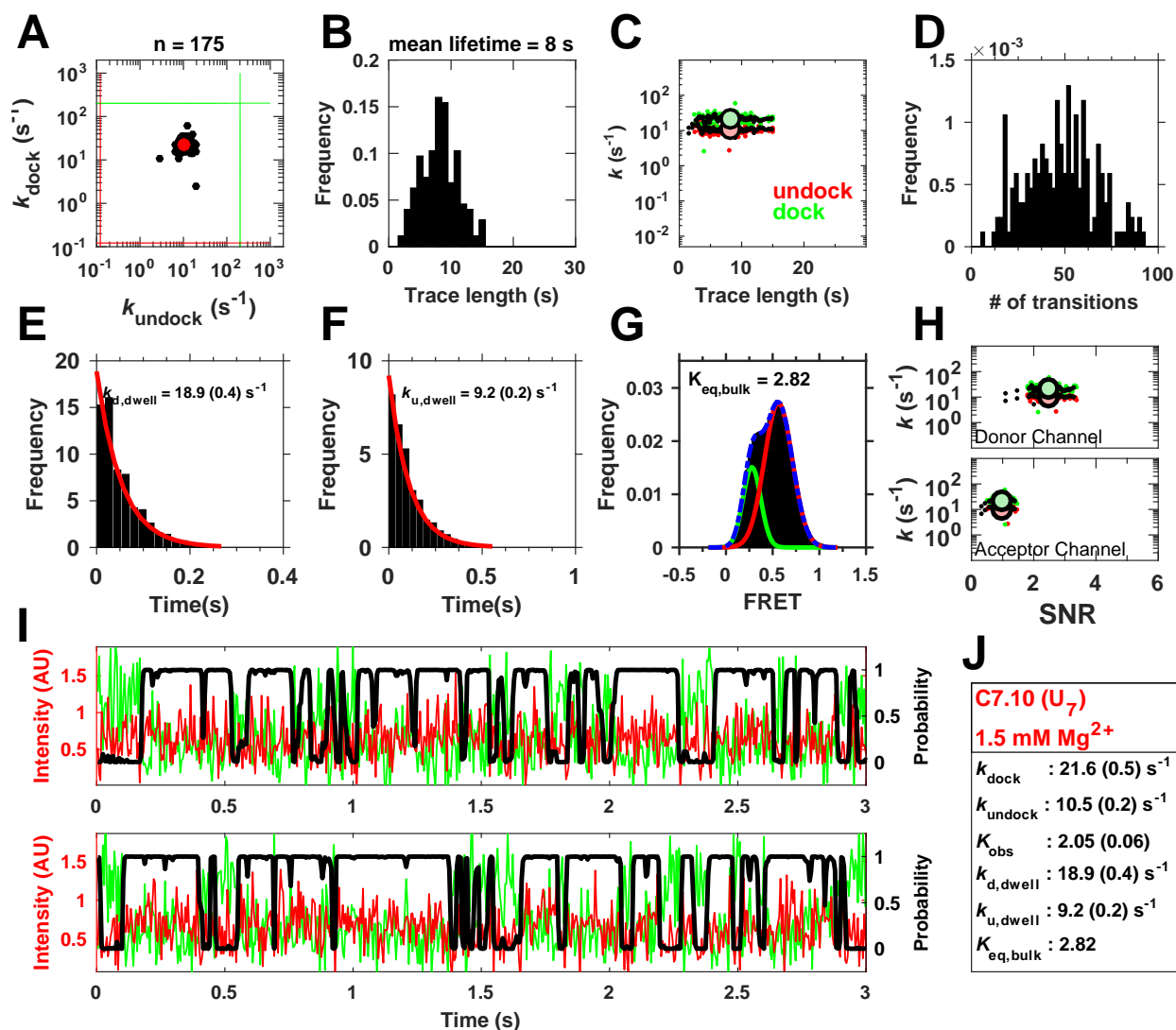


Figure S56. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

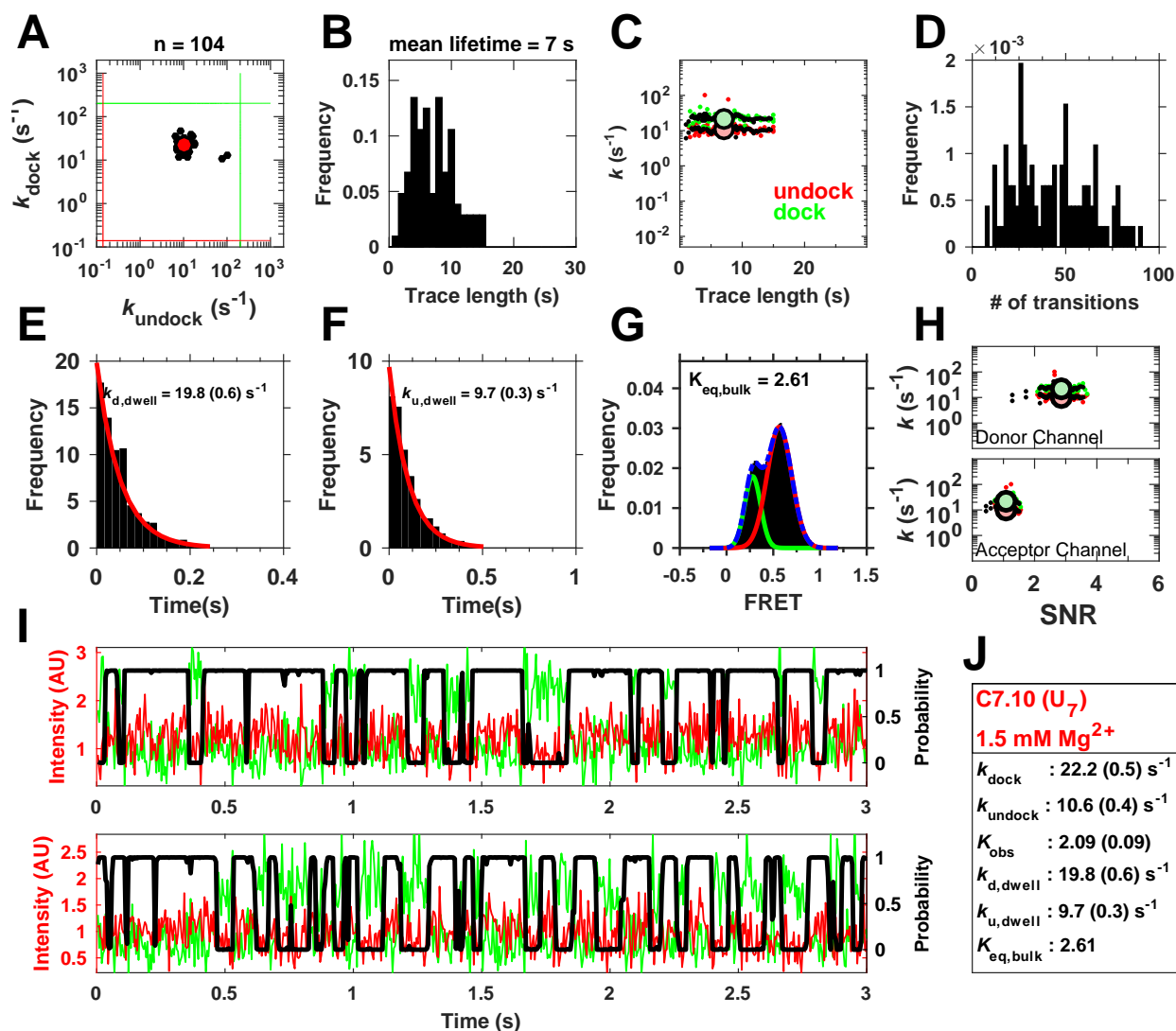


Figure S57. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

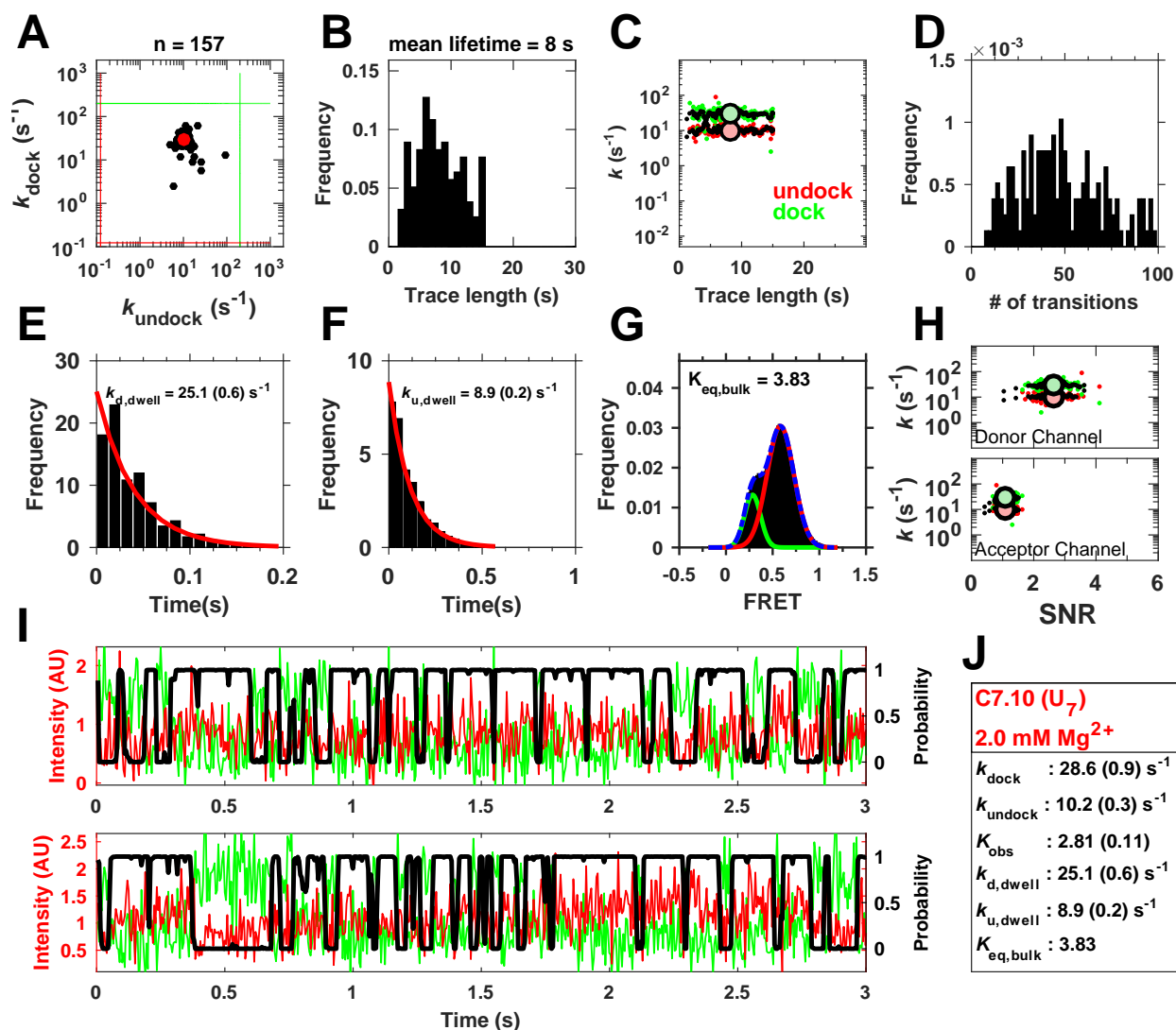


Figure S58. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

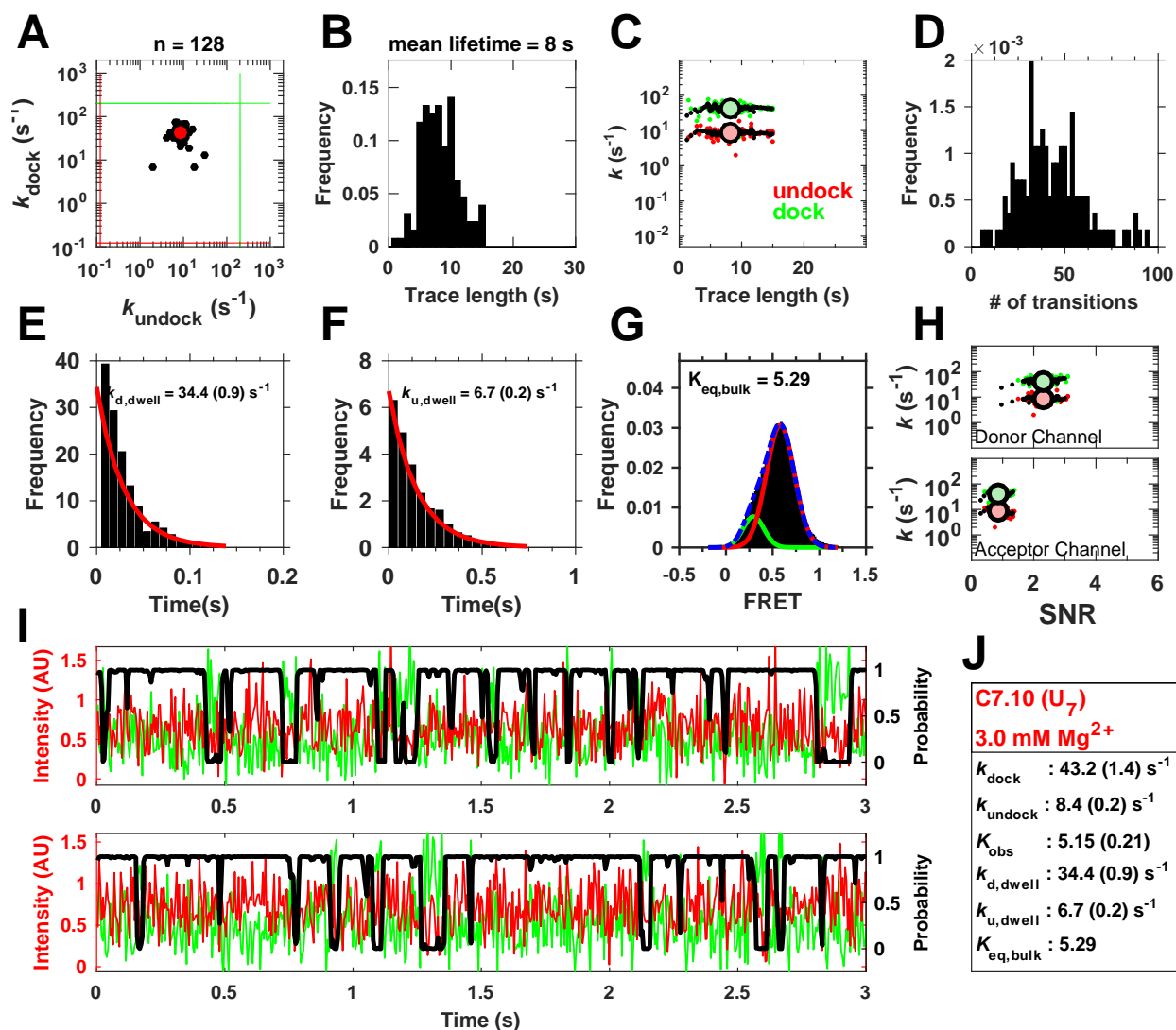


Figure S59. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

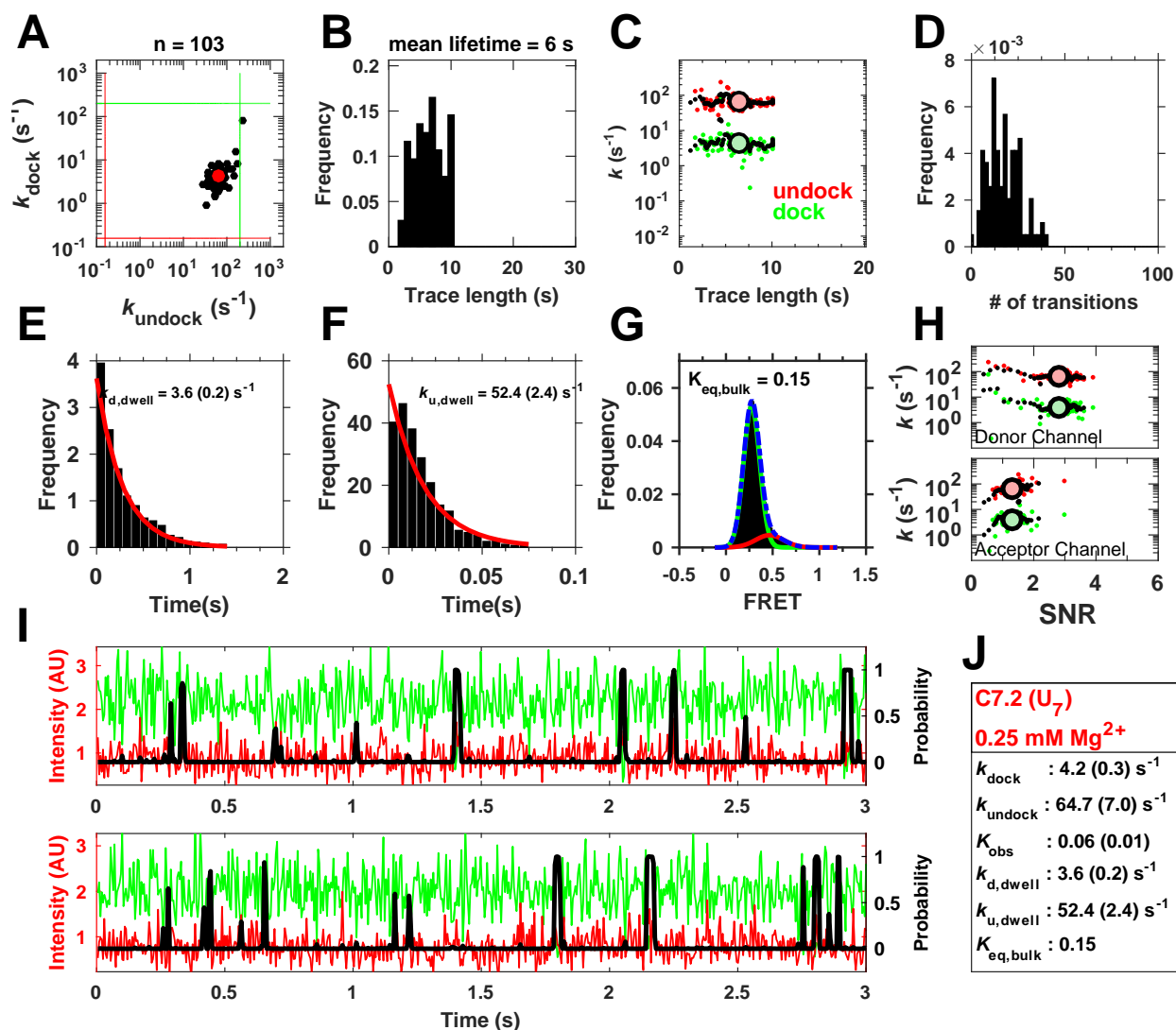


Figure S60. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

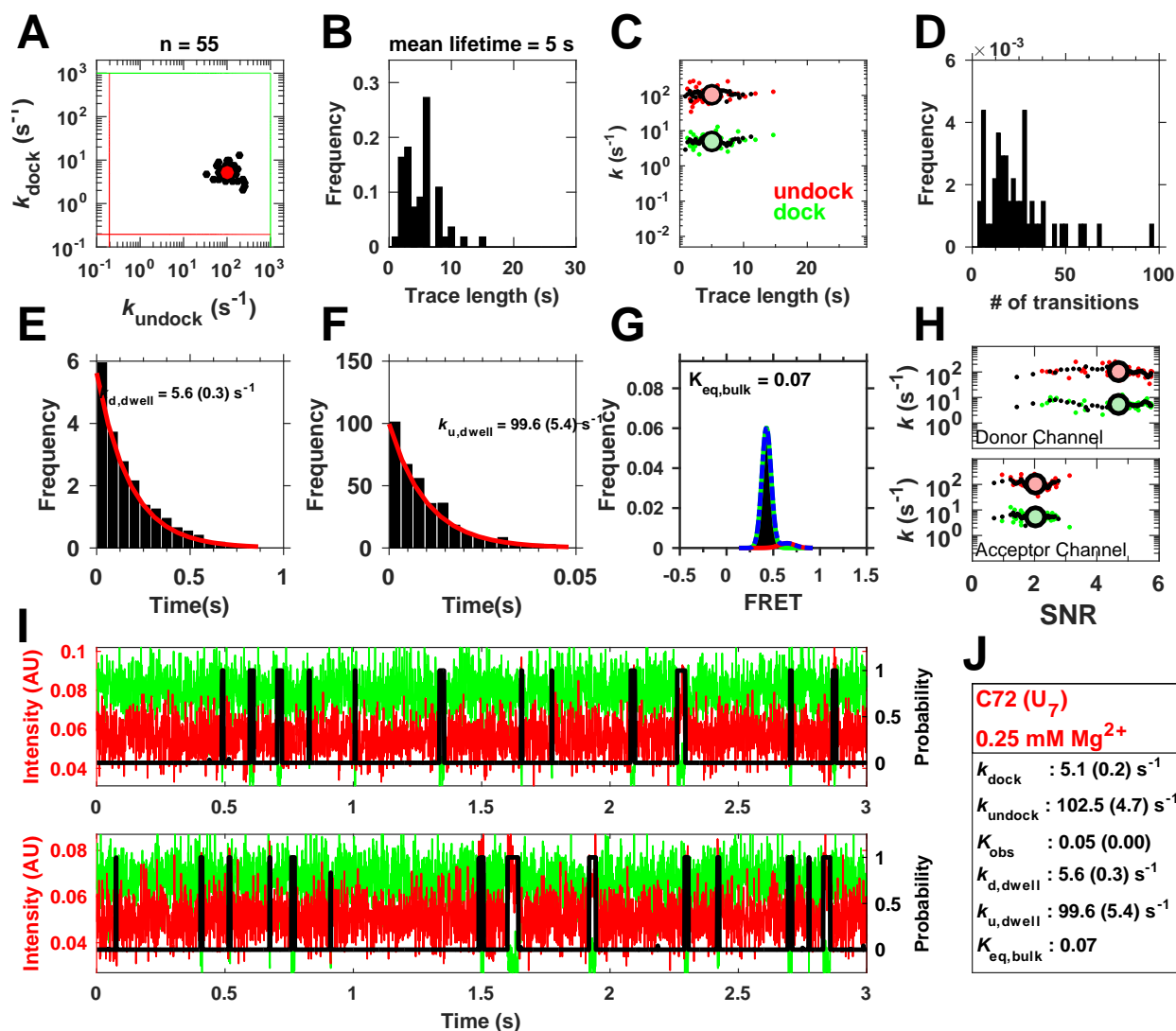


Figure S61. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

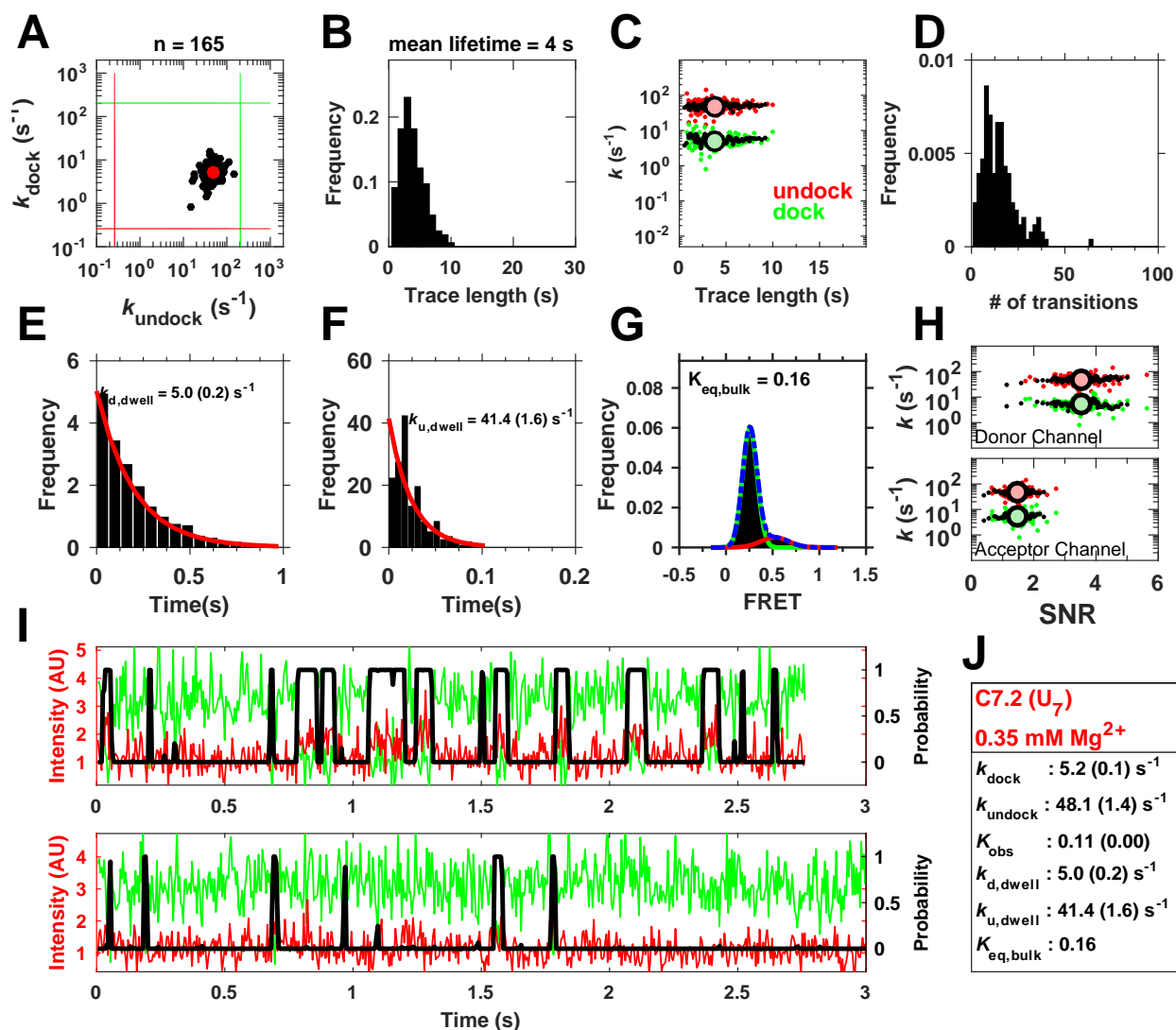


Figure S62. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

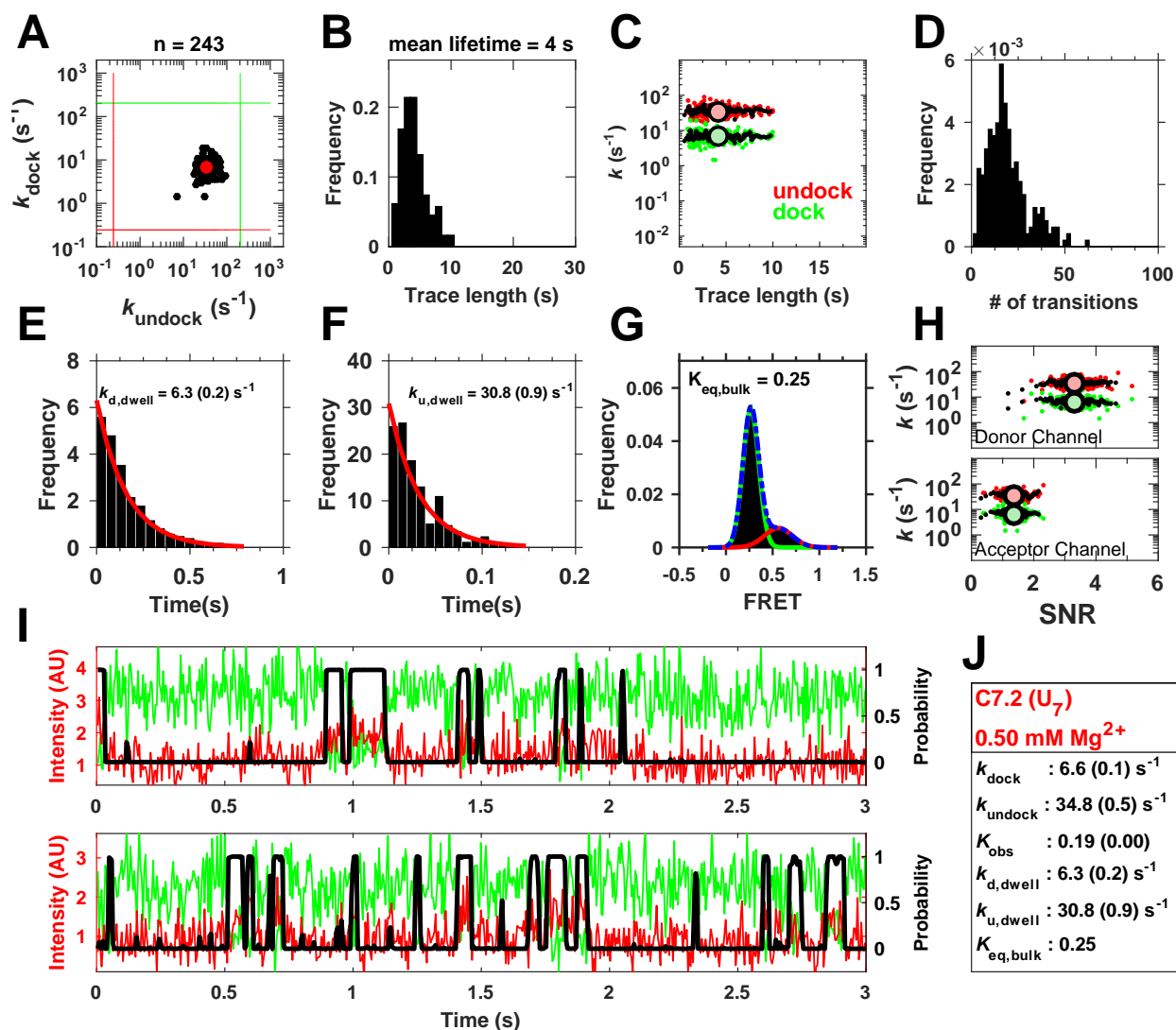


Figure S63. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

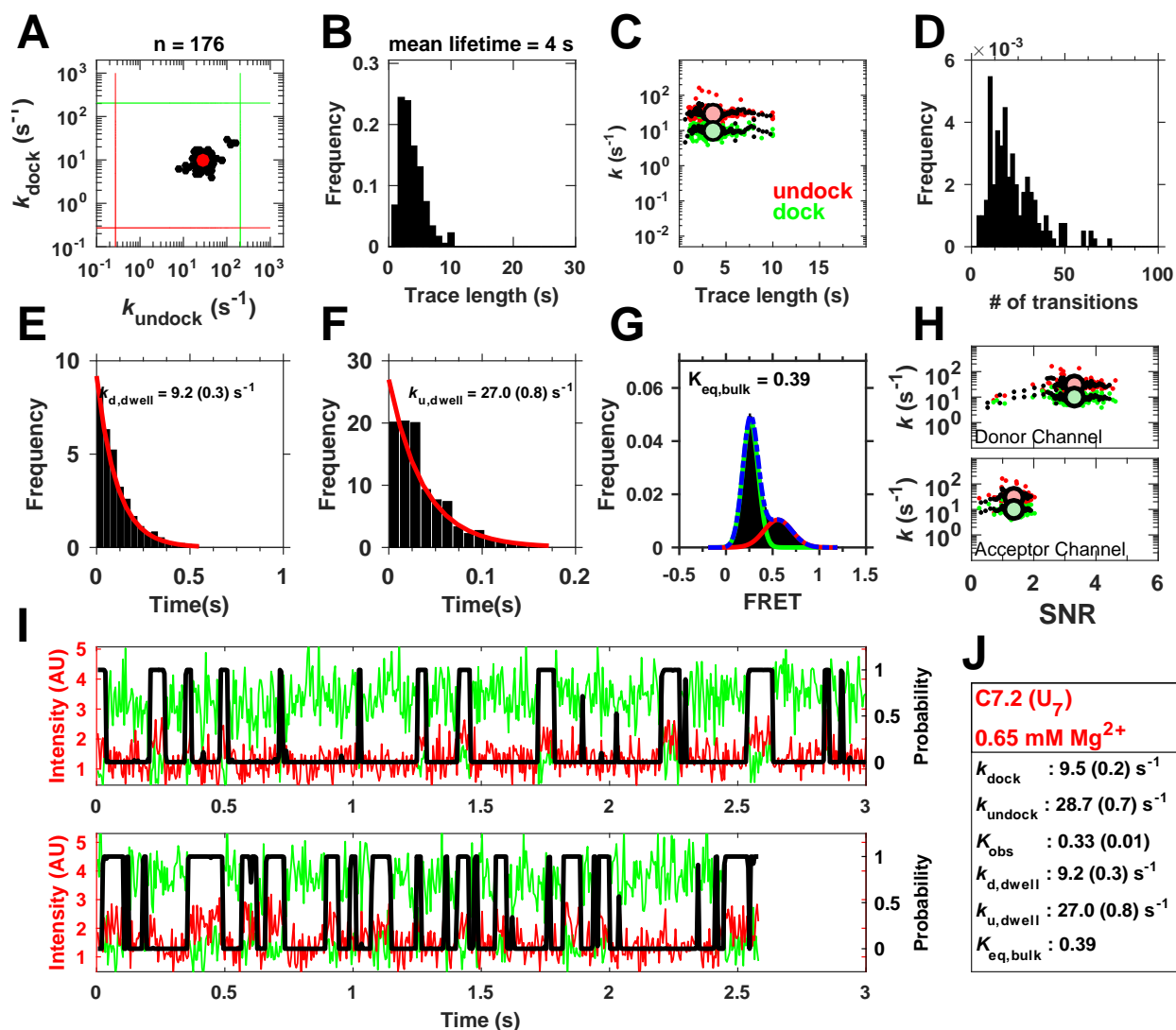


Figure S64. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

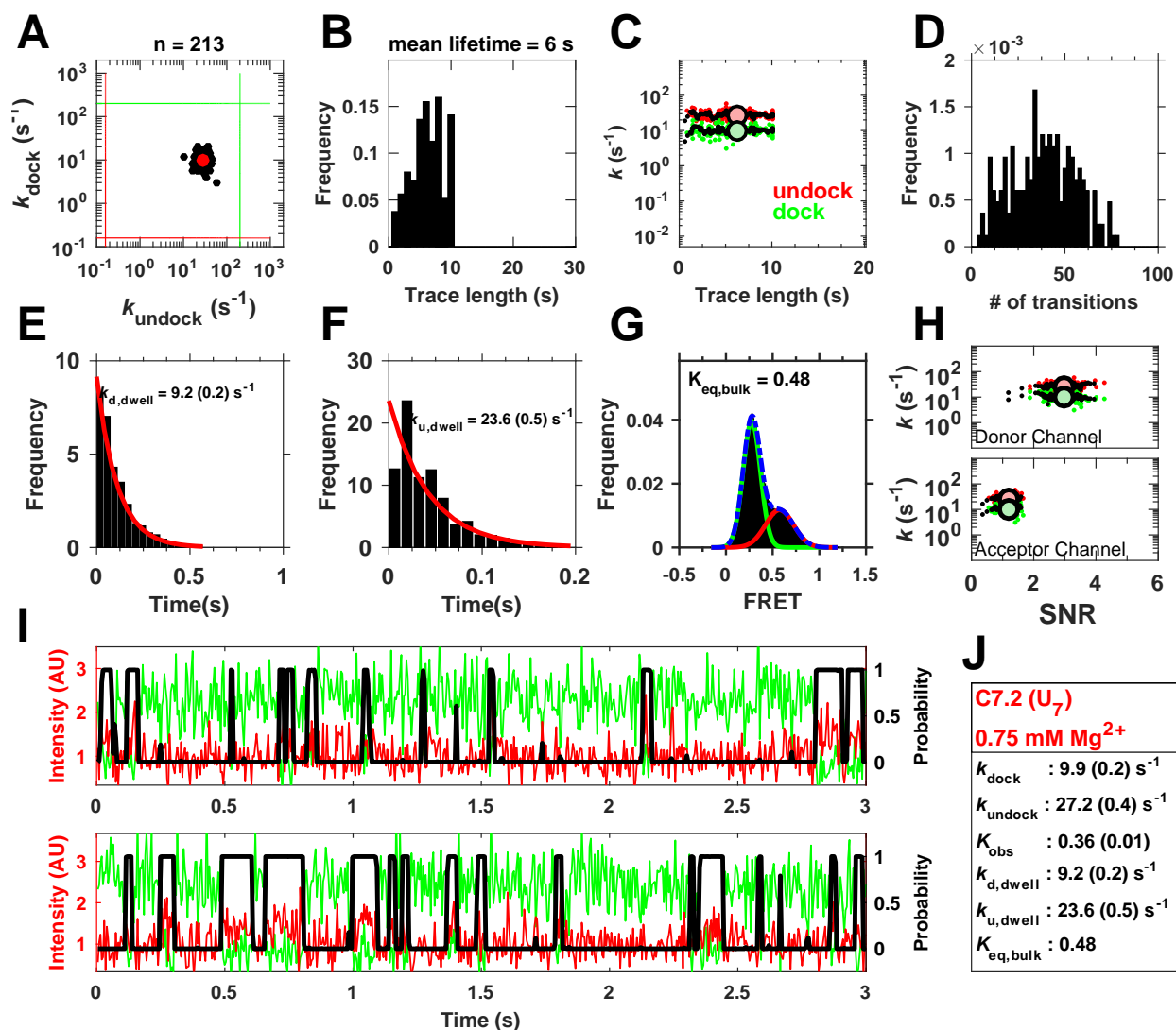


Figure S65. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

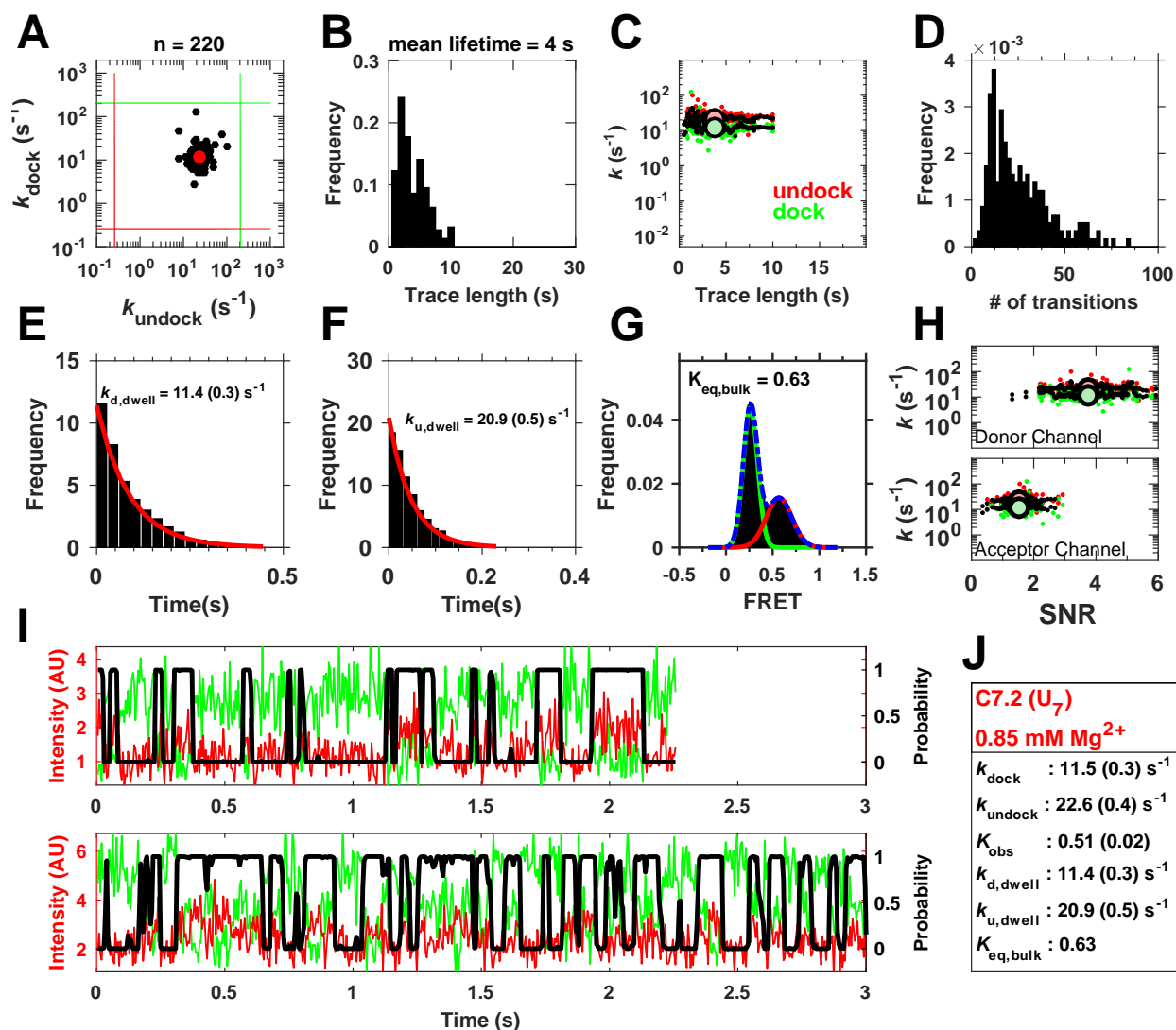


Figure S66. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

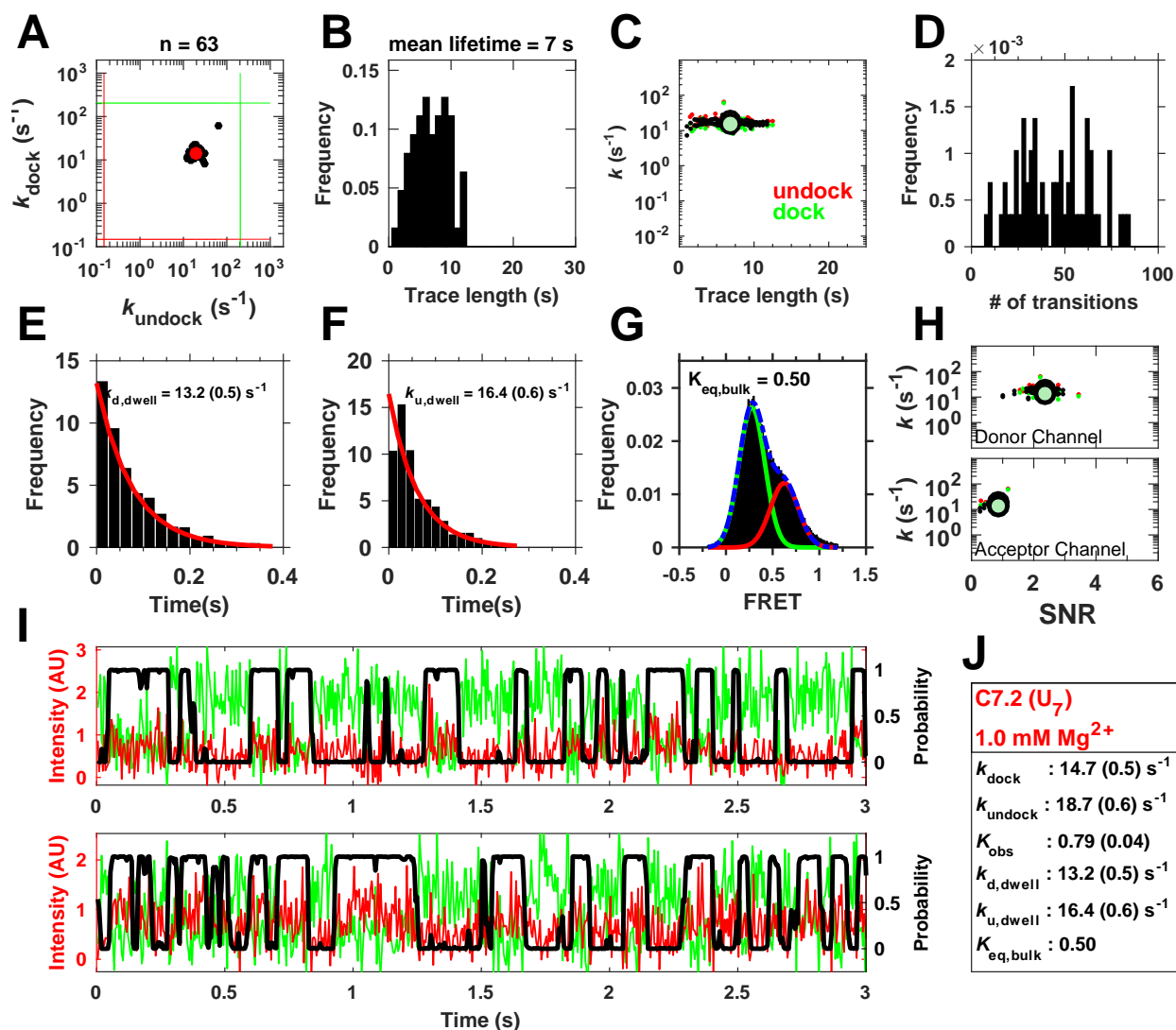


Figure S67. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

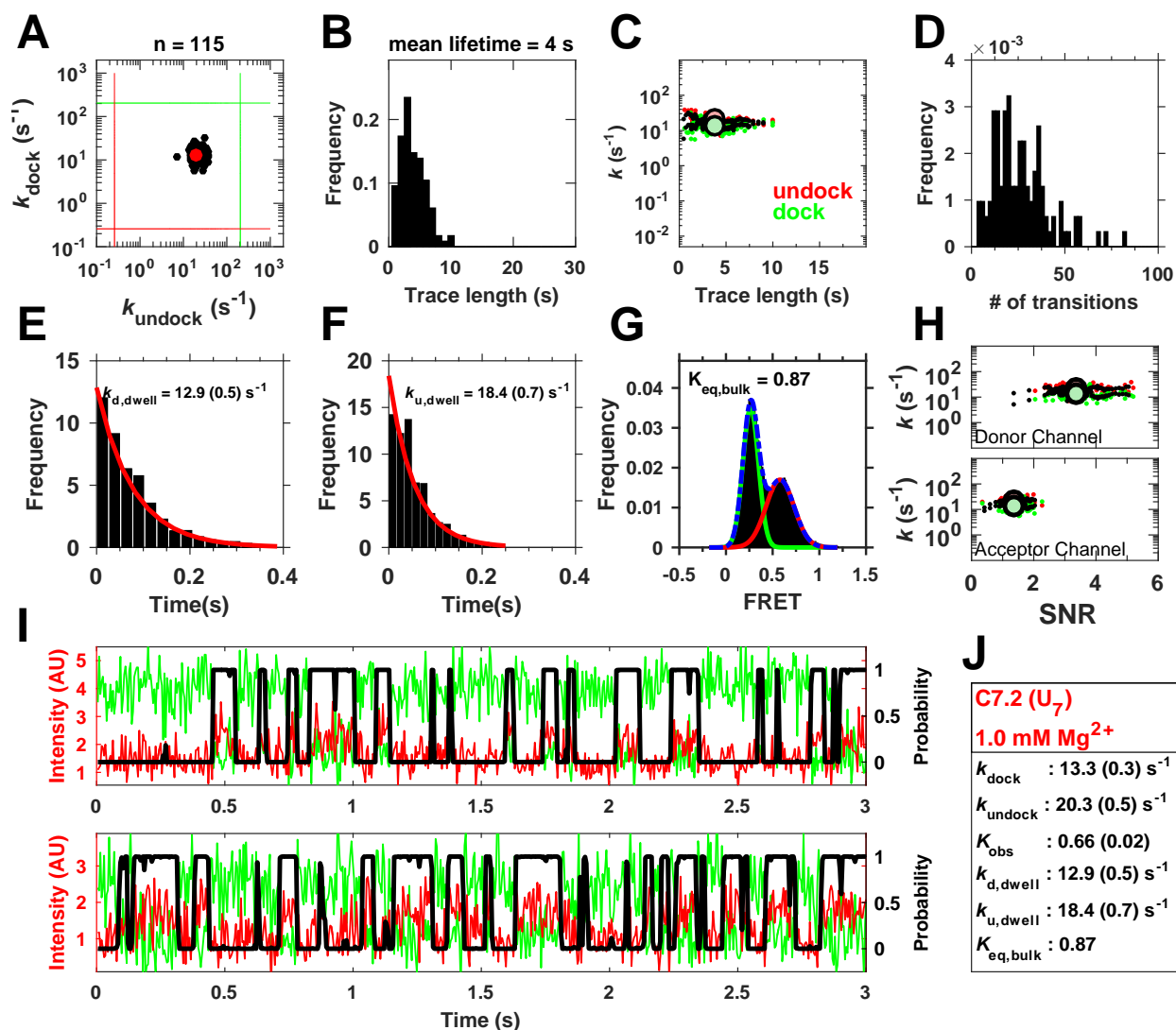


Figure S68. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

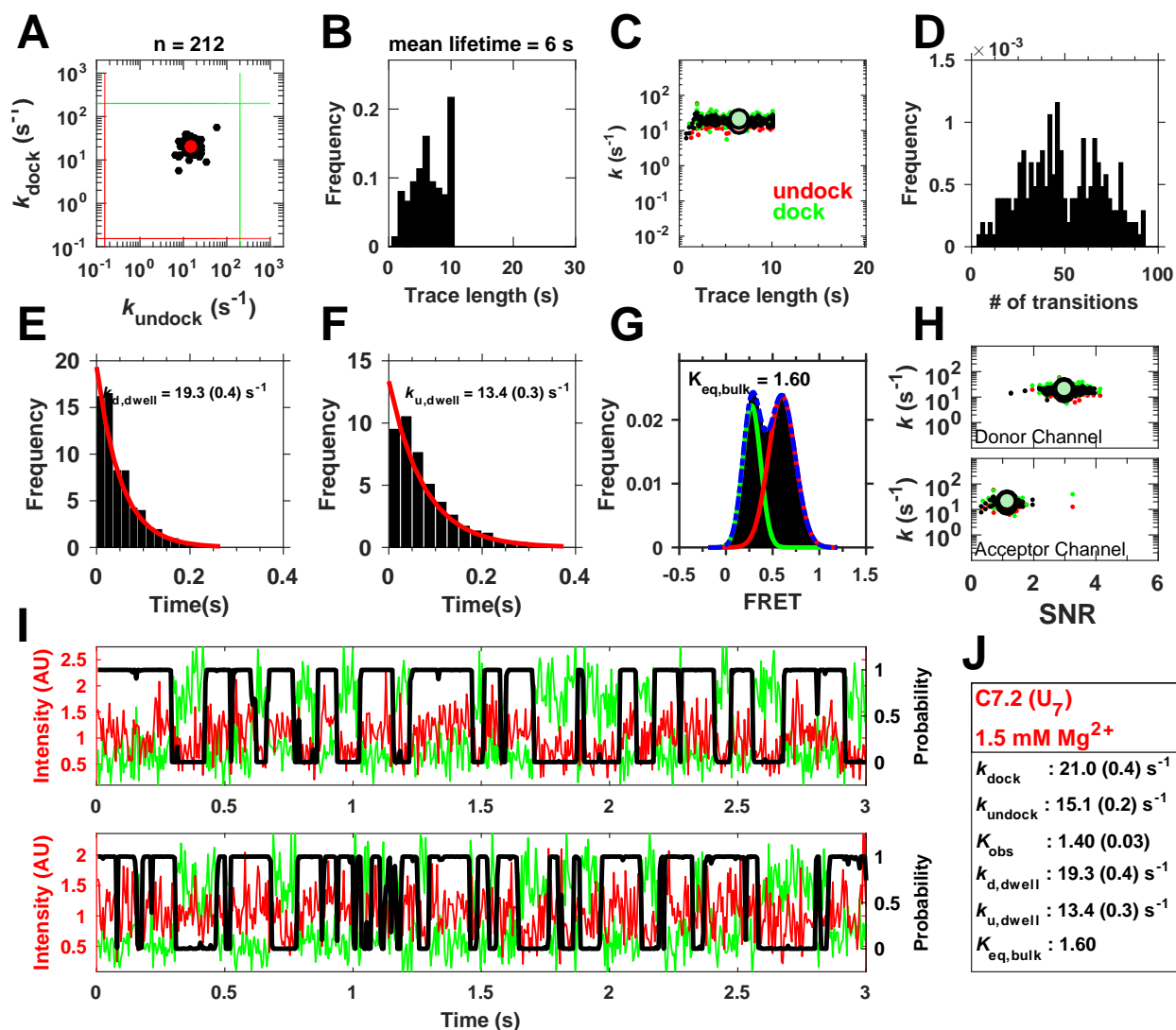


Figure S69. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

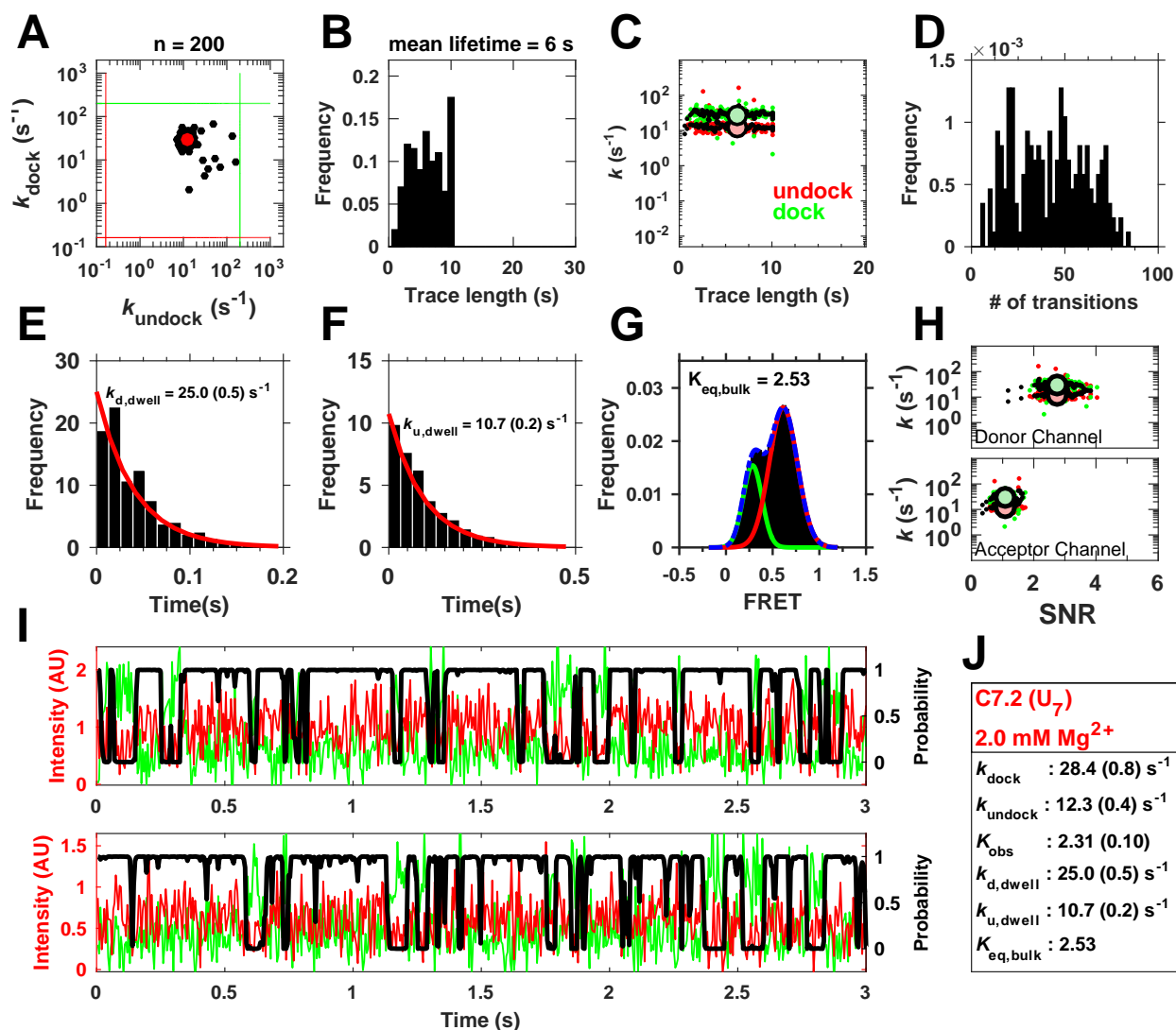


Figure S70. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

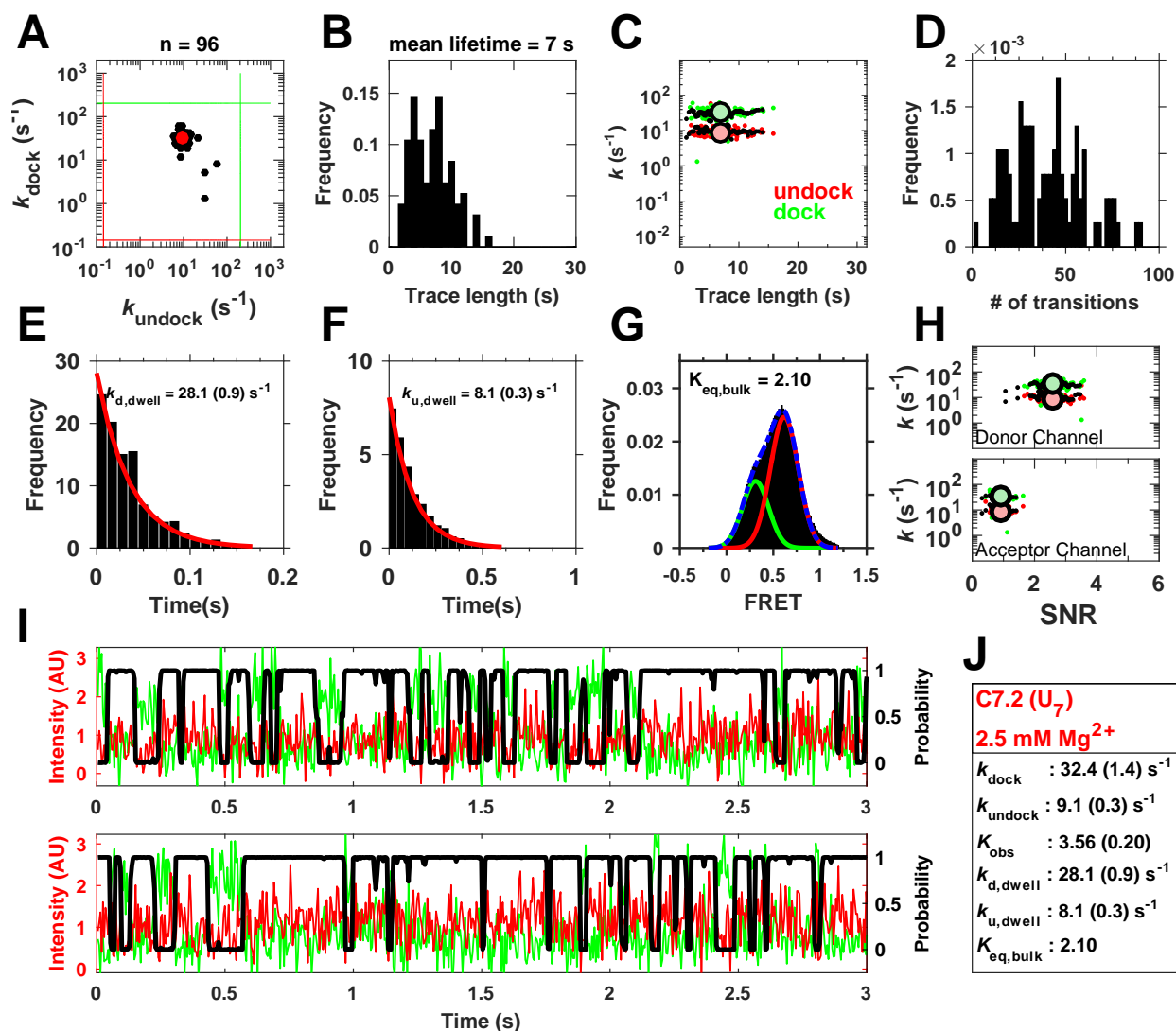


Figure S71. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

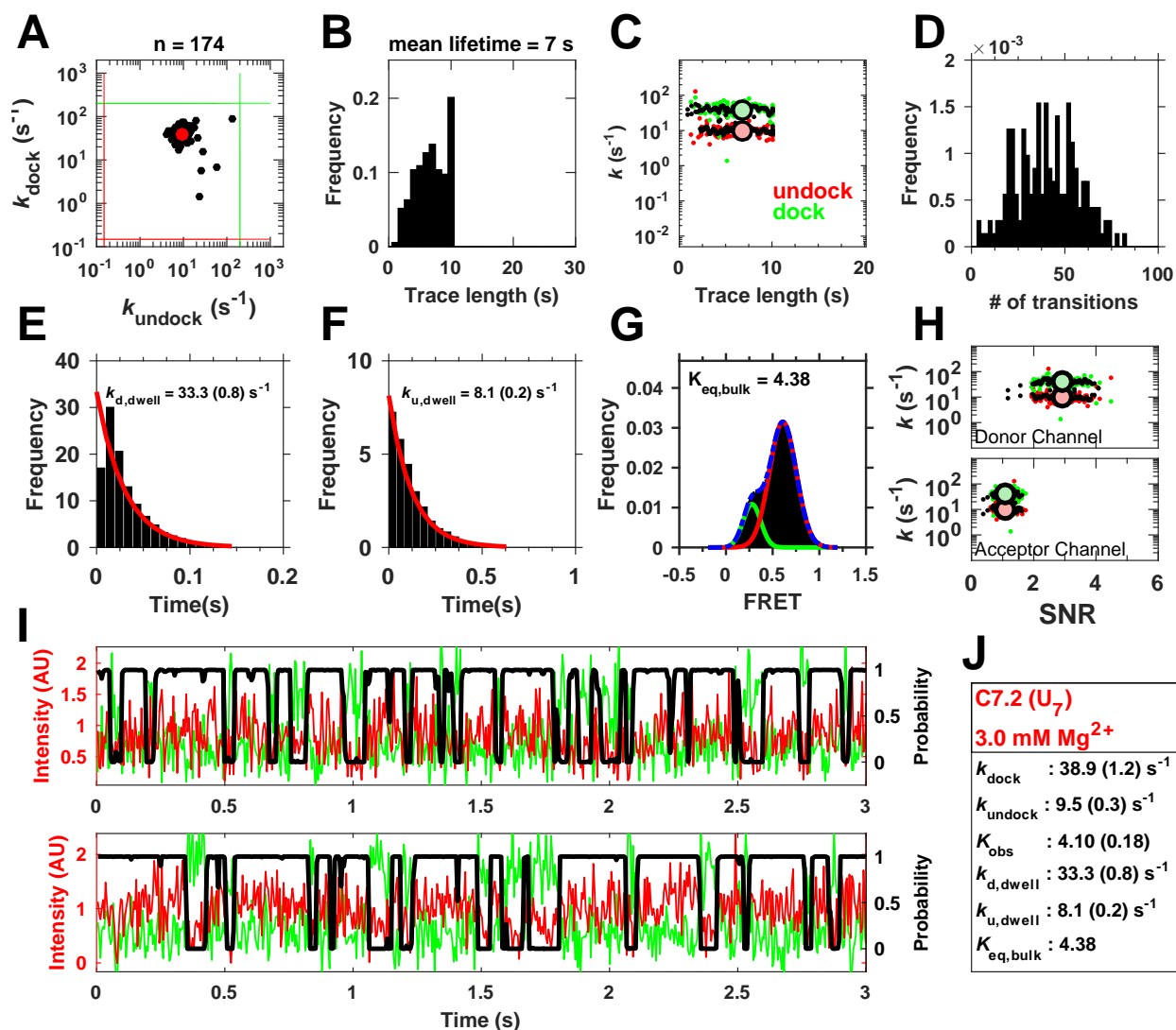


Figure S72. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

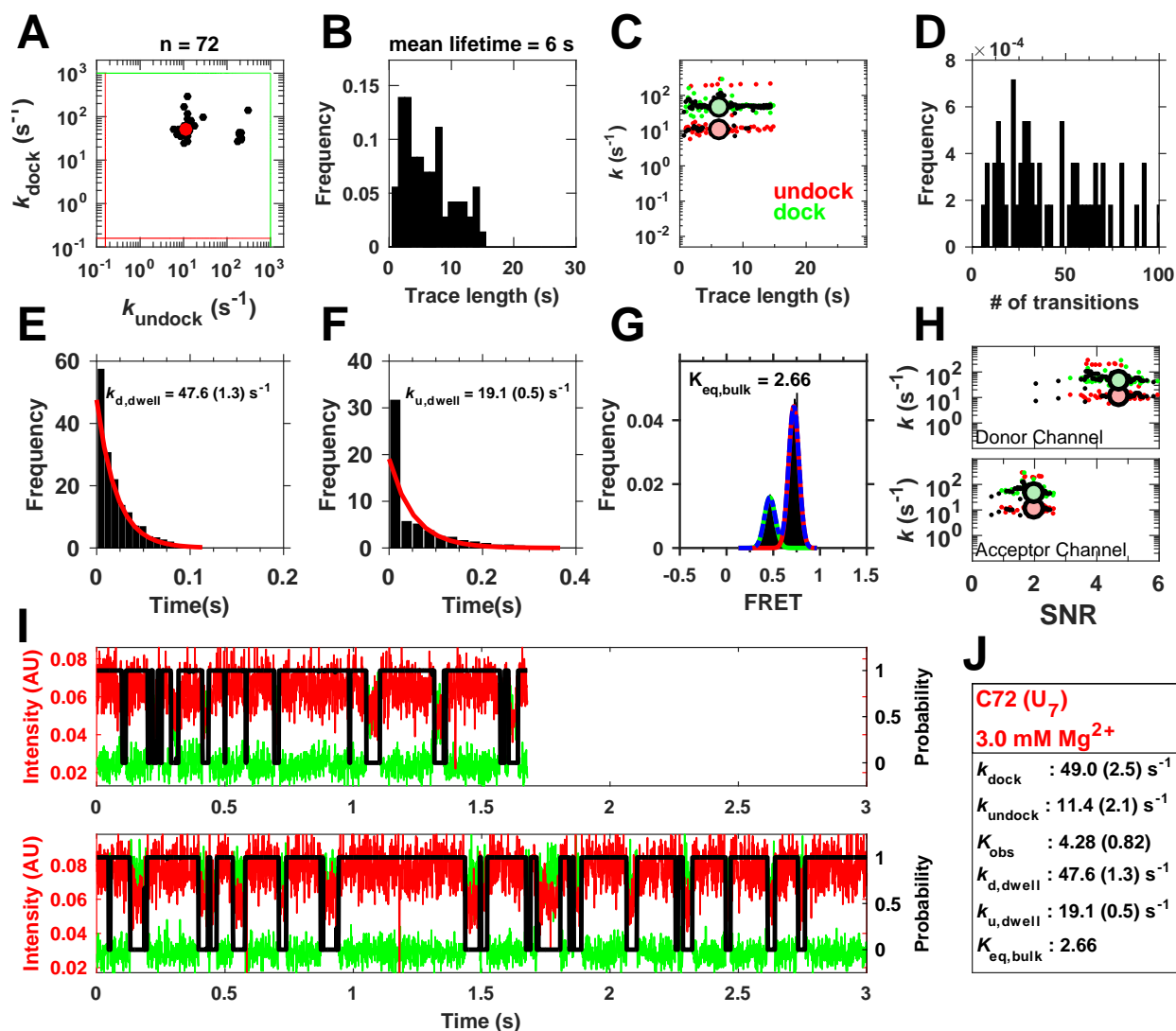


Figure S73. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

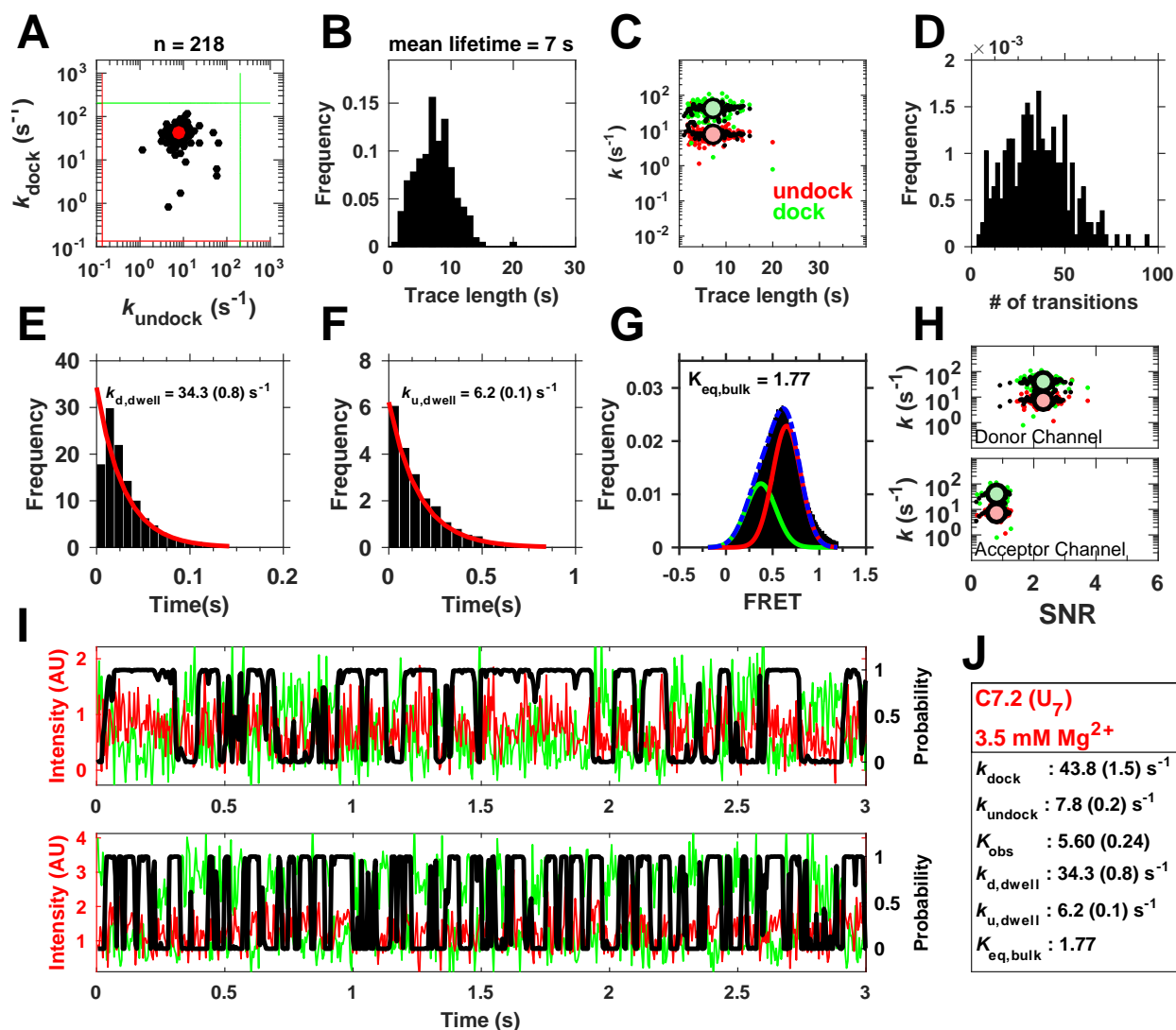


Figure S74. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

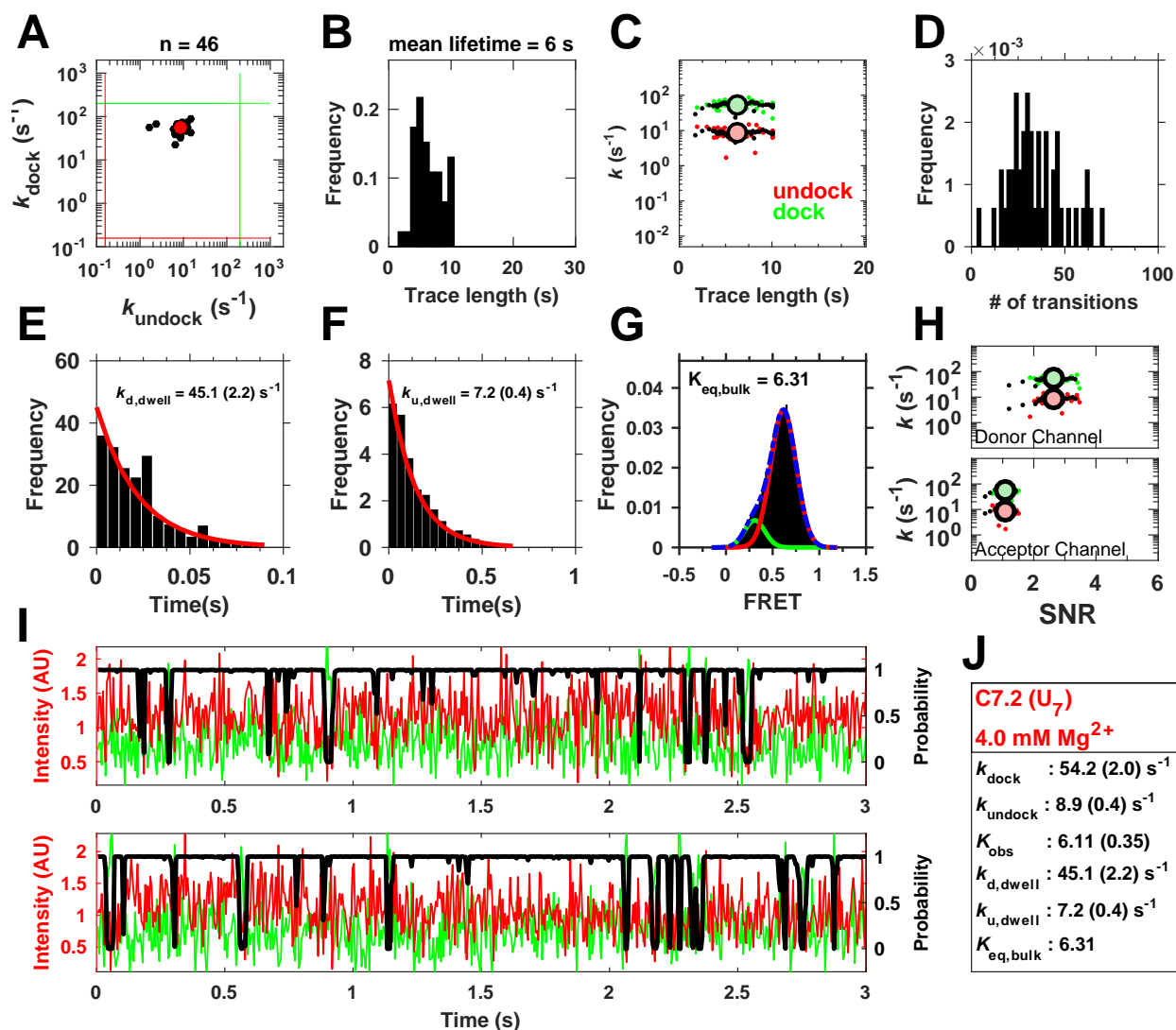


Figure S75. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

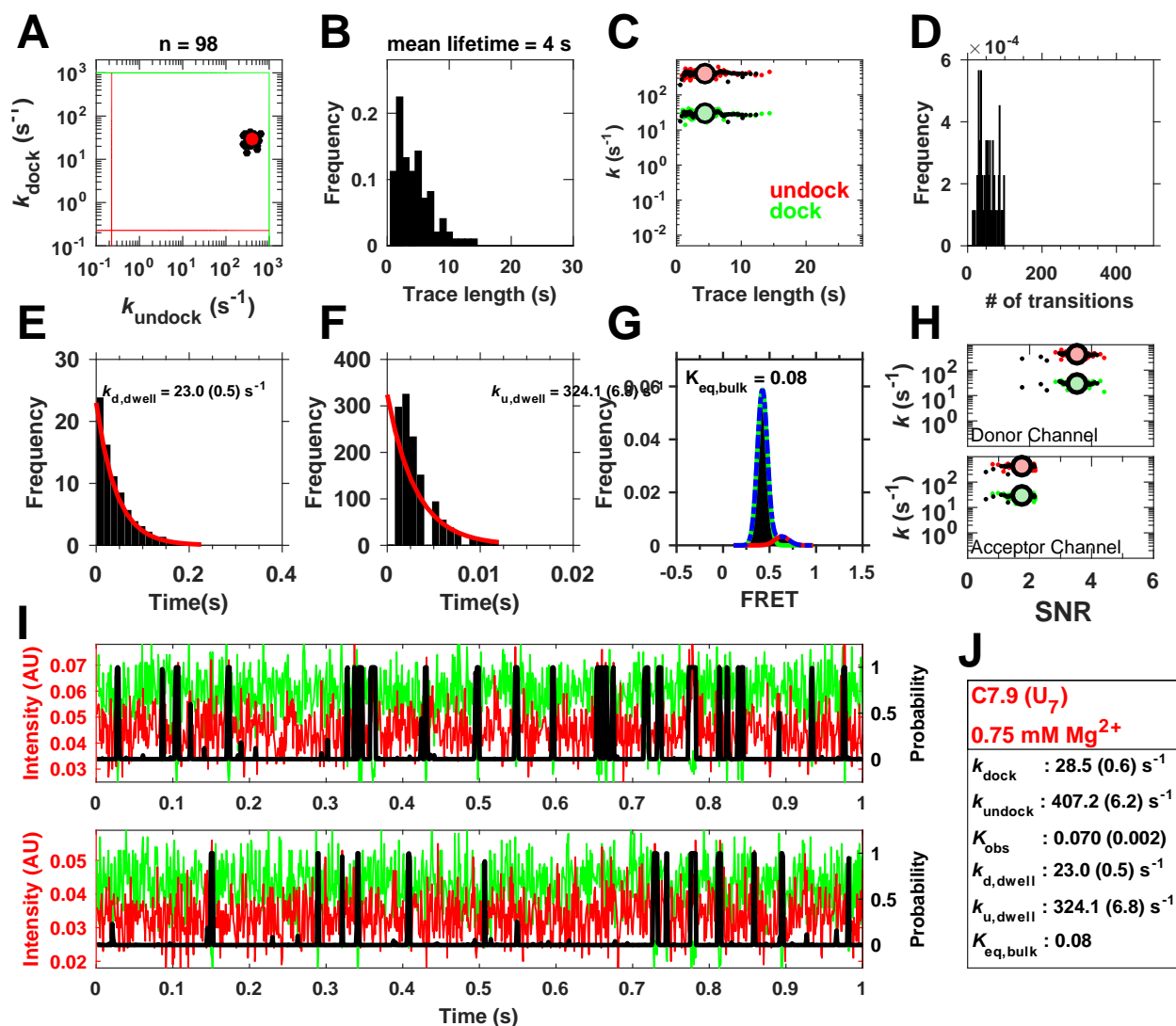


Figure S76. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

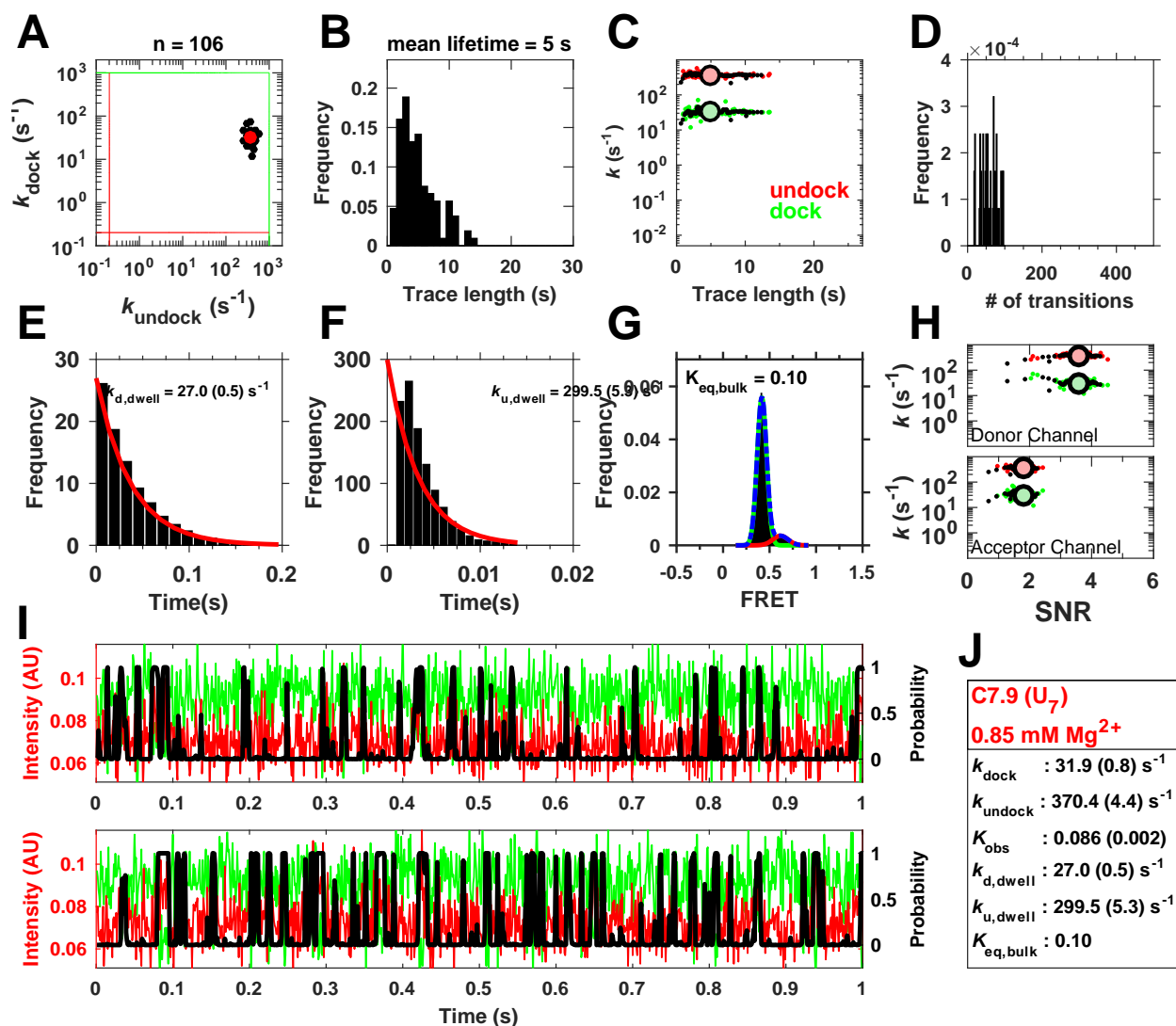


Figure S77. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

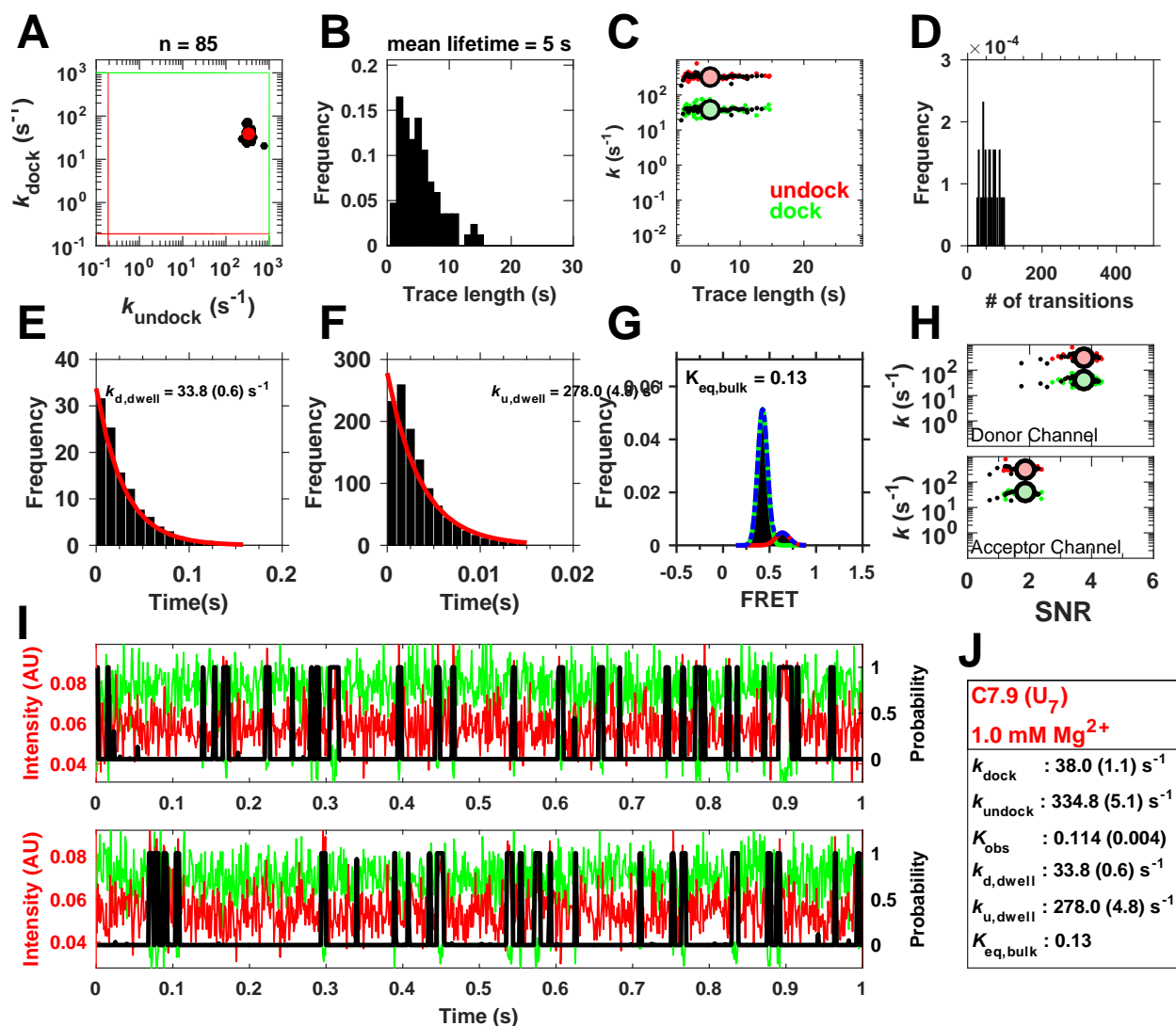


Figure S78. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

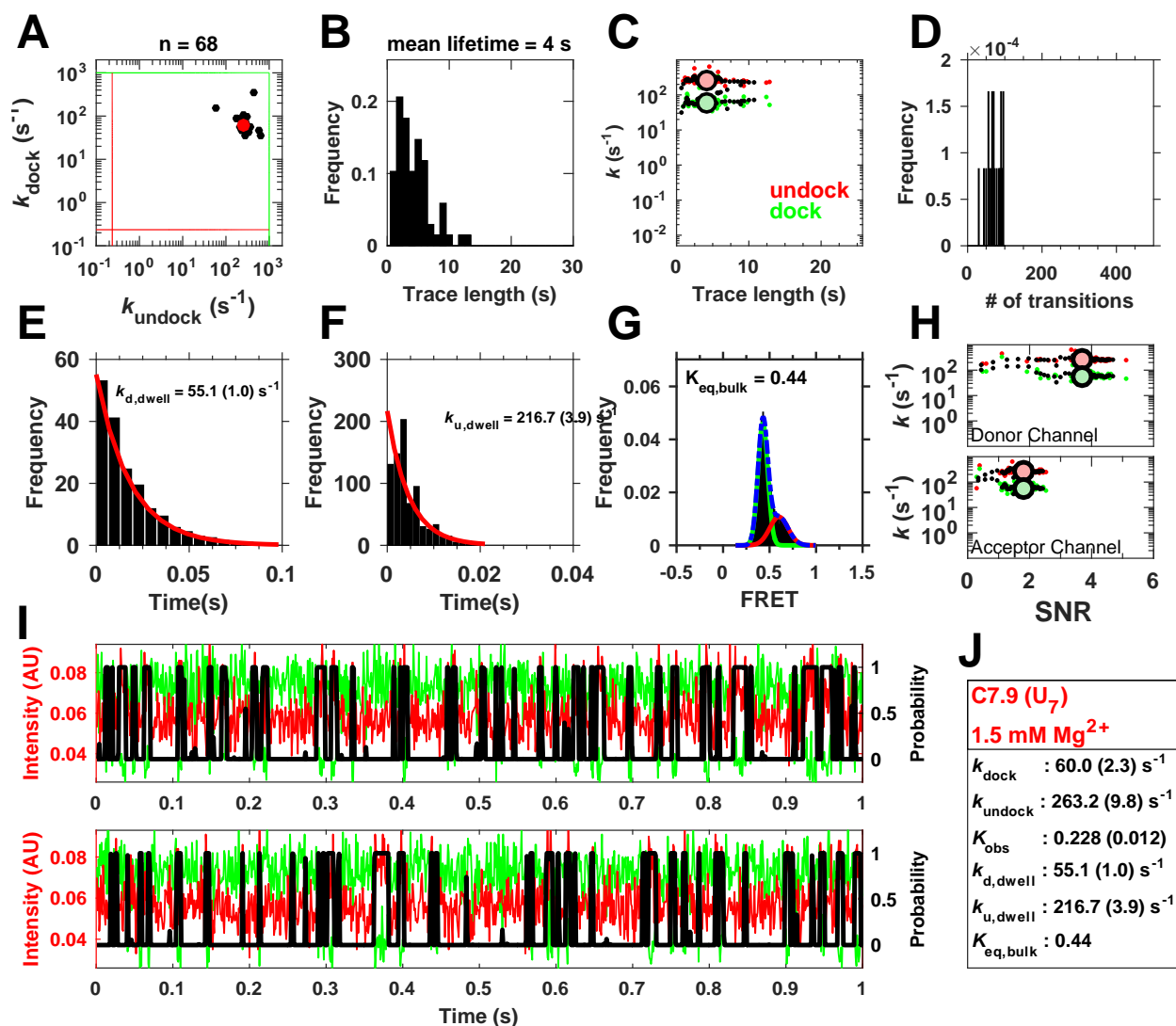


Figure S79. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

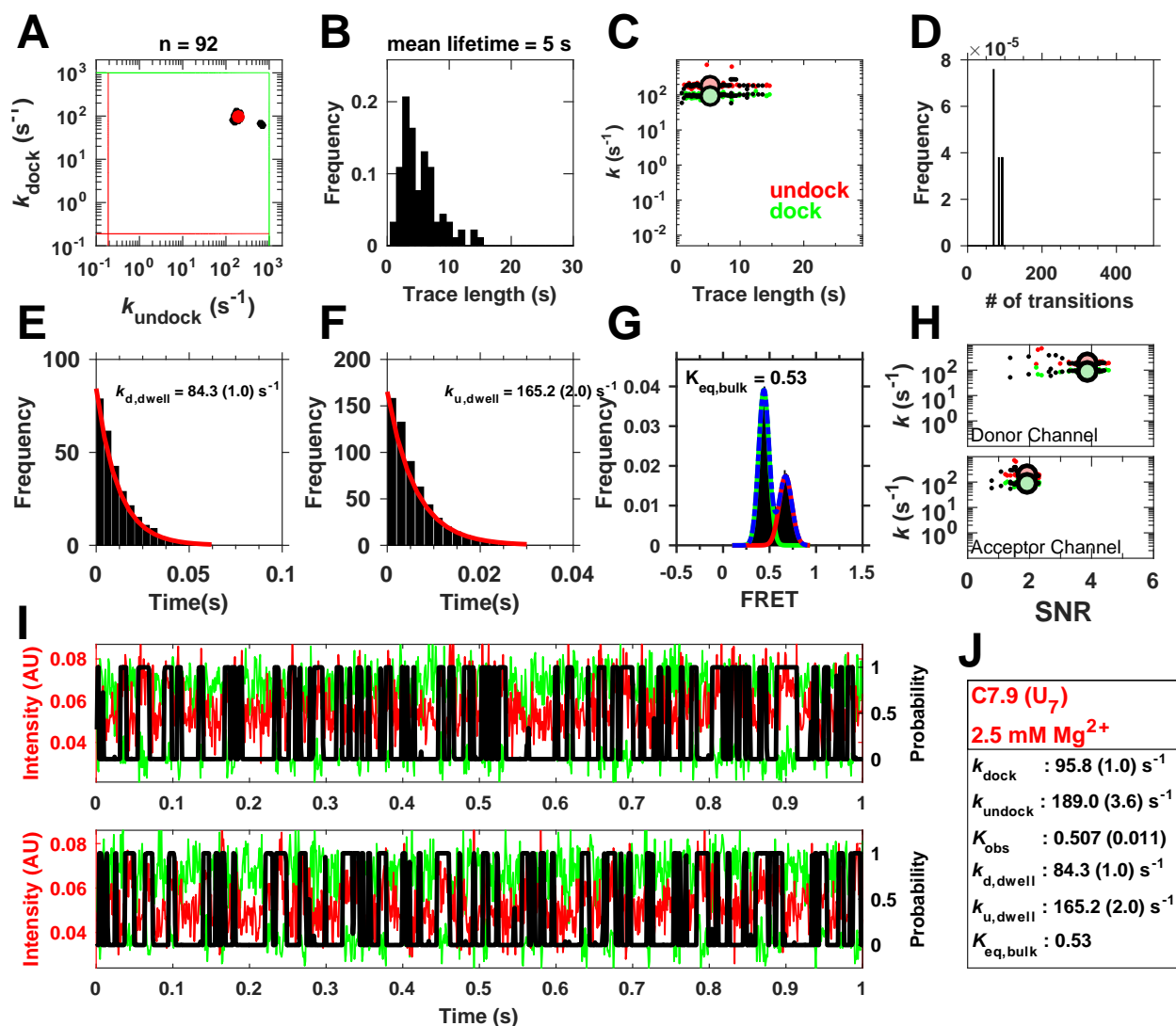


Figure S80. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

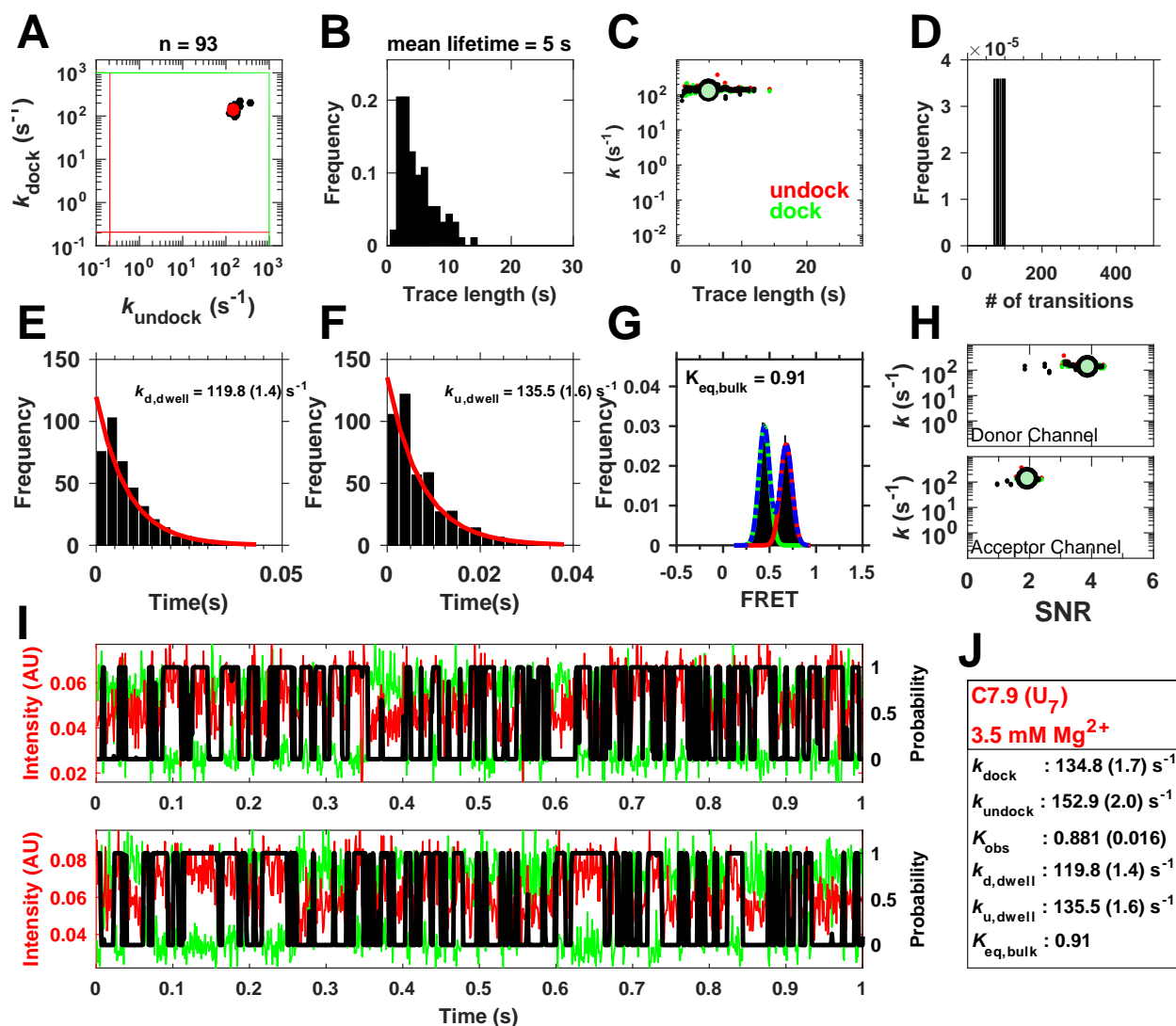


Figure S81. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

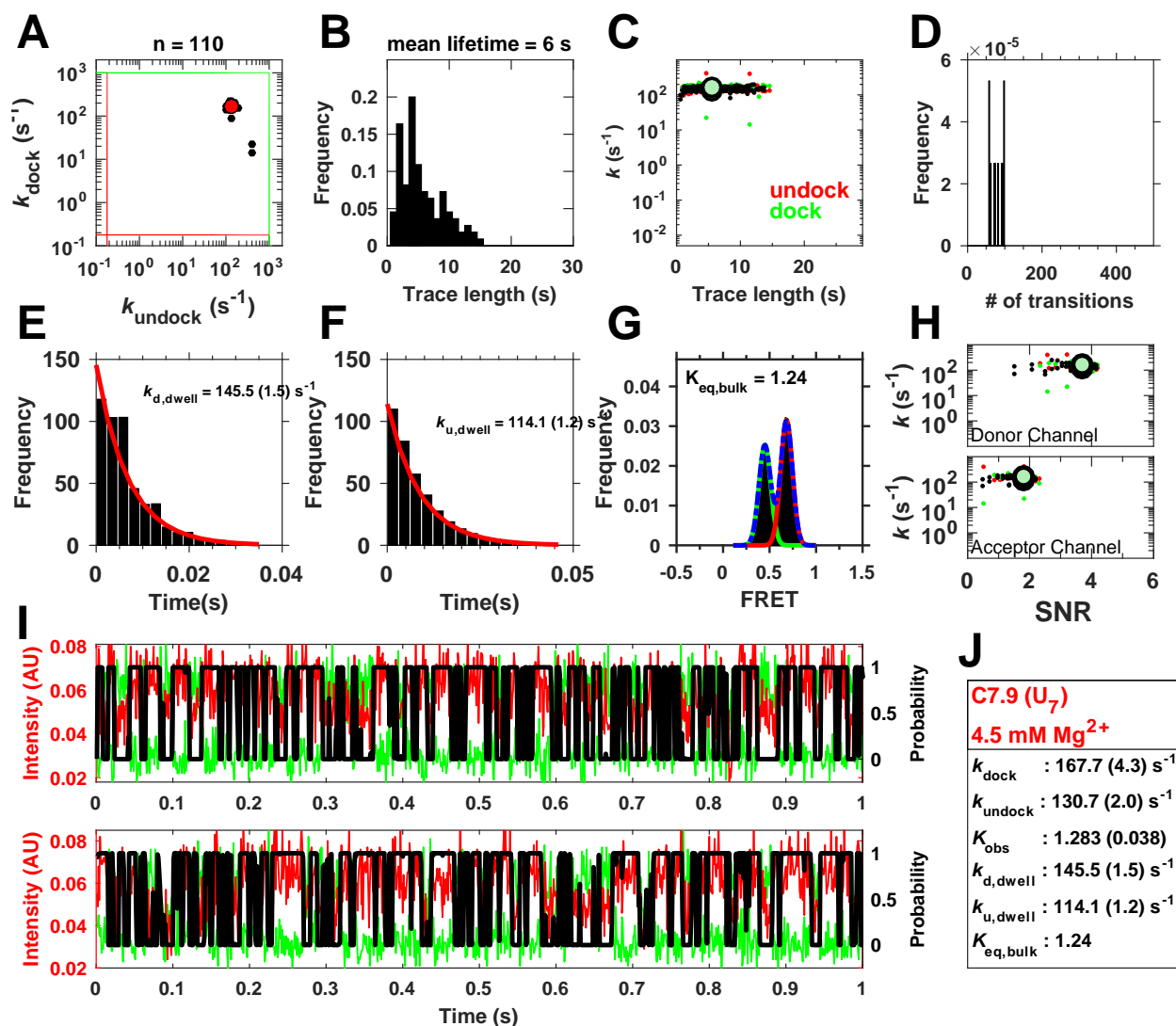


Figure S82. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

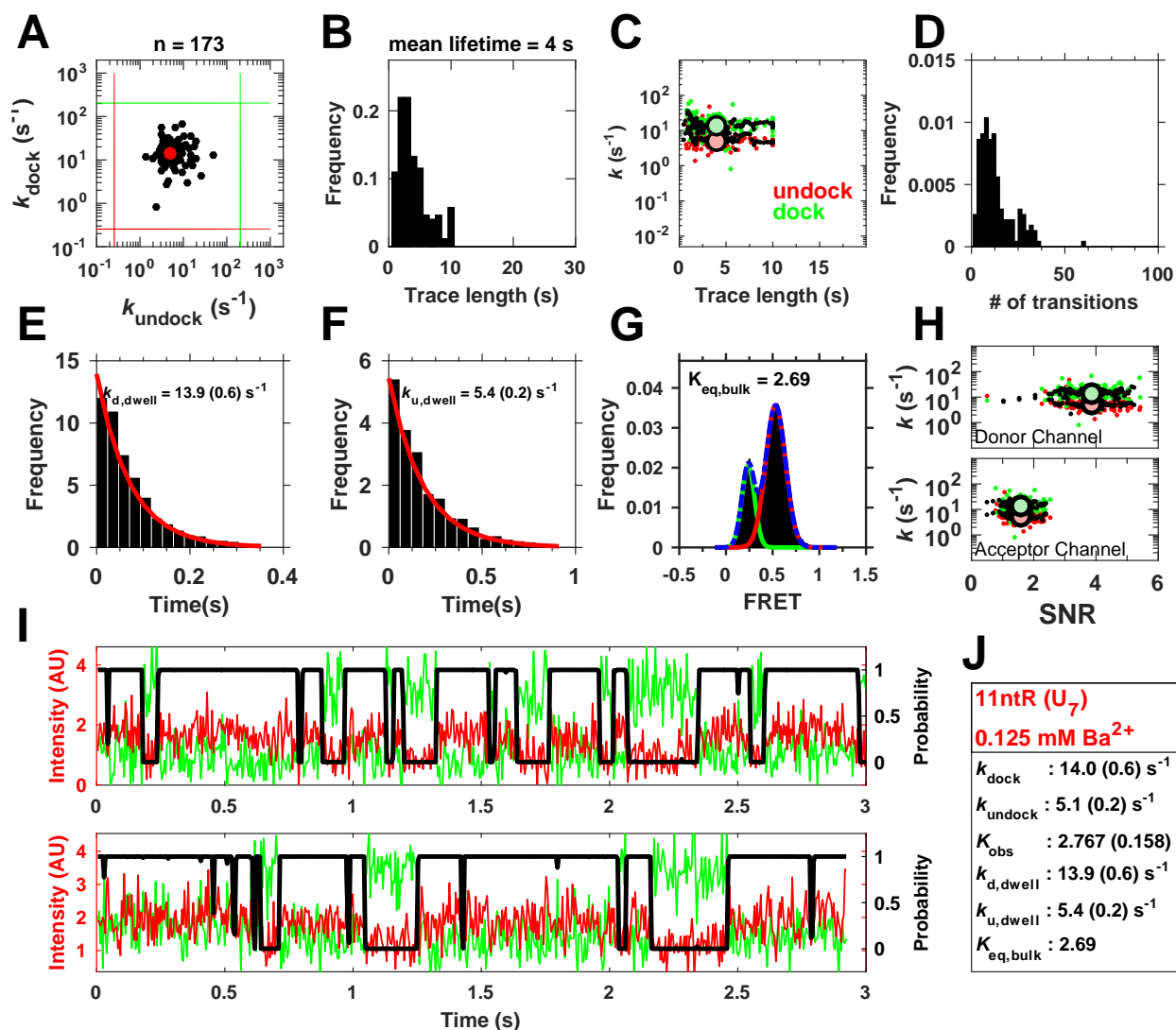


Figure S83. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

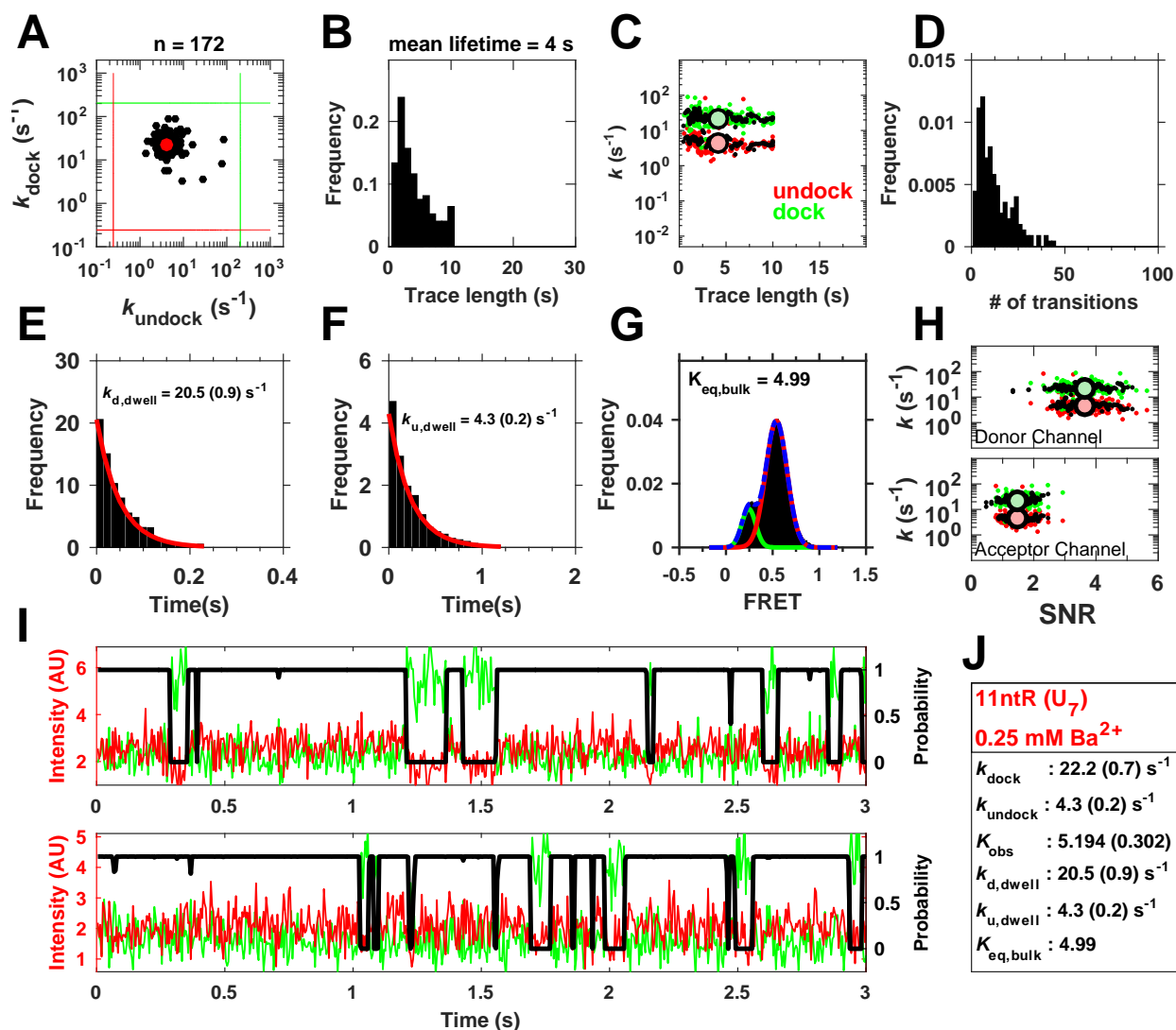


Figure S84. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

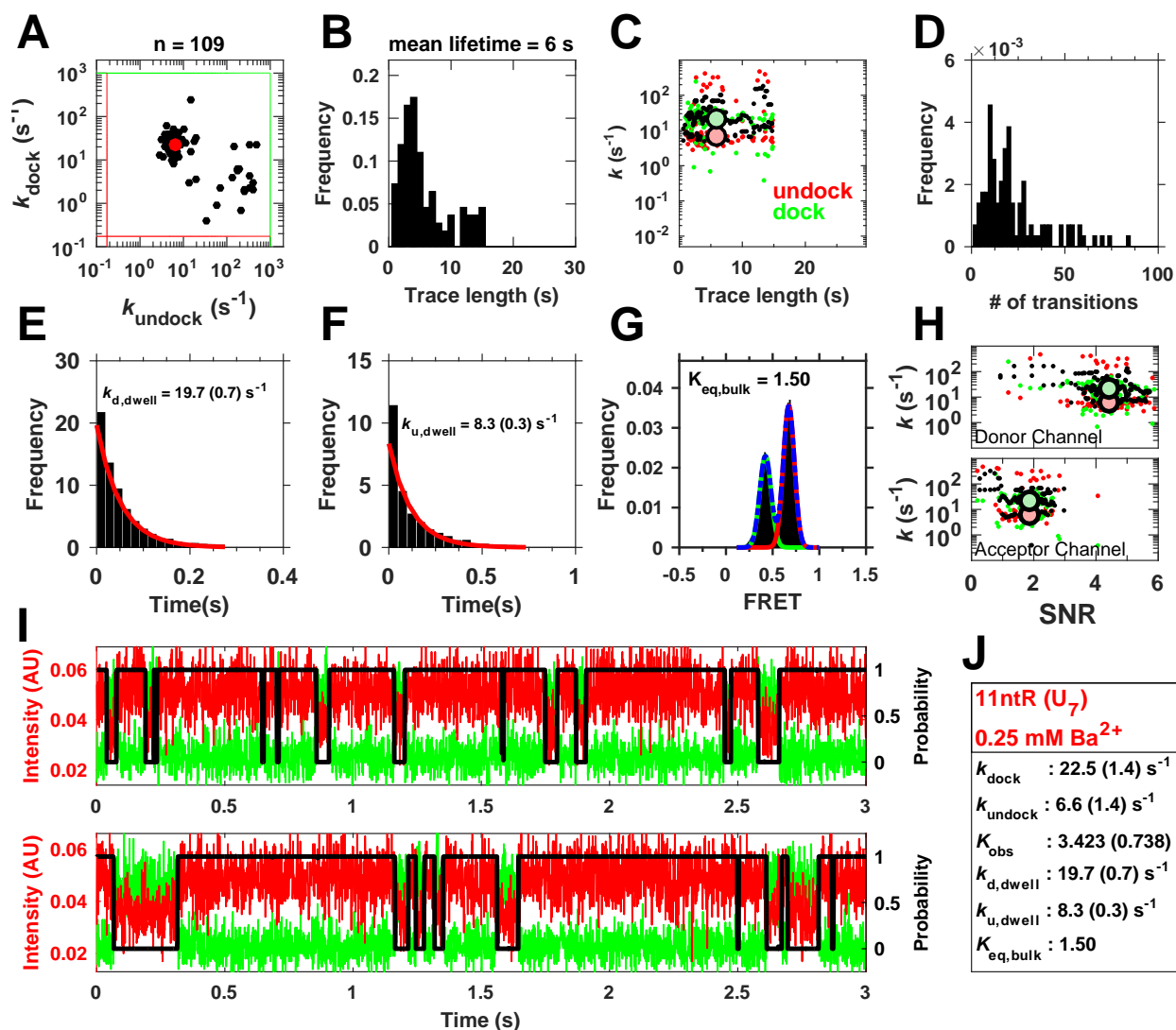


Figure S85. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

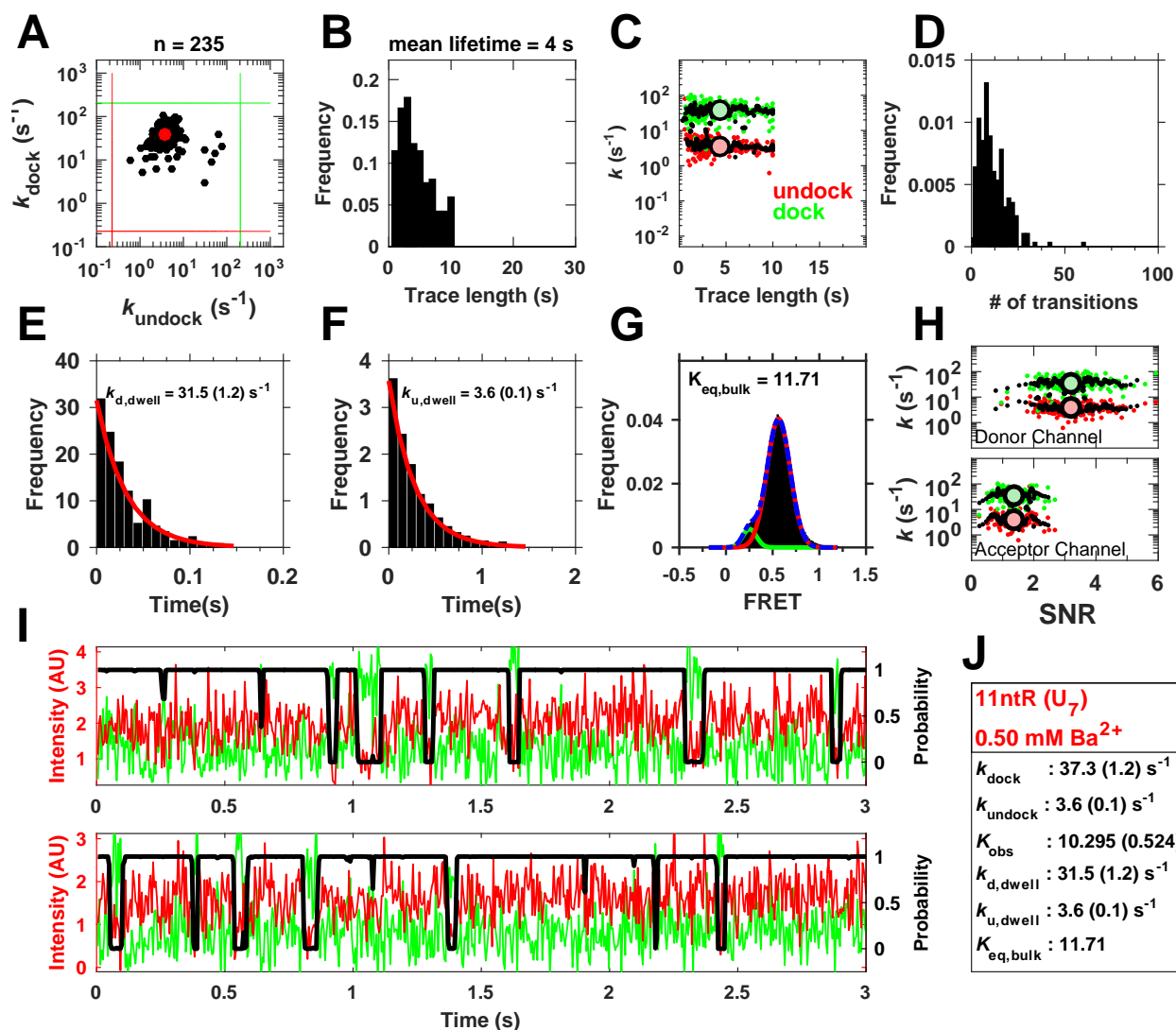


Figure S86. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

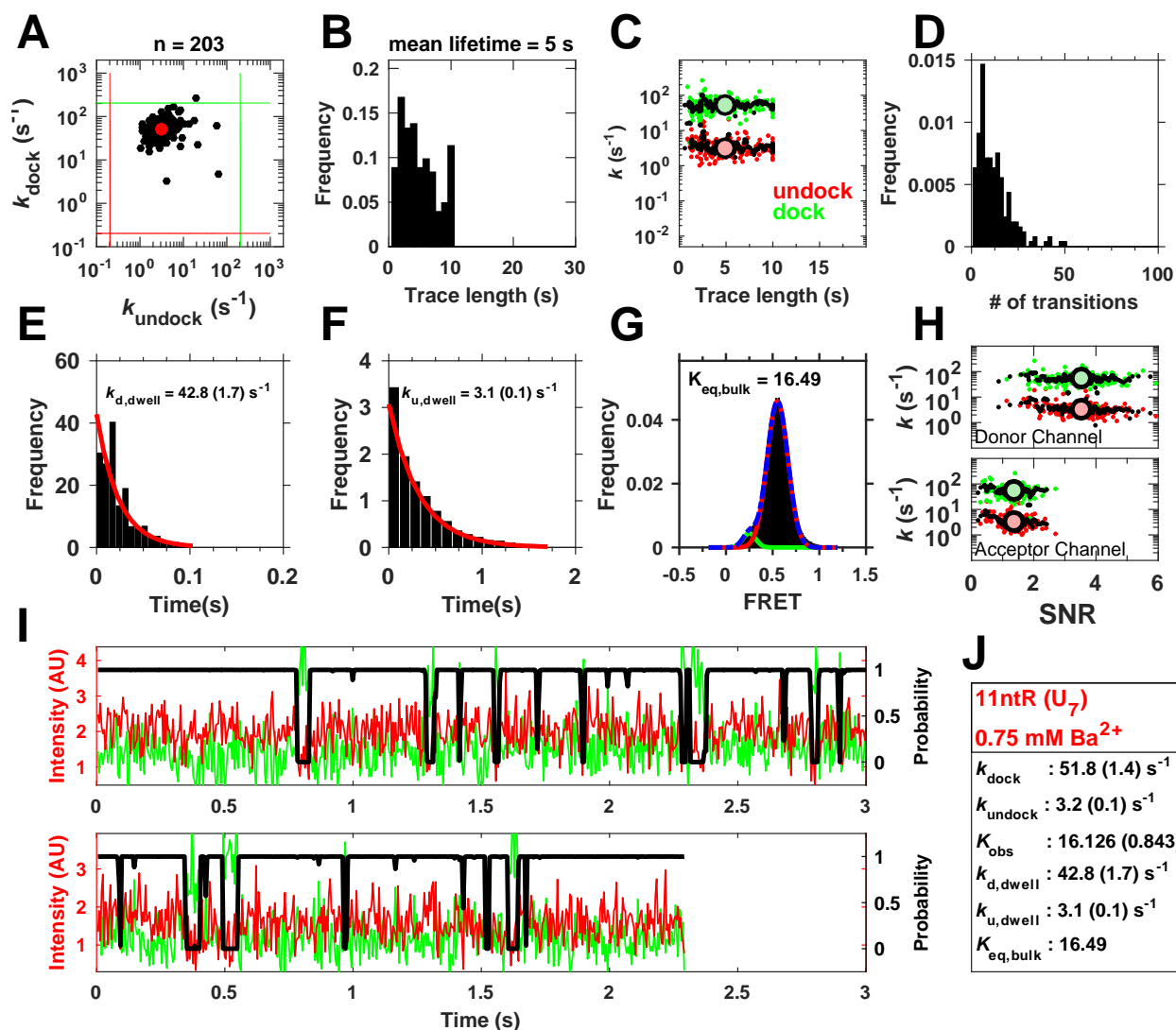


Figure S87. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

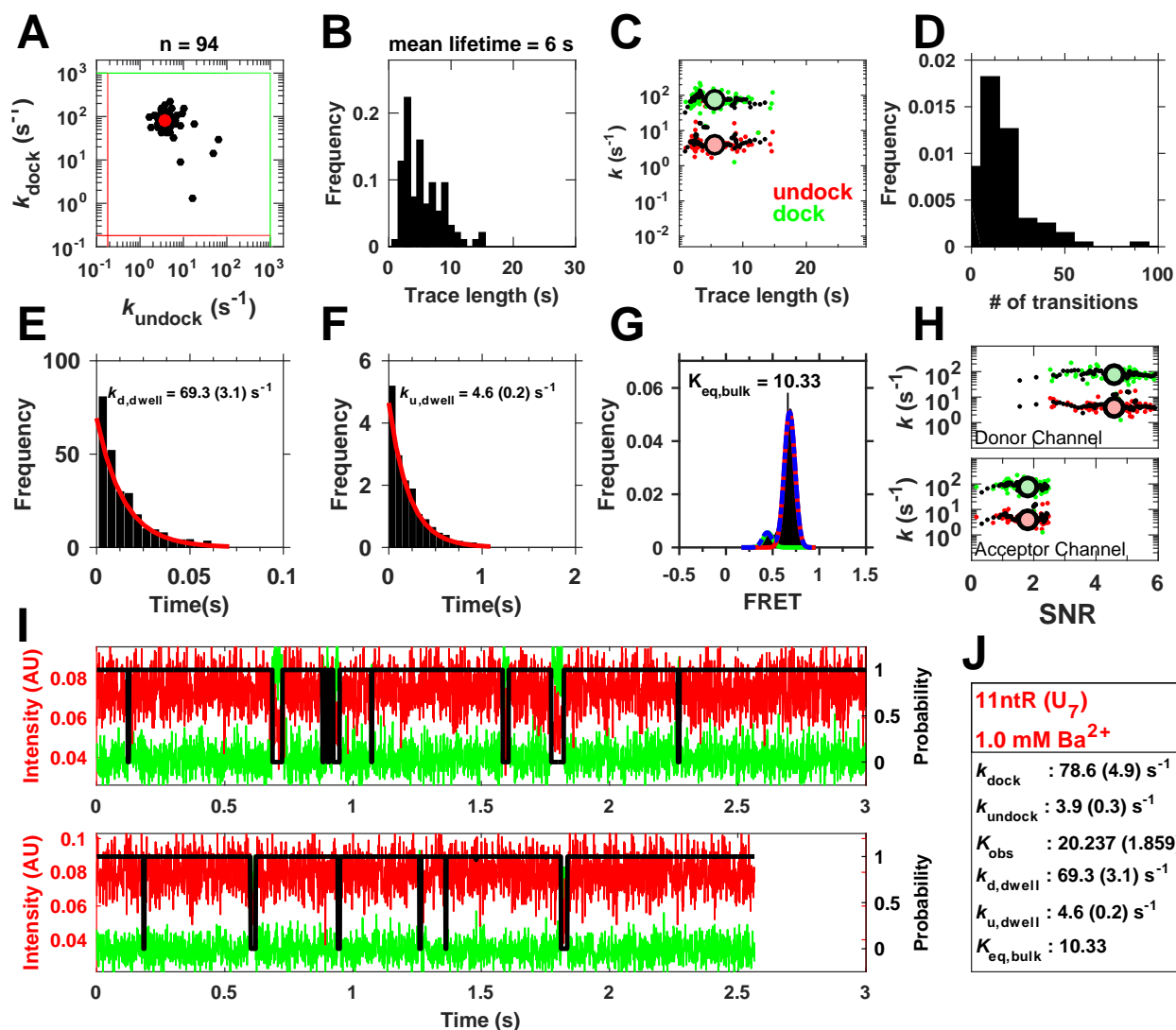


Figure S88. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

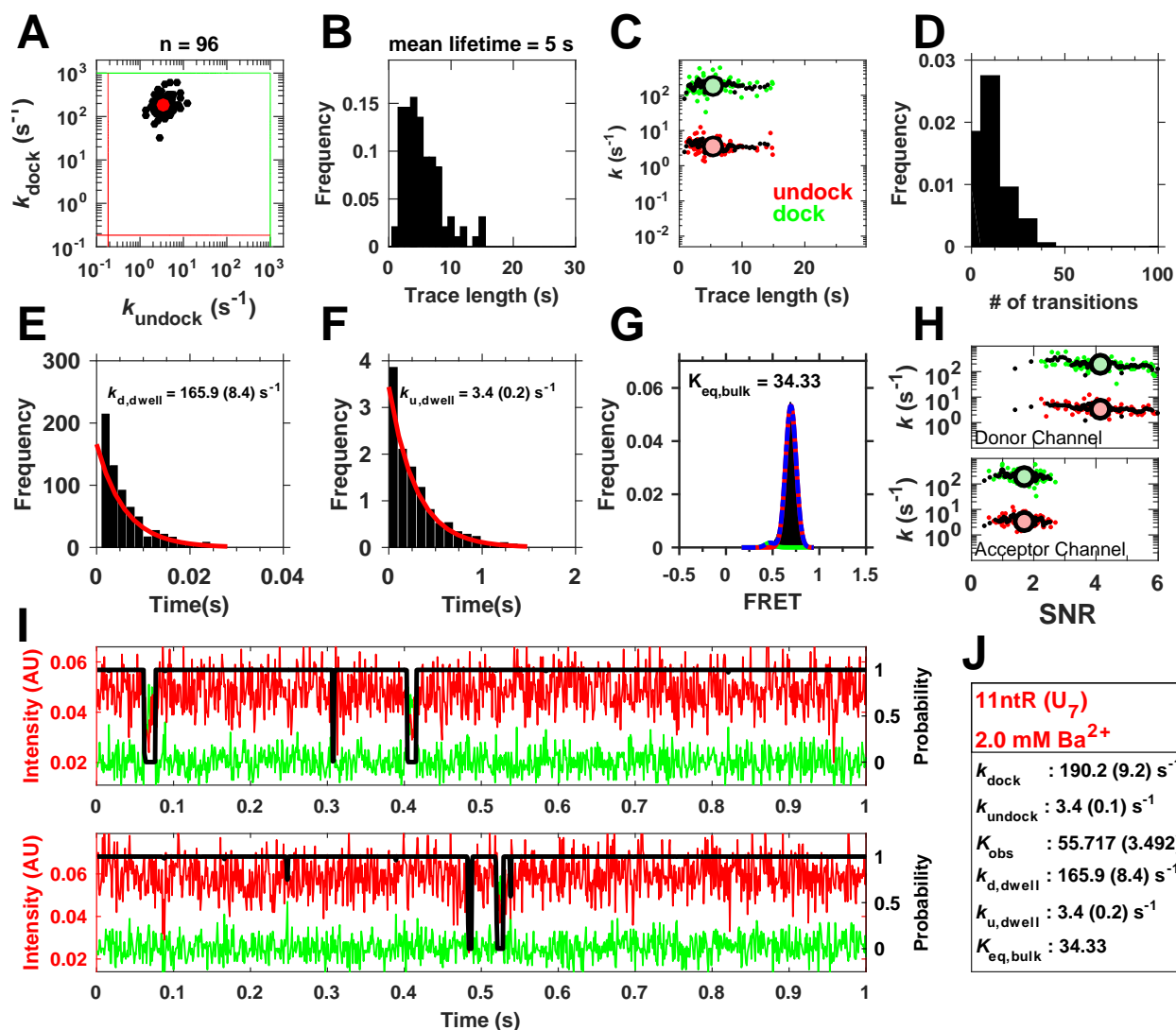


Figure S89. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

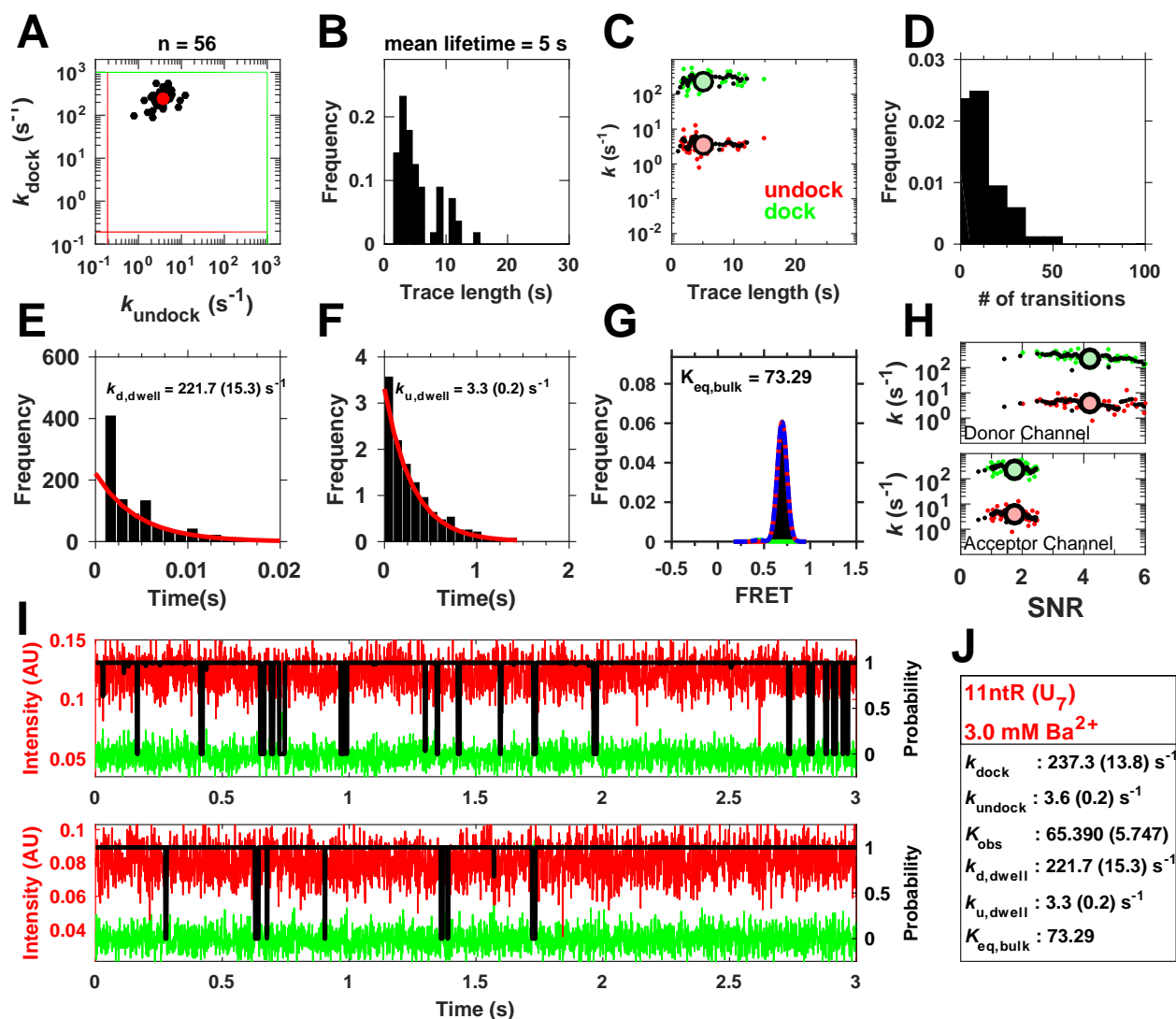


Figure S90. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

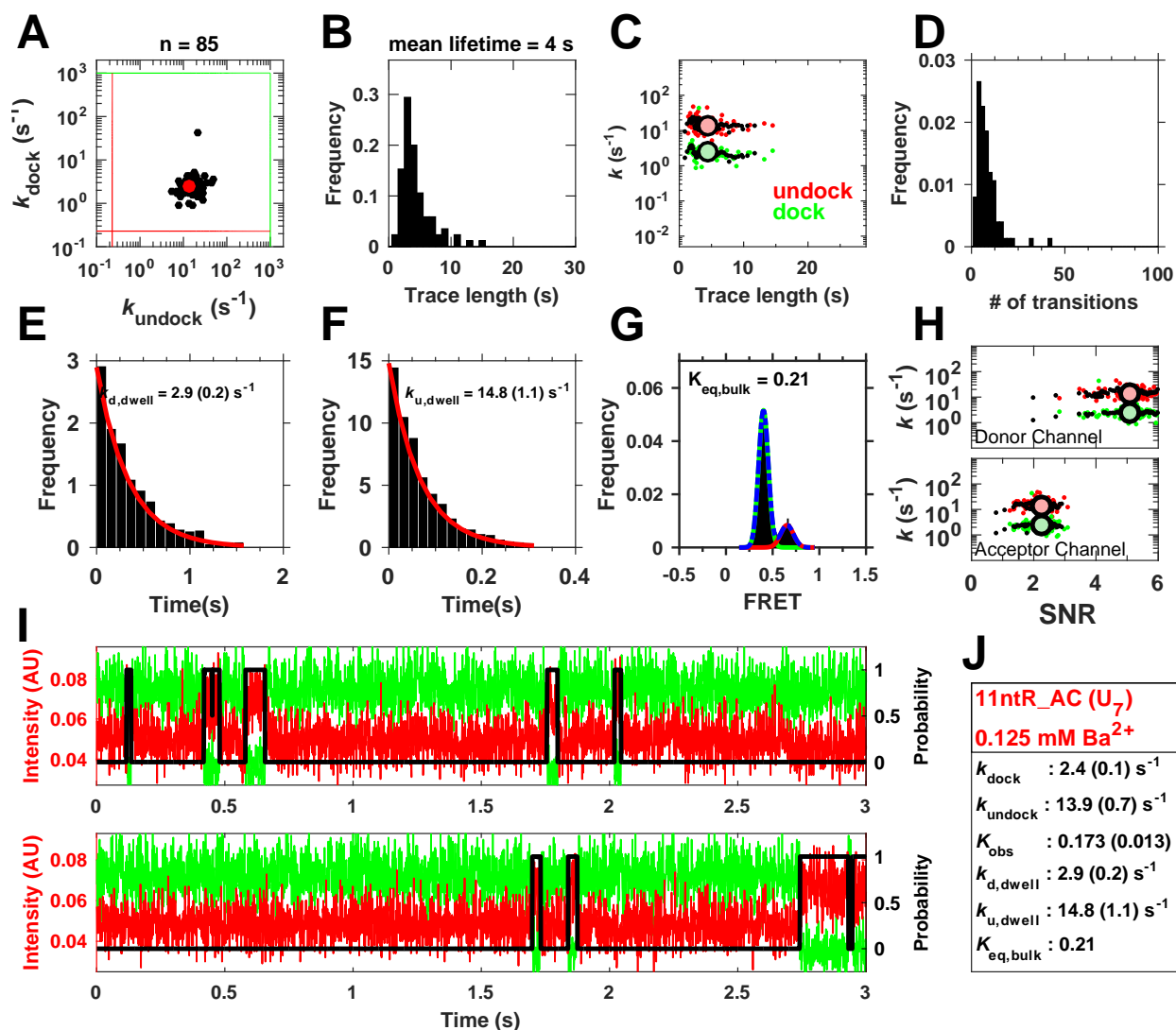


Figure S91. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

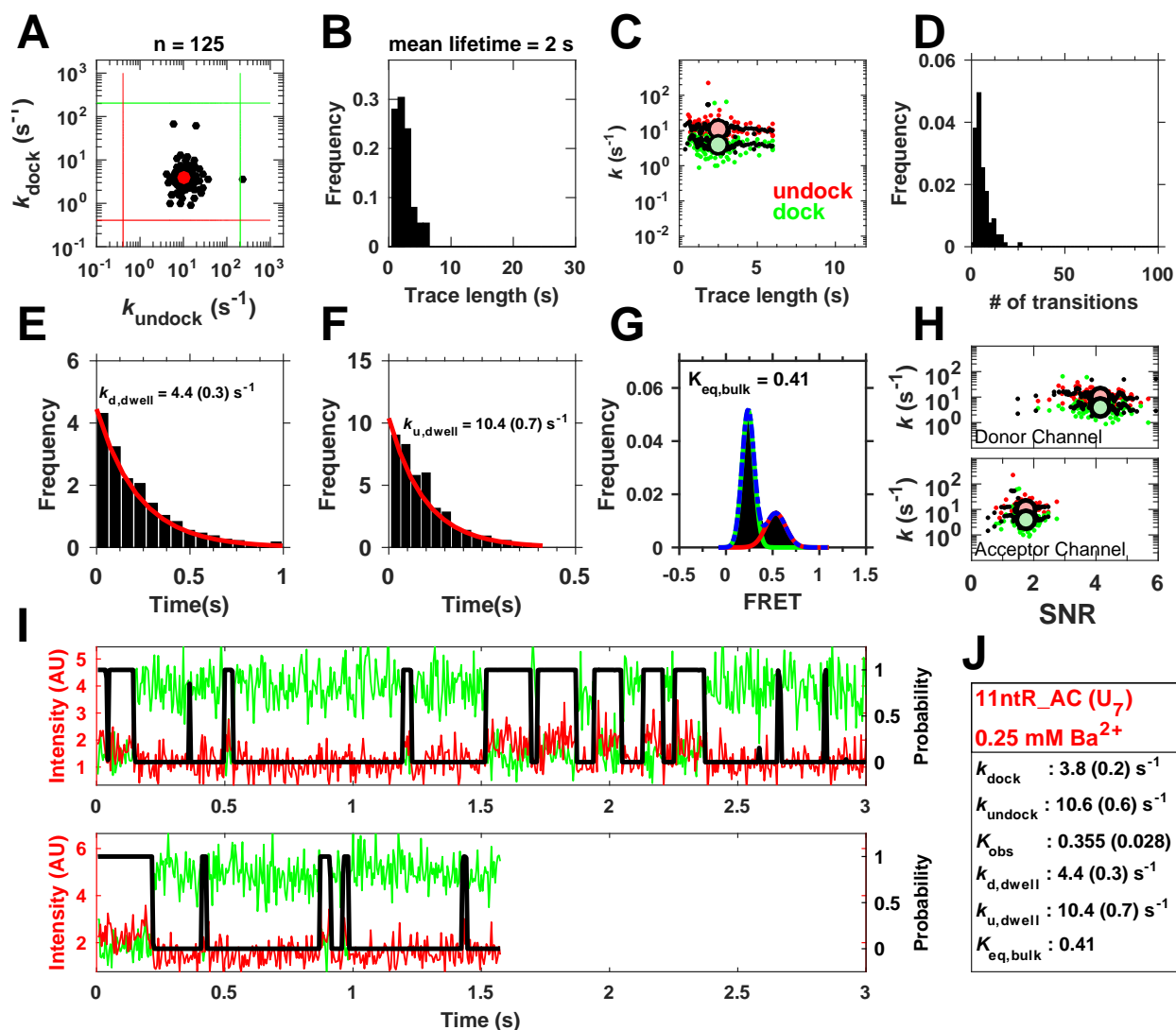


Figure S92. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

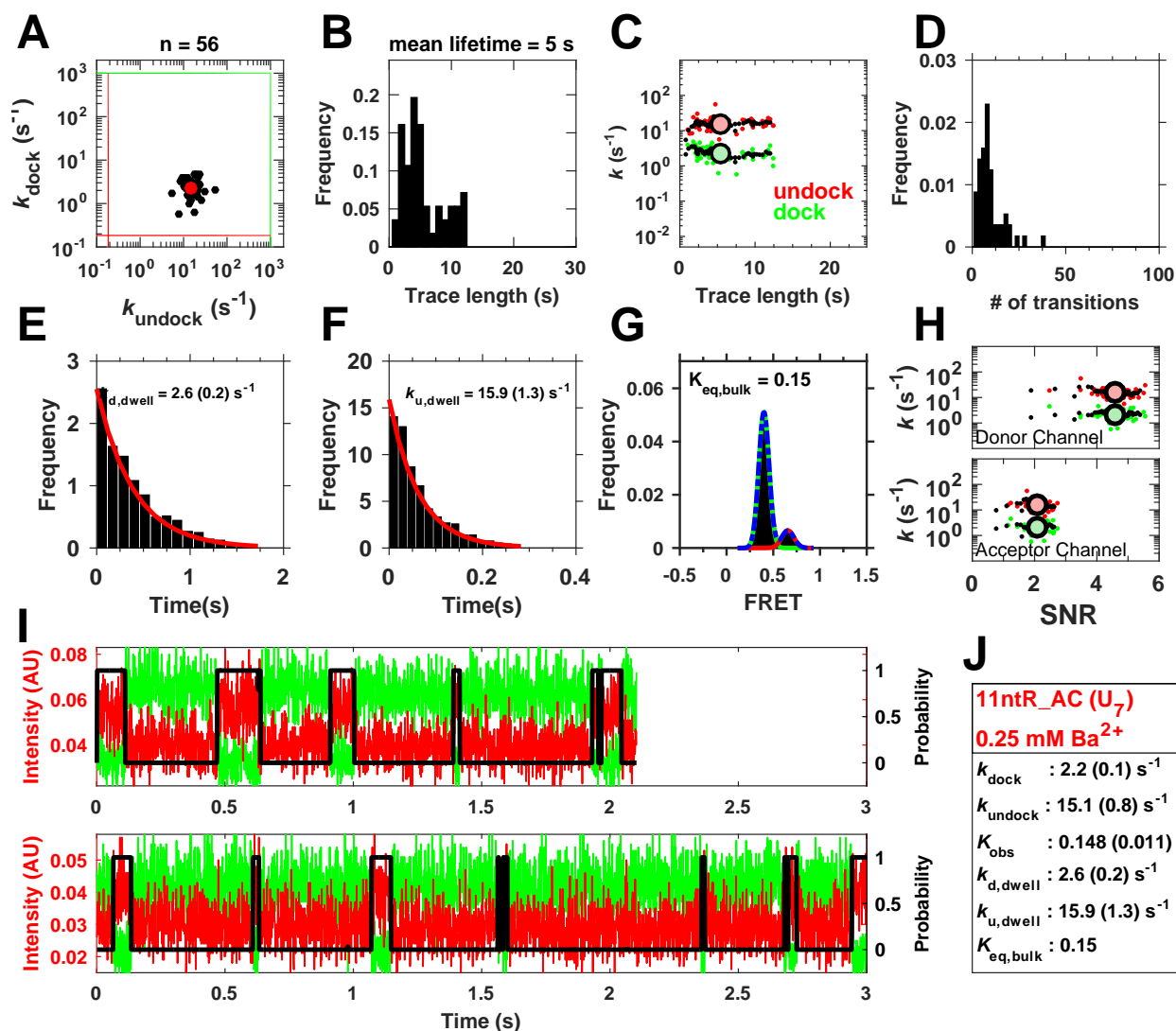


Figure S93. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

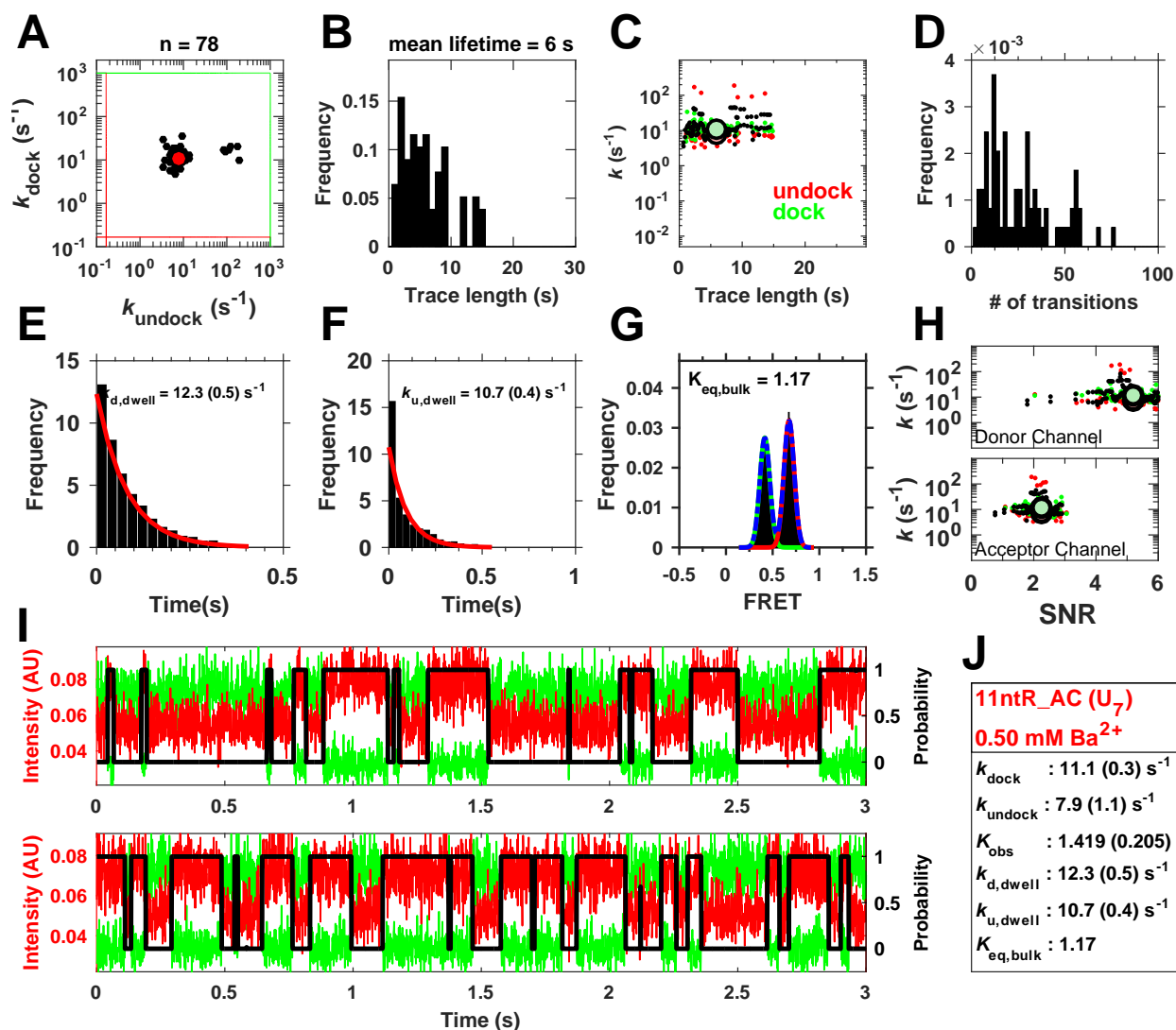


Figure S94. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

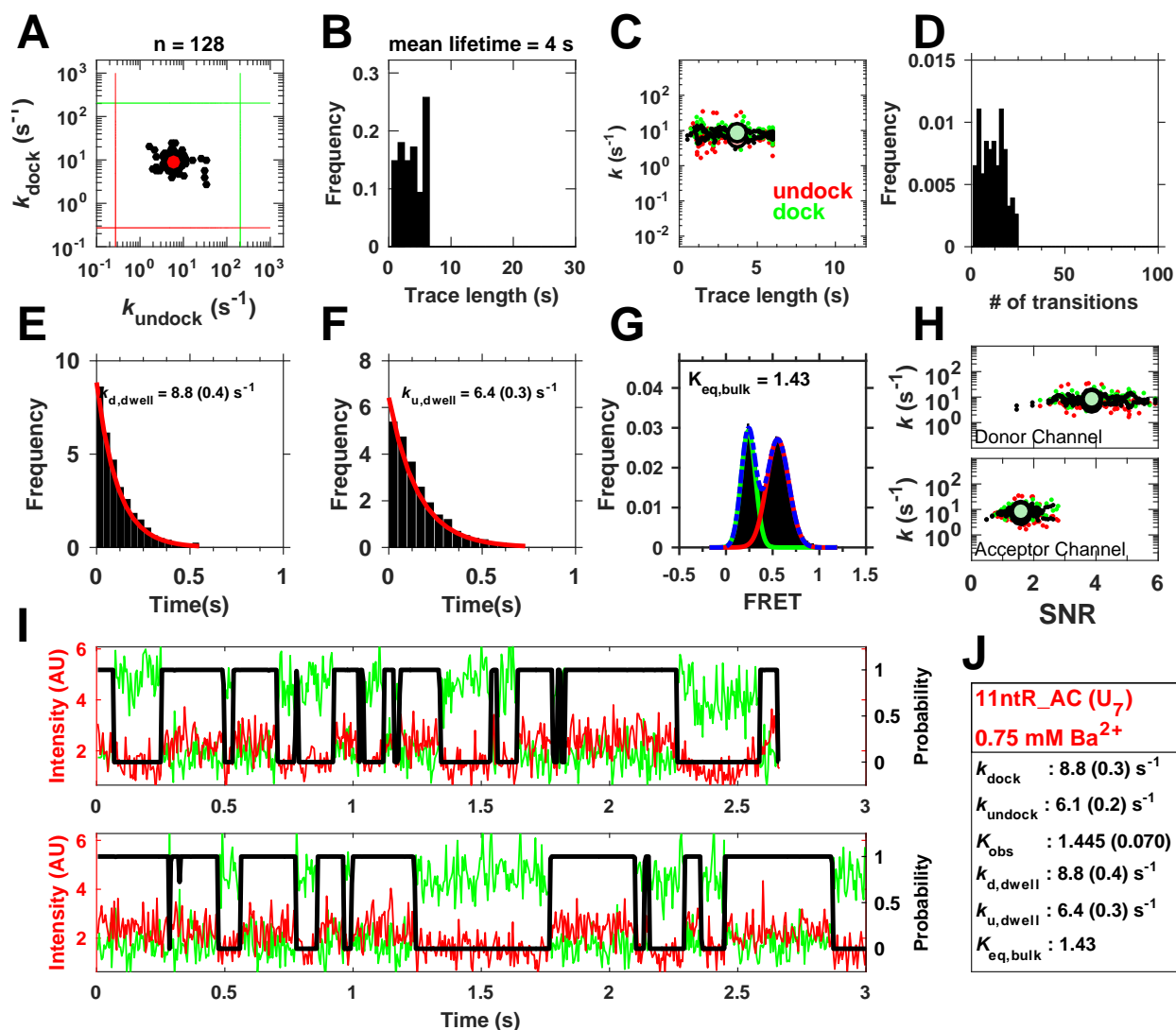


Figure S95. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

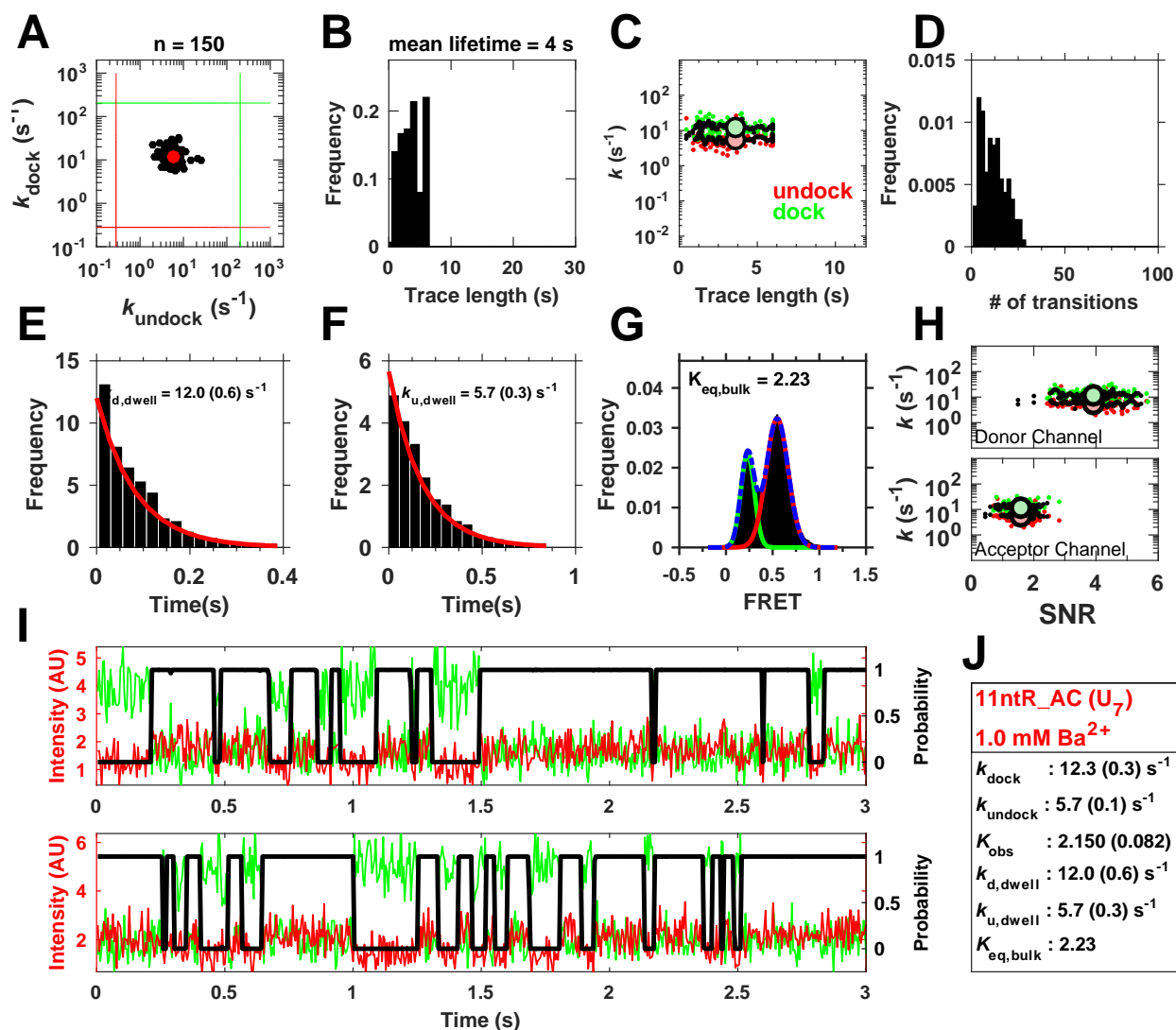


Figure S96. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

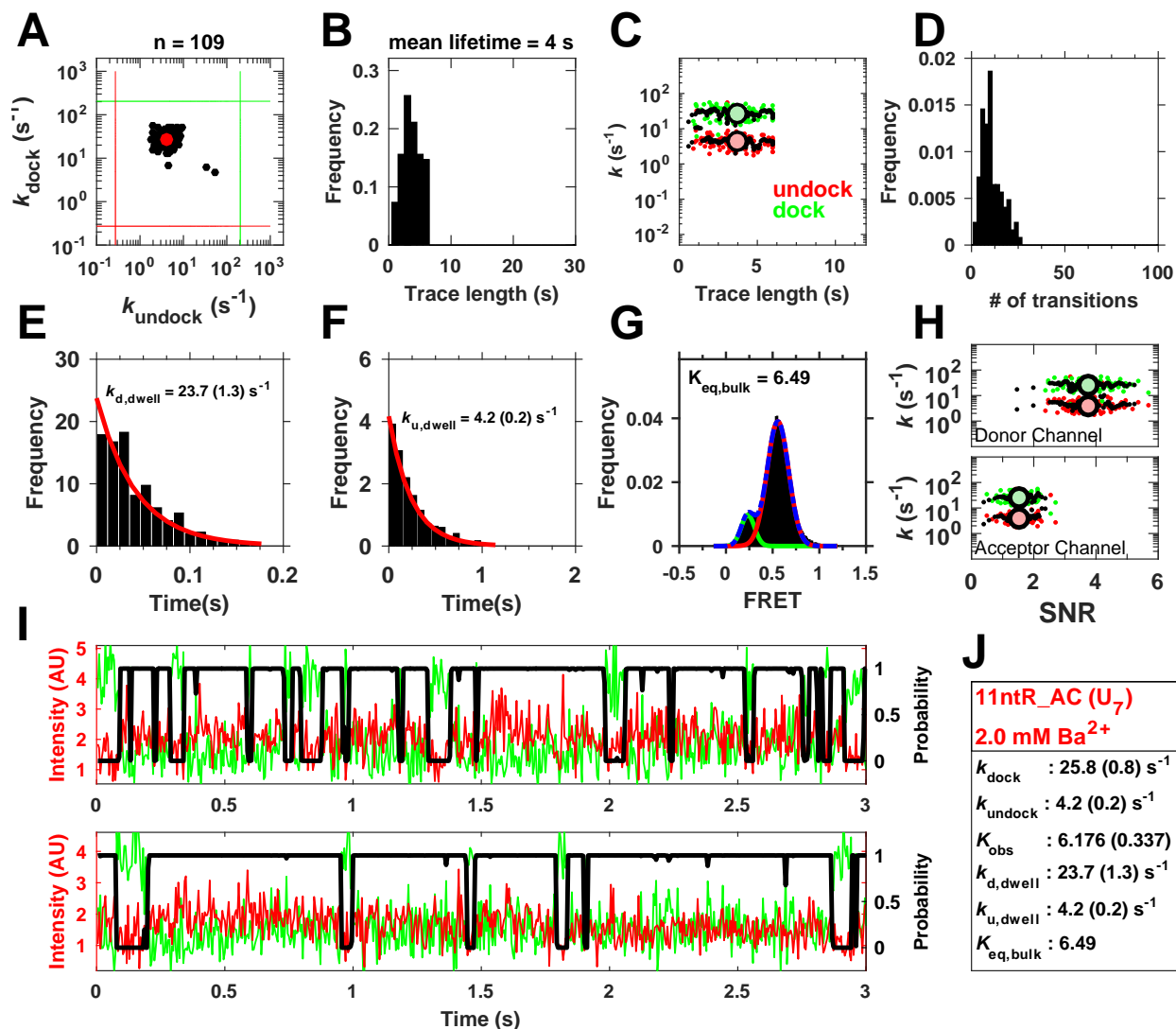


Figure S97. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

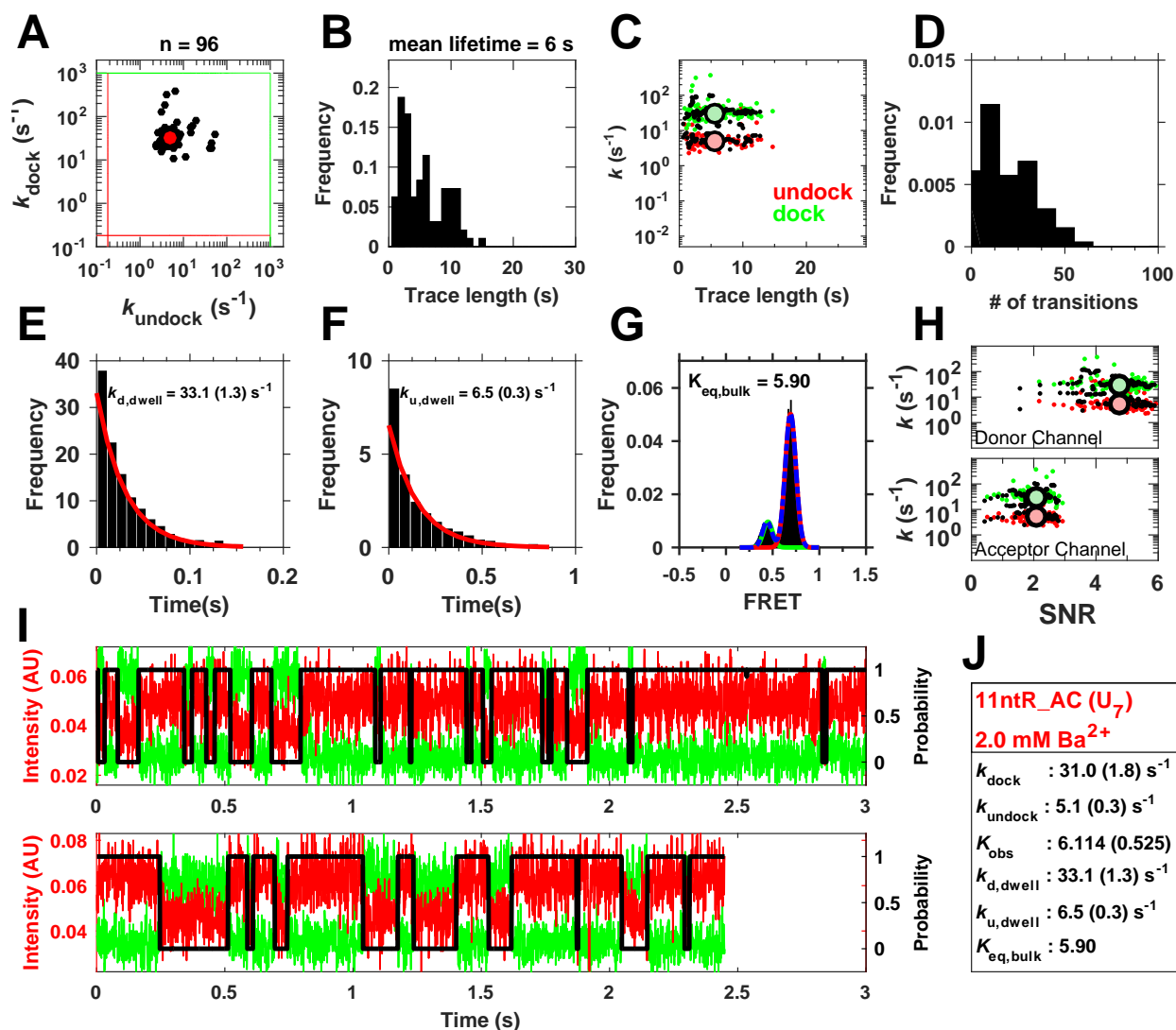


Figure S98. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

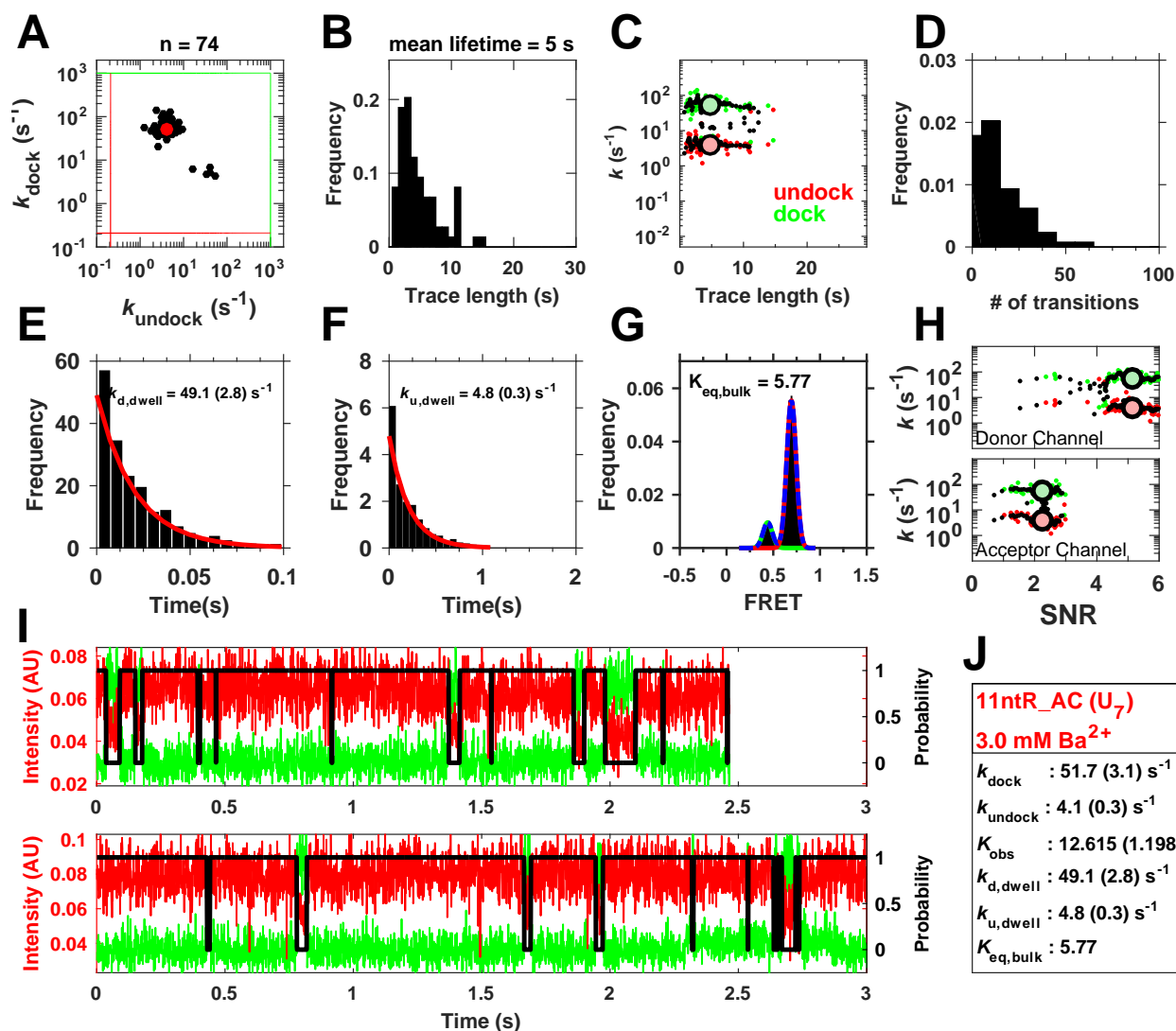


Figure S99. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

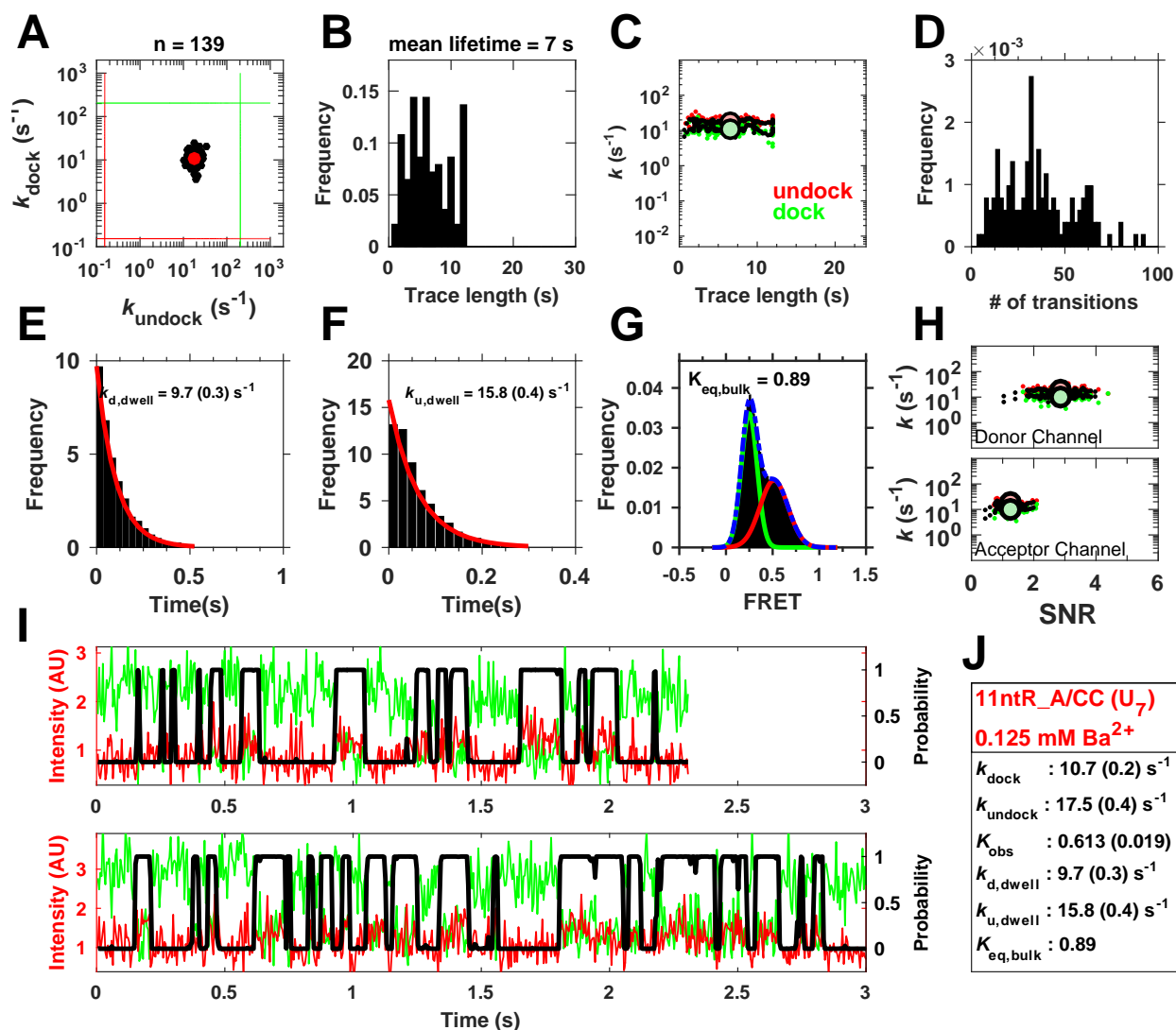


Figure S100. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

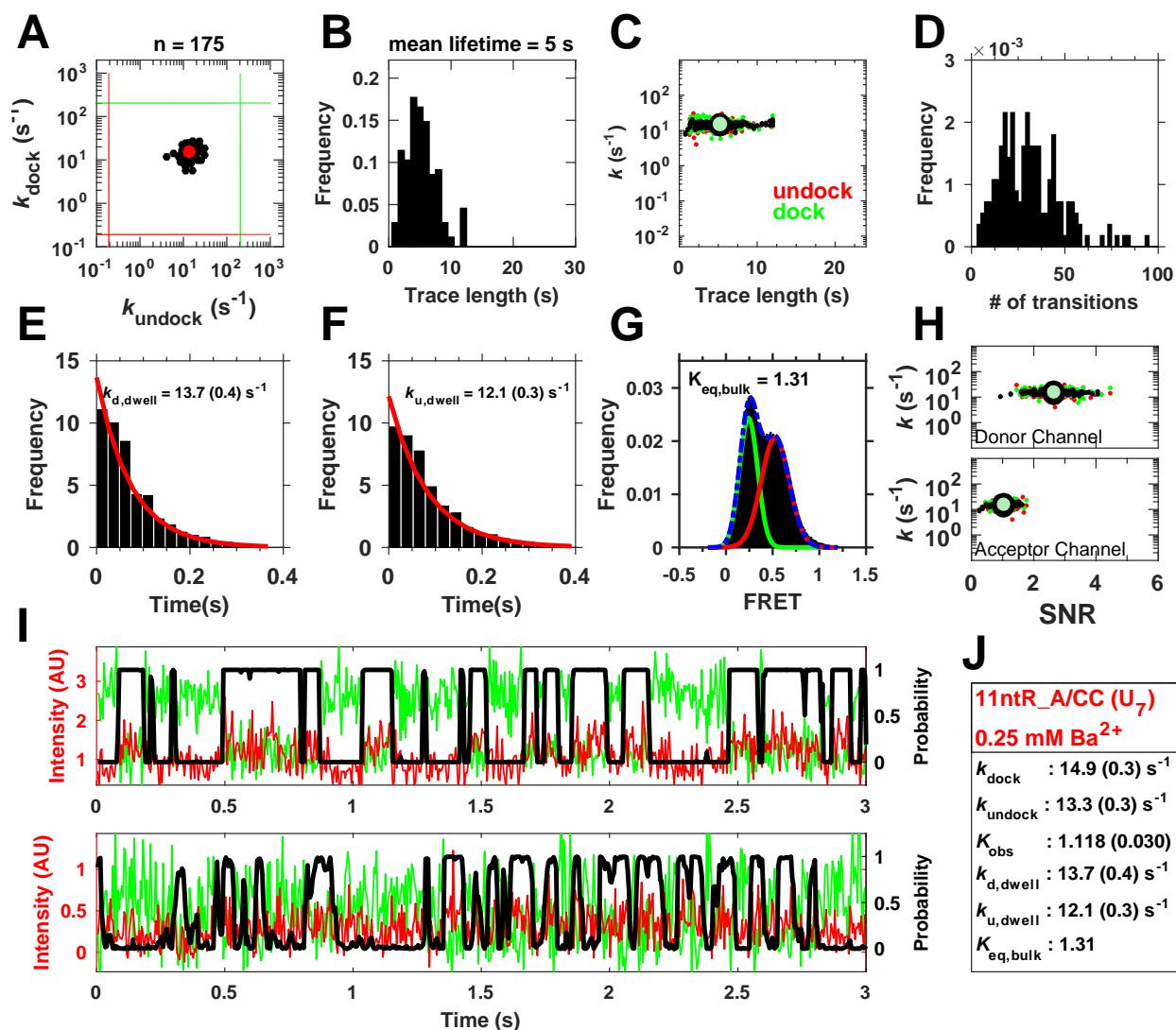


Figure S101. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

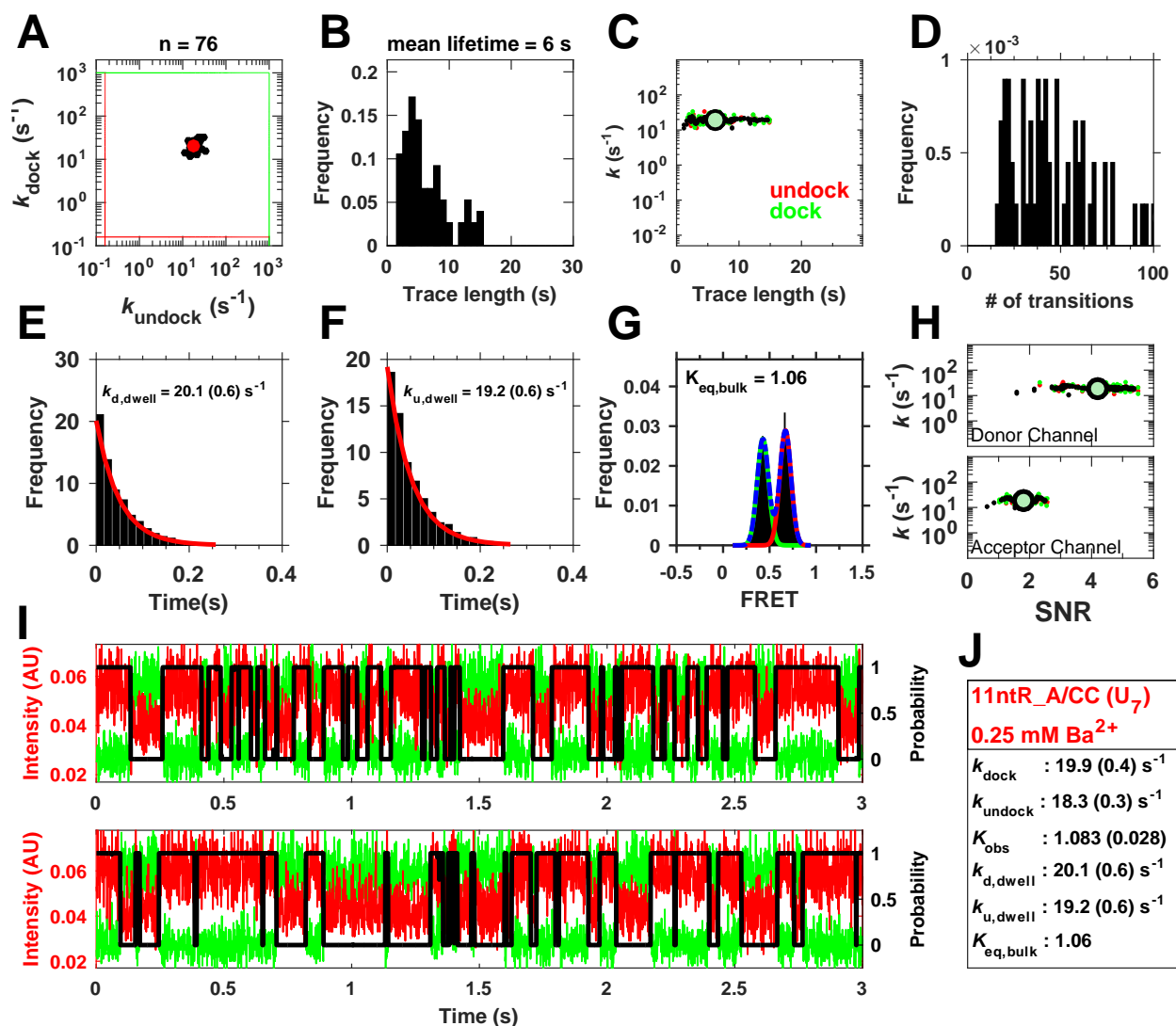


Figure S102. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

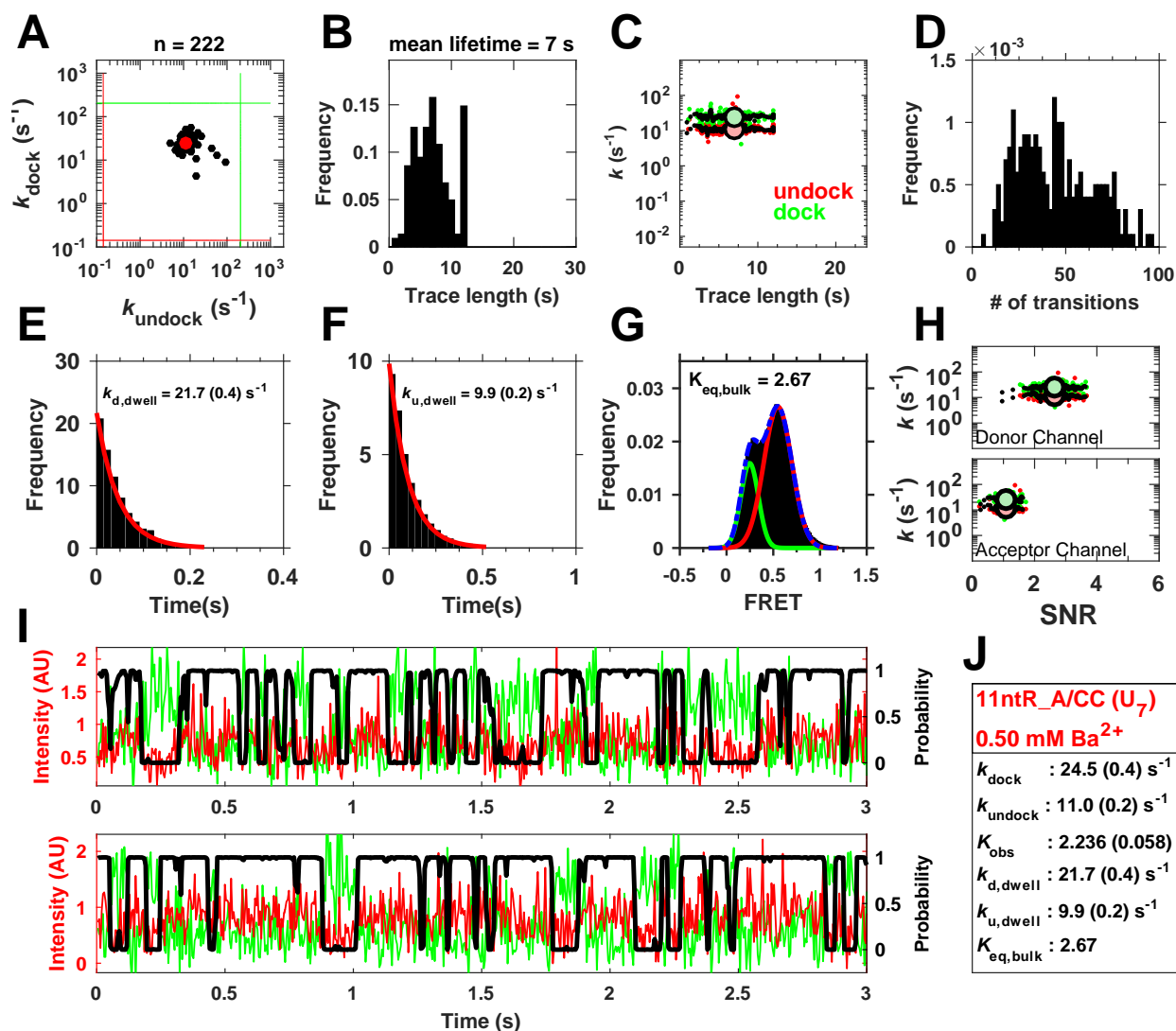


Figure S103. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

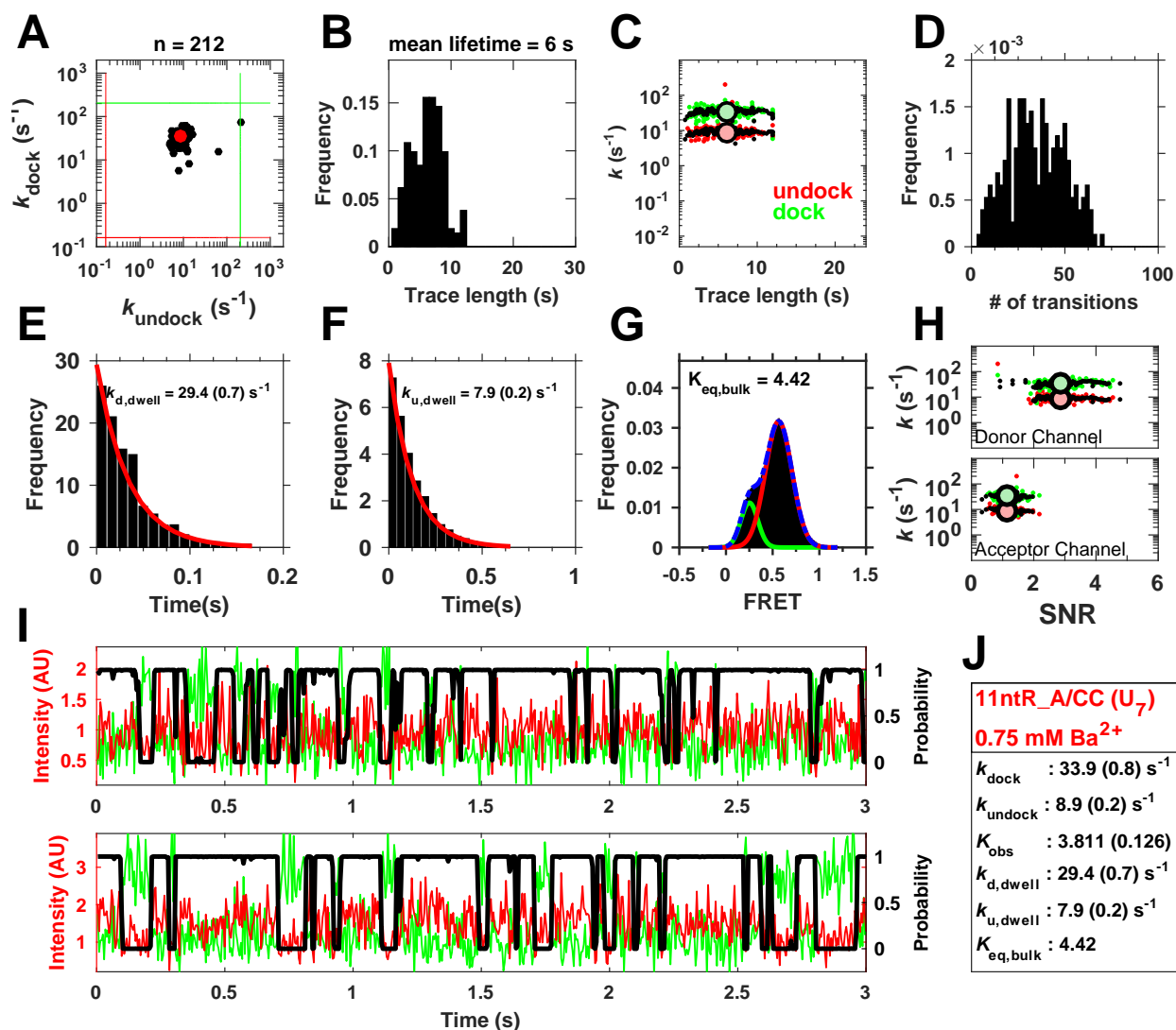


Figure S104. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

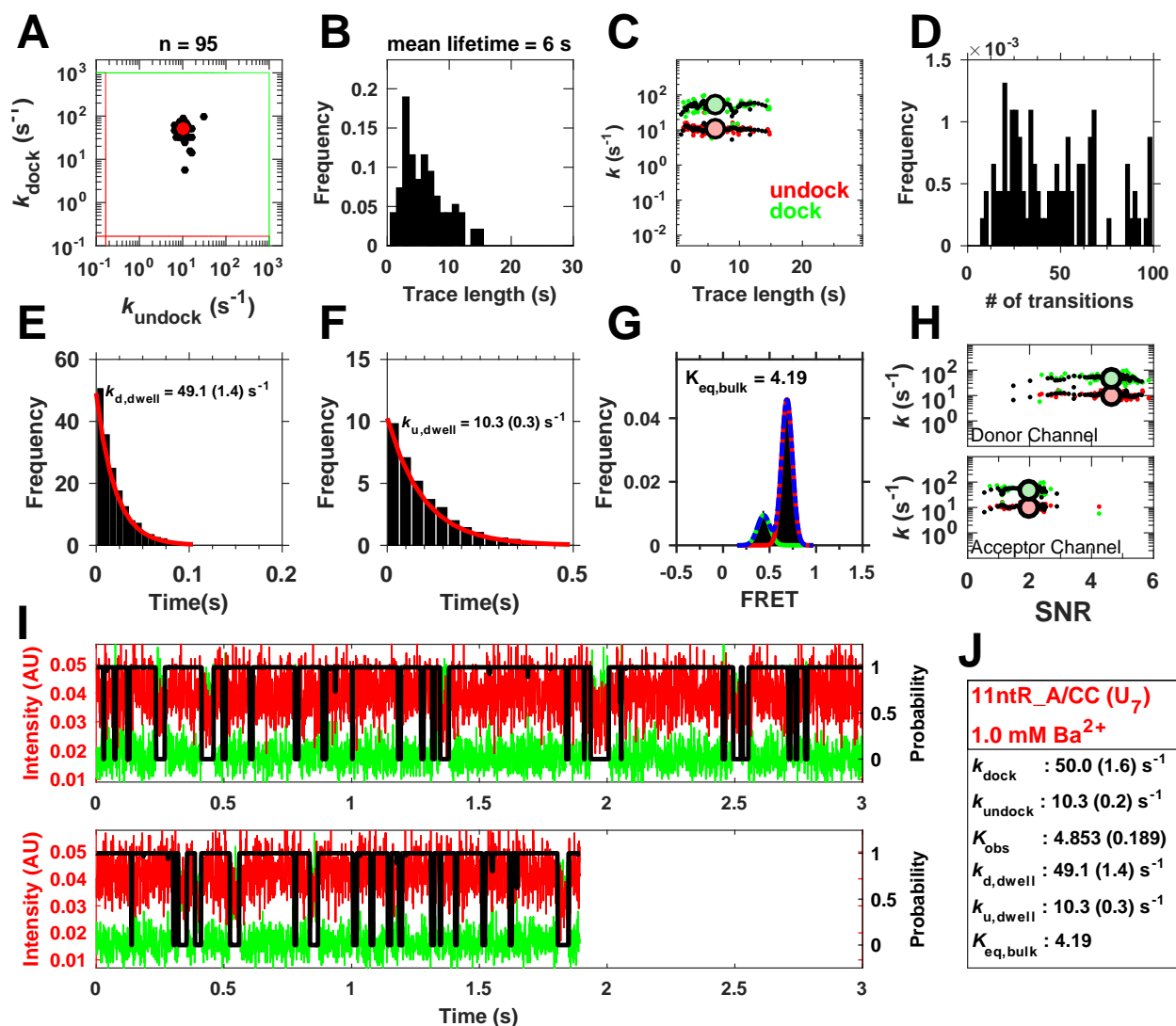


Figure S105. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

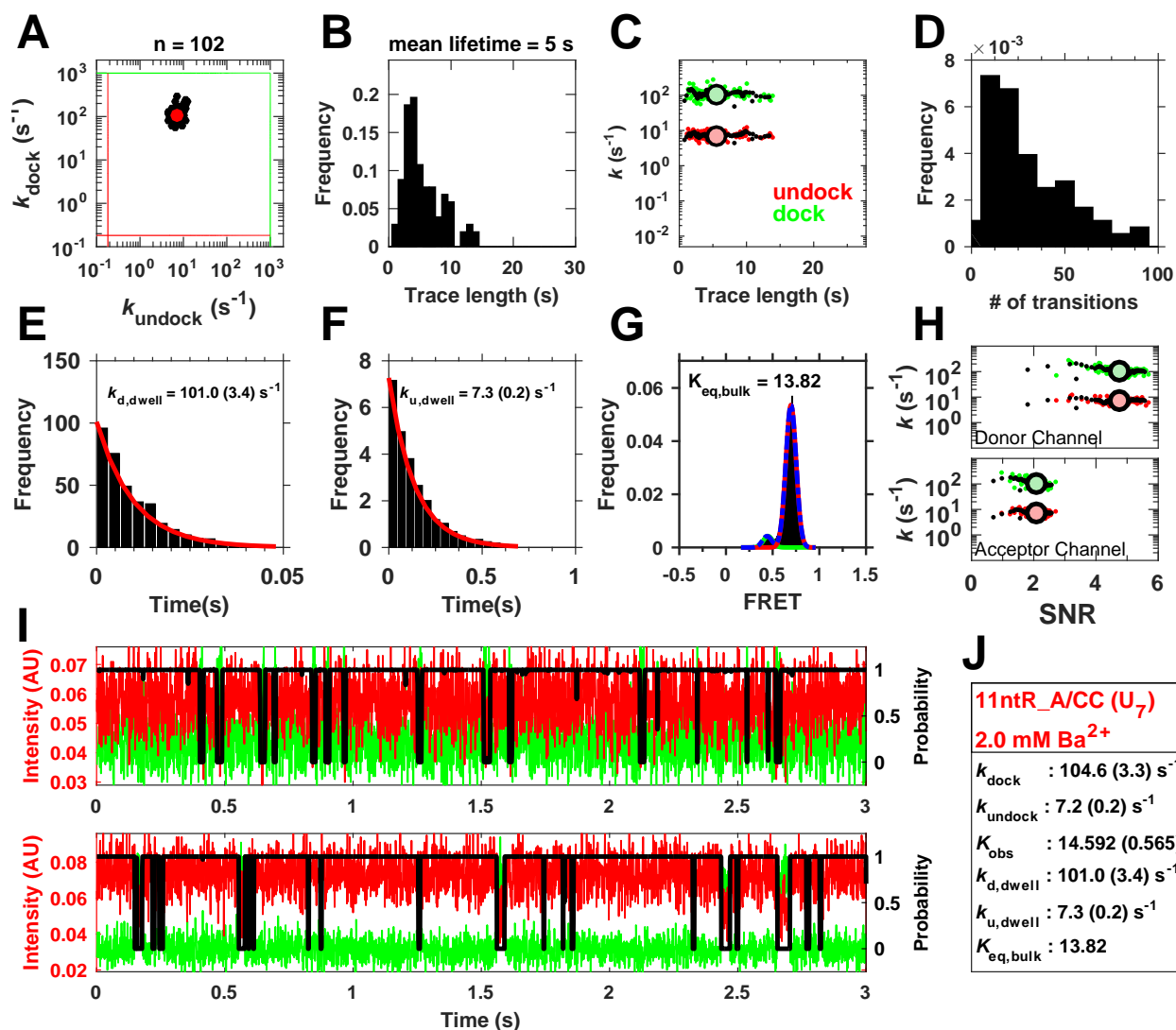


Figure S106. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

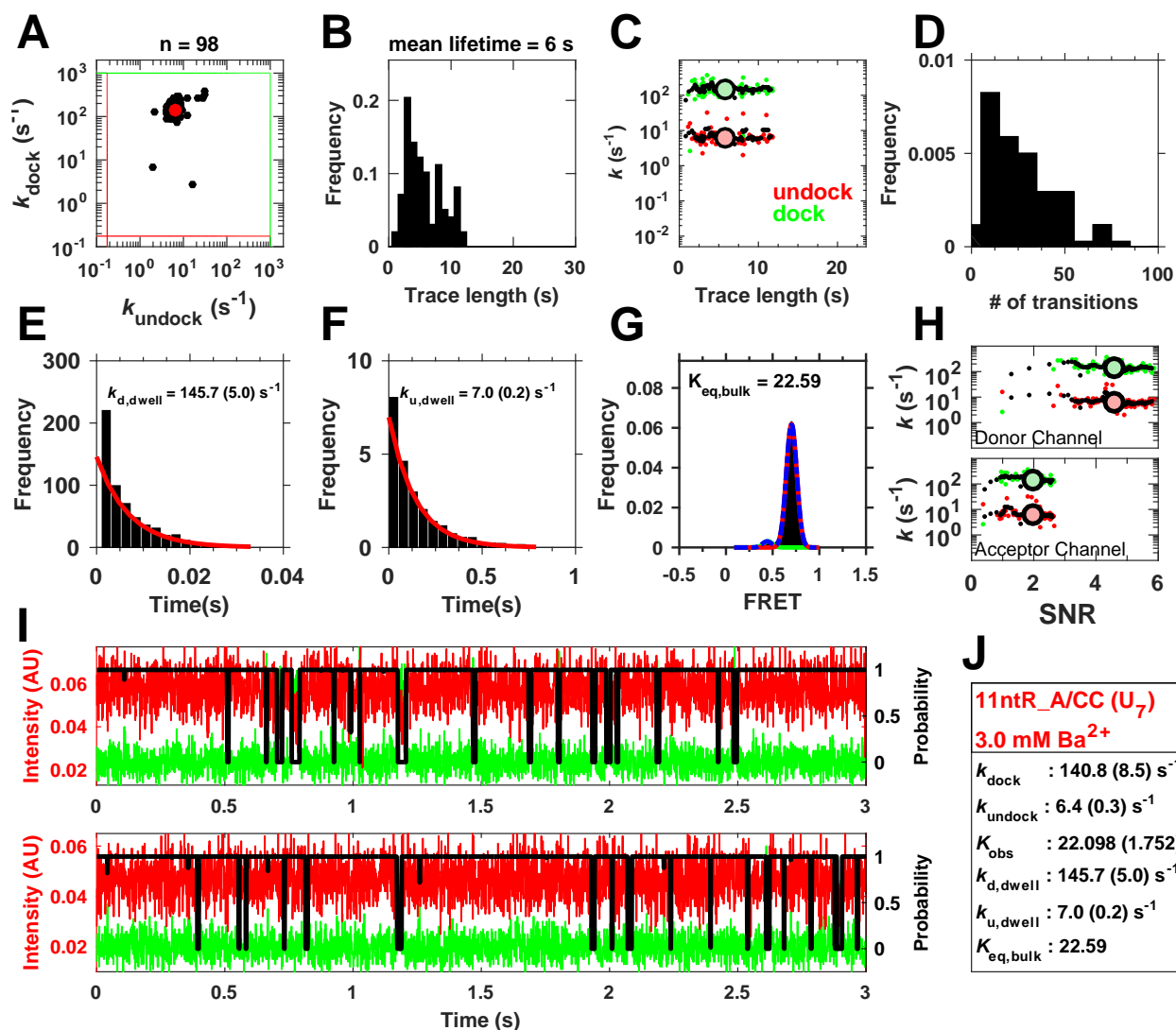


Figure S107. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

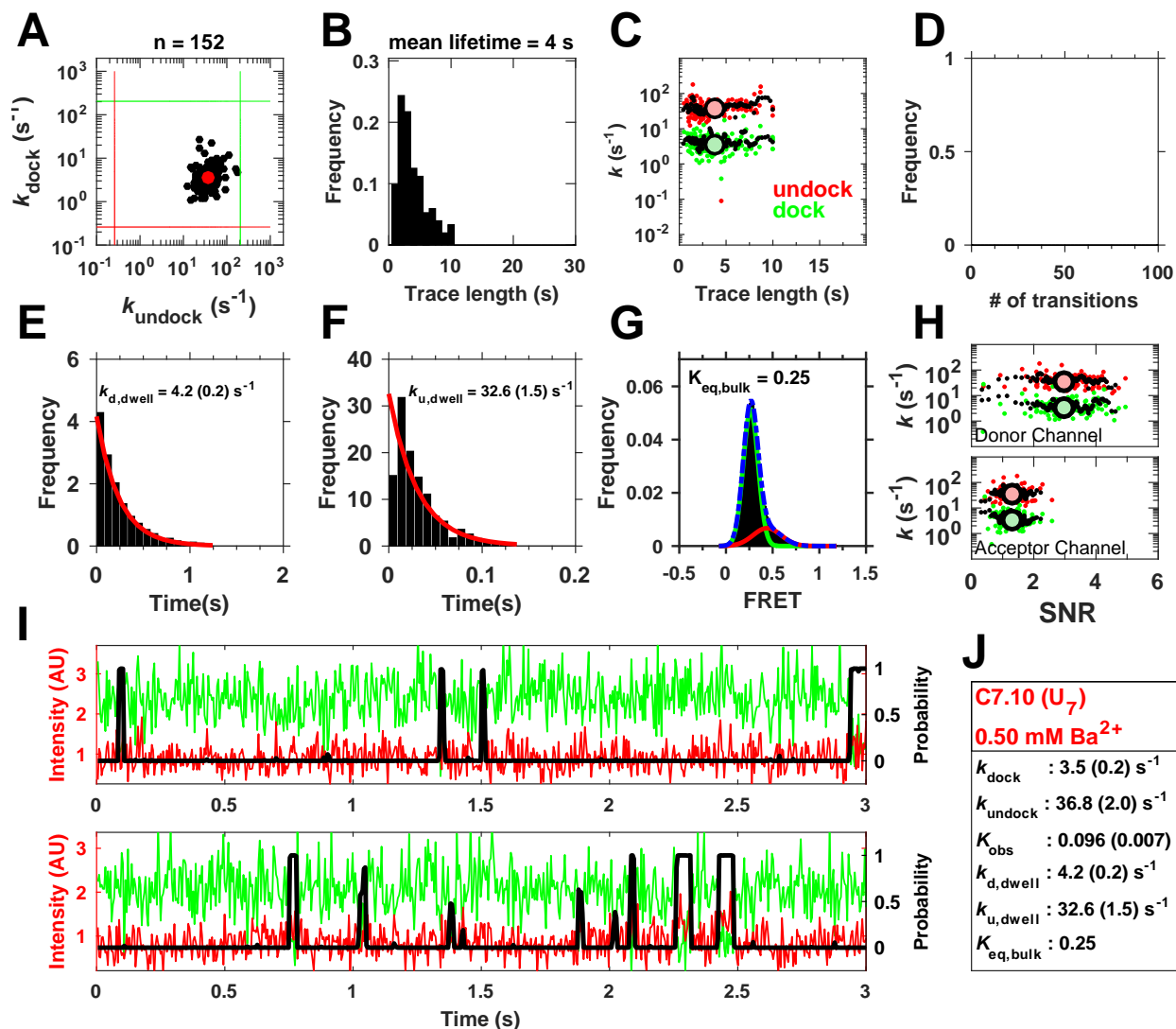


Figure S108. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

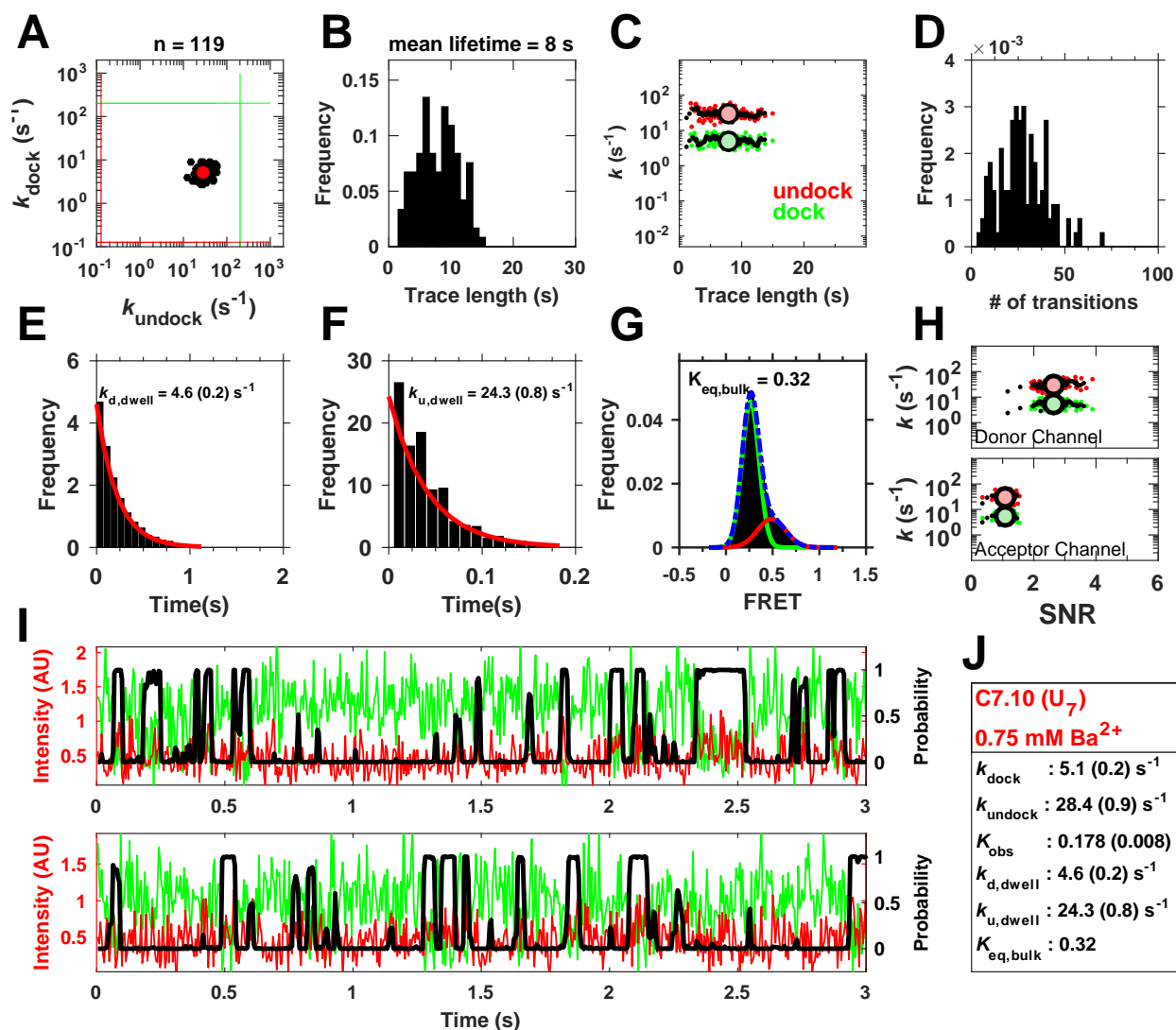


Figure S109. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

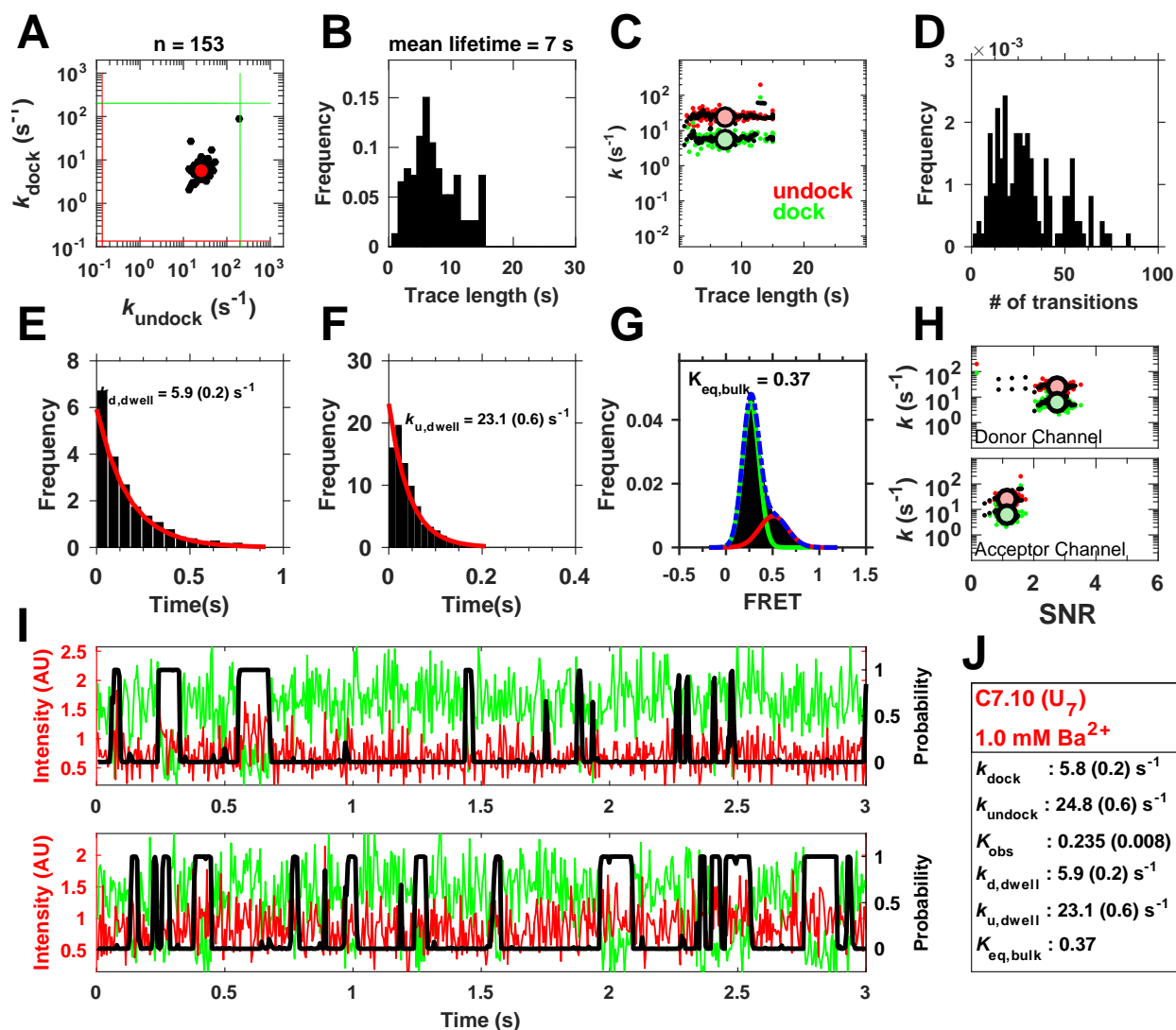


Figure S110. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

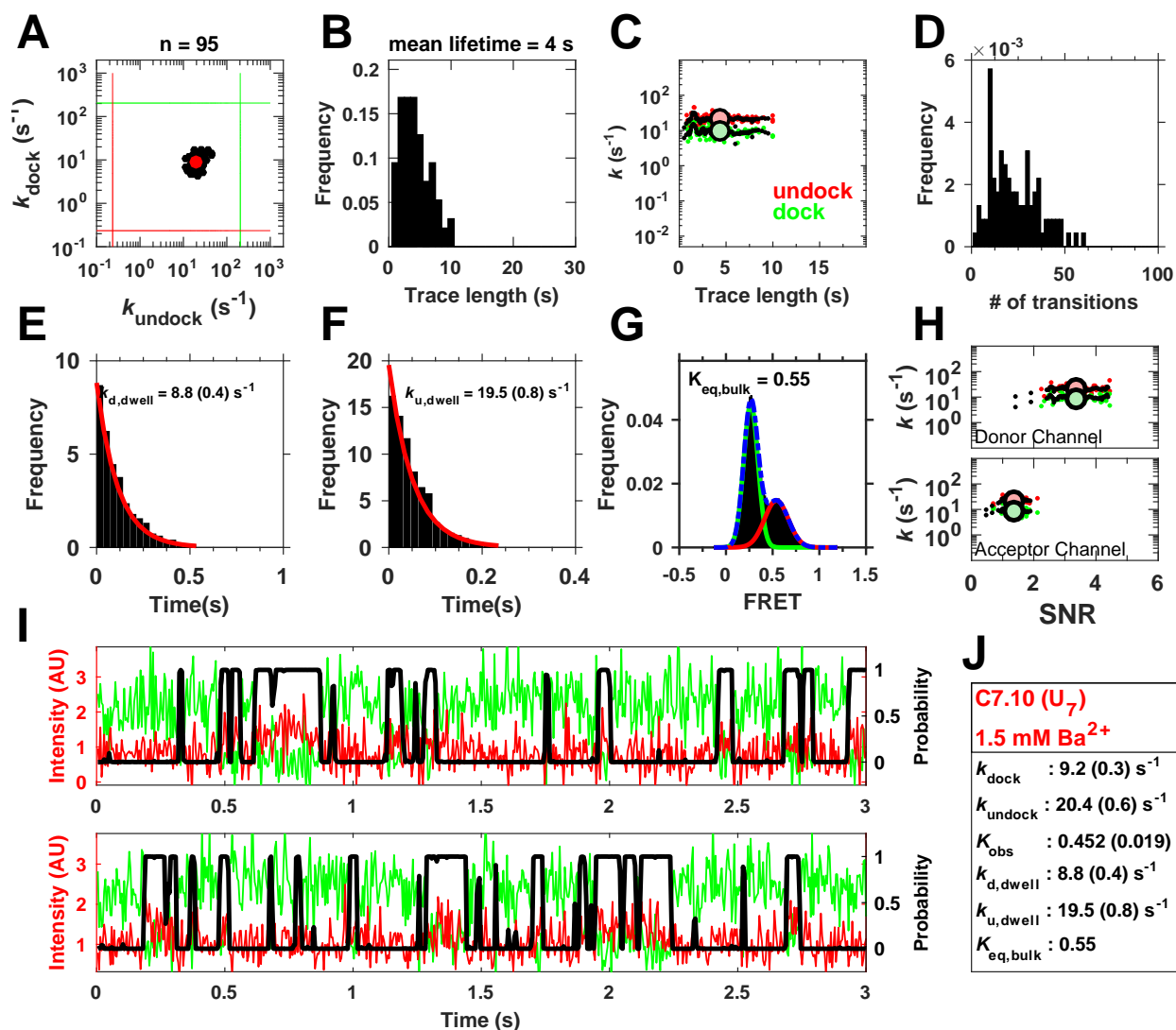


Figure S111. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

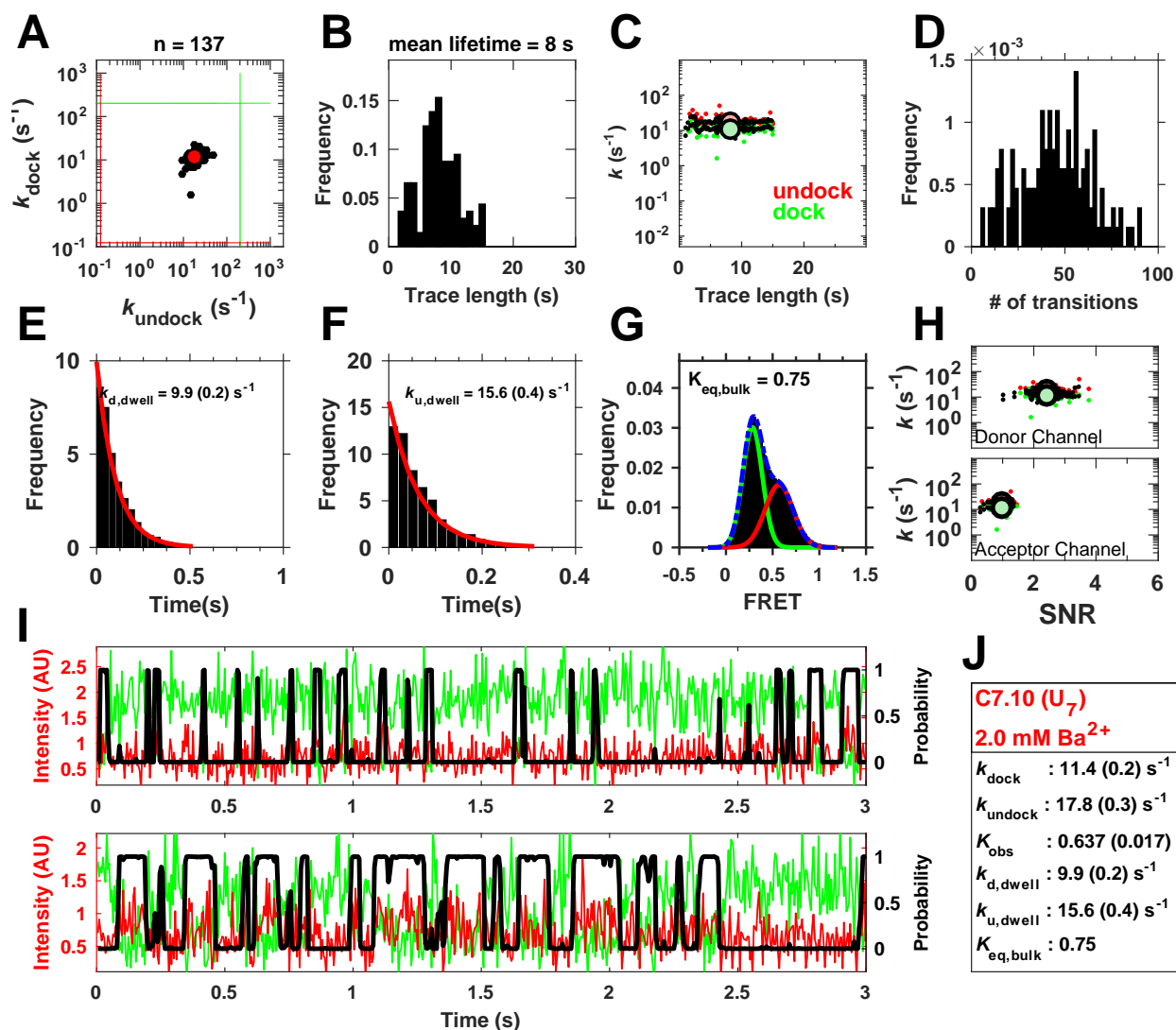


Figure S112. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

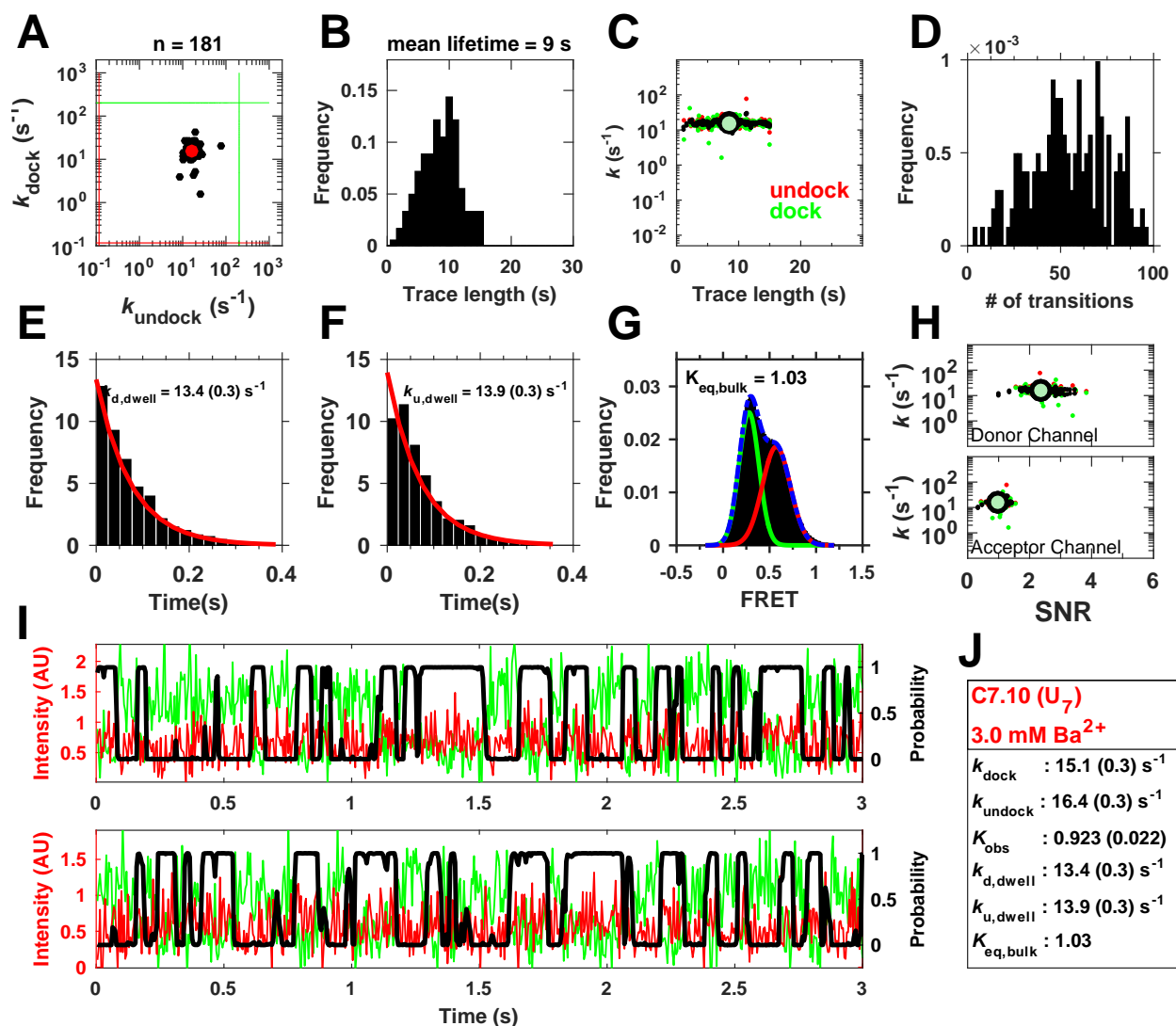


Figure S113. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

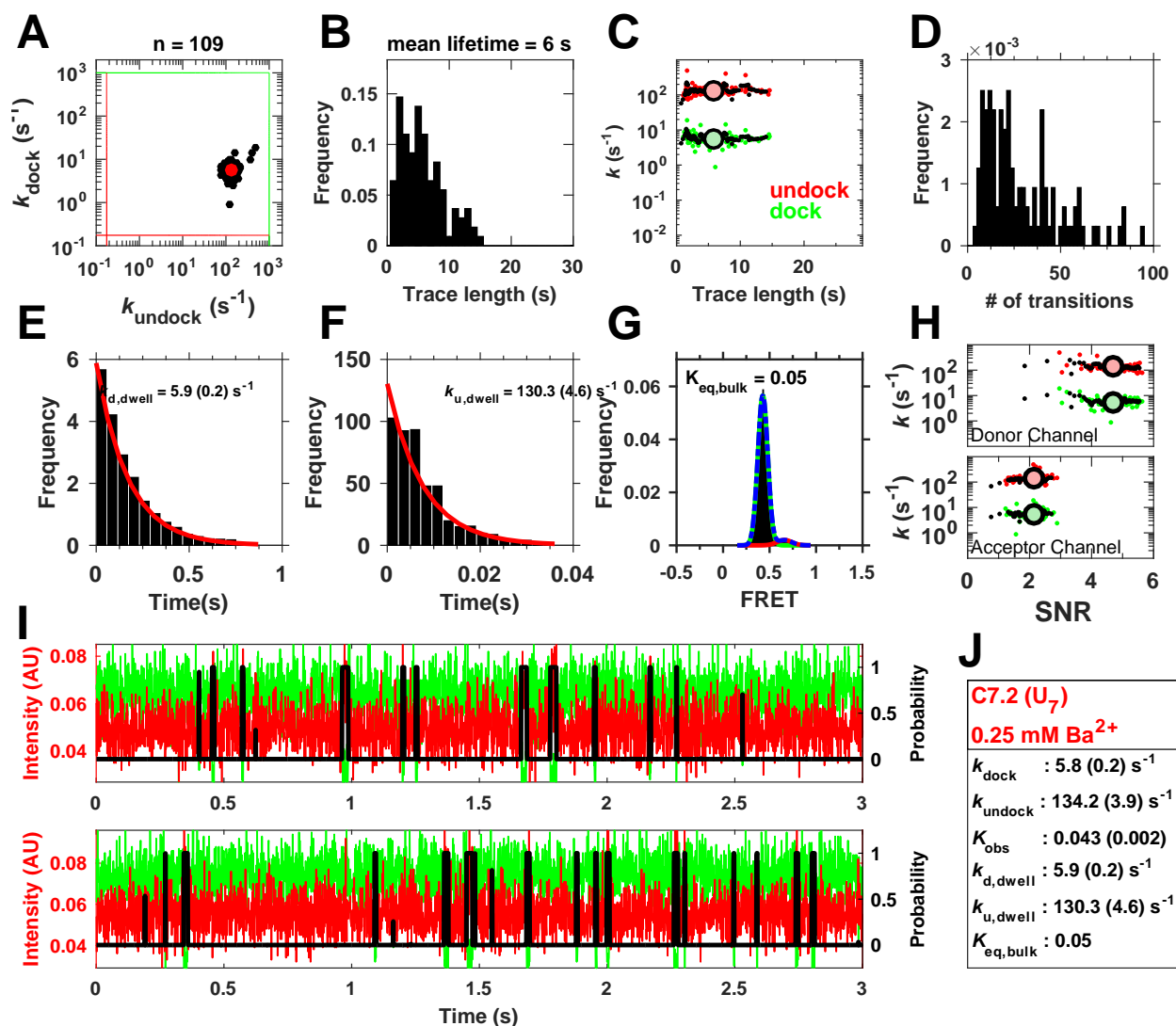


Figure S114. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

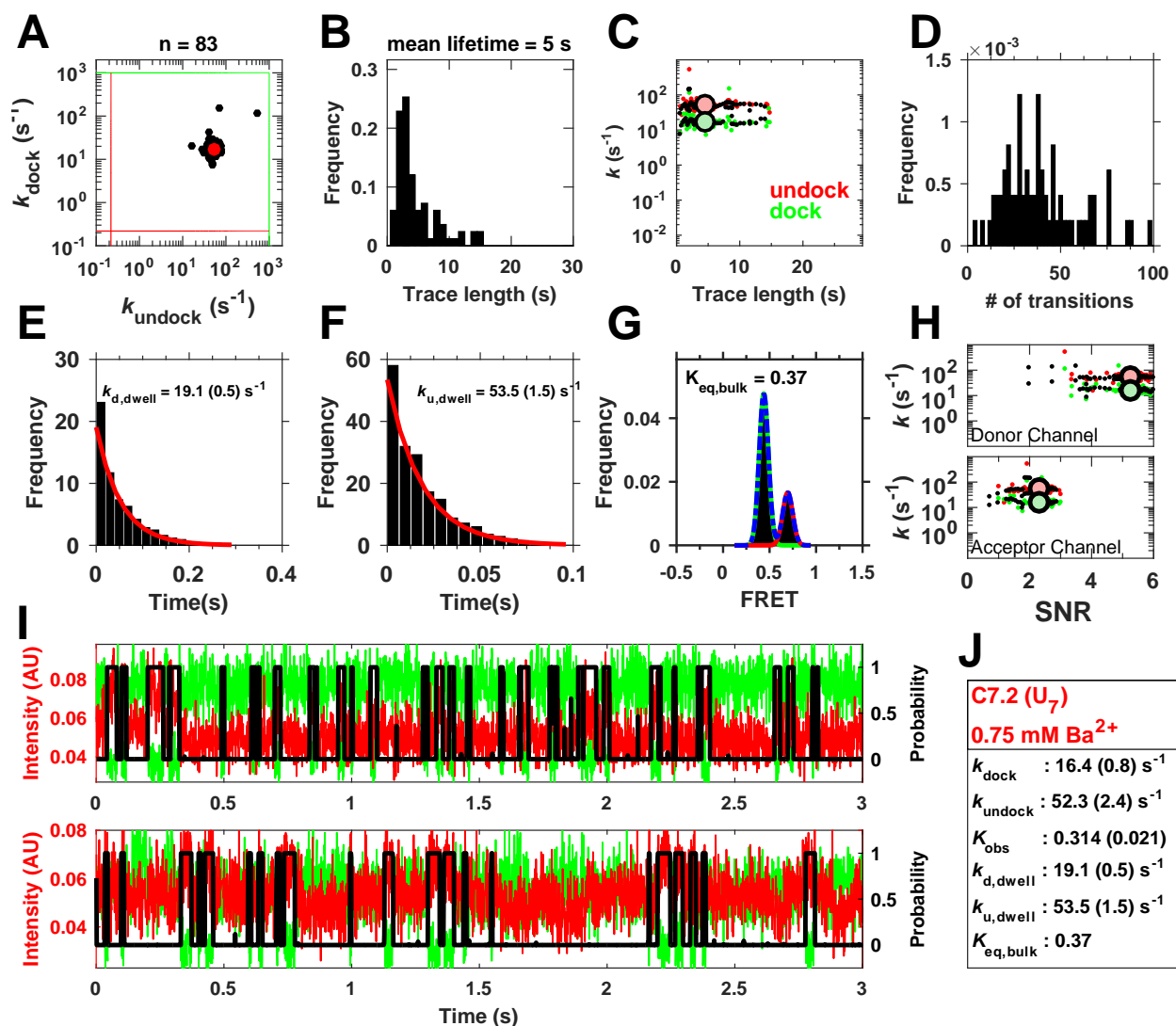


Figure S115. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

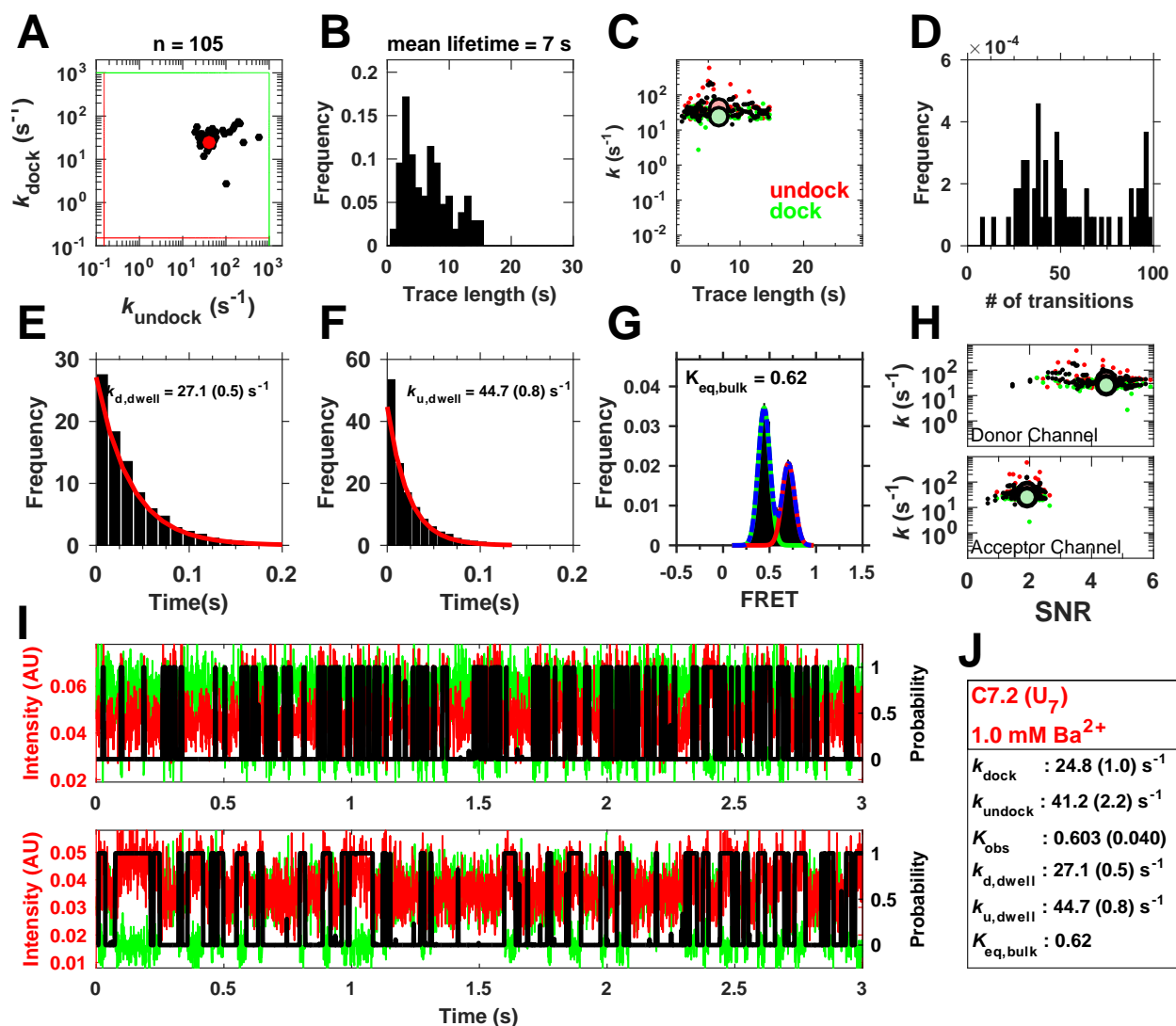


Figure S116. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

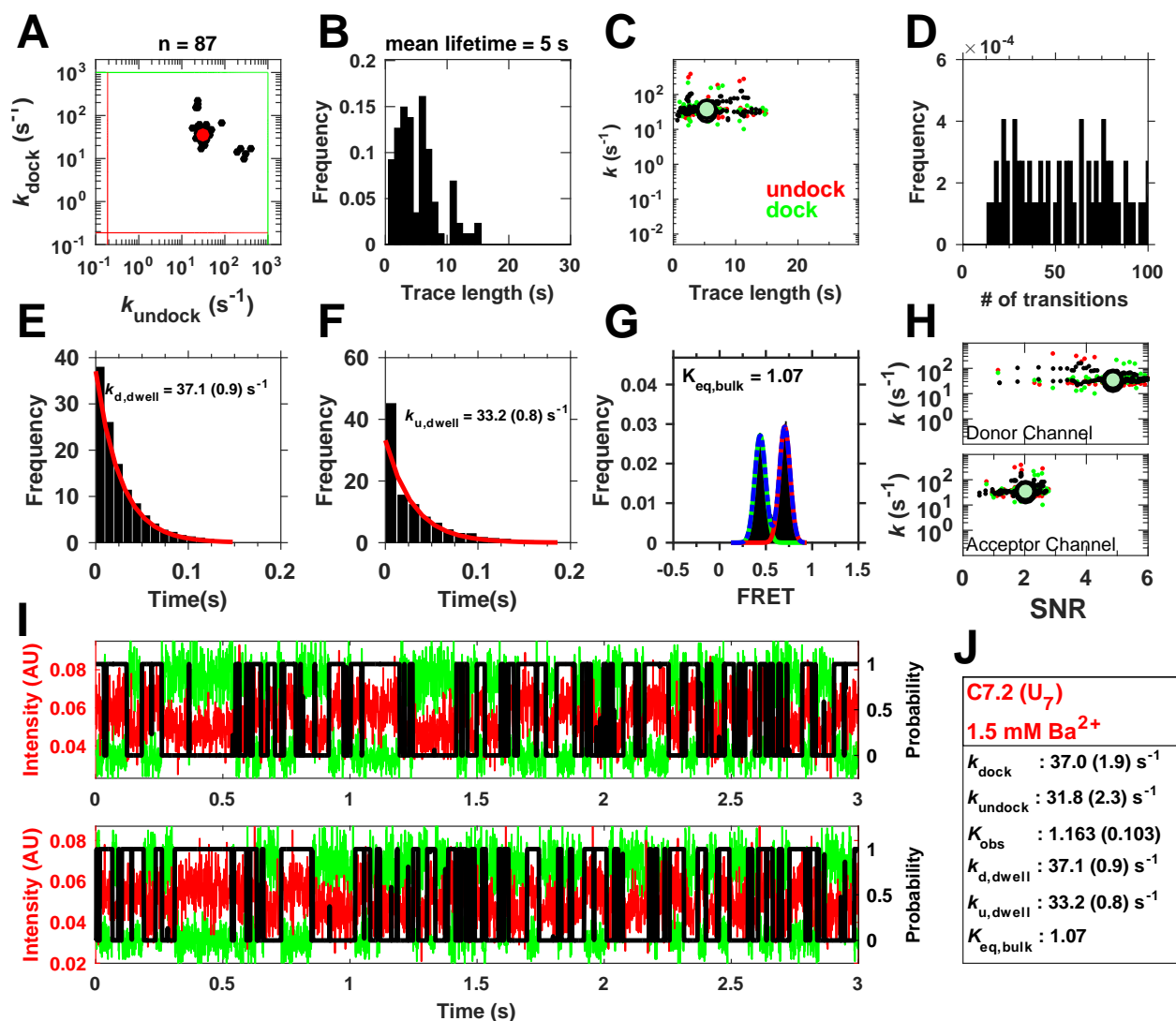


Figure S117. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

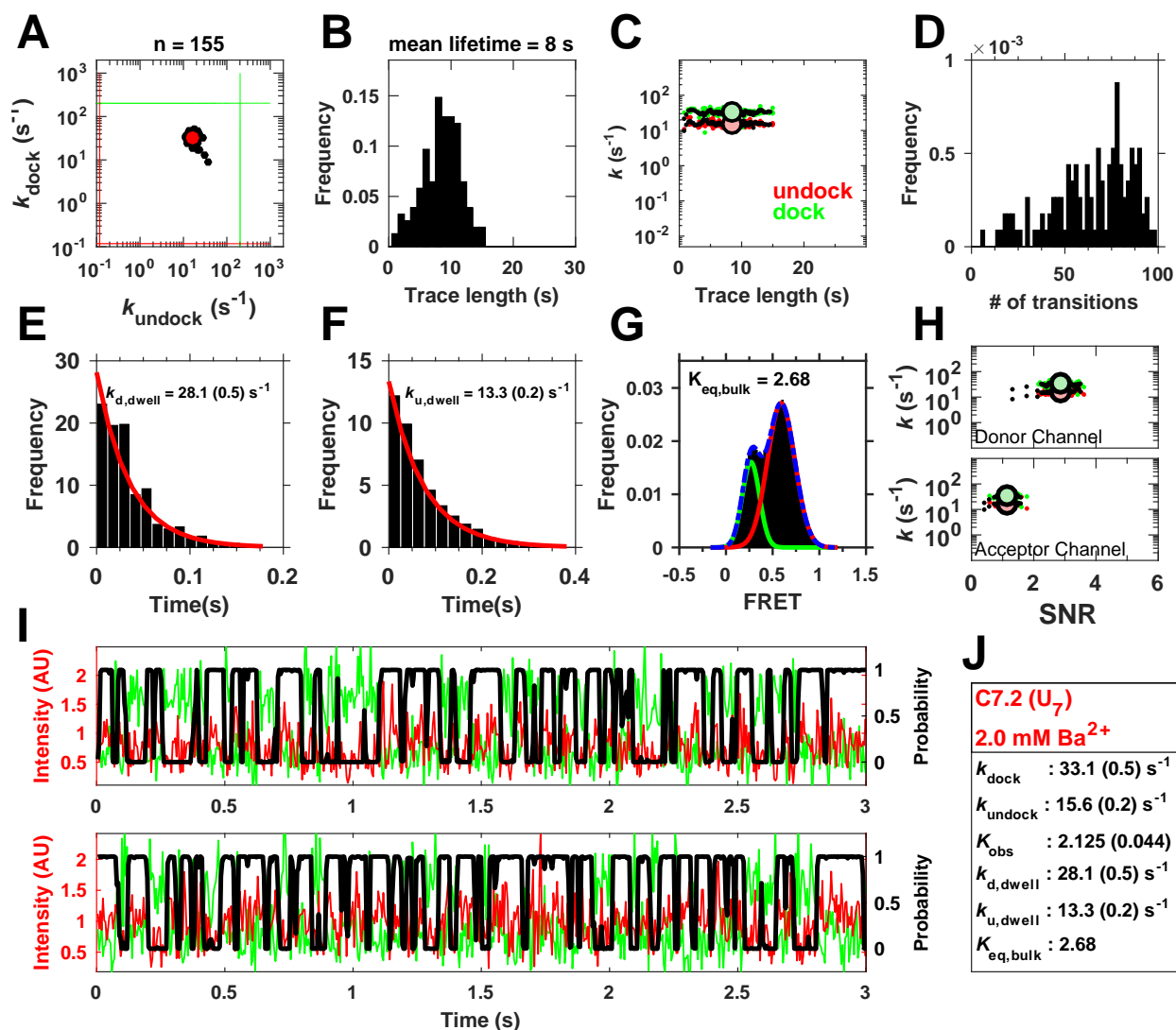


Figure S118. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

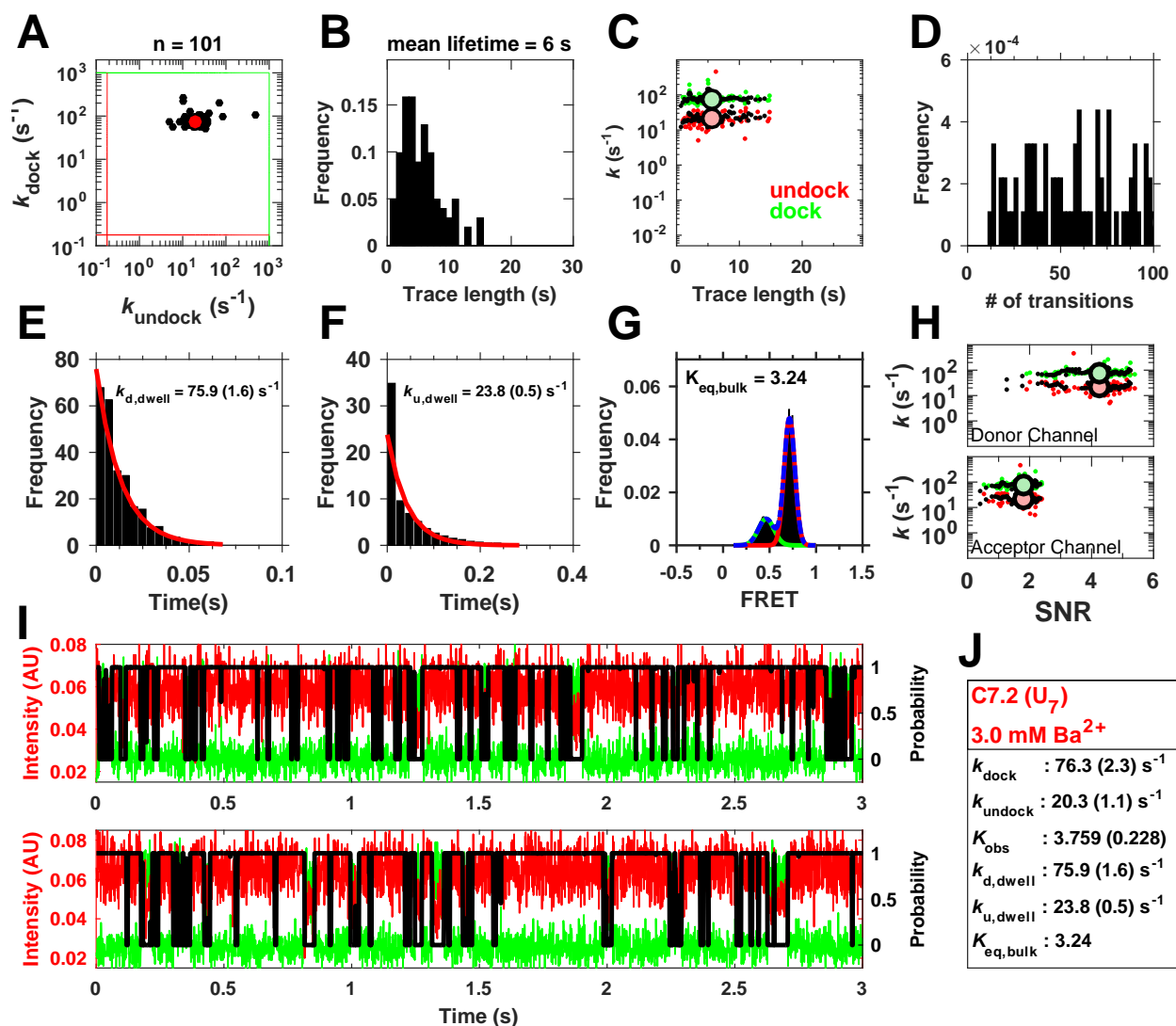


Figure S119. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

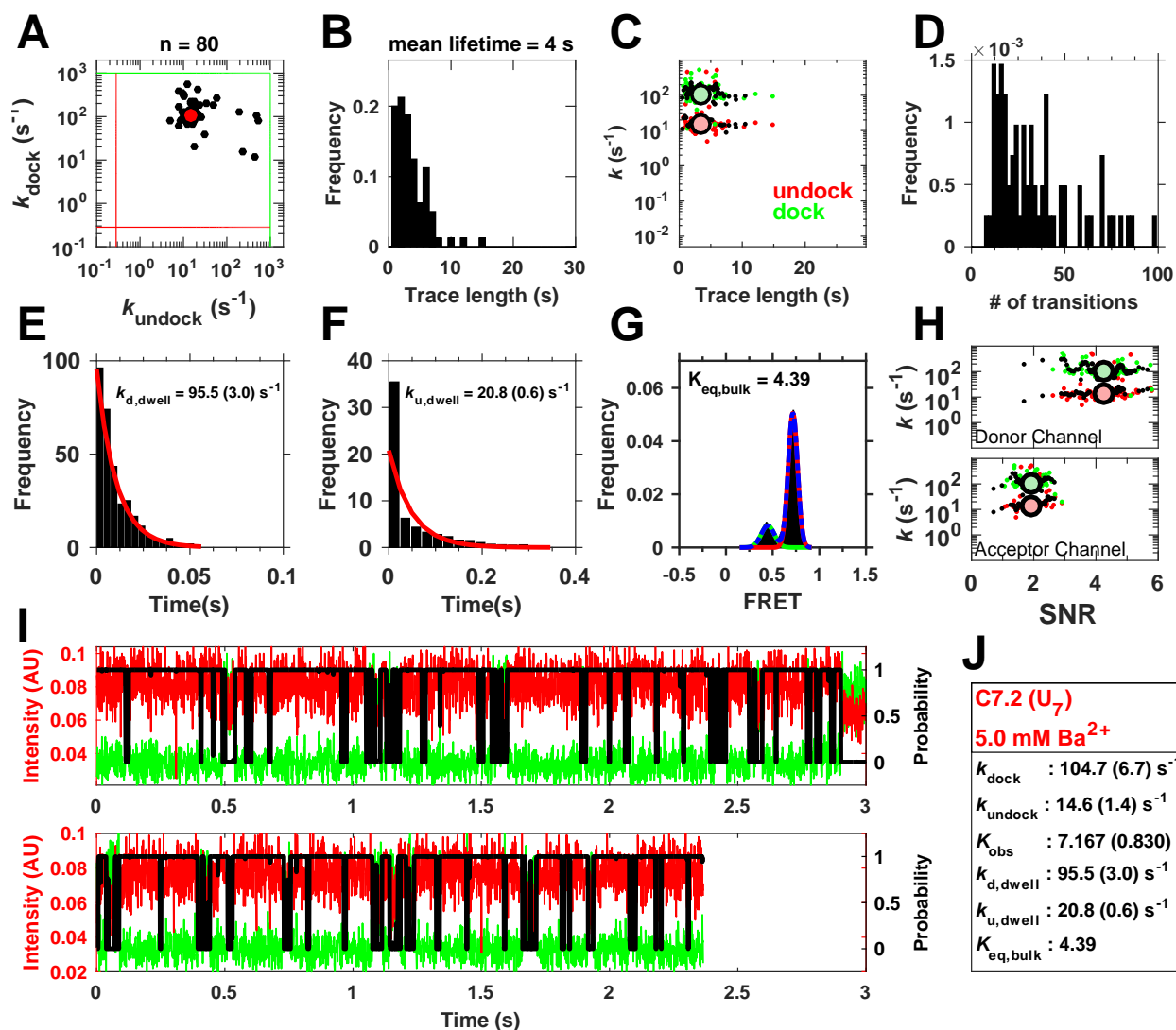


Figure S120. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

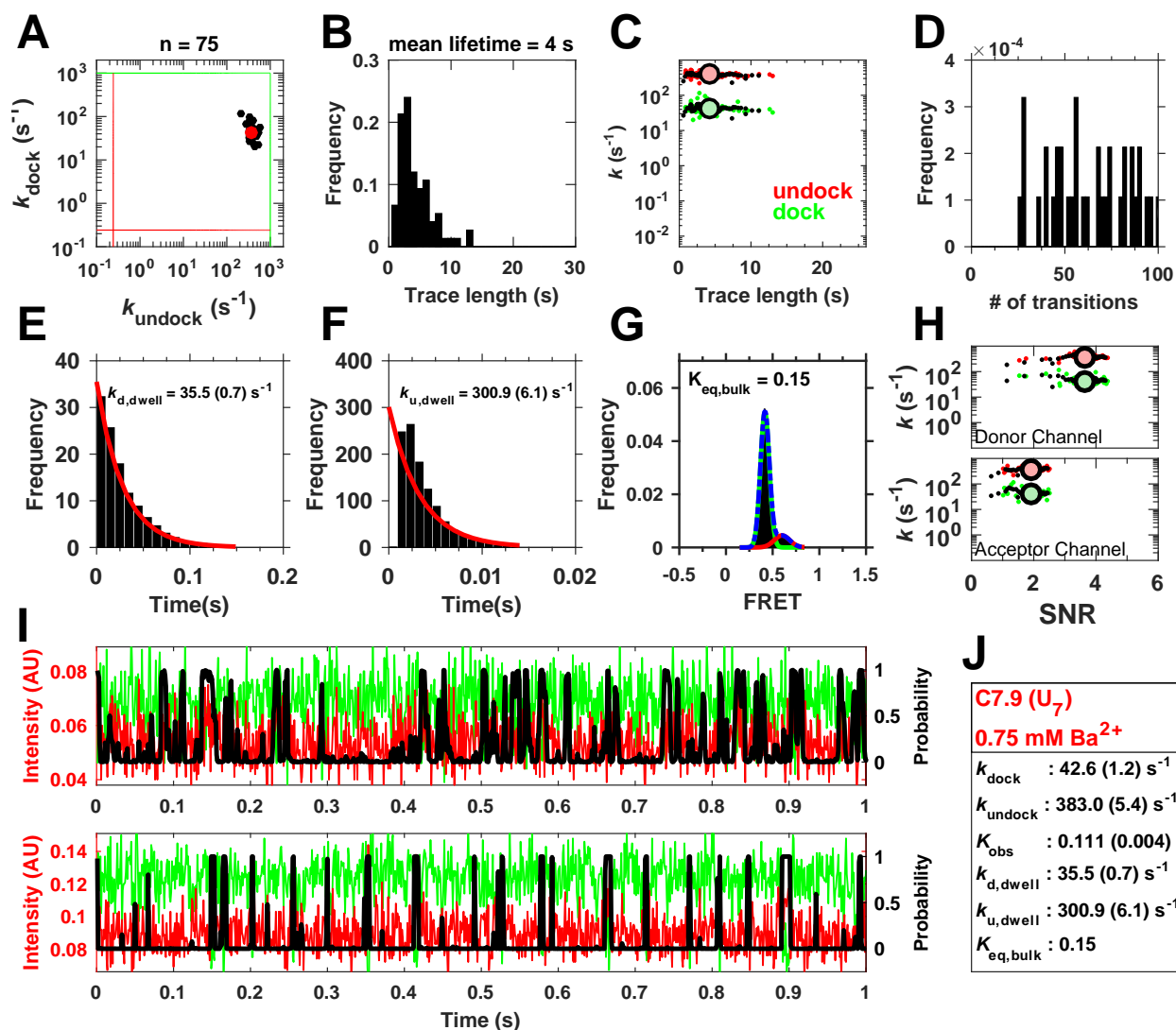


Figure S121. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

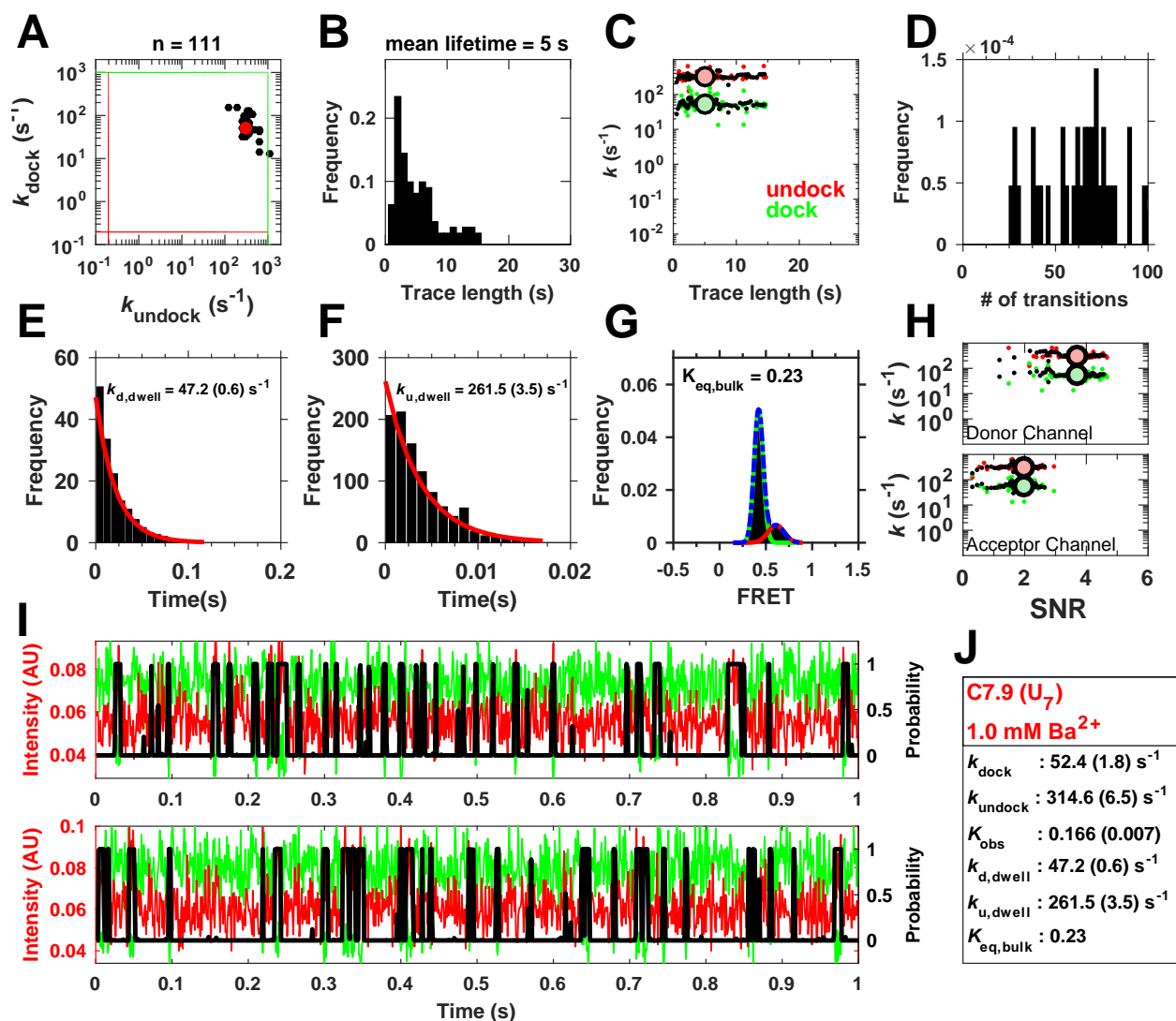


Figure S122. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

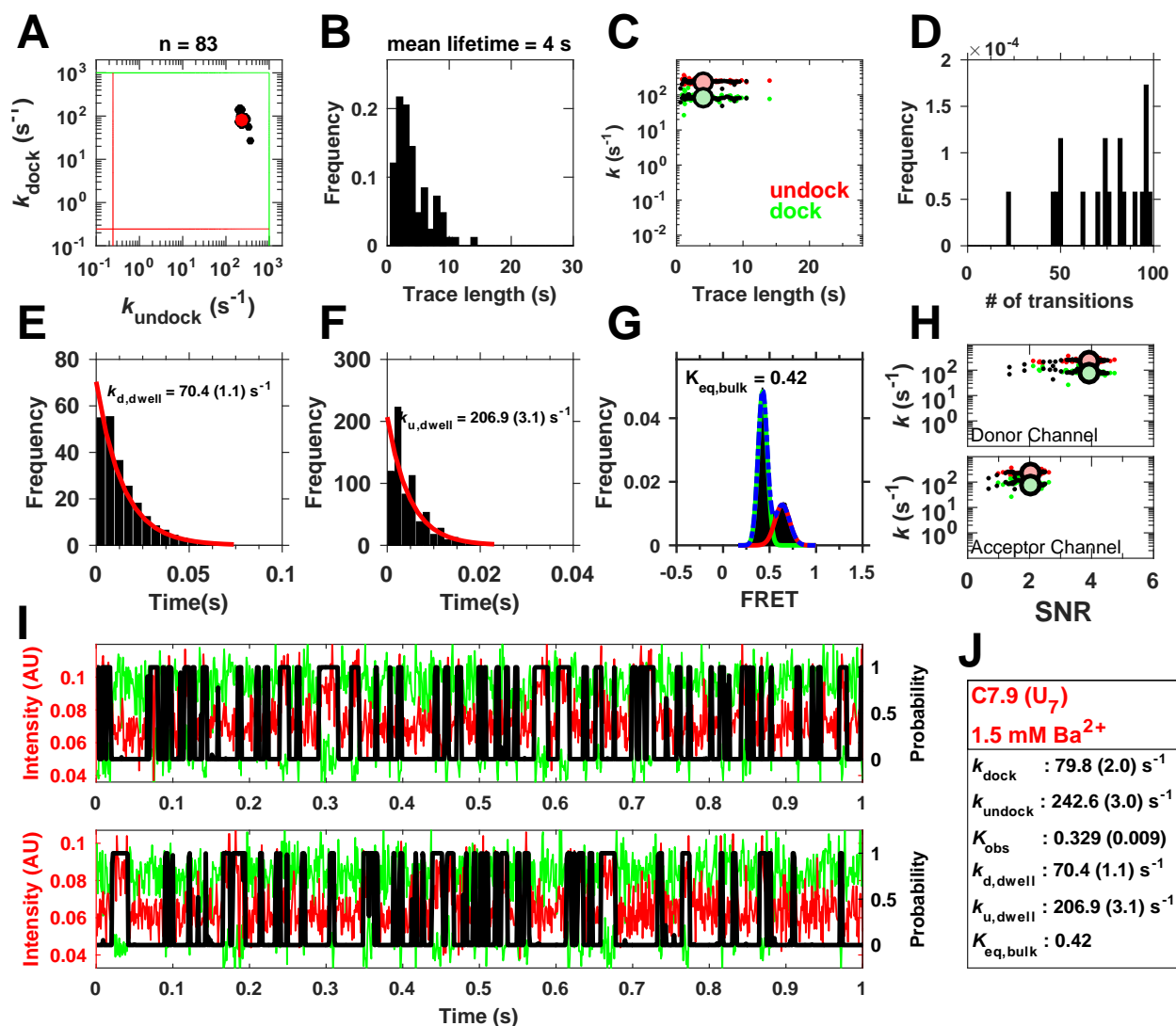


Figure S123. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

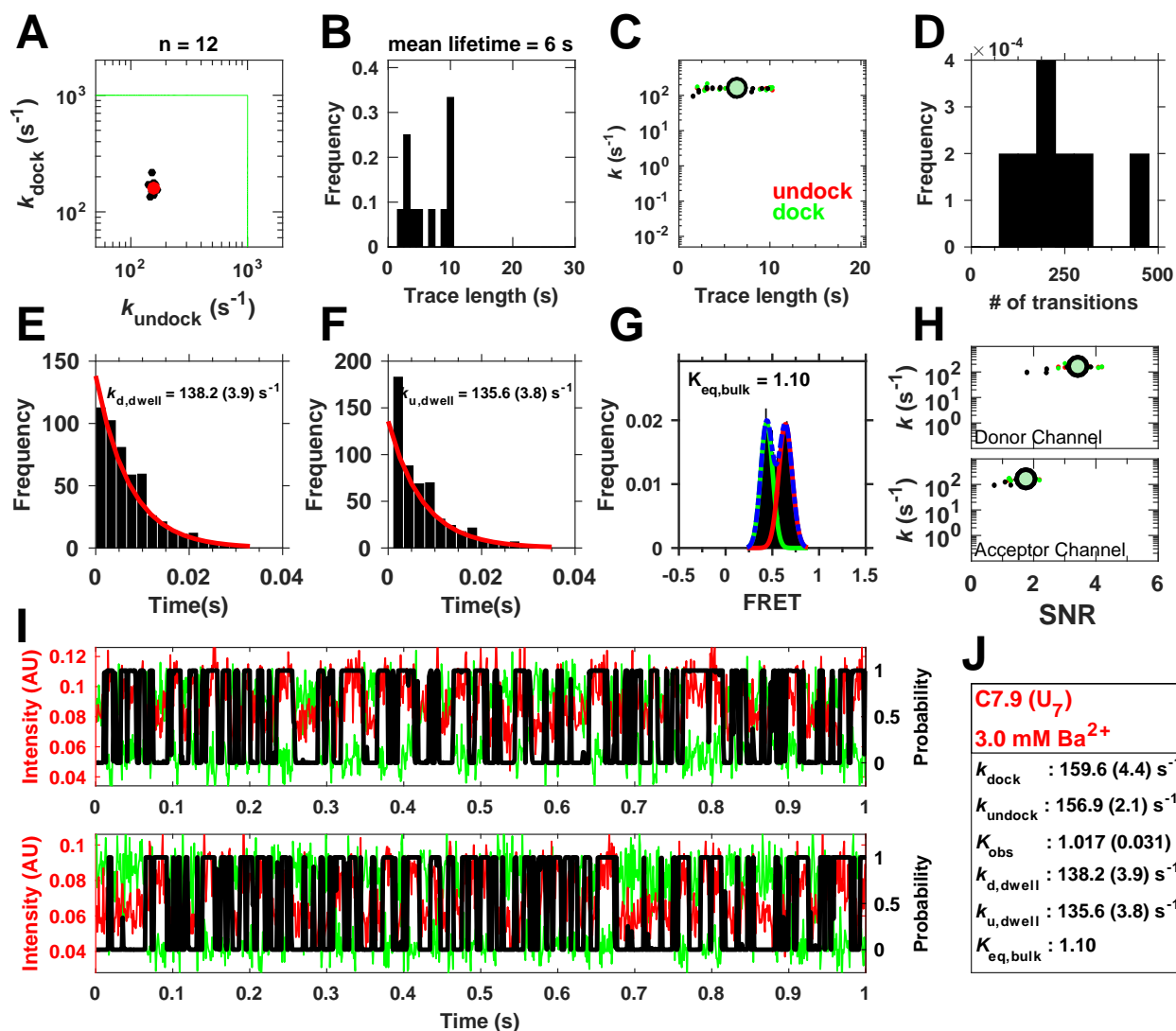


Figure S124. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

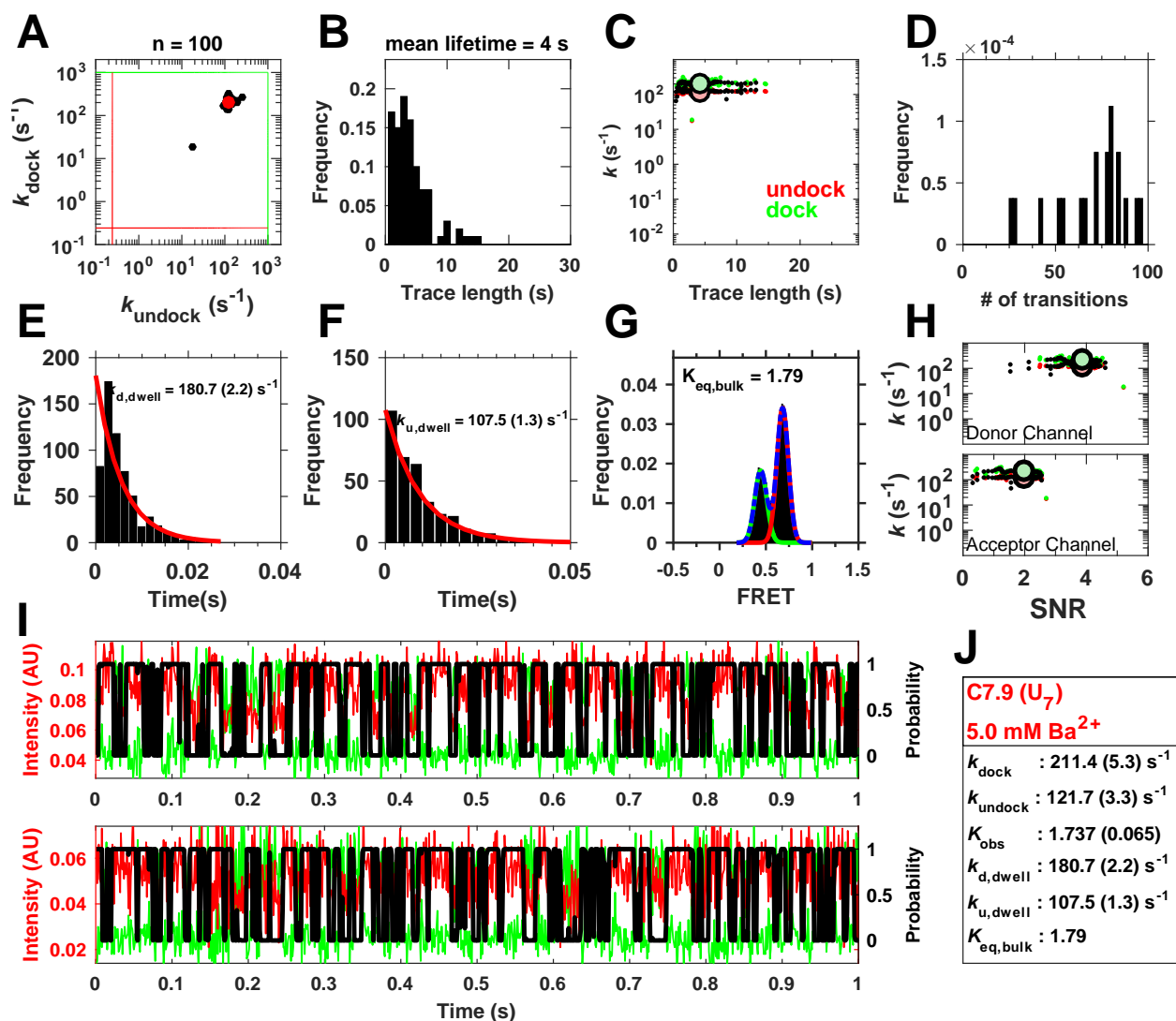


Figure S125. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

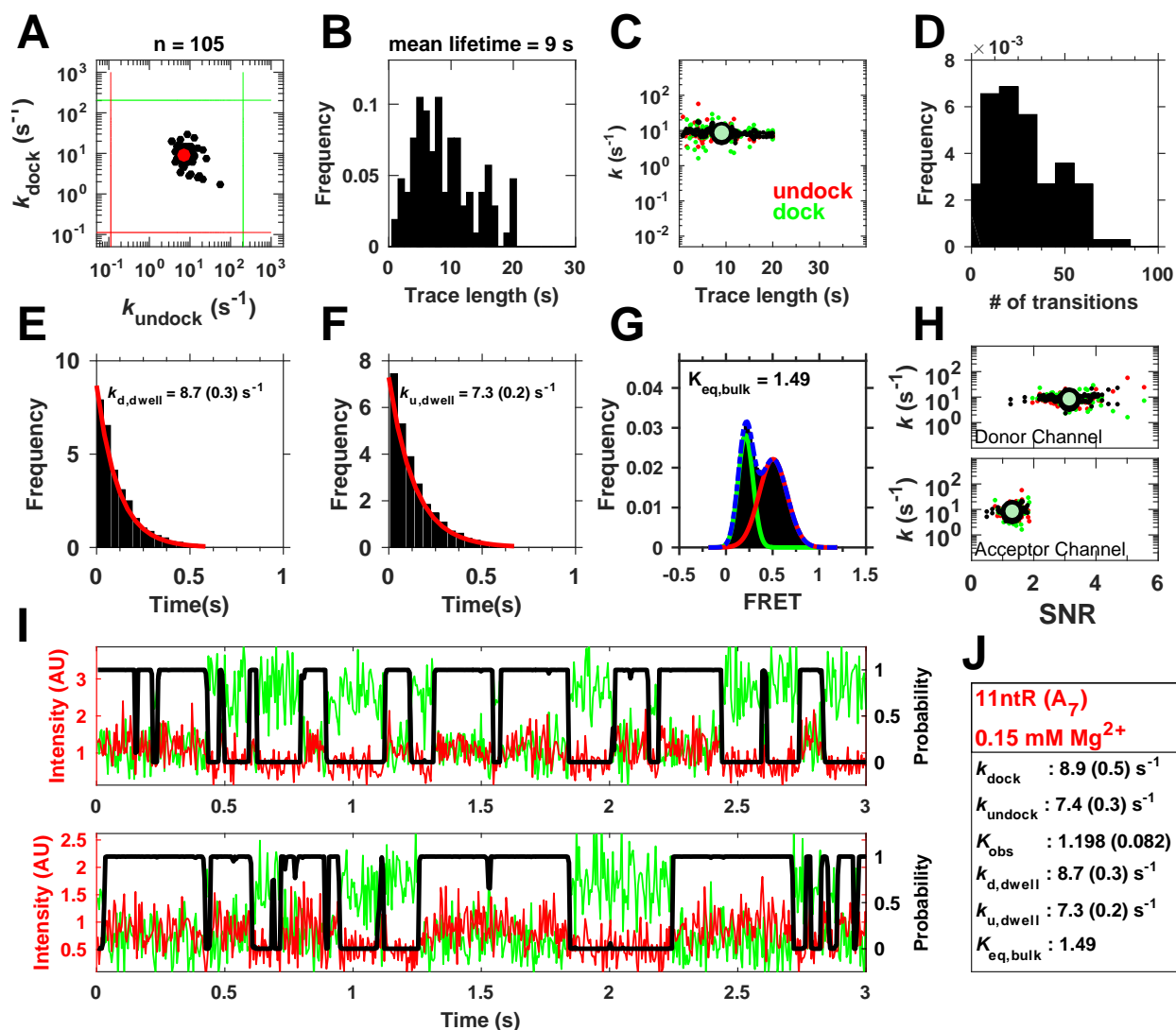


Figure S126. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

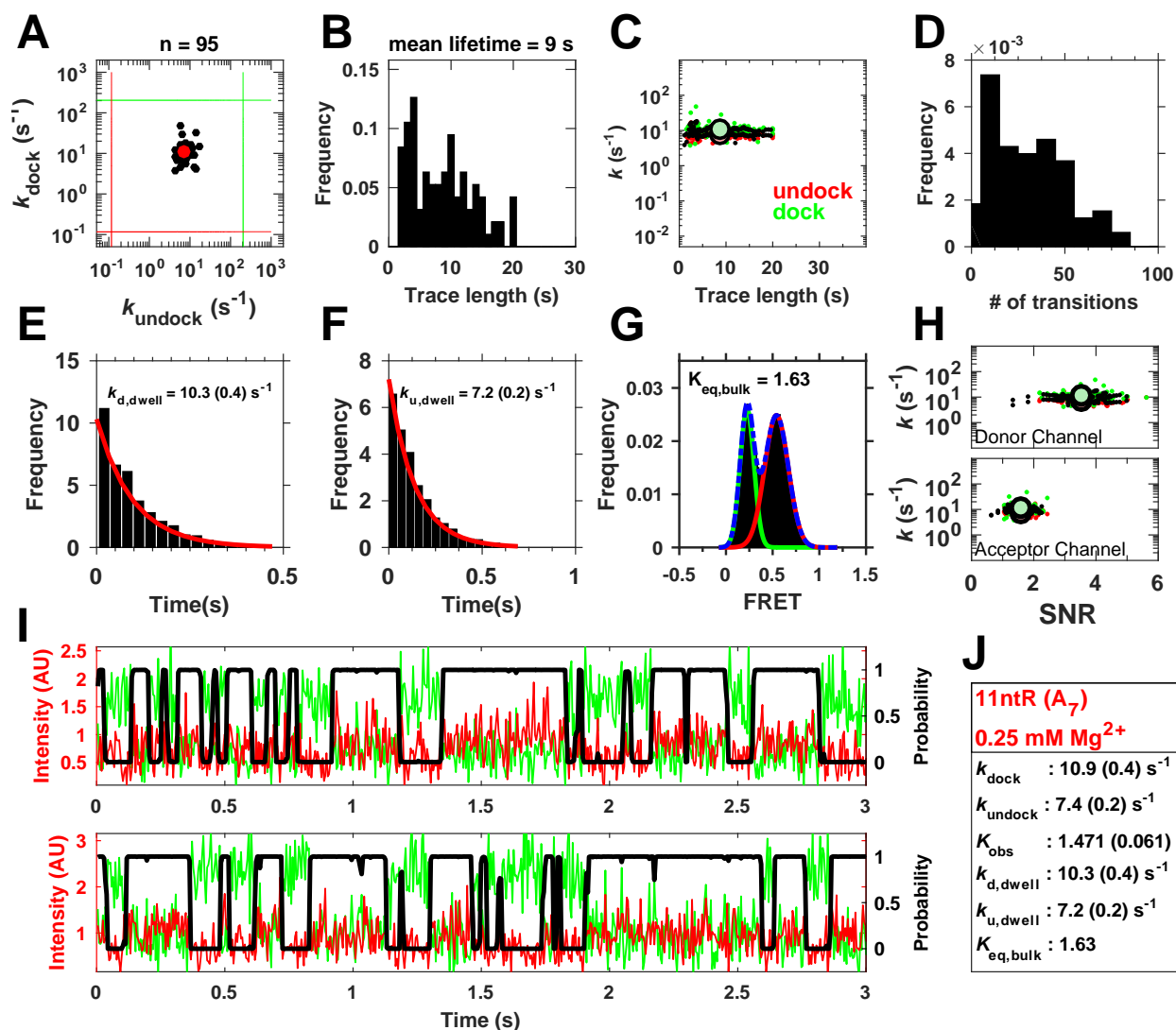


Figure S127. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

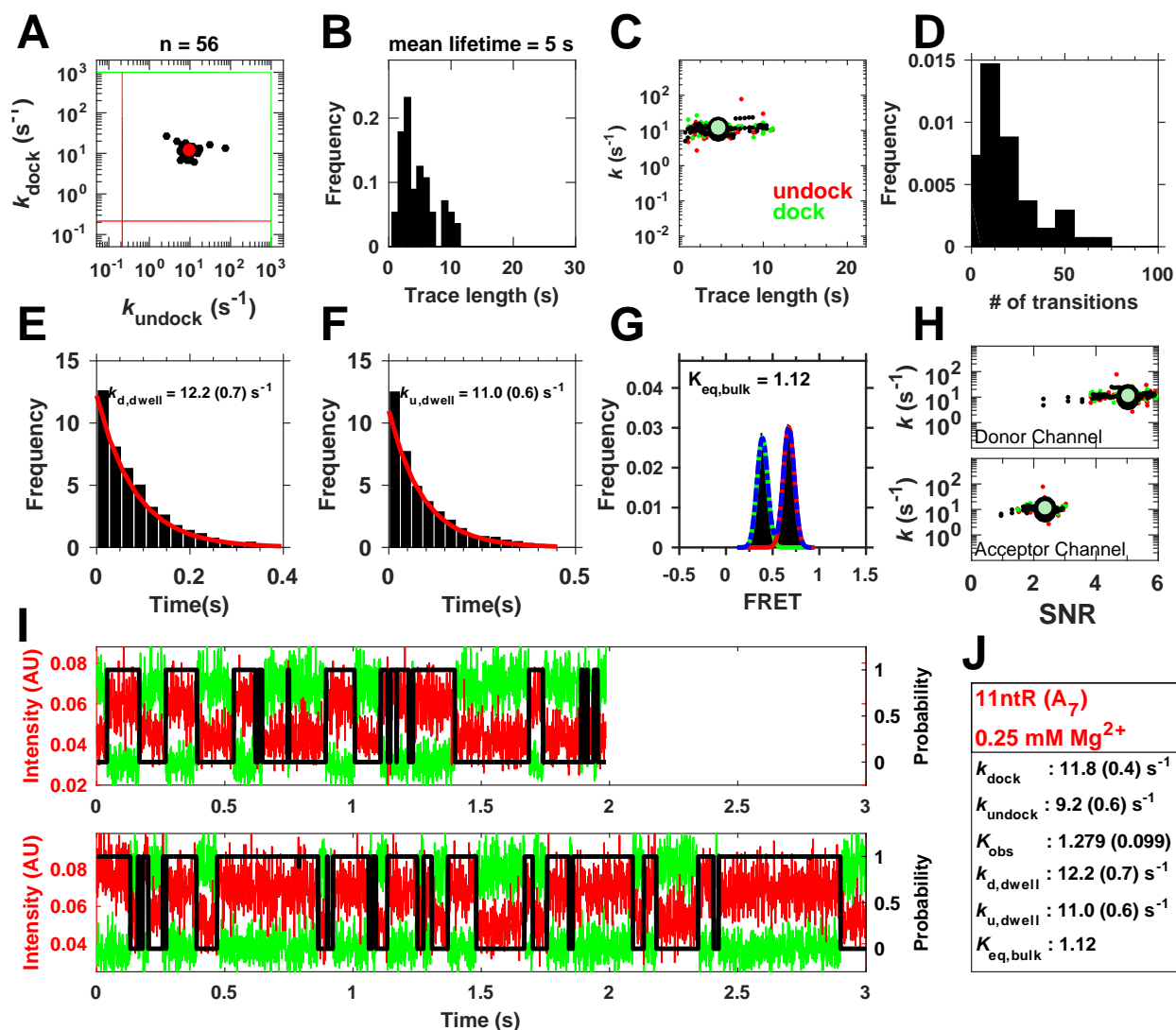


Figure S128. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

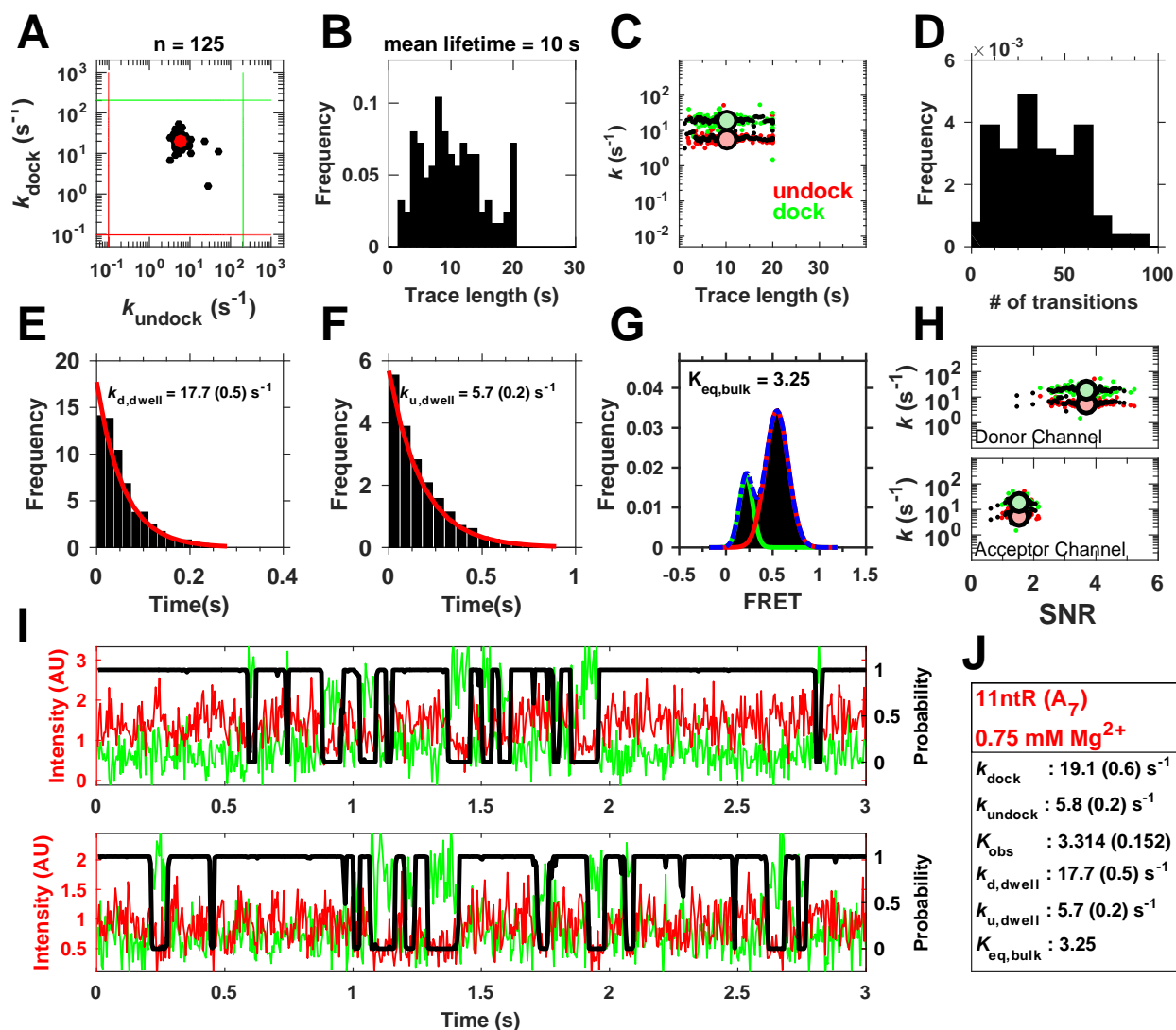


Figure S129. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

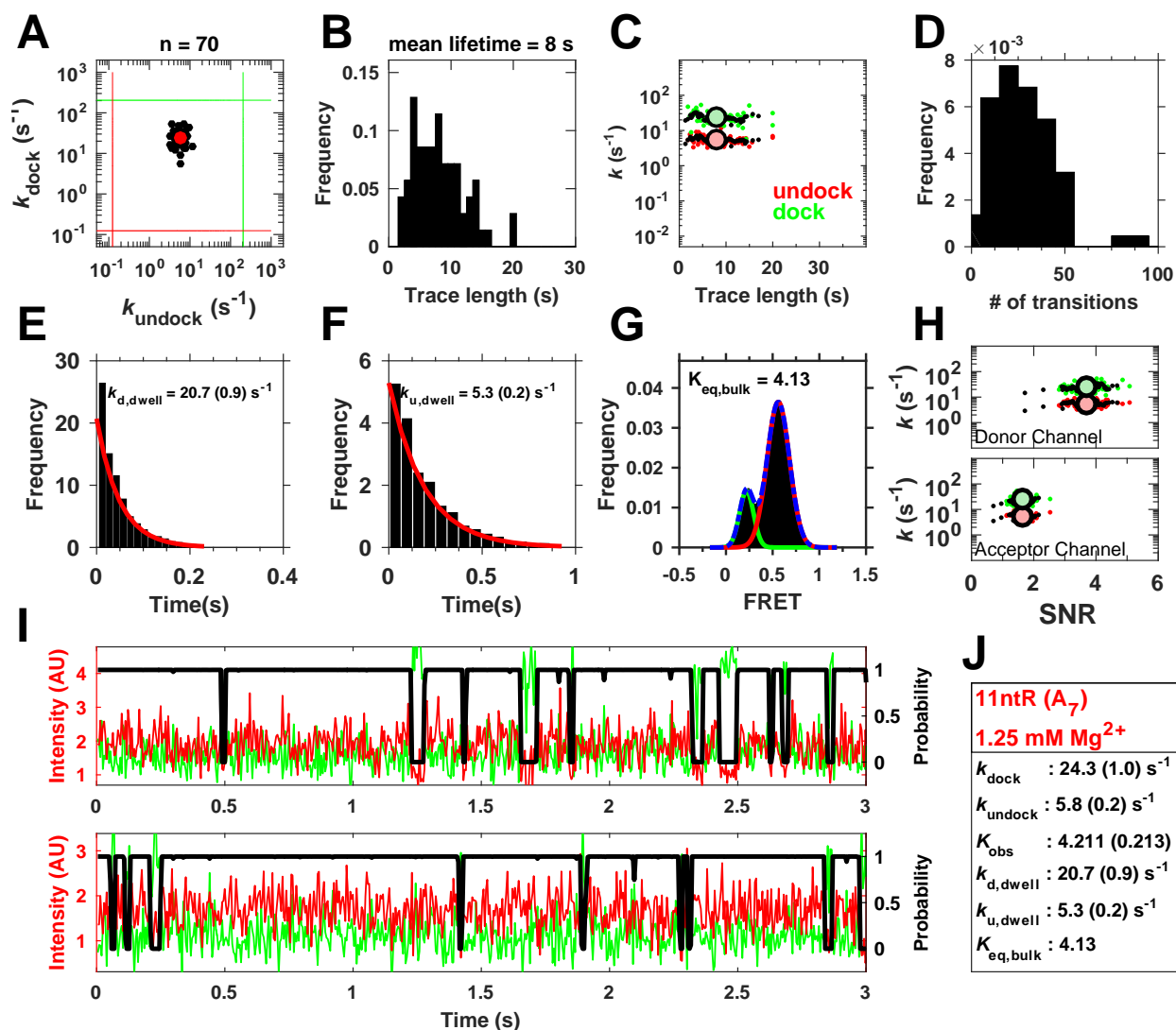


Figure S130. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

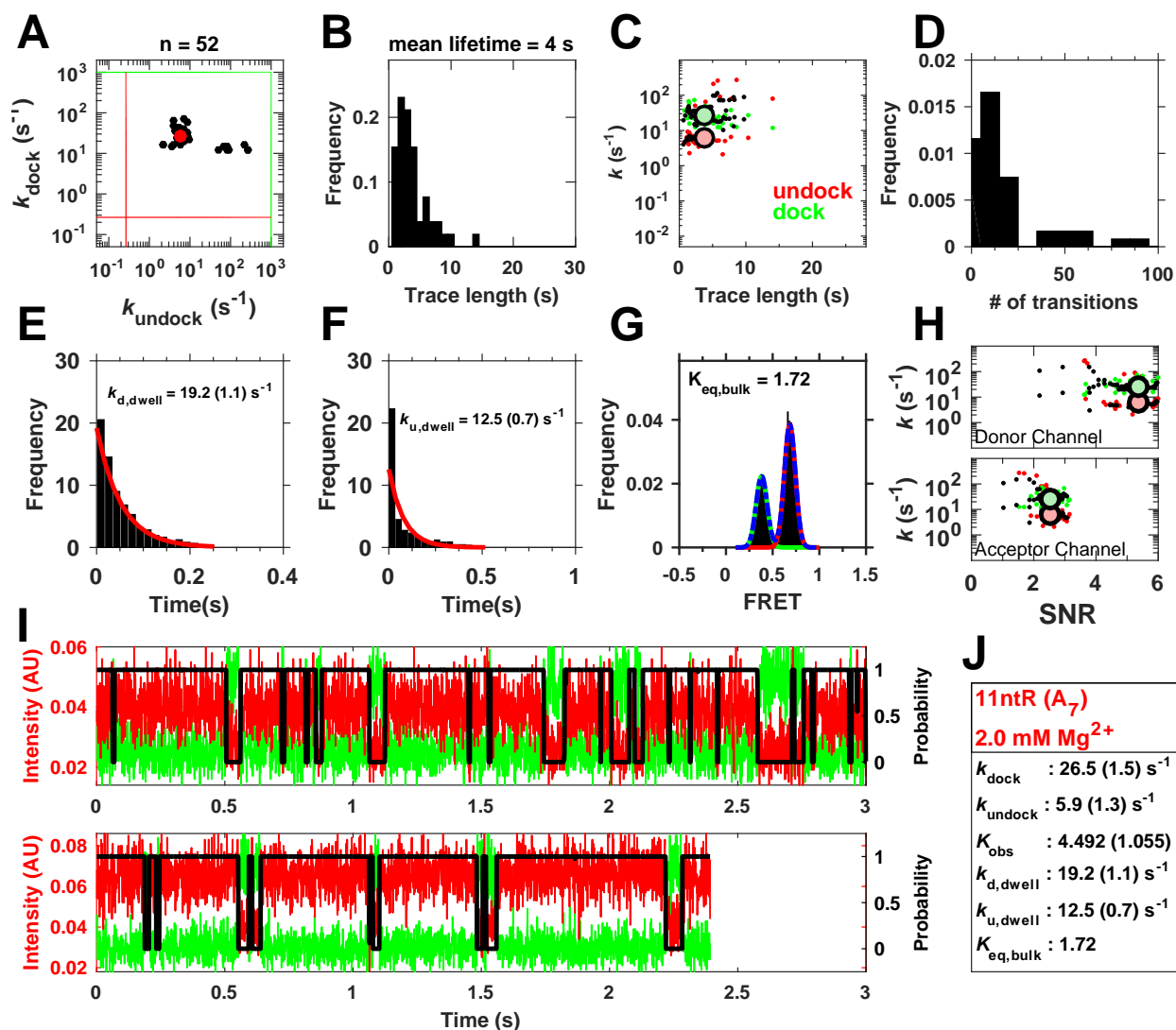


Figure S131. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

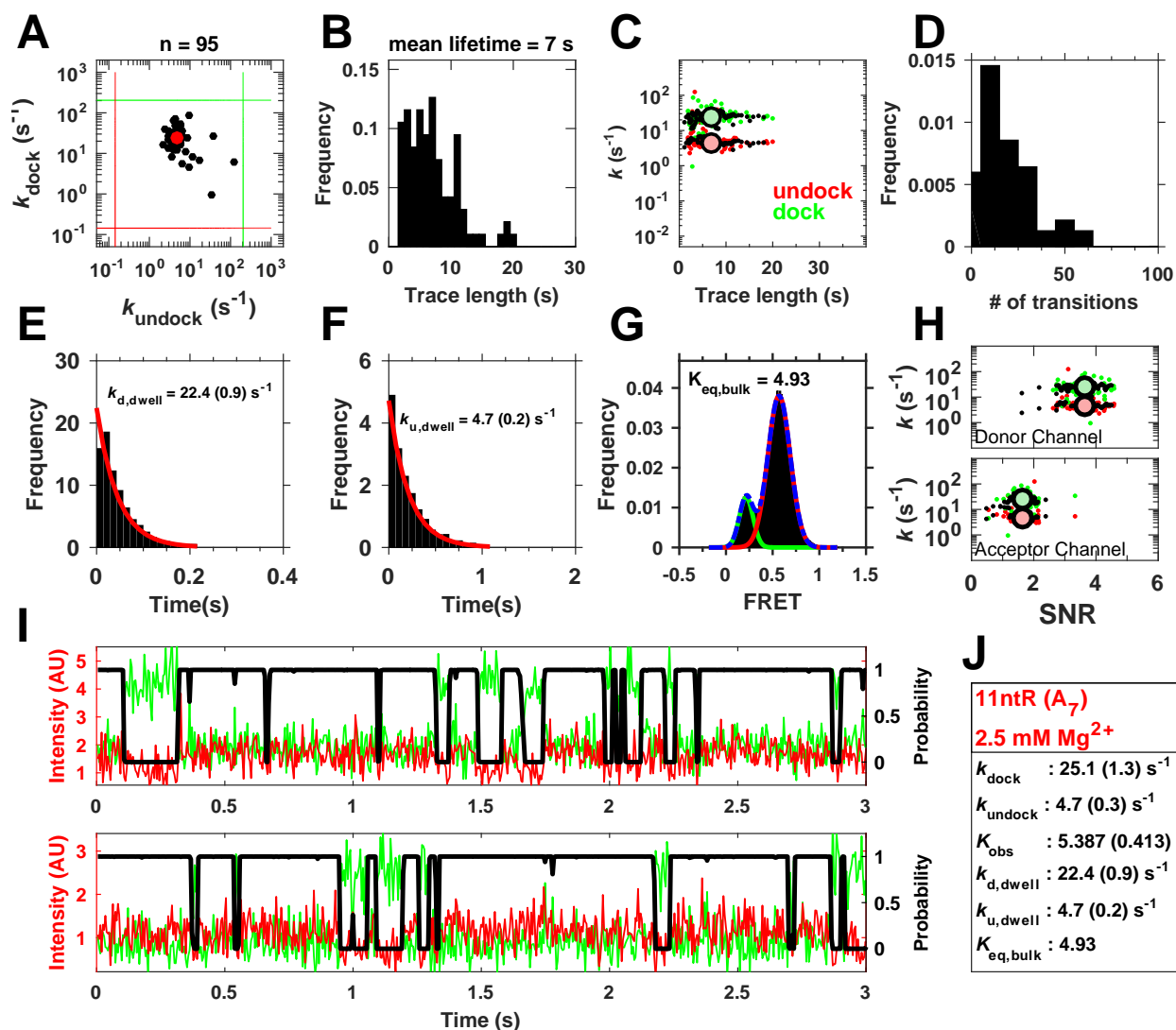


Figure S132. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

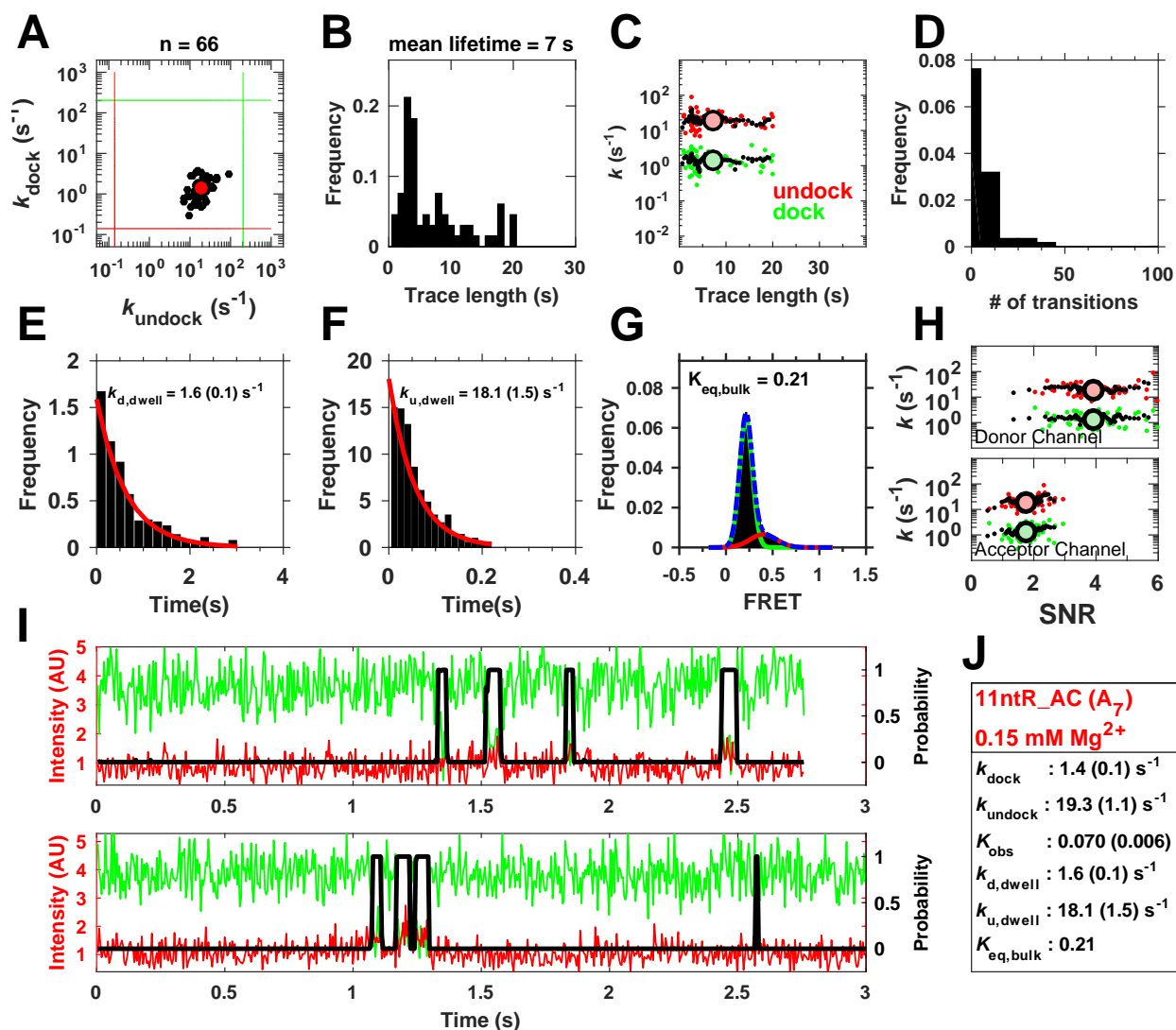


Figure S133. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

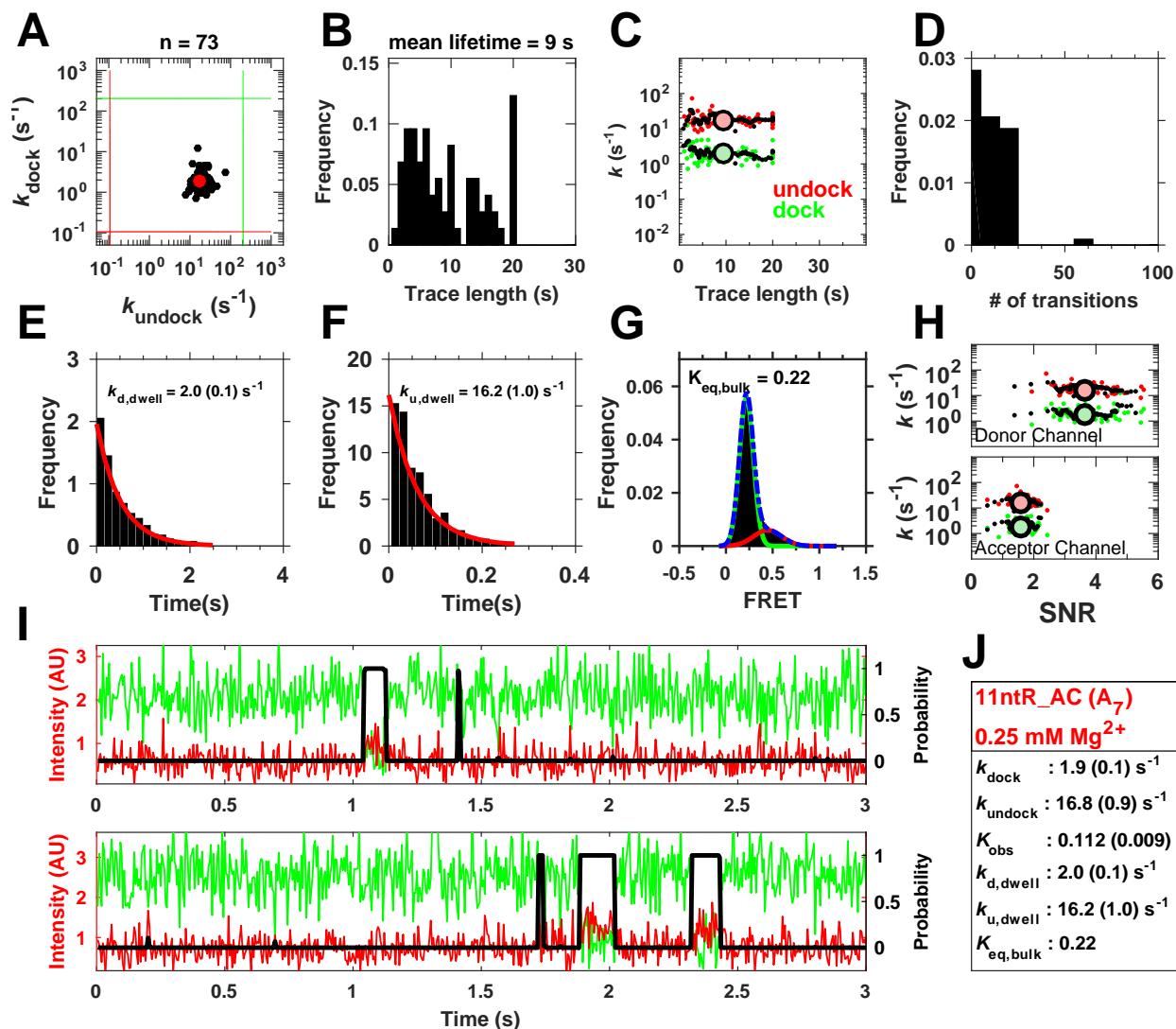


Figure S134. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

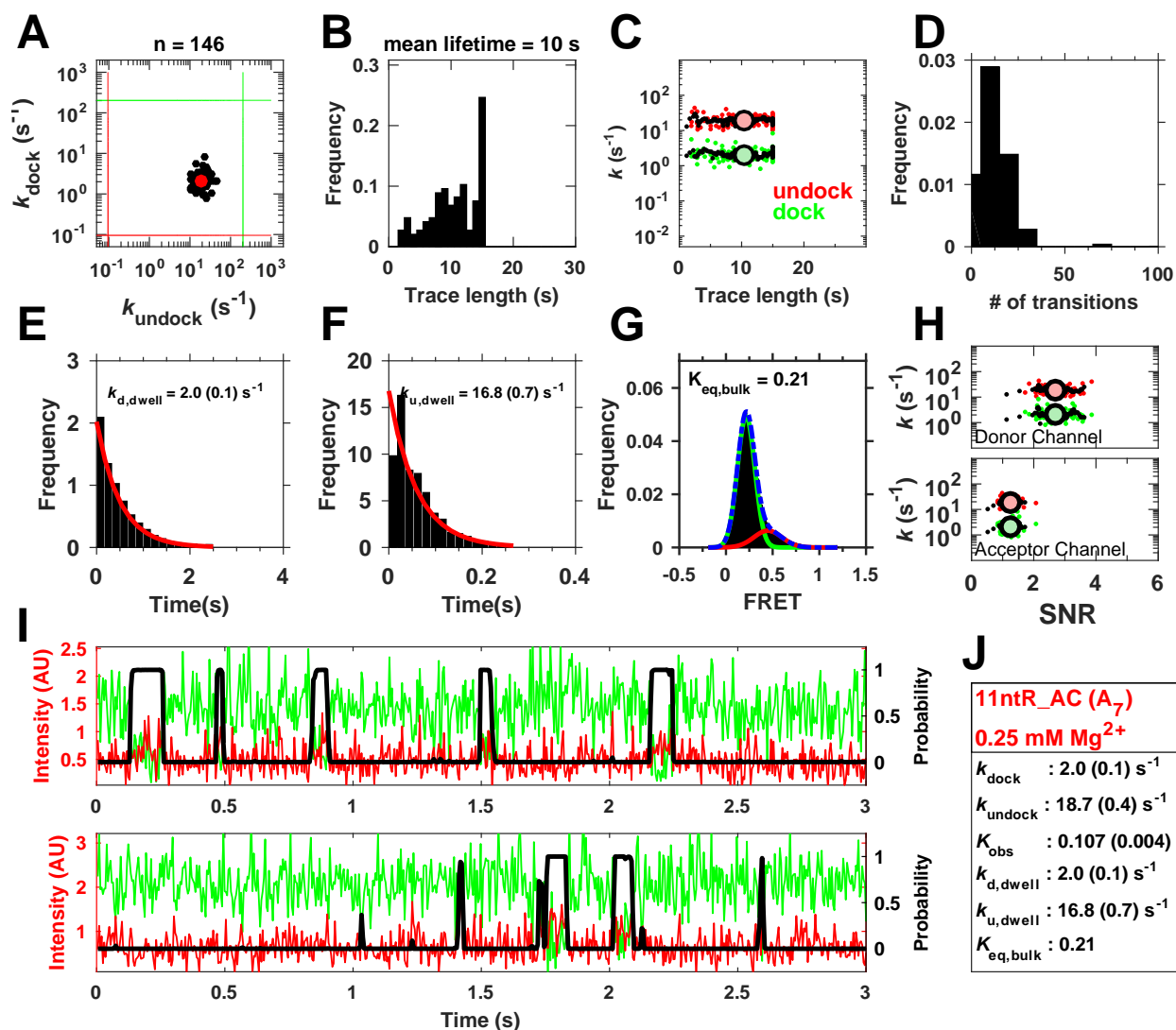


Figure S135. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

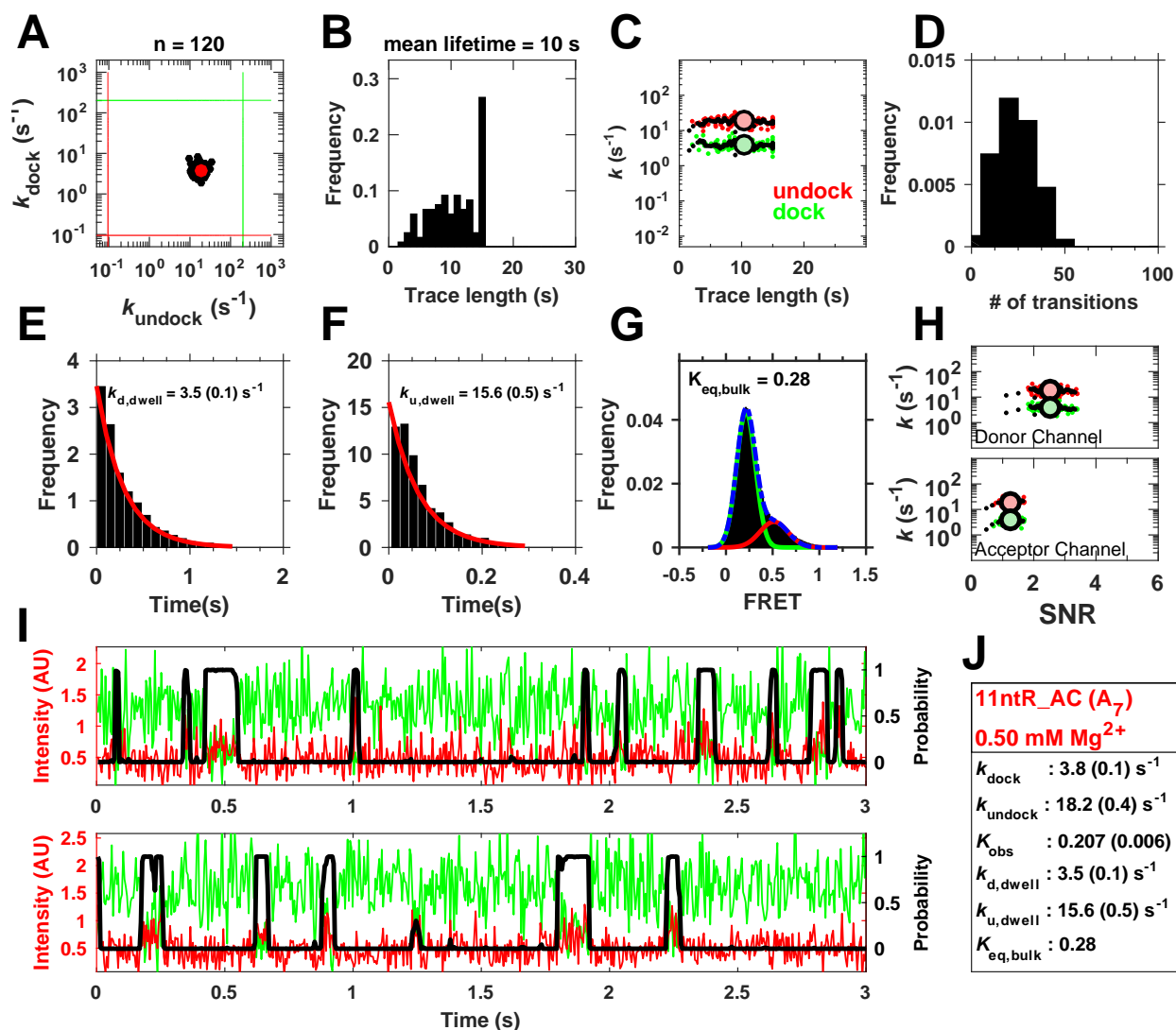


Figure S136. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.

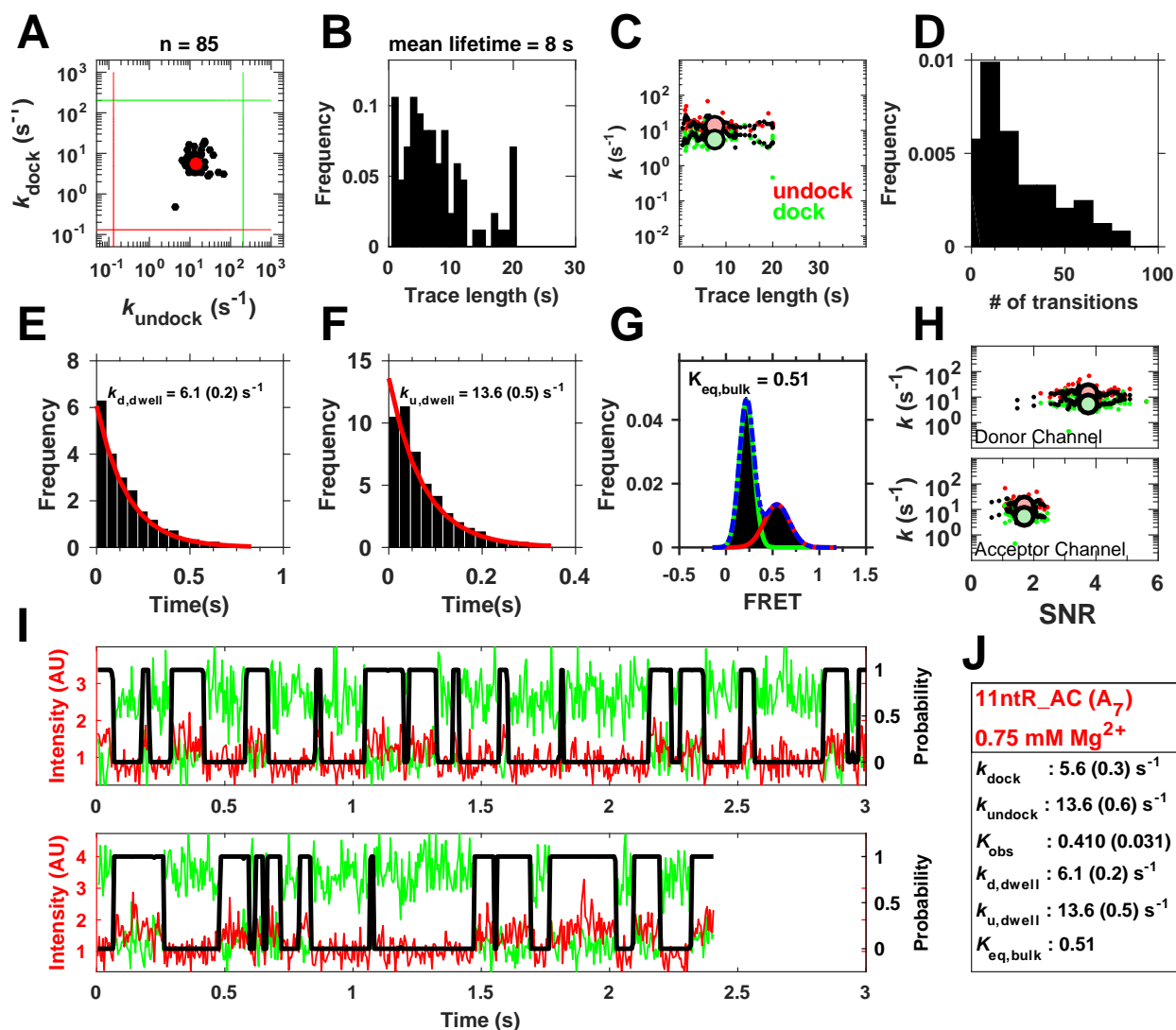


Figure S137. smFRET data summary. (A) Scatter plot of fitted values of the docking versus undocking rate constants of individual molecules. Green lines indicate the frame rate of data collection (upper limit) and red lines indicate the mean lifetime of the molecules (lower limit). Red dot is the population median. (B) Histogram of lifetimes of the smFRET traces. (C) Scatter plots of rate constants versus the length of the smFRET traces. The mean value for each rate constant is represented by a larger circle. Black dots are averages of five values and are shown to provide a visual guide. (D) Distribution of the number of transitions per trace. Distribution of dwell times in the low (E) and high (F) FRET states. Exponential fits are shown (red lines) along with the fitted single-exponential rate constants. (G) Cumulative FRET distributions. Distribution was fitted to a sum of two Gaussians (blue dashed line). The observed equilibrium constant from cumulative data was determined from the ratio of area under the high (red) and low (green) FRET components of the distribution. (H) Rate constants as functions of the signal-to-noise ratio (SNR) in the donor (top) and acceptor (bottom) channels. (I) Randomly-selected smFRET traces. Traces were truncated at one or three seconds for ease of comparison. The intensities of the donor dye (green) and the acceptor dye (red) are shown. The black line denotes the probability of occupying the high FRET state as determined by a two-state HMM model. (J) Summary table of single-molecule and cumulative kinetic and thermodynamic parameters.