
Figures and figure supplements

Real-time observation of signal recognition particle binding to actively translating ribosomes

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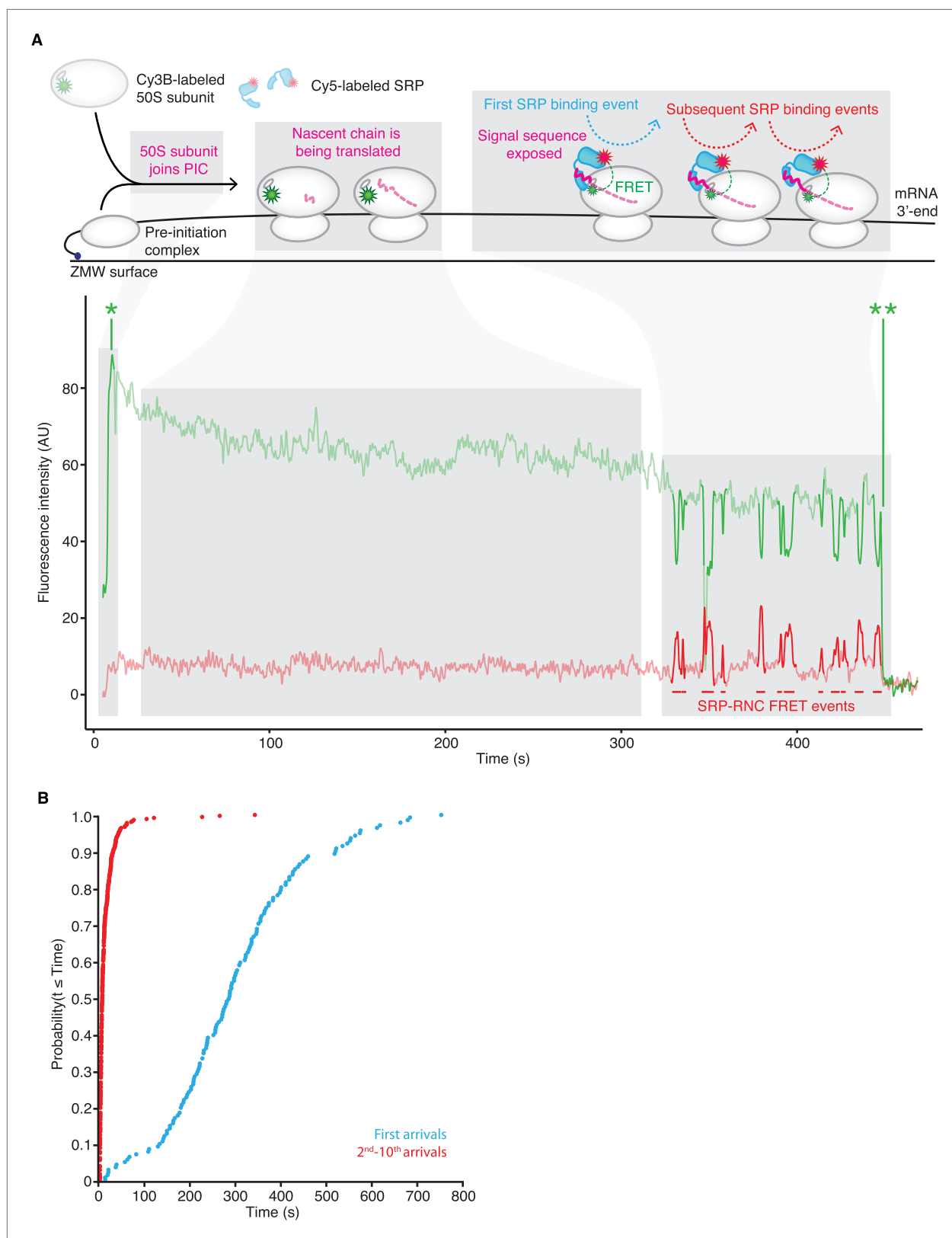


Figure 1. SRP-binding to actively translating RNCs. **(A)** Example smFRET trace of Cy5-labeled SRP, Cy3B-labeled 50S subunits, and unlabeled translation mix delivered at time = 0 to PICs pre-assembled on a truncated *lepB* mRNA (encoding the first 155 amino acids) and immobilized on ZMWs. To reduce non-specific interactions of SRP with the ZMWs, we pre-incubated the ZMWs with BSA, Blocking oligo, and unlabeled SRP, all of which were then Figure 1. Continued on next page

Figure 1. Continued

thoroughly washed away (**Figure 1—figure supplement 2**). The top panel shows a schematic representation of the molecular events throughout the trace. The bottom panel shows the fluorescence intensity of the Cy3B (green) and Cy5 (red) signal upon 532 nm excitation. 'AU' indicates 'arbitrary units'. * denotes the initial 50S ribosomal subunit joining. ** denotes photobleaching of the Cy3B dye on the 50S ribosomal subunit. **(B)** Cumulative distributions of SRP first arrival times (blue) and 2nd–10th arrival times (red) to RNCs from the experiment described in **(A)**. ($n \geq 141$ binding events).

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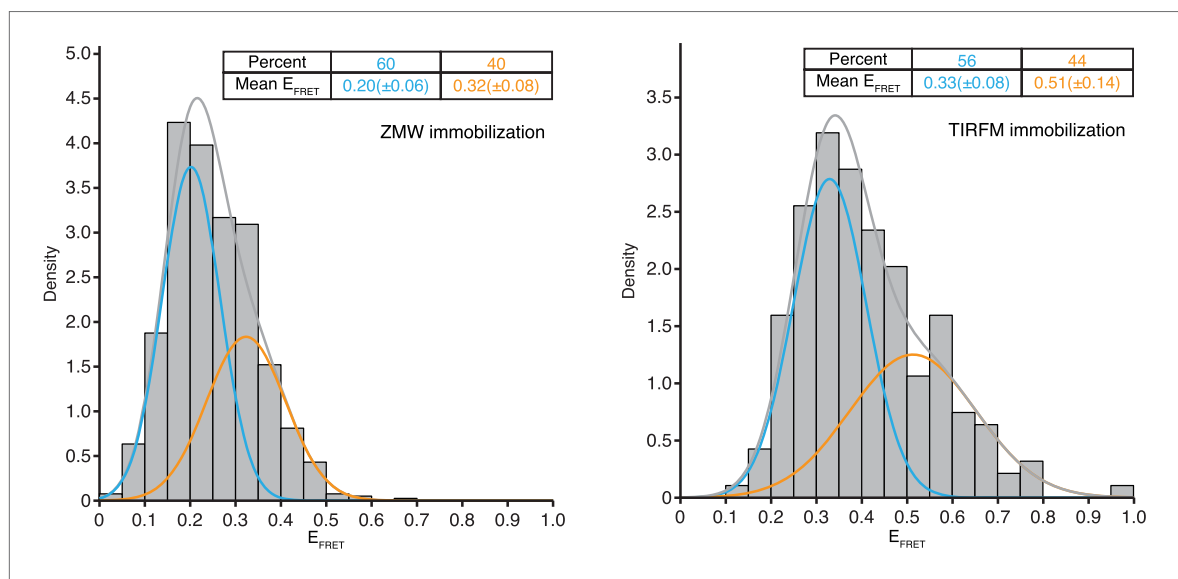


Figure 1—figure supplement 1. E_{FRET} validation. Histograms of the average E_{FRET} values of SRP-RNC binding events when Cy5-labeled SRP was delivered to Cy3B-labeled RNCs stalled with a *lepB* mRNA (encoding the first 75 amino acids) and immobilized on ZMWs (left panel, 100 nM SRP, $n = 787$ binding events) or TIRFM slides (right panel, 20 nM SRP, $n = 186$ binding events). Lines indicate the normal fits of low E_{FRET} events (blue), and high E_{FRET} events (orange). The percentage of total events and mean (\pm one standard deviation) E_{FRET} value for each are indicated. Please see 'Materials and methods' 'smFRET assay characterization' for further discussion of the figure.

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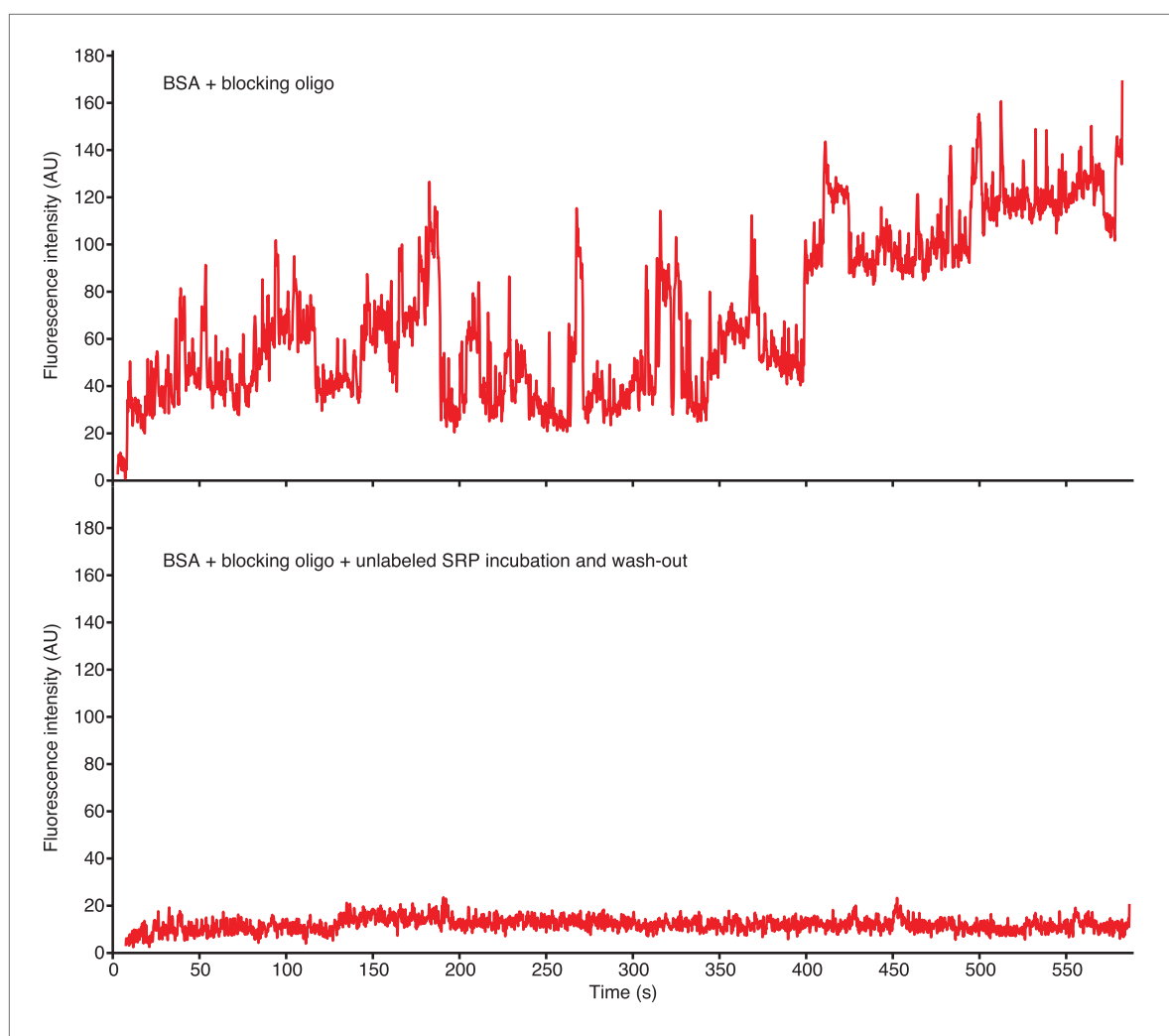


Figure 1—figure supplement 2. ZMW blocking to reduce non-specific SRP interactions. Example traces of 100 nM Cy5-labeled SRP delivered to ZMWs in the presence of BSA, and Blocking oligo in the absence (top panel) or presence of a dark SRP pre-incubation and wash-out (bottom panel). Fluorescence intensity of the Cy5 (red) signal upon 532 nm excitation is shown. 'AU' indicates 'arbitrary units'.

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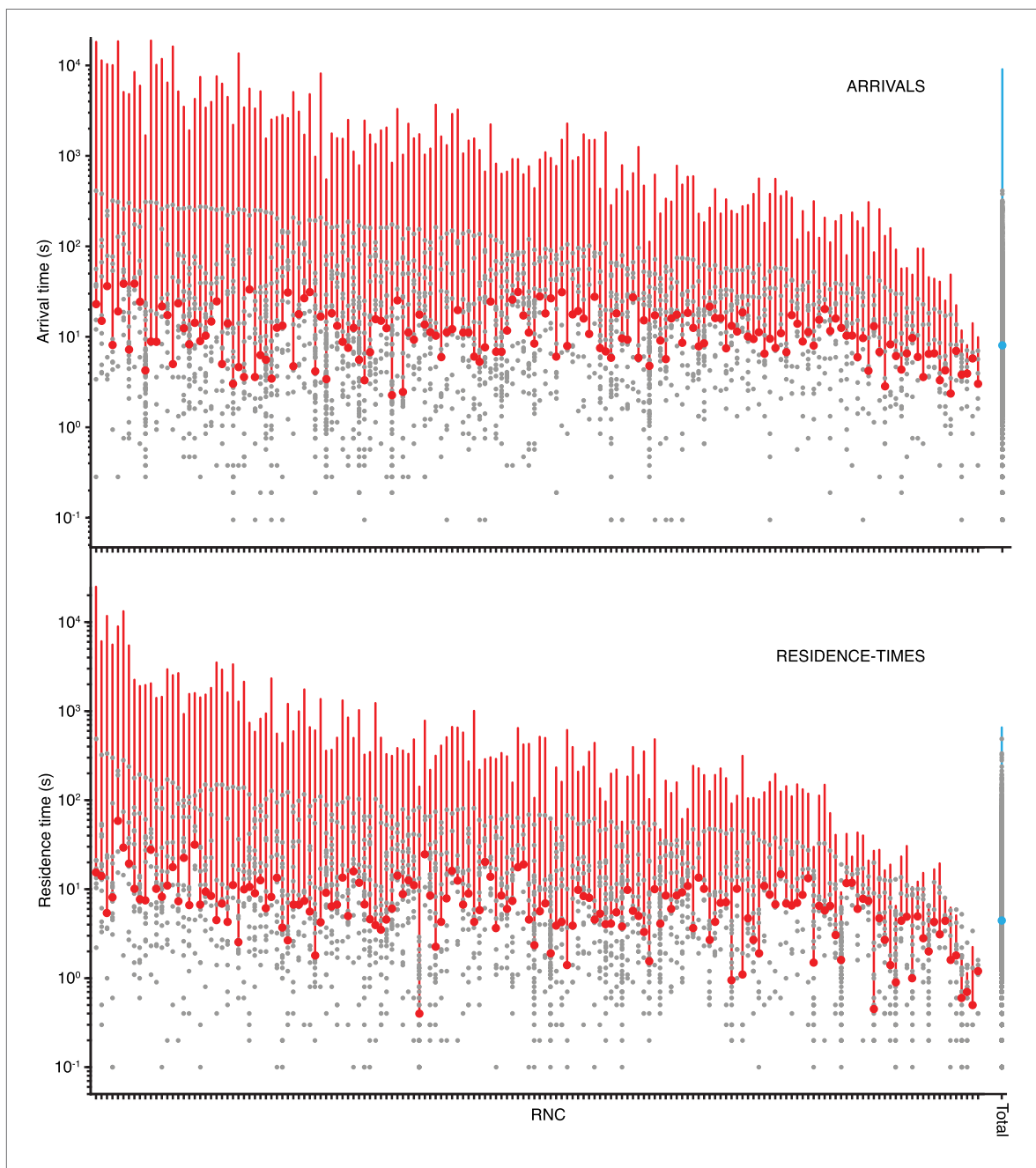


Figure 1—figure supplement 3. Wide variance in SRP arrival and residence times when RNCs are stalled in translation. Comparison of individual RNCs with more than five SRP binding events when 100 nM Cy5-labeled SRP was delivered to Cy3B-labeled RNCs stalled on a *lepB* mRNA (encoding the first 55 amino acids) and immobilized on ZMWs. Each column is an individual RNC with the grey dots indicating the arrival times (top panel, $n = 162$ RNCs) or residence times (bottom panel, $n = 162$ RNCs) of SRP-binding events. Red dots indicate median of binding events to each RNC, red line indicates variance of arrival or residence times. RNCs are arranged along the x-axis by decreasing variance. Data on the far-right labeled 'total' reflects all of the binding events analyzed together, with the blue dot and line indicating mean and variance, respectively. Note that the y-axis is in \log_{10} scale.

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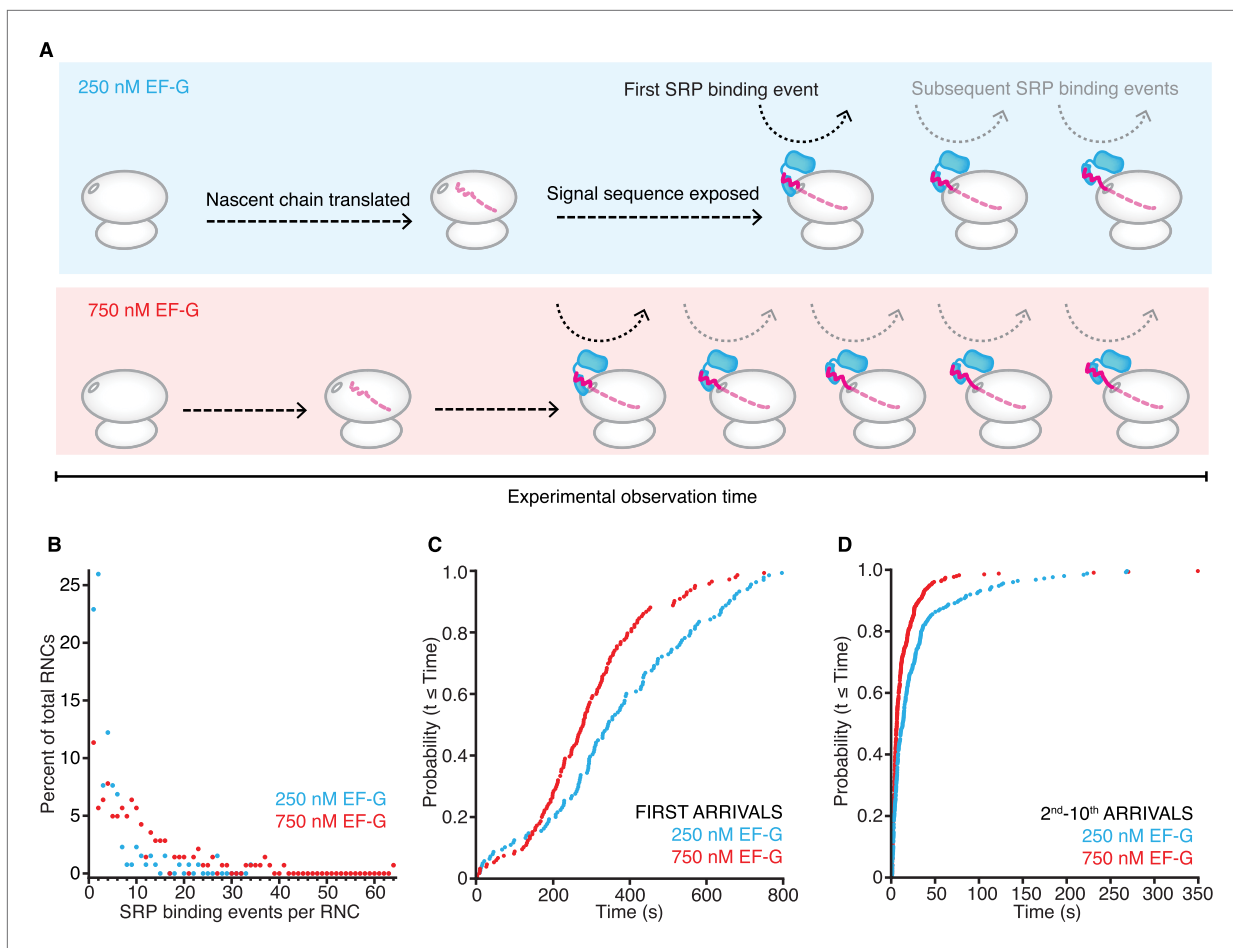


Figure 2. SRP-binding to RNCs translating at different rates. **(A)** Schematic representation of the effect on the number of SRP-RNC binding events and their first and subsequent arrival times when performing the experiment described in **Figure 1** at 250 nM (blue) and 750 nM (red) EF-G concentrations. **(B)** Comparison of SRP-binding events per RNC distributions for the experiment described in **A**. Colors as in **(A)** ($n \geq 621$ binding events) **(C–D)** Cumulative distributions of SRP first arrival times **(C)** and 2nd–10th arrival times **(D)** at 750 nM (red) and 250 nM (blue) ($n \geq 131$ binding events). DOI: [10.7554/eLife.04418.008](https://doi.org/10.7554/eLife.04418.008)

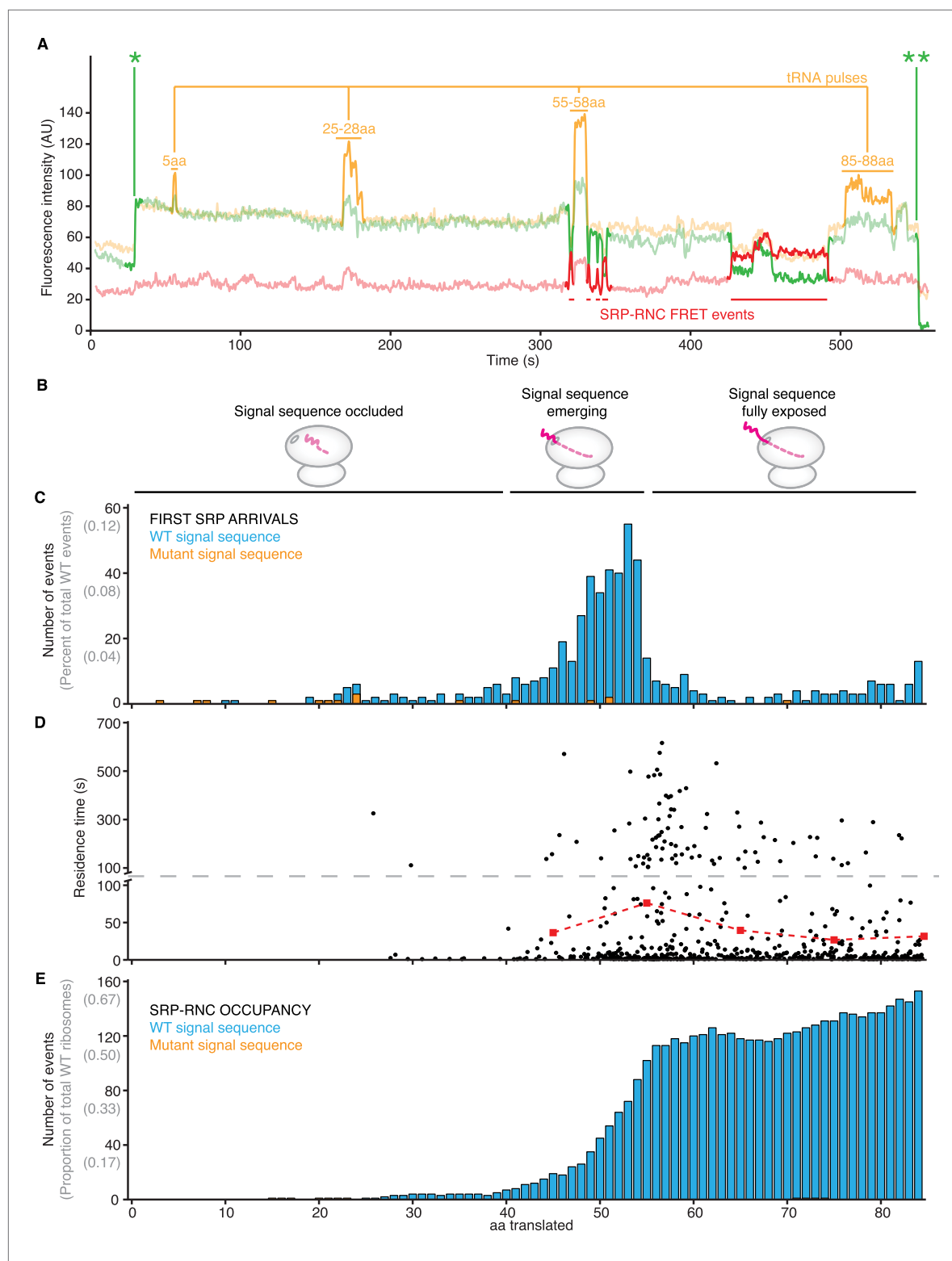


Figure 3. SRP binding to translation-calibrated RNCs. **(A)** Representative smFRET trace of Cy5-labeled SRP, Cy3B-labeled 50S subunits, Cy3.5-labeled F-tRNA and unlabeled translation mix delivered at time = 0 to PICs pre-assembled on a *lepB* cWT mRNA (encoding the first 95 amino acids) and immobilized on ZMWs (see text and **Figure 3—figure supplement 1**). Fluorescence intensity of the Cy3B (green), Cy3.5 (orange), and Cy5 (red) signal under 532 nm excitation are shown. 'AU' indicates 'arbitrary units'. * denotes the initial 50S ribosomal subunit joining. ** denotes photobleaching of the Cy3B dye on the 50S ribosomal subunit. **(B)** Schematic showing when, relative to the x-axis shared by panels **C–E**, the signal sequence is exposed from **Figure 3**. *Continued on next page*

Figure 3. Continued

RNCs. (C) Histogram showing how many amino acids have been polymerized when SRP first arrives to RNCs actively translating the lepB cWT mRNA (blue) or cMT mRNA (orange). Y-axis shows both total events, and percent of total for RNCs translating cWT mRNA. Note x-axis is shared by (C–E). (D) Scatter plot of SRP-RNC binding residence times relative to the number of amino acids polymerized when the event starts (black dots), and average lifetimes of the residence times between the tick-marks (red squares and dashed red line, with associated error bars that are too small to be seen). mRNA translated is lepB cWT. Note that for clarity the y-axis is split at 100 s, as indicated by the dashed grey line. Only traces in which four tRNA pulses were detected were included in the analysis in this panel and panel (E). (E) Histogram showing how many RNCs are occupied by SRP relative to the number of amino acids translated when RNCs are actively translating a lepB cWT mRNA (blue) or cMT mRNA (orange—with so few events that, at this y-axis scale, they are not visible). Y-axis shows both total events, and percent of total for RNCs translating cWT mRNA.

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WT sequence:	MANMF	ALILV	IATLV	TGILW	CVDKF	FFAPK	RRERQ	AAAQA	AAGDS	LDKAT	LKKVA	PKPGW
cWT sequence:	MANMF	ALILV	IATLV	TGILW	CVDKF	FFAPK	RRERQ	AAAQA	AAGDS	LDKAT	LKKVF	FFPGW
cMT sequence:	MANMF	AEIKV	IATPV	TGIRW	CVDKF	FFAPK	RRERQ	AAAQA	AAGDS	LDKAT	LKKVF	FFPGW
aa number:	5	10	15	20	25	30	35	40	45	50	55	60
WT sequence:	LETGA	SVFPV	LAIVL	IVRSF	IYEPF	QIPSG	SMMPT					
cWT sequence:	LETGA	SVLPV	LAIVL	IVRSL	IYEPF	FFPSG	SMMPT					
cMT sequence:	LETGA	SVLPV	LAIVL	IVRSL	IYEPF	FFPSG	SMMPT					
aa number:	65	70	75	80	85	90	95					

Figure 3—figure supplement 1. Translation-calibration lepB mRNA constructs. Amino acid sequences of lepB WT, cWT (for ‘calibrated WT’), or cMT (for ‘calibrated mutant signal sequence’) mRNA translation products. Blue box marks amino acids in N-terminal signal sequence. Orange boxes show expected F-tRNA pulses in calibration sequences. Red residues have been mutated relative to WT sequence.

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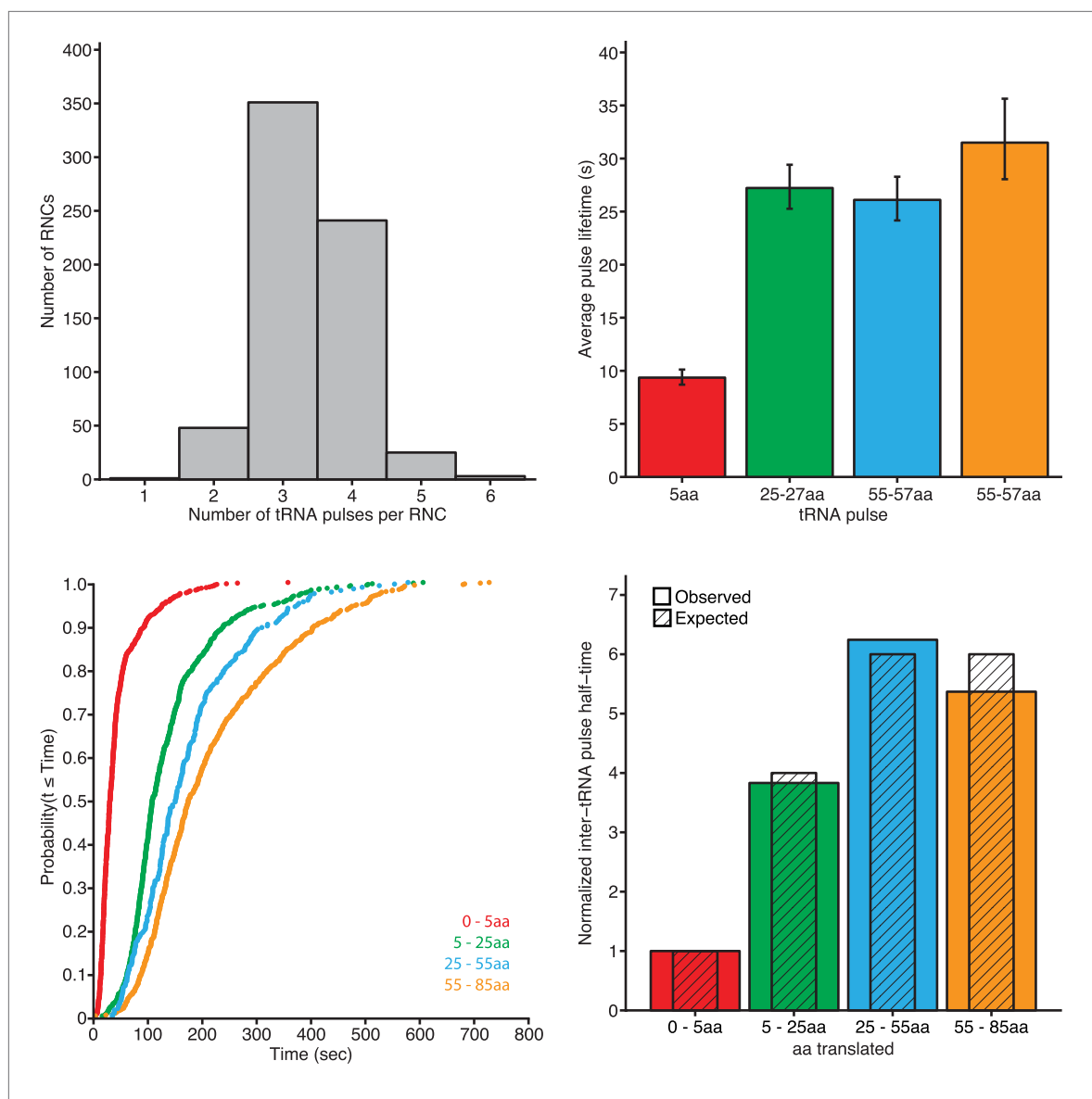


Figure 3—figure supplement 2. tRNA pulse characterization. (Upper left panel) Histogram of observed tRNA pulses per RNC with SRP-RNC binding events. (Upper right panel) Average tRNA pulse lifetimes, derived from single exponential fits to the pulse lifetimes. Error bars indicate 95% confidence of fits ($n \geq 269$). (Lower left panel) Cumulative distributions of tRNA inter-pulse times (as determined from the beginning of one to the beginning to the next). (Lower right panel) Half-times of tRNA inter-pulse times (as shown in lower-left panel, normalized to the shortest half-time (0–5 amino acids). Colored bars indicate observed values and hatched, grey bars indicate expected values based on relative number of amino acids translated.

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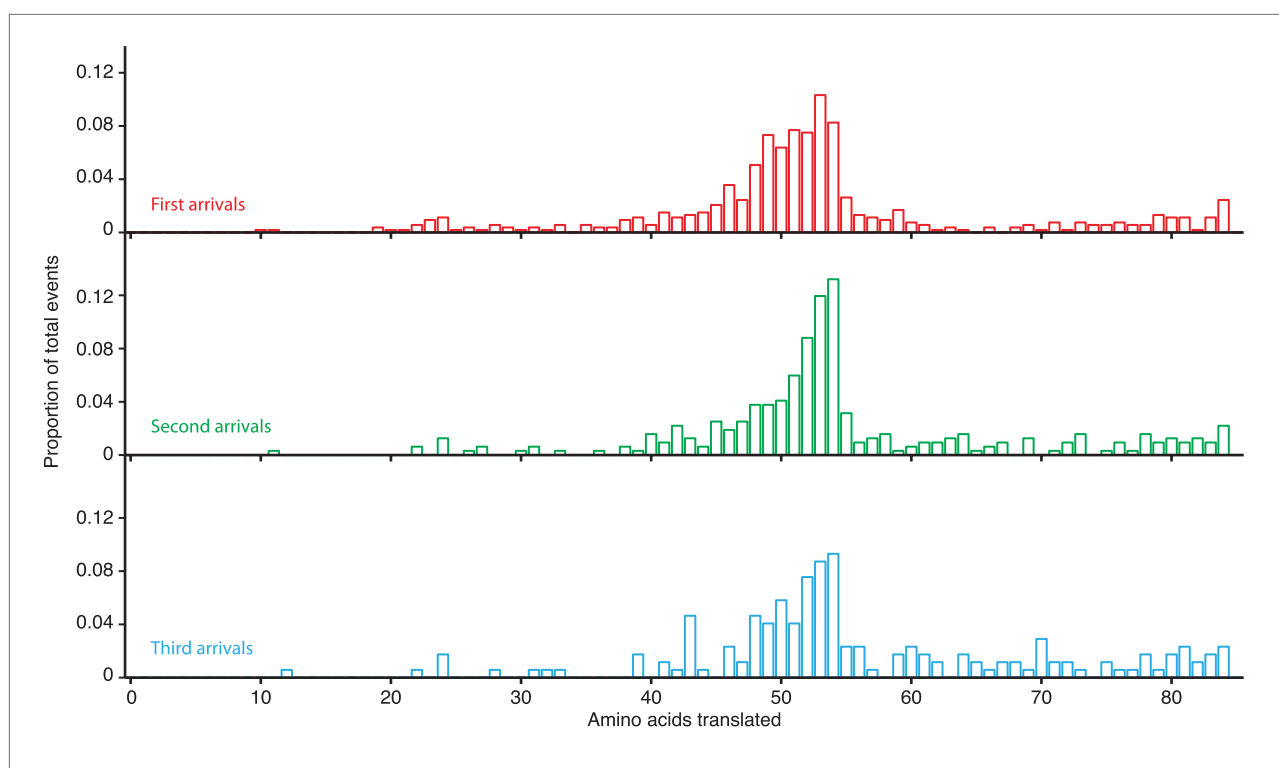


Figure 3—figure supplement 3. Translation-calibrated SRP arrivals to RNCs. Histograms showing how many amino acids have been translated when SRP arrives for the first (top panel), second (middle panel), or third (bottom panel) time to RNCs actively translating a *lepB* cWT mRNA.

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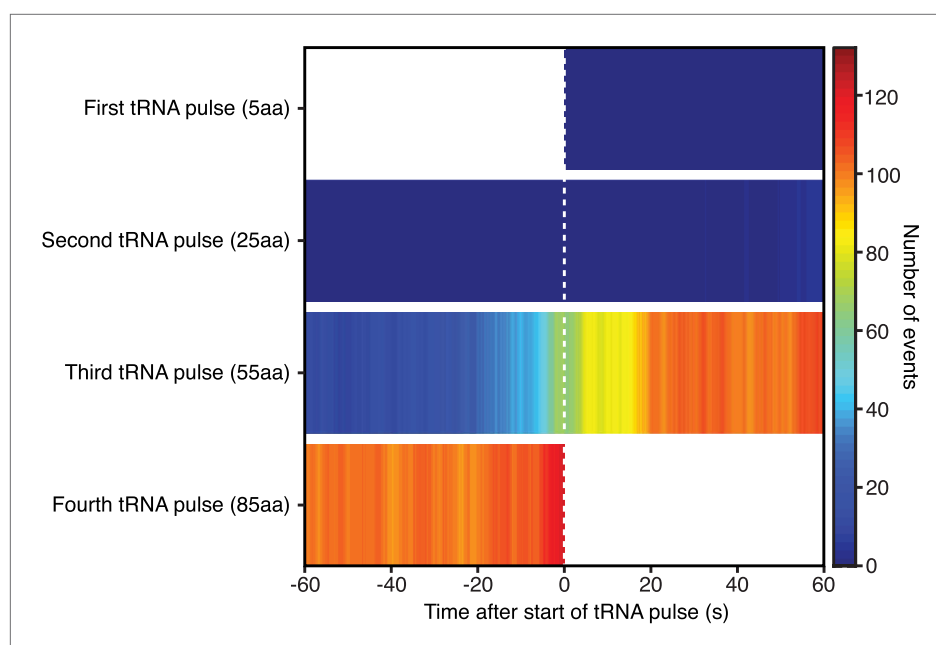


Figure 3—figure supplement 4. SRP binding before and after each tRNA pulse. Heat plot showing the number of SRP-RNC binding events 60 s before or after the start of each tRNA pulse.

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