

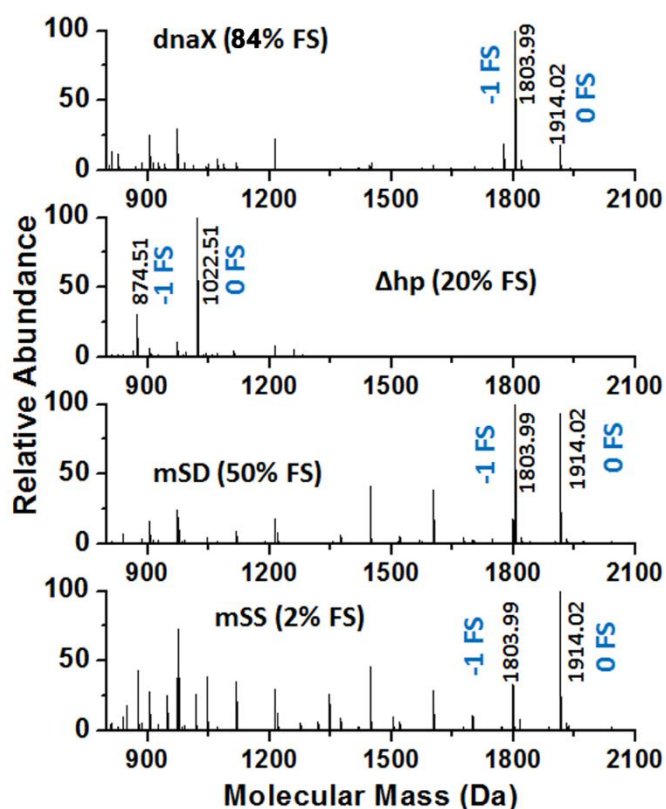
## Supplemental Information

### EF-G catalyzed translocation dynamics in the presence of ribosomal frameshifting stimulatory signals

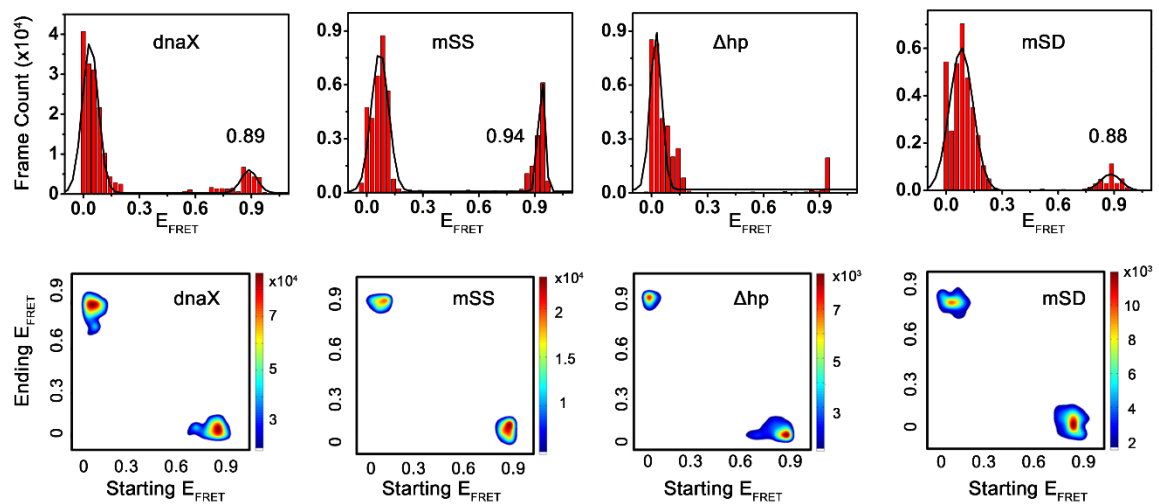
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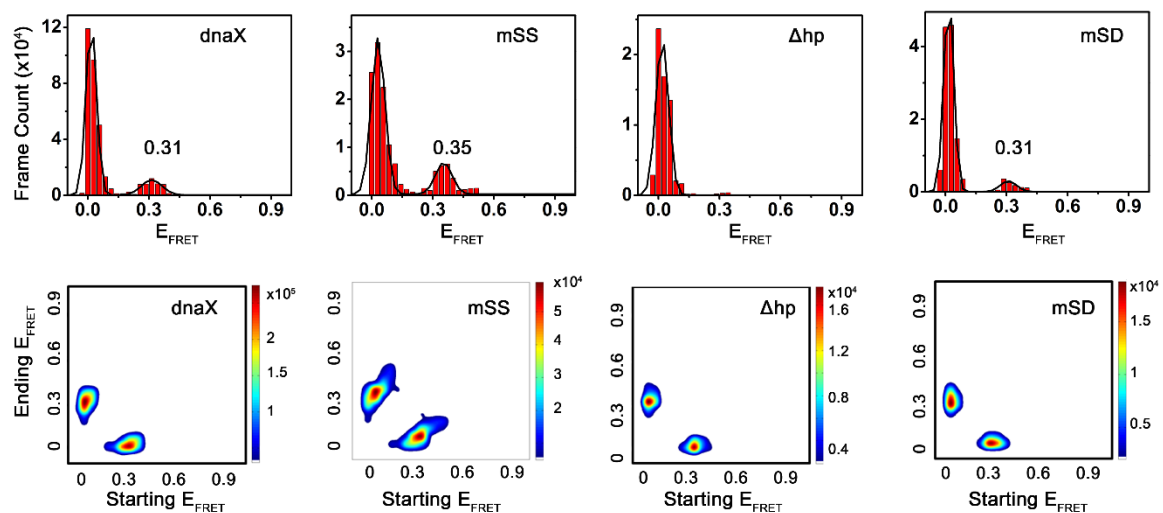
#### Supplemental Figure



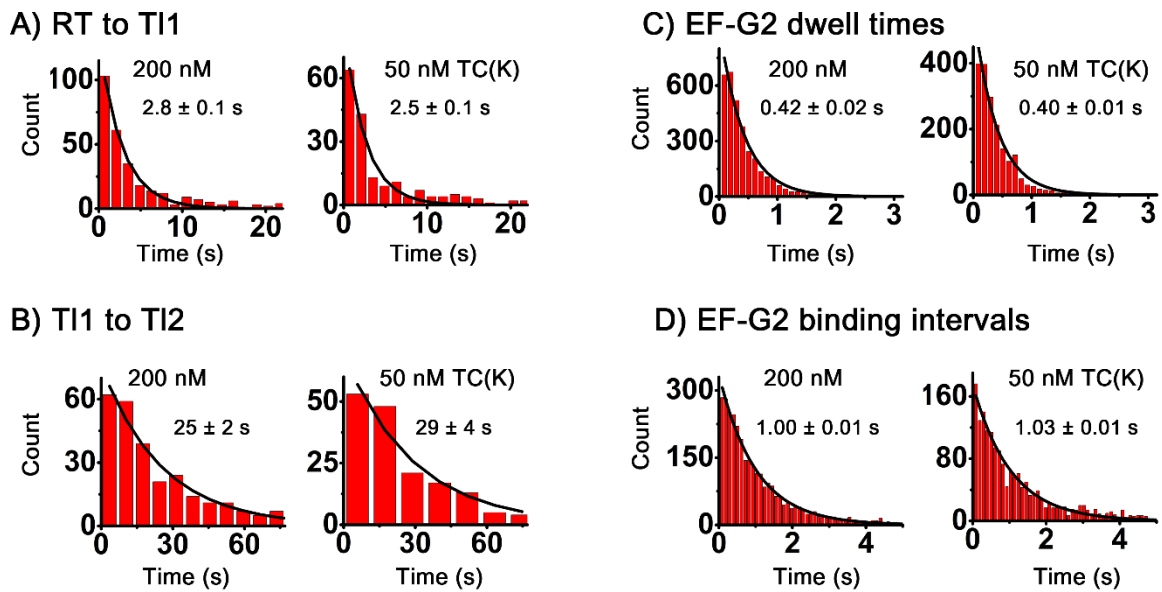
**Figure S1.** LC–mass spectrometry analysis of *in vitro* translation products from *E. coli* dnaX and mutated mRNA constructs. *In vitro* translations were carried out using a reconstituted translation system (New England Biolabs) for 2 h at 37 °C, followed by incubation with 200 μM puromycin to release uncompleted products. The polypeptide products were purified following the provided protocols. For the dnaX, mSD, and mSS constructs, the expected molecular masses of full-length products are 1914.02 Da and 1803.99 Da for in-frame (0 FS) and –1 frame (–1 FS), respectively. Those of the Δhp mRNA are 1022.51 Da for 0 FS and 874.51 Da for -1 FS. Frameshifting efficiencies, -1 FS/(-1 FS + 0 FS), calculated from the full-length polypeptide products only are 84%, 20%, 50%, 2% for the dnaX, Δhp, mSD, and mSS, respectively. Most of the unassigned peaks in the spectra correspond to uncompleted polypeptide products of the in-frame translation. Frameshifting efficiencies decrease if all the uncompleted products are considered into the 0 FS (eg. mSD: 31%, mSS: 0.7%).



**Figure S2.** FRET efficiency ( $E_{\text{FRET}}$ ) histograms per frame (upper) and transition density plots (bottom) of each mRNA construct during the first round of translocation (TI1) process (block 1). For all the four mRNA constructs, there were two major states, no FRET and  $\sim 0.9 E_{\text{FRET}}$ . They correspond to the pre-translocation ribosomal complexes (PRE-VK\*) with (Cy3)tRNA<sup>Lys</sup> at the A-site but no (Cy5)EF-G and those with both (Cy3)tRNA<sup>Lys</sup> and (Cy5)EF-G bound, respectively. Transition density plots show (Cy5)EF-G binding ( $E_{\text{FRET}}$  transition from 0 to  $\sim 0.9$ ) and dissociation ( $E_{\text{FRET}}$  transition from  $\sim 0.9$  to 0) events.

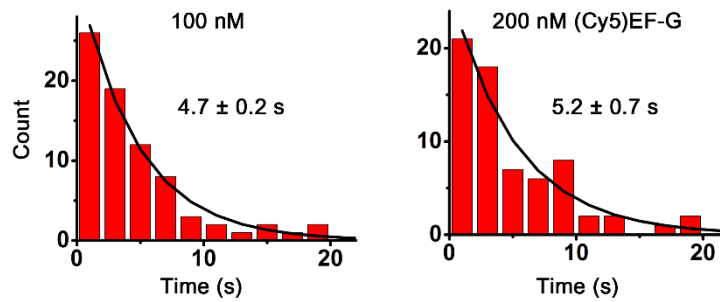


**Figure S3.** FRET efficiency ( $E_{\text{FRET}}$ ) histograms per frame (upper) and transition density plots (bottom) of each mRNA construct during after TI1 until TI2 (block 2). For all the four mRNA constructs, there were two major states, no FRET and  $\sim 0.3 E_{\text{FRET}}$ . They correspond to the pre-translocation ribosomal complexes (PRE-K\*K) with (Cy3)tRNA<sup>Lys</sup> at the P-site but no (Cy5)EF-G and those with both (Cy3)tRNA<sup>Lys</sup> and (Cy5)EF-G bound, respectively. Transition density plots show (Cy5)EF-G binding ( $E_{\text{FRET}}$  transition from 0 to  $\sim 0.3$ ) and dissociation ( $E_{\text{FRET}}$  transition from  $\sim 0.3$  to 0) events.

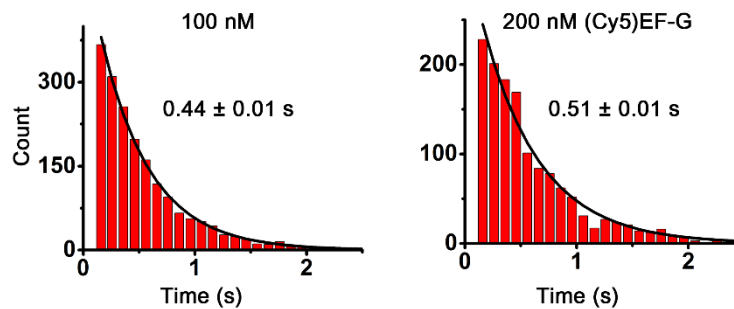


**Figure S4.** TC(K) concentration dependent reaction times of dnaX. A) reaction time from real-time (RT) delivery of (Cy5)EF-G and TC(K) to the first round of translocation event (TI1), B) from the TI1 to TI2, second round of translocation event, C) EF-G2 dwell times, and D) EF-G2 binding intervals. (Cy5)EF-G concentration was maintained to be 100 nM, while two TC(K) concentrations of 200 nM and 50 nM were examined. Mean reaction times were obtained from fitting the data to single exponential decay curves and the errors are propagated standard errors.

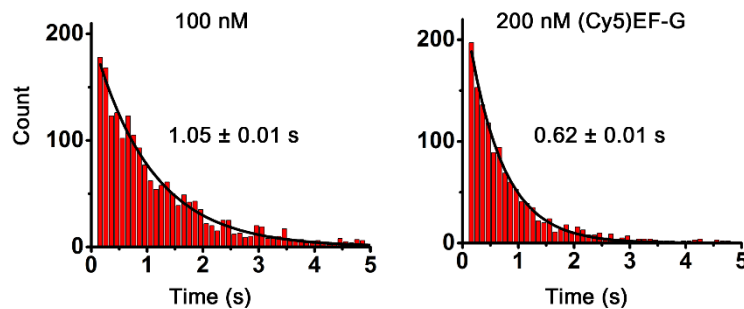
### A) T11 to 1st EF-G2



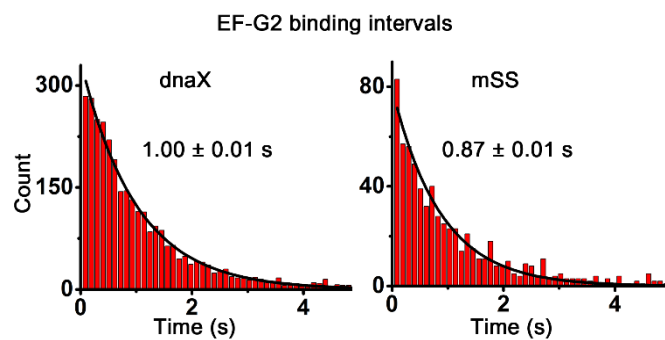
### B) EF-G2 dwells



### C) EF-G2 intervals



**Figure S5.** (Cy5)EF-G concentration dependent reaction times of dnaX. A) T11 to the first (Cy5)EF-G2 binding event, B) dwell time histograms of (Cy5)EF-G2, and C) (Cy5)EF-G2 intervals. A constant concentration of 250 nM TC(K) was used in all the experiments. Time resolution of 100 ms/frame were used with decreased excitation power (4 ms). Mean reaction times were obtained by fitting the histograms to single exponential decay curves and the errors are propagated standard errors. Upon increasing the concentration of (Cy5)EF-G from 100 nM to 200 nM, reaction time from T11 to T12 decreased with more often binding of EF-G (shorter EF-G2 intervals), while no changes were observed on the time taken from T11 to the first binding of EF-G2 and dwell times of EF-G2.



**Figure S6.** EF-G2 binding intervals between the different mRNA constructs. Mean reaction times were obtained by fitting the histograms to single exponential decay curves and the errors are propagated standard errors. EF-G2 binding intervals for mSD and  $\Delta hp$  were not obtained, as they translocated mostly via a single EF-G2 binding.

**Table S1.** Number of traces used in the data analysis.

mRNA	$n_{\text{FOV}}^*$	TI1			TI2		
		$n(\text{TI1})^\dagger$	$n(\text{EF-G1=0})^\ddagger$	$n(\text{EF-G1}\geq 1)^\S$	$n(\text{TI2})$	$n(\text{EF-G2=0})$	$n(\text{EF-G2}\geq 1)$
dnaX	62 ± 21	356	19 (5%)	337 (95%)	318	122 (38%)	196 (62%)
mSS	65 ± 10	144	6 (4%)	138 (96%)	140	59 (42%)	81 (58%)
$\Delta\text{hp}$	70 ± 17	161	21 (13%)	140 (87%)	157	114 (73%)	43 (27%)
mSD	36 ± 15	148	12 (8%)	136 (92%)	145	96 (66%)	49 (34%)

\* $n_{\text{FOV}}$  is the number of selected traces per field of view as good active single molecules. Ribosomal complexes with the mSD construct showed less number of active molecules all the time.

$^\dagger n(\text{TI})$  is the number of each round of translocation events.

$^\ddagger n(\text{EF-G=0})$  is the number of translocation events with no detected EF-G binding event.

$^\S n(\text{EF-G1}\geq 1)$  is the number of translocation events with one or more number of EF-G1 binding events. In the first round of translocation (TI1) process, evolved FRET upon binding of EF-G (EF-G1 binding) was high (~0.8) and was thus easily captured by idealization process. On the other hand, EF-G2 binding probed by the low FRET (~0.3) was difficult to be identified by the idealization program and often missed. Especially, TI2 of the  $\Delta\text{hp}$  and mSD constructs were idealized to be via no EF-G2 binding, because they often translocate via a single EF-G binding event. Therefore, the results on number of EF-G2 binding events for the  $\Delta\text{hp}$  and mSD are biased to the more number of EF-G2 binding events.