

## Mechanisms of SecM-Mediated Stalling in the Ribosome

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## Supplementary Materials

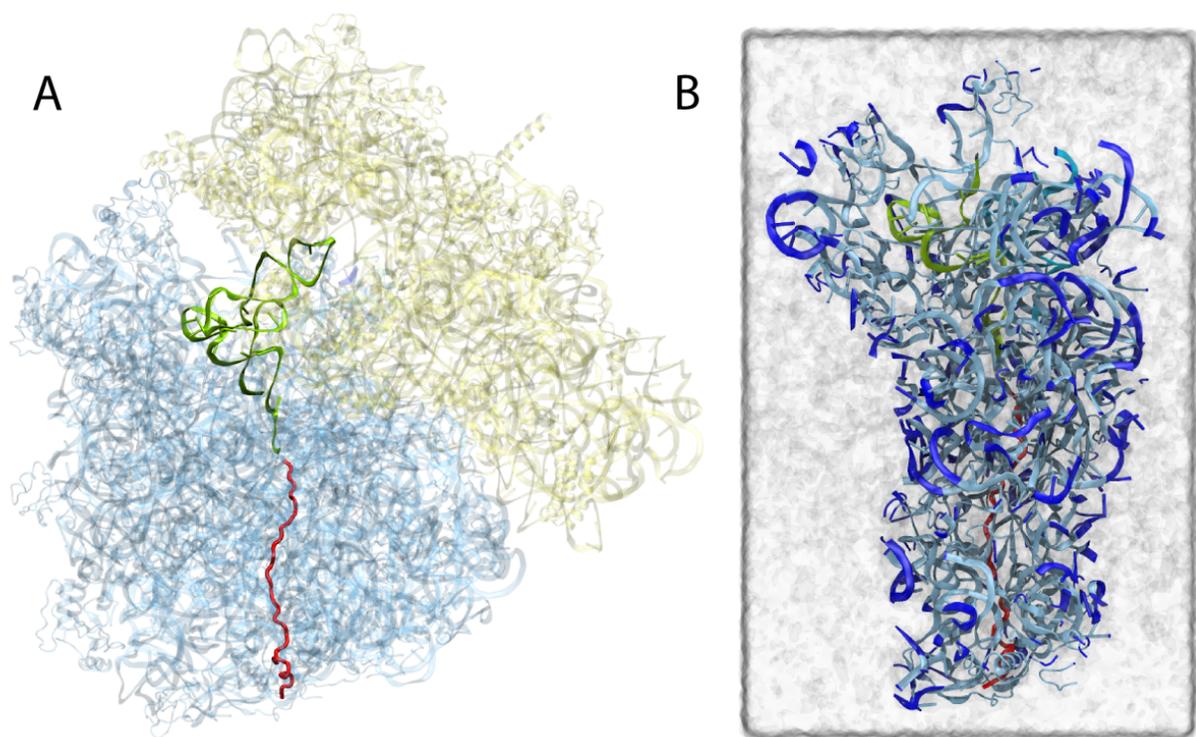


Figure S1: Exit tunnel sub-system. (A) Full system consisting of the large (blue) and small (yellow) ribosomal subunits, the P-tRNA (green) and SecM (red). (B) Sub-system used. The ribosomal residues lining the exit tunnel are shown in light blue with those portions at the edges that were restrained in dark blue. The surrounding water box is colored light gray.

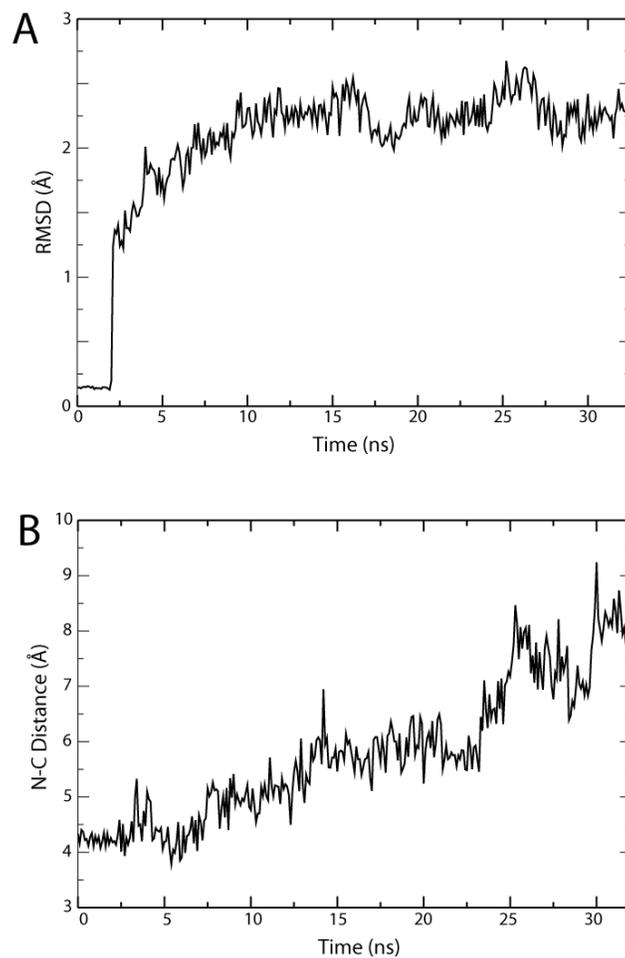


Figure S2: Metrics used to judge convergence of multi-stage fitting for the reduced subsystem (see Methods). (A) Root mean-square deviation (RMSD) for the backbone of SecM. (B) Distance between the carbonyl carbon of G165 at the P-tRNA and the nitrogen of P166 at the A-tRNA.

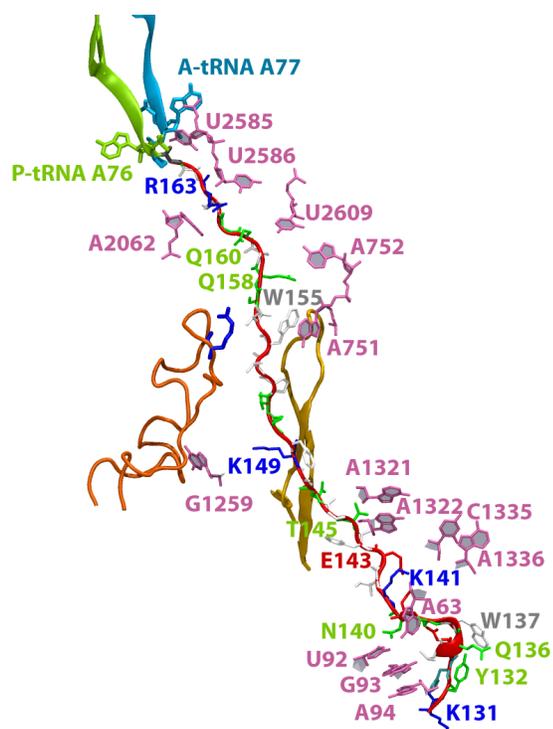


Figure S3: Zoomed-out version of Fig. 1 in the main text illustrating the full length of SecM in the exit tunnel.

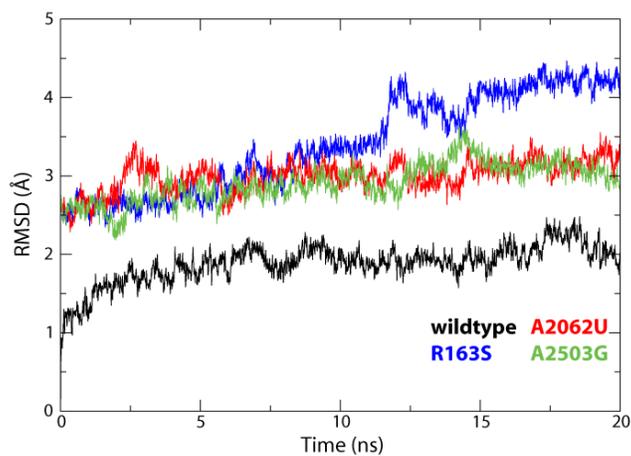


Figure S4: RMSD of SecM in the wildtype (black) and mutant (red, green, blue) simulations.

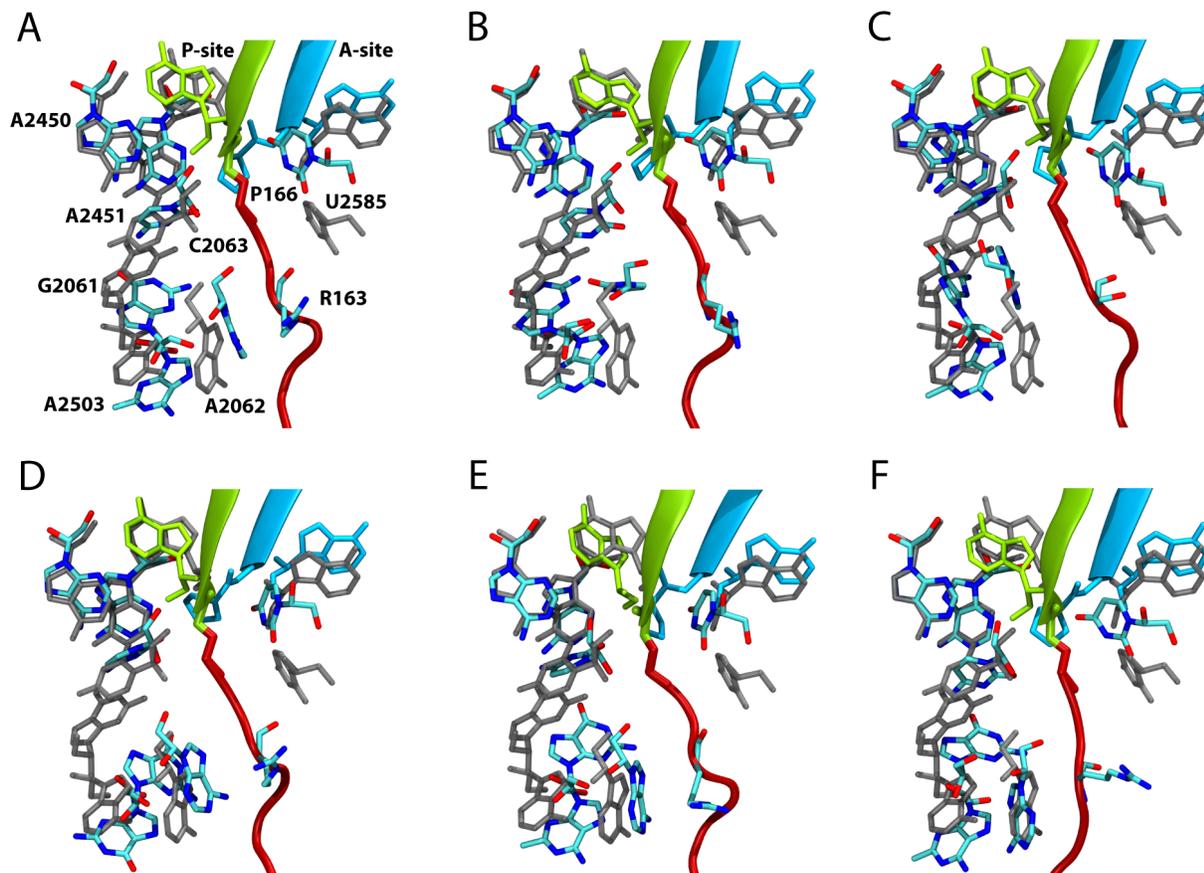


Figure S5: Disruption to the SecM-PTC relay. The key residues involved in relaying SecM's presence to the PTC are shown as in Fig. 2C, with the residues at the end of each simulation shown colored by atom type and those in 3WDK/3WDL in grey. (A) Wild-type complex after 20 ns of equilibration. (B) A2062U mutant after 20 ns. (C) R163S mutant. (D) A2503G mutant. (E) W158A mutant. (F) Relay after the application of a constant force of 1.25 nN to SecM's N-terminus.

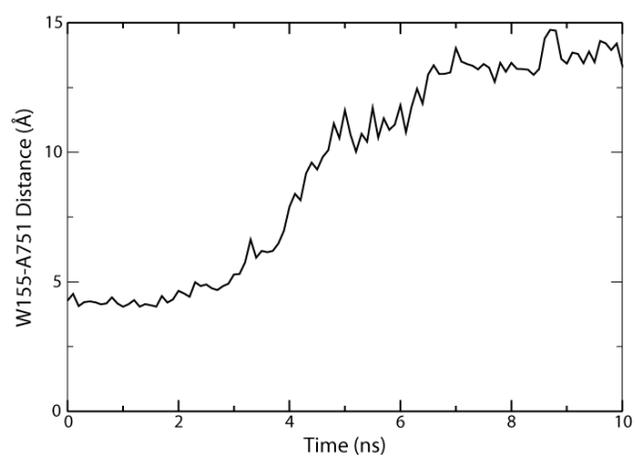


Figure S6: Distance between the centers of residues W155 of SecM and A751 of 23S during constant-force steered MD.

Res. 1	Res. 2	w/SecM	R163S	R163Q	R163K	A2062U	A2503G
SecM R162	23S A2062	0.00	0.00	0.80	0.00	0.00	0.00
SecM R163	23S A2062	0.92	0.00	0.00	0.00	0.87	0.06
SecM R163	23S U2441	0.00	0.00	0.21	0.92	0.58	0.33
SecM R163	23S U2586	0.48	0.98	0.52	0.66	0.50	0.16
SecM R163	23S A2587	0.00	0.00	0.00	0.68	0.62	0.00
23S G2061	23S C2063	0.98	0.57	0.94	0.52	0.48	0.58
23S G2061	23S A2503	1.00	0.97	0.82	0.37	1.00	0.10
23S A2062	23S A2503	0.91	0.54	0.88	0.37	0.68	1.00
23S A2062	23S G2061	1.00	1.00	1.00	0.65	0.00	1.00
23S C2063	23S A2450	0.25	0.36	0.45	0.86	0.57	0.70
23S C2063	23S A2451	0.94	0.91	0.89	0.92	0.92	0.00
23S C2063	23S C2501	1.00	0.80	1.00	1.00	1.00	0.79
23S C2501	23S A2450	1.00	1.00	1.00	1.00	1.00	1.00
23S G2583	23S G2553	1.00	0.86	0.90	0.94	1.00	0.97
23S A2450	P-tRNA A76	0.99	1.00	1.00	1.00	0.95	0.99
23S A2451	P-tRNA A76	0.91	0.96	0.67	0.20	0.60	0.21
23S G2553	A-tRNA A77	1.00	1.00	1.00	1.00	1.00	0.97
23S A2451	SecM P166	1.00	0.84	0.95	0.95	0.99	0.91
23S U2585	SecM P166	0.07	0.94	0.73	0.33	0.95	0.93

Table S1: Interactions relaying SecM-mediated stalling of the ribosome, defined as in Table 1 in the main text. The interaction frequencies highlighted in blue are especially distinct from those in the first wild-type simulation; those in red exhibit a decrease of more than 25% compared to the second wild-type simulation.