

**Supplementary Table 1: Cryo-EM data collection and model refinement (for all new structures)**

Incubation	0 minutes	30 minutes		50 minutes	
Subunit	50S	50S	70S	50S	70S
<b>Data Collection</b>					
Microscope	Titan Krios				
Camera	Gatan K2	Falcon III	Gatan K2	Falcon III	Gatan K2
Voltage (kV)	300				
Magnification	29000	127000	29000	127000	29000
Pixel size (Å.px <sup>-1</sup> )	0.858	1.1	0.858	1.1	0.858
Defocus range (µM)	(-0.7)-(-1.3)	-0.5 -(-1.50)	(-0.7)-(-1.3)	-0.5 -(-1.50)	(-0.7)-(-1.3)
Total dose (e/Å <sup>2</sup> )	40				
Micrographs collected	2074	1671	2532	2079	3951
<b>Refinement</b>					
Number of particles (autopicked)	507852	291403	494694	457948	456794
Number of particles (used for 3D reconstruction)	211046	183484	144195	145354	164877
Resolution (Å; at FSC <sup>a</sup> = 0.143)	2.65	2.48	3.11	2.73	2.86
CC (model to map fit)	0.691	0.8795	0.62	0.4175	0.84
<b>Model Quality</b>					
Bonds (°)	0.01	0.01	0.007	0.007	0.008

Angles (°)	1.08	0.803	0.926	1.041	1.046
Chirality (°)	0.055	0.05	0.081	0.079	0.066
Planarity (°)	0.007	0.005	0.009	0.009	0.008
<b>Validation</b>					
Clashscore	4.62	5.35	26.47	13.47	26.08
<b>Proteins</b>					
MolProbity score	1.55	1.80	3.55	2.22	3.66
Rotamer outliers (%)	0.64	0.20	15.47	0.39	24.26
Ramachandran favored (%)	86.15	92.11	76.08	88.7	82.33
Ramachandran allowed (%)	13.32	7.69	22.05	10.91	14.84
Ramachandran outliers (%)	0.53	0.2	1.87	0.08	2.83
<b>RNA</b>					
Correct sugar pucker (%)	99.8	99.66	98.54	98.31	98.83
Correct backbone conformation	84.35	81.95	51.97	65.23	51.38