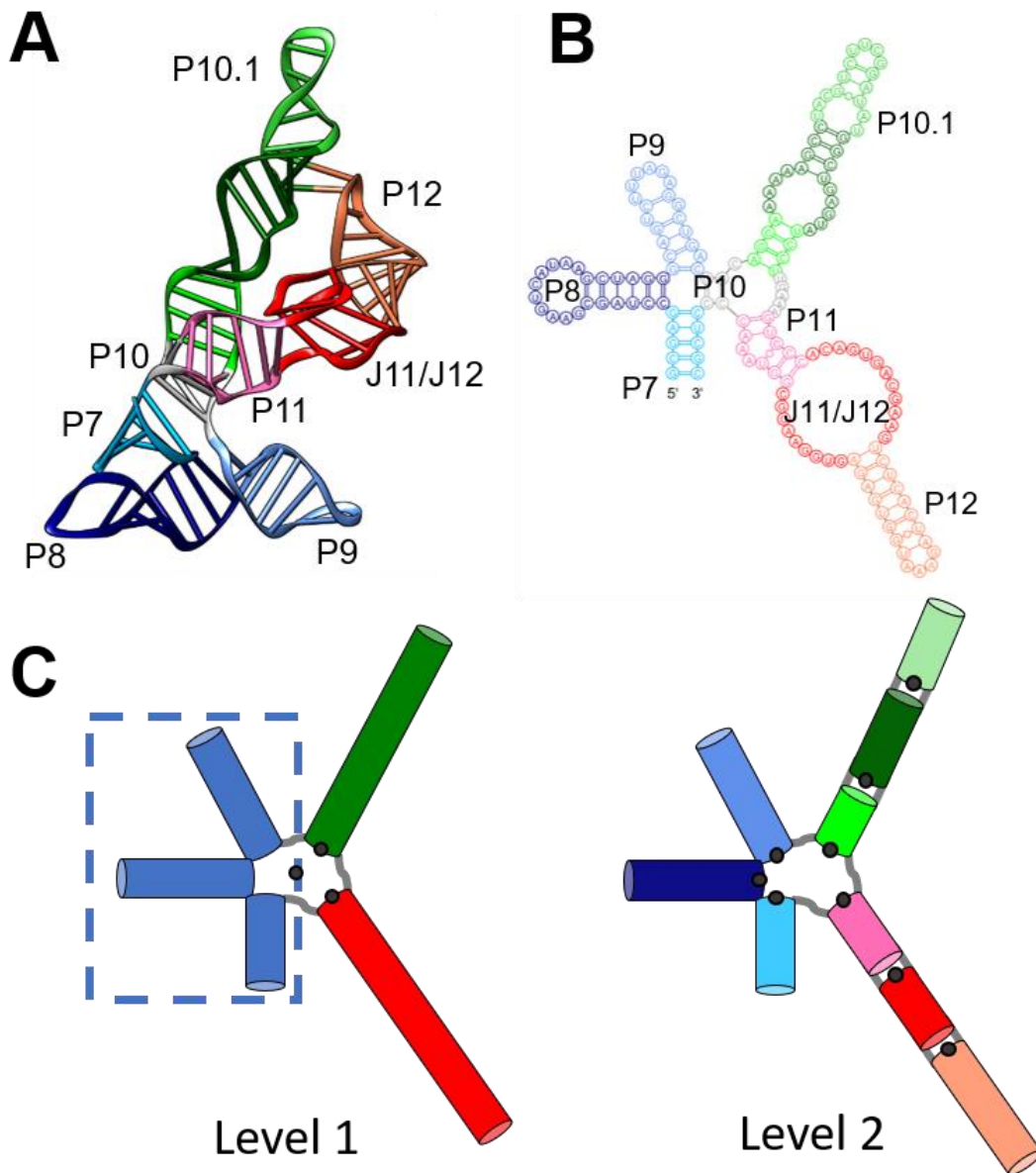**Supplementary Figure 1. Temperature-modulated HNMMC refinement of the tRNA.** The temperature, T (red), the total energy (green), and the C4' RMSD (blue) to the target structure are shown as a fluctuation against the refinement steps. The dashed line indicates the step when the structure with the lowest total energy is achieved.

Supplementary Figure 2. The DOF of the ribonuclease P S-domain (A) The crystal structure of the ribonuclease P S-domain. (B) The secondary structure of the S-domain. (C) The levels of DOF defined based on the secondary structure. Level 1 separates the S-domain into three helical arms. Helices P7, P8, and P9 are combined into one group (the blue rectangle). Level 2 further separates Level 1 into more regions for a finer refinement. In Level 2, Helices P7, P8, and P9 are independent. Helices P11, P12 and Loop J11/J12 (the red arm in level 1) are divided into three regions. The long helix P10.1 (the green arm in Level 1) is also divided into three regions.
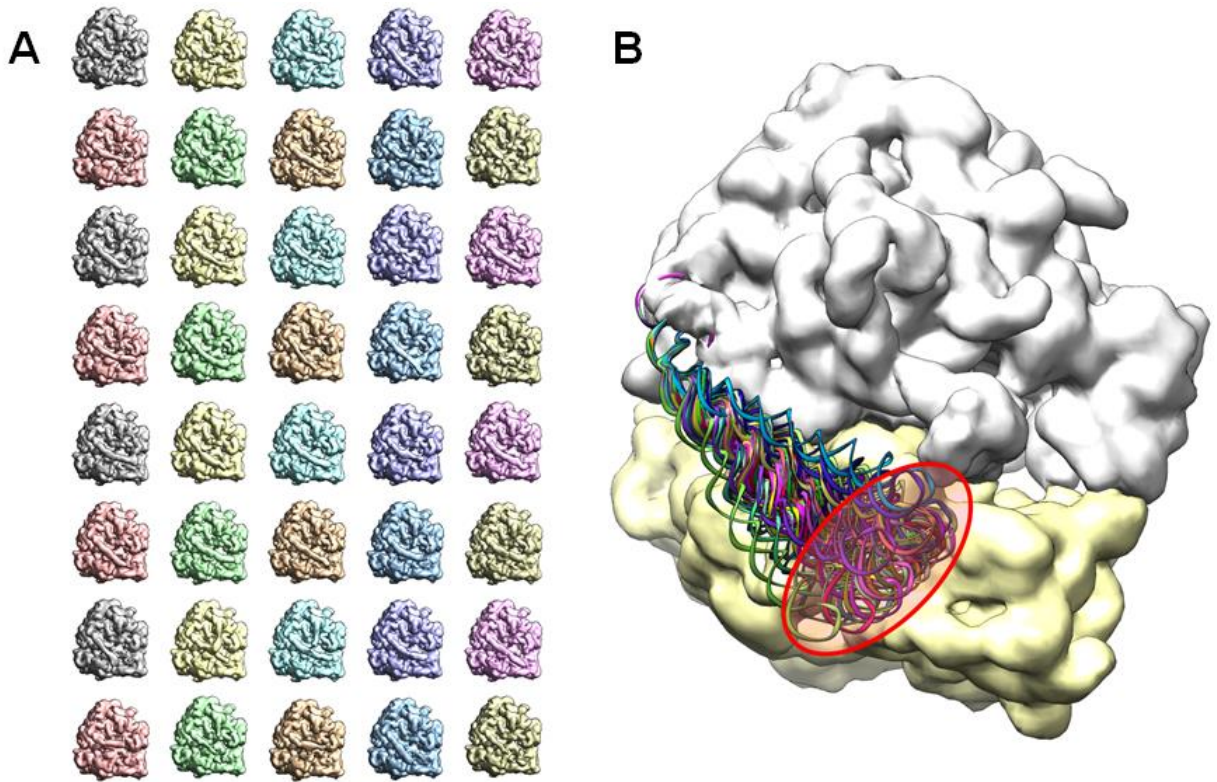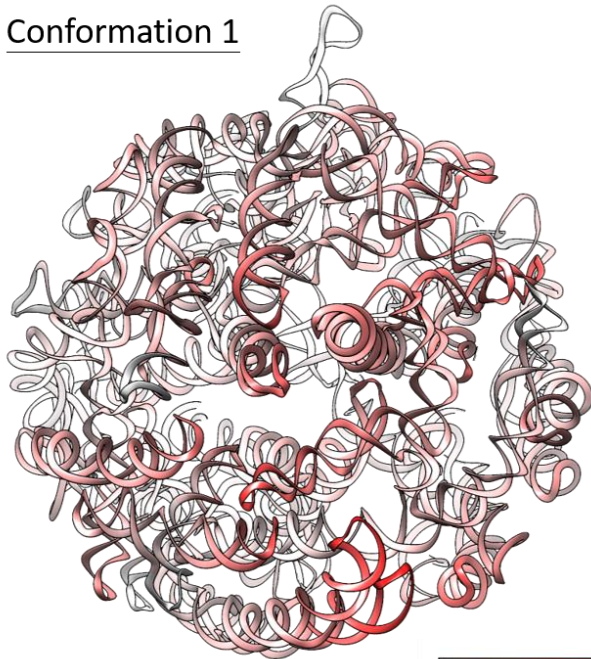
Supplementary Figure 3. Dynamics of the handle in the *Mtb* 70S ribosome depicted by HNMMC. (A) 40 cryo-EM maps of the *Mtb* 70S ribosome with different conformations of the handle. (B) 40 models of the handle are refined by HNMMC into the classified density maps. The densities of the 50S and 30S subunits are colored white and yellow, respectively. The red oval outlines the range of the motion for the tip of the handle which is around 60 Å in the short axis and 70 Å in the long axis.
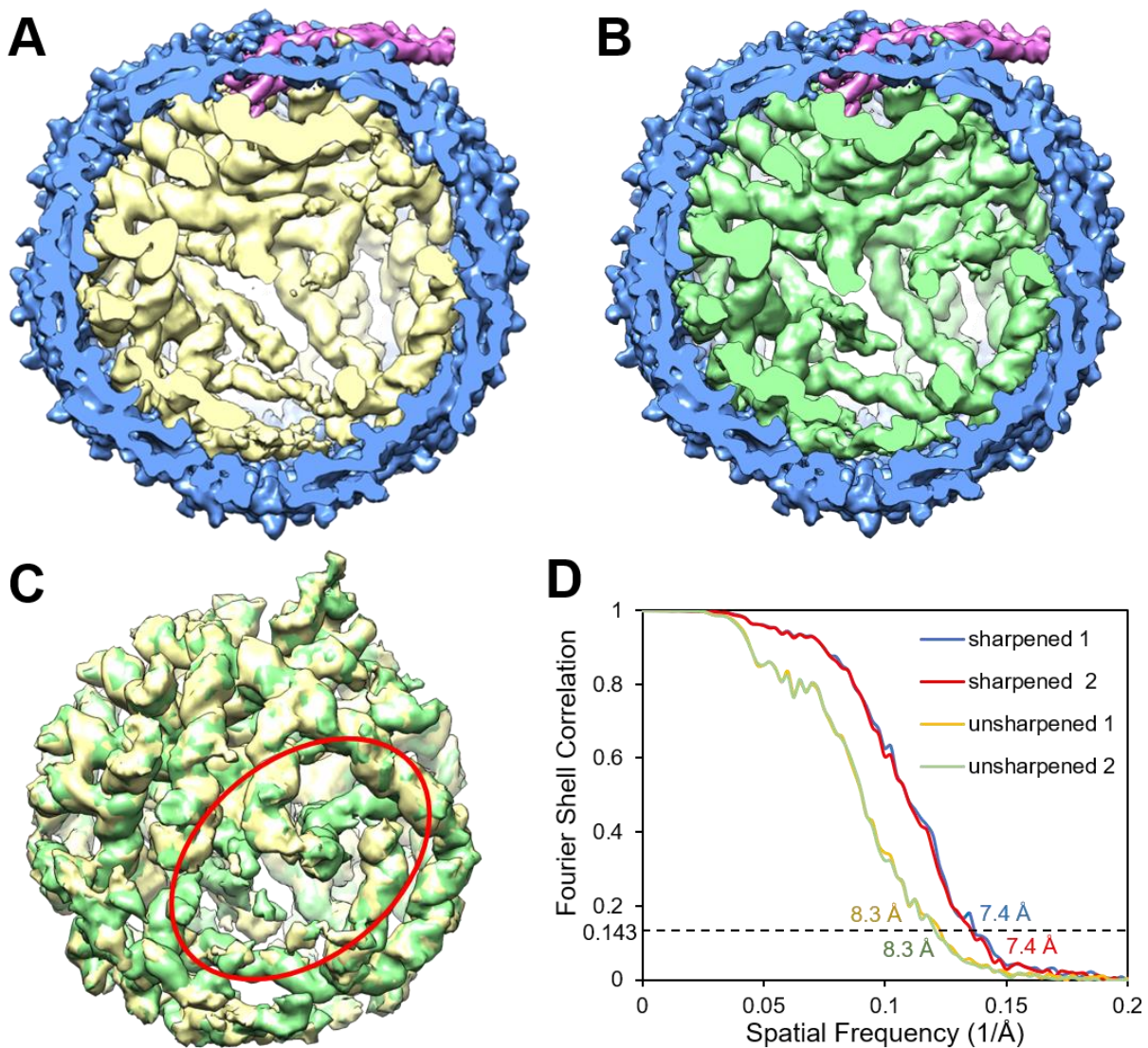
Supplementary Figure 4. Two cryo-EM maps of MS2 with different gRNA conformations.
(A) Cut-in view of the cryo-EM map showing gRNA Conformation 1. (B) Cut-in view of the cryo-EM map showing gRNA Conformation 2. (C) The comparison of two gRNA conformations. The region with the largest variation is marked by the red oval. (D) The Fourier Shell Correlation curves of the sharpened and unsharpened maps with two gRNA conformations. The curves of the sharpened maps are shown in blue and red, and the curves of the unsharpened maps are shown in yellow and green for Conformations 1 and 2, respectively. The gRNA densities (yellow and green for Conformations 1 and 2, respectively) in Panel A-C are from the unsharpened map for more completeness.
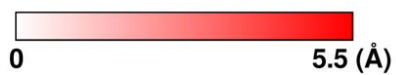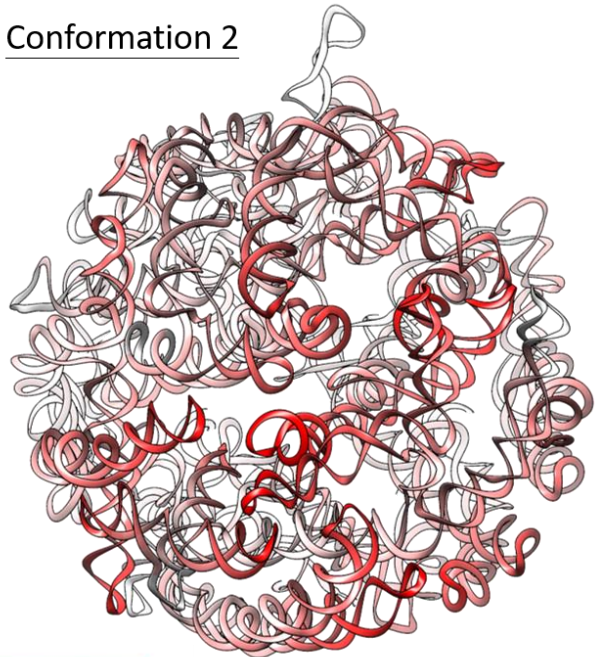
Supplementary Figure 5. Dynamics of MS2 gRNA at each nucleotide in the two conformations. For each conformation, the average C4' RMSD of each nucleotide is calculated from 20 independently HNMMC refined models and labeled by colors from white (small RMSD) to red (large RMSD).
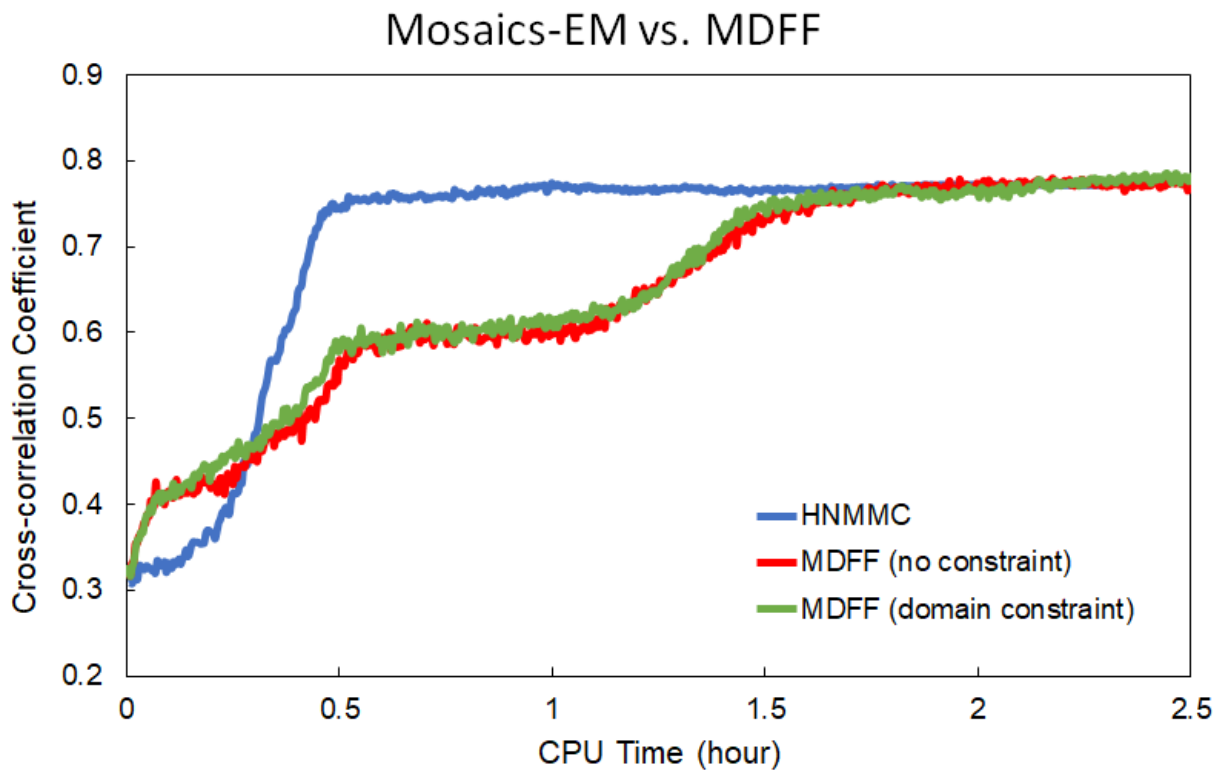
Supplementary Figure 6. The comparison between the model refinements in HNMMC and MDFF for the handle of the *Mtb* ribosome. The domain restraint in the MDFF refinement was applied based on the secondary structural information used in HNMMC. The CPU time is calculated by the time usage on a single core.

Supplementary Table 1. The C4' RMSD of the tRNA models refined by different EM weights against simulated density maps at different resolutions. The red color indicates the refined results with the lowest RMSD.

		EM weight (ω)										
		0.001	0.01	0.1	1	10	50	100	200	500	1000	∞
Map resolution (Å)	5	2.69	6.00	4.30	3.30	2.09	1.90	1.92	1.37	2.03	1.92	3.24
	10	4.55	4.72	4.94	2.87	1.77	1.87	1.65	1.80	1.97	1.99	2.21
	15	6.16	7.03	4.56	3.22	2.09	2.39	2.25	1.90	2.09	2.55	2.46
	20	4.43	4.13	4.12	3.13	2.61	3.34	2.24	2.52	2.98	2.81	2.74
	30	3.44	4.17	4.59	2.91	2.97	3.43	3.74	2.54	2.48	3.61	2.56

Supplementary Table 2. 18 representative models of the S-domain. The models are arranged through their cross-correlation score (from large to small) to the target map (transparent grey). The three helical arms are colored as Level 1 in Supplementary Figure 2.
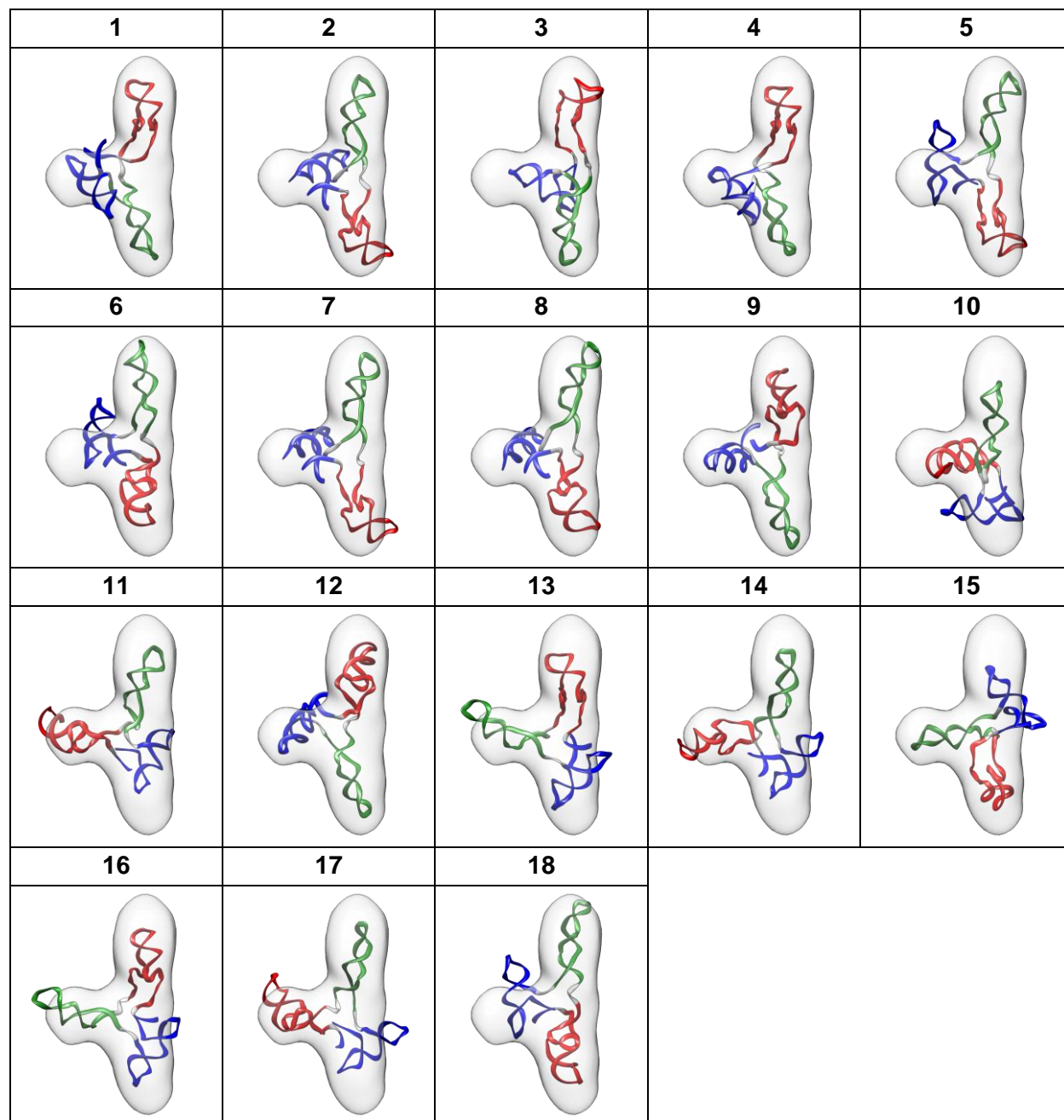
Supplementary Table 3. The refinements of the handle in 40 classified cryo-EM density maps of the *Mtb* 70S ribosome.

Map number	Cross-Correlation Coefficient (%)		Map number	Cross-Correlation Coefficient (%)	
	Initial	Refined		Initial	Refined
1	39.32	63.67	21	29.33	68.98
2	29.11	62.57	22	25.34	67.80
3	21.43	69.50	23	24.70	71.44
4	24.65	68.84	24	36.99	62.31
5	31.03	65.56	25	25.72	69.77
6	32.03	70.83	26	26.36	67.88
7	27.08	67.28	27	29.10	69.20
8	29.48	68.94	28	29.55	69.34
9	30.51	65.28	29	28.36	67.53
10	37.21	59.92	30	30.72	68.29
11	28.06	67.49	31	27.11	62.97
12	34.02	59.88	32	29.63	64.47
13	30.37	66.48	33	27.79	70.69
14	42.35	58.69	34	25.74	70.76
15	28.19	70.35	35	30.19	70.28
16	25.65	72.33	36	25.81	62.91
17	20.50	69.97	37	42.48	57.59
18	24.99	61.21	38	30.05	64.49
19	27.11	66.35	39	27.45	60.81
20	34.02	59.88	40	30.65	63.75

Supplementary Movie 1. HNMMC refinement of a distorted tRNA. The four helical stems of the tRNA are colored red, blue, green, and yellow, respectively.

Supplementary Movie 2. HNMMC refinement of the ribonuclease P S-domain with DOF 2. The three helical stems of the S-domain are colored red, blue, green, respectively. The gray color represents the unpaired RNA.

Supplementary Movie 3. HNMMC refinement of the *Mtb* handle combining all the seven levels of natural moves. The red region represents the flexible region of the *Mtb* handle. The black region which connects to the 50S main body is fixed.

Supplementary Movie 4. HNMMC refinement of the gRNA fragment 2394-2604 in MS2. The four helical stems of the gRNA fragment 2394-2604 are colored magenta, blue, green, and yellow, respectively as Level 3 in Figure 4. The black stem-loop is fixed because of the high-resolution EM structure. The gray color represents the unpaired RNA.

Supplementary Movie 5. HNMMC refinement of the entire MS2 gRNA. The gRNA model is colored according to the genes (blue: 5'UTR, red: 3'UTR, green: maturation protein, yellow: coat protein, orange: replicase).

Supplementary Movie 6. The linear morphing between two gRNA conformations of MS2. The four flexible gRNA fragments are colored yellow (fragment 614-879), green (fragment 1643-1700), blue (fragment 1992-2125) and red (fragment 2922-3087), respectively.