

Supplementary Information for

Topological Crossing in the Misfolded Tetrahymena Ribozyme Resolved by Cryo-EM

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This PDF file includes:

Figures S1 to S6

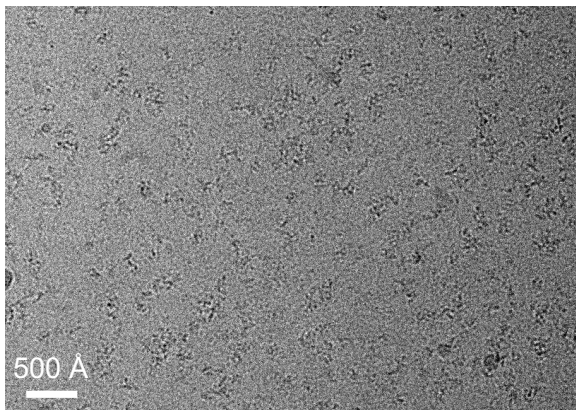
Table S1

Legends for Movies S1 to S3

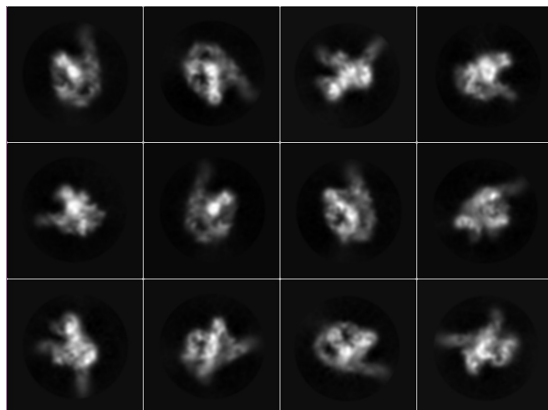
Other supplementary materials for this manuscript include the following:

Movies S1 to S3

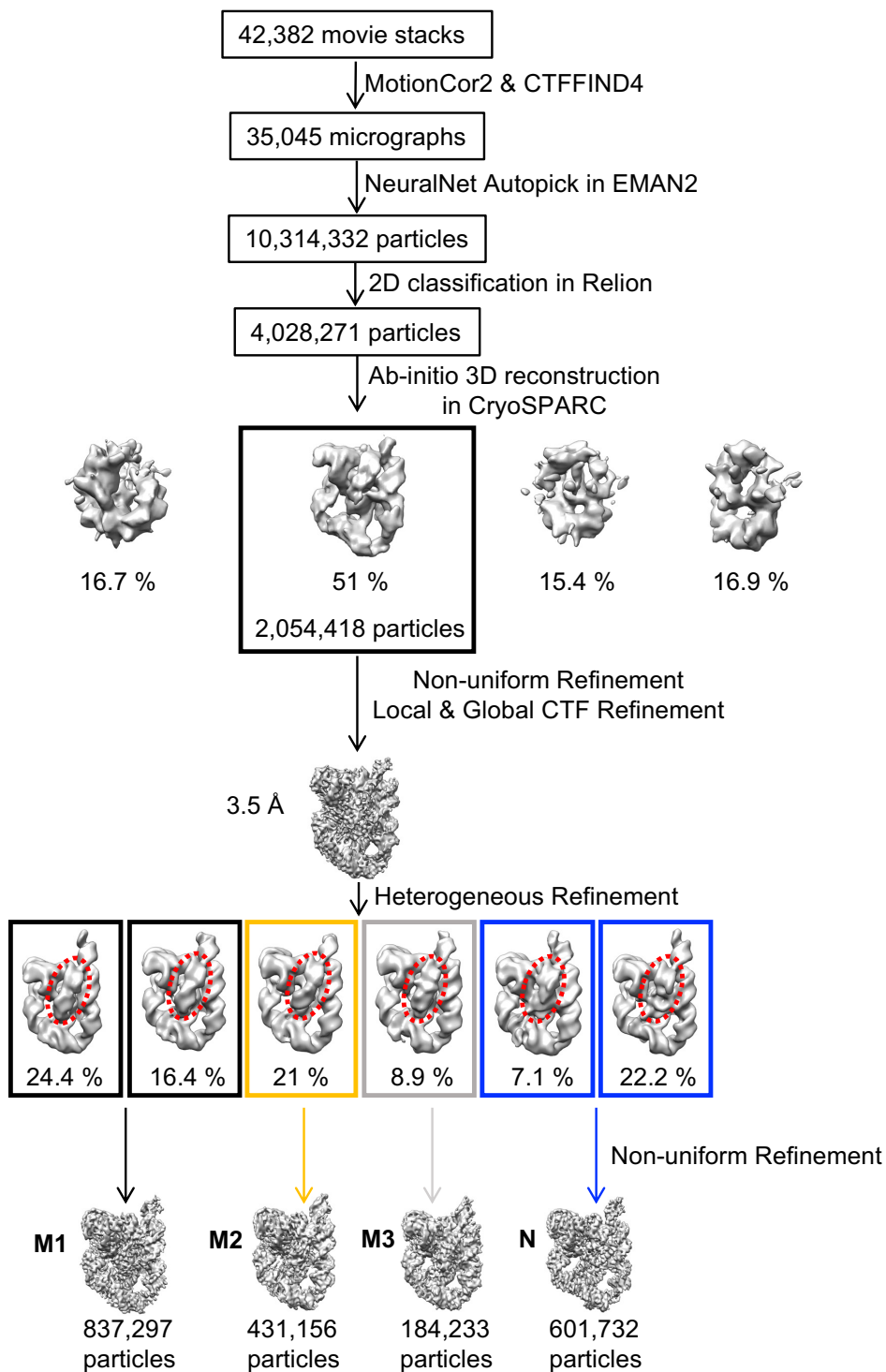
A



B

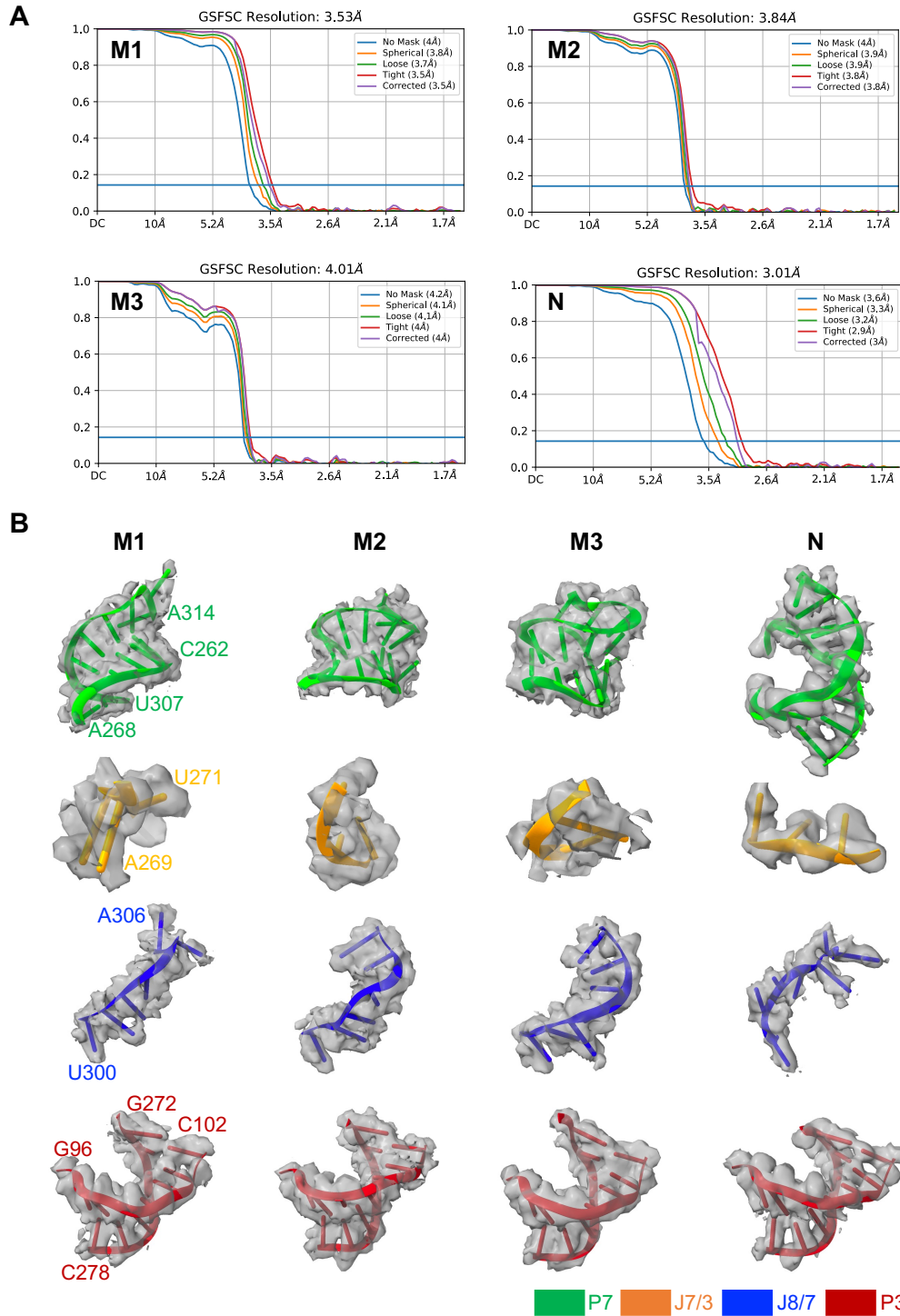


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42 **Fig. S1. Single-particle cryo-EM analysis of Tetrahymena ribozyme.** A. Representative
43 motion-corrected cryo-EM micrograph. B. Reference-free 2D class averages.



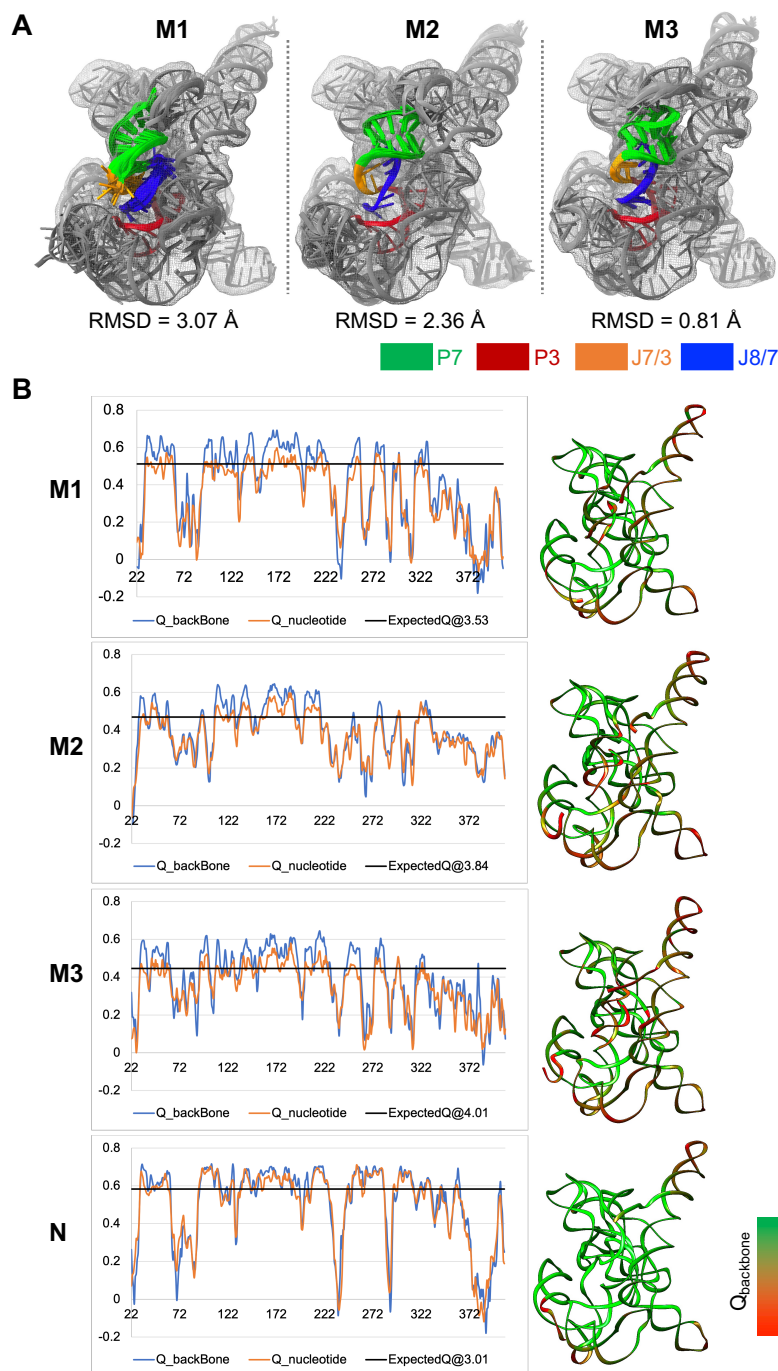
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Fig. S2. Workflow of the single-particle cryo-EM data processing. A total of 42,382 movie stacks were collected on a 300 kV Titan Krios cryo-electron microscope. cryoSPARC was used to resolve structural heterogeneity and four conformations were obtained. Detailed data processing procedures are described in “Materials and methods”.



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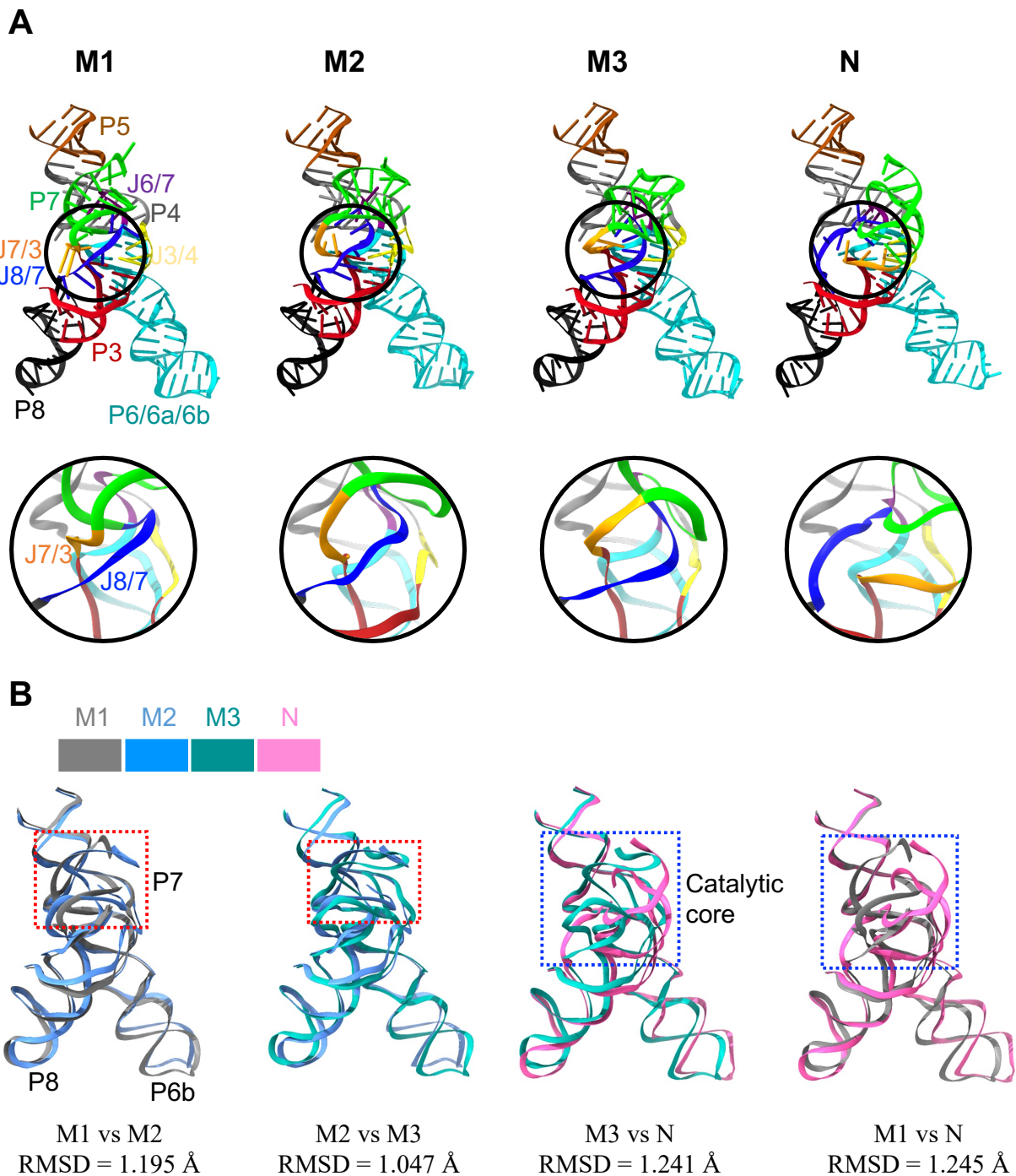
Fig. S3. Resolutions of four states of *Tetrahymena* ribozyme. A. Gold standard FSC plots calculated in cryoSPARC. B. Zoom-in views show examples of atomic models fitted to cryo-EM densities in the catalytic core.



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56 **Fig. S4. Model building of the M substates and the N state.** A. Convergence of models built by
 57 DRRAFTER. The top 10 models produced by DRRAFTER were well-converged for P7, P3, J7/3,
 58 and J8/7 regions, with RMSD labeled. B. Model validation using Q-scores. The black line
 59 represents the expected Q-score at respective resolution based on the correlation between Q-scores
 60 and map resolution. Colored models of ribozyme based on Q-score per backbone are shown on the
 61 right panel.

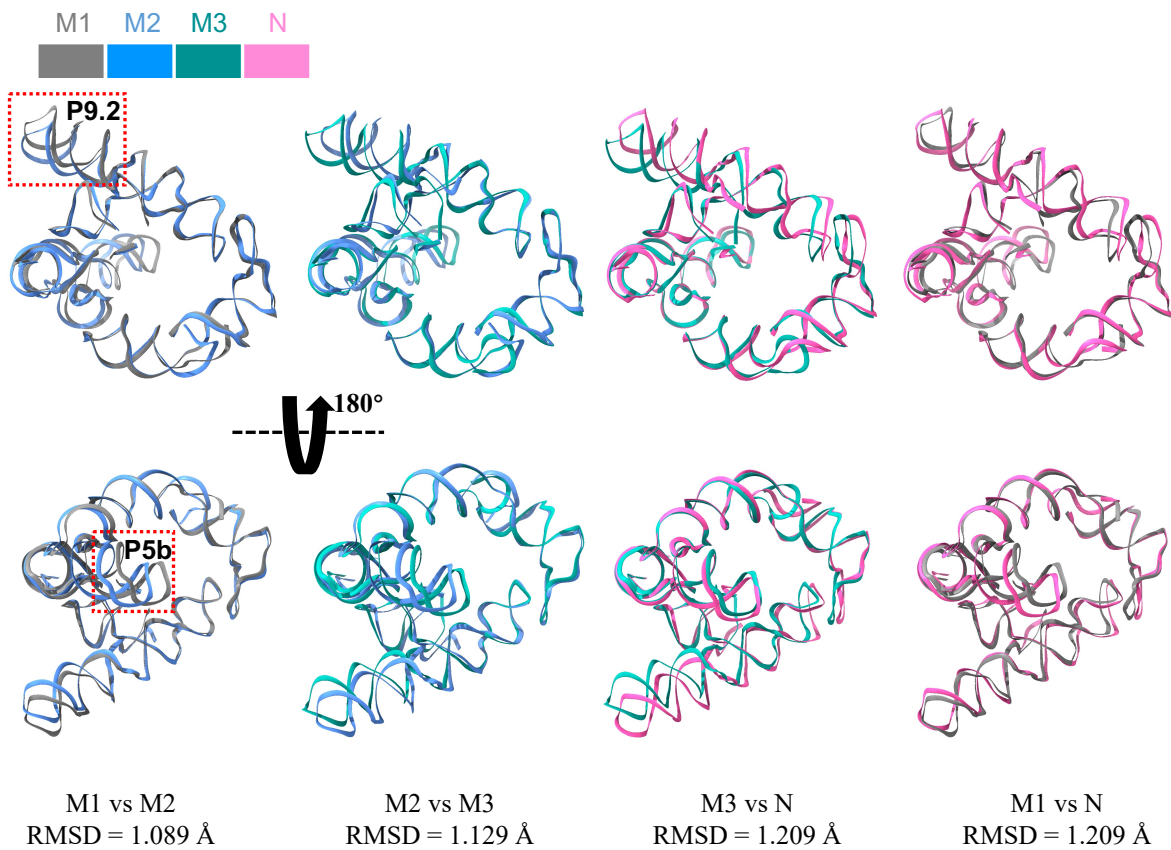
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64 **Fig. S5. Comparison of M with N at the enzyme core.** A. The enzyme core consists of eight
 65 paired and single-stranded regions shown in distinct colors. Zoom-in view illustrates the
 66 topological cross between J7/3 and J8/7. B. Overlaying cryo-EM models of M and N states reveals
 67 extensive native core with local rearrangements at the catalytic core.

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70 **Fig. S6. Comparison of M with N at the peripheral region.** Overlaying these models reveals
 71 extensive native peripheral structure.

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89 **Table S1. Cryo-EM data collection, processing, and model validation**

		<i>Tetrahymena</i> ribozyme			
Data collection and processing					
Microscope	Titan Krios G3i				
Voltage (kV)	300				
Camera	Gatan K3				
Grids Type	R2/1 Quantifoil copper grid (200 mesh)				
Sample concentration	~25 μ M				
Magnification	105,000 \times				
C2 aperture size (μ m)	70				
Objective aperture size (μ m)	100				
Pixel size (\AA)	0.82				
Total exposure ($e^-/\text{\AA}^2$)	51.3				
Exposure time (s)	3				
Number of frames per exposure	30				
Energy filter slit width (eV)	20				
Data collection software	EPU 2.7				
Number of exposures per hole	4				
Defocus range (μ m)	-1.2 - -2.8				
Number of micrographs collected	42,382				
Number of micrographs used	35,045				
Number of initial particles	10,314,332				
Conformations	M1	M2	M3	N	
Symmetry	C1	C1	C1	C1	
Number of final particles	837,297	431,156	184,233	601,732	
Resolution (0.143 gold standard FSC, \AA)	3.53	3.84	4.01	3.01	
Atomic model refinement					
Software	phenix	phenix	phenix	phenix	
Clashscore, all atoms	6.98	8.02	6.98	10.45	
MolProbity score	2.53	2.59	2.53	2.69	
Bad bonds (%)	0	0	0	0	
Bad angles (%)	0	0	0	0.01	

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Legends for Movies S1 to S3

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Movie S1. The topological difference between M and N.

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Movie S2. The similarity of three M substates including M1, M2, and M3.

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Movie S3. A possible M-to-N refolding pathway.

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