

SUPPLEMENTARY INFORMATION FILE FOR:

Cryo-specimen preparation and imaging of highly-structured and dynamic large non-coding RNAs

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This Supplemental File includes:

- Supplemental Figures S1-S6

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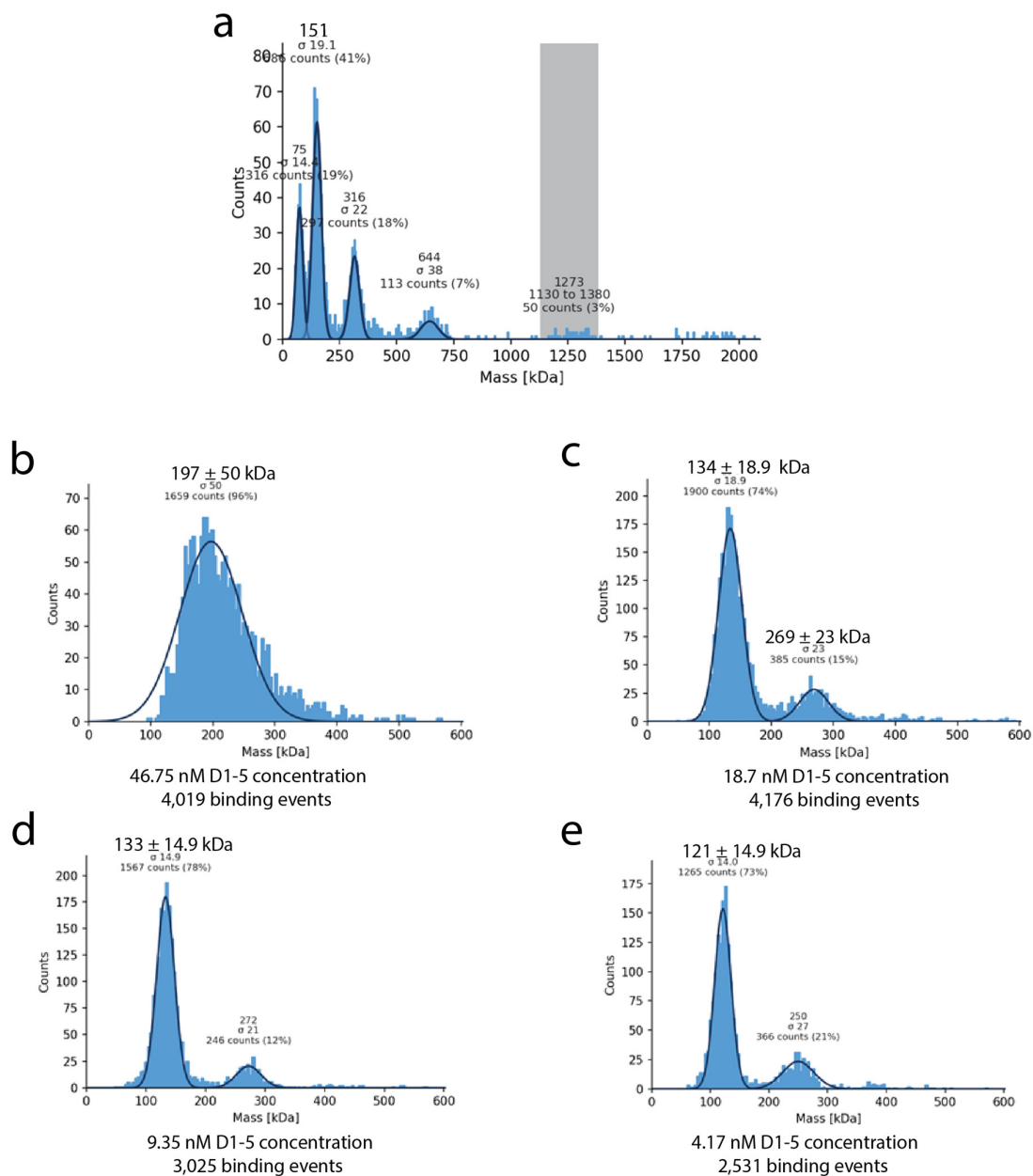


Figure S2. Mass photometry optimization. (a) Distribution of molecular weights of our RNA size ladder. From left to right, peak of 75 kDa (200 nucleotides), 151 kDa (500 nucleotides), 316 kDa (1000 nucleotides), 644 kDa (2000 nucleotides) and 1,273 kDa (4000 nucleotides). (c-f) D1-5 concentration screening. The sample concentration is indicated below each panel, along with the number of binding events observed for the estimation of the molecular weight of the sample (indicated above each panel).

1
2

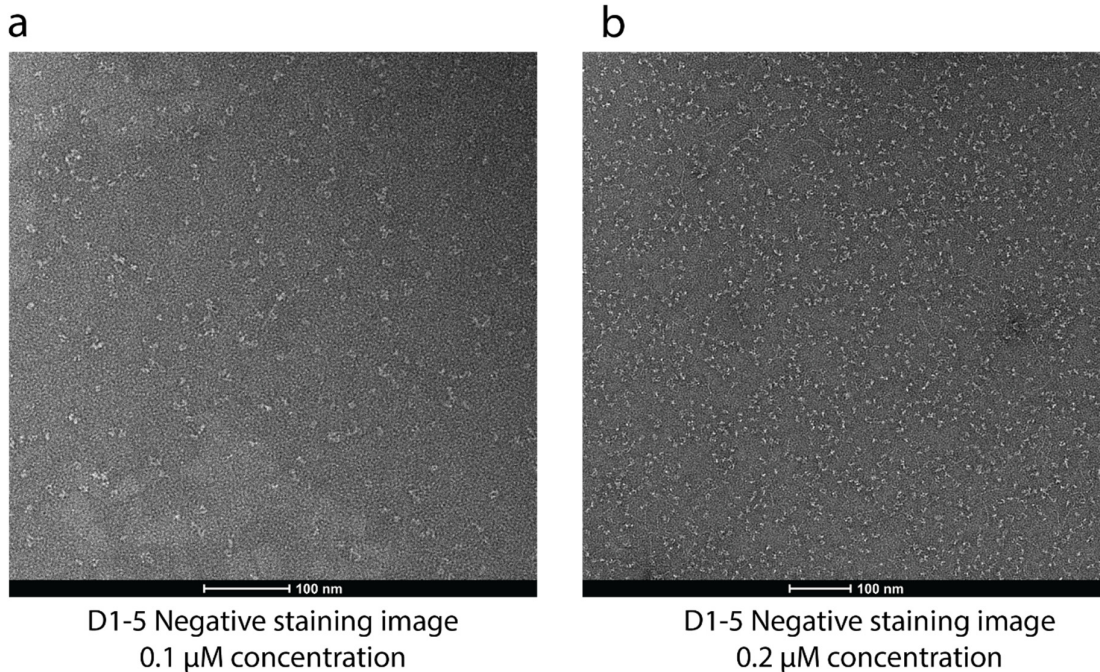
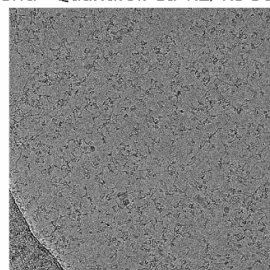


Figure S3. D1-5 negative staining images. (a) D1-5 negative staining image acquired from a grid prepared at 0.1 μ M concentration. (b) D1-5 negative staining image acquired from a grid prepared at 0.2 μ M concentration. Both images were taken at -2 μ m defocus on a Tecnai T12 microscope. The scale bar (100 nm) is indicated at the bottom of each micrograph.

1
2

D1-3, 28 μM conc.
 10 mM MgCl_2 , 150 mM KCl
 5 mM Na-MES pH 6.5
 Grid - Quantifoil Cu 1.2/1.3 300



1,695 movies
 200 keV electron gun, Flacon III camera
 pixel size - 0.94 $\text{\AA}$
 WARP motion correction,
 CTF estimation and particle picking
 174,600 particles extracted

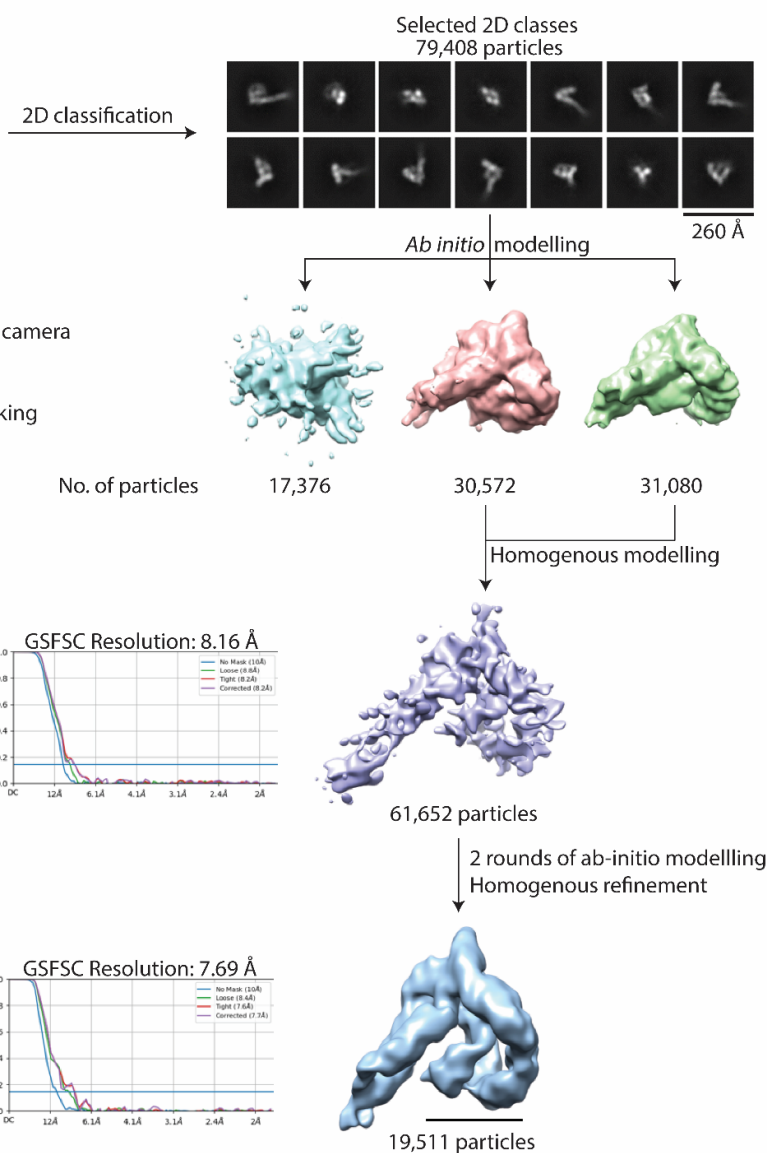


Figure S4. D1-3 data processing pipeline (dataset obtained at -9 blot force, 2 s blot time values). Grids were prepared at a sample concentration of 28 μM . The acquired micrographs were processed in WARP for motion correction, CTF estimation, particles picking and extraction with 260-pixel box size. Successive data processing, 2D classification and 3D reconstruction, was carried out in *CryoSPARC*. The scale bar of the 3D volumes represents 50 $\text{\AA}$.

1
 2



3,537 movies
200 keV electron gun, Flacoro
pixel size - 0.94 Å
WARP motion correction,
CTF estimation and particle
136,791 particles extracted

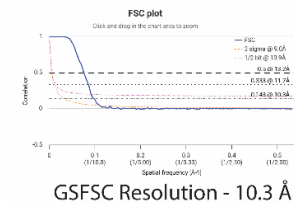


Figure S5. D1-3 data processing pipeline (dataset obtained at -10 blot force, 1 s blot time values). Grids were prepared at a sample concentration of 28 μM . The acquired micrographs were processed in WARP for motion correction, CTF estimation, particles picking and extraction with 260-pixel box size. Successive data processing, 2D classification and 3D reconstruction, was carried out in CryoSPARC. The scale bars of the 3D volumes represent 50 Å.

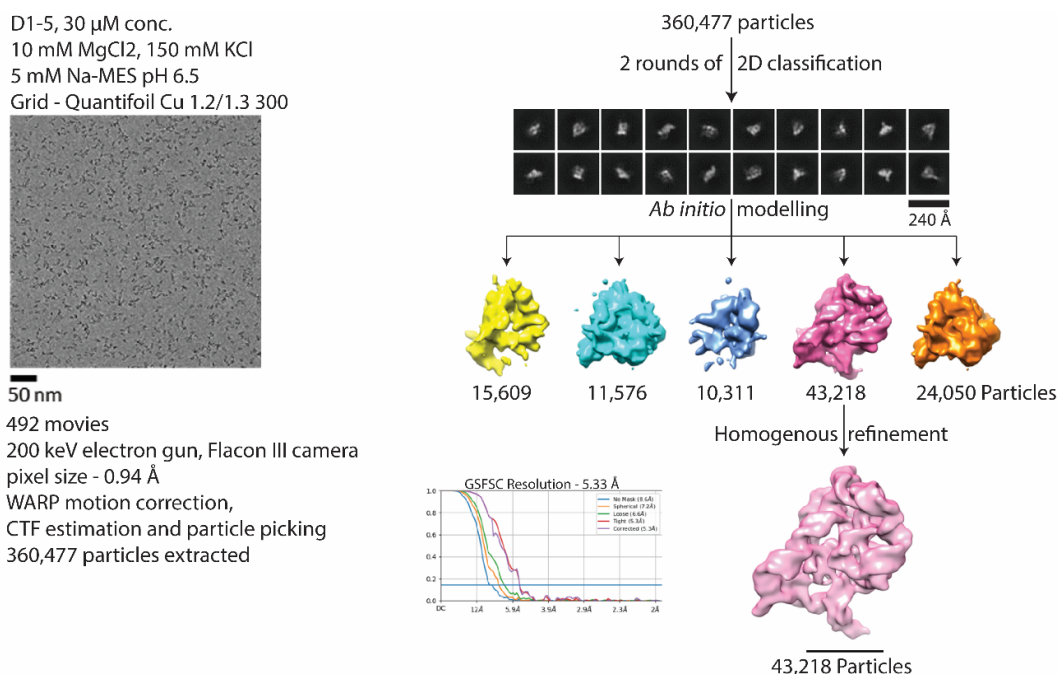


Figure S6. D1-5 data processing pipeline (dataset obtained at -7 blot force, 1 s blot time values). Grids were prepared at a sample concentration of 30 μM . The acquired micrographs were processed in WARP for motion correction, CTF estimation, particles picking and extraction with 260-pixel box size. Successive data processing, 2D classification and 3D reconstruction, was carried out in CryoSPARC. The scale bar of the 3D volumes represents 50 Å.