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Research article

The pRNA-assisted RNA imaging scheme (pARIS): a generalizable scaffold-based approach to facilitate cryo-EM structure determination of small RNAs

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Table S1. Primer sequenced used to generate pRNA-HCV: miR122.

Primer/Construct	Sequence (5′ to 3′)
HCV pRNA fwd	GCCAGCCCCC TGATGGGGGC GACACTCCAG GAGCTGTATG TTGGG GAT
miR-122 pRNA rev	CAAACACCAT TGTCACACTC CAGGAGCATT GACAACCAAT CAACAAAGTA TGTGGGCTGA ACTCA TCAG GGTTTAATCCC CAAC
T7 HCV primer fwd	TAATACGACT CACTATAGGG CCAGCCCCCT GATG
miR-122 primer rev	CAAACACCAT TGTCACACTC CAGGAGC
HCV: miR-122 pRNA construct	GCCAGCCCCC UGAUGGGGGC GACACUCCAG GAGCUGUAUG UUGGGGAUUA AACCCUGAGU UCAGCCCACA UACUUUGUUG AUUGGUUGUC AAUGCUCCGG AGUGUGACAA UGGUGUUUG

Table S2. Summary of cryo-EM image-processing parameters and reconstruction statistics for pARIS datasets.

Parameter	pRNA-tRNA	pRNA-HCV: miR-122
Initial reconstruction of entire phi29 particle		
Micrographs collected	15,356	1,860
Initial particle images	277,189	185,705
Pixel size (Å)	1.06	0.688
Box size (pixels)	800	2040
Procapsid refinement symmetry	C5	C5
Procapsid reconstruction resolution (Å)	3.0	3.7
Focused reconstruction of the target RNA		
Re-extraction performed	Yes	Yes
Re-centering at pRNA vertex	Yes	Yes
Re-extraction box size (pixels)	320	600
Fourier crop size (pixels)	Not performed	300
Final Pixel size (Å)	1.06	1.38
2D classification performed	Yes	Yes
Symmetry expansion	C5	C5
Signal subtraction	4-pRNA mask	4-pRNA mask
3D classification performed	Yes	Yes
Intermediate focused refinement resolution (Å)	5.3	6.9
Final focused refinement resolution (Å)	4.8	6.7
Final particle number	207,486	57,311
cryoSPARC version	cryoSPARC v3.3.2	cryoSPARC v4.2.1
Number of 3D classes	10	4
Local refinement job type	homogeneous	homogeneous
Rotation search extent	20°	20°
Shift search extent	10 Å	10 Å
Gaussian prior enabled	no	no

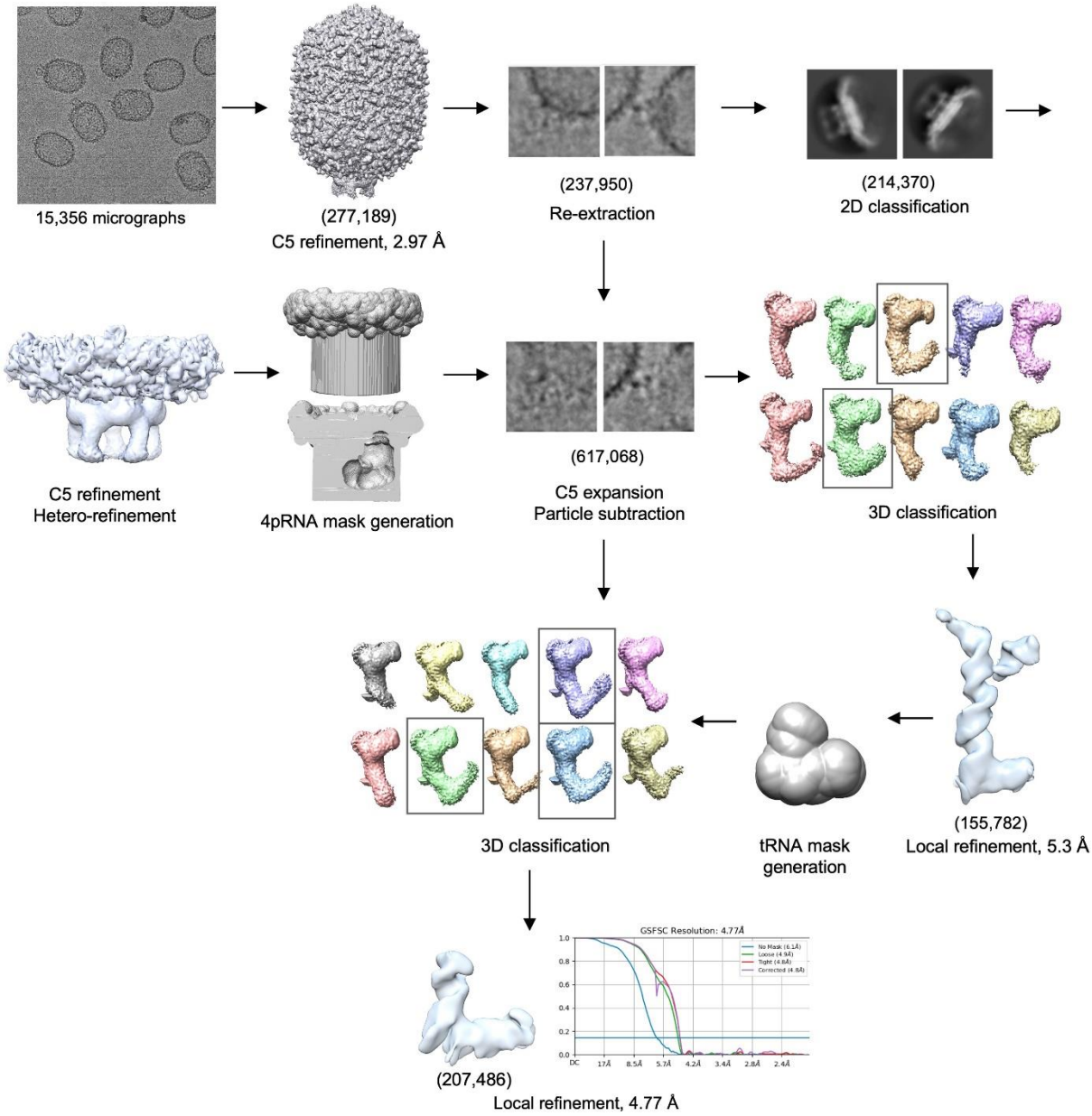

Figure S1. Workflow of tRNA structure determination using pARIS. The pRNA-tRNA construct was used as proof of principle that the pARIS method is capable of resolving the structures of small RNAs by cryo-EM. After 3D classifications, particles from selected reconstructions (boxed) were used for subsequent rounds of refinement. Following symmetry expansion and subtraction of four pRNA-tRNA subunits, focused refinement of a single pRNAtRNA subunit yielded a 5.3 Å reconstruction. Additional focused refinement using a mask excluding pRNA density yielded a final 4.77 Å reconstruction of the tRNA. Particle numbers for each step are listed in parenthesis. Final FSC curves for the tRNA molecules are shown.

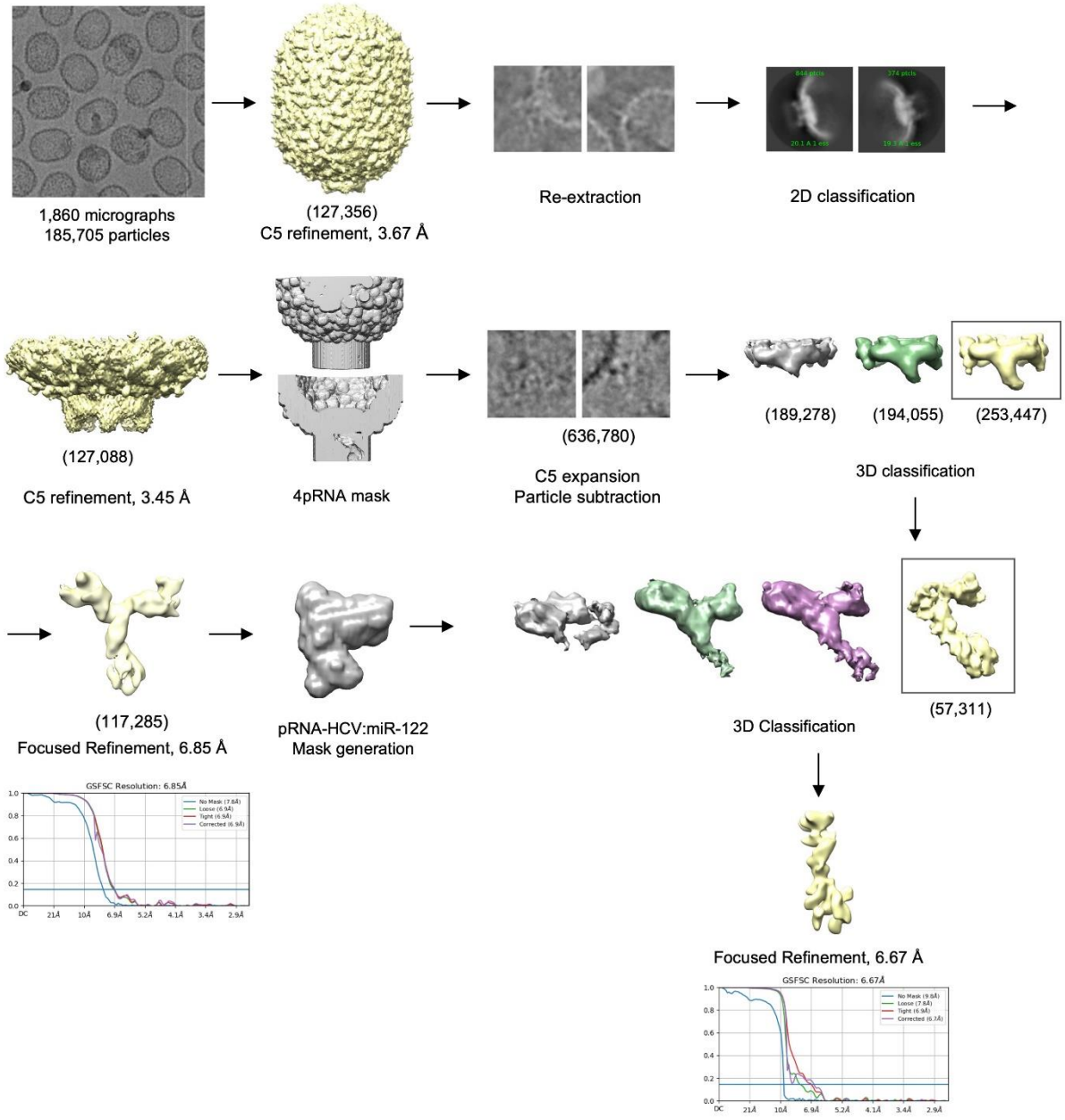

Figure S2. Workflow of structure determination of the HCV: miR-122 complex using pARIS. The pRNA-HCV: miR-122 construct was used to determine the structure of a biologically relevant RNA complex using the pARIS approach. After 3D classification, particles from selected reconstructions (boxed) were used for subsequent rounds of refinement. Following symmetry expansion and subtraction of four pRNA-HCV: miR-122 subunits, focused refinement of a single pRNA-HCV: miR-122 subunit yielded a 6.85 Å reconstruction. Additional 3D classification using a mask excluding pRNA density separated distinct conformational states of the HCV: miR-122 complex, and the largest subset of particles with a consistent SLI orientation was used for final focused refinement. Number of particles used in each step are listed in parentheses. Final FSC curves for the focused reconstructions are shown.