



NCCAT Session Report

GUP2: chameleon access

GUP2 application

Project ID: NCCAT-GUP2-KG220701

Project name: Structural basis for nuclear export of HIV-1 RNA Mediated by Rev Response Element

Primary user name: Keerthu Gottipati

eRA Commons user name: kegottip

Institution: University of Texas Medical Branch

Submission date: 07/01/2022

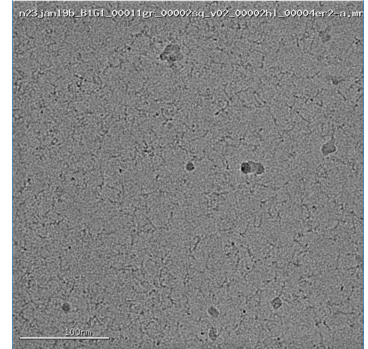
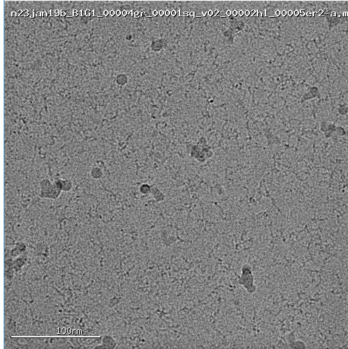
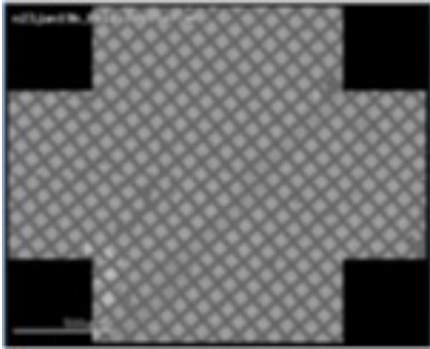
Sample information

- 1) **Name/Title of macromolecule of interest:** tRNA_RRE237
- 2) **Molecular weight:** 100 kDa
- 3) **Storage buffer:** 50 mM HEPES
- 4) **Ligands/Binding partners in sample (if applicable):**
- 5) **Standard storage temperature (in °C):** -80C
- 6) **Storage time (max time at storage temperature and/or at RT):**
- 7) **Highest soluble concentration tested (mg/mL):** 10mg/ml
- 8) **Issue the sample is facing :** ice thickness and particle density
- 9) **Additional information:**

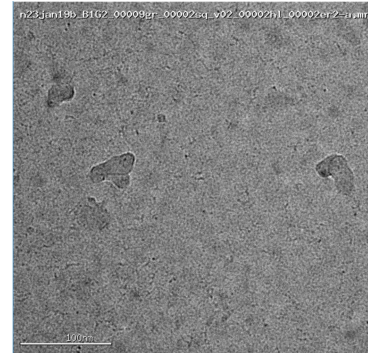
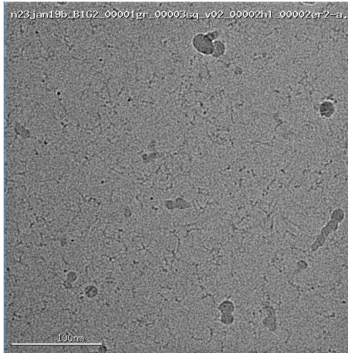
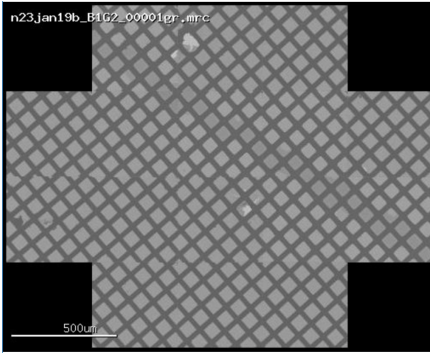
chameleon session log

Session	Grid	Notes
n23jan19b Microscope: Krios 7 Camera: Falcon 4 Dose: 47.32 e-/Å Mag: 130000 Pixel Size: 0.959 Å Number of images: 553 Concentration: 10mg/ml	B1G1	Acceptable overwicked Small collection done on this grid
	B1G2	Acceptable underwicked
n23jan20a Microscope: Krios 7 Camera: Falcon 4 Dose: 47.32 e-/Å Mag: 130000 Pixel Size: 0.959 Å Number of images: 8 Concentration: 6.66mg/ml	B2G1	Acceptable overwicked
	B2G2	Acceptable overwicked
n23jan20c Microscope: Glacios 1 Camera: Falcon 3 Dose: 51.68 e-/Å Mag: 120000 Pixel Size: 1.204 Å Number of images: 25 Concentration: 5mg/ml	B1G1	Acceptable overwicked with gradient
	B1G2	Acceptable overwicked
	B1G3	Acceptable overwicked
n23jan20e Microscope: Krios 7 Camera: Falcon 4 Dose: 47.32 e-/Å Mag: 130000 Pixel Size: 0.959 Å Number of images: 32 Concentration: BOX 2- 5mg/ml & BOX 3 – 6.66mg/ml	B2G1	Acceptable underwicked with gradient Not enough squares on stripe cannot use for collection
	B2G2	Acceptable overwicked
	B2G3	Acceptable overwicked with gradient Grid cannot be used for collection
	B2G4	Acceptable overwicked
	B3G1	Acceptable overwicked with gradient Not enough squares on stripe cannot use for collection
	B3G2	Acceptable overwicked

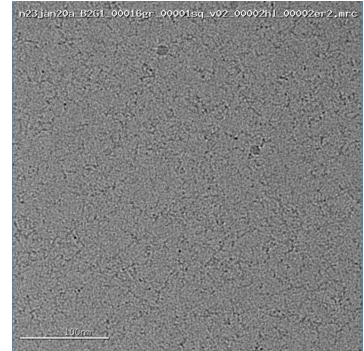
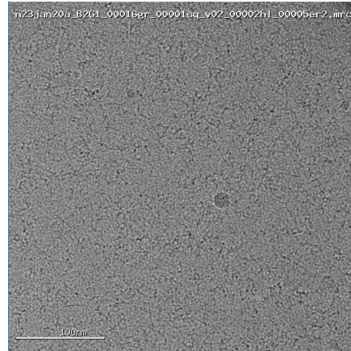
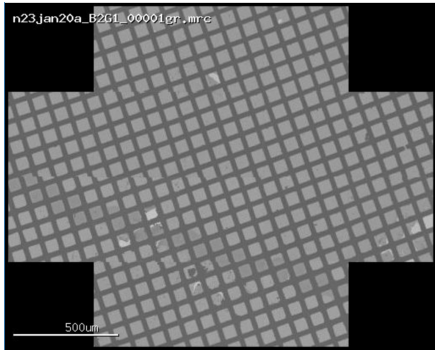
B1G1



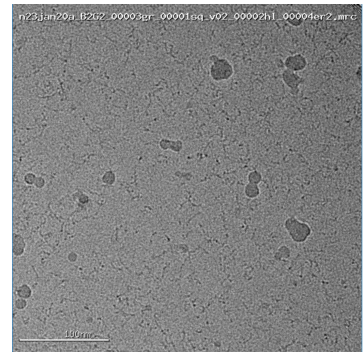
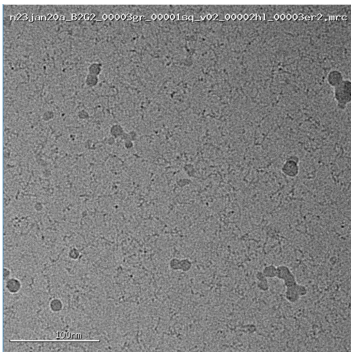
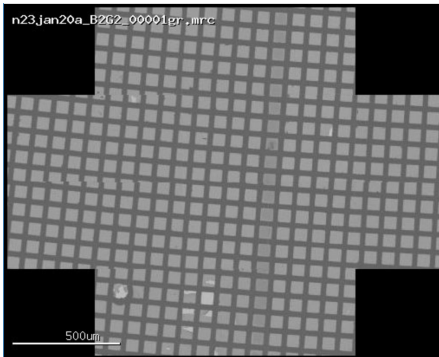
B1G2



B2G1

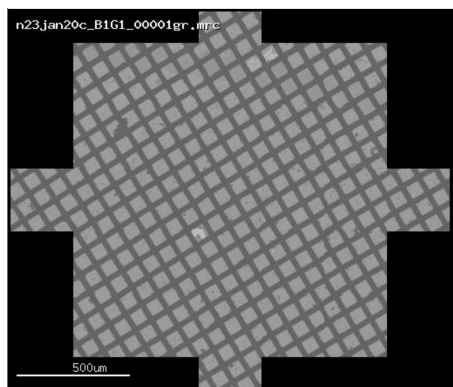


B2G2

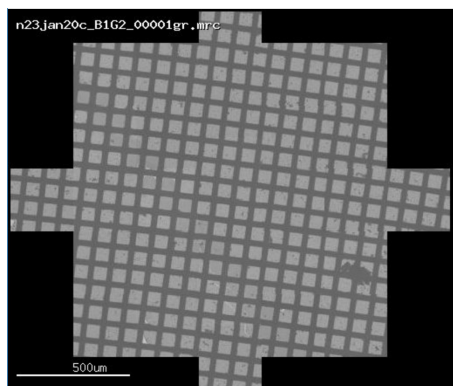


Session: n23jan20c – Glacios 1 – NCCAT-GUP2-KG220701 BOX 012023-1

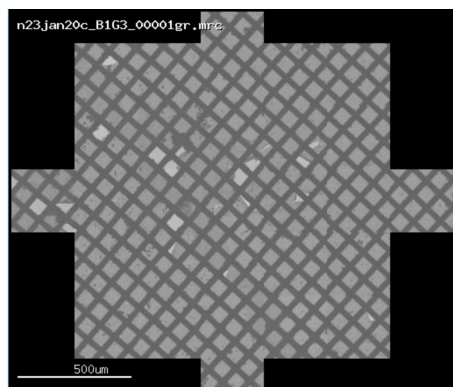
B1G1



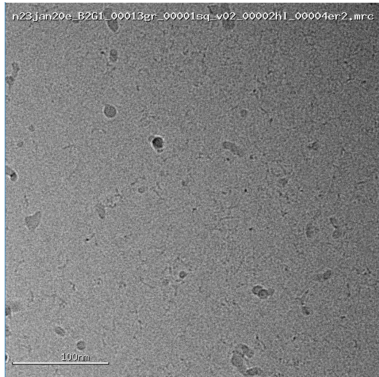
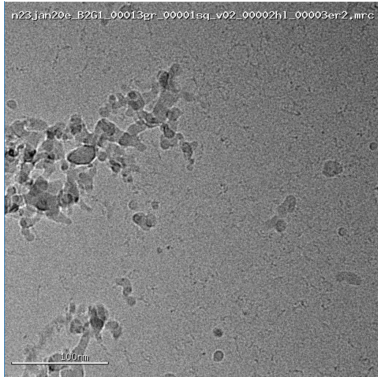
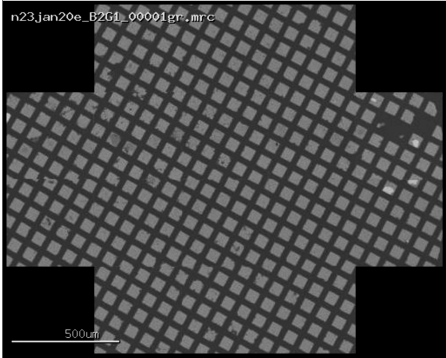
B1G2



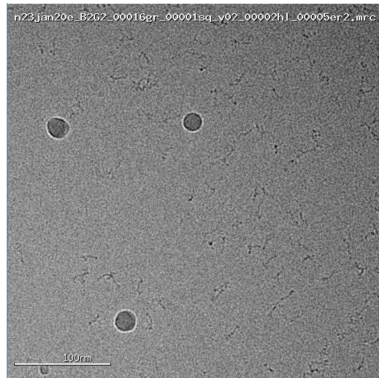
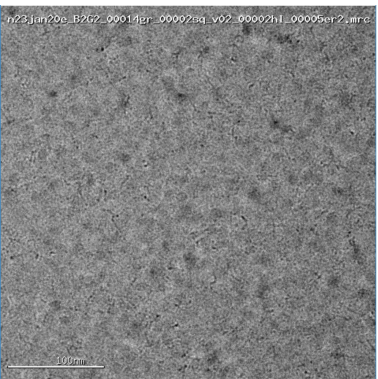
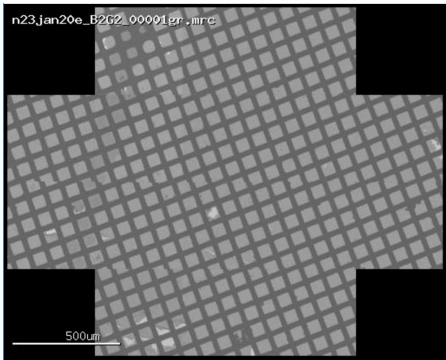
B1G3



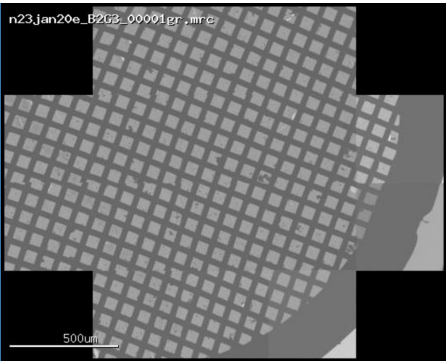
B2G1



B2G2

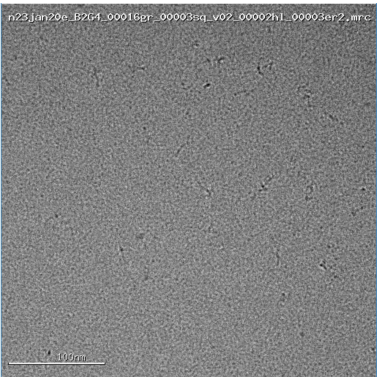
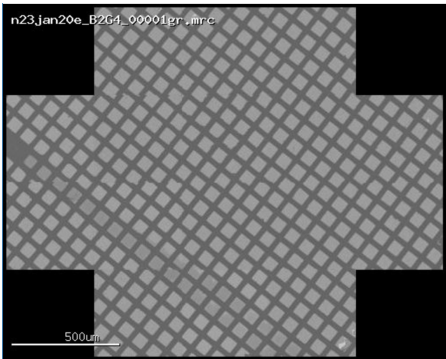


B2G3

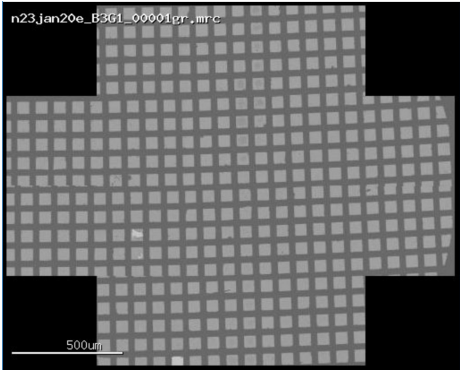


NO REPRESENTATIVE MICROGRAPHS

B2G4

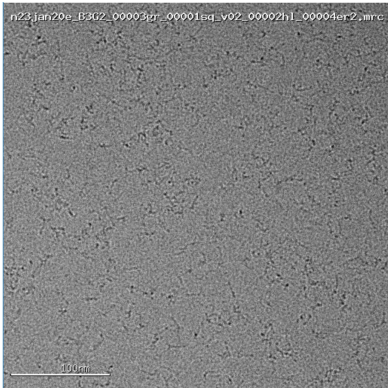
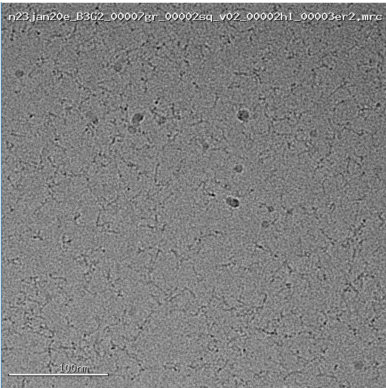
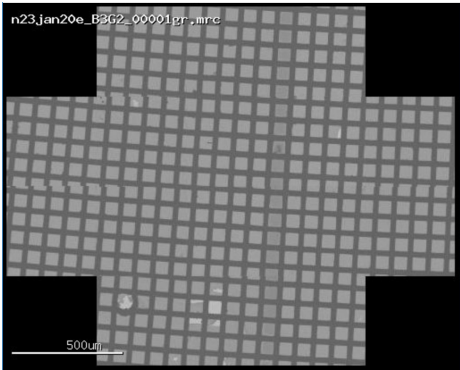


B3G1

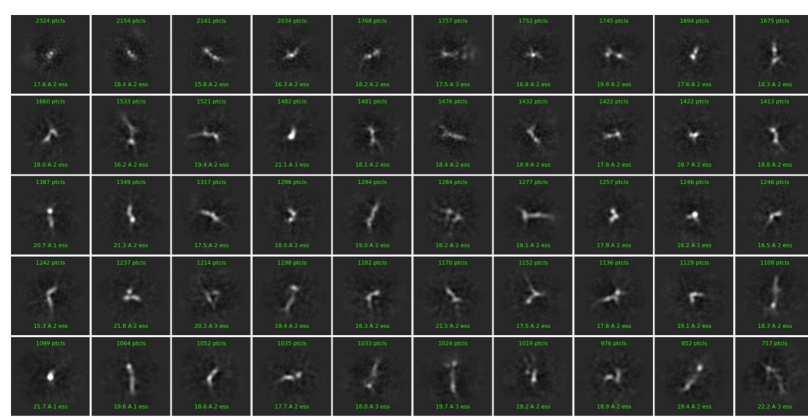


NO REPRESENTATIVE MICROGRAPHS

B3G2

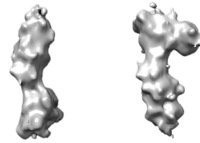
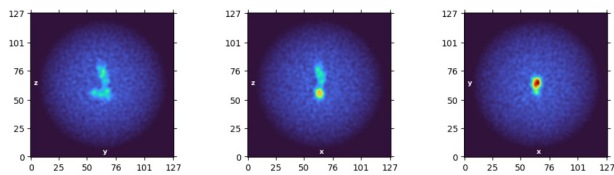


Data Processing: n23jan18b – Krios 7 – NCCAT-GUP2-KG220701 BOX 011923-1

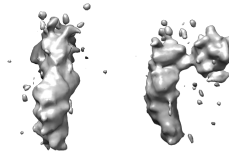
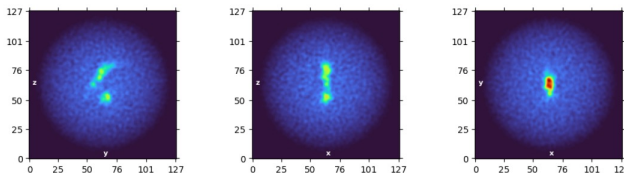


cryoSPARC v4.12
cryoSPARC live 2D classes: 635,902 particles
2nd Round 2D classes: 220,976 particles
3rd Round 2D classes: 210,708 particles
Classes selected for Ab-initio: 144,487 particles
This image are the 2D classes for the Refinement:
67,629 particles

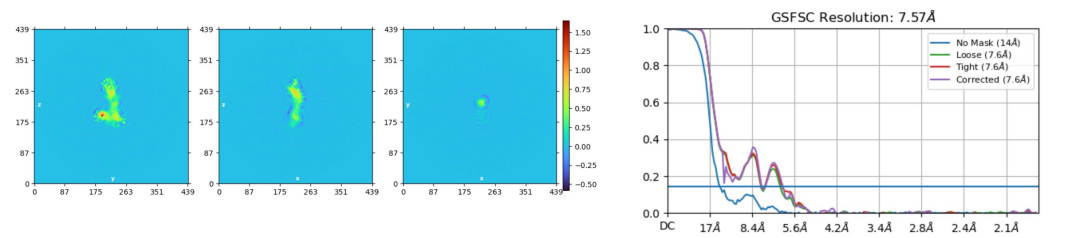
Ab-initio Class 000 (47.39%)



Ab-initio Class 001 (52.61%)



Homogenous Refinement of Ab-initio class 000



Acknowledgements

Some of this work was performed at the National Center for CryoEM Access and Training (NCCAT) and the Simons Electron Microscopy Center located at the New York Structural Biology Center, supported by the NIH Common Fund Transformative High Resolution Cryo Electron Microscopy program (U24 GM229539), and by grants from the Simons Foundation (SF349247) and NY State Assembly.