

Figure 1: Isolation of hDUS2(1-340) – tRNA-Val complex. **(a)** Mechanism of cross-linking between hDUS2 and 5-BrUrd-modified RNA. **(b)** Western blot analysis of cross-linking between 5-BrUrd-modified tRNA-Val-CAC. **(c)** Peptide-oligo adduct of hDUS2-tRNA-Val-CAC complex characterized by bottom-up proteomics. **(d)** Scheme for isolation of hDUS2-tRNA complex by mechanism-based crosslinking. **(e)** RNA-protein complex isolation by HiTrap Q anion exchange chromatography. **(f)** RNA-protein complex isolation by Superdex 200 size exclusion chromatography. **(g)** SDS-PAGE analysis of purified hDUS2 (1-340) and purified hDUS2-tRNA complex.

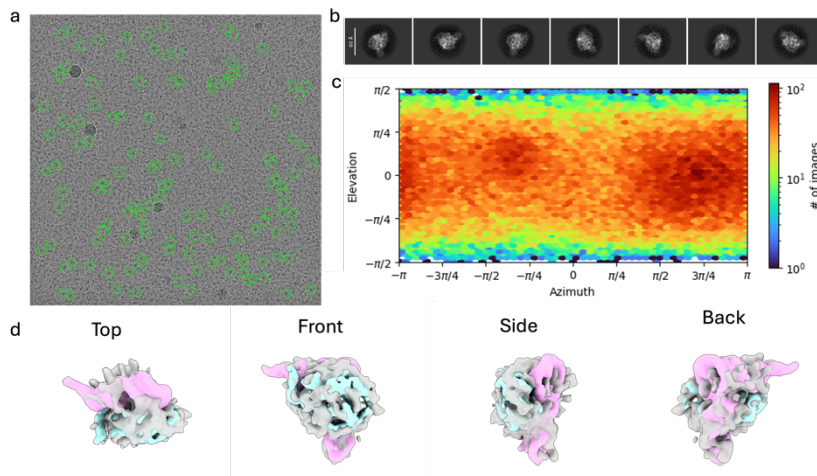


Figure 2: Preliminary Cryo-EM imaging results. **(a)** Representative micrographs (green circle = 136 Å). **(b)** Best 2D classes. **(c)** 3D map orientation distribution. **(d)** Cryo-EM maps docked with PDB 4tna (pink) and 4xp7 (teal).