
Supplementary information

NAC guides a ribosomal multienzyme complex for nascent protein processing

In the format provided by the
authors and unedited

Supplementary Table 2: Sequence of the synthetic RNAi-resistant *C. elegans* wildtype Naa15 gene (*syn-hpo-29*). Lower case = synthetic introns. Grey = 3xFLAG tag.

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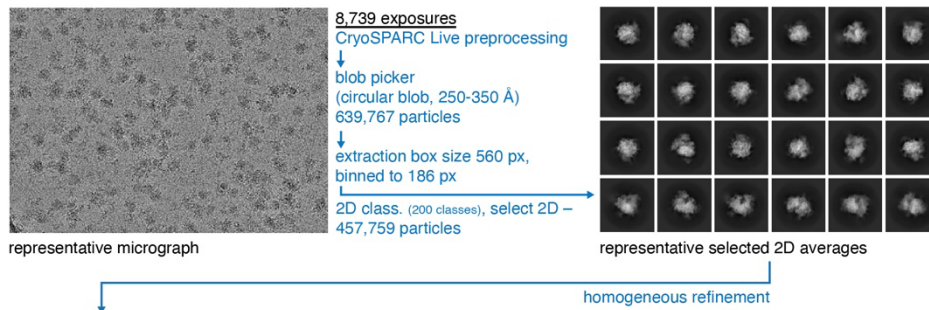
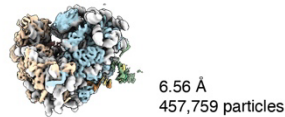
Supplementary Table 3: *C. elegans* strains

DEU192	gamSi70 [<i>icd-2p::^lSNAP25^{l2}-YFP-3xHA</i>]
DEU193	gamSi71 [<i>hpo-29p::WT-syn-hpo-29-3xFLAG; icd-2p::mCherry</i>]
DEU194	gamSi72 [<i>hpo-29p::L80A/L84A/W90A-syn-hpo-29-3xFLAG; icd-2p::mCherry</i>]
DEU195	gamSi73 [<i>icd-1p::3xFLAG-WT-syn-icd-2; icd-2p::mCherry</i>]
DEU196	gamSi74 [<i>icd-1p::3xFLAG-I186A/V187A-syn-icd-2; icd-2p::mCherry</i>]
DEU197	gamSi75 [<i>icd-1p::3xFLAG-Δ158-195-syn-icd-2; icd-2p::mCherry</i>]
DEU198	gamSi70; gamSi71 [<i>icd-2p::^lSNAP25^{l2}-YFP-3xHA; hpo-29p::WT-syn-hpo-29-3xFLAG; icd-2p::mCherry</i>]
DEU199	gamSi70; gamSi72 [<i>icd-2p::^lSNAP25^{l2}-YFP-3xHA; hpo-29p::L80A/L84A/W90A-syn-hpo-29-3xFLAG; icd-2p::mCherry</i>]
DEU200	gamSi70; gamSi73 [<i>icd-2p::^lSNAP25^{l2}-YFP-3xHA; icd-1p::3xFLAG-WT-syn-icd-2; icd-2p::mCherry</i>]
DEU201	gamSi70; gamSi74 [<i>icd-2p::^lSNAP25^{l2}-YFP-3xHA; icd-1p::3xFLAG- I186A/V187A-syn-icd-2; icd-2p::mCherry</i>]
DEU202	gamSi70; gamSi75 [<i>icd-2p::^lSNAP25^{l2}-YFP-3xHA; icd-1p::3xFLAG-Δ158-195-syn-icd-2; icd-2p::mCherry</i>]
VC440	<i>mif-3(gk228)</i> I.
DEU203	<i>mif-3(gk228)</i> I.; gamSi70 [<i>icd-2p::^lSNAP25^{l2}-YFP-3xHA</i>]

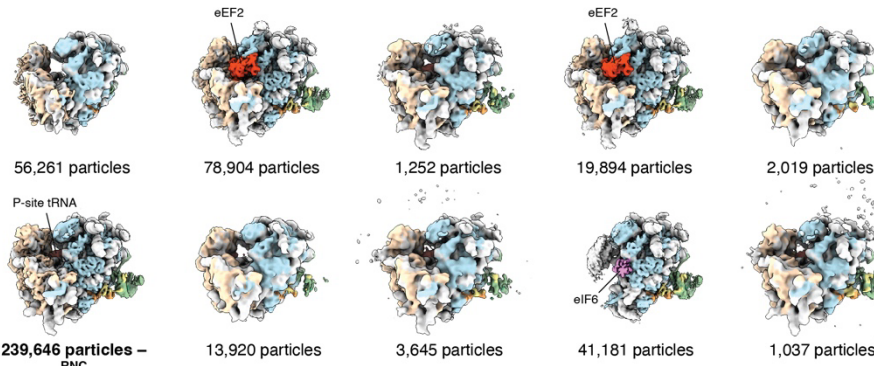
Supplementary Methods:

SI Figure 2. Cryo-EM data processing flowcharts for the datasets collected in this study. (a) The RNC_{RpL4}-NAC-NatA/E sample. Data processing steps marked in blue or red were performed using CryoSPARC or Relion, respectively. The figure lists all processing steps. Non-default parameters for every job are indicated in brackets. Particle stacks selected for further processing after 3D classifications are in bold. The rRNA in the cryo-EM maps is shown in light grey, SSU proteins are in beige, LSU proteins in light blue, the NAC heterodimer in yellow and orange, and NatA/E in hues of green. In the output of the first 3D classification, eEF2 in hibernating ribosomes is shown in red, and eIF6 on free 60S in lilac. Masks used in 3D classification and local refinement are displayed as semi-transparent green surfaces. **(b)** The RNC_{RpL4}-MetAP1-NAC-NatA/E sample. Non-default parameters for every job are indicated in brackets. Particle stacks selected for further processing after 3D classifications are in bold. The rRNA in cryo-EM maps is shown in light grey, SSU proteins in beige, LSU proteins in light blue, the NAC heterodimer in yellow and orange, NatA/E in hues of green, and MetAP1 in slate blue. In the output of the 3D variability analysis, eEF2 in hibernating ribosomes is shown in red, and eIF6 on free 60S is depicted in lilac. Masks used in 3D classification, 3D variability analysis and local refinement are displayed as semi-transparent green surfaces. **(c)** The RNC_{RpL4}-MetAP1-NAC-NatA/E-HYPK sample. Additional map segments adjacent to Naa15 were assigned to HYPK guided by docking of the free NatA/E-HYPK structure (PDB: 6PW9). In this reconstruction the EM density for MetAP1 is only resolved at low resolution, likely due to partial occupancy. All data processing was performed in CryoSPARC except for volume subtraction, which was done in ChimeraX. The respective steps are marked with a purple arrow in the flowchart. Non-default parameters for every job are indicated in brackets. Particle stacks selected for further processing after 3D classifications are in bold. The rRNA in the cryo-EM maps is shown in light grey, SSU proteins are in beige, LSU proteins in light blue, the NAC heterodimer in yellow and orange, NatA/E in hues of green, HYPK in purple, and MetAP1 in slate blue; in the output of 3D variability analysis, eEF2 in hibernating ribosomes is shown in red. The outline of the minuend volume is shown around the difference volume. Masks used in 3D classification and local refinement are displayed as semi-transparent green surfaces. See also the Methods section for details of data processing are described in.

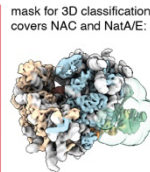
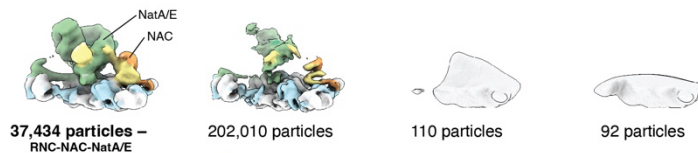
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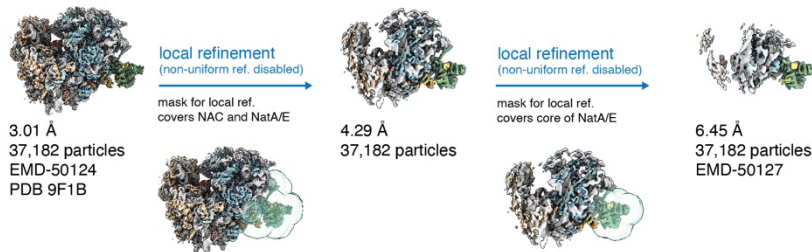
3D classification to select translating ribosomes
(initial reference lowpass filter: 40 Å;
10 classes; regularisation parameter T: 4;
mask diameter: 480 Å; no image alignment)



3D classification to select RNCs with NAC-NatA/E
(initial reference lowpass filter: 25 Å;
10 classes; regularisation parameter T: 8;
no image alignment)



homogeneous refinement, unbinned data,
homogeneous refinement
(optimize per-group CTF params;
optimize per-particle defocus)

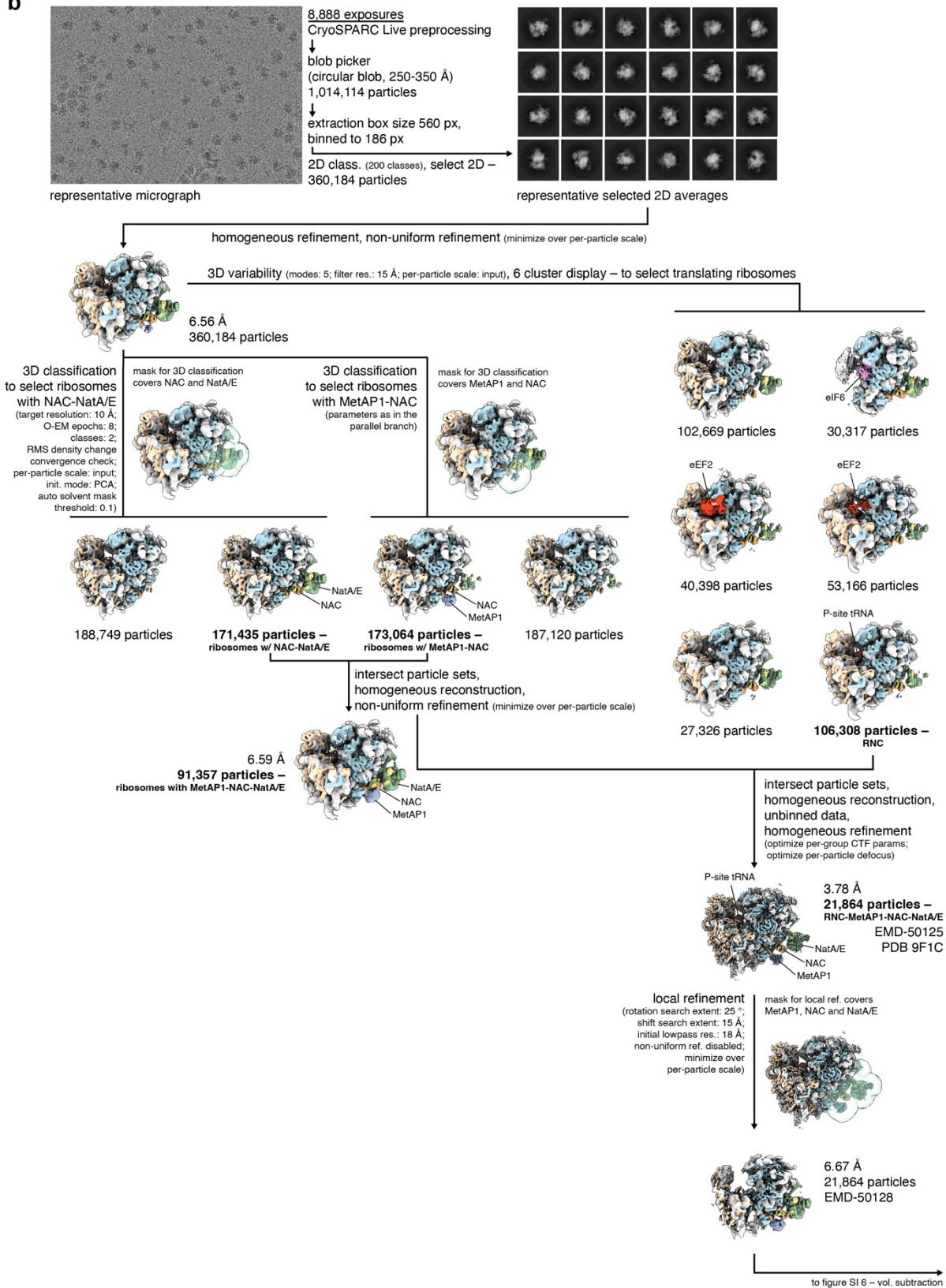


CryoSPARC

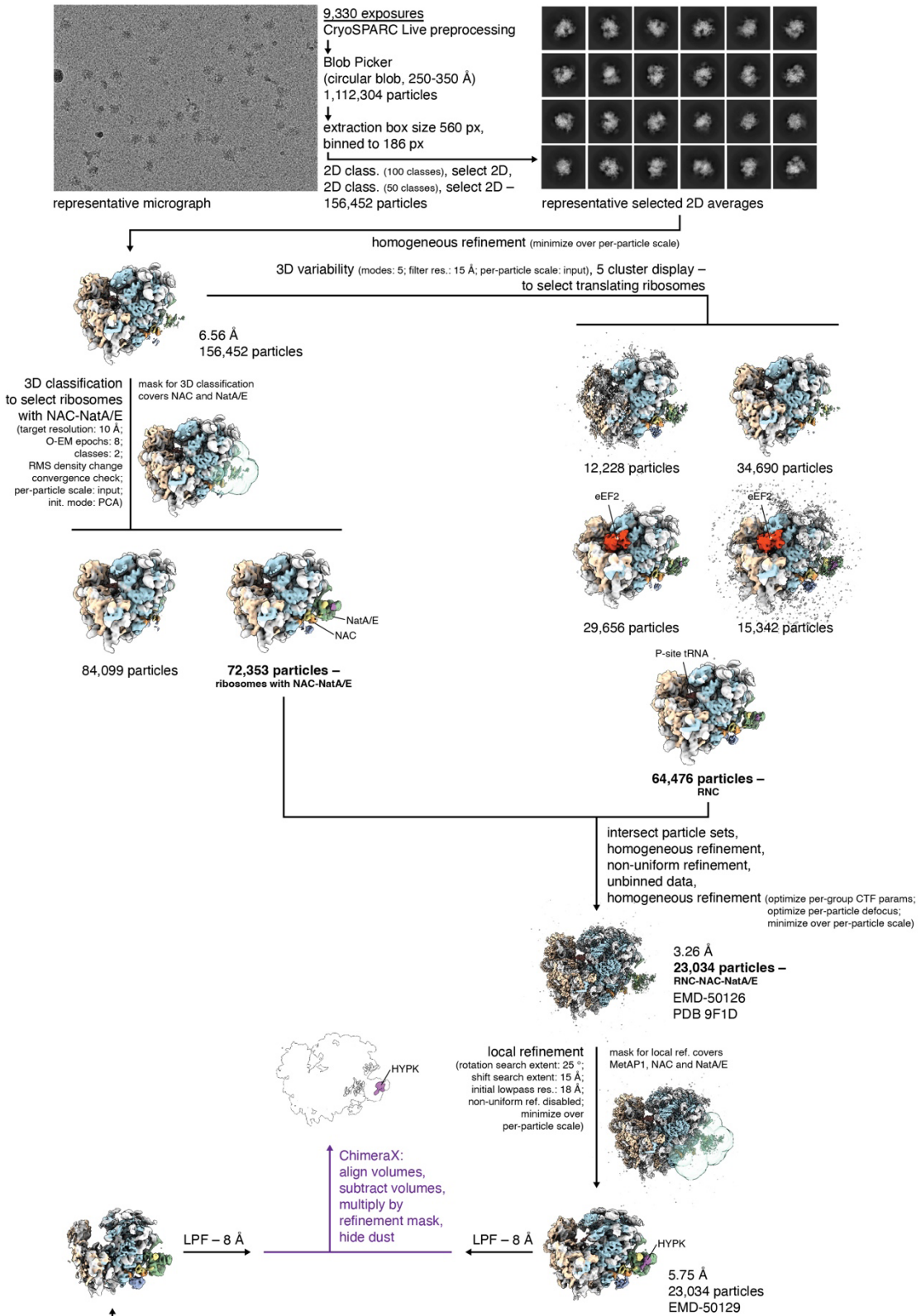
RELION

CryoSPARC

b



C

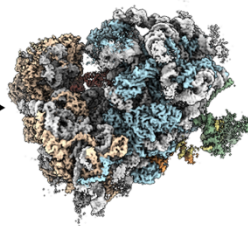


SI Figure 3. Cryo-EM data processing flowchart for the ternary 80S-NAC-NatA/E complex with improved resolution for NatA/E. (a) Cryo-EM data processing flowchart to obtain a model of NAC and NatA/E at higher resolution. Briefly, ribosome particles with NatA/E density from the RNC_{RpL4}-NAC-NatA/E dataset were used for focused 3D classification on NatA/E regardless of whether they have a nascent chain or not, and particles homogeneous with respect to NAC-NatA/E conformation were selected. The architecture of the 80S-NAC-NatA/E cryo-EM structure is the same as that of the RNC-NAC-NatA/E complex (Extended data Fig. 4) and displays finer features of NatA/E. Details of data processing are described in the Materials and Methods section. Non-default parameters of the 3D classification are indicated in brackets. The rRNA in the cryo-EM maps is shown in light grey, SSU proteins are in beige, LSU proteins in light blue, the NAC heterodimer in yellow and orange, and NatA/E in hues of green. The mask used in 3D classification is displayed as a semi-transparent green surface. The contour of the mask used to calculate FSC for the NAC-NatA/E segment of the map is displayed in orange. (b, c) FSC curves calculated for the full 80S-NAC-NatA/E map (b) and its NAC-NatA/E segment (c). (d, e) Detail of the cryo-EM map showing NatA/E and NAC coloured by protein (d) and by estimated local resolution on the scale from 2 to 10 Å (e).

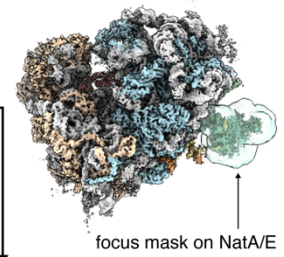
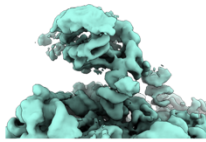
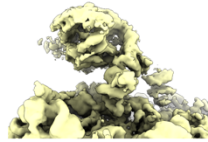
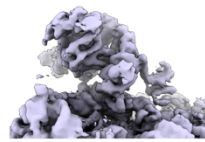
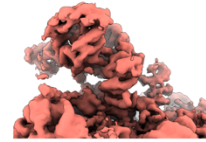
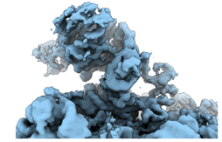
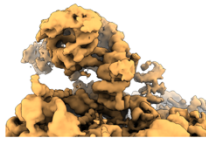
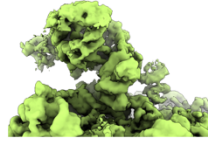
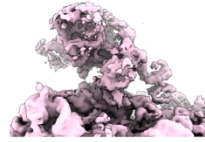
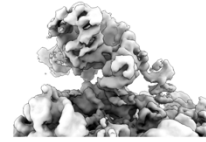
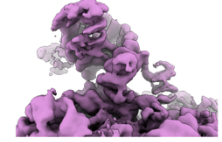
a

RPL4-RNC-NAC-NatA/E
ternary complex,
nascent chain len. – 53 aa
8,745 micrographs

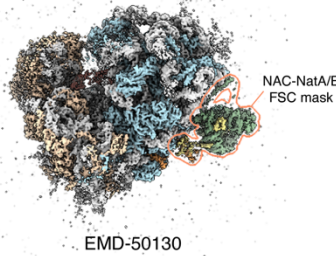
blob picker – 250-350 Å
 extract 3x binned
 2D classification
 select ribosomes – **457,759 particles**
 homogeneous refinement
 3D class. w/ mask around NAC-NatA/E
 select classes w/ NatA/E – **197,483 particles**
 unbin data
 homogeneous refinement



3D classification
 (classes: 10;
 N of O-EM epochs: 8;
 RMS density change
 convergence check;
 initialization mode: PCA;
 target resolution: 4 Å)

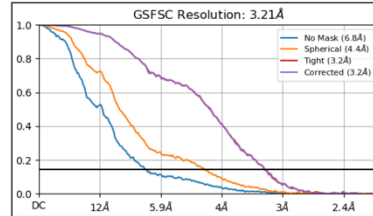


homogeneous reconstruction only



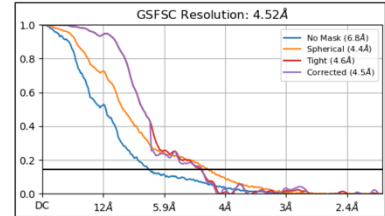
b

full map FSC

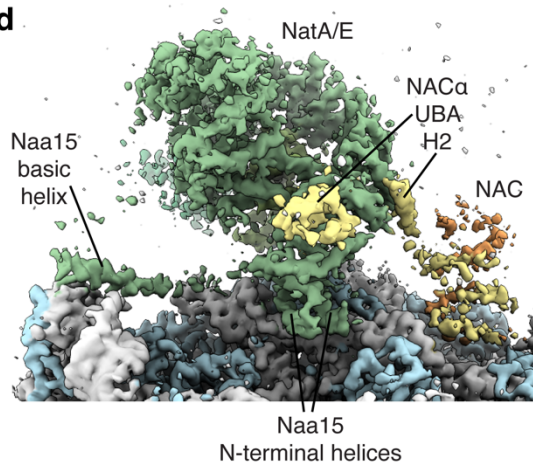


c

NAC-NatA/E FSC



d



e

