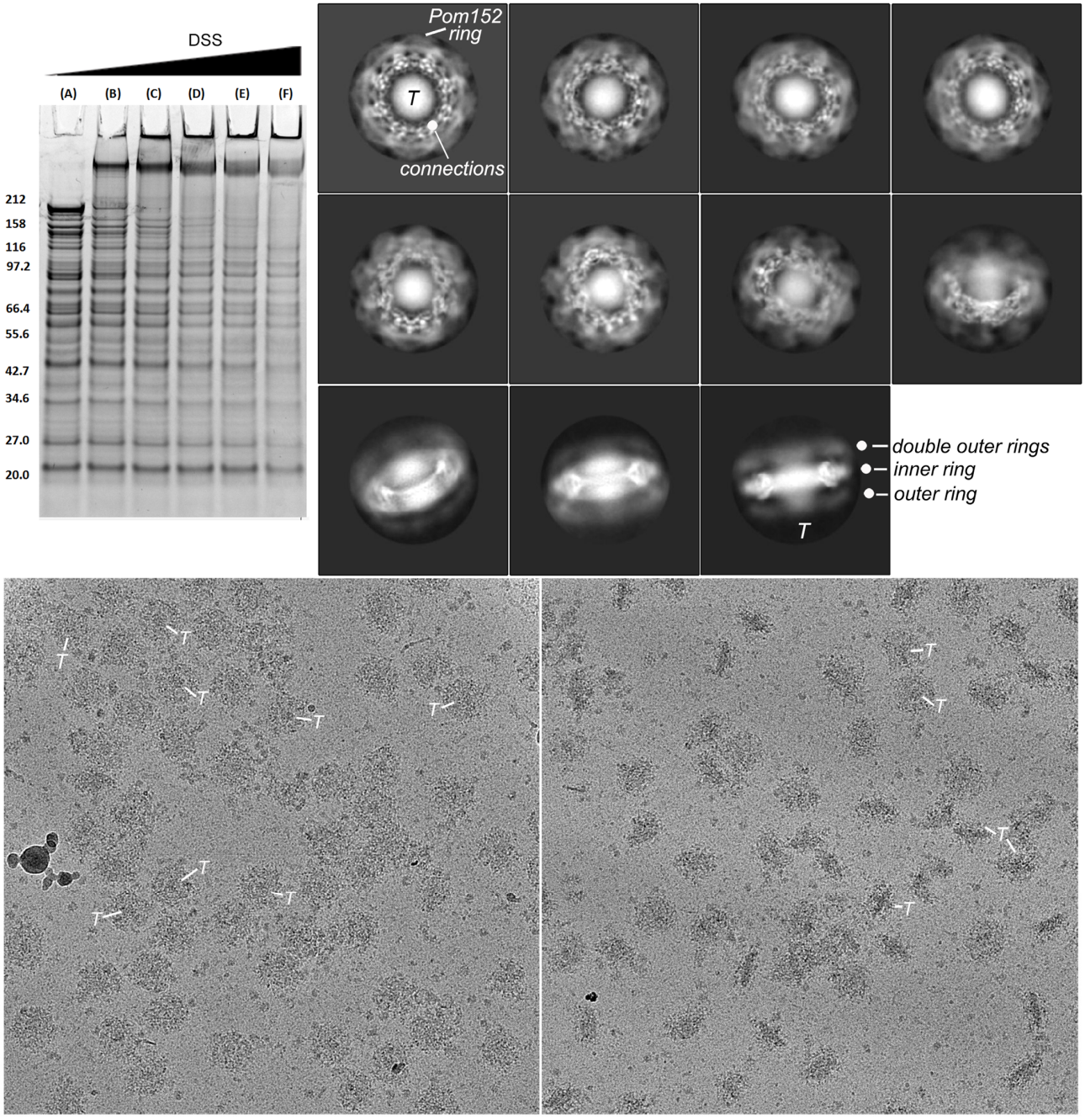
Methods S1

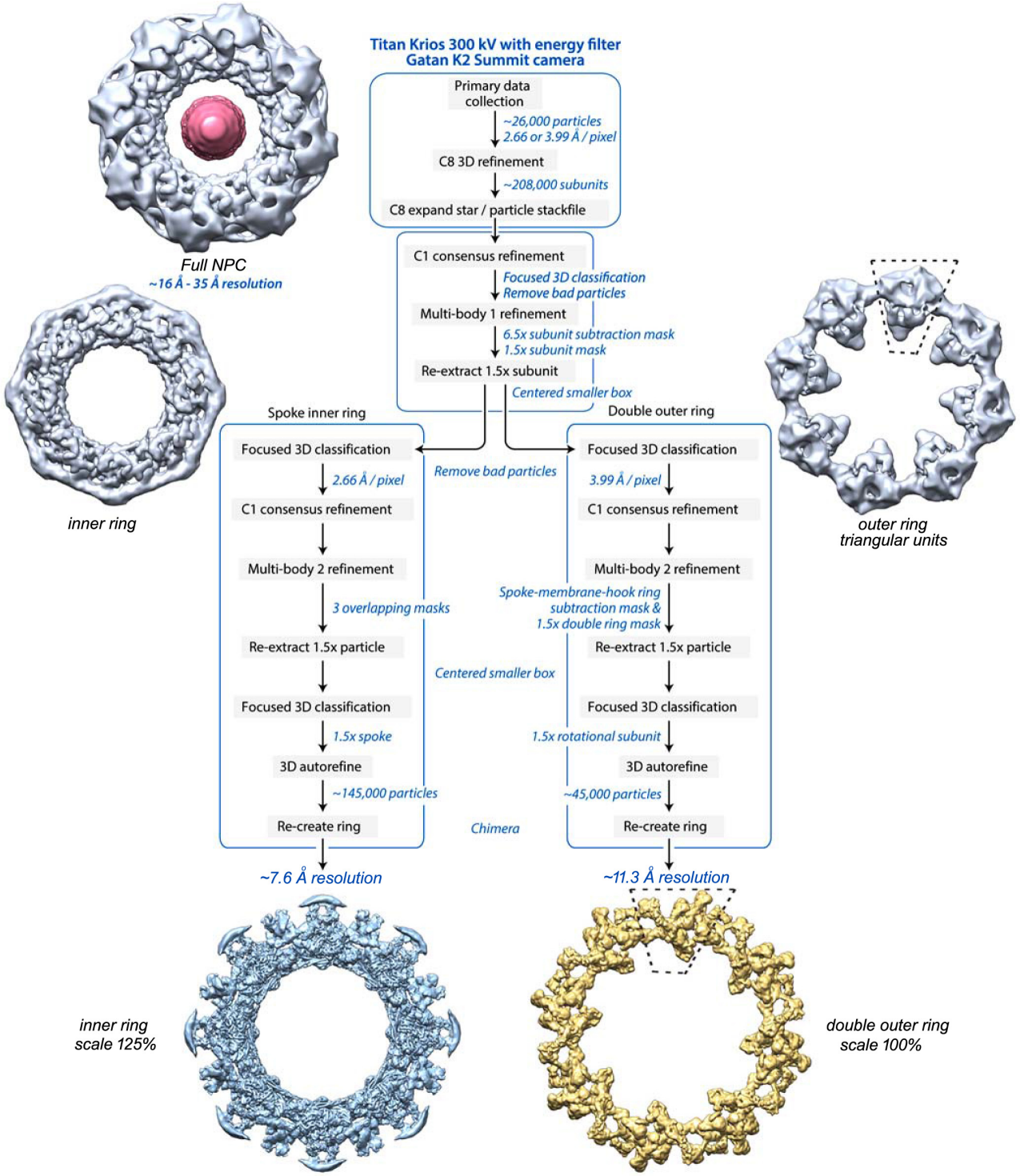
Cryo-EM single particle data processing : related to Figures 1-4,7.

Contents



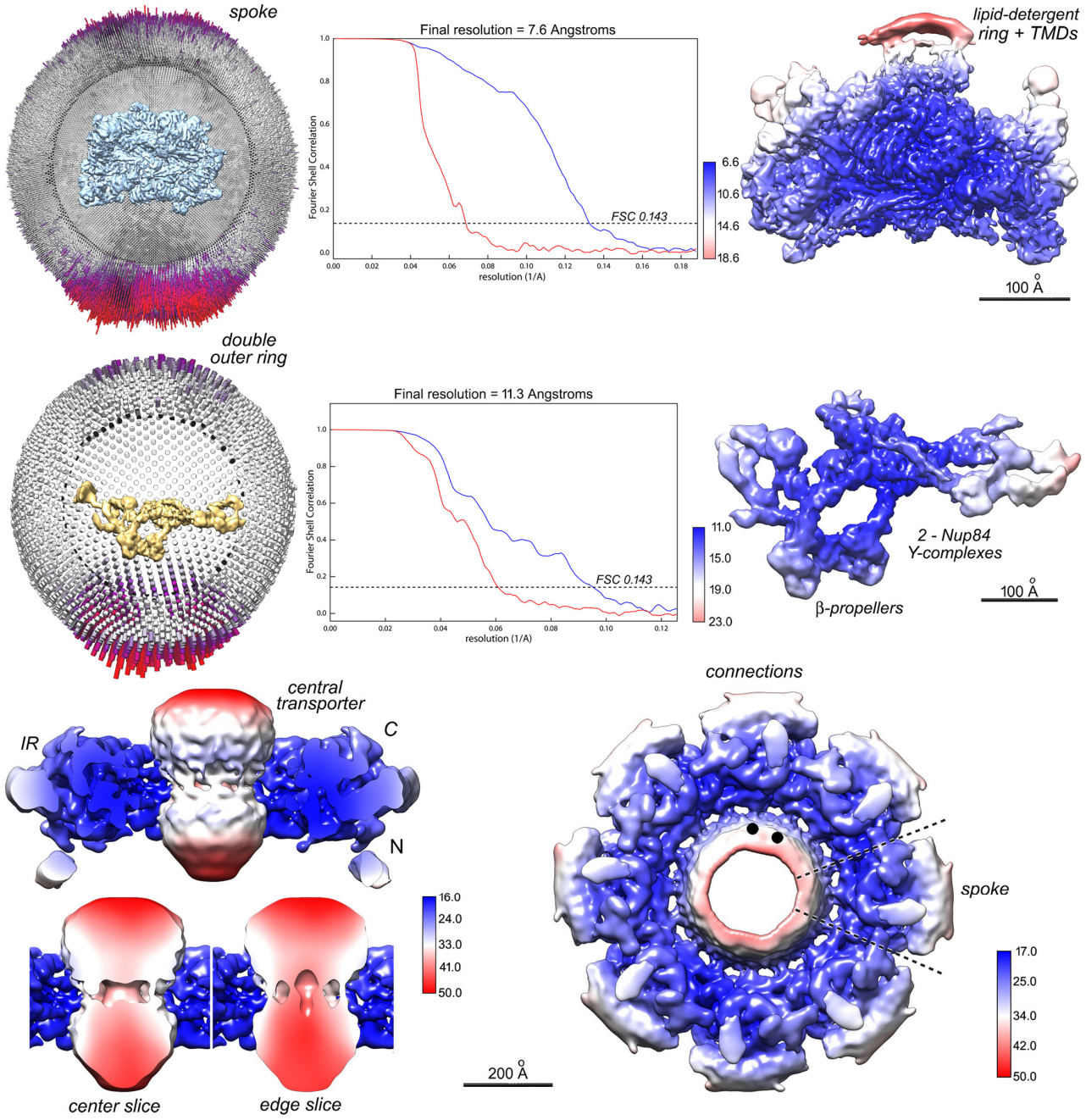
Processing scheme 1. Single particle NPCs.

(top left) Affinity purified NPCs were titrated with increasing concentrations of a bifunctional crosslinker (DSS) to stabilize flexible regions. (lower panels) Micrographs of DSS crosslinked yeast NPCs imaged on a carbon film supported over holes. Images were taken at 160 kV on a Tecnai F20 microscope. (left) NPCs in thinner ice with top and tilted views. (right) NPCs with a larger distribution of tilted and edge-on views. A central, ring-like transporter (T) is visible in most particles. (top right) 2D class averages with a range of views. Low density features may represent more flexible regions.



Processing scheme 2. Single particle analysis flow chart.

Resolution for the spoke and double outer ring improved ~3-fold after multi-body and downstream processing. (**top**) Images of an initial single particle structure of the NPC, along with the inner and outer rings aligned with C8 symmetry and a focused mask. (**bottom**) Recombined 3D maps after two iterations of multi-body processing. Note scale difference. A triangular feature (within dashed lines) in the outer ring is resolved as two overlapping V-shaped regions of Y-complexes in the double ring.



Processing scheme 3. Resolution estimates of individual component 3D maps.

(top row) Angular distribution for C1 refinement of a 1.5x spoke volume with inset map. The 8-fold axis is roughly vertical. A range of preferred orientations is apparent that may be due to basket interactions with the carbon film. However, the angular resolution is isotropic due to the distribution of poses (displayed in Chimera). (top center) Fourier shell curve for the spoke. Masked curve in blue, phase randomized curve in red. (top right) Local resolution calculated in RELION on the tilted spoke isosurface. (middle row) Angular distribution for C1 refinement of the double outer ring: inset map in gold with roughly 1.5x rotational subunits. Fourier shell curve for the double outer ring calculated with a generous mask. Local resolution calculated plotted on the double outer ring. Central dimples in each β-propeller are present (bottom left) Local resolution for a global 3D map of the central transporter and inner ring with two cross-sections through the transporter. Note the radial and vertical resolution gradient within this feature. (bottom right) Focused 3D refinement gives a local resolution of ~20-25 Å for connections to the central transporter (features above the black dots).