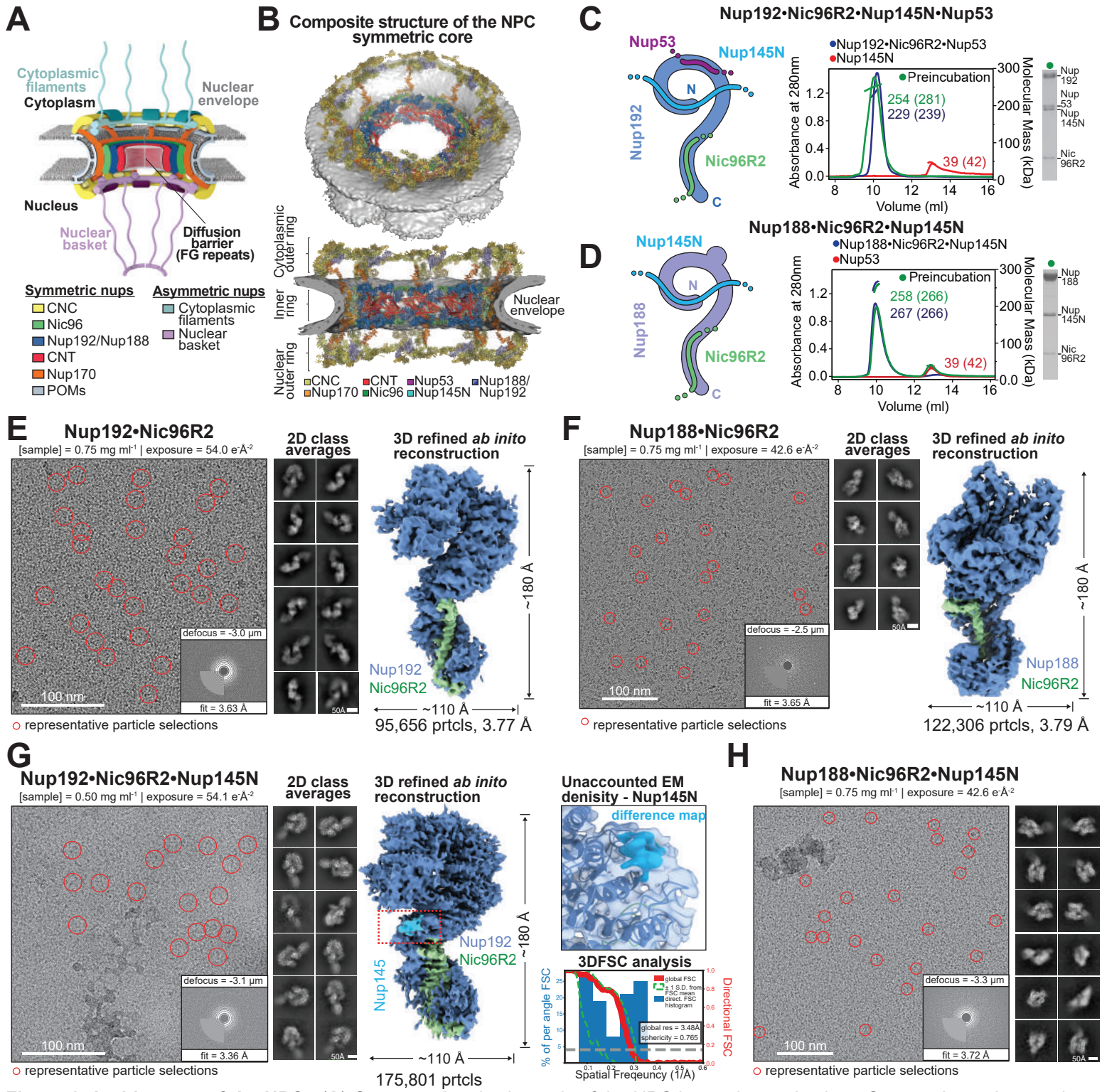

Figure 1. Architecture of the NPC. (**A**) Cross-sectional schematic of the NPC layered organization. Coat nucleoporin complexes (CNCs, yellow) form outer rings on top of the membrane on the cytoplasmic and nuclear face of the nuclear envelope. The inner ring, which supports the central transport channel and diffusion barrier, is connected to the CNC by bridging Nup170 nucleoporins (orange). Large structured domains of Nup170, Nic96 (green), Nup192/Nup188 (blue) and the channel nucleoporin trimer (CNT) organize coaxial layers within the inner ring. POMs are integral membrane proteins associated with the NPC. Asymmetric portions of the NPC are attached on either side of the nuclear envelope. (**B**) Composite structure of the NPC symmetric core generated by docking nucleoporin and nucleoporin subcomplex crystal structures into the cryo-ET reconstruction of the intact human NPC (EMD-3103). (**C**, **D**) SEC-MALS profiles and SDS-PAGE gels illustrating the reconstitution of inner ring subcomplexes organized around Nup192 and Nup188, respectively. The experimental mass measurement is indicated next to the peak, with the theoretical mass reported in parenthesis. Nup192 binds to Nup53, unlike Nup188. (**E-H**) Representative raw micrographs of inner ring subcomplexes with indicated representative particles (red circles). CTF estimate fits (bottom right) along with representative 2D class averages of the entire dataset are reported (right). When available, we report the refined *ab initio* reconstruction of the imaged complex, and report the number of particles and gold standard FSC resolution estimate. (**G**) In particular, we identify EM density unaccounted for by the Nup192 and Nic96R2 models in an anisotropic map of the Nup192•Nic96R2•Nup145N•Nup53 reconstruction that likely corresponds to Nup145N, based on previous biochemical evidence. The 3D Fourier Shell Correlation (3DFSC) analysis indicates severe anisotropy in the reconstruction.