Supplement

Structure of the c_{10} Ring of the Yeast Mitochondrial ATP Synthase in the Open Conformation

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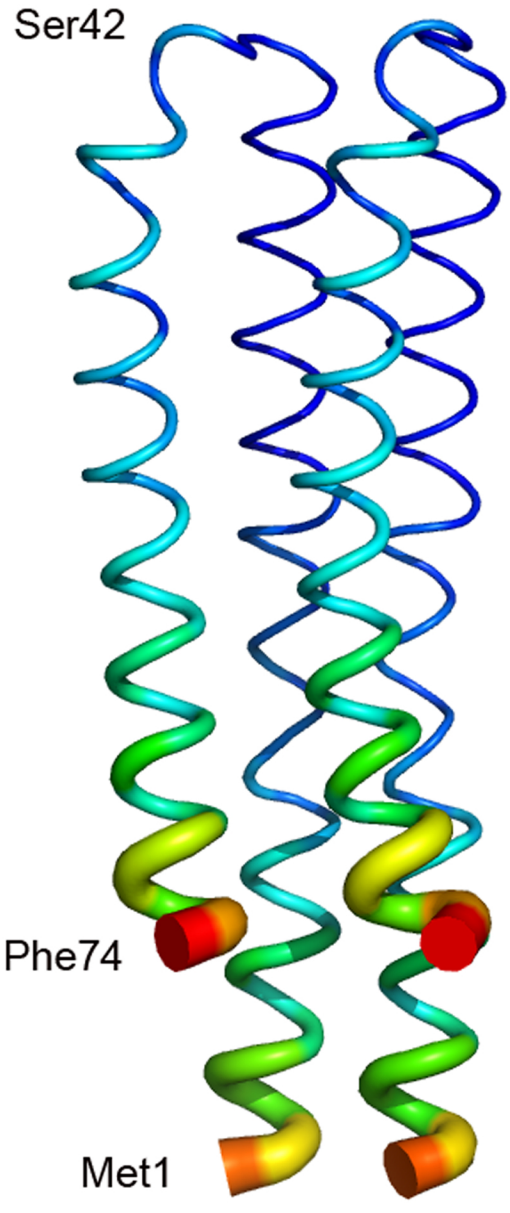
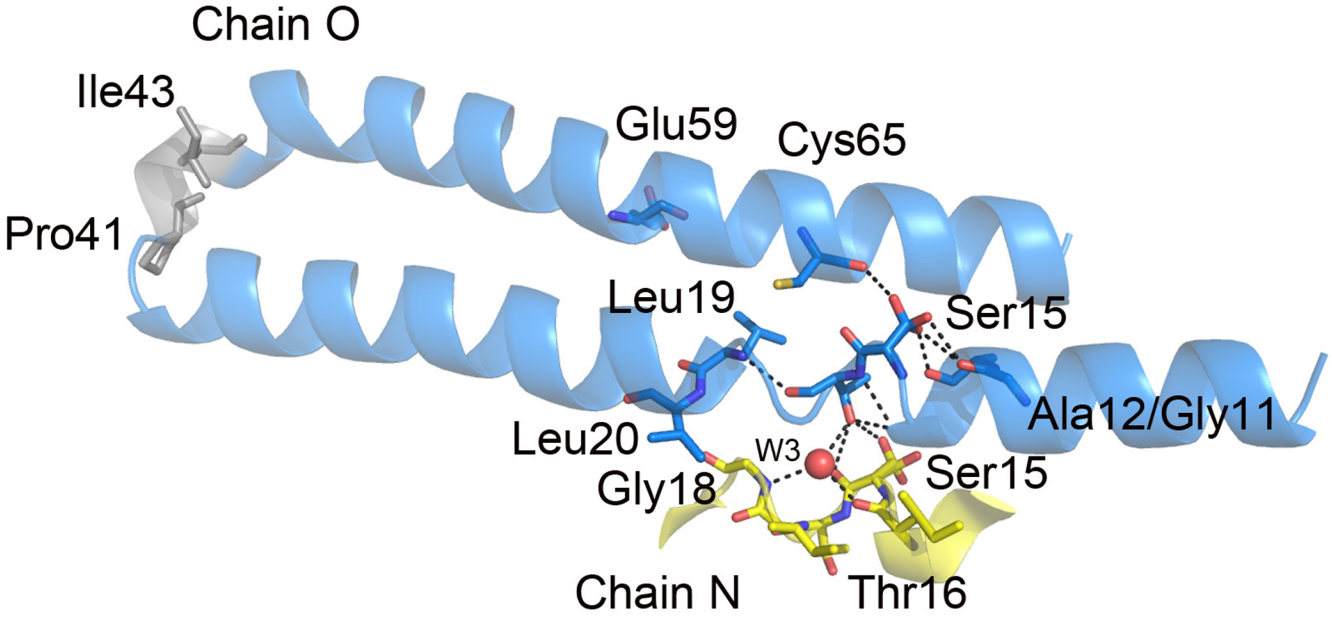
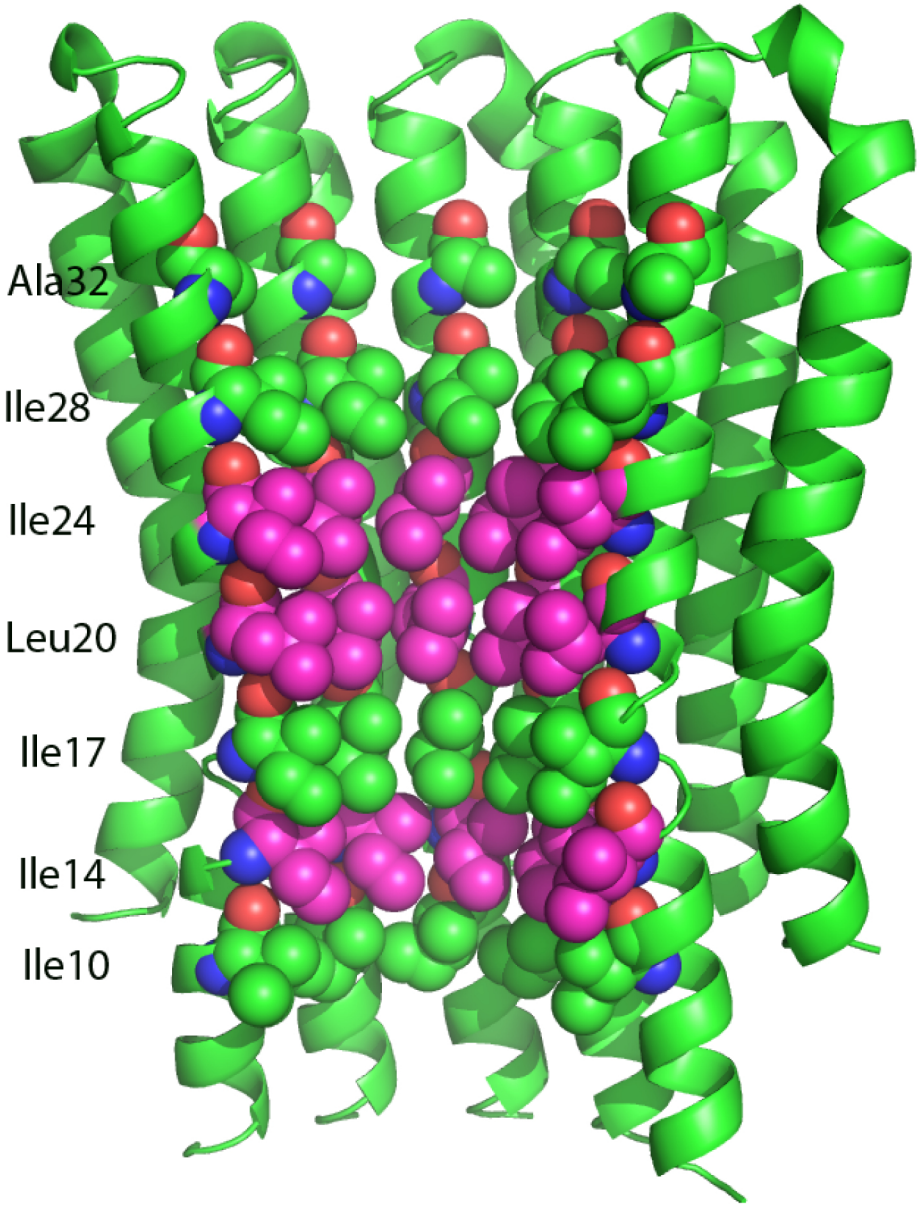


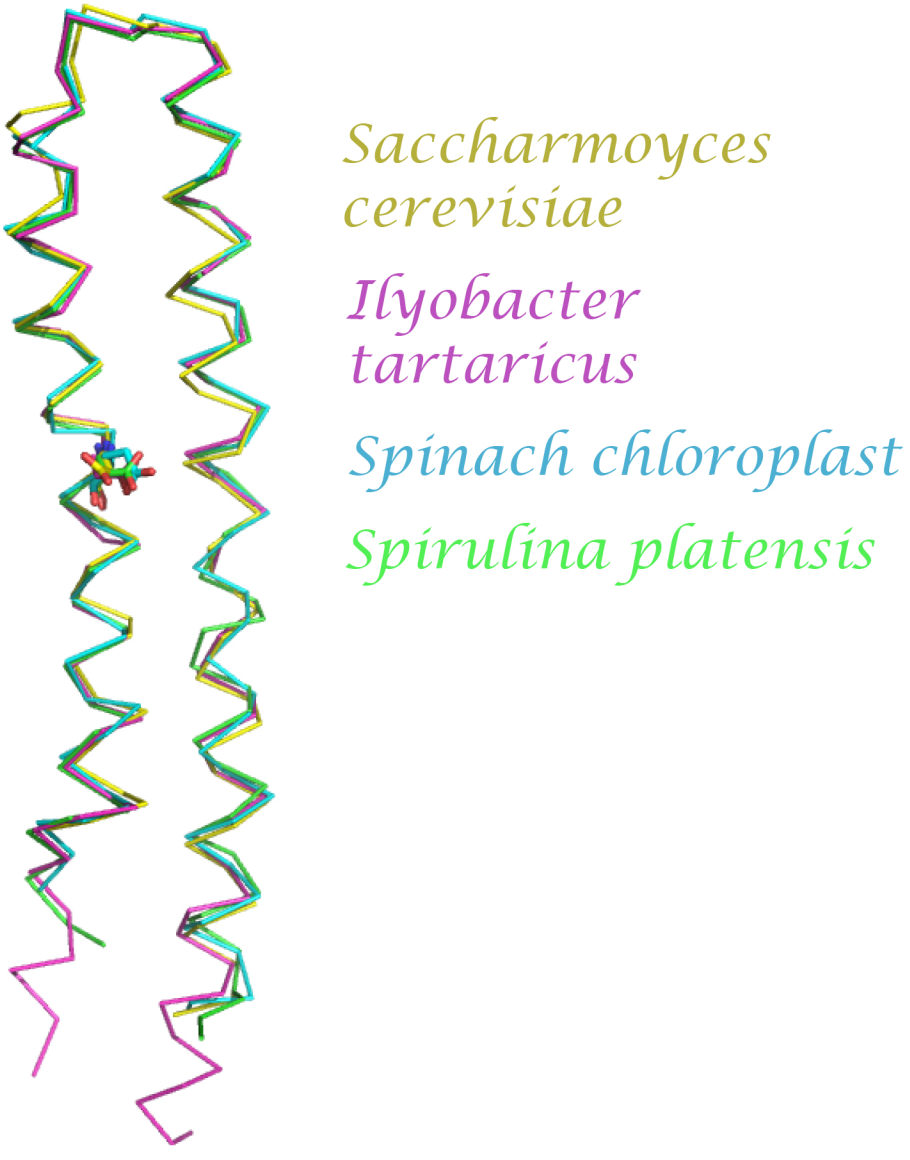
Supplement Figure 1. Distribution of B-factors along the c-subunit structure. B-factors for the main chain atoms are shown as a colored spectrum and mapped on a cartoon representation of the c-subunit, with blue representing the lowest B-factor and red the highest. In addition, the width of the cartoon is graduated with the lowest B-factor having the smallest diameter and that with the highest B-factor having the largest diameter. The B-factors is 18 for C of Ser 42 and 50 for Cα of Phe74 in chain K.



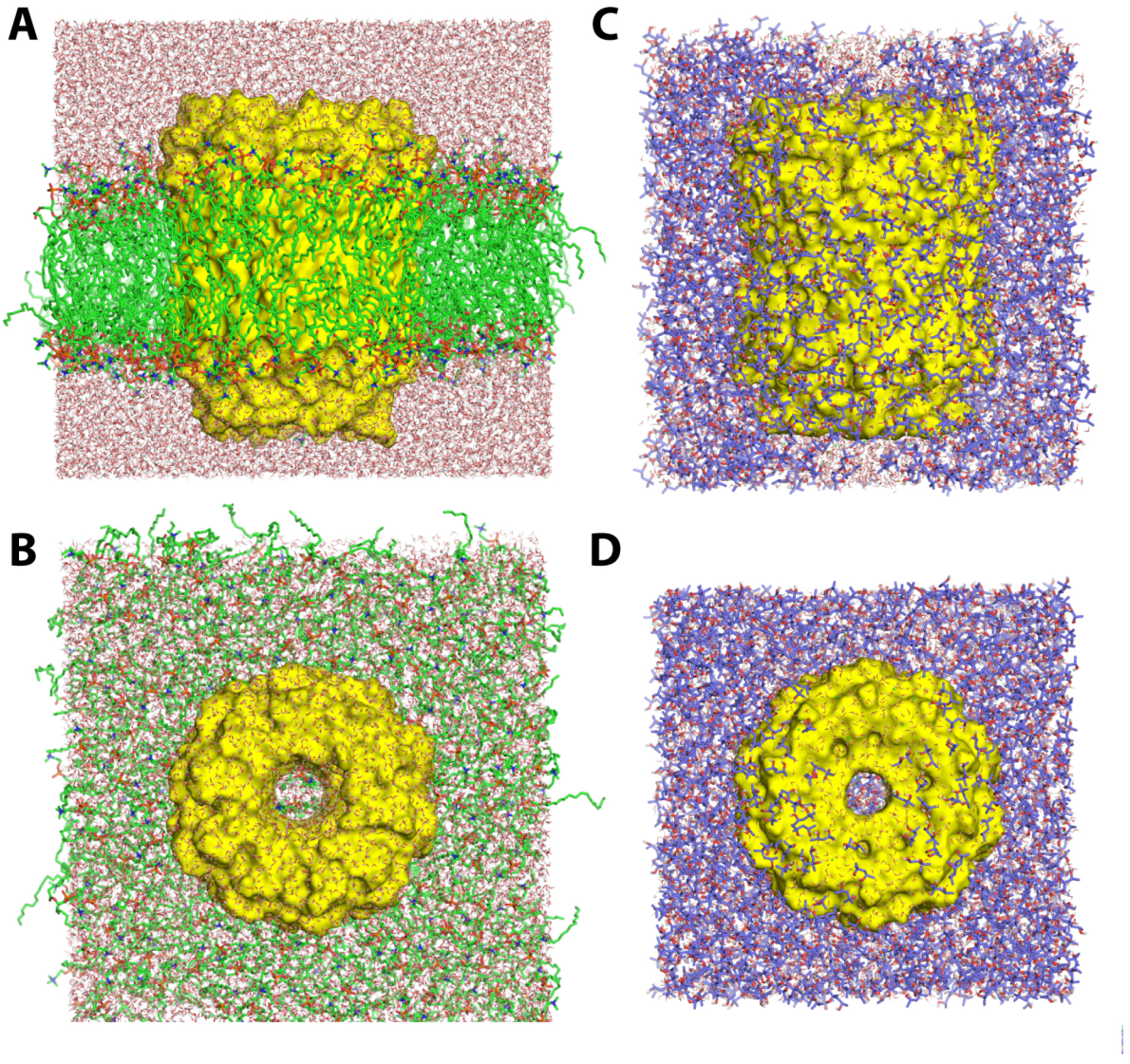
Supplement Figure 2. Secondary structure of subunit c. Subunit c consists of 4 helical fragments. The first helix extends from residues 3-14, is followed by a 3_{10} helix within residues 15-18, and by a second helix from residues 19-39. These helices form the N-terminal transmembrane span. After a sharp turn, there is a second short 3_{10} helix from residues 41-43, followed by the C-terminal helix from residues 44-73, which traverses the membrane. The H-bonded turn in the N-terminal span features H-bonding interactions to a water molecule associated with Thr16. This water molecule appears to bridge interactions between adjacent c-subunits (blue, yellow). Ser15 is shown in the three rotamer conformations revealed by the electron density.



Supplement Figure 3. Interhelical packing of the inner ring. Cartoon representation of five c-subunits, highlighting residues in the inner face of the ring that form hydrophobic contacts. The residues colored in magenta (14, 20, 24) are conserved in the animal kingdom as Ala, and postulated to be required for forming a c_8 ring[1]. Residues 10, 17, and 28 appear to be equally important in stabilizing this c-ring.



Supplement Figure 4. Conformational differences between species of c-rings. Shown is a comparison of the overall structure of the c-subunit from the c_{10} ring of yeast (yellow) with that of three other species, as indicated. The c-subunits were overlaid by fitting between the residues in the mitochondrial c-subunit with their equivalent in the other species.



Supplement Figure 5. Simulation systems. A, **B**: The c_{10} ring (yellow surface) embedded in a model phospholipid membrane, viewed laterally and from the intermembrane space, respectively. The simulation box, whose approximate dimensions are 100 × 100 × 92 Å, contains 239 lipids (green, blue, red sticks) and ~17,500 water molecules, i.e. ~96,000 atoms in total. **C**, **D**: Analogous views of the c_{10} ring, in a solution that mimics the crystallization buffer (70% MPD, 300mM NaCl). The simulation box is approx. 84 × 84 × 81 Å, and contains 1,524 MPD molecules (purple, red sticks), ~4,700 water molecules and 34 sodium and chloride ions (not shown), i.e. ~60,000 atoms in total. Non-polar hydrogen atoms are omitted in all panels, for clarity. All simulations were carried out with NAMD[2], using the CHARMM27/CMAP force field[3,4] using periodic-boundary conditions. Temperature and pressure were kept constant, at 298 K and 1 atm, respectively. In the lipid system, the surface area of the membrane was also constant (~69 Å^2 per lipid). Electrostatic interactions were computed with the Particle-Mesh-Ewald algorithm. A cut-off of 12 Å was used for van der Waals interactions.

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2. Phillips, J.C. et al. Scalable molecular dynamics with NAMD. *J Comput Chem* **26**, 1781-802 (2005).
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