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Supporting online material

Structure of the rotor ring of F-type Na^+-ATPase from *Ilyobacter tartaricus*

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Materials and Methods

ATP synthase was isolated from wild type *Ilyobacter tartaricus* cells. After disrupting the complex with N-lauroylsarcosine, pure c ring was obtained by precipication of all other

subunits of the enzyme with ammonium sulfate (*1*). The purified c ring was dialyzed against 10 mM Tris/HCl buffer (pH 8.0) to precipitate the protein by removal of the detergent. The material was collected by centrifugation and dissolved at 10 mg protein/ml in dialysis buffer containing 0.02% (w/v) NaN_3 and 0.78% (w/v) Zwittergent 3-12 (Calbiochem, La Jolla, CA, USA). Crystals were grown within 2-3 days at 17°C to a size of approximately 400 x 100 x 100 μm^3 using the vapor diffusion method in hanging drops. A drop consisted of 1 µl protein solution (passed through a 0.22 µm sterile filter) and 1 µl crystallization buffer containing 15% (v/v) polyethylene glycol 400 in 0.1 M sodium acetate buffer, pH 4.5. The crystals were flash-frozen in liquid nitrogen after an equilibration for 24 h with 30% (v/v) polyethylene glycol 400 as cryo-protectant in the reservoir buffer at 17°C. Data used for structure solution were collected at the beamline X06SA of the Swiss Light Source (SLS, Switzerland) and the structure was refined against 2.4 Å data obtained at beamline ID29 of ESRF (France).

figure legends

fig. S1. Motional flexibility within the c ring. The low frequency normal modes of the c ring were analyzed according to (*3*). The data presented show two different views of the c ring from *I. tartaricus* ATP synthase in the lowest-order nontrivial normal mode.

fig. S2. Van der Waals surface of the c ring. The surface is colored light-grey for apolar, yellow for polar, red for acidic and blue for basic surface exposed residues. A: The outer ring surface is shown. A putative DCCD-binding pocket is indicated by an arrow. B: The proximal half of the ring is removed to view the inner surface and some of the helices are shown in ribbon representation for clarity. Dashed lines near Phe^{5} and Tyr^{34} mark the putative positions of polar/apolar interfaces of the inner lipid bilayer. The cavity below the binding site is marked by an arrow.

fig. S3. Ribbon model of the *E. coli* c_{10} oligomer obtained by homology modeling (*4*) according to the *I. tartaricus* c_{11} structure. The residues which form cross-links with subunit a after cysteine substitution and the proton binding Asp^{61} are also shown.

fig. S4. Schematic model for the interconversion of the binding site in the subunit a/c interface from an alternately locked conformation to an open one. The view is from the cytoplasmic side on the section of the interface at the level of the binding sites. The side chain of Glu^{65} locks the Na^{+} ion (yellow circle) in the binding site by its orientation approximately horizontal to the membrane plane (Fig. 3). We propose that a site entering

the interface as indicated by the solid arrow encounters electrostatic interactions upon approaching Arg^{227} in two ways: the Na^+ is repelled into the cytoplasmic outlet path (i.e. in a plane perpendicular to the membrane) and the Glu^{65} is attracted, maintaining approximately its original conformation. Please note that the Glu^{65} is on the distal helix with respect to the Arg^{227} of the functional unit if rotation proceeds in the ATP synthesis direction. After rotation of the binding site to the opposite side of Arg^{227}, Glu^{65} is pulled backwards towards the arginine thus opening the gate to the periplasmic inlet channel. Na^+ ions can now pass from subunit a to subunit c (dashed arrow) and the locked conformation of the binding site reforms when the site moves on and the electrostatic attraction between Arg^{227} and Glu^{65} is attenuated. The reverse order of events takes place in the ATP hydrolysis direction.

References

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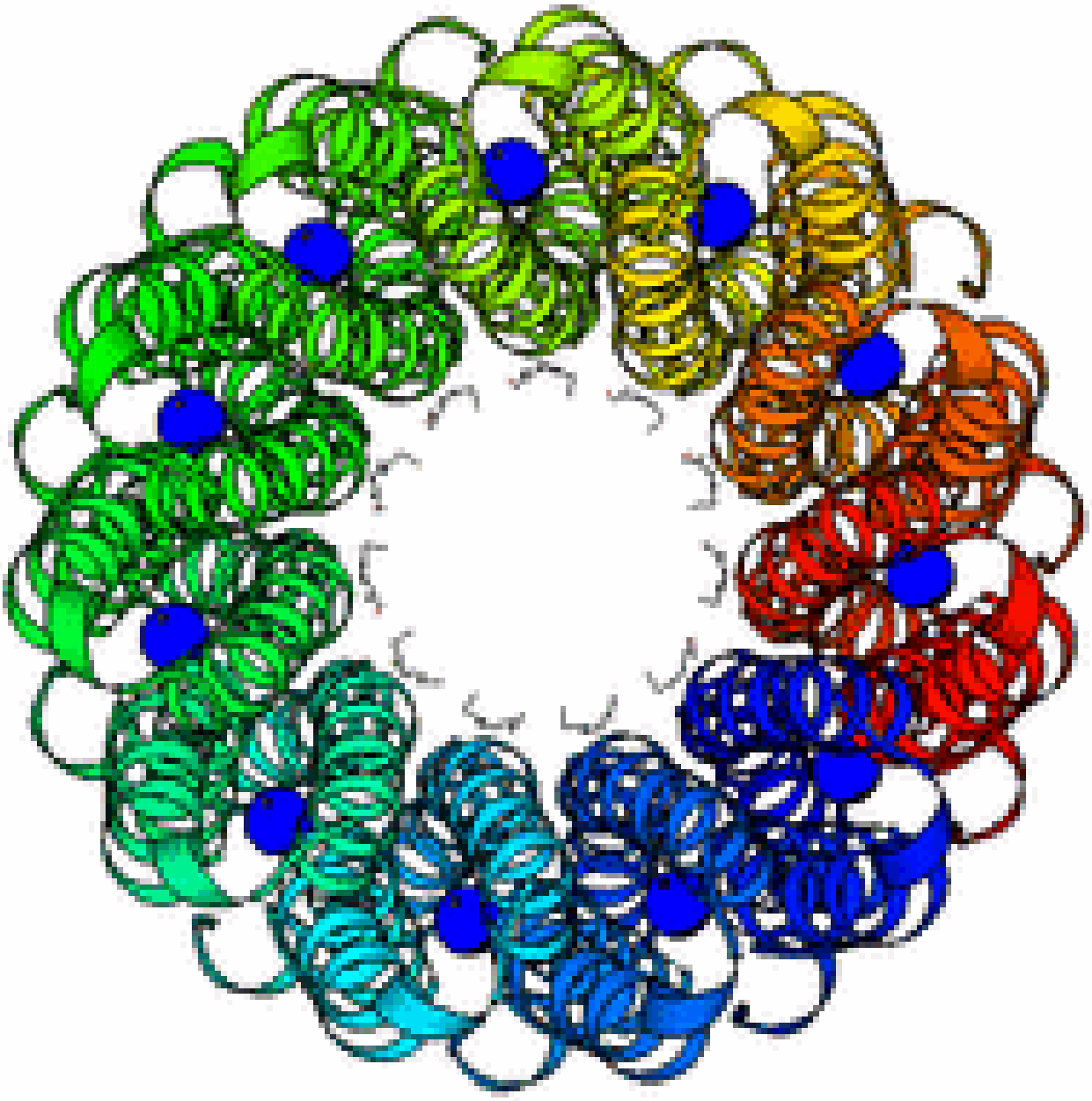
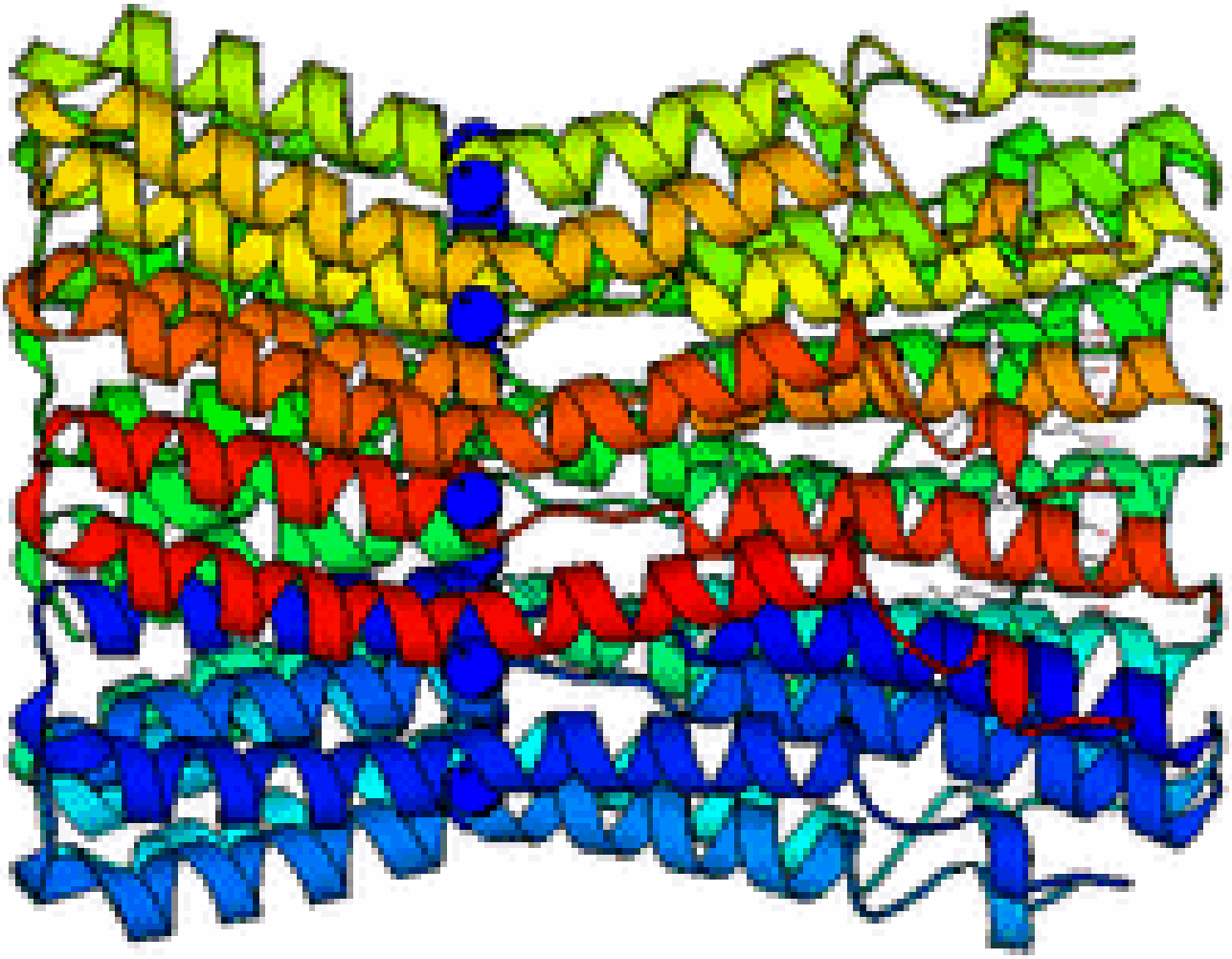
table S1. Summary of data collection and refinement statistics

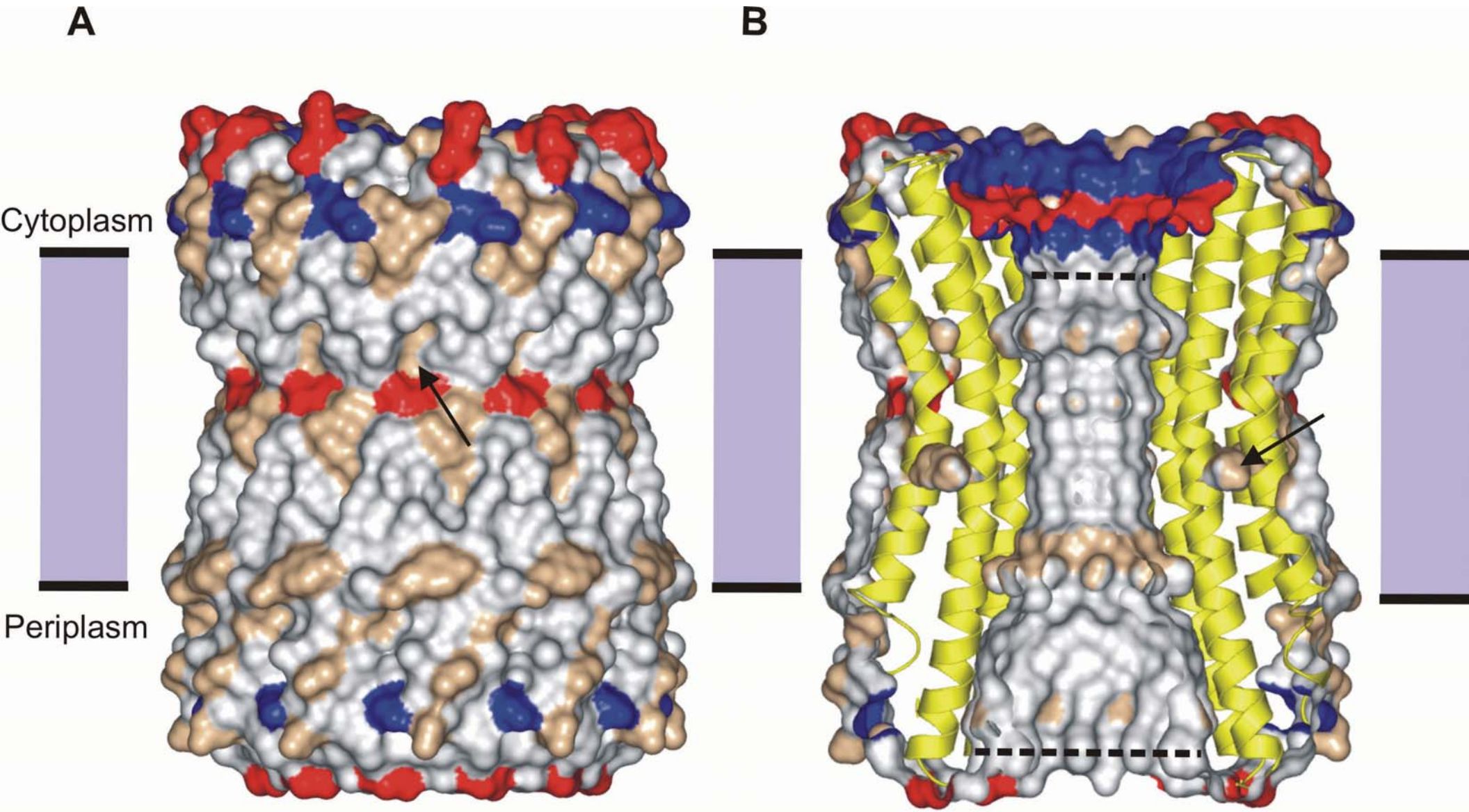
Data Processing	
Wavelength [Å]	0.9756
Spacegroup	$P2_1$
Unit cell parameters [Å]	a=147.7 b=140.0 c=153.0 α=γ=90° β=118.4°
Solvent content [%]	65.1
Resolution [Å]	10.0-2.4 [2.5-2.4]
No. of observed reflections	2284602 [165501]
No. of unique reflections	209310 [23376]
Completeness [%]	97.9 [95.2]
R_{meas} [%](*2*)	18.3 [63.5]
R_{mrgd-F} [%](*2*)	12.8 [48.7]
I/σ_I	10.0 [3.3]

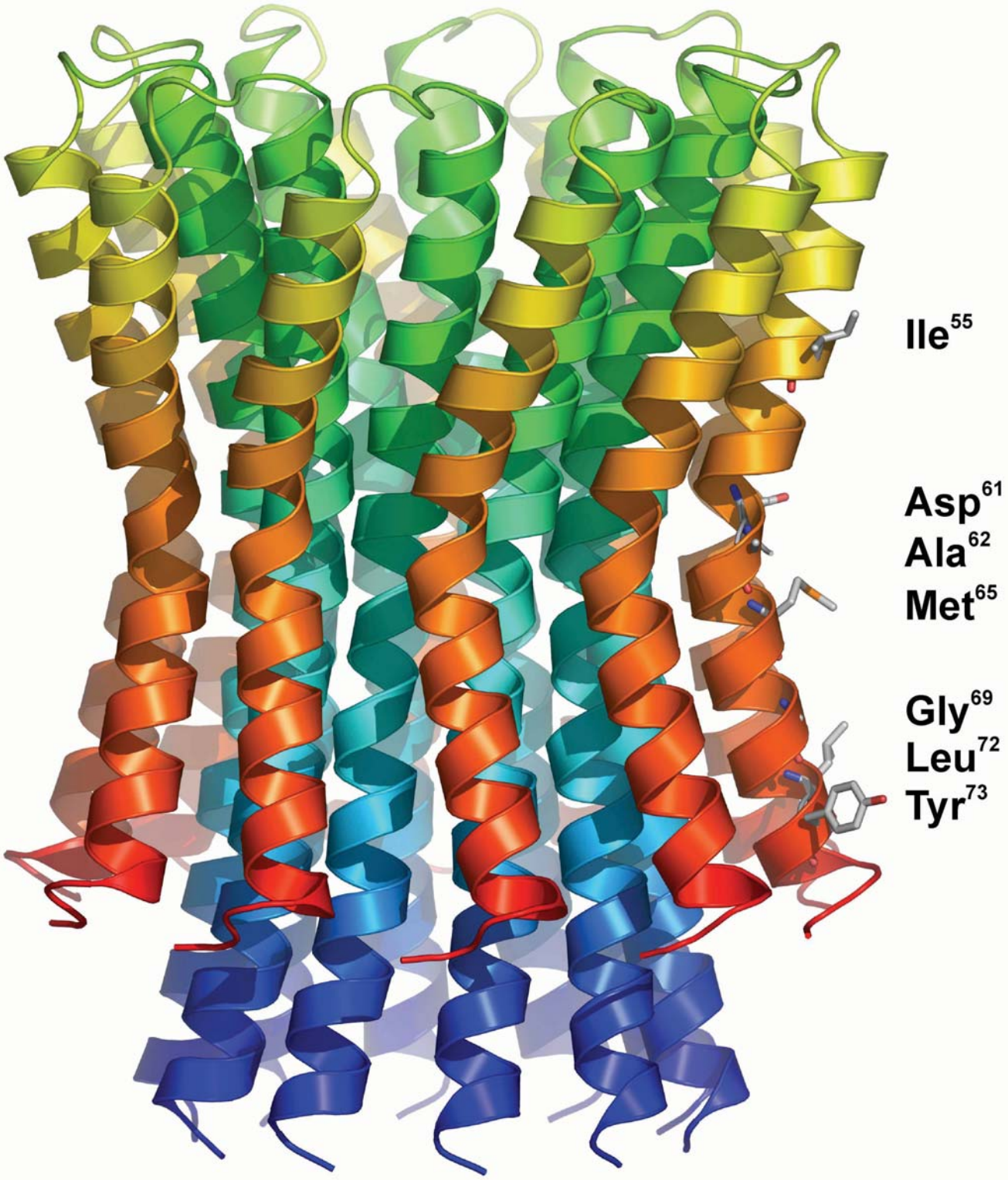
Refinement statistics	
Resolution [Å]	10.0-2.4
R-factor	20.1%
R_{free}	24.6%
No. of residues	3916
No. of solvent waters	513
No. of ions	44
No. of fatty acid chains	44
r.m.s. deviation of bond length [Å]	0.021
r.m.s. deviation of bond angles [°]	1.910

Numbers in brackets correspond to the highest resolution shell.






A
B
Cytoplasm
Periplasm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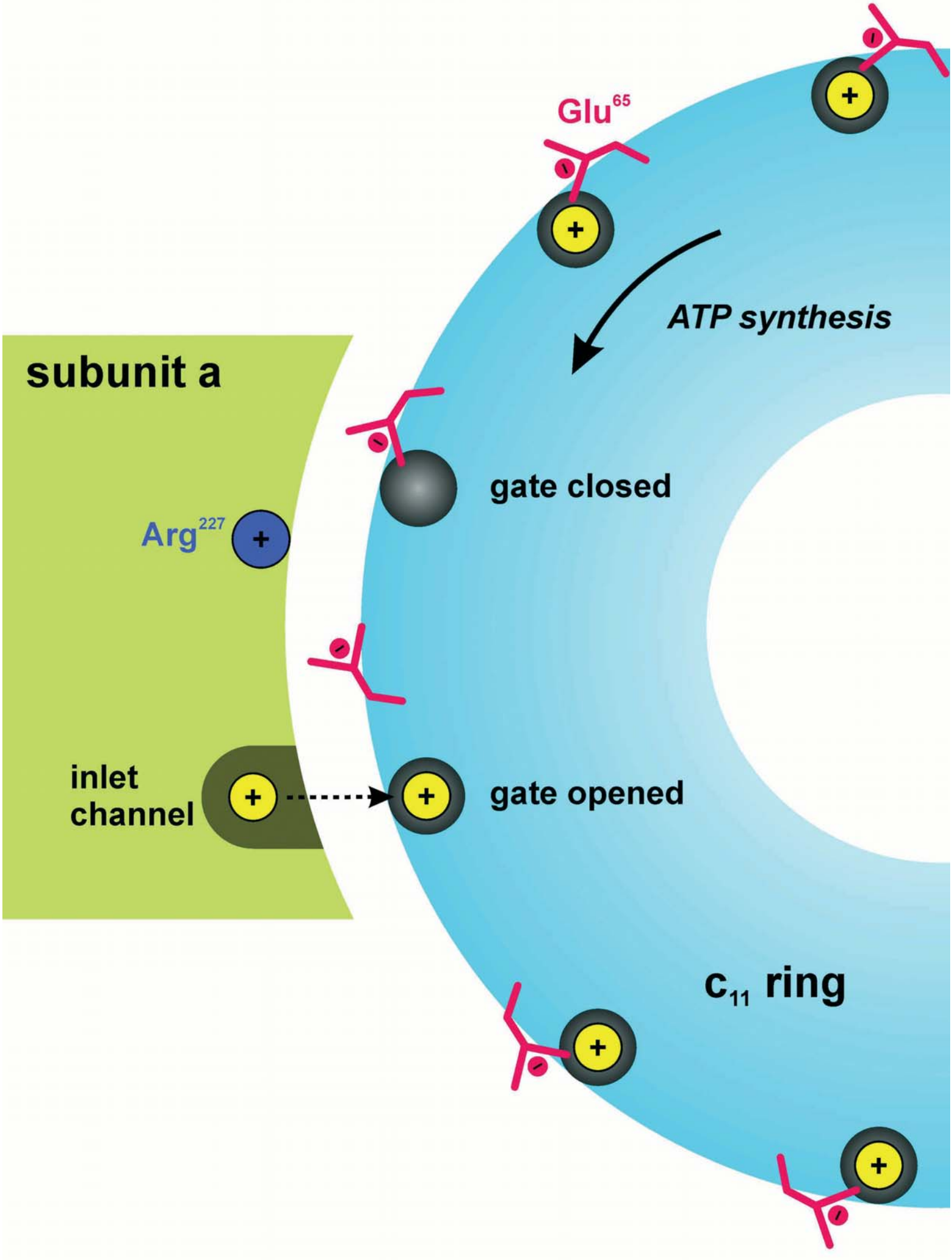
Met65
Gly69
Leu72
Tyr73


Glu65
ATP synthesis
subunit a
gate closed
Arg227
inlet
channel
gate opened
c11 ring