physiology as they control ionic conditions in the cell and energise osmotic potentials, secondary transport schemes and ionotropic signalling. A surprising finding from the $Na^+$,$K^+$-ATPase structure was the docking of two conserved tyrosine residues at the C-terminus of the alpha subunit into the transmembrane domain, hinting that this was a previously unidentified regulatory element. Several mutations causing human neurological syndromes have subsequently been mapped to the C-terminal structure element, also clearly indicating that conservation of the structure is important for pump function. Mutational analysis confirmed this and prompted our further analysis by electrophysiology and molecular dynamics simulations, which have shown a profound effect of the C-terminus on the electrogenic transport properties. We further propose that the C-terminal region forms a binding pocket that can be exploited for pharmacological intervention in cardiovascular and neurological disease.

doi:10.1016/j.bbabio.2010.04.097

## Posters

### 2P.1 The role of the N-terminus domain of $F_OF_1$ inhibitory peptide from *Saccharomyces cerevisiae*: A kinetic approach

Tiona Andrianaivomananjaona, Francis Haraux
*Service de Bioénergétique, Biologie Structurale et Mécanismes, iBiTec-S and CNRS-URA 2096, CEA Saclay, F 91191 Gif-sur-Yvette, France*
*E-mail:* tiona.andrianaivomananjaona@cea.fr

In deenergized mitochondria, a small regulatory peptide called $IF_1$ ($bIF_1$ in bovine, $yIF_1$ in yeast) binds to ATP synthase and blocks ATP hydrolysis. In crystallized bovine $F_1$-ATPase in complex with $IF_1$, residues 22–49 of bIF1 are locked at an αβ catalytic interface, while residues 8–18 mainly interact with γ [1]. It was shown that deletion of residues 1–13 of $bIF_1$ did not affect its affinity for the enzyme [2], while deletion of residues 1–17 [2] or 1–21 [3] seriously decreased it. We have examined the consequences of length changes in the N-terminus part of $yIF_1$ on the kinetics of inhibition of isolated $F_1$-ATPase. Determination of the rate constant of $IF_1$ binding to $F_1$ ($k_{on}$) [4] and of its rate constant of dissociation from the complex ($k_{off}$) allowed to discriminate effects of $yIF_1$ modifications on binding site recognition and on the inhibited complex stability. Deletion of $yIF_1$ residues 1–13 (corresponding to $bIF_1$ residues 1–18) changed neither $k_{on}$, nor $k_{off}$. Deletion of residues 1–14 did not change $k_{on}$ and slightly increased $k_{off}$. Deletion of residues 1–15 increased $k_{on}$ by a factor 2 and $k_{off}$ at least by a factor 20. We propose that residues 1–15 of $yIF_1$ do not play any role in molecular recognition and might even hamper it. On the other hand, residues 14–15 (but not residues 1–13) participate in the stabilization of $F_1$–$IF_1$ complex. Quite surprisingly, considerable lengthening of the N-terminus domain (by 44 residues) did not affect $k_{on}$ despite the expected increase of steric hindrance. It increased $k_{off}$ more than 10 fold. We conclude that $IF_1$ probably approaches the catalytic interface with its N-terminus tail folded back. Once $IF_1$ is bound by its medium domain to α and β subunits, the N-terminus spreads around the γ subunit. We are currently checking more in depth this model by attaching a small globular protein to the N-terminus domain of $yIF_1$. Our results also suggest that γ subunit, that interacts with the N-terminus part of $IF_1$, plays a minor role in the inhibition mechanism.

#### References

[1] Gledhill JR *et al.* (2007) *Proc. Natl. Acad. Sci. USA* **104:** 15671–15676.
[2] van Raaij MJ *et al.* (1996) *Biochemistry* **35:** 15618–15625.
[3] Lebowitz MS, Pedersen PL (1996) *Arch. Biochem. Biophys.* **330:** 342–354.
[4] Corvest V *et al.* (2005) *J. Biol. Chem.* **280:** 9927–9936.

doi:10.1016/j.bbabio.2010.04.098

### 2P.2 Mycobacterial ATP synthase as drug target

Anna C. Haagsma[1], Marc Hahn[1], Anil Koul[2], Holger Lill[1], Dirk Bald[1]
[1]*VU University Amsterdam, Department of Molecular Cell Biology, Faculty of Earth- and Life Sciences, The Netherlands*
[2]*Tibotec NV, Johnson & Johnson Pharmaceutical Research and Development, Department of Antimicrobial Research, Belgium*
*E-mail:* dirk.bald@falw.vu.nl

Recently, ATP synthase has come into focus as a novel antibiotic target. Previously we showed that diarylquinolines, a new class of anti-tuberculosis compounds presently in phase III clinical tests, efficiently kill *Mycobacterium tuberculosis* by inhibiting ATP synthase [1, 2]. Diarylquinolines are highly selective, we found that the lead compound of this drug class inhibits mycobacterial ATP synthesis at nanomolar concentrations ($IC_{50}<10$ nM), but has only a minimal effect on ATP synthesis in human mitochondria ($IC_{50}>200$ μM) [3]. These results demonstrate that proteins of energy metabolism, although conserved among prokaryotes and eukaryotes, can nevertheless be used as efficient antibiotic targets. The molecular basis for the observed selectivity is presently under investigation in our laboratory. The affinity of ATP synthase for diarylquinolines decreased significantly in the presence of high salt concentrations, indicating that electrostatic interactions play an important role in binding of this inhibitor. However, competition experiments showed that diarylquinolines do not directly compete with protons for the same binding site. Pinpointing the binding niche of this drug is under way in and recent progress will be reported. As affinity of several ATP synthase inhibitors depends on this enzyme's mode of action (ATP synthesis/high PMF versus ATP hydrolysis/low PMF mode) we investigated the function of ATP synthase in two mycobacterial strains. Whereas inverted membrane vesicles were clearly active in ATP synthesis, they were unable to set-up a proton motive force with ATP. These results show that mycobacterial ATP synthase is strongly blocked in ATP hydrolysis mode. The physiological function of this enzyme appears to be synthesis of ATP, not maintenance of the proton motive force.

#### References

[1] Koul *et al.* (2007) *Nat. Chem. Biol.* **3:** 323–324.
[2] Koul *et al.* (2008) *J. Biol. Chem.* **283:** 25273–25280.
[3] Haagsma *et al.* (2009) *Antimicrob. Agents Chemother.* **53:** 1290–1292.

doi:10.1016/j.bbabio.2010.04.099

### 2P.3 Comparison of high resolution structures of $F_1$ ATP synthase from mitochondria. Implications for the catalytic cycle of the enzyme

Ekaterina A. Baranova[1], Martin G. Montgomery[1], Andrew G.W. Leslie[2], John E. Walker[1]
[1]*The Medical Research Council Mitochondrial Biology Unit, Hills Road, Cambridge, CB2 2XY, UK*
[2]*The Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK*
*E-mail:* walker@mrc-mbu.cam.ac.uk

More than 20 high resolution structures of mitochondrial $F_1$-ATPase have been obtained since the first structure was described in 1994 [1]. These structures have provided the basis for the description of the conformations of the three catalytic β-subunits in the ground state [2] and in a transition state intermediate during the catalytic cycle [3]. We have compared the crystal structures of $F_1$-ATPases from bovine and yeast mitochondria, obtained with crystals with various space groups and unit cell sizes. We have examined the way that $F_1$-complexes are packed in the crystal lattices to determine whether the