Supplementary Information for:

Ionophoric effects of the antitubercular drug bedaquiline

Kiel Hards[a], Duncan G. G. McMillan[b], Lici A. Schurig-Briccio[c], Robert B. Gennis[c], Holger Lill[d], Dirk Bald[d], Gregory M. Cook[a,e*]

[a]Department of Microbiology and Immunology, School of Biomedical Sciences, University of Otago, Dunedin 9054, New Zealand

[b]Department of Biotechnology, Delft University of Technology, 2629 HZ Delft, The Netherlands

[c]Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, Illinos 61801, United States

[d]Department of Molecular Cell Biology, Amsterdam Institute for Molecules, Medicines and Systems, VU University Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

[e]Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Private Bag 92019, Auckland 1042, New Zealand

*To whom correspondence should be addressed. Email: gregory.cook@otago.ac.nz

www.pnas.org/cgi/doi/10.1073/pnas.1803723115

Supporting Materials and Methods

Bacterial strains, media and growth conditions.

In this study, either *E. coli* MG1655 (*E. coli* Genetic Stock Center (CGSC 6300); wild-type), C41(DE3) with a markerless inframe deletion in the *unc* operon ((1); C41 Δ*atp*), C41 Δ*atp* + the previously described his-tagged F_1F_o expression plasmid pBWU13 ((2); C41 Δ*atp* pBWUβHis) or DK8 Δ*atp* pBWU13 (3) were used as indicated. Strains were grown in LB medium or on LB agar (1.5 % agar) for all experiments. All bacterial growth was performed at 37 °C with agitation (200 rpm). All inoculations were performed from overnight cultures to a final optical density at 600 nm (OD_{600}) = 0.005. BDQ was a kind gift of Dr. Koen Andries, Janssen Pharmaceutical Research & Development. All BDQ stocks were prepared in filter sterilized DMSO.

Determination of cell growth inhibition.

The minimum inhibitory concentration (MIC) is defined here as the lowest concentration with no visual growth after 24 hours of growth in 200 µL LB media in a flat bottom 96 well-plate. The final DMSO concentration in all wells was 1%.

Preparation of inverted membrane vesicles (IMVs)

E. coli strains were grown in 2 L conical flasks, containing 1 L of media, overnight at 37 ˚C. Approximately 5 g wet weight of cells were collected by centrifugation (30 min, 8000 × *g*) and resuspended in 50 mL of buffer A (50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM DTT, 1 Roche cOmplete™ mini protease inhibitor tablet per 50 mL, 1 mg DNase per 50 mL). Cells were disrupted by two passages through a Stansted Fluid Power French pressure cell at 10,000 psi. Cell debris was removed by discarding the pellet after centrifugation (10 min, 15,000 × *g*). The supernatant was centrifuged (45 min, 200,000 × *g*) to collect IMVs. The membrane pellets were resuspended to a protein concentration of 25 mg mL^{-1} in buffer A + 10% glycerol and stored in aliquots at -80 °C until required.

ATP synthesis and hydrolysis assays in IMVs

End-point ATP synthesis measurements were performed using the Glucoe-6-phosphate dehydrogenase method (G6PDH) (4). A proton motive force was set-up by addition of NADH and the produced ATP was quantified with hexokinase/G6PDH. All samples contained 0.2 mg

mL^{-1} membrane protein in MOPS buffer (50mM MOPS, 2mM $MgCl_2$, pH 7.5) including protease inhibitors (Roche, EDTA free, 1 tablet per 50 ml), 11.8 U mL^{-1} hexokinase, 100 μM diadenosine pentaphosphate (Ap5a), 2 mM ADP, 25.4 mM glucose and 20 mM KH_2PO_4. Samples containing BDQ or DCCD were preincubated 15 minutes at room temperature before the start of the reaction. All reactions were initiated with 5 mM NADH (final concentration) and incubated at 37 ^oC, with agitation at 300 rpm, for 20 minutes. Afterwards, reactions were arrested with 25 mM EDTA (final concentration) and transferred to ice. Samples were boiled for 5 minutes and centrifuged in a bench-top Eppendorf centrifuge at 10000 rpm for 20 minutes. Samples were 6.5-fold diluted in MOPS buffer containing 2.5 mM $NADP^+$. Samples containing 50 μM, 100 μM and 200 μM BDQ were diluted two times before measuring. G6PDH (1.6 U mL^{-1}) was added and formation of NADPH was recorded at 340 nm.

To determine ATP hydrolysis activity, the production of inorganic phosphate was measured. All samples contained 0.2 mg mL^{-1} membrane protein and were incubated in MOPS buffer with 2 mM ATP for 10 minutes at 37 ^oC. Samples containing BDQ or DCCD were preincubated for 15 minutes at room temperature. After 10 minutes all samples were immediately transferred to ice-cold water and centrifuged at 4 ^oC 10000 rpm for 10 minutes. AMES solution (1 mL, 0.4% ammonium molybdate in 0.5 M H_2SO_4 mixed with 10% sodium ascorbate at a 6:1 v/v ratio) was added to 100 μL supernatant. After 15 minutes of incubation at room temperature, phosphate production was measured at 820 nm with a spectrophotometer.

Real time ATP synthesis measurements were performed in an Oroboros O2k fluorespirometer using the luciferase method (5). The fluorescent sensors were modified to measure luminescence by removed the light-filters, turning off the LEDs (setting intensity to 0) and increasing the gain to 1000. ATP concentrations were calculated from a standard curve performed prior to the experiment, although boluses of ATP (100 nM) were routinely added at the end of each experiment to check the integrity of the calibrations. Each assay used 0.125 mg mL^{-1} IMVs. The buffer was 20 mM HEPES-NaOH (pH 7.0), 100 mM K-Acetate, 10 mM Na_2HPO_4, 5 mM Mg-Acetate, 80 μg mL^{-1} luciferase (Sigma), 400 μM luciferin (Thermo), 200 μM Ap5A (Sigma). This concentration of luciferase did not significantly affect the O_2 concentration in the chamber.

Purification of *E. coli* F_1F_o and reconstitution into proteoliposomes

The F_1F_o ATP synthase was extracted and purified from IMVs of C41 Δ*atp* pBWUβHis according to published protocols (6). IMVs resulting from the lysis of 5 g wet weight of cells were resuspended in 10 mL extraction buffer (50 mM Tris-HCl pH 7.5, 100 mM KCl, 250 mM sucrose, 5 mM $MgCl_2$, 0.1 mM K_2-EDTA, 0.2 mM DTT, 0.8% soybean phosphatidylcholine, 1.5% octylglucopyranoside, 0.5% sodium deoxycholate, 0.5% sodium cholate, 2.5% glycerol, 30 mM imidazole, 1 Roche cOmplete™ mini protease inhibitor tablet per 50 mL) and incubated at 4 °C for 1 h with agitation. Insoluble material was removed by discarding the pellet after centrifugation (45 min, 200,000 × *g*). The supernatant was loaded on a HisTrap HP Ni-NTA column (GE Healthcare Life Sciences), washed with 10 volumes of extraction buffer and subsequently eluted with extraction buffer containing 400 mM imidazole. Reconstitution was performed immediately according to published protocols (6), with modifications. Prior to purification of F_1F_o, a 30 mg mL^{-1} solution of soybean phosphatidylcholine (Sigma, Type II-S) in buffer (10 mM HEPES-NaOH, pH 7.5, 5 mM $MgCL_2$, 100 mM KCl) was sonicated 3 x 30 s in an ice-water bath using a Sonics VibraCell (VCX-750) set to 40% amplitude. Liposomes were incubated with the purified protein and 1% sodium cholate at a 1:100 protein:lipid ratio for 30 min at room temperature. Cholate was removed using a PD-10 desalting column (GE Healthcare Life Sciences) and F_1F_o proteoliposomes were collected by centrifugation (30 min, 200,000 × *g*). This protocol has been previously demonstrated to give unilamellar liposomes with unidirectionally incorporated protein (6). Glycerol was added to 10% (final concentration) then aliquots were frozen in a dry ice-ethanol bath and stored at -80 °C until required.

Purification of *E. coli* cytochrome *bo*$_3$ and reconstitution into proteoliposomes

Cytochrome *bo*$_3$ (c*bo*$_3$) was extracted and purified from inner membranes in a method similar to that of Rumbley *et al* 1997 (7). *E. coli* inner membranes were prepared from strain GO105/pJRhisA (7) in which c*bo*$_3$ is overexpressed. *E. coli* was aerobically grown to mid-log phase at 37 °C in LB medium supplemented with 500 μM $CuSO_4$ and 100 μg ml^{-1} carbenicillin. Cells were harvested by centrifugation at 10,000 × *g* for 10 min and the pellet washed and repelleted twice with buffer B (20 mM MOPS, 30 mM Na_2SO_4, pH 7.4). Cells were then resuspended in buffer B containing 1 Roche cOmplete™ mini protease inhibitor tablet per 50 mL, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mg pancreatic DNase per mL, and lysed by two

passages through a French press at 20,000 psi. Any debris and unbroken cells were removed by centrifugation at 10,000 × *g* for 30 min. The supernatant was then ultracentrifuged (200,000 × *g*, 45 min, 4°C) and the membrane pellet resuspended in buffer C (20 mM MOPS, 30 mM Na_2SO_4, 25% w/w sucrose, pH 7.4). This was applied to the top of a 30% w/w to 55% w/w sucrose gradient and ultracentrifugation (130,000 × *g*, 16 h, 4°C) with no deceleration or breaking to separate inner membrane from outer membrane. The inner membrane fraction was removed from the sucrose gradient and washed 3 times with buffer B by ultracentrifugation (200,000 × *g*, 45 min, 4°C). Inner membranes were then resuspended in buffer B and either used immediately for purification or stored in aliquots at −80°C until use. To extract c*bo*$_3$, inner membrane vesicles were diluted to 5 mg of protein mL in solubilization buffer (20 mM Tris HCl, pH 8.0, 5 mM $MgSO_4$, 10% glycerol, 0.5% Lauryl Maltose Neopentyl Glycol (LMNG), 300 mM NaCl, 10 mM imidazole) and incubated at 30°C for 30 min with gentle inversion every 5 min. The unsolubilized material was removed by ultracentrifugation (200,000 × *g*, 45 min, 4°C), and the supernatant was applied to a Nickel-Sepharose High Performance (GE Healthcare) column that was previously washed with water and equilibrated with IMAC buffer (50 mM Tris HCl, pH 8.0, 5 mM MgSO4, 10% glycerol, 0.005% Lauryl Maltose Neopentyl Glycol (LMNG), 300 mM NaCl) containing 10 mM imidazole. To remove contaminating proteins, the resin was washed with IMAC buffer containing 30 mM imidazole and 150 mM NaCl, and c*bo*$_3$ was eluted with IMAC buffer containing with 200 mM imidazole, 150 mM NaCl, and 20% glycerol. The red c*bo*$_3$ containing fractions were pooled and concentrated to 6.57 mg mL^{-1} using an Amicon Ultra centrifugal filter devices (molecular weight cutoff ((MWCO), 100,000). Purified c*bo*$_3$ was either used immediately for reconstitution or stored in aliquots at −80°C until use. On the day of reconstitution of c*bo*$_3$, a 5 mg mL^{-1} solution of *E. coli* polar lipids (Avanti Polar Lipids) in reconstitution buffer (20 mM MOPS, pH 7.4, 30 mM Na_2SO_4, 100 mM KCl) was extruded 13 times through a 400 μM polycarbonate membrane. Liposomes were then incubated with the purified protein for 50 min at 25°C with continuous inversion.

Fluorescence quenching of cytochrome *bo*$_3$ proteoliposomes

IMVs (0.2 mg) consisting of 2% c*bo*$_3$/mass *E. coli* polar lipids doped with 1% mass ubiquinone-10 (UQ_{10}) per ml were pre-warmed to 37 °C for 15 min in 20 mM MOPS, 30 mM Na_2SO_4, pH 7.4, 1 mM DTT, and 1 μM ACMA ± 1μM valinomycin with vigorous stirring (800 rpm).

Quenching was initiated by the addition of 2.5 μM ubiquinone-0 (UQ_0) in ethanol and reversed as indicated in text. Ethanol controls had no effect on ACMA quenching.

Preparation of pyranine-containing liposomes

Pyranine-containing liposomes were prepared analogous to the method previously described for analysis of the F_o proton transport assays (6). F_1F_o proteoliposomes were stripped of the F_1 subunit by dialyzing overnight against 1000-fold volume of stripping buffer (0.5 mM Tricine-NaOH, pH 8.5, 0.5 mM Na_2-EDTA) at 4 °C, diluted 2-fold with stripping buffer and F_o liposomes were collected by centrifugation (30 min, 200,000 × *g*). The liposomes were resuspended to the original volume in incorporation buffer (2 mM MOPS-NaOH, pH 7.5, 5 mM $MgCl_2$, either 50 mM NaCl, 50 mM KCl, 50 mM Na_2SO_4 or 50 mM K_2SO_4 as indicated in text). Where specified MOPS was replaced with 2 mM MES-MOPS-Tris to avoid the introduction of undesired cations when adjusting the buffer pH. Pyranine (Sigma) was added to 1 mM final concentration and the suspension was snap frozen in a dry ice-ethanol bath, thawed in cold water and sonicated in a sonicating water bath (3 x 30 s) at room temperature. The freeze-thaw-sonicate procedure was repeated once more. Unincorporated pyranine was removed using a PD-10 desalting column (GE Healthcare), equilibrated with incorporation buffer. Eluted liposomes were collected by centrifugation (30 min, 200,000 × *g*) and the fluorescent pellet was resuspended to ~ 120 mg mL^{-1}. For protein-free liposomes, soybean phosphatidylcholine was dissolved at 30 mg mL^{-1} in incorporation buffer and sonicated 3 × 30 s on ice using a Vibra-Cell (VCX 500) set to 40% amplitude, then pyranine incorporation was performed as above. When F_o and empty liposomes were compared, empty liposomes were treated exactly the same as F_1F_o preparations (cholate insertion, dialysis etc.), except buffer was added instead of protein solution. Pyranine-containing liposomes were labeled according to the salt included in the incorporation buffer and used within 2 days with storage at 4 °C.

Analytical methods

SDS PAGE was performed as described (8), using a PageRuler™ Prestained protein ladder as a molecular weight standard. Gels were either stained with Coomassie Brilliant Blue G-250 (Sigma-Aldrich), silver staining (9) or transferred onto PVDF transfer membrane (Thermo) and western blotted using Abcam Anti-6xHis HRP linked antibodies. Chemiluminescence was

visualized using an Odyssey Fc gel imager after treatment with a Super Signal® West Pico kit (Thermo). Protein concentration was enumerated by the BCA assay (Pierce), or DC-Bradford (Thermo) where necessary, according to a BSA standard.

Table S1. Relationship of pH to fluorescence ratio ($R_{405/460}$) with different salts

Salt[1]	Best-fit relationship	n	r^2
50 mM K_2SO_4	$pH = 7.205 - 0.469\ln(R_{405/460} - 0.2796)$	7	1.0000
50 mM Na_2SO_4	$pH = 7.460 - 0.454\ln(R_{405/460} - 0.2864)$	7	1.0000
50 mM NaCl	$pH = 7.516 - 0.511\ln(R_{405/460} - 0.3572)$	24	0.9993
50 mM KCl	$pH = 7.243 - 0.370\ln(R_{405/460} - 0.7335)$	15	0.9996
10 mM KCl	$pH = 7.412 - 0.446\ln(R_{405/460} - 0.1857)$	24	0.9999
100 mM KCl	$pH = 7.174 - 0.433\ln(R_{405/460} - 0.2489)$	24	0.9998
10 mM LiCl	$pH = 6.536 - 0.431\ln(R_{405/460} - 0.8745)$	24	0.9916
10 mM NaCl	$pH = 6.528 - 0.424\ln(R_{405/460} - 0.8917)$	24	0.9973

[1]Incorporation buffer (2 mM MOPS-NaOH, 5 mM $MgCl_2$) with 50 mM of the indicated salt was used.

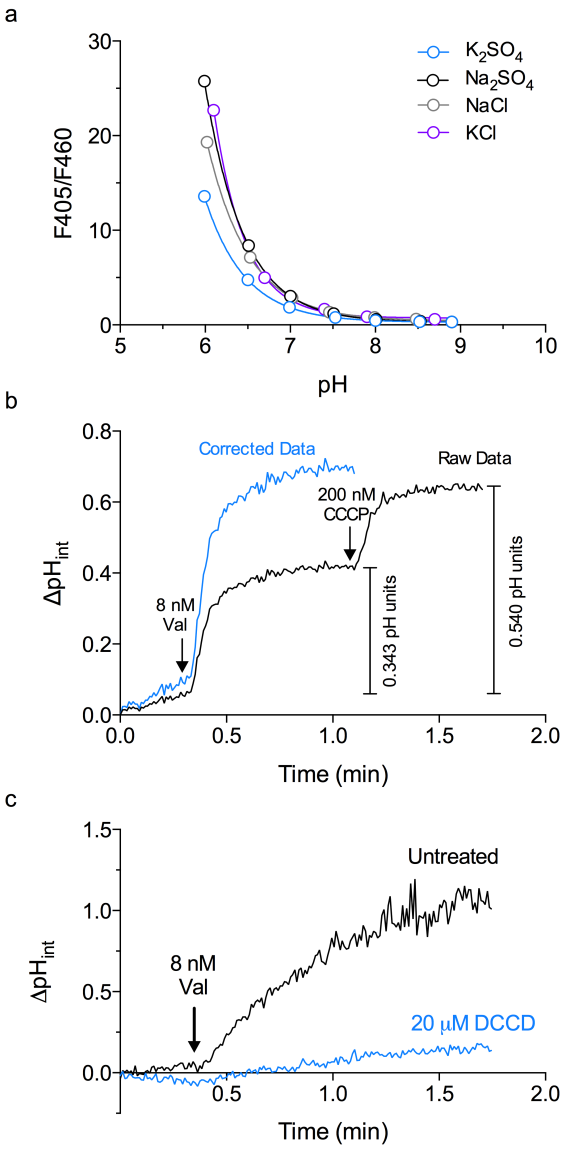

Fig. S1. Pyranine calibration and proton transport through F_o.

A) Standard curve of pyranine fluorescence vs. pH for various incorporation (2 mM MOPS, 5 mM $MgCl_2$) buffers used in pyranine transport experiments (differing by containing 50 mM of the indicated salt). Pyranine (20 nM) was added and the fluorescence ratio measured. Detailed data are given in Table 1. B) Proton transport, dependent on F_o, was initiated by an inwards K^+/Val diffusion potential (50 mM K_2SO_4 in the external 2 mM MOPS buffer, 50 mM Na_2SO_4 inside). The proportion of F_o empty proteoliposomes is calculated by the ratio of this activity and that elicited by carbonyl cyanide 3-chlorophenylhydrazone (CCCP). Multiplication of the data accounts for empty liposomes. C) Proton transport was initiated in an analogous experiment to

B), where indicated F_o proteoliposomes were pre-incubated with N,N'-dicyclohexylcarbodiimide (DCCD) for 10 minutes.

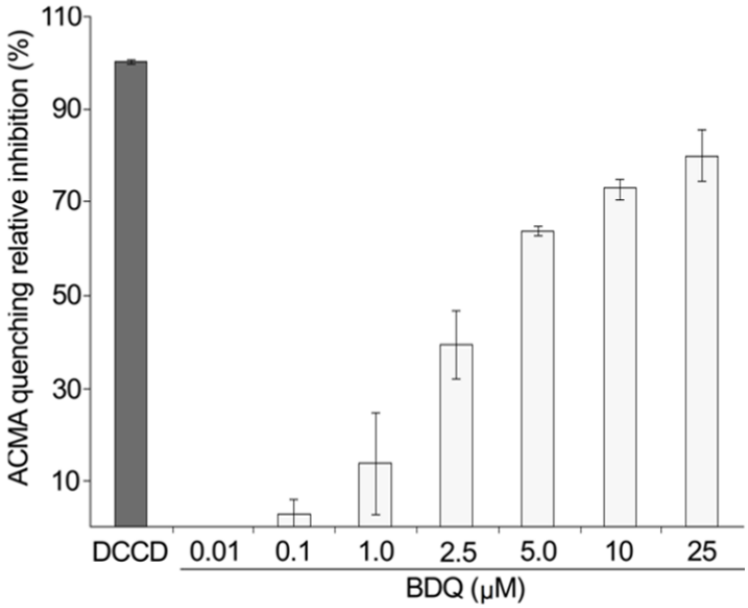

Fig. S2. Inhibition of ACMA quenching by BDQ in *E. coli* IMVs. ATP (1 mM) was added to initiate ACMA quenching, after 0.3 mM SF6847 was added to uncouple the membrane and restore fluorescence. The rate of quenching was calculated relative to sample without added ATP. End-point measurements were taken as described in the materials and methods.

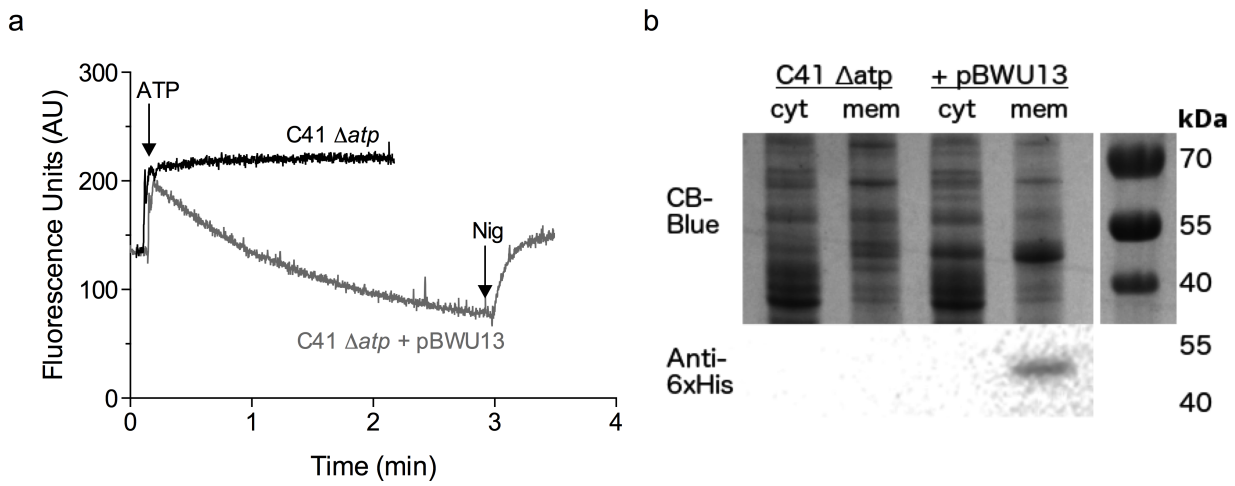

Fig. S3. Expression of F_1F_o in *E. coli* membrane vesicles. A) Proton pumping was elicited by ATP hydrolysis (0.5 mM ATP) and the proton gradient then collapsed by 1 μM nigericin (Nig) where indicated. Acridine orange buffer (10 mM HEPES pH 7.5, 100 mM KCl, 5 mM $MgCl_2$) was used. B) Coomassie SDS-PAGE (12.5%) or anti-6×His western confirms the expression of F_1F_o by detection of the his-tagged β-subunit (~50 kDa). Protein (20 μg) was loaded in each lane. cyt: cytosol, mem: membrane.

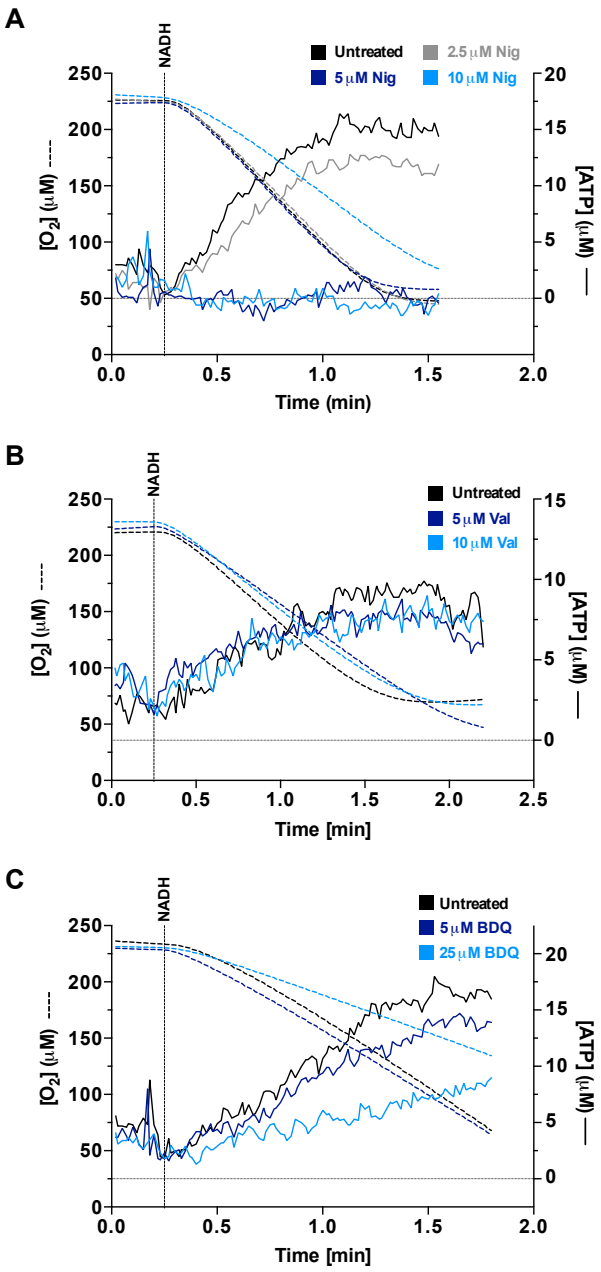

Fig. S4: Real-time ATP synthesis and oxygen consumption: 0.5 mM NADH was added at the indicated time point. Each assay used 0.125 mg mL^{-1} *E. coli* MG1655 (wild-type) inverted membrane vesicles. Samples were either untreated or preincubated with A) nigericin, B) valinomycin, C) BDQ. ATP concentrations were calculated from a standard curve performed prior to the experiment, although boluses of ATP (100 nM) were routinely added at the end of each experiment to check the integrity of the calibrations.

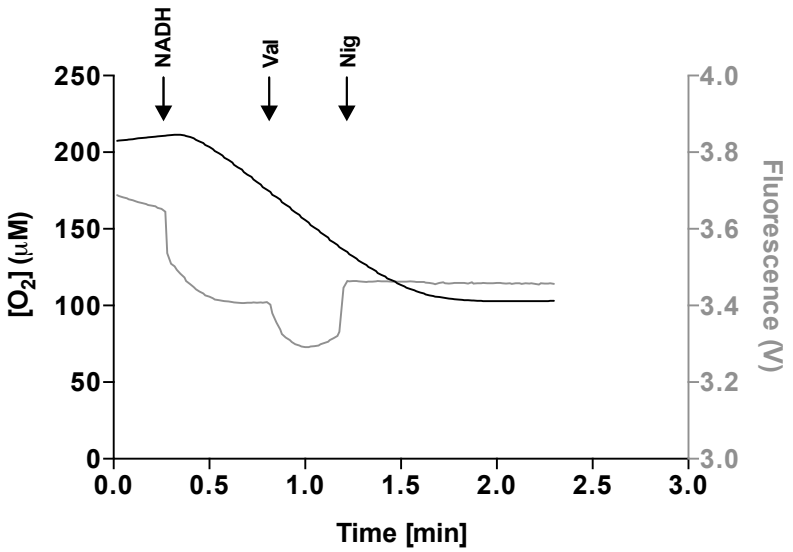

Fig. S5: Simultaneous measurement of PMF generation and oxygen consumption in the ATP synthesis buffer. The experiment was performed (using the Oroboros O2k system) with the ATP synthesis buffer except that luciferase, luciferin and Ap5a were omitted. Acridine orange (5 µM) was added to the buffer and the fluorescent attachments were fitted with the safranine filter for the LED and the magnesium green filter for the photodiode (gain: 100, intensity: 700). Reduced quenching is due to the stronger buffering capacity of the ATP synthesis buffer. NADH (0.5 mM), 10 µM valinomycin or 10 µM nigericin were added at the indicated time points. Experiment is representative of a technical triplicate.

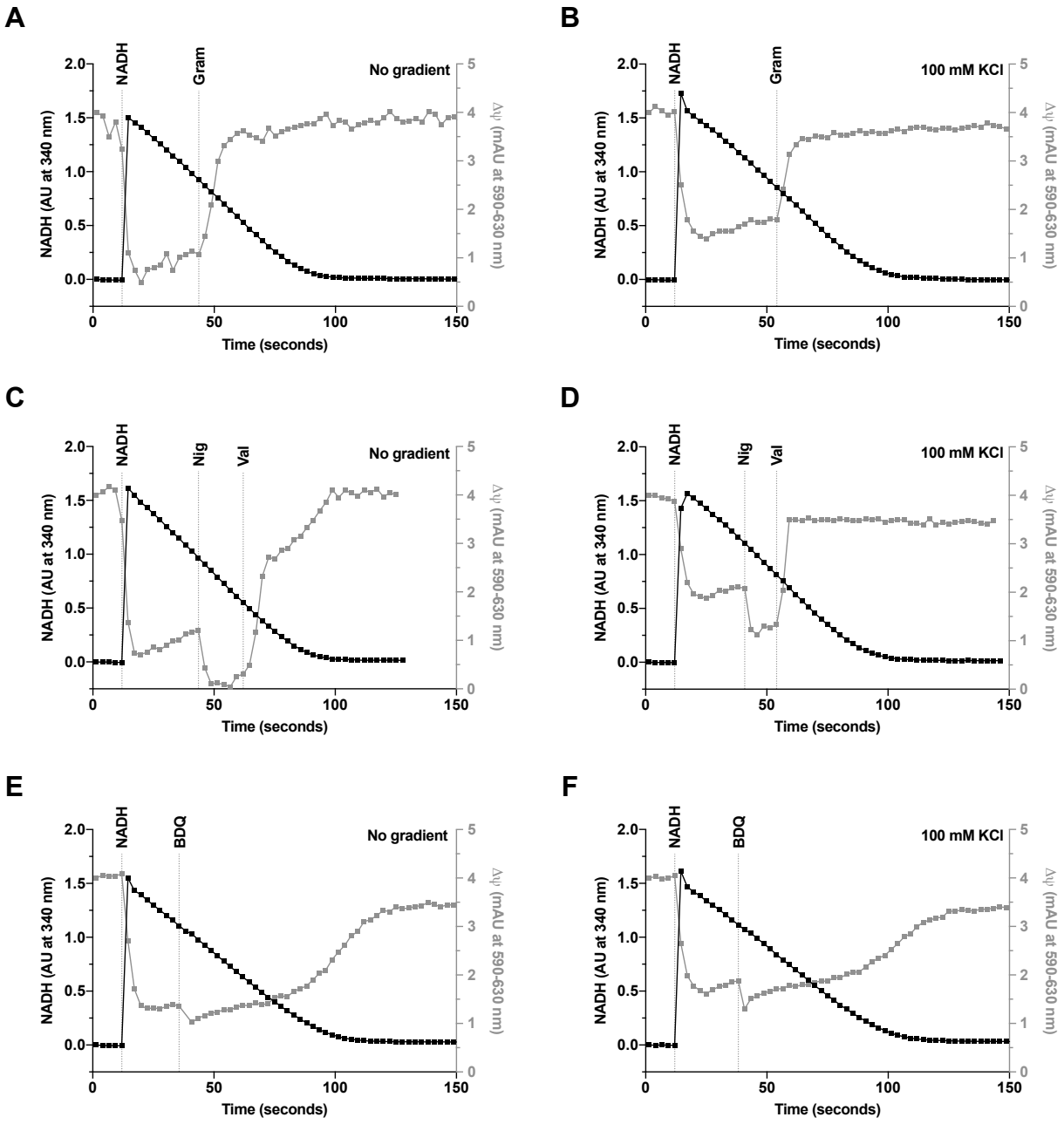

Fig. S6: Effect of uncouplers and BDQ on the membrane potential generation and NADH consumption. Experiment was performed as described in the supplementary materials and methods on *E. coli* MG1655 IMVs. In either empty (A, C, E) or KCl-containing (B, D, F) IMVs, 0.5 mM NADH was added at the indicated time points. Subsequently, either 2 μM gramicidin (A, B), 2 μM nigericin then 2 μM valinomycin (C, D) or 5 μM BDQ were added. Experiment is representative of a technical triplicate.

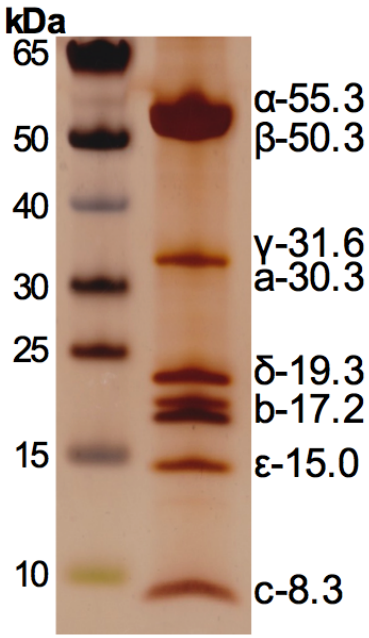

Fig S7: As-purified *E. coli* F_1F_o-ATP synthase.

Silver-stained 4-12% gradient SDS-PAGE of purified *E. coli* F_1F_o-ATP synthase (5 µg) in protein-detergent complex. The *a* subunit was visible on overloaded gels (>30 µg per lane, not shown).

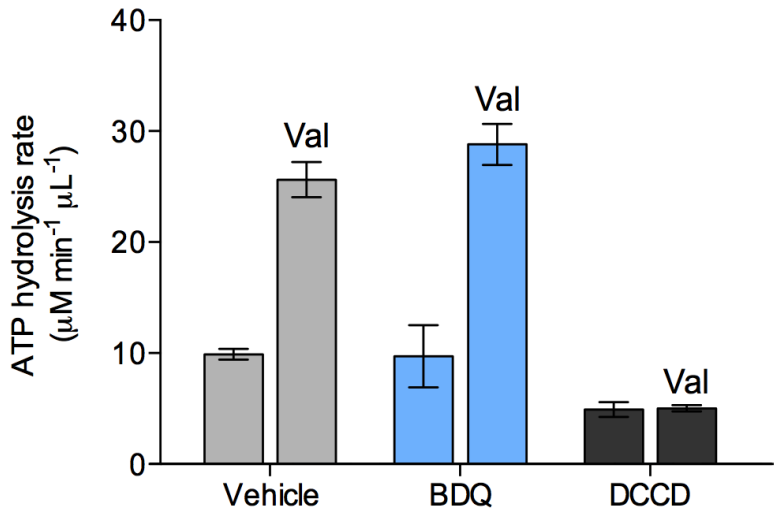

Fig S8: Effects of BDQ on ATP hydrolysis in F_1F_o-proteoliposomes.

Proteoliposomes of *E. coli* F_1F_o were prepared and measured for inhibition of ATP hydrolysis. BDQ was added at 7.5 μM and DCCD was added at 50 μM as described in the materials and methods, DMSO was used as the vehicle control. Valinomycin (Val) (10 μM) was added where indicated, otherwise EtOH was used as a second vehicle control. The buffer was 10 mM HEPES-KOH (pH 7.5), 5 mM $MgCl_2$ and 100 mM KCl. The data are derived from kinetic traces. Error bars represent standard deviation from three independent experiments.

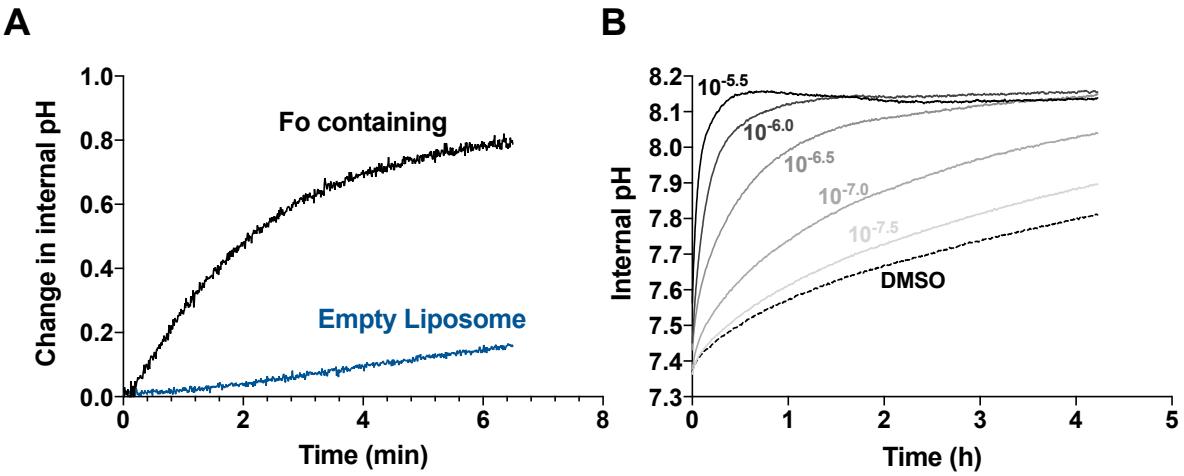

Fig S9: Long term experiments in the pyranine assay. A) Empty and F_o-containing liposomes (50 mM Na_2SO_4 inside) were diluted into buffer (50 mM K_2SO_4), in equivalent conditions as Fig. 4 (pH 7.1 either side), and measured for passive drift in internal pH. B) Empty liposomes were treated with the indicated concentrations of BDQ in moles (or DMSO vehicle control) and measured for long-term kinetics in a plate reader. The internal pH was 7.1 and the external pH was 8.1. Wells were aspirated with a pipette before measurement.

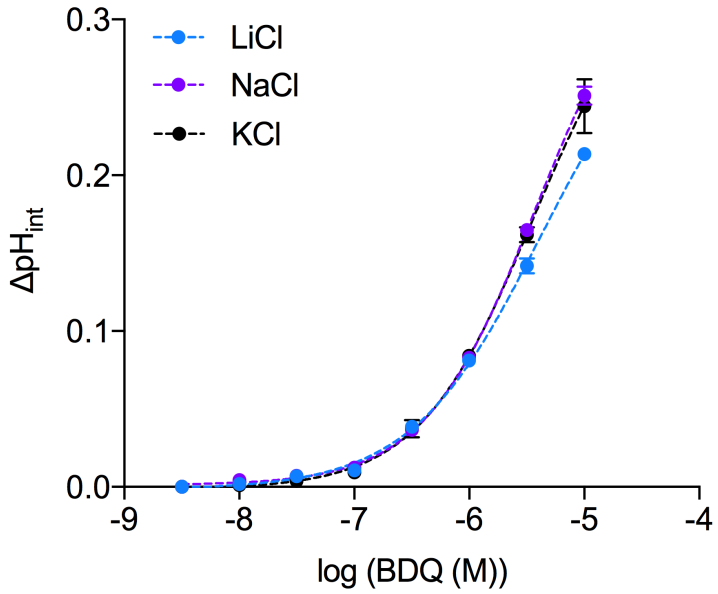

Fig. S10: Concentration dependence of salt-elicited proton movement by BDQ. LiCl, NaCl and KCl were compared for their ability to elicit proton movement upon BDQ addition. No pH gradient was imposed. The $Salt_{out}$:$Salt_{in}$ ratio was 10:1. Experiments used a 2 mM MES-MOPS-Tris buffer system. Error bars indicate standard deviation from triplicate measurements. ΔpH_{int} = change in internal pH.

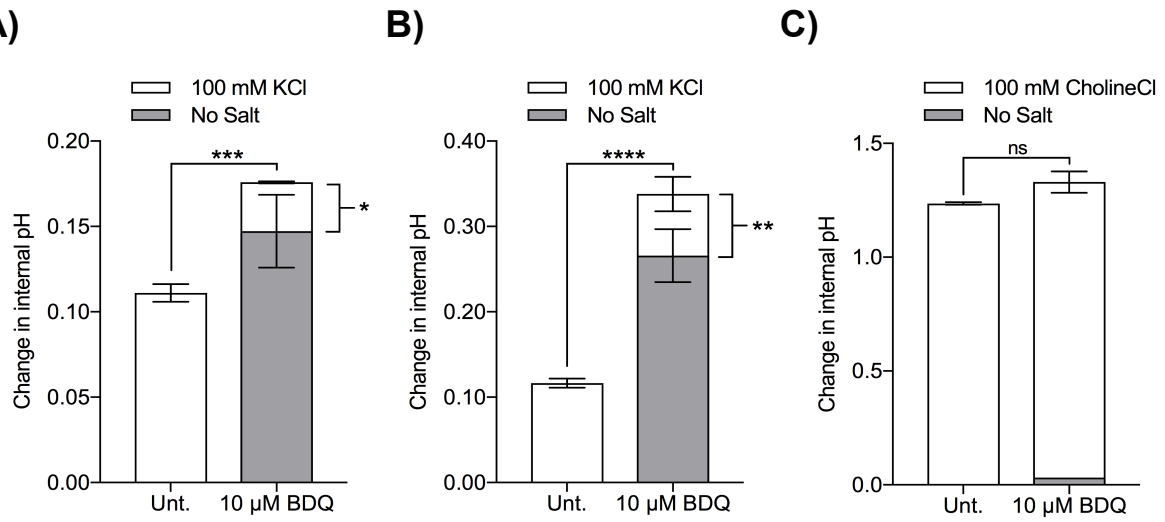

Fig. S11: Controls for pyranine-containing liposomes. The ability for salt-elicited transfer of protons by BDQ, in an analogous experiment to Fig. 2D in the main text, is assessed with the following modifications as controls: A) 5 mM K_2HPO_4-KH_2PO_4 is used instead as a buffering system, B) liposomes where prepared with 16:0-18:1 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) instead of soybean phosphatidylcholine, C) Choline chloride was used as the salt. For white bars a 10:1 $[Salt]_{out}$:$[Salt]_{in}$ gradient is established, otherwise no gradient is established (grey bars). The data are calculated relative to untreated liposomes with no salt gradient. Error bars represent standard deviation from a technical triplicate. * = $p < 0.05$, ** = $p < 0.005$, *** $p < 0.0005$, **** = $p < 0.0001$ (2-way ANOVA, Tukey's multiple comparisons test).

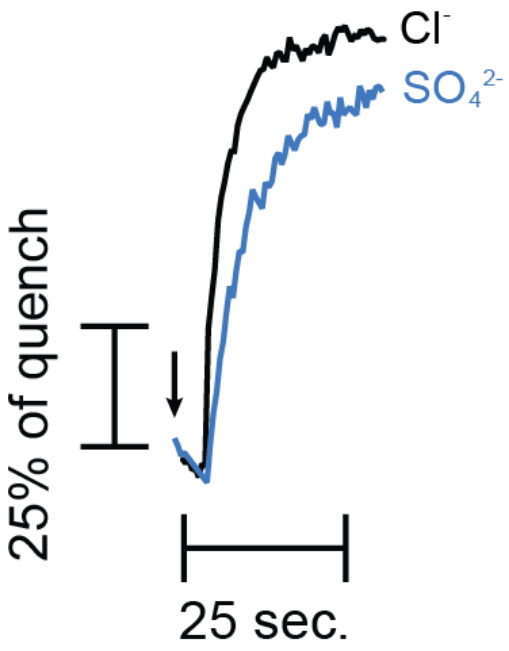

Fig. S12: Comparison of the effect of sodium anions in the cytochrome *bo*$_3$ system. The ability of BDQ to release a cytochrome *bo*$_3$-generated PMF, monitored by ACMA quenching, is compared when either 100 mM NaCl or Na_2SO_4 present both in the buffer and liposome interior. Valinomycin was not present in these experiments. BDQ (22 μM) was added at the indicated time point. Experiment is representative of triplicate experiments. Experiments used a 20 mM MOPS (pH 7.4) buffering system.

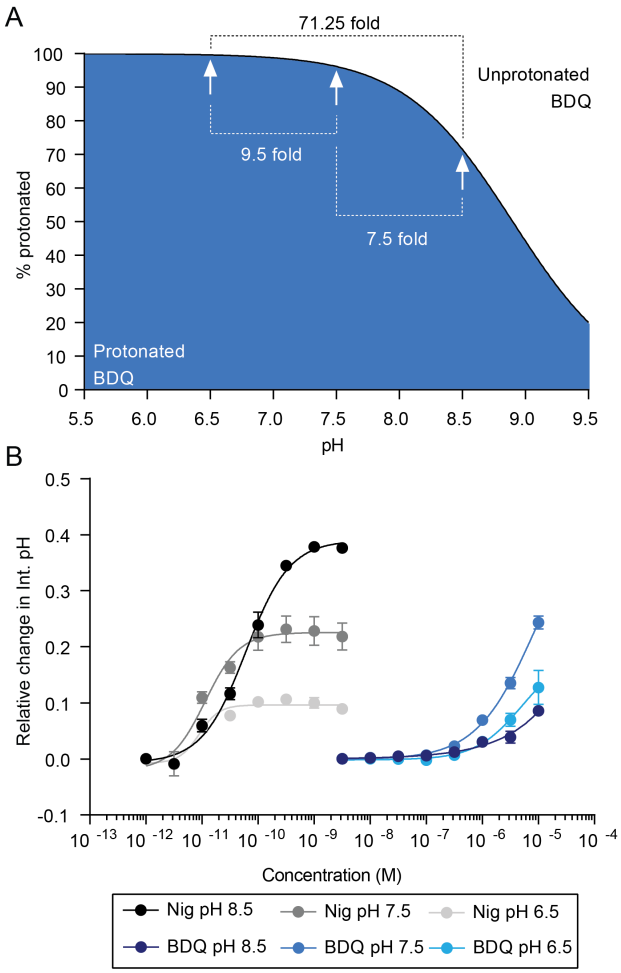

Fig. S13: Ionization-state dependency of potassium-elicited H^+ transfer. (A) The ionization state of BDQ at varying pH values, as calculated according to a pKa of 8.9 (10). (B) The concentration dependency of pH movement driven by a starting KCl gradient of 10:1 $[salt]_{out}$:$[salt]_{in}$ and initiated by the indicated compound. Assay was performed as a 30-minute end-point experiment. Error bars represent standard deviation from triplicate experiments.

Supplementary References

1. Ferguson SA, Cook GM, Montgomery MG, Leslie AGW, Walker JE (2016) Regulation of the thermoalkaliphilic F(1)-ATPase from Caldalkalibacillus thermarum. *Proc Natl Acad Sci U S A* 113(39):10860–10865.

2. Iwamoto A, et al. (1991) Mutations in Ser174 and the glycine-rich sequence (Gly149, Gly150, and Thr156) in the beta subunit of *Escherichia coli* H+-ATPase. *J Biol Chem* 266(25):16350–16355.

3. Moriyama Y, Iwamoto A, Hanada H, Maeda M, Futai M (1991) One-step purification of Escherichia coli H^+-ATPase (F_0F_1) and its reconstitution into liposomes with neurotransmitter transporters. *J Biol Chem* 266:22141–22146.

4. García JJ, Ogilvie I, Robinson BH, Capaldi RA (2000) Structure, Functioning, and Assembly of the ATP Synthase in Cells from Patients with the T8993G Mitochondrial DNA Mutation: Comparison with the enzyme in Rho0 cells completely lacking mtDNA. *J Biol Chem* 275(15):11075–11081.

5. Suzuki T, Ozaki Y, Sone N, Feniouk BA, Yoshida M (2007) The product of uncI gene in F(1)F(o)-ATP synthase operon plays a chaperone-like role to assist c-ring assembly. *Proc Natl Acad Sci U S A* 104(52):20776–20781.

6. Wiedenmann A, Dimroth P, von Ballmoos C (2008) Δpsi and ΔpH are equivalent driving forces for proton transport through isolated F0 complexes of ATP synthases. *Biochim Biophys Acta* 1777(10):1301–10.

7. Rumbley JN, Furlong Nickels E, Gennis RB (1997) One-step purification of histidine-tagged cytochrome bo3 from *Escherichia coli* and demonstration that associated quinone is not required for the structural integrity of the oxidase. *Biochim Biophys Acta - Protein Struct Mol Enzymol* 1340(1):131–142.

8. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227(5259):680–685.

9. Nesterenko M V, Tilley M, Upton SJ (1994) A simple modification of Blum's silver stain method allows for 30 minute detection of proteins in polyacrylamide gels. *J Biochem Biophys Methods* 28(3):239–242.

10. Feng X, et al. (2015) Antiinfectives targeting enzymes and the proton motive force. *Proc Natl Acad Sci* 112 (51):E7073–E7082.