Supplementary Information Text

Purification of the ratiometric pHGFP

E. coli BL21 cells containing pGEX-4T-1 pHluorin2 were lysed in lysis buffer (137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 15 % glycerol, 1 mM PMSF, 1 mM EDTA, Protease inhibitor cocktail (Roche), 5 mM DTT, 500 µg/ml Lysozyme) for 30 min on ice. Then the samples were subjected to sonication, 1 % Triton-X-100 was added and cells were incubated for 15 min on ice. The cell extract was centrifuged and glutathione agarose slurry was added to the supernatant and incubated at RT for 1 hour, with gentle rotation. The beads were washed with PBS with 15 % glycerol. Bound proteins were eluted with 50 mM reduced L-Glutathione in 100 mM Tris pH 8.0, 120 mM NaCl, 5 mM DTT, 2 % octyl β-D-glucopyranoside, 15 % glycerol, 1 mM PMSF and 1 mM EDTA for 2 hours at 4 °C.

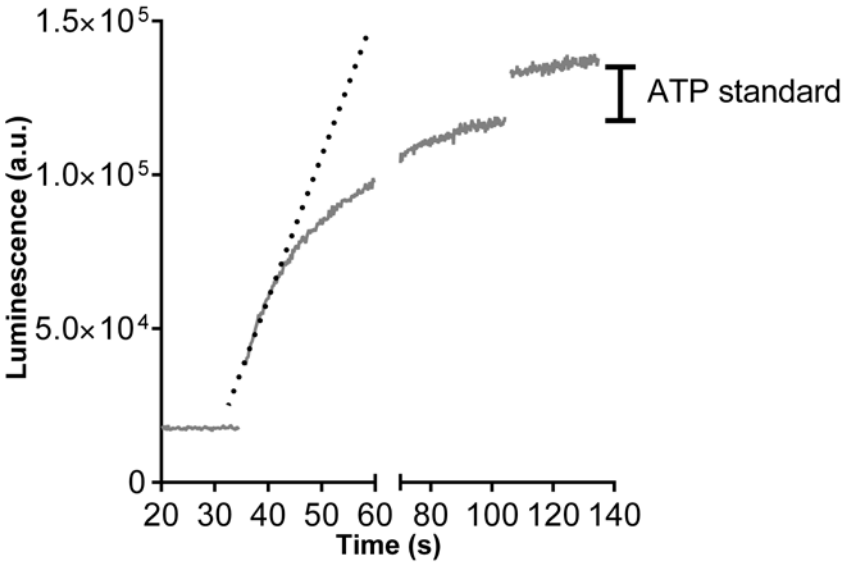
Steady state Western blot conditions

Whole cell protein extracts were prepared by harvesting an OD of 2.5 of cells at stationary phase by alkali treatment and resuspension in loading buffer. The proteins were separated in a 16 % SDS-PAGE, wet-blotted onto nitrocellulose (Rothi-NC 0.2 µm) for 90 min. The membrane was incubated with primary antibody overnight with tumbling at 4 °C. Secondary antibody was incubated for 1 hour at RT. The visualization of the bands was done by the horseradish system using WesternBright Quantum-HRP (Lifeimaging, Sweden) as substrate.

Blue Native Electrophoresis and Western blot on supercomplexes and atp20Δ strain*:*

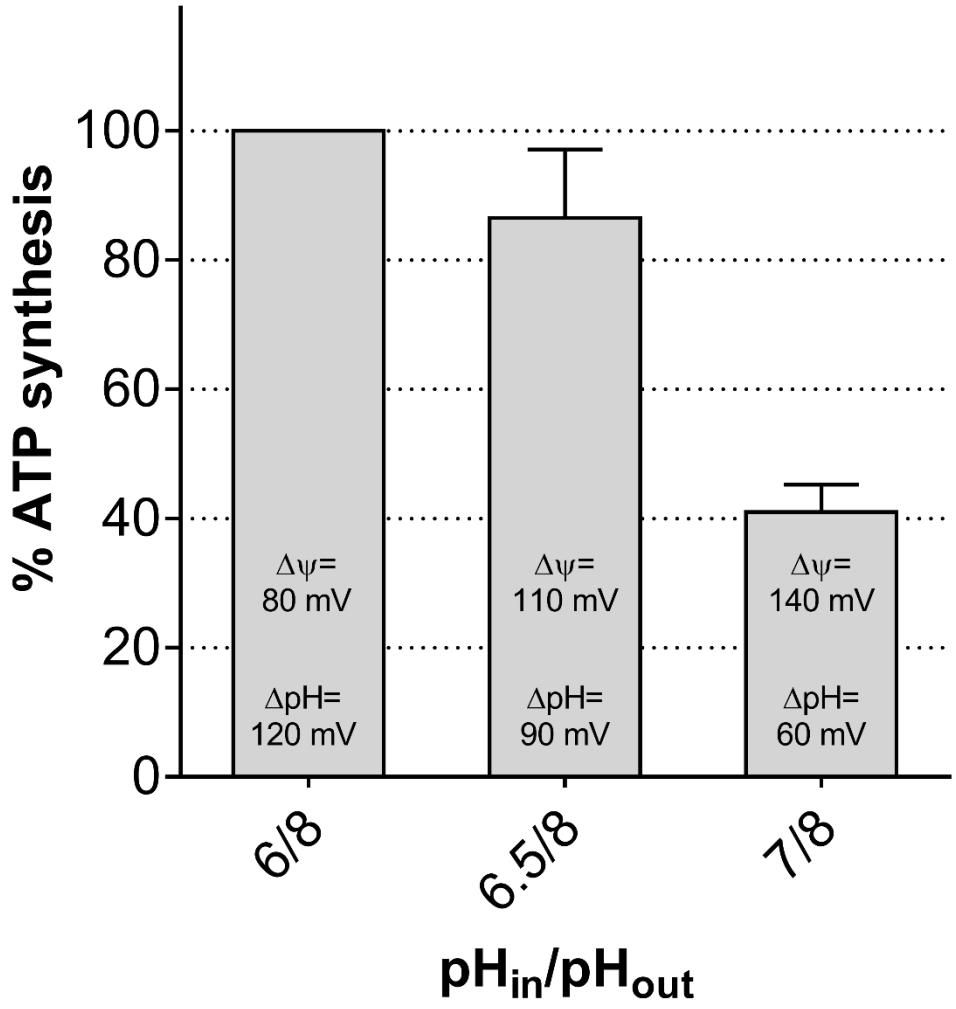
For the Blue Native electrophoresis, 100 µg of mitochondrial protein was used. The mitochondria was centrifuged down at 25000 x g for 10 min, and lysed in 1 % Digitonin (Millipore) for 10 min on ice. The lysis buffer also contained 50 mM Bis-Tris, 25 mM KCl, 2 mM Aminohexanoic acid, 12 % glycerol, 1 mM PMSF and Complete Protease Inhibitor cocktail (Roche). The samples were then subjected to a clarifying spin at 25000 g for 7 min at 4 °C. The supernatants were loaded onto a precast 3 %-12 % gel (Invitrogen). After the electrophoresis, entire lanes were excised and mounted onto SDS-PAGE. Finally, the proteins were blotted onto a nitrocellulose membrane (Carl Roth, Germany) for 90 min at 200 mA. The membranes were decorated with the indicated antibodies overnight at 4 °C. The secondary antibody (anti mouse) was incubated for 1 hour at RT. The blots were developed with Sirius WesternBright.

Supplementary Figures



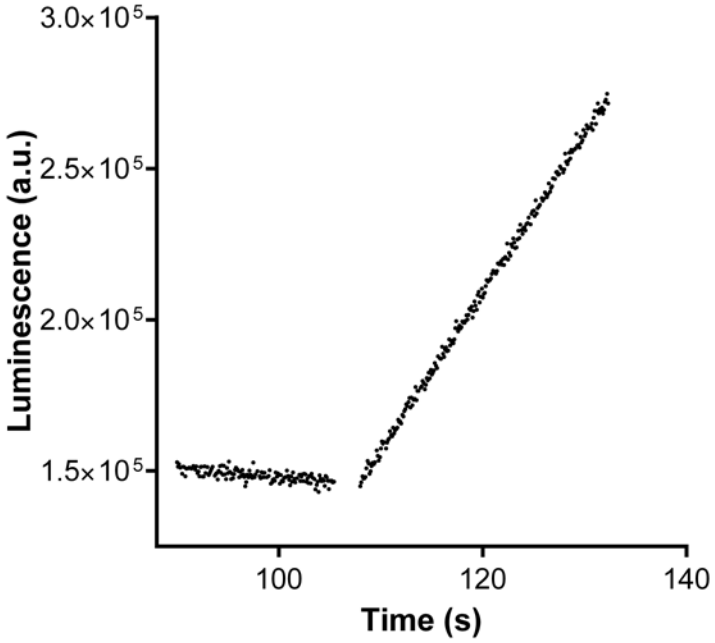
Supplementary Figure 1:

Raw trace from a typical acid-bath measurement. *N*-side buffer containing ADP, luciferin, luciferase and KCl was used as a baseline. The reaction was started by addition of liposomes (containing valinomycin) after ~35 s. After ~110 s, a defined amount of ATP was added to normalize signal. The initial rate was estimated by determining the slope over the first 2-3 s after addition of liposomes.



Supplementary Figure 2:

Influence of *P*-side pH (pH_{in}) on the initial rate of ATP synthesis. Acid-bath measurements with a constant *pmf* and *N*-side pH=8 (pH_{out}). The pH_{in} (6, 6.5, 7) and $\Delta\psi$ (80, 110, 140 mV) were varied in parallel to establish a constant *pmf* of 200 mV. Shown are the data for liposomes with 50 nm (n=3 different liposome preparations with technical duplicates). See supplementary table for further details.



Supplementary Figure 3:

ATP synthesis in liposomes with reconstituted yeast ATP synthase and *E. coli bo*$_3$ oxidase at pH 8.75. Detail of raw trace shows that addition of ubiquinone Q_1 (105 s) immediately initiates ATP synthesis without a delay for build-up of a proton gradient.

Conditions for Experiments in Figure 5B

pHin	phout	ΔpH	Δψ	total pmf (mV)	ATP (mM)	ADP (mM)	ATP/ADP	Pi (M)	Q=ATP/(ADP x Pi)	RTxln(Q)	ΔG_0 ((kJ/mol))	ΔGp ((kJ/mol))	required pmf (mV)	excess pmf (mV)
6.25	6.75	0.5	140	170	0.004	0.08	0.05	0.01	5	3.986	26	30	87	83
6.75	7.25	0.5	140	170	0.004	0.08	0.05	0.01	5	3.986	29	33	96	74
7.25	7.75	0.5	140	170	0.004	0.08	0.05	0.01	5	3.986	32	36	105	65
6.25	7.25	1	140	200	0.004	0.08	0.05	0.01	5	3.986	29	33	96	104
6.25	7.75	1.5	140	230	0.004	0.08	0.05	0.01	5	3.986	32	36	105	125
6.25	8.25	2	140	260	0.004	0.08	0.05	0.01	5	3.986	36	40	116	144
6.25	8.75	2.5	140	290	0.004	0.08	0.05	0.01	5	3.986	38	42	122	168

Conditions for Experiments in Figure 5C

pHin	phout	ΔpH	Δψ	total pmf (mV)	ATP (mM)	ADP (mM)	ATP/ADP	Pi (M)	Q=ATP/(ADP x Pi)	RTxln(Q)	ΔG_0 ((kJ/mol))	ΔGp ((kJ/mol))	required pmf (mV)	excess pmf (mV)
5.75	7.25	1.5	140	230	0.004	0.08	0.05	0.01	5	3.986	29	33	96	134
6	7.5	1.5	140	230	0.004	0.08	0.05	0.01	5	3.986	30	34	99	131
6.25	7.75	1.5	140	230	0.004	0.08	0.05	0.01	5	3.986	32	36	105	125
6.5	8	1.5	140	230	0.004	0.08	0.05	0.01	5	3.986	34	38	111	119
6.75	8.25	1.5	140	230	0.004	0.08	0.05	0.01	5	3.986	36	40	116	114
7	8.5	1.5	140	230	0.004	0.08	0.05	0.01	5	3.986	37	41	119	111
7.25	8.75	1.5	140	230	0.004	0.08	0.05	0.01	5	3.986	38	42	122	108

Conditions for Experiments in Supplementary figure 2

pHin	phout	ΔpH	Δψ	total pmf (mV)	ATP (mM)	ADP (mM)	ATP/ADP	Pi (M)	Q=ATP/(ADP x Pi)	RTxln(Q)	ΔG_0 ((kJ/mol))	ΔGp ((kJ/mol))	required pmf (mV)	excess pmf (mV)
6	8	2	80	200	0.004	0.08	0.05	0.01	5	3.986	33	37	108	92
6.5	8	1.5	110	200	0.004	0.08	0.05	0.01	5	3.986	33	37	108	92
7	8	1	140	200	0.004	0.08	0.05	0.01	5	3.986	33	37	108	92