SNARE-fusion mediated insertion of membrane proteins into native and artificial membranes

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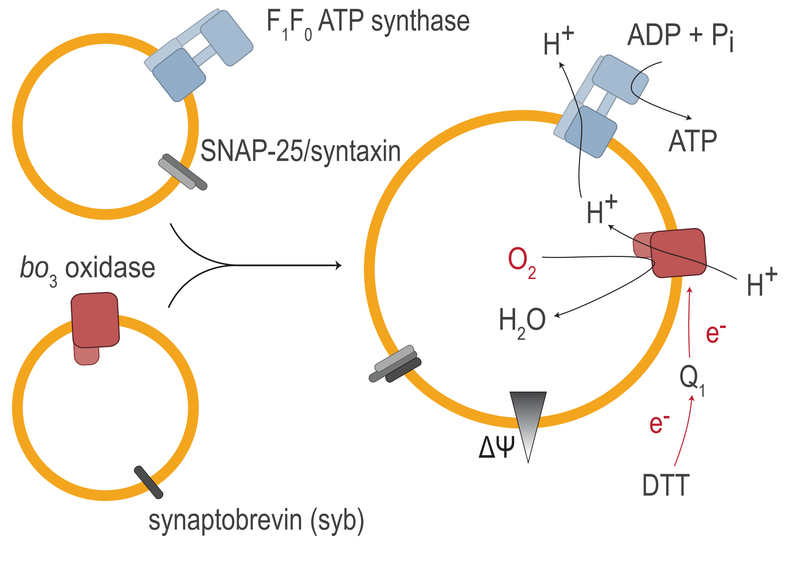
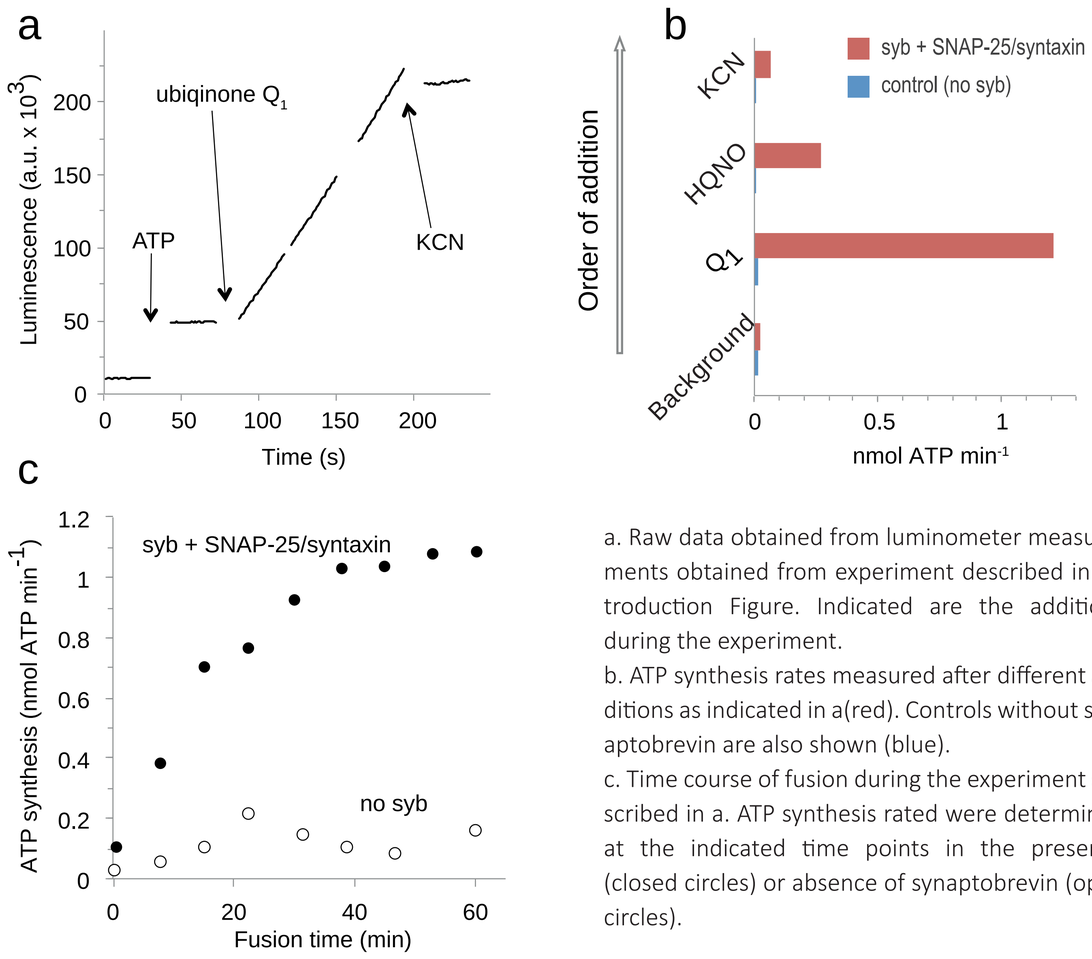
Membrane proteins carry out functions such as nutrient uptake, ATP synthesis or transmembrane signal transduction. An increasing number of reports indicate that cellular processes are underpinned by regulated interactions between these proteins. Consequently, functional studies of these networks at a molecular level require **co-reconstitution of the interacting components**. Here, we report a ***SNARE-protein based method for incorporation of multiple membrane proteins into membranes***, and for delivery of large water-soluble substrates into closed membrane vesicles. The approach is used for *in vitro* reconstruction of a ***fully functional bacterial respiratory chain from purified components***. Furthermore, the method is used for functional ***incorporation of the entire F_1F_0-ATP synthase complex into native bacterial membranes*** from which this component had been genetically removed. The novel methodology offers a tool to investigate complex interaction networks between membrane-bound proteins at a molecular level, which is expected to generate functional insights into key cellular functions.



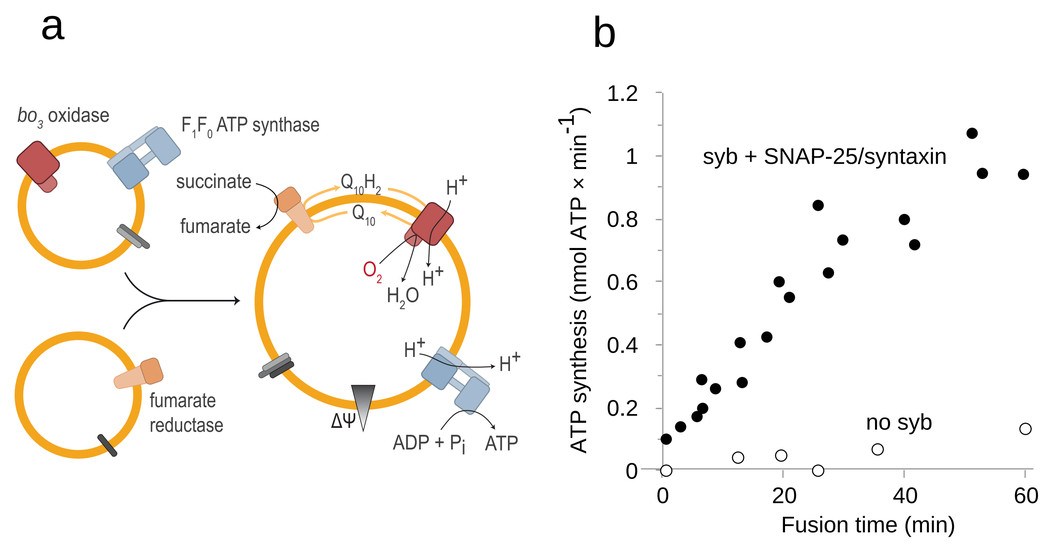
Cartoon illustrating the basic experimental setup. The two liposomes populations were allowed to fuse for 20 min and *bo*$_3$ oxidase turnover was started with the addition of DTT/Q_1.

Complex IV and ATP synthase



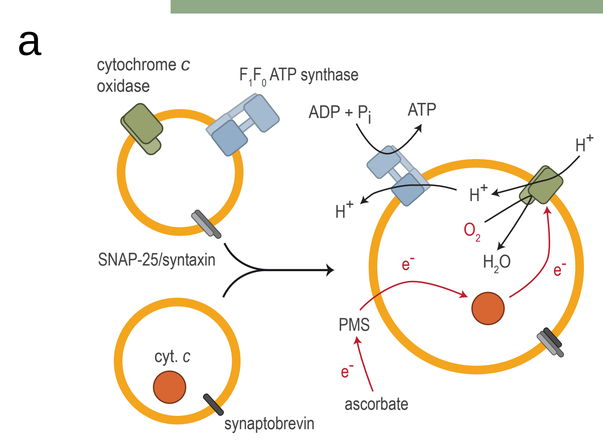
a. Raw data obtained from luminometer measurements obtained from experiment described in Introduction Figure. Indicated are the additions during the experiment.
b. ATP synthesis rates measured after different additions as indicated in a(red). Controls without synaptobrevin are also shown (blue).
c. Time course of fusion during the experiment described in a. ATP synthesis rated were determined at the indicated time points in the presence (closed circles) or absence of synaptobrevin (open circles).

Complexes II, IV and ATP synthase



Reconstitution of an intact bacterial respiratory chain.
a. Cartoon of the experimental setup. After fusion, the respiratory chain was initiated by addition of succinate, the substrate of complex II, reducing ubiquinone Q_{10} and thus energizing *bo*$_3$ oxidase.
b. Time course of the experiment described in a. ATP synthesis rated were determined at the indicated time points in the presence (closed circles) or absence of synaptobrevin (open circles).

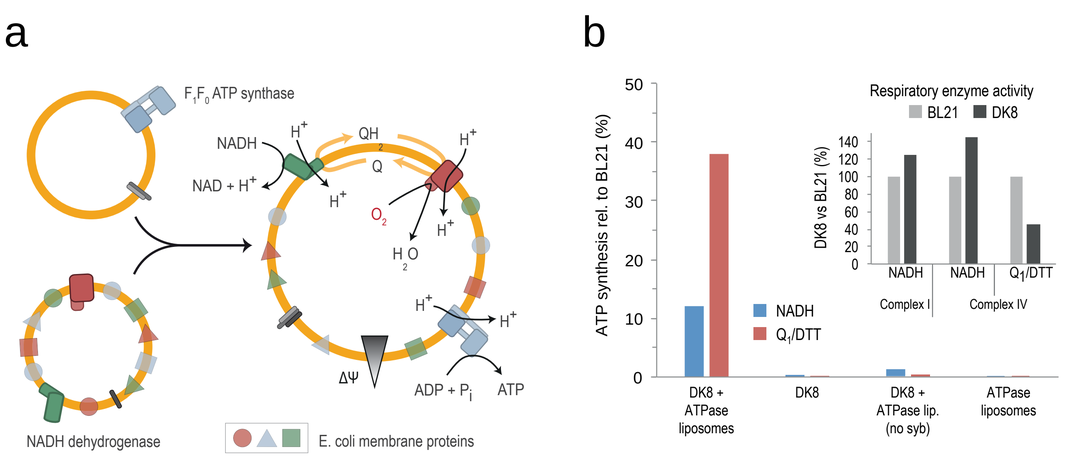
Complex IV, ATP synthase and cytc



Delivery of cytochrome c into closed vesicles.
a. Cartoon illustrating the experimental setup. Liposomes containing soluble cyt. c and synaptobrevin were mixed with proteoliposomes containing Cyt*c*O oxidase, ATP synthase and SNAP-25/syntaxin. The reaction was started by the addition of ascorbate and PMS as electron donor and mediator, respectively.

b. Time course displaying the stimulated ATP synthesis activity after fusion of the two liposome population in the presence (filled circles) and absence (open circles) of synaptobrevin. Note: The low, but constant activity found in the sample without synaptobrevin is due to direct reduction of *bo*$_3$ oxidase by ascorbate/PMS.

ATP synthase and *E. coli* membranes



Delivery of ATP synthase into inverted membranes of ATP synthase lacking *E. coli* strain
a. Cartoon illustrating the experimental setup. Inverted membrane vesicles of *E. coli* DK8 doped with synaptobrevin were mixed with liposomes containing ATP synthase and SNAP-25/syntaxin and allowed to fuse.
b. Respiratory driven ATP synthesis after fusion as described in a. The results are given compared to a similar preparation of strain BL21, normalized to the total membrane protein concentration. Shown are the relative activities after addition of NADH (blue) and DTT/Q_1 (red). Relative respiratory enzyme activities in inverted membranes of strains BL21 (light grey) and DK8 (dark grey) measured as NADH oxidation for complex I and O_2 consumption for complex IV (driven by either NADH oxidation or DTT/Q_1) are also shown (inset).

Reference:
Nordlund G., Brzezinski P., and von Ballmoos C (2014).
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