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-reconstruction 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$_{1}$F$_{0}$-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.

### Complex IV and ATP synthase

**a.** 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$_{1}$.

**b.** Reconstitution of an intact bacterial respiratory chain.

- a. Cartoon of the experimental setup. After fusion, the respiratory chain was initiated by addition of substrates, 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 rates were determined at the indicated time points in the presence (open circles) or absence of synaptobrevin (open circles).

### Complexes II, IV and ATP synthase

**a.** Cartoon illustrating the experimental setup. A similar preparation was used for complex IV (driven by either NADH oxidation or DTT/Q$_{1}$) are also shown (inset).

**b.** ATP synthase and E. coli membranes

- a. Cartoon illustrating the experimental setup. Inverted membrane vesicles of strains BL21 (light grey) and DK8 (dark grey) were measured in inverted membranes of strains BL21 (light grey) and DK8 (dark grey). The reaction was started by the addition of ascorbate and PMS as electron donor and mediator, respectively.
- b. Time course of the experiment described in a. ATP synthesis rates were determined at the indicated time points in the presence (closed circles) or absence of synaptobrevin (open circles).

### Complex IV, ATP synthase and cytc

**a.** Delivery of cytochrome c into closed vesicles.

- a. Cartoon illustrating the experimental setup. Liposomes containing soluble cytc and synaptobrevin were mixed with proteoliposomes containing CytC 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 synthase activity after fusion of the two liposome populations 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.

**b.** 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/oxb doped with synaptobrevin were mixed with liposomes containing ATP synthase and SNAP-25/syntaxin and allowed to fuse.
- b. Respiratory driven ATP synthase 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 oxb (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:


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