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,F,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. 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). Contrasts without synaptobrevin are also shown (blue).

c. Time course of fusion during 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).

Complexes II, IV and ATP synthase

a. Cartoon of the experimental setup. After fusion, the respiratory chain was initiated by addition of succinate, the substrate of complex II, reducing ubiquinone Q10, and thus energizing bo3 oxidase.

b. Time course of 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).

ATP synthase and E. coli membranes

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 cytochrome 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 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 bo3 oxidase by ascorbate/PMS.

Reference: