Cell-free Synthesis and Functional Analysis of Membrane Proteins

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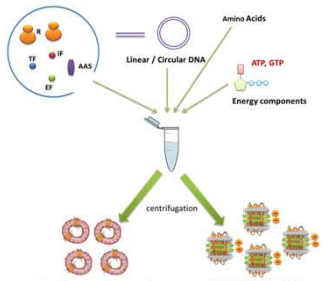
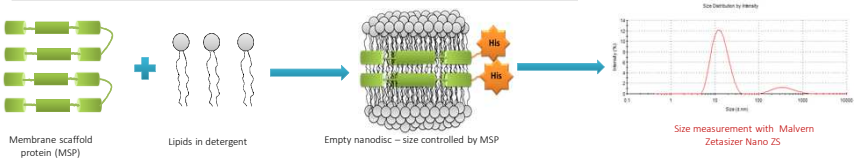
Membrane proteins (MPs) represent more than two-third of the known protein targets for drugs due to their involvement in vital cellular processes. Among them, ion channels and transporters have a crucial role in human physiology and any functional defect or overexpression of these proteins could affect the cellular activities which could often lead to a wide range of pathological conditions like channelopathies, metabolic disorders, cellular damage, and poor drug adsorption. Due to this, they are targeted by several marketed drugs and have a larger scope for future development of new drugs. Synthesis of MPs in vivo is challenging due to low yields, solubilization and purification problems, toxicity due to overexpression, and functional assessment. This method provides a complete openness with a high degree of controllability allowing direct manipulation of the reaction conditions to influence protein folding, disulfide bond formation, incorporation of unnatural amino acids and the expression of toxic proteins. Eukaryotic cell-free systems derived from *Spodoptera frugiperda* (*Sf*21) and Chinese hamster ovary (CHO) cells are used to synthesize the membrane protein of interest. The synthesized protein is incorporated cotranslationally into native endoplasmic reticulum (ER) derived microsomes. Subsequently microsomes harboring the active protein of interest are used for functional analysis. In the case of prokaryotic cell-free systems, empty nanodiscs synthesized in house are added directly into the reaction mixture. After the synthesis nanodiscs with incorporated membrane proteins are purified and used for functional analysis. Thus efficient use of CFPS helps in the economic production of functional MPs as targets for drug development. Planar bilayer electrophysiology (PLB) is used to study the ion channel activity by reconstituting them artificially into artificial lipid bilayers. Ion-channels are analyzed by planar lipid bilayer electrophysiology on a multielectrode array. PLB helps to monitor the ion channel activity after reconstitution into an artificial planar bilayer individually at a molecular level without any interfaces. In the case of transporters and pumps which have normally low turnover rates, transient currents due to activation are measured by solid supported membranes (SSM) based electrophysiology.

Cell-free Protein Synthesis



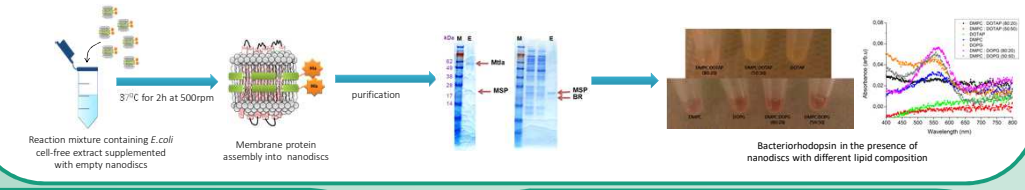
Synthesized MPs present in ER vesicles can be used directly for functional analysis. Proteoliposomes can also be prepared by mixing with artifical lipids

Step 1 Preparation and Characterization of Nanodiscs



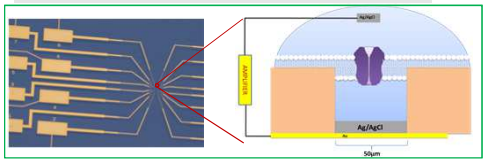
Nanodiscs were prepared by doping charged lipids (cationic DOTAP and anionic DOPG) in DMPC lipids. Purification can be performed by using His-tag attached to the nanodiscs.

Step 2 Nanodiscs for Membrane protein incorporation during Cell-free synthesis

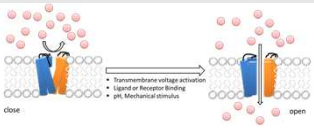


Planar bilayer electrophysiology (PLB) for ion channel functionality

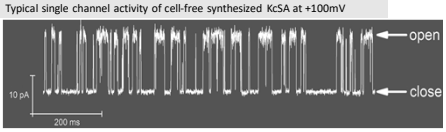
Reconstitution of ion channels into planar lipid bilayers on a multielectrode array



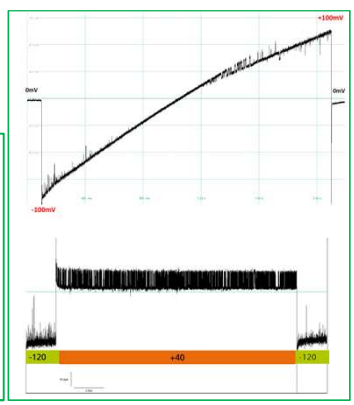
Stimulation of ion channels resulting in the opening of the gate thus permitting the ions on either side of the plasma membrane to flow down their gradient



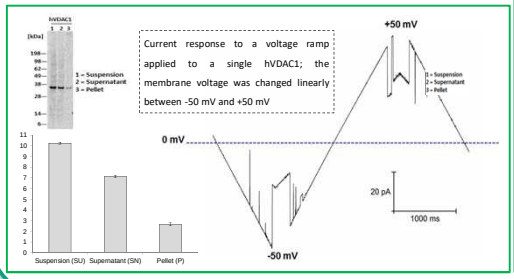
Typical single channel activity of cell-free synthesized KcSA at +100mV



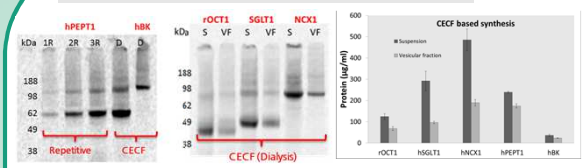
Single channel activity of cell-free synthesized KvAP measured by voltage ramp (-100mV to +100mV) and voltage switch (-120mV to +40mV)



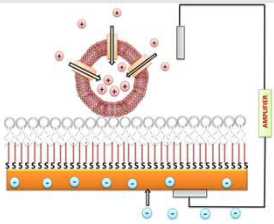
Electrical activity of cell-free synthsized hVDAC1 measured by voltage ramp (-50mV to +50mV)



Cell-free synthesis and characterization of MPs

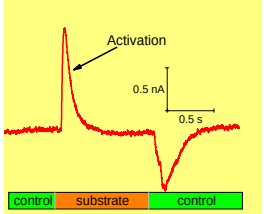


SSM-based electrophysiology



Scheme of solid supported membrane (SSM)-based electrophysiology showing the movement of substrate across the active transporter (orange cylinders)

Typical current response from oligopeptide transporter PEPT1 cell membrane patches due to the activation by dipeptide Glycyl-Glycine resulting in an activation peak.



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