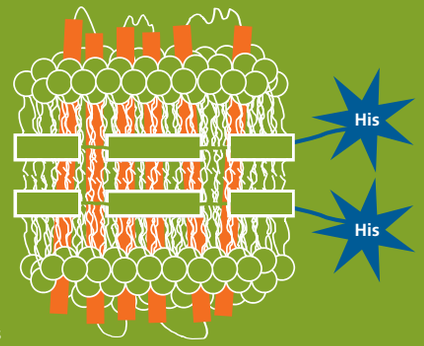

Membrane protein assembly into nanodiscs

Fraunhofer
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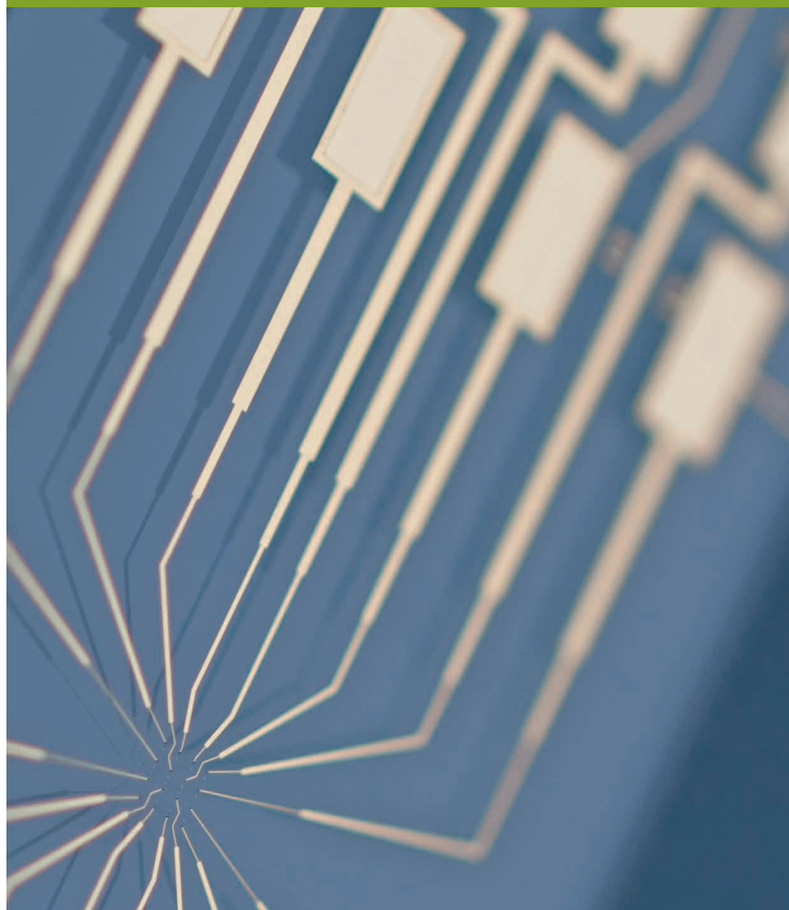
FRAUNHOFER INSTITUTE FOR CELL THERAPY AND IMMUNOLOGY, BRANCH BIOANALYTICS AND BIOPROCESSES IZI-BB

MEMBRANE PROTEIN SYNTHESIS AND ANALYSIS



APPLICATIONS

- Cell-free synthesis of membrane proteins and toxic proteins
- Nanodiscs for incorporation of membrane proteins
- Rapid functional analysis of toxic proteins immediately after synthesis by planar bilayer electrophysiology
- Screening of electrogenic transporters, pumps and ligand gated ion channels synthesized by both cell based and cell-free systems using SSM-based electrophysiology
- Screening of ion channels (voltage gated, ligand gated, mechanical regulated) synthesized by cell-free systems using planar bilayer electrophysiology
- Screening of transporter proteins synthesized by cell-free systems based on radiolabeled substrate uptake assay
- Individual protein specific optimization of the functional assay

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- Dondapati et al.: Membrane assembly of the functional KcsA potassium channel in a vesicle-based eukaryotic cell-free translation system. Biosens Bioelectron. (2014) 59:174
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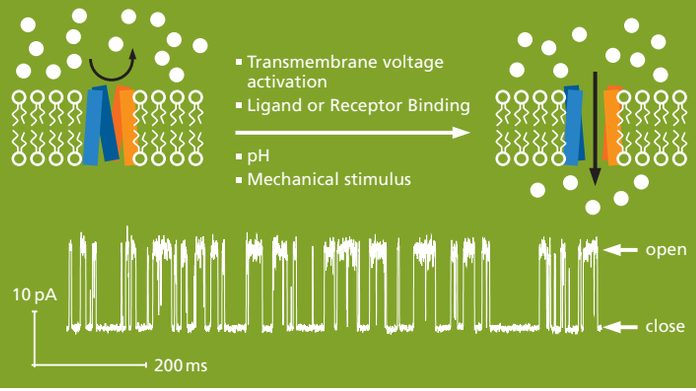
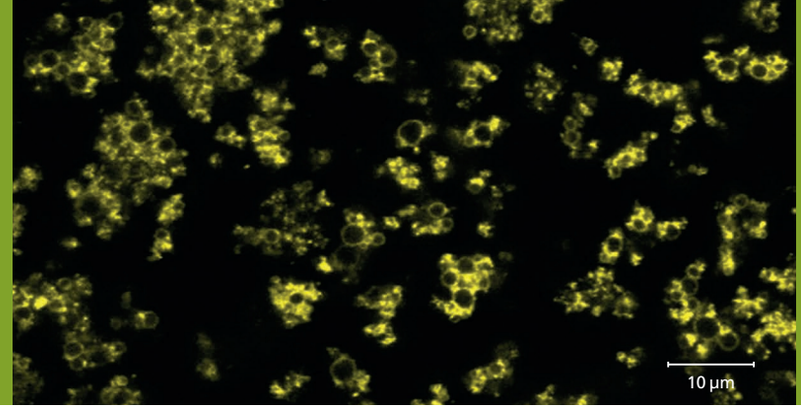
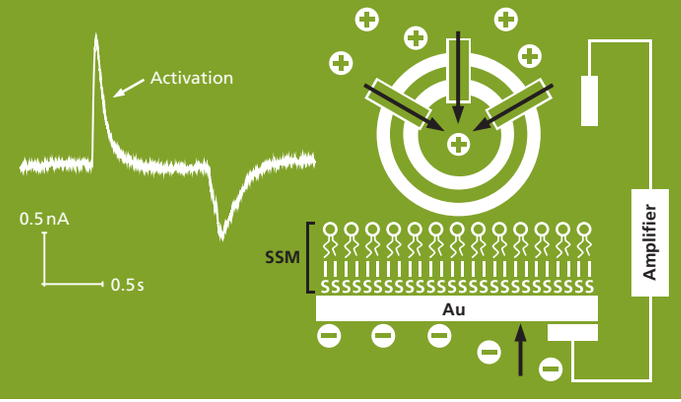




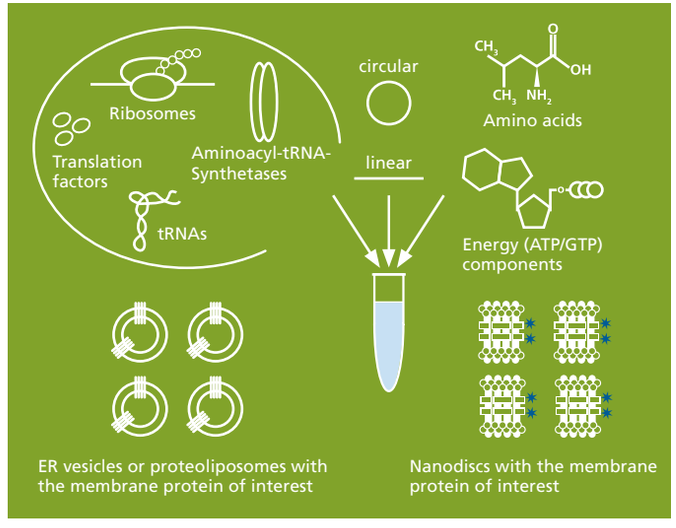


CELL-FREE MEMBRANE PROTEIN SYNTHESIS

Integral membrane proteins (MPs) represent more than two-third of the known protein targets for drugs due to their involvement in vital cellular processes. Synthesis of MPs *in vivo* is challenging due to low yields, solubilization and purification problems, toxicity due to overexpression, and functional assessment. An alternative for *in vivo* methods is cell-free synthesis of proteins. This method offers openness with a high degree of controllability allowing direct manipulation of the reaction conditions which influence protein folding, disulfide bond formation, incorporation of modified/ non-canonical amino acids and the expression of toxic proteins.

Eukaryotic cell-free lysates 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 directly into native endoplasmic reticulum (ER) derived microsomes during the cell-free synthesis. Microsomes harboring the active protein of interest can be directly used for the functional analysis. In the case of prokaryotic cell-free systems, empty nanodiscs synthesized in house are added directly in to the reaction mixture. After the synthesis, membrane protein is incorporated into the nanodiscs bilayer. Nanodiscs with incorporated membrane protein is purified and used for functional analysis.

Ion-channels and toxic porins are analyzed by planar lipid bilayer electrophysiology on a multielectrode array. Ion-channels are analyzed for their gating properties and toxic porins are analyzed for their large open pore currents induced by the insertion of oligomerized pre-pore complex into the planar lipid bilayer.
In the case of transporters which has normally a low turn over rate, transient currents due to activation is measured by solid supported membranes (SSM-based) electrophysiology.



Cell-free synthesis of lipid-embedded membrane proteins

Advantages

- Label-free electrical measurements using SSM-based electrophysiology from electrogenic transporters (symporters, exchangers and uniporters), pumps and ligand gated ion channels
- Highly stable SSM allow us to determine a wide range of kinetic properties like EC_{50}, IC_{50}, K_m, K_d, rate constants
- Parallel recordings of ion channel (voltage and ligand gated, temperature sensitive) gating properties as well as large open pore currents from toxins on microelectrode array for substantial data generation

Equipment

- Orbit mini and Orbit 16 (Nanion technologies GmbH) for parallel recording of ion channel and toxin pores
- Port-a-patch set up (Nanion technologies GmbH) for planar patch clamping of cells and GUVs
- Zetasizer (Malvern) for measuring the size of liposomes, microsomes and nanodiscs
- Vesicle Prep pro (Nanion technolgoies GmbH) for synthesizing liposomes
- Phosphoimager set up for studying radiolabeled substrate transport for metabolic transporters
- SURFE²R technology (Nanion technologies GmbH) for SSM-based electrophysiology measurements