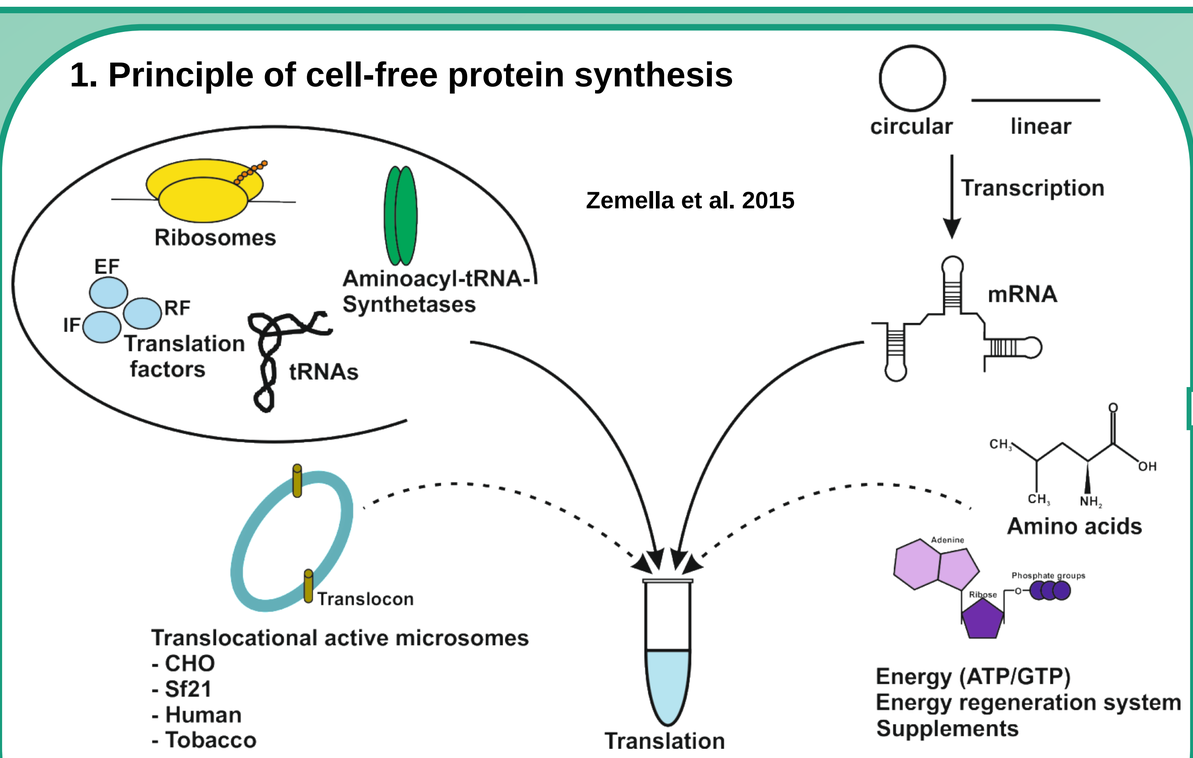
Production and Functional Characterization of G Protein-Coupled Receptors in Eukaryotic Cell-Free Systems

Anne Zemella[1], Andrei Sonnabend[1], Viola Spahn[2], Solveig Grossmann[3], Marlitt Stech[1], Lena Thoring[1], Doreen A. Wüstenhagen[1], Christoph Stein[2], Michael Schaefer[3] and Stefan Kubick[1]

[1] Fraunhofer Institute for Cell Therapy and Immunology (IZI), Branch Bioanalytics and Bioprocesses (IZI-BB), Am Mühlenberg 13, 14476 Potsdam, Germany
[2] Department of Anesthesiology and Intensive Care Medicine, Charité – Universitätsmedizin Berlin, Campus Franklin, Berlin, Germany
[3] Rudolf-Boehm-Institut für Pharmakologie und Toxikologie, Medizinische Fakultät, Härtelstraße 16-18, 04107 Leipzig, Germany

The biochemical analysis of human cell membrane proteins remains a challenging task due to the difficulties in producing sufficient quantities of functional protein. G protein-coupled receptors (GPCRs) represent a main class of membrane proteins and drug targets, which are responsible for a huge number of signaling processes regulating various physiological functions in living cells. To circumvent the current bottlenecks in GPCR studies, we established the synthesis of GPCRs in eukaryotic cell-free systems based on extracts generated from insect (*Sf*21) and Chinese Hamster Ovary (CHO) cells. Both cell lysates harbor the fully active translational and translocational machinery allowing posttranslational modifications, such as glycosylation and phosphorylation of *de novo* synthesized proteins. Here, we describe different methods to synthesize GPCRs and analyze their ligand-binding properties. The first method includes a standard “one-point” radioligand binding assay to compare different cell-free synthesized μ opioid receptors (MOR) with different initiation sequences (C = CrPV IRES, N = Spacer, T = No Spacer, F = Flag-Tag, M = Melittin) a MOR expressed in a human cell line. For the second approach a fast fluorescence-based screening method is applied to determine the localization, orientation and ligand-binding properties of the endothelin B (ET-B) receptor. Taken together, our studies underline the potential of-cell-free protein synthesis systems for the synthesis of difficult-to-express proteins and their functional characterization.

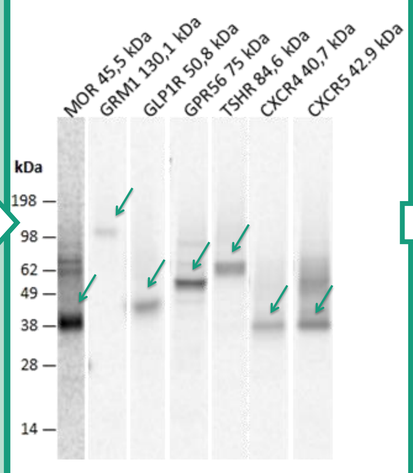
1. Principle of cell-free protein synthesis



Advantages:

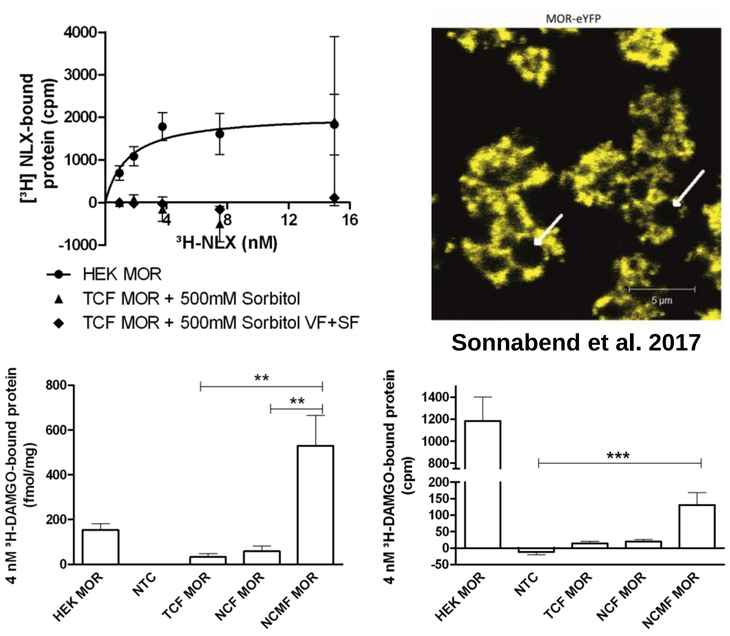
- Rapid synthesis of target protein (3h)
- PTMs (Glycosylation, Lipidation, Phosporylation)
- Optimization of reaction conditions
- HTS-compatible

2. Synthesis of different GPCRs



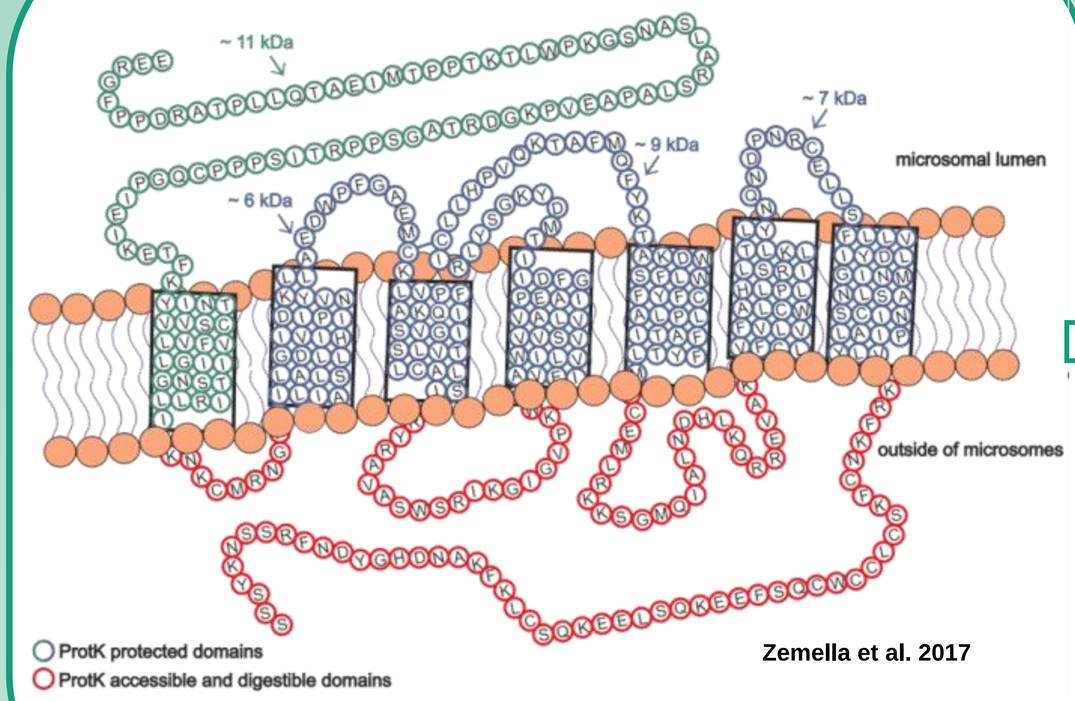
GPCRs of different types and molecular weight (40-130 kDa) were successfully synthesized in an insect-based cell-free system.

3. Radioligand binding studies of MOR



Depending on translation initiation different binding capabilities of cell-free MOR to [³H]-DAMGO are observed. Normalized to the respective applied protein concentration, cell-free MOR bound more [³H]-DAMGO compared to MOR expressed in HEK 293 cells.

4. Insertion of ET-B into microsomal membranes



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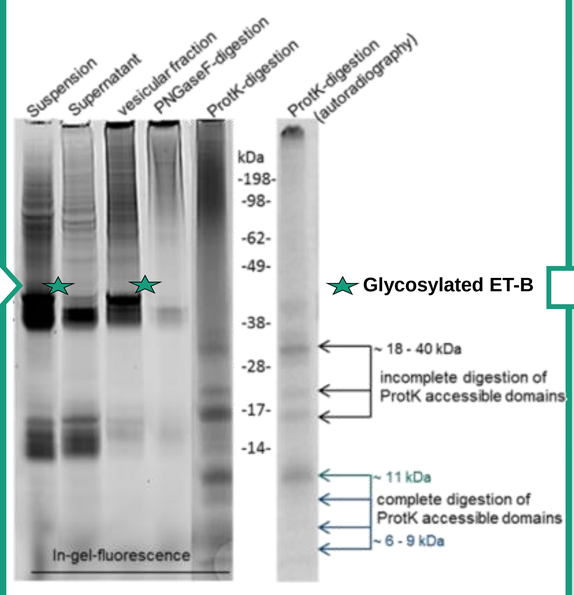
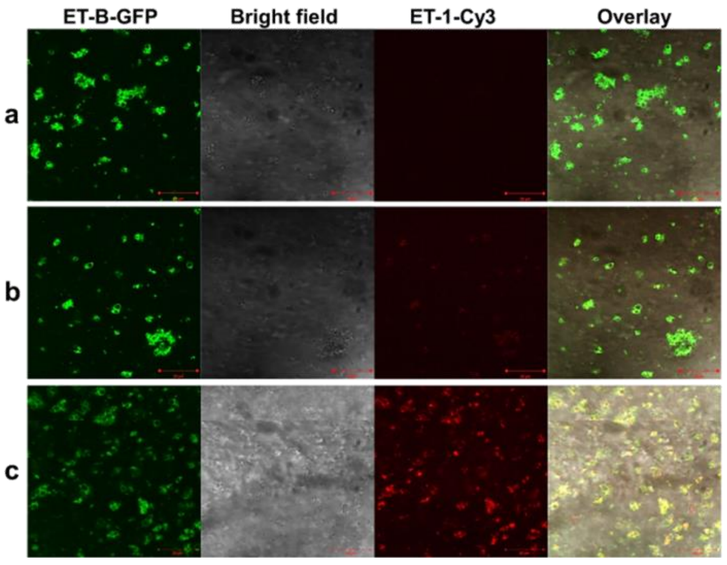
Shown in red are proteinase K acessible domains that will probably be digested by the enzyme. Proteinase K protected domains are colored in green and blue. The Ligand binding site is located in the microsomal lumen.

5. Analysis of Glycosylation and Orientation of ET-B



PNGaseF and ProtK-digestion was performed with the vesicular fraction. Green and blue arrows indicate the coressponding bands to the illustrated domains in the left panel.

6. Fluorescence based binding studies of ET-B



Determination of ligand binding using confocal laser scanning microscopy. (a) Confocal imaging of ET-B-GFP in absence of any ligands; (b) imaging of ET-B-GFP and ET-1-Cy3 in absence of any detergents; (c) Co-localization of ET-B-GFP and Cy3-ET-1 in the presence of 0,03% Brij 35;

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Contact: Anne Zemella | Fraunhofer Institute for Cell Therapy and Immunology | Branch Bioanalytics and Bioprocesses Potsdam-Golm | Cell-free and cell-based Bioproduction | Cell-free Protein Synthesis | Am Mühlenberg 13 | 14476 Potsdam | Telefon +49 331 581 87-332 | E-Mail anne.zemella@izi-bb.fraunhofer.de | www.izi.fraunhofer.de