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

CELL-FREE SERVICES

INTRODUCTION

Cell-free protein synthesis, often referred to as in vitro translation, is a fast and viable technique which, in comparison to in vivo protein synthesis, leads to the production of a target protein in a considerably less laborious way. Cell-free systems are based on lysates derived from E. coli or eukaryotic sources and they allow for a synthesis of a broad spectrum of structurally diverse and modified proteins. The path from a DNA template to the protein of interest is reduced to only a few hours of time and additionally, no genetically modified organisms (GMOs) are needed. A variety of proteins, such as certain membrane proteins (e.g. GPCRs), toxins or transcriptional and translational factors, whose synthesis in conventional in vivo systems is often associated with difficulties, can be synthesized in vitro. Only the target protein is synthesized in cell-free systems, since endogenous mRNA templates are removed during the process of lysate preparation.

The open and flexible character of cell-free systems allows the well-tuned adjustment of the reaction environment by supplementing the reaction mixture with co-factors, chaperones, detergents, rare tRNAs and buffers of varying ion composition, depending on the demands of the individual protein. The reaction conditions during protein synthesis significantly impact protein folding, protein activity and functionality.

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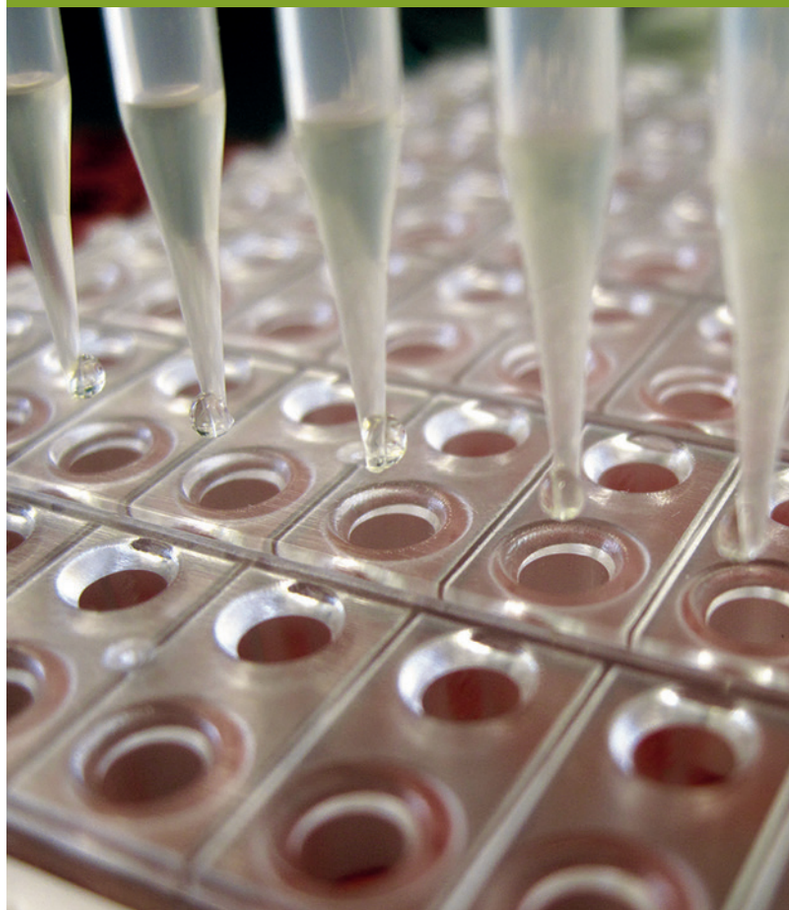
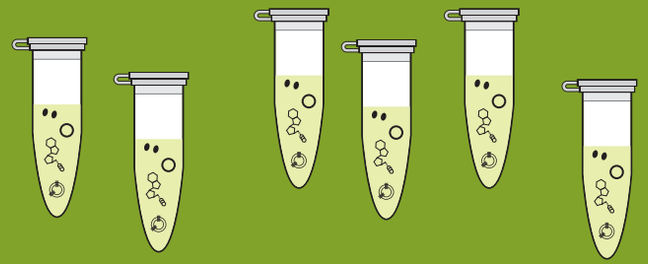
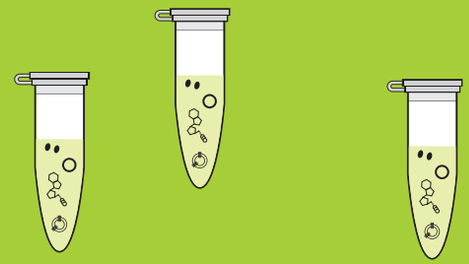




PHASE I
SCREENING CELL-FREE SYSTEMS

During phase one a first evaluation of the synthesis of the target protein is performed. Different lysates can be employed in this phase to establish the optimal system for the expression of a given target protein (based on cultivated insect, CHO or human cell lines, E. coli and wheat germ lysates).
Plasmids or mRNA can be used as template. Testing of already existing templates or design and generation of optimal templates is realized for the different systems. The introduction of mutations e.g. for protein engineering is possible.



PHASE II
SYSTEM OPTIMISATION

Further optimization of the reaction conditions in defined systems can be carried out to improve the yield or activity of newly synthesized proteins. The purification of the target protein, the performance of activity assays (e.g. ELISAs), the electrophysiological characterization of membrane proteins (e.g. ion channels) but also cell culture assays (e.g. for activity studies) can be included in this evaluation phase.

PHASE III
CELL-FREE PROTEIN SYNTHESIS USING OPTIMAL CONDITIONS

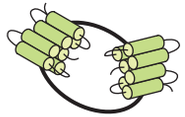
After the successful completion of protein synthesis conditions, the target protein can be synthesized in larger amounts (µg- to mg-scale).

GLP conditions
On request, cell-free protein synthesis can be carried out according to GLP guidelines.

APPLICATIONS

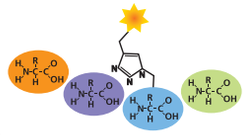
Antibodies

Membrane proteins

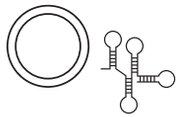


Cytotoxic proteins

Labeling of proteins



Validation of templates



Protein engineering