Protein modification in eukaryotic cell-free systems through incorporation of noncanonical amino acids

Dr. Stefan Kubick

Head of Department Cell-free Bioproduction,

Fraunhofer Institute for Cell Therapy and Immunology (IZI), Branch Bioanalytics and Bioprocesses Potsdam-Golm (IZI-BB), Am Mühlenberg 13, 14476 Potsdam

The remarkable range of functions carried out by membrane proteins as well as soluble proteins results from only 20 building blocks - the 20 canonical amino acids - together with a limited amount of additional chemistries arising from post-translational modifications and cofactors. A huge number of approaches which can be summarized as general ligation techniques and chemical aminoacylation of tRNAs, have been pursued to address this limitation given by the natural repertoire to generate proteins with enhanced or novel properties. Overcoming general restrictions of these methods, the expansion of the genetic code provides a promising tool for the efficient production of site-directed and chemoselective modified proteins enabling the introduction of unnatural characteristics to mimic or further enhance protein properties *in vivo* and *in vitro*. In this context, the cotranslational introduction of chemoselective amino acid analogues during cell-free protein synthesis represents a powerful tool to selectively equip proteins with desired characteristics. We have successfully adapted an orthogonal tRNA/synthetase pair to a cell-free system derived from cultured eukaryotic cells. In this system, we could demonstrate the efficient cotranslational incorporation of alkyne as well as azide functionalities into cell-free synthesized proteins in a site-directed manner by means of the amber suppression methodology. Subsequently, site-directed modification of proteins with a variety of different applicable reagents is possible. The *in vitro* translation system is equipped with translocationally active microsomes originating from the endoplasmic reticulum of insect cells. These microsomes provide the entire machinery for additional post-translational modifications, e.g. glycosylation and lipidation of cell-free synthesized proteins. Due to its high fidelity, this novel cell-free system provides a promising platform for the synthesis and characterization of chemically modified and functional proteins.

BRÖDEL, A.K., WÜSTENHAGEN, D:A:, KUBICK, S.: „Cell-free Protein Synthesis Systems Derived from Cultured Mammalian Cells“. Raymond J. Owens (ed.), Structural Proteomics: High-Throughput Methods, Methods in Molecular Biology, Vol. 1261, Chapter 7, DOI 10.1007/978-1-4939-2230-/_7, Springer Science and Business Media New York (2015).

DONDAPATI, S.K., KREIR, M., QUAST, R.B., WÜSTENHAGEN, D.A., BRÜGGEMANN, A., FERTIG, N., KUBICK, S.: „Membrane assembly of the functional KcsA potassium channel in a vesicle-based eukaryotic cell-free translation system”. Biosens Bioelectron. (59) (2014), 174-83.

FENZ, S.F., SACHSE, R., SCHMIDT, T., KUBICK, S.: “Cell-free synthesis of membrane proteins: tailored cell models out of microsomes”. Biochim Biophys Acta 1838(5) (2014), 1382-8.

QUAST, R.B., CLAUSNITZER, I., MERK, H., KUBICK, S., GERRITS, M.: „Synthesis and site-directed fluorescence labeling of azido proteins using eukaryotic cell-free orthogonal translation systems“. Analytical Biochemistry 451 (2014), 4-9.

SACHSE, R., DONDAPATI, S.K., FENZ, S.F., SCHMIDT, T., KUBICK, S.: “Membrane protein synthesis in cell-free systems: From bio-mimetic systems to bio-membranes”. FEBS Lett. 88 (2014), 2774-2781.

STECH, M., HUST, M., SCHULZE, C., DÜBEL, S., KUBICK, S.: “Cell-free eukaryotic systems for the production, engineering and modification of scFv antibody fragments”. Engineering in Life Sciences 14 (2014), 387-398.

STECH, M., QUAST, R.B., SACHSE, R., SCHULZE, C., WÜSTENHAGEN, D.A., KUBICK, S.: “A continuous-exchange cell-free protein synthesis system based on extracts from cultured insect cells”. PLoS One 9 (2014), e96635.