

# 2<sup>nd</sup> Glycobiology World Congress

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## Cell-free synthesis of glycoproteins

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Membrane proteins and glycoproteins have become an important focus of the current efforts in structural and functional genomics. In contrast, the molecular analysis of posttranslationally modified proteins lags far behind that of non-modified, cytosolic and soluble proteins. Preparing high quality samples of functionally folded proteins represents a major bottleneck that restricts further structural and functional studies. Cell-free protein synthesis systems, in particular those of eukaryotic origin have recently been developed as promising tools for the rapid and efficient production of a wide variety of posttranslationally modified proteins. This is mainly due to the properties of cultured eukaryotic cells, which are able to carry out many types of posttranslational modifications such as the addition of N and O-linked oligosaccharides, but also palmitoylation, myristylation and phosphorylation. Based on these versatile properties of cultured cell lines, we have developed a technique for the standardized production of translationally active eukaryotic cell lysates. Our homogenization procedure avoids any serious breakdown of membrane vesicles already existing in the cytoplasm of the prepared eukaryotic cells. We have demonstrated the functional integrity of these sub-cellular components by showing signal peptide cleavage as well as glycosylation of *in vitro* produced proteins. Moreover, we have expanded our cell-free protein synthesis system by the insertion of orthogonal tRNA/synthetase pairs to facilitate the co-translational and site directed incorporation of non-canonical building blocks. These fluorescently labeled and chemoselective moieties enable the site-specific modification of de novo synthesized glycoproteins.

## Biography

Stefan Kubick is the Head of the Department "Cell-free Bio-production" at the Fraunhofer IZI-BB. His laboratory exploits cell-free protein synthesis as a versatile tool for membrane protein and glycoprotein synthesis, as well as chip-based protein synthesis and translational regulation. In collaboration with Qiagen GmbH he developed novel eukaryotic *in vitro* translation systems. He is also a Lecturer at the Free University of Berlin and the University of Potsdam. He is an affiliate of the Technical University of Berlin and Lecturer at the University of Applied Sciences, Berlin, Germany.

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