The Expanded Genetic Code in Yeasts

Silke Grumaz, Fraunhofer IGB, Stuttgart, Germany; Yannick Bantel, IGVP, University of Stuttgart, Stuttgart, Germany; Michael Berg, Fraunhofer IGB, Stuttgart, Germany; Steffen Rupp, Fraunhofer IGB, Stuttgart, Germany; Kai Sohn, Fraunhofer IGB, Stuttgart, Germany

Synthetic Biology successfully combines aspects of engineering, chemistry, informatics and biology. Different research fields of synthetic biology have evolved over recent years including de-novo design of complete organisms, the utilization of »building bricks« that can be used to engineer tailor-made synthesis pathways for the production of platform chemicals or orthogonal biosystems that are promising tools to generate modified, synthetic proteins, for example. Orthogonal pairs comprising amber tRNAs and the corresponding aminoacyl-tRNA synthase expand the genetic code of a target organism to site-specifically integrate non-natural amino acids into proteins *in vivo*. Consequently, such artificial amino acids confer new physic-chemical properties to the corresponding protein of interest. P-azidophenylalanine is an artificial amino acid derivative of the natural amino acid phenylalanine and is particularly suitable for the study of molecular interactions or to provide unique sites for click-chemistry. The azido-group does not occur in proteins under physiological conditions, but can be activated by UV excitation to form a stable covalent bond with molecules in close vicinity. By establishing an orthogonal pair consisting of tRNA and a corresponding aminoacyl-tRNA synthase, we were able to establish an expanded genetic code in *Candida albicans*, which is the most important human pathogenic fungus. We generated synthetic proteins by position-specific integration of p-azidophenylalanine or p-benzoylphenylalanine into various proteins of *Candida albicans*, including Tup1p, which is critical for virulence. Such expanded genetic codes thus not only allows for high-resolution protein-protein interaction analyses *in vivo*, but might also enable innovative approaches to optimize therapeutic proteins in near future.