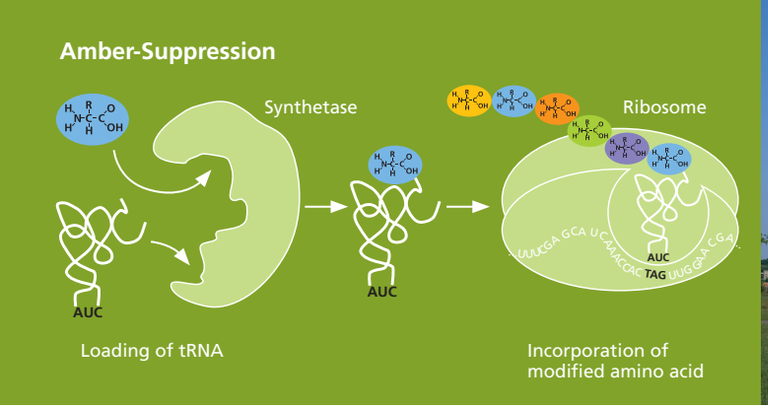

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PROTEIN LABELING AND MODIFICATION

APPLICATIONS

- Cell-free protein synthesis of difficult-to-express proteins including membrane proteins
- Tailor-made selection of positions and introduction of amber-stop-codon into the target gene sequence
- Incorporation of chemically modified amino acids at selected positions via amber suppression
- Coupling of fluorescent dyes, sugar moieties, biotin and polymers (PEGs) to the target protein
- Characterization of modified cell-free synthesized proteins by microscopic analysis and autoradiography
- Interaction studies and ligand binding assays

REFERENCES (SELECTION)

- Zemella et al.: Qualifying a eukaryotic cell-free system for fluorescence based GPCR analyses. Sci Rep. (2017) 7:3740
- Quast et al.: Cell-free synthesis of functional human epidermal growth factor receptor: Investigation of ligand-in-dependent dimerization in *Sf*21 microsomal membranes using non-canonical amino acids. Sci Rep (2016) 6:34048
- Zemella et al.: Cell-free protein synthesis: Pros and cons of prokaryotic and eukaryotic systems (2015) Chembiochem 16(17): 2420-2431

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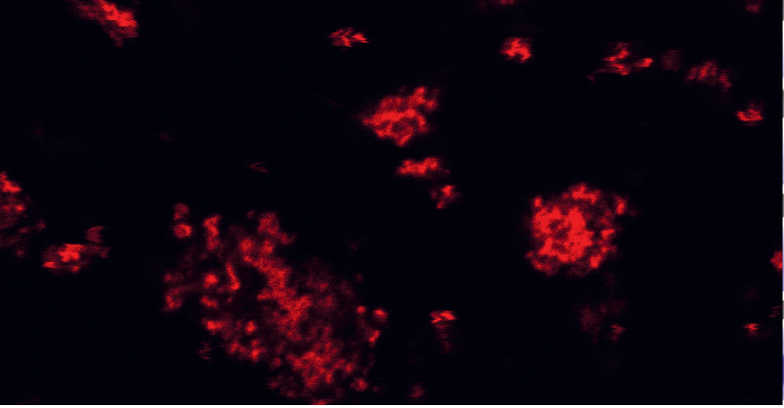
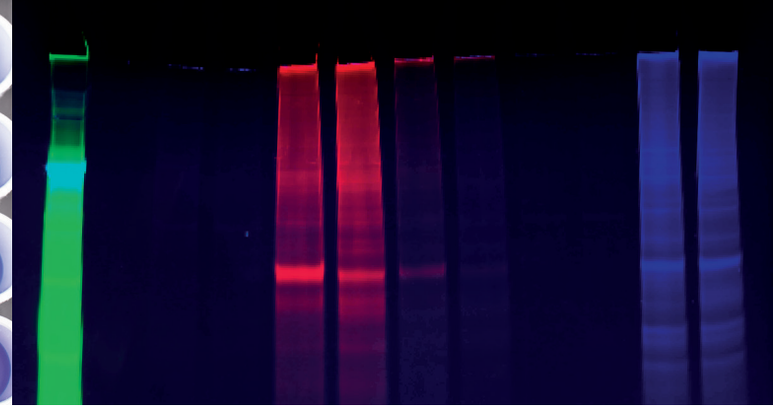




PROTEIN REMODELING

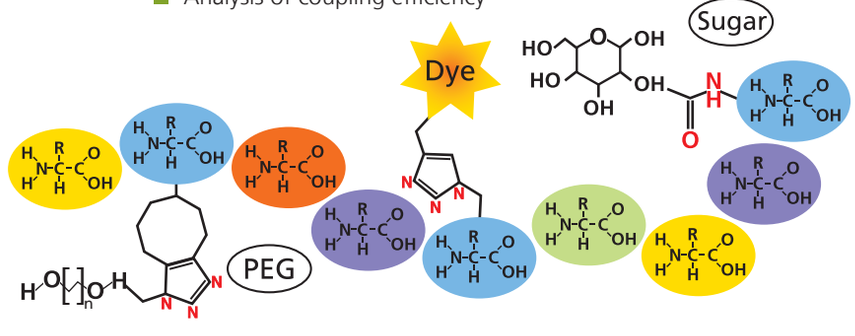
Nature has evolved a set of 20 standard amino acids that build up nearly each protein. This finite number of amino acids limits their biochemical and biophysical characteristics. The research group »Cell-free protein synthesis« provides a reliable procedure to incorporate chemically modified amino acids with diverse reactive groups into cell-free synthesized proteins.

Eukaryotic cell-free systems based on cultured insect (*Sf*21), Chinese hamster ovary (CHO) and human cells enable a time-saving and versatile alternative to *in vivo* production to remodel pharmaceutically relevant proteins. Cell-free protein synthesis is based on translationally active cell lysates that allow a protein synthesis within three hours after a DNA template was added. The incorporation of a modified amino acid is proceeded by the amber-stop-codon technology. Therefore special tRNA and aminoacyl-tRNA-synthetase pairs were established to specifically incorporate the modified amino acid at the position of the amber-stop-codon in the gene sequence.

TECHNOLOGIES

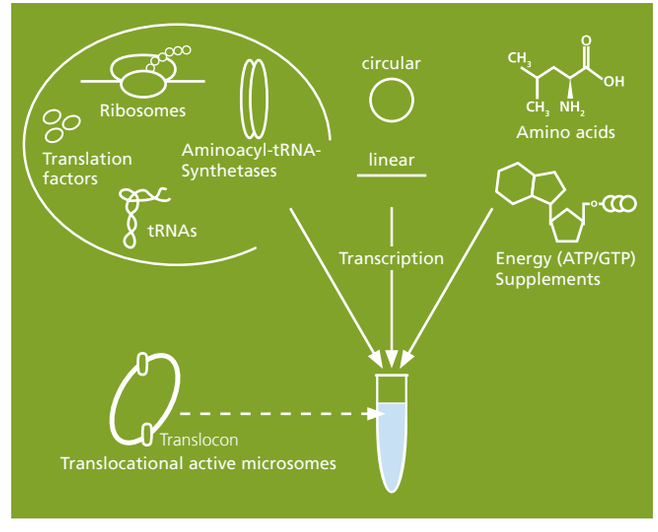
We enable the coupling of fluorescent dyes, sugar moieties, PEGylation and biotinylation to the reactive group of the incorporated amino acid by click chemistry. Our approach can be further utilized to determine specific conformations of membrane proteins, interaction partners and for the screening of novel ligands and therapeutics.

- Design of DNA templates with appropriate amber-stop-codon positions
- Evaluation of different amber-stop-codon positions by determination of incorporation efficiency
- Protein quantification
- Coupling of customized modifications with different click chemistries including copper-free click chemistry
- Analysis of coupling efficiency



EQUIPMENT

- Amersham Typhoon RGB Biomolecular Imager for autoradiography, In-gel-fluorescence, chemiluminescence
- LB 943 Mithras Monochromator Multimode Microplate Reader for luminescence, fluorescence, absorption, FRET and BRET analyses
- Confocal laser scanning microscope (Zeiss CLSM 510) for the analysis of fluorescence labeled proteins



Principle of cell-free protein synthesis