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The use of unnatural amino acids to study and engineer protein function

Petra Neumann-Staubitz[1] and Heinz Neumann[1,2]

The expansion of the genetic code for the incorporation of unnatural amino acids (UAAs) in proteins of bacteria, yeasts, mammalian cells or whole animals provides molecular and structural biologists with an amazing kit of novel tools. UAAs can be used to investigate the structure and dynamics of proteins, to study their interactions or to control their activity in living cells. Incorporation of UAAs with bioorthogonal reactivity facilitates the site-specific installation of labels for spectroscopy and microscopy. Light-activatable crosslinker UAAs can be used to trap interacting molecules in living cells with a precision almost at the structural level. Post-translational modifications such as lysine acetylation and serine phosphorylation can be directly encoded to analyse their impact on protein function, and caging groups can be installed on critical residues to create light-activatable proteins. In this review we highlight recent applications of this technology to investigate protein function.

Addresses
[1] Georg August University Göttingen, GZMB, Applied Synthetic Biology Group, Justus-von-Liebig Weg 11, 37077 Göttingen, Germany
[2] Max-Planck-Institute for Molecular Physiology, Otto-Hahn-Strasse 11, 44227 Dortmund, Germany

Corresponding author: Neumann, Heinz (heinz.neumann@mpi-dortmund.mpg.de)

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Introduction

The universally conserved genetic code has been extensively diversified in recent years. This expansion of the genetic code, which allows the incorporation of unnatural (or non-canonical) amino acids (UAAs) in proteins from bacteria to yeasts to entire animals, provides molecular biologists with amazing tools to study protein structure and function [1]. These new acquisitions in the collection of proteinogenic amino acids bring along a multitude of unprecedented useful properties.

UAAs are encoded by evolved orthogonal aminoacyl-tRNA synthetase/tRNA pairs expressed in the host system (Figure 1). The UAA is supplied with the growth medium, charged to the tRNA and utilized to decode (usually) amber codons placed into the gene of interest. Hence, the resulting protein contains the UAA at the genetically defined position. The past decade has witnessed an exponential growth in reports on applications of UAA mutagenesis to study protein function.

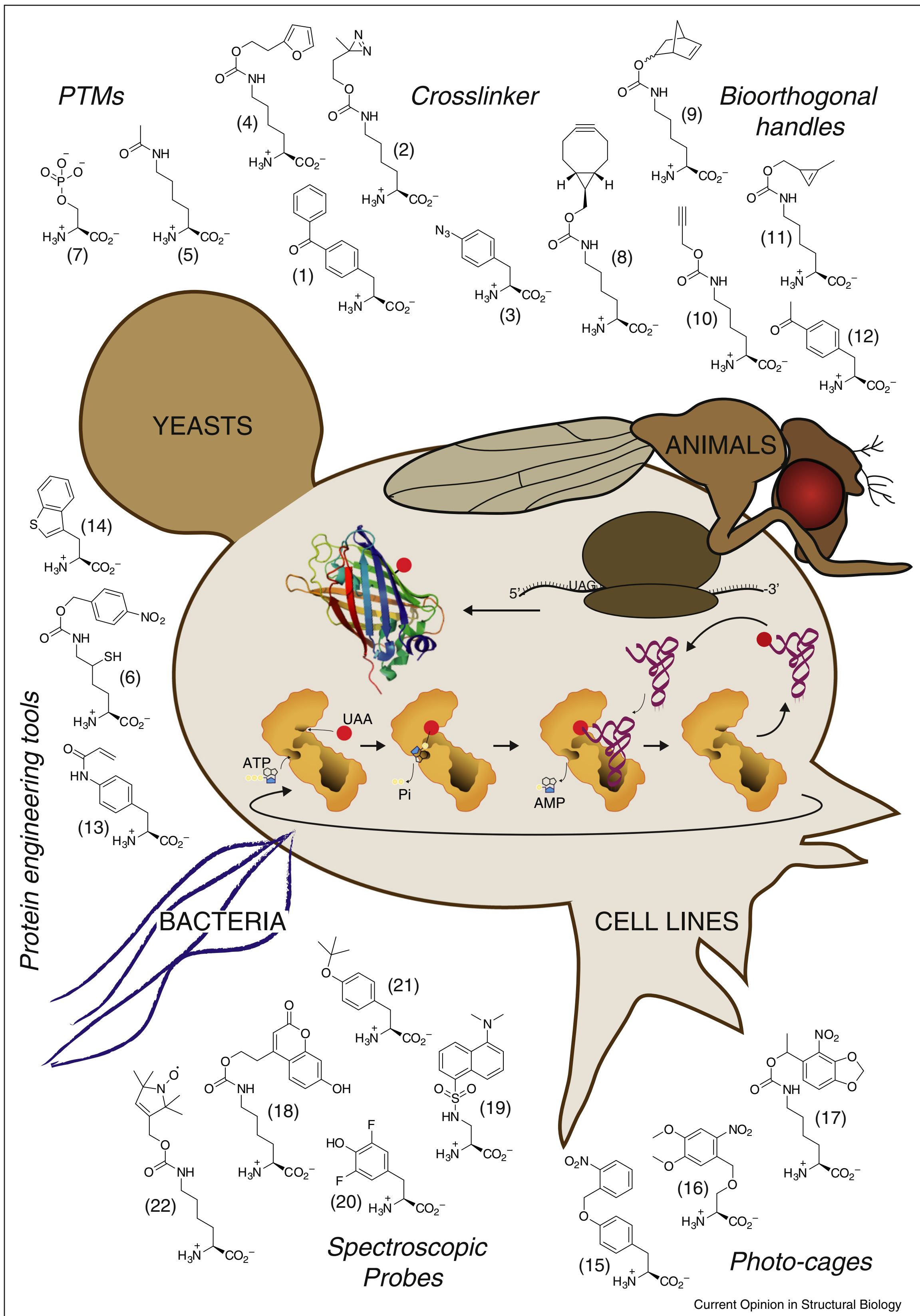
Caught in the act — trapping protein ligands with crosslinker UAAs

UV-activatable crosslinker UAAs installed at suitable positions on the surface of bait proteins can be used to trap interacting molecules. This approach has many valuable advantages over conventional chemical crosslinking technologies. Triggering the reaction by light provides perfect temporal (and spatial) control over the reaction. Different classes of photo-crosslinkers are available: *p*-benzoyl-phenylalanine (BPA, **1**) is most commonly used to trap protein–protein or protein–ligand interactions via insertion of the light-induced benzophenone diradical in C–H-bonds. Other UV-activatable crosslinkers such as diazirine-modified lysines (**2**) and tyrosines or aromatic azides (**3**) eliminate nitrogen, thereby forming a reactive carbene or nitrene intermediate, which inserts into C–H, N–H, or O–H bonds. A furan-based crosslinker (**4**) couples specifically to nucleic acids upon red-light activation [2].

BPA can also be used as a DNA crosslinker. The position of the trailing and leading edge of prokaryotic RNA-polymerase in the open complex was determined against a promoter library to identify the role of a discriminator element in start site selection by modulating the size of the transcription bubble [3]. Entire libraries of amber mutants have been used to map the interaction network of a protein of interest *in vivo*. Screening the interaction interface of the histone chaperone complex FACT (facilitates chromatin transcription) with the nucleosome in this way identified a new role of the Pob3 subunit in FACT binding to H2A–H2B [4]. A 3D model of the peptide-agonist urocortin-1 bound to its G-protein-coupled receptor (GPCR) was derived from crosslinking data using *p*-azido-phenylalanine (AzF, **3**) genetically installed at various positions along the ligand binding site [5] (Figure 2a).

Crosslinkers are of particular value in cases when information on protein structure is scarce or when the study of isolated components is insufficient to reveal function. A BPA-crosslinking survey across Tom40, the pore subunit of the active mitochondrial protein translocation gate

Figure 1



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