

A holistic approach to genetic code engineering

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Genetic code engineering is new research field that intent to reprogram protein synthesis by reassignment of specific codons to non-canonical (mainly synthetic) amino acids. The resulting proteins are alloproteins with tailor-made properties that are of outstanding interest for both, academia and industrial biotechnology. Our approach successfully combines synthetic chemistry with molecular biology and represents a paradigm for the research areas covered by synthetic biology.

We illustrate our holistic approach in this field by giving three examples demonstrating the principles and the applicability of protein engineering with synthetic amino acids.

First, in green fluorescent protein, the global replacement of ten proline residues with (4*S*)-fluoroproline substantially improves the folding and overall stability of the protein.

Second, the efficient manipulation of maturation processes is demonstrated by the design of gold fluorescent protein with unique and unprecedented spectral features. The introduction of 4-amino-tryptophan generates an entirely novel chromophore.

Third, the incorporation of synthetic protein building blocks with bioorthogonal chemical functionalities, such as terminal azide or alkyne groups, allows the chemical control of post-translational modifications. Using standard methods of organic chemistry (click chemistry), small molecules like sugars can be selectively coupled to the functionalized synthetic proteins.

Taken together, the alloproteins represent possible key players in future pharmaceutical research, materials science and drug design.

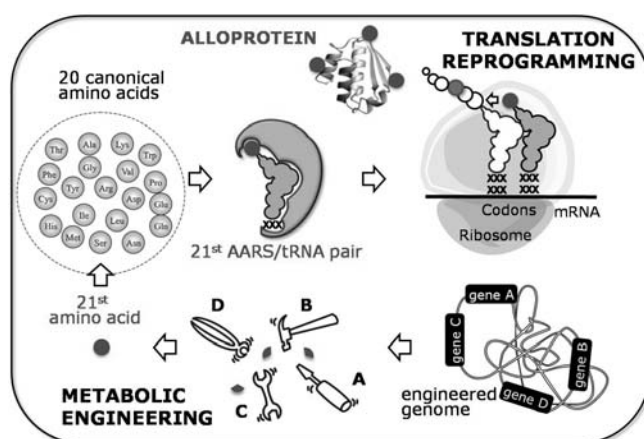


Figure 1. The *in vivo* production of alloproteins (tailor-made proteins) with synthetic amino acids is possible only with reprogrammed translation. Consequently, vital life processes of intact cells, such as amino acid uptake, activation and tRNA charging, the ribosome cycle and protein folding must be rationally manipulated. In a typical current experiment the synthetic amino acid is added to the growth medium prior to incorporation. It is highly desirable to manipulate the cells by metabolic engineering such that the synthetic amino acid can be biosynthesized intracellularly. This holistic approach is the first step towards the generation of designer cells with a new chemistry of life.

Références:

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