Noncanonical amino acids in a cell free TX-TL system.

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The canonical set of amino acids leads to an exceptionally wide range of protein functionality. Nevertheless, the set of residues still imposes limitations on potential protein applications. The incorporation of noncanonical amino acids can enlarge this scope. For this purpose we use a cell-free expression system to reassign the genetic code within the endogenous translational system. Kinetic proofreading is a process that will counteract the charging of tRNA with other amino acids than the canonical one [1,2], however, the translation machinery still accepts noncanonical amino acids as a surrogate and incorporates it at canonically prescribed locations, i.e., all occurrences of a canonical amino acid in the protein are replaced by the noncanonical one. We managed to replace L-arginine by the noncanonical amino acid L-canavanine within proteins completely [3] while the replacement of L-lysine by L-hydroxy-lysine remains a challenge because of residual L-lysine in the cell-free expression system [4,5]. We plan to study the error rate of amino-acid incorporation in competition to find out about possible non-linearities in the error correction process as opposed to the suggestions by Hopfield.

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