Expanded blueprint: Genetic incorporation of two different noncanonic amino acids into one protein

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(PhysOrg.com) -- The genetic code includes information for only 20 amino acids (AAs). If this repertoire could be expanded, it would, for example, be possible to program bacteria to produce tailored proteins with various characteristics of interest to science, technology, or medicine.

In fact, the natural protein-production mechanism can be reprogrammed, but until now it has only been possible to incorporate a single new type of AA into a protein. Wenshe Liu and his co-workers at Texas A&M University (USA) have now successfully included two different, noncanonic amino acids into the genetic material of bacteria, as they report in the journal *Angewandte Chemie.*

In order to synthesize a protein, a cell first copies a “blueprint” (mRNA) from the corresponding gene and “reads” it (translation). The genetic code for every AA consists of three “letters” (nucleotides). In addition, there is a start codon and three different codons that mean “stop”. “Transporters” (tRNA) that specifically recognize the codons are loaded with the required AA and bring it to the place where protein synthesis occurs (ribosomes). The “loaders” are special enzymes (aminoacyl tRNA synthetases).

Only 20 AAs are naturally coded; these are known as the canonical AAs. Other AAs are made accessible to organisms by modification of

individual AAs in the finished protein at a later stage. However, some bacteria that require an unusual AA as part of an enzyme used in their metabolism of methane use one of their stop codons (amber) for another purpose, so it functions as a codon for the additional AA. This method has previously been successfully emulated in the laboratory. Liu and his teams have now for the first time used two such bacterial systems in parallel. One of the tRNAs was mutated to recognize a different stop codon (ochre). By mutation, they were able to reprogram the associated aminoacyl tRNA synthetases so that they load up their tRNAs with the desired synthetic AA.

The researchers incorporated this altered genetic material into bacterial cells. As desired, these cells then incorporated two noncanonical AAs into one protein. These two AAs are constructed so that each has a specific “snap” where desired functional groups can later simply be “clicked on” (click chemistry). For example, it is possible to attach special pairs of molecules that fluoresce when they can exchange energy with each other. To do this, they must be at a specific distance and angle relative to each other. Such pairs make it possible to draw conclusions about the conformation of a protein, as well as its dynamic changes during a reaction.

More information: Wenshe Liu, A Facile System for Genetic Incorporation of Two Different Noncanonical Amino Acids into One Protein in Escherichia coli, Angewandte Chemie International Edition 2010, 49, No. 18, 3211-3214, dx.doi.org/10.1002/anie.201000465

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