

Applications of an expanded genetic code – novel methods for labeling proteins

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Abstract:

The ability to label any specific protein at a single position in the polypeptide with any desired small molecule fluorophore or probe would provide the ultimate labeling method for super resolution microscopy, FRET and single molecule imaging. In principle the genetically encoded, site-specific incorporation of custom-synthesized unnatural amino acids bearing a bioorthogonal functional group that reacts in a rapid and highly specific manner with a chemical reporter with tailored physical and biological properties could fulfill many of the requirements for an ideal protein labeling strategy.

The incorporation of designer amino acids can be achieved by making use of an expanded machinery of translation, as represented by ‘orthogonal’ aminoacyl-tRNA synthetase/tRNA pairs that direct amino acid incorporation in response to an amber stop codon (UAG) placed in a gene of interest.

We have recently discovered aminoacyl-tRNA synthetase/tRNA pairs for the efficient site-specific incorporation of several unnatural amino acids useful for site-specific protein labeling into proteins expressed in *E. coli* and mammalian cells. These unnatural amino acids, bearing strained alkene or alkyne moieties, react with tetrazine-probes in a very rapid, bioorthogonal Diels Alder reaction with inverse electron demand. We demonstrated the site-specific labeling of proteins in vitro, in *E. coli* and in live mammalian cells with tetrazine-fluorophore conjugates. (1-3) These reactions are very specific, very rapid and the tetrazine fluorophores, which are initially weakly fluorescent become strongly fluorescent once attached to the protein via the chemical reaction making the signal to noise of this labeling approach superior. This approach is being extended to site-specific protein labeling in animals and being used for several imaging studies like super resolution microscopy.

We envision that this labeling approach will impact on addressing important biological questions since it allows the non-invasive, site-specific, efficient and rapid labeling of target proteins using chemical probes with tailored physical and biological properties.

(1) Lang, K.; et al. *Nature Chemistry* 2012, 4, 298.

(2) Lang, K.; et al. *J. Am. Chem. Soc.* 2012, 134, 10317.

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