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We believe that the power of fluorescence tools in biology depends not only on pushing the limits of microscopy instrumentation, but also on our ability to engineer fluorescent dyes (labels) into proteins to make biological mechanism visible to the single molecule and superresolution technologies. Despite the fast pace of development, limitations of autofluorescent proteins (AFP) have become apparent, including their sheer size, poor photostability etc.. The need for novel protein engineering tools is emphasized by our current inability to translate in vitro studies to in cell (in vivo) studies. The unnatural amino acid (UAA) strategy (also called genetic code expansion (GCE)) is one of the most versatile protein engineering tools and requires only minimal modification to native protein structure.

We recently developed a semi-synthetic strategy based on novel UAAs that are easily and site-specifically introduced into any protein by the natural machinery of the living cell using GCE. (Nikic et al., 2014; Plass et al., 2011; Plass et al., 2012)Expressed proteins only differ from their natural counterparts by very few atoms, constituting a ring-strained cyclooctyne or cyclooctene functional group. We showed that these completely inert and non-toxic groups can be stably incorporated into any protein and readily react with commercially available single molecule fluorophores without the need of special reagents, catalysts or non-physiological buffer conditions. In particular, the fully biocompatible inverse-electron-demand Diels-Alder reaction (SPIEDAC) exhibits orders of magnitude faster reaction rates than the prototypical Huisgen-type click reaction (SPAAC). We have meanwhile discovered ultrafast reacting variants of the SPIEDAC reaction that even permit multi-color labeling of living cells.

Another important area of GCE is the production of engineered recombinant proteins, which is furthest developed in E. coli. However, many proteins and in particular eukaryotic protein complexes cannot be expressed in such simple organisms. We now present a protein engineering tool that enables site-specific introduction of unique functionalities in a recombinantly produced eukaryotic protein complex. We demonstrate the versatility of this efficient and robust protein production platform “MultiBacTAG” i) to fluorescently label target proteins and biologics using click chemistries, ii) for glycoengineering of antibodies, and iii) for structure–function studies of novel eukaryotic complexes using single molecule FRET as well as site-specific cross-linking strategies (Koehler et al., 2016).

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