Encoding new non-standard amino acid designs into proteins can address limitations of fluorescent proteins and facilitate genome editing approaches

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Project Goals: To engineer and demonstrate new cellular translation machinery in our reduced-codon *E. coli* strains to enable efficient expression of proteins containing multiple Non-Standard Amino Acids (NSAAs).

Improvements in imaging/labeling technology have often led to explosive growth of new knowledge in the basic sciences – for example, the development of fluorescent protein (FP) fusions as genetically encoded 'beacons'. However, as our ability to track the subcellular localization of single-molecules with nanometer/microsecond spatiotemporal resolutions soars, FP and FP-inspired protein labeling technologies are still lagging due to their large (>4nm) sizes and minutes-to-hours long maturation times.

Using designer fluorogenic amino acids (FgAAs) and nucleobase amino acids (NuAAs), we aim to develop new protein labeling strategies that can replace FPs in many cell biology applications. FgAAs, with their unique ability to become fluorescent only when incorporated into proteins, (i) are ~50-100 times smaller than most FPs; (ii) 'mature' instantaneously; (iii) are fluorescent in anaerobic conditions; and (iv) do not oligomerize. NuAAs will facilitate programmability of protein-DNA interactions, e.g. allowing multi-color protein imaging *via* DNA-PAINT, giving <10-nm spatial resolutions. Using growth- and translation-optimized *E. coli* C321.ΔA-based strains developed in our lab that lack UAG codons and stop factors, we are developing the ability to encode FgAAs and NuAAs into proteins of interest. These technologies promise to improve our understanding of sub-cellular organization and, *via* NuAAs, to confer DNA-like properties on proteins that will facilitate genome engineering.

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