Cheap high-level production of synthetic proteins containing non-canonical amino acids: A feasibility study

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The residue-specific incorporation of non-canonical amino acids into target proteins using amino acid auxotrophic *E. coli* strains has become a routine method for academic applications. Its prowess for the engineering of proteins with special traits was demonstrated at many examples. However, the transfer of the technology to the industrial context raises major concerns about yield, product quality, scalability and the costs for the non-canonical amino acids.

To reassess these concerns, we have performed a comprehensive study on the residue-specific labeling of proteins with a selection of non-canonical amino acids at different scales. We devised an improved protocol for the production of synthetic proteins containing non-canonical amino acids in shake-flask cultures. A palette of BL21-Gold(DE3) descendant strains that are auxotrophic for Arg, Cys, His, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp or Tyr complements the method. We successfully upscaled this approach and transferred it to the bioreactor. We were able to produce up to 2 g/L of labeled target enzymes with excellent incorporation efficiencies. Moreover, we metabolically engineered a Met auxotrophic strain for the biosynthesis of the Met analog norleucine. Under optimized cultivation conditions, this strain biosynthesized 4 g/L of norleucine and facilitated its translational incorporation into the target enzymes. This indicates that the high-level production of residue-specifically labeled proteins is feasible in cheap medium.