Abstract

Residue-specific non-natural amino acid (NNAA) incorporation has become a widely used approach to introduce bioorthogonal groups and therefore functionalities into proteins[26]. These functionalities are harnessed through click chemistry, conjugating labelled proteins to affinity or fluorescent probes. A less utilized approach involves probing the effects of bioorthogonal groups on the structure-function relationship of labelled proteins[47]. In order to investigate these relationships, there is a need to express large amounts of proteins with maximal incorporation of NNAAs.

Here, we employed photo-methionine (pMet), azidohomoalanine (AHA) and homopropargylglycine (HPG) as methionine (Met) surrogates. We investigated the impact of these NNAAs on bacterial growth of prototrophic *E. coli* BL21 or Met--auxotrophic *E. coli* B834 in Met-free MM-M9 medium. We monitored the expression of cytochrome b_5 (cyt b_5) and MBP-GFP. Using MS and LC-MS based approaches, we determined the NNAA incorporation in these recombinant proteins.

Prototrophic *E. coli* BL21 expressed significantly higher amounts of cyt b_5 compared to B834 with pMet and AHA, but the incorporation rates fell sharply after 4 hours. In contrast, Met-auxotrophic B834 expressed smaller amounts of protein, but with incorporation of pMet in 50 – 70% range and AHA about 50%, after 26 hours. Intriguingly, both strains expressed comparable amounts of cyt b_5 with HPG, with incorporation in 50 – 80% range.

Our observations offer direction towards choosing an appropriate expression system to maximize incorporation of NNAAs and highlight a discrepancy in NNAA incorporation assessment by MS and LC-MS.

Unnatural amino acids, protein modification, mass spectrometry