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Heterologous expression of mAbs in Escherichia coli

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The widespread use of monoclonal antibodies (mAbs) as a platform for therapeutic drug development in the pharmaceutical industry has led to an increased interest in robust experimental approaches for assessment of mAb structure, stability and dynamics. The ability to enrich proteins with stable isotopes is a prerequisite for the in-depth application of many structural and biophysical methods, including nuclear magnetic resonance, small angle neutron scattering, neutron reflectometry, and quantitative mass spectrometry. While mAbs can typically be produced with very high yields using mammalian cell expression, stable isotope labeling using cell culture is expensive and often impractical. The most common and cost-efficient approach to label proteins is to express proteins in *Escherichia coli* grown in minimal media. Our approaches leading to the expression and purification of a stable isotope labeled mAb will be presented. Furthermore, when expressing humanize mAbs from codon-optimized constructs in E. coli, a truncated variant of its heavy chain may be observed. N-terminal protein sequencing and mutagenesis analyses indicated that the truncation resulted from an internal translation initiation codon. The requirements for the translation initiation will be presented and the implications of the findings for E. coli protein expression and codon optimization and outline possible strategies for reducing the likelihood of internal initiation will be discussed.

Keywords: isotope labeling, NMR, SANS, neutron reflectometry, quantitative mass spectrometry, mAbs.

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