A robust and reliable method for high yield deuterated recombinant protein production using *Escherichia coli* BL21.

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We have developed a method that has proven highly reliable for the deuteration of a broad range of proteins by recombinant expression in *Escherichia coli* BL21. Typical biomass yields are 40-80 g/L wet weight, yielding 50-400 mg/L purified protein. This method uses a simple, relatively inexpensive defined medium, and routinely results in a high yield expression without need for optimisation. The key elements are: very tight control of expression, careful starter culture adaption steps, and strict maintenance of aerobic conditions ensuring exponential growth. Temperature is reduced as required to prevent biological oxygen demand exceeding maximum aeration capacity. Glycerol is the sole carbon source. We have not encountered an upper limit for the size of proteins that can be expressed, achieving excellent expression for proteins from 7-112 kDa and the quantity produced at 1L scale ensures that no SANS, NMR or neutron crystallography experiment is limited by the amount of deuterated material. Where difficulties remain, these tend to be cases of protein solubility exacerbated by high protein concentration and slightly increased stickiness of proteins in D O. There are some very few cases in which we have been unable to express a protein by our method despite unlabelled expression being reliable in rich media using induction at low OD. Few proteins tested have not expressed in deuterated medium despite unlabelled expression being reliable.