DEVELOPING BACTERIAL MICROCOMPARTMENTS FOR THE RECOMBINANT PRODUCTION OF PROTEINS

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In prokaryotes, supramolecular self-assembling protein structures, known as bacterial microcompartments (BMCs), have evolved to encapsulate proteins associated with a number of different metabolic processes, providing a physical diffusion barrier whilst increasing local enzyme concentrations. The modular nature of these structures makes them promising biological platforms for the engineering of synthetic compartmentation within the bacterial cell that have potential to be used as novel nano-bioreactors [1-3]. We are interested in the development of robust synthetic BMC technologies that can be utilised for industrially relevant applications, specifically the spatial segregation of synthetic enzyme cascades for the enhanced production of valuable chemical compounds. BMCs may also be valuable in the production of recombinant proteins. Many therapeutic proteins and antibody fragments require disulphide-bonds for correct folding and activity. Here, we are exploring the potential of BMCs to serve as synthetic cellular organelles within the bacterial cytoplasm of *E. coli* that promote correct protein folding and disulphide bond formation of recombinant proteins, providing an alternative method to traditional approaches (folding in the eukaryotic endoplasmic reticulum and in the periplasm of prokaryotes). Previously, it has been shown that proteins of interest can be compartmentalised by fusing them to targeting peptides, which direct the proteins to the microcompartment shell [4, 5]. In this work, both, the shell (PduABJKNU) and the targeting peptides (P18/ D18) are derived from the 1,2- propanediol utilisation (Pdu) BMC from *Citrobacter freundii* [4]. We first determined the effect of fusing short targeting peptides onto the *E. coli* alkaline phosphatase PhoA, a protein widely used to examine disulfide bond formation *in vivo*, and the sulfhydryl oxidase Erv1p, a catalytic enzyme for the formation of disulphide bonds. The most active fusion proteins were selected for co-production with the BMC shell. For efficient recruitment of these proteins to the BMC, gene expression levels were controlled using tunable promoters and recombinantly produced BMC variants were analysed *in vivo* and *in vitro* using biochemical and biophysical methods. We demonstrated that both, PhoA and Erv1p, are targeted to recombinant BMCs and determined disulphide bond formation of PhoA in the presence and absence of Erv1p when targeted to the microcompartments. Using this approach, a range of other proteins of industrial interest will be tested and the potential for the production and purification of bio-therapeutic proteins and antibody fragments will be determined.

References:

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