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4-3-2022

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SELECTIVE *IN VITRO* LOADING OF PROTEINS INTO PROTEIN NANOCOMPARTMENTS FOR APPLICATIONS IN THE BIOINDUSTRY

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Key Words: Nanocompartments, Encapsulins, Enzymes, *in vitro* loading

Spatial segregation is the basis of biological complexity. Protein compartments offer definitive structures with a large potential design space. One family of protein compartments, encapsulins, are simple prokaryotic nanocompartments that self-assemble from a single protomer into selectively permeable cages of between 24 and 50 nm (1). Over the last 14 years encapsulins have been developed for a diverse application portfolio utilising their defined cargo loading mechanisms and repetitive surface display (2). Control over the self-assembly of the quaternary capsids and conformational size of these capsids is of vital importance for commercial quality assurance, effective cargo loading and delivery and possible generation of various types of proteinaceous biomaterials. Here we will describe the efficiency and control of reversible disassembly of 2 size classes of encapsulins under a range of denaturants, and the development of a selective *in vitro* cargo loading strategy using native encapsulin targeting peptides over passive loading. This work was carried out first with the model protein sfGFP, followed by an industrially relevant enzymatic cargo, transketolase. Following on from this, we aim to address the key question of how loading of proteins into a robust shell affects enzyme stability and activity. This work supports the move towards fully harnessing structural, spatial, and functional control in synthetic biological systems with applications in enzyme stabilisation and/or multistage biocatalysis.

References

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