Pore structure controls stability and molecular flux of engineered protein cages

Protein cages are a common architectural motif used by living organisms to compartmentalise and control biochemical reactions.[1] While engineered protein cages have recently been featured in the construction of nanoreactors and synthetic organelles,[2, 3] relatively little is known about the underlying molecular parameters that govern cage stability and molecular flux through their pores.

In this talk, I will describe the design of 24 variants of the 'encapsulin' protein cage from *Thermotoga maritima*, each featuring pores of different size and charge. Twelve variants were successfully assembled, purified, and analysed for thermal stability. In collaboration with the ACMM and the University of Michigan, the cryo-EM structures of seven variants were determined with resolutions between 2.5 and 3.6 Å. The diffusion kinetics of cations into seven variants were then investigated using a lanthanide-based luminescence assay. Through these experiments, we examine the factors that contribute to protein cage assembly and stability, the challenges associated with examining and quantifying small molecule diffusion in protein cages, and provide guidance for the design and characterisation of future nanoreactors.

References

1. Diekmann, Y.; Pereira-Leal, J. B., Evolution of intracellular compartmentalization. *Biochemistry Journal* **2013,** *449*, 319-331.
2. Giessen, T. W.; Silver, P. A., Encapsulation as a Strategy for the Design of Biological Compartmentalization. *J Mol Biol* **2016,** *428* (5), 916-927.
3. Lau, Y. H.; Giessen, T. W.; Altenburg, W. J.; Silver, P. A., Prokaryotic nanocompartments form synthetic organelles in a eukaryote. *Nature Communications* **2018,** *9* (1), 1311.