Applications of Microscopy to Bio-synthetic systems

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The synergies between Bacterial Microcompartments (BMCs) and synthetically engineered self-assembling peptide systems are being explored. The protein shell of a BMC is like a scaffold that supports encapsulation of peptides which target and enfold enzymes involved in a metabolic reaction. We have applied EM and CLEM (Correlative Light and Electron Microscopy) techniques to study and visualise our manipulation of the Pdu (Propanediol utilization) metabalosome BMC. CryoEM is proving a useful tool to gain structural folding information on the PduA shell proteins (Fig1), which when over expressed in a slightly more stable modified form, form filaments of 20nm diameter. When expressed in E-coli these filaments form a cyto scaffold (Ref 1) , which we have visualised in 3d by Electron tomography (Fig 2) . CLEM is used to show that the scaffold can be targeted with our de novo coiled-coiled peptide linker, which can be made fluorescent, bound to an enzyme and directed to a specific cell location to increase chemical production (Fig3).

Furthermore, synthetic self assembling peptide cage structures and sequences have been designed to mimic the function of BMCs. These designs are checked with simple negative stain TEM and SEM. Further application of CryoTEM and High Angle Annular Dark Field STEM imaging will further underpin our understanding of these bio-engineered designs, providing accurate data for the mathematical modelling and further improved bio- synthetic systems which can be harnessed as cell factories, for vaccines or drug delivery applications.

1. Lee, M.J, Mantell, J, Hodgson, L , Alibhai, D, Fletcher, J.M, Brown I.R , Frank, S, Xue W-F", Verkade, P, Woolfson D.N, & Warren, M.J, Engineered synthetic scaffolds for organizing proteins within the bacterial cytoplasm *Chemical Biology* doi: 10.1038/nchembio.2535 (2017)

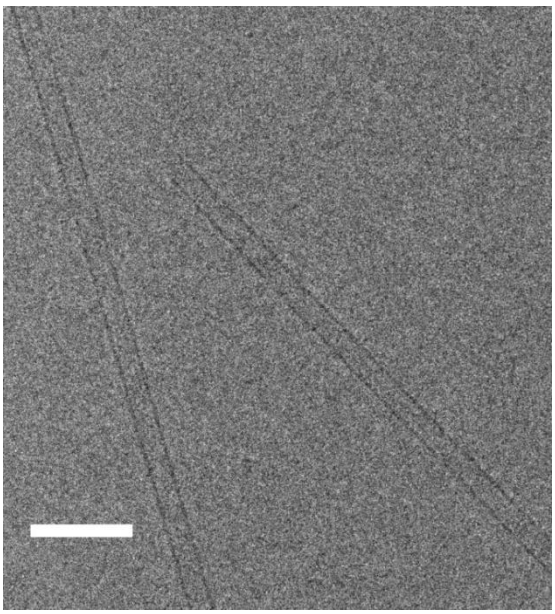

Figure 1: CryoTEM image of PduA filaments. Scale Bar = 100nm

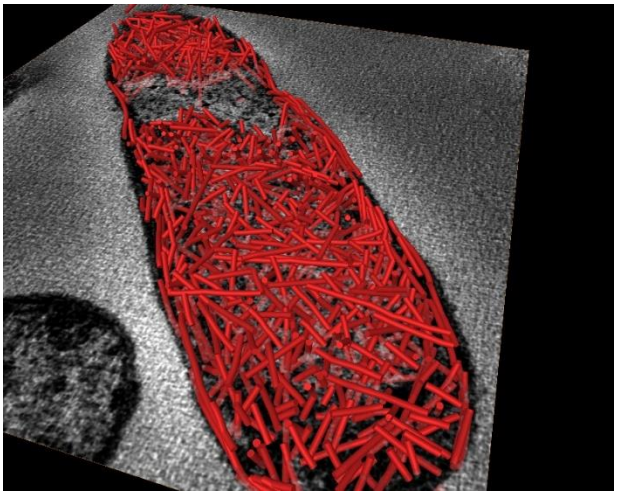

Figure 2: Segmented tomography reconstruction to show the cyto scaffold formed by the filaments within E-coli

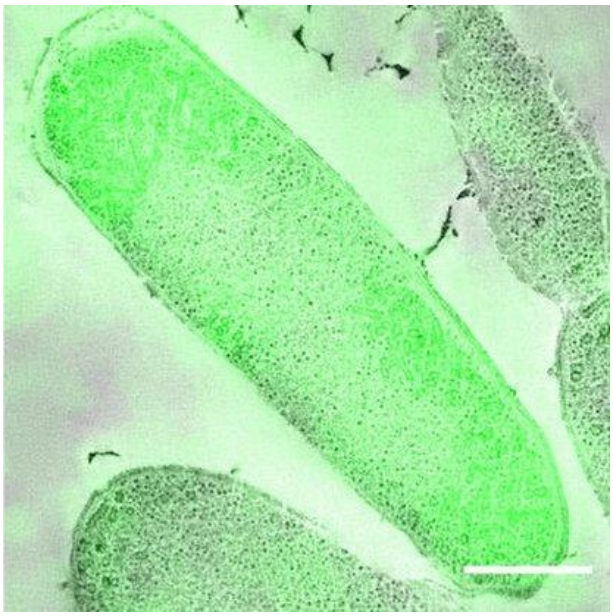

Figure 3: CLEM experiment shows the PduA tubes can be targeted with a synthetic dimer expressing a fluorescent GFP tag. Scale bar is 500 nm

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