# Development of a Bioassay to Detect Inhibition in Bacterial Microcompartment Shell Protein Crosslinking

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## Background

Bacterial microcompartments (BMCs) are intracellular protein-based organelles, which consist of selectively permeable protein shells and encapsulated enzymes. BMCs are typically 100-200nm in diameter, and can be classified into metabolosomes and carboxysomes according to their functions.

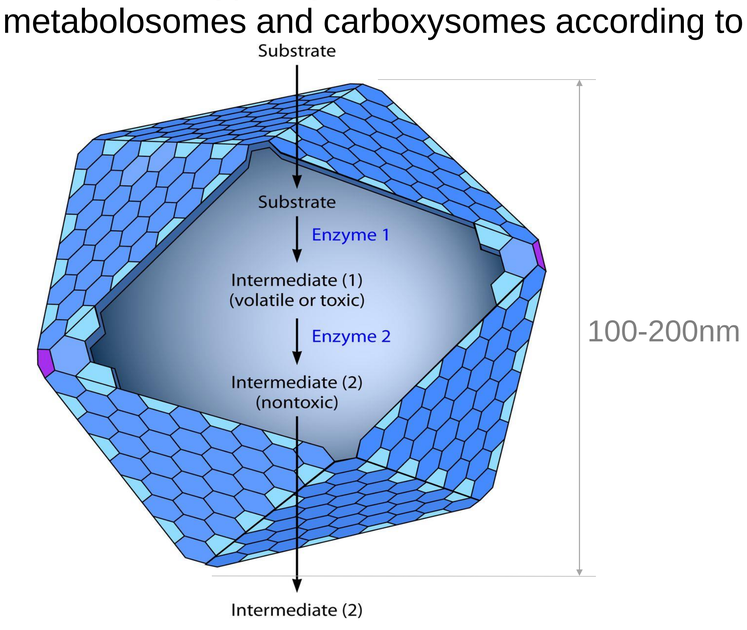


**Figure 1. Bacterial microcompartments are protein shells encapsulating short metabolic pathways**
(Kerfeld.,2018)

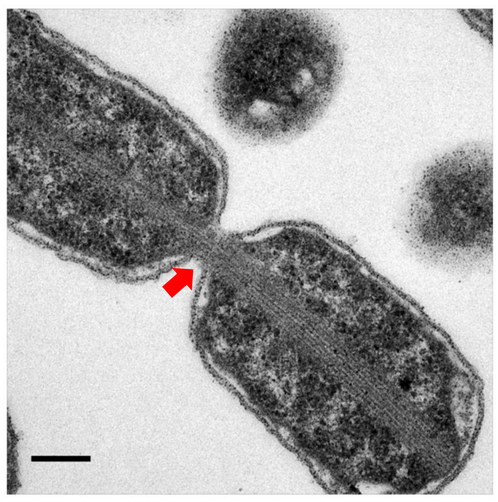

**Figure 2. TEM analysis of thin-sectioned E.coli cells overexpressing BMC-H protein PduA**
scale bar shows 200nm. Arrow indicates nanotubes
(Uddin *et al*., 2018)

There are three classes of building block proteins which self-assemble to form the shell: BMC-H, BMC-T, and BMC-P. The BMC-H and BMC-T form hexamers or pseudohexamers, while BMC-P forms pentamers. Hydrogen bonds between lysine residues on the edges of the hexamers or pseudohexamers are key factors linking hexamers together to make protein sheets. A well-studied type of BMC is the 1,2-propanediol utilization bacterial microcompartment (Pdu) composed of a mixture of BMC-H and one type BMC-P. Overexpression of single Pdu BMC-H shell proteins produces intracellular protein nanotubes (also requiring hexamer crosslinking to make curved sheets), which prevent cells from dividing, and causes the formation of elongated cells (Parsons et al., 2008, Kennedy *et al*., 2021) .

Small molecules predicted to bind cross-linking residues of BMC-H in sheet form were sought . We hypothesized that these small molecules could hinder the formation of nanotubes, and that we can detect this effect by light microscopy of *E. coli* over-expressing a BMC-H protein. These molecules may be useful for manipulating microcompartment function by affecting the formation of the protein shell.

## Aim

Using non-pathogenic *E. coli* cells to over-express BMC-H proteins, establish metrics of a cross-linking proxy phenotype by measuring cell length and morphology (percentage of cells with kebab-like morphology as in Fig.2) using light microscopy. Test positive controls using antibiotics known to affect cell length. Test the effect of addition of small molecules with predicted high-affinity to Lysine cross-linking sites on the cross-linking metrics.

## Methods

The ISAMBARD modelling program was used to generate a list of small molecules with high ligand efficiency scores (predicted binding energy (kJ/mol)/number of heavy atoms) to bind the key residues required for cross linking of Pdu BMC-H proteins. Plasmid pET3a-PduA encoding Citrobacter PduA (BMC-H) was transformed into *E. coli* BL21 DE3. The bioassay comprises incubation of transformed cells at 37°C overnight for 16-18h, then inoculate in LB medium 1:100. Meanwhile, small molecules at different concentrations (MolPort, Latvia) are added to see the inhibition effect on elongation. When the OD reaches 0.4, add IPTG and small molecule cross linking inhibitors, then incubate for 8-10 hours at 19°C. Then use crystal violet to stain a wet preparation under a cover slip, and 100X light microscope to visualize the elongated cells. Capture jpg photos and use ImageJ to analyze the pictures. For each tube, the mean single cell (non cross-linking cell) length (L) is obtained by averaging the length of 120 single cells. Count and measure the length of elongated cells showing indentations indicating failed division (kebab cells) and single *E. coli* cells with no indentations, across three high power fields, using three biological replicates and cross-linking inhibitor-free controls. Cross linking proxy metrics are mean cell length of single cells (L), and the percentage of cellular units counted incorporated in kebab cells (C).

C= $(\frac{K}{L})/(\frac{K}{L}+s)*100$ . Where K is the total length of kebab cells counted and S is the number of single cells counted. Percentage of visible cells incorporated in elongated cells compared by one-way ANOVA , Bonferroni correction for multiple groups, Using RStudio 2022.07.0, R 4.2.1

## Results

*E. coli* cells with induced PduA have higher percentages of crosslinking (metric C), an average at about 95%, while the crosslinking rate of PduA non-induced cells is around 73%. 2 chemicals (C1, C2) shown significant effect on crosslinking inhibition, and they do not interfere with the cell growth according to a growth test. While the other 2 chemicals (C3, C4 ) did not show a significant effect on cell crosslinking. Although there is no dose-related response detected in these experiments, when the concentration is reduced to 12.5μM, there is no significant difference in crosslinking detected among all molecules.

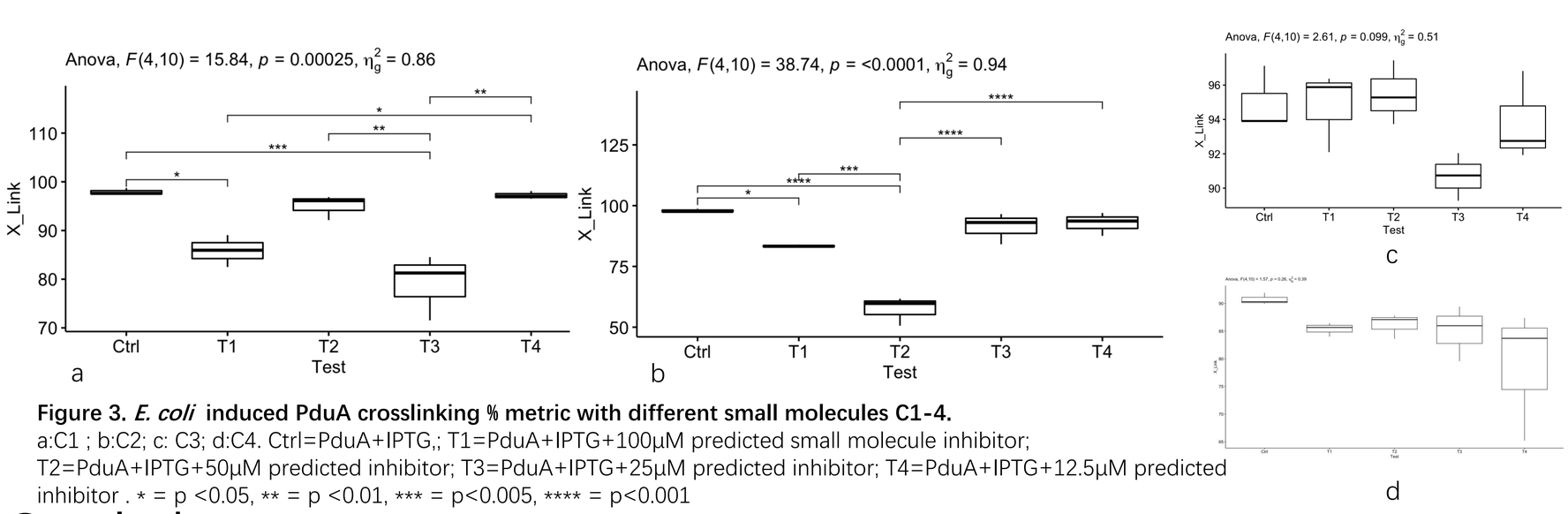


**Figure 3. *E. coli* induced PduA crosslinking % metric with different small molecules C1-4.**
a:C1 ; b:C2; c: C3; d:C4. Ctrl=PduA+IPTG,; T1=PduA+IPTG+100μM predicted small molecule inhibitor; T2=PduA+IPTG+50μM predicted inhibitor; T3=PduA+IPTG+25μM predicted inhibitor; T4=PduA+IPTG+12.5μM predicted inhibitor . * = p <0.05, ** = p <0.01, *** = p<0.005, **** = p<0.001

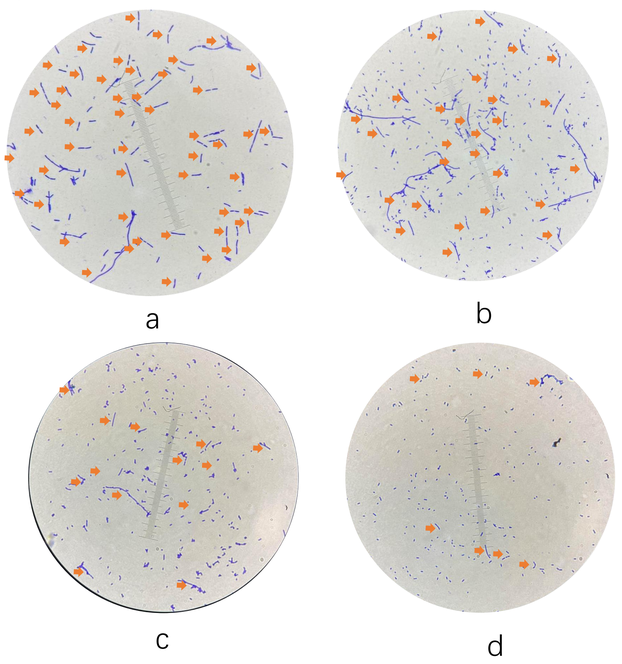


**Figure 4. *E. coli* light microscopy (100X objective)**
a: PduA+IPTG; b:PduA; c: PduA+IPTG+C2;
d:PduA+IPTG+C1. Crystal violet stain.
*All crosslinked cells highlighted by ➡

## Conclusion

Induction of PduA is effective for generating the crosslinking proxy assessable by light Microscopy.
The addition of small molecules has a significant effect on the inhibition of cell crosslinking.

## Subsequent plan

Electron microscopy will be applied to confirm intracellular microtubules as a cause for the elongated cells.
The optimal type and concentration of small molecules will be determined by further experiments. Tests will be applied to confirm that small molecules do not interfere with the inductor IPTG-induced PduA expression. Effects on microtubule formation in a cell-free system will be determined by electron microscopy with collaborators at Queen Mary, University of London, UK

## Acknowledgement

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References: Parsons *et al*., JBiolChem.2008, 283(21)14366-14375; Kennedy *et al*., J Mol Biol. 2021 Jan 22;433(2):166721; Uddin *et al*., Small 2018, 14, 1704020; Kerfeld *et al.,* Nat Rev Microbiol. 2018,16, 277–290