ENZYMATIC INITIATION AND POLYMERIZATION OF GROUP 2 BACTERIAL CAPSULES

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Capsule polysaccharides (CPS) and wall teichoic acid (WTA) are the most important surface glycans in Gram-negative and Gram-positive bacteria, respectively, and are essential for survival during host colonization [1,2]. CPS present structurally diverse antigens key for the development of glycoconjugate vaccines against pathogenic bacteria such as *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae* [3]. The enzymes catalyzing capsule assembly are potential drug targets and valuable biotechnological tools to synthesize vaccine antigens. It remains unknown how structurally variable capsule polymers of Gram-negative pathogens are linked to the conserved glycolipid that anchors these virulence factors to the bacterial membrane. Here, we identify two enzymes that synthesize this linker in a large group of Gram-negative pathogens. Using chemically synthesized analogs of the glycolipid, we reconstructed the entire biosynthesis of the capsule polymer, demonstrating that the two enzymes not only produce the linker between the glycolipid and capsule polymer, but also stimulate the capsule polymerase to produce more and longer polymers. We identify the linker as a wall teichoic acid (WTA) type I homolog, demonstrating similarity between the biosynthesis of Gram-positive WTA and Gram-negative capsules. Moreover, our X-ray crystallographic structure of the capsule polymerase highlights two catalytic sites, CgaT and CgoT, for the processive galactose and glycerol-3-P addition of the repeating unit respectively, and a tetratricopeptide repeat (TPR) domain as central element (**Figure 1**), crucial for recognizing the linker and, in turn, mediating processive elongation and modulating the activity of the three enzymes.

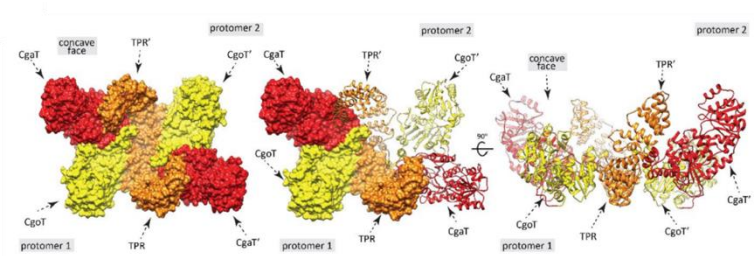

Figure 1. Overall structure of Cps3D surface and ribbon representation of the multimodular architecture of Cps3D as observed in the crystal structure. Each protomer of the homodimer es colored as follos: CgaT red, CgoT yellow, TPR orange.

References:
1. Whitfield, C., *et al.*, *Annu. Rev. Microbiol.,* **2020**, *74*, 521–543.
2. Sande, C. & Whitfield, C., *EcoSal. Plus,* **2021**, *9*, eESP00332020.
3. Willis, L. M. & Whitfield, C., *Carbohydr. Res.*, **2013**, *378,* 35–44.