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Electron Crystallography of the *E. coli* Outer Membrane Protein Wza_{K30}

C.M. Hill[*], J. Nesper[**], C. Whitfield[**] and G. Harauz[*]

[*]Department of Molecular Biology and Genetics and [**]Department of Microbiology, University of Guelph, Ontario, Canada, N1G 2W1

The surface-exposed outer membrane lipoprotein Wza_{K30} of *E. coli* translocates the K30 capsular polysaccharide to the exterior face of the cell. Transmission electron microscopy of negatively stained Wza_{K30} preparations has shown that Wza_{K30} peptides assemble into a multimeric ring structure with an apparent central pore large enough to permit the passage of a filamentous polysaccharide [1]. Here we present structural data obtained by using a two-dimensional crystallization approach for integral membrane proteins developed by Lévy et al. [2].

A recombinant form of Wza_{K30} incorporating a C-terminal hexahistidine tag was purified from sodium dodecyl sulphate-solubilised outer membranes via Ni^{+2} affinity chromatography and solubilised in the detergent SB3-14. Planar crystals of Wza_{K30} multimers were then obtained by incubating the preparation under a monolayer incorporating an equal proportion of *E. coli* lipids and a lipid containing a nickel-chelating headgroup. Binding of the hexahistidine tags to the nickel ions presented by these latter lipids permitted concentration of Wza_{K30} at the monolayer surface. Detergent was then removed in the presence of free *E. coli* lipid by the incorporation of polystyrene BioBeads®. It is proposed that this method results in the replacement of detergent with lipids from the aqueous phase, reconstituting the protein within a bilayer matrix [2].

Upon mounting, dehydration and staining with 2% uranyl acetate, planar arrays of Wza_{K30} exhibiting varying degrees of regularity were observed. The individual ring multimers were 7.6 nm in diameter (Figure 1) and were arranged in a square lattice with spacings of 1 nm (Figure 2). These crystals were readily reproducible and work is ongoing to collect a large number of micrographs for averaging and extraction of structural detail by the IMAGIC-5 image processing software package [3].

References and Acknowledgements

[1] J. Drummelsmith and C. Whitfield, *EMBO J.* 19 (2000) 57.
[2] D. Lévy *et al*., *J. Struct. Biol.* 127 (1999) 44
[3] M. van Heel *et al*., *J. Struct. Biol*. 116 (1996) 17.
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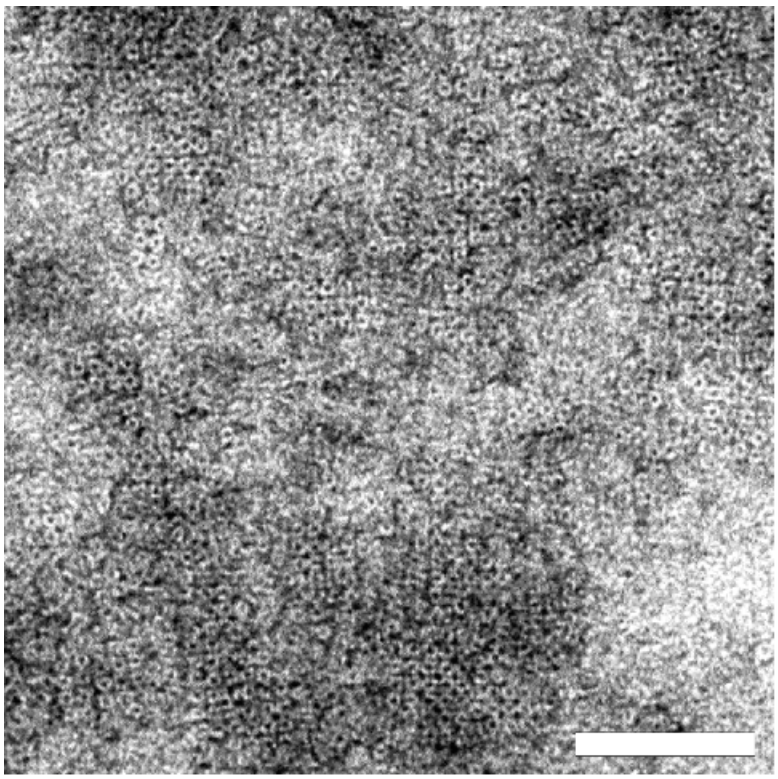

Figure 1
Planar arrays of anchored wza_{K30}. The protein appears as a ring with a stain filled central core.
Nominal magnification 20,000x. Scale bar represents 100 nm.

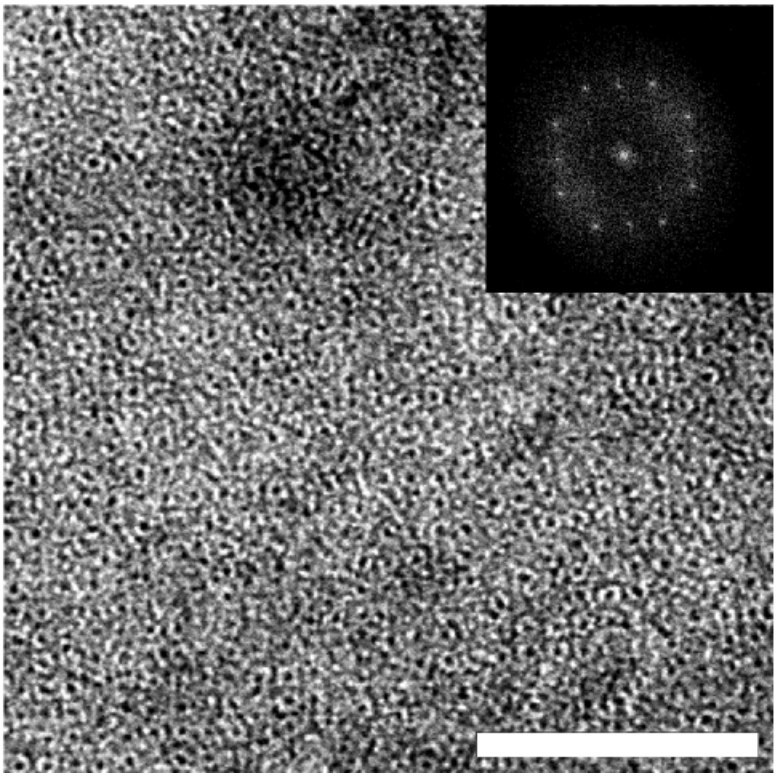

Figure 2
Ordered planar arrays of anchored wza_{K30}. *Inset:* Fourier transform analysis reveals two orders of regularity.
Nominal magnification 31,500x. Scale bar represents 100 nm.

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