Growth-dependent molecular order in the *E. coli* outer membrane

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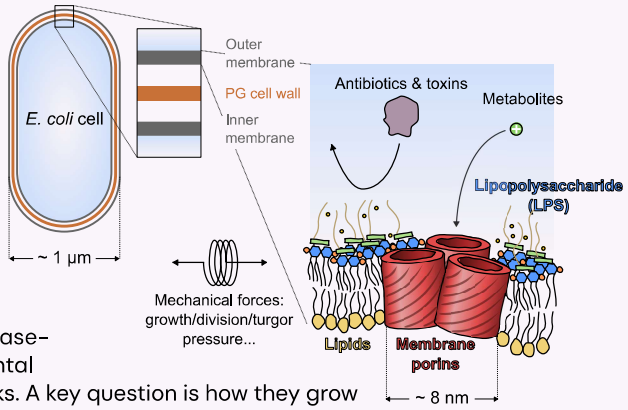
Introduction

Gram-negative bacteria are a key risk to public health and dominate the World Health Organisation's list of critical and high-priority strains to monitor. This is due, in part, to their outer membrane (OM), an additional barrier containing β-barrel OM proteins (OMPs), phospholipids and lipopolysaccharides (LPS). Collectively, these provide a robust molecular sieve that protects against antibiotics while permitting nutrient ingress [1], bears mechanical loads [2] and also allows for rapid growth and division [3].



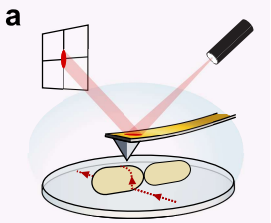
In *E. coli*, trimeric OMPs organise into networks that exhibit phase-separated domains rich in LPS [4]. As yet, we lack a fundamental understanding of the origin and implications of these networks. A key question is how they grow and evolve over the cell cycle and in different conditions and how such dynamic heterogeneity is linked to cell viability or membrane integrity [3].

The outer membrane is a heterogeneous network of OMP trimers and LPS

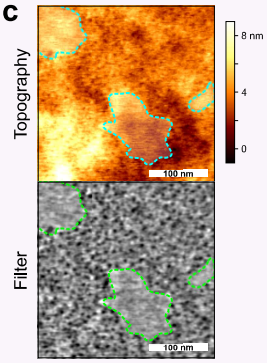
We use atomic force microscopy (AFM, **a**) to image the outer membrane of living, growing *E. coli* cells (**b**) in biological conditions. We map the organisation of the outer membrane at the scale of single OMP trimers across the entire accessible surface (**c**).

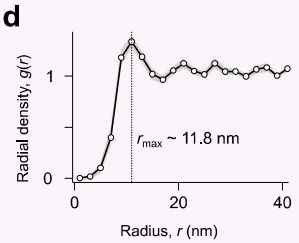
The trimers appear as densely-packed depressions in the outer membrane and form a glassy network defined by short-range order on the scale of ~12 nm (**d**), with interactions mediated by LPS.

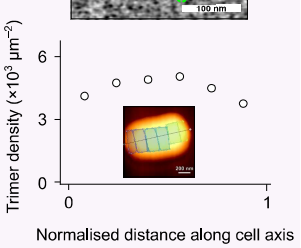
These porin networks are pseudo-static, with diffusion observed only on the timescale of cell growth. Molecular motion is therefore almost entirely determined by biogenesis. Indeed, analysis across the cell backbone (**f**) shows a smooth reduction in porin density away from the midpoint, which points to an intriguing spatial variation of the OM insertion machinery.



In-liquid AFM of living *E. coli* cells

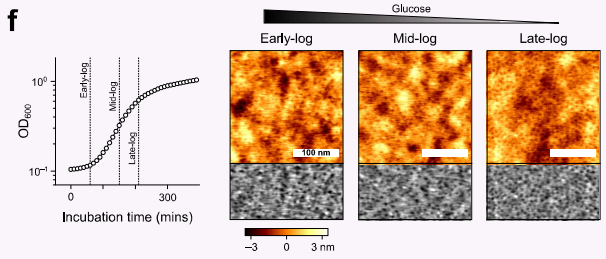




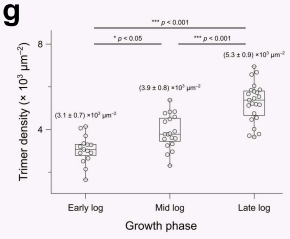


Porin organisation depends sensitively on growth phase and environment

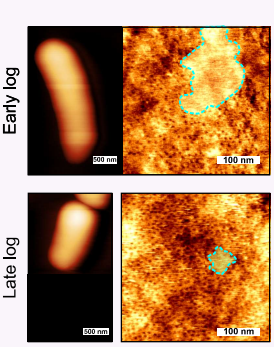
The global porin content is sensitively linked to the cells' growth phase (**f**), with density increasing by > 170% as the cells move from early-log growth (rapid growth rate, high glucose levels) to late-log/stationary phase (arrested division, low glucose) (**g**).

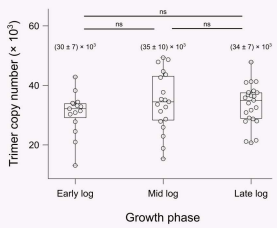


This density change is precisely compensated by a change in cell surface area (**h**), resulting in conserved OMP copy numbers (**i**) that reflect the tightly-regulated porin insertion and biogenesis machinery.



These results illustrate how finely-tuned the outer membrane order and composition must be in order to balance the competing needs of cell growth, nutrient intake and protection from environmental insults.





References

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