PERSPECTIVE
https://doi.org/10.1071/MA25064

The bacterial cell envelope as a morphogenetic code for biofilm development

Jilong Qin[A], Robert Kinobe[B], Patrick M. Schaeffer[C] and Yaoqin Hong[C,D,*]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Yaoqin Hong
Biomedical Sciences and Molecular Biology, College of Medicine and Dentistry, James Cook University, Townsville, Qld, Australia
Email: yaoqin.hong@jcu.edu.au

Published: 13 November 2025

Cite this: Qin J *et al.* (2025) The bacterial cell envelope as a morphogenetic code for biofilm development. *Microbiology Australia* **46**(4), 227–230. doi:10.1071/MA25064

Introduction

Most microbes in nature, such as bacteria, rarely exist as solitary, free-floating cells. Instead, they organise into structured and densely populated communities known as biofilms. A biofilm can be thought of as a microbial city, in which the inhabitants are the bacteria that are within a protective scaffold called the extracellular matrix (ECM) produced by themselves. The ECM is composed of polysaccharides, proteins, lipids, and extracellular DNA.[1]

The ECM provides far more than structural support. It creates a formidable barrier against both physical and chemical disturbances, limiting the penetration of antimicrobial agents.[1] This often establishes antibiotic concentration gradients, exposing subsets of cells to sublethal or subinhibitory levels that promote adaptation and the evolution of resistance.[2] In addition, dormant subpopulations within the biofilm are intrinsically tolerant to chemical inhibition, further contributing to persistence.[3] In clinical contexts, this recalcitrance presents a major challenge, allowing pathogens such as *Pseudomonas aeruginosa*, a leading cause of chronic lung infections in cystic fibrosis patients, and *Escherichia coli*, the most common agent of urinary tract infections, to establish difficult-to-treat infections.

Biofilms are not inherently detrimental. Their mechanochemical stability and cooperative behaviour between bacterial members can be harnessed for beneficial applications. Examples of these include the production of polymeric substances that trap contaminants and aid microbial degradation during wastewater treatment, biomineralisation for metal recovery, and the industrial production of valuable biopolymers.[4,5] This duality, as a threat to human or animal health and a useful biotechnological tool, calls for a better understanding and control of biofilm development in microbiology.

The bacterial cell envelope

One promising strategy to influence biofilm behaviour is to target the bacterial cell envelope. This complex, multilayered structure not only defines the cell but also mediates its interactions with the environment. In Gram-negative bacteria such as the Enterobacterales, the envelope comprises three principal components. The outer membrane serves as the anchoring surface for a plethora of cell surface polysaccharides, as well as acting as a robust permeability barrier, regulating solute transport while excluding toxic compounds, including antibiotics.[6,7] The innermost cytoplasmic membrane is a dynamic lipid bilayer that governs a series of fundamental processes including energy generation, nutrient transport, and macromolecular biosynthesis.[8] Between these lies the periplasm, a gel-like compartment enriched with proteins that drive key biochemical reactions, such as oxidative protein folding.[9]

The cell envelope is a responsive and adaptive interface that senses environmental cues, transduces signals, and coordinates metabolic responses to cope with stress.[10] This dynamism opens the door to engineering biofilm architecture, mechanochemical resilience, and the functionality of biofilms by tuning cell envelope composition and the sensory-adaptive pathway that regulate it.

How does the cell envelope govern biofilm formation?

The envelope controls biofilm construction at multiple levels.[11] Briefly, specific examples of this interaction are:

(a) Cell–cell adhesion: the envelope is decorated with adhesive factors such as curli fibres (extracellular, amyloid-like protein filaments) and surface polysaccharides. These act as

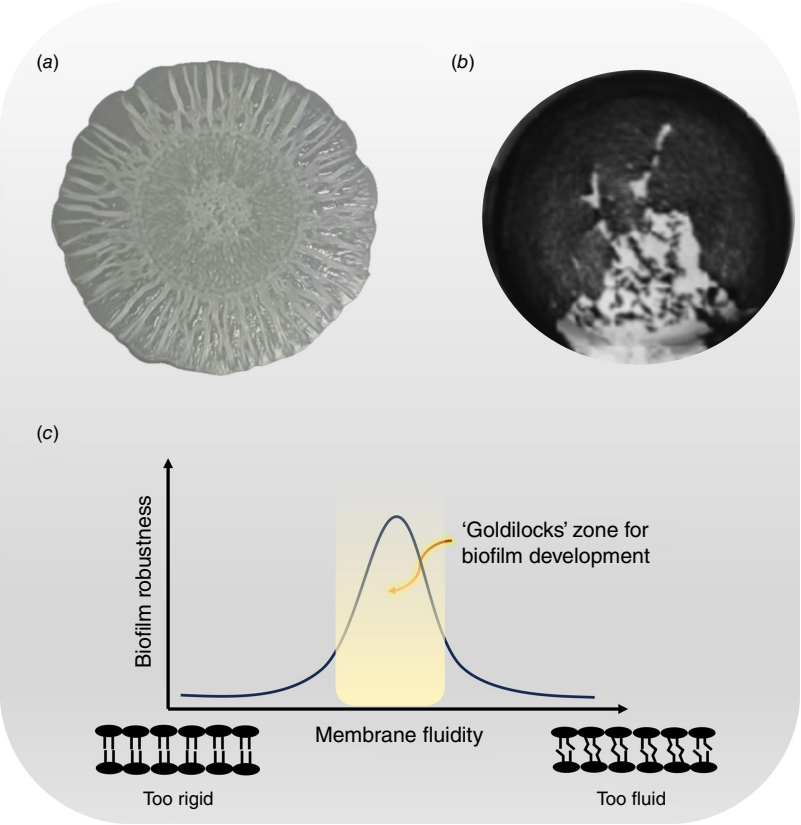

Fig. 1. The 'Goldilocks' zone for membrane fluidity required for biofilm development. (*a*) A rugose macrocolony biofilm produced by a strain of *E. coli* K-12 AR3110. (*b*) *E. coli* K-12 AR3110 pellicle grown at the liquid-air interface (stained with 0.1% crystal violet). (*c*) Conceptual model of the 'Goldilocks' zone for membrane fluidity in biofilm development. When the membrane is too rigid (left) or too fluid (right), bacterial communities collapse into fragile and poorly structured assemblies. Only within an optimal 'Goldilocks' zone of fluidity (highlighted peak) does bacteria produce resilient, multicellular biofilms with characteristic architecture. This proposal provides a generalisable model for how physical state variables at the molecular scale govern emergent community-level organisation.

biological glue, enabling cells to attach to one another and initiate the formation of multicellular communities.

(b) Cell–ECM interactions: envelope-derived components determine how tightly cells integrate into the ECM scaffold. This influences the mechanical properties of the biofilm, producing surface textures that range from rough to smooth, and from brittle to flexible.

(c) Environmental sensing: the envelope harbours signal transduction systems that detect chemical and physical stress. For instance, the Rcs phosphorelay functions as a stress-response circuit that senses envelope damage or antibiotic exposure and, in turn, activates protective ECM production.

A well-studied biofilm example is the *E. coli* macrocolony (Fig. 1*a*). When grown on solid media, certain strains (e.g. AR3110) produce large, wrinkled, rugose colonies with skin-like elasticity.[11] This complex architecture arises from envelope-mediated coordination of cellulose, curli fibres and other exopolysaccharides.[12] Thus, the cell envelope functions as a morphogenetic hub, converting molecular-scale signals into emergent, community-level architecture. Among these envelope-mediated processes, membrane fluidity has recently emerged as a unifying physical parameter with profound consequences for biofilm organisation.[13] Bacteria may utilise this relationship to adapt to hostile environment, resist antibiotics and persist on surfaces.

Membrane fluidity: the 'Goldilocks' principle

Membrane fluidity is largely governed by the relative abundance of saturated fatty acids, which pack tightly to rigidify the membrane and unsaturated fatty acids, which contain cis-double bonds that interfere with packing and increase fluidity (Fig. 1*c*).[13]

We recently showed that *E. coli* requires a 'Goldilocks' zone of membrane fluidity, not too rigid and not too fluid, but just the right amount, for robust biofilm formation.[13] Within this optimal zone, the bacterium produces highly structured communities, including wrinkled, rugose macrocolonies on agar surfaces and elastic pellicles at the liquid-air interface. When fluidity strays outside this range, however, macrocolonies collapse into smooth, fragile colonies lacking their characteristic architecture.[13] Similarly, in pellicles (biofilms at the liquid-air interface; Fig. 1*b*), we observed the same behaviour, indicating broader applicability of the 'Goldilocks' fluidity model (Fig. 1*c*).

By modulating fluidity, bacteria achieve the balance of elasticity and mechanical strength required for stable community life. The adjustment to reach optimal fluidity range desirable for biofilm formation appeared to be species-specific. In *Klebsiella pneumoniae*, increasing fluidity enhances rather than destabilises biofilm robustness,[14] while in *Salmonella enterica*, biofilm-associated cells exhibit a higher proportion of saturated fatty acids compared with their planktonic counterparts, i.e. increased rigidity appeared beneficial.[15] Such apparently paradoxical observations may reflect the distinct fatty acid profiles each species favours in the planktonic state, which necessitate different adjustments when transitioning to the biofilm lifestyle. Additional works will be required to refine the molecular fluidity handles used in biofilm development.

Programmable biofilm morphology and its applications

Viewing the envelope as a morphogenetic interface suggests that small, targeted changes in envelope chemistry can trigger large-scale architectural shifts in entire communities. Indeed, unpublished work within our group indicates that a single gene manipulation altering the cell envelope can create biofilms with strikingly different structural patterns, with distinctive architectures and mechanical properties. In this view, biofilm morphology becomes programmable, steerable by rational re-engineering of the envelope.

In conclusion, the bacterial cell envelope is not merely a defensive shell but a morphogenetic switch that translates molecular-scale changes into community-scale architecture. By tuning many parameters such as membrane fluidity, a single genetic manipulation can switch between fragile smooth films and resilient multicellular assemblies with remarkable functionalised properties. This perspective carries two practical implications. Clinically, destabilising pathogenic biofilms by pushing their envelopes outside the biofilm zone may offer new therapeutic avenues. For industrial applications, cell envelope engineering could enable the design of ultra-stable biofilms for high-value chemical productions, biomineralisation, or bioremediation.

More broadly, biofilms offer a tractable model for a fundamental question: how do individual cells coordinate in space and time to build organised multicellular societies? We will integrate genetic approaches and biochemical interventions to decode this language. Overall, this line of attack will uncover strategies that to police biofilm formation, while illuminating how living systems construct complexity.

References

1. Sauer K, Stoodley P, Goeres DM, Hall-Stoodley L, Burmølle M, Stewart PS, Bjarnsholt T. The biofilm life cycle: expanding the conceptual model of biofilm formation. *Nat Rev Microbiol* 2022; 20: 608–620. doi:10.1038/s41579-022-00767-0
2. Dayton H, Kiss J, Wei M, Chauhan S, LaMarre E, Cornell WC, Morgan CJ, Janakiraman A, Min W, Tomer R, Price-Whelan A, Nirody JA, Dietrich LEP. Cellular arrangement impacts metabolic activity and antibiotic tolerance in *Pseudomonas aeruginosa* biofilms. *PLoS Biol* 2024; 22: e3002205. doi:10.1371/journal.pbio.3002205
3. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999; 284: 1318–1322. doi:10.1126/science.284.5418.1318
4. Vishwakarma V. Impact of environmental biofilms: industrial components and its remediation. *J Basic Microbiol* 2020; 60: 198–206. doi:10.1002/jobm.201900569
5. Li X, Chopp DL, Russin WA, Brannon PT, Parsek MR, Packman AI. Spatial Patterns of Carbonate Biomineralization in Biofilms. *Appl Environ Microbiol* 2015; 81: 7403–7410. doi:10.1128/AEM.01585-15
6. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol Mol Biol Rev* 2003; 67: 593–656. doi:10.1128/MMBR.67.4.593-656.2003
7. Hong Y, Qin J, Verderosa AD, Hawas S, Zhang B, Blaskovich MAT, Cronan JE, Totsika M. Loss of β-ketoacyl acyl carrier protein synthase III activity restores multidrug-resistant *Escherichia coli* sensitivity to previously ineffective antibiotics. *mSphere* 2022; 7: e00117-22. doi:10.1128/msphere.00117-22
8. Strahl H, Errington J. Bacterial Membranes: Structure, Domains, and Function. *Annu Rev Microbiol* 2017; 71: 519–538. doi:10.1146/annurev-micro-102215-095630
9. Heras B, Shouldice SR, Totsika M, Scanlon MJ, Schembri MA, Martin JL. DSB proteins and bacterial pathogenicity. *Nat Rev Microbiol* 2009; 7: 215–225. doi:10.1038/nrmicro2087
10. Mitchell AM, Silhavy TJ. Envelope stress responses: balancing damage repair and toxicity. *Nat Rev Microbiol* 2019; 17: 417–428. doi:10.1038/s41579-019-0199-0
11. Serra DO, Hengge R. Bacterial Multicellularity: The Biology of *Escherichia coli* Building Large-Scale Biofilm Communities. *Annu Rev Microbiol* 2021; 75: 269–290. doi:10.1146/annurev-micro-031921-055801
12. Serra DO, Richter AM, Klauck G, Mika F, Hengge R. Microanatomy at cellular resolution and spatial order of physiological differentiation in a bacterial biofilm. *mBio* 2013; 4: e00103-13. doi:10.1128/mBio.00103-13
13. Hong Y, Qin J, Totsika M. Not too rigid nor too wobbly: defining an optimal membrane fluidity range essential for biofilm formation in *Escherichia coli*. *mLife* 2025; 4: 461–464. doi:10.1002/mlf2.70024
14. Dramé I, Rossez Y, Krzewinski F, Charbonnel N, Ollivier-Nakusi L, Briandet R, Dague E, Forestier C, Balestrino D. FabR, a regulator of membrane lipid homeostasis, is involved in *Klebsiella pneumoniae* biofilm robustness. *mBio* 2024; 15: e01317-24. doi:10.1128/mbio.01317-24
15. Dubois-Brissonnet F, Trotier E, Briandet R. The Biofilm Lifestyle Involves an Increase in Bacterial Membrane Saturated Fatty Acids. *Front Microbiol* 2016; 7: 1673. doi:10.3389/fmicb.2016.01673

Data availability. Data sharing is not applicable as no new data were generated or analysed during this study.

Conflicts of interest. The authors declare that they have no conflicts of interest.

Declaration of funding. YH is supported by Grant Submission Schemes from James Cook University.

Author affiliations

[A]Institute for Molecular Bioscience, University of Queensland, St Lucia, Qld, Australia.

[B]Veterinary Sciences, College of Science and Engineering, James Cook University, Townsville, Qld, Australia.

[C]Biomedical Sciences and Molecular Biology, College of Medicine and Dentistry, James Cook University, Townsville, Qld, Australia.

[D]Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, Qld, Australia.

Biographies

Jilong Qin received his PhD from the University of Adelaide in 2020 and is currently a Research Fellow at the Institute for Molecular Bioscience, The University of Queensland. His research focuses on the biogenesis of bacterial cell envelope, specifically peptidoglycan, outer membrane proteins and lipopolysaccharides, and how these components coordinate to maintain cell integrity to govern bacterial survival and virulence.

Robert Kinobe is an Associate Professor of pathophysiology, pharmacology and toxicology in Preclinical Veterinary Sciences, College of Science and Engineering, James Cook University. Robert acquired a Bachelor of Veterinary Medicine and Surgery with Honours at Makerere University in 1996, worked as a veterinarian and then completed a PhD in Pharmacology and Toxicology at the University of Queensland in 2004. Robert investigates causes, mechanisms and effects of antimicrobial resistance along a One Health continuum. He is also a qualified member and past president for the Veterinary Pharmacology Chapter of the Australian and New Zealand College of Veterinary Scientists, and a Diplomate of the American Board of Veterinary Toxicology.

Patrick M. Schaeffer is an associate professor at the College of Medicine and Dentistry, James Cook University. He holds advanced degrees from the University of Strasbourg (MSc) and the University of Basel (PhD). His research expertise lies in macromolecular interactions and their applications in biotechnology. Notably, he has designed and developed several groundbreaking diagnostic and sensing technologies with diverse applications. A significant achievement includes creating the first melioidosis immunodiagnostic capable of distinguishing between LPS serotypes, showcasing his innovative approach to addressing complex health challenges.

Yaoqin Hong is a Lecturer at the College of Medicine and Dentistry, James Cook University. He is an alumnus of the University of Sydney, where he received his PhD in 2014 for establishing the specificity of lipid-linked oligosaccharide flippase as a functional checkpoint governing the structural fidelity of polysaccharide synthesis. His research focuses on the functional analysis of bacterial cell envelope synthesis to maintain physiological fitness, how these interactions have evolved, and how they may be leveraged as antimicrobial strategies.