



HAL
open science

Two temperature-dependent membrane fluidity regimes in Gram-positive bacteria

Aurelien Barbotin, Dimitri Juillot, Paprapach Wongdontree, Rut Carballido Lopez

► To cite this version:

Aurelien Barbotin, Dimitri Juillot, Paprapach Wongdontree, Rut Carballido Lopez. Two temperature-dependent membrane fluidity regimes in Gram-positive bacteria. 2026. <hal-05477619>

HAL Id: hal-05477619

<https://hal.science/hal-05477619v1>

Preprint submitted on 26 Jan 2026

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Two temperature-dependent membrane fluidity regimes in Gram-positive bacteria

Aurélien Barbotin, Dimitri Juillot, Paprapach Wongdontree and Rut Carballido-López

Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350, Jouy-en-Josas, France

*Correspondence to Aurélien Barbotin aurelien.barbotin@outlook.fr and to R. Carballido-López rut.carballido-lopez@inrae.fr

Abstract

It is widely accepted that bacterial cells maintain a constant membrane fluidity in response to temperature changes. This process, known as membrane fluidity homeostasis, occurs through remodeling of plasma membrane composition. We tested this using an assay based on total internal reflection-fluorescence correlation spectroscopy (TIR-FCS) that directly quantifies membrane fluidity as the diffusion speed of a membrane marker in *Bacillus subtilis* and two other Gram-positive bacteria, *Streptococcus pneumoniae* and *Staphylococcus aureus*, across a temperature range of 20°C to 37°C. Instead of the expected constant membrane fluidity, we identified a two-component regime: membrane fluidity is maintained at low temperatures (<26°C), but freely increases at higher temperatures.

Significance statement

Temperature changes affect the physical properties of the bacterial plasma membrane. Typically, with a reduction in temperature comes a loss of membrane fluidity. It is known that bacteria like *Bacillus subtilis* adapt their membrane composition when this happens, and it was thought to maintain membrane fluidity constant. We found that this is not true: fluidity is maintained only in a low (<26°C) temperature regime, while at higher temperatures fluidity is not maintained. We found this to be true for several Gram+ bacteria.

Introduction

Membrane fluidity changes have been linked to a wide variety of processes in bacteria, for example responses to physical or chemical stresses, protein folding, respiration¹ and antibiotic resistance². One well-studied aspect of membrane fluidity in bacteria is membrane fluidity homeostasis in response to changes in temperature. In Gram-negative bacteria like *E. coli*, membrane fluidity homeostasis was measured as a gradual increase of membrane FA unsaturation as temperature decreased³. The mechanism of adaptation in Gram-positive bacteria like *B. subtilis*⁴ is more complex: changes of temperature are detected by the two-component system DesK/DesR, which initiates the transcription of the FA desaturase Des upon cold shock⁵. Long-term membrane adaptation involves FA branching and shortening⁴. Membrane FA analysis has long remained the gold standard to evaluate membrane fluidity

homeostasis, despite growing evidence of the role of other actors like lipid headgroups⁶ or proteins⁷ on the physical properties of the membrane. Another standard approach, which consists in using environment-sensitive probes such as diphenylhexatriene (DPH) to probe local membrane properties, produced conflicting results⁸. This is not entirely surprising, as environment-sensitive probes report on complex signals⁹ that correlate with membrane fluidity only under specific biophysical assumptions that are often not verified in biological experiments¹⁰.

Recent quantitative measurements revealed that plasma membrane fluidity in *B. subtilis* is approximately two-fold lower at low temperatures (20-23°C) than at 37°C^{11,12}. This is in disagreement with the substantial literature that uncovered mechanisms of thermoadaptation in this bacterium. Here, we sought to resolve this apparent contradiction using a technique based on total internal reflection-fluorescence correlation spectroscopy (TIR-FCS) that we recently developed¹² to directly measure membrane fluidity at a range of temperatures in three different Gram-positive bacteria. We found two distinct operating regimes: at low temperatures (< 26°C), membrane fluidity is maintained constant by the established homeostatic feedback mechanisms; in contrast, at higher temperatures, membrane fluidity increases linearly with temperature.

Results

We quantified membrane fluidity as the diffusion speed of the membrane marker Nile Red in live wild-type *B. subtilis* cells using TIR-FCS¹² (Fig. 1A). We first measured membrane fluidity shortly (25-70 mins, after complete cold shock recovery¹²) after cells were transferred from their growth temperature (37°C) to a target temperature between 20°C and 37°C. To our surprise, we observed that membrane fluidity in *B. subtilis* was neither constant nor proportional to temperature. Instead, we observed two operating regimes: membrane fluidity was constant below 26°C, and evolved linearly with temperature above this temperature (Fig. 1B). We then verified that removing a gene of the well-studied thermosensing pathway of *B. subtilis* could alter this phenotype. These experiments being performed shortly after the change in temperature, we decided to remove the fatty acid desaturase Des, the fast responder known to immediately desaturate membrane fatty acids after a cold shock⁴. We measured membrane fluidity in a Δdes mutant across the same temperature range applied to wild-type cells. We observed that the fluidity was no longer constant below 26°C (Fig. 1C), confirming the role of the Des enzyme in membrane fluidity homeostasis at low temperatures. Using membrane FA extraction and analysis, we measured the relative proportion of the most varying FA between exponentially growing cells at 37°C and 45 mins after a cold shock at 22°C. The only notable difference between both strains was the apparition of unsaturation in WT cells after cold shock explaining the different phenotype of both strains at low temperature. We observed in both strains a similar reduction in overall carbon chain length (Fig. 1D) after cold shock, as previously observed¹³, which was likely the beginning of the long-term membrane fluidity adaptation.

Measuring the dynamics of cold shock recovery in both wild-type and Δdes cells, we observed as expected a much lower recovery of membrane fluidity with time in the Δdes mutant than in the WT (Fig. 1D). From this, we concluded that the Δdes mutant loses its membrane homeostasis phenotype at low temperatures because of its inability to quickly increase its membrane fluidity. We verified that this inhibition was transient, as predicted by the literature that describes long-term membrane homeostasis to be performed by FA branching and shortening and not unsaturation. For this, we compared membrane fluidity measurements in

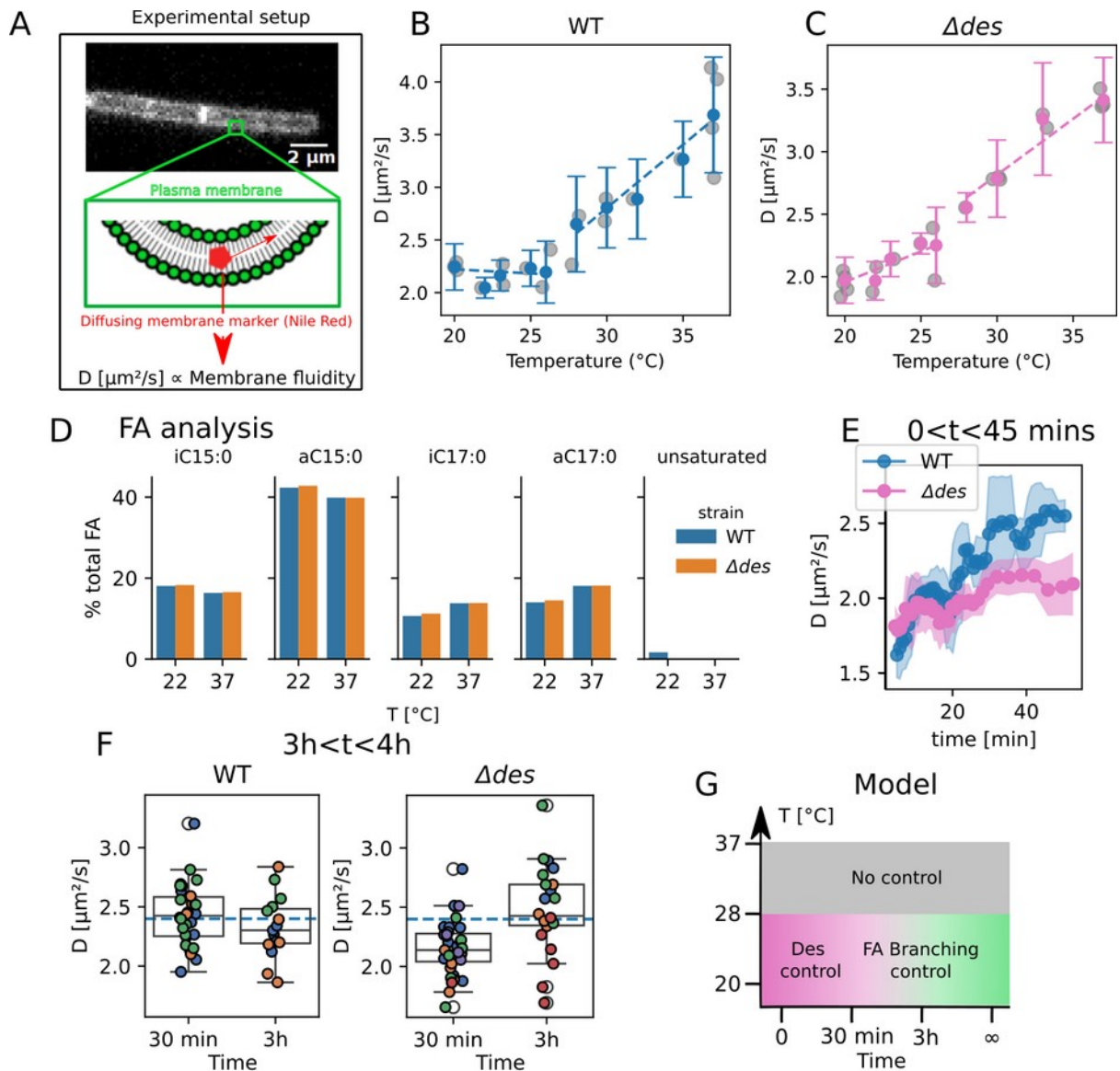


Figure 1. Temperature-dependent membrane fluidity homeostasis in *B. subtilis*. (A) schematic of the methodology. Membrane fluidity is measured as the diffusion coefficient of the membrane marker Nile Red in the plasma membrane. (B-C) Membrane fluidity (mean \pm s.d., gray dots: median diffusion coefficients of individual replicates) in wild-type (WT) (B) and Δdes *B. subtilis* cells (C) measured 25-70 mins after transfer at the target temperature. (D) Relative FA composition of WT and Δdes strains at 37°C and 45 mins after cold shock at 22°C. FA types represented are iso (iC) or anteiso (aC) with saturated carbon chains of length 15 or 17, and unsaturated FA (mean of n=2). (E) Time-dependent membrane fluidity recovery in WT (blue) and Δdes (pink) cells after a transfer from 37°C to 20°C. (F) steady-state membrane fluidity in WT (left) and Δdes (right) ~30 min after cold shock or ~3h after cold shock. (G) Proposed model of temperature-dependent membrane fluidity control in *B. subtilis*.

WT and Δdes strains, immediately after cold shock and after approximately 3 hours (~ 1 generation) spent at 20°C. While WT cells exhibited the same membrane fluidity regardless of time spent at 20°C, Δdes cells recovered the WT fluidity after 3 hours at 20°C (Fig. 1E). Membrane fluidity of WT cells remained comparatively lower at 20°C than at 37°C even for much longer times spent at 20°C: the diffusion coefficient of Nile Red remained below 2.5 $\mu\text{m}^2/\text{s}$ even in cells that grew for 24h at 20°C (data not shown). These observations led us to propose a new model of membrane fluidity homeostasis, to complete the current model and reconcile conflicting observations (Fig. 1F). Our observations do not contradict that the two

mechanisms uncovered so far of FA unsaturation and branching are indeed activated at different timescales after a cold shock to maintain membrane fluidity. However, our data revealed that above a critical temperature, which we estimate around 26°C (Fig. 1B), membrane fluidity is not controlled.

To verify whether this model is specific to *B. subtilis* or extends to other Gram-positive bacteria, we performed TIR-FCS temperature scans in two Gram-positive mesophilic pathogens, *Staphylococcus aureus* and *Streptococcus pneumoniae* (Fig. 2). These data clearly show that both bacterial species exhibit a membrane fluidity profile similar to that observed in *B. subtilis*: membrane fluidity remains constant below a certain critical temperature, and increases linearly with temperatures above this threshold. The exact value of this critical temperature seems to be around 26°C in both *S. pneumoniae* and *S. aureus* as in *B. subtilis*, yet the noisier TIR-FCS measurements in these two species precludes drawing a definitive conclusion. Notably, the diffusion coefficients across all three bacteria are remarkably similar (ranging from 2-2.5 $\mu\text{m}^2/\text{s}$ at lower temperatures to $\sim 3.5 \mu\text{m}^2/\text{s}$ at higher temperatures). This could hint at a conserved membrane fluidity control mechanism across all three bacteria, in accordance with recent findings that identified thermosensor proteins similar to those of *B. subtilis* in *S. aureus* and *Bacillus anthracis*¹⁴.

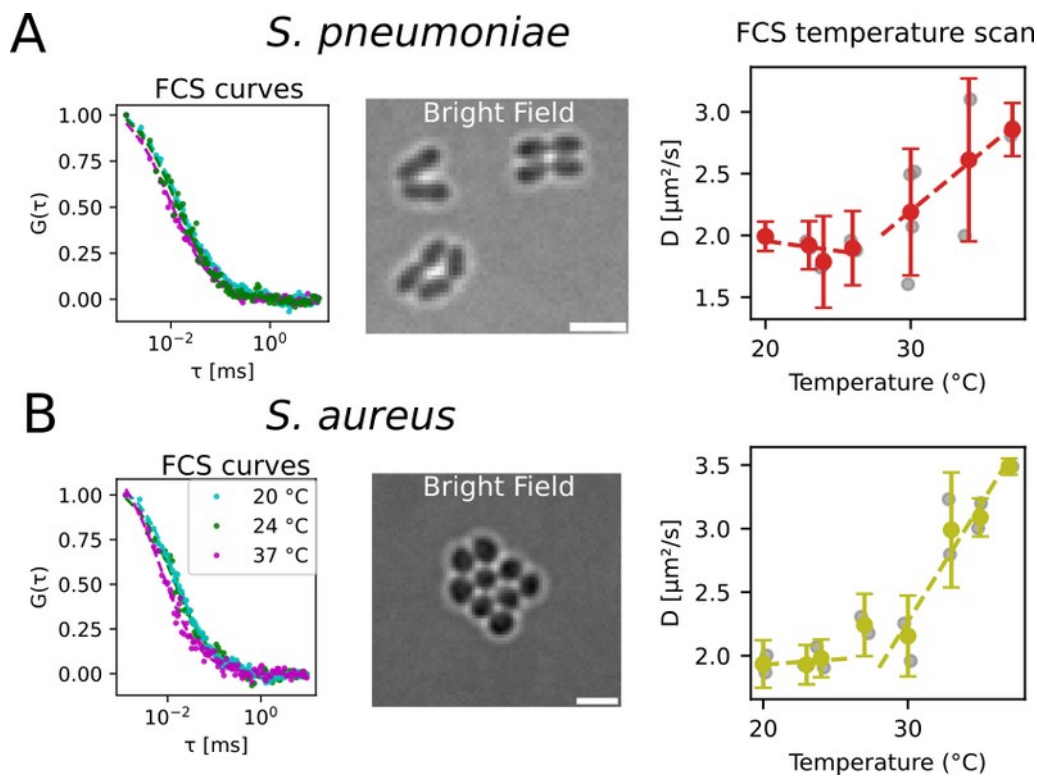


Figure 2. FCS temperature scans in *S. pneumoniae* (A) and *S. aureus* (B). Left, Representative FCS curves at different temperatures. Middle, bright-field images (scale bar: 2 μm). Right, temperature-dependent membrane fluidity.

Discussion

Through quantitative measurements of membrane fluidity, we uncovered the existence of two distinct operating regimes of membrane fluidity in several Gram-positive bacteria, instead of one as previously thought. This discovery does not contradict previous data, in particular measured changes in membrane composition as a response to cold, but rather gives it a new

perspective. The temperature threshold between the two regimes, which we estimate to be around 26°C in *B. subtilis* based on our data, is in line with the previously reported activation temperature of the thermosensor desK¹⁵.

We believe that our finding raises many interesting questions. What is the point of these two operating regimes? Based on membrane FA analysis³, it is likely that Gram-negative bacteria do not exhibit this behaviour in this range of temperatures: why and what does this imply for the functions regulated by membrane properties? We observed the same regimes in three Gram-positive mesophilic bacteria, but what about psychrophilic bacteria? In *B. subtilis*, the thermosensor desK is thought to measure membrane thickness and not fluidity directly⁴. We can reasonably expect that membrane thickness will follow the same two-component behaviour and it is likely to be the case for many other physical parameters of the membrane. Finally, this work highlights that caution is required when quantifying motion of membrane-associated objects at physiological temperatures, as minor changes in temperatures can lead to substantial measurement bias. When the highest precision is required, higher stability can be attained by working in the membrane-fluidity controlled regime.

Materials and Methods

Diffusion speed of membrane marker Nile Red was measured with TIR-FCS¹². Each acquisition of 50 000 frames was about one minute long. Raw diffusion coefficients were divided by a factor specific to the shape of each bacterium (*B. subtilis*: 1.54, *S. aureus* 2.38 and *S. pneumoniae* 2.19) estimated from simulations. Detailed methods can be found in the appendix.

Acknowledgements

We thank Drs Charlène Cornilleau and Armand Lablaine and the members of the ProCeD laboratory for their support and helpful discussions. We also thank Dr Daniela Albanesi for useful discussion. We thank Drs Alexandra Gruss and Florence DUbois-Brissonnet for their help with FA extraction and analysis. This project was funded by a Consolidator grant from the European Research Council (ERC) under the Horizon 2020 research and innovation program (ERC-2017-CoG-772178 to R.C.-L.) and under the Marie Skłodowska-Curie grant agreement no. 101030628.

Competing interest

The authors declare no competing interests

References

- (1) Budin, I.; De Rond, T.; Chen, Y.; Chan, L. J. G.; Petzold, C. J.; Keasling, J. D. Viscous Control of Cellular Respiration by Membrane Lipid Composition. *Science* **2018**, *362* (6419), 1186–1189. <https://doi.org/10.1126/science.aat7925>.
- (2) Boudjemaa, R.; Cabriel, C.; Dubois-Brissonnet, F.; Bourg, N.; Dupuis, G.; Gruss, A.; Lévêque-Fort, S.; Briandet, R.; Fontaine-Aupart, M.-P.; Steenkeste, K. Impact of Bacterial Membrane Fatty Acid Composition on the Failure of Daptomycin To Kill *Staphylococcus Aureus*. *Antimicrob. Agents Chemother.* **2018**, *62* (7), e00023-18. <https://doi.org/10.1128/AAC.00023-18>.

- (3) Marr, A. G.; Ingraham, J. L. EFFECT OF TEMPERATURE ON THE COMPOSITION OF FATTY ACIDS IN *ESCHERICHIA COLI*. *J. Bacteriol.* **1962**, *84* (6), 1260–1267. <https://doi.org/10.1128/jb.84.6.1260-1267.1962>.
- (4) Mendoza, D. D. Temperature Sensing by Membranes. *Annu. Rev. Microbiol.* **2014**, *68* (1), 101–116. <https://doi.org/10.1146/annurev-micro-091313-103612>.
- (5) Svobodová, J.; Julák, J.; Pilař, J.; Svoboda, P. Membrane Fluidity in *Bacillus Subtilis*. Validity of Homeoviscous Adaptation. *Folia Microbiol. (Praha)* **1988**, *33* (3), 170–177. <https://doi.org/10.1007/BF02925901>.
- (6) Seu, K. J.; Cambrea, L. R.; Everly, R. M.; Hovis, J. S. Influence of Lipid Chemistry on Membrane Fluidity: Tail and Headgroup Interactions. *Biophys. J.* **2006**, *91* (10), 3727–3735. <https://doi.org/10.1529/biophysj.106.084590>.
- (7) Zielińska, A.; Savietto, A.; de Sousa Borges, A.; Martinez, D.; Berbon, M.; Roelofsen, J. R.; Hartman, A. M.; de Boer, R.; Van der Klei, I. J.; Hirsch, A. K.; Habenstein, B.; Bramkamp, M.; Scheffers, D.-J. Flotillin-Mediated Membrane Fluidity Controls Peptidoglycan Synthesis and MreB Movement. *eLife* **2020**, *9*, e57179. <https://doi.org/10.7554/eLife.57179>.
- (8) Heřman, P.; Konopásek, I.; Plášek, J.; Svobodová, J. Time-Resolved Polarized Fluorescence Studies of the Temperature Adaptation in *Bacillus Subtilis* Using DPH and TMA-DPH Fluorescent Probes. *Biochim. Biophys. Acta BBA - Biomembr.* **1994**, *1190* (1), 1–8. [https://doi.org/10.1016/0005-2736\(94\)90028-0](https://doi.org/10.1016/0005-2736(94)90028-0).
- (9) Amaro, M.; Reina, F.; Hof, M.; Eggeling, C.; Sezgin, E. Laurdan and Di-4-ANEPPDHQ Probe Different Properties of the Membrane. *J. Phys. Appl. Phys.* **2017**, *50* (13), 134004. <https://doi.org/10.1088/1361-6463/aa5dbc>.
- (10) Poojari, C.; Wilkosz, N.; Lira, R. B.; Dimova, R.; Jurkiewicz, P.; Petka, R.; Kepczynski, M.; Róg, T. Behavior of the DPH Fluorescence Probe in Membranes Perturbed by Drugs. *Chem. Phys. Lipids* **2019**, *223*, 104784. <https://doi.org/10.1016/j.chemphyslip.2019.104784>.
- (11) Lucena, D.; Mauri, M.; Schmidt, F.; Eckhardt, B.; Graumann, P. L. Microdomain Formation Is a General Property of Bacterial Membrane Proteins and Induces Heterogeneity of Diffusion Patterns. *BMC Biol.* **2018**, *16* (1), 97. <https://doi.org/10.1186/s12915-018-0561-0>.
- (12) Barbotin, A.; Billaudeau, C.; Sezgin, E.; Carballido-López, R. Quantification of Membrane Fluidity in Bacteria Using TIR-FCS. *Biophys. J.* **2024**, *123* (16), 2484–2495. <https://doi.org/10.1016/j.bpj.2024.06.012>.
- (13) Beranova, J.; Mansilla, M. C.; Mendoza, D. de; Elhottova, D.; Konopasek, I. Differences in Cold Adaptation of *Bacillus Subtilis* under Anaerobic and Aerobic Conditions. *J. Bacteriol.* **2010**, *192* (16), 4164–4171.
- (14) Fernández, P.; Díaz, A. R.; Ré, M. F.; Porrini, L.; De Mendoza, D.; Albanesi, D.; Mansilla, M. C. Identification of Novel Thermosensors in Gram-Positive Pathogens. *Front. Mol. Biosci.* **2020**, *7*, 592747. <https://doi.org/10.3389/fmolb.2020.592747>.
- (15) Albanesi, D.; Martín, M.; Trajtenberg, F.; Mansilla, M. C.; Haouz, A.; Alzari, P. M.; De Mendoza, D.; Buschiazzo, A. Structural Plasticity and Catalysis Regulation of a Thermosensor Histidine Kinase. *Proc. Natl. Acad. Sci.* **2009**, *106* (38), 16185–16190. <https://doi.org/10.1073/pnas.0906699106>.