



UvA-DARE (Digital Academic Repository)

Molecular dynamics guided analysis of *Bacillus subtilis* spore germination mechanisms

Chen, L.

Publication date
2026

[Link to publication](#)

Citation for published version (APA):

Chen, L. (2026). *Molecular dynamics guided analysis of Bacillus subtilis spore germination mechanisms*. [Thesis, fully internal, Universiteit van Amsterdam].

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, P.O. Box 19185, 1000 GD Amsterdam, The Netherlands. You will be contacted as soon as possible.

9 Summary

The germination of *Bacillus subtilis* spores triggered by L-alanine is initiated by sensing of the germinant by the germinant receptor subunit GerAB. This subunit belongs to the APC transporter super family and has previously been shown to contain an L-alanine binding pocket and to form a water channel. To characterize the functional mechanism of GerAB, we employed molecular simulations to obtain atomic-level spatial insight with picosecond temporal resolution. The molecular hypotheses based on simulations were then by site-directed mutagenesis followed by spore germination assays.

The workflow of this thesis begins with characterizing water channel formation in GerAB. In **Chapter 3**, the water channel was initially explored using steered molecular dynamics (SMD) simulations, in which water molecules were pulled through GerAB to reveal a preliminary water permeation pathway. These simulations identified residues with bulky side chains, Y97, L199, and F342, as potential blockades along this pathway. To experimentally test whether these residues indeed obstruct water passage, we mutated them to alanine to remove their side chains, obstructing the single mutants Y97A, L199A, and F342A, as well as the triple mutant triA (Y97A/L199A/F342A). Spores harbouring the L199A, F342A, and triA mutations were unable to germinate in response to L-alanine, whereas Y97A showed reduced sensitivity to L-alanine. In addition, germination induced by the AGFK mixture (L-asparagine, D-glucose, D-fructose, and KCl) was perturbed in all mutants. Immunoblotting revealed an absence of GerAA in the L199A, F342A, and triA mutants, and a reduced GerAA level in Y97A compared to wild-type spores. These results indicate that GerAB in the L199A, F342A, and triA mutants fails to assemble with its partner subunits, whereas assembly is partially compromised in Y97A. Together, these findings suggest that these residues are structurally important for GerAB and that mutations in GerAB affect germinosome functionality, as reflected by impaired GerB and GerK activity. The data in this chapter did not provide direct experimental evidence that the predicted residues form a functional water channel in GerAB.

Chapter 4 continued the investigation of water channel formation in GerAB using molecular dynamics (MD) simulations. Water permeation events were identified across ten parallel 1- μ s simulations, with the number of water passage events ranging from 121 to 1 per microsecond across individual runs. By combining all simulations, we calculated contact frequencies between passed water molecules and GerAB residues, identifying eight residues, T30, R33, N62, Y97, E105, S197, E202, and S335, that frequently contacted water via their side chains. These residues are located on transmembrane helices TM1, TM3, TM6, TM8, and TM10 and line the predicted water pathway by **Chapter 3**. Each residue was substituted with a similarly sized hydrophobic residue both *in silico* and *in vivo* (T30V, R33L, N62L, Y97F, E105L, S197A,

E202L, and S335A). Most of these mutants showed little water permeation in simulations. Germination assays revealed that all mutants were defective in L-alanine-induced germination, while AGFK-induced germination was impaired to varying degrees. Immunoblotting showed the absence of GerAA in all mutants, indicating that these GerAB variants are unstable or unable to assemble with their partner subunits GerAA and GerAC. The impairment of GerB and GerK function further supports the conclusion that mutations in GerAB disrupt germinosome integrity. Collectively, these results identify water-contacting residues as structural hotspots within the GerA receptor and the germinosome, while leaving the direct physiological role of the water channel unresolved. Remarkably, we observed that mutant G25A, previously described as stable, was also unstable in our hands, which may have been related to the fact that two copies of the mutant gene were present in the genome of the cells.

Chapter 5 focused on deepening our understanding of GerAB dynamics using molecular simulations alone. We identified the C-terminal segment of transmembrane helix 6 (TM6C) as a gating element for water passage in GerAB and identified residue pairs between TM6C and the rest of the protein that potentially regulate water gating through close contacts between one and other. Pulling the contact map of the potential gating residues from high to low, mimicking channel opening, resulted in water passage in seven out of nine simulations. We further characterized TMI bending behaviour, reported for other APC transporters as one of the markers for outward-facing (OF) and inward-facing (IF) conformations transition. Docking L-alanine into three GerAB structures with different TMI bending states and TM6C contact numbers, followed by MD simulations, showed that L-alanine escaped from the previously identified binding pocket in two out of three parallel simulations for each structure. Notably, the L-alanine dissociation pathway differed depending on the TMI bending state. In summary, this chapter identifies two key molecular mechanisms in GerAB: TM6C-mediated gating of water passage and TMI bending-dependent regulation of L-alanine dissociation.

Chapter 6, conducted in collaboration with the University of Connecticut (USA) and the University of Cambridge (UK), focused on lithium-ion-triggered germination in spores from *Bacillus* species. In *B. subtilis*, Li^+ induces spore germination via the GerA receptor, but with markedly slower dynamics (~60 h) compared to L-alanine-induced germination, which completes within ~2 h. Li^+ -induced germination can be inhibited by Na^+ but not by K^+ , and neither Na^+ nor K^+ alone triggers germination. MD simulations showed that Li^+ exhibits higher proximity to GerAB than K^+ , with the most interactions occurring at extracellular loops. RMSD and RMSF analyses revealed reduced structural fluctuations in GerAB in Li^+ simulations compared to K^+ simulations, especially on its transmembrane regions (TM2, 4, 5, 6, 7, 10) and loops (EL1, 2, 3 and IL1, IL2). The high proximity between Li^+ and the extracellular loops

likely stabilizes these regions, providing a possible explanation for the slower dynamics of Li⁺-induced germination. However, the detailed molecular mechanism underlying Li⁺-triggered germination and its competition with Na⁺ remains unresolved.

Chapter 7 describes the development of a multidisciplinary teaching module for first-year undergraduate students based on the research presented in this thesis. This six-week, hands-on research project guides students through investigating the structure–function relationship of GerAB using a combination of molecular simulations and phase-contrast microscopy. Students are introduced to the full research workflow of a target protein, beginning with structure prediction using AlphaFold, followed by membrane system construction for MD simulations and trajectory analysis using residue–residue pairwise distance to formulate functional hypotheses. This computational work is completed by experimental monitoring of germination in wild-type and mutant spores using phase-contrast microscopy. By integrating simulation and experimental results, students learn how mutations in GerAB affect protein structure and, consequently, germination efficiency. Through this project, students gain foundational knowledge of *B. subtilis* germination, protein structure prediction, and the execution of a complete multidisciplinary research workflow, enriching multidisciplinary education in the undergraduate programme Science Technology and Innovation at the University of Amsterdam.

In summary, GerAB is predicted by MD simulations to regulate water passage during spore germination. MD simulations identify Y97, L199 and F342 as potential blockades and reveal high water contact frequencies for residues T30, R33, N62, Y97, E105, S197, E202, and S335 located on transmembrane regions TM1, TM3, TM6, TM8, and TM10. Mutations at these sites destabilize GerAB or prevent its assembly with GerAA and GerAC, thereby affecting L-alanine-induced *B. subtilis* spore germination and compromising germinosome integrity, as evidenced by reduced GerB and GerK functionality. Although mutagenesis could not directly confirm a functional water channel, simulations indicate that TM6C gates water passage in GerAB via interaction with the rest of the protein. Moreover, we also identified that TM1 bending state is correlated to different L-alanine dissociation pathway. Furthermore, GerAB mediates Li⁺-triggered germination, with high proximity between Li⁺ at extracellular loops that likely reduce protein flexibility and slower germination dynamics.