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

Chen, L.

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List of Publications

Chapter 3: **L. Chen**, H. Beekhuis, C. van den Bosch, G. Vinay, G. Korza, J. Vreede, P. Setlow, S. Brul, GerAB residues predicted to interfere with water passage based on steered molecular dynamics are key to germinosome functionality. *Front. Microbiol.* **16**, 1656964 (2025).

Chapter 4: **L. Chen**, S. van Buuren, G. Korza, J. Vreede, P. Setlow, S. Brul, GerAB residues predicted to interact with water based on MD simulations mediate germinosome stability in *Bacillus subtilis* spores. *Front. Microbiol.* *Under review*

Chapter 5: **L. Chen**, P. Setlow, S. Brul, J. Vreede, Decoding Dynamic Details of Bacillus subtilis Germinant Receptor Subunit GerAB with Molecular Simulations. *In preparation*

Chapter 6: J. Wicander, J. Gorsuch, **L. Chen**, R. Caldbeck, G. Korza, S. Brul, G. Christie, and P. Setlow. Germination of *Bacillus* spores by LiCl. *J. Bacteriol.* **207**, e00510-24 (2025).

Chapter 7: **L. Chen**, S. Brull, I. Ilie, J. Vreede, Combining in silico and in vivo Approaches: A Multidisciplinary Practical Module for Undergraduate Students. *In preparation*

Bibliography

1. Bonilla, C. Y. Generally stressed out bacteria: Environmental stress response mechanisms in gram-positive bacteria. *Integr. Comp. Biol.* **60**, 126–133 (2020).
2. Egan, M., Dempsey, E., Ryan, C. A., Ross, R. P. & Stanton, C. The sporobiota of the human gut. *Gut Microbes* **13**, 1863134 (2021).
3. Waldrop, M. P. *et al.* microbial ecology of permafrost soils: populations, processes, and perspectives. *Permafrost. Periglac. Process.* **36**, 245–258 (2025).
4. Bankar, A., Patil, S., Shinde, M., Shinde, S. & Kowligi, B. Microbial extremozymes. 89–109 (2022) doi:10.1016/b978-0-12-822945-3.00016-6.
5. Shcherbakova, V. A. *et al.* Novel psychrophilic anaerobic spore-forming bacterium from the overcooled water brine in permafrost: description *Clostridium algorithilum* sp. nov. *Extremophiles* **9**, 239–246 (2005).
6. Bovallius, A., Bucht, B., Roffey, R. & Anäs, P. Long-range air transmission of bacteria. *Appl. Environ. Microbiol.* **35**, 1231–1232 (1978).
7. Lazar, C. S. *et al.* The endolithic bacterial diversity of shallow bedrock ecosystems. *Sci. Total Environ.* **679**, 35–44 (2019).
8. Lipkens, B., Dionne, J., Costolo, M., Stevens, A. & Rietman, E. Separation of bacterial spores from flowing water in macro-scale cavities by ultrasonic standing waves. *J. Acoust. Soc. Am.* **128**, 2338–2338 (2010).
9. Stelma, G. N. Use of bacterial spores in monitoring water quality and treatment. *J. Water Heal.* **16**, 491–500 (2018).
10. Walker, J. J. & Pace, N. R. Endolithic microbial ecosystems. *Annu. Rev. Microbiol.* **61**, 331–347 (2007).
11. Isticato, R. & Ricca, E. Spore surface display. *Microbiol. Spectr.* **2**, 10.1128/microbiolspec.tbs-0011–2012 (2014).
12. Hong, H. A. *et al.* *Bacillus subtilis* isolated from the human gastrointestinal tract. *Res. Microbiol.* **160**, 134–143 (2009).
13. Tetz, V. & Tetz, G. Draft genome sequence of a strain of *Bacillus intestinalis* sp. nov., a new member of sporobiota isolated from the small intestine of a single patient with intestinal cancer. *Genome Announc.* **5**, e00489-17 (2017).
14. Kotiranta, A., Lounatmaa, K. & Haapasalo, M. Epidemiology and pathogenesis of *Bacillus cereus* infections. *Microbes Infect.* **2**, 189–198 (2000).
15. Rhee, K.-J., Sethupathi, P., Driks, A., Lanning, D. K. & Knight, K. L. Role of Commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire. *J. Immunol.* **172**, 1118–1124 (2004).

Bibliography

16. Lopetuso, L. R., Scalfaferrri, F., Petito, V. & Gasbarrini, A. Commensal *Clostridia*: leading players in the maintenance of gut homeostasis. *Gut Pathog.* **5**, 23 (2013).
17. Frank, D. N. *et al.* Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc. Natl. Acad. Sci.* **104**, 13780–13785 (2007).
18. Ricca, E., Baccigalupi, L., Cangiano, G., Felice, M. D. & Istitico, R. Mucosal vaccine delivery by non-recombinant spores of *Bacillus subtilis*. *Microb. Cell Factories* **13**, 115 (2014).
19. Mattosovich, R. *et al.* Conversion of xylan by recyclable spores of *Bacillus subtilis* displaying thermophilic enzymes. *Microb. Cell Factories* **16**, 218 (2017).
20. Gomis-Cebolla, J. & Berry, C. *Bacillus thuringiensis* as a biofertilizer in crops and their implications in the control of phytopathogens and insect pests. *Pest Manag. Sci.* **79**, 2992–3001 (2023).
21. Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J. & Setlow, P. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol. Mol. Biol. Rev.* **64**, 548–572 (2000).
22. Tocheva, E. I. *et al.* Peptidoglycan remodeling and conversion of an inner membrane into an outer membrane during sporulation. *Cell* **146**, 799–812 (2011).
23. McCormick, J. R. & Flårdh, K. Signals and regulators that govern *Streptomyces* development. *FEMS Microbiol. Rev.* **36**, 206–231 (2012).
24. Müller, F. D., Schink, C. W., Hoiczky, E., Cserti, E. & Higgs, P. I. Spore formation in *Myxococcus xanthus* is tied to cytoskeleton functions and polysaccharide spore coat deposition. *Mol. Microbiol.* **83**, 486–505 (2012).
25. Iwańska, O. *et al.* Translation in *Bacillus subtilis* is spatially and temporally coordinated during sporulation. *Nat. Commun.* **15**, 7188 (2024).
26. Tibocho-Bonilla, J. D., Lyda, J., Riley, E., Pogliano, K. & Zengler, K. Deciphering metabolic differentiation during *Bacillus subtilis* sporulation. *Nat. Commun.* **16**, 129 (2025).
27. Christie, G. & Setlow, P. *Bacillus* spore germination: Knowns, unknowns and what we need to learn. *Cell Signal* **74**, 109729 (2020).
28. Stülke, J., Gruppen, A., Bramkamp, M. & Pelzer, S. *Bacillus subtilis*, a Swiss Army Knife in Science and Biotechnology. *J. Bacteriol.* **205**, e00102-23 (2023).
29. Errington, J. Regulation of endospore formation in *Bacillus subtilis*. *Nat. Rev. Microbiol.* **1**, 117–126 (2003).
30. Hutchison, E. A., Miller, D. A. & Angert, E. R. Sporulation in bacteria: beyond the standard model. *Microbiol. Spectr.* **2**, 10.1128/microbiolspec.tbs-0013-2012 (2014).
31. Piggot, P. J. & Hilbert, D. W. Sporulation of *Bacillus subtilis*. *Curr. Opin. Microbiol.* **7**, 579–586 (2004).
32. Setlow, P. Spore resistance properties. *Microbiol. Spectr.* **2**, 10.1128/microbiolspec.tbs-0003-2012 (2014).

33. Glasset, B. *et al.* *Bacillus cereus*, a serious cause of nosocomial infections: Epidemiologic and genetic survey. *PLoS ONE* **13**, e0194346 (2018).
34. Setlow, P. & Christie, G. If ATP and macromolecular synthesis are needed for dormant spores of *Bacillota* species to trigger spore germination, where do the energy and precursors come from? *J. Appl. Microbiol.* **lxaf177** (2025) doi:10.1093/jambio/lxaf177.
35. Gao, Y. *et al.* Bacterial spore germination receptors are nutrient-gated ion channels. *Science* **380**, 387–391 (2023).
36. Knudsen, S. M. *et al.* Water and small-molecule permeation of dormant *Bacillus subtilis* spores. *J. Bacteriol.* **198**, 168–177 (2015).
37. Paidhungat, M. & Setlow, P. Role of ger proteins in nutrient and nonnutrient triggering of spore germination in *Bacillus subtilis*. *J. Bacteriol.* **182**, 2513–9 (2000).
38. Ragkousi, K., Eichenberger, P., Ooij, C. van & Setlow, P. Identification of a new gene essential for germination of *Bacillus subtilis* spores with Ca²⁺-dipicolinate. *J. Bacteriol.* **185**, 2315–29 (2003).
39. Wuytack, E. Y., Boven, S. & Michiels, C. W. Comparative study of pressure-induced germination of *Bacillus subtilis* spores at low and high pressures. *Appl. Environ. Microbiol.* **64**, 3220–3224 (1998).
40. Rode, L. J. & Foster, J. W. Germination of bacterial spores with alkyl primary amines. *J. Bacteriol.* **81**, 768–779 (1961).
41. Setlow, B., Cowan, A. E. & Setlow, P. Germination of spores of *Bacillus subtilis* with dodecylamine. *J. Appl. Microbiol.* **95**, 637–648 (2003).
42. Popham, D. L., Helin, J., Costello, C. E. & Setlow, P. Muramic lactam in peptidoglycan of *Bacillus subtilis* spores is required for spore outgrowth but not for spore dehydration or heat resistance. *Proc. Natl. Acad. Sci.* **93**, 15405–15410 (1996).
43. Shah, I. M., Laaberki, M.-H., Popham, D. L. & Dworkin, J. A Eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments. *Cell* **135**, 486–496 (2008).
44. Artzi, L. *et al.* Dormant spores sense amino acids through the B subunits of their germination receptors. *Nat Commun* **12**, 6842 (2021).
45. Blinker, S., Vreede, J., Setlow, P. & Brul, S. Predicting the Structure and Dynamics of Membrane Protein gerab from *Bacillus subtilis*. *Int J Mol Sci* **22**, 3793 (2021).
46. Li, Y. *et al.* Structural and functional analyses of the N-terminal domain of the A subunit of a *Bacillus megaterium* spore germinant receptor. *Proc. Natl. Acad. Sci.* **116**, 11470–11479 (2019).
47. Amon, J. D., Artzi, L. & Rudner, D. Z. Genetic Evidence for Signal Transduction within the *Bacillus subtilis* GerA Germinant Receptor. *J. Bacteriol.* **204**, e00470-21 (2021).
48. Griffiths, K. K., Zhang, J., Cowan, A. E., Yu, J. & Setlow, P. Germination proteins in the inner membrane of dormant *Bacillus subtilis* spores colocalize in a discrete cluster. *Mol. Microbiol.* **81**, 1061–1077 (2011).

Bibliography

49. Li, Y. *et al.* Role of a SpoVA protein in dipicolinic acid uptake into developing spores of *Bacillus subtilis*. *J. Bacteriol.* **194**, 1875–1884 (2012).
50. Tovar-Rojo, F., Chander, M., Setlow, B. & Setlow, P. The products of the spoVA operon are involved in dipicolinic acid uptake into developing spores of *Bacillus subtilis*. *J. Bacteriol.* **184**, 584–587 (2002).
51. Gao, Y. *et al.* The SpoVA membrane complex is required for dipicolinic acid import during sporulation and export during germination. *Genes Dev.* **36**, 634–646 (2022).
52. Boland, F. M., Atrih, A., Chirakkal, H., Foster, S. J. & Moir, A. Complete spore-cortex hydrolysis during germination of *Bacillus subtilis* 168 requires SleB and YpeB. *Microbiology* **146**, 57–64 (2000).
53. Chirakkal, H., O'Rourke, M., Atrih, A., Foster, S. J. & Moir, A. Analysis of spore cortex lytic enzymes and related proteins in *Bacillus subtilis* endospore germination. *Microbiology* **148**, 2383–2392 (2002).
54. Moir, A. & Cooper, G. Spore Germination. *Microbiol. Spectr.* **3**, (2015).
55. Cooper, G. R. & Moir, A. Amino Acid Residues in the GerAB Protein Important in the Function and Assembly of the Alanine Spore Germination Receptor of *Bacillus subtilis* 168. *J. Bacteriol.* **193**, 2261–2267 (2011).
56. Collier, T. A., Piggot, T. J. & Allison, J. R. Molecular dynamics simulation of proteins. *Methods Mol Biology Clifton N J* **2073**, 311–327 (2020).
57. Dodson, G. G., Lane, D. P. & Verma, C. S. Molecular simulations of protein dynamics: new windows on mechanisms in biology. *Embo Rep* **9**, 144–150 (2008).
58. Sztain, T. *et al.* A glycan gate controls opening of the SARS-CoV-2 spike protein. *Nat Chem* **13**, 963–968 (2021).
59. Konovalov, K. A., Unarta, I. C., Cao, S., Goonetilleke, E. C. & Huang, X. Markov state models to study the functional dynamics of proteins in the wake of machine learning. *JACS Au* **1**, 1330–1341 (2021).
60. Afshinpour, M., Parsi, P. & Mahdiuni, H. Investigation of molecular details of a bacterial cationic amino acid transporter (GkApcT) during arginine transportation using molecular dynamics simulation and umbrella sampling techniques. *J. Mol. Model.* **29**, 260 (2023).
61. Krämer, A. *et al.* Membrane permeability of small molecules from unbiased molecular dynamics simulations. *J Chem Phys* **153**, 124107 (2020).
62. Orlando, M. A., Pouillon, H. J. T., Mandal, S., Kroos, L. & Orlando, B. J. Substrate engagement by the intramembrane metalloprotease SpoIVFB. *Nat. Commun.* **15**, 8276 (2024).
63. Kermani, A. A. A guide to membrane protein X-ray crystallography. *FEBS J.* **288**, 5788–5804 (2021).
64. Sgro, G. G. & Costa, T. R. D. Cryo-EM Grid Preparation of membrane protein samples for single particle analysis. *Front. Mol. Biosci.* **5**, 74 (2018).
65. Nakane, T. *et al.* Single-particle cryo-EM at atomic resolution. *Nature* **587**, 152–156 (2020).

66. Hong, M., Zhang, Y. & Hu, F. Membrane protein structure and dynamics from NMR spectroscopy. *Phys. Chem.* **63**, 1–24 (2012).
67. Källberg, M. *et al.* Template-based protein structure modeling using the RaptorX web server. *Nat. Protoc.* **7**, 1511–1522 (2012).
68. Burley, S. K. *et al.* RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences. *Nucleic Acids Res.* **49**, D437–D451 (2020).
69. Baker, D. & Sali, A. Protein structure prediction and structural genomics. *Science* **294**, 93–96 (2001).
70. Pereira, J. *et al.* High-accuracy protein structure prediction in CASP14. *Proteins: Struct., Funct., Bioinform.* **89**, 1687–1699 (2021).
71. Jumper, J. *et al.* Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589 (2021).
72. Wayment-Steele, H. K. *et al.* Predicting multiple conformations via sequence clustering and AlphaFold2. *Nature* **625**, 832–839 (2024).
73. Shaffer, P. L., Goehring, A., Shankaranarayanan, A. & Gouaux, E. Structure and mechanism of a Na⁺-independent amino acid transporter. *Science* **325**, 1010–1014 (2009).
74. Henzler-Wildman, K. & Kern, D. Dynamic personalities of proteins. *Nature* **450**, 964–972 (2007).
75. Faham, S. *et al.* The Crystal structure of a sodium galactose transporter reveals mechanistic insights into Na⁺/Sugar Symport. *Science* **321**, 810–814 (2008).
76. Adelman, J. L. *et al.* Stochastic steps in secondary active sugar transport. *Proc. Natl. Acad. Sci.* **113**, E3960–E3966 (2016).
77. Hohl, M., Briand, C., Grütter, M. G. & Seeger, M. A. Crystal structure of a heterodimeric ABC transporter in its inward-facing conformation. *Nat. Struct. Mol. Biol.* **19**, 395–402 (2012).
78. Izrailev, S., Stepaniants, S., Balsera, M., Oono, Y. & Schulten, K. Molecular dynamics study of unbinding of the avidin-biotin complex. *Biophys. J.* **72**, 1568–1581 (1997).
79. Izrailev, S. *et al.* Computational Molecular Dynamics: Challenges, Methods, Ideas. *Lect. Notes Comput. Sci. Eng.* 39–65 (1999) doi:10.1007/978-3-642-58360-5_2.
80. Jarzynski, C. Equilibrium free energy differences from nonequilibrium measurements: a master equation approach. *arXiv* (1997) doi:10.48550/arxiv.cond-mat/9707325.
81. Park, S. & Schulten, K. Calculating potentials of mean force from steered molecular dynamics simulations. *J. Chem. Phys.* **120**, 5946–5961 (2004).
82. Hummer, G. & Szabo, A. Free energy reconstruction from nonequilibrium single-molecule pulling experiments. *Proc. Natl. Acad. Sci.* **98**, 3658–3661 (2001).

Bibliography

83. Xu, Y. *et al.* Steered molecular dynamics simulations of protein-ligand interactions. *Sci. China Ser. B: Chem.* **47**, 355–366 (2004).
84. Kunst, F. *et al.* The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. *Nature* **390**, 249–256 (1997).
85. Smith, M. C. M. Molecular biological methods for *Bacillus*. *FEBS Lett.* **287**, 227–227 (1991).
86. Hong, K.-Q., Liu, D.-Y., Chen, T. & Wang, Z.-W. Recent advances in CRISPR/Cas9 mediated genome editing in *Bacillus subtilis*. *World J. Microbiol. Biotechnol.* **34**, 153 (2018).
87. Westbrook, A. W., Moo-Young, M. & Chou, C. P. Development of a CRISPR-Cas9 tool kit for comprehensive engineering of *Bacillus subtilis*. *Appl. Environ. Microbiol.* **82**, 4876–4895 (2016).
88. Mongkolthanaruk, W., Cooper, G. R., Mawer, J. S. P., Allan, R. N. & Moir, A. Effect of amino acid substitutions in the gerAA protein on the function of the alanine-responsive germinant receptor of *Bacillus subtilis* spores. *J. Bacteriol.* **193**, 2268–2275 (2011).
89. Burnette, W. N. Protein blotting and detection, methods and protocols. *Methods Mol. Biol. (Clifton, NJ)* **536**, 5–8 (2009).
90. Towbin, H., Staehelin, T. & Gordon, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci.* **76**, 4350–4354 (1979).
91. Ramirez-Peralta, A., Zhang, P., Li, Y. & Setlow, P. Effects of sporulation conditions on the germination and germination protein levels of *Bacillus subtilis* spores. *Appl. Environ. Microbiol.* **78**, 2689–2697 (2012).
92. Zernike, F. How I Discovered phase contrast. *Science* **121**, 345–349 (1955).
93. Zernike, F. Phase contrast, a new method for the microscopic observation of transparent objects. *Physica* **9**, 686–698 (1942).
94. Zernike, F. Phase contrast, a new method for the microscopic observation of transparent objects part II. *Physica* **9**, 974–986 (1942).
95. Kong, L. *et al.* Characterization of bacterial spore germination using phase-contrast and fluorescence microscopy, Raman spectroscopy and optical tweezers. *Nat. Protoc.* **6**, 625–39 (2011).
96. Setlow, P. Spore germination. *Curr. Opin. Microbiol.* **6**, 550–556 (2003).
97. Omardien, S. *et al.* Evaluating novel synthetic compounds active against *Bacillus subtilis* and *Bacillus cereus* spores using Live imaging with SporeTrackerX. *Sci. Rep.* **8**, 9128 (2018).
98. Pandey, R. *et al.* Live cell imaging of germination and outgrowth of individual *Bacillus subtilis* spores; the effect of heat stress quantitatively analyzed with SporeTracker. *Plos One* **8**, e58972 (2013).
99. Genovese, M., Poulain, E., Doppler, F., Toussaint, R. & Boyer, M. *Bacillus* spore enumeration using flow cytometry: A proof of concept for probiotic application. *J. Microbiol. Methods* **190**, 106336 (2021).

100. Sunde, E. P., Setlow, P., Hederstedt, L. & Halle, B. The physical state of water in bacterial spores. *Proc. Natl. Acad. Sci.* **106**, 19334–19339 (2009).
101. Yi, X. & Setlow, P. Studies of the commitment step in the germination of spores of *Bacillus* species. *J. Bacteriol.* **192**, 3424–33 (2010).
102. Zuberi, A. R., Moir, A. & Feavers, I. M. The nucleotide sequence and gene organization of the gerA spore germination operon of *Bacillus subtilis* 168. *Gene* **51**, 1–11 (1987).
103. Cabrera-Martinez, R.-M., Tovar-Rojo, F., Vepachedu, V. R. & Setlow, P. Effects of overexpression of nutrient receptors on germination of spores of *Bacillus subtilis*. *J. Bacteriol.* **185**, 2457–64 (2003).
104. Smart, O. S., Neduvilil, J. G., Wang, X., Wallace, B. A. & Sansom, M. S. P. HOLE: A program for the analysis of the pore dimensions of ion channel structural models. *J. Mol. Graph.* **14**, 354–360 (1996).
105. Wilson, M. J., Carlson, P. E., Janes, B. K. & Hanna, P. C. Membrane topology of the *Bacillus anthracis* GerH germinant receptor proteins. *J. Bacteriol.* **194**, 1369–1377 (2012).
106. Maginn, E. J. Atomistic Simulation of the thermodynamic and transport properties of ionic liquids. *Acc. Chem. Res.* **40**, 1200–1207 (2007).
107. Madeira, F. *et al.* The EMBL-EBI Job Dispatcher sequence analysis tools framework in 2024. *Nucleic Acids Res.* **52**, W521–W525 (2024).
108. Gao, X. *et al.* Changes in the Spore proteome of *Bacillus cereus* in response to introduction of plasmids. *Microorganisms* **10**, 1695 (2022).
109. Yi, X., Liu, J., Faeder, J. R. & Setlow, P. Synergism between different germinant receptors in the germination of *Bacillus subtilis* spores. *J. Bacteriol.* **193**, 4664–4671 (2011).
110. Alamo, D. del, Meiler, J. & Mchaourab, H. S. Principles of alternating access in LeuT-fold transporters: commonalities and divergences. *J. Mol. Biol.* **434**, 167746 (2022).
111. Jack, D. L., Paulsen, I. T. & Saier, M. H. The amino acid/polyamine/organocation (APC) superfamily of transporters specific for amino acids, polyamines and organocations. *Microbiology* **146**, 1797–1814 (2000).
112. Alamo, D. del, Meiler, J. & Mchaourab, H. S. Principles of alternating access in LeuT-fold transporters: commonalities and divergences. *J. Mol. Biol.* **434**, 167746 (2022).
113. Choe, S., Rosenberg, J. M., Abramson, J., Wright, E. M. & Grabe, M. Water permeation through the sodium-dependent galactose cotransporter vSGLT. *Biophys. J.* **100**, 248a (2011).
114. Abraham, M. J. *et al.* GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* **1**, 19–25 (2015).
115. Aguayo, D., González-Nilo, F. D. & Chipot, C. Insight into the properties of cardiolipin containing bilayers from Molecular Dynamics simulations, Using a Hybrid All-Atom/United-Atom Force Field. *J. Chem. Theory Comput.* **8**, 1765–1773 (2012).

Bibliography

116. Mark, P. & Nilsson, L. Structure and dynamics of the TIP3P, SPC, and SPC/E water models at 298 K. *J. Phys. Chem. A* **105**, 9954–9960 (2001).
117. Bussi, G., Donadio, D. & Parrinello, M. Canonical sampling through velocity rescaling. *J. Chem. Phys.* **126**, 014101 (2007).
118. Parrinello, M. & Rahman, A. Polymorphic transitions in single crystals: A new molecular dynamics method. *J. Appl. Phys.* **52**, 7182–7190 (1981).
119. Nosé, S. & Klein, M. L. Constant pressure molecular dynamics for molecular systems. *Mol. Phys.* **50**, 1055–1076 (1983).
120. Darden, T., York, D. & Pedersen, L. Particle mesh Ewald: An $N \cdot \log(N)$ method for Ewald sums in large systems. *J. Chem. Phys.* **98**, 10089–10092 (1993).
121. Tribello, G. A., Bonomi, M., Branduardi, D., Camilloni, C. & Bussi, G. PLUMED 2: New feathers for an old bird. *Comput. Phys. Commun.* **185**, 604–613 (2014).
122. Bonomi, M. *et al.* PLUMED: a portable plugin for free-energy calculations with molecular dynamics. *arXiv* (2009) doi:10.48550/arxiv.0902.0874.
123. Bonomi, M. *et al.* Promoting transparency and reproducibility in enhanced molecular simulations. *Nat. Methods* **16**, 670–673 (2019).
124. Humphrey, W., Dalke, A. & Schulten, K. VMD: visual molecular dynamics. *J. Mol. Graph.* **14**, 33–8, 27–8 (1996).
125. McGibbon, R. T. *et al.* MDTraj: A Modern open library for the analysis of molecular dynamics trajectories. *Biophys. J.* **109**, 1528–1532 (2015).
126. Conte, A. D. *et al.* RING-PyMOL: residue interaction networks of structural ensembles and molecular dynamics. *Bioinformatics* **39**, btad260 (2023).
127. Wen, J., Pasma, R., Manders, E. M. M., Setlow, P. & Brul, S. Visualization of germinosomes and the inner membrane in *Bacillus subtilis* spores. *J. Vis. Exp. : JoVE* (2019) doi:10.3791/59388.
128. Setlow, P., Wang, S. & Li, Y.-Q. Germination of spores of the orders *Bacillales* and *Clostridiales*. *Annu. Rev. Microbiol.* **71**, 1–19 (2016).
129. Camilleri, E. *et al.* Properties of aged spores of *Bacillus subtilis*. *J. Bacteriol.* **201**, 10.1128/jb.00231-19 (2019).
130. Chen, L. *et al.* GerAB residues predicted to interfere with water passage based on steered molecular dynamics are key to germinosome functionality. *Front. Microbiol.* **16**, 1656964 (2025).
131. Cameron, A. D., Beckstein, O. & Henderson, P. J. F. Encyclopedia of Biophysics. 1481–1485 (2013) doi:10.1007/978-3-642-16712-6_772.
132. Sansom, M. S. P. & Law, R. J. Membrane proteins: Aquaporins — channels without ions. *Curr. Biol.* **11**, R71–R73 (2001).

133. Choe, S., Rosenberg, J. M., Abramson, J., Wright, E. M. & Grabe, M. Water permeation through the sodium-dependent galactose cotransporter vSGLT. *Biophys. J.* **99**, L56–L58 (2010).
134. Taylor, P. M. Amino acid transporters: éminences grises of nutrient signalling mechanisms? *Biochem. Soc. Trans.* **37**, 237–241 (2009).
135. Lee, J. *et al.* CHARMM-GUI Input Generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/OpenMM Simulations Using the CHARMM36 Additive Force Field. *J. Chem. Theory Comput.* **12**, 405–413 (2016).
136. Cheatham, T. E. I., Miller, J. L., Fox, T., Darden, T. A. & Kollman, P. A. Molecular dynamics simulations on solvated biomolecular systems: the particle mesh ewald method leads to stable trajectories of DNA, RNA, and proteins. *J. Am. Chem. Soc.* **117**, 4193–4194 (1995).
137. Hoover, W. G. Canonical dynamics: Equilibrium phase-space distributions. *Phys. Rev. A* **31**, 1695–1697 (1985).
138. Paidhungat, M., Setlow, B., Driks, A. & Setlow, P. Characterization of Spores of *Bacillus subtilis* which lack dipicolinic Acid. *J. Bacteriol.* **182**, 5505–5512 (2000).
139. Hopf, T. A. *et al.* Three-Dimensional Structures of Membrane Proteins from Genomic Sequencing. *Cell* **149**, 1607–1621 (2012).
140. Marks, D. S. *et al.* Protein 3D Structure computed from evolutionary sequence variation. *PLoS ONE* **6**, e28766 (2011).
141. Abramson, J. *et al.* Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* **630**, 493–500 (2024).
142. Yan, R. *et al.* Mechanism of substrate transport and inhibition of the human LAT₁-4F2hc amino acid transporter. *Cell Discov.* **7**, 16 (2021).
143. Yan, R., Zhao, X., Lei, J. & Zhou, Q. Structure of the human LAT₁-4F2hc heteromeric amino acid transporter complex. *Nature* **568**, 127–130 (2019).
144. Chen, L. *et al.* GerAB residues predicted to interact with water based on MD simulations mediate germinosome stability in *Bacillus subtilis* spores. *bioRxiv* 2026.01.10.698761 (2026) doi:10.64898/2026.01.10.698761.
145. Heesch, T. van, Bolhuis, P. G. & Vreede, J. Decoding dissociation of sequence-specific protein–DNA complexes with non-equilibrium simulations. *Nucleic Acids Res.* **51**, 12150–12160 (2023).
146. Eberhardt, J., Santos-Martins, D., Tillack, A. F. & Forli, S. AutoDock Vina 1.2.0: New docking methods, expanded force field, and python bindings. *J. Chem. Inf. Model.* **61**, 3891–3898 (2021).
147. Trott, O. & Olson, A. J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **31**, 455–461 (2010).
148. Fan, J., Fu, A. & Zhang, L. Progress in molecular docking. *Quant. Biol.* **7**, 83–89 (2019).

Bibliography

149. Pansar, T. & Poso, A. Binding affinity via docking: Fact and Fiction. *Mol. : A J. Synth. Chem. Nat. Prod. Chem.* **23**, 1899 (2018).
150. HU, L. A. & KING, S. C. Membrane topology of the *Escherichia coli* γ -aminobutyrate transporter: implications on the topography and mechanism of prokaryotic and eukaryotic transporters from the APC superfamily. *Biochem. J.* **336**, 69–76 (1998).
151. Mikros, E. & Diallinas, G. Tales of tails in transporters. *Open Biol.* **9**, 190083 (2019).
152. Setlow, P. & Christie, G. New thoughts on an old topic: secrets of bacterial spore resistance slowly being revealed. *Microbiol. Mol. Biol. Rev.* **87**, e00080-22 (2023).
153. Setlow, P. & Johnson, E. A. Food microbiology. 23–63 (2019) doi:10.1128/9781555819972.ch2.
154. Swarge, B. N. *et al.* "One-Pot" sample processing method for proteome-wide analysis of microbial cells and spores. *Proteom. Clin. Appl.* **12**, 1700169 (2018).
155. Rode, L. J. & Foster, J. W. Ionic and non-ionic compounds in the germination of spores of bacillus megaterium texas. *Arch. für Mikrobiol.* **43**, 201–212 (1962).
156. Rode, L. J. & Foster, J. W. Ionic germination of spores of bacillus megaterium QM B1551. *Arch. für Mikrobiol.* **43**, 183–200 (1962).
157. Christie, G. & Lowe, C. R. Role of Chromosomal and plasmid-borne receptor homologues in the response of *Bacillus megaterium* QM B1551 spores to germinants. *J. Bacteriol.* **189**, 4375–4383 (2007).
158. Korza, G., Goulet, M., DeMarco, A., Wicander, J. & Setlow, P. Role of *Bacillus subtilis* spore core water content and pH in the accumulation and utilization of spores' large 3-phosphoglyceric acid depot, and the crucial role of this depot in generating ATP early during spore germination. *Microorganisms* **11**, 195 (2023).
159. Vary, J. C. & Halvorson, H. O. Kinetics of germination of *Bacillus* spores. *J. Bacteriol.* **89**, 1340–1347 (1965).
160. Korza, G. *et al.* Expression of the *zduf* protein in wild-type *Bacillus subtilis* spores stabilizes inner membrane proteins and increases spore resistance to wet heat and hydrogen peroxide. *J. Appl. Microbiol.* **134**, lxado40 (2023).
161. Yu, B. *et al.* Identification and characterization of new proteins crucial for bacterial spore resistance and germination. *Front. Microbiol.* **14**, 1161604 (2023).
162. Khan, S. *et al.* Resistance and germination of spores of *Bacillus* species lacking members of a spore integral inner membrane protein family and locations of these proteins in spores. *J. Bacteriol.* **207**, e00217-25 (2025).
163. Clements, M. O. & Moir, A. Role of the *gerI* operon of *Bacillus cereus* 569 in the response of spores to germinants. *J. Bacteriol.* **180**, 6729–6735 (1998).
164. Ghosal, S., Leighton, T. J., Wheeler, K. E., Hutcheon, I. D. & Weber, P. K. spatially resolved characterization of water and ion incorporation in *Bacillus* spores. *Appl. Environ. Microbiol.* **76**, 3275–3282 (2010).
165. WARBURG, R. J., MOIR, A. & SMITH, D. A. influence of alkali metal cations on the germination of spores of wild-type and GerD mutants of *Bacillus subtilis*. *Microbiology* **131**, 221–230 (1985).

166. Ramírez-Guadiana, F. H., Meeske, A. J., Wang, X., Rodrigues, C. D. A. & Rudner, D. Z. The *Bacillus subtilis* germinant receptor GerA triggers premature germination in response to morphological defects during sporulation. *Mol. Microbiol.* **105**, 689–704 (2017).
167. Luo, Y. *et al.* Properties of spores of *Bacillus subtilis* with or without a transposon that decreases spore germination and increases spore wet heat resistance. *J. Appl. Microbiol.* **131**, 2918–2928 (2021).
168. Gao, Y. *et al.* SpoVAF and FigP assemble into oligomeric ion channels that enhance spore germination. *Genes Dev.* **38**, 31–45 (2024).
169. Ray, S. *et al.* High-resolution structures with bound Mn²⁺ and Cd²⁺ map the metal import pathway in an Nramp transporter. *eLife* **12**, (2023).
170. Koo, B.-M. *et al.* Construction and Analysis of Two Genome-Scale Deletion Libraries for *Bacillus subtilis*. *Cell Syst.* **4**, 291-305.e7 (2017).
171. Setlow, P. Observations on research with spores of *Bacillales* and *Clostridiales* species. *J. Appl. Microbiol.* **126**, 348–358 (2019).
172. Gupta, S. *et al.* Investigating the functional hierarchy of *Bacillus megaterium* PV361 Spore Germinant Receptors. *J. Bacteriol.* **195**, 3045–3053 (2013).
173. Ghosh, S. & Setlow, P. Isolation and characterization of super dormant spores of *Bacillus* species. *J. Bacteriol.* **191**, 1787–1797 (2009).
174. Igarashi, T., Setlow, B., Paidhungat, M. & Setlow, P. Effects of a gerF (lgt) mutation on the germination of spores of *Bacillus subtilis*. *J. Bacteriol.* **186**, 2984–2991 (2004).
175. Anteghini, M., Gualdi, F. & Oliva, B. How did we get there? AI applications to biological networks and sequences. *Comput. Biol. Med.* **190**, 110064 (2025).
176. Zhang, Z., Ou, C., Cho, Y., Akiyama, Y. & Ovchinnikov, S. Artificial intelligence methods for protein folding and design. *Curr. Opin. Struct. Biol.* **93**, 103066 (2025).
177. Jänes, J. & Beltrao, P. Deep learning for protein structure prediction and design—progress and applications. *Mol. Syst. Biol.* **20**, 162–169 (2024).
178. Chen, L. *et al.* AI-driven deep learning techniques in protein structure prediction. *Int. J. Mol. Sci.* **25**, 8426 (2024).
179. Karplus, M. & McCammon, J. A. Molecular dynamics simulations of biomolecules. *Nat. Struct. Biol.* **9**, 646–52 (2002).
180. Markowitz, F. All biology is computational biology. *PLoS Biol.* **15**, e2002050 (2017).
181. Kitano, H. Systems biology: A brief overview. *Science* **295**, 1662–1664 (2002).
182. Aloy, P. & Russell, R. B. Structural systems biology: modelling protein interactions. *Nat. Rev. Mol. Cell Biol.* **7**, 188–197 (2006).

Bibliography

183. You, H. S. Why Teach science with an interdisciplinary approach: history, trends, and conceptual frameworks. *J. Educ. Learn.* **6**, 66 (2017).
184. Attwood, T. K., Blackford, S., Brazas, M. D., Davies, A. & Schneider, M. V. A global perspective on evolving bioinformatics and data science training needs. *Brief. Bioinform.* **20**, 398–404 (2017).
185. Moir, A., Corfe, B. M. & Behravan, J. Spore germination. *Cell. Mol. Life Sci. CMLS* **59**, 403–409 (2002).
186. Krathwohl, D. R. A revision of Bloom's taxonomy: an overview. *Theory Pr.* **41**, 212–218 (2002).
187. Ludvigsson, J. F. Biggs' teaching for quality learning at university. *J. Pediatr. Gastroenterol. Nutr.* **36**, 513–514 (2003).
188. Murzyn, K., Róg, T. & Pasenkiewicz-Gierula, M. Phosphatidylethanolamine-phosphatidylglycerol bilayer as a model of the inner bacterial membrane. *Biophys. J.* **88**, 1091–1103 (2005).
189. Hunter, J. D. Matplotlib: A 2D graphics environment. *Comput. Sci. Eng.* **9**, 90–95 (2007).
190. Wang, Y. *et al.* Visualization of germination proteins in putative *Bacillus cereus* germinosomes. *Int. J. Mol. Sci.* **21**, 5198 (2020).
191. Hollingsworth, S. A. & Dror, R. O. Molecular dynamics simulation for all. *Neuron* **99**, 1129–1143 (2018).
192. Wuyun, Q. *et al.* Recent progress of protein tertiary structure prediction. *Molecules* **29**, 832 (2024).