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Development and application of cryo-EM tools to study the ultrastructure of microbes in changing environments

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REFERENCES

REFERENCES

1. Dubochet, J., Adrian, M., Chang, J., Homo, J., Lepault, J., McDowell, A. W., and Schultz, P. (1988) Cryo-electron microscopy of vitrified specimens. *Q. Rev. Biophys.* **21**, 129–228
2. Turk, M., and Baumeister, W. (2020) The promise and the challenges of cryo-electron tomography. *FEBS Lett.* **594**, 3243–3261
3. Yonekura, K., Braunfeld, M. B., Maki-Yonekura, S., and Agard, D. A. (2006) Electron energy filtering significantly improves amplitude contrast of frozen-hydrated protein at 300 kV. *J. Struct. Biol.* **156**, 524–536
4. Tivol, W. F., Briegel, A., and Jensen, G. J. (2008) An improved cryogen for plunge freezing. *Microsc. Microanal.* **14**, 375–379
5. Moor, H. (1987) Theory and Practice of High Pressure Freezing. *Cryotech. Biol. Electron Microsc.* 10.1007/978-3-642-72815-0_8
6. Al-Amoudi, A., Chang, J. J., Leforestier, A., McDowell, A., Salamin, L. M., Norlén, L. P. O., Richter, K., Blanc, N. S., Studer, D., and Dubochet, J. (2004) Cryo-electron microscopy of vitreous sections. *EMBO J.* **23**, 3583–3588
7. Chlanda, P., and Sachse, M. (2014) Cryo-electron microscopy of vitreous sections. *Methods Mol. Biol.* **1117**, 193–214
8. Schaffer, M., Pfeffer, S., Mahamid, J., Kleindiek, S., Laugks, T., Albert, S., Engel, B. D., Rummel, A., Smith, A. J., Baumeister, W., and Plitzko, J. M. (2019) A cryo-FIB lift-out technique enables molecular-resolution cryo-ET within native *Caenorhabditis elegans* tissue. *Nat. Methods.* **16**, 757–762
9. Kuba, J., Mitchels, J., Hovorka, M., Erdmann, P., Berka, L., Kirmse, R., König, J., De Bock, J., Goetze, B., and Rigort, A. (2021) Advanced cryo-tomography workflow developments – correlative microscopy, milling automation and cryo-lift-out. *J. Microsc.* **281**, 112–124
10. Wanner, A. A., Genoud, C., and Friedrich, R. W. (2016) 3-dimensional electron microscopic imaging of the zebrafish olfactory bulb and dense reconstruction of neurons. *Sci. Data.* **3**, 1–15
11. Tsang, T. K., Bushong, E. A., Boassa, D., Hu, J., Romoli, B., Phan, S., Dulcis, D., Su, C.-Y., and Ellisman, M. H. (2018) High-quality ultrastructural preservation using cryofixation for 3D electron microscopy of genetically labeled tissues. *Elife.* **7**, e35524
12. Pattison, D. I., and Davies, M. J. (2006) Actions of ultraviolet light on cellular structures. in *Cancer: Cell Structures, Carcinogens and Genomic Instability* (Bignold, L. P. ed), pp. 131–157, Birkhäuser Verlag/Switzerland, 10.1007/3-7643-7378-4_6
13. Albers, S. V., and Meyer, B. H. (2011) The archaeal cell envelope. *Nat. Rev. Microbiol.* **9**, 414–426
14. Skennerton, C. T., Haroon, M. F., Briegel, A., Shi, J., Jensen, G. J., Tyson, G. W., and Orphan, V. J. (2016) Phylogenomic analysis of Candidatus “Izimaplasma” species: Free-living representatives from a Tenericutes clade found in methane seeps. *ISME J.* **10**, 2679–2692
15. Gan, L., Chen, S., and Jensen, G. J. (2008) Molecular organization of Gram-negative peptidoglycan. *Proc. Natl. Acad. Sci.* **105**, 18953–18957
16. Bharat, T. A. M., Kureisaite-Ciziene, D., Hardy, G. G., Yu, E. W., Devant, J. M., Hagen, W. J. H., Brun, Y. V., Briggs, J. A. G., and Löwe, J. (2017) Structure of the hexagonal surface layer on *Caulobacter crescentus* cells. *Nat. Microbiol.* **2**, 1–6
17. Danilova, O. V., Suzina, N. E., Van De Kamp, J., Svenning, M. M., Bodrossy, L., and

- Dedysh, S. N. (2016) A new cell morphotype among methane oxidizers: A spiral-shaped obligately microaerophilic methanotroph from northern low-oxygen environments. *ISME J.* **10**, 2734–2743
18. Jiang, C., Brown, P. J. B., Ducret, A., and Brun, Y. V. (2014) Sequential evolution of bacterial morphology by co-option of a developmental regulator. *Nature*. **506**, 489–493
 19. Wanger, G., Onstott, T. C., and Southam, G. (2008) Stars of the terrestrial deep subsurface: A novel “star-shaped” bacterial morphotype from a South African platinum mine. *Geobiology*. **6**, 325–330
 20. Briegel, A., Ortega, D. R., Huang, A. N., Oikonomou, C. M., Gunsalus, R. P., and Jensen, G. J. (2015) Structural conservation of chemotaxis machinery across Archaea and Bacteria. *Environ. Microbiol. Rep.* **7**, 414–419
 21. Szwedziak, P., Wang, Q., Bharat, T. A. M., Tsim, M., and Löwe, J. (2014) Architecture of the ring formed by the tubulin homologue FtsZ in bacterial cell division. *Elife*. **3**, e04601
 22. Beeby, M., Ribardo, D. A., Brennan, C. A., Ruby, E. G., Jensen, G. J., and Hendrixson, D. R. (2016) Diverse high-torque bacterial flagellar motors assemble wider stator rings using a conserved protein scaffold. *Proc. Natl. Acad. Sci. U. S. A.* **113**, E2759
 23. Daum, B., Vonck, J., Bellack, A., Chaudhury, P., Reichelt, R., Albers, S. V., Rachel, R., and Kühlbrandt, W. (2017) Structure and in situ organisation of the pyrococcus furiosus archaeellum machinery. *Elife*. **6**, 1–21
 24. Briegel, A., and Jensen, G. (2017) Progress and Potential of Electron Cryotomography as Illustrated by Its Application to Bacterial Chemoreceptor Arrays. *Annu. Rev. Biophys.* **46**, 1–21
 25. Schrempf, H., Koebsch, I., Walter, S., Engelhardt, H., and Meschke, H. (2011) Extracellular Streptomyces vesicles: Amphorae for survival and defence. *Microb. Biotechnol.* **4**, 286–299
 26. Reichow, S. L., Korotkov, K. V., Hol, W. G. J. J., and Gonen, T. (2010) Structure of the cholera toxin secretion channel in its closed state. *Nat. Struct. Mol. Biol.* **17**, 1226–1232
 27. Nans, A., Kudryashev, M., Saibil, H. R., and Hayward, R. D. (2015) Structure of a bacterial type III secretion system in contact with a host membrane in situ. *Nat. Commun.* **6**, 10114
 28. Ghosal, D., Chang, Y.-W., Jeong, K. C., Vogel, J. P., and Jensen, G. J. (2016) Structure of the Legionella Dot/Icm type IV secretion system in situ by electron cryotomography. *EMBO Rep.* **10.1101/085977**
 29. Chang, Y.-W., Rettberg, L. A., and Jensen, G. J. (2017) In vivo structures of an intact type VI secretion system revealed by electron cryotomography. *EMBO Rep.* **10.1101/108233**
 30. Chang, Y.-W. W., Kjær, A., Ortega, D. R., Kovacicova, G., Sutherland, J. A., Rettberg, L. A., Taylor, R. K., and Jensen, G. J. (2017) Architecture of the Vibrio cholerae toxin-coregulated pilus machine revealed by electron cryotomography. *Nat. Microbiol.* **2**, 16269
 31. Perras, A. K., Daum, B., Ziegler, C., Takahashi, L. K., Ahmed, M., Wanner, G., Klingl, A., Leitinger, G., Kolb-Lenz, D., Gribaldo, S., Auerbach, A., Mora, M., Probst, A. J., Bellack, A., and Moissl-Eichinger, C. (2015) S-layers at second glance? Altiarchaeal grappling hooks (hami) resemble archaeal S-layer proteins in structure and

REFERENCES

- sequence. *Front. Microbiol.* 10.3389/fmicb.2015.00543
32. Adams, D. W., and Errington, J. (2009) Bacterial cell division: Assembly, maintenance and disassembly of the Z ring. *Nat. Rev. Microbiol.* **7**, 642–653
33. Caccamo, P. D., and Brun, Y. V. (2018) The Molecular Basis of Noncanonical Bacterial Morphology. *Trends Microbiol.* **26**, 191–208
34. Ghosal, D., Chang, Y., Jeong, K. C., Vogel, J. P., and Jensen, G. J. (2017) *In situ* structure of the *Legionella* Dot/Icm type IV secretion system by electron cryotomography. *EMBO Rep.* **18**, 726–732
35. Green, E. R., and Mecsas, J. (2016) Bacterial Secretion Systems: An Overview. *Microbiol. Spectr.* **4**, 1–19
36. Hospenthal, M. K., Costa, T. R. D., and Waksman, G. (2017) A comprehensive guide to pilus biogenesis in Gram-negative bacteria. *Nat. Rev. Microbiol.* **15**, 365–379
37. Kearns, D. B. (2010) A field guide to bacterial swarming motility. *Nat. Rev. Microbiol.* **8**, 634–644
38. Kim, J. H., Lee, J., Park, J., and Ghoo, Y. S. (2015) Gram-negative and Gram-positive bacterial extracellular vesicles. *Semin. Cell Dev. Biol.* **40**, 97–104
39. Press Release. (2017) The Nobel Prize in Chemistry 2017. NobelPrize.org. Nobel Media AB 2020. Available at: <https://www.nobelprize.org/prizes/chemistry/2017/press-release/> (26 Jan 2020)
40. Doerr, A. (2017) Cryo-electron tomography. *Nat. Methods.* **14**, 34
41. Stuart, D. I., Subramaniam, S., and Abrescia, N. G. A. (2016) The democratization of cryo-EM. *Nat. Methods.* **13**, 607–608
42. Alewijnse, B., Ashton, A. W., Chambers, M. G., Chen, S., Cheng, A., Ebrahim, M., Eng, E. T., Hagen, W. J. H., Koster, A. J., López, C. S., Lukyanova, N., Ortega, J., Renault, L., Reyntjens, S., Rice, W. J., Scapin, G., Schrijver, R., Siebert, A., Stagg, S. M., Grum-Tokars, V., Wright, E. R., Wu, S., Yu, Z., Zhou, Z. H., Carragher, B., and Potter, C. S. (2017) Best practices for managing large CryoEM facilities. *J. Struct. Biol.* **199**, 225–236
43. Dandey, V. P., Wei, H., Zhang, Z., Tan, Y. Z., Acharya, P., Eng, E. T., Rice, W. J., Kahn, P. A., Potter, C. S., and Carragher, B. (2018) Spotiton: New features and applications. *J. Struct. Biol.* **202**, 161–169
44. Rubinstein, J. L., Guo, H., Ripstein, Z. A., Haydaroglu, A., Au, A., Yip, C. M., Trani, J. M. Di, Benlekbi, S., and Kwok, T. (2019) Shake-it-off: A simple ultrasonic cryo-EM specimen preparation device. *bioRxiv.* 10.1101/632125
45. Comolli, L. R., Duarte, R., Baum, D., Luef, B., Downing, K. H., Larson, D. M., Csencsits, R., and Banfield, J. F. (2012) A portable cryo-plunger for on-site intact cryogenic microscopy sample preparation in natural environments. *Microsc. Res. Tech.* **75**, 829–836
46. Chen, S., Beeby, M., Murphy, G. E., Leadbetter, J. R., Hendrixson, D. R., Briegel, A., Li, Z., Shi, J., Tocheva, E. I., Müller, A., Dobro, M. J., and Jensen, G. J. (2011) Structural diversity of bacterial flagellar motors. *EMBO J.* **30**, 2972–2981
47. Briegel, A., Ladinsky, M. S., Oikonomou, C., Jones, C. W., Harris, M. J., Fowler, D. J., Chang, Y. W., Thompson, L. K., Armitage, J. P., and Jensen, G. J. (2014) Structure of bacterial cytoplasmic chemoreceptor arrays and implications for chemotactic signaling. *Elife.* **2014**, 1–16
48. Wang, J., Brackmann, M., Castaño-Díez, D., Kudryashev, M., Goldie, K. N., Maier, T., Stahlberg, H., and Basler, M. (2017) Cryo-EM structure of the extended type VI secretion system sheath-tube complex. *Nat. Microbiol.* 10.1038/s41564-017-

- 0020-7
49. Hand, E. (2020) 'We need a people's cryo-EM.' Scientists hope to bring revolutionary microscope to the masses. *Science* (80-.). 10.1126/science.aba9954
 50. Ali, M., Nelson, A. R., Lopez, A. L., and Sack, D. A. (2015) Updated global burden of cholera in endemic countries. *PLoS Negl. Trop. Dis.* 10.1371/journal.pntd.0003832
 51. Halpern, M., and Izhaki, I. (2017) Fish as hosts of *Vibrio cholerae*. *Front. Microbiol.* 10.3389/fmicb.2017.00282
 52. Laviad -Shitrit, S., Lev-Ari, T., Katzir, G., Sharaby, Y., Izhaki, I., and Halpern, M. (2017) Great cormorants (*Phalacrocorax carbo*) as potential vectors for the dispersal of *Vibrio cholerae*. *Sci. Rep.* 7, 7973
 53. Butler, S. M., and Camilli, A. (2005) Going against the grain: chemotaxis and infection in *Vibrio cholerae*. *Nat. Rev. Microbiol.* 3, 611–620
 54. Krebs, S. J., and Taylor, R. K. (2011) Protection and attachment of *Vibrio cholerae* mediated by the toxin-coregulated pilus in the infant mouse model. *J. Bacteriol.* 193, 5260–5270
 55. Utada, A. S., Bennett, R. R., Fong, J. C. N., Gibiansky, M. L., Yildiz, F. H., Golestanian, R., and Wong, G. C. L. (2014) *Vibrio cholerae* use pili and flagella synergistically to effect motility switching and conditional surface attachment. *Nat. Commun.* 5, 1–8
 56. Tacket, C. O., Taylor, R. K., Losonsky, G., Lim, Y. U., Nataro, J. P., Kaper, J. B., and Levine, M. M. (1998) Investigation of the roles of toxin-coregulated pili and mannose- sensitive hemagglutinin pili in the pathogenesis of *Vibrio cholerae* O139 infection. *Infect. Immun.* 66, 692–695
 57. Conner, J. G., Teschler, J. K., Jones, C. J., and Yildiz, F. H. (2016) Staying Alive: *Vibrio cholerae*'s Cycle of Environmental Survival, Transmission, and Dissemination. *Microbiol. Spectr.* 4, VMBF-0015-2015
 58. Peterson, K. M., and Gellings, P. S. (2018) Multiple intrainestinal signals coordinate the regulation of *Vibrio cholerae* virulence determinants. *Pathog. Dis.* 10.1093/femspd/ftx126
 59. Nelson, E. J., Chowdhury, A., Flynn, J., Schild, S., Bourassa, L., Shao, Y., Larocque, R. C., Calderwood, S. B., Qadri, F., and Camilli, A. (2008) Transmission of *Vibrio cholerae* is antagonized by lytic phage and entry into the aquatic environment. *PLoS Pathog.* 4, e1000187
 60. Almagro-Moreno, S., Pruss, K., and Taylor, R. K. (2015) Intestinal Colonization Dynamics of *Vibrio cholerae*. *PLoS Pathog.* 11, 1–11
 61. Merrell, D. S., Butler, S. M., Qadri, F., Dolganov, N. A., Alam, A., Cohen, M. B., Calderwood, S. B., Schoolnik, G. K., and Camilli, A. (2002) Host-induced epidemic spread of the cholera bacterium. *Nature.* 417, 642–645
 62. Bachmann, V., Kostiuik, B., Unterweger, D., Diaz-Satizabal, L., Ogg, S., and Pukatzki, S. (2015) Bile salts modulate the mucin-activated type vi secretion system of pandemic *vibrio cholerae*. *PLoS Negl. Trop. Dis.* 9, 1–22
 63. Bartlett, T. M., Bratton, B. P., Duvshani, A., Miguel, A., Sheng, Y., Martin, N. R., Nguyen, J. P., Persat, A., Desmarais, S. M., VanNieuwenhze, M. S., Huang, K. C., Zhu, J., Shaevitz, J. W., and Gitai, Z. (2017) A Periplasmic Polymer Curves *Vibrio cholerae* and Promotes Pathogenesis. *Cell.* 168, 172–185
 64. Brenzinger, S., Van Der Aart, L. T., Van Wezel, G. P., Lacroix, J. M., Glatter, T., and Briegel, A. (2019) Structural and proteomic changes in viable but non-culturable

REFERENCES

- vibrio cholerae. *Front. Microbiol.* **10**, 1–15
65. Shi, H., Westfall, C. S., Kao, J., Odermatt, P. D., Anderson, S. E., Cesar, S., Sievert, M., Moore, J., Gonzalez, C. G., Zhang, L., Elias, J. E., Chang, F., Huang, K. C., and Levin, P. A. (2021) Starvation induces shrinkage of the bacterial cytoplasm. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.2104686118
66. Runft, D. L., Mitchell, K. C., Abuaita, B. H., Allen, J. P., Bajer, S., Ginsburg, K., Neely, M. N., and Withey, J. H. (2014) Zebrafish as a natural host model for *Vibrio cholerae* colonization and transmission. *Appl. Environ. Microbiol.* **80**, 1710–1717
67. Murdoch, C. C., and Rawls, J. F. (2019) Commensal Microbiota Regulate Vertebrate Innate Immunity-Insights From the Zebrafish. *Front. Immunol.* **10**, 1–14
68. Millet, Y. A., Alvarez, D., Ringgaard, S., von Andrian, U. H., Davis, B. M., and Waldor, M. K. (2014) Insights into *Vibrio cholerae* Intestinal Colonization from Monitoring Fluorescently Labeled Bacteria. *PLoS Pathog.* 10.1371/journal.ppat.1004405
69. Pham, L. N., Kanther, M., Semova, I., and Rawls, J. F. (2008) Methods for generating and colonizing gnotobiotic zebrafish. *Nat. Protoc.* **3**, 1862–1875
70. Depelteau, J. S., Koning, G., Yang, W., and Briegel, A. (2020) An Economical, Portable Manual Cryogenic Plunge Freezer for the Preparation of Vitrified Biological Samples for Cryogenic Electron Microscopy. *Microsc. Microanal.* **26**, 413–418
71. Zheng, S. Q., Keszthelyi, B., Branlund, E., Lyle, J. M., Braunfeld, M. B., Sedat, J. W., and Agard, D. A. (2007) UCSF tomography: An integrated software suite for real-time electron microscopic tomographic data collection, alignment, and reconstruction. *J. Struct. Biol.* **157**, 138–147
72. Mastronarde, D. N. (2005) Automated electron microscope tomography using robust prediction of specimen movements. *J. Struct. Biol.* **152**, 36–51
73. Kremer, J. R., Mastronarde, D. N., and McIntosh, J. R. (1996) Computer visualization of three-dimensional image data using IMOD. *J. Struct. Biol.* **116**, 71–76
74. Mastronarde, D. N., and Held, S. R. (2017) Automated tilt series alignment and tomographic reconstruction in IMOD. *J. Struct. Biol.* **197**, 102–113
75. Oikonomou, C. M., and Jensen, G. J. (2016) A new view into prokaryotic cell biology from electron cryotomography. *Nat. Rev. Microbiol.* **14**, 205–220
76. Briegel, A., Ortega, D. R., Tocheva, E. I., Wuichet, K., Li, Z., Chen, S., Muller, A., Iancu, C. V., Murphy, G. E., Dobro, M. J., Zhulin, I. B., and Jensen, G. J. (2009) Universal architecture of bacterial chemoreceptor arrays. *Proc Natl Acad Sci U S A.* **106**, 17181–17186
77. Rapisarda, C., Cherrak, Y., Kooger, R., Schmidt, V., Pellarin, R., Logger, L., Cascales, E., Pilhofer, M., Durand, E., and Fronzes, R. (2019) *In situ* and high-resolution cryo-EM structure of a bacterial type VI secretion system membrane complex. *EMBO J.* 10.15252/embj.2018100886
78. Dobro, M. J., Oikonomou, C. M., Piper, A., Cohen, J., Guo, K., Jensen, T., Tadayon, J., Donermeyer, J., Park, Y., Solis, B. A., Kjær, A., Jewett, A. I., McDowall, A. W., Chen, S., Chang, Y. W., Shi, J., Subramanian, P., Iancu, C. V., Li, Z., Briegel, A., Tocheva, E. I., Pilhofer, M., and Jensen, G. J. (2017) Uncharacterized bacterial structures revealed by electron cryotomography. *J. Bacteriol.* **199**, 1–14
79. Ferreira, J. L., Gao, F. Z., Rossmann, F. M., Nans, A., Brenzinger, S., Hosseini, R.,

- Wilson, A., Briegel, A., Thormann, M., Rosenthal, P. B., and Beeby, M. (2019) γ - proteobacteria eject their polar flagella under nutrient depletion , retaining flagellar motor relic structures. *PLoS Biol.* **17**, e3000165
80. Alam, A., LaRocque, R. C., Harris, J. B., Vanderspurt, C., Ryan, E. T., Qadri, F., and Calderwood, S. B. (2005) Hyperinfectivity of human-passaged *Vibrio cholerae* can be modeled by growth in the infant mouse. *Infect. Immun.* **73**, 6674–6679
 81. Tamayo, R., Patimalla, B., and Camilli, A. (2010) Growth in a biofilm induces a hyperinfectious phenotype in *Vibrio cholerae*. *Infect. Immun.* **78**, 3560–3569
 82. Bourassa, L., and Camilli, A. (2009) Glycogen contributes to the environmental persistence and transmission of *Vibrio cholerae*. *Mol. Microbiol.* **72**, 124–138
 83. von Kruger, W. M. A., Lery, L. M. S., Soares, M. R., de Neves-Manta, F. S., Batista e Silva, C. M., Neves-Ferreira, A. G. da C., Perales, J., and Bisch, P. M. (2006) The phosphate-starvation response in *Vibrio cholerae* O1 and *phoB* mutant under proteomic analysis : Disclosing functions involved in adaptation , survival and virulence. *Protein Sci.* **6**, 1495–1511
 84. Harapin, J., Börmel, M., Sapra, K. T., Brunner, D., Kaeck, A., and Medalia, O. (2015) Structural analysis of multicellular organisms with cryo-electron tomography. *Nat. Methods.* **12**, 634–636
 85. Medeiros, J. M., Böck, D., and Pilhofer, M. (2018) Imaging bacteria inside their host by cryo-focused ion beam milling and electron cryotomography. *Curr. Opin. Microbiol.* **43**, 62–68
 86. Juraszek, J., Rutten, L., Blokland, S., Bouchier, P., Voorzaat, R., Ritschel, T., Bakkers, M. J. G., Renault, L. L. R., and Langedijk, J. P. M. (2021) Stabilizing the closed SARS-CoV-2 spike trimer. *Nat. Commun.* **12**, 1–8
 87. Ma, J., Su, D., Sun, Y., Huang, X., Liang, Y., Fang, L., Ma, Y., Li, W., Liang, P., and Zheng, S. (2021) Cryo-EM structure of S-Trimer, a subunit vaccine candidate for COVID-19. *J. Virol.* 10.1128/jvi.00194-21
 88. Wolff, G., Limpens, R. W. A. L., Zevenhoven-Dobbe, J. C., Laugks, U., Zheng, S., de Jong, A. W. M., Koning, R. I., Agard, D. A., Grünewald, K., Koster, A. J., Snijder, E. J., and Bárcena, M. (2020) A molecular pore spans the double membrane of the coronavirus replication organelle. *Science (80-)*. **369**, 1395–1398
 89. Yao, H., Song, Y., Chen, Y., Wu, N., Xu, J., Sun, C., Zhang, J., Weng, T., Zhang, Z., Wu, Z., Cheng, L., Shi, D., Lu, X., Lei, J., Crispin, M., Shi, Y., Li, L., and Li, S. (2020) Molecular Architecture of the SARS-CoV-2 Virus. *Cell.* **183**, 730-738.e13
 90. Subramaniam, S. (2020) COVID-19 and cryo-EM. *IUCrJ.* **7**, 575–576
 91. Jin, Q., Vogt, S., Lai, B., Chen, S., Finney, L., Gleber, S. C., Ward, J., Deng, J., Mak, R., Moonier, N., and Jacobsen, C. (2015) Ultraviolet germicidal irradiation and its effects on elemental distributions in mouse embryonic fibroblast cells in x-ray fluorescence microanalysis. *PLoS One.* **10**, 1–14
 92. Welch, D., Buonanno, M., Grilj, V., Shuryak, I., Crickmore, C., Bigelow, A. W., Randers-Pehrson, G., Johnson, G. W., and Brenner, D. J. (2018) Far-UVC light: A new tool to control the spread of airborne-mediated microbial diseases. *Sci. Rep.* **8**, 1–7
 93. Boyce, J. M., and Donskey, C. J. (2019) Understanding ultraviolet light surface decontamination in hospital rooms: A primer. *Infect. Control Hosp. Epidemiol.* **40**, 1030–1035
 94. Britt, A. B. (1996) DNA damage and repair in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 75–100

REFERENCES

95. Durchschlag, H., Fochler, C., Feser, B., Hausmann, S., Seroneit, T., Swientek, M., Swoboda, E., Winklmaier, A., Wlček, C., and Zipper, P. (1996) Effects of X-and UV-irradiation on proteins. *Radiat. Phys. Chem.* **47**, 501–505
96. Krisko, A., and Radman, M. (2010) Protein damage and death by radiation in *Escherichia coli* and *Deinococcus radiodurans*. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 14373–14377
97. Silva-Valenzuela, C. A., and Camilli, A. (2019) Niche adaptation limits bacteriophage predation of *Vibrio cholerae* in a nutrient-poor aquatic environment. *Proc. Natl. Acad. Sci.* **116**, 1627–1632
98. Hagen, W. J. H., Wan, W., and Briggs, J. A. G. (2017) Implementation of a cryo-electron tomography tilt-scheme optimized for high resolution subtomogram averaging. *J. Struct. Biol.* **197**, 191–198
99. Diebolder, C. A., Dillard, R. S., and Renault, L. L. R. (2021) From tube to structure: SPA cryo-EM workflow using apoferritin as an example. *Methods Mol. Biol.* **2305**, 229–256
100. Xiong, Q., Morpew, M. K., Schwartz, C. L., Hoenger, A. H., and Mastronarde, D. N. (2009) CTF determination and correction for low dose tomographic tilt series. *J. Struct. Biol.* **168**, 378–387
101. Castaño-Díez, D., Kudryashev, M., and Stahlberg, H. (2017) Dynamo Catalogue: Geometrical tools and data management for particle picking in subtomogram averaging of cryo-electron tomograms. *J. Struct. Biol.* **197**, 135–144
102. Castaño-Díez, D., Kudryashev, M., Arheit, M., and Stahlberg, H. (2012) Dynamo: A flexible, user-friendly development tool for subtomogram averaging of cryo-EM data in high-performance computing environments. *J. Struct. Biol.* **178**, 139–151
103. Kucukelbir, A., Sigworth, F. J., and Tagare, H. D. (2014) Quantifying the local resolution of cryo-EM density maps. *Nat. Methods.* **11**, 63–65
104. Scheres, S. H. W. (2012) A bayesian view on cryo-EM structure determination. *J. Mol. Biol.* **415**, 406–418
105. Zhang, K. (2016) Gctf: Real-time CTF determination and correction. *J. Struct. Biol.* **193**, 1–12
106. Zheng, S. Q., Palovcak, E., Armache, J. P., Verba, K. A., Cheng, Y., and Agard, D. A. (2017) MotionCor2: Anisotropic correction of beam-induced motion for improved cryo-electron microscopy. *Nat. Methods.* **14**, 331–332
107. Chen, Z., Sun, L., Zhang, Z., Fokine, A., Padilla-Sanchez, V., Hanein, D., Jiang, W., Rossmann, M. G., and Rao, V. B. (2017) Cryo-EM structure of the bacteriophage T4 isometric head at 3.3-Å resolution and its relevance to the assembly of icosahedral viruses. *Proc. Natl. Acad. Sci. U. S. A.* **114**, E8184–E8193
108. Zivanov, J., Nakane, T., Forsberg, B. O., Kimanius, D., Hagen, W. J. H., Lindahl, E., and Scheres, S. H. W. (2018) New tools for automated high-resolution cryo-EM structure determination in RELION-3. *Elife.* **7**, 1–22
109. Rohou, A., and Grigorieff, N. (2015) CTFFIND4: Fast and accurate defocus estimation from electron micrographs. *J. Struct. Biol.* **192**, 216–221
110. Zivanov, J., Nakane, T., and Scheres, S. H. W. (2019) A Bayesian approach to beam-induced motion correction in cryo-EM single-particle analysis. *IUCrJ.* **6**, 5–17
111. Zivanov, J., Nakane, T., and Scheres, S. H. W. (2020) Estimation of high-order aberrations and anisotropic magnification from cryo-EM data sets in RELION-3.1. *IUCrJ.* **7**, 253–267
112. Goddard, T. D., Huang, C. C., Meng, E. C., Pettersen, E. F., Couch, G. S., Morris, J.

- H., and Ferrin, T. E. (2018) UCSF ChimeraX: Meeting modern challenges in visualization and analysis. *Protein Sci.* **27**, 14–25
113. Barth, Z. K., Netter, Z., Angermeyer, A., Bhardwaj, P., and Seed, K. D. (2020) A Family of Viral Satellites Manipulates Invading Virus Gene Expression and Can Affect Cholera Toxin Mobilization. *mSystems*. **5**, 1–18
 114. Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S. A. A., Ballard, A. J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., Back, T., Petersen, S., Reiman, D., Clancy, E., Zielinski, M., Steinegger, M., Pacholska, M., Berghammer, T., Bodenstein, S., Silver, D., Vinyals, O., Senior, A. W., Kavukcuoglu, K., Kohli, P., and Hassabis, D. (2021) Highly accurate protein structure prediction with AlphaFold. *Nature*. 10.1038/s41586-021-03819-2
 115. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., and Ferrin, T. E. (2004) UCSF Chimera - A visualization system for exploratory research and analysis. *J. Comput. Chem.* **25**, 1605–1612
 116. Singharoy, A., Teo, I., McGreevy, R., Stone, J. E., Zhao, J., and Schulten, K. (2016) Molecular dynamics-based refinement and validation for sub-5 Å cryo-electron microscopy maps. *Elife*. **5**, 1–33
 117. Phillips, J. C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R. D., Kalé, L., and Schulten, K. (2005) Scalable molecular dynamics with NAMD. *J. Comput. Chem.* **26**, 1781–1802
 118. Humphrey, W., Dalke, A., and Schulten, K. (1996) VMD: Visual Molecular Dynamics. *J. Mol. Graph.* **14**, 33–38
 119. Caspar, D. L., and Klug, A. (1962) Physical Principles in the Construction of Regular Viruses. *Cold Spring Harb Symp Quant Biol.* **27**, 1–24
 120. Ferraro, G., Ciambellotti, S., Messori, L., and Merlino, A. (2017) Cisplatin Binding Sites in Human H-Chain Ferritin. *Inorg. Chem.* **56**, 9064–9070
 121. Buonanno, M., Welch, D., Shuryak, I., and Brenner, D. J. (2020) Far-UVC light (222 nm) efficiently and safely inactivates airborne human coronaviruses. *Sci. Rep.* **10**, 1–8
 122. IES Photobiology Committee (2020) *IES Committee Report : Germicidal Ultraviolet (GUV) – Frequently Asked Questions*
 123. Berger, C., Ravelli, R. B. G., López-Iglesias, C., Kudryashev, M., Diepold, A., and Peters, P. J. (2021) Structure of the Yersinia injectisome in intracellular host cell phagosomes revealed by cryo FIB electron tomography. *J. Struct. Biol.* 10.1016/j.jsb.2021.107701
 124. Yang, W., Keith Cassidy, C., Ames, P., Diebolder, C. A., Schulten, K., Luthey-Schulten, Z., Parkinson, J. S., and Briegel, A. (2019) In situ conformational changes of the escherichia coli serine chemoreceptor in different signaling states. *MBio*. 10.1128/mBio.00973-19
 125. Muok, A. R., Ortega, D. R., Kurniyati, K., Yang, W., Maschmann, Z. A., Sidi Mabrouk, A., Li, C., Crane, B. R., and Briegel, A. (2020) Atypical chemoreceptor arrays accommodate high membrane curvature. *Nat. Commun.* **11**, 1–13
 126. Arnold, J., Mahamid, J., Lucic, V., De Marco, A., Fernandez, J. J., Laugks, T., Mayer, T., Hyman, A. A., Baumeister, W., and Plitzko, J. M. (2016) Site-Specific Cryo-focused Ion Beam Sample Preparation Guided by 3D Correlative Microscopy. *Biophys. J.* **110**, 860–869
 127. Bokhari, H., Ali, A., Noreen, Z., Thomson, N., and Wren, B. W. (2017) Galleria

REFERENCES

- mellonella is low cost and suitable surrogate host for studying virulence of human pathogenic *Vibrio cholerae*. *Gene*. **628**, 1–7
128. Schaffer, M., Mahamid, J., Engel, B. D., Laugks, T., Baumeister, W., and Plitzko, J. M. (2017) Optimized cryo-focused ion beam sample preparation aimed at in situ structural studies of membrane proteins. *J. Struct. Biol.* **197**, 73–82
129. Sartori, N., Richter, K., and Dubochet, J. (1993) Vitrification depth can be increased more than 10-fold by high-pressure freezing. *J. Microsc.* **172**, 55–61
130. Henderson, R. (1995) The Potential and Limitations of Neutrons, Electrons and X-Rays for Atomic Resolution Microscopy of Unstained Biological Molecules. *Q. Rev. Biophys.* **28**, 171–193
131. Eltsov, M., Grewe, D., Lemercier, N., Frangakis, A., Livolant, F., and Leforestier, A. (2018) Nucleosome conformational variability in solution and in interphase nuclei evidenced by cryo-electron microscopy of vitreous sections. *Nucleic Acids Res.* **46**, 9189–9200
132. Bharat, T. A. M., Hoffmann, P. C., and Kukulski, W. (2018) Correlative Microscopy of Vitreous Sections Provides Insights into BAR-Domain Organization In Situ. *Structure*. **26**, 879-886.e3
133. Couture-Tosi, E., Ranck, J.-L., Haustant, G., Pehau-Arnaudet, G., and Sachse, M. (2010) CEMOVIS on a pathogen: analysis of *Bacillus anthracis* spores. *Biol. Cell.* **102**, 609–619
134. Mielanczyk, L., Matysiak, N., Michalski, M., Buldak, R., and Wojnicz, R. (2014) Closer to the native state. Critical evaluation of cryo-techniques for Transmission Electron Microscopy: Preparation of biological samples. *Folia Histochem. Cytobiol.* **52**, 1–17
135. Melia, C. E., Bolla, J. R., Katharios-Lanwermyer, S., Mihaylov, D. B., Hoffmann, P. C., Huo, J., Wozny, M. R., Elfari, L. M., Böhning, J., Owens, R. J., Robinson, C. V., O'Toole, G. A., and Bharat, T. A. M. (2021) Architecture of cell-cell junctions in situ reveals a mechanism for bacterial biofilm inhibition. *Proc. Natl. Acad. Sci.* **118**, 2021.02.08.430230
136. Hohenberg, H., Tobler, M., and Müller, M. (1996) High-pressure freezing of tissue obtained by fine-needle biopsy. *J. Microsc.* **183**, 133–139
137. Hohenberg, H., Mannweiler, K., and Muller, M. (1994) High-pressure freezing of cell suspensions in cellulose capillary tubes. *J. Microsc.* **175**, 34–43
138. Nyholm, S. V., and McFall-Ngai, M. J. (2021) A lasting symbiosis: how the Hawaiian bobtail squid finds and keeps its bioluminescent bacterial partner. *Nat. Rev. Microbiol.* 10.1038/s41579-021-00567-y
139. Sarkar, P., Bosneaga, E., Yap, E. G., Das, J., Tsai, W. T., Cabal, A., Neuhaus, E., Maji, D., Kumar, S., Joo, M., Yakovlev, S., Csencsits, R., Yu, Z., Bajaj, C., Downing, K. H., and Auer, M. (2014) Electron tomography of cryo-immobilized plant tissue: A novel approach to studying 3d macromolecular architecture of mature plant cell walls in situ. *PLoS One*. **9**, e106928
140. Meijer, A. H., and Spaink, H. P. (2011) Host-pathogen interactions made transparent with the zebrafish model. *Curr. Drug Targets.* **12**, 1000–17
141. Gitai, Z. (2005) The new bacterial cell biology: Moving parts and subcellular architecture. *Cell*. **120**, 577–586
142. Klose, K. E. (2000) The suckling mouse model of cholera. *Trends Microbiol.* **8**, 189–191
143. Lutz, C., Erken, M., Noorian, P., Sun, S., and McDougald, D. (2013) Environmental

- reservoirs and mechanisms of persistence of *Vibrio cholerae*. *Front. Microbiol.* **4**, 1–15
144. Singh, A., and Barnard, T. G. (2016) Surviving the acid barrier: responses of pathogenic *Vibrio cholerae* to simulated gastric fluid. *Appl. Microbiol. Biotechnol.* **100**, 815–824
 145. Yang, J. H., Wu, U. I., Tai, H. M., and Sheng, W. H. (2019) Effectiveness of an ultraviolet-C disinfection system for reduction of healthcare-associated pathogens. *J. Microbiol. Immunol. Infect.* **52**, 487–493
 146. Weiss, G. L., Kieninger, A.-K., Maldener, I., Forchhammer, K., and Pilhofer, M. (2019) Structure and function of a bacterial gap junction analog. *Cell.* **178**, 374–384
 147. Rigort, A., and Plitzko, J. M. (2015) Cryo-focused-ion-beam applications in structural biology. *Arch. Biochem. Biophys.* **581**, 122–130
 148. Romero-Brey, I. (2018) 3D Electron Microscopy (EM) and Correlative Light Electron Microscopy (CLEM) Methods to Study Virus-Host Interactions. in *Influenza Virus: Methods and Protocols* (Yamauchi, Y. ed), pp. 213–236, Springer Nature, **1836**, 213–236
 149. Cyrklaff, M., Frischknecht, F., and Kudryashev, M. (2017) Functional insights into pathogen biology from 3D electron microscopy. *FEMS Microbiol. Rev.* **41**, 1–26
 150. Cho, I., and Blaser, M. J. (2012) The human microbiome: At the interface of health and disease. *Nat. Rev. Genet.* **13**, 260–270
 151. Cho, J. Y., Liu, R., Macbeth, J. C., and Hsiao, A. (2021) The Interface of *Vibrio cholerae* and the Gut Microbiome. *Gut Microbes.* 10.1080/19490976.2021.1937015
 152. Ligthart, K., Belzer, C., de Vos, W. M., Tytgat, H. L. P. P., Vos, W. M. De, and Tytgat, H. L. P. P. (2020) Bridging Bacteria and the Gut: Functional Aspects of Type IV Pili. *Trends Microbiol.* **28**, 340–348
 153. Sokol, N. S., and Newton, I. L. G. (2020) The Microbiome Sets the Stage for Cholera. *Trends Microbiol.* **28**, 430–432
 154. Rubinsky, B., Perez, P. A., and Carlson, M. E. (2005) The thermodynamic principles of isochoric cryopreservation. *Cryobiology.* **50**, 121–138
 155. Fuest, M., Schaffer, M., Nocera, G. M., Galilea-Kleinsteuber, R. I., Messling, J. E., Heymann, M., Plitzko, J. M., and Burg, T. P. (2019) In situ Microfluidic Cryofixation for Cryo Focused Ion Beam Milling and Cryo Electron Tomography. *Sci. Rep.* **9**, 1–10
 156. Sergey, G., Denis, K., Ava, H., Gediminas, G., Viola, O., Owen L., K., Ruby H.P., L., Moira O', B., Roger, P., James C., W., and Alex, de M. (2018) Oxygen plasma focused ion beam scanning electron microscopy for biological samples. *bioRxiv.* 10.1101/457820
 157. Gorelick, S., Buckley, G., Gervinskas, G., Johnson, T. K., Handley, A., Caggiano, M. P., Whisstock, J. C., Pocock, R., and de Marco, A. (2019) PIE-scope, integrated cryo-correlative light and FIB/SEM microscopy. *Elife.* **8**, 1–15
 158. Wilfling, F., Lee, C. W., Erdmann, P. S., Zheng, Y., Sherpa, D., Jentsch, S., Pfander, B., Schulman, B. A., and Baumeister, W. (2020) A Selective Autophagy Pathway for Phase-Separated Endocytic Protein Deposits. *Mol. Cell.* **80**, 764–778.e7