Using cryo-EM methods to uncover structure and function of bacteriophages

Ouyang, R.

Citation

Ouyang, R. (2023, December 5). *Using cryo-EM methods to uncover structure and function of bacteriophages*. Retrieved from https://hdl.handle.net/1887/3666064

Version:	Publisher's Version
License:	[Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden]()
Downloaded from:	https://hdl.handle.net/1887/3666064

Note: To cite this publication please use the final published version (if applicable).

Publications

Ouyang, R., Costa, A. R., Cassidy, C. K., Otwinowska, A., Williams, V. C., Latka, A., ... & Briegel, A. (2022). High-resolution reconstruction of a Jumbo-bacteriophage infecting capsulated bacteria using hyperbranched tail fibers. Nature Communications, 13(1), 7241; doi: https://doi.org/10.1038/s41467-022-34972-5

References

1 Williams, D. B., Carter, C. B., Williams, D. B. & Carter, C. B. *The transmission electron microscope*. (Springer, 1996).

2 Murata, K. & Wolf, M. Cryo-electron microscopy for structural analysis of dynamic biological macromolecules. *Biochimica et Biophysica Acta (BBA)-General Subjects* **1862**, 324-334 (2018).

3 Turk, M. & Baumeister, W. The promise and the challenges of cryo-electron tomography. *FEBS letters* **594**, 3243-3261 (2020).

4 Kaplan, M. *et al.* in *CryoEM* 83-111 (Springer, 2021).

5 Zhang, P. Advances in cryo-electron tomography and subtomogram averaging and classification. *Current opinion in structural biology* **58**, 249-258 (2019).

6 Mahamid, J. *et al.* Visualizing the molecular sociology at the HeLa cell nuclear periphery. *Science* **351**, 969-972 (2016).

7 Januliene, D. & Moeller, A. Single-particle Cryo-EM of membrane proteins. *Structure and Function of Membrane Proteins*, 153-178 (2021).

8 Nygaard, R., Kim, J. & Mancia, F. Cryo-electron microscopy analysis of small membrane proteins. *Current opinion in structural biology* **64**, 26-33 (2020).

9 Thangaratnarajah, C., Rheinberger, J. & Paulino, C. Cryo-EM studies of membrane proteins at 200 keV. *Current Opinion in Structural Biology* **76**, 102440 (2022).

10 Hiraizumi, M., Yamashita, K., Nishizawa, T. & Nureki, O. Cryo-EM structures capture the transport cycle of the P4-ATPase flippase. *Science* **365**, 1149-1155 (2019).

11 Lubbe, L., Sewell, B. T., Woodward, J. D. & Sturrock, E. D. Cryo-EM reveals mechanisms of angiotensin I-converting enzyme allostery and dimerization. *The EMBO Journal* **41**, e110550 (2022).

12 Mazhab-Jafari, M. T. & Rubinstein, J. L. Cryo-EM studies of the structure and dynamics of vacuolar-type ATPases. *Science Advances* **2**, e1600725 (2016).

13 López-Alonso, J. P. *et al.* CryoEM structural exploration of catalytically active enzyme pyruvate carboxylase. *nature communications* **13**, 6185 (2022).

14 Zhang, K. *et al.* Cryo-EM, protein engineering, and simulation enable the development of peptide therapeutics against acute myeloid leukemia. *ACS Central Science* **8**, 214-222 (2022).

15 Murphy, E. L. *et al.* Cryo-electron microscopy structure of the 70S ribosome from Enterococcus faecalis. *Scientific Reports* **10**, 16301 (2020).

16 Qin, B. *et al.* Cryo-EM captures early ribosome assembly in action. *Nature Communications* **14**, 898 (2023).

17 Fica, S. M. & Nagai, K. Cryo-electron microscopy snapshots of the spliceosome: structural insights into a dynamic ribonucleoprotein machine. *Nature structural & molecular biology* **24**, 791-799 (2017).

18 Fica, S. M. Cryo-EM snapshots of the human spliceosome reveal structural adaptions for splicing regulation. *Current Opinion in Structural Biology* **65**, 139-148 (2020).

19 Fontana, P. *et al.* Structure of cytoplasmic ring of nuclear pore complex by integrative cryo-EM and AlphaFold. *Science* **376**, eabm9326 (2022).

20 Maimon, T., Elad, N., Dahan, I. & Medalia, O. The human nuclear pore complex as revealed by cryo-electron tomography. *Structure* **20**, 998-1006 (2012).

21 Huang, G. *et al.* Cryo-EM structure of the nuclear ring from Xenopus laevis nuclear pore complex. *Cell Research* **32**, 349-358 (2022).

22 Tai, L., Yin, G., Sun, F. & Zhu, Y. Cryo-electron microscopy reveals the structure of the nuclear pore complex. *Journal of Molecular Biology*, 168051 (2023).

23 Harrison, J. J. E. *et al.* Cryo-EM structure of the HIV-1 Pol polyprotein provides insights into virion maturation. *Science Advances* **8**, eabn9874 (2022).

24 Lee, J. H., Ozorowski, G. & Ward, A. B. Cryo-EM structure of a native, fully glycosylated, cleaved HIV-1 envelope trimer. *Science* **351**, 1043-1048 (2016).

25 Passos, D. O. *et al.* Cryo-EM structures and atomic model of the HIV-1 strand transfer complex intasome. *Science* **355**, 89-92 (2017).

26 Chang, S. *et al.* Cryo-EM structure of influenza virus RNA polymerase complex at 4.3 Å resolution. *Molecular cell* **57**, 925-935 (2015).

27 Liu, Y. *et al.* CryoEM Structure of an Influenza Virus Receptor-Binding Site Antibody–Antigen Interface. *Journal of molecular biology* **429**, 1829-1839 (2017).

28 Huang, Q. J. *et al.* Quantitative structural analysis of influenza virus by cryo-electron tomography and convolutional neural networks. *Structure* **30**, 777-786. e773 (2022).

29 Sirohi, D. *et al.* The 3.8 Å resolution cryo-EM structure of Zika virus. *Science* **352**, 467-470 (2016).

30 Sevvana, M. *et al.* Refinement and analysis of the mature Zika virus cryo-EM structure at 3.1 Å resolution. *Structure* **26**, 1169-1177. e1163 (2018).

31 Agip, A.-N. A., Chung, I., Sanchez-Martinez, A., Whitworth, A. J. & Hirst, J. Cryo-EM structures of mitochondrial respiratory complex I from Drosophila melanogaster. *Elife* **12**, e84424 (2023).

32 Guan, S., Zhao, L. & Peng, R. Mitochondrial Respiratory Chain Supercomplexes: From Structure to Function. *International Journal of Molecular Sciences* **23**, 13880 (2022).

33 Sousa, J. S., D'Imprima, E. & Vonck, J. Mitochondrial respiratory chain complexes. *Membrane protein complexes: structure and function*, 167-227 (2018).

34 Wrapp, D. *et al.* Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* **367**, 1260-1263 (2020).

35 Juraszek, J. *et al.* Stabilizing the closed SARS-CoV-2 spike trimer. *Nature communications* **12**, 1-8 (2021).

36 Cerutti, G. *et al.* Cryo-EM structure of the SARS-CoV-2 Omicron spike. *Cell reports* **38**, 110428 (2022).

37 Cai, Y. *et al.* Distinct conformational states of SARS-CoV-2 spike protein. *Science* **369**, 1586-1592 (2020).

38 Yin, W. *et al.* Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. *Science* **368**, 1499-1504 (2020).

39 Wang, Q. *et al.* Structural basis for RNA replication by the SARS-CoV-2 polymerase. *Cell* **182**, 417-428. e413 (2020).

40 Hillen, H. S. *et al.* Structure of replicating SARS-CoV-2 polymerase. *Nature* **584**, 154-156 (2020).

41 Malone, B. *et al.* Structural basis for backtracking by the SARS-CoV-2 replication–transcription complex. *Proceedings of the National Academy of Sciences* **118**, e2102516118 (2021).

42 Yang, H. & Rao, Z. Structural biology of SARS-CoV-2 and implications for therapeutic development. *Nature Reviews Microbiology* **19**, 685-700 (2021).

43 Iancu, C. V. *et al.* Electron cryotomography sample preparation using the Vitrobot. *Nature protocols* **1**, 2813-2819 (2006).

44 Tivol, W. F., Briegel, A. & Jensen, G. J. An improved cryogen for plunge freezing. *Microscopy and Microanalysis* **14**, 375-379 (2008).

45 Rheinberger, J., Oostergetel, G., Resch, G. P. & Paulino, C. Optimized cryo-EM data-acquisition workflow by sample-thickness determination. *Acta Crystallographica Section D: Structural Biology* **77**, 565-571 (2021).

46 Nogales, E. & Scheres, S. H. Cryo-EM: a unique tool for the visualization of macromolecular complexity. *Molecular cell* **58**, 677-689 (2015).

47 Nakane, T. *et al.* Single-particle cryo-EM at atomic resolution. *Nature* **587**, 152-156 (2020).

48 Yip, K. M., Fischer, N., Paknia, E., Chari, A. & Stark, H. Atomic-resolution protein structure determination by cryo-EM. *Nature* **587**, 157-161 (2020).

49 Castaño-Díez, D. & Zanetti, G. In situ structure determination by subtomogram averaging. *Current opinion in structural biology* **58**, 68-75 (2019).

50 Mastronarde, D. N. Automated electron microscope tomography using robust prediction of specimen movements. *Journal of structural biology* **152**, 36-51 (2005).

51 Thompson, R. F., Iadanza, M. G., Hesketh, E. L., Rawson, S. & Ranson, N. A. Collection, pre-processing and on-the-fly analysis of data for high-resolution, single-particle cryo-electron microscopy. *Nature protocols* **14**, 100-118 (2019).

52 Carragher, B. *et al.* Leginon: an automated system for acquisition of images from vitreous ice specimens. *Journal of structural biology* **132**, 33-45 (2000).

53 Scheres, S. H. RELION: implementation of a Bayesian approach to cryo-EM structure determination. *Journal of structural biology* **180**, 519-530 (2012).

54 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nature methods* **14**, 290-296 (2017).

55 Kimanius, D., Dong, L., Sharov, G., Nakane, T. & Scheres, S. H. New tools for automated cryo-EM single-particle analysis in RELION-4.0. *Biochemical Journal* **478**, 4169-4185 (2021).

56 Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. *Acta crystallographica section D: biological crystallography* **60**, 2126-2132 (2004).

57 Afonine, P. V. *et al.* Real-space refinement in PHENIX for cryo-EM and crystallography. *Acta Crystallographica Section D: Structural Biology* **74**, 531-544 (2018).

58 Wang, R. Y.-R. *et al.* Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta. *Elife* **5**, e17219 (2016).

59 Croll, T. I. ISOLDE: a physically realistic environment for model building into low-resolution electron-density maps. *Acta Crystallographica Section D: Structural Biology* **74**, 519-530 (2018).

60 Peplow, M. (ACS Publications, 2020).

61 Joseph, A. P. *et al.* Comparing cryo-EM reconstructions and validating atomic model

fit using difference maps. *Journal of chemical information and modeling* **60**, 2552-2560 (2020).

62 Gupta, M. *et al.* CryoEM and AI reveal a structure of SARS-CoV-2 Nsp2, a multifunctional protein involved in key host processes. *bioRxiv* (2021).

63 Jumper, J. *et al.* Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583-589 (2021).

64 Cramer, P. AlphaFold2 and the future of structural biology. *Nature Structural & Molecular Biology* **28**, 704-705, doi:10.1038/s41594-021-00650-1 (2021).

65 Tunyasuvunakool, K. *et al.* Highly accurate protein structure prediction for the human proteome. *Nature* **596**, 590-596 (2021).

66 Humphreys, I. R. *et al.* Computed structures of core eukaryotic protein complexes. *Science* **374**, eabm4805 (2021).

67 Dejnirattisai, W. *et al.* SARS-CoV-2 Omicron-B. 1.1. 529 leads to widespread escape from neutralizing antibody responses. *Cell* (2022).

68 Meng, B. *et al.* Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity. *Nature* **603**, 706-714 (2022).

69 McGreevy, R., Teo, I., Singharoy, A. & Schulten, K. Advances in the molecular dynamics flexible fitting method for cryo-EM modeling. *Methods* **100**, 50-60 (2016).

70 Lopéz-Blanco, J. R. & Chacón, P. iMODFIT: efficient and robust flexible fitting based on vibrational analysis in internal coordinates. *Journal of structural biology* **184**, 261-270 (2013).

71 Saibil, H. R. Cryo-EM in molecular and cellular biology. *Molecular Cell* **82**, 274-284 (2022).

72 Liedtke, J., Depelteau, J. S. & Briegel, A. How advances in cryo-electron tomography have contributed to our current view of bacterial cell biology. *Journal of Structural Biology: X* **6**, 100065 (2022).

73 Lučić, V., Rigort, A. & Baumeister, W. Cryo-electron tomography: the challenge of doing structural biology in situ. *Journal of Cell Biology* **202**, 407-419 (2013).

74 Khanna, K. & Villa, E. Revealing bacterial cell biology using cryo-electron tomography. *Current Opinion in Structural Biology* **75**, 102419 (2022).

75 Turoňová, B., Schur, F. K., Wan, W. & Briggs, J. A. Efficient 3D-CTF correction for cryo-electron tomography using NovaCTF improves subtomogram averaging resolution to 3.4 Å. *Journal of structural biology* **199**, 187-195 (2017).

76 Kremer, J. R., Mastronarde, D. N. & McIntosh, J. R. Computer visualization of three-dimensional image data using IMOD. *Journal of structural biology* **116**, 71-76 (1996).

77 Chen, M. *et al.* Convolutional neural networks for automated annotation of cellular cryo-electron tomograms. *Nature methods* **14**, 983-985 (2017).

78 Liu, Y.-T. *et al.* Isotropic reconstruction of electron tomograms with deep learning. *bioRxiv* (2021).

79 Chung, J. M., Durie, C. L. & Lee, J. Artificial Intelligence in Cryo-Electron Microscopy. *Life* **12**, 1267 (2022).

80 Bepler, T., Kelley, K., Noble, A. J. & Berger, B. Topaz-Denoise: general deep denoising models for cryoEM and cryoET. *Nature communications* **11**, 5208 (2020).

81 Li, H. *et al.* Noise-Transfer2Clean: denoising cryo-EM images based on noise

modeling and transfer. *Bioinformatics* **38**, 2022-2029 (2022).

82 Wang, F. *et al.* DeepPicker: A deep learning approach for fully automated particle picking in cryo-EM. *Journal of structural biology* **195**, 325-336 (2016).

83 Zhu, Y., Ouyang, Q. & Mao, Y. A deep convolutional neural network approach to single-particle recognition in cryo-electron microscopy. *BMC bioinformatics* **18**, 1-10 (2017).

84 Bepler, T. *et al.* Positive-unlabeled convolutional neural networks for particle picking in cryo-electron micrographs. *Nature methods* **16**, 1153-1160 (2019).

85 Tegunov, D. & Cramer, P. Real-time cryo-electron microscopy data preprocessing with Warp. *Nature methods* **16**, 1146-1152 (2019).

86 Wagner, T. *et al.* SPHIRE-crYOLO is a fast and accurate fully automated particle picker for cryo-EM. *Communications biology* **2**, 218 (2019).

87 Gupta, H., McCann, M. T., Donati, L. & Unser, M. CryoGAN: A new reconstruction paradigm for single-particle cryo-EM via deep adversarial learning. *IEEE Transactions on Computational Imaging* **7**, 759-774 (2021).

88 Zhong, E. D., Bepler, T., Berger, B. & Davis, J. H. CryoDRGN: reconstruction of heterogeneous cryo-EM structures using neural networks. *Nature methods* **18**, 176-185 (2021).

89 Punjani, A. & Fleet, D. J. 3D flexible refinement: Structure and motion of flexible proteins from cryo-EM. *BioRxiv*, 2021.2004. 2022.440893 (2021).

90 Cardone, G., Heymann, J. B. & Steven, A. C. One number does not fit all: mapping local variations in resolution in cryo-EM reconstructions. *Journal of structural biology* **184**, 226-236 (2013).

91 Kucukelbir, A., Sigworth, F. J. & Tagare, H. D. Quantifying the local resolution of cryo-EM density maps. *Nature methods* **11**, 63-65 (2014).

92 Vilas, J. L. *et al.* MonoRes: automatic and accurate estimation of local resolution for electron microscopy maps. *Structure* **26**, 337-344. e334 (2018).

93 Ramirez-Aportela, E., Mota, J., Conesa, P., Carazo, J. M. & Sorzano, C. O. S. DeepRes: a new deep-learning-and aspect-based local resolution method for electron-microscopy maps. *IUCrJ* **6**, 1054-1063 (2019).

94 Maddhuri Venkata Subramaniya, S. R., Terashi, G. & Kihara, D. Protein secondary structure detection in intermediate-resolution cryo-EM maps using deep learning. *Nature methods* **16**, 911-917 (2019).

95 He, J., Lin, P., Chen, J., Cao, H. & Huang, S.-Y. Model building of protein complexes from intermediate-resolution cryo-EM maps with deep learning-guided automatic assembly. *Nature Communications* **13**, 4066 (2022).

96 Zeng, X. & Xu, M. Aitom: Open-source ai platform for cryo-electron tomography data analysis. *arXiv preprint arXiv:1911.03044* (2019).

97 Harshitha, N. *et al.* Bacteriophages: Potential Biocontrol Agents and Treatment Options for Bacterial Pathogens. *Clinical Microbiology Newsletter* **44**, 41-50 (2022).

98 Rogovski, P. *et al.* Uses of Bacteriophages as Bacterial Control Tools and Environmental Safety Indicators. *Frontiers in Microbiology* **12**, 3756 (2021).

99 Moye, Z. D., Woolston, J. & Sulakvelidze, A. Bacteriophage applications for food production and processing. *Viruses* **10**, 205 (2018).

100 Zinke, M., Schröder, G. F. & Lange, A. Major tail proteins of bacteriophages of the order Caudovirales. *Journal of Biological Chemistry* **298** (2022).

101 Nobrega, F. L. *et al.* Targeting mechanisms of tailed bacteriophages. *Nature Reviews Microbiology* **16**, 760-773 (2018).

102 Dion, M. B., Oechslin, F. & Moineau, S. Phage diversity, genomics and phylogeny. *Nature Reviews Microbiology* **18**, 125-138 (2020).

103 Luque, D. & Castón, J. R. Cryo-electron microscopy for the study of virus assembly. *Nature chemical biology* **16**, 231-239 (2020).

104 Hardy, J. M., Dunstan, R. A., Lithgow, T. & Coulibaly, F. Tall tails: cryo-electron microscopy of phage tail DNA ejection conduits. *Biochemical Society Transactions* **50**, 459-422W (2022).

105 Conners, R. *et al.* Cryo-electron microscopy of the f1 filamentous phage reveals insights into viral infection and assembly. *Nature Communications* **14**, 2724 (2023).

106 Leiman, P. G. *et al.* Morphogenesis of the T4 tail and tail fibers. *Virology journal* **7**, 1-28 (2010).

107 Ackermann, H.-W. Phage classification and characterization. *Bacteriophages: Methods and protocols, volume 1: Isolation, characterization, and interactions*, 127-140 (2009).

108 Turner, D. *et al.* Abolishment of morphology-based taxa and change to binomial species names: 2022 taxonomy update of the ICTV bacterial viruses subcommittee. *Archives of Virology* **168**, 74 (2023).

109 Samuel, A. D. *et al.* Flagellar determinants of bacterial sensitivity to χ-phage. *Proceedings of the National Academy of Sciences* **96**, 9863-9866 (1999).

110 Taslem Mourosi, J. *et al.* Understanding Bacteriophage Tail Fiber Interaction with Host Surface Receptor: The Key "Blueprint" for Reprogramming Phage Host Range. *International Journal of Molecular Sciences* **23**, 12146 (2022).

111 Montemayor, E. J. *et al.* Flagellar structures from the bacterium caulobacter crescentus and implications for phage ϕ CbK predation of multiflagellin bacteria. *Journal of Bacteriology* **203**, e00399-00320 (2021).

112 Zampara, A. *et al.* Exploiting phage receptor binding proteins to enable endolysins to kill Gram-negative bacteria. *Scientific reports* **10**, 12087 (2020).

113 González-García, V. A. *et al.* Conformational changes leading to T7 DNA delivery upon interaction with the bacterial receptor. *Journal of Biological Chemistry* **290**, 10038-10044 (2015).

114 Boeckaerts, D. *et al.* Predicting bacteriophage hosts based on sequences of annotated receptor-binding proteins. *Scientific Reports* **11**, 1467 (2021).

115 Labrie, S. J., Samson, J. E. & Moineau, S. Bacteriophage resistance mechanisms. *Nature Reviews Microbiology* **8**, 317-327 (2010).

116 Kutter, E. & Sulakvelidze, A. *Bacteriophages: biology and applications*. (Crc press, 2004).

117 Walter, M. *et al.* Structure of the receptor-binding protein of bacteriophage det7: a podoviral tail spike in a myovirus. *Journal of virology* **82**, 2265-2273 (2008).

118 Efimov, A. D. *et al.* RB49-like Bacteriophages Recognize O Antigens as One of the Alternative Primary Receptors. *International Journal of Molecular Sciences* **23**, 11329

(2022).

119 Šiborová, M. *et al.* Tail proteins of phage SU10 reorganize into the nozzle for genome delivery. *Nature Communications* **13**, 5622 (2022).

120 Yap, M. L. & Rossmann, M. G. Structure and function of bacteriophage T4. *Future microbiology* **9**, 1319-1327 (2014).

121 Esteves, N. C. & Scharf, B. E. Flagellotropic Bacteriophages: Opportunities and Challenges for Antimicrobial Applications. *International Journal of Molecular Sciences* **23**, 7084 (2022).

122 Geiben-Lynn, R., Sauber, K. & Lutz, F. Flagellin inhibits Myoviridae phage φCTX infection of Pseudomonas aeruginosa strain GuA18: purification and mapping of binding site. *Archives of microbiology* **176**, 339-346 (2001).

123 Dunstan, R. A. *et al.* The flagellotropic bacteriophage YSD1 targets Salmonella Typhi with a Chi-like protein tail fibre. *Molecular Microbiology* **112**, 1831-1846 (2019).

124 Baldvinsson, S. B., Sørensen, M. C. H., Vegge, C. S., Clokie, M. R. & Brøndsted, L. Campylobacter jejuni motility is required for infection of the flagellotropic bacteriophage F341. *Applied and environmental microbiology* **80**, 7096-7106 (2014).

125 Meynell, E. W. A phage, ?χ, which attacks motile bacteria. *Microbiology* **25**, 253-290 (1961).

126 Sertic, V. & Boulgakov, N. Bacteriophages Specific for Flagellated Forms of Bacteria. *Compte rendu des seances de la Societe de biologie* **123**, 887-888 (1936).

127 Esteves, N. C., Porwollik, S., McClelland, M. & Scharf, B. E. The multidrug efflux system AcrABZ-TolC is essential for infection of Salmonella typhimurium by the flagellum-dependent bacteriophage Chi. *Journal of virology* **95**, e00394-00321 (2021).

128 Lotz, W., Acker, G. & Schmitt, R. Bacteriophage 7-7-1 Adsorbs to the Complex Flagella of Rhizobium lupini H13–3. *Journal of General Virology* **34**, 9-17 (1977).

129 Gonzalez, F., Helm, R. F., Broadway, K. M. & Scharf, B. E. More than rotating flagella: Lipopolysaccharide as a secondary receptor for flagellotropic phage 7-7-1. *Journal of bacteriology* **200**, e00363-00318 (2018).

130 Gonzalez, F. & Scharf, B. E. Identification of receptor binding proteins in flagellotropic Agrobacterium phage 7-7-1. *Viruses* **13**, 1267 (2021).

131 Jollick, J. & Wright, B. L. A flagella specific bacteriophage for Caulobacter. *Journal of General Virology* **22**, 197-205 (1974).

132 Guerrero-Ferreira, R. C. *et al.* Alternative mechanism for bacteriophage adsorption to the motile bacterium Caulobacter crescentus. *Proceedings of the National Academy of Sciences* **108**, 9963-9968 (2011).

133 You, L., Suthers, P. F. & Yin, J. Effects of Escherichia coli physiology on growth of phage T7 in vivo and in silico. *Journal of bacteriology* **184**, 1888-1894 (2002).

134 Golomidova, A. K. *et al.* Branched lateral tail fiber organization in T5-like bacteriophages DT57C and DT571/2 is revealed by genetic and functional analysis. *Viruses* **8**, 26 (2016).

135 Latka, A., Leiman, P. G., Drulis-Kawa, Z. & Briers, Y. Modeling the architecture of depolymerase-containing receptor binding proteins in Klebsiella phages. *Frontiers in microbiology* **10**, 2649 (2019).

136 Prokhorov, N. S. *et al.* Function of bacteriophage G7C esterase tailspike in host cell

adsorption. *Molecular microbiology* **105**, 385-398 (2017).

137 Witte, S. *et al.* Structural and functional characterization of the receptor binding proteins of Escherichia coli O157 phages EP75 and EP335. *Computational and Structural Biotechnology Journal* **19**, 3416-3426 (2021).

138 Leiman, P. G. *et al.* The structures of bacteriophages K1E and K1-5 explain processive degradation of polysaccharide capsules and evolution of new host specificities. *Journal of molecular biology* **371**, 836-849 (2007).

139 Niu, Y. D. *et al.* Genomic, proteomic and physiological characterization of a T5-like bacteriophage for control of Shiga toxin-producing Escherichia coli O157: H7. *PLoS one* **7**, e34585 (2012).

140 Savalia, D. *et al.* Genomic and proteomic analysis of phiEco32, a novel Escherichia coli bacteriophage. *Journal of molecular biology* **377**, 774-789 (2008).

141 Schwarzer, D. *et al.* A multivalent adsorption apparatus explains the broad host range of phage phi92: a comprehensive genomic and structural analysis. *Journal of virology* **86**, 10384-10398 (2012).

142 Pickard, D. *et al.* A conserved acetyl esterase domain targets diverse bacteriophages to the Vi capsular receptor of Salmonella enterica serovar Typhi. *Journal of bacteriology* **192**, 5746-5754 (2010).

143 Ouyang, R. *et al.* High-resolution reconstruction of a Jumbo-bacteriophage infecting capsulated bacteria using hyperbranched tail fibers. *Nature Communications* **13**, 7241 (2022).

144 Plattner, M. *et al.* Structure and function of the branched receptor-binding complex of bacteriophage CBA120. *Journal of molecular biology* **431**, 3718-3739 (2019).

145 Hu, B., Margolin, W., Molineux, I. J. & Liu, J. Structural remodeling of bacteriophage T4 and host membranes during infection initiation. *Proceedings of the National Academy of Sciences* **112**, E4919-E4928 (2015).

146 Estrada Bonilla, B. *et al.* Genomic characterization of four novel bacteriophages infecting the clinical pathogen Klebsiella pneumoniae. *DNA Research* **28**, dsab013 (2021).

147 Buttimer, C. *et al.* Things are getting hairy: Enterobacteria bacteriophage vB_PcaM_CBB. *Frontiers in microbiology* **8**, 44 (2017).

148 Abbasifar, R. *et al.* Supersize me: Cronobacter sakazakii phage GAP32. *Virology* **460**, 138-146 (2014).

149 Pan, Y.-J. *et al.* Klebsiella phage ΦK64-1 encodes multiple depolymerases for multiple host capsular types. *Journal of virology* **91**, e02457-02416 (2017).

150 Imam, M. *et al.* vB_PaeM_MIJ3, a novel jumbo phage infecting Pseudomonas aeruginosa, possesses unusual genomic features. *Frontiers in Microbiology* **10**, 2772 (2019).

151 Attai, H. *et al.* Larger than life: isolation and genomic characterization of a jumbo phage that infects the bacterial plant pathogen, Agrobacterium tumefaciens. *Frontiers in microbiology*, 1861 (2018).

152 Murray, C. J. *et al.* Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, doi:10.1016/s0140-6736(21)02724-0 (2022).

153 Tacconelli, E. *et al.* Discovery, research, and development of new antibiotics: the WHO

priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases* **18**, 318-327 (2018).

154 Ryan, K. J. & Ray, C. G. Medical microbiology. *McGraw Hill* **4**, 370 (2004).

155 Ko, K. S. The contribution of capsule polysaccharide genes to virulence of Klebsiella pneumoniae. *Virulence* **8**, 485-486 (2017).

156 Follador, R. *et al.* The diversity of Klebsiella pneumoniae surface polysaccharides. *Microbial genomics* **2** (2016).

157 Šimoliūnas, E. *et al.* Klebsiella phage vB_KleM-RaK2—A giant singleton virus of the family Myoviridae. *PLoS One* **8**, e60717 (2013).

158 Hendrix, R. W. in *Lesser Known Large dsDNA Viruses* 229-240 (Springer Berlin Heidelberg, 2009).

159 Yuan, Y. & Gao, M. Jumbo bacteriophages: an overview. *Frontiers in microbiology* **8**, 403 (2017).

160 Bonilla, B. E. *et al. Genomic characterization of four novel bacteriophages infecting the clinical pathogen Klebsiella pneumoniae* (Cold Spring Harbor Laboratory, 2021).

161 Rosenthal, P. B. & Henderson, R. Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *Journal of molecular biology* **333**, 721-745 (2003).

162 Krylov, V., Dela Cruz, D., Hertveldt, K. & Ackermann, H.-W. "φKZ-like viruses", a proposed new genus of myovirus bacteriophages. *Archives of virology* **152**, 1955-1959 (2007).

163 Effantin, G. *et al.* Cryo-electron microscopy three-dimensional structure of the jumbo phage ΦRSL1 infecting the phytopathogen Ralstonia solanacearum. *Structure* **21**, 298-305 (2013).

164 Neumann, E. *et al.* 3D structure of three jumbo phage heads. *J Gen Virol* **101**, 1219-1226, doi:10.1099/jgv.0.001487 (2020).

165 Suhanovsky, M. M. & Teschke, C. M. Nature′ s favorite building block: Deciphering folding and capsid assembly of proteins with the HK97-fold. *Virology* **479**, 487-497 (2015).

166 Duda, R. L. & Teschke, C. M. The amazing HK97 fold: versatile results of modest differences. *Current opinion in virology* **36**, 9-16 (2019).

167 Helgstrand, C. *et al.* The refined structure of a protein catenane: the HK97 bacteriophage capsid at 3.44 Å resolution. *Journal of molecular biology* **334**, 885-899 (2003).

168 Gan, L. *et al.* Capsid conformational sampling in HK97 maturation visualized by X-ray crystallography and cryo-EM. *Structure* **14**, 1655-1665 (2006).

169 Wikoff, W. R. *et al.* Topologically linked protein rings in the bacteriophage HK97 capsid. *Science* **289**, 2129-2133 (2000).

170 Kamiya, R. *et al.* Acid-stable capsid structure of Helicobacter pylori bacteriophage KHP30 by single-particle cryoelectron microscopy. *Structure* **30**, 300-312. e303 (2022).

171 Sun, C., Gonzalez, B. & Jiang, W. Helical Indexing in Real Space. *Scientific Reports* **12**, 8162, doi:10.1038/s41598-022-11382-7 (2022).

172 Goddard, T. D. *et al.* UCSF ChimeraX: Meeting modern challenges in visualization and analysis. *Protein Science* **27**, 14-25 (2018).

173 Robert, X. & Gouet, P. Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Research* **42**, W320-W324, doi:10.1093/nar/gku316 (2014).

174 Aksyuk, A. A. *et al.* Structural conservation of the myoviridae phage tail sheath protein fold. *Structure* **19**, 1885-1894 (2011).

175 Zheng, W. *et al.* Refined cryo-EM structure of the T4 tail tube: exploring the lowest dose limit. *Structure* **25**, 1436-1441. e1432 (2017).

176 Ge, P. *et al.* Atomic structures of a bactericidal contractile nanotube in its pre- and postcontraction states. *Nat Struct Mol Biol* **22**, 377-382, doi:10.1038/nsmb.2995 (2015).

177 Heymann, J. B. *et al.* Three-dimensional structure of the toxin-delivery particle antifeeding prophage of Serratia entomophila. *Journal of Biological Chemistry* **288**, 25276-25284 (2013).

178 Clemens, D. L., Ge, P., Lee, B.-Y., Horwitz, M. A. & Zhou, Z. H. Atomic structure of T6SS reveals interlaced array essential to function. *Cell* **160**, 940-951 (2015).

179 Kudryashev, M. *et al.* Structure of the type VI secretion system contractile sheath. *Cell* **160**, 952-962 (2015).

180 Maghsoodi, A., Chatterjee, A., Andricioaei, I. & Perkins, N. C. How the phage T4 injection machinery works including energetics, forces, and dynamic pathway. *Proceedings of the National Academy of Sciences* **116**, 25097-25105, doi:10.1073/pnas.1909298116 (2019).

181 Leiman, P. G. & Shneider, M. M. Contractile tail machines of bacteriophages. *Viral molecular machines*, 93-114 (2012).

182 King, J. & Mykolajewyoz, N. Bacteriophage T4 tail assembly: proteins of the sheath, core and baseplate. *Journal of molecular biology* **75**, 339-358 (1973).

183 Jacques, D. A. *et al.* HIV-1 uses dynamic capsid pores to import nucleotides and fuel encapsidated DNA synthesis. *Nature* **536**, 349-353 (2016).

184 Leiman, P. G. *et al.* Type VI secretion apparatus and phage tail-associated protein complexes share a common evolutionary origin. *Proceedings of the National Academy of Sciences* **106**, 4154-4159 (2009).

185 Records, A. R. The type VI secretion system: a multipurpose delivery system with a phage-like machinery. *Molecular plant-microbe interactions* **24**, 751-757 (2011).

186 Basler, M. Type VI secretion system: secretion by a contractile nanomachine. *Philosophical Transactions of the Royal Society B: Biological Sciences* **370**, 20150021, doi:10.1098/rstb.2015.0021 (2015).

187 Zheng, S. Q. *et al.* MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. *Nature methods* **14**, 331-332 (2017).

188 Rohou, A. & Grigorieff, N. CTFFIND4: Fast and accurate defocus estimation from electron micrographs. *Journal of structural biology* **192**, 216-221 (2015).

189 Russo, C. J. & Henderson, R. Ewald sphere correction using a single side-band image processing algorithm. *Ultramicroscopy* **187**, 26-33, doi:https://doi.org/10.1016/j.ultramic.2017.11.001 (2018).

190 Singharoy, A. *et al.* Molecular dynamics-based refinement and validation for sub-5 Å cryo-electron microscopy maps. *Elife* **5**, e16105 (2016).

191 Phillips, J. C. *et al.* Scalable molecular dynamics with NAMD. *Journal of*

computational chemistry **26**, 1781-1802 (2005).

192 Huang, L. & Roux, B. Automated force field parameterization for nonpolarizable and polarizable atomic models based on ab initio target data. *Journal of chemical theory and computation* **9**, 3543-3556 (2013).

193 Humphrey, W., Dalke, A. & Schulten, K. VMD: visual molecular dynamics. *Journal of molecular graphics* **14**, 33-38 (1996).

194 Potter, S. C. *et al.* HMMER web server: 2018 update. *Nucleic Acids Research* **46**, W200-W204, doi:10.1093/nar/gky448 (2018).

195 dmpelt/jumbo-bacteriophage: Initial release (v1.0.0) (2022).

196 Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *Journal of molecular biology* **215**, 403-410 (1990).

197 Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N. & Sternberg, M. J. The Phyre2 web portal for protein modeling, prediction and analysis. *Nature protocols* **10**, 845-858 (2015).

198 Baek, M. *et al.* Accurate prediction of protein structures and interactions using a three-track neural network. *Science* **373**, 871-876 (2021).

199 Weigele, P. R., Scanlon, E. & King, J. Homotrimeric, β-stranded viral adhesins and tail proteins. *Journal of bacteriology* **185**, 4022-4030 (2003).

200 Yan, J., Mao, J. & Xie, J. Bacteriophage polysaccharide depolymerases and biomedical applications. *BioDrugs* **28**, 265-274 (2014).

201 Cornelissen, A. *et al.* The T7-related Pseudomonas putida phage φ15 displays virion-associated biofilm degradation properties. *PloS one* **6**, e18597 (2011).

202 Latka, A., Maciejewska, B., Majkowska-Skrobek, G., Briers, Y. & Drulis-Kawa, Z. Bacteriophage-encoded virion-associated enzymes to overcome the carbohydrate barriers during the infection process. *Applied microbiology and biotechnology* **101**, 3103-3119 (2017).

203 Seul, A. *et al.* Bacteriophage P22 tailspike: structure of the complete protein and function of the interdomain linker. *Acta Crystallographica Section D: Biological Crystallography* **70**, 1336-1345 (2014).

204 Zimmermann, L. *et al.* A completely reimplemented MPI bioinformatics toolkit with a new HHpred server at its core. *Journal of molecular biology* **430**, 2237-2243 (2018).

205 Pelt, D. M. & Sethian, J. A. A mixed-scale dense convolutional neural network for image analysis. *Proceedings of the National Academy of Sciences* **115**, 254-259 (2018).

206 Hagen, W. J., Wan, W. & Briggs, J. A. Implementation of a cryo-electron tomography tilt-scheme optimized for high resolution subtomogram averaging. *Journal of structural biology* **197**, 191-198 (2017).

207 Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis. *Nature methods* **9**, 676-682 (2012).

208 Pelt, D. M., Batenburg, K. J. & Sethian, J. A. Improving tomographic reconstruction from limited data using mixed-scale dense convolutional neural networks. *Journal of Imaging* **4**, 128 (2018).

209 Segev-Zarko, L.-a. *et al.* Cryo-electron tomography with mixed-scale dense neural networks reveals key steps in deployment of Toxoplasma invasion machinery. *PNAS Nexus* (2022).

210 Kingma, D. P. & Ba, J. Adam: A method for stochastic optimization. *arXiv preprint arXiv:1412.6980* (2014).

211 Ahrens, J., Geveci, B. & Law, C. Paraview: An end-user tool for large data visualization. *The visualization handbook* **717** (2005).

212 Finn, R. D., Clements, J. & Eddy, S. R. HMMER web server: interactive sequence similarity searching. *Nucleic acids research* **39**, W29-W37 (2011).

213 Gabler, F. *et al.* Protein sequence analysis using the MPI bioinformatics toolkit. *Current Protocols in Bioinformatics* **72**, e108 (2020).

214 Raimondo, L. M., Lundh, N. P. & Martinez, R. J. Primary adsorption site of phage PBS1: the flagellum of Bacillus subtilis. *Journal of virology* **2**, 256-264 (1968).

215 Gábor, M. Transformation of streptomycin markers in rough strains of Rhizobium lupini. II. The relation between the determinant of streptomycin dependence and those for streptomycin resistance and sensitiveness. *Genetics* **52**, 905 (1965).

216 Gelvin, S. B. Agrobacterium-mediated plant transformation: the biology behind the "gene-jockeying" tool. *Microbiology and molecular biology reviews* **67**, 16-37 (2003).

217 Escobar, M. A. & Dandekar, A. M. Agrobacterium tumefaciens as an agent of disease. *Trends in plant science* **8**, 380-386 (2003).

218 Lai, C.-C. *et al.* Clinical and microbiological characteristics of Rhizobium radiobacter infections. *Clinical infectious diseases* **38**, 149-153 (2004).

219 Edmond, M. B., Riddler, S. A., Baxter, C. M., Wicklund, B. M. & Pasculle, A. W. Agrobacterium radiobacter: a recently recognized opportunistic pathogen. *Clinical Infectious Diseases* **16**, 388-391 (1993).

220 Adnan, M., Khan, S., Patel, M., Al-Shammari, E. & Ashankyty, I. M. Agrobacterium. *Reviews and Research in Medical Microbiology* **24**, 94-97 (2013).

221 Lotz, W. & Mayer, F. Electron microscopical characterization of newly isolated Rhizobium lupini bacteriophages. *Canadian Journal of Microbiology* **18**, 1271-1274 (1972).

222 Kropinski, A. M. *et al.* Genome and proteome analysis of 7-7-1, a flagellotropic phage infecting Agrobacterium sp H13-3. *Virology journal* **9**, 1-8 (2012).

223 Bradley, D. E. Ultrastructure of bacteriophage and bacteriocins. *Bacteriological reviews* **31**, 230 (1967).

224 Van Raaij, M. J., Mitraki, A., Lavigne, G. & Cusack, S. A triple β-spiral in the adenovirus fibre shaft reveals a new structural motif for a fibrous protein. *Nature* **401**, 935-938 (1999).

225 Xiang, Y. & Rossmann, M. G. Structure of bacteriophage ϕ 29 head fibers has a supercoiled triple repeating helix-turn-helix motif. *Proceedings of the National Academy of Sciences* **108**, 4806-4810 (2011).

226 Cheng, L. *et al.* Cryo-EM structures of two bovine adenovirus type 3 intermediates. *Virology* **450**, 174-181 (2014).

227 Zheng, J. *et al.* A Capsid Structure of Ralstonia solanacearum podoviridae GP4 with a Triangulation Number T= 9. *Viruses* **14**, 2431 (2022).

228 Geisselsoder, J., Sedivy, J., Walsh, R. & Goldstein, R. Capsid structure of satellite phage P4 and its P2 helper. *Journal of ultrastructure research* **79**, 165-173 (1982).

229 Choi, K. H. *et al.* Insight into DNA and protein transport in double-stranded DNA

viruses: the structure of bacteriophage N4. *Journal of molecular biology* **378**, 726-736 (2008).

230 Cui, N. *et al.* Capsid structure of anabaena cyanophage A-1 (L). *Journal of Virology* **95**, e01356-01321 (2021).

231 Tao, Y. *et al.* Assembly of a tailed bacterial virus and its genome release studied in three dimensions. *Cell* **95**, 431-437 (1998).

232 Xu, J., Wang, D., Gui, M. & Xiang, Y. Structural assembly of the tailed bacteriophage ϕ29. *Nature communications* **10**, 2366 (2019).

233 Leonard, K., Kleinschmidt, A., Agabian-Keshishian, N., Shapiro, L. & Maizel Jr, J. Structural studies on the capsid of Caulobacter crescentus bacteriophage φCbK. *Journal of molecular biology* **71**, 201-216 (1972).

234 Kalatzis, P. G., Bastías, R., Kokkari, C. & Katharios, P. Isolation and characterization of two lytic bacteriophages, φSt2 and φGrn1; phage therapy application for biological control of Vibrio alginolyticus in aquaculture live feeds. *PloS one* **11**, e0151101 (2016).

235 Frank, H. & Moebus, K. An electron microscopic study of bacteriophages from marine waters. *Helgoländer Meeresuntersuchungen* **41**, 385-414 (1987).

236 Nicklin, S. A., Wu, E., Nemerow, G. R. & Baker, A. H. The influence of adenovirus fiber structure and function on vector development for gene therapy. *Molecular Therapy* **12**, 384-393 (2005).

237 Zubieta, C., Schoehn, G., Chroboczek, J. & Cusack, S. The structure of the human adenovirus 2 penton. *Molecular cell* **17**, 121-135 (2005).

238 Chroboczek, J., Ruigrok, R. & Cusack, S. Adenovirus fiber. *The Molecular Repertoire of Adenoviruses I: Virion Structure and Infection*, 163-200 (1995).

239 Von Seggern, D. J., Huang, S., Fleck, S. K., Stevenson, S. C. & Nemerow, G. R. Adenovirus vector pseudotyping in fiber-expressing cell lines: improved transduction of Epstein-Barr virus-transformed B cells. *Journal of virology* **74**, 354-362 (2000).

240 Krasnykh, V. N., Mikheeva, G. V., Douglas, J. T. & Curiel, D. T. Generation of recombinant adenovirus vectors with modified fibers for altering viral tropism. *Journal of virology* **70**, 6839-6846 (1996).

241 Gall, J., Kass-Eisler, A., Leinwand, L. & Falck-Pedersen, E. Adenovirus type 5 and 7 capsid chimera: fiber replacement alters receptor tropism without affecting primary immune neutralization epitopes. *Journal of virology* **70**, 2116-2123 (1996).

242 Yen, J. Y., Broadway, K. M. & Scharf, B. E. Minimum requirements of flagellation and motility for infection of Agrobacterium sp. strain H13-3 by flagellotropic bacteriophage 7-7-1. *Applied and environmental microbiology* **78**, 7216-7222 (2012).

243 Trifan, A. *et al.* Intelligent resolution: Integrating Cryo-EM with AI-driven multi-resolution simulations to observe the severe acute respiratory syndrome coronavirus-2 replication-transcription machinery in action. *The International Journal of High Performance Computing Applications* **36**, 603-623 (2022).

244 Wagner, F. R. *et al.* Preparing samples from whole cells using focused-ion-beam milling for cryo-electron tomography. *Nature protocols* **15**, 2041-2070 (2020).

Curriculum Vitae

Ruochen Ouyang was born on 10 May 1995 in Lanzhou, the capital city of Gansu province in northwest China. From 2013 to 2017, he studied at the School of Physical Science and Technology in Lanzhou University. In 2017, he obtained a bachelor’s degree in physics and got a recommendation to enter Xi’an Jiaotong University as a direct-PhD candidate (long academic system).

From 2017 to 2019, he studied in the MOE Key Laboratory for Nonequilibrium Synthesis and Modulation of Condensed Matter at the School of Physics in Xi'an Jiaotong University, and was supervised by Prof. Shengli Zhang and Prof. Lei Zhang. During this period, he learned some scientific research skills including the preparation of frozen samples, basic cryo-EM operations, and utilization of scientific software to reconstruct proteins by single-particle analysis (SPA) and cryo-electron tomography (Cryo-ET) methodologies.

In 2019, Ruochen Ouyang was selected and joined the Innovative Talent Joint Training Program between Xi'an Jiaotong University and Leiden University, Netherlands. After obtaining funding from China Scholarship Council (CSC), he began his study in the Institute of Biology Leiden (IBL) at Leiden University under the supervision of Prof. Ariane Briegel, as a joint training PhD candidate.

This thesis covers the work of the Ph.D. research during his study at Leiden University.