Evolutionary adaptability of β-lactamase: a study of inhibitor susceptibility in various model systems

Alen, I. van
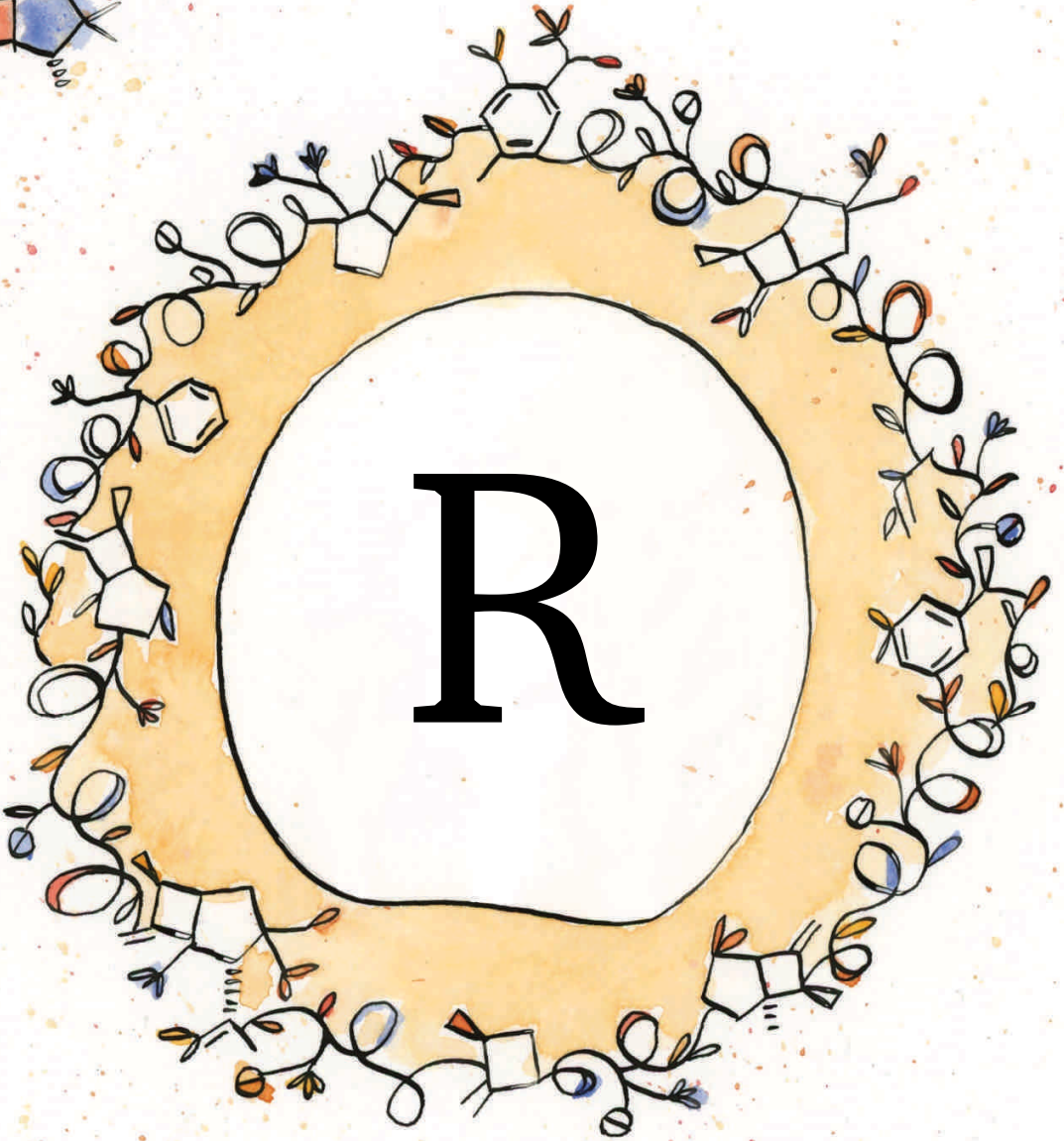
References

(1) Govindarajan, S., and Goldstein, R. A. (1997) Evolution of model proteins on a foldability landscape. *Proteins: Structure, Function, and Genetics 29*, 461–466.

(2) Bloom, J. D., Wilke, C. O., Arnold, F. H., and Adami, C. (2004) Stability and the evolvability of function in a model protein. *Biophys J 86*, 2758–2764.

(3) Gutin, A. M., Abkevich, V. I., and Shakhnovich, E. I. (1995) Evolution-like selection of fast-folding model proteins. *Proceedings of the National Academy of Sciences 92*, 1282–1286.

(4) Mélin, R., Li, H., Wingreen, N. S., and Tang, C. (1999) Designability, thermodynamic stability, and dynamics in protein folding: A lattice model study. *J Chem Phys 110*, 1252–1262.

(5) Bar-Even, A., Noor, E., Savir, Y., Liebermeister, W., Davidi, D., Tawfik, D. S., and Milo, R. (2011) The moderately efficient enzyme: Evolutionary and physicochemical trends shaping enzyme parameters. *Biochemistry 50*, 4402–4410.

(6) Wagner, A. (2008) Robustness and evolvability: A paradox resolved. *Proceedings of the Royal Society B: Biological Sciences 275*, 91–100.

(7) Firnberg, E., and Ostermeier, M. (2013) The genetic code constrains yet facilitates Darwinian evolution. *Nucleic Acids Res 41*, 7420–7428.

(8) Pál, C., Papp, B., and Hurst, L. D. (2001) Highly expressed genes in yeast evolve slowly. *Genetics 158*, 927–931.

(9) Rocha, E. P. C., and Danchin, A. (2004) An analysis of determinants of amino acids substitution rates in bacterial proteins. *Mol Biol Evol 21*, 108–116.

(10) Subramanian, S., and Kumar, S. (2004) Gene expression intensity shapes evolutionary rates of the proteins encoded by the vertebrate genome. *Genetics 168*, 373–381.

(11) Wright, S. I., Yau, C. B. K., Looseley, M., and Meyers, B. C. (2004) Effects of gene expression on molecular evolution in *Arabidopsis thaliana* and *Arabidopsis lyrata*. *Mol Biol Evol 21*, 1719–1726.

(12) Pál, C., Papp, B., and Lercher, M. J. (2006) An integrated view of protein evolution. *Nat Rev Genet 7*, 337–348.

(13) Drummond, D. A., Bloom, J. D., Adami, C., Wilke, C. O., and Arnold, F. H. (2005) Why highly expressed proteins evolve slowly. *Proceedings of the National Academy of Sciences 102*, 14338–14343.

(14) Fraser, H. B., Hirsh, A. E., Steinmetz, L. M., Scharfe, C., and Feldman, M. W. (2002) Evolutionary rate in the protein interaction network. *Science (1979) 296*, 750–752.

(15) Bloom, J. D., and Arnold, F. H. (2009) In the light of directed evolution: Pathways of adaptive protein evolution. *Proceedings of the National Academy of Sciences 106*, 9995–10000.

(16) Zuckerlandl, E., and Pauling, L. (1965) Evolutionary divergence and convergence in proteins, in *Evolving Genes and Proteins* (Bryson, V., and Vogel, H. J., Eds.), pp 97–166. Springer, New York, NY.

(17) Stimple, S. D., Smith, M. D., and Tessier, P. M. (2020) Directed evolution methods for overcoming trade-offs between protein activity and stability. *AIChE Journal 66*, e16814.

(18) Storz, J. F. (2018) Compensatory mutations and epistasis for protein function. *Curr Opin Struct Biol 50*, 18–25.

(19) Kimura, M. (1968) Evolutionary rate at the molecular level. *Nature 217*, 624–626.

(20) Bershtein, S., Goldin, K., and Tawfik, D. S. (2008) Intense neutral drifts yield robust and evolvable consensus proteins. *J Mol Biol 379*, 1029–1044.

(21) Weinreich, D. M., Delaney, N. F., DePristo, M. A., and Hartl, D. L. (2006) Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science (1979) 312*, 111–114.

(22) Podgornaia, A. I., and Laub, M. T. (2015) Pervasive degeneracy and epistasis in a protein-protein interface. *Science (1979) 347*, 673–677.

(23) Hartman, E. C., and Tullman-Ercek, D. (2019) Learning from protein fitness landscapes: A review of mutability, epistasis, and evolution. *Curr Opin Syst Biol 14*, 25–31.

(24) Campbell, J. H., Lengyel, J. A., and Langridge, J. (1973) Evolution of a second gene for β-galactosidase in *Escherichia coli*. *Proceedings of the National Academy of Sciences 70*, 1841–1845.

(25) Hall, B. G. (1978) Experimental evolution of a new enzymatic function. II. Evolution of multiple functions for *Ebg* enzyme in *E. coli*. *Genetics 89*, 453–465.

(26) Fasan, R., Chen, M. M., Crook, N. C., and Arnold, F. H. (2007) Engineered alkane-hydroxylating cytochrome P450$_{BM3}$ exhibiting nativelike catalytic properties. *Angewandte Chemie - International Edition 46*, 8414–8418.

(27) Abraham, E. P., and Chain, E. (1940) An enzyme from bacteria able to destroy penicillin. *Nature 146*, 837–837.

(28) Harper, G. J. (1943) Inhibition of penicillin in routine culture media. *The Lancet 242*, 569–571.

(29) Ungar, J. (1944) Penicillinase from *B. subtilis*. *Nature 154*, 236–237.

(30) Duthie, E. S. (1944) The production of penicillinase by organisms of the subtilis group. *Br J Exp Pathol 25*, 96–100.

(31) Kirby, W. M. M. (1944) Extraction of a highly potent penicillin inactivator from penicillin resistant staphylococci. *Science (1979) 99*, 452–453.

(32) Goffin, C., and Ghuysen, J.-M. (1998) Multimodular penicillin-binding proteins: An enigmatic family of orthologs and paralogs. *Microbiol. Mol. Biol. Rev. 62*, 1079–1093.

(33) Yocum, R. R., Waxman, D. J., Rasmussen, J. R., and Strominger, J. L. (1979) Mechanism of penicillin action: penicillin and substrate bind covalently to the same active site serine in two bacterial D-alanine carboxypeptidases. *Proc Natl Acad Sci U S A 76*, 2730–2734.

(34) Waxman, D. J., and Strominger, J. L. (1980) Sequence of active site peptides from the penicillin-sensitive D-alanine carboxypeptidase of *Bacillus subtilis*. Mechanism of penicillin action and sequence homology to β-lactamases. *Journal of Biological Chemistry 255*, 3964–3976.

(35) Massova, I., and Mobashery, S. (1998) Kinship and diversification of bacterial penicillin-binding proteins and β-lactamases. *Antimicrob Agents Chemother 42*, 1–17.

(36) Kelly, J. A., Dideberg, O., Charlier, P., Wery, J. P., Libert, M., Moews, P. C., Knox, J. R., Duez, C., Fraipont, C., Joris, B., Dusart, J., Frère, J. M., and Ghuysen, J. M. (1986) On the origin of bacterial resistance to penicillin: Comparison of a β-lactamase and a penicillin target. *Science (1979) 231*, 1429–1431.

(37) Hall, B. G., and Barlow, M. (2004) Evolution of the serine β-lactamases: Past, present and future. *Drug Resistance Updates 7*, 111–123.

(38) Smith, J. D., Kumarasiri, M., Zhang, W., Hesek, D., Lee, M., Toth, M., Vakulenko, S., Fisher, J. F., Mobashery, S., and Chen, Y. (2013) Structural analysis of the role of *Pseudomonas aeruginosa* penicillin-binding protein 5 in β-lactam resistance. *Antimicrob Agents Chemother 57*, 3137–3146.

(39) Ambler, R. P. (1980) The structure of β-lactamases. *Philosphical Transactions of the Royal Society B 289*, 321–331.

(40) Hall, B. G., and Barlow, M. (2005) Revised Ambler classification of β-lactamases. *Journal of Antimicrobial Chemotherapy 55*, 1050–1051.

(41) Jaurin, B., and Grundström, T. (1981) AmpC cephalosporinase of *Escherichia coli* K-12 has a different evolutionary origin from that of β-lactamases of the penicillinase type. *Proceedings of the National Academy of Sciences 78*, 4897–4901.

(42) Ouellette, M., Bissonnette, L., and Roy, P. H. (1987) Precise insertion of antibiotic resistance determinants into Tn21-like transposons: nucleotide sequence of the OXA-1 beta-lactamase gene. *Proc Natl Acad Sci U S A 84*, 7378–7382.

(43) Bush, K. (1989) Characterization of β-Lactamases. *Antimicrob Agents Chemother 33*, 259–263.

(44) Bush, K., and Jacoby, G. A. (2010) Updated functional classification of β-lactamases. *Antimicrob Agents Chemother 54*, 969–976.

(45) Kasik, J. E. (1965) The nature of mycobacterial pencilinnase. *Am Rev Respir Dis 91*, 117–119.

(46) Finch, R. (1986) β-Lactam antibiotics and mycobacteria. *Journal of Antimicrobial Chemotherapy 18*, 6–8.

(47) Bush, K., Jacoby, G. A., and Medeiros, A. A. (1995) A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrobioal Agents and Chemotherapy 39*, 1211–1233.

(48) Datta, N., and Kontomichalou, P. (1965) Penicillinase synthesis controlled by infectious R factors in enterobacteriaceae. *Nature 208*, 239–241.

(49) Ambler, R. P., and Scott, G. K. (1978) Partial amino acid sequence of penicillinase coded by *Escherichia coli* plasmid R6K. *Proc Natl Acad Sci U S A 75*, 3732–3736.

(50) Pitton, J.-S. (1972) Mechanisms of bacterial resistance to antibiotics, in *Ergebnisse der Physiologie, Biologischen Chemie und experimentellen Pharmakologie* (Adrian, R. H., Helmreich, E., Holzer, H., Jung, R., Kramer, K., Krayer, O., Lynen, F., Miescher, P. A., Rasmussen, H., Renold, A. E., Trendelenburg, U., Vogt, W., and Weber, H. H., Eds.), pp 17–93. Springer Berlin.

(51) Matthew, M., Hedges, R. W., and Smith, J. T. (1979) Types of beta-lactamase determined by plasmids in gram-negative bacteria. *J Bacteriol 138*, 657–662.

(52) Bauernfeind, A., Schweighart, S., and Grimm, H. (1990) A new plasmidic cefotaximase in a clinical isolate of *Escherichia coli*. *Infection 18*, 294–298.

R

(53) Wang, F., Cassidy, C., and Sacchettini, J. C. (2006) Crystal structure and activity studies of the *Mycobacterium tuberculosis* β-lactamase reveal its critical role in resistance to β-lactam antibiotics. *Antimicrob Agents Chemother 50*, 2762–2771.

(54) Egesborg, P., Carlettini, H., Volpato, J. P., and Doucet, N. (2015) Combinatorial active-site variants confer sustained clavulanate resistance in BlaC β-lactamase from *Mycobacterium tuberculosis*. *Protein Science 24*, 534–544.

(55) Hackbarth, C. J., Unsal, I., and Chambers, H. F. (1997) Cloning and sequence analysis of a class A β-lactamase from *Mycobacterium tuberculosis* H37Ra. *Antimicrob Agents Chemother 41*, 1182–1185.

(56) Olsen, G. J., and Woese, C. R. (1993) Ribosomal RNA: a key to phylogeny. *The FASEB Journal 7*, 113–123.

(57) Jackson, M. (2014) The mycobacterial cell envelope - lipids. *Cold Spring Harb Perspect Med 4*, a021105.

(58) Berks, B. C. (1996) A common export pathway for proteins binding complex redox cofactors? *Mol Microbiol 22*, 393–404.

(59) McDonough, J. A., Hacker, K. E., Flores, A. R., Pavelka, M. S., and Braunstein, M. (2005) The twin-arginine translocation pathway of *Mycobacterium smegmatis* is functional and required for the export of mycobacterial β-lactamases. *J Bacteriol 187*, 7667–7679.

(60) McDonough, J. A., McCann, J. R., Tekippe, E. M., Silverman, J. S., Rigel, N. W., and Braunstein, M. (2008) Identification of functional Tat signal sequences in *Mycobacterium tuberculosis* proteins. *J Bacteriol 190*, 6428–6438.

(61) Yahr, Timothy. L., and Wickner, W. T. (2001) Functional reconstitution of bacterial Tat translocation *in vitro*. *EMBO J 20*, 2472–2479.

(62) Sitthidet Tharinjaroen, C., Intorasoot, S., Anukool, U., Phunpae, P., Butr-Indr, B., Orrapin, S., Sangboonruang, S., Arunothong, S., Chaiyasirinroj, B., Kunyanone, N., Kasinrerk, W., and Tragoolpua, K. (2016) Novel targeting of the *lepB* gene using PCR with confronting two-pair primers for simultaneous detection of *Mycobacterium tuberculosis* complex and *Mycobacterium bovis*. *J Med Microbiol 65*, 36–43.

(63) Smith, G. T., Sweredoski, M. J., and Hess, S. (2014) O-linked glycosylation sites profiling in *Mycobacterium tuberculosis* culture filtrate proteins. *J Proteomics 97*, 296–306.

(64) Birhanu, A. G., Yimer, S. A., Kalayou, S., Riaz, T., Zegeye, E. D., Holm-Hansen, C., Norheim, G., Aseffa, A., Abebe, M., and Tønjum, T. (2019) Ample glycosylation in membrane and cell envelope proteins may explain the phenotypic diversity and virulence in the *Mycobacterium tuberculosis* complex. *Sci Rep 9*, 2927.

(65) Ambler, R. P., Coulson, A. F. W., Frère, J. M., Ghuysen, J. M., Joris, B., Forsman, M., Levesque, R. C., Tiraby, G., and Waley, S. G. (1991) A standard numbering scheme for the class A β-lactamases. *Biochemical Journal 276*, 269–270.

(66) Jelsch, C., Mourey, L., Masson, J. M., and Samama, J. P. (1993) Crystal structure of *Escherichia coli* TEM1 beta-lactamase at 1.8 Å resolution. *Proteins 16*, 364–83.

(67) Maveyraud, L., Pratt, R. F., and Samama, J. P. (1998) Crystal structure of an acylation transition-state analog of the TEM-1 β-lactamase. Mechanistic implications for class A β-lactamases. *Biochemistry 37*, 2622–2628.

(68) Minasov, G., Wang, X., and Shoichet, B. K. (2002) An ultrahigh resolution structure of TEM-1 β-lactamase suggests a role for Glu166 as the general base in acylation. *J Am Chem Soc 124*, 5333–5340.

(69) Golemi-Kotra, D., Meroueh, S. O., Kim, C., Vakulenko, S. B., Bulychev, A., Stemmler, A. J., Stemmler, T. L., and Mobashery, S. (2004) The importance of a critical protonation state and the fate of the catalytic steps in class A β-lactamases and penicillin-binding proteins. *Journal of Biological Chemistry 279*, 34665–34673.

(70) Elings, W., Gaur, A., Blok, A. J., Timmer, M., van Ingen, H., and Ubbink, M. (2020) β-Lactamase of *Mycobacterium tuberculosis* shows dynamics in the active site that increase upon inhibitor binding. *Antimicrob Agents Chemother 64*, e02025-19.

(71) Hermann, J. C., Hensen, C., Ridder, L., Mulholland, A. J., and Höltje, H.-D. (2005) Mechanisms of antibiotic resistance: QM/MM modeling of the acylation reaction of a class A β-lactamase with benzylpenicillin. *J Am Chem Soc 127*, 4454–4465.

(72) Hermann, J. C., Pradon, J., Harvey, J. N., and Mulholland, A. J. (2009) High level QM/MM modeling of the formation of the tetrahedral intermediate in the acylation of wild type and K73A mutant TEM-1 class A β-lactamase. *Journal of Physical Chemistry A 113*, 11984–11994.

(73) Tomanicek, S. J., Blakeley, M. P., Cooper, J., Chen, Y., Afonine, P. v., and Coates, L. (2010) Neutron diffraction studies of a class A β-lactamase Toho-1 E166A/R274N/R276N triple mutant. *J Mol Biol 396*, 1070–1080.

(74) Swarén, P., Maveyraud, L., Guillet, V., Masson, J.-M., Mourey, L., and Samama, J.-P. (1995) Electrostatic analysis of TEM1 β-lactamase: effect of substrate binding, steep potential gradients and consequences of site-directed mutations. *Structure 3*, 603–613.

(75) Meroueh, S. O., Fisher, J. F., Schlegel, H. B., and Mobashery, S. (2005) Ab initio QM/MM study of class A β-lactamase acylation: Dual participation of Glu166 and Lys73 in a concerted base promotion of Ser70. *J Am Chem Soc 127*, 15397–15407.

(76) Nichols, D. A., Hargis, J. C., Sanishvili, R., Jaishankar, P., Defrees, K., Smith, E. W., Wang, K. K., Prati, F., Renslo, A. R., Woodcock, H. L., and Chen, Y. (2015) Ligand-induced proton transfer and low-barrier hydrogen bond revealed by x-ray crystallography. *J Am Chem Soc 137*, 8086–8095.

(77) Strynadka, N. C. J., Adachi, H., Jensen, S. E., Johns, K., Sielecki, A., Betzel, C., Sutoh, K., and James, M. N. G. (1992) Molecular structure of the acyl-enzyme intermediate in β-lactam hydrolysis at 1.7 Å resolution. *Nature 359*, 700–705.

(78) Lewandowski, E. M., Lethbridge, K. G., Sanishvili, R., Skiba, J., Kowalski, K., and Chen, Y. (2018) Mechanisms of proton relay and product release by Class A β-lactamase at ultrahigh resolution. *FEBS Journal 285*, 87–100.

(79) Vandavasi, V. G., Weiss, K. L., Cooper, J. B., Erskine, P. T., Tomanicek, S. J., Ostermann, A., Schrader, T. E., Ginell, S. L., and Coates, L. (2016) Exploring the mechanism of β-lactam ring protonation in the class A β-lactamase acylation mechanism using neutron and x-ray crystallography. *J Med Chem 59*, 474–479.

(80) Herzberg, O., and Moult, J. (1987) Bacterial resistance to β-lactam antibiotics: *Staphylococcus aureus*. *Science (1979) 236*, 694–701.

R

(81) Elings, W., Tassoni, R., Van Der Schoot, S. A., Luu, W., Kynast, J. P., Dai, L., Blok, A. J., Timmer, M., Florea, B. I., Pannu, N. S., and Ubbink, M. (2017) Phosphate promotes the recovery of *Mycobacterium tuberculosis* β-lactamase from clavulanic acid inhibition. *Biochemistry 56*, 6257–6267.

(82) Ellerby, L. M., Escobar, W. A., Fink, A. L., Mitchinson, C., and Wells, J. A. (1990) The role of lysine-234 in β-lactamase catalysis probed by site-directed mutagenesis. *Biochemistry 29*, 5797–5806.

(83) Murphy, B. P., and Pratt, R. F. (1988) Evidence for an oxyanion hole in serine β-lactamases and DD-peptidases. *Biochemical Journal 256*, 669–672.

(84) Knox, J. R., Moews, P. C., and Frère, J.-M. (1996) Molecular evolution of bacterial beta-lactam resistance. *Chem Biol 3*, 937–947.

(85) Tremblay, L. W., Fan, F., and Blanchard, J. S. (2010) Biochemical and structural characterization of *Mycobacterium tuberculosis* β-lactamase with the carbapenems ertapenem and doripenem. *Biochemistry 49*, 3766–3773.

(86) Chow, C., Xu, H., and Blanchard, J. S. (2013) Kinetic characterizations of nitrocefin, cefoxitin, and meropenem hydrolysis by β-lactamase from *Mycobacterium tuberculosis*. *Biochemiistry 52*, 4097–4104.

(87) Therrien, C., and Levesque, R. C. (2000) Molecular basis of antibiotic resistance and β-lactamase inhibition by mechanism-based inactivators: perspectives and future directions. *FEMS Microbiol Rev 24*, 251–262.

(88) Brown, A. G., Butterworth, D., Cole, M., Hanscomb, G., Hood, J. D., Reading, C., and Rolinson, G. N. (1976) Naturally occurring β-lactamase inhibitors with antibacterial activity. *J Antibiot (Tokyo) 29*, 668–669.

(89) Reading, C., and Cole, M. (1977) Clavulanic acid: a β-lactamase-inhibiting β-lactam from *Streptomyces clavuligerus*. *Antimicrob Agents Chemother 11*, 852–857.

(90) Brown, R. P. A., Aplin, R. T., and Schofield, C. J. (1996) Inhibition of TEM-2 β-lactamase from Escherichia coli by clavulanic acid: Observation of intermediates by electrospray ionization mass spectrometry. *Biochemistry 35*, 12421–12432.

(91) Tremblay, L. W., Hugonnet, J.-E., and Blanchard, J. S. (2008) Structure of the covalent adduct formed between *Mycobacterium tuberculosis* β-lactamase and clavulanate. *Biochemistry 47*, 5312–5316.

(92) Fisher, J., Belasco, J. G., Charnas, R. L., Khosla, S., and Knowles, J. R. (1980) β-Lactamase inactivation by mechanism-based reagents. *Philosphical Transactions of the Royal Society B 289*, 309–319.

(93) Aronoff, S. C., Jacobs, M. R., Johenning, S., and Yamabe, S. (1984) Comparative activities of the β-lactamase inhibitors YTR 830, sodium clavulanate, and sulbactam combined with amoxicillin or ampicillin. *Antimicrob Agents Chemother 26*, 580–582.

(94) Drawz, S. M., and Bonomo, R. A. (2010) Three decades of β-lactamase inhibitors. *Clin Microbiol Rev 23*, 160–201.

(95) Hugonnet, J.-E., and Blanchard, J. S. (2007) Irreversible inhibition of the *Mycobacterium tuberculosis* β-lactamase by clavulanate. *Biochemistry 46*, 11998–12004.

(96) Imtiaz, U., Billings, E. M., Knox, J. R., and Mobashery, S. (1994) A structure-based analysis of the inhibition of class A β-lactamases by sulbactam. *Biochemistry 33*, 5728–5738.

(97) Li, R., Feng, D., and Feng, S. (2009) Computational study on decarboxylation mechanism of β-lactamases inhibitors: Clavulanate vs. sulbactam. *Chem Phys Lett 472*, 248–253.

(98) Totir, M. A., Helfand, M. S., Carey, M. P., Sheri, A., Buynak, J. D., Bonomo, R. A., and Carey, P. R. (2007) Sulbactam forms only minimal amounts of irreversible acrylate-enzyme with SHV-1 β-lactamase. *Biochemistry 46*, 8980–8987.

(99) Li, R., Liao, J. M., Gu, C. R., Wang, Y. T., and Chen, C. L. (2011) Theoretical investigation on reaction of sulbactam with wild-type SHV-1 β-lactamase: Acylation, tautomerization, and deacylation. *Journal of Physical Chemistry B 115*, 10298–10310.

(100) Kuzin, A. P., Nukaga, M., Nukaga, Y., Hujer, A., Bonomo, R. A., and Knox, J. R. (2001) Inhibition of the SHV-1 β-lactamase by sulfones: Crystallographic observation of two reaction intermediates with tazobactam. *Biochemistry 40*, 1861–1866.

(101) Padayatti, P. S., Helfand, M. S., Totir, M. A., Carey, M. P., Hujer, A. M., Carey, P. R., Bonomo, R. A., and van den Akker, F. (2004) Tazobactam forms a stoichiometric trans-enamine intermediate in the E166A variant of SHV-1 β-Lactamase: 1.63 Å crystal structure. *Biochemistry 43*, 843–848.

(102) Xu, H., Hazra, S., and Blanchard, J. S. (2012) NXL104 irreversibly inhibits the β-lactamase from *Mycobacterium tuberculosis. Biochemistry 51*, 4551–4557.

(103) Ehmann, D. E., Jahić, H., Ross, P. L., Gu, R. F., Hu, J., Kern, G., Walkup, G. K., and Fisher, S. L. (2012) Avibactam is a covalent, reversible, non-β-lactam β-lactamase inhibitor. *Proc Natl Acad Sci U S A 109*, 11663–11668.

(104) Tassoni, R., Blok, A., Pannu, N. S., and Ubbink, M. (2019) New conformations of acylation adducts of inhibitors of β-lactamase from *Mycobacterium tuberculosis. Biochemistry 58*, 997–1009.

(105) Bonnefoy, A., Dupuis-Hamelin, C., Steier, V., Delachaume, C., Seys, C., Stachyra, T., Fairley, M., Guitton, M., and Lampilas, M. (2004) *In vitro* activity of AVE1330A, an innovative broad-spectrum non-β-lactam β-lactamase inhibitor. *Journal of Antimicrobial Chemotherapy 54*, 410–417.

(106) Ehmann, D. E., Jahić, H., Ross, P. L., Gu, R. F., Hu, J., Durand-Réville, T. F., Lahiri, S., Thresher, J., Livchak, S., Gao, N., Palmer, T., Walkup, G. K., and Fisher, S. L. (2013) Kinetics of avibactam inhibition against class A, C, and D β-lactamases. *Journal of Biological Chemistry 288*, 27960–27971.

(107) Das, C. K., and Nair, N. N. (2020) Elucidating the molecular basis of avibactam-mediated inhibition of class A β-lactamases. *Chemistry – A European Journal 26*, 9639–9651.

(108) Blizzard, T. A., Chen, H., Kim, S., Wu, J., Bodner, R., Gude, C., Imbriglio, J., Young, K., Park, Y. W., Ogawa, A., Raghoobar, S., Hairston, N., Painter, R. E., Wisniewski, D., Scapin, G., Fitzgerald, P., Sharma, N., Lu, J., Ha, S., Hermes, J., and Hammond, M. L. (2014) Discovery of MK-7655, a β-lactamase inhibitor for combination with Primaxin®. *Bioorg Med Chem Lett 24*, 780–785.

(109) Morinaka, A., Tsutsumi, Y., Yamada, M., Suzuki, K., Watanabe, T., Abe, T., Furuuchi, T., Inamura, S., Sakamaki, Y., Mitsuhashi, N., Ida, T., and Livermore, D. M. (2015) OP0595, a

R

new diazabicyclooctane: Mode of action as a serine β-lactamase inhibitor, antibiotic and β-lactam "enhancer." *Journal of Antimicrobial Chemotherapy 70*, 2779–2786.

(110) Hecker, S. J., Reddy, K. R., Totrov, M., Hirst, G. C., Lomovskaya, O., Griffith, D. C., King, P., Tsivkovski, R., Sun, D., Sabet, M., Tarazi, Z., Clifton, M. C., Atkins, K., Raymond, A., Potts, K. T., Abendroth, J., Boyer, S. H., Loutit, J. S., Morgan, E. E., Durso, S., and Dudley, M. N. (2015) Discovery of a cyclic boronic acid β-lactamase inhibitor (RPX7009) with utility vs class A serine carbapenemases. *J Med Chem 58*, 3682–3692.

(111) Liu, B., Trout, R. E. L., Chu, G., McGarry, D., Jackson, R. W., Hamrick, J. C., Daigle, D. M., Cusick, S. M., Pozzi, C., de Luca, F., Benvenuti, M., Mangani, S., Docquier, J.-D., Weiss, W. J., Pevear, D. C., Xerri, L., and Burns, C. J. (2020) Discovery of taniborbactam (VNRX-5133): A broad-spectrum serine- and metallo-β-lactamase inhibitor for carbapenem-resistant bacterial infections. *J Med Chem 63*, 2789–2801.

(112) Cahill, S. T., Cain, R., Wang, D. Y., Lohans, C. T., Wareham, D. W., Oswin, H. P., Mohammed, J., Spencer, J., Fishwick, C. W. G., McDonough, M. A., Schofield, C. J., and Brem, J. (2017) Cyclic boronates inhibit all classes of β-lactamases. *Antimicrob Agents Chemother 61*, 1–13.

(113) Krajnc, A., Brem, J., Hinchliffe, P., Calvopiña, K., Panduwawala, T. D., Lang, P. A., Kamps, J. J. A. G., Tyrrell, J. M., Widlake, E., Saward, B. G., Walsh, T. R., Spencer, J., and Schofield, C. J. (2019) Bicyclic boronate VNRX-5133 inhibits metallo- and serine-β-lactamases. *J Med Chem 62*, 8544–8556.

(114) Cahill, S. T., Tyrrell, J. M., Navratilova, I. H., Calvopiña, K., Robinson, S. W., Lohans, C. T., McDonough, M. A., Cain, R., Fishwick, C. W. G., Avison, M. B., Walsh, T. R., Schofield, C. J., and Brem, J. (2019) Studies on the inhibition of AmpC and other β-lactamases by cyclic boronates. *Biochim Biophys Acta Gen Subj 1863*, 742–748.

(115) Tooke, C. L., Hinchliffe, P., Krajnc, A., Mulholland, A. J., Brem, J., Schofield, C. J., and Spencer, J. (2020) Cyclic boronates as versatile scaffolds for KPC-2 β-lactamase inhibition. *RSC Med Chem 11*, 491–496.

(116) Soroka, D., de La Sierra-Gallay, I. L., Dubée, V., Triboulet, S., van Tilbeurgh, H., Compain, F., Ballell, L., Barros, D., Mainardi, J. L., Hugonnet, J. E., and Arthur, M. (2015) Hydrolysis of clavulanate by *Mycobacterium tuberculosis* β-lactamase BlaC harboring a canonical SDN motif. *Antimicrob Agents Chemother 59*, 5714–5720.

(117) Soroka, D., Ourghanlian, C., Compain, F., Fichini, M., Dubée, V., Mainardi, J. L., Hugonnet, J.-E., and Arthur, M. (2017) Inhibition of β-lactamases of mycobacteria by avibactam and clavulanate. *Journal of Antimicrobial Chemotherapy 72*, 1081–1088.

(118) Elings, W., Chikunova, A., van Zanten, D. B., Drenth, R., Ahmad, M. U. D., Blok, A. J., Timmer, M., Perrakis, A., and Ubbink, M. (2021) Two β-lactamase variants with reduced clavulanic acid inhibition display different millisecond dynamics. *Antimicrob Agents Chemother 65*, e02628-20.

(119) Kurz, S. G., Wolff, K. A., Hazra, S., Bethel, C. R., Hujer, A. M., Smith, K. M., Xu, Y., Tremblay, L. W., Blanchard, J. S., Nguyen, L., and Bonomo, R. A. (2013) Can inhibitor-resistant substitutions in the *Mycobacterium tuberculosis* β-lactamase BlaC lead to clavulanate

resistance?: A biochemical rationale for the use of β-lactam-β-lactamase inhibitor combinations. *Antimicrob Agents Chemother 57*, 6085–6096.

(120) Chaibi, E. B., Péduzzi, J., Farzaneh, S., Barthélémy, M., Sirot, D., and Labia, R. (1998) Clinical inhibitor-resistant mutants of the β-lactamase TEM-1 at amino-acid position 69. Kinetic analysis and molecular modelling. *Biochim Biophys Acta 1382*, 38–46.

(121) Prinarakis, E. E., Miriagou, V., Tzelepi, E., Gazouli, M., and Tzouvelekis, L. S. (1997) Emergence of an inhibitor-resistant β-lactamase (SHV-10) derived from an SHV-5 variant. *Antimicrob Agents Chemother 41*, 838–840.

(122) Bermudes, H., Jude, F., Chaibi, E. B., Arpin, C., Bebear, C., Labia, R., and Quentin, C. (1999) Molecular characterization of TEM-59 (IRT-17), a novel inhibitor- resistant TEM-derived β-lactamase in a clinical isolate of *Klebsiella oxytoca*. *Antimicrob Agents Chemother 43*, 1657–1661.

(123) Thomas, V. L., Golemi-Kotra, D., Kim, C., Vakulenko, S. B., Mobashery, S., and Shoichet, B. K. (2005) Structural consequences of the inhibitor-resistant Ser130Gly substitution in TEM β-lactamase. *Biochemistry 44*, 9330–9338.

(124) Curley, K., and Pratt, R. F. (2000) The oxyanion hole in serine β-lactamase catalysis: Interactions of thiono substrates with the active site. *Bioorg Chem 28*, 338–356.

(125) Feiler, C., Fisher, A. C., Boock, J. T., Marrichi, M. J., Wright, L., Schmidpeter, P. A. M., Blankenfeldt, W., Pavelka, M., and DeLisa, M. P. (2013) Directed evolution of *Mycobacterium tuberculosis* β-lactamase reveals gatekeeper residue that regulates antibiotic resistance and catalytic efficiency. *PLoS One 8*, e73123.

(126) Stec, B., Holtz, K. M., Wojciechowski, C. L., and Kantrowitz, E. R. (2005) Structure of the wild-type TEM-1 β-lactamase at 1.55 Å and the mutant enzyme Ser70Ala at 2.1 Å suggest the mode of noncovalent catalysis for the mutant enzyme. *Acta Crystallogr D Biol Crystallogr 61*, 1072–1079.

(127) Chikunova, A., and Ubbink, M. (2022) The roles of highly conserved, non-catalytic residues in class A β-lactamases. *Protein Science 31*, e4328.

(128) World Health Organization. (2020) Global Tuberculosis Report 2020.

(129) Singh, R., Dwivedi, S. P., Gaharwar, U. S., Meena, R., Rajamani, P., and Prasad, T. (2020) Recent updates on drug resistance in *Mycobacterium tuberculosis. J Appl Microbiol 128*, 1547–1567.

(130) Chambers, H. F., Moreau, D., Yajko, D., Miick, C., Wagner, C., Hackbarth, C., Kocagoz, S., Rosenberg, E., Hadley, W. K., and Nikaido, H. (1995) Can penicillins and other β-lactam antibiotics be used to treat tuberculosis? *Antimicrob Agents Chemother 39*, 2620–2624.

(131) Kurz, S. G., and Bonomo, R. A. (2012) Reappraising the use of β-lactams to treat tuberculosis. *Expert Rev Anti Infect Ther 10*, 999–1006.

(132) Dhar, N., Dubeé, V., Ballell, L., Cuinet, G., Hugonnet, J. E., Signorino-Gelo, F., Barros, D., Arthur, M., and McKinney, J. D. (2015) Rapid cytolysis of *Mycobacterium tuberculosis* by faropenem, an orally bioavailable β-lactam antibiotic. *Antimicrob Agents Chemother 59*, 1308–1319.

R

(133) Diacon, A. H., van der Merwe, L., Barnard, M., von Groote-Bidlingmaier, F., Lange, C., García-Basteiro, A. L., Sevene, E., Ballell, L., and Barros-Aguirre, D. (2016) β-Lactams against tuberculosis — new trick for an old dog? *New England Journal of Medicine 375*, 392–393.

(134) Bush, K. (1988) β-Lactamase inhibitors from laboratory to clinic. *Clin Microbiol Rev 1*, 109–123.

(135) Papp-Wallace, K. M., and Bonomo, R. A. (2016) New β-lactamase inhibitors in the clinic. *Infect Dis Clin North Am 30*, 441–464.

(136) English, A. R., Retsema, J. A., Girard, A. E., Lynch, J. E., and Barth, W. E. (1978) CP-45,899, a β-lactamase inhibitor that extends the antibacterial spectrum of β-lactams: Initial bacteriological characterization. *Antimicrob Agents Chemother 14*, 414–419.

(137) Lee, N., Yuen, K.-Y., and Kumana, C. R. (2003) Clinical role of β-lactam/β-lactamase inhibitor combinations. *Drugs 63*, 1511–1524.

(138) Zhang, D., Wang, Y., Lu, J., and Pang, Y. (2015) *In vitro* activity of β-lactams in combination with β-lactamase inhibitors against multidrug-resistant *Mycobacterium tuberculosis* isolates. *Antimicrob Agents Chemother 60*, 393–399.

(139) Tremblay, L. W., Xue, H., and Blanchard, J. S. (2010) Structures of the Michaelis-complex (1.2 Å) and the covalent acyl-intermediate (2.0 Å) of cefamandole bound in the active sites of the *Mycobacterium tuberculosis* β-lactamase K73A and E166A mutants. *Biochemistry 49*, 9685–9687.

(140) Gu, S., Chen, J., Dobos, K. M., Bradbury, E. M., Belisle, J. T., and Chen, X. (2003) Comprehensive proteomic profiling of the membrane constituents of a *Mycobacterium tuberculosis* strain. *Mol Cell Proteomics 2*, 1284–1296.

(141) Philippon, A., Slama, P., and Labia, R. (2016) A structure-based classification of class A β-lactamases, a broadly diverse family of enzymes. *Clin Microbiol Rev 29*, 29–57.

(142) Hussain, M., Pastor, F. I. J., and Lampen, J. O. (1987) Cloning and sequencing of the *blaZ* gene encoding β-lactamase III, a lipoprotein of *Bacillus cereus* 569/H. *J Bacteriol 169*, 579–586.

(143) Antunes, N. T., Frase, H., Toth, M., and Vakulenko, S. B. (2012) The class A β-lactamase FTU-1 is native to *Francisella tularensis*. *Antimicrob Agents Chemother 56*, 666–671.

(144) Kuzin, A. P., Nukaga, M., Nukaga, Y., Hujer, A. M., Bonomo, R. A., and Knox, J. R. (1999) Structure of the SHV-1 β-lactamase. *Biochemistry 38*, 5720–5727.

(145) Ourghanlian, C., Soroka, D., and Arthur, M. (2017) Inhibition by avibactam and clavulanate of the β-Lactamases KPC-2 and CTX-M-15 harboring the substitution N132G the conserved SDN motif. *Antimicrob Agents Chemother 61*, e02510-16.

(146) Brennan, P. J. (2003) Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis*. *Tuberculosis 83*, 91–97.

(147) Smeets, L. C., Becker, S. C., Barcak, G. J., Vandenbroucke-Grauls, C. M. J. E., Bitter, W., and Goosen, N. (2006) Functional characterization of the competence protein DprA/Smf in *Escherichia coli*. *FEMS Microbiol Lett 263*, 223–228.

(148) Chikunova, A., Manley, M. P., Ud Din Ahmad, M., Bilman, T., Perrakis, A., and Ubbink, M. (2021) Conserved residues Glu37 and Trp229 play an essential role in protein folding of β-lactamase. *FEBS J 288*, 5708–5722.

(149) Eaton, J. W., Bateman, D., Hauberg, S., and Wehbring, R. (2019) GNU Octave version 5.2.0 manual: a high-level interactive language for numerical computations.

(150) Pervushin, K., Riek, R., Wider, G., and Wüthrich, K. (1997) Attenuated T2 relaxation by mutual cancellation of dipole–dipole coupling and chemical shift anisotropy. *Proc Natl Acad Sci U S A 94*, 12366–12371.

(151) Schanda, P., Van Melckebeke, H., and Brutscher, B. (2006) Speeding up three-dimensional protein NMR experiments to a few minutes. *J Am Chem Soc 128*, 9042–9043.

(152) Vranken, W. F., Boucher, W., Stevens, T. J., Fogh, R. H., Pajon, A., Llinas, M., Ulrich, E. L., Markley, J. L., Ionides, J., and Laue, E. D. (2005) The CCPN data model for NMR spectroscopy: Development of a software pipeline. *Proteins: Structure, Function and Genetics 59*, 687–696.

(153) Newman, J., Egan, D., Walter, T. S., Meged, R., Berry, I., Jelloul, M. Ben, Sussman, J. L., Stuart, D. I., and Perrakis, A. (2005) Towards rationalization of crystallization screening for small- to medium-sized academic laboratories: The PACT/JCSG+ strategy. *Acta Crystallogr D Biol Crystallogr 61*, 1426–1431.

(154) Kabsch, W. (2010) XDS. *Acta Crystallogr D Biol Crystallogr 66*, 125–132.

(155) Evans, P. R. (2011) An introduction to data reduction: Space-group determination, scaling and intensity statistics. *Acta Crystallogr D Biol Crystallogr 67*, 282–292.

(156) Winn, M. D., Ballard, C. C., Cowtan, K. D., Dodson, E. J., Emsley, P., Evans, P. R., Keegan, R. M., Krissinel, E. B., Leslie, A. G. W., McCoy, A., McNicholas, S. J., Murshudov, G. N., Pannu, N. S., Potterton, E. A., Powell, H. R., Read, R. J., Vagin, A., and Wilson, K. S. (2011) Overview of the CCP4 suite and current developments. *Acta Crystallogr D Biol Crystallogr 67*, 235–242.

(157) Joosten, R. P., Long, F., Murshudov, G. N., and Perrakis, A. (2014) The PDB-REDO server for macromolecular structure model optimization. *IUCrJ 1*, 213–220.

(158) Chen, V. B., Arendall III, W. B., Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S., and Richardson, D. C. (2010) MolProbity: All-atom structure validation for macromolecular crystallography. *Acta Crystallogr D Biol Crystallogr 66*, 12–21.

(159) Sobolev, O. V., Afonine, P. V., Moriarty, N. W., Hekkelman, M. L., Joosten, R. P., Perrakis, A., and Adams, P. D. (2020) A global Ramachandran score identifies protein structures with unlikely stereochemistry. *Structure 28*, 1249–1258.

(160) Ibuka, A. S., Ishii, Y., Galleni, M., Ishiguro, M., Yamaguchi, K., Frère, J. M., Matsuzawa, H., and Sakai, H. (2003) Crystal structure of extended-spectrum β-lactamase Toho-1: Insights into the molecular mechanism for catalytic reaction and substrate specificity expansion. *Biochemistry 42*, 10634–10643.

(161) Alsenani, T. A., Viviani, S. L., Kumar, V., Taracila, M. A., Bethel, C. R., Barnes, M. D., Papp-Wallace, K. M., Shields, R. K., Nguyen, M. H., Clancy, C. J., Bonomo, R. A., and van den Akker, F. (2022) Structural characterization of the D179N and D179Y variants of KPC-2 β-lactamase: Ω-Loop destabilization as a mechanism of resistance to ceftazidime-avibactam. *Antimicrob Agents Chemother 66*, e02414-21.

R

(162) Vakulenko, S. B., Tóth, M., Taibi, P., Mobashery, S., and Lerner, S. A. (1995) Effects of Asp-179 mutations in TEM$_{pUC19}$ β-lactamase on susceptibility to β-lactams. *Antimicrob Agents Chemother 39*, 1878–1880.

(163) Vakulenko, S. B., Taibi-Tronche, P., Tóth, M., Massova, I., Lerner, S. A., and Mobashery, S. (1999) Effects on substrate profile by mutational substitutions at positions 164 and 179 of the class A TEM$_{pUC19}$ β-lactamase from *Escherichia coli. Journal of Biological Chemistry 274*, 23052–23060.

(164) Gonzalez, C. E., Roberts, P., and Ostermeier, M. (2019) Fitness effects of single amino acid insertions and deletions in TEM-1 β-lactamase. *J Mol Biol 431*, 2320–2330.

(165) Firnberg, E., Labonte, J. W., Gray, J. J., and Ostermeier, M. (2014) A comprehensive, high-resolution map of a gene's fitness landscape. *Mol Biol Evol 31*, 1581–1592.

(166) Barnes, M. D., Winkler, M. L., Taracila, M. A., Page, M. G., Desarbre, E., Kreiswirth, B. N., Shields, R. K., Nguyen, M.-H., Clancy, C., Spellberg, B., Papp-Wallace, K. M., and Bonomo, R. A. (2017) *Klebsiella pneumoniae* Carbapenemase-2 (KPC-2), substitutions at Ambler position Asp179, and resistance to ceftazidime-avibactam: Unique antibiotic-resistant phenotypes emerge from β-lactamase protein engineering. *mBio* (Bush, K., Ed.) *8*, e00528-17.

(167) Levitt, P. S., Papp-Wallace, K. M., Taracila, M. A., Hujer, A. M., Winkler, M. L., Smith, K. M., Xu, Y., Harris, M. E., and Bonomo, R. A. (2012) Exploring the role of a conserved class A residue in the Ω-loop of KPC-2 β-lactamase: A mechanism for ceftazidime hydrolysis. *Journal of Biological Chemistry 287*, 31783–31793.

(168) Stojanoski, V., Chow, D. C., Hu, L., Sankaran, B., Gilbert, H. F., Prasad, B. V. V., and Palzkill, T. (2015) A triple mutant in the Ω-loop of TEM-1 β-lactamase changes the substrate profile via a large conformational change and an altered general base for catalysis. *Journal of Biological Chemistry 290*, 10382–10394.

(169) Papp-Wallace, K. M., Becka, S. A., Taracila, M. A., Winkler, M. L., Gatta, J. A., Rholl, D. A., Schweizer, H. P., and Bonomo, R. A. (2016) Exposing a β-lactamase "twist": The mechanistic basis for the high level of ceftazidime resistance in the C69F variant of the *Burkholderia pseudomallei* PenI β-lactamase. *Antimicrob Agents Chemother 60*, 777–788.

(170) Sawyer, L., and James, M. N. G. (1982) Carboxyl–carboxylate interactions in proteins. *Nature 295*, 79–80.

(171) Lin, J., Pozharski, E., and Wilson, M. A. (2017) Short carboxylic acid-carboxylate hydrogen bonds can have fully localized protons. *Biochemistry 56*, 391–402.

(172) van Alen, I., Chikunova, A., Safeer, A. A., Ahmad, M. U. D., Perrakis, A., and Ubbink, M. (2021) The G132S mutation enhances the resistance of *Mycobacterium tuberculosis* β-lactamase against sulbactam. *Biochemistry 60*, 2236–2245.

(173) Castanheira, M., Arends, S. J. R., Davis, A. P., Woosley, L. N., Bhalodi, A. A., and MacVane, S. H. (2018) Analyses of a ceftazidime-avibactam-resistant *Citrobacter freundii* isolate carrying *bla$_{KPC-2}$* reveals a heterogenous population and reversible genotype. *mSphere 3*, e00408-18.

(174) Bonomo, R. (1997) OHIO-1 β-lactamase mutants: Asp179Gly mutation confers resistance to ceftazidime. *FEMS Microbiol Lett 152*, 275–278.

(175) Winkler, M. L., Papp-Wallace, K. M., and Bonomo, R. A. (2015) Activity of ceftazidime/avibactam against isogenic strains of *Escherichia coli* containing KPC and SHV β-lactamases with single amino acid substitutions in the Ω-loop. *Journal of Antimicrobial Chemotherapy 70*, 2279–2286.

(176) Majiduddin, F. K., and Palzkill, T. (2003) An analysis of why highly similar enzymes evolve differently. *Genetics 163*, 457–466.

(177) Patel, M. P., Hu, L., Stojanoski, V., Sankaran, B., Prasad, B. V. V., and Palzkill, T. (2017) The drug-resistant variant P167S expands the substrate profile of CTX-M β-lactamases for oxyimino-cephalosporin antibiotics by enlarging the active site upon acylation. *Biochemistry 56*, 3443–3453.

(178) Taracila, M. A., Bethel, C. R., Hujer, A. M., Papp-Wallace, K. M., Barnes, M. D., Rutter, J. D., VanPelt, J., Shurina, B. A., van den Akker, F., Clancy, C. J., Nguyen, M. H., Cheng, S., Shields, R. K., Page, R. C., and Bonomo, R. A. (2022) Different conformations revealed by NMR underlie resistance to ceftazidime/avibactam and susceptibility to meropenem and imipenem among D179Y variants of KPC β-lactamase. *Antimicrob Agents Chemother 66*, e02124-21.

(179) Herzberg, O., Kapadia, G., Blanco, B., Smith, T. S., and Coulson, A. (1991) Structural basis for the inactivation of the P54 mutant of β-lactamase from *Staphylococcus aureus* PC1. *Biochemistry 30*, 9503–9509.

(180) Clabbers, M. T. B., Gruene, T., Parkhurst, J. M., Abrahams, J. P., and Waterman, D. G. (2018) Electron diffraction data processing with DIALS. *Acta Crystallogr D Struct Biol 74*, 506–518.

(181) Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D., and Higgins, D. G. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol 7*, 539.

(182) Chen, Y., Delmas, J., Sirot, J., Shoichet, B., and Bonnet, R. (2005) Atomic resolution structures of CTX-M β-lactamases: Extended spectrum activities from increased mobility and decreased stability. *J Mol Biol 348*, 349–362.

(183) Ke, W., Bethel, C. R., Thomson, J. M., Bonomo, R. A., and Van Den Akker, F. (2007) Crystal structure of KPC-2: Insights into carbapenemase activity in class A β-lactamases. *Biochemistry 46*, 5732–5740.

(184) Swarén, P., Maveyraud, L., Raquet, X., Cabantous, S., Duez, C., Pédelacq, J.-D., Mariotte-Boyer, S., Mourey, L., Labia, R., Nicolas-Chanoine, M.-H., Nordmann, P., Frère, J.-M., and Samama, J.-P. (1998) X-ray Analysis of the NMC-A β-Lactamase at 1.64-Å resolution, a class A carbapenemase with broad substrate specificity. *Journal of Biological Chemistry 273*, 26714–26721.

(185) Escobar, W. A., Miller, J., and Fink, A. L. (1994) Effects of site-specific mutagenesis of tyrosine 105 in a class A β-lactamase. *Biochemical Journal 303*, 555–558.

(186) Doucet, N., De Wals, P.-Y., and Pelletier, J. N. (2004) Site-saturation mutagenesis of tyr-105 reveals its importance in substrate stabilization and discrimination in TEM-1 β-lactamase. *Journal of Biological Chemistry 279*, 46295–46303.

(187) Krishnan, N. P., Nguyen, N. Q., Papp-Wallace, K. M., Bonomo, R. A., and Van Den Akker, F. (2015) Inhibition of *Klebsiella* β-lactamases (SHV-1 and KPC-2) by avibactam: A structural study. *PLoS One 10*, 1–13.

(188) Lahiri, S. D., Mangani, S., Durand-Reville, T., Benvenuti, M., De Luca, F., Sanyal, G., and Docquier, J.-D. (2013) Structural insight into potent broad-spectrum inhibition with reversible recyclization mechanism: Avibactam in complex with CTX-M-15 and *Pseudomonas aeruginosa* AmpC β-Lactamases. *Antimicrob Agents Chemother 57*, 2496–2505.

(189) World Health Organization. (2021) Global Tuberculosis Report 2021.

(190) Russell, D. G. (2011) *Mycobacterium tuberculosis* and the intimate discourse of a chronic infection. *Immunol Rev 240*, 252–268.

(191) Myllymäki, H., Bäuerlein, C. A., and Rämet, M. (2016) The zebrafish breathes new life into the study of tuberculosis. *Front Immunol 7*.

(192) Cronan, M. R., and Tobin, D. M. (2014) Fit for consumption: Zebrafish as a model for tuberculosis. *DMM Disease Models and Mechanisms 7*, 777–784.

(193) Matty, M. A., Roca, F. J., Cronan, M. R., and Tobin, D. M. (2015) Adventures within the speckled band: heterogeneity, angiogenesis, and balanced inflammation in the tuberculous granuloma. *Immunol Rev 264*, 276–287.

(194) Varela, M., and Meijer, A. H. (2022) A fresh look at mycobacterial pathogenicity with the zebrafish host model. *Mol Microbiol 117*, 661–669.

(195) Parikka, M., Hammarén, M. M., Harjula, S. K. E., Halfpenny, N. J. A., Oksanen, K. E., Lahtinen, M. J., Pajula, E. T., Iivanainen, A., Pesu, M., and Rämet, M. (2012) *Mycobacterium marinum* causes a latent infection that can be reactivated by gamma irradiation in adult zebrafish. *PLoS Pathog 8*.

(196) Stinear, T. P., Seemann, T., Harrison, P. F., Jenkin, G. A., Davies, J. K., Johnson, P. D. R., Abdellah, Z., Arrowsmith, C., Chillingworth, T., Churcher, C., Clarke, K., Cronin, A., Davis, P., Goodhead, I., Holroyd, N., Jagels, K., Lord, A., Moule, S., Mungall, K., Norbertczak, H., Quail, M. A., Rabbinowitsch, E., Walker, D., White, B., Whitehead, S., Small, P. L. C., Brosch, R., Ramakrishnan, L., Fischbach, M. A., Parkhill, J., and Cole, S. T. (2008) Insights from the complete genome sequence of *Mycobacterium marinum* on the evolution of *Mycobacterium tuberculosis*. *Genome Res 18*, 729–741.

(197) Clay, H., Davis, J. M., Beery, D., Huttenlocher, A., Lyons, S. E., and Ramakrishnan, L. (2007) Dichotomous role of the macrophage in early *Mycobacterium marinum* infection of the zebrafish. *Cell Host Microbe 2*, 29–39.

(198) Swaim, L. E., Connolly, L. E., Volkman, H. E., Humbert, O., Born, D. E., and Ramakrishnan, L. (2006) *Mycobacterium marinum* infection of adult zebrafish causes caseating granulomatous tuberculosis and is moderated by adaptive immunity. *Infect Immun 74*, 6108–6117.

(199) van der Sar, A. M., Abdallah, A. M., Sparrius, M., Reinders, E., Vandenbroucke-Grauls, C. M. J. E., and Bitter, W. (2004) *Mycobacterium marinum* strains can be divided into two distinct types based on genetic diversity and virulence. *Infect Immun 72*, 6306–6312.

(200) Meijer, A. H. (2016) Protection and pathology in TB: learning from the zebrafish model. *Semin Immunopathol 38*, 261–273.

(201) Yang, C. T., Cambier, C. J., Davis, J. M., Hall, C. J., Crosier, P. S., and Ramakrishnan, L. (2012) Neutrophils exert protection in the early tuberculous granuloma by oxidative killing of mycobacteria phagocytosed from infected macrophages. *Cell Host Microbe 12*, 301–312.

(202) Ramakrishnan, L. (2012) Revisiting the role of the granuloma in tuberculosis. *Nat Rev Immunol 12*, 352–366.

(203) Davis, J. M., and Ramakrishnan, L. (2009) The role of the granuloma in expansion and dissemination of early tuberculous infection. *Cell 136*, 37–49.

(204) Matty, M. A., Knudsen, D. R., Walton, E. M., Beerman, R. W., Cronan, M. R., Pyle, C. J., Hernandez, R. E., and Tobin, D. M. (2019) Potentiation of P2RX7 as a host-directed strategy for control of mycobacterial infection. *Elife 8*.

(205) Boland, R., Heemskerk, M. T., Forn-Cuní, G., Korbee, C. J., Walburg, K. V., Esselink, J. J., Carvalho dos Santos, C., de Waal, A. M., van der Hoeven, D. C. M., van der Sar, E., de Ries, A. S., Xie, J., Spaink, H. P., van der Vaart, M., Haks, M. C., Meijer, A. H., and Ottenhoff, T. H. M. (2023) Repurposing tamoxifen as potential host-directed therapeutic for tuberculosis. *mBio* (Buchrieser, C., Ed.) *14*.

(206) Bhandari, M., Soria-Carrera, H., Wohlmann, J., Dal, N.-J. K., de la Fuente, J. M., Martín-Rapún, R., Griffiths, G., and Fenaroli, F. (2023) Subcellular localization and therapeutic efficacy of polymeric micellar nanoparticles encapsulating bedaquiline for tuberculosis treatment in zebrafish. *Biomater Sci 11*, 2103–2114.

(207) Meijers, A. S., Troost, R., Ummels, R., Maaskant, J., Speer, A., Nejentsev, S., Bitter, W., and Kuijl, C. P. (2020) Efficient genome editing in pathogenic mycobacteria using *Streptococcus thermophilus* CRISPR1-Cas9. *Tuberculosis 124*, 101983.

(208) Papp-Wallace, K. M., Winkler, M. L., Taracila, M. A., and Bonomo, R. A. (2015) Variants of β-lactamase KPC-2 that are resistant to inhibition by avibactam. *Antimicrob Agents Chemother 59*, 3710–3717.

(209) Mehta, A. C., Hart-Davies, S., Paynet, J., and Lacey, R. W. (1994) Stability of amoxycillin and potassium clavulanate in co-amoxiclav oral suspension. *J Clin Pharm Ther 19*, 313–315.

(210) Peace, N., Olubukola, O., and Moshood, A. (2012) Stability of reconstituted amoxicillin clavulanate potassium under simulated in-home storage conditions. *J Appl Pharm Sci 2*, 28–31.

(211) Zimmermann, W., and Rosselet, A. (1977) Function of the outer membrane of *Escherichia coli* as a permeability barrier to beta-lactam antibiotics. *Antimicrob Agents Chemother 12*, 368–372.

(212) Jarlier, V., and Nikaido, H. (1990) Permeability barrier to hydrophilic solutes in *Mycobacterium chelonei. J Bacteriol 172*, 1418–1423.

(213) Parish, T., and Brown, A. C. (2008) Mycobacteria protocols: Second edition.

(214) Camacho, L. R., Constant, P., Raynaud, C., Lanéelle, M. A., Triccas, J. A., Gicquel, B., Daffé, M., and Guilhot, C. (2001) Analysis of the phthiocerol dimycocerosate locus of *Mycobacterium tuberculosis*. Evidence that this lipid is involved in the cell wall permeability barrier. *Journal of Biological Chemistry 276*, 19845–19854.

R

(215) Ortalo-Magné, A., Lemassu, A., Elle, M.-A. L., Bardou, F., Silve, G., Gounon, P., Marchal, G., and Daffé, M. (1996) Identification of the surface-exposed lipids on the cell envelopes of *Mycobacterium tuberculosis* and other mycobacterial species. *J Bacteriol 178*, 456–461.

(216) Rennekamp, A. J., and Peterson, R. T. (2015) 15 years of zebrafish chemical screening. *Curr Opin Chem Biol 24*, 58–70.

(217) Bootorabi, F., Manouchehri, H., Changizi, R., Barker, H., Palazzo, E., Saltari, A., Parikka, M., Pincelli, C., and Aspatwar, A. (2017) Zebrafish as a model organism for the development of drugs for skin cancer. *Int J Mol Sci 18*, 1–15.

(218) Cassar, S., Adatto, I., Freeman, J. L., Gamse, J. T., Iturria, I., Lawrence, C., Muriana, A., Peterson, R. T., van Cruchten, S., and Zon, L. I. (2020) Use of zebrafish in drug discovery toxicology. *Chem Res Toxicol 33*, 95–118.

(219) Gustafson, A. L., Stedman, D. B., Ball, J., Hillegass, J. M., Flood, A., Zhang, C. X., Panzica-Kelly, J., Cao, J., Coburn, A., Enright, B. P., Tornesi, M. B., Hetheridge, M., and Augustine-Rauch, K. A. (2012) Inter-laboratory assessment of a harmonized zebrafish developmental toxicology assay - Progress report on phase I. *Reproductive Toxicology 33*, 155–164.

(220) Ordas, A., Raterink, R. J., Cunningham, F., Jansen, H. J., Wiweger, M. I., Jong-Raadsen, S., Bos, S., Bates, R. H., Barros, D., Meijer, A. H., Vreeken, R. J., Ballell-Pages, L., Dirks, R. P., Hankemeier, T., and Spaink, H. P. (2015) Testing tuberculosis drug efficacy in a zebrafish high-throughput translational medicine screen. *Antimicrob Agents Chemother 59*, 753–762.

(221) Habjan, E., Ho, V. Q. T., Gallant, J., van Stempvoort, G., Jim, K. K., Kuijl, C., Geerke, D. P., Bitter, W., and Speer, A. (2021) An anti-tuberculosis compound screen using a zebrafish infection model identifies an aspartyl-tRNA synthetase inhibitor. *Dis Model Mech 14*.

(222) Dubée, V., Bernut, A., Cortes, M., Lesne, T., Dorchene, D., Lefebvre, A. L., Hugonnet, J. E., Gutmann, L., Mainardi, J. L., Herrmann, J. L., Gaillard, J. L., Kremer, L., and Arthur, M. (2014) β-Lactamase inhibition by avibactam in *Mycobacterium abscessus*. *Journal of Antimicrobial Chemotherapy 70*, 1051–1058.

(223) Lefebvre, A. L., Le Moigne, V., Bernut, A., Veckerlé, C., Compain, F., Herrmann, J. L., Kremer, L., Arthur, M., and Mainardi, J. L. (2017) Inhibition of the β-lactamase Bla$_{Mab}$ by avibactam improves the *in vitro* and *in vivo* efficacy of imipenem against *Mycobacterium abscessus*. *Antimicrob Agents Chemother 61*, e02440-16.

(224) Jabila Mary, T. R., Kannan, R. R., Muthamil Iniyan, A., Carlton Ranjith, W. A., Nandhagopal, S., Vishwakarma, V., and Prakash Vincent, S. G. (2021) β-lactamase inhibitory potential of kalafungin from marine *Streptomyces* in *Staphylococcus aureus* infected zebrafish. *Microbiol Res 244*, 126666.

(225) Ramakrishnan, L., and Falkow, S. (1994) *Mycobacterium marinum* persists in cultured mammalian cells in a temperature-restricted fashion. *Infect Immun 62*, 3222–3229.

(226) Huff, J., Czyz, A., Landick, R., and Niederweis, M. (2010) Taking phage integration to the next level as a genetic tool for mycobacteria. *Gene 468*, 8–19.

(227) Takaki, K., Davis, J. M., Winglee, K., and Ramakrishnan, L. (2013) Evaluation of the pathogenesis and treatment of *Mycobacterium marinum* infection in zebrafish. *Nat Protoc 8*, 1114–1124.

(228) Stirling, D. R., Suleyman, O., Gil, E., Elks, P. M., Torraca, V., Noursadeghi, M., and Tomlinson, G. S. (2020) Analysis tools to quantify dissemination of pathology in zebrafish larvae. *Sci Rep 10*, 3149.

(229) Mood, A. M. (1954) On the asymptotic efficiency of certain nonparametric two-sample tests. *The Annals of Mathematical Statistics 25*, 514–522.

(230) Stiffler, M. A., Hekstra, D. R., and Ranganathan, R. (2015) Evolvability as a function of purifying selection in TEM-1 β-lactamase. *Cell 160,* 882–892.

(231) Steinberg, B., and Ostermeier, M. (2016) Shifting fitness and epistatic landscapes reflect trade-offs along an evolutionary pathway. *J Mol Biol 428*, 2730–2743.

(232) Imtiaz, U., Billings, E., Knox, J. R., Manavathu, E. K., Lerner, S. A., and Mobashery, S. (1993) Inactivation of class A β-lactamases by clavulanic acid: the role of arginine-244 in a proposed nonconcerted sequence of events. *J Am Chem Soc 115*, 4435–4442.

(233) Mendonça, N., Manageiro, V., Robin, F., Salgado, M. J., Ferreira, E., Caniça, M., and Bonnet, R. (2008) The Lys234Arg substitution in the enzyme SHV-72 is a determinant for resistance to clavulanic acid inhibition. *Antimicrob Agents Chemother 52*, 1806–1811.

(234) Benard, E. L., van der Sar, A. M., Ellett, F., Lieschke, G. J., Spaink, H. P., and Meijer, A. H. (2012) Infection of Zebrafish Embryos with Intracellular Bacterial Pathogens. *Journal of Visualized Experiments* e3781.

(235) Deshpande, D., Srivastava, S., Chapagain, M., Magombedze, G., Martin, K. R., Cirrincione, K. N., Lee, P. S., Koeuth, T., Dheda, K., and Gumbo, T. (2017) Ceftazidime-avibactam has potent sterilizing activity against highly drug-resistant tuberculosis. *Sci Adv 3*.

(236) Jain, P., Hsu, T., Arai, M., Biermann, K., Thaler, D. S., Nguyen, A., González, P. A., Tufariello, J. M., Kriakov, J., Chen, B., Larsen, M. H., and Jacobs, W. R. (2014) Specialized Transduction Designed for Precise High-Throughput Unmarked Deletions in Mycobacterium tuberculosis. *mBio* (Rubin, E. J., Ed.) *5*.

(237) Liang, Y. C., Berton, S., Reeks, C., and Sun, J. (2022) An in vivo biosafety-level-2-compatible model of Mycobacterium tuberculosis infection for drug susceptibility testing. *STAR Protoc 3*, 101575.

(238) Bush, K., and Bradford, P. A. (2019) Interplay between β-lactamases and new β-lactamase inhibitors. *Nat Rev Microbiol 17*, 295–306.

(239) Jalde, S. S., and Choi, H. K. (2020) Recent advances in the development of β-lactamase inhibitors. *Journal of Microbiology 58*, 633–647.

R