Minireview

Influence of substrates and inhibitors on the structure of *Klebsiella pneumoniae* carbapenemase-2

Ben A Shurina and Richard C Page (ORCID)

Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056, USA
Corresponding author: Richard C Page. Email: pagerc@MiamiOH.edu

Impact statement

The work herein is important to the field as it provides a clear and succinct accounting of available KPC-2 structures. The work advances the field by collecting and analyzing differences and similarities across the available structures. This work features new analyses and interpretations of the existing structures which will impact the field in a positive way by making structural insights more widely available among the beta-lactamase community.

Abstract

The hydrolysis of last resort carbapenem antibiotics by *Klebsiella pneumoniae* carbapenemase-2 (KPC-2) presents a significant danger to global health. Combined with horizontal gene transfer, the emergence KPC-2 threatens to quickly expand carbapenemase activity to ever increasing numbers of pathogens. Our understanding of KPC-2 has greatly increased over the past decade thanks, in great part, to 20 crystal structures solved by groups around the world. These include apo KPC-2 structures, along with structures featuring a library of 10 different inhibitors representing diverse structural and functional classes. Herein we focus on cataloging the available KPC-2 structures and presenting a discussion of key aspects of each structure and important relationships between structures. Although the available structures do not provide information on dynamic motions with KPC-2, and the family of structures indicates small conformational changes across a wide array of bound inhibitors, substrates, and products, the structures provide a strong foundation for additional studies in the coming years to discover new KPC-2 inhibitors.

Keywords: Beta-lactamase, KPC-2, inhibitors

Introduction

The development, mass production, and administration of antimicrobial drugs were a turning point in modern medicine.[1] At the start of the 20th century, infectious bacterial diseases were the leading causes of human mortality in the United States.[2] The clinical success of Penicillin G prompted an explosion of development of β-lactam drugs[3] that was immediately met with the spread of bacterial expression of β-lactamases, enzymes capable of hydrolyzing, and thereby inactivating, β-lactam drugs. Of the thousands of known β-lactamases,[4] carbapenemases are of particular concern due to their ability to hydrolyze last resort drugs.[5] *Klebsiella pneumoniae* carbapenemase-2 (KPC-2) is a serine β-lactamase that poses a significant threat to global health.[6,7] This review details structural elements of KPC-2 and the nuanced structural changes observed for complexes of KPC-2 with inhibitors and natural substrates.

KPC-2 structural features

KPC-2 is a 293 amino acid protein in length exhibiting a mixed α and β structure. Although the mature protein is 28 kDa, the first 24 residues of the N-terminus comprise a signal peptide that designates the protein for secretory export. The overall architecture of KPC-2 (Figure 1) is consistent with other class-A β-lactamases such as TEM β-lactamase (named after the initials of the original patient) and the sulfhydryl variant of TEM β-lactamase (SHV).[8] KPC-2 has two major sub-domains: one cluster of α-helices, and the other an $\alpha+\beta$ sandwich with a core of five β-strands.[9] The β-sheet of the $\alpha+\beta$ sandwich is formed by both the N and C terminal regions of the protein, while the α-helical subunit for KPC-2 sequentially lies between the outermost strands, 2 and 3, of the β-sheet. The $\alpha+\beta$ sandwich and α-helical cluster subdomains are tethered by a conserved disulfide bridge between C69 and C238 that is thought to contribute substrate specificity for
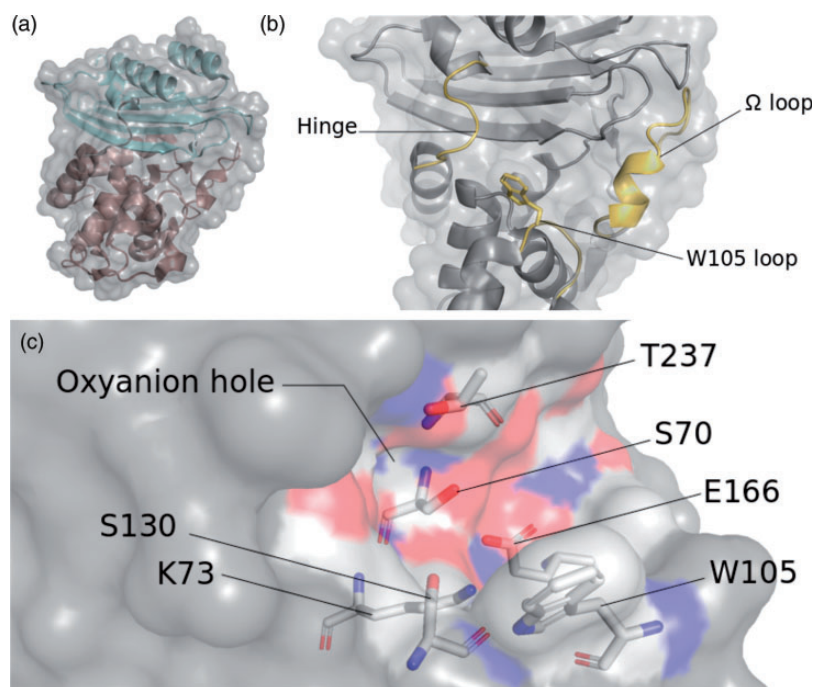
Figure 1. Panel A shows the two sub-domains of KPC-2, the $\alpha+\beta$ sandwich (*blue*) and the α-helical cluster (*red*). Panel B highlights the three loop regions near the active site. The sidechain of W105 has been rendered with stick representation to highlight the "gating" role it plays for the active site. Panel C is a rendering of the active site with the surface of the active site colored by element and select active site residues shown as sticks. The KPC-2 structure with PDB code 2OV5 was used as the basis for all representations in this figure. (A color version of this figure is available in the online journal.)

class-A β-lactamases[10] as the resulting cleft between the $\alpha+\beta$ sandwich and α-helical cluster subdomains forms the active site of the enzyme. The active site itself is flanked by three loop regions. The loop region comprised of residues N214-H219 between α-helices $\alpha10$ and $\alpha11$ is homologous to the "hinge" region in TEM-1.[11] A second loop between α-helices $\alpha3$ and $\alpha4$ contains residue W105 at the periphery of the active site. The side chain of W105 engages in π–π stacking interactions with the penam rings of substrates.[12] While tryptophan is not conserved at position 105 across Class-A β-lactamases, these π–π stacking interactions play a role in substrate binding and specificity[13] and these interactions are conserved as W105 is replaced by histidine in *Serratia marcescens* enzyme 1 (SME-1) and tyrosine in both TEM-1 and SHV-1.[14,15] A third loop comprised of residues R164-D179 is known as the Ω-loop and is critical for catalytic activity and substrate specificity.[16,17] The Ω-loop is highly conserved across Class-A β-lactamases[18] and is predicted to be a "hot spot" for mutations as bacteria respond to the evolutionary pressures of antibiotic use. The active site serine at position 70, by Ambler numbering,[19] is in close spatial proximity to K73, S130, and E166, residues identified as catalytically important for β-lactam hydrolysis.[20] S130 itself is part of the "SDN" loop, highly conserved throughout the Class-A β-lactamases, consisting of residues S130-N132.[21,22] S130 protonates the β-lactam nitrogen after the β-lactam bond is broken and is reprotonated through a proton shuttling mechanism between K73 and E166.[20] N132 stabilizes the positive charge on K73 during catalysis and participates in hydrogen bonds that maintain the structural integrity of the active site.[20,21] D131 coordinates an extensive hydrogen bond network that stabilizes the overall

architecture of the active site. In the apo crystal structure of KPC-2, the sidechain of D131 interacts with residues in the $\alpha4$ and $\alpha5$ helices, thereby contributing to the position and stabilization of the loop containing W105.[9]

The oxyanion hole (Figure 1), relatively conserved across the serine β-lactamases and thought to interact with the β-lactam ring carbonyl to facilitate orientation for hydrolysis,[23] is formed by the backbone nitrogen atoms of S70 and T237.[9] For KPC-2 and Ambler Class-A β-lactamases, the oxyanion hole is shallower compared to those of other serine β-lactamases, allowing KPC-2 and Ambler Class-A β-lactamases to accommodate a broader and bulkier spectrum of substrates.[9,12] Immediately preceding T237 is the conserved KTG motif, found throughout Class-A β-lactamases, at positions K234-G236 on the $\beta7$ strand.[24] This KTG motif is typical of penicillin binding proteins[25,26] and is spatially near an SXXK motif, Ambler positions 70–73, which harbors active site residues S70 and K73.[13]

Interactions with substrates and inhibitors and observed structural changes

X-ray crystal structures for KPC-2 deposited in the PDB can grouped into the following categories: (1) apo structures of KPC-2 including those without inhibitors or natural substrates and those that contain incidental ligands from crystallization conditions, (2) structures of KPC-2 complexed with boronic acid transition state analog inhibitors, (3) structures of KPC-2 with β-lactam substrates or hydrolyzed products, and (4) structures with diazabicyclooctane inhibitors. Table 1 lists details of KPC-2 structures deposited in the PDB and discussed in this review. We also direct the reader to the excellent review of β-lactamase inhibition

Table 1. List of KPC-2 structures, active site ligands, and the sub-grouping in which they have been categorized.

PDB ID	Year released	Resolution (Å)	Structure type	Active site ligand	Potency or binding data
2OV5	2007	1.85	Apo	Bicine[a]	N.A.[b]
3DW0	2008	1.6	Apo	None	N.A.[b]
3C5A	2008	1.23	Apo	Citrate[a]	N.A.[b]
3E2K	2009	2.1	BLIP bound	BLIP 45[c]	K_i 84 ± 3 pM[14]
3E2L	2009	1.87	BLIP bound	BLIP 45[c]	K_i 84 ± 3 pM[14]
3RXW	2012	1.26	β-lactam containing	PSR3-226[d]	K_m 3.8 ± 0.4 μM[28]
3RXX	2012	1.62	Boronic acid	3-NPBA[d]	K_m 1.0 ± 0.1 μM[28]
4ZBE	2016	1.8	Diazabicyclooctanes	Avibactam[d]	K_m 1.0 ± 0.1 μM[29]
5EEC	2016	1.87	Boronic acid	S02030[e]	IC_{50} 0.080 ± 0.002 μM[30]
5UJ3	2017	1.45	β-lactam containing	Cefotaxime[d]	K_m 120 ± 14 μM[12]
5UJ4	2017	1.4	β-lactam containing	Faropenem[d]	N.A.[b]
5UL8	2017	1.15	Apo	None	N.A.[b]
5LL7	2018	1.4	Boronic acid	Phenyl boronic acid derivative	N.A.[b]
5MGI	2018	1.5	Boronic acid	Phenyl boronic acid derivative	N.A.[b]
6B1F	2018	1.44	Diazabicyclooctanes	WCK 4234[d]	$K_{i\ app}$ 0.32 ± 0.03 μM[31]
6B1H	2018	1.8	Diazabicyclooctanes	WCK 4234[e]	$K_{i\ app}$ 0.32 ± 0.03 μM[31]
6B1J	2018	1.6	Diazabicyclooctanes	zidebactam(WCK 5107)[d]	$K_{i\ app}$ 4.5 ± 0.5 μM[31]
6B1W	2018	1.73	Diazabicyclooctanes	Zidebactam(WCK 5107)[e]	$K_{i\ app}$ 4.5 ± 0.5 μM[31]
6B1X	2018	1.45	Diazabicyclooctanes	WCK 5153[d]	$K_{i\ app}$ 7.8 ± 0.8 μM[31]
6B1Y	2018	1.8	Diazabicyclooctanes	WCK 5153[e]	$K_{i\ app}$ 7.8 ± 0.8 μM[31]

[a]Buffer molecule co-crystallized with enzyme.
[b]Data not available.
[c]The structures BLIP-KPC-2 complexes[14] were included for sake of completeness, but are not discussed in this review.
[d]Inhibitor-bound structure obtained by soaking.
[e]Inhibitor-bound structure obtained by co-crystallization.

by Drawz and Bonomo[27] which provides a more extensive history of most of the chemical families involved and further details for the molecular basis of their inhibitory properties.

Apo structures of KPC-2

The first crystal structure of KPC-2 became available in 2007 at 1.85 Å resolution.[9] Aside from being the first deposited, this structure was notable for the bicine molecules occupying the active site. The bicine carboxylates form hydrogen bonds with T235, T237, and S130 in KPC-2,[9] analogous to the interactions between the cefoxitin carboxylate and T316 observed in multiple structures of cefoxitin bound to the ampC β-lactamase (AmpC).[32] A similar interaction is utilized by the inhibitor SA2-13 for binding to SHV-1.[33] A year later in 2008, two additional crystal structures of KPC-2 were published at 1.6 Å and 1.23 Å resolution, respectively,[34] followed nine years later by a fourth apo KPC-2 structure deposited in 2017 at 1.15 Å resolution.[35] The 2008 structure at 1.23 Å revealed a citrate anion in the active site featuring carboxylate group interactions between the ligand and T237, echoing the interactions discussed above. Similar active site interactions are also observed in other β-lactamase structures, often to sulfate anions as in the structures of *Staphylococcus aureus* penicillinase 1 (PC1),[36] TEM-1,[11] and *Pseudomonas aeruginosa* strain RNL-1 β-lactamase (PER-1).[37] Across the family of four KPC-2 structures, slight differences in the width of the active site are seen compared to the initial structure solved by Ke *et al*.[9] Petrella *et al*.[34] rationalized these differences as the result of crystal packing interactions and alignment of the four apo structures revealed a small C_α RMSD

of 0.42 Å. The differences between the four structures occur primarily at the N- and C-termini, with exception structure 5UL8[35] which also exhibits an altered conformation for G239 and a small shift of helix α3. Consistent between the structures is the relative accessibility of the catalytic S70 and the shallow active site, features which have been proposed as explanations for the broad spectrum of hydrolyzable substrates for KPC-2.[9,34]

Boronic acid transition state analogs

Boronic acids and their derivatives are tetrahedral mimetics that have been explored as possible transition state analog inhibitors since the 1970s.[38,39] Ambler Class-C β-lactamases can be inhibited using boronic acids,[40–42] and later research was conducted on boronic acids analogs of natural β-lactamase substrates[43] for several Class-A enzymes such as RTEM-1 (R-factor encoded TEM-1),[44] TEM-1,[45] and SHV.[46] The first published structure of a boronic acid transition state analog inhibitor (BATSI) for KPC-2 was a crystal structure of 3-nitrophenyl boronic acid (3-NPBA) complexed with KPC-2 at 1.62 Å (Figure 2(a)).[28] Aligning the 3-NPBA complex with the apo KPC-2 structures results in a C_α RMSD values less than 0.574 Å, respectively.[28] Although the structural changes upon ligand binding for these structures are small, relative to crystal structures of inhibitors and drugs bound to serine β-lactamases, the 3-NPBA bound structure reveals some differences. Compared to the prior structure of the AmpC/3-NPBA complex,[47] the orientation of the 3-NPBA 3-nitro group was suggested as incompatible with the active site of KPC-2 due to the orientation of N170.[28] In 2016, a second KPC-2 BATSI complex was solved, and this time resulting in a 1.87 Å structure
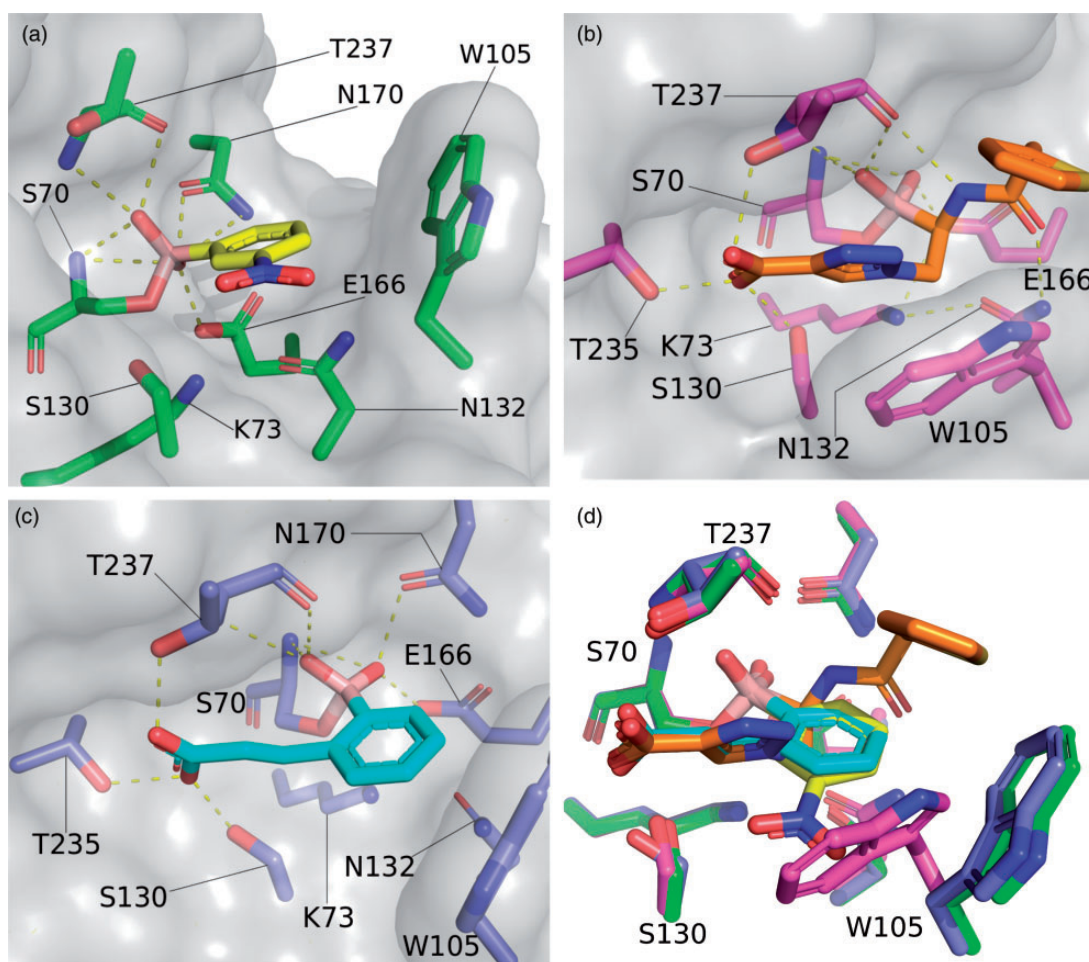
Figure 2. Panel A is a rendering of the active site of the KPC-2 3NPBA complex (PDB ID 3RXX). Select active site residues (*green*) are shown as sticks. The boron of 3NPBA is covalently bonded to the side chain oxygen atom of catalytic S70, and the oxyanion hole is occupied. Panel B is a rendering of the active site of KPC-2 with S02030 (PDB ID 5EEC), though for clarity, only one conformation of S02030 is shown. The carboxyl moiety does not interact with T235 or T237 in the second conformation (not shown). Panel C is a rendering of the active site of KPC-2 in complex with a phenyl boronic acid with a prop-2-enoic substituent (PDB ID 5LL7). Like in PDB ID 3RXX, the side chain of W105 is positioned such that the active site cleft is open compared to the positioning in PDB ID 5EEC. Panel D is an overlay of the three structures from panels A–C. The most dramatic changes are in the positioning of W105. (A color version of this figure is available in the online journal.)

with S02030 bound (Figure 2(b)).[30] S02030 was of particular interest as it had shown inhibitory effects on the Class-C β-lactamase ADC-7.[48] Compared to structures of SHV-1 in complex with S02030, the KPC-2/S02030 complex exhibited two distinct conformers for the carboxyl-triazole moiety.[30] While SHV-1 has an arginine at position 244 which forms a salt bridge to the carboxyl-triazole, KPC-2 instead makes contacts with the carboxyl-triazole via S130, T235, and T237. Structures of one additional BATSI complexed with KPC-2 were deposited to the PDB in 2016 and released in 2018 under two separate accession codes (PDB codes: 5LL7 and 5MGI). The structures provide KPC-2 in complex with a phenyl boronic acid featuring a prop-2-enoic substituent (Figure 2(c)). The carboxy moiety on the prop-2-enoic substituent makes contacts with the KPC-2 KTG motif, as well as S130. Cα alignment of 5LL7 with apo KPC-2 structure 3DW0 exhibits an RMSD value of 0.191 Å indicating minor structural changes upon BATSI complexation. Across these structures, the primary difference within for active site residues occurs for W105 which, when in complex with S02030, occupies a side chain rotamer that closes off the

active site (Figure 2(d)) relative to the orientations seen for KPC-2 in complex with 3-NPBA or the prop-2-enoic substituted phenylboronic acid.

β-lactam containing substrates and inhibitors

PSR-3–226 is a penam sulfone, a class of compounds including the drugs sulbactam[49] and tazobactam.[50] Studies of serine β-lactamase acylation by tazobactam discovered an imine or enamine intermediate as a key step,[51,52] suggesting that a stabilized trans enamine might trap the enzyme in an inhibited state.[33] Thus, PSR-3–226 was intended to be an improvement on the SA2-13 inhibitor[53] that exploited this stabilized trans enamine.[33,54] The KPC-2/PSR-3–226 complex was one of the first solved crystal structures of KPC-2 with an inhibitor. Cα alignment of the KPC-2/PSR-3–226 structure with 3DW0 produced an RMSD of 0.158 Å[28] indicating that, with the notable exception of W105, the structure of KPC-2 with PSR-3–226 bound exhibited only minor changes relative to apo KPC-2. While movement of the W105 sidechain was pronounced, this

rotation is consistent with a gating role for residues at this position (Figure 3(a)).[15] The KPC-2/PSR-3–226 structure has served as a guidepost for further development of inhibitors for KPC-2 and other carbapenemases. In 2017, two additional structures of KPC-2 with hydrolyzed forms of β-lactam antibiotics were solved (Figure 3). These structures, solved at 1.45 Å and 1.4 Å resolution, respectively, represented the first enzyme-product complexes of a wild type serine β-lactamase.[35] Although prior structures of KPC-2 featured ligands acylated to S70, crystal structures for KPC-2 in complex with the hydrolyzed cephalosporin cefotaxime, and the hydrolyzed penem faropenem represented final products and were not covalently linked to KPC-2. Interestingly, for the KPC-2/hydrolyzed cefotaxime and KPC-2/hydrolyzed faropenem structures, the sidechain of the catalytic S70 has shifted into the oxyanion hole.[35] In comparison, prior apo structures found the sidechain of S70 is typically hydrogen bonded to K73, a residue suggested to function as a base in the initial acylation.[55,56] The high energy conformation adopted by S70 shifted into the oxyanion hole was taken as evidence in support of previous proposed mechanisms for the expulsion of products

from the active sites of other Class-A β-lactamases.[35] In this conformation, steric clashes and electrostatic repulsions of the newly generated hydroxyl group on hydrolyzed products had been proposed as primary drivers of product expulsion.[57,58] Compared to acylated intermediates,[59] the KPC-2/hydrolyzed cefotaxime structure[35] and product complexes of active site mutants[60] indicate that the loss of additional hydrogen bonds to N132 may promote product expulsion and substrate turnover.[35] The rotation of S70 into the oxyanion hole in the KPC-2/hydrolyzed cefotaxime complex was also observed in the KPC-2 hydrolyzed Faropenem structure. Additionally, the rotation of the faropenem hydroxyethyl substituent away from N132 is of particular interest as the hydrogen bond between the hydroxyethyl substituent and N132 is cited as key factor in stabilization of carbapenems.[61–63] Faropenem complexes with noncarbapenamases, such as *Citrobacter sedlakii* β-lactamase (SED-1) (PDB code: 3BFF), feature hydrogen bonds with N132 and the active site deacylating water, preventing the carbapenem from being deacylated from the enzyme.[62,64,65] In the KPC-2, the faropenem hydroxyethyl group is able to rotate away from N132 due to the relatively
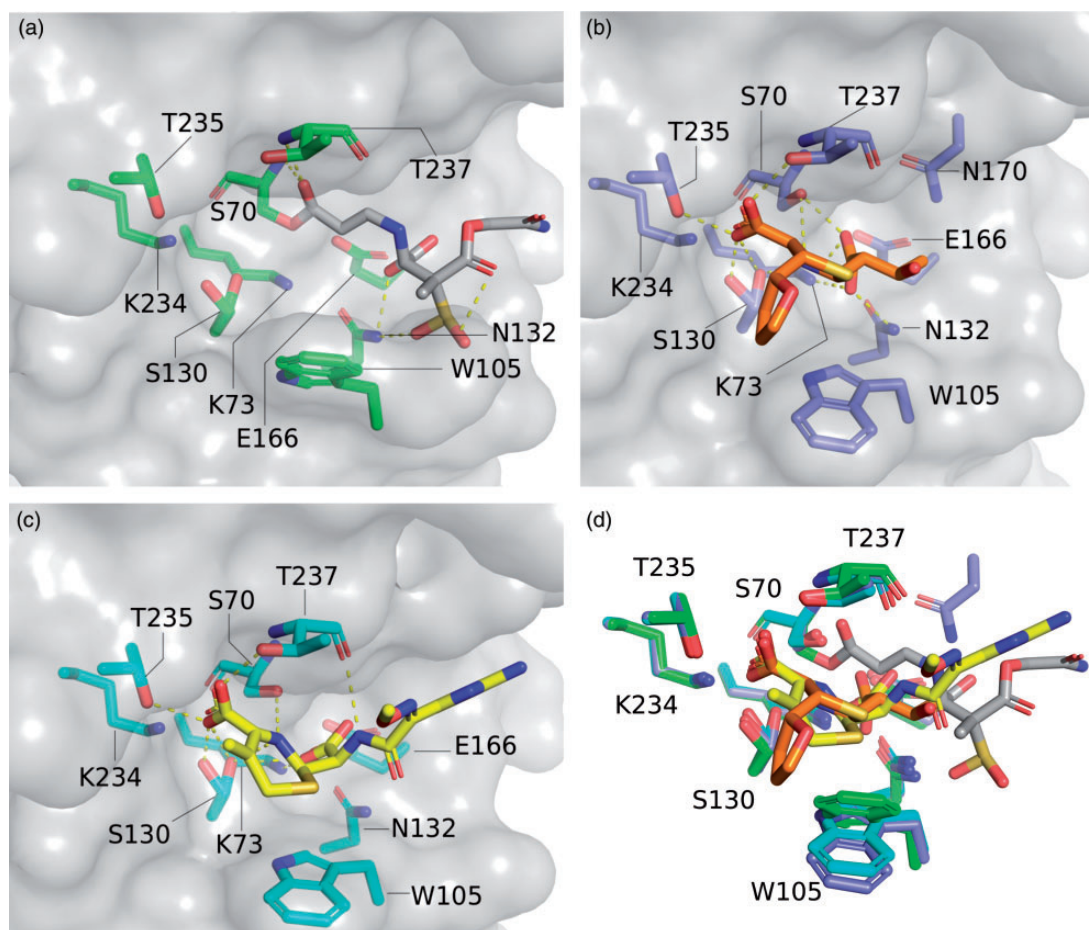

Figure 3. Panel A shows the active site of the PSR3–226 complex (PDB ID 3RXW). For clarity, the citrate molecules are not shown, and for W105 only the side chain rotamer corresponding to bound PSR3-226 is depicted. Panel B is a rendering of the hydrolyzed faropenem product (PDB ID 5UJ4). Two conformers for S130 are shown and the side chain of S70 is rotated into the oxyanion hole. Panel C is a rendering of the cefotaxime hydrolyzed product (PDB ID 5UJ3). The side chain of S70 is rotated into the oxyanion hole, which is thought to assist in expulsion of product from the active site. The wide and shallow active site of KPC-2 is readily accommodating of the comparatively bulky substituents within cefotaxime. Panel D is an overlay of the three structures from panels A–C. (A color version of this figure is available in the online journal.)

enlarged active site and favorable van der Waals contacts with L167, a residue conserved across class A carbapenemases.[15]

Diazabicyclooctane β-lactamase inhibitors

Diazabicyclooctane (DABCO) compounds are a novel class of non β-lactam inhibitors of β-lactamases. Early development began in the mid-1990s[66] with initial focus placed on co-administration of DABCO inhibitors with conventional β-lactam-containing antibiotics,[67–70] but recent DABCO β-lactamase inhibitors have also shown modest inhibition of penicillin binding proteins.[71] The first X-ray crystal structure of KPC-2 complexed with a DABCO, avibactam, was published in 2015.[72] Aside from the bridged DABCO center, avibactam was unique in that it lacked the carboxy

group present in many of the ligands complexed with the active site in other KPC-2 structures (Figure 4).[73] Avibactam is able to deacylate and recyclize, effectively regenerating an active inhibitor,[73] and is capable of inhibiting Class-A, -C, and -D β-lactamases, including KPC-2.[74] KPC-2 is unique relative to other β-lactamases in that KPC-2 also is capable of desulfating avibactam,[74] though the desulfation pathway is typically slow enough that effective treatment with avibactam is possible in bacterial infections expressing KPC-2.[72,74] Potential changes in chirality about N1 in avibactam and a lack of hydrogen bonds between KPC-2 and N6 have been postulated as leaving avibactam prone to desulfation.[72] In 2015, Papp-Wallace *et al.*[29] found KPC mutants exhibiting signs of resistance to inhibition by avibactam. In the KPC-2/avibactam structure, the avibactam carbonyl occupies the oxyanion hole of KPC-2, while the
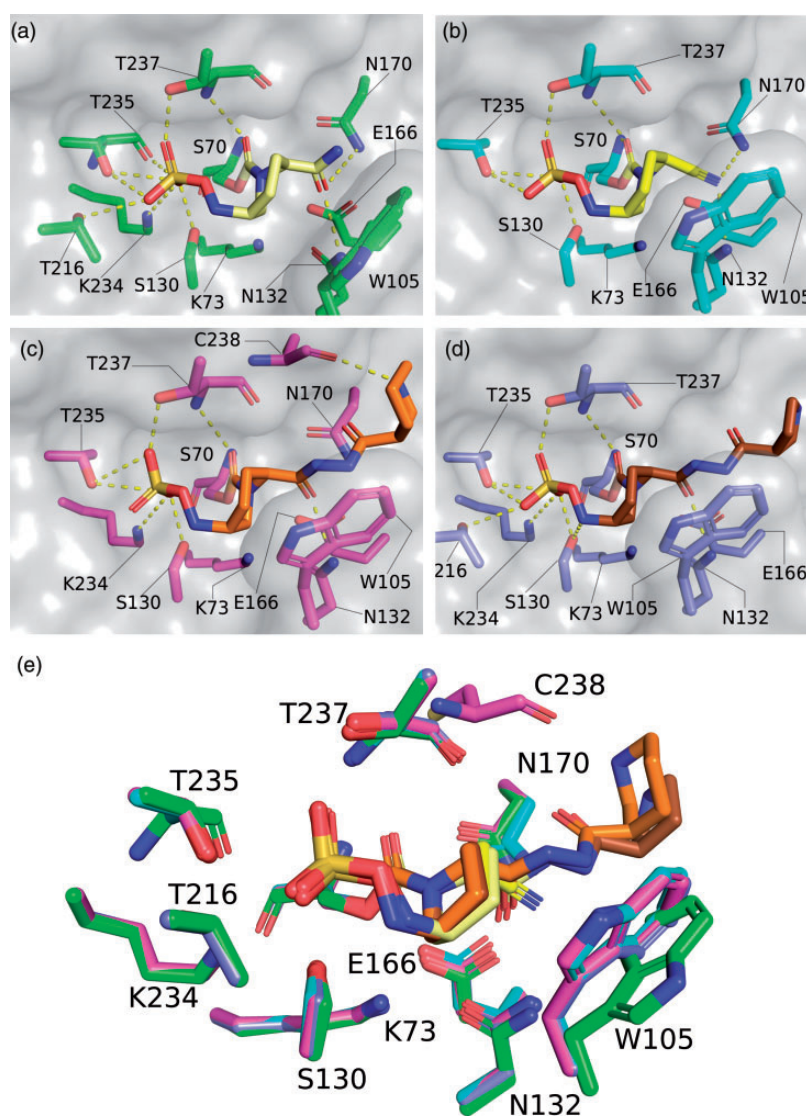

Figure 4. Panel A shows the active site of the KPC-2 Avibactam complex (PDB ID 4ZBE). The sulfate group of avibactam interacts with residues from the KTG motif and T216. Panel B shows the active site in complex with WCK 4234 (PDB ID 6B1H). The positioning of W105 is consistent for the WCK DABCO compounds, though each are different than the W105 rotamer observed in the avibactam bound KPC-2 structure (PDB ID 4ZBE). Panel C shows the active site of the WCK 5107 complex (PDB ID 6B1H). Notable amongst the WCK compounds, the piperidine ring interacts with the carbonyl of C238. Further functionalization might yield other stabilizing interactions. Panel D shows the active site of the WCK 5153 complex (PDB ID 6B1X). The pyrrolidine ring does not have the same interaction with the carbonyl of C238 as seen in 6B1H. Panel E overlays all four of DABCO-bound structures in panels A–D. Consistent with the structures displayed in Figures 2 and 3, the most dramatic changes in the active site are seen for the side chain of W105. (A color version of this figure is available in the online journal.)

sulfate group makes contacts with S130, T235, T237 much in the manner observed with prior structurally characterized ligands.

Six additional structures of KPC-2 complexed with three DABCO β-lactamase inhibitors were published in 2018 using both co-crystallization and soaking methods.[31] Two of the structures obtained through co-crystallization showed evidence of desulfation of the DABCO in the active site, whereas the soaking experiments did not exhibit desulfation. An overlay of each of the three new DABCO compounds with the KPC-2/avibactam structure showed similar active site architecture and binding geometries as those seen for the KPC-2/avibactam complex indicating a similar, if not identical mode of interaction with KPC-2.[31] Of note, however, is the added interaction between the carbonyl of C238 and the piperidine ring in zidebactam (previously known as WCK 5107).

Conclusions and outlook

The pursuit of structural information for KPC-2 and its interactions with antibiotics and inhibitors, spanning just over a decade, has been a clear success. With 20 KPC-2, X-ray crystal structures solved and deposited in the PDB, representing complexes of inhibitors that span multiple approaches of inhibition, and the field has been buoyed by a wealth of structural data for guiding the development of new β-lactamase inhibitors. Small structural variations between the active site of KPC-2 and other serine β-lactamases have enabled a broad swath of structures on different serine β-lactamases to provide compelling explanations as to how KPC-2 and similar carbapenamases are able to hydrolyze a broad spectrum of β-lactam-containing antibiotics and β-lactam-containing inhibitors. Subtle differences among these structures have even suggested mechanisms through which KPC-2 degrades non-β-lactam inhibitors such as Avibactam.[72] Despite this progress, there are several problems that remain unsolved due to the nature of KPC-2 and the limitations of crystallographic studies. First, the active site of KPC-2 is uniquely troublesome based on the tremendous number of substrates it is able to hydrolyze or otherwise degrade. The wide, shallow active site with an accessible catalytic serine is not readily perturbed by mainline inhibitors. Second, most of the observed structural changes in the active site of KPC-2 have been minute,[9,28,30,31,35,72] often differing by only a single hydrogen bond or polar contact. While these insights from X-ray crystallography are important and have assisted in the development of new drugs, the library of current structures does not provide information on the dynamic motions of these structures, bonds, and interactions. Thankfully there are well established techniques for measuring dynamic parameters, such as NMR spectroscopy.[75,76] Prior NMR dynamics experiments have been conducted on Class-A β-lactamases to investigate backbone dynamics,[77,78] picosecond-nanosecond motions of active site residues,[76,79,80] and substrate binding.[81] Such approaches may assist in the development of allosteric β-lactamase inhibitors[82–84] for KPC-2, and when teamed with the extensive library of KPC-2 crystals, structures may open avenues

to new inhibitors that target active site dynamics for inhibition. The future of KPC-2 inhibitor development for the next decade builds off a strong foundation of structural data, providing scientists with a crucial edge in the battle to combat carbapenem resistance.

ORCID iD

Richard C Page ⓘD https://orcid.org/0000-0002-3006-3171

REFERENCES

1. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J Infect Dis* 2008;**197**:1079–81
2. Cohen ML. Changing patterns of infectious disease. *Nature* 2000;**406**:762–7
3. Babic M, Hujer AM, Bonomo RA. What's new in antibiotic resistance? Focus on beta-lactamases. *Drug Resist Updat* 2006;**9**:142–56
4. Naas T, Oueslati S, Bonnin RA, Dabos ML, Zavala A, Dortet L, Retailleau P, Iorga BI. Beta-lactamase database (BLDB) – structure and function. *J Enzyme Inhib Med Chem* 2017;**32**:917–9
5. Livermore DM, Woodford N. The beta-lactamase threat in *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter*. *Trends Microbiol* 2006;**14**:413–20
6. Chen LF, Anderson DJ, Paterson DL. Overview of the epidemiology and the threat of *Klebsiella pneumoniae* carbapenemases (KPC) resistance. *Infect Drug Resist* 2012;**5**:133–41
7. Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *Lancet Infect Dis* 2009;**9**:228–36
8. Walther-Rasmussen J, Hoiby N. Class A carbapenemases. *J Antimicrob Chemother* 2007;**60**:470–82
9. Ke W, Bethel CR, Thomson JM, Bonomo RA, van den Akker F. Crystal structure of KPC-2: insights into carbapenemase activity in class A beta-lactamases. *Biochemistry* 2007;**46**:5732–40
10. Raquet X, Lamotte-Brasseur J, Bouillenne F, Frere JM. A disulfide bridge near the active site of carbapenem-hydrolyzing class A beta-lactamases might explain their unusual substrate profile. *Proteins* 1997;**27**:47–58
11. Jelsch C, Mourey L, Masson JM, Samama JP. Crystal structure of Escherichia coli TEM1 beta-lactamase at 1.8 A resolution. *Proteins* 1993;**16**:364–83
12. Papp-Wallace KM, Bethel CR, Distler AM, Kasuboski C, Taracila M, Bonomo RA. Inhibitor resistance in the KPC-2 beta-lactamase, a preeminent property of this class A beta-lactamase. *Antimicrob Agents Chemother* 2010;**54**:890–7

13. Papp-Wallace KM, Taracila M, Hornick JM, Hujer AM, Hujer KM, Distler AM, Endimiani A, Bonomo RA. Substrate selectivity and a novel role in inhibitor discrimination by residue 237 in the KPC-2 beta-lactamase. *Antimicrob Agents Chemother* 2010;**54**:2867–77

14. Hanes MS, Jude KM, Berger JM, Bonomo RA, Handel TM. Structural and biochemical characterization of the interaction between KPC-2 beta-lactamase and beta-lactamase inhibitor protein. *Biochemistry* 2009;**48**:9185–93

15. Majiduddin FK, Palzkill T. Amino acid residues that contribute to substrate specificity of class A beta-lactamase SME-1. *Antimicrob Agents Chemother* 2005;**49**:3421–7

16. Banerjee S, Pieper U, Kapadia G, Pannell LK, Herzberg O. Role of the omega-loop in the activity, substrate specificity, and structure of class A beta-lactamase. *Biochemistry* 1998;**37**:3286–96

17. Petrosino JF, Palzkill T. Systematic mutagenesis of the active site omega loop of TEM-1 beta-lactamase. *J Bacteriol* 1996;**178**:1821–8

18. Levitt PS, Papp-Wallace KM, Taracila MA, Hujer AM, Winkler ML, Smith KM, Xu Y, Harris ME, Bonomo RA. Exploring the role of a conserved class A residue in the Omega-Loop of KPC-2 beta-lactamase: a mechanism for ceftazidime hydrolysis. *J Biol Chem* 2012;**287**:31783–93

19. Ambler RP. The structure of beta-lactamases. *Philos Trans R Soc Lond B Biol Sci* 1980;**289**:321–31

20. Hermann JC, Hensen C, Ridder L, Mulholland AJ, Holtje HD. Mechanisms of antibiotic resistance: QM/MM modeling of the acylation reaction of a class A beta-lactamase with benzylpenicillin. *J Am Chem Soc* 2005;**127**:4454–65

21. Jacob F, Joris B, Lepage S, Dusart J, Frère JM. Role of the conserved amino acids of the 'SDN' loop (Ser[130] Asp[131] and Asn[132]) in a class A β-lactamase studied by site-directed mutagenesis. *Biochem J* 1990;**271**:399–406

22. Parker AC, Smith CJ. Genetic and biochemical analysis of a novel Ambler class A beta-lactamase responsible for cefoxitin resistance in Bacteroides species. *Antimicrob Agents Chemother* 1993;**37**:1028–36

23. Murphy BP, Pratt RF. Evidence for an oxyanion hole in serine beta-lactamases and DD-peptidases. *Biochem J* 1988;**256**:669–72

24. Girlich D, Poirel L, Nordmann P. Novel ambler class A carbapenem-hydrolyzing beta-lactamase from a Pseudomonas fluorescens isolate from the Seine River, Paris, France. *Antimicrob Agents Chemother* 2010;**54**:328–32

25. Joris B, Ledent P, Dideberg O, Fonze E, Lamotte-Brasseur J, Kelly JA, Ghuysen JM, Frere JM. Comparison of the sequences of class A beta-lactamases and of the secondary structure elements of penicillin-recognizing proteins. *Antimicrob Agents Chemother* 1991;**35**:2294–301

26. Spratt BG, Cromie KD. Penicillin-binding proteins of Gram-negative bacteria. *Rev Infect Dis* 1988;**10**:699–711

27. Drawz SM, Bonomo RA. Three decades of beta-lactamase inhibitors. *Clin Microbiol Rev* 2010;**23**:160–201

28. Ke W, Bethel CR, Papp-Wallace KM, Pagadala SR, Nottingham M, Fernandez D, Buynak JD, Bonomo RA, van den Akker F. Crystal structures of KPC-2 beta-lactamase in complex with 3-nitrophenyl boronic acid and the penam sulfone PSR-3-226. *Antimicrob Agents Chemother* 2012;**56**:2713–8

29. Papp-Wallace KM, Winkler ML, Taracila MA, Bonomo RA. Variants of beta-lactamase KPC-2 that are resistant to inhibition by avibactam. *Antimicrob Agents Chemother* 2015;**59**:3710–7

30. Nguyen NQ, Krishnan NP, Rojas LJ, Prati F, Caselli E, Romagnoli C, Bonomo RA, van den Akker F. Crystal structures of KPC-2 and SHV-1 beta-lactamases in complex with the boronic acid transition state analog S02030. *Antimicrob Agents Chemother* 2016;**60**:1760–6

31. Papp-Wallace KM, Nguyen NQ, Jacobs MR, Bethel CR, Barnes MD, Kumar V, Bajaksouzian S, Rudin SD, Rather PN, Bhavsar S, Ravikumar T, Deshpande PK, Patil V, Yeole R, Bhagwat SS, Patel MV, van den Akker F, Bonomo RA. Strategic approaches to overcome resistance against gram-negative pathogens using beta-lactamase inhibitors and beta-lactam enhancers: activity of three novel diazabicyclooctanes WCK 5153, zidebactam (WCK 5107), and WCK 4234. *J Med Chem* 2018;**61**:4067–86

32. Beadle BM, Trehan I, Focia PJ, Shoichet BK. Structural milestones in the reaction pathway of an amide hydrolase: substrate, acyl, and product complexes of cephalothin with AmpC beta-lactamase. *Structure* 2002;**10**:413–24

33. Padayatti PS, Sheri A, Totir MA, Helfand MS, Carey MP, Anderson VE, Carey PR, Bethel CR, Bonomo RA, Buynak JD, van den Akker F. Rational design of a beta-lactamase inhibitor achieved via stabilization of the trans-enamine intermediate: 1.28 A crystal structure of wt SHV-1 complex with a penam sulfone. *J Am Chem Soc* 2006;**128**:13235–42

34. Petrella S, Ziental-Gelus N, Mayer C, Renard M, Jarlier V, Sougakoff W. Genetic and structural insights into the dissemination potential of the extremely broad-spectrum class A beta-lactamase KPC-2 identified in an Escherichia coli strain and an *Enterobacter cloacae* strain isolated from the same patient in France. *Antimicrob Agents Chemother* 2008;**52**:3725–36

35. Pemberton OA, Zhang X, Chen Y. Molecular basis of substrate recognition and product release by the *Klebsiella pneumoniae* carbapenemase (KPC-2). *J Med Chem* 2017;**60**:3525–30

36. Herzberg O. Refined crystal structure of β-lactamase from *Staphylococcus aureus* PC1 at 2.0 Å resolution. *J Mol Biol* 1991;**217**:701–19

37. Tranier S, Bouthors AT, Maveyraud L, Guillet V, Sougakoff W, Samama JP. The high resolution crystal structure for class A beta-lactamase PER-1 reveals the bases for its increase in breadth of activity. *J Biol Chem* 2000;**275**:28075–82

38. Bone R, Shenvi AB, Kettner CA, Agard DA. Serine protease mechanism: structure of an inhibitory complex of alpha-lytic protease and a tightly bound peptide boronic acid. *Biochemistry* 1987;**26**:7609–14

39. Lindquist RN, Terry C. Inhibition of subtilisin by boronic acids, potential analogs of tetrahedral reaction intermediates. *Arch Biochem Biophys* 1974;**160**:135–44

40. Beesley T, Gascoyne N, Knott-Hunziker V, Petursson S, Waley SG, Jaurin B, Grundstrom T. The inhibition of class C beta-lactamases by boronic acids. *Biochem J* 1983;**209**:229–33

41. Chen CC, Rahil J, Pratt RF, Herzberg O. Structure of a phosphonate-inhibited beta-lactamase. An analog of the tetrahedral transition state/intermediate of beta-lactam hydrolysis. *J Mol Biol* 1993;**234**:165–78

42. Crompton IE, Cuthbert BK, Lowe G, Waley SG. Beta-lactamase inhibitors. The inhibition of serine beta-lactamases by specific boronic acids. *Biochem J* 1988;**251**:453–9

43. Caselli E, Powers RA, Blaszczak LC, Wu CY, Prati F, Shoichet BK. Energetic, structural, and antimicrobial analyses of beta-lactam side chain recognition by beta-lactamases. *Chem Biol* 2001;**8**:17–31

44. Strynadka NC, Adachi H, Jensen SE, Johns K, Sielecki A, Betzel C, Sutoh K, James M. Molecular structure of the acyl-enzyme intermediate in β-lactam hydrolysis at 1.7 Å resolution. *Nature* 1992;**359**:700

45. Strynadka NC, Martin R, Jensen SE, Gold M, Jones JB. Structure-based design of a potent transition state analogue for TEM-1 beta-lactamase. *Nat Struct Biol* 1996;**3**:688–95

46. Thomson JM, Prati F, Bethel CR, Bonomo RA. Use of novel boronic acid transition state inhibitors to probe substrate affinity in SHV-type extended-spectrum β-lactamases. *Antimicrob Agents Chemother* 2007;**51**:1577–9

47. Powers RA, Shoichet BK. Structure-based approach for binding site identification on AmpC beta-lactamase. *J Med Chem* 2002;**45**:3222–34

48. Powers RA, Swanson HC, Taracila MA, Florek NW, Romagnoli C, Caselli E, Prati F, Bonomo RA, Wallar BJ. Biochemical structural analysis of inhibitors targeting the ADC-7 cephalosporinase of *Acinetobacter baumannii*. *Biochemistry* 2014;**53**:7670–9

49. Campoli-Richards DM, Brogden RN. Sulbactam/ampicillin. A review of its antibacterial activity, pharmacokinetic properties, and therapeutic use. *Drugs* 1987;**33**:577–609

50. Bryson HM, Brogden RN. Piperacillin/tazobactam. A review of its antibacterial activity, pharmacokinetic properties and therapeutic potential. *Drugs* 1994;**47**:506–35

51. Bush K, Macalintal C, Rasmussen BA, Lee VJ, Yang YJ. Kinetic interactions of tazobactam with beta-lactamases from all major structural classes. *Antimicrob Agents Chemother* 1993;**37**:851–8

52. Yang Y, Janota K, Tabei K, Huang N, Siegel MM, Lin YI, Rasmussen BA, Shlaes DM. Mechanism of inhibition of the class A beta-lactamases PC1 and TEM-1 by tazobactam. Observation of reaction products by electrospray ionization mass spectrometry. *J Biol Chem* 2000;**275**:26674–82

53. Rodkey EA, Winkler ML, Bethel CR, Pagadala SR, Buynak JD, Bonomo RA, van den Akker F. Penam sulfones and beta-lactamase inhibition: SA2-13 and the importance of the C2 side chain length and composition. *PLoS One* 2014;**9**:e85892

54. Totir MA, Padayatti PS, Helfand MS, Carey MP, Bonomo RA, Carey PR, van den Akker F. Effect of the inhibitor-resistant M69V substitution on the structures and populations of trans-enamine beta-lactamase intermediates. *Biochemistry* 2006;**45**:11895–904

55. Meroueh SO, Fisher JF, Schlegel HB, Mobashery S. Ab initio QM/MM study of class A beta-lactamase acylation: dual participation of Glu166 and Lys73 in a concerted base promotion of Ser70. *J Am Chem Soc* 2005;**127**:15397–407

56. Nichols DA, Hargis JC, Sanishvili R, Jaishankar P, Defrees K, Smith EW, Wang KK, Prati F, Renslo AR, Woodcock HL, Chen Y. Ligand-induced proton transfer and low-barrier hydrogen bond revealed by X-ray crystallography. *J Am Chem Soc* 2015;**137**:8086–95

57. Delmas J, Leyssene D, Dubois D, Birck C, Vazeille E, Robin F, Bonnet R. Structural insights into substrate recognition and product expulsion in CTX-M enzymes. *J Mol Biol* 2010;**400**:108–20

58. Leyssene D, Delmas J, Robin F, Cougnoux A, Gibold L, Bonnet R. Noncovalent complexes of an inactive mutant of CTX-M-9 with the substrate piperacillin and the corresponding product. *Antimicrob Agents Chemother* 2011;**55**:5660–5

59. Shimamura T, Ibuka A, Fushinobu S, Wakagi T, Ishiguro M, Ishii Y, Matsuzawa H. Acyl-intermediate structures of the extended-spectrum class A beta-lactamase, Toho-1, in complex with cefotaxime, cephalothin, and benzylpenicillin. *J Biol Chem* 2002;**277**:46601–8

60. Lewandowski EM, Skiba J, Torelli NJ, Rajnisz A, Solecka J, Kowalski K, Chen Y. Antibacterial properties and atomic resolution X-ray complex crystal structure of a ruthenocene conjugated beta-lactam antibiotic. *Chem Commun* 2015;**51**:6186–9

61. Fonseca F, Chudyk EI, van der Kamp MW, Correia A, Mulholland AJ, Spencer J. The basis for carbapenem hydrolysis by class A beta-lactamases: a combined investigation using crystallography and simulations. *J Am Chem Soc* 2012;**134**:18275–85

62. Nukaga M, Bethel CR, Thomson JM, Hujer AM, Distler A, Anderson VE, Knox JR, Bonomo RA. Inhibition of class A beta-lactamases by carbapenems: crystallographic observation of two conformations of meropenem in SHV-1. *J Am Chem Soc* 2008;**130**:12656–62

63. Stewart NK, Smith CA, Frase H, Black DJ, Vakulenko SB. Kinetic structural requirements for carbapenemase activity in GES-type beta-lactamases. *Biochemistry* 2015;**54**:588–97

64. Maveyraud L, Mourey L, Kotra LP, Pedelacq J-D, Guillet V, Mobashery S, Samama J-P. Structural basis for clinical longevity of carbapenem antibiotics in the face of challenge by the common class A *β*-lactamases from the antibiotic-resistant bacteria. *J Am Chem Soc* 1998;**120**:9748–52

65. Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: past, present, and future. *Antimicrob Agents Chemother* 2011;**55**:4943–60

66. Coleman K. Diazabicyclooctanes (DBOs): a potent new class of non-beta-lactam beta-lactamase inhibitors. *Curr Opin Microbiol* 2011;**14**:550

67. Bonnefoy A, Dupuis-Hamelin C, Steier V, Delachaume C, Seys C, Stachyra T, Fairley M, Guitton M, Lampilas M. In vitro activity of AVE1330A, an innovative broad-spectrum non-beta-lactam beta-lactamase inhibitor. *J Antimicrob Chemother* 2004;**54**:410–7

68. Livermore DM, Mushtaq S, Warner M, Zhang J, Maharjan S, Doumith M, Woodford N. Activities of NXL104 combinations with ceftazidime and aztreonam against carbapenemase-Producing Enterobacteriaceae. *Antimicrob Agents Chemother* 2011;**55**:390–4

69. Mushtaq S, Warner M, Livermore DM. In vitro activity of ceftazidime+NXL104 against *Pseudomonas aeruginosa* and other non-fermenters. *J Antimicrob Chemother* 2010;**65**:2376–81

70. Walkty A, DeCorby M, Lagace-Wiens P, Karlowsky J, Hoban D, Zhanel G. In vitro activity of ceftazidime combined with NXL104 versus *Pseudomonas aeruginosa* isolates 241 obtained from patients in Canadian hospitals (CANWARD 2009 study). *Antimicrob Agents Chemother* 2011;**242**:2992–4

71. King AM, King DT, French S, Brouillette E, Asli A, Alexander JA, Vuckovic M, Maiti SN, Parr TR, Jr., Brown ED, Malouin F, Strynadka NC, Wright GD. Structural and kinetic characterization of diazabicyclooctanes as dual inhibitors of both serine-beta-lactamases and penicillin-binding proteins. *ACS Chem Biol* 2016;**11**:864–8

72. Krishnan NP, Nguyen NQ, Papp-Wallace KM, Bonomo RA, van den Akker F. Inhibition of Klebsiella beta-Lactamases (SHV-1 and KPC-2) by avibactam: a structural study. *PLoS One* 2015;**10**:e0136813

73. Ehmann DE, Jahić H, Ross PL, Gu R-F, Hu J, Kern G, Walkup GK, Fisher SL. Avibactam is a covalent, reversible, non–*β*-lactam *β*-lactamase inhibitor. *Proc Natl Acad Sci U S A* 2012;**109**:11663–8

74. Ehmann DE, Jahic H, Ross PL, Gu RF, Hu J, Durand-Reville TF, Lahiri S, Thresher J, Livchak S, Gao N, Palmer T, Walkup GK, Fisher SL. Kinetics of avibactam inhibition against Class A, C, and D beta-lactamases. *J Biol Chem* 2013;**288**:27960–71

75. Palmer Iii AG. Enzyme dynamics from NMR spectroscopy. *Acc Chem Res* 2015;**48**:457–65

76. Perez A, Morrone JA, Simmerling C, Dill KA. Advances in free-energy-based simulations of protein folding and ligand binding. *Curr Opin Struct Biol* 2016;**36**:25–31

77. Fisette O, Morin S, Savard PY, Lague P, Gagne SM. TEM-1 backbone dynamics-insights from combined molecular dynamics and nuclear magnetic resonance. *Biophys J* 2010;**98**:637–45

78. Savard PY, Gagne SM. Backbone dynamics of TEM-1 determined by NMR: evidence for a highly ordered protein. *Biochemistry* 2006;**45**:11414–24

79. Bos F, Pleiss J. Multiple molecular dynamics simulations of TEM beta-lactamase: dynamics and water binding of the omega-loop. *Biophys J* 2009;**97**:2550–8

80. Doucet N, Savard PY, Pelletier JN, Gagne SM. NMR investigation of Tyr105 mutants in TEM-1 beta-lactamase: dynamics are correlated with function. *J Biol Chem* 2007;**282**:21448–59

81. Fisette O, Gagne S, Lague P. Molecular dynamics of class A beta-lactamases-effects of substrate binding. *Biophys J* 2012;**103**:1790–801

82. Cortina GA, Hays JM, Kasson PM. Conformational intermediate that controls KPC-2 catalysis and beta-lactam drug resistance. *ACS Catal* 2018;**8**:2741–7

83. Galdadas I, Lovera S, Perez-Hernandez G, Barnes MD, Healy J, Afsharikho H, Woodford N, Bonomo RA, Gervasio FL, Haider S. Defining the architecture of KPC-2 Carbapenemase: identifying allosteric networks to fight antibiotics resistance. *Sci Rep* 2018;**8**:12916

84. Llarrull LI, Testero SA, Fisher JF, Mobashery S. The future of the beta-lactams. *Curr Opin Microbiol* 2010;**13**:551–7