

1 **Title:** OXA-66 structure and oligomerisation of OXAAb enzymes

2

3 **Authors:** Yuiko Takebayashi^{a,b}, Sara R. Henderson^{c,d}, Dimitri Y. Chirgadze^e, Philip J. Warburton^{a,f},
4 Benjamin A. Evans^{a,c*}

5

6 **Affiliations:** ^aDepartment of Biomedical and Forensic Science, Anglia Ruskin University, Cambridge,
7 UK; ^bSchool of Cellular and Molecular Medicine, University of Bristol, Bristol, UK; ^cNorwich Medical
8 School, University of East Anglia, Norwich, UK; ^dInstitute of Microbiology and Infection, College of
9 Medical and Dental Sciences, University of Birmingham, Birmingham, UK; ^eDepartment of
10 Biochemistry, University of Cambridge, Cambridge, UK; ^fSchool of Biomedical Sciences, Faculty of
11 Health, University of Plymouth, Plymouth, UK

12

13 **Corresponding author:** benjamin.evans@uea.ac.uk

14

15 **Keywords:** Beta-lactamase, carbapenemase, Acinetobacter, antibiotic resistance

16

17 **Repositories:** The crystal structure of OXA-66 has been deposited in the Protein Data Bank (PDB)
18 with the PDB ID: 6T1H.

19

20

21

22

23

24

25

26

27

28

29

30

31 **Abstract**

32 The OXA β -lactamases are responsible for hydrolysing β -lactam antibiotics and contribute to the
33 multidrug-resistant phenotype of several major human pathogens. The OXAAb enzymes are intrinsic
34 to *Acinetobacter baumannii* and can confer resistance to carbapenem antibiotics. Here we determined
35 the structure of the most prevalent OXAAb enzyme, OXA-66. The structure of OXA-66 was solved at
36 a resolution of 2.1 Å and found to be very similar to the structure of OXA-51, the only other OXAAb
37 enzyme that has had its structure solved. Our data contained one molecule per asymmetric unit, and
38 analysis of positions responsible for dimer formation in other OXA enzymes suggest that OXA-66
39 likely exists as a monomer.

40

41 **Data Summary**

42 The crystal structure of OXA-66 has been deposited in the Protein Data Bank (PDB) with the PDB ID:
43 6T1H. The coordinate files, diffraction data and validation report can be downloaded from the PDB,
44 DOI: 10.2210/pdb6t1h/pdb.

45

46 **Introduction**

47 The OXA-type β -lactamases are enzymes that hydrolyse the β -lactam antibiotics. They are commonly
48 found in Gram-negative bacteria that cause serious infections in humans, including *Pseudomonas*
49 *aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* (1). While some
50 groups of OXA enzymes, such as OXA-48, have been mobilised on plasmids and become globally
51 spread in highly successful multidrug-resistant bacterial lineages, others represent intrinsic enzymes
52 belonging to specific bacterial species and are encoded on the chromosome. One such group are the
53 OXAAb enzymes that are intrinsic to *A. baumannii*. It has recently been shown that these intrinsic
54 enzymes are capable of conferring resistance to the carbapenem antibiotics (2), and it is therefore
55 important to understand the molecular mechanisms behind this. To date, only the structure of the
56 OXAAb enzyme OXA-51 has been solved (3). However, by far the most prevalent OXAAb enzyme is
57 OXA-66 due to its association with the predominant *A. baumannii* Global Clone 2. We therefore
58 sought to determine the structure of OXA-66 and compare it to OXA-51.

59

60 Although the OXA-type enzymes have been reported to have a high degree of structural similarity
61 despite their divergence in sequence, there have been contradictory reports suggesting that the
62 oligomerisation of these enzymes may not be universal. For example, some enzymes have been
63 reported to be monomers such as OXA-1 where an elongated Ω -loop is postulated to inhibit the
64 process of dimerization (4), or OXA-25 where an inward bend of the β -3 strand is suggested to
65 destabilise dimerization (5). In contrast, OXA-48 was determined to be a dimer with a series of salt
66 bridges stabilising an alternative dimeric interface (6, 7). A third group, including OXA-10 and OXA-
67 14, are only dimeric in the presence of metal ions (8). Furthermore, for those enzymes that require
68 metal ions for dimerization, it has been determined that the dimer form is more active than the
69 monomeric form, but dimerization is destabilised by the presence of the substrate (8). Currently there
70 has been no report of any OXA-51-like enzymes having their oligomerisation state confirmed, with
71 only the N-terminal fragment of OXA-58 from *A. baumannii* being classed and found to be a
72 monomer, although there is no evidence of the oligomerisation state of the full length enzyme (9).

73

74 Here we present a new structure of the most prevalent OXAAb enzyme, OXA-66, and compare it to
75 the previously solved structures of OXA-51. Additionally we propose, based on similarity and
76 structural models, that this sub-group of enzymes is of a monomeric nature.

77

78 **Methods**

79 **Macromolecule production**

80 For crystal structure determination, OXA-66 was amplified by PCR without its signal peptide, using
81 the OXA-66-BamHI forward and reverse primers (Table 1). The signal peptide was predicted using
82 the SignalP 4.1 Server (10, 11). The insert was subcloned into pGEM-T Easy (Promega, United
83 Kingdom) and confirmed by sequencing with the universal T7 Promoter primer. For expression of
84 OXA-66 fused with a glutathione S-transferase (GST) tag, the insert was digested with BamHI, ligated
85 into the protein expression vector pGEX-6P-1 (GE Healthcare, United Kingdom) and transformed into
86 *E. coli* DH5 α . Transformants were selected with ampicillin (100 mg/L) and confirmed by PCR using
87 pGEX Sequencing Primers (GE Healthcare Life Sciences, United Kingdom). The recombinant
88 plasmid was transformed into *E. coli* BL21 (DE3) (Biolone, United Kingdom) following manufacturer
89 guidelines.

90

91 **Table 1:** Macromolecule production information

Source organism	<i>Acinetobacter baumannii</i>
OXA-66-BamHI Forward primer	AAAGGATCCATGAATCCAAATCACAGC
OXA-66-BamHI Reverse primer	AAAGGATCCCTATAAAAATACCTAATTGTTC
Cloning vector	pGEM-T Easy
Expression vector	pGEX-6P-1
Expression host	<i>E. coli</i> BL21 (DE3)

92

93 BL21 (DE3) pGEX-6P-1 OXA-66 was grown at 37 °C to an OD600 of 0.8-1.0 in LB broth before
94 inducing with a final IPTG concentration of 0.1 mM for 6 h. A total of 11.5 g of cell pellet was yielded
95 for purification. OXA-66 was purified according to the column chromatography method outlined by the
96 GST-Bind Kit (Novagen, United Kingdom), up to the first flow through fraction collection.
97 Subsequently, the manufacturer protocol for PreScission Protease cleavage of GST-tagged protein
98 bound to the column (GE Healthcare, United Kingdom) was followed to remove the GST-tag and elute
99 OXA-66. Homogeneity of the purified protein was confirmed by running the eluted sample on a 10%
100 Bis-Tris SDS-PAGE gel (Life Technologies, United Kingdom). The purified protein appeared as a
101 single band (data not shown).

102 **Crystallization**

103 Octahedron crystals were grown under conditions described in table 2. Crystals used for X-ray
104 diffraction experiments were harvested at 8 days in the precipitant supplemented with 26% (v/v)
105 ethylene glycol with liquid nitrogen.

106 **Data collection and processing**

107 The X-ray diffraction dataset was collected a wavelength of 1.5418 Å using a copper rotating anode
108 X-ray diffraction system equipped with confocal mirror monochromator, a kappa geometry
109 goniometer, and Platinum 135 CCD detector (PROTEUM X8, Bruker AXS, Ltd) at a temperature of
110 100K (Oxford Cryosystems, Ltd). The exposure time was set to 60 sec for a single phi-oscillation
111 image of 1 degree, and the total of 1,398 oscillation images were collected in 8 different kappa
112 geometry orientations. The dataset was indexed, scaled and merged using PROTEUM2 data
113 processing software (12). The resultant data-collection statistics are summarized in table 3.

114

115 **Table 2:** Crystallization conditions

Method	Sitting drop
Plate type	MRC crystallisation plates (SWISSCI, Wokingham, UK)
Temperature (K)	292
Protein concentration (mg ml ⁻¹)	22
Buffer composition of protein solution	20 mM HEPES, 50 mM NaCl, pH 7.5
Composition of reservoir solution	0.1 M MES pH 6.5, 22.85% (v/v) PEG MME 550, 10 mM ZnSO ₄
Volume and ratio of drop	400 nl 1:1
Volume of reservoir (μl)	70

116

117 **Structure solution and refinement**

118 The OXA-66 crystal structure was solved by the Molecular Replacement (MR) method. The crystal
 119 structure of OXA-51 beta-lactamase (PDB-ID: 4ZDX) was used as the MR search probe. The
 120 sequence identity between the search probe and OXA-66 is 97%. All MR calculations were performed
 121 in PHASER, part of the PHENIX crystallographic software suite (13, 14). The obtained model was
 122 subjected to several rounds of alternating manual rebuilding performed in the molecular graphics
 123 software suite COOT and crystallographic refinement calculations in PHENIX crystallographic
 124 software suite (13, 15). Final refinement and validation statistics are summarized in table 4. All
 125 molecular graphics and structural analyses were carried out within the CCP4MG and CCP4 suite (16).

126

127

128

129

130

131

132

133

134

135

136

137

Diffraction source	In-house, Copper rotating anode.
Wavelength (Å)	1.5418
Temperature (K)	100
Detector	Platinum 135 CCD (PROTEUM X8, Bruker AXS, Ltd)
Rotation range per image (°)	1
Total rotation range (°)	1398
Exposure time per image (s)	60
Space group	$P4_322$
a, b, c (Å)	87.54, 87.54, 90.12
α, β, γ (°)	90, 90, 90
Resolution range (Å)	45.06 – 2.10 ¹ (2.20-2.10) ²
No. of unique reflections	21092 (2689)
Completeness (%)	100 (100)
Redundancy	75.4 (45.7)
$\langle I/\sigma(I) \rangle$	28.17 (3.36)
$R_{p.i.m.}$ (%) ³	1.9 (11.2)
Overall B factor from Wilson plot (Å ²)	29.1
Number of molecules per asymmetric unit	1
Matthews Coefficient (V_m)	2.82

139 ¹The resolution cut-off criteria was based on the data strength, i.e. only the resolution shells with
140 $\langle I/\sigma(I) \rangle > 3$ were included. ²Values for the outer resolution shell are given in parentheses. ³ $R_{p.i.m.} = (\sum$
141 $hkl [1/(N-1)]^{1/2} \sum_i |I_i(hkl) - I_{mean}(hkl)|) / \sum hkl \sum_i I_i(hkl)$, where N is redundancy (p.i.m. – precision-
142 indicating R-factor).

143

144 **Results and discussion**

145 We present here the crystal structure of the most prevalent member of the OXAAb group, OXA-66, at
146 a maximum resolution of 2.1 Å, solved by molecular replacement using the OXA-51 structure (PDB
147 ID: 4ZDX) as the molecular replacement model with an amino acid sequence identity of 97%. As
148 expected, there is as high degree of structural similarity between these enzymes, with an R.M.S.D
149 value of 0.48 Å on 239 c-alpha atoms with the 4ZDX apo structure of OXA-51. OXA-66 differs from
150 OXA-51 by 6 amino acids – T5A, E36V, V48A, Q107K, P194Q, D225N (17), but this variation in the
151 observed sequences does not appear to affect the main chain or give rise to any significantly altered
152 charge interactions in or near the active site (Figure 1). This is consistent with the very similar levels

153 of phenotypic resistance to the carbapenem antibiotics that these two enzymes have been shown to
154 confer (2).

155

156 **Table 4:** Structure solution and refinement

Resolution range (Å)	43.7720–2.1000 (2.1526–2.1001) ¹
Completeness (%)	99.5 (99.5)
σ cutoff	$F > 1.43\sigma(F)$
No. of reflections, working set	18,970 (1,316)
No. of reflections, test set	1,990 (140)
Final R_{cryst}	0.188 (0.2470)
Final R_{free}	0.224 (0.2954)
No. of non-H atoms	
Protein	1,917
Ligand	0
Solvent	229
Total	2,150
R.m.s. deviations	
Bonds (Å)	0.007
Angles (°)	0.843
Average B factors (Å ²)	
Protein	27.2
Ligand	29.1
Ramachandran plot	
Most favoured (%)	98.33
Allowed (%)	1.67

157 ¹Values for the outer resolution shell are given in parentheses.

158

159 Similar to the active site in the Apo OXA-51 structures (5ZKH and 4ZDX), we observe that the
160 tryptophan at position 222 lies in a conformation disfavoured the substrate from binding in the active
161 site (supplementary figure 1) (3, 18). However, an alternative conformation is observed in the
162 Doripenem bound OXA-51 structure (5L2F) whereby the tryptophan adopts a position pointing away
163 from the substrate coordinating with a water molecule to the substrate to enable binding (18). This
164 has previously been suggested as an explanation into the weak activity of this sub-class of enzymes
165 (3).

166

167 One of the interesting features of the OXA enzymes is the varying reports of these highly similar
168 enzymes being either monomeric or dimeric, although at present no OXAAb enzyme has currently
169 been classified as either a dimer or monomer. We therefore compared our OXA-66 structure with
170 confirmed monomeric and dimeric enzymes to determine the possible oligomeric state. Three
171 schemes of salt bridges have been identified as being important for dimerization previously with
172 several key residues identified (7, 19, 20). These three systems have been identified in OXA-10,
173 OXA-13 and OXA-48. Comparing the sequence of OXA-66 with each of these systems we observe
174 the likely absence of most or all the salt bridges in OXA-66. Both OXA-13 and OXA-48 form native
175 dimers utilising 5-6 salt bridges on an interface distant to the active site (6, 7, 19). Comparison to the
176 OXA-66 sequence suggests that these salt bridges are likely to be abolished or significantly
177 weakened throughout, suggesting OXA-66 may be monomeric. For example, the glutamic acid at
178 position 86 in OXA-13 forms salt bridges to both Lys182 and Asp176, and the equivalent Glu89 in
179 OXA-48 forms interactions with Arg189. However, in the OXA-66 structure a threonine is present at
180 this position in 3D-space which is much shorter and cannot reach across the gap efficiently to form a
181 salt bridge, especially as the alternative position for Arg189 is a negatively charged aspartic acid
182 residue hence there is likely repulsion at this site (Figure 2). Likewise, any conserved charges present
183 around this region do not appear to be in a position that would enable salt bridge formation.

184

185 OXA-10 differs from OXA-13 and OXA-48 in that the OXA-10 salt bridges require the octahedral
186 coordination of a metal ion to enable dimerization. Comparing the sequence and structure of OXA-66
187 to that of OXA-10, the hydrophobic interactions that help to promote dimerization are not present in
188 OXA-66 (Figure 3). Additionally, OXA-10 requires the presence of a divalent metal ion coordinating in
189 an octahedral orientation with E190 and E227 from one chain and H203 from the other alongside
190 several water atoms (20). Although our structure contains two zinc ions, these are not bound at the
191 expected dimer interact and the arrangement of charges for co-ordinating a metal ion at the dimer
192 interface is not conserved in OXA-66. Instead, a serine, tyrosine and valine are present at this site
193 resulting in disrupted charge interactions suggesting that OXA-66 may not form dimers in the
194 presence of metal ions (Figure 3). Previously, it was thought that the β -3 and Ω -loops played an
195 important role in the dimerization process (4, 5). However, on comparing this interface and the salt
196 bridges, we conclude that these reports are unlikely to affect the overall dimerization of the enzyme

197 and instead are more likely to play a mechanistic role. Previous crystal structures of OXA-51 have
198 been crystallised with 1 or 4 molecules within an asymmetric unit (3, 18) , while our structure contains
199 just one molecule per asymmetric unit which corresponds to Matthews Coefficient of 2.82 and
200 crystal's solvent content of 56.4%. In contrast the asymmetric units of dimeric OXAs such as OXA-10,
201 OXA-13 and OXA-48 commonly contain multiple copies of the respective enzymes (19-21). While not
202 definitive, this suggests that dimerization may be less favoured in the OXAAb enzymes. Further work
203 using appropriate analytical methods such as size exclusion chromatography with multiple angle light
204 scattering (SEC-MALS), or dynamic light scattering (DLS), is needed to determine the true oligomeric
205 state of this group of enzymes.

206

207 Overall, we demonstrate that the most prevalent OXAAb enzyme OXA-66 has structural similarity to
208 OXA-51. On the bases of sequence and structural analyses, we also suggest that residues at the
209 dimeric interface suggest the OXAAb enzymes may exist as monomers.

210

211 **Authors and contributors**

212 Conceptualisation – BAE; Methodology – YT, DYC, PJW, BAE; Investigation – YT, DYC; Formal
213 Analysis – SRH, DYC; Visualisation – SRH; Writing – Original Draft Preparation – SRH, BAE; Writing
214 – Review and Editing – YT, SRH, DYC, PJW, BAE; Supervision – PJW, BAE; Funding – PJW, BAE.

215

216 **Conflicts of interest**

217 The authors declare that there are no conflicts of interest.

218

219 **Funding information**

220 The experiments described in this paper were funded by Anglia Ruskin University. SRH was funded
221 by a grant from the Wellcome Trust awarded to BAE (grant reference 213979/Z/18/Z).

222

223 **Acknowledgements**

224 We thank Dr Shintaro Aibara (Max Planck Institute for Biophysical Chemistry, Gottingen) for valuable
225 discussion on protein purification.

226

227 **References**

- 228 1. Evans BA, Amyes SG. OXA β -lactamases. *Clin Microbiol Rev.* 2014;27(2):241-63.
- 229 2. Takebayashi Y, Findlay J, Heesom KJ, Warburton PJ, Avison MB, Evans BA. Variability in
230 carbapenemase activity of intrinsic OxaAb (OXA-51-like) beta-lactamase enzymes in *Acinetobacter*
231 *baumannii*. 2020.
- 232 3. Smith CA, Antunes NT, Stewart NK, Frase H, Toth M, Kantardjieff KA, et al. Structural Basis
233 for Enhancement of Carbapenemase Activity in the OXA-51 Family of Class D beta-Lactamases. *ACS*
234 *Chemical Biology.* 2015;10(8):1791-6.
- 235 4. Sun T, Nukaga M, Mayama K, Braswell EH, Knox JR. Comparison of beta-lactamases of
236 classes A and D: 1.5-angstrom crystallographic structure of the class D OXA-1 oxacillinase. *Protein*
237 *Science.* 2003;12(1):82-91.
- 238 5. Santillana E, Beceiro A, Bou G, Romero A. Crystal structure of the carbapenemase OXA-24
239 reveals insights into the mechanism of carbapenem hydrolysis. *Proceedings of the National Academy*
240 *of Sciences of the United States of America.* 2007;104(13):5354-9.
- 241 6. Docquier JD, Calderone V, De Luca F, Benvenuti M, Giuliani F, Bellucci L, et al. Crystal
242 Structure of the OXA-48 beta-Lactamase Reveals Mechanistic Diversity among Class D
243 Carbapenemases. *Chemistry & Biology.* 2009;16(5):540-7.
- 244 7. Lund BA, Thomassen AM, Nesheim BHB, Carlsen TJO, Isaksson J, Christopeit T, et al. The
245 biological assembly of OXA-48 reveals a dimer interface with high charge complementarity and very
246 high affinity. *Febs Journal.* 2018;285(22):4214-28.
- 247 8. Danel F, Frere JM, Livermore DM. Evidence of dimerisation among class D beta-lactamases:
248 kinetics of OXA-14 beta-lactamase. *Biochimica Et Biophysica Acta-Protein Structure and Molecular*
249 *Enzymology.* 2001;1546(1):132-42.
- 250 9. Verma V, Testero SA, Amini K, Wei W, Liu J, Balachandran N, et al. Hydrolytic Mechanism of
251 OXA-58 Enzyme, a Carbapenem-hydrolyzing Class D beta-Lactamase from *Acinetobacterbaumannii*.
252 *Journal of Biological Chemistry.* 2011;286(43):37292-303.
- 253 10. Nielsen H, Engelbrecht J, Brunak S, vonHeijne G. Identification of prokaryotic and eukaryotic
254 signal peptides and prediction of their cleavage sites. *Protein Engineering.* 1997;10(1):1-6.
- 255 11. Nielsen H. Predicting Secretory Proteins with SignalP. *Methods Mol Biol.* 2017;1611:59-73.
- 256 12. AXS B. PROTEUM 2 User Manual. 2010.
- 257 13. Adams PD, Afonine PV, Bunkoczi G, Chen VB, Davis IW, Echols N, et al. PHENIX: a
258 comprehensive Python-based system for macromolecular structure solution. *Acta Crystallographica*
259 *Section D-Biological Crystallography.* 2010;66:213-21.
- 260 14. McCoy AJ, Grosse-Kunstleve RW, Adams PD, Winn MD, Storoni LC, Read RJ. Phaser
261 crystallographic software. *Journal of Applied Crystallography.* 2007;40:658-74.
- 262 15. Emsley P, Lohkamp B, Scott WG, Cowtan K. Features and development of Coot. *Acta*
263 *Crystallographica Section D-Biological Crystallography.* 2010;66:486-501.
- 264 16. McNicholas S, Potterton E, Wilson KS, Noble MEM. Presenting your structures: the CCP4mg
265 molecular-graphics software. *Acta Crystallographica Section D-Structural Biology.* 2011;67:386-94.
- 266 17. Brown S, Amyes SG. The sequences of seven class D beta-lactamases isolated from
267 carbapenem-resistant *Acinetobacter baumannii* from four continents. *Clin Microbiol Infect.*
268 2005;11(4):326-9.
- 269 18. June CM, Muckenthaler TJ, Schroder EC, Klamer ZL, Wawrzak Z, Powers RA, et al. The
270 structure of a doripenem-bound OXA-51 class D beta-lactamase variant with enhanced
271 carbapenemase activity. *Protein Science.* 2016;25(12):2152-63.
- 272 19. Pernot L, Frenois F, Rybkine T, L'Hermite G, Petrella S, Delettre J, et al. Crystal structures of
273 the class D beta-lactamase OXA-13 in the native form and in complex with meropenem. *Journal of*
274 *Molecular Biology.* 2001;310(4):859-74.
- 275 20. Paetzel M, Danel F, de Castro L, Mosimann SC, Page MGP, Strynadka NCJ. Crystal structure of
276 the class D beta-lactamase OXA-10. *Nature Structural Biology.* 2000;7(10):918-25.

277 21. Lund BA, Christopeit T, Guttormsen Y, Bayer A, Leiros HKS. Screening and Design of Inhibitor
278 Scaffolds for the Antibiotic Resistance Oxacillinase-48 (OXA-48) through Surface Plasmon Resonance
279 Screening. Journal of Medicinal Chemistry. 2016;59(11):5542-54.

280

281 **Figure 1:** Structural alignment of our OXA-66 structure (blue) with the apo OXA-51 structure 4ZDX
282 (gold), displaying the key active site residues.

283

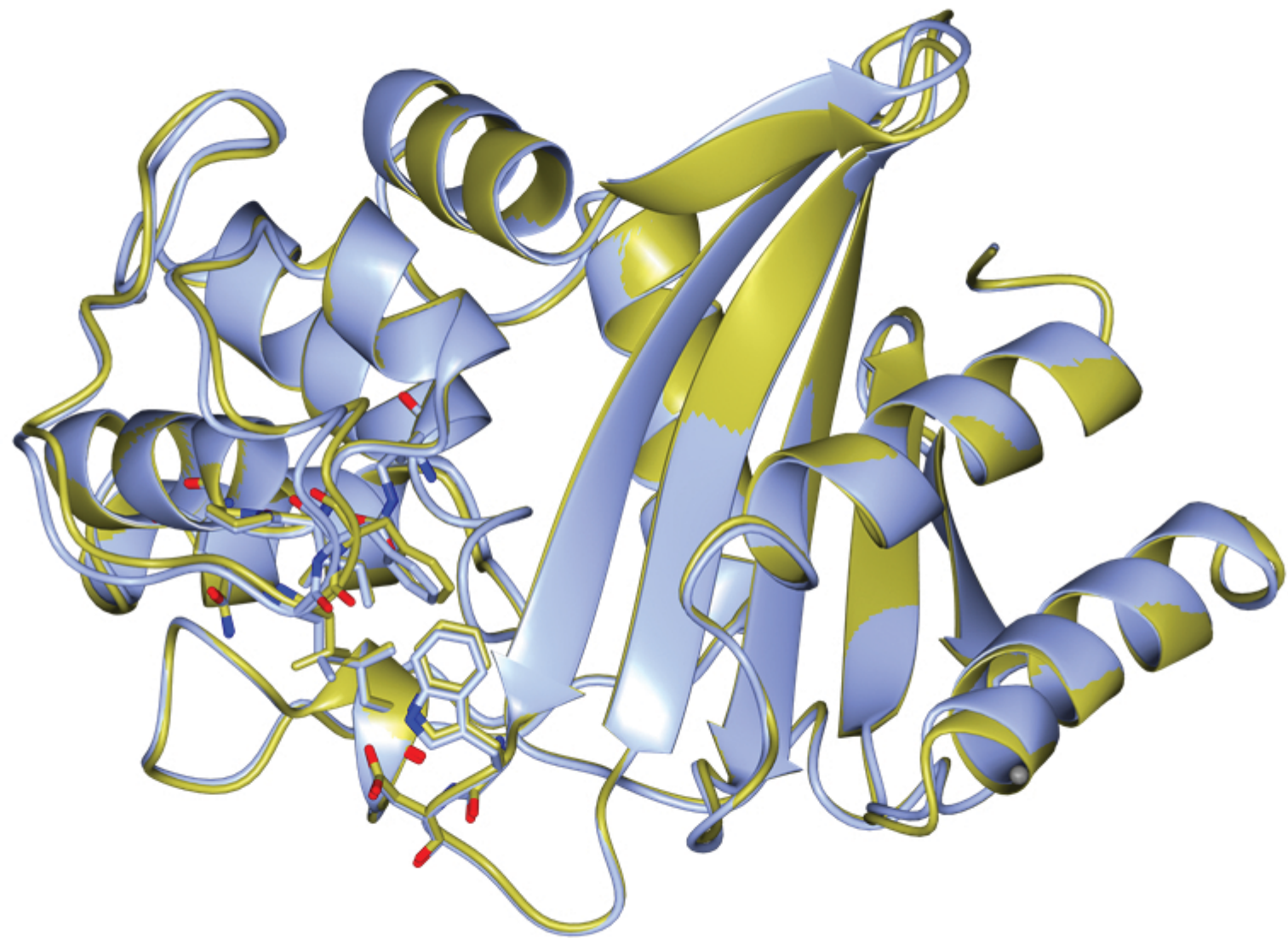
284 **Figure 2:** View of part of the dimer interface of (left) OXA-13 (pink, PDB ID: 1H8Z) and (right) OXA-48
285 (grey, PDB ID: 5DTK) aligned with 2 copies of OXA-66 (blue). Ionic interactions and bonding
286 distances are represented. No ionic interactions were found at the OXA-66 interface.

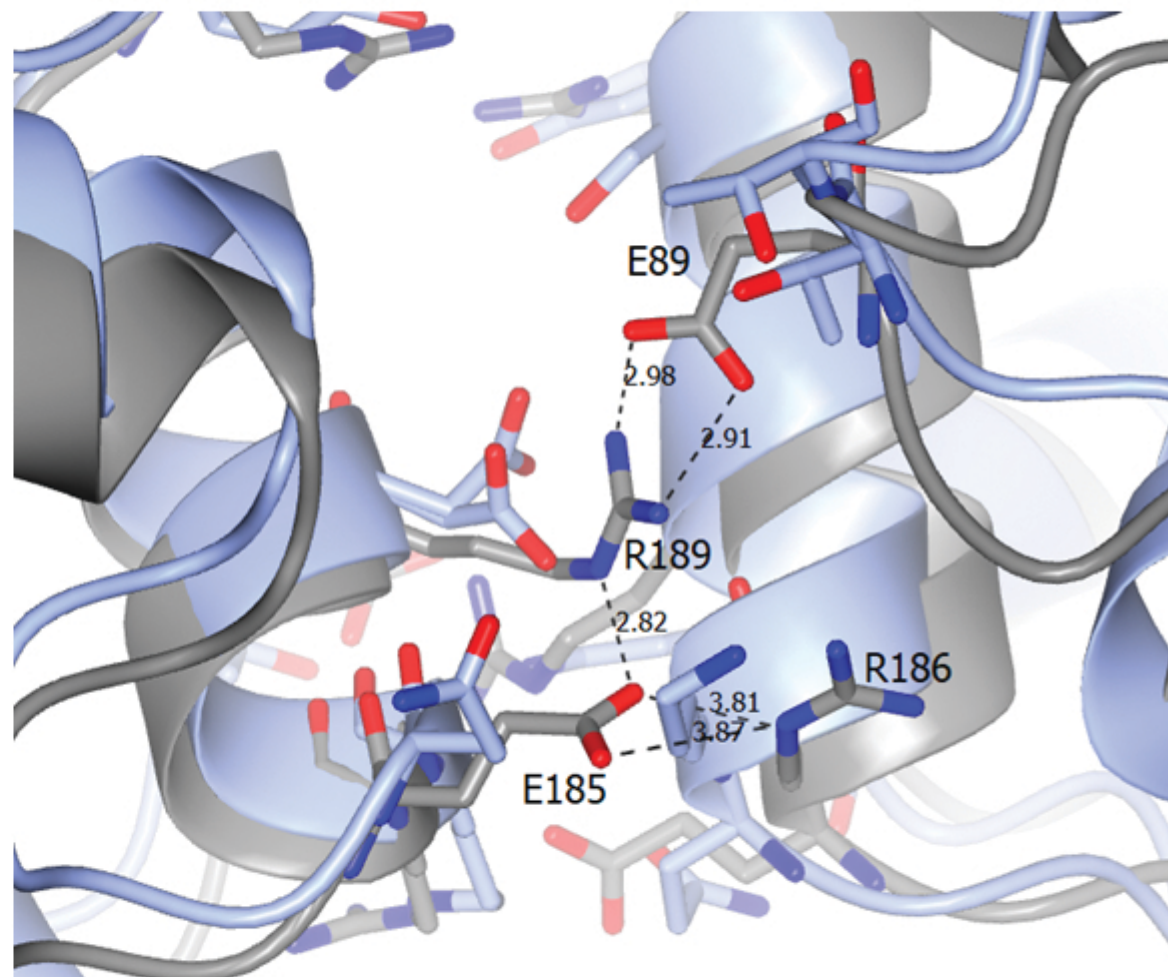
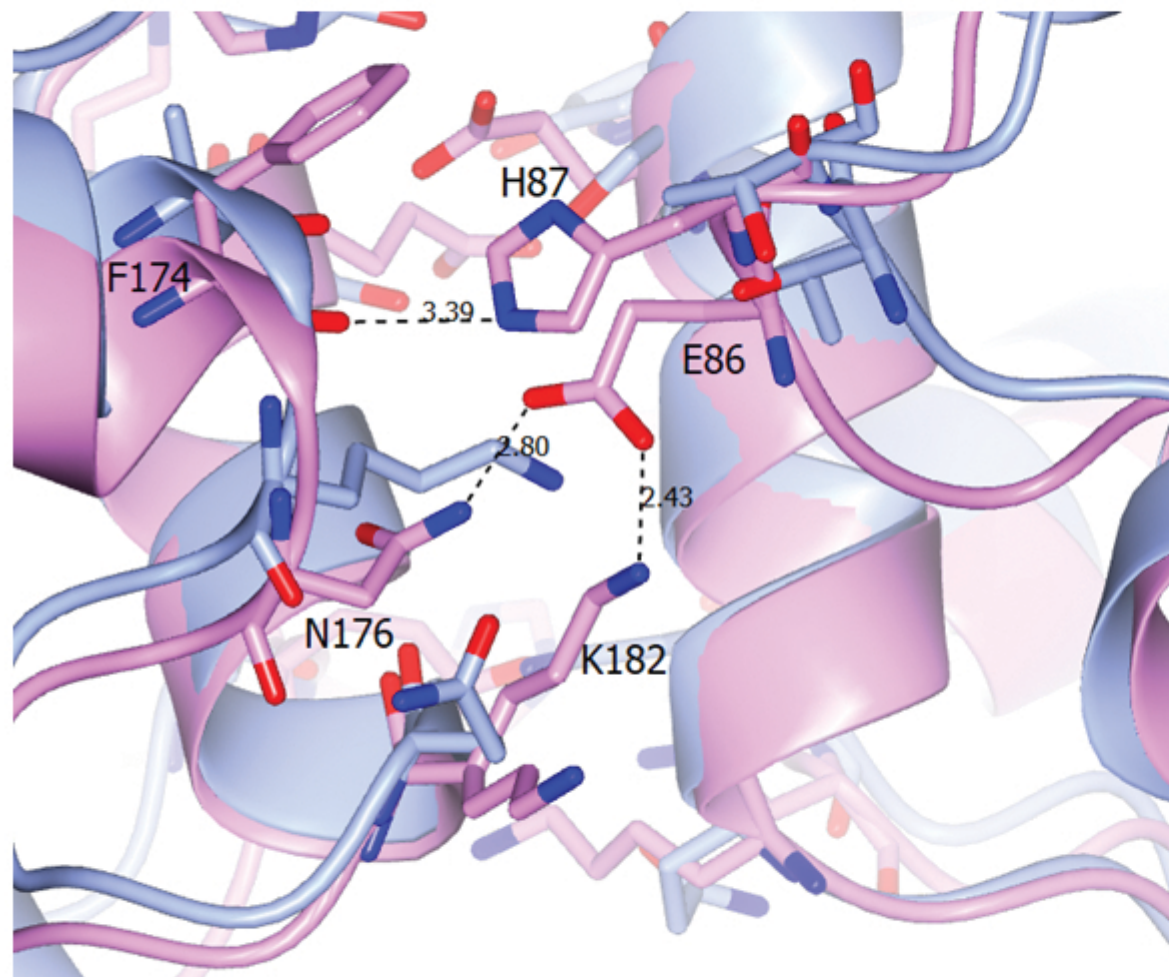
287

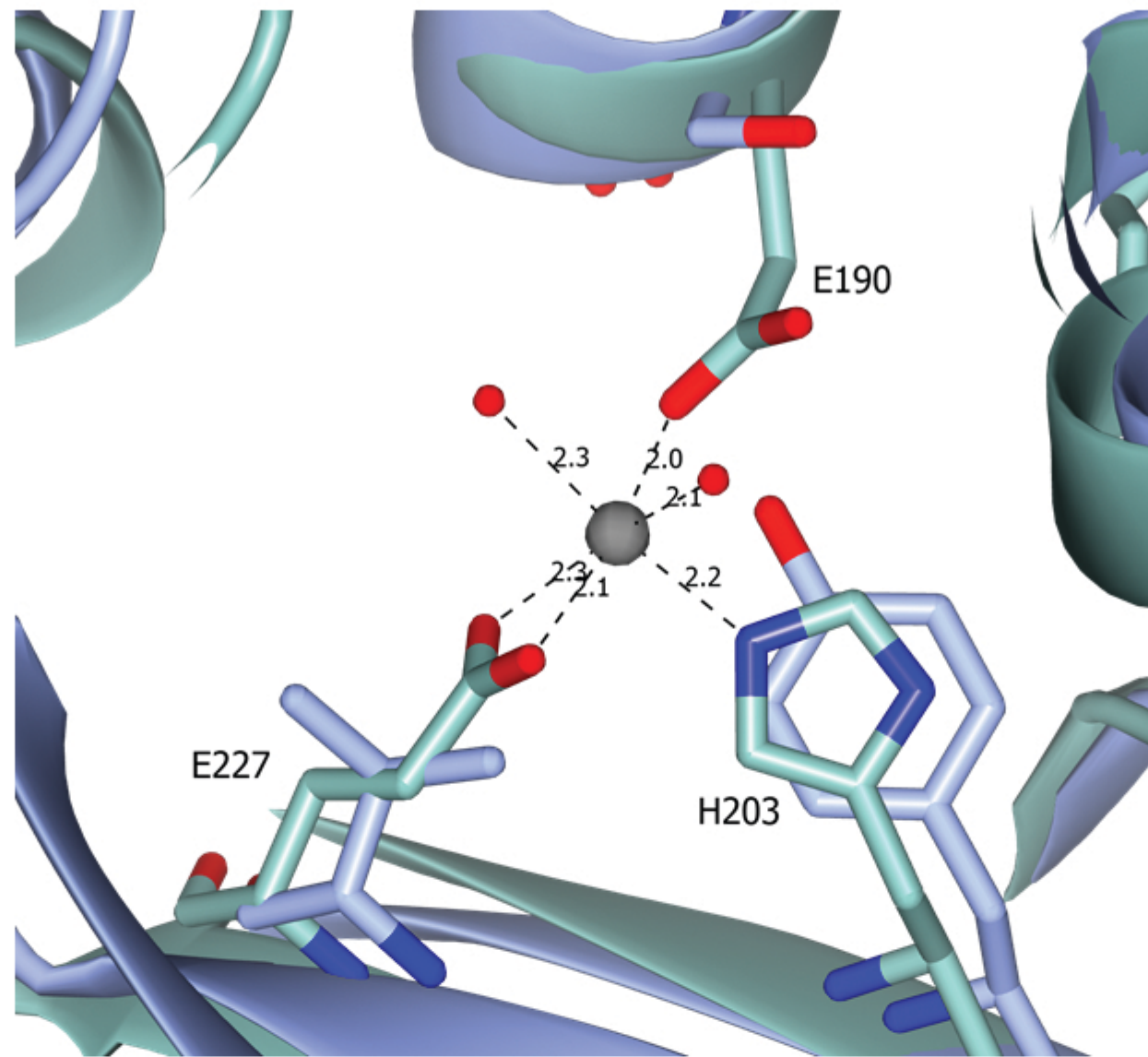
288 **Figure 3:** Top: Metal coordination forming dimer interaction in OXA-10 (green), overlaid with 2 copies
289 of OXA-66 (blue), where no salt bridge or metal co-ordination is observed in comparison to the OXA-
290 10 metal coordination. Numbering refers to OXA-10 structure. Bottom: Sequence alignment of OXA-
291 66-Ab with OXA-10-Pa demonstrating the absence of charge conservation at key residues involved in
292 metal coordination.

293

294







```

OXA-66-Ab      MNIKALLLITSAIFISACSPYIVTANPNHSASKSDVKAEKIKNLFNEAHTTGVLVIQQGQ  60
OXA-10-Pa      -----MKTFAAYVIIACLSSTALA-----GSITENTSWNKEFSAEAVNGVFLCKSS  47
                :  :*  .*  **   .  *           *  .:  .  ::  *.   ..**:::  ...

OXA-66-Ab      TQQSYGNDLARASTEYVPASTFKMLNALIGLEHHKA-TTTEVFKWDGKKRLFPWEKDMT  119
OXA-10-Pa      SKSCATNDLARASKEYLPASTFKIPNAIIGLETGVIKNEHQVFKWDGKPRAMQWERDLT  107
                :...  *****.*::*****:  **::****   .  :***** *  :  :***:*

OXA-66-Ab      LGDAMKASAI PVYQDLARRIGLELMSKEVKRVGYGNADIGTQVDNFWLVGPLKITPQOEA  179
OXA-10-Pa      LRGAIQVSAVPVFQQIAREVGEVRM QKYLKKFSGYGNQISGGIDKFWLEGQLRISAVNQV  167
                *  .*:::.*::**::**::**::**::**  *.*  :*::..***  :*.  :*::***  *  *:::  :..

OXA-66-Ab      QFAYKLANCTL PFSQKVQDEVQ SMLFIEEKNGN--K IYAKSGWGDVNPQVGWLTGWVV  236
OXA-10-Pa      EFLESLYLNKLSASKENQLIVK EALVTEAAPEYLV HSKTGFSGVGTESNPGVAWVVGWVE  227
                :*  .*  :.*  *::  *  *::  *.  *           .  .  **  *  :  **  *.  .***

OXA-66-Ab      QPQGNIVAFSLNLEMKKGIPSSVRKEITYKSLEQLGIL--  274
OXA-10-Pa      KET-EVYFFAFNMDIDNESKPLRKS IPTKIMESEGIIGG  266
                :  ::  *::*:::..:  ;**.*  *  :*.  **:
```

Supplementary Material

Figure S1: Active site of OXA-66 (blue) and the previously solved OXA-51 structure (gold) co-crystallized with Doripenem (green) (PDB ID: 5L2F).

