SUPPLEMENTARY MATERIAL FOR:

Structural studies on bacterial topoisomerase inhibitors: trapping a DNA-cleaving machine in motion

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Table S1 Coordinates of biological complexes derived from 23 xtal structures of the *S. aureus* DNA gyrase GyrBA fusion truncate – on ***'Research'*** tab at https://www.cardiff.ac.uk/people/view/1141625-bax-ben

Co-ordinates for biological complexes are available (click to upload) in the columns labelled 'Coordinates for first (or second) complex in asymmetric unit'. If the complex has twofold disorder around the axis of the complex two complexes are available, representing the two orientations of the biological complex observed in the crystal structure. *Note most structures have one or two complexes in the asymmetric unit; but in the two apo structures (2xco and 2xcq, the GyrBA dimer sits on a crystallographic twofold and there is half a dimer in the asymmetric unit), in 6qx2 there are six complexes in the asymmetric unit.

No	PDB code/ Res.	Inhibitor	Inhibitor pockets occupied						Crystal coords. (BA-x numb.), Space-group [cell (a,b,c Å, and α,β,γ °)]	Coordinates for first complex in asym. unit*	Coordinates for second complex in asym. unit*
			1	1'	2 D	2 A	3	3'			
1	2xcq 2.98	none	-	-	-		-	-	2xcq-BA-x.pdb, $P6_122$, 90,90,416 90,90,120	2xcq-c1.pdb	
2	2xco 3.1	none	-	-	-		-	-	2xco-BA-x.pdb $P6_122$, 90,90,411 90,90,120	2xco-c1a.pdb 2xco-c1b.pdb	
3	6fqv 2.6	none	-	-	-		-	-	6fqv-BA-x.pdb $P2_1$, 93,125,155 90,96,90	6fqv-c1.pdb	6fqv-c2.pdb
4	5cdr 2.65	none	-	-	-		-	-	5cdr-BA-x.pdb $P6_1$, 93,93,411 90,90,120	5cdr-c1.pdb	
5	5iwi 1.98	'237	-	-	X	X	-	-	5iwi-BA-x.pdb $P6_1$, 93,93,411 90,90,120	5iwi-c1a.pdb 5iwi-c1b.pdb	
6	2xcs 2.1Å	'423	-	-	X	X	-	-	2xcs-BA-x.pdb, $P6_1$, 93,93,413 90,90,120	2xcs-c1a.pdb 2xcs-c1b.pdb	
7	6qtk 2.31Å	gepo'	-	-	X	X	-	-	6qtk-BA-x.pdb		
8	6qtp 2.37Å	gepo'	-	-	X	X	-	-	6qtp-BA-x.pdb		
9	5iwm 2.5Å	'237	-	-	X	X	-	-	5iwm-BA-x.pdb $P6_1$, 94,94,413 90,90,120	5iwm-c1a.pdb 5iwm-c1b.pdb	
10	4bul 2.6Å	'587	-	-	X	X	-	-	4bul-BA-x.pdb $P6_1$, 94,94,416 90,90,120	4bul-c1a.pdb 4bul-c1b.pdb	
11	2xcr 3.5Å	'423	-	-	X	X	-	-	2xcr-BA-x.pdb $P2_12_12_1$ 113,165,308 90,90,90	2xcr-c1a.pdb 2xcr-c1b.pdb	2xcr-c2a.pdb 2xcr-c2b.pdb
12	5npp 2.22Å	'237 + Thp2	-	-	X	X	X	X	5npp-BA-x.pdb $P6_1$, 93,93,410 90,90,120	5npp-c1a.pdb 5npp-c1b.pdb	
13	5npk 1.98Å	Thp1	-	-	-	-	X	X	5npk-BA-x.pdb $P2_1$, 89,121,169 90,90.1,90	5npk-c1.pdb	5npk-c2.pdb
14	6qx1 2.65	Benzois'3	-	-	-	-	X	X	6qx1-BA-x.pdb		
15	6qx2 3.4	Benzois'3	-	-	-	-	X	X	6qx2-BA-x.pdb		
16	5cdp 2.45Å	Etop.	X	-	-		-	-	5cdp-BA-x.pdb $P6_1$, 93,93,411 90,90,120	5cdp-c1.pdb	
17	5cdm 2.5Å	QPT-1	X	X	-		-	-	5cdm-BA-x.pdb $P6_1$, 94,94,412 90,90,120	5cdm-c1.pdb	
18	5cdn 2.8Å	Etop.	X	X	-		-	-	5cdn-BA-x.pdb $P2_1$, 90, 170, 125, 90, 102, 90	5cdn-c1.pdb	
19	5cdq 2.95Å	Moxi.	X	X	-		-	-	5cdq-BA-x.pdb $P2_1$, 88, 171,126, 90, 103, 90		
20	6fqm 3.06Å	IPY-t1	X	X	-		-	-	6fqm-BA-x.pdb $P2_1$ 88, 172, 125, 90, 103, 90	6fqm-c1.pdb	
21	6fqS 3.11Å	IPY-t3	X	X	-		-	-	6fqs-BA-x.pdb $P6_1$, 94,94,420 90,90,120	6fqs-c1a.pdb 6fqs-c1b.pdb	
22	5cdo 3.15Å	QPT-1	X	X	-		-	-	5cdo-BA-x.pdb $P2_1$, 91,170, 125, 90, 103, 90	5cdo-c1.pdb	
23	2xct 3.35	Cipro.	X	X	-		-	-	2xct-v2-BA-x.pdb $P2_1$, 89,123,170 90,90.3,90 90	2xct2-v2-c1.pdb	2xct2-v2-c2.pdb

Table S2 Comparison of DNA sequences used in published *S. aureus* DNA-gate crystal structures.

DNAs are self-complementary and form 20 base-pair duplexes. The three heteroduplexes crystallised with taps-NBTIs, 20-21/20-21cmp, 20-23/20-23cmp., and 20-12-8^{23}/20-23cmp, had static disorder and were observed in two orientations related by the twofold axis of the complex. The **20-12p-8** DNA duplex has an artificial nick in the DNA at each cleavage site, and the 5' nucleotide of the 12mer includes a 5' phosphate (indicated by a ^{P}). DNAs observed to have been cleaved by the enzyme are indicated Y^{P}G (the Y^{P} representing the phophotyrosine bond between Tyr 123 and the DNA). Uncleaved DNA sequences have a ____ at cleavage sites. PDB codes are coloured as, black (binary complex, no compound), blue (NBTIs - 2D and 2A pockets), red (pocket 1 compounds), green (pocket 3 compounds).

PDB codes (and resolution in Å) for structures with this DNA	**DNA DUPLEX NAME**	**DNA SEQUENCE** (Note all DNAs, when annealed, form 20 base-pair duplexs. Note DNAs are listed in order: (i) uncleaved (ii) nicked (with artificial nicks at one or both cleavage sites) and (iii) cleaved. Note 447T DNA is seen in both states (i) and (iii)
	Position* 5'-3'	-8 -5 -4 **-1** **1** 2 3 4 5 8 9 12 (Ile)
	3'-5'	12 9 8 5 4 3 2 **1** **-1** -4 -5 -8 (Ile)
6fqv (2.6)	20-447T 5'-3'	GAGC GTAC ____ G G C C GTAC GCTC
	20-447T 3'-5'	CTCG CATG C C G G ____ CATG CGAG
2xcs(2.1), 2xcr (3.5), *5bs3(2.65), 4plb(2.69)*	20-20 5'-3'	AGCC GTAG ____ G **G** **C** C CTAC GGCT
	20-20 3'-5'	TCGG CATC C **C** **G** G ____ GATG CCGA
5iwm (2.5)	20-21 5'-3'	TGTG CGGT ____ G **A** **A** C CTAC GGCT
	20-21cmp. 3'-5'	ACAC GCCA C **T** **T** G ____ GATG CCGA
4bul(2.6)	20-23 5'-3'	TGTG CGG**T** ____ **G** **T** **A** C CTAC GGCT
	20-23cmp. 3'-5'	ACAC GCCA C **A** **T** **G** ____ **G**ATG CCGA
5iwi (1.98)	20-12-8^{23} 5'-3'	TGTG CGG**T** **G** **T** **A** C CTAC GGCT
	20-23cmp. 3'-5'	ACAC GCCA C **A** **T** G ____ GATG CCGA
2xct (3.35)*	20-12-8^{23} 5'-3'	TGTG CGG**T** **G** **T** **A** C CTAC GGCT
	20-12^{23c}-8^{23c} 3'-5'	ACAC GCCA C **A** **T** **G** **G**ATG CCGA
6qx2 (3.4)	20-444T 5'-3'	GAGC GTAC ____ A **G** **C** T GTAC GCTT
	20-444T 3'-5'	TTCG CATG T **C** **G** A ____ CATG CGAG
5npp(2.2), 5npk(1.98), 6qtk (2.3), 5cdp(2.45), 5cdr(2.65), 6qx1(2.65)	20-12p-8 5'-3'	AGCC GTA**G** **^{P}G** **T** **A** C CTAC GGCT
	20-12p-8 3'-5'	TCGG CATC C **A** **T** **G^{P}** **G**ATG CCGA
5cdn (2.8)	20-447 5'-3'	GAGC GTAC Y^{P}G G C C GTAC GCTC
	20-447 3'-5'	CTCG CATG C C G G^{P}Y **C**ATG CGAG
5cdm(2.5), 5cdo(3.15)	20-447T 5'-3'	GAGC GTAC Y^{P}G G C C GTAC GCTC
	20-447T 3'-5'	CTCG CATG C C G G^{P}Y **C**ATG CGAG
5cdq(2.95)	20-448T 5'-3'	GAGC GTAT Y^{P}G G C C ATAC GCTT
	3'-5'	TTCG CATA C C G G^{P}Y **T**ATG CGAG
6fqm(3.06), 6fqs(3.11)	20-448TU 5'-3'	GAGA GTAT Y^{P}G G C C ATAC UCTT
	3'-5'	TTCU CATA C C G G^{P}Y **T**ATG AGAG

By convention cleavage sites for topo2A DNA sequences are numbered relative to the cleavage position between -1 and 1 nucleotides. In the above crystal structures there is no ambiguity as to the register of oligos (because there is clear electron density for twenty nucleotides in at least one strand). However, we note that the same oligo can be cleaved by a topo2 at different positions [1]; in some low-resolution structures of type IIA topos the DNA might be bound in different registers.

Table S3. Refinement statistics from re-refinement of two structures: 2XCT, 3L4K – comparison with original refinement statistics.

Original PDB code - comment	2XCT – 3.35-Å ciprofloxacin complex with *S. aureus* gyrase and DNA [2]. Twinned.		3L4K – 2.98-Å binary complex of *S. cerevisae* topo II with one DNA strand with artificial nick and the other cleaved (with 3´ sulfur) [3]. Static disorder around crystallographic twofold	
	Re-refined structure	'Original' coordinates	Re-refined structure	'Original' coordinates
PDB CODE	xxxx	2XCT	yyyy	3L4K
Space group	$P2_1$	$P2_1$	$P2_122_1$	$P2_122_1$
Cell dimensions (a,b,c in Å, α,β,γ in degrees)	a = 89.0 b = 123.2 c = 170.4 90, 90.25,90	a = 89.0 b = 123.2 c = 170.4 90, 90.25,90	a = 86.1 b = 91.9 c = 116.0 90,90,90	a = 86.1 b = 91.9 c = 116.0 90,90,90
Resolution (Å)	3.35	3.35	2.98	2.98
No. of complexes in asym. unit	2	2	0.5	0.5
Refinement*				
Resolution (Å)	25.0-3.34 (3.42-3.34)	25.0-3.35 (3.41-3.35)	49.0-2.98 (3.06-2.98)	49.0-2.98 (3.08-2.98)
No. reflections	52172 (3314)	52173 (2628)	17618 (1283)	17619 (2722**)
R_{work}/R_{free} (%)	16.1/20.6 (18.9/29.5)	16.6/23.6 (21.1/32.9)	19.1/25.7 (33.0/39.8)	23.9/27.3 (33.7/39.4)
No. Atoms[1]				
Protein	21715	20805	5989	5937
DNA	1592	1461	1063	1059
Active site metal ions (Mn^{2+} - 2XCT, or ZN^{2+} - 3L4K)	4 (each with occupancy 1.0)	4 (each with occupancy 1.0)	2 (each with occupancy 0.5)	2 (each with occupancy 1.0)
Waters coordinating active site metals	16	None	6 (each with occupancy 0.5)	None
B-factors				
Protein	77.5	73.7	83.8	87.5
DNA	90.2	76.8	95.0	96.4
Active site metal ions (Mn^{2+} - 2XCT, or ZN^{2+} - 3L4K)	68.7	74.0	71.1	133.5
Waters coordinating active site metals	68.7	N/A (no waters)	68.4	N/A (no waters coordinating active site metals)
R.m.s deviations				
Bond lengths (Å)	0.0150	0.024	0.0125	0.0068
Bond angles (°)	1.70	1.44	1.68	1.04
Ramachandran favoured	96.02%	79.00%	91.32%	95.54%
Ramachandran outliers	0.18%	4.80%	0.96%	0.62%

*Highest resolution shell is shown in parenthesis. [1] The number of atoms does not include hydrogens and B-factors do not include hydrogens. Structures are not close to atomic resolution. **The version of phenix (1.5_2) used in original 3L4K refinement counts both F(+) and F(-) in resolution bins but only FP in overall number of reflections.

Table S4 Coordinates of biological complexes for the deposited and re-refined crystal structures of 3L4K (available from https://www.cardiff.ac.uk/people/view/1141625-bax-ben - click on 'Research' tab)

Because 3L4K sits on a crystallographic twofold axis, the observed 2.98-Å electron density is effectively a convolution of two structures superposed, related by the crystallographic twofold axis. This makes refinement and interpretation of the electron density more challenging, and more ambiguous than would be the case for a 2.98-Å X-ray crystal structure not suffering from such static disorder. Below are presented coordinates from the two interpretations of the data: 3lk4.pdb and the derived complexes, 3l4k-c1a.pdb and 3l4k-c1b.pdb are the originally published interpretation [3], while RR-3l4k.pdb and RR-3l4k-c1a.pdb and RR-3l4k-c1b.pdb are from the re-refinement described in this paper. We make all coordinates available from the web-site http:/, so that interested parties may download them.

PDB file	Active site 1			Active site 2			Crystallographic coordinates	Coordinates for biological complex
	Metal site occupancies		WHD Tyr 782	Metal site occupancies		WHD Tyr 782'		
	A	B		A	B			
Original 3L4K	1.0	1.0	Tyr	1.0	1.0	Tyr	3l4k.pdb	3l4k-c1a.pdb 3l4k-c1b.pdb
Re-refined RR-3L4K	0.5	0.5	Tyr	0.5	0.5	Tyr	RR-3l4k.pdb	RR-3l4k-c1a.pdb RR-3l4k-c1b.pdb

Table S5 Number of electrons observed in atoms at full occupancy and half occupancy

The number of electrons for different atoms/ions is shown, as well as the number of electrons that should be observed for that atom/ion if it is present at half occupancy.

Atom/ Ion	No. of ELECTRONS (full occupancy)	No of electrons observed at HALF OCCUPANCY
Mn^{2+}	23	11.5
Zn^{2+}	28	14
Mg^{2+}	10	5
Na^{+}	10	5
H_2O	10	5
OH^{-}	10	5
S	16	8
P	15	7.5
O	8	4
N	7	3.5
C	6	3
H	1	0.5

Supplementary methods

Re-refining Mn^{2+}, Mg^{2+} and Zn^{2+} ions in low (>3 Å), medium-low (2.5-2.99 Å), medium-high (2.01-2.49 Å) and high (>2 Å) resolution X-ray crystal structures of type IIA topoisomerases.

One solution to the problem of refining low resolution crystals structures with metal ions, is to use the information from related higher resolution crystal structures where the coordination geometry is reasonably well defined (as well as restraints derived from small molecule crystal structures [4, 5].). This is the approach we adopt in this paper.

The definitions of resolution used in this paper [low (>3 Å), medium-low (2.5-2.99 Å), medium-high (2.01-2.49 Å) and high (>2 Å)] are based on how easy it is to determine the coordination geometry of a metal ion at that resolution. A typical 3.0 Å X-ray protein crystal structure contains relatively few waters, whereas at 2.0 Å many waters are visible. The coordination sphere of a metal ion often includes waters and is usually reasonably well defined in a 2.0 Å structure, but at 3.0 Å the coordination sphere is usually difficult to determine from the X-ray data alone. The coordination geometries that can be adopted by a particular metal ion can be defined from an analysis of small molecule crystal structures (which typically have a resolution better than 0.83 Å, and can be refined without geometric restraints [6]). For example, an analysis of some 1,586 small molecule crystal structures containing Mg^{2+} ions, and some 3,198 small molecule crystal structures containing Mn^{2+} ions [5] from the CSD gave clear indications of the possible coordination geometries adopted by these ions when coordinated by oxygen and nitrogen. The most common coordination geometry observed for both Mg^{2+} and Mn^{2+} ions when coordinated by six oxygens is octahedral, with bond distances of 2.073(3) Å for Mg^{2+} ions and 2.172 (4) Å for Mn^{2+} ions, with O--M^{2+}--O angles of 90 (3) degrees. We note that because a Mg^{2+} ion has ten electrons, the same number as a Na^{+} ion, a water and an OH^{-} ion (supplementary table S5), models of low resolution X-ray crystal structures which include Mg^{2+} ions without proper coordination geometry are not easy to believe in.

In vivo type IIA topoisomerases are believed to use Mg^{2+} ions in the two DNA-cleavage and two DNA-religation steps of the catalytic cycle [7]. However, *in vitro* Mn^{2+} can be used in place of Mg^{2+} ions [7]. Most *S.aureus* DNA gyrase crystal structures contain Mn^{2+} ions (table 1, 2) because the protein is purified in the presence of 5mM Mn^{2+} ions [8]. In the 2.1Å crystal structure of *S. aureus* DNA gyrase with GSK299423 (pdb code: 2XCS) a single Mn^{2+} ion is seen at the 'A' or 3' site [2], coordinated by six oxygens (Fig 4b, Fig 6a, supplementary Fig 3a), the coordination geometry is defined by the data (see Fig 5 in Bax *et al.,* 2010; [2]). In the 2.16Å crystal structure of human topo IIβ with DNA and etoposide (pdb code: 3qx3) a single metal ion is seen at the B site [9], coordinated by six oxygens. This B site configuration is also seen in, for example, the 2.5Å and 1.98 Å crystal structures of *S.aureus* gyrase with QPT-1 and a thiophene (supplementary Fig. 4). We note that in *S.aureus* gyrase crystal structures the B-site does not exist when a metal is bound at the A-site, and vice-versa (supplementary Fig. 2).

Type IIA topoisomerases are flexible enzymes and many early structures with DNA were obtained at low (>3.0 Å) or medium low resolution (2.5-2.99 Å). In this paper we re-refine two of these early DNA complexes (2xcs, 3qx3) using information from higher resolution crystal structures – to show that early ambiguous structures can be interpreted consistently with subsequently determined higher resolution unambiguous structures (Table S3).

We note that resolution is important in how a structure is refined, and in what details will be revealed by the data. Blundell and Johnson (1976)[10] state that: 'Thus it is assumed that the smallest detail that can be faithfully imaged in such electron density maps correspond to features separated by no more than 0.715dm'. This suggests that theoretically at 3 Å it is not be possible to resolve features separated by less than 2.145Å, while at 2 Å features would not be resolvable if they are less than 1.43 Å apart.

We also note that because a Mg^{2+} ion has 10 electrons (the same number of electrons as a water – supplementary table S5) it can be difficult to definitively locate an Mg^{2+} ion in a low resolution electron density map. A Mn^{2+} ion has some 23 electrons and Zn^{2+} ion has 28 electrons (table S5), so that they can usually be located in a 3.0 Å electron density map. However a 3.0 Å electron density map does not usually clearly define the coordination geometry of a Mg^{2+} ion or a Mn^{2+} ion, and because the metal ion is not always clearly resolved from its coordinating atoms the metal and coordinating atoms can sometimes scatter in phase (at low resolution). Peak heights in electron density maps for structures where data extend to atomic resolution structures (better than 1.2 Å), can be used to distinguish between carbons, nitrogens and oxygens (6, 7 or 8 electrons). And at 2 Å peak heights in electron density maps can be used to estimate occupancies of metal ions, which can then be refined. We have used information from small molecule crystal structures of quinolones [11], and restraints derived small molecules crystal structures containing Mg^{2+} in the CSD [5], to propose a model for the binding of moxifloxacin (pdb code: 2xkk; [12]), including the water metal ion bridge, with only low resolution (3.25Å**)** data. However, in refining lower resolution crystal structure great care needs to be taken, as at low resolution (less than 3 Å), the data alone cannot clearly define the coordination sphere of a magnesium ion.

Refining Occupancies and B values at low and medium/low resolution

It is often tricky to identify the identity of atoms and/or their occupancies. At relatively high resolution one can refine occupancies with sufficient reliability. At low resolution (3 Å or lower) the problem is trickier. By analysing B values of the local neighbourhood of atoms one can get some feeling about potential occupancy. We assume that atoms have been identified correctly (i.e. element name for the atoms are correct), individual B values of atoms have been refined. Let neighbouring atoms have B

value – B_n and our atom of interest have B value – B. In order peak height of equal atoms to be same as an atom with B value similar to neighbourhood the occupancy of this atom should be adjusted to:

$$c = (\frac{B_n}{B})^{3/2} \frac{-\sqrt{B} s_{max} e^{-B s_{max}^2/4} + \sqrt{\pi}\, erf(\sqrt{B} s_{max}/2)}{-\sqrt{B_n} s_{max} e^{-B_n s_{max}^2/4} + \sqrt{\pi}\, erf(\sqrt{B_n} s_{max}/2)}$$

Where s_{max} is the maximum resolution of the data in $Å^{-1}$ units. This formula assumes that B values have been refined accurately and noise effect is negligible. Obviously in reality because of experimental noise and inaccuracy of atom models' peak height will be reduced even further. However, this formula can be used as a rule of thumb for making decision about atom identity and occupancies.

For example if the resolution is 2.9 Å, the occupancy of the atom (atom1) of interest is 120 $Å^{-2}$ and neighbouring atoms have average B equal to 70 $Å^{-2}$ then occupancy of the atom1 should be changed to 0.54 to make its B value similar to that of neighbours.

Static disorder in type IIA topoisomerase crystal structures.

Two common types of disorder encountered in X-ray crystallography are dynamic and static disorder [13-15]. In dynamic disorder atoms are free to move and are not visible in electron density maps. For example in the *S. aureus* DNA gyrase structures discussed in this paper, the two apo crystal structures show a region of dynamic disorder at the C-terminus of GyrB and the N-terminus of GyrA (including Bα9, Bα10 and the N-terminal region of Aα1).

Most protein crystals have some regions of static disorder: that is parts of molecules in different unit cells do not occupy exactly the same position and do not have exactly the same orientation. Perhaps the commonest type of static disorder observed by protein crystallographers is the multiple orientations seen for many side-chains in high resolution crystal structures, which at low resolution are sometimes interpreted as a single distorted conformer [16].

One problem that has been encountered in a number of crystal structures of type IIA topoisomerases is that an asymmetric DNA duplex is bound with static disorder about the twofold axis of the complex. This type of static disorder of the DNA has been observed both when the twofold axis of the complex is crystallographic [3], or non-crystallographic [17-19]. It is not clear if, in a number of low resolution type IIA topoisomerase crystal structures with asymmetric DNAs, the DNA suffers from this kind of static disorder; as the resolution of the data are not sufficient to clearly show whether different bases are occupying the same position in such low resolution crystal structures [20-22].

The problem with static disorder about the twofold axis is also encountered with NBTI bacterial topoisomerase inhibitors. These compounds sit on the twofold axis of the complex, occupying two pockets, one in the centre of the DNA, and one between the two GyrA subunits. In high resolution and medium high resolution crystal structures with NBTIs two orientations of the

compound are clearly observed [2, 18, 23], however in two medium-low resolution crystal structures only a single orientation for the NBTI has been modelled [24, 25]. Electron density maps suggest that for these two medium-low structures (4plb, 5bs3) a second NBTI orientation may be present in the crystal, but the modest resolution of the data have allowed the authors not to model it.

The published interpretations of several early low resolution or medium low resolution type IIA topoisomerase crystal structures [2, 3, 20, 21] seem questionable. A full refinement of all questionable type IIA topoisomerase crystal structures is beyond the scope of this review. Here we re-refine two crystal structures to show that the metal ions in them can be re-refined consistently with subsequently published higher resolution, less ambiguous structures. We note that while we can refine all structures which are complicated by static disorder at the metal binding sites on the Toprim domains consistently with a single moving metal mechanism, this does not and cannot prove that such a moving metal mechanism is true. However, we do not believe that the debate about type IIA topoisomerase mechanism should be driven by ambiguous blurred structures with questionable interpretations, when there are clear unambiguous structures available.

Supplementary Results.

1. Re-refinement of a 3.35A S.aureus DNA gyrase complex with ciprofloxacin (2XCT).

A low resolution (3.35 Å) crystal structure of ciprofloxacin with *S. aureus* DNA gyrase and DNA (2xct), originally deposited in 2010 contained metals without proper coordination geometry. The 2XCT structure was deliberately originally deposited with poor metal coordination geometry to indicate that it was not a well-defined structure; it was only deposited to indicate the positions of ciprofloxacin is different to that of GSK299423 [2]. To correct this error in the coordinates of 2XCT we now have re-refined and redeposit 2xct, to include the water metal-ion bridge [22] which links the quinolone (ciprofloxacin) to the protein, and also B-site metals with reasonable coordination geometry. The data for this structure are twinned and the electron density maps are not of very good quality. The reinterpreted structure is now consistent with other subsequently published fluoroquinolone structures in having the water metal ion bridge [19, 22, 26] which links the compound to the protein and is also consistent with subsequently published biochemical data on the water metal ion bridge [27-31]. The re-refined 2XCT structure also has a B site metal on the Toprim domain with reasonable coordination geometry (Table S3).

2. **Re-refinement of a 2.98 Å yeast complex (3L4K) soaked with Zn^{2+} ions**.

A medium/low resolution **(2.98Å)** structure of yeast topo II with one nicked DNA strand and one cleaved DNA strand soaked with zinc ions was published in 2010 [3]. This 2.98-Å structure is complicated by static disorder around a twofold axis. To the best of our knowledge this is the only type IIA topoisomerase structure that has been interpreted in terms of a two metal mechanism [3]. The originally deposited coordinates for 3L4K do not have any waters at the zinc coordination sites, and each zinc of the two zincs at the active sites was refined with single occupancy (Table S3, Table S4, supplementary Fig S5.). We have re-refined this structure to have metal binding geometry consistent with all other published crystal structures for type IIA topoisomerase (Table S3, Table S4, supplementary Fig S5.). We think the new interpretation of 3L4K is more probable than the original interpretation. Coordinates for both interpretations (Table S4) are available for

download from a web-site (https://www.cardiff.ac.uk/people/view/1141625-bax-ben - click on 'Research' tab – and scroll down to find relevant table).

Supplementary Discussion - A moving water-metal ion model for DNA cleavage by *S.aureus* DNA gyrase

Introduction a standard BA-x nomenclanture for inhibitors, catalytic metals and associated waters in *S. aureus* gyrase^{core} crystal structures.

To facilitate comparison of multiple *S. aureus* DNA gyrase structures we have adopted a common nomenclature for protein subunits, DNA, and metal ions and associated waters – so that catalytic metals and associated waters have the same names in different crystal structures and inhibitors binding in the same pockets have the same residue name (see figure 4). In *S. aureus* DNA gyrase^{CORE} structures with a single complex in the asymmetric unit the chains are named as B (GyrB) and A (GyrA) from the first gyrase^{CORE} fusion truncate subunit, and D (GyrB) and C (GyrA) in the second subunit (we call this nomenclature BA-x for GyrB/GyrA extended numbering). DNA strands have ChainIDs as E and F.

Compounds (inhibitors) are given chain ID I (for inhibitor) if they are sitting in a defined pocket; compounds whose binding sites are at crystal contacts and are not thought to be biologically relevant are not labelled with the I chain identifier. Compounds that sit in the DNA-cleavage site (pocket 1) are numbered I 1 for the first pocket (near the B subunit) and I 201 for the second cleavage site (pocket 1' near D GyrB subunit). taps-NBTIs are numbered I 2 indicating that they are inhibitors that bind in both pockets 2D and 2A on the twofold axis. Compounds binding in the hinge pocket (pocket 3) between the B and A subunits are numbered I3 (or I 203 – between the D and C subunits). Inhibitor associated waters and metal ions are numbered I11, 112 etc. if associated with a pocket 1 inhibitor (e.g waters in the water-metal ion bridge of fluoroquinolones), I 21, I 22 if associated with a taps-NBTI, and I31, 32 if associated with an inhibitor in pocket 3 (the hinge pocket).

TOPRIM bound metals are given ChainID of the TOPRIM domain to which they are bound and the number 5081 (i.e B5081 – for catalytic metal bound to the B GyrB chain, D5081 for the metal

bound to the D GyrB chain). This nomenclature is used to indicate that in all *S. aureus* DNA gyrase structures solved to date Asp 508 from GyrB, moves to coordinate the metal ion whether it occupies the A or B site (Figure 6). One water, which appears conserved between the A and B sites is named 5091. The coordination sphere for the A site includes only two waters, 5091 and 5090 (the second water contacts GyrB Asp510 and is displaced by it at the B site). In contrast the B site metal co-ordination sphere has four metals, 5091 and 5093, 5094 and 5095. We have only observed metals binding at the B site when water 5093 contacts the phosphate before the scissile phosphate. If the B site metal coordination sphere is rotated onto the A site, by superposing the three conserved atoms, the metal ion, water 5091 and the coordinating oxygen of Asp 508 (see figure 6), then water 5093 occupies a similar position to the 3' oxygen of the scissile phosphate, water 5094 occupies a similar position to the coordinating non-bridging oxygen from the scissile phosphate and water 5095 from the B site occupies a similar position to an oxygen from Glu 435. This observation allows us to propose a fully dissociative mechanism for DNA-cleavage by *S.aureus* DNA gyrase (supplementary figures 9 and 10).

A fully dissociate moving water-metal ion model for DNA cleavage by *S.aureus* DNA gyrase.

In this proposed mechanism (supplementary Fig. 10) the metal ion (usually Mg^{2+}) initially binds at the B-site (see supplementary Figure 9 – panel b and d) before being attracted towards the A-site. In order for a metal ion to be bound at the B-site, the phosphate before the scissile phosphate needs to be close enough to form a hydrogen bond with water 5093 (supplementary Fig. 10a). When the metal is attracted from the B-site towards the A-site it moves water 5093 to protonate the 3' oxygen and thus cleave the DNA, allowing the catalytic tyrosine (Tyr 123), which is in a tyrosinate form and moves with the metal ion, to capture the metaphosphate-like cleaved phosphate.

This mechanism is informed by Frey and Hegman [32], and previously proposed mechanisms for phosphotranfer reactions Agrawal [33], Bax, Chung, Edge [34]. We note the deprotonation enthalpies for hydrates (e.g. $Mg^{2+}[OH_2] \rightarrow Mg^{2+}[OH^-] + H^+$). can be quite small when metal ions have six liganding oxygens [35].

Supplementary References

1. Infante Lara, L., et al., *Coupling the core of the anticancer drug etoposide to an oligonucleotide induces topoisomerase II-mediated cleavage at specific DNA sequences.* Nucleic Acids Res, 2018. **46**(5): p. 2218-2233.
2. Bax, B.D., et al., *Type IIA topoisomerase inhibition by a new class of antibacterial agents.* Nature, 2010. **466**(7309): p. 935-940.
3. Schmidt, B.H., et al., *A novel and unified two-metal mechanism for DNA cleavage by type II and IA topoisomerases.* Nature, 2010. **465**(7298): p. 641-644.
4. Engh, R.A.a.H., R. , *Accurate bond and angle parameters for X-ray protein structure refinement.* Acta Crystallog. sect. A, 1991. **47**: p. 392-400.
5. Bock, C.W., et al., *Manganese as a Replacement for Magnesium and Zinc: Functional Comparison of the Divalent Ions.* J. Am. Chem. Soc, 1999. **121**: p. 7360-7372.
6. Wilson, K.S., et al., *Who checks the checkers? Four validation tools applied to eight atomic resolution structures. EU 3-D Validation Network.* J Mol Biol, 1998. **276**(2): p. 417-36.
7. Deweese, J.E. and N. Osheroff, *The use of divalent metal ions by type II topoisomerases.* Metallomics, 2010. **2**(7): p. 450-459.
8. Srikannathasan, V., et al., *Crystallization and preliminary X-ray crystallographic analysis of covalent DNA cleavage complexes of Staphyloccocus Aureus DNA Gyrase with QPT-1, Moxilfloxacin and Etoposide.* Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun, 2015. **71**: p. 1242-1246.
9. Wu, C.C., et al., *Structural basis of type II topoisomerase inhibition by the anticancer drug etoposide.* Science, 2011. **333**(6041): p. 459-462.
10. Blundell, T.L. and L.N. Johnson, *Protein Crystallography*. 1976, New York, London, San Francisco: Academic Press.
11. Drevensek, P., et al., *X-Ray crystallographic, NMR and antimicrobial activity studies of magnesium complexes of fluoroquinolones - racemic ofloxacin and its S-form, levofloxacin.* J Inorg Biochem, 2006. **100**(11): p. 1755-63.
12. Wohlkonig, A., et al., *Structural basis of quinolone inhibition of type IIA topoisomerases and target-mediated resistance.* Nature structural & molecular biology, 2010. **17**(9): p. 1152.
13. Ladd, M.F.C.a.P., R.A., *Structure Determination by X-ray Crystallography*. Third Edition ed. 1994, New York and London: Plenum Press.
14. Drenth, J., *Principles of Protein X-ray Crystallography* Spinger Advanced Texts in Chemistry. , ed. C.R. Cantor. 1994, New York Springer.
15. Sherwood, D. and J. Cooper, *Crystals, X-rays and Proteins: Comprehensive Protein Crystallography*. 2011, Oxford: Oxford Univerity Press.
16. MacArthur, M.W. and J.M.W. Thornton, *Protein side-chain conformation: a systematic variation of χ1 mean values with resolution–a consequence of multiple rotameric states?.* Acta Crystallographica Section D: Biological Crystallography, 1999. **55**: p. 994-1004.
17. Miles, T.J., et al., *Novel hydroxyl tricyclics (e.g., GSK966587) as potent inhibitors of bacterial type IIA topoisomerases.* Bioorg. Med. Chem. Lett, 2013. **23**(19): p. 5437-5441.
18. Miles, T.J., et al., *Novel tricyclics (e.g., GSK945237) as potent inhibitors of bacterial type IIA topoisomerases.* Bioorg. Med. Chem. Lett, 2016.
19. Blower, T.R., et al., *Crystal structure and stability of gyrase-fluoroquinolone cleaved complexes from Mycobacterium tuberculosis.* Proc. Natl. Acad. Sci. U. S. A, 2016. **113**(7): p. 1706-1713.
20. Laponogov, I., et al., *Structural basis of gate-DNA breakage and resealing by type II topoisomerases.* PLoS. One, 2010. **5**(6): p. e11338.
21. Laponogov, I., et al., *Structural insight into the quinolone-DNA cleavage complex of type IIA topoisomerases.* Nat. Struct. Mol. Biol, 2009. **16**(6): p. 667-669.
22. Wohlkonig, A., et al., *Structural basis of quinolone inhibition of type IIA topoisomerases and target-mediated resistance.* Nat. Struct. Mol. Biol, 2010. **17**(9): p. 1152-1153.
23. Gibson, E.G., et al., *Mechanistic and Structural Basis for the Actions of the Antibacterial Gepotidacin against Staphylococcus aureus Gyrase.* ACS Infect Dis, 2019.

24. Singh, S.B., et al., *Tricyclic 1,5-naphthyridinone oxabicyclooctane-linked novel bacterial topoisomerase inhibitors as broad-spectrum antibacterial agents-SAR of left-hand-side moiety (Part-2).* Bioorg. Med. Chem. Lett, 2015. **25**(9): p. 1831-1835.
25. Singh, S.B., et al., *Oxabicyclooctane-linked novel bacterial topoisomerase inhibitors as broad spectrum antibacterial agents.* ACS Med. Chem. Lett, 2014. **5**(5): p. 609-614.
26. Chan, P.F., et al., *Structural basis of DNA gyrase inhibition by antibacterial QPT-1, anticancer drug etoposide and moxifloxacin.* Nat. Commun, 2015. **6**: p. 10048.
27. Aldred, K.J., et al., *Role of the water-metal ion bridge in mediating interactions between quinolones and Escherichia coli topoisomerase IV.* Biochemistry, 2014. **53**(34): p. 5558-5567.
28. Aldred, K.J., R.J. Kerns, and N. Osheroff, *Mechanism of quinolone action and resistance.* Biochemistry, 2014. **53**(10): p. 1565-1574.
29. Aldred, K.J., et al., *Topoisomerase IV-quinolone interactions are mediated through a water-metal ion bridge: mechanistic basis of quinolone resistance.* Nucleic Acids Res, 2013. **41**(8): p. 4628-4639.
30. Aldred, K.J., et al., *Drug interactions with Bacillus anthracis topoisomerase IV: biochemical basis for quinolone action and resistance.* Biochemistry, 2012. **51**(1): p. 370-381.
31. Aldred, K.J., et al., *Overcoming Target-Mediated Quinolone Resistance in Topoisomerase IV by Introducing Metal-Ion-Independent Drug-Enzyme Interactions.* ACS Chem. Biol, 2013. **8**: p. 2660-2668.
32. Frey, P.A. and A.D. Hegeman, *Enzymatic Reaction Mechanisms*. 2007, New York: Oxford University Press.
33. Agrawal, A., et al., *Mycobacterium tuberculosis DNA gyrase ATPase domain structures suggest a dissociative mechanism that explains how ATP hydrolysis is coupled to domain motion.* Biochemical Journal, 2013. **456**(2): p. 263-273.
34. Bax, B., C.W. Chung, and C. Edge, *Getting the chemistry right: protonation, tautomers and the importance of H atoms in biological chemistry.* Acta Crystallogr D Struct Biol, 2017. **73**(Pt 2): p. 131-140.
35. Trachtman, M., et al., *Interactions of metal ions with water: ab initio molecular orbital studies of structure, vibrational frequencies, charge distributions, bonding enthalpies, and deprotonation enthalpies. 2. Monohydroxides.* Inorg Chem, 2001. **40**(17): p. 4230-41.