The high-resolution crystal structure for class A β-lactamase PER-1 reveals the bases for its increase in breadth of activity.

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RUNNING TITLE

New fold of the Ω-loop in the PER-1 class A β-lactamase
SUMMARY

The treatment of infectious diseases by β-lactam antibiotics is continuously challenged by the emergence and dissemination of new β-lactamases. In most cases, the cephalosporinase activity of class A enzymes results from a few mutations in the TEM and SHV penicillinases. The PER-1 β-lactamase was characterized as a class A enzyme displaying a cephalosporinase activity. This activity was however insensitive to the mutations of residues known to be critical for providing extended substrate profiles to TEM and SHV. The X-ray structure of the protein, solved at 1.9 Å resolution, reveals that two of the most conserved features in class A β-lactamase are not present in this enzyme: the fold of the Ω-loop and the cis conformation of the peptide bond between residues 166 and 167. The new fold of the Ω-loop and the insertion of four residues at the edge of strand S3 generate a broad cavity that may easily accomodate the bulky substituents of cephalosporin substrates. The trans conformation of the 166-167 bond is related to the presence of an aspartic acid at position 136. Selection of class A enzymes based on the occurrence of both Asp136 and Asn179 identifies a subgroup of enzymes with high sequence homology.
INTRODUCTION

*Pseudomonas aeruginosa*, a prevalent micro-organism responsible for several infections in human, is intrinsically resistant to many antibiotics (1). This resistance simultaneously arises from the low permeability of its outer membrane (2), the existence of an active efflux system (3,4), and the production of a chromosomally encoded inducible class C β-lactamase (5). This bacterium reinforces its resistance to β-lactam antibiotics by expressing a chromosomally encoded class A cephalosporinase, PER-1 (for *Pseudomonas* Extended Resistance).

PER-1 was first detected in 1993 in a strain isolated from a Turkish patient (6). It was subsequently identified in nosocomial strains of *Salmonella typhimurium* and *Acinetobacter baumanii* in Turkey (7,8) and more recently in France (9). PER-2, a closely related enzyme sharing 86% homology, has been found in South America (10). These enzymes hydrolyze efficiently penicillins and cephalosporins, but not cephamycins or carbapenems, and are susceptible to clavulanic acid inhibition (6,10). PER enzymes belong to the class A β-lactamases (10-12) but the sequence identity with the TEM and SHV enzymes is only 27% (11). It was proposed that PER enzymes and various β-lactamases from Bacteroides species (13-15) may constitute a subgroup of the class A enzymes (11).

Class A β-lactamases hydrolyze β-lactam antibiotics through a double displacement mechanism with a transient acylation of the catalytic Ser70 residue. Eight X-ray structures of apo-enzymes, TEM-1 and TOHO-1 from *Escherichia coli* (16,17), PC1 from *Staphylococcus aureus* (18), SHV from *Klebsiella pneumoniae* (19), NMC-A from *Enterobacter cloacae* (20), MFO from *Mycobacterium fortuitum* (PDB entry: 1MFO), BLIC from *Bacillus licheniformis* (21), and SAG from *Streptomyces albus* G (22) have been solved. These enzymes display a very similar fold and the detailed comparisons of the structures were helpful in relating some
significant differences in substrate profile to local structural features (23,24). The structural impact of point mutations leading to some 100 extended spectrum enzymes in the TEM and SHV families is less documented than the possible function of the invariant residues of the catalytic machinery (25-28). Several studies supported the hypothesis that the cephalosporinase activity of these extended substrate enzymes was related to an increased flexibility of the Ω-loop region and to alterations of the S3 strand, two of the regions lining the active site (24,29-33).

Surprisingly, site-directed mutagenesis on the PER-1 enzyme showed that none of the residues responsible for the cephalosporinase activity in the TEM and SHV families (104, 164, 179, 238, and 240) were implicated in the substrate profile of this enzyme (34,35). The 1.9 Å X-ray structure of PER-1 presented in this report reveals a completely new fold of the Ω-loop region, so far one of the most conserved feature in the class A enzymes. Structure and sequence analysis suggest that PER-1 defines a group of class A β-lactamases which can be recognized by the presence of an aspartic acid residue at position 136. The insertion of four amino acids in the 238-242 region, together with the Ω-loop fold, lead to a significant increase in size of the substrate binding pocket.
EXPERIMENTAL PROCEDURES

Crystallization

The PER-1 \(\beta\)-lactamase was expressed in \textit{E. coli} and purified as previously described (34,35). Crystals were obtained using the vapour diffusion method: an 8 \(\mu\)l drop, made of 2 \(\mu\)l of protein (20 mg/ml in Tris/HCl 20 mM, DTT 0.5 mM, NaN3 0.06 %, pH 7.60) and of 6 \(\mu\)l of reservoir solution was equilibrated against 330 \(\mu\)l of reservoir solution containing 18\% (v/v) saturated ammonium sulfate, 0.1 M sodium acetate (pH 4.7) and 1 mM dithiothreitol. After 4 to 7 days at 8°C, parallelepipedic crystals (300\(\mu\)m x 80\(\mu\)m x 80\(\mu\)m) were formed. They belong to spacegroup \(P4_3\) with cell parameters \(a = b = 84.4\ \text{Å}, c = 46.8\ \text{Å}\). There is one molecule per asymmetric unit.

Data collection and phasing

Diffracted intensities were measured on the W32 beam line at LURE (Orsay, France) on a large MAR Research imaging plate. The data of the native and of the first HgCl\(_2\) derivative (HgCl\(_2\) \textit{1}) were collected at 20°C. This derivative was obtained by soaking the crystal for 64 h in a capillary at a final concentration of 10 mM. The data for the second HgCl\(_2\) derivative (HgCl\(_2\) \textit{2}) were collected at –160°C. Soaking for the HgCl\(_2\) \textit{2} derivative was performed during 69 h in a drop of mother liquor at a final concentration of 5 mM. For cryocooling, the crystal was immersed for 10 seconds in the reservoir solution complemented with 20\% ethylene-glycol (w/v) prior to flash-cooling in a stream of nitrogen gas.

Intensities were processed with DENZO (36), and scaled and reduced with the CCP4 suite of programs (37) (Table 1). The single heavy atom site was determined from the Harker sections of the difference Patterson map. Heavy atom parameters refinement and phasing were conducted
with SHARP (38) using the full native and derivatives data sets (Table 1). Improvement of the 2.8 Å electron density map and phase extension to 2.5 Å were performed using SOLOMON (39), assuming a solvent content of 44% in the asymmetric unit.

**Model building and crystallographic refinement**

The modified 2.5 Å electron-density map allowed tracing of 92% of the PER-1 main chain atoms and of 52% of side chain atoms. The structure was refined by the maximum likelihood method as implemented in CNS (40), including a bulk-solvent correction, and manual fitting into SIGMAA-weighted electron-density maps with TURBO FRODO. Ten percent of the available reflections were randomly selected for the calculation of the free-R factor (41). A first round of refinement was performed at 2.5 Å. All data to 1.9 Å resolution were included in the next refinement steps. The final model comprises 2198 non-hydrogen atoms of the PER-1 enzyme, one sulfate ion, and 155 water molecules. The crystallographic R and Rfree values were 0.14 (for 23174 reflections) and 0.18 (for 2533 reflections), respectively. The average B factors are 20.17 Å² for all atoms and 33.51 Å² for water molecules. No defined electron density could by assigned to Gln25, to residues 298 to 300 and to 3 solvent exposed side chains (His170, Asp173 and Gln174) which were assigned a null occupancy. Ser35, Gln93, Asp90, Ser116, Gln120, Ser126, Met183, Glu195, and Ser295 have alternate conformations.

**Protein sequences analysis**

The sequences of representative class A β-lactamases were aligned using CLUSTAL W (42). The alignment was optimized manually using SEAVIEW (43) and according to the structural homology defined by the program MAPS (Lu, unpublished). The resulting alignment
was displayed using the program ESPript (44). Phylogenetic analysis was performed by the neighbor-joining method with the bootstrap tree option using the CLUSTAL X program (45) and allowing for 1000 bootstrap iterations. The tree was displayed using NJPLOT (Gouy, unpublished).

The assignment of secondary structure elements was made using STRIDE (46). Three-dimensional structure were superposed with LSQKAB (47) from the CCP4 package. Global superposition matrices were derived from 140 equivalent Cα atoms, as determined by MAPS, whereas active site superposition matrices were obtained using all side chain atoms from the catalytically important residues Ser70, Lys73, Ser130, Glu166, and Lys234 and the main chain nitrogen atoms of residues 70 and 237 which constitute the oxyanion hole. Root mean square deviations after both superposition strategies are given in Table 2.
RESULTS

Structure determination

The quality of the SIRAS electron density map computed at 2.5 Å resolution, using the two data sets of the mercury derivative for phasing, allowed an unambiguous main chain tracing except for residues 104–109, 162–164 and 170–176. One half of the side chain were built at that stage. Inclusion of the high resolution data followed by a few rounds of refinement led to $R$ and $R_{\text{free}}$ values of 0.14 and 0.18, respectively at 1.9 Å resolution. The refined structure of PER-1 includes 274 residues and 155 water molecules. The first N-terminal (Gln25) and the last three C-terminal residues (Ser298, Pro299, and Asn300) have no electron density. The average temperature factors was 20.17 Å$^2$, close to the value estimated from the Wilson plot (16.9 Å$^2$) (48). Coordinates error was evaluated to be 0.16 Å from a Luzzati plot (49).

Overall structure and catalytic machinery

The PER-1 β-lactamase (274 residues) is made of two domains. The first one (residues 26–62 and 218–297) folds as a five stranded anti-parallel β-sheet with both N- and C-terminal α-helices packed on one side of the sheet. The second domain (residues 69–215) is mainly built of α-helices and loops. The active site is defined by the interface between the two domains (Figure 1).

The side chains of the essential catalytic residues, Ser70, Lys73, Ser130, and Glu166, the atoms forming the oxyanion hole (the main chain nitrogen atoms of Ser70 and Thr237) and the deacylating water molecule (Figure 2) are found in identical relative positions compared to other class A β-lactamases (rmsd = 0.350 ± 0.110 Å), suggesting a conserved catalytic mechanism. A water molecule occupies the oxyanion hole, at 2.8 and 3.2 Å of Thr237N and Ser70N, respectively. A sulfate anion, likely provided by the crystallization medium, is found at hydrogen
bond distance to Ser130, Thr235 and Thr236. A sulfate ion bound in a similar position was also observed in the TEM structure (16).

**Insertions**

Sequence alignment of PER-1 with the class A β-lactamases pointed out the occurrence of three insertions (11,34). Two of them (Q103a and N103b, Q112a and G112b ) are located in the loop connecting helices H2 and H4 (Figure 1). They likely contribute to the different conformation of this region when compared to that of the TEM enzyme (rmsd on common main chain atoms is 2.6 Å) (Figure 3). This conformation nevertheless preserves the Cα positions of residues 104 and 105, two residues known to be important in class A enzymes for substrate binding and catalysis (50,51). In PER-1, the γ-hydroxyl group of the Thr104 side chain is hydrogen bonded (2.9 Å) to the side chain of the invariant Asn132 residue (Figures 3 and 4). This conformation of residue104 has never been observed in class A enzymes where the polar interaction to Asn132 was always provided by the main chain oxygen atom of residue 104 (Figure 3). Trp105 (an aromatic residue in most class A enzymes, Figure 4) has the same location as Tyr105 in TEM. In this protein, the aromatic ring is at van der Waals distance to the bound substrate in the crystal structures of enzyme-ligand complexes (52-54).

The third insertion occurs in a region where the sequence and the local fold were related to the extension of substrate profile in the TEM and SHV β-lactamases (28,32,33). In PER-1, four amino acids are inserted after residue 240 (Lys240a, Ala240b, Gly240c, and Lys240d). The loop 238-242 that connects strands S3 and S4 protrudes in the solvent, away from the Ω-loop region (Figure 1). As a consequence, the adjacent loop between strand S5 to helix H11 is short, and helix H11 starts at residue 272. It could be noticed that the start of helix H11 (at position 272 or 276) and therefore the length of the S5-H11 connection (long or short, respectively) partition
the class A enzymes into two groups. It would seem from structures examination that the long connection, when it occurs, shields from solvent the aromatic residue found at position 241 (TOHO-1, SAG, MFO, BLIC, PC1, NMCA) (Figure 4).

The Ω-loop fold

The Ω-loop is an idiosyncrasy of class A β-lactamases. Its fold and the cis peptide bond between residues 166 and 167 were typical and invariant features observed in the 8 structures solved to date (16-20,22,55). This loop presents Glu166 into the active site, in a position where it promotes activation of the hydrolytic water molecule for hydrolysis of the acyl-enzyme intermediate (25,26,52). In PER-1, the fold of the Ω-loop is quite different and the 166–167 peptide bond has a trans conformation (Figures 1 and 5). The trans conformation apparently results from the replacement of the highly conserved asparagine at position 136 by an aspartic acid in PER-1 (Figure 4). In the structure of the other class A β-lactamases, the cis conformation is stabilized by the hydrogen bonds provided by the amide group of Asn136 to the main chain nitrogen and oxygen atoms of Glu166 (3.1 Å in TEM) (16,56) (Figure 5). This hydrogen bond pattern cannot be fulfilled with a carboxylate group. In the trans conformation, hydrogen bonds are established between the oxygen atoms of the carboxylate group of Asp136 and the main chain nitrogen atoms of Glu166 and of Ala167 (2.9 Å) (Figure 5).

The Ω-loop region in PER-1 is highly organized in secondary structure elements. The edges of this loop (residues 159–161 and 180–182) form a small antiparallel beta sheet. Between residues Ala164 and Asn179, two short helices (residues 166–171 and 173–178) run nearly orthogonal to each other (Figure 5). The amide group of Asn179 is buried and hydrogen bonded to the main chain nitrogen atoms of residues 163 and 164, and to the main chain oxygen atom of Ala164. There is no salt-bridge in PER-1 in striking contrast to the other class A β-lactamases.
(16-20,22,55) where the invariant interaction between Arg164 and Asp179 is directly implicated in the substrate profile of these enzymes (Figure 5).

The fold of the Ω-loop in PER-1 maintains Glu166 in the right location for the catalytic function and for binding the deacylating water molecule. This fold however brings His170 8 Å away from the position of the homologous residue in the other class A enzymes. In these structures, Asn170, a highly conserved amino acid in this family (Figure 4) is the second ligand of the deacylating water molecule. In PER-1, this function is achieved by Gln69 (Figure 2).
DISCUSSION

Although PER-1 is a typical class A enzyme with respect to the conservation of the catalytic machinery, its three dimensional structure revealed that this super-family is not as homogeneous in structure as it was thought. It was unexpected to find a new fold of the Ω-loop, a region considered to be a canonical motif in these enzymes. This finding suggested to perform a selection of the β-lactamases only based on the occurrence of an aspartic acid at position 136. Several enzymes with significant sequence identity (40%) were identified, that form a subgroup in the class A super-family (Figures 4 and 6). These “PER-like” enzymes (11) comprise PER-2 from *Salmonella typhimurium* (10), VEB-1 from *E. coli* (57), CME-1 from *Chryseobacterium (flavobacterium) meningosepticum* (58), TLA-1 from *E. coli* (59), CBLA from *Bacteroides uniformis* (15), CEPA from *B. fragilis* (13) and CFXA from *B. vulgatus* (14). The phylogenetic tree shown in Figure 6 indicates that these proteins constitute a distinct cluster of enzymes, and the X-ray structure of PER-1 suggests that this partition reflects major structural differences compared to the TEM-like β-lactamases. On the contrary, there is no major partition between the penicillinases and the NMC-A, IMI-1 (60) and Sme-1 (61) carbapenemases, which may be identified by the presence of cysteine residues at positions 69 and 238 (Figure 4). It agrees with the recent structure determination of NMC-A (20) which revealed that the carbapenemase activity was associated with subtle structural modifications. For reasons discussed in the following paragraphs, we suggest that the presence of both Asp136 and Asn179 should identify any new β-lactamase as member of the PER- subgroup.

Previous studies on class A β-lactamases, including X-ray structure determinations, led to the conclusion that the *cis* conformation of the 166–167 peptide bond was mandatory for the proper location of Glu166 in the active site, and therefore for catalysis. The Asn136Ala mutant of
the PC1 enzyme was found to accumulate acyl-enzyme adducts, but had no hydrolytic activity against penicillin and cefotaxime (29). Recent kinetic analysis of randomly generated \( \Omega \)-loop TEM mutants concluded that the isomerisation of the 166–167 peptide bond controlled one conformation of this loop that was compatible with fast acylation of the protein (27). The PER-1 structure presented here reveals that a functional enzyme can accommodate the \textit{trans} conformation of the 166-167 peptide bond through interactions with Asp136, and maintains the position of Glu166 that preserve the molecular basis of the catalytic mechanism.

The \textit{trans} conformation goes with a different sequence of the 161-181 (\( \Omega \)-loop) region which display a new fold compared to the typical class A enzymes. In those cases, the \( \Omega \)-loop is stabilized by salt bridge interactions, and the Arg164Ser mutation is frequently observed in naturally occurring TEM and SHV mutants with extended-spectrum (62). The disruption of the Arg164-Asp179 salt-bridge is assumed to increase the flexibility of the \( \Omega \)-loop thereby favoring the binding of third generation cephalosporins (63,64). In PC1, the \( \beta \)-lactamase from \textit{Staphylococcus aureus}, disruption of this single salt-bridge by the Asp179Asn mutation lead to a disordered conformation of the \( \Omega \)-loop (29,65). There are no salt bridges in PER-1 where the stability of the \( \Omega \)-loop fold stems from the presence of secondary structure elements complemented by the hydrogen bond interactions of the side chain of Asn179 to the main chain nitrogen atoms of Val 163 and Ala164, and to the main chain oxygen atom of Ala164 (Figure 5).

In retrospect and in view of the major structural differences between the PER-1 and the TEM enzymes, it is not surprising that site directed mutagenesis of PER-1 (34,35) could not relate the cephalosporinase activity of PER-1 to the residue type at positions 164, 179, 238 and 240. These residues, which provide extended spectrum profiles in the TEM and SHV enzymes, have different spatial location and environments in these three dimensional structures. Protein
engineering in PER-1 nevertheless identified two residues whose mutations had significant kinetic effects. Replacement of Thr104 with Glu completely abolished the catalytic activity on penicillins and reduced the kcat values for cephalosporins by a factor of 50 to 700 (34). According to the X-ray structure, this mutation should in any case disrupt the interaction of the γ-hydroxyl group of Thr104 with Asn132. These observations shed light on the previously suggested importance for catalysis in class A enzymes of the hydrogen bond interaction between residue 104 and Asn132 (50,66). It involves the main chain oxygen atom of residue 104 in all structures except PER-1, and its contribution could evidently not be demonstrated by protein engineering in these enzymes. Replacement of Thr237 by an alanine residue increased the kcat/Km of the mutant protein on cephalosporin substrates by 10 to 100 fold (35). Interestingly, it is the reverse mutation, Ala237Thr, which improved hydrolysis on cephalosporin substrates in the TEM enzyme (67).

The relocation of the side chain of residue 104, the fold of the Ω-loop and the conformation of the 238–242 region in PER-1 seems in direct relationship with the cephalosporinase activity of this enzyme. Indeed, these folding features generates a cavity, filled with several water molecules in the apo-enzyme, which is precisely located in the area expected to bind the bulky side chain of third and fourth generation cephalosporins (33) (Figure 7). According to the superposed TEM and PER-1 structures (Figure 8), this observation supports the proposal that the displacement of the Ω-loop in the TEM enzyme should favor the binding of cephalosporin substrates.

The structure determinations of the PER-1 β-lactamase breaks the assumption of a unique fold of the class A enzymes. This finding promoted the suggestion that class A β-lactamases may be categorized into three subgroups according to their kinetic properties as penicillinases
(TEM/SHV group), cephalosporinases (PER group) and carbapenemases (NMC-A group). These groups have sequence signatures that should help assigning any new enzymes into either of them. From the microbiological point of view, the simultaneous occurrence in bacteria of extended spectrum TEM or SHV, and PER enzymes should complicate identification of the resistance enzymes based on the antibiogram patterns.
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FIGURE LEGENDS

Figure 1. Overall topology of the PER-1 β-lactamase (black) superposed to the Cα trace of the TEM-1 enzyme (red). In PER-1, every tenth Cα atoms is indicated with a black dot, and inserted residues and the Ω-loop are in green.

Figure 2. Stereo view of the active site in PER-1 (main chain in thick lines, side chain in thin lines, water molecules as black spheres). The sulfate ion is also shown.

Figure 3. Detailed view of the conformation of the 100-115 region in PER-1 (black) and in TEM-1 (red). Two insertions in PER-1 occur in this area. Residues 104, 105, and 132 are displayed with thick lines. The amide group of Asn132 is hydrogen bonded to the γ-hydroxyl group of Thr104 in PER-1 and to the main chain oxygen of residue 104 in TEM-1.

Figure 4. Sequence alignment of representative class A β-lactamases (see text). Three groups are distinguished based on sequence, structure and substrate similarities (i) cephalosporinases (PER) (ii) penicillinases (TEM), (iii) carbapenemases (NMC-A). Strictly conserved residues across the groups are indicated by a red background. Yellow background highlights residues discussed in text. Residues indicated with red letters denotes conservation within a group. Secondary structure elements, as defined by STRIDE, are indicated for PER (red), TEM (green), and NMCA (blue). Regions that are considered by MAPS as equivalent in all structures of class A enzymes are framed in black.
Figure 5. Stereo views of the $\Omega$-loop regions in PER-1 (A) and TEM-1 (B). The main chains are represented as thick lines and the side chains as thin lines. Selected polar interactions are indicated with hatched lines.

Figure 6. Phylogenetic tree for 15 class A $\beta$-lactamases (see text for abbreviations). Asterisks indicate enzymes of known 3D-structures.

Figure 7. Stereo view of the solvent accessible surface area in a modeled PER-1-cefotaxime acyl-enzyme complex. It illustrates the large cavity where the substituents of third generation cephalosporins bind to the protein. The surface was computed using MSMS (68) and displayed with DINO (Philippsen A., University of Basel, Switzerland).

Figure 8. The superposition of the active sites in PER-1 (black) and TEM (red) shows the significant enlargement of the substrate binding cavity in PER-1.
Table 1. Diffraction data and phasing statistics

|                        | Native (293 K) | HgCl\textsubscript{2} 1 (293 K) | HgCl\textsubscript{2} 2 (100 K) |
|------------------------|----------------|---------------------------------|-------------------------------|
| Resolution (Å)\textsuperscript{a} | 24.18 – 1.90   | 24.11 – 2.80                    | 29.88 – 2.87                   |
|                         | (2.00 – 1.90)  | (2.95 – 2.80)                   | (2.95 – 2.87)                 |
| Nb. observation         | 66,657         | 13,399                          | 47,986                        |
| Unique reflections      | 25,029         | 7,193                           | 7,644                         |
| Multiplicity            | 2.7            | 1.9                             | 6.3                           |
| Bijvoet pairs           | -              | 3,517                           | 6,824                         |
| \(R_{\text{sym}}\)\textsuperscript{b} | 0.052 (0.122)  | 0.072 (0.156)                   | 0.058 (0.116)                 |
| Completeness (%)        | 94.3 (93.7)    | 87.5 (88.8)                     | 91.7 (85.1)                   |
| \(<I/>\sigma\(I)\>)   | 9.2 (5.6)      | 6.0 (3.9)                       | 11.2 (6.5)                    |
| \(R_{\text{iso}}\)\textsuperscript{c} | -              | 0.216                           | 0.312                         |
| \(R_{\text{anom}}\)\textsuperscript{d} | -              | 0.074 (0.147)                   | 0.038 (0.069)                 |
| \(f'/f^{\text{ref}}\) (e-\textsuperscript{e}) | -              | -12.14/7.04                     | -8.54/5.91                    |
| Occupancy\textsuperscript{e} | -              | 1.39                            | 0.79                          |
| Isomorphous \(R_{\text{Cullis}}\)\textsuperscript{e} (centric/acentric) | - | 0.71/0.76                       | 0.78/0.82                     |
| Anomalous \(R_{\text{Cullis}}\)\textsuperscript{e} | -              | 0.40                            | 0.81                          |
| Iso. Phasing power\textsuperscript{e} (centric/acentric) | - | 1.81/1.40                      | 1.69/1.38                     |
| Ano. Phasing power\textsuperscript{e} | -              | 1.00                            | 1.51                          |
| Figure of merit\textsuperscript{e} (centric/acentric) | - | 0.51/0.42                      |

\textsuperscript{a} parenthesised numbers denote the respective values for the highest resolution shell

\textsuperscript{b} \(R_{\text{sym}} = \frac{\Sigma \Sigma |<I>-|I_i|/\Sigma \Sigma I_i}{\Sigma \Sigma I_i}\)

\textsuperscript{c} \(R_{\text{iso}} = \frac{\Sigma |F_{\text{PH}}-F_{\text{P}}|/\Sigma |F_{\text{P}}|}{\Sigma |F_{\text{P}}|}\)

\textsuperscript{d} \(R_{\text{anom}} = \frac{\Sigma |<I>^+-<I>|/\Sigma (<I>^+-<I>)}{\Sigma (<I>^+-<I>)}\)

\textsuperscript{e} as provided by the program SHARP (38)
The high-resolution crystal structure for class A beta-lactamase PER-1 reveals the bases for its increase in breadth of activity
Samuel Tranier, Anne-Typhaine Bouthors, Laurent Maveyraud, Valérie Guillet, Wladimir Sougakoff and Jean-Pierre Samama

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