Crystal structure of an extracellular superoxide dismutase from *Onchocerca volvulus* and implications for parasite-specific drug development

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Superoxide dismutases (SODs) are metalloproteins that are responsible for the dismutation of superoxide anion radicals. SODs are consequently protective against oxidative damage to cellular components. Among other protective mechanisms, the filarial parasite *Onchocerca volvulus* has a well developed defense system to scavenge toxic free radicals using SODs during migration and sojourning of the microfilariae and adult worms in the human body. *O. volvulus* is responsible for the neglected disease onchocerciasis or ‘river blindness’. In the present study, an extracellular Cu/Zn-SOD from *O. volvulus* (*Ov*EC-SOD) was cloned, purified and crystallized to obtain structural insight into an attractive drug target with the potential to combat onchocerciasis. The recombinant *Ov*EC-SOD forms a dimer and the protein structure was solved and refined to 1.55 Å resolution by X-ray crystallography. Interestingly, a sulfate ion supports the coordination of the conserved copper ion. The overall protein shape was verified by small-angle X-ray scattering. The enzyme shows a different surface charge distribution and different termini when compared with the homologous human SOD. A distinct hydrophobic cleft to which both protomers of the dimer contribute was utilized for a docking approach with compounds that have previously been identified as SOD inhibitors to highlight the potential for individual structure-based drug development.

1. Introduction

The filarial parasite *Onchocerca volvulus* is the causative agent of human onchocerciasis, an infectious disease characterized by skin lesions, acute and chronic dermatitis, and depigmentation (Brattig et al., 1994). It is the second leading cause of infectious blindness worldwide. At least 220 million people are estimated to have required preventive chemotherapy against onchocerciasis. 99% of infected people live in Africa and 1.15 million people are visually impaired or blind according to the World Health Organization (World Health Organization, 2022). Onchocerciasis causes chronic disability with long-term complications and socio-economic problems, particularly in developing countries. A number of strategies to control onchocerciasis have aimed at targeting the transmitting vector, *i.e.* the blackfly, and/or fighting *O. volvulus* in the human host itself. Among various approaches, the drug ivermectin has frequently been used (Basáñez et al., 2008). However, ivermectin is only effective against microfilariae (Borsboom et al., 2003), has adverse effects and emerging resistance has also...
been reported due to its massive utilization (Keiser et al., 2002; Osei-Atweneboana et al., 2007). Hence, there is an urgent need for the development of drugs that also target adult worms since they resume the production of microfilariae (Churche et al., 2009). Alternative treatments using medicinal plants have been also put in place, using *Onchocerca ochengi* and *Caenorhabditis elegans* as model organisms (Cho-Ngwa et al., 2010; Ndjokka et al., 2011). Moreover, substantial work has focused on targeting *Wolbachia* bacteria, which coexist in symbiosis with filarial worms (Harcus et al., 2004; Wanji et al., 2009). Although these attempts have been successful in decreasing the number of cases, onchocerciasis has by no means been eliminated and continues to be a major public health concern. One additional approach focuses on the identification of excretory/secretory products (ESPs) that are essentially involved in parasite–host interaction. Their diversity and extracellular accessibility render them attractive drug targets (Hewitson et al., 2008; Lustigman et al., 2002). The secretion of antioxidant enzymes by parasites is thought to predominantly protect them against toxic reactive oxygen species (ROS) released by immune effector cells as a host defense mechanism (Hewitson et al., 2008). *O. volvulus* responds to ROS by producing antioxidant enzymes such as thioredoxin peroxidase (Chandrashekar et al., 1998), glutathione S-transferases (Liebau et al., 2008) and superoxide dismutases (SODs; Henkle-Dührsen et al., 1997; Lizotte-Waniewski et al., 2000). SODs are metalloenzymes that catalyse the disproportion redox reaction of superoxide anions to oxygen and hydrogen peroxide, i.e. $2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$. In addition to oxidative stress, this is also relevant for peroxide signaling (Montllor-Albalate et al., 2019). Since $H_2O_2$ is a rather inert and small oxidant, it can freely diffuse through cell membranes and possesses multiple physiological effects, as discussed elsewhere (Storz & Imlayt, 1999; Rahbari et al., 2017). SODs are distinguished based on their cellular localization and the metal cofactor(s) in their active sites. All SOD subgroups have been characterized in humans in great detail (Petersen et al., 2004, 2008), as well as, for example, in fungi (Robinett et al., 2018; Mohsin et al., 2021) and to a limited extent in nematodes (Dabin et al., 2008; Henkle-Dührsen et al., 1994; James et al., 1994; Ou et al., 1995). A number of copper(II) complexes are capable of mimicking SOD activity (Siqueira et al., 2020). Nanocomposite-based materials with SOD activity are being considered for pharmaceutical use in the treatment of stress-related diseases (Pavlovic et al., 2021). Some high-resolution structures of SODs have been determined, among which is the recently reported structure of a Cu/Zn-SOD from the fungus *Chaetomium thermophilum* (Mohsin et al., 2021).

The first characterization of a SOD from *O. volvulus* was reported by Henkle et al. (1991), but additional SOD activity was found in *vitro* culture supernatants of *Onchocerca* microfilariae and adult worms (James et al., 1994). The suggestion that there is another secretory or excretory form of this enzyme was supported by a study that detected a SOD in larval and adult stages (Henkle-Dührsen et al., 1997). It was first predicted that the individual N-terminal signal peptide (SP) of this extracellular SOD (*Ov*EC-SOD; EC 1.15.1.1) is cleaved off between Asn42 and Gly43 of the preprocessed protein (James et al., 1994). Although computational tools for the detection of SPs have continuously improved, the detection of cleavage sites remains challenging. This is especially critical when preparing the recombinant production of putative ESPs for structural studies.

In terms of our investigations, we successfully designed a soluble *Ov*EC-SOD construct starting from Gly43 of the preprocessed protein. The structure of *Ov*EC-SOD was solved at 1.55 Å resolution, comprising a homodimer with 156 residues and one copper and one zinc ion in the active site of each protomer. Despite the conserved fold, the overall sequence identities compared with human cytosolic and extracellular SODs are only 45% and 55%, respectively. Inhibitors of *Taenia solium* SOD, which potentially target a widely non-conserved clef close to the dimerization interface, were docked to *Ov*EC-SOD. The overall structure of *Ov*EC-SOD and its dimerization were verified in solution. Structural insights into *Ov*EC-SOD may shed light on the structural diversity of SODs and may potentially further be explored in future drug-discovery approaches to treat onchocerciasis with improved specificity.

### 2. Materials and methods

#### 2.1. Cloning, protein production and purification

The open reading frame encoding *Ov*EC-SOD starting from Ala44 was amplified from *O. volvulus* genomic DNA by PCR (Table 1). The N-terminus of the protein is supplemented with an OmpA signal peptide mediating secretion to the periplasmic space, a Strep-tag II and a TEV protease cleavage site (ENLYFQG). *Escherichia coli* BL21 (DE3) cells were transformed and grown in Luria–Bertani medium containing 50 µg ml$^{-1}$ ampicillin at 310 K. When the optical density at 600 nm reached approximately 0.6, overexpression was disrupted by sonication and the supernatant was loaded onto a
StrepTactin Sepharose column (IBA, Germany) pre-equilibrated with lysis buffer. Protein was eluted using 2.5 mM desthiobiotin in the same buffer. The Strep-tag II was cleaved off by TEV protease at a molar ratio of 1:50 using the combined fractions containing OvEC-SOD. Subsequent size-exclusion chromatography allowed estimation of the oligomeric state of OvEC-SOD using a calibrated HiLoad 16/600 Superdex 200 column equilibrated with 50 mM Tris–HCl, 200 mM NaCl pH 8.0. The homogeneity and the optimal solution composition were verified by dynamic light scattering using a SpectroLight 300 instrument (XtalConcepts, Germany) in preparation for crystallization experiments. The identity and integrity of the purified protein were confirmed by SDS-PAGE (Laemmli, 1970) as shown in Supplementary Fig. S1(a).

### 2.2. Crystallization and crystal handling

OvEC-SOD was initially screened against 400 distinct crystallization conditions (Qiagen, Germany) applying the sitting-drop vapor-diffusion method using a Honeybee 961 dispensing robot (Genomic Solutions, UK) at 293 K combined with a 2-well MRC plate. A 500 nl droplet of protein solution was mixed with the same volume of reservoir solution and equilibrated against 50 μl reservoir solution. Brick-shaped crystals appeared after one week using a reservoir solution consisting of 0.01 mM calcium chloride, 0.1 mM sodium acetate pH 4.0, 60% (w/v) MPD. Unfortunately, these crystals only diffracted to approximately 6 Å resolution. To optimize the crystal quality, the N-terminal tag was cleaved off and otherwise identical crystallization experiments were performed. Crystals appeared using the conditions specified in Table 2 and grew to full size after five months. The crystal morphology is shown in Supplementary Fig. S1(b).

Crystals were prepared for data collection by manual harvesting using nylon loops. They were briefly immersed in mother liquor supplemented with 10% glycerol as a cryoprotectant and were flash-cooled in liquid N2 for subsequent data collection.

### 2.3. Data collection, processing and refinement

Diffraction data were collected on EMBL beamline P13 at DESY, Hamburg, Germany. Further details are specified in Table 3. Indexing of the data was carried out with XDS (Kabsch, 2010). The crystal structure was solved by molecular replacement with MOLREP (Vagin & Teplyakov, 2010) using the crystal structure of a C. elegans SOD (PDB entry 3kbe; O. N. Pakhomova, A. B. Taylor, J. P. Schuermann, V. L. Culotta & P. J. Hart, unpublished work) as a search model. REFMACS (Kovalenko et al., 2018) from the CCP4 suite version 4.2 (Winn et al., 2011) was used for iterative refinement in combination with Coot (Emsley et al., 2010) for manual model building. Model building resulted in an overall R of 15.9% and Rfree of 18.1% using all data in the resolution range 25.80–1.55 Å. Data-collection, indexing and refinement statistics are shown in Tables 3 and 4, respectively. The structure was deposited in the Protein Data Bank with PDB code 5in2.
2.4. Structural investigation of OvEC-SOD in solution

Monodisperse solutions containing pure OvEC-SOD were applied to small-angle X-ray scattering (SAXS) to verify the dimerization of OvEC-SOD and to analyze its shape. Data were collected on EMBL beamline P12 at the PETRA III storage ring, DESY, Hamburg, Germany as further specified in Supplementary Table S1. Four solute concentrations in the range 0.5–7.5 mg ml\(^{-1}\) were exposed to the beam. The obtained scattering amplitudes were averaged over all 40 exposures per sample for 45 ms each and the averaged buffer scattering of 40 buffer exposures was subtracted. Data were normalized to the transmitted beam intensity. The scattering profiles were plotted and evaluated using PRIMUS as part of the ATSAS suite (Manalastas-Cantos et al., 2021). The Guinier approximation (Guinier, 1939) was utilized to determine the radius of gyration (\(R_g\)). The pair distance-distribution function was calculated using GNOM. \(Ab\ \textit{initio}\) models were calculated using GASBOR (Svergun et al., 2001). Furthermore, CRYSOL (Svergun et al., 1995) and SREFLEX (Panjkovich & Svergun, 2016), which considers additional conformational flexibility of a given high-resolution structure in solution, allowed the comparison of the scattering data to known high-resolution crystal structure coordinates.

2.5. Docking

Using \(FT\text{map}\) (Kozakov et al., 2015), potential binding sites for the default docking library of small molecules were identified. These binding sites and the individual pattern of binding sites on the surface of related SODs are considered to be useful in preparation for fragment-based drug-development approaches and also for identifying potential binding sites of a given ligand. The underlying probe library consists of 16 molecules, \(i.e\). acetamide, acetonitrile, acetone, acetaldehyde, methylamine, benzaldehyde, benzene, isobutanol, cyclohexane, \(N, N\)-dimethylformamide, dimethyl ether, ethanol, ethane, phenol, 2-propanol and urea.

The compounds for the docking of putative inhibitors were selected based on a previous docking study as well as activity and specificity assays on \(T.\ \textit{solium}\) Cu/Zn-SOD (García-Gutiérrez et al., 2011). These authors utilized the LeadQuest library, which contains 51 068 drug-like compounds, reduced the size of the screen using Lipinski-like rules for docking and finally tested 50 candidate compounds \textit{in vitro}. The molecular weights of the library compounds range from 200 to 700 Da. Based on this previous study, which indicated inhibitor binding outside the active site (García-Gutiérrez et al., 2011), and agreement with \(FT\text{map}\) indicating potentially druggable binding sites, the \textit{in silico} docking analysis was prepared. \textit{AutoDock 4.2.3} (Morris et al., 2009) was used for compound docking to the OvEC-SOD dimer applying the Lamarckian genetic algorithm (LGA). For each docking, the grid size was set to \(60 \times 60 \times 60\ \text{Å}\) with a grid spacing of 0.675 Å centered at the putative hydrophobic compound binding cleft in proximity to the dimerization interface of the OvEC-SOD dimer [grid center coordinates \((x, y, z): -9.3, -3.8, -13.5\)]. Step sizes of 1 Å for translation and 60° for rotation were chosen, the maximum number of energy evaluations was set to 150 000 and 150 runs were performed. Ligand-binding site plots were prepared using \textit{LigPlot+} version 2.2 (EMBL–EBI).

3. Results and discussion

3.1. Overall crystal structure

The major differences among Cu/Zn-type SODs at the sequence level are the length and composition of the large nonconserved turns and loops, as visualized as an alignment in Supplementary Fig. S2. The crystal structure of OvEC-SOD contains one molecule per asymmetric unit; the respective data processing is summarized in Table 4. The biological assembly, however, is a dimer with two distinct independent active sites. Every monomer contains three \(\alpha\)-helices, accounting for approximately 9% of the secondary structure, and nine \(\beta\)-sheets representing 37% of the secondary structure. The OvEC-SOD structure shows typical features of SODs that are highly conserved throughout the different kingdoms of organisms, including the Greek-key \(\beta\)-barrel motif (Fig. 1a). Closely related homologues from \textit{Homo sapiens} share an overall r.m.s.d. of \(<1\ \text{Å}\) for \(C^\beta\) positions with OvEC-SOD (Fig. 1b). The common feature is a large cylindrical barrel comprising nine extended sheets with an entirely antiparallel structure. The rest of the protomer structure contains two loops of a nonrepetitive type. The first loop, \(i.e\). residues 47–84, has two distinct parts. The first part is a disulfide loop, which is connected to the \(\beta\)-barrel motif by a disulfide bond. The second part is predominantly hydrophilic and contributes to coordination of the Zn\(^{2+}\) ion. The second, and also the second largest, loop is hydrophilic and is referred to as the electrostatic loop. The dimerization interface mainly consists of hydrophobic interactions, involving only four hydrogen bonds. The dimer, as displayed in Fig. 1(c), has a dimerization interface area of approximately 730 Å\(^2\).

3.2. The active site of OvEC-SOD and its geometry

The active site contains one Cu\(^{2+}\) ion relevant for catalysis and one structural Zn\(^{2+}\) ion. The identity of the metal ions is supported by the \textit{CMM} server (Zheng et al., 2014) based on their coordination geometries. Specifically, \(B\) factors of 21.4 and 18.3 Å\(^2\) were determined for the copper and zinc ions, respectively. Further, the gRMSD, \(i.e\). the determined deviation from ideal coordination angles (ligand–metal–ligand) for the respective metal ion (Zheng et al., 2014), is 9.4° for the copper ion and 7.9° for the zinc ion. The coordination spheres of the Cu\(^{2+}\) and Zn\(^{2+}\) ions are defined by the invariant residues His46, His48, His63 and His120 and His63, His71, His80 and Asp83, respectively (Fig. 1d). The Zn\(^{2+}\) ion is coordinated by three histidine residues and one aspartate residue in a tetragonal geometry. The Cu\(^{2+}\) ion is coordinated by three histidine residues arranged in a distorted tetrahedral geometry. Additionally, many high-resolution SOD structures possess a water molecule that is involved in the coordination. To our knowledge, for the first time in a Cu/Zn-SOD structure a larger anion consisting of multiple atoms was identified in this
position; although, it is generally not easy to crystallographically distinguish sulfate from phosphate. The sulfate ion interacts via one of its O atoms, which is at a distance of 2.06 Å from the Cu\(^{2+}\) ion and appears to displace water (Fig. 1d and Supplementary Fig. S3). As MOPS and HEPES are sulfonic acids, and sulfate (and also phosphate) was not part of the original crystallization solution and buffer composition, one can consider that the sulfate electron density might be part of a disordered buffer molecule or could alternatively originate from the *E. coli* culture or the purification environment. The copper ion is not only involved in the redox cycle of the catalytic activity but along with the zinc ion also contributes to the stability of the typical SOD β-barrel motif (Assfalg et al., 2003). Hence, it might be hypothesized that the observed ligand ion of the complex could stabilize the protein at an elevated stress level in *E. coli*, although the requirements for formation of the copper complex are not fully understood in *vivo*. In human SOD1 in *E. coli* cells copper was reported to be absent in the context of NMR experiments (Banci et al., 2011).

The catalytic activity of OvEC-SOD seems to be independent of the presence of a conserved water molecule (Banci et al., 1989). In OvEC-SOD, His63 forms a ‘bridge’ between the Cu\(^{2+}\) and Zn\(^{2+}\) ions, with distances of 3.2 and 2.0 Å, respectively. The Cu–His63–Zn imidazolate bridge is intact in the oxidized form of the enzyme. In the reduced form of the enzyme this bond is broken and the catalytic metal becomes three-coordinated (Ascone et al., 1997). In the oxidized form, the typical distance between copper and zinc should be approximately 6.0 Å, while in the reduced form of the enzyme this distance should be around or greater than 6.5 Å. The crystal structure of OvEC-SOD revealed that this bond was broken at a distance of 6.9 Å between the metal ions (Fig. 1d).

The active-site channel is formed in part by the disulfide loop, *i.e.* residues 48–62, and in part by the electrostatic loop,
i.e. residues 130–146. The latter provides the electrostatic potential to drive the substrate to the reaction site with a major regulatory potential depending on charge and conformation (García-Gutiérrez et al., 2011). However, the entrance of the channel to the active site varies in sequence and also in structure when comparing OvEC-SOD with the homologous human enzyme. In human Cu/Zn-SOD this electrostatic loop is positively charged overall, while OvEC-SOD contains more nonpolar and negatively charged residues. In OvEC-SOD the loop is ordered, with well defined $2F_o - F_c$ electron density. The active-site channel is maintained by the conserved intramolecular disulfide bond. The side chain of Arg146, which is the residue responsible for the correct orientation of superoxide in the catalytic cavity and is highly conserved, is stabilized by hydrogen bonding to the carbonyl O atom of Cys57.

### 3.3. SAXS

Monodisperse solutions containing purified OvEC-SOD after affinity-tag cleavage were used in SAXS measurements to confirm the crystallographic dimer and analyze the solution structure (Fig. 2). There was no indication of concentration-dependent oligomerization. The scattering amplitudes recorded at different concentrations were averaged, resulting in the scattering pattern displayed in Fig. 2(a). SAXS data-collection parameters and characteristics of the protein are summarized in Supplementary Table S1.

The molecular mass of 38 ± 2 kDa was estimated from the forward scattering using bovine serum albumin as a reference protein. This approximation indicates that OvEC-SOD is dimeric in solution. An experimental $R_g$ of $2.55 \pm 0.01$ nm and a $D_{max}$ of $9 \pm 1$ nm were determined. The $ab\ initio$ structure was calculated using scattering vectors up to $s = 0.4$ Å$^{-1}$ and indicated a rhomboidal shape that was superposed with the crystal structure. The fit curve of this $ab\ initio$ model is shown in Fig. 2(a). Comparison of the experimental SAXS scattering data with theoretical scattering curves calculated using the crystal structure of the OvEC-SOD dimer resulted in the optimized fit curves shown in Fig. 2(b). Considering the additional conformational flexibility of OvEC-SOD in solution,
the experiment confirms that the OvEC-SOD dimer seen in the crystal resembles the overall structure of the protein in solution well. The core of the solution structure is formed by the presumably more rigid $\beta$-sheets surrounded by loop regions and helices at both ends of the elongated dimer, rendering the $ab$ initio structure shown in Fig. 2(c). The scattering data including the described fitting is available in the Small Angle Scattering Biological Data Bank (SASBDB) as entry SASDPF2.

According to Muñoz et al. (2005), the dimerization of SODs in solution is typical and vital for their catalytic function. Dimer-destabilizing mutations of Cu/Zn-SODs have been related to a number of degenerative motor neurone diseases (Téllez-Valencia et al., 2004; Hough et al., 2004), with a very complex maturation pathway of the dimeric functional holoenzyme, as recently summarized for SOD1 (Trist et al., 2021).

3.4. Previously identified inhibitors and their potential binding sites

50 compounds from the LeadQuest library were selected $in silico$ and screened $in vitro$ for inhibition of $T. solium$ SOD in a previous approach (García-Gutiérrez et al., 2011). A set of polyaromatic compounds within this library showed inhibition of $T. solium$ SOD $in vitro$, with IC$_{50}$ values in the micromolar range. They were predicted to bind outside the highly conserved active site with specificity for $T. solium$ SOD over the human homologue by $in silico$ docking (García-Gutiérrez et al., 2011). Docking scores are summarized in Supplementary Table S2. Since the residues directly or indirectly involved in metal binding are conserved among Cu/Zn-SODs, binding outside the narrow core active site in regions with lower sequence conservation is of particular interest for the development of highly species-specific SOD inhibitors. García-Gutiérrez and coworkers searched for potentially druggable binding cavities $in silico$, which may indicate an option to interfere with the catalytic activity of $T. solium$ SOD. Similarly, we considered $in silico$ predictions provided by the FTmap server utilizing small-molecule docking across the entire protein surface. FTmap predicted a wide central cleft, which is formed by both protomers of the OvEC-SOD dimer, as a ‘hotspot’ for interaction with small molecules from the default FTmap library. The patterns of small-molecule interaction are notably different for the three homologue proteins that were analyzed despite the similarity of the proteins (Supplementary Fig. S4). This wide cleft spanning halfway around the narrow side of the dimer close to the dimerization site overlaps with a binding site considered and previously used in docking studies by García-Gutiérrez and coworkers. Utilizing this cleft as the definition of the region of interest, three of the previously analyzed $T. solium$ Cu/Zn-SOD inhibitors were selected for a similar docking approach with OvEC-SOD. Specifically, the targeted core of this cleft comprises amino acids 62–64, 104–113 and 152 of the OvEC-SOD structure. This location is separated from the active site and hence the docking does not interfere with the metal-coordination sites. García-Gutiérrez and coworkers hypothesize that one explanation for the observed inhibition could be local restrictions of loop movement in close proximity to the abovedescribed cleft, resulting in reduced substrate delivery to the active site upon compound binding.

The three docked compounds are indicated to specifically interact with partly differing sections of the targeted cleft, as shown in Figs. 3(a)–3(c). The putative mechanism of binding is mainly assured through hydrophobic interactions and involves both protomers of the dimer, both of which are in agreement with FTmap predictions.
with the binding sites reported for \( T. \textit{solium} \) SOD. A hydrogen bond to compounds 1545-7806 and 1502-3317 is formed via the backbone of the conserved Leu106 of \( \textit{Ov} \textit{EC-SOD} \). Favoring species specificity, Arg3 and Arg107 of \( \textit{Ov} \textit{EC-SOD} \), which contribute hydrophobic interactions with all three described ligands, as well as several amino-acid side chains between positions 107 and 115, which hydrophobically interact with at least one of the compounds, are not conserved. Previously, in the comparison of \( T. \textit{solium} \) SOD and the homologue from \( H. \textit{sapiens} \), the partly nonconserved residues Thr107 and Ser111 were highlighted by García-Gutiérrez and coworkers; however, \( T. \textit{solium} \) and \( \textit{O. volvulus} \) share a serine at position 111. Detailed two-dimensional ligand-binding site plots for the docked compounds are displayed in Supplementary Fig. S5.

The docking scores obtained for \( \textit{Ov} \textit{EC-SOD} \) are slightly different from the values obtained for \( T. \textit{solium} \) Cu/Zn-SOD, as summarized in Supplementary Table S2. However, for both homologues a higher affinity compared with the human SOD homologue is indicated \textit{in silico}. A careful verification of these predictions by further \textit{in vitro} experiments and by means of structural biology is nonetheless desirable, also considering the challenges in state-of-the-art docking approaches as summarized by Zev and coworkers in the context of a protease (Zev \textit{et al.}, 2021). Further understanding of the mode of action of the compounds, potential off-target effects and options for allosteric specific inhibition of SODs would provide a solid perspective for structure-based optimization of the compounds based on the presented crystallographic data.

In summary, the widely conserved structure of \( \textit{Ov} \textit{EC-SOD} \) was solved and compared with its solution structure, which comprises an elongated dimer. Differences in metal-ion coordination are discussed. Based on the individual surface areas of \( \textit{Ov} \textit{EC-SOD} \) and related enzymes, initial suggestions for achieving species specificity in inhibitor development remain to be expanded.

Acknowledgements

We acknowledge DESY and EMBL, Hamburg, Germany; parts of this research were carried out on EMBL beamlines P12 and P13 at PETRA III. We particularly thank Al Kikhney for highly valuable support in operating the SAXS beamline P12 and the beamline scientists at the MX beamline P13. Open access funding enabled and organized by Projekt DEAL.

Funding information

We acknowledge financial support from the Cluster of Excellence ‘Advanced Imaging of Matter’ of the Deutsche Forschungsgemeinschaft (DFG; EXC 2056 – project ID 390715994), German–African Cooperation Projects in Infectiology (PAK296) and the Joachim-Herz-Stiftung Hamburg (Project Infecto-Physics). Amr Moustafa would like to thank the German Academic Exchange Service (DAAD) for financial support via grant No. A/11/92506 and the Egyptian Ministry of Higher Education for partial funding.

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