Mechanism of phosphoribosyl-ubiquitination mediated by a single *Legionella* effector

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Ubiquitination is a post-translational modification that regulates many cellular processes in eukaryotes1–4. The conventional ubiquitination cascade culminates in a covalent linkage between the C terminus of ubiquitin (Ub) and a target protein, usually on a lysine side chain5,6. Recent studies of the *Legionella pneumophila* SidE family of effector proteins revealed a ubiquitination method in which a phosphoribosyl ubiquitin (PR-Ub) is conjugated to a serine residue on substrates via a phosphodiester bond5–7. Here we present the crystal structure of a fragment of the SidE family member SdeA that retains ubiquitination activity, and determine the mechanism of this unique post-translational modification. The structure reveals that the catalytic module contains two distinct functional units: a phosphodiesterase domain and a mono-ADP-ribosyltransferase domain. Biochemical analysis shows that the mono-ADP-ribosyltransferase domain-mediated conversion of Ub to ADP-ribosylated Ub (ADPR-Ub) and the phosphodiesterase domain-mediated ligation of PR-Ub to substrates are two independent activities of SdeA. Furthermore, we present two crystal structures of a homologous phosphodiesterase domain from the SidE family member SdeD3 in complexes with Ub and ADPR-Ub. The structures suggest a mechanism for how SdeA processes ADPR-Ub to PR-Ub and AMP, and conjugates PR-Ub to a serine residue in substrates. Our study establishes the molecular mechanism of phosphoribosyl-linked ubiquitination and will enable future studies of this unusual type of ubiquitination in eukaryotes.

A variety of microbial pathogens exploit the eukaryotic ubiquitination pathway during their respective infections10,11. The intracellular pathogen *L. pneumophila* injects more than 300 effectors into host cells during its infection, including at least ten proteins that are involved in ubiquitin manipulation12. These effectors include HECT-like13,14 and F- or U-box-containing Ub ligases15–18 as well as novel Ub ligases of the SidE family, such as SdeA, that act independently of canonical E1 and E2 enzymes6–8. SdeA first uses its mono-ADP-ribosyltransferase (mART) activity to catalyse the transfer of ADP-ribose from NAD+ to the side chain of R42 on Ub to generate ADPR-Ub. Subsequently, SdeA uses its phosphodiesterase (PDE) activity to catalyse the conjugation of ADPR-Ub to a serine residue on substrates to generate a protein–PR-Ub product. Alternatively, in the absence of substrates, the SdeA PDE domain will catalyse the hydrolysis of ADPR-Ub to generate PR-Ub and AMP (Fig. 1a, Extended Data Fig. 1). The molecular mechanism of this unique ubiquitination pathway is still unknown.

To determine the mechanism of phosphoribosyl-linked ubiquitination, we determined the crystal structure of a portion of SdeA (amino acids 211–910, hereafter called SdeA-core; Extended Data Table 1). The structure is composed of two distinct domains, the PDE and mART domains (Fig. 1b, c). A calculation of the surface electrostatic potential revealed no notably charged areas on the surface of SdeA other than a deep and highly positively charged groove on the PDE domain (Fig. 1d, e). Analogous to other PDEs19, the active site is likely to be harboured in this deep groove (Extended Data Fig. 2a–c). Indeed, a sequence alignment of PDE domains showed that most of the conserved residues reside in this groove, consistent with their forming the PDE active site (Extended Data Figs. 2d, 3).

The mART domain is composed of two lobes, an N-terminal α-helical lobe (amino acids 592–758) and a main lobe (amino acids 759–911). The main lobe contains a β-sandwich core and harbours the three catalytic motifs: the (F/Y)-(R/H), STS and EXE motifs (Extended Data Figs. 4a–f, 5), that are conserved in other mART proteins, such as the *Pseudomonas syringae* effector HopU1 and the *Clostridium perfringens* iota-toxin20–22. A structural comparison of the α-helical lobe with its counterparts in other mARTs revealed that although the total number and the length of α-helices are variable, three α-helices form a structural core that is conserved in most mART proteins (Extended Data Fig. 4g–i). Although it packs in close contact with the main lobe in other mARTs, the α-helical lobe is extended away from the main lobe in our SdeA-core crystal structure (Extended Data Fig. 6a, b). The extended conformation observed in our crystal structure is consistent with the conformation in solution as detected by small-angle X-ray scattering (SAXS) and does not change in the presence of NAD+ (Extended Data Fig. 6c–f). However, the α-helical lobe adopts a closed conformation and mediates contact with NAD+ in a structure of iota-toxin21. Moreover, the α-helical lobe is enriched with highly conserved residues (including N723, Q727 and R729) that form a cluster on its surface, as revealed by an analysis of surface residue conservation using the ConSurf server23 (Extended Data Figs. 5, 7a). Thus, we hypothesized that the α-helical lobe may have a similar role in SdeA catalysis. Indeed, an α-helical lobe deletion in SdeA (SdeA-Δα-lobe), as well as N723A, Q727A or R729A point mutations in the α-helical lobe completely abrogated ADP-ribosylation activity (Extended Data Fig. 7b, c). A mutation in a residue that is not conserved but is close to the conserved surface patch (F719A), yielded a substantial impairment of activity, whereas mutation of a conserved residue that is away from the patch (D622A) resulted in an activity level comparable to wild-type SdeA. Taken together, our data show that the α-helical lobe is crucial for ADP-ribosylation of Ub, and that a surface patch composed of highly conserved residues may mediate the binding of NAD+ during catalysis. These observations further suggest that the closed conformation of the α-helical lobe is required for the mART activity of SdeA. An accompanying paper describing the crystal structure of a longer construct of SdeA in complex with both NAD+ and Ub reports that the α-helical lobe is indeed observed in a closed conformation24.

The main lobe of the mART domain is packed against the PDE domain in the SdeA structure. The two catalytic sites face in opposite directions and are separated by a distance of over 55 Å (Fig. 1b), which raises the question of how the activities of the two domains are coordinated. To address this question, we performed assays with SdeA fragments that retain only mART or PDE activity (Fig. 2a). Similar to wild-type SdeA-core, reactions that contain both SdeA-PDE and SdeA-mART efficiently generate PR-Ub and ubiquitinate the substrate (Extended Data Figs. 2d–f). These observations suggest a mechanism for how SdeA processes ADPR-Ub to PR-Ub to wild-type SdeA-core, mediated by a single *Legionella* effector.
Ub as evidenced by NMR-peak perturbations. We then successfully determined the structures of SdeD, both on its own and in complex with Ub (Extended Data Fig. 8d–f). Notably, two Ub molecules are in contact with a single PDE domain in the crystal. One Ub (Ub2) binds on the opposite side to the catalytic groove, making the physiological significance of this binding mode unclear (Extended Data Fig. 8g). The other Ub (Ub1) binds to a flat surface at the opening of the catalytic groove (Fig. 3a). Similar to the Ub surface area mapped by NMR titration experiments in solution (Extended Data Fig. 8c), three regions of Ub1 contact the PDE domain: the loop region around residue T9, the C terminus and a region that includes R42 (Fig. 3a). At the T9 loop region, in addition to the hydrophobic interactions mainly contributed by L8, residue K6 of Ub1 forms electrostatic interactions with E251 on SdeD (Fig. 3b). At the C terminus of Ub1, in addition to hydrophobic interactions mediated by L73, R72 of Ub1 forms salt bridges with E242 on SdeD (Fig. 3c). Notably, the R42 side chain of Ub1 extends into the catalytic groove and forms hydrogen bonds and electrostatic interactions with the conserved residues Q52 and E126 at the PDE catalytic site (Fig. 3d). To test whether the PDE domain of SdeA interacts with Ub in a manner that is similar to SdeD, we modelled Ub binding by the PDE domain of SdeA on the basis of the SdeD–Ub1 complex (Fig. 3e). The model predicts that E465 and E454 in SdeA would have analogous roles in Ub binding to E251 and E242 in SdeD, respectively (Fig. 3a, e). Consistent with this prediction, PDE activity was substantially impaired in SdeA E465A and E454A mutants as evidenced by the marked reduction of both the Pro-Q staining signal and ubiquitination of RAB33B (Fig. 3f, g). In addition, a V414Y mutant designed to sterically block the access of ADP-Ub to the catalytic site also largely impaired the PDE activity (Fig. 3e–g). All three SdeA mutants were able to cause a band shift of Ub on native gels (Fig. 3e, top) indicating that the mART activity of these mutants remained intact. Together, these data support the notion that the SdeA PDE domain recognises Ub in a manner that is similar to the strategy observed for SdeD, although the interaction is markedly weaker as evidenced by the NMR-titration analysis.

To further address the question of how the ADP moiety of ADP-Ub fits in the active-site groove of the PDE domain, we determined the structure of a catalytically inactive SdeD mutant (H67A) in complex with ADP-Ub. The binding mode of ADP-Ub is similar to Ub1 with the ADP moiety nestled in the catalytic groove (Extended Data Fig. 9a–d). ADP sits atop several invariant residues, including H67A, H189 and E126, and engages in extensive interactions, with a large number of conserved residues within the catalytic groove (Fig. 4a–c, Extended Data Fig. 9e). To test the role of the ADP-ubiquitinating residues within the catalytic groove, we mutated several corresponding residues in SdeA. PDE activity was completely abolished in the H277A, H407A, and E340A mutants, as indicated by the lack of both the Pro-Q staining signal and RAB33B ubiquitination (Fig. 4c, d). The activity of the R413A mutant was substantially impaired, whereas H281A and W394A mutations showed little or no effect on PDE activity.

Based on our results, we propose a two-step reaction mechanism for the transfer of Ub to a substrate (Fig. 4e). In the first step, negatively charged E340 helps to position R42 of ADP-Ub and the PDE domain of SdeA on the basis of the SdeD–Ub1 complex (Fig. 3e). The model predicts that E465 and E454 in SdeA would have analogous roles in Ub binding to E251 and E242 in SdeD, respectively (Fig. 3a, e). Consistent with this prediction, PDE activity was substantially impaired in SdeA E465A and E454A mutants as evidenced by the marked reduction of both the Pro-Q staining signal and ubiquitination of RAB33B (Fig. 3f, g). In addition, a V414Y mutant designed to sterically block the access of ADP-Ub to the catalytic site also largely impaired the PDE activity (Fig. 3e–g). All three SdeA mutants were able to cause a band shift of Ub on native gels (Fig. 3e, top) indicating that the mART activity of these mutants remained intact. Together, these data support the notion that the SdeA PDE domain recognises Ub in a manner that is similar to the strategy observed for SdeD, although the interaction is markedly weaker as evidenced by the NMR-titration analysis.

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Based on our results, we propose a two-step reaction mechanism for the transfer of ADP-Ub to PR-Ub or to ubiquitinate RAB33B. However, the presence of both SdeA-core and SdeA-PDE successfully catalysed the production of PR-Ub and the ubiquitination of RAB33B. Moreover, SdeA-PDE alone can catalyse phosphoribosyl-linked ubiquitination of RAB33B when purified ADPR-Ub is supplied (Fig. 2d). This interaction could enhance the nucleophilicity of H277 through the orientation of the molecule is the same as shown in b. c. An orthogonal view of d. Molar surface model of SdeA. The surface is coloured on the basis of electrostatic potential with positively charged regions in red and negatively charged surfaces in red. The orientation of the molecule is the same as shown in b. e. An orthogonal view of d.

Fig. 1 | Overall structure of SdeA. a. Schematic of the phosphoribosyl-ubiquitination reaction. b. Overall structure of SdeA-core in ribbon representation. This portion of SdeA has two distinct domains: the PDE (green) and mART (gold) domains. The active site residues of both the mART and PDE domains are shown as red spheres. The linear distance between these two active sites is approximately 55 Å. c. An orthogonal view of b, d. Molecular surface model of SdeA. The surface is coloured on the basis of electrostatic potential with positively charged regions in red and negatively charged surfaces in red. The orientation of the molecule is the same as shown in b. e. An orthogonal view of d.
The interaction between Ub and the PDE domains of SdeD and SdeA. a, Overall view of the binding of Ub (Ub1) with the PDE domain of SdeD. The PDE domain residues within Van der Waals distance of Ub1 are coloured in light blue. Three interacting regions of Ub1 that contact SdeD are marked by dashed outlines. b–d, Expanded views of the three Ub1–SdeD interacting regions outlined in a. e, Surface representation of the PDE domain of SdeA. Ub-binding was modelled the SdeD–Ub1 complex structure and the potential Ub-interacting surface is highlighted in dark green. Three key residues (E465, E454 and V414) at the potential Ub-interacting interface are shown in stick representation. The PDE active site is shown in red. f, g, In vitro Ub-modification (f) and phosphoribosyl-ubiquitination assays (g) of SdeA mutants at the potential Ub binding site is shown in red.

Fig. 2 | ADP-ribosylation of Ub and phosphoribosyl-linked ubiquitination of serine are two independent activities of SdeA. a, Schematic of SdeA constructs. SdeA has an N-terminal deubiquitinase (DUB) domain, followed by PDE, mART and C-terminal coiled coil (CC) domains. b, In vitro Ub-modification assays. The modification of Ub to ADPR-Ub or PR-Ub was monitored by the band-shift of Ub in native PAGE with Comassie staining (top). The production of PR-Ub was visualized by SDS-PAGE and phosphoprotein staining with Pro-Q Diamond (bottom). ADPR-Ub and PR-Ub migrate at the same position on a native gel (labelled as modified Ub), however, only PR-Ub is visible by Pro-Q phosphoprotein stain. c, In vitro phosphoribosyl-ubiquitination assay of RAB33B by indicated the SdeA proteins. IB, immunoblot. d, In vitro phosphoribosyl-ubiquitination assay of RAB33B in the presence of purified ADPR-Ub. e, Intracellular-ubiquitination assays of RAB33B by SdeA. Data shown in b–d are representative of four independent experiments. GFP, green fluorescent protein. f, Similar results were obtained from three independent experiments. g–h, Uncropped gels and blots are shown in Supplementary Fig. 1. WT, wild type.
a eukaryotic enzyme system will advance our understanding of the versatile Ub code.

Online content
Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0147-6.

Received: 25 September 2017; Accepted: 18 April 2018; Published online 23 May 2018.

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Acknowledgements We acknowledge L. Pollack’s group for SAXS data collection. This work is supported by National Institute of Health (NIH) grants 5R01GM116964 (Y.M.), R01AI127465 (Z.-Q.L.), R01GM088055 (R.E.K.), 1R01GM098503-05 (P.S.B.), and 1F32 GM120797 (K.H.R.). The X-ray data were collected at Cornell High Energy Synchrotron Source, CHESS is supported by the NSF and NIH/National Institute of General Medical Sciences (NIGMS) via NSF award DMR-1332208, and the MacCHESS resource is supported by NIH/NIGMS award GM-103485. Some SAXS data were collected at Stanford Synchrotron Radiation Lightsource. Use of the Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory is supported by the US Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. DE-AC02-76SF00515. The SSRL Structural Molecular Biology Program is supported by the DOE Office of Biological and Environmental Research, and by the NIH, NIGMS (including P41GM103393).

ReviewersNature thanks K. Gehring and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Author contributions A.A. and D.J.W. performed crystallization, X-ray data collection, structural determination and phosphoribosyl-ubiquitination analysis; X.W. performed protein purification and crystallization; Y.L. and Y.Z. performed the mutagenesis and bacterial infection experiments; Y.Z. and J.Q. performed the ubiquitination-by-co-expression experiments; K.H.R. performed the SAXS experiment; P.S.B. performed the NMR experiment; Z.-Q.L. analysed the data; K.H.R., P.S.B., R.E.K. and Y.M. analysed the data and wrote the manuscript.

Competing interests The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41586-018-0147-6.

Supplementary information is available for this paper at https://doi.org/10.1038/s41586-018-0147-6.

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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessment.

Cloning and mutagenesis. DNA fragments encoding the SdeA-core and SdeD(Δ1–341) were amplified from *L. pneumophila* genomic DNA. The PCR products were digested with BamHI and XhoI restriction enzymes and inserted into a pET28a-based vector in-frame with an N-terminal 6x-His-SUMO tag for protein overexpression in bacteria cells. Amino acid substitutions of SdeA and SdeD were introduced by site-directed mutagenesis using oligonucleotide primer pairs containing the appropriate base changes. The Ub gene was subcloned into a PET21a vector. All constructs were confirmed by DNA sequencing.

Protein expression and purification. Relevant plasmids (containing *Legionella* protein constructs or RAB33B) were transformed into *E. coli* BL21(DE3) cells. Cultures derived from single colonies were grown in Luria–Bertani medium supplemented with 50 μg ml−1 kanamycin or 100 μg ml−1 ampicillin to mid-log phase. Protein expression was induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 12 h at 18 °C. Collected cells were resuspended in a lysing buffer containing 20 mM Tris-HCl (pH 8.0) and 150 mM NaCl and lysed by sonication. Insoluble cellular debris was pelleted by centrifugation at 31,000 g for 30 min at 4 °C, and the clarified lysate was incubated with cobalt resin (Gold-Bio) for 1.5 h at 4 °C. Proteins bound to the resin were extensively washed with lysis buffer. The SUMO-specific protease Ulp1 was then added to the resin slurry to release the expressed protein from the His-SUMO tag. Eluted protein samples were further purified by fast protein liquid chromatography (Superdex 16/60, GE Lifesciences) in 150 mM NaCl, 20 mM Tris pH 7.5. Peak fractions were collected, pooled and concentrated. Protocols for Ub expression and purification were adapted from the published literature. Briefly, collected cells were resuspended in 20 mM ammonium acetate, pH 5.1. Cells were lysed by sonication and cell lysate was clarified by centrifugation (31,000g for 30 min). The pH of the clarified lysate was lowered to 4.8 using glacial acetic acid. The decrease in pH caused the lysate to turn milky white (a result of precipitated proteins), and the solution was again centrifuged at 31,000g for 30 min at 4 °C to remove the precipitated protein fraction. The pH of the remaining soluble fraction was adjusted to 5.1 by the addition of NaOH. The soluble fraction was then loaded onto a Hitrap SP cation exchange column (GE Healthcare) in 20 mM ammonium acetate pH 5.1, and eluted in a continuous gradient of 500 mM ammonium acetate pH 5.1. Fractions containing the ubiquitin peak were pooled and further purified using size exclusion chromatography in 150 mM NaCl, 20 mM Tris pH 7.5. Ubiquitin-containing fractions were pooled and concentrated.

To generate ADPR-Ub for both biochemical assays and crystallographic trials, 1 μM SdeA-core and S. *pneumoniae* ΔthiM, which lacks PDE activity, was incubated with 25 μM Ub and 1 mM NAD+ for 1 h at 37 °C. ADPR-Ub was purified by size exclusion chromatography (Superdex 200 Increase PC 3.2/30 (GE Healthcare)) in 20 mM ammonium acetate, pH 5.1. Concentrations of other protein components varied from 35–300 μM. Two independent experiments were collected for the ADPR-Ub SdeD PDE domain complex. Each experiment used different stocks of Ub and PDE. Four separate samples containing Ub and different concentrations of SdeD were prepared to collect spectra monitoring the interaction between SdeD and Ub (Ub = 150 μM; SdeD = 37.5, 75, 150 and 300 μM).

SAXS data collection. SAXS experiments were performed on beamline 4-2 at the Stanford Synchrotron Radiation Lightsource (SSRL). Concenated SdeA-core protein samples were buffer exchanged into 20 mM HEPES pH 7.5, 150 mM NaCl, and stored at 4 °C before data collection. Fifty microfilters of SdeA-core (7 mg ml−1) were injected onto a Superdex 200 Increase PC 3.2/30 (GE Healthcare) column in 20 mM HEPES pH 7.5, 150 mM NaCl, 5 mM DTT, 0.02% NaN3, with a flow rate of 0.05 ml min−1 for online SEC–SAXS. Data were collected using a Pilatus3 × 1 M detector with a 2.5 s sample-to-detector distance and X-ray beam energy of 12.4 keV (wavelength, λ = 1 Å), with 1-s exposures collected every 5 s. The first 100 images were averaged as buffer scattering data and subtracted from the corresponding protein scattering data. SAXS patterns, the radius of gyration (Rg), the maximal particle dimension (Dmax), and the pairwise distance distribution histogram (P(r) plot) and Kratky plot were analysed using the ATSAS software suite. The AllosMod–FOXUS server was used for the comparison of solution and X-ray structure conformations. The X-ray–determined open structure and modelled closed conformations were used as input structures. AllosMod generated one hundred static structures, using MODELLER, which were similar to the input X-ray determined (open) or modelled (closed) structures of SdeA-core.

The solvent accessible surface profile and complete SAD data were collected and complemented by the SAXS data using FOXUS rigid-body modelling as previously described, with a maximal q value of 0.25. The mean and s.d. in χ2 amongst the five best-fitting models were examined for fit comparisons.

Computational analysis and graphical presentation of protein sequence and structure. Sequences homologous to SdeA were selected from results generated by the BLAST server (NCBI). Edited sequences were aligned with Clustal Omega and coloured using the Multiple Align Show online server (http://www.bioinformatics.org/sms/index.html). Protein surface conservation was calculated using the online ConSurf server (http://consurf.tau.ac.il). All structural figures were generated using PyMOL (The PyMOL Molecular Graphics System, v.1.8, Schrödinger, LLC) except for the difference Fourier electron density map figure (Extended Data Fig. 9e), which was generated in Coot. The electrostatic surface potential is calculated using the APBS program (http://www.coombsboltzmann.org). The surface is coloured on the basis of electrostatic potential with positively charged regions in blue (+4 kcal per electron) and negatively charged surfaces in red (−4 kcal per electron).

Ubiquitin-modification and RAB33B-ubiquitination assays. Ub-modification reactions were carried out by mixing 1 μM of SdeA-core or SdeA-∆M(5,653–910) with 25 μM ubiquitin in a reaction buffer containing 50 mM NaCl and 50 mM Tris pH 7.5, in the presence or absence of 1 mM NAD+. The reactions were incubated for 1 h at 37 °C and reaction products were assessed using both 8% native PAGE and 12% SDS–PAGE. Native gels were stained with Coomassie and SDS–PAGE gels were stained with Pro-Q Diamond phosphoprotein stain (Invitrogen) to assay for PDE activity. ADPR-Ub and PR-Ub migrate to the same position on a native gel (labelled as modified Ub), however, only PR-Ub is visible by Pro-Q phosphoprotein stain owing to its free phosphoryl group.
cellular phosphoribosyl-ubiquitination assay of RAB33B, plasmids expressing Flag–RAB33B, GFP alone or the indicated GFP-tagged SdeA were co-transfected in NIH HEK293T cells. Whole cell lysates were subjected to immunoprecipitation with Flag beads and the products were analysed using anti-Flag western blot. The expression of GFP–SdeA constructs was analysed with an anti-GFP western blot. 

**Reporting summary.** Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability.** Atomic coordinates and structure factors for the reported structures have been deposited into the Protein Data Bank under the accession codes 6B7Q (Hg-bound SdeA), 6B7P (Se–SdeD), 6B7M (SdeD–Ub) and 6B7O (SdeD–Ub–ADPR-Ub). The data supporting the findings of the study are available within the paper and the Extended Data figures and tables. Further data are available from the corresponding author upon reasonable request. The raw images of electrophoreses and western blots can be found in Supplementary Fig. 1.

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Extended Data Fig. 1 | Chemical structure of phosphoribosyl-linked ubiquitination catalysed by SdeA. Phosphoribosyl-linked ubiquitination catalysed by SdeA involves two enzymatic activities of SdeA. First, using its mART activity, SdeA catalyses the ADP-ribosylation of Ub to generate ADPR-Ub by consuming an NAD\(^+\) molecule. Second, SdeA catalyses the conjugation of ADPR-Ub to a serine residue of substrate proteins via its PDE activity to generate protein–PR-Ub and AMP. In the absence of substrate proteins, the PDE domain of SdeA can simply hydrolyse ADPR-Ub to PR-Ub and AMP using a water molecule.
Extended Data Fig. 2 | Structure of the PDE domain of SdeA. a, Model of the PDE domain of SdeA in ribbon representation. Two invariable histidine residues (H277 and H407) are shown in stick representation and labelled. b, Surface representation of the PDE domain. The two invariable histidine residues (shown in red) are situated at the bottom of a deep groove. c, The PDE domain from a Legionella effector (lpg1496). Notably the all α-helical structural core of the PDE domains is easy to superimpose onto that of SdeA with a root mean square deviation (r.m.s.d.) of 1.9 Å over 225 aligned Cα atoms. A prominent difference between the two PDE domains is that some loops (indicated by dashed outlines) connecting the α-helices vary both in primary sequence and in length (Extended Data Fig. 3). d, Surface residue conservation analysis of the PDE domain. The conservation is calculated using the ConSurf server with the most conserved residues coloured in purple and the least conserved residues in cyan. Note that the catalytic groove is enriched with the most conserved residues.
Extended Data Fig. 3 | Multiple sequence alignment of selected PDE domains from the SidE family effectors. Representative sequences corresponding to the PDE domain of SdeA (amino acids 222–502) were aligned using the MultAlin online server (http://wwwbioinformatics.org/sms/index.html). Secondary structural elements are drawn above the alignment. The numbering for the SdeA sequence is marked on the top of the alignment and the numbering for the SdeD sequence is marked below. Variable loop regions are outlined with dashed squares. Conserved residues located within the catalytic groove are highlighted with purple dots. In particular, three essential catalytic residues (H277, H407 and E340) are highlighted with red stars below the sequences. SdeD residues that are in close contact with Ub1 (Fig. 3a) are marked by blue triangles on the top of the sequences. Amongst the potential Ub1-interacting residues, V414, E454 and E465 of SdeA used in mutagenesis studies in Fig. 3f, g are marked with solid red triangles. Entrez database accession numbers are as follows: SdeA, GI: 1064303039; SidE, GI: 52840489; SdeB, GI: 52842367; SdeC, GI: 52842370; lpg2154, GI: 52842368; and SdeD, GI: 52842717.
Extended Data Fig. 4 | Structural comparison of the SdeA mART domain with other mART domains from bacterial toxins. a, Model of the main lobe of the SdeA mART domain in ribbon representation. The main lobe is composed of two nearly perpendicular β-sheets forming a two-layered β-sandwich core. Residues comprising the three mART catalytic signature motifs: (F/Y)-(R/H), STS and EXE motif are shown in sticks. b, HopU1 from *P. syringae* (PDB ID: 3U0J) in ribbon representation. c, Structural superimposition of the mART domains from SdeA (gold) and HopU1 (blue). d, Iota-toxin from *C. perfringens* (PDB ID: 4H03). e, Iota-toxin in complex with NAD⁺ (red spheres). f, Structural overlay of the mART domains from SdeA (gold) and iota-toxin (cyan). g, A cartoon diagram of the α-helical lobe of the SdeA mART domain. The α-helical lobe consists of eight α-helices. Three structurally conserved α-helices (α6–8) are coloured in brown. h, A cartoon diagram of the α-helical lobe of HopU1, the three equivalent α-helices (α4–6) are highlighted in blue. i, Structural overlay of the α-helical lobe of SdeA and HopU1.
Extended Data Fig. 5 | Multiple sequence alignment of the mART domains. Representative sequences corresponding to the mART domains of SdeA (amino acids 593–904) were aligned using MultAlin. Secondary structural elements (cyan for the α-helical lobe and gold for the main lobe of the mART domain) are drawn above the alignment. The numbering for the SdeA sequence is marked on the top of the alignment. Residues comprising the catalytically important (F/Y)-(R/H), STS and EXE motifs are marked with red stars. Residues in the α-helical lobe, which form—or are close to—the conserved surface patch and are essential for the mART activity (Extended Data Fig. 7), are marked with purple triangles. D622, which is conserved but has no effect on the mART activity is marked with a green triangle. Entrez database accession numbers are as follows: SdeA, GI: 1064303039; SidE, GI: 52840489; SdeB, GI: 52842367; SdeC, GI: 52842370; SidE _Legionella cincinnatiensis_, GI: 966421657; LLO_3095, GI: 489730495; SidE _Legionella gratiana_, GI: 966468332; SidE _Legionella santicrucis_, GI: 966496250; LLO_0424, GI: 502743808.
Extended Data Fig. 6 | The \(\alpha\)-helical lobe of SdeA mART domain has an extended conformation compared to other mART proteins. 

a. Structural superimposition of SdeA onto the HopU1 structure referenced on the main lobe of the mART domain. SdeA is coloured using the same scheme as Fig. 1b. The main lobe of HopU1 is coloured in blue and its \(\alpha\)-helical lobe is in grey. The \(\alpha\)-helical lobe of the SdeA mART is extended away from the main lobe whereas its counterpart in HopU1 packs in close contact with the main lobe. 

b. Structural model of SdeA with the \(\alpha\)-helical lobe in a closed conformation. The positioning of the \(\alpha\)-helical lobe was based on a structural overlay of the three structurally conserved \(\alpha\) helices identified in all mART domains (Extended Data Fig. 4g–i). 

c. Experimental and theoretical SAXS curves for SdeA-core and the resulting best-fit AllosMod structure for the determined structure (open) and modelled closed conformation, with residual plots shown below. Best fit \(\chi^2\) values are indicated. 

d. Overlay of the determined SdeA-core structure (PDE, green; mART main lobe and \(\alpha\)-helical lobe, yellow) and best-fit AllosMod structures for the open (magenta) and closed (cyan) conformations. 

e. Summary of the experimentally derived SAXS parameters for SdeA-core, AllosMod derived best-fit \(R_g\) and average FOXS \(\chi^2\) for the five best-fitting AllosMod models compared to the experimental SAXS curve. The program Primus was used to calculate the radius of gyration (\(R_g\)) and maximum linear dimension (\(D_{max}\)). Kratky plot (\(I(q)q^2\) versus \(q\)), and distance-distribution plot \(P(r)\) obtained from GNOM are shown. 

f. Overlay of SdeA-core SAXS curves in the presence of 4.7 mM NAD\(^+\) (10\(\times\) protein concentration), with corresponding Guinier \(R_g\) values. Data shown in e, c and f are representative of two biologically independent experiments.
Extended Data Fig. 7 | The α-helical lobe of SdeA mART domain is indispensable for Ub ADP-ribosylation. a, Surface representation of residue conservation of SdeA (the most conserved residues are shown in purple and the least conserved residues in cyan). Surface residue conservation was calculated using the ConSurf server. An expanded view of a surface cluster that consists of the most conserved residues on the α-helical lobe is shown on the right. b, Analysis of in vitro ubiquitin-modification assays by SdeA mutants carrying mutations on the α-helical lobe. The reaction products were analysed using native PAGE with Coomassie blue stain (top) and SDS–PAGE with Pro-Q phosphoprotein stain (bottom). c, SDS–PAGE analysis of the proteins in the reaction mixture. Data shown in b and c are representative of three independent experiments. Uncropped gels are shown in Supplementary Fig. 1.
Extended Data Fig. 8 | The interaction between Ub and the SdeD PDE domain. a, NMR $^2$H-$^{15}$N HSQC TROSY spectral overlay of 150 μM Ub (black) in the presence or absence of 300 μM SdeA PDE domain (cyan). Ub binds very weakly to SdeA as manifested by minimal changes in $^{15}$NH peaks of Ub. b, Spectral overlay of 150 μM Ub (black) with 75 μM SdeD PDE. Ub binds with higher affinity to SdeD as evidenced by peak broadening and/or disappearance of Ub resonances. c, Residues whose resonances are most affected by the presence of SdeD are mapped in red on a cartoon structure of Ub. d, PDE domain of SdeD (grey) shown in ribbon representation. Two invariable histidine residues (H67 and H189) are shown in stick representation (cyan). The variable loop unique to SdeD is outlined. e, Structural overlay of the PDE domain of SdeD (grey) and the PDE domain of SdeA (green). The overall structures of these two PDE domains are very similar with an r.m.s.d. of 1.73 Å over 251 overlaid Ca atoms. f, Two orthogonal views of the SdeD PDE domain in complex with two Ub molecules in ribbon representation: Ub1 (cyan) and Ub2 (blue). Ub1 binds at the opening of the PDE catalytic groove with its R42 side chain sticking into the groove. Ub2 binds a region on the opposite side of the catalytic groove. g, Structural superimposition of SdeA onto the SdeD PDE–Ub complex referenced on the PDE domain. The PDE domain of SdeA is shown in green and the mART domain is shown in gold. Note that Ub1 shows no conflicting contacts against the superimposed SdeA molecule whereas the Ub2 binding site largely overlaps with the space occupied by the mART domain in SdeA. This analysis suggests that the binding of the PDE domain of SdeD to Ub1 is probably applicable to the PDE domain of SdeA; however, the second Ub-binding site observed in SdeD might not exist in SdeA. Experiments in a and b were repeated independently two times.
Extended Data Fig. 9  | Crystal structure of the PDE domain of SdeD in complex with ADPR-Ub and Ub.  

a, SdeD PDE domain H67A mutant in complex with both ADPR-Ub and unmodified Ub. The crystal was obtained by mixing the SdeD PDE H67A mutant, ADPR-Ub, and Ub in a 1:2:3 molar ratio (see the 'Protein crystallization' section of the Methods for details). The PDE domain is shown in grey, the bound ADPR-Ub is shown in cyan and the unmodified Ub is shown in blue. The unmodified Ub binds a region identical to Ub2 found in the SdeD–Ub complex shown in Extended Data Fig. 7d. ADPR-Ub binds in a mode that is similar to that of Ub1 in the SdeD–Ub complex with the ADPR moiety fitting into the catalytic groove. 

b, An orthogonal view of a. 

c, d, Two orthogonal views of the complex shown in a in surface representation. Note that the ADPR-moiety shown in light green fits deeply into the catalytic groove. 

e, The density was generated by refinement against the structural model without the ADPR portion. The $F_o - F_c$ difference map is shown in green and contoured at 1$\sigma$. 

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## Extended Data Table 1 | X-ray data collection and structural refinement statistics.

| Data collection                                      | SdeA          | SdeD          | SdeD-Ub        | Sde-D-ADPRUB-Ub |
|-------------------------------------------------------|---------------|---------------|----------------|-----------------|
| Synchrotron beam lines                                | MCChem F1     | MCChem A1     | MCChem F1      | MCChem F1       |
| Wavelength (Å)                                        | 0.9789        | 0.68          | 0.9789         | 0.9789          |
| Space group                                           | P21           | R3            | P21            | P21             |
| Cell dimensions                                       |               |               |                |                 |
| $a, b, c$ (Å)                                         | 69.8, 80.6, 85.6 | 154.4, 154.4, 89.6 | 64.8, 58.6, 74.1 | 64.7, 58.8, 75.1 |
| $\alpha, \beta, \gamma$ (°)                          | 90, 109.8, 90 | 90, 120, 90   | 90, 114.6, 90  | 90, 114.2, 90   |
| Maximum resolution (Å)                                | 2.2           | 1.51          | 1.73           | 1.88            |
| Observed reflections                                  | 61,395        | 634,900       | 363,307        | 281,813         |
| Unique reflections                                    | 18,728        | 124,885       | 108,100        | 43,941          |
| Completeness (%)                                      | 99.3          | 99.5          | 99.4           | 100             |
| Redundancy*                                           | 3.4(3.3)      | 5.1(2.9)      | 3.4(2.2)       | 6.4(5.9)        |
| <$\langle \sigma \rangle$ /$\langle \sigma \rangle$* | 7.98(0.87)    | 29.2(1.52)    | 25.4(1.54)     | 19.28(1.18)     |
| $R_{sym}$ (%)                                         | 0.122(0.759)  | 0.07(0.622)   | 0.078(0.798)   | 0.093(1.105)    |

| Refinement                                            | SdeA          | SdeD          | SdeD-Ub        | Sde-D-ADPRUB-Ub |
| Resolution (Å)*                                       | 80.51(2.20)   | 77.174(1.51)  | 67.36(1.70)    | 68.93(1.85)     |
| $R_{crys}$ / $R_{free}$ (%)                          | 0.192/0.241   | 0.167/0.195   | 0.172/0.28     | 0.210/0.249     |
| No. atoms                                             |               |               |                |                 |
| Protein                                               | 5338          | 4932          | 3721           | 3765            |
| Ligand/ion                                            | 10            | --            | --             | --              |
| Water                                                 | 170           | 448           | 228            | 211             |
| $B$-factors                                           |               |               |                |                 |
| Protein                                               | 49.728        | 24.998        | 28.036         | 33.327          |
| Ligand/ion                                            | 61.563        | --            | --             | --              |
| Water                                                 | 46.001        | 31.136        | 30.525         | 34.580          |
| R.m.s deviations                                      |               |               |                |                 |
| Bond length (Å)                                        | 0.023         | 0.027         | 0.03           | 0.028           |
| Bond angles (°)                                        | 2.29          | 2.47          | 2.47           | 2.43            |

*Values in parentheses are for the highest-resolution shell.
Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a  Confirmed

☐  The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement

☐  An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

☐  The statistical test(s) used AND whether they are one- or two-sided

☐  *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*

☐  A description of all covariates tested

☐  A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons

☐  A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)

☐  For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted

☐  Give P values as exact values whenever suitable.

☐  For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

☐  For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☐  Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

☐  Clearly defined error bars

☐  *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

| ADX data collection software at MacCHESS synchrotron beamline F1 |

Data analysis

| HKL2000_v716.1-Linux; HKL2MAP v0.4; Coot 0.8.2; Refmac 5.5; CCP4 suite v7.0; NMRpipe; NMRViewJ; Modeller 9.19; Pymol v1.8.X |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Atomic coordinates and structure factors for the reported structures have been deposited into the Protein Data Bank under the accession codes 6B7Q (Hg-bound SdeA), 6B7P (Se-SdeD), 6B7M (SdeD-Ub), and 6B7O (SdeD-Ub-ADPR-Ub)
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No sample size calculation was performed for all the experiments. The crystals obtained were reproducible. The SAXS NMR data were reproducible at different protein concentrations. The enzymatic assays were repeated independently at least three times and were all repeatable with similar results. |
| Data exclusions | No data was excluded from the analyses |
| Replication | The SAXS data and NMR data were collected at n >= 2 different protein concentrations. Enzymatic assays were repeated independently n >=3 times for each experiments. All attempts of replication were successful. |
| Randomization | not relevant. no statistics was used in all data collection and processing. |
| Blinding | not relevant. no statistics was used in all data collection and processing. We checked the quality of the crystals based on the diffraction data. |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
| --- | --- |
| ■ | Unique biological materials |
| ■ | Antibodies |
| ■ ■ | Eukaryotic cell lines |
| ■ | Palaeontology |
| ■ ■ | Animals and other organisms |
| ■ ■ | Human research participants |

Methods

| n/a | Involved in the study |
| --- | --- |
| ■ | ChIP-seq |
| ■ | Flow cytometry |
| ■ | MRI-based neuroimaging |

Antibodies

Antibodies used

- anti-Flag antibody (sigma-Aldrich) M2 clone; Cat No. F3165. 1000x dilution was used for western blot.

Validation

- same batch of antibody was used in previous publication (see ref No. 6 of the manuscript: Qiu et al., Nature 2016).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

- HEK293T cells from ATCC

Authentication

- this cell line is not authenticated

Mycoplasma contamination

- Yes, it’s free of mycoplasma contamination

Commonly misidentified lines (See ICLAC register)

- no commonly misidentified cell lines were used.