A shape-shifting redox foldase contributes to *Proteus mirabilis* copper resistance

Emily J. Furlong1, Alvin W. Lo2,3, Fabian Kurth1,†, Lakshmanane Premkumar1,2,†, Makrina Totsika2,3,†, Maud E.S. Achard2,3,†, Maria A. Halili1, Begoña Heras4, Andrew E. Whitten1,†, Hassanul G. Choudhury1,†, Mark A. Schembri2,3 & Jennifer L. Martin1,5

Copper resistance is a key virulence trait of the uropathogen *Proteus mirabilis*. Here we show that *P. mirabilis* ScsC (*PmScsC*) contributes to this defence mechanism by enabling swarming in the presence of copper. We also demonstrate that *PmScsC* is a thioredoxin-like disulfide isomerase but, unlike other characterized proteins in this family, it is trimeric. *PmScsC* trimerization and its active site cysteine are required for wild-type swarming activity in the presence of copper. Moreover, *PmScsC* exhibits unprecedented motion as a consequence of a shape-shifting motif linking the catalytic and trimerization domains. The linker accesses strand, loop and helical conformations enabling the sampling of an enormous folding landscape by the catalytic domains. Mutation of the shape-shifting motif abolishes disulfide isomerase activity, as does removal of the trimerization domain, showing that both features are essential to foldase function. More broadly, the shape-shifter peptide has the potential for ‘plug and play’ application in protein engineering.
The Escherichia coli periplasmic Dsb protein family is the best characterized bacterial oxidative folding system, E. coli DsbA is a monomeric thioredoxin-fold oxidase \(^1\) that introduces disulfide bonds into protein substrates \(^2\), whereas E. coli DsbC is a dimeric V-shaped thioredoxin-fold protein disulfide isomerase \(^3\) that proof-reads and shuffles incorrect disulfide bonds \(^4\). E. coli DsbG is also a dimeric V-shaped thioredoxin-fold protein disulfide isomerase and cysteine reducing system \(^5\) that protects cysteines of periplasmic proteins from inappropriate oxidation \(^6\).

Here, we characterize a putative DsbA-like protein from P. mirabilis and show it plays a key role in virulence by enabling swarming during copper stress. We find that it is not DsbA-like, as it does not catalyse disulfide bond formation. Rather, it is a disulfide isomerase that shuffles incorrect disulfide bonds in proteins. Further, we show that this thioredoxin fold protein is unlike any other characterized to date—it is trimeric—and we demonstrate that its function depends on a shape-shifting motif that could potentially be used as a ‘plug and play’ peptide module.

**Results**

Proteus mirabilis UniProt C2LPE2 is a predicted thioredoxin-fold protein, and the purified protein exhibits redox properties characteristic of the thioredoxin fold family. It is highly oxidizing (\(-108 \text{ mV}\)), has an acidic active site cysteine (\(pK_a = 3.1\); compared to a typical cysteine thiol \(pK_a\) of \(\approx 8-9\) ) and the oxidized disulfide form of the active site destabilizes the protein compared with the reduced form (Supplementary Fig. 1a–c).

The encoded protein plays an important role in P. mirabilis virulence. Copper intoxication is a component of nutritional immunity having a number of detrimental effects on bacterial cells. Copper can react with host-generated hydrogen peroxide to generate free radicals, which damage biological molecules \(^7\); cycling between Cu(I) and Cu(II) can lead to detrimental redox reactions in bacteria; and copper can bind to protein thiols in place of other metal co-factors \(^8,9\). Overcoming these antibacterial effects of copper is a key bacterial defence mechanism. The gene encoding P. mirabilis C2LPE2 is located within a cluster of four predicted suppressor of copper sensitivity (Scs) genes and we proposed this the encoded protein (hereafter called PmScsC) would contribute to bacterial copper resistance. Indeed, inactivation of scsC in two independent P. mirabilis clinical isolates significantly inhibited swarming motility (a key virulence trait of P. mirabilis) in the presence of copper (Fig. 1b, Supplementary Figs 2–4). This phenotype could be complemented by introduction of a plasmid encoding wild-type PmScsC, but not a plasmid encoding an inactive PmScsC (Fig. 1a,b).

Although it is annotated DsbA-like, PmScsC is a powerful protein disulfide isomerase (Fig. 1c) that is able to refold and reanimate the scrambled disulfide form of the model substrate RNase A just as rapidly as the archetypal disulfide isomerase E. coli DsbC. It has negligible DsbA-like dithiol oxidase activity (Supplementary Fig. 1d).

The best-characterized protein disulfide isomerases—EcDsbC and eukaryotic protein disulfide isomerase (PDI)—each have two thioredoxin-fold catalytic domains. DsbC is a dimer, and PDI is a modular 4-domain protein with two catalytically active thioredoxin domains. The presence of two thioredoxin catalytic domains is thought to contribute to highly efficient disulfide shuffling activity \(^10,11\). The amino acid sequence of PmScsC encodes a single thioredoxin fold, and we therefore expected that its disulfide isomerase activity would be a consequence of dimerization to generate the necessary two catalytic domains. We also expected that the protein would adopt the same dimeric...
architecture as V-shaped EcDsbC. However, we were wrong on both counts.

Unexpectedly, evidence from chemical cross-linking (Supplementary Fig. 5), small angle X-ray solution scattering (Fig. 2a,b) and multi-angle light scattering (Fig. 2c) all indicated that PmScsC is trimeric. We confirmed that PmScsC is a trimer by determining three independent crystal structures. The three crystal structures—which we refer to as compact, transitional and extended (Fig. 3a–c; Table 1)—reveal an extraordinary range of motion in this protein. Importantly, the compact and transitional structures have multiple protomers in the asymmetric unit that adopt the same overall structures in each case (8 compact trimers, RMSD ~1.5 Å for 645 C atoms; two transitional trimers, RMSD 0.5 Å for 653 C atoms) so that crystal symmetry does not appear to impact on the observed conformation. Moreover, the compact and transitional conformations were determined from crystals grown under similar conditions (2.85 M sodium malonate pH 5.8, 20 °C with either 0.1 M cobalt or copper added). Nevertheless, comparison of the eight compact and the two transitional trimers in these two crystal structures, reveals an unprecedented level of conformational re-arrangement (RMSDs > 20 Å). The third structure was determined from crystals grown in the presence of 2 mM copper chloride (32% Jeffamine M-600, 0.1 M HEPES pH 8, 20 °C) and reveals a fully extended trimer with one protomer in the asymmetric unit.

PmScsC also exhibits dynamic conformational flexibility in solution. No single PmScsC crystal structure is consistent with the experimentally determined SAXS scattering curve. However, an ensemble of structures fits the experimental data closely (Fig. 2a,b) supporting the notion that the trimer is highly dynamic in solution. Moreover, the solution scattering curve is unchanged by modification of the pH (range 6.0–8.0) or ionic strength (150–1,500 mM NaCl) (Supplementary Fig. 6) indicating the dynamic motion is an inherent property of the protein.

We can pinpoint the region responsible for the extraordinary range of motion in this protein, to an 11-residue peptide linking the trimerization and catalytic domains (residues 39-KADEQ-QAQFRQ-49) (Fig. 3). The 31 crystallographically characterized PmScsC protomers have the same thioredoxin-fold catalytic domains (Fig. 4a,b) (RMSD 0.2–1.2 Å for 129 C atoms). Similarly, all 31 PmScsC protomers share the same trimeric right-handed coiled coil stalk formed from the N-terminal residues (Fig. 3a–c).

At the secondary structure level, it is only the 11-residue linker peptide that changes significantly across the crystal structures (Fig. 4c, Supplementary Movie 1).

The linker is a shape-shifting peptide that can adopt helical, strand or loop conformations. In the 24 protomers present in the compact PmScsC crystal structure, the linker forms a loop that positions the three catalytic domains close to the trimerization stalk (Figs 3a and 4c). In the extended crystal structure the linker is helical, rotating and translating the catalytic domain away from the trimerization domain relative to the compact structure (Figs 3c and 4c). In the transitional PmScsC crystal structure, each of the two trimers incorporates protomers in three conformations: compact, extended-like and intermediate (Fig. 3b). The two compact protomer conformations of the transitional structure are similar to those in the compact crystal structure (RMSD 0.9–1.8 Å for 188 C atoms). The two extended-like protomer conformations of the transitional structure are similar to the protomer in the extended crystal structure though they bend at different points in the linker helix, giving rise to somewhat different catalytic domain placements (Fig. 4c). The two intermediate conformations in the transitional crystal structure have a linker that forms a short β-strand, and positions the catalytic domain directly above the trimerization stalk (Fig. 4c).

Figure 2 | SAXS and MALLS of PmScsC. (a) Small-angle X-ray scattering data collected from wild type PmScsC (grey) and the calculated scattering profile of the ensemble model overlayed in black (SASDB: SASDB94). The predicted scattering profile of each of the crystal structures is also shown (dashed lines: PDB: 4XVW compact, red; PDB: 5IDR transitional, blue; PDB: 5ID4 extended, green). The agreement between the experimental data and the ensemble model is excellent, yielding χ² = 1.0 (compared to χ² = 863.9 (compact); χ² = 1222 (transitional); χ² = 348.2 (extended)). The Guinier region (inset) of the scattering data is linear, consistent with a monodisperse solution. (b) Pair distance distribution function derived from the scattering data, showing the maximum dimension of the particles in solution is 105 Å. Also shown is the calculated p(r) for each of the crystal structures (dashed lines: compact; red; transitional; blue; extended; green), showing a maximum dimension of 90, 105 and 100 Å, respectively. The p(r) generated from the extended structure (green) is most similar to the experimentally derived p(r), while the other p(r) curves are markedly different. (c) MALLS profile of PmScsC and PmScsCΔN. PmScsC eluted faster (21.5 min) than PmScsCΔN (27.2 min): experimentally determined molecular masses are 74 ± 0.3 kDa for PmScsC (theoretical trimer mass 74.3 kDa) and 18.3 ± 0.3 kDa for PmScsCΔN (theoretical monomer mass 18.1 kDa).
Figure 3 | PmScsC crystal structures. (a) Compact (PDB: 4XVW), (b) Transitional (PDB: 5IDR) and (c) Extended (PDB: 5ID4) crystal structures of PmScsC. Upper panels: side view, secondary structure with catalytic domains in green, 11-residue peptide linker in cyan and trimerization domains in magenta or white. Height in this orientation is indicated. In each case, one trimerization domain is shown in magenta for comparison of the conformational changes across the three crystal structures. Lower panels: top view, surface representation (catalytic TRX fold domains green, trimerization domains white), maximum dimension in this orientation is labelled. Active site positions are indicated in orange for each protomer.

Table 1 | PmScsC crystal structure statistics.

|                        | Compact (4XVW) | Transitional (5IDR) | Extended (5ID4) |
|------------------------|----------------|---------------------|-----------------|
| **Data collection**    |                |                     |                 |
| Space group            | P 2_1          | I4                  | H3_2            |
| Cell dimensions (Å)    | 137.5, 163.9, 181.9 | 193.1, 193.1, 105.8 | 86.7, 86.7, 330.9 |
| a, b, c (Å)            | 90, 90, 90     | 90, 90, 90          | 90, 90, 120     |
| Resolution (Å)         | 91.15–2.60 (2.74–2.60) | 136.51–2.56 (2.57–2.56) | 110.29–2.92 (2.93–2.92) |
| Rmerge                 | 0.072 (0.617)  | 0.083 (0.741)       | 0.059 (0.625)   |
| Completeness (%)       | 98.6 (95.4)    | 99.4 (100.0)        | 99.2 (100.0)    |
| Redundancy             | 3.8 (3.7)      | 4.1 (4.1)           | 4.1 (4.2)       |
| **Refinement**         |                |                     |                 |
| Resolution (Å)         | 91.15–2.60     | 42.82–2.56          | 40.36–2.92      |
| No. reflections        | 243409         | 62069               | 10652           |
| Rwork/Rfree (%)        | 24.8/28.2      | 17.1/22.2           | 25.1/26.3       |
| No. atoms              | 40850          | 10262               | 1720            |
| Protein                | NA             | NA                  | NA              |
| Ligand/ion             | NA             | NA                  | NA              |
| Water                  | 281            | 82                  | 0               |
| B factors (Å²)         | 59.7           | 50.6                | 122.2           |
| Protein                | 41.5           | 43.0                | NA              |
| Ligand/ion             | NA             | NA                  | NA              |
| Water                  |                |                     |                 |
| RMS deviations         | 0.006          | 0.008               | 0.010           |
| Bond length (Å)        | 1.21           | 1.05                | 1.17            |

Single crystals were used to collect each data set. Values for the highest resolution shell are shown in parentheses.
As a consequence of the linker peptide flexibility, the three crystal structures describe extraordinarily different PmScsC quaternary shapes and dimensions. The eight compact PmScsC trimers are mushroom-shaped with dimensions $80 \times 80 \times 50 \text{ Å}^3$ (Fig. 3a). The two intermediate PmScsC crystal structures are asymmetric flat triangles with dimensions $105 \times 40 \times 70 \text{ Å}^3$ (Fig. 3b). The extended PmScsC crystal structure is three-leaf-clover shaped with dimensions $75 \times 80 \times 80 \text{ Å}^3$ (Fig. 3c).

We hypothesized that the dynamic motion suggested by the crystal structures, and supported by solution data, is critical to the mechanism of PmScsC. Indeed, replacement of the shape-shifting peptide with a rigid helical peptide (PmScsC RHP, 39-AEAAAKEAAA-KA-50)\textsuperscript{12} reduced the flexibility of the protein as evidenced by small angle scattering data (Supplementary Fig. 7), and abolished activity in the isomerase assay (Fig. 1c). Furthermore, we showed that trimerization of PmScsC is critical for activity because removal of the N-terminal residues (PmScsCAN) also abolishes in vitro disulfide isomerase activity (Fig. 1c) and in vivo complementation assays (swarming in the presence of copper) (Fig. 1b).

Curiously, Nature has also performed the ScsCAN experiment. PmScsC shares 58% sequence identity with *Salmonella enterica* serovar Typhimurium StScsC\textsuperscript{13} (Supplementary Fig. 8) and both proteins contribute to copper resistance. Whereas we showed above that PmScsC is required for swarming motility under copper stress, StScsC is required for growth of *S. Typhimurium* in rich media in the presence of copper. By contrast, deletion of *scsC* in *P. mirabilis* had no effect on growth in rich media whether copper was present or absent (Supplementary Fig. 2c). These findings imply that the two proteins have different target substrates and/or different molecular functions. Indeed, StScsC and PmScsC are very different proteins. StScsC lacks the N-terminal residues of PmScsC, it is monomeric rather than trimeric, and it has no isomerase activity. Intriguingly, the catalytic domains of the two proteins (as assessed by crystal structures) are very similar (PDB: 4GXZ) (RMSD 1.0–1.6 Å for PmScsC and StScsC, respectively) (Supplementary Fig. 1d).

These two proteins, trimeric disulfide isomerase PmScsC and monomeric dithiol oxidase StScsC, are encoded in similar loci and are both associated with copper resistance. Yet, they have very different architectures, and different molecular and cellular functions. To examine if the region encoding the N-terminal extension of PmScsC is broadly conserved in *P. mirabilis*, the *scsC* gene was PCR amplified from 25 randomly selected clinical isolates and sequenced. We found that the *pmcssc* sequence was conserved in all 25 isolates—including the region encoding the trimerization domain and shape-shifting motif—for example, a single synonymous mutation in the signal sequence. This high degree of conservation suggests a highly conserved functional role. *Caulobacter crescentus* SscS also has an extended N-terminal region and is predicted to be dimeric\textsuperscript{14}. Altogether with our work, this suggests that the N-terminal region of this protein is necessary for oligomerization and that there are at least two very different molecular and functional classes of SscS.

---

**Figure 4 | PmScsC protomer structure.** (a) Cartoon representation of the PmScsC catalytic domain (equivalent to a DsbA fold, green). Secondary structure elements and N- and C- termini are labelled. Position of the active site CXXC motif is shown as an orange oval shape. (b) PmScsC topology diagram showing the N-terminal helices z1/2/3 that precede the catalytic domain (magenta, flexible linker shown in cyan), and the DsbA domain (green) and CXXC active site (orange dots). Secondary structure elements and N- and C- termini are labelled. (c) The PmScsC protomers are structurally diverse: compact, intermediate, extended-like and extended. Colour scheme is as for **b**, N-terminal helices z1/2/3 and angles between the helices are labelled. N- and C- termini are also labelled.
How does the trimeric and highly dynamic molecular architecture of PmScsC compare with other structurally characterized disulfide isomerases? Until this point, highly efficient disulfide isomerases were thought to function through the presence of two TRX domains (each with a CXXC active site) that can simultaneously interact with two substrate cysteines\(^1\). The two active sites of V-shaped DsbC and U-shaped PDI are embedded within a dynamic framework that enable shuffling of substrate disulfides\(^1\)\(^-\)\(^4\)\(^-\)\(^9\) (Fig. 5a,b). Trimeric PmScsC also has the potential to interact with multiple substrate cysteines simultaneously. However, the evidence presented here shows a much more expansive range of motion than observed previously for DsbC or PDI, as depicted in morphing movies (Supplementary Movies 2–4) and static structural comparison (Fig. 5c). For example, the distance between active sites of DsbC (PDB: 1ZJ2-1E1)\(^3\)\(^,\)\(^6\) or PDI (PDB: 4EKZ-4EL1)\(^1\)\(^8\) in reported crystal structures ranges between 31–39 Å (26% increase) and 28–40 Å (43%), respectively. By comparison, this distance ranges from 16 to 52 Å (225%) in PmScsC.

**Discussion**

Why is it that other characterized disulfide isomerases do not have the highly flexible, trimeric structure of PmScsC? How does the monomeric S. Typhimurium ScsC—not a disulfide isomerase—confer copper resistance? How do the very diverse Scs proteins (monomeric, dimeric or trimeric) form redox relay systems with a predicted partner protein ScsC\(^1\)\(^4\) that appears to be highly conserved in bacterial Scs gene clusters? The likely explanation for the diversity in architecture and flexibility of Scs proteins may relate to differences in their target specificity.

The structural diversity among ScsC homologues may simply reflect the fact that each ScsC homologue has evolved to optimize disulfide reduction and/or isomerization of a distinct protein substrate or set of substrates that are sensitive to copper-induced damage. Identifying and characterizing these specific substrates, and understanding why these are not refolded or reduced by other periplasmic TRX-fold proteins is key questions that will inform our understanding of bacterial copper resistance mechanisms.

In summary, PmScsC is a unique and highly dynamic disulfide isomerase. The N-terminal residues bring three catalytic domains together into a trimer, and a unique flexible linker enables extraordinary twisting and extending motions that impact on the catalytic domain placement. Crystal structure snapshots supported by X-ray scattering data from solution, indicate an almost doubling in length of the protein in one dimension, in concert with considerable catalytic domain rotation. The combination, extent and diversity of these motions would enable misfolded substrates bound to PmScsC to explore a broad folding landscape, consistent with the redox-foldase activity of this key P. mirabilis copper resistance protein. These findings show in much greater detail than ever before how redox proteins with multiple active sites are able to shuffle incorrectly folded substrates. It is unclear whether PmScsC directly interacts with copper or how it interacts with other proteins required for virulence under copper stress. Nevertheless, the structural data may provide a suitable basis for drug discovery targeting a central defence mechanism of an important human pathogen\(^2\)\(^,\)\(^2\)\(^1\). Importantly, the shape-shifting motif may also represent a modular component for dynamic motion that could be used in ‘plug and play’ protein engineering applications.

**Methods**

**Bacterial strains and growth conditions.** BLAST searches of DsbA homologues were performed on the genome of Proteus mirabilis HI4320 (ref. 22). Clinical isolates of P. mirabilis were cultured from the urine or blood cultured with urinary tract infection or bacteremia. The isolates were sourced from the Princess Alexandra Hospital or Sullivan Nicolaides Pathology (Brisbane, Australia). E. coli Top10 (Invitrogen) was used for plasmid manipulations. E. coli and P. mirabilis were cultured in Luria-Bertani (LB) broth (10 g l\(^{-1}\) tryptone, 5 g l\(^{-1}\) yeast extract, 10 g l\(^{-1}\) NaCl) on LB agar (LB medium containing 15 g l\(^{-1}\) agar). To prevent swarming of P. mirabilis, LB medium containing 30 g l\(^{-1}\) agar and without NaCl was used. Media were supplemented with chloramphenicol (30 mg l\(^{-1}\)), ampicillin (100 µg ml\(^{-1}\)) or kanamycin (25 µg ml\(^{-1}\)) as required.

**Construction of Proteus mirabilis scsC mutants.** Mutation of the scsC gene in P. mirabilis PM38 and PM54 was performed using the TargetRon gene knockout system (Sigma-Aldrich) as per the manufacturer’s instructions. Briefly, optimal intron insertion sites and primer sequences (5827, 5′-aaaaagctttatcctttgctttgt, 5828, 5′-capggattggatatcagttgtgtagatagtgg, 5829, 5′-tagacgtattagcatagagttgtgtagatagtgg, 5830, 5′-ttgtagattaggatatcagttgtgtagatagtgg, and 5831, 5′-ttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt...
introducing plasmids pScsC, pScsC AXCC, pScsCΔAN or pSU2718 (vector control) into PM54scsC.

Swarming motility in the presence of CuSO4

Overnight cultures of P. mirabilis were diluted in fresh LB broth to an OD600 of 0.1. Swarming on LB agar was measured after 14 h incubation, and every one or two hours thereafter for a total of 10h. Swarming of PM54scsC containing pSU2718 (vector control) or complementation plasmids (pScsC, pScsC AXCC and pScsCΔAN) was performed as described above, but with the addition of chlormephonol (30 μg·mL⁻¹) and 1 mM isopropyl-β-D-thiogalactopyranoside for ScsC induc- tion. Swarming motility at 37°C on LB agar was measured after 6 and 8 h of incubation. Three replicate experiments were performed for all assays. Swarming motility was expressed as the diameter of a swarming zone in the presence of CuSO₄

Western blotting

ScsC expression in P. mirabilis wild-type, mutant and complemented strains was examined by western blot analysis. Crude cell lysates were prepared from standard overnight cultures. Samples were resolved by SDS-polyacrylamide gel electrophoresis and transferred onto a poly-vinylidene difluoride membrane. Blots were probed using a PmScsC specific polyclonal antiserum raised in rabbits (WEHI Antibody Facility) at 1:1 000 dilution in phosphate buffer saline with 0.05% v/v Tween-20.

Protein production

Codon-optimized PmScsC (see Supplementary Note 1 for the sequence) lacking the first 21 amino acids (which correspond to the predicted linker peptide in PmScsC (residues 39-KADEQQAQFRQ-49) was inserted into pMCSG7 (Midwest Center for Structural Genomics) by ligation-independent cloning. PmScsC and PmScsCΔAN were expressed in BL21(DE3)pLysS cells (Novagen) at 30°C for 16-20 h using ZYP-3052 autoinduction medium25.

Selenomethionine-labelled PmScsC (mature PmScsC contains four native methionine sites) was expressed in BL21(DE3)pLysS cells grown in minimal media (M63) supplemented with 0.05 mg·mL⁻¹ D/L-selenomethionine. Recombinant protein expression was induced by 0.1 mM isopropyl-β-D-thiogalactopyranoside overnight at 30°C.

After expression, all cells were centrifuged by centrifugation in lysis buffer (25 mM Tris pH 7.4, 150 mM NaCl) with protease inhibitor (1:1 000 dilution into lysis, BioPioneer, Inc., USA) and DNase I (3.3 μg·mL⁻¹). The lysate was clarified by centrifugation (40,000 × g, 30 min, 4°C) and the supernatant was run on a non-reducing SDS–PAGE and subjected to Coomassie staining. Aliquots were removed at various time points and mixed with (NH₄)HCO₃ (50 mM final), to stop the reaction, through hydrolysis of remaining functional ester groups in DTSSP. The samples were then run on a non-reducing SDS–PAGE and subjected to Coomassie staining. As control, 50 mM DTT was added to selected samples, which led to cleavage of the internal disulfide bond in DTSSP, and consequently disruption of cross-links between ScsC protomers.

MALLS

A combined approach, using analytical size exclusion chromatography (SEC) and multiangle laser light scattering (MALLS) was utilized to determine and compare the stoichiometry of PmScsC with PmScsCΔAN in solution. The setting consisted of an L20 high-performance liquid chromatography (HPLC) system (Shimadzu, Rydalmere, Australia) and a DAWN HELEOS II laser light detector (Wyatt Technology, USA). A Superdex 200 10/300 GL analytical column (GE Healthcare, USA) was connected to the L20 HPLC system and equilibrated with 25 mM Tris and 150 mM NaCl, pH 7.5 overnight. Purified proteins were injected (500 μL of 3 mg·mL⁻¹ at a flow rate of 1.0 mL·min⁻¹) into the SEC analysis system for analysis. The detector system for the two columns was a refractive index detector (Shimadzu, Rydalmere, Australia) and a UV detector (Mitsubishi Electric, Tokyo, Japan) measuring absorbance at 280 nm.

SAXS

Small angle X-ray scattering data were collected on the SAXS-WAXS beamline at the Australian Synchrotron30. Immediately before loading, all samples were centrifuged at 10 000 × g to remove large particles from the solution, and radiation damage was minimized by allowing samples to stream in a liquid nitrogen cryostream in 15 mm quartz capillaries (Hampton Research). Data reduction was carried out using the Australian Synchrotron Scatterbrain software37 correcting for sample transmission and solvent scattering.

Scattering data were collected on reduced PmScsC wildtype and RHP mutant prepared in 25 mM HEPES buffer pH 7.5 50 mM NaCl, 1 mM DTT. Data were also collected on oxidized wildtype PmScsC, but as no significant differences in the scattering were observed, only data from the reduced forms are presented. To confirm that the pH and ion strength of the crystallization conditions do not induce conformational changes in PmScsC, scattering data were collected from wildtype PmScsC prepared at 0.50 mg·mL⁻¹ in a gradient of 25 mM HEPES pH 6.0, 6.5, 7.0, 7.5 and 8.0 and NaCl (150, 300, 600, 900 and 1 500 mM). No significant systematic change in the structural parameters was observed at any point of the gradient, hence, it was concluded that neither ion strength nor pH influence the conformation of the complex in the ranges measured.

The quality was assessed by the log of the integrated intensity values in the RI domain of the data (qRI<1.3), measured molecular mass of the protein complex, and concentration dependence of the scattering. The estimated molecular mass was determined as outlined in29, where the contrast and partial specific volume were estimated from the protein sequence28. The pair-distance distribution function (p(r) was generated from the experimental data using GNOM26 from which Rₐ and Dmax were determined. Rigid body modelling of the scattering data from PmScsC wildtype and mutant proteins was performed using CORAL31. C₂ symmetry was assumed, and the starting model was oriented such the 3-fold axis was parallel with the z-axis, and passed through the centre of the oligomerization domain. Two rigid bodies were then defined for each monomer: residues 1-44 (oligomerization domain); 47-224 (catalytic domain). The position of the oligomerization domain was fixed, and the position and orientation of the catalytic domain was then optimized against the measured scattering data. The program was run 16 times for each protein, but the models with the lowest penalty function still showed significant errors (residuals all ≤7 5%). This was resolved by using an improved beamline and an increased number of data points. Given the crystal structures show...
significant structural diversity, it is likely that these systematic deviations arise from the fact that there is an ensemble of structures present in solution. Hence, ensemble optimization was also performed with the program EOM33. From an initial pool of 1,000 structures (the oligomerization domain possessed C₅ symmetry in all structures, but the entire trimer was permitted to adopt either Cₛ or Cᵥ symmetry), a final pool of random structures was the same for both WT and RHP optimizations, and the number of structures in the final ensemble was not artificially limited. The reduction in size of the ensemble (from 6 for WT to 4 for RHP) is consistent with the RHP mutation rigifying the flexible helix, and reducing the conformational space sampled by the protein. Details of the data collection and structural parameters are summarized in Supplementary Table 1.

Crystalization and structure determination. Crystalization screenings were performed at the UQ ROX crystal facility at the University of Queensland (uqroxcim.b.uq.edu.au) using commercial screens and the hanging-drop vapour diffusion method.

Compact structure. Selenium-methionine-labelled PmScsC crystals were grown at 20 °C from a drop comprising 200 nl of 30 mg ml⁻¹ purified protein in 10 mM HEPES pH 7.4 and 200 nl of well solution, 2.85 M sodium malonate pH 5.8 containing 0.1 M chloride hexahydrate. MUSTANG (PDB: 4GXZ) in MOLREP37. Manual selection of solutions based on observed packing, allowed placing of six molecules in the asymmetric unit. This solution was improved in the subsequent MR runs in MOLREP37, allowing the addition of eight molecules in the second trial and a total of nine molecules in the third run. A SAD-MR approach in PHENIX30, by combining SAD phasing and the partial MR solution (nine molecules), and combining SAD phasing and the partial MR solution (nine molecules) was used. The advantage of this approach is that it can be solved even if the protein is not perfectly crystalline, which is consistent with the presence of two trimers. Manual building of the trimerization domain was performed in Coot and rounds of autobuild using Buccaneer46 in CCP4 and refinement using REFMAC42 and BUSTER54 were performed to improve the structure. Several rounds of manual adjustment in Coot40 and refinement in PHENIX38, including the use of TLS, were performed to yield final R/Refree values of 25.1 and 26.3%. Validation in MolProbity48 was used throughout the refinement process and the final Ramachandran favoured/outlier statistics for the structure are 98.9%/0.5%. A stereo image of representative electron density for this structure is shown in Supplementary Fig. 9c.

a-Helical extended structure. Crystals were grown at 20 °C from drops containing 1 µl of 20 mg ml⁻¹ oxidized PmScsC in 10 mM HEPES pH 7.4, 150 mM NaCl and 2.5 mM copper (II) chloride (added to protein solution immediately before crystallization) and 1 µl of 32% jeffamine M-600 pH 7 in 0.1 M HEPES pH 8. Crystal formation occurred within minutes of set up and crystals continued to grow overnight. Crystals were cryoprotected with 32% jeffamine M-600 pH 7, 0.1 M HEPES pH 8 and 20% ethylene glycol and frozen in liquid nitrogen. Data were collected using the BluIce software34 on the MX2 beamline of the Australian Synchrotron at a wavelength of 0.9537 Å under cryogenic conditions (100 K). Using the autoPROC framework43 the data were processed with XDS35 and scaled with AIMLESS36. The Wilson B factor for the data was calculated (high (3.7) for most of the data and lower for the other two crystal structures). The space group was determined to be H₂J₃ and molecular replacement using PHASER57 and refinement with BUSTER54 to improve the structure. Several rounds of manual adjustment in Coot40 and refinement in PHENIX38, including the use of TLS, were performed to yield final R/Refree values of 25.1 and 26.3%. Validation in MolProbity48 was used throughout the refinement process and the final Ramachandran favoured/outlier statistics for the structure are 98.9%/0.5%. A stereo image of representative electron density for this structure is shown in Supplementary Fig. 9c.

Residues in all three crystal structures were numbered based on their position after removal of the cleavage site from the construct. MUSTANG (PDB: 4GXZ) in MOLREP37 for structure alignment and RMSD calculations and PyMOL was used to create images of the structures and perform other measurements. The distance range between active sites in DsbC, PDI and PmScsC structures was measured using the CB positions of the more N-terminal of the two catalytic cysteines. Supplementary Movie 1 was created in PyMOL by generating morphs between each of the PmScsC crystal structures, as well as morphs between each of the available structures for E. coli DsbC (PDB: 1JZD-1EE1) and human PDI (PDB: 4EKZ-4EL1).

Measurement of PmScsC redox potential and pKₐ values. The redox potential of PmScsC was determined by incubating 2 µM PmScsC with 1 mM GSSG in combination with varying concentrations of GSH (4–15 mM) for ~12 to 24 h at 20 °C in 100 mM sodium phosphate buffer, pH 7.0 and 1 mM EDTA. Trichloroacetic acid, 10% v/v, was used to precipitate the protein samples and the pellets were washed with ice-cold acetone. The samples were then treated with 2 mM 4-acetamido-4'-maleimidylstilbene-2,2'-disulfonate (AMS-22) which blocks free thiols and increases the weight of the reduced protein by ~1 kDa. The reduced and oxidized forms of PmScsC were separated on a 12% non-reducing SDS–PAGE (NuPAGE) and stained with Coomassie Brilliant Blue. The fraction of reduced protein, R, was determined via densitometric analysis of the scanned SDS–PAGE gel using ImageJ, and the equilibrium constant Kₑq was calculated via R = [GSH]²/[GSSG]/([Kₑq] + ([GSH]²/([GSSG])²)³)². The Nernst equation, E = F / (R GSH/GSSG) × log Kₑq where F / (R GSH/GSSG) is the standard potential of GSH/GSSG and Kₑq was used to calculate the redox potential of PmScsC.

The pH-dependent absorbance of the catalytic thiolate anion of PmScsC was determined at 240 nm (ref 54) using a CARY 50 UV/VIS spectrophotometer (Agilent Technologies, USA). The pH titration measurements of oxidized or reduced PmScsC (40 µM) were conducted at 22 °C in 2 ml reaction buffer (10 mM Tris, 10 mM sodium citrate, 10 mM K₂HPO₄, 10 mM KH₂PO₄, 200 mM KCl and 1 mM EDTA). Absorbance (A = 240 and 280 nm) was measured between pH 6.5 and 1.5 in 0.25 increments. The pKₐ value was calculated from the fitted curves of three replicates using the Henderson–HasSELbach equation (pH = pKₐ + log ([A²]/[A²]) of [A²]/[A²] at pH).

Relative stability of oxidized and reduced PmScsCAN. Unfolding of PmScsCAN was monitored using the change in far-ultraviolet circular dichroism (CD) signal32. The largest difference in molar ellipticity for oxidized and reduced enzymes was calculated from initial far-ultraviolet CD spectra (from 250 to 190 nm) recorded at 25 and 95 °C using a Jasco J-810 circular dichroism (CD) spectropolarimeter (Jasco, USA), respectively. The unfolding of oxidized and reduced PmScsCAN (225.5 µM, PmScsCANred = 219.5 µM) was monitored at a constant heat rate of 1 °C min⁻¹ starting from 25 °C and increasing to 95 °C in 6 min at the wavelengths of 219.5 and 219.5 nm in 100 mM NaH₂PO₄/NaHPO₄, 1 mM EDTA at pH 7.0. Reduced enzyme samples contained 0.75 mM DTT. The CD data were converted to a fraction of folded protein via θobs (θ folded) = (θ (θ unfolded) (θ folded - θ unfolding))/θ unfolding and θobs (θ unfolded) was then fitted using a Boltzmann sigmoid function f = 1/(1 + e⁻ⁿθ₀) where Tm is the temperature at which the net absorbance is 50% of the final maximum absorbance. The CD unfolding experiment was repeated three times for both the oxidized and reduced protein.
Protein disulfide isomerase assay. The bovine pancreatic ribonuclease RNase A contains four disulfide bonds that are necessary for its enzymatic function. Starting with scrambled RNase A (in which the cysteines have formed non-native disulfide bonds), one can determine the ability of other enzymes to restore its function by spectrophotometrically monitoring the hydrolysis of 3',5'-cyclic monophosphate (cCMP), a synthetic substrate of the ribonuclease. Thus, in vitro disulfide isomerase activity of PmScsC was monitored by using scrambled RNase A as substrate. Inactive scrambled RNase A was produced by treating 70 mg of native RNase A (Sigma-Aldrich) with 6 M guanidinium chloride and 150 mM DTT in 50 mM Tris pH 8, incubated at room temperature for 16 h. After adjusting the solution to pH 8, the unfolded protein was then desalted and buffer exchanged into 100 mM acetic acid/NaOH pH 4 and incubated for 11.5 mg mL⁻¹ for use in assays. The absence of reduced cysteines was confirmed, as described above. The total reaction volume for the scrambled RNase A assay was 750 μL containing native RNase A and scrambled RNase A in the absence of any other enzyme served as positive controls. As negative control, EcDsbA served as positive control. As negative control, 150 μM DTT and 10 μM PpmScsC. PmScsC, PmScsC RHP, EcDsbC or EcDsbA and 40 μM of scrambled RNase A. At various time intervals, 50 μL sample was removed and mixed with 150 μL of 3 mM cysteine 3',5'-cyclic monophosphate (cCMP) to measure RNase A activity. Hydrolysis of the cyclic phosphate ester bond in cCMP was monitored at 290 nm using a Synergy H1 multimode plate reader (BioTek, USA). Samples containing native RNase A and scrambled RNase A in the absence of any other enzyme served as positive and negative controls, respectively. The disulfide isomerase assays were repeated twice for each protein.

PmScsC dithiol oxidation activity. The potential of PmScsC/PmScsCCan to catalyse disulfide bond formation in vitro was assessed using a synthetic model peptide (QQQGPGDGTQNSCK), as described before.37 Dithiol oxidation was determined fluorometrically, using a Synergy H1 multimode plate reader (BioTek Instrument Inc., USA) with the excitation wavelength set to 340 nm and emission to 615 nm. A 150 μs delay before reading and 100 μs reading time were used for time-resolved fluorescence. The reaction buffer contained 50 mM MES, 50 mM NaCl and 2 mM EDTA at pH 5.5. The reaction volume was 50 μL in each well of a white 384-well plate (Perkin Elmer OptiPlate-384, Part #: 6007296), including increasing concentrations (0–320 μM) of EcDsbA, EcDsbC, PmScsC, or PmScsC RHP and 2 mM glutathione (GSSG) as oxidant. At last, 8 μM peptide substrate was added to initiate the reaction. EcDsbA served as positive control. As negative control, reactions in the absence of proteins were used. The initial linear portion of the raw data was used to calculate the rate of oxidation as fluorescence increase per minute. The dithiol oxidation activity experiments were repeated three times for each protein.

Data availability. The UniProt accession code for the P. mirabilis ScsC protein described in this paper is CL2P2E and the accession codes for other proteins described in this study are H9LA41 (StScsC), P0AEG4 (EcDsbA) and P0AEG6 (EcDsbC). Coordinates and structure factors for the three ScsC crystal structures were deposited in the protein data bank (PDB) with accession codes 4XVW (complex) and 5DIF (extended). In addition to the PDB accession codes listed above, PDB accession code 4GZK (StScsC) was used as a molecular replacement template and 1E1F (EcDsbC), and 4KEK and 4ELI (hPDI) were used to generate Fig. 5. Scattering data and models have been accession codes listed above, PDB accession code 4GXZ (StScsC) was used as a (compact), 5IDR (transitional) and 5ID4 (extended). In addition to the PDB structures of the DsbG disulfide isomerase reveal an unstable disulfide. 18549–5601 (2016). 9. Hodgkinson, V. & Petris, M. J. Copper homeostasis at the host-pathogen interface. J. Biol. Chem. 287, 13549–13555 (2012). 10. Sun, X. & Wang, C. C. The N-terminal sequence (residues 1-65) is essential for dimerization, activities, and peptide binding of Escherichia coli DsbC. J. Biol. Chem. 275, 22743–22749 (2000). 11. Zhao, Z., Peng, Y., Hao, S. F., Zeng, Z. H. & Wang, C. C. Dimerization by domain hybridization bestows Escherichia coli and isomerase activities. J. Biol. Chem. 278, 43292–43298 (2003). 12. Chen, X., Zaro, J. L. & Shen, W. C. Fusion protein linkers: property, design and functionality. Adv. Drug Deliv. Rev. 65, 1357–1369 (2013). 13. Shepherd, M. et al. Structural and functional characterization of ScsC, a periplasmic thioredoxin-like protein from Salmonella enterica serovar Typhimurium. Antioxid. Redox Signal. 19, 1494–1506 (2013). 14. Cho, S. H. et al. A new family of membrane electron transporters and its substrates, including a new cell envelope peroxiredoxin, reveal a broadened reductive capacity of the oxidative bacterial cell envelope. mBio. 3, 1–11 (2012). 15. Pan, J. L. & Bardwell, J. C. The origami of thioredoxin-like folds. Protein Sci. 15, 2217–2227 (2006). 16. Haebel, P. W., Goldstone, D., Katzen, F., Beckwith, J. & Metcalf, P. C. The disulfide bond isomerase DsbA is activated by an immunoglobulin-fold thiol oxidoreductase: crystal structure of the DsbC-DsbDalpha complex. EMBO J. 21, 4774–4784 (2002). 17. Tian, G. et al. The catalytic activity of protein-disulfide isomerase requires a conformationally flexible molecule. J. Biol. Chem. 283, 33630–33640 (2008). 18. Wang, C. C. Structural insights into the redox-regulated dynamic conformations of human protein disulfide isomerase. Antioxid. Redox Signal. 19, 36–45 (2013). 19. Tian, G., Xiang, S., Noiva, R., Lennarz, W. J. & Schindelin, H. The crystal structure of yeast protein disulfide isomerase suggests cooperativity between its active sites. Cell 124, 61–73 (2006). 20. Adams, L. A. et al. Application of fragment-based screening to the design of inhibitors of Escherichia coli DsbA. Angew. Chem. Int. Ed. Engl. 54, 2179–2184 (2015). 21. Duprez, W. et al. Peptide inhibitors of the Escherichia coli DsbA oxidative machinery essential for bacterial virulence. J. Med. Chem. 58, 577–587 (2015). 22. Pearlman, M. M. et al. Complete genome sequence of urapathogenic Proteus mirabilis, a master of both adherence and motility. J. Bacteriol. 190, 4027–4037 (2008). 23. Studier, F. W. Protein production by auto-induction in high-density shaking cultures. Protein Expr. Purif. 41, 207–234 (2005). 24. Gasteiger, E. et al. in The Proteinomics Protocols Handbook (ed. Walker, J. M.) 571–607 (Humana Press, 2005). 25. Heckman, K. L. & Pease, L. R. Gene splicing and mutagenesis by PCR-driven overlap extension. Nat. Protoc. 2, 924–932 (2007). 26. Kirby, N. M. et al. A low-background-intensity focusing small-angle X-ray scattering undulator beamline. J. Appl. Crystallogr. 46, 1670–1680 (2013). 27. Scatterbrain—Software for acquiring, processing and viewing SAXS/WAXS data at the Australian Synchrotron. (Australian Synchrotron, Clayton, VIC). 28. Orthaber, D., Bergmann, A. & Glatter, O. SAXS experiments on absolute scale with Kratky systems using water as a secondary standard. J. Appl. Crystallogr. 33, 218–225 (2000). 29. Whitten, A. E., Cat, S. & Trethewlla, J. MULCh: modules for the analysis of small-angle neutron contrast variation data from biomolecular assemblies. J. Appl. Crystallogr. 41, 222–226 (2008). 30. Svergun, D. I. Determination of the regularization parameter in indirect-transform methods using perceptual criteria. J. Appl. Crystallogr. 25, 495–503 (1992). 31. Parakhov, M. V. et al. New developments in the program package for small-angle scattering data analysis. J. Appl. Crystallogr. 45, 342–350 (2012). 32. Franke, D., Jeffries, C. M. & Svergun, D. I. Correlation Map, a goodness-of-fit test for one-dimensional X-ray scattering spectra. Nat. Methods 12, 419–422 (2015). 33. Tria, G., Mertens, H. D., Kakaha, M. & Svergun, D. I. Advanced ensemble modeling of flexible macromolecules using X-ray solution scattering. IUCrJ 2, 207–217 (2015). 34. McPhillips, T. M. et al. Blu-Ice and the distributed control system: software for data acquisition and instrument control at macromolecular crystallography beamlines. J. Synchrotron Radiat. 9, 401–406 (2002). 35. Kabsch, W. XDS. Acta Crystallogr. D Biol. Crystallogr. 66, 125–132 (2010). 36. Evans, P. R. & Murshudov, G. N. How good are my data and what is the resolution? Acta Crystallogr. D Biol. Crystallogr. 69, 1204–1214 (2013). 37. Vagin, A. & Tepliyakov, A. An approach to multi-copy search in molecular replacement. Acta Crystallogr. D Biol. Crystallogr. 56, 1622–1624 (2000).
38. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D Biol. Crystallogr. 66, 213–221 (2010).

39. McCoy, A. J. et al. Phaser crystallographic software. J. Appl. Crystallogr. 40, 658–674 (2007).

40. Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60, 2126–2132 (2004).

41. Dauter, Z. Estimation of anomalous signal in diffraction data. Acta Crystallogr. D Biol. Crystallogr. 60, 2184–2195 (2004).

42. Vagin, A. A. et al. REFMAC5 dictionary: organization of prior chemical knowledge and guidelines for its use. Acta Crystallogr. D Biol. Crystallogr. 60, 687–876 (2004).

43. Winn, M. D. et al. Overview of the CCP4 suite and current developments. Acta Crystallogr. D Biol. Crystallogr. 67, 235–242 (2011).

44. Cowtan, K. The Buccaneer software for automated model building. 1. Tracing protein chains. Acta Crystallogr. D Biol. Crystallogr. 62, 1002–1011 (2006).

45. Smart, O. S. et al. Exploiting structure similarity in refinement: automated NCS and target-structure restraints in BUSTER. Acta Crystallogr. D Biol. Crystallogr. 68, 368–380 (2012).

46. Bricogne, G. et al. BUSTER version 2.10.1 (Global Phasing Ltd., 2011).

47. Chen, V. B. et al. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr. D Biol. Crystallogr. 66, 12–21 (2010).

48. Konagurthu, A. S., Whistock, J. C., Stuckey, P. J. & Lesk, A. M. MUSTANG: a multiple structural alignment algorithm. Proteins 64, 559–574 (2006).

49. Konagurthu, A. S. et al. MUSTANG-MR structural sieving server: applications in protein structural analysis and crystallography. PLoS ONE 5, e10048 (2010).

50. Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675 (2012).

51. Schüler, C. A., Robert, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675 (2012).

52. Wunderlich, M. & Glockshuber, R. In vivo control of redox potential during protein folding catalyzed by bacterial protein disulfide-isomerase (DsbA). J. Biol. Chem. 268, 24547–24550 (1993).

53. Gilbert, H. F. Thioldisulfide exchange equilibria and disulfidebond stability. Methods Enzymol. 251, 8–28 (1995).

54. Nelson, J. W. & Creighton, T. E. Reactivity and ionization of the active site cysteine residues of DsbA, a protein required for disulfide bond formation in vivo. Biochemistry 33, 5974–5983 (1994).

55. Heras, B. et al. Staphylococcus aureus DsbA does not have a destabilizing disulfide. A new paradigm for bacterial oxidative folding. J. Biol. Chem. 283, 4261–4271 (2008).

56. Hillson, D. A., Lambert, N. & Freedman, R. B. Formation and isomerisation of disulfide bonds in proteins: protein disulfide-isomerase. Methods Enzymol. 107, 281–294 (1984).

57. Vivian, J. P. et al. Structure and function of the oxidoreductase DsbA1 from Neisseria meningitidis. J. Mol. Biol. 394, 931–943 (2009).

58. Valentin, E., Kühn, A. G., Pavlakis, G. J., Jeffries, C. M. & Svergun, D. I. SASSDb, a repository for biological small-angle scattering data. Nucleic Acids Res. 43, D357–D363 (2015).

Acknowledgements
We acknowledge use of the UQ ROCX Diffraction Facility and of the MX1, MX2 and SAXS/WAXS beamlines at the Australian Synchrotron. We thank the support teams at both facilities for advice and expert assistance and we are grateful to G. King for chemical cross-linking support. This work was supported by the Australian Research Council through a Laureate Fellowship (FL0992138, J.L.M.), Future Fellowships (FT100100662 MAS; FT130100580, B.H.) and DECRA Fellowship (DE130101169, M.T.); and the National Health and Medical Research Council (Senior Research Fellowships to J.L.M., GNT455829 and to M.A.S., GNT1106930; project grant to M.A.S., GNT1106590).

Author contributions
E.J.F. and F.K. performed molecular cloning of ScsC constructs for protein purification, produced the ScsC protein for biochemical and structural studies. E.J.F., F.K., L.P. and H.G.C. collected diffraction data and solved the structures, and together with J.L.M. refined the structures and analysed the data. E.J.F., F.K., B.H. and M.A.H. performed the in vitro assays for the wildtype protein. E.J.F. designed and generated the rigid helical linker mutant and performed the in vitro assays for this protein. A.W.L. constructed the P. mirabilis mutants, generated the complementation plasmids, performed the motility assays and examined ScsC expression by western blotting. M.E.S.A. tested the growth of wild-type and mutant strains in the presence and absence of copper. F.K. and M.T. evaluated the clinical P. mirabilis strains. A.E.W. designed the SAXS experiments and collected, analysed and modelled the SAXS data. J.L.M. and M.A.S. designed and directed the project. H.G.C., L.P., A.E.W., M.A.S. and J.L.M. jointly supervised the research. All authors contributed to writing the paper.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Furlong, E. J. et al. A shape-shifting redox foldase contributes to Proteus mirabilis copper resistance. Nat. Commun. 8, 16065 doi: 10.1038/ncomms16065 (2017).

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.