The Name's Bond…….Disulfide Bond

Begoña Heras[#], Mareike Kurz[#], Stephen R. Shouldice[#] and Jennifer L. Martin[#]*

Institute for Molecular Bioscience, University of Queensland,

Brisbane, Queensland 4072 Australia

[#]All authors contributed equally

*Corresponding author: Institute for Molecular Bioscience,

University of Queensland, Brisbane, Queensland 4072 Australia

Phone: +61 7 3346 2016

Fax: +61 7 3346 2101

Email: j.martin@imb.uq.edu.au

Email addresses of all authors: b.heras@imb.uq.edu.au, m.kurz@imb.uq.edu.au, s.shouldice@imb.uq.edu.au, j.martin@imb.uq.edu.au

SUMMARY

A repeating theme in the structural biology of disulfide oxidants and isomerases is the extraordinary architectural similarity between functionally related proteins from prokaryotes and eukaryotes. The recently determined structure of full-length yeast protein disulfide isomerase reveals a U-shaped molecule with two redox active sites. It bears a remarkable resemblance to the V-shaped, but dimeric, bacterial disulfide isomerases DsbC and DsbG. Similarly, the much-anticipated structure of the bacterial membrane protein DsbB, the redox partner of DsbA, comprises a flexible redox loop embedded in an anti-parallel four-helix bundle. This architecture is similar to that of soluble eukaryotic Ero1p and Erv2p proteins, the redox partners of protein disulfide isomerase. Importantly, the DsbB crystal structure is a complex with DsbA, providing our first view of the molecular interactions between these two proteins.

INTRODUCTION

A vital step in the protein folding process is the formation of disulfide bonds between cysteine side chains (Figure 1). Organisms from bacteria to humans encode elaborate enzymatic pathways to catalyze this step [1,2]. Major advances have been made in our understanding of oxidative protein folding recently, through the structure determination of some of the most challenging systems in the field. These include structures of key disulfide-catalyzing proteins from bacteria, archaea and eukaryotes.

BACTERIA

In Gram-negative cells, disulfide bond formation occurs in the periplasm. Two disulfide pathways co-exist in *Escherichia coli*, the DsbA-DsbB oxidative pathway and the DsbC-DsbD isomerization pathway (Figure 1). Disulfide bonds are introduced into proteins rapidly, but non-specifically, by the soluble and highly-oxidizing protein

DsbA. In turn, DsbA is re-oxidized by the inner membrane protein DsbB. The isomerase pathway plays a proof-reading role; DsbC (or DsbG) are thought to be important for rearranging incorrectly paired cysteines introduced by DsbA, and is in turn maintained in a reduced form by the inner membrane protein DsbD (Figure 1). These two redox pathways transport electrons in opposite directions in the periplasm, and large kinetic barriers prevent cross-talk [3,4].

Oxidative Pathway

DsbA from *Escherichia coli* was the first disulfide-catalyzing protein to be structurally characterized [5], revealing a thioredoxin fold with an inserted helical domain (Figure 2). Like other redox-active members of the thioredoxin-fold family [6], *E. coli* DsbA has a redox active site CXXC motif that interconverts from disulfide to dithiol, and an adjacent *cis*-Pro motif involved in substrate interactions [7]. *E. coli* DsbA is characterized by a surface groove near the redox active site that is suggestive of a substrate-binding site. Recent NMR analysis indicates that the reduced form of DsbA is more flexible than oxidized (active) DsbA, suggesting that dynamics may play a role in the catalytic cycle [8]. This inference is supported by the crystal structure of the C33A mutant of DsbA which shows structural changes at both the redox active site and at the groove adjacent to the active site [9]. Many substrates of DsbA have been identified [10], but to date no structural information is available for a DsbA:substrate complex. Consequently, the molecular mechanism by which DsbA recognises and oxidizes a wide variety of proteins remains an active area of structural research.

After donating its disulfide to a substrate protein, DsbA is re-oxidized to carry out further disulfide catalysis. This re-oxidation step is catalyzed by the membrane-bound

protein DsbB, using quinones as the electron acceptors that deliver the electrons ultimately to molecular oxygen. The crystal structures of Erv2p and Ero1p, soluble eukaryotic proteins that are functionally analogous to DsbB, have been determined previously [11,12] (Figure 3, see also below). Sequence analysis led to the proposal that DsbB shares a core structure with these eukaryotic analogs, comprising four anti-parallel α-helices, a CXXC motif, aromatic amino acids for cofactor binding, and a second pair of cysteines [13]. For *E. coli* DsbB, this second cysteine pair was predicted to be in a mobile periplasmic loop, and to act as a disulfide shuttle between the DsbB active site and that of DsbA [13].

A major advance in the field was the recent crystal structure determination of the *E. coli* DsbB-DsbA complex [14]. This structure showed, as predicted, that DsbB comprises an antiparallel four-helix bundle (Figure 2). Furthermore, the structure revealed that the periplasmic loop of DsbB does indeed interact with DsbA (Figure 3). The redox-active DsbB Cys104 residue on the periplasmic loop forms an intermolecular disulfide with DsbA Cys30, necessitating movement of DsbB Cys104 away from its DsbB redox partner Cys130 [14].

Isomerase Pathway

E. coli encodes two disulfide isomerases, DsbC and DsbG, that share just 24% sequence identity. Both are homodimers with each monomer comprising a catalytic thioredoxin domain connected by a linker α-helix to a dimerization domain [15,16]. Together, the two subunits form a V-shape (Figure 2) with an extensive hydrophobic surface on the inner surface of the V, that is a likely binding site of substrate proteins. The structures of oxidized [17] and reduced [18] *E. coli* DsbC, *H. influenzae* DsbC

[19] and of the complex between *E. coli* DsbC and nDsbD [20] suggest substantial flexibility in the angle of the V, to accommodate different substrates and binding partners. DsbC is required for the folding of at least three *E. coli* periplasmic proteins, AppA, MepA and RNase I [10,21].

The structure of *E. coli* DsbG differs from *E. coli* DsbC both in the dimerisation domain [22] and in the putative substrate binding cleft, the latter indicating different substrate specificity [16]. Indeed, DsbG does not catalyze the folding of proteins that are known substrates of DsbC. However, a laboratory evolution approach, combined with structural studies, identified four DsbG residues that, when mutated, converted DsbG to a more DsbC-like structure with more DsbC-like function [23].

The inner membrane redox-active protein DsbD maintains DsbC, DsbG and CcmG (also called DsbE, a specific disulfide reductase involved in *c*-type cytochrome maturation in the periplasm) in their catalytically-active reduced form (Figure 4). DsbD has two periplasmic domains, an immunoglobulin-like N-terminal domain (nDsbD) and a thioredoxin-like C-terminal domain (cDsbD), linked to a central transmembrane domain (tDsbD) (Figures 2, 4). Each of the three domains has two reactive cysteines, and these three cysteine pairs are required for the relay of electrons from cytoplasmic thioredoxin through tDsbD then to cDsbD, nDsbD and finally to substrate proteins.

There is no structure of a full-length DsbD so the precise details of the electron transfer process across the bacterial inner membrane remains uncertain. However, genetic, biochemical and structural studies provide important clues. Mutational

analysis and redox state determination of nDsbD, cDsbD and DsbC allowed identification of residues in tDsbD involved in the cytoplasm-periplasm electron transfer process [24]. The C-terminal domain, cDsbD, has a thioredoxin-like fold [25] and unusual properties including similar thermodynamic stabilities of the oxidized and reduced forms and a very high pK_a (9.3) of the solvent exposed catalytic cysteine. These features are indicative of a role for cDsbD as a rigid electron shuttle between tDsbD and nDsbD [25,26]. Recent NMR analysis confirmed the rigidity of cDsbD during the redox cycle, and demonstrated that acidic residues in the active site contribute to the unusually high pK_a of the catalytic cysteine [27].

The structures of complexes of nDsbD–DsbC [20], nDsbD–cDsbD [4] and nDsbD–CcmG [28] show that nDsbD uses a common interaction surface for all three of its thioredoxin-like substrates, with additional regions contributing to the specific recognition of each binding partner (Figure 4). The structures also indicate that nDsbD probably undergoes large movements relative to tDsbD, to catalyze electron transfer to its substrates [4,26].

As noted above, most structural work on bacterial disulfide catalysts have focused on *E. coli* proteins, with few exceptions. The first Gram-positive Dsb disulfide catalyst protein to be structurally and functionally characterized was *Mycobacterium tuberculosis* DsbE [29] (Figure 2). Surprisingly, whereas the structure resembles that of the specific reductant CcmG (DsbE) from *E coli* [30] and *Bradyrhizobium japonicum* [31], *Mtb* DsbE was found to be an oxidant rather than a reductant, based on physicochemical and functional studies [29].

ARCHAEA, THERMOPHILIC BACTERIA AND POXVIRUS

Archaea and thermophilic bacteria do not have an extracellular compartment for disulfide bond formation. Instead, they encode *intracellular* protein disulfide oxidoreductases (PDOs). These proteins catalyze the formation of disulfide bonds in the cytoplasm, and play a critical role in the adaptation of these organisms to extreme conditions [32]. The structures of three PDOs have been solved; *Pf*PDO (from the archaeal thermophile *Pyrococcus furiosus*, [33]) (Figure 2), *Aa*PDO (from the thermophilic bacteria *Aquifex aeolicus*, [34]) and *Ap*PDO (from the hyperthermophilic archaeon *Aeropyrum pernix* K1 [35]). All three catalyze reduction, oxidation and isomerization of disulfide bonds and all three have similar structures comprising two CXXC-containing thioredoxin domains joined by a short linker. The two thioredoxin domains are functionally comparable to the *a* and *a'* domains of eukaryotic protein disulfide isomerase (see below) so that PDOs are considered to be bacterial PDI-like oxidoreductases [32,34].

Poxviruses also encode a disulfide relay pathway that enables disulfide formation in the normally reducing environment of the host cell cytoplasm. The poxvirus G4 protein catalyzes the introduction of disulfides into poxvirus proteins that are critical for virus maturation. The crystal structure of vaccinia virus G4 [36] reveals a modified thioredoxin fold with a CXXC active site motif (Figure 2).

EUKARYOTES

Eukaryotic cellular compartments such as the cytosol, mitochondria and plant chloroplasts each possess their own folding machinery. However, most protein folding occurs in the endoplasmic reticulum (ER), prior to translocation within the cell or extracellular secretion. Protein disulfide isomerases (PDIs) catalyze disulfide

bond formation in the ER, and 17 human PDI family members have been identified [37], with varying numbers and arrangements of catalytically active and inactive thioredoxin domains (Figure 5). A possible reason for the profusion of PDI enzymes is the need for discrete substrate specificities. For example, the substrates of ERp57 were found to be heavily glycosylated proteins that share a common structural (EGF-like) domain [38].

The most abundant mammalian PDI comprises four thioredoxin domains, two of which are redox-active (a, a') and two inactive (b, b'). These are arranged in the order a, b, b′, a′, followed by a C-terminal extension (c domain) with an ER-retention signal. A crucial breakthrough in our understanding of eukaryotic oxidative protein folding came with the crystal structure determination of full-length yeast PDI [39]. The structure showed that the four thioredoxin domains are arranged in a twisted "U" shape, with the catalytic a and a′ domains facing each other across the U, much like the two thioredoxin domains in the V-shaped DsbC and DsbG structures [40] (Figure 2, 5). The a′ domain of PDI functions as a disulfide oxidase whereas the a domain is thought to be a more efficient disulfide isomerase [41]. The non-catalytic b and b′ domains form the rigid base of the U-shaped molecule; the same structural arrangement for b and b' is observed in the crystal structure of truncated ERp57 (engineered to remove the a and a' domains, leaving the b and b′ domains only) [42].

In a manner analogous to the DsbA-DsbB oxidase pathway in prokaryotes, PDI is re-oxidized by the membrane-associated ER oxidoreductin 1 protein (Ero1p). Recent evidence suggests that two disulfide pairs that anchor an unstructured cap to the Ero1p helical core play a key regulatory role [43]. If the ER becomes too oxidizing

these regulatory cysteine pairs become disulfide bonded, blocking the active site, decreasing Ero1p activity and reducing the flow of oxidizing equivalents to protein thiols in the ER. If free thiols in the ER rise, the reverse reaction occurs resulting in increased Ero1p activity [43].

Ero1p transfers electrons from thiol substrates to molecular oxygen, resulting in the formation of H_2O_2 *in vitro* [44]. Under anaerobic conditions, flavins such as FAD serve as the electron acceptors. A second thiol oxidase, Erv2p, has been identified in yeast. As noted above, Ero1p and Erv2p and DsbB share similar structural features despite very low sequence identity [12-14]. These features include a FAD or quinone cofactor binding site within a bundle of four antiparallel α-helices. A redox-active CXXC motif is located near the cofactor and a second (relay) redox-active disulfide that interacts with the partner protein (PDI or DsbA, as appropriate) is located on a distal flexible loop.

Although most oxidative protein folding research in eukaryotes has focussed on proteins in the ER, a very exciting development has been the recent description of a disulfide relay system in mitochondria [45,46]. The mitochondrial disulfide machinery involves the protein Mia40 and FAD-bound Erv1p. Small mitochondrial inter-membrane space proteins bind to Mia40, in a step that is crucial for their transport across the outer membrane. A highly conserved CXC-CX_9C-CX_9C motif in Mia40 is vital for the import and proper folding of essential mitochondrial proteins. The structure of Mia40 is likely to be very different to that of the PDI and Dsb proteins, in that there is no obvious thioredoxin domain. The Mia40 structure will

represent another landmark in the field, by revealing the molecular details of eukaryotic oxidative protein folding in a non-ER compartment.

CONCLUSIONS

Recent structures of yeast protein disulfide isomerase and of the *E. coli* DsbB-DsbA complex have transformed our understanding of how redox proteins operate and interact. Furthermore, structures of disulfide catalysts and complexes (Ero1p, Erv2p, DsbA, DsbB, DsbD, DsbC, PDI) have highlighted the role that protein motion plays in disulfide catalysis. Indeed, motion may turn out to be a ubiquitous feature of these proteins. A common, but not quite ubiquitous, feature of disulfide catalyst structures is the presence of a thioredoxin fold. This domain is also found in disulfide-reducing proteins, but the features that give rise to specific redox activities (reducing, oxidizing or isomerising) are not yet completely understood.

ACKNOWLEDGEMENTS

This work was supported by an Australian Research Council grant (DP0556707) to BH, an International Postgraduate Research Scholarship to MK, a University of Queensland Postdoctoral Fellowship to SRS, an Australian Research Council grant (DP0664564) to JLM and a National Health and Medical Research Senior Research Fellowship (455829) to JLM.

REFERENCES

1. Kadokura H, Katzen F, Beckwith J: **Protein disulfide bond formation in prokaryotes**. *Annu Rev Biochem* 2003, **72**:111-135.

2. Tu BP, Weissman JS: **Oxidative protein folding in eukaryotes: mechanisms and consequences**. *J Cell Biol* 2004, **164**:341-346.

3. Rozhkova A, Glockshuber R: **Kinetics of the intramolecular disulfide exchange between the periplasmic domains of DsbD**. *J Mol Biol* 2007, **367**:1162-1170.

4. Rozhkova A, Stirnimann CU, Frei P, Grauschopf U, Brunisholz R, Grutter MG, Capitani G, Glockshuber R: **Structural basis and kinetics of inter- and intramolecular disulfide exchange in the redox catalyst DsbD**. *EMBO J* 2004, **23**:1709-1719.

5. Martin JL, Bardwell JC, Kuriyan J: **Crystal structure of the DsbA protein required for disulphide bond formation in vivo**. *Nature* 1993, **365**:464-468.

6. Martin JL: **Thioredoxin-a fold for all reasons**. *Structure* 1995, **3**:245-250.

7. Kadokura H, Tian H, Zander T, Bardwell JC, Beckwith J: **Snapshots of DsbA in action: detection of proteins in the process of oxidative folding**. *Science* 2004, **303**:534-537.

8. Horne J, d'Auvergne EJ, Coles M, Velkov T, Chin Y, Charman WN, Prankerd R, Gooley PR, Scanlon MJ: **Probing the flexibility of the DsbA oxidoreductase from Vibrio cholerae - a 15N-1H heteronuclear NMR relaxation analysis of oxidised and reduced forms of DsbA**. *J. Mol. Biol.* 2007, **accepted**.

9. Ondo-Mbele E, Vives C, Kone A, Serre L: **Intriguing conformation changes associated with the trans/cis isomerization of a prolyl residue in the active site of the DsbA C33A mutant**. *J Mol Biol* 2005, **347**:555-563.

10. Hiniker A, Bardwell JC: **In vivo substrate specificity of periplasmic disulfide oxidoreductases**. *J Biol Chem* 2004, **279**:12967-12973.

11. Gross E, Sevier CS, Vala A, Kaiser CA, Fass D: **A new FAD-binding fold and intersubunit disulfide shuttle in the thiol oxidase Erv2p**. *Nat Struct Biol* 2002, **9**:61-67.

12. Gross E, Kastner DB, Kaiser CA, Fass D: **Structure of Ero1p, source of disulfide bonds for oxidative protein folding in the cell**. *Cell* 2004, **117**:601-610.

*13. Sevier CS, Kadokura H, Tam VC, Beckwith J, Fass D, Kaiser CA: **The prokaryotic enzyme DsbB may share key structural features with eukaryotic disulfide bond forming oxidoreductases**. *Protein Sci* 2005, **14**:1630-1642.

Using a structural bioinformatics approach, the authors define three classes of bacterial DsbB proteins, and identify features that suggest structural convergence with functionally equivalent (but soluble) eukaryotic proteins. This prediction was subsequently confirmed with the publication of the DsbB:DsbA structure (reference 14).

14. Inaba K, Murakami S, Suzuki M, Nakagawa A, Yamashita E, Okada K, Ito K: **Crystal structure of the DsbB-DsbA complex reveals a mechanism of disulfide bond generation. *Cell* 2006, **127**:789-801.

This paper describes the first structure of the membrane protein DsbB, revealing that is has a similar architecture to the eukaryotic, but soluble, Ero1p and Erv2p proteins. A stable complex of DsbB with DsbA was formed by mutating specific cysteines in each protein to alanine. The resulting structure

shows how a mobile redox loop in DsbB relays oxidizing equivalents to the active site of DsbA.

15. Messens J, Collet JF: **Pathways of disulfide bond formation in Escherichia coli**. *Int J Biochem Cell Biol* 2006, **38**:1050-1062.

16. Heras B, Edeling MA, Schirra HJ, Raina S, Martin JL: **Crystal structures of the DsbG disulfide isomerase reveal an unstable disulfide**. *Proc Natl Acad Sci U S A* 2004, **101**:8876-8881.

17. McCarthy AA, Haebel PW, Torronen A, Rybin V, Baker EN, Metcalf P: **Crystal structure of the protein disulfide bond isomerase, DsbC, from Escherichia coli**. *Nat Struct Biol* 2000, **7**:196-199.

18. Banaszak K, Mechin I, Frost G, Rypniewski W: **Structure of the reduced disulfide-bond isomerase DsbC from Escherichia coli**. *Acta Crystallogr D Biol Crystallogr* 2004, **60**:1747-1752.

19. Zhang M, Monzingo AF, Segatori L, Georgiou G, Robertus JD: **Structure of DsbC from Haemophilus influenzae**. *Acta Crystallogr D Biol Crystallogr* 2004, **60**:1512-1518.

20. Haebel PW, Goldstone D, Katzen F, Beckwith J, Metcalf P: **The disulfide bond isomerase DsbC is activated by an immunoglobulin-fold thiol oxidoreductase: crystal structure of the DsbC-DsbDalpha complex**. *EMBO J* 2002, **21**:4774-4784.

21. Berkmen M, Boyd D, Beckwith J: **The nonconsecutive disulfide bond of Escherichia coli phytase (AppA) renders it dependent on the protein-disulfide isomerase, DsbC**. *J Biol Chem* 2005, **280**:11387-11394.

22. Yeh SM, Koon N, Squire C, Metcalf P: **Structures of the dimerization domains of the Escherichia coli disulfide-bond isomerase enzymes DsbC and DsbG**. *Acta Crystallogr D Biol Crystallogr* 2007, **63**:465-471.

23. Hiniker A, Ren G, Heras B, Zheng Y, Laurinec S, Jobson R, Stuckey J, Martin J, Bardwell J: **Laboratory evolution of one disulfide isomerase to resemble another**. *Proc Natl Acad Sci U S A* 2007:in press.

24. Cho SH, Beckwith J: **Mutations of the membrane-bound disulfide reductase DsbD that block electron transfer steps from cytoplasm to periplasm in Escherichia coli**. *J Bacteriol* 2006, **188**:5066-5076.

25. Stirnimann CU, Rozhkova A, Grauschopf U, Bockmann RA, Glockshuber R, Capitani G, Grutter MG: **High-resolution structures of Escherichia coli cDsbD in different redox states: A combined crystallographic, biochemical and computational study**. *J Mol Biol* 2006, **358**:829-845.

26. Stirnimann CU, Grutter MG, Glockshuber R, Capitani G: **nDsbD: a redox interaction hub in the Escherichia coli periplasm**. *Cell Mol Life Sci* 2006, **63**:1642-1648.

27. Mavridou DA, Stevens JM, Ferguson SJ, Redfield C: **Active-site Properties of the Oxidized and Reduced C-terminal Domain of DsbD Obtained by NMR Spectroscopy**. *J Mol Biol* 2007, **370**:643-658.

28. Stirnimann CU, Rozhkova A, Grauschopf U, Grutter MG, Glockshuber R, Capitani G: **Structural basis and kinetics of DsbD-dependent cytochrome c maturation**. *Structure* 2005, **13**:985-993.

29. Goulding CW, Apostol MI, Gleiter S, Parseghian A, Bardwell J, Gennaro M, Eisenberg D: **Gram-positive DsbE proteins function differently from**

Gram-negative DsbE homologs. A structure to function analysis of DsbE from Mycobacterium tuberculosis. *J Biol Chem* 2004, **279**:3516-3524.

30. Ouyang N, Gao YG, Hu HY, Xia ZX: **Crystal structures of E. coli CcmG and its mutants reveal key roles of the N-terminal beta-sheet and the fingerprint region**. *Proteins* 2006, **65**:1021-1031.

31. Edeling MA, Guddat LW, Fabianek RA, Thony-Meyer L, Martin JL: **Structure of CcmG/DsbE at 1.14 A resolution: high-fidelity reducing activity in an indiscriminately oxidizing environment**. *Structure* 2002, **10**:973-979.

32. Beeby M, O'Connor BD, Ryttersgaard C, Boutz DR, Perry LJ, Yeates TO: **The genomics of disulfide bonding and protein stabilization in thermophiles**. *PLoS Biol* 2005, **3**:e309.

33. Ren B, Tibbelin G, de Pascale D, Rossi M, Bartolucci S, Ladenstein R: **A protein disulfide oxidoreductase from the archaeon Pyrococcus furiosus contains two thioredoxin fold units**. *Nat Struct Biol* 1998, **5**:602-611.

34. Pedone E, D'Ambrosio K, De Simone G, Rossi M, Pedone C, Bartolucci S: **Insights on a new PDI-like family: structural and functional analysis of a protein disulfide oxidoreductase from the bacterium Aquifex aeolicus**. *J Mol Biol* 2006, **356**:155-164.

35. D'Ambrosio K, Pedone E, Langella E, De Simone G, Rossi M, Pedone C, Bartolucci S: **A novel member of the protein disulfide oxidoreductase family from Aeropyrum pernix K1: structure, function and electrostatics**. *J Mol Biol* 2006, **362**:743-752.

36. Su HP, Lin DY, Garboczi DN: **The structure of G4, the poxvirus disulfide oxidoreductase essential for virus maturation and infectivity**. *J Virol* 2006, **80**:7706-7713.

37. Van Anken E, Braakman I: **Versatility of the endoplasmic reticulum protein folding factory**. *Crit. Rev. Biochem. Mol. Biol.* 2005, **40**:191-228.

38. Jessop CE, Chakravarthi S, Garbi N, Hammerling GJ, Lovell S, Bulleid NJ: **ERp57 is essential for efficient folding of glycoproteins sharing common structural domains**. *EMBO J* 2007, **26**:28-40.

39. Tian G, Xiang S, Noiva R, Lennarz WJ, Schindelin H: **The crystal structure of yeast protein disulfide isomerase suggests cooperativity between its active sites. *Cell* 2006, **124**:61-73.

The long-awaited structure of full-length PDI reveals that the four thioredoxin domains adopt a twisted U shape with the two catalytically active thioredoxin domains facing each other across the U. This architecture is remarkably similar to the V-shaped architectures of bacterial DsbC and DsbG proteins.

40. Gruber CW, Cemazar M, Heras B, Martin JL, Craik DJ: **Protein disulfide isomerase: the structure of oxidative folding**. *Trends Biochem Sci* 2006, **31**:455-464.

41. Kulp MS, Frickel EM, Ellgaard L, Weissman JS: **Domain architecture of protein-disulfide isomerase facilitates its dual role as an oxidase and an isomerase in Ero1p-mediated disulfide formation**. *J Biol Chem* 2006, **281**:876-884.

42. Kozlov G, Maattanen P, Schrag JD, Pollock S, Cygler M, Nagar B, Thomas DY, Gehring K: **Crystal structure of the bb' domains of the protein disulfide isomerase ERp57**. *Structure* 2006, **14**:1331-1339.

*43. Sevier CS, Qu H, Heldman N, Gross E, Fass D, Kaiser CA: **Modulation of cellular disulfide-bond formation and the ER redox environment by feedback regulation of Ero1**. *Cell* 2007, **129**:333-344.

An elegant study that identifies noncatalytic cysteine pairs in Ero1p as potential redox sensors that regulate Ero1p activity thus maintaining a redox homeostasis in the ER.

44. Gross E, Sevier CS, Heldman N, Vitu E, Bentzur M, Kaiser CA, Thorpe C, Fass D: **Generating disulfides enzymatically: reaction products and electron acceptors of the endoplasmic reticulum thiol oxidase Ero1p**. *Proc Natl Acad Sci U S A* 2006, **103**:299-304.

*45. Mesecke N, Terziyska N, Kozany C, Baumann F, Neupert W, Hell K, Herrmann JM: **A disulfide relay system in the intermembrane space of mitochondria that mediates protein import**. *Cell* 2005, **121**:1059-1069.

This paper and the following paper, describe the protein Mia40, that catalyzes disulfide bond formation in a pathway that is critical for mitochondrial protein import.

*46. Chacinska A, Pfannschmidt S, Wiedemann N, Kozjak V, Sanjuan Szklarz LK, Schulze-Specking A, Truscott KN, Guiard B, Meisinger C, Pfanner N: **Essential role of Mia40 in import and assembly of mitochondrial intermembrane space proteins**. *EMBO J* 2004, **23**:3735-3746.

Figure Legends

Figure 1. Disulfide bond formation. A. The general reaction for oxidation and reduction of sulfhydryl (-SH) groups is shown. In the cell, this reaction can be catalyzed in either direction, depending on the enzyme and compartment. Here we focus on enzymes that catalyze the introduction and shuffling, or isomerization, of disulfides. **B.** In bacteria, the introduction of disulfide bridges into newly translocated proteins occurs in the periplasm and is catalyzed by the oxidant DsbA. Reduced DsbA is reoxidized by the inner membrane protein DsbB. DsbA can introduce non-native disulfides into proteins; non-native disulfides are shuffled to the correct form by the periplasmic isomerases DsbC and DsbG. The inner membrane protein DsbD maintains DsbC and DsbG in the active reduced state. In eukaryotes, PDI catalyzes both oxidation and isomerization steps, and it is maintained in the oxidized form by Ero1p or Erv2p.

Figure 2. Structures of disulfide-catalyzing proteins. Representative structures of disulfide oxidants and disulfide isomerases are shown: *E coli* DsbA (PDB code 1DSB); *E coli* DsbB (2HI7); yeast PDI (2B5E); *Saccharomyces cerevisiae* Ero1p (1RP4); *S. cerevisiae* Erv2p (1JR8); *E coli* DsbC (1EEJ); *E coli* nDsbD (1L6P); *E coli* cDsbD (2FWH); *E coli* DsbG (1V57); MTb DsbE (1LU4), *Pyrococcus furiosus* PDO (1A8L) and the vaccinia virus G4 monomer (2G2Q). The thioredoxin domain (blue) is a commonly-used motif in these proteins. Where more than one thioredoxin domain is present in a protein structure, additional thioredoxin domains are shown in white. Non-thioredoxin domains are shown in orange, and dimeric proteins (Erv2p, DsbC and DsbG) are shown with one of the two subunits in pale orange. Redox active disulfides are shown as yellow spheres. The bacterial inner membrane is shown in

schematic form for DsbB and DsbD. The tDsbD domain, not yet structurally characterized, is also shown in schematic form.

Figure 3. The DsbA-DsbB complex. A. The recently-determined crystal structure of the complex of the membrane protein DsbB (green) with the disulfide oxidant DsbA (magenta) [14]. Cysteine residues are shown as yellow spheres and the DsbB-bound quinone is shown as atom-coloured spheres. **B**. The flexible redox loop of DsbB (green) forms a mixed-disulfide (yellow) with the redox-active site of DsbA (yellow), and interacts with the surface hydrophobic groove adjacent to the DsbA active site.

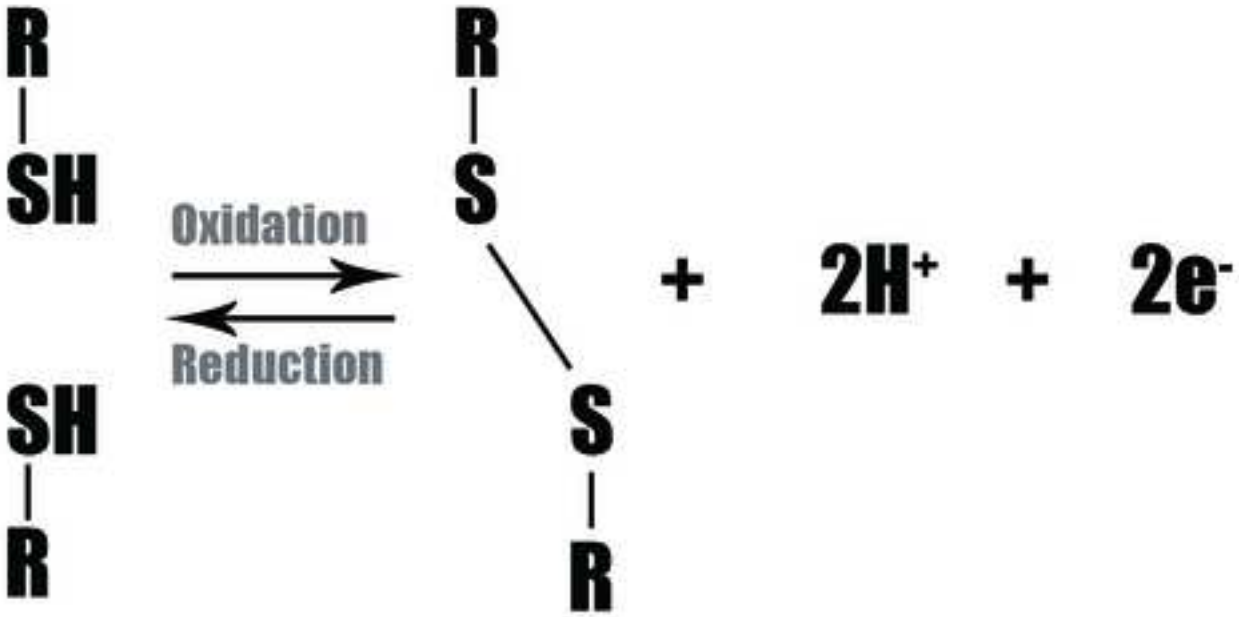
Figure 4. Disulfide shuffling in the bacterial periplasm. DsbD plays a central role in transferring electrons from the reducing cytoplasm to the oxidative environment of the periplasm. DsbD has an N-terminal immunoglobulin-like domain nDsbD (grey, PDB ID code 1JPE), a transmembrane domain tDsbD (8 transmembrane helices) and a C-terminal thioredoxin-like domain cDsbD (salmon, PDB ID code 2FWH). DsbD utilizes NADPH as a source of reducing power; two electrons flow from NADPH via thioredoxin reductase and thioredoxin (green, PDB ID code 2TRX) to tDsbD, then successively to cDsbD and nDsbD (cDsbD-nDsbD complex in grey and salmon, PDB ID code 1VRS). Reduced nDsbD reduces substrates including the disulfide isomerases DsbC and DsbG (oxidized and reduced DsbC shown in dark blue, PDB ID code 1EEJ and 1TJD; nDsbD-DsbC complex shown in grey and dark blue, PDB ID code 1JZD) and CcmG, a membrane anchored protein involved in cytochrome *c* maturation (nDsbD-CcmG complex in grey and cyan, PDB ID code 1Z5Y). nDsbD must undergo re-orientations in order to interact with these different substrates. The cysteines of tDsbD involved in electron transfer are represented as yellow dots and

the active sites cysteines of the other proteins are shown in yellow.

Figure 5. Human PDI-like proteins localized to the ER. (**A**) Domain arrangement of human PDI family members. Signal peptides are shown in brown; a-like domains (thioredoxin domains with a CXXC-like motif) are in light blue; b-like domains (thioredoxin domains without a redox-active centre) are in red; acidic c-domains are shown in green; DnaJ domains are shown in grey; calsequestrin-like thioredoxin domains are yellow; and α-helical d-domain is in dark blue. For the **t**rans**m**embrane TR**X**-related (TMX) proteins, the transmembrane domain is indicated in orange, the cytoplasmic domain in black and the internal ER domain in pink (adapted from [37]). (**B**) Ribbon diagram of a model of human PDI based on the crystal structure of yeast PDI [39]. The colours used for each domain are the same as those used in panel A.

Figure

Click here to download high resolution image



Figure

Click here to download high resolution image

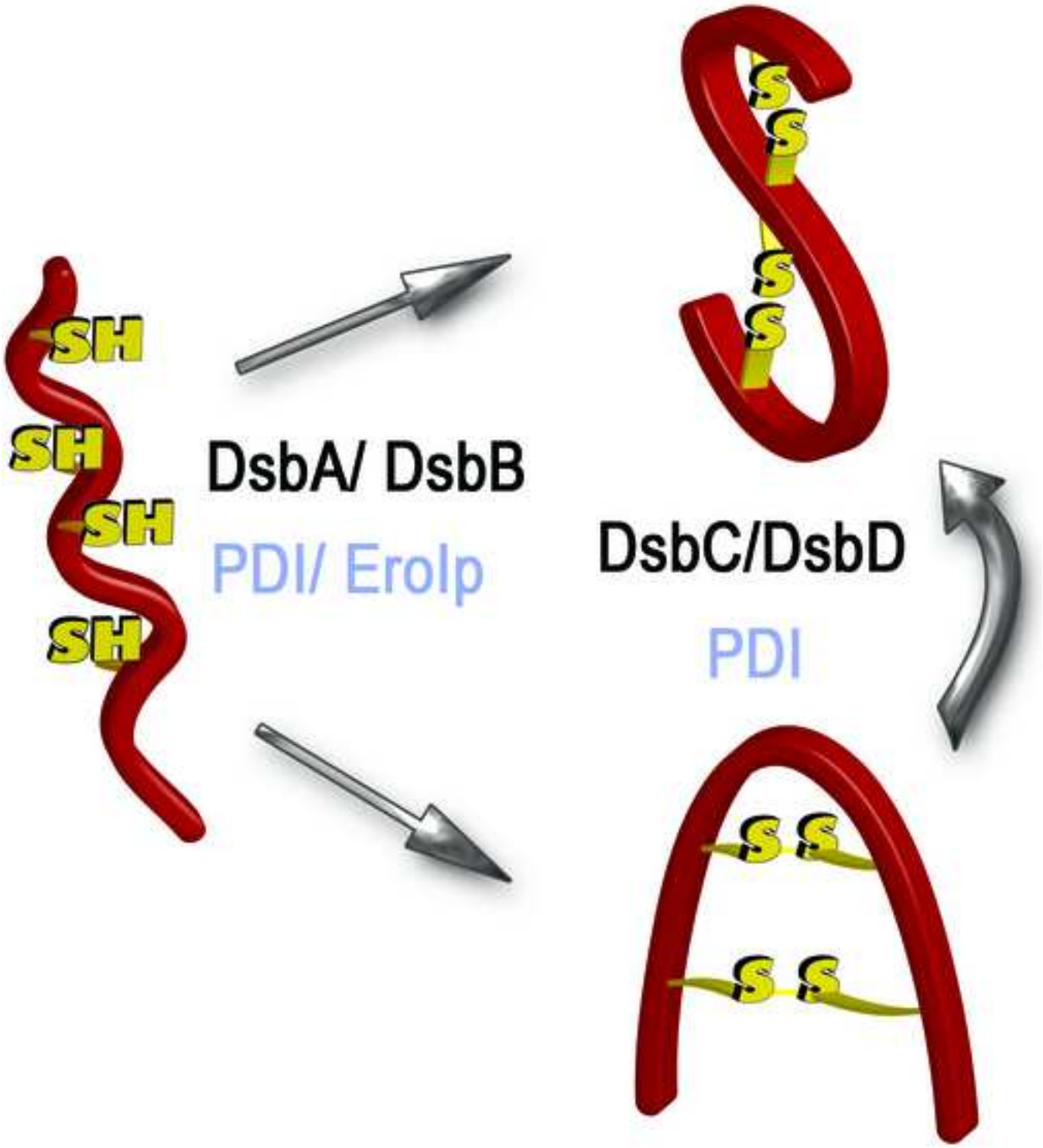


Figure
Click here to download high resolution image

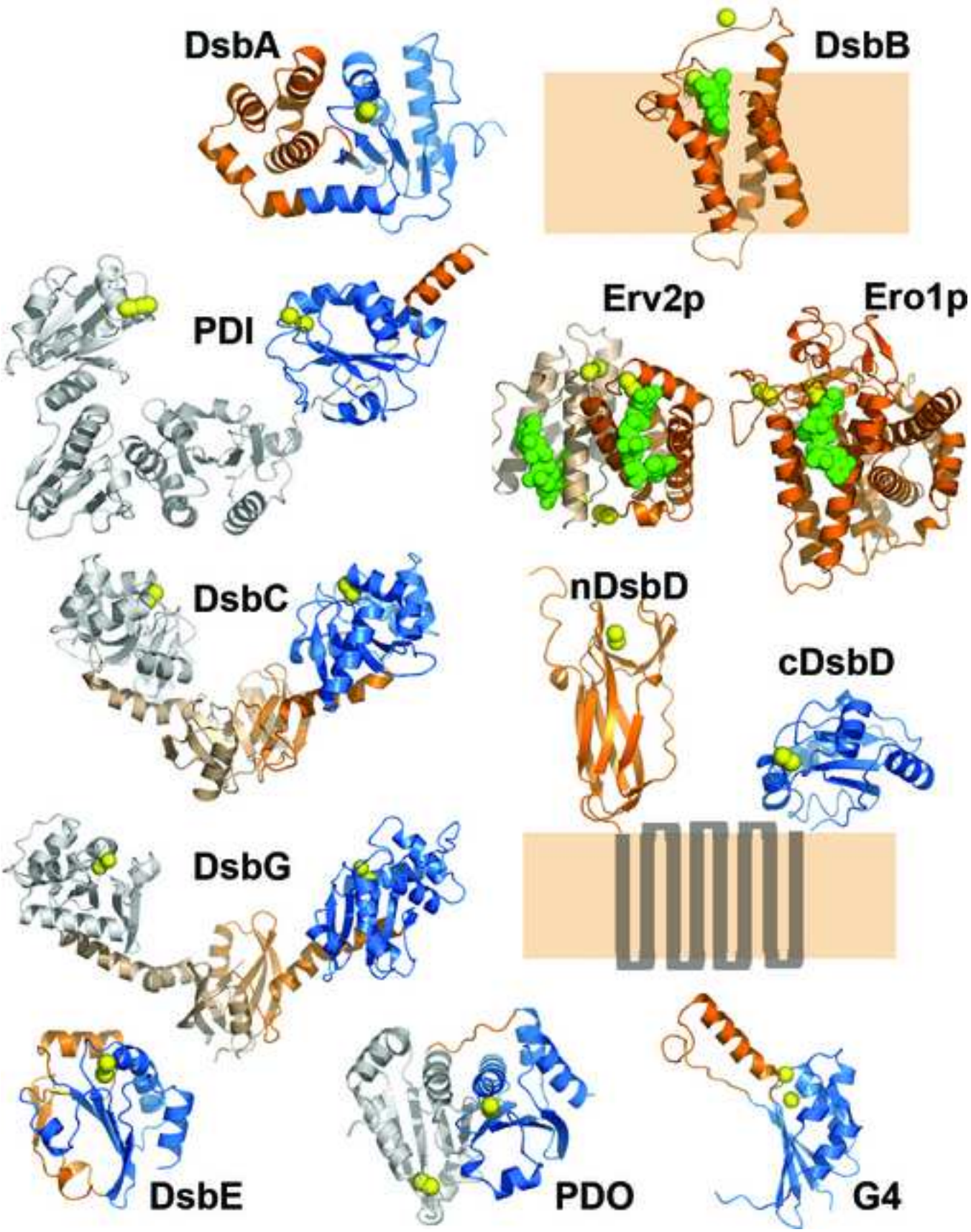
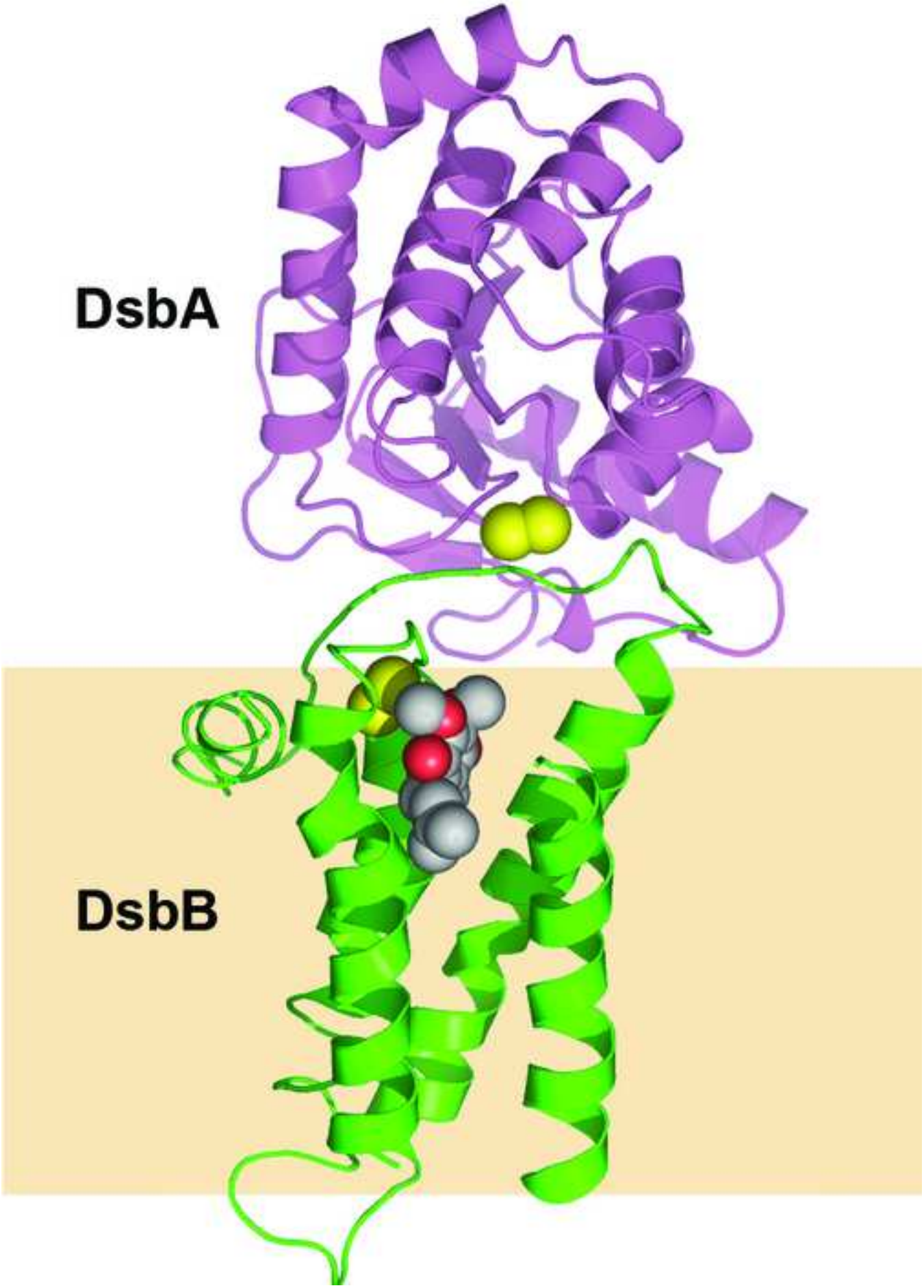
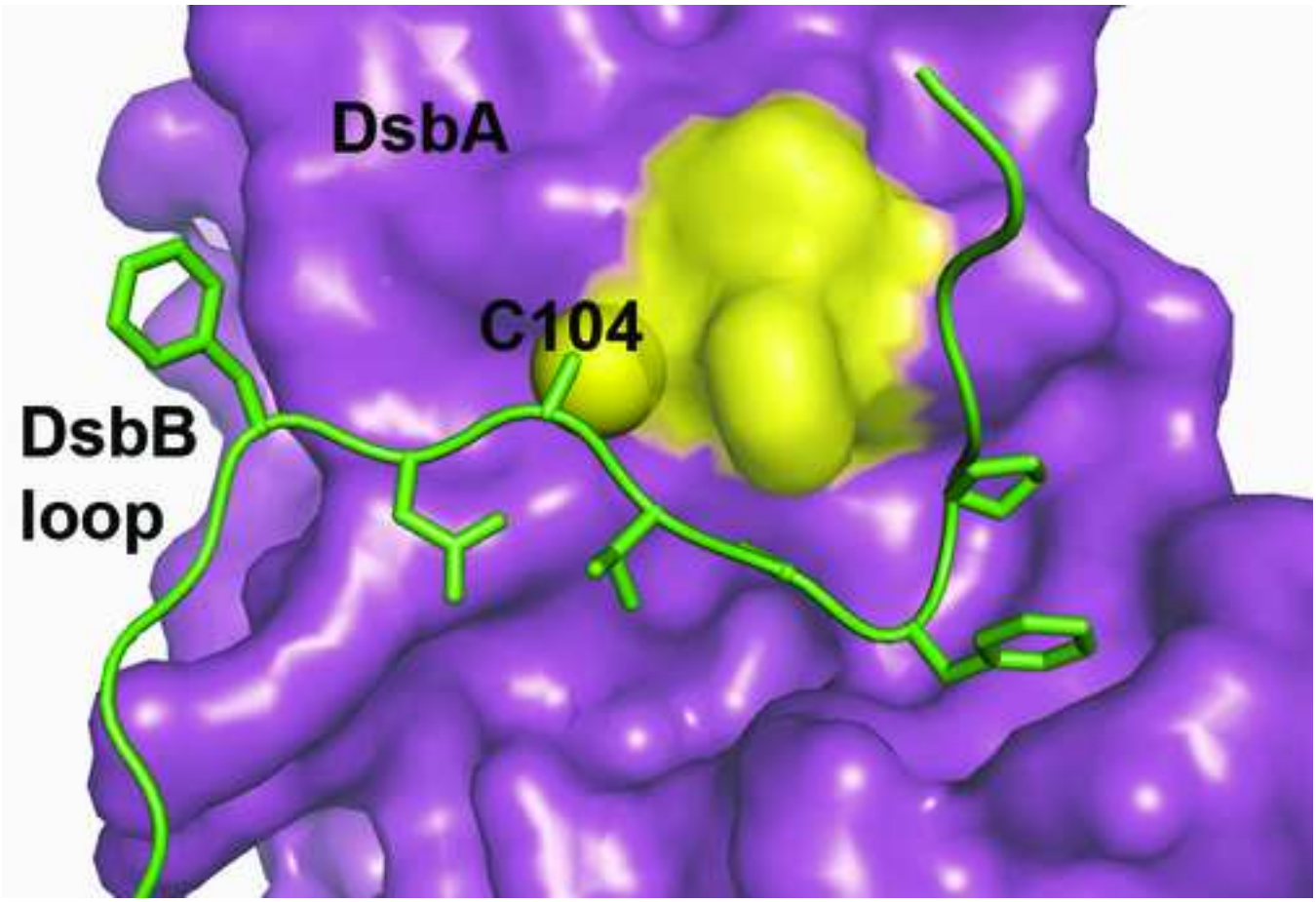


Figure
Click here to download high resolution image



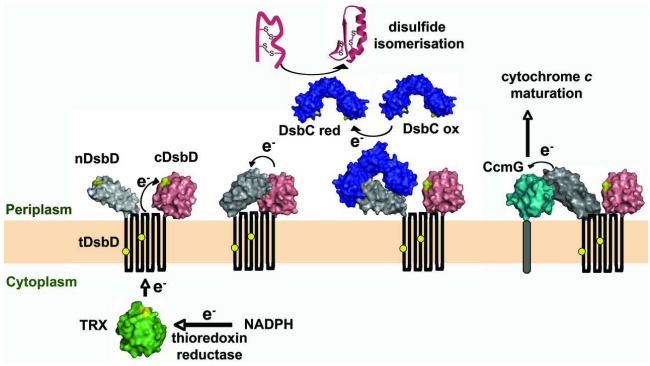
Figure

Click here to download high resolution image



Figure

Click here to download high resolution image



Figure

Click here to download high resolution image