Allosteric disulfides: Sophisticated molecular structures enabling flexible protein regulation

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Protein disulfide bonds link pairs of cysteine residues in polypeptide chains. Many of these bonds serve a purely structural or energetic role, but a growing subset of cleavable disulfide bonds has been shown to control the function of the mature protein in which they reside. These allosteric disulfides and the factors that cleave these bonds are being identified across biological systems and life forms and have been shown to control hemostasis, the immune response, and viral infection in mammals. The discovery of these functional disulfides and a rationale for their facile nature has been aided by the emergence of a conformational signature for allosteric bonds. This post-translational modification mostly occurs extracellularly, making these chemical events prime drug targets. Indeed, a membrane-impermeable inhibitor of one of the cleaving factors is currently being trialed as an antithrombotic agent in cancer patients. Allosteric disulfides are firmly established as a sophisticated means by which a protein's shape and function can be altered; however, the full scope of this biological regulation will not be realized without new tools and techniques to study this regulation and innovative ways of targeting it.

Disulfide bonds are one of the first elements of protein architecture we learn about in biology. Although they seem simple at first glance, they are a key part of what made human life possible. An analysis of the time of acquisition and conservation of 5,181 protein disulfide bonds across 29 eukaryotic species remarkably showed that about three-quarters of disulfide bonds in human proteins were acquired in vertebrate ancestors (1). Moreover, the acquisition of a disulfide bond coincided with the evolution of a new protein in most cases, and there was a positive trend in the rate of disulfide acquisition and complexity of the organism. Once a disulfide bond appeared in a protein, it was very rarely lost thereafter. These findings suggest that accrual of disulfide bonds has contributed to the higher complexity of vertebrates. It is of interest that the frequency of cysteine in the proteome of modern species is increasing (2, 3), which implies that disulfide acquisition is ongoing in our evolution.

For a long time, disulfide bonds were assumed to be inert in the mature protein. However, we now know that disulfide bonds play both structural and functional roles. Structural disulfides are those that are formed during protein folding and either impact the folding process and/or stabilize the resultant protein architecture. There are two types of functional disulfide: the catalytic disulfides that manipulate disulfides in other proteins, and allosteric disulfides that control the function of the protein in which they reside when cleaved or formed (4, 5). Allosteric control of protein function is defined as a change in one site–the allosteric site–that influences another site by exploiting the protein's flexibility (6). An allosteric disulfide bond thus represents the "allosteric site," and the conformational change triggered by cleavage of the bond alters protein function. Although there is some ambiguity in the structural *versus* functional designation, it is helpful to define disulfide bonds in this way. For instance, while the "structural" disulfides are inert they can contribute to protein function by stabilizing the structural integrity of the native conformation and associated binding sites.

The first disulfide bond implicated in protein control was identified in the mid-1990s in the adhesive glycoprotein thrombospondin-1 (TSP-1). TSP-1 is secreted by mammalian cells and participates in cell–cell and cell–matrix interactions. Ligand binding to TSP-1 was shown to be controlled by protein-disulfide isomerase (PDI)[2] that manipulates a disulfide bond in the C-terminal domain of the protein (7–11). About 40 allosteric disulfides have now been described (Table 1) where we know the identity of the disulfide and the consequences for protein function when the bond is cleaved. The bonds have been identified in viruses, bacteria, plants, and mammals. They have been arranged in Table 1 according to the type of change in the protein upon cleavage of the disulfide: ligand binding, substrate hydrolysis, proteolysis, or oligomerization. There are many other allosteric disulfides under investigation that have yet to be defined at this level of understanding.

This review focuses on allosteric disulfides and the factors that cleave them. The concept of allosteric disulfides was intro-

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This article contains Table S1.

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[2] The abbreviation used is: PDI, protein-disulfide isomerase; ER, endoplasmic reticulum; IPA, 2-iodo-*N*-phenylacetamide; PDB, Protein Data Bank; SNP, single nucleotide polymorphism; ALS, amyotrophic lateral sclerosis; TG2, transglutaminase-2.

Table 1
Examples of allosteric disulfides grouped according to the type of change in protein function associated with cleavage of the bonds
Cleavage of the indicated disulfide in the protein can result in increase or decrease in ligand binding to the protein, change in the efficiency of substrate hydrolysis if the protein is an enzyme, increase or decrease in subsequent proteolysis of the protein, or formation of inter-molecular disulfide bonds and homo- or hetero-oligomerization of the protein.

Change	Protein	Disulfide Cys	Reference
ligand binding	β2-glycoprotein I	288-326	(76)
	β3 integrin	177-184	(51)
	HIV gp120	126-196 296-331 385-418	(77-82)
	CD4	130-159	(64)
	CD44	81-101	(83)
	Glycoprotein 1b	4-17 209-248	(84)
	Interleukin-4	46-99	(70)
	Interleukin receptor subunit gamma	160-209	(85)
	Thrombospondin-1	974-?	(7,11)
	Tissue Factor	186-209	(68,86)
	Vitronectin	137-161 274-453	(87)
	von Willebrand factor	1669-1670	(69)
substrate hydrolysis	Adenosine-5'-phosphosulfate kinase	86-119	(88)
	Arylsulfate sulfotransferase	418-424	(89)
	Botulinum neurotoxins	436-445	(90)
	QueF	55-99	(91)
	Thiolase	88-378	(92)
	hTryptaseβ	191-220	(93)
	Methionine aminopeptidase 2	228-448	(94)
	Prolyl cis-trans isomerase, AtFKBP13	106-111	(95)
	Transglutaminase 2	370-371 230-370	(42)
	Lon protease	617-691	(96)
	CgDapF	83-221	(97)
	SAMHD1	341-350	(98)
proteolysis	Angiotensinogen	18-138	(99)
	Factor XI	362-482	(100)
	MICA	202-259	(101)
	Plasmin(ogen)	512-536 462-541	(57)
oligomerisation	CD4	130-159	(64)
	Vascular endothelial growth factors C & D	156-165	(58)
	LYVE-1	201-201	(103)

duced in 2006 (4). At that time, we knew of only a few allosteric disulfides, had a rudimentary understanding of why and how they were cleaved, and had no *in vivo* evidence for their physiological relevance. We now know of several dozen allosteric bonds, have a good handle on why and how they are cleaved, and have compelling *in vivo* evidence for their importance. The first effort to target this post-translational modification for the treatment of disease is being tested in patients with cancer. This review describes our current understanding of these bonds, how to study and target them, and what needs to happen for this research field to accelerate and realize its potential.

Where disulfide bonds are formed

Most disulfide bonds form during protein folding (Fig. 1). The thermodynamics of this spontaneous process are governed by Gibbs free energy (G) and is dominated by the second law, $\Delta G = \Delta H - T\Delta S$, which states that the entropy of a closed system cannot decrease. Protein folding is a process where the enthalpy (ΔH) and entropy ($-T\Delta S$) terms compete with one another to achieve a net negative ΔG, which is required for a spontaneous reaction. Although the change in entropy of the protein as it folds is negative, the change in entropy of the surrounding water molecules is positive, *i.e.* reductions in conformational entropies are balanced by gains in entropy due to release of solvent molecules. The enthalpic contributions include hydrogen bonding of the protein and water molecules, ionic salt bridges, van der Waals forces and, of course, disulfide bond formation (Fig. 1). Heat is released during formation of these bonds.

In eukaryotic cells, disulfide bonds form in the ER, Golgi complex, post-Golgi complex vesicles, and mitochondrial inter-membrane space (12). In bacteria, these bonds form in the periplasmic space (13). The formation of disulfide bonds in all these compartments is assisted by many protein factors and small molecules, primarily the oxidoreductases, chaperones and GSH. The information required for the folding of a protein is encrypted within its primary amino acid sequence (14, 15). The role that disulfide bonds play in folding has been debated, and different theories have been proposed. A popular concept is that disulfide bonds direct protein folding in a stepwise fashion where a protein achieves its native structure via stable disulfide intermediates that are partially folded (16–18). Another model says that disulfide bonds stabilize the native conformation rather than facilitate folding (19–21). It is based on conformational sampling of the native structure, either locally or globally, and lastly formation of disulfide bonds that stabilize the mature conformation. It has also been proposed that disulfide formation drives protein folding (22), *i.e.* the local changes induced by the formation of a single disulfide bond directs global folding of the protein in a cooperative fashion. It is likely that all models are in play to some extent and that their relative contributions to the native structure will vary from protein to protein.

Where disulfide bonds are found

Over 180,000 disulfide bonds are present in Protein Data Bank structures (23). Many disulfides are represented more than once in different structures of the same protein. For example, immunoglobulin disulfides are represented thousands of times. A subset of 13,031 unique disulfide bonds in 4,895 nonredundant human proteins is shown in Table S1 along with a number of features of the bonds. About half of these disulfide bonds are in membrane proteins (plasma or organelle membranes) and about half in proteins containing a secretion signal sequence (secreted or function in the ER, Golgi, and endosome). Interestingly, 298 proteins that function in the cytoplasm and/or nucleus contain 509 structurally-defined disulfide bonds. These disulfide bonds can be grouped in Table S1 by sorting on the "Subcellular location" column. This group is notable as the cytoplasm and nucleus are cellular compartments traditionally not thought to support disulfide bond formation because they are considered too reducing. How many of these disulfides form in the proteins in their native environment remains to be determined, although it seems likely that several of them will. For instance, we know that disulfide bonds form in some prokaryote and eukaryote transcription factors (24). We also know that different intracellular environments have different redox potentials (25) that will influence disulfide bond stability. Formation or cleavage of a protein disulfide bond is a lot about the protein being in the right place at the right time.

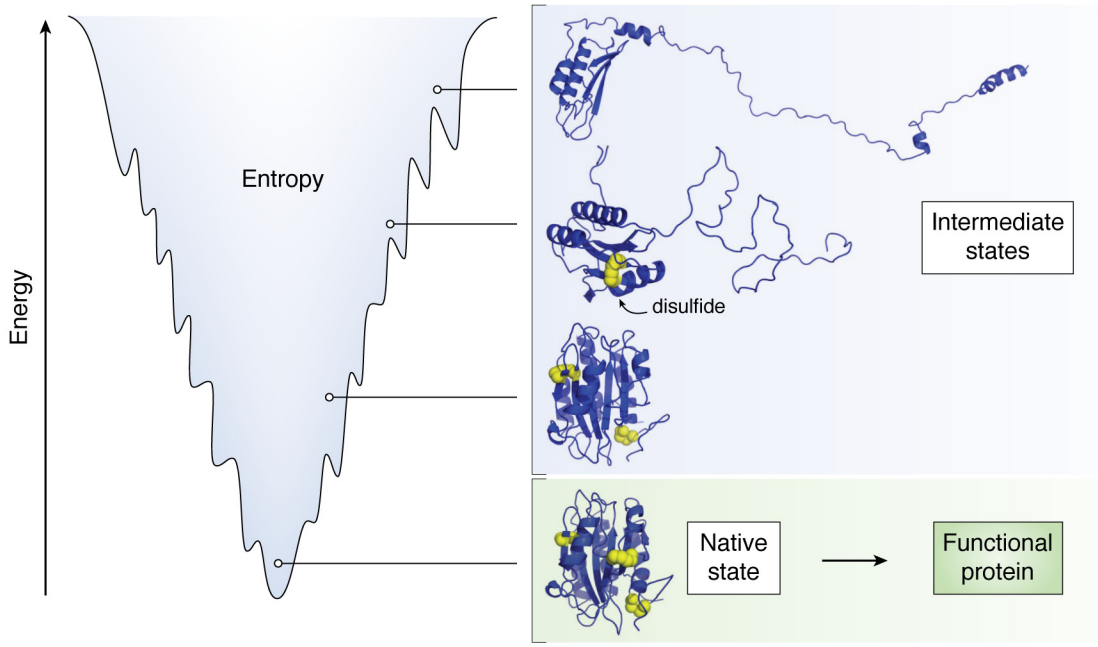

Figure 1. Disulfide bonds form as proteins fold. Protein folding is associated with reduction in conformational entropy that is overcome by favorable enthalpic contributions and by an increase in solvent entropy. Folding of a model protein (*blue ribbon*) containing disulfide bonds (*yellow spheres*) is shown. The intermediate states contain zero, one, or two disulfide bonds. The native functional protein contains three disulfide bonds.

How allosteric disulfides are cleaved

Protein disulfide bonds can be cleaved by oxidoreductases and by thiol–disulfide exchange (Fig. 2). Another mechanism, recently discovered in an engineered system, is hydrolysis of the disulfide. All three mechanisms of disulfide bond cleavage are potentially reversible.

Oxidoreductases

Humans make more than 20 different oxidoreductases (26, 27), and many of them are exported by activated cells. Seven members of the PDI family (26), two members of the thioredoxin family, and glutaredoxin-1 (27) are secreted into the blood (28), whereas transmembrane TMX1, TMX3, ERp44, and ERp29 have been detected on the platelet surface (29–31). An important fundamental question is how the secretion of oxidoreductases is achieved and regulated. They are not exported by the classical secretion pathway.

The active sites of oxidoreductases contain a reactive dithiol/disulfide that reduces or oxidizes a substrate disulfide bond (Fig. 2*A*). For example, an active-site dithiol will reduce a substrate disulfide bond and become oxidized in the process. For the oxidized oxidoreductase to cleave another substrate disulfide, it needs to be first reduced in another reaction. Oxidoreductases are not catalysts in the true sense of the word as the bonds are consumed during reaction and require regeneration. The suffix 'ase' is used to designate an enzyme, although the oxidoreductases may be more correctly considered factors rather than enzymes. A conundrum in the field is how or if these factors turn over in the extracellular space. There are mechanisms in the ER and cytoplasm that regenerate spent oxidoreductases. For instance, cytoplasmic thioredoxin is reduced by cytoplasmic thioredoxin reductase using electrons from NADPH (27). Such reactions have not been identified

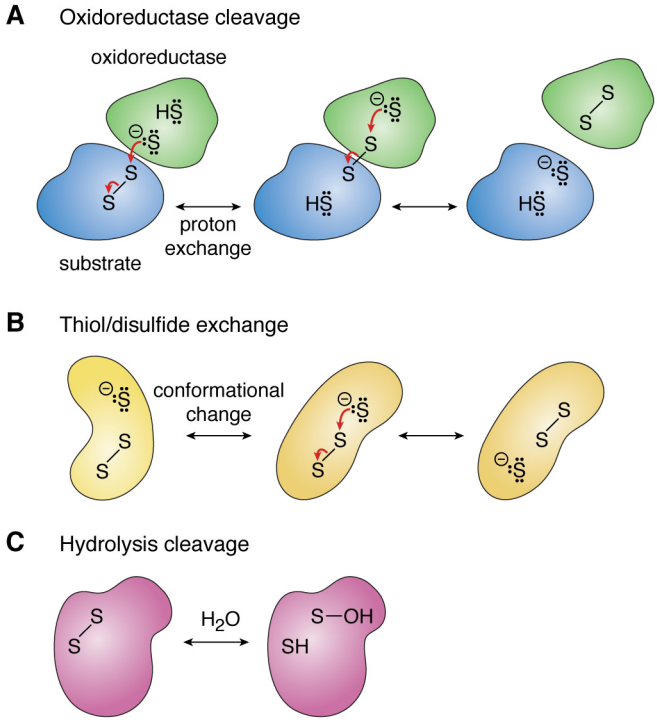

Figure 2. Mechanisms of cleavage of allosteric disulfides. Disulfide cleavage is a highly directional chemistry. The sulfur ion nucleophile of the oxidoreductase and the two sulfur atoms of the substrate disulfide bond must be in-line for cleavage to proceed. *A,* cleavage by an oxidoreductase. An active-site sulfur ion of the oxidoreductase attacks one of the allosteric bond sulfur atoms. An intermediate bond between the attacked and the attacking sulfur atoms decomposes and leaves the allosteric disulfide bond reduced and the oxidoreductase active-site sulfurs oxidized. *B,* cleavage by thiol/disulfide exchange. A conformational change brings an unpaired sulfur ion nucleophile in-line with an allosteric disulfide. The sulfur ion attacks the disulfide bond, cleaving it. *C,* cleavage by hydrolysis. This mechanism results in formation of a cysteine sulfenic acid and thiol. This mechanism has been observed in one instance, but the biological significance remains to be determined.

outside the cell. A mechanism of oxidation of an oxidoreductase has been described– oxidation of PDI by platelet surface Ero1α (30)–but no mechanism of reduction of the factors has been found thus far. It is possible that some oxidoreductases act as single turnover reductants in the extracellular space (32), *i.e.* a reduced oxidoreductase is secreted and cleaves one substrate disulfide bond. Although this scenario seems a waste of energy in protein production and secretion, it may be an energy cost that cells will tolerate. The flip side is that the activity of single turnover enzymes is readily controlled–they act once and are then cleared. This situation avoids the need for endogenous inhibitors of the factors, which have not been identified for secreted oxidoreductases to date.

Secreted oxidoreductases appear to have many substrates in the extracellular milieu (33). The following general parameters likely govern substrate specificity of the oxidoreductases. As for proteases and their substrates, lock-and-key–type interactions of the substrates with the binding pockets of the factors will be important, particularly considering the stereochemistry of disulfide bond cleavage. Reduction of a disulfide bond proceeds via an S_n2 reaction mechanism where the sulfur ion nucleophile of the oxidoreductase and the two sulfur atoms of the substrate disulfide bond must line up at an ~180° angle for cleavage to proceed (34, 35). In fact, distorting a protein influences the rate of disulfide bond cleavage by changing the alignment of the sulfur atoms (36, 37). Other factors that will influence substrate specificity are spatial (right place) and temporal (right time) considerations and matching the energetics of the oxidoreductase and the substrate disulfides. An oxidoreductase that exhibits a lower redox potential (more negative value) will only donate electrons to or cleave the disulfide of a substrate with a higher redox potential (more positive value).

Transglutaminase-2 (TG2) provides one recent example where location and redox potentials are critical elements in defining disulfide bond regulation. TG2 is ubiquitously expressed in mammals and has a wide range of functions. Inside the cell, TG2 functions as a GTPase and participates in cell signaling. Outside the cell, TG2 converts glutamine residues on substrate proteins to glutamate. The enzyme is also an autoantigen in celiac disease (38). Enzyme assays coupled with structural studies have defined an allosteric disulfide linking cysteines 370 and 371 as a switch that turns TG2 on (reduced) or off (oxidized) in the extracellular matrix (Fig. 3*A*). *Ex vivo* and *in vivo* studies have shown that the bond is reduced by thioredoxin (39, 40) and oxidized by ERp57 (41) in the extracellular space. The protein conformational transitions triggered by cleavage or formation of the bond mediate allosteric control of enzyme activity (42). The Cys-370–Cys-371 disulfide has a standard redox potential of −184 mV (39), which fits with the redox potentials of the switching oxidoreductases. Thioredoxin has a redox potential of −270 mV (43), which is in accordance with its function as a TG2 reductant, whereas the redox potentials of the two catalytic dithiols/disulfides of ERp57 are −167 mV (*a*-domain) and −156 mV (*a′*-domain) (44), which is consistent with its role as TG2 oxidizer.

Vascular thiol isomerases—The role of oxidoreductases and their substrates in thrombosis and hemostasis is a particularly active area of research. This activity has been stimulated by findings that the extracellular actions of five oxidoreductases, the vascular thiol isomerases PDI (45), ERp57 (46), ERp5 (47), ERp72 (48), and TMX1 (31), are essential for normal thrombosis in mice (31, 49). PDI, ERp5, ERp57, and ERp72 are soluble proteins secreted by activated platelets and endothelial cells, whereas TMX1 is an intrinsic membrane protein of the platelet plasma membrane. Interestingly, the soluble vascular thiol isomerases are pro-thrombotic *in vivo*, whereas TM1 is antithrombotic. Platelet-specific knockouts of the individual soluble vascular thiol isomerases all result in decreased platelet incorporation into growing thrombi, whereas knockout of TMX1 increases platelet incorporation.

A function of one of the vascular thiol isomerases, ERp5, was recently elucidated. Blood vessel damage leads to blood platelet aggregation at the injury site. The aggregation is largely mediated by cross-linking of platelet αIIbβ3 integrin receptors on different platelets by the bivalent circulating ligand, fibrinogen. It is critical that platelet aggregation is spatially constrained so that the platelet plug does not occlude the blood vessel and damage the tissue supplied by the vessel. One mechanism by which this process appears to be controlled is through secretion of ERp5 from activated platelets (47, 50). ERp5 binds to the β3 subunit of activated αIIbβ3 integrin and cleaves the βI-domain Cys-177–Cys-184 disulfide bond at the rim of the fibrinogen-binding pocket (51). Cleavage of the disulfide changes the conformation of the binding pocket and triggers release of fibrinogen (Fig. 3*B*).

It is not clear at present whether the five vascular thiol isomerases work independently or cooperatively to control thrombosis. It has been proposed that they may function as a redox chain (49), shuffling electrons among themselves and to substrate disulfides. The PDI family factors have been shown to exchange electrons with each other (52), so this scenario is feasible. Examination of the redox potentials of the soluble vascular thiol isomerases provides, in principle, support for this idea (Table 2). It is conceivable that electrons flow from thioredoxin to ERp72 to ERp5 to PDI to ERp57, and to substrate disulfides at any point in the chain (Fig. 4). It is also possible that electrons could skip ERp5 and flow directly from ERp72 to PDI, for example. Outstanding questions for this model are the original source of the electrons and whether there are other as yet unknown factors in the chain.

Pathological thrombosis is the first human disease where allosteric disulfides are being targeted for treatment. The flavonoid quercetins were discovered in a high-throughput screen of an annotated library of known bioactive compounds to be inhibitors of the vascular thiol isomerase, PDI. Quercetin–3-rutinoside blocks PDI activity by binding to the substrate-binding pocket and inducing a conformational change that impairs protein substrate binding (53). Isoquercetin, which has a glycoside at the third position on the C ring, has improved bioavailability in humans and was selected for clinical development (54). It is membrane-impermeable, orally-available, inhibits PDI activity and thrombus formation in mice and plasma PDI activity and platelet-dependent thrombin generation in humans. Isoquercetin is currently being tested as an antithrombotic in a cancer thrombosis clinical trial (54).

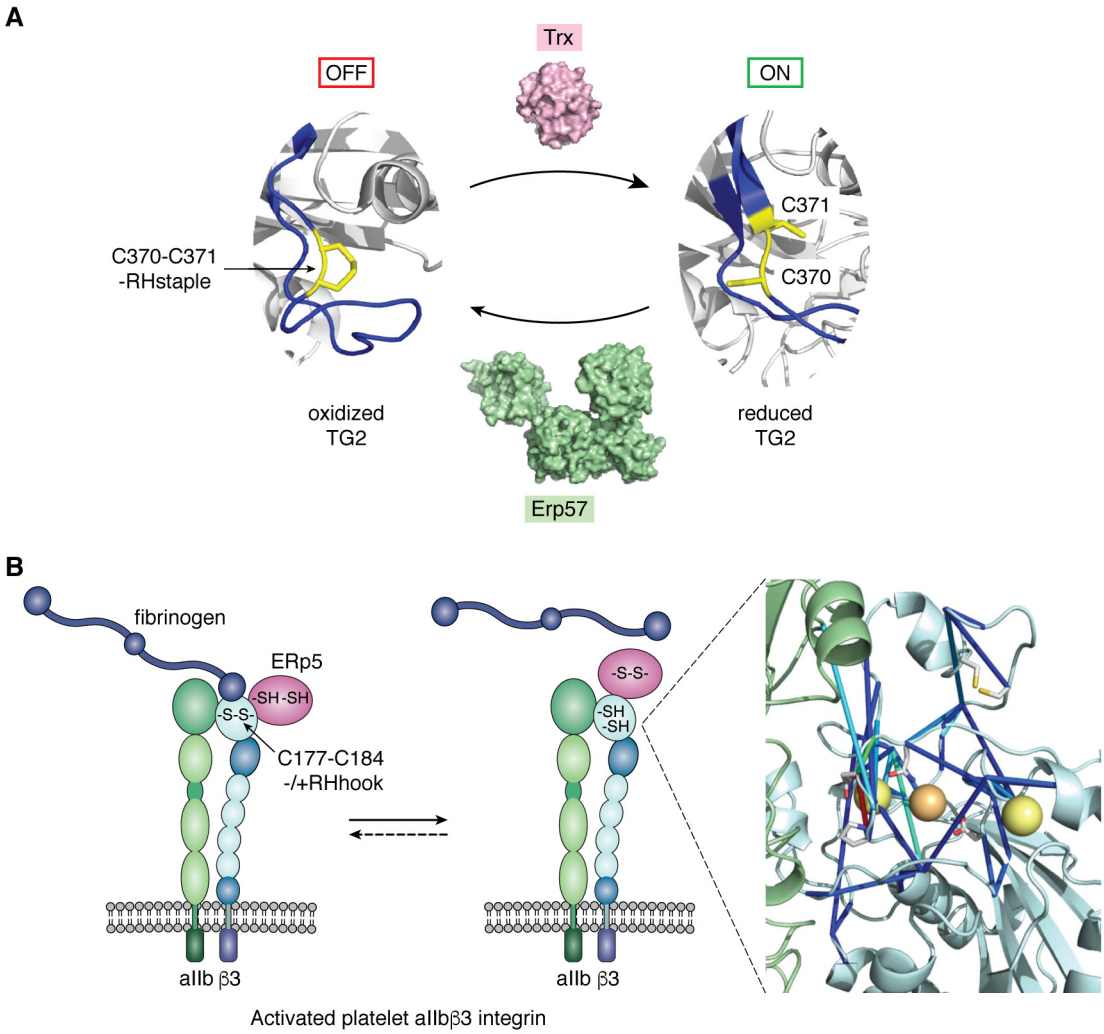

Figure 3. Allosteric disulfides in transglutaminase-2 and αIIbβ3 integrin. *A,* Cys-370–Cys-371 disulfide is a switch that turns TG2 "on" (reduced) or "off" (oxidized) in the extracellular matrix. The bond is reduced by thioredoxin and oxidized by ERp57. Control of enzyme activity is via conformational transitions in the protein upon cleavage or formation of the disulfide. The disulfide cysteines are in *yellow*, and local conformational changes are highlighted in *blue ribbon*. The oxidized TG2 structure is of PDB identifier 2q3z (42) and reduced structure of 1kv3 (105). The surface representation of the thioredoxin and ERp57 structures are of PDB identifiers 1aiu (106) and 3f8u (107), respectively. *B,* ERp5 (*red oval*) triggers fibrinogen release from activated platelet αIIbβ3 integrin by cleaving the βI-domain (*cyan oval*) Cys-177–Cys-184 disulfide bond. Molecular dynamics simulations show that cleavage of the disulfide results in long-range allosteric effects within the βI-domain, including in the metal-binding sites that are critical for fibrinogen binding. The *blue sticks* represent the allosteric signaling network measured by force distribution analysis (51). The network involves both cysteines, Asp-119 that is critical for ligand binding, and Asp-217 and Asn-214 that are involved in positioning of the calcium (*yellow spheres*) and magnesium (*orange sphere*) ions. The αIIb β-propeller domain is the *green cartoon* and the βI-domain the *cyan cartoon*.

Table 2
Redox potentials of human oxidoreductase catalytic disulfides

Oxidoreductase	Domain	Disulfide	E'_o	Refs.
			mV	
Thioredoxin		Cys-32–Cys-35	−270	43
ERp72	*a*	Cys-91–Cys-94	−217	[a]
	a′	Cys-206–Cys-209	−215	
	a″	Cys-555–Cys-558	−220	
ERp5	*a*	Cys-55–Cys-58	−206	51
	a′	Cys-190–Cys-193	−211	
PDI	*a*	Cys-53-Cys-56	−191	104
	a′	Cys-397-Cys-400	−190	
ERp57	*a*	Cys-57–Cys-60	−167	44
	a′	Cys-406–Cys-409	−156	

[a] J. Chiu and P. Hogg, unpublished data.

Oxidoreductase SNPs and disease—Evidence for involvement of oxidoreductases in human disease, whether detrimental or protective, has mainly been correlative thus far. The first molecular evidence for a causative role came from exome sequencing that identified rare SNPs in oxidoreductases associated with amyotrophic lateral sclerosis (ALS) (55). A total of 16 novel SNPs in PDI and ERp57 were identified in patients with familial or sporadic ALS that are not present or are rarely found in healthy controls.

Of the SNPs, four variants (PDID292N, PDIR300H, ERp57D271N, and ERp57Q481K) have been studied with respect to effect on PDI and ERp57 function in protein maturation in the ER and a role in pathogenesis of ALS (56). Expression of the mutant oxidoreductases in murine motoneuron cell lines impairs dendritic branching and thus neuronal outgrowth. The functional consequences of three of the mutations have been studied. The PDI D292N mutation alters PDI interaction with its ER substrates by restricting substrate access to the binding pocket, whereas the R300H mutation has the opposite effect. The ERp57 D271N mutation creates a new *N*-linked

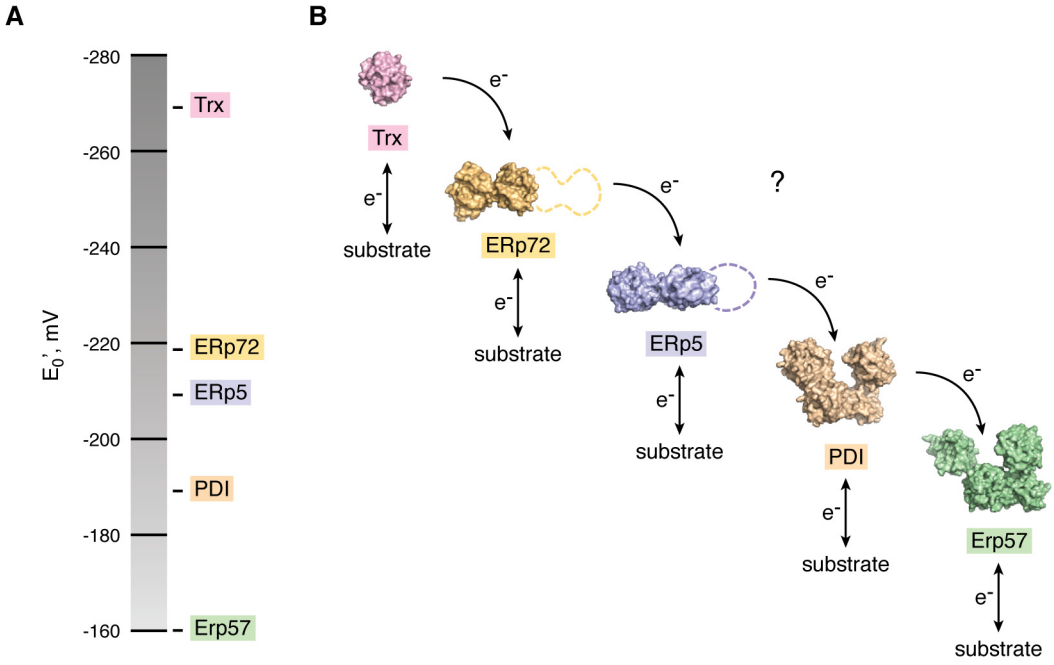

Figure 4. Possible redox chain of the vascular thiol isomerases. The vascular thiol isomerases that include PDI, ERp57, ERp5, and ERp72 are required for normal thrombosis in mice. *A,* standard redox potentials of the vascular thiol isomerases (Table 2). *B,* it is possible that the vascular thiol isomerases function as a redox chain delivering electrons to each other and the substrate disulfides. Their relative redox potentials are consistent with this notion. The surface representations of the oxidoreductases are from the following PDB identifiers: 1aiu for thioredoxin (106); 3idv for ERp72 (108); 3w8j (*a*-domain (109)) and 4gwr (*a′*-domain) for ERp5; 4ekz for PDI (102); and 3f8u for ERp57 (107). The *dashed lines* in ERp72 and ERp5 represent an unknown structure.

glycosylation site in the protein that impacts ERp57 function in ER homeostasis. It is not known whether these SNPs impair function of the secreted oxidoreductases and their cleavage of allosteric disulfides, although it is likely that they will.

Thiol–disulfide exchange

Cleavage of disulfides by thiol–disulfide exchange occurs within or between proteins or between proteins and small molecules. In contrast to oxidoreductases, this cleavage does not require additional electrons (Fig. 2*B*). For intramolecular reactions, all that is required is a conformational change in the substrate protein to bring the three sulfur atoms involved in-line (*i.e.* the sulfur ion nucleophile and the two sulfur atoms of the disulfide bond). This mechanism of cleavage could be triggered by ligand binding and mechanical forces, for instance. Allosteric disulfide bonds in plasminogen (57) and vascular endothelial growth factors C and D (58), for example, are cleaved by thiol–disulfide exchange.

Hydrolysis

A hydrolytic mechanism of cleavage of a disulfide bond (Fig. 2*C*) was recently discovered in an engineered protein (59). A tetrameric metalloprotein with disulfide bonds at the subunit interfaces was constructed, and removal of the tetramer's four Zn^{2+} ions resulted in cleavage of one of the bonds. A sulfenic acid/thiol pair was resolved in the X-ray structure of the Zn^{2+}-depleted protein, which implies hydrolytic cleavage. This finding is the first example of this mechanism of cleavage, and its significance for native disulfide bonds remains to be determined.

Why allosteric disulfides are cleaved

As mentioned above, the reduction of a disulfide bond depends on geometry of the atoms involved and the energetics of the disulfide bond to be cleaved. A protein's backbone and the geometry of the disulfide bonds that link it can change shape (60). For instance, the Cys-119–Cys-205 disulfide bond in the HIV envelope glycoprotein, gp120, can adopt five different conformations in different structures of the protein (60). However, the current indications are that allosteric bonds strongly favor one conformation only. To appreciate the significance this finding, we must first discuss how disulfide conformation is classified and how conformation influences bond cleavage.

Our laboratory devised a classification system for disulfide bonds when we first described allosteric disulfides in 2006 (4). We thought at the time that allosteric bonds might prefer certain conformations, and we needed a scheme to classify disulfides based on conformation. The knowledge gained since 2006 supports this hypothesis. The classification system is founded on the geometry of the five dihedral or χ angles that describe the cystine residue (4). The dihedral angle in chemistry is the angle between planes through two sets of three atoms, having two atoms in common. For example, the S–S bond dihedral angle is the angle between the planes created by the $C\beta$, $S\gamma$, and $S\gamma'$ atoms, and the $C\beta'$, $S\gamma'$, and $S\gamma$ atoms (Fig. 5*A*). The signs of the central three dihedral angles define whether the bonds are spirals (−,−,− or +,+,+), hooks (+,−,− or +,+,−), or staples (−,+,− or +,−,+). The S–S bond dihedral angle determines whether the conformation is right-handed (+) or left handed (−). The signs of the two $C\alpha$–$C\beta$ dihedral angles determine

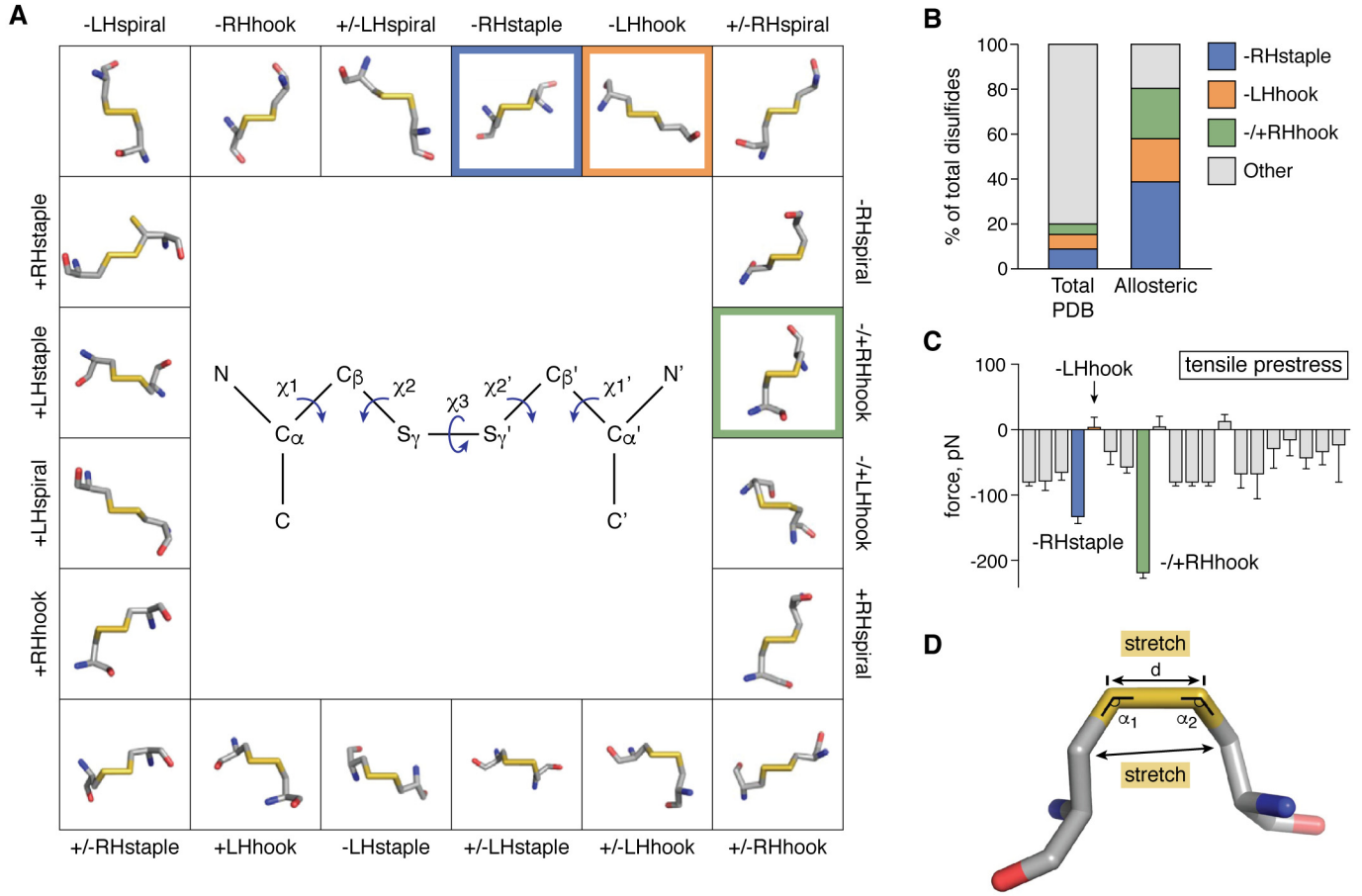

Figure 5. Classification of disulfide bonds and allosteric conformations. *A,* classification of disulfide bonds using the five dihedral angles of the cystine residue (*center*). There are 20 possible disulfide conformations using these angles, and example structures of each from the PDB are shown. *B,* incidence of the three allosteric disulfide conformations. The −RHstaple, −LHhook, and −/+RHhook conformations constitute ~20% of all nonredundant disulfide bonds in the PDB, but ~80% of the structurally defined allosteric bonds ($n = 32$ (61)). *C,* allosteric −RHstaple and −/+RHhook conformations are naturally strained. The pairwise forces between the cysteine residues of the 20 different disulfide conformations were calculated using force distribution analysis (65). The stresses on the −RHstaple and −/+RHhook disulfide bonds are significantly higher than the other 18 conformations. The bonded pre-stress (or tensile pre-stress) measure comprises all force terms involving the sulfur atoms of the disulfide, so is the relevant indicator for disulfide reactivity. Mean tensile pre-stress of the 20 disulfide conformations was adapted from Fig. 2*D* of Zhou *et al.* (65). *D,* tensile pre-stress of the −RHstaple and −/+RHhook bonds is a result of stretching of the sulfur–sulfur bond length (indicated by *d*) and neighboring α angles.

whether the bond is termed − (both angles are negative), + (both angles are positive), −/+, or +/−. Twenty possible disulfide bond conformations are possible using this convention, and all 20 are represented in protein structures (Fig. 5*A*).

The −LHspiral conformation accounts for about one-quarter of all disulfide bonds and is often associated with stable, typically structural bonds (4). The disulfides at the active sites of oxidoreductases are nearly always +/−RHhooks because they occur in the same relatively rigid thioredoxin-domain fold. Allosteric disulfide bonds are most often associated with one of three conformations (Fig. 5*B*). The −RHstaple is the archetypal allosteric conformation and accounts for 14 of the 36 structurally characterized allosteric disulfides to date (61, 62). There are eight examples of −LHhooks and seven examples of −/+RHhooks, so far. The protein secondary structures linked by the −RHstaple bonds have some recurring features. These disulfides often link adjacent strands in antiparallel β-sheets, also known as cross-strand bonds (63, 64), adjacent (vicinal) cysteines in the polypeptide chain and constrain β-loop structures. The secondary structures linked by −LHhooks and −/+RHhook bonds, however, are more variable, and no pattern is apparent at this time. For example, the TG2 Cys-370–Cys-371 disulfide links adjacent cysteines and has a −RHstaple conformation (42), whereas the αIIbβ3 integrin Cys-177–Cys-184 bond links a hydrogen-bonded turn and a loop structure and has a −/+RHhook conformation (Fig. 3) (51). The reasons why the −RHstaple and −/+RHhook conformations are associated with allosteric function are beginning to emerge.

In essence, the protein's structure prevents the −RHstaple and −/+RHhook disulfides from fully relaxing into the state of minimal potential energy, so the bonds carry a "tensile prestress" (Fig. 5*C*) (4, 65). The stress mostly derives from stretching of the S–S bond and neighboring α angles (Fig. 5*D*) and is expected to fine-tune disulfide cleavage and thus the function of the protein. In support of this scenario, stretching of S–S bonds has been demonstrated experimentally to increase their susceptibility to cleavage (35–37, 66). The natural strain of the −RHstaple and −/+RHhook disulfides may have contributed to their evolution as allosteric bonds. Their propensity for cleavage coupled with a positive functional change would have resulted in evolutionary pressure to retain the bonds. In contrast, the −LHhook conformation is no more stressed than the

other 18 conformations. It is an open question why this bond type associated with allosteric function. It is possible the −LHhook conformation is better suited to one type of cleavage, such as thiol/disulfide exchange (Fig. 2*B*), but this hypothesis remains to be examined.

Interestingly, structural and molecular dynamics studies of the tissue factor (67, 68), CD4 (64, 65), von Willebrand factor (69), and integrin β3 (51) allosteric disulfides show that they always adopt the same conformation. The tissue factor, CD4, and von Willebrand factor disulfides always have a −RHstaple conformation, whereas the integrin β3 allosteric bond is always a −/+RHhook. This situation is due to topological features that constrain the secondary protein structure in which the bond resides. Such constraints likely set the bond up for cleavage and also enable it to reform when required. In contrast, dynamic isomerization of certain allosteric disulfide bonds is possibly an important component of their function, *i.e.* cleavage of the bond may only occur when the disulfide adopts a particular conformation in response to events such as ligand binding or mechanical forces. For instance, the Cys-46–Cys-99 disulfide in interleukin-4 that is cleaved by extracellular thioredoxin to inactivate the cytokine (70) can adopt 10 different conformations in NMR structures of the soluble protein, including an allosteric −/+RHhook conformation. This particular scenario has yet to be tested experimentally.

Tools and techniques for exploring allosteric disulfides

As mentioned above, TSP-1 was the first protein discovered to have an allosteric disulfide. The key ingredient to this discovery was the identification of a mAb that recognizes different disulfide-bonded forms of TSP-1. The antibody was used to show that cultured mammalian cells make the different TSP-1 forms and that PDI changes the balance of the forms. Of course, techniques have advanced since the 1990s, but the identification and study of functional disulfide bonds are being held back by technical hurdles. There are some simple tools that point to the presence of a redox-active disulfide in a protein, such as reporter-tagged thiol-reactive compounds that react with the cysteine thiols of reduced allosteric bonds. Biotin-linked maleimides are often used and protein labeling measured using streptavidin-based probes. Change in incorporation of the label is an indication of cleavage or formation of an allosteric bond. A shortcoming of this technique is that it does not provide a quantitative measure of disulfide cleavage or formation in the protein population. Does the event occur in 0.1 or 50% of the protein molecules? If it is occurring in only a minor fraction of the protein, it may not be relevant. Also, if the protein being studied has more than one disulfide bond, which is often the case, this technique does not indicate which is being cleaved.

To properly analyze an allosteric disulfide, it is important to quantify its redox state in the protein population and how this state shifts under different conditions relevant to the protein in question. Unfortunately, this measurement is far from routine at present. The best method currently is a differential cysteine alkylation and MS technique (51, 71). Reduced disulfide bond cysteines in the protein are first alkylated with thiol-specific 2-iodo-*N*-phenylacetamide ([^{12}C]IPA). The protein is then resolved on SDS-PAGE, the disulfide bonds reduced with DTT, and the disulfide cysteines alkylated with a carbon-13 isotope of IPA ([^{13}C]IPA). The protein is digested, the peptides separated using LC and their identity determined by MS. The LC peak areas of peptides containing the disulfide bond cysteines labeled with either [^{12}C]IPA or [^{13}C]IPA are calculated. The ratio of [^{12}C]IPA to [^{12}C]IPA + [^{13}C]IPA for the peptides represents the fraction of the disulfide in the protein population that is in the reduced state. The advantage of this pair of cysteine alkylators is that they have the same efficiency of reactivity and the same structure, which enhances the reliability of alkylation and resolution of the alkylated peptides by LC and their detection by MS. A mass difference of 6 Da is the only change in a cysteine-containing peptide labeled with [^{12}C]IPA or [^{13}C]IPA. This method was used to measure the redox state of 24 of the 28 disulfide bonds in the β3 subunit of platelet αIIbβ3 integrin (51). The downside is that the method takes several months to perfect for a new user and needs to be optimized for each protein.

What the field needs is a method to quantify disulfide bond cleavage that is as easy and quick to use as the routine method to quantify peptide bond cleavage. The study of proteolytic control of protein function languished until the advent of SDS-PAGE that enabled precise quantification of this chemical event. The equivalent of an SDS-PAGE–type method to quantify allosteric disulfide bonds in complex protein mixtures would enable rapid advances in our understanding of and appreciation for this post-translational modification.

How to target allosteric disulfides

As most allosteric disulfides function outside the cell, they are excellent drug targets. Inhibitors do not need to cross the plasma membrane to function, which also markedly reduces the chances of off-target effects. Allosteric disulfides can be targeted by both biologicals and small molecules. Monoclonal antibodies could take out either the reduced or oxidized form of a protein or the factor that cleaves or forms the bond. Small molecules can be designed to cleave an allosteric disulfide or prevent one from forming. They can also be designed to inhibit the cleaving factors, such as the PDI inhibitor isoquercetin (see above).

Cleavage of an allosteric disulfide bond can be achieved using targeted cysteines. For example, an allosteric disulfide bond in the HIV envelope glycoprotein, gp120, has been targeted using this approach (72, 73). HIV gp120 binds to CD4 and a chemokine receptor on immune cells that leads to infection of the cell. CD4 mimetics that contain a cysteine sulfur ion nucleophile bind to gp120 and cleave the Cys-126–Cys-196 allosteric disulfide, which results in a stable mixed disulfide linking the two molecules and inhibition of HIV infection.

The reduced form of an allosteric disulfide can be targeted using thiol-reactive moieties that will prevent the bond from re-forming (74). Co-location of an allosteric protein cysteine thiol with a poorly reactive thiol alkylator will result in specific alkylation of the allosteric cysteine, rendering it unable to reform the disulfide. Thiol alkylators containing weak electrophilic moieties such as chloroacetyl, acrylamide, or aminomethyl methyl acrylate are useful for this purpose. These compounds react very slowly with unpaired cysteines unless they

contain a targeting moiety that brings them into close proximity with the allosteric cysteine. For instance, specific covalent coupling of a ligand containing a chloroacetyl group with a cysteine in an antibody-binding pocket was achieved in mouse ascites fluid (110).

The future of allosteric disulfides

Allosteric disulfides are proving to be an important aspect of protein function across biological systems and life forms. It is only relatively recently that investigators have become aware of these bonds and are finding more and more each year. Considering that about 1 in 5 disulfides in proteins in the Protein Data Bank have 1 of the 3 allosteric conformations (Fig. 5*B*), there may be hundreds, if not thousands, of allosteric disulfides. Indeed, it may be that as many disulfide bonds are cleaved in proteins as peptide bonds. As a cleaved disulfide bond can reform, as seen in the example of TG-2, allosteric disulfides have the potential to reform, unlike peptide bonds. This reversibility provides for a more sophisticated control of protein function compared with proteolysis and is perhaps one of the reasons why disulfide bonds have accrued faster in complex organisms (1).

It is a helpful starting point to consider that a potential allosteric disulfide exists in an equilibrium between cleaved and bound forms in the protein population and that perturbation of the equilibrium state one way or the other has different functional consequences. When studying an allosteric disulfide, it is therefore wise to consider factors that will cleave or form the bond and not presuppose which event is more relevant. However, some cleaved disulfides will not reform due to downstream effects, such as proteolysis of the protein. This situation is the case for the allosteric disulfides in plasmin (57).

A surprise is that disulfide bonds are not limited to secreted proteins, which was traditionally thought to be their purview. About 300 proteins that function in the cytoplasm and/or nucleus contain about 500 structurally-defined disulfide bonds (Table S1) (61), and some of these bonds appear to have an allosteric role. How these bonds form in the cytoplasm and nucleus and how their redox state is controlled in these environments are important questions. PDI, the archetypal oxidoreductase that was originally thought to reside only in ER, has been found associated with the actin cytoskeleton (75). This observation suggests that PDI may facilitate disulfide bond formation in the cytosol, as it does in the ER.

Allosteric disulfides are a supermarket of new drug targets. The most advanced drug in this class is isoquercetin, the PDI inhibitor that is in Phase II testing in a cancer thrombosis trial (54). This trial is an important milestone in the field and is the first of what will likely be many attempts to target this post-translational modification for the treatment of disease. We anticipate that the next 2 decades will see therapeutics that target allosteric disulfides being approved for treatment of unwanted thrombosis and other human conditions.

References

1. Wong, J. W., Ho, S. Y., and Hogg, P. J. (2011) Disulfide bond acquisition through eukaryotic protein evolution. *Mol. Biol. Evol.* **28,** 327–334 CrossRef Medline
2. Brooks, D. J., Fresco, J. R., Lesk, A. M., and Singh, M. (2002) Evolution of amino acid frequencies in proteins over deep time: inferred order of introduction of amino acids into the genetic code. *Mol. Biol Evol.* **19,** 1645–1655 CrossRef Medline
3. Jordan, I. K., Kondrashov, F. A., Adzhubei, I. A., Wolf, Y. I., Koonin, E. V., Kondrashov, A. S., and Sunyaev, S. (2005) A universal trend of amino acid gain and loss in protein evolution. *Nature* **433,** 633–638 CrossRef Medline
4. Schmidt, B., Ho, L., and Hogg, P. J. (2006) Allosteric disulfide bonds. *Biochemistry* **45,** 7429–7433 CrossRef Medline
5. Hogg, P. J. (2003) Disulfide bonds as switches for protein function. *Trends Biochem. Sci.* **28,** 210–214 CrossRef Medline
6. Monod, J., Wyman, J., and Changeux, J. P. (1965) On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* **12,** 88–118 CrossRef Medline
7. Hotchkiss, K. A., Chesterman, C. N., and Hogg, P. J. (1996) Catalysis of disulfide isomerization in thrombospondin 1 by protein disulfide isomerase. *Biochemistry* **35,** 9761–9767 CrossRef Medline
8. Hotchkiss, K. A., Matthias, L. J., and Hogg, P. J. (1998) Exposure of the cryptic Arg-Gly-Asp sequence in thrombospondin-1 by protein disulfide isomerase. *Biochim. Biophys. Acta* **1388,** 478–488 CrossRef Medline
9. Hogg, P. J., Hotchkiss, K. A., Jiménez, B. M., Stathakis, P., and Chesterman, C. N. (1997) Interaction of platelet-derived growth factor with thrombospondin 1. *Biochem. J.* **326,** 709–716 CrossRef Medline
10. Speziale, M. V., and Detwiler, T. C. (1990) Free thiols of platelet thrombospondin. Evidence for disulfide isomerization. *J. Biol. Chem.* **265,** 17859–17867 Medline
11. Sun, X., Skorstengaard, K., and Mosher, D. F. (1992) Disulfides modulate RGD-inhibitable cell adhesive activity of thrombospondin. *J. Cell Biol.* **118,** 693–701 CrossRef Medline
12. Braakman, I., and Bulleid, N. J. (2011) Protein folding and modification in the mammalian endoplasmic reticulum. *Annu. Rev. Biochem.* **80,** 71–99 CrossRef Medline
13. Nakamoto, H., and Bardwell, J. C. (2004) Catalysis of disulfide bond formation and isomerization in the *Escherichia coli* periplasm. *Biochim. Biophys. Acta* **1694,** 111–119 CrossRef Medline
14. Anfinsen, C. B. (1973) Principles that govern the folding of protein chains. *Science* **181,** 223–230 CrossRef Medline
15. Anfinsen, C. B., Haber, E., Sela, M., and White, F. H., Jr. (1961) The kinetics of formation of native ribonuclease during oxidation of the reduced polypeptide chain. *Proc. Natl. Acad. Sci. U.S.A.* **47,** 1309–1314 CrossRef Medline
16. Staley, J. P., and Kim, P. S. (1994) Formation of a native-like subdomain in a partially folded intermediate of bovine pancreatic trypsin inhibitor. *Protein Sci.* **3,** 1822–1832 CrossRef Medline
17. van Mierlo, C. P., Kemmink, J., Neuhaus, D., Darby, N. J., and Creighton, T. E. (1994) ^{1}H NMR analysis of the partly-folded non-native two-disulphide intermediates (30–51,5–14) and (30–51,5–38) in the folding pathway of bovine pancreatic trypsin inhibitor. *J. Mol. Biol.* **235,** 1044–1061 CrossRef Medline
18. Creighton, T. E., Darby, N. J., and Kemmink, J. (1996) The roles of partly folded intermediates in protein folding. *FASEB J.* **10,** 110–118 CrossRef Medline
19. Creighton, T. E. (1977) Kinetics of refolding of reduced ribonuclease. *J. Mol. Biol.* **113,** 329–341 CrossRef Medline
20. Creighton, T. E. (1979) Intermediates in the refolding of reduced ribonuclease A. *J. Mol. Biol.* **129,** 411–431 CrossRef Medline
21. Frech, C., and Schmid, F. X. (1995) Influence of protein conformation on disulfide bond formation in the oxidative folding of ribonuclease T1. *J. Mol. Biol.* **251,** 135–149 CrossRef Medline
22. Welker, E., Wedemeyer, W. J., Narayan, M., and Scheraga, H. A. (2001) Coupling of conformational folding and disulfide-bond reactions in oxidative folding of proteins. *Biochemistry* **40,** 9059–9064 CrossRef Medline
23. Wong, J. W., and Hogg, P. J. (2010) Analysis of disulfide bonds in protein structures. *J. Thromb. Haemost.* **8,** 2345 CrossRef
24. Antelmann, H., and Helmann, J. D. (2011) Thiol-based redox switches and gene regulation. *Antioxid. Redox Signal.* **14,** 1049–1063 CrossRef Medline

25. Go, Y. M., and Jones, D. P. (2008) Redox compartmentalization in eukaryotic cells. *Biochim. Biophys. Acta* **1780,** 1273–1290 CrossRef Medline
26. Kozlov, G., Määttänen, P., Thomas, D. Y., and Gehring, K. (2010) A structural overview of the PDI family of proteins. *FEBS J* **277,** 3924–3936 CrossRef Medline
27. Holmgren, A. (1989) Thioredoxin and glutaredoxin systems. *J. Biol. Chem.* **264,** 13963–13966 Medline
28. Farrah, T., Deutsch, E. W., Omenn, G. S., Campbell, D. S., Sun, Z., Bletz, J. A., Mallick, P., Katz, J. E., Malmstrom, J., Ossola, R., Watts, J. D., Lin, B., Zhang, H., Moritz, R. L., and Aebersold, R. (2011) A high-confidence human plasma proteome reference set with estimated concentrations in PeptideAtlas. *Mol. Cell. Proteomics* **10,** M110.006353 CrossRef Medline
29. Holbrook, L. M., Watkins, N. A., Simmonds, A. D., Jones, C. I., Ouwehand, W. H., and Gibbins, J. M. (2010) Platelets release novel thiol isomerase enzymes which are recruited to the cell surface following activation. *Br. J. Haematol.* **148,** 627–637 CrossRef Medline
30. Swiatkowska, M., Padula, G., Michalec, L., Stasiak, M., Skurzynski, S., and Cierniewski, C. S. (2010) Ero1α is expressed on blood platelets in association with protein-disulfide isomerase and contributes to redox-controlled remodeling of αIIbβ3. *J. Biol. Chem.* **285,** 29874–29883 CrossRef Medline
31. Zhao, Z., Wu, Y., Zhou, J., Chen, F., Yang, A., and Essex, D. W. (2019) The transmembrane protein disulfide isomerase TMX1 negatively regulates platelet responses. *Blood* **133,** 246–251 CrossRef Medline
32. Butera, D., Cook, K. M., Chiu, J., Wong, J. W., and Hogg, P. J. (2014) Control of blood proteins by functional disulfide bonds. *Blood* **123,** 2000–2007 CrossRef Medline
33. Stopa, J. D., Baker, K. M., Grover, S. P., Flaumenhaft, R., and Furie, B. (2017) Kinetic-based trapping by intervening sequence variants of the active sites of protein-disulfide isomerase identifies platelet protein substrates. *J. Biol. Chem.* **292,** 9063–9074 CrossRef Medline
34. Fernandes, P. A., and Ramos, M. J. (2004) Theoretical insights into the mechanism for thiol/disulfide exchange. *Chemistry* **10,** 257–266 CrossRef Medline
35. Wiita, A. P., Ainavarapu, S. R., Huang, H. H., and Fernandez, J. M. (2006) Force-dependent chemical kinetics of disulfide bond reduction observed with single-molecule techniques. *Proc. Natl. Acad. Sci. U.S.A.* **103,** 7222–7227 CrossRef Medline
36. Baldus, I. B., and Gräter, F. (2012) Mechanical force can fine-tune redox potentials of disulfide bonds. *Biophys. J.* **102,** 622–629 CrossRef Medline
37. Wiita, A. P., Perez-Jimenez, R., Walther, K. A., Gräter, F., Berne, B. J., Holmgren, A., Sanchez-Ruiz, J. M., and Fernandez, J. M. (2007) Probing the chemistry of thioredoxin catalysis with force. *Nature* **450,** 124–127 CrossRef Medline
38. Dieterich, W., Ehnis, T., Bauer, M., Donner, P., Volta, U., Riecken, E. O., and Schuppan, D. (1997) Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat. Med.* **3,** 797–801 CrossRef Medline
39. Jin, X., Stamnaes, J., Klöck, C., DiRaimondo, T. R., Sollid, L. M., and Khosla, C. (2011) Activation of extracellular transglutaminase 2 by thioredoxin. *J. Biol. Chem.* **286,** 37866–37873 CrossRef Medline
40. Plugis, N. M., Palanski, B. A., Weng, C. H., Albertelli, M., and Khosla, C. (2017) Thioredoxin-1 selectively activates transglutaminase 2 in the extracellular matrix of the small intestine: implications for celiac disease. *J. Biol. Chem.* **292,** 2000–2008 CrossRef Medline
41. Yi, M. C., Melkonian, A. V., Ousey, J. A., and Khosla, C. (2018) Endoplasmic reticulum-resident protein 57 (ERp57) oxidatively inactivates human transglutaminase 2. *J. Biol. Chem.* **293,** 2640–2649 CrossRef Medline
42. Pinkas, D. M., Strop, P., Brunger, A. T., and Khosla, C. (2007) Transglutaminase 2 undergoes a large conformational change upon activation. *PLoS Biol.* **5,** e327 CrossRef Medline
43. Krause, G., Lundström, J., Barea, J. L., Pueyo de la Cuesta, C., and Holmgren, A. (1991) Mimicking the active site of protein disulfide-isomerase by substitution of proline 34 in *Escherichia coli* thioredoxin. *J. Biol. Chem.* **266,** 9494–9500 Medline
44. Frickel, E. M., Frei, P., Bouvier, M., Stafford, W. F., Helenius, A., Glockshuber, R., and Ellgaard, L. (2004) ERp57 is a multifunctional thiol–disulfide oxidoreductase. *J. Biol. Chem.* **279,** 18277–18287 CrossRef Medline
45. Cho, J., Furie, B. C., Coughlin, S. R., and Furie, B. (2008) A critical role for extracellular protein disulfide isomerase during thrombus formation in mice. *J. Clin. Invest.* **118,** 1123–1131 Medline
46. Holbrook, L. M., Sasikumar, P., Stanley, R. G., Simmonds, A. D., Bicknell, A. B., and Gibbins, J. M. (2012) The platelet-surface thiol isomerase enzyme ERp57 modulates platelet function. *J. Thromb. Haemost.* **10,** 278–288 CrossRef Medline
47. Passam, F. H., Lin, L., Gopal, S., Stopa, J. D., Bellido-Martin, L., Huang, M., Furie, B. C., and Furie, B. (2015) Both platelet- and endothelial cell-derived ERp5 support thrombus formation in a laser-induced mouse model of thrombosis. *Blood* **125,** 2276–2285 CrossRef Medline
48. Zhou, J., Wu, Y., Chen, F., Wang, L., Rauova, L., Hayes, V. M., Poncz, M., Li, H., Liu, T., Liu, J., and Essex, D. W. (2017) The disulfide isomerase ERp72 supports arterial thrombosis in mice. *Blood* **130,** 817–828 CrossRef Medline
49. Flaumenhaft, R., and Furie, B. (2016) Vascular thiol isomerases. *Blood* **128,** 893–901 CrossRef Medline
50. Jordan, P. A., Stevens, J. M., Hubbard, G. P., Barrett, N. E., Sage, T., Authi, K. S., and Gibbins, J. M. (2005) A role for the thiol isomerase protein ERP5 in platelet function. *Blood* **105,** 1500–1507 CrossRef Medline
51. Passam, F., Chiu, J., Ju, L., Pijning, A., Jahan, Z., Mor-Cohen, R., Yeheskel, A., Kolšek, K., Thärichen, L., Aponte-Santamaría, C., Gräter, F., and Hogg, P. J. (2018) Mechano-redox control of integrin de-adhesion. *Elife* **7,** e34843 CrossRef Medline
52. Oka, O. B., Yeoh, H. Y., and Bulleid, N. J. (2015) Thiol-disulfide exchange between the PDI family of oxidoreductases negates the requirement for an oxidase or reductase for each enzyme. *Biochem. J.* **469,** 279–288 CrossRef Medline
53. Lin, L., Gopal, S., Sharda, A., Passam, F., Bowley, S. R., Stopa, J., Xue, G., Yuan, C., Furie, B. C., Flaumenhaft, R., Huang, M., and Furie, B. (2015) Quercetin-3-rutinoside inhibits protein disulfide isomerase by binding to its b'x domain. *J. Biol. Chem.* **290,** 23543–23552 CrossRef Medline
54. Stopa, J. D., Neuberg, D., Puligandla, M., Furie, B., Flaumenhaft, R., and Zwicker, J. I. (2017) Protein disulfide isomerase inhibition blocks thrombin generation in humans by interfering with platelet factor V activation. *JCI Insight* **2,** e89373 Medline
55. Gonzalez-Perez, P., Woehlbier, U., Chian, R. J., Sapp, P., Rouleau, G. A., Leblond, C. S., Daoud, H., Dion, P. A., Landers, J. E., Hetz, C., and Brown, R. H. (2015) Identification of rare protein disulfide isomerase gene variants in amyotrophic lateral sclerosis patients. *Gene* **566,** 158–165 CrossRef Medline
56. Woehlbier, U., Colombo, A., Saaranen, M. J., Pérez, V., Ojeda, J., Bustos, F. J., Andreu, C. I., Torres, M., Valenzuela, V., Medinas, D. B., Rozas, P., Vidal, R. L., Lopez-Gonzalez, R., Salameh, J., Fernandez-Collemann, S., *et al.* (2016) ALS-linked protein disulfide isomerase variants cause motor dysfunction. *EMBO J.* **35,** 845–865 CrossRef Medline
57. Butera, D., Wind, T., Lay, A. J., Beck, J., Castellino, F. J., and Hogg, P. J. (2014) Characterization of a reduced form of plasma plasminogen as the precursor for angiostatin formation. *J. Biol. Chem.* **289,** 2992–3000 CrossRef Medline
58. Chiu, J., Wong, J. W., Gerometta, M., and Hogg, P. J. (2014) Mechanism of dimerization of a recombinant mature vascular endothelial growth factor C. *Biochemistry* **53,** 7–9 CrossRef Medline
59. Churchfield, L. A., Medina-Morales, A., Brodin, J. D., Perez, A., and Tezcan, F. A. (2016) De novo design of an allosteric metalloprotein assembly with strained disulfide bonds. *J. Am. Chem. Soc.* **138,** 13163–13166 CrossRef Medline
60. Schmidt, B., and Hogg, P. J. (2007) Search for allosteric disulfide bonds in NMR structures. *BMC Struct. Biol.* **7,** 49 CrossRef Medline
61. Pijning, A. E., Chiu, J., Yeo, R. X., Wong, J. W. H., and Hogg, P. J. (2018) Identification of allosteric disulfides from labile bonds in X-ray structures. *R. Soc. Open Sci.* **5,** 171058 CrossRef Medline
62. Pijning, A. E., and Hogg, P. J. (2018) Allosteric Disulphide Bonds. In *Oxidative Folding of Proteins and Peptides* (Feige, M., ed) pp. 152–174, The Royal Society of Chemistry, London
63. Wouters, M. A., Lau, K. K., and Hogg, P. J. (2004) Cross-strand disulphides in cell entry proteins: poised to act. *Bioessays* **26,** 73–79 CrossRef Medline

64. Matthias, L. J., Yam, P. T., Jiang, X. M., Vandegraaff, N., Li, P., Poumbourios, P., Donoghue, N., and Hogg, P. J. (2002) Disulfide exchange in domain 2 of CD4 is required for entry of HIV-1. *Nat. Immunol.* **3,** 727–732 CrossRef Medline
65. Zhou, B., Baldus, I. B., Li, W., Edwards, S. A., and Gräter, F. (2014) Identification of allosteric disulfides from prestress analysis. *Biophys. J.* **107,** 672–681 CrossRef Medline
66. Li, W., and Gräter, F. (2010) Atomistic evidence of how force dynamically regulates thiol/disulfide exchange. *J. Am. Chem. Soc.* **132,** 16790–16795 CrossRef Medline
67. Zhou, B., Hogg, P. J., and Gräter, F. (2017) One-way allosteric communication between the two disulfide bonds in tissue factor. *Biophys. J.* **112,** 78–86 CrossRef Medline
68. Chen, V. M., Ahamed, J., Versteeg, H. H., Berndt, M. C., Ruf, W., and Hogg, P. J. (2006) Evidence for activation of tissue factor by an allosteric disulfide bond. *Biochemistry* **45,** 12020–12028 CrossRef Medline
69. Butera, D., Passam, F., Ju, L., Cook, K. M., Woon, H., Aponte-Santamaría, C., Gardiner, E., Davis, A. K., Murphy, D. A., Bronowska, A., Luken, B. M., Baldauf, C., Jackson, S., Andrews, R., Gräter, F., and Hogg, P. J. (2018) Autoregulation of von Willebrand factor function by a disulfide bond switch. *Sci. Adv.* **4,** eaaq1477 CrossRef Medline
70. Plugis, N. M., Weng, N., Zhao, Q., Palanski, B. A., Maecker, H. T., Habtezion, A., and Khosla, C. (2018) Interleukin 4 is inactivated via selective disulfide-bond reduction by extracellular thioredoxin. *Proc. Natl. Acad. Sci. U.S.A.* **115,** 8781–8786 CrossRef Medline
71. Pasquarello, C., Sanchez, J. C., Hochstrasser, D. F., and Corthals, G. L. (2004) *N-t*-Butyliodoacetamide and iodoacetanilide: two new cysteine alkylating reagents for relative quantitation of proteins. *Rapid Commun. Mass Spectrom.* **18,** 117–127 CrossRef Medline
72. Cerutti, N., Mendelow, B. V., Napier, G. B., Papathanasopoulos, M. A., Killick, M., Khati, M., Stevens, W., and Capovilla, A. (2010) Stabilization of HIV-1 gp120-CD4 receptor complex through targeted interchain disulfide exchange. *J. Biol. Chem.* **285,** 25743–25752 CrossRef Medline
73. Martin, G., Burke, B., Thaï, R., Dey, A. K., Combes, O., Ramos, O. H., Heyd, B., Geonnotti, A. R., Montefiori, D. C., Kan, E., Lian, Y., Sun, Y., Abache, T., Ulmer, J. B., Madaoui, H., Guérois, R., *et al.* (2011) Stabilization of HIV-1 envelope in the CD4-bound conformation through specific cross-linking of a CD4 mimetic. *J. Biol. Chem.* **286,** 21706–21716 CrossRef Medline
74. Hogg, P. J. (2013) Targeting allosteric disulphide bonds in cancer. *Nat. Rev. Cancer* **13,** 425–431 CrossRef Medline
75. Sobierajska, K., Skurzynski, S., Stasiak, M., Kryczka, J., Cierniewski, C. S., and Swiatkowska, M. (2014) Protein disulfide isomerase directly interacts with β-actin Cys374 and regulates cytoskeleton reorganization. *J. Biol. Chem.* **289,** 5758–5773 CrossRef Medline
76. Ioannou, Y., Zhang, J. Y., Passam, F. H., Rahgozar, S., Qi, J. C., Giannakopoulos, B., Qi, M., Yu, P., Yu, D. M., Hogg, P. J., and Krilis, S. A. (2010) Naturally occurring free thiols within β2-glycoprotein I *in vivo*: nitrosylation, redox modification by endothelial cells, and regulation of oxidative stress-induced cell injury. *Blood* **116,** 1961–1970 CrossRef Medline
77. Auwerx, J., Isacsson, O., Söderlund, J., Balzarini, J., Johansson, M., and Lundberg, M. (2009) Human glutaredoxin-1 catalyzes the reduction of HIV-1 gp120 and CD4 disulfides and its inhibition reduces HIV-1 replication. *Int. J. Biochem. Cell Biol.* **41,** 1269–1275 CrossRef Medline
78. Barbouche, R., Miquelis, R., Jones, I. M., and Fenouillet, E. (2003) Protein-disulfide isomerase-mediated reduction of two disulfide bonds of HIV envelope glycoprotein 120 occurs post-CXCR4 binding and is required for fusion. *J. Biol. Chem.* **278,** 3131–3136 CrossRef Medline
79. Gallina, A., Hanley, T. M., Mandel, R., Trahey, M., Broder, C. C., Viglianti, G. A., and Ryser, H. J. (2002) Inhibitors of protein-disulfide isomerase prevent cleavage of disulfide bonds in receptor-bound glycoprotein 120 and prevent HIV-1 entry. *J. Biol. Chem.* **277,** 50579–50588 CrossRef Medline
80. Ou, W., and Silver, J. (2006) Role of protein disulfide isomerase and other thiol-reactive proteins in HIV-1 envelope protein-mediated fusion. *Virology* **350,** 406–417 CrossRef Medline
81. Reiser, K., François, K. O., Schols, D., Bergman, T., Jörnvall, H., Balzarini, J., Karlsson, A., and Lundberg, M. (2012) Thioredoxin-1 and protein disulfide isomerase catalyze the reduction of similar disulfides in HIV gp120. *Int. J. Biochem. Cell Biol.* **44,** 556–562 CrossRef Medline
82. Azimi, I., Matthias, L. J., Center, R. J., Wong, J. W., and Hogg, P. J. (2010) Disulfide bond that constrains the HIV-1 gp120 V3 domain is cleaved by thioredoxin. *J. Biol. Chem.* **285,** 40072–40080 CrossRef Medline
83. Kellett-Clarke, H., Stegmann, M., Barclay, A. N., and Metcalfe, C. (2015) CD44 binding to hyaluronic acid is redox regulated by a labile disulfide bond in the hyaluronic acid binding site. *PLoS ONE* **10,** e0138137 CrossRef Medline
84. Li, J., Kim, K., Jeong, S., Chiu, J., Xiong, B., Petukhov, P. A., Dai, X., Li, X., Andrews, R. K., Du, X., Hogg, P. J., and Cho, J. (2018) Platelet protein disulfide isomerase promotes glycoprotein Ibα-mediated platelet-neutrophil interactions under thromboinflammatory conditions. *Circulation* **2018,** CrossRef 30586735
85. Metcalfe, C., Cresswell, P., and Barclay, A. N. (2012) Interleukin-2 signalling is modulated by a labile disulfide bond in the CD132 chain of its receptor. *Open Biol.* **2,** 110036 CrossRef Medline
86. Ahamed, J., Versteeg, H. H., Kerver, M., Chen, V. M., Mueller, B. M., Hogg, P. J., and Ruf, W. (2006) Disulfide isomerization switches tissue factor from coagulation to cell signaling. *Proc. Natl. Acad. Sci. U.S.A.* **103,** 13932–13937 CrossRef Medline
87. Bowley, S. R., Fang, C., Merrill-Skoloff, G., Furie, B. C., and Furie, B. (2017) Protein disulfide isomerase secretion following vascular injury initiates a regulatory pathway for thrombus formation. *Nat. Commun.* **8,** 14151 CrossRef Medline
88. Ravilious, G. E., Nguyen, A., Francois, J. A., and Jez, J. M. (2012) Structural basis and evolution of redox regulation in plant adenosine-5′-phosphosulfate kinase. *Proc. Natl. Acad. Sci. U.S.A.* **109,** 309–314 CrossRef Medline
89. Malojcić, G., and Glockshuber, R. (2010) The PAPS-independent aryl sulfotransferase and the alternative disulfide bond formation system in pathogenic bacteria. *Antioxid. Redox Signal.* **13,** 1247–1259 CrossRef Medline
90. Swaminathan, S., and Eswaramoorthy, S. (2000) Structural analysis of the catalytic and binding sites of *Clostridium botulinum* neurotoxin B. *Nat. Struct. Biol.* **7,** 693–699 CrossRef Medline
91. Mohammad, A., Bon Ramos, A., Lee, B. W., Cohen, S. W., Kiani, M. K., Iwata-Reuyl, D., Stec, B., and Swairjo, M. A. (2017) Protection of the queuosine biosynthesis enzyme QueF from irreversible oxidation by a conserved intramolecular disulfide. *Biomolecules* **7,** E30 Medline
92. Kim, S., Jang, Y. S., Ha, S. C., Ahn, J. W., Kim, E. J., Lim, J. H., Cho, C., Ryu, Y. S., Lee, S. K., Lee, S. Y., and Kim, K. J. (2015) Redox-switch regulatory mechanism of thiolase from *Clostridium acetobutylicum*. *Nat. Commun.* **6,** 8410 CrossRef Medline
93. Cook, K. M., McNeil, H. P., and Hogg, P. J. (2013) Allosteric control of bII-tryptase by a redox active disulfide bond. *J. Biol. Chem.* **288,** 34920–34929 CrossRef Medline
94. Chiu, J., Wong, J. W., and Hogg, P. J. (2014) Redox regulation of methionine aminopeptidase 2 activity. *J. Biol. Chem.* **289,** 15035–15043 CrossRef Medline
95. Gopalan, G., He, Z., Balmer, Y., Romano, P., Gupta, R., Héroux, A., Buchanan, B. B., Swaminathan, K., and Luan, S. (2004) Structural analysis uncovers a role for redox in regulating FKBP13, an immunophilin of the chloroplast thylakoid lumen. *Proc. Natl. Acad. Sci. U.S.A.* **101,** 13945–13950 CrossRef Medline
96. Nishii, W., Kukimoto-Niino, M., Terada, T., Shirouzu, M., Muramatsu, T., Kojima, M., Kihara, H., and Yokoyama, S. (2015) A redox switch shapes the Lon protease exit pore to facultatively regulate proteolysis. *Nat. Chem. Biol* **11,** 46–51 CrossRef Medline
97. Sagong, H. Y., and Kim, K. J. (2017) Structural basis for redox sensitivity in *Corynebacterium glutamicum* diaminopimelate epimerase: an enzyme involved in L-lysine biosynthesis. *Sci. Rep.* **7,** 42318 CrossRef Medline
98. Mauney, C. H., Rogers, L. C., Harris, R. S., Daniel, L. W., Devarie-Baez, N. O., Wu, H., Furdui, C. M., Poole, L. B., Perrino, F. W., and Hollis, T. (2017) The SAMHD1 dNTP triphosphohydrolase is controlled by a redox switch. *Antioxid. Redox Signal.* **27,** 1317–1331 CrossRef Medline

99. Zhou, A., Carrell, R. W., Murphy, M. P., Wei, Z., Yan, Y., Stanley, P. L., Stein, P. E., Broughton Pipkin, F., and Read, R. J. (2010) A redox switch in angiotensinogen modulates angiotensin release. *Nature* **468,** 108–111 CrossRef Medline
100. Giannakopoulos, B., Gao, L., Qi, M., Wong, J. W., Yu, D. M., Vlachoyiannopoulos, P. G., Moutsopoulos, H. M., Atsumi, T., Koike, T., Hogg, P., Qi, J. C., and Krilis, S. A. (2012) Factor XI is a substrate for oxidoreductases: enhanced activation of reduced FXI and its role in antiphospholipid syndrome thrombosis. *J. Autoimmun.* **39,** 121–129 CrossRef Medline
101. Kaiser, B. K., Yim, D., Chow, I. T., Gonzalez, S., Dai, Z., Mann, H. H., Strong, R. K., Groh, V., and Spies, T. (2007) Disulphide-isomerase-enabled shedding of tumour-associated NKG2D ligands. *Nature* **447,** 482–486 CrossRef Medline
102. Wang, C., Li, W., Ren, J., Fang, J., Ke, H., Gong, W., Feng, W., and Wang, C. C. (2013) Structural insights into the redox-regulated dynamic conformations of human protein disulfide isomerase. *Antioxid. Redox Signal.* **19,** 36–45 CrossRef Medline
103. Banerji, S., Lawrance, W., Metcalfe, C., Briggs, D. C., Yamauchi, A., Dushek, O., van der Merwe, P. A., Day, A. J., and Jackson, D. G. (2016) Homodimerization of the lymph vessel endothelial receptor LYVE-1 through a redox-labile disulfide is critical for hyaluronan binding in lymphatic endothelium. *J. Biol. Chem.* **291,** 25004–25018 CrossRef Medline
104. Bekendam, R. H., Bendapudi, P. K., Lin, L., Nag, P. P., Pu, J., Kennedy, D. R., Feldenzer, A., Chiu, J., Cook, K. M., Furie, B., Huang, M., Hogg, P. J., and Flaumenhaft, R. (2016) A substrate-driven allosteric switch that enhances PDI catalytic activity. *Nat. Commun.* **7,** 12579 CrossRef Medline
105. Liu, S., Cerione, R. A., and Clardy, J. (2002) Structural basis for the guanine nucleotide-binding activity of tissue transglutaminase and its regulation of transamidation activity. *Proc. Natl. Acad. Sci. U.S.A.* **99,** 2743–2747 CrossRef Medline
106. Andersen, J. F., Sanders, D. A., Gasdaska, J. R., Weichsel, A., Powis, G., and Montfort, W. R. (1997) Human thioredoxin homodimers: regulation by pH, role of aspartate 60, and crystal structure of the aspartate 60 → asparagine mutant. *Biochemistry* **36,** 13979–13988 CrossRef Medline
107. Dong, G., Wearsch, P. A., Peaper, D. R., Cresswell, P., and Reinisch, K. M. (2009) Insights into MHC class I peptide loading from the structure of the tapasin-ERp57 thiol oxidoreductase heterodimer. *Immunity* **30,** 21–32 CrossRef Medline
108. Kozlov, G., Azeroual, S., Rosenauer, A., Määttänen, P., Denisov, A. Y., Thomas, D. Y., and Gehring, K. (2010) Structure of the catalytic a(0)a fragment of the protein disulfide isomerase ERp72. *J. Mol. Biol.* **401,** 618–625 CrossRef Medline
109. Sato, Y., Kojima, R., Okumura, M., Hagiwara, M., Masui, S., Maegawa, K., Saiki, M., Horibe, T., Suzuki, M., and Inaba, K. (2013) Synergistic cooperation of PDI family members in peroxiredoxin 4-driven oxidative protein folding. *Sci. Rep.* **3,** 2456 CrossRef Medline
110. Chmura, A. J., Orton, M. S., and Meares, C. F. (2001) Antibodies with infinite affinity. *Proc. Natl. Acad. Sci. U.S.A.* **98,** 8480–8484 CrossRef Medline