Redox Potentials of Disulfide Bonds in LOXL2 Studied by Nonequilibrium Alchemical Simulation

Lirui Lin1,2, Haiying Zou1,3, Wenjin Li4, Li-Yan Xu3,5*, En-Min Li1,3* and Geng Dong1,2*

1Department of Biochemistry and Molecular Biology, Shantou University Medical College, Shantou, China, 2Medical Informatics Research Center, Shantou University Medical College, Shantou, China, 3Key Laboratory of Molecular Biology in High Cancer Incidence Coastal Area of Guangdong Higher Education Institutes, Shantou University Medical College, Shantou, China, 4Institute for Advanced Study, Shenzhen University, Shenzhen, China, 5Cancer Research Center, Shantou University Medical College, Shantou, China

Lysyl oxidase-like 2 (LOXL2) is a metalloenzyme that catalyzes the oxidative deamination ε-amino group of lysine. It is found that LOXL2 is a promotor for the metastasis and invasion of cancer cells. Disulfide bonds are important components in LOXL2, and they play a stabilizing role for protein structure or a functional role for regulating protein bioactivity. The redox potential of disulfide bond is one important property to determine the functional role of disulfide bond. In this study, we have calculated the reduction potential of all the disulfide bonds in LOXL2 by non-equilibrium alchemical simulations. Our results show that seven of seventeen disulfide bonds have high redox potentials between −182 and −298 mV and could have a functional role, viz., Cys573–Cys625, Cys579–Cys695, Cys657–Cys673, and Cys663–Cys685 in the catalytic domain, Cys351–Cys414, Cys464–Cys530, and Cys477–Cys543 in the scavenger receptor cysteine-rich (SRCR) domains. The disulfide bond of Cys351–Cys414 is predicted to play an allosteric function role, which could affect the metastasis and invasion of cancer cells. Other functional bonds have a catalytic role related to enzyme activity. The rest of disulfide bonds are predicted to play a structural role. Our study provides an important insight for the classification of disulfide bonds in LOXL2 and can be utilized for the drug design that targets the cysteine residues in LOXL2.

Keywords: LOXL2, disulfide bond, redox potential, non-equilibration simulation, alchemical method

INTRODUCTION

Lysyl oxidase-like 2 (LOXL2) is a copper-dependent amine oxidase enzyme, which belongs to the lysyl oxidase (LOX) family (Smith-Mungo and Kagan, 1998; Jourdan-Le Saux et al., 1999; Csizsar, 2001). It catalyzes the oxidation of collagen and elastin to promote cross-linking, leading to the stiffening of the extracellular matrix (ECM) (Smith-Mungo and Kagan, 1998). Aside from its basic enzyme function, LOXs are found to be significant to human diseases, e.g., several types of cancer (Kagan, 2000; Barker, Cox, and Erler, 2012; Johnston and Lopez, 2018). The members of the LOX family have complex and paradoxical roles of both tumor suppressor and metastasis promoter (Barker, Cox, and Erler, 2012; Johnston and Lopez, 2018). For LOXL2, it is highly expressed in tumors (Barry-Hamilton et al., 2010) and proposed to act as a metastasis promoter (Kirschmann et al., 2002; Peinado et al., 2008; Barry-Hamilton et al., 2010). Thus, many studies are targeting LOXL2 to inhibit the metastasis/invasion of cancer (Hutchinson et al., 2017; Chopra et al., 2020; Kлепыш, et al., 2020).
LOXL2 is synthesized as an 87-kDa proenzyme of 774 amino acids. In 1997, the LOXL2 gene was recognized as a reduced transcript in kinds of non-adherent tumor cell lines compared to adherent tumor cell lines (Saito et al., 1997). LOXL2 has a conserved catalytic region that contains a copper-binding domain as well as a quinone cofactor, which is formed by highly conserved lysine and tyrosine residues. There are four scavenger receptor cysteine-rich (SRCR) domains linking to the catalytic domain. The crystal structure of human LOXL2 in a precursor state was first determined in 2018 (Zhang et al., 2018), but SRCR1 and SRCR2 domains are missing (Figure 1A). In addition, the catalytic center is occupied by a zinc ion instead of a copper ion. Recently, the complete structure of LOXL2 is published in the AlphaFold Protein Structure Database (AlphaFold) (Figure 1B), which is predicted by AlphaFold2 (Jumper et al., 2021; Bershtein et al., 2021). It can be seen from the alignment of the X-ray structure and AlphaFold structure that the predicted structure has a good overlap with the experimental structure with RMSD of 0.62 Å (Figure 1C).

To determine the redox potential of the disulfide bond in protein, maleimide-biotin (MPB) labeling of free cysteine thiols and Western blot densitometry are suitable experimental ways (Liang et al., 2011; Cook et al., 2013; Chiu et al., 2014), and differential cysteine labeling, tandem mass spectrometry, and the
Redox potential low as −470 mV

Redox potential from −89 mV to −330 mV

FIGURE 2 | The classification of disulfide bonds. Reduction potential of the disulfide bond is one key indicator to classify its biological role in proteins. The disulfide bond with a structural role simply maintains the structure of protein. For disulfide bond with an allosteric role, it can alter the substrate protein structure to regulate protein function or activity by being cleaved, and the disulfide bond with a catalytic role performs the functional role without change in the substrate protein structure.

METHODS

System Setup

In this study, the structure of LOXL2 was divided into 5 systems (Figure 3): SRCR1−4 and catalytic domains. The structures of SRCR1 and 2 domains were predicted by AlphaFold2 in the AlphaFold Protein Structure Database (Bershtein et al., 2021; Jumper et al., 2021), and the remaining parts were taken from the 2.60-Å resolution of X-ray crystal structure (PDB ID: 5ZE3) (Huang et al., 2018). Systems 1−5 include residues 50−165, 166−321, 322−431 and 432−546, and 547−762, respectively.

The protonation states of all the residues are determined by using the pKa value from PROPKA (Olsson et al., 2011), a comprehensive evaluation for solvent accessibility, hydrogen-bond pattern around His residues, and possible formation of ionic pairs. All Arg, Lys, Asp, and Glu residues were assumed to be charged. Among the His residues, His 67, 91, 94, 221, 271, 623, 652, and 739 were assumed be protonated on ND1, whereas the remaining parts were modeled as doubly protonated.

All systems were solvated in a cubic box of TIP3P water with a minimum distance of 12 Å from protein structures to the box boundary by using the genbox module in GROMACS-4.5.5 (Jorgensen et al., 1983; Hess et al., 2008; Gillan et al., 2016). A salt concentration of 0.1 mol/l was used to mimic physiological conditions and to neutralize the systems.

MD Simulation

All MD simulations were carried out using the GROMACS-4.5.5 package (Hess et al., 2008). Temperature was kept at 300 K by using velocity rescaling (Grest and Kremer, 1986; Bussi et al., 2007); Pressure was kept at 1 bar by using isotropic pressure coupling with Parrinello–Rahman barostat and a coupling constant of 1.0 ps (Parrinello and Rahman, 1981). For all systems, energy minimization was first performed by using the steepest descent algorithm. Next, a 100-ps NVT simulation with restraints is performed for heavy atoms of protein using a spring
constant of 1,000 kJ/(mol·nm²) (Hess, 2008). Finally, a 50-ns production simulation in the NPT ensemble was performed. Sampling structures were saved every 25 ps, which gave 2,000 structures for each system. Non-bonded interactions were calculated within a cutoff of 1.0 nm. Electrostatic interactions beyond 1.0 nm were treated with particle-mesh Ewald (PME) with a grid spacing of 0.12 nm (Essmann et al., 1995). The periodic boundary condition was used for all simulations. In addition, for the catalytic system, two metal ions Ca²⁺ and Zn²⁺ and their coordinated residues were restrained at crystal structure positions. All amino acids were described by the CHARMM27 force field. A more detailed description for the parameters can be found in the previous studies (Gräter and Li, 2019).

Non-Equilibrium Free Energy Calculation
To estimate the free energy differences between reduced and oxidized states in the redox reaction, non-equilibrium transition was constructed based on the previous study (Pohorille et al., 2010; Li et al., 2015; Gräter and Li, 2019). As is shown in Figure 4, the disulfide bond is formed in the oxidized state, and two normal Cys residues present the reduced state. For the two end states, 0.5-ns NVT equilibrium simulations are carried out, followed by 50-ns NPT production simulations. Subsequently, 500 snapshots were extracted every 55 ps from each production trajectory (the first 20 ns from the trajectories were discarded). Finally, 2 ns × 500 replicas of non-equilibrium simulations (backward and forward directions, $\Delta \lambda = 0.00002$ fs) were carried out to alchemically morph between the oxidized and reduced states. Free energy differences were calculated by the pmx program (Gapsys et al., 2015), in which the work values were used to estimate the free energy differences basing on the Crooks fluctuation theorem (Crooks, 1999) and utilizing the Bennett acceptance ratio as a maximum likelihood estimator (Shirts et al., 2003).

For the transformation between cystine and two free cysteines, hybrid cysteine/cystine topologies were constructed based on our previous protocol (Gräter and Li, 2019). As is shown in Figure 5, a new cysteine residue type is added for the disulfide-bonded cysteine (CYS2), which is named residue CYD, and it includes one dummy atom HUD and two virtual sites (Vc and Vs, respectively). Notably, the dummy atom and virtual sites do not have bonded and Lennard–Jones interactions, and its description is based on the CHARMM27 force field. A more detailed description for the parameters can be found in the previous studies (Gräter and Li, 2019).

For alchemical MD simulations, a single topology approach was used (Gao et al., 1989; Axelsen and Li, 1998). When $\lambda = 0$ (oxidized state), there is no charge on the dummy atom of HUD and two virtual sites (Vc and Vs), and no interaction between the dummy HUD atom and the two virtual sites. Thus, CYD is the same as CYS2 in the CHARMM27 force field. HUD is switched to a hydrogen atom and presents entire bonded interactions when $\lambda = 1$. Thus, CYD is a standard free cysteine residue. In addition, more bond interactions present in the oxidized state, i.e., the bond S1–S2, angles CB1–S1–S2, and related dihedral angles (subscripts 1 and 2 refer to the atoms in CYD1 and CYD2 in Figure 5). These bonded interactions are absent in two independent cysteine residues at $\lambda = 1$. As is shown in Figure 6, we performed non-equilibrium transition for the seventeen disulfide bonds in LOXL2, and the oxidized and reduced states are presented. The disulfide bonds link three types of secondary structure of LOXL2, i.e., $\alpha$ helix, $\beta$ sheet, and loop. The $\alpha$ helix and $\beta$ sheet usually form a related conserved structure for specific function, while the loop is usually kept at a flexible state, which provides more space for interaction with adjacent areas (Pijnings and Hogg, 2019).

Redox Potential Calculation
Finally, the reduction potential ($E^0$) was calculated based on Nernst’s equation,

$$E^0 = -\Delta G/nF$$

where $\Delta G$ is the free energy difference between the reduced and oxidized states in a redox reaction, $n$ is the number of electrons transferred, and $F$ is the Faraday constant. We use the Crooks Gaussian Intersection (CGI) method to calculate free energy
differences between reduced and oxidized states in redox reactions. The method has been described in detail in the literature (Goette and Grubmuller, 2009).

RESULTS AND DISCUSSION

The SRCR1 and SRCR2 Domains of LOXL2

For the LOX family, the crystal structure is only available for LOXL2, but without the SRCR1 and SRCR2 domains (Zhang et al., 2018). The structures of missing domains were predicted by AlphaFold2 (Bershtein et al., 2021; Jumper et al., 2021) and 1-TASSA (Roy et al., 2010) servers. The structures from the three methods are compared with the crystal structure, and the AlphaFold2 structure was used in this study due to its smallest RMSD value of 0.62 Å (Figure 1; Supplementary Figure S1). Then, we constructed an entire system of LOXL2 by combining the crystal structure (PDB ID: 5ZE3) with the AlphaFold2 structure.

For the structures of SRCR domains in LOXL2, we make a multiple-sequence alignment by MUSCLE (Edgar, 2004) and a structure alignment by PyMOL (Schrödinger, 2010), visualized in Figure 7. In Figure 7A, the alignment of SRCR1 and SRCR2 presents an RMSD of 2.43 Å, and the RMSDs are respectively 0.49 and 1.63 Å when the SRCR1 and SRCR2 domains are aligned with M2BP, which is a standard SRCR of group A in the SRCR family (Hohenester et al., 1999). SRCR1 has almost the same structure with SRCR3 (RMSD = 0.85 Å). Overall, SRCR domains are highly conserved.

The sequence alignment results (Figures 5C, 7B) show the residue characters of SRCR 1-4, including conservation, quality, consensus, and occupancy evaluations. On account of the deep degree of cysteine, it can be seen that disulfide bonds have a high conservation degree. The phylogenetic tree via the cladogram rectangle shows the correlations between SRCR 1-4 and M2BP, in which SRCR domains 1 and 3 are closer to M2BP in the branch, whereas SRCR2 and 4 are at the third level. This might be one reason why RMSD values are large when the SRCR2 and SRCR4 domains aligned to M2BP. In this view, SRCR2 might inherit the function of SRCR1 in LOXL2, while evolving to get some new role compared to SRCR1. Accordingly, SRCR4 is evolved to be more versatile than SRCR3.

Reduction Potentials for the Disulfide bonds in LOXL2

Disulfide bonds are formed by cysteines and have two roles in protein, i.e., one role is structural and the other one is functional (Cook, 2019). Previous studies demonstrated that the disulfide bonds with a structural role normally have a low reduction potential (<−470 mV), indicating that the bond is very stable and hard to be broken down. On the contrary, high reduction potentials (−89—−330 mV) of disulfide bonds often present a functional role, and the bond distance is slightly longer than that of structural bonds (Cook, 2019; Pijning and Hogg, 2019). To distinguish the role of disulfide bonds in LOXL2, we calculated the reduction potential for all the disulfide bonds through nonequilibrium free energy simulations. In order to find a functional role of disulfide bonds, disulfide bonds with redox potentials in the range of −89—−330 mV are deemed to have a functional role, whereas others are excluded to have a structural role.

According to our previous study, the reduction potentials have a correlation with experimental data, $E_{exp} = 1.5 \times E_{cal} - 43$ mV (Li, Baldus, and Gräte, 2015). We then added this correction to our results. All computational data are collected in Table 1. For the disulfide bonds of Cys351–Cys414, Cys464–Cys530, Cys477–Cys543, Cys573–Cys625, Cys579–Cys695, Cys657–Cys673, and Cys663–Cys685, they are predicted to be functional bonds. Furthermore, the remaining bonds are classified as structural bonds. It should be noted that two functions were found for LOXL2; i.e., one is the enzyme function related to amine oxidase activity, and the other one is the promoting effect of migration and invasion to cancer cells (Zou et al., 2020). The disulfide bond with a catalytic role is related to the enzymatic function of LOXL2, while the disulfide bond with an allosteric role is related to the function of migration and invasion in LOXL2 (Hogg, 2009; Friedl and Alexander, 2011).

Discussion of the Function Role of Disulfide Bonds in LOXL2

For the disulfide bonds in the SRCR 1 domains (⊙, ⊙, and ⋄ in Figure 8), all these three bonds have a reduction potential in the range of −335 to −420 mV, indicating that they have a structural role for LOXL2. Based on our previous study, LOXL2 without the SRCR1 domain (LOXL2ΔSRCR1) has an approximate catalytic activity compared with wild-type LOXL2 (Zou et al., 2020). It is shown in Figure 8A that the SRCR1 domain is located far from the catalytic domain; it would be easy to understand that it owns less correlation to the catalytic reaction. On the other hand, LOXL2ΔSRCR1 had little promotion effects on esophageal squamous cell carcinoma (ESCC) cellular mobility than wild-type LOXL2 in bioactive assay (Zou et al., 2020). Thus, it can be
**FIGURE 6** The oxidized and reduced states of each disulfide bond in LOXL2. (A-E) show each domain in LOXL2, respectively. The dotted circles present oxidized state, solid circles present reduced state, and disulfide bond and Cys are shown as stick. The font color of residues stands for the secondary structure type of each disulfide bond connected: red: helix, blue: sheet, and green: loop.
FIGURE 7 | (A) Structure alignment for SRCR 1–4 of LOXL2 by using PyMOL (Schrödinger, 2010). (B) Multiple-sequence alignment by MUSCLE (Edgar, 2004). The depth of color indicates the conservation degree of residue, and the deeper color indicates more conservative. The histograms indicate the level in each character, score from 0 to 8. (C) Phylogenetic tree via cladogram rectangle by MUSCLE.

TABLE 1 | Data collection for the disulfide bonds in LOXL2.

| Domain | Disulfide bond | Work | Reduction potential | Corrected reduction potential* |
|--------|----------------|------|---------------------|-------------------------------|
| SRCR 1 | Cys84–Cys148   | 37.20| −201.11             | −348.67                      |
| SRCR 1 | Cys97–Cys158   | 46.58| −249.13             | −420.70                      |
| SRCR 1 | Cys128–Cys138  | 36.22| −191.67             | −334.51                      |
| SRCR 2 | Cys218–Cys291  | 36.68| −192.07             | −335.11                      |
| SRCR 2 | Cys231–Cys301  | 49.78| −260.67             | −430.00                      |
| SRCR 2 | Cys265–Cys275  | 39.19| −205.22             | −354.82                      |
| SRCR 3 | Cys351–Cys414  | 28.59| −148.16             | −269.24                      |
| SRCR 3 | Cys364–Cys424  | 47.06| −243.87             | −412.81                      |
| SRCR 3 | Cys395–Cys405  | 24.63| −112.09             | −215.13                      |
| SRCR 4 | Cys464–Cys530  | 21.63| −112.09             | −215.13                      |
| SRCR 4 | Cys477–Cys543  | 28.66| −148.52             | −269.78                      |
| SRCR 4 | Cys511–Cys521  | 48.71| −252.84             | −425.63                      |
| Catalytic | Cys573–Cys585 | 18.08| −93.69              | −187.54                      |
| Catalytic | Cys579–Cys585 | 28.67| −149.61             | −271.41                      |
| Catalytic | Cys657–Cys673 | 17.36| −89.96              | −181.94                      |
| Catalytic | Cys663–Cys685 | 32.31| −167.44             | −298.15                      |
| Catalytic | Cys732–Cys746 | 43.58| −225.84             | −385.76                      |

*Corrected reduction potential is $E_{corr} = 1.5 \times E_{cal} - 43 \text{ mV}$. 
concluded that disulﬁde bonds of ①, ②, and ③ do not show an apparent effect to enzyme activity as well as the migration and invasion function, and they are structural disulﬁde bonds.

For the disulﬁde bonds in SRCR 2 domains (④, ⑤, and ⑥ as shown in Figure 8, the reduction potentials are lower than the threshold value −330 mV, and they are predicted to have a structural role. Similarly to LOXL2 ΔSRCR1, our previous bioactive assay showed that LOXL2 ΔSRCR2 does not change the oxidative activity and has no effects on ESCC cellular mobility (Zou et al., 2020). In addition, LOXL2 without SRCR1 and 2 shows indistinctive amine oxidase activity after proteolytic processing (Lopez-Jimenez et al., 2017), meaning that there might not be a functional role of disulﬁde bond in the SRCR1 and 2 domains of LOXL2. Thus, ④, ⑤, and ⑥ are evaluated to be structural disulﬁde bonds.

For the disulﬁde bonds in SRCR3 domains (⑦, ⑧, and ⑨ as shown in Figure 8, Cys351–Cys414 has a high reduction potential of −269 mV, indicating that it could be a functional bond. For the other two disulﬁde bonds, they are predicted to have a structural role. Structurally, Cys351–Cys414 are located on the surface of the SRCR3 domain and link two loops (loopG348–L357, loopD404–G421, as shown in Figure 9A). Compared to the α-helix and β-sheet, the loop owns higher ﬂexibility (Liljas et al., 2017), and it expands the interaction between the disulﬁde bond and the adjacent area. Experimentally, LOXL2 ΔSRCR3 had distinct decrease effects on the migration of ESCC cells than LOXL2 WT, but little effect on the oxidase activity of LOXL2 (Zou et al., 2020). In another study, knockout of SRCR domains 1–3 did not affect the oxidation activity of LOXL2 remarkably, which demonstrates that the SRCR1-3 domains are not indispensable for the enzyme activity (Xu et al., 2013). Therefore, the functional disulﬁde bond ⑦ is related to the migration and invasion of cells and should be an allosteric disulﬁde bond. To explore how disulﬁde bond ⑦ inﬂuences the conformation changes of LOXL2, we perform MD simulation with the reduced state of disulﬁde bond ⑦. It is shown in Figure 10A that, after cleavage of disulﬁde bond ⑦, the conformation of LOXL2 changes remarkably. The RMSD between oxidized and reduced states is 2.7 Å. Secondary structure changes are found on some residues. As is shown in Figures 10B,C, loop A formed by G348–V350 changes

FIGURE 8 | Classification of disulﬁde bonds in LOXL2. (A) The structure of LOXL2. (B) The reduction potential distribution of disulﬁde bonds. Blue area: structural function; orange area: allosteric or catalytic function. Icon for the role of disulﬁde bond: circle for structural role, triangle for allosteric role, and rhomb for catalytic role. The front color of the residue stands for the secondary structure type of each disulﬁde bond connected: red: helix, blue: sheet, and green: loop.
Cys464 be the structural role). Structurally (as shown in Figure 9B), Cys511 did not have the catalytic domain, had a similar activity in ESCC to be functional role, Cys521 is functional role (disulfide bond). Thus, the two disulfide bonds in SRCR4 show higher activity in ESCC cell migration compared with wild-type LOXL2, indicating that the migration function of LOXL2 is independent of the catalytic domain (Zou et al., 2020). On the other hand, it can be seen from the spatial conformation that the four disulfide bonds are close to the catalytic center. Intriguingly, the reduction potentials for the five disulfide bonds in the catalytic domain are correlated with the distances between the locations of disulfide bonds and catalytic site of metal Zn (in Figure 9C), i.e., short distance (5–18 Å) corresponds to a high reduction potential (∼ 182–298 mV) which corresponds to a functional role.

In Figure 9C, based on structure alignment, the β-sheet structures in the catalytic domain are aligned with the β-galactosidase type; those aligned commendably apart are defined as structure core (colored yellow), and the nonoverlapping regions are defined as extra segments (ES1–3, colored orange) (Zhang et al., 2018). In the core area, the catalytic site is surrounded by 4 pairs of disulfide bonds (B, D, E, and F) in different extents of distances. Moreover, disulfide bond D links the edge of the core area and ES3. ES1 (residues 568–580) contains a α-helix, which links to the core through disulfide bond D. The major structure element of ES2 (residues 614–634) is a β-hairpin, which accommodates a highly conserved copper-binding motif. ES2 is linked to ES1 through disulfide bond D, which may act its stabilization function to this crucial β-hairpin. ES3 (residues 745–774) is at the C terminus of the catalytic domain and is responsible for the interaction with the SRCR3 domain. In a whole view, beside structural characters, disulfide bonds B, D, E, and F are preferred to have a catalytic role, while G has a structural role.

In summary, seven disulfide bonds are predicted to have a functional role in LOXL2. The disulfide bond of Cys351–Cys414 in SRCR3 is most likely to act its allosteric function. In the catalytic domain, four disulfide bonds play a role in catalytic function. The remaining two catalytic function bonds are located in the SRCR4 domain, which is close to the catalytic domain.

**The Performance of Work Distributions in Non-Equilibrium Free Energy Calculations**

In this study, all the work values for cleavage of disulfide bonds were calculated by the pnmx program (GapSys et al., 2015), as shown in...
The $\delta H/\delta \lambda$ curve as a function of $\lambda$ in typical forward and backward transformations is shown in Supplementary Figure S2, indicating the high convergence of transformation. Independent work values for 500 snapshots of forward and backward transitions are generated, and the total free energy difference $\Delta G$ for each disulfide bond is shown on the top right of each picture.

In our study, $\Delta G$ values of disulfide bonds in LOXL2 were calculated by the BAR estimator in the pmx program. The accuracy of results can be assessed in various ways. One indicator of accuracy is the convergence of systematic dynamics of work value (Jarzynski, 2006). In Figure 11 and Supplementary Figure S3, the work values do not have a large drift, especially in the forward distribution. In addition, the distributions of work values of 500 snapshots for all disulfide bonds are concentrated and can be fitted to Gaussian distribution. Second, the overlap of the forward and backward work distribution is important to the binding free energy (Mey et al., 2020). The accuracy of the CGI and BAR estimator is sensitive to the overlap. We can see an apparent overlap for each disulfide bond as shown in Figure 11 and Supplementary Figure S3. In our study, 500 replicas of non-equilibrium simulations are performed to generate a free energy difference. Third, the standard errors of $\Delta G$ are less than 1.4 kJ/mol in all our calculations, indicating that our results are stable (Shirts et al., 2003). Thus, all these confirm that the $\Delta G$s are reliable.

CONCLUSION

Disulfide bonds exist in many proteins and play important roles in the structure and function of proteins (Pijning and Hogg, 2019).
LOXL2, seventeen disulfide bonds are formed. The functional bonds can be divided into two types: catalytic and allosteric functions. The allosteric function of the disulfide bond in LOXL2 corresponds to the ability of metastasis and invasion of cancer cells, and the catalytic function corresponds to the enzyme activity. In this paper, we determine the role of each disulfide bond by reduction potential calculation with nonequilibrium alchemical simulations. Seven disulfide bonds are predicted to have a functional role, whereas others have a structural role. One of the functional disulfide bonds (Cys351–Cys414) is the allosteric bond in SRCR3. Two catalytic functions of disulfide bonds (Cys464–Cys530 and Cys477–Cys543) are found in the SRCR4 domain, which is close to the catalytic domain. In the catalytic domain, four disulfide bonds are predicted to be of catalytic function, i.e., Cys573–Cys625, Cys579–Cys695, Cys657–Cys673, and Cys663–Cys685. Notably, the reduction potentials for the five disulfide bonds in the catalytic domain are correlated with the distances between the location of disulfide bonds and catalytic sites, i.e., short distance leads to high reduction potential. In addition, no functional disulfide bond is found in the SRCR 1 and SRCR2 domains.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding authors.

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AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

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