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Analysis of the Interactions Between Thioredoxin and 20 Selenoproteins in Chicken

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Abstract Thioredoxin (Trx) is a small molecular protein with complicated functions in a number of processes, including inflammation, apoptosis, embryogenesis, cardiovascular disease, and redox regulation. Some selenoproteins, such as glutathione peroxidase (Gpx), iodothyronine deiodinase (Dio), and thioredoxin reductase (TR), are involved in redox regulation. However, whether there are interactions between Trx and selenoproteins is still not known. In the present paper, we used a Modeller, Hex 8.0.0, and the KFC2 Server to predict the interactions between Trx and selenoproteins. We used the Modeller to predict the target protein in objective format and assess the accuracy of the results. Molecular interaction studies with Trx and selenoproteins were performed using the molecular docking tools in Hex 8.0.0. Next, we used the KFC2 Server to further test the protein binding sites. In addition to the selenoprotein physiological functions, we also explored potential relationships between Trx and selenoproteins beyond all the results we got. The results demonstrate that Trx has the potential to interact with 19 selenoproteins, including iodothyronine deiodinase 1 (Dio1), iodothyronine deiodinase 3 (Dio3), glutathione peroxidase 1 (Gpx1), glutathione peroxidase 2 (Gpx2), glutathione peroxidase 3 (Gpx3), glutathione peroxidase 4 (Gpx4), selenoprotein H (SelH), selenoprotein I (SelI), selenoprotein M (SelM), selenoprotein N (SelN), selenoprotein T (SelT), selenoprotein U (SelU), selenoprotein W (SelW), selenoprotein 15 (Sep15), methionine sulfoxide reductase B (Sepx1), selenophosphate synthetase 1 (SPS1), TR1, TR2, and TR3, among which TR1, TR2, TR3, SPS1, Sep15, SelN, SelM, SelI, Gpx2, Gpx3, Gpx4, and Dio3 exhibited intense correlations with Trx. However, additional experiments are needed to verify them.

Keywords Trx · Selenoproteins · Modeller · Hex · KFC2 Server

Abbreviation

Dio1	Iodothyronine deiodinase 1
Dio2	Iodothyronine deiodinase 2
Dio3	Iodothyronine deiodinase 3
Gpx1	Glutathione peroxidase 1
Gpx2	Glutathione peroxidase 2
Gpx3	Glutathione peroxidase 3
Gpx4	Glutathione peroxidase 4
SelH	Selenoprotein H
SelI	Selenoprotein I
SelM	Selenoprotein M
SelN	Selenoprotein N
SelO	Selenoprotein O
Sep15	Selenoprotein 15
SelU	Selenoprotein U
SelW	Selenoprotein W
Sepx1/MrsB	Methionine sulfoxide reductase B
SPS1	Selenophosphate Synthetase 1
SPS2	Selenophosphate Synthetase2
TR1	Thioredoxin reductase 1
TR2	Thioredoxin reductase 2
TR3	Thioredoxin reductase 3
Trx	Thioredoxin
Sec	Selenocysteine
GSH	Glutathione
ER	Endoplasmic Reticulum
Rdx	Radixin

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Introduction

Thioredoxin (Trx) is a small (12 kDa) multifunctional protein that acts as an essential cofactor (hydrogen donor) for ribonucleotide reductase, was first found in 1964 in *Escherichia coli*, and is widely distributed in bacteria, plants, mammals, and humans [1]. Trx is located in the nucleus, cytoplasm, chloroplasts, and mitochondria in higher organisms. The structures of Trx in different organisms display similarity and consist of a β-sheet core in the middle of the structure that is encircled by four α-helixes and has a highly conserved active site sequence Cys-X-X-Cys [2]. The Trx system consists of NADPH, thioredoxin reductase (TR), and Trx; electrons are transferred from NADPH to Trx via TR, which is a type of selenoprotein and is involved in many signaling pathways [3]. In mammalian cytosolic and mitochondrial Trx systems, TRs are high molecular weight selenoenzymes that can effectively modulate the cellular redox environment [4]. Glutathione peroxidase (Gpx) and TR selenoenzymes are also major members in intracellular redox systems and glutathione- and thioredoxin-dependent systems. They also participate in redox regulation, apoptosis, inflammation, various signaling pathways, transcription, and immunological responses [5, 6]. A growing number of studies in recent years have been focused on the role of Trx in oxidative stress, programmed cell death, and apoptosis.

Micronutrient selenium is an essential trace element in mammals that is essential for human physiological health [7–9]. Selenium in proteins is part of the rare amino acid selenocysteine (Sec) that is present during protein translation [10]. Sec-containing proteins, which are known as selenoproteins, are widely found in all three kingdoms of life. Furthermore, Sec-containing forms of Trx have been found in bacteria, such as *Treponema denticola* [11]. Selenoproteins might participate extensively in key biological processes, such as protein folding, cellular differentiation, cellular response to oxidative stress, and immune response [12, 13].

Since the initial discovery of Sec, there have been significant developments in understanding how Sec is synthesized and inserted into selenoproteins. Thus far, various families of selenoproteins have been identified, including selenoproteomes in human (25), chicken (25), zebrafish (38), and mouse (25). In our laboratory, we have shown that selenium deficiency mainly influences the expression of antioxidative selenoproteins [14–16] and endoplasmic reticulum-resident selenoproteins in chicken [17]; it also leads to an imbalance in calcium regulation-related genes in broiler hearts [18]. However, the complete molecular characterization of the individual selenoproteins in chicken, and their influence on each other, still needs to be investigated. Therefore, we designed this study to identify possible interactions between Trx and other selenoproteins. Modeller 9.11 (Andrej Sali, San Francisco, USA) was used to establish 3D structures, which could help in designing new effective compounds and inhibitors [19]. The interactions were analyzed using Hex 8.0.0 (David W. Ritchie, Vandoeuvre-les-Nancy, France) and further confirmed with their binding hot spots in the KFC2 Server [20]. This study may aid in unveiling the interplay between Trx and other selenoproteins, further explain their biological and biomedical roles in cells, and identify the importance of Trx signaling pathways in diseases.

Materials and Methods

Ligand Preparation

The 25 selenoproteins (Dio1, Dio2, Dio3, Gpx1, Gpx2, Gpx3, Gpx4, SelH, SelI, SelM, SelN, SelS, SelT, SelU, SelO, SelP, SelK, SelW, Sep15, Selpb, Sepx1, SPS1, TR1, TR2, and TR3) and 1 target protein (Trx) in chicken had no significant structures present in the Protein Data Bank (PDB) database and were therefore modeled using different modeling techniques. Based on the BLAST score obtained for each individual gene, the 3D structure of the gene products was established using Modeller, a computer program for comparative protein structure modeling [21]. To successfully model the 3D structure of the target protein, the sequence alignment requires the template structures, the template atomic coordinates of the templates, and a simple script file [22]. Subsequently, models containing non-hydrogen atoms were calculated automatically within minutes on a modern PC without user intervention. In chicken, five selenoproteins (Dio2, SelK, SelO, Sepp1, and Selpb) did not show sequence similarity in the PDB database and therefore were not considered in the modeling and following processes. Additionally, SPS1 was used instead of SPS2 because there was no data for SPS2.

Protein Receptor Preparation

The 3D structure of the receptor was not found in the PDB database; therefore, 3D models were developed using Modeller. The method was the same as adopted for ligand preparation.

Ligand-Protein and Receptor-Protein Docking

Hex is a program that can dock interactive proteins and superpose molecules. It utilizes FFT correlation with the Gaussian density representation of the protein shape and spherical polar coordinates. In this study, we used the Hex 8.0.0 software (http://hex.loria.fr) for rigid docking to compute possible interaction sites between Trx and 20 selenoproteins, including Gpx1, Gpx2, Gpx3, Gpx4, Dio1, Dio3, SelH, SelM, SelN, SelT, SelW, SelS, SPS1, Sep15, TR1, TR2, TR3, SelI, Sepx1, and SelU.

KFC2 Server Searching for Hot Spots

The original KFC2 Server (Knowledge-based FADE and Contacts) was used to predict protein-protein interface binding "hot spots" by recognizing structural features indicative of important binding contacts. The server can analyze several chemical and physical features surrounding an interface residue and predict the residue classification using a model trained with prior experimental data [16]. In this study, we used the KFC2 Server to calculate hot spots on the protein-protein complex surfaces.

Results

Ligand Preparation

3D structures were built using Modeller and then assessed. The results showed high-definition modeling with the final results being displayed in Fig. 1.

Trx Protein Receptor

The 3D structure of the receptor protein Trx was also predicted and assessed using Modeller. The best result is presented in Fig. 2.

Ligand-Protein and Receptor-Protein Docking

To understand the Trx inhibition mechanism and its interactions with selenoproteins as well as generate as many near-native complex structures (hits) as possible, basic docking calculations were performed with Hex 8.0.0. The program uses PDB files to analyze protein binding conformations. The analysis was based on the E_{total} energy or free energy of binding. The results of the docking are presented in Fig. 3. The binding energies of the structures are summarized in Table 1.

We can clearly see that the binding in this harmonic surface and the binding energy actually reflect the opportunity to bind. In fact, based on molecular thermodynamics, lower E_{total} values correlate with easier and firmer binding to other proteins.

KFC2 Server Complex Hot Spot Calculation

The best model was selected from the overlapping results and then analyzed with the KFC2 Server to identify the hot spots among the protein-protein interactions. The data for all the residues in the target proteins and details are shown in Table 2. The E_{total} is the total calculated interaction energy of the system. Further analysis was based on the E_{total} or free energy of binding, which is the lowest docked energy. Lower E_{total} values generally correlate with stronger binding.

After docking, the KFC2 Server was used to further calculate the complex hot spots. The calculated results are summarized in Table 2, while the KFC2 Server observations are presented in Fig. 4. The E_{total} for Trx and most selenoprotein binding ranges from −497.67 to −753.39 cal/mol, suggesting strong interactions with Trx. In strong contrast, the E_{total} for SelS to bind Trx is −43.07 cal/mol, which shows the mismatch between the two binding surfaces. The binding hot spots are summarized in Table 2, and the KFC2 Server results are presented in Fig. 4.

Based on the amino acid combinations, which are important for forming binding hot spots, Trx potentially exists interactions with TR1, TR2, TR3, SPS1, Sep15, SelN, SelM, SelI, Gpx1, Gpx2, Gpx3, Gpx4, Dio1, Dio3, SelH, SelT, SelW, and Sepx1. However, based on the previous results, SelS lacks features necessary to bind Trx. Further details are shown in Table 2.

Discussion

Together with other redox systems, such as GSH, Trx is responsible for regulating oxidation and redox center mechanisms that help to regulate the energy metabolism, a variety of signaling pathways, the biological function of transcription factors [23], and the immune response, which is required to maintain normal cell function. However, the interactions between Trx and selenoproteins remain enigmatic. Thus, we designed this study to computationally elucidate the mechanistic roles of Trx in conjunction with selenoproteins in organisms.

Gpx

The Gpx family, which includes Gpx1, Gpx2, Gpx3, and Gpx4, is found in the three domains of life [24]. Due its role in removing toxic hydrogen peroxide in cells, Gpx has gained a great deal of attention. Gpx1 can be expressed in all types of cells, though its highest expression is in the liver and kidney [25].

In strong contrast, Gpx2 and Gpx3 are expressed in specific tissues. Gpx2 is mainly found in the gastrointestinal epithelium, and Gpx3, the major form of Gpx in plasma, is secreted by the kidney. Gpx4 can be expressed in many different cell and tissue types [26]. Several studies have proven that Gpx1 knockout mice show an increase in susceptibility to oxidative stress [27, 28]. Gpx2 was also shown to be involved in the development of cancer [29]. This indicates that Gpx2 is similar to TR1 and Sep15 (discussed in the following sections), which might be essential during later stages of cancer development and in preventing and promoting tumor cell growth. Because knockout of the Gpx4 gene in mice led to embryonic lethality, it was inferred that Gpx4 plays an essential role in inhibiting lipid peroxidation during the early period of embryo development [30].

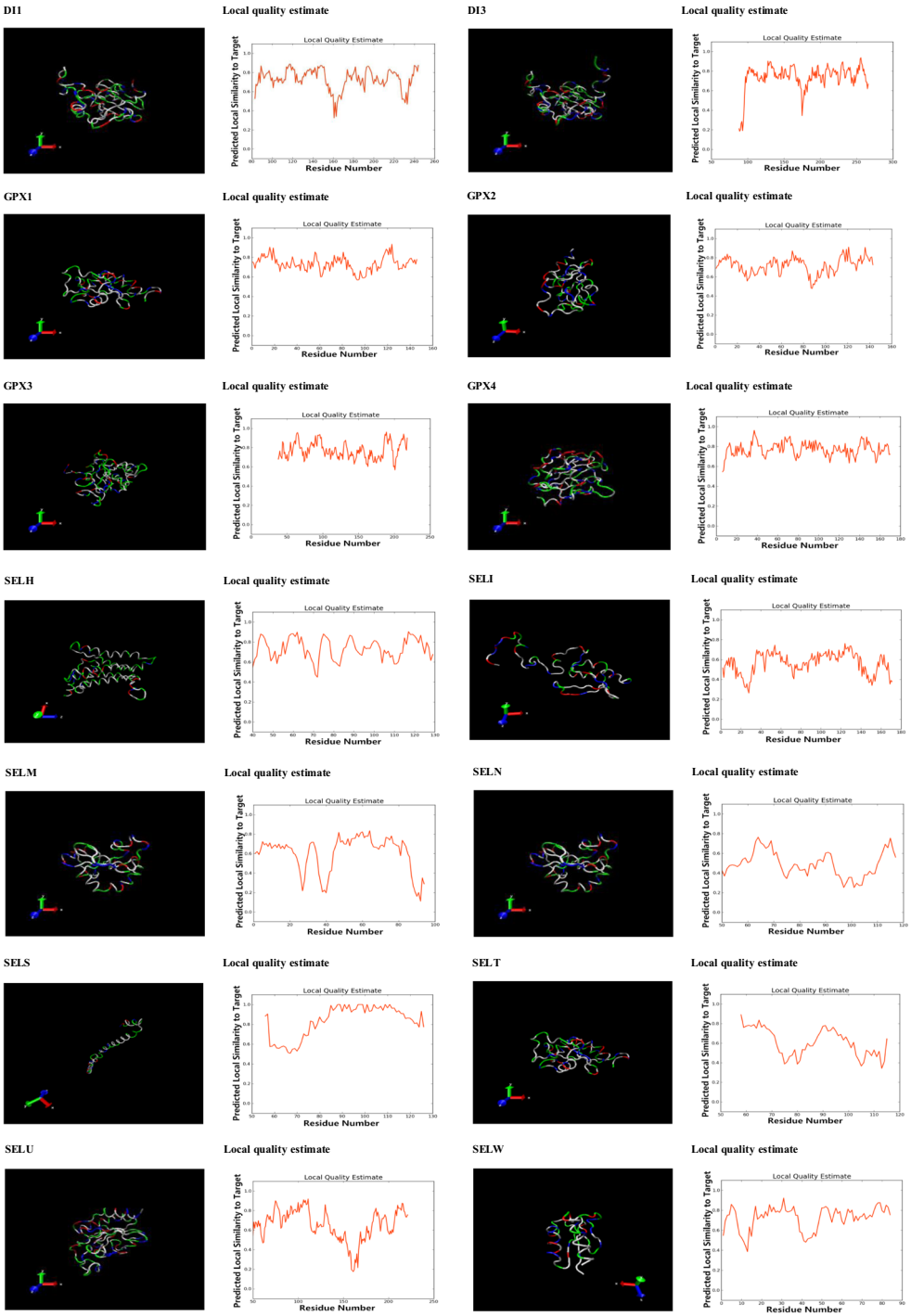

Fig. 1 The results of simulate Modeller and the mode quality estimate of ligand

The E_{total} for Trx-Gpx1, Trx-Gpx2, Trx-Gpx3, and Trx-Gpx4 binding is −547.24, −588.10, −596.46, and −574.58 cal/mol, respectively. Gpx1, Gpx2, Gpx3, and Gpx4 binding hot spots include VAL123, PRO124, and PHE125; HIS78, LEU79, and PRO80; LEU69, VAL70, and VAL71; and SER104, LYS105, and ILE106, respectively. The hot spot details are shown in Table 2. These results showed that there are potential relationships between Trx and Gpxs. The interactions between Trx and Gpxs have been demonstrated.

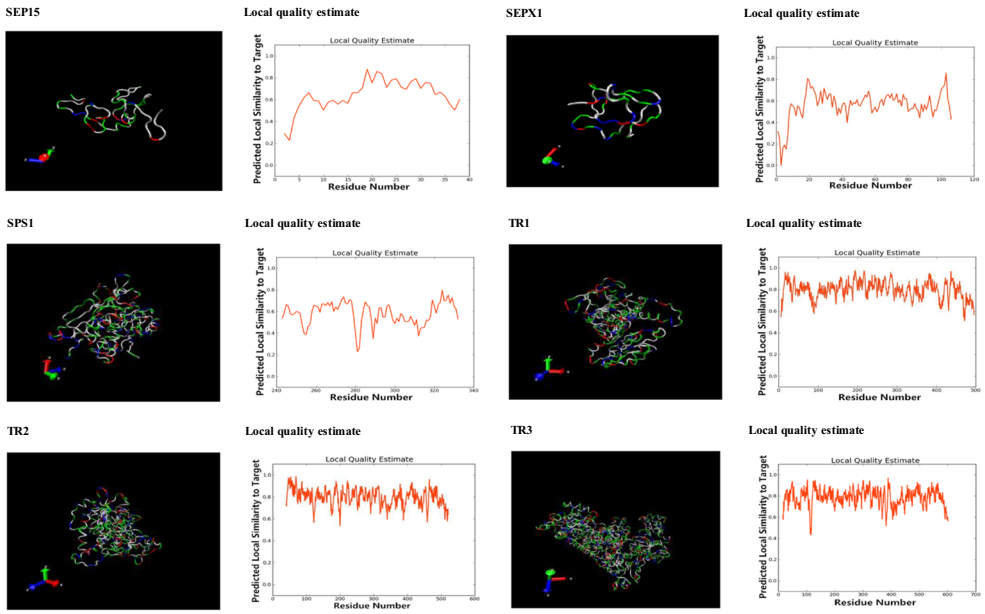

Fig. 1 (continued)

DI (Dio)

In mammals, the iodothyronine deiodinase family is present in three forms (DI1, DI2, and DI3); these proteins are selenoproteins and are involved in regulating thyroid hormone activity. DI1 and DI3 are found on the plasma membrane, while DI2 is found on the ER. They are found in simple eukaryotes, bacteria, vertebrates. They are also involved in maintaining thyroid hormone levels and activities. DI1 primarily regulates circulating levels of thyroid hormone. By contrast, DI2 and DI3 play important roles in regulating intracellular T3 concentrations [31]. Lacking DI2 obviously affects the growth and normal functions of skeletal muscle tissue in organisms. DI1 and DI3 exude their functions by accurately regulating thyroid hormone concentrations.

The E_{total} for Trx-DI1 and Trx-DI3 binding is −531.84 and −631.64 cal/mol, respectively. ILE152, GLU153, and GLU154 and TYR222, GLY223, and TYR225 play vital roles in binding as DI1 and DI3 hot spots, respectively (Table 2). Based on the data, there are potential relationships between Trx and DIs.

TR

The essential disulfide reduction system in cells consists of TRs and Trx. There are three types of TR isozymes in mammalian cells. TR1, also known as TrxR1 and TxnRd1, is mainly present in the cytosol and nucleus. A number of low molecular weight compounds can be reduced by TR1 [32].

TR2, which also known as Txnrd2 and TrxR2, is present in the mitochondria and involved in the reduction of mitochondrial Trx [32]. The third member is TR2 or TrxR3. TR1 and TR3 are present in all vertebrates, and knockout of either protein leads to mouse embryonic lethality [33, 34]. As a major protein disulfide reductase in the cell, TR1 plays an important role in the reduction of Trx1, which is also involved in controlling physiological processes, such as antioxidant defense, DNA repair, apoptosis, and transcription factor regulation, and serves as an electron donor for redox-active enzymes [35–38].

The E_{total} for Trx-TR1, Trx-TR2, and Trx-TR3 binding is −676.40, −753.39, and −562.23 cal/mol, respectively. PRO344, VAL345, and ILE347; TYR97, SER100, and LEU101; and LYS564, CYS565, and GLY566 are TR1, TR2, and TR3 hot spots, respectively (Table 2). Based on the data, there are potential relationships between Trx and TRs.

SelT, SelH, and SelW

SelW, SelT, and SelH are all members of the Rdx family [39]. They all possess a thioredoxin-like fold and redox-related functions.

SelT is mainly present in the ER and Golgi during embryonic development and is also present in adult tissues [35]. In some studies, the lack of SelT was shown to upregulate SelW expression. More recently, SelT was proven to be involved in pancreatic β-cell functions and glucose homeostasis regulation

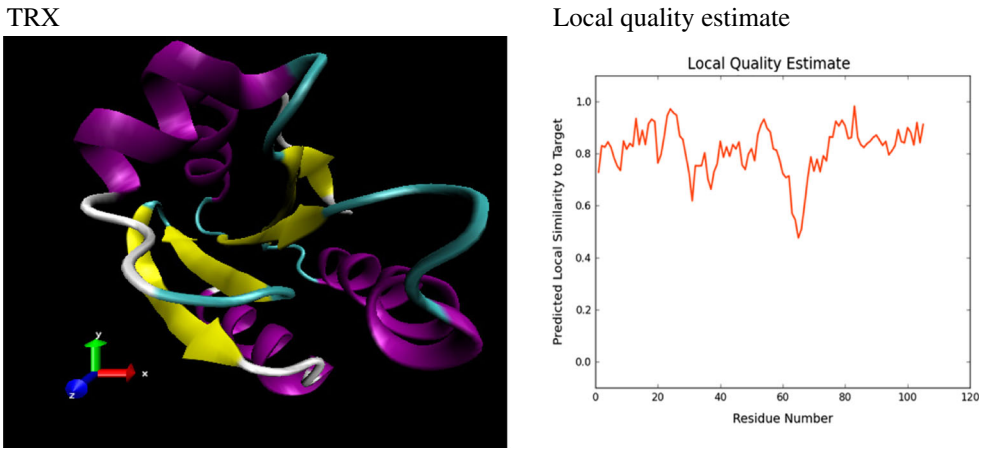

Fig. 2 The results of Modeller simulating and the mode quality estimate of receptor

[40]. Knocking down SelT in mouse fibroblasts resulted in changes in cell structure and cell adhesion properties [41]. The E_{total} for Trx-SelT binding is −547.24 cal/mol, and VAL123, PRO124, and PHE125 are SelT hot spots. Based on the data, there is a potential relationship between Trx and SelT.

SelW contains a highly reactive selenocysteine; thus, it appears to be involved in regulating the cellular redox state [42]. It has also been suggested to be involved in muscle growth and differentiation by protecting developing myoblasts from oxidative stress [43]. The E_{total} for Trx-SelW binding is −497.67 cal/mol, and GLU49, VAL50, and THR51 are SelW hot spots. Based on the data, there is a potential relationship between Trx and SelW [44].

SelH was first identified in fruit flies and has been shown to bind to stress response elements [45, 46]. SelH possesses glutathione peroxidase activity and is also involved in regulating transcription genes that participate in the de novo glutathione synthesis and the second phase of detoxification enzymes [47]. The E_{total} for Trx-SelH binding is −521.93 cal/mol, and LEU104, GLY105, and LYS106 are SelH hot spots (Table 2). Based on the data we have got, there is a potential relationship between Trx and SelH; this relationship has been mentioned in another study.

SelI

SelI is a recently evolved protein found only in vertebrates. Its COOH-terminal contains a Sec residue whose function is currently not known. When the Sec residue is absent from the SelI protein, it has been shown to have ethanolamine phosphotransferase activity [48]. Nevertheless, its functions in organisms and the role of the Sec residue in this protein needed to be further determined. The E_{total} for Trx-SelI binding is −643.21 cal/mol, and VAL89, LEU92, and LEU93 are SelI hot spots (Table 2). Based on the data, there is a potential relationship between Trx and SelI.

Sep15 and SelM

Sep15 was identified by experiments as a 15-kDa selenoprotein in 1998; it was later suggested to participate in cancer prevention by mediating dietary Se [49, 50]. It has also been proven to regulate redox homeostasis in the ER [51]. Sep15 can be expressed in a wide range of mammalian tissues but is highest in the kidney, liver, prostate, and testis. In a recent study, it suggested that the Sep15 gene is important for a variety of processes related to AIDS [52].

The E_{total} for Trx-Sep15 binding is −617.79 cal/mol, and LEU39, GLY40, and PHE41 are Sep15 hot spots. Based on the data, there is a potential relationship between Trx and Sep15.

SelM was identified by bioinformatics approaches that also proved that it is a Sep15 homolog [53]. Because SelM is highly expressed in the brain, several studies have investigated its possible function in neuroprotection. SelM overexpression can prevent oxidative damage caused by H_2O_2 treatment in neuronal cells, while SelM knockdown leads to decreased cell viability and even result in a significant apoptotic cell death [54]. SelM overexpression has also been shown to be related to preventing Alzheimer's disease [55]. However, the exact function of SelM and its mechanism for protecting the brain and other tissues remains unclear.

The E_{total} for Trx-Sep15 to binding is −617.79 cal/mol, and PRO92, ARG93, ARG94, and ALA98 are SelM hot spots (Table 2). Based on the data, there is a potential relationship between Trx and SelM.

SelS

The SelS gene has been shown to regulate the levels of IL-6, IL-1β, TNF-α, and several cytokines and be involved in coronary heart disease, gastric cancer, colorectal cancer,

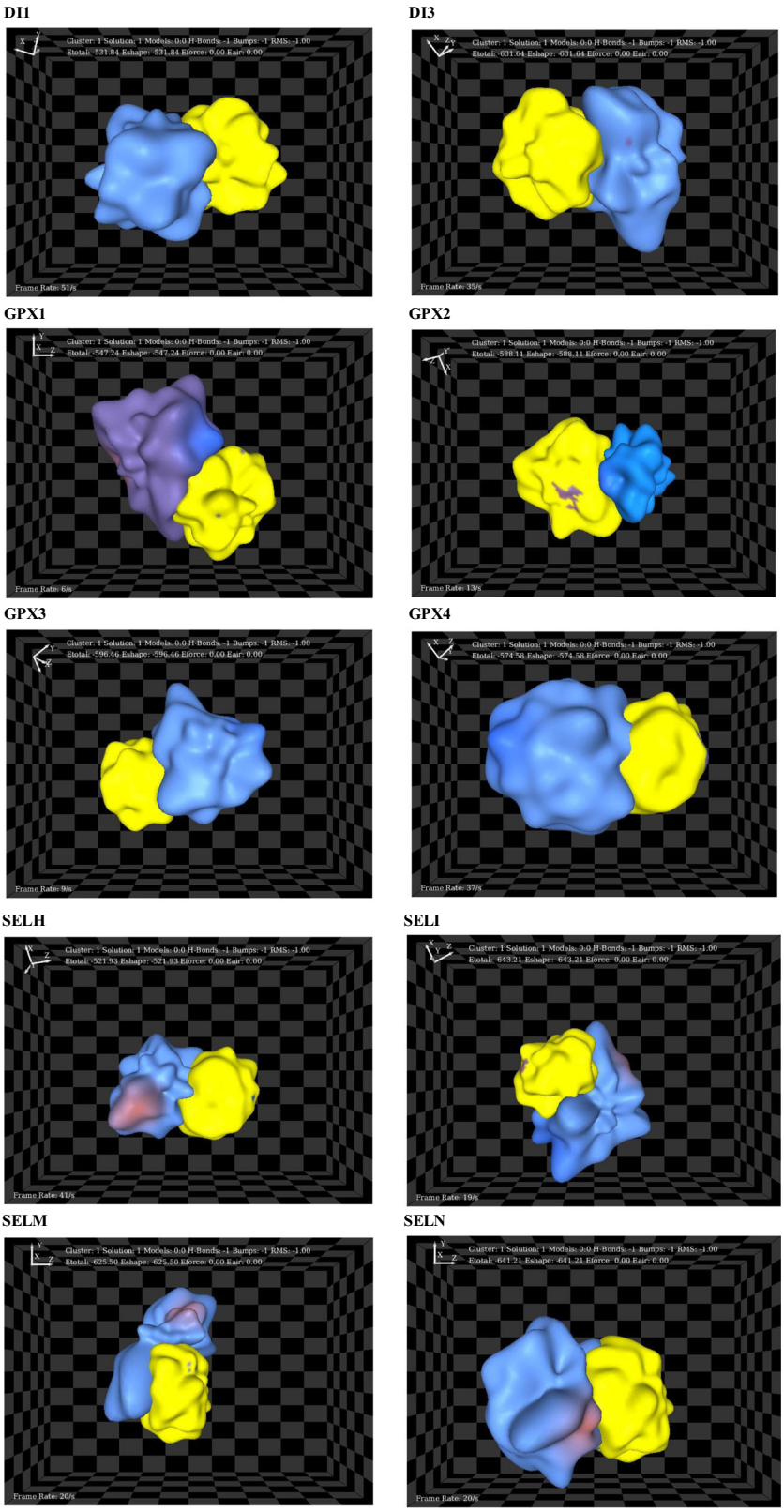

Fig. 3 Docking results of Trx and target selenoproteins

and other diseases related to inflammation [56–58]. Nevertheless, further investigation is needed to understand how SelS molecular mechanisms influence immune function and inflammatory reactions.

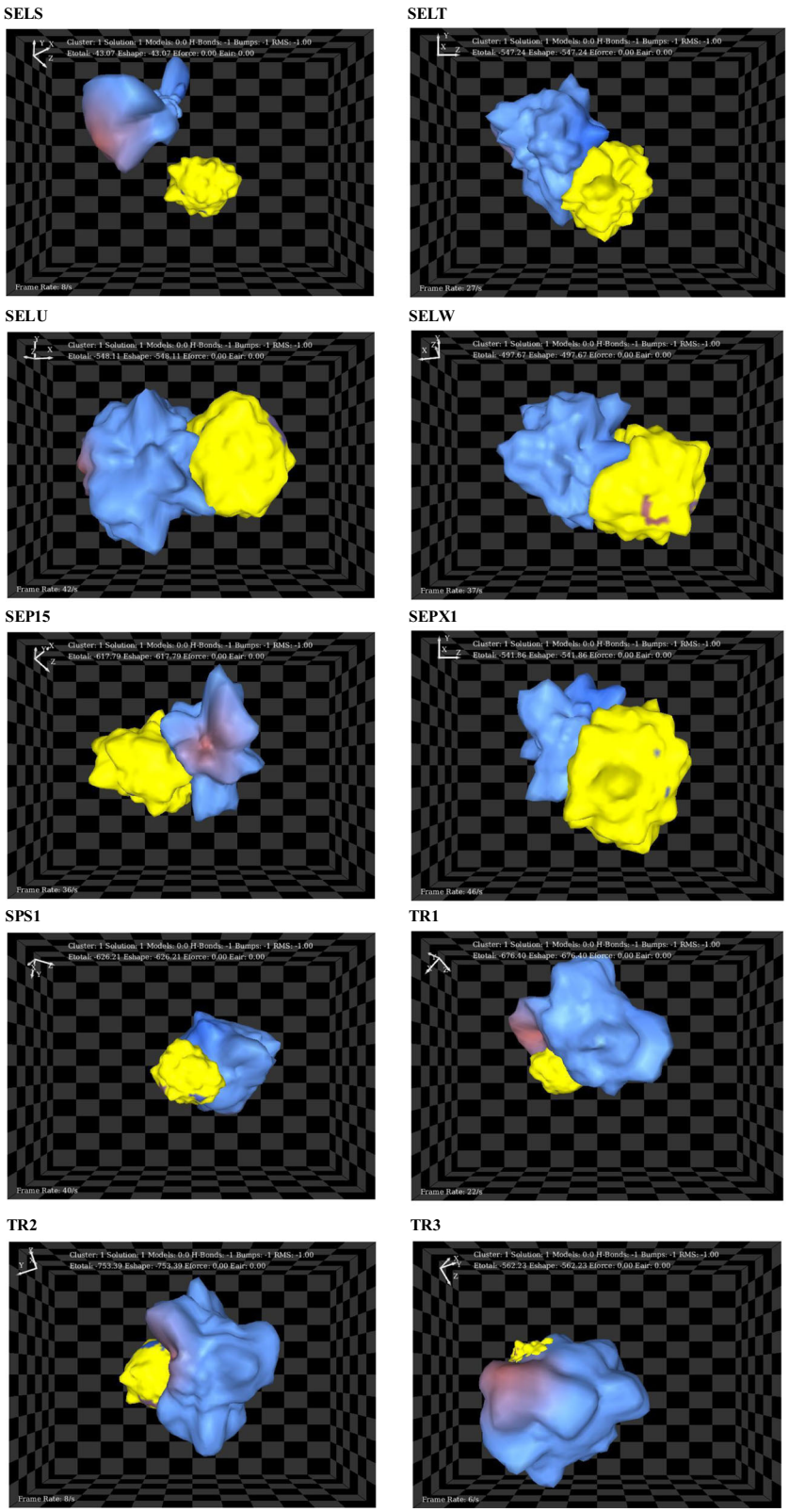

Fig. 3 (continued)

The E_{total} for Trx-SelS binding is −617.79 cal/mol, and TYR49, LEU50, and GLN53 are SelS hot spots (Table 2). Based on the data, it is believed that SelS lacks enough interactions to bind to or have a relationship with Trx.

Table 1 Total energy value of Trx and selenoproteins

Protein	E_{total}	Protein	E_{total}
DI1	−531.84	TR1	−676.40
DI2	–	TR2	−753.39
DI3	−631.64	TR3	−562.23
GPX1	−547.24	SELS	−43.07
GPX2	−588.10	SELT	−547.24
GPX3	−596.46	SELU	−548.11
GPX4	−574.58	SELW	−497.67
SELH	−521.93	SEP15	−617.79
SELI	−643.21	SEPX1	−541.86
SELK	–	SPS1	−626.21
SELM	−625.50	Selpb	–
SELN	−641.23	SELP	–
SELO	–		

SelN

SelN was one of the first identified selenoproteins and promotes early-onset muscle disorders when the gene is mutated [59]. SelN is located on the ER and is highly expressed during embryonic development, though its expression is lower in adult tissues, such as skeletal muscle [60]. A zebrafish model has recently proven that SelN is essential during the period of early muscle growth and differentiation [61, 62]. Further studies are required to better understand the role of mechanisms in normal muscle function.

The E_{total} for Trx-SelN binding is −641.23 cal/mol, and ALA354, ALA355, and GLN356 are SelN hot spots (Table 2). Based on the data, there is a potential relationship between Trx and SelN.

Sepx1

Sepx1 belongs to the MsrB family and functions as a repair enzyme to protect proteins from damage caused by oxidative stress by catalyzing the reduction of methionine-*R*-sulfoxides to methionines. It is highly expressed in the liver and kidney and is localized in the nucleus and cytosol.

The E_{total} for Trx-Sepx1 binding is −541.86 cal/mol, and CYS23, ALA24, and ARG25 are Sepx1 hot spots (Table 2). Based on the data, there is a potential relationship between Trx and Sepx1.

SelU

The functions of SelU are not yet known, though its function has been predicted based on its protein structure from modeling and docking. The E_{total} for Trx-Sepx1 binding is −541.11 cal/mol, and GLU50, ALA51, and ILE52 are SelU hot spots (Table 2). Based on the data, there is a potential relationship between Trx and SelU.

SPS1

SPS1 is an enzyme in humans encoded by the SEPHS1 gene. It is involved in synthesizing selenocysteine as a selenium donor and also plays an essential role in regulating redox homoeostasis in mammals [63]. SPS1 has been shown to recycle L-selenocysteine in the human lung, while SPS2 has been proven to participate in selenite assimilation [64].

SPS2 is enzymatic in nature, can regulate its own production with SelS, and is involved in SelS biosynthesis [65]. However, data for SPS2 in chicken, which are not present in NCBI, showed that its function in organisms is not clear. To predict interactions between Trx and selenophosphate synthetase (SPS) and potential processes that SPS may participate in, we considered the similarity between SPS1 and SPS2 and used SPS1 to establish the model.

ASN293, PHE295, and GLY296 are SPS1 hot spots (Table 2). Based on the data, there is a potential relationship between Trx and SPS1. Considering the similarity between SPS1 and SPS2, further experiments are needed to prove this hypothesis.

In this study, we established a 3D model of Trx and 20 selenoproteins for the first time to identify whether there are possible interactions between these proteins. We searched for protein sequences in the NCBI database and then used Modeller to establish the structure of target proteins. The specific substrate binding modes were determined using molecular docking with the Hex 8.0.0 and the KFC2 Server to confirm the specific hot spots, which are the major binding sites for protein-protein interactions. Interestingly, there are many interactions between Trx and selenoproteins. By comparing the TR data, we can reasonably assume that Trx has a close relationship with selenoproteins. The docking results were studied in further detail to identify the hot spot residues responsible for the interactions.

Several amino acid residues in Trx were identified to exclusively contributive to selenoprotein binding. Based on the modeling and docking results, we can reasonably suggest that Trx potentially interacts with selenoproteins. We can reasonably hypothesize that Trx may participate in many signal pathways that are regulated by selenoproteins. Additionally, the generated homology model is expected to be useful for understanding structure-based signaling pathways and physiological processes in organisms.

Table 2 Hot spots analysed by the KFC2 Server

	Hot spot of Trx	Target protein
DI1	MET1, LYS39, PRO40, PHE41, PHE42, HIS43, ASP47, LYS94	GLU83, ILE152, GLU153, GLU154, ALA155, ASN201, ALA208, LEU210, PRO211, GLU212, ARG213
DI3	HIS68, ASP70, TYR80, LYS84, LYS85, VAL86, GLU88, GLU99, THR100, SER103, LEU104	ASP92, SER96, ILE166, TYR222, GLY223, TYR225, PHE226, ARG228, TYR230, GLN240, ARG243
GPX1	MET1, LYS3, ASP10, GLU12, ALA13, GLU14, ALA17, LYS21, CYS46, VAL53	LEU82, PRO83, HIS86, VAL123, PRO124, PHE125, ARG126, ASP140, LEU143
GPX2	PRO75, GLN87, GLU88, PHE89, SER90, ASN93, LYS96, THR100, LEU104	HIS78, LEU79, PRO80, ALA81, VAL83, ALA86, PRO121, PHE122, ARG123, ARG124
GPX3	ASP70, LYS72, CYS73, PRO75, GLU88, PHE89, SER90, GLY91	THR22, GLU27, ILE29, TYR34, GLN61, LEU69, VAL70, VAL71, LEU72, VAL104, ASN106, GLN108
GPX4	MET1, VAL2, LYS3, SER4, VAL5, ASN7, ASP10, HIS43, GLU56, ILE57, ASP58, ASP6	ASP6, ALA19, ARG20, SER104, LYS105, ILE106, GLU107, ASP111, GLY112, HIS114, PRO115
SELI	GLU12, CYS73, PRO75, LYS85, VAL86, GLN87, GLU88, PHE89, GLY91, LYS96, LEU104	ILE37, PHE41, SER53, LEU57, LEU64, ASN84, VAL89, LEU92, LEU93, ILE96
SELM	MET1, VAL2, LYS3, SER4, GLY6, ASN7, ASP10, GLU14, GLU56, ILE57, ASP58, VAL59, ASP60, ASP61, GLN63, ASP64	HIS19, PHE38, PRO74, VAL75, GLN80, PRO92, ARG93, ARG94, ALA98, ASP99, LYS101, GLN106, LYS108
SELN	MET1, VAL2, LYS3, SER4, VAL5, GLY6, ASP10, ALA13, GLU14, ALA17, PHE42, CYS46, VAL53, ASP58, ASP61, ASP62	ALA354, ALA355, GLN356, LYS357, LEU358, TYR363, TYR370, LEU371, PRO372, LEU432, VAL433, LYS434 GLU435, PHE447
SELH	VAL2, SER4, ALA29, LYS39, PHE42, HIS43, GLU56, ILE57, ASP58	GLU46, ARG82, GLU87, GLN99, ILE104, GLY105, LYS106, GLY107
SELU	HIS68, CYS69, ASP70, LYS72, CYS73, TYR80, LYS85, VAL86, GLN87, GLU88, PHE89, SER90, LYS96, LEU104	GLU50, ALA51, ILE52, ARG144, LYS145, ARG146, LYS147, PHE163, ARG164
SELW	TRP31, PRO34, VAL59, LYS72, CYS73, MET74, PRO75,	THR6, LEU8, THR41, TRP47, GLU49, VAL50, THR51, SER54, LEU56
SEP15	SER4, VAL5, GLY6, ASN7, ASP10, ALA29, THR30, LYS36, MET37, LYS39, ASP58, VAL59, ASP60, ASP61	LEU15, LEU39, GLY40, PHE41, CYS50, LEU52, GLN55, GLN67, LEU75, ARG78, LEU86
SPS1	GLU12, ALA66, THR67, HIS68, ASP70, VAL71, LYS72, GLN78, TYR80, LYS84, LYS85, VAL86, GLU88	THR14, PRO19, ASP21, LEU257, GLN261, ASN293, PHE295, GLY296, MET298, HIS299, THR301, ASP353
TXNRD1	MET1, LYS3, CYS46, ASP47, LYS48, PHE49, GLY50, ASP51, VAL52, CAL53, LYS102	ILE65, LYS67, TYR116, PRO344, VAL345, ILE347, GLN348, ARG351, LEU352, GLU447, GLN450
TXNRD2	THR30, TRP31, CYS32, GLY33, PRO34, LYS36, MET37, VAL59, ASP60, ALA66, LYS72, CYS73, MET74, PRO75, SER90, GLY91, ASN93	ALA15, LYS18, CYS48, ILE54, TYR97, SER100, LEU101, TRP103, GLY104, HIS105, VAL107, GLN108, PRO331, THR332, ILE334, ALA335, LYS338, PRO358, PHE362, GLN437, ALA440, LEU441, LYS444
TXNRD3	PHE11, HIS68, CYS69, ASP70, LYS72, TYR80, LYS84, LYS85, GLN87, GLU88, PHE89, SER90, LYS96, THR100	ASP538, ARG541, LYS564, CYS565, GLY566, THR568, LEU571, GLU574
SELS	GLU56, ILE57, ASP58, ASP61, ALA62	VAL46, TYR49, LEU50, GLN53, PRO57, TYR58, ARG60, MET61
SEPX1	MET1, VAL2, LYS3, SER4, ASP10, GLU14, LYS39, PHE42, VAL53, PHE55, GLU56, ILE57, ASP58	TYR21, CYS23, ALA24, ARG25, CYS26, GLY27, HIS51, GLU52, ASN74
SELT	MET1, LYS3, ASP10, ALA13, GLU14, LYS16, ALA17, ALA18, LYS21, CYS46, VAL53	LEU82, PRO83, HIS86, VAL123, PRO124, PHE125, ARG126, TYR128, ASP140, LEU143

Conclusions

In conclusion, among the 20 selenoproteins examined in this study, TR1, TR2, TR3, SPS1, Sep15, SelN, SelM, SelI, Gpx2, Gpx3, Gpx4, and DI3 exhibited intense correlations with Trx. DI1, Gpx1, SelH, SelT, SelW, and Sepx1 also showed strong correlations with Trx. By contrast, SelS did not show an obvious relationship with Trx. Currently, the exact interactions between these proteins is still unknown, meaning that further investigations to confirm these binding interactions are needed in the future.

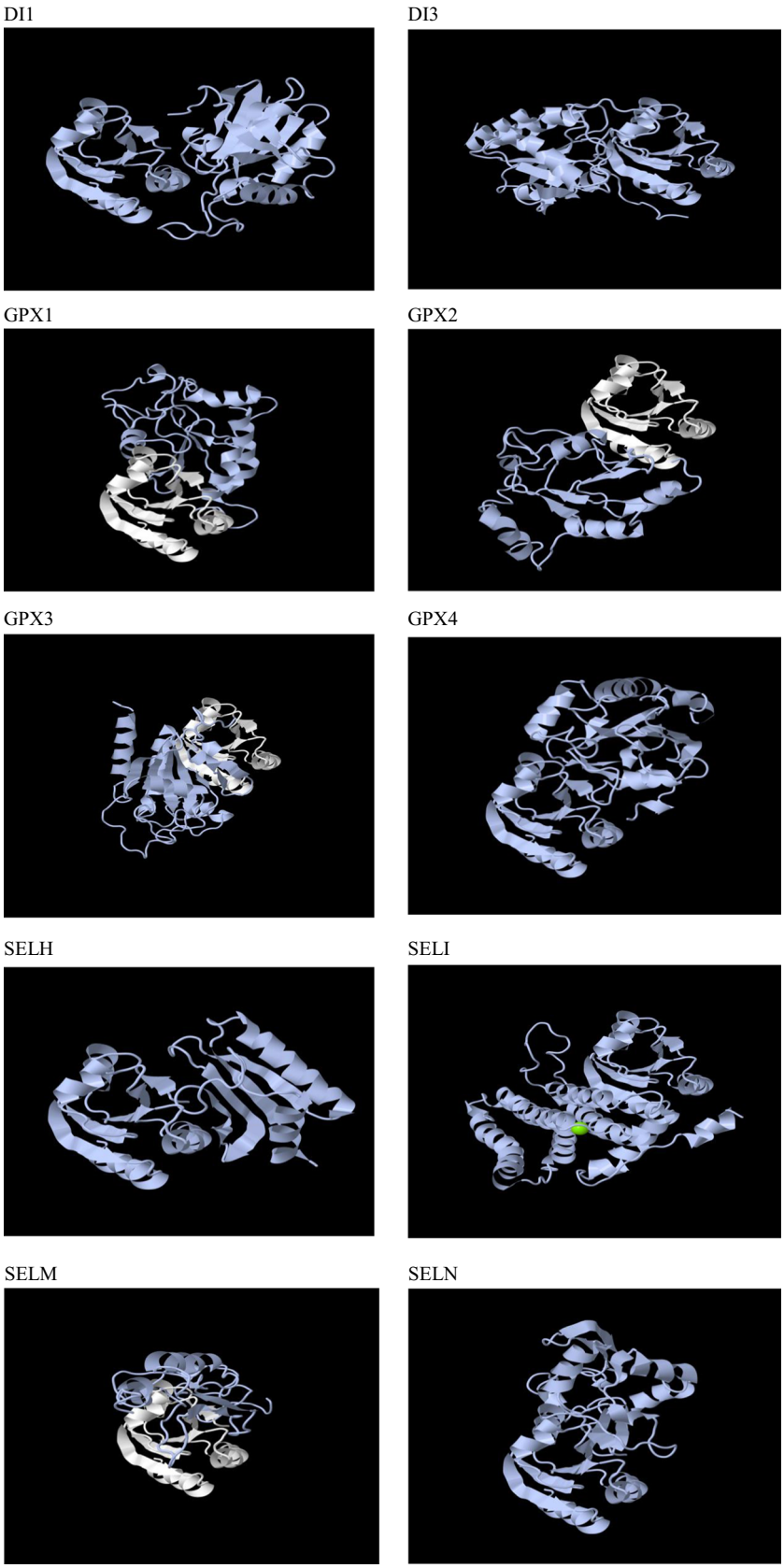

Fig. 4 Results of the KFC2 Server

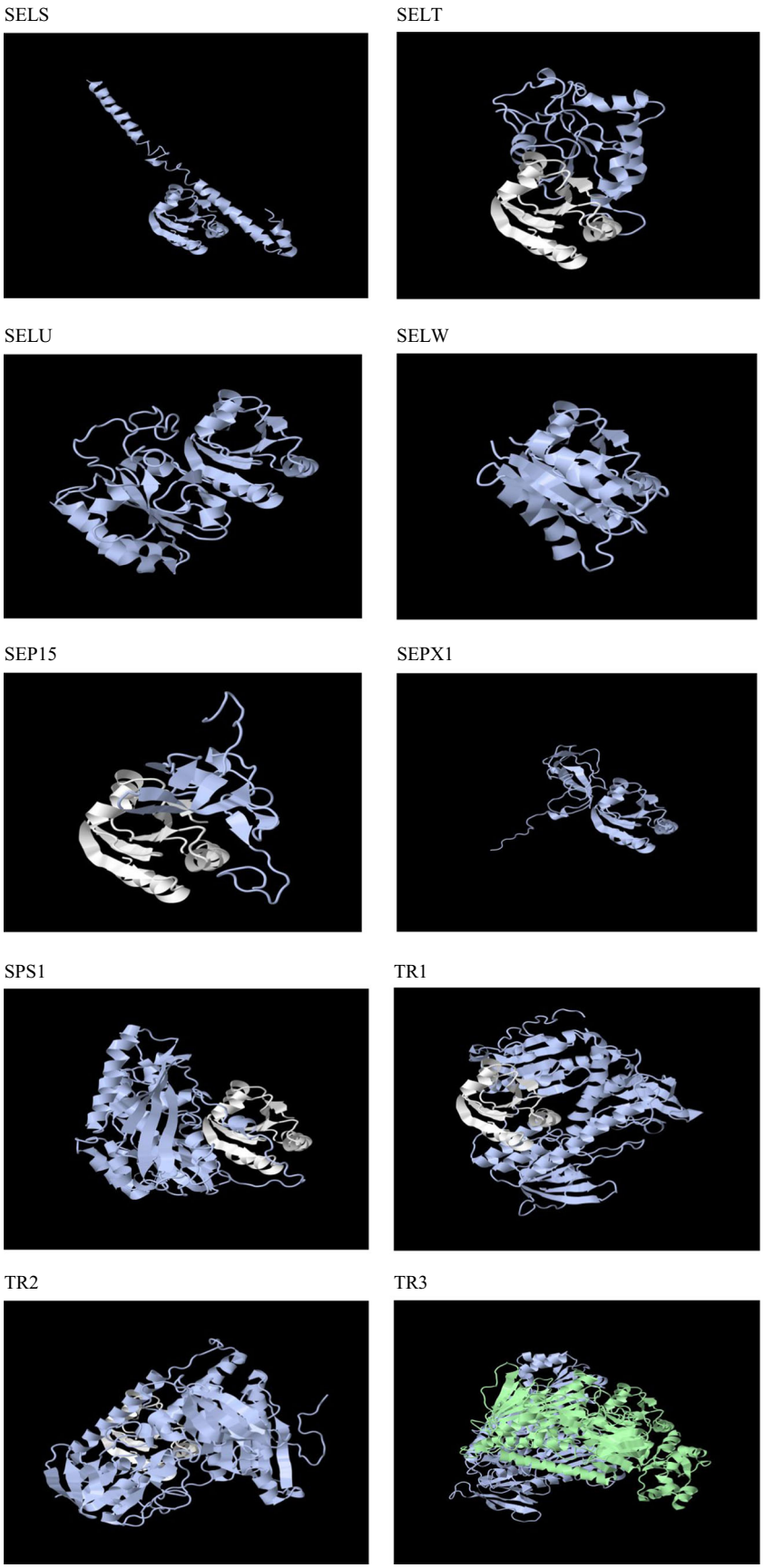

Fig. 4 (continued)

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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