ISSN 2581 – 4303

Available Online at www.ijpba.info
International Journal of Pharmaceutical & Biological Archives 2019; 10(1):52-59

RESEARCH ARTICLE

In Silico Modeling and Docking Studies on Methionine Sulfoxide Reductase A Protein

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Received: 28 December 2018; Revised: 28 January 2018; Accepted: 20 February 2019

ABSTRACT

Alzheimer disease (AD) is a neurodegenerative disorder including continuously progressive cognitive and functional deficits as well as behavioral changes and is related with amassing of amyloid and tau depositions in the brain. Subjective side effects of AD most ordinarily incorporate deficits in short-term memory, executive and visuospatial dysfunction, and praxis. Mammalian methionine sulfoxide reductase A is encoded by a single gene and is found in both cytosol and mitochondria. Biologically active compounds from different plants have been used to treat various ailments. In the present study, mitochondrial peptide methionine sulfoxide reductase protein sequence from Homo sapiens was retrieved from UniProt and selected structure of the peptide methionine sulfoxide reductase from *Escherichia coli* (Protein Data Bank [PDB] id: 1FF3) was used as template. The homology model was developed by using Modeller 9.20 version. Molecular docking studies were performed using Autodock4.2. 20 natural compounds were docked against modeled protein. All the compounds exhibited good binding energy. Campesterol showed with lesser energy of −9.0 Kcal/mol.

Keywords: Docking, homology modeling, methionine sulfoxide reductase A, natural compounds

INTRODUCTION

Age-related dementia is most commonly caused by Alzheimer's disease (AD). It is characterized by cognitive impairment and severe neurodegeneration. The dementia typically begins with subtle and poorly recognized failure of memory and slowly becomes more severe and eventually, incapacitating. Different discoveries incorporate disarray, misguided thinking, dialect unsettling influence, tumult, withdrawal, and mental trips. Infrequent seizures, Parkinsonian highlights, expanded muscle tone, myoclonus, incontinence, and mutism happen. Passing as a rule results from general inanition, unhealthiness, and pneumonia. The regular clinical length of the ailment is 8–10 years, with a range from 1 to 25 years.

Therefore, AD remains one of the principal health issues for the aging population and is destined to grow remarkably in the coming decades. AD is characterized by the deposition of extracellular amyloid-beta (Aβ) peptide,[1] which is generated from the amyloid precursor protein (APP) or β-APP,[2,3] forming senile plaques and intracellular abnormally hyperphosphorylated tau protein, and forming neurofibrillary tangles.[4]

Alzheimer's remains one of the most pressing principle health issues for the aging problem and is destined to grow remarkably in coming times. What makes it a challenging interest among scientists is that a promising treatment is not yet at the horizons. Accumulating studies have investigated the molecular factors by which oxidative stress is a major deleterious mechanism in Alzheimer's and other neurodegenerative disorders as well as in normal aging process. Oxidative stress is created and maintained by inflammation surrounding the amyloid plaques including activated microglia and astrocytes.[5] Mitochondrial functions are disrupted by oxidative stress which causes reduced metabolic activity due to oxidative damage to vital mitochondrial regions which is seen in patients with AD. AD is linked to oxidative stress.[6] Methionine sulfoxide reductase system can affect any process that involves oxidative damage.[7] Methionine is highly

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susceptible to oxidation *in vivo*, particularly under conditions of oxidative stress.[8]

In the present study, *in silico* studies were performed due to the absence of crystal structure for mitochondrial peptide methionine sulfoxide reductase protein. The homology model of the protein was developed using Modeller 9.20 and validated using Procheck. To study the binding affinity of protein-ligand and molecular interactions of mitochondrial peptide, methionine sulfoxide reductase docking studies were performed using Autodock4.2.

METHODOLOGY

Sequence alignment and structure prediction

The amino acid sequence of mitochondrial peptide methionine sulfoxide reductase (UniProt accession number: Q9UJP8) from the species *Homo sapiens* was retrieved from the UniProtKB database.[9] A Basic Local Alignment Search Tool (BLAST) search was performed to select the template. Structure of the peptide methionine sulfoxide reductase from Escherichia coli (PDB ID: 1FF3_A)[10] was selected as a template. The three-dimensional structure was generated using Modeller 9.20. The respective templates were retrieved from protein database like PDB.[11] When choosing the template, it is important to consider the sequence identity and resolution of the template. When both parameters are high, the resulting model would be sufficiently good to allow structural and functional research.

MODELLER 9.20 was then used to generate satisfactory models; an automated approach to homology modeling by satisfaction of spatial restraints. Sequence alignments using the protein and template sequences was then carried out using platforms such as Clustal X and Clustal W[12] [Figure 1]. Homology models for the chosen protein were then constructed using modeler programs like Modeller 9.20.[13] After manually modifying the alignment input file in MODELLER 9.20 to match the query and template sequence, 20 models were generated. The best model is determined by the lowest value of the Modeller objective function. The stereochemical quality of the given models was then evaluated using software like PROCHECK,[14] and the model can be used for further structural or functional study. PROCHECK generated a Ramachandran plot which explains residue by residue listing that facilitates the in-depth calculation of Psi/Phi angles and the backbone conformation of the models. The root-mean-square deviation (RMSD) was calculated by superimposing (1FF3_A) over the generated model to access the accuracy and reliability of the generated model using SPDBV.[15]

Docking methodology

Identification of active site pockets: The active site prediction was carried out using Tripo's Sybyl6.7.[16] It showed three active site pockets. The amino acids in pocket one were Asn140, Tyr219, Gly221, Leu222, Gly223, Cys74, Ala78, and Lys81.

In total, 20 natural compounds were retrieved from NCBI. All the molecules were sketched in sybyl6.7 and minimized by adding Gasteiger-Huckel charges and saved in.mol2 format. Molecular docking studies were performed on all the natural compounds separately using AutoDock4.2[17] program, using the Lamarckian genetic algorithm, and empirical free energy function was implemented. Initially, the modeled mitochondrial peptide methionine sulfoxide reductase protein was loaded and hydrogens were added before saving it in PDBQT format. Later,

Figure 1: Sequence alignment of mitochondrial peptide methionine sulfoxide reductase protein and template 1FF3

the ligand was loaded and conformations were set and saved in PDBQT format. The grid parameters were selected and calculated using AutoGrid. For all the dockings, a grid-point spacing of 0.375 Å was applied and grid map with 60 × 60 × 60 points was used. X, Y, and Z coordinates were selected on the basis of the amino acids present in the active site predicted in sybyl6.7 biopolymer module. Default parameters were used to run the Autodock.

RESULTS AND DISCUSSION

Homology modeling and model evaluation

The present study reports that the template protein (PDB ID: 1FF3_A) having high degree of homology with Q9UJ68 protein was used as a template with good atomic resolution of its crystal structure. The target sequence of mitochondrial peptide methionine sulfoxide reductase (UniProt accession number: Q9UJ68_Human) bearing 235 amino acid residues was retrieved from the UniProt protein sequence database with Accession No. Q9UJ68. Using BLAST, PDB ID 1FF3_A was identified and selected as a template to predict the model. The structure was modeled using Modeller9.20. The generated structure was validated using the protein structure and by PROCHECK. The generated model showed that 90.3% of amino acid residues in core region with 176 amino acids, 8.2% of amino acid residues in additionally allowed region having 16 amino acids, 1.5% of the amino acid residues in the generously allowed region with three amino acids, and there are no amino acids present in disallowed region. The template PDB shows that 90.1% of amino acids in core region, 9.9% of the amino acid residues in additionally allowed region, and there are no amino acid residues in generously allowed region and disallowed region. Cartoon model of secondary structure of the modeled protein is shown in Figure 2 and Ramachandran plot is shown in Figure 3. RMSD was calculated for template and generated model using SPDBV. Both the models were loaded and superimposed using the alpha carbon and RMSD was calculated. It showed RMSD of 0.32Å, which indicates that the generated model shows similarity to the template [Figure 4].

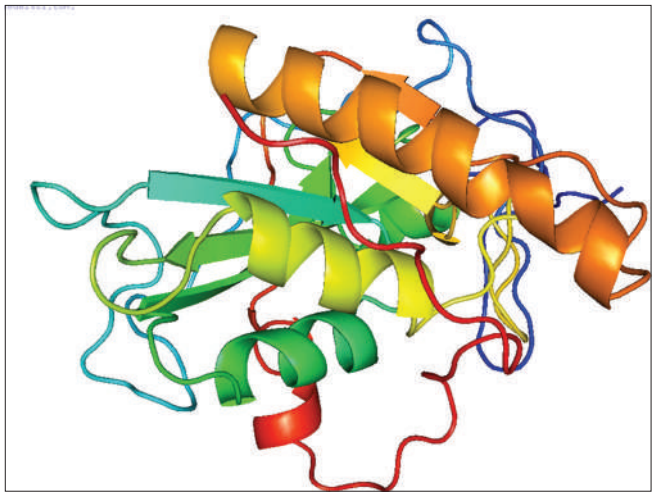

Figure 2: The cartoon model of mitochondrial peptide methionine sulfoxide reductase modeled protein

Molecular docking results

Molecular docking is the most extensively used method for the calculation of protein-ligand interactions. It is an efficient method to predict the potential ligand interactions. In the present study, the native plant secondary metabolites (ligands) have been identified as potent mitochondrial peptide methionine sulfoxide reductase inhibitors. AutoDock4.2 uses (genetic algorithm) binding free energy assessment to assign the best binding conformation**.** Further, the activity of docked ligand molecules was compared to that of standard drugs which were controls. In total, 20 natural compounds were docked against modeled mitochondrial peptide methionine sulfoxide reductase.

However, the compounds campesterol and xanthostigmine showed better interactions and lower free energy values, indicating more thermodynamically favored interactions. Both the compounds exhibited binding energy of <−9.0 Kcal/mol. Specifically, campesterol exhibited the highest binding energy of value −9.09 K.cal/mol while interacting with Glu117 and artocarpin exhibited binding energy of −8.54 K.cal/mol with interacting Glu117. When compared to the standard drugs, i.e. memantine, donepezil, tacrine, and xanthostigmine campesterol exhibited highest binding energy. Xanthostigmine exhibited binding energy of −9.00 Kcal/mol while interacting with Tyr105. All the compounds showed good binding energy with modeled protein. Three compounds exhibited binding energy <−8.00 Kcal/mol, six compounds exhibited binding energy of <−7.00

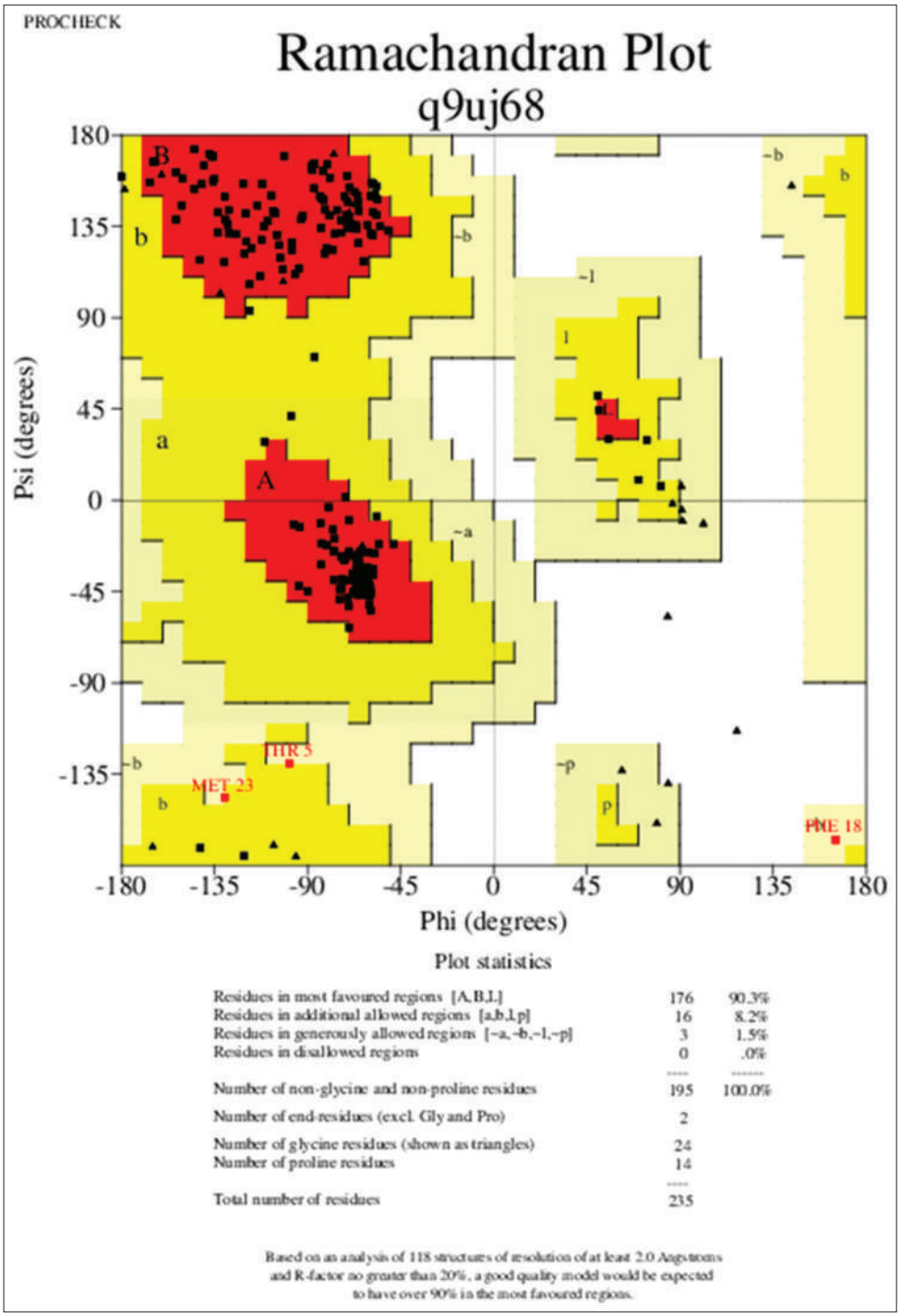

Figure 3: Ramachandran plot of the modeled mitochondrial peptide methionine sulfoxide reductase protein exhibited 90.3% amino acid residues in the most favored region

KCal/mol. The natural compounds with their corresponding interactions and binding energies are shown in Table 1 and Figure 5.

CONCLUSION

The sequence obtained from UniProt does not contain the crystal structure (three-dimensional structure) in the PDB database. The crystal structure was built by homology modeling using Modeller 9.20. Modeled protein was validated using Procheck. The generated model showed 90.3% of amino acid residues in the most favored region. The generated model was then docking with 20 natural compounds and also docked already existing drugs as controls. Natural compounds showed better binding energies than already existing drugs. Campesterol exhibited highest binding energy of −9.09 Kcal/mol with interacting Glu117. The study explains that natural compounds are more potent than already existing drugs for Alzheimer's.

Table 1: Binding energy, dissociation constant and interacting amino acids of twenty natural compounds and four existing drugs

C. No	Compound name	Interacting amino acids	Binding energy	Dissociation constant (ΔG)
1	Apingenin	Gly97, Gly98	−5.61	77.73 µM
2	Eugenol	Gly221	−5.44	103.47 µM
3	Campesterol	Glu117	−9.09	216.41 nM
4	Gallic acid	Cys74, Trp76, Tyr105, Glu117	−5.93	45.33 µM
5	Isoquercetin	Asp142, Asp152, Cys220, Gly224	−6.61	14.36 µM
6	Kaempferol	Glu17, Tyr157	−7.22	5.08 µM
7	Quercetin	Asp152, Gly223, Gly224	−6.95	8.11 µM
8	Nimbin	Asn151	−8.17	1.03 µM
9	Eriodictyol	Met23, Glu117	−6.97	7.73 µM
10	Luteolin	Asp152, Gly223, Gly224	−7.33	4.22 µM
11	Castin	Asp142, Asn151	−6.58	15.06 µM
12	Artocarpin	Glu117	−8.54	551.59 nM
13	Baicalein	Asn151, Asp152	−7.11	6.12 µM
14	Cudraflavone	Met23, Glu117	−8.16	491.59 nM
15	Macakurzin	Glu117, Asp152	−7.28	4.59 µM
16	Curcumin	Cys74	−7.93	1.54 µM
17	Genkwanin	Cys74	−6.87	9.14 µM
18	Yanuthone	Asn151, Arg148	−7.48	3.28 µM
19	Bilobide	Tyr105	−6.18	29.53 µM
20	Bilobol	Met23, Tyr105	−5.70	66.51 µM
21	Memantine	Tyr105, Tyr157	−6.67	13.0 µM
22	Donepezil	Gly221	−8.98	262.74 µM
23	Tacrine	Asp152, Tyr219	−6.43	19.46 µM
24	Xanthostigmine	Tyr105	−9.00	253.17 nM

Table 2: Compound structures

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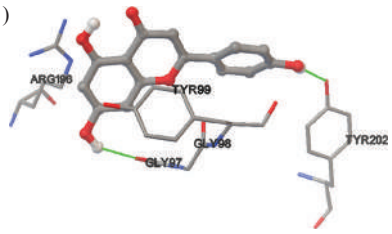

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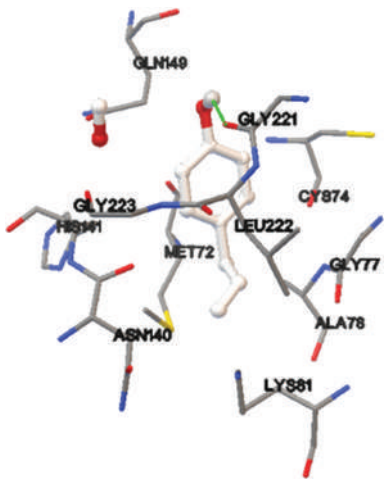

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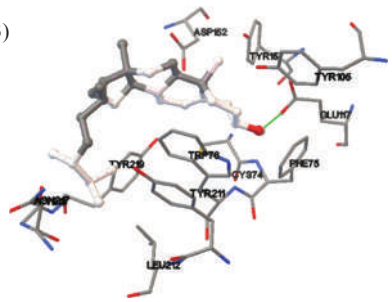

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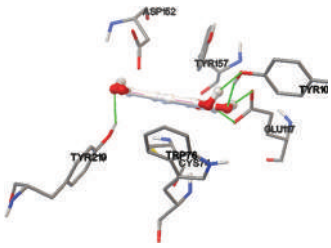

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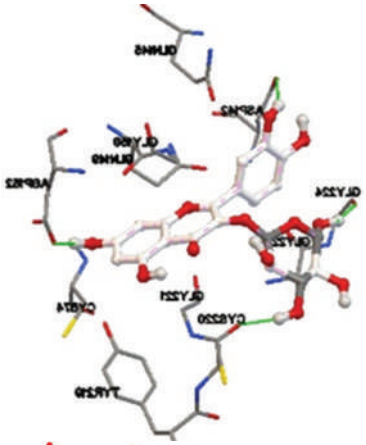

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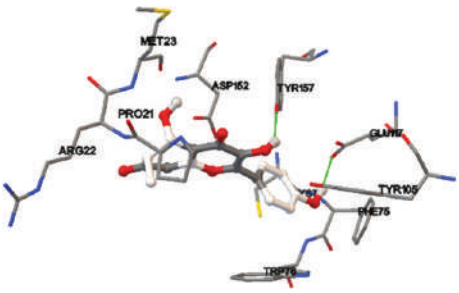

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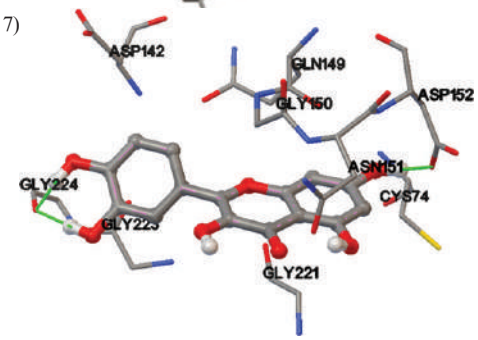

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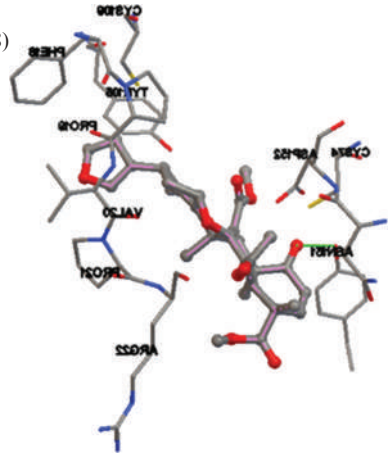

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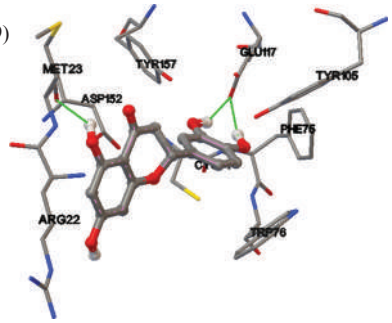

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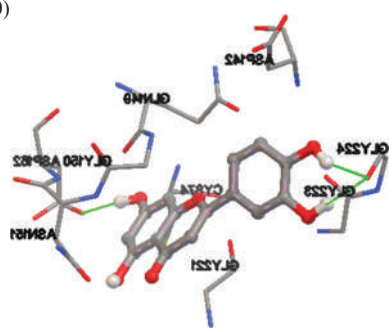

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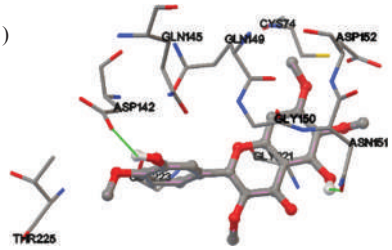

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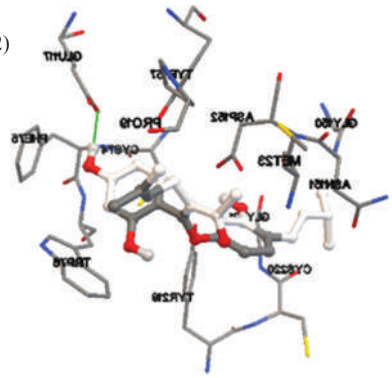

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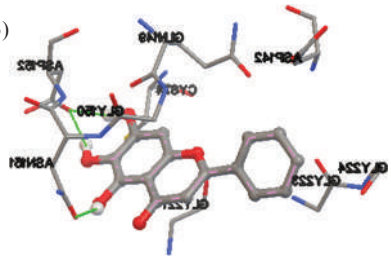

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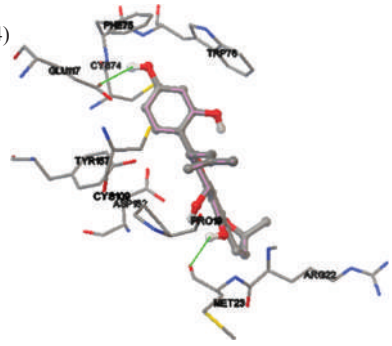

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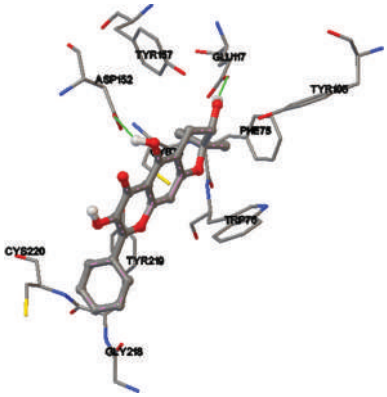

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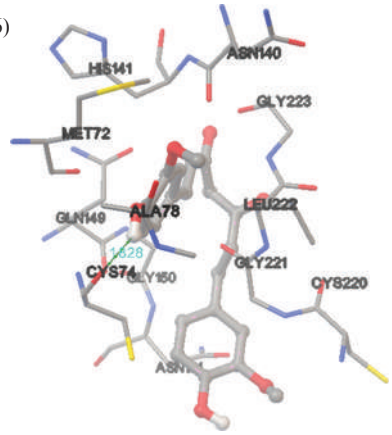

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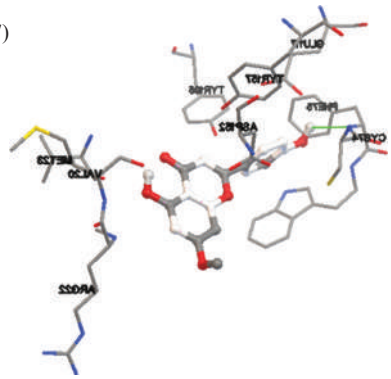

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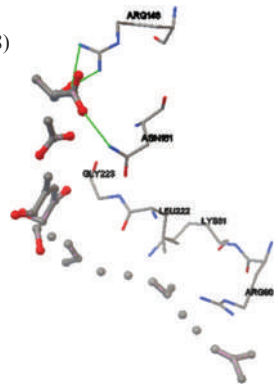

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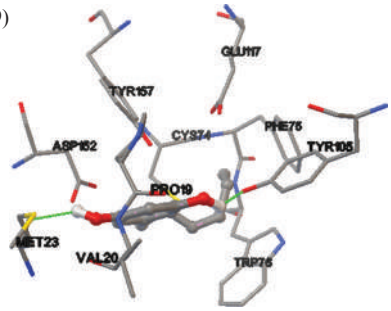

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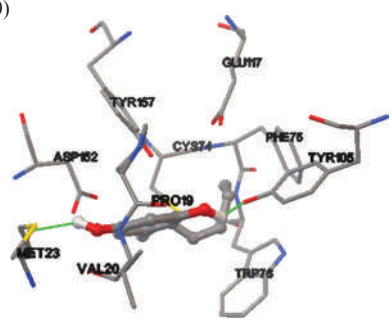

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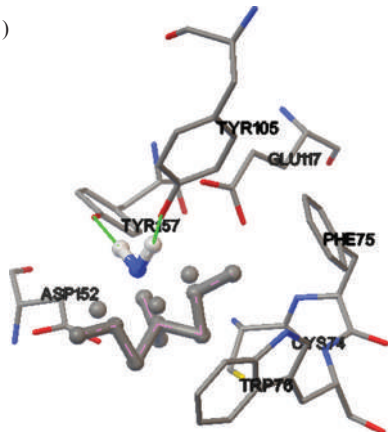

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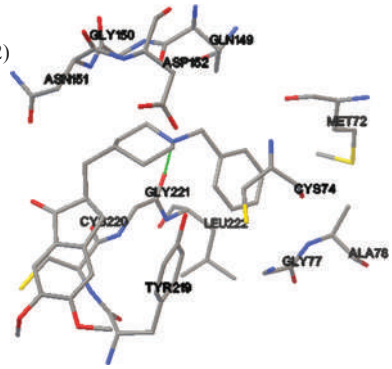

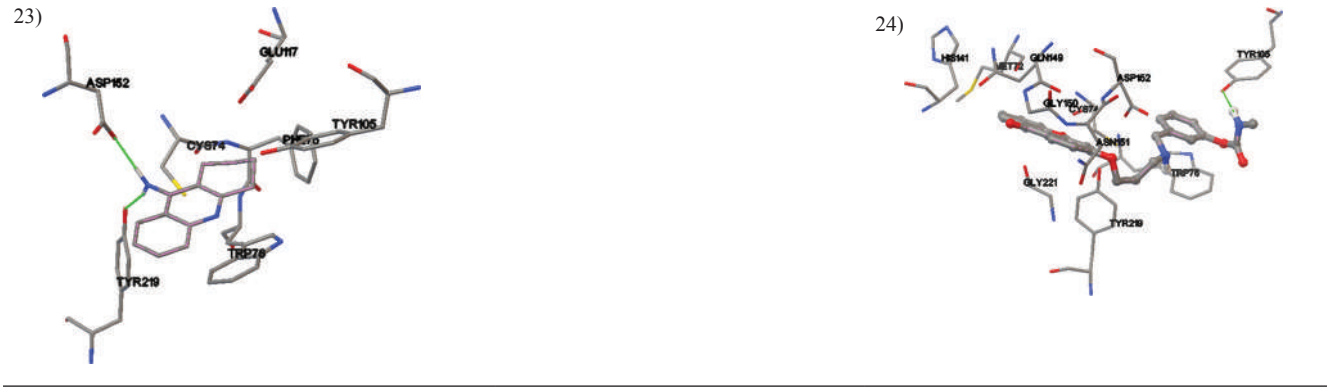

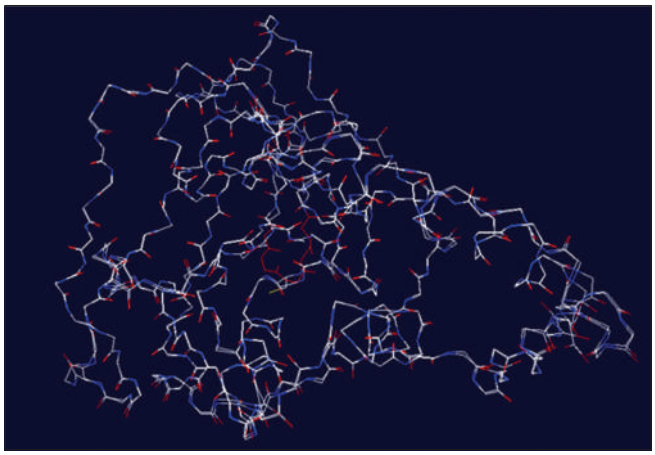

Figure 4: Superimposed model of modeled mitochondrial peptide methionine sulfoxide reductase protein and template protein

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