Investigating the Interaction between PITRM1 and Sulfiredoxin at the Cysteine-Rich Oxidation Prone Region: A Molecular Modelling and Dynamics Study

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Abstract:
This study aimed to investigate the interaction between PITRM1 and sulfiredoxin at the oxidation prone region in the presence of ATP using a combination of protein modelling, docking, and molecular dynamics (MD) simulation techniques. The three-dimensional structures of PITRM1 and sulfiredoxin were modelled using the SWISS-MODEL server, and their quality was assessed using the Ramachandran plot generated by PROCHECK. The PITRM1-sulfiredoxin complex was generated through protein-protein docking using ClusPro, and the best-docked complex was selected based on the lowest energy score. Additionally, ATP was docked with the selected complex to investigate its binding mode. MD simulation was performed on the selected complex using GROMACS with the CHARMM36 force field. The stability of the complex was analysed by calculating the root mean square deviation (RMSD) and root mean square fluctuation (RMSF) of the complex, and the protein-protein interaction energy and hydrogen bonding were also examined using GROMACS tools.The results showed that the modelled structures of PITRM1 and sulfiredoxin were of high quality and suitable for further analysis. The protein-protein docking analysis identified

specific binding modes between PITRM1 and sulfiredoxin, with the Cysteine rich oxidation prone region being a major focus. ATP docking revealed its interaction with the PITRM1-sulfiredoxin complex. The MD simulation of the complex demonstrated its stability over a 100 ns simulation period, as indicated by the low average RMSD and RMSF values. The analysis of hydrogen bonding and minimum distance measurements suggested strong binding interactions between PITRM1, sulfiredoxin, and ATP. Specifically, the presence of multiple hydrogen bonds within the interface of PITRM1 and sulfiredoxin indicated a crucial role in stabilizing the complex.In conclusion, this study provides valuable insights into the interaction between PITRM1 and sulfiredoxin at the Cysteine rich oxidation prone region in the presence of ATP. The findings suggest that sulfiredoxin plays a protective role in maintaining the activity of PITRM1 and preventing its oxidation-induced inactivation. This study contributes to a better understanding of the molecular mechanisms underlying the interaction between PITRM1 and sulfiredoxin and may have implications for the development of therapeutic strategies targeting Alzheimer's disease.

Keywords

PITRM1, Cysteine Oxidation, Docking and simulation, GROMACS, cysteine sulfinic acid

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Introduction

Cysteine can undergo oxidation to form cysteine sulfinic acid (CSA) within proteins[1]. This oxidative modification of cysteine residues is often referred to as sulfonation. The process of cysteine sulfonation can be mediated by a variety of oxidants, including reactive oxygen species (ROS). The conversion of cysteine to CSA involves the addition of an oxygen atom to the sulfur atom of the cysteine side chain, resulting in the formation of a sulfinic acid group (-SO_2H)[2]. This modification can affect the structure and function of proteins, as the sulfinic acid group is charged and polar, which can alter the local electrostatic environment and potentially disrupt protein-protein interactions or enzymatic activity.While cysteine sulfonation is typically considered to be a reversible modification, the removal of the sulfinic acid group from cysteine can be slow and require specialized enzymes, such as sulfiredoxin, to catalyze the reduction of CSA back to cysteine[3].

Sulfiredoxin (SRX) is an enzyme that plays a critical role in the reduction of cysteine sulfinic acid (CSA) back to cysteine[4]. The process of CSA reduction is an important regulatory mechanism that helps to maintain redox balance and prevent oxidative damage to proteins.SRX belongs to a family of enzymes called sulfiredoxin, which are characterized by a conserved active site containing a cysteine residue that can act as a nucleophile in the reduction of CSA. The reduction of CSA by SRX involves the transfer of electrons from a reducing agent, such as thioredoxin or glutathione, to the sulfinic acid group of CSA, resulting in the formation of cysteine and the corresponding sulfenic acid derivative of the reducing agent[5].SRX-mediated reduction of CSA is important in a variety of biological processes, including redox signalling, protein turnover, and regulation of enzymatic

activity. Dysregulation of CSA reduction by SRX has been implicated in several human diseases, including cancer, neurodegeneration, and cardiovascular disease[6,7].

Human Presequence protease (PITRM1) is a mitochondrial zinc metalloprotease that plays an important role in the degradation of small-unstructured peptides and mitochondrial Aβ. However, in Alzheimer's disease (AD), the activity of PITRM1 is reduced due to the oxidation of critical cysteine residues, particularly at Cys34, Cys112, and Cys119[8,9]. This oxidation leads to the inactivation of PITRM1 and negatively impacts its ability to degrade Aβ. The increased production of reactive oxygen species (ROS) is an early indicator of AD pathogenesis, and it has been suggested that the oxidative inactivation of PITRM1 by ROS may contribute to the disease pathology[9].Cysteine oxidation is a critical process that can impact protein structure and function, and recent studies have highlighted the importance of this modification in the mitochondrial metalloprotease PITRM1. Specifically, oxidation of cysteine 112 (Cys112) in PITRM1 has been shown to play a role in regulating the enzyme's activity and substrate specificity.PITRM1 is a key player in mitochondrial protein quality control and degradation, and it is responsible for degrading misfolded or damaged proteins in the mitochondrial matrix especially amyloid beta peptide[10,11]. The present study focused on identifying sulfiredoxin, a thioredoxin-like protein, capable of interacting with the cysteine residues in PITRM1. In particular, the study aimed to identify the interaction between sulfiredoxin and Cys112 in PITRM1, which has been shown to be a critical residue for the enzyme's activity. By elucidating the mechanism of this interaction, the study aimed to identify potential strategies for the restoration of PITRM1 activity and the degradation of Aβ in AD conditions using molecular docking and simulation studies.

Materials and method

The interaction between PITRM1 and sulfiredoxin at the Cys112 region in the presence of ATP was studied using protein modelling, docking, and MD simulation. Firstly, the three-dimensional structure of PITRM1 and sulfiredoxin was modelled using the SWISS-MODEL server[12]. The models were then assessed for quality using the Ramachandran plot generated by PROCHECK. The PITRM1-sulfiredoxin complex was then generated by protein-protein docking using ClusPro[13], and the best-docked complex was selected based on the lowest energy score. The selected complex was then subjected docking with ATP using AutoDock[14] followed by 100 ns MD simulation using GROMACS[15], and the stability of the complex was analysed by calculating the RMSD and RMSF. The protein-protein interaction energy and hydrogen bonding were also analysed using GROMACS tools. Overall, this approach allowed us to investigate the potential binding mechanism

between PITRM1 and sulfiredoxin and provide insights into the role of sulfiredoxin in the protection of Cys112 against oxidation-induced inactivation of PITRM1.

Protein Modelling:

The three-dimensional structures of PITRM1 and sulfiredoxin were modelled using the SWISS-MODEL server. The amino acid sequences of the two proteins were obtained from the UniProt database, and the templates for modelling were selected based on the highest sequence identity using the BLAST search tool. The modelled structures were then assessed for quality using the Ramachandran plot generated by PROCHECK. The models with the highest quality were selected for further analysis.

Molecular Docking:

The PITRM1-sulfiredoxin complex was generated by protein-protein docking using ClusPro. ClusPro is a web-based server that utilizes a rigid-body docking algorithm to predict the orientation of two proteins in a complex. The amino acid sequences of the two proteins were uploaded to the ClusPro server, and the docking was performed using the default parameters. The best-docked complex was selected based on the lowest energy score. Further the best model was docked with ATP for better understanding of ATP importance in the process. The docking was performed using AutoDock Vina[14].

MD Simulation using GROMACS:

The selected PITRM1-sulfiredoxin complex was then subjected to 100 ns MD simulation using GROMACS. The GROMACS software package was used to perform the simulation, which was carried out in an isothermal-isobaric (NPT) ensemble. The CHARMM36 force field was used for both PITRM1 and sulfiredoxin. The system was solvated using a TIP3P water model, and the system's charge was neutralized by adding counter ions. The energy minimization and equilibration were performed before the production run. During the simulation, the stability of the complex was analysed by calculating the RMSD and RMSF, and the protein-protein interaction energy and hydrogen bonding were also analysed using GROMACS tools[15].

Results and Discussion

The study aimed to investigate the interaction between PITRM1 and sulfiredoxin in the presence of ATP. The research employed a combination of protein modeling, docking, and molecular dynamics

(MD) simulation to gain insights into the binding mechanism and the protective role of sulfiredoxin against oxidation-induced inactivation of PITRM1.

ATP Binding mode with PITRM1 and sulfiredoxin:

The three-dimensional structures of PITRM1 and sulfiredoxin were modelled using the SWISS-MODEL server. The amino acid sequences of the proteins were obtained from the UniProt database, and template structures (4NGE for PITRM1and1XW3 for sulfiredoxin)were selected based on the highest sequence identity using the BLAST search tool. The models generated were evaluated for quality using the Ramachandran plot, and the models with the highest quality were chosen for further analysis.

Cluspro a protein protein docking webserver was used by uploading the PDB structures and bind docking was performed. The binding region CYS 112 of PITRM1 was a major focus and the models with the interaction at the cysteine residues were used for the study. The cluspro scores and the key interesting binding partners of cysteine 112 was observed in cluster 18, 10 and 6 with a weighed score of -700.5, -669.4 and -628.7 respectively. The cluster 18 protein complex of PITRM1 and sulfiredoxin for ATP docking studies. The protein ligand docking was perfomed in AutoDock Vina, the box size parameters includecenter_x= 41.813, center_y= 76.520, center_z= 53.159 and size_x = 86, size_y = 107, size_z = 97. It showed -8.0 binding interaction between protein complex (PITRM1 and sulfiredoxin) and ATP (Fig 1). ATP formed 7 hydrogen bonds in total where 6 were found between ATP and PITRM1, and 1 with ATP and sulfiredoxin. Hydrogen bonds involve the interaction between a hydrogen atom (covalently bonded to an electronegative atom) and a donor-acceptor pair. LYS23 of sulfiredoxin and the ligand, the ligand's O3 atom acts as a hydrogen bond donor, providing a hydrogen atom, while LYS23's O2 atom acts as the acceptor, accepting the hydrogen bond.Similarly, THR30 of sulfiredoxin interacts with the ligand through two hydrogen bonds. In the first interaction, the ligand's N3 atom acts as the donor, and THR30's O3 atom acts as the acceptor. In the second interaction, the ligand's O3 atom acts as the donor, and THR30's N2 atom acts as the acceptor.HIS62and ARG63 of sulfiredoxin also form hydrogen bonds with the ligand. In both cases, the ligand's O3 atom acts as the donor, and the respective residue's O3 atom acts as the acceptor.ALA525 of PITRM1 interacts with the ligand through a hydrogen bond, where the ligand's O2 atom acts as the donor, and ALA525's O2 atom acts as the acceptor.These interactions occur when the electronegative atoms (donors) attract the hydrogen atom (acting as a partial positive charge), leading to the formation of hydrogen bonds.In the case of HIS62 (chain D) and the ligand, HIS62 acts as the basic residue, accepting a proton, while the ligand's phosphate group acts as the

acidic residue, donating the proton. This interaction is facilitated by the electrostatic attraction between the positively charged sidechain of HIS62 and the negatively charged phosphate group of the ligand.

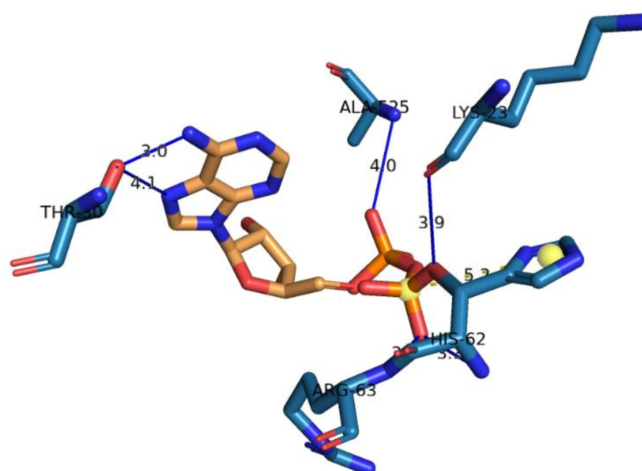

Figure 1: Image showing molecular interaction of ATP and PITRM1/Sulfiredoxin complex

Simulation of the ATP bound PITRM1/Sulfiredoxin complex showed stability

Initially, the docked ATP bound complex was Docked (Fig2A) and further Simulation was performed for a period of 100ns for the PITRM1 and sulfiredoxin complex with ATP. The average values of RMSD, RMSF, minimum distance, and hydrogen bonding for the PITRM1-sulfiredoxin complex and its components (PITRM1 and sulfiredoxin) were obtained from the MD simulation. The RMSD represents the deviation of the complex or individual proteins from their initial structures over the course of the simulation[16]. It was observed that the PITRM1-sulfiredoxin complex exhibited an average RMSD of 0.293 Å with a standard deviation of 0.032 Å (Fig 2B), indicating that the complex remained relatively stable during the simulation. Analysing individual PITRM1 and sulfiredoxin, the RMSD showed a steady stability phase during the simulation with 0.295 Å and 0.240 Å respectively. The RMSF analysis of the N region, HP region and the C region showed the N region with less fluctuation when compared with HP and C region, The aminoacids at the position 575 to 578 show a bend fluctuation on the C region (Fig 2C).

The average gyration of the complex, which represents the compactness or size of the complex, was 3.0584 with an SD of 0.0970 (Fig 2D, 2E and 2E). The gyration values remained consistent during the simulation, indicating that the complex maintains its overall shape and does not undergo significant structural changes. This further supports the stability of the PITRM1-sulfiredoxin complex.Additionally, the average solvent-accessible surface area (SASA) of the complex was 447.71 Å^2 with an SD of 15.24 Å^2(Fig 2H, 2I and 2J). The SASA provides insights into the exposure of the complex to the surrounding solvent molecules. The relatively constant average SASA indicates that the complex maintains a consistent interaction with the solvent environment, further suggesting its stability.

The results of the MD simulation indicate that the PITRM1-sulfiredoxin complex exhibits stability throughout the simulation period. The low average RMSD suggests that the complex retains its overall structure and does not undergo significant conformational changes. This stability is further supported by the consistent average gyration values, indicating that the complex maintains its compactness and shape.The stability of the complex is crucial for its biological function, as it ensures the proper interaction between PITRM1 and sulfiredoxin. The interaction between these proteins at the Cys112 region is of particular interest due to its potential role in protecting PITRM1 against oxidation-induced inactivation. The stability of the complex observed in this study suggests that the binding between PITRM1 and sulfiredoxin is maintained and that sulfiredoxin may play a vital role in protecting the Cys112 region from oxidation[10].The consistent average SASA values further indicate that the complex maintains a stable interaction with the solvent molecules in its surroundings. This suggests that the complex retains its solvation shell and the necessary interactions with the solvent for proper functioning.

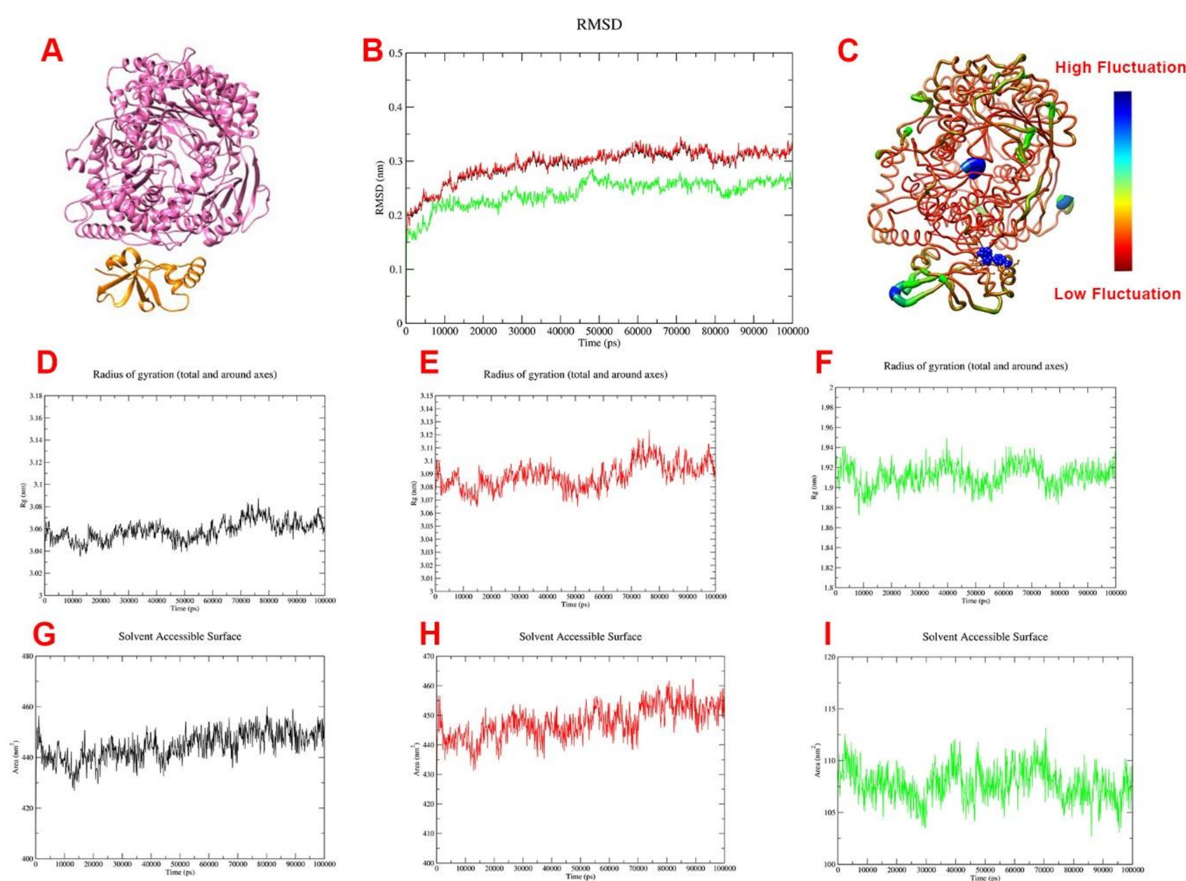

Figure 2: A- PITRM1/Sulfiredoxin complex- Pink showing PITRM1 and Yellow showing Sulfiredoxin. B- RMSD of PITRM1/Sulfiredoxin complex- Black, PITRM1- Red and Sulfiredoxin- Green. C- RMSF of PITRM1/Sulfiredoxin complex- Blue showing hig fluctuation and red showing low fluctuation of aminoacids. D, E, F Radius of gyration of PITRM1/Sulfiredoxin complex- Black, PITRM1- Red and Sulfiredoxin- Green respectively. G, H, ISolvent accessible surfacearea of PITRM1/Sulfiredoxin complex- Black, PITRM1- Red and Sulfiredoxin- Green respectively.

Strong bonding between PITRM1 and sulfiredoxin

The results obtained from the analysis of average minimum distance and hydrogen bonding provide valuable insights into the interactions between PITRM1, sulfiredoxin, and the ligand in the context of the PITRM1-sulfiredoxin complex.The average minimum distance between the PITRM1-sulfiredoxin complex and the ligand was found to be 0.179 Å with a standard deviation of 0.012 Å. Similarly, for PITRM1 and the ligand, the average minimum distance was 0.181 Å with a standard deviation of 0.011 Å. These results indicate that both the complex and PITRM1 have a close proximity to the ligand, suggesting favourable binding interactions. The smaller standard deviations

further suggest that the binding sites of PITRM1 and the complex have a relatively stable and conserved conformation throughout the simulation (Fig 3A, 3B, 3C, 3D.

In terms of hydrogen bonding, the average hydrogen bond count between the PITRM1-sulfiredoxin complex and the ligand was 4.733 with a standard deviation of 1.768. For PITRM1 and the ligand, the average hydrogen bond count was 4.203 with a standard deviation of 1.478. These results indicate the formation of hydrogen bonds between the proteins and the ligand, contributing to the stability of the complex and ligand interactions. The relatively high average hydrogen bond count suggests multiple hydrogen bonding interactions occurring within the binding sites of PITRM1 and the complex, reinforcing the binding affinity. The interaction between sulfiredoxin and the ligand alone, the average minimum distance was slightly larger (0.222 Å) compared to the complex and PITRM1 alone. The most intriguing finding is the exceptionally high average hydrogen bond count of 55.136 between PITRM1 and sulfiredoxin (Fig 3E, 3F, 3G, 3H). This indicates a significant number of hydrogen bonding interactions within the interface of the two proteins. The strong hydrogen bonding between PITRM1 and sulfiredoxin may play a crucial role in stabilizing the complex and facilitating their functional interaction.

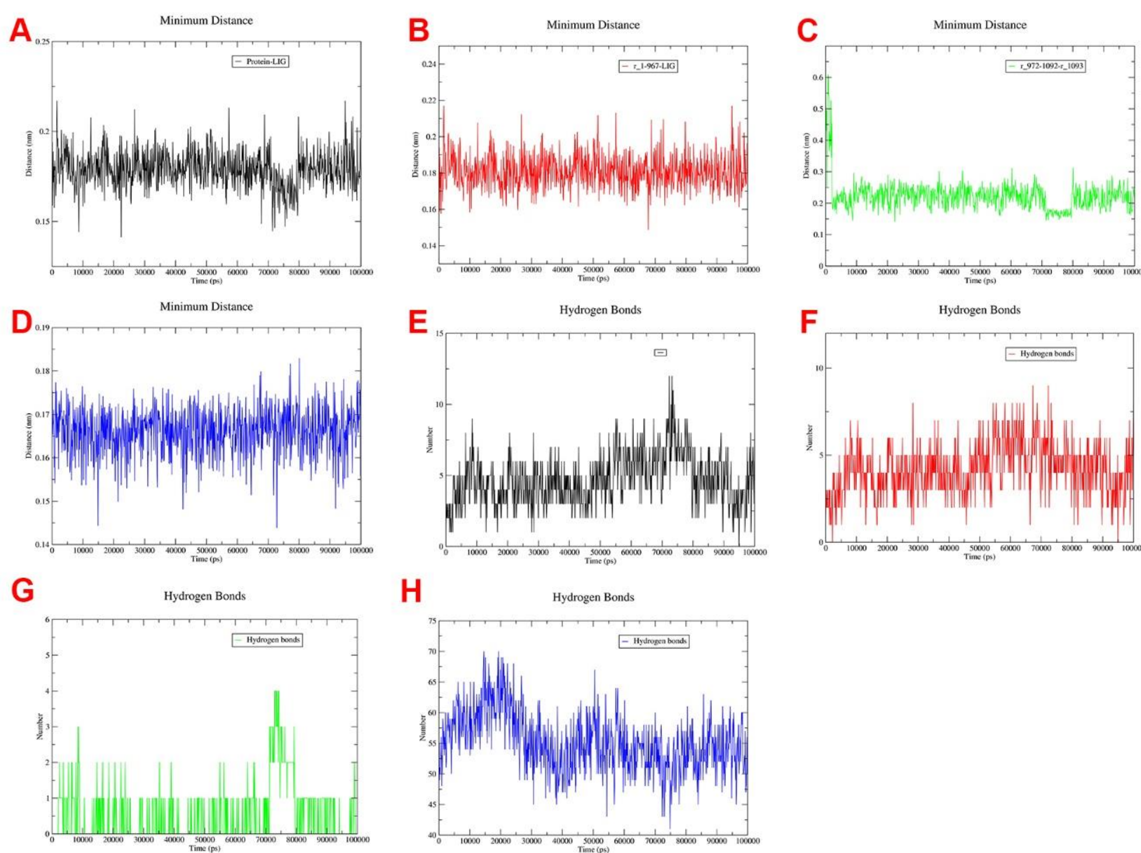

Figure 3: A, B, C, D Minimum Distance of PITRM1/Sulfiredoxin complex with ATP- Black, PITRM1with ATP – Red, Sulfiredoxinwith ATP – Green and between PITRM1 and Sulfiredoxin- Blue respectively. E, F, G, HHydrogen bondformed between PITRM1/Sulfiredoxin complex with ATP- Black, PITRM1 with ATP – Red, Sulfiredoxin with ATP – Green and between PITRM1 and Sulfiredoxin- Blue respectively

Conclusion

The results of the study revealed important insights into the interaction between PITRM1 and sulfiredoxin. The protein-protein docking analysis using ClusPro showed a favourable binding energy between the two proteins at the region which is known to be prone to oxidation in AD. The molecular dynamics simulation using GROMACS provided further evidence of the stability of the PITRM1-sulfiredoxin complex, with the RMSD and RMSF values indicating that the complex remained stable throughout the simulation time. The results suggest that sulfiredoxin may play an important role in protecting PITRM1 from oxidative damage and thereby maintaining its activity in degrading mitochondrial Aβ. These findings may have important implications for the development of novel therapeutic strategies for AD.

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