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In silico analysis of free heme action on the dimerization and activity of the human redox sensor PARK7

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Protein PARK7 (Parkinson disease protein 7) has several enzymatic activities and also functions as a redox sensor,copper chaperone andtranscription regulator. Under oxidative stress PARK7promotes cell survival by activating the ERK1/2 and the PIK3 signaling. PARK7 inactivation causes the reactive oxygen species accumulation and oxidative stress progression. PARK7 disorders has been revealed under neurodegenerative diseases, diabetes and other pathologies. PARK7 functioning is based on the redox changes of conserved Cys106 in the active center. In some neurodegenerative diseases, such as Parkinson's disease, the superoxidation of redox-activeCys106is the basis of the disorder. The removal of 15 amino acid residues from C-end is an obligatory step for the proteolytic active center formation. Modifications observed under oxidative stress affect dimerization of PARK7 necessary for protein maximum activation.

Erythrocyte lysis is known to result in a significant heme accumulation but heme effect on the activity of PARK7 has not yet been investigated. Therefore, the potential heme-binding sites in PARK7 and heme binding effect on the amino acid residues were analyzed.

Structural alignment of PARK7 mutant forms with Cys53 and Cys106 substitutions and wild type has not revealed significant differences (RMSD<0.2Å). Two areas have been found as probable targets for heme binding in PARK7: near the C-terminal region (175-189) that is cleaved for protein activation and in the redox-center with Cys106 and His126. Heme binding to the PARK7 protein could potentially affect its activity by several mechanisms. C-end heme binding can prevent peptide removal necessary for catalytic activity. Cys106 oxidative modification to sulfinic acid could occur under low free heme level and activate PARK7 as a cytoprotector. Significant heme accumulation can result in cysteine superoxidation to sulfonic acid and disrupt PARK7 functionality. Free iron ions as products of heme degradation can compete with copper ions for Cys106 and Glu18 residues therefore inhibit PARK7 activity as SOD1 chaperon. Heme attachment to the sites of oxidation (Cys46, 53 and 106) or sumoylation (Lys130) could disrupt the regulation of PARK7 under stress. Some of the potential heme binding sites of PARK7 protein are involved in protein dimerization, so hemecan block the formation of functional PARK7 dimers.

Therefore free heme accumulation could have multiple negative effect on PARK7 functioning and be one of the mechanisms of PARK7-dependent neurological disorders.

Keywords: *PARK7, heme, redox regulation, dimerization, molecular docking, neurodegeneration*

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Introduction

Protein PARK7 (Parkinson disease protein 7) has multiple regulatory functions as a redox sensor, metal chaperone, antioxidant ortranscription co-activator (Sun, Zheng, 2023) and also reveals various enzymatic activities (EC 3.5.1.124, EC 4.2.1.130). The protective role of PARK7under oxidative stress is associated with its effect on signaling pathways. It promotes cell survival and proliferation by activating the ERK1/2 pathway and the phosphatidylinositol 3-kinase pathway. It attenuates cell death signaling by inhibiting the activation of apoptosis-signaling kinase 1 (Liao et al., 2022). PARK7 inactivation decreases the level of Nrf2-dependent antioxidants and causes the reactive oxygen species accumulation (Neves et al, 2022). The disorders of PARK7 functioning has been revealed under neurodegenerative and oncological diseases, diabetes, heart disorders and other pathologies (Skou et al., 2024; Wilson, 2011).

PARK7 belongs to the DJ-1/ThiJ/PfpI protein family, contains β-sheets arranged in a flavodoxin-like Rossmann fold but has a C-terminal helix (α9) required for dimerization (Smith, Wilson, 2017) PARK7

functioning is based on the redox changes of conserved Cys106 in the active center. Cys106 could be in three states - reduced (-S^-), oxidized (-SO_2H) and superoxidized (-SO_3H), that affects the whole protein isoelectric point and activity (Wilson, 2011). The highest activity of PARK7 is reached under the intermediate degree of Cys106 reversible oxidation till Cys-SO_2H (sulfinic acid). The further oxidation tillsulfonic acid (Cys-SO_3H) results in the conformation changes, the increase of contact area with solvent, destabilization of the dimer and loss of defense functions (Percio et al., 2024). It has been suggested that overoxidation may cause additional loss of function through structural destabilization of the dimeric state and the active conformation of PARK7. In some neurodegenerative diseases, such as Parkinson's disease, the severe oxidation of cysteine makes a crucial contribution to the disease progression.Cys106 can undergo persulfidation forming CysS-SH, which prevents uncontrolled S-oxygenation under stress conditions (Galardon et al, 2022).

The removal of 15 amino acid residues from C-end (175-189 positions)is an obligatory step for the protease activity and active center formation with Cys106 and His126 (Chen et al., 2010). Two other cysteines (Cys46 andCys53) could undergo S-nitrosylation and Lys130 is the site for sumoylation. Oxidative modifications and excessive sumoylation are observed under oxidative stress and affect dimerization of PARK7 with further loss of protein activity (Skou et al., 2024)

PARK7 is capable to bindheavy metals (Björkblom et al., 2013), particularly copper, using Cys106 and Glu18 as sites for coordination. PARK7 is considered to act as a copper chaperone, activating SOD1 (Girottoet al., 2014). Positions 149-150 of PARK7 are the cleavage site for CASP6 associated with neurodegeneration (Cao et al., 2012).

Oxidative stress in mammals is usually accompanied by erythrocyte lysis and hemoproteins degradation known to result in the significant accumulation of free heme in tissues (Belcher et al., 2010). But heme effect on the activity of PARK7 has not yet been investigated. Therefore, in this study, we analyzed the potential heme-binding sites in PARK7 and heme binding effect on the amino acid residues important for PARK7 dimerization and functioning.

Materials and methods

Protein annotations and sequence in *fasta format were downloaded from UniProt knowledgebase (http://www.uniprot.org/Q99497/). Sequence analysis for cysteine accessibility and modifications was carried out by pCysMod server (Li et al, 2021, http://pcysmod.omicsbio.info/)

Structures of PARK7 monomers and dimers (Table 1) in pdb format were downloaded from Protein Data Bank (PDB, http://www.rcsb.org/). Currently, 64 structures are known for the PARK7 protein. Three monomeric and four homodimeric structures with high resolution with or without cysteine mutations were selected for further analysis (Table 1).

Table 1. Characteristics of selected structures of human PARK7 monomers and dimers (by PDB and UniProt data, method – X-rays)

PDB ID	Resolution	Positions	Mutations	Ligands and modifications
Monomers (chain A)				
1P5F	1.10 Å	1-189	No	-
4N0M	1.95 Å	1-189	53Cys→Ala	Ligands: copper (II) ion, pentaethylene glycol
6AF7	1.30 Å	1-189	106Cys→Ser	Ligands: pentaethylene glycol, chloride ion
Dimers (chains A/B)				
1UCF	1.95 Å	1-189	No	
2R1U	1.50 Å	2-188	No	
3SF8	1.56 Å	1-189	No	Cys106 modified in chain A
2R1T	1.70 Å	2-188	Chains A, B 106Cys→Ala	Cys53 modified in chain B

Protein inter-chain contacts, hydrogen bonds and salt bridges between interacting amino acid residues in PARK7 dimers were analyzed by PDBsum server (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/). TM-Align tool was used for structural alignment of the selected protein models (http://zhanglab.ccmb.med.umich.edu/TM-align/). Structural similarity was estimated byRMSD and TM-score.

Molecular docking of heme as a ligand to the protein pdb structures was performed by two online tools. CB-Dock2 (Cavity-detection guided Blind Docking) reveals cavities and predicts docking poses by AutoDock Vina scoring (Liu et al., 2022; https://cadd.labshare.cn/cb-dock2). COACH-D predicts protein-ligand binding sites using templates (Wu et al., 2018; https://yanglab.nankai.edu.cn/COACH-D/).

Heme b structural file was downloaded from PubeChem (https://pubchem.ncbi.nlm.nih.gov/). Visualization of known and modeled structures in pdb-format was performed by UCSF CHIMERA (Pettersen et al., 2004).

Results and Discussion

Contact interface analysis in PARK7 dimers. According to PDBsum data, minimum 13 (in 2R1T) and maximum 15 (in 1UCF) hydrogen bonds are formed between protein chains in PARK7 dimers.There aretwosalt bridges in 3SF8 structure that has Cys106 mutation, three - in 1UCF and four – in 2R1Tand2R1U structures. In all selected dimers Cys53 and His126 as well as C-end residues were involved in the hydrogen inter-chain bonds formation.

Structural alignment of selected PARK7 structures. Since cysteine residues are important for the functioning of PARK7, the protein structures with cysteine mutations and modifications were compared with wild type variants. For all selected structures very high similarity was shown. RMSD for monomers was in the range from 0,11Å for 1P5F and 6AF7 till 0.22 Å for 4N0M and 6AF7. Dimers comparison has also revealed very high similarity with minimal RMSD (0,12 Å) between 1UCF and 2R1T and maximum (0.24 Å) for pair 2R1U and 3SF8.Therefore the replacement of cysteine by small amino acids serine and alanine had no effect on PARK7 structure independently on the polarity of amino acid.

Analysis of the amino acids accessibility. PARK7 amino acid sequence analysis by pCysMod has revealed all cysteines to be poorly accessible from the surface (Fig.1). The lowest coefficient of accessibility has been shown for Cys106 and Cys53. His126 which is also the part of the active center is much more accessible. Taking into account the involvement of these amino acids not only in redox and enzymatic activity but also in the inter-chain hydrogen bonds, they could become less accessible for binding of ligands after the formation of PARK7 dimers.

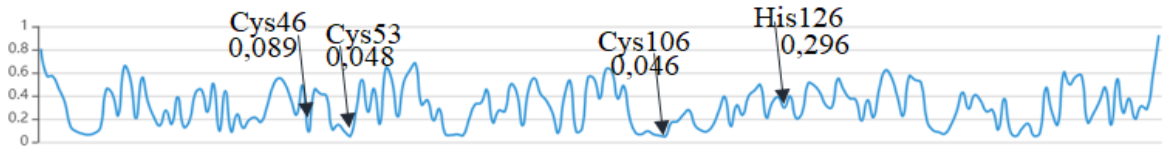

Fig. 1. The coeffecients of the amino acid residues accessibility to the solvent by the analysis of PARK7 sequence (pCysMod). Cysteine residues and histidine of active site are marked

Molecular docking of heme to PARK7 monomers. Two main variants of heme binding sites have been predicted for all analyzed monomeric PARK7 structures (Table 2).

First docking area modeled by CB-Dock2 is located near cleavage site for CASP6 (positions 149-150) and carboxy-end peptide 175-189 need to be removed for activation. Leu166 mutated under neurodegeneration has also been revealed in this contact area. Second docking variant predicted by both algorithms contains two residues able to coordinate heavy metals (Glu18 and redox-active Cys106), His126 involved in enzymatic activity and Lys130 that is the site of sumoylation. Cysteines 46 and 53 have not been predicted to bind heme. No significant differences have been revealed between models with mutations (4N0M, 6AF7) and wild type protein (1P5F). The amino acids necessary for inter-chain bonds formation in PARK7 dimers (Glu15, Arg48, His126 and Arg145) have been found in both heme binding variants. According to docking results free heme accumulation could inhibit PARK7 functioning by direct blocking the enzymatic center as well as by preventing PARK7 dimerization or C-end peptide removal.

Molecular docking of heme to PARK7 dimers. PARK7 has maximum activity as a homodimer (Lv et al., 2025). Heme docking to the dimeric PARK7 structures (Table 3) has not revealed principally new docking areas comparing with monomers.

Prevalently heme binding has been asymmetrical with more contact sites in one of the chains. Some of solutions have heme bound to only one chain (data not shown). But the highest scores and the biggest contact area have been calculated for heme docking to both chains.

The mostly represented docking variant is heme binding to the active site with Cys106 and His126. Arg48 which forms hydrogen inter-chain bonds is also in heme proximity. The docking pose near C-end is possible only before the removal of the C-end peptide and could negatively affect PARK7 activation.

It's worth mentioning that cysteine, histidine and tyrosine are known to have the most ability for heme stable coordination. Cys-Pro motives are considered as heme regulatory sites and are found in various heme-sensitive proteins (Rathod et al., 2023; Zhang, 1995). In PARK7 protein 53Cys-Pro motif has not been predicted to bind heme as well as His137 and any of three tyrosine residues. His115 and Cys46 have been found in docking results, in 2R1U and 3SF8 respectively. His126 is the most close to heme residues in the majority of docking variants. Taking into account a very high affinity of histidine to heme b molecule, this type of interaction could provide the prolonged inhibition of PARK7 enzymatic activity.

One more site for potential heme binding together with Cys106 is involved in copper chaperon activity: Glu18. These copper ligands in PARK7 are necessary for SOD1 activation therefore superoxide

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elimination under oxidative stress. Competition of heme and copper for regulatory sites could enhance prooxidant reactions in tissues. PARK7 is known to be abundant in nervous tissue, kidneys and heart.

The alternative way for PARK7 inactivation under excess heme prooxidant action is over-oxidation of Cys106 till sulfonic acid with full inactivation of protein antioxidant properties.

Table 2. Putative sites of heme binding to PARK7 monomers (chain A). The variants with the highest scores of binding energy and size of the contact area predicted by two algorithms. Amino acid residues involved in the covalent modifications or inter-chain binding are marked in bold, residues of the C-end peptide are underlined

PDB ID, docking site		Residues predicted to contact with heme	Online tool and docking solution
1P5F	1	Lys122 Glu143 Asn144 Arg145 Val146 Glu147 Lys148 **Asp149** Leu153 Phe162 Glu163 Phe164 Leu166 Ala167 Ile168 Glu170 Ala171 Lys175 Ala178 Ala179 Lys182 Leu187	CB-Dock2: Vina score – 8,0; Cavity volume 147 Å^3
	2	Gly13 Glu15 **Glu18** Ser47 **Arg48** Gly75 Asn76 Leu77 Ala79 Gln80 Asn81 Ser83 Glu84 **Cys106** Ala107 Thr110 Ala111 **His126** Pro127 Leu128 Ala129 **Lys130** Asp131 Lys132 Met133 Gly157 Pro158	CB-Dock2: Vina score – 5,2; Cavity volume 786 Å^3
		Glu18 Gly74 Gly75 Asn76 **Cys106** Ala107 **His126** Leu128 Ala129 Lys132 Pro158	COACH-D**: C-Score** 0.65, **Cluster size** 41
4N0M	1	Glu143 Asn144 **Arg145** Val146 Glu147 Lys148 Leu153 Gly159 Phe162 Glu163 Leu166 Ala167 Glu170 Ala171 Lys175 Ala178 Ala179 Lys182 Leu187 Lys188	CB-Dock2: Vina score – 7,1; Cavity volume 124 Å^3
	2	Gly13 Glu15 **Glu18** Ser47 **Arg48** Gly74 Gly75 Asn76 Leu77 Gln80 **Cys106** Ala107 Thr110 **His126** Pro127 Leu128 Ala129 Lys130 Asp131 Lys132	CB-Dock2: Vina score – 6.4; Cavity volume 319 Å^3
		Glu18 Gly74 Gly75 Asn76 **Cys106** Ala107 **His126** Leu128 Ala129 Lys132 Pro158	COACH-D**: C-Score** 0.67; **Cluster size** 40
6AF7	1	Asn144 **Arg145** Val146 Glu147 Lys148 **Asp149** Gly150 Leu153 Phe162 Glu163 Leu166 Ala167 Ile168 Glu170 Ala171 Leu172 Lys175 Ala178 Ala179 Lys182 Leu187 Lys188	CB-Dock2: Vina score – 7,6; Cavity volume 85 Å^3
	2	Lys12 Gly13 Ala14 Glu15 Met17 **Glu18** Ser47 Arg48 Gly74 Gly75 Asn76 Leu77 Gly78 Ala79 Gln80 Asn81 Ser83 ***Ser106*** Ala107 Thr110 **His126** Leu128 Ala129 Lys132 Arg156 Gly157 Pro158 Gly159	CB-Dock2: Vina score – 6,0; Cavity volume 799 Å^3
		Glu18 Gly74 Gly75 Asn76 ***Ser106*** Ala107 **His126** Leu128 Ala129 Lys132 Pro158	COACH-D: **C-Score**0.42; **Cluster size** 39

Cys106 modification (3SF8) or substitution (2R1T) have not significantly affect location of the heme binding sites but could weaken heme coordination. There are two main binding area for heme: docking site near Arg145 with C-end peptide in proximity. And the combination of N-end amino acid residues including Glu18 able to bind heavy metals as well Cys106 and His126 important for formation of active site together with Arg28 and Arg48 necessary for the interchain hydrogen bonds. N-end amino acids have been found in heme contact area much often in monomers but not in dimers.

Therefore, two main variants of heme docking have been predicted for PARK7 both monomers and dimers – near active center and near C-end peptide (Fig.2). But the binding energy as well as contact surface area are much more favorable for heme binding by PARK7 dimers.

Heme has a plane rigid tetrapyrrol ring with Fe-ion able to electron transfer. Four coordination places are occupied by the nitrogen and two more are available for coordination with proteins or small ligands. Free heme acts as a detergent and prooxidant and can bind hydrophobic structures and oxidize cell components under hemeprotein degradation. Proteins coordinate heme iron through histidine, tyrosine or cysteine (Wißbrock et al., 2019).

Docking areas have revealed mostly hydrophobic and positively charged amino acids such as lysine, arginine, and histidine, able to attract carboxyl groups of heme. However, in the absence of residues that bind heme strongly (cysteine, histidine, tyrosine), the interaction through hydrophobic residues is unstable. When heme is attached to the PARK7 dimer, it binds to the chains not symmetrically: with one chain more than with another. By attaching to residues that interact between monomers during dimerization, heme can probably prevent the formation of dimers. This can lead to structural instability, which will negatively affect the activity of the protein.

Table 3. Putative sites of heme binding to PARK7 dimers (chains A/B). The variants with the high binding energy and the biggest size of the contact area predicted by CB-Dock2 and COACH-D

PDB ID, docking site		Residues predicted to contact with heme (chain A / B)	Online tool and docking scores
1UCF	1	**A:** Ile21 Glu143 Asn144 **Arg145** Val146 Pro158 Gly159 Thr160 Ser161 Phe162 Glu163 Leu166 Ala167 Glu170 Lys182 Leu187 Lys188 **B**: Ile21 **Arg145** Pro158 Gly159 Thr160 Ser161 Phe162 Glu163 Leu166Lys182 Leu187 Lys188	CB-Dock2: Vina score –7,1; Cavity volume 287 Å^3
	2	**A**: Arg28 Gln180 Pro184 Leu185 **B**: Gly13 Glu15 **Glu18** Ser47 **Arg48** Gly74-75 Asn76 Leu77 Gly78 Ala79 Gln80 Asn81 Ser83 Glu84 **Cys106** Ala107 **His126** Pro127 Leu128 Ala129 **Lys130** Asp131 Lys132 Pro158	CB-Dock2: Vina score –5,6; Cavity volume 1029 Å^3
		A: Arg28 Pro184 **B**: Glu15 **Glu18** Gly74 Asn76 **Cys106** Ala107 **His126** Leu128 Pro158	COACH-D: **C-Score** 0.74; **Cluster size** 81
2R1U	1	**A**: Lys188 **B**: Asn144 **Arg145** Val146 Glu147 Lys148 Leu153 Gly159 Phe162 Glu163 Phe164 Leu166 Ala167 Glu170 Lys175 Ala178 Ala179 Lys182 Leu187 Lys188	CB-Dock2: Vina score –6,8; Cavity volume 324 Å^3
	2	**A**: **Arg28** Gln180 Pro184 Leu185 **B**: Gly13 Glu15 **Glu18** Ser47 **Arg48** Gly74-75 Asn76 Leu77 Gly78 Ala79 Gln80 Ser83 Glu84 **Cys106** Ala107 Thr110 Leu113 Ala114 **His115** Glu116 **His126** Leu128 Ala129 Asp131 Lys132 Asn135 Pro158	CB-Dock2: Vina score –5.; Cavity volume 1190 Å^3
		A: Glu15 **Glu18** Gly74-75 Asn76 **Cys106His126** Leu128 Ala129 Pro158 **B**: Arg28 Pro184	COACH-D**: C-Score** 0.74; **Cluster size** 75
3SF8	1	**A:** Arg28 Val181 Pro184 Leu185 **B:** Gly13 Ala14 Glu15 **Glu18** Ser47 **Arg48** Gly74-75 Asn76 Leu77 Gly78 Ala79 Gln80 **Cys106** Ala107 Thr110 **His126** Pro127Leu128 Ala129 **Lys130** Asp131 Lys132 Met133 Pro158	CB-Dock2: Vina score –7,9; Cavity volume 877 Å^3
		A: Arg28 Pro184 **B:** Glu15 **Glu18** Gly74-75 Asn76 **Cys106** Ala107 **His126** Leu128 Pro158	COACH-D: **C-Score** 0.74, **Cluster size**75
	2	**A:** Glu143 Asn144 **Arg145** Val146 Glu147 Gly159 Phe162 Glu163 Phe164 Leu166 Ala167 Glu170 Lys175 Ala178-179 Lys182 Leu187 Lys188 Asp189 **B:** Glu143 Asn144 **Arg145** Val146 Phe162 Glu163 Phe164 Leu166 Ala167 Lys182 Leu187 Lys188	CB-Dock2: Vina score –6,6; Cavity volume 378 Å^3
2R1T (106 C→A)	1	**A**: Ile21 **Arg145** Gly157 Pro158 Gly159 Thr160 Ser161 Phe162 Glu163 Leu166 Lys182 Val186 Leu187 Lys188 **B**: Arg145 Gly159 Thr160 Phe162 Glu163 Phe164 Leu166 Ala167 Lys182 Val186 Leu187 Lys188	CB-Dock2: Vina score –7,6; Cavity volume 85 Å^3
	2	**A**: Gly13 Glu15 **Glu18** Ser47 **Arg48** Gly74-75 Asn76 Leu77 Gly78 Ala79 Gln80 Asn81 Ser83 Glu84 ***Ala106*** Ala107 Thr110 **His126** Leu128 Ala129 **Lys130** Asp131 Lys132 Met133 Asn135 Arg156 Gly157 Pro158 **B**: Arg28 Pro184 Leu185	CB-Dock2: Vina score –6,0; Cavity volume 799 Å^3
		A: Glu15 **Glu18** Gly75 Asn76 ***Ala106*****His126** Leu128 Ala129 Pro158 **B**: Arg28 Pro184	COACH-D: **C-Score** 0.74, **Cluster size** 75

Summarizing the results of molecular docking studies, it could be assumed that heme binding to the PARK7 protein could potentially affect its activity by several mechanisms. First, the C-terminus in the full sequence of the PARK7 protein that is a putative heme binding site according to docking results, may prevent protein activation through proteolysis. Second target is Cys106. Under conditions of insignificant hemolysis and low oxidative stress heme can perform a signaling role and activate PARK7 through moderate modification of redox-active cysteine to sulfinic acid, that contributes to its cytoprotective activity. However, increased intravascular hemolysis and significant accumulation of free heme in tissues together with high level of reactive oxygen species, results in cysteines oxidation to sulfonic acid. Cys106 peroxidation will lead to a disruption of PARK7 functionality as a redox sensor and block the activation of Akt- and Nrf2-dependent signaling pathways, critically important for antioxidant functions. A similar inhibition can be caused by free iron as products of heme degradation. The iron ions could bind to the Cys106 and Glu18 residues competing with copper ions therefore inhibiting PARK7 chaperon activity towards antioxidant enzyme SOD1. An additional mechanism of heme effect is the inhibition of post-

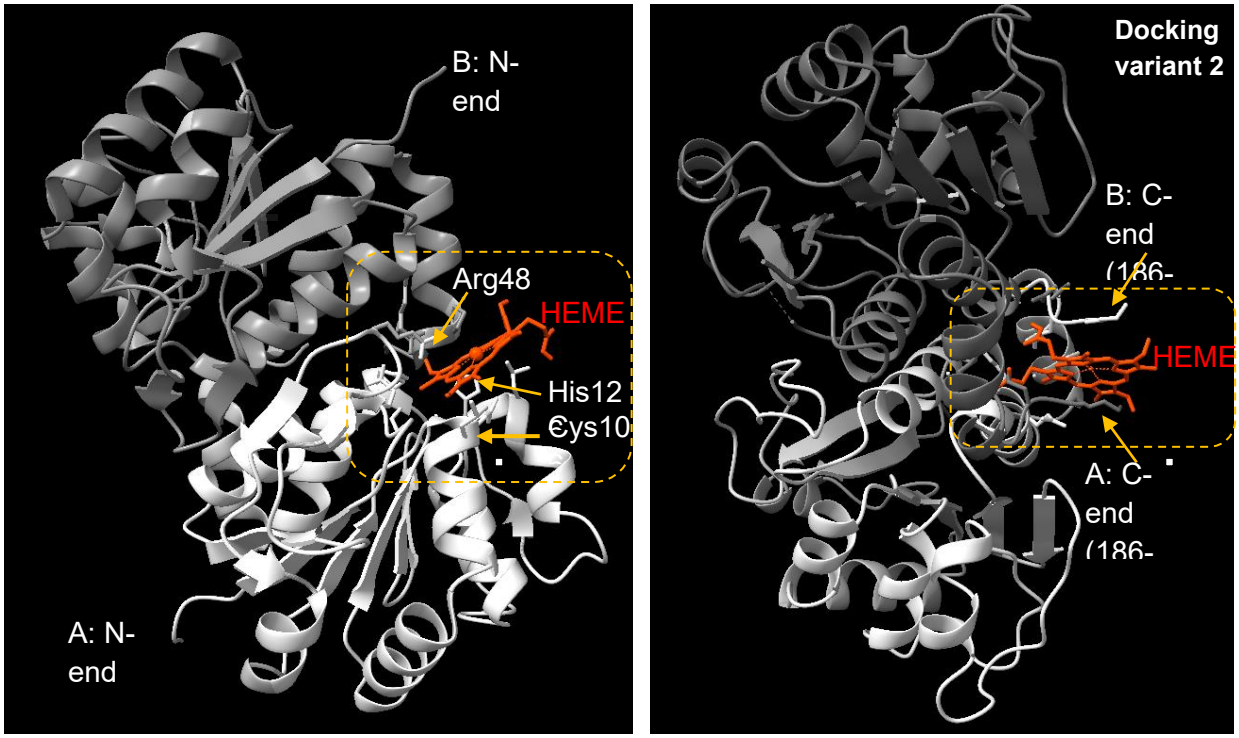

Fig. 2. Two main variants of the heme binding sites predicted for PARK7 dimers. 1UCF structure used as an example

translational modifications of the PARK7 protein. Heme attachment to the sites of phosphorylation or sumoylation could interfere normal maturation and regulation of PARK7 under stress.

Conclusions

Structural alignment of PARK7 protein mutant forms with Cys53 and Cys106 substitutions with PARK7 wild type without modifications has not revealed significant differences (RMSD<0.2Å).

Two areas are the most probable targets for heme binding in PARK7. First one is near the C-terminal region so heme may prevent the cleavage of this region necessary for protein activation. Second binding site is in the redox-center with Cys106 and His126. Partial oxidation of redox-active Cys106till sulfinic acid could take place under low heme level and provide PARK7 activation. Under intense hemolysis and high concentrations of reactive oxygen species, Cys106 could be further oxidized till sulfonic acid that makes PARK7 totally inactivated.

Some of the potential heme binding sites of PARK7 protein are involved in protein dimerization, so hemeaccumulationcould prevent the formation of functional PARK7 dimers.

Heme attachment at the sites of PARK7 post-translational modifications or in proximity to them could destabilize the regulation of protein activity.

Therefore, in general PARK7 activity could be dependent on free heme concentration. A long-term heme accumulation could have multiple negative effects on PARK7 functioning and be one of the mechanisms of PARK7-dependent neurological disorders.

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In silico аналіз дії вільного гему на димеризацію та активність редокс-сенсора PARK7 людини

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Білок PARK7 (білок хвороби Паркінсона 7) має кілька ферментативних активностей, а також функціонує як редокс-сенсор, мідний шаперон і регулятор транскрипції. В умовах оксидативного стресу PARK7 сприяє виживанню клітин шляхом активації ERK1/2 і PIK3 сигналінга. Інактивація PARK7 викликає накопичення активних форм кисню та прогресування оксидативного стресу. Порушення пов'язані з PARK7 виявлені при нейродегенеративних захворюваннях, діабеті та інших патологіях. Функціонування PARK7 базується на окисно-відновних змінах консервативного Cys106 вактивному центрі. При деяких нейродегенеративних захворюваннях, таких як хвороба Паркінсона, суперокислення редокс-активного Cys106 є основою розладу. Видалення 15 амінокислотних залишків

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з С-кінця є обов'язковим етапом формування протеолітичного активного центру. Модифікації, що відбуваються під час оксидативного стресу, впливають на димеризацію PARK7, необхідну для максимальної активації білка.
Відомо, що лізис еритроцитів призводить до значного накопичення гему, але вплив гему на активність PARK7 на цей момент не досліджено. Отже, у роботі були проаналізовані потенційні сайти зв'язування гема в PARK7 і ефект зв'язування гема з амінокислотними залишками.
Структурне вирівнювання мутантних формPARK7 із замінами Cys53 і Cys106 з білком дикого типу не показалозначущих відмінностей (RMSD<0,2Å). За результатами молекулярного докінгу дві області є ймовірними мішенями для зв'язування гему уPARK7: поблизу С-кінцевої ділянки (175-189), яка видаляється для активації білка, і в редокс-центрі з Cys106 і His126. Зв'язування гему з білком PARK7 потенційно може впливати на його активність за допомогою кількох механізмів. Зв'язування гема на С-кінці може запобігти видаленню пептида, що необхідно для каталітичної активності. Окислювальна модифікація Cys106 до сульфінової кислоти може відбуватися за низького рівня вільного гему та активувати PARK7, який буде діяти як цитопротектор. Значне накопичення гему може призвести до суперокислення цистеїну до сульфонової кислоти та порушити функціональність PARK7. Вільні іони заліза як продукти деградації гему можуть конкурувати з іонами міді за залишки Cys106 і Glu18, і таким чином інгібувати активність PARK7 як шаперона SOD1. Приєднання гему до сайтів окислення (Cys46, 53 і 106) або сумоїлювання (Lys130) може порушити регуляцію PARK7 під час стресу. Деякі з потенційних сайтів зв'язування гема білком PARK7 беруть участь у димеризації, тому гем може блокувати утворення функціональних димерів PARK7.
Отже накопичення вільного гему може мати множинний негативний вплив на функціонування PARK7 і бути одним із механізмів PARK7-залежних неврологічних розладів.

***Ключові слова*:** *PARK7, гем, редокс-регуляція, димеризація, молекулярний докінг, нейродегенерація*

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