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In silico аналіз потенційних гем-зв'язувальних сайтів у протеїнових комплексах, що містять repulsive guidance molecule BMP co-receptor B (RGMB) людини

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BMP сигналінг і рівень гему задіяні у регуляції метаболізму заліза, розвитку оксидативного стресу і запалення. Накопичення вільного гему внаслідок деструкції гемопротеїнів веде до пошкоджень клітин та/або модуляції сигналінгу. Передбачення потенційних гем-зв'язувальних сайтів у протеїнових комплексах, що містять repulsive guidance molecule BMP co-receptor B (RGMB), було проведено за допомогою онлайн програм PatchDock і HemeBIND з використанням даних щодо структури RGMB комплексів з неогеніном (PDB ID 4bq6) та/або BMP2 (PDB ID 4uhz і 4ui2). Молекулярний докінг виявив, що природний тетрамерний комплекс неогеніну та його корецептору RGMB надає декілька сайтів для зв'язування гему поруч з ділянками контакту ланцюгів, в той час як взаємодія гему з мономерними компонентами менш вірогідна. Тільки окремі передбачені сайти містили амінокислоти, здатні формувати стабільні зв'язки з гемом (His, Cys або Tyr), але неспецифічне приєднання гему до декількох ланцюгів комплексу одночасно могло б спричинити короткочасний конформаційний ефект. Беручи до уваги прозапальну дію BMP сигналінгу на ендотелій, зв'язування гему з BMP рецепторним комплексом може обговорюватись як неспецифічний механізм судинного патогенезу при накопиченні гему внаслідок стресу.

Ключові слова: *зв'язування гему, метаболізм заліза, RGMB, неогенін, BMP2, молекулярний докінг.*

In silico analysis of the potential heme binding sites in the protein complexes containing human repulsive guidance molecule BMP co-receptor B (RGMB)

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Both BMP signalling and heme level are involved in the regulation of iron metabolism, development of oxidative stress and inflammation. Free heme accumulation due to hemoproteins destruction results in cells damage and/or signalling modulation. Prediction of potential heme-binding sites in protein receptor complexes containing repulsive guidance molecule BMP co-receptor B (RGMB) was performed by online tools PatchDock and HemeBIND using structural data on RGMB complexes with neogenin (PDB ID 4bq6) and/or BMP2 (PDB ID 4uhz and 4ui2). Molecular docking revealed that natural tetrameric complex of neogenin and its co-receptor RGMB provided several sites for heme binding near interchain contacting areas while heme interaction with monomeric components was less probable. Only few predicted sites contained amino acids capable to form stable bonds with heme (His, Cys or Tyr) but non-specific heme attachment to several chains of the protein complex simultaneously could have short-term conformational effect. Taking into account the proinflammatory action of BMP signalling on endothelium, heme binding to BMP receptor complex can be discussed as non-specific mechanism of vascular pathogenesis under stress-derived heme accumulation.

Key words: *heme binding, iron metabolism, RGMB, neogenin, BMP2, molecular docking.*

In silico анализ потенциальных гем-связывающих сайтов в белковых комплексах, содержащих repulsive guidance molecule BMP co-receptor B (RGMB) человека

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BMP сигналинг и уровень гема задействованы в регуляции метаболизма железа, развития оксидативного стресса и воспаления. Накопление свободного гема вследствие деструкции гемопротеинов ведет к повреждениям клеток и/или модуляции сигналинга. Предсказание потенциальных гем-связывающих сайтов в белковых комплексах, содержащих repulsive guidance molecule BMP co-receptor B (RGMB), было проведено с помощью онлайн программ PatchDock и HemeBIND с использованием данных о структуре RGMB комплексов с неогенином (PDB ID 4bq6) и/или

BMP2 (PDB ID 4uhz и 4ui2). Молекулярный докинг выявил, что природный тетрамерный комплекс неогенина и его корецептора RGMB предоставляет несколько сайтов для связывания гема возле участков контакта цепей, в то время как взаимодействие гема с мономерными компонентами менее вероятно. Только отдельные предсказанные сайты содержали аминокислоты, способные формировать стабильные связи с гемом (His, Cys или Tyr), но неспецифическое прикрепление гема к нескольким цепям комплекса одновременно могло бы оказать кратковременный конформационный эффект. Принимая во внимание провоспалительное действие BMP сигналинга на эндотелий, связывание гема с BMP рецепторным комплексом может обсуждаться как неспецифический механизм сосудистого патогенеза при накоплении гема вследствие стресса.

Ключевые слова: *связывание гема, метаболизм железа, RGMB, неогенин, BMP2, молекулярный докинг.*

Introduction

Iron balance maintains the normal rate of hemoproteins and iron-sulfur complexes biosynthesis essential for many vitally important functions. The main role in the regulation of iron metabolism in mammals belongs to the BMP/SMAD signalling pathway that adopts gene expression in response to iron levels (Frazer et al., 2012; Siebold et al., 2017). BMP signalling plays important role in regulating vascular oxidative stress and inflammation (Derwall et al., 2012) and is linked to the cardiovascular and muscles pathologies (Cai et al., 2012). So the correction of BMP signalling axis is one of prospective strategies for vascular diseases therapy (Liu et al., 2016). Membrane anchored members of the repulsive guidance molecule (RGM) family interact with neogenin (NEO1) as co-receptors potentiating the BMP pathway (Siebold et al., 2017). Repulsive guidance molecule BMP co-receptor B (RGMB) expression in various tissues suggests its wide functionality as the regulator with undetermined functions (Corradini et al., 2009).

Free heme accumulation under hemoproteins destruction can raise heme concentration in several orders (Chiabrando et al., 2014). Circulating erythrocytes are the main reservoir of heme in mammals so hemolysis-derived heme at intoxications or trauma may be indirectly involved in pathogenesis including cardiovascular disorders (Immenschuh et al., 2017). Non-specific heme binding can lead to damage of cell structures because of heme prooxidant and detergent-like properties (Rother et al., 2005) while heme attachment to sensor proteins is one of the signalling event and heme regulatory motifs (HRM), such as Cys-Pro, have been described in transcription factors, ion channels and enzymes (Mense, Zhang, 2006).

The sequences of RGMB protein and its partner NEO1 also have Cys-Pro motifs, but their heme-binding capacity has not been described yet, so the investigation of heme binding to BMP receptor complex acquires particular relevance. The objective of this study was *in silico* analysis of potential heme-binding sites in the complexes of RGMB with BMP2 protein and/or with neogenin.

Materials and methods

The amino acid (AA) sequences and protein annotations (Table 1) were downloaded from UniProt knowledgebase (http://www.uniprot.org/). GPI-anchored glycoprotein RGMB has two binary extracellular interactions: with BMP2 protein that is secreted homodimeric disulfide-linked glycoprotein and neogenin (NEO1) that is transmembrane 1-pass receptor for BMP2.

RGMB is active as homooligomer or as heterotetramer with NEO1 in 2:2 stoichiometry. The data on protein structures of RGMB complexes (Table 2) was downloaded from Protein Data Bank (PDB) knowledgebase (http://www.rcsb.org/pdb/home/home.do). All PDB structures used in this study contained the proteins fragments with extracellular location, NEO1 fragments had no cysteines (Table1). Free online tool HemeBIND (http://mleg.cse.sc.edu/hemeBIND/; Liu, Hu, 2011) was used for RGMB sequence and PDB-coordinates analysis for heme-binding propensity of potential HRM (Cys-Pro/ Pro-Cys motifs).

Docking of heme as a ligand to the protein fragments in PDB-structures was carried out by on-line tool PatchDock, Beta 1.3 Version (Schneidman-Duhovny, 2005; http://bioinfo3d.cs.tau.ac.il/PatchDock/) with clustering RMSD 1,5 Å as it was recommended for protein-ligand docking. First 20 docking solutions with the highest scores for each target PDB-structure were analyzed for amino acids arranged in close proximity to heme ring. The variants with highest scores and highest number of RGMB residues close to heme but not crossing the heme ring were selected. Among variants with close scores the solutions with heme bound to two or three chains simultaneously were preferred. Selected solution of the previous round was used for the next round of docking. Scoring was based on both geometric fit and atomic desolvation energy (Schneidman-Duhovny et al., 2005).

For analysis of single protein chain the PDB-file was edited for removal of the other components of the complex: RGMB (C/D chains) or NEO1 (A chain) were extracted from 4bq6.pdb; BMP2 (A chain) was taken from 4uhz.pdb. Structure PDB-file for heme molecule was downloaded from PubeChem (http://www.ebi.ac.uk/pdbe-srv/pdbechem/chemicalCompound/show/HEM).

Table 1.

Selected UniProt data for the proteins analyzed in the study

Protein name (gene symbol)	UniProt ID	Length	Number of Cys, CP/PC motifs, disulfide bonds, topology
RGM domain family member B (RGMB)	Q6NW40	437 AA	Total – 17 cysteines; CP motifs: C26 in signal peptide and C316 in CPL motif; 2 disulfides: C139–C226 (PC139) and C163–C312. GPI-linked through asparagine 413.
Neogenin (NEO1)	Q92859	1461 AA, Isoform 1	Total – 13 cysteines: C20 in signal peptide, 4 disulfides in extracellular domain 34–1105: C74–C129, C173–C221, C270–C320 (motif PC with C270), C362–C410. Sequence region 411-1126 has no cysteines. Region 1106–1126 has transmembrane and 1127-1451 – cytosolic location.
Bone morphogenetic protein 2 (BMP2)	P12643	396 AA	Total – 7 cysteines; 6 in intrachain disulfide bonds: C296–C361; C325–C393; C329–C395; (CP motif with C329); C360 in the interchain disulfide. No cysteines in propeptide (24-282), C7 in signal peptide (1-23).

Table 2.

PDB data for the crystal structures of RGMB complexes analyzed in the study: 4bq6 (Bell et al., 2013); 4uhz and 4ui2 (Healey et al., 2015). Method for all three structures: X-ray diffraction

PDB ID (resolution)	Chain (Protein)	Sequence region	Mutations/ ligands
4BQ6 (2,3 Å)	A, B (NEO1)	883-1101 (fibronectin-type III domains 5 and 6)	Ligand: N-acetyl-D-glucose-amine
	C, E (RGMB)	50-168 ectodomain	
	D, F (RGMB)	169-410 ectodomain	Mutation: E225G
4UHZ (2,85 Å)	A (BMP2)	283-396 (C-terminal signaling domain)	Ligand: sulfate ion
	B (RGMB)	52-137 (N-terminal domain)	
4UI2 (3,15 Å)	A (NEO1)	883-1101 (fibronectin-type III domains 5 and 6)	Ligands: N-acetyl-D-glucose-amine; β-D-mannose
	B (BMP2)	283-396 (C-terminal signaling domain)	Ligands: S,R-mesotartaric acid, acetate ion
	C (RGMB)	50-168 ectodomain	
	D (RGMB)	169-240 ectodomain	Mutation: E225G

Visualization of PDB-structures was carried out by the help of PyMOL (The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC); analysis of structures (including the selection of residues within certain distance to ligand or other protein chain) was carried out by SwissProtViewer 4.1.0 (http://spdbv.vital-it.ch).

Results and discussion

It was revealed that complex of RGMB and NEO1 fragments (4bq6.pdb) bound heme mostly in the cavities made by partner proteins so that heme contacted with at least two chains simultaneously (Table 3, Fig. 1B). In most variants heme tended to bind RGMB chain near glutamine Q284 in the region with hydrophobic (Val, Leu, Pro) and charged amino acids (Asp, Arg) (Table 3). Only when this site was occupied by other heme molecule the next one bound to NEO1 in polar region with serine S1012 in proximity to heme iron (chain B) or other RGMB chain (docking rounds 2 and 3, Table 3).

Polar AA prevailed in heme-binding sites predicted in RGMB-NEO1 complex (4bq6), but no cysteines were revealed among them (Table 3). More stable heme binding through iron ion is known to be provided by heterocyclic His or aromatic Tyr as well as by Cys residues in certain motifs (Li et al., 2011). But also hydrophobic interactions with porphyrin ring or electrostatic binding through propionate residues with positively charged AA groups are possible. Type of interaction revealed in our study could not provide stable bonds, but might be enough for short-term regulatory effect.

Table 3.

Representative results of heme docking to the fragments of RGMB complex with NEO1 (PDB ID 4bq6, PatchDock). Amino acids most close to heme iron are marked by bold font

Docking round (solution)	Total Score	Contact area	Number of AA in contact	Chain (protein)	Amino acids in heme neighborhood (predicted to be within 6 Å to heme iron)
1st round (solution 4)	6614	767	14 AA	A (NEO1)	P931, T934, K935, K937
				B (NEO1)	N933
				F (RGMB)	V235, T236, D237, D238, L239, **Q284**, V285, G286, R287
2nd round (solution 5)	6532	836	14 AA	B (NEO1)	K952; S987; K990; P1011; **S1012**; E1013
				D (RGMB)	N214; V235; T236; D237; R287
				F (RGMB)	N233
3rd round (solution 1)	7314	847	12 AA	B (NEO1)	T934, K935; Y936; K937
				D (RGMB)	D237; D238; L239; R283; **Q284**; V285; G286; R287

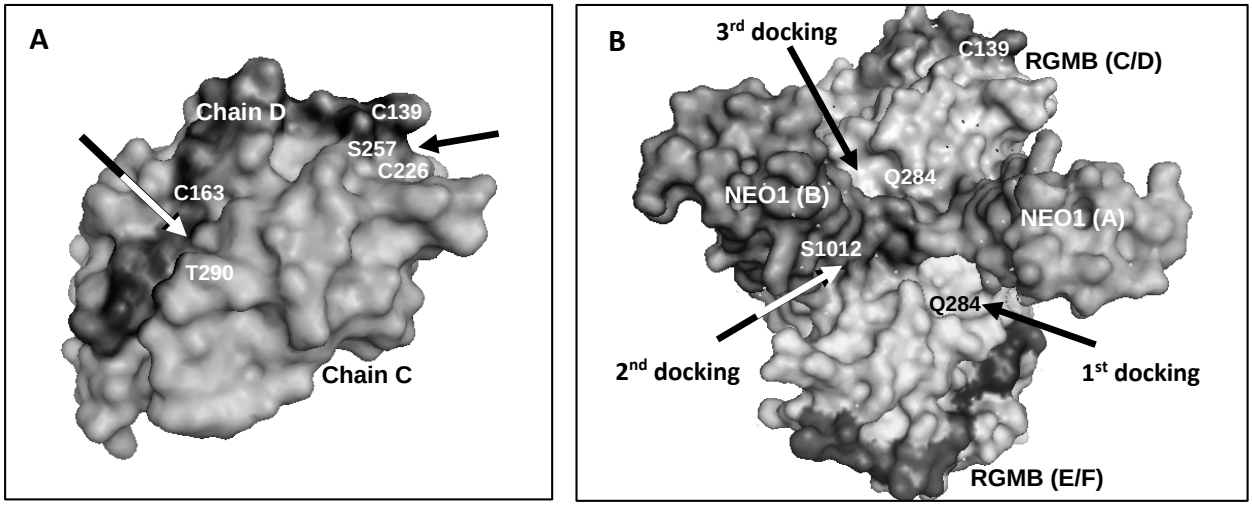

Fig. 1. Surface view (PyMol) of monomeric RGMB fragment (A) and the complex of RGMB with NEO1 (B) with predicted heme binding sites (PatchDock). Arrows show the location of heme docking variants. Chains C/D in the left and right parts of the figure are in the same projection but different scale. Protein chains are shown in parenthesis

The search of heme-binding sites in RGMB protein monomer was performed using the chains C and D (sequence regions 138–168 and 169–321) extracted from 4bq6.pdb (Fig. 1A). Two protein regions were predicted as putative heme-binding sites (Table 4): the first one was near to Tyr268 and disulfide bond between Cys163 and Cys312 with Thr290 or Leu291 most close to Fe ion (solutions N6, N15 and N18) and the second site was arranged near disulfide bond Cys226–Cys139 with Ser257 in the close proximity to Fe (solution N16).

To analyze heme-binding propensity of RGMB Cys-Pro motif HemeBind tool was also applied for protein sequence and structures of C and D chains. HemeBIND uses structural and sequence information

about binding interfaces based on the analysis of heme-protein complexes (Liu, Hu, 2011) while PatchDock algorithm is oriented on molecular shape complementarity (Schneidman-Duhovny et al., 2005). HemeBind predicted mostly hydrophobic (12 of 21 AA) residues as potential heme-binding sites. Chain C fragment with C163 was predicted as heme-binding region by both programs (Table 4). Binding sites predicted by two program tools in the chain D differed and didn't contain free cysteines.

Table 4.

Prediction of potential heme binding sites in RGMB fragment by two program tools. PatchDock data on two representative sites of heme docking to C/D chains of 4bq6. Amino acids predicted by PatchDock as most close to heme iron are marked by bold font

Program	Amino acids in close proximity to heme
PatchDock	(chain C): P138; C139; N140; C163; L165; F166; (chain D): G225; C226; D254; A255; K256; **S257**; L258; Y268; F281; V282; R283; V285; **T290**, L291; A292; I293
HemeBind	(chain C): Y160; F162; C163; L165; F166; G167; (chain D): H170; L171; F174; F178; C181; G185; W187; Q198; V199; V204; A210; T211; M295; L311; C312

In silico analysis of RGMB in the complexes with BMP2 protein (4uhz and 4uk2) revealed neogenin and BMP2 as probable heme targets in the first docking rounds (Table 5). BMP2 was predicted to surround heme molecule by polar environment with glutamic acid in close proximity to iron ion, several cysteines were at short distance of heme but all of them are inside disulfide bonds (Table 1). In dimeric complex (4uhz) RGMB protein was predicted to bind heme only if BMP2 sites were occupied.

Table 5.

Representative results of heme docking to the fragments of RGMB complexes with BMP2 and/or NEO1 (PatchDock)

Docking round (solution)	Total Score	Contact area	Number of AA in contact	Chain (protein)	Amino acids in heme neighborhood (predicted to be within 6 Å to heme iron)
PDB ID 4uhz (BMP2 and RGMB)					
1st round (solution 4)	5690	776	11 AA	A (BMP2)	K293, S294, S295, C296, H326, G327, **E328**, C329, K358, A359, C361
2nd round (solution 2)	6094	810	7AA	B (RGMB)	A89; T92; Q93; S96; **Y105;** H106; V109
3rd round (solution 1)	5542	808	11AA	B (RGMB)	R55, K58; C59, C91; T92; Q93; R94; **T95**; S96; K97, A98
PDB ID 4ui2 (BMP2, NEO1 and RGMB)					
1st round (solution 2)	5740	727	12 AA	A (NEO1)	P931; I930; K952; P953; T955; P1011; **S1012**; Q1013; A1014; N1015
				D (RGMB)	Q233; V244
2nd round (solution 6)	5796	748	10 AA	A (NEO1)	W905; A906; D907, N908; L910, P911, K912**, H913**, Q914
				B (BMP2)	D307
3rd round (solution 5)	5712	790	9 AA	A (NEO1)	Q914; I916
				B (BMP2)	**Y302**; V303; D304; P318; G319; Y320, H321

Analysis of docking to the tertiary complex (4ui2) revealed BMP2 and neogenin but not RGMB residues in heme iron proximity. Taking into account heme affinity to certain amino acids (Li et al., 2011), His913 of NEO1 or Tyr302 of BMP2 protein (Fig. 2) could provide more prolonged heme binding under accumulation of free heme. These residues are arranged near the contact area of protein chains (Fig. 3) so the oligomeric complex of studied proteins is more preferable heme target than monomeric RGMB. It is worth mentioning that RGMB fragment used for analysis had no big cavities and most AA predicted to contact with heme were not at the protein surface and were not involved in oligomerization.

Comparison of the scoring results for different structures used as heme targets in PatchDock revealed similar scores in the case of monomeric chains and RGMB complexes with BMP and NEO1 containing only one copy of RGMB fragment (Table 6). But RGMB–NEO1 complex with dimeric fragments revealed much higher scores in all three rounds of docking ($p<0,05$).

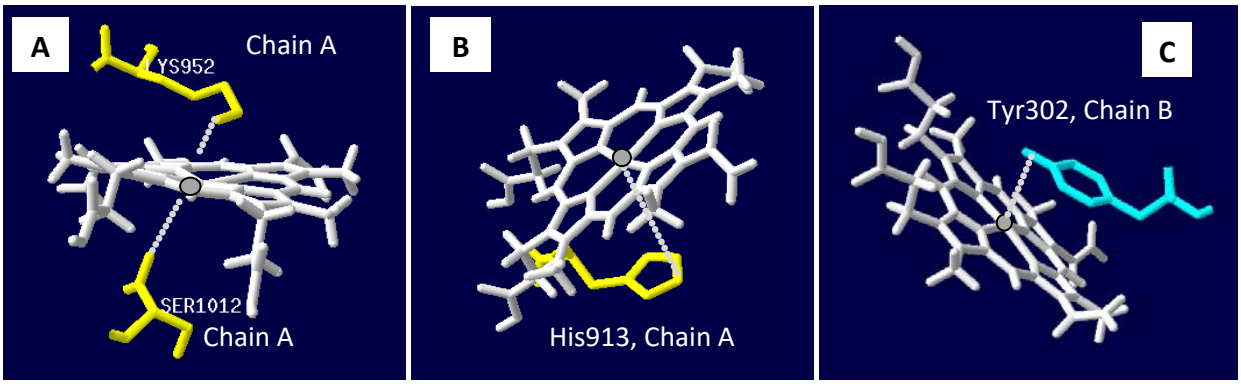

Fig. 2. Amino acids side chains arranged in proximity to heme iron according to 1st (A), 2nd (B) and 3rd (C) rounds of heme docking to 4ui2 (by PatchDock). Chain A – NEO1, chain B – BMP2. Visualization and analysis – SwissPDBViewer. Dots visualize distance between Fe and AA side chain

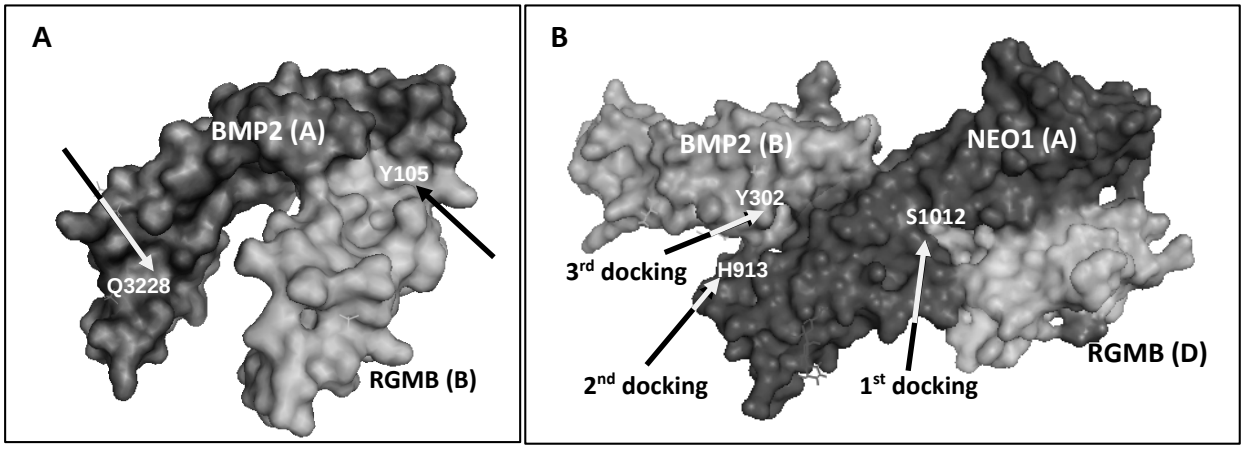

Fig. 3. Surface view in PyMol of (A) RGMB complex with BMP2 (4uhz) and (B) the complex of BMP2, RGMB and NEO1 (4ui2) with predicted heme binding sites (PatchDock). Arrows show the variants of heme docking. Protein chains are shown in parenthesis

Table 6.

Scoring results of heme docking to different target proteins. Mean and standard deviation were calculated by the data for the best 20 solutions for each docking round (PatchDock)

Target	Scores (1st round)	Scores (2nd round)	Scores (3rd round)	Predicted heme targets (chains) with Cys, CP motifs, His or Tyr residues in docking areas
4bq6	6641±138	6534±112	6514±297	NEO1 (B): Y936
4uhz	5668±179	5568±193	5368±108	BMP2 (A): disulfide C296-C361, C329 (CP); H326; RGMB (B): C59, C91, Y105, H106
4ui2	5650±72	5636±68	5615±86	NEO1 (A): H913; BMP2 (B): Y302, Y320, H321
4bq6-CD	5092±124	4762±103	–	RGMB (C, D): C139-C226 (PC139), C163, Y268
4bq6-A	5281±126	–	–	NEO1 (A): Y957
4uhz-A	5274±156	–	–	BMP2 (A): C296-C361, C329 (CP); H326

So molecular docking revealed higher scores for heme binding to the tetrameric complex of neogenin and its co-receptor RGMB near interchain contact areas while heme interaction with monomeric components was less probable. Only few predicted sites contained amino acids capable to form stable bonds with heme (cysteine, histidine or tyrosine), major sites could provide only short-term interaction. Part of AA residues predicted to interact with heme in neogenin and RGMB monomers was found at the surfaces involved in oligomerization so complex formation might be affected under heme accumulation. Non-specific heme attachment simultaneously to several chains of the signalling protein complex also could have short-term conformational effect. Taking into account the proinflammatory action of BMP signalling on endothelial cells (Cai et al., 2012), heme binding to BMP receptor complex can be discussed as non-specific mechanism of vascular pathogenesis under stress-derived heme accumulation.

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