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Research Article

Proteins with the HAEE tetrapeptide motif as potential targets for beta-amyloid

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Abstract

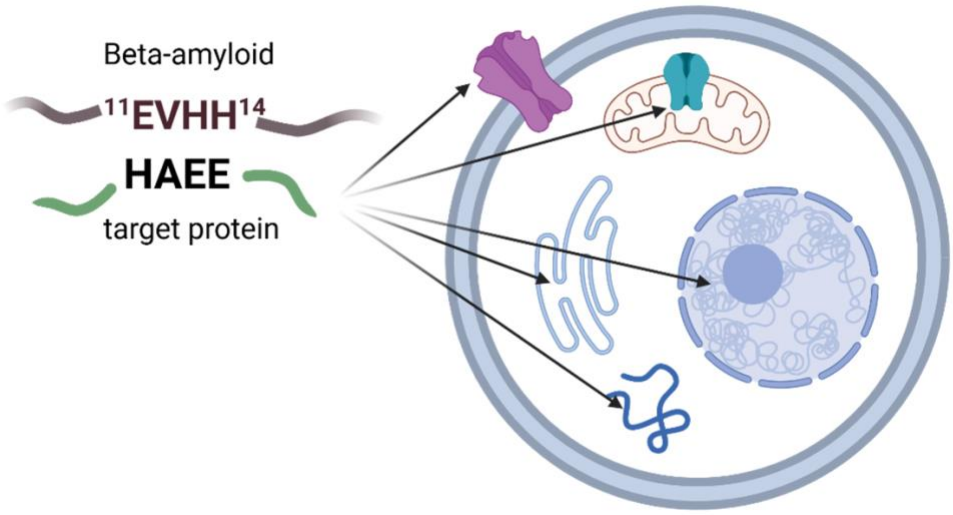
Introduction: Beta-amyloid (Aβ) is involved in numerous physiological and pathophysiological processes and is one of the key players in the pathogenesis of Alzheimer's disease. Aβ interacts with the 35-HAEE-38 site of the α4 subunit of the α4β2 nicotinic acetylcholine receptor. The synthetic tetrapeptide HAEE effectively inhibits the aggregation of endogenous Aβ. HAEE specifically binds to the 11-EVHH-14 site of Aβ both in the absence and presence of zinc ions, leading to the formation of stable complexes. We hypothesized that the HAEE motif could represent a universal binding site for Aβ within the human proteome.

Materials and Methods: To test this hypothesis, a large-scale search for all amino acid sequences containing the HAEE motif in the human (*Homo sapiens*) proteome was performed using our in-house PepString server (http://pepstring.eimb.ru/). The conservation of the identified sites was analyzed across jawed vertebrates using BLAST. Protein localization and structural features were determined based on data from UniProt, PDB, and AlphaFold.

Results: We identified 85 proteins (including 200 isoforms) containing the HAEE motif. Of these, 26 proteins are membrane proteins, including receptors, ion channels, and transporters (e.g., CACNA1B, MRS2, SLC15A2), and 59 are intracellular proteins, mostly nuclear transcription factors (including 13 zinc finger proteins). Structural analysis revealed that the HAEE motif is often located within functionally important domains, such as cytoplasmic loops of transmembrane proteins or DNA-binding domains.

Conclusion: Given that Aβ acts as an extracellular ligand and can also penetrate various intracellular compartments, all identified proteins with the HAEE motif are considered potential physiological and pathophysiological targets for Aβ. The most promising candidates are proteins whose HAEE sites are structurally similar to that in α4β2-nAChR and/or coordinate zinc ions. These findings enhance our understanding of the molecular mechanisms of Aβ function and open new avenues for the search of therapeutic targets in AD.

Graphical Abstract



Keywords

Alzheimer's disease, beta-amyloid, molecular targets, short linear motifs, bioinformatic analysis, proteome, HAEE

Introduction

Alzheimer's disease (AD) is a leading cause of dementia worldwide and is characterized by the formation of soluble oligomers and insoluble aggregates from endogenous amyloid-β (Aβ) molecules, which exist in a dynamic equilibrium (Cohen et al. 2013). Insoluble Aβ aggregates are found both on the walls of blood vessels and on the surface of neurons in the brain (Querfurth and LaFerla 2010). Aβ aggregates and oligomers trigger neuroinflammation and other pathological processes in AD (Long and Holtzman 2019; Livingston et al. 2020; Walker 2020).

Aβ is a polypeptide consisting of 38-43 amino acid residues (Surguchov et al. 2023), generated through the proteolytic cleavage of the amyloid precursor protein (APP) (Golde et al. 2018). The Aβ peptide is present in both brain tissues and peripheral organs (Wang et al. 2017), where it is primarily produced in blood platelets (Cadoni et al. 2024) and circulates across the blood-brain barrier (Wang et al. 2017). The concentration of Aβ in the blood of both healthy individuals and patients diagnosed with the sporadic form of AD is in the picomolar range (Roher et al. 2009). Known physiological functions of Aβ include suppressing microbial infections, regulating synaptic plasticity, aiding in recovery from brain injury, sealing the blood-brain barrier, and potentially inhibiting the proliferation of cancer cells (Brothers et al. 2018; Jeong et al. 2022).

The physiological and pathophysiological molecular targets of Aβ are highly diverse (Chen et al. 2017), and new molecular partners of Aβ are continually being identified. Recently, using frog oocytes, we obtained evidence that the primary event in AD pathogenesis is the formation of a complex composed of Aβ and the α4 subunit of the α4β2 nicotinic acetylcholine receptor (α4β2-nAChR) (Barykin et al. 2020; Mitkevich et al. 2023). The formation of this complex involves the 11-EVHH-14 fragment of Aβ and the 35-HAEE-38 fragment of the receptor, which jointly chelate a zinc ion. An anti-amyloid effect of the synthetic tetrapeptide HAEE has also been established. The interaction of Aβ with HAEE leads to the formation of stable complexes that, in turn, block the formation and spread of Aβ aggregates.

We propose that the tetrapeptide HAEE motif in proteins represents a universal site for complex formation with Aβ. Therefore, this study aimed to search for HAEE sites within the human proteome to identify potential targets of Aβ action, thereby improving our understanding of the physiological role and functional mechanisms of Aβ in humans.

Materials and Methods

Identification of proteins containing the HAEE sequence using the PepString server

We have previously developed a specialized tool for the rapid and convenient search of protein sequences containing short fragments and their combinations – the PepString server (http://pepstring.eimb.ru) (Kozin et al. 2025). Using this server, we entered HAEE into the sequence field and limited the search by selecting the taxon *Homo sapiens*. We identified 200 isoforms of 85 human proteins containing the HAEE fragment in their sequences. The conservation of this fragment for each protein among jawed vertebrates was determined using the BLAST program (Zaru et al. 2023) (see Appendix Table S1). For this purpose, we selected the 1000 most similar protein sequences of jawed vertebrates from the UniProt database for each protein and calculated the percentage of cases where the HAEE fragment was present at the corresponding position in the multiple sequence alignment.

Conservation calculations using BLAST

The conservation of the HAEE site was calculated based on homologous protein sequences from other species among jawed vertebrates using the BLAST program (Altschul et al. 1990). For each UniProt protein identifier from Table S1, 1000 homologous sequences were retrieved. The number of sequences containing the HAEE site (the first number in the "Conservation" column in Table S1) was divided by the number of sequences with all variants of this site (the second number in the "Conservation" column in Table S1) and multiplied by 100 to obtain the Conservation value as a percentage.

HAEE site variants

A short program was written in C++ to parse FASTA sequence files and count all variants of the HAEE sites. The source code of this program can be found in the Article Appendix (Kozin et al. 2025).

Results and Discussion

Using the PepString server, 200 isoforms of 85 human proteins containing the HAEE fragment were identified in the human proteome. Appendix Table S1 compiles information on the conservation of the HAEE fragment in the protein sequences and the position of this fragment within the protein structure.

For 18 out of the 85 sequences, experimental protein structures with a resolved HAEE site were found. In cases where the HAEE site was missing in the experimental structures or no experimental structure was available at all, we utilized AlphaFold predictions (63 proteins). For four proteins, no structure – either experimental or predicted – could be found.

In 39 out of the 81 protein structures, the HAEE region adopted an α-helix conformation; in 24 out of 85, it was in a disordered conformation; in 11 proteins, it was located in a bend (with only 2 of these being a specific β-turn); 2 were in an extended conformation; and 5 were in a β-strand conformation (see Appendix Table S1).

Proteins containing the most conserved HAEE sites are predominantly localized within the nucleus or in the cytoplasm. However, 26 of them are embedded in various membranes: 3 in neuronal cell membranes, 1 in the mitochondrial membrane, and 22 in other membranes (Table 1).

The transmembrane proteins of neuronal cells include: Neuronal acetylcholine receptor subunit alpha-4 (CHRNA4), located in the synaptic cell membrane (Gao et al. 2021), for which the receptor interaction of Aβ with the HAEE site has been demonstrated both *in vitro* and *in vivo*; Voltage-dependent N-type calcium channel subunit alpha-1B (CACNA1B), involved in signaling between neurons; and SHC-transforming protein 4 (SHC4), located in the postsynaptic cell membrane (Table 1, Fig. 1). In CHRNA4, the HAEE site (residues 35-38) is situated on the outer surface of the extracellular domain, resembling a classical receptor binding site (Fig. 1A). In contrast, in CACNA1B, the HAEE segment (430-433) is located in the cytoplasm within a disordered loop that is absent from experimental structures (Fig. 1B), making it unlikely to function as a receptor site. While there is no experimentally determined structure for SHC4, which is located in the postsynaptic membrane, indirect evidence suggests that its HAEE site (365-368) is positioned at the boundary of the PID domain (residues 186-369). This domain interacts with phosphorylated muscle and skeletal receptor tyrosine-protein kinase MUSK (via an NPXY motif). The structure of the HAEE region is predicted by AlphaFold to be disordered (Fig. 1C). Activation of MuSK by agrin binding at the neuromuscular junction induces clustering and tyrosine phosphorylation of acetylcholine receptors, which are essential for synaptic transmission (Jones et al. 2007).

Table 1. Human transmembrane proteins containing the HAEE site in their sequence.

№	Conservativity	Uniprot IDs	Protein name	Position in sequence	Sequence context
Neuronal cell membrane					
1	715/900 (79.4%)	P43681	Neuronal acetylcholine receptor subunit alpha-4, synaptic membrane	35-38	AHAEER
2	691/996 (69.4%)	Q00975	Voltage-dependent N-type calcium channel subunit alpha-1B, synaptic membrane	430-433	IHAEEG
3	96/988 (9.7%)	Q6S5L8	SHC-transforming protein 4, postsynaptic membrane	365-368	SHAEER
Mitochondrion inner membrane					
4	784/909 (86.2%)	Q9HD23	Magnesium transporter MRS2 homolog, mitochondrial	288-291	DHAEEM
Endoplasmic reticulum membrane					
5	641/776 (82.6%)	Q15363	Transmembrane emp24 domain-containing protein 2	28-31	AHAEEC
6	381/998 (38.2%)	Q6AZY7	Scavenger receptor class A member 3	392-395	THAEEL
7	25/127 (19.7%)	Q9H6L5	Reticulophagy regulator 1	9-12	EHAEEG
8	119/977 (12.2%)	P31513	Flavin-containing monooxygenase 3	46-49	DHAEEG
Cell membrane					
9	925/938 (98.9%)	Q8NDI1	EH domain-binding protein 1	89-92	PHAEEF
10	980/1000 (98.0%)	Q9H0B6	Kinesin light chain 2	414-417	MHAEER
11	668/808 (82.7%)	Q9Y4D8	Probable E3 ubiquitin-protein ligase HECTD4	933-936	EHAEEY
12	650/939 (69.2%)	Q7Z6B0	Coiled-coil domain-containing protein 91	333-336	AHAEER
13	309/559 (55.3%)	P58743	Prestin	3-6	DHAEEN
14	541/994 (54.4%)	Q9UBT7	Alpha-catulin	469-472	IHAEET
15	23/56 (41.1%)	Q9BVW6	Small integral membrane protein 2	73-76	CHAEED
16	144/410 (35.1%)	Q02388	Collagen alpha-1(VII) chain	2838-2841	SHAEEE
17	218/966 (22.6%)	P49815	Tuberin	1348-1351	LHAEEL
18	188/994 (18.9%)	Q16348	Solute carrier family 15 member 2	176-179	KHAEER
19	174/1000 (17.4%)	O14638	Ectonucleotide pyrophosphatase/phosphodiesterase family member 3	552-555	SHAEEV
20	93/993 (9.4%)	P54852	Epithelial membrane protein 3	121-124	IHAEEI
21	58/924 (6.3%)	Q9NY59	Sphingomyelin phosphodiesterase 3	611-614	LHAEEG
22	1/17 (5.9%)	Q96LA6-4	Fc receptor-like protein 1	192-195	GHAEEV
23	21/364 (5.8%)	Q7Z6P3	Ras-related protein Rab-44	783-786	AHAEEQ
24	1/418 (0.2%)	Q7Z7H3	Ciliogenesis-associated TTC17-interacting protein	235-238	VHAEEG
Other membranes					
25	40/438 (9.1%)	Q86UP6	CUB and zona pellucida-like domain-containing protein 1	557-560	THAEET
26	7/971 (0.7%)	Q6NUT3	Major facilitator superfamily domain-containing protein 12	246-249	PHAEEP

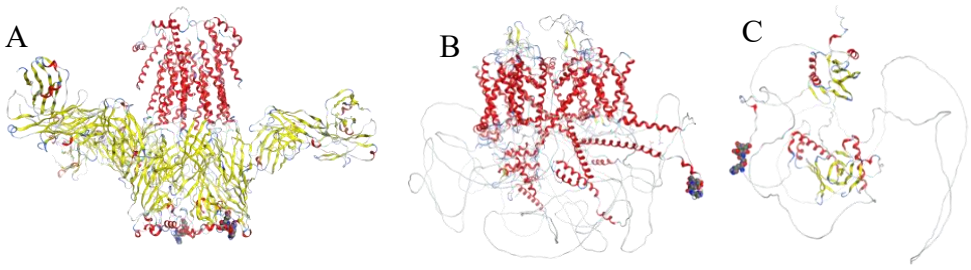


Figure 1. Structures of neuronal transmembrane proteins containing the HAEE site in their sequence. **A.** Experimental structure (PDB ID 8ST4) of the Neuronal acetylcholine receptor subunit alpha-4 (CHRNA4). The HAEE site (residues 35-38) is located on the external surface of the receptor's extracellular domain. **B.** Predicted structure AF-Q00975-F1 of the Voltage-dependent N-type calcium channel subunit alpha-1B (CACNA1B). The HAEE segment (430-433) is located in the cytoplasm within a disordered loop that is absent in experimental structures. **C.** Predicted structure AF-Q6S5L8-F1 of the Postsynaptic cell membrane SHC-transforming protein 4 (SHC4). The HAEE site (365-368) is positioned at the boundary of the PID domain (186-369) (Jones et al. 2007) and is predicted to be disordered.

Another protein, besides CHRNA4, in which the HAEE site resembles a classical receptor binding site is the mitochondrial homolog of the Magnesium transporter MRS2 (Fig. 2) (Zsurka et al. 2001). The magnesium transporter mediates the influx of magnesium into the mitochondrial matrix and regulates magnesium metabolism (Piskacek et al. 2009). According to cryo-electron microscopy data, the HAEE site (288-291) is located in the mitochondrial matrix and faces the interior of the mitochondrion (Lai et al. 2023).

It is known that mitochondrial dysfunction is a hallmark of Aβ-induced neuronal toxicity in AD. Aβ interacts with various mitochondrial targets, including the outer mitochondrial membrane, intermembrane space, inner mitochondrial membrane, and matrix (Pagani and Eckert n.d.). It blocks the transport of nuclear-encoded mitochondrial proteins into mitochondria, interacts with mitochondrial proteins, disrupts the electron transport chain, increases the production of reactive oxygen species, causes mitochondrial damage, and impairs normal neuronal function (Chertkova et al. 2017). However, the molecular mechanism of Aβ action on mitochondria remains unclear. The potential protein target of Aβ identified in our study may shed light on the mechanism of mitochondrial dysfunction in AD.

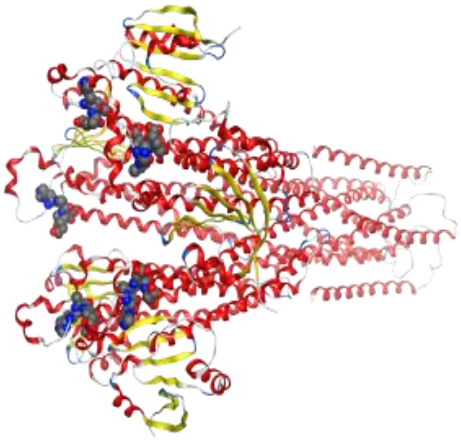


Figure 2. Experimental structure (PDB ID 8IP5) of the mitochondrial homolog of the Magnesium transporter (MRS2). The HAEE site (amino acid residues 288-291) is in an α-helical conformation and resembles a classical receptor binding site. It is located in the mitochondrial matrix and faces the interior of the mitochondrion (Lai et al. 2023).

Four of the identified transmembrane proteins containing the HAEE site are localized to the endoplasmic reticulum membrane. These are Transmembrane emp24 domain-containing protein 2 (TMED2), Scavenger receptor class A member 3 (SCARA3) (Han et al. 1998), Reticulophagy regulator 1 (RETREG1) (Khaminets et al. 2015), and Flavin-containing monooxygenase 3 (FMO3) (Table 1, Fig. 3). TMED2 is involved in vesicular protein trafficking. It acts as a cargo receptor on the luminal side for the incorporation of secretory cargo molecules into transport vesicles and is involved in vesicle coat formation on the cytoplasmic side (Anwar et al. 2022). The HAEE site (28-31) was found in a β-strand conformation by X-ray analysis (Fig. 3A). SCARA3 appears to protect cells by scavenging oxidative molecules or harmful oxidation products (Han et al. 1998). Its HAEE site (392-395) is predicted to be in an α-helical conformation (Fig. 3B). RETREG1 is an endoplasmic reticulum (ER)-anchored autophagy regulator that mediates ER delivery into lysosomes by sequestering it into autophagosomes (Khaminets et al. 2015). It is required for the long-term survival of nociceptive and autonomic ganglion neurons (Kurth et al. 2009; Khaminets et al. 2015). The HAEE site (9-12) is predicted to be in a disordered conformation (Fig. 3C). FMO3 is an essential hepatic enzyme that catalyzes the oxygenation of a wide variety of nitrogen- and sulfur-containing compounds, including drugs and dietary compounds (Rawden et al. 2000). Specifically, it catalyzes the production of taurine from hypotaurine (Veeravalli et al. 2020) and trimethylamine (TMA) from trimethylamine N-oxide (TMAO) (Lang et al. 1998). The HAEE site (46-49) is predicted to be in an extended conformation (Fig. 3D).

Among the 16 transmembrane proteins of the basal membrane, a structure – either experimental or predicted – could not be found for only one protein, Collagen alpha-1(VII) chain (COL7A1). COL7A1 forms anchoring fibrils that contribute to the organization and adherence of the epithelial basement membrane by interacting with extracellular matrix (ECM) proteins such as type IV collagen. It is a secreted component of the extracellular matrix and basement membrane. We have no structural information regarding the conformation of its HAEE site (2838-2841). The structures of the remaining 15 membrane proteins are presented in Figure 4A-O. Two proteins are of particular note: Solute carrier family 15 member 2 (SLC15A2) (Fig. 4I) and Epithelial membrane protein 3 (EMP3) (Fig. 4K). In these proteins, the HAEE sites adopt an α-helical conformation and are located at the end of an α-helical bundle, resembling classic receptor sites.

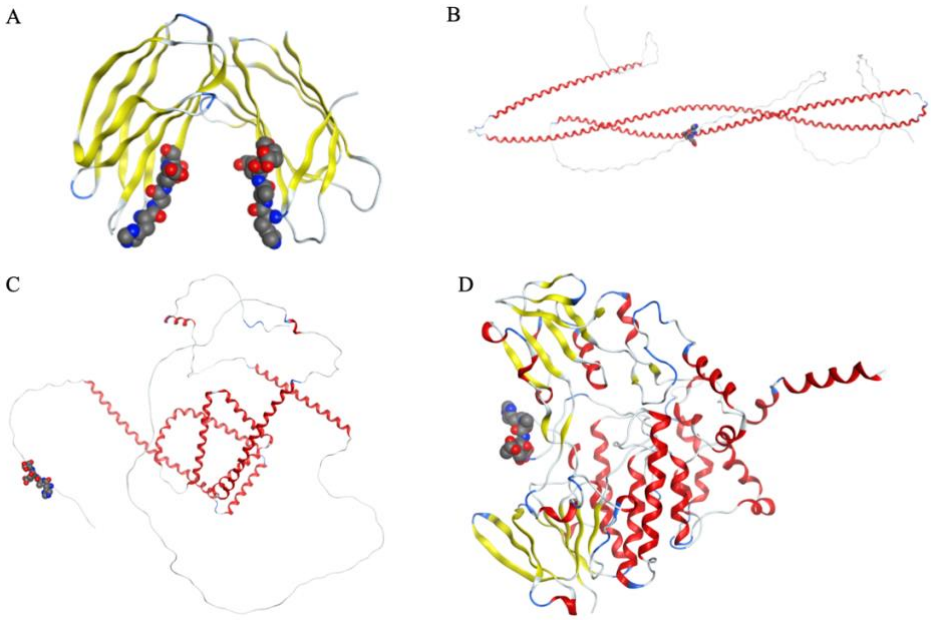


Figure 3. Structures of endoplasmic reticulum transmembrane proteins containing the HAEE site in their sequence. **A.** X-ray structure (PDB ID 5AZW, residues 20-113) of the luminal domain of Transmembrane emp24 domain-containing protein 2 (TMED2). The HAEE site (28-31) is in a β-strand conformation. **B.** Predicted structure AF-Q6AZY7-F1 of Scavenger receptor class A member 3 (SCARA3). The HAEE site (392-395) is in an α-helical conformation. **C.** Predicted structure AF-Q9H6L5-F1 of Reticulophagy regulator 1 (RETREG1). The HAEE site (9-12) is in a disordered conformation. **D.** Predicted structure AF-P31513-F1 of Flavin-containing monooxygenase 3 (FMO3). The HAEE site (46-49) is in an extended conformation.

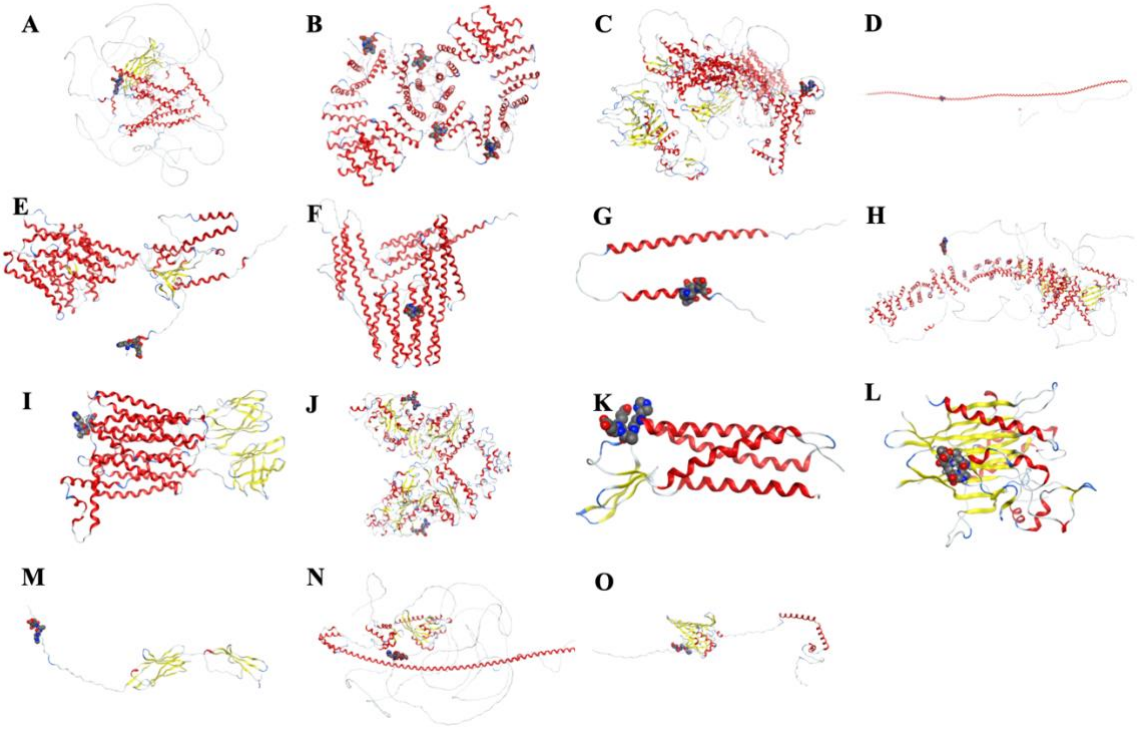


Figure 4. Structures of cell membrane proteins containing the HAEE site in their sequence. **A.** Predicted structure AF-Q8NDI1-F1 of EH domain-binding protein 1 (EHBP1). HAEE (89-92) is predicted to form a turn. **B.** Crystal structure PDB ID 3EDT of kinesin light chain 2 (KLC2). HAEE (414-417) is part of an α-helix. **C.** Predicted structure AF-Q9Y4D8-2 of probable E3 ubiquitin-protein ligase (HECTD4). HAEE (933-936) is predicted to be α-helical. **D.** Predicted structure AF-Q7Z6B0-F1 of Coiled-coil domain-containing protein 91 (CCDC91). HAEE (333-336) is predicted to be α-helical. **E.** Predicted structure AF-P58743-F1 of prestin (SLC26A5). HAEE (3-6) is predicted to be α-helical. **F.** Predicted structure AF-Q9UBT7-F1 of alpha-catulin (CTNNAL1). HAEE (469-472) is predicted to be α-helical. **G.** Predicted structure AF-Q9BVW6-F1 of Small integral membrane protein 2 (SMIM2). HAEE (73-76) is predicted to form an α-helix. **H.** Predicted structure AF-P49815-F1 of Tuberin (TSC2). HAEE (1348-1351) is predicted to be disordered. **I.** Electron microscopy structure (7PMY) of solute carrier family 15 member 2 (SLC15A2). HAEE (176-179) is α-helical and located in the cytoplasmic domain. **J.** Crystal structure (PDB ID 6C02) of Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3). HAEE (552-555) is in an extended conformation. **K.** Predicted structure AF-P54852-F1 of Epithelial membrane protein 3 (EMP3). HAEE (121-124) is predicted to be α-helical. **L.** Crystal structure PDB ID 5UVG of Sphingomyelin phosphodiesterase 3 (SMPD3). HAEE (611-614) forms a β-strand. **M.** Predicted structure AF-Q96LA6-4-F1 of Fc receptor-like protein 1 (FCRL1). HAEE (192-195) is predicted to be disordered. **N.** Predicted structure AF-Q7Z6P3-F1 of Ras-related protein Rab-44 (RAB44). HAEE (783-786) is predicted to be disordered. **O.** Predicted structure AF-Q7Z7H3-F1 of Ciliogenesis-associated TTC17-interacting protein (CATIP). HAEE (235-238) is predicted to be disordered.

In addition to the extracellular production pathway, Aβ is also generated inside cells (Hartmann 1999), where it can interact with nuclear and cytoplasmic proteins. Therefore, the remaining 59 potential protein targets of Aβ identified in our study may be involved in regulating genome function, endoplasmic reticulum transport, and vesicular trafficking. Our attention was drawn to two Cysteine and glycine-rich proteins localized in the nucleus, one with a conservation of 98.8% and the other – 65.8%. Interestingly, the solution structure of Cysteine and glycine-rich protein 3 (PDB ID 2O10) contains two zinc ions. We have previously shown that the synthetic HAEE analog forms a zinc-mediated binding site with Aβ (Mitkevich et al. 2023). Zinc is also an essential component of 13 zinc finger proteins identified in our search (Table 2). The simultaneous presence of both zinc and the HAEE site within a protein structure creates the necessary preconditions for high-affinity interaction with Aβ. It should be noted that in the identified zinc finger proteins, the HAEE site in ZNF658 and ZNF355P is specific only to humans. In proteins ZNF480, ZNF354A, ZNF732, ZIM3, and ZNF676, the HAEE site is found only in primates. In contrast, the HAEE site is present in proteins ZNF268, ZCCHC8, PRDM2, PLAGL1, ZNF316, and CASZ1 across a wider range of species – from mammals to birds and some fish.

Table 2. Thirteen zinc finger proteins containing the HAEE site in their sequence.

№	Conservativity	Uniprot IDs	Gene name	Protein name	Position in sequence	Sequence context	Animals with the same site HAEE in homologues proteins
18	572/782 (73.1%)	Q6NZY4 Q6NZY4-2	ZCCHC8	Zinc finger CCHC domain-containing protein 8	275-278	YHAEEV	Mammals, some birds, some fish, monitor lizards, turtles and snakes
23	578/902 (64.1%)	Q13029 Q13029-2 Q13029-3 Q13029-5	PRDM2	PR domain zinc finger protein 2	1156-1159	IHAEEW	
48	179/1000 (17.9%)	Q9UM63 Q9UM63-2	PLAGL1	Zinc finger protein PLAGL1	142-145	AHAEEK	
54	109/999 (10.9%)	A6NFI3	ZNF316	Zinc finger protein 316	825-828	AHAEEK	
55	84/787 (10.7%)	Q86V15 Q86V15-2	CASZ1	Zinc finger protein castor homolog 1	135-138	DHAEEP	
67	49/1000 (4.9%)	Q14587 Q14587-2	ZNF268	Zinc finger protein 268	298-301	THAEEK	Primates, all cats, wild and domestic pigs, bats, meerkats, and manatees
70	42/1000 (4.2%)	Q8WV37 Q8WV37-2 Q8WV37-3	ZNF480	Zinc finger protein 480	309-312	IHAEEK	Aotus nancymaae, Callithrix jacchus, Cebus imitator, Cercocebus atys, Gorilla gorilla gorilla, Homo sapiens, Macaca fascicularis, Macaca mulatta, Macaca nemestrina, Mandrillus leucophaeus, Nomascus leucogenys, Pan paniscus, Pan troglodytes, Papio anubis, Piliocolobus tephrosceles, Pongo abelii, Rhinopithecus bieti, Rhinopithecus roxellana, Sapajus apella, Theropithecus gelada
72	36/1000 (3.6%)	O60765	ZNF354A	Zinc finger protein 354A	320-323	IHAEEN	Homo sapiens, Aotus nancymaae, Callithrix jacchus, Carlito syrichta, Cebus imitator, Cercocebus atys, Chlorocebus sabaeus, Colobus angolensis palliatus, Gorilla gorilla gorilla, Macaca fascicularis, Macaca mulatta, Macaca nemestrina, Mandrillus leucophaeus, Otolemur garnettii, Pan paniscus, Pan troglodytes, Papio anubis, Piliocolobus tephrosceles, Pongo abelii, Prolemur simus, Rhinopithecus bieti, Rhinopithecus roxellana, Saimiri boliviensis boliviensis, Sapajus apella, Theropithecus gelada
76	21/1000 (2.1%)	B4DXR9	ZNF732	Zinc finger protein 732	272-275	IHAEEK	Homo sapiens, Carlito syrichta, Cercocebus atys, Chlorocebus sabaeus, Colobus angolensis palliatus, Gorilla gorilla gorilla, Macaca fascicularis, Macaca mulatta, Macaca nemestrina, Nomascus leucogenys, Pan troglodytes, Pongo abelii, Rhinopithecus bieti, Rhinopithecus roxellana, Theropithecus gelada
79	7/1000 (0.7%)	Q96PE6	ZIM3	Zinc finger imprinted 3	217-220	THAEER	Homo sapience, Pan paniscus, Pan troglodytes, Gorilla gorilla gorilla, Pongo abelii, Callithrix jacchus
81	6/1000 (0.6%)	Q8N7Q3	ZNF676	Zinc finger protein 676	471-474	IHAAEK IHAAEK	Homo sapience, Pan troglodytes, Pongo abelii, Nomascus leucogenys, Gorilla gorilla gorilla
83	3/999 (0.3%)	Q5TYW1 Q5TYW1-2	ZNF658	Zinc finger protein 658	183-186	AHAEEK	Homo sapience
85	1/1000 (0.1%)	Q9NSJ1	ZNF355P	Putative zinc finger protein 355P	285-288	VHAEEK	Homo sapience

Conclusion

This large-scale bioinformatic investigation of the human proteome has revealed an extensive pool of 85 proteins containing the tetrapeptide motif HAEE, which we previously characterized as an Aβ-binding site within the α4β2-nAChR. Analysis of the identified proteins demonstrated that the HAEE motif is highly conserved in many of them, indicating its functional importance. Several groups of the identified potential Aβ targets appear to be particularly significant: synaptic proteins such as CACNA1B and SHC-transforming protein 4, interaction with which could directly mediate Aβ-induced synaptic dysfunction; MRS2, which may be a novel target explaining the mechanism of mitochondrial dysfunction in AD; transmembrane proteins SLC15A2 and EMP3, in which the HAEE site is positioned in a classical "receptor-like" conformation at the end of a transmembrane bundle; and nuclear proteins, especially zinc-binding proteins (cysteine and glycine-rich proteins and zinc finger proteins). The simultaneous presence of both zinc ions and the HAEE motif in their structure creates the prerequisites for high-affinity binding to Aβ.

Thus, the HAEE motif represents a novel, structurally determined site for predicting Aβ interaction with target proteins. Given that Aβ can act both outside and inside the cell, none of the discovered proteins can be excluded from the list of potential targets. This study lays the groundwork for the targeted experimental validation of the identified targets, which could ultimately lead to the discovery of new pathways in Alzheimer's disease pathogenesis and the development of strategies for their therapeutic intervention.

Additional Information

Conflict of interest

The authors declare the absence of a conflict of interests.

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Data availability

All of the data that support the findings of this study are available in the main text.

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