East European Journal of Physics. **1.** 236-240 (2023)
DOI:10.26565/2312-4334-2023-1-31 ISSN 2312-4334

INTERACTIONS OF FIBRILLAR PROTEINS WITH LIPIDS: A MOLECULAR DOCKING INSIGHT[†]

Valeriya Trusova*, Uliana Tarabara, Olga Zhytniakivska, Kateryna Vus, Galyna Gorbenko
Department of Medical Physics and Biomedical Nanotechnologies, V.N. Karazin Kharkiv National University
4 Svobody Sq., Kharkiv, 61022, Ukraine
**Corresponding Author: valerija.trusova@karazin.ua*
Received January 9, 2023, revised February 11, 2023; accepted February 13, 2023

The aggregation of misfolded proteins into specific ordered aggregates, amyloid fibrils, associated with more than forty human diseases, currently attracts great research attention in biomedical and nanotechnological aspects. These aggregates and their oligomeric intermediates are thought to exert their toxic action predominantly at the level of cell membranes. In addition, membrane lipids were found in many amyloid deposits in vivo suggesting that lipid molecules are able to incorporate into fibril structure affecting their morphology and mechanical properties. However, the biological implications and structural prerequisites of fibril-lipid interactions still remain unclear. In the present study the molecular docking techniques was employed to explore the interactions between the amyloid fibrils and lipids in the model systems containing the fibrillar forms of lysozyme, insulin, Aβ (1-42) peptide and N-terminal (1-83) fragment of apolipoprotein A-I, as a protein component and cholesterol, cardiolipin or phosphatidylcholine as a lipid component. Using the PatchDock web server and BIOVIA Discovery Studio software, the structural peculiarities of fibril-lipid associates were uncovered. The van der Waals and alkyl/π-alkyl interactions were found to prevail in stabilization of all types of fibril-lipid complexes. The analysis of most energetically favorable docking positions revealed a preferable surface location of lipids and partial penetration of acyl chains of cardiolipin and phosphatidylcholine into fibril grooves.
Keywords: *amyloid fibrils; lysozyme; insulin; Aβ (1-42) peptide; apolipoprotein A-I; fibril-lipid complex; molecular docking*
PACS: 87.14.C++c, 87.16.Dg

Among a variety of conformational and aggregational states of polypeptide chain the states of amyloid fibrils and their oligomeric precursors are currently regarded as the most dangerous, since they are associated with more than forty of human diseases [1,2]. The amyloid-related cytotoxicity is thought to be primarily determined by the impairment of cell membrane integrity through either detergent-like mechanism of membrane damage (lipid extraction from the bilayer) [3-7] or membrane permeation for ions via the formation of pore-like structures [8-10]. Moreover, it is becoming increasingly apparent that lipids are an integral part of most fibrillar deposits formed *in vivo* or amyloid fibrils grown *in vitro* in the presence of lipids [11]. The analysis of lipids extracted from various deposits of amyloid fibrils by high-performance thin layer chromatography and mass spectrometry revealed that they may contain more than 10 % of lipid (by dry weight), with major lipid constituents such as cholesterol and sphingomyelin, and minor fraction of sulfatides, cholesterol esters and fatty acids [12]. In the paired helical filaments of tau protein formed in Alzheimer disease the bound lipids were represented by the galactocerebrosides and phosphatidylcholines [13]. It is hypothesized that the presence of lipids as an integral part of many amyloid deposits *in vivo*, is fundamental for biological activity of fibrillized proteins and is essential for the nucleation, morphology, and mechanical properties of fibrils [11]. In the onset of amyloid formation the protein-lipid interactions can favor fibril nucleation, but the lipid uptake is limited to the transfer of a fatty acyl group to serine or lysine residues [14], or transfer of a reactive aldehyde fragment produced by oxidative damage to a lipid [15]. Lipids are most likely to associate with amyloid assembly at the stages of protein oligomerization and protofilament formation [1]. Despite extensive studies of amyloid cytotoxicity, the mechanisms by which lipids may become incorporated into fibril structure still remain poorly understood. In view of this, the aim of the present study was to elucidate the molecular details of fibril-lipid interactions. The molecular docking technique was employed to explore the complex formation in the systems containing the model fibrils of lysozyme, insulin, Aβ (1-42) peptide and N-terminal (1-83) fragment of apolipoprotein A-I as a protein component and cholesterol, cardiolipin or phosphatidylcholine as a lipid one.

METHODS

To predict the most favorable modes of interactions between the amyloid fibrils and lipids, the molecular docking was performed using the PatchDock algorithm built upon a geometry-based shape complementarity principle. The implementation of this algorithm involves the segmentation of molecular shape into patches of different geometry; matching of these patches and evaluation of the docking positions by geometric shape complementarity fit and atomic desolvation energy scoring function [20]. The model amyloid fibrils of hen egg white lysozyme, Aβ and apoliporotein A-I were constructed using the CreateFibril tool based on the translational and rotational affine transformations providing

[†] ***Cite as:*** V. Trusova, U. Tarabara, O. Zhytniakivska, K. Vus, and G. Gorbenko, East Eur. J. Phys. **1**, 236 (2023), https://doi.org/10.26565/2312-4334-2023-1-31

several copies of a certain fragment of fibril core, whose subsequent stacking produces the elongated fibrillar aggregate [21]. To this end, the following amino acid sequences from fibril core were used: i) K-peptide of lysozyme, GILQINSRW, residues 54-62 of wild-type protein (LzF); ii) the 24-50 fragment from G26R-mutated apolipoprotein A-I, DSRRDYVSQFEGSALGKQLNLKLLDNW (apoA); iii) the 1-42 fragment from Aβ protein, LVFFAEDVGSNKGAIIGLMVGGVVIA (Aβ). The input structures for CreateFibril were generated from the monomers in the β-strand conformation with PatchDock (LzF, apoA) or were obtained from the Protein Data Bank (Aβ tetramer, PDB code 2BEG). The twisting angle was taken as 15°, the value recovered previously for the most stable conformations of fibrillar LzF, Aβ and apoA. The selected docking poses were visualized with the UCSF Chimera software (version 1.14) and analyzed with BIOVIA Discovery Studio Visualizer, v21.1.0.20298, San Diego: Dassault Systemes; 2021. The prominent binding sites were also predicted through the DoGSiteScorer server (https://proteins.plus/2ozr#dogsite).

RESULTS AND DISCUSSION

While analyzing the predictions of PatchDock tool, the fibril-lipid binding modes were ranked according to the global binding energy (GBE), so that the complexes with the lowest binding energy were selected for deeper evaluation (Fig. 1). Further analysis of the docked structures was focused on the following characteristics: i) the location of lipid binding sites on/within amyloid fibril and interacting amino acid residues; ii) the types of interactions accounting for stabilization of the most energetically favorable amyloid-lipid complexes; iii) the atomic contact energy (ACE).

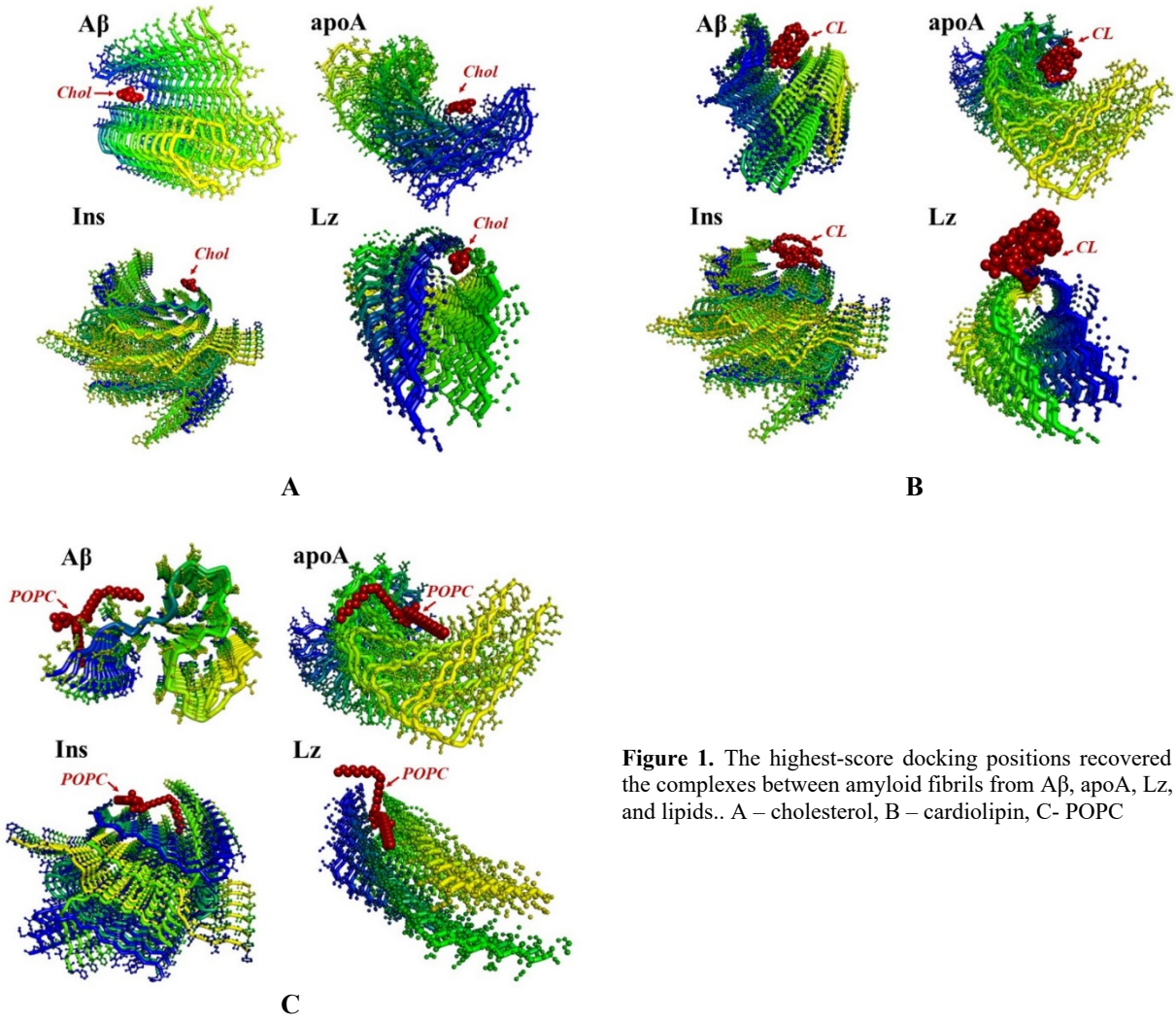

Figure 1. The highest-score docking positions recovered for the complexes between amyloid fibrils from Aβ, apoA, Lz, Ins and lipids.. A – cholesterol, B – cardiolipin, C- POPC

As seen in Table 1, the global energy of lipid-fibril binding falls in the range from -69.25 to -7.26 kcal/mol. It was found that or all examined systems the values of GBE decrease in the row Chol → CL → POPC, indicating that the neutral phospholipid POPC forms the most stable complexes with fibrillar proteins. Remarkably, analogous tendencies were revealed also for atomic contact energies, i.e., the lowest ACE values were observed for POPC, while the highest – for Chol.

For all fibrillar proteins under consideration cholesterol was found to display some penetration into the amyloid grooves with the shallowest location of sterol being observed for the lysozyme fibrils (Fig. 1, A). Furthermore, as follows from the Discovery Studio analysis, all amyloid-cholesterol complexes are stabilized by van der Waals and alkyl/π-alkyl

interactions. Specifically, Chol participates in the strong alkyl/π-alkyl interactions with His, Leu, Ile amino acid residues of Aβ amyloid fibrils, Tyr of Ins fibrils and Leu of fibrillar Lz. In turn, van der Waals protein-sterol contacts are ensured by Asn residues of apoA and Ins fibers, and predominantly by Gly and Leu residues of Aβ(1-42) and Lz. Additionally, π-σ contacts between His residues of Aβ(1-42) and Chol favor the intercalation of sterol into the amyloid fibril.

Table 1. Parameters of molecular docking of amyloid fibrils with lipid molecules

Protein	Lipid	GBE, kcal/mol	ACE, kcal/mol	Amino acid residues involved in complexation
Aβ	Chol	-33.19	-12.28	Gly, Leu, Ile, His
	CL	-44.39	-13.14	Gln, His, Leu, Ile, Gly, Val
	POPC	-63.18	-17.89	Lys, Gln, His
apoA-83	Chol	-7.26	-4.36	Asn, Lys, Gln
	CL	-13.84	-4.25	Gln, Asn, Ala, Lys
	POPC	-29.74	-4.32	Ser, Leu, Gly, Gln, Asn, Lys
Ins	Chol	-15.87	-1.89	Asn, Tyr
	CL	-28.24	-7.78	Asn, Glu, Tyr, Phe, Val
	POPC	-69.25	-10.34	Gln, Phe, Leu, Tyr, Glu, Asn
Lz	Chol	-29.11	-16.83	Gly, Trp, Leu
	CL	-32.12	-17.83	Trp, Gly
	POPC	-42.16	-22.38	Trp, Ser, Leu, Gly

As shown in Fig. 1, B cardiolipin are characterized mostly by superficial location in the complexes with amyloid fibrils which can be explained by the steric restrictions arising from the bulky structure of this phospholipid containing four acyl chains. No specific interactions were revealed for the association of Aβ(1-42) and Ins fibers with CL acyl tails, where amyloid-lipid assemblies were found to be stabilized predominantly by van der Waals and alkyl interactions (Fig. 1, B). Furthermore, hydrogen bonds were formed between His of Aβ(1-42) and CL headgroup. In turn, the binding of fibrillar apoA-83 and Lz to anionic cardiolipin exhibited more complex behavior. Specifically, apart from van der Waals, H-bond and alkyl contacts, Asn residues of apoA-83 are involved in the metal-acceptor and unfavorable positive-positive interactions with CL. The complexation of CL with Lz amyloid fibers is stabilized by a set of van der Waals, attractive charge, and unfavorable positive-positive contacts. Interestingly, Trp residues were found to be responsible for all these types of interactions.

Shown in Fig. 1, C are the docking poses of the fibril complexes with POPC which demonstrate the preferable surface location of POPC with some protrusion of its acyl chains into fibril grooves. Similar to Chol and CL, the association of POPC with all types of amyloid fibers studied here was found to be stabilized by van der Waals and alkyl/π-alkyl interactions. Additionally, conventional and carbon hydrogen bonds were formed between Gln, Gly, Leu, Tyr amino acid residues and fibrillar Aβ(1-42), Ins and Lz. Furthermore, attractive charge and metal-acceptor contacts were revealed for apoA and Lz amyloid fibrils, while the covalent bond between Tyr residue of Ins and phosphate group of POPC was formed in the case of Ins fibers. Interestingly, unfavorable positive-positive interactions were found to contribute also into POPC complexation with fibrillar Lz. In the present context, it is noteworthy that unfavorable interactions may appear due to several limitations of molecular docking protocol: i) unaccounted flexibility of protein backbone, ii) insufficient conformational sampling of the protein because of repeated fibrillar structure, iii) steric restrictions.

Table 2. Structural characteristics of binding pockets for lipids within the amyloid fibrils predicted by DoGSiteScorer

Protein	Lipid	Volume, Å^3	Surface, Å^2	Amino acid residues participating in the binding
Aβ (1-42)	Chol	57.4	181.8	Ala, Asn, Asp, Gly, Ile, Leu, Phe, Ser
	CL	137.3	265.3	Gly, Ala, Leu, Ile, Val, Gln
	POPC	85.5	197.2	Asn, Leu, Ile, Lys, Phe, His
apoA-83	Chol	80.8	304.5	Gly, Ala, Asn, Ile, Lys, Gln
	CL	112.3	567.1	Ala, Gln, Lys, Ser, Asn
	POPC	99.3	434.6	Lys, Leu, Ser, Gly, Asn
Ins	Chol	75.1	115.2	Asn, Cys, Gln, Leu, Tyr, Val
	CL	104.6	347.9	Ile, Glu, Gln, Tyr, Val, Asn, Phe
	POPC	90.4	262.6	Phe, Val, Asn, Cys, Tyr, Glu, Leu
Lz	Chol	26.5	234.4	Ser, Trp, Arg, Gly, Leu, Ile, Asn
	CL	118.6	438.2	Arg, Gly, Ile, Trp, Ser
	POPC	97.2	311.1	Gly, Ile, Leu, Arg, Trp, Ser

Importantly, most of the interacting amino acid residues defined by the Discovery Studio based on the PatchDock analysis, were found to be within the binding pockets predicted by the DoGSiteScorer server. The DoGSiteScorer is a grid-based method that uses a difference of Gaussian filter to detect potential binding pockets based on three-dimensional structure of the protein and ligand molecules. Specifically, the calculations of the amyloid fibril structures via DoGSiteScorer with respect to every lipid allowed us to identify from 2 to 8 binding pockets. However, for every amyloid-lipid complex the binding pocket with the highest score, calculated through the DoGSiteScorer, almost coincided with the binding site, revealed by the molecular docking. The structural properties of the binding pockets are shown in Table 2.

CONCLUSIONS

In summary, the present study provides additional arguments in favor of the possibility of direct interaction of individual lipid molecules with amyloid fibrils. The model fibril-lipid systems whose protein component was represented by the fibrillar lysozyme, insulin, Aβ (1-42) peptide and N-terminal (1-83) fragment, while a lipid component involved cholesterol, cardiolipin or phosphatidylcholine were investigated using the molecular docking tool. The analysis of the results obtained with PatchDock showed that Asn, Gly, Glu, Leu and Trp are the most common amino acid residues in the formation of van der Waals amyloid-lipid contacts for all types of lipids and proteins studied in the present contribution. In turn, a predominant role of Leu, Tyr and Trp among other residues in providing the alkyl/π-alkyl interactions was demonstrated. Further studies in this direction are important for uncovering the determinants of amyloid biological activity and the development of therapeutics for amyloid diseases.

Acknowledgements

This work was supported by the Ministry of Education and Science of Ukraine (the project "Development of novel means of medical diagnostics by biomedical nanotechnologies and modern ultrasonic and fluorescence methods").

ORCID IDs

Valeriya Trusova, https://orcid.org/0000-0002-7087-071X; **Uliana Tarabara,** https://orcid.org/0000-0002-7677-0779
Olga Zhytniakivska, https://orcid.org/0000-0002-2068-5823; **Kateryna Vus,** https://orcid.org/0000-0003-4738-4016
Galyna Gorbenko, https://orcid.org/0000-0002-0954-5053

REFERENCES

[1] M.G. Iadanza, M.P. Jackson, E.W. Hewitt, N.A. Ranson, and S.E. Radford, Nat. Rev. Mol. Cell. Biol. **19**, 755 (2018). https://doi.org/10.1038/s41580-018-0060-8
[2] T. Knowles, M. Vendruscolo, and C. Dobson, Nat. Rev. Mol. Cell. Biol. **15**, 384 (2014). https://doi.org/10.1038/nrm3810
[3] D.C. Bode, M. Freeley, J. Nield, M. Palma, and J.H. Viles, J. Biol. Chem. **294**, 7566 (2019). https://doi.org/10.1074/jbc.AC118.007195
[4] K. Sasahara, K. Morigaki, and K. Shinya, Phys. Chem. Chem. Phys. **15**, 8929 (2013). https://doi.org/10.1039/C3CP44517H
[5] N.P. Reynolds, A. Soragni, M. Rabe, D. Verdes, E. Liverani, S. Handschin, R. Riek, and S. Seeger, J. Am. Chem. Soc. **133**, 19366 (2011). https://doi.org/10.1021/ja2029848
[6] M.F. Engel, L. Khemtémourian, C.C. Kleijer, H.J. Meeldijk, J. Jacobs, A.J. Verkleij, B. de Kruijff, A. Killian, and J.W. Höppener, Proc. Natl. Acad. Sci. U.S.A. **105**, 6033−6038 (2008). https://doi.org/10.1073/pnas.0708354105
[7] E. Sparr, M.F. Engel, D.V. Sakharov, M. Sprong, J. Jacobs, B. de Kruijff, and J.W. Höppener, A. Killian, J. FEBS Lett. **577**, 117 (2004). https://doi.org/10.1016/j.febslet.2004.09.075
[8] N. Arispe, E. Rojas, and H.B. Pollard, Proc. Natl. Acad. Sci. U.S.A. **90**, 567 (1993). https://doi.org/10.1073/pnas.90.2.56
[9] N. Arispe, H.B. Pollard, E. Rojas, Mol. Cell. Biochem. **140**, 119 (1994). https://doi.org/10.1007/BF00926750
[10] N. Arispe, H.B. Pollard, E. Rojas, Proc. Natl. Acad. Sci. U.S.A. **90**, 10573 (1993). https://doi.org/10.1073/pnas.90.22.10573
[11] J.M. Sanderson, J. Biol. Chem. **298**, 102108 (2022). https://doi.org/10.1016/j.jbc.2022.102108
[12] G.P. Gellermann, T.R. Appel, A. Tannert, A. Radestock, P. Hortschansky, V. Schroeckh et al. Proc. Natl Acad. Sci. U.S.A. **102**, 6297 (2005). https://doi.org/10.1073/pnas.0407035102
[13] G.P. Gellermann, T.R. Appel, P. Davies, S. Diekmann, Biol. Chem. **387**, 1267 (2006). https://doi.org/10.1515/BC.2006.157
[14] J. M. Sanderson, Far from inert: membrane lipids possess intrinsic reactivity that has consequences for cell biology. BioEssays, **42**, el900147 (2020). https://doi.org/10.1002/bies.201900147
[15] M. Babych, P.T. Nguyen, M. Côte-Cyr, N. Kihal, N. Quittot, and M. Golizeh et al. Biochemistry, **60**, 2285 (2021). https://doi.org/10.1021/acs.biochem.1c00308
[16] A.E. Saghir, G. Farrugia, N. Vassailo, Chem. Phys. Lipids 234, 105010 (2021). https://doi.org/10.1016/j.chemphyslip.2020.105010
[17] J.R. Brender, U.H. Durr, D. Heyl, M.B. Budarapu, and A. Ramamoorthy, Biochim. Biophys. Acta, **1768**, 2026 (2007). https://doi.org/10.1016/j.bbamem.2007.07.001
[18] M. Grey, S. Linse, H. Nilsson, P. Brundin, E. Sparr, J. Parkinson's Dis. **1**, 359 (2011). https://doi.org/10.3233/JPD-2011-11067
[19] E. Hellstrand, A. Nowacka, D. Topgaard, S. Linse, and E. Sparr, PLoS One **8**, e77235 (2013). https://doi.org/10.1371/journal.pone.0077235
[20] C. Zhang, G. Vasmatzis, J.L. Cornette, and C. De Lisi, J. Mol. Biol. 267, 707 (1997). https://doi.org/10.1006/jmbi.1996.0859
[21] D. Schneidman-Duhovny, Y. Inbar, R. Nussimov, and H. Wolfson, Nucl. Acid Res. **33**, W363 (2005). https://doi.org/10.1093/nar/gki481

ВЗАЄМОДІЯ ФІБРИЛЯРНИХ БІЛКІВ З ЛІПІДАМИ: ДОСЛІДЖЕННЯ МЕТОДОМ МОЛЕКУЛЯРНОГО ДОКІНГУ

Валерія Трусова, Уляна Тарабара, Ольга Житняківська, Катерина Вус, Галина Горбенко

Кафедра медичної фізики та біомедичних нанотехнологій, Харківський національний університет імені В.Н. Каразіна м. Свободи 4, Харків, 61022, Україна

Агрегація неправильно згорнутих білків з утворенням специфічних впорядкованих агрегатів, амілоїд них фібрил, пов'язаних з більш ніж 40 захворювань людини, наразі привертає велику увагу дослідників у біомедичному та нанотехнологічному аспектах. Згідно з сучасними уявленнями, ці агрегати та їх олігомерні інтермедіати здійснюють їх токсичний вплив переважно на рівні клітинних мембран. Окрім цього, мембранні ліпіди були виявлені в багатьох амілоїдних депозитах in vivo, що є свідченням здатності ліпідних молекул вбудовуватись в структуру фібрил та впливати на їх морфологію і механічні властивості. Однак, біологічна роль та структурні передумови фібрил-ліпідних взаємодій залишаються нез'ясованими. У даній роботі методом молекулярного докінгу було проведено дослідження взаємодії між амілоїдними фібрилами та ліпідами в модельних системах, що містили фібрилярні форми лізоциму, інсуліну, Aβ (1-42) пептиду та N-термінального (1-83) фрагменту аполіпопротеїну А-І у якості білкового компоненту, та холестерин, кардіоліпін і фосфатидилхолін у якості ліпідного компоненту. З використанням web-серверу PatchDock та програмного пакету BIOVIA Discovery Studio були охарактеризовані структурні особливості фібрил-ліпідних асоціатів. Показано, що ван-дер-ваальсові та алкіл/π-алкіл взаємодії домінують у стабілізації всіх типів фібрил-ліпідних комплексів. Аналіз найбільш енергетично вигідних структур, отриманих методом докінгу, свідчить про переважно поверхневу локалізацію ліпідів та часткове проникнення ацильних ланцюгів кардіоліпіну та фосфатидилхоліну у фібрилярні груви.

Ключові слова: *амілоїдні фібрили; лізоцим; інсулін; Aβ (1-42) пептид; аполіпопротеїн А-І; комплекс фібрила-ліпід; молекулярний докінг*