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MULTIPLE DOCKING OF FLUORESCENT DYES TO FIBRILLAR INSULIN[†]

Uliana Tarabara*, Olga Zhytniakivska, Kateryna Vus, Valeriya Trusova, Galyna Gorbenko
Department of Medical Physics and Biomedical Nanotechnologies, V.N. Karazin Kharkiv National University
4 Svobody Sq., Kharkiv, 61022, Ukraine
** Corresponding Author: uliana.tarabara@karazin.ua*
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The co-localization of the donor and acceptor fluorophores capable of transferring the energy by the Förster mechanism, on the molecular scaffold of amyloid fibrils offers new opportunities not only for refinement of the amyloid detection and structural analysis, but also for designing photonic nanodevices on their basis. The assembly of these systems involves the non-covalent dye-protein interactions which can hardly be characterized in terms of a precise dye location within the fibril structure that is required for fabricating the FRET-based light harvesting systems or photonic nanowires. In view of this, the dye-fibril binding process deserves a detailed *in silico* study. In the previous molecular docking studies of the FRET donors and acceptors interacting with the insulin model fibrils we considered only one ligand during the simulation procedure. However, the real situation is much more complicated, when the multiple ligands can compete for the same binding site, a direct complexation between the dyes on the fibril scaffold can take place, the spatial distribution of the bound fluorophores can be unfavorable for the energy transfer, etc. In addition, the mutual orientation of the donor and acceptor molecules essentially contribute to the efficiency of the Förster resonance energy transfer (FRET) in the investigated systems. The present study was undertaken to gain molecular docking insight into the binding of the donor (Thioflavin T) and acceptor (Congo Red or a phosphonium dye TDV) fluorophores to the insulin amyloid fibrils using the multiple docking approach. The employed PatcDock and SwissDock webserves provided evidence for the preferable association of all dyes with the fibril grooves. The protein-ligand interaction profiler (PLIP) was employed for analyzing the (InsF + ThT +CR) and (InsF + ThT + TDV) systems. The revealed binding modes and the types of the dye-fibril interactions may be of importance for a more detailed analysis of the FRET process in amyloid systems and may serve as a background for further *in silico* studies of the cascade FRET on the amyloid fibril scaffold.
Keywords: fibrillar insulin, Thioflavin T, Congo Red, phosphonium dye, molecular docking study
PACS: 87.14.C++c, 87.16.Dg

Amyloid fibrils, a special class of the β-structured protein aggregates, are now receiving considerable attention in view of their link with numerous human disorders [1] and a promising potential as a novel type of nanomaterials [2]. A peculiar architecture of amyloid assemblies in which the solvent-exposed grooves lined with amino acid side chains extend along the fibril axis, accounts for specific binding of amyloid-sensitive dyes, among which the most widely used are the benzothiazole dye Thioflavin T (ThT) and the azo dye Congo red (CR) [3-5]. In our previous study we demonstrated that ThT and CR can be employed jointly as a donor-acceptor pair in the fluorescence amyloid assay based on the Förster resonance energy transfer (FRET) [6]. Later on, we found that amyloid fibrils can serve as a molecular scaffold not only for one-step energy transfer, but also for multi-step FRET (msFRET) in the three- and four- chromophore systems [7-10]. In the examined FRET chains ThT was recruited as an input donor, squaraine dyes SQ1/SQ2/SQ3 as terminal acceptors, while dimethylaminochalcone [8], phosphonium [7, 10], benzanthrone dyes [9] and squaraine (SQ4) dyes play the role of relay fluorophores. These studies open the way not only for improvement of the amyloid detection assays, but also for the use of amyloid assemblies in nanophotonics. The revealed significant enhancement of FRET in the amyloid protein fold was interpreted in terms of the increased quantum yields and greater donor-acceptor spectral overlap in the fibril-bound state, particular spatial and orientational arrangement of the fluorophores constituting the energy transfer chain. In view of a high amyloid sensitivity of the FRET process, it seems reasonable to take a deeper look on the determinants of fibril-fluorophore interactions using the *in silico* tools. Among these, one of the most widespread is the molecular docking modulation technique capable of predicting the best way of the ligand-receptor interactions and affinity of small molecules to the target macromolecule [11]. During the simulation procedure the orientation and conformation of the ligand are changed to reach the global minimum of the ligand-macromolecule binding energy [12]. The molecular docking can be implemented by two ways: i) geometric search for the receptor surface regions complementary to ligand) [13], ii) docking simulation through evaluating the minimum in energy landscape by considering each binding mode) [14, 15]. A more sophisticated docking technique, multiple ligand simultaneous docking (MLSD), allows to perform the docking of several ligands to the same target, that is more realistic since water molecules and a variety of cofactors, substrates, ions can bind to biomacromolecules along with the main ligand [16, 17]. The optimized MLSD algorithm has been developed [16] that provides a sensitivity of ligands to the presence of each other. Since the two or more dyes are involved in the energy transfer, it is sensible to analyze the binding behavior of the donor and acceptor fluorophores using the multiple docking approach. In view of this, the aim of the present study was to

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evaluate the possibility of simultaneous docking of the classic amyloid markers (ThT and CR) and one donor-acceptor pair from the previously examined msFRET chains (ThT – TDV) to the insulin amyloid fibrils.

METHODS

Figure 1. Structural formulas of the examined dyes

The structures of three dyes under study (Fig. 1) were built in MarvinSketch (version 18.10.0) [18] and optimized in Avogadro (version 1.1.0) [19]. The counterions were not added to the dye structures in order to save the molecular charges. The model of the human insulin fibril (InsF) was obtained from http://people.mbi.ucla.edu/sawaya/jmol/fibrilmodels/. The input structure of the ThT complexes with fibrillar insulin for the next docking run (Fig. 2) was performed using the SwissDock server [20]. The free energy of the ThT-InsF binding (ΔG) was estimated to be -8.36 kcal/mol. To perform the docking of CR or TDV to the complex (InsF+ThT) we tried to use the two different webservers: PatchDock and SwissDock. The molecular docking algorithm underlying the PatchDock server target on the finding the maximal surface shape complementarity along with the minimal amounts of steric clashes [21]. The EADock DSS algorithm behind the SwissDock server computes and clustered the most favorable binding modes using the rigid docking procedure [20]. In order to identify the amino acid residues constituting the binding sites and to analyze the types of the dye-protein contacts, the protein-ligand interaction profiler (PLIP, https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index) was employed. The selected docking poses were visualized with the UCSF Chimera software (version 1.14) [22].

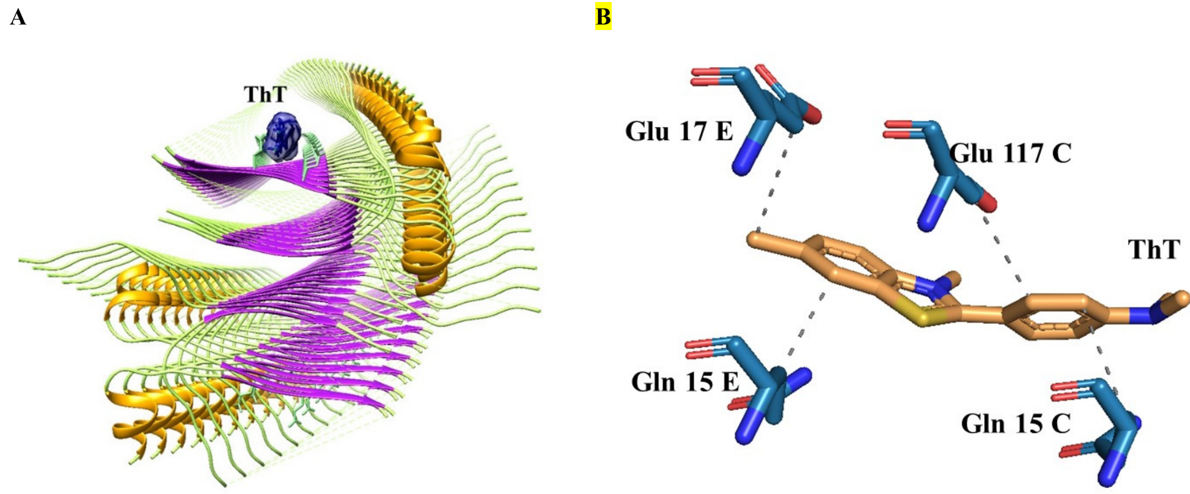

Figure 2. The (InsF + ThT) complex that was selected as input structure for the next docking run, visualized by the UCSF Chimera software (A) and analyzed in PLIP (B). The β-sheets and α-helixes are colored in violet and goldenrod, respectively, while ThT and the residues Gln15, Glu17 are marked by navy blue and light green, respectively (A). The gray dashed lines on the panel B represent the hydrophobic interactions between the dye molecule and the insulin residues.

RESULTS AND DISCUSSION

The PLIP analysis of the ThT complex with the groove Gln 15 – Glu 17 of the insulin fibrils revealed a predominant role of hydrophobic interactions between the dye and the residues (Gln 15 C, Gln 15 E, Glu 17E, Glu 117 C) (Fig. 2 B), that is in accord with the experimental studies of the ThT location within fibril structure [23, 24]. All the PatchDock solutions provided evidence for the CR binding in the cross-strand ladder formed by B 17 leucine residues located on the dry steric zipper fibril interface (Fig. 3 A). The obtained results are similar to those previously reported for the complexes of cyanines [25, 26], DMC and ThT [8, 26] with the fibrillar insulin. The global energy for the most energetically favorable model was -132.95 kcal/mol, revealing both a very high stability of this complex *in silico* and a high probability of such mode of the dye-fibril association *in vitro*. The hydrophobic interactions between CR and the nonpolar residues (Ile 102 of S, U, W chains and Leu 306 of U, W, Y chains) of the fibrillar insulin, along with the hydrogen bonds between sulfonate and amino groups of the CR and both nonpolar (Val 218 X) and polar (Cys 219 T, Cys 307 W) residues were also uncovered by the PLIP analysis (Fig. 3 B).

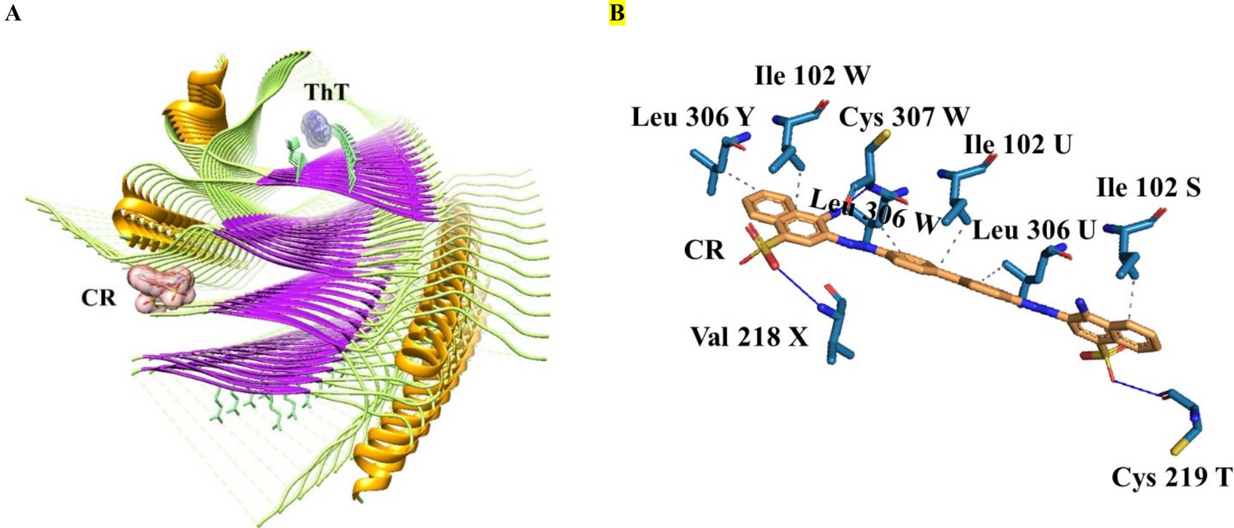

Figure 3. The energetically most favorable CR associate with the complex (InsF + ThT), obtained using the PatchDock webserver (A) and analyzed in PLIP (B). The CR and ThT are marked by red and navy blue, respectively. The gray dashed lines on the panel B represent the hydrophobic interactions between the dye molecule and the insulin residues, while the blue solid line displays the hydrogen bonds.

In contrast to the PatchDock results, the most CR docking poses revealed by the SwissDock are located near the groove Gln 15 – Glu 17 on the wet fibrillar surface and close to the residue Gln 204 belonging to the native-like N-terminus of the insulin B chain. One binging mode of this dye was in Q15 – E17 groove demonstrating the disposition of the CR along the long fibril axis, parallel to the selected ThT binding pose (Fig. 4 A, red). This complex with $\Delta G = -8.59$ kcal/mol is stabilized mainly by the hydrophobic contacts with the groove residues of the D, F, H chains and hydrogen bonds between functional groups of the dye and Leu 16, Gln 204, in addition to Gln 15 and Glu 17 (Fig. 4 B). The binding mode with the minimal free energy of binding ($\Delta G = -9.36$ kcal/mol) involves a partial intercalation of Congo Red in the groove by the naphthalene ring (Fig. 4 A, yellow). Therefore, the possibility of almost perpendicular dye orientation relative to ThT cannot be excluded. A significant amount of hydrogen bonds (Gln 15 L, Glu 117 J, Phe 201 H, Gln 204 J) were found to participate in the formation of this complex (Fig. 4 C). The distinguishable and non-specific binding site located at the native-like N-terminus of the insulin B chain is represented in Figs. 4 A (green CR structure) and 4 D. According to this model, Congo Red molecules are buried between Gln 304 and Leu 306 interacting with them and the neighboring His 305 by both hydrophobic and electrostatic (hydrogen bonds and salt bridges) forces. This binding mode can be explained by the rigid structure of receptor during the docking procedure, while the great flexibility of these non-extended N-terminal tails of the chain B (in comparison to the native insulin) can be expected *in vitro*, that with high probability may lead to the disruption of the cavity Gln 304 - Leu 306 [27].

It should be noted that the ThT molecule seems to be removed in the SwissDock output files, indicating that this server is not suitable for the multiple ligand simultaneous docking. Therefore, a more appropriate technique like AutoDock 4.0 [28, 16] is planned to be used in the further studies for a more precise analysis of the simultaneous docking results and, ideally, description of the ligand-ligand interactions. Nevertheless, the differences in the algorithms, technical realization, numerous docking runs provide a good opportunity for considering the dye-protein interactions more comprehensively.

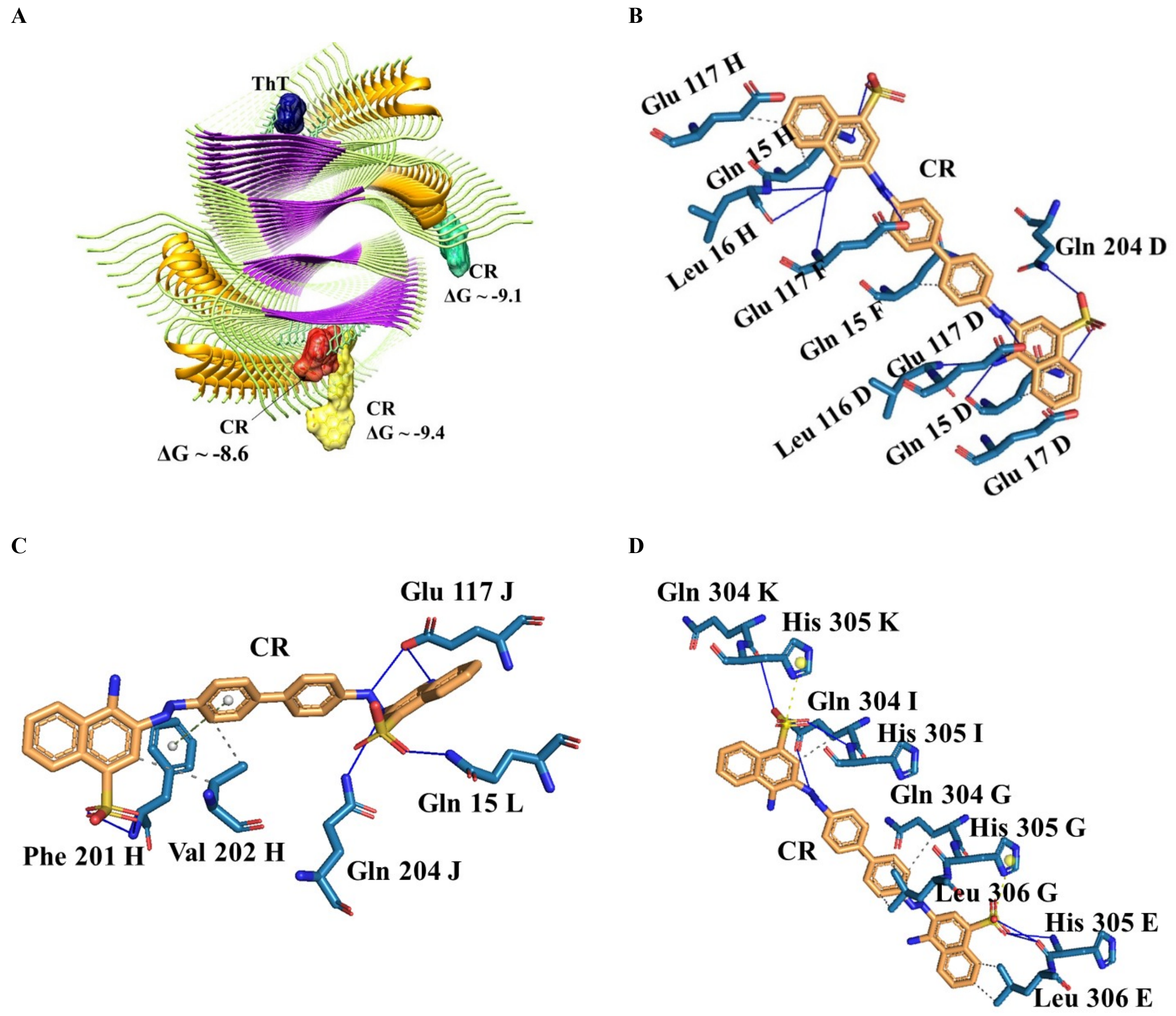

Figure 4. Representative modes of interactions between CR and the complex (InsF + ThT), obtained using the SwissDock server (A) and analyzed in PLIP (B -D). ThT is marked by navy blue (A). The docking energies for selected complexes are -8.59 kcal/mol (CR is marked by red on the panel A, PLIP results are depicted on the panel B), -9.36 kcal/mol (yellow, C), -9.07 kcal/mol (green, D). The gray dashed lines on the panels B, C and D represent the hydrophobic interactions between the dye molecule and the insulin residues, while the green dashed line, yellow dashed line and blue solid line display the π-stacking contacts, salt bridges and hydrogen bonds, respectively.

At the next step of study, the interaction of the phosphonium dye TDV with the complex (InsF + ThT) was explored using the above approaches. According to the PatchDock, this dye can be located in the grooves Gln 15 – Glu 17 (Fig. 5 A red) and Tyr19 – Asn21 (Fig. 5 A green), as well as can reside in the area between the native-like N-terminal tails of the chain B and outer β-sheet formed by the LYQLENY segment from the A chain. The binding mode with higher value of the global energy (-37.19 kcal/mol) is depicted in Figure 5 A (violet). This complex is stabilized by a large number of hydrophobic contacts between TDV and nonpolar (Ile 2 D, Leu 13 D, Leu 13 F, Val 202 D, Leu 206 D), polar (Gln 204 B, Gln 204 B, Gln 204 D), aromatic (Phe 201 B, Phe 201 D) and positively charged (His 205 B) residues (Fig. 5 D). The π-stacking contact (His 205 B) was also observed, while the other selected docking poses (Fig. 5 B, C) are stabilized mainly by hydrophobic interactions with the groove residues.

Most binding modes obtained by the SwissDock suggest that TDV is localized on the wet surface of fibril structure (data not shown). The more energetically favorable complexes ($\Delta G \leq -8.4$ kcal/mol) revealed the TDV orientation in which one benzene ring of phosphonium group is directed to the groove Q15 – E17, while the rest of the dye molecule is intercalated between N-terminal parts of the chains constituting the fibril periphery. The PLIP analysis indicates that the complex (InsF + TDV) is stabilized by hydrophobic contacts, hydrogen bonds and π-stacking interactions preferably with the residues of the non-extended fibril part such as Gly 1, Ile 2, Val 3, Gln 5, Phe 201, Val 202, Gln 204, His 205) than Gln 15 and Glu 17.

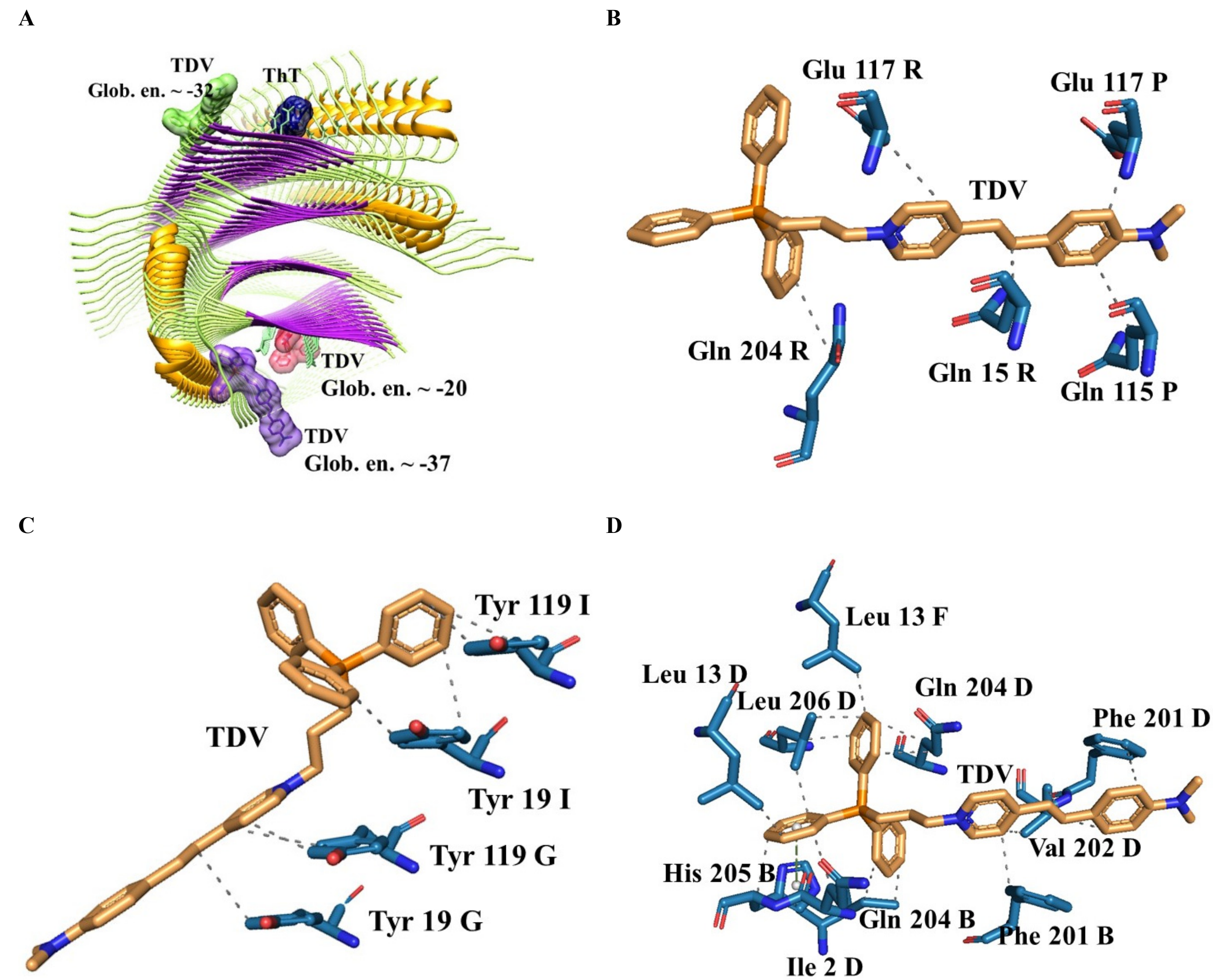

Figure 5. Representative modes of interactions between TDV and the complex (InsF + ThT), obtained using the PatchDock webserver (A) and analyzed in PLIP (B -D). ThT is marked by navy blue (A). The global energies for selected complexes are -20.4 kcal/mol (TDV is marked by red on the panel A, PLIP results are depicted on the panel B), -31.99 kcal/mol (green, C), -37.19 kcal/mol (violet, D). The gray dashed lines on the panels B, C and D represent the hydrophobic interactions between the dye molecule and the insulin residues, while yellow dashed line displays the salt bridges.

CONCLUSIONS

To summarize, the present study was focused on the investigation of the interactions between the FRET acceptor dyes CR or TDV and the insulin amyloid fibrils hosting the donor dye ThT using the two webservers: PatchDock and SwissDock. The binding sites of the (InsF+ThT) complex for the examined dyes, as well as the types of the dye-protein interactions were identified. Based on the acquired results, the following assumptions can be made: i) regardless of the cationic nature of ThT and TDV molecules, and the negative charge of CR, the dye-protein complexes are stabilized by hydrophobic, rather than electrostatic interactions; ii) the dye location along the surface side-chain grooves Q15 - E17 (CR, TDV), Y19 – N21 (TDV) is in line with the previously proposed binding mode and provides almost parallel orientation of the fluorophore transition dipoles; iii) the existence the binding site such as native-like N-terminus and cross-strand ladder on the dry steric zipper interface that is characterized by the lower docking energy than the fibril grooves does not rule out the other orientations of the donor and acceptor transition dipoles, as well as point to the existence of different binding sites for ThT and the FRET acceptors such as CR and TDV, reducing the probability of the competition between the dyes. Overall, our findings may be useful for a deeper understanding of the factors controlling the binding and orientational behavior of the donor and acceptor fluorophores involved in the energy transfer on the amyloid fibril scaffold.

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ORCID IDs
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; **Valeriya Trusova,** https://orcid.org/0000-0002-7087-071X
Galyna Gorbenko, https://orcid.org/0000-0002-0954-5053

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МУЛЬТИМОЛЕКУЛЯРНИЙ ДОКІНГ ФЛУОРЕСЦЕНТНИХ БАРВНИКІВ З ФІБРИЛЯРНИМ ІНСУЛІНОМ

У. Тарабара, О. Житняківська, К. Вус, В. Трусова, Г. Горбенко
Кафедра медичної фізики та біомедичних нанотехнологій, Харківський національний університет імені В.Н. Каразіна
м. Свободи 4, Харків, 61022, Україна

Колокалізація донорів та акцепторів флуорофорів, здатних переносити енергію за механізмом Фьорстера, на молекулярній матриці амілоїдних фібрил, відкриває нові можливості не тільки для вдосконалення детекції амілоїдних фібрил та структурного аналізу, але і для розробки фотонних нанопристроїв на їх основі. Збірка цих систем передбачає нековалентні взаємодії барвник-білок, які складно охарактеризувати з точки зору точного розташування барвника в структурі фібрили, необхідного для виготовлення світлозбиральних систем чи фотонних нанодротів на основі Фьорстерівського резонансного переносу енергії (ФРПЕ). З огляду на це, процес зв'язування барвників з фібрилами доцільно детально проаналізувати *in silico*. У попередніх дослідженнях донорів та акцепторів ФРПЕ, що взаємодіяли з модельними фібрилами інсуліну, методом молекулярного докінгу, під час процедури моделювання ми розглядали лише один ліганд. Однак реальна ситуація набагато складніша, оскільки кілька лігандів можуть конкурувати за один і той же сайт зв'язування, може відбуватись пряме комплексоутворення між барвниками на матриці фібрил, просторовий розподіл зв'язаних флуорофорів може бути несприятливим для передачі енергії тощо. Крім того, взаємна орієнтація молекул донора та акцептора вносить суттєвий вклад в ефективність Фьорстерівського резонансного переносу енергії у досліджуваних системах. Дана робота була проведена, щоб отримати уявлення про зв'язування донорних (Тіофлавін Т) і акцепторних (Конго Червоний або фосфонієвий барвник TDV) флуорофорів з амілоїдними фібрилами інсуліну за допомогою підходу мультимолекулярного докінгу. Використані веб-сервери PatcDock і SwissDock надали докази переважної асоціації всіх барвників із жолобками фібрил. Для аналізу систем (InsF + ThT +CR) і (InsF + ThT + TDV) використовувався protein-ligand interaction profiler (PLIP). Виявлені сайти зв'язування та типи взаємодій між барвниками та фібрилою можуть бути важливими для більш детального аналізу процесу ФРПЕ в амілоїдних системах і можуть слугувати основою для подальших досліджень *in silico* каскаду ФРПЕ на матриці амілоїдних фібрил.

Ключові слова: фібрилярний інсулін, Тіофлавін Т, Конго Червоний, фосфонієвий барвник, молекулярний докінг