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MULTIPLE LIGAND SIMULTANEOUS DOCKING OF ANTIVIRAL DRUGS AND CYANINE DYES WITH PROTEINS[†]

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Protein nanoparticles are currently regarded as promising biocompatible and biodegradable systems for targeted delivery of different types of pharmacological agents. Prior to fabricating such kind of drug nanocarriers it is reasonable to evaluate the drug-protein binding affinity and possible interaction modes using the computational tools, particularly, the molecular docking technique. The present study was undertaken to evaluate the possibility of creating the protein nanoparticles carrying the antiviral drugs and cyanine dyes as visualizing agents. The components of the examined systems included endogenous functional proteins cytochrome c, serum albumin, lysozyme and insulin, antiviral drugs favipiravir, molnupiravir, nirmatrelvir and ritonavir, mono- and heptamethinecyanine dyes. Using the multiple ligand simultaneous docking technique, it was demonstrated that: i) the drugs and the dyes occupy different binding sites on the protein molecule and do not interfere with each other; ii) the heptamethines AK7-5 and AK7-6 possess the highest affinity for the proteins; iii) among the examined systems the strongest complexes are formed between the heptamethine dyes and serum albumin. Taken together, the results obtained indicate that albumin-based nanoparticles functionalized by the heptamethine cyanine dyes can be used for targeted delivery of the explored antiviral agents.
Keywords: *Antiviral agents; Protein nanoparticles; Drug nanocarriers; Cyanine dyes; Molecular docking*
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During the last decades the nanostructured drug delivery systems (DDS) such as liposomes, polymeric, lipidic, protein and inorganic nanoparticles, nanotubes, fullerenes, dendrimers and many others, have become a subject of considerable research interest in biomedical field [1-3]. Particular attention is devoted to protein nanoparticles which have numerous advantages among which are: i) biocompatibility and biodegradability; ii) a weak or negligible immune response; iii) controlled size and stability; iv) a possibility of drug protection from enzymatic degradation and renal clearance; v) mild preparation and drug encapsulation conditions; vi) an opportunity for surface modification and functionalization with tissue-specific ligands; vii) protein abundance in natural sources; viii) easy synthesis process and cost-effectiveness [4-6]. The use of proteins makes it possible to deliver the drugs with low aqueous solubility. The first endogenous protein utilized for preparing the nanoparticles was the blood serum albumin. A remarkable ability of human serum albumin to reversibly bind hydrophobic molecules provided a basis for obtaining the albumin-bound formulation of the antitumor low soluble drug paclitaxel (Abraxane) [7]. Due to their amphiphilic nature, proteins can interact with both hydrophilic and hydrophobic drugs. Along with albumin, the nanoparticles were fabricated from a variety of animal (gelatin, collagen, casein, silk fibroin) and plant (zein, gliadin, lectin, soy protein) [4,8]. Protein nanoparticles are currently used in cancer therapy, lung treatment, vaccine production, etc [9]. To create the protein-based drug delivery system it is necessary to ascertain whether the protein can simultaneously interact with a drug and functionalizing molecules, such as targeting, stabilizing or visualizing agents. The cyanine dyes fluorescing in the near-infrared region, in the optical windows of biological samples are among the most promising visualizing agents for DDS. In view of this, it seems of interest to evaluate the possibility of concurrent binding of antiviral drugs and cyanine fluorescent dyes. A series of the examined endogenous proteins included cytochrome c (Ct), serum albumin (SA), lysozyme (Lz), insulin (Ins). These proteins play important roles in the electron transport (Ct), blood circulation (SA), innate immune (Lz) and endocrine (Ins) systems. The antiviral drugs were represented by favipiravir, molnupiravir, nirmatrelvir and ritonavir. The aim of in the present study was to characterize the ternary complexes protein-drug-dye using the technique of multiple ligand simultaneous docking (MLSD).

MATERIALS AND METHODS

Molecular docking studies

The set of the investigated pharmaceuticals included favipiravir, molnupiravir, nirmatrelvir and ritonavir [10]. The examined drug structures as well as monomethine and heptamethine cyanine dyes (Figure 1) were built in MarvinSketch (version 18.10.0) and optimized in Avogadro (version 1.1.0) using the Universal Force Field with the steepest descent algorithm [11,12]. The counterions were not added to the dye structures in order to save the molecular charges. The three-dimensional X-ray crystal structures of the examined proteins were obtained from the Protein Data Bank using the PDB IDs 1REX, 3I40, 3ZCF, 6M4R for lysozyme, insulin, cytochrome c and serum albumin, respectively. The proteins were

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taken in the native monomeric form. The SA structural model was prepared using the DockPrep module of UCSF Chimera molecular software by removing the water molecules and addition of polar hydrogen atoms and Kollman charges [13]. The blind docking of the drugs with the proteins was performed using the PatchDock server (http://bioinfo3d.cs.tau.ac.il/PatchDock/php.php) in which the molecular docking algorithm is focused on the finding of maximal surface shape complementarity along with the minimal amounts of steric clashes [14]. Subsequently, the top-scored docked drug-protein complexes were considered as a receptor for docking of the second ligand, one representative of the monomethine or heptamethine cyanine dyes, using the PatchDock server. The control dye-protein systems were also considered. In order to identify the amino acid residues constituting the binding sites and to analyse the types of the drug-protein and the dye-protein contacts, the protein-ligand interaction profiler (PLIP, https://plip-tool.biotec.tudresden.de/plip-web/plip/index) was employed [15]. The selected docking poses were visualized with the UCSF Chimera software (version 1.14) in which the docking models with the best geometric shape complementary were combined in the same picture to achieve the best visibility of the binding sites [16].

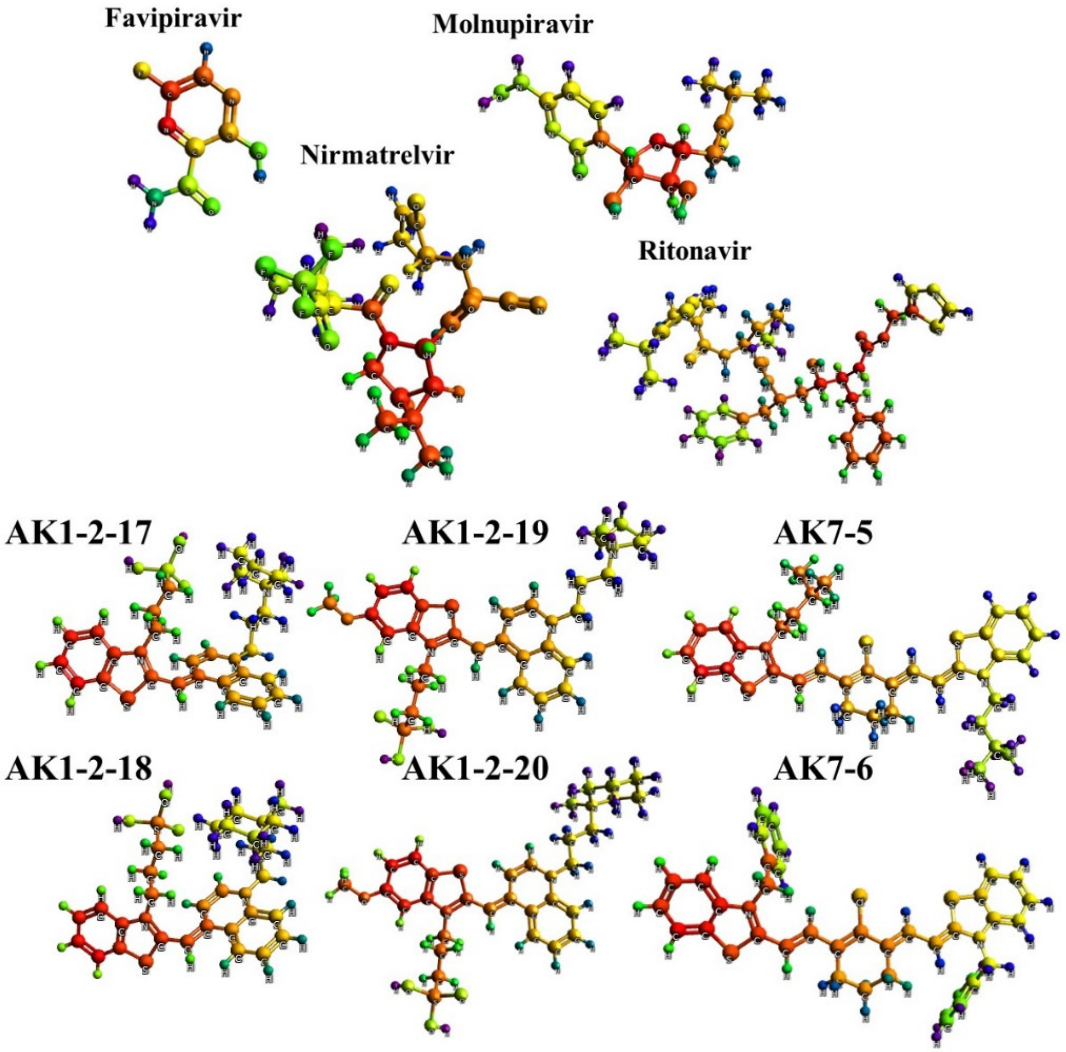

Figure 1. Structural formulas of the examined antiviral drugs and cyanine dyes

RESULTS AND DISCUSSION

Shown in Figs 2-5 are the highest-score ternary complexes protein-drug-dye recovered for cytochrome c (Fig.2), albumin (Fig. 3), lysozyme (Fig. 4) and insulin (Fig. 5). It can be seen that in the cases of Ct, Lz and Ins the binding sites for the investigated mono- and heptamethines are located rather close to each other in the vicinity of the protein surface. Meanwhile, the drugs penetrate deeper into the interior of Ct, Lz and Ins, occupying the sites different from that of the cyanine dyes. In the case of albumin, the antiviral agents and the dyes reside in the distinct protein pockets.

The analysis of the geometric shape complementarity scores and approximate interface areas derived for the cytochrome *c*-drug-dye complexes (Table 1) revealed that: i) the drugs do not exert influence only on the dye-protein affinity and interface area, only in the cases of AK-1-2-20 for all drugs and AK7-6 for molnupiravir these parameters

slightly decrease; ii) the dye-protein affinity follows the order: AK7-5 > AK7-6 > AK-1-2-20 > AK-1-2-19 > AK-1-2-17 > AK-1-2-18; iii) the interface area decreases in the order: AK7-6 > AK7-5 > AK-1-2-19 > AK-1-2-20 > AK-1-2-17 > AK-1-2-18 for all drugs except molnupiravir for which AK7-5 has higher area than AK7-6.

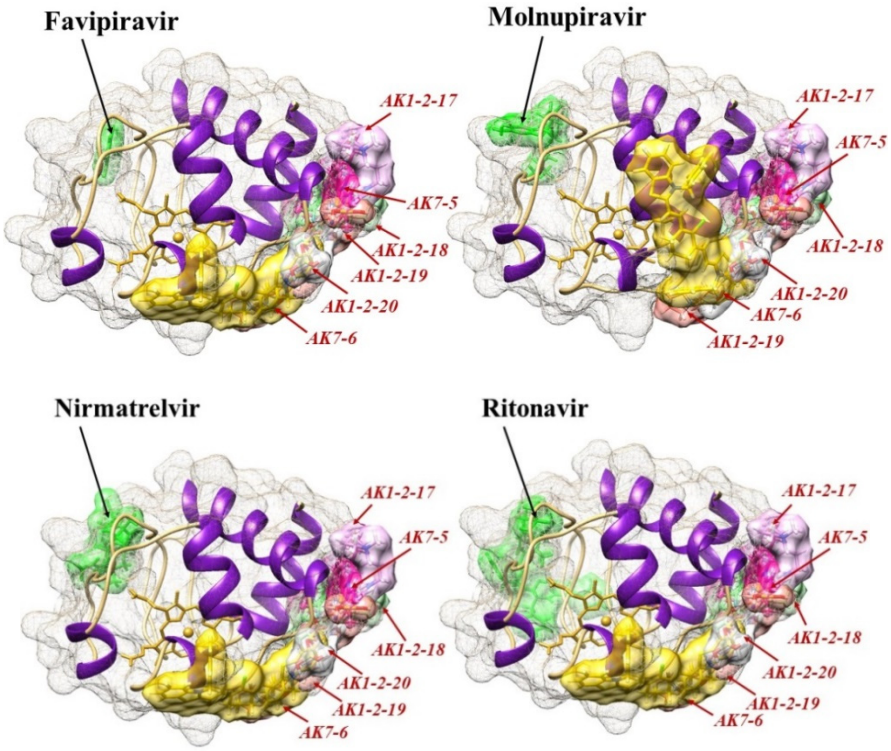

Figure 2. The highest-score docking positions obtained for cytochrome c using the MLSD in PatchDock.

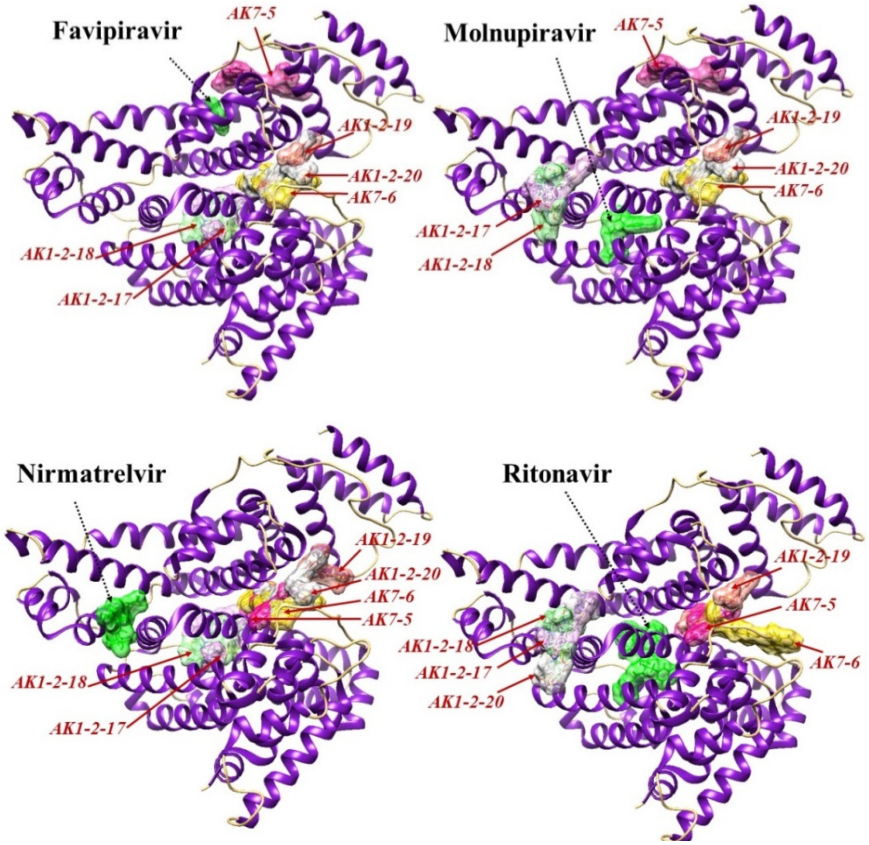

Figure 3. The highest-score docking positions obtained for albumin using the MLSD in PatchDock.

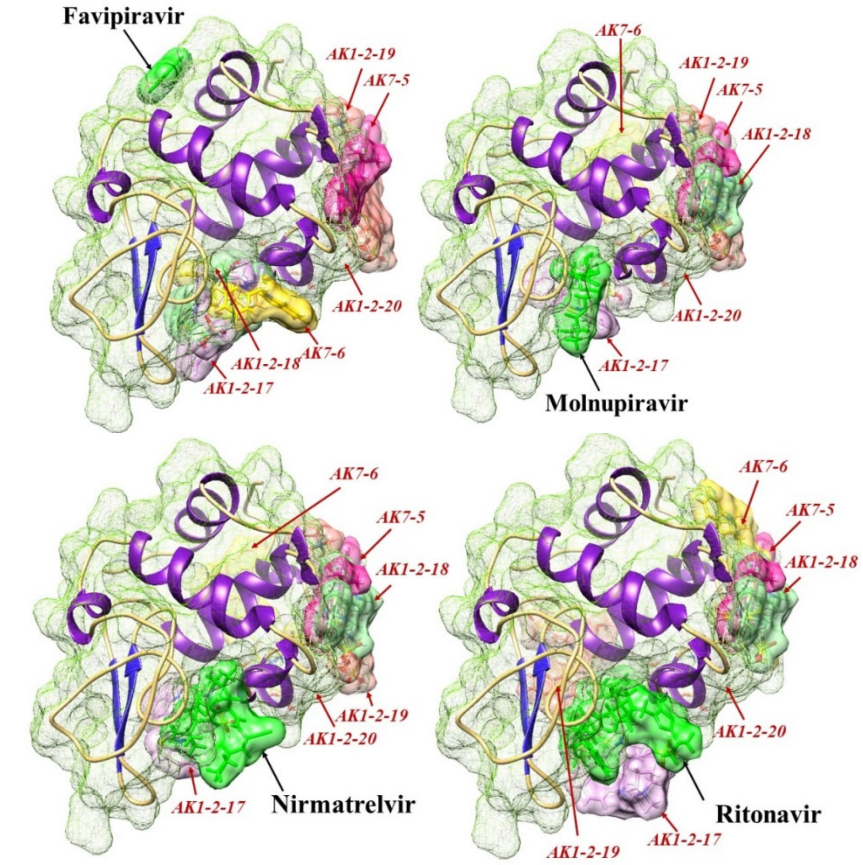

Figure 4. The highest-score docking positions obtained for lysozyme using the MLSD in PatchDock.

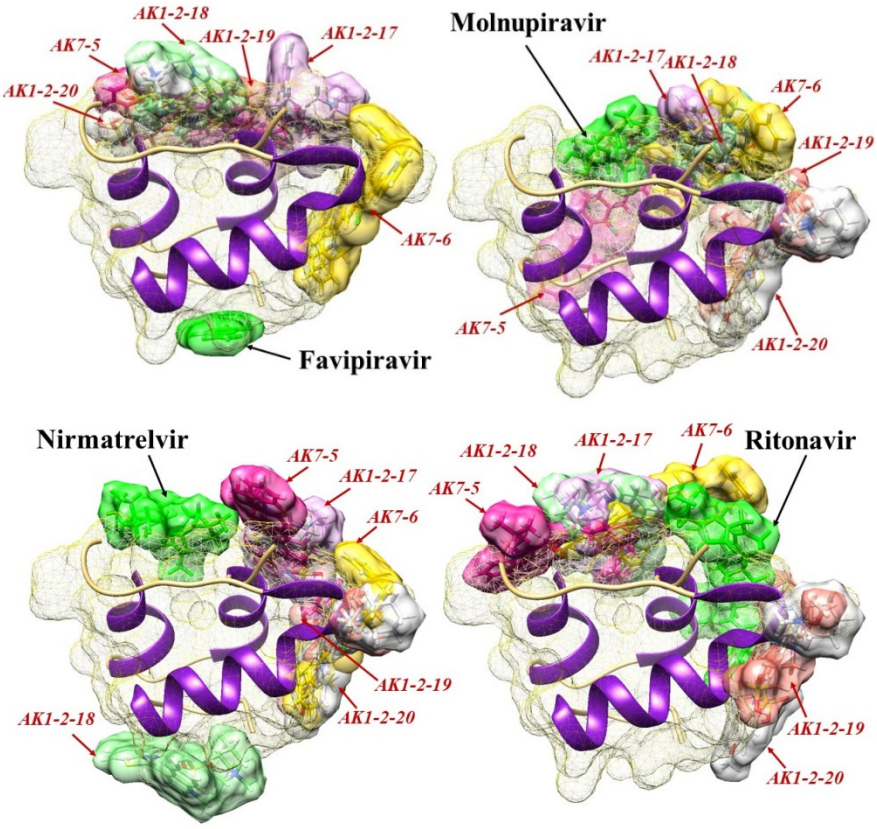

Figure 5. The highest-score docking positions obtained for insulin using the MLSD in PatchDock.

Table 1. The geometric shape complementarity score and approximate interface area of the complex derived for the cytochrome *c*-drug-dye systems (F- Favipiravir, M – Molnupiravir, N - Nirmatrelvir, R – Ritonavir).

Cytochrome *c*										
Dye	**Score**					**Approximate interface area of the complex, A^2**				
	F	**M**	**N**	**R**	**-**	**F**	**M**	**N**	**R**	**-**
-	2220	3648	4292	6062		237.40	384.30	576.30	797.80	
AK-1-2-17	4642	4642	4642	4642	4642	608.50	608.50	608.50	608.50	608.50
AK-1-2-18	4572	4544	4572	4572	4572	580.00	544.00	580.00	580.00	580.00
AK-1-2-19	5414	5414	5414	5414	5414	744.10	744.10	744.10	744.10	744.10
AK-1-2-20	5476	5476	5476	5476	5582	716.20	716.20	716.20	716.20	757.70
AK7-5	6174	6174	6174	6174	6174	819.80	819.80	819.80	819.80	819.80
AK7-6	5842	5716	5842	5842	5842	846.40	732.90	846.40	846.40	846.40

The data acquired for the albumin-drug-dye complexes (Table 2) show a greater variability. The dye-protein affinity remains the same for the systems AK-1-2-17 + F/N, AK-1-2-18 + F/N, AK-1-2-19 + N, AK-1-2-20 + F/M/N. AK7-5 + N, AK7-6 + F/M/N; decreases in the systems AK-1-2-18 + M/R, AK-1-2-20 + R, AK7-5 + F/M/R, AK7-6 + R; and increases in the systems AK-1-2-19 + F/M/R. The highest albumin-dye affinity was observed for AK7-6 (F, M, N) and AK7-5 (N, R).

Table 2. The geometric shape complementarity score and approximate interface area of the complex derived for the albumin-drug-dye systems (F- Favipiravir, M – Molnupiravir, N - Nirmatrelvir, R – Ritonavir).

Serum albumin										
Dye	**Score**					**Approximate interface area of the complex, A^2**				
	F	**M**	**N**	**R**	**-**	**F**	**M**	**N**	**R**	**-**
-	2900	5054	6212	8412		343.60	553.10	719.50	1036.60	
AK-1-2-17	6444	6052	6444	6058	6444	747.70	756.70	747.70	754.60	747.70
AK-1-2-18	6522	6420	6522	6420	6522	740.90	758.00	740.90	758.00	740.90
AK-1-2-19	6936	6936	6790	6932	6790	886.60	886.60	867.40	885.60	867.40
AK-1-2-20	6924	6924	6924	6804	6924	935.80	935.80	930.40	766.70	930.40
AK7-5	6942	6940	7204	7016	7204	768.80	768.20	950.10	920.10	950.10
AK7-6	7022	7022	7022	6954	7022	891.80	891.80	891.80	916.90	891.80

In the lysozyme-drug-dye systems (Table 3) the dye-protein affinity was found to follow the order: AK7-5 > AK-1-2-20 > AK7-6 > AK-1-2-19 > AK-1-2-18 > AK-1-2-17, while in the case of insulin (Table 4) the highest affinity for the protein was observed for AK-1-2-19 (F), AK7-6 (M), AK7-5 (N, R). The PLIP analysis of the driving forces of the cyanine-protein binding revealed essential role of hydrophobic interactions for all dye-protein complexes under study. The other types of interactions involve hydrogen bonds, salt and water bridges, as well as more rarely occurring π-stacking and **π-**cation interactions**.** Notably, the strongest complexes formed by heptamethines with Cyt, SA, Lz and Ins are stabilized exclusively by hydrophobic interactions.

Table 3. The geometric shape complementarity score and approximate interface area of the complex derived for the lysozyme-drug-dye systems (F- Favipiravir, M – Molnupiravir, N - Nirmatrelvir, R – Ritonavir).

Lysozyme										
Dye	**Score**					**Approximate interface area of the complex, A^2**				
	F	**M**	**N**	**R**	**-**	**F**	**M**	**N**	**R**	**-**
-	2184	3628	4690	5922		245.40	-54.84	522.30	702.70	
AK-1-2-17	4986	4636	4612	4668	4986	527.70	553.60	518.20	527.40	527.70
AK-1-2-18	5366	4874	4874	4874	5366	600.10	555.30	555.30	555.30	600.10
AK-1-2-19	5432	5432	5432	5314	5432	666.10	666.10	666.10	691.30	666.10
AK-1-2-20	5760	5760	5760	5752	5760	789.00	789.00	789.00	789.00	789.00
AK7-5	5886	5886	5886	5886	5886	739.30	739.30	739.30	739.30	739.30
AK7-6	5734	5472	5472	5492	5734	663.80	692.80	692.80	727.10	663.80

Table 4. The geometric shape complementarity score and approximate interface area of the complex derived for the insulin-drug-dye systems (F- Favipiravir, M – Molnupiravir, N - Nirmatrelvir, R – Ritonavir).

Insulin										
Dye	Score					Approximate interface area of the complex, A^2				
	F	**M**	**N**	**R**	**-**	**F**	**M**	**N**	**R**	**-**
-	1944	2826	3544	4582		244.40	327.80	410.40	619.30	
AK-1-2-17	3888	3802	3784	4030	3888	443.20	416.20	457.90	472.30	443.20
AK-1-2-18	4000	3898	3862	4130	4022	454.80	489.00	493.20	490.90	475.10
AK-1-2-19	5022	4954	4954	5102	5022	652.60	578.70	578.70	601.90	652.60
AK-1-2-20	4886	4920	4920	5024	4886	613.70	591.30	591.30	578.50	613.70
AK7-5	4816	5096	5048	5554	4816	613.00	638.00	570.80	708.70	613.00
AK7-6	4858	5274	4858	4738	4858	649.40	688.10	649.40	557.00	649.40

CONCLUSIONS

To summarize, in the present study the multiple ligand simultaneous docking technique was used to investigate the interactions between four functionally important proteins (cytochrome c, serum albumin, lysozyme and insulin), four antiviral drugs (favipiravir, molnupiravir, nirmatrelvir and ritonavir) and cyanine dyes (four monomethines and two heptamethines). It was found that: i) in most systems the drugs and the dyes interact with different binding sites on the protein molecule suggesting that there is no marked interference between the drugs and the dyes; ii) the heptamethines AK7-5 and AK7-6 form the strongest complexes with the proteins; iii) among the examined proteins the highest affinity binding of heptamethines is observed for albumin molecule where the dyes are located within the protein cavities. Overall, our findings suggest that the systems containing albumin and heptamethines are the most prospective for fabricating the protein nanoparticles for targeted delivery of the investigated antiviral drugs.

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ОДНОЧАСНИЙ ДОКІНГ ПРОТИВІРУСНИХ АГЕНТІВ ТА ЦІАНІНОВИХ БАРВНИКІВ З БІЛКАМИ

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Білкові наночастинки наразі розглядаються як перспективні біосумісні та біодеградовні системи для цілеспрямованої доставки фармакологічних агентів різних класів. Перед створенням наносіїв ліків такого типу доцільно здійснити оцінку спорідненості лікарських препаратів до білків та охарактеризувати можливі способи взаємодії з використанням комп'ютерних підходів, зокрема, методу молекулярного докінгу. Дане дослідження було проведене з метою оцінки можливості створення білкових наночастинок, навантажених противірусними препаратами та ціаніновими барвниками у якості візуалізуючих агентів. Компоненти досліджуваних систем були представлені ендогенними функціональними білками цитохромом с, сироватковим альбуміном, лізоцимом та інсуліном; противірусними агентами фавіпіравіром, молнупірівіром, нірматрелвіром і ритонавіром; моно- та гептаметиновими ціаніновими барвниками. З використанням методу одночасного молекулярного докінгу багатьох лігандів було продемонстровано, що: і) лікарські препарати та барвники займають різні центри зв'язування на білковій молекулі; іі) гептаметини АК7-5 та АК7-6 мають найвищу спорідненість до білків; ііі) серед досліджених систем найміцніші комплекси утворюються між гептаметиновими барвниками та сироватковим альбуміном. В цілому, отримані результати свідчать про те, що наночастинки на основі альбуміну, функціоналізовані гептаметиновими ціаніновими барвниками, можуть бути застосовані для таргетної доставки досліджуваних противірусних агентів.

Ключові слова: *противірусні агенти; білкові наночастинки; наноносії ліків; ціанінові барвники; молекулярний докінг*