SIMULTANEOUS DOCKING OF ANTIVIRAL DRUGS AND CYANINE DYES WITH PROTEINS USING MULTIPLE LIGAND APPROACH

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The protein-based nanosystems for targeted drug delivery of a wide array of substances, ranging from small drugs and therapeutic proteins to nucleic acids and genes, attract increasing attention due to their biocompatibility and biodegradability, extraordinary binding capacity for different ligands, accessibility from natural sources, effective drug protection and gentle encapsulation conditions. Due to the multitude of binding pockets and functional groups on the protein surface, these nanocarriers seem to be highly efficient multifunctional nanotheranostic systems that could incorporate both a therapeutic drug and a visualizing agent. This integration serves multiple purposes, including the regulation of drug release, monitoring the alterations at the target site in response to treatment, and offering crucial insights into the efficacy of the intervention in its early stages. The development of these advanced nanosystems necessitates a thorough comprehension of the potential interactions within these intricate systems. In the present study we assessed the potential of six trimethine and seven pentamethine cyanine dyes to serve as visualizing agents in the drug-protein-dye systems which include functionally significant proteins (cytochrome c, serum albumin, lysozyme and insulin) and four antiviral drugs, viz. favipiravir, molnupiravir, nirmatrelvir and ritonavir. The ternary systems with the highest dye-protein surface shape complementarity were established for all groups of the examined cyanine dyes. The influence of the cyanine dye structure on the stability of the drug-protein-dye complexes was assessed. The obtained results indicate that the dye-protein affinity is not solely dependent on the length of the polymethine chain. It was found that the most prospective drug delivery systems containing the trimethines and pentamethines as visualizing agents are AK5-6-, AK5-8- and AK3-11-drug-albumin complexes.

Keywords: Protein-drug-dye complexes, Antiviral agents, Protein nanoparticles, Drug nanocarriers, Cyanine dyes, Multiple molecular docking

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During the past decades the field of engineering materials for drug delivery applications in cancer therapy [1-3], central nervous system indications [4,5], antiviral therapy [6,7], inflammatory [8,9] and cardiovascular diseases [10,11] has witnessed a growing interest in utilizing the nanostructured drug delivery systems (DDS). The evolution of these second-generation DDSs offers a range of advantages that aim to tackle numerous challenges associated with conventional therapies including: i) enhanced stability and solubility of drugs; ii) reduced drug toxicity; iii) uniform dosing; iv) improved drug pharmacokinetics and distribution, to name only a few [12-15]. Among the plethora of biomaterials and synthetic polymers explored as fundamental blocks for creating nanopharmaceuticals tailored for targeted drug delivery of a wide array of substances, ranging from small drugs and therapeutic proteins to nucleic acids and genes, particular attention is devoted to the protein-based nanosystems [13-15]. Their appeal lies in numerous advantageous attributes, such as: i) biocompatibility and biodegradability; ii) extraordinary binding capacity for various drugs; iii) abundance of proteins available from natural sources; iv) drug protection from enzymatic degradation and rapid renal excretion; v) gentle formulation and drug encapsulation requirements; vi) capability of surface covering with ligands specific to target tissues, vii) streamlined synthesis procedures with cost-effective outcomes [13-16]. Moreover, owing to the presence of numerous binding pockets and functional groups within the proteins, protein nanocarriers are especially promising for the development of effective multifunctional nanotheranostic systems merging both the therapeutic and diagnostic properties [17-19]. Theranostic nanomedicines assume the simultaneous integration of a therapeutic drug and a visualizing agent for control of drug release, monitoring the changes at the target site in response to the treatment, and providing valuable insights into the effectiveness of the intervention at an early stage [17-18]. The fabrication of these advanced second-generation nanosystems requires a comprehensive understanding of the possible interactions within the complex systems.

In our previous work, we employed the multiple ligand simultaneous docking technique to investigate the interactions among four functionally significant proteins (cytochrome c, serum albumin, lysozyme and insulin), four antiviral drugs (favipiravir, molnupiravir, nirmatrelvir and ritonavir) and a series of cyanine dyes represented by four monomethines and two heptamethines. Our primer focus was to identify the most suitable systems for creating the protein nanoparticles carrying both antiviral drugs and cyanine dyes as visualizing agents [20]. The obtained results indicate that the albumin-based nanosystems functionalized by the heptamethine cyanine dyes can serve as effective carriers for targeted delivery of the explored antiviral agents. In continuation of our previous work, in the present study we extended our investigation to other cyanine dyes (six trimethines and seven pentamethines). The main was threefold: i) to delve
into the interactions within the ternary protein-dye-drug complexes using the multiple ligand simultaneous docking (MLSD) technique; ii) to identify the most promising candidates for the development of protein-based theranostic drug delivery nanoplatforms; iii) to determine the structural features of the cyanine dyes responsible for their loading in the multicomponent protein-based drug delivery nanosystems.

MATERIALS AND METHODS

Molecular docking studies

The three-dimensional X-ray crystal structures of the examined proteins in their native monomeric form were obtained from the Protein Data Bank using the PDB IDs 1REX, 3I40, 3ZCF, 6M4R for lysozyme (Lz), insulin (Ins), cytochrome c (Ct) and serum albumin (SA), respectively. The structural model for serum albumin was prepared by employing the DockPrep module of UCSF Chimera molecular software [21]. This involved the removal of water molecules and the addition of polar hydrogen atoms and Kollman charges [21]. The structure of the antiviral drugs (favipiravir, molnupiravir, nirmatrelvir and ritonavir) [20] and the investigated cyanine dyes (Figure 1) were constructed using the MarvinSketch (version 18.10.0) and optimized in Avogadro (version 1.1.0) using the Universal Force Field with the steepest descent algorithm [22,23]. Notably, counterions were omitted from the dye structures to retain molecular charges. Initially, the blind docking of the drugs or dyes (control dye-protein systems) with the proteins was carried out using the PatchDock server (http://bioinfo3d.cs.tau.ac.il/PatchDock/php.php) which focuses on finding the maximum surface shape complementarity while minimizing the steric clashes [24]. Subsequently, the top-scored docked drug-protein complexes were utilized as a receptor for docking of the second ligand, which represents either a trimethine or pentamethine cyanine dye, using the PatchDock server. To characterize the possible interactions involved in the formation of composite drug-dye-protein systems, the protein-ligand interaction profiler (PLIP, https://plip-tool.bioc.ucd.ie/plip-web/plip/index) was employed [25]. The selected docking poses were visualized using the UCSF Chimera software (version 1.14), combining the docking models with the best geometric shape complementarity in the same image to optimize visibility of the binding sites [26].

RESULTS AND DISCUSSION

The ternary complexes with the highest scores, comprising proteins, drugs, and dyes are depicted in Fig.2 (the drugs and dyes binding modes were identified for cytochrome c (Fig. 2), albumin (Fig. 3), lysozyme (Fig. 4) and insulin (Fig. 5). The examined tri- and pentamethines as well as previously reported mono- and heptamethines [20] are situated in close proximity to each other near the surfaces of cytochrome c, lysozyme and insulin. The binding sites for favipiravir,
molnupiravir, nirmatrelvir and ritonavir did not change compared to our previous findings [20]. However, the distinct binding pockets of albumin are observed for the drugs and the cyanine dyes examined here.

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

**Figure 3.** The highest-score docking poses obtained for albumin using the MLSD in PatchDock
Next, the geometric shape complementarity scores and approximate interface areas for the protein-drug-dye systems were analyzed. In the cytochrome c-drug-dye systems the antiviral agents have no impact on the docking positions compared to the protein-dye complexes (Table 1). This observation does not hold true only in the specific cases of AK3-
The dye influence on the docking parameters in the albumin-cyanine systems was found to be more pronounced (Table 2). The docking score remains the same for the systems AK3-1 + F/M/N, AK3-3 + N, AK3-5 + N, AK3-7 + N, AK3-8 + N, AK3-11 + N, AK5-1 + N, AK5-2 + N, AK5-3 + all drug systems, AK5-4 + M/R, AK5-8 + M/R, AK5-9 + M/R; decreases in the systems AK3-5 + F/M/R, AK3-7 + F/M/R, AK3-8 + N, AK3-11 + N, AK5-1 + F/M, AK5-2 + F/M, AK5-3 + all drug systems, AK5-4 + M/R; and increases in the systems AK3-1+R, AK3-3 + F/M/R, AK5-1+ R, AK5-2 + R, AK5-4 + M/R, AK5-6 + R. The ranking of the explored complexes according to aforementioned parameter appeared to be as follows: AK7-5 (N) > AK7-6 > AK-1-2-20 (F, M, N) > AK-1-2-19 (N) > AK3-11 (F, M, R) > AK5-8 (F, N, R) > AK-1-2-18 (F, N) > AK5-2 (F, M, N) > AK5-3 > AK5-4 (F, M, R) > AK-1-2-17 (F, N) > AK5-1 (F, M, N) > AK5-2 (N). The highest albumin-cyanine interface area was observed for AK7-5 (N, R), AK-1-2-20 (F, M, N), AK7-6, AK-1-2-19, AK5-6, AK5-8, AK3-11.

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)
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**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).

<table>
<thead>
<tr>
<th>Lysozyme</th>
<th>Score</th>
<th>Approximate interface area of the complex, Å²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>AK3-1</td>
<td>2184</td>
<td>3628</td>
</tr>
<tr>
<td>AK3-2</td>
<td>4212</td>
<td>4212</td>
</tr>
<tr>
<td>AK3-3</td>
<td>4520</td>
<td>4646</td>
</tr>
<tr>
<td>AK3-4</td>
<td>4634</td>
<td>4708</td>
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<td>AK3-5</td>
<td>4334</td>
<td>4344</td>
</tr>
<tr>
<td>AK3-6</td>
<td>4872</td>
<td>4866</td>
</tr>
<tr>
<td>AK3-7</td>
<td>5176</td>
<td>5198</td>
</tr>
<tr>
<td>AK3-8</td>
<td>4526</td>
<td>4680</td>
</tr>
</tbody>
</table>

**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).

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Score</th>
<th>Approximate interface area of the complex, Å²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>AK3-1</td>
<td>4096</td>
<td>4096</td>
</tr>
<tr>
<td>AK3-2</td>
<td>4202</td>
<td>4202</td>
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<td>AK3-3</td>
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<td>AK3-5</td>
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<td>AK3-7</td>
<td>4268</td>
<td>4202</td>
</tr>
<tr>
<td>AK3-8</td>
<td>4276</td>
<td>4300</td>
</tr>
</tbody>
</table>

The docking score remains the same for all lysozyme-favipiravir-dye complexes (Table 3) and for the systems: AK3-1 + M/N, AK3-7 + N, AK5-4 + R; decreases in the systems: AK3-3 + N, AK3-5 + N/R, AK3-8 + M/N/R, AK3-11 + N, AK3-1 + N, AK5-2 + N/R, AK5-3 + M/N/R, AK5-4 + M/N, AK5-6 + M/N/R, AK5-8 + M/N/R, AK5-9 + M/N/R; and increases in the systems: AK3-1 + R, AK3-3 + M/R, AK3-5 + M, AK3-7 + M/R, AK3-11 + M/R, AK3-15 + M/R, AK5-2 + M. The dye-lysozyme affinity was found to follow the order: AK7-5 > AK-1-2-20 > AK7-6 > AK5-6 > AK1-2-19 > AK1-2-18 > AK5-8 > AK3-11 > AK5-9 > AK1-2-17 > AK3-8 > AK5-3 > AK5-4 > AK5-2 > AK5-1 > AK3-7 > AK3-1, while the highest protein-cyanine interface area was observed in the case of AK1-2-20, AK7-5, AK1-2-19, AK7-6, AK5-6, AK3-11. The favipiravir do not exert influence on the dye-protein affinity and interface area in the cases of insulin and lysozyme (Table 4). The score remains the same for the complexes: AK3-1 + M/N, AK3-3 + M, AK3-7 + M/N, AK3-11 + M/N; decreases in the systems: AK3-1 + R, AK3-3 + R, AK3-5 + M/N, AK3-8 + M/N, AK3-1 + M/N/R, AK3-5 + M/R, AK3-8 + R, AK5-9 + M/N/R; and increases in the systems: AK3-3 + N, AK3-5 + R, AK3-7 + N, AK3-11 + R, AK5-3 + R, AK5-4 + M/N/R, AK5-6 + M/N/R, AK5-8 + N. The dye-insulin affinity decreases in the order: AK1-2-19 > AK1-2-20 > AK7-6 > AK5-6 > AK1-2-19 > AK1-2-18 > AK5-8 > AK3-11 > AK5-9 > AK1-2-17 > AK3-8 > AK5-3 > AK5-4 > AK5-2 > AK5-1 > AK3-7 > AK3-1, while the highest protein-cyanine interface area was observed in the order: AK1-2-19 > AK7-6 > AK1-2-20 > AK7-5 > AK1-2-17 > AK3-8 > AK5-3 > AK5-4 > AK5-2 > AK5-1 > AK3-7 > AK3-1, while the interface area follows the order: AK1-2-19 > AK7-6 > AK1-2-20 > AK7-5 > AK1-2-17 > AK3-8 > AK5-3 > AK5-4 > AK5-2 > AK5-1 > AK3-7 > AK3-1. The crucial contribution of hydrophobic interactions in all examined dye-protein complexes was revealed by the PLIP analysis. The other types of interactions involved hydrogen bonds, salt and water bridges.

**CONCLUSIONS**

In conclusion, the present study was focused on the use of multiple ligand simultaneous docking technique to investigate the interactions between the four functionally significant proteins (cytochrome c, serum albumin, lysozyme, and...
and insulin, four antiviral drugs (favipiravir, molnupiravir, nirmatrelvir and ritonavir) and the cyanine dyes (six trimethines and seven pentamethines). The comparison with ternary complexes from our previous work (that included four monomethines and two heptamethines) was conducted. The obtained results indicate that the dye-protein affinity seems to be not directly dependent on the polymethine chain length. The strongest complexes with the proteins were formed by the heptamethines (AK7-5, AK7-6), monomethines with CH2O substitution (AK-1-2-20, AK-1-2-19), pentamethines with CH2C6H6 and C3H4N substitution (AK5-6, AK5-8), trimethines with OC2H5 and CH3 substitution (AK3-11, AK3-8). Among the examined proteins, the cyanine dyes showed the highest affinity binding to the albumin molecule, while the lowest values of the docking score were observed for insulin. The other results include: i) the cyanines and drugs occupy the different binding sites, except the lysozyme cavity that seems to be suitable for accommodation of both ligands (in the case of molnupiravir, nirmatrelvir and ritonavir); ii) the obtained complexes are predominantly stabilized by hydrophobic forces.

Overall, the most prospective drug delivery systems with the trimethines and pentamethines as visualizing agents are AK5-6-, AK5-8- and AK3-11-drug-albumin complexes, but, generally, the albumin-based nanosystems functionalized by the heptamethine cyanine dyes seem to be the most effective carriers for targeted delivery of the explored antiviral agents.

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REFERENCES

Одночасний докінг противірусних препаратів та ціанінових барвників з білками з використанням мультилігандного підходу

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Наносистеми на основі білків для цільової доставки широкого спектру лікарських засобів, почаючи від невеликих ліків і терапевтичних білків до нуклеїнових кислот і генів, привертають все більшу увагу завдяки свої біосумісності та здатності до біодеградації, надзвичайною стабільністю до зв’язування різних лігандів, доступності з природних джерел, ефективному захисту ліків і м’яким умовам інкапсуляції тощо. Завдяки численним центрам зв’язування та функціональним групам на поверхні білків, ці наносистеми є високо-ефективними багатофункціональними нанотерапевтичними системами, які можуть включати як терапевтичний препарат, так і візуалізуючий агент. Ця інтеграція служить багатьох цілям, включаючи регулювання вив’язування ліків, моніторинг змін у цільовій ділянці у відповідь на лікування та оцінку ефективності втручання на ранніх стадіях. Розробка цих передових наносистем другого покоління вимагає детального розуміння потенційних взаємодій у цих складних системах. У даній роботі ми оцінили потенціал шести триметинових та семи пентаметинових ціанінових барвників як потенційних візуалізуючих агентів в системах білок-лікарський препарат, що включали функціонально важливі білки (цитохром c, сироватковий альбумін, лізоцим та інсулін) та чотири противірусні препарати (фавіпіравір, моліпіра́вір, нірматрелвір і рітонавір). Для всіх груп досліджуваних ціанінових барвників визначені потрібні системи з найвищою комплементарністю форми поверхні барвник-білок, а також типи стабілізуючих взаємодій. Проведено оцінку впливу структури ціанінових барвників на стабільність комплексів лікарський препарат-білок-зонд. Отримані результати вказують на те, що спорідненість барвник-білок не залежить безпосередньо від дожини поліметинового ланцюга. Виявлено, що найбільш перспективними системами доставки ліків, що містять розглянуті триметини та пентаметини як візуалізуючі агенти, є комплекси АК3-6, AK5-8 та AK3-11-ліки-альбумін.

Ключові слова: комплекси білок-лікарський препарат-барвник; противірусні агенти; білкові наноочищники; наносистеми; ціанінові барвники; одночасний молекулярний докінг