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INTERACTIONS OF PERTECHNETATE WITH PROTEINS: AN IN-SILICO STUDY

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Technetium 99m is a radionuclide extensively used in clinical practice due to a range of its properties among which are short half-life, reduced radiation exposure and toxicity, short labeling time, high target to non-target ratio and low cost. In its highest oxidation state +VII, technetium exists in the form of pertechnetate ($[TcO_4]^-$) that serves as an effective imaging agent. One important determinant of pharmacokinetics and bioavailability of pertechnetate involves the possibility of its complexation with blood proteins. In the present work we performed in silico study of the pertechnentate complexes with three blood proteins, deoxyhemoglobin, albumin and transferrin. The molecular docking of $[TcO_4]^-$ to the examined proteins provided evidence for pertechnetate localization in the protein structural cavities containing positively charged amino acid residues, with the highest binding affinity being obverved for deoxyhemoglobin. At the same time, the molecular dynamics simulations indicated that, in contrast to deoxyhemoglobin, only the complexes of pertechnetate with plasma proteins albumin and transferrin remain stable and do not show significant variations in root mean square deviation of atomic positions, solvent accessible surface area, radius of gyration and secondary structure per residue. The results obtained may help in better understaning of pertechnetate pharmacokinetic behavior and enhancing its efficiency as an imaging agent.
Keywords: *Technetium 99m; Pertechnetate; Hemoglobin; Albumin; Transferrin; Molecular docking; Molecular dynamics*
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Among a great variety radionuclides currently used in medical diagnostics one of the most widespread is technetium 99m (^{99m}Tc) [1]. This radionuclide is highly suitable for performing the single-photon emission computerized tomography (SPECT) due to a range of its favorable for nuclear medicine properties such as short half-life ~ 6 h, gamma photon energy 140 keV, reducing the radiation hazard, short labeling time, low cost, availability from ^{99}Mo/^{99m}Tc generator, high limit of accumulation by target organ and high target to non-target ratio, low toxicity, etc. [1-3]. Technetium is a transition metal in the group VII B with 8 oxidation states from – 1 to +VII, and in the highest oxidation state it commonly exists as pertechnetate, $^{99m}TcO_4^-$, which is the most stable form of Tc in aqueous solution [3]. This compound has been employed as an imaging agent in the scintigraphy of thyroid [4,5], salivary gland [6] and Meckel diverticulum [7]. Likewise, in radiochemical labeling $^{99m}TcO_4^-$ may appear as an undesirable impurity worsening the image resolution. Obviously, the biodistribution of pertechnetate is affected by the extent and nature of its interactions with biomolecules, particularly, with blood proteins. In our recent work we quantitatively examined the binding of $^{99m}TcO_4^-$ to structurally and functionally different proteins, human serum albumin, lysozyme and insulin using the precipitation-ultracentrifugation assay followed by radioactivity measurements [8]. It was demonstrated that the extent of pertechnetate association with blood plasma protein albumin is significantly higher than that of lysozyme and insulin taken for comparison. In a logical continuation of this research, the aim of the present study was to assess the stability of $^{99m}TcO_4^-$ complexes with three blood proteins, deoxyhemoglobin, albumin and transferrin, using the molecular docking and molecular dynamics techniques. Albumin is the predominant protein in human plasma, with a number of important functions among which are transport of a variety of substances, maintenance of osmotic pressure, regulation of immune response, etc. [9]. Hemoglobin is the main component of red blood cells responsible for transport of oxygen and carbon dioxide, modulation of erythrocyte metabolism, heat transduction, etc. [10]. Transferrin, a blood plasma glycoprotein, accounts for iron transport into cells through binding to specific transferrin receptors [11].

METHODS

The crystal structures of the functional proteins were taken from the Protein Data Bank (https://www.rcsb.org/) using the following PDB IDs:1AO6 (human serum albumin in its dimeric form), 2DN2 (human deoxyhemoglobin), 1D3K (human transferrin). To stabilize protein conformation in water, each examined protein structure was subjected to 2 ns molecular dynamics relaxation and then the relaxed structures were docked with pertechnetate using the HDOCK server (https://hdock.phys.hust.edu.cn/), which integrates a template-based modeling and free docking via a fast FFT-based algorithm [13]. The most energetically favorable docking complexes were visualized with the VMD software.

The MD simulations of the model systems pertechnetate – proteins were performed using the GROMACS software (version 2024.2) with the modified CHARMM36 force field. The parameterization of the CHARMM36 force field for the Tc metal center was made through introducing the metal−ligand bond lengths, bond angles and the force constants.

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To add $[TcO_4]^-$ as a new residue to the force field, the modifications were made in the following files: aminoacids.rtp, atomtypes.atp, ffnonbonded.itp, ffbonded.itp, residuetype.dat. More specifically, in ffnonbonded.itp the Lennard-Jones parameters for Tc were taken as those for Ru (II) (ε = 1.748 kJ/mol, σ = 0.262 nm [14]), by analogy with the work of de Andrade and coauthors [12]. The parameters such as equilibrium distance b0, force constant kb, valence angle theta0, averaged force constant of valence-angle harmonic potential ktheta were taken from [15-17], and included in the file ffbonded.itp (sections bondtypes and angletypes). The mol2 file for $[TcO_4]^-$ was taken from supporting info [12] and converted to pdb format in UCSF Chimera. In the file aminoacids.rtp the following partial charges were introduced: Tc1 – 1.398377, O2 – -0.599668, O3 – -0.599521, O4 – -0.599668, O5 – -0.599521 calculated in [12] from ChelpG fitting. The best score docking complexes between the proteins and $[TcO_4]^-$ were placed in a rectangular water box containing neutralizing ions, with a minimum distance of 10 Å from the protein to the box edges. The calculations were conducted at a temperature of 310 K using the TIP3P water model, Nose-Hoover thermostat and Verlet barostat. The Particle Mesh Ewald method was used for correct treatment of the long-range electrostatic interactions. The minimization and equilibration of the systems were carried out during 50000 steps. The time interval for MD calculations was 10 ns. The MD trajectories were corrected using the gmx trjconv GROMACS command. The analysis of MD data was performed with the GROMACS commands gmx rms, gmx gyrate, gmx sasa and VMD plugin Timeline, to explore time evolution of root mean square deviation of atomic positions (RMSD), gyration radius, sovent accessible surface area SASA) and protein secondary structure per residue. The visualization of the snapshots of MD runs was performed in VMD.

RESULTS AND DISCUSSION

Presented in Fig. 1 are the top-scored docking positions for the complexes of pertechnetate with deoxyhemoglobin, albumin and transferrin. As can be seen, in all cases $[TcO_4]^-$ is located in the cavities in protein structures. A more detailed analysis of the docking results revealed that the binding affinity decreases in the row: deoxyhemoglobin > transferrin > albumin (Table 1).

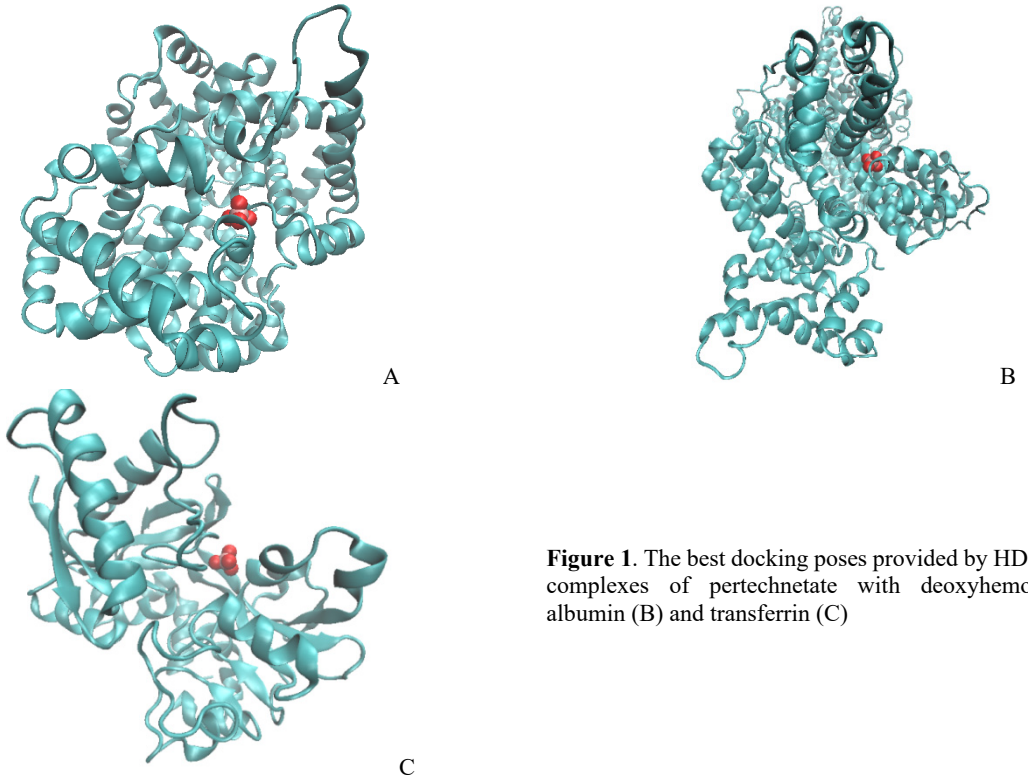


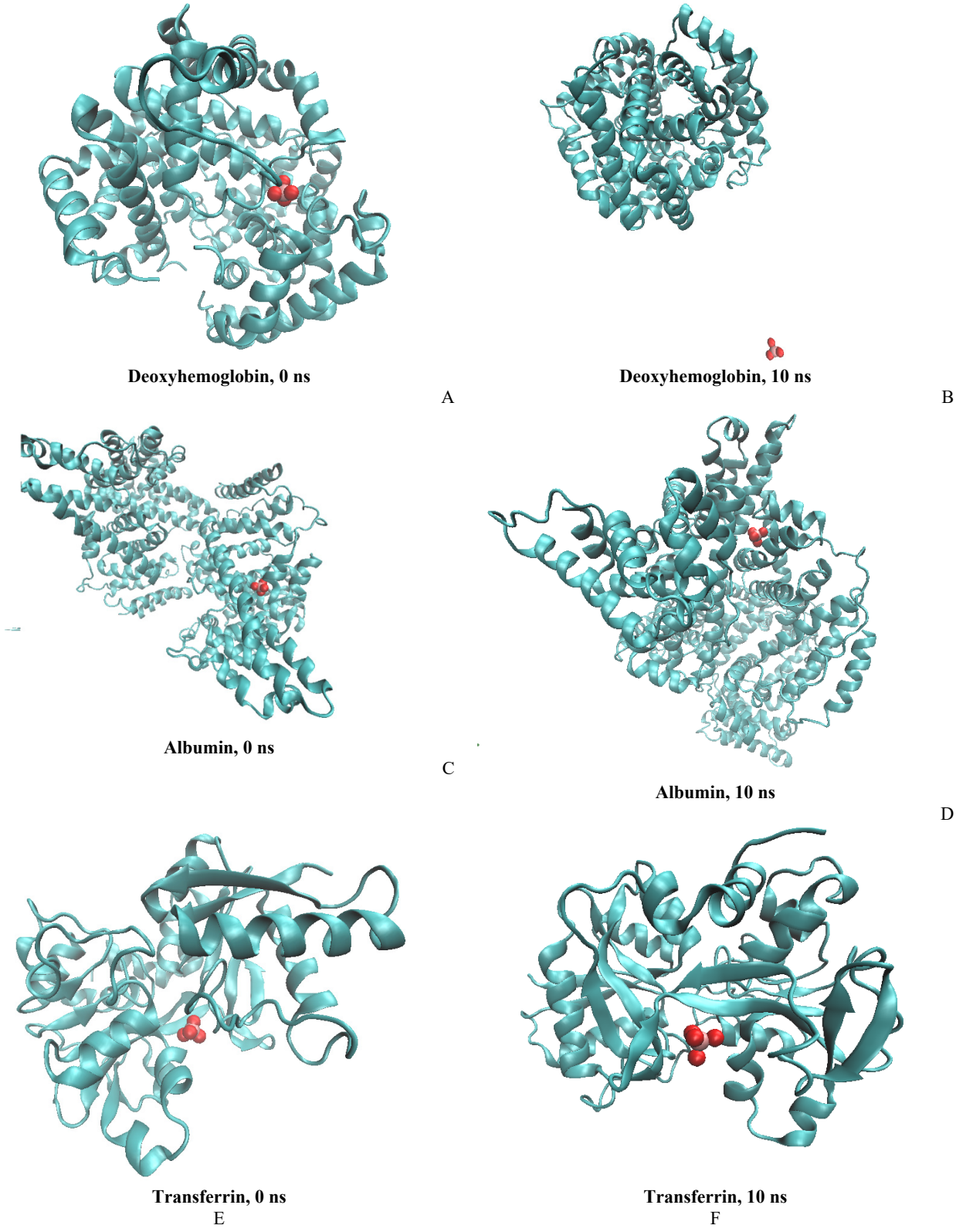
Figure 1. The best docking poses provided by HDOCK for .the complexes of pertechnetate with deoxyhemoglobin (A), albumin (B) and transferrin (C)

Notably, the sites accommodating $[TcO_4]^-$ contain positively charged amino acid residues (ARG_{40B} and ARG_{92C} for deoxyhemoglobin, ARG_{348A} , ARG_{484A} and ARG_{485A} for albumin, LYS_{296} for transferrin) suggesting that electrostatic interactions enhance the association of pertechnetate with proteins.

Table 1. Docking scores characterizing the binding affinities of pertechnetate for functional proteins and interface residues in the protein - $[TcO_4]^-$ complexes

Protein	Docking score	Interface residues
Deoxyhemoglobin	-68.30	TRP_{37B} THR_{38B} ARG_{40B} PHE_{41B} ASP_{99B} ASN_{102B} TYR_{42C} ARG_{92C} VAL_{93C} ASP_{94C}
Albumin	-57.77	VAL_{344A} ARG_{348A} GLU_{450A} ASP_{451A} LEU_{453A} SER_{454A} LEU_{457A} LEU_{481A} VAL_{482A} ASN_{483A} ARG_{484A} ARG_{485A} PRO_{486A}
Transferrin	-59.49	TYR_{85} TYR_{95} TYR_{96} GLN_{206} HSD_{207} SER_{208} GLU_{212} LYS_{296} SER_{298}

Next, to compare the stabilities of the examined complexes, we performed a series of 10 ns molecular dynamics simulations. Shown in Fig. 2 are the snapshots of MD simulations taken at the time points 0 ns and 10 ns.



Deoxyhemoglobin, 0 ns A

Deoxyhemoglobin, 10 ns B

Albumin, 0 ns C

Albumin, 10 ns D

Transferrin, 0 ns E

Transferrin, 10 ns F

Figure 2. The snapshots of MD simutations of the complexes between pertechnetate and functional proteins

It appeared that within the time interval of MD simulation $[TcO_4]^-$ remains attached to albumin and transferrin, but dissociates from its complexes with deoxyhemoglobin, despite higher binding affinity to this protein compared to albumin and transferrin. The subsequent analysis of MD data obtained for albumin and transferrin revealed that the $[TcO_4]^-$ complexes with these proteins retain its stability during the simulation time, as judged from insignificant changes of the parameters such as RMSD, radius of gyration and total SASA (Fig. 3).

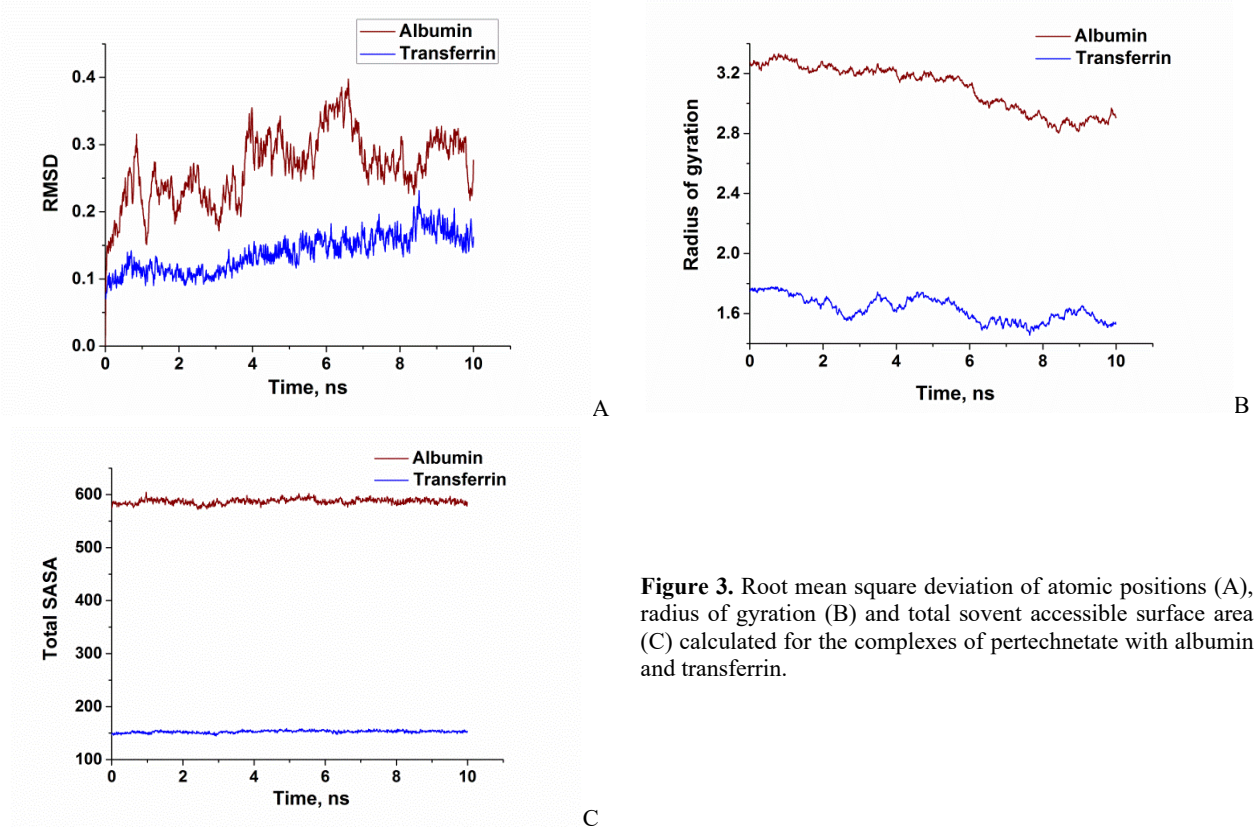


Figure 3. Root mean square deviation of atomic positions (A), radius of gyration (B) and total sovent accessible surface area (C) calculated for the complexes of pertechnetate with albumin and transferrin.

Accordingly, VMD Timeline analysis showed that the secondary structure of interfacial residues remain virtually unchanged during the simulation time in the complexes of pertechnetate with albumin and transferrin (Fig. 4).

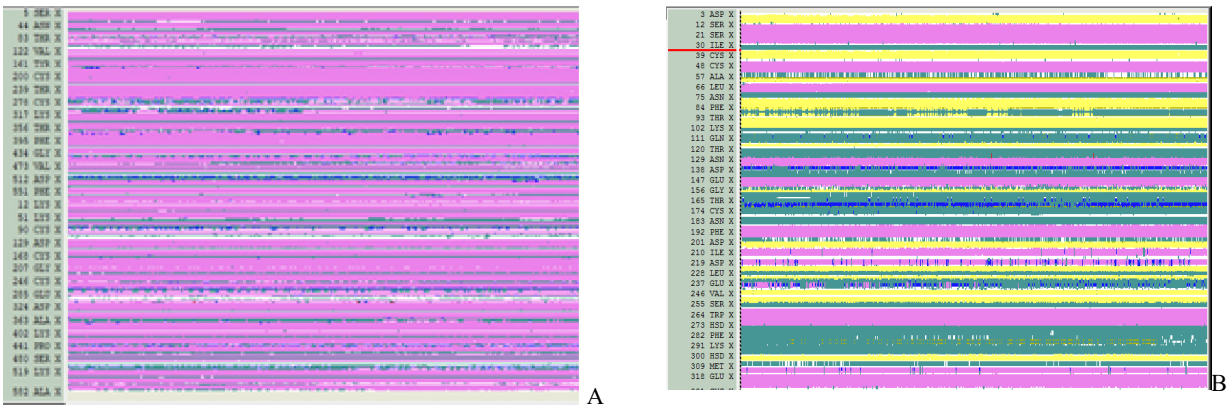


Figure 4. Time evolution of the protein secondary structure per residue provided by VMD Timeline plugin for albumin (A) and transferrin (B).

It should be mentioned that MD results presented here are in accordance with our recent experimental study showing that the binding capacity of albumin for pertechnetate is about two-fold higher than that of lysozyme and insulin [9]. Likewise, this work demonstrates the necessity of validating the docking data by subsequent MD simulation. According to our docking results, the strongest binding of $[TcO_4]^-$ was observed for deoxyhemoglobin, but the stability of this complex was not confirmed by MD calculations, in contrast to albumin and transferrin.

CONCLUSIONS

In summary, the present study was undertaken to gain further insights into the structural details of interactions between pertechnetate and three blood proteins, deoxyhemoglobin, albumin and transferrin using in silico approaches. The molecular docking data suggest that $[TcO_4]^-$displays binding preference for the cavities in protein structures, with the binding affinity being higher for deoxyhemoglobin compared to albumin and transferrin. The presence of positively charged amino acid residues in the binding sites points to the importance of ionic contacts for stabilization of the $[TcO_4]^-$ complexes with proteins. However, the molecular dynamics simulations evidenced that only the complexes of pertechnetate with plasma proteins, albumin and transferrin, are stable and do not dissociate within the MD calculation

time. The analysis of MD results in terms of the parameters such as RMSD, SASA, radius of gyration and secondary structure per residue provided the arguments in favor of structural stability of pertechnetate complexes with albumin and transferrin. These findings may have implications for the clinical use of pertechnetate and improvement of imaging procedures.

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ВЗАЄМОДІЯ ПЕРТЕХНЕТАТУ З БІЛКАМИ: ДОСЛІДЖЕННЯ IN SILICO

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Технецій 99m – радіонуклід, який широко застосовується в клінічній практиці завдяки таким його властивостям, як короткий період напівжиття, допустимий рівень опромінення та низька токсичність, швидке мічення, високий ступінь накопичення в органі-мішені та низька вартість При найвищому ступені окислення +VII технецій існує у формі пертехнетату ($[TcO_4]^-$) який є ефективним візуалізуючим агентом. Одним із важливих детермінантів фармакокінетики та біодоступності пертехнетату є можливість його асоціації з білками крові. У даній роботі було проведене in silico дослідження комплексів пертехнетату з трьома білками крові – дезоксигемоглобіном, альбуміном та трансферином. Методом молекулярного докінгу між $[TcO_4]^-$ та досліджуваними білками були отримані докази локалізації пертехнетату в структурних порожнинах, що містять позитивно заряджені амінокислотні залишки, а найвища афінність була виявлена для дезоксигемоглобіну. В той же час, молекулярно-динамічні розрахунки показали, що, на відміну від дезоксигемоглобіну, тільки комплекси пертехнетату з білками плазми крові, альбуміном та трансферином, залишаються стабільними та не характеризується значущими варіаціями таких параметрів як середньоквадратичне відхилення атомних позицій, площа поверхні, доступна розчиннику, радіус інерції та вторинна структура амінокислотних залишків. Отримані результати можуть бути корисними для більш глибокого розуміння фармакокінетичної поведінки пертехнетату та підвищення його ефективності як візуалізуючого агента.

Ключові слова: *технецій 99m; пертехнетат; гемоглобін; альбумін; трансферин; молекулярний докінг; молекулярна динаміка*