Molecular Level *in Silico* Studies for Oncology. Direct Models Review

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Abstract. The combination of therapy and diagnostics in one process "theranostics" is a trend in a modern medicine, especially in oncology. Such an approach requires development and usage of multifunctional hybrid nanoparticles with a hierarchical structure. Numerical methods and mathematical models play a significant role in the design of the hierarchical nanoparticles and allow looking inside the nanoscale mechanisms of agent–cell interactions. The current position of *in silico* approach in biomedicine and oncology is discussed. The review of the molecular level *in silico* studies in oncology, which are using the direct models, is presented.

THERANOSTICS AND THE HIERARCHICAL NANOPARTICLES

Recently, more and more researchers have noted that to treat serious illnesses such as cancer an interdisciplinary approach is required [1, 2]. It is necessary to involve specialists from various non-traditional fields, including physicists and mathematicians. Mathematical models and computer simulations allow both to deeply analyze experimental and statistical data and to obtain additional qualitative and quantitative data from the numerical experiments at almost any time and spatial scales.

Combining therapy and diagnostics into a united procedure is a trend in modern biomedicine and, especially, in oncology. A new hybrid term that is used to define this complex approach is "theranostics", namely meaning therapy plus diagnostics. Targeted cancer therapy and imaging using hybrid multifunctional nanoparticles (NP) with core-shell structure proposed by Li et al. [3] is a vivid example of the theranostic approach. The key role in this case is played by the hierarchical nanoparticles. The hierarchy includes three functional levels. The first (most inner) level is a core made of ferromagnetic metal supra-particles. Magnetic property provides nano-agent with many functions as ability being concentrated within the necessary tissue area using external magnetic field [4], ability to enhance contrast in magnetic resonance imaging (MRI) of tumors, and also allows using magnetic separation during preparation. The second level is a shell made of Ni-Al layered double hydroxide (LDH) nanosheets hosting drug molecules. LDH is ceramics having layered nanostructure with host-guest architecture, where staked positively charged nanosheets of metal hydroxide are "glued" together by guest water molecules and charge balancing anions. The third (outer) level is modified anticancer drug doxorubicin having deprotonated carboxylic group (DOX-COO$^-$) as a guest anion between LDH nanosheets. The formation of LDH-drug nanohybrid is possible *via* anion-exchange intercalation mechanism due to competitive ion adsorption onto LDH nanosheets.

The surface of hierarchical NP was functionalized by (iminodiacetic acid)-modified folate *via* the chelating interaction, enabling the NP to target HeLa cells. After the cellular uptake of the hierarchical NP by cancerous cell, modified DOX-COO$^-$, being in the acidic cytoplasm, takes the cationic form. Further, due to electrostatic repulsion, the cationic therapeutic agent releases from the interlayer space of LDH.

Not only doxorubicin but many other anticancer drugs can be intercalated within hydrated gallery space of LDH, forming stable nanohybrid. The nanohybrids containing camptothecin (CPT) [5], 10-hydroxycamptothecin (HCPT) [6], 5-fluorouracil (5-FU) [7, 8], methotrexate (MTX) [9–12], doxifluridine (DFUR) [13] and podophyllotoxin

(PPT) [14] are among experimentally synthesized ones. Moreover, the formation of stable LDH-based nanohybrids with intercalated antibiotics, vitamins, liposomes, genes, amino acids, antioxidants and many other bio-molecules was reported in many publications.

The theranostic anticancer system composed of a multifunctional endoscope and hierarchical nanoparticles was proposed by Lee et al. [15]. The hierarchical NP with core-shell structure had a gold nanorod as the core, and the double shell. The inner shell was made of mesoporous silica with fluorescent dyes, photodynamic compounds and the doxorubicin embedded. The second layer of the shell, composed of thermosensitive poly-(N-iso-propyl-acryl-amid), plays the role of "crust", preventing DOX release from the NP. The surface of NP was functionalized with specific anti-bodies Cetuximab, which allow targeting of HT-29 cell line of colon cancer. These anti-bodies interact with the receptors of the epidermal growth factor, which are abnormally high expressed on the colon cancer cell membrane. During endoscopic examination, fluorescent dye (rhodamine B) allows revealing cancerous areas, which further undergo an irradiation of red (670 nm) and near infrared (808 nm) lasers. Under the lasers treatment hierarchical NPs directly generate reactive oxygen species (photodynamic therapy), warms up cancer cells (photoinduced hyperthermia) and releases doxorubicin in a controlled way after the outer polymeric shell thermodestruction.

The multifunctional NPs on a base of magnetic iron oxide with controlled release of gemcitabine (GEM) for targeted therapy and magnetic resonance imaging (MRI) of pancreatic cancer is also a vivid example of the theranostics approach in oncology [16]. After the internalization of such a nanoparticle in the endosome or lysosome a tetrapeptide GFLG linker (degradable under the acidic pH and in the presence of cathepsin B), which covalently binds core with therapeutic agent, breaks up, releasing free GEM into the cancer cell. The rest part of the particle acts as MRI contrast agent.

Summarizing, the further progress in the theranostic approach requires the development of multifunctional hierarchical nanoparticles (MHNP).

IN SILICO APPROACH IN THE PROBLEMS OF THE ONCOLOGY

In block-scheme the MHNP development process may be represented as a sequence of design and synthesis, as well as *in vitro, in vivo* testing and clinical trials. *In vitro* studies are performed with cell models, *in vivo* studies utilize animal (mammalian) models. Studies "*in silico*", meaning literally "on silicon chips", deal with numerical models (Fig. 1).
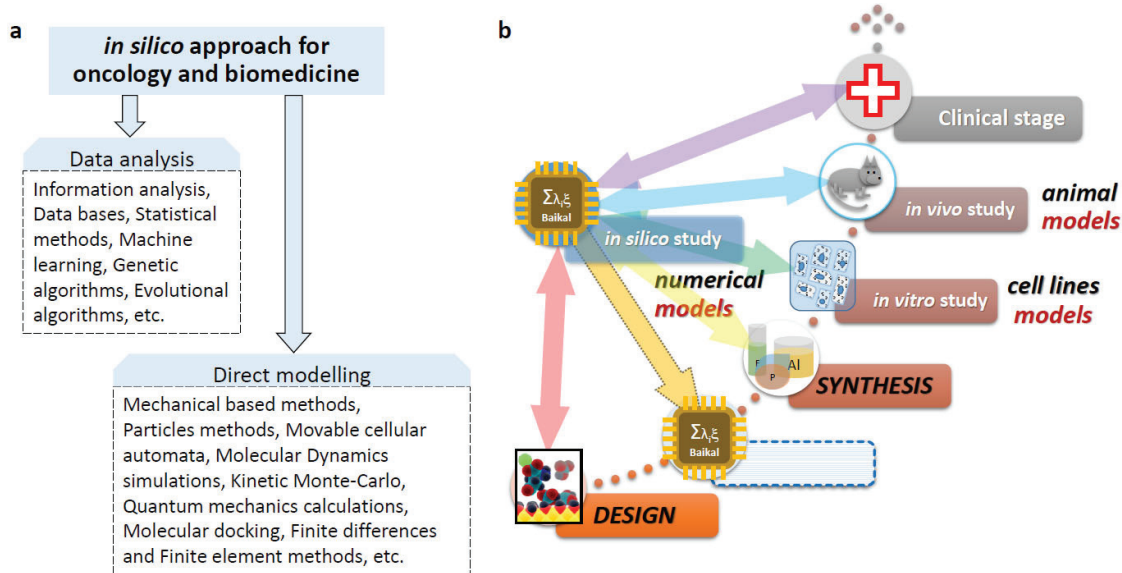

FIGURE 1. (a) *In silico* approach in the oncology and biomedicine in the broad sense of this term. (b) A rough scheme of the MHNP development sequence. Orange arrow shows *in silico* block position accordingly to most common meaning in oncology, but actual position is aside, having connections with almost all other blocks (explanation is in the text)

Traditionally, *in silico* approach in oncology combines the information analysis methods as statistical methods, data-base processing, machine learning, genetic and evolutional algorithms, which are usually used at the computer-aided drug design stage (Fig. 1a, *first block*). However, the "*in silico*" term is wider and can also "absorb" all numerical methods, including direct modeling, based on mathematical physics: particle dynamics methods as movable cellular automata, all-atom and coarse-grained molecular dynamics, kinetic Monte-Carlo and molecular docking as well as quantum mechanics calculations, finite element and finite differences methods, etc. (Fig. 1a, *second block*). Moreover, *in silico* experiments may assist not only nano-agent design but, for example, its synthesis, simulating reactions and estimating optimal synthesis parameters, or estimating energy characteristics of organic-inorganic nanohybrid formation, showing the conditions of nanocomplex stability (Fig. 1b, yellow arrow). In particular, estimating from the *in silico* experiments Gibbs free energy of DOX molecule binding with Fe-Ag surfaces, the conditions of the drug release from nanocomposite can be found, which is quite important for the development and fabrication of biodegradable implants encapsulated with anticancer therapeutics [17]. The same is true for *in vitro* testing, where *in silico* approach, for instance, may shed some light on the molecular level interaction of MHNP with the cell membrane and membrane proteins, mechanisms of endosome formation, lipid bilayer disruption etc. (Fig. 1b, green arrow).

The use of computer technologies in oncology studies has been developing rapidly over the past decades due to many reasons, including the development of computational techniques, and the increase in supercomputer performance [1, 2, 18, 19]. The first block methods, roughly called here "data analysis", are widely used in oncology [20–22]. Direct models at the molecular level for the anticancer nano-agent development, illustrating second block methods, are considered in the next section.

MOLECULAR LEVEL IN SILICO STUDIES IN ONCOLOGY

In silico investigation on DNA nanotubes as drug delivery vehicle for four anticancer therapeutics agents was conducted by Liang et al. using the molecular dynamics (MD) modeling [23]. In the study doxorubicin (DOX), daunorubicin (ADR), taxol (TAX) and vinblastine (VIN) were considered as model drug molecules. These anticancer agents are hydrophobic molecules and possess poor water solubility, moreover, they are non-selective and can exhibit a toxic effect to the normal cells. Therefore, the development of the hybrid nanoparticles for selective/targeted delivery and the controlled release of such hydrophobic drugs are quite important. The current progress in DNA-nanotechnologies provides the unprecedented opportunities for the manufacture of DNA nanostructures with precise geometry and universal functionality, in particular, self-assembled DNA nanotubes (DNTs), which are inherently non-toxic and biocompatible. In addition, they can be labeled with folic acid to target specific receptors in the cancer cell membrane [24].

The results of the MD simulation series demonstrated that the formation of multimolecular nanocomplexes of DNT oligomers with each of drugs molecules considered is possible. The model DNA nanotubes were built from four, six and eight double strands DNA with the poly-(adenine-thymine)$_{20}$ sequence. The analysis of the molecular level absorption mechanism of anticancer drugs by DNT showed that both electrostatic interaction and van der Waals forces play the role in the nanocomplex formation, but the contribution of the second ones is higher. Liang and co-workers found that this reduces the aggregation of anticancer drugs in water and at the same time increases the stability of the DNA-nanotubes themselves.

Another drug delivery vehicle based on the carbon nanotube was investigated using *in silico* approach by Mousavi et al. [25]. The all-atom level molecular dynamics simulations of the hierarchical NP translocation through cell membrane were performed. Nanoparticle has host-guest structure, where single-walled carbon nanotube (CNT) with opened ends contained anticancer drug paclitaxelum (PTX). The core of the PTX molecule is polar. Four steered MD simulations were conducted with different values of the velocity constant. The orientation of the CNT was constrained to be perpendicular to the lipid bilayer plane during pulling process. The PTX molecule was initially placed near the second CNT end, which is more distant from the membrane. The greatest resistance to penetration was observed when the PTX@CNT nanocomplex was passing through the lipid tail region, which is associated with the hydrophobic interactions between the lipophilic groups of the lipids and the outer surface of CNT. It was shown that during PTX@CNT translocation several lipids and the water molecules were captured inside nanotube. The amount of lipids entering the CNT inner space decreases with increasing the speed of the NP translocation. Furthermore, the amount of lipids that are completely removed out from the membrane by the NPs also decreases. It was noted that for the retention of the PTX molecule inside the CNT, an especial role is played by

van der Waals forces between PTX and the inner wall of CNTs, as well as by hydrogen bonds formation between PTX and the water molecules, which were trapped inside the nanotube.

Using single-walled CNT as a nanocarrier for another anticancer therapeutic agent—vinblastine was investigated *in silico* utilizing several theoretical tools such as molecular dynamics, quantum mechanics and Monte-Carlo methods, aiming to analyze the stability and conformation of the drug molecule inserted into CNT [26].

Aronda and co-authors conducted a comprehensive *in silico* study, including molecular docking followed by the molecular dynamics simulations and the post-dynamic analysis to understand whether the nelfinavir (NFV) and eight other HIV-1 protease inhibitors could act as anticancer drug [27]. It was previously hypothesized that the known anticancer activity of NFV is due to its inhibitory effect on heat shock protein 90 (Hsp90), which is a promising target for anticancer therapy. Thus, to justify the assumption and to understand possible mechanism, the free energy of nine HIV-1 protease inhibitors binding with Hsp90 were estimated. The obtained results showed that all of nine molecules exhibit affinity to Hsp90, moreover, the binding of NFV with Hsp90 has the largest gain in energy ($\Delta G = -38.5$ kJ/mol) in comparison with other protease inhibitors considered: the binding affinity of indinavir, saquinavir and ritonavir with protein are characterized by free energy change $\Delta G = -37.7$, -36.0 and -35.6 kJ/mol, respectively. It means that NFV and eight other HIV-1 protease inhibitors may possess the same action with respect of heat shock protein of the cancer cell. It was also found that the most dominant role in the binding of the drug is played by the hydrophobic interactions, especially with Val534 and Met602 protein residues.

Two-dimensional carbon nanomaterials such as pristine graphene nanosheets, graphene oxide (GO), PEGylated GO (functionalized with polyethylene glycol) [28], as well as nitrogen-doped graphene [29] are also attracted attention as the prominent base for the development of the anticancer drug delivery system. Wang et al., using all-atom MD, found that the binding of finite graphene nanosheets with anticancer drugs CE6, DOX, MTX and SN38 is favorable when the drug molecule and the nanosheet have comparable sizes, since in this case the deformation of graphene nanosheet is minimized [28]. The estimates of the average binding energy vary from 40 to 290 kJ/mol. It was also found that the nanosheet boundaries restrict the movement of the drug molecules adsorbed. Besides, the PEGylated GO nanosheets bind the drug molecules more strongly as compared to pristine one. Azizi and Ebrahimi conducted *in silico* study of pristine graphene nanosheet and N-doped one binding with anticancer drug paclitaxel (PTX), using MD [29]. It was demonstrated that content of nitrogen atoms influences adsorption of PTX at the nanosheet. Moreover, the optimal value of N atoms concentration in N-doped graphene nanosheet was estimated as 6.8%.

In this context, it is also pertinent to mention our *in silico* study, which is not directly associated with the oncology, but devoted to the formation of organic-inorganic nanohybrid based on LDH, which can be used for the development of the drug delivery system [30]. Using all-atom level MD models, the interaction of organic anions with the single nanosheet of Mg/Al-LDH was characterized. The typical configurations of "soft-matter" organic anions on the LDH surface in the adsorbed state were found and most common binding sites. Quantitative estimations for non-covalent bonds formation were obtained. Using constant velocity steered MD technique, the free energies of adsorption for aspartic and glutamic amino acid anions, bicarbonate-anion and chlorine on the LDH nanosheet were evaluated, characterizing the competitive adsorption sequence for modeled anions:

$$Cl^- < HCO_3^- < Asp^- \approx Glu^-,$$
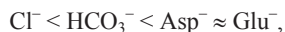
where in the estimates of the free energy wells depths for the anionic amino acids residues are quite large—about 50 kJ/mol. Strong interaction energy provides the formation of hybrid multimolecular complexes. The outcome for the theranostics could be the possibility of non-covalent functionalization of the LDH-based hierarchical NPs with receptor binding peptides, contained in the structure the anionic amino acid residues Asp^- and Glu^-. The obtained competitive adsorption series means that no one of extracellular anions (Cl^- and HCO_3^-) can release Asp^- or Glu^- amino acids from nanoparticle surface before cellular uptake of the NP.

SUMMARY

A new stage of *in silico* approach for solution of oncology problems based on development of direct modeling (and not just the information analysis) started. Direct modeling provides the understanding of the mechanisms at the nanoscale level that cannot be investigated employing the known methods.

Further development of numerical modeling methods and computational technologies will allow solving multiscale problems, providing the possibility to predict the biomedical action of drugs, including adverse side

effects, directly based on their structure. This would allow rapid design and production of the novel theranostic nano-agents for the treatment of complex diseases, such as cancer.

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