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How can molecular dynamics simulations assist with gene medicines?

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A B S T R A C T

Molecular Dynamics (MD) simulations can provide a glimpse of complex atomistic and molecular events at the interface of biomaterials and biosystems. Gene therapy efforts that deploy biomaterial mediated delivery of nucleic acids could benefit immensely from such MD simulations. These efforts most commonly employ supramolecular assembly whose structure is highly dynamic and influential in the final outcomes. By careful analysis of the behavior of constituting elements, one can visualize the assembly as it makes its way though biosystems. We highlight the beneficial information to be gained from MD studies in this short perspective and outline a vision for future activity in the field.

Perspective

Biomaterials have become an integral part of recent gene therapy efforts to realize the clinical benefit of nucleic acids. The initial attempts to deliver plasmid DNA (pDNA) based expression systems have evolved into delivery of a range of nucleic acids with different functionalities. The latter include short DNA based antisense oligonucleotides (ASO), short interfering RNA (siRNA) and aptamers, as well as larger DNA and RNA products in the form of linear DNAs and messenger RNAs (mRNA). There is an increasing need to deliver more complex mixtures of nucleic acids, in the form of DNA/RNA combinations and other mixtures of nucleic acids to enable CRISPR (clustered regularly interspaced short palindromic repeats) mediated gene editing [1]. The biomaterials designed to interact with nucleic acids facilitate formation of supramolecular complexes in the form of nanoparticles (NPs), whose physicochemical and biological features dictate the efficiency of delivery and resulting functional outcomes in target cells. Biomaterial efforts have predominantly relied on experimental approaches to assess and elucidate the underlying mechanism of operation for designed gene medicines. An integrated set of experimental systems has now become the norm in assessing the features of NPs in order to reveal the operational principles of new systems. However, experimentation has its limits; it is often slow and expensive, the outcomes may depend on minor variations on the manipulations of reagent conditions and may not always have the resolution to reveal all that is necessary to understand about the investigated biosystem. Computational studies, in particular all-atom molecular dynamics (MD) simulations, offer a fruitful alternative approach in this pursuit.

In a typical MD simulation, atoms are modeled as soft balls and bonds as elastic sticks. The forces on the atoms are calculated from intra- and intermolecular interactions defined in the form of a potential energy. The potential energy of the simulated system is a function of geometrical variables such as atom distances and bond angles, as specified in a validated force field. Equations of motion for the atoms are integrated with respect to time to update their coordinates and velocities until equilibrium is reached. MD simulations can provide detailed atomistic information not accessible by experiments: structural information such as conformational changes, formation and breaking of physical bonds such as ionic interactions and other reactive states [2,3], molecular aggregation and assembly; and energetic information such as free energy of binding and potential of mean force. MD simulations are intrinsically limited by the computational power and algorithm efficiency. The current state-of-the-art all-atom MD simulations can simulate millions of atoms for microseconds, which is still much smaller than practical systems studied in experiments. In addition, validity of the MD results relies on the accuracy of the force field [6], which governs the force calculations, computationally the most costly part of an MD simulation. Nevertheless, with the fast-growing computing power [4], integration with novel algorithms such as machine learning, great efforts on developing advanced and more reliable force fields, MD simulations hold immense potential towards modeling realistic time and length scales.

Several aspects of the nucleic acid NPs have been intimately probed with the MD simulations. The supramolecular assembly process is initiated with the functional groups on biomaterials that can interact with functional groups on nucleic acids; while charged species are the primary determinants of such interactions, the contributions of polar groups and hydrogen bonding (H-bonding) are additional features that can be illuminated. The ability to condense the nucleic acids and assembly into a cohesive nanoparticle will rely on such interactions, so that biomaterial features can be optimized (and guide the synthetic efforts) based on the outcomes of MD simulations of such systems. Once

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assembled in NPs, the details of the final structure, as well as various molecular motions of the constituents can shed light on the stability of the particle. Charge neutralization in local regions, overall charge of the NP as well as the bridging interactions among the constituent molecules can be illuminated with such simulations. We have illuminated, for example, the role of lipid moieties on the assembly and stability of nucleic acid NPs in this way [5]. An unexpected observation on the effect of a particular functional group (i.e., propionic acid) on experimental determined gene transfer efficiency was revealed with MD simulations that suggested significant gradients in the intraparticle features, which was difficult to probe experimentally.

An important requirement for gene medicines is the effective and intact transport of NPs through cellular membranes. Unlike coarse-grained simulations that avoid the molecular motion at atomistic details, all-atom MD simulations are revealing the highly dynamic nature of the internalization process from the moment of first interactions to penetration and release from the lipid-rich phase. It has been possible to visualize compression and expansion of nucleic acid NPs and motion of the NP constituents during the transport in the lipid phase. The beneficial effects of structural components that improve stability during lipid membrane transport has been suggested from the experimental standpoint, and now MD simulations are providing specific clues that enhance NP stability for improved membrane transport. New insights have been possible on the role of nanoparticle features and the strength of interactions with zwitterionic membranes not obvious from experimental observations and with possible underlying mechanism(s) [6]. The force calculations are providing quantitative parameters to estimate the degree of resistance encountered by the NPs during the transport. The exchange of membrane lipids between the inner and outer surface of lipid leaflets, so called flip-flops [7], has been observed; this may have significant bearing on integrity of cell membranes (which needs to control the leakage of intracellular components) as well as cell fate on the long run, which will be adversely affected on the display of intracellular lipids on external surfaces. The MD simulations in these studies utilized relatively simple membranes with a few constituent lipids. Natural membranes with dozens of major lipids, and especially in their segregated structures, are bound to behave differently; more realistic investigations of NP transport will require more sophisticated lipid membrane models. The NP transport are bound to be drastically different between the 'fluid' and 'solid' phases of the membrane and MD simulations could probe details of the transport not accessible to experimental approaches. It is additionally desirable to explore the role of proteoglycans especially on lipid membranes, since they are expected to strongly interact with NPs, which were suggested to be the primary source of destabilization for NPs. Towards this goal, proteoglycan (heparin) models have been build that probed the role of free heparin interactions with nucleic acid NPs, elucidating functional groups that strongly participate or do not participate in interactions with NP constituents [8]. Immobilizing such proteoglycans on lipid membranes should allow more realistic simulations of membrane transport.

Further developments and dedicated efforts are needed to improve the impact of MD simulations. An obvious limitation is the relatively short duration of simulations that can be currently attempted (usually $<\mu s$). The computational power needed for longer simulations is simply lacking. Due to the fact that computational expertise has traditionally evolved separated from the experimental biomaterial science, it has been difficult to set up integrated studies where the predictions from MD simulations could be directly validated by experimental measurements. Such studies will go a long way to inspire confidence in computational approaches in solving the problems of traditionally experimentally evolved biomaterials science. Beyond the simple binding and transport through lipid membranes, critical steps in functional performance of nucleic acid NPs remains to be explored; (i) the changes in the state of the NPs as the particles encounter different pH environments remains to be explored. This will be vital to illuminate the endosomal transport and escape of the NPs; (ii) the interactions of the competing species in cell cytoplasm with the NP constituents will be critical to better understand unpacking of the nucleic acids on their way to functional sites. This will help us to identify the species responsible for release of nucleic acids and may help to design improved carriers more responsive to intracellular milieu of the cell; (iii) nuclear uptake, the critical step for gene expression, remains to be unprobed with MD simulations. Is the transport through plasmid membrane (or endosomal membranes) same as nuclear membrane? It is expected that these detailed studies will pave the way for increased adaptation of MD simulation in nanoparticulate delivery of exogenous cargo to cellular systems.

There are specific physiological processes that cannot be addressed now by MD simulations due to less than predictable nature of many interactions between synthetic NPs and highly dynamic cellular substructures [9], so that experimentation is indispensable in this regard at the moment. However, one could envision the possibility of the whole process of delivery and implementation of functional activity of gene medicines to be simulated one day. Metabolism of exogenous nucleic acids may be followed up based on simulations of chemical changes analogous to peptide bonds. This will be a significant leap from today's activity where the simulations are typically restricted to illuminating the atomistic details of delivery and functioning. Realistic cellular models amenable for MD simulations [10] combined with significantly expanded computation power may replace traditionally experimental biomaterials science and engineering.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] K C RB, Thapa B, Valencia-Serna J, Aliabadi HM, Uludağ H. Nucleic acid combinations: a new frontier for cancer treatment. J Control Release 2017;256:153–69.
[2] Senftle TP, et al. The ReaxFF reactive force-field: development, applications and future directions. npj Comp. Mat. 2016;2:15011.
[3] Salsbury AM, Lemkul JA. Recent developments in empirical atomistic force fields for nucleic acids and applications to studies of folding and dynamics. Curr Opin Struct Biol 2020;67:9–17.
[4] https://arxiv.org/abs/2005.00223
[5] Meneksedag-Erol D, RB KC, Tang T, Uludağ H. A delicate balance when substituting a small hydrophobe onto low molecular weight polyethylenimine to improve its nucleic acid delivery efficiency. ACS Appl Mater Interfaces 2015;7:24822–32.
[6] Gurtovenko AA. Molecular-level insight into the interactions of DNA/polycation complexes with model cell membranes. J Phys Chem B 2019;123:6505–14.
[7] Saito H, Morishita T, Mizukami T, et al. Free energy profiles of lipid translocation across pure POPC and POPC/CHOL bilayer: all-atom molecular dynamics study. J Phys: Conf Series 2019;1290:012020.
[8] Meneksedag-Erol D, Tang T, Uludağ H. Mechanistic insights into the role of glycosaminoglycans in delivery of polymeric nucleic acid nanoparticles by molecular dynamics simulations. Biomaterials 2018;156:107–20.
[9] Hall A, Lächelt U, Bartek J, Wagner E, Moghimi SM. Polyplex evolution: understanding biology, optimizing performance. Mol Ther 2017;25:1476–90.
[10] http://www.digizyme.com/cst_landscapes.html