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Perspective

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All-Atom Molecular Dynamics Simulations of Whole Viruses

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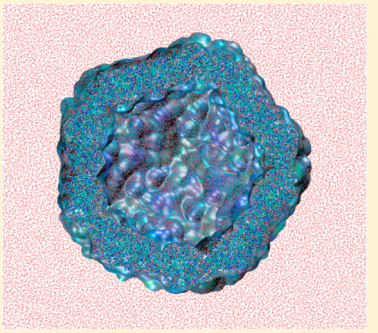
ABSTRACT: Classical molecular dynamics modeling of whole viruses or their capsids in explicit water is discussed, and known examples from the literature are analyzed. Only works on all-atom modeling in explicit water are included. Physical chemistry of the whole system is the focus, which includes the structure and dynamics of the biomolecules as well as water and ion behavior in and around the virus particle. It was demonstrated that in most investigations molecular phenomena that currently can not be studied experimentally are successfully reproduced and explained by the simulations. These include, for example, transport and distribution of ions inside viruses that ultimately connected to their stability, the hydrodynamic pressure in the capsid related to viruses' elastic properties, the role of metal ions in virus swelling, and others. Current and future tendencies in the development of all-atom virus simulations are outlined.



State-of-the-art experimental capabilities to measure the atomistic structure of very large molecular systems (a recent example presents the cryo-electron microscopy (cryo-EM)-measured structure of a 125 nm diameter virus capsid[1]) rely critically on sophisticated modeling techniques that fit known chemical structures to three-dimensional density maps obtained from the experimental data. Currently these modeling techniques are predominantly concerned with *structural* fitting, which is especially difficult for the parts of the density where ambiguity is possible because of lower resolution or absence of data. However, the atomistic *dynamics* is very important, both for better quality of fitting (for example, by indicating flexible regions of molecules where experimental data is of poor quality by definition) and for subsequent use of the obtained atomistic models (providing, for example, short-lived conformations unobtainable in the experiment). "Modeling atomistic dynamics" means classical molecular dynamics (MD). Therefore, MD simulations of very large, by current MD standards, systems are useful and important despite the technical difficulties caused by the sheer number of atoms in the system.

Among various large molecular systems with known atomistic structure, viruses are particularly convenient for MD modeling. This is because they exist in solution as isolated molecular entities, interacting only with water and ions. Complicated and poorly known interactions with other viruses, cell membranes, other cell organelles, etc. can be excluded from simulation, at least for some stages of the virus life cycle. Also, the size of viruses varies over a large interval, from ∼17 nm in diameter, amenable to rather routine MD simulations, to hundreds of nanometers, currently out of reach for MD. Despite the diversity of their structure and function, it is possible to compile a set of viruses with overall similar construction. For example, they would all consist of nothing else but a capsid formed by a repeating copy of one (or a small group of) medium sized protein and a genome of relatively short DNA or RNA strand packed inside this capsid. A set of such viruses of various sizes can serve as convenient models for tuning and validating MD simulations.

This Perspective is devoted to all-atom MD simulations of whole viruses or their complete capsids. Importantly, we consider only works on atomistic modeling, excluding (much more numerous) publications on coarse grained simulations of viruses. We concentrate on the physical chemistry side of modeling rather than the biological aspects of the studied viruses. From this point of view, the interaction with water and ions is particularly important, and it usually receives significantly less attention (if any) in biological literature. We, therefore, selected mostly simulations with explicit solvent (Figure 1), that is where water molecules and ions are included in the model at atomistic representation,[2] rather than a structureless continuum or any other approximate external correction.

Only a few years ago did publications on all-atom whole virus modeling with explicit solvent start to appear. The first work was published in 2006 by the Klaus Schulten group,[3] in which satellite tobacco mosaic virus (STMV) was modeled. This virus consists of an RNA genome enclosed in an icosahedral capsid of approximately 17 nm diameter. STMV is a "nonenveloped" virus; that is, the virus shell consists of only one layer made of proteins, in contrast to the "enveloped" viruses where in addition there is another outer layer of lipids and proteins. The capsid was built from 60 copies of a single protein, the initial structure of which was taken from

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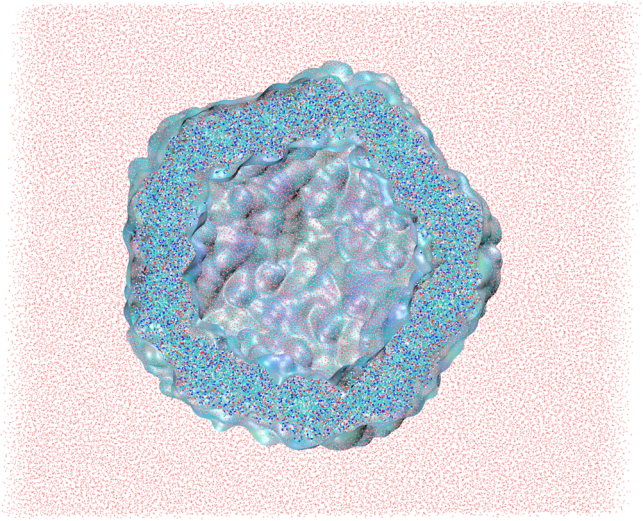

Figure 1. Cross-cut through the all-atom model of PCV2 capsid without genome immersed in a simulation box filled with explicit water.[5]

crystallographic measurements. An empty capsid was modeled as well as the whole virus with artificial RNA, built with the assumption that its structure consists of 30 double-stranded stems (which backbone was resolvable from the crystallographic data) and arbitrarily packed single-stranded regions connecting the resolved double-stranded ones;[4] the sequence of the RNA was artificial, and its length (949 nucleotides) was similar to the native genome (1058). This RNA core was also modeled separately, without the capsid.

The results of modeling showed that water can pass through the capsid's wall. The authors also calculated the diffusion constant of chloride ions in the system. More interesting results for biological applications concerned the stability of the system. The capsid containing RNA and RNA separately turned out to be stable during all time of modeling (13 and 10 ns). However, the empty capsid was unstable, where the protein blocks shifted toward the capsid's center, which led to overall deformation of the capsid. The fact of an unstable empty capsid agreed with experiment. These findings had implications for the virus assembly mechanism emphasizing the role of RNA in the process.

For the stable entire virion, an interesting observation on the dynamics of the protein units was found. The units around an equatorial "belt" exhibited correlated motion, while the units at the opposite ends were in anticorrelated motion. This is one of the results that was possible to obtain only in all-atom MD simulation of an entire virus.

The results of the publication allowed the conclusion that the correct assembly of the virus proceeds in the presence of RNA, while empty capsids are not formed. We have also observed this behavior of capsid deformation for a different virus, PCV2,[5] when the genome is absent inside the capsid. We have shown that such instability is caused by incorrect distribution of ions on the capsid's surfaces (inside and outside). In contrast to STMV, however, empty capsids of PCV2 are known to exist experimentally.[6]

Three years after the first publication, a system of 4.5 million atoms was modeled,[7] with a focus on mechanical properties of the capsid of southern bean mosaic virus. In this work, the force probe MD simulation was applied to obtain the distribution of mechanical properties (elastic constant and yielding forces) on viral capsid at the atomistic level. The approach allowed the analysis of the viral shell properties with the variation of the probe velocity of the forcing tip-sphere and its size. The authors observed three deformation regimes under the load. They also did not find any water flow through the capsid wall even under tip-sphere indentation that resulted in substantial change of the inside volume.

It is generally assumed that the removal of calcium ions from the capsid's structure of southern bean mosaic virus leads to its swelling, which is the initial stage of cell infection during which the genome is released.[8] In their subsequent publication, the authors[9] continued the investigation of the capsid's mechanical properties of the virus with the force probe MD simulation at atomistic resolution with the aim of investigating how the mechanical properties of the capsid are changed when calcium is removed. They also investigated the distribution of the elastic constant and yielding forces on the outer surface as well as on the inner surface of the capsid, which is not possible to do experimentally for the inner surface. In the work, they noted that after calcium removal the mechanical properties changed before the changes in the structure. The found weakening along the 5-fold axes of the capsid inspired the hypothesis that the pentamers that form the capsid's structure serve as possible ports for RNA release.

For some viruses, the increase of their diameter or "swelling" was observed when metal ions (for example, Ca^{2+}) were removed from the capsid's structure.[10,11] This property plays an important role in the infection process. MD modeling allows studying the dynamics and the structural features of the capsid connected with swelling, as it was done in ref 12 for another satellite virus, satellite tobacco necrosis virus (STNV). Modeling was performed for capsids containing structural Ca^{2+} ions and the capsids after the removal of ions in explicit water solutions for 1 μs each; RNA was not included into the simulation.

The authors analyzed how the water permeability of the capsid changes after calcium removal, and they found that the capsid's calcium-binding sites play different roles in the STNV structure: some are responsible for the swelling after removing the calcium ions, while others play a structural role and do not take part in swelling.

In ref 13, the authors performed MD simulation of the native and swollen states of the cowpea chlorotic mottle virus (CCMV) in vacuum and showed that the study of structural transitions in the swollen capsid requires long time simulation. They applied their multiscale molecular dynamics/order parameter extrapolation (MD/OPX) approach[14] for the long time (200 ns) simulation of the swollen CCMV capsid in vacuum that allowed the investigation of its structural transition mechanisms.

In their next work,[15] the authors demonstrated the application of their MD/OPX methodology to the investigation of the CCMV capsid swelling in a solution-mimicking environment. Their simulation results showed that the swelling of the capsid, as well as the shrinking notable in simulation in vacuum, was observed accompanied by the loss of symmetry first locally and then in the whole capsid.

The conformation of unstructured, dynamic fragments of the capsid are impossible to measure experimentally, but they can be reconstructed, and their structure and dynamics can be studied in simulation, as was done in ref 16 for poliovirus. The poliovirus capsid includes four proteins—VP1, VP2, VP3, and

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VP4—that form a single structural unit (block); 60 copies of this unit make the whole capsid. X-ray analysis could not determine the structure of the first 20 amino acids of the N-terminal domain of VP1, which is involved in the infection process. Modeling was performed for four systems: whole virus with RNA, empty capsid, pentamer, and isolated N-terminal domain. The size of the modeled systems reached 4 million atoms. The modeling was performed for 10 ns for each system in NaCl solution of 0.154 mM concentration. Also, in the system with RNA, Mg^{2+} ions were added for stabilizing the structure. It was observed that in the detached domain the α-helix structure is preserved for the whole 10 ns of simulation; this domain is more flexible in the empty capsid than in the complete virus. The behavior of ions in full virion was investigated, and it was found that Na^+ and Cl^- ions diffuse across the capsid wall through a small crack in the 2-fold axis region. Na^+ ions diffuse inward, Cl^-ions diffuse outward, while Mg^{2+} ions remain bound by the RNA.

The properties of this virus were also investigated in 2014 at longer times, up to 200 ns.[17] In their work the authors used sodium phosphate buffer at pH 7.4 for reproducing experimental conditions. The total system size was 6.5 million atoms. After long equilibration for 100 ns, water molecule diffusion through the capsid's wall was observed. At equilibrium, the time for total replacing of water inside the capsid with the outside water was 25 μs. This result explains experimental data on the ability of poliovirus to withstand high pressure and pressure shocks:[18] water diffuses fast enough to equalize the pressure without destroying the capsid. Results were also obtained on the possibility of water evaporation from the inside of the capsid. These results are important for developing methods of viral deactivation. Despite water diffusion, ions did not pass through the capsid for all 200 ns of the simulation time. This classifies the capsid as a semipermeable membrane. Negative pressure was observed inside the empty capsid, which was explained by Coulombic interaction of the solution inside the capsid with the capsid excess charges.

To date, contributions have demonstrated successful application of MD to whole viruses, the analysis of their stability at various conditions, and interaction with the environment and (in some rare cases) with the genome.

Similar results on the capsid functioning as a semipermeable membrane were found in ref 19 for porcine circovirus type 2 (PCV2). In the work, an all-atom modeling of an empty PCV2 capsid in an environment similar to physiological conditions with explicit water model was conducted. The capsid was modeled by two methods: the classical MD and a hybrid MD–hydrodynamics approaches. The system size was 1.9 million atoms, and the simulation time was 10 ns. It was found that the capsid was stable after 10 ns. The water and ion flows were analyzed and showed that while water could cross the capsid wall in both directions, the ions could not.

In ref 20, the authors modeled an empty capsid of the HIV-1 virus and investigated its physical chemical properties. The simulated time was 1.2 μs, and the system size reached 64 million atoms. The modeling was performed at physiological conditions with 150 mM NaCl explicit water solution. The authors investigated the stability of the capsid, both overall and locally; calculated the rate of water exchange between the inside and outside of the capsid; and determined the location of the ions on the capsid's surfaces. Electrostatic properties as well as acoustic properties and modes were analyzed.

It was found that the capsid can translocate ions with high specificity, which could play a role during the infection by assisting in filtering particular molecules. The sodium ions were transferred through the capsid wall via special channels. It was found that the chloride ions form a layer on the inner surface of the capsid, while sodium ions form a layer on the outer surface. Similar layers were found on the surfaces of PCV2.[5]

A large part of the analysis was devoted to the acoustic modes on the surface of the capsid. Oscillatory behavior was found that propagates through the capsid similar to capillary waves common to membranes and fluids. Interestingly, the frequencies of these waves were found to lie in the ultrasound region (2.38–11.9 MHz). Moreover, waves of different frequencies were found to be localized at different regions of the capsid (for example, the highest frequency was associated with the tip of the capsid, etc.). It was hypothesized that this behavior plays an important role in the infection process as it facilitates the transfer of information through very large distances.

All-atom MD simulation was performed for hepatitis B virus capsid.[21] The simulations were performed for 1.1 μs in explicit water and 150 mM NaCl; thus, the size of the system was 6 million atoms. The results demonstrate high flexibility of the capsid and the acceptance of asymmetric distortion that may result in the limitation of structural determination with cryo-EM. The HBV capsid is also permeable for water molecules; sodium ions can move through the capsid wall faster than chloride ions. The authors assumed that this slow translocation of the chloride ions may play important function in the gradualness of structural distortion of the capsid. Ions of a physiological solution could play an important part in mediating specific interactions.

At the moment all-atom modeling of viruses using MD is at the beginning of active development, which is shown by the small number of publications in the field. These works demonstrate successful application of MD to whole viruses, the analysis of their stability at various conditions, interaction with the environment and (in some rare cases) with the genome. The developed models allow investigations of macroscopic properties of viruses at some stages of their life cycle starting from the fundamental microscopic dynamics. The fact of the existence of stable empty capsids is important as it suggests that such capsids could exist in nature under proper conditions and, perhaps, play a role in the virus self-assembly. However, because modeling of such large systems is a new field of research, it is important to pay close attention to correct preparation of the system.

One of the most intriguing questions in this field for both experimentalists and modellers is the structure of the genome inside the capsid and its interaction with the capsid. To date there are very few attempts to reconstruct the genome because experimental data on its structure is fragmented at best.

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However, rapid progress in structure determination leads us to believe that all-atom modeling of the genome in viruses will be one of the most actively developed directions of research for the next few years.

The questions of viral self-assembly[22,23] remain largely open. They are related to the analysis of specific interactions between the capsid's units as well as between the genome and the capsid. Such interactions are of key importance for the process of self-assembly and genome packing. All-atom MD modeling will be the instrumental methodology in studying the details of these interactions, thus indirectly contributing significantly to reconstructing the biological mechanisms of viral self-assembly. Research activity in this direction is likely to be the trend in all-atom MD simulation of viruses.

Overall, all-atom modeling of entire viruses in explicit water has proven its usefulness, but at the moment it is at the beginning of its active use. The tendencies in experimental techniques, computer developments, and questions posed by state-of-the-art biology are such that these simulations are likely to see active developments in the near future.

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Notes

The authors declare no competing financial interest.

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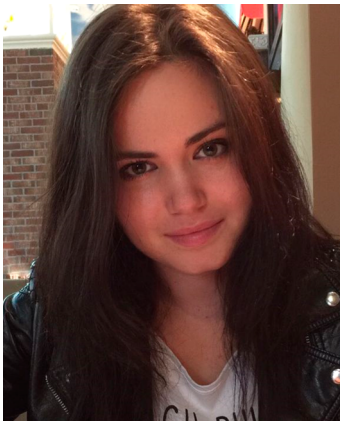

Elvira Tarasova (Ph.D. in physical chemistry, 2018) works as a JSPS postdoctoral fellow at RIKEN Institute in Japan. She also did research at Immanuel Kant Baltic Federal University, Russia and Aston University, U.K. Her scientific interests are in computational chemistry of biomolecules, including very large systems such as viruses, drug discovery for antimicrobial resistance, and physicochemical properties and mechanisms of the viral life cycle.

Dmitry Nerukh. After a number of postdoctoral positions at various Universities in the U.K. and United States, Dr. Nerukh worked at Department of Chemistry, Cambridge University, U.K., before accepting a permanent position at Aston University. His work has led to the development of an original numerical method for Bohmian quantum dynamics and fundamentally new approaches for the complex dynamics of classical molecular systems that applies state-of-the-art mathematical theory of complexity of dynamical systems. His most recent research includes the development of multiscale hybrid molecular dynamics–hydrodynamics framework for modelling large biomolecular systems at several spatial and temporal scales simultaneously. Such systems include all-atom models of entire viruses in solution that are modelled with a very large number of atoms using high-performance supercomputers.

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