Perspectives of 1,4-DHP-lipid Molecular Dynamics

Vita Duka[1,2], Cezary Czaplewski[3], Adam Liwo[3], Gunars Duburs[2], Inta Liepina[2], Anda Zeidmane[1]

[1] Latvia University of Agriculture, Liela str. 2, Jelgava, LV3001, Latvia
[2]Latvian Institute of Organic Synthesis, Aizkraukles str. 21, Riga LV1006, Latvia
[3]University of Gdansk,ul. Sobieskiego 18, 80-952 Gdansk, Poland
vita.duka@llu.lv ; inta@osi.lv; anda.zeidmane@llu.lv

Abstract: *This paper focuses on software aided methodology of molecular dynamics using classical modeling cycle that can be used for investigation of complex lipid system molecular dynamics such as the cationic amphiphilic lipid type compound 1,1'-{[3,5-bis(dodecyloxycarbonyl)-4-phenyl-1,4-dihydropyridin-2,6-diyl]dimethylene} bispyridinium dibromide (1,4-DHP lipid). We summarized and systematized the molecular dynamics simulation process, and provided a list of software tools that can be successfully used for different purposes in different steps of molecular dynamics simulation. We show that molecular dynamics simulation as a computer modeling method complies with the assumptions of the mathematical modeling cycle. That was proved with the successful molecular dynamics studies of 1,4-DHP lipid system. The cycle of 1,4-DHP lipid system modeling was accomplished with the verification of results, that is excellent result although it opens a perspectives for further analysis of this system.*

Keywords: molecular modeling, molecular dynamics, lipid, gene transfection agent.

Introduction

Molecular modeling, also called computational chemistry, is the science that studies molecular structures through model building and uses principles of computer science to assist in solving chemical problems. Molecular modeling encompasses variety of computer based methods in order to understand and predict the behavior of molecular systems at the molecular level. This is a rapidly progressing area due to the development of theoretical methods based on classical, statistical mechanics and quantum mechanics, rapid increment in computer speed and memory, algorithm efficiency and steady improvements in force field development. Modeling and simulation of chemical and biological systems is a truly multidisciplinary challenge. Schlich writes, biologists describe the cellular picture; chemists fill in the atomic and molecular details; physicists extend these views to the electronic level and the underlying forces; mathematicians analyze and formulate appropriate numerical models and algorithms; and computer scientists and engineers provide the crucial implementational support for running large computer programs on high-speed and extended-communication platforms (Schlich, 2010).

The role of computer science becomes constantly even more important as computer simulations become so precise and accessible that they can support and even substitute real experiments. Although nowadays, modeling of large and complex systems as proteins, nucleic acids, and lipids has been made available, computational intensity still remains as a problem (Gubbins, et.al., 2011). Molecular modeling has a wide range of applications also in various disciplines of engineering sciences, such as material science, chemical engineering, biomedical engineering, etc. Knowledge provided by molecular modeling, is essential for understanding the behavior of nanosystems and it forms the route to the nanosciences and nanotechnology. (Mashaghi, et.al., 2013)

Lipids are organic molecules that include fats, waxes, sterols, fat-soluble vitamins, monoglycerides, diglycerides, triglycerides, phospholipids, and others. Phospholipids - the dominant lipids in biomembranes are molecules with hydrophobic tails and hydrophilic head groups. The head groups can be charged (positively or negatively) or neutral. Thanks to hydrophobic nature of their tails, in solution lipids can self-assemble into different nano-structures such as bilayers, liposomes, micelles or reversed micelles. (Alberts et al., 1994) The main lipid biological functions are energy storage, signaling, and acting as "building blocks" of cell membranes.(Fahy et.al.,2009, Subramaniam et.al.,2011) Lipids have many applications in cosmetic and food industries as well as in nanotechnology.(Mashaghi et.al., 2013) Recent researches show that lipids work as nanocarriers in drug delivery using lipid nanotechnology for cancer and tumor treatment. (Selvamuthukumar and Velmurugan, 2012). There are also studies of drug transdermal diffusion by modeling molecule transfer through lipid bilayer - compounds that are soluble in oil, can be delivered by the intercellular lipid layer. (Rim et.al., 2009)

This paper focuses on software aided methodology of molecular dynamics using classical modeling cycle that can be used for investigation of complex lipid system molecular dynamics such as the cationic amphiphilic lipid type compound 1,1'-{[3,5-bis(dodecyloxycarbonyl)-4-phenyl-1,4-dihydropyridin-2,6-diyl]dimethylene} bispyridinium dibromide (1,4-DHP lipid). This work is continuation of our earlier studies, where it was confirmed that 1,4-DHP lipid has the gene transfection activity. (Liepina et al., 2011)

Materials and methods

In this paper we focus on two main research objectives:

- to summarize and classify molecular dynamics software that can be used for 1,4-DHP lipid system molecular dynamics;
- to provide the worked out software aided methodology for molecular dynamics using classical modeling cycle.

As it is not possible to cover the whole range of accessible software for molecular dynamics, authors' selected and listed software choice is subjective and based on scientific analysis and evaluation of a number of information sources and reports connected with molecular modeling and dynamics and also on the authors' reflection and research experience.

Molecular mechanics. Molecular mechanics uses classical mechanics to study small molecules as well as large biological systems or material assemblies with many thousands to millions of atoms. All-atomistic molecular mechanics methods have the following properties: each atom is simulated as a single particle; each particle is assigned a radius, polarizability, and a constant net charge; bonded interactions are treated as "springs" with an equilibrium distances and angles equal to the experimental or calculated values. Main branches of molecular mechanics applications are energy minimization and molecular dynamics.

In molecular dynamics, trajectories of the molecular systems that consist of atoms are generated by integrating Newton's laws of motion. The result is a trajectory that specifies how the positions and velocities of the atom in the system change in time. The trajectory of *i-th* atom is obtained by solving the differential equation embodies in Newton's second law (*F=ma*):

$$\frac{d^2 x_i}{dt^2} = \frac{F_{x_i}}{m_i}, (i = 1 \ldots n) \tag{1}$$

where m_i mass of atom *i*;

x_i – one coordinate of atom *i*;

F_{x_i} – force that acts on the atom *i* in x_i direction.

Equation (1) describes the motion of an atom *i* of mass m_i along one coordinate x_i with force F_{x_i}. The force that acts on the atoms depends on its positions relative to the other atoms. Here the motion is often very difficult, sometimes impossible, to describe analytically, due to the coupled nature of the atoms' motions. The force on each atom will change whenever the atom change its position, or whenever any of the other atoms with which is interacts changes position. These interactions are described by the force field. (Leach, 2001)

Force field. The usage of the term "force field" in molecular modeling differs from the standard usage in physics. Here it is a system of potential energy functions rather than the gradient of potential, as defined in physics. Molecular mechanics ignore the electronic motions that are used in quantum mechanical methods and calculate the energy of system as a function of atoms nuclear positions only. This gives the opportunities to perform the calculations on systems containing significantly larger number of atoms. Molecular mechanics is based upon a rather simple approximation model of the atom interactions within system that includes such processes as bond stretching, opening and closing of angles and rotation about single bonds. (Leach, 2001) A force field is built up from two distinct components to describe the interaction between atoms:

- the set of equations, also called the potential functions, used to generate the potential energies and their derivatives, the forces;
- the parameters that are used in this set of equations.

One functional form for such a force field that can be used to model single molecules or systems of atoms is:

$$V(r_N) = \sum_{\text{bonds}} k_b (l - l_0)^2 + \sum_{\text{angles}} k_a (\theta - \theta_0)^2 + \sum_{\text{torsions}} \frac{1}{2} V_n [1 + \cos(n\omega - \gamma)] + \sum_{j=1}^{N-1} \sum_{i=j+1}^{N} \left\{ \eta_{i,j} \left[\left(\frac{r_{0ij}}{r_{ij}} \right)^{12} - 2 \left(\frac{r_{0ij}}{r_{ij}} \right)^{6} \right] + \frac{q_i q_j}{4\pi\eta_0 r_{ij}} \right\} \tag{2}$$

$V(r_N)$ denotes the potential energy, that is function of the positions r (usually is three dimensional Cartesian space) of N atoms. The first term in equation (2) models interactions between pairs of bonded atoms and is expressed by harmonic potential that gives the increase in energy as the bond length l_i deviates from the equilibrium value l_0. The second term in (2) is a summation over all valence angles that are formed between

three atoms and expressed also in the form of harmonic potential. The third term in (2) is rotational potential that models how the energy changes when torsion angles change. The fourth contribution is the non-bonded term. This is calculated between all pairs of atoms (*i* and *j*) that are in different molecules or are separated in one molecule by at least with three bonds. Non-bonded terms are usually modeled with Lennard-Jones potential for van der Waals interactions and Coulomb potential for electrostatic interactions.

From the potential energy function, mathematical equations, is obtained empirical force field, equations and parameters that relate chemical structure and conformation to energy. All force fields are based on numerous approximations and derived from different types of experimental data. Therefore they are called empirical. There are three main types of force fields: all atom - parameters provided for every atom within the system, united atom – some atoms are excluded, coarse grained - an abstract representation of molecules are created by grouping several atoms into one unit. In the literature (Mackerell, 2004), often is proposed classification as Class I (or classical) Class II and other specific force field models. Some force fields are specifically developed for some biological molecule types and system sizes. AMBER (Case et.al., 2012), OPLS, CHARMM (Vanommeslaeghe et.al.,2010) and GROMOS (Hess et al., 2008) have been developed primarily for molecular dynamics of macromolecules. These are classical, all atom force fields and developers provide information about their force field parameterization strategy. But as they are different, parameters from one force field cannot usually be used in another force field. (Leach, 2011, Ramachandran et.al., 2008, Schlich, 2010, Griebel et.al.,2007)

Molecular dynamics simulation. Due to the complexity of the force field, equation of motion (1) is integrated using finite difference method – integration is broken down into many small stages, each separated in time by a fixed time δt . The wide variety of integration schemes are available - *Verlet algorithm, velocity Verlet method, Beeman's algorithm, Gear predictor – corrector algorithm, etc.* (Leach, 2001)Various factors should be taken account before deciding which method is the most appropriate. It is clear that large computational effort is required for complex system integration, but for best methods high-speed computation is as valued as trajectory precision.

Before running the molecular dynamics simulation, initial configuration of the system should be established. The initial configuration can be obtained from the experimental data or from the theoretical model using energy minimization techniques. It is necessary to assign initial velocities for particles in the system and that can be done by randomly selecting initial velocities from Maxwell-Boltzmann distribution at the temperature of interest. Molecular dynamics is performed in the constant microcanonical ensemble, depending on which state variables are kept fixed – energy E, volume V, temperature T, number of particles N. Two most common alternative ensembles from the traditional constant NVE (number of particles, volume and energy) and constant NVEP (number of particles, volume, energy and pressure) are constant NVT (number of particles, volume and temperature) and constant NPT (number of particles, pressure and temperature) ensembles. (Ramachandran et.al., 2008)

Results and discussion

Software aided methodology of molecular dynamics. The fact that molecular modeling includes a whole set of theoretical and computational methods that are used to investigate and simulate behavior of molecular systems, should be taken into account when talking about proper software tools. There are available many self-sufficient software tools that can be used by molecular modelers from beginners to advanced scientists. Some of them include many methods covering a wide range, while others are concentrating on a very specific range or even a single method. By authors' vision, there is no strict classification for molecular modeling software provided in the literature. Of course all software highly depends on the developers. Some of them have made more successful implementations of methods than others however some of them historically or by other assumptions are more popular among users. Authors propose to systematize molecular modeling software by some qualitative features*:*

- *Functionality* – what methods that are implemented in software, e.g., molecular model building, visualization, energy minimization, molecular dynamics, stochastic molecular dynamics, protein folding, protein structure prediction, etc.
- *Supported biomolecules* – what biological systems are supported in the software, e.g., nucleotides, proteins, lipids or saccharides.
- *Type of graphical interface* – e.g., graphical user interface, command line, batch interface.
- *Type of license* – e.g., commercial software, GNU general public licence, open source, etc.
- *Supported operating system (OS)* – various Windows OS, Macintosh OS, Linux OS, Unix based.

In further analysis only software suitable for 1,4-DHP lipid molecular dynamics process will be discussed. Molecular dynamics simulation as a computer modeling method complies with the assumptions of the mathematical modeling cycle. Classical mathematical modeling competences and cycle more detailed is described by Duka (Duka, 2012). Molecular dynamics modeling cycle consists of five steps: real world problem,

mechanical molecular model, computer model, molecular dynamics simulation, conclusions. These five steps can be repeated cyclically until best model for real world problem representation is chosen. (Fig. 1)

The beginning of molecular dynamics simulation cycle is the *model building part*. From the real world problem the mechanical molecular model and after then also computer model is formed. Mechanical molecular model follows from the theoretical background of molecular dynamics method and molecular modeling theory. As a molecular modeling is an interdisciplinary field, model in molecular dynamics encompass not only mathematical but also physical, chemical, biological theories. Then mechanical molecular model is transferred in computer model development. Simple computer model, that is suitable for molecular dynamics simulation, is data file with descriptions of atom types, atomic coordinates in three dimensional Cartesian coordinate space, atomic connectivity, etc. These computer models are often prepared in the Protein Data Bank (pdb) file format or XYZ file format. There are many ways how to create initial computer models of molecular system. Model building can be carried out manually, by hand, but software assistance as, for example, 3D graphical molecular builders and editors can be used. Most of the model building software that handle large molecular system building are commercial and supported also with graphical user interface, e.g., MOE (Molecular Operating Environment, 2012), MacroModel (MacroModel, 2012). Detailed description of software list in (Table 1)

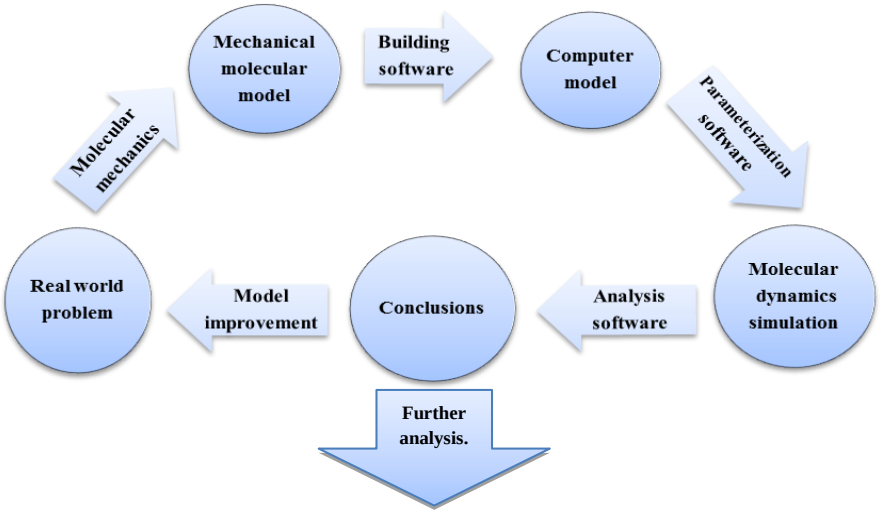

Fig. 1. **Modeling cycle of molecular dynamics simulation.**

After creation of molecular system computer model, it should be prepared for molecular dynamics simulation. In preparation process initial structure is solvated in periodic box, octahedron of, for example, water and then constructed force field files for this system. After such preparation, from initial system (usually one file) several data files are created where the information about topology, trajectory and simulation parameters is going to be kept. Different software tools are used for preparing input files for the simulation programs. For standard molecular dynamics simulation, common known molecular dynamics software developers have grown together with their provided force fields and parameters. The transfer between softwares and force fields is not recommended during single simulation process, therefor preparation, parameterization and simulation of molecular system is usually done using tools from the same developer. Must be noted that for standard molecular structures force field parameters are assigned from known databases, but for non-standard systems even for common used GROMOS, AMBER, and CHARMM force fields, this derivation often takes the form of various quantum mechanical calculations. Also for this reason, automated tools are greatly preferred. For each force field, there are methodologies or software programs for assigning parameters for molecular structures, compatible with various force fields. Using AMBER software for molecular dynamics, Antechamber and Leap packages from Amber Tools software (Case et.al., 2012) prepares the molecular systems and applies all atom AMBER force field to the molecule. CGenFF can be used for generalized force field assignment for CHARMM (Brooks, B. R., et.al., 2009). For molecular dynamics simulation in GROMOS87/GROMOS96 force fields with GROMACS molecular dynamics software, also PRODRG 2.5 (Schüttelkopf and Aalten, 2004) ATB (Automated Topology Builder) (Malde et.al., 2011) web server online services can be used as an automated servers for topology generation. Molecular dynamics simulations in these force fields can be calculated also using other softwares, such as Abalone, NAMD (Phillips et.al., 2005), Ascalph, Maestro (Maestro, 2012), MOE, Desmond (Bowers, 2006). Some of them are more advanced than another and direct graphical dynamics can be very illustrative for demonstrations. But when the research work comes to large biological systems, then the fact that molecular dynamics calculations are time and resource demanding must be noted, and then molecular dynamics

simulations are carried out on a remote Unix based servers in computer centres or laboratories using command line and batch interface. Detailed description of software list in (Table 1)

Following the modelling cycle principles (Duka, 2012), after a molecular dynamics simulation, result analysis should be carried out for making decent conclusions about the behaviour and structure of investigated molecular system. Analysis, like mean energy, density of the system, RMS difference between two structures etc., can be performed manually using different scripts, that reads and represents the molecular dynamics information from large number of data files or specific analysis tools for different purposes can be used. For graphical representations of molecular system and obtained dynamics – systems trajectory, a list of visualization softwares can be found, Vega ZZ (Pedretti, 2004),VMD (Humphrey et.al., 1996), RASMOL (Sayle and Milner-White, 1995, Bernstein, 2000), MOIL (West et.al., 2007), MOE, Maestro, MacroModel, Abalone, Ascalph. Most of the tools for molecular dynamics come together with functions for static or dynamical graphical representations and analysis possibilities, such as MOE, Maestro, MacroModel, some of these tools are only for representation, like Rasmol. Detailed description of software list in (Table 1)

Very important part of molecular dynamics simulation is verification process. Thanks to the many research groups that have been working and developing molecular dynamics, this method has become as independent research method with high precision. However, for every computer simulation result testing should be done either by literature review or comparison with experimental data.

Advantages of modeling is in its possibilities - going beyond visible, seeing further and discovering more using simplified model and computer technologies. The role of molecular dynamics is highly valued especially for the possibility to notice and prove regularities that could not have been seen with any other microscopic methods. Analysis, hypothesis testing and prediction come as another important part of molecular dynamics cycle.

Molecular modeling of 1,4-DHP lipid. This study of molecular dynamics simulation is offered as an example of successful computer experiment. Molecular model of one 1,4-DHP lipid was built using MOE software and lipid bilayer consisting of 72 molecules of 1,4-DHP-lipid was created manually using self-written coordinate transformation script written in programming language C++. With software package Leap from Amber Tools 8.0 1,4-DHP lipid system initially transformed into a periodic lipid bilayer-water box, with 10 Å water over the solute and with a small amount of excessive water on the lipid edges to ensure the mobility of lipid molecules. There were 72 DHP-lipid molecules, 144 counterions of chlorine ions and 4401 water molecules in the system DHP-lipid-water box. The total number of atoms in the system is 22491.

After the 1,4-DHP-lipid-water box were subjected to molecular dynamics, (AMBER 8.0 (f99) force field, version 8.0 [14-16], NTP protocol (constant number of particles, constant temperature, constant pressure). The temperature was increased gradually from T = 10 K by step of 10 degrees till 300 K. Calculations were performed for 326212 ps. Molecular dynamics simulations were started from the temperature T = 10 K and risen gradually till 300 K. Analysis of molecular dynamics results were proceeded with VMD, visualization were made with RASMOL.

The1,4-DHP-lipid-water box system kept the initial bilayer organization at the beginning of the MD simulation, but during MD run lipid molecules squeezed from one bilayer into another, finally forming worm-like micellae. Results of 1,4-DHP lipid MD simulation show that 1,4-DHP lipid in natural conditions does not form a lipid bilayer, but one of its structures is a tubular worm-like micellaes. We could expect that such the micellaes are capable to form a functional structure for the DNA transfection. Result was confirmed with the studies electron microscopy showing extended, worm-like structures as one of the possible 1,4-DHP lipid structures.

Table 1

List of software for performing complete modeling cycle of lipid molecular dynamics simulation

Name	MB(1)	MD(2)	GR(3)	L(4)	Developer	Supported OS	Interface	License
Abalone	+	+	+		Agile Molecule	Windows XP	Graphical user interface	Commercial
AMBER, AMBER Tools	+	+		+	AMBER developer project	Linux, Various Unix workstations	Command line, Batch interface	Commercial
Ascalaph	+	+	+		Agile Molecule	Windows 2K/XP	Graphical user interface	GNU General Public License

Atomistix ToolKit	+	+	+	+	Quantum Wise	Windows XP/Vista/7, Linux	Graphical user interface, Command line	Commercial
CGenFF	+				Harvard University	Linux and Various UNIX workstations	Command line. Batch interface	Commercial, Open source
CHARMM		+		+	Harvard University	Linux and Various UNIX workstations	Command line. Batch interface	Commercial, Open source
Desmond		+			D.E.Shaw Research	Linux and Various UNIX workstations	Command line Batch interface	GNU General Public License, Open Source
GROMACS		+			GROMACS project group	Solaris, Linux, OS-X, Windows Various UNIX workstations	Command line. Batch interface	GNU General Public License Open Source.
MAESTRO MacroModel	+	+	+	+	Schrödinger	Windows XP/Vista/7, Linux, Mac OS X	Graphical user interface, Command line	Commercial
MOE	+	+	+	+	Chemical Computing Group	Windows XP/Vista/7, Linux, Mac OS X	Graphical user interface, Command line	Commercial
MOIL			+		MOIL team	Windows, MacOsX, Linux (Fedora)	Graphical user interface, Command line	GNU General Public License, Open Source
Rasmol			+		Roger A. Sayle, Herbert J.Bernstein	Windows, MacOS, UNIX, VMS systems, etc	Graphical user interface, command line.	GNU General Public License, RASLIC license
VMD NAMD		+	+	+	University of Illinois at Urbana-Champaign	MacOS X, Unix, or Windows	Graphical user interface, Command line, Batch interface	GNU General Public License, Open Source
Vega ZZ		+	+	+	Drug Design Laboratory	Windows Linux and Various UNIX workstations	Graphical user interface, Command line	GNU General Public License, Open Source

(1) MB – Software for molecular model building
(2) MD – Software for molecular dynamics simulation
(3) GR – Software for graphical representations of molecular systems
(4) L – Applicable for lipid analysis

Conclusion

We summarized and systematized the molecular dynamics simulation process, and provided a list of software tools that can be successfully used for different purposes in different steps of molecular dynamics simulation. We showed that molecular dynamics simulation as a computer modeling method complies with the assumptions of the mathematical modeling cycle. That was proved with the successful molecular dynamics studies of 1,4-DHP lipid system. The cycle of 1,4-DHP lipid system modeling was accomplished with the verification of results, that is excellent result although it opens a perspectives for further analysis of this system, while the last step of modeling cycle - analysis and prediction, is still neglected. Further studies promise challenges in the field of availability of the molecular modeling software, while 1,4-DHP lipid system deviated from the standard lipid bilayer structure and formed tubular, worm-like structure. Tubular structure lipid systems are investigated less with molecular modeling methods and software tools.

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