University Babeş-Bolyai
Faculty of Physics

PhD Thesis

Summary

Numerical simulations of complex biomolecular processes

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2018

Keywords: molecular dynamics simulations, CHARMM force-field, polyethyleneimine, polyplexes, Ras proteins, conformational states.

Contents

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Introduction

The priorities of societal challenge areas (notably bio-economy, security, energy, transport, space, eco-innovation) will ultimately depend on the development in the health sector. Since key research areas for health research are rapidly changing and evolving, cancer research remains a hot topic. Modern biophysics and molecular biology provide important insights in medicine (a central challenge is to investigate the behavior of biomolecules). One of the most successful approaches to describe biological phenomena is to characterize the structural dynamics of the molecular systems. The discovery, development, and delivery of drugs, to create new therapeutic strategies for human diseases, using the interface of physics and biology to gain a better understanding of molecules and cell signaling, have a great impact on major global health challenges.

Molecular dynamics (MD) can reveal information about the experimentally inaccessible characterization of dynamic processes close to atomic resolution. Furthermore, owing to the availability of this simulation methodology, new insights into disease mechanisms and improved target conditions are beginning to be available. On the other hand, MD simulations have the potential to significantly enhance the knowledge on the target mechanisms for biomolecules and the behavior of cancer-related proteins. Very often, mutated proteins are identified but it is not clear how they are linked to the specific diseases.

This thesis is organized as follows. Chapter 1 represents the introduction. Chapter 2 deals with the main aspects of MD simulation methodology. A detailed description of the equations of motion and different thermodynamic ensembles used to model real-life conditions is

presented. We specifically mention MD software packages widely used by researchers to simulate complex biomolecular systems. We describe the defining features of molecular mechanics force fields.

Chapter 3 describes the potential energy function of the CHARMM force field and, in particular, the force field development for polyethyleneimine (PEI). The all-atom force field was parameterized to reproduce the structural properties and dynamics of polymer chains in solution. We present the entire set of procedures for the adjustment of valence bond, bond angle and dihedral parameters applied in the course of the force field development process. Atomistic MD simulations allowed us to study the influence of PEI chain size and protonation fraction on the dynamical structure described in terms of gyration radius, end-to-end distance, persistence length, radial distribution functions, and diffusion coefficients. The results are in very good agreement with the experimental data.

Chapter 4 focuses on simulations of cationic polymers as gene carriers, aimed to reduce the number of experiments required in the gene vector development cycle. Here, we considered an alternatively protonated PEI chain as a representative polymer that was employed to describe the complexation between DNA strand and PEI chains. MD simulations of PEI/DNA mixtures were performed at an atomic level in order to understand the processes involved in gene delivery. We described the binding pattern of DNA with multiple PEI chains, which are related to electrostatic interactions of the positively charged amine groups (PEI chains) with the negatively charged phosphate groups (DNA strand).

In Chapter 5, the structure and composition of biological membranes are described. We focus on lipids and proteins, which account for almost all the mass of membranes. Here, we comparatively present three types of membrane models from simple to complex and we motivate the use of simpler versus more complex models in the study of proteins anchored in the membrane. A detailed description of nanodomains, nanoclusters and the signal transduction mechanism is presented in this chapter. Subsequently, we focus on Ras proteins, summarizing the general features and studying the conformational and orientational sampling of the NRas protein.

Simulations and results from these three chapters were published or submitted for publication. The general layout of the published or submitted articles is maintained in thesis chapters, so that each chapter contains a brief introduction, simulation details, results, conclusions, and bibliography. In Chapter 6, we summarize the contributions of the thesis and the impact of the reported research, and discuss the future prospects.

2

Molecular dynamics

Molecular dynamics (MD) is one of the principal tools used to study the time-dependent behavior of the systems at the microscopic level. This computational methodology is used as a complement to NMR and X-ray crystallography measurements in order to investigate the structural flexibility of molecular systems. Within classical MD simulations, the integration of the equation of motion provides trajectories that allow studying the many-particle systems. From these trajectories, a large variety of properties can be evaluated, including thermodynamic and other macroscopic quantities, which can be validated by comparison with experimental results.

Atomistic representation

Atomistic representation is a detailed atom-level description of a large system, which models the collective behavior of its composing atoms. Each atom in a molecule is considered as a point with corresponding mass and partial charge. The force fields differ in the way they are parameterized (derived from experimental data and/or from high-qualiy quantum-mechanical calculations). In order to reduce the number of parameters and to develop force fields that are transferable, the parameters are calculated from small molecules that are subunits of the molecules of interest.

The potential energy function of the CHARMM force field [1] is given by the sum of bonded and non-bonded contributions.

The bonded terms are:

$$U_{bonded} = \sum_{bonds} k_b \left(b - b_0\right)^2 + \sum_{angles} k_\theta \left(\theta - \theta_0\right)^2 + \sum_{dihedrals} k_\psi \left[1 + \cos(n\psi - \delta)\right] + \sum_{impropers} k_\omega \left(\omega - \omega_0\right)^2 + \sum_{Urey-Bradley} k_{UB} \left(b^{1,3} - b_0^{1,3}\right)^2, \quad (2.1)$$

The non-bonded contributions are:

$$U_{non-bonded} = \sum_{atomsi,j} \frac{q_i q_j}{\epsilon_0 r_{ij}} + \varepsilon_{ij} \left[\left(\frac{r_{ij}^{min}}{r_{ij}} \right)^{12} - 2 \left(\frac{r_{ij}^{min}}{r_{ij}} \right)^{6} \right], \quad (2.2)$$

$$r_{ij}^{min} = \left(r_i^{min} + r_j^{min}\right)/2, \;\; \epsilon_{ij} = \sqrt{\epsilon_i \epsilon_j}. \quad (2.3)$$

Coarse-grained representation

Complex biomolecular systems, including proteins inserted in membranes, are difficult investigate by atomistic simulations. The time scales of biological processes require the simulation time to be dramatically extended. In this regard, coarse-grained (CG) simulations use a simplified representation of the system, which enables microsecond-long simulations. CG models derived from atomistic force fields are able to fairly reproduce the physical behavior of the all-atom models. CG description of a biomolecular system regards pseudo atom sites (beads) which are represented at a lower resolution than individual atoms. Each CG bead is composed of entire groups of atoms and reduces the number of interaction sites, thereby improving the simulation speed. The effective CG energy function defines the interaction between beads.

References

[1] K. Vanommeslaeghe, E. Hatcher, C. Acharya, S. Kundu, S. Zhong, J. Shim, E. Darian, O. Guvench, P. Lopes, I. Vorobyov, MacKerell, A. D., J. Comput. Chem., 31 (2010), 671.

[2] S. J. Marrink, H. J. Risselada, S. Yefimov, D. P. Tieleman, A. H. de Vries, J. Phys. Chem. B, 111 (2007), 7812.

3

Polyethyleneimine

Parameterization of the CHARMM force field

In molecular modeling, a force field is constructed by parameterizing the potential function using ab initio quantum mechanical calculations, experimental data, or both. As an indispensable step toward performing realistic PEI simulations, CHARMM FF parameters for protonated PEI were derived by fitting the molecular mechanics (MM) potential energy function to generated quantum mechanical (QM) target data. In order to develop CHARMM-compatible parameters, the ffTK (Force Field Tool Kit) [1] plugin distributed as a part of molecular modeling software package VMD [2] was used to manage the FF parameter adjustment process. The FF development implies defining residues and atom types, which have a characteristic name, a specific mass and charge. Two linear tetramer models were used in the parameterization process: PEI4p0—unprotonated (see Fig. 3.1a) and PEI4p1— protonated at the central nitrogen (see Fig. 3.1b).

The definition of the new atom types in the FF construction is crucial for describing the chemical environments accurately: NH2 – nitrogen atom of NH2 group; HN2 – hydrogen atom of NH2 group; NH1 – nitrogen atom of NH group; HN1 – hydrogen atom of NH1 group; NH2P – nitrogen atom of $NH2^+$ group; HN2P – hydrogen atom of $NH2^+$ group; CH2 – carbon atom adjacent to NH1; CH2P – carbon atom adjacent to NH2P; CH2X – carbon atom adjacent to CH2P; HC2 – hydrogen atom adjacent to CH2, CH2P, or CH2X.

The custom residue types for PEI chains that we defined are: PEI – unprotonated CH2-

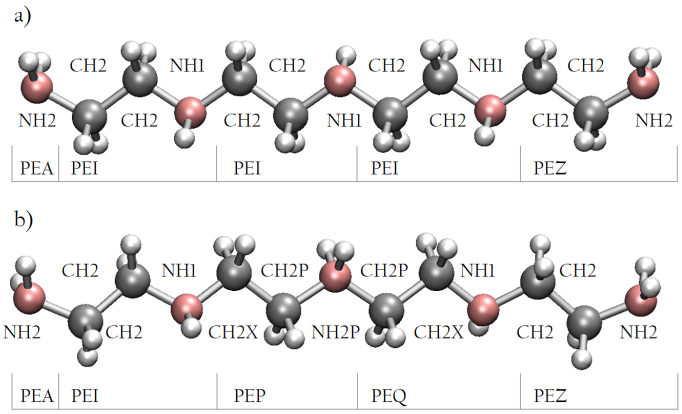

Figure 3.1. PEI models used in the parametrization of the CHARMM force field: a) PEI4p0 unprotonated and b) PEI4p1 protonated at the central nitrogen. The first model contains three unprotonated PEI residues, one PEA starting residue and the terminal PEZ patch. The second model was constructed using the PEA residue, one unprotonated PEI residues, one protonated PEP residue, one unprotonated monomer PEQ that connects the PEI and PEP residues, and the PEZ patch. PEI models are represented with solid spheres (the nitrogen atom is represented with pink color, carbon atoms with gray and corresponding hydrogen atoms with white).

CH2-NH monomer; PEP – protonated CH2-CH2-NH2+ monomer; PEQ – unprotonated monomer that connects PEI and PEP residues; PEA – NH2 group starting a chain, PEZ – patch ending a chain and replacing NH by NH2. The residues PEP and PEQ provide a gradual transition of protonated charge to unprotonated PEI, where the protonated NH_2^+ group is modeled as a PEP-PEQ sequence, (CH2X-CH2P-NH2)-(CH2P-CH2X-NH1).

For each of the two linear models all the parameters (charges, bonds, angles, and dihedrals) were optimized following the automatic fitting procedure implemented in ffTK. The common parameters of the unprotonated PEI4p0 and the protonated PEI4p1 models were considered from the unprotonated model.

Lennard Jones parameters

Nonbonded interaction parameters were assigned to each atom type based on similar types provided in the CGenFF [3] to reproduce bulk phase properties.

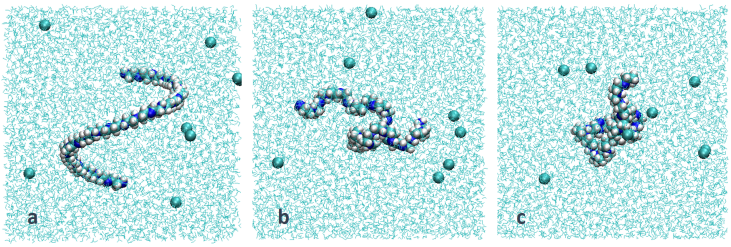

Figure 3.2. (a) Initial configuration, respectively (b), (c) intermediate configurations of a typical trajectory of solvated helicoidal PEI26-mer with 1/3 protonation fraction. The PEI chain and the chloride ions are represented with solid spheres and the water molecules with lines.

Partial atomic charges

The CHARMM FF partial atomic charges were derived to reproduce QM interactions with single TIP3P [4] water molecules placed in representative positions. By following the CHARMM convention, the water-accessible atoms of PEI models are assigned to a list of hydrogen bond acceptors, donors or both. The interactions profiles were calculated by characterizing interactions of complexes between PEI and a single water molecule, each started with an optimized PEI and a single water test molecule opposing one of the PEI atoms and were based on Gaussian geometry optimization at the HF/6–31G(d) level of theory. The charges resulting from optimization are modified within 5%, in order to build independent functional residues of integer charge. The protonated residue PEP and the connector residue PEQ were constructed essentially to have a continuous and uniform charge distribution. The cumulated charge of PEP and PEQ residues amounts to 1.

Optimized bond and angle parameters

The comparison between QM and MM Hessian matrices calculated in internal coordinates (IC) provides the equilibrium values for bonds and angles. The computation of QM Potential Energy Surface (PES) was done by generating small distortions along bonds and angles. The local PES was evaluated in MM using the trial bond and angle parameters for each conformations.

Optimized dihedral parameters

The torsion potential energy profiles were determined by scanning the dihedrals of interest. Dihedral parameters were calculated starting from a fully relaxed Potential Energy Surface

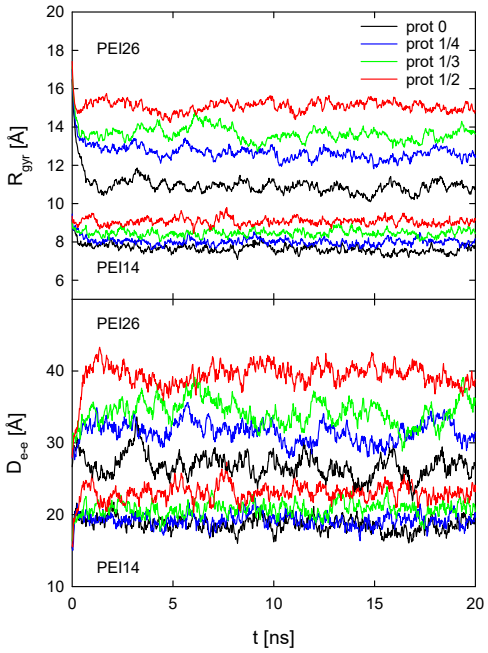

Figure 3.3. Evolution in time of ensemble-averaged gyration radius and, respectively, end-to-end distance for PEI 14-mers and 26-mers at different protonation fractions.

(PES) scan using the QM Hamiltonian and MM Hamiltonian about a single dihedral angle. The difference between the QM and the MM PES was minimized in optimization process using simulating annealing.

Linear PEI chains in aqueous solution

The initial helicoidal configurations with uniform protonation fractions 0, 1/4, 1/3, and 1/2 for PEI 14, 26, and 50-mers were used in MD simulations. Figure 3.2 presents snapshots from a typical run for a 1/3-protonated 26-mer, illustrating the initial helicoidal and two intermediate confgurations of PEI chains.

Spatial PEI chains extent

The ensemble-averaged time dependences for R_g and D_{e-e} for different protonation fractions (equal to 0 (unprotonated) - black, 1/4 (one-in-four) - blue, 1/3 (one-in-three) - green, and 1/2 (alternatively protonated) - red) are represented in Fig. 3.3, where the most compact chain configurations correspond to unprotonated PEI chains. Overall, the R_g and D_{e-e} values

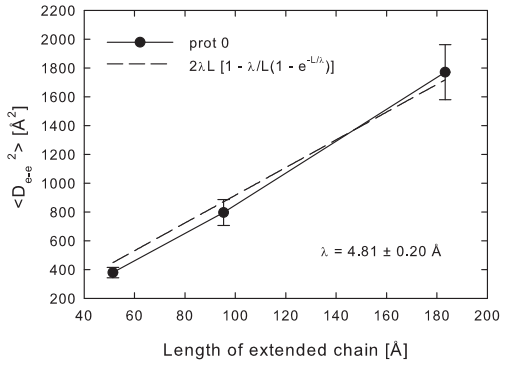

Figure 3.4. Mean square end-to-end distance dependence on the fully extended lengths for unprotonated PEI chains obtained from MD simulations and fitting profile. The worm-like chain model [5] is appropriate to describe the unprotonated PEI chains.

can be seen to substantially increase with chain size, and for a given length, with protonation fraction.

Persistence length

The simulations yield a persistence length for linear PEI equal to 4.88 Å . The persistence length value is in good agreement with the value obtained by Lee et al. [6] for polyethylene oxide (4.3 Å). Figure 3.4 shows that the worm-like chain model [5] can be used to describe unprotonated polymer chains. In the case of the protonated PEI, this model does not adequately reproduce the behavior of the polymer chains.

Radial distribution functions

The spherical environment of the polymer backbone atoms is described by means of the radial distribution function (RDF). The RDF gives the description of the averaged local organization around the atoms. From the RDF profiles depicted in Fig. 3.5, we easily distinguish the different affinities of the carbon and nitrogen atoms for the oxygen atoms of water. The first and second solvation shells are very pronounced for nitrogen species, but for carbon species the second peak is not clearly defined. The oxigen atoms of water are situated on average closer to the nitrogen species than to the carbon species.

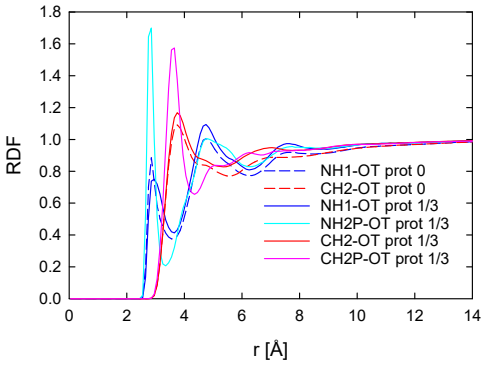

Figure 3.5. Radial distribution functions for the nitrogen atoms and carbon atoms for PEI 26-mers with oxygen atoms of water (unprotonated chains - dashed line and 1/3 protonated chains - solid line).

Diffusion coefficients

The diffusion coefficient is a useful quantity for describing the dynamics of flexible polymers in solution. The mean squared displacement (MSD) was calculated as average deviation over time, between the position of the center of mass and a reference position. The resulted MSD was used to calculate the diffusion coefficient using Einstein's relation. Specifically, the diffusion coefficients of the PEI chains decrease both with the chain length and protonation ratio.

Summary

The parametrization of the additive atomistic CHARMM force field for polyethyleneimine was reported based on the published journal article [7]. We determined the force field parameters compatible with the CHARMM36 force field using the ffTK parameterization procedure. High-quality ab initio calculations on tetramer PEI models provided the entire set of target data. The partial atomic charges and all the bond-, angle-, and torsion terms were consistently adjusted in the parameterization process.

Effective gene delivery systems based on cationic polymers are essential for using gene therapy in clinical applications. In this regard, molecular dynamics simulations were employed to investigate the linear PEI chains solvation behavior in different protonation states. The dynamic structuring was characterized by gyration radius, end-to-end distance, persistence

length, radial distribution function, and diffusion coefficient. The gyration radius and the end-to-end distance increase with chain size and protonation.

The orientation of water molecules in regard to the backbone atoms of the polymer chains is given by the radial distribution functions. The water molecules are predominantly oriented with the dipole moment away from the nitrogen atoms composing the protonated amine groups. The center-of-mass diffusion coefficients decrease both with chain size and protonation fraction and describe very well earlier experimental results.

References

[1] C. G. Mayne, J. Saam, K. Schulten, E. Tajkhorshid, J. C. Gumbart, Rapid parameterization of small molecules using the Force Field Toolkit, J. Comput. Chem., 34 (2013), 2757.

[2] W. Humphrey, A. Dalke, K. Schulten, VMD: Visual molecular dynamics, J. Mol. Graph., 14 (1996), 33.

[3] K. Vanommeslaeghe, E. Hatcher, C. Acharya, S. Kundu, S. Zhong, J. Shim, E. Darian, O. Guvench, P. Lopes, I. Vorobyov, A. D. MacKerell Jr., CHARMM General Force Field: A Force field for Drug-Like Molecules Compatible with the CHARMM All-Atom Additive Biological Force Field, J. Comput. Chem., 31 (2010), 671.

[4] W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, M. L. Klein, Comparison of simple potential functions for simulating liquid water, J. Chem. Phys., 79 (1983), 926.

[5] J. E. Mark, P. J. Flory, The Configuration of the Polyoxyethylene Chain, J. Am. Chem. Soc., 87 (1965), 1415.

[6] H. Lee, R. M. Venable, A. D. Mackerell Jr., R.W. Pastor, Molecular Dynamics Studies of Polyethylene Oxide and Polyethylene Glycol, Biophysical Journal, 95 (2008), 1590.

[7] T. A. Beu, A. Farcaş, CHARMM force field and molecular dynamics simulations of protonated polyethylenimine, J. Comput. Chem, 38 (27) (2017), 2335.

4

Formation of DNA-polyethyleneimine complexes

Gene therapy

Scientists have been trying for decades to provide clinical implementation of gene delivery, yet only a few patients have received effective treatment. Gene therapy is designed to introduce nucleic acids into cells to treat cancer and different disorders (immunological, neurological and cardiovascular) [1].

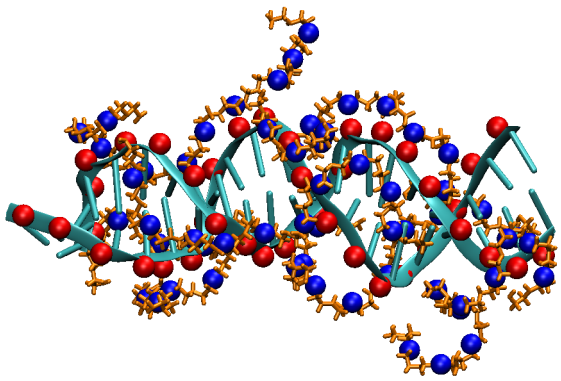

Figure 4.1. Binding of PEI chains to the DNA complex (polyplexes). Nitrogens from the protonated amine groups are represented with vdW spheres in blue and phosphorus atoms of DNA in red. PEI chains are illustrated with licorice representation and DNA strand with cartoon representation

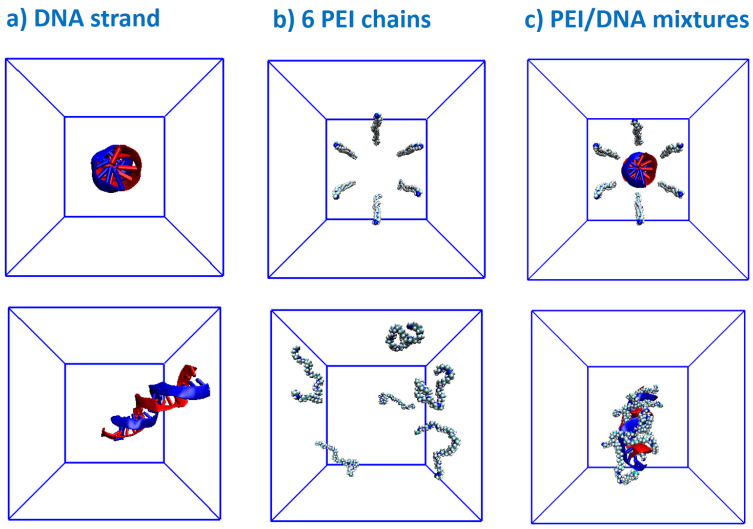

Figure 4.2. Initial/intermediate configurations for a) DNA strand (chain A - red, chain B -blue using cartoon representation), b) PEI chains (vdW spheres) and c) PEI/DNA mixtures during typical trajectory. The water molecules and counterions are excluded for clarity.

Nonviral gene delivery vectors

Nonviral vectors provide a safe delivery of genetic material into host cells [2]. Among different nonviral gene vectors, cationic polymers are the most utilized non-viral vectors, which combine efficiency and safety. Mixtures of cationic polymers with DNA form nanosized complexes called polyplexes (see Fig. 4.1). PEI is considered the most attractive cationic polymer utilized for in vivo/vitro gene transfer. Due to the high density of polymer amine groups, the transfection is very efficient (proton sponge effect). In this work, we performed all-atom MD simulations to study the interaction of PEI chains with DNA.

Formation of complexes between nucleic acids and synthetic polymers

We considered three types of systems, respectively, (a) solvated DNA strand (see Fig. 4.2a), (b) solvated PEI chains (see Fig. 4.2b), and (c) solvated DNA-PEI (see Fig. 4.2c) mixture.

The time dependence of the gyration radius (R_g) for free PEI20-mers with 1/2 uniform protonation fraction in solution is shown in Fig. 4.3, where the average R_g is represented with black line. This figure indicates that the polymer is basically rigid and become more compact as time elapses. Our value for alternatively protonated PEI20-mers, namely 1.25 ± 0.50 nm,

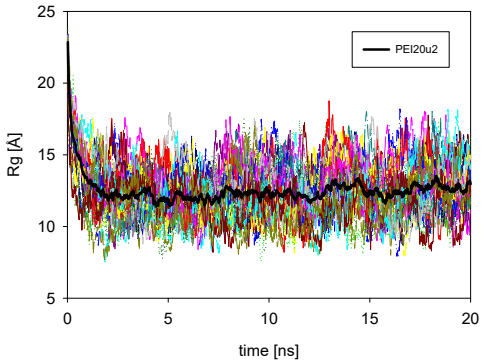

Figure 4.3. Time evolution of the gyration radius for 6 alternatively protonated PEI 20-mers in 4 independent trajectories. The average profile is represented with black.

compares very well with the value of 1.27 ± 0.56 nm obtained by Choudhury et al. [3] from MD simulations. The average R_g of PEI chains interacting with DNA is 12.65 Å and the difference in Rg between the bare PEI chains and PEI chains interacting with DNA values is less than 0.1 Å .

To provide a characterization of the spatial extent for free PEI chains in solution, and respectively, of PEI chains interacting with DNA, we compare the probability distribution of the time-averaged R_g of polymer chains. Free PEI chains in solution exhibit a single peak corresponding to an arbitrary folded configuration. The distribution profile for DNA-interacting PEI chains shows at aproximately 14 Å, an additional peak, which corresponds to R_g values of PEI chains that interact with DNA (PEI/DNA polyplexes). Polyplexes also contain PEI chains free in solution and these contribute to enhancing the gene therapy efficiency, as suggested by experiments [4].

To describe the electrostatics around the DNA strand and the role of PEI charge content, were calculated cumulative charge distributions around DNA. We found that more Na^+ ions reside around bare DNA in solution than around complexed DNA, the latter attracting more Cl^- ions. A clear decrease in the number of attracted Na^+ evidenced the screening effect of PEI on the phosphate groups of DNA.

Summary

In this chapter, the dominant pathways of the PEI/DNA self-assembly process were described using molecular dynamics simulations. The simulations confirm that PEI chains and DNA

can form PEI-DNA polyplexes driven by the electrostatic interactions between the positively charged amine groups and the negatively charged phosphate groups.

We focus on the comparative study between free PEI chains, respectively free DNA in solution and PEI/DNA mixtures. Our results reveal that some of the alternatively PEI chains in free form bind to DNA and exhibit a considerably higher radius of gyration in comparison with those in free form. We find that fewer sodium counterions are attracted by the polyplexes than by free uncomplexed DNA.

The reported force field enables the atomic-level understanding of how PEI chains interact with DNA strands. To show the impact of the polymer protonation on the complexation between anionic DNA and PEI chains, we determined the number of the protonated/unprotonated amine groups in direct contact with the DNA strand. We conclude that the improvement of the gene delivery systems requires to adjust the PEI-based nonviral vectors by precisely controlling parameters such as the protonation and length of the polymer chains.

References

[1] M. A. Mintzer, E. E. Simanek, Nonviral vectors for gene delivery, Chem. Rev., 109 (2009), 259.

[2] D. W. Pack, A. S. Hoffman, S. Pun, P. S. Stayton, Design and development of polymers for gene delivery, Nature Rev. Drug Discov., 4 (2005), 581.

[3] C. K. Choudhury, S. Roy, Structural and dynamical properties of polyethylenimine in explicit water at different protonation states: a molecular dynamics study, Soft Matter, 7 (2013), 2269.

[4] Y. Yue, F. Jin, R. Deng, J. Cai, Y. Chen, M. C. M. Lin, H. F. Kung, C. Wu, Revisit Complexation between DNA and Polyethylenimine - Effect of Uncomplexed Chains Free in the Solution Mixture on Gene Transfection, J Controlled Release, 155 (2011), 67.

5

Lipid-Anchored NRas signalling proteins

Ras signalling proteins are important regulators of a series of signal transduction pathways involved in highly diverse biological processes, including cell differentiation, growth and proliferation [1]. Point mutations found in components of these signaling cascades are associated with approximately 30% of all human cancers. To date there have been few studies examining the role of NRAS in cancer within the context of the specific genetic alterations. To expand and motivate the existent knowledge, MD simulations are used to show their application in the study of NRas proteins that are anchored to the membrane.

NRas is a membrane-associated Ras protein attached to the inner part of the membrane by two post-translational modification (palmitoylated cysteine and farnesylated cysteine). Because the previous studies focused on to HRas and KRas, the structural behavior of the NRas protein-membrane system is not well understood. The NRas mutation-dependent orientations relative to the membrane, involves changes in lipid-protein interactions, leading to different distributions of conformational states. Our investigations are focused on G12V mutation, which is predominant in NRas.

Ras proteins belong to a class called small GTPase, which control the activity of multiple signaling pathways (e.g. mitogen-activated protein kinase - MAPK) involved in diverse cellular processes. Ras proteins function as binary molecular switches, cycling between GTP-bound active state, that turn on several effectors to activate downstream pathways and the GDP-bound inactive state. The Ras activity is regulated by two classes of signaling proteins: guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) [2].

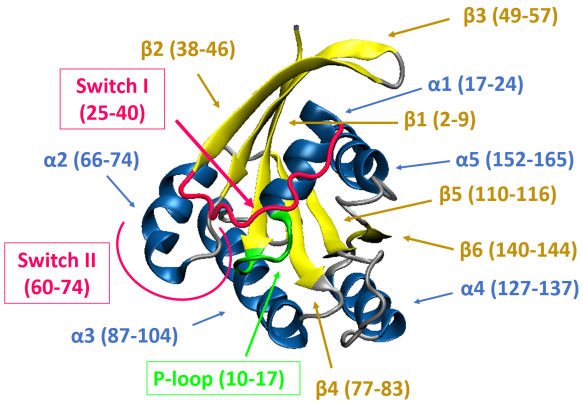

Figure 5.1. NRas protein G-domain secondary structure is colored in blue for α-helices, yellow for β-sheets and gray for loops. The functionally important regions, switch I (magenta) and switch II (magenta), together with the phosphate-binding P-loop (green) are illustrated.

Ras structure

Ras proteins are composed of two subunits: G-domain and hypervariable region (HVR). The G-domain of Ras comprises 166 amionacids necessary for binding guanosine diphosphate (GDP) or guanosine triphosphate (GTP), GAP or GEF, and effector proteins. Ras G-domain secondary structure [3] contains six β sheets and five α helices (see Fig. 5.1). Additionally, three loop regions mediate cycling between the active GTP-bound state and the inactive GDP-bound state [4]. P-loop (G1 region) is comprised of residues 10-17, being responsible for binding the beta phosphate of GDP and GTP. Switch I (G2 region) is a loop comprised of residues 25-40 and contains residue THR35, which binds the terminal phosphate of GTP and the magnesium ion. Switch II is composed of a loop and helix α2 (60-74) and mediates the Ras functionality as a molecular switch protein.

Activation mechanism of Ras involves binding of GEF proteins which destabilize the interaction of Ras with GDP and Mg^{2+} [5]. GTP concentration in the cytoplasm is 10 times higher than that of GDP, which determines GTP to bind to the nucleotide-free form. Ras protein is transformed in active site and allows binding of effector proteins to G-domain, which triggers distinct signaling cascades.

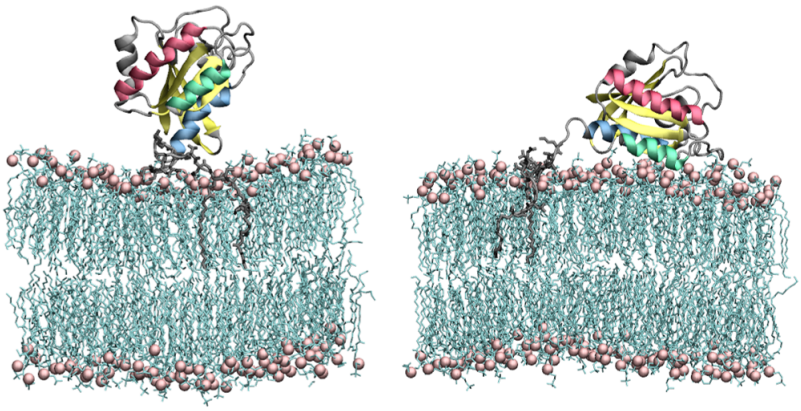

Figure 5.2. The two initial starting configurations of NRas in GDP-native (left) conformation (at approximately 45° with the membrane) and GTP-native (right) conformation (quasi-parallel to the membrane). The five α-helices are colored in different colors, β-sheets in yellow, loops in grey, cysteine palmitoyl and farnesyl lipid modifications in gray licorice representation, the membrane model in cyan, with P atoms (pink) in bead representation.

Ras point mutations

A point mutation represents a substitution in the protein of one amino acid with another. The point mutations in Ras proteins are associated with different types of cancer. Mutations of the Ras gene occur in over 30% of all human cancers. The most common mutation in NRas is G12V, represented by the amino acid substitution at position 12, from GLY to VAL.

NRas orientation relative to the membrane

The trajectories related to native NRas (WT) and NRas with G12V mutation (G12V) were analyzed by studying the structural properties. The HRas representative structures [6] (parallel orientation dominant in GTP-bound HRas and perpendicular orientation in the GDP-bound HRas of the G-domain represented in Fig. 5.2) were used as starting conformations for all MD simulations. NRas WT shows a predominant parallel orientation relative to the lipid bilayer and NRas G12V exhibits and orientation of 45^o-60^o with respect to the membrane. These orientations correspond to GTP-, respectively GDP-bound conformations obtained both from HRas simulations [6], and from IRRA spectroscopy experiments of NRas inserted in a similar bilayer (DPPC:DOPC:CHOL 50:25:25) [7, 8].

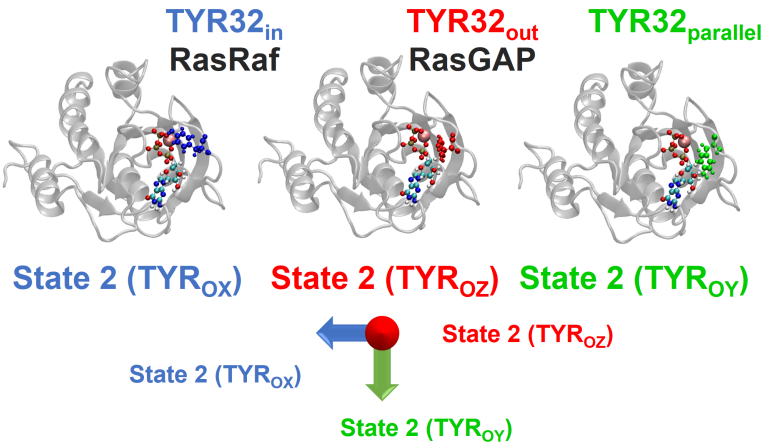

Figure 5.3. NRas exhibits three conformational substates of state 2, which are characterized by the quasi-perpendicular orientations of Tyr32. Recently was proposed that TYR_{OX} (blue) corresponds to Ras-Raf binding (signal-transduction), while TYR_{OZ} (red) to Ras-GAP binding (enhanced GTP hydrolysis) [9]. We propose that TYR_{OY} (green) corresponds to an effector binding state. Protein is shown in secondary structure representation in grey, TYR32 residue and GTP molecule in vdW spheres representation. The arrows indicate the quasi-90° orientation of the TYR32 in the three substates, with the arrow head from the backbone towards the ring of the aminoacid.

WT and G12V conformational states

The three Ras isomers adopt two different conformations, known as State 1 (inactive) and State 2 (active) [10]. The active/inactive form refers to specific interaction between Ras and the effector molecules. Recently, Li and co-workers [9] showed that WT NRas proteins have two substates of State 2: $TYR32_{in}$ and $TYR32_{out}$, where first is associated with interaction between proteins and effectors (like Raf) and the second with affinity for GAP. Our simulations sampled all currently known states (State 1 and State 2 ($TYR32_{in}$ - TYR_{OX}, $TYR32_{out}$ - TYR_{OZ})). We identified a new substate of State 2, TYR_{OY} (see Fig. 5.3). The schematic NRas conformations of State 2 are represented in Fig. 5.3, where the 3 substates of State 2 are characterized by the residue TYR32 quasi 90° orientation: TYR_{OX} blocks the GTP pocket, TYR_{OZ} is oriented outwards and TYR_{OY} is parallel to the GTP pocket.

The alternation between the TYR_{OX} and TYR_{OZ} substates disappears, NRas G12V protein being blocked in TYR_{OY} substate (with residue TYR32 oriented toward residue ALA18). This substate occurs frequently in the presence of membrane-bound NRas G12V mutation. Since G12V is an oncogenic mutation, we propose that the newly discovered NRas

substate TYR_{OY} is linked to Ras effectors, blocking NRas G12V into its permanently active form. This, in turn, prevents the transition to state TYR_{OZ}, the binding of GAP to Ras and, therefore, the enhanced GTP hydrolysis and Ras inactivation.

Summary

In this chapter, we performed MD simulations of a ternary symmetric, simple and complex asymmetric bilayer. Ternary symmetric lipid bilayers are the simplest model membrane systems that exhibit phase separation and are perfectly suited to study the NRas proteins that are anchored to the membrane.

Specifically, we investigate the orientational and conformational sampling GTP-bound NRas proteins. We show that G-domain orientation of NRas WT and NRas G12V correspond to GTP-/GDP-bound H-/N-Ras WT. NRas WT has a predominant parallel orientation relative to the membrane and NRas G12V exhibits an orientation of 45°-60° relative to the membrane.

We sampled all previously known Ras conformational states: state 2 (active) and state 1 (inactive). Our results showed that a new substate of state 2 with TYR32 residue oriented parallel to the GTP pocket appears. The residue TYR32 for the three substates of state 2 is quasi 90° oriented. The new substate apears frequently in the presence of G12V mutation in membrane-bound NRas. Because G12V is an oncogenic mutation and we found that the GAP-binding conformation is not sampled in NRas G12V membrane-bound simulations, we propose the new substate to be an effector binding conformation. Hence, without the GAP-enhanced GTP hydrolysis NRas G12V remains permanently active.

References

[1] M. Barbacid, ras genes, Annu. Rev. Biochem., 56 (1987), 779.

[2] J. Tcherkezian, N. Lamarche-Vane, Current knowledge of the large RhoGAP family of proteins, Biol. Cell, 99 (2007), 67.

[3] E. F. Pai, U. Krengel, G. A. Petsko, R. S. Goody, W. Kabsch, A. Wittinghofer, Refined crystal structure of the triphosphate conformation of H-ras p21 at 1.35 A resolution: implications for the mechanism of GTP hydrolysis, EMBO J., 9 (1990), 2351.

[4] M. Spoerner, C. Herrmann, I. R. Vetter, H. R. Kalbitzer, A. Wittinghofer, Dynamic properties of the Ras switch I region and its importance for binding to effectors, Proc Natl Acad Sci U S A., 98 (2001), 4944.

[5] J. L Bos, H. Rehmann, A. Wittinghofer, GEFs and GAPs: critical elements in the control of small G proteins, Cell, 129 (2007), 865.

[6] A. A. Gorfe, M. Hanzal-Bayer, D. Abankwa, J. F. Hancock, J. A. McCammon, Structure and Dynamics of the Full-Length Lipid-Modified H-Ras Protein in a 1,2-Dimyristoylglycero-3-phosphocholine Bilayer, J. Med. Chem., 50 (2007), 674.

[7] S. Kapoor, G. Triola, I. R. Vetter, M. Erlkamp, H. Waldmann, R. Winter, Revealing conformational substates of lipidated N-Ras protein by pressure modulation, Proc. Natl. Acad. Sci. USA, 109 (2012), 460.

[8] S. Kapoor, K. Weise, M. Erlkamp, G. Triola, H. Waldmann, R. Winter, The role of G-domain orientation and nucleotide state on the Ras isoform-specific membrane interaction, Eur. Biophys. J., 41 (2012), 801.

[9] Y. Li, Y. Zhang, S. Stephan, S. Cui, C. Kotting, F. Xia, K. Gerwert, Specific Substates of Ras to Interact with GAPs and Effectors: Revealed by Theoretical Simulations and FTIR Experiments, J. Phys. Chem. Lett., 9 (2018), 1312.

[10] M. Geyer, T. Schweins, C. Herrmann, T. Prisner, A. Wittinghofer, H.R. Kalbitzer, Conformational transitions in p21ras and in its complexes with the effector protein Raf-RBD and the GTPase activating protein GAP, Biochemistry, 35 (1996),10308.

6

Conclusions

The thesis is divided into three main parts. The first part presents a novel parameterization of the CHARMM force field for polyethyleneimine (PEI). We developed a realistic fine-grained force field for PEI, rigorously derived from high-quality quantum mechanical calculations. The ability of this force field for PEI to describe both the unprotonated and protonated states enables accurate simulations of PEI chains in conjunction with nucleic acids, lipids or proteins. We investigated the dynamic structuring of solvated PEI chains in different protonation states, in terms of synthetic parameters. The developed force field and the insight gained are meant to be a stepping stone for future efforts of developing atomistic force fields that accurately describe a broad range of complex biomolecular systems.

In the second part, we provided new insight into the atomistic behavior of DNA/PEI polyplexes which is expected to bolster improved design of PEI-based nonviral vectors by using molecular dynamics simulations. The particular charge distribution of PEI affects both the electrostatic interaction between the negatively-charged DNA and positively charged PEI chains and polyplexes formation. The investigated binding patterns of the charged interactions sites of PEI chains and DNA strand extend the knowledge in the field of cationic polymers, enabling an improved design of gene delivery protocols.

The third part deals with NRas proteins which are the main cause of melanoma, thyroid cancer and leukemia. We used molecular dynamics simulations to investigate orientational and conformational sampling for the GTP-bound form of the membrane-anchored NRas protein. Wild-type NRas is parallel to the membrane, whereas the oncogenic NRas G12V is at

an angle, which unexpectedly corresponds to inactive GDP-bound NRas' orientation. Our molecular dynamics simulations sampled all previously known conformational states: state 1 (inactive) and state 2 (active). We identified a novel substate of state 2, and determined that the tyrosine residue at position 32 for the three substates of this active state has different orientations: one perpendicular to the GTP pocket, one oriented towards the bulk water, and the new substate oriented parallel to the GTP pocket and in close contact with the alanine residue at position 18. Moreover, we found that the sampling of the new substate of membrane-bound protein appears predominantly in the presence of the G12V mutation. Considering that this mutation is oncogenic and that in our simulations the substate corresponding to the GTP hydrolysis is not present in G12V (as opposed to wild-type), we postulate that the new substate is signal transmitting.

Outlook

The coarse-grained force field development for PEI will extend the scientific knowledge base of the area of synthetic polymers by enabling large-scale molecular dynamics simulations. The coarse-grained investigations of DNA condensation are aimed to enhance the efficiency of condensation process. In addition, the all-atom force field development for branched and grafted PEI will provide estimates for optimal architectures of cationic polymers, in order to exploit their potential as non-viral vector systems.

Along the last research line, we intend to develop a new approach on identifying potential medications against oncogene Ras proteins. We will investigate dynamics of association for NRas in full GTP-Ras conformation to determine association binding sites with their corresponding binding strengths. The results could be used to design novel drugs to prevent association of oncogenic Ras proteins. Another research direction will include the free energy profile calculations, namely the potential of mean force (PMF) profile for transition between the active NRas substates.

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Acknowledgments

First and foremost, I would like to express my sincere gratitude to my supervisor Prof. Dr. Titus A. Beu for introducing me to this exciting field of science and for his guidance, constant dedication and tremendous support. Besides my advisor, I would like to thank to my co-advisor Dr. Lóránt Jánosi for his constant support. Further, I would like to thank the other members of my thesis committee: Prof. Dr. Zoltán Néda and Prof. Dr. Vasile Chiş for their invaluable feedback.

Many thanks to Andrada-Elena Ailenei and Dr. Roxana Pasca for proof-reading my thesis.

This work was supported by grants of the Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI), project numbers: PN-III-P4-IDPCE-2016-0474, PN-II-RU-TE-2014-4-2418, PN-III-P1-1.1-TE-2016-0032.

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List of publications

Journal Articles

1. T. A. Beu, A. Farcaş, " Tight-binding normal mode analysis of suspended single-wall carbon nanotubes", EPL 113, 37004 (2016).
   ISI: 1.957
   AIS = 0.8

2. T. A. Beu, A. Farcaş, " CHARMM force field and molecular dynamics simulations of protonated polyethylenimine", J. Comput. Chem. 38(27), 2335 (2017).
   ISI: 3.229
   AIS = 1.4

3. T. A. Beu, A. Farcaş, " Structure and Dynamics of Solvated Polyethylenimine Chains", AIP Conference Proceedings 1916, UNSP 020001 (2017).

4. A. Farcaş, T. A. Beu, " Complexation of DNA with cationic polymers", STUDIA UBB CHEMIA, LXIII, 2, 165 (2018).
   ISI: 0.305
   AIS = 0.1

5. T. A. Beu, A. E. Ailenei, A. Farcaş, " CHARMM Force Field for Protonated Polyethyleneimine", J. Comput. Chem., accepted (2018), DOI:10.1002/jcc.25637.

ISI: 3.221
AIS = 1.4

Journal Articles (submitted or in preparation)

1. A. Farcaş, L. Janosi, " Distribution and Dynamics of N-Ras Conformational States and Substates are Modulated by Membrane and Point Mutation", in preparation.

2. T. A. Beu, A. Farcaş, A. E. Ailenei, " MARTINI Force Field for Protonated Polyethylenimine", in preparation.

Conference contributions

1. A. Farcaş, L. Buimaga Iarinca, C. Floare, L. Janosi, " Ras proteins-Mammalian membrane interactions and dynamics using coarse-grained models", National Conference in Biophysics (CNB), 2-4 June 2016, Cluj-Napoca, Romania, poster presentation.

2. A. Farcaş, L. Buimaga Iarinca, C. Floare, L. Janosi, " Plasma membrane model dynamics in the presence of Ras protein nanoclusters", International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences (ICANMBES), 29 June-1 July 2016, Brasov, Romania, poster presentation.

3. T. A. Beu, A. Farcaş, " Force Field Modeling and Molecular Dynamics Simulations of Cationic Polymers", Molecular Modeling in Chemistry and Biochemistry (MOLMOD), 13-15 November 2016, Cluj-Napoca, Romania, oral presentation.

4. A. Farcaş, T. A. Beu, " Molecular Dynamics Simulations of Cationic Polymers", 19th International Union of Pure and Applied Biophysics (IUPAB) and 11th European Biophysical Societies' Association (EBSA) Congress, 16-20 July 2017, Edinburgh, UK, poster presentation, best poster award.

5. A. Farcaş, L. Buimaga Iarinca, C. Floare, L. Janosi, " Influence of G12V mutation on NRas proteins' aggregation", 19th International Union of Pure and Applied Biophysics (IUPAB) and 11th European Biophysical Societies' Association (EBSA) Congress, 16-20 July 2017, Edinburgh, UK, poster presentation, best poster award.

6. L. Janosi, C. Floare, A. Farcaş, L. Buimaga Iarinca, " In silico study of Ras-binding peptides' self-association", 19th International Union of Pure and Applied Biophysics (IUPAB) and 11th European Biophysical Societies' Association (EBSA) Congress, 16-20 July 2017, Edinburgh, UK, poster presentation.

7. L. Buimaga Iarinca, C. Floare, A. Farcaş, A. S. Porav, L. Janosi, " Use of complementary molecular modeling approaches in search of peptides binding to oncogenic Ras", 19th International Union of Pure and Applied Biophysics (IUPAB) and 11th European Biophysical Societies' Association (EBSA) Congress, 16-20 July 2017, Edinburgh, UK, poster presentation.

8. T. A. Beu, A. Farcaş, " CHARMM force field and molecular dynamics simulations of polyethylenimine chains", 4th International Conference on Physical and Theoretical Chemistry, 18-19 September 2017, Dublin, Ireland, oral presentation.

9. A. Farcaş, L. Buimaga Iarinca, C. Floare, L. Janosi, " Multiscale Models of Wildtype and G12V Mutant NRas Oncogenic Systems", 11th International Conference on Processes in Isotopes and Molecules (PIM), 27-29 September 2017, Cluj-Napoca, Romania, poster presentation.

10. A. Farcaş, T. A. Beu, " Cationic Polymers as Drug Delivery Systems", International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences (IC-ANMBES), 23-25 May 2018, Brasov, Romania, oral presentation.

11. A. Farcaş, L. Buimaga Iarinca, A. S. Porav, C. Floare, L. Janosi, " Structural Features and Aggregation of NRas Proteins and it's Oncogenic Mutations", International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences (IC-ANMBES), 23-25 May 2018, Brasov, Romania, poster presentation.

12. L. Buimaga Iarinca, A. Farcaş, L. Janosi, " Use of Molecular Docking as a Tool for Comparative Binding Analysis of Large Peptides to Ras Wild Type and Oncogenic Proteins", International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences (IC-ANMBES), 23-25 May 2018, Brasov, Romania, poster presentation.

13. T. A. Beu, A. Farcaş, A. E. Ailenei, " Atomistic and Coarse-Grained Modelling of Gene Delivery Polymers", 12th Joint Conference on Mathematics and Computer Science (12th MaCS), 14-17 June 2018, Săcuieu, Romania, oral presentation.

14. T. A. Beu, A. Farcaş, A. E. Ailenei, " Atomistic and Coarse-Grained Modelling of Polyethylenimine for Gene Delivery Applications", Polymer World Congress (PWC), 3–6 September 2018, Stockholm, Sweden, Romania, oral presentation.

Other contributions

1. A. Farcaş, C. Floare, L. Buimaga Iarinca, L. Janosi, " Mammalian membrane model behavior in the presence of Ras protein nanoclusters", FEBS JOURNAL 283, 236-236 (2016).

2. A. Farcaş, L. Buimaga Iarinca, C. Floare, L. Janosi, " Effect of complex mammalian membrane models with multiple membrane components on Ras protein nanoclustering", FEBS JOURNAL 283, 235-235 (2016).