Résumé

1. *Name:* Maciej Długosz

2. *Diplomas, academic degrees:*
   - Ph.D. in physics, University of Warsaw, Warsaw 2006, thesis *"Constant-pH molecular dynamics algorithms and their applications to model compounds and small peptides"*
   - M.Sc. in physics (biophysics), University of Warsaw, Warsaw 2002, thesis *"Methods of analysis of stopped-flow spectrophotometry experiments"*

3. *Employment history:*
   - since 01/2012 University of Warsaw, Centre of New Technologies, adiunkt naukowy
   - 05/2007 - 06/2012 University of Warsaw, Interdisciplinary Centre for Mathematical and Computational Modelling / Centre of New Technologies, post-doc position, FOCUS program, Foundation for Polish Science
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4. *Scientific achievement referred to in art. 16, paragraph 2 of the act of 14 march 2003 Law of Academic Degrees and Title and Degrees and Title in the Arts (Official Journal of Laws of 2003, No. 65, item 595, as amended):* a series of six papers under the common title:

"Brownian dynamics modelling of biomolecular diffusion and association - effects of anisotropy, hydrodynamic interactions and molecular crowding"

A. Długosz, M.; Trylska, J. *"Diffusion in crowded biological environments. Applications of Brownian dynamics"*, BMC Biophysics, 2011, 4:3, review article

B. Długosz, M.; Zieliński, P.; Trylska, J. *"Brownian dynamics simulations on CPU and GPU with BD_BOX"*, Journal of Computational Chemistry, 2011, 32, 2734-2744

C. Długosz, M.; Antosiewicz, J. M.; Zieliński, P.; Trylska, J. *"Contributions of far-field hydrodynamic interactions to the kinetics of electrostatically driven molecular association"*, Journal of Physical Chemistry B, 2012, 116, 5437-5447

D. Długosz, M.; Antosiewicz, J. M. *"Hydrodynamic effects on the relative rotational velocity of associating proteins"*, Journal of Physical Chemistry B, 2013, 117, 6165-6174

E. Długosz, M.; Antosiewicz, J. M. *"Anisotropic diffusion effects on the barnase-barstar encounter kinetics"*, Journal of Chemical Theory and Computation, 2013, 9, 1667-1677

F. Długosz, M.; Antosiewicz, J. M. *"Evaluation of protein's rotational diffusion coefficients from simulations of their free Brownian motion in volume-occupied environments"*, Journal of Chemical Theory and Computation, 2014, 10, 481-491

On modelling of diffusion in biological systems

Most processes that occur in cells of living organisms at low Reynolds number are either governed by diffusion or involve stages that are diffusion-controlled (1). Transport, formation of macromolecular complexes, enzymatic reactions, signal propagation - these are just a few examples of diffusion-mediated processes. This ubiquity of diffusion and its fundamental role in cell biology motivates experimental and theoretical studies.

Our knowledge on molecular basis of functioning of cells of living organisms results predominantly from *in vitro* studies of dilute solutions of biomolecules with known and controlled composition. Even though it has been widely known that biological processes occur in complex heterogeneous media (cell's interior, intercellular space) in which the total concentration of molecules falls in the range between 50 and 400 mg/ml with molecules occupying up to 40% of the accessible volume, this issue has been ignored for a long time (2, 3). Theoretical and experimental studies from the end of the 20th century and from the beginning of the 21st century have proved that conditions that occur in cells modify molecular diffusion and quantitatively and qualitatively alter biological processes in comparison with their *in vitro* counterparts. Significant progress in the field of experimental techniques has made possible both *in vitro* and *in vivo* studies of macromolecular systems, for example with NMR (7, 8) or fluorescence methods (9, 10). However, to achieve the complete understanding of mechanisms of diffusion and biological processes under different conditions, computer simulations are required. Their major advantages are the possibility to observe molecular systems at virtually any resolution, simultaneous observation of different molecular species and the possibility to investigate the impact of different factors (ionic strength, pH, molecular composition of the solution, concentrations of molecular species) and interactions (intermolecular, for instance excluded volume, polar and nonpolar, or solvent-mediated hydrodynamic interactions) on the studied system.

Brownian dynamics (11) is one of the many computer simulation techniques that can be applied to study complex biological systems. It allows (potentially) for simulations of systems containing tens, hundreds or even thousands of molecules, with length scales of nano- or micrometers and time scales of micro- or even miliseconds, either at the atomic level of detail or with coarse-grained models. Brownian dynamics algorithms, their limitations and their exemplary applications are described in the review article (A) with a particular focus on molecular diffusion and association in crowded interiors of living cells.

Originally, the most popular applications of Brownian dynamics in the field of biophysics were studies on the kinetics of diffusional association. In such studies

bimolecular association rate constants are derived based on Brownian dynamics simulations of two binding partners immersed in an infinite solvent (11, 12). In a last few years, Brownian dynamics has been also used to model molecular diffusion and association under *in vivo*-like conditions (A). However, these Brownian dynamics simulations are far from being routine. On the one hand, this is a technical issue, as Brownian dynamics software that allows for such simulations is not readily available. On the other hand, models and algorithms that are employed in such simulations are often based on *ad hoc* approximations, which are not always justifiable, and may potentially falsify or limit conclusions derived from simulations. Development and verification of models and simulation algorithms are important for the improvement of tools of modern biophysics as well as for our understanding of fundamental aspects of biological processes.

The focus of my studies that employ the Brownian dynamics technique is on factors which, while often neglected in Brownian dynamics simulations, influence diffusion-mediated biological processes. Here I present a few published research works related to effects of solvent-mediated hydrodynamic interactions and hydrodynamic anisotropies of arbitrarily-shaped biomolecules.

New tools of molecular modelling

Research topics outlined above require new molecular modelling tools to be created and applied. One of the results of my research is a freely available[1] Brownian dynamics package. Models and algorithms implemented in the package, as well as its functionality are described in article (B). Original version of the package allowed for coarse-grained simulations of multicomponent biomolecular systems accounting for intermolecular interactions, hydrodynamic interactions (modelled within the framework of the two-body, far-field approximation) and conformational flexibility of simulated objects. Its subsequent versions were extended to allow for rigid-body Brownian dynamics

[1] currently available at www.browniandynamics.org

simulations of hydrodynamically anisotropic molecules described at the atomic level of detail. Studies described in articles (C, E, F) employed this tool.

Effects of hydrodynamic interactions on the diffusional association of molecules

Formation of macromolecular complexes is usually preceded by a stage during which molecules search for binding partners by means of diffusion. This is often the rate-limiting step of molecular reactions with the relative diffusion of molecules controlling the overall kinetics. Effects of hydrodynamic interactions on the diffusional association of molecules are the subject of articles (C, D).

In article (C) effects of hydrodynamic interactions on the kinetics of association of model isotropic and anisotropic molecules are evaluated. Associating partners are oppositely charged and their direct electrostatic interactions are screened by ions of dissolved salt. It is shown that hydrodynamic interactions decrease the association rate constant. The relative magnitude of this decrease does not depend on the ionic strength for the association of isotropic objects and for nonspecific association of anisotropic objects. Interestingly, in the case of the orientation-specific association of anisotropic objects, the relative magnitude of the hydrodynamic interactions effect on the association rate constant becomes a nonmonotonic function of ionic strength and the maximal effect of hydrodynamic interactions is observed at physiological values of ionic strength. It is also observed that some orientations of anisotropic molecules are hydrodynamically favourable during their mutual approach, and that such molecules can be hydrodynamically steered toward a particular relative orientation. This hydrodynamic orientational steering is impeded in the case of strong electrostatic interactions or steric hindrance, an effect of possible importance under *in vivo* conditions.

While Brownian dynamics simulations described in article (C) were performed for somewhat primitive models of molecules their results allowed for a few interesting observations. Studies on hydrodynamic effects on the diffusional association of molecules were thus continued as described in article (D) and primitive models of molecules were replaced with detailed molecular and hydrodynamic models of proteins barnase and

barstar. Hydrodynamic interactions were evaluated using a rigorous model that accounts for their long-range and many-body character. However, due to its high in the studied case computational cost, direct Brownian dynamics simulation were not possible. Thus, only the relative rotational velocity of proteins moving toward the configuration observed in their crystallographic complex under the influence of forces and moments of forces arising from their electrostatic interactions was evaluated. In article (D), the complicated relationship between shapes of proteins, electrostatic and hydrodynamic interactions, and their impact on the relative rotational movement of proteins during the formation of the complex was analysed. The picture emerging from this study is much more complicated than in the case of simplified model systems.

Due to the high computational cost of hydrodynamic interactions, approximations in which molecules are treated as spheres are applied in Brownian dynamics simulations of complex systems with large numbers of molecular components (13, 14). Findings described in papers (C) and (D) seem to contradict this approach. However, it is not currently possible to model hydrodynamic interactions in Brownian dynamics simulations of complex molecular systems in a rigorous and, at the same time, efficient manner.

Consequences of anisotropy of molecules

Shapes of biomolecules are often quite complicated, which results in their anisotropic diffusion. However, this issue is usually ignored in Brownian dynamics simulations. Diffusion of molecules is assumed to be isotropic and instead of diffusion tensors their average translational and rotational diffusion coefficients are used.

In paper (E) the role of hydrodynamic anisotropy in diffusional association of proteins is considered. Based on atomistically-detailed Brownian dynamics simulations of barnase and barstar it was shown that relatively small hydrodynamic anisotropies of proteins influence their association. Association rate constants obtained from Brownian dynamics simulations that ignore hydrodynamic anisotropies of the binding partners are even 20% smaller than association rate constants derived from Brownian dynamics

simulations that correctly account for hydrodynamic anisotropies of proteins. It was also shown that hydrodynamically anisotropic objects are able to dissociate faster from the complex. The effect of hydrodynamic anisotropy manifests itself through steric interactions which might be of particular importance under *in vivo* conditions.

Connection between the hydrodynamic anisotropy and steric interactions described in paper (E) motivated in part the research described in paper (F) which is dedicated to the rotational dynamics of hydrodynamically anisotropic molecules under crowded conditions. Based on Brownian dynamics simulations of lysozyme solutions with concentrations of up to 250 mg/ml, it is shown in paper (F) that the anisotropy of diffusional rotations of hydrodynamically anisotropic objects increases with an increasing degree of crowding - an effect that is potentially of biological importance that could not be observed with an isotropic description of simulated molecules. Additionally, in paper (F) a method of analysis based on the free diffusion model is proposed that allows one to derive rotational diffusion tensors of molecules from their trajectories resulting from molecular simulations.

Summary

Studies presented here are related to fundamental properties of biomolecules and their interactions and are only a prelude to modelling of the complex phenomena that occur in biological cells. However, reliable modelling of biological systems requires a systematic approach, in which by considering problems and systems of an increasing complexity, we gradually gain knowledge about the mechanisms that govern the studied systems. Such an approach, combined with results of experimental studies will eventually allow us to describe factors and interactions affecting biological processes and resolve to what extent the behaviour of molecules observed in a test tube resembles their behaviour inside living cells.

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3. Ellis, R. J. *"Macromolecular crowding: obvious but under appreciated"*, TRENDS in Biochemical Sciences, 2001, 26, 597-604
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13. Ando, T.; Skolnick, J. *"Crowding and hydrodynamic interactions likely dominate in vivo molecular motion"*, Proceedings of the National Academy of Sciences USA, 2010, 107, 18457-18462

14. Merghetti, P.; Wade, R. C. *"Atomic detail Brownian dynamics simulations of concentrated protein solutions with a mean field treatment of hydrodynamic interactions"*, Journal of Physical Chemistry B, 2012, 116, 8523-8533

5. Discussion of other scientific and research achievements

Kinetics of molecular reactions - stopped-flow spectrophotometry

1. Długosz, M.; Bojarska, E.; Antosiewicz, J. M. *"A procedure for analysis of stopped-flow transients for protein-ligand association"*, Journal of Biochemical and Biophysical Methods, 2002, 51, 179-193

2. Długosz, M.; Błachut-Okrasińska, E.; Bojarska, E.; Darżynkiewicz, E.; Antosiewicz, J. M. *"Effects of pH on kinetics of binding of mRNA-cap analogs by translation initiation factor eIF4E"*, European Biophysics Journal, 2003, 31, 608-616

3. Długosz, M.; Antosiewicz, J. M.; Bzowska, A. *"Stopped-flow studies of guanine binding by calf spleen purine nucleoside phosphorylase"*, Biophysical Chemistry, 2005, 115, 67-76

4. Wielgus-Kutrowska, B.; Antosiewicz, J. M.; Długosz, M.; Holy, A.; Bzowska, A. *"Towards the mechanism of trimeric purine nucleoside phosphorylases: stopped-flow studies of binding of multisubstrate analogue inhibitor – 2-amino-9-[2-(phosphonomethoxy)-ethyl]-6- sulfanylpurine"*, Biophysical Chemistry, 2007, 125, 260-268

5. Antosiewicz, J. M.; Wielgus-Kutrowska, B.; Długosz, M.; Holy, A.; Bzowska, A.*"Kinetics of binding of multisubstrate analogue inhibitor (2-amino-9-*

[2(phosphonomethoxy)ethyl]-6-sulfanylpurine) with trimeric purine nucleoside phosphorylase", Nucleosides, Nucleotides and Nucleic Acids, 2007, 26, 969-974

Stopped-flow method can be applied to evaluate the kinetics of molecular reactions in the liquid phase. The reaction is initiated by rapid mixing of solutions of the reactants. The progress of the reaction is measured using optical spectroscopy methods. For example, in a stopped-flow experiment, the fluorescence of the mixture as a function of time elapsed from the mixing of the reactants can be recorded and on this basis reaction rate constants can be determined. As molecular reactions often involve several steps the analysis of stopped-flow experimental data can be difficult. The situation is further complicated by the fact that the recorded signal can come from all components of the mixture - reagents and reaction products, whose concentrations change in the course of the observed reaction. Analysis of stopped-flow experiments is possible by using methods of numerical integration of differential equations corresponding to reactions observed in experiments, together with an optimisation of reaction rates with constraints inflicted by the observed in the experiment time-dependent signal. Development and applications of such methods (1-5) was the subject of my M.Sc. thesis.

Effects of pH on structure and dynamics of molecules - constant-pH molecular dynamics

6. Długosz, M.; Antosiewicz, J. M. *"The impact of protonation equilibria on protein structure"*,Journal of Physics: Condensed Matter, 2005, 17, S1607-S1616

7. Długosz, M.; Antosiewicz, J. M. *"Effects of solute-solvent proton exchange on polypeptide chain dynamics: a constant pH molecular dynamics study"*, Journal of Physical Chemistry B, 2005, 109, 13777-13784

8. Długosz, M.; Antosiewicz, J. M. *"pKas In dicarboxylic acids by constant-pH molecular dynamics simulations"*, Zeitschrift für Naturforschung, 2004, 59a, 873-874

9. Długosz, M.; Antosiewicz, J. M. *"Constant-pH molecular dynamics simulations: a test case of succinic acid"*, Chemical Physics, 2004, 302, 161-170

10. Długosz, M.; Antosiewicz, J. M.; Robertson, A. D. *"Constant-pH molecular dynamics study of protonation-structure relationship In a heptapeptide derived from ovomucoid third domain"*, Physical Review E, 2004, 69, 1-10

Molecules of living cells occur and react in aqueous solutions. pH of the solution (quantity related to the concentration of hydronium ions that varies in different organs or tissues of the human body) affects the stability, structural properties and functions of molecules. The source of this pH dependence is the presence in macromolecules of the so-called titratable groups that are able to exchange protons with the surrounding aqueous solvent. Protonation and deprotonation equilibria of titratable groups depend on the pH. Changes in protonation states of titratable groups modify the charge distribution within the molecule as well as its overall electrostatic charge. Long-range nature of electrostatic interactions causes these local pH-dependent changes in the distribution of charges to have a fundamental impact on the properties and functions of biomolecules. The traditional approach used in molecular dynamics simulations is based on the assumption that titratable groups of molecules can be assigned fixed protonation states, most probable at a given pH. However, such an approach is not alway correct. Protonation states of titratable groups depend on the conformation of a molecule. Assigning specific states to titratable groups of a molecule and keeping these states fixed during the simulation when the conformation of the molecule is changing may restrict the conformational space of the molecule. The subject of my Ph.D. dissertation was the development of an algorithm which allows for molecular dynamics simulations of solvated molecules in which the solution pH is an external parameter and protonation states of titratable groups of molecules change dynamically during simulations. The resulting algorithm of molecular dynamics at constant pH combines classical molecular dynamics with the Poisson-Boltzmann model (for the evaluation of free energy differences between protonation states) and Monte Carlo method in the ensemble of protonation states. This method was

applied to organic compounds and small peptides allowing to illustrate how protonation equilibria of titratable groups of molecules affect their conformations and dynamics (6-10) .

Molecular modelling and NMR experiments

11. Nowakowski, M.; Długosz, M.; Taraszewska, J.; Wójcik, J. *"Complexation of aminoglutethimide with native and modified cyclodextrins"*, Journal of Physical Organic Chemistry, 2009, 22, 948-953

12. Wojtkielewicz, A.; Długosz, M.; Maj, J.; Morzycki, J. W.; Nowakowski, M.; Renkiewicz, J.; Strnad, M.; Swaczynova, J.; Wilczewska, A. Z.; Wojcik, J. *"New analogues of the potent cytotoxic saponin OSW-1"*, Journal of Medicinal Chemistry, 2007, 50, 3667-3673

While working at the Institute of Biochemistry and Biophysics PAS I participated in studies that utilised methods of molecular modelling as techniques complementing NMR experiments. In my research I applied quantum-mechanical calculations and classical molecular dynamics to investigate structural properties of complexes of drugs with chiral selectors (11) and conformational preferences of organic compounds with therapeutic potential (12).

Brownian dynamics studies of molecular association

13. Długosz, M.; Antosiewicz, J. M.; Trylska, J. *"Association of aminoglycosidic antibiotics with the ribosomal A-site studied with Brownian dynamics"*, Journal of Chemical Theory and Computation, 2008, 4, 549-559

14. Długosz, M.; Trylska, J. *"Aminoglycoside association pathways with the 30S ribosomal subunit"*, Journal of Physical Chemistry B, 2009, 113, 7322-7330

15. Długosz, M.; Huber, G. A.; McCammon, J. A.; Trylska, J. *"Brownian dynamics study of the association between the 70S ribosome and the elongation factor G"*, Biopolymers, 2011, 95, 616-627

16. Grant, B. J.; Gheorghe, D.; Zheng, W.; Alonso, M.; Huber, G.; Długosz, M.; McCammon, J. A.; Cross, R. A. *"Electrostatically biased binding of kinesin to microtubules"*, PLOS Biology, 2011, 9, e1001207

During my post-doc I was involved in studies of interactions of aminoglycoside antibiotics (13, 14) and protein factors (elongation factor G) with the bacterial ribosome (15) which employed the Brownian dynamics technique. Application of this method allowed for estimation of association rate constants, prediction of antibiotics binding sites on the ribosome, prediction of structures of molecular complexes and description of association pathways. In collaboration with the group of prof McCammon (University of California at San Diego) I also participated in studies that employed the Brownian dynamics technique to simulate interactions of motor proteins with microtubules (16).

Molecular dynamics studies of protein aggregation

17. Długosz, M.; Trylska, J. *"Secondary structures of native and pathogenic huntingtin N-terminal fragments"*, Journal of Physical Chemistry B, 2011, 115, 11597–11608

One of the several projects that I was involved in during my post-doc was related to the process of aggregation of the huntingtin protein which accompanies the neurodegenerative Huntington's disease. I applied molecular dynamics to predict / determine structural preferences of N-terminal fragments of huntingtin containing glutamine sequences of lengths found in the native protein and in its pathogenic variant (17). The predicted structure of the native N-terminal fragment agrees with the published crystallographic structure of the huntingtin first exon. The predicted structure of the

pathogenic fragment adheres to an aggregation model that is mediated by the first 17 amino acids of huntingtin proposed by others (17).

Evaluation of the similarity of proteins based on their physicochemical properties

18. Długosz, M.; Trylska, J. *"Electrostatic similarity of proteins: application of 3D spherical harmonic decomposition"*, Journal of Chemical Physics, 2008, 129, 015103

Comparison of physicochemical properties of biomolecules and their classification on the basis of these properties is often required in studies of molecular complexes, drug design, or in attempts to construct various molecular databases. In addition to comparing the chemical composition of molecules, there is also a need to compare their shapes, their electrostatic potentials and properties of their surfaces. However, such comparisons are often ambiguous as orientations of molecules need to be aligned prior to the comparison. During my post-doc I developed a method for comparison of various properties of molecules (shapes, electrostatic potentials, hydrophobicity) that is based on rotation-invariant descriptors and thus does not require any alignment of compared objects (18).

Effects of pH on the kinetics of the diffusion-controlled association of proteins

19. Długosz, M.; Antosiewicz, J. M.; Trylska, J. *"pH-dependent association of proteins. The test case of monoclonal antibody HyHEL-5 and its antigen hen egg white lysozyme"*, Journal of Physical Chemistry B, 2009, 113, 15662-15669

pH-dependent protonation equilibria of proteins determine both local distribution of proteins charges as well as their total charge. As a result, the solution pH affects not only the structure and dynamics of individual molecules, but also their interactions with other molecules and the kinetics of their diffusional association. Acts of the protonation and deprotonation of titratable groups accompanying the binding of molecules cause the

association rate constant to be dependent on the solution pH. In my research on pH-dependent association of proteins I employed the transition complex model that allows for determination of rate constants of the diffusional association of interacting molecules. This model was modified to include the pH of the solution in calculations of the electrostatic energy of interactions of associating proteins and to account for the fact that at a given pH molecules sample many possible protonation states with different probabilities and these probabilities can be affected by interactions between the binding partners. The complex of proteins HyHEL5 and HEWL was considered as a test system. The modified transition complex model gives a much better qualitative agreement with experimental data than the traditional approach in which constant protonation states of the binding partners are assumed (19).

Maciej Długosz