Doctoral thesis:

The theoretical and simulation studies on the channel transport of potassium ions across biological membranes.

Ion channels are a group of transport proteins, which can be found, among others, in human organism and are able to transport particular ion types through the biological membranes effectively and selectively. Their activity plays an important role in a number of physiological processes like: neurotransmitter release, smooth muscle spasms, regulation of action potential in neurons. In spite of many years of extensive research on mechanisms of ion channel's activity, some aspects of their activation (tendency to retain conducting conformation in presence of a particular stimulus) and conformational dynamics (fluctuations of channel states between conducting and non-conducting conformations in a fixed external conditions) remain still unrevealed. Finding a detailed description of functioning of particular channel groups can enable to design effective drugs, acting as ion channel modulators, against many diseases.

The main aim of current research was to propose models of Kv 1.2 and BK channel activity in different external conditions which can influence their transport properties. Proposed models were formulated by means of stochastic processes in diffusive space and potential field which reflect the crucial structural and physicochemical properties of the real system (channel protein and its surroundings), or by geometrical calculations based on the available structural data.

Our structure-based approach shows the possibility of theoretical description which directly refers to the properties of a biological system, and, what is most important, enables to make substantive inferences about mechanisms of channel activation by different stimuli. In that manner, our research complements many of theoretical approaches introduced hitherto, which neither correspond to particular structural elements of a channel nor to the magnitudes of their mutual interactions resulting in occurrence of the observed channel states.

Our essential requirement for proposed models was simplicity of calculations and possibility of simulation in a relatively long time scale, which allows to reflect system's behavior in experimental terms, where single channel currents can be recorded even for several minutes by the patch-clamp method. This makes a notable difference between our approach and modelling by methods of Molecular Dynamics which allow for a detailed description but due to high computational effort (resulting from considering interactions between all atoms forming a macromolecule) they allow for description in a very short time scale (femto- or picoseconds), and their results may depend on the applied force field and on "all-atom" or "course-grained" variant of model.

The proposed models were validated by comparison between the statistics of surrogate data and experimental ones, which were obtained in form of time series of single channel currents recorded by patch-clamp method.

The obtained results allowed for:

1. Statistical analysis of considered process.
2. Answer the question: what kind of stochastic process – Markovian or non-Markovian and under what assumptions can describe ion channel activity and allow to reflect experimental dependence of open state probability on activation stimuli, dwell-time distributions of open and closed states, and a long-range memory effect - that is well worth noticing because popular models are not able to reflect that feature.
3. Discussion of probable mechanisms of interactions between channel's functional subunits during its activation by particular stimuli (membrane depolarization, membrane strain). This aspect was crucially important due to the existence of several hypotheses about possible scenario of voltage-activation (helical-screw, helical-twist, paddle model) which differ distinctively by the assumptions about range and type of motion of the S4 helix (main voltage sensor). On the base of performed simulations of channel activation by membrane depolarization we indicated the most probable mechanism of that process.
4. Emphasizing the role of geometry changes during voltage-activation in observed channel conductance and open state probability.
5. Indication of the possibility of channel regulation by external factors: membrane fluctuations, rotations of side chains of the amino acids constituting S6 helices which form channel pore, susceptibility of S6 helices to bending.
6. Investigation of the impact of physicochemical factors (temperature, difference of pressure between the opposite sides of cell membrane) on the conformational dynamics of channel gate. This part of our studies allowed not only to describe thermo- and mechanosensitivity of the BK channels, but also to evaluate: net activation energy, changes of entropy and Gibbs free energy during the macroconformational change from non-conducting (closed) to a conducting (open) state of a channel. The obtained results enabled to infer that the channel activation by membrane depolarization is a entropy-driven process and to emphasize the important role of cytoskeleton morphology in ion channel activity.