Załącznik 3. Tłumaczenie autoreferatu na język angielski

Table of Contents

1. Name and surname

Bartosz Jan Różycki

2. Education and degrees

- PhD in physics
  Faculty of Physics, University of Warsaw, November 20, 2006
  Dissertation title: *Stochastic models of cell membrane adhesion away from thermodynamic equilibrium*
  Supervisor: Prof. Marek Napiórkowski
- MSc in physics
  Faculty of Physics, University of Warsaw, June 14, 2002
  MSc theses: *Exact solution of a two-dimensional model for the wetting phenomenon*
  Supervisor: Prof. Marek Napiórkowski

3. Information on employment

- Since October 2012: assistant professor at the Institute of Physics, Polish Academy of Sciences, Warsaw, Poland.
- October 2011 – October 2012: postdoctoral associate at the Max Planck Institute of Colloids and Interfaces, Potsdam, Germany. Scientific advisor: Prof. Reinhard Lipowsky.
- October 2008 – October 2011: visiting fellow at the National Institutes of Health, Bethesda, Maryland, USA. Scientific advisor: Prof. Gerhard Hummer.
- November 2006 – September 2008: postdoctoral researcher at the Max Planck Institute of Colloids and Interfaces, Potsdam, Germany. Scientific advisor: Dr. Thomas Weikl.

4. Bibliometric summary of scientific publications

My publication record includes **33** scientific articles (excluding conference proceedings) published in journals listed in the Journal Citation Reports (JCR). The total impact factor of these publications (calculated according to the JCR list with the assumption that the impact factor in 2017 is the same as in 2016) is **206.1**.

Bibliometric data according to the Web of Science database on January 11, 2018:
- Researcher ID: B-7005-2009
- Number of articles with citation data: **40**
- Total number of citations: **805**
- Hirsh index: **16**
- i-10 index: **20**

Bibliometric data according to the Web of Science database on January 11, 2018:
- Total number of citations: **1017**
- Hirsh index: **18**
- i10 index: **20**

Most of my research articles have been published in journals dedicated to physics (two publications in *Physical Review Letters,* one publication in *Physical Review E*, one publication in *New Journal of Physics*, one publication in *Journal of Physics: Condensed Matter,* three publications in *Europhysics Letters* and one publication in *European Physics Journal E*), in particular

mathematical physics (one publication in *Journal of Physics A: Mathematical and General*), statistical physics (one publication in *Journal of Statistical Mechanics*), chemical physics (three publications in *Journal of Chemical Physics* and one publication in *Physical Chemistry Chemical Physics*) and soft matter physics (two publications in *Soft Matter*). Since the subjects of my work lie on the frontiers of physics, chemistry and biology, the results of my research have also been published in journals dedicated to chemistry (one publication in *Journal of American Chemical Society*), chemical biology (one publication in *Nature Chemical Biology*), molecular biology (one publication in *EMBO Reports*), structural biology (three publications in *Structure* and two publications in *Journal of Structural Biology*), computational biology (one publication in *PLoS Computational Biology*) and cell biology (two publications in *Cell*). Several of my papers have also been published in journals devoted to molecular biophysics and biochemistry (one publication in *Proteins: Structure, Function, Bioinformatics* and one publication in *Molecular BioSystems*) and sciences in general (one publication in *Proceedings of the National Academy of Sciences of the United States of America* and two publications in *PLoS ONE*). The diversity of journals publishing my work is due to the interdisciplinary nature of my research as well as my scientific collaborations not only with physicists but also with biochemists and biologists.

5. Scientific achievement being the basis of the habilitation procedure

The scientific achievement, in accordance with art.16 paragraph 2 of the Act of March 14th, 2003, concerning the scientific degrees and titles (Dz. U. item no. 882, 2016, with amendments in Dz. U. item no. 1311, 2016), is a series of related publications.

5.1 Title of the scientific achievement

Conformational dynamics of multi-domain proteins within the framework of coarse-grained models

5.2 List of publications constituting the scientific achievement

H1. <u>Bartosz Różycki</u>, Young C. Kim, Gerhard Hummer. *SAXS ensemble refinement of ESCRT-III CHMP3 conformational transitions*. Structure **19**: 109-116 (2011). *Impact Factor* in 2011: 6,347. Over 100 citations according to the Web of Science database.

H2. Evzen Boura, <u>Bartosz Różycki</u>, Dawn Z. Herrick, Hoi Sung Chung, Jaroslav Vecer, William A. Eaton, David S. Cafiso, Gerhard Hummer, James H. Hurley. *Solution structure of the ESCRT-I complex by small angle X-ray scattering, EPR, and FRET spectroscopy*. Proc. Natl. Acad. Sci. USA **108**: 9437-9442 (2011). *Impact Factor* in 2011: 9,681.

H3. Evzen Boura*, <u>Bartosz Różycki</u>*, Hoi Sung Chung, Dawn Z. Herrick, Bertram Canagarajah, David S. Cafiso, William A. Eaton, Gerhard Hummer, James H. Hurley. *Solution structure of the ESCRT-I and -II supercomplex: implications for membrane budding and scission*. Structure **20**: 874-886 (2012). *Impact Factor* in 2012: 5,994.

H4. <u>Bartosz Różycki</u>, Evzen Boura, James H. Hurley, Gerhard Hummer. *Membrane-elasticity model of coatless vesicle budding induced by ESCRT complexes*. PLoS Comp. Biol. **8**: e1002736 (2012). *Impact Factor* in 2012: 4,867.

H5. <u>Bartosz Różycki</u>**, Marek Cieplak, Mirjam Czjzek. *Large conformational fluctuations of the multi-domain xylanase Z of Clostridium thermocellum*. J. Struct. Biol. **191**: 68-75 (2015). *Impact Factor* w 2015 r.: 2,570.

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 * equal contributions
 ** corresponding author

H6. <u>Bartosz Różycki</u>**, Marek Cieplak. *Stiffness of the C-terminal disordered linker affects the geometry of the active site in endoglucanase Cel8A*. Mol. BioSyst. **12**: 3589-3599 (2016). *Impact Factor* in 2016: 2,781.

H7. <u>Bartosz Różycki</u>, Pierre Andre Cazade, Shane O'Mahony, Damien Thompson, Marek Cieplak. *The length but not the sequence of peptide linker modules exerts the primary influence on the conformations of protein domains in cellulosome multi-enzyme complexes*. Phys. Chem. Chem. Phys. **19**: 21414-21425 (2017). *Impact Factor* in 2016: 4,123.

H8. <u>Bartosz Różycki</u>**, Lukasz Mioduszewski, Marek Cieplak. *Unbinding and unfolding of adhesion protein complexes through stretching: Interplay between shear and tensile mechanical clamps*. Proteins: Struct., Funct., Bioinf. **82**: 3144-3153 (2014). *Impact Factor* in 2014: 2,627.

H9. <u>Bartosz Różycki</u>**, Marek Cieplak. *Citrate synthase proteins in extremophilic organisms: Studies within a structure-based model*. J. Chem. Phys. **141**: 235102 (2014). *Impact Factor* in 2014: 2,952.

5.3 Description of the scientific objectives and the research results presented in publications H1-H9, together with the discussion of their possible applications

Proteins constitute a huge family of organic compounds. They are found in all living organisms and viruses. They are involved in virtually all functions of biological cells. Common examples are replication, transcription and translation of genetic code, metabolism, signaling, transport and shaping of cells [1]. An amazing fact is that the basis for this great variety and complexity of functions performed by the proteins is their amino acid sequence.

Some proteins perform metabolic functions. These proteins are enzymes that use nutrients to harness energy that is needed to sustain life processes in cells. Other proteins mediate signal transduction. They control chains of biochemical reactions that allow the cell to react to changes in its environment. Cells also use proteins as building material. For example, the cytoskeleton – which is the molecular 'scaffolding' that gives shape to eukaryotic cells – is built entirely of proteins. Proteins also act as pumps and channels in cell membranes. In fact, proteins organize the vast majority of activities of living cells [2]. Understanding the molecular principles of life requires therefore an in-depth understanding of the physical and chemical properties of proteins.

The pioneering works of John Kendrew and Max Perutz (Nobel Prize in chemistry in 1962 for solving the first atomic structures of proteins using X-ray crystallography) led to a groundbreaking discovery – a given protein performs its biological functions owing to a strictly defined, stable structure, which is called the native structure. Determining the native structure of a given protein usually leads to an explanation of how the protein performs its biological functions. Amongst the most important, recent examples are the structures of G-protein-coupled receptors, which explain how signal transduction occurs and how hormones and opioids work [3,4]. Other important examples are the structures of whole viruses, such as enterovirus 71, which have helped to explain the mechanisms of cell infection and have given rise to rational drug design [5]. An excellent example of rational drug design is the recent work of Balbas and co-workers [6]. They have used X-ray crystallography and molecular dynamics simulations to rationally select screening tests that have identified chemical compounds which inhibit the growth of drug-resistant prostate cancer cells. It is argued that the use of structural biology methods for rational drug design will play an increasingly important role in medicine [7].

Research on protein structures leads to the understanding of basic processes of life at the molecular level. It started in the 1950s and 1960s with X-ray crystallography studies. Contemporary

structural biology [8] often uses also such methods as nuclear magnetic resonance (NMR), cryo-electron microscopy (cryo-EM), Förster resonance energy transfer (FRET), electron paramagnetic resonance (EPR), and small angle X-ray scattering (SAXS). All these methods, including X-ray crystallography, have their roots in condensed matter physics. However, they have been gradually adapted for the studies of structures of proteins and other macromolecules.

Even a small protein is made up of thousands of atoms coupled together by a variety of covalent and non-covalent bonds. Therefore, in the description of the three-dimensional structures of proteins, it is helpful to distinguish several levels of organization [1]. The amino acid sequence (i.e., the sequence given from the N-terminus to the C-terminus) of the polypeptide chain is the so-called primary structure. Determination of the polypeptide chain segments forming α helices and β sheets yields the so-called secondary structure. The three-dimensional conformation, which attains the folded polypeptide chain, is called the tertiary structure.

In the description of structures and functions of proteins, it often is helpful to distinguish their domains. A protein domain is formed by a part of the polypeptide chain (usually between 50 and 350 amino acids) folded into a packed, stable and functional spatial structure. Domains usually contain several α helices and/or β sheets and are constituent parts of many large proteins. Usually, different domains in a given protein perform different functions.

The non-covalent interactions that drive protein folding also cause proteins to bind one another, leading to the formation of larger protein structures, which are called protein complexes. Each polypeptide chain in a protein complex is called a subunit. To characterize structures of protein complexes, one introduces yet another level of organization. Namely, the relative position of subunits in a protein complex gives the quaternary structure.

Until the end of the twentieth century, there was a paradigm saying that a unique, tertiary structure is required for a protein to perform its biological functions. This paradigm was challenged in the early 2000s by the discovery of intrinsically disordered proteins which, despite their lack of stable tertiary structure under physiological conditions, remain fully functional [9]. The native state of this type of proteins is a conformational ensemble rather than a single conformation.

An important class of intrinsically disordered proteins are multi-domain proteins in which separate, folded domains are linked by disordered polypeptide chain segments [10]. Although such proteins are widespread and perform many important functions in biological cells, they are often very difficult to study using conventional methods of structural biology. Namely, they are not directly accessible to X-ray crystallography due to the presence of the disordered inter-domain linkers (only structures of the individual, constituent domains or subunits can be solved). They are not accessible to protein NMR because of their large sizes (usually in the range of hundreds of kDa). Therefore, structural studies on this type of proteins increasingly use the so-called hybrid methods, which employ advanced computational techniques to combine and interpret data from a wide variety of complementary experiences [8]. My contribution to this research area was the development and implementation of the Ensemble Refinement of SAXS (EROS) method [H1], which combines computer simulations of multi-domain proteins with the results of SAXS experiments. I have been gradually improving and modifying the EROS method. At present, it can be used to model conformation ensembles of multi-domain proteins based on data from SAXS, EPR and FRET [H2,H3] experiments. I have used it to structurally characterize ESCRT protein complexes [H1,H2,H3], protein kinases [S1], and kinases in dynamic complexes with phosphatases [S2,S3].

The endosomal sorting complexes required for transport (ESCRT) are involved in intracellular transport [11,12,13]. Their main function is to shape and organize the endosomes.

(Endosomes are intracellular membrane-bound compartments, or organelles, responsible for sorting the material internalized from the plasma membrane). My contribution to the research on conformationals [H1,H2,H3] and mechanics [H3,H4] of ESCRT proteins is presented in section 5.3.1. This section also describes the essential components of the EROS method, which is founded on such basic concepts of statistical mechanics as the maximum entropy method, Boltzmann distribution and Monte Carlo simulations.

The EROS method combines coarse-grained simulations with data from SAXS experiments. I have used an analogous combination to determine and analyze the conformational ensemble of a multi-domain enzyme that is a constituent part of cellulosomes secreted by thermophilic bacteria *Clostridium thermocellum* [H5]. Cellulosomes are multi-enzyme complexes degrading plant-cell-wall polysaccharides – cellulose and hemicellulose – down into simple sugars [14,15]. A common feature of cellulosomes and ESCRTs is their molecular architecture, i.e., many domains and subunits linked by disordered and flexible polypeptide segments of different sequences and lengths. My research on cellulosomal proteins has dealt mainly with the properties [H5,H7] and functions [H6,H7] of these disordered linkers. Numerical studies involving both coarse-gained and all-atom models show that disordered polypeptide segments between protein domains do not only play a role of linkers but also affect the conformations of the adjacent domains [H6,H7]. The scale of this effect strongly depends on the length and stiffness of the linkers [H7]. These results suggest that the amino-acid sequence of the disordered linkers may in a non-trivial way modulate the enzymatic activity of cellulosomes [H6]. My contribution to the research on cellulosomal multi-domain proteins is described in section 5.3.2.

My contribution to the research on multi-domain proteins and protein complexes has been concerned not only with conformational ensembles [H1,H2,H3,H5] and molecular mechanisms underlying biological functions [H3,H4,H6,H7] but also with mechanical [H8] and thermal [H9] stability. My contribution to the studies on stability of multi-domain proteins and protein complexes is described in section 5.3.3.

As for the mechanical stability, it should be noted that certain types of proteins are subjected to external forces under physiological conditions. Examples are adhesion proteins. Their trans-membrane domains are embedded into the plasma membrane and their extracellular domains bind to proteins in the outer membrane of another cell [1,2]. Adhesion proteins mediate cellular interactions that occur, for example, during tissue formation and in the recognition of surrounding cells by leukocytes. In order to remain functional, adhesion proteins must retain their structures when external forces are applied to adhering cells. I have conducted studies on the mechanostability of complexes of adhesion proteins using molecular dynamics methods within the framework of a coarse-grained Go-type model [H8], which is based on a map of amino-acid contacts identified in the native structure. The results of these simulations are summarized in section 5.3.3.

As for the thermal stability of proteins, it is worth noting that living organisms are adapted to different temperature ranges occurring on the Earth. For example, cryophilic bacteria *Planococcus halocryphilus* thrive at temperatures as low as -15°C. Their metabolism is maintained even at -25°C [16]. On the other hand, thermophilic archaeobacteria *Geogemma barossii*, discovered in deep sea hydrothermal vents, grow and reproduce at temperatures up to 120°C [17]. Interestingly, the proteins of thermophilic organisms remain functional at temperatures in which proteins of other organisms are denatured. This observation provokes the following questions: what types of structural and topological features and what kind of physicochemical properties determine the stability of the protein structure in different temperature ranges? This subject has been studied for over three decades. My contribution [H9] to this research area concerns the thermostability of a

multi-domain enzyme – which is a homodimer, i.e., a complex of two identical proteins – found in thermophilic, mesophilic and cryophilic organisms. I have conducted this research using molecular dynamics methods within the framework of the coarse-grained Go-type model. The results of these simulations are summarized in section 5.3.3.

5.3.1 Conformations and mechanics of ESCRT proteins (publications H1, H2, H3, H4)

The endosomal sorting complexes required for transport (ESCRT) is a group of proteins that includes the complexes denoted as ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III [13,18]. ESCRTs are cytoplasmic proteins – they are present in the intracellular fluids and bind to specific components of cellular membranes. For example, the ESCRT-0 and ESCRT-II complexes contain domains that bind phosphatidylinositol 3-phosphate (PI3P), which is a phospholipid present in the plasma membrane and in the membrane of endosomes. In addition, ESCRT-0, ESCRT-I and ESCRT-II contain ubiquitin-binding domains. (Ubiquitin is a small protein covalently bound to these proteins on the surface of endosomes that are designed for degradation in organelles called lysosomes). The process of maturation of endosomes occurs by successive binding of ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III to their surfaces [13].

ESCRT proteins have been identified in a variety of organisms ranging from yeast to human. In addition to the coordination of endosome activity, other functions of the ESCRTs have been discovered [11]. For example, they participate in the final stage of cell division, when the membrane of the mother cell is separated from the membrane of the daughter cell. Also, certain viruses, including HTLV and HIV-1, hijack ESCRT proteins and use them to get out of the host cells.

A prominent feature of the ESCRT proteins is the effect of their interactions with cellular membranes. Namely, they induce budding of the endosomal membrane toward the interior of the endosome; they also induce budding of the cytoplasmic membrane towards the outside of the cell [13,19]. In these processes, the membrane gets deformed in the direction opposite to the direction from which the ESCRT proteins bind to the membrane (as I illustrated it in Fig. 1 in Ref. [H4]). Even more interesting is the fact that ESCRT proteins have been observed only in the neck interconnecting the newly formed membrane bud with the mother membrane [20,21]. Despite intensive research in this area, the molecular mechanisms underlying the ESCRT-driven membrane budding have not been elucidated so far.

ESCRT-0, ESCRT-I and ESCRT-II are present in the cytosol as three separate protein complexes [18]. ESCRT-III subunits, however, do not bind one another in the intracellular fluid. Instead, the ESCRT-III complexes are formed only on the membranes. The ESCRT-III subunits are thought to occur in the intracellular fluid in an inactive, or 'closed', state in which they cannot bind to one other [22]. On the membrane surface, however, they attain an active, or 'open', conformation and only then the ESCRT-III complex is formed [22]. The transition between the closed conformation and the open conformation is likely to be caused by electrostatic interactions. This hypothesis is supported by SAXS experiments on CHMP3 in aqueous solutions of different salt concentrations [23]. (The CHMP3 protein is one of the major components of ESCRT-III). The results of these SAXS experiments show that when the salt concentration in the solution is not higher than the physiological concentration (150mM), CHMP3 assumes a closed conformation. On the other hand, when the salt concentration in the solution is significantly higher than the physiological concentration and, thus, when the electrostatic interactions are effectively screened at distances smaller than 1 nm, CHMP3 adopts an open conformation. Thus, the electrostatic screening has a significant effect on the spatial distribution of the CHMP3 domains.

SAXS is commonly used to measure the characteristic sizes and shapes of proteins in aqueous solutions. Leaving aside some technical aspects, the procedure is as follows. SAXS measurements on macromolecules in a solution result in the so-called scattering curve, which is the dependence of X-ray intensity on the scattering angle. The reverse Fourier transform of the scattering curve gives the pair-distance distribution function, i.e., the distribution of distances between the pairs of atoms in the protein under study. Based on this distribution and on the amino acid sequence of the protein, and assuming a uniform distribution of mass within the protein molecule, one can derive the so-called molecular envelope, which represents the space occupied by the protein [24,25]. Molecular envelopes provide information about the characteristic sizes of the protein under study and also help visualize its shape. However, they do not yield information on the atomic structure of the protein and, for this reason, SAXS is commonly considered as a method of low spatial resolution.

In Ref. [23], molecular envelopes of CHMP3 at low and high salt concentrations have been determined. These envelopes clearly indicate differences between the open and closed conformations but they do not provide information about rearrangements in molecular structures. To fill this knowledge gap, I performed computer simulations of the CHMP3 protein [H1]. Since significant conformational changes of multi-domain proteins are very difficult to sample in all-atom molecular dynamics simulations, for the purpose of the CHMP3 study I used a coarse-grained model that has been introduced by Kim and Hummer [26]. The main elements of this model – which I will refer to as the KM model – are summarized in the paragraph below.

The KM model is a coarse-grained model in which amino acid residues are represented by single 'grains' or 'beads'. Protein domains with known structures (i.e. structures solved by X-ray crystallography or NMR) are represented by rigid bodies formed of these beads. The rigid bodies are only subject to translations and rotations in the course of simulations. Disordered loops and linkers, on the other hand, are represented by chains of amino-acid beads. The potential energy of these chains includes terms describing their local stretching, bending and torsions. Physical interactions between the beads that form both the rigid domains and the flexible inkers are described by a potential energy consisting of two components: (i) Miyazawa-Jernigan-type statistical potential and (ii) electrostatic potential within the Debye-Hückel approximation. By changing the Debye length, which describes the effective range of electrostatic interactions in aqueous solutions, it is possible to simulate proteins in solutions with different ionic strengths. As shown in a series of Monte Carlo simulations, the KH potential energy correctly predicts both the structures and the binding affinities of many protein complexes [26]. This result indicates that Monte Carlo simulations within the KH model sample the relevant conformations of protein complexes.

Within the KM approach, the coarse-grained model of the CHMP3 protein contains three rigid bodies: (i) the helical core, formed by helices α1-α4, whose structure has been solved by x-ray crystallography (PDB code: 3FRT), (ii) helix α5 and (iii) helix α6. The three rigid domains are linked by two disordered polypeptide segments (as illustrated in Figure 3 in Ref. [H1]). The CHMP3 simulations, which I have performed within the KH model using Monte Carlo methods, lead to results that are qualitatively consistent with the SAXS data on CHMP3 [H1]. For the Debye lengths larger than 1 nm, which correspond to low salt concentrations, both the α5 helix and the disordered linkers form frequent contacts with the core of CHMP3 whereas the α6 helix remains in the vicinity of the core. These conformations correspond to the closed state. For the Debye lengths smaller than 1 nm, which correspond to high salt concentrations, both the α5 helix and the α6 helix are separated from the CHMP3 core. These conformations correspond to the open state.

In order to quantitatively compare the CHMP3 simulation results with the results of the SAXS experiments on CHMP3, I have calculated the scattering curves for the conformational ensembles obtained in the simulations with low and high ionic strengths. The calculated scattering curves are overall consistent with the SAXS experimental data but there are also some discrepancies between them (as I presented it in Fig. 2 in Ref. [H1]). In order to improve the agreement between the simulated ensembles and the results of the SAXS experiments, I introduced and implemented an ensemble refinement method, which is called EROS and which is summarized in the paragraphs below.

Modeling of protein conformations within the EROS method proceeds in two stages. In the first stage, computer simulations of a particular protein are performed, resulting in a pool of structural models. In this way, an initial, or 'original', conformational ensemble is obtained. In the second step, the conformational ensemble is refined to achieve agreement with the available experimental data and, at the same time, make as little modifications as possible to the original ensemble that has been obtained from the simulations. This is done as follows:

- The structural models obtained from the simulations are sorted and grouped into clusters on the basis of their mutual similarity. A given cluster consists of structural models (usually from several to several dozen of models) that are mutually similar. In order to divide the pool of models into disjunctive clusters, standard clustering algorithms such as k-means or quality threshold (QT) are used. As a measure of similarity between the structural models, the distance root-mean-square (DRMS) is used.

- For each of the structural models individually, the scattering curve is computed. An important factor that needs to taken into account in the computation of the scattering curves for the structural models is the hydration shell on the protein surface. Next, scattering curves are assigned to the clusters of structural models. The scattering curve assigned to a given cluster is obtained by averaging the scattering curves determined for the structural models within this cluster.

- Statistical weights are assigned to the clusters of structural models. Also, it is assumed that the weighted average over the clusters represents the average over the statistical ensemble. This implies that the scattering curve averaged over the conformational ensemble, which is meant to be compared directly to the results of the SAXS experiments, depends on the statistical weights assigned to the clusters of structural models. Therefore, by selecting appropriate statistical weights of the clusters, it is usually possible to reach an agreement between the experimental and theoretical results (i.e. agreement between the quantities measured in experiments and the values computed on the basis of the structural models after averaging with appropriate weights).

- To prevent over-fitting, and to avoid the problem of ambiguity in choosing the appropriate statistical weights, the fitting of the theoretical values to the experimental data is based on the maximum entropy method. This method comes to a numerical minimization of a difference of two functions. The first of these two functions, $\chi 2$, describes the degree of agreement between the theoretical predictions and the experimental results. It depends in a complicated manner on the statistical weights assigned to the clusters of the simulation structures. The second of these two functions, S, is analogous to the Shannon entropy and increases with changes in the statistical weights of the clusters. As a result of minimization of the difference of the two functions, one obtains a conformational ensemble (i.e. clusters of structural models with associated statistical weights) that is consistent with the results of the SAXS experiments and, at

the same time, is as similar as possible to the conformational ensemble obtained directly from the simulation (i.e. the pool of structural models with no statistical weights).

The procedure described above is introduced and discussed in details in the original work [H1]. Importantly, it can be generalized and applied to modeling protein conformations based on other experiments, not only SAXS, in which the measurement signal is averaged over the statistical ensemble.

I have used the method described above to determine the conformational ensembles of CHPM3 in solutions with different ionic strengths [H1]. In contrast, for the construction of conformational ensembles of the ESCRT-I [H2], ESCRT-II and ESCRT-I-II [H3] protein complexes, I have used a simpler method that comes to selecting the smallest set of clusters of structural models that gives an agreement between the theory and the experiment. The advantage of this approach is that the resulting conformational ensemble can be usually represented by only several structures, which can be easily visualized and interpreted. However, by neglecting a significant portion of the structural models obtained in molecular simulations, the 'minimal ensemble' method does not fully utilize the predictive capabilities of molecular simulations, which are based on physics and chemistry.

The conformational ensembles of ESCRT-I, ESCRT-II and ESCRT-I-II [H2,H3] have been determined using not only the coarse-grained simulations and the SAXS experiments but also two spectroscopy methods that rely on site-directed labeling, i.e., Förster resonance energy transfer (FRET) and electron paramagnetic resonance (EPR). The physical phenomena underlying these two methods are very different – energy transfer between two chromophores (FRET) and electron paramagnetic resonance (EPR). However, their basic application in structural characterization of proteins is based on their common feature – both of these methods can provide information about the distances between pairs of labels that are covalently bound to the protein surface. In one case (FRET), these are the distances between the electrical dipoles of the fluorescent labels. In the other case (EPR), these are the distances between the unpaired electrons of the paramagnetic labels. The EPR spectroscopy method that can provide information on the paramagnetic label distances is the double electron-electron resonance (DEER) method.

The distances between pairs of labels provide fragmentary information about the structure of the labeled protein. However, in combination with data from complementary experiments, such as SAXS, they can be very useful for modeling protein conformations. The results of FRET and DEER measurements imply 'local' restrictions on the distances between different protein domains or subunits, and data from SAXS experiments provide 'global' information on the size and shape of the protein under study. Therefore, a combination of SAXS, FRET and/or DEER experiments with computer simulations (molecular dynamics or Monte Carlo) provides an effective and reliable way to determine conformations of multi-domain proteins and protein complexes.

Contemporary studies on protein structure often employ molecular modeling. Determination of protein structures on the basis of the data from crystallography or NMR experiments requires modeling all the atoms that constitute a particular protein molecule. In contrast, coarse-grained approaches are sufficient to structurally interpret SAXS data because the spatial resolution of the SAXS method (usually in the range between 1 and 2 nm) is not sufficient to resolve single amino acid residues. For the spectroscopy methods based on site-directed labeling of proteins, atom-level modeling of the labels is crucial to correctly interpret the experimental spectra. The EROS method uses coarse-grained simulations because it has been developed to study large multi-domain proteins and multi-protein complexes that can exhibit significant conformational changes. However, the atomic structures of fluorescent and paramagnetic labels are used in the modified EROS method

[H2,H3] in the ensemble refinement procedure when the simulation results are compared to data from FRET and DEER experiments.

The conformational ensemble can be derived by fitting structural models either to raw experimental data, such as a scattering curve, or to 'processed' data, such as the pair-distance distribution function, which is the Fourier transform of the scattering curve. However, to avoid introducing any regularization-dependent artifacts into the ensemble refinement, the simulation structures are fitted directly to experimental data in the EROS method [H1] and its modifications [H2,H3]. It is also important to cross-validate the conformational ensemble with independent data sets excluded from the refinement procedure [H2, H3].

Works [H2,H3] have provided important information on the conformations and dynamics of the ESCRT-I and ESCRT-II protein complexes. The ESCRT-I complex consists of four subunits denoted by Vps28, Vps37, Vps23 and Mvb12 (Fig. 1 in Ref. [H2]). The core of ESCRT-I contains portions of all of the four subunits. Three domains are linked to the ESCRT-I core: (i) the CTD domain of Vps28 that binds to one of the proteins of the ESCRT-II complex, (ii) the UEV domain of Vps23 that binds ubiquitin, and (iii) the NTH domain of Vps37 that binds – probably due to electrostatic interactions – to lipid membranes. The structures of all these domains have been solved and deposited in the PDB. The Mvb12 subunit has a long, disordered segment of the polypeptide chain at the N-terminus. The entire ESCRT-I complex is built of almost one thousand amino acids and its molecular mass is approximately 108 kDa.

I have performed Monte Carlo simulations of the full-length ESCRT-I complex within the KH model [H2]. Interestingly, out of tens of thousand of structural models generated in these simulations, none of them gives a scattering curve consistent with the data obtained from the SAXS experiments on ESCRT-I. However, two clusters of structural models, taken together with equal statistical weights, give very good agreement with the results of SAXS measurements (Fig. 2 in Ref. [H2]). One of these clusters of structural models corresponds to a closed conformation in which the CTD and UEV domains are located near the ESCRT-I core. In contrast, the other cluster of structural models corresponds to an open conformation in which the CTD and UEV domains do not form direct contacts with the core. Interestingly, the minimal ensemble that fits both the SAXS and DEER experimental data (Fig. 3 in Ref. [H2]) consists of six clusters of structural models (Fig. 4 in Ref. [H2]). Three of them correspond to the open conformation. The remaining three correspond to the closed conformation in which the CTD and UEV domains form different contacts with the ESCRT-I core. These results, taken together, show that ESCRT-I adopts a spectrum of diverse configurations while the interactions between the different domains are weak and transient.

The ESCRT-II complex (Fig. 2 in Ref. [H3]) comprises one subunit of Vps36, one subunit of Vps22 and two subunits of Vps25. (The stoichiometry of this complex is 1:1:2). The core of the ESCRT-II complex consists of both of the Vps25 subunits, the whole Vps22 subunit, and a portion of the Vps36 subunit. The Vps36 protein contains also the GLUE domain and two zinc-binding domains (NZF1 and NZF2). The GLUE domain binds to PI3P, which is a phospholipid found in the plasma membrane and in the membrane of endosomes. The NZF1 domain binds to the CTD domain of the Vps28 protein belonging to the ESCRT-I complex. The NZF2 domain binds ubiquitin. The GLUE, NZF1 and NZF2 domains are linked to the ESCRT-II core by a very long, disordered segment of the Vps28 polypeptide chain. The structures of all these four domains have been solved and deposited in the PDB.

I have simulated the full-length ESCRT-II complex using the KH [H3] model. Based on the results of these simulations, I have determined a minimal conformational ensemble that is consistent with both the scattering curves obtained from SAXS experiments (Fig. 1 in Ref. [H3])

and with FRET efficiency histograms obtained from single-molecule FRET measurements (Fig. 3 in Ref. [H3]). This conformational ensemble is represented by 15 structural models (Fig. 5 in Ref. [H3] work). The vast majority of them correspond to a closed conformation with a relatively small radius of gyration as for such a large protein complex. In these conformations, the GLUE domain makes direct contacts with the ESCRT-II core. Interestingly, there seem to be many ways in which the GLUE domain can make contacts with the core. These results lead to the conclusion that interactions between the different domains of the ESCRT-II complex are weak and transient, similarly as is the case of the ESCRT-I complex.

Using the KH approach I have also performed Monte Carlo simulations of the full-length ESCRT-I-II complex, which is formed by binding the CTD domain of Vps28 to the NZF1 domain of Vps36 (Fig. 1 in Ref. [H2] and Fig. 2 in Ref. [H3]). On the basis of the structural models generated in the simulations, I have identified a minimal conformational ensemble that is consistent with the results of SAXS, FRET and DEER experiments altogether. It is represented by 18 structural models, which differ considerably (Fig. 6 in Ref. [H3]). Interestingly, most of them have the shape of a crescent, which is often characteristic of proteins that target to membranes and induce curvature. The curvature characterizing these ESCRT-I-II conformations is close to the curvature of the neck of the endosomal membrane during the budding process. This observation leads to a hypothesis about how the ESCRT-I-II complexes are positioned on a budding endosome (Fig. 7 in Ref. [H3] work). Since the structural models of ESCRT-I-II are appropriately curved, the ESCRT domains that bind PI3P and ubiquitin are directed towards the curved membrane of the budding endosome. These results fit well into the research on the structure-function relationships of ESCRT proteins.

The above-described studies [H3] have been continued in work [H4], where I have introduced a physical model for membrane budding induced by the ESCRT-I-II protein complexes. The assumptions underlying this model are based on literature observations that have been made in experiments using fluorescence microscopy techniques. Firstly, ESCRT-I and ESCRT-II complexes bind to lipid membranes with endosome-like compositions, i.e., membranes containing sphingomyelin, PI3P and cholesterol [20,21,27]. Lipid membranes with this type of composition exhibit two fluid phases, i.e., liquid-ordered and liquid-disordered phases. Secondly, the binding of the ESCRT-I and ESCRT-II complexes to the lipid membrane results in the separation of the two lipid phases [27]. The membrane areas occupied by the ESCRT-I-II complexes are found to be in the fluid-ordered phase. Thirdly, the binding of the ESCRT-I-II complexes to the membrane causes membrane budding [21]. Fourthly, the ESCRT-I-II proteins are found to be localized only in the neck connecting the newly formed membrane bud with the budding membrane [19,20,21].

The physical model introduced in Ref. [H4] is cast in the framework of membrane elasticity theory. It is given by an energy function that depends both on the shape of the lipid membrane and on the distribution of the ESCRT-I-II proteins on the membrane surface. The energy function takes into account deformations of the lipid membrane and the four aforementioned experimental observations. I have solved the model numerically using a Monte Carlo simulated annealing algorithm. I have also found an analytical solution of a simplified model in which the surface of the budding membrane is approximated a spherical cap. This solution explains why the ESCRT-I-II complexes are capable of inducing either endosomal buds with a diameter of about 50 nanometers or much larger, two-micrometer membrane buds in giant unilamellar vesicles (GUVs), as observed in fluorescence microscopy experiments. The model solution also explains another interesting experimental observation, namely, a relatively narrow distribution of diameters of membrane buds in a given experimental system.

I have determined the range of model parameters in which the ESCRT-I-II proteins are located only in the neck of the membrane bud. In this parameter range, the shapes of the budding membranes obtained from the numerical calculations are consistent with those seen in the fluorescence images [21]. Based on the results of the numerical and analytical calculations, I have also identified a three-stage mechanism of spontaneous membrane budding in the presence of the membrane-bound ESCRT-I-II proteins. The three-stage budding process is spontaneous in the sense that no energy barriers are present on the corresponding path in the configuration space of the model system. These results, taken together, provide a theoretical description of the membrane budding process controlled by the ESCRT-I-II proteins.

The main function of the ESCRT proteins in cells is the control of the endosomal pathway [13]. The mechanical process that is required for completing this cellular function is the budding of endosomal membranes [19]. Therefore, my research has provided not only new structural information on the ESCRT complexes [H1,H2,H3] but also results that contribute towards the understanding how the ESCRT machinery performs its biological function [H4]. In addition, a 'by-product' of my research on the ESCRT proteins is the EROS method [H1] and its modifications [H2,H3], which contribute to the development of the so-called hybrid methods of structural biology.

In works [H1-H4] I have used selected methods of statistical and computational physics, including replica exchange Monte Carlo simulations [H1-H3], clustering [H1-H3], maximum entropy [H1], Fourier analysis [H4], and simulated annealing [H4]. In addition, in the research described in publications [H1-H3], I have applied the theory of X-ray scattering, and in work [H4] I have used the membrane elasticity theory. The diversity of theoretical and computational methods, which I have used in my research, reflects the complexity of the undertaken problems.

My contribution to publication [H1] was the following: (i) I participated in deciding about the research directions and in formulating the research objectives; (ii) I performed the replica exchange Monte Carlo simulations of the coarse-grained model of the CHMP3 protein for several different Debye lengths; (iii) I computed the scattering curves based on the CHMP3 structures obtained from the coarse-grained simulations; (iv) I developed the EROS method, i.e., a method for refining conformational ensembles based on SAXS experimental data; (v) I applied the EROS method to the CHMP3 simulation results and obtained conformational ensembles of the CHMP3 protein in solutions of different ionic strengths; (vi) I analyzed the conformational changes of CHMP3; (vii) I wrote the article and prepared all of the figures; and (viii) I participated in responding to the reviewers' comments and in revising the article accordingly.

My contribution to publication [H2] was the following: (i) I participated in formulating the research objectives and methods; (ii) I applied the KH model to the ESCRT-I protein complex and performed the replica exchange Monte Carlo simulations; (iii) I computed the scattering curves for the ESCRT-I structures obtained from the coarse-grained simulations; (iv) I introduced a method for modeling paramagnetic labels (MTSL) and fluorescence labels (Alexa Fluor) at specified, surface-exposed sites of protein structures in the coarse-grained representation; (v) I performed simulations of the DEER signal for pairs of paramagnetic labels on the surfaces of the ESCRT-I structures obtained from the coarse-grained simulations; (vi) I developed a method for determining a minimal conformational ensemble consistent with data from both SAXS and DERR experiments; (vii) I applied the minimum ensemble method to the ESCRT-I protein complex; (viii) For this minimal conformational ensemble, I computed FRET efficiency histograms for pairs of fluorescent labels; (ix) I validated the minimal conformational ensemble on the basis of data from single-molecule FRET experiments on the ESCRT-I complex; (x) I participated in discussing the results, writing the article and preparing figures.

My contribution to publication [H3] was the following: (i) I participated in conceiving and designing the research; (ii) Using the KH coarse-grained approach, I performed the replica exchange Monte Carlo simulations of the ESCRT-II and ESCRT-I-II protein complexes, (iii) Based on the structural models obtained from the coarse-grained simulations, I determined the minimal conformational ensembles consistent with the results of SAXS, DEER and single-molecule FRET experiments performed on the ESCRT-II and ESCRT-I-II protein complexes; (iv) I participated in discussing the results and in writing the article; (v) I produced most of the figures.

My contribution to publication [H4] was the following: (i) I contributed to discussions with co-authors about the literature on the interactions of ESCRT proteins with lipid membranes, (ii) I proposed the physical model of membrane deformations driven by the ESCRT-I-II protein complexes; (iii) I performed all the analytical and numerical calculations within the proposed model; (iv) I had a major contribution to the analysis and interpretation of the calculation results; (v) I wrote substantial parts of the article and made all the figures; (vi) I actively participated in responding to reviewers' comments and in revising the article accordingly.

5.3.2 Properties and functions of disordered linkers in cellulosomal proteins (publications H5, H6, H7)

Cellulosomes are multi-enzyme complexes that break down polysaccharides forming plant-cell walls down to simple sugars [14,15,28]. They mainly catalyze the reactions of hydrolysis of hemicellulose to xylose and cellulose to glucose. They are secreted by cellulolytic microorganisms (certain bacteria and fungi) that use the products of these reactions (glucose and xylose) as a source of nutrients. The best-studied microorganism producing cellulosomes is a thermophilic bacterium *Clostridium thermocellum*.

Cellulosomes are large multi-protein complexes with huge molecular weights – hundreds or even thousands of kDa – depending on the microorganism [29,30]. A protein complex constituting a cellulosome is composed of many subunits that usually contain several distinct domains, which are linked by disordered polypeptide segments [29,30]. This type of molecular architecture is a common feature of ESCRTs and cellulosomes.

The basic structural element of the cellulosome is a protein called scaffoldin [28]. It consists of a number of protein domains called cohesins. The individual cohesins are linked in series, one to another, by disordered segments of the scaffoldin polypeptide chain. The function of the cohesins is the specific binding to various enzymatic subunits – mainly cellulases, glucanases and xylanases of various types. Each of these catalytic subunits contains a protein domain called dockerin. These are the dockerins that bind tightly to the complementary cohesins that make up the scaffoldin [28]. In addition to the cohesin domains, scaffoldins typically contain also carbohydrate-binding modules (CBMs). The CBMs are also present in some of the catalytic subunits.

It turns out that such a complex molecular architecture makes the cellulosomes much more active catalytically than their constituent enzymatic subunits acting independently [15,28]. The molecular mechanisms underlying the cooperative action of cellulosomal subunits remain unexplained. One hypothesis, however, is that the synergy effect is due to the inter-domain linkers.

The disordered inter-domain linkers in cellulosomal proteins differ significantly [29,30]. Some of them are short, built of only a few amino acids. Others are very long and contain several dozens or even several hundred amino-acid residues. Many of them are abundant in proline and threonine. The amino acid sequences of others seem to be quite random. The properties and functions of these disordered segments of polypeptide chains remain largely unexplored. To fill this

knowledge gap, I have conducted a series of theoretical studies [H5,H6,H7] using coarse-grained and full-atom models.

In work [H5] I have studied conformations of xylanase Z, which is a multi-domain enzyme and one of the main components of the CipA cellulosome from *C. thermocellum*. Xylanase Z contains four domains – carbohydrate esterase family 1 (CE1), carbohydrate binding module family 6 (CBM6), type I dockerin and glycoside hydrolase family 10 (GH10) – which are linked in series with three disordered segments of the polypeptide chain (Fig. 1 in Ref. [H5]). The catalytic domain CE1 is involved in braking down chemical bonds between hemicellulose chains. The CBM6 domain binds to carbohydrates – cellulose and other β-glucans – which results in anchoring the xylanase to the plant-cell wall polysaccharides. The dockerin binds tightly to the complementary cohesin domain, which anchors the xylanase in the scaffoldin and, thus, integrates it with the cellulosome. The catalytic domain GH10 breaks down linear xylanes into xylose, thereby contributing to the hemicellulose degradation. The three disordered linkers between the CE1, CBM6, dockerin and GH10 domains are formed by 12, 6 and 24 amino acids, respectively.

The results of SAXS experiments on xylanase Z and also on xylanase Z in complex with a complementary cohesin were published in 2012 [31]. Three years later, [H5] I performed simulations of the xylanase Z using the KH approach. I calculated the theoretical scattering curves for the structural models obtained from the simulations. The scattering curve averaged over the ensemble sampled in these simulations is consistent with the data from SAXS experiments on xylanase Z in an aqueous solution (Fig. 7A in Ref. [H5]). This agreement validates the results obtained from the coarse-grained simulations. Using the KH approach, I have also simulated xylanase Z forming a complex with a cohesin domain. The conformational ensemble generated in these simulations yields a scattering curve that is fully consistent with the data from SAXS experiments on the xylanase-cohesin complex in an aqueous solution (Fig. 3A in Ref. [H5]). This consistency validates the coarse-grained simulation results.

A careful analysis of the aforementioned simulation results, which I have described in detail in Ref. [H5], leads to the following conclusions. Firstly, xylanase Z appears to be flexible in the sense that it adopts diverse conformations – ranging from rather compact ones to very extended ones – both in the presence and absence of the cohesin domain (Figs. 3, 4 and 7 in Ref. [H5]). Secondly, the direct contacts between the individual domains are transient and unstable (Fig. 5 in Ref. [H5]). Xylanase Z is kept as a unit due to the disordered polypeptide chain segments that interconnect the adjacent domains. Thirdly, the end-to-end distance distributions for the disordered polypeptide segments can be accounted for by excluded volume effects (Fig. 6 and 8 in Ref. [H5]). These results, taken together, provide a detailed picture of the conformational ensemble of xylanase Z in an aqueous environment.

Work [H5] demonstrates that xylanase Z attains diverse conformations, which is caused by weak inter-domain interactions and by flexibility of the three disordered linkers. The conformational flexibility of the xylanase is essential for the biological function of this enzyme. In fact, the individual domains of xylanase Z must simultaneously make direct contacts with different portions of the plant-cells wall polysaccharides. These contacts would be impossible to take place if the enzyme had a single stable conformation. Thus, the conformational diversity and flexibility of xylanase Z is of biological significance.

My contribution to publication [H5] was the following: (i) I co-initiated the study; (ii) I proposed and implemented the research methods; (iii) I performed the replica exchange Monte Carlo simulations of xylanase Z within the KH model; (iv) I computed the scattering curves of the structures obtained from the coarse-grained simulations; (v) I compared the computed scattering

curves with the literature results of SAXS experiments on xylanase Z in an aqueous solution (the experimental SAXS data were provided by Prof. Mirjam Czjzek, a co-author on this article); (vi) I analyzed the simulation results; (vii) I wrote the paper and produced all the figures; (viii) I responded to the reviewers' reports and revised the article according to the reviewers' comments.

One of the subjects of publication [H6] concerns conformations of a designer cellulosome that is composed of endoglucanase Cel8A and a mini-scaffoldin ScafT. Both of these proteins are derived from *C. thermocellum*. The endoglucanase Cel8A is formed of a catalytic domain and a dockerin (Fig. 1 Ref. [H6]). These domains are linked by a disordered polypeptide segment. The scaffoldin ScafT is composed of a CBM and a cohesin (Fig. 1 in Ref. [H6]). These two domains are linked by a long, disordered segment of the polypeptide chain. The Cel8A-ScafT complex is formed by tight, non-covalent binding of the dockerin to the cohesin.

I have applied the KH framework to theoretically study conformations of the Cel8A-ScafT mini-cellulosome. Using the replica exchange Monte Carlo simulation method, I have determined the end-to-end distance distributions for the two disordered linkers (Fig. S1 in Ref. [H6]). I have also determined the probabilities of occurrence of amino-acid contacts between the distinct domains of Cel8A and ScafT (Tab. SI in Ref. [H6]). These results demonstrate that direct interactions between the individual protein domains are weak and transient, and that the Cel8A-ScafT mini-cellulosome exhibits large conformational diversity.

I have performed analogous simulations for the scaffoldin ScafT bound by the cohesin-dockerin complex with a four-point mutant of endoglucanase Cel8A. The four-point mutant has been shown experimentally to exhibit increased thermal stability and enhanced enzymatic activity. This mutant is denoted as Cel8A*. It differs from the native enzyme, Cel8A, by four amino acid residues (K276R, G283P, S329G and S375T) within the catalytic domain [32]. Since the atomic structure of the catalytic domain of the mutant enzyme is not known, I have assumed – for the sake of the coarse-grained simulations – that it is analogous to the structure of the native enzyme catalytic domain, i.e., that the mutual positions of the main chain atoms are the same in the native (Cel8A) and mutated (Cel8A*) catalytic domains.

Simulations of the Cel8A-ScafT and Cel8A*-ScafT protein complexes within the KH model yield the same results within the statistical error (Fig. S1 and Tab. SI in Ref. [H6]). In other words, the conformational ensembles of the Cel8A-ScafT and Cel8A*-ScafT complexes are practically indistinguishable within the KH approach. This result follows from the assumption that the main chains of the catalytic domains of Cel8A and Cel8A* have the same structures, and from a simplified representation of protein domains by rigid domains within the KH model. In reality, the catalytic domain of the endoglucanase may, however, undergo non-local structure changes upon the four-site mutation, which is not taken into account within the KH model.

To investigate the structural differences between the native (Cel8A) and mutated (Cel8A*) catalytic domains of the endoglucanase under study, I have performed molecular dynamics (MD) simulations within an all-atom model. Specifically, I have used the NAMD package [33] equipped with the CHARMM force-filed [34,35] with the CMAP correction [36] and the TIP3P water model [37]. The simulation system is the catalytic domain of the investigated endoglucanase (native Cel8A or mutated Cel8A*) bound to a substrate molecule (an oligosaccharide consisting of five glucose residues and being a product of cellulose hydrolysis). A detailed description of the full-atom MD simulation methods is given in section 3.3 in Ref. [H6].

The main result of the all-atom MD simulations is the characterization of the non-local effects of the four-site mutation on the structure of the enzyme. Although the mutated residues are located far away from the active site of the enzyme, the direct contacts between the catalytic

domain and the substrate molecule are found to be different in Cel8A and in Cel8A* (Fig. 7 in Ref. [H6]). This result is consistent with the experimental observation that Cel8A and Cel8A* show different enzymatic activities.

In work [H7] I have used the KH simulation approach to examine a group of about 25 peptides that form disordered linkers in cellulosomes from three organisms: *C. thermocellum*, *A. cellulolyticus* and *B. cellulosolvens*. The quantities determined in the replica exchange Monte Carlo simulations are the end-to-end distances, *d*, for the individual peptides. Since the end-to-end distance distributions, *P(d)*, obtained from the simulations are unimodal, they can be described by the mean value and the variance, which is inversely proportional to the elastic modulus, κ, characterizing a given peptide. The results of the coarse-grained simulations show that κ depends primarily on the number of amino acid residues forming a given peptide, *n*, while the specific sequence of the peptide is a less important factor [H7]. They also show that κ decreases with *n* and $\kappa \sim 1/n^2$ for *n*>10.

Publication [H7] includes also results of all-atom molecular dynamics simulations of twelve peptides comprising *n* = 5, 10 and 15 amino acid residues. The same peptides have been characterized within the KH model. The results obtained on the basis of the KH model are consistent with the results of the all-atom model (Figs. 2 and 3 in Ref. [H7]). This comparison validates the KH model for short peptides.

Many of the disordered linkers in cellulosomes are abundant in prolines. However, the peptides examined in Ref. [H7] have been found to be significantly less rigid than polyprolines (i.e. peptides containing only the proline residues). The results obtained for polyprolines (with *n* = 15, 20, 25, 30 and 35) within the KH model are fully consistent with the literature results of FRET experiments on the polyprolines [38]. This comparison (shown Fig. 2 in Ref. [H7]) provides additional validation of the KH model for peptides.

In work [H7] I have investigated also the influence of the linker stiffness on conformations of the individual domains forming Cel8A-ScafT min-cellulosome. I have conducted this research using a different coarse-grained model, which – unlike the KH model – takes into account the conformational dynamics of protein domains. This model, which I refer to as the Go-type model, is based on a map of residue contacts identified in the atomic structure of the protein under study. The Go-type model has been introduced by Cieplak and co-workers [39,40]. The main assumptions and components of this model are summarized in the following three paragraphs.

The input required to construct the Go-type model is the atomic structure of the protein under study. The atomic structure is used to determine native contacts between pairs of amino acid residues. The native contacts are identified using an overlap criterion, in which heavy atoms are represented by van der Waals spheres increased by a factor that corrects for interactions between atoms.

Within the framework of the coarse-grained Go-type model, each amino acid residue is represented by one 'grain', or 'bead', whose center coincides with the position of the α carbon atom. The neighboring beads along the polypeptide chain are bonded one to another by a strong harmonic potential with a minimum in the native state, i.e., at a distance of about 0.38 nm for each pair of the bonded beads. In addition, a harmonic potential for the polypeptide chain chirality is applied. This potential has the global minimum in the native state. It describes a local stiffness of the polypeptide chain. The remaining interactions between the beads are divided into two groups – native and non-native. The interactions of the native contacts are described by the Lennard-Jones potential, while the non-native interactions are purely repulsive and short-ranged (they vanish at a distance of 0.4 nm). For each pair of beads forming a native contact, the value of the distance

parameter of the Lennard-Jones potential is chosen so that the location of the potential minimum corresponds to the native distance between the beads. The depth of the well of the Lennard-Jones potential is the same for all native contacts.

Within the coarse-grained Go-type model, all beads are assigned with the same mass, and their dynamics is given by the Langevin equation, which introduces the thermostat and describes some dynamic effects of the water environment on the protein motions. However, this description does not take into account long-range hydrodynamic effects. Within the Langevin dynamics, the force acting on each of the beads is a sum of the three components: (i) the force of direct interactions between the beads, which is determined by the potential energy described in the preceding paragraph, (ii) the damping force which is proportional to the velocity of the bead, and (iii) the random force representing thermal noise. The latter force has a zero mean value and its dispersion is such that the fluctuation-dispersion relationship is fulfilled. The Langevin equations are solved using the fifth-order predictor-corrector algorithm. More information on the Go-type model is given in Refs. [H6-H9] and the literature cited therein.

In work [H7] I have used a modified version of the coarse-grained Go-type model to investigate the effects of the disordered linkers on the conformations of the individual domains forming the Cel8A-ScafT mini-cellulosome. One of the main conceptual challenges associated with this study is that the native contact map of the Cel8A-ScafT mini-cellulosome is undetermined because both Cel8A and ScafT contain intrinsically disordered linkers and the Cel8A-ScafT complex as a whole does not assume a single, native, quaternary structure. However, the atomic structures of the individual domains of the Cel8A-ScafT mini-cellulosome (i.e. the catalytic domain of Cel8A, the CBM domain of ScafT, and the cohesin-dockerin complex) are known. Therefore, it has been assumed in Ref. [H7] that the contact map assigned to the full-length Cel8A-ScafT complex contains only the native contacts formed within the individual domains. All the amino-acid contacts involving the residues forming the disordered linkers as well as all the possible contacts between the protein domains are excluded from the contact map.

In the framework of the modified Go-type model, I have performed MD simulations of the Cel8A-ScafT mini-cellulosome. In these simulations, I have determined the average lengths of the native contacts, i.e., the time-averaged distances between these pairs of beads that are identified with the native contacts. I have also performed analogous MD simulations of the individual protein domains separately, i.e., simulations of the catalytic domain alone, of the CBM domain alone, and of the cohesin-dockerin complex alone. The results of these simulations show that some of the native contacts have a different average length in an isolated domain than in the Cel8A-ScafT complex (Fig. 6 in Ref. [H7]). These contacts are localized within about 1.5 nm from the anchoring sites of the linkers. The observed differences in the contact average lengths are small in magnitude (0.1 Angstrom), but the number of modified contacts is significant (over 40 contacts in the entire Cel8A-ScafT complex). This results means that the presence of the disordered polypeptide segments between the protein domains affects the conformations of the linked domains. The MD simulations of the Cel8A-ScafT mini-cellulosome with shorted linkers show that the scale of this effect strongly depends on the length of the linkers (Fig. 6 and 7 in Ref. [H7]). These observations have been confirmed by all-atom MD simulations of a system of two cohesins linked by a disordered polypeptide segment (Fig. 8 in Ref. [H7]).

In work [H6] I have also studied the influence of the linker stiffness on the conformations of the catalytic domain of the Cel8A endoglucanase that is anchored *via* the substrate molecule to a cellulose chain. In these studies, I have used molecular dynamics methods within the coarse-grained Go-type model. The effect of the anchoring of the Cel8A endoglucanase both to the cellulose chain

and to the scaffoldin has been taken into account in the MD simulations as follows. A harmonic potential imitating the bond between the substrate molecule and the cellulose chain has been applied to the bead representing the first monomer of the substrate molecule. Another harmonic potential imitating the linker between the catalytic domain and the rest of the cellulosome has been applied to the bead representing the C-terminal amino-acid residue of the catalytic domain. The rigidity of this linker, κ, is given by the spring constant of the introduced harmonic potential.

I have performed the coarse-grained Go-type MD simulations of the above-described system for different values of parameter κ. The results of these simulations show that the average lengths of certain native contacts vary with the changes in parameter κ (Fig. 3 in Ref. [H6]). These contacts are located in the vicinity of the two sites where the harmonic potentials are applied (Fig. 2 in Ref. [H6]). Interestingly, changes in κ are also accompanied by changes in the average lengths of certain contacts between the catalytic domain and the substrate molecule. In other words, based on the results of the MD simulations, I have identified a group of contacts between the enzyme active site and the substrate molecule whose average lengths vary with the changes in the linker stiffness. This result suggests that the stiffness of the disordered linkers may have an effect on the enzymatic activity of the Cel8A endoglucanase.

In summary, the results presented in publications [H6] and [H7] show that the disordered linkers between the adjacent domains affect the conformations of the linked domains. The scale of this effect is determined by the stiffness of linkers, κ, which depends mainly on the number of amino acid residues forming the linker, *n*, and to a smaller extent on the amino acid sequence of the linker, especially for *n*>10. Thus, the sequential length of the disordered linkers is likely to be a factor that affects the enzymatic activity of cellulosomes.

My contribution to publication [H6] was the following: (i) I proposed most of the research directions; (ii) I developed the main theoretical concepts; (iii) Using the KH approach, I performed the replica exchange Monte Carlo simulations of the Cel8A-ScafT complex; (iv) I performed – within both the all-atom model and the coarse-grained Go-type model – the molecular dynamics simulations of the catalytic domain of the Cel8A endoglucanase as well as its mutant Cel8A*; (v) I analyzed and discussed the results of the coarse-grained and full-atom simulations; (vi) I wrote substantial parts of the article and produced all the figures; (vii) I responded to the reviewers' comments and revised the article accordingly.

My contribution to publication [H7] was the following: (i) I was actively involved in initiating the research subject; (ii) I was actively involved in selecting and implementing the research methods; (iii) I performed the replica exchange Monte Carlo simulations of a group of about 25 peptides forming disordered linkers in cellulosomal proteins; (iv) Using the coarse-grained Go-type methods, I performed molecular dynamics simulations of the Cel8A-ScafT complex; (v) I analyzed and interpreted the results of all the coarse-grained simulations; (vi) I assisted in analyzing the results of the all-atom molecular dynamic simulations, which were performed by Dr. Pierre-Andre Cazade; (vii) I wrote the great majority of the text of the article; (viii) I produced most of the figures; (ix) I responded to the reviewers' comments and revised the article accordingly; (x) I led a research project supported by the National Science Center, Poland, which included the research presented in Ref. [H7].

5.3.3 Mechanical and thermal stability of multi-domain proteins (publications H8, H9)

Adhesion proteins are usually multi-domain proteins. Their trans-membrane domains are embedded in the cell membrane and their extracellular domains bind to proteins in the membrane of another cell [2]. Complexes of adhesion proteins form connections between adhering cells. In order for the

adhesion proteins to perform their biological functions, the individual domains must exhibit adequate mechanical stability, i.e., retain the native structure when external forces are applied to the adhering cells. Publication [H8] concerns the mechanical stability of the CD48-2B4 adhesion protein complex. The CD48-2B4 complex controls the activity of natural killer cells. It is thus involved in immune responses against cancer cells as well as cells infected by viruses.

In work [H8], I have investigated the mechanical response of the ectodomain of the CD48-2B4 protein complex (Fig. 1 in Ref. [H8]) to atomic-force-microscope (AFM) stretching at constant velocity (Fig. 2 in Ref. [H8]). In these studies, I have used the molecular dynamics methods within the coarse-grained Go-type model. The most important results of these simulations are as follows. Firstly, the dissociation process of the CD48-2B4 complex strongly depends on the direction in which the stretching forces are applied, and may take place in different pathways (Figs. 3-5 in Ref. [H8]). In some cases, CD48 and 2B4 separate without any noticeable deformations or structural changes (Figs. 3 in Ref. [H8]). In other cases, CD48 unfolds partially before it dissociates from 2B4 (Figs. 4 and 5 in [H8]). Secondly, the native contacts between CD48 and 2B4 can be divided into three distinct groups, each of which acts as a unit when resisting the stretching forces (Fig. 1 Ref. [H8]). Thirdly, the characteristic mechanostability forces that the CD48-2B4 complex exhibits (Fig. 10 in Ref. [H8]) need not be associated with the tensile forces involved in the act of dissociation of the protein complex because prior shear-involving unraveling within the individual proteins may be more costly mechanically (Fig. 7 in Ref. [H8]). Therefore, inferring information about adhesion forces from AFM stretching experiments may not be straightforward without employing MD simulations.

My contribution to publication [H8] was the following: (i) I proposed the research on the mechanostability of the CD48-2B4 complex; (ii) I adapted the coarse-grained Go-type model and performed the great majority of the molecular dynamics simulations of the CD48-2B4 stretching in the AFM geometry; (iii) I had a major contribution to the analysis and interpretation of the simulation results; (iv) I supervised an undergraduate student (Łukasz Mioduszewski) working on this project within the framework of his summer internship; (v) I wrote the article and produced all the figures; (vi) I revised the article according to the reviewers' comments; (vii) I led the research project that was supported by the National Science Center, Poland, and resulted in publication [H8].

In work [H9] I have studied thermodynamic and kinetic properties of citrate synthase from various organisms (thermophilic, mesophilic and cryophilic) and in different conformations (open and closed). In these studies, I have used the molecular dynamics methods within the coarse-grained Go-type model. I have modified the Go-type model for the purpose of simulating the aforementioned enzyme. Namely, I have replaced the chirality potential by appropriate potentials for bond angles and dihedral angles. These potentials take the minimal values in the native states of the proteins under study. The modification of the potential energy of polypeptide chains in the Go-type model [H9] has been introduced in such a way that the root-mean-square fluctuations (RMSF) obtained in the MD simulations are consistent with the temperature-factors determined by X-ray crystallography (Fig. 4 in Ref. [H9]).

Citrate synthase is an enzyme functioning in almost all living organisms [41]. It is a homodimer, as I have illustrated it in Fig. 1 in Ref. [H9]. Although the crystal structures of citrate synthase from different organisms are almost identical, the Go-type model can distinguish the properties of these proteins in agreement with experimental evidence. It turns out that the proteins that from thermophilic organisms show, within the Go-type model, higher thermal stability than their homologue forms from mesophilic and cryophilic organisms. This correlation has been observed both in the dependence of the native state probability on temperature (Figs. 2 and 3 in Ref.

[H9]) and in the kinetics of thermal denaturation (Figs. 8 and 9 in Ref. [H9]). Interestingly, the level of thermal stability appears to be positively correlated with the average coordination number of the native contacts, and with the degree of structural compactness of the protein under study. The distribution of positional fluctuations of amino acid residues – as measured by RMSF – is different in the open and closed conformations, especially in the vicinity of the active site (Figs. 4-6 in Ref. [H9]). The collective motions of the residues forming the active site are distinctly different in the open and closed conformations (Fig. 7 in Ref. [H9]). In conclusion, the coarse-grained MD simulation results show that the precise location of amino acid contacts in the native structure seems to be crucial to explain the differences and similarities in thermodynamic properties, local elasticity and active-site collective motions between the different forms of citrate synthase.

My contribution to work [H9] was the following: (i) I was actively involved in formulating the research objectives and tasks; (ii) I adapted the coarse-grained Go-type model and performed the molecular dynamics simulations of citrate synthase from several different organisms; (iii) I analyzed and discussed the simulation results; (iv) In close cooperation with Prof. Marek Cieplak, I develop the theoretical concepts needed to interpret the simulation results; (v) I wrote extensive parts of the article and produced all the figures; (vi) I responded to reviewers' comments and revised the article according to the reviewers' recommendations; (vii) I led the research project that was supported by the National Science Center, Poland, and resulted in publication [H9].

The results of my research on the mechanostability of adhesion protein complexes [H8] contribute to a better understanding of how biological cells can withstand mechanical stresses that are enormous on molecular scales. They will also be useful for proper interpretation of the results of future AFM experiments on protein complexes. The results of my research on citrate synthase [H9] contribute to a better understanding of the factors determining the degree of thermal stability of multi-domain proteins. Therefore, they may be used in future research to predict mutations increasing thermal stability of multi-domain proteins and enzymes in particular.

5.3.4. Summary of the significance of publications H1-H9

Publications [H1-H4] contribute to the research on the structure-function relationships of ESCRT proteins. The EROS [H1] method and its modifications [H2,H3], which I have introduced and implemented in the context of my research on the ESCRT proteins, make a significant contribution to the development of the so-called hybrid methods of structural biology. The results presented in Refs. [H5-H7] contribute to the understanding of the physical properties and biological functions of the disordered linkers in cellulosomes. Works [H8,H9] are contributions to the research on thermal and mechanical stability of multi-domain proteins and protein complexes.

In works [H1-H9] I have used a number of distinct computational methods, which have their roots in statistical physics, namely, molecular dynamics (within both the coarse-grained Go-type model [H6-H9] and all-atom models [H6,H7]) Monte Carlo methods [H1-H7] (including replica exchange Monte Carlo simulations [H1,H2,H3,H5,H6]), the maximum entropy method [H1] and clustering [H1-H3]. In works [H1,H2,H3,H5] I have used the classical theory of X-ray scattering. In work [H4] I have applied the theory of phase transitions in classical fluids as well as the theory of membrane elasticity. The need for this variety of computational methods and physical theories is due to the complexity of the investigated problems related to the conformational dynamics of multi-domain proteins.

My work on the conformations [H1,H2,H3] and the mechanisms of action [H3,H4] of the ESCRT proteins have been published in renowned scientific journals dedicated to structural biology (publications [H1,H3] in *Structure*), computational biology (publication [H4] in *PLoS*

Computational Biology) and natural sciences in general (publication [H2] in *Proceedings of the National Academy of Sciences of the United States of America*). Publications [H1-H4] together have been cited over 200 times according to the Web of Science database.

My research on disordered linkers in cellulosomal proteins [H5,H6,H7] has been published in scientific journals dedicated to structural biology (publication [H5] in *Journal of Structural Biology*), biophysics and molecular biochemistry (publication [H6] in *Molecular BioSystems*) and physical chemistry and chemical physics (publication [H7] in *Physical Chemistry Chemical Physics*). My works on the stability of multi-domain proteins [H8,H9] have been published in journals dedicated to protein research (publication [H8] in *Proteins: Structure, Function and Bioinformatics*) and chemical physics (publication [H9] in *Journal of Chemical Physics*). This diversity of journals publishing the results of my work demonstrates the interdisciplinary nature of my research that lies at the crossroads of physics, chemistry and biology.

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6. Discussion of other scientific achievements

6.1 Description of the research not contributing directly to the habilitation theses

6.1.1 Research conducted prior to obtaining PhD in physics

During my master studies and doctoral studies at the Faculty of Physics, University of Warsaw, I specialized in statistical physics and soft-matter physics. The subject of my master thesis was the wetting phenomenon. I studied the finite-size effects on the wetting transition. Using the transition matrix method I obtained, within the equilibrium statistical mechanics, an exact solution of a lattice model of a one-dimensional interface (line) confined between two parallel walls in two dimensions [D1]. At the beginning of my doctoral studies, I modified the aforementioned model for the case of a one-dimensional membrane (line) interacting *via* adhesion molecules with two parallel surfaces [D2].

During my doctoral studies, I spent three semesters as a visiting student at the Theory Department of the Max Planck Institute of Colloids and Interfaces (Potsdam, Germany, summer semesters in 2003, 3004 and 2005). My supervisors were Dr. Thomas Weikl and Prof. Reinhard Lipowsky, who introduced me to the research on membrane adhesion.

Adhesion of cell membranes results from non-covalent binding of adhesion molecules (so-called receptors) embedded in the plasma membrane with specific molecules (so-called ligands) contained in the opposing membrane of another cell. Membrane adhesion facilitates interactions between cells, which is important in many biological processes, including tissue formation and immune response processes of multicellular organisms.

My PhD dissertation concerns the application of the theory of stochastic processes to the theoretical description of cell membrane adhesion. The adhesion of cell membranes has been modeled and studied theoretically for several decades but mainly within the framework of equilibrium statistical physics. Cellular membranes, however, are dynamic systems that are maintained away from the state of thermodynamic equilibrium. In particular, certain adhesion receptors (e.g. integrins) are coupled to processes or reactions – such as ATP hydrolysis – which are kept by living cells away from the chemical equilibrium. This type of coupling of adhering membranes with their environment excludes the possibility of describing the system under study in the framework of equilibrium statistical physics. It is the necessity of characterizing non-equilibrium states that constitutes the original research subject of my dissertation. The subject matter of my PhD theses are non-equilibrium steady states of membranes interacting by active adhesion molecules, i.e., molecules whose conformational changes are driven by external stimuli [D3,D4,D5]. I have analyzed such steady states by modeling the dynamics of the interacting membranes within the framework of the theory of stochastic processes.

During my doctoral studies I also collaborated with Dr. Mesfin Asfaw. (At that time, he was a PhD student at the Theory Department of the Max Planck Institute for Colloids and Interfaces). Our collaboration was focused on the application of equilibrium statistical mechanics to the theoretical description of adhesion of multi-component membranes [D6]. The phenomena under study were, firstly, membrane unbinding transitions and, secondly, phase separation processes occurring in adhering membranes.

Works published prior to obtaining my PhD or publications directly related to my dissertation:
D1. Bartosz Różycki, Marek Napiórkowski. *The RSOS model for a slit with different walls*. J. Phys. A: Math. Gen. **36**: 4551-4559 (2003). *Impact Factor* in 2003: 1,357.

D2. Bartosz Różycki, Marek Napiórkowski. *Phase transitions in multicomponent string model*. Europhys. Lett. **66**: 35-40 (2004). *Impact Factor* in 2004: 2,120.

D3. Bartosz Różycki, Reinhard Lipowsky, Thomas R. Weikl. *Adhesion of membranes with active stickers*. Phys. Rev. Lett. **96**: 048101 (2006). *Impact Factor* in 2006: 7,072.

D4. Bartosz Różycki, Thomas R. Weikl, Reinhard Lipowsky. *Adhesion of membranes via switchable molecules*. Phys. Rev. E **73**: 061908 (2006). *Impact Factor* in 2006: 2,438.

D5. Bartosz Różycki, Thomas R. Weikl, Reinhard Lipowsky. *Stochastic resonance for adhesion of membranes with active stickers*. Eur. Phys. J. E **22**: 97-106 (2007). *Impact Factor* in 2007: 2,025.

D6. Mesfin Asfaw, Bartosz Różycki, Reinhard Lipowsky, Thomas R. Weikl. *Membrane adhesion via competing receptor/ligand bonds*. Europhys. Lett. **76**: 703-706 (2006). *Impact Factor* in 2006: 2,229.

Summary of my contributions to publications D1-D6:

- My contribution to publication [D1] was the following: (i) I formulated the theoretical problem and found its solution using the transition matrix method; (ii) I performed all the calculations; (iii) I analyzed and discussed the calculation results; (iv) I described both the methods and the results, and I produced all the figures; (v) I was involved in responding to the reviewers' comments and in revising the article according to the reviewers' suggestions.

- My contribution to publication [D2] was the following: (i) I proposed the research topic; (ii) I formulated the theoretical problem and found its analytical solution; (iii) I performed all the calculations; (iv) I analyzed and discussed the calculation results; (v) I wrote the article and made all the figures; (vi) I contributed to revising the article according to reviewers' comments.

- My contribution to publication [D3] was the following: (i) I was actively involved in formulating the research subject; (ii) I performed the dynamic Monte Carlo (DMC) simulations of the lattice model of fluid membranes interacting *via* active adhesion molecules; (iii) I performed the analytical calculations that helped in interpreting the stochastic resonance effect observed in the DMC simulations; (iv) I studied the dependence of the membrane unbinding transition on the rate of switching of the active adhesion molecules; (v) I analyzed and discussed the simulation results; (vi) I contributed to the writing of the article and to the production of the figures.

- My contribution to publication [D4] was the following: (i) I was actively involved in designing the research; (ii) I performed the DMC simulations of the lattice model of fluid membranes bound by active adhesion molecules; (iii) I performed analytical calculations showing how the DMC dynamics converges to the Langevin dynamics; (iv) I found an analytical solution of the Langevin equations in a harmonic approximation, and I compared this solution to the DMC simulation results; (v) I analyzed and discussed the simulation results; (vi) I wrote substantial parts of the article and produced all the figures.

- My contribution to publication [D5] was the following: (i) I was actively involved in formulating the research directions and objectives; (ii) I performed the numerical simulations of the lattice model of membranes interacting *via* active adhesion molecules; (iii) I derived the Fokker-Planck equation equivalent to the stochastic dynamics of the aforementioned membrane model; (iv) I solved the Fokker-Planck equation numerically within a mean-filed approximation, and I compared this numerical solution to the simulation results of the lattice

model; (v) I analyzed and discussed all the numerical results; (vi) I wrote essential parts of the article and produced all the figures.

- My contribution to publication [D6] was the following: (i) I had numerous discussions with co-authors about phase transitions in adhering multi-component membranes; (ii) In collaboration with Mesfin Asfaw, I performed the majority of analytical calculations; (iii) I helped to derive equations (10) and (12), which were the main results of Ref. [D6].

6.1.2 Research conducted after obtaining PhD in physics

6.1.2.A Research on adhesion of lipid membranes and multicomponent membranes

During my postdoctoral research at the Department of Theory and Bio-Systems, Max Planck Institute of Colloids and Interfaces, Germany, 2006-2008, I conducted research on membrane adhesion [A1-A7] and phase separation in multi-component membranes [A1,A6,A7]. In these studies, I used various methods of classical statistical mechanics (including Monte Carlo methods) as well as the membrane elasticity theory. My research supervisors were Dr. Thomas Weikl and Prof. Reinhard Lipowsky. The work on membrane adhesion [A4-A7] was, to some extent, a continuation and extension of the research I conducted during my doctoral studies in collaboration with Dr. Mesfin Asfaw [D6]. In contrast, publications [A1-A3] were not related to the research I conducted during my PhD studies. Publication [A1] deals with phenomena occurring in multi-component lipid membranes adhering to corrugated surfaces of solid substrates. Publications [A2,A3] concern membrane adhesion that results from nanoparticle adsorption from the membrane environment.

My contributions the research on membrane adhesion and phase separation in membranes:

A1. <u>Bartosz Różycki</u>, Thomas R. Weikl, Reinhard Lipowsky. *Stable patterns of membrane domains at corrugated substrates*. Phys. Rev. Lett. **100**: 098103 (2008). *Impact Factor* in 2008: 7,180.

A2. <u>Bartosz Różycki</u>, Reinhard Lipowsky, Thomas R. Weikl. *Effective surface interactions mediated by adhesive particles*. EPL **84**: 26004 (2008). *Impact Factor* in 2008: 2,203.

A3. <u>Bartosz Różycki</u>, Reinhard Lipowsky, Thomas R. Weikl. *Adhesion of surfaces via particle adsorption: exact results for a lattice of fluid columns*. J. Stat. Mech. P11006 (2009). *Impact Factor* in 2009: 2,670.

A4. Heinrich Krobath, <u>Bartosz Różycki</u>, Reinhard Lipowsky, Thomas R. Weikl. *Binding cooperativity of membrane adhesion receptors*. Soft Matter **5**: 3354-3361 (2009). *Impact Factor* in 2009: 4,869.

A5. Thomas R. Weikl, Mesfin Asfaw, Heinrich Krobath, <u>Bartosz Różycki</u>, Reinhard Lipowsky. *Adhesion of membranes via receptor-ligand complexes: Domain formation, binding cooperativity, and active processes*. Soft Matter **5**: 3213-3224 (2009). *Impact Factor* in 2009: 4,869.

A6. <u>Bartosz Różycki</u>, Reinhard Lipowsky, Thomas R. Weikl. *Segregation of receptor-ligand complexes in cell adhesion zones: phase diagrams and the role of thermal membrane roughness*. New J. Phys. **12**: 095003 (2010). *Impact Factor* in 2010: 3,849.

A7. Heinrich Krobath, <u>Bartosz Różycki</u>, Reinhard Lipowsky, Thomas R. Weikl. *Line tension and stability of domains in cell-adhesion zones mediated by long and short receptor-ligand complexes*. PLoS ONE **6**: e23284 (2011). *Impact Factor* in 2011: 4,092.

Summary of publications A1-A7:

- Publication [A1] is about formation of domains of the liquid-ordered (Lo) and liquid-disordered (Ld) phases in a multi-component lipid membrane adhering to a corrugated surface. This phenomenon has been discovered in fluorescence microscopy experiments where it has been observed that, under certain conditions, the shapes of the Lo and Ld domains reflect the topography of the solid substrate to which the membrane adheres. Publication [A1] explains and quantitatively describes this phenomenon on the basis of the classical thermodynamics and the theory of elasticity of fluid membranes. The theory introduced in publication [A1] takes into account such parameters as the line tension between the Lo and Ld phases, the differences in the rigidity of the membrane domains in the Lo and Ld phase, the adhesion energy of the lipid membrane to the solid substrate, and the shape of the corrugated substrate. The main results of publication [A1] are the phase diagrams indicating the parameter regimes in which stable, disjoint Lo and Ld domains are present in the corrugated lipid membrane.

  My contribution to this work was the following: (i) I had a major contribution to the development of the theory explaining the fluorescence microscopy experiments; (ii) I performed all the analytical calculations and all the Monte Carlo simulations; (iii) I built the phase diagrams; (iv) I wrote most of the article and produced all of the figures.

- Publication [A2] concerns the adhesion of membranes that is caused by adsorption of nanoparticles from the membrane environment. The system under study consists of two opposing membranes and nanoparticles in the aqueous solution surrounding the membranes. The nanoparticles bind non-covalently to the membranes, which leads to effective interactions between the two membranes. Publication [A2] introduces a lattice model describing interactions of the nanoparticles with the membrane surfaces. I have solved this model analytically within the framework of the classical statistical mechanics in the limit of small concentrations of the nanoparticles. The main result of this calculation is the dependence of the effective membrane interactions on the bulk concentration of the nanoparticles. This dependence turns out to be non-monotonic. The maximum energy of the effective membrane interactions occurs at the nanoparticle concentrations for which a single, separate membrane is covered by the nanoparticles in about half of its area. It depends on the Boltzmann factor with the binding energy of a single nanoparticle to the membrane surface.

  My contribution to publication [A2] was the following: (i) I participated in formulating the theoretical model of the membrane system under study; (ii) I performed all the calculations presented in Ref. [A2]; (iii) I actively participated in the analysis and interpretation of the calculation results; (iv) I actively participated in the writing of the article, and I produced Figs. 2, 3 and 4.

- Publication [A3] is a continuation of the work published in Ref. [A2]. Namely, it contains an exact solution of the lattice model introduced in publication [A2]. This solution approaches the results of publication [A2] in the limit of small concentrations of the adhesive nanoparticles. My contribution to publication [A3] was the following: (i) I developed the theoretical studies on the effect of the nanoparticle adsorption on the effective membrane interactions; (ii) I performed and presented all the calculations; (iii) I analyzed and discussed the calculation results; (iv) I wrote substantial parts of the article and produced Figs. 2-6; (v) I was actively involved in responding to reviewers' reports and in revising the article according to reviews' comments.

- Publication [A4] concerns the effect of thermal fluctuations on the binding of membrane adhesion molecules. The system under study consists of two opposing membranes. One of them contains adhesion molecules (receptors). The other one contains complementary adhesion

molecules (ligands) that bind non-covalently to the receptors in the opposite membrane, which leads to membrane adhesion. The receptors and ligands diffuse within the membranes in which they are anchored. The shapes of the membranes are subject to local changes due to thermal excitations. The binding of a receptor to a ligand depends not only on the energy of their direct interactions, but also on the local separation between the membranes and, thus, also on the location of the receptor-ligand complexes that have already been formed. This effect is the source of the cooperative receptor-ligand binding.

The system under study is cast in the framework of a lattice model in which the energy of membrane deformations is described by the Helifrich Hamiltonian. The energy of interactions of the receptors with the ligands depends on the local separation between the membranes. The model system is described within the grand-canonical ensemble, which makes it possible to sum out the degrees of freedom of the receptors and ligands in the partition function. As a result, the theoretical problem is reduced to considering homogeneous, mono-component membranes interacting *via* an effective potential, which depends implicitly on the concentrations of the receptors and ligands.

The introduced lattice model has been solved within the framework of equilibrium statistical mechanics. The analytical calculations have been supported and supplemented by Monte Carlo simulations. The main result of this work is Eq. (3), which quantitatively describes the cooperativity of the receptor-ligand binding. My contribution to this publication was the following: (i) I had an essential contribution to the development of the theory of cooperative bonding of adhesion molecules; (ii) I performed most of the analytical calculations and, in particular, derived Eq. (3), which is the main result of publication [A4]; (iii) I participated in the analysis of the numerical simulations performed by Dr. Heinrich Krobath; (iv) I participated in the writing of the article.

- Article [A5] is a review on theoretical works about membrane adhesion. My contribution to publication [A5] was focused on two issues: (i) adhesion of membranes interacting *via* active adhesion molecules, and (ii) fluctuation-induced cooperative binding of adhesion receptors with their ligands in an opposing membrane.

- Publication [A6] is, to some extent, related to publication [A4]. It concerns the influence of thermal fluctuations on the segregation of adhesion protein complexes in the membrane adhesion zone. The system under study consists of two opposing membranes. One o them represents a segment of a vesicle, or a cell, and contains membrane adhesion molecules (receptors). The other membrane is supported on a flat substrate and contains two types of complementary adhesion molecules (ligands of types L1 and L2) that differ in size (one type of ligands is longer than the other one) and interacts with receptors in the opposing membrane. This system is illustrated in Fig. 1 in Ref. [A6].

The non-covalent binding of the receptors to the ligands leads to the adhesion of the two opposing membranes. Since the ligands L1 and L2 vary in length, the receptor-ligand complexes also have different lengths. The presence of the 'long' and 'short' receptor-ligand complexes in the membrane adhesion zone results in deformations of the membrane containing the receptor molecules. If the average membrane deformation energy is sufficiently large, the receptor-ligand complexes get separated spatially. One can then distinguish a thermodynamic phase enriched in the 'long' receptor-ligand complexes and a thermodynamic phase enriched in the 'short' receptor-ligand complexes. Publication [A6] contains a theoretical description of this phenomenon based the classical statistical physics. As in publication [A4], the considered

system is cast in the framework of a lattice model that takes into account the energy of membrane deformations as well as the energy of receptor-ligand interactions. However, the analytical calculations are performed in the canonical ensemble, not in the grand-canonical one, because the total number of the adhesion receptors on the vesicle surface is fixed.

The main results of publication [A6] are illustrated in the phase diagrams shown in Fig. 5. Importantly, these diagrams account for thermal fluctuations in the system of interacting membranes. Figure 5a refers to a situation in which the ligands L1 and L2 bind to the same type of receptors. Figure 5b refers to a situation in which the ligands L1 bind to the receptors R1, and the ligands L2 bind to the receptors R2. Depending on the values of the model parameters, the phase enriched in the 'long' receptor-ligand complexes or the phase enriched in the 'short' receptor-ligand complexes may occupy the membrane adhesion zone. These two phases can coexist in a certain range of model parameters. The phase diagrams shown in Fig. 5 contain also a critical point above which the two phases become indistinguishable. The analytical expressions for the phase coexistence lines are derived in sections 4.3 and 5.2 of Ref. [A6].

My contribution to publication [A6] was the following: (I) I participated in formulating the theoretical approach to the physical problem and in developing appropriate computational methods; (ii) I performed all of the analytical calculations and all of the numerical simulations; (iii) I constructed the phase diagrams in Fig. 5; (iv) I was involved in writing the article and in responding to reviewers' comments.

- Publication [A7] is closely related to publication [A6]. It is about the stability of membrane domains formed by the adhesion protein complexes in the membrane adhesion zone. The physical system under study is analogous to the system considered in Ref. [A6]. It can exhibit the phase enriched in the 'long' receptor-ligand complexes or the phase enriched in the 'short' receptor-ligand complexes. These phases can coexist under certain conditions. The free energy per unit length of the line separating the coexisting phases is called the line tension. In work [A7], the line tension has been determined as a function of the model parameters: the bending rigidity modulus of the receptor-containing membrane; the energy of binding a receptor molecule R1 to its ligand L1; the energy of binding a receptor molecule R2 to its ligand L2; and the lengths and concentrations of the receptors and ligands. These results provide much information, for example, about the nucleation of the membrane domains.

My contribution to this work was the following: (i) I performed a dimensional analysis (Appendix S1 to the article), which helped to solve the statistical-physical model; (ii) I designed a novel Monte Carlo method that allowed for an effective determination of the line tension on the basis of the classical nucleation theory; (iii) I participated in the analysis and discussions of the simulation results; (iv) I was involved in the writing of the article.

6.1.2.B Research on small angle X-ray scattering on proteins in aqueous solutions

During my postdoctoral studies in the Laboratory of Chemical Physics at the National Institute of Diabetes, Digestive and Kidney Diseases (NIH, USA, 2008-2011) I was involved in research on structures, dynamics and functions of proteins. My scientific supervisor was Prof. Gerhard 5(now director of the Max Planck Institute for Biophysics, Frankfurt, Germany), who is a world-class expert in biological and chemical physics (Hirsh index = 75, over 19000 citations, according to the Web of Science database).

The research on protein structures is motivated mainly by the aspiration to explain the basic life processes at the molecular level. Determining the three-dimensional structure of a given protein often leads to the explanation of how the protein performs its biological functions. Among the

proteins that are nowadays most difficult to characterize structurally are those, which contain multiple domains linked by disordered polypeptide segments. Although such proteins are abundant and critically important in biological cells (examples are the ESCRT proteins, described in section 5.3.1), they are a white spot in the field of structural biology. They cannot be studied comprehensively by X-ray crystallography methods due to the presence of the disordered linkers (only structures of the individual domains can be solved). They cannot be studied by nuclear magnetic resonance due to their large size (usually of the order of hundreds of kDa). Therefore, complementary methods have to be used in structural studies of this type of proteins. In particular, small angle X-ray scattering (SAXS) is increasingly used to complement X-ray crystallography and NMR.

X-ray crystallography and nuclear magnetic resonance (NMR) are considered to be the main driving forces of structural biology. However, these methods obviously have their limitations. Namely, X-ray crystallography is optimally suited to study the proteins that take a single, stable, well-defined structure – i.e. the native structure – which enables the growth of mono-crystals of these proteins. The current NMR methods are applicable to structural studies of proteins with molecular masses not exceeding ~50 kDa. The SAXS method is not subject to these restrictions and gives the possibility of structural characterization of proteins in aqueous environments. Firstly, unlike X-ray crystallography, the SAXS method does not require protein crystals. Therefore, it can be used, for example, to study intrinsically disordered proteins. Secondly, unlike NMR, the SAXS method is not limited by the protein size. It can be used for large protein complexes as well as small proteins or peptides. The quality of the scattering curves obtained from SAXS experiments does not depend directly on the size or 'softness' of the proteins. Furthermore, the SAXS method can be used to study proteins in a wide range of concentrations, temperatures, pH values, ionic strengths, and so on.

A weak point of the SAXS method is the low spatial resolution – usually in the range of 1 to 2 nm. One can also say that a complex, three-dimensional structure of a protein molecule is reduced to a one-dimensional scattering curve in SAXS experiments. However, despite the resulting loss of structural information, careful analyses and interpretation of data from SAXS experiments may provide important information on the protein structure-function relationships.

Protein crystallography is being increasingly supplemented by SAXS to determine structures of multi-domain proteins and protein complexes [S4]. In addition, methods combining NMR and SAXS can be used to determine protein conformations in aqueous solutions [S4]. This combination seems to be particularly effective because NMR and SAXS provide complementary structural information. Namely, data from NMR measurements impose 'local' restrains on the distances between atoms and/or dihedral angles between chemical bonds in the protein under study, while data from SAXS experiments provide 'global' information about the size and shape of the protein.

Data from SAXS experiments are often used to determine the so-called molecular envelopes that represent the protein shapes and sizes [S4]. However, if a given protein is disordered under physiological conditions – i.e., it does not have a single, stable, tertiary structure – then a single molecular envelope cannot represent its shape. Therefore, molecular modeling of this type of proteins requires a different strategy, which gives the possibility to describe the protein state as a conformational ensemble rather than a single structure [H1,H2,H3,H5]. In this approach, the native state of the protein is represented by a group of structural models, which as a whole must be consistent with the available experimental data. My contribution to the research on intrinsically disordered proteins is the development the EROS method, which combines molecular simulations

with SAXS experiments to determine conformational ensembles [H1]. I have used the EROS method to characterize conformations of protein kinase C [S1] as well as kinases forming dynamic complexes with phosphatases [S2,S3]. These results have provided important information about the molecular mechanisms underlying the biological functions of these enzymes, and have been published in prestigious journals dedicated to cell biology (*Cell*) [S1], chemistry (*Journal of the American Chemical Chemistry*) [S2] and biochemistry (*Nature Chemical Biology*) [S3].

Having accomplished the post-doctoral training in the group of Prof. Gerhard Hummer, I continued the research on interpretation of SAXS experimental data. In work [H5], I have used coarse-grained simulations and available SAXS data to characterize the conformational ensemble of a cellulosomal multi-domain enzyme. In article [S4], I have reviewed the hybrid methods of structural biology that are being developed to determine conformational ensembles of multi-domain and partially disordered proteins. An important ingredient of these hybrid methods is small angle X-rays scattering. In work [S5], I have helped in developing a novel computational method for structure refinement of peptides and small proteins based on SAXS experimental data. In work [S6], I have used coarse-grained simulations and data from SAXS experiments to determine conformations of the PI4KB-14-3-3 complex, i.e., conformations of phosphatidylinositol 4-kinase IIIβ (PI4KB) in complex with a 14-3-3 protein. Based on the structural models of the PI4KB-14-3-3 complex, which I have determined using molecular simulations and SAXS data, three hypotheses have been made about the interaction between the 14-3-3 protein and the PI4KB enzyme, and about the molecular mechanisms underlying the biological function the PI4KB-14-3-3 complex. These hypotheses have been tested and verified in biochemical experiments.

My contributions to the research on protein structure-function relationships using computer simulations and data from SAXS experiments:

S1. Thomas A. Leonard, <u>Bartosz Różycki</u>, Layla F. Saidi, Gerhard Hummer, James H. Hurley. *Crystal structure and allosteric activation of protein kinase C β-II*. Cell **144**: 55-66 (2011). *Impact Factor* in 2011: 32,403. Over 100 citations according to the Web of Science database.

S2. Dana M. Francis*, <u>Bartosz Różycki</u>*, Antoni Tortajada, Gerhard Hummer, Wolfgang Peti, Rebecca Page. *Resting and active states of the ERK2:HePTP complex*. J. Am. Chem. Soc. **133**: 17138-17141 (2011). *Impact Factor* in 2011: 9,907.

S3. Dana M. Francis, <u>Bartosz Różycki</u>, Dorothy Koveal, Gerhard Hummer, Wolfgang Peti, Rebecca Page. *Structural basis of p38-α regulation and specificity by hematopoietic tyrosine phosphatase*. Nature Chem. Biol. **7**: 916-924 (2011). *Impact Factor* in 2011: 14,690.

S4. <u>Bartosz Różycki</u>, Evzen Boura. *Large, dynamic, multi-protein complexes: A challenge for structural biology*. J. Phys.: Condens. Matter **26**: 463103 (2014). *Impact Factor* in 2014: 2,346.

S5. Kecheng Yang, <u>Bartosz Różycki</u>, Fengchao Cui, Ce Shi, Wenduo Chen, Yunqi Li. *Sampling enrichment toward target structures using hybrid Molecular Dynamics-Monte Carlo simulations*. PLoS ONE **11**: e0156043 (2016). *Impact Factor* in 2016: 2,806.

S6. Dominika Chalupska, Andrea Eisenreichova, <u>Bartosz Różycki</u>, Lenka Rezabkova, Jana Humpolickova, Martin Klima, Evzen Boura. *Structural analysis of phosphatidylinositol 4-kinase IIIβ (PI4KB) – 14-3-3 protein complex reveals internal flexibility and explains 14-3-3 mediated protection from degradation in vitro*. J. Struct. Biol. **200**: 36-44 (2017). *Impact Factor* in 2016: 2,767

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* equal contributions

Summary of publications S1-S6:

- Publication [S1] is a study on the structure-function relationship of protein kinase C (PKC) of type β-II. The atomic structure of a large part of the multi-domain PKC β-II enzyme has been determined by X-ray crystallography methods. This structure has captured an unexpected intermediate state in the activation pathway of this enzyme. The results of SAXS experiments on PKC β-II in an aqueous environment provided a low-resolution structural model of this enzyme in a closed conformation. The structural studies taken together show how the PKC β-II enzyme is allosterically regulated in two steps (Fig. 6 in Ref. [S1]), with the second step defining a novel protein kinase regulatory mechanism as discovered in work [S1].

  My contribution to this work was the following: (i) Using the KH coarse-grained approach, I performed replica exchange Monte Carlo simulations of the multi-domain PKC β-II enzyme; (ii) I computed the scattering curves for the low-resolution PKC β-II structures obtained from the coarse-grained simulations; (iii) I selected representative structural models consistent with the results of SAXS experiments on PKC β-II in an aqueous environment; (iv) I participated in discussing the SAXS results and in producing Fig. 5.

- Work [S2] concerns the mitogen-activated protein kinase ERK2 in complex with the tyrosine phosphatase HePTP. I have used an EROS-type method to determine the conformations of the ERK2-HePTP protein complex in the resting and active states. These results show that the resting-state ERK2-HePTP complex exhibits diverse conformations with an elongated shape (Fig. 1 in Ref. [S2]). However, in the active state, ERK2 is bound closely to HePTP (Fig. 2 in Ref. [S2]). The ERK2-HePTP complex takes a much more compact form in the active state than in the resting state. Therefore, the phosphorylation and de-phosphorylation of ERK2 results in very large conformational changes of the ERK2-HePTP complex (Fig.3 in Ref. [S2]). These results contribute to the research on molecular mechanisms of regulation of protein kinase activity.

  My contribution to publication [S2] was the following: (i) I adapted the KH model and performed replica exchange Monte Carlo simulations of the ERK2-HePTP complex both in the active and resting states; (ii) Based on the ERK2-HePTP conformations obtained from the coarse-grained simulations, I determined the minimal ensembles consistent with the results of the SAXS experiments on the ERK2-HePTP complex in the resting and active states; (iii) I described the computational methods and results; (iv) I participated in producing all of the figures.

- Publication [S3] concerns a physicochemical and structural characterization of interactions between the mitogen-activated protein kinase p38α and the tyrosine phosphatase HePTP. I have used a modified EROS method to integrate data from SAXS, NMR and X-ray crystallography experiments and, thus, determine the conformations of the p38α-HePTP protein complex. Here, X-ray crystallography has been used to determine structures of the individual protein domains that form the p38α-HePTP complex; chemical shift perturbations (as determined in NMR experiments) have provided information on the amino acid residues that form inter-domain contacts; and data from SAXS experiments have helped to position the constituent protein domains in space one relative to another.

  My contribution to this work was the following: (i) I modified the KH approach by introducing a method for biasing replica exchange Monte Carlo simulations by NMR data (namely, by chemical shift perturbations), and applied this novel method to the p38α-HePTP complex; (ii) I analyzed the results of these simulations and determined a conformational

ensemble consistent with the SAXS data; (iii) I described the computational methods and the results obtained; (iv) I participated in producing Fig. 5.

- Article [S4] is a review on the so-called hybrid methods of structural biology. One of the main objectives of this work is to discuss the usage of computer simulations as well as other computational methods for interpretation of data from SAXS experiments on multi-domain and partially disordered proteins. My contribution to publication [S4] was the following: (i) I participated in preparing the review on the subject matter discussed in the article, (ii) I wrote extensive parts of the article and made most of the figures; (iii) I edited the entire text of the article; (iv) I revised the article according to reviewers' comments.

- In publication [S5], a novel method of utilizing SAXS data for protein structure refinement has been introduced and tested. The method is based on molecular dynamics simulations that are biased by SAXS experimental data using a Monte Carlo procedure. By combining molecular dynamics simulations with SAXS experiences, this method yields better predictions of protein and peptide structures. My contribution to this work was the following: (I) I consulted with co-authors on the theory of X-ray scattering on proteins in aqueous solutions; (ii) I participated in interpreting the simulation results and in writing the article.

- Publication [S6] provides an analysis of the structure-function relations of phosphatidylinositol 4-kinase IIIβ (PI4KB) in complex with a 14-3-3 protein. The PI4KB enzyme is involved in the synthesis of phosphatidylinositol 4 phosphate (PI4P), which is a phospholipid involved in signaling in eukaryotic cells. PI4KB is strictly regulated by many proteins. In particular, it is known that the 14-3-3 protein, after phosphorylation by protein kinase D, binds to PI4KB. However, the molecular mechanisms underlying the PI4KB recognition by the 14-3-3 proteins have been unknown.

  In work [S6], it has been demonstrated – by using analytical ultracentrifugation – that proteins 14-3-3 and PI4KB form a complex in the 2:2 stoichiometry. Also SAXS experiments have been performed on the PI4KB-14-3-3 complex in aqueous solutions. The results of these experiments have been interpreted on the basis of the PI4KB-14-3-3 simulations, which I have performed within the KH model. These results together have led to proposing a three-dimensional model of the PI4KB-14-3-3 complex in the 2:2 stoichiometry. This model predicts that both the active site and the ACBD3-binding site (ACBD3 stands for acyl-CoA-binding domain-containing protein-3) in PI4KB are located far away from the 14-3-3 protein dimer. Therefore, on the basis of the proposed structural model of PI4KB-14-3-3, the following three hypotheses have been made: Firstly, the 14-3-3 protein does not directly affect the PI4KB enzymatic activity. Secondly, the binding of the 14-3-3 protein to the PI4KB enzyme does not affect the recruitment of PI4KB to intracellular membranes by the ACBD3 protein. Thirdly, the function of the 14-3-3 protein is to protect disordered segments of PI4KB from proteolysis. All these hypotheses have been tested and confirmed in biochemical experiments.

  My contribution to publication [S6] was the following: (i) I performed replica exchange Monte Carlo simulations of the PI4KB-14-3-3 complex within the coarse-grained KH model; (ii) I computed the scattering curves for the structural models obtained from the coarse-grained simulations; (iii) I analyzed the data obtained from the SAXS experiments on the PI4KB-14-3-3 complex in aqueous solutions; (iv) I determined the PI4KB-14-3-3 structural models consistent with the SAXS data; (v) I analyzed and mutually compared the determined models of PI4KB-14-3-3; (vi) I participated in deducing the functions that the 14-3-3 protein performs when bound to the PI4KB enzyme; (vii) I participated in writing the article, and I produced Fig. 3.

6.1.2.C Research on membrane proteins and lipid membrane deformations

Since October 2012, I have been employed as assistant professor at the Institute of Physics, Polish Academy of Sciences, Warsaw. The main subjects of my current research are multi-domain proteins and protein complexes [H5-H9], in particular cellulosomal proteins [H5-H7]. In this research area, I collaborate closely with Prof. Marek Cieplak. My side projects concern, firstly, the structures and functions of membrane proteins [M1,M2,M3] and, secondly, the mechanisms of lipid membrane deformations [M3,M4,M5]. I conduct my research on membrane proteins [M1,M2] in collaboration with Dr. Evzen Boura of the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences. In the area of research on deformations of lipid bilayers [M4, M5], I collaborate with Prof. Reinhard Lipowsky of the Max Planck Institute of Colloids and Interfaces. The common denominator of these two research directions is provided by the physicochemical properties of lipid membranes. The studies on both membrane proteins and lipid membranes are motivated by the aspiration to understand the molecular mechanisms that underlie the functioning of cell membranes.

Cell membranes separate the interior of biological cells from their environment. They are built of a lipid bilayer and various proteins. Certain types of proteins are only loosely bound to the lipid membrane, while others are embedded or anchored in the lipid bilayer. Lipid membranes also form the boundaries of intracellular organelles. The chemical composition and shapes of these membranes must be actively modified and controlled by specialized proteins. This activity is necessary to maintain a variety of life processes. Therefore, cell membranes are very dynamic supramolecular systems.

The chemical composition of cell membranes is modified, for instance, by the activity of membrane enzymes. Some of them act on membrane proteins whereas others act on lipids. Examples of enzymes that chemically modify lipid molecules are phosphatidylinositol 4-kinases (PI4K), which are the research subject of publication [M1]. Some types of PI4K bind to the multi-domain ACBD3 protein (acyl-CoA-binding domain-containing protein-3), which is essential for the proper functioning of the Golgie apparatus. ACBD3 is also used by some viruses for replication in host cells, which is the leading theme of publication [M2].

Not only the lipid composition but also the shapes of cell membranes are modified by various proteins. Examples are the ESCRT protein complexes, as discussed in Section 5.3.1, which cause the endosomal membranes to bud towards the interior of endosomes [H3,H4,M3]. Article [M3] is a literature review on cell membrane budding. It compares the relative contributions of proteins and lipids in various cellular processes in which membrane budding occurs.

The shapes of lipid membranes can be altered not only by the action of proteins but also by small molecules or ions. Such phenomena are obviously caused the physical interactions between the lipids forming the bilayer and the small molecules or ions present in the aqueous environment surrounding the membrane. For example, the adsorption of small molecules or ions on one of the two lipid layers causes an asymmetric distribution of stresses across the lipid membrane, which leads to generation of membrane curvature [M4]. A similar effect can be expected if the interactions between the lipids forming the membrane and the small molecules or ions in their aqueous environment are repulsive [M5]. The main objective of works [M4,M5] is to explain – on the solid basis of statistical physics, fluid mechanics and molecular mechanics – how fluid lipid membranes are deformed by interactions with small molecules or ions.

My contributions to the research on membrane proteins [M1-M3] and membrane deformations [M3-M5]:

M1. Adriana Baumlova, Dominika Chalupska, <u>Bartosz Różycki</u>, Marko Jovic, Eva Wisniewski, Martin Klima, Anna Dubankova, Daniel P Kloer, Radim Nencka, Tamas Balla, Evzen Boura. *The crystal structure of the phosphatidylinositol 4‒kinase II-α*. EMBO Reports **15**: 1085-1092 (2014). *Impact Factor* in 2014: 9,055.

M2. Martin Klima, Dominika Chalupska, <u>Bartosz Różycki</u>, Jana Humpolickova, Lenka Rezabkova, Jan Silhan, Adriana Baumlova, Anna Dubankova, Evzen Boura. *Kobuviral non-structural 3A proteins act as molecular harnesses to hijack the host ACBD3 protein*. Structure **25**: 219-230 (2017). *Impact Factor* in 2016: 4,945.

M3. James H. Hurley, Evzen Boura, Lars-Anders Carlson, <u>Bartosz Różycki</u>. *Membrane budding*. Cell **143**: 875-887 (2010). *Impact Factor* in 2010: 32,406. Over 100 citations according to the Web of Science database.

M4. <u>Bartosz Różycki</u>, Reinhard Lipowsky. *Spontaneous curvature of bilayer membranes from molecular simulations: Asymmetric lipid densities and asymmetric adsorption*. J. Chem. Phys. **142**: 054101 (2015). *Impact Factor* in 2015: 2,894.

M5. <u>Bartosz Różycki</u>, Reinhard Lipowsky. *Membrane curvature generated by asymmetric depletion layers of ions, small molecules, and nanoparticles*. J. Chem. Phys. **145**: 074117 (2016). *Impact Factor* in 2016: 2,965.

Summary of publications M1-M5:

- In work [M1], the physical interactions of phosphatidylinositol 4-kinase (PI4K) of type II-α with lipid membranes have been studied. PI4K enzymes are involved in the synthesis of phosphatidylinositol 4-phosphate (PI4P), which is a phospholipid with important signaling functions in eukaryotic cells. The structure of PI4K II-α in complex with two ATP molecules has been solved by collaborators from the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences. The structure has revealed a non-typical kinase fold that could be divided into N- and C-lobes with the ATP-binding groove located in between. Interestingly, a second ATP-binding site has been found in the lateral hydrophobic pocket of the C-lobe. Having solved the atomic structure of PI4K II-α, it has been possible to simulate the full-length enzyme anchored to the lipid membrane. The computer simulations, which I have performed myself, in combination with mutagenesis experiments depict the positioning of PI4K II-α on the lipid membrane (Fig. 3A and 3B in Ref. [M1]) and indicate a possible function of the lateral pocket in the C-lobe. These results presented in publication [M1] suggest the mechanisms of recruitment, regulation and action of the PI4K II-α enzyme on the cellular membranes. They provide important information on how the PI4P lipids are synthesized in the membranes of the Golgi apparatus.

    My contribution to this work was to simulate a coarse-gained model of PI4K II-α anchored to a lipid membrane *via* four palmitoylated cystine residues. Based on the analysis of the simulation results, I characterized the spatial arrangement of the enzyme on the lipid membrane, and proposed a group of amino acid residues contributing to the binding of PI4K II-α to the membrane.

- Publication [M2] is related to publication [M1] in the sense that it also concerns proteins interacting with the membranes of the Golgi apparatus. Publication [M2], however, is focused on the molecular basis of picornavirus replication. Picornaviruses constitute a family of viruses

that cause a number of human diseases – from the common cold to hepatitis A and polio. One of the common features of all the viruses belonging to this family is that they replicate in host cells on specific membrane platforms, which are called the replication organelles. To create a replication organelle, picornaviruses use several types of host cell proteins, including the ACBD3 protein (acyl-CoA-binding domain-containing protein-3), with which a viral protein, 3A, interacts. The 3A protein lacks the tertiary structure and contains a trans-membrane helix that anchors the 3A-ACBD3 protein complex in the membrane of the replication organelle. The Golgi dynamics (GOLD) domain of the ACBD3 protein in complex with an extra-membrane portion of the 3A viral protein has been crystallized and solved by collaborators from the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences. Using this crystal structure as input, I have performed molecular dynamics simulations of the 3A-GOLD complex within a lipid membrane. The simulation results show how the 3A-GOLD complex is anchored to the membrane and which of the amino acid residues on the GOLD domain surface contribute to the protein-lipid interactions. The site-directed mutagenesis has confirmed the simulation results. Publication [M2] provides the structural information needed to understand how the virus and host proteins (3A-GOLD) are organized spatially on the replication organelles. These results can be used to identify potential antiviral therapies.

My contribution to publication [M2] was the following: (i) I performed the all-atom molecular dynamics simulations of the full-length 3A protein in complex with the GOLD domain anchored in the lipid membrane *via* a N-terminal myristoyl moiety; (ii) I analyzed the simulation results, on the basis of which I identified a group of amino acid residues contributing to the binding of the GOLD domain to the lipid membrane. The identified residues were the basis for the site-directed mutagenesis experiments.

- Publication [M3] is a review article on cell membrane budding. The biological and biophysical literature reports more and more studies in which various cellular processes involving membrane budding have been reconstructed in vitro. These studies provide reliable basis for explaining the membrane budding processes at the molecular level. It has been now recognized that the budding of cell membranes is usually caused by coating membrane portions with specific proteins. In these cases, the protein coat imposes its shape on the membrane, which drives the budding processes. A well-studied example is a protein called clathrin, which coats intracellular vesicles formed from the plasma membrane in the process of endocytosis. The clathrin molecules bind through adapter protein complexes to the membrane portion to be deformed, bend it into a bud, and then transform it into a vesicle. Upon reaching their destination, the vesicles are stripped of the clathrin coat, and then are fused with the target membrane [M3].

An exception to the aforementioned rule is provided by the ESCRT-I-II protein complexes, which probably do not coat the membrane buds. In fact, they have been observed to be localized only in the necks connecting the membrane buds to the mother membrane. Therefore, the ESCRT proteins are likely to utilize other mechanisms of membrane remodeling – which remain largely unexplained – rather than membrane coating [H3,H4,M3].

Publication [M3] discusses also the budding of multi-component lipid bilayers, which under certain conditions occurs spontaneously – with no proteins involved – as demonstrated in fluorescence microscopy experiments on giant unilamellar vesicles (GUVs) whose diameter is of the order of 10 μm. The GUV spontaneous budding can be easily explained on the basis of thermodynamics and mechanics of fluid lipid membranes. In fact, the separation of the liquid-

ordered and liquid-disordered phases in a multi-component membrane induces a line tension acting on the boundaries between the two liquid phases. If the line tension forces exceed the elastic forces required to deform the lipid membrane, the budding process occurs spontaneously [M3].

A comparison of these three factors – clathrin coat, ESCRT protein complexes, and line tension – provokes the following question: Which molecules contribute to the budding processes more – the lipids forming the membranes, or the proteins acting on the membranes? An attempt to answer to this question is the motivation for publication [M3], which focuses on the comparison of the roles of proteins and lipids in various cellular processes in which the membrane budding occurs. My contribution to publication [M3] was dedicated mainly to the sections about the mechanics and thermodynamics of lipid membranes.

- Publications [M4,M5] concern deformations of lipid bilayers that are caused by interactions of the lipids forming the bilayers with small molecules or ions in the aqueous environment. The key quantity studied in Refs. [M4,M5] is the spontaneous curvature, which is one of the mesoscopic parameters of the membrane elasticity theory. This parameter is related to the asymmetry between the two layers of a given membrane. It quantitatively describes the tendency of the membrane to attain curved spatial configurations. Although the concept of spontaneous curvature has been discussed in the context of continuous membrane models, it has been largely ignored in molecular dynamics simulations of lipid membranes – mainly because of a conceptual problem of appropriate boundary conditions. In works [M4,M5], I have conducted the first, systematic, molecular dynamics studies on the spontaneous curvature generated, firstly, by the asymmetry of lipid packing in the opposite layers of the membrane [M4], secondly, by the asymmetry of the coverage of the opposite membrane layers by the small molecules or ions adsorbed from the aqueous environment [M4] and, thirdly, by the difference in concentrations of the small molecules or ions present in the aqueous solutions on opposite sides of the membrane layers [M5]. I have performed the molecular dynamics simulations within the framework of a coarse-grained DPD model. The simulation results have provided an in-depth understanding of the aforementioned systems and, thus, facilitated their theoretical analysis on the basis of statistical physics and fluid mechanics. As a result of these numerical and analytical studies – and in close collaboration with Prof. Reinhard Lipowsky of the Max Planck Institute of Colloids and Interfaces, Germany – I have derived simple and general relationships between (i) the spontaneous curvature, (ii) the asymmetry of distribution of the small molecules or ions on the opposite membrane layers, and (iii) the surface tension of these membrane layers.

I have used two different methods to determine the spontaneous curvature in the molecular dynamics simulations. The first one – based on the 1981 work of Helfrich – requires computing the distribution of local stresses across the membrane in the state of zero mechanical tension. The second method, introduced in Ref. [M4], is based on representing the membrane as two parallel water-lipid interfaces, and determining the surface tension of these interfaces. These two methods have given equivalent results for all of the investigated systems.

My contribution publications [M4,M5] was the following: (i) I participated in defining the research directions, in formulating the physical problems, and in developing the appropriate theoretical tools; (ii) I performed all of the molecular dynamics simulations as well as substantial parts of the analytical calculations; (iii) I analyzed and interpreted the simulation

results; (iv) I wrote the first versions of the articles, produced the great majority of the figures, and actively participated in revising the articles.

6.2 Publications in journals not included in the Journal Citation Reports

6.2.1 Book chapter

- Wolfgang Peti, Rebecca Page, Evzen Boura, <u>Bartosz Różycki</u>. „Structures of dynamic protein complexes: Hybrid techniques to study MAP kinase complexes and the ESCRT system". Chapter 17 in „Protein NMR: Methods and Protocols", Ranajeet Ghose (ed). Humana Press, New York, NY. *Methods in Molecular Biology* **1688**: 375-389 (2018).

This chapter is a review on the so-called hybrid methods of contemporary structural biology. My contribution to this review was the following: (i) I wrote the entire section 3.3; (ii) I produced Fig. 1 illustrating the EROS method described in section 3.3; (iii) I participated in writing the introduction and section 3.2.

6.2.2 Review and popular science articles in Polish language

- <u>Bartosz Różycki</u>. „Konformacje białek wielodomenowych i częściowo nieustrukturyzowanych – symulacje i eksperymenty". *Postępy Biochemii* **63**: 132-136 (2017).

This publication is a brief review about the contemporary research on multi-domain and intrinsically disordered proteins. It is focused on hybrid methods of structural biology.

- <u>Bartosz Różycki</u>. „Postęp w pomiarach białek nieustrukturyzowanych". *Wszechświat* **117**: 295-304 (2016).

This work is a popular-science article about intrinsically disordered proteins.

6.3 Invited lectures at conferences, symposia and colloquia

Invited lectures at international conferences

- Biomolecules and Nanostructures 6, Podlesice, Poland, May 11, 2017. Lecture title: „Assembling cellulosomes by molecular simulations and SAXS experiments".
- Biomembrane Days 2014, Berlin, Germany, September 1, 2014. Lecture title: „Spontaneous curvature of bilayer membranes from asymmetric lipid and adsorbate densities".
- 9th European Conference on Mathematical and Theoretical Biology, Göteborg, Sweden, June 16, 2014. Lecture title: „Adhesion of membranes via receptor-ligand complexes: Binding cooperativity, domain formation and line tension effects".
- International Conference on Computational Science and Technology, Warszawa, Poland, May 16, 2014. Lecture title: „Ensembles of multiprotein complexes in simulation and experiment".

Invited lectures at symposia

- Symposium on „Physics Under Extreme Conditions", International PhD School at the Institute of Physics of the Polish Academy of Sciences, Warsaw, Poland, November 3, 2016. Lecture title: „Life at extreme temperatures".
- ASBMB Symposium on Biochemistry and Cell Biology of ESCRTs in Health and Disease, Snowbird, Utah, USA, October 15, 2010. Lecture title: „Coatless vesicle budding by ESCRTs".

Invited lectures at colloquia

- Colloquium of the International Graduate Research Training Group 1524, Technical University, Berlin, Germany, February 10, 2015. Lecture Title: „Assembling multi-protein complexes by SAXS, FRET, EPR and molecular simulations".

6.4 Lectures at Seminars

- Condensed Matter Physics Seminar, Institute of Physics of the Polish Academy of Sciences, Warsaw, Poland, 17.01.2017. Lecture title: "Small angle x-ray scattering as a method to study multi-domain proteins".
- Skype Seminar on Life Science, Institute for Computational Science and Technology in Vietnam, Warsaw, Poland, 26.06.2015. Lecture title: "Spontaneous curvature of bilayer membranes from molecular simulations: Asymmetric lipid densities and asymmetric adsorbate concentrations".
- Seminar of the State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, China, Changchun, 17.06.2015. Lecture title: "Spontaneous curvature of bilayer membranes from molecular simulations".
- Polymer Science Lecture Series, State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, China, Changchun, 15.06.2015. Lecture title: "Assembling protein complexes with intrinsic disorder by simulation and experiment".
- Seminar on Biological Physics and Bioinformatics, Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, Institute of Physics of the Polish Academy of Sciences, and Department of Biophysics of the University of Warsaw, Warsaw, Poland, 22.10.2014. Lecture title: "Spontaneous curvature of bilayer membranes: Asymmetric lipid densities and asymmetric adsorption".
- Skype Seminar on Life Science, Institute for Computational Science and Technology in Vietnam, Warsaw, Poland, 28.02.2014. Lecture title: "Assembling the multi-protein ESCRT complexes by simulation and experiment".
- Seminar on Biological Physics and Bioinformatics, Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, Institute of Physics of the Polish Academy of Sciences, and Department of Biophysics of the University of Warsaw, Warsaw, Poland, 23.10.2013. Lecture title: "Adhesion of cellular membranes: cooperate binding of adhesion receptors and formation of domains in the immunological synapse".
- Seminar of the Biochemistry Department, Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic, 04.10.2013. Lecture title: "Assembling multi-protein complexes by simulation and experiment".
- Seminar on Modern Concepts in Structural Biology, Max F. Perutz Laboratories, Department of Structural and Computational Biology, Vienna, Austria, 31.01.2013, Lecture title: "Assembling multi-protein complexes with intrinsic disorder by simulation and experiment: application to the ESCRT system".
- Seminar on Biological Physics and Bioinformatics, Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, Institute of Physics of the Polish Academy of Sciences, and Department of Biophysics of the University of Warsaw, Warsaw, Poland, 12.12.2012. Lecture title: "Conformations of multi-domain proteins and multi-protein complexes studied by combining molecular simulations with SAXS, EPR, FRET and NMR experiments".
- Department Workshops, Max Planck Institute of Colloids and Interfaces, Department of Theory and Bio-systems, Ringberg, Germany, 12.03.2012. Lecture title: "The ESCRT machinery".
- Theory and Bio-systems Department Seminar, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, 12.12.2011. Lecture title: "Ensemble refinement of multi-domain proteins with disordered segments".

- LCP Seminar, National Institutes of Health, NIDDK, Laboratory of Chemical Physics, Bethesda, MD, USA, 01.12.2009. Lecture title: "Binding cooperativity of membrane adhesion receptors".
- LCP Seminar, National Institutes of Health, NIDDK, Laboratory of Chemical Physics, Bethesda, MD, USA, 17.12.2008. Lecture title: "Stable Patterns of Membrane Domains at Corrugated Substrates".
- Department Workshops, Max Planck Institute of Colloids and Interfaces, Department of Theory and Bio-systems, Semlin, Germany, 11.03.2008. Lecture title: "Stable Patterns of Membrane Domains at Corrugated Substrates".
- Department Workshops, Max Planck Institute of Colloids and Interfaces, Department of Theory and Bio-systems, Malta, 16.03.2007. Lecture title: "Lipid membranes: adhesion via soluble stickers and curvature-modulated phase separation".
- Statistical Physics Seminar, Department of Physics of the University of Warsaw, Institute of Theoretical Physics, Warsaw, Poland, 09.06.2006. Lecture title: "Stochastic models of cell membrane adhesion away from thermodynamic equilibrium".
- Seminar on Biological Physics and Bioinformatics, Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, Institute of Physics of the Polish Academy of Sciences, and Department of Biophysics of the University of Warsaw, Warsaw, Poland, 24.04.2006. Lecture title: "Adhesion of membranes with active components".
- Statistical Physics Seminar, Department of Physics of the University of Warsaw, Institute of Theoretical Physics, Warsaw, Poland, 07.10.2005. Lecture title: "Brownian Motors".
- "Theory of Soft and Biomatter: Irreversible and Active Processes" Seminar, Max Planck Institute of Colloids and Interfaces, International Max Planck Research School on Biomimetic Systems, Potsdam, Germany, 13.06.2005. Lecture title: "Brownian ratchets".
- Department Workshops, Max Planck Institute of Colloids and Interfaces, Department of Theory, Ringberg, Germany, 16.03.2005. Lecture title: "Adhesion control of membranes by active stickers".
- "Theory of Soft and Biomatter" Seminar, Max Planck Institute of Colloids and Interfaces, International Max Planck Research School on Biomimetic Systems, Potsdam, Germany, 10.05.2004. Lecture title: "Unfolding and Jarzynski's equality".
- Statistical Physics Seminar, Department of Physics of the University of Warsaw, Institute of Theoretical Physics, Warsaw, Poland, 07.11.2003. Lecture title: "Stochastic models for the active components of biological membranes".
- Statistical Physics Seminar, Department of Physics of the University of Warsaw, Institute of Theoretical Physics, Warsaw, Poland, 25.10.2002. Lecture title: "Effects of finite system sizes on the wetting transition".
- Theory Department Seminar, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, 22.07.2002. Lecture title: "Finite-size effects on wetting transitions".

6.5 Active participation in conferences and meetings

- Poster presentation at the Gordon Research Conference on Cellulases and Other Carbohydrate-Active Enzymes, Proctor Academy, Andover, NH, USA, 23-28.07.2017. Poster title: "Assembling cellulosomes by molecular simulations and SAXS experiments".
- Poster presentation at the Biophysical Society Thematic Meeting on "Conformational Ensembles from Experimental Data and Computer Simulations", Berlin, Germany, 25-

29.08.2017. Poster title: "Large, dynamic, multi-protein complexes - molecular simulations and SAXS experiments".

- Poster presentation at the conference „Biomembrane Days 2016", Berlin, Germany, 05-07.09.2016. Poster title: „Spontaneous curvature of biomembranes from molecular simulations".
- Contributed talk at the Polish Physical Society Meeting, Session 7: Biophysics, Kielce, Poland, 07.09.2015. Title: "Conformational diversity of the multi-domain xylanase Z".
- Poster presentation at the conference "Biomolecules and Nanostructures 5", Jaroszowice, Poland, 15.05.2015. Poster title: "Conformational ensemble of the multi-domain xylanase Z of Clostridium thermocellum".
- Chairing the "Networks" session at the conference "From Soft Matter to Bio-Systems", Potsdam, Germany, 21.11.2013.
- Poster presentation at the conference "Biomolecules and Nanostructures 4", Pułtusk, Poland, 15-19.05.2013. Poster title: "Protein adsorption and deformations on solid surfaces".
- Contributed talk at the conference "Multi-Pole Approach to Structural Biology", Warsaw, Poland, 18.11.2011. Title: "Ensembles of proteins with disordered segments".
- Invited talk at the Alumni Meeting of the Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, 03.06.2010. Title: "Conformations of ESCRT CHMP3: insights from simulations and SAXS experiments".
- Contributed talk at the German Physical Society Meeting, Berlin, Germany, 25-29.02.2008. Title: "Phase separation in membranes supported on corrugated substrates".
- Poster presentation at the Alumni Meeting of the Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, 08.06.2007. Poster title: "Adhesion via soluble crosslinkers".
- Contributed talk at the German Physical Society Meeting, Regensburg, Germany, 26.03.2007. Title: "Adhesion of membranes with active stickers".
- Poster presentation at the Alumni Meeting of the Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, 27.05.2005. Poster title: "Adhesion of membranes with active stickers".
- Poster presentation at the German Physical Society Meeting, Berlin, 04.03.2005. Poster title: "Adhesion control of membranes by active stickers".
- Poster presentation at the 15th Marian Smoluchowski Symposium on Statistical Physics: Fundamentals and Applications, Zakopane, Poland, 07-12.09.2002, Poster title: "Exact solution of the two-dimensional RSOS model for the wetting transition".

6.6 National and foreign collaboration documented by scientific publications

- Prof. Marek Cieplak, Institute of Physics, Polish Academy of Sciences. Research collaboration on conformational dynamics of multi-domain and partially disordered proteins (publications H5-H9), including cellulosomal proteins (publications H5, H6, H7).
- Prof. Reinhard Lipowsky, Max Planck Institute of Colloids and Interfaces, Germany. Research collaboration on deformations of lipid membranes (publications M4, M5) and adhesion of bio-mimetic and cellular membranes (publications D3-D6 and A1-A7).
- Dr hab. Thomas Weikl, Max Planck Institute of Colloids and Interfaces, Germany. Research collaboration on membrane adhesion (publications D3-D6, A1-A7) and adhesion proteins – within the project entitled "Multi-domain proteins with disordered linkers in cellulose decomposition and cell adhesion" and supported by the National Science Center, Poland,

program *Opus 11*.

- Prof. Gerhard Hummer, Max Planck Institute of Biophysics, Germany. Research collaboration on multi-domain and partially disordered proteins, in particular the ESCRT proteins (publications H1-H4), protein kinases (publication S1) and kinases in complexes with phosphatases (publications S2, S3).
- Dr. Evzen Boura, Institute of Organic Chemistry and Biochemistry, Czech Republic. Research collaboration on structure-function relationships of membrane proteins (publications M1-M3), remodeling of cellular membranes (publications H4 and M3), and application of the SAXS method to study conformations of multi-domain proteins (publications H2, H3, S4, S6).
- Prof. James H. Hurley, University of California, Berkeley, USA. Research collaboration on ESCRT proteins (publications H2, H3, H4), cellular membranes (publication M3), and protein kinase C (publication S1).
- Prof. Wolfgang Peti, University of Arizona, USA. Research collaboration on multi-domain kinases and phosphatases (publications S2 and S3).
- Dr. Yunqi Li, Changchun Institute of Applied Chemistry, Chinese Academy of Science, China. Research collaboration on novel computational methods to bias molecular dynamics simulations using SAXS data (publication S5).
- Prof. Mirjam Czjzek, Station Biologique de Roscoff, CNRS, France. Research collaboration on cellulosomal proteins (publication H5).
- Dr. Pierre-Andre Cazade, University of Limerick, Ireland. Research collaboration on properties and functions of linkers in cellulosomal proteins (publication H7).

6.7 Leader in research projects

- "Multi-domain proteins with disordered linkers in cellulose decomposition and cell adhesion". Ongoing project within the framework of the *Opus 11* program supported by the National Science Center, Poland. Proposal evaluation results: second position on the ranking list in the NZ1 panel. Financial support: 278 400 PLN. Start date: 12.01.2017. Planned end date: 11.01.2020.
- "Multidomain and multimeric proteins: structural and mechanical insights". Project within the framework of the *Opus 3* program supported by the National Science Center, Poland. Proposal evaluation results: first position on the ranking list in the NZ1 panel. Financial support: 287 056 PLN. Start date: 06.03.2013. End date: 05.06.2015.
- "Proteins with the F-BAR domain in the processes of endocytosis and tabulation of lipid membranes". Project within the framework of the *Iuventus Plus* program supported by the Ministry of Science and Higher Education, Poland. Proposal evaluation results: 68/70 points. Financial support: 169 000 PLN. Start date: 08.07.2013. End date: 07.07.2015.

6.8 Fellowships and awards

- Outstanding Young Scientist Award granted by the Ministry of Science and Higher Education, Poland (November 1, 2013 – October 31, 2016)
- Marie Skłodowska-Curie Outgoing Fellowship within the 7th Framework Programme of the European Community (October 15, 2009 – October 14, 2012)

7. Service to the academic community

7.1 Reviewer activity

7.1.1 Reviews of research projects

- In 2017, work for National Science Center, Poland. Member of the Expert Team in Panel NZ1:
  - reviews of sixteen research proposals submitted to the National Science Center, Poland, in the 23th edition of the *Opus*, *Preludium* and *Sonata* calls,
  - participation in two panel meetings (February 13-14 and April 10, 2017).
- In 2016, review of one research proposal for the National Research, Development and Innovation Office of Hungary (NKFIH).

7.1.2 Reviews of scientific publications for journals on the JCR list

- *Physical Review E*, 2008-2017, 22 reviews
- *Physical Review Letters*, 2009-2016, 20 reviews
- *Biophysical Journal*, 2008-2017, 18 reviews
- *Journal of Physics D: Applied Physics*, 2014-2017, 9 reviews
- *Soft Matter*, 2012-2017, 7 reviews
- *Scientific Reports*, 2015-2017, 4 reviews
- *Journal of Biomechanics*, 2014-2016, 4 reviews
- *Physical Biology*, 2015-2016, 4 reviews
- *Journal of Chemical Information and Modeling*, 2017, 3 reviews
- *EPL* (*Europhysics Letters*), 2012-2017, 3 reviews
- *Physica A: Statistical Mechanics and its Applications*, 2015-2016, 3 reviews
- *Science Advances*, 2017, 2 reviews
- *Chemical Physics Letters*, 2017, 2 reviews
- *Biomedical Materials*, 2017, 2 reviews
- *BBA Biomembranes*, 2016, 2 reviews
- *Journal of Biological Systems*, 2015-2016, 2 reviews
- *Biophysical Reviews and Letters*, 2008-2012, 2 reviews
- *Journal of Chemical Physics*, 2016, 1 review
- *Structure*, 2015, 1 review
- *Journal of Statistical Mechanics: Theory and Experiment*, 2015, 1 review
- *Molecular and Cellular Proteomics*, 2014, 1 review
- *Biopolymers*, 2014, 1 review
- *BMC Biophysics*, 2014, 1 review
- *Cell*, 2012, 1 review
- *New Journal of Physics*, 2010, 1 review
- *Journal of Theoretical Biology*, 2007, 1 review
- *Langmuir*, 2007, 1 review

7.2 Teaching activities

7.2.1 Teaching PhD students at the Institute of Physics, Polish Academy of Sciences

- four two-hour lectures within a course "Introduction to Biophysics", summer term of 2014/2015
- six two-hour lectures within a course "Statistical thermodynamics in soft matter and biological

physics", summer term of 2015/2016

7.2.2 Teaching activities at the Faculty of Physics, University of Warsaw

- tutorials on *Phenomenological Thermodynamics*, supplementing the lecture course given by Prof. Marek Napiórkowski (30 teaching hours in the winter term of 2002/2003

- tutorials on *Mathematics*, *Level III A*, supplementing the lecture course given by Prof. Jerzy Kamiński (60 teaching hours in the winter term of 2002/2003)

- tutorials on *Statistical Mechanics*, supplementing the lecture course given by Prof. Marek Napiórkowski (45 teaching hours in the winter term of 2003/2004)

- tutorials on *Mathematics*, *Level III A*, supplementing the lecture course given by Prof. Ernest Bartnik (60 teaching hours in the winter term of 2003/2004)

- tutorials on *Statistical Physics*, supplementing the lecture course given by Prof. Marek Napiórkowski (45 teaching hours in the winter term of 2004/2005)

- tutorials on *Classical Mechanics*, supplementing the lecture course given by Prof. Wojciech Kopczyński (30 teaching hours in the winter term of 2004/2005)

7.2.3 Supervision of intern students

- Institute of Physics, Polish Academy of Sciences, Warsaw, Poland, July-September 2013, two intern students

- Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, July-August 2007, one intern student

7.3 Activities popularizing science

- Popular science article, written in Polish, about intrinsically disordered proteins. Published in *Wszechświat*, which is a journal devoted to popularizing sciences. Reference: *Wszechświat*, vol. 117, no. 10-12/2016, pp. 295-304.

- Popular science lecture "Life at extreme temperatures" given on November 3, 2016, at the symposium entitled "Physics Under Extreme Conditions", which was organized within the International PhD Program of the Institute of Physics, Polish Academy of Sciences.

- Interview for the *Projects* magazine, which disseminates information on European projects in the area of research and development. Joshua Howgego, "Spaghetti-like protein structures untangled", *Projects* **29**: 90-91 (2012).

Bartosz Różycki