The hidden complexity in RNA molecules

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Time-resolved single-molecule FRET (smFRET) experiments are versatile tools, which yield an unrivaled amount of detailed information on biomolecular systems. In particular, they have the potential to simultaneously reveal structural and dynamical information of the molecule under study. However, this information is hidden in the photon traces because the conformations of molecules typically overlap in their FRET-efficiencies and because of measurement errors. Opposed to conventional analysis tools, stochastic methods, such as hidden Markov models (HMM), have the power to extract long-lived (conformational) states and transition rates from a stochastic signal trace. We develop and use smFRET estimators based on HMMs to explore the folding of the ribozyme Diels-Alderase. The aim is to understand the conformational substates of the molecule, including their structural interpretation and to link this information to the kinetics of folding. We also assess how these methods can be used for the design of new experiments.

Graph Measures Reveal Fine Structure of Complexes Forming in Multi-Particle Simulations

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Modern simulation techniques are beginning to study the dynamic assembly and disassembly of multi-protein systems. In these many-particle simulations it can be very tedious to monitor the formation of specific structures such as fully assembled protein complexes or virus capsids above a background of monomers and partial complexes. However, such analyses can be performed conveniently when the spatial configuration is mapped onto a dynamically updated interaction graph. On the example of Monte Carlo simulations of spherical particles with either isotropic or directed mutual attractions we demonstrate that this combined strategy allows for an efficient and also detailed analysis of complex formation in many-particle systems [Lauck, Geyer, Helms, J. Chem. Theory Comput. 5 (2009) 641].

Spatiotemporal model of particle diffusion in the primary rod vision signal transduction

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Rod cell phototransduction is a prime example of a functional module whose properties may strongly depend on its specific spatial embedding. The rod outer segment has a highly regular layered geometry comprising disc membranes that are densely filled with the photon-collecting Rhodopsins and their G-proteins Transducin. Despite a wealth of functional studies on rod cell phototransduction and a rather complete knowledge of the proteins involved in the process, the spatiotemporal mechanism of the activation cascade is poorly understood. Since recently, the existence Rhodopsin patterns on the disc and their possible effects on functional properties of photoactivation are highly debated. In the present study we conduct spatiotemporal simulations of the two-dimensional reaction-diffusion photoactivation processes on the disc membrane with all protein copies explicitly resolved. We investigate the effects of crowding, the spatiotemporal evolution of the activation, and different settings of the reaction rates of the physicochemical events such as the dissociation of G-protein subunits. Finally, we compare free diffusion of the involved proteins with a situation where attractive interactions favor Rhodopsin-Rhodopsin aggregations. In order to compare our results to a well-defined experimental test system, the simulations are set up on a spherical membrane mimicking experimentally prepared disc membrane vesicles for which extensive kinetic studies exist. Our analyses yield insight into which space-time mechanisms in the phototransduction activation module are possible and allow a number of highly debated questions concerning pattern formation on the disc membrane to be reconsidered.

Analysis Tool for Brownian Dynamics Simulations

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The association of two proteins can be described by a simple picture where free proteins diffuse randomly in solution until they enter into the electrostatic field of the other. Brownian dynamics (BD) simulation has proven to be a suitable tool for the analysis of protein-protein association. The computed rates for protein are in good agreement with experimental results (Gabdoulline, R. R. and Wade, R. C. On the Protein-Protein Diffusional Encounter Complex. J. Mol. Recogn. 1999). The generated trajectories describe the relative conformations of the two simulated proteins along their association path. The aim of this project was the reimplementation of the analysis method for BD simulations, which was first presented by Alexander Spaar et al.(Spaar et al. Free Energy Landscape of Protein-Protein Encounter Resulting from Brownian Dynamics Simulations of Barnase:Barstar, J. Chem. Theory Comput., 2005) in 2005 with improved efficiency, user-friendliness and additional options and features. During the simulations, the positions and orientations of the molecules are recorded on occupancy maps. Additionally, the electrostatic- and desolvation- energies are stored as separate maps. Due to the high number of trajectories, good statistics are obtained so that the occupancy map can be interpreted as a probability distribution from which the entropy landscape can be calculated. This implementation allows users to use this analysis method in combination with the BD software package SDA (http://projects.eml.org/mcm/software/SDA) without any restriction on the version of SDA used. Only Open Source programs are used for the analysis. Using this tool allows users to identify reaction pathways for the simulated association and allows the encounter complex to be defined at its minimum of energy. The created maps can be visualized in three-dimensions, providing a good picture of the regions most occupied by the diffusing protein. In addition, a two-dimensional representation of the occupancy maps can also be created with this tool. For this, the Cartesian coordinates are converted to spherical coordinates and the occupancy of every position is represented by a color.

Multiscale modeling in rabbit ventricular myocytes

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The architecture of the transverse tubular system (t-system) and the arrangement of associated proteins are central to the function of ventricular cardiomyocytes. The objective of our research is to study structure-function relationships of the t-system and its associated proteins, specifically by modelling the spatiotemporal features of $Ca2+$ signaling, buffering and diffusion. Our recent results in rat myocytes (Lu et al., IEEE EMB 2009; Cheng et al., PLoS Comp Biol 2010) indicate that the more accurate knowledge of transverse-axial t-tubule microanatomy and protein distributions along the cell membrane is important to better understand the mechanisms regulating cardiac excitation-contraction coupling. The results demonstrate that $Ca2+$ movement from the cell membrane to the cell interior relies also strongly on the presence of mobile and stationary $Ca2+$ buffers. The modelling approach has since been applied to reconstructed three-dimensional models of the rabbit myocyte. Specifically, we have investigated the $Ca2+$ diffusion properties for a larger, multi-tubule domain, and have examined the role of tubule constrictions in generating local inhomogeneities in the $Ca2+$ distribution.

Protein Diffusion in Live E. coli

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Fluorescence recovery after photobleaching (FRAP) has been used to study the diffusive motion of proteins in the cytoplasm and periplasm of E. coli. By varying osmotic conditions, we have characterized translational diffusion of cytoplasmic GFP for different degrees of crowding and immediately following moderate osmotic upshift. The seven-fold reduction of Dgfp from buffer to cytoplasm, the wide variation in Dgfp from cell to cell under identical conditions, and the 50-fold decrease in < Dgfp> after osmotic upshift are not well explained by simple hard-sphere crowding models. Fluorescence anisotropy measurements suggest an approximate three-fold increase in the rotational correlation time of GFP from buffer to cytoplasm at 0.28 Osm growth osmolality. On average, periplasmic GFP diffuses 1.5-3.5 times more slowly than cytoplasmic GFP, depending on growth osmolality. GFP-labeled RNA polymerase in the cytoplasm exhibits comparable mobile and immobile fractions on a 30-60 s timescale. For the mobile fraction, presumed to be engaged in

“hopping” and “sliding”, <Drnap> = 0.24 um2-s-1. This is remarkably similar to measured 1D sliding diffusion coefficients of RNAP on ds-DNA in vitro. We suggest that the immobile fraction comprises RNAP copies that are actively transcribing or stalled at transcription initiation sites. Cell-to-cell heterogeneity in both Drnap and f(mobile) is substantial. Finally, recent single-molecule tracking studies of the protein Kaede in the E. coli cytoplasm reveal spatial heterogeneity in Dkaede, with faster diffusion in the nucleoid region. Time permitting, we will also present a statistical model that explains nucleoid-ribosome segregation in the E. coli cytoplasm.

Spatio-temporal simulations of the JAK2/STAT5 pathway in CFU-E and NIH3T3 cells

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Cellular geometries can vary significantly, how they influence signaling remains largely unknown. Here, we investigate the influence of cell shape on the Janus kinase (JAK) signal transducer and activator of transcription (STAT) pathway by data-based mathematical modeling, numerical simulations and parameter identification. As cellular model systems we use spherical shaped erythroid progenitor cells at the colony-forming unit erythroid stage (CFU-E) and NIH3T3 fibroblast cells. The key components of the pathway are modeled with a system of ordinary differential equations (ODE) to estimate the parameters that can not be measured experimentally. The ODE model is then enlarged by the transport of STAT5 through the cytoplasm. A mixed system of differential equations (PDE + ODE) with Robin boundary conditions is obtained which is solved with the in-house software Gascoigne based on Finite Elements. Realistic cell geometries for the simulations are obtained by three-dimensional reconstruction of microscopy data. Numerical algorithms are explored to reduce the long computing time caused by the fine mesh and a small time step size necessary due to fast diffusion combined with slow activation and deactivation kinetics of STAT5. We use Implicit Euler, Crank- Nicholson time stepping method and discretization of the stationary equations by Q1, Q2 Finite Elements. The resulting linear system is solved with multigrid methods. We analyze the influence of the cell shape on the gene response to the activated pathway and do some in silico experiments. For similar linear models in signal transduction we can conclude which geometry and which diffusion coefficient is necessary so that diffusion plays a significant role in the dynamics of the observed pathway.

Elucidating the targeting mechanism of a membrane binding domain using Brownian dynamics simulations

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The recruitment of signalling proteins to the surface of the plasma membrane is an essential part of signal transduction. Often, peripheral proteins are recruited by phosphoinositides (PIs): polyvalent, negatively charged lipid species present in low concentrations at the membrane surface. Many signalling proteins have a well-defined PI binding domain as part of their sequence to facilitate membrane association [1]. However, the existence of other negatively charged lipid species like phosphatidylserine in the cytoplasmic leaflet of the cell membrane raises questions about how the protein is able to locate its target PI against this backdrop of negative charge [2]. The general receptor for phosphoinositides isoform 1 (GRP1) is a key component of the phosphatidylinositol-3-kinase (PI3K) signalling pathway, and contains a pleckstrin homology (PH) domain that allows it to bind reversibly and with high affinity to phosphatidylinositol-3,4,5-trisphosphate (PI(3,4,5)P3) in the plasma membrane [3,4]. GRP1 is a nucleotide exchange factor for ADP-ribosylation factor 6 (ARF6), which is involved in membrane-trafficking and endocytosis. GRP1 has been shown to be autoinhibited in the cytosol, only becoming functionally competent when bound to the plasma membrane via its PH domain [5]. To investigate the mechanism of lipid targeting by membrane-binding domains, we have performed exploratory Brownian dynamics simulations of GRP1-PH encountering a PI(3,4,5)P3-containing membrane. Association of proteins with other, membrane-bound proteins has previously been studied using Brownian dynamics simulations [6]. It is shown that targeting can be disrupted by introducing additional negative charges on the surrounding lipid headgroups, and that the orientation of the protein is influenced by the net charge carried by the membrane surface. The results suggest that the presence of a background negative charge impairs the protein’s ability to locate its cognate ligand in the membrane. Simultaneously, however, this background charge also appears to enhance electrostatic steering of the protein into an orientation more suitable for binding. The possibility of using BD simulations to generate encounter complexes as precursors for atomistic molecular dynamics simulations is also explored. [1] Lemmon, M.A. (2008) Nat. Rev. Mol. Cell Biol. 9:99-111 [2] Corbin, J.A., Dirkx, R.A., and Falke, J.J. (2004) Biochemistry 43:16161-73 [3] Lietzke, S.E., Bose, S., Cronin, T., Klarlund, J., Chawla, A., Czech, M.P., and

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A structure determination of the Cysteine Synthase complex by Brownian dynamics simulation

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Plants and bacteria assimilate and incorporate inorganic sulfur into organic compounds such as the amino acid cysteine. Cysteine biosynthesis involves a bienzyme complex, the Cysteine Synthase (CS) complex. The CS complex is composed of the enzymes serine acetyl transferase (SAT) and O-acetyl-serine-(thiol)-lyase (OAS-TL). Although it is experimentally known that formation of the CS complex influences cysteine production, the exact biological function of the CS complex, the mechanism of reciprocal regulation of the constituent enzymes and the structure of the complex are still poorly understood. Here, we used docking techniques to construct a model of the CS complex from mitochondrial Arabidopsis thaliana. Diffusional encounter complexes of SAT and OAS-TL were generated by rigid-body Brownian dynamics (SDA) simulation. By incorporating experimental constraints during Brownian dynamics simulation, we identified complexes consistent with experiments.

Twist propagation in nucleosome arrays: A Monte Carlo and Brownian Dynamics study

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The distribution and progagation of twist in nucleosome arrays subjected to torsional forces is studied using two methods: Brownian Dynamics (BD) and Monte Carlo (MC) simulations. We present results from a study of a two-nucleosome array using a mesoscopic model that accounts for the nucleosome/linker geometry along with bending and twisting mechanics of the DNA linkers. A Monte Carlo approach is used to simulate the conformation of the linkers under quasi-static twisting conditions. Our simulations reveal an intriguing finding: the magnitude and sign of the twist measured on one linker relative to the imposed twist at the other linker on the other side of the nucleosome depends strongly on the relative orientation of the entering and exiting linkers, or the DNA wrapping angle in nucleosomes. We have constructed a ``phase diagram" characterizing the relative sign of the twist in the two linkers as a function of various geometrical parameters. Our simulations also reveal a ``buckling" phenomena, whereby nucleosomes sometimes undergo sudden flipping in response to twisting, leading to drastic changes in the entry/exit conformation of the DNA linker. We also present results from an ongoing Brownian Dynamics study of twist propagation in large nucleosomes arrays in which we examine the twist / extension relationship and dynamics of nucleosomes subjected to applied torsion. Our results thus provide insights into the underlying mechanisms by which torsional stress impact chromatin organization and subsequent biological activities and suggests ways by which such torsional stresses could be relieved to promote genomic integrity.

Mobility of proteins in the chromatin network

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Accessing specific DNA sequences in the genome is essential for the function of proteins involved in gene regulation and DNA processing. The interior of the cell nucleus is a 'sticky tangle' composed primarily of chromatin, but also other filamentous and globular proteins. How enzymes can access their specific target sites in this polymer network is not yet fully understood. Using a combination of in-vivo diffusion measurements by fluorescence correlation spectroscopy and modeling of diffusion inside a porous medium by continuum or lattice-based simulations, we can define limits of accessibility of the cell nucleus for different size proteins and understand the role of chromatin dynamics in this process.

Single molecule tracking in individual living bacterial cells

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(1) Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden (2) University of Tartu, Institute of Technology, Tartu, Estonia (3) Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Delft, The Netherlands We have developed a single molecule fluorescence assay to directly observe the stringent response in individual living E. coli cells. For this purpose, we have created chromosomal fusions of both RelA and three ribosomal proteins with a photoconvertible fluorescent protein. The stringent factor RelA binds to a small fraction of ribosomes, where it synthesizes the global transcriptional regulator ppGpp in response to amino acid deprivation. Our objective is to study the binding kinetics of individual RelA molecules to the ribosome in living cells and to observe how its kinetics changes during a nutritional downshift. While E. coli contains on average about 100 RelA molecules and 20000 ribosomes, using a photoconvertible fluorescent probe we can activate only a few fluorescent molecules per cell at any given time. We induce stringent response by rapid addition of amino acid hydroxamates. Since our fluorescent tag is photoconvertible, we can repeat tracking experiments many times in the same E. coli cell. We record trajectories of individual RelA molecules diffusing in living E. coli cells with a laser exposure time of 2 ms, a frame time of 20 ms, and a spatial precision of 30 nm. The high resolution of the experiments makes it possible to characterize RelA binding kinetics under varying growth conditions. When the cell grows exponentially, RelA trajectories closely resemble trajectories of fluorescently tagged ribosomal proteins. After nutritional downshift, RelA binding kinetics changes rapidly. Our results suggest that under amino acid starvation, RelA is only transiently bound to the ribosome. The data is consistent with an order of magnitude drop in affinity to the ribosome.