SYSM 1: Single molecules

Time: Tuesday 14:00–17:00 Location: H 0105

Invited Talk SYSM 1.1 Tue 14:00 H 0105

Two-Focus Fluorescence Correlation Spectroscopy: A versatile tool for precise measurements of molecular diffusion — •Jörg Enderlein[1], Anastasia Loman[1], Thomas Dertinger[2], Iris von der Hocht[3], Bernd Müller[4], Victor Pacheco[2], Konstantin Komolov[5,6], Karl-Wilhelm Koch[5], and Ingo Gregor[3] — [1]Eberhard-Karls-Universität Tübingen, Germany — [2]Forschungszentrum Jülich, Germany — [3]RWTH Aachen, Germany — [4]University of California, Los Angeles, USA — [5]Universität Oldenburg, Germany — [6]Moscow State University, Moscow, Russia

Thermally induced translational diffusion is one of the fundamental properties exhibited by molecules within a solution. Via the Stokes-Einstein relation it is directly coupled with the hydrodynamic radius of the molecules [1]. Any change in that radius will change the associated diffusion coefficient of the molecules. Such changes occur to most biomolecules - in particular proteins, RNA and DNA - when interacting with their environment (e.g. binding of ions or other biomolecules) or performing biologically important functions (e.g. enzymatic catalysis) or reacting to changes in environmental parameters such as pH, temperature, or chemical composition (e.g. protein unfolding). Therefore, the ability to precisely measure diffusion coefficients has a large range of potential applications, for monitoring e.g. conformational changes in proteins upon ion binding or unfolding. However, many biologically relevant conformational changes are connected with rather small changes in hydrodynamic radius on the order of Ångstrøms (see e.g. [2]). To monitor these small changes, it is necessary to measure the diffusion coefficient with an accuracy of better than a few percent.

An elegant technique capable of measuring diffusion coefficients of fluorescent molecules at nanomolar concentrations is Fluorescence Correlation Spectroscopy (FCS) which was originally introduced by Elson, Magde and Webb in the early seventies [3]. In its original form it was invented for measuring diffusion, concentration, and chemical/biochemical interactions/reactions of fluorescent or fluorescently labelled molecules at nanomolar concentrations in solution. However, standard FCS is prone to a wide array of optical and photophysical artefacts which make precise quantitative and absolute measurements of e.g. diffusion coefficients rather difficult [4]. The main problem of standard FCS is the absence of a reliable extrinsic length scale in the measurements, which is, however, necessary for obtaining absolute values of the diffusion coefficient.

Here, we report on our recently developed new technique of 2-focus fluorescence-correlation spectroscopy [5], allowing for measuring the hydrodynamic radius of molecules at pico- and nanomolar concentrations with sub-Angstrom precision. In 2fFCS, the problem of an extrinsic length scale is solved by generating two excitation foci with well defined distance from each other. Several applications of 2fFCS are presented, for example monitoring conformational changes of proteins upon ion binding, or monitoring protein unfolding curves upon chemical and thermal denaturation.

References: [1] A. Einstein Investigations on the Theory of the Brownian Movement, Dover, New York, 1985. [2]. A. M. Weljie et al., Protein Science 12 (2003) 228-236 [3]. D. Magde et al. Phys. Rev. Lett. 29 (1972) 705-8. [4] J. Enderlein et al. ChemPhysChem. 6 (2005) 2324-36. [5] T. Dertinger et al. ChemPhysChem. 8 (2007) 433-443.

Invited Talk SYSM 1.2 Tue 14:30 H 0105

Tracking and Manipulating Single Molecule Diffusion in Liquids — •Frank Cichos — Universität Leipzig, Leipzig, Germany

Single molecule optical detection provides the tools to follow the motion of molecules and other nano-objects in solution in real time. It allows to identify spatial differences in single molecule dynamics, as a result of local variations in chemical and physical conditions. As a consequence of this, control of local chemical and physical environments especially at surfaces allows to modify and manipulate molecular dynamics in a well defined way opening new concepts for nanofluidic applications. We report on recent experiments which are aimed to guide and trap the motion of single molecules and nanoparticles in liquids by local chemical structures and thermal fields. Our experiments are based on confined geometries, where the structure and dynamics of a liquid is largely determined by interfacial interactions. Micro contact printing of chemical structures is used to modulate these interfacial interactions to achieve anisotropic single molecule diffusion. Gold nanoparticles are immobilized on surfaces and employed as local nano-heatsources to induce controlled thermal gradient flows directing single molecule motion.

Invited Talk SYSM 1.3 Tue 15:00 H 0105

Single Molecule Studies on Myosin Motors — •Claudia Veigel — National Institute for Medical Research, Mill Hill, London, UK

Many types of cellular motility are based on the myosin family of motor proteins. There are now known to be at least 18 different classes of myosins, involved in muscle contraction, intracellular transport processes, exocytosis or even signal transduction in vision or hearing. Using a combined approach of recombinant protein expression and single molecule techniques including optical tweezers we study the basic mechanisms of force production and movement of these molecular machines on the single molecule level. In this talk we will report on our recent studies on myosins class V and VI which transport cargo, such as cyplasmic vesicles, processively over micrometer distances along the actin cytoskeleton in the cell.

30 min. break

Invited Talk SYSM 1.4 Tue 16:00 H 0105

Real-time observation of bacteriophage T4 gp41 helicase reveals unwinding mechanism — M. Manosa, T. Lionnet, M. M. Spiering, S. J. Benkovic, D. Bensimon, and •V. Croquette — Laboratoire de Physique Statistique, Ecole Normale Supérieure, 24 rue Lhomond, 75005 Paris, France

Helicases are enzymes that couple ATP hydrolysis to the unwinding of double-stranded (ds) nucleic acids. The bacteriophage T4 helicase (gp41) is a hexameric helicase that promotes DNA replication within a highly coordinated protein complex termed the replisome. Despite recent progress, the gp41 unwinding mechanism and regulatory interactions within the replisome remain unclear. Here we use a single tethered DNA hairpin as a real-time reporter of gp41-mediated dsDNA unwinding and single-stranded (ss) DNA translocation with 3-bp resolution. Whereas gp41 translocates on ssDNA as fast as the in vivo replication fork (˜400 bp/s), its unwinding rate extrapolated to zero force is much slower (˜30 bp/s). Taken together, our results have two implications: first, gp41 unwinds DNA through a passive mechanism; and second, this weak helicase cannot efficiently unwind the T4 genome alone. Our results suggest that important regulations occur within the replisome in order to achieve rapid and processive replication. We have also investigate Rec-Q from E-coli which is a monomeric helicase involved in DNA repair. This enzyme behaves very differently from gp41 clearly demonstrating an active unwinding behavior.

Invited Talk SYSM 1.5 Tue 16:30 H 0105

From valleys to ridges: Exploring the dynamic energy landscape of single membrane proteins — •Daniel Müller — Technische Universität Dresden, Germany

Membrane proteins are involved in essential biological processes such as energy conversion, signal transduction, solute transport and secretion. Every biological process such as those involving membrane proteins is steered by molecular interactions. Molecular interactions guide the folding and stability of membrane proteins, determine their assembly, switch their functional states or mediate signal transduction. The sequential steps of molecular interactions driving these processes can be described by dynamic energy landscapes. The conceptual landscape allows to follow the complex reaction pathways of membrane proteins while its modifications describe why and how pathways are changed. Single-molecule force spectroscopy (SMFS) detects, quantifies and locates interactions within and between membrane proteins. SMFS helps to determine how these interactions change with temperature, point mutations, oligomerization, and the functional states of membrane proteins. Applied in different modes, SMFS explores the coexistence and population of reaction pathways in the energy landscape of the protein and thus reveals detailed insights into local mechanisms determining its structure and function relationship. Here we review how SMFS extracts with high precision the defining parameters of an energy landscape such as the barrier position, the potential well width, reaction kinetics and roughness.