DNA, proteins, membranes

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2020

document version
Publisher's PDF, also known as Version of record

Summary

**DNA, proteins, membranes:
exploring the physics behind biomolecular processes**

Biomolecular processes represent the fundamental interactions occurring at the nanoscale which are vital for any biological system to function. The complexity of these events and the spatial/temporal scale at which they occur, dictate the biggest challenge in experimental biophysics: unravelling such processes and their working mechanism. This thesis investigates several biomolecular processes driven by the interactions between three major macromolecules of life, namely DNA, proteins and membranes. Thanks to state-of-the-art single-molecule instruments we catch, manipulate and observe one macromolecule at a time.
The experiments in this thesis are performed with three main techniques which complement each other in the information that can be extracted from each studied system. i) Optical tweezers combined with confocal fluorescence microscopy (OT&C) allows to catch single DNA molecules and to observe proteins binding, unbinding and moving along the DNA. At the same time the DNA can be manipulated and its force response provides information on the induced mechanical properties. OT&C was also used to trap single membrane-coated beads and to observe and quantify the interactions induced by membrane-binding proteins. ii) Acoustic force spectroscopy (AFS) also allows to perform proteins-DNA interactions experiments, with the advantage of parallel measurements using short DNA molecules and of applying stable low forces. iii) Atomic force microscopy (AFM) is a gentle technique as it permits to image biological particles (soft particles, such as liposomes) placed on a surface by scanning over them. At the same time AFM was used to push and/or break them, allowing to get insights into their material and mechanical properties. See **Chapter 1** for more details.

Here a short summary follows for every experimental chapter presented in this thesis.

Chapter 3

1D-sliding assists σ70-dependent
promoter binding by E.coli RNA polymerase

The flow of genetic information from DNA to RNA is of central importance to living systems and it can only start after an RNA polymerase has found a promoter site. But how does this enzyme find its promoter sites on DNA in the first place? In recent years, the controversial debate on this topic seems to lean in favor of a promoter search mechanism that is dominated by 3D-diffusion, rather than by 1D-sliding along DNA. We designed an improved single-molecule assay that unambiguously revealed extensive 1D-sliding of RNAP. An intriguing

implication of our quantitative analysis is that around the RNAP concentration that is present in living cells, the 1D and 3D-search mechanisms are elegantly balanced, suggestive of a buffering mechanism.
For the direct observation of single E. coli σ^{70}-RNAP, we performed confocal fluorescence imaging of linear DNA molecules and reproduced all previous key findings such as binding kinetics and sequence-dependent binding affinity. One crucial element of our experimental configuration is that we hold single DNA molecules fully suspended in solution between two optically trapped beads, which permits full and unimpeded 1D-sliding of RNAP along DNA. Indeed, we directly observed single RNAPs sliding over thousands of basepairs. This is in contrast to the lack of evidence for 1D sliding in previous surface-bound assays where RNAP inevitably encounters a substrate while it undergoes rotation-coupled 1D-diffusion along DNA.
Our results demonstrate a powerful single-molecule fluorescence assay that avoids potential surface-associated perturbations. This assay is applicable to a wide range of DNA-protein interactions and is perhaps even essential for analysing the mobility of DNA-bound species. Foremost, our results provide new insights into the promoter search mechanism. This is essential for our fundamental understanding of gene expression and it provides us with the elementary insights that are required to start interpreting/addressing situations with elevated complexity such as the impact of crowding on target search processes.

Chapter 4

Real-time assembly of an artificial virus-like particle elucidated at the single-particle level

In this study we investigated in real-time the dynamics of assembly of virus-like particles, which remains one of the poorly understood key steps in the viral life cycle. Using a powerful combination of single-molecule techniques, such as confocal fluorescence microscopy of optically trapped DNA molecules and acoustic force spectroscopy we scrutinized this process. We observe directly and in real time the critical early stages (i.e. protein nucleation) of the assembly pathway of an artificial virus-like particle. Furthermore, we followed the growth of single nuclei and revealed the binding kinetics and dynamics of single capsid proteins, while monitoring the DNA mechanical changes such as its time-dependent compaction. We complemented the study with observations from atomic force microscopy and bulk assays. Our findings not only contribute to a fundamental understanding of the complex, dynamic processes occurring during viral self-assembly, but also support the development of virus-like particles in order to design effective and controlled novel artificial genetic carriers. Moreover, our study demonstrates how the powerful combination of single-molecule fluorescence assays with high temporal/spatial resolution is well suited to scrutinize proteinaceous structure assembly.

Chapter 5

synaptotagmin-1 and Doc2b exhibit distinct membrane remodeling mechanisms

Synaptotagmin-I (Syt1) is a calcium sensor protein that is vital for neural activity, for example its deletion in mice leads to death within 48 hours after birth. Due to its central importance during neurotransmitter release, Syt1 has been extensively studied by various molecular and structural approaches. Nevertheless, many questions remain on how Syt1 mediates membrane fusion. In particular, there is extensive debate in the field on the membrane-bound configuration of Syt1 before and during fusion. This debate is hard to settle due to a lack of appropriate experimental tools.
In a previous publication our lab demonstrated that optical tweezers combined with confocal fluorescence can be used to study protein-membrane interactions. Here we successfully expand the capacity of these tools to directly observe and quantitatively measure the interactions between single vesicles with an unprecedented resolution and a degree of control that is impossible to reach with other techniques. Using this instrument, we have now succeeded to forge a breakthrough into the understanding of the mechanism of fusion-mediation by Syt1. First, we address the long standing question of the binding configuration between Syt1 and membranes, focusing on discriminating between binding to an opposing membrane or to another copy of the protein in the opposing membrane, i.e. establishing if Syt1 binds symmetric or asymmetric to trigger fusion. Our results clearly show, on the bases of quantitative measurements of the binding strength and frequency, that Syt1 binds significantly more strongly to membranes rather than to other Syt1 molecules. This striking result helps resolve a long-standing controversy in the field for the mode of Syt1 action during fusion. Second, we succeeded to quantitatively and directly demonstrate, using AFM nanoindentation, that the binding of Syt1 to membranes results in membrane softening. This lowering of the energy required for membrane deformation likely contributes significantly to fusion facilitation by calcium sensor proteins, and may be a general feature of C2AB domains. The finding that calcium sensor proteins directly modify the mechanical properties of membranes in order to trigger membrane fusion was speculated about but never directly demonstrated before. This study thus sheds new light on the mechanism of Ca^{2+} induced fusion triggering, which is essential for a fundamental understanding of neurotransmitter secretion.

Chapter 6

Multilamellar nanovesicles show distinct mechanical properties depending on their degree of lamellarity

Nanoscale vesicles are currently in use as drug delivery vehicles, and are widely studied for their broader potential in therapeutic applications. It has been shown that small multilamellar vesicles (sMLVs) can have important benefits for drug delivery, such as slower release kinetics and larger storage volume for hydrophobic drugs. Recently, it has also become clear that the mechanical properties of vesicles affect important processes, such as cellular uptake. Our lab recently introduced a new approach and model to determine and describe the mechanics of nanovesicles. Here we expand that approach and present the first study of the mechanical properties of multilamellar (nano)vesicles. Having a quantitative understanding of these properties will help with the rational design of sMLVs for drug delivery applications. In this study, we use atomic force microscopy (AFM) nanoindentation to obtain this quantitative information on multilamellar nanovesicles. We scrutinize the break events that occur during nanoindentations, and show that their size corresponds well to the lipid bilayer thickness. Moreover, we show that we can determine the lamellarity of individual vesicles and distinguish between vesicles with 1 to 5 lipid bilayers. To validate these results, we obtained an independent measurement of the lamellarities present in the vesicle population using cryoEM. We then quantified the vesicle geometry and mechanical properties as a function of their lamellarity, and find that for each additional bilayer adherent vesicles stay in a more spherical shape and are stiffer. Surprisingly, the stiffness scales only weakly with the amount of lipid bilayers: every added bilayer makes the vesicle only ~20% stiffer. We provide an explanation, based on our previous observation that adherent vesicles are osmotically pressurized. In summary, here we show that AFM can be used to determine the lamellarity of single vesicles and we made a big step in understanding the physical properties of multilamellar vesicles. Importantly, the observed more spherical shape and higher stiffness of multilamellar vesicles could be beneficial for uptake by cells, suggesting that the lamellarity of vesicles can be leveraged to tune vesicle behavior for drug delivery purposes.