an array of gold nanostructures by chemically and mechanically robust bonds provides a unique way to carry out spatially controlled, repeatable measurements of single molecules.

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Model for Harnessing the Device Stiffness in Dynamic Single-Molecule Force Spectroscopy

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Single-molecule force spectroscopy methods use optical traps or tiny cantilevers to impose controlled forces to individual molecules for studying their mechanical behavior and transitions along specific paths called reaction coordinates. A powerful application of these sophisticated approaches is the extraction of activation energy barriers and intrinsic rates of transition from measurements of the rupture force, i.e., when the molecules stretched at a speed undergoes sudden unfolding.

Existing analyses of force measurements relies heavily on theoretical models for reliable extraction of kinetics and energetic properties. Despite significant advances, there remain large gaps in fully exploiting the experiments and their analyses. Specifically, the effect of pulling device stiffness or compliance has not been comprehensively captured. Hence, the best models for extracting molecular parameters can only be applied to measurements obtained from soft pulling devices (e.g., optical tweezers) and result in well-documented discrepancies when applied to stiff devices (e.g., AFM). This restriction makes pulling speed the only control parameter in the experiments, making reliable extraction of molecular properties problematic and prone to error.

Here we present an analytical model derived from physical principles for extracting the intrinsic rates and activation free energies from rupture force measurements that is applicable to the entire range of pulling speeds and device stiffnesses. The model therefore is not restricted to the analyses of force measurements performed with soft pulling devices only. On the contrary, the model allows better design of experiments that specifically exploit device stiffness as a control parameter in addition to pulling speed for a more reliable estimation of energetic and kinetic parameters. The model also helps explain previous discrepancies noted in rupture forces measured with devices of different effective stiffnesses and provides a framework for modeling other stiffness-related issues in single-molecule force spectroscopy.

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Extracting Complex Network and Effective Free Energy Landscape of Protein Fluctuation from Single-Molecule Time Series

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The complexity in kinetics observed in single molecule measurements arises from the morphological feature inherent to the underlying multidimensional energy landscape or, in general, state space. However, how can one extract such dynamic information from a set of single molecule time series? Recently we developed two methodologies for extracting an effective free energy landscape composed of local equilibrium states [1,2] and a multiscale state space network (SSN) from single molecule time series [3,4]. Both are designed as free from a priori assumption such as local equilibration. The state is defined not by the value of the observable at each time but by a set of subsequences of the observable. These methods enable us to lift degeneracy_different physical states having the same value for a measured observable_as much as possible under the limitation of scalar quantity. The morphological feature of the free energy landscape and the SSN naturally depends on the time scale of observation. The length of the subsequence constructing the states in the multiscale SSN can tell us the extent to which the memory of the system can predict the next state. We present the brief overview with some examples such as strange diffusion kinetics in conformation fluctuation of Flavin-Enzyme System (H. Yang *et al Science*, **302**, 262 (2003)) and show the multiscale SSN buried in the observation.

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Influence of the Experimental Set-Up on Single Molecule DNA Dynamics When Analyzed by Tethered Particle Motion

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Among the various experimental techniques now available to explore the interaction between a chosen protein and a double stranded DNA, the single molecule technique of Tethered Particle Motion (TPM) is the only one to permit the observation of the dynamics of the DNA polymer at mechanical equilibrium as it consists in tracking the movement of a bead tethered to the glass surface by a DNA molecule. Relatively easy to implement on a microscope, the details of the experimental set-up might nevertheless have some strong influence on the collected data. Considering that the DNA molecules under studies have a size varying between a few hundred to a few thousand base pairs, they are assimilated to semi-flexible polymers. With the help of both experiments and simulations, we show that the beads used for the labelling, whose diameters are usually about a few hundred of nanometres, can slow down the observed dynamics due to their own drag force. We also quantify the bias resulting from the detector averaging effects in this case of TPM experiments.

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DNA Origami as a Nanoscopic Ruler For Super-Resolution Microscopy

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The possibility of highly parallel formation of nanostructures using self-assembly of DNA molecules provides a powerful tool for bottom-up fabrication. The DNA origami technique, involves folding a long single-stranded DNA scaffold using short DNA staple strands that can only bind at particular points along this scaffold. With this technique large numbers of identical structures can be assembled simultaneously in a single experiment. One of the most attractive features of the origami technique is the precise addressability of the DNA structures formed. Each staple strand can serve as an attachment point for many different kinds of molecules or other objects.

Due to their small size, DNA nanostructures are commonly imaged using atomic force microscopy or electron microscopy, but with recent advances in far-field fluorescence microscopy beyond the diffraction limit (super-resolution microscopy), structures in the sub-200 nm regime become amenable also to optical analysis.

We here show that the distance between fluorescently labeled staple strands bound on specific positions of rectangular DNA origami structures can be accurately determined using a variety of super-resolution techniques such as single-molecule high-resolution imaging with photo-bleaching (SHRImP), direct stochastic optical reconstruction microscopy (dSTORM), and Blink-Microscopy.

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Near-Field Fluorescence Correlation Spectroscopy Approach to the Study of Living Cell Membrane Dynamics

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We developed a near-field (NF) microscopy-based fluorescent correlation spectroscopy (FCS) approach that allows to measure protein and lipid mobility on living cells plasma membrane at sub-diffraction scale. The near-field excitation is obtained by means of an aluminum-coated optical fiber with sub-wavelength aperture (<100 nm in diameter), effectively reducing the illumination area of about one order of magnitude compared to standard confocal FCS. The use of this kind of probe also provides capability for dual-color FCS and fluorescence cross-correlation spectroscopy (FCCS) and guarantees the overlap of the excitation areas. The optical fiber is attached to an oscillating tuning fork and a shear force based feedback keeps the probe in the close proximity of the cell, preventing the membrane from fluctuating outside the evanescent field volume while minimizing probe-membrane interactions.

We demonstrated the feasibility of the dual-color NF-FCS approach by measuring the diffusion of phosphoethanolamine or sphingomyelin simultaneously with GPI-anchored protein on the plasma membrane of living CHO cells. The comparison of these results with those obtained by confocal FCS highlights the advantages of the reduced illumination area in detecting anomalous or heterogeneous diffusion.

Although other techniques have been shown to provide comparable illumination sizes, the NF-FCS approach offers the further advantage of dual-color FCS and FCCS and therefore represents a powerful tool to unravel the details of a variety of membrane processes occurring at the nanometric scale.