feeling. Cocoa butter consists mainly of three triglycerides (TAGs): 1,3-di-palmitoyl-2-oleoylglycerol (POPG), 1-palmitoyl-2-oleoyl-3-stearoylglycerol (POS) and 1,3-dioleoyl-2-oleoylglycerol (SOS). Besides, smaller amounts of mono-, di-glycerides and free fatty acids are present.

The TAG composition of cocoa butter or a fat in general is one of the most important parameters since it governs the physical properties and determines its polymorphic form. Polymorphism is defined here as the ability of the TAG molecules to crystallize in different molecular packing arrangements corresponding to different unit cell structures. Fat polymorphs are typically classified by three main forms (α, β, γ and variations within these types). The different polymorphs determine the physical-chemical properties and hence the texture, long-term stability and consequently shelf-life of the products used in technological applications. In a combined synchrotron small- and wide-angle x-ray scattering (SAXS/WAXS) study, we have explored the use of the thermodynamic parameter pressure in modulating and controlling these properties, and to determine the temperature-pressure phase diagram of a triglyceride system.

1942-Pos Board B672
Unraveling the Heparin-Induced Protofibril Structure of GAPDH
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Citotoxicity in Parkinson disease has been linked to an oligomeric arrangement of the protein α-synuclein (α-SN), which can alter the membrane permeability. In this work we could demonstrate the ability of heparin-induced Gliceraldehyde-3-phosphate dehydrogenase (GAPDH) aggregation to modulate the effect of oligomers with an average length of 22 nm and a diameter of 12 nm are able to sequester species with an average length of 22 nm and a diameter of 12 nm are able to sequester α-SN oligomers. Using biochemical techniques we obtained the first all-atm model of the GAPDH protofibril capable to satisfy experimental restrictions deduced from small angle X-ray scattering and mass spectrometry. We also propose a fibrillation pathway for the heparin-induced GAPDH aggregation. Upon heparin binding to GAPDH, the tetrameric state of the enzyme is lost and native-like dimer species appeared. The formed dimers are the building block of higher orders aggregates, which in a very fast way assemble to hexamers that piling up allowing the formation of the protofibril species.

1943-Pos Board B673
Optical Scattering of Liposomes Suspended above a Surface
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Lipid bilayers exhibit a different refractive index parallel and perpendicular to their surface. This optical birefringence is characteristic for the size, shape and orientation of the lipid molecules in the membrane [1]. A new optical model to investigate the optical birefringence of liposomes suspended above a surface is presented. The solution to Maxwell’s equation for particles suspended above a surface, as presented by Bobbert and Vlieger [2], is extended to investigate the optical scattering properties of liposomes suspended above a surface. Numerical simulations demonstrate that ellipsometry is highly sensitive to the distance of liposomes above a surface and that ellipsometry can be applied to determine the optical anisotropy of the liposome lipid bilayer, creating opportunities for the use of ellipsometry in the investigation of the supported lipid bilayer formation process.

The optical model also allows for the analysis of more complex structures such as nanoparticles coated with a lipid bilayer, or liposomes whose lumen contains a spherical nanoparticle. The influence of the presence of a lipid bilayer and its optical anisotropy on ellipsometry measurements is presented. Also, it is investigated how model parameters such as lipid bilayer anisotropy can be experimentally separated from other model parameters such as e.g. the overall particle radius.

12345-Pos Board B674
The Effect of Magnesium on the Thermodynamics of Nucleic Acid Tertiary Contact Formation
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Functional RNAs fold into compact, well-defined tertiary structures despite strong electrostatic repulsion both within and between helices. To achieve these compact structures, many RNAs employ structural divalent cations, typically Mg2+. The simplest tertiary contact in nucleic acids is two helices, joined by some non-helical contact, that gives the fundamental characteristics of tertiary contact formation in nucleic acids we studied a system of two DNA helices tethered via a short PEG linker by both computational and experimental methods. Computationally, we predict the electrostatic repulsion between these helices as a function of Mg2+ and Na+ concentration. Experimentally, we linked the distal termini of these helices via a disulfide linker. Using small-angle x-ray scattering, we measured the fraction of intact disulfide bonds as a function of reducing strength of the buffer at a range of Mg2+ and Na+ concentration. Using these data we can extrapolate the magnitude of the strain on the disulfide bond, and thus the repulsion between the helices. Previous results show that the conformational ensemble is narrowly distributed around an extended, co-linear conformation at low salt and becomes more relaxed at high salt, but is unable to isolate a conformation in which the helices are stacked. Furthermore, previous results also suggest that specific interactions between Mg2+ and the phosphate backbone strongly are more important than simple ionic strength in determining the magnitude of the repulsive potential between the helices. In this project, we hope to experimentally demonstrate the energy of helix stacking and the different role of ionic strength and ionic specific interactions.

1945-Pos Board B675
Asymmetric Illumination of Optically Anisotropic Beads for Detecting Rotational Motion
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Many biological molecules, including RNA polymerases and ATP synthases, undergo rotational motion during their biological processes. Characterizing these processes at the single molecule level with high temporal and spatial resolution can reveal valuable information about their mechanical and dynamical properties. We are particularly interested in the dynamics of conformational changes of DNA molecules under torsion. Toward this long term goal we have developed a methodology that enables us to directly record the angular displacement of particles undergoing rotational motion by using asymmetric illumination of optically anisotropic beads. The method is implemented by illuminating a partially metal-coated bead with a laser beam coupled into the back side of the objective. The laser beam illuminating beads in the sample plane is oriented at an oblique angle to form asymmetric illumination. We observe that the scattering signal of the bead changes with the angular displacement of the coating on the bead relative to the illuminating laser beam. We use an optoelectronic system to detect scattering signal which is the direct high-speed measurement of the angular displacement of the bead. We conclude that our method is able to map angular displacements to electrical signal and we can determine the angular displacement of the biological molecule when conjugated to the bead. Our method obviates image acquisition and image processing procedures commonly used in previous studies, and it has the potential to significantly enhance the bandwidth of detection. We envision usage of this method in a range of biophysical measurements including magnetic trapping, tethered particle motion. We plan to use this rotational tracking method in combination with magnetic tweezers, a single-molecule technique that enables the application of torsional stress to twisted single DNA molecules to extract high bandwidth torsional mechanical properties and dynamics of the molecules.

Force Spectroscopy

1946-Pos Board B676
Investigating Protein-Protein Interaction Networks with Force Spectroscopy
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Colicins are antimicrobial proteins produced by bacteria. Their highly active mode of killing make colicins of interest as a potential new class of antibiotics. However, the precise mechanism of cytotoxicity is not understood thus limiting their potential to biotechnological applications. To explore the fundamental characteristics of specific interactions.

It is thought that colicin intoxication occurs via a series of protein-protein interactions (PPIs) that span the periplasm (Housden et al. Science, 2013). Mechanical force may play a role in this process by an inside-out energy transduction mechanism. Periplasmic proteins that are subverted by colicins undergo rotational motion during their biological processes. Characterizing these processes at the single molecule level with high temporal and spatial resolution can reveal valuable information about their mechanical and dynamical properties. We are particularly interested in the dynamics of conformational changes of DNA molecules under torsion. Toward this long term goal we have developed a methodology that enables us to directly record the angular displacement of particles undergoing rotational motion by using asymmetric illumination of optically anisotropic beads. The method is implemented by illuminating a partially metal-coated bead with a laser beam coupled into the back side of the objective. The laser beam illuminating beads in the sample plane is oriented at an oblique angle to form asymmetric illumination. We observe that the scattering signal of the bead changes with the angular displacement of the coating on the bead relative to the illuminating laser beam. We use an optoelectronic system to detect scattering signal which is the direct high-speed measurement of the angular displacement of the bead. We conclude that our method is able to map angular displacements to electrical signal and we can determine the angular displacement of the biological molecule when conjugated to the bead. Our method obviates image acquisition and image processing procedures commonly used in previous studies, and it has the potential to significantly enhance the bandwidth of detection. We envision usage of this method in a range of biophysical measurements including magnetic trapping, tethered particle motion. We plan to use this rotational tracking method in combination with magnetic tweezers, a single-molecule technique that enables the application of torsional stress to twisted single DNA molecules to extract high bandwidth torsional mechanical properties and dynamics of the molecules.

Force Spectroscopy
In this work we study the colicin E9 system and the PPIs that remodel during its translocation across the outer and inner membranes of *E. coli*. Through a series of *in-vitro* single molecule force spectroscopy experiments performed using an atomic force microscope, we identify a mechanism by which dissociation of the highly avid colicin E9-immunity protein interaction (*M* affinity, half-life days) can take place via the application of small forces (<20 pN) during colicin translocation (Farrance et al. PLoS Biol. 2013). Such a mechanism could account for the disparity between measured lifetimes of colicin-immunity protein interactions and the timescale on which colicins kill (days vs. minutes) for which immunity protein release is a prerequisite.

Further and ongoing pulling experiments on other PPIs formed during colicin E9 translocation involving periplasmic proteins from the Tol complex also show interesting behaviour under applied forces. These results suggest that the PPI network formed during E9 translocation would be able to support and transduce significant forces and these may be crucial in understanding the mechanism by which colicins gain access to the cellular interior and ultimately kill their target.

1947-Pos Board B677
**Single Molecule Force Spectroscopy Reveals the Molecular Mechanical Anisotropy of the FeS4 Metal Center in Rubredoxin**

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Mechanical anisotropy is an important feature of materials. Depending on the pulling direction, a material can exhibit very different mechanical properties. Mechanical anisotropy on the microscopic scale has also been observed for individual elastomeric proteins. Depending upon the pulling direction, a protein can unfold via different mechanical unfolding pathways and exhibit different mechanical stability. However, it remains to be demonstrated if the concept of mechanical anisotropy can be extended to molecular scale for small molecular objects containing only a few chemical bonds. Here, we choose the iron-sulfur center FeS4 in rubredoxin as a model system to demonstrate the molecular level mechanical anisotropy. We used single molecule force spectroscopy to investigate the mechanical rupture of the FeS4 center along different pulling directions. The FeS4 cluster is a simple molecular object with defined three-dimensional structure, where a ferric ion and four coordinating cysteinyln ligands are arranged into a distorted tetrahedral geometry. Mutating two specific residues in rubredoxin to cysteines provides anchoring points that enable us to stretch and rupture the FeS4 center along five distinct and precisely controlled directions. Our results showed that mechanical stability as well as the rupture mechanism and kinetics of the FeS4 center are strongly dependent upon the pulling direction, a protein can unfold via different mechanical unfolding pathways and exhibit different mechanical stability. However, it remains to be demonstrated if the concept of mechanical anisotropy can be extended to molecular scale for small molecular objects containing only a few chemical bonds. Here, we choose the iron-sulfur center FeS4 in rubredoxin as a model system to demonstrate the molecular level mechanical anisotropy. We used single molecule force spectroscopy to investigate the mechanical rupture of the FeS4 center along different pulling directions. The FeS4 cluster is a simple molecular object with defined three-dimensional structure, where a ferric ion and four coordinating cysteinyln ligands are arranged into a distorted tetrahedral geometry. Mutating two specific residues in rubredoxin to cysteines provides anchoring points that enable us to stretch and rupture the FeS4 center along five distinct and precisely controlled directions. Our results showed that mechanical stability as well as the rupture mechanism and kinetics of the FeS4 center are strongly dependent upon the pulling direction.

References:

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**Force Spectroscopy of Tip Link Proteins: A Study of Inner-Ear Biophysics**

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Hair cells in the inner ear convert mechanical stimuli, in the vestibular and auditory systems, into electrical signals which can be processed by the brain. Hair cells are highly polarized with a unique elaboration of modified microvilli at their apical surface known as stereocilia. Sensory stimuli such as sound waves of a particular frequency will result in oscillations of the stereociliary bundle at that frequency. Movements of this bundle couple mechanically to the channel. This force acts to gate the channel in turn resulting in a mechano-electrical transduction. The force is relayed to the channel by filaments known as tip links. Tip links are made of two atypical cadherins, protocadherin-15 and cadherin-23 at the lower and upper ends respectively. The tip link is held together by a non-covalent interaction between two anti-parallel pairs of EC domains. Although much has been discerned about the biophysics of this system the lack of adequate technology has prevented the direct measurement of many of these properties. We have developed molecular tools to facilitate the single molecule study of these properties. Self-assembled DNA nanoswitches are functionalized with protocadherin 15 and cadherin 23 fragments using the enzyme sortase. In order to preserve protein function, protein-DNA coupling is performed under physiological conditions. In this two-step process, a small synthetic peptide is first coupled to a DNA oligo. Next, utilizing the enzyme sortase, the protein is coupled to the DNA-peptide chimera under physiological conditions. This strategy frontloads all of the protein-incompatible chemistry so that it is performed on an oligo and a synthetic peptide, which are far more tolerant of non-physiological conditions. Once assembled, the nanoswitches are then used in an optical tweezer system to measure the rupture forces of the tip link ununder different conditions.

1949-Pos Board B679
**Probing the Unbinding Kinetics of DNA-H-NS-DNA Protein Complexes by a High-Speed and High-Throughput Single-Molecule Pulling Assay using Atomic Force Microscopy**

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We demonstrate an atomic force microscopy (AFM) pulling assay to measure the unbinding kinetics of DNA-protein interactions. Here we use this technique to investigate the interactions between DNA and the abundant, non-specific nucleoid-associated protein H-NS (Histone-like Nucleoid-Structuring protein). In particular, we probe the unbinding kinetics of the DNA-H-NS-DNA bridges under the influence of one environmental factor, Mg²⁺ ions. In this experiment, biotinylated DNA molecules are attached on an avidin-coated mica surface. Then thiolated DNA molecules and H-NS proteins are added to the sample solution to form DNA-H-NS-DNA complexes. Individual DNA-protein complexes are then pulled by a gold-coated AFM tip. By measuring the force-distance curve, we gain information on the strength with which H-NS forms bridges between DNA molecules. Here, we probe both single DNA-H-NS-DNA complexes and sample many complexes by fast scanning of the AFM tip over the sample’s surface and performing approach/pulling cycles for each position. This experimental approach leads to high-throughput measurements with single-molecule resolution and is widely applicable to other DNA-bridging proteins and receptor-ligand interactions.

References:

1950-Pos Board B680
**Instrument Free Biomolecular Interaction Measurement with DNA Nanoswitches**

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The quantification of molecular interactions is a cornerstone of almost all aspects of biomedical research, from fundamental cell biology to drug discovery. Yet progress has been impeded by challenges implicit in current approaches: high-cost of equipment and reagents, low throughput of many approaches, and requisite technical expertise. Here we present a new method that solves these challenges, replacing standard equipment with a nanoscale molecular tool. This is accomplished by using DNA origami to place molecules on a strand of DNA effectively creating a “molecular laboratory” that allows us to control both local concentration and binding stoichiometry at a single-molecule level. The binding state of these molecules alters the geometry of the DNA, which can be easily readout using gel electrophoresis. This allows us to make standard binding kinetics measurements in a highly parallel way at low cost. Furthermore these DNA “nanoswitches” provide new capabilities such as the ability to readout the binding state of multi-component systems such as bispecific antibodies or allosteric drugs.

1951-Pos Board B681
**Simultaneous Measurement of Forces and Currents using an AFM-FET Hybrid Sensor for Studying Single Biomolecular Interactions**

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The majority of biological functions are carried out at the molecular level by interactions between various biomolecules. Because these interactions are often the target of pharmaceutical agents, drug screening techniques must