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Mapping Mechanostable Pulling Geometries of Protein-Ligand Complexes

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1745-Pos

Anticalin is a non-immunoglobulin protein scaffold with potential as an alternative to monoclonal antibodies for nanoparticle-based drug delivery to cells displaying cytotoxic T-lymphocyte antigen 4 (CTLA-4). In this context, one limiting factor is the resistance of the anticalin:CTLA-4 complex to mechanical forces exerted by fluid shear stress. Here, we used single-molecule AFM force spectroscopy to screen residues along the anticalin backbone and determine the optimal pulling point that achieves maximum mechanical stability of the anticalin:CTLA-4 complex. We used non-canonical amino acid incorporation by amber suppression in the anticalin combined with click chemistry to attach an Fgβ peptide at internal residues of the anticalin. We then used the Fgβ peptide as a handle to mechanically dissociate anticalin from CTLA-4 by applying tension at 8 different anchor residues, and measure the unbinding energy landscape for each pulling geometry. We found that pulling from amino acid position 60 on the anticalin resulted in ~100% higher mechanical stability of the complex as compared with either the N- or C-terminus. Molecular dynamics (MD) simulations using the coarse-grained Martini force field showed strong agreement with experiments and help explain the mechanisms underlying the geometric dependency of mechanical stability in this therapeutic molecular complex. These results demonstrate that the mechanical stability of receptor-ligand complexes can be optimized by controlling the loading geometry without making any changes to the binding interface.

ligand binding. We demonstrate the observed differences in the ligand modulation arise due to differences in binding site and binding mode of the two complexes. Our simulations reveal that the transition state structure of SUMO1 gets perturbed on binding with S12. In contrast, the binding of CUE-2 has minimal structural impact of ubiquitin. Thus our study demonstrates that a direct interaction with the β-clamp does not necessarily modulate the mechanical stability of proteins, an observation in contrast with previous studies. Instead ligand binding far from the β-clamp can reinforce the mechanical stability of the proteins. Our study highlights the importance of ligand binding site in modulating mechanical stability of ubiquitin family proteins.

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1746-Pos

Exploring the Doxorubicin-Dna Interaction by Dna Stretching using Optical Tweezers

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As a chemotherapeutic agent heavily used since the 1970s, Doxorubicin's (DOX) mission is well known and carried out, to halt cancerous cell replication and cause cancerous cells to die. DOX accomplishes this by binding to DNA and inhibiting topoisomerase II during the replication of a cell causing cellular apoptosis. The necessary concentration for this to happen and the way in which these interactions are carried out at the molecular level is still under debate. We use dual-beam optical tweezers to trap and isolate a single DNA molecule. Stretching the DNA molecule in the presence of various concentrations of DOX allows us to quantify the binding and better understand this complex interaction. We have explored the binding of DOX previously by stretching and holding the DNA at a constant force allowing the drug binding to reach equilibrium. We are now exploring the binding equilibrium by stretching the DNA in small steps in the presence of DOX and waiting an extended amount of time at each step to assure equilibrium. The results confirm that DOX does in fact bind to dsDNA through intercalation. Unlike past literature stating that DOX binds to DNA in the micromolar range, we clearly see it intercalating DNA at concentrations as low as 0.5 nM in the presence of force. Further force-dependent measurements will allow us to accurately determine the zero-force binding affinity. In addition, progressive stretching through the melting transition shows that melting facilitates the binding of DOX.

1747-Pos

Does ATP Modulate Protein Nanomechanics

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ATP has a crucial role in cell biology. One of its well-known functions is intracellular energy transport to fuel many different cellular processes. However, another role for this molecule has been recently described. ATP has been proposed to be also a biological hydrotrope, preventing protein aggregation and dissolving already formed aggregates. The heart muscle is constantly contracting and relaxing for a lifetime, which means, that it is a highly energy-demanding organ. This contractility is carried out by the sarcomeres, functional units made of proteins with mechanical functions, such as titin. The immunoglobulin-like domains of this protein are able to unfold and refold under force during contraction. We hypothesized that the mechanical properties of these domains may be affected by the hydrotropic function of ATP. To test this hypothesis, we measured how I27 domain of titin unfolds and refolds in absence and presence of ATP using Atomic Force Spectroscopy (AFS). We subjected polyproteins composed of eight domains of I27 to unfolding-quench-probe experiments. These experiments inform both about the mechanical stability of I27 and its ability to refold. Contrary to our initial hypothesis, we have found that the presence of 10 mM ATP does not affect the mechanical stability or the folding rate of titin I27 domain.

Posters: Biosensors

1748-Pos

Nucleic Acid Sequence Detection by a Multi-Technique Approach

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The development of highly sensitive, specific, cheap and rapid laboratory diagnostics is an increasingly important challenge. Diagnosis of virus or various cancer diseases can be achieved through nucleic acid sequence detection, able to recognize specific short DNA or RNA sequences. DNA-based biosensors are excellent candidates, thanks also to their reusable property. A careful multi-technique characterization of DNA self-assembled monolayers (SAMs) represents a crucial step in order to optimize a sturdy sequence detection scheme based on helix-helix hybridization. In our work, we exploit molecular self-assembly on gold of single 22/28-bases DNA strands complementary to specific disease-related RNA or DNA sequences. The characterization is based on microscopic (Atomic Force Microscopy, AFM), spectroscopic (Spectroscopic Ellipsometry, SE, and X-rays Photoelectron Spectroscopy, XPS) and mass-sensitive (Quartz-Crystal Microbalance with Dissipation, QCM-D) methods, in order to investigate structural, optical and packing properties of DNA films. By AFM nanolithography, regularly defined micro-areas were depleted from molecules under a high tip load: the depth of the shaved area provides an accurate estimate of the film thickness. Specific sequence recognition has therefore been detected by monitoring changes in the film thickness upon hybridization. Through SE experiments it has been possible to monitor the optical thickness (influenced by both thickness and refractive index) and the fingerprints of the 260 nm DNA molecular absorption, allowing to evaluate the hybridization state of a DNA monolayer through a non-destructive and rapid method. Finally, XPS measurements provided useful information about surface coverage, allowing to distinguish between single strand and double strand DNA monolayers, while by QCM-D we could follow the dynamic process and, from the changes in mass/area ratio, evaluate the molecular density.

1749-Pos

Chemically-Tuned Solid-State Nanopores for Single-Molecule Biophyics

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Solid-state nanopores (SSNs) are nanofluidic conduits fabricated through synthetic materials such as silicon nitride, silicon dioxide, graphene, etc. Intrapore variations, temporal fluctuations in open-pore current and analyte sticking are legacy limitations associated with SSNs which have challenged its footprint in single molecule science. We report a modification to the Controlled Dielectric Breakdown (CDB) method of nanopore fabrication on silicon nitride (SiN_x) membranes where a blend of electrolyte and sodium hypochlorite is used as the conducting solution. These chemically tuned SSNs are devoid of the fundamental challenges associated with SSNs. The change in surface chemistry, with