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New Obligate Folding Intermediate of an RNA Pseudoknot Observed Using Atomic Force Microscopy Based Force Spectroscopy with 10 μs Resolution

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Programmed −1 frameshifting (−1 PRF) is an important biological process for the modification of gene expression enabled by the presence of RNA secondary and tertiary structures. However, the mechanism for this process is still under active study. Here, we investigate the role of mechanical force in −1 PRF by characterizing mechanically induced folding and unfolding of RNA pseudoknots using an enhanced atomic force microscopy (AFM) based single-molecule force spectroscopy (SMFS) assay featuring ∼10 μs resolution. The pioneering SMFS study of RNA psuedoknots associated with −1 PRF used a custom-built optical trap. Unexpectedly, this study indicated PRF efficiency was correlated with the presence of alternative folding pathways (rather than with average unfolding force), indicating a complex role of RNA psuedoknots in −1 PRF involving folding dynamics. Here, we found a new folding intermediate in an RNA pseudoknot associated with the sugarcane yellow leaf virus (ScYLV) that was not observed in the original optical-trapping based assay. We speculate that the shorter linkers and stiffer force probe in our AFM assay (relative to an optical trap) are the primary reasons for this enhanced state resolution. Our initial measurements of contour length and folding kinetics indicate this folding intermediate is an RNA hairpin that is part of the overall pseudoknot structure. We observed this intermediate every time the pseudoknot folds, indicating that this intermediate is obligate for folding. Overall, our results indicate that the folding dynamics of RNA pseudoknots are significantly more complex than previously observed. Because of the role of the folding dynamics of RNA pseudoknots in −1 PRF, we expect these new insights into RNA pseudoknot folding dynamics will provide a deeper understanding into the mechanisms of −1 PRF.

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Measuring the Effect of Antimicrobial Peptides on the Biophysical Properties of Bacteria using Atomic Force Microscopy

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With the increase in bacteria developing resistance to traditional antibiotics, there is pressing need for new antibiotics. Antimicrobial peptides are currently being explored as just such an option. These peptides are found as part of the immune system of many animals, and are typically small peptides that kill bacteria in one of two mechanisms. In the first mechanism, multiple peptide subunits join together to form pores in the bacterial membranes. In the second, the peptide crosses the membrane and binds to some molecule in the cell, preventing its action. Here, we use atomic force microscopy to characterize the biophysical changes that occur in the cell when *Escherichia coli* are exposed to antimicrobial peptides.

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Temperature-Dependent Nanomechanics and Topography of Bacteriophage T7

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Viruses are nanoscale infectious microorganisms which may be inactivated by heat treatment. Although heat inactivation is thought to be caused by the release of genetic material from the capsid, the thermally-induced structural changes in viruses are little known. In this work we measured the heat-induced changes in the properties of T7 bacteriophage particles. T7 is a non-enveloped, short-tailed icosahedral *E.coli* phage that contains a 40 kbp genomic DNA. T7 samples were exposed to two-stage (65 °C and 80 °C) thermal effect, and the structural changes were investigates by using AFM-based nanomechanical and topographical measurements. The structural features of the T7 capsids were extracted from the AFM images with particle analysis. We found that exposure to 65 °C causes the release of genomic DNA due to the loss of the capsid tail which leads to a destabilization of the T7 particles. Further heating to 80 °C surprisingly leads to an increase in mechanical stability due to partial denaturation of the capsomeric proteins kept within the global capsid arrangement. Revealing this structural arrangement may assist in understanding the remarkable resilience of viral capsids against harsh environmental exposures.

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Biomechanical Characterization of Protein-Based Hydrogels using a Force-Clamp Rheometer

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Here we introduce a new force spectroscopy method to study the mechanics of protein-based hydrogels under controlled force conditions. Our technique relies on exposing cylindrical hydrogels to mechanical force by tethering them between a voice coil motor and a force sensor. Our unique approach uses an analog PID controller to continuously adjust the hydrogel extension, such that the experienced force follows predefined protocols. We demonstrate this technique by using Bovine Serum Albumin (BSA) hydrogels ranging from 0.7mM to 5mM, crosslinked via a photoactivated reaction. When exposed to linear controlled-stress/relaxation, BSA-based hydrogels show a hysteresis associated to unfolding/ refolding of protein domains. This hysteresis decreases with BSA concentration while the measured Young's moduli had an opposite behavior in Tris/glycerol solution. Furthermore, in the presence of glycerol solution, hydrogels stiffen, while when immersed in guanidinium chloride (GuHCl) 6M, hydrogels soften and lose their hysteresis. Under constant force mode, the length of BSA-based hydrogels changes continuously with time due to the viscoelastic effects resulting from unfolding/refolding of protein domains. These constant force measurements enable the direct separation between elastic and viscoelastic behaviors, represented by gel network response and protein unfolding/refolding events, respectively. In constant force mode, BSA-based hydrogels also showed smaller extension in glycerol 50% and a higher extension in GuHCl 6M than in Tris solution. Additionally, BSA-based hydrogels show a purely elastic behavior in GuHCl 6M. In conclusion, this new method is a critical step toward establishing a new class of experiments to study protein nanomechanics in an ensemble mode and will open new routes for smart biomaterials discovery.

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Probing Elastic Properties of Mouse Articular Cartilage across Tissue Thickness

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Cartilage matrix is composed of a dense collagen matrix and of the highly-charged glycoprotein, aggrecan that is responsible for tissue osmotic swelling. The interplay of the two is presumably critical for the cushioning of bones at the joints. The relationship between osmotic and mechanical properties and composition is poorly understood and mapping of the related parameters through tissue depth is complicated by several issues relating to the need to section the tissue to gain access. Tissue damage and intrinsic roughness at the sectioned surface, and aggrecan diffusional loss from the section are some of those issues. In our experiments, aggrecan loss was minimized by mild fixation which, however, did not prevent thickness changes of the cut sections. We performed high-resolution (1um) elasticity mapping of the cartilage matrix of newborn mouse across tissue depth. The indentations were performed on thin (∼12um) cartilage sections cut parallel to the bone longitudinal axis. We optimized the size of the spherical indentation probe (5um) and applied relatively deep indentations. We developed a novel data analysis method that determines a virtual contact point, and a fitting protocol that ensures that we are probing the bulk matrix properties and are minimizing the effects of surface roughness, tissue inhomogeneity and corrected for the finite sample thickness. We demonstrated the robustness and effectiveness of our method and we compared its performance to the standard methods used in the field. Thickness changes upon sectioning, and the high cell density, characteristic of mouse cartilage, that leaves narrow matrix septa between cells are two factors that may cause underestimation of the measured elastic moduli. Fixation is possibly causing over-estimation. We discuss the implications of these uncertainties and future directions to address them.

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Micropipette Geometry-Induced Electrostatic Trapping of Nanoparticles

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Trapping and manipulation of small objects have been of great interest for a range of applications. Here we report on an electrostatic trapping of charged nanoparticle between the aperture of a nanopipette and a glass substrate in