data verified this structure, thus establishing the existence of the first internal tRNA-like structure discovered. Molecular dynamics simulations showed the stability of the entire structure as well as flexibility of some of its elements, in agreement with the in-line structure probing data. The model with its structure-implied functionality has led to further experimental determination of how the interconnected 3′ UTR sequence and structure elements participate in the processes of translation and replication. The structure element was found to bind the 60S ribosomal subunit, and thus act as a translation enhancer. This is the first such interaction in a 3′ UTR with the large subunit discovered. We also found that this tRNA-like element is a major part of a structural switch determining if the viral template is translated or replicated. Similar mechanisms may exist in other viruses and genomes, as the structural elements similar to the tRNA-like structure in the TCV have also been identified in several viruses.

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Pre-stressed Tensegrity Structures built from DNA

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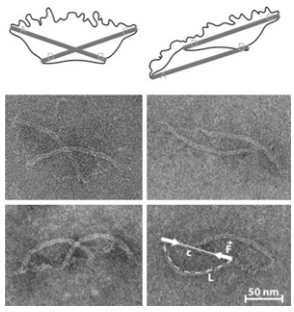
Tensegrity is a building principle that utilizes a network of tensed elements balanced by internal compression struts to create a self-equilibrated mechanical structure that requires tensile prestress for its mechanical stability. The concept of tensegrity has helped to understand the physical behavior of viruses, cells, tissues, organs and organisms [1] from the nano to the macro scale. This biological design principle also has been leveraged to construct artificial, static tensegrity structures using DNA nanotechnology approaches [2].

Here, we use the DNA origami approach [3] to engineer prestressed 2D and 3D nanoscale tensegrities assembled from compression-resistant DNA six-helix bundles connected by long sections of single-stranded DNA (several hundred bases long) that act as tensed entropic springs, which prestress the entire structure. The introduction of spring elements as building components for DNA nanostructures allows for the design of flexible nanostructures and nanoscopic lever spring balances. To prove the potential application of DNA tensegrity structures as force sensors and actuators, basic force experiments were performed with the 2D 'kite' structure.



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The Role of Sequence-Dependent Mechanics in DNA Looping

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The formation of protein mediated DNA loops are a key component in many biological regulatory functions. The binding of LacI protein within the Lac Operon of E.coli serves as the canonical example in which loop regulated transcription is understood. This fundamental looping motif consists of one protein simultaneously bound to two DNA operator binding sites. We calculate the free energy cost of loop formation using a Hamiltonian constructed about the looped state as well as the intrinsically open state of the DNA. The shape of the inter-operator DNA loop in mechanical equilibrium with the protein, is determined using a non-linear mechanical rod model. Our rod model captures the effect of sequence dependent curvature, sequence dependent persistence length, including any anisotropic bending. The equilibrium DNA-protein binding orientations are inferred from LacI protein crystallized with DNA operator segments.

Our Hamiltonian describes the change in bending energy of the DNA due to linear perturbations about either the looped or open state. We now calculate the normal modes of the Hamiltonian in order to characterize thermal fluctuations of the loop. Comparing the change stiffness we then calculate the Stockmayer J-factor(looping probability), free energy as well as the entropic contributions, of loop formation. Our works shows that these entropic contributions can play a significant role in determining loop stability and formation.

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Chromatin Organization in E.coli

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Prokaryotic organisms must strike a balance between DNA accessibility and condensation: facilitating the genetic processes of transcription, replication, and DNA repair while simultaneously enabling the structural and physical processes of chromosome condensation and segregation in rapidly dividing cells. Recent studies have revealed that prokaryotic chromosomes are intricately structured but the mechanism for this physical organization is not yet understood. In this study, we describe the construction of a large number of E.coli strains carrying three spectrally distinct, fluorescently labeled genetic loci. The origin and terminus of replication as well as a random position on the chromosome are labeled and tracked simultaneously in live cells. These labeled loci are identified, counted and tracked automatically using custom MATLAB software which enables the analysis of a large number of nucleoid conformations. Positions of the labeled loci are determined with respect to the cell body and used to compute both the distribution of locations of single loci within the cell (thus revealing "chromosome territories") as well as the correlations between fluctuations in the positioning of different loci. This data is analyzed in the context of a simple polymer model of the E.coli chromosome and it provides quantitative support for a nucleoid-centered mechanism of chromosome organization.

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Structure and Dynamics of the Bacterial Chromosome

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Despite the great deal of studies on bacteria, and especially E coli, our understanding of the spatio-temporal organization of bacterial chromosomes is minimal, largely because their dynamics have been difficult to observe directly. Even more remarkable is bacteria's ability to control the topology of the 1.5 mm-long DNA in the confined environment of the cell. The objective of our project is to study dynamics of chromosome structure during the process of cell division at the single-cell level, in the bacterium E. coli. Using a unique microcolony growth technique, we track cell growth and with fluorescent-protein techniques we can monitor chromosome folding and quantify gene expression levels through fluorescence microscopy. We have developed a bacterial strain containing fluorescent gfp-fusion versions of a chromosome-folding protein, Fis, under inducible control. This strain is used to visualize bacterial chromosome conformation during cell growth and division. Our further objective will be quantitatively analyze the coupling of nucleoid protein level and chromosome folding to gene expression, and to directly examine nucleoid mechanical properties as a function of protein levels using micromanipulation methods.

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DNA Strand Exchange on Liposome Surfaces

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The mechanism of DNA strand exchange, performed in vivo by proteins in the Rec A family, is despite extensive studies not understood in full detail. We therefore want to study the molecular parameters involved in the exchange using an in vitro platform. Positively charged liposomes have been widely used as non-viral gene-carriers, where the positive charges attract the negatively charged DNA. We here show that DNA strand exchange is significantly enhanced, both in rate and yield, on the surface of cationic liposomes.

We study the strand exchange reaction by adding an excess of non-labeled single strands to liposome bound DNA duplexes labeled with a FRET pair and monitor the exchange as an increase in donor emission. The fastest exchange is seen for liposomes containing 35% cationic lipid and when the positive charges on the outer leaflet of the liposomes equal the amount of negative charges on the added DNA. Both increasing and decreasing the amount of DNA slows down the exchange rate, either due to competition for the binding sites or due to that the single-strands on average are further away from the duplexes. By studying the exchange of mismatched DNA we conclude that the DNA opens in a ''zipper-like'' manner on the liposome surface, since a mismatched base-pair in the end of the sequence affects the exchange rate much more than a mismatch base-pair in the middle of the sequence. The liposome surface can be easily functionalized with lipids bearing specific functionalities and we intend to modify the surface to study the effects of hydrogen bonding and/or hydrophobic interactions.