Restriction enzymes (REs) are the central part of the defence system of bacteria against invading viruses. The protein complexes recognize viral DNA by the methylation state of their target sequence and destroy it by cleaving it into pieces. For this, the majority of REs need to interact with two distant target sites. This long-range inter-site communication can be accomplished either by passive 3D diffusive looping or by 1D motion along the DNA contour. Among the different classes of REs, Type I and Type III REs play a special role due to their helicase domains, which are key to the inter-site communication.
For Type I REs it is well established that the helicase domain acts as a dsDNA translocating motor. Cleavage is triggered after a pure 1D communication process, when two translocating motors from distant target sites collide.
In comparison, the communication mechanism for Type III REs has not been accurately defined and conflicting models including 3D diffusion and 1D translocation have been proposed. Using single-molecule DNA stretching based on magnetic tweezers, we provide evidence for a pure 1D communication mechanism in the absence of any 3D diffusive looping. Furthermore, we exclude translocation for inter-site communication due to the low ATPase rates and the observation that the enzymes move bidirectionally along DNA. From this we conclude that Type III REs use 1D diffusion to communicate between their distant target sites.
In order to test the diffusion hypothesis we have started to track the movement of Type I and III REs along DNA using a setup combining magnetic tweezers with single-molecule fluorescence (total-internal reflection fluorescence microscopy).

2142-Pos Board B112
Bacteriophage Phi29 Negatively Twists DNA During Packaging
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Bacteriophage phi29 employs a homomeric ring of RecA-like ATPases in order to package its dsDNA genome into the capsid at near-crystalline density. Previous single-molecule measurements of packaging have revealed the coordination of motor subunits, the step size of the motor, and the sensitivity of the motor to substrate modifications, thereby suggesting structural and kinetic models for the mechanism of translocation.
We directly observe that phi29 negatively supercoils DNA during packaging against applied force by monitoring rotation of a bead attached to the side of the substrate DNA in a laser tweezers. Simultaneously, torque generation and response to applied torque are measured. In this way, we probe the details of force and torque generation by the packaging motor. Combining these measurements with angstrom-scale laser tweezers observations of motor stepping suggests specific geometric models for the interaction of the motor and DNA during translocation.
The magnetic rotor bead technique introduced here allows simple application of torque and straightforward measurement of twist in a laser tweezers apparatus at arbitrary forces. We propose that this method can be applied with ease to a number of existing single-molecule experiments.

2143-Pos Board B113
Sequence-Dependent Kinetics of One-Dimensional Diffusion of p53 on DNA
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Proteins such as transcription factors that must locate and bind to one or a small number of cognate sites on the genome have been suggested to undergo alternating rounds of one-dimensional (1D) and three-dimensional (3D) diffusion. It has been argued that a single protein-DNA energy landscape as a function of the position of the protein on DNA cannot be both smooth enough for a rapid search process and rugged enough so that the protein binds stably to its cognate site (Slutsky and Mirny, Biophys. J. 87 (2004) 4021). We have offered instead a model of kinetic pre-selection whereby proteins diffuse with low friction along a search landscape and pause on cognate sites before adopting transitioning to a recognition landscape which accounts for the rapid localization process observed experimentally (submitted).
The transcription factor p53's role in tumor suppression gives it a biological need to locate and bind its promoters quickly. We developed a single-molecule fluorescence imaging approach to directly observe the protein diffusing in 1D on stretched DNA in vitro (Tafvizi et al., Biophys. J. 95 (2008) L01). By using total internal reflection microscopy to image fluorescently labeled p53 diffusing on stretched duplex DNA, we obtain information on the protein's diffusional properties as a function of its position on the DNA. Using a bioinformatics approach, we have identified a number of sites on our substrate DNA that closely resemble known p53 promoters. We present initial results that demonstrate the feasibility of this approach to correlate high-resolution information on diffusional properties with the positions of these binding sites.

2144-Pos Board B114
Caught in the Act: Single Molecule Structure-Function Studies of Telomerase
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Telomerase is a cellular ribonucleoprotein reverse transcriptase catalyzing the addition of telomeric repeats onto chromosome ends. Given its role in maintaining genomic stability and replicative capacity of cells, telomerase is implicated in cancer development and ageing. Mechanistically, telomerase negotiates a complex set of catalytic substeps, likely requiring exquisite structural rearrangements, to promote processive elongation of chromosome ends. Due to the inherent asynchronicity in enzymatic reactions, traditional biochemical tools are ill-suited for studying how telomerase structure and conformational dynamics enable function. To circumvent this shortcoming, we have developed a single molecule FRET based structure-function assay for telomerase activity and processivity. This assay allows us to capture enzyme-primer interaction (i.e. binding events) and the relevant structural dynamics in real time and to evaluate the catalytic outcome of such interaction. To visualize binding, FRET dye pairs are site-specifically placed at key positions within telomerase to report structural dynamics within the enzyme when bound to surface-immobilized DNA primer. Subsequently, a novel FRET based hybridization approach utilizing a partially complementary detection oligonucleotide (DO) is used to determine the length of the primer previously bound by the labeled enzyme. For an appropriately designed DO, the primer length is revealed through the binding energetics between primer and DO. Proof of principle experiments performed with primers of known lengths show that this detection scheme has single nucleotide sensitivity and low error rates. Since the activity and processivity of telomerase is manifested in its ability to increase the length of a given primer, such scheme allows the determination of enzyme activity one binding event at a time. Using this assay, we have characterized how the structure and conformational dynamics of various Tetrahymena thermophila telomerase RNA motifs such as the pseudoknot and stemloop IV contribute to telomerase function.

2145-Pos Board B115
Conformation of Telomerase RNP Established through Footprinting and Single-Molecule FRET
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Telomerase, a ribonucleoprotein (RNP) expressed in all highly proliferating cells, is comprised of telomerase RNA, telomerase reverse transcriptase (TERT) and other protein cofactors. The complex compensates for the chromosomal shortening that arises with each round of DNA replication. Since mutations altering telomerase expression, assembly and regulation cause multiple human diseases, it is of utmost importance to understand telomerase at the structural level. However, while the structure of TERT in the absence of RNA has been recently published, the full RNP structure remains unknown.
Using a combination of single-molecule FRET and ensemble footprinting, we are probing the functional structure of telomerase RNP. In particular, we have determined the active conformation adopted by two conserved regions of telomerase RNA - the stemloop IV and the pseudoknot - upon assembly with TERT and other protein cofactors.
The pseudoknot, unformed in naked telomerase RNA, was folded in active RNPs. Proper pseudoknot folding was required for catalysis, as demonstrated by mutations that abolished telomerase activity when formation of either pseudoknot stem was disallowed. Conversely, compensatory mutations that reinstated basepairing in the pseudoknot region also restored telomerase activity.
Binding of TERT, in addition to allowing pseudoknot formation, brought loop IV into the proximity of the pseudoknot without affecting its conformation. Mutations in the loop IV region that abolished TERT binding and telomerase activity resulted in an extended RNA conformation with a substantially larger loop IV-pseudoknot distance, as well as protection of the pseudoknot by RNP proteins. Interestingly, the telomerase holoenzyme protein p65 could compensate for these effects of loop IV mutations, restoring the loop IV-TERT interaction, the folded conformation of stemloop IV and telomerase activity.
These results suggest that proper conformations of the pseudoknot and stemloop IV of telomerase RNA are critical for enzyme activity.