Theoretical and computer modeling of the time evolution of multicellular systems provides valuable insight and guidance to in vitro experiments relevant to embryogenesis, tumor growth, angiogenesis, cell sorting and self-assembly of artificial cell aggregates that are used in bioprinting. The cellular particle dynamics (CPD) computer simulation method is designed to describe and predict the time evolution of multicellular systems that behave like viscoelastic liquids. In CPD each cell is modeled as an ensemble of interacting cellular particles. The time evolution of the multicellular system is determined by recording the 3D trajectories of the cellular particles by integrating their equations of motion. To test the CPD method we applied it to

(i) cell sorting in spherical aggregates composed of cells with different adhesion strengths and
(ii) the fusion of two identical spherical cellular aggregates.

For both cases the results of the CPD simulations agree well with the experimental data. In particular, we compared the time evolution of the interfacial area between the two fusing aggregates obtained from experiment, a CPD simulation, and an analytical calculation based on a continuum theory for viscous fluids. We used the comparison to relate the simulation time and the experimental time, and we demonstrate how the comparison can be used to relate cellular level CPD parameters to tissue level biophysical quantities such as surface tension and viscosity. As an application, we used the obtained model parameters in large scale CPD simulations to predict the formation of complex 3D cellular structures as a results of the fusion of bioprinted cell aggregates.

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Platform AB: Single Molecule Biophysics - I

985-Plat Single-molecule Fret Observations And Large-scale Simulations Of Ribosome Dynamics

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High-spatial and -time resolution single-molecule fluorescence resonance energy transfer measurements have been used to probe the structural and kinetic parameters of transfer RNA (tRNA) movements within the ribosome. Our investigation of tRNA motions, quantified on wild-type, mutant, and L1-depleted ribosome complexes, reveals a dynamic exchange between multiple metastable tRNA configurations. This new dynamic information, together with supporting evidence, will be presented in the context of a framework in which the formation of intermediate states in the translocation process is achieved through global conformational rearrangements of the ribosome particle. All-atom explicit solvent simulations performed on the 70S ribosome have been used to investigate the metastable states visited during the translocation reaction coordinate. These simulations shed light on the stereochemical feasibility of various models, including independent vs. simultaneous movement of the aminoacyl- and peptidyl-tRNAs. The relationship between the movement of tRNA, the small subunit, and the L1 stalk will be explored in detail.

986-Plat Single-Molecule Tracking of mRNA Exiting from RNA Polymerase II

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Single-pair fluorescence resonance energy transfer was used to track RNA exiting from RNA polymerase (Pol II) in elongation complexes. Measuring the distance between the RNA 5'-end and three known locations within the elongation complex allows us to determine its position by means of triangulation.

RNA leaves the polymerase active center cleft via the previously proposed exit tunnel. When the RNA reaches lengths of 26 and 29 nucleotides, its 5'-end associates with Pol II at the base of the dock domain.

TFIIB changes the position of the nascent RNA presumably due to a competition for the binding site on the dock domain. Thus exiting RNA may contribute to TFIIB displacement during the transition from initiation to elongation and may prevent TFIIB reassociation during elongation.

A more general implication is that the applied single-molecule triangulation technique combining multiple FRET measurements with high resolution crystallographic data provides an accurate tool for determining the positions of flexible domains in large multiprotein complexes.

987-Plat Assembly And Activity Of Transcription Complexes Detected At The Single-molecule Level

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Initiation of messenger RNA (mRNA) transcription in eukaryotes involves assembly of RNA polymerase II (Pol II), general transcription factors, activators, and co-activators on a promoter, unwinding of the promoter, initiation of mRNA synthesis, and promoter escape by Pol II. The mechanism of assembly of the components of the preinitiation complex on promoter remains poorly understood. We have built an instrument that combines total-internal-reflection microscopy with magnetic nanomanipulation of single DNA molecules. The instrument is designed to directly detect assembly of Pol II transcription complexes from fluorescently labeled components, and to detect promoter unwinding activity of Pol II, all in the same experiment. As a proof of principle, using our instrument we have detected binding by fluorescently labeled *E coli* RNA polymerase (RNAP) to a single, immobilized DNA molecule, and, simultaneously, detected promoter unwinding by RNAP. Thus, the rate of isomerisation of "closed" RNAP-promoter complex into "open" RNAP-promoter complex can be measured at the single-molecule level. To demonstrate that our instrument can detect

assembly and activity of Pol II transcription complexes, we have detected specific promoter binding of the human transcription factor IID labeled with quantum dots. In a separate experiment, we have detected single-molecule promoter unwinding activity of Pol II transcription complexes.

988-Plat Activity and Processivity Measurements on Single Telomerase Reverse Transcriptase Enzymes

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The telomerase ribonucleoprotein (RNP) is a specialized reverse transcriptase that solves the end replication problem and maintains chromosome stability by adding short telomere DNA repeats to chromosome termini. Telomerase operates by a unique reverse transcription mechanism, wherein a region of the integral telomerase RNA serves as the template for DNA synthesis. Structural dynamics of telomerase RNA are thought to be required for processive telomere repeat synthesis. However, due to the lack of appropriate assays which can directly correlate telomerase structure with catalytic function, the precise nature of these conformational changes remains speculative. Here, we describe a highly sensitive single-molecule telomerase activity assay that reports on substrate-enzyme interaction kinetics, enzyme structural dynamics, and the catalytic outcome of individual telomerase-DNA primer binding events. Direct detection of telomerase reaction product length by DNA hybridization provides single nucleotide sensitivity and an accurate measure of enzyme processivity, as compared with conventional ensemble telomerase assays. Using the single-molecule telomerase assay, we have revealed heterogeneities in the DNA binding kinetics of catalytically active telomerase enzymes, and an increase in the stability of telomerase-DNA interaction in the presence of nucleotide cofactors. The integration of single-molecule telomerase activity detection with our recently reported assay for measuring structural dynamics within individual enzymes(1) provides an unprecedented opportunity to directly evaluate the structure-function relationship of the telomerase motor.

References

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989-Plat Splicing of Single pre-mRNA Molecules in Whole Cell Extract

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The excision of introns from nascent eukaryotic transcripts is catalyzed by the spliceosome, a highly complex and dynamic macromolecular machine composed of RNA and protein. Because of its complexity, biochemical analysis of the spliceosome has been previously limited to bulk assays in largely unfractionated cell extracts. We now report development of a novel system for studying the splicing of isolated single pre-mRNA molecules in real time. In this system, a fluorescently tagged pre-mRNA is tethered to a glass surface via its 3′-end. Splicing can be observed in Saccharomyces cerevisiae whole cell extract by monitoring loss of intron-specific fluorescence with a multi-wavelength total internal reflection fluorescence (TIRF) microscope. To prolong fluorophore lifetime, two enzyme-based O2 scavenging systems compatible with splicing were also developed. This work provides a powerful new approach for elucidating the mechanisms of spliceosome function and demonstrates the feasibility of utilizing TIRF microscopy for biochemical studies of single molecules in highly complex environments.

990-Plat Intranuclear Dynamics of Single Balibiani Ring mRNP Particles in Living Salivary Gland Cells

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The salivary gland cells of the dipteran *Chironomus tentans* provide an elegant model system for the analysis of specific messenger ribonucleoprotein particles, the Balbiani Ring (BR) mRNPs. BR2 mRNPs contain long RNA transcripts (∼35–40 kb) and are roughly 50 nm in diameter. Although their biogenesis was thoroughly analysed, up to now very little is known about the intranuclear dynamics of the BR mRNPs *in vivo*. We analysed the intranuclear mobility of BR particles by single particle tracking of fluorescence labelled BR2 mRNPs in living gland cells. Labelling was accomplished by microinjection of fluorescent oligonucleotides, which were complementary to a highly repetitive sequence on the BR mRNA. Using high speed laser microscopy we could for the first time follow the intranuclear pathways of native mRNPs. They exhibited a very complex *in vivo* dynamics, but did not show any sign of directed motion. Fast particles moved with a diffusion coefficient of up to 4 $\mu m^2/s$, slower particles with 0.6 and 0.2 $\mu m^2/s$. A forth fraction was almost, but not completely immobile with 0.015 $\mu m^2/s$. Using inert fluorescence nanoparticles and quantum dots the nuclear viscosity was measured as ∼ 4 cP. Thus, the fast mRNPs could move as rapid as expected by hydrodynamics of non-interacting 50 nm particles. Hence, they can cover the distance from the transcription site to the nuclear envelope within the huge salivary gland cell nuclei with diameters of 60 μm within 1 minute only. Slow mRNPs were supposed either to be transiently retarded or comprised in slow, bulky supramolecular complexes. Functionalized fluorescent quantum dots were used to study the transient mRNP interactions.

991-Plat Spinning DNA and its Implications for Cellular Function

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The double-stranded nature of the DNA helix has important implications for the build-up of torque in biological processes that unravel the helix to read out the genetic code. Indeed, excess torque can lead to the formation of supercoils in the DNA which must subsequently be removed.

Using single-molecule techniques, we have studied the physics of supercoil removal to assess the rotational drag of large plectonemic structures. On bare DNA, this rotational drag is found to be quite small, indicating a low resistance to diffusion of supercoils along the DNA and implying that supercoils may be removed via annihilation on a bare circular plasmid (Crut et al., PNAS 2007). However, the rotational drag of DNA inside the nucleus is likely to be significantly larger as a consequence of protein binding, requiring the activity of topoisomerases for supercoil removal. In earlier work, we quantified the mechanism of type 1B topoisomerases and demonstrated that these enzymes use a stochastic process of religation that is torque-dependent (Koster et al., Nature 2005). Recently, we have generalized this torque-dependence of religation to include additional enzymes such as DNA ligases.

We have also examined the effect of chemotherapeutic drugs on the rate of supercoil removal and observed a dramatic reduction in the rate of supercoil removal in the presence of topotecan, a drug in clinical use. This reduction was particularly strong for the removal of positive supercoils. We have consequently investigated whether positive supercoils accumulate in yeast cells and demonstrate such an accumulation in both the G1- and S-phases of the cell cycle. These experiments provide a unique link between single-molecule studies on the one hand, and cellular processes on the other (Koster et al., Nature 2007).

992-Plat DNA Tension Can Regulate Gene Expression

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We have investigated the effect of DNA tension upon the association and dissociation of the T7 RNA polymerase/promoter DNA interaction. Using two optically trapped beads, we suspended a single DNA molecule containing the T7-phi13 promoter above a 3rd surface-immobilized bead that bears active molecules of T7 RNAP. The DNA was brought into contact with the surface bead, while applying a 50 Hz triangle oscillation to the bead upstream of the promoter. Binding of T7 RNAP at the promoter was detected as the decoupling of motion of the two optically trapped beads: oscillations of the downstream bead are reduced or cease altogether upon binding. The DNA tension upon binding is directly proportional to the displacement of the downstream bead from the center of the optical trap. By altering both the mean tension in the DNA - changing trap stiffness or the mean bead-to-bead distance - we have been able to observe promoter binding across the range of force 1-12 pN. From these data we find clear evidence that the mean lifetime of the promoter/T7 RNAP complex decreases with increasing tension as indicated by an increasing dissociation rate constant, k_{off}, from 3.5 s^{-1} at 1.5 pN up to 73 s^{-1} at >8 pN. From these observations, we propose that tension within a DNA molecule is able to regulate gene expression, at least in the case of the bacteriophage T7 enzyme. Based on the recent structural data of the T7 RNAP initiation complex we speculate on the mechanism by which force may affect the dissociation kinetics.

Platform AC: RNA Folding

993-Plat Temperature-Dependent Kinetics of an RNA Tertiary Interaction using Single-Molecule FRET

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The functional diversity of ribozymes lies in RNA's ability to fold into complex structures governed by multiple tertiary interactions. However, little information is available on the kinetic and thermodynamic contributions of individual tertiary motifs to the global RNA fold. In this study, we isolate a single tertiary binding motif, the GAAA tetraloop and its 11 nucleotide tetraloop receptor from the P4–P6 domain of the Tetrahymena ribozyme. By combining

(i) time-correlated single-photon counting,
(ii) confocal microscopy and
(iii) single-molecule FRET, we explore the kinetics and thermodynamics of RNA folding due to the GAAA tetraloop-receptor interaction.

The distance between the tetraloop and receptor in single RNA constructs is interrogated via real-time FRET with undocked and docked states clearly distinguished by low and high FRET efficiencies (E_{FRET}), respectively. Previously, the [Mg^{2+}]-dependence of tetraloop-receptor docking and undocking rate constants (k_{dock} and k_{undock}) was reported [1]. In the present work, we employ precise temperature-control methods to probe the thermodynamics of the tetraloop-receptor interaction at the single-molecule level in both surface-immobilized and freely diffusing RNA, yielding the temperature dependence of k_{dock}, k_{undock}, and K_{dock} at a given [Mg^{2+}]. At 1mM Mg^{2+} the entropy of reaction is -50 ± 1 cal mol^{-1} K^{-1} and the enthalpy of reaction is -17 ± 2 kcal mol^{-1} as determined from both single-molecule trajectories and freely diffusing E_{FRET} population distributions. Preliminary results suggest the tetraloop-receptor RNA folds via a barrierless transition with entropy loss in the docked state dictating the docking rate. Furthermore we explore the effect of temperature on kinetic heterogeneity in the tetraloop-receptor system, i.e. the temperature-dependence of RNA subpopulations with different folding kinetics.

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994-Plat Combining Simulation And Experiment To Investigate The Structural Dynamics Of The S-adenosylmethionine Riboswitch

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