transfer to the hemes by using time-resolved absorption spectroscopy and pH indicator dyes. By comparison of proton uptake and release kinetics observed for solubilized COX and COX-containing liposomes, we conclude that the 1-μs-electron-injection into Cu_A, close to the positive membrane side (P-side) of the enzyme, already results in H^+-uptake from *both* the P- and N (negative)-side (1.5 H^+ and 1 H^+, respectively). The following 10-μs-transfer of the electron to heme *a* is accompanied by the release of one proton from the P-side to the aqueous bulk phase, leaving about 0.5 H^+ at this side to electrostatically compensate the charge of the electron. With about 200 μs, all but 0.3 H^+ at the N-side are released to the bulk, and the remaining proton is transferred towards the hemes to a so called "pump site". Thus, this proton may already be taken up by the enzyme as early as during the first electron transfer to Cu_A. The results of continuum electrostatic calculations support our conclusions by identifying residues which undergo protonation changes and may act as primary proton acceptor sites.

68-Minisymp A Serine to Aspartate Mutation Close to Heme a Results in a Slow, Proton Dependent Electron Transfer from CuA to Heme a and an Altered EPR Spectrum

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The difference observed in the heme *a* EPR spectra between cytochrome *c* oxidases (C*c*O) from mammalian mitochondria and various bacteria can be explained in part as due to a different amino acid, $G30_I$ in bovine C*c*O vs. $S44_I$ in *Rhodobacter sphaeroides* (*Rs*) C*c*O, hydrogen bonded to one of the heme *a* ligands, $H102_I$. Analysis of two site-directed mutants of *Rs*C*c*O, S44G and S44D, shows that S44G has native characteristics, but displays a shifted heme *a* EPR signal, closer to that of the bovine C*c*O. The S44D mutant has lower activity than wild-type and a shifted, split heme *a* EPR signal moved to a lower value, further from bovine. The splitting of the EPR signal is sensitive to pH, as is the intrinsic rate of electron transfer from Cu_A to heme *a.* At pH 8, the heme *a* EPR signal is most intense at g_z=2.72, and the electron transfer from Cu_A to heme *a* is less than 90 sec^{-1}, compared to 90,000 sec^{-1} for wild-type; whereas at pH 5.5, the g_z=2.77 peak is more intense, and close to 50% of the Cu_A to heme *a* rate is wild-type, while the rest remains slow. It is proposed that the protonated/deprotonated states of D44 result in drastically different redox potentials of heme *a,* by as much as 300 mV. This accounts for the markedly different electron transfer rates from Cu_A to heme *a*, where the slow rate is dependent on proton uptake to the internal S44D residue. The source of the proton is under investigation.

The different protonation states of residue D44, and an otherwise unaltered structure, are supported by preliminary X-ray crystallographic analysis.

Platform F: DNA, RNA, Structure & Conformation

69-Plat Correlation Between Mechanical Strength Of mRNA Pseudoknots And Ribosomal Frameshifting

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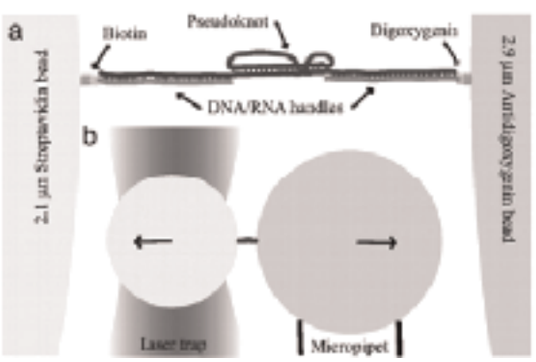
Programmed ribosomal frameshifting is often used by viral pathogens including HIV. Slippery sequences present in some mRNAs cause the ribosome to shift reading frame. Although the mechanism is not well understood, frameshifing is known to be stimulated by an mRNA structure such as a pseudoknot. Here, we show that the efficiency of frameshifting relates to the mechanical strength of the pseudoknot. Two pseudoknots derived from the Infectious bronchitis Virus were used, differing by one base pair in the first stem. These two pseudoknots caused framsshifting frequencies that differed by a factor of two. We used optical tweezers to unfold the pseudoknots. The pseudoknot giving rise to the highest degree of frameshifting required a nearly 2 fold larger unfolding energy than the other. We propose that the degree of ribosomal frameshifting is related to the mechanical strength of RNA pseudoknots (PNAS vol. 104 p. 5830, 2007). Our observations support the 9 Å model that predicts some physical barrier is needed to force the ribosome into the 1−1 frame. The result has implicatiomns for the understanding of genetic regulation and unwinding of mRNA secondary structures by ribosomes.



70-Plat Translation by Single Ribosomes One Codon at a Time

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The ribosome and the mechanism of translation have been extensively studied by biochemical, genetic, and structural approaches. To explore the dynamic nature of translation, we have used optical

tweezers to follow the real-time motion of single ribosomes along single messenger RNAs. The messenger RNAs were designed to form either a 60-bp or a 274-bp hairpin. The RNA was held at a force below its unfolding transition, and translation was followed by the increase in end-to-end distance when the hairpin was unwound by the translating ribosome. Translation occurs by a series of successive translocation-and-pause steps. The distribution of pause durations, with a median value of 2.8 s, indicates that at least two rate-determining processes control the pause. Each translocation step measured three bases - one codon - and occurred in less than 0.1 s. Analysis of the times required for translocation reveals that there are three substeps in the process. Increasing force applied to the ends of the RNA hairpin destabilized secondary structures and decreased pause durations, but did not affect translocation times. The presence of internal Shine-Dalgarno-like sequences in the messenger RNA tended to cause translation arrest. The underlying molecular bases and biological meanings of these discoveries remain to be investigated.

71-Plat Real-time Kinetics and Efficiency of Nascent Polypeptide Folding Following Synchronous Ribosome Release

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In mammalian cells, protein folding is influenced and often limited by ribosome-associated translation machinery and the relatively slow rate of synthesis (5–7 aa/sec). However, the mechanism(s) by which folding and synthesis are coupled is poorly understood since the complex biochemical systems needed to support translation are generally not amenable to traditional biophysical analysis. To circumvent this problem, we used variant green and red fluorescent proteins (FPs) to monitor real-time folding kinetics of nascent polypeptides following synchronized ribosome release. mRNAs of eight FP-fusion proteins were truncated and expressed in vitro to generate uniform cohorts of [^{14}C]lysine-labeled ribosome-nascent chains (RNCs) that retain an intact peptidyl-tRNA bond and thus mimic native translation intermediates. RNCs were isolated by gel filtration, quantitated based on radiolabel incorporation, and FP folding was determined as a function of fluorescence per fmol of FP synthesized. Sequestration of C-terminal residues in the ribosome exit tunnel strongly inhibited chromophore formation and likely, proper folding of the 11 stranded FP β-barrel. FPs truncated 15–22 aa beyond the C-terminus remained stably trapped in an unfolded, but folding-competent, state. This allowed folding to be measured in real-time when the nascent FP was released from the ribosome. Remarkably, FPs showed striking differences in the effect of C-terminus sequestration on the overall folding efficiency. Unlike GFP variants (eCFP and Venus), the RFP variant mCherry folded significantly more efficiently when the unfolded intermediate was released post-translationally from the ribosome (tether length < 22 aa) than if the entire FP exited the ribosome cotranslationally (tether length $>$ 46 aa). These findings define a novel method for analyzing nascent protein folding and indicate that the cotranslational folding environment can impact post translational protein maturation even for proteins with a highly conserved fold.

72-Plat Single-Molecule Structural Dynamics of Active Telomerase

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Telomerase is a ribonucleoprotein expressed in all highly proliferating cells - e.g., cancer and stem cells. It prevents chromosomal end shortening arising with each round of DNA replication and mutations altering its expression, assembly and regulation are the basis for multiple human diseases. Therefore, it is of utmost importance to characterize this enzyme in structural and functional detail.

Telomerase consists of telomerase RNA, telomerase reverse transcriptase (TERT) and other protein cofactors. Telomerase RNA provides a template sequence and structural domains essential for assembly, activity and processivity of the enzyme, while TERT carries out catalysis. It remains difficult to recombinantly express telomerase, and only a handful of small segments of the telomerase RNA and TERT are available at atomic resolution. We developed a single-molecule FRET assay where FRET donors and acceptors are attached to several key elements of the telomerase RNA, allowing us to probe its structure and dynamics region by region. Previously, we showed that telomerase assembles hierarchically via several steps of protein-mediated RNA folding.

Here, we show that distant domains of the RNA move into the vicinity of the catalytic site. In particular, we find that StemLoop4, a key region for enzyme processivity, undergoes a significant conformational change. Originally positioned far from the active site, Loop4 is brought into its close proximity upon assembly with telomerase proteins. Mutations in Loop4 eliminated this compaction and severely reduced telomerase activity and processivity. Furthermore, upon addition of the telomeric primer substrate, Loop4 alternates between the compacted conformation and another, in which it is farther from the active site. We also found another catalytically important, protein-induced structural change in the pseudoknot region of the RNA. Our FRET data provide the basis for a structural model of telomerase RNA and help elucidate functional dynamics vital for activity.

73-Plat Single Molecule Detection Of A Conformational Change Within The Active Site Of The Tetrahymena Group I Ribozyme

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The precise alignment of substrates and active site groups required for enzymatic reactions often involve conformational transitions that occur after the binding of substrates. Prior functional analysis of

the Tetrahymena ribozyme indicated that a conformational transition is induced by tertiary docking of the substrate-containing P1 duplex and strengthened by guanosine (G) binding. This cooperative conformational change apparently allows contacts between a site-bound metal ion within the active site and both the substrate and G, which position them for reaction. Using a single molecule fluorescence resonance energy transfer (smFRET) approach, data from individual ribozyme molecules reveal that most of the time is spent in the previously-characterized docked, conformation (FRET value ~ 0.8), but we find that the ribozyme fluctuates transiently to a structure that gives a lower FRET (~ 0.6), as well as occasionally undocking the P1 helix (FRET value ~ 0.2). This conformer with an intermediate FRET value does not give cleavage of substrate, as indicated by analysis of time traces, but it does reflect a docked structure, as its formation is inhibited by a specific 2′ methoxy substitution within the substrate. Addition of saturating G stabilizes the fully docked state but not the 'alternative' docked state, indicating cooperativity between G binding and the conformational transition. Together these results suggest that the lower FRET value represents a conformation in which the P1 helix is docked in the active site, but the contacts that bridge and align P1 and G are not yet formed. The ability to detect this unaligned docked state and directly follow its formation and decay in individual molecules allows a strikingly clear view of the kinetics and thermodynamics of physical steps that are required for catalysis by this ribozyme.

74-Plat Characterization of DNA Conformational Dynamics In Site-specific Recombination Events

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DNA supercoiling is a feature of almost all DNA molecules inside the cell. It is a powerful thermodynamic force that drives and directs many DNA associated processes in vivo. The level of supercoiling or DNA spatial conformation is constantly changing due to the activities of proteins and the environmental conditions of the cell. Proteins such as DNA topoisomerases are responsible for maintaining the steady state of supercoiling essential for the cell viability. However, local and temporal changes in DNA supercoiling affect many cellular processes such as replication, transcription recombination and chromosome organization.

In prokaryotes, DNA supercoiling is expected to play an important role in site-specific recombination, a fundamental process to achieve resolution of dimeric chromosomes, allowing plasmids and chromosome segregation and consequently cell division. During this process, DNA undergoes to multiple conformational changes due to the activity of Xer tyrosine recombinases and accessory proteins like FtsK translocase, that so far have not been characterized.

Here we use a combination of biochemical and biophysical techniques to study the role of DNA topology in recombination. In vitro, we demonstrate the topology dependence of the different steps in site-specific recombination events using DNA substrates with different superhelical density.

Using high-resolution amplitude modulation atomic force microscopy (AM-AFM) in physiological buffer we characterize at the single molecule level the nature of the forces that drive relevant DNA conformational changes by it self or after protein interaction in each step of site-specific recombination events. Additionally, we observe for the first time the dynamics of DNA and the conformational changes of DNA during XerC/D site-specific recombination events imaged by high-speed AFM at time resolutions up to 20 ms and sub-nm spatial resolution.

75-Plat Anomalous Small-Angle X-ray Scattering Study of Trivalent Mediated DNA-DNA Interactions through Ion Competition

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Trivalent cations are known to condense highly negatively charged DNA strands into compact structures. We have probed the onset of these DNA-DNA interactions through trivalent-monovalent ion competition studies. Utilizing Anomalous Small-Angle X-ray Scattering (ASAXS), we have elucidated the monovalent and trivalent ion atmospheres independently while simultaneously measuring inter-DNA behavior. DNA-DNA attraction was observed and the ion atmospheres at the onset of attraction were measured. Competition results were compared with predictions by the Poisson-Boltzmann equation and were found to be in surprising agreement when accounting for ion size effects. From these data, an estimate of the minimum number of trivalent ions needed for DNA-DNA interaction was obtained.

76-Plat Measuring Nanometer Distances In Nucleic Acids Using A Sequence Independent Nitroxide Probe

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In site-directed spin labeling (SDSL), a stable nitroxide radical is attached at a specific location within a macro-molecule, and structural and dynamic information at the labeling site is obtained via electron paramagnetic resonance (EPR) spectroscopy. I will present work on development and application of SDSL for mapping global structures of nucleic acids. These studies center on an R5 nitroxide probe that can be attached, in an efficient and cost-effective manner, at a specific nucleotide of any given RNA and DNA. State-of-art pulse EPR techniques have been used to successfully measure nanometer distances (20 – 50 Å) between a pair of R5's attached in DNA and RNA, and an efficient program, called NASNOX, has been established for correlating the inter-R5 distances to the parent nucleic acid structure. Together, the R5 probe,

the pulse EPR method, and the NASNOX program constitute a tool-kit for un-restricted global structural mapping of nucleic acids and protein/nucleic acid complexes. This tool-kit is being utilized to map the global structure of the packaging RNA (pRNA) in the phi29 bacteriophage DNA packaging motor, which is the strongest known bio-molecular motor. The pRNA forms a ring-shaped complex that is indispensable in motor ATPase activity, yet structural information on pRNA is lacking. We are using the SDSL tool-kit to obtain pRNA inter-helical distances. These constraints are used to determine the spatial packing of the helices and define the global structure of functionally relevant pRNA complexes. This represents the first application of SDSL to study an RNA with unknown structure.

Platform G: Cardiac Electrophysiology

77-Plat KCNE2 Transcript Levels Are Directly Correlated With Heart Estrogen Concentrations

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We have previously shown that the mouse KCNE2 gene is an estrogen-responsive gene and that its transcripts are highly upregulated by estrogen (E2) in ovariectomized (ovx) mice. As male and female have different levels of E2, we investigated whether KCNE2 transcripts are differentially regulated in males and in females at different estral stages: estrus (under the influence of E2 hours after the proestrus E2 surge) and diestrus2 (after a prolonged exposure to low estrogen levels). Real time PCR showed that KCNE2 transcript levels were significantly higher in estrus (10 fold) compared to diestrus as can be expected for KCNE2 being an E2 responsive gene. Interestingly, KCNE2 transcripts were similar in males and in feamles at estrus. Based on these unexpected findings, we speculated that male heart could have high E2 levels. This assumption is conceivable as heart has all the machinery to synthesize locally E2 from testosterone by aromatase CYP450. Plasma and heart E2 levels were measured with the radioimmunoassay technique in males, females at estrus and diestrus, ovx mice and ovx mice treated with E2. Male heart has significantly higher E2 levels than plasma (35±3 pg/ml, n=6 *vs.* 12±0.9 pg/ml, n=5) favoring the hypothesis that male hearts can locally synthesize E2. In fact, male hearts had twice as much E2 levels than females (diestrus 20.2±1.5 n=4; estrus 17.2±0.9 pg/ml, n=4). E2 treatment of ovx mice increased heart E2 concentration from 16±1.4 to 62.7±2.9 pg/ml, n=3. We conclude that:

1. local heart E2 biosynthesis leads to a higher E2 concentration in male hearts;
2. heart KCNE2 transcript levels are directly correlated with the local heart E2 concentrations, and
3. KCNE2 expression is indeed regulated by E2 *in vivo*.

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78-Plat Embryonic Cardiomyocytes Can Functionally Integrate Into Infarcted Myocardium And Reduce The Vulnerability To Post-infarct Arrhythmias

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Transplantation of progenitors or stem cells into the infarcted myocardium is thought to improve the contractile function of the heart, which lacks regenerative capacity. However, proper function of engrafted cells in the infarct requires their electrical coupling with the native myocardium.

To test the coupling of transplanted cells *in vivo* we have used the Ca^{2+}-sensitive circular-permutated GFP (GCaMP2). Embryonic cardiomyocytes (eCM) were harvested from GCaMP2 positive embryos or eCM transduced with a lentivirus encoding GCaMP2 under control of the CMV promoter. GCaMP2 positive eCM showed spontaneous beating and concomitant Ca^{2+} transients *in vitro*. Histological analysis of hearts two weeks after cryoinfarction and injection of GCaMP2 positive eCM revealed stable engraftment and differentiation. Electrical coupling of the transplanted cells was assessed by parallel ECG recording and *in vivo* imaging of cytosolic Ca^{2+} in GCaMP2 positive eCM. For this purpose, the mouse was intubated, ventilated and the chest wall removed. After exposing and stabilizing the heart, GCaMP2 fluorescence was recorded with a macroscope and an EMCCD camera. At physiological heart rates 2:1 or 4:1 coupling of Ca^{2+} transients from engrafted eCM with the native heartbeat could be observed indicating loose electrical coupling. In addition, we could also observe GCaMP2 positive eCM which were not coupled to the native myocardium but displayed slow conduction between grafted eCM within the infarct.

We next wondered whether engrafted eCM affect post-infarct arrhythmia and therefore assessed electrical vulnerability two weeks after infarction by *in vivo* transvenous electrophysiological investigation. VT could be evoked in 96.4% of infarcted mice, however transplantation of eCM reduced VT inducibility to 35.7%, comparable to non-infarcted control mice (38.9%).

Thus, transplanted eCM stably engraft, can electrically couple with the native myocardium and reduce the vulnerability to post-infarct arrhythmia.

79-Plat Characterization of Ion Channels in Human Cardiac Fibroblasts

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Electrophysiology is well established in cardiac contractile myocytes; however, information for ion channels is poorly understood in