channel (KATP) in INS 832/13 cells. Membrane potential was monitored under the whole-cell current clamp mode. NE hyperpolarized the cell membrane, an effect that was abolished by tolbutamide. The effect of NE on KATP channels was investigated in parallel using outside-out single channel recording. This revealed that NE enhanced the open activities of the KATP channels ~2 fold without changing the single channel conductance, demonstrating that NE-induced hyperpolarization was mediated by activation of the KATP channels. The NE effect was abolished cells in cells preincubated with pertussis toxin, indicating coupling to heterotrimeric Gi/Go proteins. To identify the G proteins involved, antisera raised against a and b subunits (anti-Ga-common, anti-Gb, anti-Gai-1/2/3 and anti-Gao) were used. Anti-Ga-common totally blocked the effects of NE on membrane potential and KATP channels. Individually, anti-Gai-1/2/3 and anti-Gao only partially inhibited the action of NE on KATP channels. However, the combination of both completely eliminated the action. Antibodies against Gb had no effects. To confirm these results and to further identify the G-protein subunits involved the blocking effects of peptides containing the eleven amino acids at the C-termini of the a-subunits were used. The data obtained were similar to those derived from the antibody work with the additional information that Gai-3, Gao-1 were not involved. In conclusion, both Gi and Go proteins are required for the full effect of norepinephrine to activate the KATP channel.

**Protein-Nucleic Acid Interactions - I**

## 1982-Pos Kinetic Cooperativity In 30S Ribosome Assembly Detected With 2-Photon Fluorescence Fluctuation Spectroscopy In Microfluidics

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**Board B97**

The bacterial 30S ribosomal subunit is able to self-assemble in vitro into a functional macromolecular machine. The assembly process is thought to be dominated by folding of the core 16S rRNA, while binding of the 20 small-subunit proteins facilitates folding via a tertiary-structure capture mechanism. As monitored by protein binding, the assembly process exhibits significant thermodynamic cooperativity. However, bulk kinetic experiments have elucidated relatively few ordered binding events and so to fully examine kinetic cooperativity we are observing assembly on the level of individual assembly intermediates.

To gain single-intermediate resolution, we have built a 2-photon microscope with single-molecule sensitivity. Three spectrally-distinct fluorophores are simultaneously excited at ~880nm, and photons are recorded and stored for offline analysis. Adaptations of FCS/Coincidence Analysis allow us to extract kinetic traces with ~1second resolution over the duration of the hour-long reaction. 30S Assembly reactions are initiated by mixing three fluorescently-labeled ribosomal proteins with varying amounts of 16S rRNA and unlabeled recombinant ribosomal proteins. From three auto-correlations, three cross-correlations and a triple-correlation, we can monitor eight assembly intermediates and thus detect kinetic cooperativity between any given trio of proteins. Since the technique does not require FRET it can probe interactions between distant domains of the 30S.

Early simulations of reactions highlighted the need for a large dataset of kinetic traces prepared from a combinatorial array of initial reagent concentrations, as well as the need for multiple-start reactions. To facilitate this task, we have designed and built an automated microfluidic reactor which can precisely prepare and initiate the requisite large number of reactions. We are presently investigating late stages of 30S assembly in the 3′Major domain which are thought to involve resolution of misfolded 16S rRNA.

## 1983-Pos Helicase Superfamily 1 and 2 ATPase Mechanisms

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**Board B98**

Helicases catalyze the unwinding of double-stranded DNA or RNA for a variety of functions through various mechanisms. Helicases are classified into six superfamilies (SF) through conserved sequences. The ATPase mechanisms of two contrasting helicases, one from the highly characterised SF1, and the other from the largest and most diverse family, SF2, will be compared. The SF1 bacterial helicase PcrA is a monomeric enzyme with a role in plasmid replication. This is one of the most characterised helicases and it is known to translocate single-stranded DNA (ssDNA) and move with discrete steps of one base per ATP. The SF2 RecG is a monomeric bacterial helicase which brings about replication fork reversal through the atypical translocation of double stranded DNA. Fork reversal allows repair of an ssDNA lesion which caused the replication fork arrest. A translocation step size of 2–4 bp per ATP has been observed for RecG by determination of the translocation kinetics with oligonucleotide junctions. A variety of biophysical techniques have been applied to determine the ATPase kinetic mechanism and the individual rate constants. This includes utilising fluorescent analogues of ATP, 2′ (3′)-mantATP/ADP (2′ (3′)-*O*-*N*-methylanthraniloyl-ATP/ADP), in rapid-reaction experiments, allowing the nucleotide binding and release kinetics to be explored. These analogues are further used for analysis of the hydrolysis step using quenched-flow measurements. Additionally, the fluorescent phosphate binding protein (MDCC-PBP) measures the phosphate release kinetics. Oxygen exchange experiments, using $^{18}$O-substituted ATP or phosphate, allow the kinetics of reversal of the hydrolytic cleavage step and phosphate binding to be probed.

# 1984-Pos Molecular Motor Translocation: Application of Monte Carlo Computer Simulations to Determine Microscopic Kinetic Parameters

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**Board B99**

The translocation of motor proteins along DNA or protein filaments is often studied by monitoring the total production of ADP or $P_i$ by the motor during translocation along the filament, the arrival/departure of the molecular motor at/from a specific position on the filament (often one end of the molecule), or the dissociation of the motor from the filament. Quantitative analysis of the kinetic time courses associated with these processes generally uses sequential "*n*-step" mechanisms to provide estimates of the magnitude and periodicity of the rate-limiting step in the translocation mechanism as well as the "kinetic step-size". Sequential *n*-step mechanisms assume a uniform repetition of irreversible rate-limiting steps, ignoring any non-uniform motion such as pauses or backwards motion. Using Monte Carlo computer simulations of translocation time courses, we examine here the effects of such non-uniform motion during translocation on the estimates of the macroscopic kinetic parameters (translocation rate and processivity) as well as the microscopic kinetic parameters (kinetic step-size and the rate constant for the rate limiting step) obtained from analysis using a simple *n*-step sequential mechanism. We further present a novel method of analysis using Monte Carlo computer simulations that has the potential to resolve whether non-uniform motion occurs during translocation. This approach is demonstrated for the analysis of the kinetic mechanism of single-stranded DNA translocation by the monomeric *E. coli* UvrD translocase.

# 1985-Pos Molecular Recognition of AT-Rich DNAs by HMGA2

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**Board B100**

The Mammalian high mobility group AT-hook 2 (HMGA2) is a transcriptional factor involved in cell differentiation and transformation. Disruption of its normal expression pattern is directly linked to oncogenesis and obesity. HMGA2 contains three "AT-hook" DNA binding motifs, which specifically binds to the minor groove of AT-rich sequences. Using a PCR-based SELEX procedure, we identified two consensus sequences for HMGA2: 5′-ATATTCGCGAWWATT-3′ and 5′-ATATTGCGCAWWATT-3′. These two consensus sequences have a unique feature: the first 5 bp are AT-rich, the middle 4 bp are GC-rich, and the last 6 bp are AT-rich. Our results showed that all three segments are critical for high affinity binding of HMGA2 to DNA. For example, if one of the AT-rich sequences was mutated to a non-AT-rich sequence, the DNA binding affinity of HMGA2 was reduced at least 100-fold. Intriguingly, if the GC-segment was replaced by an AT-rich segment, the binding affinity of HMGA2 was reduced approximately 5-fold. Identification of the consensus sequences for HMGA2 represents an important step towards finding its binding sites within genome. In this study, we also investigated enthalpy-entropy compensation of HMGA2 binding to different AT-rich DNAs using ITC. Two DNA hairpins were used: 5′-CCAAAAAAAAAAAAAAAGCCCCCGCTTTTTTTTTTTTTTT-3′ (FL-AT-1) and 5′-CCATATATATATATAGCCCCCGCTATATATATATATGG-3′ (FL-AT-2). Under a physiologically relevant condition (1×BPE and 200 mM NaCl), HMGA2 binding to FL-AT-1 is entropy-driven and to FL-AT-2 is entropy-driven at 25 °C. Interestingly, the binding free energies for HMGA2 binding to both AT-rich DNAs are almost independent of temperature; however, the enthalpy and entropy changes are highly dependent on temperature. The heat capacity change ($\Delta C_p$) for HMGA2 binding to AT-rich DNAs are almost identical (−350 cal/mol/deg), indicating that $\Delta C_p$ is a signature property for HMGA2 binding to DNA.

# 1986-Pos Energetics and Mechanisms of DNA Supercoil Relaxation by Human Topoisomerase I Revealed Through Molecular Dynamics Simulations

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**Board B101**

Topoisomerases are enzymes which play a crucial role in maintaining the topology and physical properties of DNA, which in turn affects essential cellular processes such as translation, transcription, and replication. Human topoisomerase I removes supercoil stress by breaking one phosodiester bond, covalently attaching to the 3′ end of the nicked strand, and allowing the downstream DNA to rotate followed by religation of the DNA backbone. Using molecular dynamics simulations and umbrella sampling we have calculated free energy profiles for the rotation of downstream DNA to mimic the release of both positive and negative supercoils which confirms previous computational and experimental work suggesting these mechanisms progress along distinct pathways. Additionally we have performed simulations with the ternary complex of topoisomerase, DNA, and the chemotherapeutic drug topotecan (which is thought to inhibit both strand rotation and religation) and we note important differences in the mechanisms for DNA relaxation in this system which could account for the decreased rate of supercoil relaxation observed in experiments. We also present evidence for a "semi-open" state which would facilitate rotations after the initial one as a result of biasing the protein into a conformation more favorable to strand rotation than the clamped state required for nicking of the DNA.

## 1987-Pos RNA-dependent RNA Polymerase Stalling On The Template RNA Molecules And Its Effects On The Elongation Rates

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**Board B102**

RNA-dependent RNA polymerases (RdRP) are responsible for the transcription and replication of the genomes of many RNA viruses that pose high health risks (e.g. hepatitis). In recent years, this class of nucleic acid polymerases has also emerged as an integral part of the RNA interference mechanism in some organisms. The viral RdRPs share many of the structural features with other types of nucleic acids polymerases. However, their ability to perform both the replication and transcription reactions sets them apart from more commonly known polymerases such as DNA-dependent RNA polymerases. We have applied single-molecule magnetic tweezers and bulk electrophoretic techniques to study the polymerization kinetics of the model RdRp from Bacteriophage Φ6. We have compared elongation rates when the RdRP was allowed to polymerize the complimentary strand with and without prior stalling. First, we show an efficient stalling of RdRP on the template strands provided that the enzyme is present in molar excess. However, the comparison of the elongation rates of the stalled and unstalled RdRP reveals that the stalling decreases the elongation rates of the viral RdRP by an order of magnitude. The fraction of the RdRP population slowed down by the stalling step depends on the concentration of the nucleotides present during the stalling step. In addition, the stalling of the RdRp on the template strand affected the temperature dependence of the RdRP elongation rates to make them insensitive to the temperature changes between 16 $^{o}$C and 30 $^{o}$C. We will comment on the generality of the stalling effect on the elongation rates of other polymerases and discuss the effect of stalling in the context of other experiments that require the stalling of nucleic acid polymerases.

## 1988-Pos Nucleic Acid Chaperone Activity of Retrovirus Nucleocapsid Protein Variants

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**Board B103**

The nucleocapsid protein (NC) plays an important role in the retroviral life cycle, in part, by facilitating numerous nucleic acid rearrangements throughout the reverse transcription process. The nucleic acid chaperone activity of the human immunodeficiency virus type-1 (HIV-1) NC has been extensively studied, and duplex destabilization, nucleic acid aggregation, and rapid protein binding kinetics have been identified as major components of the activity of this highly basic protein (pI ~10). The chaperone activity of other retrovirus NC, such as human T-cell leukemia virus type-1 (HTLV-1) NC, is less well understood. HTLV-1 NC protein possesses a unique C-terminal acidic domain and has an overall pI of ~7. We used bulk (gel-shift annealing, nucleic acid binding, and sedimentation) and single molecule (DNA stretching) techniques to characterize the activity of wild-type and C-terminally mutated HTLV-1 NC. In the single molecule experiments, protein-induced duplex destabilization was measured directly as an average decrease of the force-induced melting free energy, while NC's ability to facilitate strand annealing was determined by the amount of hysteresis in the DNA stretch-relax cycle. By studying different HTLV-1 NC mutants, the role of C-terminal domains to the chaperone activity was elucidated. Measurements of DNA force-induced melting showed significantly more rapid protein binding kinetics for some variant. In addition, bulk experiments showed that this mutant exhibited much stronger nucleic acid binding, aggregation, and annealing activities. These observations suggest that certain amino acid sequence on the carboxyl terminus of HTLV-1 NC strongly affect its nucleic acid binding properties and chaperone function. Investigation of NC protein of Rous sarcoma virus (RSV) and Moloney murine leukemia virus (MoMLV) indicates different Chaperone requirement of each replication system.

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## 1989-Pos Structural Analysis of the HSV-1 ICP27 N-terminal RNA binding motif

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**Board B104**

The Herpes Simplex Virus type 1 (HSV-1) immediate-early protein ICP27 is a multifunctional protein that is essential for lytic virus replication. ICP27 inhibits host cell splicing, prevents apoptosis and cell cycle progression while promoting the expression of viral early and late gene products. ICP27 preferentially binds HSV-1 intronless RNA through its N-terminal RGG box in vivo and promotes their export from the nucleus and interacts with several cellular and viral proteins. We have investigated the interactions of ICP27 with HSV-1 RNAs by EMSA and NMR. To identify native HSV-1 RNAs that interact with ICP27, overlapping 30mers corresponding to the HSV-1 glycoprotein C (gC) RNA, a late RNA that had been found previously to interact with ICP27 in UV cross linking experiments, were tested in EMSA experiments. ICP27 N-terminus was able to strongly shift several of these 30mers. HSQC NMR on the ICP27 N-

terminus alone showed that the N-H peaks are not well dispersed in the proton dimension, suggesting that the protein may not be rigidly folded. HSQC analysis performed in the presence of high affinity gC substrates, identified by EMSA, revealed some shifted peaks. However, the majority of peaks did not shift suggesting only a few residues are involved in gC binding. Interestingly, the same peaks shifted upon addition of several high affinity gC substrates, suggesting that a single binding site is interacting with the gC RNA. This is in agreement with EMSA competition experiments performed with the same gC sequences. 3D HSQC-NOESY analysis showed that most of the backbone N-H signals are exchanging with water and lack long range NOEs. However, 15N-NOE analysis revealed that most of the protein peaks give positive NOE signals, which is consistent with a compact state, not random coil or fully unfolded.

## 1990-Pos Dps from *M. smegmatis* : Molecular insight on protein *Surface to Interface*

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**Board B105**

We used frequency domain phase-modulation fluorescence spectroscopy and Förster Resonance Energy Transfer (FRET) to monitor the *in vitro* oligomeric switch of *M. smegmatis* Dps (Ms-Dps) protein, from a trimer to a dodecamer and to elucidate the structure of DNA-Dps dodecamer complex. The single mutated Cysteine at $169^{th}$ position is subsequently labeled with IAEDANS and FITC is placed at the N-terminus, as crystal structure of the protein reveals several side-chain interactions between these two termini, both exposed towards the "*surface*" of the protein. We have shown the N-$C_{169}$ distance distribution in the oligomers both in the presence and absence of DNA through discrete lifetime coupled with Maximum Entropy Method analysis. We have also shown a tandem linear array of Dps dodecamers over DNA with the help of time-resolved fluorescence anisotropy measurements. So far the novelty of Dps, as characterized by the DNA binding activity of the dodecameric form is confined on the surface of this spherically symmetric molecule. We are interested to look at the "*interface*" region, so as to construct some natural mutant(s) of Dps protein, *in vitro*, and isolate a trimer or a monomeric form and hence a protein incapable of binding to DNA. Several single and double amino acid mutants are constructed in the interface region with the help of a computational Graph Theory approach followed by Site Directed Mutagenesis. We are finally able to generate a single mutant, F47E, being unable to dodecamerize and highly stable as a trimer and a double mutant E146AF47E, which is a native monomer under high salt condition. A new dimeric interface, predicted from graph theory combining two trimeric interfaces is thought to provide significant contribution towards building up the "cage" like dodecameric structure.

## 1991-Pos Post-translation Modification Effects To The Histone Tail Dynamics

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**Board B106**

Nucleosome core particles (NCP) are the basic building blocks of chromatin. Each NCP includes histone proteins and nucleosomal DNA. The tail domains of histone proteins, which are referred to as 'histone tails', play important roles in gene regulation and nucleosome packing. The tail residues are subjected to post-translation modifications. In 'histone code' hypothesis, gene activation and repression depend on the nature of those modifications. Despite their significance, the dynamics of histone tails and the modification effects remain poorly understood.

In this study, all-atom molecular dynamics simulations are used to study the dynamics of histone tails at different modification states in NCP systems. The simulations reveal that histone tails are very mobile and have a tendency to bind to the NCP core region, especially to the nucleosomal DNA in a nonspecific and dynamic manner. The post-translation modifications affects the binding pattern and affinities of histone tails. Interestingly, the H3 N-terminal tails and H2A C-terminal tails all tend to bind to the DNA entry-exit site and the bindings are influenced by the modifications. This may have implications to the 'histone code' mechanism because the chromatin structures are influenced by the H3 and H2A tails. The changes of the chromatin will affect the access of other related co-factors. Additionally, the global motions of NCP are not affected by post-translation modifications significantly, according to the principal component analysis and normal mode study. This MD simulation study provides a clear picture of histone tail dynamics, as well as post-translation modification effects. Such information is very valuable for the understanding of nucleosome packing in chromatin and the mechanism of 'histone code'.

## 1992-Pos Structural Bioinformatics Modeling Of Gene Regulation Through Protein-DNA Complexes

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**Board B107**

Gene regulation is achieved by complex interactions involving protein-protein and protein-DNA interactions. Very often, gene regulation involves more than one transcription factors at the same time, or these transcription factors are binding other proteins. Here we present a structural bioinformatics approach to build the complexes that regulate gene expression. First we map gene regulation into protein-protein interaction maps. The protein interactions that include at least one that bind DNA will be selected. Then we build

the structure models of the proteins that are interacting use complex templates where available. Third, we map the DNA sequences on the DNA binding protein complexes. For those DNA binding proteins that are transcription factors, we apply the statistical potentials for TFBS prediction. These statistical potentials are built from experimentally identified protein-DNA X-ray structures. We have showed by combining the protein complex in gene regulation TFBS identification, the false positive rate is dramatically reduced, while improving the ranking of the true binding sites.

## 1993-Pos Influence Of HIV-1 Nucleocapsid Protein on Nucleic Acid Secondary Structure Invasion by Complementary Strands

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**Board B108**

The HIV-1 nucleocapsid protein (NC) catalyzes different strand-exchange reactions during reverse transcription. The role of NC in these processes is linked to its nucleic acid chaperone activity, by means of which nucleic acids are rearranged into their thermodynamically most stable structure. To probe NC's chaperone activity we used an optical assay based on quadruplex formation upon strand displacement. We investigated short DNA and RNA molecules having different secondary structures: duplexes containing mismatches, quadruplexes of different stability, and hairpins. The reactions were studied as a function of temperature, which revealed that strand replacement/invasion proceeds through different pathways: dissociative and sequential displacement. The former requires complete unfolding of initial secondary structure followed by rapid formation of product duplex, and the latter pathway assumes that the chase strand initially base pairs to the reactant molecule at the most unstable part followed by a fast displacement process. As expected, the activation energy of the sequential displacement pathway is lower than that of the dissociative pathway. NC generally accelerates the kinetics by 1–2 orders of magnitude, and lowers the activation energies by 10–25 kcal/mol.

## 1994-Pos A High-affinity Inner-sphere Divalent Cation stabilizes Conformational Change in the *B. subtilis* RNase P Holoenzyme·pre-tRNA Complex

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**Board B109**

Ribonuclease P (RNase P) is a ribonucleoprotein complex that catalyzes the 5′ maturation of precursor tRNA. The *B. subtilis* RNase P holoenzyme (E) consists of a catalytically-active RNA component and a small protein component that directly interacts with the 5′ leader of the pre-tRNA substrate and enhances metal affinity for catalysis. Fluorescence titration and transient kinetics data indicate conformational rearrangements occur both after pre-tRNA (S) association and prior to product dissociation. The conformational change in the RNase P holoenzyme·pre-tRNA (ES) complex is stabilized by one or more divalent cations (Ca(II) or Mg (II)) that can form inner-sphere complexes at μM concentrations. Time-resolved FRET experiments of the ES complex indicate that the conformational change upon addition of Ca(II) causes global movements of RNA domains. These data demonstrate that metal binding to the ES complex is coupled to a conformational change that enhances substrate affinity and organizes the active site in the ES complex. However, one or more divalent cations with lower affinity are required to activate catalytic activity.

## 1995-Pos Genetically Engineered M13 Bacteriophages as Standalone Tethers for Probing Single-Molecule Protein-DNA Interactions

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**Board B110**

Zinc fingers, the highly conserved, widespread, and multifunctional DNA binding domains for transcription factors, are paradigm proteins for studying protein-DNA interactions. Furthermore, as regulators of eukaryotic gene expression, the ability of these proteins to bind their complementary DNA substrate is crucial to cellular proliferation and development. Mutations in zinc finger proteins often lead to serious diseases, such as in the $Cys_2$-$His_2$ superfamily, which have been implicated in certain cancers, Huntington's disease, and hereditary neuropathies. The biochemical features of this superfamily of proteins have been well documented and their crystal structures solved. However, biophysical investigations on the single-molecule level are lacking, perhaps due to an inability to build single-molecule assays around them. We report the genetic engineering of M13 filamentous bacteriophage into a standalone tether, specifically by expressing the DNA binding domain of Zif268/Egr1 directly on coat proteins of one end and biotin acceptor peptide (BAP) on coat proteins of the other end. We then probed the binding interaction between Zif268/Egr1 and its 9 base-pair GC-rich DNA target in a single-molecule optical trap setup. Specifically, unbinding experiments between short DNA strands, affixed to coverslip surfaces with biotin-streptavidin linkages, and zinc fingers, coupled to polystyrene beads via the genetically customized M13 handle, were conducted. This scheme

allows us to build zinc finger binding specificity through high resolution measurements, with potential implications for gene therapy applications and a better understanding of transcription events involving low-level expression systems. Furthermore, our genetically encoded tether provides a testbed for mutation studies that could offer a glimpse of the single-molecule binding physics that underlie important disease states.

## 1996-Pos Building blocks of protein-RNA interactions

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**Board B111**

Prediction of protein-RNA interactions is crucial for our ability to interfere with processes such as gene expression and regulation. Here we investigate protein binding pockets that accommodate extruded nucleotides not involved in RNA base pairing. We observed that 86% of protein interacting nucleotides are part of a consecutive fragment of at least two nucleotides, whose rings have a significant interaction with the protein. Moreover, many of these share the same protein binding cavity and almost 30% are pi-stacked with each other. Consequently, we suggest that in many cases consideration of pairs of consecutive RNA nucleotides may be essential for the correct prediction of protein-RNA interactions.

We present a novel classification of known RNA nucleotide and dinucleotide protein binding sites and identify the common types of shared 3D physico-chemical binding patterns. These are recognized by a new classification methodology which is based on spatial multiple alignment. The shared patterns reveal novel similarities between dinucleotide binding sites of proteins with different overall sequences, folds and functions. We use these patterns for the prediction of the sequence and structure of RNA fragments that can bind to a protein of interest. Specifically, given a novel target protein we search it for regions similar to known binding patterns and achieve a success rate of 80%. By combining the dinucleotides bound to known patterns we obtain a prediction of a new RNA fragment which is useful for applications such as RNA aptamer design. In addition, by searching a database of all known small molecule binding sites for regions similar to nucleotide and dinucleotide binding patterns, we can suggest fragments and scaffolds that target nucleotide binding sites.

This project has been funded in whole or in part with Federal funds from the NCI, NIH, under contract number NO1-CO-12400.

## 1997-Pos Characterizing The Attachment Of A DNA Molecular Spring To A Protein

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**Board B112**

Protein-Nucleic Acid complexes play essential roles in important biological processes, such as nucleoprotein-primed viral replication and gene regulation and translation. Accordingly, artificial protein-DNA conjugates (Protein-DNA Chimera) could serve as powerful tools for the study of biological phenomena, and for the developments of new bio-techniques and smart drugs. Recently, Protein-DNA Chimeras were constructed to provide mechanical tension on proteins to allosterically control the conformation and thus the activity of proteins and enzymes.

In an effort to develop a standard protocol for constructing site-specific protein-DNA chimeras, we investigate quantitatively the factors, such as the rate of protein polymerization and the speed of crosslinker-protein coupling, affecting the conjugation. We also describe certain non-mechanical protein-DNA interactions for one specific chimera.

## 1998-Pos How Proteins Find And Recognize Their Targets On Dna

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**Board B113**

Protein-DNA interactions play a fundamental role in all biological processes. Proteins typically start its functioning after finding and recognizing specific sequences or sites on DNA. From experiments it is known that some proteins are capable to find their targets by 10–100 times faster than predicted by simple three-dimensional diffusion-limited rate. Current theoretical views suggest that protein molecules perform a facilitated diffusion search, moving part of the time along the DNA and part of the time in the bulk. However, this theoretical picture assumes that diffusion constants for 1D and 3D motions are of the same order, which contradicts latest single-molecule experiments. We present a theoretical approach which describes some aspects of the target search and recognition. In our approach the search process is viewed as a sequence of cycles, with each cycle consisting of 3D and 1D tracks. Our analysis shows that the acceleration in the search time can be reached by parallel scanning for the target by many proteins, and because of the increased local concentration of proteins due to nonspecific interactions with DNA. We also show how the complementarity of the charge patterns on a target sequence and on the protein may result in electrostatic recognition of a specific track on DNA and subsequent protein pinning. We estimate the depth and width of the potential well near the recognition region as well as the typical time that a protein spends in the well. Our theoretical analysis agrees with current experimental single-molecule observations and bulk chemical kinetic studies.

## 1999-Pos Searching and Binding of Nucleic Acid Fragments: Kinetics and Stretching

Buddhapriya Chakrabarti, Buddhapriya Chakrabarti, Tom Chou

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**Board B114**

How do short strands of nucleic acids recognize their complementary segments on long target strands? One proposed mechanism is that proteins slide along the target strand via one-dimensional diffusion. These studies have mostly neglected the role of sequence heterogeneity, kinetics of binding-unbinding of probe and target strands, and mechanical stretching in complementary search. In this talk we include these features in a model and compute the mean time taken by a probe strand to find its homologous pair on the target strand. Unlike protein-DNA binding, the search and recognition of short complementary strands depends on the mechanisms binding and unbinding of the strands and greatly influences the homology search time. We also find that stretching of base pairs due to protein binding e.g. RecA increases the effective target size and search efficiency.

## 2000-Pos The Sliding Rate of the Restriction Nuclease EcoRI along DNA

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**Board B115**

Many specific sequence DNA binding proteins locate their target sequence by first binding to DNA nonspecifically, then linearly diffusing along DNA until either the protein dissociates from the DNA or it finds the recognition sequence. Our extensive measurements of dissociation rates and specific-nonspecific relative binding constants of the restriction nuclease EcoRI enable us to determine sliding rate of the nonspecifically bound protein along the DNA from the ratio of dissociation rates of EcoRI from DNA fragments containing one and two specific binding sites. By varying the distance between the two binding sites we are able to confirm a linear diffusion mechanism. The sliding rate is relatively insensitive to salt concentration and osmotic pressure indicating the protein moves smoothly along the DNA certainly not 'hopping' on and off as has been suggested. EcoRI is able to diffuse an average of 100 base pairs along DNA in about 10 milliseconds. This rate is more than 100-fold slower than the diffusion of the free protein in water. Two possibilities could account for this activation energy barrier:

1. the salt bridges between the DNA and protein are transiently broken or
2. the water structure at the protein-DNA interface is disrupted as the two surfaces move past one another.

## 2001-Pos Direct Observation Of EcoRV Sliding And Jumping On Non-cognate DNA

Andreas Biebricher[1], Isabelle Bonnet[1], Claude Loverdo[2], Raphaël Voituriez[2], Olivier Bénichou[2], Wolfgang Wende[3], Alfred Pingoud[3], Pierre Desbiolles[1]

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**Board B116**

Many DNA-binding proteins are able to locate their target site within long non-cognate sequences faster than can be accounted for by simple diffusion models. Generally, this "facilitated diffusion" is attributed to a sliding mechanism, in which the enzyme binds nonspecifically to the DNA and diffuses to the target site in a 1D restricted motion. However, recent studies indicate that jumping, a mechanism involving enzyme detachment from the DNA and subsequent rebinding at a remote site after brief 3D diffusion, plays an important role in effective target search. We present data from single molecule experiments regarding the interaction of the restriction endonuclease EcoRV with non-cognate DNA. In our assay, we tracked the motion of fluorescently-labeled EcoRV along a DNA strand, which was attached to a glass surface specifically by its ends. Our results reveal that the facilitated diffusion of EcoRV comprises both sliding and jumping motion along the DNA. From our data, we obtained both the 1D diffusion constant and the jump length distribution of the enzyme on DNA. Comparison of the obtained distribution with theoretical calculations confirmed not only the 3D nature of the observed jumps, but indicated also the existence of a large number of small jumps undetectable by optical means.

In addition, recent experimental improvements will be presented that include labeling of the enzyme with semiconductor nanocrystals and the incorporation of double optical tweezers for DNA handling. This approach, which circumvents photobleaching and allows for a controlled DNA stretching, will be used to study the dynamics of specific site recognition by the enzyme.

## 2002-Pos Mechanism Of PKR Activation By dsRNA

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**Board B117**

PKR (protein kinase R) is a central component of the interferon antiviral defense pathway. Upon binding dsRNA, PKR undergoes autophosphorylation reactions that activate the kinase leading it to phosphorylate eIF2a, thus inhibiting protein synthesis in virally-infected cells. PKR contains an N-terminal dsRNA binding domain (dsRBD) and a C-terminal kinase domain. We have characterized the stoichiometries and affinities for PKR binding to a series of dsRNA sequences of increasing length and correlate RNA binding with enzymatic activation. In fluorescence anisotropy and sedimen-

tation equilibrium measurements performed at 200 mM NaCl, only a single PKR binds to a 20 bp dsRNA. A second PKR binds to the 30 bp sequence, and the stoichiometries increase with dsRNA length, consistent with a nonspecific binding mode with a site size of ~ 13 bp. At lower salt (75 mM NaCl), a second PKR binds to the 20 bp dsRNA. In autophosphorylation assays, the 20–25 bp sequences fail to activate, the 30 bp dsRNA activates weakly and peak activity increases with dsRNA length. Thus, the ability of dsRNAs to function as PKR activators is correlated with binding of two or more PKR monomers that are resistant to dissociation in 200 mM NaCl. Sedimentation velocity experiments indicate that PKR binds to the 20 bp dsRNA weakly, with Kd ~ 1 uM. Consistent with expected statistical effects, the Kd decreases to ~ 50 nM for the activating 30 bp dsRNA and a second PKR binds about 10-fold more weakly. Thus, PKR assembles on an activating dsRNA without apparent cooperativity. These data support a dimerization model for PKR activation, where dsRNA functions as a template to bring multiple PKR monomers into close proximity in a manner that is analogous to dimerization of the free enzyme that occurs at high protein concentrations.

## 2003-Pos Determination Of Structure And Dynamics Of The RepA-DNA Complex Using Fluorescence

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**Board B118**

Time-resolved and steady-state fluorescence studies on the replication initiator protein RepA from *Pseudomonas* are presented. This protein binds DNA either as dimer or as monomer, depending on the sequence of the cognate DNA. Effects of DNA binding on domain orientation and hydrodynamics are described. A strategy for a fast and general fluorescence anisotropy and FRET based method for the study of the three-dimensional organisation of protein-DNA complexes in solution is discussed.

## 2004-Pos Multiplexed single-molecule measurements of DnaB

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**Board B119**

The replicative helicase of E. coli, DnaB, is a ring-shaped hexameric motor protein capable of unwinding double-stranded DNA at a fork. It is thought to do this through 'steric-occlusion,' in which DnaB encircles and translocates along one single-strand of DNA, forcing the other single-strand (the occluded strand) to pass outside the ring. Using magnetic tweezers, we have performed single-molecule measurements of the unwinding activity of DnaB in which either the occluded or encircled strand is held under tension. We will report on progress of measurements of the velocity of the motor in each case, and interpret the results in terms of DnaB's possible translocation mechanism. Finally, to facilitate our data acquisition, we have developed a magnetic tweezer system in which multiple beads can be tracked at once, effectively allowing us to perform up to ~15 single-molecule measurements simultaneously. We will show how we have optimized the system for maximum data throughput while maintaining sufficient tracking accuracy.

## 2005-Pos Kinetic Mechanism of ssDNA translocation by E. coli RecB and RecBC

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**Board B120**

*E. coli* RecBCD is a bipolar DNA helicase consisting of two motor subunits with opposite translocation polarities: RecB (3′ to 5′) and RecD (5′ to 3′). In the holoenzyme, these two helicase subunits function in unison to unwind dsDNA in the same net direction processively by acting on opposite DNA ends. The RecBC enzyme, which possesses a single motor subunit, still functions as a DNA helicase. We have determined the minimal kinetic mechanism by which RecBC initiates DNA unwinding from DNA duplexes possessing preformed 5′-$(dT)_6$, 3′-$(dT)_6$ tails, the end structure to which RecBC binds with optimal affinity. These results indicate that RecBC unwinds DNA in a series of repeated rate limiting steps with equal rate constant $k_{obs}$ ($79 \pm 11$ $sec^{-1}$) during which an average of $m$ base-pairs ($4.4 \pm 0.1$ bp) are unwound ($mk_{obs} = 348 \pm 5$ bp/sec). Because translocation along the filament is essential for processive DNA unwinding, we have designed DNA substrates to examine the ssDNA translocation activities of both the RecB and RecBC enzymes. RecB single-turnover ssDNA translocation kinetic time-courses can be described by a simple $n$-step sequential mechanism in which on average $4.5 \pm 0.2$ nucleotides are translocated between successive rate liming steps with a stepping rate constant of $206 \pm 7$ $sec^{-1}$, resulting in a macroscopic translocation rate ($mk_{obs}$) of $934 \pm 10$ nt/sec. RecBC translocates along ssDNA with a similar rate ($mk_{obs} = 920 \pm 6$ nt/sec), which is about two times faster than the macroscopic DNA unwinding rate.

(This work is supported by NIH grant GM45948)

## 2006-Pos A Two-state Model For Helicase Translocation And Unwinding: Theory And Application To A Flashing-ratchet Mechanism For NS3 Helilcase

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**Board B121**

Helicases are molecular motors that unwind double-stranded nucleic acids. Typically a helicase translocates along one of the NA

single strands while unwinding. A focus of current helicase research is the coupling of the ATP hydrolysis cycle to helicase binding and motion. Here we develop a quantitative discrete model of unwinding by a helicase that can switch between two states, which could represent two different points in the ATP hydrolysis cycle. We calculate the speed of unwinding of the double-stranded NA and fluctuations around the average unwinding velocity. To test the model, we compare to the flashing-ratchet mechanism recently proposed for hepatitis C virus NS3 helicase. The model predictions capture some features of the data on NS3.

## 2007-Pos Kinetic mechanism of single-stranded DNA translocation by *E. coli* Rep and RepΔ2B monomers

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**Board B122**

The *E. coli* Rep protein is an SF1 DNA helicase with 3′ to 5′ single-stranded DNA translocase and double stranded DNA helicase activity. Rep is required for replication of bacteriophage ϕX174 and is involved in removing nascent lagging strands from stalled replication forks *in vivo*. Although a Rep monomer is a rapid and processive ss-DNA translocase *in vitro*, an oligomeric form of Rep is needed for processive helicase activity *in vitro.* Previously, the crystal structure of monomeric Rep bound to single-stranded DNA revealed two distinct conformations, differing by a ~130$^{o}$ rotation of its 2B subdomain about a hinge region connected to the 2A subdomain. Based upon this observation, it was proposed that the 2B domain might serve a role in Rep oligomerization. Based on crystallographic studies of two structurally similar SF1 helicases, UvrD and PcrA, it has been proposed that the 2B subdomains of PcrA and UvrD monomers facilitate DNA unwinding through direct interactions with duplex DNA. However, monomers of UvrD and PcrA are not processive helicases *in vitro,* and the 2B subdomain of Rep is auto-inhibitory for monomer helicase activity in that deletion of the 2B subdomain activates a limited helicase activity within the monomer. Towards elucidate the role of the 2B subdomain in Rep helicase and translocase activity, we have compared the single-stranded DNA translocase activity of both Rep and RepΔ2B monomers *in vitro*. Global nonlinear least squares analysis of full kinetic time-courses revealed that all three monomeric motors are rapid and processive ss-DNA translocases. Interestingly, the macroscopic translocation rate of RepΔ2B is two to three fold greater than for either Rep or UvrD monomers, hence deletion of the 2B subdomain increases the ss-DNA translocation rate suggesting that it may play a regulatory role.

## 2008-Pos Mechanism of NTP Hydrolysis by DnaC-DnaB Complex in Presence of Nucleic Acid

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**Board B123**

In *E. coli* cells, interactions between the primary replicative DnaB helicase and the essential replication factor DnaC protein are absolutely necessary for the initiation and elongation stages of DNA replication. However, in spite of the paramount role of the DnaC-DnaB interactions in the *E. coli* DNA replication little is known about the mechanism by which DnaC exerts this control. Here, kinetic mechanism of nucleotide hydrolysis by the DnaB helicase-DnaC protein complex has been quantitatively examined using the rapid quench-flow technique. As DnaC protein has adenosine specificity and any NTP binds to the DnaB helicase with similar affinities, we used CTP and GTP as cofactor to perform the experiment of the NTP hydrolysis by the DnaB helicase where the NTP was bound exclusively to the helicase hexamer of the DnaC-DnaB complex to see how NTPase active site of the DnaB helicase is affected by the DnaC protein binding to the helicase. Kinetic studies of NTP hydrolysis indicate that bimolecular association of CTP with the helicase active site is followed by the reversible hydrolysis of nucleotide triphosphate and subsequent conformational transition of the enzyme-product complex. Thus, the simplest mechanism, which describes the data, is a three-step sequential process. Analysis of relaxation times and amplitudes of the reaction allowed us to estimate all rate and equilibrium constants of partial steps of the proposed mechanism. Here we show the detailed study for NTP hydrolysis by the DnaB helicase in presence and absence of nucleic acid and then we also showed how the kinetic parameters (rate constants and partial equilibrium constants) are affected by DnaC binding to the DnaC-DnaB complex.

## 2009-Pos Kinetic Analysis of the DEAD-Box Protein A (DbpA) rRNA Helicase ATPase Cycle

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**Board B124**

The *Escherichia coli* DEAD-box protein DbpA is an ATP-dependent RNA helicase that catalyzes duplex RNA unwinding. The DbpA ATPase activity is specifically activated by 23S ribosomal RNA. The defined RNA specificity in the absence of accessory proteins makes DbpA an excellent model system to study the molecular function and mechanism of DExD/H proteins participating in RNA metabolism. In this work, we solved the complete enzymatic reaction scheme for DbpA ATPase cycling involving interactions among DbpA, RNA and nucleotides (ATP, ADP, ADP-$P_i$). The exact solutions from solving the enzymatic scheme were used to analyze and interpret the experimental data obtained from the steady-state ATPase, nucleotides (ATP, ADP) binding, ATP resynthesis, phosphate release and RNA binding measurements.

## 2010-Pos A Biophysical Characterization Of The Mechanism Of The DExH Helicase Nph-II

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**Board B125**

NPH-II is an essential RNA helicase involved in early transcriptional termination of Vaccinia virus. NPH-II tracks along the backbone of an RNA substrate in a processive, unidirectional 3′ → 5′ manner and efficiently strips away bound material from the tracking strand. Although a detailed kinetic framework for unwinding has been elucidated, a rigorous physical mechanistic understanding of NPH-II's helicase activity is still lacking. In particular the exact meaning of the "kinetic step size" obtained through traditional helicase unwinding assays remains ambiguous. Here we report the results of a modified approach to that recently developed by Serebrov and Pyle (*Nature* 2004, **430**, 476–480) that allows us to obtain site specific unwinding kinetics of product release and each translocation event. Implications for stepping behavior, initiation, translocation, duplex destabilization and product release are discussed.

## 2011-Pos Translocation of the NS3 Helicase from Hepatitis C Virus

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**Board B126**

The NS3 helicase, which is essential for Hepatitis C viral replication, is a member of the DExH proteins that constitute a subgroup of the Superfamily 2 helicases. Proteins from this superfamily are involved in all aspects of RNA metabolism. The NS3 helicase is a processive molecular motor that can unwind both RNA and DNA duplexes. It does so by utilizing the energy of NTP hydrolysis to translocate along its nucleic acid substrate. Our previous single molecule FRET study was designed to observe the relative motion of nucleic acid strands during unwinding have shown how DNA duplex separation is achieved in regular 3bp steps within larger periodic top strand release steps[1]. However, the motion of NS3 relative to its substrate remains unclear. Using site specific fluorescence labeling of the NS3 protein we have been able to monitor NS3 translocation directly in bulk and at the single molecule level on DNA and RNA to better understand the motion of NS3 relative to its tracking strand as well as the top strand. By correlating the motion of NS3 relative to its substrate with strand separation, our understanding of the overall mechanism of unwinding has become clearer.

### References

[1]. Myong et al. 2007 *Science*, ***317***(5837), 513–6.

**Protein-Nucleic Acid Interactions - II**

## 2012-Pos The ATPase Cycle Mechanism Of The DEAD-box rRNA Helicase Dbpa

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**Board B127**

DEAD-box RNA helicases ATPase enzymes that couple the free energy of ATP binding, hydrolysis and product release to the unwinding of duplex RNA. However, limited quantitative information regarding the rate and equilibrium constants defining the ATPase cycle of RNA helicases is available, including the distribution and flux of populated biochemical intermediates, the catalytic step that limits the enzymatic reaction cycle and how ATP utilization and RNA interactions are linked. We present in this study a complete, quantitative kinetic and equilibrium characterization of the rRNA-activated ATPase cycle mechanism of DbpA, a DEAD-box rRNA helicase implicated in ribosome biogenesis. rRNA activates the ATPase activity of DbpA by promoting ATP hydrolysis. ATP hydrolysis by DbpA-rRNA occurs via a gamma phosphate attack mechanism and is readily reversible. The rRNA-activated steady-state ATPase cycle of DbpA is limited *both* by ATP hydrolysis (or a step preceding) and $P_i$ release, which occur with comparable rates. Consequently, the predominantly populated biochemical states during steady-state cycling are the ATP- and ADP-$P_i$-bound intermediates. The ADP-bound states are favored under physiological nucleotide concentrations. The steady-state parameters ($k_{cat}$, $K_{m,RNA}$, $K_{m,ATP}$) predicted from the rate constants are comparable to the experimentally determined values. ATP or ADP and rRNA binding are weakly coupled. In contrast, ADP-$P_i$ and RNA binding display strong thermodynamic coupling which causes DbpA-ADP-$P_i$ to bind rRNA with $>$ 10-fold higher affinity than in the absence of nucleotide, suggesting that DbpA undergoes significant conformational change. The data are consistent with unwinding being coupled to strong rRNA binding. The quantitative knowledge of the DEAD-box helicase ATPase cycle is critical for developing mechanistic models of ATP utilization and helicase activity, and will help develop testable hypotheses regarding the cellular functions of these molecular motor enzymes.

## 2013-Pos Comparative Kinetic Studies on Three Forms of TATA-binding Protein Reveal the Nature of the Structural and Energetic Changes Associated with DNA Binding

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