1262-Pos Board B172
Investigating the Binding of Insulin to G-Quadruplex DNA by Differential Scanning Calorimetry
Christine Timmer, Kumar Sinniah.
The formation of guanine (G)-quadruplex in the guanine-rich tandem repeats of the insulin-linked polymorphic region (ILPR) is linked to transcriptional effects on the insulin gene. Recent studies demonstrate that these G-quadruplexes can bind insulin, and while this may impact the transcription of insulin, little is known about the binding mechanism. We have performed differential scanning calorimetry to characterize the binding interaction between insulin and G-quadruplex DNA. The consensus quadruplex ILPR sequence shows an unfolding temperature at 93.2 degrees celsius, while this peak is absent in a scrambled version of this sequence. The unfolding temperature of an insulin-G-quadruplex mix showed ~ 3 degrees celsius change in the Tm for the insulin peak in comparison with the unfolding of insulin alone, and a ~1 degree celsius change from insulin-scrambled DNA mix. These results provide additional support for the binding of insulin to G-quadruplex DNA. Experiments are currently in progress to measure the binding constant of this interaction by isothermal titration calorimetry.

1263-Pos Board B173
The RNA Chaperone Hfq Makes a Transient Ternary Complex with RNA Strands to Facilitate RNA Annealing
Subrata Panja, Julia Frances Hopkins, Sarah A. Woodson.
In bacteria, small anti-sense RNAs (sRNAs) help control the cell's response to environmental stress by binding complementary sequences in their mRNA targets. Hfq protein is required for the regulatory function of anti-sense sRNAs, and facilitates the formation of sRNA-mRNA complexes. Hfq is a ring-shaped homohexamer that preferentially binds single-stranded U-rich RNA on its proximal face and A-rich RNA on its distal face. Although Hfq has been shown to accelerate RNA base pairing and strand exchange in vitro, how Hfq does this is still unknown. To model the annealing mechanism of Hfq, we used a U-rich16-nt RNA (D16). Stopped-flow FRET measurements using fluorescein-labeled D16 RNA and Cy3-labeled Hfq showed that Hfq binds single-stranded RNA rapidly ($\geq 7 \bullet 10^7 M^{-1}s^{-1}$). Upon the addition of a complementary RNA, Hfq is rapidly released from the RNA complex ($k_{obs} \sim 30\ s^{-1}$). Stopped-flow experiments with a fluorescent molecular beacon showed that Hfq accelerates RNA annealing and strand exchange one hundred times. However, annealing is slower than Hfq release, suggesting the protein dissociates before the duplex is fully formed. Hfq mutations and competition experiments suggest that D16 RNA and the RNA beacon interact with the proximal and distal faces of Hfq, respectively, as expected. When a DNA beacon that binds Hfq weakly (1.4 μM) is used instead of an RNA beacon, Hfq-mediated RNA annealing is faster, but saturates at a higher concentration of Hfq. These results are consistent with rapid binding of RNA to the distal and proximal faces of Hfq, forming a transient ternary complex. We propose that Hfq leaves the complex before RNA hybridization is complete, after which the RNA strands form a stable duplex or dissociate and rebind Hfq as single-strands for the next round of annealing.

1264-Pos Board B174
The Role of Sm-Like Protein Hfq in RNA Annealing
Kevin W. Doxzen.
Small RNAs (sRNAs) regulate bacterial response and adaptation to environmental stress. This RNA regulation mechanism requires the Sm-like binding protein, Hfq, which promotes annealing of complimentary RNAs. The distal face of Hfq interacts with poly(A) sequences, while the proximal face interacts with poly(U) sequences. The sRNA DsrA binds to the *rpoS* mRNA, opening an inhibitory stem loop in the mRNA, and consequently initiating translation of the RpoS sigma stress response factor in *Escherichia coli*. Hfq binding to an $(AAN)_4$ repeat element upstream of the inhibitor stem loop on *rpoS* mRNA has been shown to be required to promote DsrA annealing. To further understand the role of Hfq in RNA annealing, we studied the formation of the ternary complex between Hfq, sRNA DsrA, and mRNA *rpoS* mRNA. Two mutations were chosen to disrupt RNA binding to either the proximal or distal face of the Hfq hexamer. Fluorescence Anisotropy experiments were used to study the binding affinity of Hfq mutants to poly(U) oligomers. Y25D, on the distal face of Hfq, showed only a two fold decrease in binding affinity; whereas K56A, on the proximal face, showed a 5-fold decrease. Native gel mobility shift assays showed that only the K56A mutant formed a ternary complex with DsrA and *rpoS* RNAs. In contrast, the Y25D mutation inhibits *rpoS* mRNA binding and also fails to form a ternary complex. These results support previous results showing that Hfq binding to A-rich sequences in *rpoS* mRNA is critical for *rpoS* regulation.

1265-Pos Board B175
Dynamic Competition for the Proximal RNA Binding Site of Hfq Drives Efficient Annealing of Small Noncoding RNA and mRNA
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Hfq, a bacterial RNA chaperone, stimulates specific RNA-RNA interactions by inducing RNA unwinding or RNA-RNA annealing. However, it is still unclear how Hfq can selectively achieve two completely opposite functions–annealing, and unwinding–depending on RNA substrates. Also, basic properties like an oligomeric state of an active form is under debate. Here we describe single-molecule fluorescence studies on Hfq-mediated annealing of DsrA, a small noncoding regulatory RNA of *E. coli*, and its mRNA target *rpoS*, which encodes the σ^Stranscription factor. Our results reveal that a single hexamer of Hfq stimulates DsrA-*rpoS* annealing by simultaneously binding both RNAs using same RNA binding site on the proximal surface of Hfq. The competition for the same binding site of the two RNAs makes the RNA-Hfq interaction dynamic, but drives more efficient annealing. When the Hfq-binding sequence exists only in one of the two RNAs, RNA unwinding can occur due to the reduced stability of the RNA caused by the partial unwinding of the RNA upon Hfq binding on the specific binding site.

1266-Pos Board B176
Probing the RNA Binding Surface of the HIV-1 Nucleocapsid Protein by Site-Directed Mutagenesis
Wei Ouyang, Philip N. Borer.
The HIV-1 nucleocapsid protein is highly conserved and plays roles in many stages of HIV infection. NC binds genomic RNA and distinguishes it from other RNA's in order to form new virus particles. NC recognizes a packaging signal known as SL3 in the 5'-UTR of genomic RNA. Previous NMR work on the NCp7-SL3 complex has provided detailed information, including the protein side chains that contact RNA. Site-directed mutagenesis was used to construct 18 mutants of NCp7 to eliminate RNA-side chain contacts or to switch salt-bridge partners. The protein variants were subjected to MALDI mass spectrometry and analytical ultracentrifugation for integrity and homogeneity checks. The dissociation constants of the mutants for SL3 RNA were measured using a Trp-fluorescence assay and/or isothermal titration calorimetry. A few of the variants we tested, such as F16A, showed dramatic reductions in affinity, and others showed moderate reductions, including I24A and Q45A. The affinity of most variants, including N5A, V13A and N17A, were not significantly affected by the mutation, even though the replaced side-chains are predicted to interact directly with RNA. The bridge-switch mutants showed small to moderate reductions in affinity, confirming that the residues involved contribute mainly to stabilizing the protein internal structure through salt bridges, and that the disposition of the side chains is less important. As most mutations have relatively minor effects on stability of the complex, we conclude that (1) In wild-type SL3-NC, many of the H-bond and steric contacts either contribute little to stability or can be compensated by new contacts in the mutants, and (2) Conservation of many of these residues arise from NC's interactions with other proteins or in other aspects of its many roles in HIV infection. Supported in part by NIH grant R01 GM32691.

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HIV-1 NC Protein Modifies Opening and Closing Kinetics of TAR RNA Hairpin
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Retroviral nucleocapsid (NC) proteins are nucleic acid chaperones that play a key role in the viral life cycle, including reverse transcription, where NC destabilizes the transactivation response RNA (TAR RNA) hairpin. To quantify the interaction of HIV-1 NC and TAR RNA, we used optical tweezers to exert tension upon the free ends of the individual TAR hairpin, forcing the hairpin open and then allowing it to close. In the absence of NC, the TAR hairpin opens and closes at forces that depend upon the hairpin stability, as well as the rate of pulling and relaxation. Surprisingly, once saturated with NC, the opening forces increase, suggesting an apparent stabilization of the structure. However, the higher opening rate in the presence of NC is only observed at high pulling rates, while extrapolation of the measured TAR opening rate vs pulling rate to infinitely slow pulling yields ~1000-fold faster opening with NC. This ability of NC to facilitate opening of the TAR hairpin is equivalent to ~4 kcal/mol decrease in the TAR opening barrier. The stronger pulling rate dependence of TAR opening in the presence of NC indicates that fewer TAR base pairs unzip prior to complete hairpin stem destabilization. Specifically, of the 24 TAR hairpin stem base pairs it is sufficient to unzip 12 bp in the absence of but only 6 bp in the presence of NC to cause complete TAR unfolding. This result quantitatively characterizes the ability of NC to moderately destabilize every nucleic acid base pair.