## Symposium 5: RNAs Large and Small

**1027-Symp**
**Principles of RNA Tertiary Structural Organization Revealed by Group II Intron Crystal Structures**
**Anna Pyle**.
Yale Univ, New Haven, CT, USA.
No Abstract.

**1028-Symp**
**Structural Insights into Metabolite-Sensing Messenger RNAs**
**Robert Batey**.
Univeristy of Colorado, Boulder, Boulder, CO, USA.
Riboswitches are structured elements found in the 5'-untranslated regions of mRNAs that directly bind a small molecule to regulate gene expression in a cis-fashion. These riboregulatory elements typically consist of two domains: an aptamer domain that binds a cellular metabolite and an expression platform that directs expression of the mRNA. Our laboratory is primarily focused upon understanding the three-dimensional structures of these mRNA elements in complex with their effector ligand and how this binding event leads to regulatory control. We have solved the X-ray crystal structures of the aptamer domains of a number of riboswitches in complex with their cognate ligands, each revealing a complex tertiary architecture that scaffolds the ligand-binding pocket. In each case, the RNA directly or indirectly senses almost all of the functional groups of the ligand, resulting in high binding specificity. Complementing these structures, we have used biochemical methods to probe the nature of the unliganded form of the aptamer domains, illuminating aspects of their ligand-dependent folding. These studies reveal that regulation is achieved through a series of ligand-induced tertiary structural changes in the RNA that serve to stabilize a helix that forms part of a secondary structural switch with the expression platform.

**1029-Symp**
**Biophysical Analysis of Gene Regulation Pathways Controlled by Bacterial Non-Coding RNAs**
Nilshad Salim, Dandan Li, Martha Faner, **Andrew L. Feig.**
Wayne State Univ, Detroit, MI, USA.
Small non-coding RNAs are transcribed in bacteria in response to a variety of cellular stimuli. These sRNAs often work in conjunction with the RNA binding protein Hfq to regulate gene expression, control stress response pathways, and modulate virulence. Using a combination of biophysics and genetics we have deconstructed these pathways and developed an integrated model for the molecular handoffs that control each step in the regulatory cycle. We will present our work on DsrA and OxyS, sRNAs associated with cold shock and oxidative stress respectively, as well as the mRNAs they regulate and the proteins they bind. While much of the work in this area has focused on post-transcriptional regulation of gene expression, our data lead us to propose a new model for these pathways that requires both co-transcriptional and post-transcriptional processes inducing structural rearrangements at both the RNA and DNA levels in response to these environmental stimuli.

**1030-Symp**
**Translation Factor Control of Ribosome Dynamics During Protein Synthesis**
**Ruben L. Gonzalez Jr.**.
Columbia University, New York, NY, USA.
Conformational rearrangements of the ribosome, its transfer RNA (tRNA) substrates, and its associated translation factors are hypothesized to play important mechanistic roles throughout all stages of protein synthesis. In order to determine how the structural dynamics of the translational machinery couple to the molecular mechanisms underlying protein synthesis, we are using single-molecule Förster resonance energy transfer (smFRET) to directly characterize these dynamics and their regulation during translation. Here we report a series of distinct smFRET signals which have allowed us to directly characterize the intrinsic conformational dynamics of a highly-mobile ribosomal domain, the L1 stalk, as well as the coupling between L1 stalk and tRNA dynamics, throughout protein synthesis. Our smFRET data reveal that the translating ribosome exists in a dynamic equilibrium between two global conformational states, global state 1 (GS1) and global state 2 (GS2), and that transitions between GS1 and GS2 involve coupled movements of the L1 stalk and the ribosome-bound tRNAs that are accompanied by a rotation of the small ribosomal subunit relative to the large subunit. Most importantly, we find that translation factors involved in the elongation, termination, and ribosome recycling stages of protein synthesis specifically recognize GS1 or GS2 and precisely regulate the rates of transitions between GS1 and GS2 as part of their mechanisms of action. Thus, our results support the view that specific regulation of the global state of the ribosome is a fundamental characteristic of all translation factors and a unifying theme throughout protein synthesis.

## Symposium 6: The Proton Gets Channeled

**1031-Symp**
**The Hv1 Voltage-Gated Proton Channel: Are Two Pores Better than One?**
**Francesco Tombola**.
Univ California, Irvine, Irvine, CA, USA.
Voltage-gated proton channels serve different functions in a variety of excitable and non-excitable cells, such as acid extrusion from neurons, muscles, and epithelial cells, and regulation of the NADPH oxidase in phagocytic cells. The first -and so far only- cloned voltage-gated proton channel, Hv1 (also known as VSOP), belongs to the family of proteins containing voltage-sensing domains (VSDs). In all other known channels of this family, the function of ion conduction is performed by a pore domain permeable to sodium, potassium or calcium ions. In Hv1, there is no pore domain, and the function of proton conduction is performed by the VSD itself. The Hv1 protein was recently shown to form dimers, in which each subunit has its own proton pore and gate, and the channel was found to function also when dimerization was prevented. This posed an interesting problem. If the two subunits of Hv1 can work separately, why do they form dimers? Do they gain any new functional feature by dimerization? Here, I will describe our attempts to answer these questions, focusing on the study of cooperativity between Hv1 subunits. I will also review our current understanding of the structural organization of Hv1 channels, and discuss the relationship between the gating of Hv1 and the gating of ion-conducting pores recently discovered in the VSDs of mutant voltage-gated potassium and sodium channels.

**1032-Symp**
**Voltage-Dependent Activation and Proton Permeation in Hv1**
**I. Scott Ramsey**.
Virginia Commonwealth Univ, Richmond, VA, USA.
The hallmark biophysical features of voltage-gated proton conductances ($G_{vH+}$) include exquisite $H^+$ selectivity and activation by both voltage and the transmembrane pH gradient. Heterologous expression of the voltage sensor domain (VSD) protein Hv1 is sufficient to reconstitute $G_{vH+}$, and the *Hvcn1* gene that encodes Hv1 is required for $G_{vH+}$ and murine phagocytic leukocytes. Hv1 is therefore likely to represent the long-sought molecular correlate of $G_{vH+}$. In contrast to tetrameric voltage-dependent cation channels, each VSD subunit forms a distinct $H^+$ permeation pathway in dimeric Hv1 channels. Rather than mediating transmembrane ion flux by dehydrating permeant ions in a pore domain selectivity filter, Hv1is likely to achieve rapid proton-selective transmembrane $H^+$ transfer by a Grötthuss-type hydrogen-bonded chain (HBC) mechanism. In order to investigate the mechanism of proton permeation in Hv1, we performed charge-neutralizing mutagenesis of candidate $H^+$-titratable residues that we expected would be required for formation of the HBC. Whereas mutations of conserved acidic and basic residues in Hv1 dramatically affected both voltage- and pH gradient-dependent activation, we were unable to identify any single neutralizing mutation that was sufficient to abrogate expressed $H^+$ currents. Our data therefore suggest that $H^+$-selective permeation in Hv1 is likely to require $H^+$ transfer in a water wire rather than by side-chain titration. We interpret our mutagenesis data in the context of an Hv1 open-state homology model and describe a hypothetical mechanism for $H^+$ permeation in Hv1.

**1033-Symp**
**Structure and Mechanism of Influenza Proton Channels**
**James J. Chou**[1], Rafal M. Pielak[1], Jason R. Schnell[2], Junfeng Wang[1].
[1]Harvard Med School, Boston, MA, USA, [2]University of Oxford, Oxford, United Kingdom.
pH-activated, proton selective channels are required during viral entry and replication of influenza viruses. We determined the high resolution structures of

proton channels from influenza A and B viruses by solution NMR spectroscopy. The channel structures reveal pore features that are important for proton gating and proton relay. Structural details of the anti-influenza drug, rimantadine, bound to the channel suggests an unexpected allosteric mechanism of drug inhibition and drug resistance, which has been verified by thorough functional and mutagenesis experiments.
The work is supported by NIH grant AI067438.

**1034-Symp**
**Proton Transport Through Channels: Insights and Surprises from Molecular Simulation**
**Gregory A. Voth**.
University of Utah, Salt Lake City, UT, USA.
The behavior of hydrated excess protons passing into and through transmembrane proton channels will be presented, based on the results of a novel multi-scale computer simulation methodology. The unique electrostatics related to the dynamic delocalization of the excess proton charge defect will be elaborated, as well as its effect on the channel proton transport and selectivity properties. The often opposing and asymptotic viewpoints related to electrostatics on one hand and Grotthuss proton shuttling on the other will be reconciled and unified into a single conceptual framework. Specific simulation results will be given for various channel systems, including the M2 channel of influenza A, proton selective mutant aquaporin-1 channels, the ClC $Cl^-/H^+$ antiporter, and models of the Hv1 voltage gated proton channel. Comparison to experimental results will be discussed where possible.

## Platform L: Protein Folding Pathways

**1035-Plat**
**Protein Folding: Independent Unrelated Pathways or Predetermined Pathway with Optional Errors**
**Krishna Mallela**[1], S. Walter Englander[2].
[1]University of Colorado Denver, Aurora, CO, USA, [2]University of Pennsylvania, Philadelphia, PA, USA.
There is a fundamental conflict between two different views of how proteins fold. Kinetic experiments and theoretical calculations are often interpreted in terms of different population fractions folding through different intermediates in independent unrelated pathways (IUP model). However, detailed structural information indicates that all of the protein population folds through a sequence of intermediates predetermined by the foldon substructure of the target protein and a sequential stabilization principle. These contrary views can be resolved by a predetermined pathway_optional error (PPOE) hypothesis. The hypothesis is that any pathway intermediate can incorporate a chance misfolding error that blocks folding and must be reversed for productive folding to continue. Different fractions of the protein population will then block at different steps, populate different intermediates, and fold at different rates, giving the appearance of multiple unrelated pathways. A test of the hypothesis matches the two models against kinetic folding results for two proteins, hen lysozyme and staphylococcal nuclease, which have been interpreted previously in terms of independent parallel pathways. Folding kinetics of both proteins fit equally well to the two models, indicating that the measured experimental data does not require alternative parallel pathways. The fitted PPOE reaction scheme leads to known folding behavior, whereas the IUP properties are contradicted by experiment. The appearance of a conflict with multipath theoretical models seems to be due to their different focus, namely on multitrack microscopic behavior versus cooperative macroscopic behavior. The integration of three welldocumented principles in the PPOE model (cooperative foldons, sequential stabilization, optional errors) provides a unifying explanation for how proteins fold and why they fold in that way.

**1036-Plat**
**Multiple Routes and Milestones in the Folding of HIV-1 Protease Monomer**
**Massimiliano Bonomi**[1], Alessandro Barducci[1], Francesco L. Gervasio[2], Michele Parrinello[1].
[1]ETH Zurich, Lugano, Switzerland, [2]Spanish National Research Centre, Madrid, Spain.
Evolution has lead proteins to display funneled energy landscapes with small degrees of ruggedness. However, a funneled landscape does not preclude the presence of multiple kinetically relevant folding routes. Here we show that for an extremely relevant biological case, the monomer of HIV type 1 protease (HIV-1-PR), multiple pathways and milestones can coexist along the folding process.
We provide a comprehensive picture of the folding mechanism of HIV-1-PR monomer using a variety of theoretical and computational techniques. These include all-atom molecular dynamics simulations in explicit solvent, an analysis of the network of structure clusters found in multiple high-temperature unfolding simulations and a complete characterization of the free energy surface carried out using an all-atom structure based potential and a combination of metadynamics and parallel tempering.
Our results confirm that the monomer in solution is stable and show unambiguously that at least two (un)folding pathways exist. Moreover, we demonstrate how the formation of a hydrophobic core can be considered a milestone in the folding process which must occur along all the routes that lead towards the protein's native state. These results also provide a theoretical framework that is able to rationalize both the experimental evidences and the evolutionary data for HIV-1-PR monomer. Finally, our characterization of the ensemble of possible folding routes substantiates a rational drug design strategy based on inhibiting the folding of each of the subunits that build the HIV-1 protease homo-dimer.

**1037-Plat**
**Characterizing Energy Landscapes of Proteins and Identifying Shape-Determining Factors**
Yue Li, Gary Tyson, **Jinfeng Zhang.**
Florida State University, Tallahassee, FL, USA.
Protein folding is a long standing problem in biology, whose mechanism is still not completely understood. Funnel-shape energy landscape has been proposed as a plausible folding mechanism. However, the factors that determine the funnel-shape energy landscapes is largely unknown. In this study, we use hydrophobic-hydrophilic (HP) model to investigate the factors that affect the funnel-shape of protein energy landscapes. We designed a clustering method based on graph theory to analyze the conformations sampled using a recently developed Monte Carlo method, FRESS. We found that the way conformations move from one to another defined by a particular sampling method (move set) has a significant effect on the shape of protein energy landscapes. To our surprise, both protein-like sequences and random sequences with around 50% hydrophobic residues have a stable state represented by a single dominant cluster, consisting of a large number of similar conformations. The energy landscapes resemble a funnel, where there are many paths to minimum energy conformations in the dominant cluster from conformations of higher energies. We also found that sequences with hydrophobic residues above or below the optimal range around 50% do not have a single stable state. In stead, there are many much smaller clusters, representing multiple local energy minima. Our finding is consistent with the compositions of hydrophobic and polar residues in globular proteins (fold to unique structures) and intrinsically disordered proteins (IDPs). Our study suggests that in computational simulations, move sets affect significantly the shape of protein energy landscapes; hydrophobic interaction is likely a major force leading to the funnel-shape energy landscape of proteins; and the composition of hydrophobic and polar residues is an important sequence feature for the formation of funnel-shape of protein energy landscapes.

**1038-Plat**
**Common Folding Mechanism of a Peptide Revealed by Multiple MD Simulations**
**Lipi Thukral**[1], Jeremy C. Smith[2], Isabella Daidone[3].
[1]University of Heidelberg, Heidelberg, Germany, [2]Oak Ridge National Laboratory, Oak Ridge, TN, USA, [3]University of L'Aquila, Via Vetoio, Coppito (AQ), Italy.
Here, we report on the folding of a 15 residue beta-hairpin peptide (Peptide 1) using multiple unbiased, atomistic molecular dynamics (MD) simulations. Fifteen independent MD trajectories, each 2.5 microseconds-long for a total of 37.5 microseconds are performed in explicit solvent, at room temperature and without the use of enhanced sampling techniques. The computed folding time of 1-1.5 microseconds obtained from the simulations is in good agreement with experiment. A common folding