state and may interact with the inactivated states of Nav1.7 and Nav1.8 channels. The state-and use-dependent modulation of hNav1.7 and rNav1.8 Na^+ channels by ranolazine could lead to an increased effect of the drug at high firing frequencies, as in injured neurons.

67-Plat
Insecticide Binding to Voltage-gated Sodium Channels
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DDT and the pyrethroid class of insecticides target voltage-gated sodium channels. Their binding stabilises the channel open state, inducing prolonged tail currents associated with insect paralysis ('knockdown') and death. Target-site mutations conferring resistance, while a challenge for pest control programs, have provided valuable information on the location of the elusive insecticide-binding site. Homology modelling of the housefly sodium channel and automated ligand docking studies have identified a binding site consistent with resistance-associated mutagenesis data, structure-activity relationships of insecticides and their state-dependent binding activity [O'Reilly et al (2006) Biochem. J. 396:255-263]. The putative binding site, delimited by the domain II S4-S5 linker, S5 & S6 helices and domain III S6 helix, interfaces the lipid bilayer and is therefore accessible to lipid-soluble insecticide ligands. The model is supported by recent experimental results from voltage-clamp electrophysiology studies on mutant fruitfly sodium channels [Usherwood et al (2007) FEBS Letters 581:5485-5492]. The mutation T929I, predicted to inhibit DDT binding through steric hindrance, abolishes the effects of DDT on channel activity. M918, a residue predicted to form a binding contact with pyrethroids but not DDT, decreased deltamethrin potency without effecting DDT potency when mutated. We have developed methods based on circular dichroism spectroscopy to identify ligand binding to a non-insect channel system, namely the voltage-gated sodium channel NaChBac from *Bacillus halodurans* [Nurani et al (2008) Biochemistry 31:8114-8121], which will be used as a further test of the binding of various insecticides as predicted by the model.
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Symposium 3: The Biophysics of HIV

68-Symp
Nucleic Acid Chaperone Activity of Retroviral Gag and Nucleocapsid Proteins
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Retroviral reverse transcription involves multiple nucleic acid rearrangements catalyzed by the nucleocapsid protein (NC). Ensemble and single-molecule studies have been used to gain mechanistic insights into the chaperone activity of retroviral NC proteins. The Gag polyprotein also appears to be a nucleic acid chaperone protein, a property that is likely to facilitate RNA genome dimerization and tRNA primer annealing. Using point mutations, truncated constructs, and individual domains of Gag, we have investigated the role of Gag's structural domains in chaperone function. HIV Gag mediates tRNA annealing at a reduced rate relative to NC. The NC domain is essential for Gag-mediated annealing, while the matrix (MA) domain appears to inhibit Gag's chaperone activity. Interestingly, inositol phosphates (IPs), which are known to bind to basic residues within MA and facilitate Gag particle assembly *in vitro*, stimulate the chaperone activity of Gag. Stimulation by IPs was shown to depend on the presence of MA residues K30 and K32, and the maximum effect was achieved at a 1:1 Gag:IP ratio. Taken together with previous data, these results suggest that IP or membrane binding by MA results in a conformational switch that stimulates Gag's ability to facilitate annealing of the tRNA primer. This work was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

69-Symp
Assembly of Ribonucleoproteins Involved in Viral RNA Trafficking
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Oligomerization of the HIV-1 protein Rev on the Rev Response Element (RRE) regulates nuclear export of genomic viral RNA and partially spliced viral mRNAs encoding for structural proteins. Single-molecule fluorescence imaging has been used to dissect the multi-step assembly pathway of this essential ribonucleoprotein under physiologically relevant conditions, revealing dynamic intermediates and the mechanism of assembly. Assembly is initiated by binding of Rev to a high-affinity site in stem-loop IIB of the RRE and proceeds rapidly by addition of single Rev monomers, facilitated by cooperative Rev-Rev interactions on the RRE. Dwell time analysis of fluorescence trajectories recorded during individual Rev-RRE assembly reactions has revealed the microscopic rate constants for several of the Rev monomer binding and dissociation steps. The high-affinity binding of multiple Rev monomers to the RRE is achieved on a much faster time scale than reported in previous bulk kinetic studies of Rev-RRE association, indicating that oligomerization is an early step in complex assembly. In addition to Rev, a variety of cellular proteins are also required for nuclear export of the viral mRNA. Hence, the single-molecule imaging system has also been used to monitor Rev-RRE complex assembly in the presence of selected cellular cofactors.

70-Symp
Insights Into The Mechanism Of Retroviral Genome Packaging And Assembly
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In HIV-1 infected cells, newly synthesized retroviral Gag polyproteins are directed to specific cellular membranes where they assemble and bud to form immature virions. Membrane binding is mediated by Gag's matrix (MA) domain, a 132-residue polypeptide containing an N-terminal myristyl group that can adopt sequestered and exposed conformations. Membane specificity was recently shown to be regulated by phosphatidylinositol-(4,5)-bisphosphate ($PI(4,5)P_2$), a cellular factor abundant in the inner leaflet of the plasma membrane (PM). We now show that phosphoinositides, including soluble analogs of $PI(4,5)P_2$ with truncated lipids, bind HIV-1 MA and trigger myristate exposure. The phosphoinositol moiety and one of the fatty acid tails binds to a cleft on the surface of the protein. The other fatty acid chain of $PI(4,5)P_2$ and the exposed myristyl group of MA bracket a conserved basic surface patch implicated in membrane binding. Our findings indicate that $PI(4,5)P_2$ acts as both a trigger of the myristyl switch and as a membrane anchor, and suggest a structure-based mechanism for the specific targeting HIV-1 Gag to $PI(4,5)P_2$-enriched membranes. Retroviral genomes contain elements within their 5′-untranslated regions (UTRs) that regulate multiple essential functions, including splicing, nuclear export, translational activation, genome packaging, and reverse transcription, among others. A number of studies suggest that these processes may be differentially regulated by RNA conformational changes. To gain insights into the structural basis for these processes, we have initiated NMR studies of intact retroviral packaging elements, including the native, dimeric 200 nucleotide core encapsidation signal (Ψ^{CES}) of the Moloney murine leukaemia virus (MLV) and the intact, dimeric 748 nucleotide 5′-UTR of the human immunodeficiency virus Type-1 (HIV-1). Progress toward the implementation of these data as restraints for structure refinement of the dimeric MLV Ψ^{CES} will be presented.

71-Symp
Mechanistic Studies of HIV Budding
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The HIV Gag protein coordinates viral trafficking, membrane binding, assembly, cofactor packaging, budding, and maturation. Late in the infectious cycle, Gag assembles on plasma membranes and forms enveloped particles that bud through the membrane. Efficient HIV budding depends on the actions of at least two cellular proteins that bind directly to conserved elements within the C-terminal p6 region of Gag: TSG101 and ALIX. Both of these proteins normally function as part of a multi-cellular pathway termed the, ESCRT pathway (Endosomal Sorting Complex Required for Transport). In the cell, the ESCRT pathway helps to sort ubiquitylated protein cargos into vesicles that bud into late endosomal multivesicular bodies (MVB), and also helps mediate the final step of cytokinesis (called abscission). Thus, HIV and many other enveloped RNA viruses have evolved to usurp the cellular ESCRT pathway and utilize its intrinsic membrane remodeling activities to bud from cells.
Recent studies have suggested that late-acting ESCRT pathway factors, including subunits of the ESCRT-III complex and the AAA ATPase, VPS4, may constrict the neck of the budding vesicle and/or mediate membrane fission. I will review evidence suggesting that ESCRT-III subunits can assemble into ''rings'' that surround the necks of budding particles, and then describe our structural, biophysical, and biochemical studies that indicate how: 1) ESCRT-III proteins change conformation as they are deposited from the cytoplasm onto the membrane, 2) ESCRT-III proteins bind and recruit VPS4 ATPases, and 3) VPS4 complexes assemble and act on their ESCRT-III substrates. These studies, together complementary studies from other laboratories, are providing a framework for understanding the mechanics of HIV budding.