3192-Plat
Improving Protein Structure Prediction by Smoothing Energy Functions
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We present a method for post-processing protein structure predictions by smoothing the free energy landscape. This method captures the idea that similar structures should have similar scores. We smooth the energy landscape by solving the diffusion equation on the free energy surface implicitly defined by the structural ensemble. By smoothing the energy landscape we improve the correlation between energy and RMSD. This method typically allows us to choose better structures than using the scoring function directly.

3193-Plat
DE/SERVED Protein Analysis: Micro Scale Detection of Subtle Protein Stability Changes Using a Fluorescent Reporter
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Detection of protein 3D structural changes normally requires the use of advanced and expensive biophysical equipment. We are developing ways to make simple analysis available to a wider community. The aim is to detect changes in the relative foldedness of samples such as mutant or modified proteins so that the decision can be made whether a more detailed biophysical analysis is needed. We present a Simple, Rapid, Small volume Detection (SERVED) method to investigate small changes to a protein's non covalent structure. The method combines the well established use of fluorescent dye binding to detect the destabilisation of the protein structure with modern low sample volume fluorescent methods. We demonstrate that using 2 ul samples different structural states can be easily differentiated within five minutes. Using surface mutations of the pore forming domain of colicin A we show that mildly destabilising mutations can be easily differentiated from wild type. Furthermore differences in the stability of mutant thermophilic proteins, which are difficult to measure by normal means, become clearer by this method. Finally differences in natively unfolded proteins, otherwise visible by AUC or HSQC-NMR, are shown to provide small but significant signals in this assay. Finally, in Depth Exploitation of the SERVED-analysis (DESERVED) can generate relative stability profiles of protein samples for side-by-side comparison up to ten times quicker and using ten times less protein than standard biophysical analyses.

3194-Plat
Interactions between Phospolipid Membranes and SOD1 Protein: Effect of Charge Changing fALS Mutations
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The neurodegenerative disease amyotrophic lateral sclerosis (ALS) is closely connected to point mutations scattered across Cu/Zn superoxide dismutase (SOD1) protein, whose conversion into misfolded aggregates is a hallmark of familial ALS. Here, we explore the impact of net charge changing SOD1 mutations on their ability to interact with mitochondrial membranes and the consequences for their folding behaviour. Using biophysical methods we monitored the conformational changes of the wildtype SOD1, and SOD1 mutations with increasing and decreasing net charge species, in their respective metal-free, monomeric apo-statse in the presence of increasing amounts of negatively charged model lipids bilayers.. The results clearly indicate an electrostatically driven association process, where the binding event induces a pronounced increase in the helical character of the pWT and the SOD1 mutants, in correlation with their respective charge and protein stability. Nevertheless, how charge mutations influence the stability of different structural intermediates of the various SOD1 species and their ability to interact with mitochondrial membrane is a very complex process with yet unknown consequences for the molecular mechanisms behind familial ALS disease.

Platform AX: Membrane Fusion II

3195-Plat
Diverse Enveloped Viruses Fail to Undergo Complete Fusion with the Cell Plasma Membrane
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Recent work from our laboratory (Miyauchi et al. 2009, Cell 137:433-444) provided functional evidence for HIV-1 entry via an endocytic pathway. In contrast, HIV-1 fusion with the plasma membrane (PM) did not proceed beyond the lipid mixing step. We have therefore assessed the ability of other enveloped viruses to fuse with the PM of permissive cells. Imaging of single amphotropic Murine Leukemia Virus particles revealed complete endoplasmic fusion with occasional lipid mixing at the PM. Next, we tested whether low pH-dependent viruses can be forced to fuse with the PM by lowering the external pH. When retroviral cores pseudotyped with the Semliki Forest Virus E1E2 glycoproteins were bound to cells and exposed to acidic pH, lipid mixing, but not viral content transfer was observed. Likewise, Avian Sarcoma and Leukosis Virus (ASLV) that enters target cells via a receptor-mediated endocytosis followed by low pH-induced fusion with endosomes failed to release its content at the PM upon reducing the pH. These results demonstrate a surprisingly strong preference for endosomal fusion among pH-dependent and pH-independent viruses, suggesting the existence of a fusion block at the PM and/or the involvement of an endosomal factor(s) facilitating the viral fusion. Consistent with the latter mechanism, inhibition of the dynamin function blocked endosomal fusion of HIV-1, ASLV and several other viruses. Together, these results are consistent with the involvement of cellular factors in facilitating the viral entry via endosomes. Supported by NIH grants GM054787 and AI053668.

3196-Plat
Disseminating the HIV Fusion Mechanism: gp41 Structural Details in Membranes
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Membrane fusion is the initial step of HIV infection, and is carried out by the viral gp41 protein through direct interaction with target cells at physiologic pH and conformational change. The N-terminal fusion peptide (FP) region of gp41 is involved in both target membrane insertion and perturbation, and its trimeric assembly and depth of membrane insertion have been correlated with fusion fitness. Key gp41 fusion conformations include early and late, and are characterized by trimeric coiled-coil and low energy six-helix bundle structure, respectively. Recent findings point to early gp41 as fusion active, with late gp41 involved in membrane bilayer stabilization. We present solid-state NMR structural analysis of constructs modeling early and late gp41 fusion conformations in membranes which suggests that specific FP structure is not correlated with gp41 fusion action or inaction, and that fusion arrest may be due to withdrawal of the FP from membranes for the late gp41 six-helix bundle conformation. Specifically, we observe populations of helical and β-sheet FP structure for both early and late gp41. β-sheet FP structure in early and late gp41 is predominant and analogous to structure observed in minimal FP fragments, with antiparallel strand arrangement crossing at approximately Leucine-7. Helical structure in coiled-coil and six-helix bundle regions is preserved upon membrane interaction, and induces partial helical structure in downstream FP for early and late gp41, respectively. FP structure is sensitive to membrane cholesterol only in early fusion active gp41, which suggests FP insertion into membranes as shown for minimal FP fragments, and lack of FP insertion for late, fusion inactive gp41. Additional solid-state NMR findings are presented examining the membrane location of the gp41 apolar FP region. Our structural analysis provides new insight into the mechanism of membrane fusion induced by HIV gp41.

3197-Plat
Point Contact between Membranes Precursory to Fusion
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Membranes do not fuse easily under normal circumstances. Spontaneous fusion between membranes is prevented by energy barriers. The role of fusion proteins is to lower these barriers at the appropriate time and place. One much discussed barrier is that for the initial contact between bilayers, precursory to hemifusion. It has been speculated some specific actions by proteins (e.g. creating nipples or micro-protrusions) is required for this initial contact to occur, in addition to the force bringing two membranes into close proximity. Since the main energy barrier for a contact between two lipid bilayers is the hydration force, we applied osmotic pressure to a stack of multiple-bilayers and observed the consequence on the bilayer configuration by X-ray diffraction. We found a new lipid phase between the lamellar phase and the stalk (rhombohedral) phase discovered in 2002. The new phase was tetragonal symmetry. The resulted electron-density distribution (Figure) showed two adjacent lipid bilayers curved toward each other to form point contact. Lipid bilayers abruptly transformed to this point contact from a parallel configuration when the P-P distance was 1.20nm, right before the phase transition. No specific force is necessary to create curvature for a point contact.