Quite surprisingly, N- and the C-termini remained in close proximity at high denaturant concentrations for ca. 40% of the conformations, suggesting that DrkN-SH3behaves at least partially like a disordered circular chain.
Reference:
1. J.A. Marsh and J.D. Forman-Kay, Proteins 80(2):556-572 (2012)

979-Plat
De Novo Generation of Bound Structures for an Intrinsically Disordered Protein using Only Alpha Carbon Chemical Shifts
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Intrinsically disordered proteins (IDPs) challenge our traditional notions of protein structure/function relationships because they are highly dynamic in their native state and do not form tertiary structures. To design new drugs for IDPs it is essential to generate realistic structural ensembles for these proteins. We have recently developed a method called broad ensemble generation with re-weighting (BEGR) where a million or more diverse IDP structures are generated and then re-weighted to fit experimental data. Results show that bound state structures of the disordered p53 transactivation domain (p53TAD) are reproducibly generated, even though the BEGR method is using experimental data for unbound p53TAD. These bound state structures were found for wild type p53TAD and a mutant that increases the transient helical secondary structure of one of the binding sites using only the alpha carbon chemical shift data.

980-Plat
Conditions for Stabilizing Structure in an Intrinsically Disordered Peptide under Confinement: A Molecular Dynamics Study
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Over the last few decades, the discovery of intrinsically disordered proteins (IDPs) has challenged conventional wisdom by demonstrating that they play biologically important roles while not holding any well-defined structure. The experimental characterization of their behaviour is complicated by the fact that they do not have a folded native state. In addition, an understanding of the mechanisms by which they operate in vivo is further obscured by the fact that cellular environments are confined spaces in which they perform their function: a daunting problem since even the folding of regular proteins in confined environments is still not yet fully understood. Here, we address the dynamics and possible structure stabilization of an IDP under confinement by using all-atom molecular dynamics simulations in explicit water to study the sporadic formation of secondary structure in the decapeptide fragment of the full-length amyloid β-protein (implicated in Alzheimer's disease), the Aβ(21-30) decapeptide. Metastable β-hairpin structures found in this decapeptide, and characterized by a lifetime in bulk water, are shown to become more stable or unstable when confined inside small pores with either polar or nonpolar surfaces. For progressively smaller pores the stability of these β-hairpin structures is shown to depend on the nature of this surface rather than on the effects of confinement on the solvent water. Results are also presented using a familial mutation of the Aβ, the Iowa mutation, responsible for a more radical form of the disease.

Platform: Membrane Structure I

981-Plat
Integrin Sequestering in Raft-Mimicking Lipid Mixtures: The Role of Bilayer Asymmetry and Cholesterol Content
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It is widely accepted that lipid microdomains play an important functional role in plasma membranes. However, small size and transient nature of lipid/membrane heterogeneities in the plasma membrane make a characterization of microdomains and microdomain-related membrane processes in cellular systems quite challenging. To address this important problem, we recently introduced a powerful model membrane system that allows the investigation of membrane protein sequestering and oligomerization in raft-mimicking lipid mixtures using combined confocal fluorescence spectroscopy, photon counting histogram (PCH), and epifluorescence microscopy (1). Our experiments on bilayer-spanning domains showed that αvβ3 and α5β1 integrins predominantly exist as monomers and sequester preferentially to the liquid-disordered (ld) phase in the absence of ligands. Notably, addition of vitronectin (αvβ3) and fibronectin (α5β1) caused substantial translocations of integrins into the liquid-ordered (lo) phase without altering receptor oligomerization state. Here we expand our previous studies and report on the sequestering and oligomerization state of αvβ3 and α5β1 in asymmetric bilayer compositions containing coexisting lo and ld phases located exclusively in the top leaflet of the bilayer (bottom leaflet shows only ld phase). Remarkably, in such a membrane environment, both integrins show a higher affinity for the top leaflet-restricted lo domains in the absence of their respective ligands. This sequestering behavior of integrins was only slightly modified after addition of their respective native ligands. Our findings show that cholesterol content has a substantial influence on integrin sequestering and oligomerization in raft-mimicking lipid mixtures. The described experimental results highlight the potential importance of membrane asymmetry and lipid composition in the sequestering of membrane proteins in biological membranes.
(1) Siegel, A. P. et al. (2011) Biophys J 101(7): 1642-1650.

982-Plat
Molecular Dynamics and Concentration in Raft and Boundary Domains in Actin-Depleted Plasma Membrane Vesicles as Revealed by Single-Molecule Imaging
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Compartmentalization of the cellular plasma membrane has been considered a critical mechanism for regulating molecular interactions occurring in the plane of the membrane, by varying local molecular concentration and dynamics within and across the compartments. Among various compartments, raft domains, enriched in sterols, sphingolipids, and proteins anchored by saturated aliphatic lipid tails, have been drawing extensive attention but remained elusive due to their nano-meso-scale sizes. Recently developed plasma membrane vesicles (PMVs), which are largely depleted of the actin filaments (actin-based membrane skeleton) but contain virtually the full complement of lipids and proteins of native membranes, provide a unique platform for investigating raft domains because, by lowering the temperature, micron-sized raft-like, liquid-ordered-phase (Lo)-like domains can be induced. Here, using these PMVs with coexisting domains and single-molecule imaging-tracking methods, we examined molecular dynamics and concentration of various molecules in raft and boundary domains. One of the most interesting findings is that GPI-APs, although they preferentially partition into the Lo-like raft domains, continually move back and forth between Lo-like domains and the bulk domain, showing very dynamic partitioning, without any particular concentration in the boundary domain. Their diffusion coefficient within the boundary region has been measured for the first time: it was in the middle of the values for the Lo-like domain and the bulk domain. Other observations using molecules with various levels of raft affinity will be reported and discussed in the context of signal transduction.

983-Plat
Cell-To-Cell Variability in Plasma Membrane Lipid Rafts
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In recent years compelling evidence pointed to an important role of cholesterol enriched microdomains, so-called lipid rafts in miscellaneous cellular functions. Supposably, this lateral sub-compartmentalization facilitates selective protein-protein interactions by a local enrichment of the components involved. To study lipid rafts in the context of virus assembly, we produced several variants of two viral proteins, the Influenza virus transmembrane protein hemagglutinin and the Human Immunodeficiency Virus glycoprotein gp41. Fluorescence lifetime imaging microscopy was used to report Förster Resonance Energy Transfer (FRET) between a raft marker labelled with a cyan fluorescent protein and viral chimeras in living cells. Since it is highly distance dependent, occurring FRET indicates a co-clustering of both fluorescent protein species in membrane microdomains. Both viral proteins were found to be associated with plasma membrane lipid rafts and interestingly we observed a significant cell-to-cell variability in our samples exhibiting two distinct cell-populations with clearly differing raft related properties. To verify this