Glycans on glycoproteins play a number of roles in mammalian physiology including quality control in protein folding, stabilization of structure, regulation of composition, and direct modulation of function. The complex-type biantennary N-glycan on the IgG Fc fragment modulates cellular responses of the adaptive immune system, even turning inflammatory responses to anti-inflammatory ones through subtle modifications of glycan structure. The underlying mechanism of this extreme example remains elusive as existing structural data suggest the glycans cannot directly engage cell surface receptors from their position deeply buried between the two polypeptide chains of the Fc dimer. Solution NMR has now been used to characterize the dynamics and structural disposition of galactose and sialic acid residues at the glycan termini. Methods include relaxation dispersion studies to characterize dynamics and long-range paramagnetic perturbation from a lanthanide carrying, Fc binding, peptide to characterize disposition. Contrary to previous conclusions based on x-ray crystallography, the terminal residues of both glycan branches are found to be highly dynamic exchanging between states in which the cavity is fully and partially occupied on a 500 µs timescale. MD simulations of the partially occupied states suggest a close coupling of glycan position and protein conformation. New strategies for sparse labeling of glycoproteins such as IgG promise to allow testing of this suggestion.
Protein Dynamics from NMR Spectroscopy and MD Simulations
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NMR spectroscopy is a powerful experimental approach for characterizing protein conformational dynamics on multiple time scales, while molecular dynamics (MD) simulation is the only method capable of describing full atomistic details of protein dynamics. Back calculation of NMR chemical shifts and relaxation parameters, such as spectral density functions and generalized order parameters, allows detailed validation of MD simulations. The validated trajectories in turn allow detailed assessment of atomistic mechanisms linking protein conformational dynamics with molecular recognition of ligands and substrates. These approaches will be illustrated using the enzyme ribonuclease HI from bacteria adapted to different thermal environments¹⁴ and the bZip DNA-binding domain of the yeast transcription factor GCN4.⁵

References:
Targeting Protein-Protein Interaction Networks
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The NESG is one of four Large-Scale Centers (LSCs) for structure production funded by the NIH NIGMS Protein Structure Initiative (PSI-Biology). The goals of the PSI LSCs are to generate 3D structures for large numbers of proteins selected using broad biological, genomic, and bioinformatics criteria, together with targets selected from specific biological theme projects.

Our efforts span three classes of protein targets: (i) proteins nominated in collaborations with PSI Biology Partnerships, (ii) proteins nominated by the general biomedical research community, and (iii) proteins defined by the NESG Biomedical Theme of ‘Networks of Proteins Associated with Human Cancer and Developmental Biology’.

NESG technology development emphasizes three key areas: (i) new technologies for production of eukaryotic proteins and their complexes, (ii) protein NMR methods directed to proteins and complexes in the 20 – 50 kDa range, (iii) hybrid approaches exploiting synergies of crystallography, SAX, NMR, and computational prediction for determining structures of proteins.

The PSI-Biology program has provided the driving force for the development of improved methods using NMR for determining atomic resolution structures. These include the use of sparse NMR restraints obtained for perdeuterated proteins in the 25 – 50 KDa range, 19F NMR, and the development of molecular modeling methods utilizing NMR data, including Rosetta-NMR. I will present progress in developing new sample preparation and NMR spectroscopic methods for addressing the challenges presented by larger (20 – 50 kDa) proteins, protein-ligand complexes, and protein-protein complexes.
NMR with Multiple Receivers

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The advent of multiple receivers that allow recording of free induction decays (FID-s) simultaneously from several nuclear species can be exploited to increase the efficiency of spectrometer use. At the same time, development of cryogenically cooled probes has significantly improved the sensitivity of NMR experiments. This opens an exciting possibility of changing the traditional paradigm of experiment design. We show two main approaches for exploiting the multi-receiver technology in NMR experiments - parallel and sequential acquisition. The sequential acquisition method has been exploited in a number of applications. Notably, one application, PANACEA has been used for structure determination of small molecules from a single measurement. Three important NMR pulse sequences, INADEQUATE, HSQC and 3D J-HMBC have been combined into a single entity PANACEA - Parallel Acquisition NMR: an All-in-one Combination of Experimental Applications - to provide reliable structural information about a small molecule in a single pass. In labelled bio-molecules, e.g. proteins, the weak signal that remains after $^{13}$C detected experiments (the $^{13}$C “afterglow”) is measured with high sensitivity by proton detection in a combined dual-receiver 2D (HA)CACO / 3D (HA)CA(CO)NNH experiment. The utility of the parallel acquisition is established through the introduction of the H-1 and C-13 detected 2D HSQC and 3D HNCA experiments in which pairs of 2D and 3D spectra are recorded simultaneously. With the anticipated further increases in cryogenic probe sensitivity it is expected that multiple receiver experiments will become an important approach for efficient recording of NMR data.
NMR continues to be an extraordinary technology for improving our understanding of biology in terms of fundamental chemical and physical properties.

Crystallography of course tells us great stories in structural biology, but sometimes there are inconsistencies. An example is the changes on natural mutation in basal activity of the kinases associated with fibroblast growth factor signaling, where there is insignificant structural modification between the mutant forms, by crystallography. NMR reveals that a two-state equilibrium is actually present and the time scale on which it occurs. The scaling of conformational fluctuation and basal enzymatic activity correlates with the developmental conditions observed in human patients (Chen et al., 2013).

NMR has contributed substantially to our current understanding of protein folding, and provides a benchmark to molecular dynamics simulation. The self-association and structural features of the reactions of split inteins play roles in their applicability and efficiency in protein engineering. Chemical shift mapping and MD have permitted the identification of how extein residues play an intimate role in protein trans-splicing (Shah et al., 2013).

The role of intrinsically disordered proteins is increasingly recognized. In addition to the well-characterized group in which secondary structure formation plays a role in function, we suggest on the basis of NMR and other methods that functional activity in the filtering function in the nuclear pore is associated with very fast interactions specifically without selecting secondary structure formation (Hough et al., 2013).

