

PROJECT SELECTION

There are a couple of decisions you need to make regarding project selection:

1. What type of problem are you interested in?
2. What molecular system would you like to study?
3. What research skills would you like to apply/learn?

The types of research problems we wish to address are:

a. *Solving structures of novel proteins and protein complexes.* In these problems, little may be known about the proteins thus may require a substantial amount of biochemical characterization in preparation for NMR work. Not all unknowns will ultimately lead to NMR projects, since they may have unfavorable solution behavior. These projects are also quite comprehensive, including components of molecular biology, protein purification, heteronuclear NMR and structure calculations. In general, the goal of these types of projects is to use NMR-derived information to learn about structure and function.

The molecular systems in the lab that fall under this category are:

RNase P proteins. We seek to determine the solution structures of several of the protein subunits from the archaeon *Methanothermobacter thermoautotrophicus* and study the protein-protein and protein-RNA interactions in the holoenzyme.

Chamberlain, et al., 1998, Purification and characterization of the nuclear RNase P holoenzyme complex reveals extensive subunit overlap with RNase MRP, *Genes Dev*, **12**:1678-90. Andrews, et al., 2001, Characterization of RNase P holoenzymes from *Methanococcus jannaschii* and *Methanothermobacter thermoautotrophicus*, *Biol Chem*, **382**:1171-7. Eder, et al., 1997, Characterization of two scleroderma autoimmune antigens that copurify with human ribonuclease P, *Proc Natl Acad Sci U S A*, **94**:1101-6. Guerrier-Takada, et al., 2002, Purification and characterization of Rpp25, an RNA-binding protein subunit of human ribonuclease P, *Rna*, **8**:290-5. Hall and Brown, 2002, Archaeal RNase P has multiple protein subunits homologous to eukaryotic nuclear RNase P proteins, *RNA*, **8**:296-306. Jiang, et al., 2001, Protein-RNA interactions in the subunits of human nuclear RNase P, *Rna*, **7**:937-41. Jarrous, 2002, Human ribonuclease P: subunits, function, and intranuclear localization, *Rna*, **8**:1-7.

Nit2/Nit4. These two DNA-binding proteins from *Neurospora crassa* are responsible for transcriptional control of nitrate metabolism. Our interest is in deciphering the protein-protein interactions that mediate specific gene recognition.

Fu and Marzluf, 1987, Characterization of nit-2, the major nitrogen regulatory gene of *Neurospora crassa*, *Mol Cell Biol*, **7**:1691-6. Fu, et al., 1989, Isolation of nit-4, the minor nitrogen regulatory gene which mediates nitrate induction in *Neurospora crassa*, *J Bacteriol*, **171**:4067-70. Yuan, et al., 1991, nit-4, a pathway-specific regulatory gene of *Neurospora crassa*, encodes a protein with a putative binuclear zinc DNA-binding domain, *Mol Cell Biol*, **11**:5735-45. Feng and Marzluf, 1998, Interaction between major nitrogen regulatory protein NIT2 and pathway-specific regulatory factor NIT4 is required for their synergistic activation of gene expression in *Neurospora crassa*, *Mol Cell Biol*, **18**:3983-90.

TRAP. This oligomeric RNA-binding protein controls the expression of the Trp biosynthesis genes in several bacilli. We seek to determine the solution structure of this protein using NOEs and residual dipolar couplings in the free state and bound to Trp and RNA.

Baumann, et al., 1996, Kinetic and thermodynamic analysis of the interaction between TRAP (trp RNA-binding attenuation protein) of *Bacillus subtilis* and trp leader RNA, *J Biol Chem*,

271:12269-74., Antson, et al., 1994, 11-fold symmetry of the trp RNA-binding attenuation protein (TRAP) from *Bacillus subtilis* determined by X-ray analysis, *J Mol Biol*, **244**:1-5., Antson, et al., 1995, The structure of trp RNA-binding attenuation protein, *Nature*, **374**:693-700., Elliott, et al., 2001, The mechanism of RNA binding to TRAP: initiation and cooperative interactions, *Rna*, **7**:85-93., Chen, et al., 1999, Regulatory features of the trp operon and the crystal structure of the trp RNA-binding attenuation protein from *Bacillus stearothermophilus*, *J Mol Biol*, **289**:1003-16.

AT (Anti-TRAP). This multimeric small protein binds TRAP and inhibits RNA binding. No structural information is available for this protein.

Valbuzzi, et al., 2002, The anti-trp RNA-binding attenuation protein (Anti-TRAP), AT, recognizes the tryptophan-activated RNA binding domain of the TRAP regulatory protein, *J Biol Chem*, **277**:10608-13., Valbuzzi and Yanofsky, 2001, Inhibition of the *B. subtilis* regulatory protein TRAP by the TRAP- inhibitory protein, AT, *Science*, **293**:2057-9.

Zpr. These putative zinc binding proteins appear to have roles in receptor-mediated signal transduction, as well as RNA splicing. There are homologs in eukarya and archaea. Preliminary NMR analysis suggests aggregation problems need to be addressed.

Galcheva-Gargova, et al., 1996, Binding of Zinc Finger Protein ZPR1 to the Epidermal Growth Factor Receptor, *Science*, **272**:1795-7., Galcheva-Gargova, et al., 1998, The cytoplasmic zinc finger protein ZPR1 accumulates in the nucleolus of proliferating cells, *Mol Biol Cell*, **9**:2963-71., Gangwani, et al., 1998, Interaction of ZPR1 with translation elongation factor-1alpha in proliferating cells, *J Cell Biol*, **143**:1471-84., Gangwani, et al., 2001, Spinal muscular atrophy disrupts the interaction of ZPR1 with the SMN protein, *Nat Cell Biol*, **3**:376-83.

Adenovirus 14.7K. This is an adenoviral gene product that inhibits apoptosis in an as-yet determined mechanism. The protein is oligomeric and rather insoluble. May not be suitable for NMR.

Kim and Foster, 2002, Characterization of Ad5 E3-14.7K, an adenoviral inhibitor of apoptosis: structure, oligomeric state, and metal binding, *Protein Sci*, **11**:1117-28., Wold, 1993, Adenovirus genes that modulate the sensitivity of virus-infected cells to lysis by TNF, *J Cell Biochem*, **53**:329-35.

b. *Studying protein dynamics using NMR and computer methods.* In these problems, the function and structure of the protein may be known but the relationship between a protein's mobility/dynamics and its function is not well characterized or understood. One goal of this work is to attempt to correlate changes in dynamics with binding of ligands. These projects will involve NMR spectroscopy to generate resonance assignments for protein targets and measure relaxation parameters. Another approach may be to use computational molecular dynamics to attempt to correlate simulated molecular motions with NMR measurements. There is quite a bit of fundamental theoretical development (physics, mathematics and pulse program design) that still must be done to extract motional parameters from NMR data, and you would be expected to learn and perhaps help to develop the theory.

The systems that fall under this category are:

Bacteriophage lambda integrase catalytic domain. We seek to correlate loop motion/dynamics with substrate recognition and catalysis.

Kwon, et al., 1997, Flexibility in DNA recombination: structure of the lambda integrase catalytic core, *Science*, **276**:126-31. Tirumalai, et al., 1997, The catalytic domain of lambda site-specific recombinase, *Proceedings of the National Academy of Sciences of the United States America*, **94**:6104-9.

Cre recombinase. Similar objectives as for lambda integrase, except that the crystal structure of the DNA complex is available, but not that of the free protein.

Hoess, et al., 1990, DNA specificity of the Cre recombinase resides in the 25 kDa carboxyl domain of the protein, *J Mol Biol*, **216**:873-82. Guo, et al., 1999, Asymmetric DNA bending in the Cre-loxP site-specific recombination synapse, *Proc Natl Acad Sci U S A*, **96**:7143-8. Gopaul, et al., 1998, Structure of the Holliday junction intermediate in Cre-loxP site-specific recombination, *Embo J*, **17**:4175-87, Guo, et al., 1997, Structure of Cre recombinase complexed with DNA in a site-specific recombination synapse, *Nature*, **389**:40-6.

Peptide deformylase: here we seek to dissect dynamic and thermodynamic contributions to ligand binding by analysis of NMR relaxation and other techniques.

Chan, et al., 1997, Crystal structure of the Escherichia coli peptide deformylase, *Biochemistry*, **36**:13904-9. Meinnel, et al., 1997, Structure-function relationships within the peptide deformylase family. Evidence for a conserved architecture of the active site involving three conserved motifs and a metal ion, *J Mol Biol*, **267**:749-61. Rajagopalan, et al., 1997, Purification, characterization, and inhibition of peptide deformylase from Escherichia coli, *Biochemistry*, **36**:13910-8. Meinnel, et al., 1996, A new subclass of the zinc metalloproteases superfamily revealed by the solution structure of peptide deformylase, *J Mol Biol*, **262**:375-86.

TRAP (see above). We are interested in characterizing via experiment and simulation the molecular motions that result in ligand exchange/protein activation and to explain the lack of cooperativity in Trp binding.

c. Methods development/implementation

Chemical shift calculations

Wishart and Case, 2001, Use of chemical shifts in macromolecular structure determination, *Methods Enzymol*, **338**:3-34.

Xu and Case, 2001, Automated prediction of ¹⁵N, ¹³Calpha, ¹³Cbeta and ¹³C' chemical shifts in proteins using a density functional database, *J Biomol NMR*, **21**:321-33.

Structure calculation of symmetrical macromolecules from rDC data

Bolon, et al., 1999, Residual dipolar coupling derived orientational constraints on ligand geometry in a 53 kDa protein-ligand complex, *J Mol Biol*, **293**:107-15., Delaglio, et al., 2000, Protein Structure Determination Using Molecular Fragment Replacement and NMR Dipolar Couplings, *J Am Chem Soc*, **122**:2142-3., Zweckstetter and Bax, 2001, Characterization of molecular alignment in aqueous suspensions of Pf1 bacteriophage, *J Biomol NMR*, **20**:365-77., Bax, et al., 2001, Dipolar couplings in macromolecular structure determination, *Methods Enzymol*, **339**:127-74. Tjandra, et al., 1997, Use of dipolar ¹H-¹⁵N and ¹H-¹³C couplings in the structure determination of magnetically oriented macromolecules in solution, *Nature Structural Biology*, **4**:732-8, Tjandra and Bax, 1997, Direct measurement of distances and angles in biomolecules by NMR in a dilute liquid crystalline medium, *Science*, **278**:1111-4.

MD (molecular dynamics) simulations of enzymatically relevant conformational transitions

Nicholson, et al., 1995, Flexibility and function in HIV-1 protease, *Nature Structural Biology*, **2**:274-80. Radkiewicz and Brooks III, 2000, Protein Dynamics in Enzymatic Catalysis: Exploration of Dihydrofolate Reductase, *J Am Chem Soc*, **122**:225-231, Chatfield, et al., 1997, Molecular Dynamics of Staphylococcal Nuclease: Comparison of Simulation with ¹⁵N and ¹³C NMR Relaxation Data, *J Am Chem Soc*, **120**:5301-5311. Ma, et al., 2000, A dynamic model for the allosteric mechanism of GroEL, *J Mol Biol*, **302**:303-13.

In addition, there are a number of papers that refer to relevant methodology and theory that may be useful:

NMR structure determination

Stein, et al., 1997, Torsion-angle molecular dynamics as a new efficient tool for NMR structure calculation, *J Magn Reson*, **124**:154-64, Güntert, et al., 1997, Torsion angle dynamics for NMR structure calculation with the new program DYANA, *J Mol Biol*, **273**:283-98, Rohl and Baker, 2002, De novo determination of protein backbone structure from residual dipolar couplings using rosetta, *J Am Chem Soc*, **124**:2723-9., Andrec, et al., 2001, Protein structural motif recognition via NMR residual dipolar couplings, *J Am Chem Soc*, **123**:1222-9., Tolman, et al., 2001, Structural and dynamic analysis of residual dipolar coupling data for proteins, *J Am Chem Soc*, **123**:1416-24. Bax, et al., 2001, Dipolar couplings in macromolecular structure determination, *Methods Enzymol*, **339**:127-74, Mueller, et al., 2000, A method for incorporating dipolar couplings into structure calculations in cases of (near) axial symmetry of alignment, *J Biomol NMR*, **18**:183-8., Mueller, et al., 2000, Global folds of proteins with low densities of NOEs using residual dipolar couplings: application to the 370-residue maltodextrin-binding protein, *J Mol Biol*, **300**:197-212.

Extracting motional parameters from heteronuclear relaxation measurements:

Dayie, et al., 1996, Theory and practice of nuclear spin relaxation in proteins, *Annu Rev Phys Chem*, **47**:243-82. Palmer III, et al., 1996, Nuclear Magnetic Resonance Studies of Biopolymer Dynamics, *J. Phys. Chem.*, **100**:13293-13310. Mandel, et al., 1995, Backbone dynamics of Escherichia coli ribonuclease HI: correlations with structure and function in an active enzyme, *J Mol Biol*, **246**:144-63. Lee, et al., 1997, Rotational diffusion anisotropy of proteins from simultaneous analysis of ¹⁵N and ¹³C alpha nuclear spin relaxation, *J Biomol NMR*, **9**:287-98. Palmer III, 1997, Probing molecular motion by NMR, *Curr Opin Struct Biol*, **7**:732-7. Yang, et al., 1998, Contributions to protein entropy and heat capacity from bond vector motions measured by NMR spin relaxation, *Nat Struct Biol*, **5**:156-63. Yang and Kay, 1996, Contributions to conformational entropy arising from bond vector fluctuations measured from NMR-derived order parameters: application to protein folding, *Journal of Molecular Biology*, **263**:369-82. Yang, et al., 1997, Contributions to protein entropy and heat capacity from bond vector motions measured by NMR spin relaxation, *J Mol Biol*, **272**:790-804. Yang, et al., 1998, A study of protein side-chain dynamics from new ²H auto-correlation and ¹³C cross-correlation NMR experiments: application to the N-terminal SH3 domain from drk, *J Mol Biol*, **276**:939-54. Farrow, et al., 1997, Characterization of the backbone dynamics of folded and denatured states of an SH3 domain, *Biochemistry*, **36**:2390-402. Kay, et al., 1996, Correlation between dynamics and high affinity binding in an SH2 domain interaction, *Biochemistry*, **35**:361-8. Tjandra, et al., 1996, Protein Backbone Dynamics and ¹⁵N Chemical Shift Anisotropy from Quantitative Measurement of Relaxation Interference Effects, *J Am Chem Soc*, **118**:6986-6991. Tjandra, et al., 1997, Defining long range order in NMR structure determination from the dependence of heteronuclear relaxation times on rotational diffusion anisotropy, *Nat Struct Biol*, **4**:443-9. Tjandra, et al., 1996, Anisotropic rotational diffusion of perdeuterated HIV protease from ¹⁵N NMR relaxation measurements at two magnetic fields, *Journal of Biomolecular NMR*, **8**:273-84. Tjandra, et al., 1995, Rotational dynamics of calcium-free calmodulin studied by ¹⁵N-NMR relaxation measurements, *Eur J Biochem*, **230**:1014-24.

Correlating dynamics and function

Bosco, et al., 2002, Catalysis of cis/trans isomerization in native HIV-1 capsid by human cyclophilin A, *Proc Natl Acad Sci U S A*, **99**:5247-52., Eisenmesser, et al., 2002, Enzyme dynamics during catalysis, *Science*, **295**:1520-3. Nicholson, et al., 1995, Flexibility and function in HIV-1 protease, *Nature Structural Biology*, **2**:274-80. Mulder, et al., 1999, Altered flexibility in the substrate-binding site of related native and engineered high-alkaline Bacillus subtilisins, *J Mol Biol*, **292**:111-23, Miller and Agard, 1999, Enzyme specificity under dynamic control: a normal mode analysis of alpha-lytic protease, *J Mol Biol*, **286**:267-78. Kay, et al., 1998, Correlation between binding and dynamics at SH2 domain interfaces, *Protein Sci*, **7**:336-41.