Summary

- Energy of transition and the Boltzmann distribution
- Relaxation
- Vector and Product Operator formalisms
- Polarization transfer
- Creation of a 2\textsuperscript{nd} dimension
- NOE
- COSY
What’s It Good For?

- Structure
- Kinetics
- Dynamics
- Ligand interactions
- Folding
- Metabolism (whole cell)

Structure
Strategy for the Structure Determination of Biomolecules via Heteronuclear NMR Methods

- Generation of mg quantities of uniformly labeled ($^{15}$N/ $^{13}$C) molecule
- Sequence specific resonance assignment
- Extraction of structural information (coupling constants, NOE volumes)
- Calculation of structures using covalent information, distance and angle constraints
- Refinement
Torsion Angle Information

\[ J_{P-C4'} = A \cos^2 \varepsilon + B \cos \varepsilon + C \]
Is It Doable?

- **Size**
  - Proteins up to \( \approx 450 \) aa; Complexes\(^*\) < 200 kDa
  - Nucleic acids \( \approx 75 \) nt

- **Stability**
  - Purification (Re-foldability?)

- **Solubility**
  - \( \geq 0.20 \) mM (3D structure)
  - \( \geq 0.20 \) \( \mu \)M (“other” studies)

- **Relaxation properties**
  - size, solvent composition, temperature
  - \( (^2H \) incorporation?)

- **Sample adjustment**
  - mono-, di-valent cations and anions (\( \text{Mg}^{+2}, \text{Ca}^{+2} \))
  - buffer, pH
  - detergents, micelles
  - mutations/truncations
  - post-translational/transcriptional modifications
Preparing the Sample

- **Sources of paramagnetism**: transition metal ions: Mn$^{+2}$, Fe$^{+3}$ (EDTA, chelation resins) 
  O$_2$, degas, N$_2$ or Ar purge

- **pH**: > 7.0, accelerates x-change at NH & NH$_2$

- **Buffers**: Protonated, deuterated, inorganic 
  Tris, KPi, cacodylate, MOPS, acetate... 
  select for the appropriate pKa

- **Volume**: ~150 - 600 µl 
  typically, the limitation is concentration, not quantity

- **Concentration**: Goal dependent 
  3D structure, ≥ 0.20 mM 
  ligand binding via heteronuclear NMR, ≥ 2.0 µM
$^1$H Based Sequence Specific Assignment Strategy Made Simple

**Proteins**

NOESY: inter- and intra-residue correlations
COSY: intra-residue correlations

**Nucleic Acids**

NOESY: inter- and intra-residue correlations
COSY: intra-residue correlations
1H-31P HetCor: inter- and intra-residue correlations