From PISA Wheels to High Resolution Protein Structure

Taking Liberties with The Leaning Tower of PISA

- **The α-helix casts a projection onto the plane of PISA**
- **PISA - Polar Index Slant Angles**
- **The helix is stabilized by hydrogen bonds between the carbonyl of residue ‘i’ and the amide nitrogen of residue ‘i+4’**

Wang et al., 2000 JMR 144:162-167.
Marassi & Opella, 2000 JMR 144: 150-155.

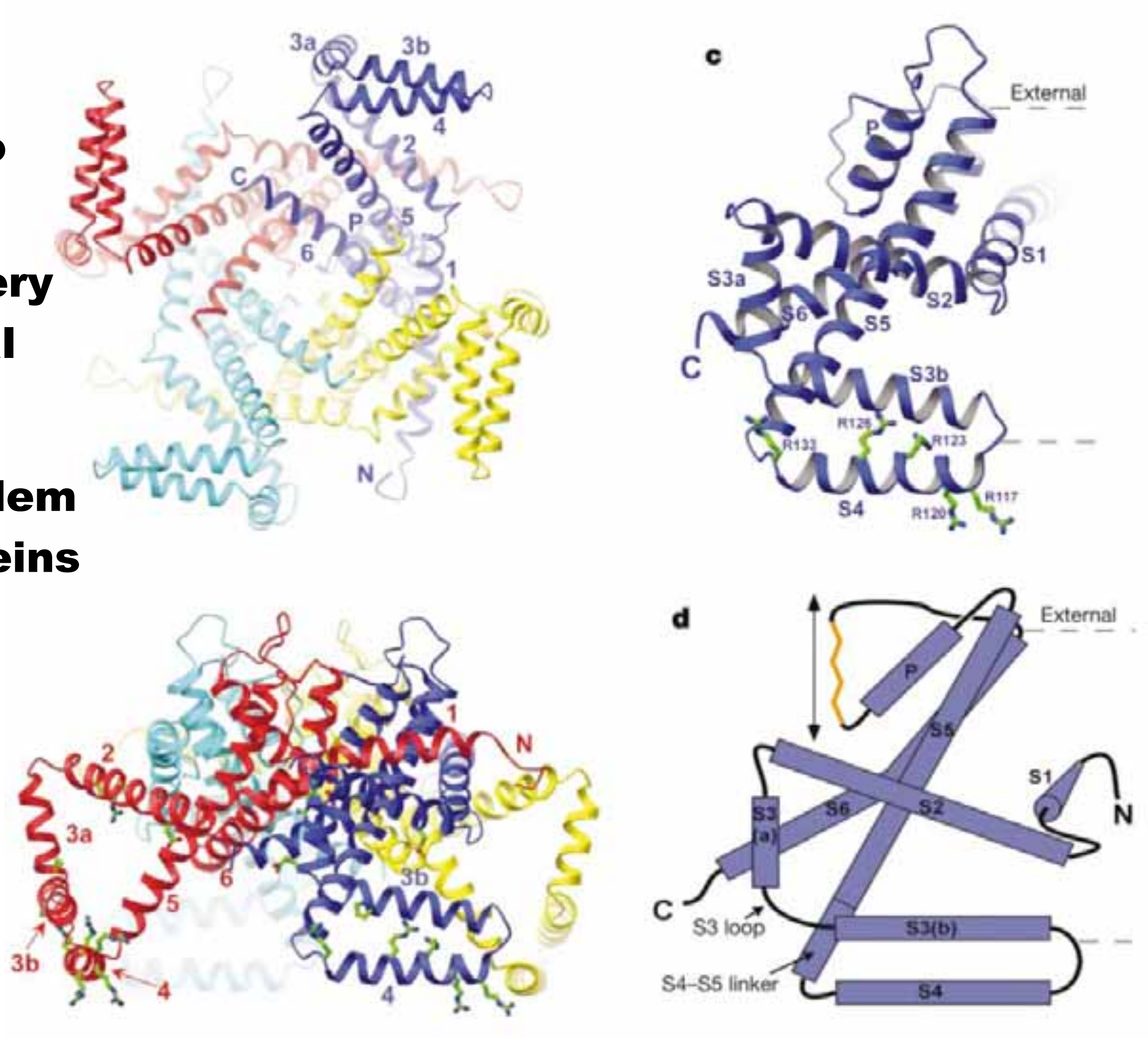
Aligned Sample Techniques: PISA Wheels & Structural Refinement

1. **PISA wheels their use in initial structure and implications for membrane protein biophysics.**

2. **Simulated Annealing and structural refinement from assigned orientational restraints - the challenges of working with high precision restraints**

Membrane Proteins: So why NMR?

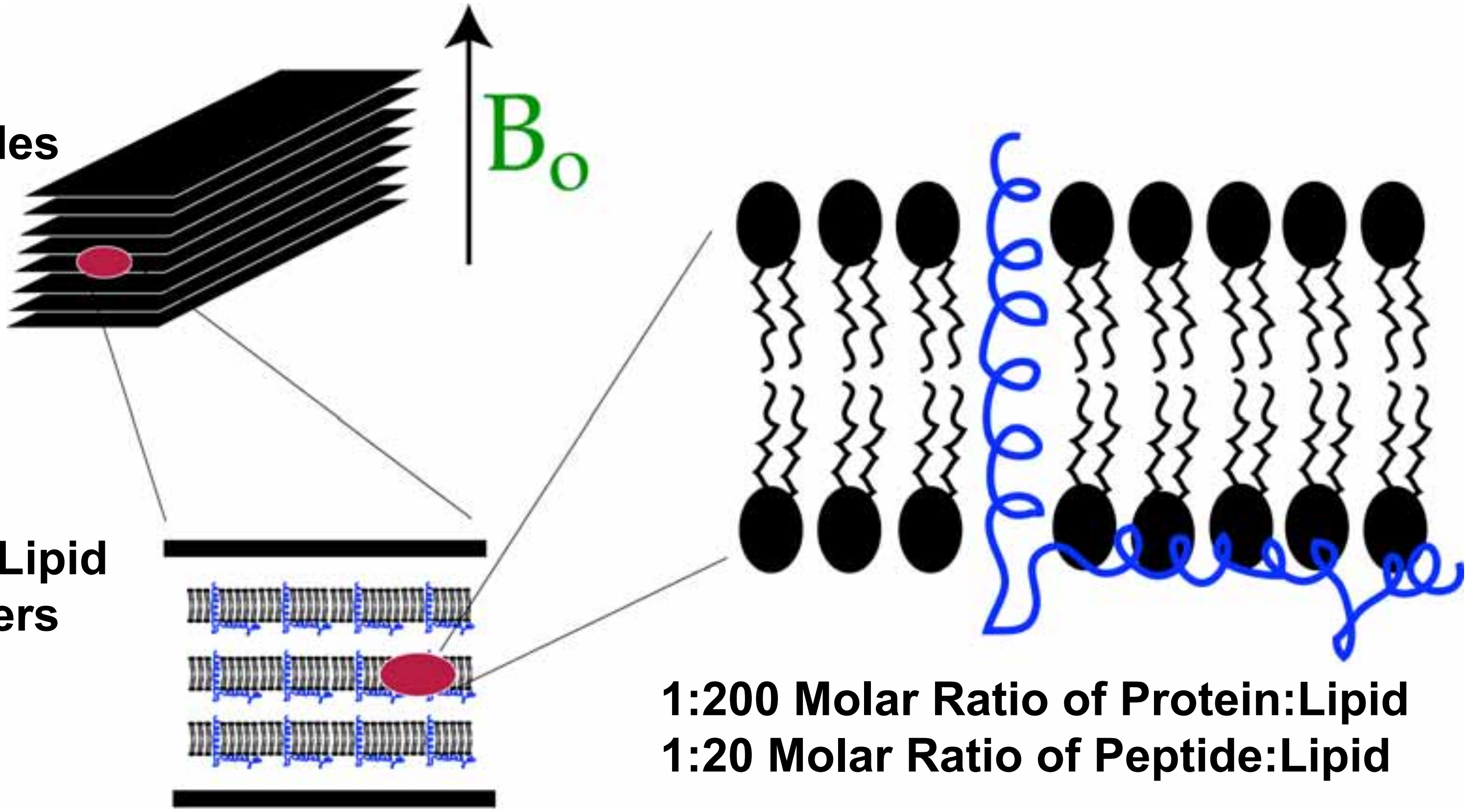
- **X-ray crystallography has provided most of the MP structures we have to date.**
- **But such structures are very easily distorted by crystal packing forces**
- **In particular this is a problem for small membrane proteins**
- **Small is good for NMR**
- **Membrane proteins have multiple structural conformations dependent on environment**
- **NMR can readily change the membrane protein's environment**



The crystal structure of the tetrameric K+ Channel, KvAP (Jiang et al., 2003)

Uniformly Aligned Samples

Oriented Samples for Solid State NMR Spectroscopy:
Hydrated Lipid Bilayer Preparations of Membrane Proteins and Polypeptides

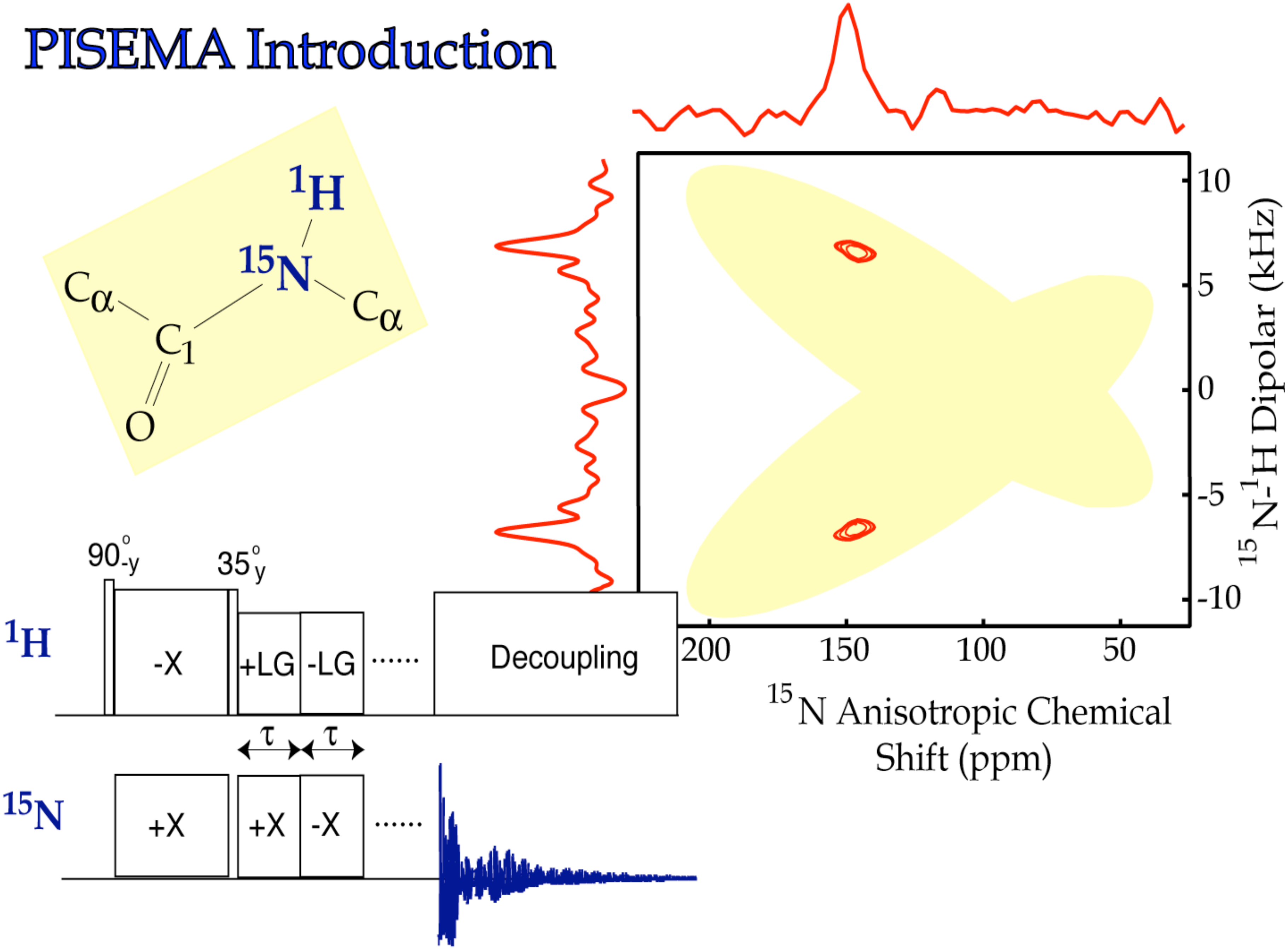


50 Slides

6000 Lipid
Bilayers

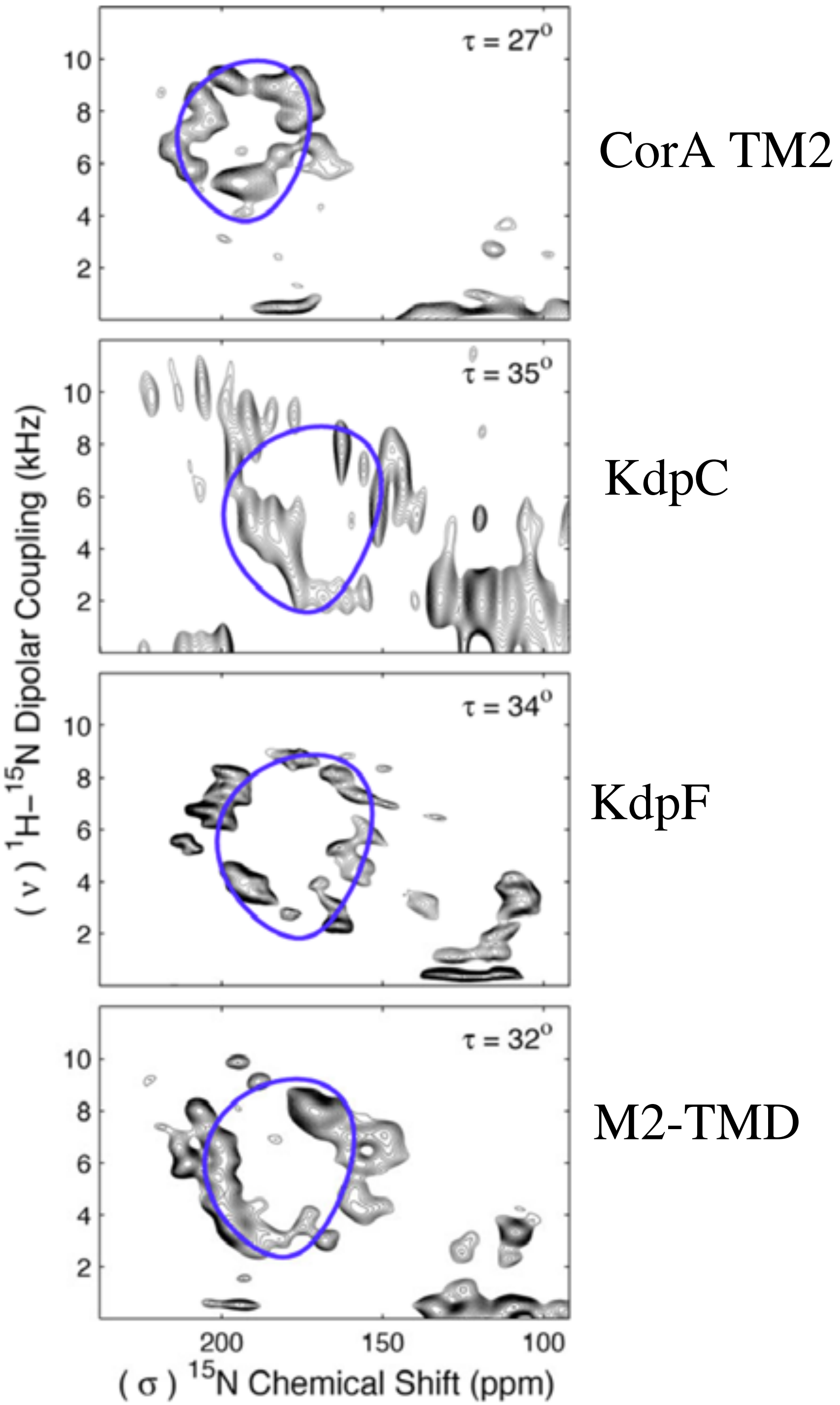
1:200 Molar Ratio of Protein:Lipid
1:20 Molar Ratio of Peptide:Lipid

PISEMA Introduction



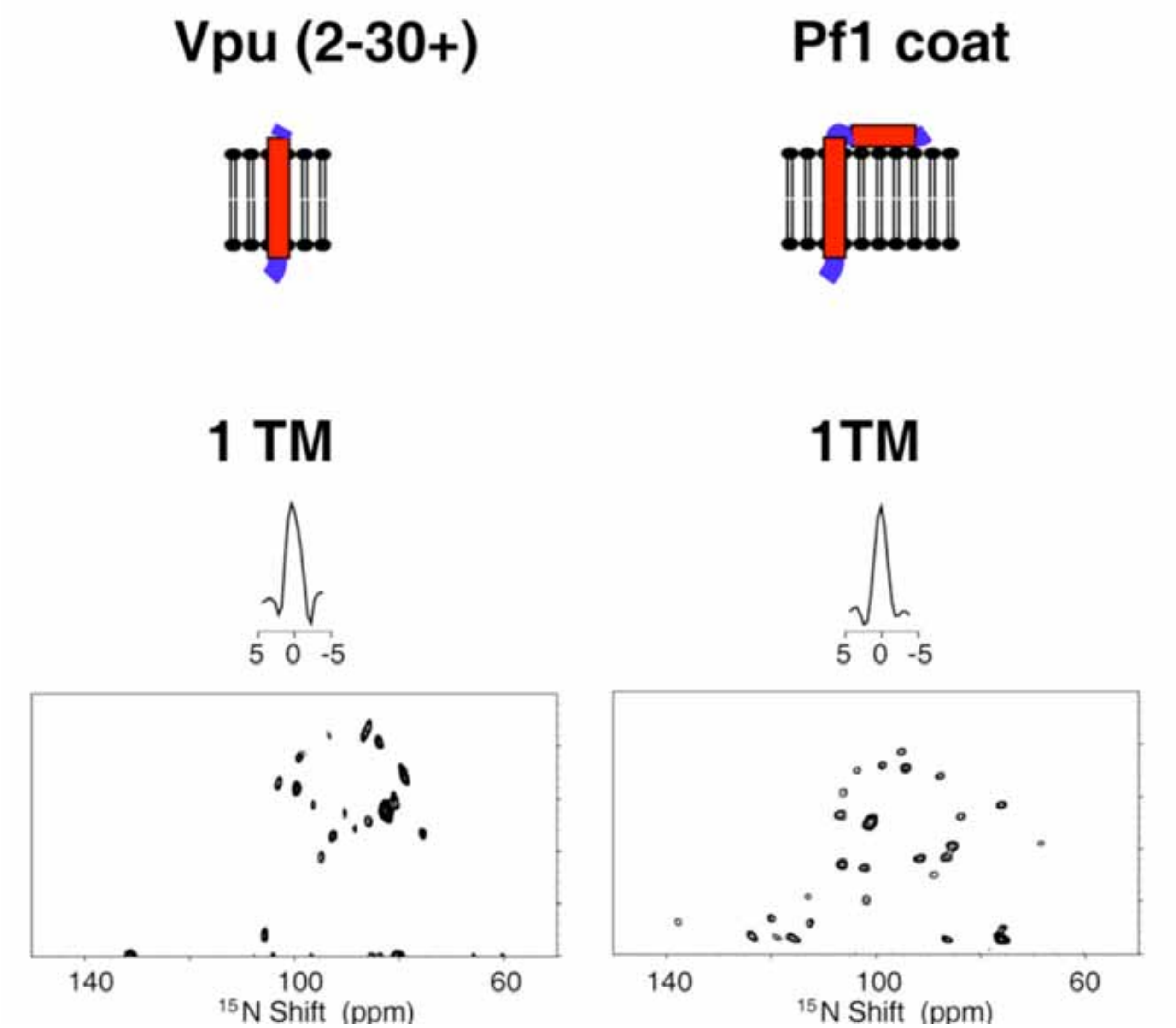
PISA Wheels observed in Peptides and Proteins

- CorA TM2: The second of two transmembrane helices from the Mg^{2+} transporter from *M. tuberculosis*. The native protein is pentameric but this peptide is monomeric
- KdpC: An 18 kDa protein with a single transmembrane helix that forms part of the Kdp K^{+} transport system in *Mtb* - this is a spectrum of the full length protein.
- KdpF: Another gene product for the Kdp complex - this one is just 4 kDa and this is the spectrum of the full length protein
- M2-TMD: This is a spectrum of the transmembrane helix from M2 protein of influenza A virus that forms a tetrameric bundle.



Membrane Proteins: Solid State NMR

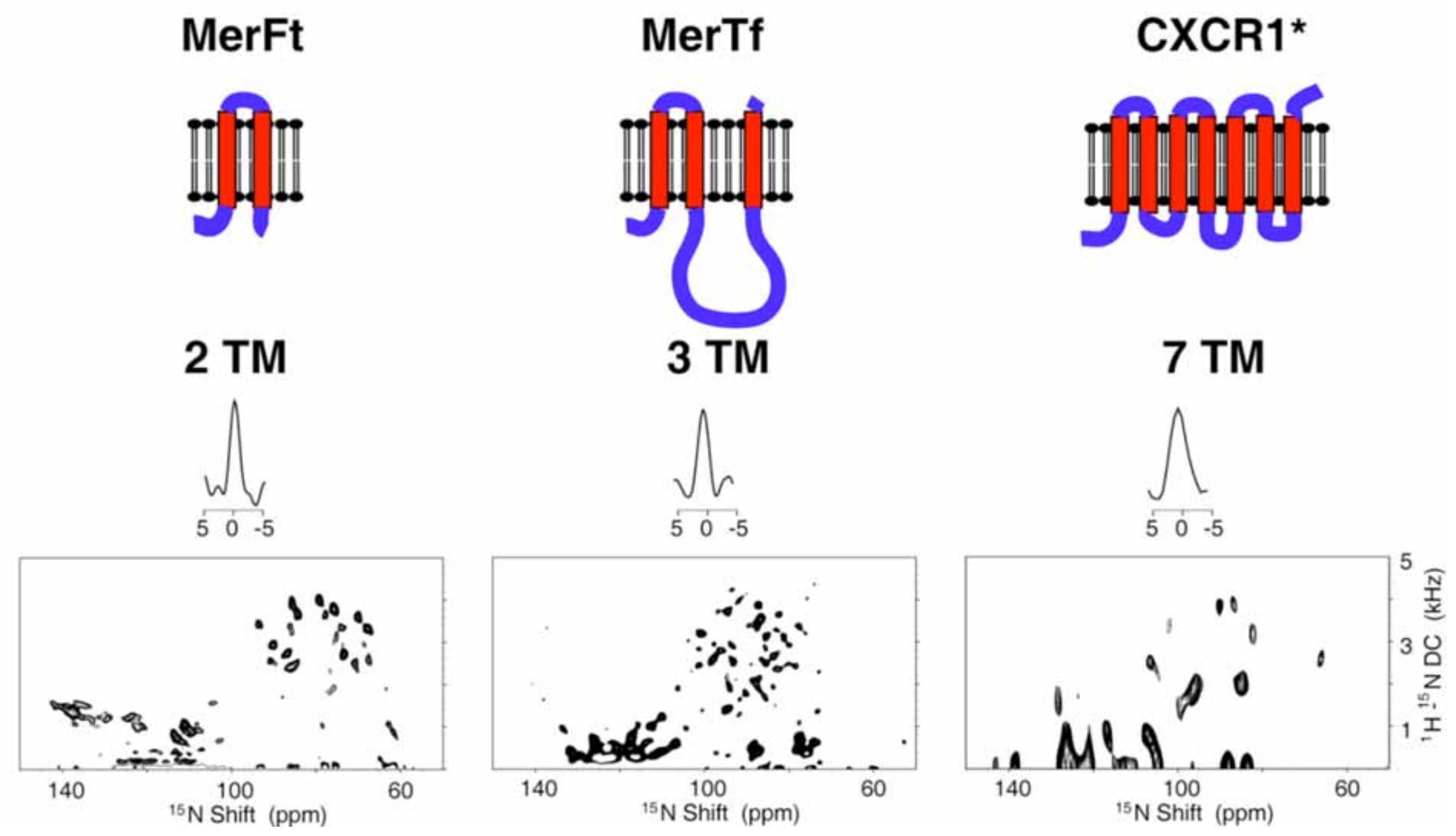
- **Sample Preparation is everything**
- **Homogeneous and Uniformly Aligned Preparation**
- **Bicelle Preparations**



Spectra from Opella and coworkers in uniformly aligned bicelles

Membrane Proteins: Solid State NMR

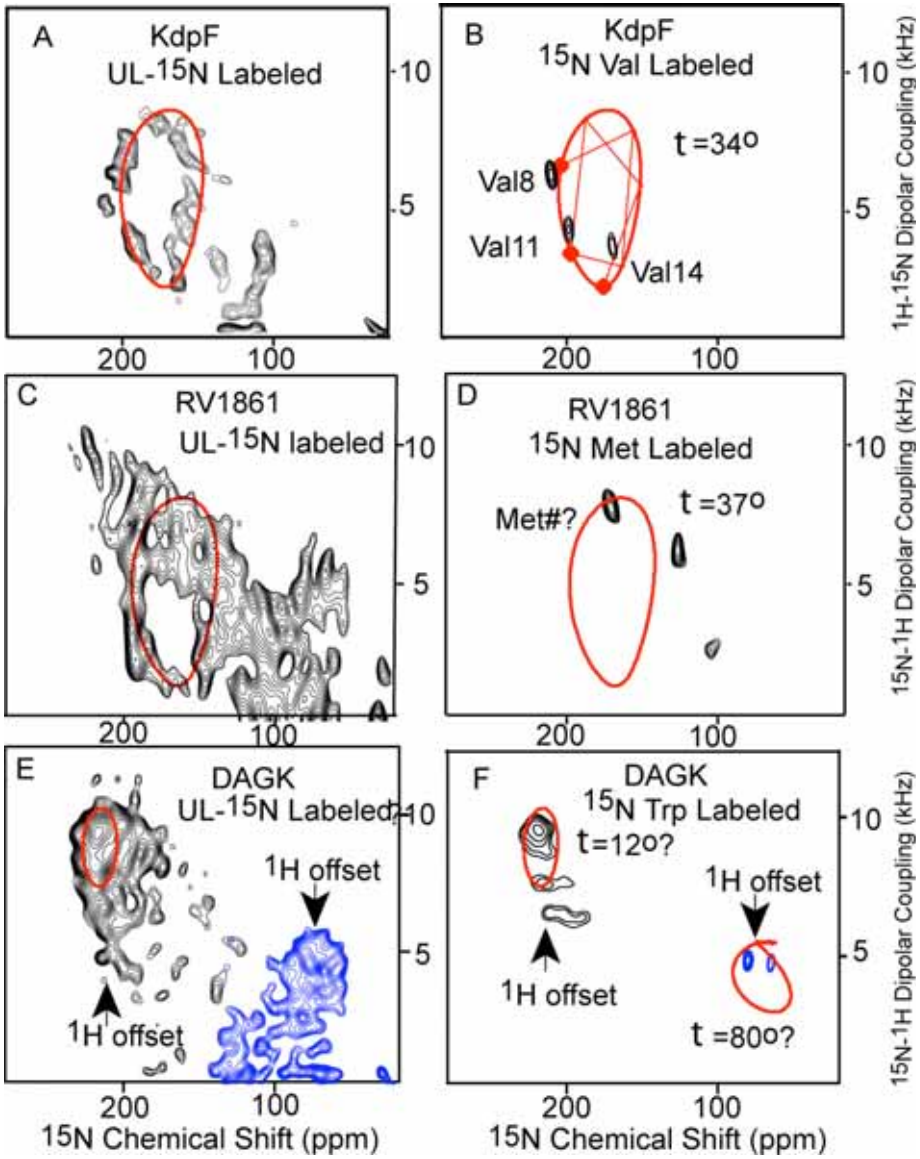
- **More Bicelle Preparations**



All spectra from Opella and coworkers in uniformly aligned bicelles

Membrane Proteins: Solid State NMR

- **Sample Preparation is everything**
- **Homogeneous and Uniformly Aligned Preparation**
- **Spectral Resolution**
- **Long Term Stability**
- **Utilized Liquid-crystalline Lipid Bilayers**
- **Requires isotopic labeling**
- **Can obtain structural information from 1st spectrum**
- **1st Spectrum includes Structural Restraints, but need more**
- **Finally build a Structural Model**



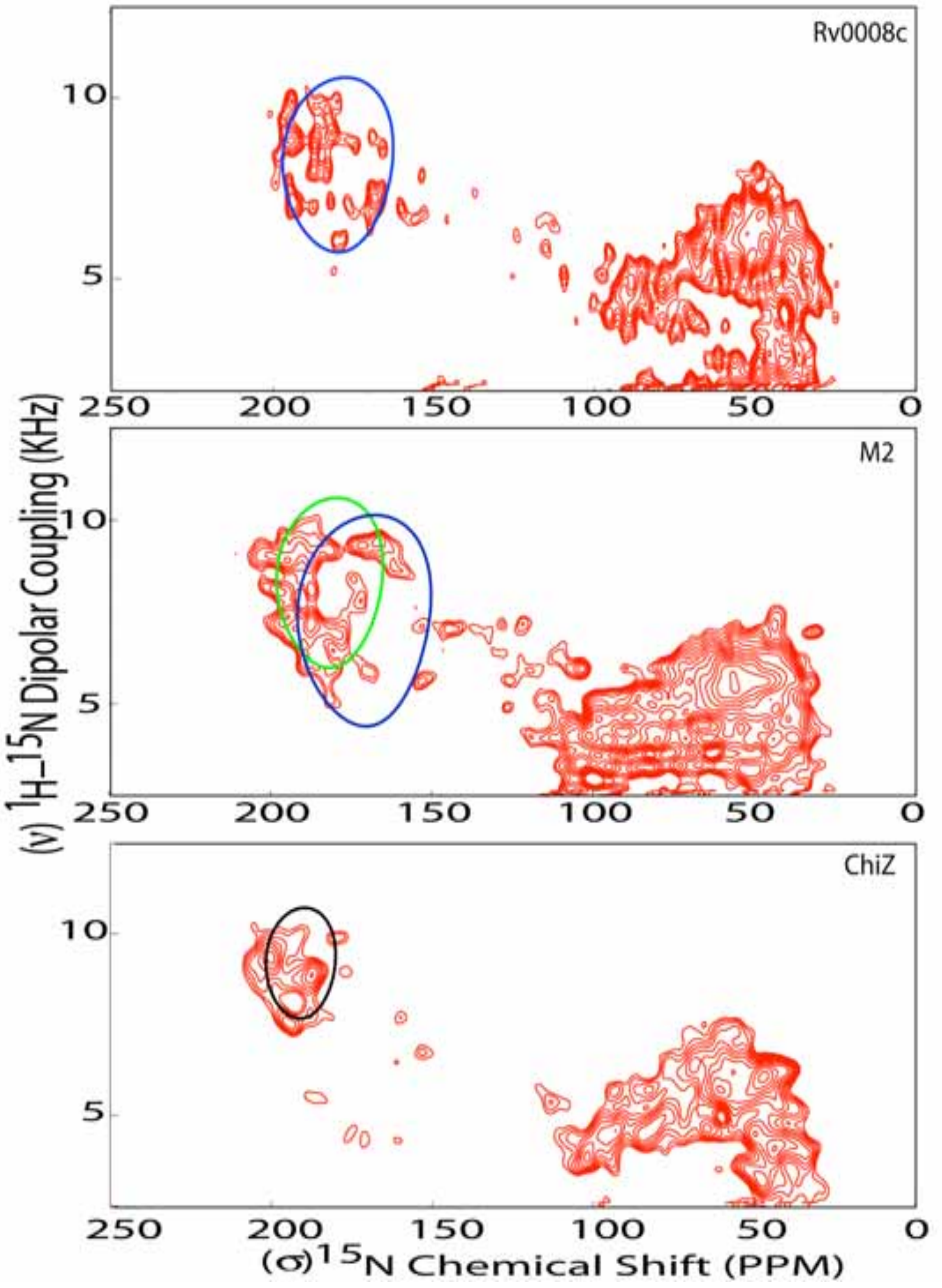
Spectra of three full length MPs in Uniformly Aligned Liquid-Crystalline Lipid Bilayers - Li et al., JACS 2007

Membrane Proteins: Solid State NMR

- **Rv0008c is an 18kDa protein with a single TM and a large water soluble domain**

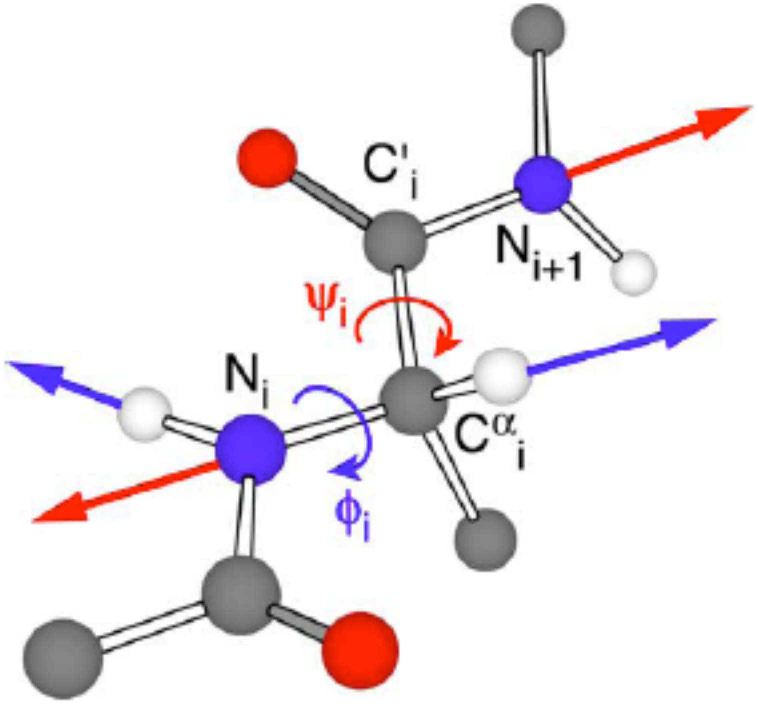
- **Full length M2 protein with the antiviral drug Amantadine bound. The heliix appears kinked with similar tilt angles as observed with the isolated transmembrane domain.**

- **Chiz is also 18 kDa with a single TM and two moderately sized water soluble terminal domains.**



Spectra of another three full length MPs in Uniformly Aligned Liquid-Crystalline Lipid Bilayers

Torsion Angles in Protein Backbone

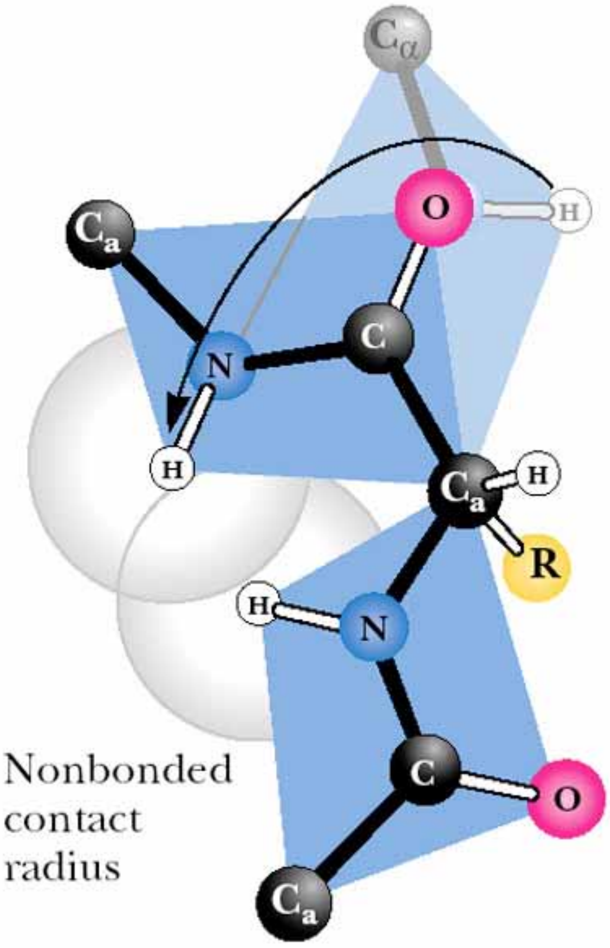


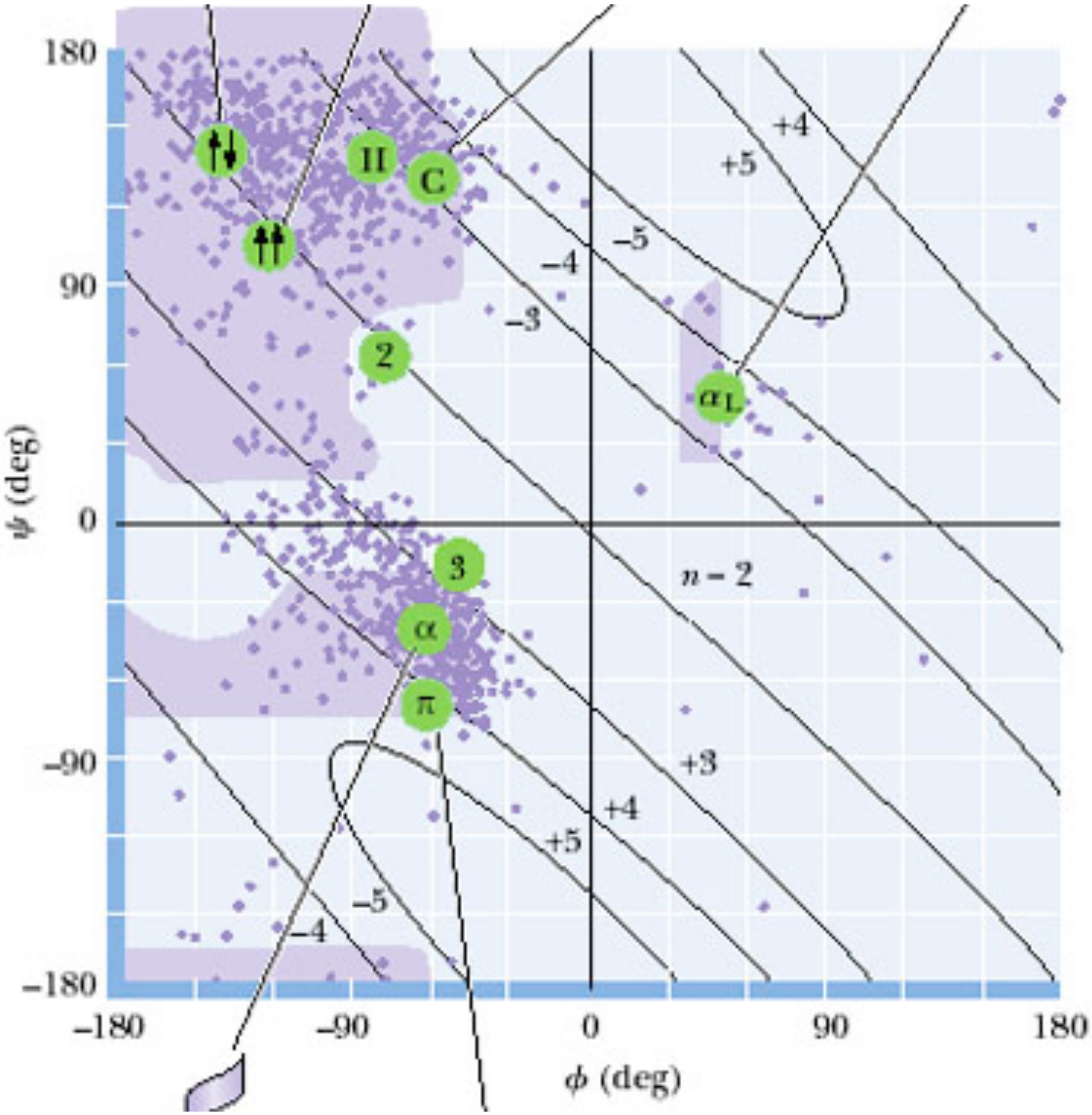
- Note the two planes on either side of the α-carbon, with ϕ angle between the α-carbon and the N of one amide and the ψ angle between the α-carbon and the carbonyl C of the next amide.

- The ϕ angle is defined incorrectly in 90% of all Biochemistry texts even though the definition was changed in 1972!!

Thanks to Chris Jaroniec for correctly Defining ϕ and ψ

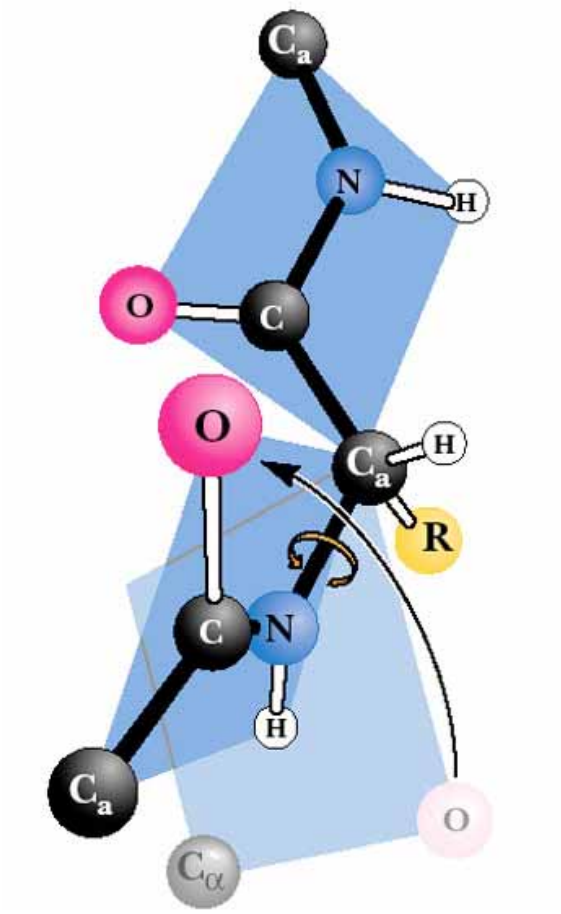
Rhamachandran Diagram

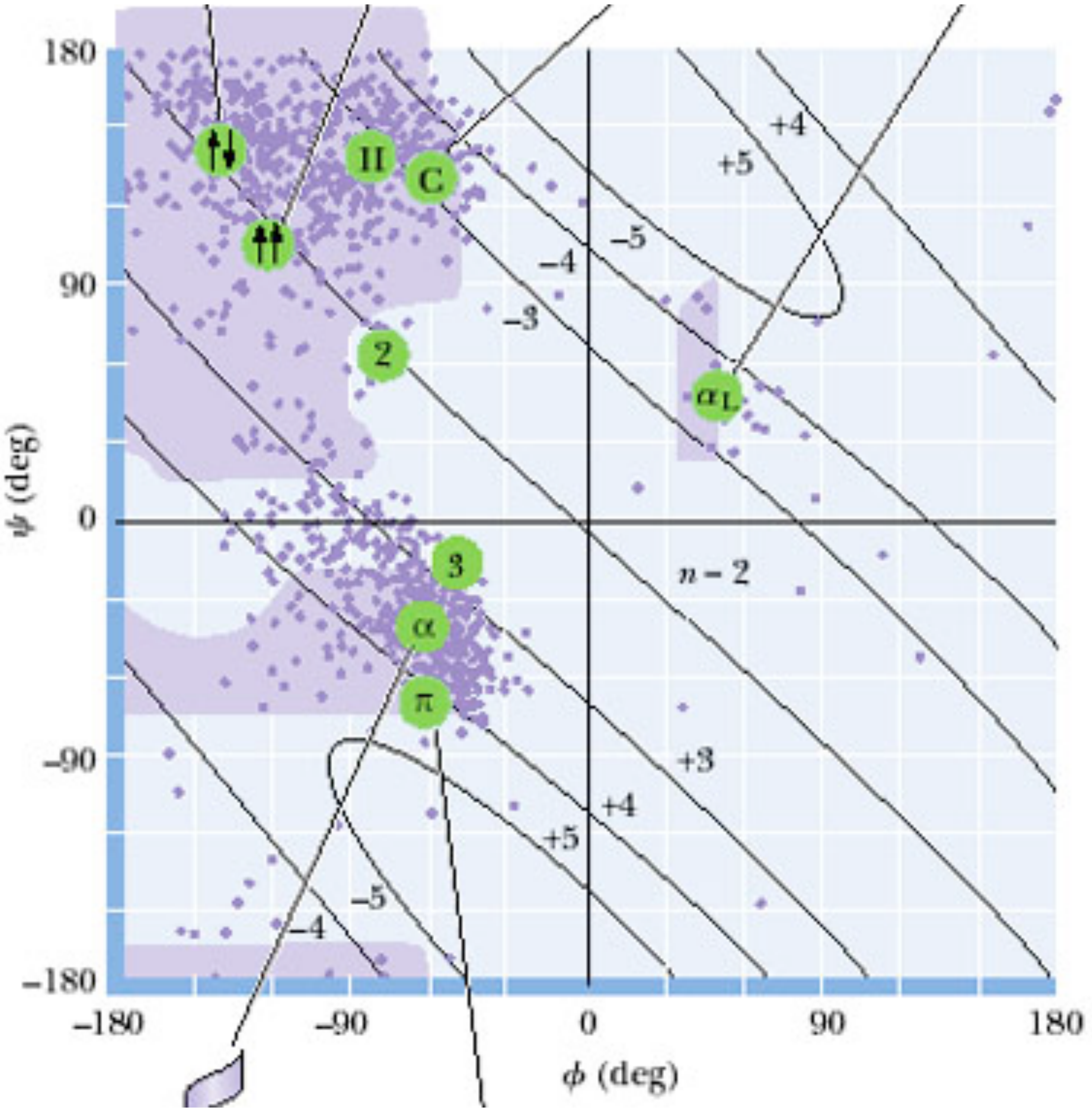




ϕ=180°, ψ=0°

Rhamachandran Diagram

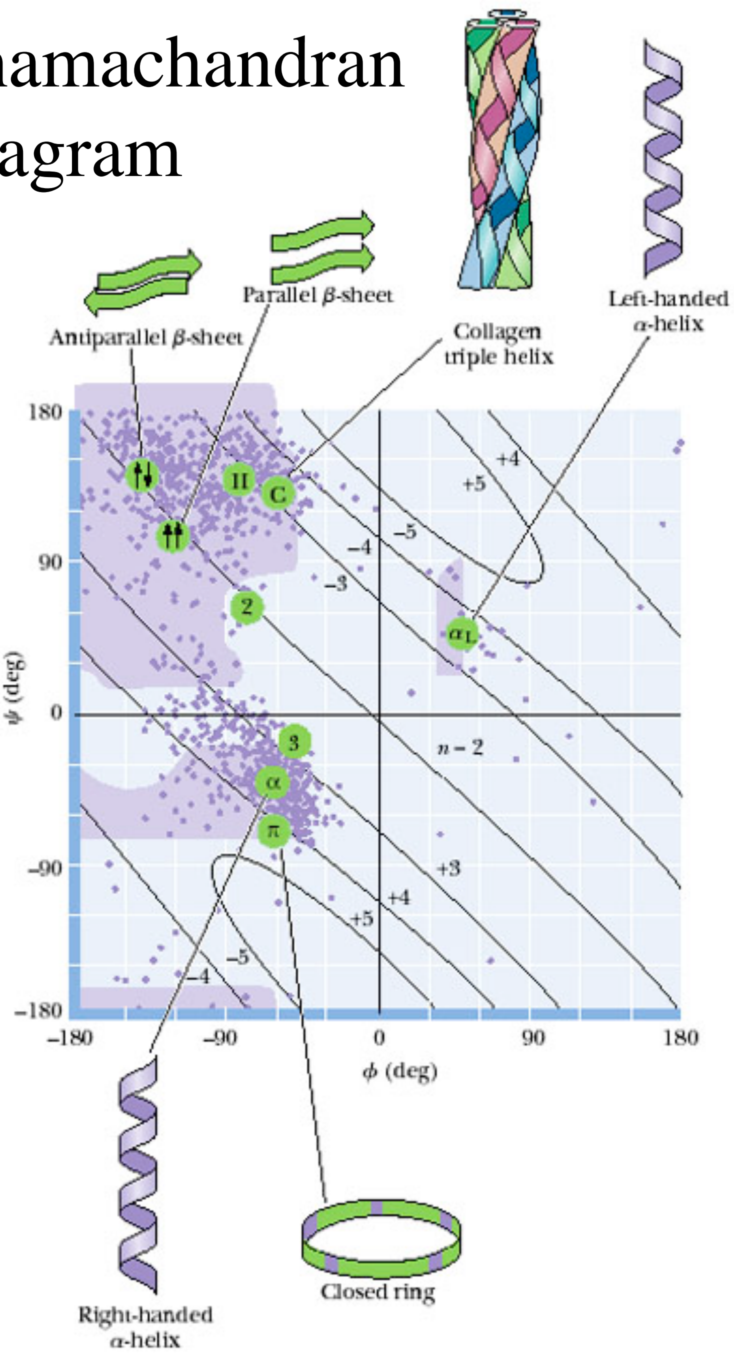




$\phi=-60^\circ$, $\psi=0^\circ$

A rotation of 120° in ϕ results in removing the steric hindrance between the carbonyl and the side-chain

Rhamachandran Diagram



- Many secondary structures have a repeating set of phi/psi torsion angles.

- However, this does not mean that the torsion angles are precisely the same.

- The dark purple spots indicate a distribution of torsion angles - even for the helical region the distribution is large.

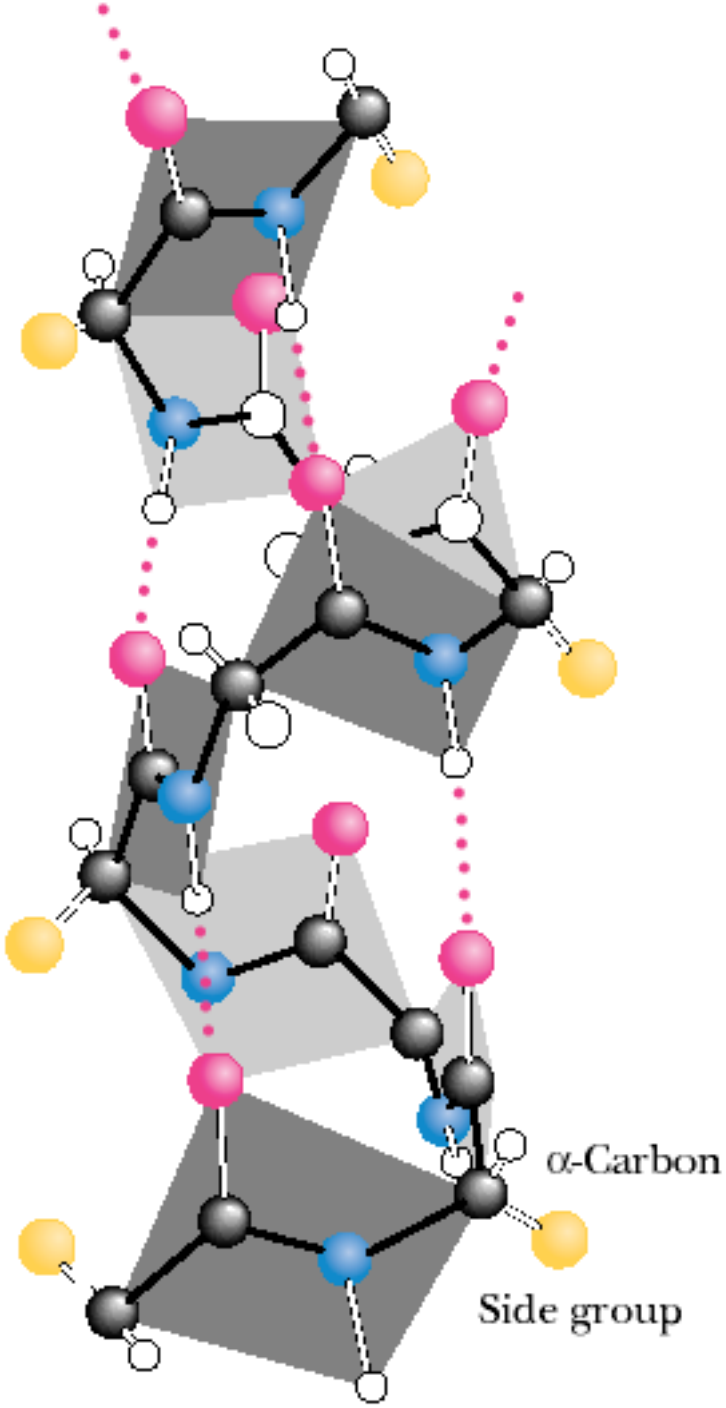
- Why might they not be precisely the same?

α-Helix

Note that:

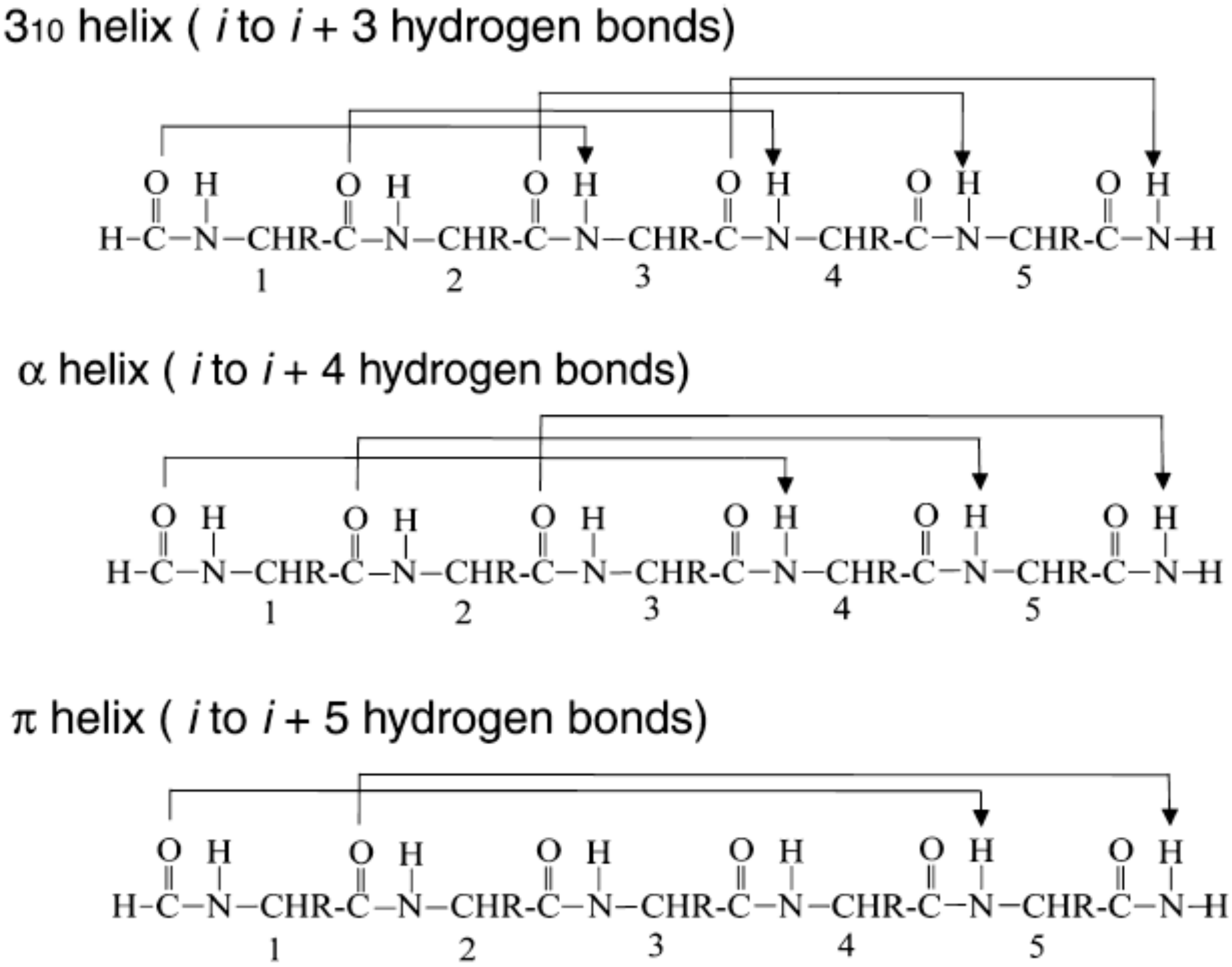
- The helix can be viewed as a stacked array of peptide planes hinged at the α-carbons and approximately, but not quite parallel to the helix.

- To achieve this secondary structure the approximate torsion angles are Φ = -65°; Ψ = -40° based on thousands of water soluble protein structures.



Helices

- Residues & Atoms per turn:
  - α-Helix: 3.6 residues & 13 atoms (3.6_{13} helix)
  - 3_{10}-Helix: 3.2 residues & 10 atoms (3.2_{10} helix)
  - π-Helix: 4.4 residues & 16 atoms (4.4_{16} helix)



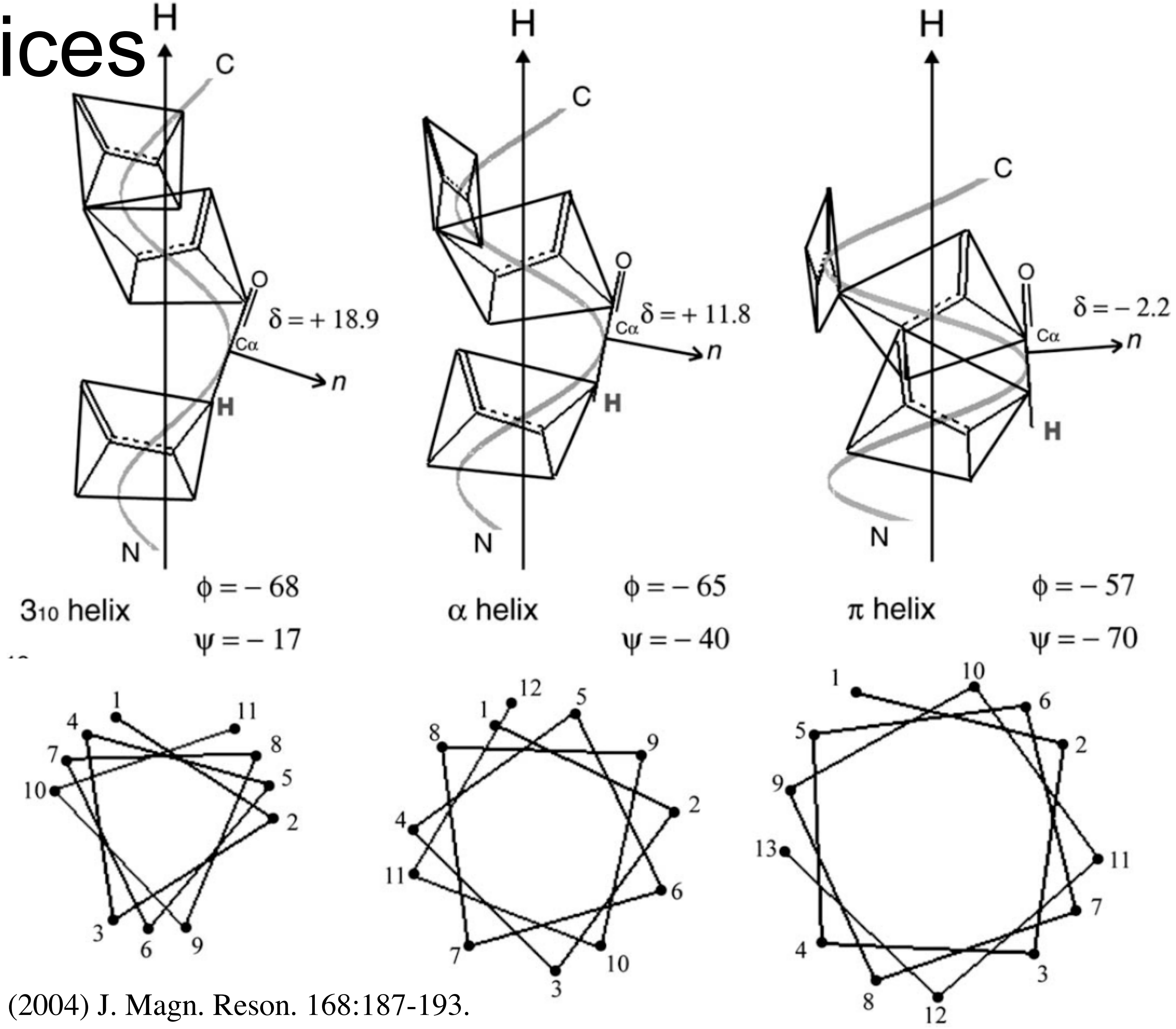
3_{10} helix (*i* to *i* + 3 hydrogen bonds)

α helix (*i* to *i* + 4 hydrogen bonds)

π helix (*i* to *i* + 5 hydrogen bonds)

Kim & Cross (2004) J. Magn. Reson. 168: 187-193.

Helices



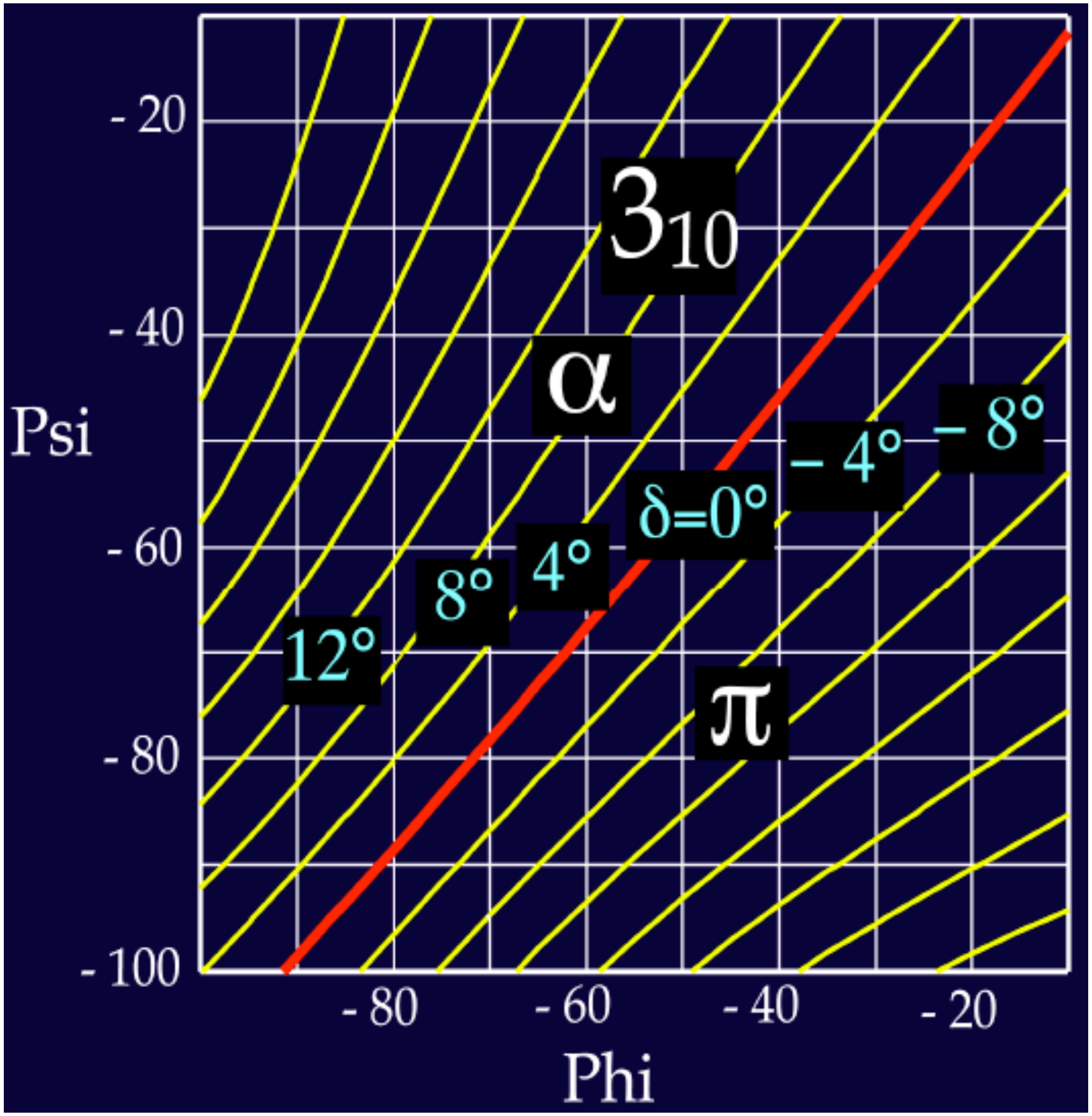
Kim & Cross (2004) J. Magn. Reson. 168:187-193.

The Rhamachandran-delta diagram

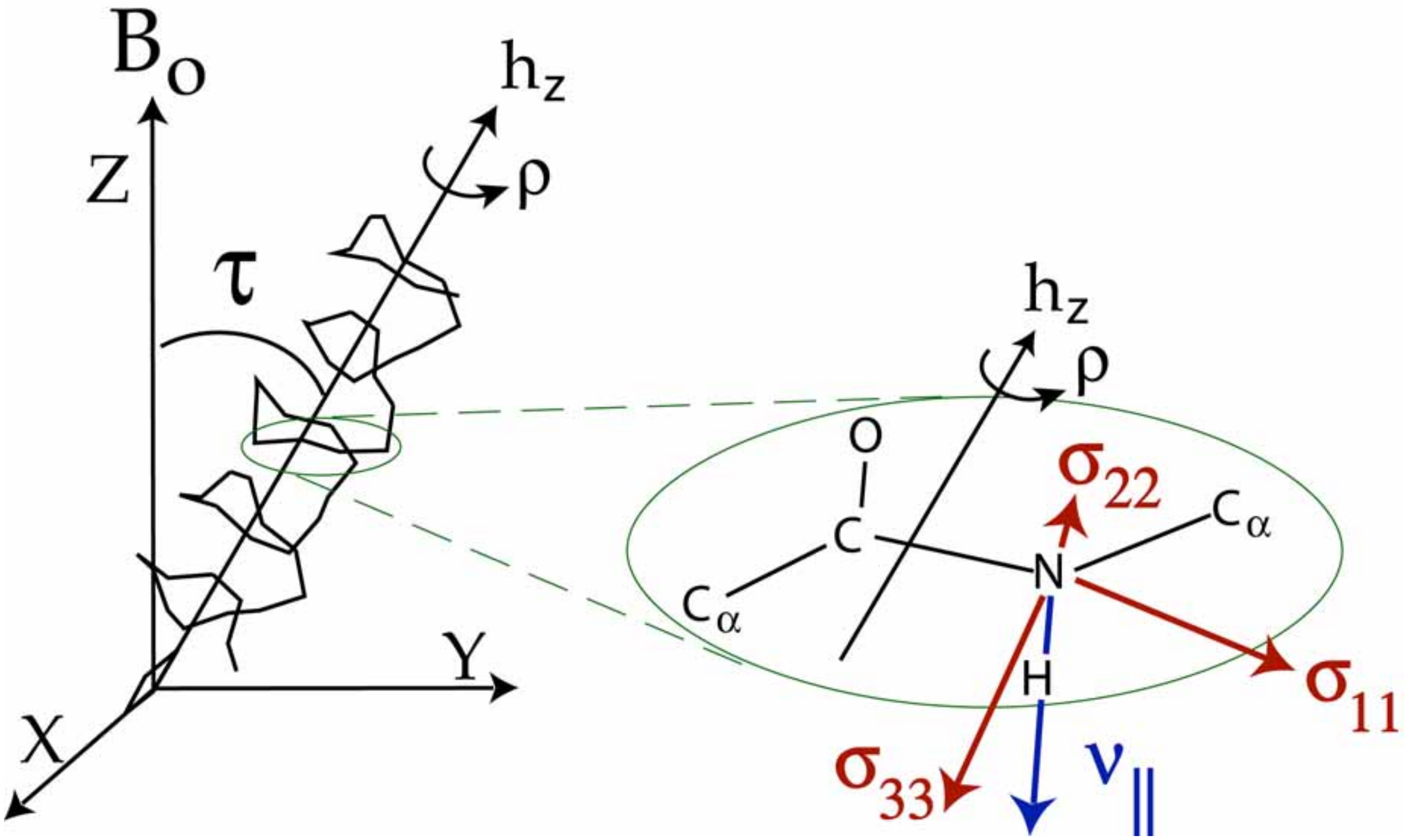
- **For regular helical structures it is possible to draw lines of constant peptide plane tilt angle onto the Rhamachandran diagram.**

- **The very approximate positions of 3_{10}, α and π helices is displayed**

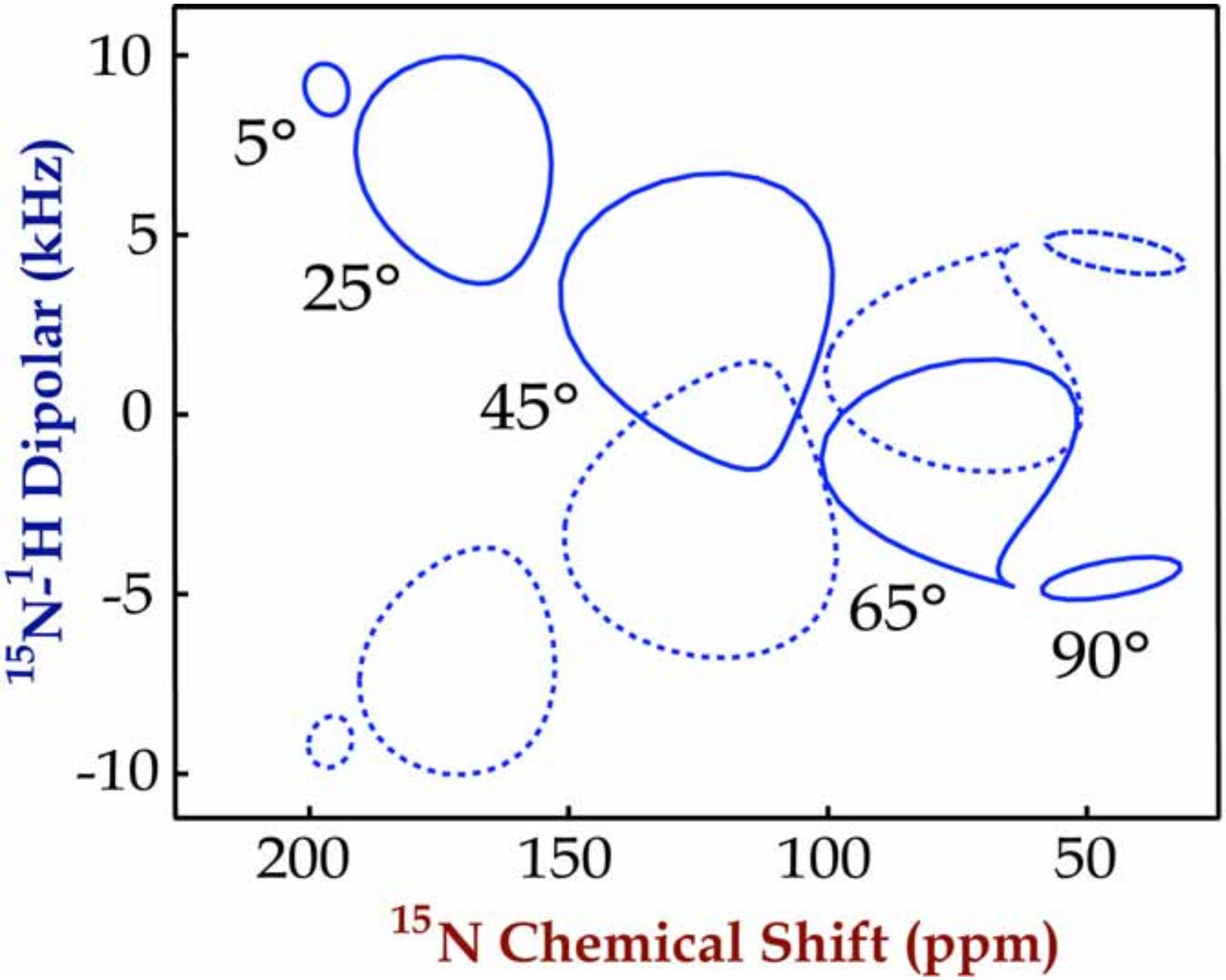
- **Note that the π helix can have a negative peptide plane tilt angle**



Calculation of PISA Wheels

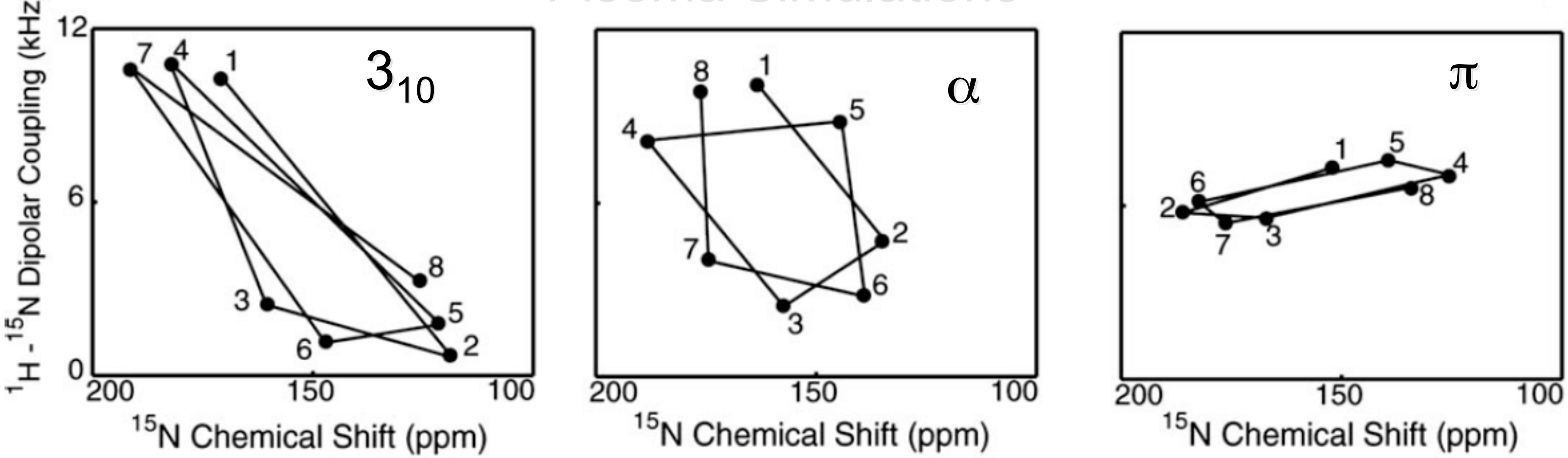


Calculation of PISA Wheels

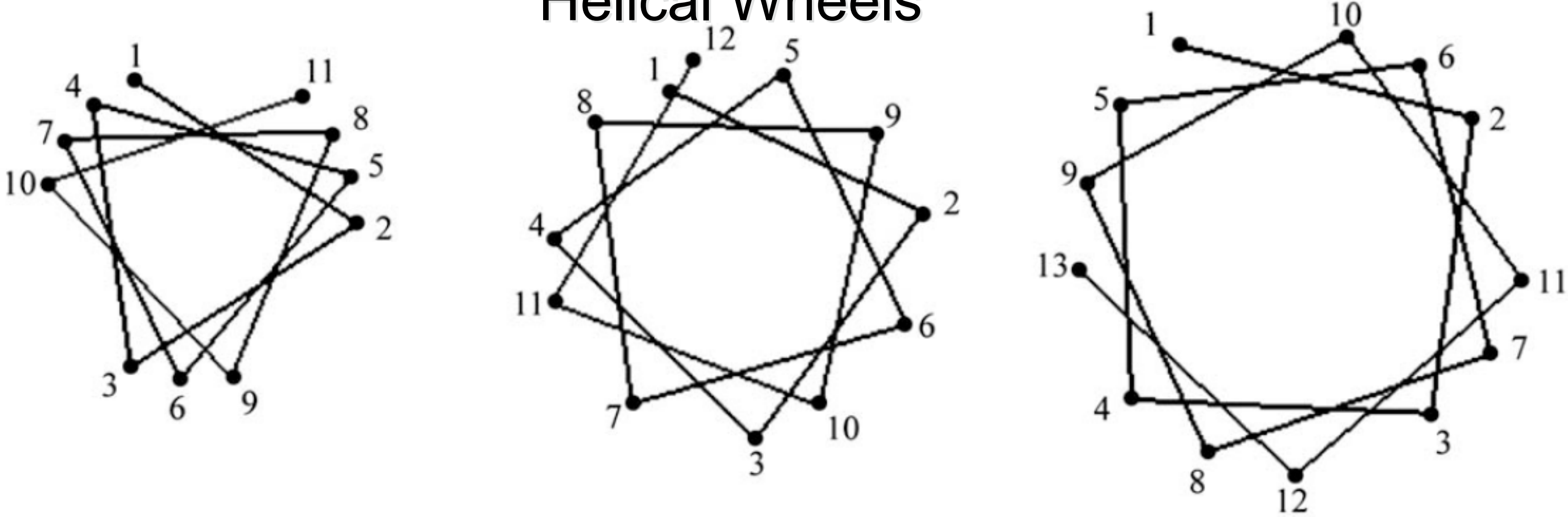


Helices

Pisema Simulations



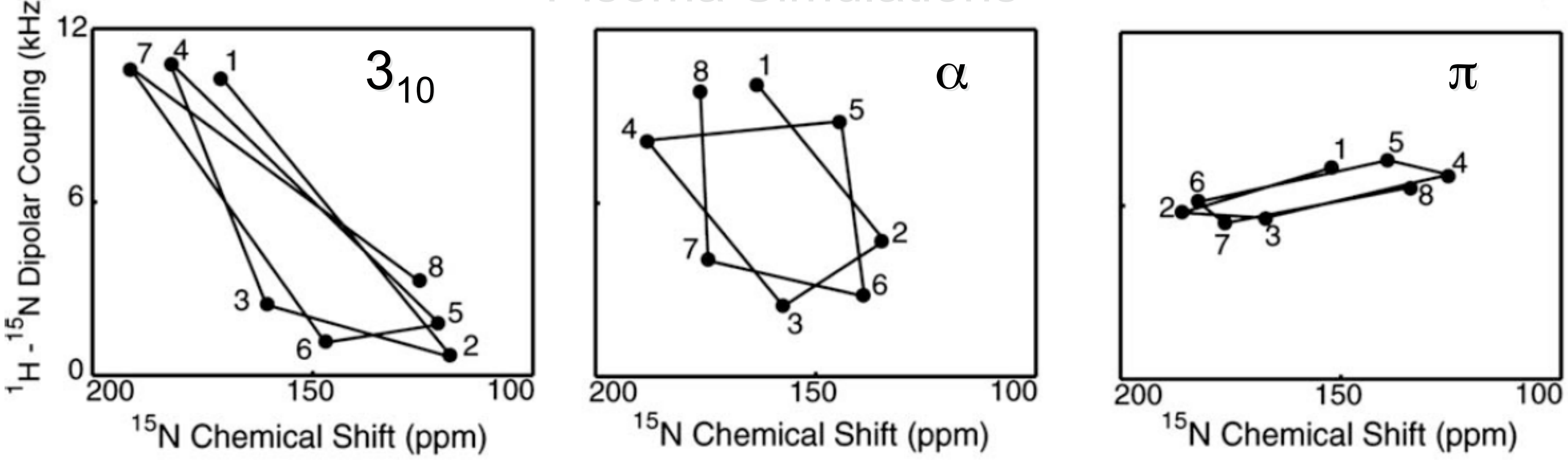
Helical Wheels

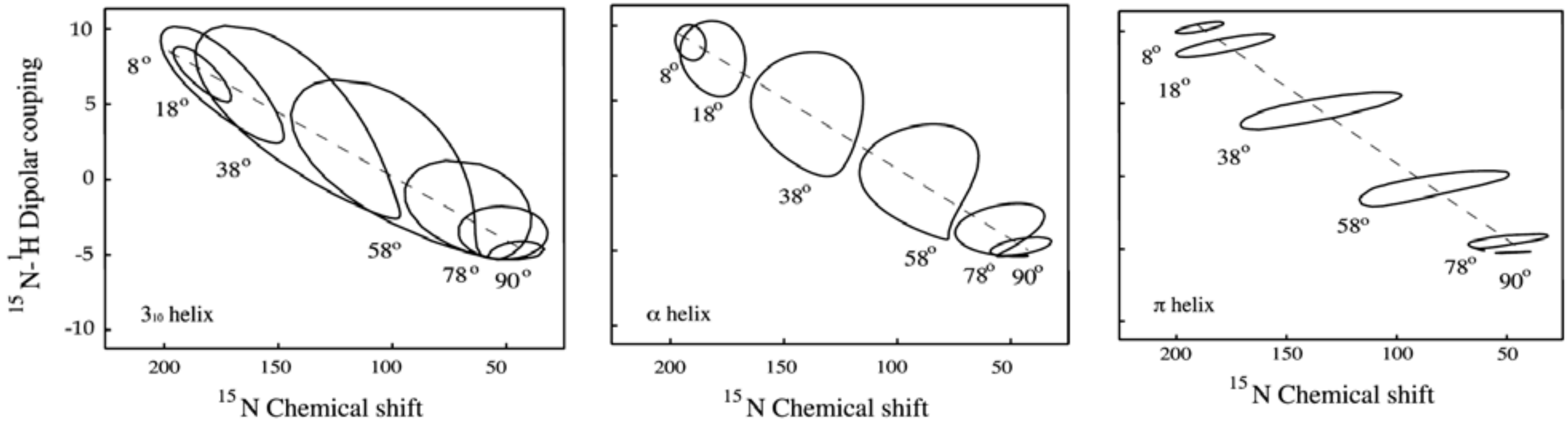


Kim & Cross (2004) J. Magn. Reson. 168:187-193.

Helices

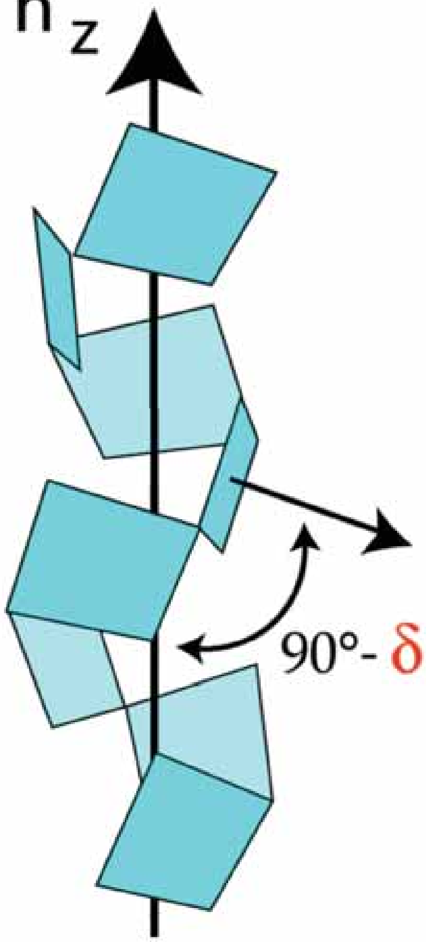
Pisema Simulations

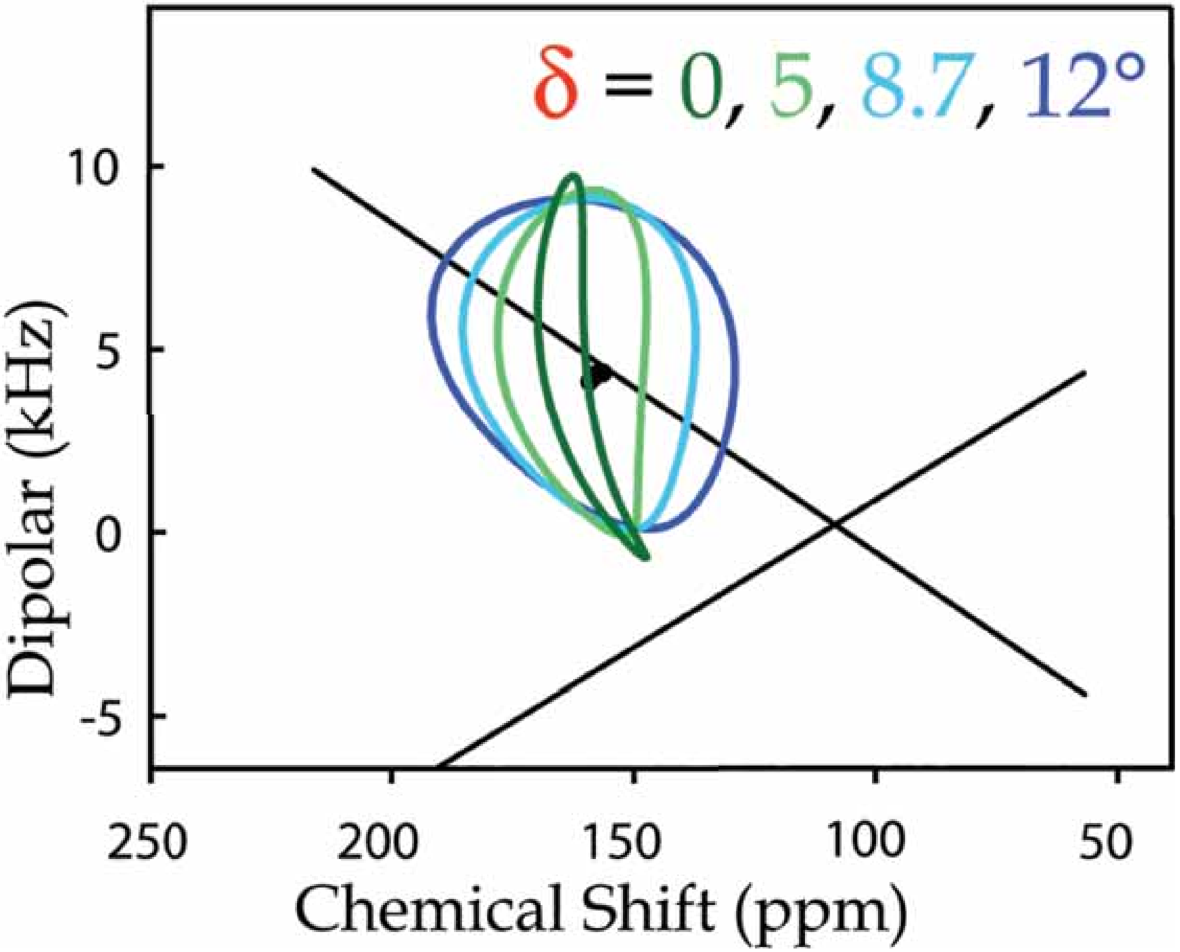




Kim & Cross (2004) J. Magn. Reson. 168:187-193.

Effect of Peptide Plane Tilt on PISA Wheel

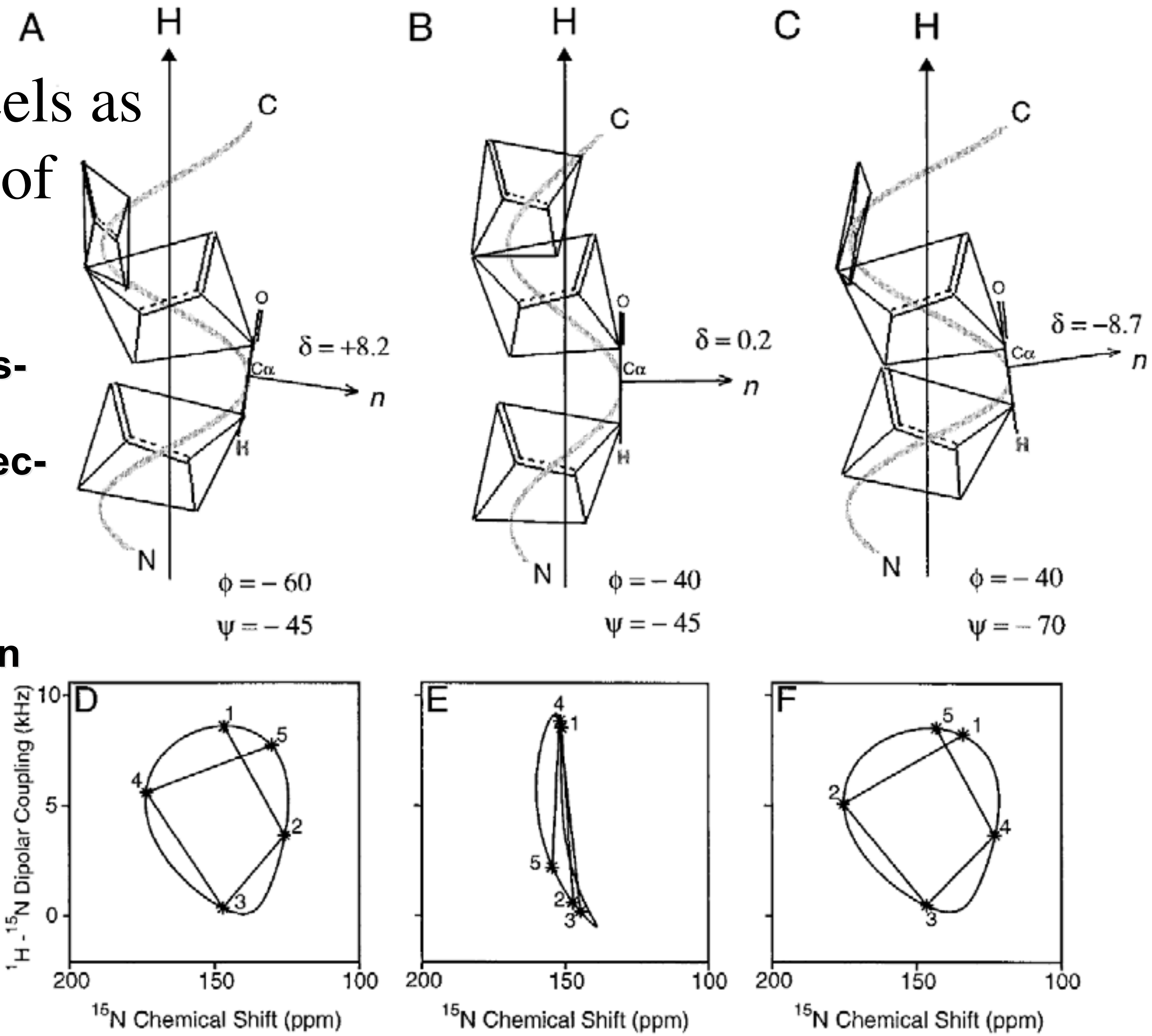




- The wheel continuously expands with increasing delta value

PISA Wheels as a function of delta

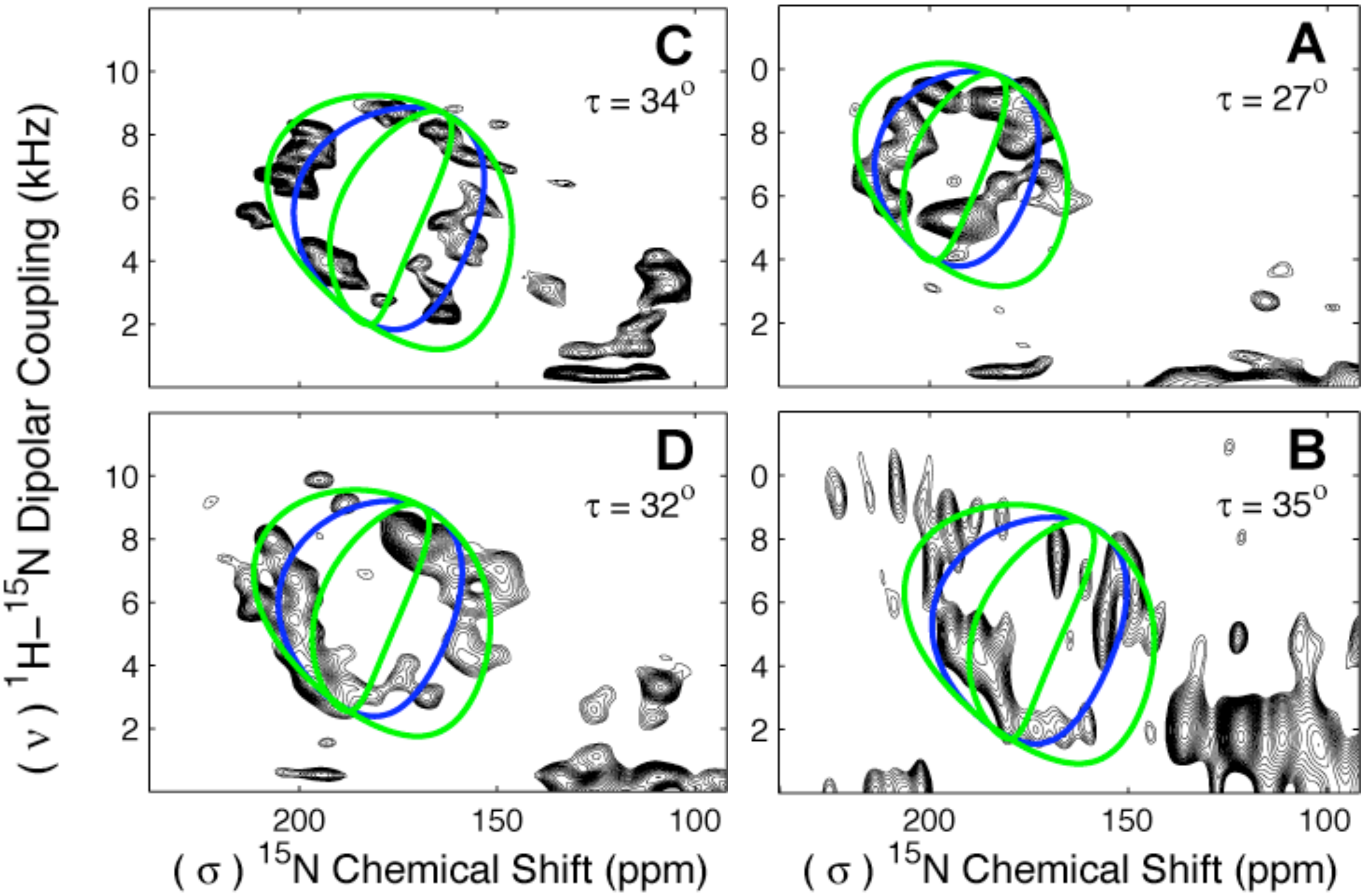
- **Here we illustrate that the rotational direction of the sequential pattern reverses when the delta value becomes negative.**



Kim & Cross (2002) J. Magn. Reson. 168:187-193.

Observed PISA Wheels: δ = 9±4°

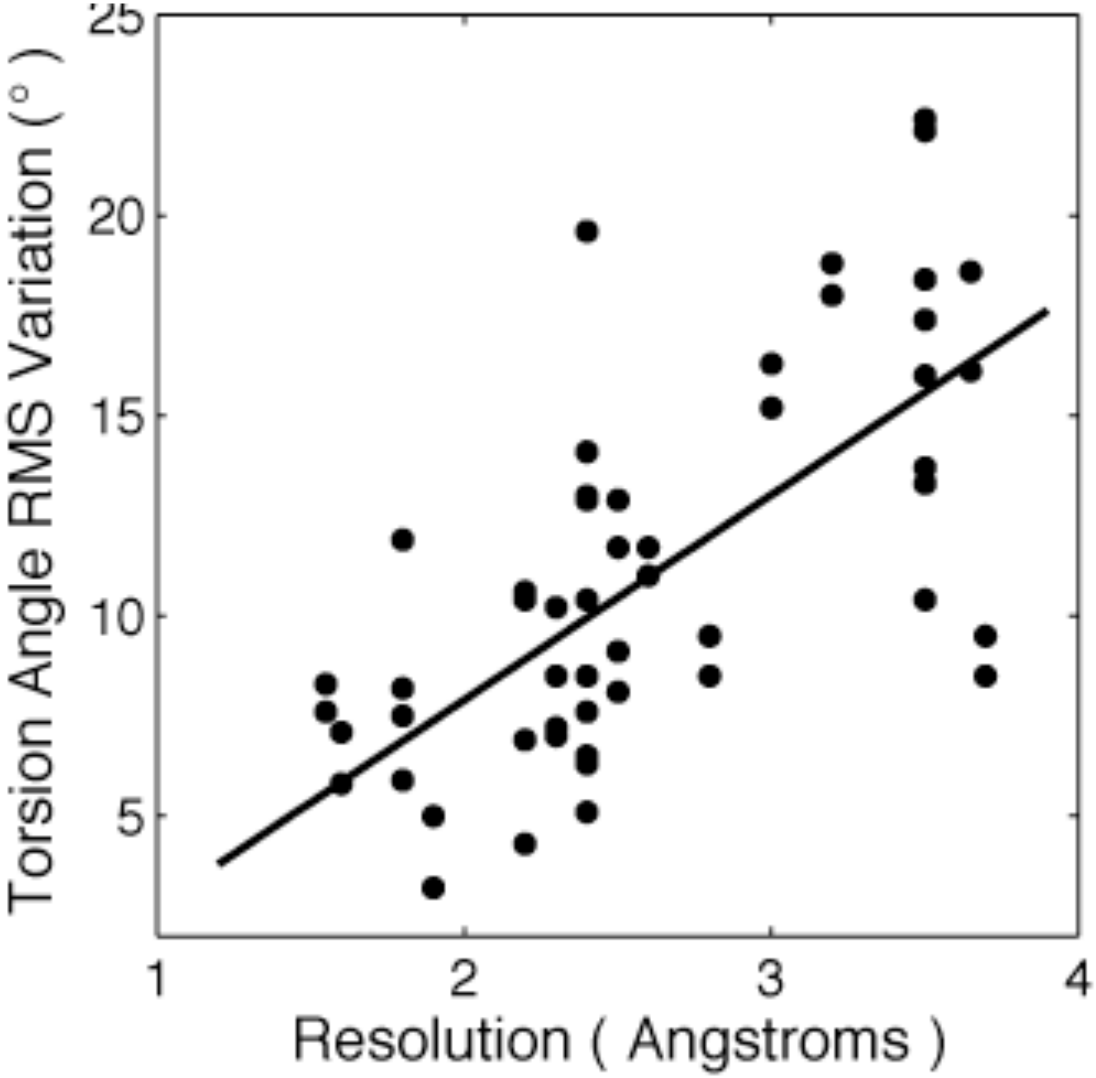
»» It is clear that the the torsion angles are very uniform in a membrane environment for a variety of proteins



Torsion Angle Data for Membrane Proteins from x-ray crystallographic data

- Data for trans-membrane α-helices as a function of crystallographic resolution.

»» High resolution crystal data supports very uniform torsion Angles in sharp contrast with the water soluble proteins.

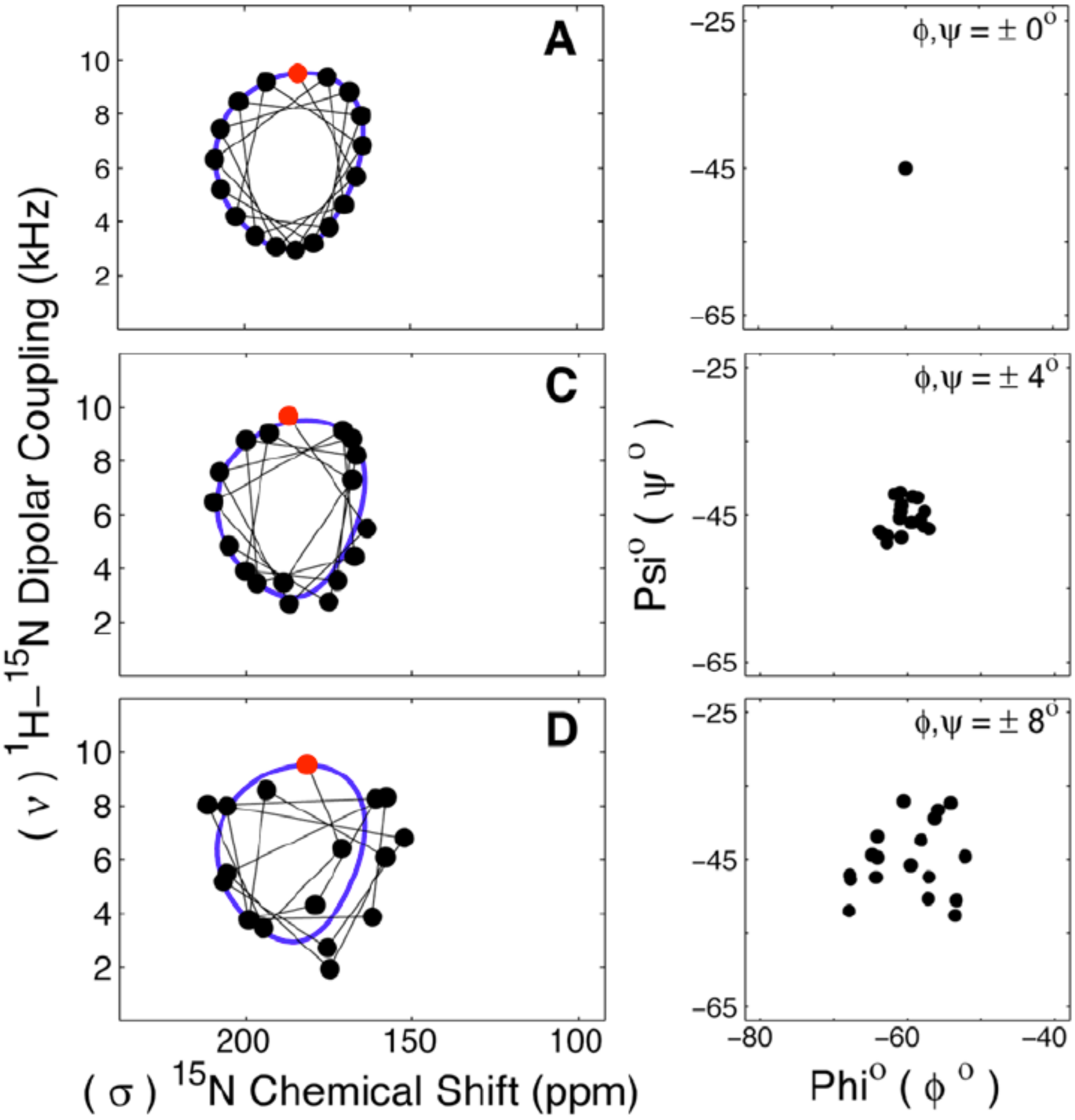


Dependence of PISA Wheels on Local Conformation

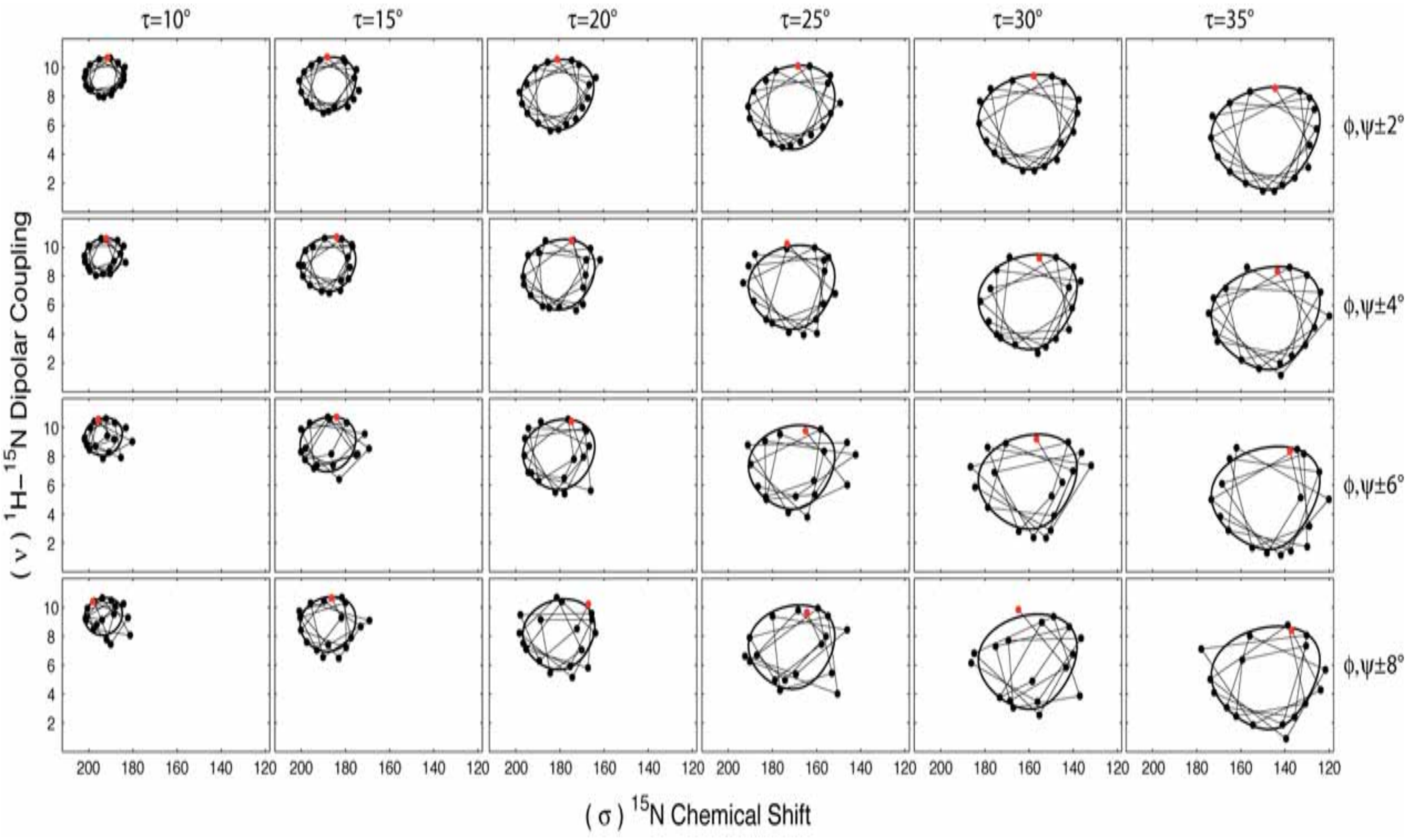
$\phi = -45 \pm 0^\circ$
$\psi = -60 \pm 0^\circ$

$\phi = -45 \pm 4^\circ$
$\psi = -60 \pm 4^\circ$

$\phi = -45 \pm 8^\circ$
$\psi = -60 \pm 8^\circ$

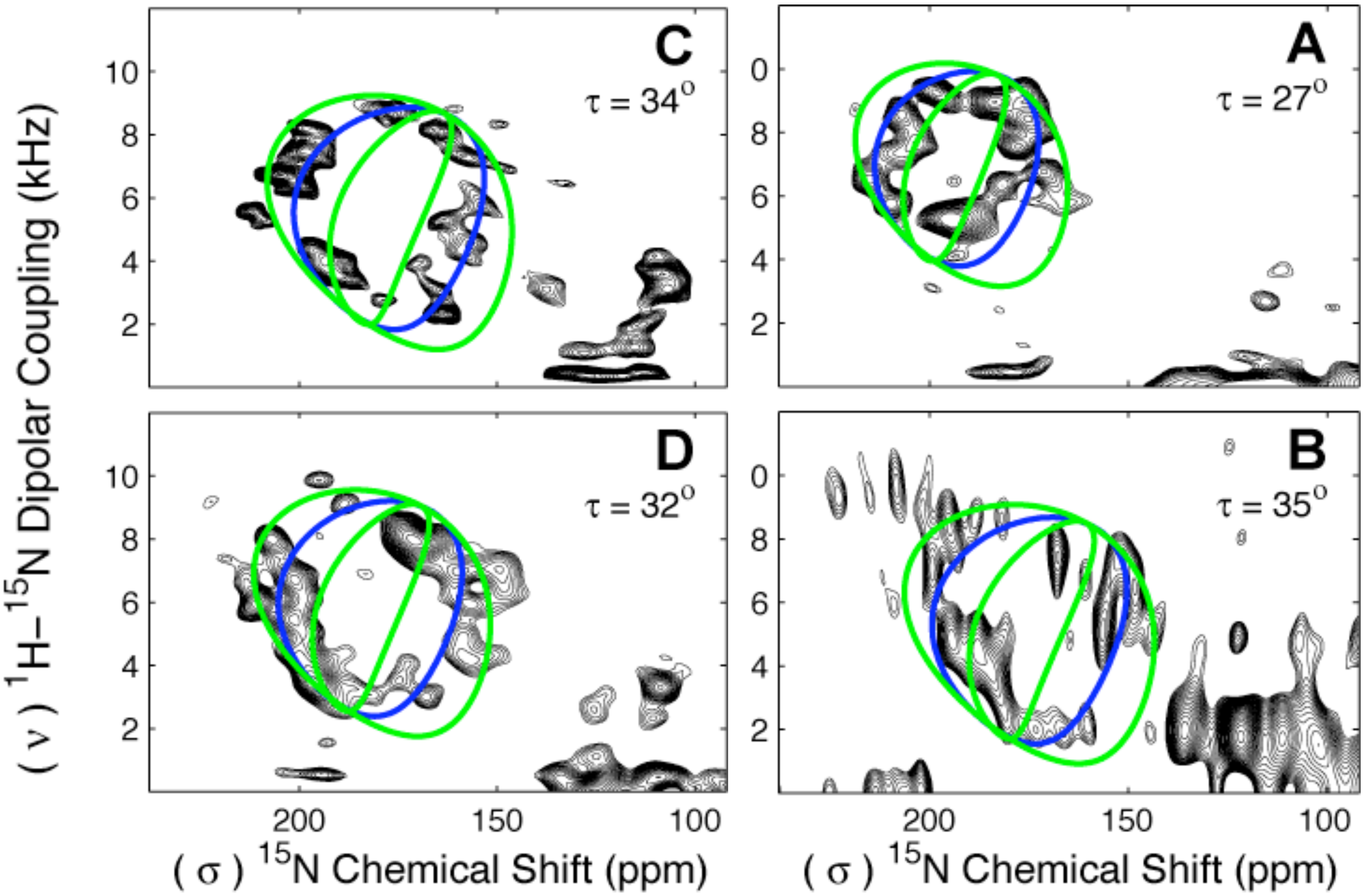


Calculation of PISA Wheels



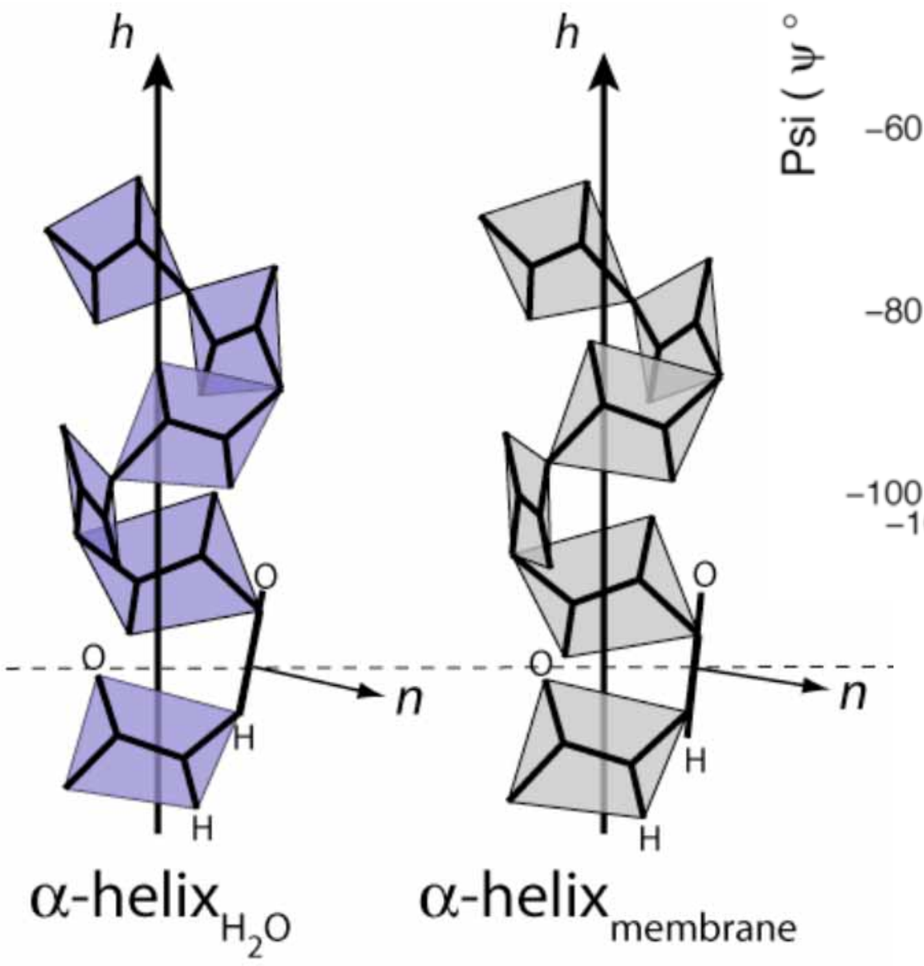
PISA Wheels: δ = 8±4°

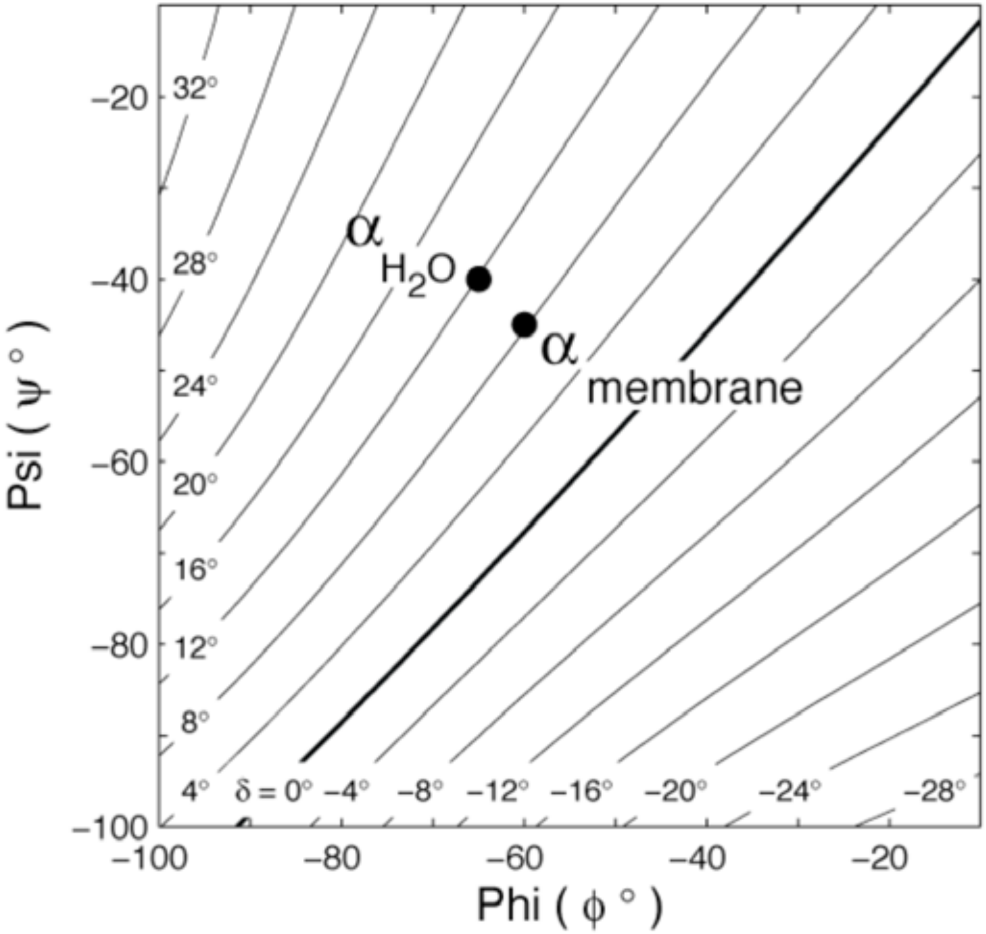
»» It is clear that the δ ≠ 12° - the torsion angles are different in a membrane environment



Helical Torsion Angles

»» The difference between ϕ,ψ angles of -40°, -65° and -45°, -60° is quite small, but the carbonyl oxygen becomes less exposed.





»» Rhamachandran diagram showing the d values for the tilt of the carbonyl bond with respect to the helix axis

Hydrogen Bond geometry

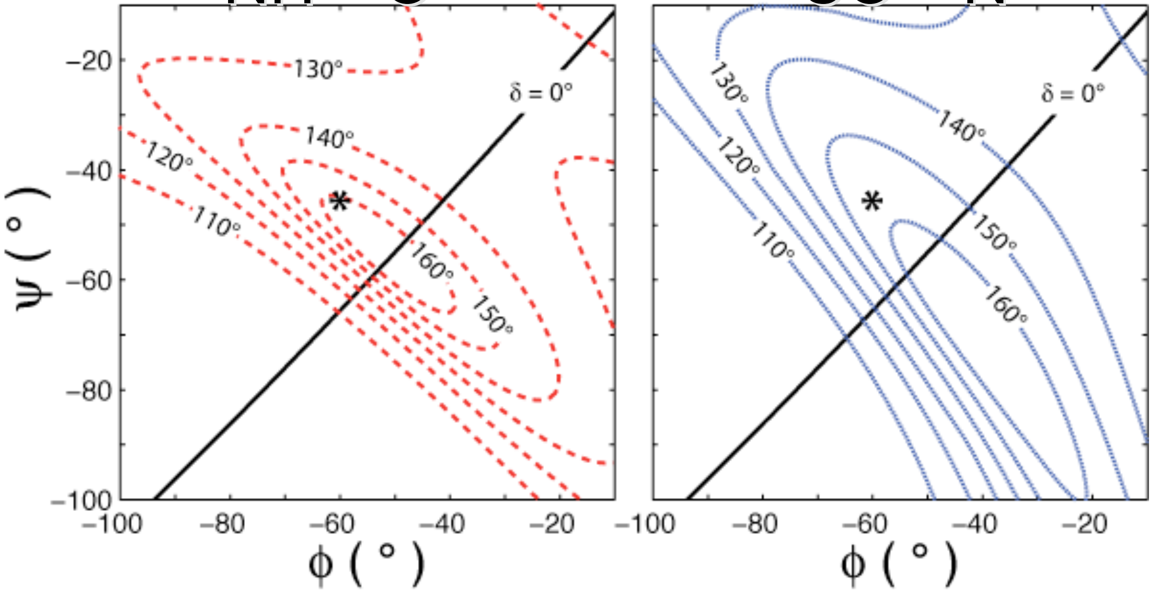
- NH•••O angles are typically ≥140°

- CO•••N angles are typically between 140 and 160°

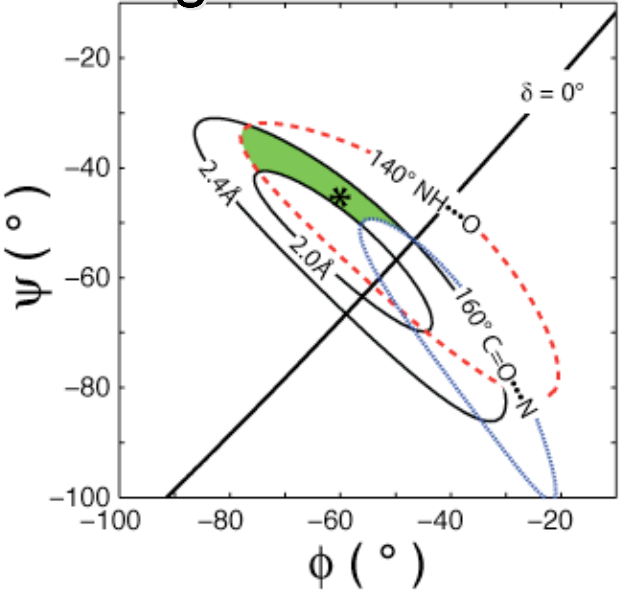
- H•••O distances are typically between 2.0 and 2.4Å

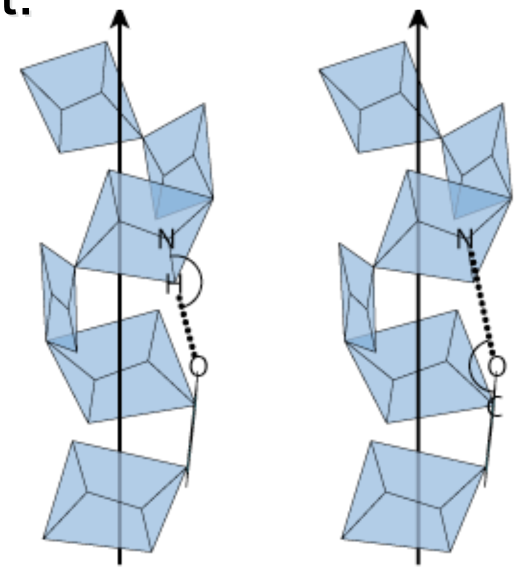
»» A preferred region of ϕ/ψ space is defined

NH•••O CO•••N

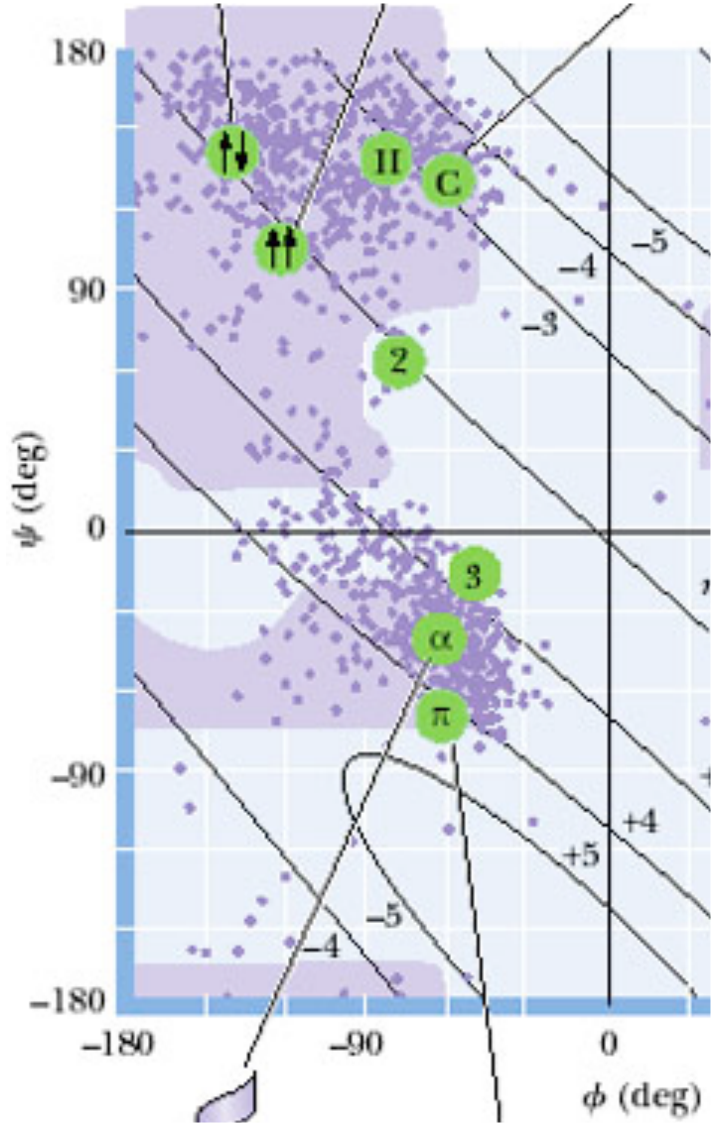


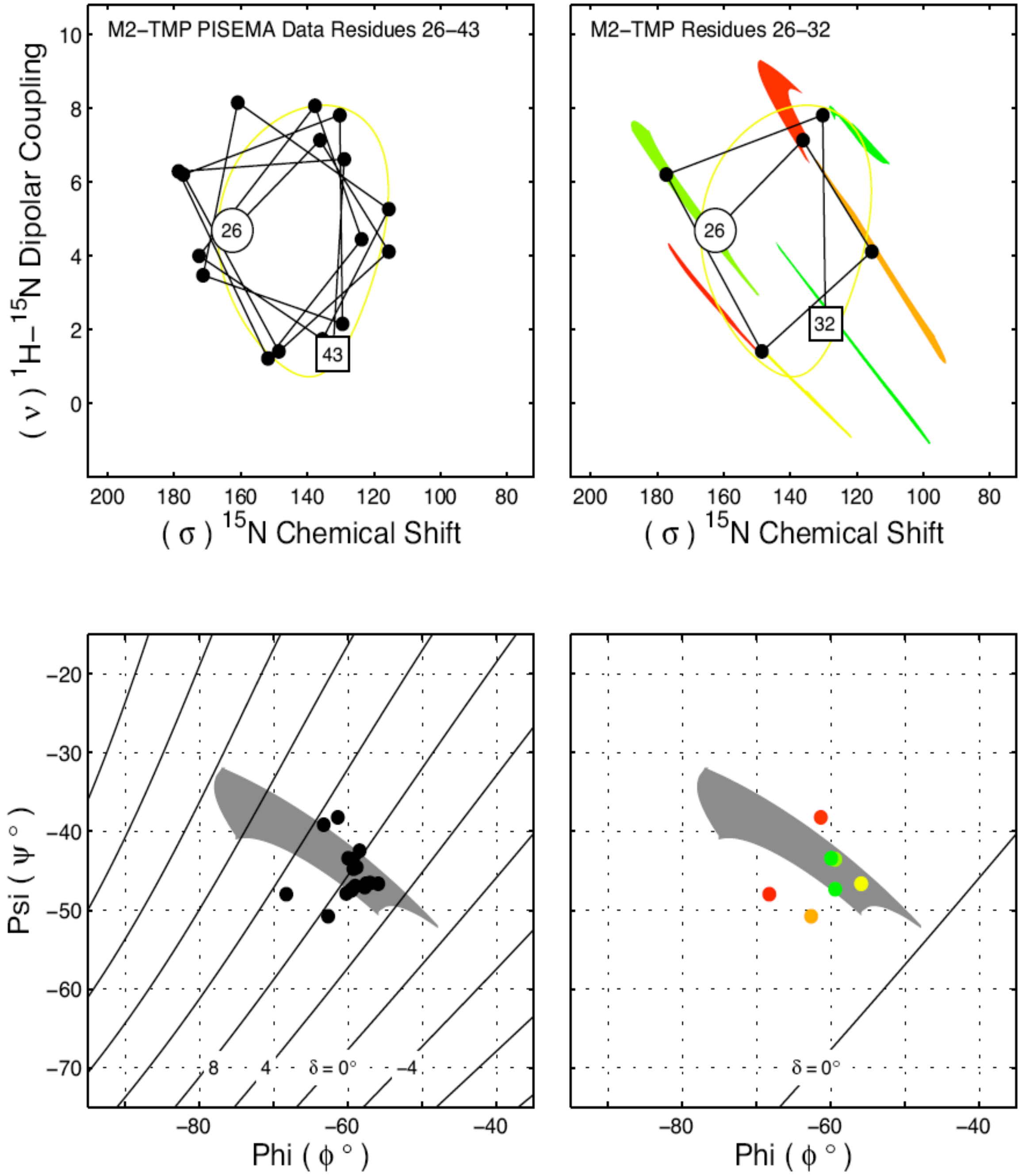
Angles + H•••O dist.



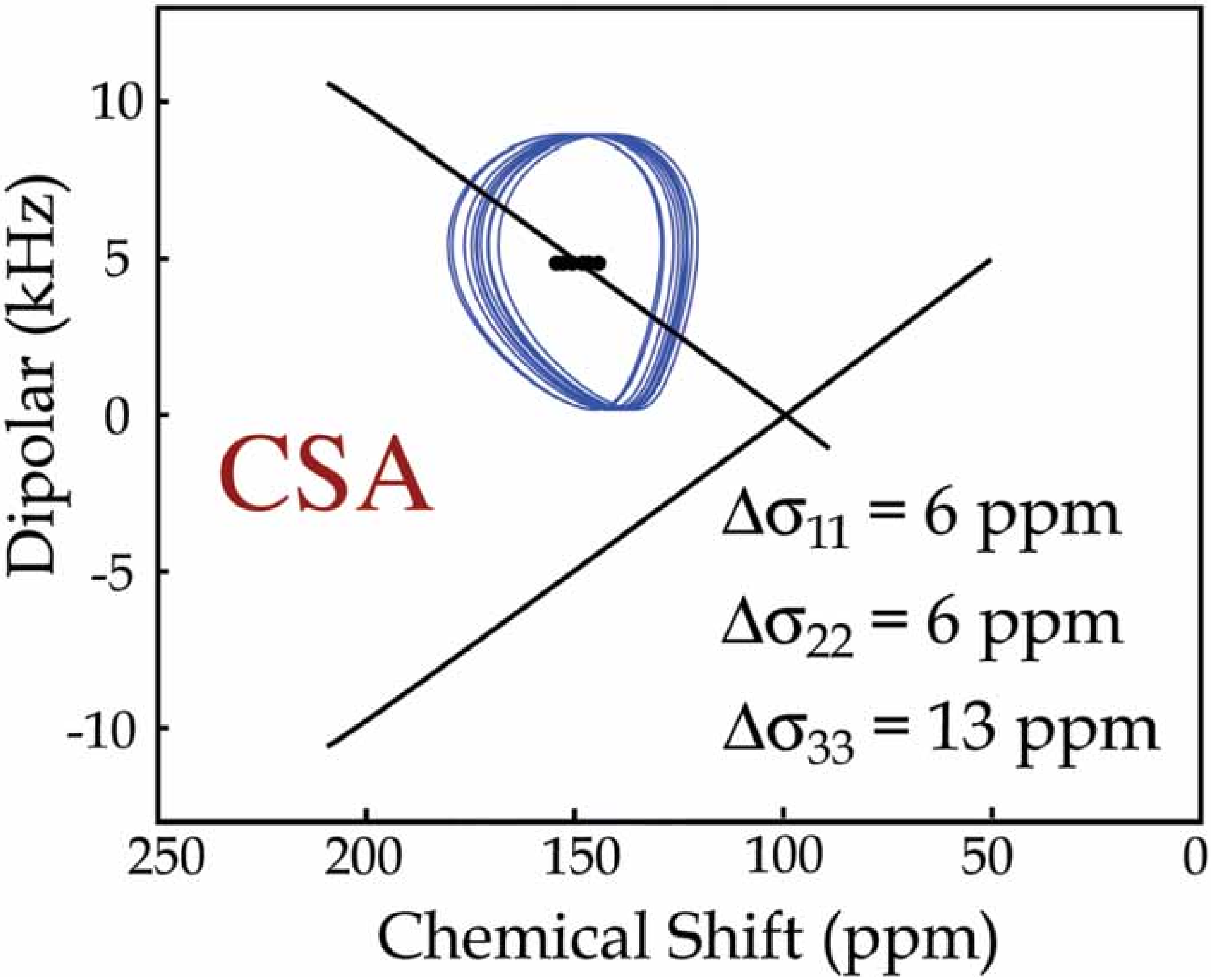


Conformational Variation does not explain all of the data scatter





Effect of Chemical Shift Anisotropy Variation



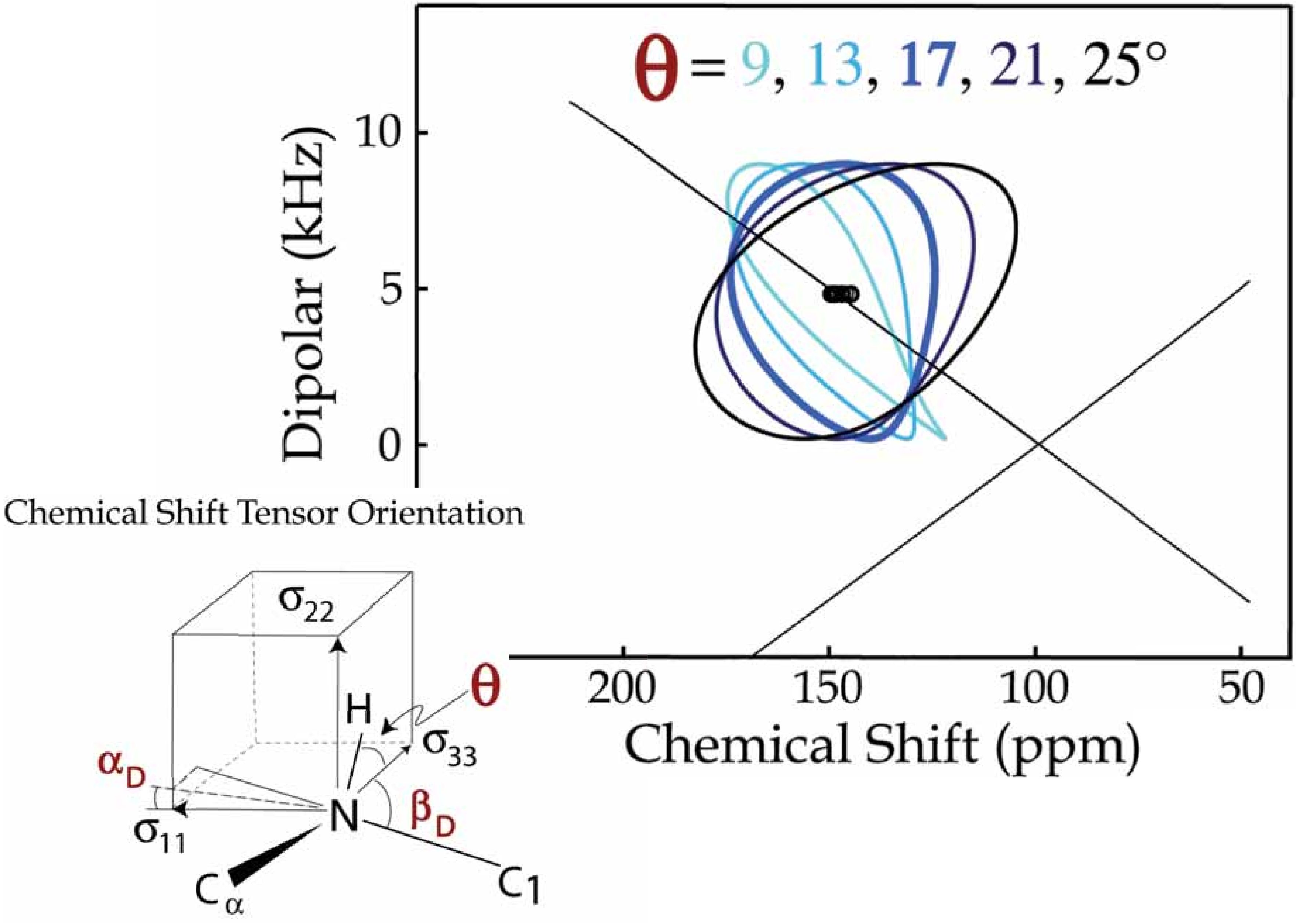
CSA

$\Delta\sigma_{11} = 6$ ppm

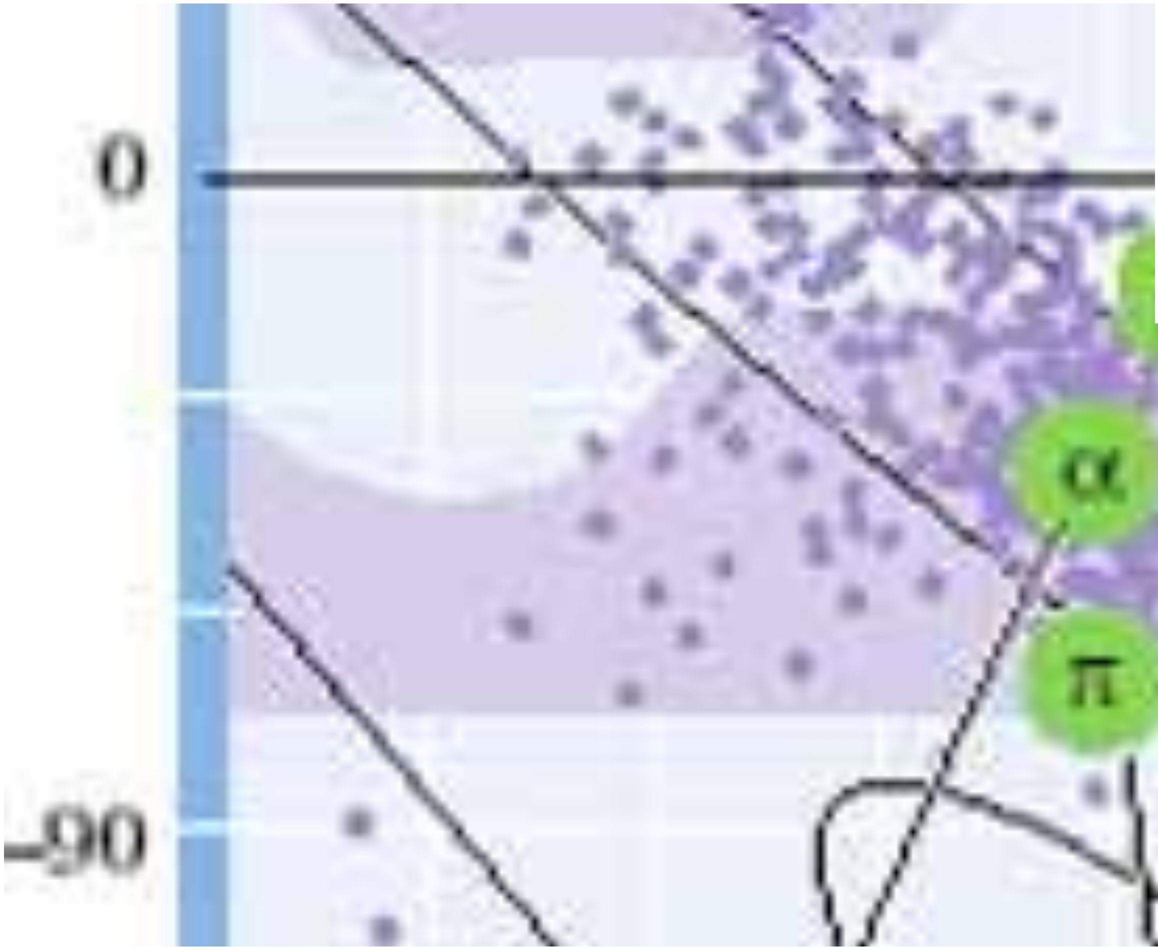
$\Delta\sigma_{22} = 6$ ppm

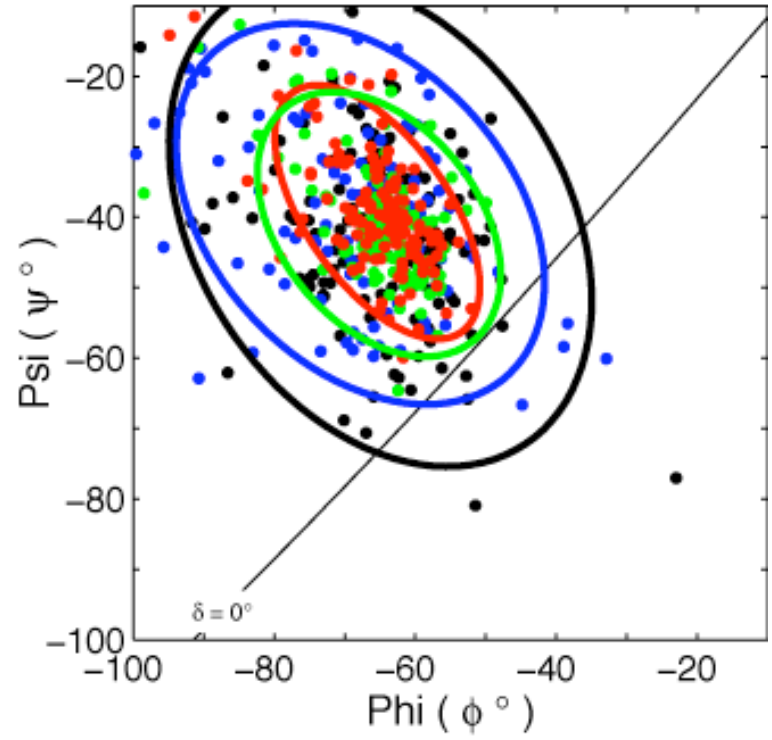
$\Delta\sigma_{33} = 13$ ppm

Effect of the Chemical Shift Tensor Orientation



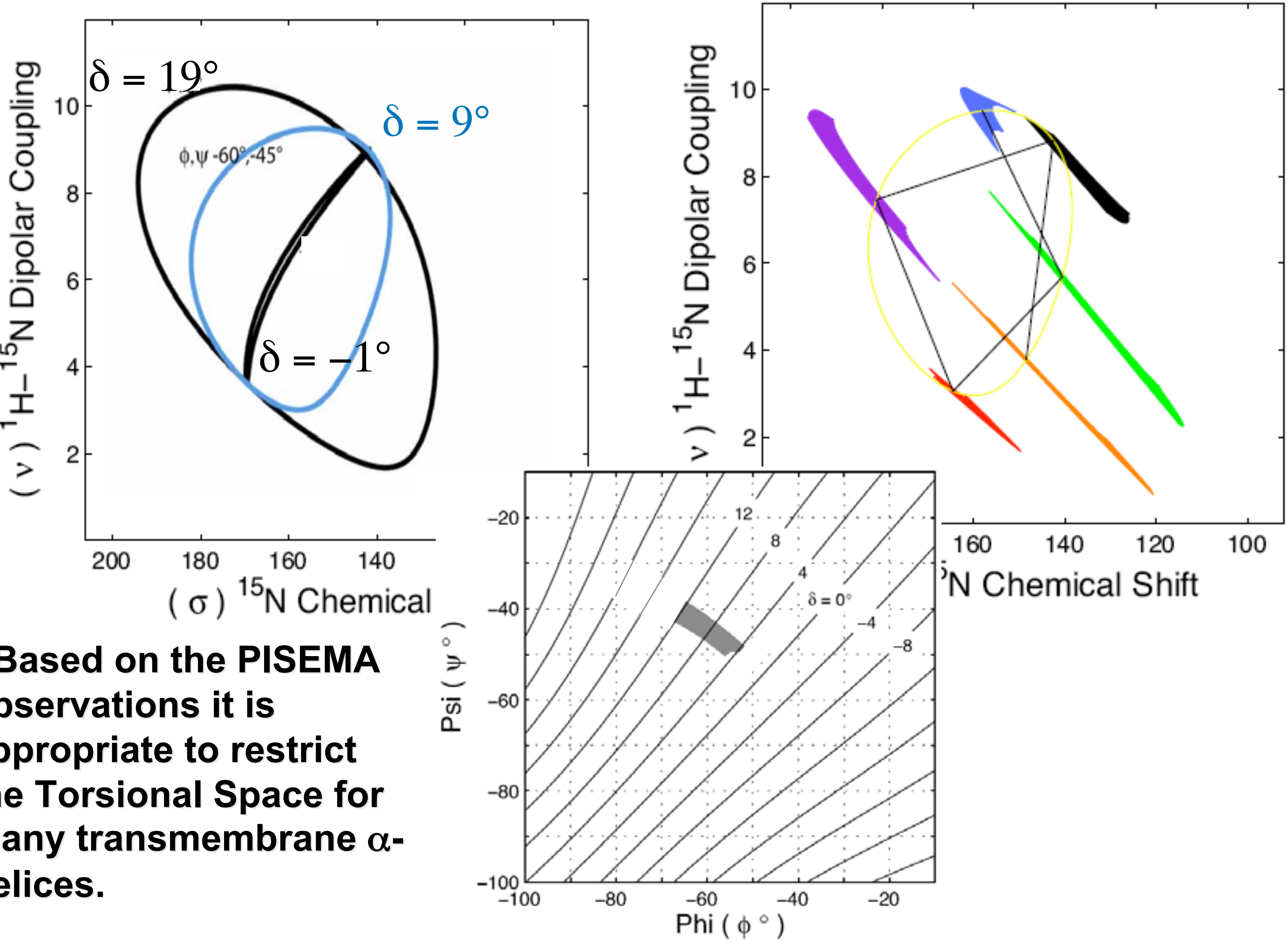
A Dramatic Reduction in Torsion Angle Scatter for High Res Structures





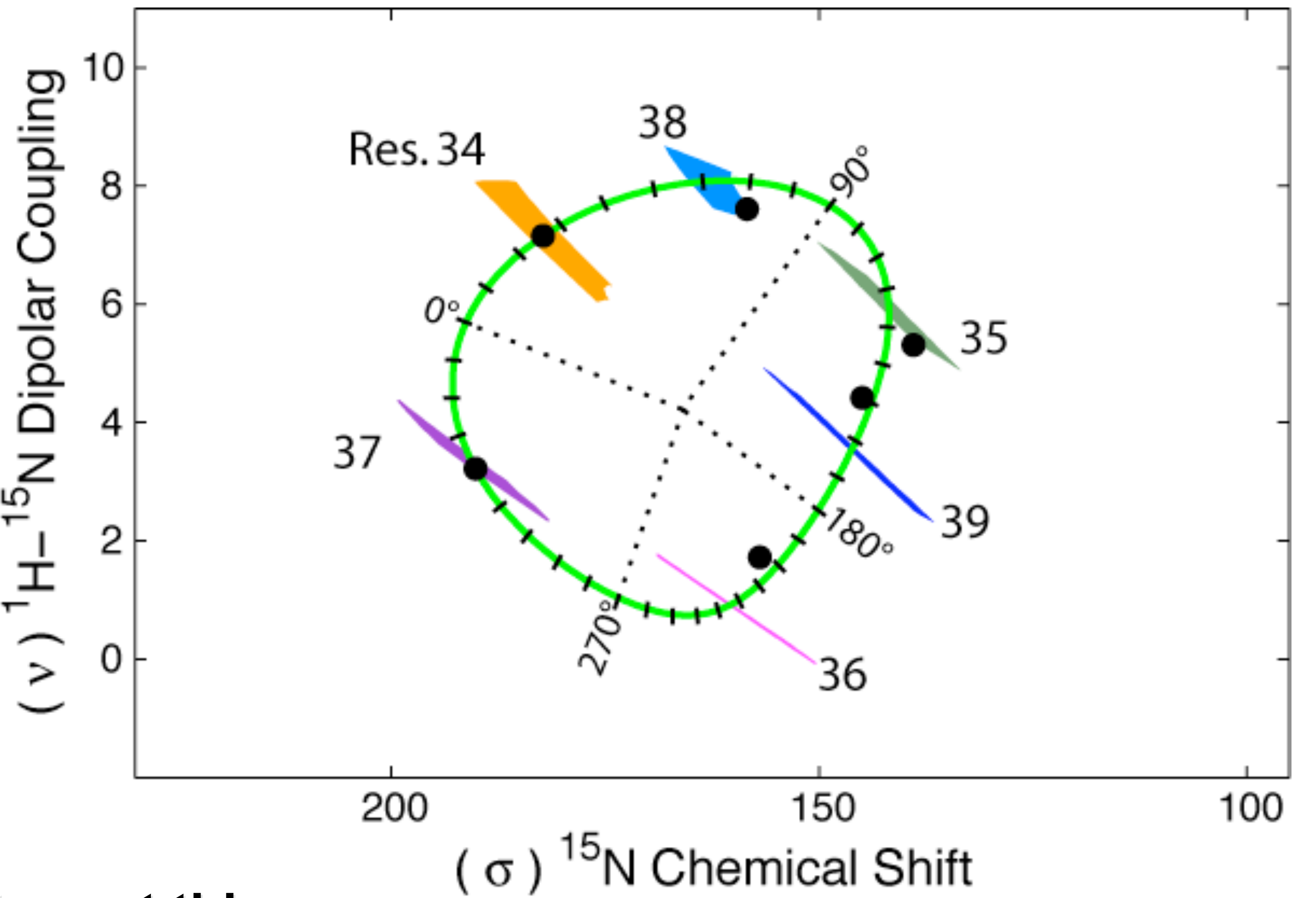
»» Approximately a factor of four reduction in scatter for moderate resolution structures

Defining a more Realistic Torsional Space



- **Based on the PISEMA observations it is appropriate to restrict the Torsional Space for many transmembrane α-helices.**

Revised Torsion Angle Space



»» Two ways to interpret this -

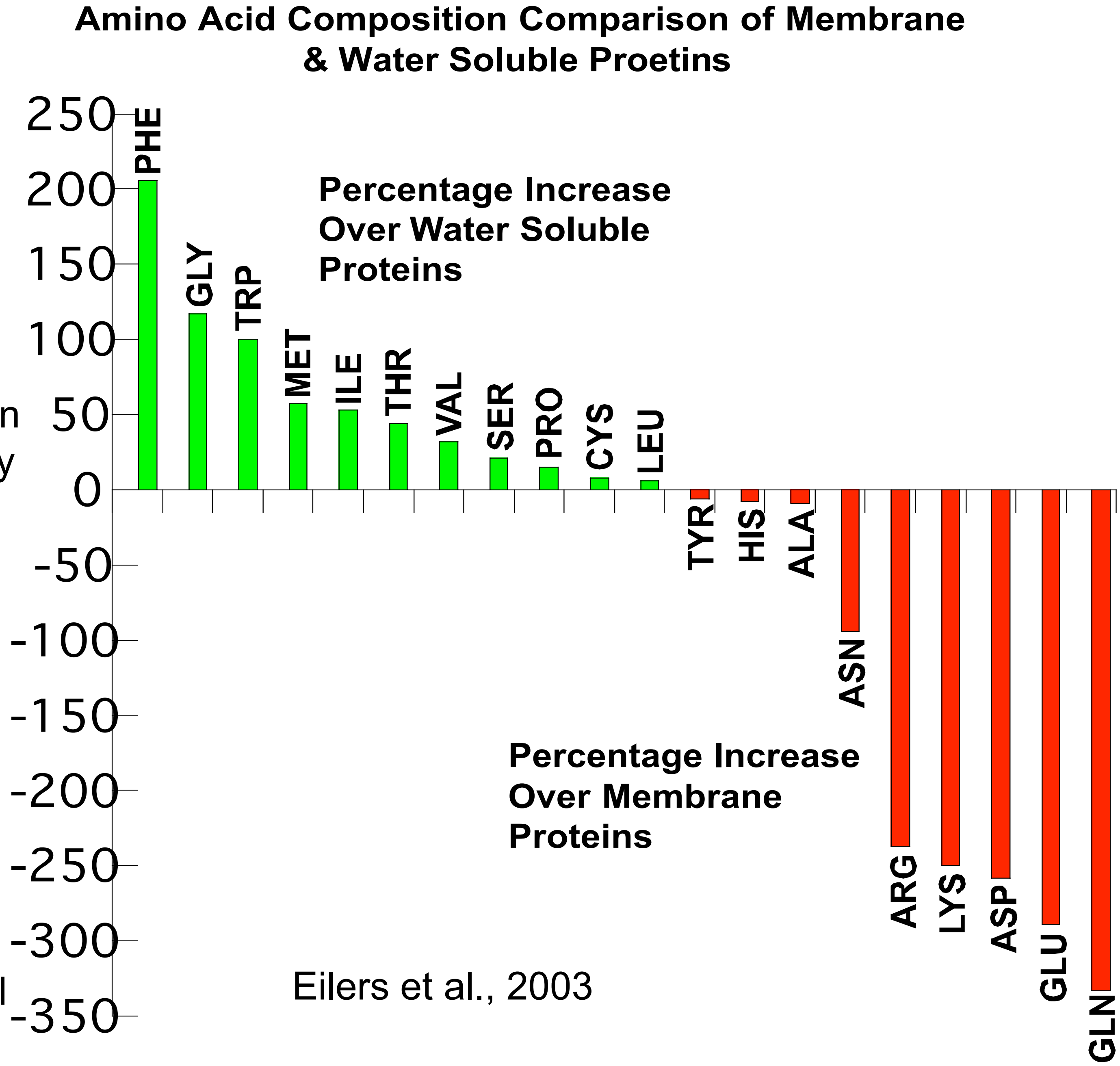
1) a new constraint - δ is restricted to 8±4° or

2) the NH•••O angle is greater than 150°, as opposed to greater than 140°. Moreover, NC•••O angles tend toward 160° and away from 120 and 140°.

»» The result is that the hydrogen bond geometry is dominated by electrostatic terms.

Amino Acids

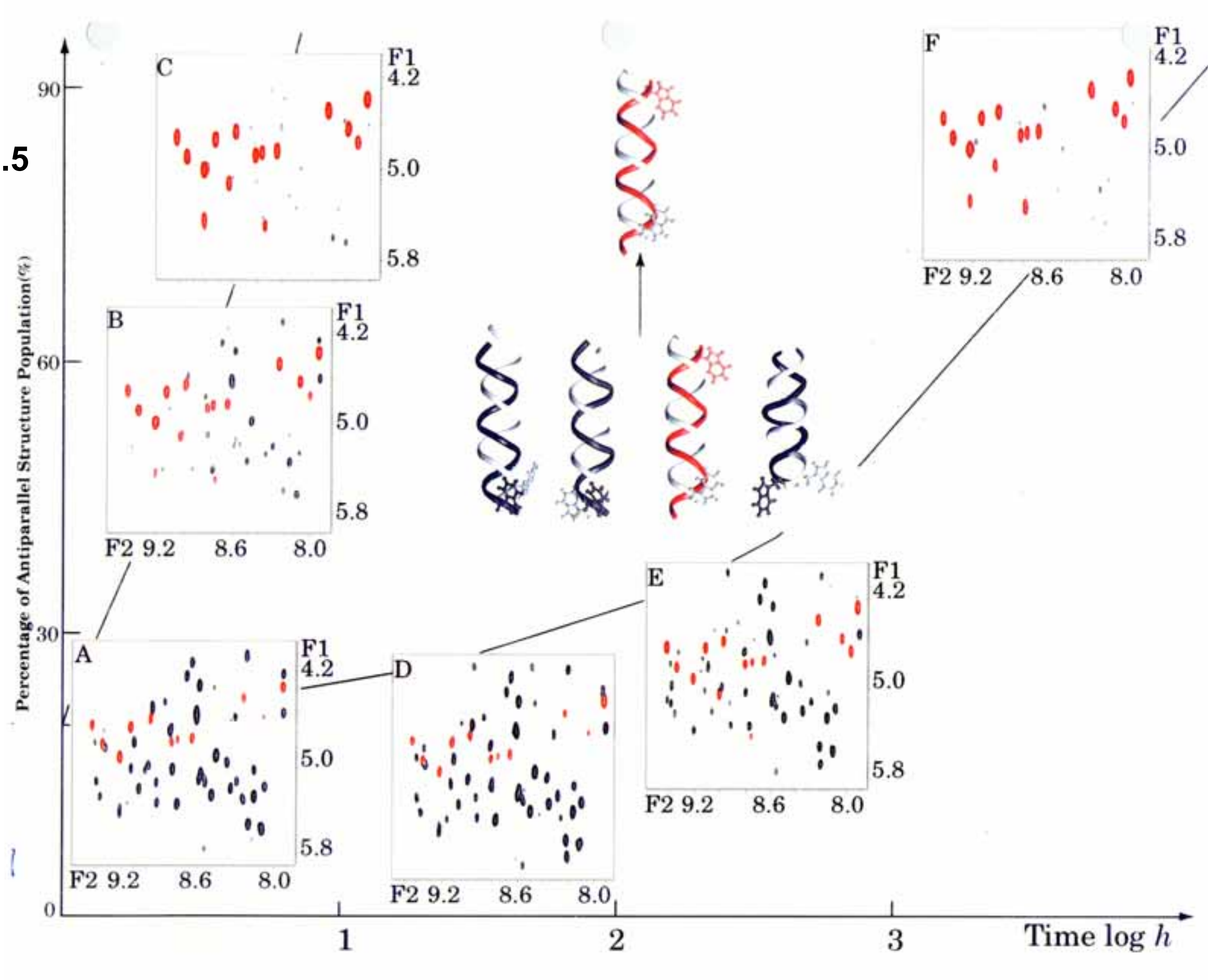
- This is the composition for the TM regions
- Non polar AA is more common in MP
- Some polar AA are also more common in MP e.g. Trp, Gly
- But others are not such as Asn and Gln
- The only charged residue that is as common in MP is His
- Many of these stand-out amino acids have special functions in MP



Eilers et al., 2003

Water Facilitates Conformational Interconversion

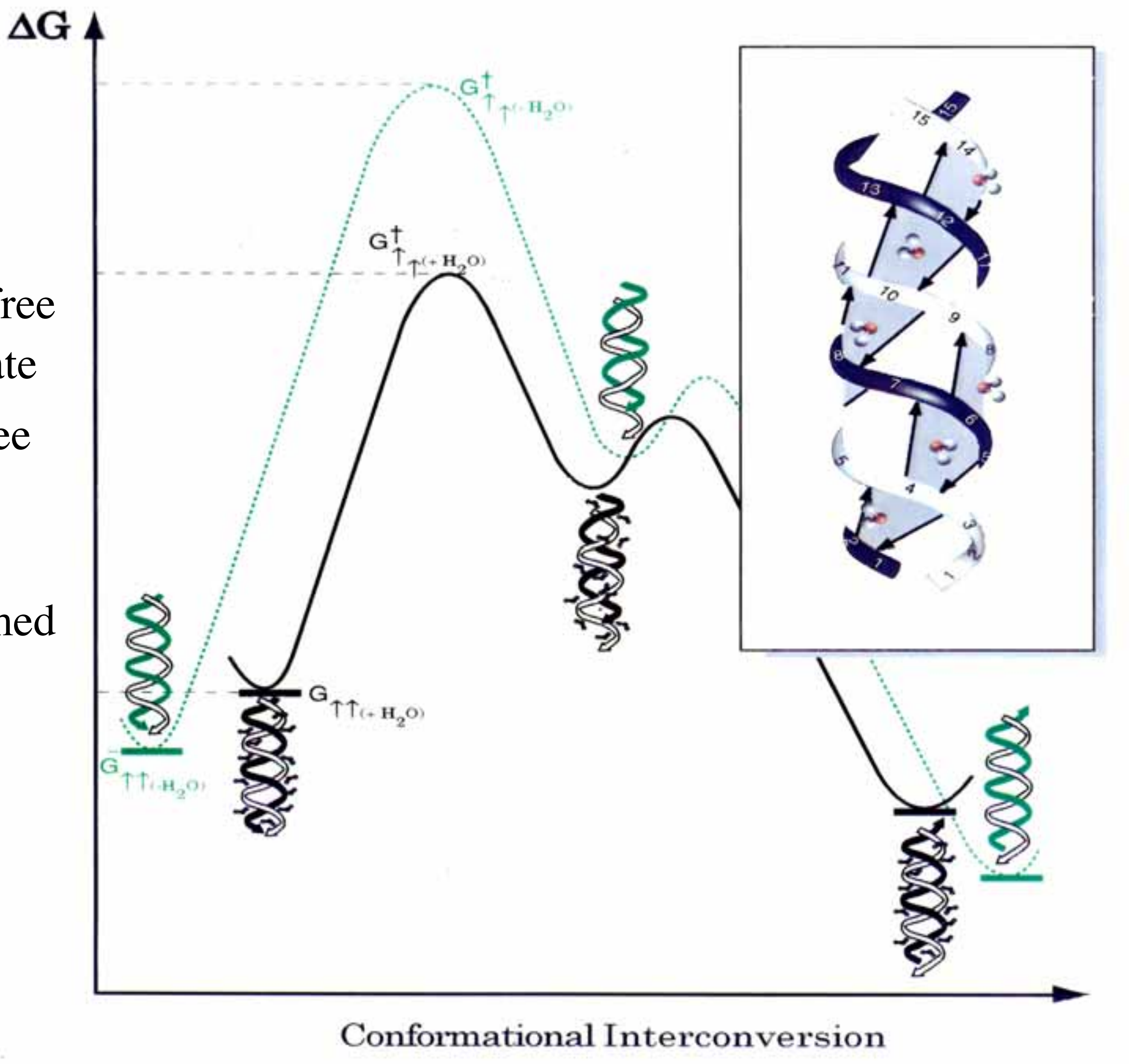
$v \; \alpha \; [H_2O]^{6.5}$



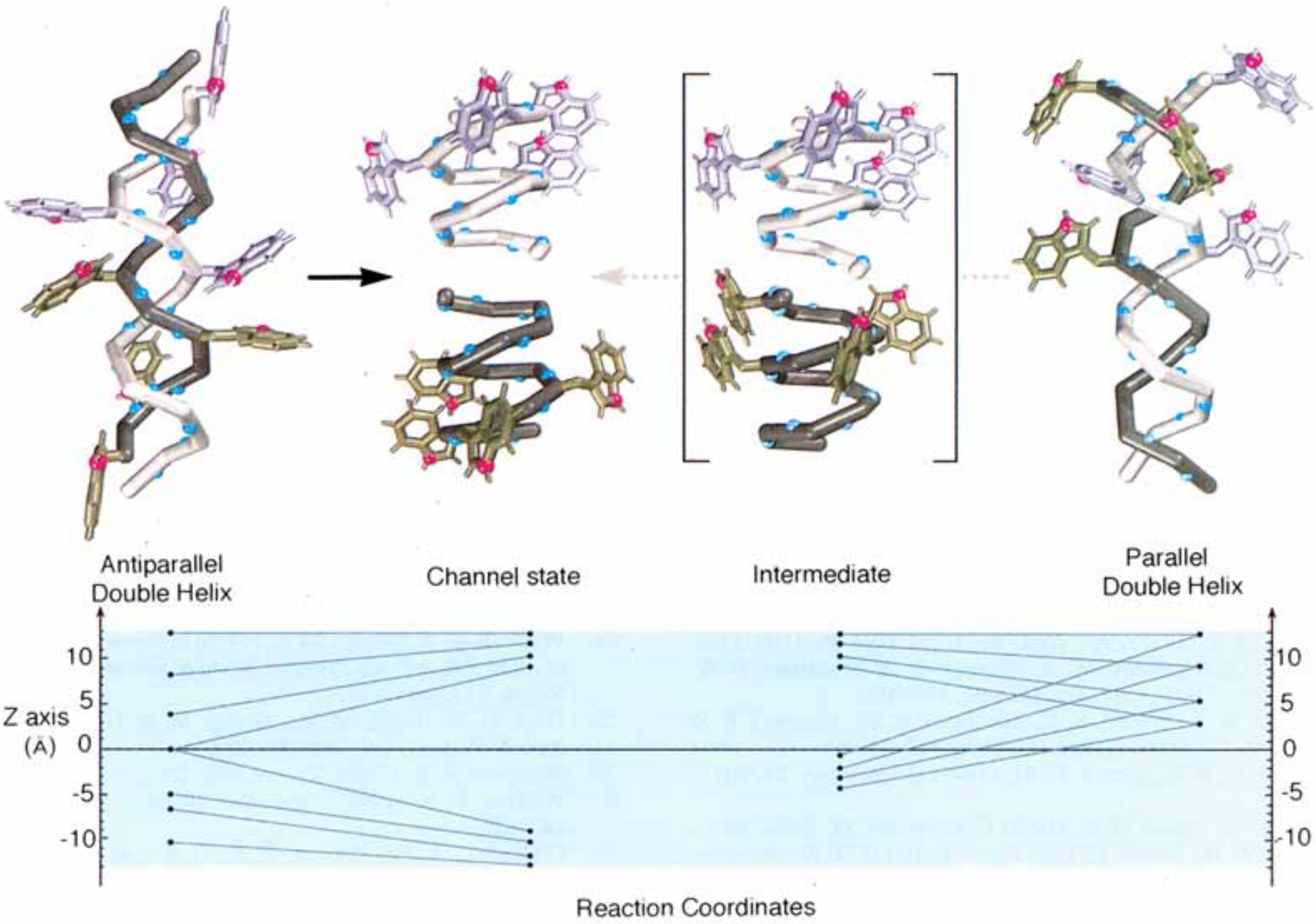
Water is a Catalyst for H-bond Exchange

v α $[H_2O]^{6.5}$

- »» Water elevates the free energy of the ground state
- »» Water lowers the free energy of the potential energy barrier
- »» Water is not consumed in the process
- »» Hence water is a catalyst



Trapped Conformation in a Lipid Bilayer



- The antiparallel structure readily (< 10 min) rearranges to form the channel state
- The parallel structure rearranges slowly (>5000 min) to form the channel state

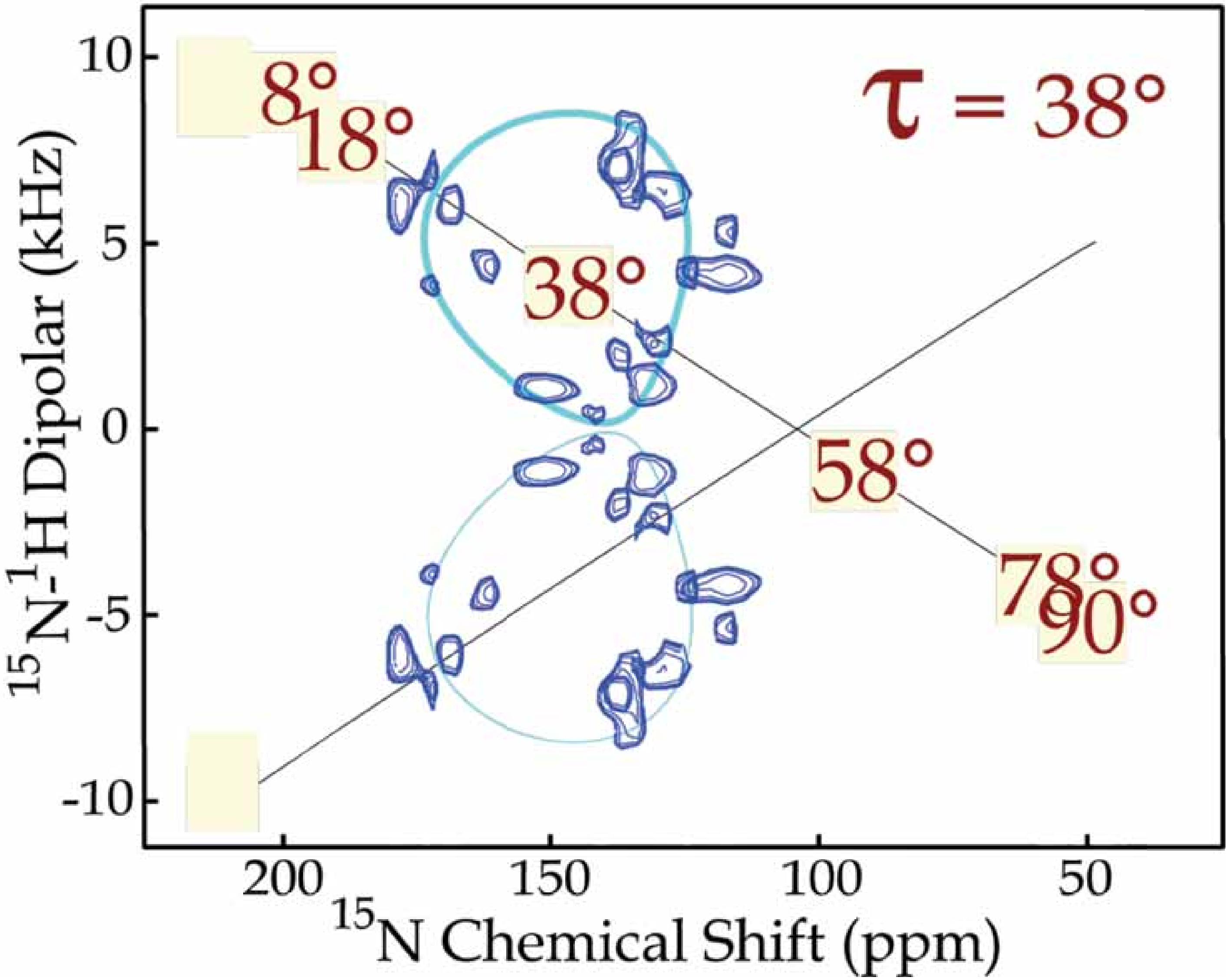
Structural Characterization: The Challenge of Using Precise Structural Restraints

1. **Orientational Restraints from uniformly aligned samples are high precision data with error bars of 1 to a few degrees at most.**

2. **The orientational restraints result in degenerate solutions such as the orientational restraints used in solution NMR**

3. **The overall problem is a conformational space separated by high energy barriers**

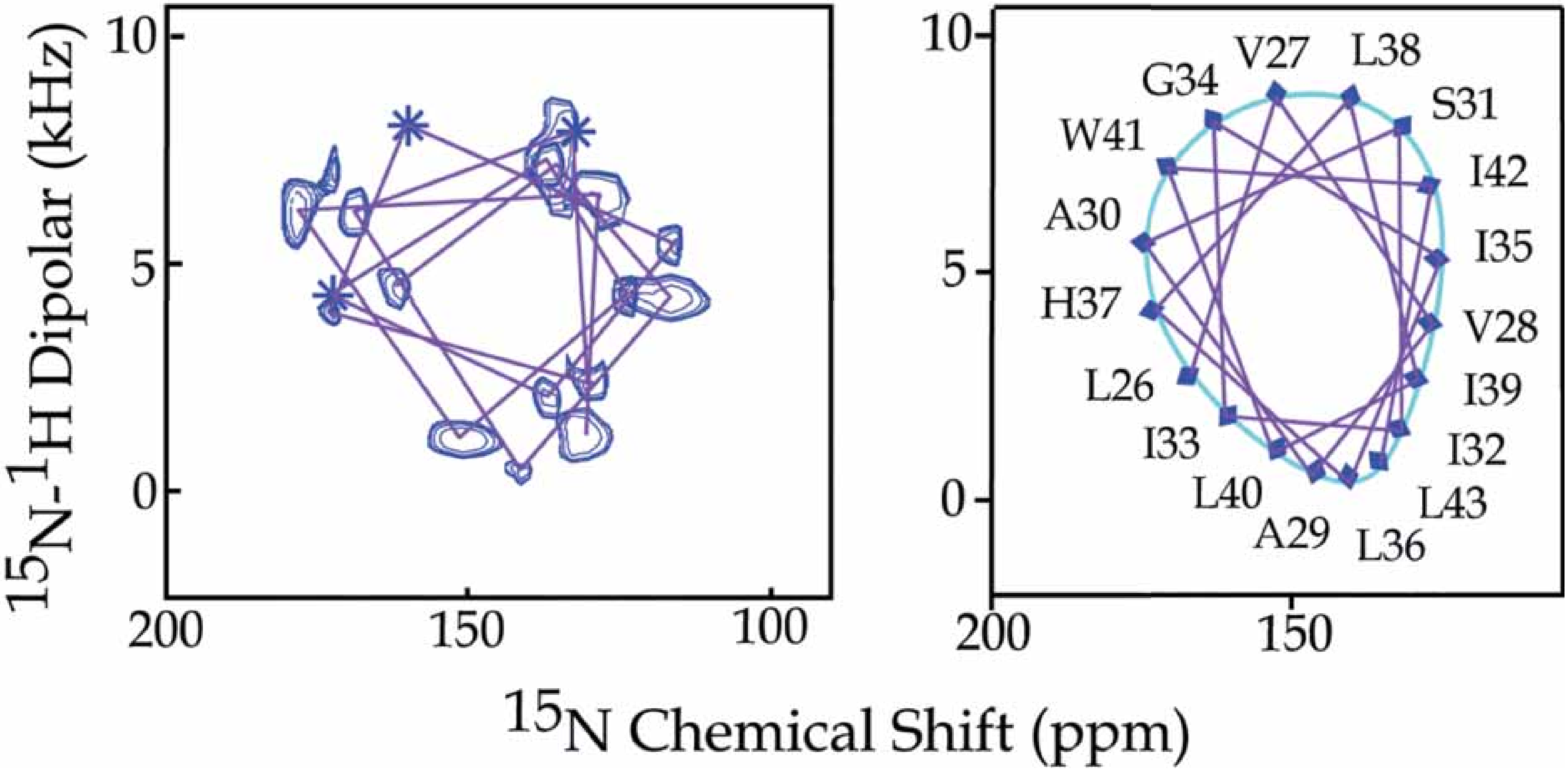
Determination of Tau



»» **Data from the M2 transmembrane domain of the Proton Channel from Influenza A virus**

Experimental and Theoretical Comparison

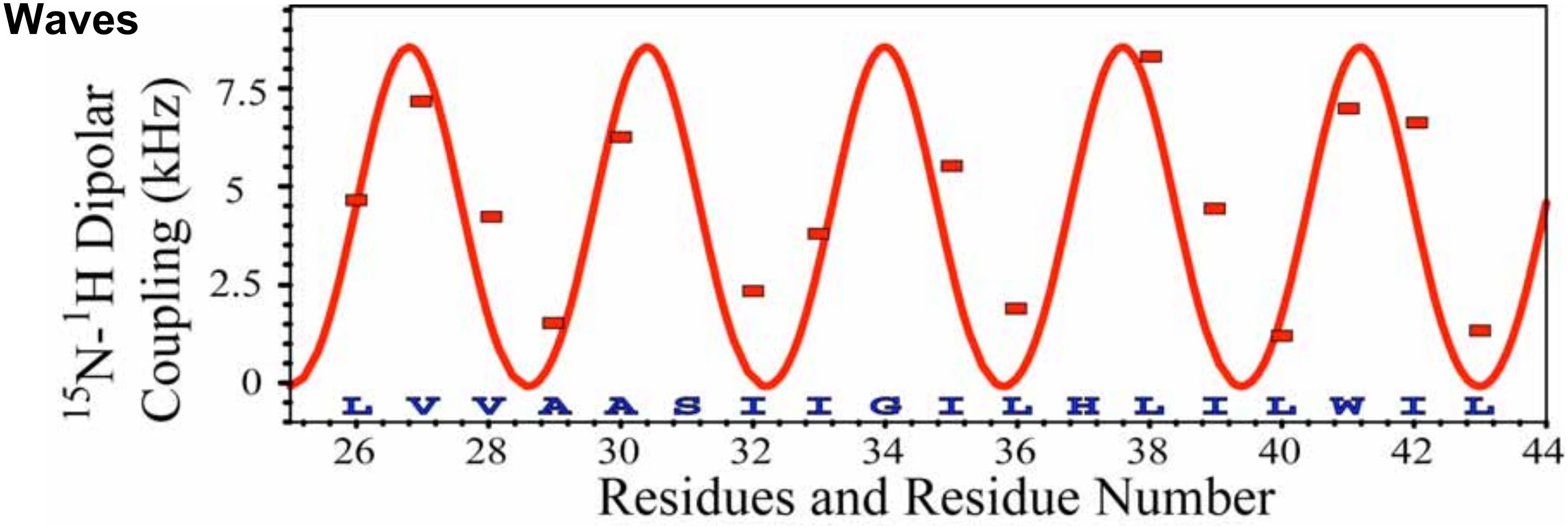
$\rho_o = -50°\ \ \tau = 38°$



»» The assignments were achieved by brute force - amino acid specific labeling; the stars indicate expected resonance positions for experimental data that was not obtained.

PISEMA Data for Backbone of the M2 TM Domain

Dipolar Waves



»» The same data can be plotted as dipolar waves. Here both the tilt (given by the amplitude of the dipolar fluctuations) and rotational orientation (given by the phase of the sinusoidal wave) are clearly defined.

Nishimura et al., (2002) Biochemistry 41:13170

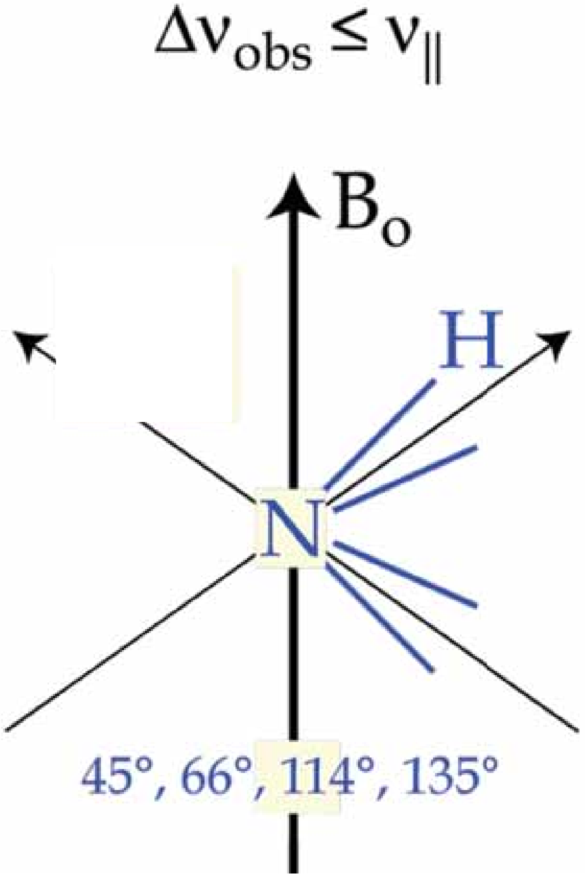
Initial Structure

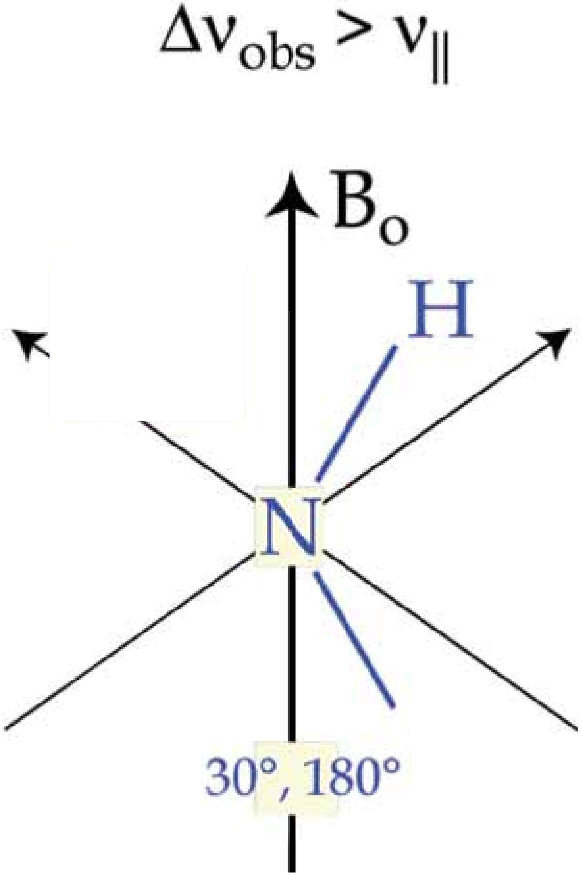
- hydrogen bonding pairs can be uniquely defined
- helical sense can be determined
- in modest systems orientational restraints alone can lead to a unique fold

but,

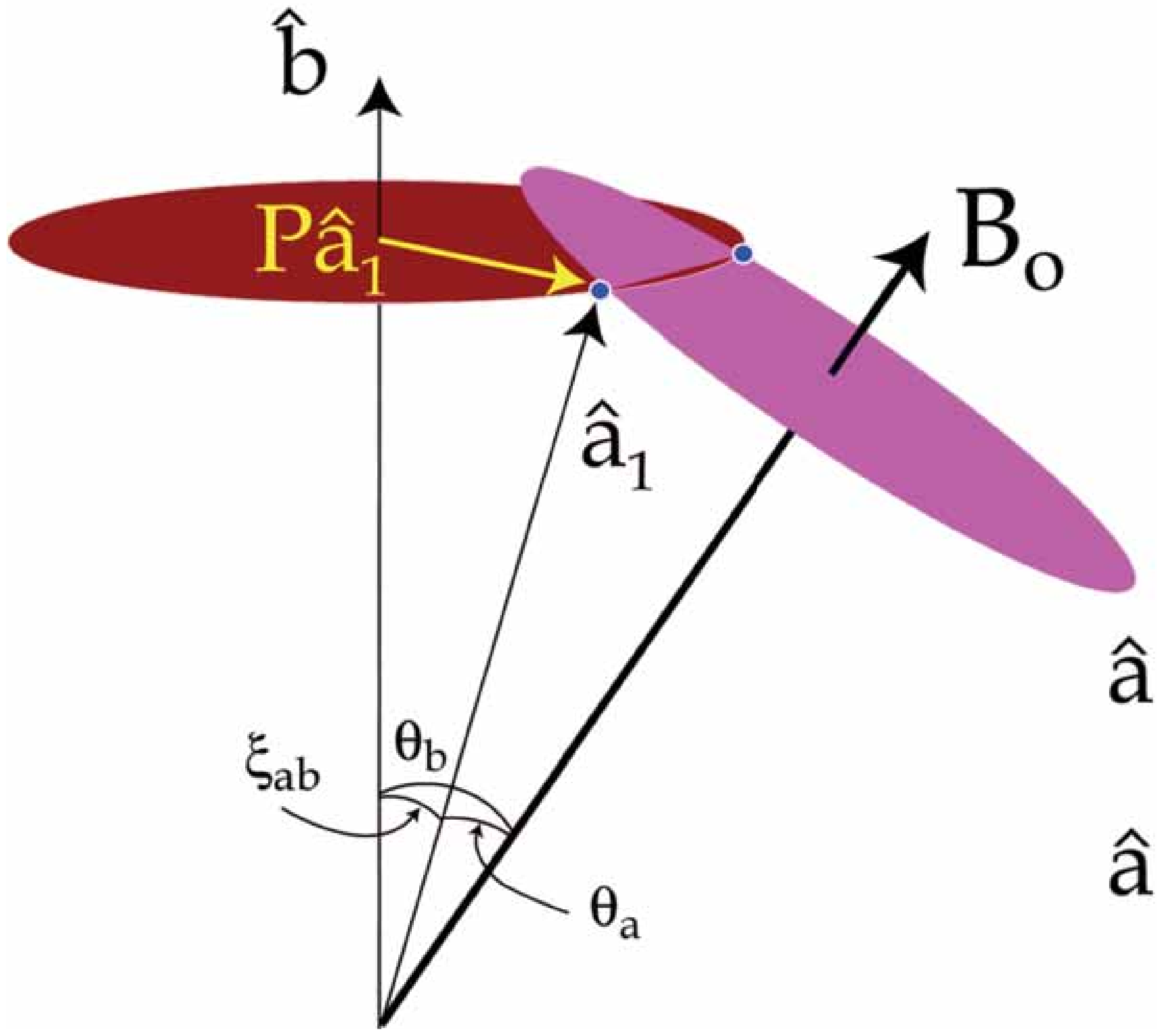
- orientational restraints have inherent ambiguities
- unique solutions can be found through
  - multiple restraints and
  - restraint correlations

Ambiguities in Dipolar Observations





Ambiguities in Peptide Plane Characterizations

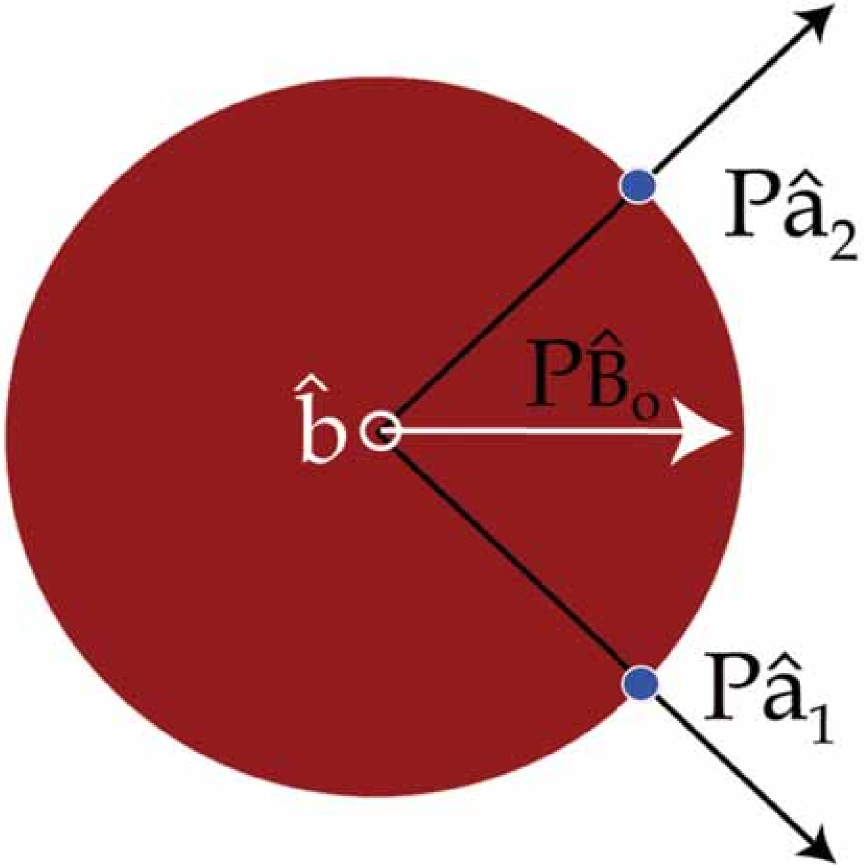


»» where a and b are unit vectors representing bonds in the peptide plane

$$\hat{a} \cdot \hat{b} = \cos \xi_{ab}$$

$$\hat{a} \cdot \hat{B}_o = \cos \theta_a$$

Ambiguities in Peptide Plane Characterizations

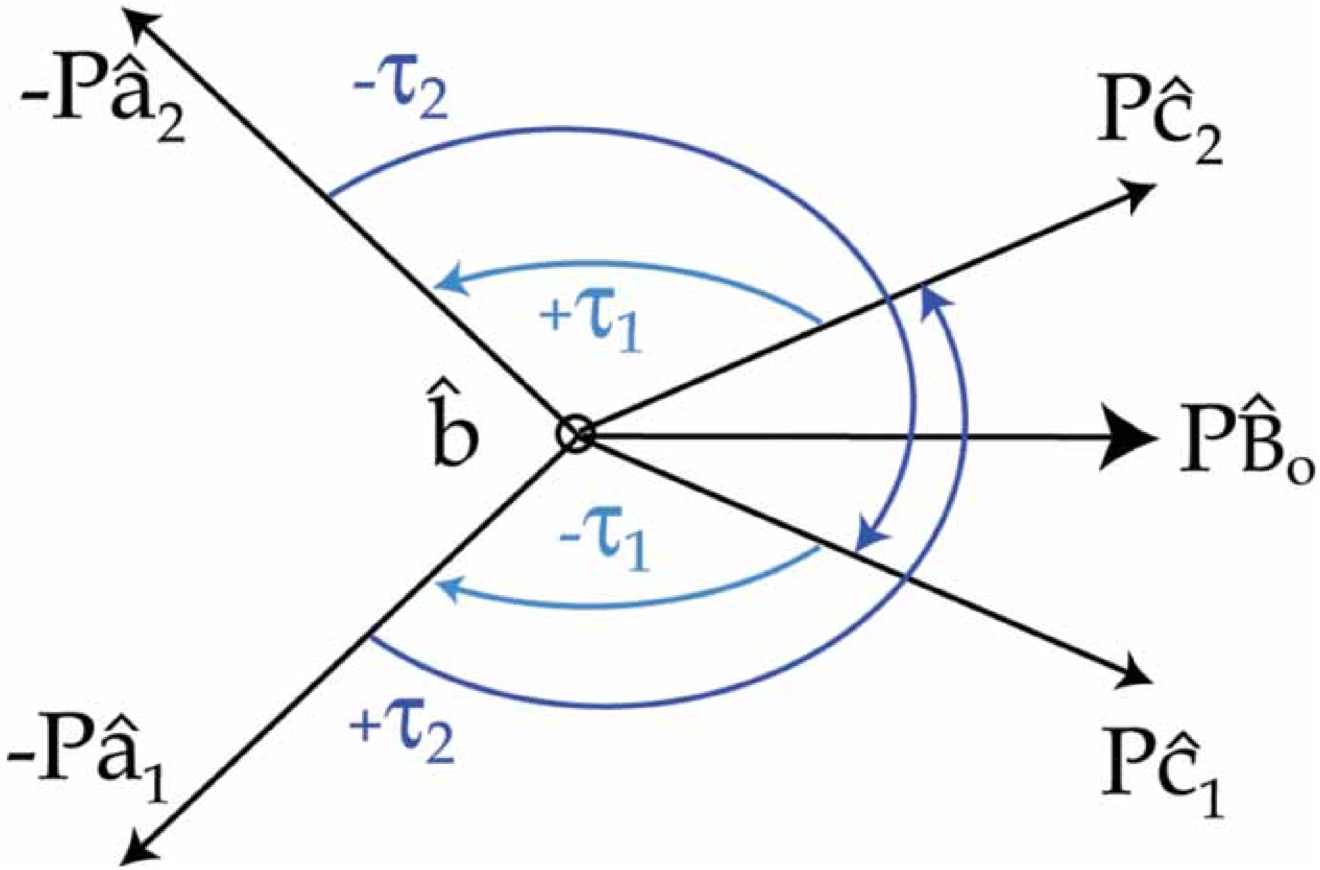


$\hat{b} \cdot (\hat{B}_o \times \hat{a}_2) > 0$

A Chirality Ambiguity

$\hat{b} \cdot (\hat{B}_o \times \hat{a}_1) < 0$

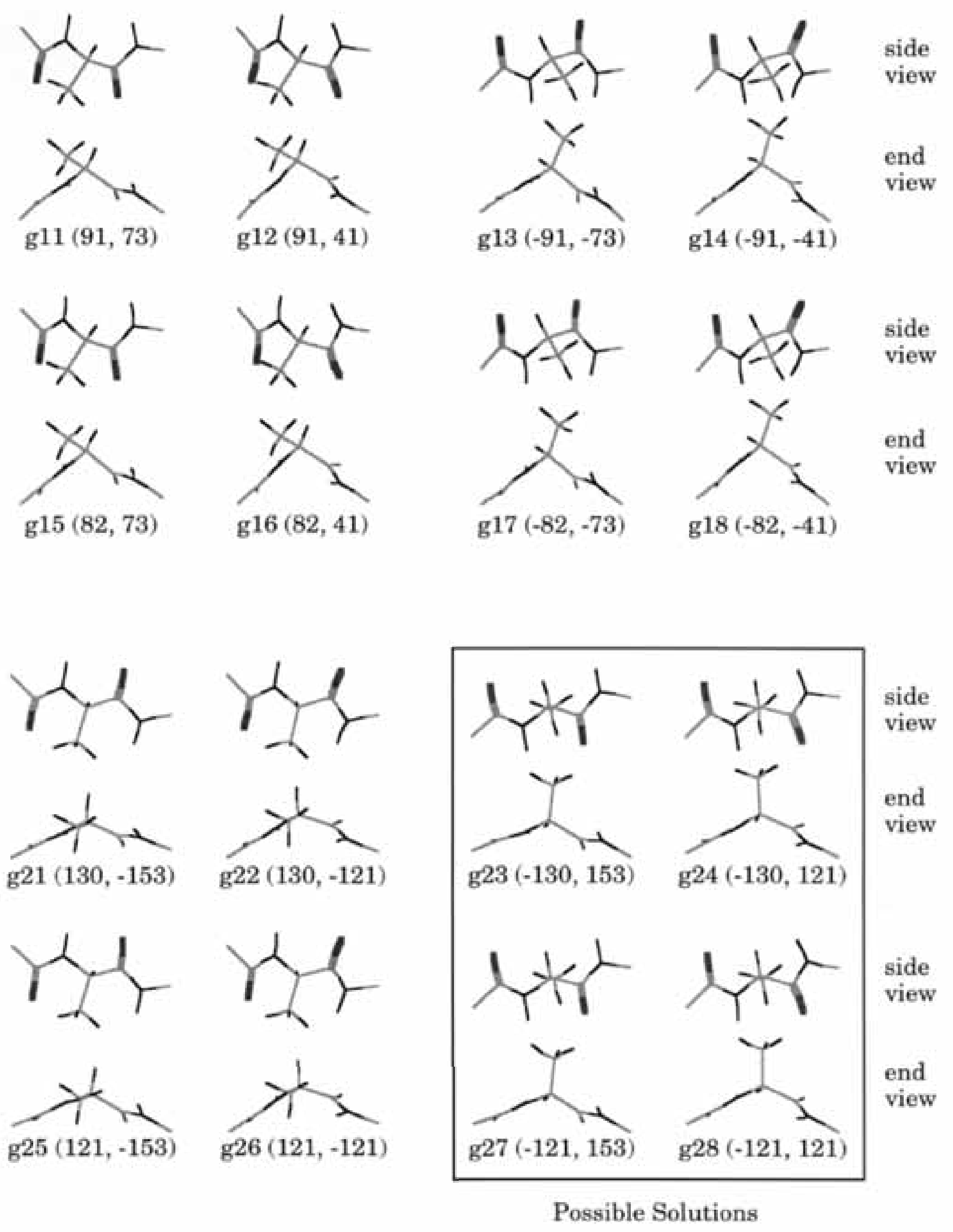
Ambiguities in Torsion Angles ($\hat{a}$, $\hat{b}$, $\hat{c}$)



Ambiguities in Diplanes using:

- ^{15}N Chemical Shift
- ^{15}N-^{1}H Dipolar
- ^{15}N-^{13}C Dipolar

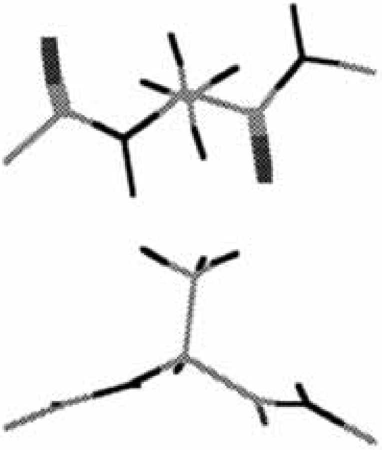
»» These are diplane solutions from Gramicidin A. Most of the ambiguities were resolved with a characterization of the Cα-H vectors through the use of ^{2}H NMR

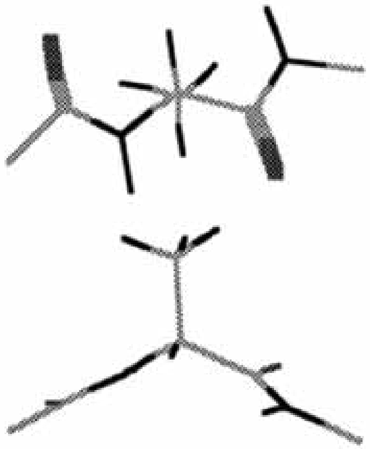


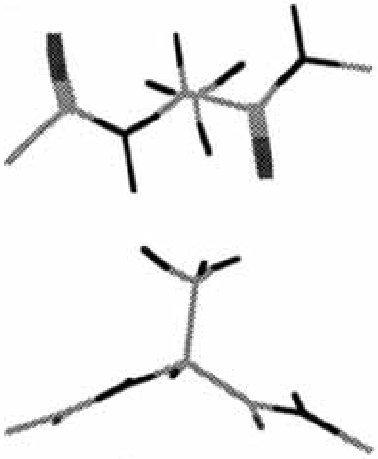
Ambiguities in Diplanes using:

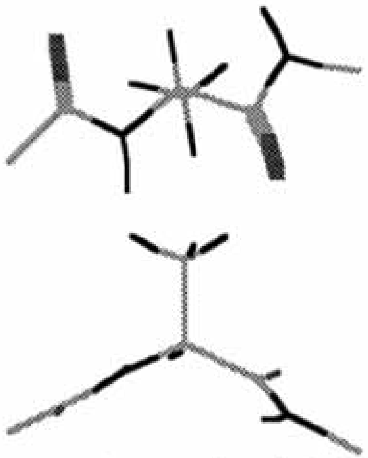
- ^{15}N Chemical Shift
- ^{15}N-^{1}H Dipolar
- ^{15}N-^{13}C Dipolar
- ^{2}H-C$_{\alpha}$ Quadrupolar

»» All of the remaining ambiguities are consistent with a β-strand structure with a 9° difference for ϕ and a 32° difference for ψ

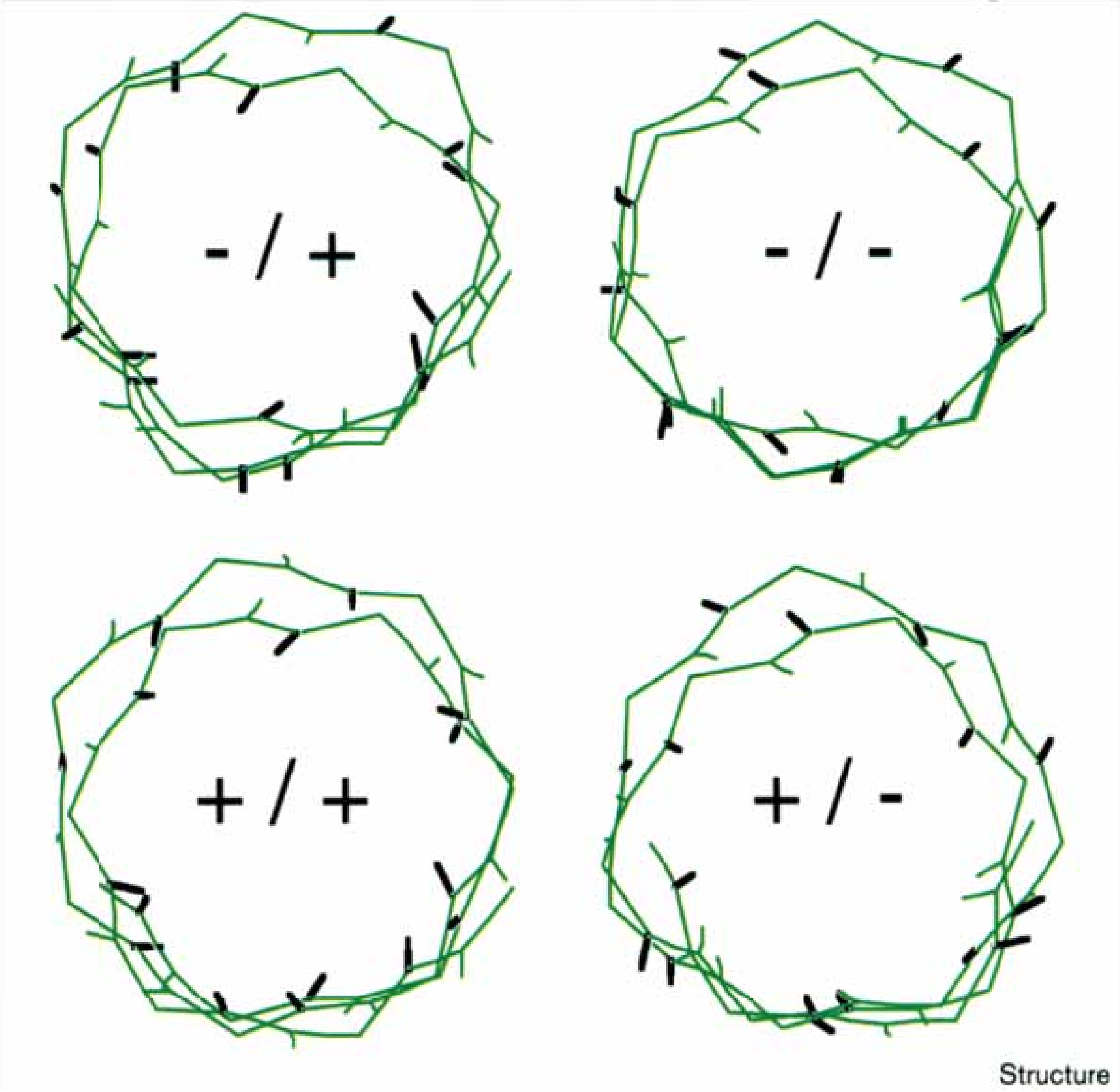

g23 (-130, 153)


g24 (-130, 121)


g27 (-121, 153)


g28 (-121, 121)

Initial Gramicidin Structures of Varying Chirality Ambiguities

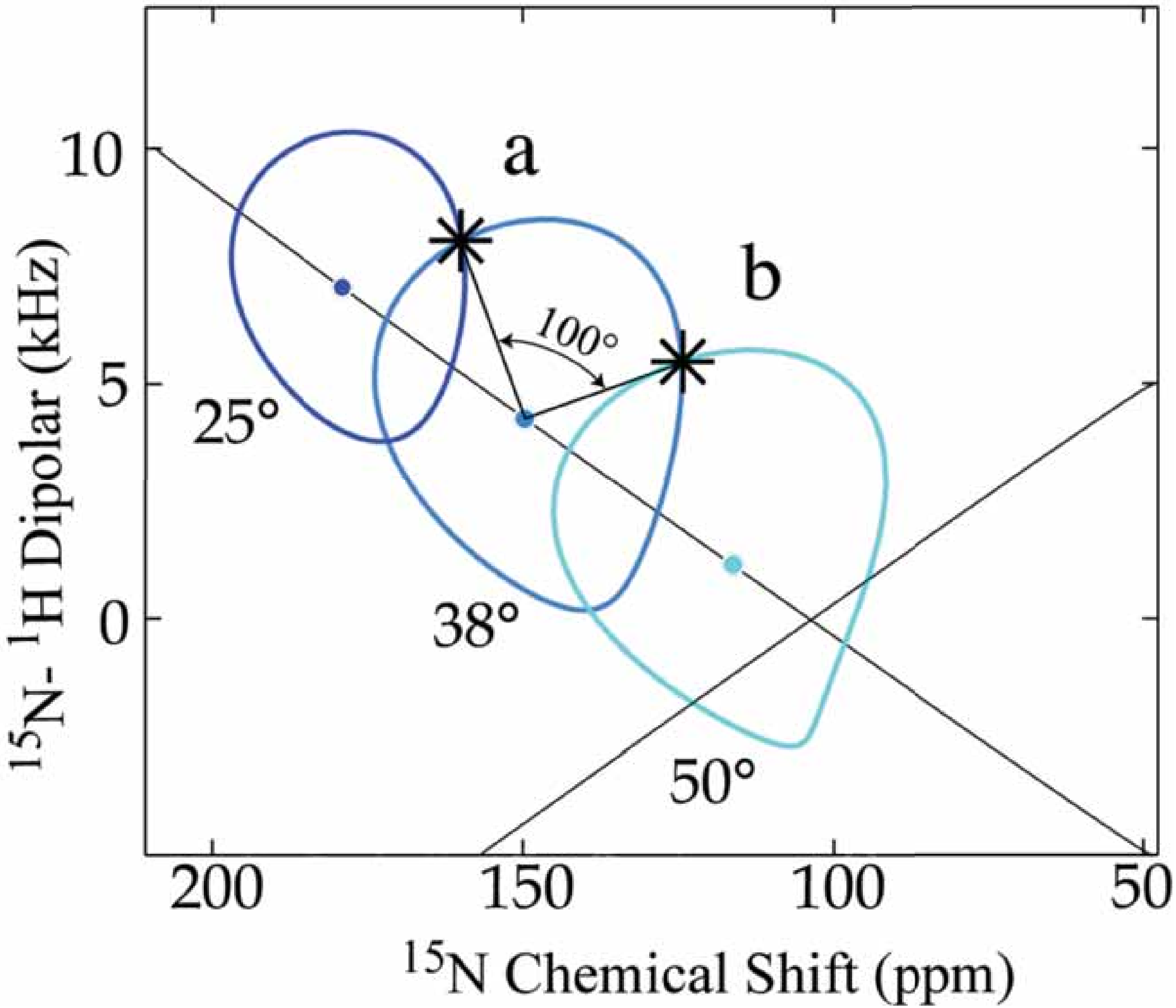


»» The structures have a set of ambiguities in the carbonyl oxygens that can be represented as

1) **Always pointing out (-/-)**
2) **Always pointing in (+/+)**
3) **Alternating in and out (-/+) or**
4) **Alternating out and in (+/-)**

Resonance Correlations in Helices

- »» The situation is much better for α-helices
- »» Helices can be recognized easily in the spectra and their structure is typically very uniform - even if a heliix has a kink the two fragments are uniform in conformation.



»» Here the correlations between resonances define the common PISA wheel and eliminate degenerate solutions. An initial structure is characterized

Refinement

- to relax covalent structure
- to optimize energetics, e.g. van der Waals, H-bonds, etc.
- to solve chirality ambiguities

but,

- substantial conformational space needs to be searched
- global orientation must be maintained
- a global energy is used
- cross validation is performed

Penalty Function for Refinement

$$\text{Total Penalty} = \sum_{i=1}^{M} (\lambda \cdot \text{Structural Penalty}_i) + \lambda_e \cdot \text{Energy}$$

$$\text{Structural Penalty} = \sum_{j=1}^{N} \frac{1}{2} \left(\frac{\text{Calculated}_j - \text{Observed}_j}{\text{Experimental Error}_j} \right)^2$$

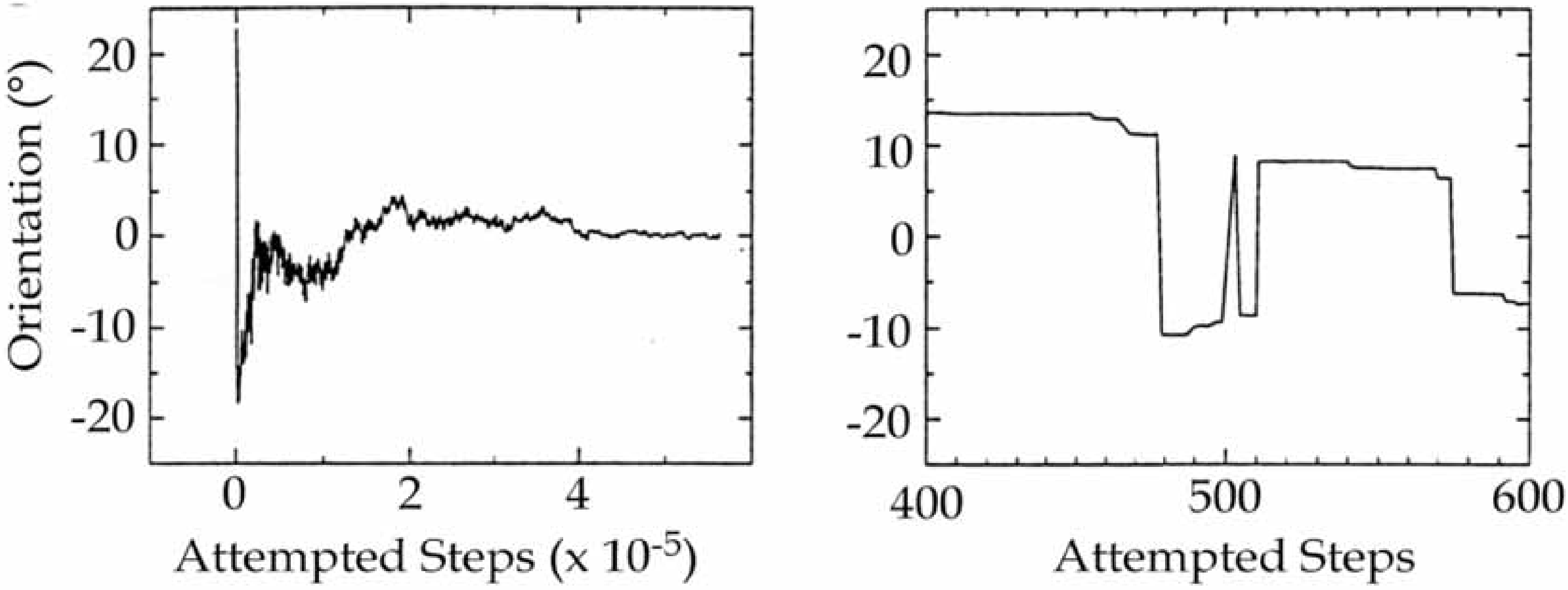
»» λ **is a weighting factor for the various restraints**

»» **Dividing by the Experimental error generates a unitless structural penalty that normalizes the different types of restraints. It is possible to use individual error bars for each restraint or for each class of restraints**

Simulated Annealing: Three types of Atom Moves

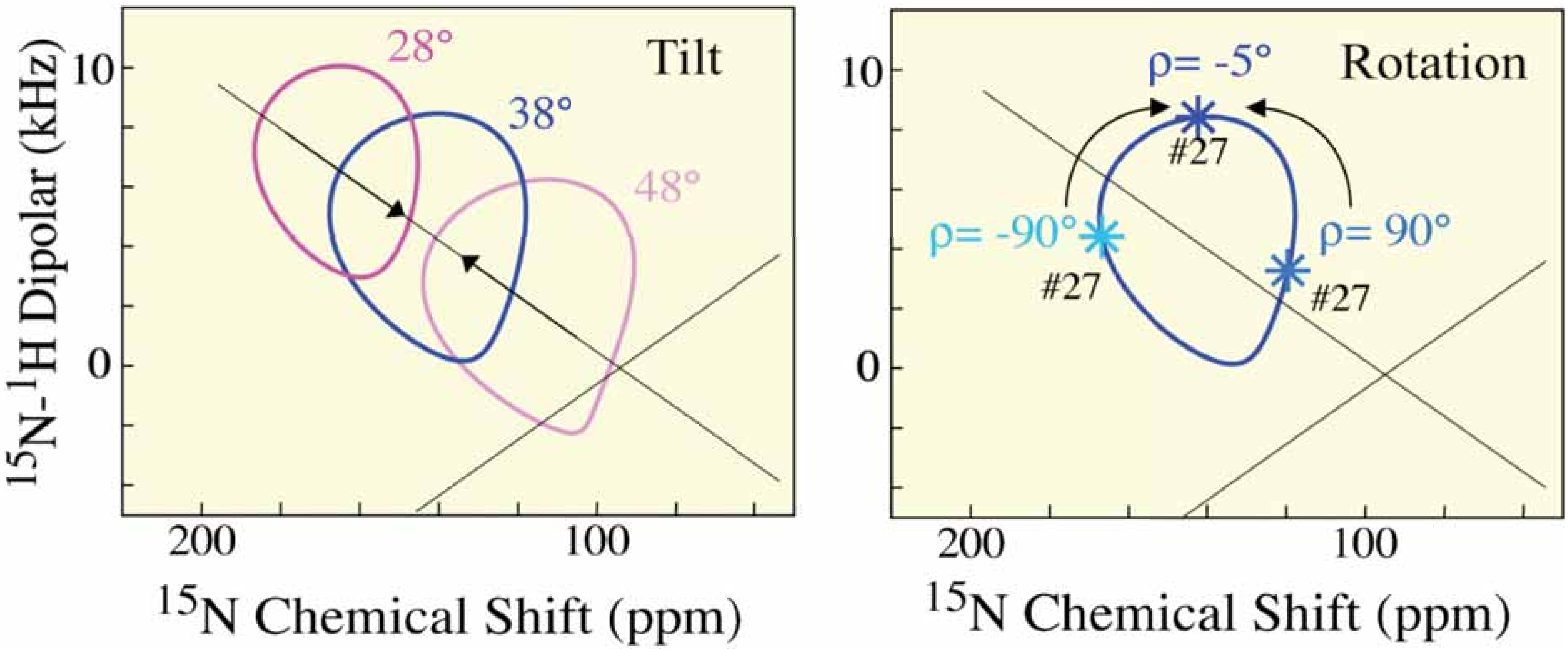
1) **Atom displacements between ±0.001 Å**
2) **Tortional moves ±3° for ϕ,ψ and ±0.1° for ω**
3) **Tortional moves that inverts the peptide plane about a plane formed by the $C\alpha$-$C\alpha$ axis and B_o**

Peptide Plane Tunneling



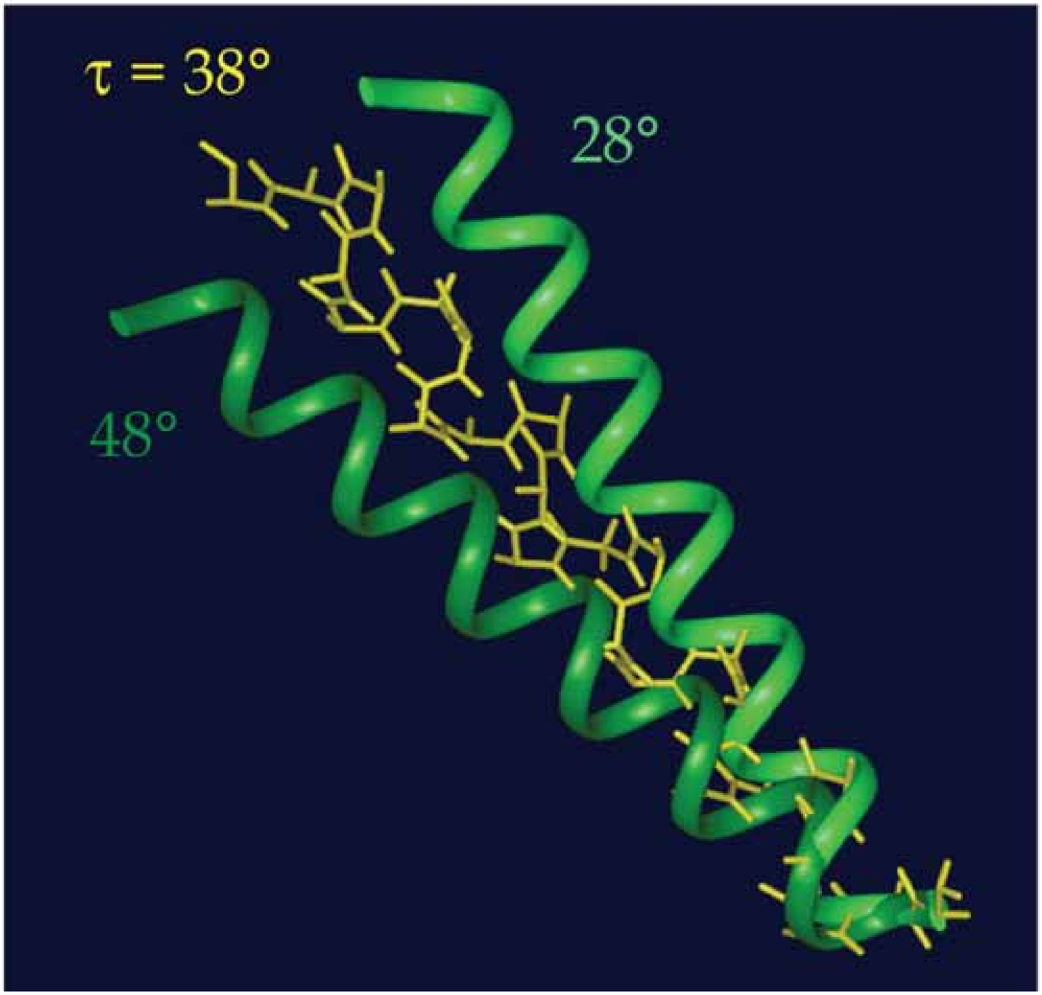
»» The tunneling moves were not necessary for the α-helical refinement

Global Reorientation in Structural Refinement without Rigid Body Moves

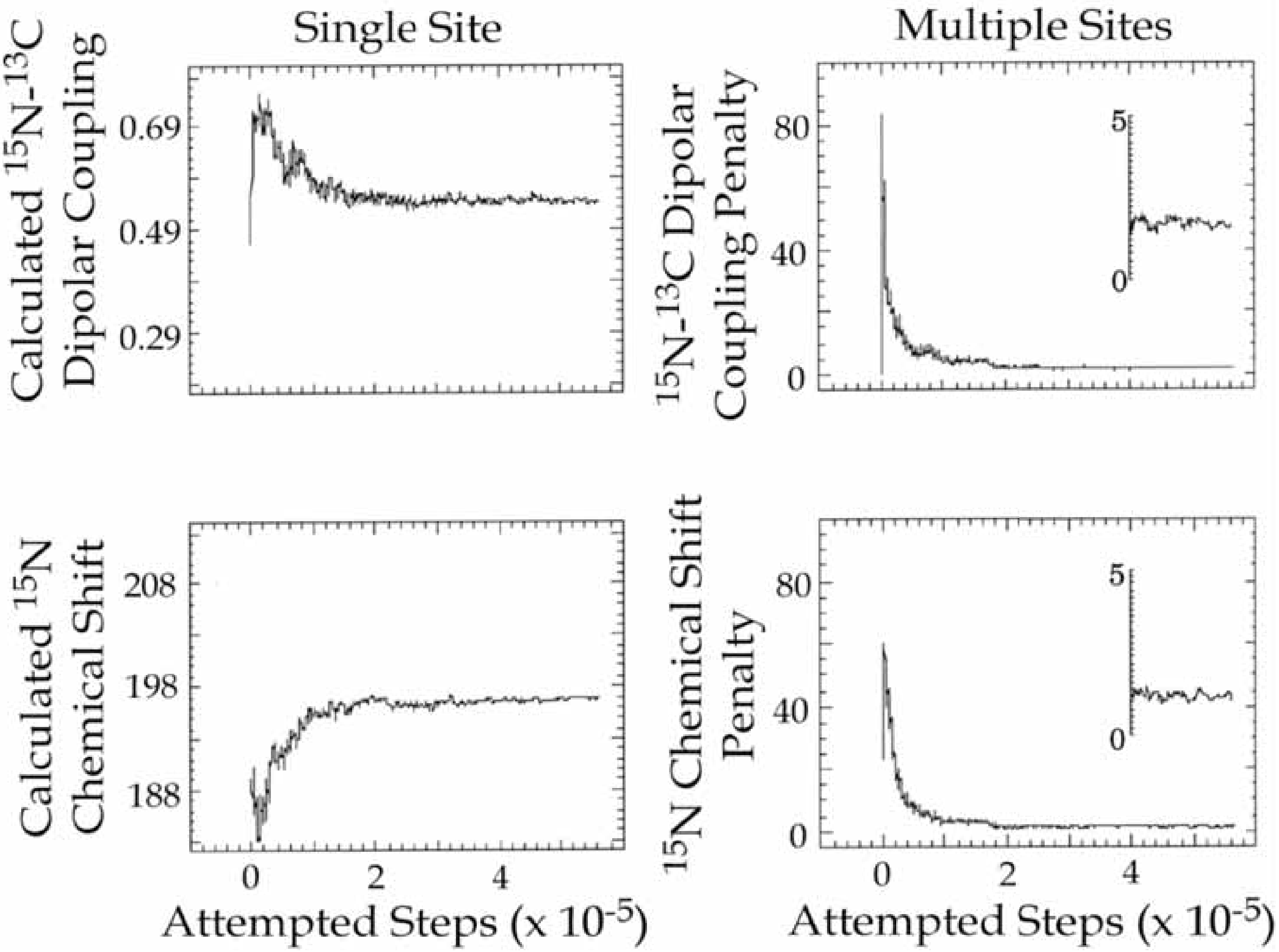


››› Global search of conformational space was achieved without rigid body moves - the torsional moves were adequate.

Global Reorientation of the M2-TMP Helix Upon Refinement

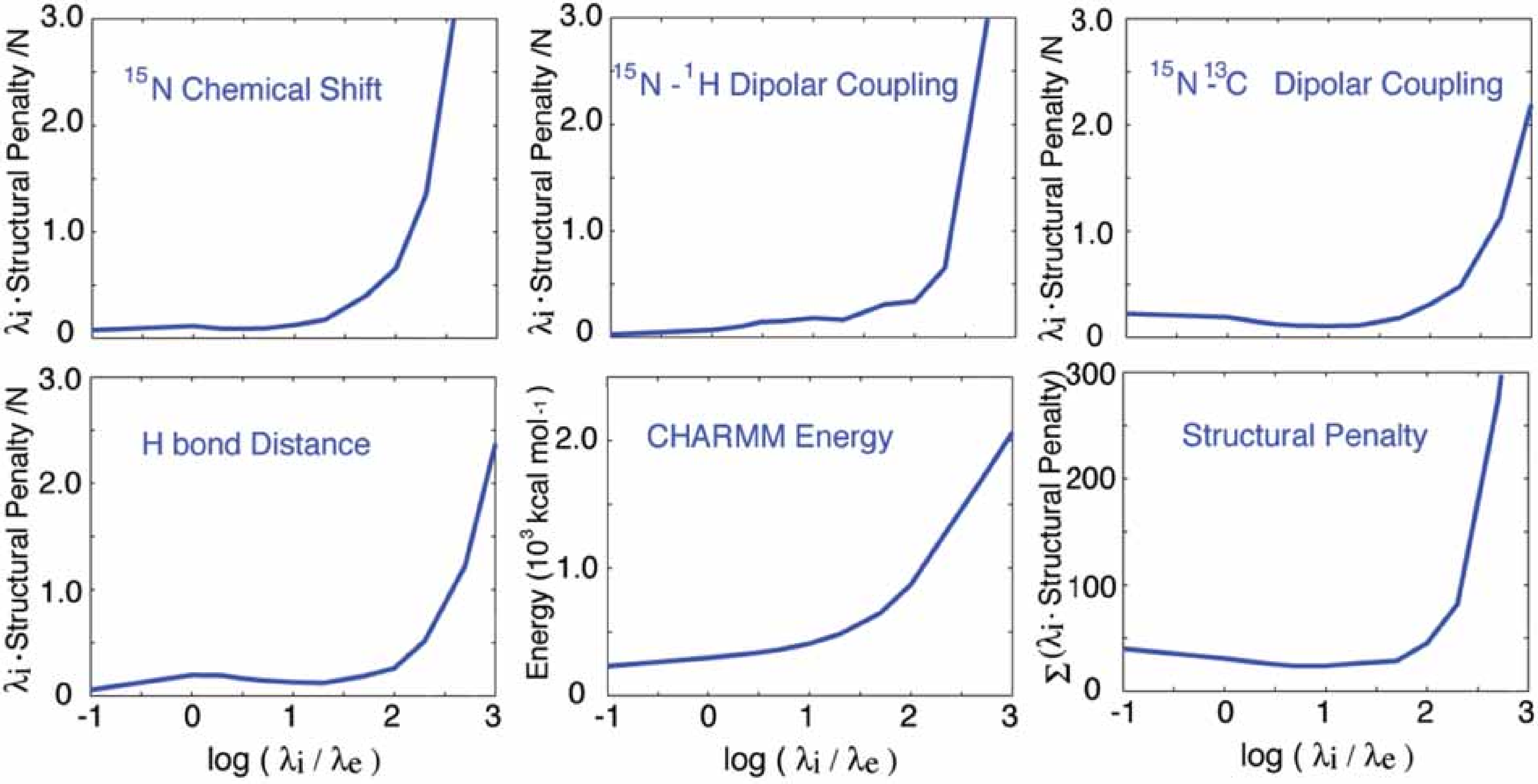


Refinement Trajectories



»» Flat well potentials were originally used, but were later found not to be essential. The experimental data was well fit while avoiding poor geometry.

Weighting Factor Influence on Penalty Components

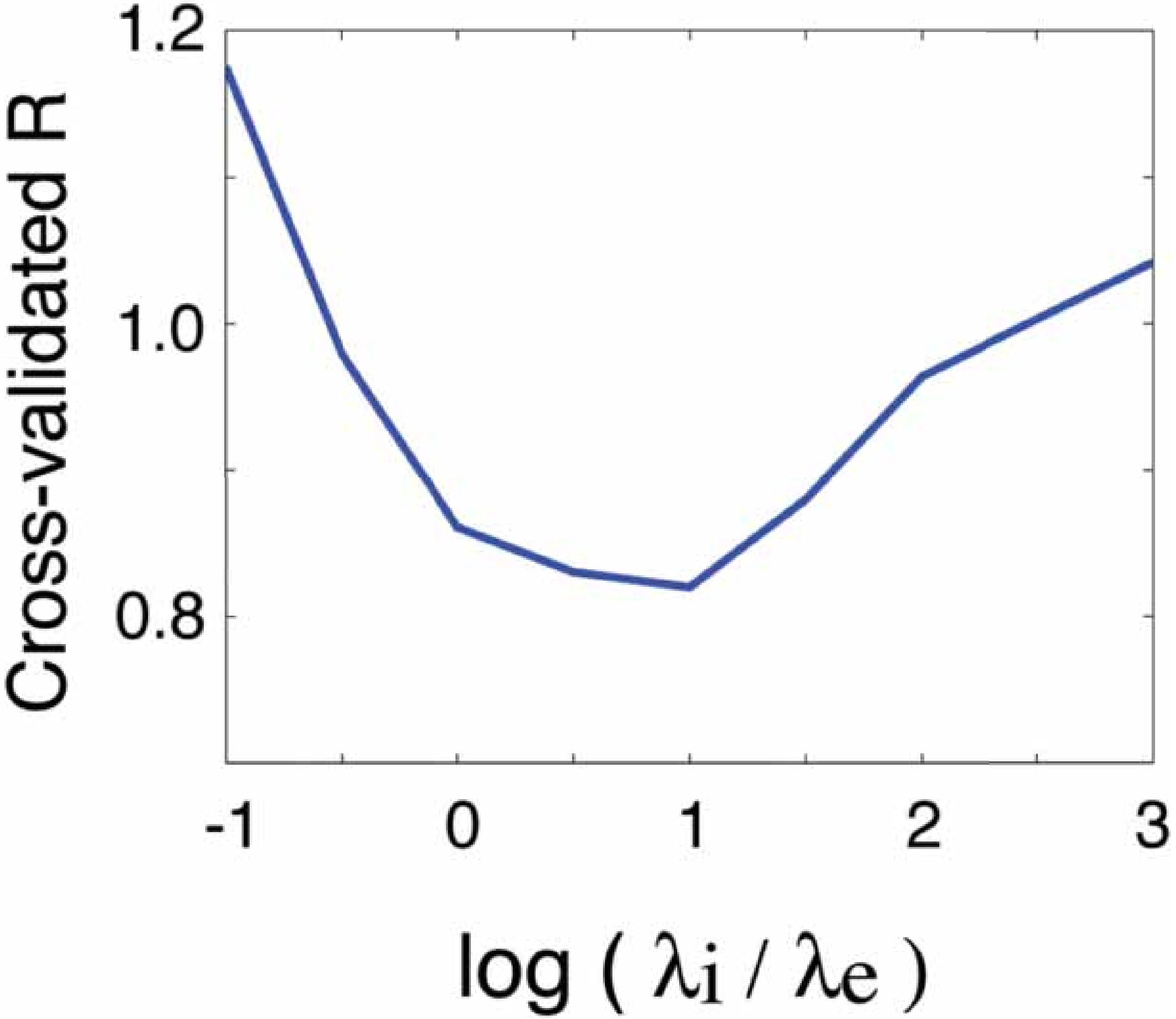


»» The structural penalty had a minimum that was well defined by a complete cross validation approach.

CHARMM Energy Terms

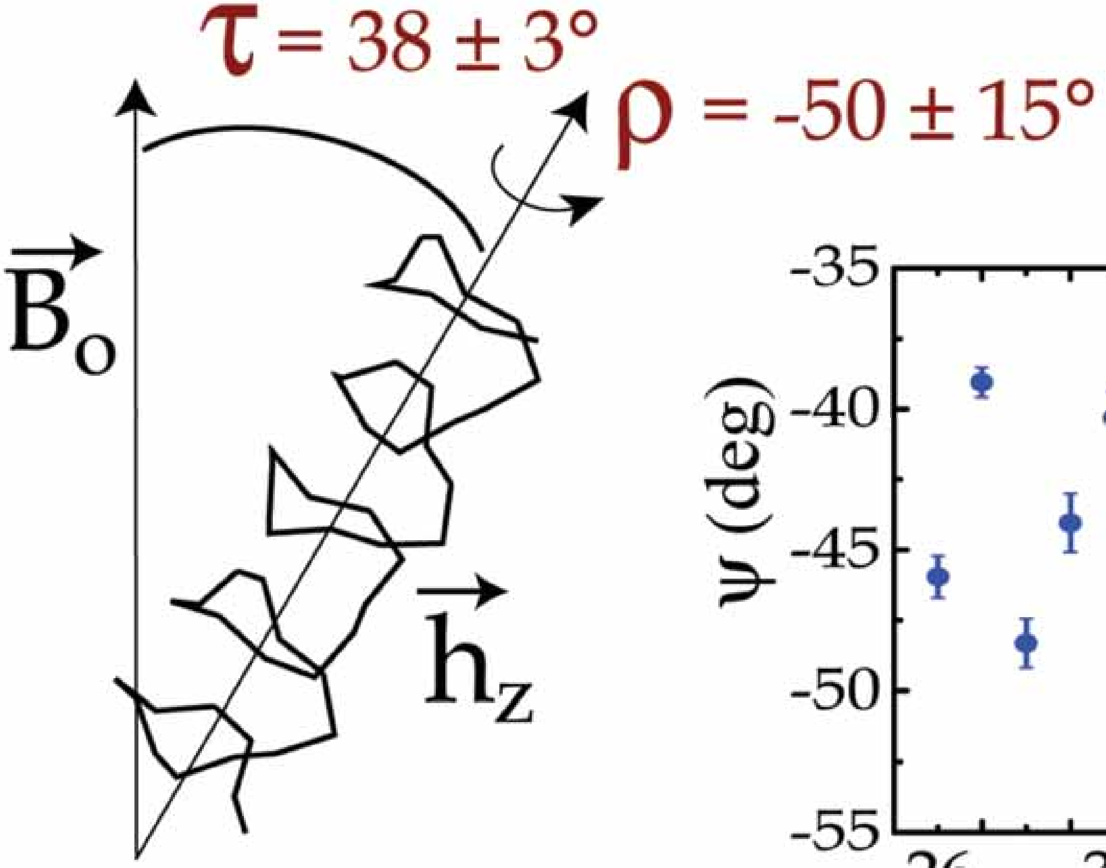
- Bonds
- van der Waals
- van der Waals (image)
- Electrostatics
- Electrostatics (image)
- Angles
- Urey-Bradley
- Dihedrals
- Impropers
- Center of Mass Orientation

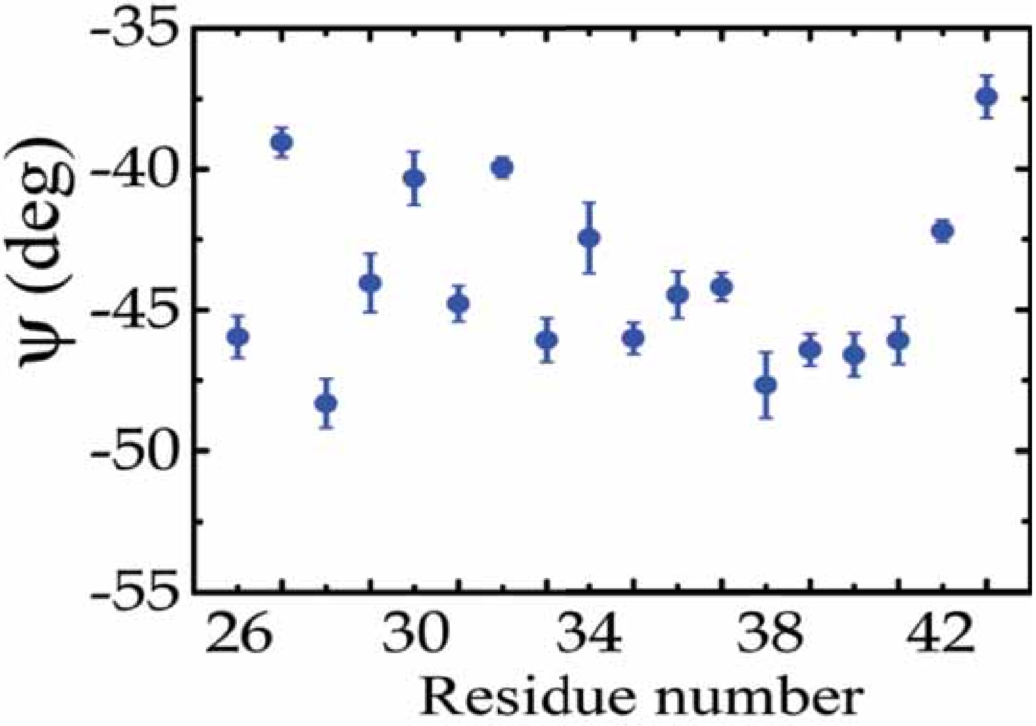
Cross-Validated R



»» The cross-validated minimum that defines the optimal balance between the experimental restraints and the empirical force field.

From Topology to High Resolution

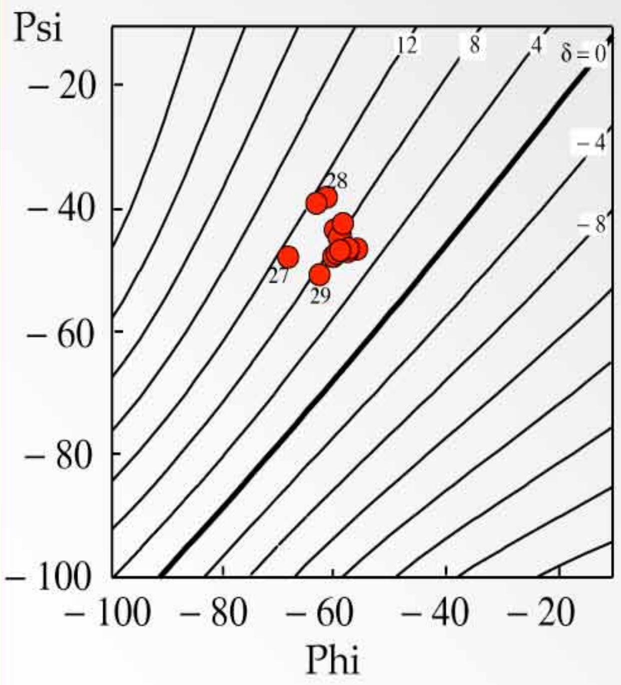




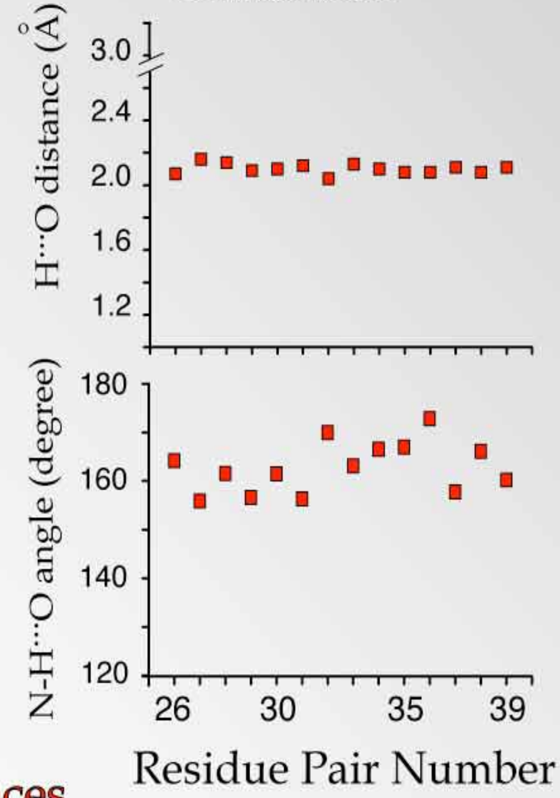
»» The overall result are very well defined torsion angles, but note that there is some scatter in the ψ torsion angle - similar results were obtained for the ϕ torsion angle.

Characterization of the Refined Solid State NMR M2-TM Domain Structure

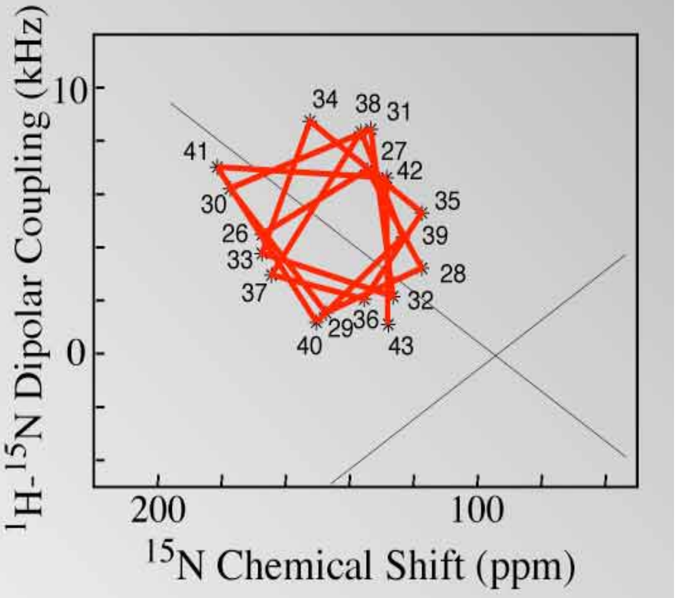
Rhamachandran Diagram



Hydrogen Bond Distances



Experimental PISA Wheel



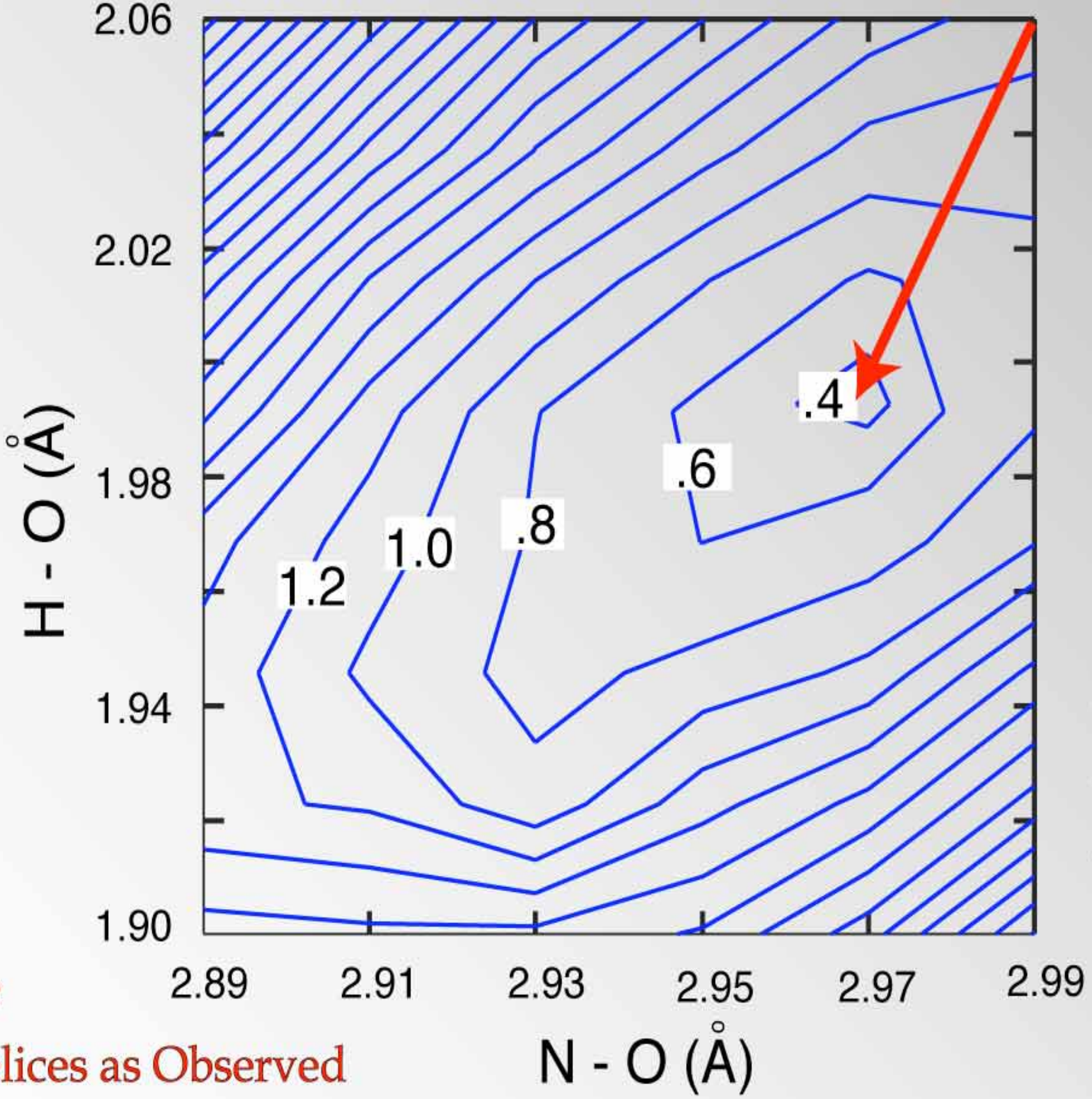
»» Uniform H-bond Distances

»» Slightly Different Torsion Angles

»» Uniorm Helical Structures - Not a Coiled Coil Structure

Kim et al., (2002) Biophys. J. 83:2084-2095

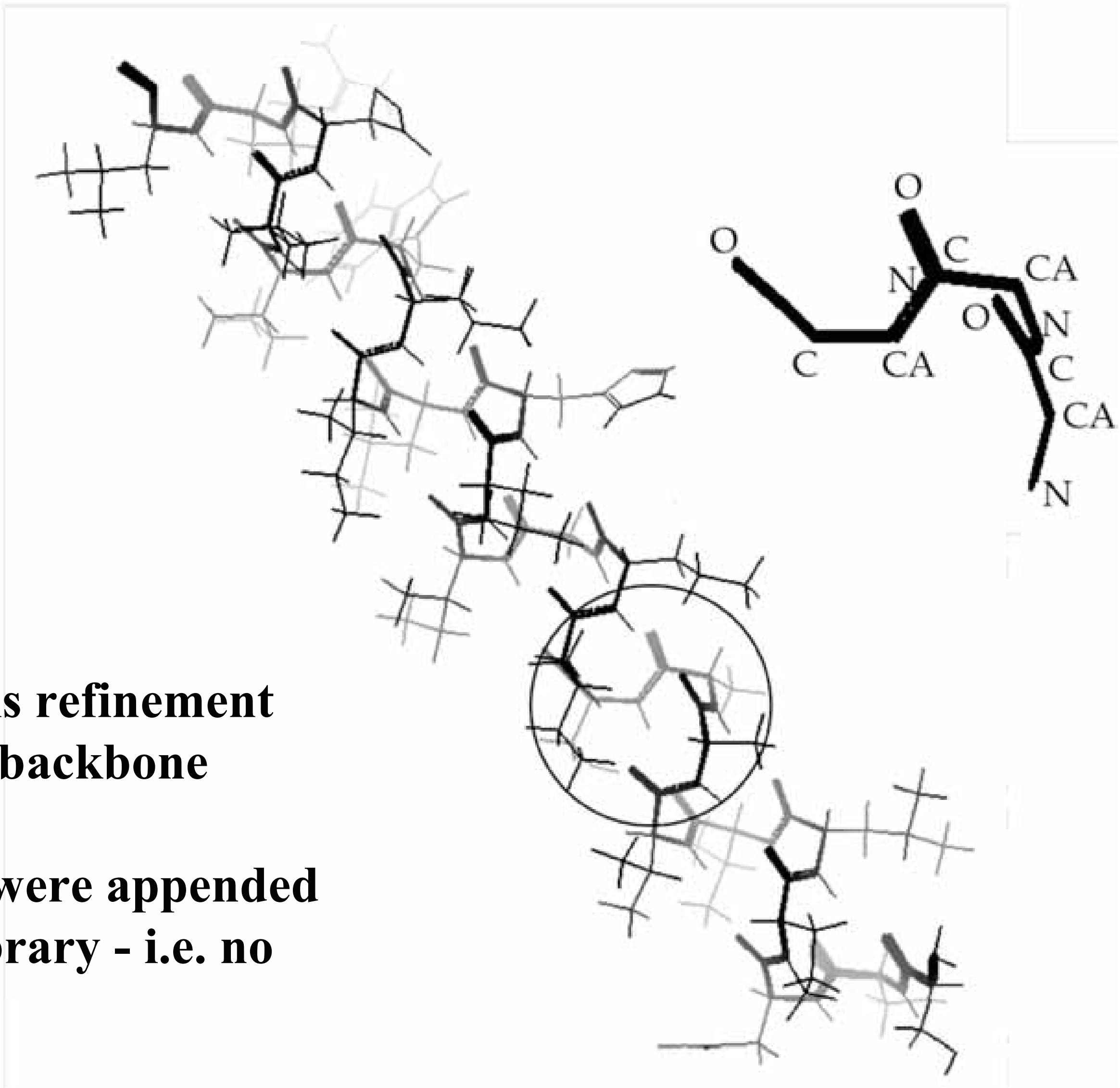
Hydrogen Bond Geometry in M2 TM Domain



Kim et al., (2002) Biophys. J. 83:2084-2095

- »» H-Bonds Reflect Increased Electrostatic Interactions
- »» Suggests More Uniform α-Helices as Observed in Bacteriorhodopsin

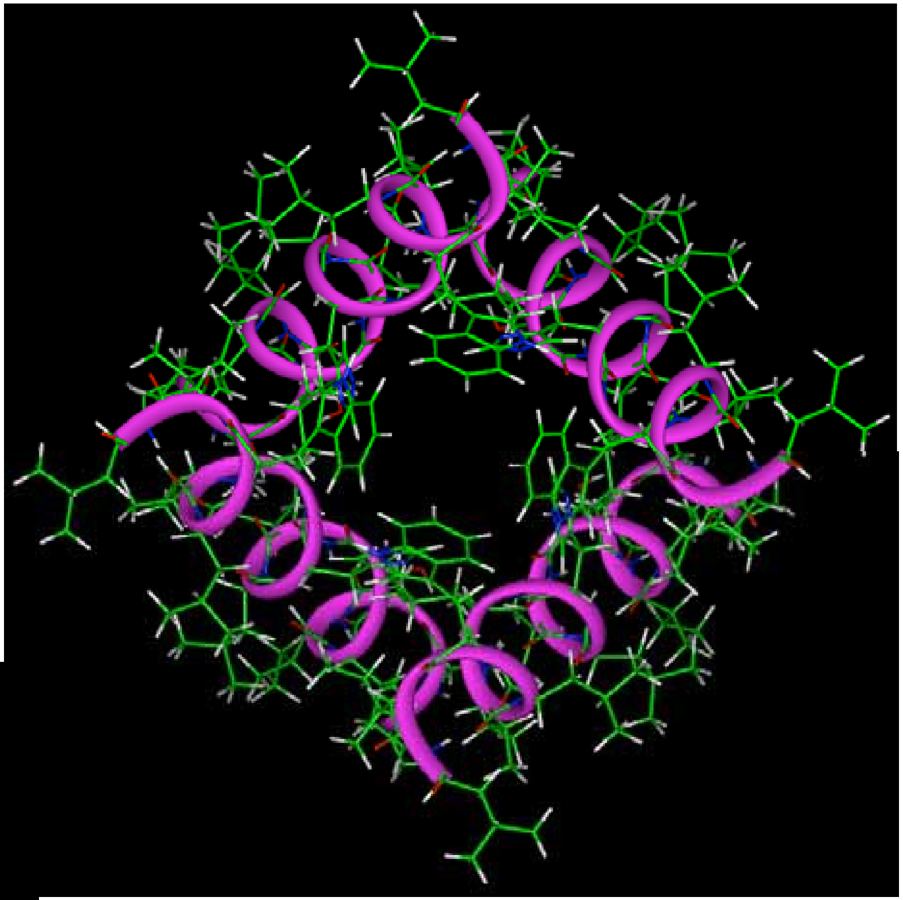
Refined Backbone Structure for M2-TMP

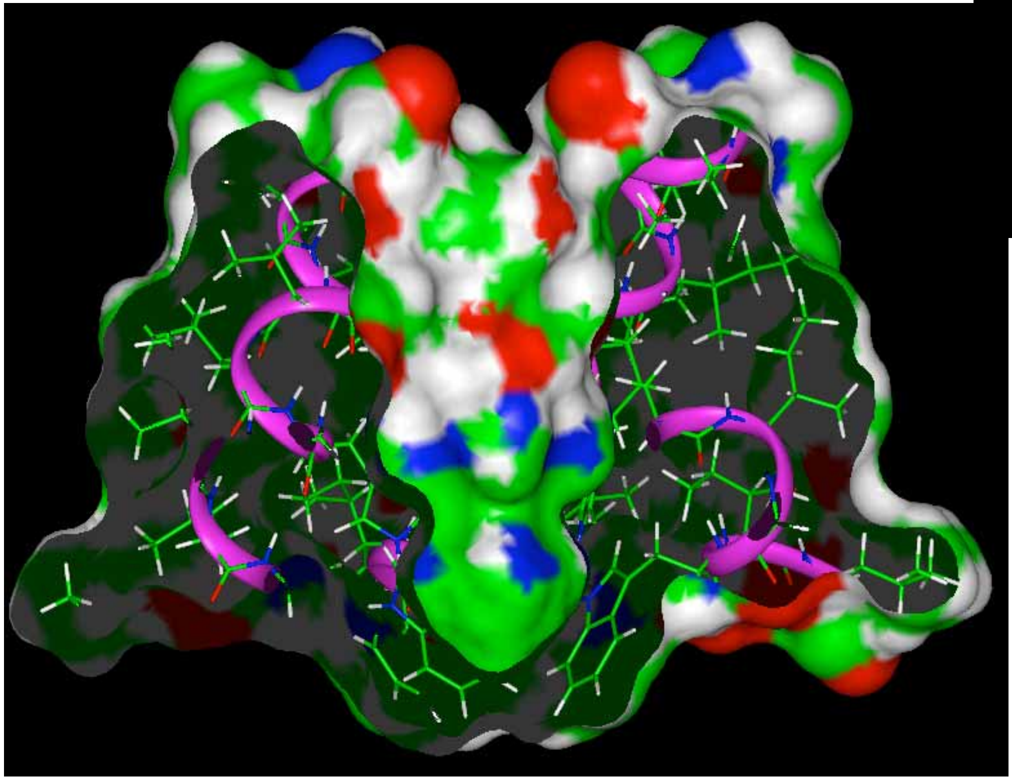


»» The result of this refinement was a well defined backbone structure

»» The sidechains were appended using a rotamer library - i.e. no experimental data

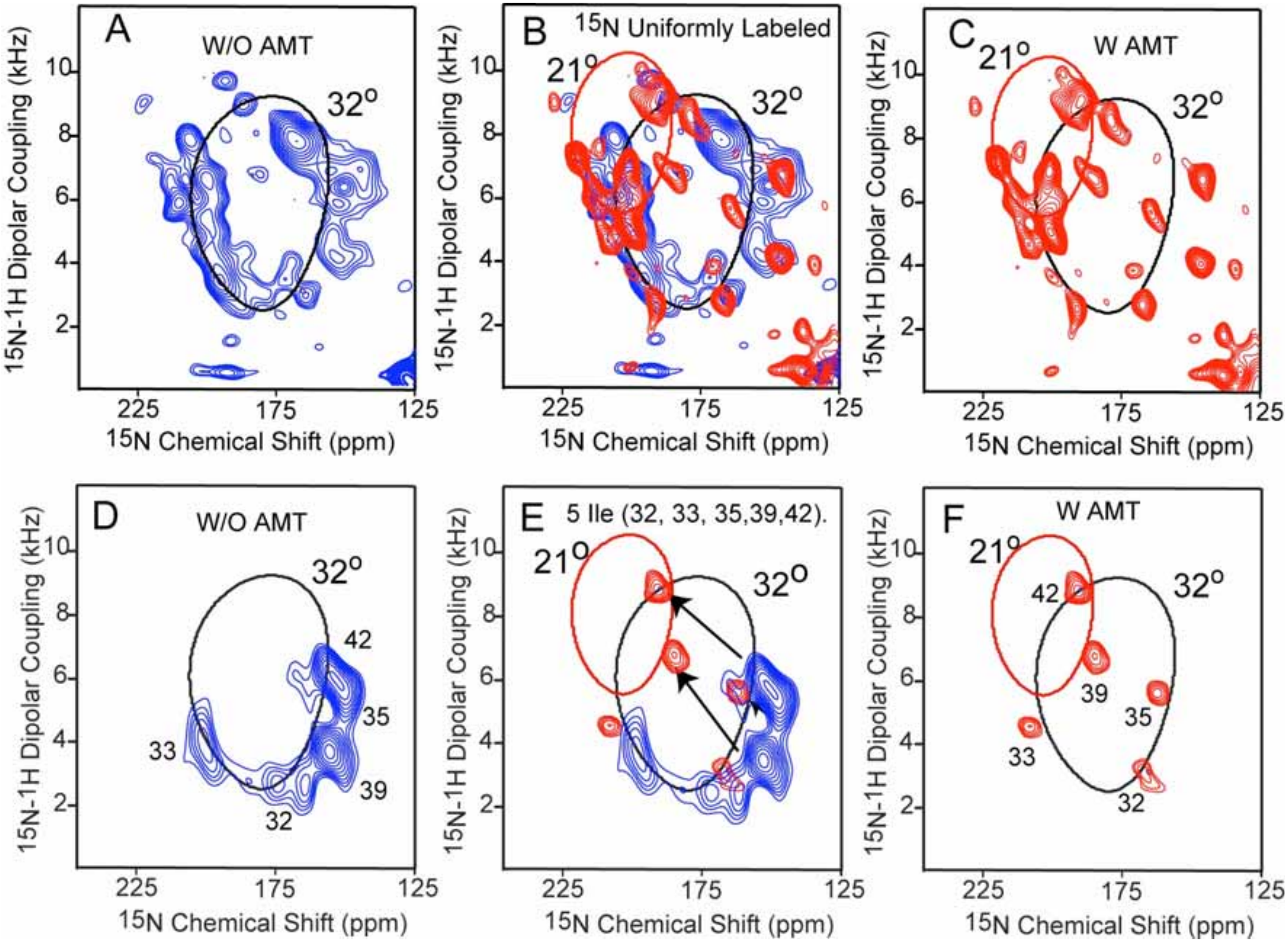
Backbone Structure of the Closed State of the M2 Transmembrane Domain Characterized by Orientational and Distance Restraints - Derived from Solid-State NMR





A pore exists on the Proton Channel axis formed by this Tetrameric Structure.
The Pore is lined with a functionally Important tetrad of histidines and Tryptophan sidechains.

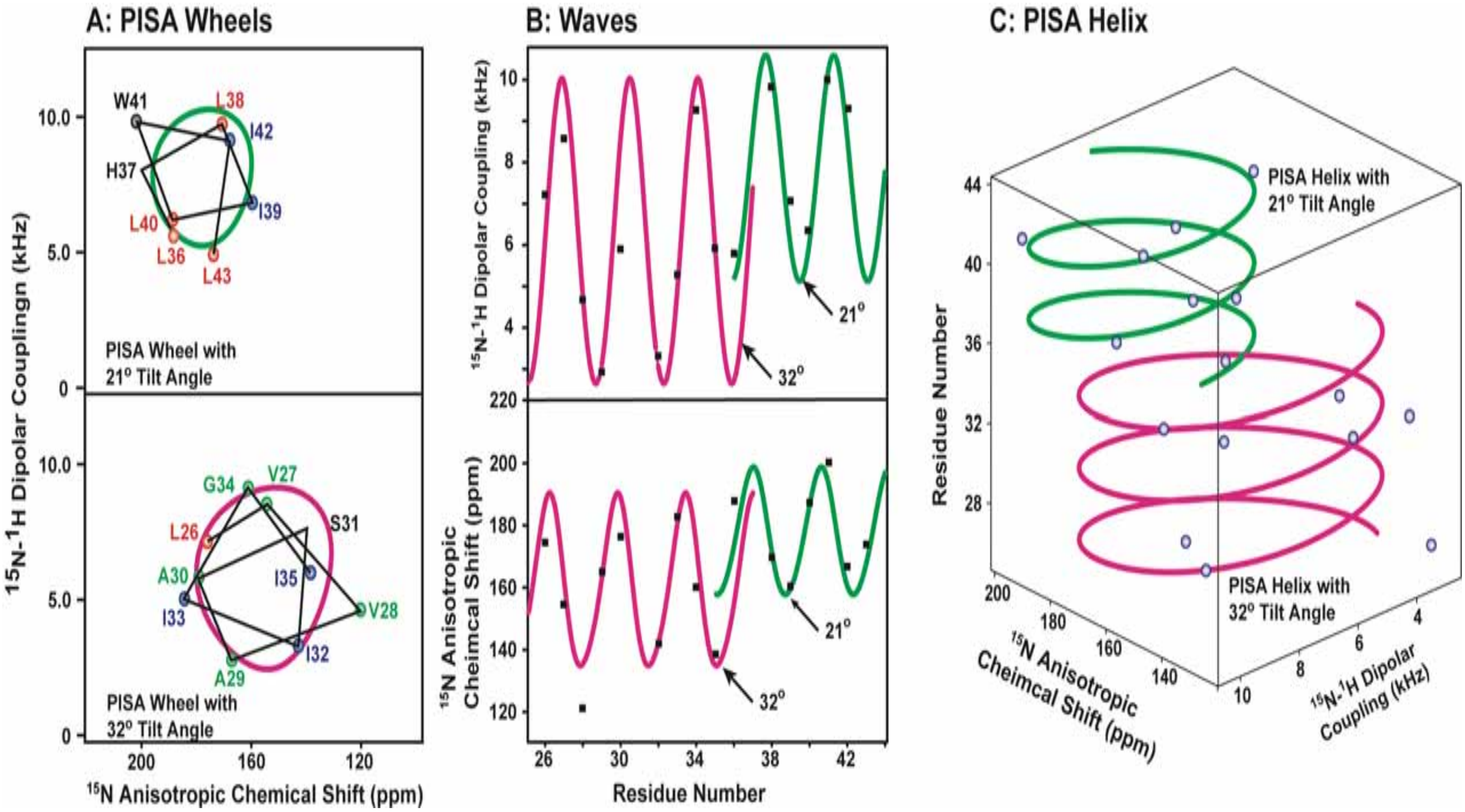
M2 Transmembrane Domain with & without Amantadine



Conggang Li, Jun Hu

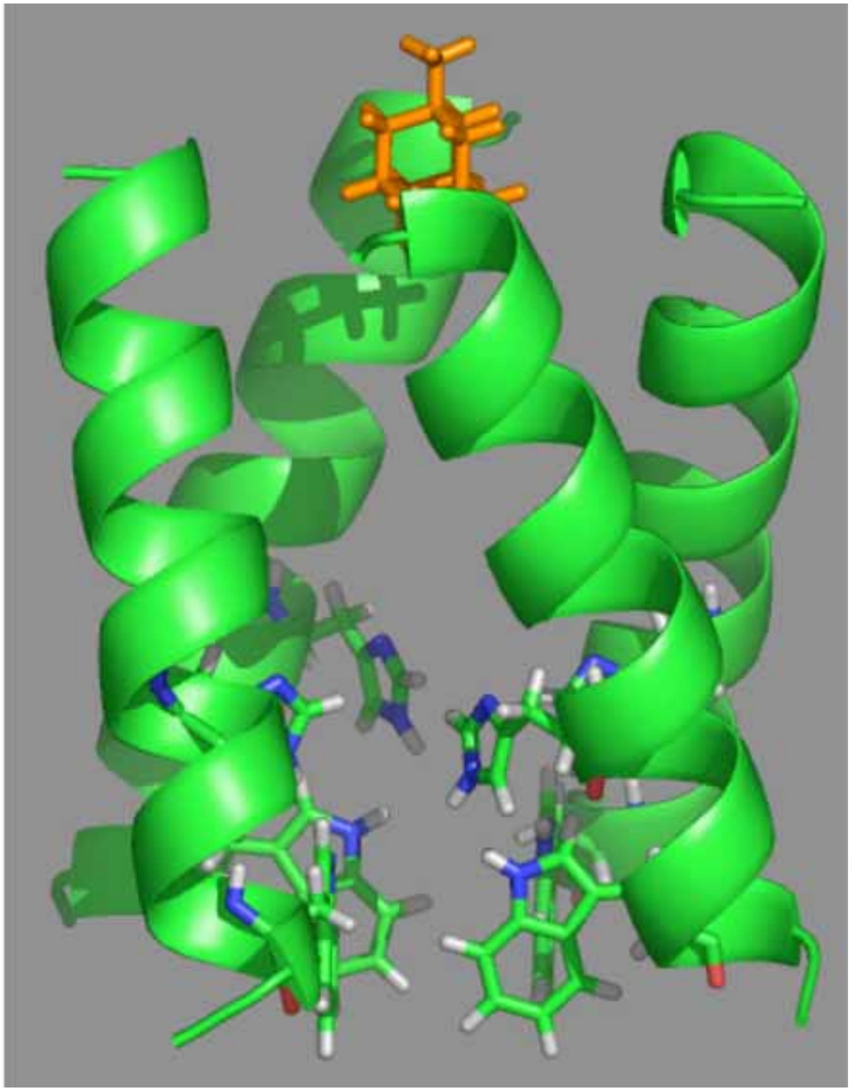
Analyses of the PISEMA Data for M2-TMD in the Presence of Amantadine

Jun Hu
Conggang Li

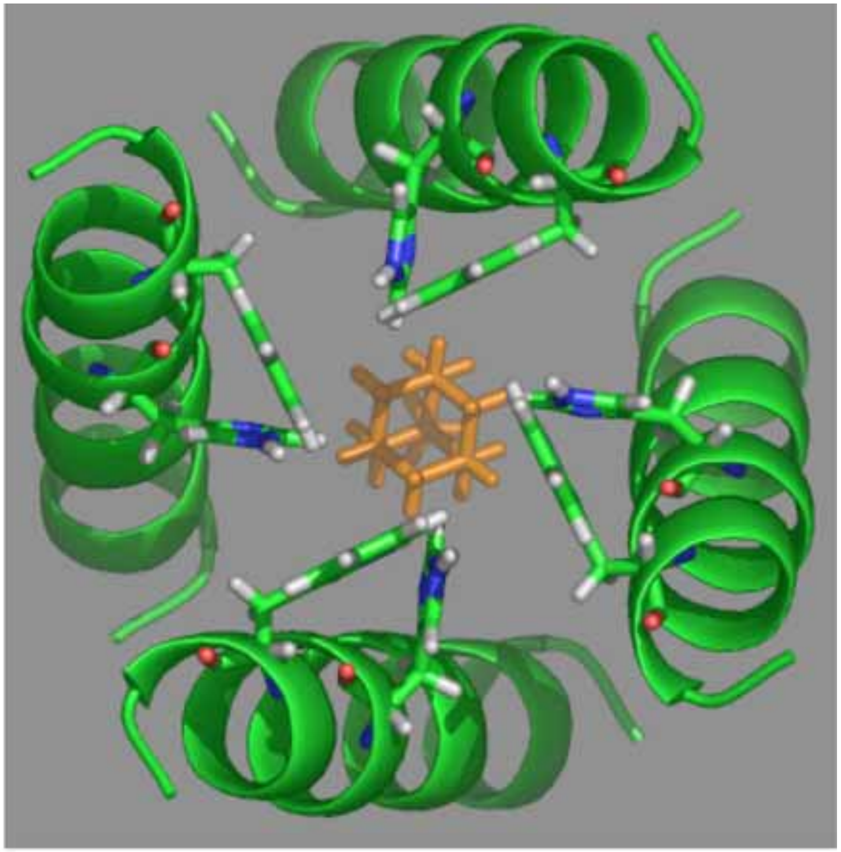


M2-TMD Backbone Model w/AMT

Side View



C-terminal End View



Hu, Asbury et al., (submitted) Biophys J.

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