Chemical Shift Anisotropy & Multidimensional Recoupling for Uniformly Labeled Proteins

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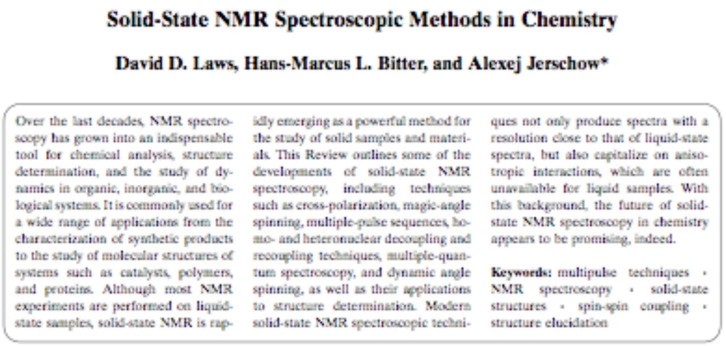
Winter School on Biomolecular Solid State NMR
Jan. 20-25, 2008
Stowe, Vermont

Outline

- A. Basic Concepts
  - What is CSA?
  - Why is CSA important, interesting, and/or useful?
  - Can it be calculated accurately?
- B. CSA Measurement Methods
  - Single crystal goniometer experiments
  - Slow magic-angle spinning
  - Recoupling in 2D and 3D
- C. Interpretation and Utility
  - Relationships to *ab initio* quantum calculations
  - Structure determination

Principal References

- Oldfield, *Ann. Rev. Phys. Chem.*, 2002, 53:349
- Laws et al., *Ang. Chem.* 2002, 41:3096
- Wylie Ph.D. thesis & recent pubs (*J. Chem. Phys.*, in press)

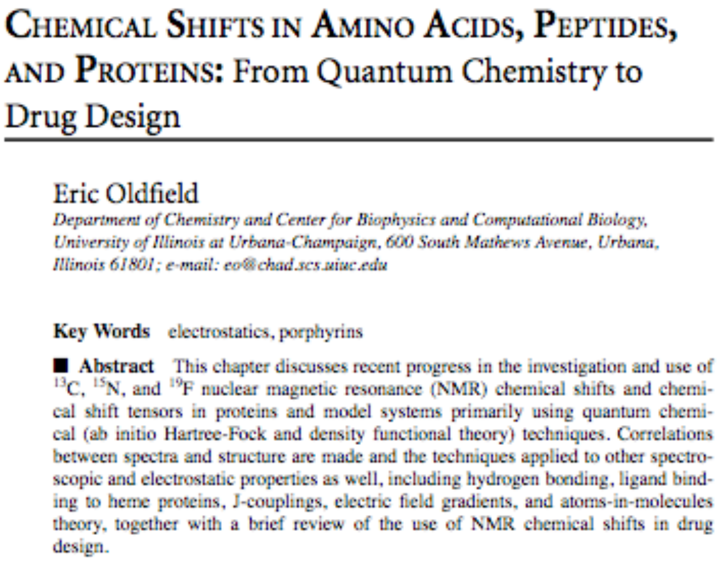


Solid-State NMR Spectroscopic Methods in Chemistry

David D. Laws, Hans-Marcus L. Bitter, and Alexej Jerschow*

Over the last decades, NMR spectroscopy has grown into an indispensable tool for chemical analysis, structure determination, and the study of dynamics in organic, inorganic, and biological systems. It is commonly used for a wide range of applications from the characterization of synthetic products to the study of molecular structures of systems such as catalysts, polymers, and proteins. Although most NMR experiments are performed on liquid-state samples, solid-state NMR is rapidly emerging as a powerful method for the study of solid samples and materials. This Review outlines some of the developments of solid-state NMR spectroscopy, including techniques such as cross-polarization, magic-angle spinning, multiple-pulse sequences, homo- and heteronuclear decoupling and recoupling techniques, multiple-quantum spectroscopy, and dynamic angle spinning, as well as their applications to structure determination. Modern solid-state NMR spectroscopic techniques not only produce spectra with a resolution close to that of liquid-state spectra, but also capitalize on anisotropic interactions, which are often unavailable for liquid samples. With this background, the future of solid-state NMR spectroscopy in chemistry appears to be promising, indeed.

Keywords: multipulse techniques · NMR spectroscopy · solid-state structures · spin-spin coupling · structure elucidation



CHEMICAL SHIFTS IN AMINO ACIDS, PEPTIDES, AND PROTEINS: From Quantum Chemistry to Drug Design

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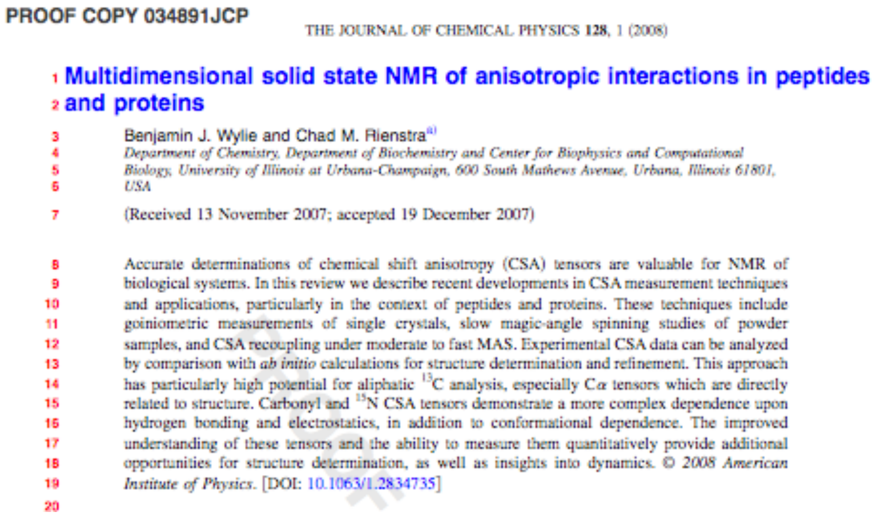
Key Words electrostatics, porphyrins

■ **Abstract** This chapter discusses recent progress in the investigation and use of ^{13}C, ^{15}N, and ^{19}F nuclear magnetic resonance (NMR) chemical shifts and chemical shift tensors in proteins and model systems primarily using quantum chemical (ab initio Hartree-Fock and density functional theory) techniques. Correlations between spectra and structure are made and the techniques applied to other spectroscopic and electrostatic properties as well, including hydrogen bonding, ligand binding to heme proteins, J-couplings, electric field gradients, and atoms-in-molecules theory, together with a brief review of the use of NMR chemical shifts in drug design.



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THE JOURNAL OF CHEMICAL PHYSICS **128**, 1 (2008)

Multidimensional solid state NMR of anisotropic interactions in peptides and proteins

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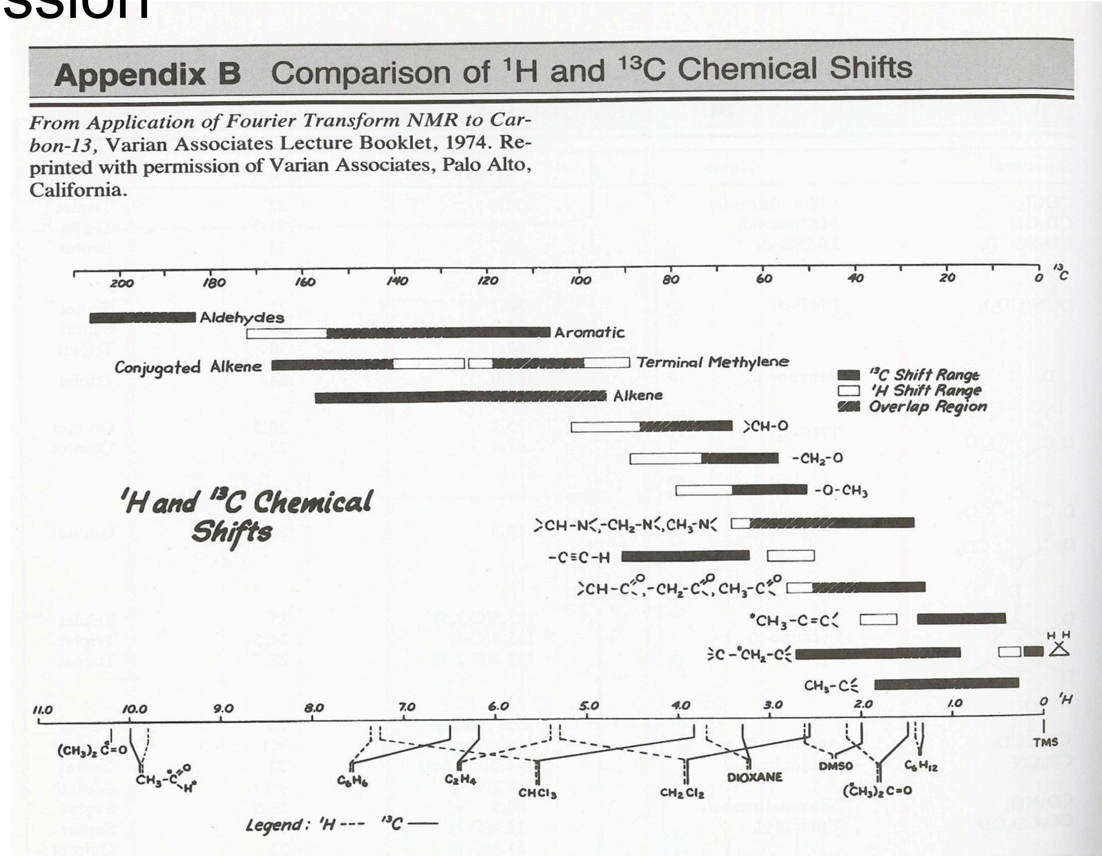
(Received 13 November 2007; accepted 19 December 2007)

Accurate determinations of chemical shift anisotropy (CSA) tensors are valuable for NMR of biological systems. In this review we describe recent developments in CSA measurement techniques and applications, particularly in the context of peptides and proteins. These techniques include goiniometric measurements of single crystals, slow magic-angle spinning studies of powder samples, and CSA recoupling under moderate to fast MAS. Experimental CSA data can be analyzed by comparison with *ab initio* calculations for structure determination and refinement. This approach has particularly high potential for aliphatic ^{13}C analysis, especially Cα tensors which are directly related to structure. Carbonyl and ^{15}N CSA tensors demonstrate a more complex dependence upon hydrogen bonding and electrostatics, in addition to conformational dependence. The improved understanding of these tensors and the ability to measure them quantitatively provide additional opportunities for structure determination, as well as insights into dynamics. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2834735]

The “Chemical Shift”

- Not called “NMR emission frequencies”
- More useful than that!
- Explicit, predictable relationship to electronic structure



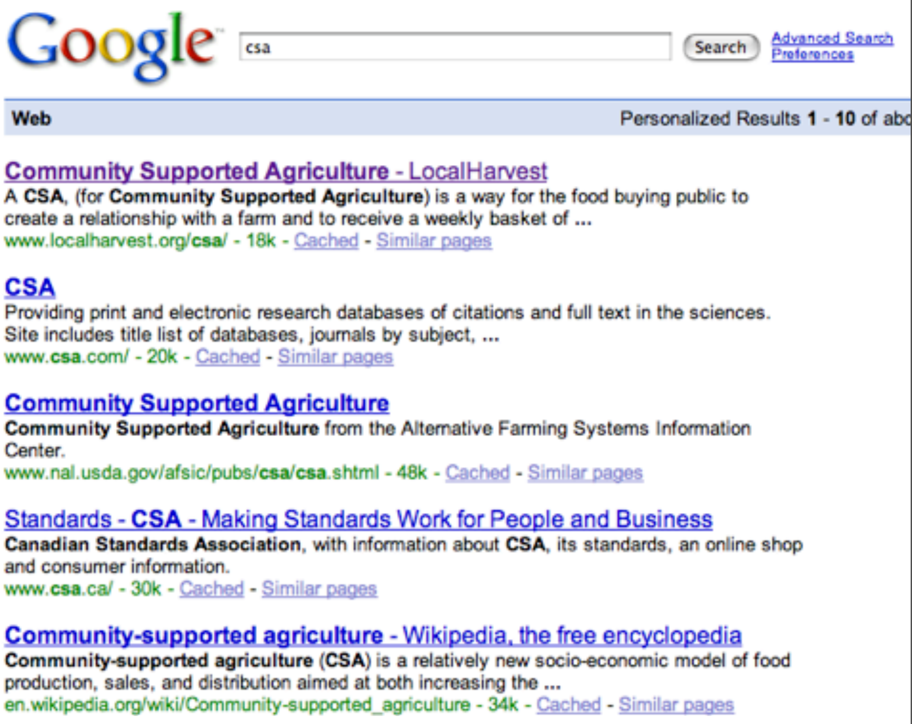
Appendix B Comparison of ^1H and ^{13}C Chemical Shifts

From Application of Fourier Transform NMR to Carbon-13, Varian Associates Lecture Booklet, 1974. Reprinted with permission of Varian Associates, Palo Alto, California.

Silverstein, Bassler, and Morrill, *Spectrometric Identification of Organic Compounds,* Appendix B

Some things the CSA is not

- Community-supported agriculture
- Certified senior advisors
- Confederate States of America (a Spike Lee joint)
- Controlled substance act
- Canadian space agency
- Continuous symmetry analysis



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A **CSA**, (for **Community Supported Agriculture**) is a way for the food buying public to create a relationship with a farm and to receive a weekly basket of ...
www.localharvest.org/**csa**/ - 18k - Cached - Similar pages

CSA
Providing print and electronic research databases of citations and full text in the sciences. Site includes title list of databases, journals by subject, ...
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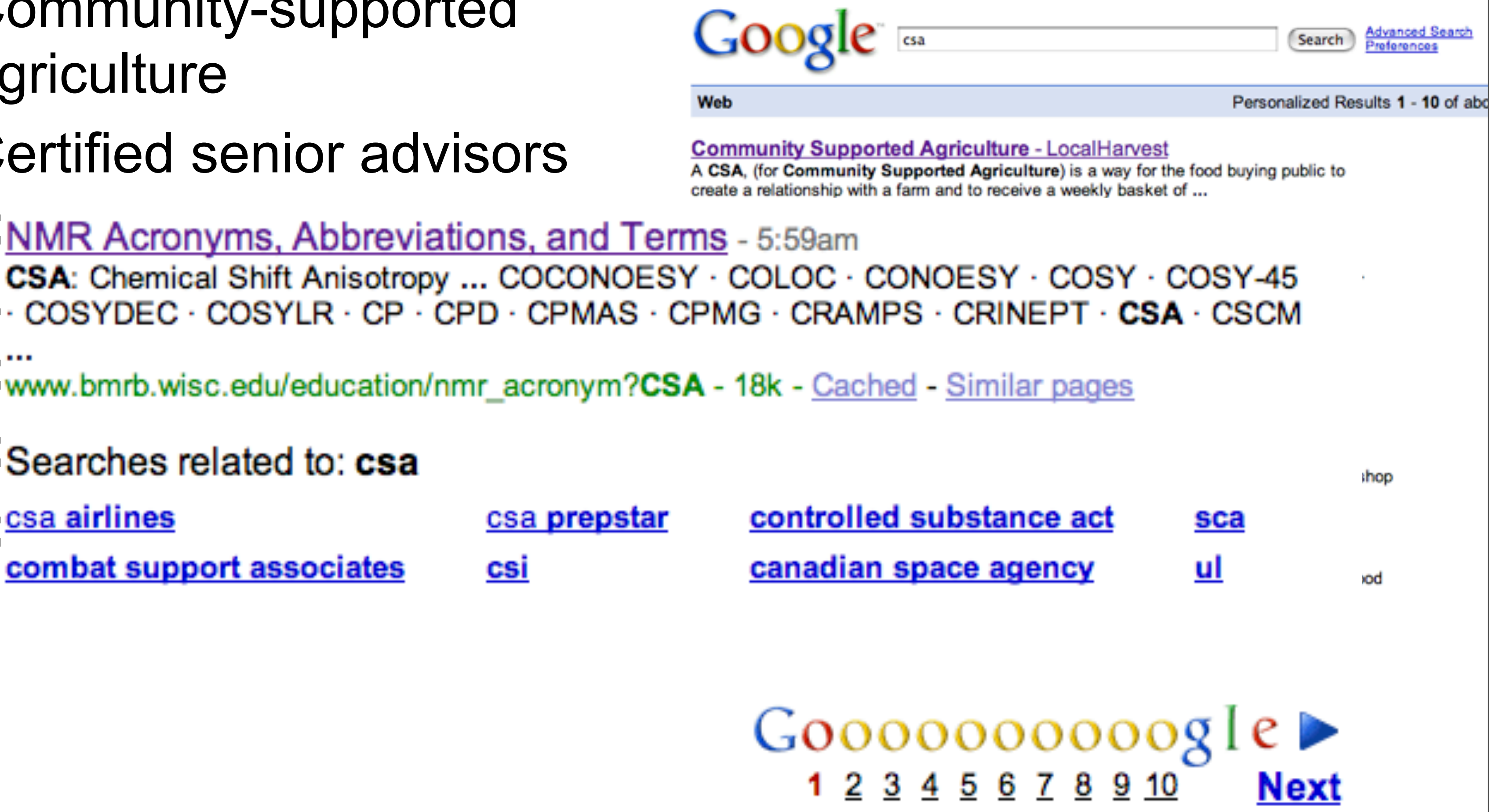
Community Supported Agriculture
Community Supported Agriculture from the Alternative Farming Systems Information Center.
www.nal.usda.gov/afsic/pubs/**csa**/**csa**.shtml - 48k - Cached - Similar pages

Standards - **CSA** - Making Standards Work for People and Business
Canadian Standards Association, with information about **CSA**, its standards, an online shop and consumer information.
www.**csa**.ca/ - 30k - Cached - Similar pages

Community-supported agriculture - Wikipedia, the free encyclopedia
Community-supported agriculture (**CSA**) is a relatively new socio-economic model of food production, sales, and distribution aimed at both increasing the ...
en.wikipedia.org/wiki/Community-supported_agriculture - 34k - Cached - Similar pages

Some things the CSA is not

- Community-supported agriculture
- Certified senior advisors
- C A
- C
- C
- C a



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A **CSA**, (for **Community Supported Agriculture**) is a way for the food buying public to create a relationship with a farm and to receive a weekly basket of ...

NMR Acronyms, Abbreviations, and Terms - 5:59am
CSA: Chemical Shift Anisotropy ... COCONOESY · COLOC · CONOESY · COSY · COSY-45 · COSYDEC · COSYLR · CP · CPD · CPMAS · CPMG · CRAMPS · CRINEPT · **CSA** · CSCM ...
www.bmrb.wisc.edu/education/nmr_acronym?**CSA** - 18k - Cached - Similar pages

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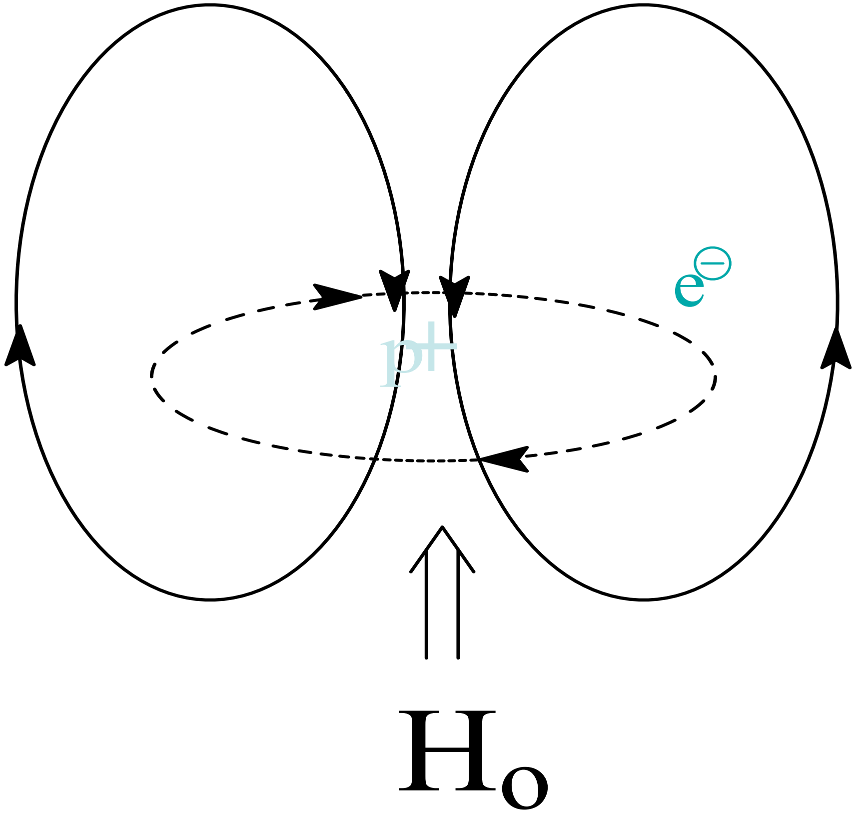
csa **airlines**	csa **prepstar**	**controlled substance act**	**sca**
combat support associates	**csi**	**canadian space agency**	**ul**

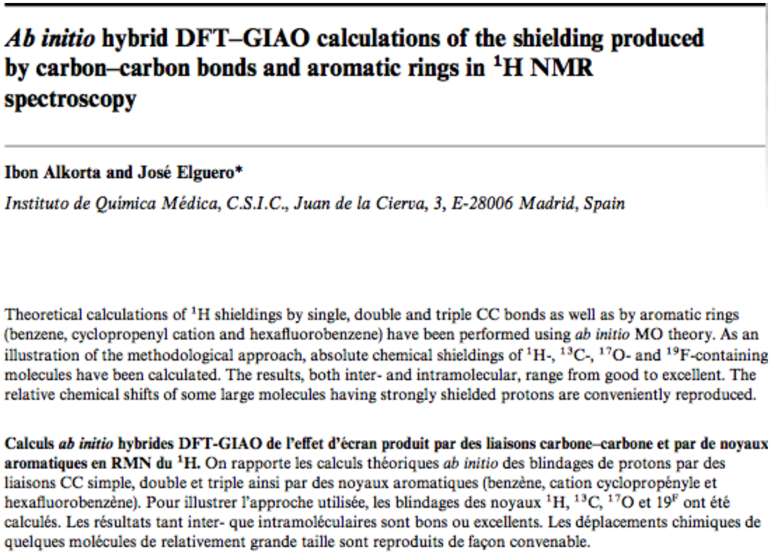
Basic Qualitative Description

- In a magnetic field (Ho) valence electrons are induced to circulate, which generates a secondary magnetic field that opposes the applied field near the nucleus. Thus a higher field is needed to achieve resonance. This is shielding.



Extreme Shielding, Stowe Edition

- *New J. Chem.*, 1998, 331



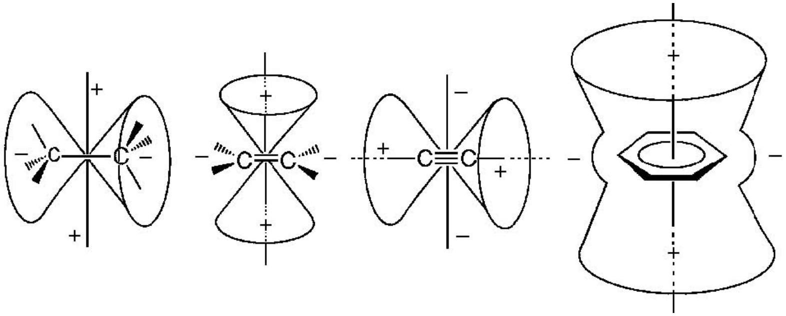
***Ab initio* hybrid DFT–GIAO calculations of the shielding produced by carbon–carbon bonds and aromatic rings in ^{1}H NMR spectroscopy**

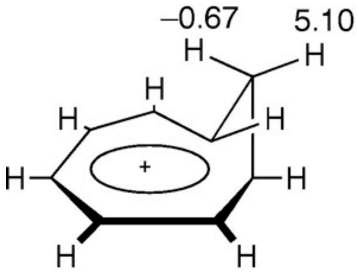
Ibon Alkorta and José Elguero*

Instituto de Química Médica, C.S.I.C., Juan de la Cierva, 3, E-28006 Madrid, Spain

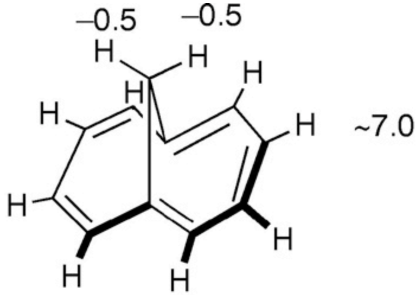
Theoretical calculations of ^{1}H shieldings by single, double and triple CC bonds as well as by aromatic rings (benzene, cyclopropenyl cation and hexafluorobenzene) have been performed using *ab initio* MO theory. As an illustration of the methodological approach, absolute chemical shieldings of ^{1}H-, ^{13}C-, ^{17}O- and ^{19}F-containing molecules have been calculated. The results, both inter- and intramolecular, range from good to excellent. The relative chemical shifts of some large molecules having strongly shielded protons are conveniently reproduced.

Calculs *ab initio* hybrides DFT-GIAO de l'effet d'écran produit par des liaisons carbone–carbone et par de noyaux aromatiques en RMN du ^{1}H. On rapporte les calculs théoriques *ab initio* des blindages de protons par des liaisons CC simple, double et triple ainsi par des noyaux aromatiques (benzène, cation cyclopropényle et hexafluorobenzène). Pour illustrer l'approche utilisée, les blindages des noyaux ^{1}H, ^{13}C, ^{17}O et 19^F ont été calculés. Les résultats tant inter- que intramoléculaires sont bons ou excellents. Les déplacements chimiques de quelques molécules de relativement grande taille sont reproduits de façon convenable.

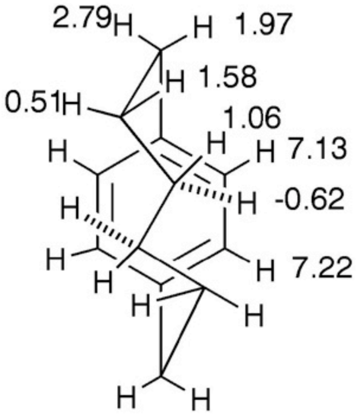




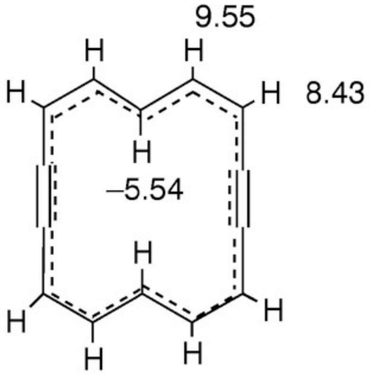
8, $[C_8H_9]^+$



9, $C_{11}H_{10}$



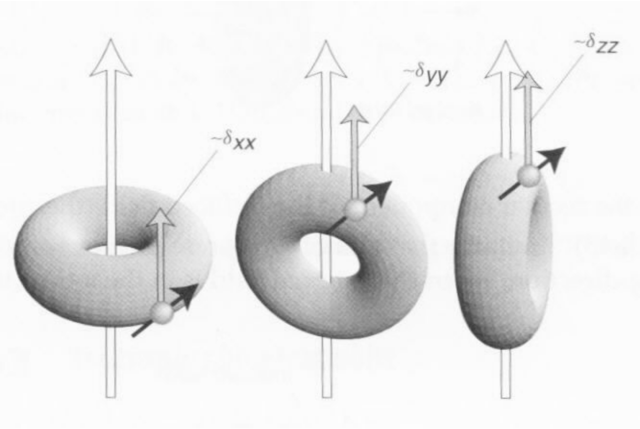
10, $C_{12}H_{16}$



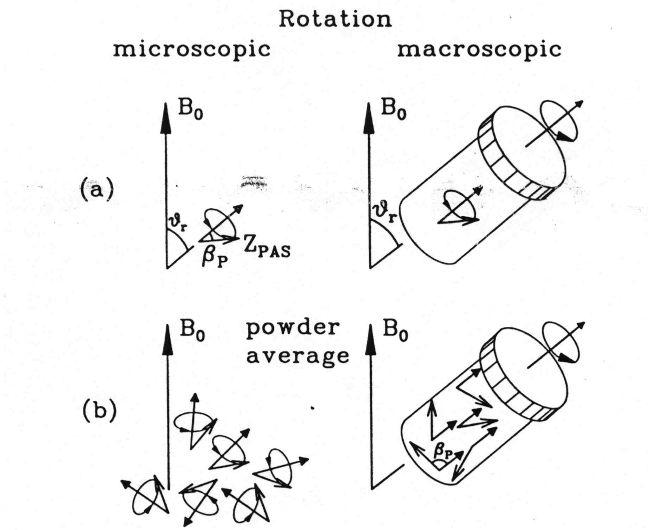
11, $C_{14}H_{10}$

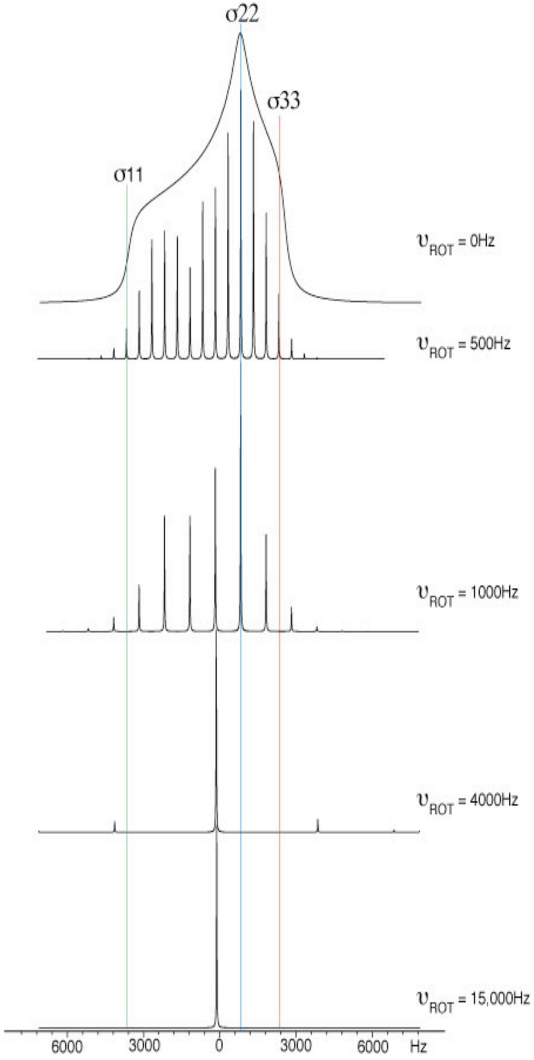
8: homotropylium cation
9: 1,6-methano[10]annulene
11: 1,2,8,9-tetrahydro[14]annulene

Origins of CSA



Elements of the Chemical Shift Tensor



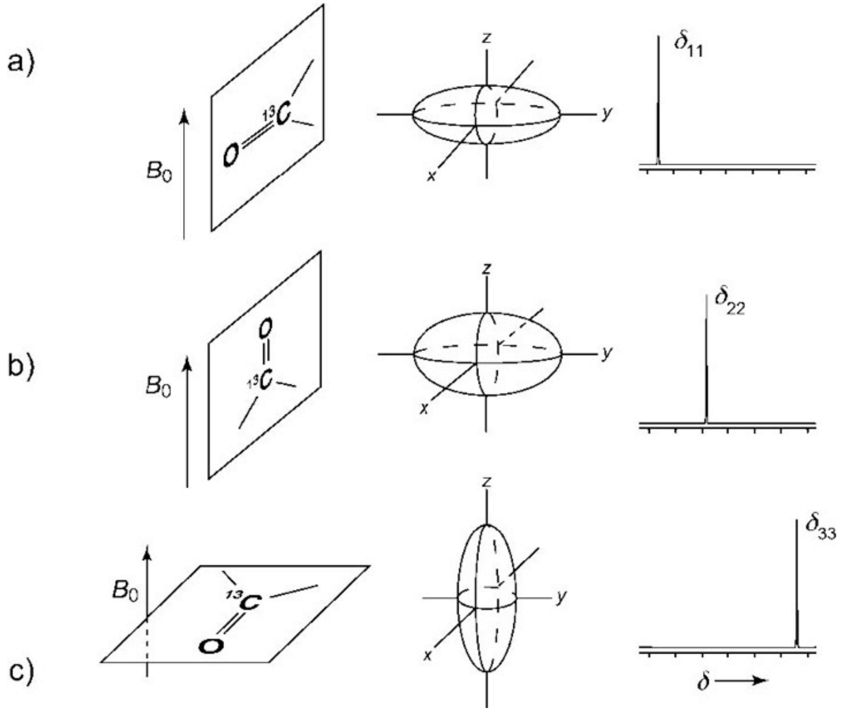


Spinning Sidebands under MAS

Schmidt-Rohr and Spiess (1994)

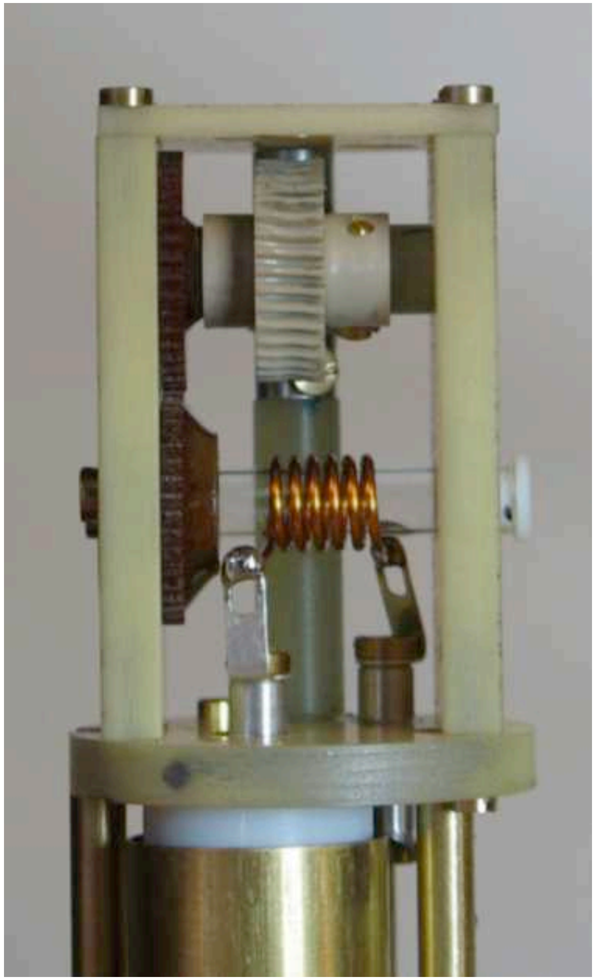
Chemical Shift Anisotropy

- CSA means orientation dependence of the chemical shift, and arises due to the fact that in a nuclei, the charge distribution is rarely spherically symmetrical.
- The degree to which electron density affects resonance frequency (also known as shielding) of a nucleus depends on the orientation of the electron cloud.



Goniometer Probes

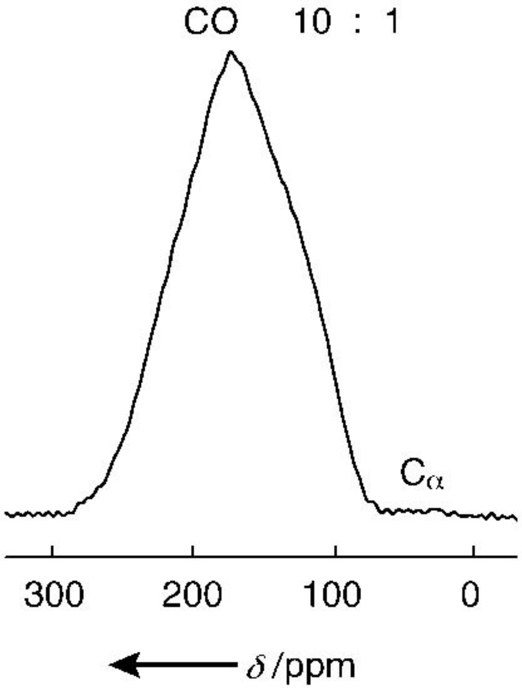
- Must have single crystal
- Shift is a function of orientation, as discussed by KWZ yesterday
- Useful for small molecules
- Can determine absolute orientation to the crystal frame
- Impractical for larger molecules

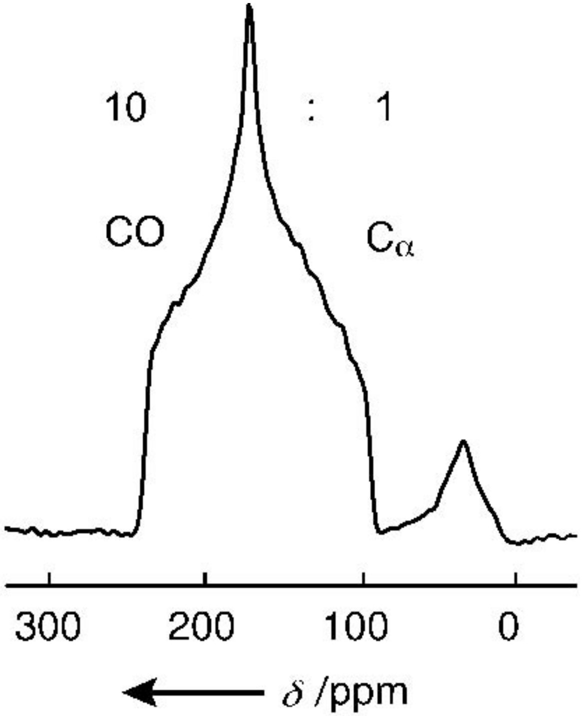


http://www.nmr-service.de/?Solids_NMR_Probes:Low_Temp._NMR%2FNQR

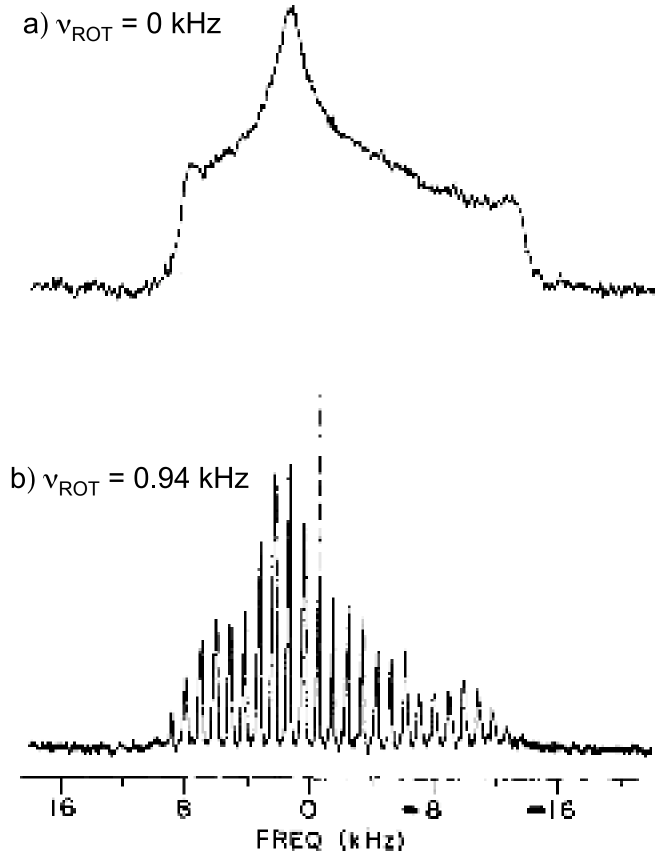
Static Powder Lineshape

- Resolution limited: glycine (1-^{13}C, as below)
- Overlapping lineshapes

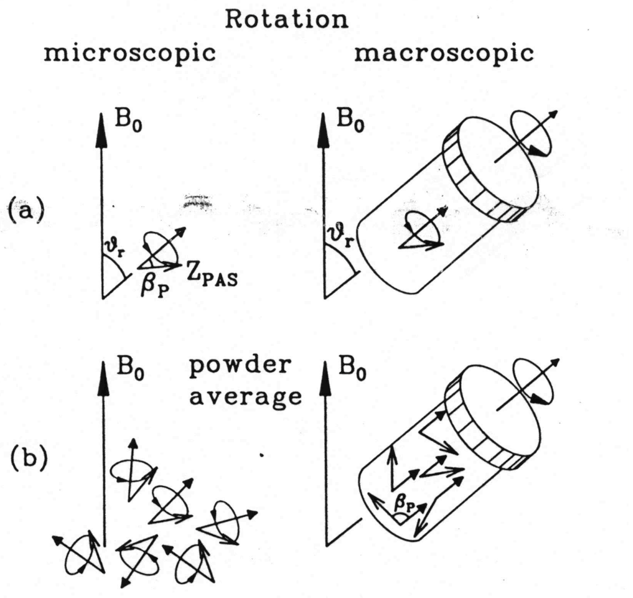




CSA under Magic Angle Spinning



- Slow spinning produces spinning sidebands spaced ω_r from the isotropic line in the spectra



J. Herzfeld, A. E. Berger, *J. Chem. Phys.*, **1980**, 73, 6021- 6030.
Schmidt-Rohr, Spiess, *Multidimensional Solid State NMR and Polymers*; Academic Press, **1994**

Herzfeld-Berger Convention

Principal Components, δ_{11}, δ_{22} and δ_{33} are ordered from highest to lowest frequency:

$$\delta_{11} \geq \delta_{22} \geq \delta_{33}$$

Isotropic chemical shift:

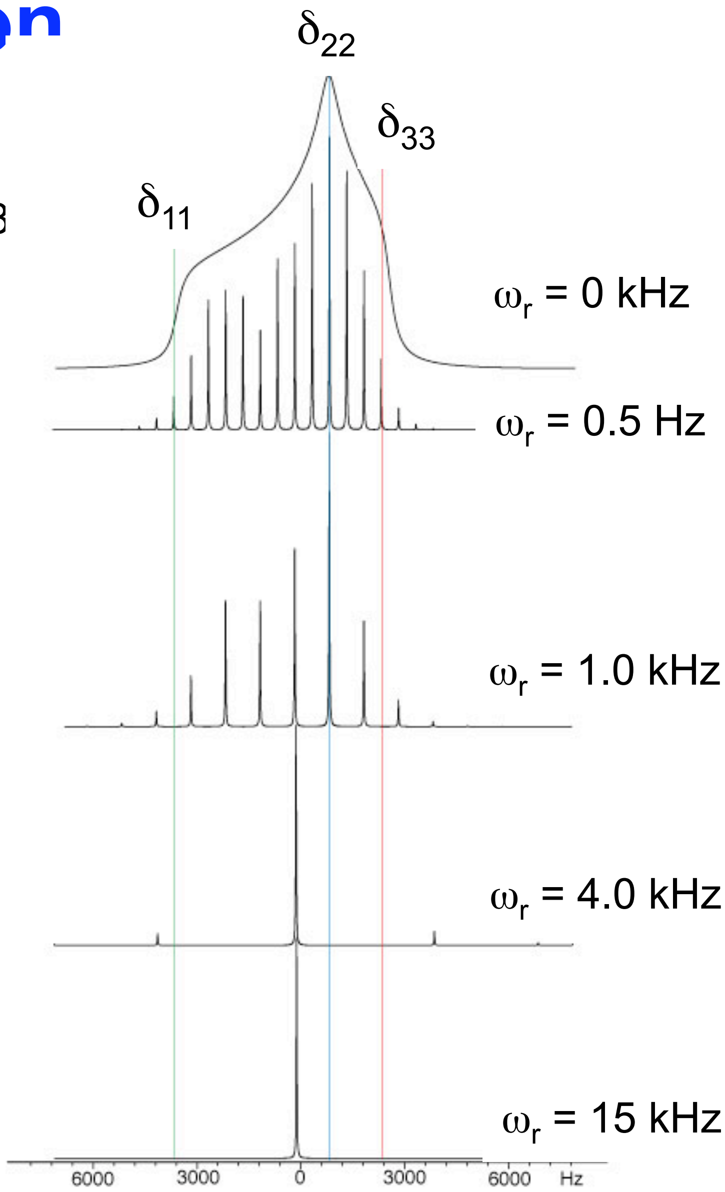
$$\delta_{iso} = (\delta_{11} + \delta_{22} + \delta_{33})/3$$

Span:

$$\Omega = \delta_{11} - \delta_{33}$$

Skew:

$$\kappa = 3(\delta_{22} - \delta_{iso})/\Omega \quad (-1 \leq \kappa \leq +1)$$



J. Herzfeld, A. E. Berger, *J. Chem. Phys.*, **1980**, 73, 6021- 6030.
Schmidt-Rohr, Spiess, *Multidimensional Solid State NMR and Polymers*; Academic Press, **1994**

Haeberlen Convention

- Principal Components are ordered according to their magnitude in the traceless representation:

$$|\delta_{zz} - \delta_{iso}| \geq |\delta_{xx} - \delta_{iso}| \geq |\delta_{yy} - \delta_{iso}|$$

- Isotropic Chemical Shift: $\delta_{iso} = (\delta_{xx} + \delta_{yy} + \delta_{zz})/3$
- Reduced Anisotropy: $\delta = \delta_{zz} - \delta_{iso}$
- Chemical Shift Anisotropy: $\Delta\delta = \delta_{zz} - (\delta_{xx} + \delta_{yy})/2 = 3/2\ \delta$
- Simplifies simulations in the basis of spherical irreducible tensors

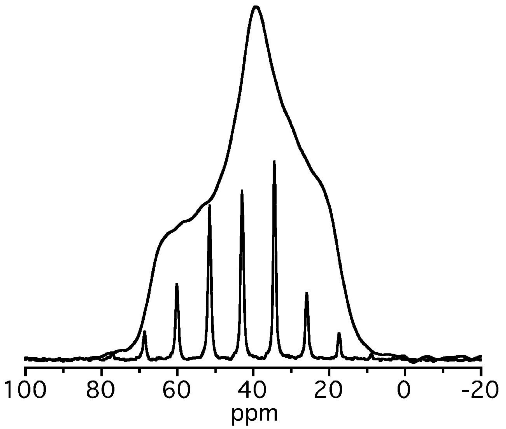
$$\rho_{00} = \delta_{iso}, \rho_{20} = \sqrt{\frac{3}{2}}\delta, \rho_{2\pm 2} = \frac{1}{2}\eta\delta$$

Haeberlen, U. *High Resolution NMR in Solids: Selective Averaging*; Academic Press, **1976**.

Measurement Strategies

- Single crystal goniometer
    - Extremely precise and accurate
    - Orientation relative to crystal frame
- Powder lineshapes (directly measured)
    - High accuracy and precision
    - Low signal-to-noise and resolution
- Slow MAS: Herzfeld Berger
    - Slightly reduced accuracy and precision
    - Resolution for ~10 to 20 sites; good sensitivity
- Recoupling methods
    - High accuracy and precision (if done well)
    - Resolution for hundreds of sites

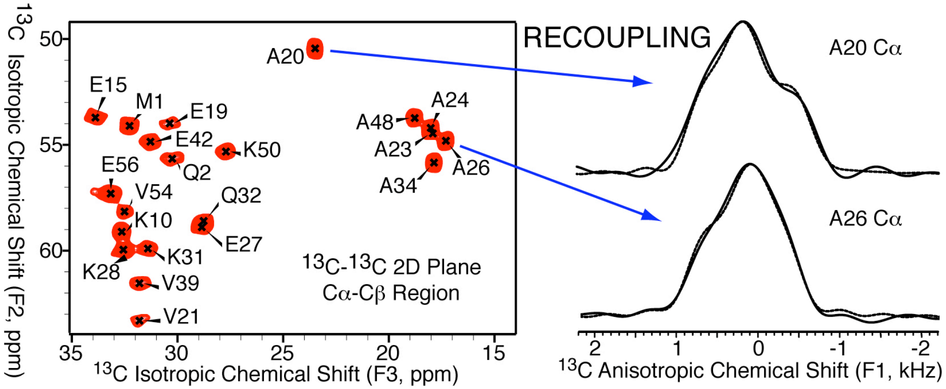
Methods to Measure CSA Parameters

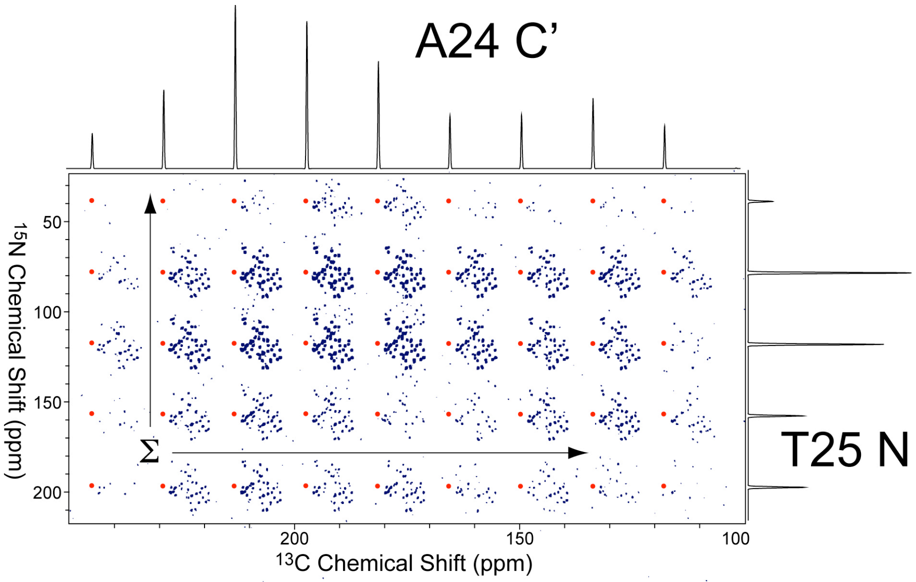


Static lineshape and sideband manifold

Goal: measure CSA throughout protein

Site resolution is essential



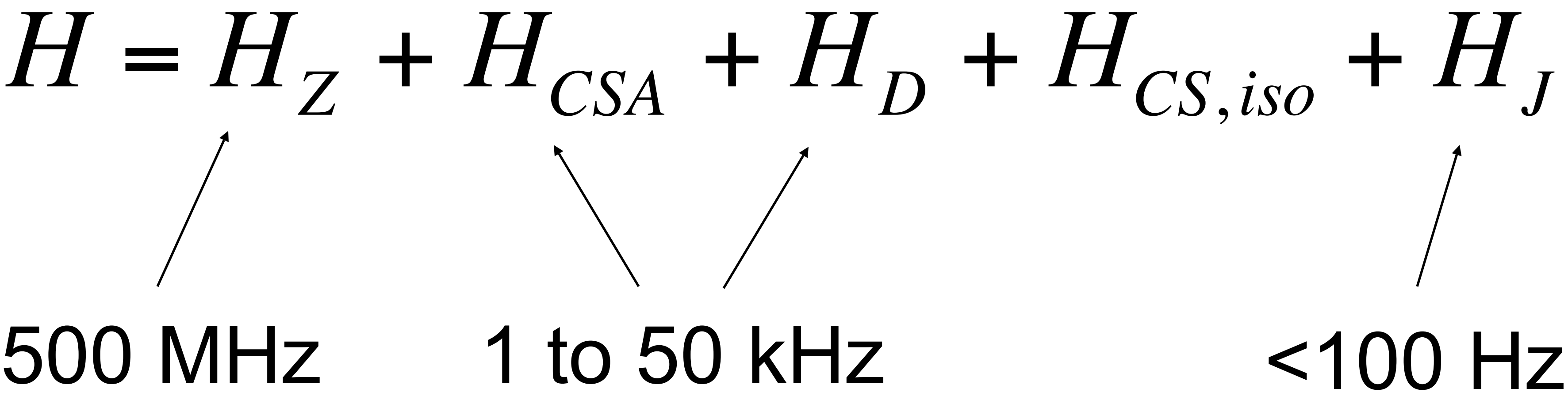


Recoupling

Slow MAS

Wylie, Franks, Graesser, Rienstra, *JACS* **2005**, *127*, 11946-11947.
Wylie, Sperling, Frericks, Shah, Franks, Rienstra, *JACS* **2007**, *129*, 5318-19.

SSNMR Spin Hamiltonian



$$H = H_Z + H_{CSA} + H_D + H_{CS,iso} + H_J$$

500 MHz

1 to 50 kHz

<100 Hz

Rotating frame eliminates Zeeman term

Internal terms of the Hamiltonian

- Anisotropic terms (solid): ~1 to 50 kHz
- Isotropic terms (solution): ~1 to 100 Hz

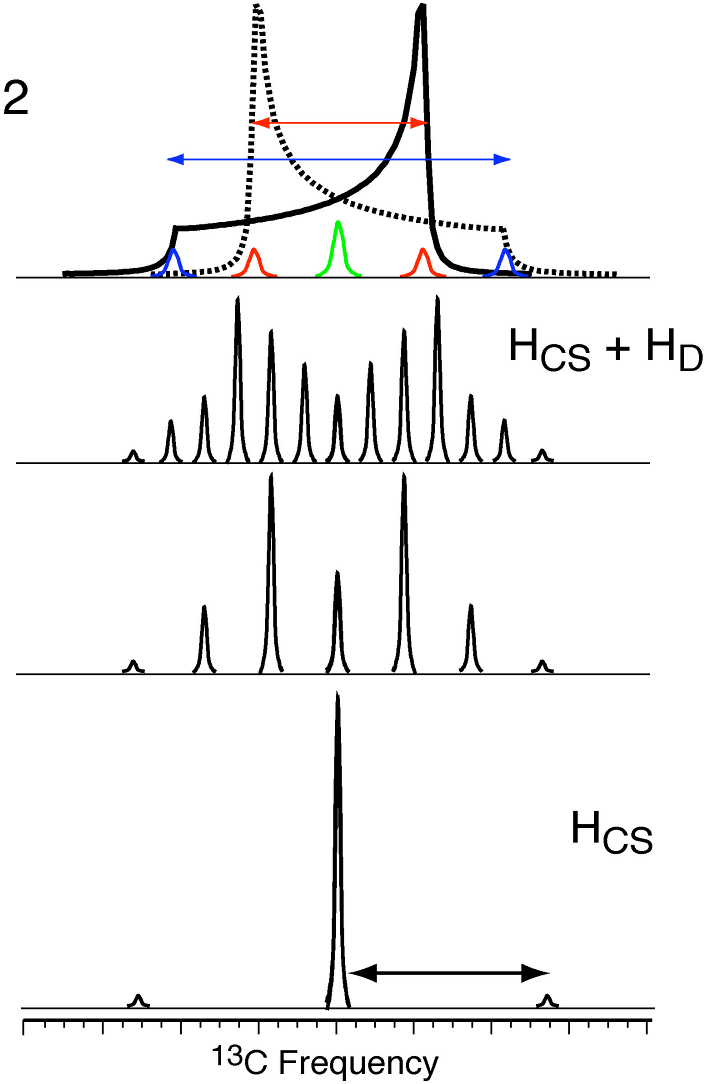
Experimental control

- Radio frequency pulses: Up to 125 kHz

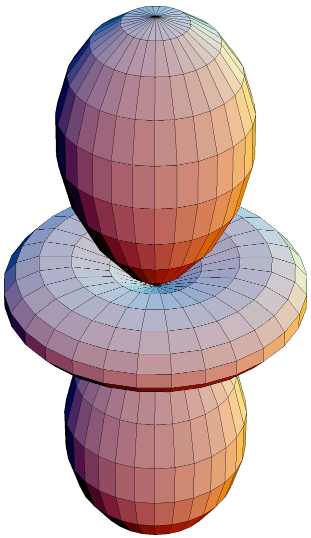
Magic-Angle Spinning (MAS)

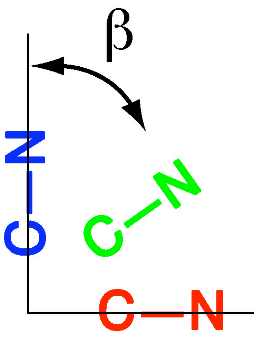
$$\omega_D = \omega_{max}\,(3\cos^2\beta - 1)/2$$

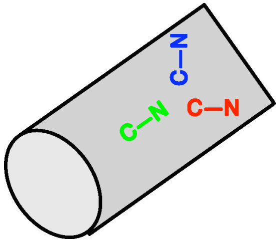
$$\omega_{max} = \kappa\,\frac{\gamma_I\,\gamma_S}{r^3}$$



maximum
structural
information



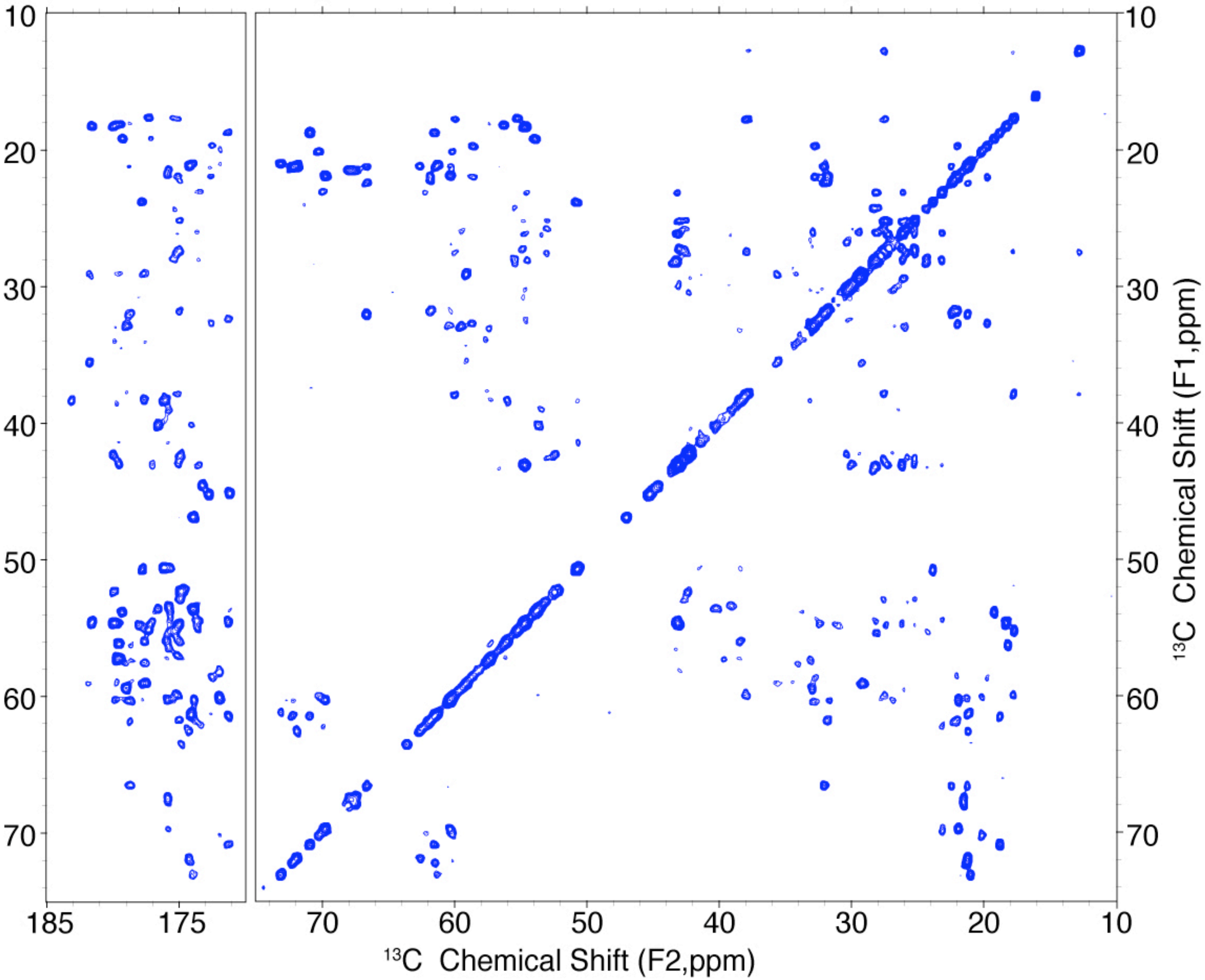




maximum
resolution
and
sensitivity

Lowe, I.J., *Phys. Rev. Lett.* **1959**, *2*, 285.
Andrew, Bradbury, Eades, *Nature* **1958**, *182*, 1659.

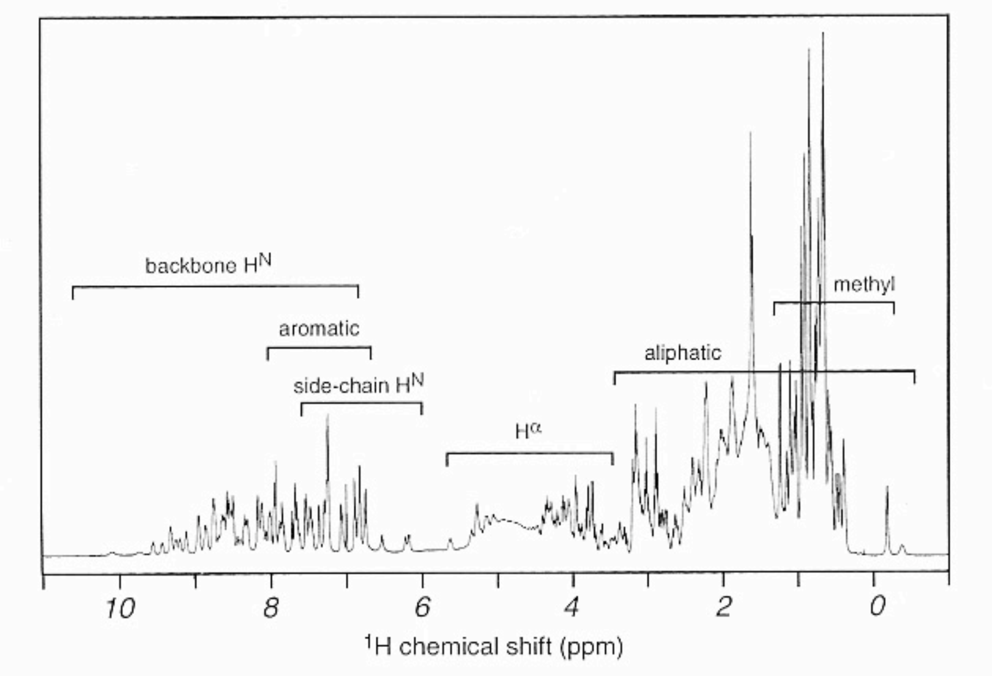
High-Resolution Protein SSNMR

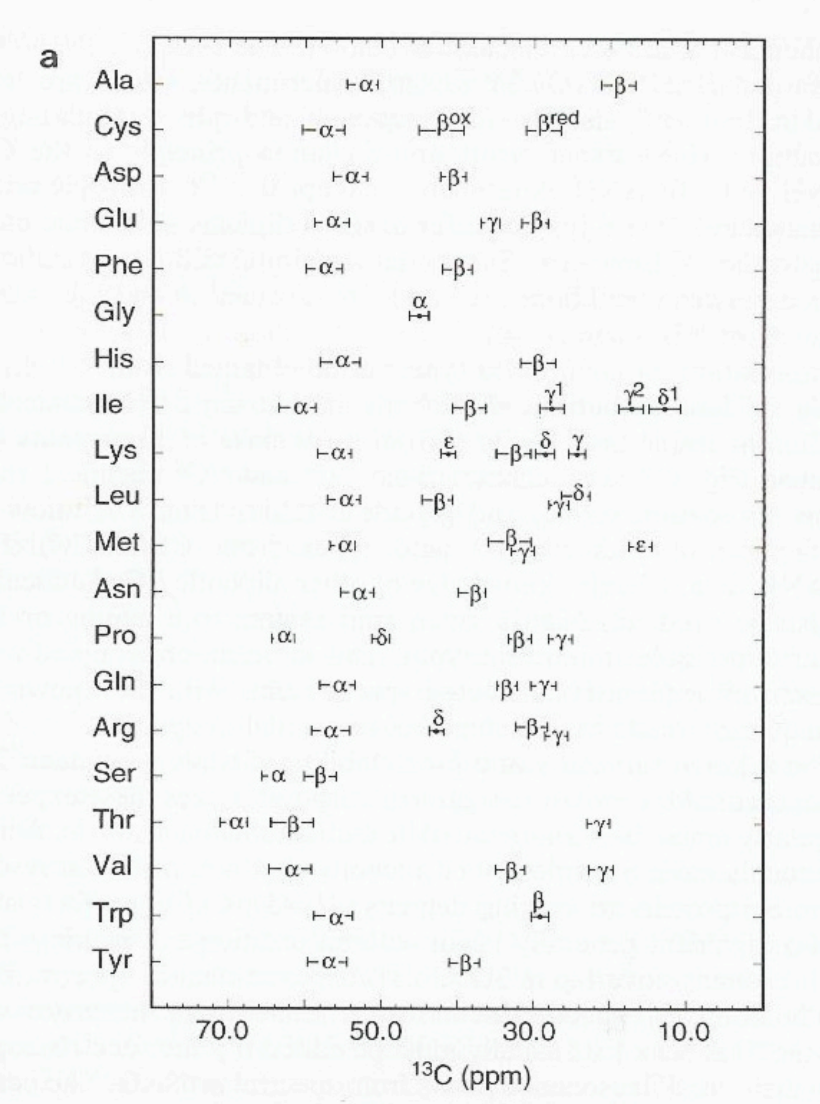


56 residue protein (GB1), 6 kDa, 750 MHz ^{1}H frequency; ~380 peaks shown

Heather Frericks Schmidt and Donghua Zhou

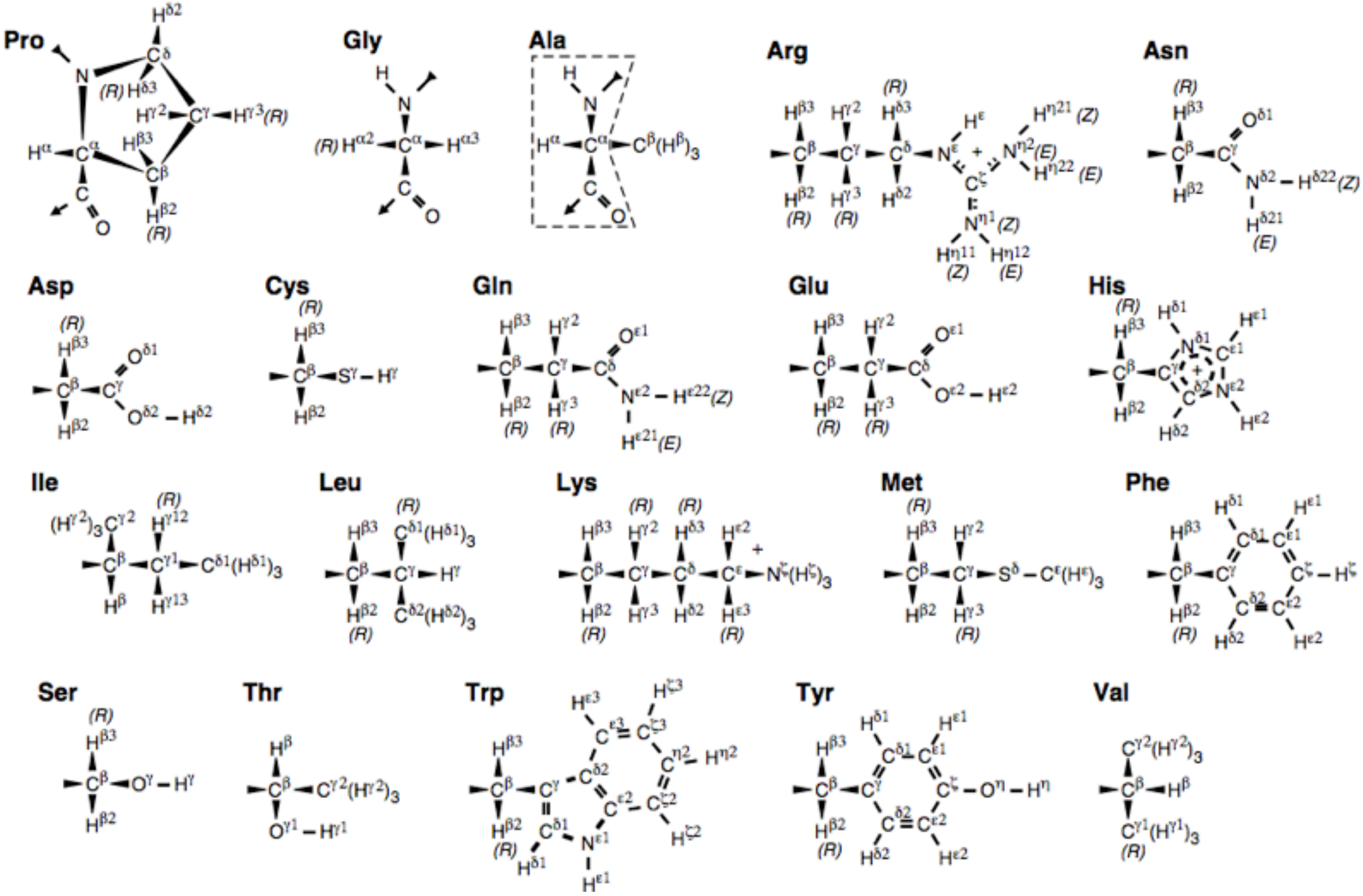
Unique Chemical Shifts in Proteins





- ^{1}H and ^{13}C shifts depend on:
  - 1. amino acid residue type (~20 ppm)
  - 2. conformation (~5 ppm)
  - 3. residue type of neighbors (~2 ppm)
  - 4. hydrogen bonding (varies)

Amino Acid Structures



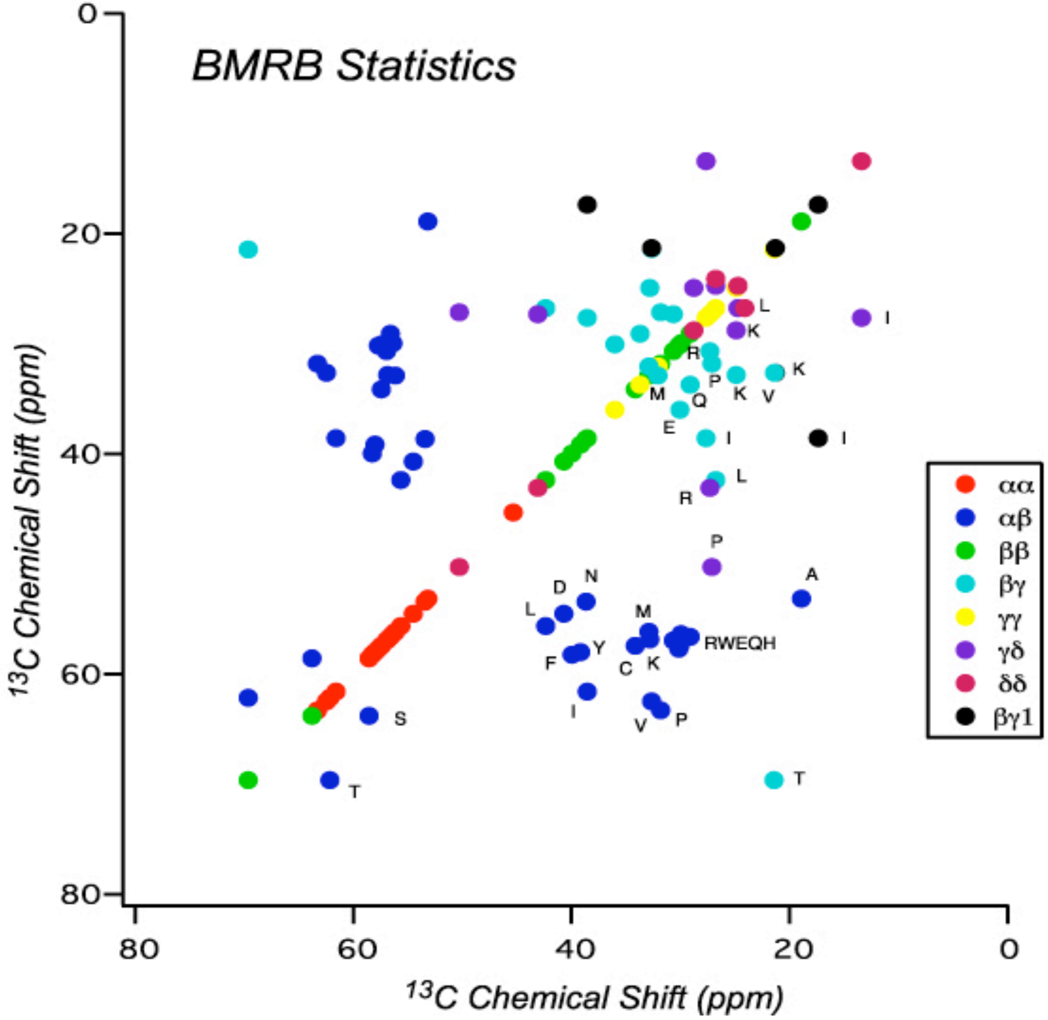
Residue Type Dependence

Always clear:
G, A, S, T, P, I

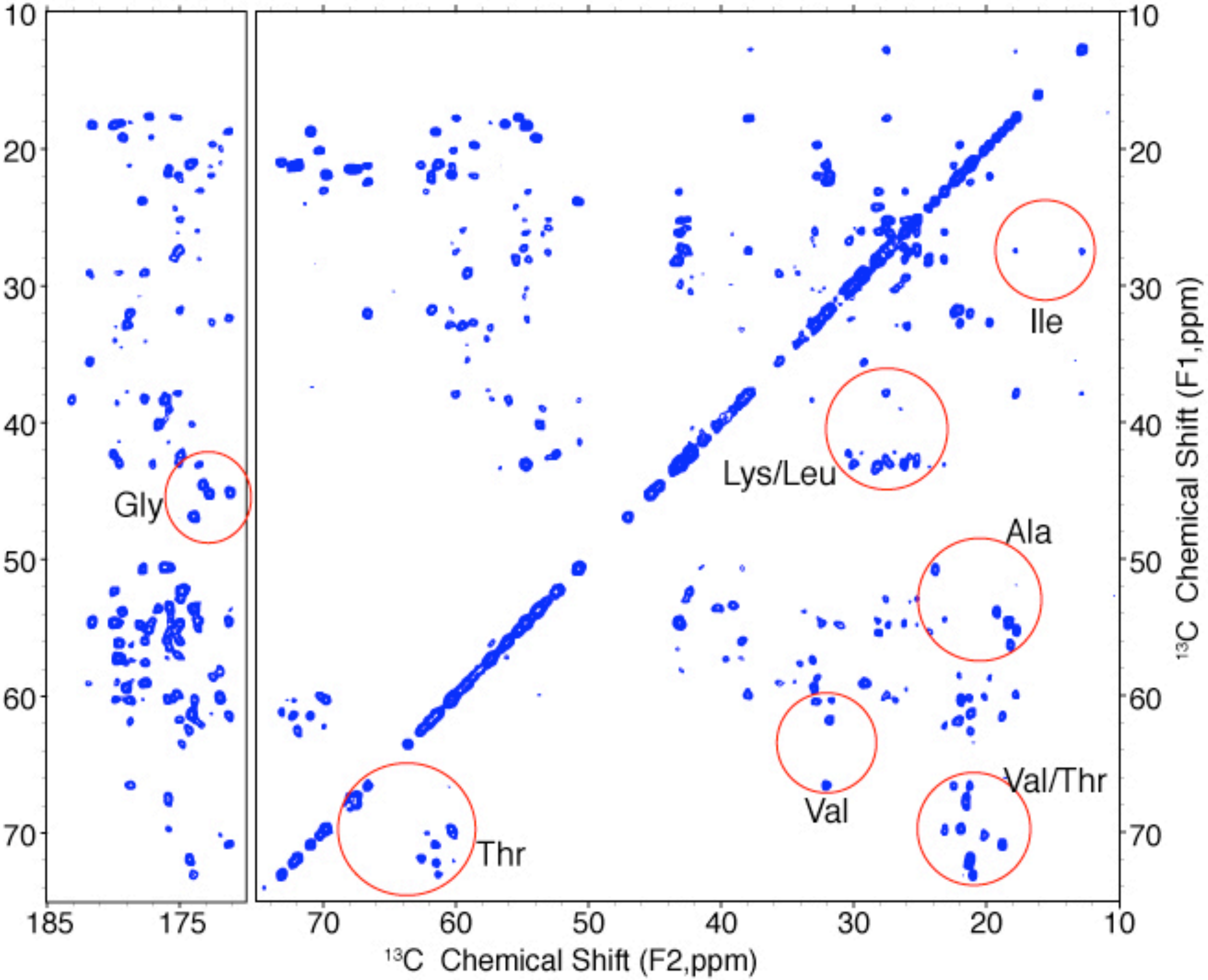
Usually clear:
L, V, D/N

Aromatics:
use Cβ-Cγ to distinguish

Ambiguous:
C, M, K, R, E/Q



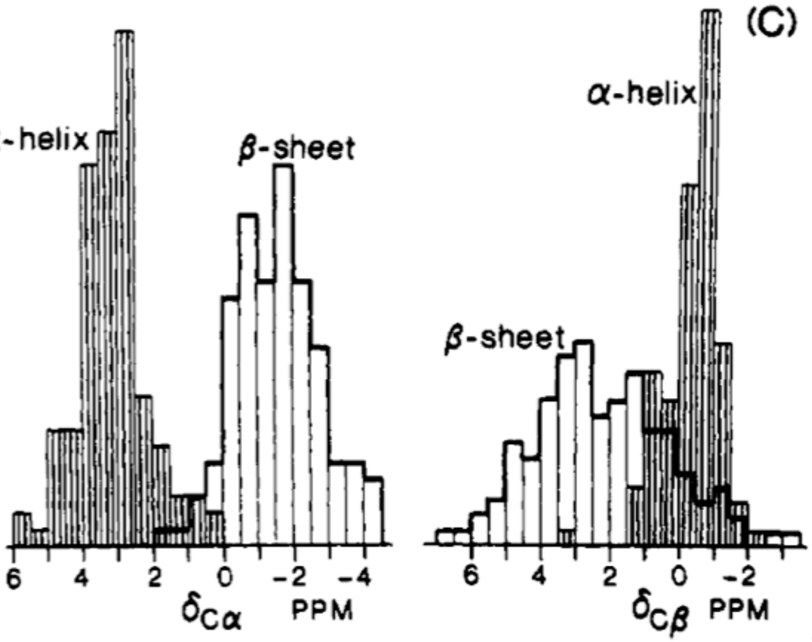
Signal Patterns by Amino Acid Type



56 residue protein (GB1), 6 kDa, 750 MHz ^{1}H frequency; ~380 peaks shown

Heather Frericks Schmidt and Donghua Zhou

Secondary Chemical Shifts



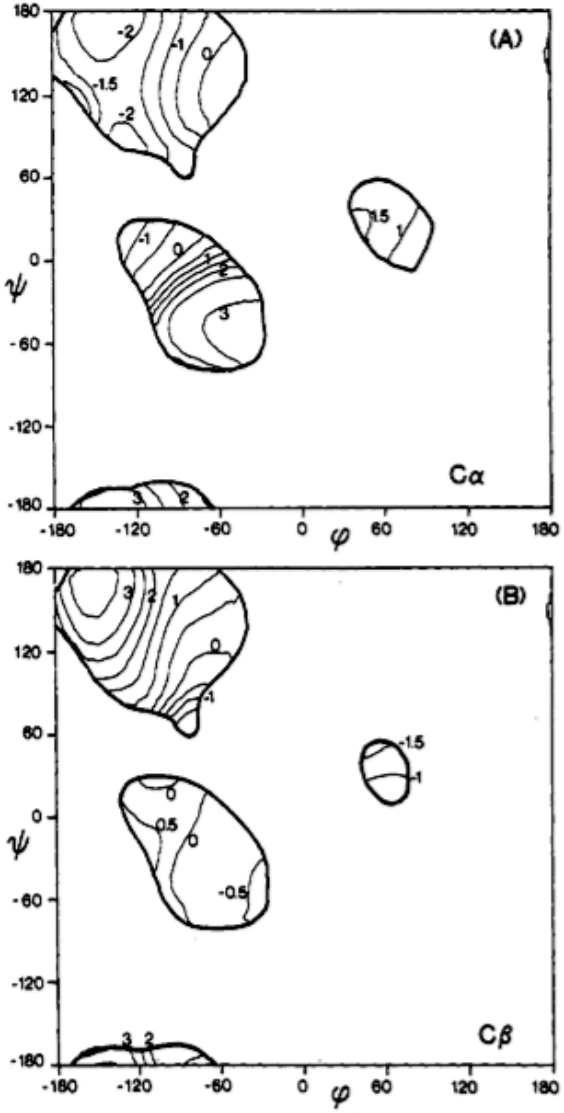
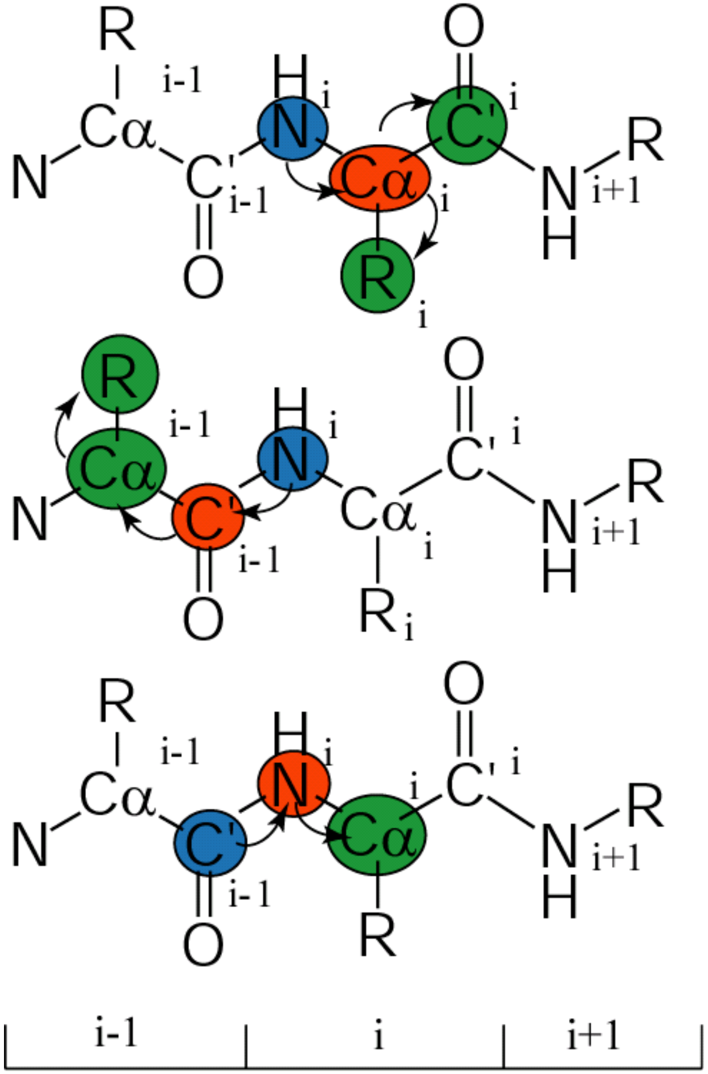


Figure 1. Contour plots of the average secondary shift, $\Delta(\phi,\psi)$, of (A) $C\alpha$ and (B) $C\beta$ resonances and (C) histograms of secondary shift distribution in α-helix and β-sheet. The $\Delta(\phi,\psi)$ surface is calculated by convolution of each of the $\delta(\phi_k,\psi_k)$ values with a Gaussian function, prior to addition and normalization: where the summations extend over all

- Spera & Bax, JACS 1991

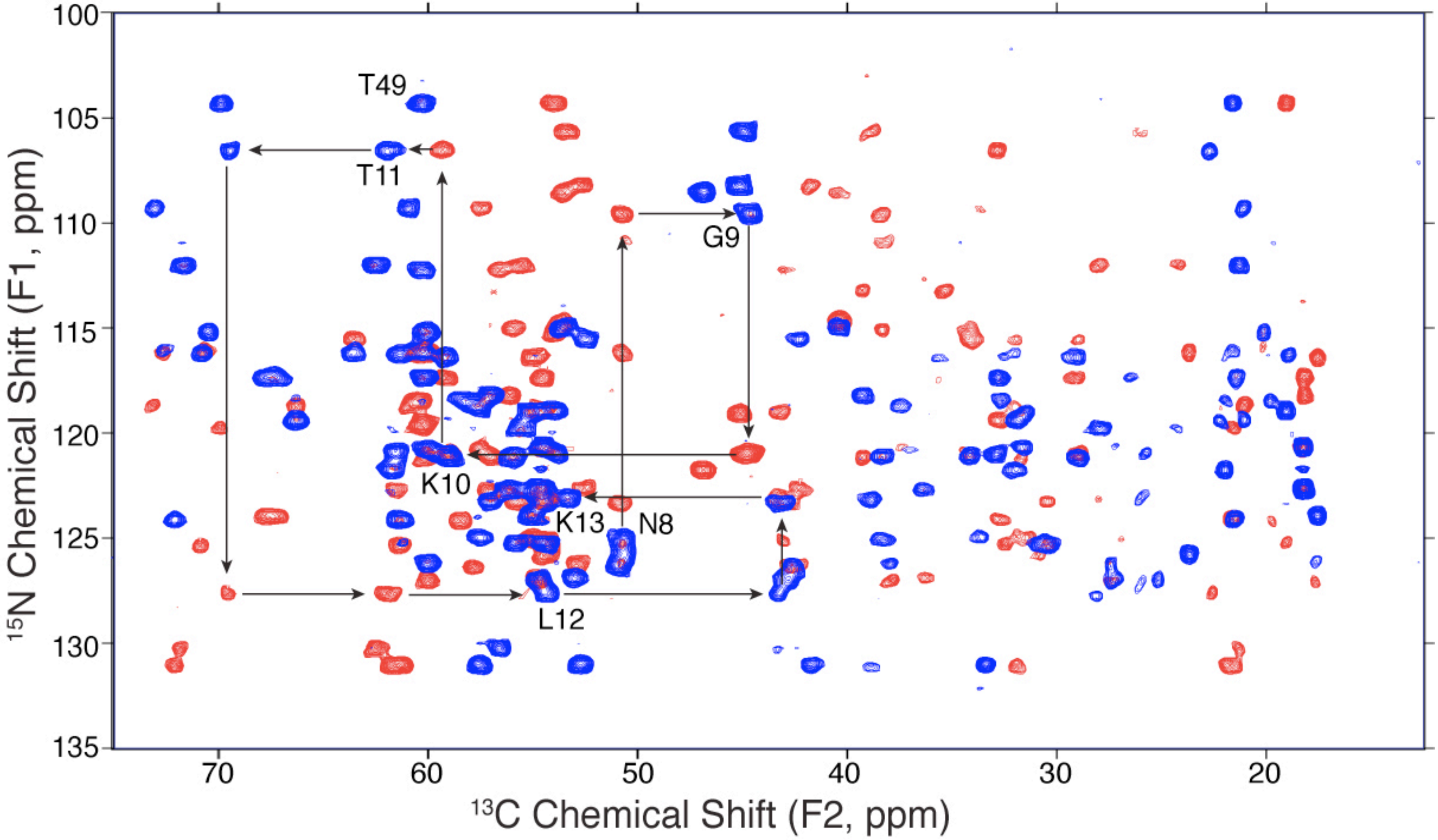
Assignments: Backbone Walk



Solid	Solution
N-CA-CX	H-N-CA
N-CO-CX	H-N-CO H-N-(CO)-CA
CO-N-CA/CB	H-N-CA/CO H-N-CA/CB

Bennett, Rienstra, Auger, Lakshmi, Griffin, *J. Chem. Phys.* **1995**, *103*, 6951. Hohwy, Rienstra, Jaroniec, Griffin, *J. Chem. Phys.* **1999**, *110,* 7983-7992 and **2002**, *117, 4973.* Baldus et al., *Mol. Phys.* **1998**, *95,* 1197. Morcombe and Zilm, *JACS* **2004**, *126*, 7196.

GB1 Chemical Shift Assignments



Franks, Zhou, Wylie, Money, Graesser, Frericks, Sahota, Rienstra, *JACS* **2005**, *127*, 12291-12305.
Wylie, Franks, Graesser, Rienstra, *JACS* **2005**, *127*, 11946-11947.

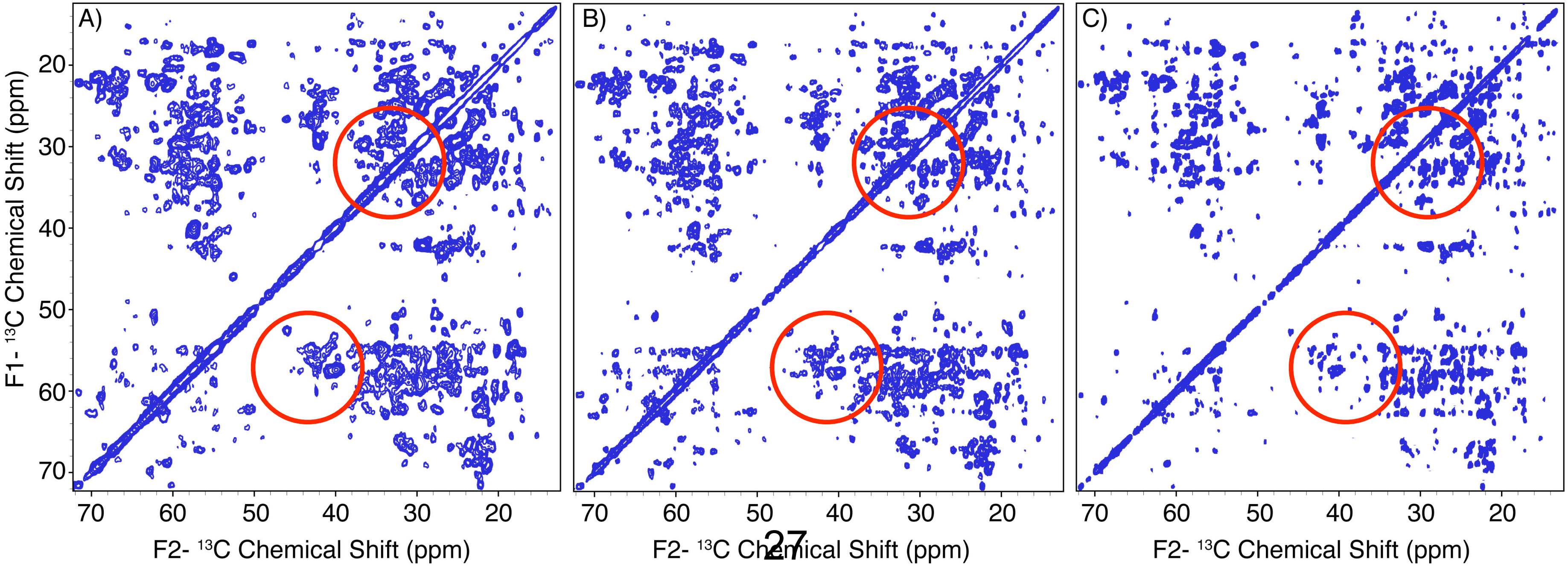
DsbA, 21 kDa Microcrystals

Microcrystalline

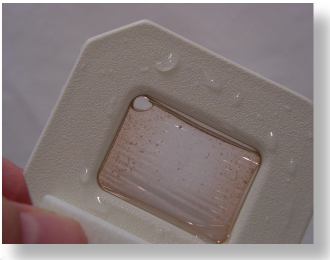
Linewidths ~ $1/B_0$

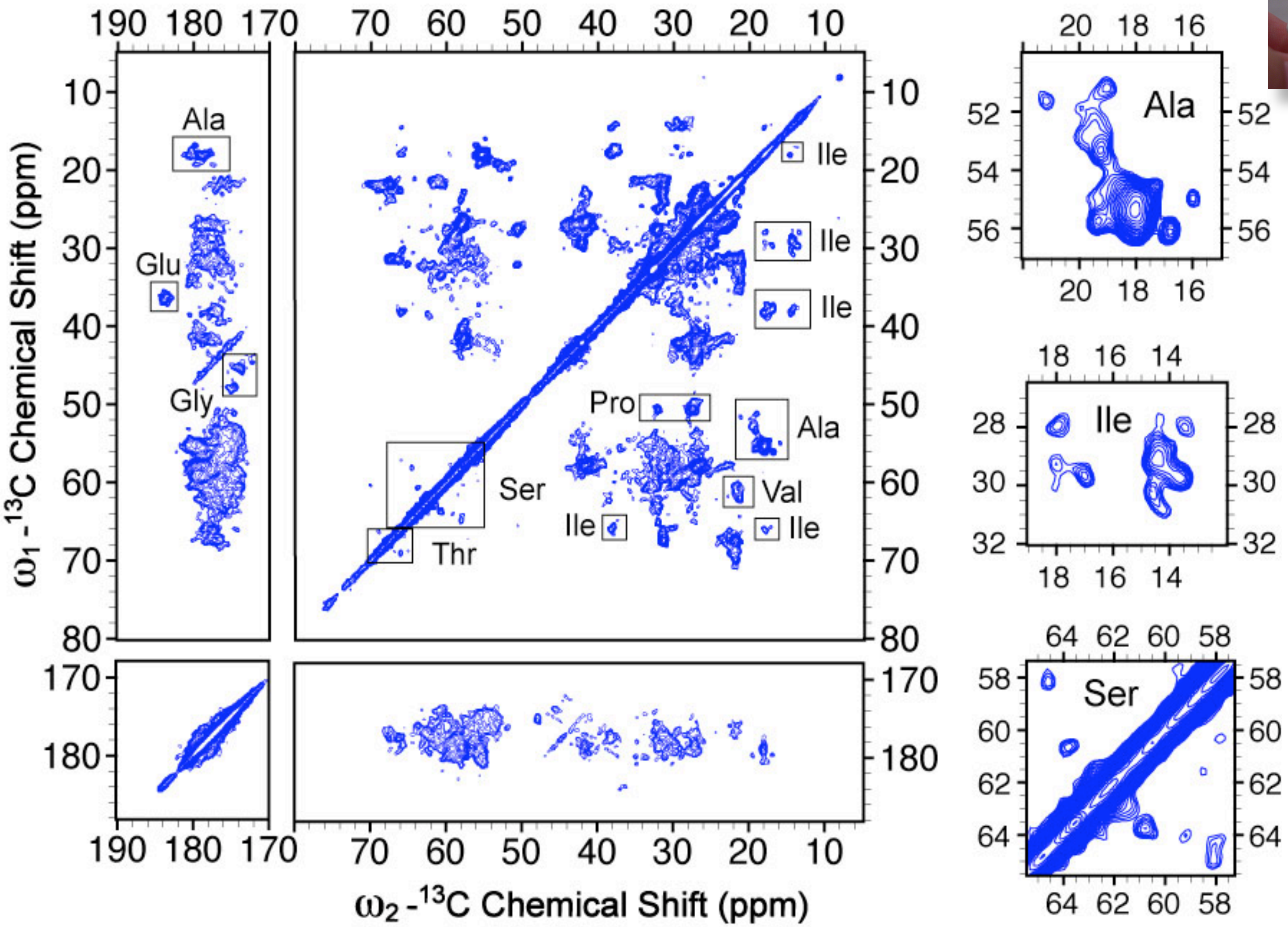
Quadratic benefit in 2D ^{13}C-^{13}C spectra

F(^{1}H):	500 MHz	750 MHz	900 MHz
DARR:	50 ms	100 ms	200 ms



DsbB: 20 kDa, Membrane Protein

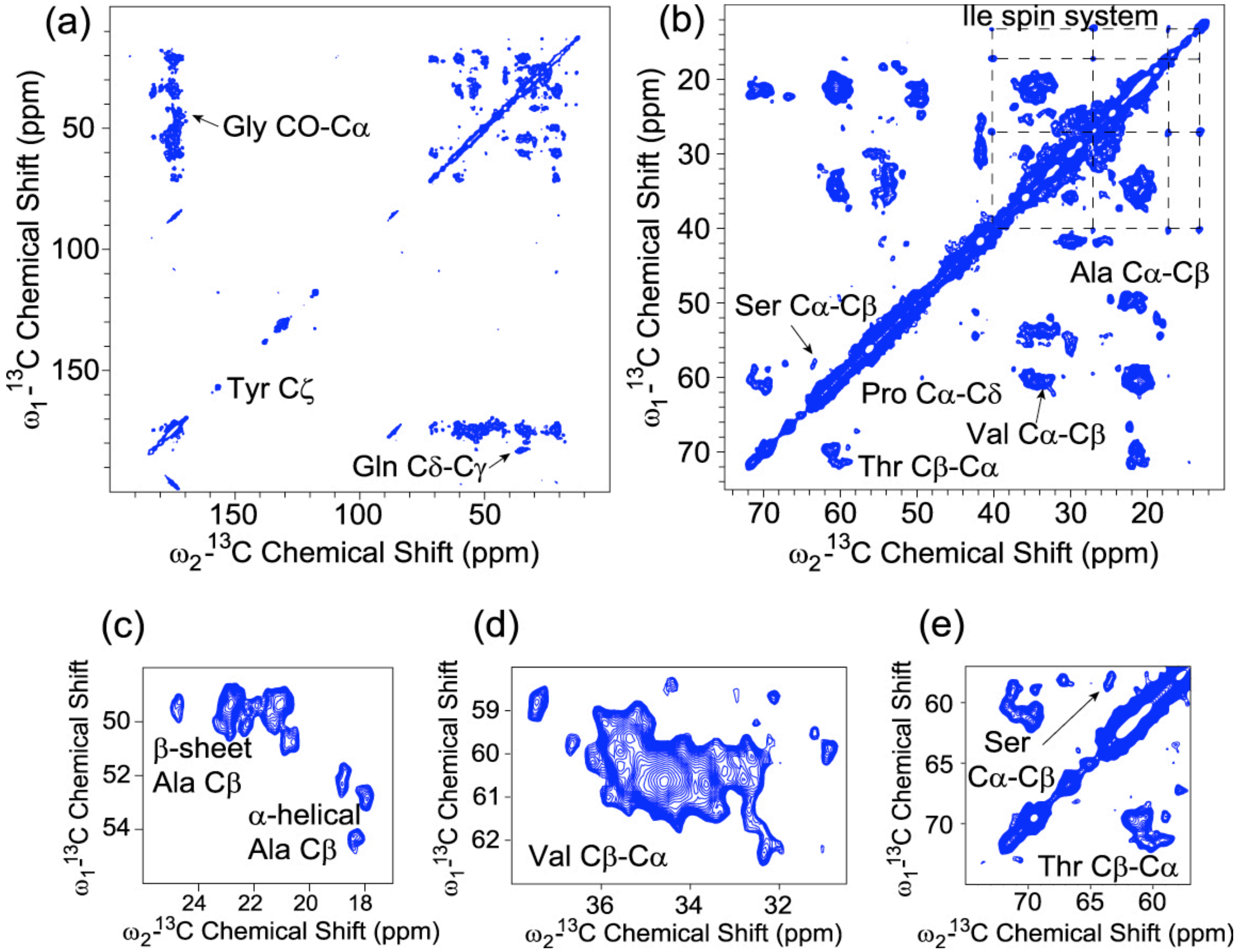




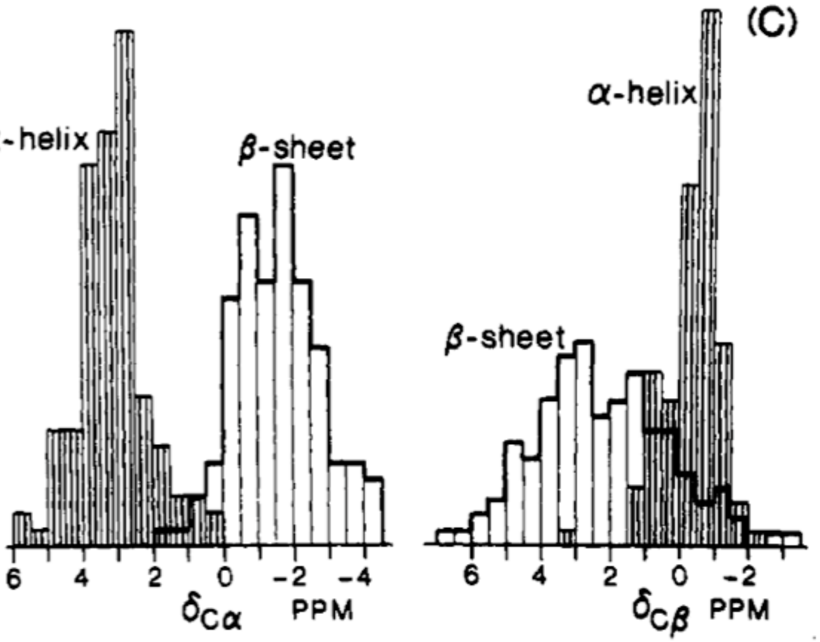
Li, Berthold, Frericks, Gennis, Rienstra, *ChemBioChem* **2007**, *8*, 434-442.

Li, Berthold, Gennis, Rienstra, *Protein Sci.*, in press.

αS Fibrils: ^{13}C-^{13}C 2D Spectra



Secondary Chemical Shifts



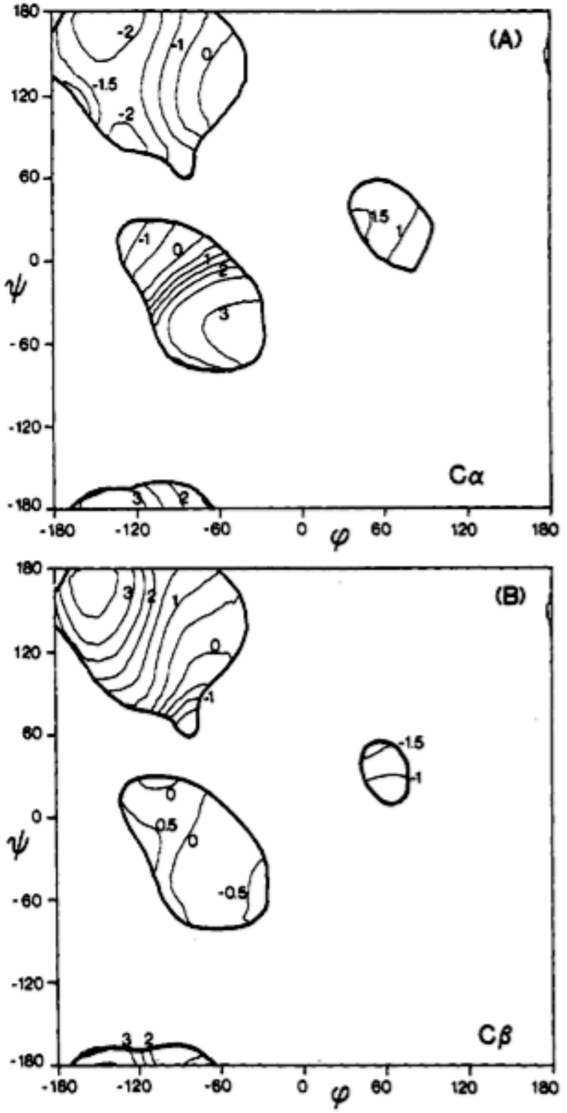
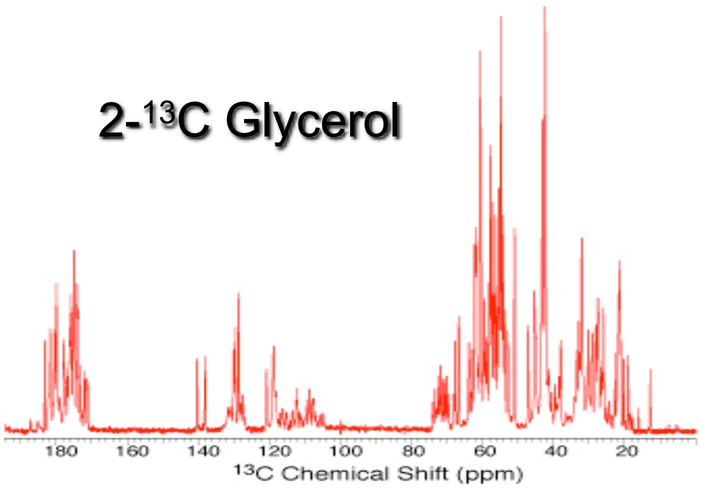


Figure 1. Contour plots of the average secondary shift, $\Delta(\phi,\psi)$, of (A) $C\alpha$ and (B) $C\beta$ resonances and (C) histograms of secondary shift distribution in α-helix and β-sheet. The $\Delta(\phi,\psi)$ surface is calculated by convolution of each of the $\delta(\phi_k,\psi_k)$ values with a Gaussian function, prior to addition and normalization: where the summations extend over all

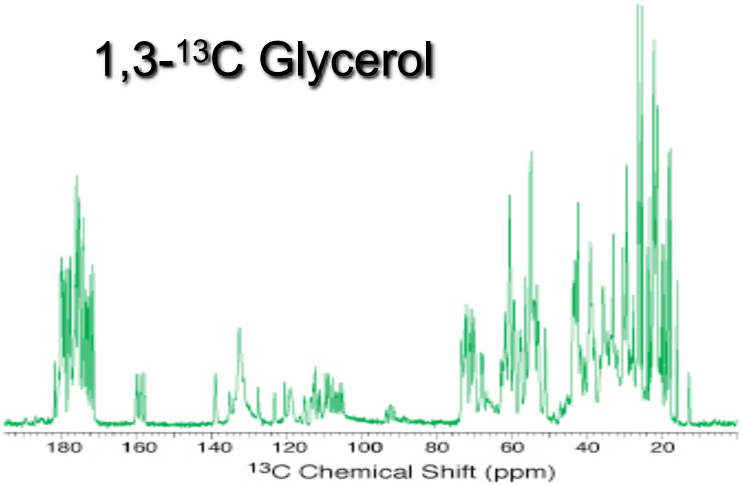
- Spera & Bax, JACS 1991

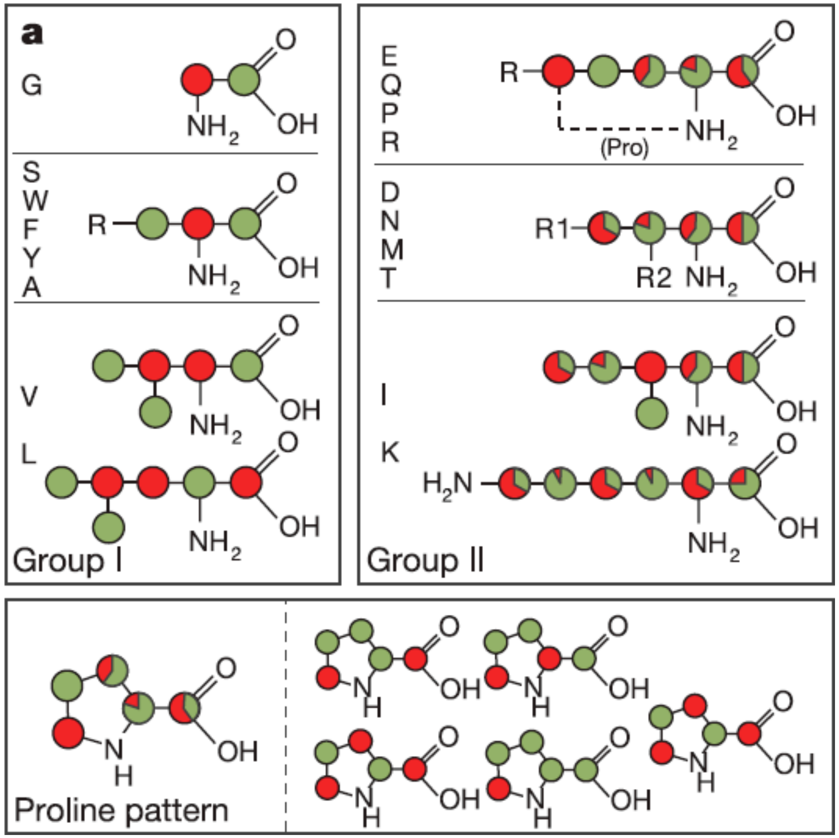
Glycerol Labeling Scheme

2-^{13}C Glycerol



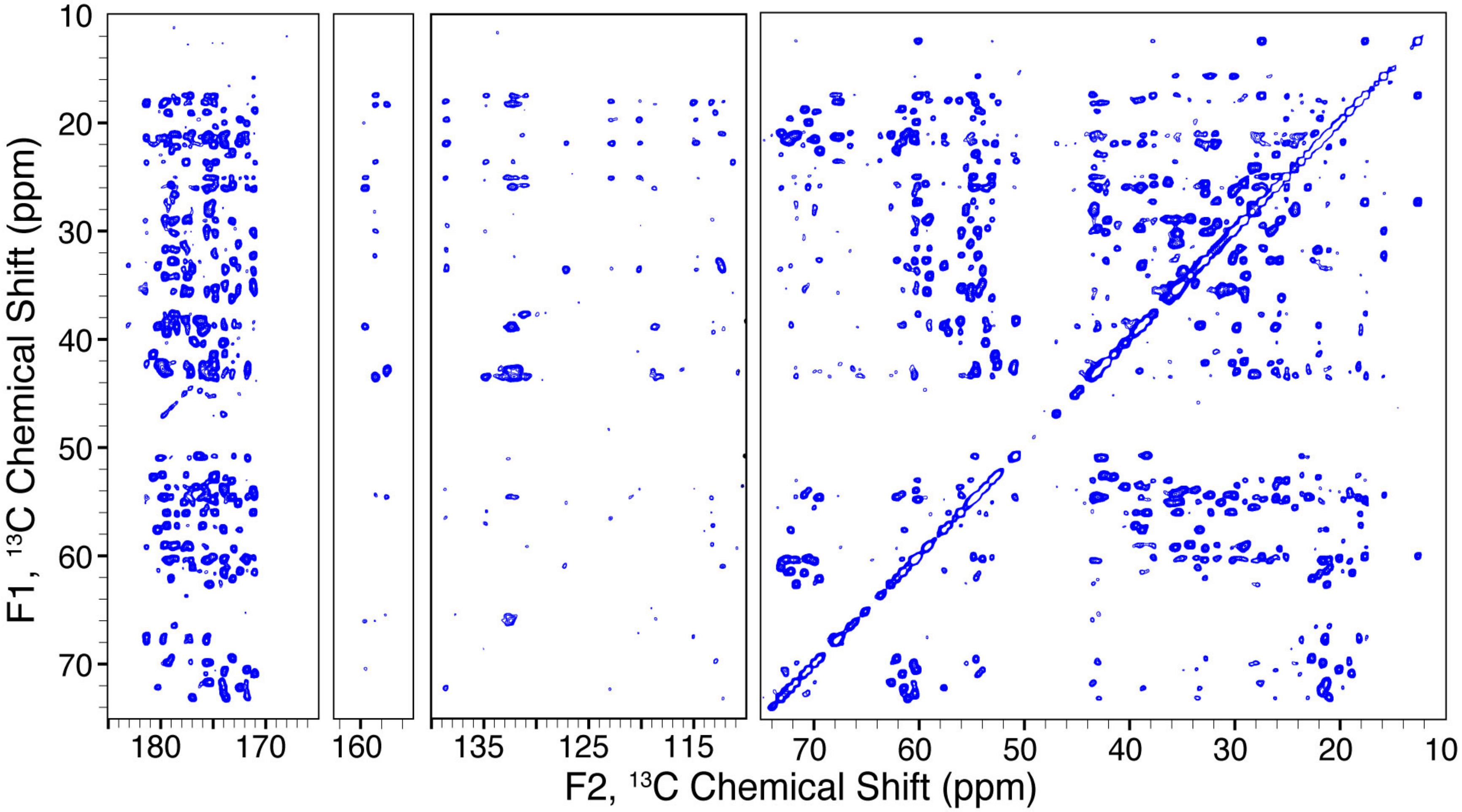
1,3-^{13}C Glycerol





Castellani et. al., *Nature* 420, 98-102 (2002)

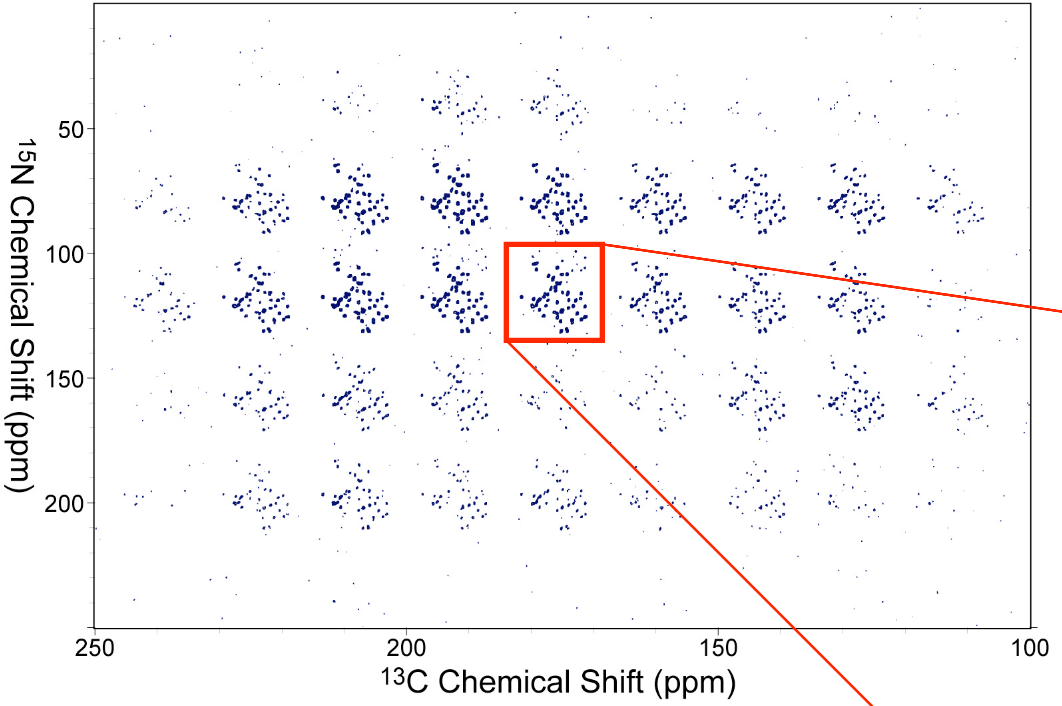
High-Resolution 2D ^{13}C-^{13}C (GB1)

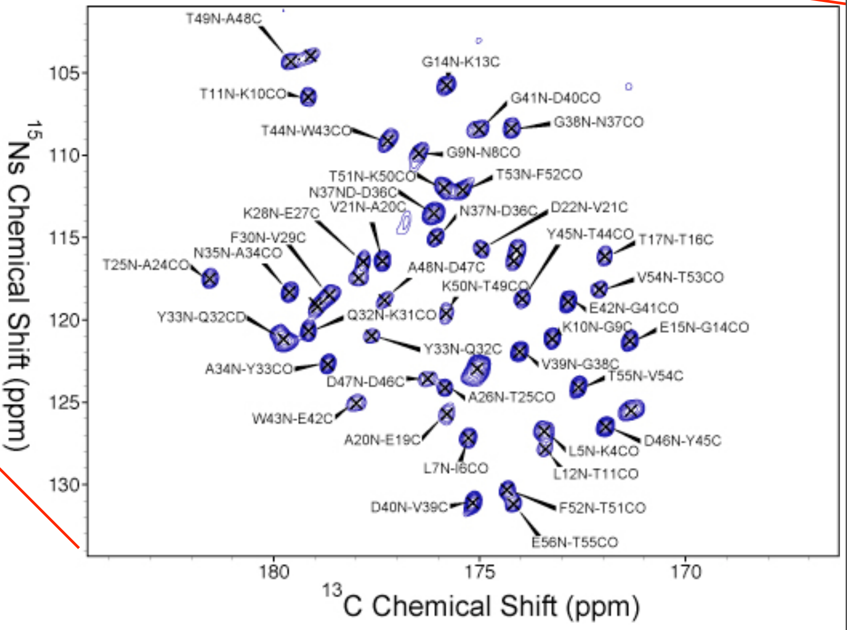


Scroll resonator probe: Stringer, Bronnimann, Mullen, Zhou, Stellfox, Li, Williams, Rienstra, *J. Magn. Reson.* **2005**, *173*, 40-48.

^{15}N-^{13}C’ Site Resolution

Slow MAS of ^{15}N,^{13}C labeled GB1 grown in 1,3-^{13}C-glycerol provides a 2D manifold of ^{15}N and ^{13}C sidebands

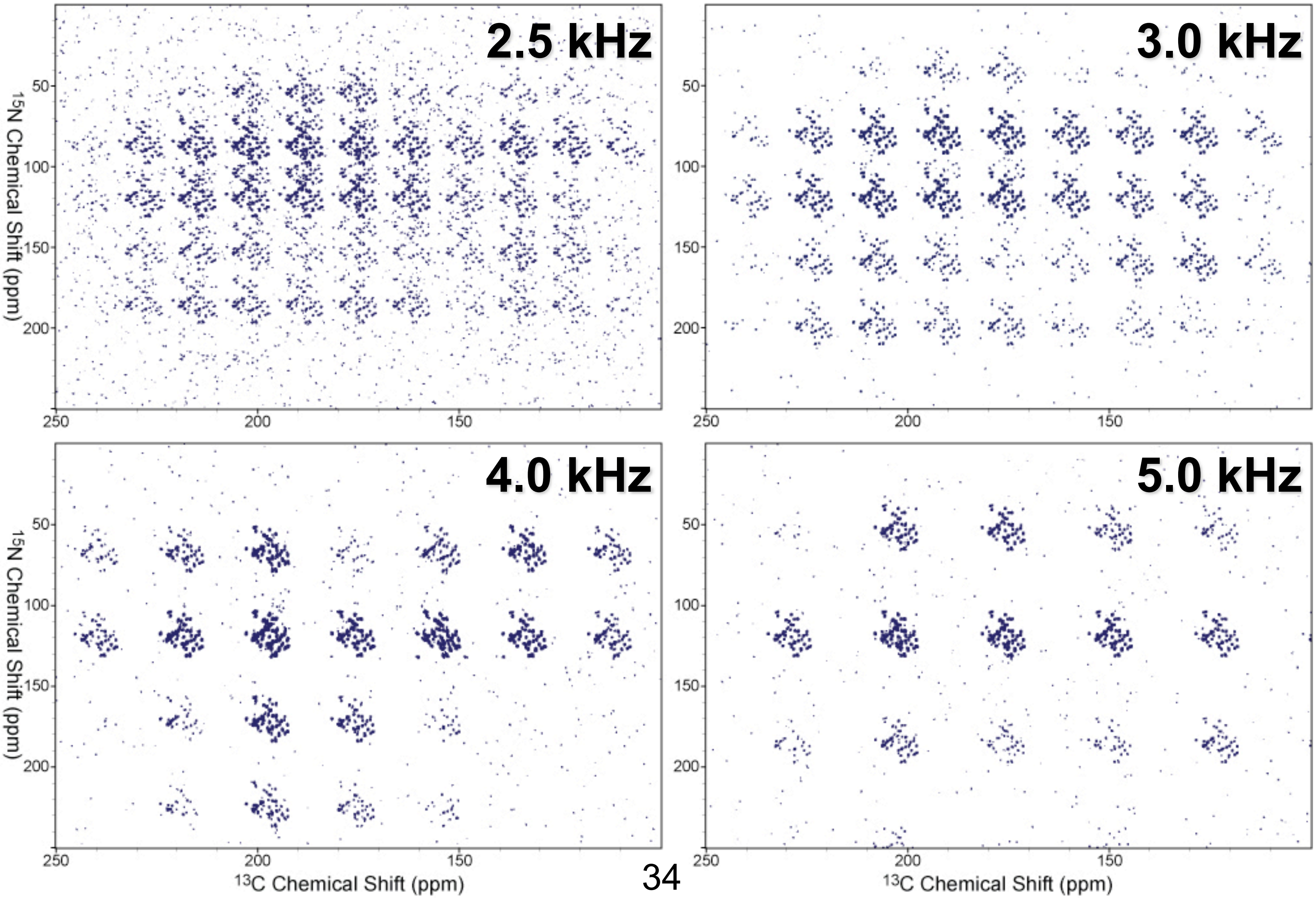




Each piece of the checker board exhibits site-resolved peaks with line widths of ~0.2 to 0.3 ppm.

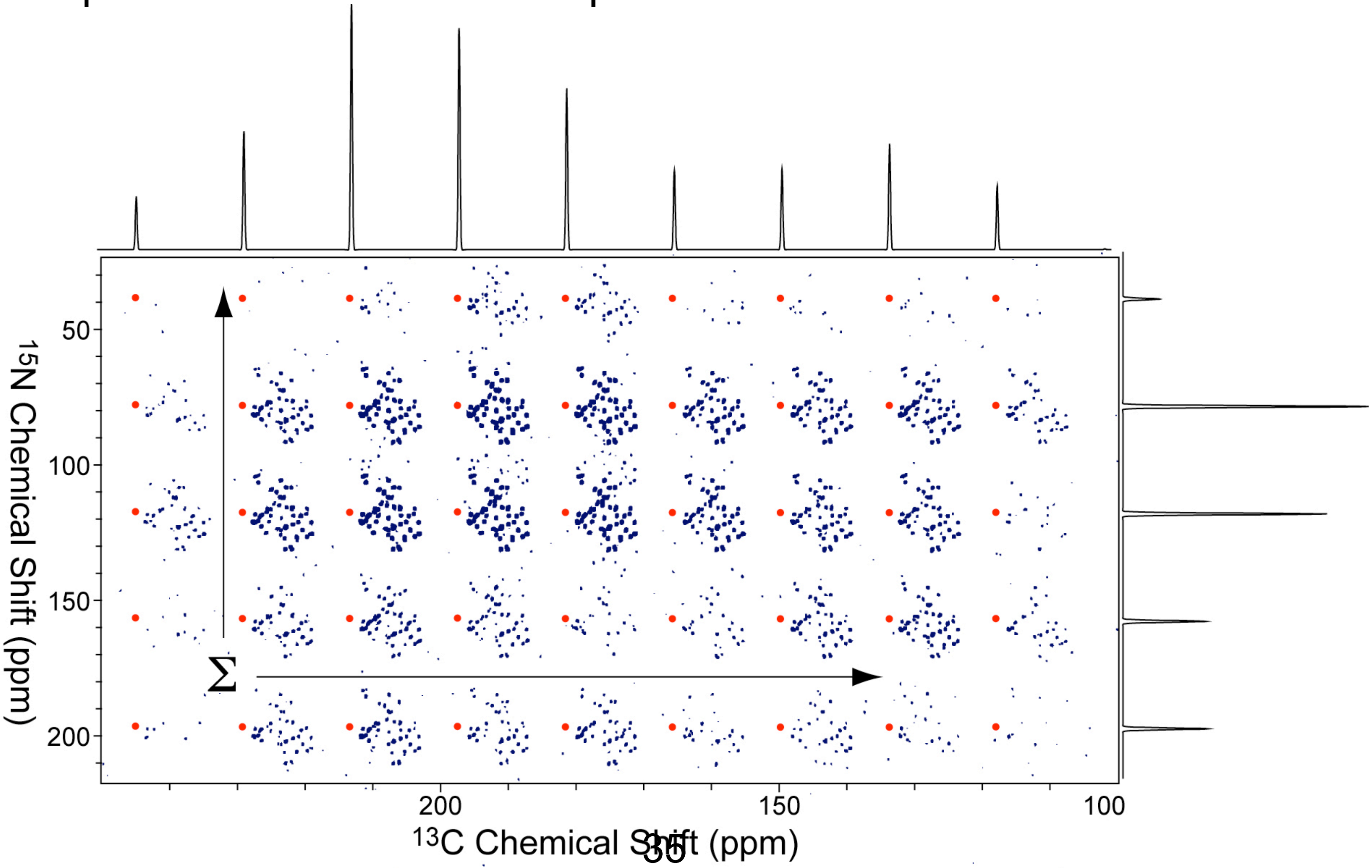
Wylie, Sperling, Frericks, Shah, Franks, Rienstra, *JACS* **2007**, *129*, 5318-19.

Slow Spinning ^{15}N-$^{13}C'$ 2D



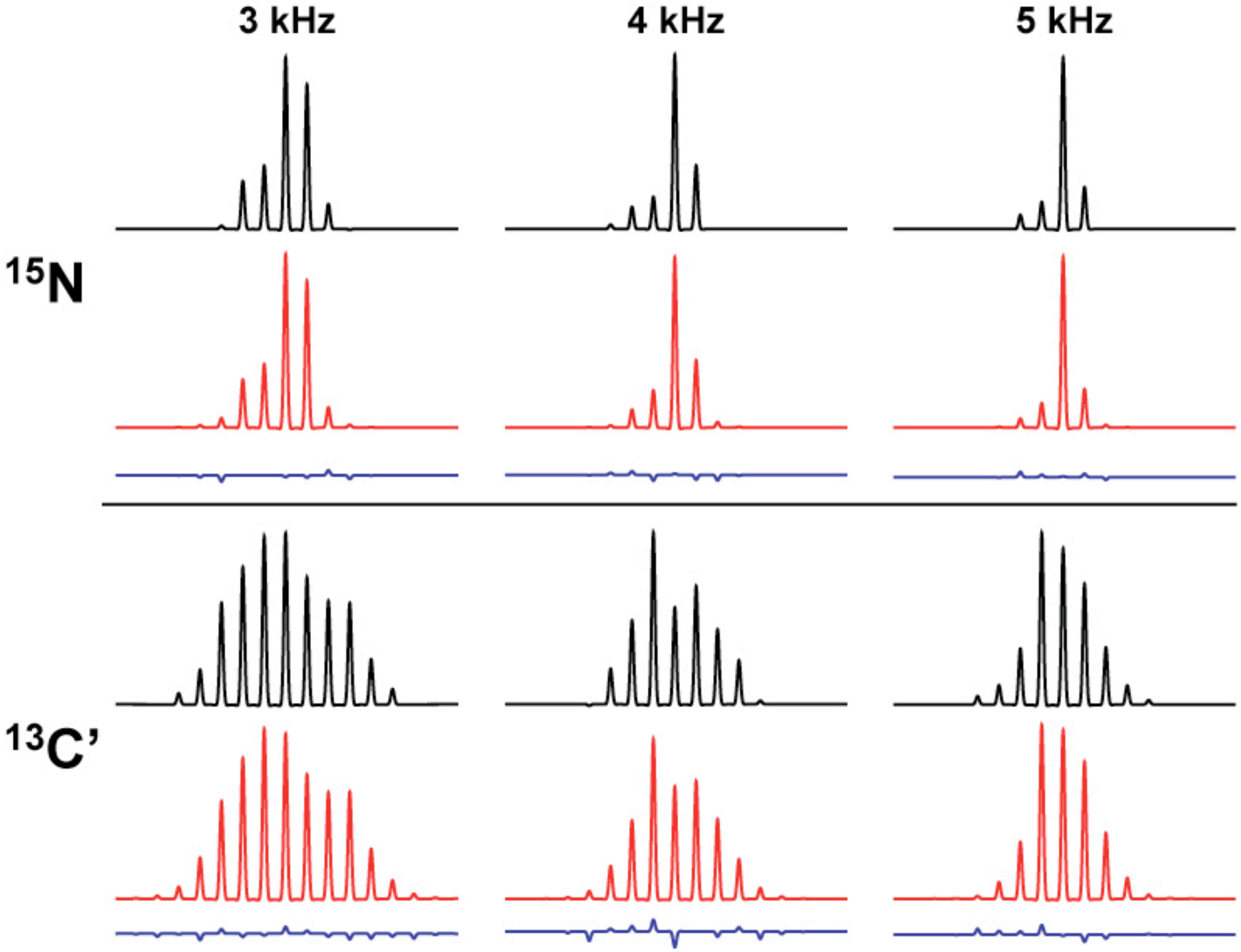
Slow Spinning Analysis

- Each peak in the spectrum is integrated
- A 1D manifold is reconstructed by summation along each dimension
- Repeated for 42 sites in the protein



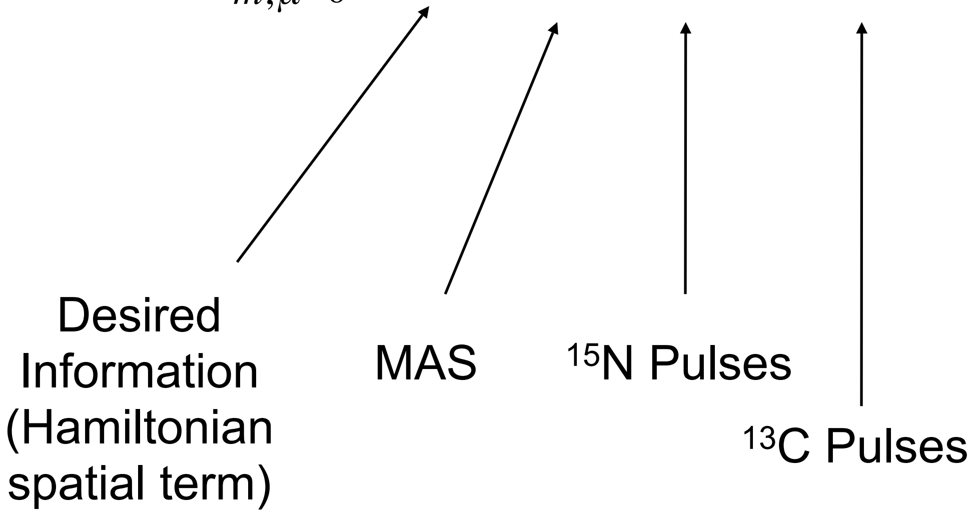
1D Fits From SPINEVOLUTION

- Results from D40N-V39C’ cross peaks



Dipolar Recoupling

$$\overline{H}^{(0)} = \sum_{m,\mu}\int_0^\tau \omega_{m,\mu} e^{im\omega_r t} e^{i\mu\omega_{1N} t} e^{i\mu\omega_{1C} t}\,dt$$

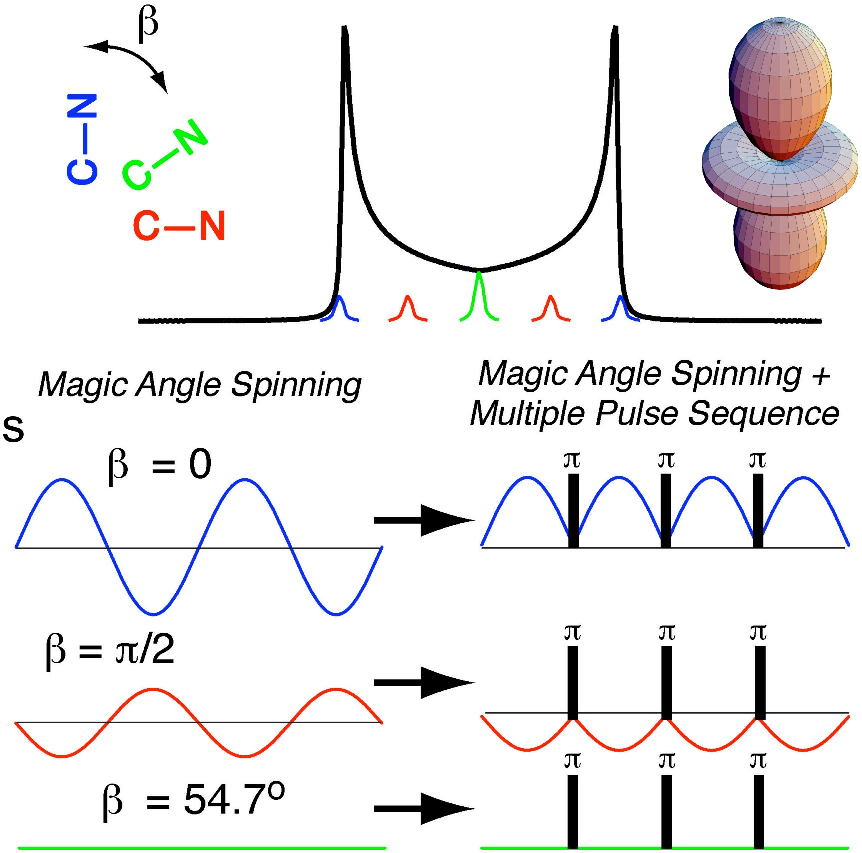


Desired Information (Hamiltonian spatial term)

MAS

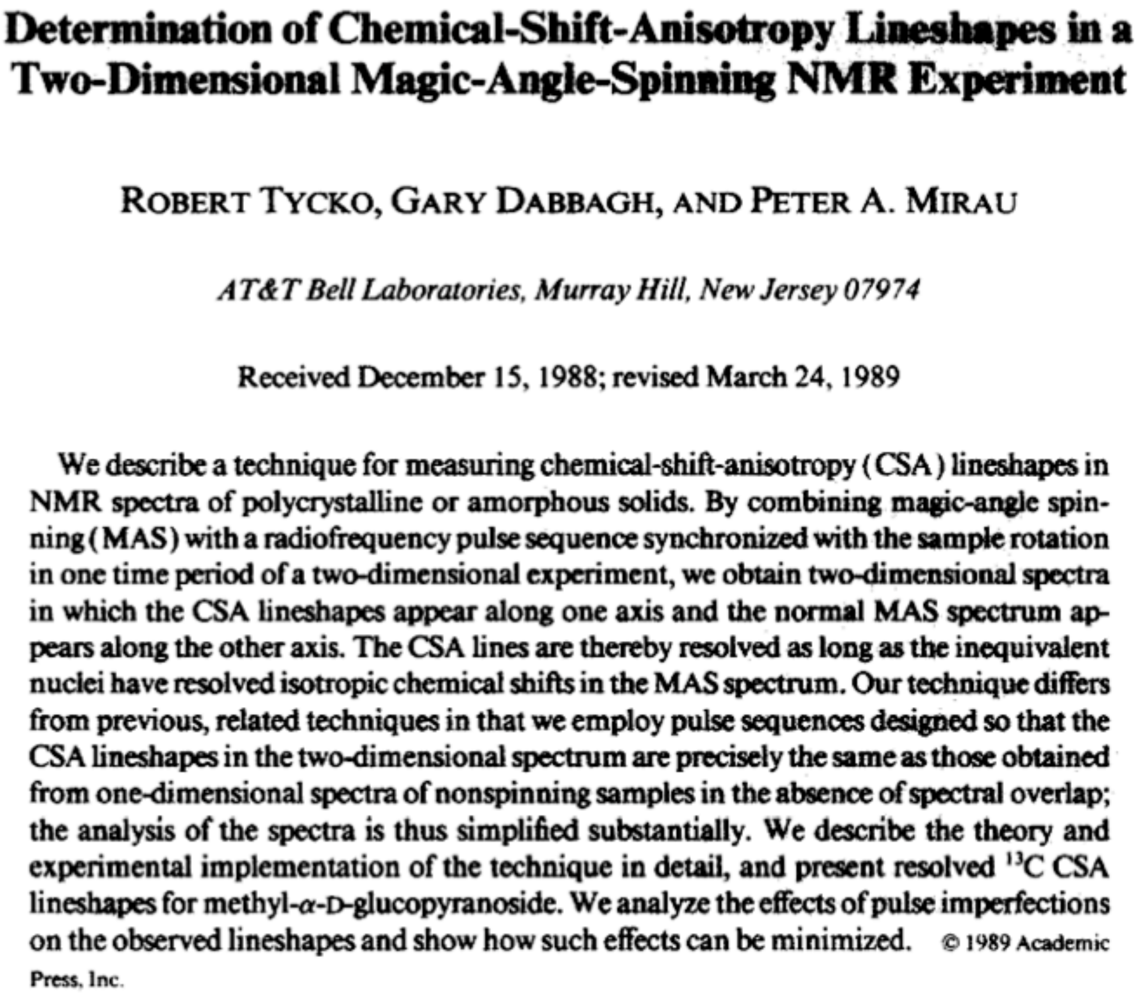
^{15}N Pulses

^{13}C Pulses



- MAS averages couplings to zero
- Multiple pulse sequence restores dipolar couplings

Tycko's "CRAMA" Experiment



Determination of Chemical-Shift-Anisotropy Lineshapes in a Two-Dimensional Magic-Angle-Spinning NMR Experiment

ROBERT TYCKO, GARY DABBAGH, AND PETER A. MIRAU

AT&T Bell Laboratories, Murray Hill, New Jersey 07974

Received December 15, 1988; revised March 24, 1989

We describe a technique for measuring chemical-shift-anisotropy (CSA) lineshapes in NMR spectra of polycrystalline or amorphous solids. By combining magic-angle spinning (MAS) with a radiofrequency pulse sequence synchronized with the sample rotation in one time period of a two-dimensional experiment, we obtain two-dimensional spectra in which the CSA lineshapes appear along one axis and the normal MAS spectrum appears along the other axis. The CSA lines are thereby resolved as long as the inequivalent nuclei have resolved isotropic chemical shifts in the MAS spectrum. Our technique differs from previous, related techniques in that we employ pulse sequences designed so that the CSA lineshapes in the two-dimensional spectrum are precisely the same as those obtained from one-dimensional spectra of nonspinning samples in the absence of spectral overlap; the analysis of the spectra is thus simplified substantially. We describe the theory and experimental implementation of the technique in detail, and present resolved ^{13}C CSA lineshapes for methyl-α-D-glucopyranoside. We analyze the effects of pulse imperfections on the observed lineshapes and show how such effects can be minimized.

Tycko’s CSA Recoupling Sequence

- *JMR* 1989, 85:265
- 2D correlation
- π pulse schemes
- Scaling factor depends on π pulse duration

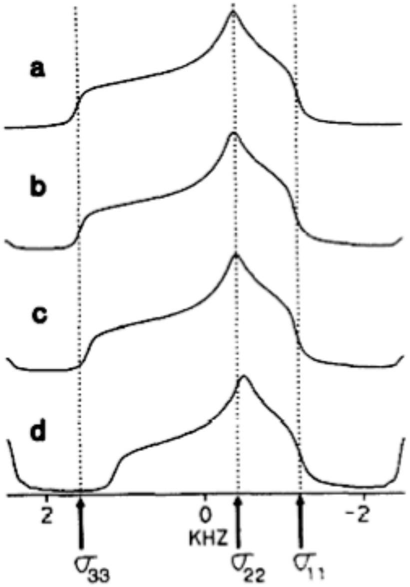
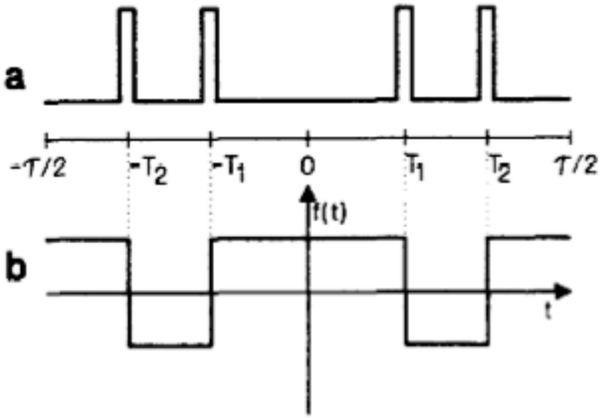
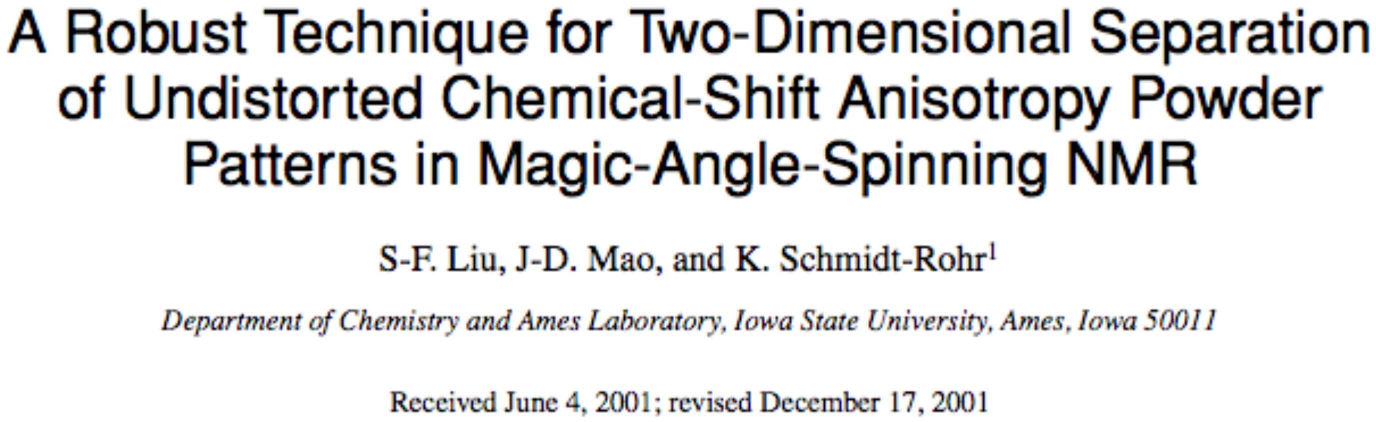


FIG. 3. Simulated powder pattern spectra from calculations of nuclear spin dynamics under the pulse sequence in Fig. 2, illustrating the effects of finite pulse amplitudes and pulse length errors. Isolated spin-$\frac{1}{2}$ nuclei with chemical-shift-anisotropy principal values $\sigma_{11} = 2$ kHz, $\sigma_{22} = 4$ kHz, and $\sigma_{33} = 9$ kHz (with respect to the carrier frequency) and random orientations are assumed. The sequence with four π pulses with theoretical anisotropy scaling factor $\chi = 0.393$ and offset scaling factor $\xi = 0.000$ is used. Arrows indicate the positions of the principal values expected in the limit of delta function π pulses. Lengths and flip angles for the actual nominal π pulses are (a) 1.00 μs, 180.0°; (b) 8.00 μs, 180.0°; (c) 8.00 μs, 189.5°; (d) 8.00 μs, 200.0°.



SUPER (K. Schmidt-Rohr et al.)



A Robust Technique for Two-Dimensional Separation of Undistorted Chemical-Shift Anisotropy Powder Patterns in Magic-Angle-Spinning NMR

S-F. Liu, J-D. Mao, and K. Schmidt-Rohr[1]

Department of Chemistry and Ames Laboratory, Iowa State University, Ames, Iowa 50011

Received June 4, 2001; revised December 17, 2001

- **S**eparation of **U**ndistorted **P**owder patterns by **E**ffortless **R**ecoupling
- *JMR* 2002, 155:15-28
- Better stability than π pulse schemes

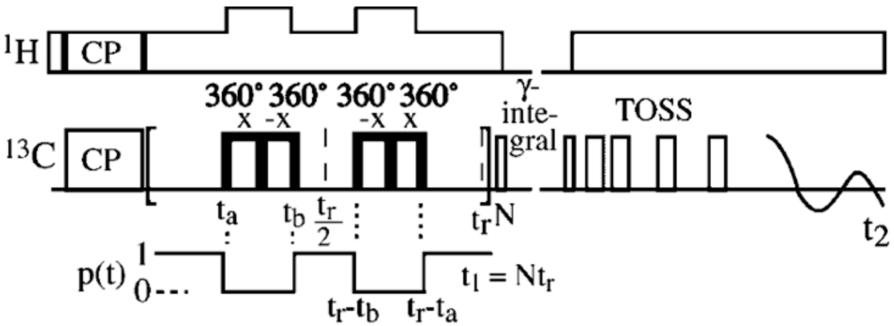
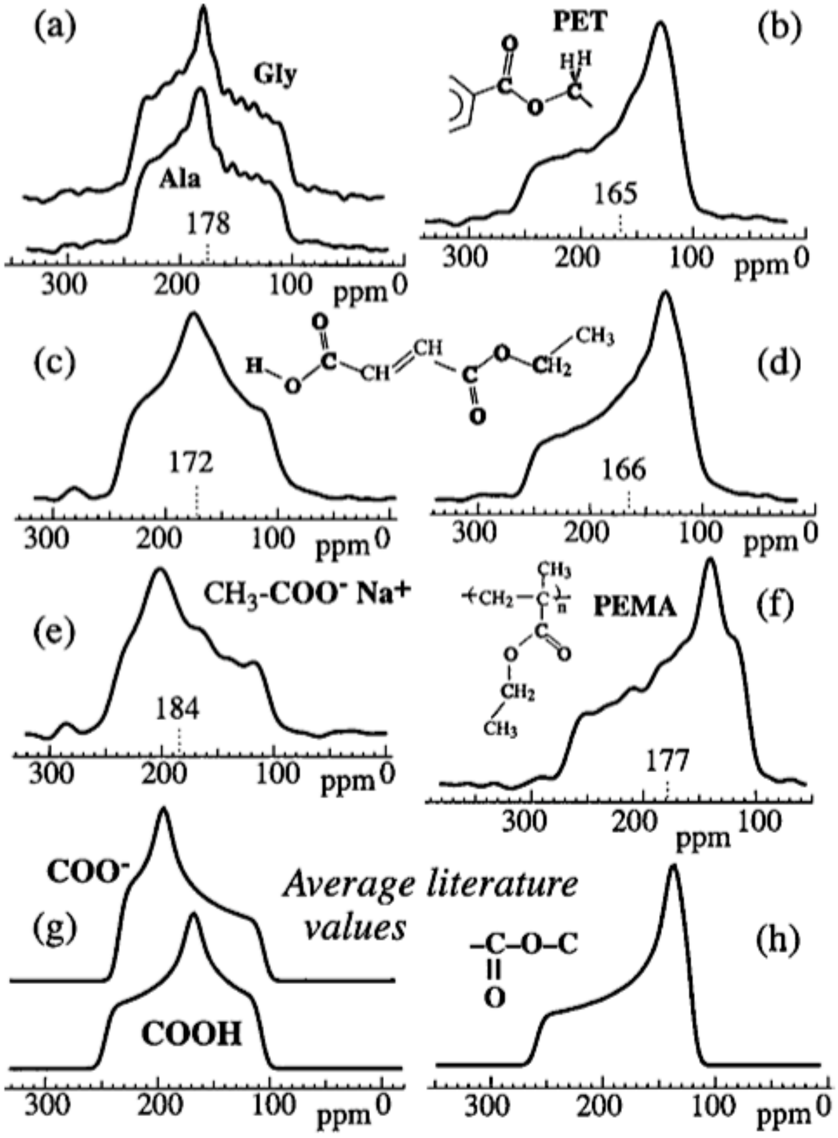


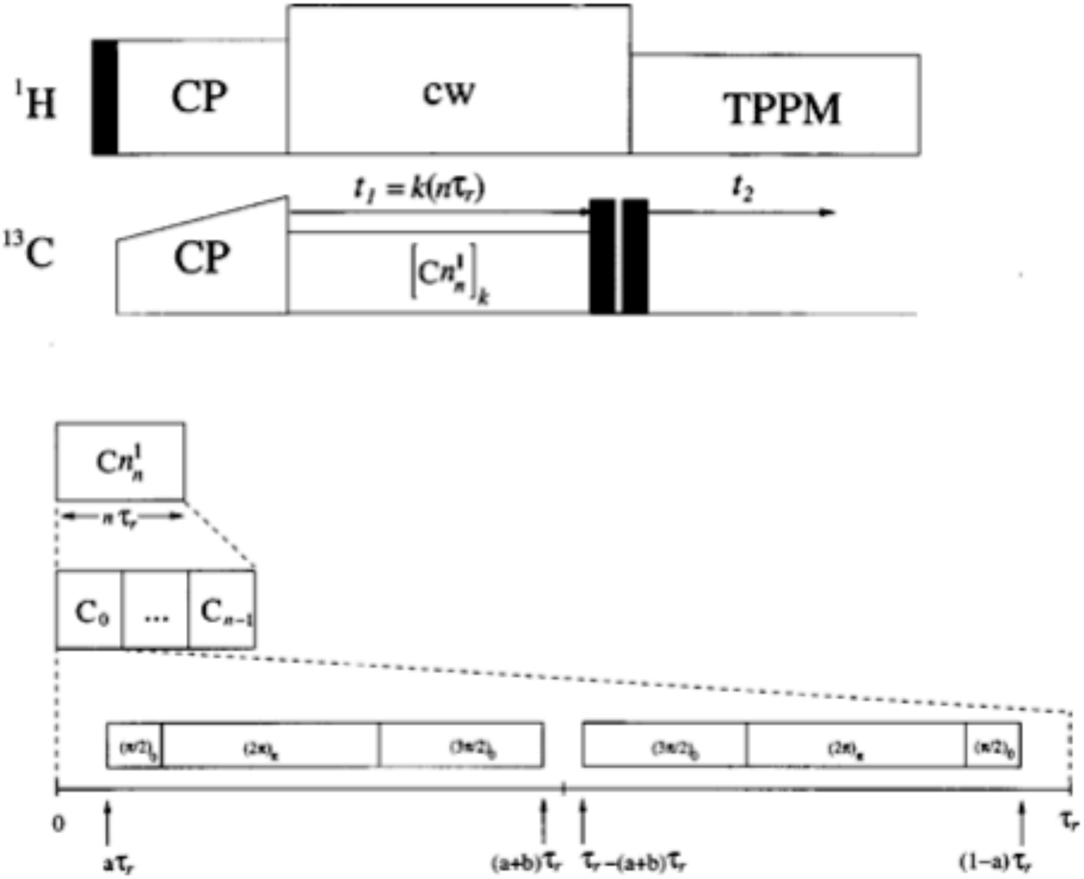
FIG. 1. Pulse sequence of the SUPER NMR experiment. The increment of the evolution time t_1 is one rotation period t_r. Each of the two pulse blocks in a given rotation period consists of two 360° pulses (or two 180° pulses flanking a 360° pulse; see Fig. 3). At the bottom of the figure, the function $p(t)$ multiplying the instantaneous frequency is shown; see text for more details. (It should not be confused with a trigger for rotor synchronization, which is not required in this experiment.)

SUPER Lineshapes

- Robust with respect to variations in RF amplitude
- 2π rotations on ^{13}C require less ^{1}H decoupling power (factor of 2 mismatch; π pulses require factor of 3)
- Disadvantage: homonuclear coupling still present



ROCSA: Symmetry-Based Sequence



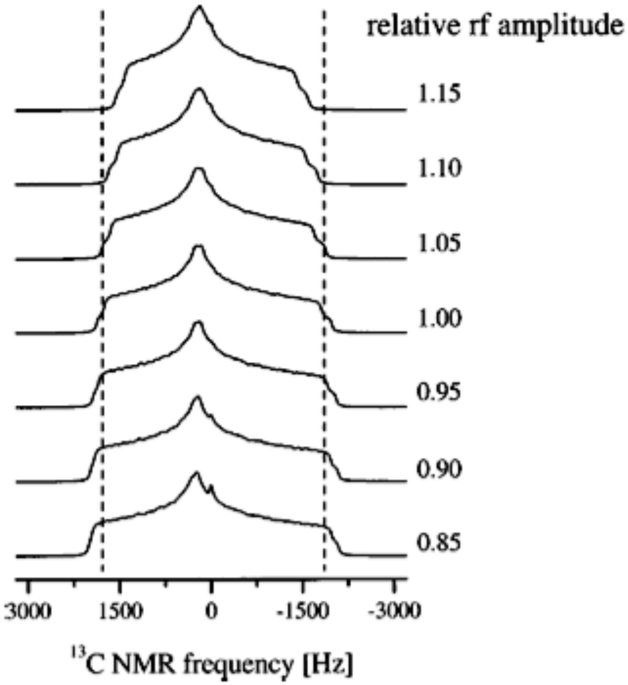
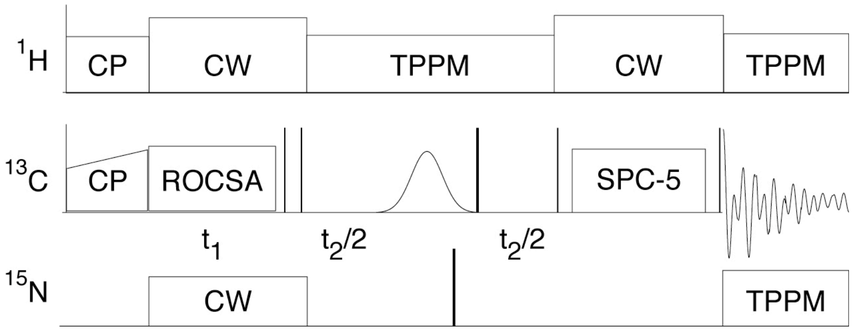
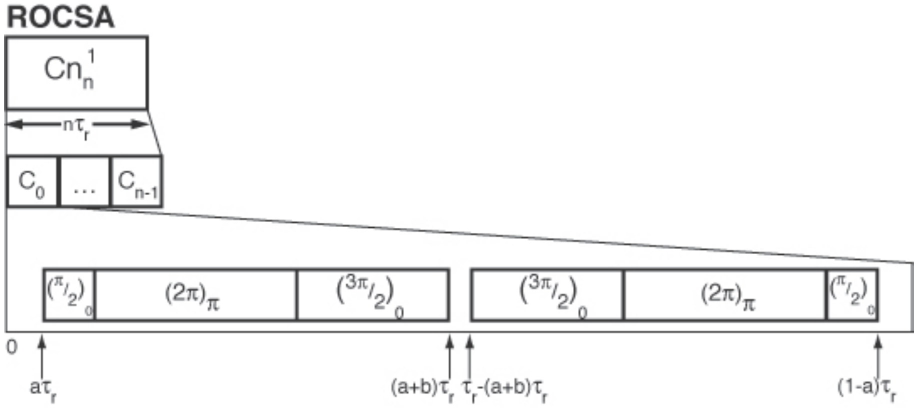


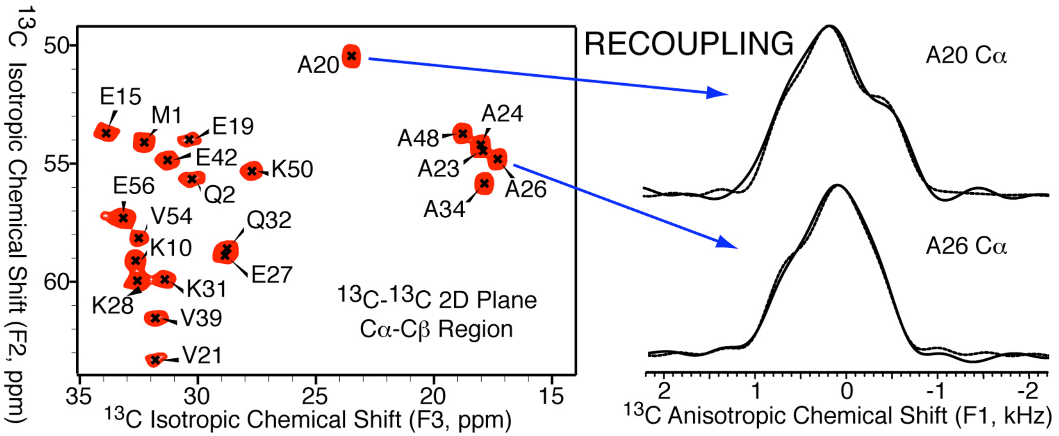
FIG. 4. Simulations of the effects of rf inhomogeneity on the ROCSA spectra calculated for C′ in L-alanine, including coupling to C_α as in Fig. 3. The rf field amplitude is varied from 0.85 to 1.15 times the ideal value.

- Chan & Tycko, *J. Chem. Phys.* 2003, 118:8378

3D ROCSA Experiments





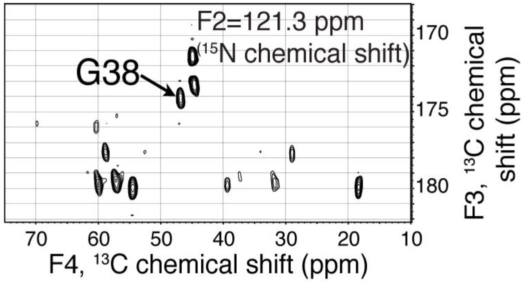


- Cross peaks from SPC5 mixing
- Gaussian pulse improves resolution
- ROCSA trajectories extracted from third dimension

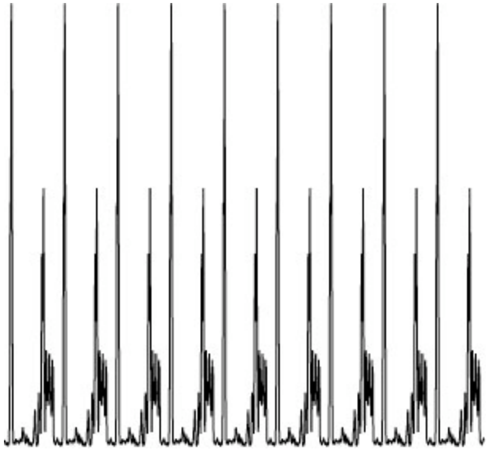
Chan, J.C.C.; Tycko, R.; *J. Chem Phys* **2003** 118 8378-8389
Wylie, B.J., Franks W. T., Graesser, D.T., Rienstra, C.M; *JACS* **2005**, 127, 11946-11947.

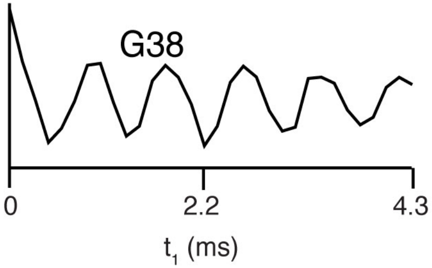
Stable Amplifiers & Air Compressors

$$\overline{H}^{(0)} = \sum_{m,\mu} \int_0^{\tau} \omega_{m,\mu} e^{im\omega_r t} e^{i\mu\omega_{1N} t} e^{i\mu\omega_{1C} t} dt$$



$$S(t_1 = 0) = 1$$

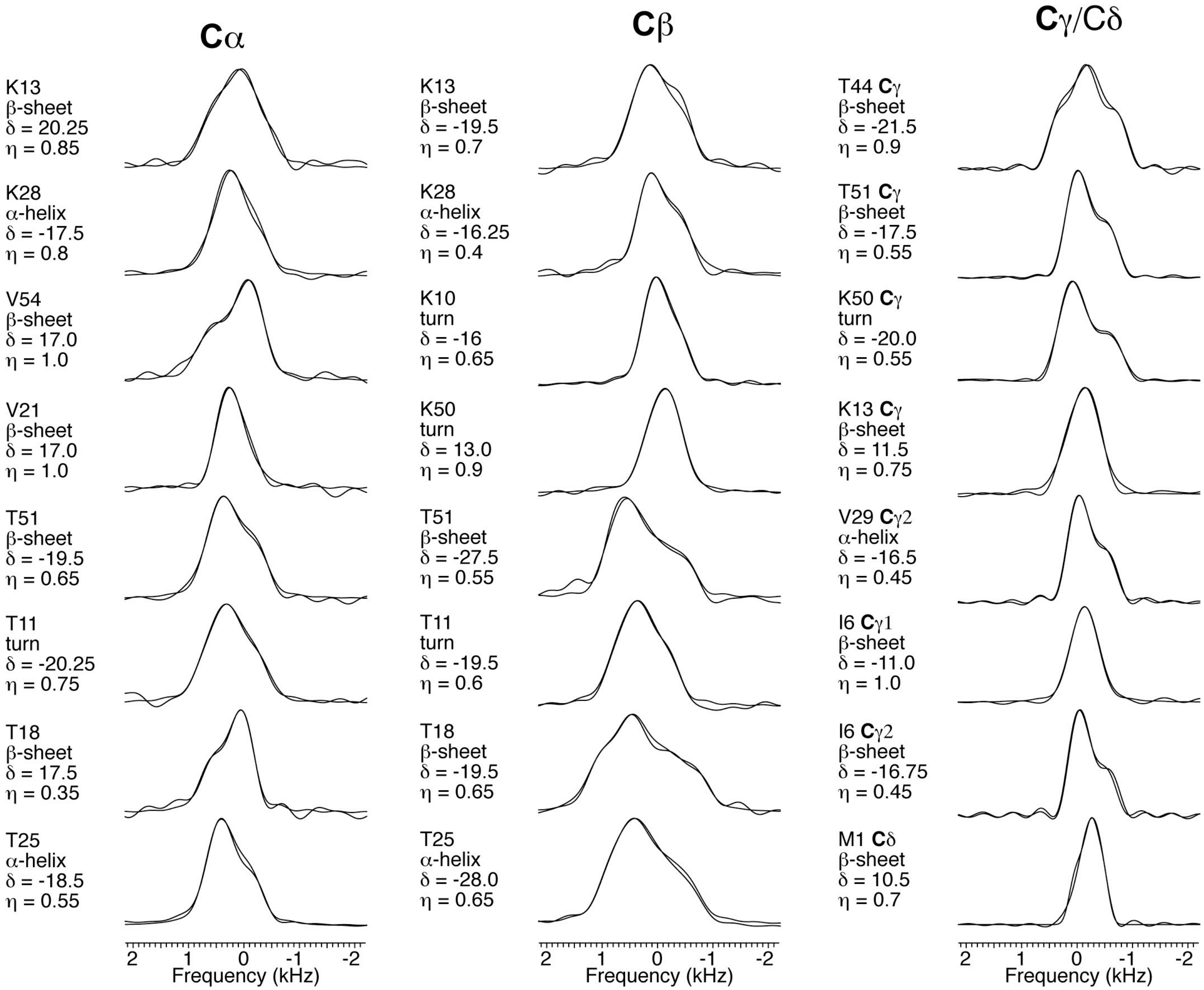




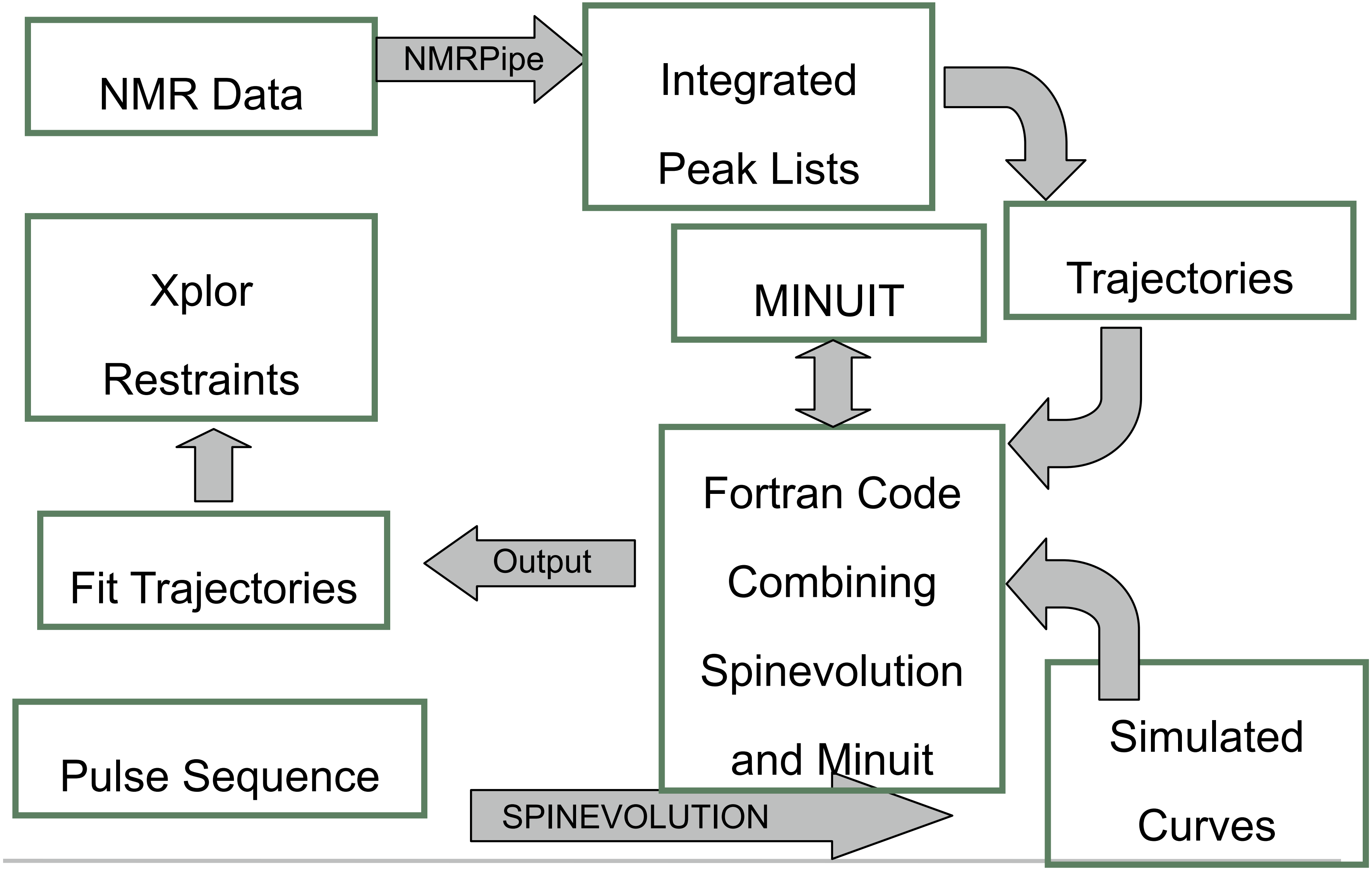
$$S(t_1) = \cos(\omega_{m,\mu} t_1) e^{-t_1/T_2}$$

Franks, Kloepper, Wylie, Rienstra, *J. Biomol. NMR* **2007**, *39*, 107-131.

^{13}C ROCSA Lineshapes



Fitting Strategy: Spectra to Structure



NMR Data

NMRPipe

Integrated Peak Lists

Trajectories

MINUIT

Xplor Restraints

Fortran Code Combining Spinevolution and Minuit

Fit Trajectories

Output

Simulated Curves

Pulse Sequence

SPINEVOLUTION

Veshtort, M. Griffin, R. *JMR*, **178**, 248-282, 2006. F. James and M. Winkler. CERN, Geneva. 2004.

Lineshape Acquisition and Analysis

Chemical shift anisotropy

Aliphatic ^{13}C

- Local conformation
- Very good QM methods

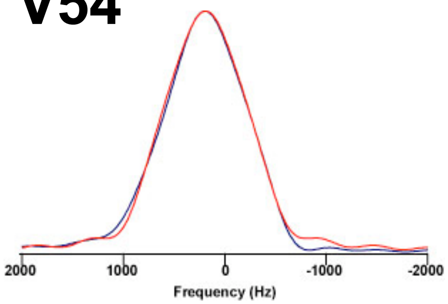
Carbonyl ^{13}C

- Hydrogen bond length
- Harder to calculate

Amide ^{15}N

- Critical for NMR dynamics
- Benchmarks needed for QM

V54



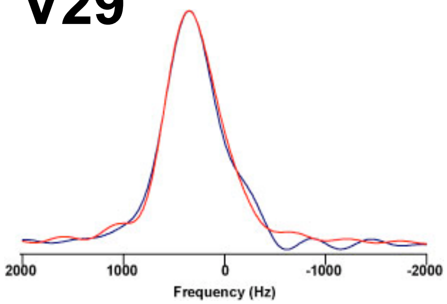
δ **= 17.0,** η **= 1.0**

ϕ **= -121,** ψ **= 140**

gauche +

(β-sheet)

V29

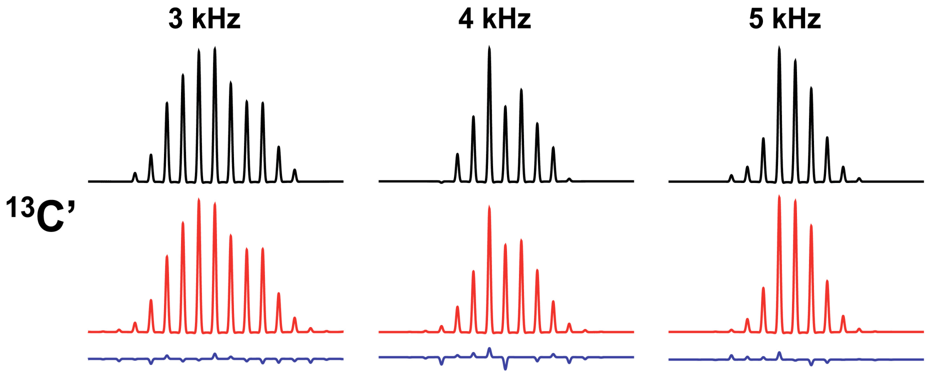


δ **= -10.8,** η **= 0.4**

ϕ **= -62,** ψ **= -49**

trans

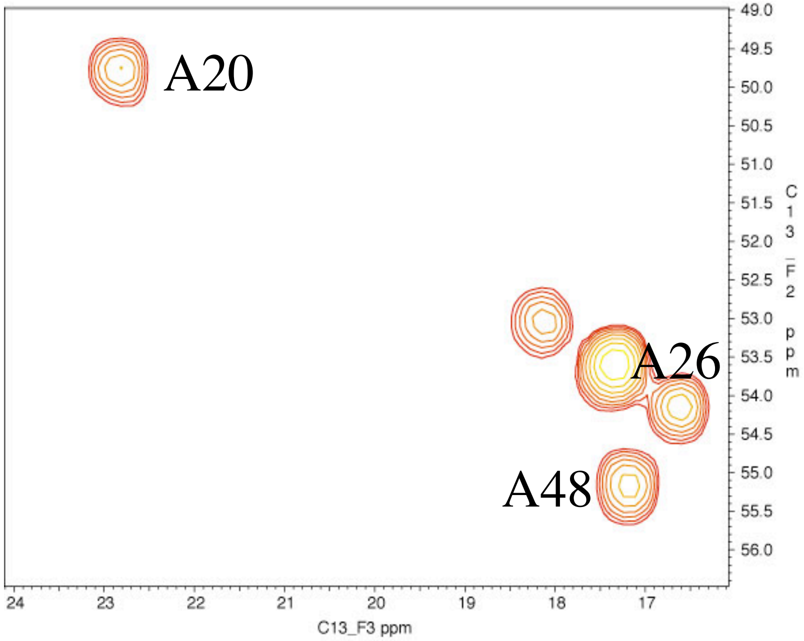
(α-helix)

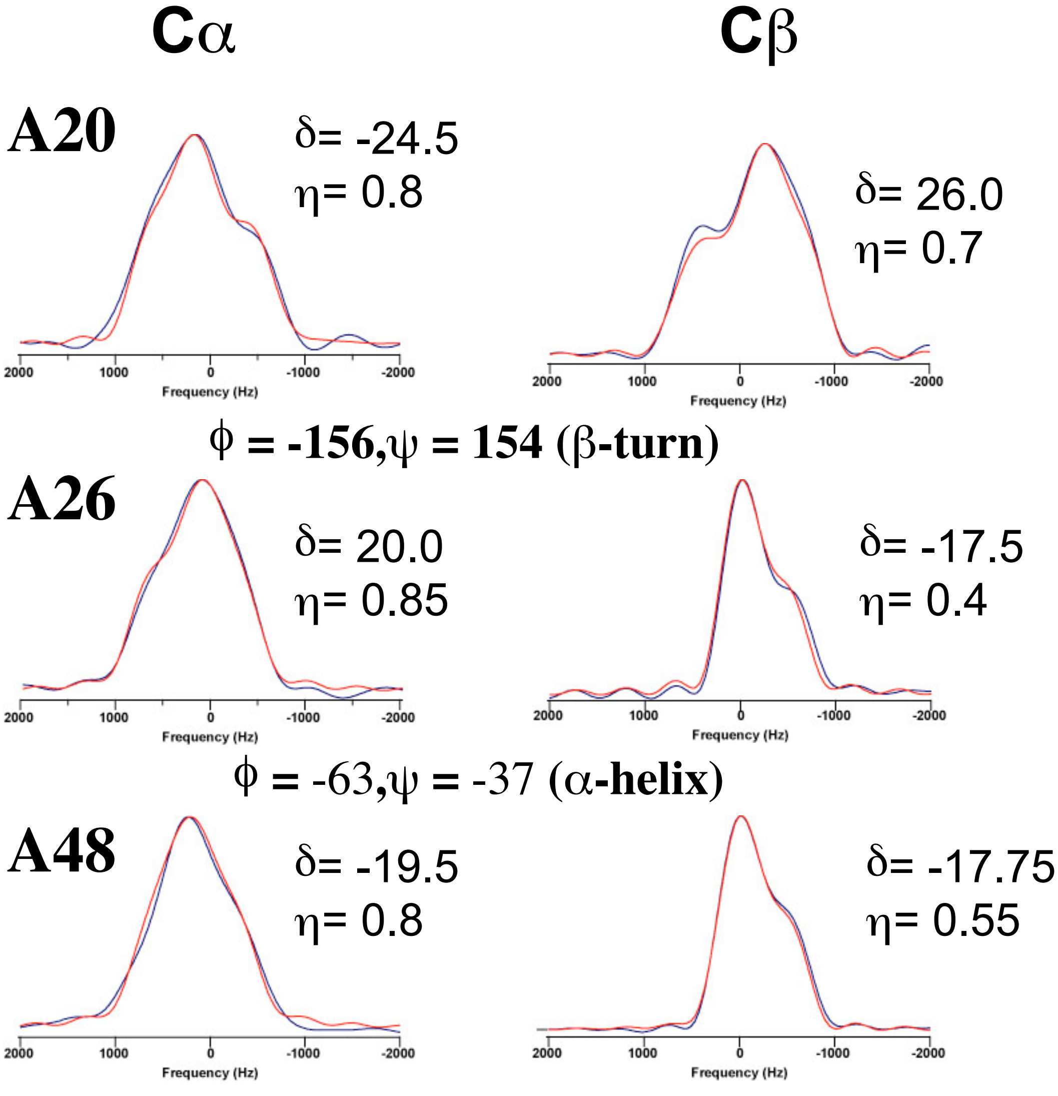


Wylie, Franks, Graesser, Rienstra*, JACS* **2005**, *127*, 11946-11947.

Sun, Sanders, Oldfield, *JACS* **2002**, *124*, 5486-5495.

Alanine Cα and Cβ Tensors





A20

Cα: δ= -24.5, η= 0.8

Cβ: δ= 26.0, η= 0.7

ϕ = -156,ψ = 154 (β-turn)

A26

Cα: δ= 20.0, η= 0.85

Cβ: δ= -17.5, η= 0.4

ϕ = -63,ψ = -37 (α-helix)

A48

Cα: δ= -19.5, η= 0.8

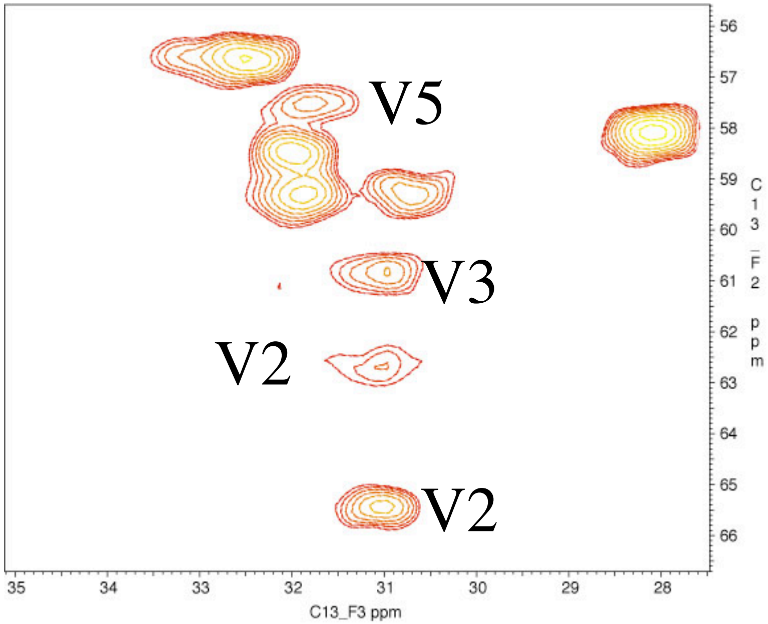
Cβ: δ= -17.75, η= 0.55

ϕ = -59,ψ = -24 (turn)

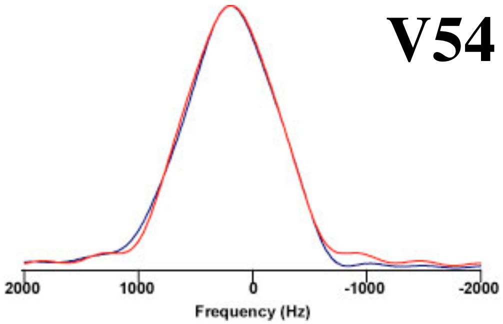
- $\delta = \delta_{zz} - \delta_{iso}$
- δ larger in β-sheet relative to α-helix

Wylie, B.J., Franks W. T., Graesser, D.T., Rienstra, C.M; *JACS* **2005**, 127, 11946-11947.

Valine Cα Tensors



- δ and η greater in β-sheet relative to α-helix
- Also depends upon χ_1 for β-branched residues

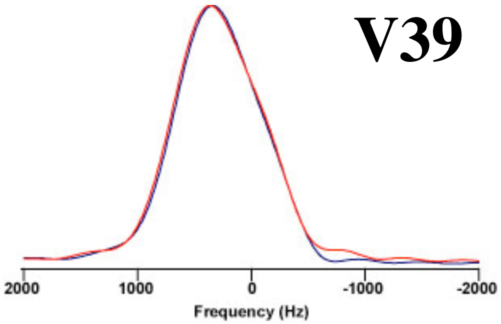


$\delta = 17.0, \ \eta = 1.0$

$\phi = -121, \psi = 140$

gauche +

(β-sheet)

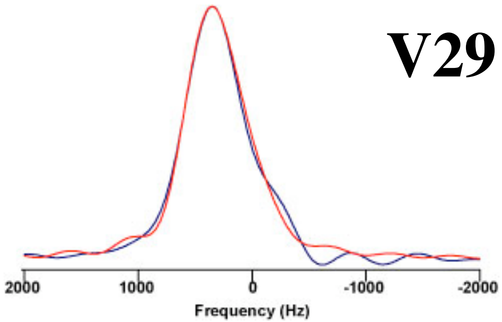


$\delta = -16.75, \ \eta = 0.8$

$\phi = -91, \psi = 140$

trans

(turn)

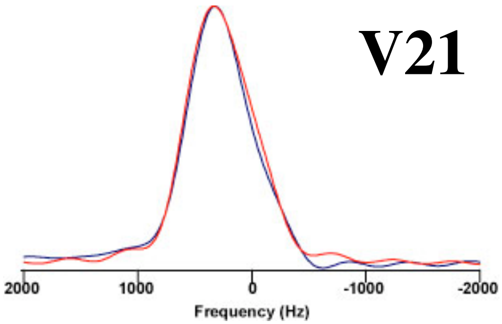


$\delta = -10.75, \ \eta = 0.4$

$\phi = -62, \psi = -49$

trans

(α-helix)



$\delta = -11.75, \ \eta = 0.6$

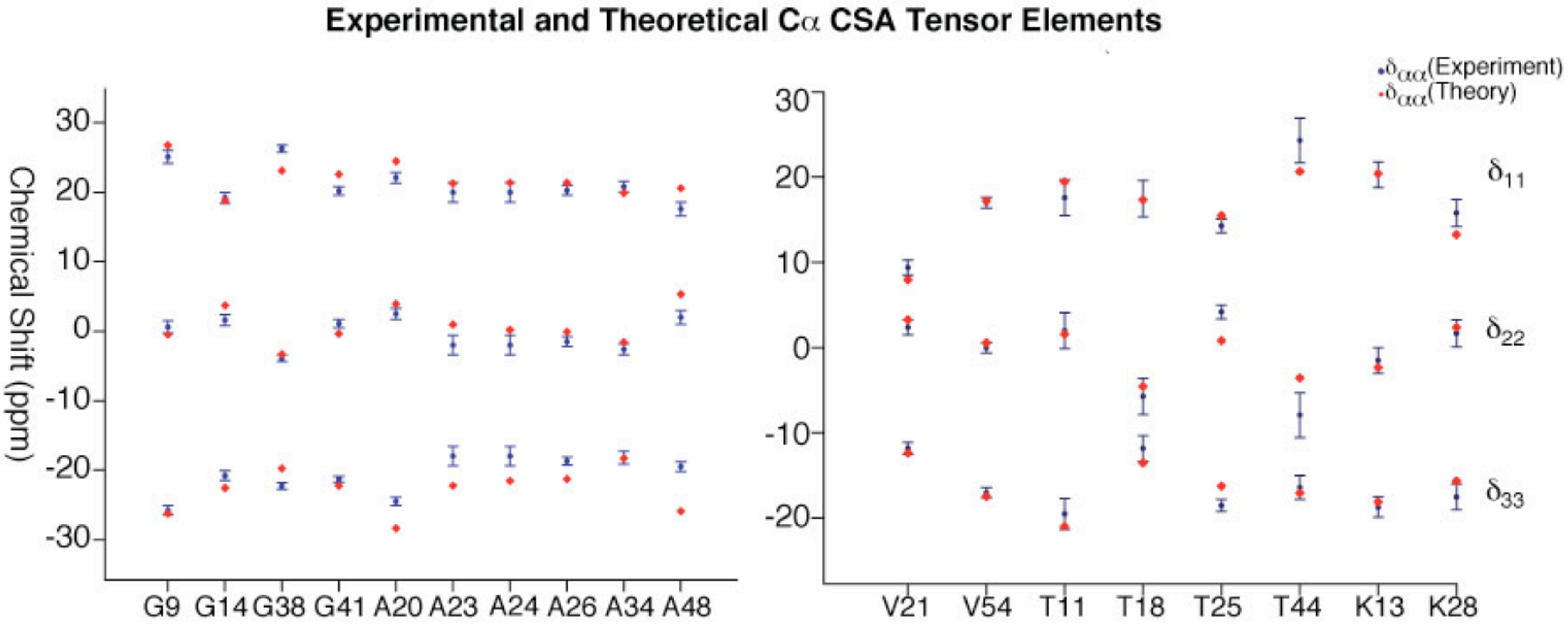
$\phi = -60, \psi = -38$

rotameric hopping

(turn)

Wylie, B.J., Franks W. T., Graesser, D.T., Rienstra, C.M; *JACS* 2005, 127, 11946-47.

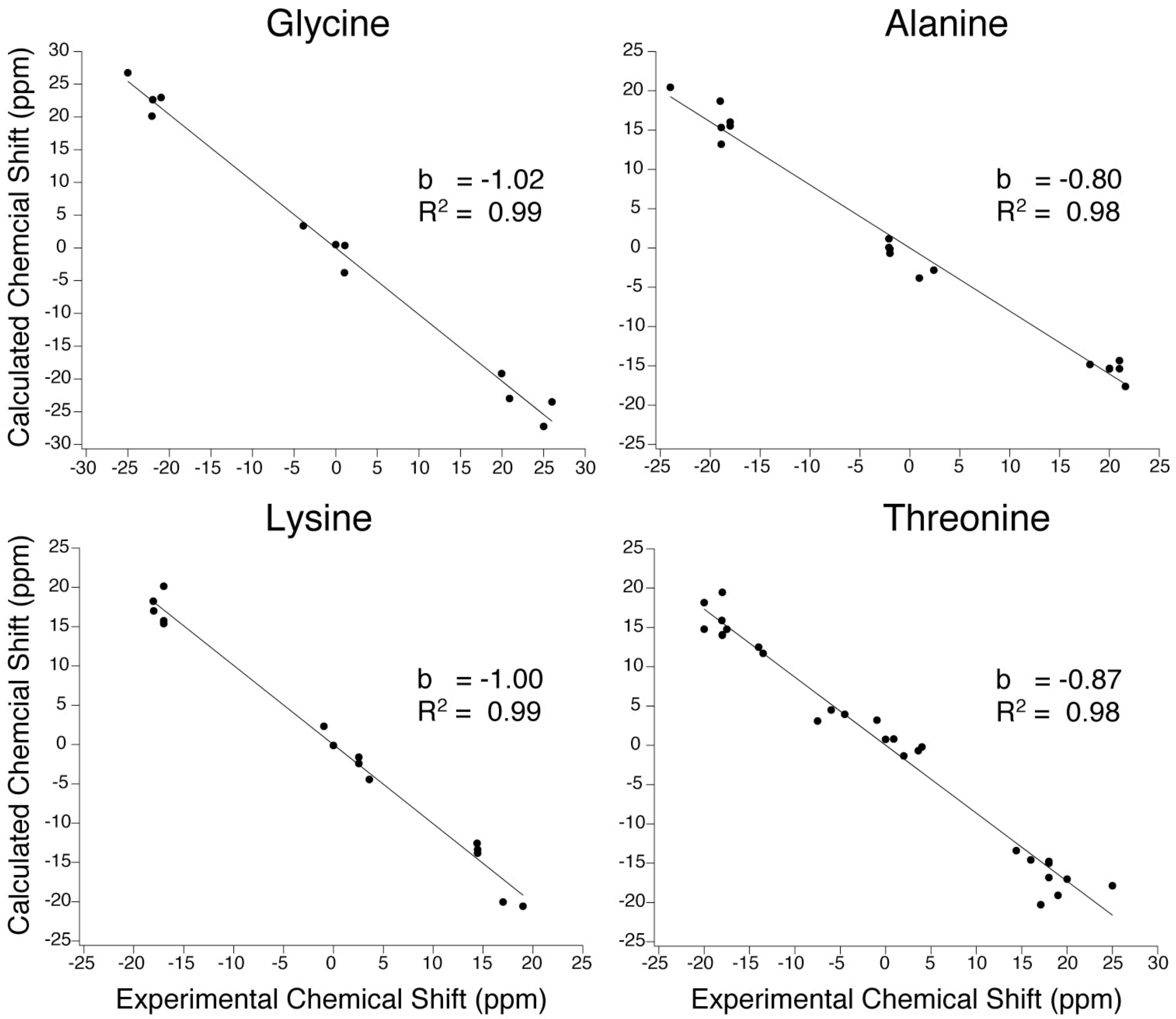
Elements of CSA tensors of Cα Resonances



- Traceless representation
- Theoretical values assume 1PGA crystal structure geometry
- Val and Thr show the greatest variation

Aliphatic ^{13}C CSA Agreement with Theory

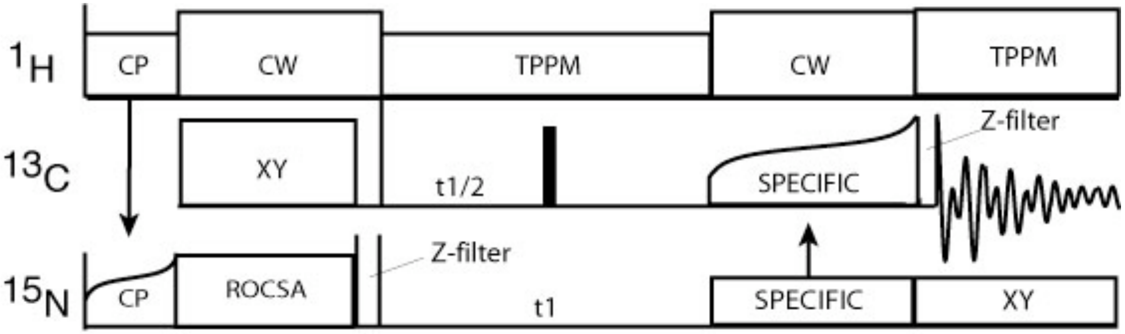
Regression Analysis of Calculated Tensor Elements vs. Experimental Values



Wylie, Franks, Graesser, Rienstra, *JACS* **2005**, *127*, 11946-11947.

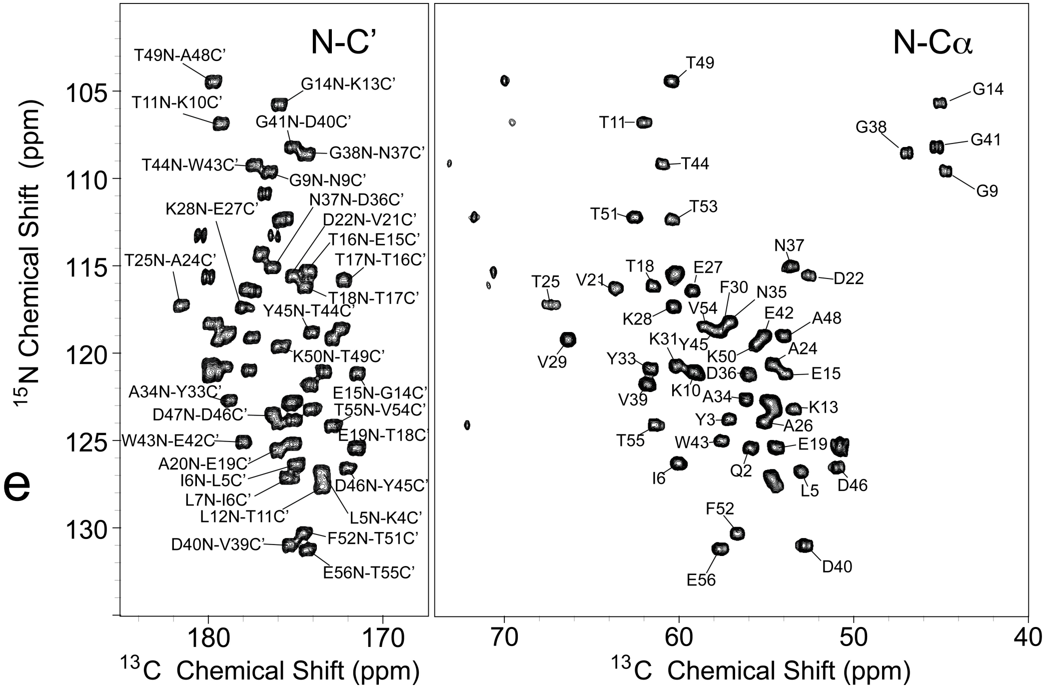
Sun, Sanders, Oldfield, *JACS* **2002**, 124, 5486-5495.

^{15}N ROCSA



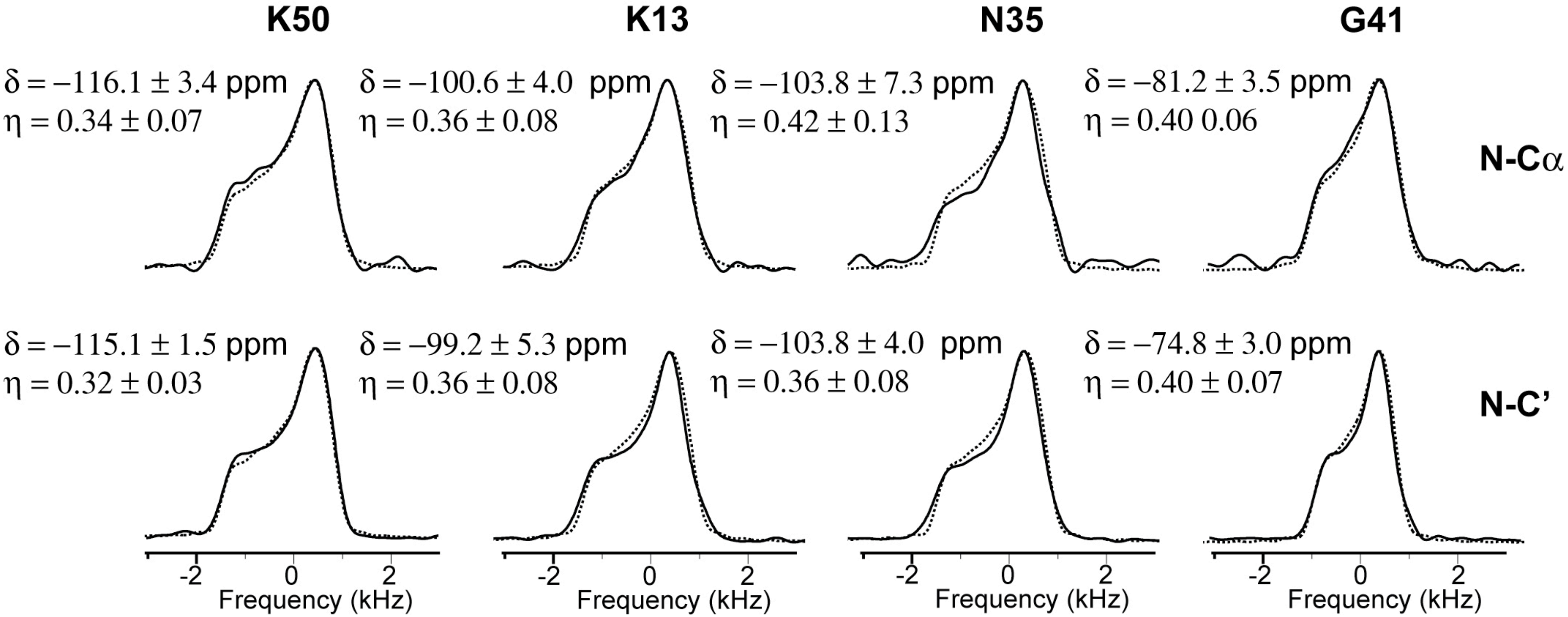
ROCSA incorporated into ^{15}N-^{13}C correlation experiment

- U-^{15}N,^{13}C sample
- 50 ^{15}N sites resolved
- ^{15}N CSA trajectories extracted from third dimension and fit in the time domain



Wylie, B. J.; Franks, T.; Rienstra, C. M., *J. Phys. Chem. B* **2006,** *110*, 10926-10936.

Variations in ^{15}N Tensors



Wylie, B. J.; Franks, T.; Rienstra, C. M., *J. Phys. Chem. B* **2006,** *110*, 10926-10936.

^{15}N CSA Tensor Results

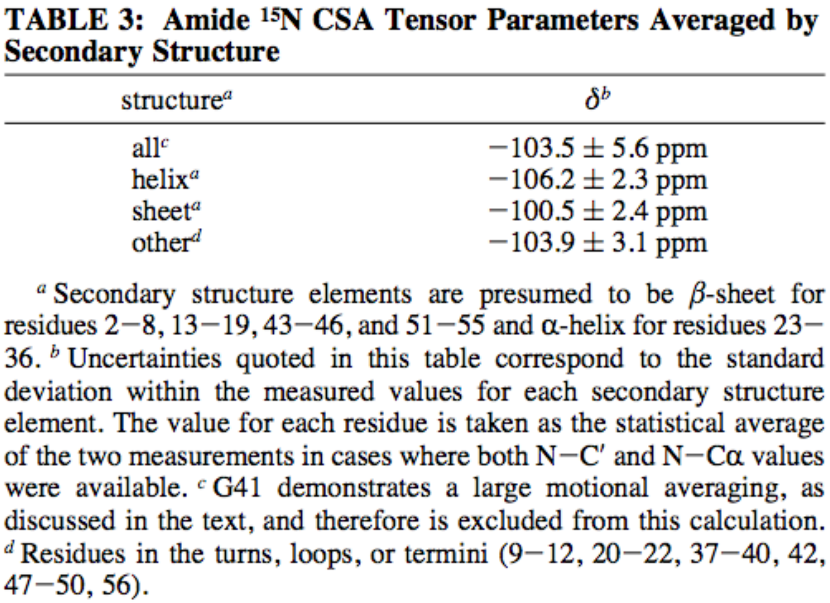


TABLE 3: Amide ^{15}N CSA Tensor Parameters Averaged by Secondary Structure

structure[a]	δ[b]
all[c]	−103.5 ± 5.6 ppm
helix[a]	−106.2 ± 2.3 ppm
sheet[a]	−100.5 ± 2.4 ppm
other[d]	−103.9 ± 3.1 ppm

[a] Secondary structure elements are presumed to be β-sheet for residues 2−8, 13−19, 43−46, and 51−55 and α-helix for residues 23−36. [b] Uncertainties quoted in this table correspond to the standard deviation within the measured values for each secondary structure element. The value for each residue is taken as the statistical average of the two measurements in cases where both N−C′ and N−Cα values were available. [c] G41 demonstrates a large motional averaging, as discussed in the text, and therefore is excluded from this calculation. [d] Residues in the turns, loops, or termini (9−12, 20−22, 37−40, 42, 47−50, 56).

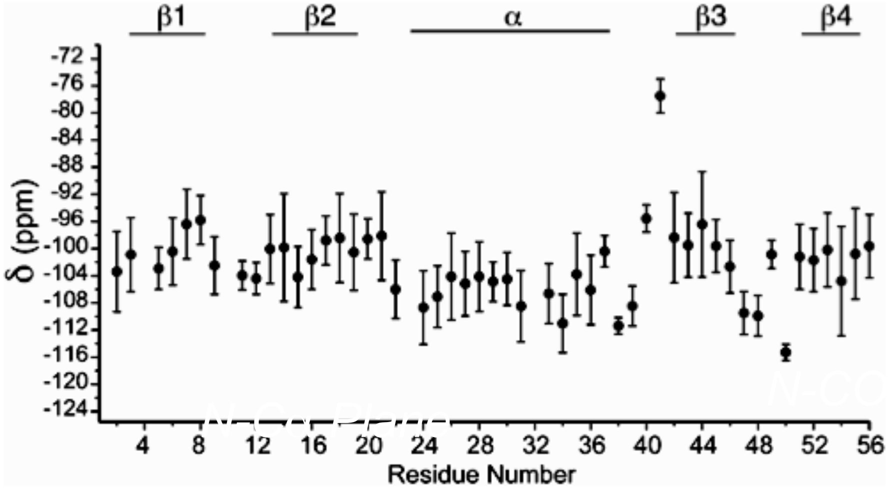
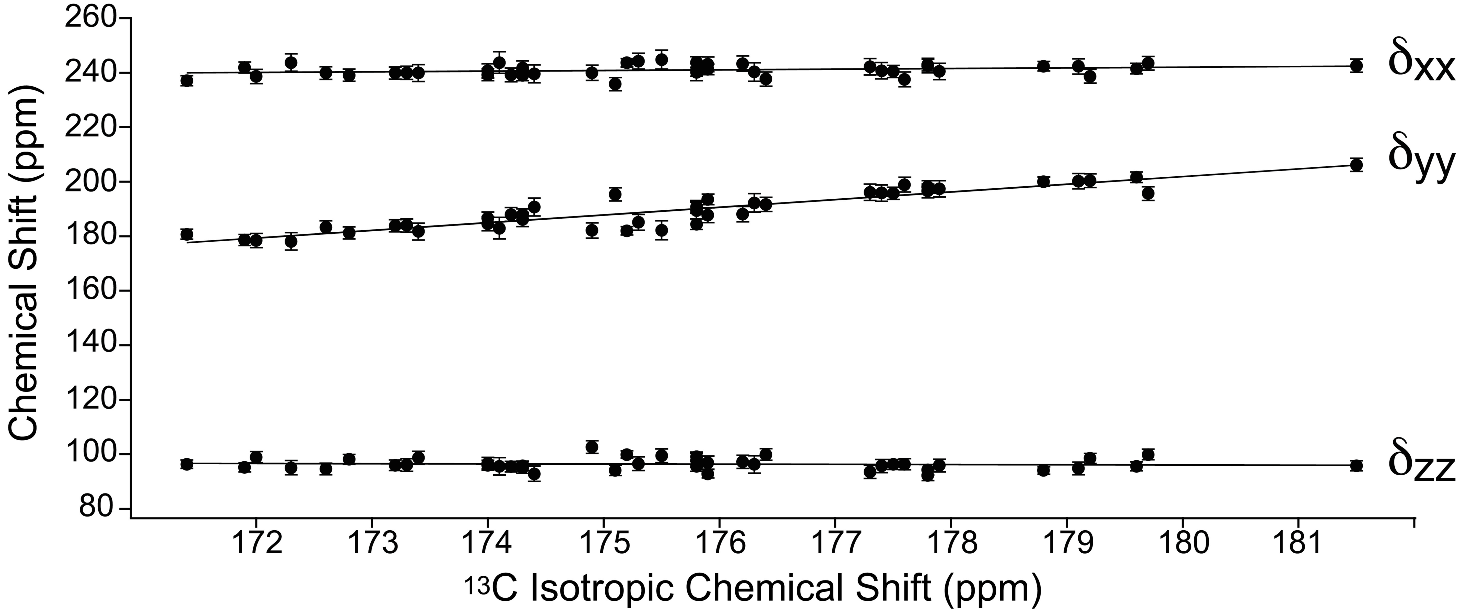


Figure 8. Plot of the anisotropy parameter, δ, versus residue number. Secondary structural elements are labeled at the top of each column. The absolute value of δ is greater in the α-helix compared to the β-sheets by an average value of 6 ppm, as discussed further within the text.

- Reduced anisotropy magnitude varies with structure
- Magnitude greater in helix, although dependence is complex
- Backbone ^{15}N tensors are most used probes of motion in NMR
- Asymmetry, η, slightly smaller in α-helix compared to β-strands

Wylie, Franks, Rienstra, *J. Phys. Chem. B* **2006**, *110*, 10926-10936.
Wylie, Sperling, Frericks, Shah, Franks, Rienstra, *JACS* **2007**, *129*, 5318-19.

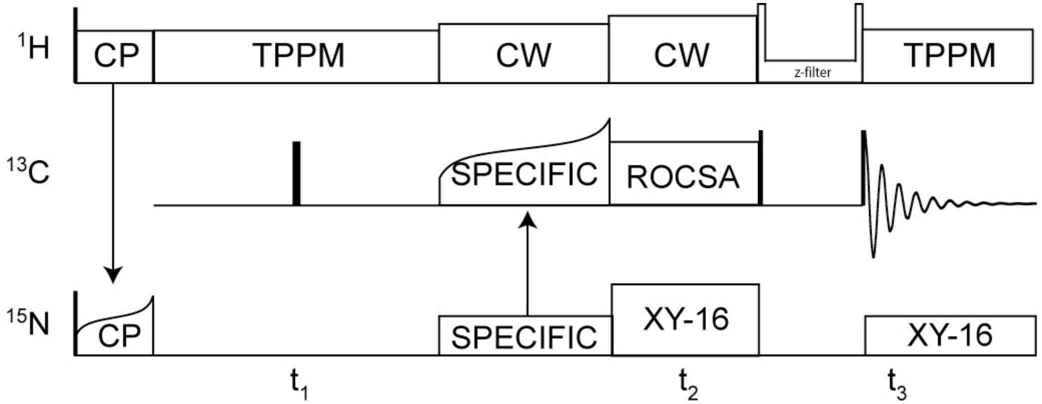
Carbonyl Tensor Sensitive to H-Bonding

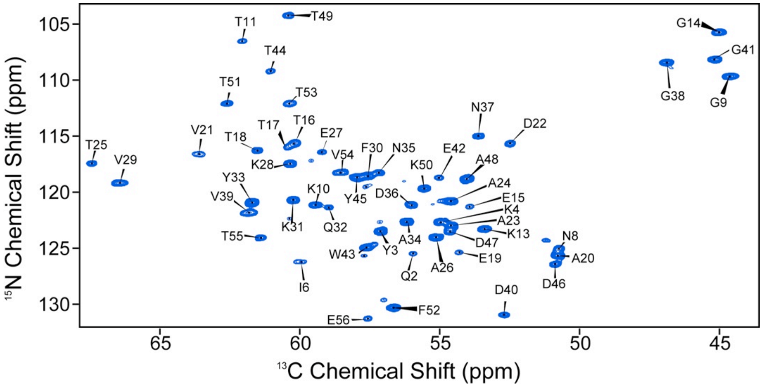


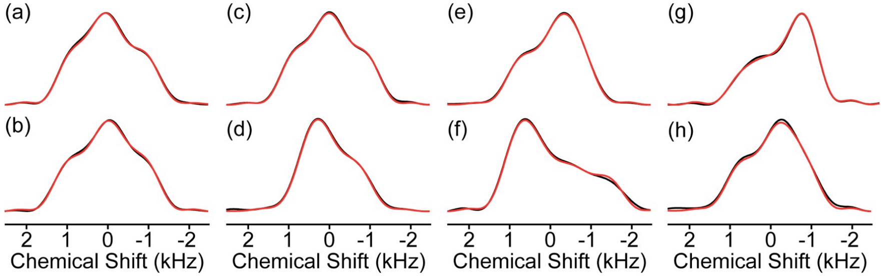
- Tensors depend upon secondary structure
- δ_{yy} tensor element increases linearly with isotropic shift
- C’ chemical shift correlated to H-bond length

Wylie, Sperling, Frericks, Shah, Franks, Rienstra, *JACS* **2007**, *129*, 5318-19.

Computing Structure from CSA

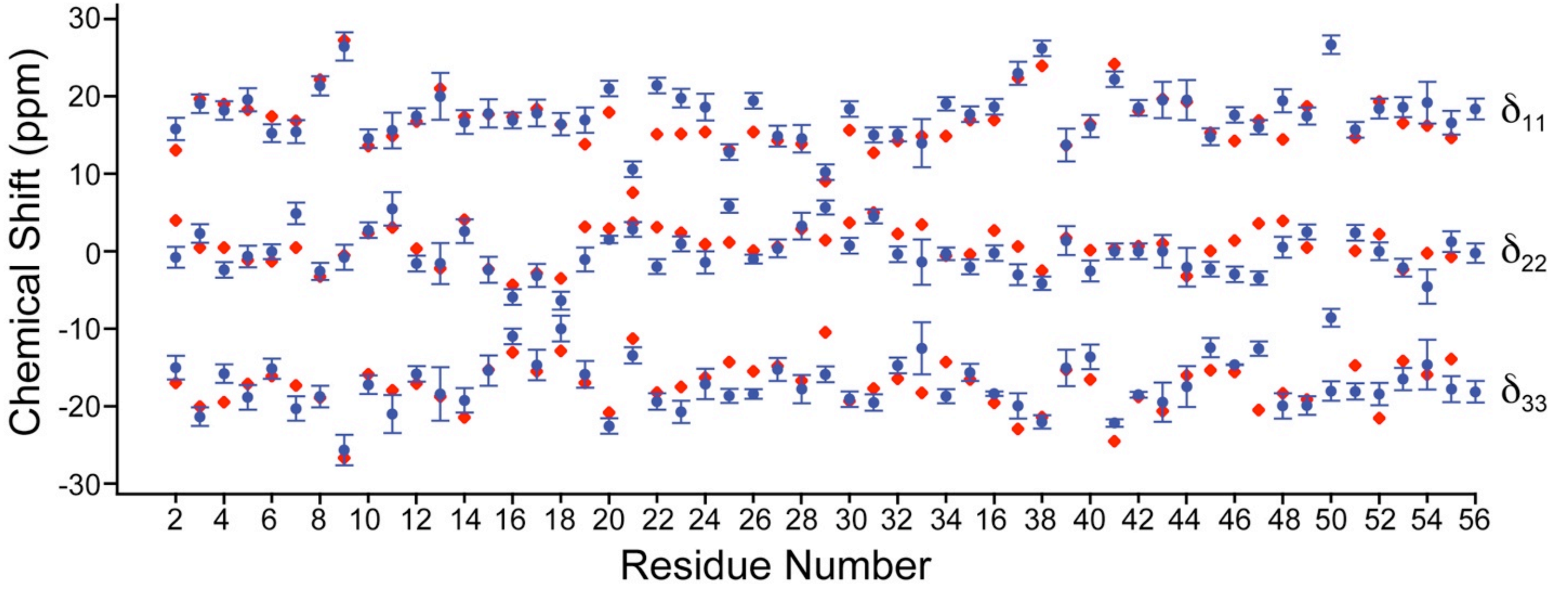






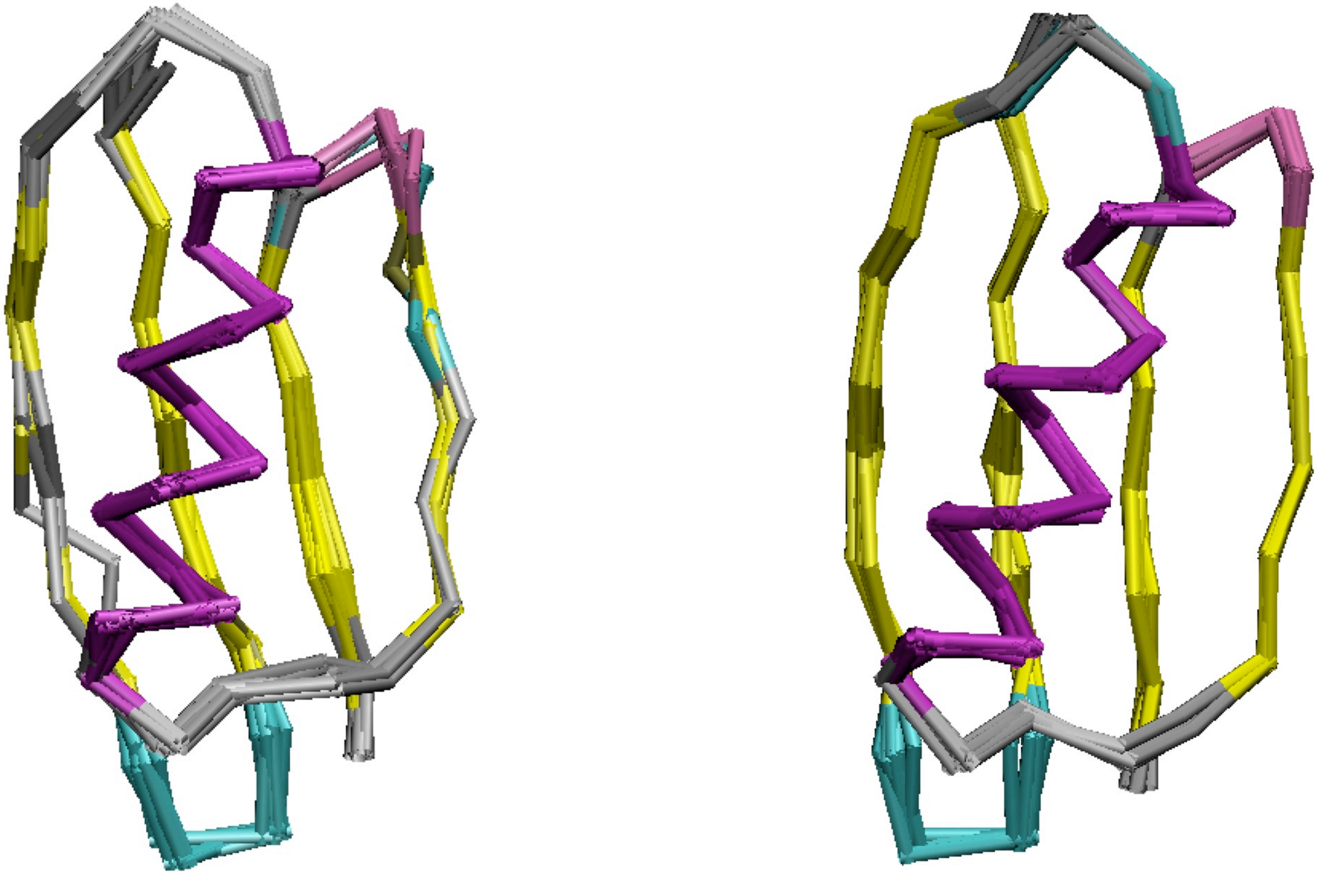
- Wylie et al., unpublished

Comparison with Crystal Structure



- RMSD = ~3 ppm (variation between predicted and observed)
- Noise in data ~1 ppm

Wylie’s CSA Refined GB1 Structure



- Before (left) and after (right) CA CSA refinement

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- Eric Oldfield