Advanced topics in solid state NMR, R. Tycko

(presented at Winter School on Biomolecular Solid State NMR, Stowe, Vermont, 1/25/08)

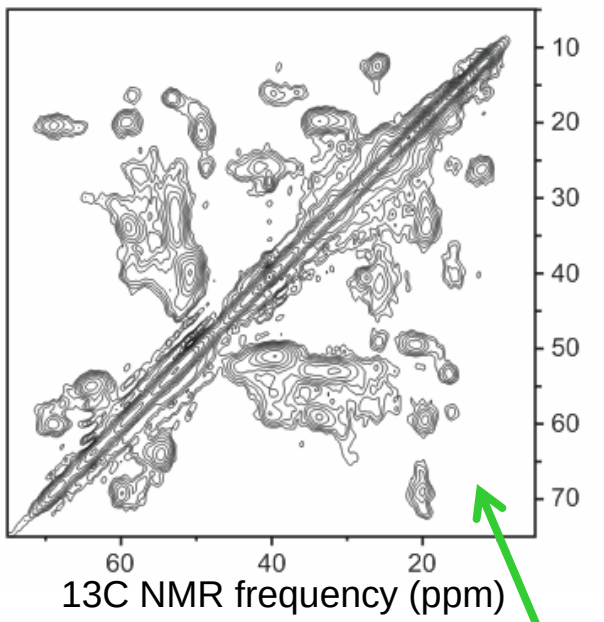
1. Isotopic labeling strategies and sample preparations for amyloid fibrils (and other systems?)

2. Low-temperature MAS

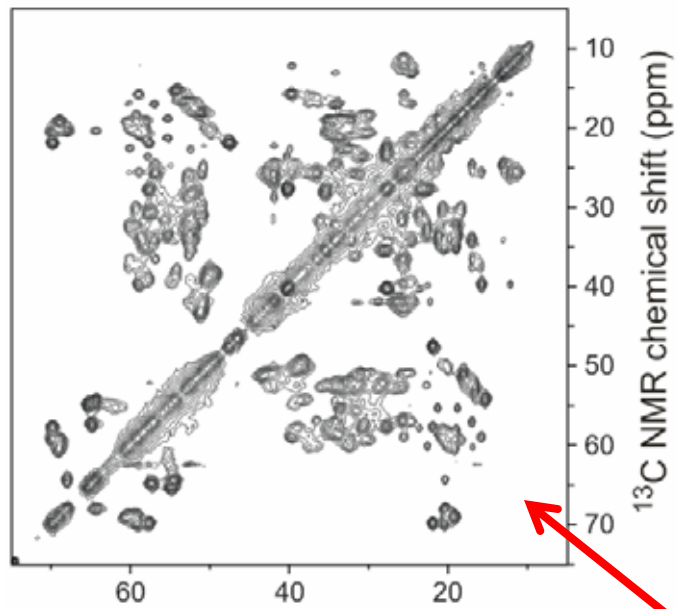
3. Quantitative distance measurements by homonuclear recoupling in uniformly or multiply labeled samples

1. Isotopic labeling strategies, etc.

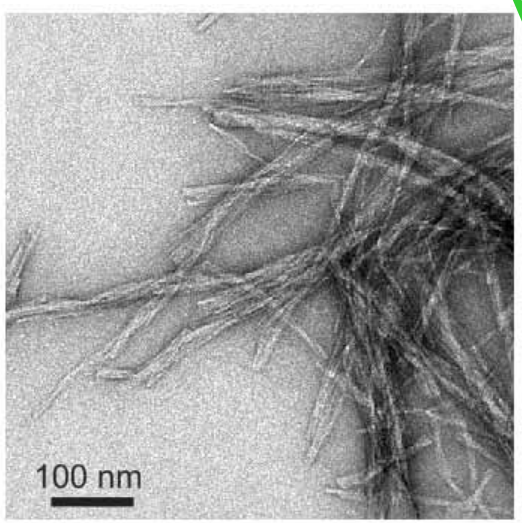
U-labeled Ure2p$_{1\text{-}89}$ fibrils



U-labeled HET-s$_{218\text{-}289}$ fibrils



Note: lyophilized, then rehydrated in the MAS rotor



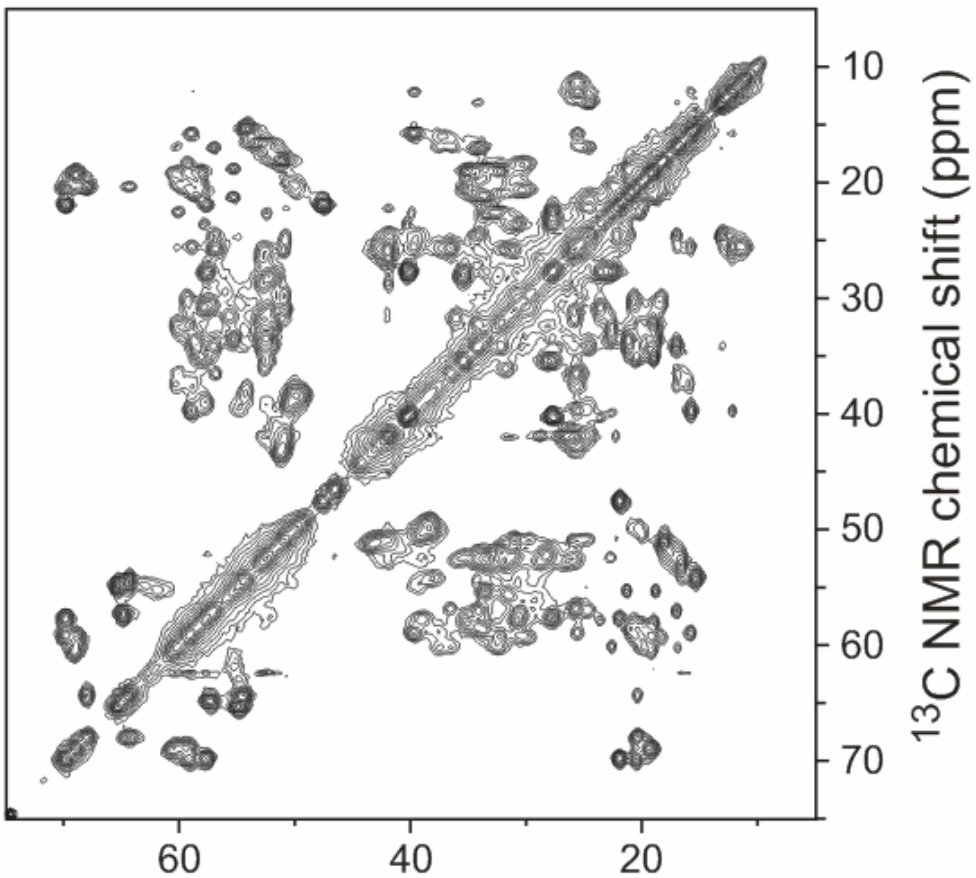
Can make great progress towards full 3D structure determination (B. Meier, R. Riek, et al.)

Can not determine full 3D structure, but still can address real scientific questions.

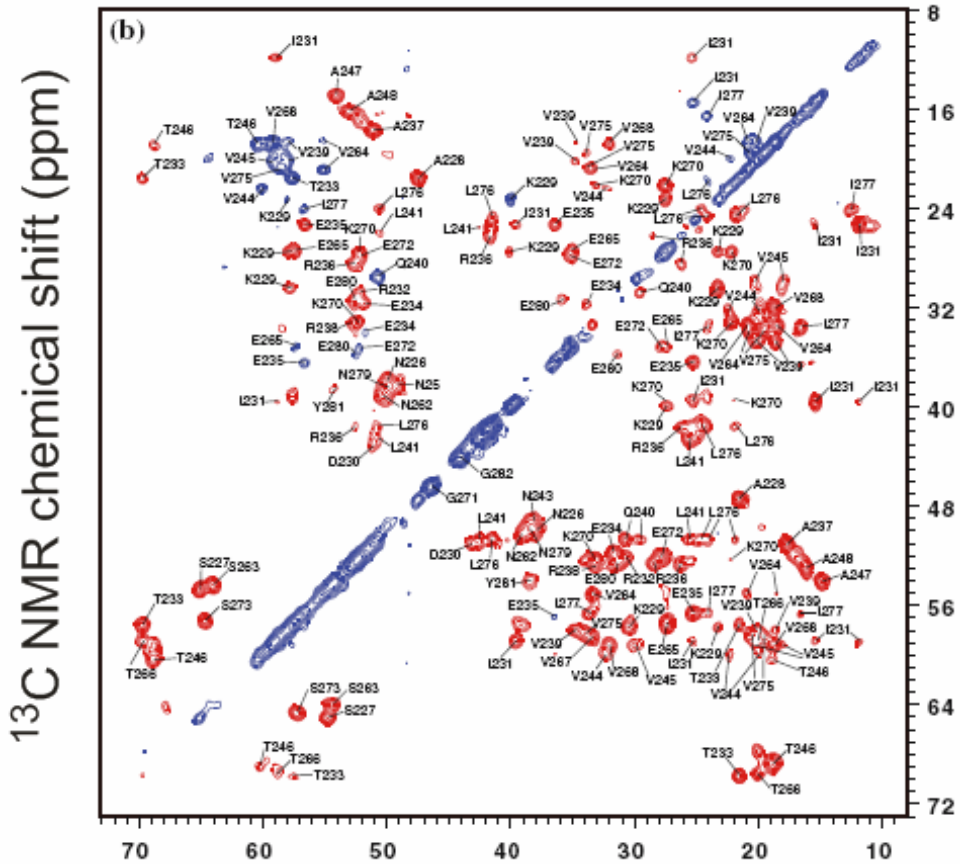
Baxa et al., Biochemistry 2007

1. Isotopic labeling strategies, etc.

U-labeled HET-$s_{218-289}$ fibrils



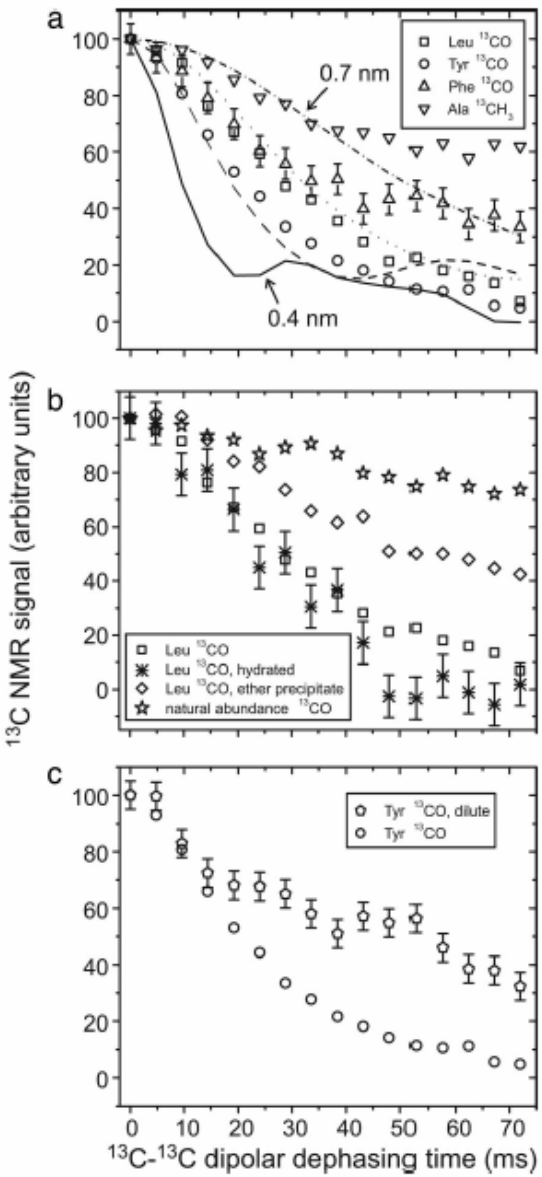
lyophilized, then rehydrated (Baxa et al., Biochemistry 2007)

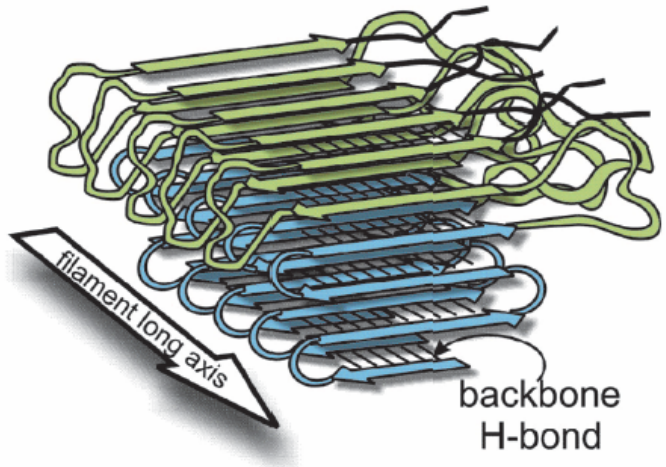


wet pellet, never lyophilized (Siemer et al., J. Biomolec. NMR 2006)

1. Isotopic labeling strategies, etc.

Sup35NM fibrils, selective labeling of amino acid types

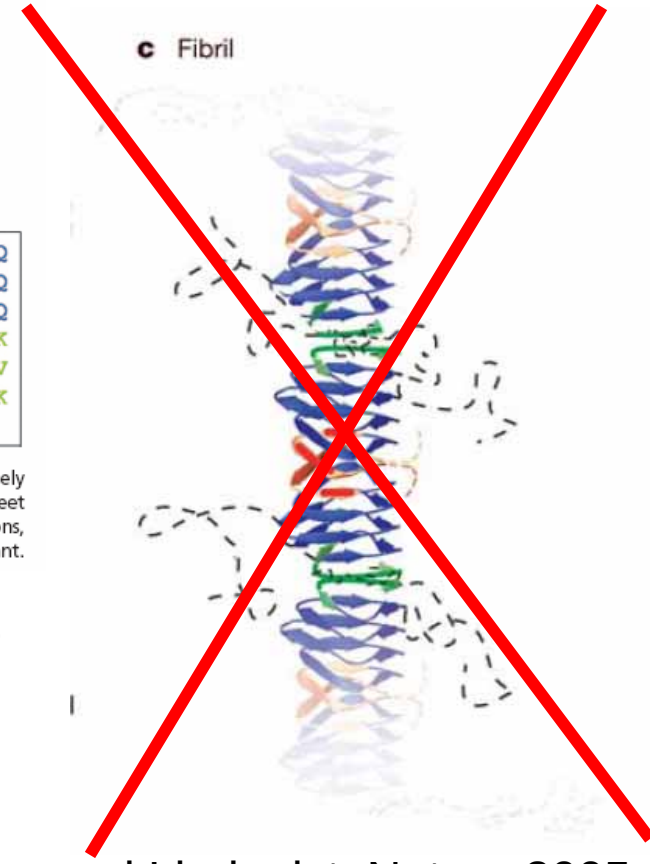




MSDSNQGNNQ QNYQQYSQNG NQQQGNNRYQ GYQAYNAQAQ
PAGGYYQNYQ GYSGYQQGGY QQYNPDAGYQ QQYNPQGGYQ
QYNPQGGYQQ QFNPQGGRGN YKNFNYNNNL QGYQAGFQPQ
SQGMSLNDFQ KQQKQAAPKP KKTLKLVSSS GIKLANATKK
VGTKPAESDK KEEEKSAETK EPTKEPTKVE EPVKKEEKPV
QTEEKTEEKS ELPKVEDLKI SESTHNTNNA NVTSADALIK
EQEEEVDDEV VND

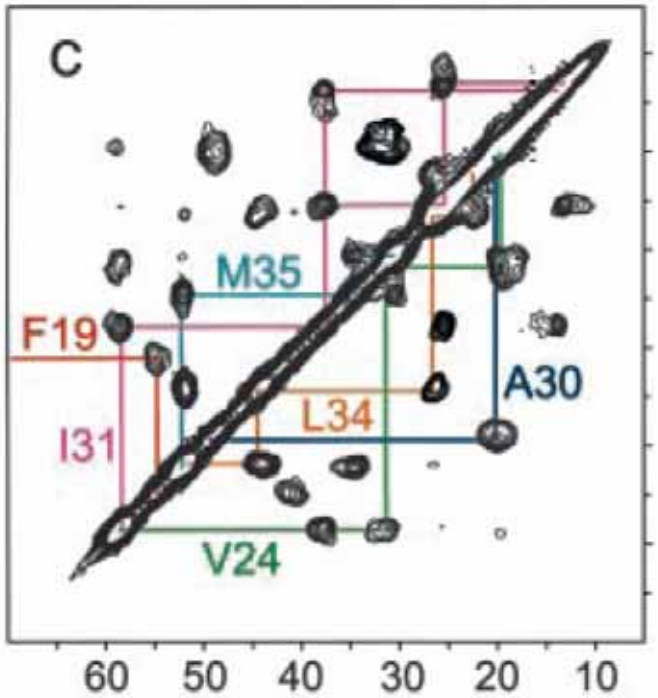
Fig. 4. Tentative model of Sup35NM amyloid. The N domain (blue) is largely β-sheet, whereas the M domain (green) consists of both β-sheet and non-β-sheet regions. The model is schematic and does not pretend to portray the locations, number, or lengths of β-strands or loops, which may depend on the prion variant.

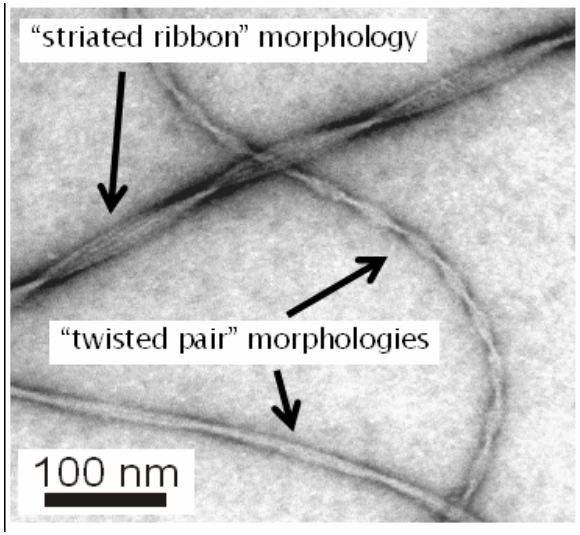
Shewmaker et al., PNAS 2006



Krishnan and Lindquist, Nature 2005

1. Isotopic labeling strategies, etc.





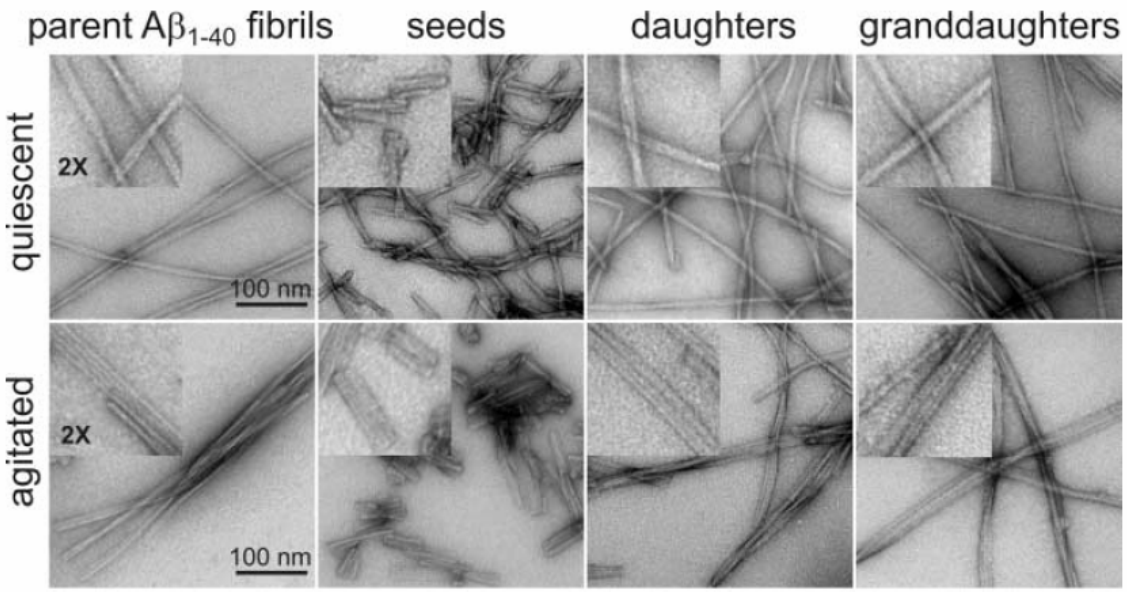


Fig. 1. TEM images of amyloid fibrils formed by the Aβ1–40 peptide, negatively stained with uranyl acetate. Parent fibrils were prepared by incubation of Aβ1–40 solutions either under quiescent dialysis conditions or in a closed polypropylene tube with gentle agitation. Daughter and granddaughter fibrils were grown under dialysis from solutions that were seeded with sonicated fragments of parent and daughter fibrils, respectively.

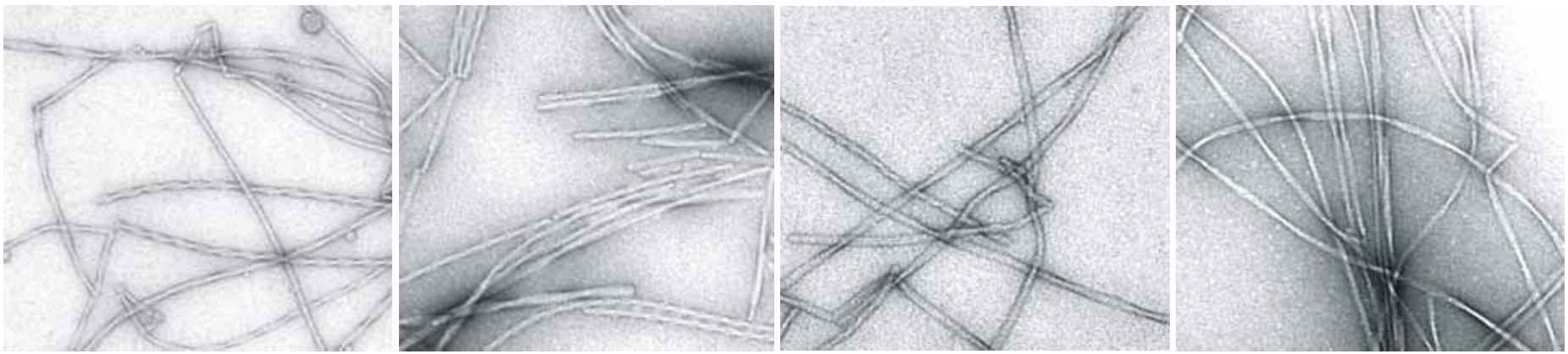
$A\beta_{1\text{-}40}$ fibrils, U-labeled at specific sites by solid-phase synthesis. Dry lyophilized, <u>somewhat polymorphic</u> (Petkova et al., PNAS 2002)

linewidths ~ 2.0-2.5 ppm

Petkova et al., Science 2005

Self-purification by multiple rounds of sonication/seeding/growth (Anant Paravastu)

1 2 5 12 rounds

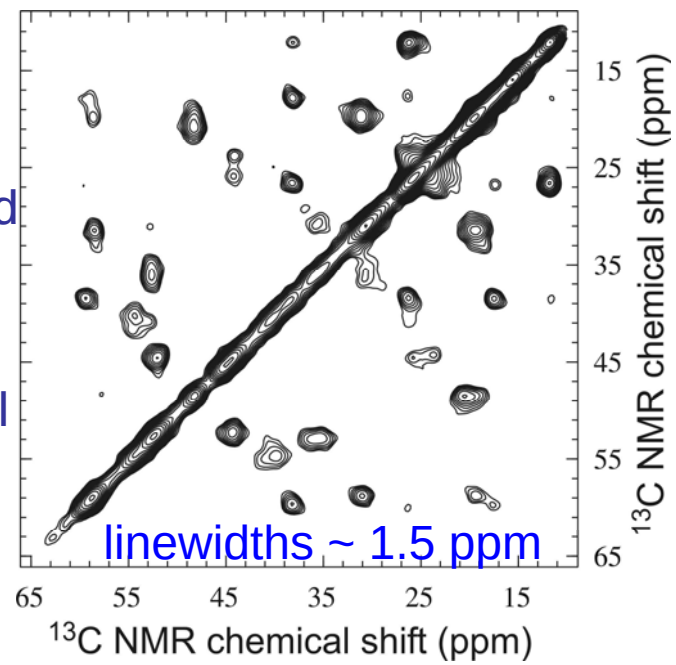


Solid state NMR, U-labeled F19, V24, G25, A30, I31, L34, M35

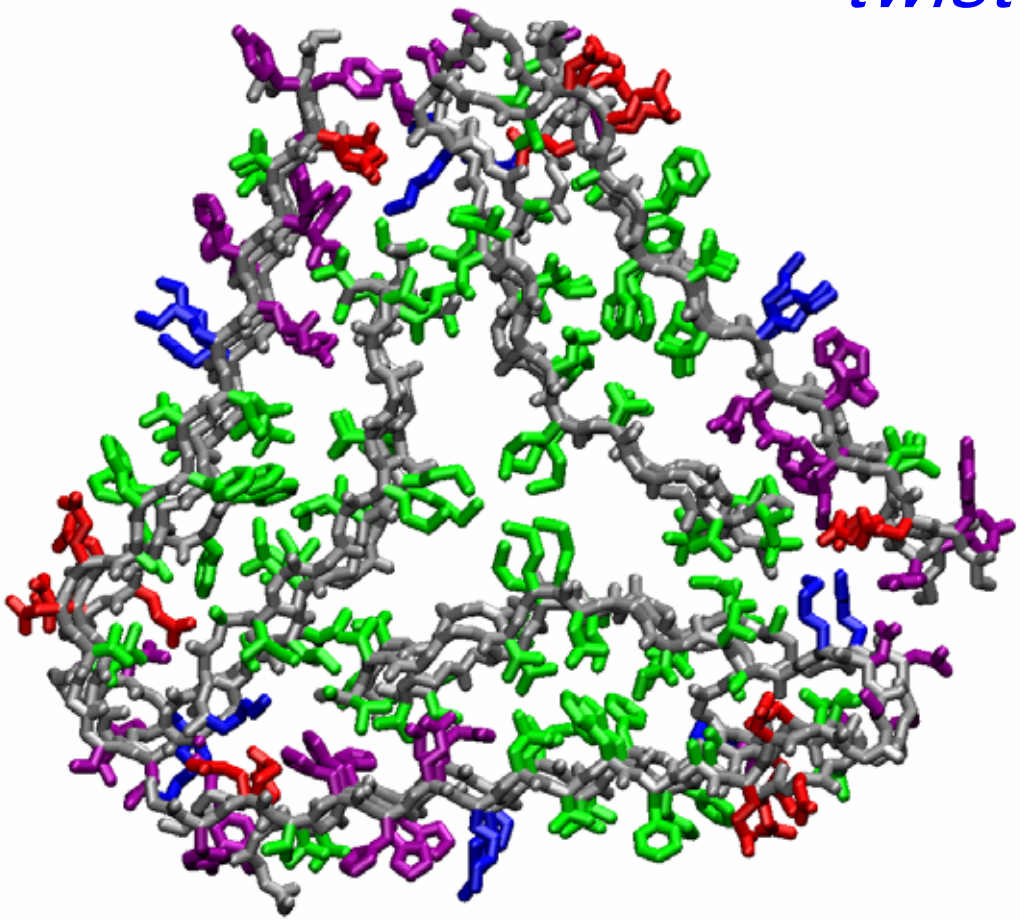
Single, sharp line for each ^{13}C-labeled site.

All molecules have the same conformation and the same structural environment.

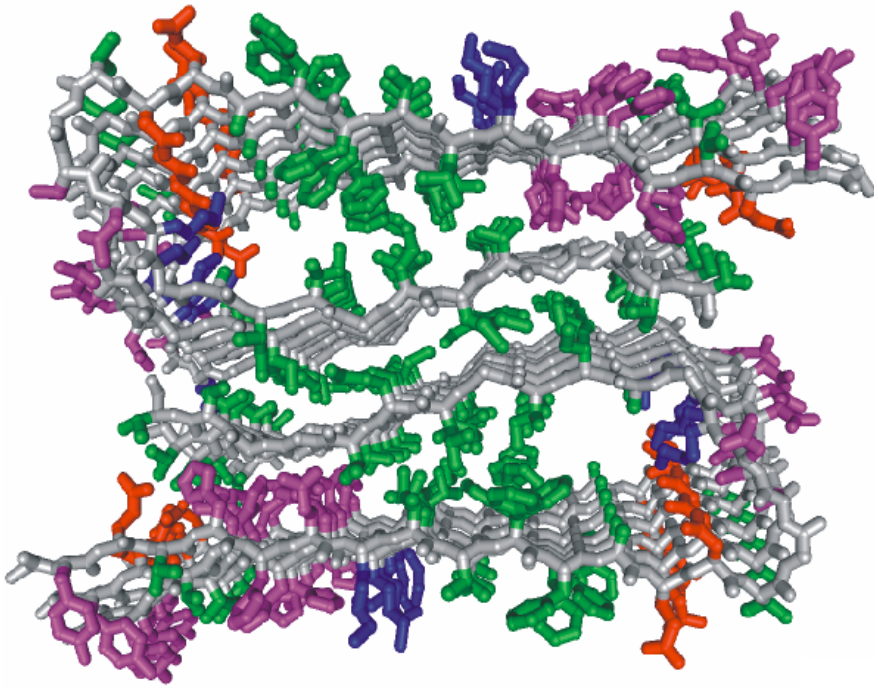
IMPLIES 3-FOLD SYMMETRY.



"twisted pair" $A\beta_{1-40}$ fibril structure

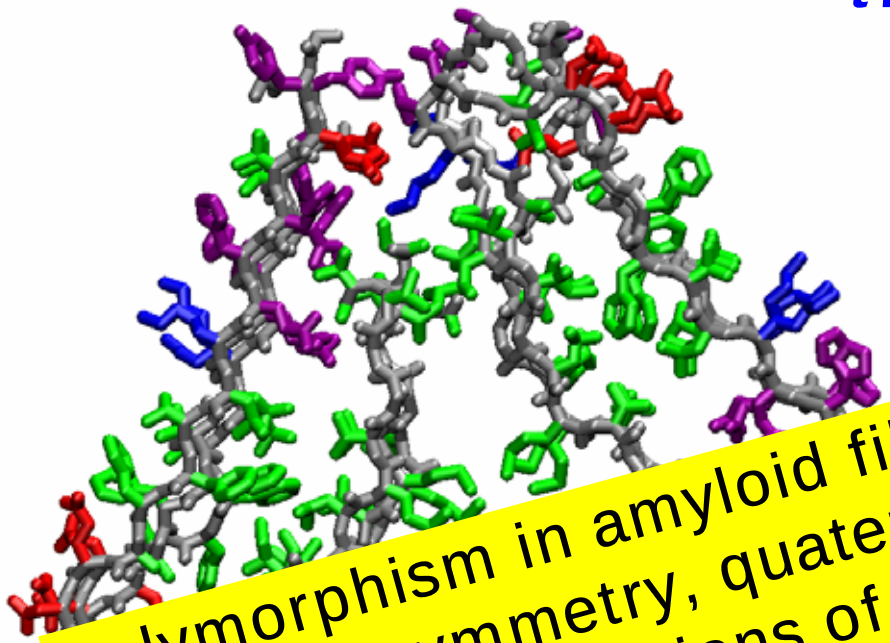


- hydrophobic
- negatively charged
- positively charged
- polar



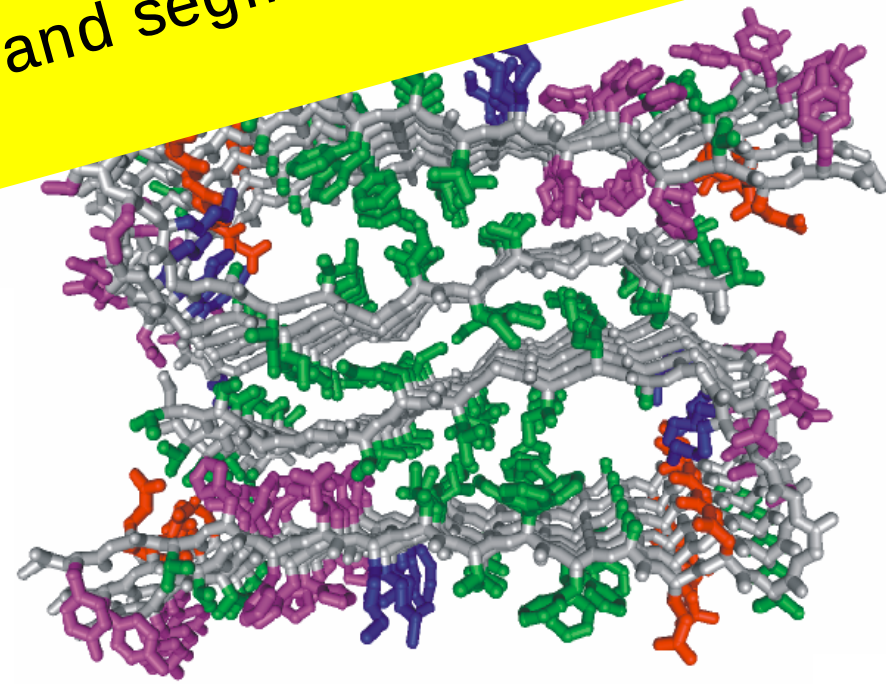
"striated ribbon" $A\beta_{1-40}$ fibril structure

"twisted pair" $A\beta_{1-40}$ fibril structure



Polymorphism in amyloid fibrils arises from variations in overall symmetry, quaternary structure and the detailed conformations of non-β-strand segments.

(but not the location of β-strand segments or the type of β-sheets)

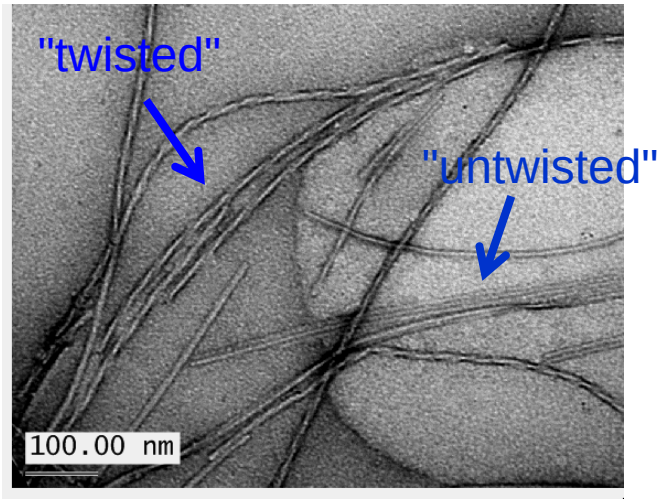


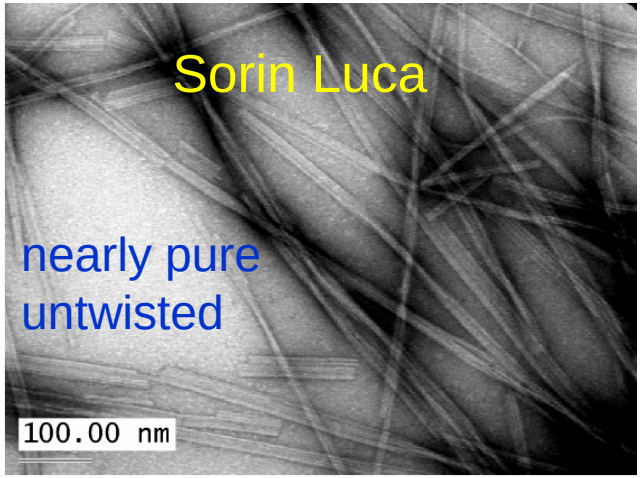
"striated ribbon" $A\beta_{1-40}$ fibril structure

Universality of amyloid structure

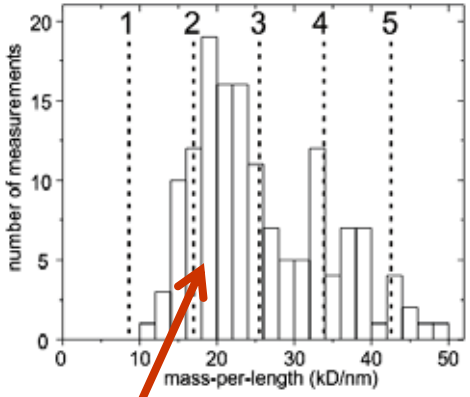
amylin (a.k.a. islet amyloid polypeptide, IAPP):

KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY

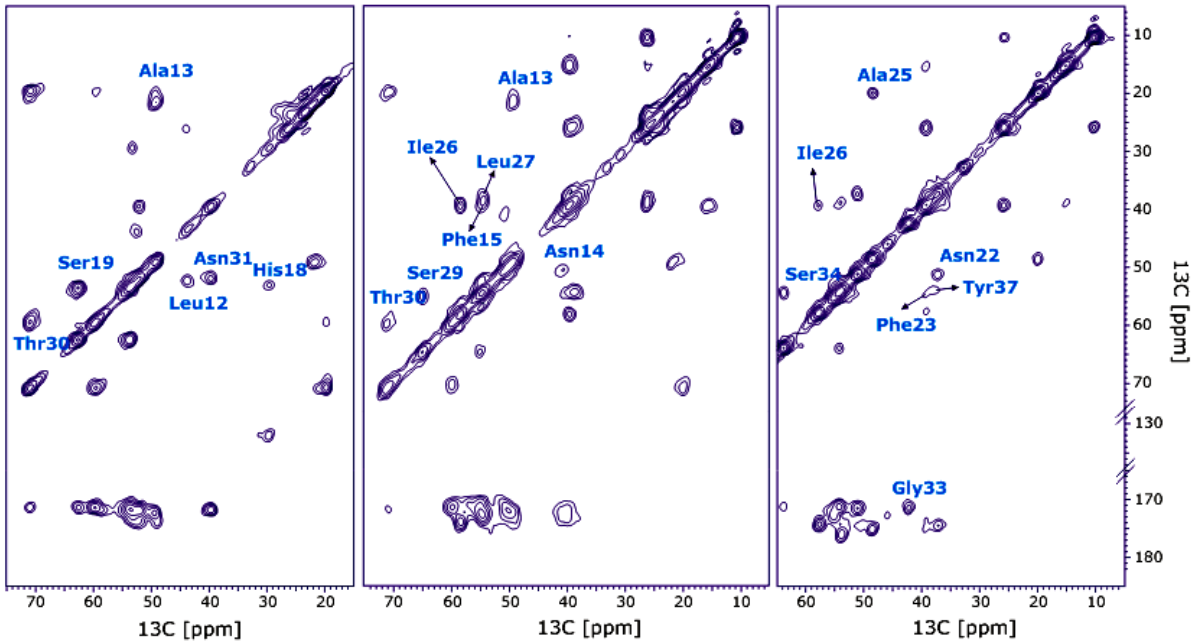




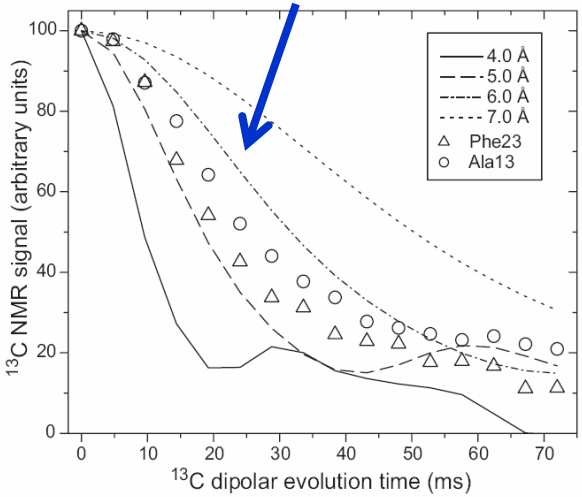
STEM data



amylin protofilament contains two cross-β units



parallel β-sheets

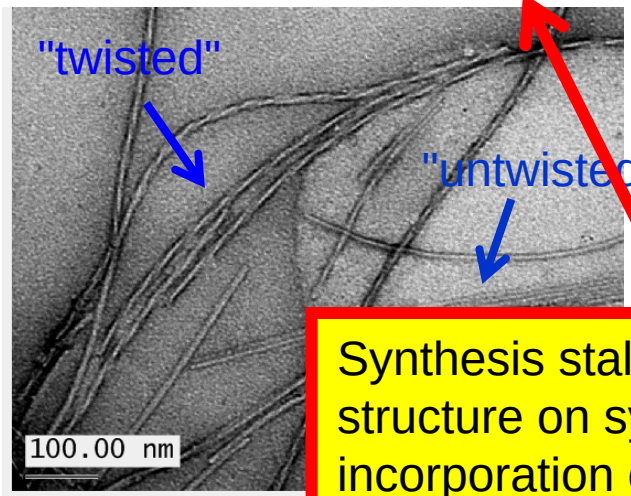


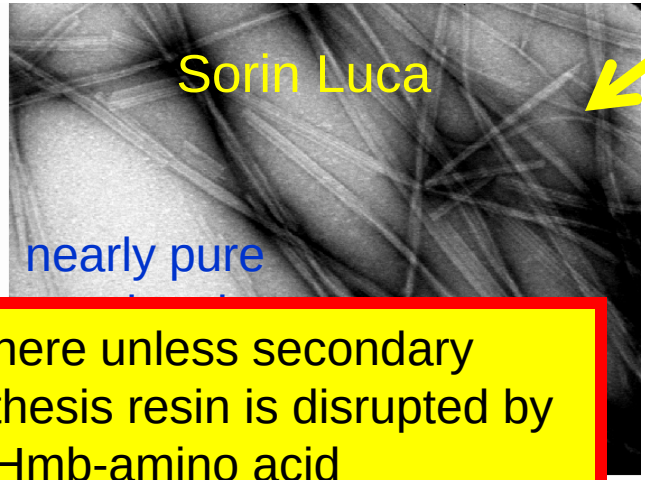
Universality of amyloid structure

amylin (a.k.a. islet amyloid polypeptide, I

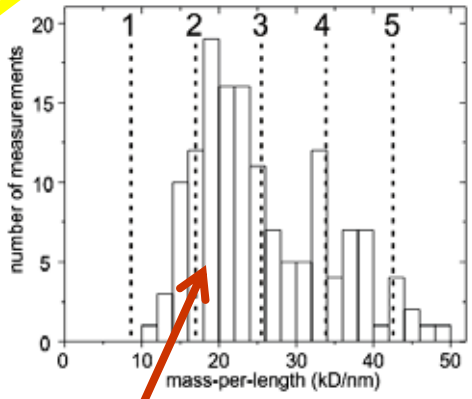
KCNTATCATQRLANFLVHSSNNFGAILSST

purification of monomers by gel filtration, seeded growth





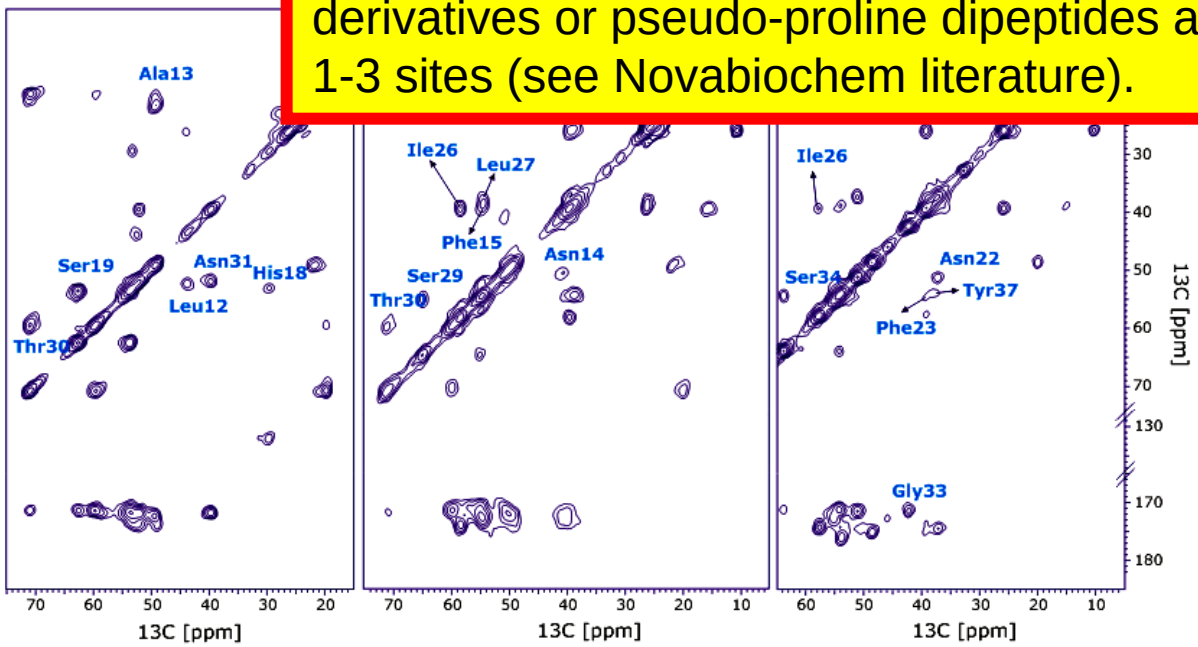
STEM data

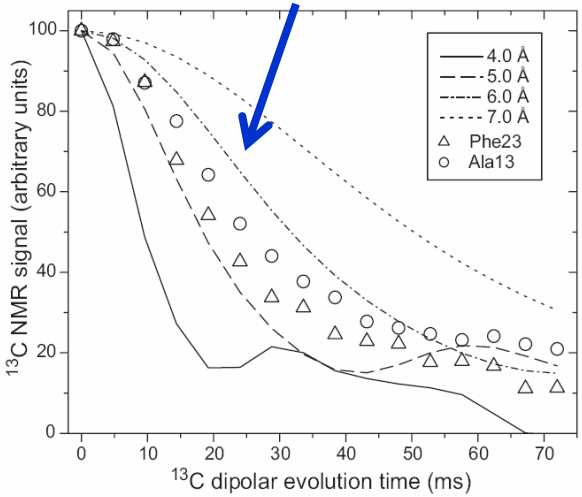


Synthesis stalls here unless secondary structure on synthesis resin is disrupted by incorporation of Hmb-amino acid derivatives or pseudo-proline dipeptides at 1-3 sites (see Novabiochem literature).

amylin protofilament contains two cross-β units

parallel β-sheets





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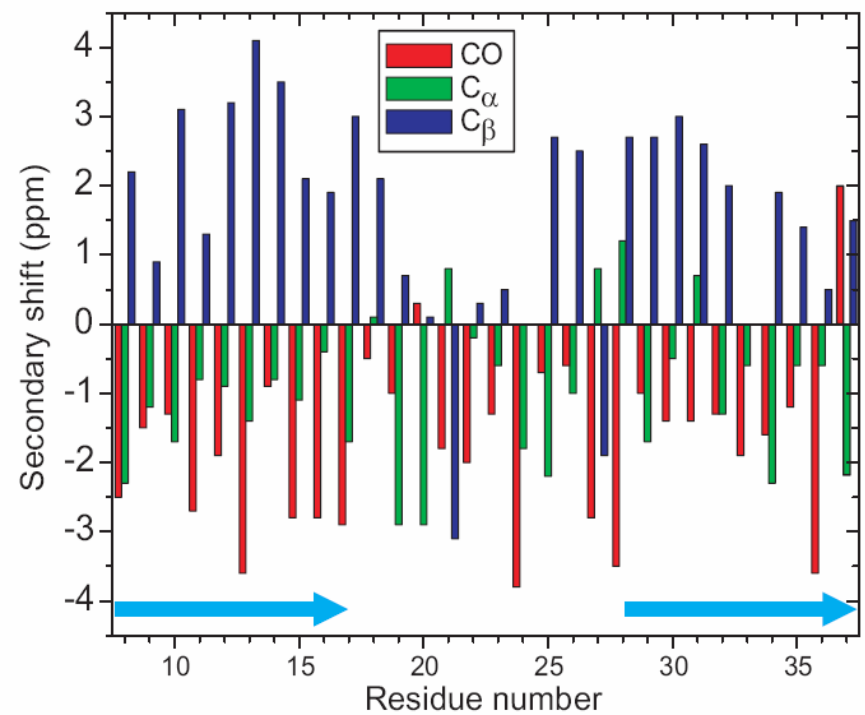
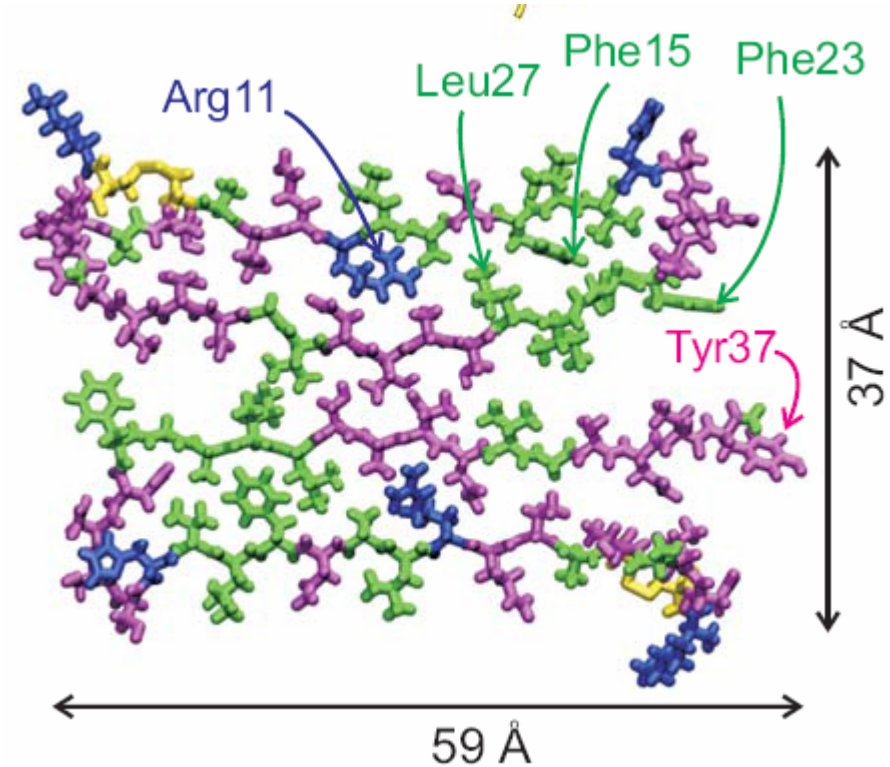


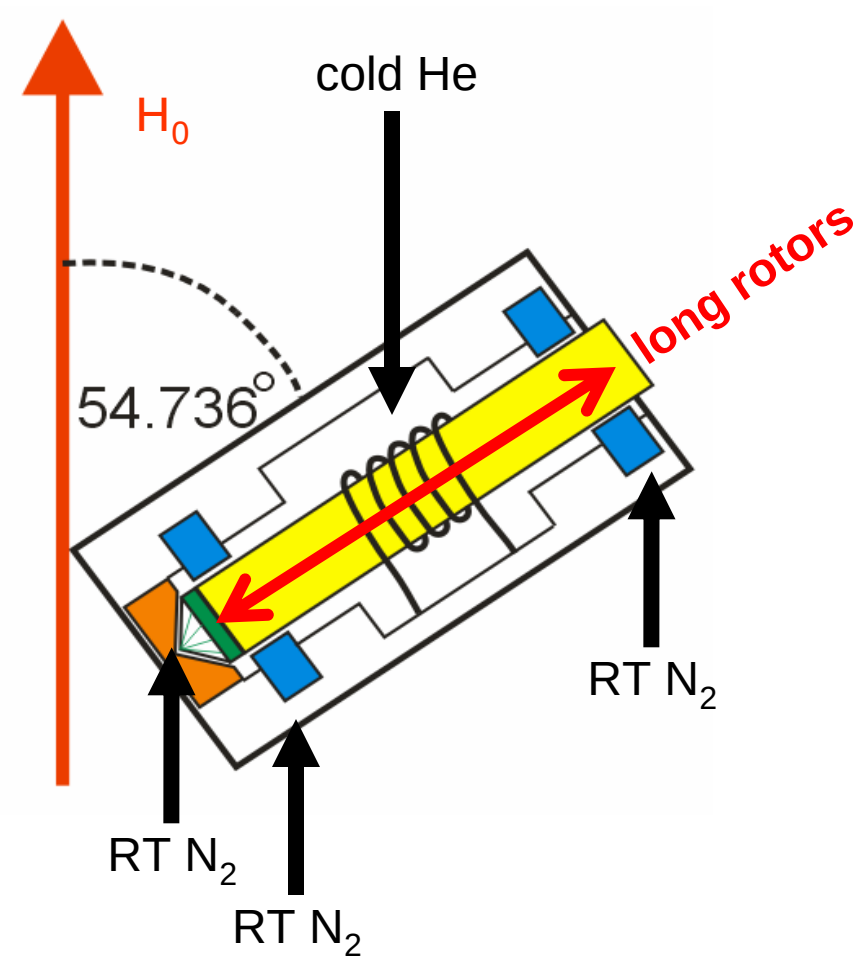


- disordered, disulfide-bridged N-terminal segment
- two β-strand segments, forming parallel β-sheets
- two-fold symmetry about fibril axis
- closely resembles $A\beta_{1\text{-}40}$ fibril structure

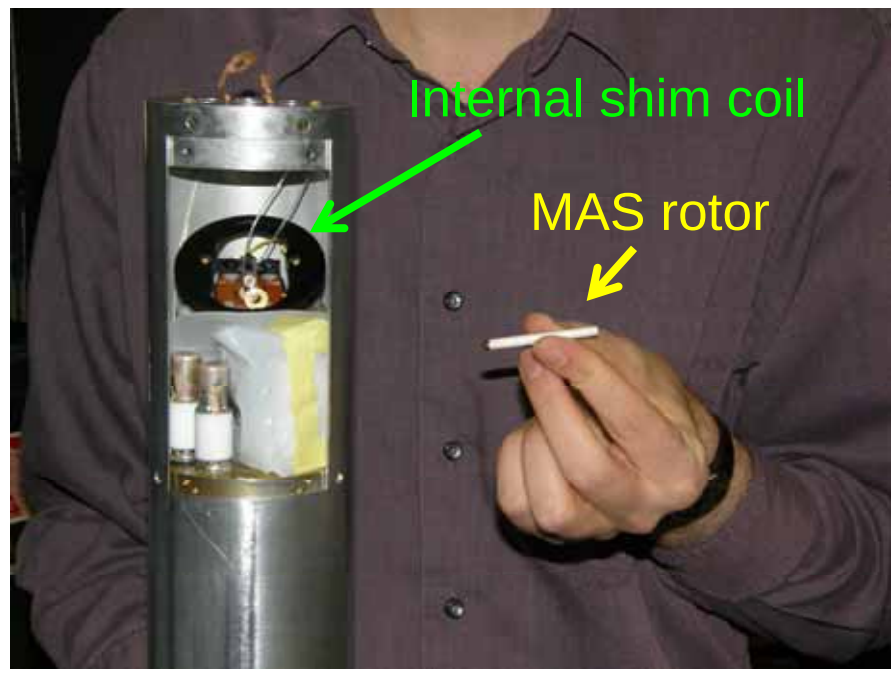
hydrophobic
negatively charged
positively charged
polar

Luca et al., Biochemistry 2007

2. Low-temperature MAS



experiment time ~ $1/T^2 - 1/T^3$



Current Probe Properties

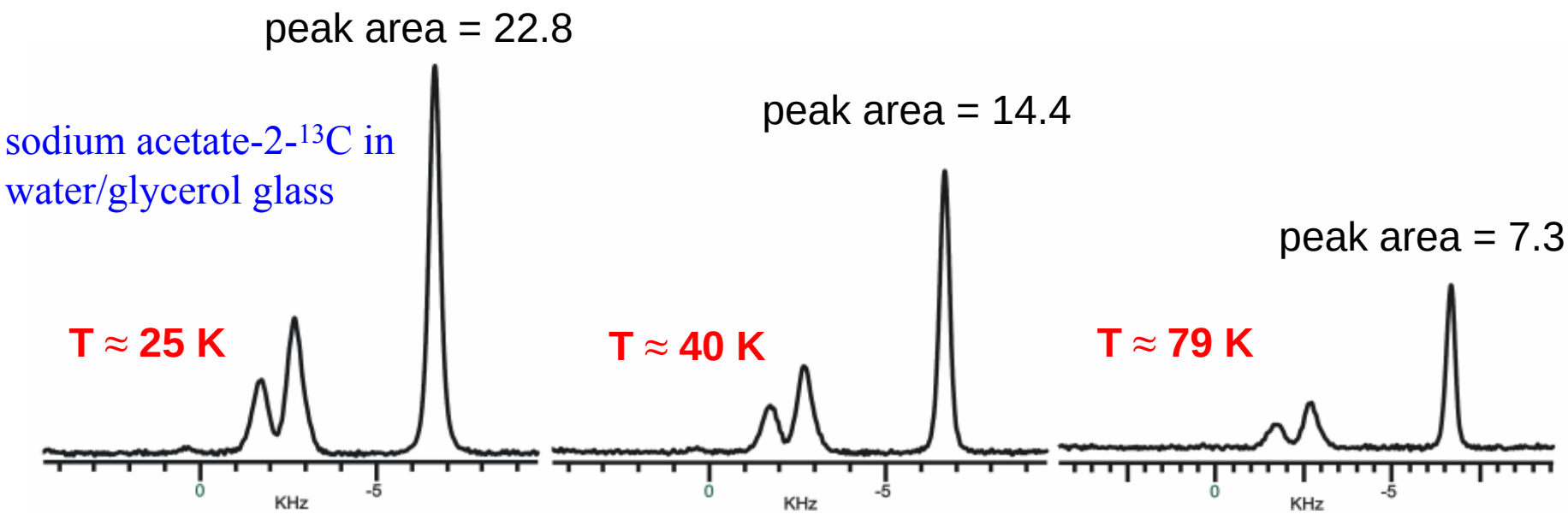
25 K sample temperature at 6.7 kHz spinning and 3 liters/hour liquid He

Sample volume 50ul, 1H decoupling 75 kHz, 100 kHz usable for a few ms.

4 mm outer diameter rotor, based on Varian spinner housing with longer rotor. Liquid He cooling sample, room temperature nitrogen bearing and drive gas. Teflon coated wire used for coil, Teflon enclosure around coil and sample region insulates helium cooled region from nitrogen gas.

Spinning stable (5 Hz) (runs have currently lasted up to ~10 hours.)

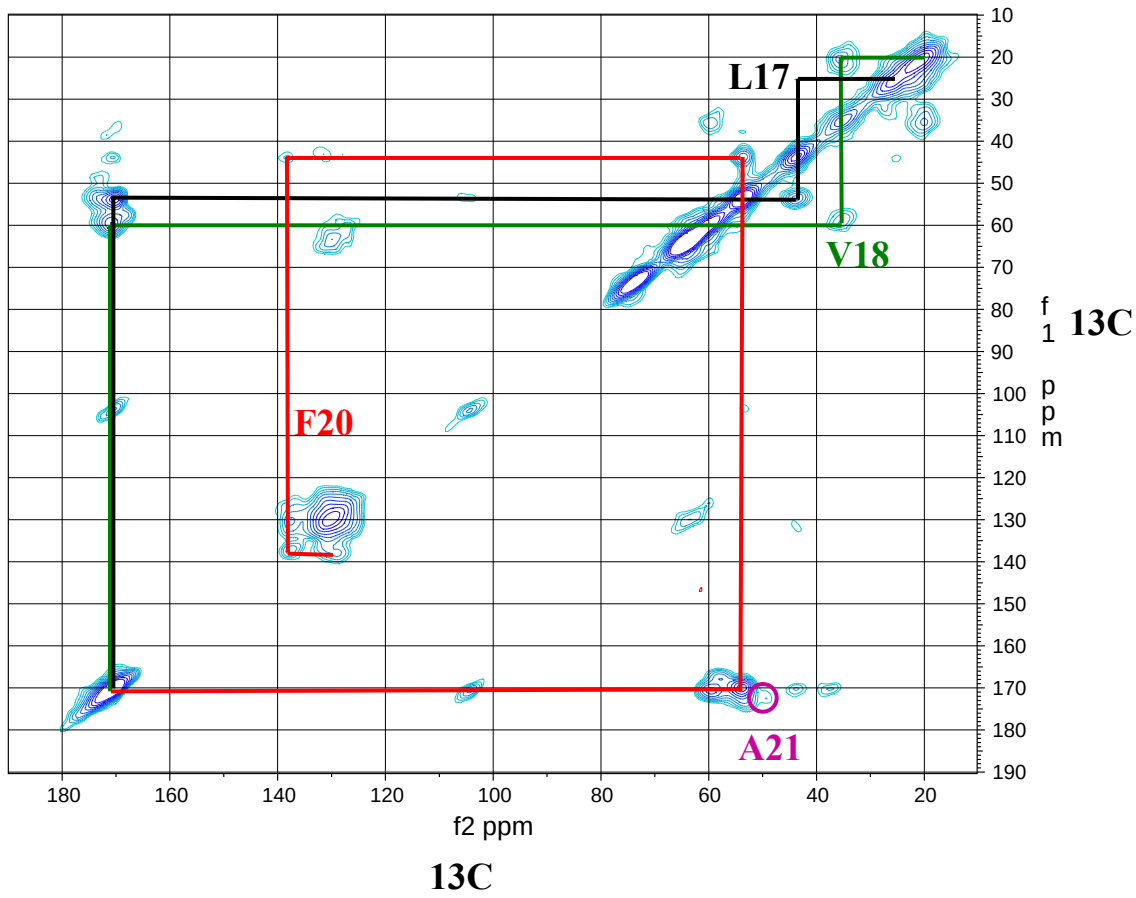
Dysprosium EDTA used to provide fluctuating electron spin to have reasonable 1H relaxation rate at low temperatures.



Magic-angle spinning at 7.00 kHz
Proton decoupling at 105 kHz
Proton $T_1 \approx 6$ s at 25 K, with 200 μM Dy-EDTA
NMR linewidths < 1.4 ppm
Helium consumption ~ 2 liters per hour

Abeta 14-23 amyloid fibrils

2D 13C-13C correlation by RFDR pulse sequence



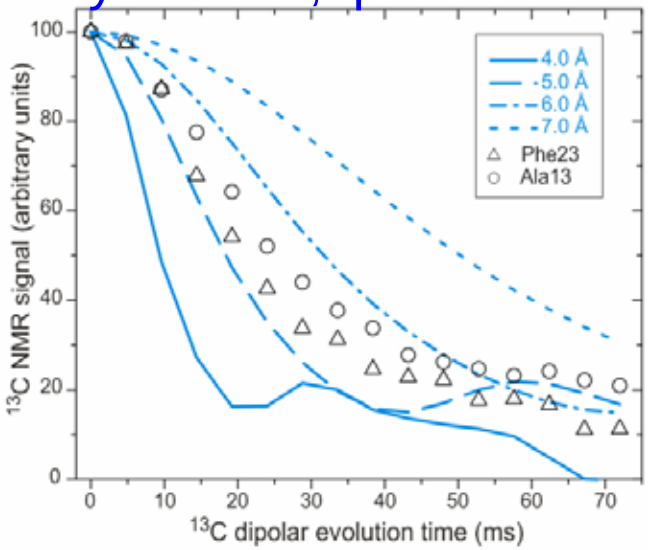
25 K, 6.7 kHz spin, 2080 total scans for 2D,
1H T1 = 4 sec, repeat rate 6 sec,
time required = 3.5 hours

Abeta 14-23
amino acid sequence: HQKLVFFAED
1.7 mg of sample labeled at V18, F20
1.0 mg of sample labeled at L17, A21
hydrated with 10ul of 200uM DyEDTA in
25 mol% glycerol.

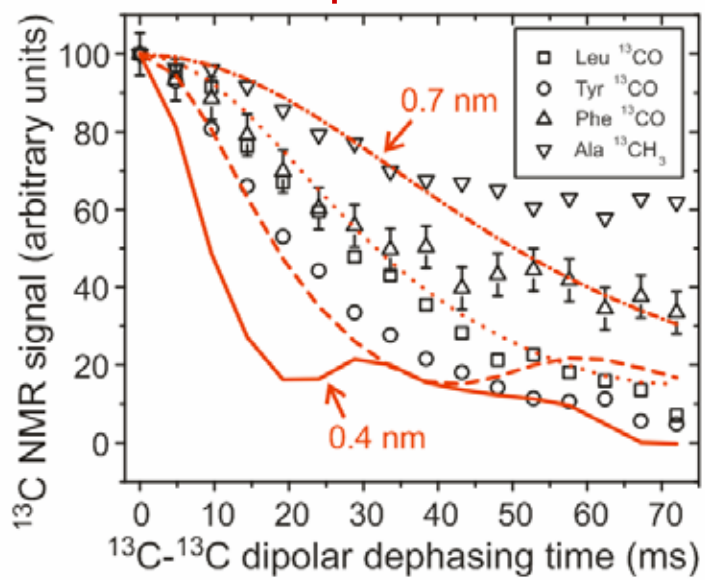
3. Quantitative distance measurements with homonuclear dipolar recoupling

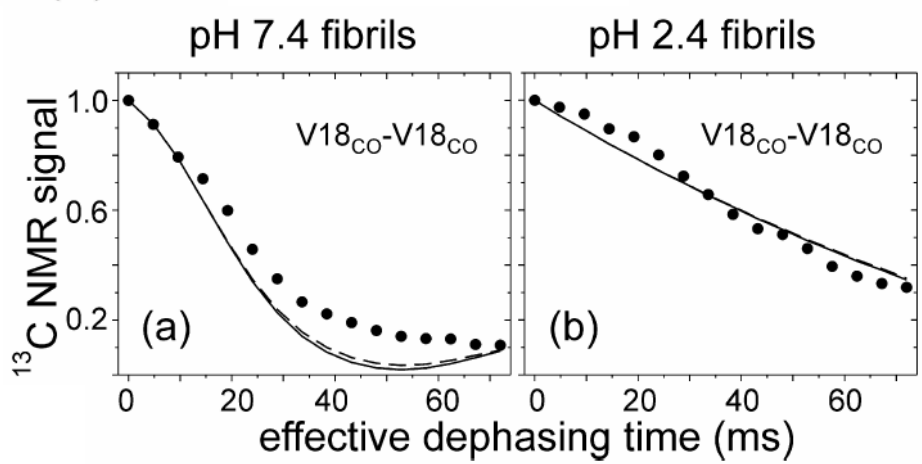
works well for selectively labeled samples:

amylin fibrils, fpRFDR-CT data



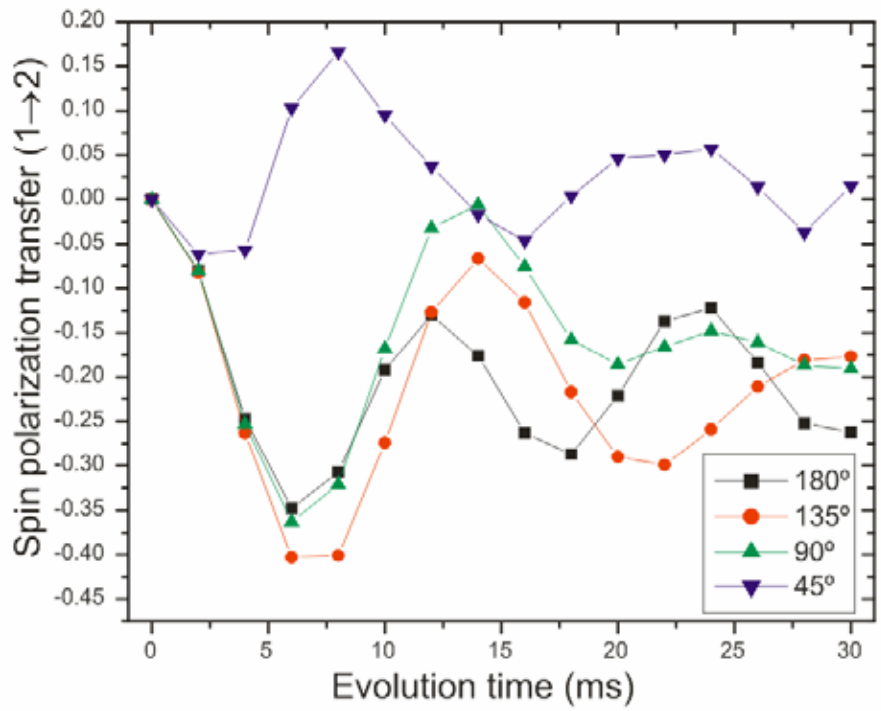
Sup35NM fibrils





A$\beta_{11\text{-}25}$ fibrils

In uniformly or multiply labeled solids, recoupling data depend on the full geometry, not only the pairwise internuclear distances. This is a consequence of the non-commutivity of pairwise dipole-dipole couplings.

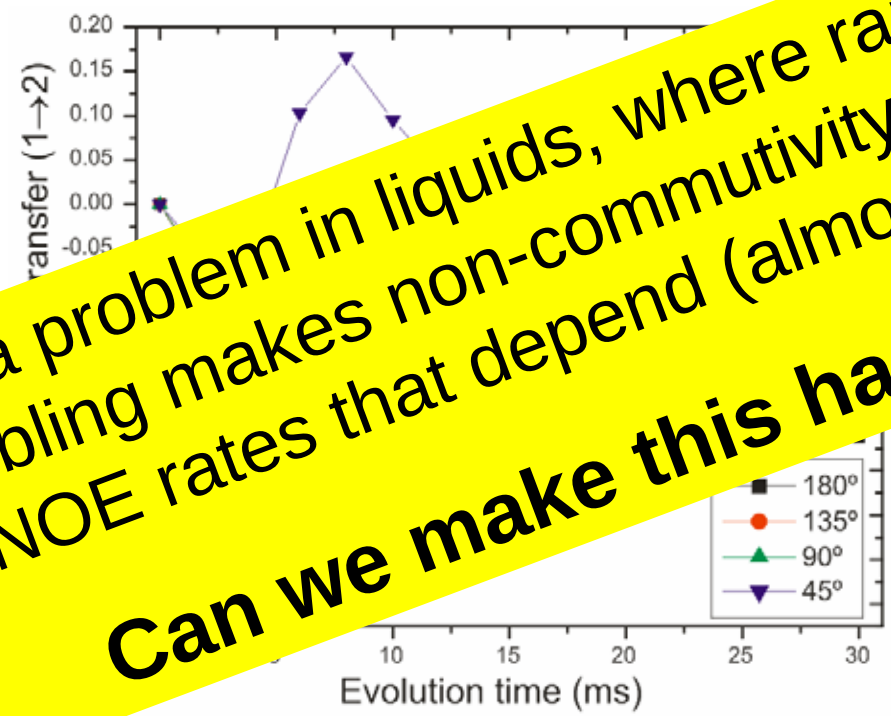


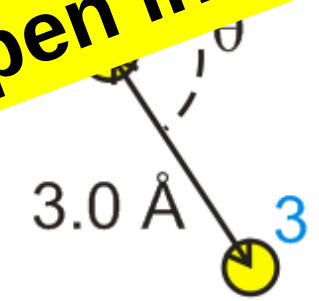
simulations, POST-C7 recoupling, MAS at 8 kHz, ^{13}C NMR at 14.1 T, isotropic shifts = 20 ppm, -20 ppm, 5 ppm

In uniformly or multiply labeled solids, recoupling data depend on the full geometry, not only the pairwise internuclear distances. This is a consequence of the non-commutivity of pairwise dipole-dipole

Not a problem in liquids, where random molecular tumbling makes non-commutivity unimportant, leading to NOE rates that depend (almost) purely on distances.

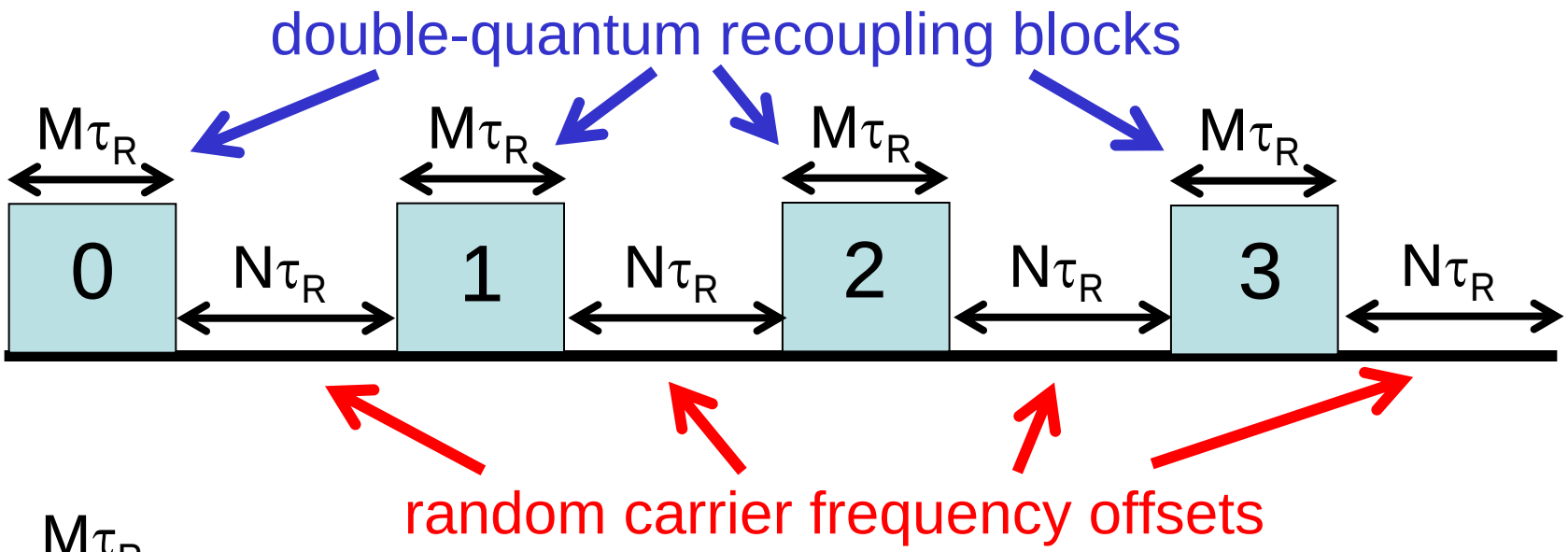
Can we make this happen in solids??

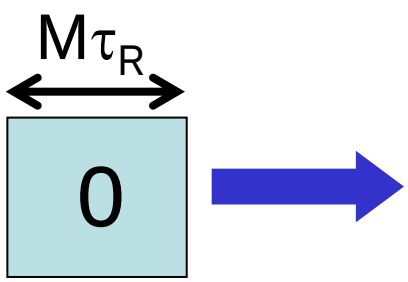




simulations, POST-C7 recoupling, MAS at 8 kHz, ^{13}C NMR at 14.1 T, isotropic shifts = 20 ppm, -20 ppm, 5 ppm

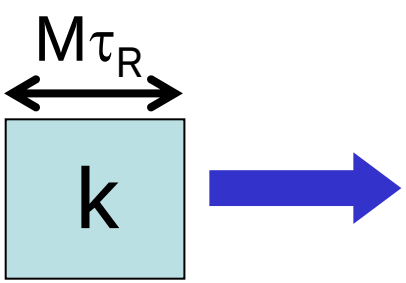
Stochastic homonuclear dipolar recoupling





$$H_{eff}^{(0)} = dI_+S_+ + d^*I\_S\_$$

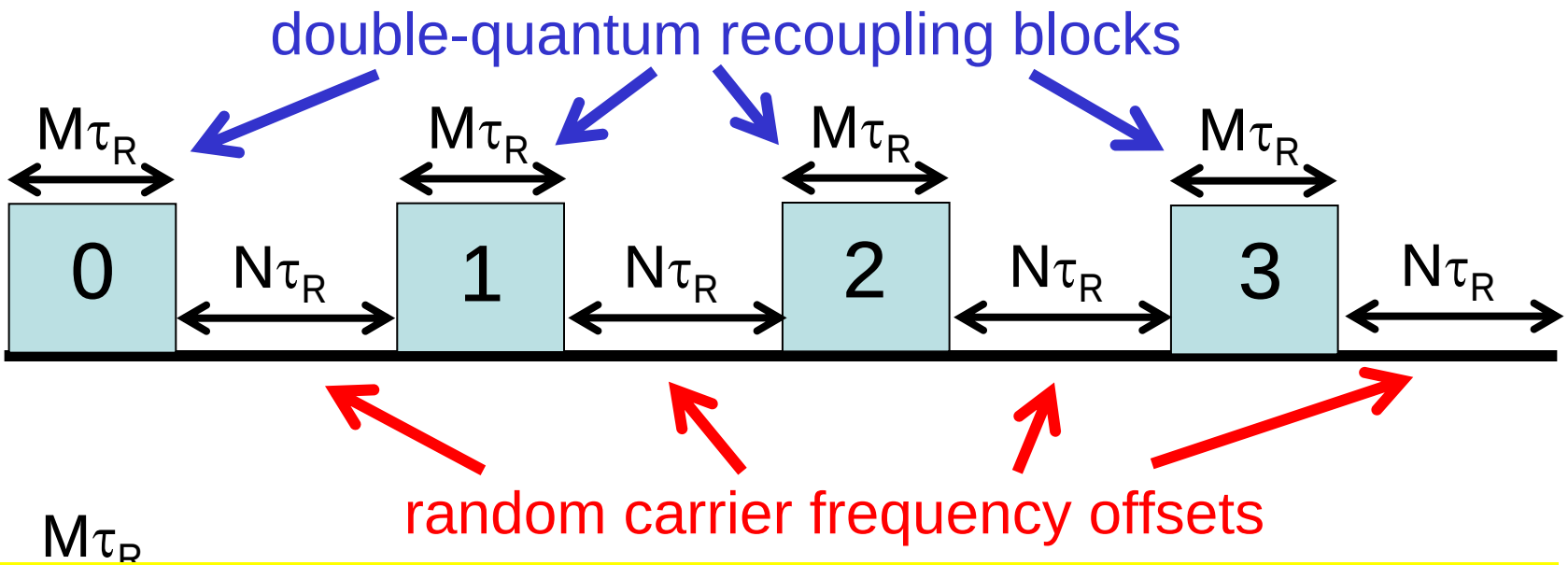
random phase modulation



$$H_{eff}^{(k)} = dI_+S_+e^{-i\phi_{IS}(k)} + d^*I\_S\_e^{i\phi_{IS}(k)}$$

where $\phi_{IS}(k) = \sum_{j=1}^{k} N[\Delta\omega_I(j) + \Delta\omega_S(j)]\tau_R$

Stochastic homonuclear dipolar recoupling



$M\tau_R$

All pairwise couplings average to zero, but in a stochastic and (almost) uncorrelated way.

Coherent polarization transfers become incoherent, described by rates that are proportional to $1/R^6$ (NOE-like).

e

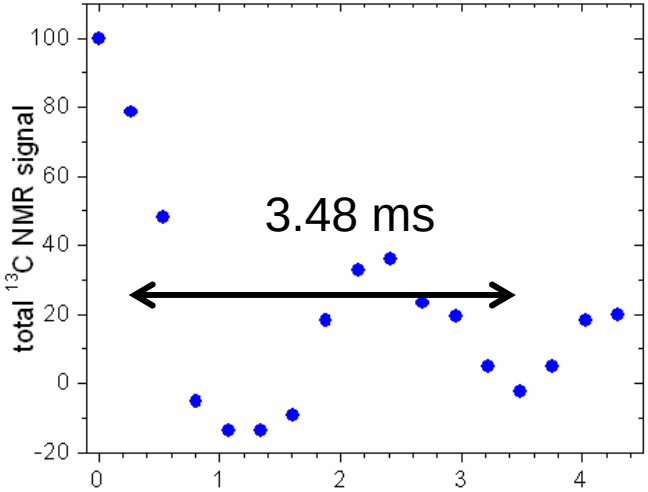
$_S(k)$

where $\phi_{IS}(k) = \sum_{j=1} N[\Delta\omega_I(j) + \Delta\omega_S(j)]\tau_R$

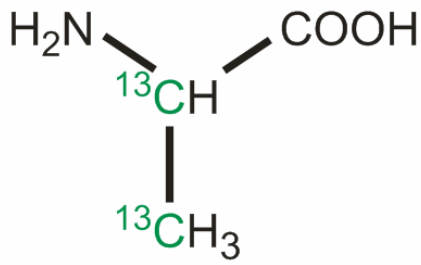
Experimental demonstrations of stochastic recoupling, two-spin systems

coherent

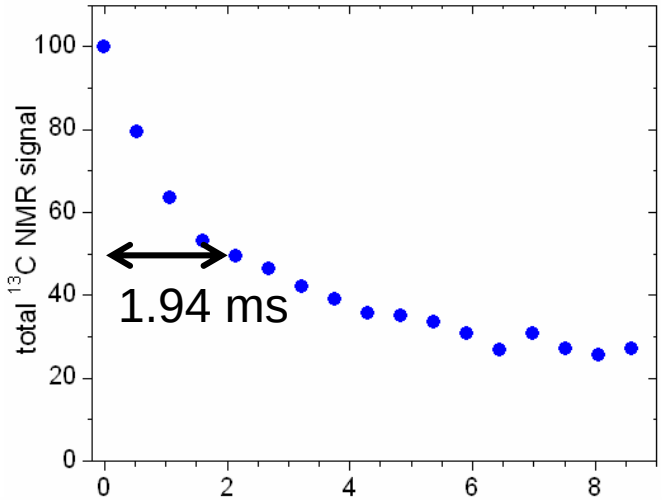
stochastic

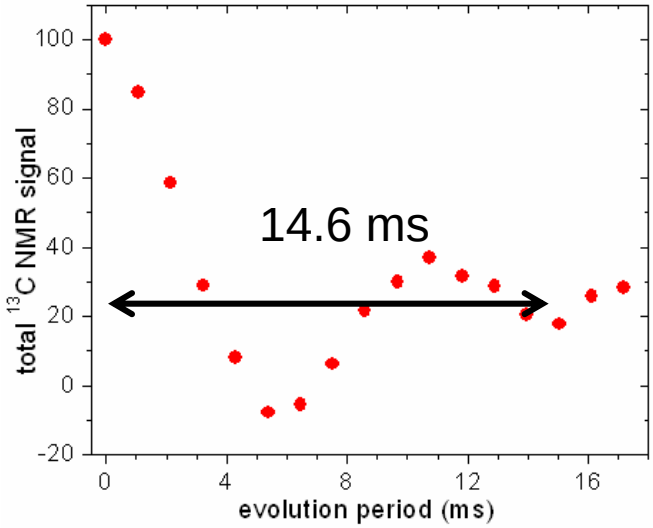


L-alanine

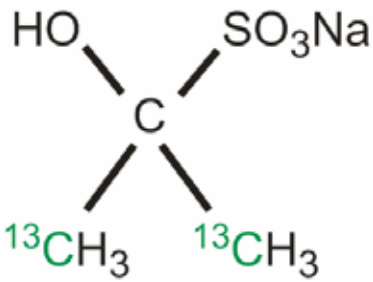


R = 1.54 Å

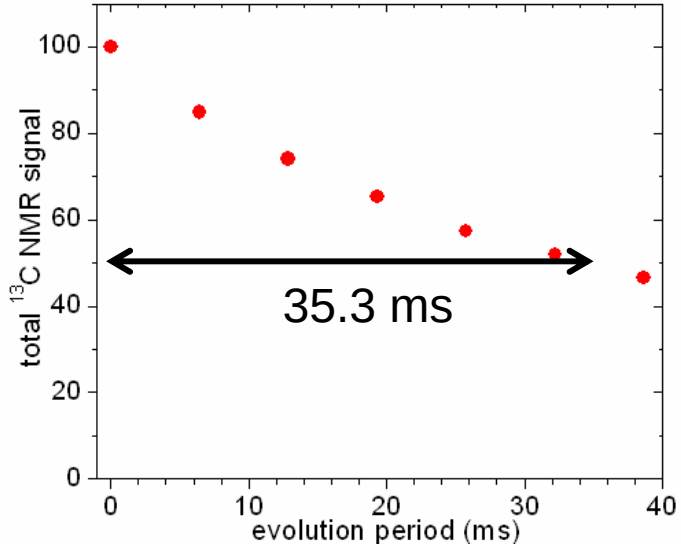




bisulfite adduct of acetone



R = 2.51 Å



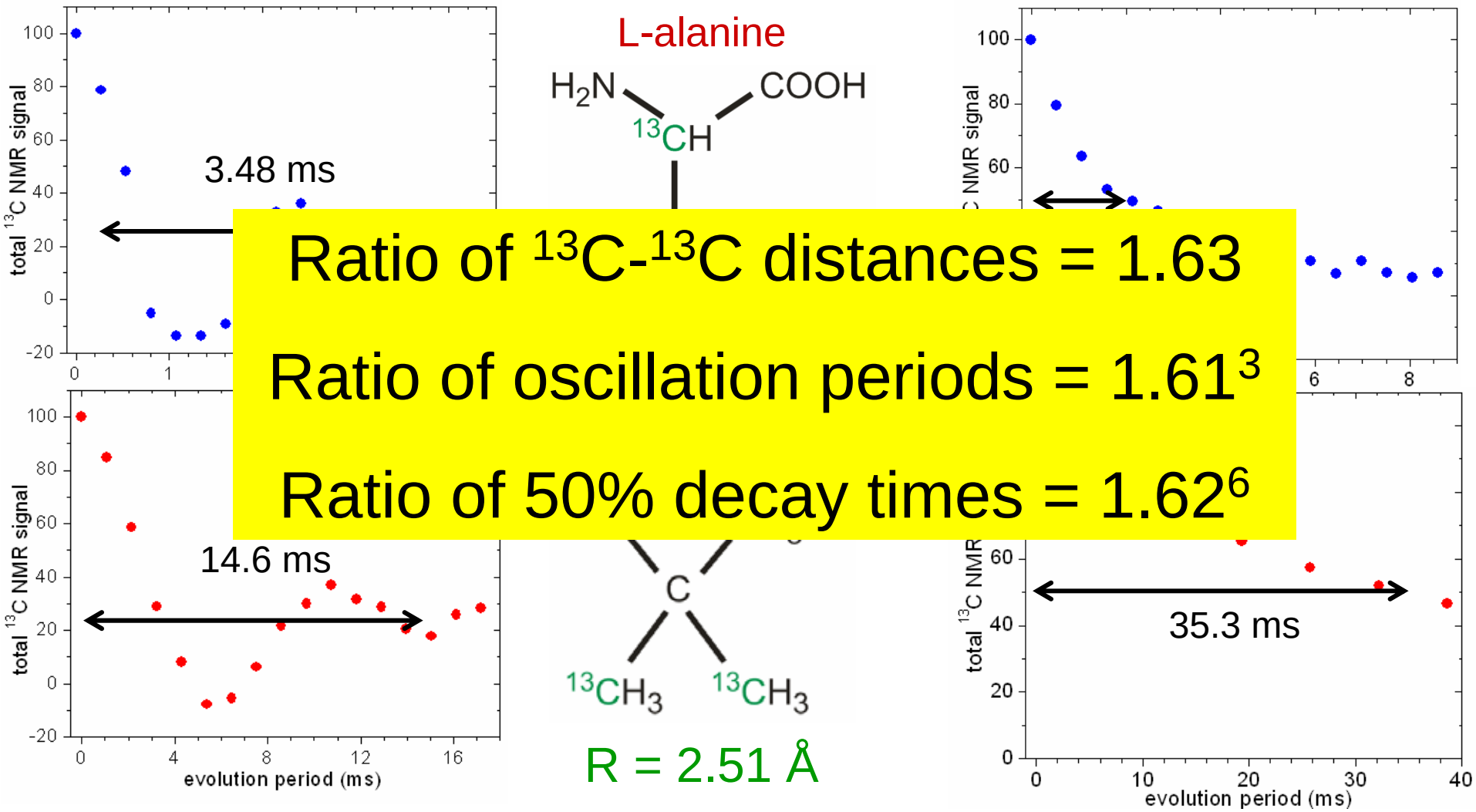
(POST-C7 recoupling, 7.45 kHz MAS, 9.4 T)

Experimental demonstrations of stochastic recoupling, two-spin systems

coherent

stochastic

L-alanine



R = 2.51 Å

Ratio of ^{13}C-^{13}C distances = 1.63

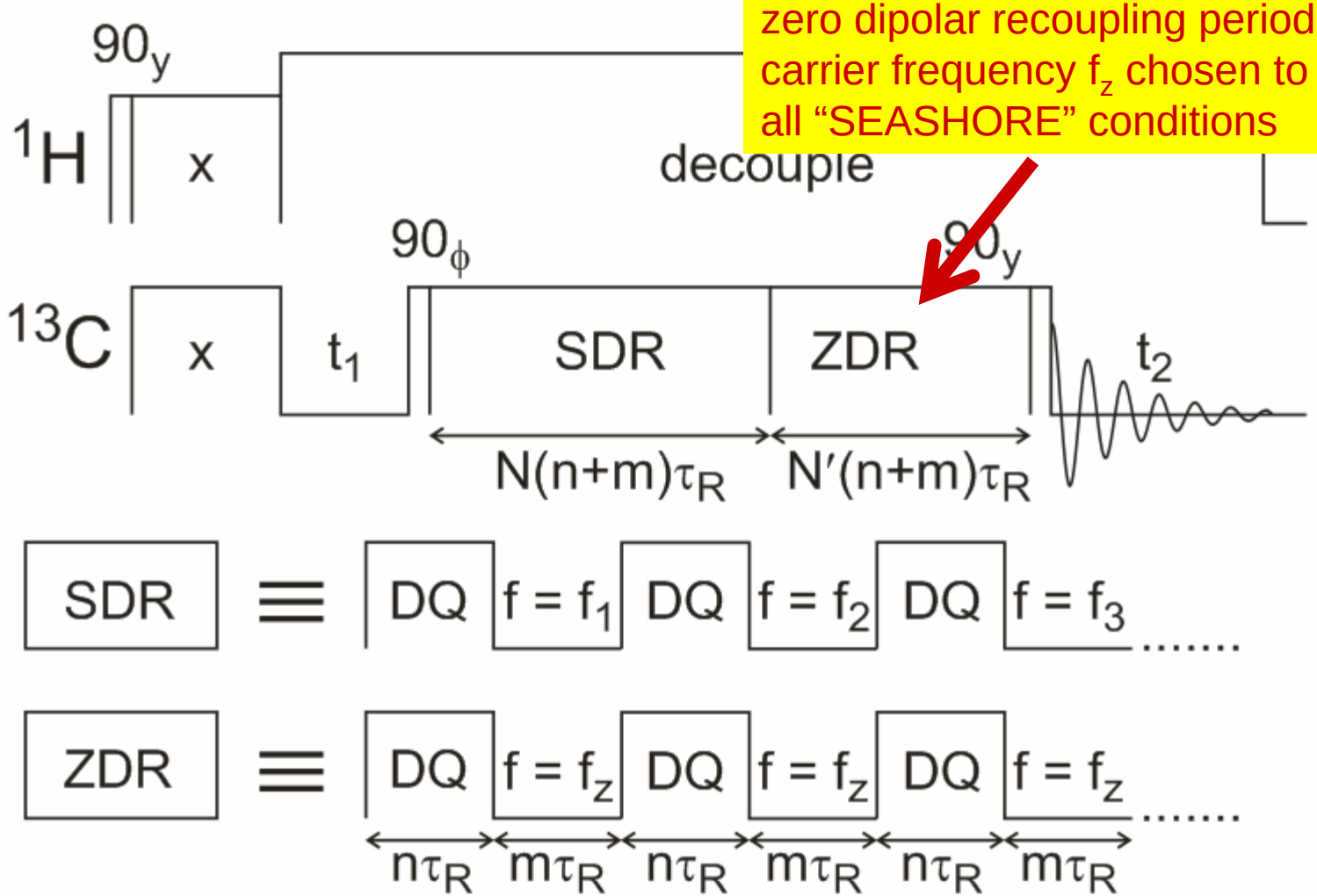
Ratio of oscillation periods = 1.61^3

Ratio of 50% decay times = 1.62^6

(POST-C7 recoupling, 7.45 kHz MAS, 9.4 T)

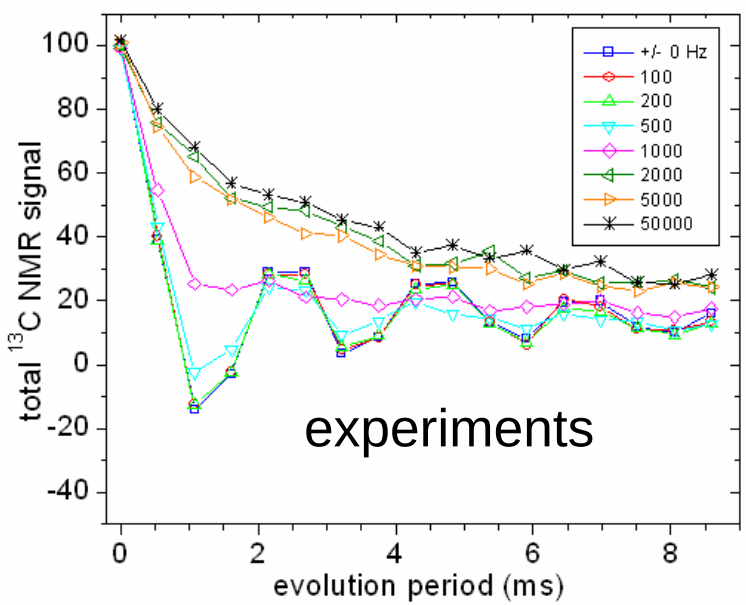
“Constant-time” stochastic dipolar recoupling

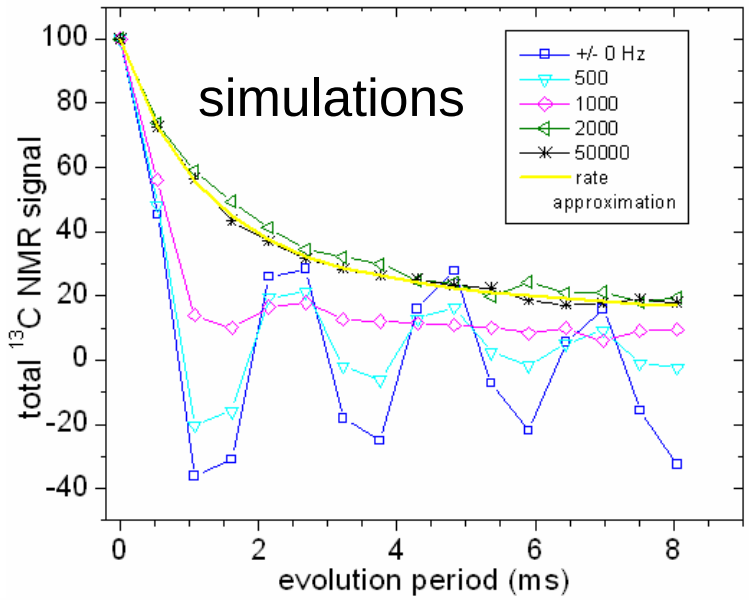
zero dipolar recoupling period: carrier frequency f_z chosen to avoid all “SEASHORE” conditions

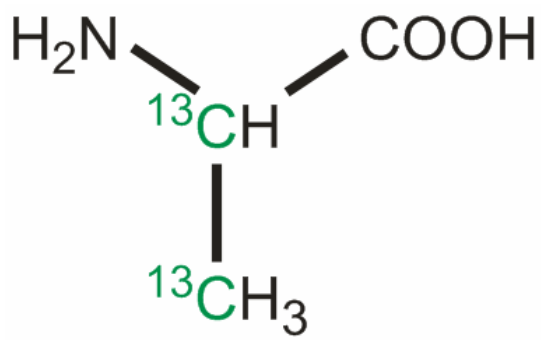


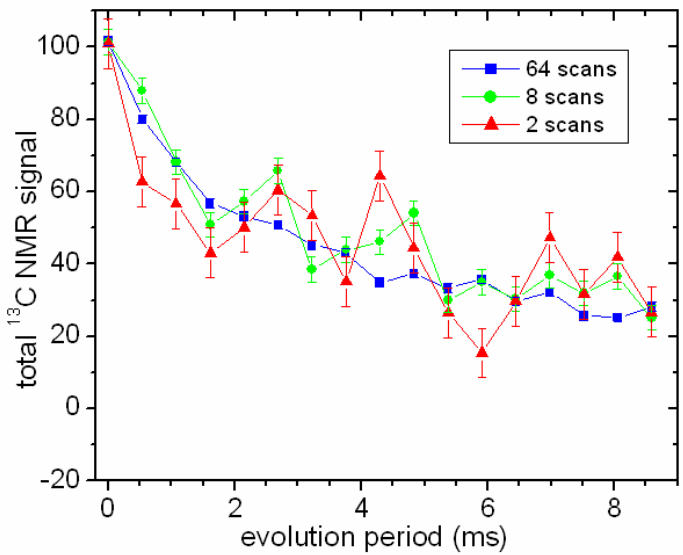
Experimental demonstrations of stochastic recoupling, two-spin systems

dependence on the range of carrier jumps between recoupling blocks



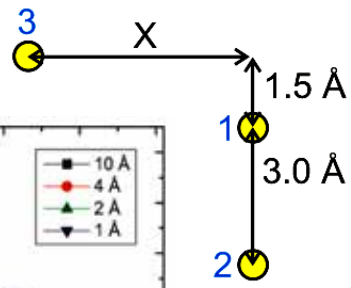


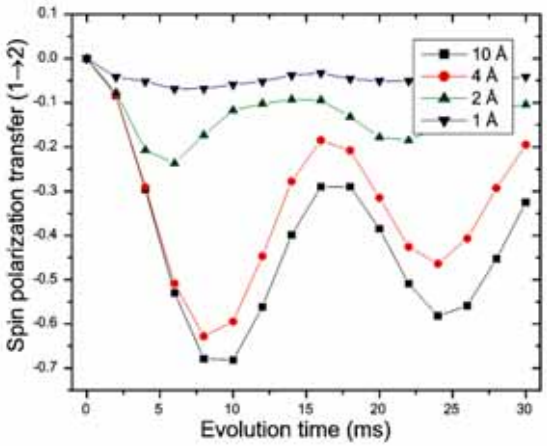


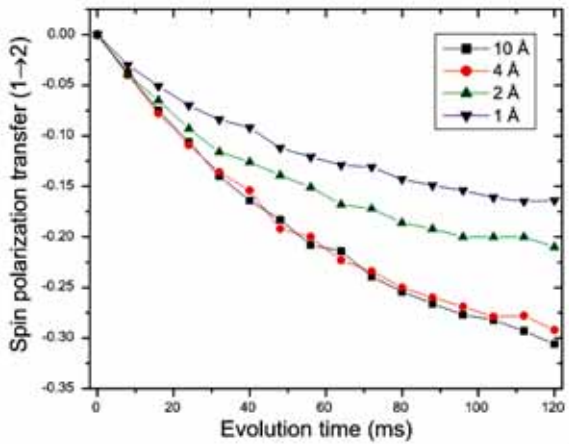


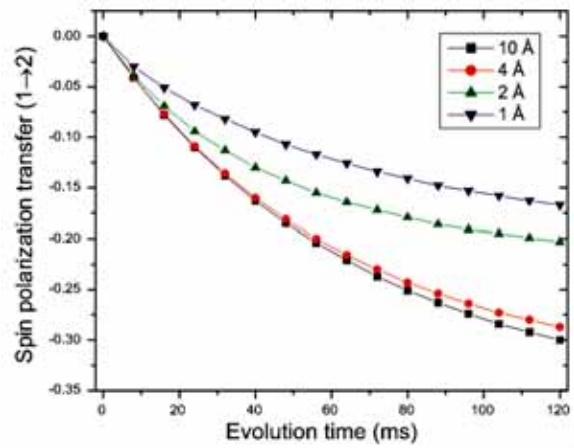
dependence on the number of scans per point

Simulations for three-spin systems

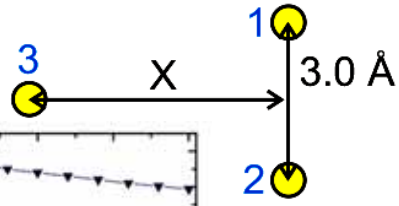


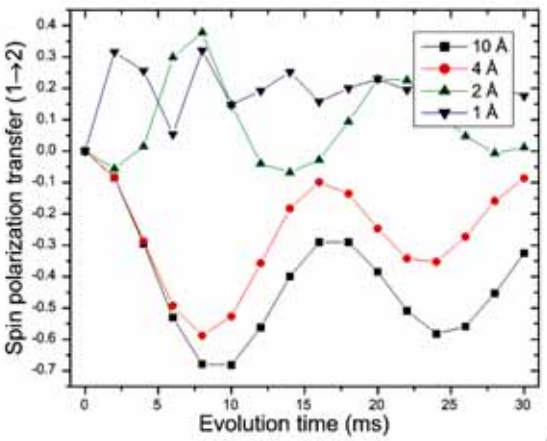


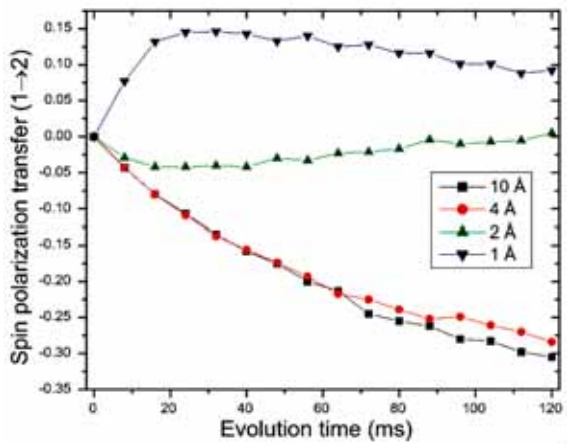


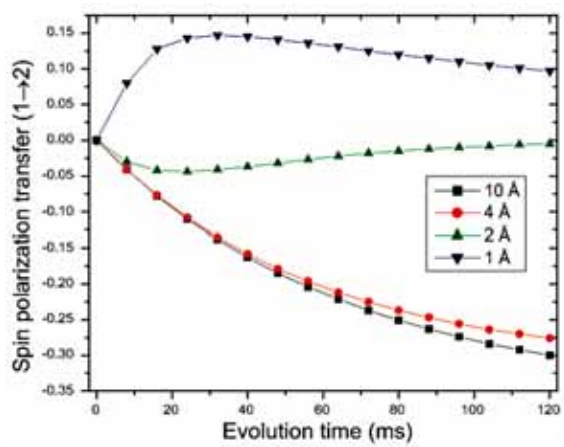


coherent stochastic rate approximation

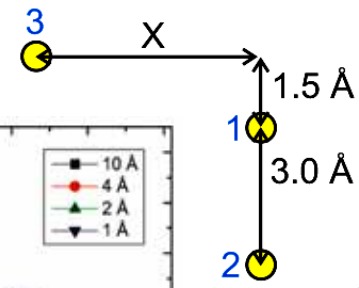


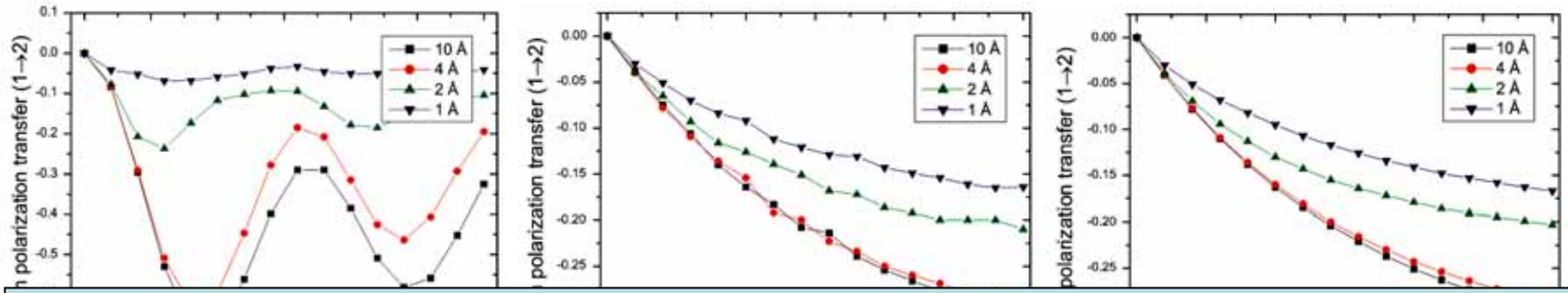






Simulations for three-spin systems





Rate approximation for spin polarization transfers after N recoupling blocks:

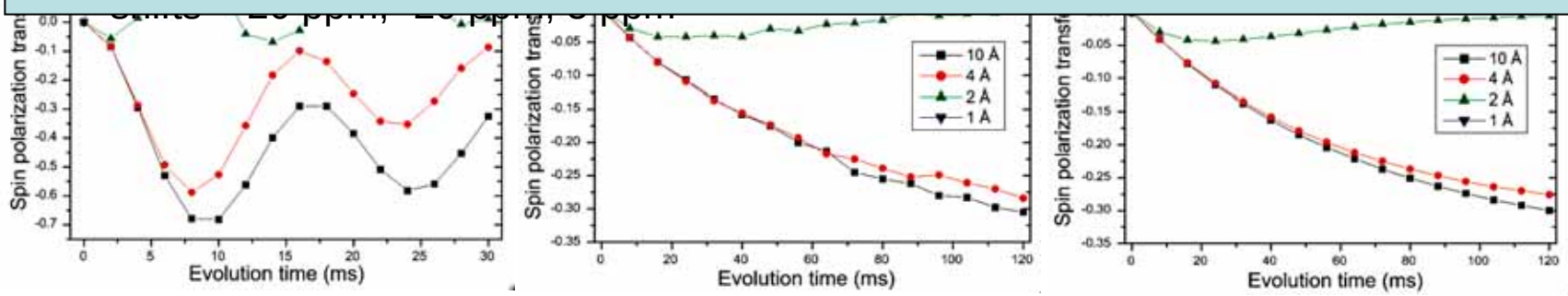
$$\mathbf{p}(\mathrm{N}) = \mathbf{W}^{\mathrm{N}} \bullet \mathbf{p}(0)$$

where **p**(N) is an n-dimensional polarization vector

and **W** is an n X n "rate matrix" with elements

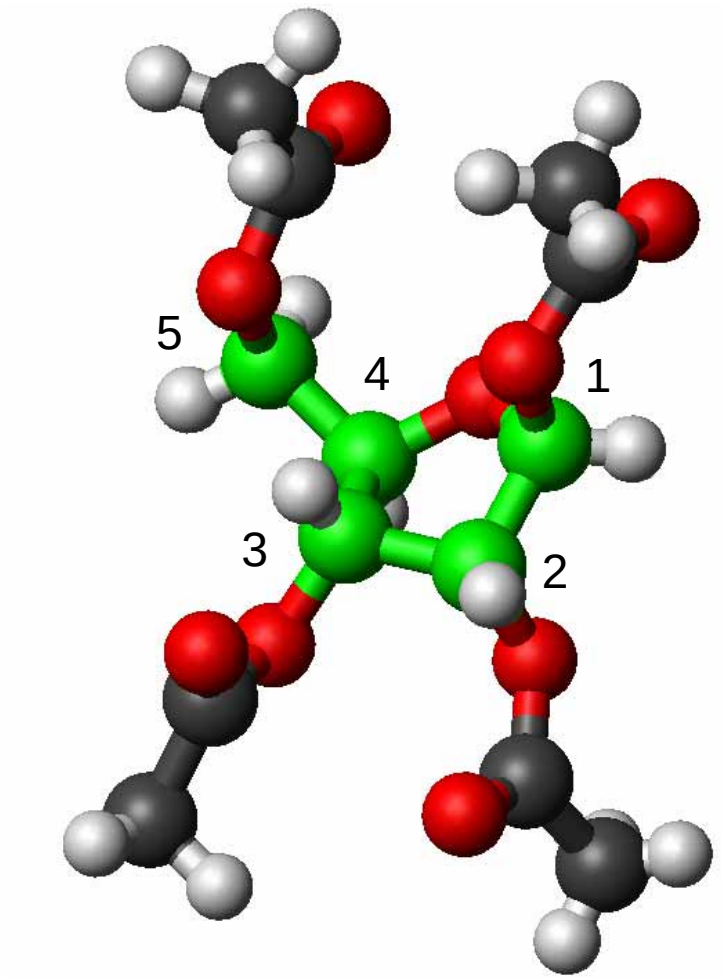
$$W_{ij} = -\sin^2 \frac{1}{2} n d_{ij} \tau_R$$

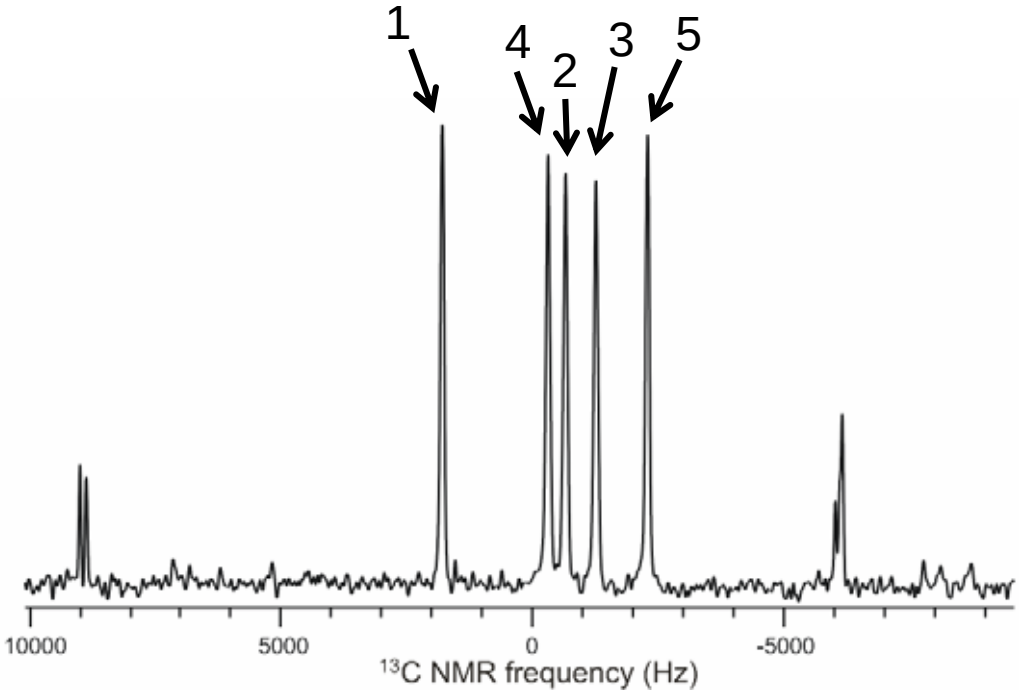
$$W_{jj} = 1 + \sum_{i \neq j} W_{ij}$$



Stochastic recoupling, 5-spin system

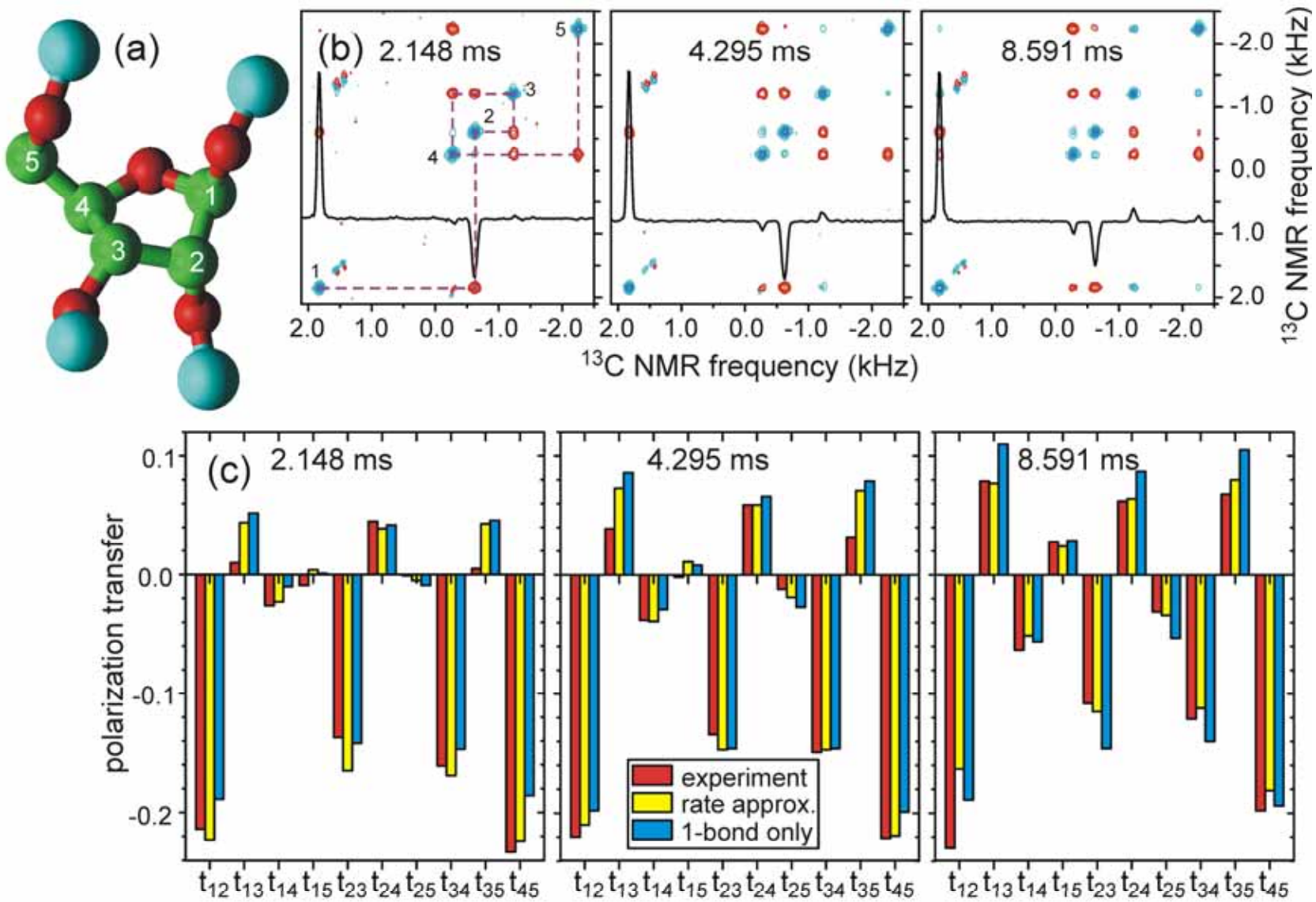
β-D-ribofuranose tetraacetate (rfta)





(9% $^{13}C_5$-rfta, diluted in unlabeled rfta)

Stochastic recoupling, 5-spin system



see Phys. Rev. Lett. ***99****, 187601 (2007)*

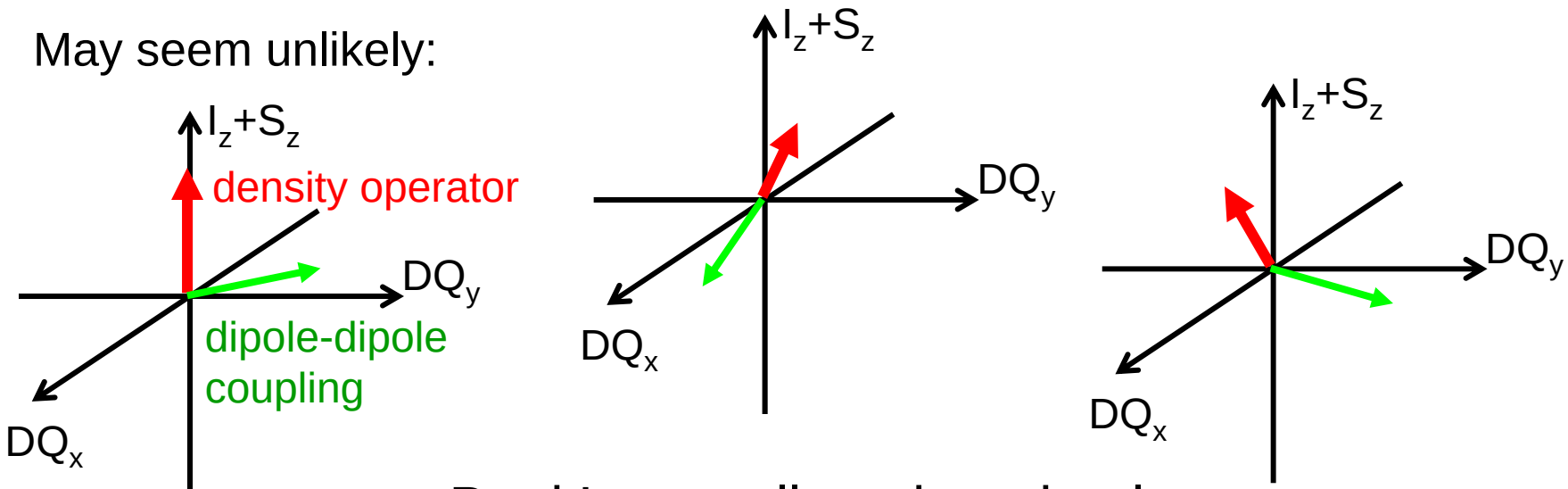
Analytical theory of SDR for two-spin systems

In the fully coherent limit ($f_{max} = 0$), have oscillatory spin dynamics.

In the fully incoherent limit ($f_{max}\tau_R >> 1$), have exponential approach to equilibrium.

Is there an analytical expression that describes the SDR data for arbitrary f_{max}?

May seem unlikely:



But it's actually rather simple …

During the SDR pulse sequence, the effective spin Hamiltonian alternates between

$$\tilde{H}_D^{(0)} = \frac{\gamma^2\hbar}{R^3}(gI_{+1}I_{+2} + g^*I_{-1}I_{-2})$$

dipole-dipole coupling

isotropic chemical shifts

and $$\tilde{H}_{CS}^{(0)}(k) = \frac{1}{2}(\delta_1 + \delta_2 + 2f_k)(I_{z1} + I_{z2}) + \frac{1}{2}(\delta_1 - \delta_2)(I_{z1} - I_{z2})$$

With $\delta_1+\delta_2 \equiv 0$ at $f_k = 0$, the net evolution operator after N blocks can then be written as

$$U(N) = \exp[-iNm(\delta_1 - \delta_2)\tau_R J_\Delta]\exp(iJ_\Sigma\alpha)$$

x rotation

$$\times\left\{\prod_{k=1}^{N}\{\exp(-i2mf_k\tau_R J_\Sigma)\exp(-ind_{12}\tau_R J_x)\}\right\}\exp(-iJ_\Sigma\alpha)$$

random z rotation

$$J_\Sigma \equiv \frac{1}{2}(I_{z1} + I_{z2}) \qquad J_\Delta \equiv \frac{1}{2}(I_{z1} - I_{z2})$$

where the "fictitious spin-1/2 operators" are defined by

$$J_x \equiv \frac{1}{2}(I_{+1}I_{+2} + I_{-1}I_{-2}) \qquad J_y \equiv -\frac{i}{2}(I_{+1}I_{+2} - I_{-1}I_{-2})$$

and where $$g = |g|e^{i\alpha}$$

$$d_{12} \equiv \frac{2\gamma^2\hbar}{R^3}|g|$$

Assume that spins 1 and 2 are initially polarized along z:

$$\rho(0) = p_1(0)I_{z1} + p_2(0)I_{z2} = p_\Delta(0)J_\Delta + p_\Sigma(0)J_\Sigma$$

The density operator after N blocks must then have the form

$$\rho(N) = p_\Delta(0)J_\Delta + p_\Sigma(0)[u(N)J_x + v(N)J_y + w(N)J_\Sigma]$$

The quantity of interest, which determines the SDR NMR signals, is:

$$\langle w(N)\rangle = \left\langle (0,0,1)\cdot\prod_{k=1}^{N} R_\Sigma(\theta_\Sigma(k))R_x(\theta_x)\cdot\begin{pmatrix}0\\0\\1\end{pmatrix}\right\rangle$$

$$= (0,0,1)\cdot\prod_{k=1}^{N}\langle R_\Sigma(\theta_\Sigma(k))\rangle R_x(\theta_x)\cdot\begin{pmatrix}0\\0\\1\end{pmatrix}$$

This can be calculated explicitly, because the random "Σ" rotations are all drawn from the same distribution.

Notes: --Does not assume that the two spins are spin-1/2 nuclei.

--This is a "Liouville" description. Equivalent "Schrodinger" description seems intractable.

The rotation matrices are:

$$R_x(\theta_x) = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\theta_x & -\sin\theta_x \\ 0 & \sin\theta_x & \cos\theta_x \end{pmatrix}$$

$$\langle R_\Sigma(\theta_\Sigma)\rangle = \begin{pmatrix} \langle\cos\theta_\Sigma\rangle & -\langle\sin\theta_\Sigma\rangle & 0 \\ \langle\sin\theta_\Sigma\rangle & \langle\cos\theta_\Sigma\rangle & 0 \\ 0 & 0 & 1 \end{pmatrix} = \begin{pmatrix} \frac{1}{\theta_{max}}\sin\theta_{max} & 0 & 0 \\ 0 & \frac{1}{\theta_{max}}\sin\theta_{max} & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

which implies that

$$\langle w(N)\rangle = (0,0,1)\cdot\begin{pmatrix} \frac{1}{\theta_{max}}\sin\theta_{max} & 0 & 0 \\ 0 & \frac{1}{\theta_{max}}\sin\theta_{max}\cos\theta_x & -\frac{1}{\theta_{max}}\sin\theta_{max}\sin\theta_x \\ 0 & \sin\theta_x & \cos\theta_x \end{pmatrix}^N \cdot \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix}$$

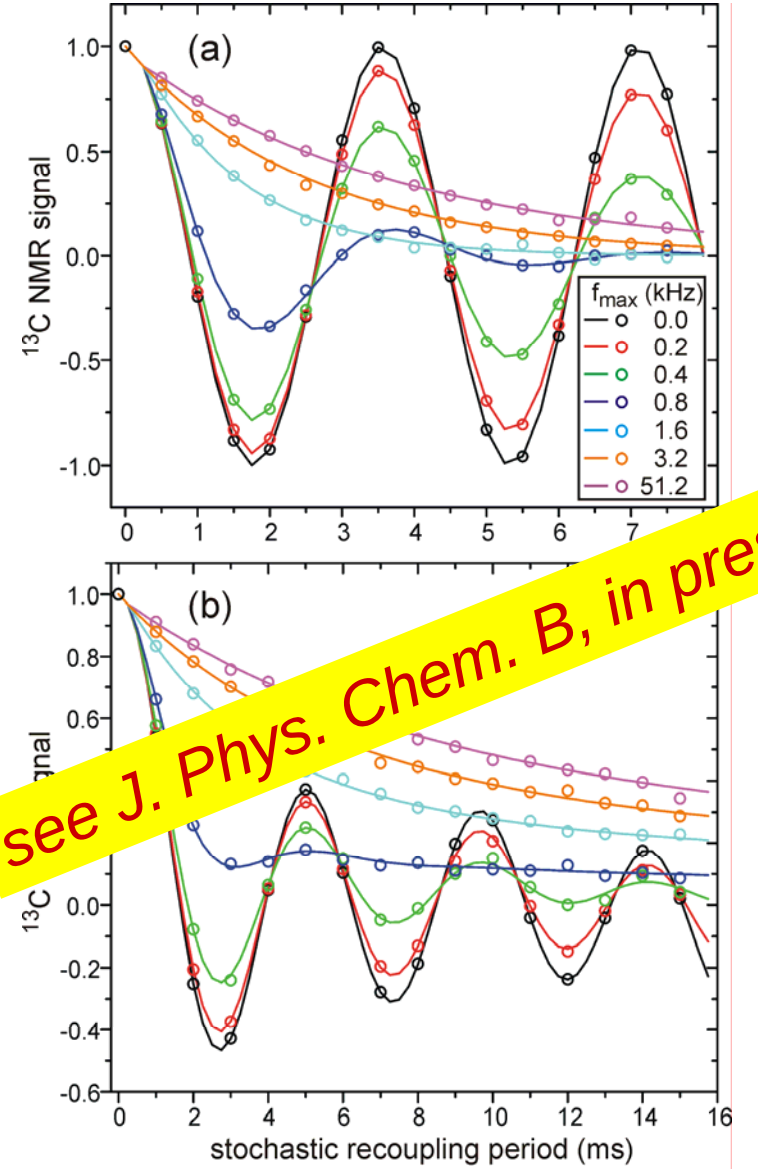
By diagonalizing the non-Hermitian 2 X 2 matrix (with complex eigenvalues and non-orthogonal eigenvectors):

final result

$$\langle w(N)\rangle = \frac{\varepsilon_+{}^N(\cos\theta_x - \varepsilon_-) - \varepsilon_-{}^N(\cos\theta_x - \varepsilon_+)}{\varepsilon_+ - \varepsilon_-}$$

$$\varepsilon_\pm = \tfrac{1}{2}\left(1 + \frac{\sin\theta_{max}}{\theta_{max}}\right)\cos\theta_x \pm \tfrac{1}{2}\sqrt{\left(1 + \frac{\sin\theta_{max}}{\theta_{max}}\right)^2\cos^2\theta_x - 4\frac{\sin\theta_{max}}{\theta_{max}}}$$

Comparison of exact (lines) and numerical (circles) calculations

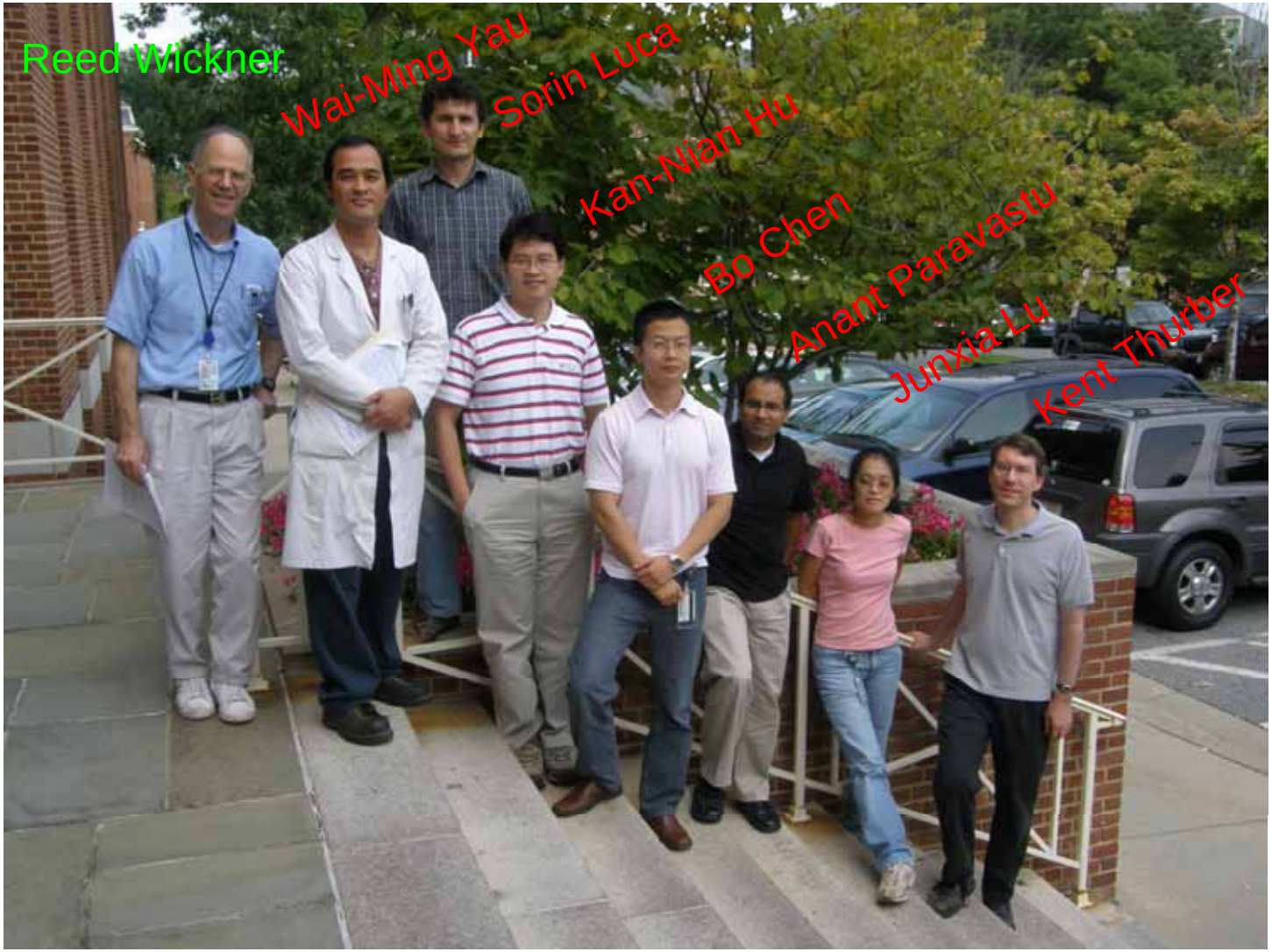


^{13}C spin pairs, 3.0 Å distance, g = ½, τ_R = 125 μs, n = 2, and m = 1.

^{13}C spin pairs, 2.0 Å internuclear distance, POST-C7 sequence, τ_R = 125 μs, n = 2, and m = 1. Exact calculations use orientation-dependent g values extracted from the numerically determined propagator for DQ periods.

see J. Phys. Chem. B, in press (Attila Szabo festschrift)

National Institute of Diabetes & Digestive & Kidney Diseases



NATIONAL INSTITUTES OF HEALTH

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