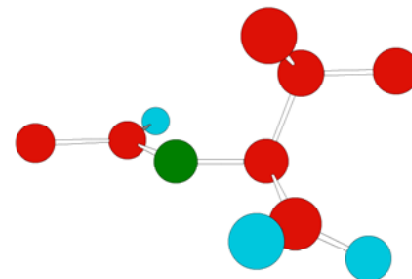
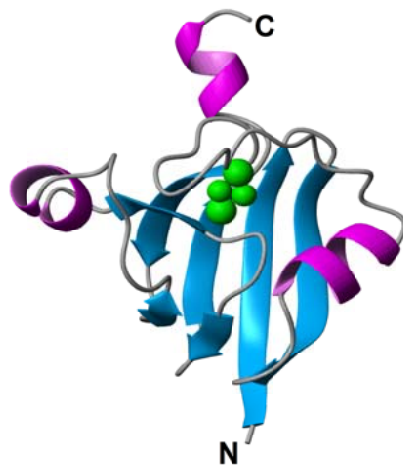
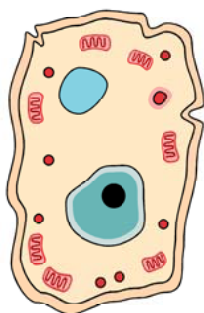
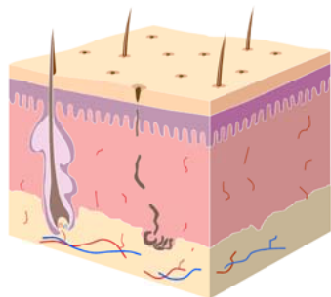
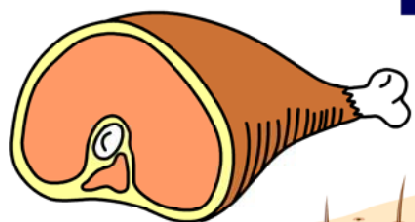


# 固体NMRによる構造決定

## オンGSTロームからミクロンまで

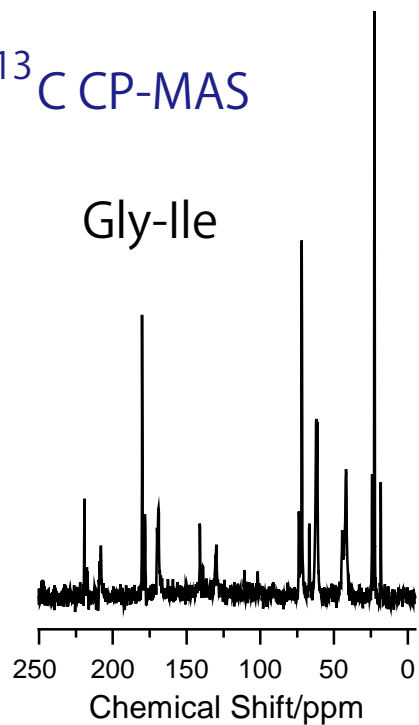
京大・理 竹腰



# いろいろなスペクトル

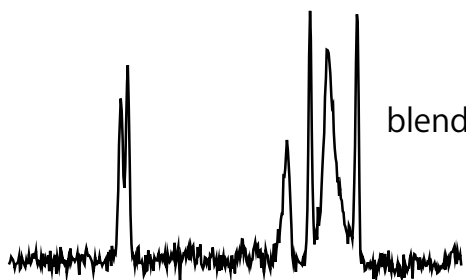
$^{13}\text{C}$  CP-MAS

Gly-Ile

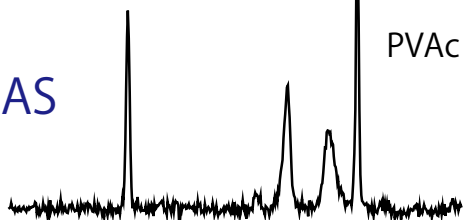


$^{13}\text{C}$  CP-MAS

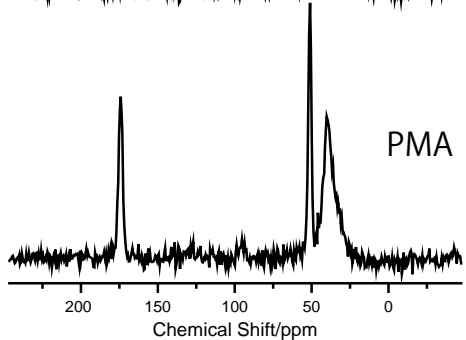
blend



PVAc



PMA

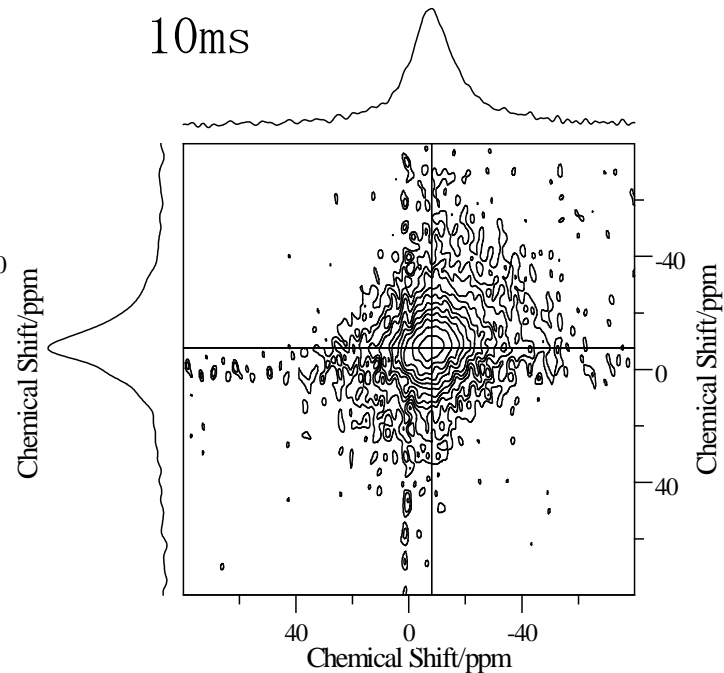
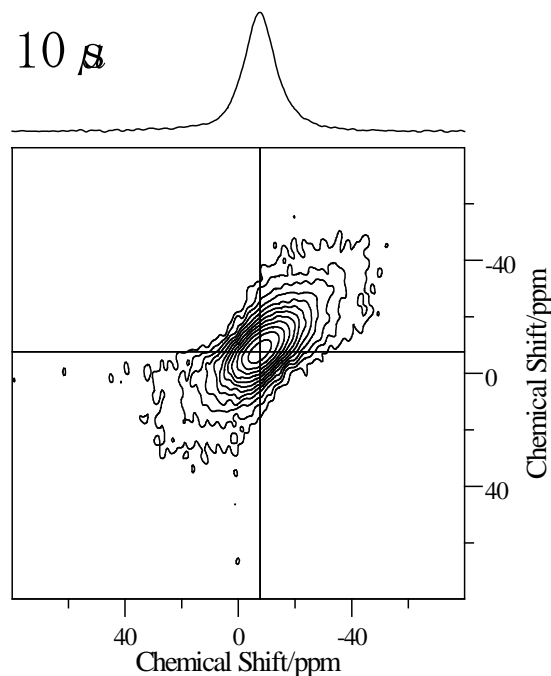


$^7\text{Li}$  2D exchange NMR

Li doped PAS

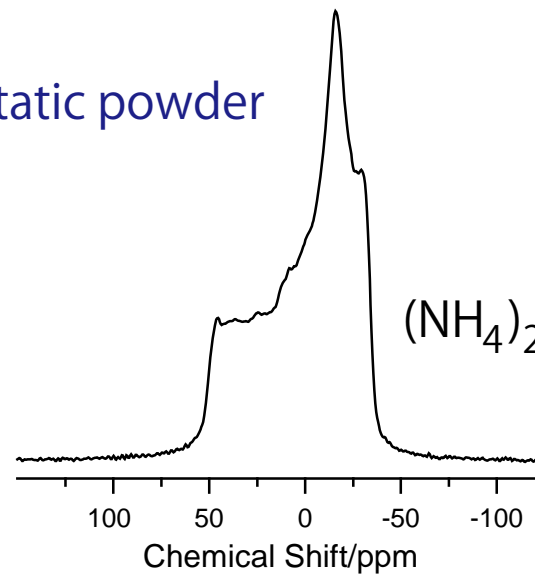
10  $\mu$ s

10ms

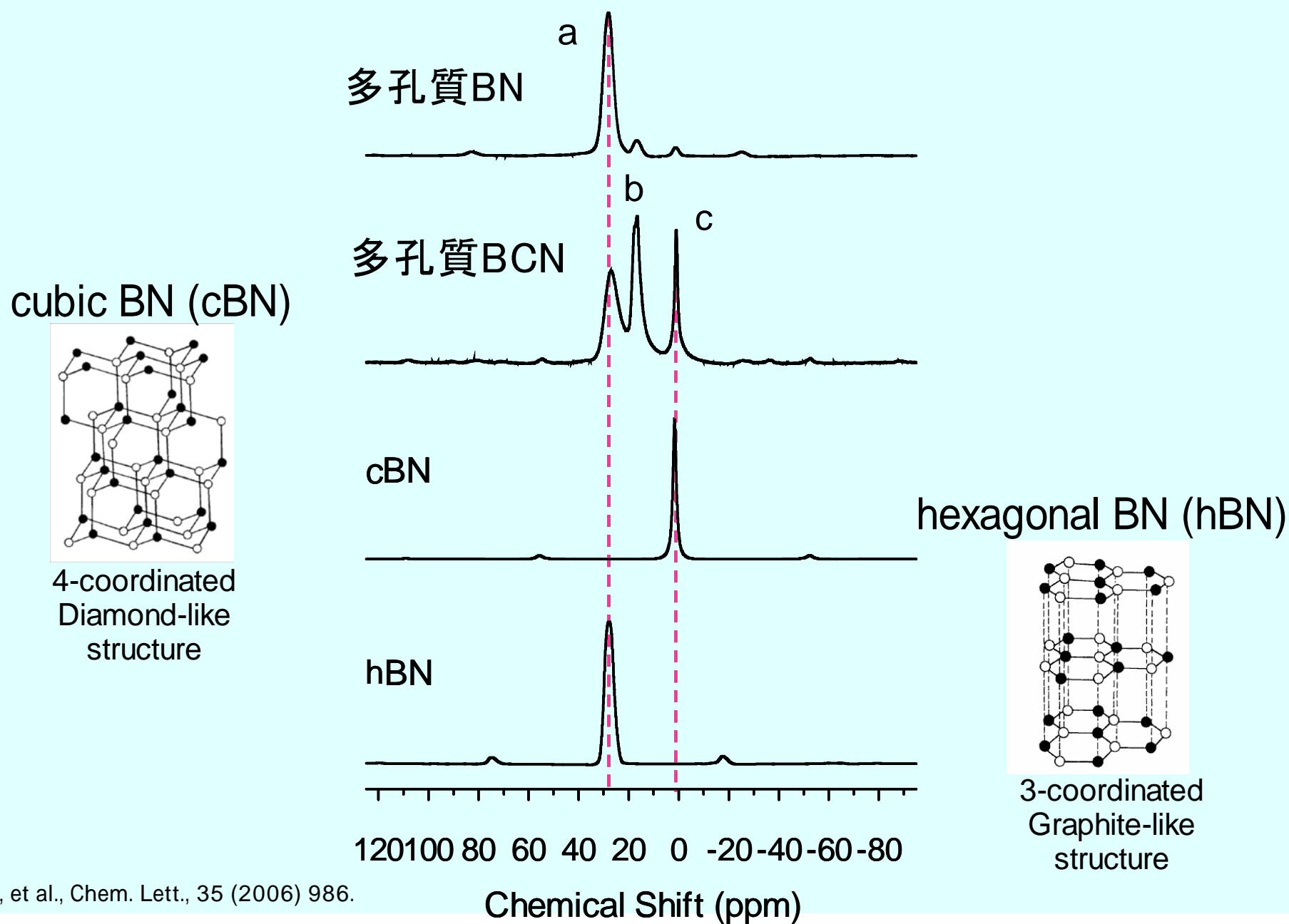


$^{31}\text{P}$  static powder

$(\text{NH}_4)_2\text{HPO}_2$



# $^{11}\text{B}$ MAS spectra of amorphous B-N compounds



# Solid-NMR : Why?

Crystal : OK  
Powder : OK  
Fiber : OK  
Amorphous : OK  
.....

Distance between spins  
Angles between anisotropies  
Diffusion (molecular, spin)

High precision  
for local structure

Today's Special!



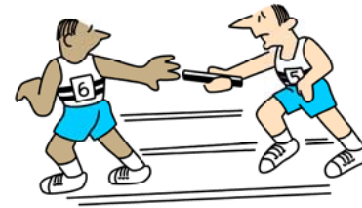
Structures from nm to mm ...

# 固体NMRの観測量と距離

0.1 1 10 100 1000 10000 / nm



粉末線形・磁化移動

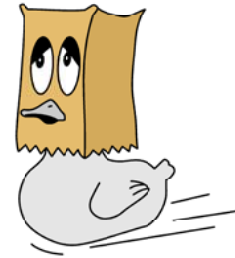


$^1\text{H}$   $T_1$   $\rho$



Spin diffusion

$^1\text{H}$   $T_1$



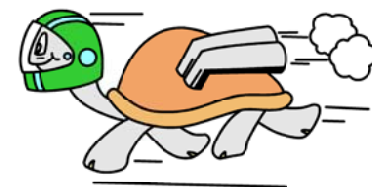
Molecular diffusion

$^{129}\text{Xe}$  1D spectra



$^{129}\text{Xe}$  2D spectra

# 溶液におけるスピン相互作用



分子の等方的な  
回転拡散の速度

$d=10\text{nm} \rightarrow k=10^{11}\text{ Hz}$   
 $d=1\text{nm} \rightarrow k=10^{13}\text{ Hz}$

スピン相互作用 **溶液中の分子の等方回転による平均化**

化学シフト相互作用	～数10 kHz		等方値
双極子相互作用	～数10 kHz	▶▶▶▶	0!
四重極相互作用	～数10 kHz～数MHz		0!

結論：  
溶液のNMRの観測値からは  
距離や角度などの構造を反映する  
情報は得られない・・・エッ！？



# NMRで構造決定・・・ 構造決定に核スピンの相互作用を使おう！

スピンの相互作用・・・って？

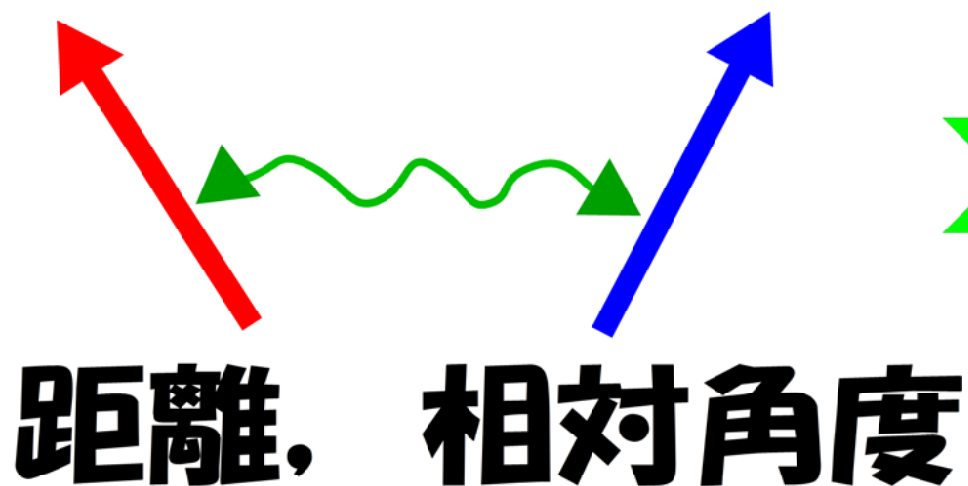
相互: なにかとなにか

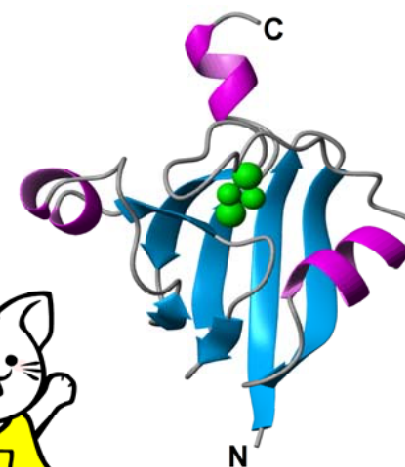
一方は**スピン**だ！

もう一方は？



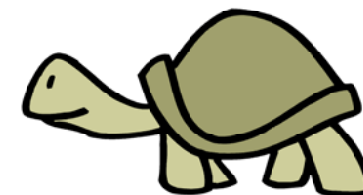
磁場 : 化学シフト相互作用  
スピン : 双極子相互作用  
電場勾配 : 四重極相互作用

  
距離, 相対角度



# 固体におけるスピン相互作用

スピン相互作用 **運動による平均化はない**



化学シフト相互作用    ~ 数10 kHz  
双極子相互作用        ~ 数10 kHz  
四重極相互作用        ~ 数10 kHz ~ 数MHz

結論：  
NMRの観測値からは距離や角度などの  
構造を反映する情報を得ることが出来る。  
しかし...

**スピン相互作用により線形が複雑に！**

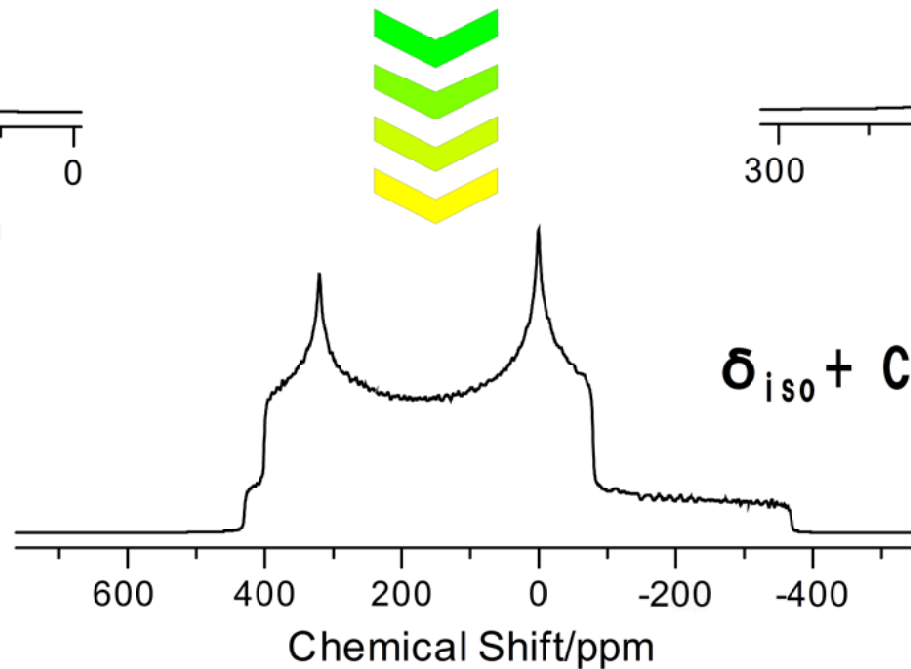
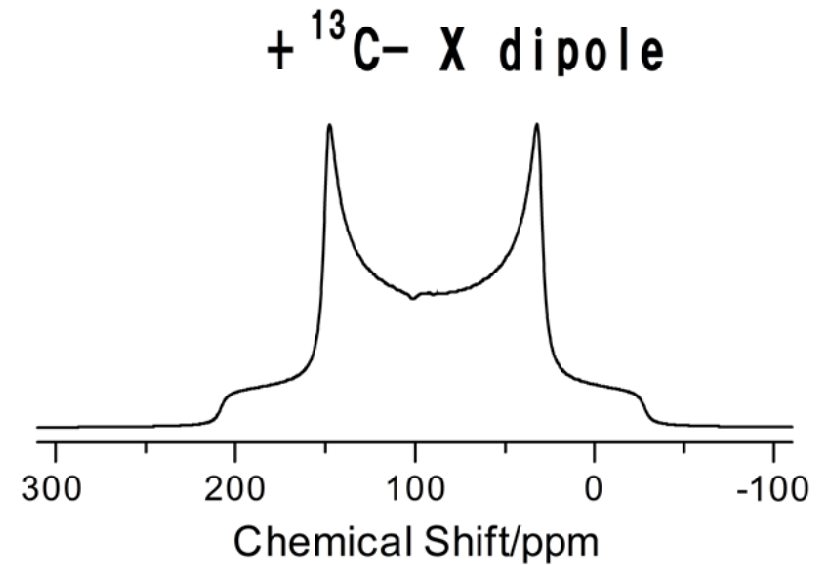
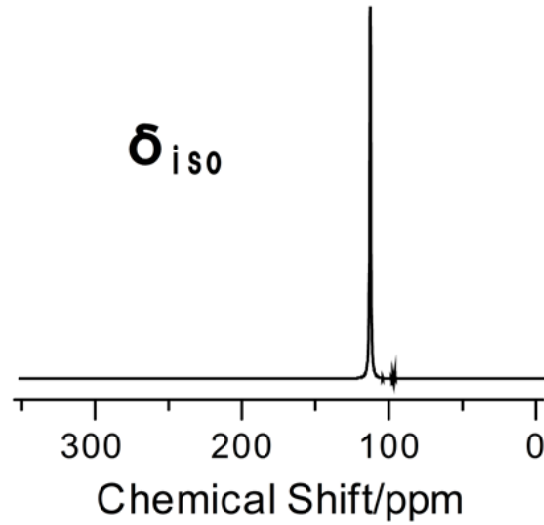
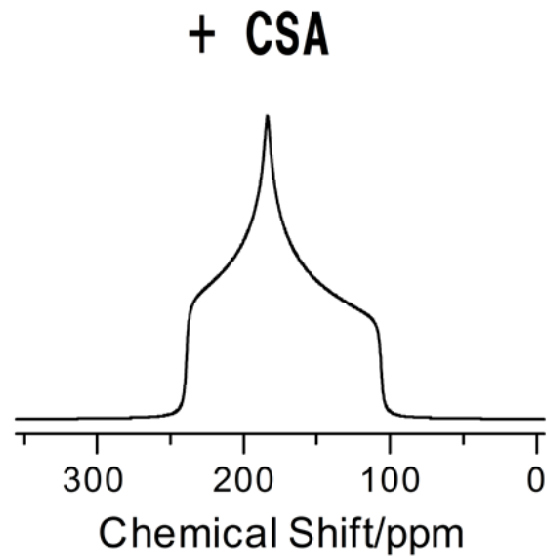
特に、<sup>1</sup>Hはダメ！





# $^{13}\text{C}$ の場合

X- $^{13}\text{C}$ =O powder spectra (calculated)



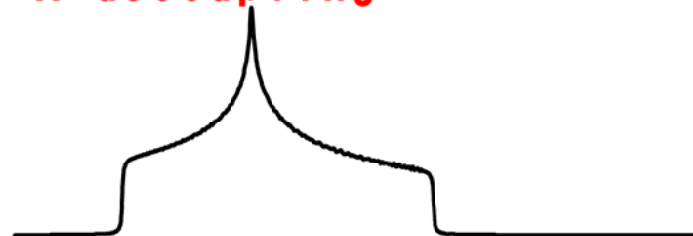
# Powder NMR spectra of spin=1/2 (rare-nuclei case), eg. $^{13}\text{C}$

No gadgets



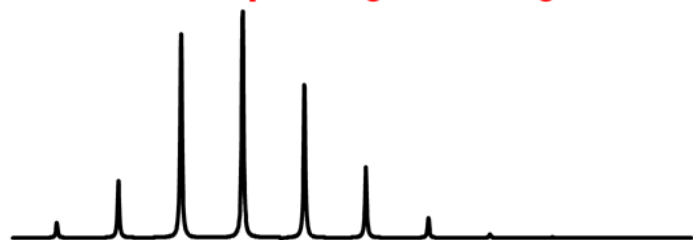
$^{13}\text{C}$  chemical-shift anisotropy  
 $^1\text{H}$ - $^{13}\text{C}$  heteronuclear dipolar interactions

$^1\text{H}$  decoupling



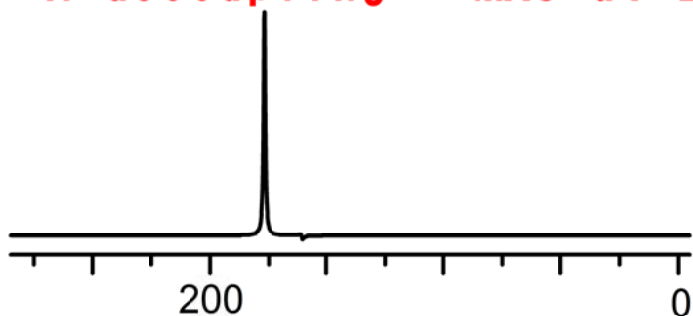
$^{13}\text{C}$  chemical-shift anisotropy

$^1\text{H}$  decoupling + Magic-angle spinning at 2 kHz



$^{13}\text{C}$  isotropic shift + spinning sidebands

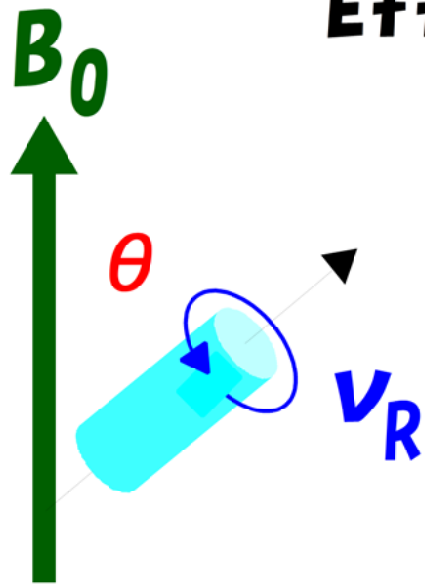
$^1\text{H}$  decoupling + MAS at 20 kHz



$^{13}\text{C}$  isotropic shift



# Effect of sample spinning



$H_0$

Chemical shift anisotropy  
Dipolar interaction



$$H(t) = (3\cos^2\theta - 1)H_0 + \frac{H(2\pi\nu_R t)}{\nu_R}$$



Scaling

Spinning  
sidebands

Magic angle

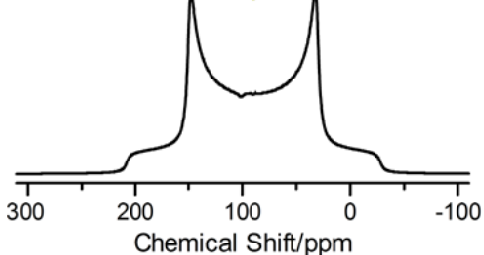
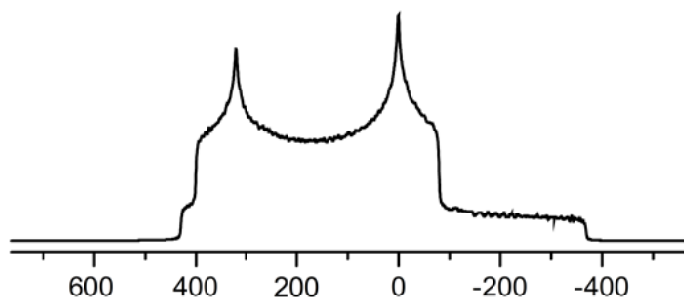
$$3\cos^2\theta_M - 1 = 0$$

# MASの問題点：欲しい相互作用も消えてしまう

溶液NMRと同じだ！構造決定出来ない！

## 問題

化学シフト異方性＋双極子相互作用から  
化学シフト異方性を除いて、双極子だけにしなさい



距離情報

MASでいったん全部消して、  
双極子相互作用だけを  
復活させるとか・・・？

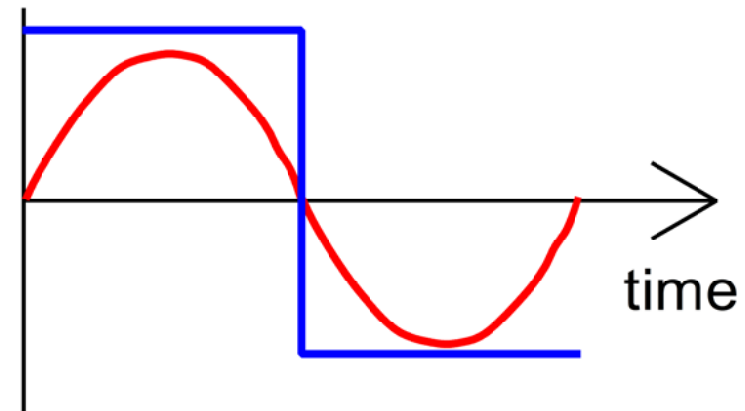
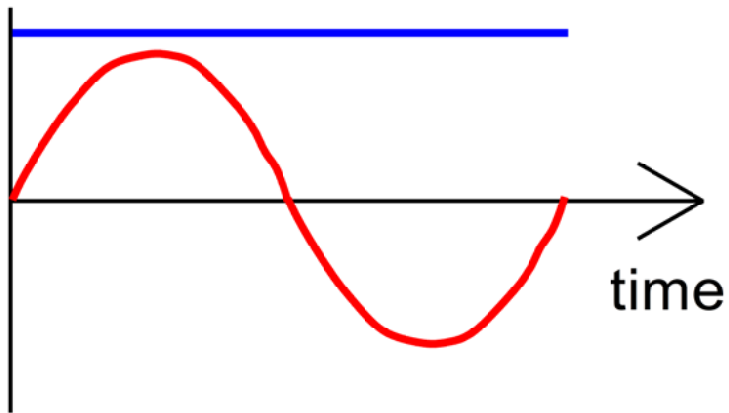


# MAS下で双極子相互作用を復活させる

$$H(t) = D(t) \times A(\text{Spin部分})$$

MAS  $\rightarrow \overline{D(t)} = 0$

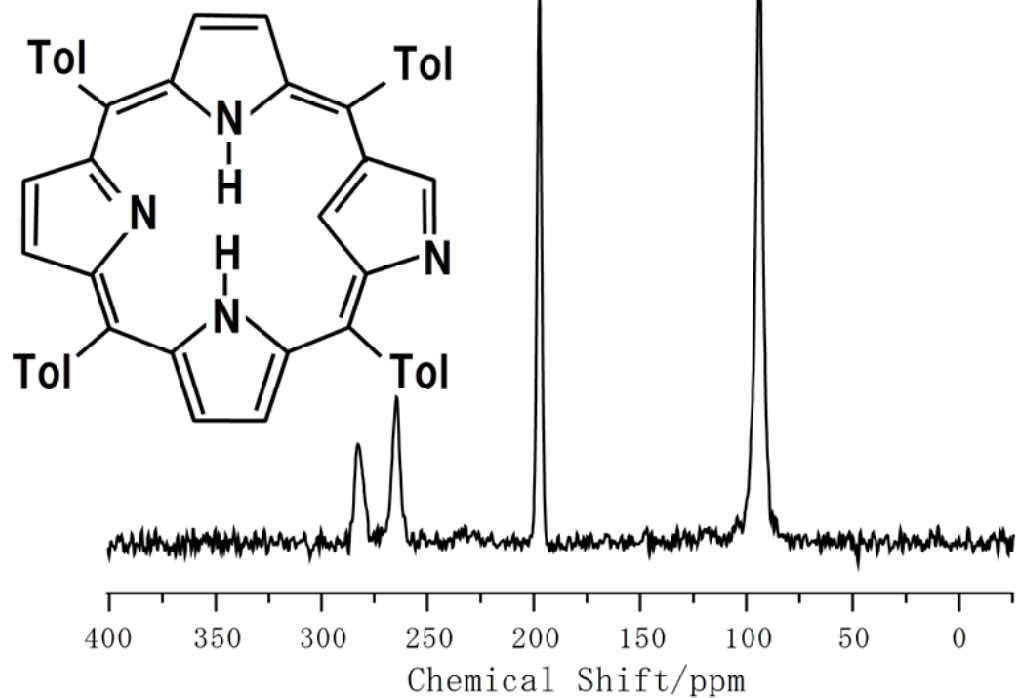
ラジオ波照射  $A \rightarrow A(t)$



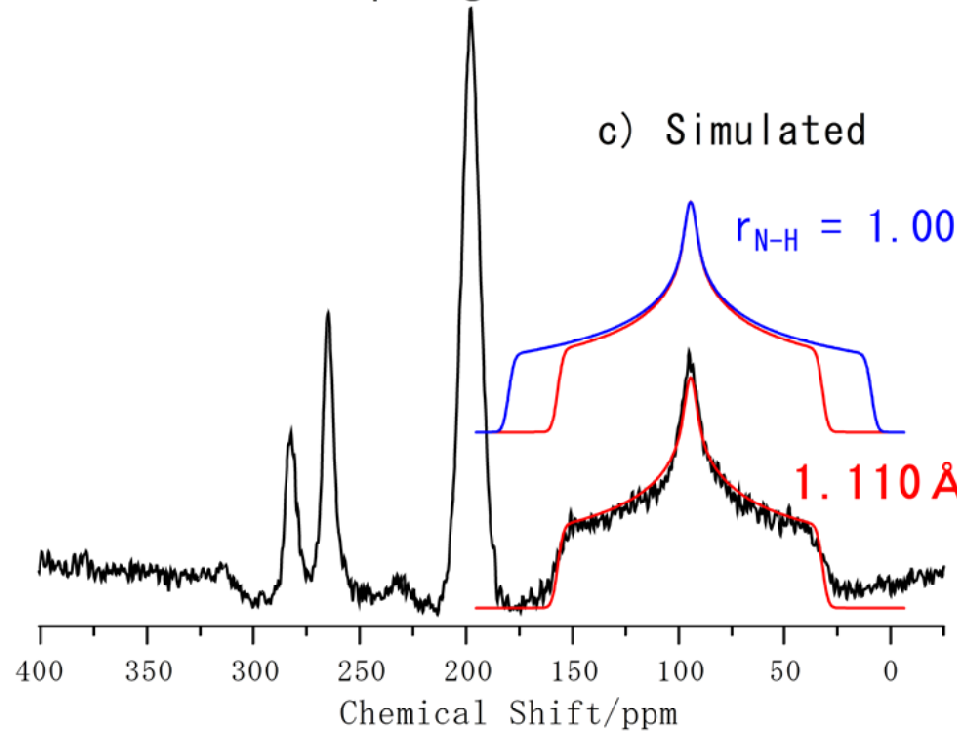
$$\overline{H(t)} \neq 0$$

# $^{15}\text{N}$ CP/MAS spectra of TolyI体

a) Under  $^{15}\text{N}$ - $^1\text{H}$  decoupling



b) Under  $^{15}\text{N}$ - $^1\text{H}$  recoupling



c) Simulated

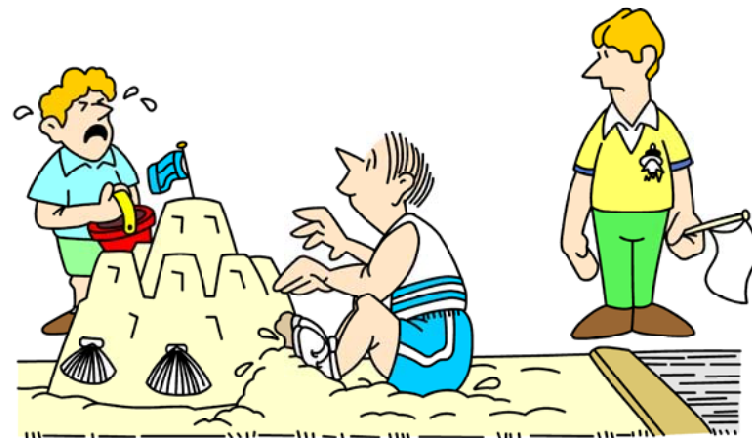
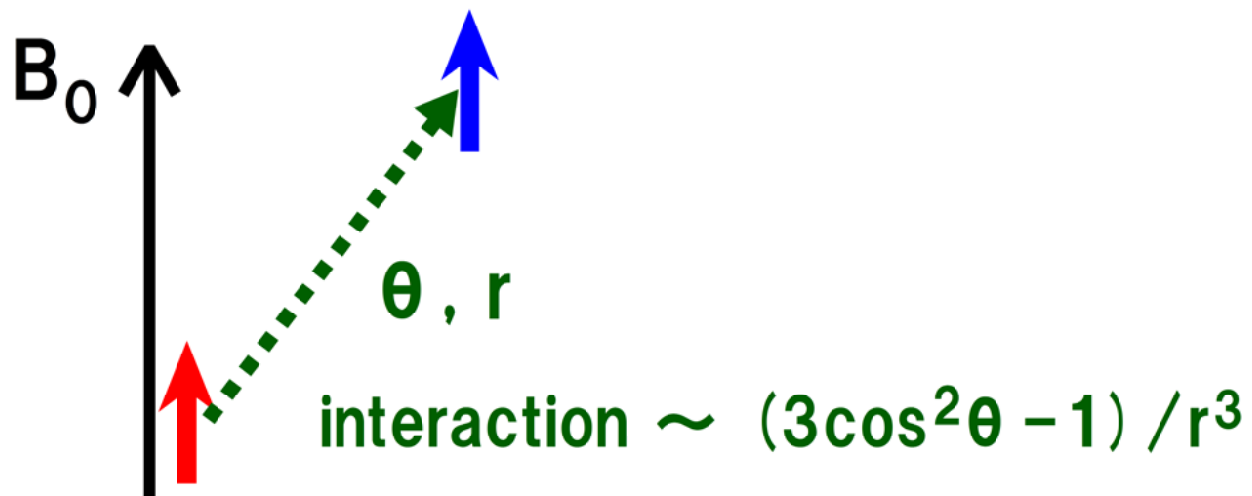
$r_{\text{N-H}} = 1.000 \text{ \AA}$

$1.110 \text{ \AA}$

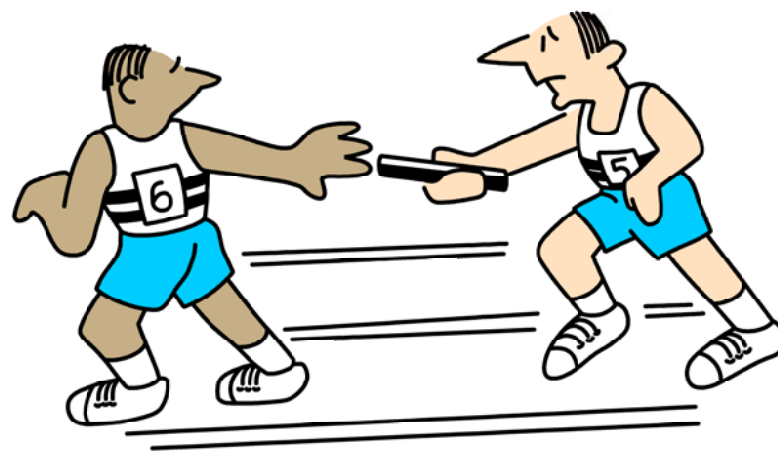
**Recoupling by MORE**

# 粉末試料での距離測定

## 1) From dipolar-broadened powder lineshapes

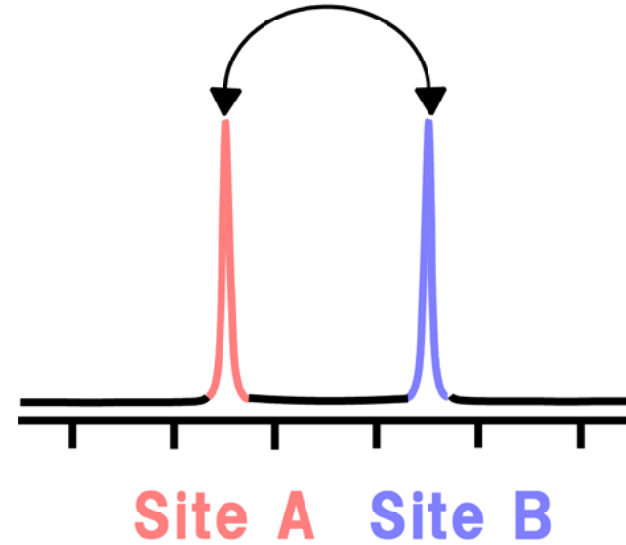
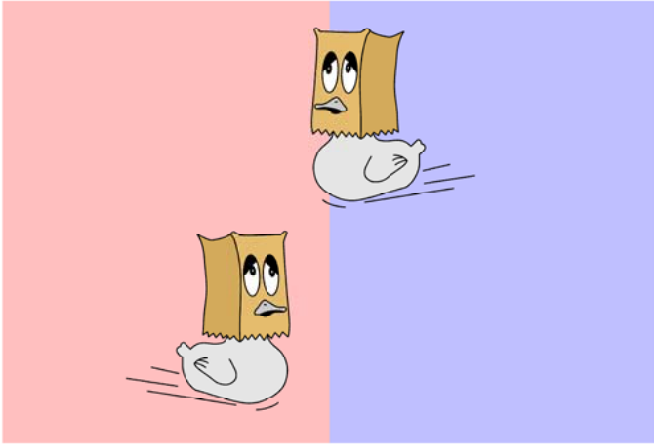


## 2) From flip-flop transition rates

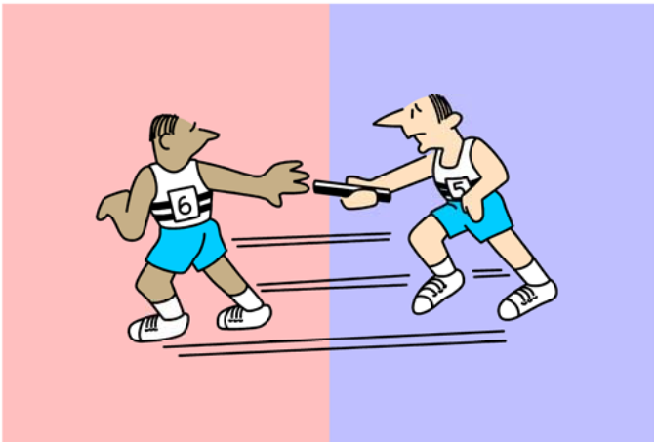


# Longer distances by diffusion

## 1) Molecular Diffusion



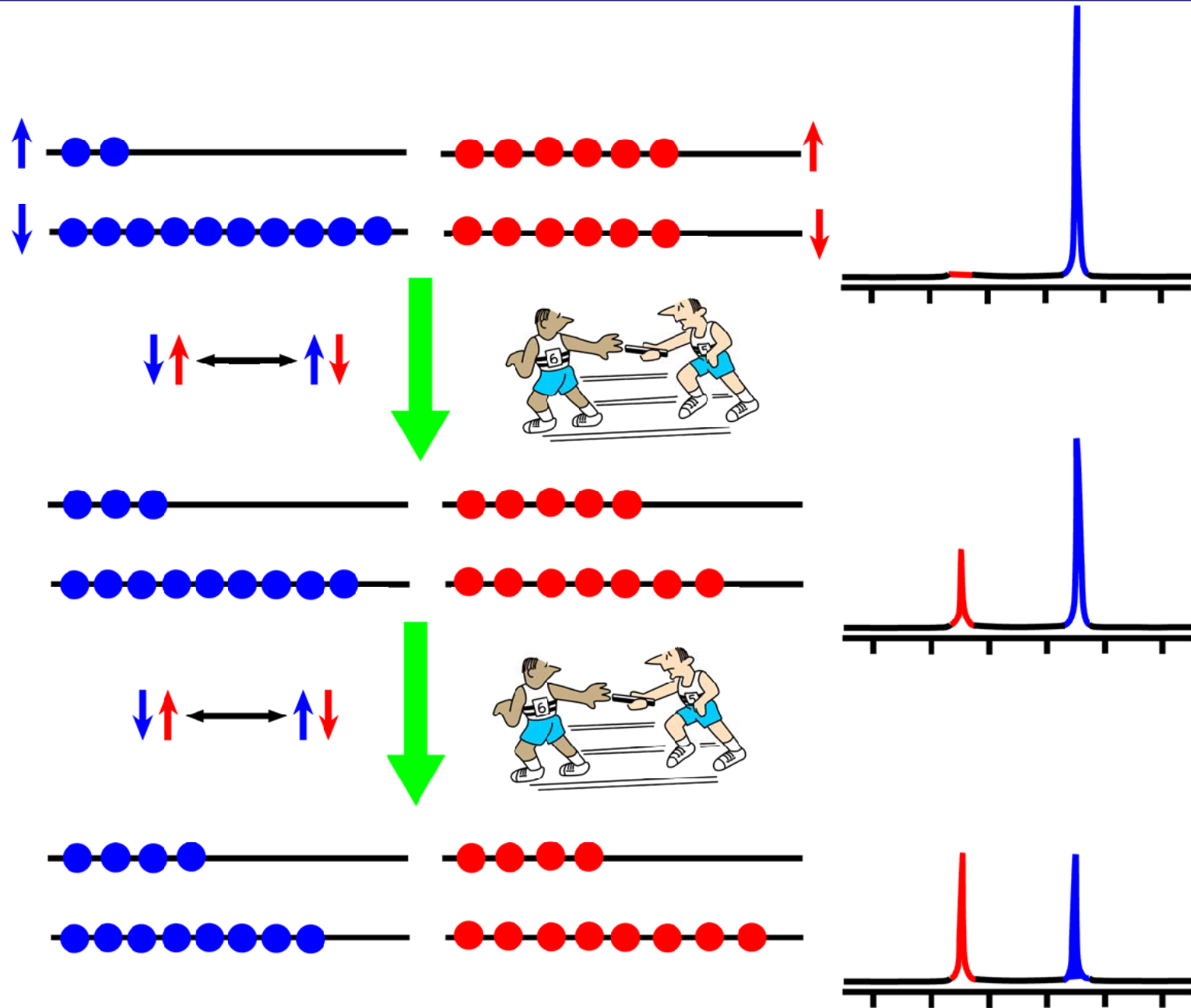
## 2) Spin Diffusion



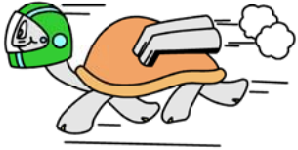
Studies on domain sizes  
in inhomogeneous solids



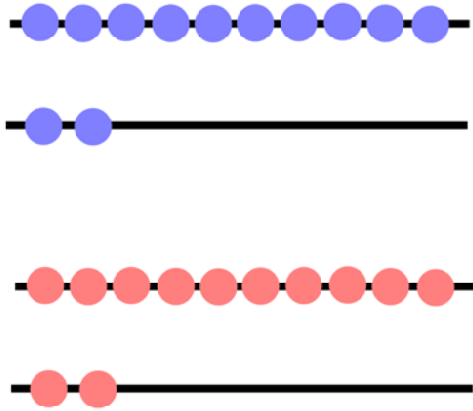
# Polarization transfer/spin diffusion by flip-flop motion



# Spin diffusion and relaxation



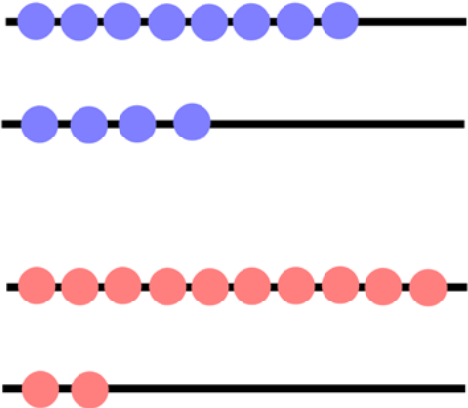
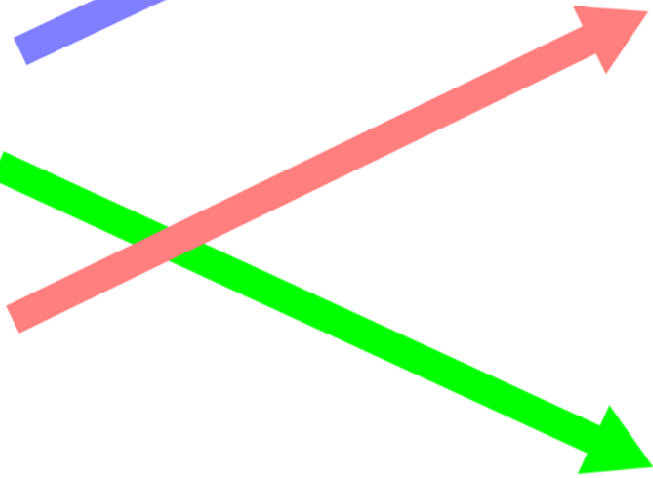
Short relaxation time



Long relaxation time

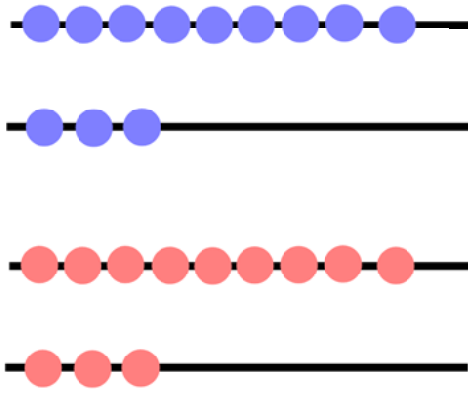


No diffusion



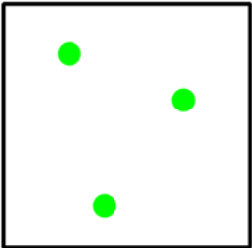
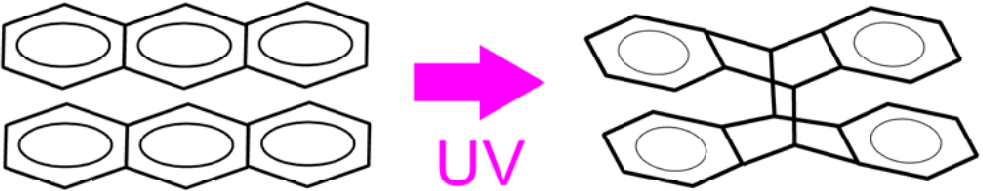
Relax individually

Fast spin diffusion



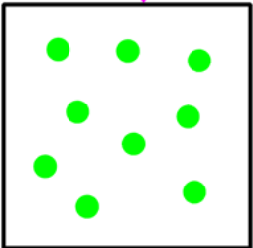
Relax together

# Photo-dimerization of solid anthracene



**Crystal sites**  
**Random distribution**

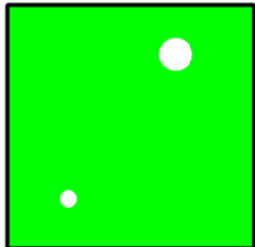
**Defect sites**  
**Form domains**



?



**Spin diffusion : fast**  
**i.e.**  
**Equal relaxation rates**



**Spin diffusion : slow**  
**i.e.**  
**Unequal rates**

$^{13}\text{C}$

分解能がいい



but!

天然存在比が1% つまり、つまり、スピン拡散しない



$^1\text{H}$

分解能は悪い

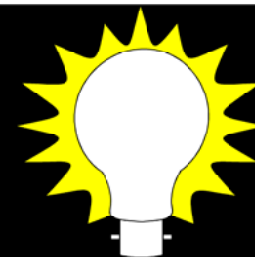


but

天然存在比は100% つまり、スピン拡散する

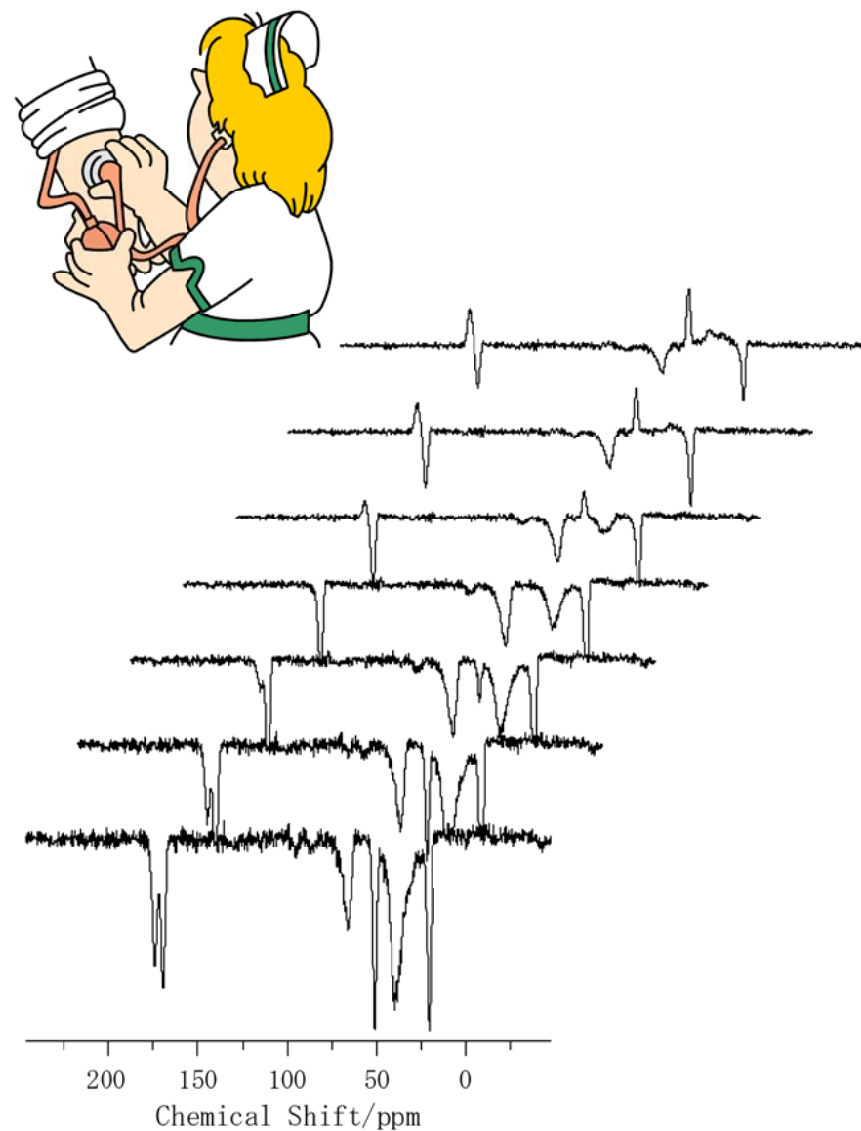
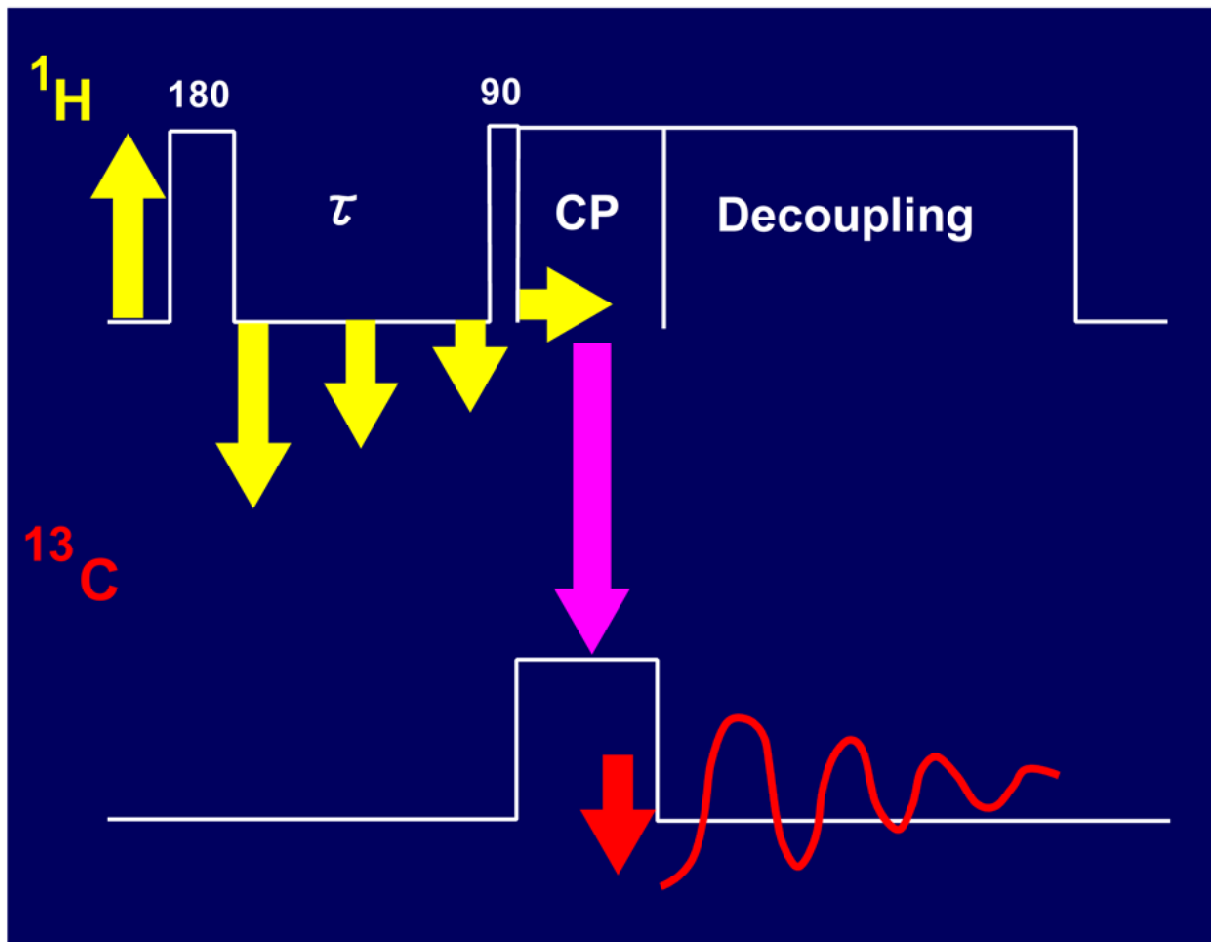


$^1\text{H}-^1\text{H}$  のスピン拡散を



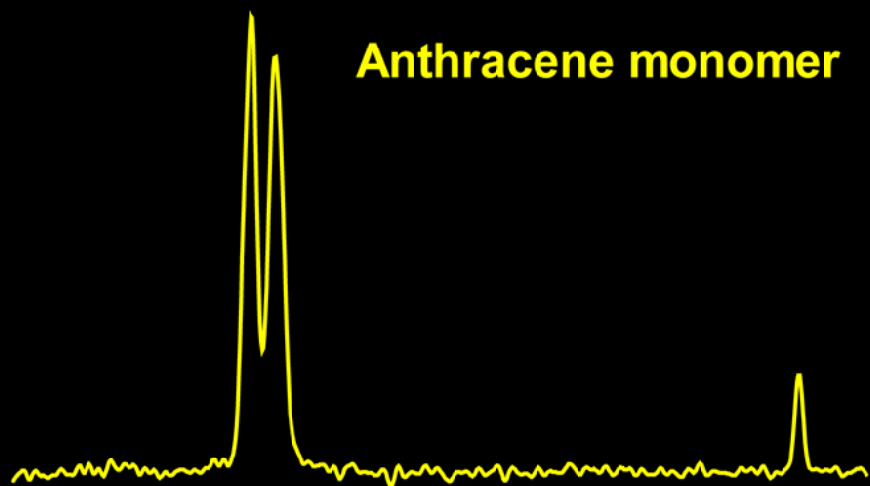
$^{13}\text{C}$ の信号で間接測定しよう!

# Indirect $^{13}\text{C}$ observation of $^1\text{H}$ spin-lattice relaxation

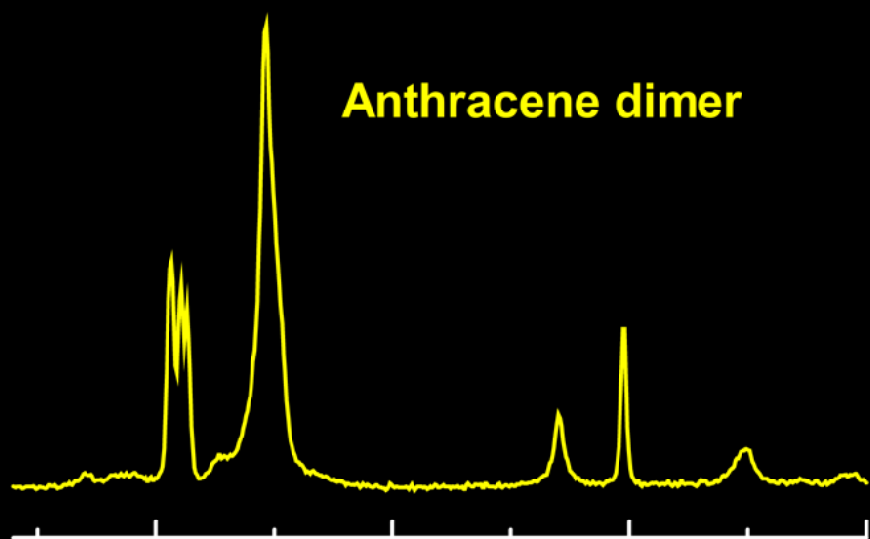


# $^{13}\text{C}$ MAS spectra

**Anthracene monomer**



**Anthracene dimer**



150

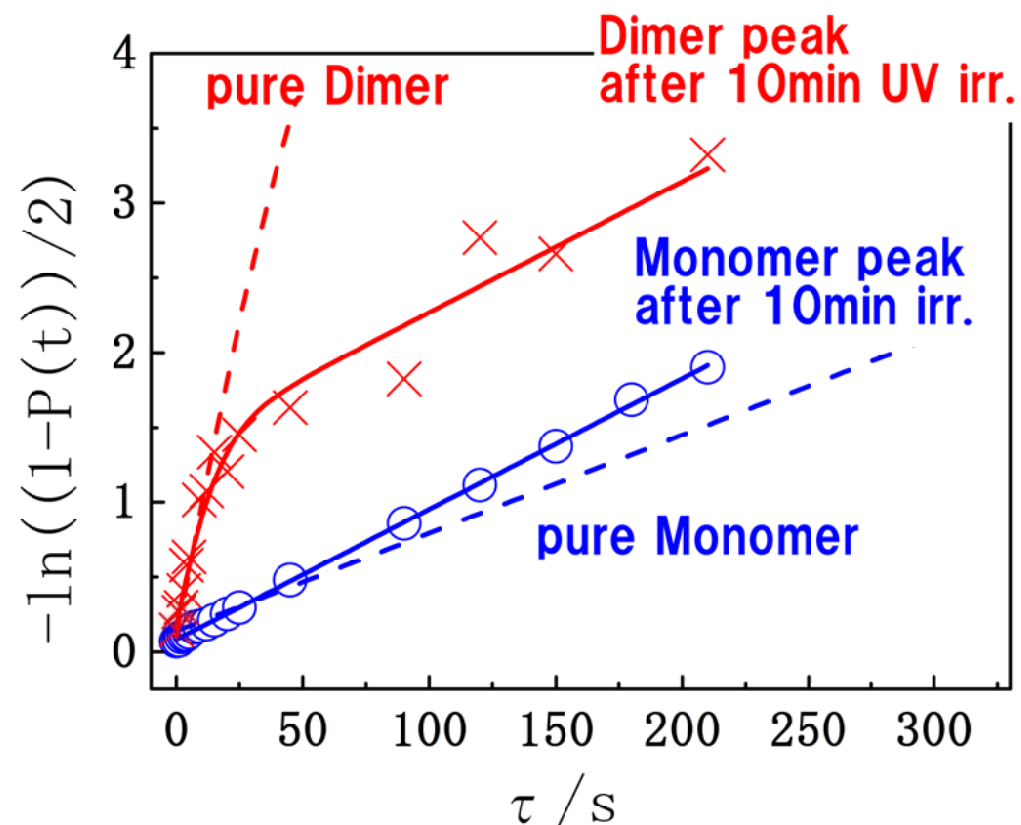
100

50

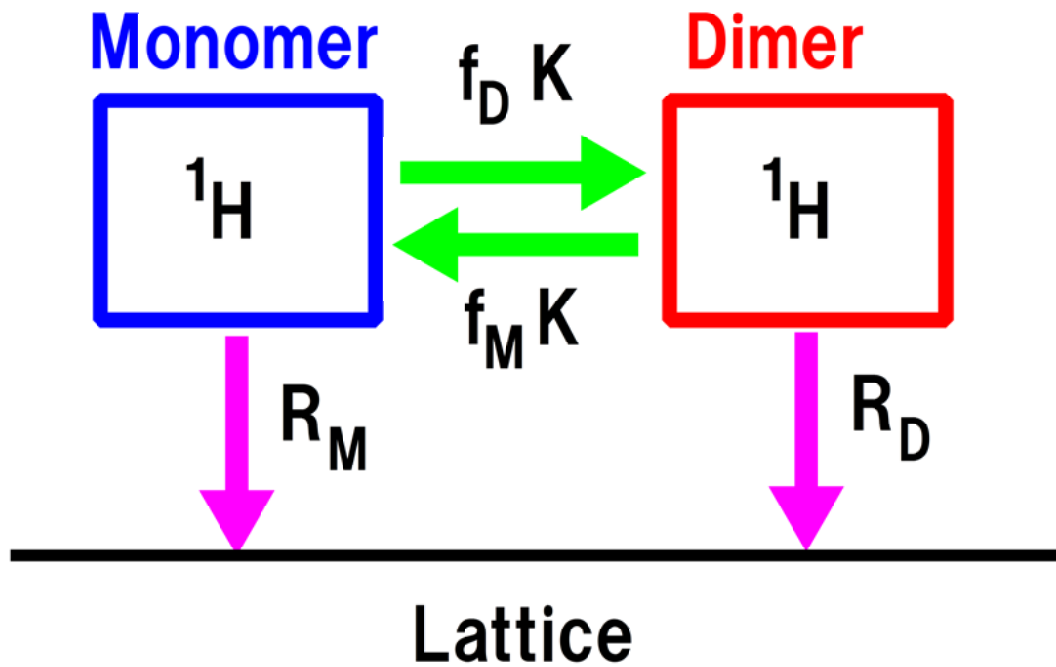
0

Chemical Shift/ppm

$^1\text{H}$  relaxation curves observed  
via  $^{13}\text{C}$  signals

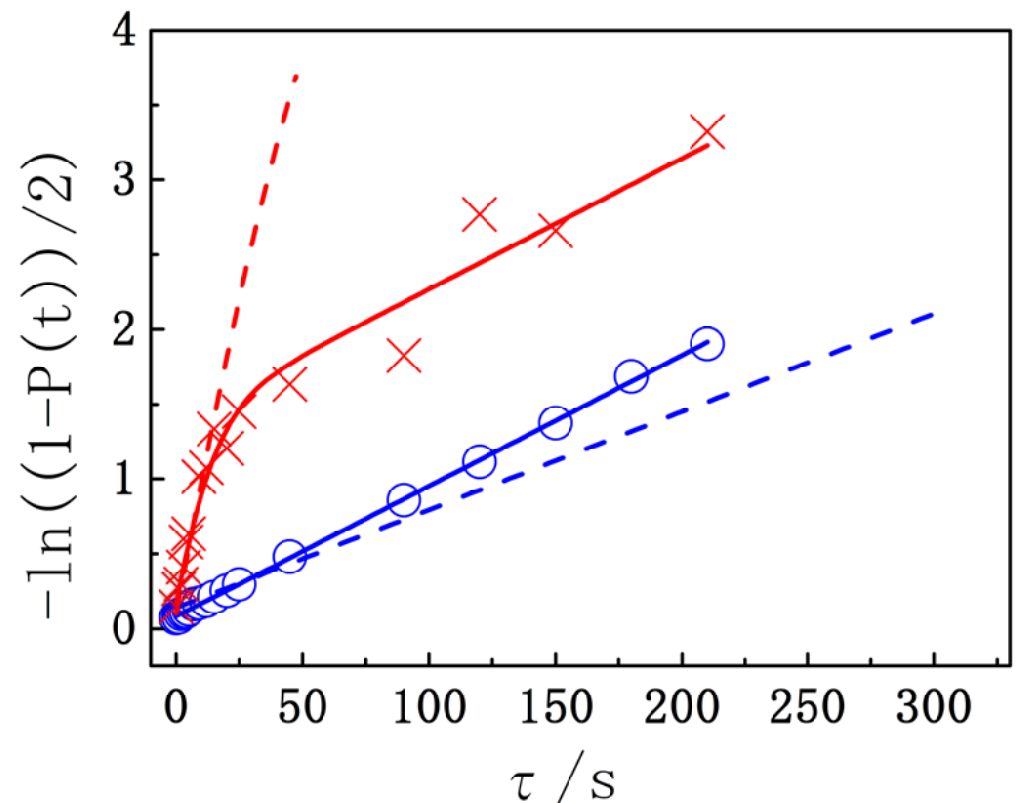


## Flow of spin energy

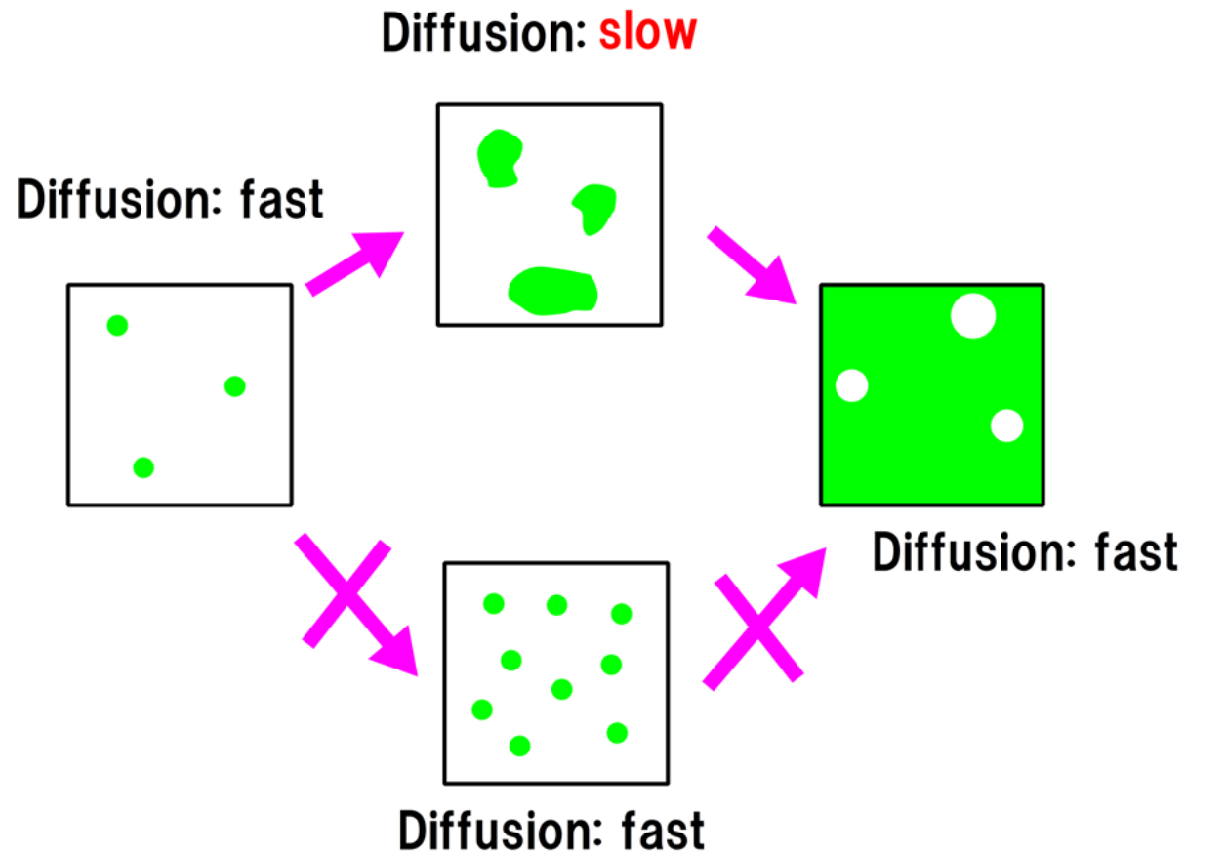
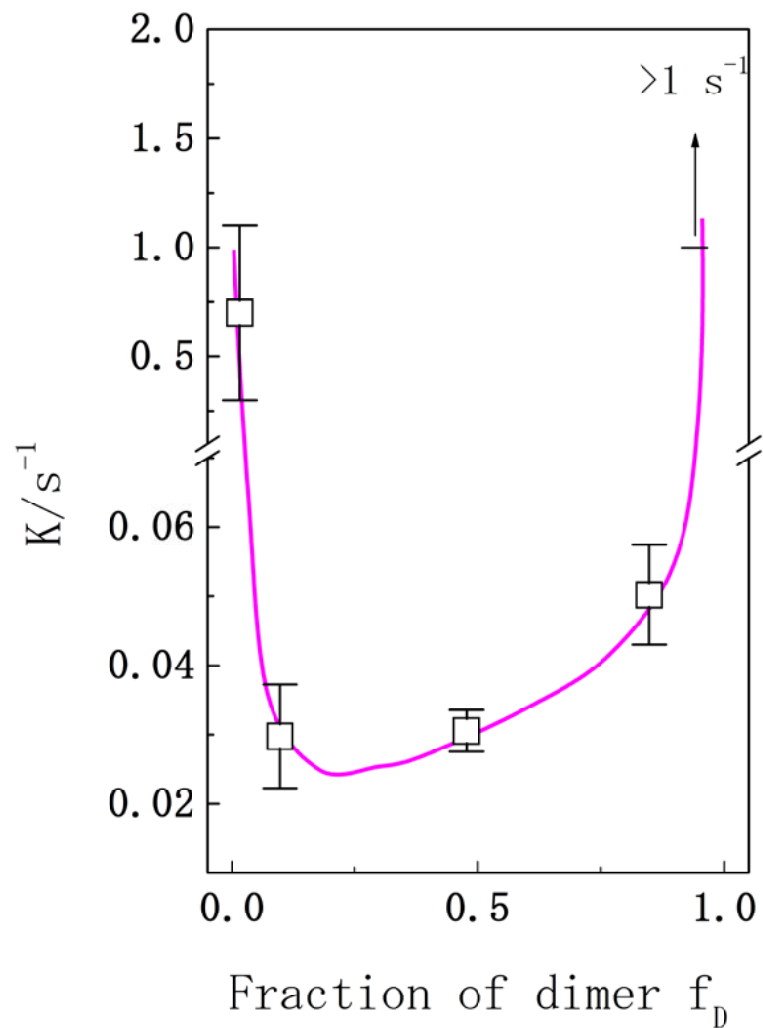


$$\dot{M}(t) = -(R_M + f_D K) M(t) + f_M K D(t)$$

$$\dot{D}(t) = -(R_D + f_M K) D(t) + f_D K M(t)$$



# Spin-diffusion rates vs fraction of dimer



**Reaction** →



## Estimation of the domain size

Assume spin diffusion from a point source

$$\langle l^2 \rangle = 6Dt$$

Assume  $t \sim K^{-1} = (0.03)^{-1} \text{ s}$

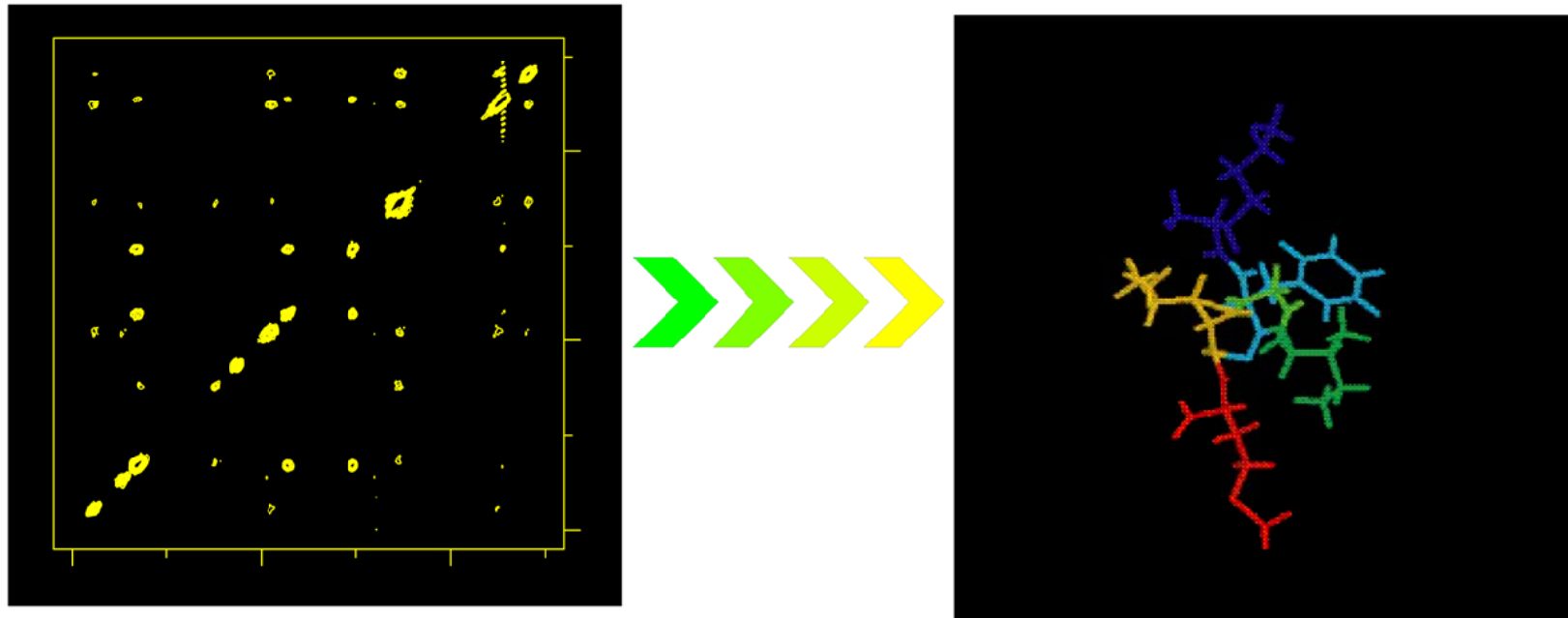
Estimate the diffusion constant as

$$D \sim 2r_0/T_2 \sim 5.5 \times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$$

$$r_0 = 0.117 \text{ nm}$$

$$\sqrt{\langle l^2 \rangle} \sim 300 \text{ nm}$$

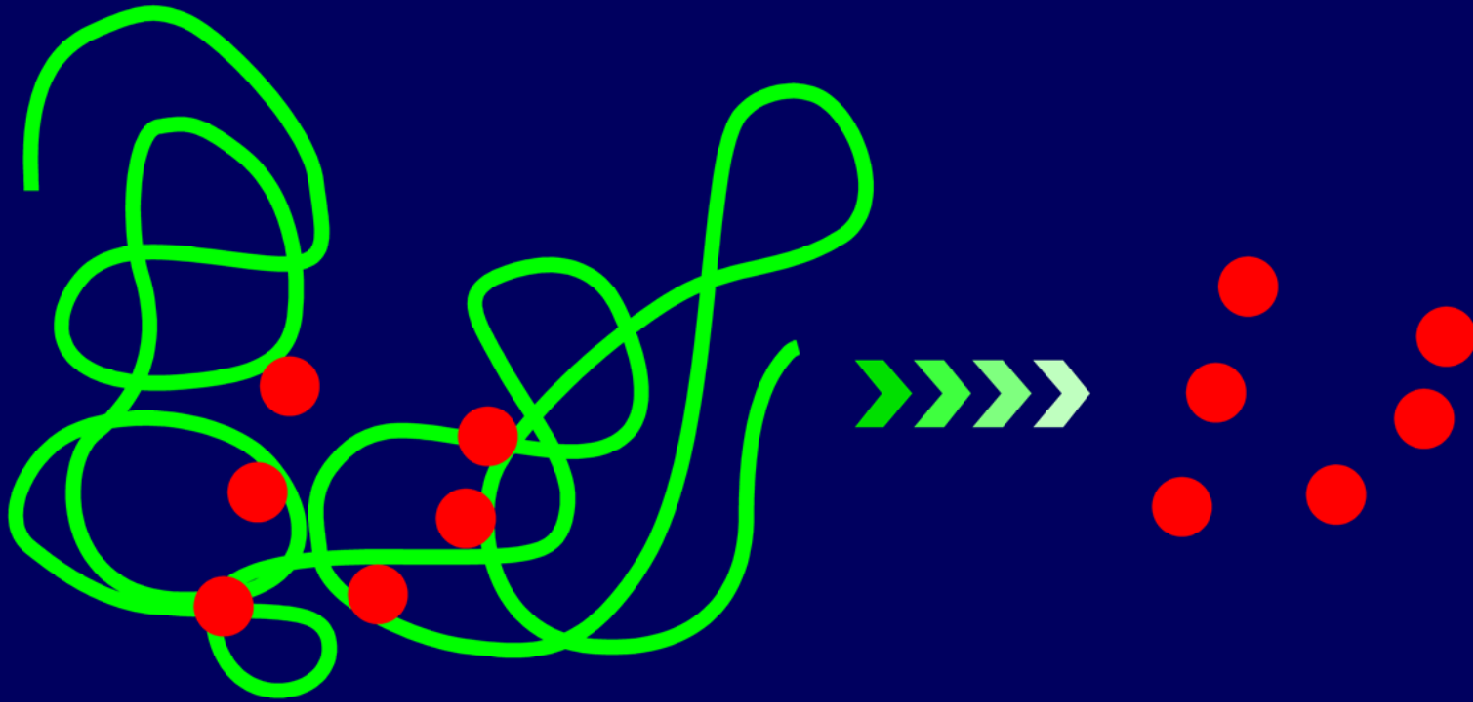
# Structural determination by solid NMR



Resolution of  $^1\text{H}$  resonances is too bad....

We replace  $^{12}\text{C}$  and  $^{14}\text{N}$  by  $^{13}\text{C}$  and  $^{15}\text{N}$   
to achieve high resolution...

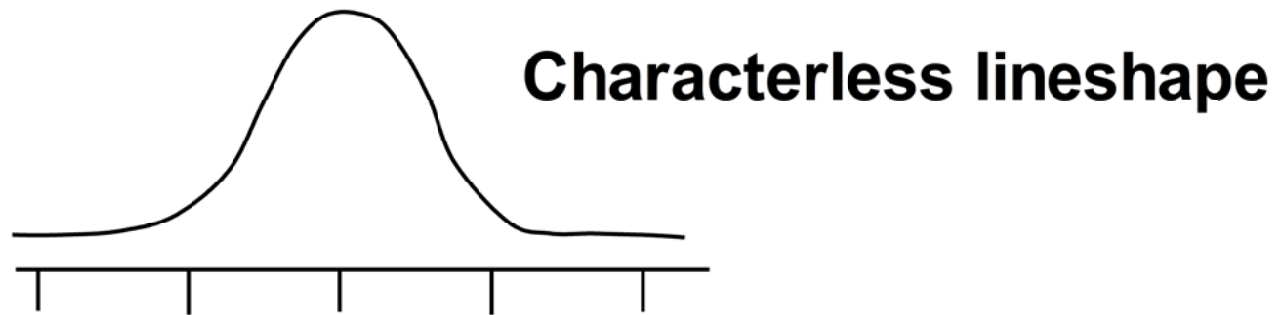
# High-lighting local structure by isotope labeling



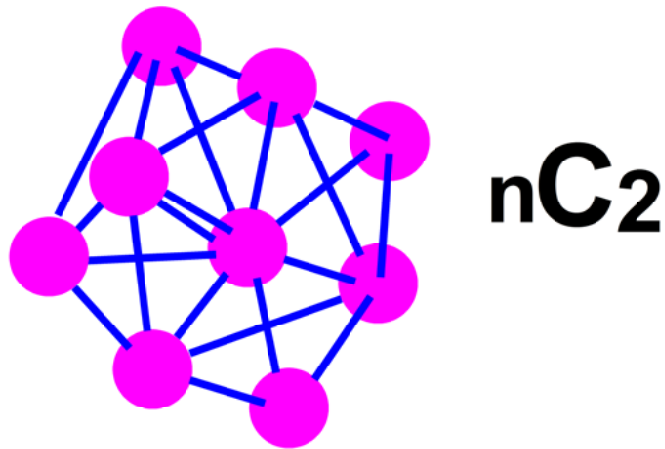
# Excessive $^{13}\text{C}$ labeling

1) Spectral overlap due to anisotropic line broadening

Chemical shift anisotropy & Dipole-dipole coupling



2) Complex-coupling network



**Use doubly labeled samples!**

**Selective observation of dipolar & CSA under MAS**

Distance between spins

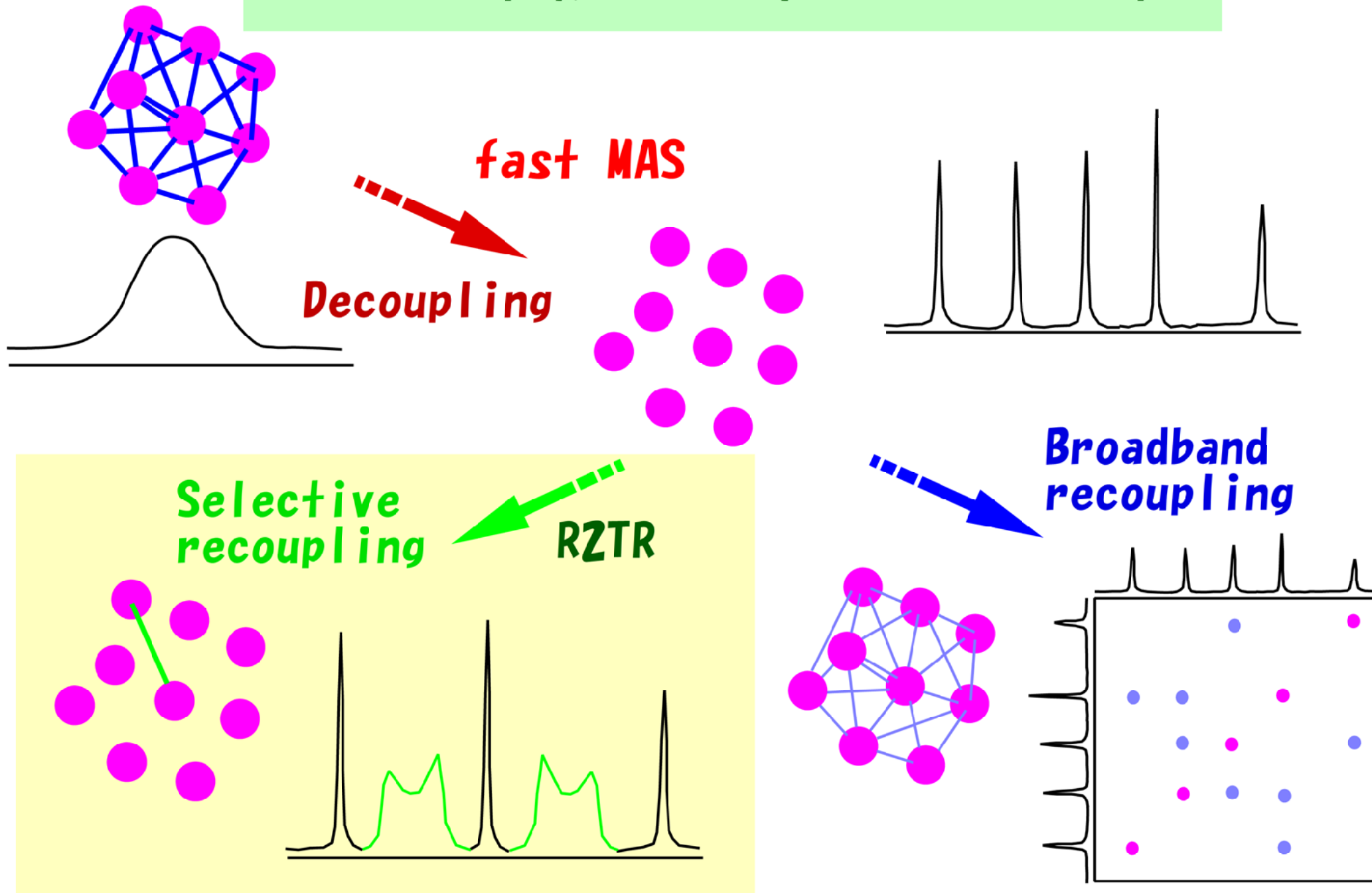
Angles between anisotropies

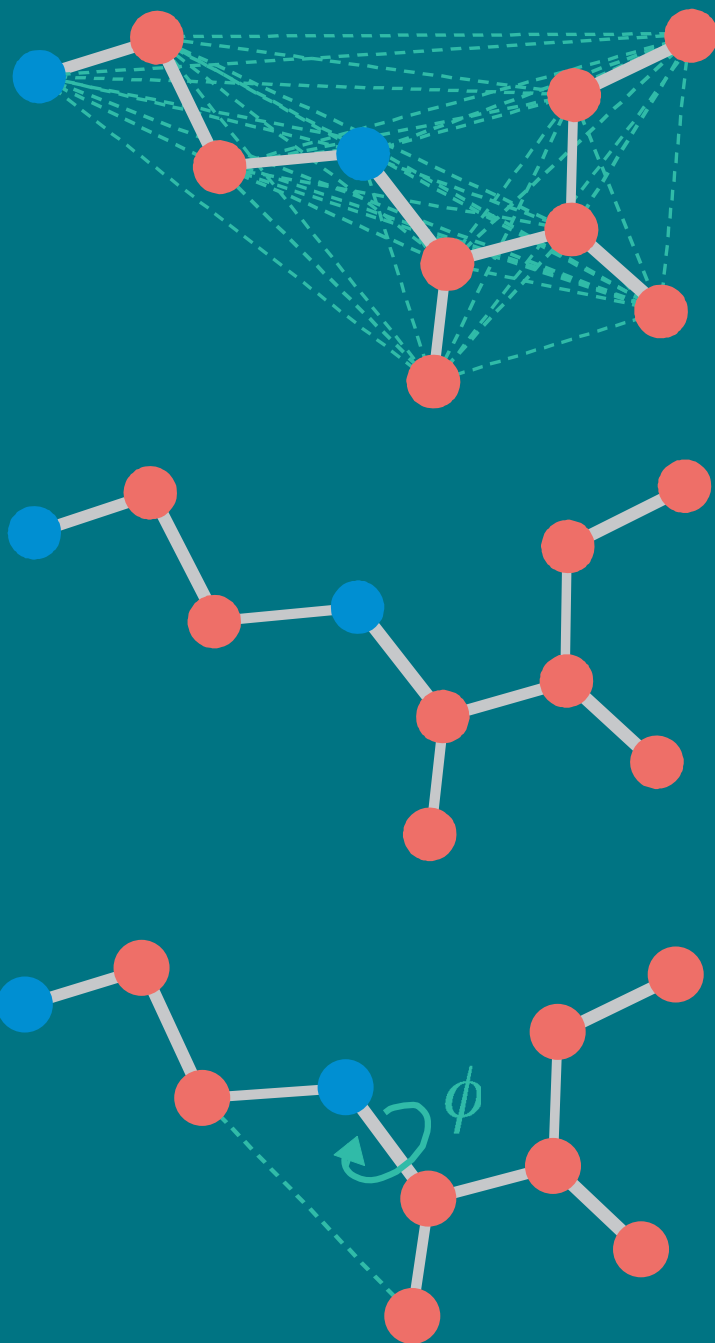
**High precision for local structure**

**Using one multiply labeled sample ...**



For a multiply/uniformly  $^{13}\text{C}$ -labeled sample





**Uniform**  
Labeling  
by  $^{13}\text{C}$  /  $^{15}\text{N}$

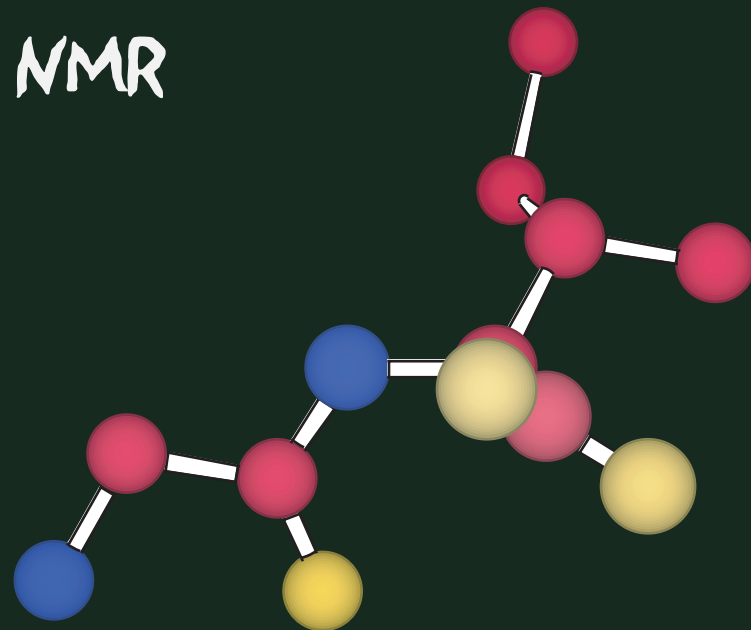


**Uniform**  
Decoupling  
by MAS



**Selective**  
Recoupling  
by R2TR

NMR



$\psi_G$  180

$\omega$  180

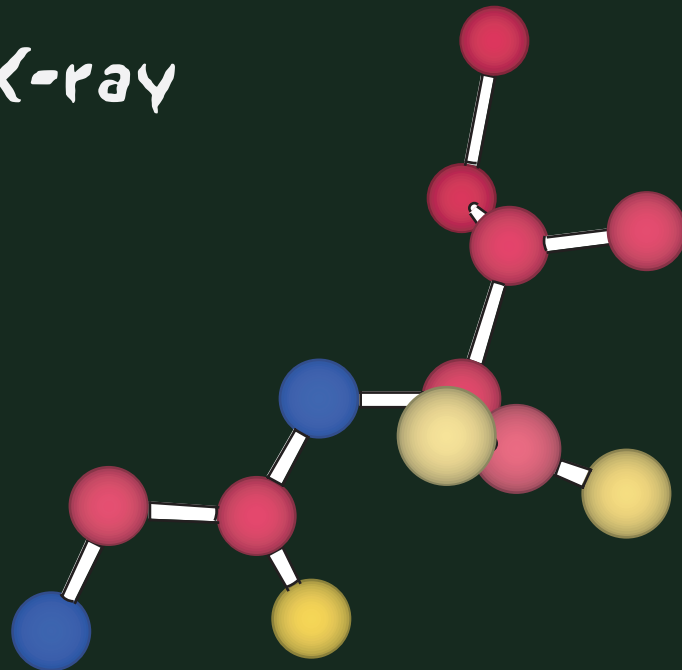
$\phi_I$  -76

$\psi_I$  144

$\chi_I^1$  179

$\chi_I^2$  160

X-ray



$\psi_G$  165

$\omega$  170

$\phi_I$  -70

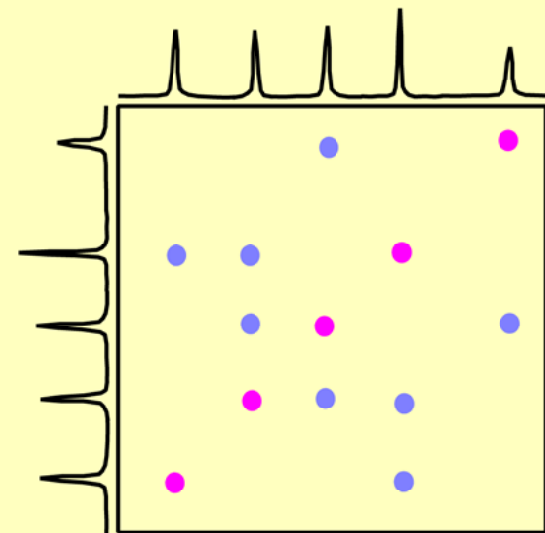
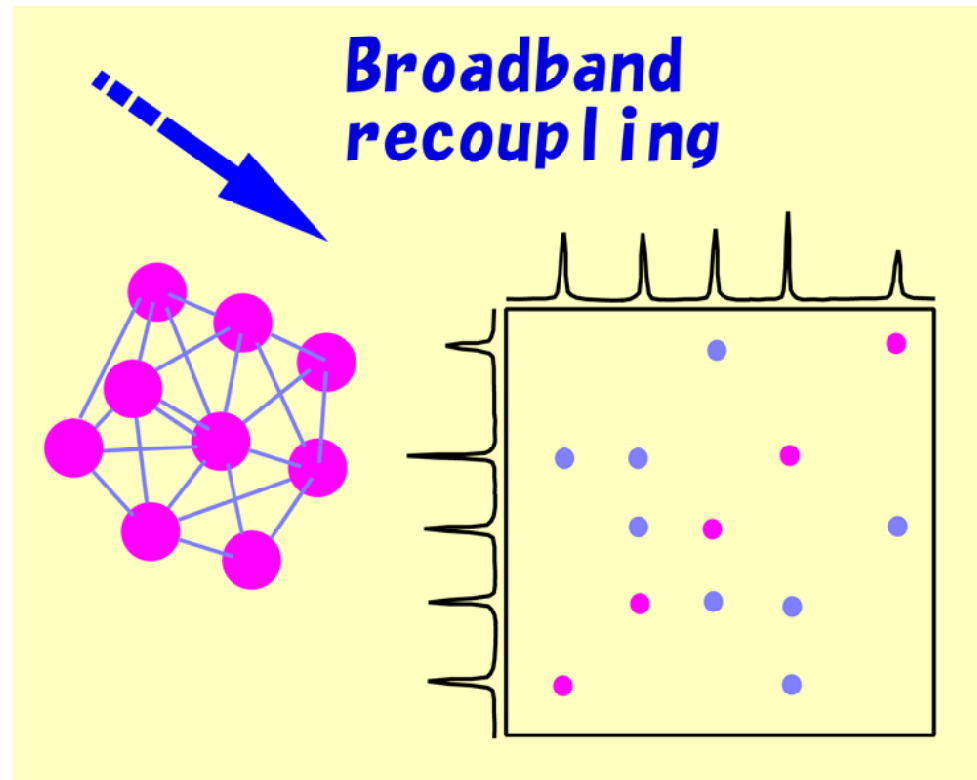
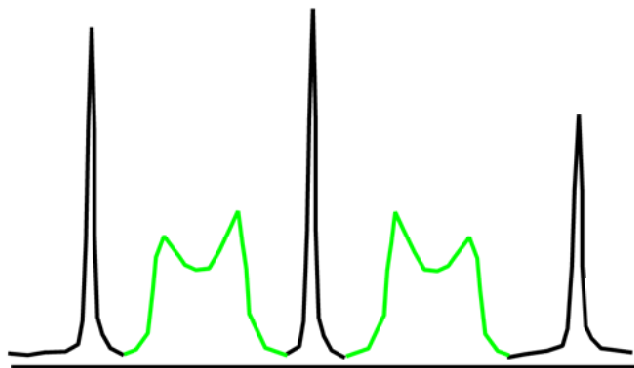
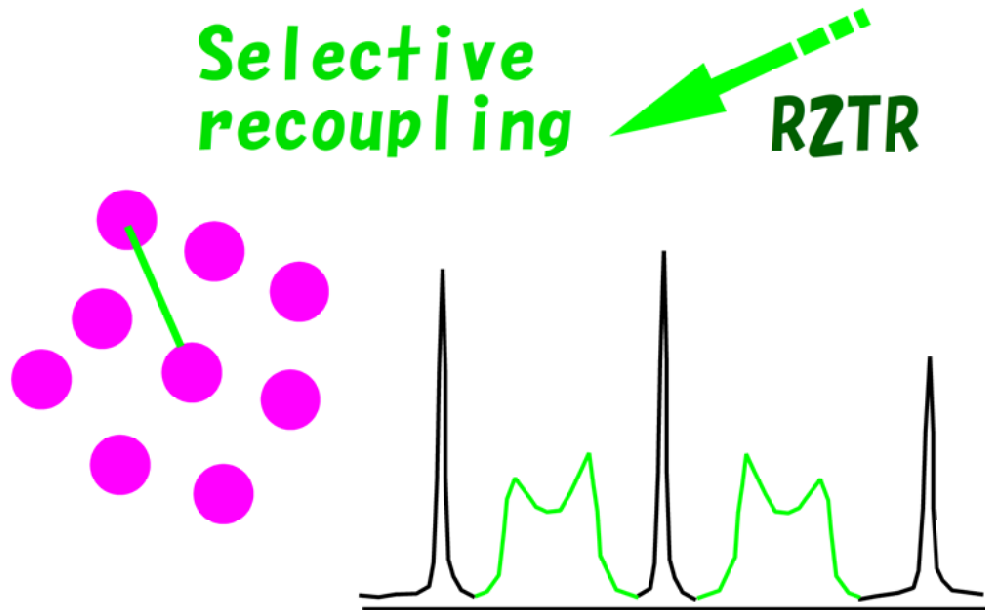
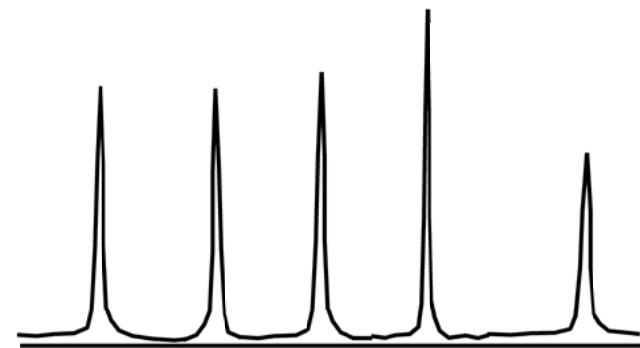
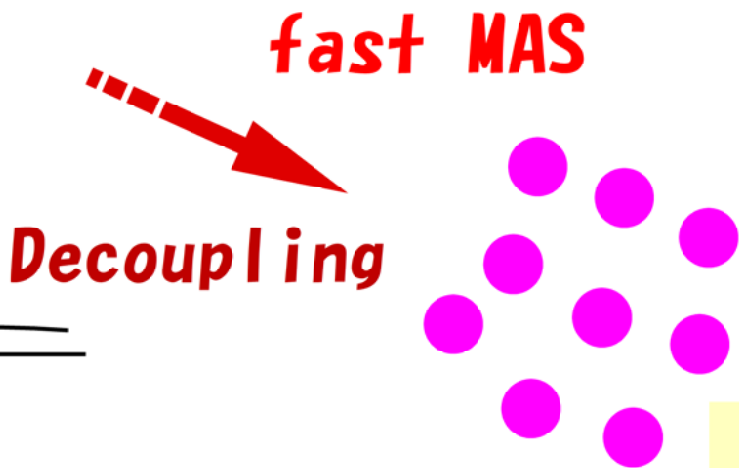
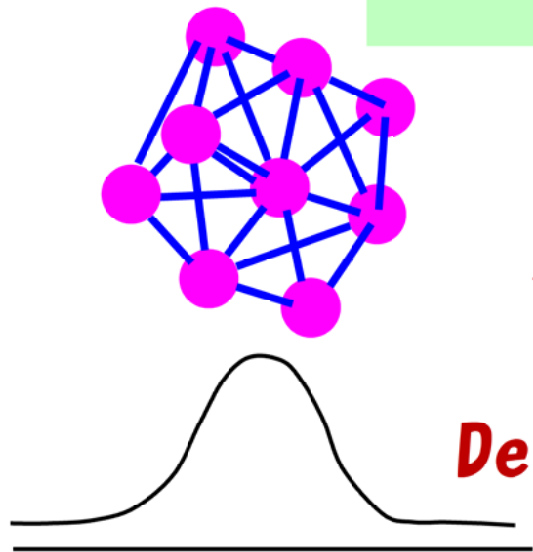
$\psi_I$  153

$\chi_I^1$  178

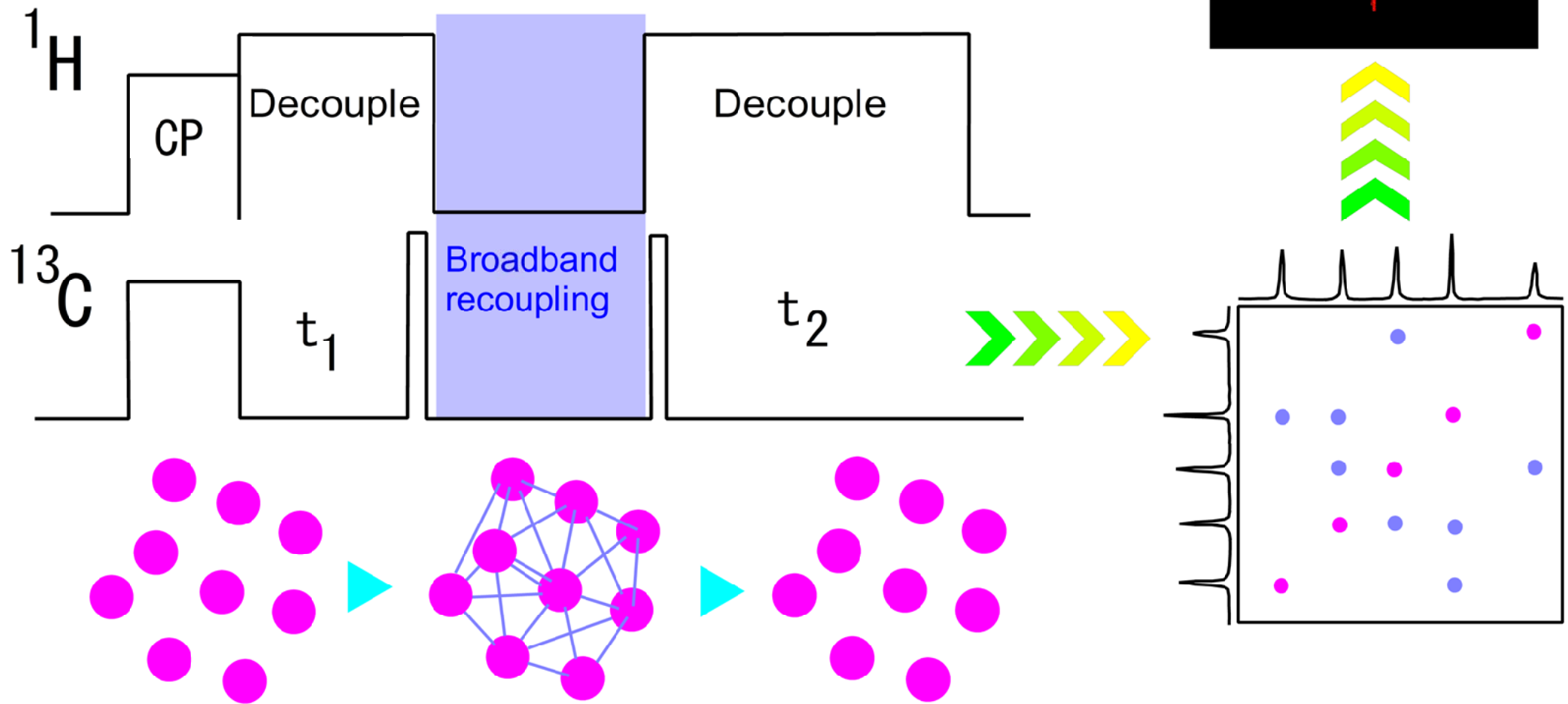
$\chi_I^2$  170



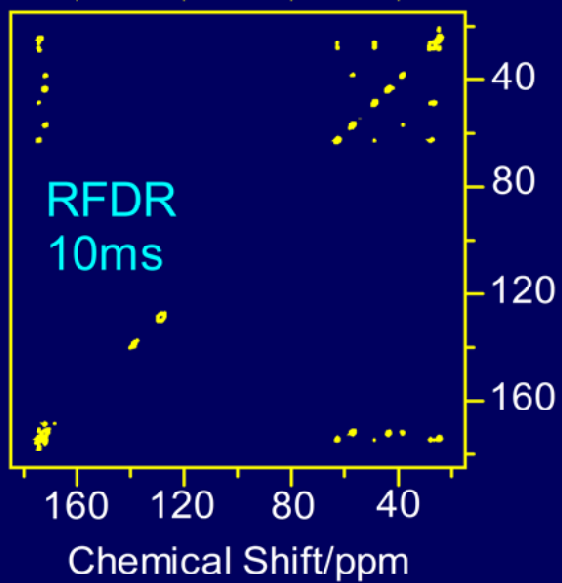
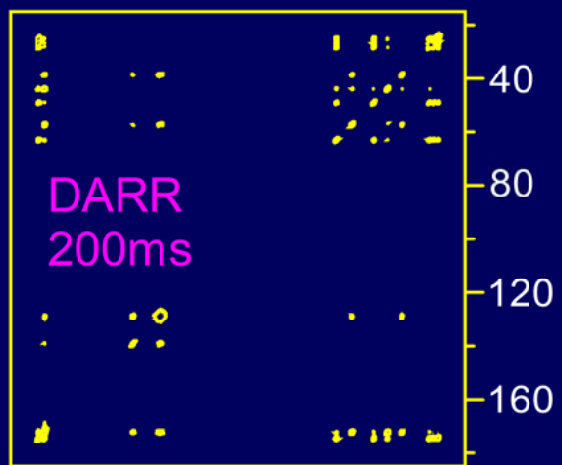
For a multiply/uniformly  $^{13}\text{C}$ -labeled sample



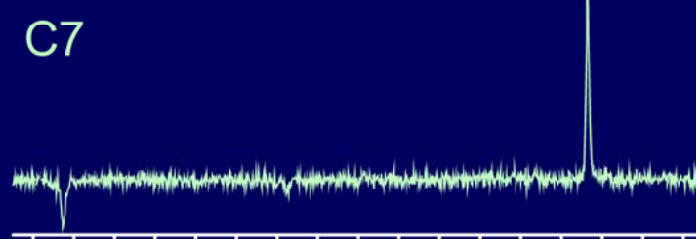
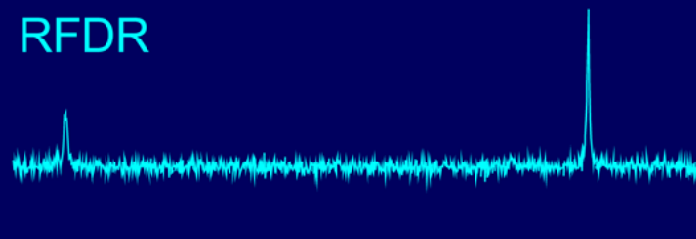
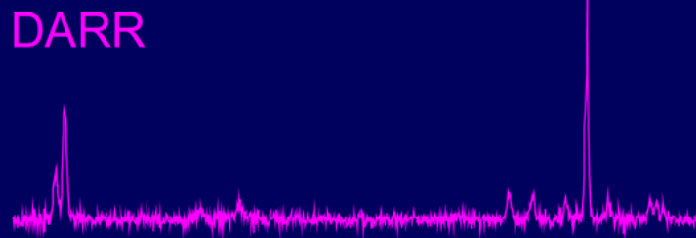
# A 2D $^{13}\text{C}$ - $^{13}\text{C}$ exchange experiment



# $^{13}\text{C}$ - $^{13}\text{C}$ polarization spectra of $^{13}\text{C}$ -labeled Ac-Pro-Gly-Phe

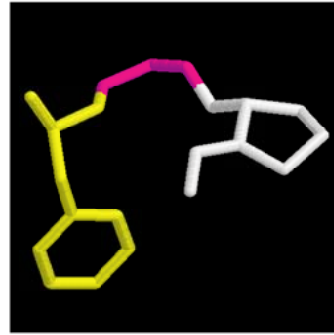


Slices at Gly-  $\alpha$

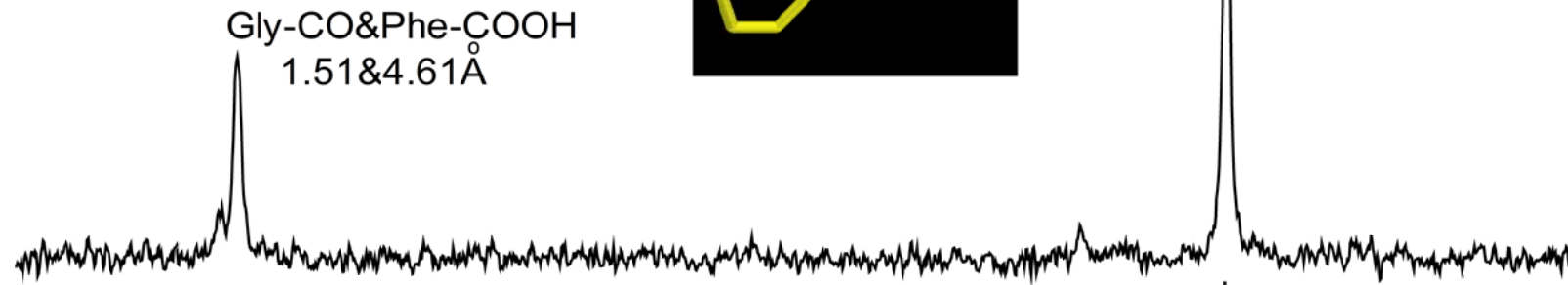


Chemical Shift/ppm

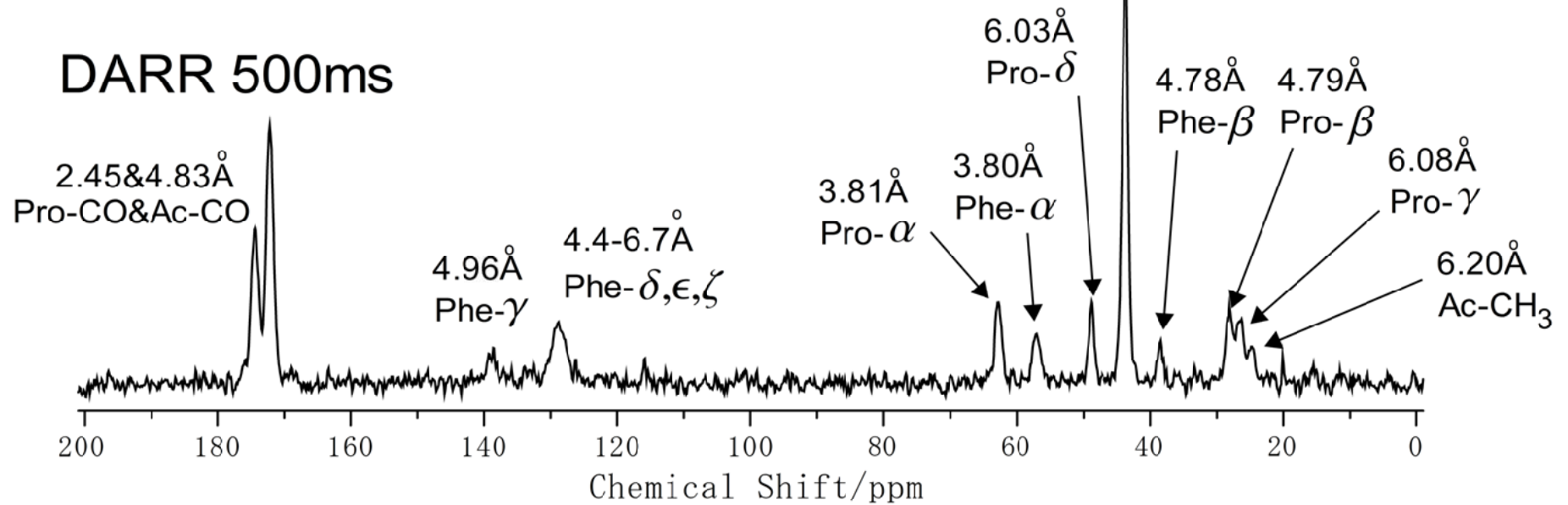
Cross-section spectra of 2D RFDR/DARR  $^{13}\text{C}$ - $^{13}\text{C}$  exchange spectra  
[u- $^{13}\text{C}$ ] AcProGlyPhe



RFDR 10ms



DARR 500ms



# Alzheimer's disease

**1906**

**Auguste, D. died.  
Her characteristic neuropathology was  
first observed by Alzheimer, A.**

*(Neurologisches Centralblatt 1906, 23, 1129-1136)*



**1984**

**Identification of  $\beta$  amyloid ( $A\beta$ )**

**2000**

**4 million patients in US  
(4-6% of over-65 age people)**



**Alois Alzheimer  
(1864~1915)**

痴呆度テスト (改訂-長谷川式簡易知能評価スケール)

質問内容	配点
1. お歳はいくつですか? (2歳までの誤差は正解)	..... 0 1
2. 今日は何年の何月何日ですか? 何曜日ですか?	..... 0 1
	年 ..... 0 1
	月 ..... 0 1
	日 ..... 0 1
	曜日 ..... 0 1
3. 私たちが今いるところはどこですか? (自発的に出れば2点, 5秒おいて, 家ですか? 病院ですか? 施設ですか? の中から正しい選択をすれば1点)	..... 0 1 2
4. これから言う3つの言葉を言ってみてください。 あとでまた聞きますのでよく覚えておいてください。 (以下の系列のいずれか1つで, 採用した系列に○印をつけておく)	..... 0 1
1: a) 桜 b) 猫 c) 電車      2: a) 梅 b) 犬 c) 自動車	..... 0 1
5. 100から7を順番に引いてください。 (100-7? それから また7を引くと? と質問する。 (93) ..... 0 1 最初の答えが不正解の場合, 打ち切る) (86) ..... 0 1	
6. 私がこれから言う数字を逆から言ってください。 (6-8-2, 3-5-2-9)      2-8-6 ..... 0 1 (3桁逆唱に失敗したら打ち切る)      9-2-5-3 ..... 0 1	
7. 先ほど覚えてもらった言葉をもう一度言ってみてください。 (自発的に回答があれば各2点, もし回答がない場合, 以下の ヒントを与えて正解であれば1点)	a ..... 0 1 2 b ..... 0 1 2 c ..... 0 1 2
8. これから5つの品物を見せます。それを隠しますので何が あったか言ってください。 (時計, 鍵, タバコ, ペン, 硬貨など必ず相互に無関係なもの)	..... 0 1 2 3 4 5
9. 知っている野菜の名前をできるだけ多く言ってください。 (答えた野菜の名前を右側に記入する。途中で詰まり, 約10秒 待っても出ない場合にはそこで打ち切る) ..... 0 1 2 3 4 5 × 5個までは0点, 6個=1点, 7個=2点, 8個=3点, 9個=4点, 10個=5点	

**20 ~ 30 点** ..... 異常なし

**16 ~ 19 点** ..... 認知症の疑いあり

**11 ~ 15 点** ..... 中程度の認知症

**5 ~ 10 点** ..... やや高度の認知症

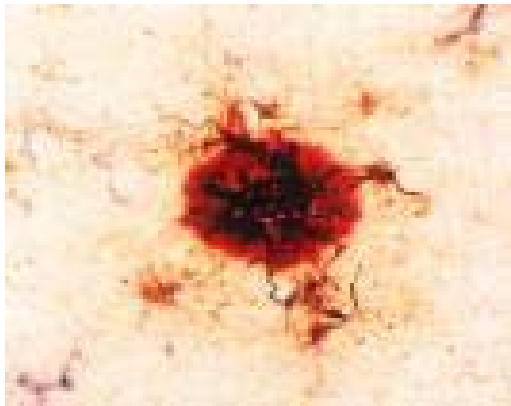
**0 ~ 4 点** ..... 高度の認知症

# Pathology of Alzheimer's disease



**AD brain**

## **Senile plaque**



40- and 42-mer  $A\beta$  peptides

## **Neurofibrillary tangles**

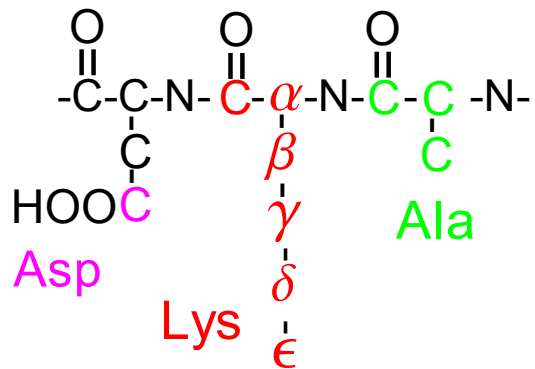


Phosphorylated tau

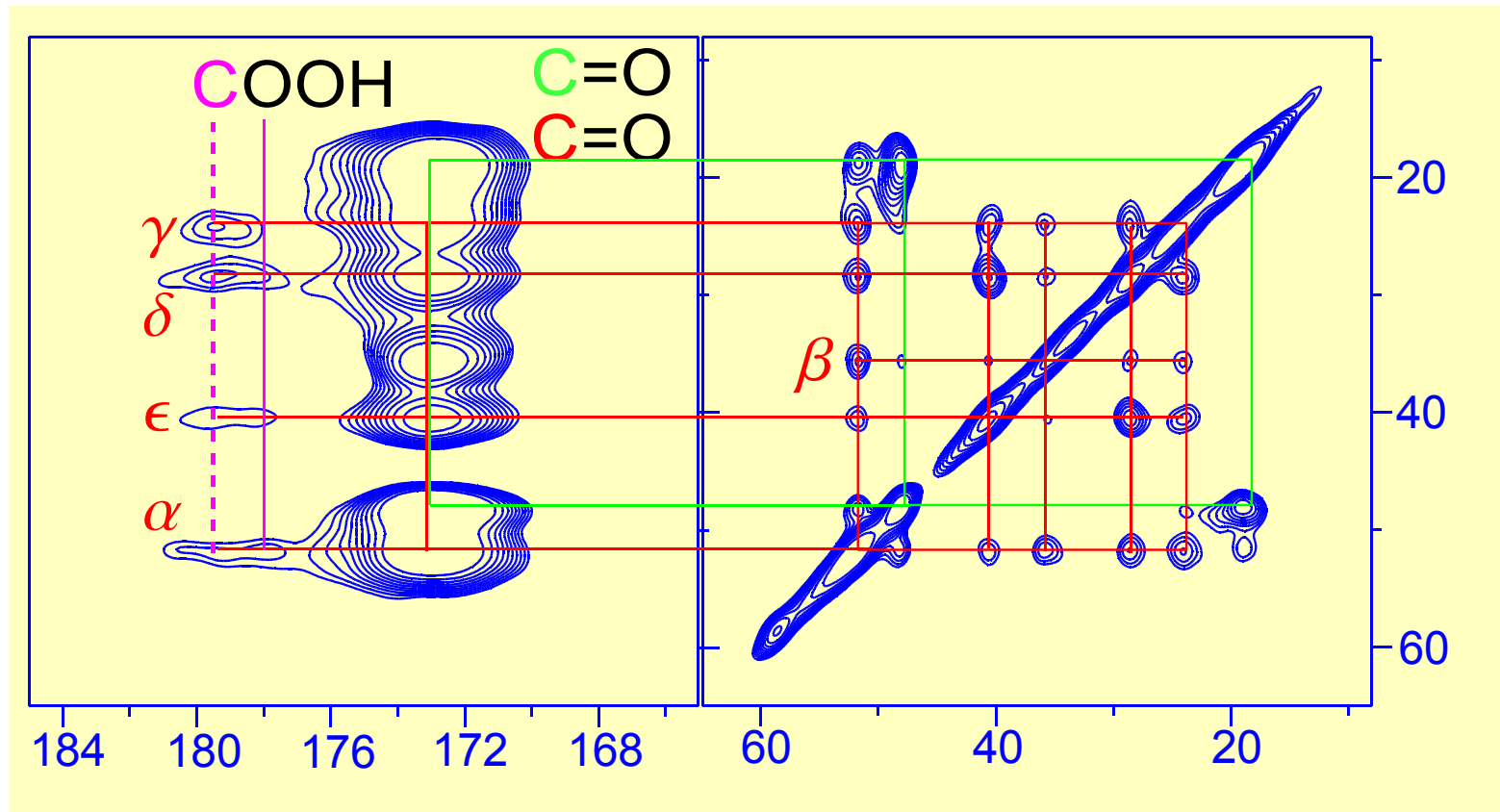




$^{13}\text{C}$ - $^{13}\text{C}$  DARR  
 $\tau = 500\text{ms}$



Selectively labeled  
 E22K-A $\beta$ 42



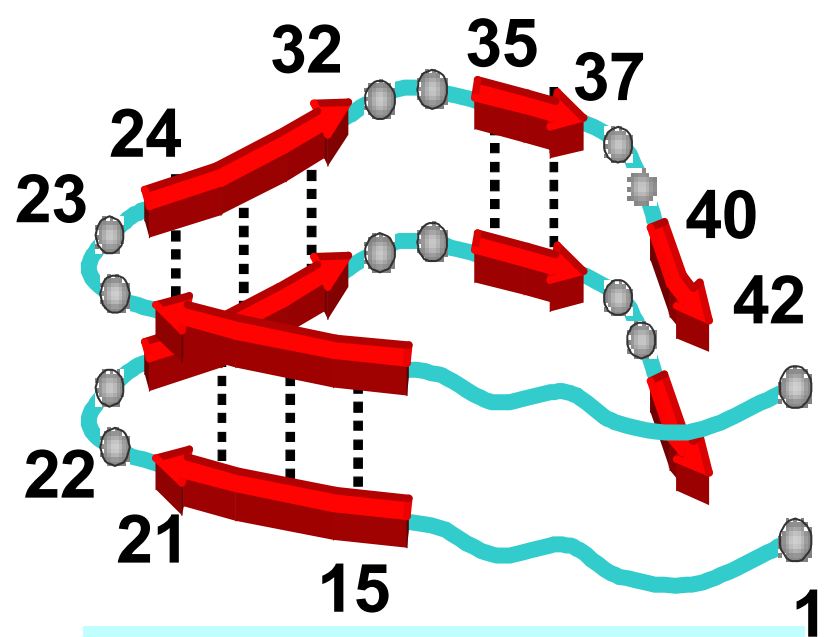
Cross peak between

- 1) Asp-COOH (major) & CH of Val or Ala?
- 2) Asp-COOH & side-chain of Lys?

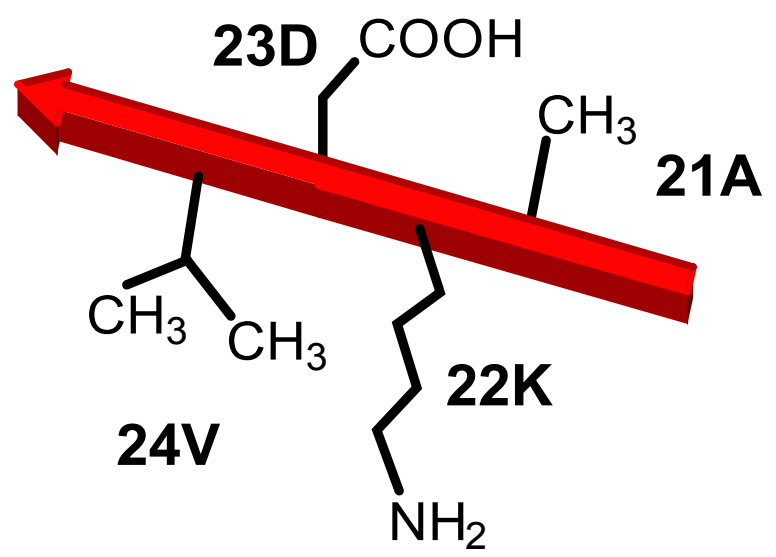
Asp-COOH(major) & Val-CH<sub>3</sub>

Asp-COOH(minor) & side-chain of Lys

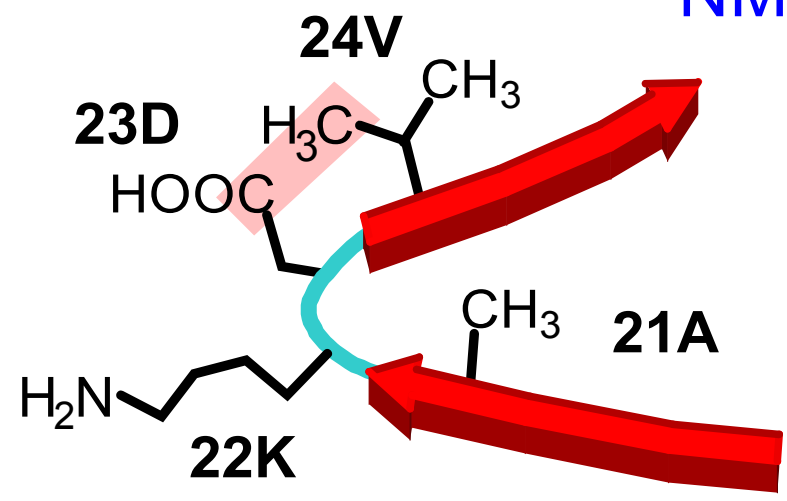
Side chain directions in parallel  $\beta$ -sheet



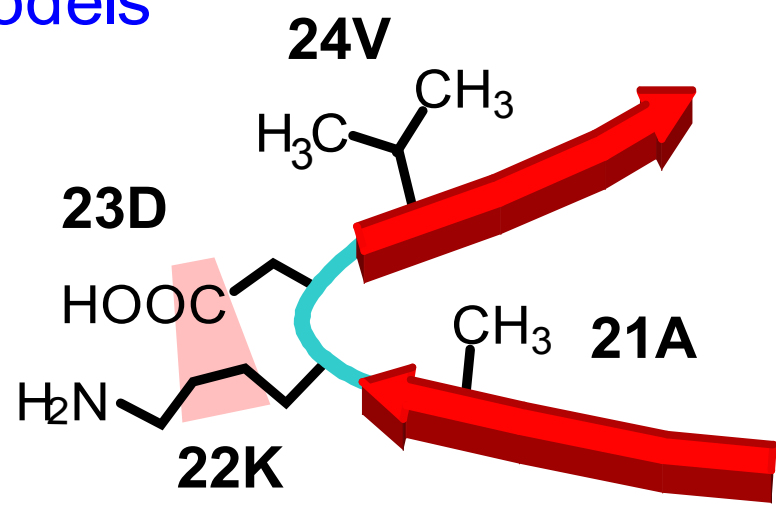
Irie's model based on proline replacement exptl.



NMR models



major conformer



minor conformer