Advantages and disadvantages of a high magnetic field NMR and ^{13}C detection

2012 Jan. 25-26th
Akihabara Satellite campus
of Tokyo Metropolitan Univ.
The 13th RRR-workshop 2011/12

Institute for Protein Research
Osaka University
Takahisa IKEGAMI
池上貴久

Advantages of a higher magnetic field NMR

Higher sensitivity $\propto B_0^{3/2}$

magnetic moment $\propto B_0$

Larmor frequency $\propto B_0$

noise $\propto B_0^{1/2}$

$(S/N)_{950MHz} / (S/N)_{600MHz} = 2$

$$\bar{M}_z = \frac{N\gamma^2\hbar^2 I(I+1)}{3kT} B_0$$

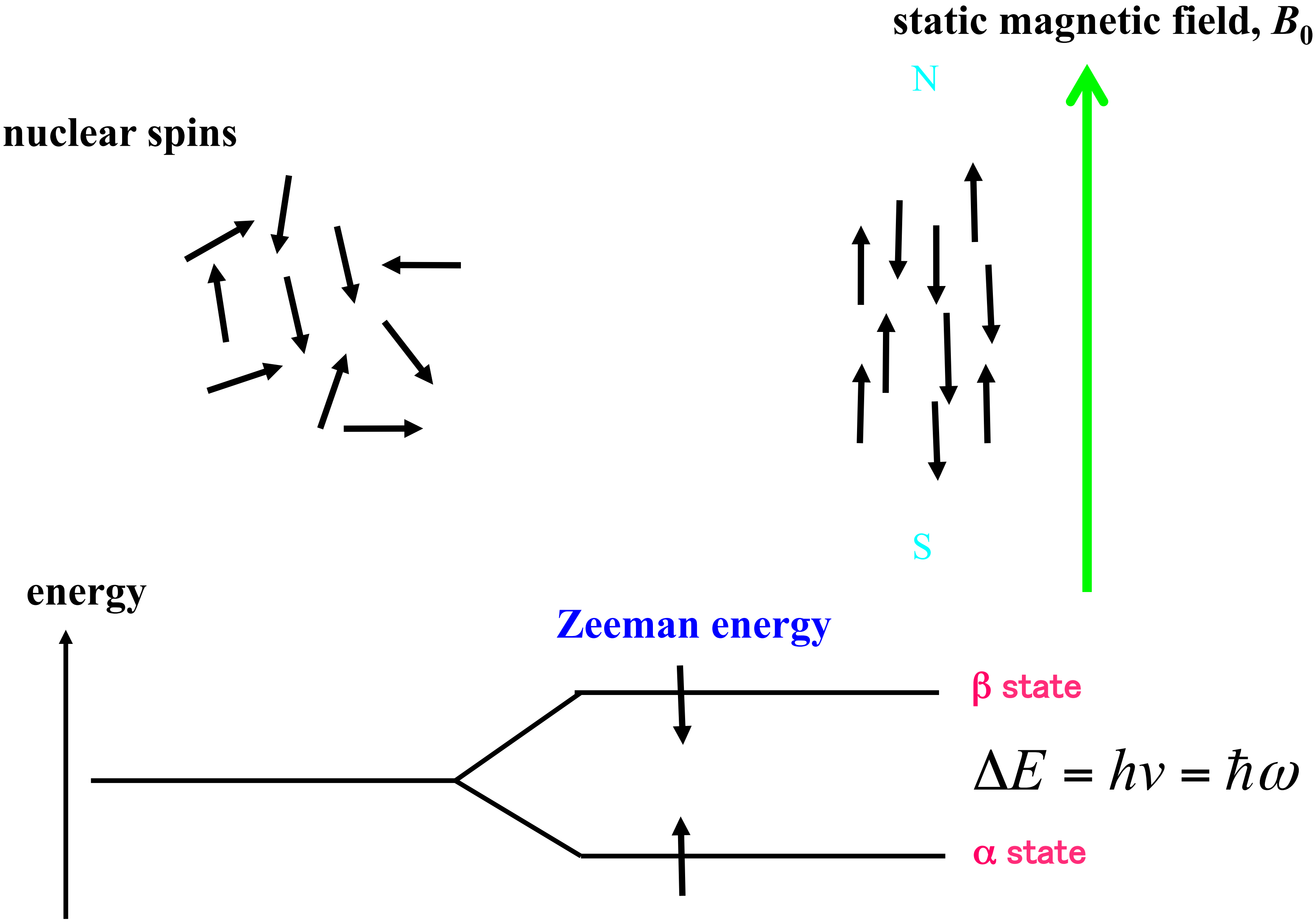
$$\omega_0 = -\gamma B_0$$

Higher resolution in the direct-detection dimension (FID)

The ^{1}H–^{15}N TROSY cross correlated relaxation effect

A higher degree of alignment by anisotropic magnetic susceptibility

Nuclear magnetic moments



static magnetic field, B_0

nuclear spins

energy

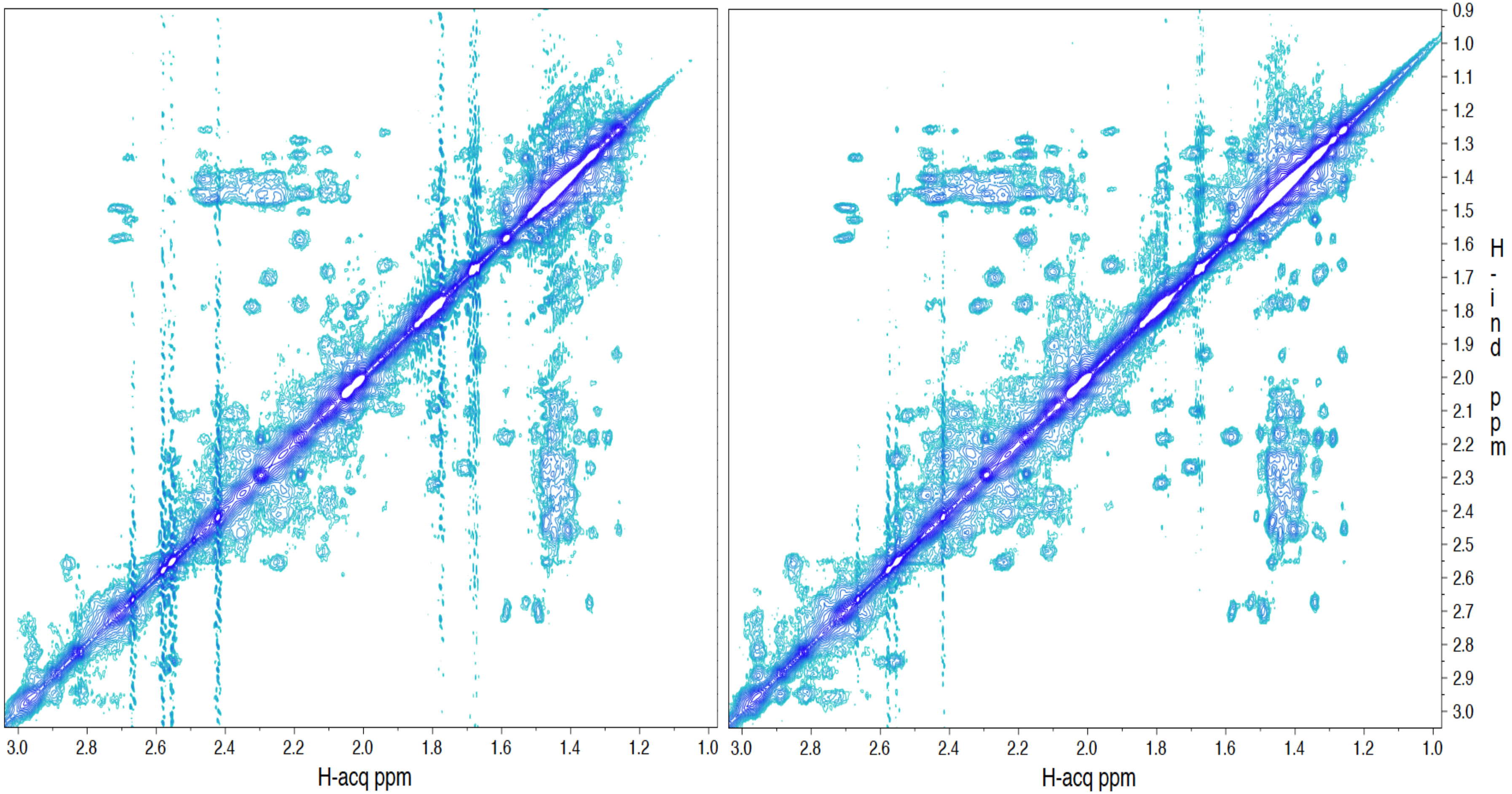
Zeeman energy

β state

$$\Delta E = h\nu = \hbar\omega$$

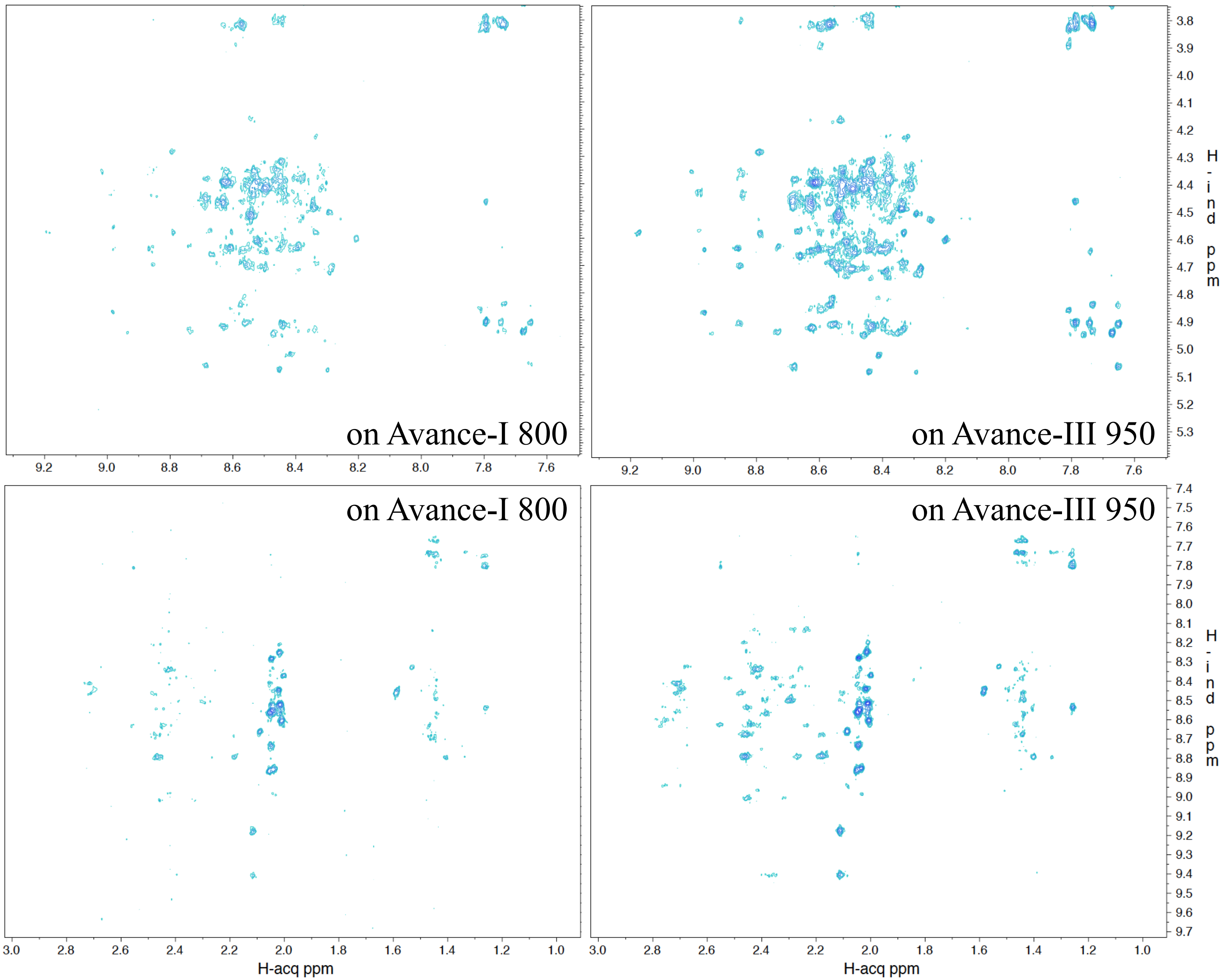
α state

Comparison of particular regions in 2D NOESY spectra

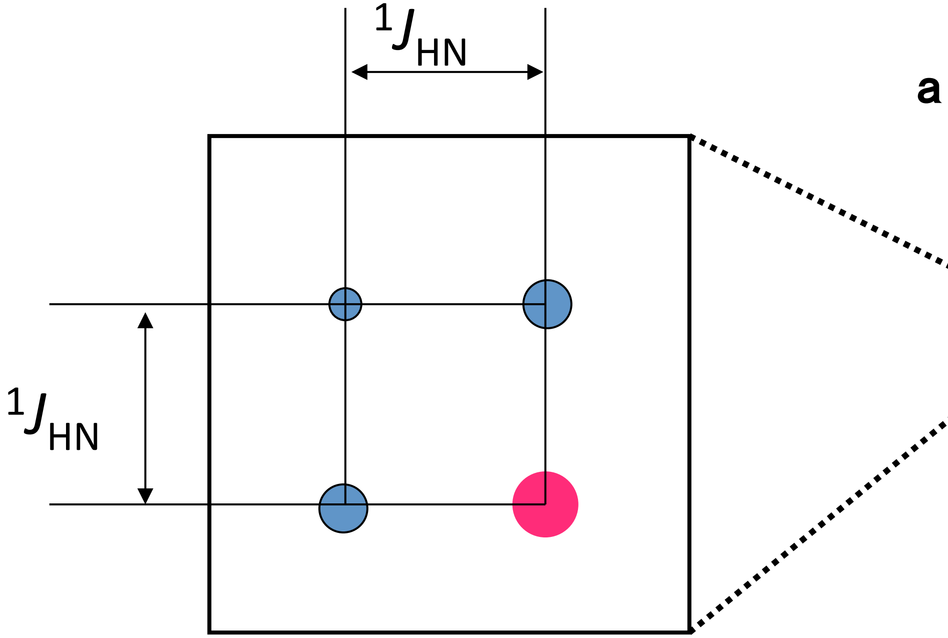


on Avance-I 800
116.7ms (t_1) × 239.6ms (t_2)
Ns = 16

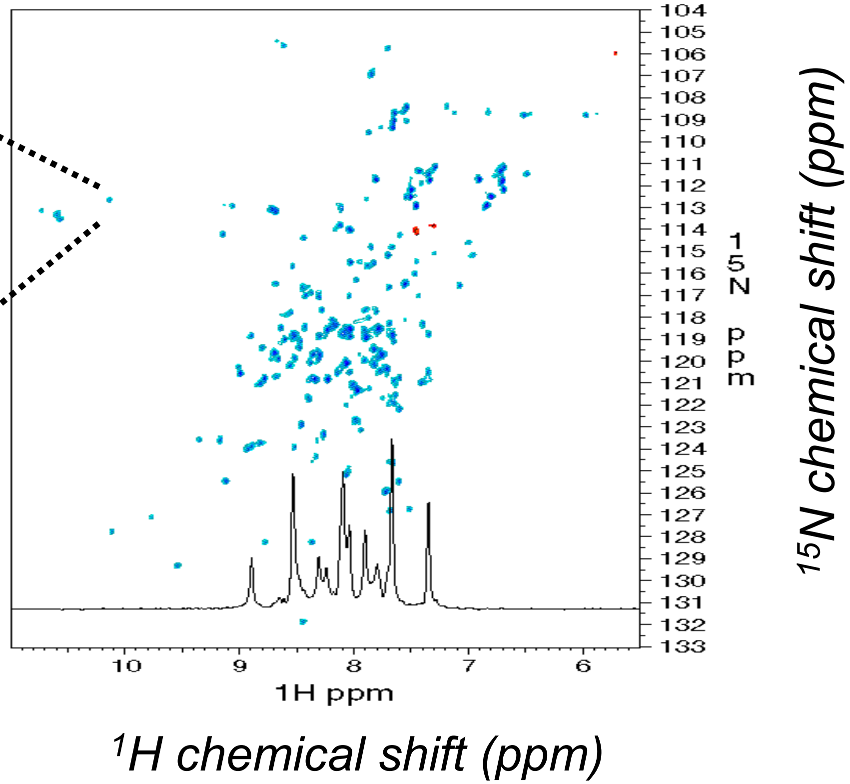
on Avance-III 950
98.3ms (t_1) × 240.3ms (t_2)
Ns = 16


on Avance-I 800
on Avance-III 950
on Avance-I 800
on Avance-III 950
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.5
4.6
4.7
4.8
4.9
5.0
5.1
5.2
5.3
H-ind ppm
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
H-acq ppm
7.4
7.5
7.6
7.7
7.8
7.9
8.0
8.1
8.2
8.3
8.4
8.5
8.6
8.7
8.8
8.9
9.0
9.1
9.2
9.3
9.4
9.5
9.6
9.7
H-ind ppm

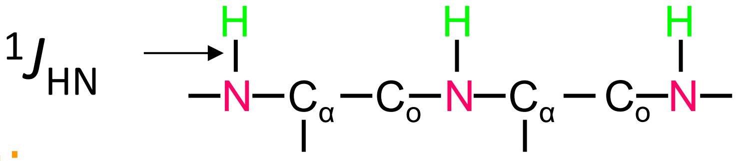
Detection of large proteins by TROSY



a 2D ^{1}H–^{15}N correlation spectrum

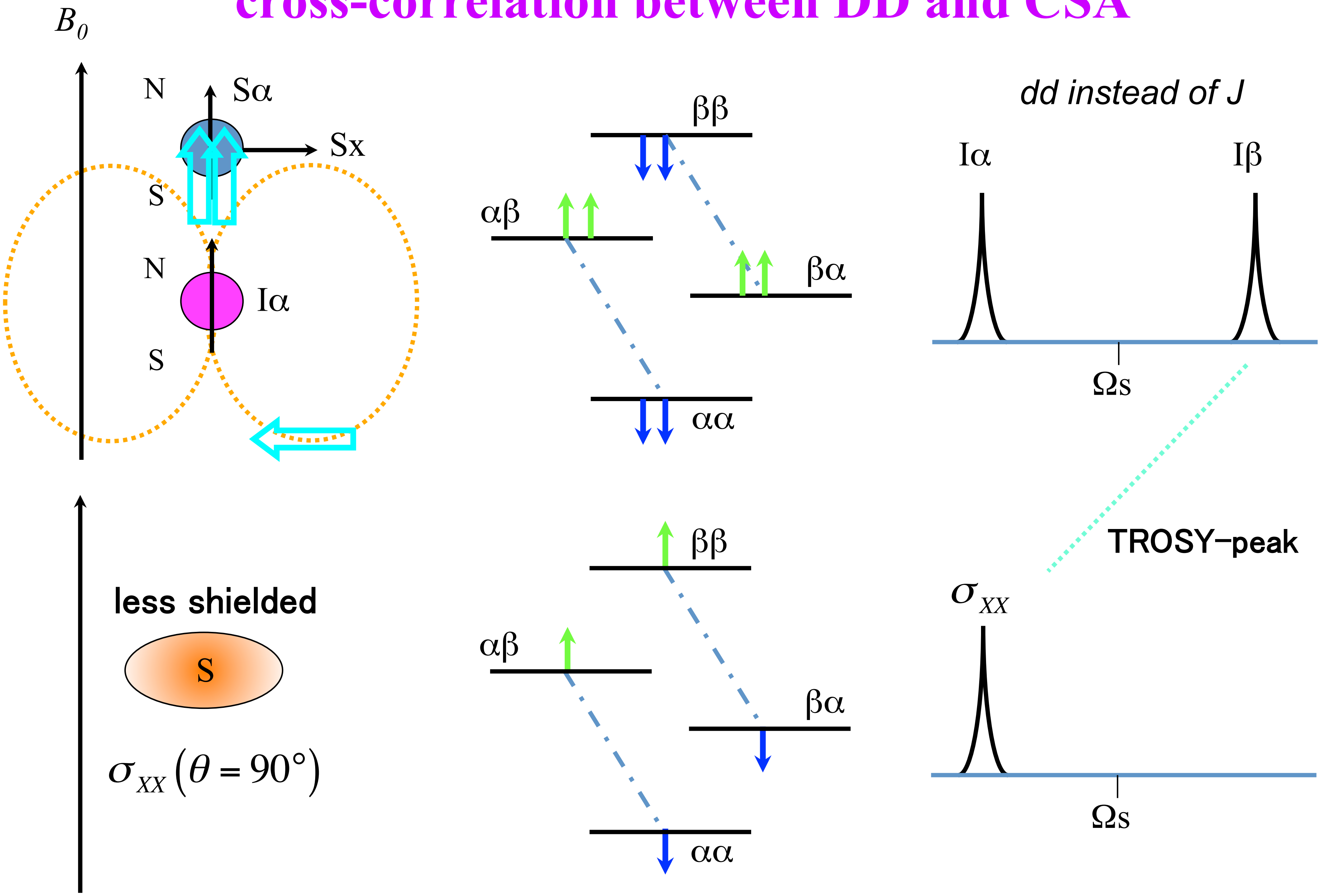


available to 800 kDa?
the maximum on 1 GHz NMR?

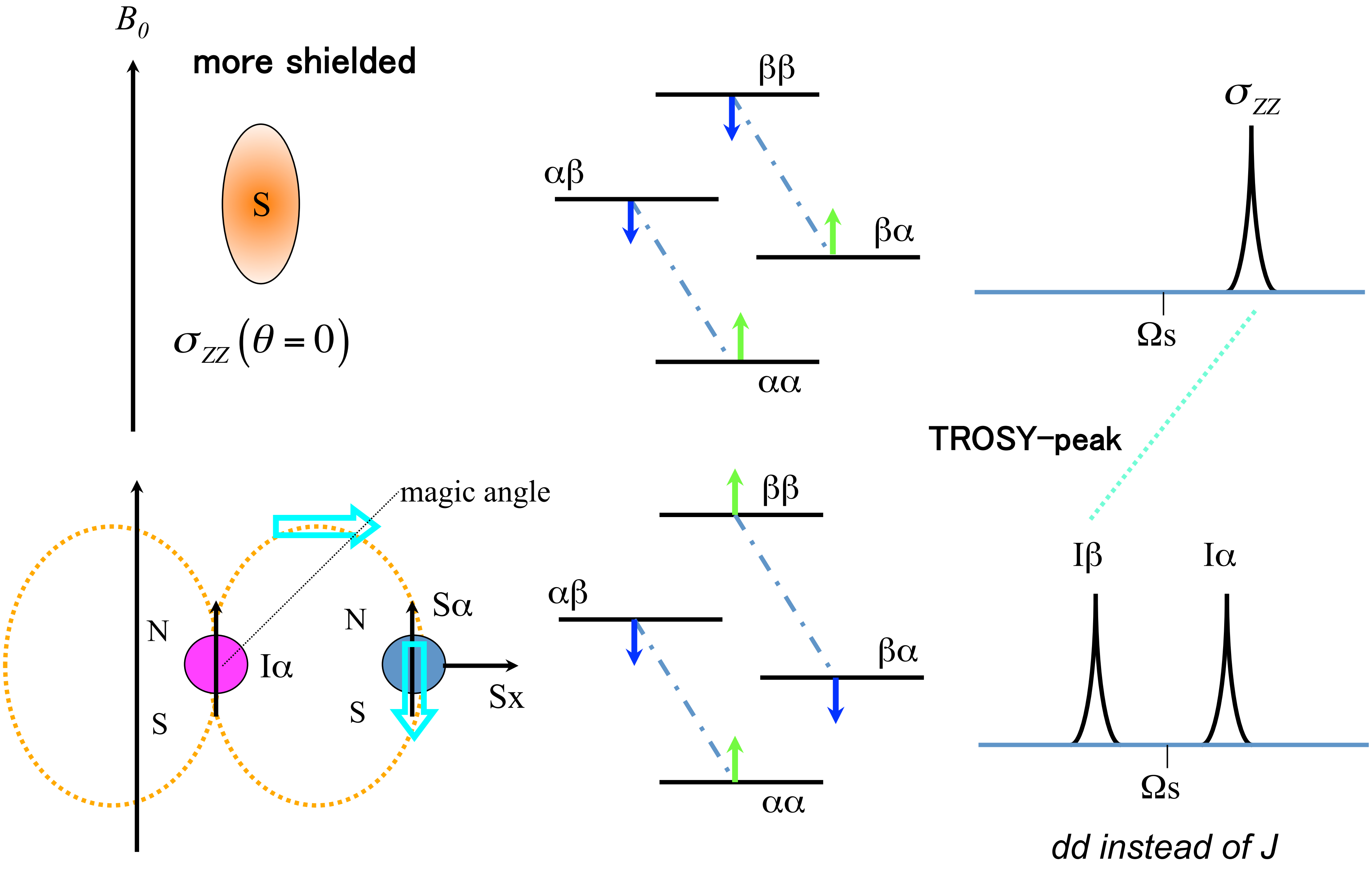


applicable to amide, methyl, and aromatic groups

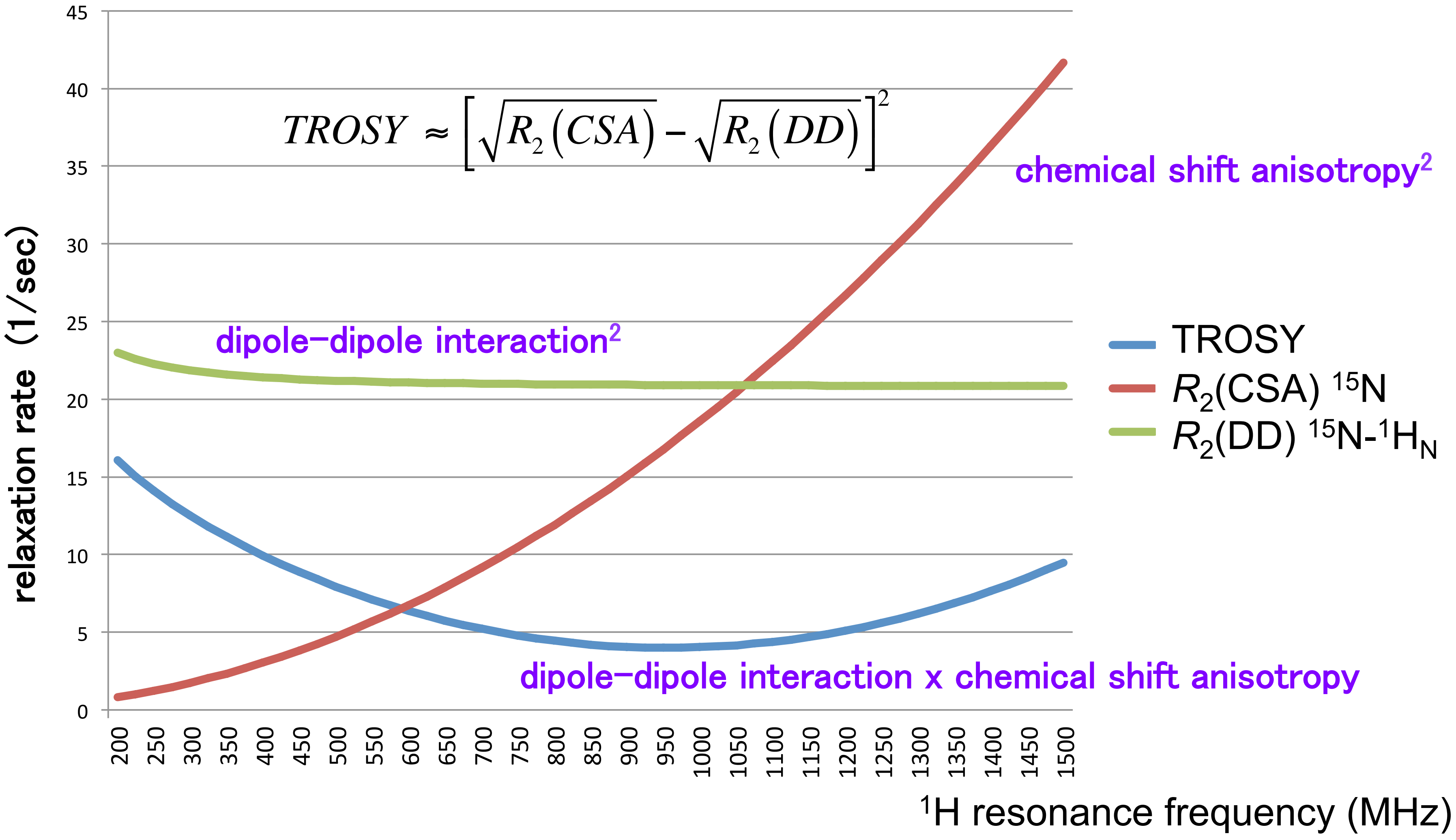
cross-correlation between DD and CSA



when the molecule rotates by 90° (I-S on the horizontal)



The DD/CSA TROSY effect of ^{15}N–^{1}H depends on B_0.



$$TROSY \approx \left[\sqrt{R_2(CSA)} - \sqrt{R_2(DD)}\right]^2$$

chemical shift anisotropy^2

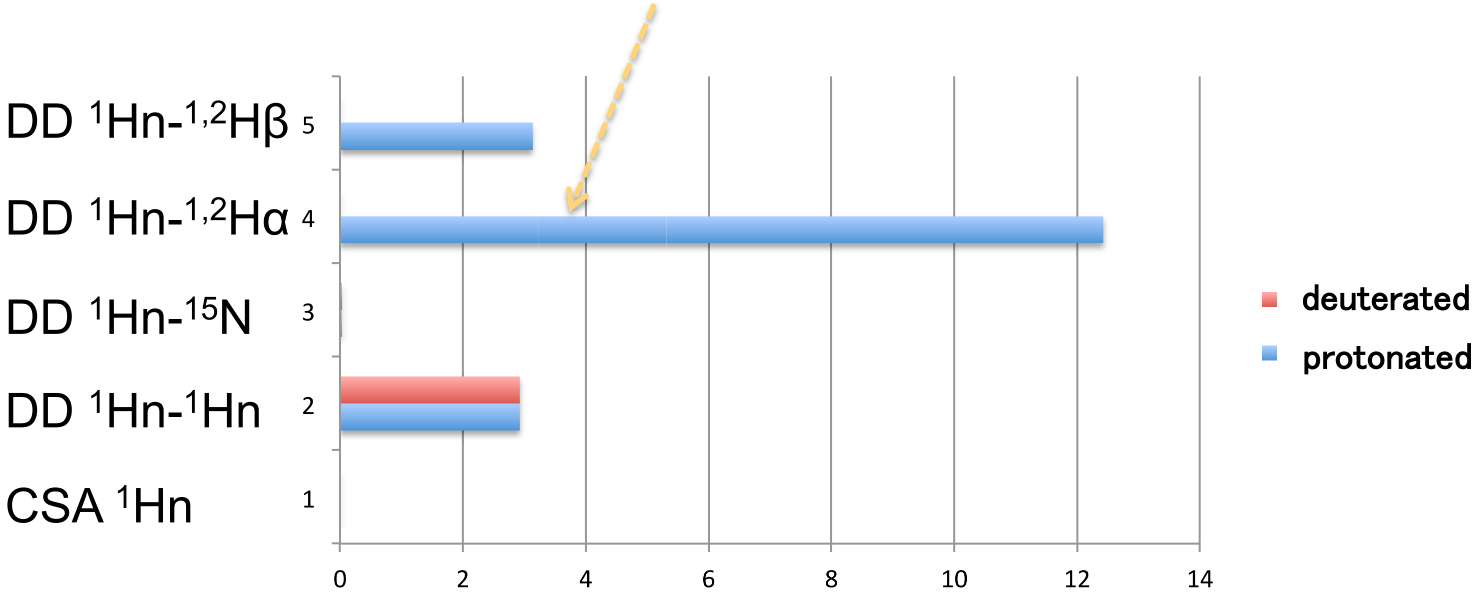
dipole–dipole interaction^2

dipole–dipole interaction x chemical shift anisotropy

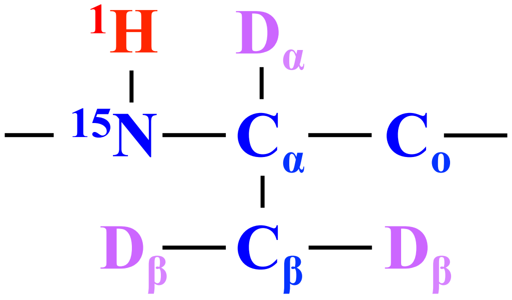
τ_r = 20 ns (~50 kDa), $\theta_{csa\text{-}dd}$ = 15°

The T_1 relaxation of ^1H_N becomes slower in deuterated proteins.

The *dd* relaxation rate with ^{2}Hα is ~1/7000 ?
A longer repetition-delay is required.



longitudinal auto-relaxation rate ρ_1 (1/sec)
(assuming no cross-relaxation as in SOFAST HMQC)
(500 MHz ^{1}H) τ_r =20 ns (~50 kDa)



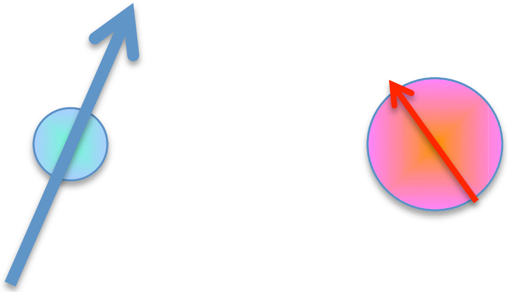
Good for TROSY, since the α and β states of ^1H_N are maintained for a long time.

Advantages and disadvantages of ^{13}C-direct detection (FID)

$$\gamma_{1H} = 26.75\times10^{7}\ (\frac{1}{T\cdot s})$$

$$\gamma_{13C} = 6.73\times10^{7}\ (\frac{1}{T\cdot s})$$

$\mu_{1H} : 2.79\mu_N$ $\mu_{13C} : 0.70\mu_N$



The gyromagnetic ratio of γ_{13C} is about 1/4 of γ_{1H}.

$$\mu_N : the\ nuclear\ Borh\ magneton = 5.05\times10^{-27}\left(\frac{J}{T}\right)$$

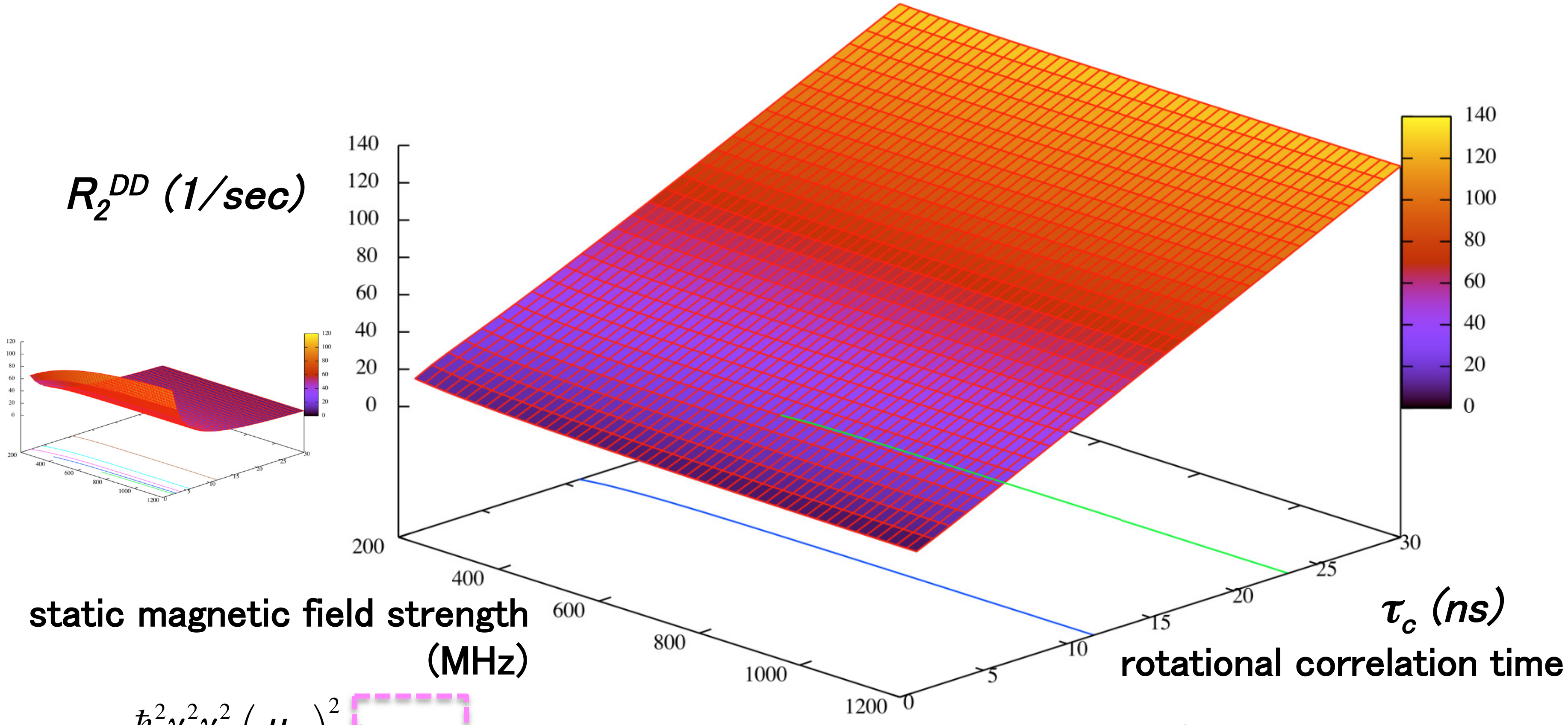
Good → The dipole-dipole T_2 relaxation is slow, particularly when ^{2}H is bound. Therefore, the ^{13}C detection provides narrower line-widths, being suitable for large and metallo-proteins.

$$R(dd) \propto \gamma_I^2\cdot\gamma_S^2\cdot S(S+1)$$

Bad → The sensitivity is low.

→ Slower T_1 relaxation requires a longer repetition-delay.

The T_2 relaxation by DD does not depend so much on B_0.



$$R_2^{DD} = \frac{\hbar^2\gamma_I^2\gamma_S^2}{8r^6}\left(\frac{\mu_0}{4\pi}\right)^2\left\{\boxed{4J(0)} + J(\omega_I-\omega_S) + 3J(\omega_I) + 6J(\omega_S) + 6J(\omega_I+\omega_S)\right\}$$

$$J(\omega) = \frac{2}{5}\frac{\tau_c}{1+\omega^2\tau_c^2}$$

I (^{13}Cα) is observed.

If ^{13}C FID is sampled for a sufficiently long time, the resolution represented by ppm is higher under a higher static magnetic field.

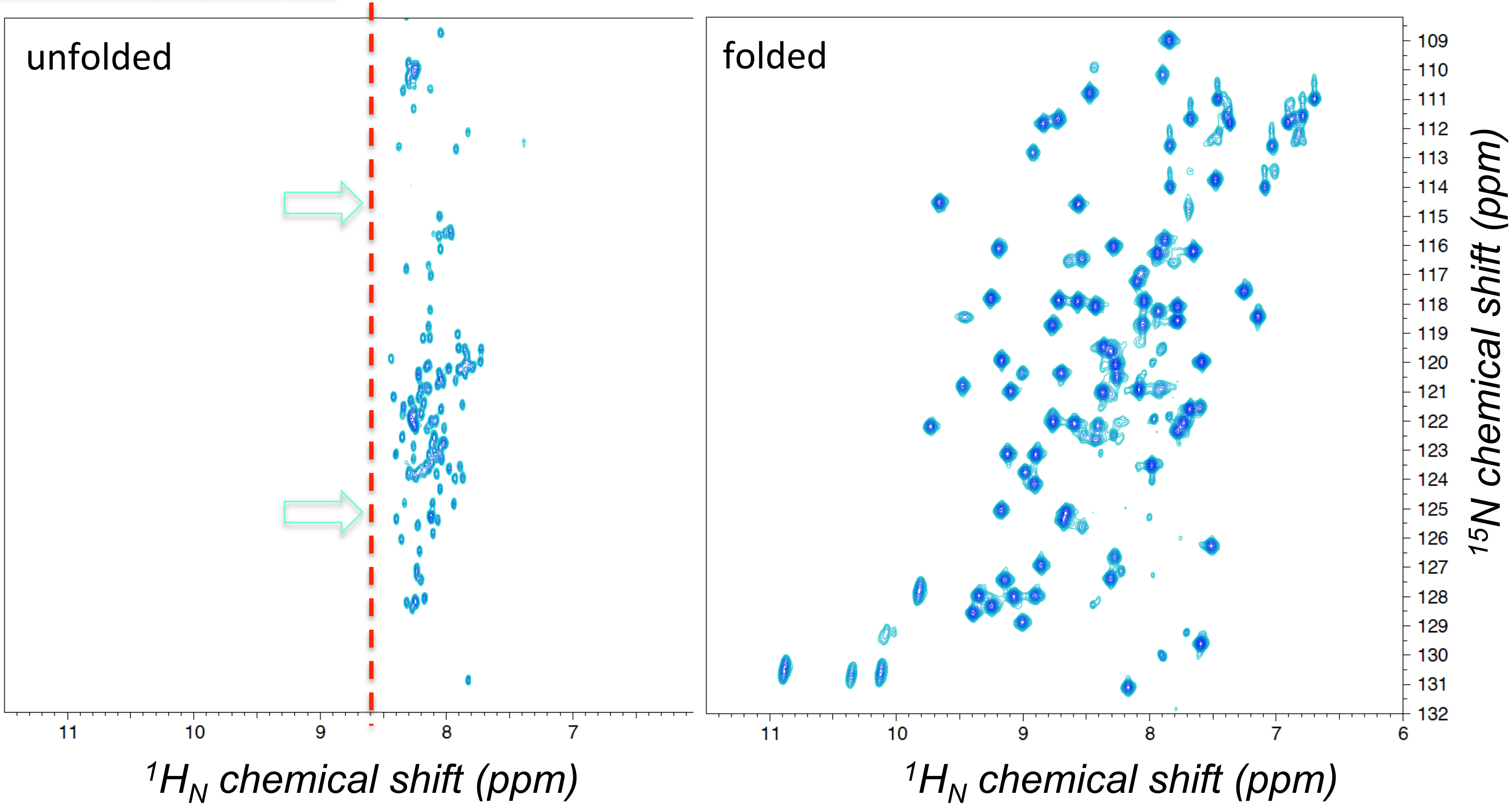
^{13}Cα-^{1}Hα 2-spin system 1.09 Å
DD alone is considered.

Disordered proteins exhibit a narrower distribution of ^1H_N chemical shifts.

寒川作

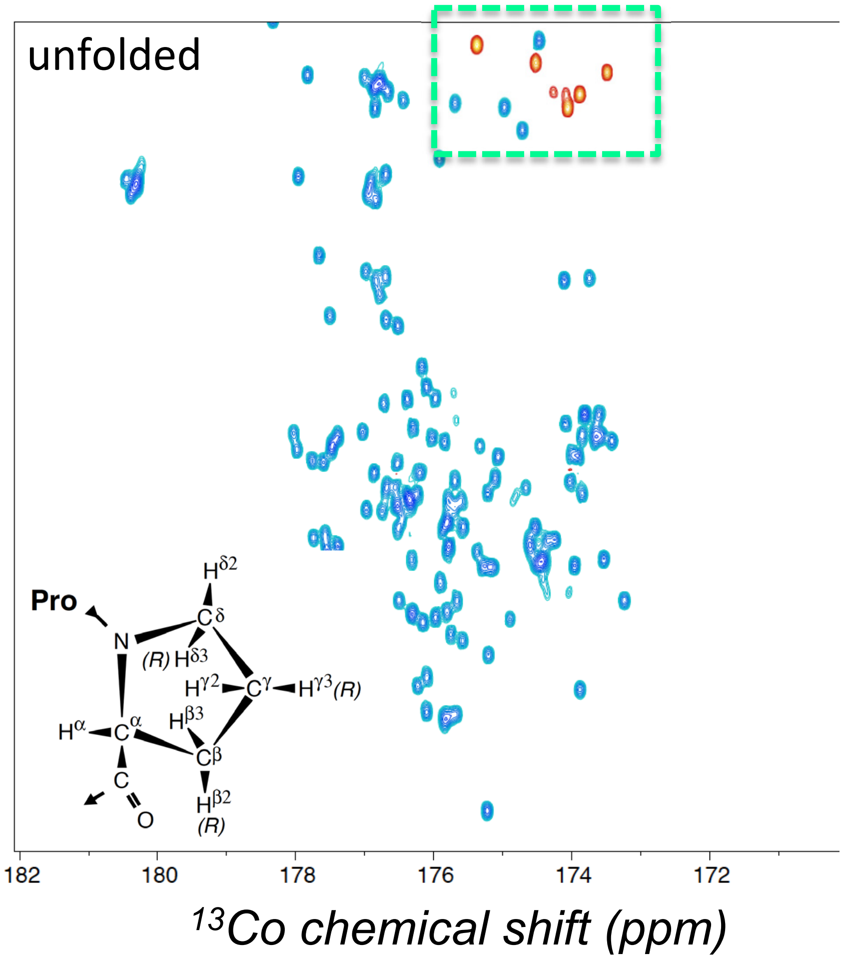
2D ^1H–^{15}N HSQC

a cliff at 8.6ppm

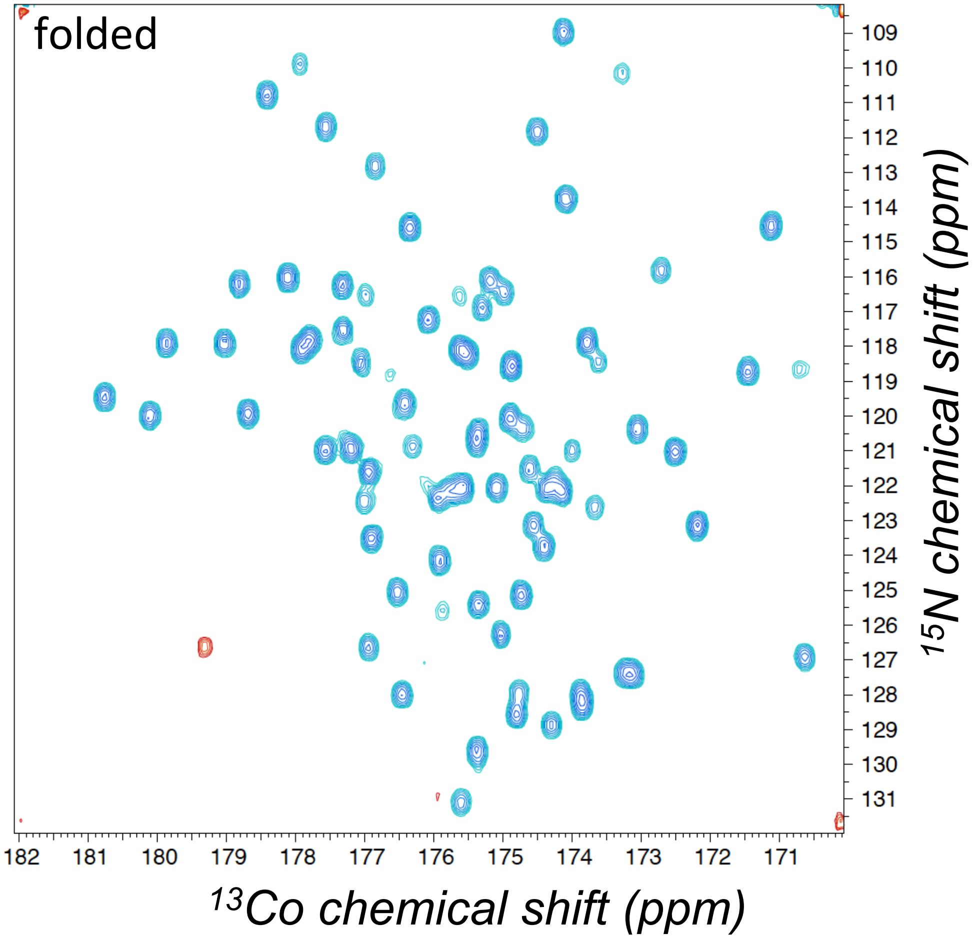


The hetero-nuclei like ^{15}N and ^{13}C have wider C.S. distributions even in unfolded proteins.

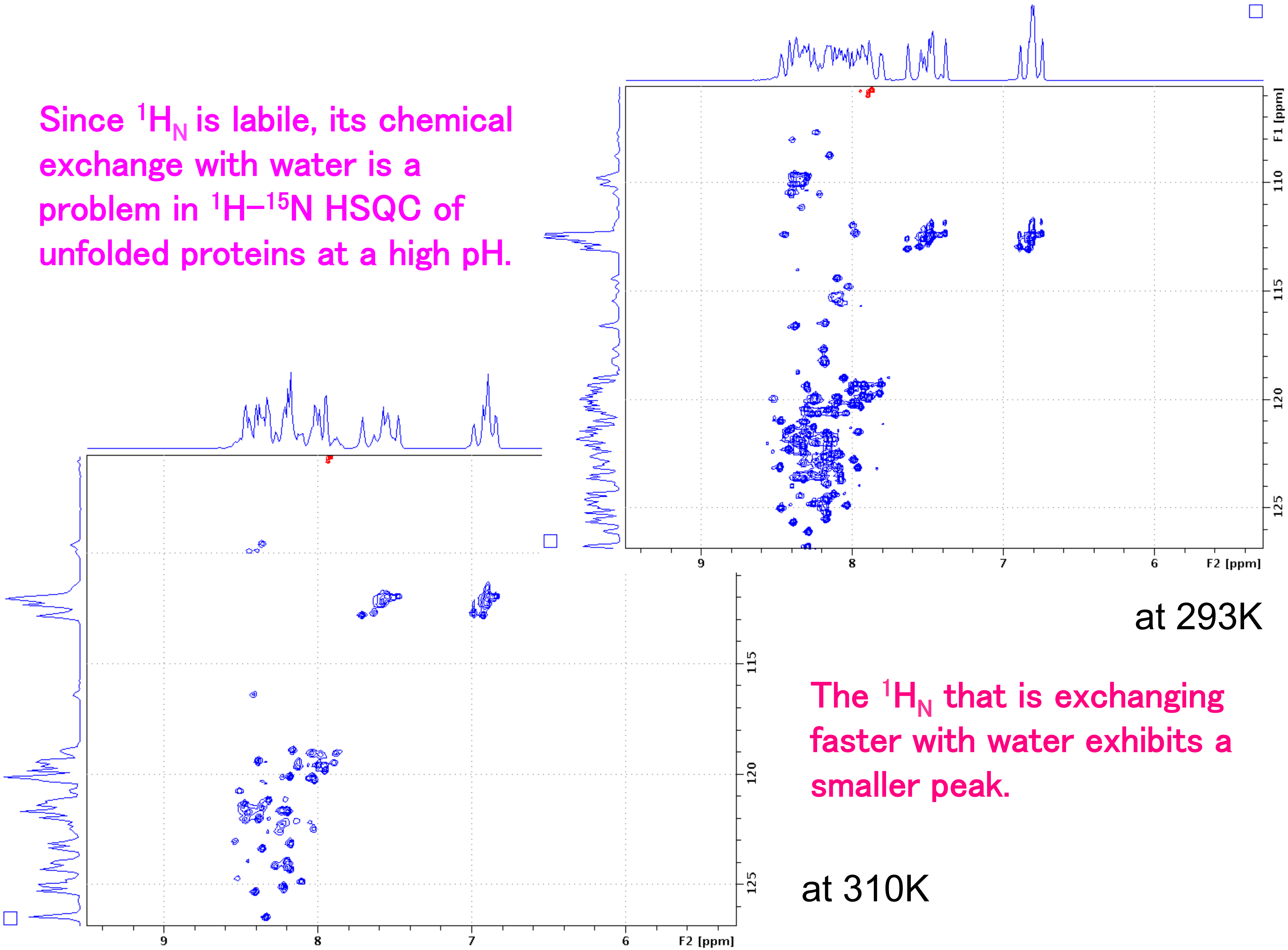
2D CONCO



The projection along the $^{1}H_N$ dimension in 3D HNCO



Since $^{1}H_{N}$ is labile, its chemical exchange with water is a problem in ^{1}H–^{15}N HSQC of unfolded proteins at a high pH.



at 293K

The $^{1}H_{N}$ that is exchanging faster with water exhibits a smaller peak.

at 310K

Low sensitivity in the ^{13}C detection

$$\frac{S}{N} \propto Conc \cdot \gamma_{exc} \cdot \gamma_{obs}^{\frac{3}{2}} \cdot B_0^{\frac{3}{2}} \cdot N_{scan}^{\frac{1}{2}}$$

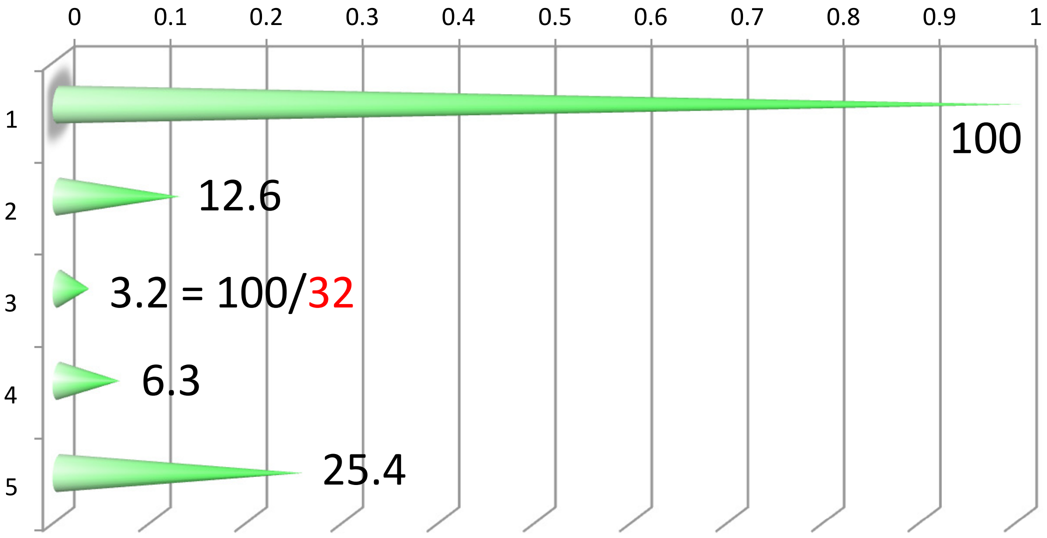
start with ^{1}H
end (FID) with ^{1}H
B_0: 600 MHz
a normal probe

end (FID) with ^{13}C

start with ^{13}C

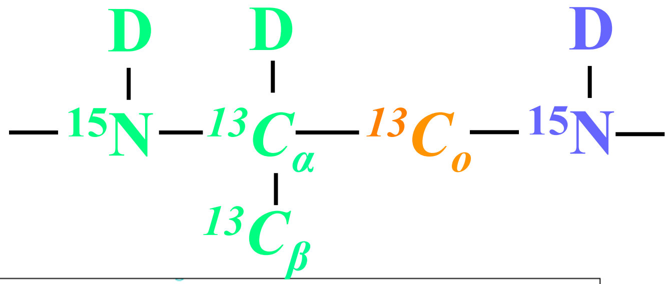
B_0: 950 MHz

a cryogenic probe (inverse)

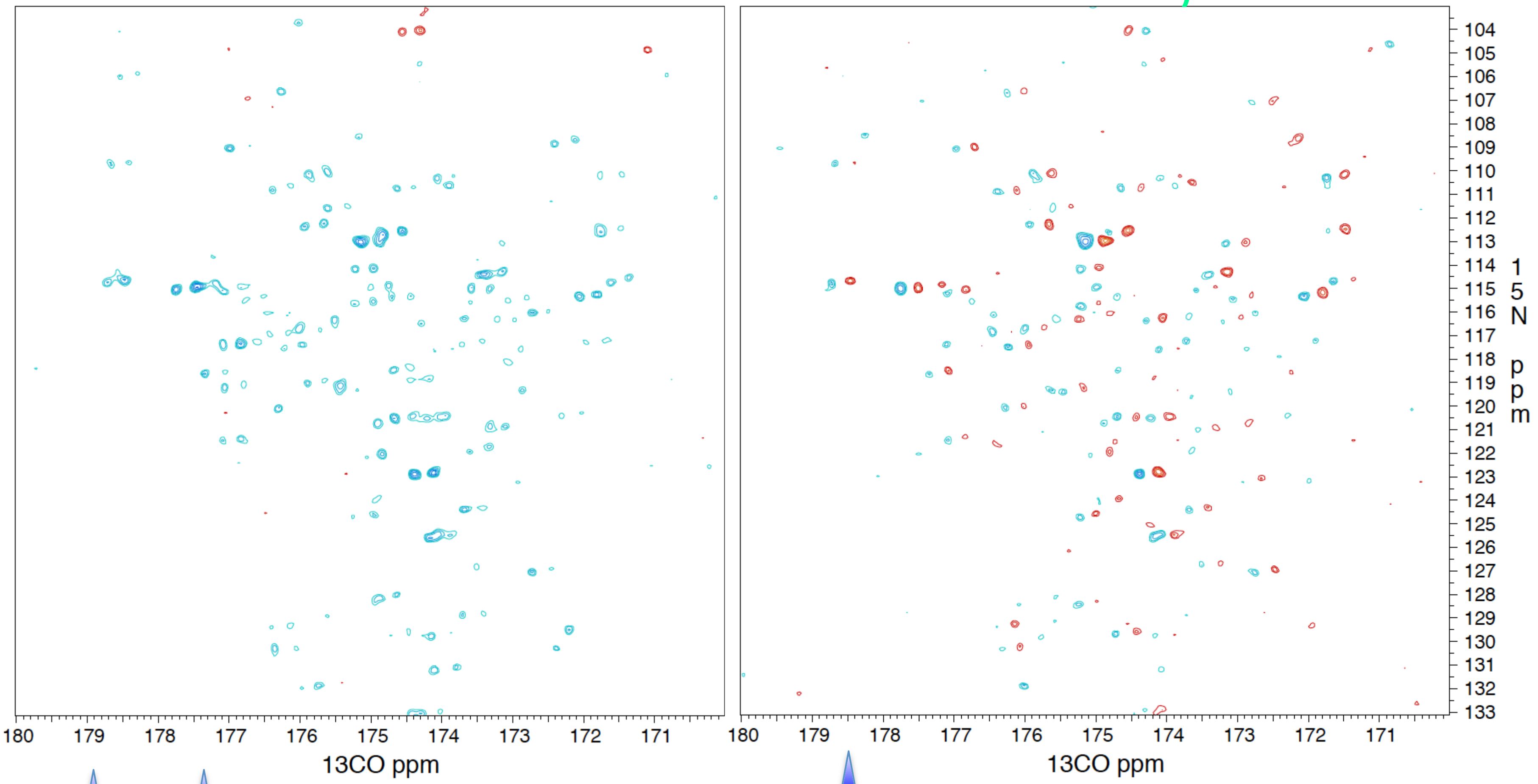


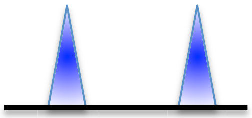
4 x higher concentration
or
16 x data accumulation (NS)
or
starting with ^{1}H (not applicable to 100% deuteration)

IPAP-process in ^{13}C detection

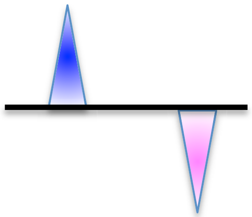


$$-{}^{15}\mathrm{N}(\mathrm{D})-{}^{13}C_{\alpha}(\mathrm{D})({}^{13}C_{\beta})-{}^{13}C_{o}-{}^{15}\mathrm{N}(\mathrm{D})-$$



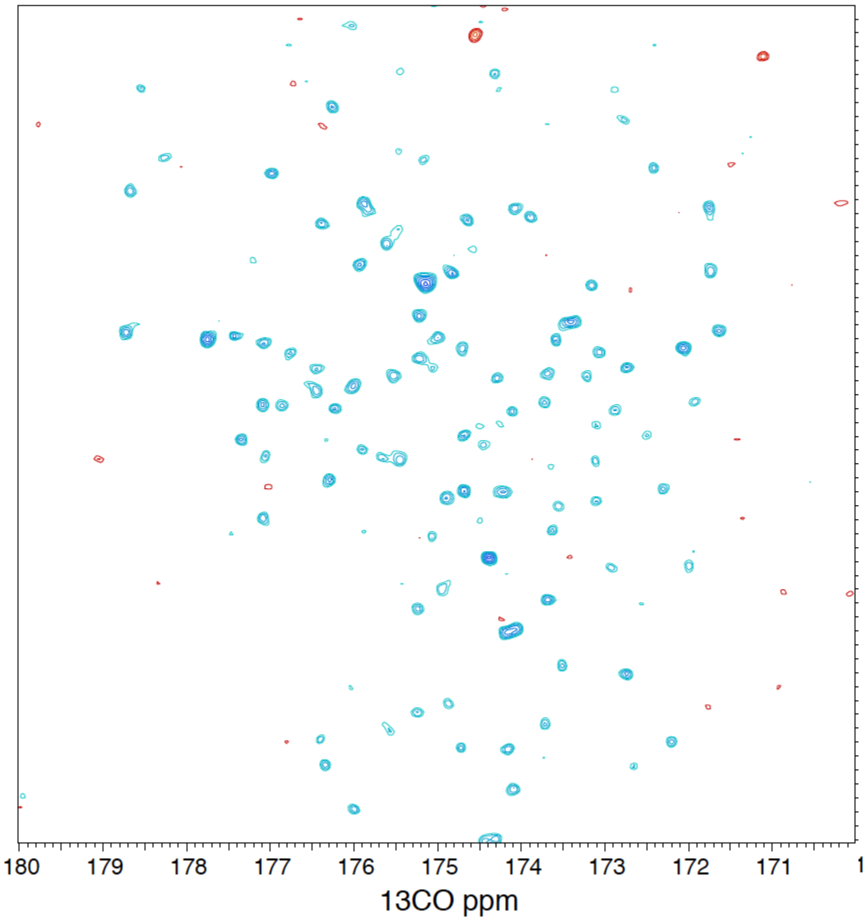


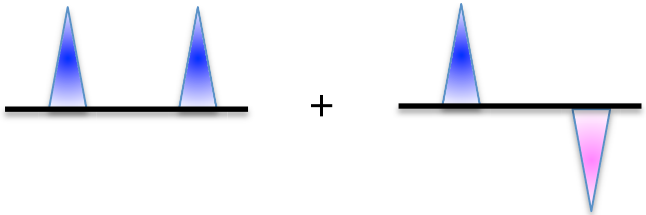
in-phase



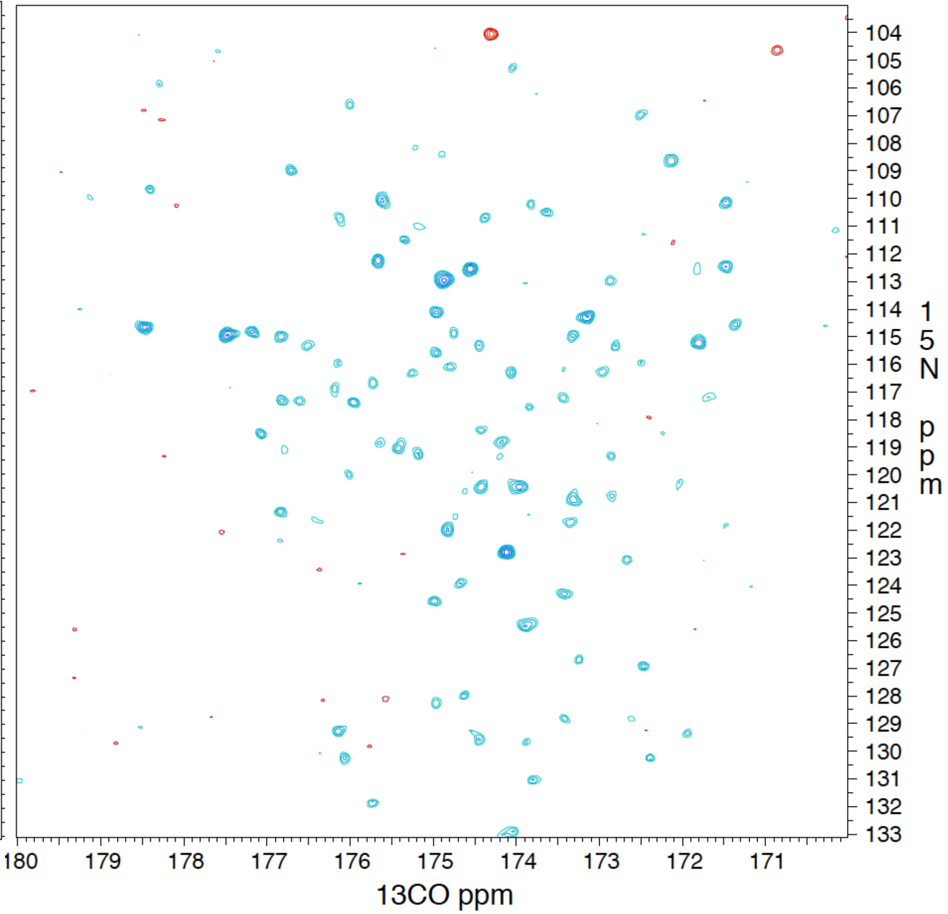
anti-phase

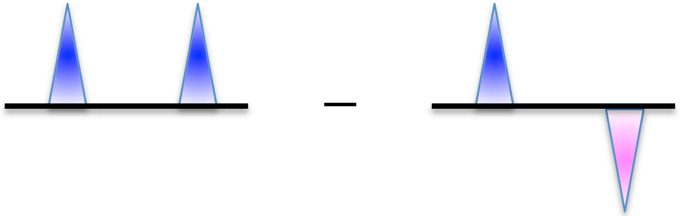
in-phase + anti-phase



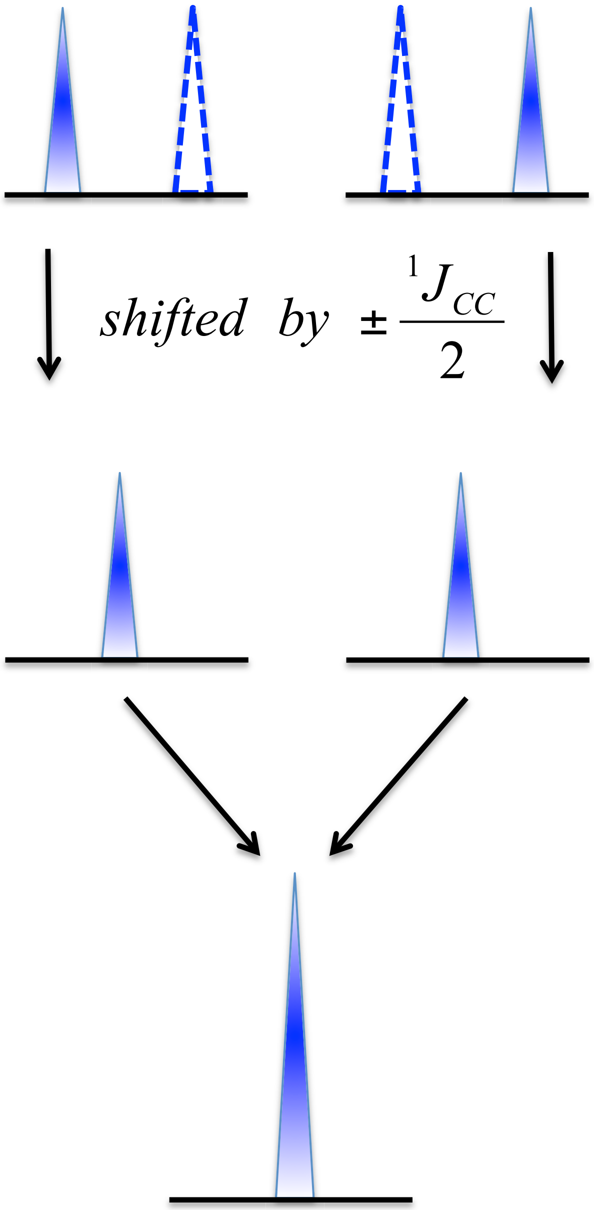


in-phase - anti-phase

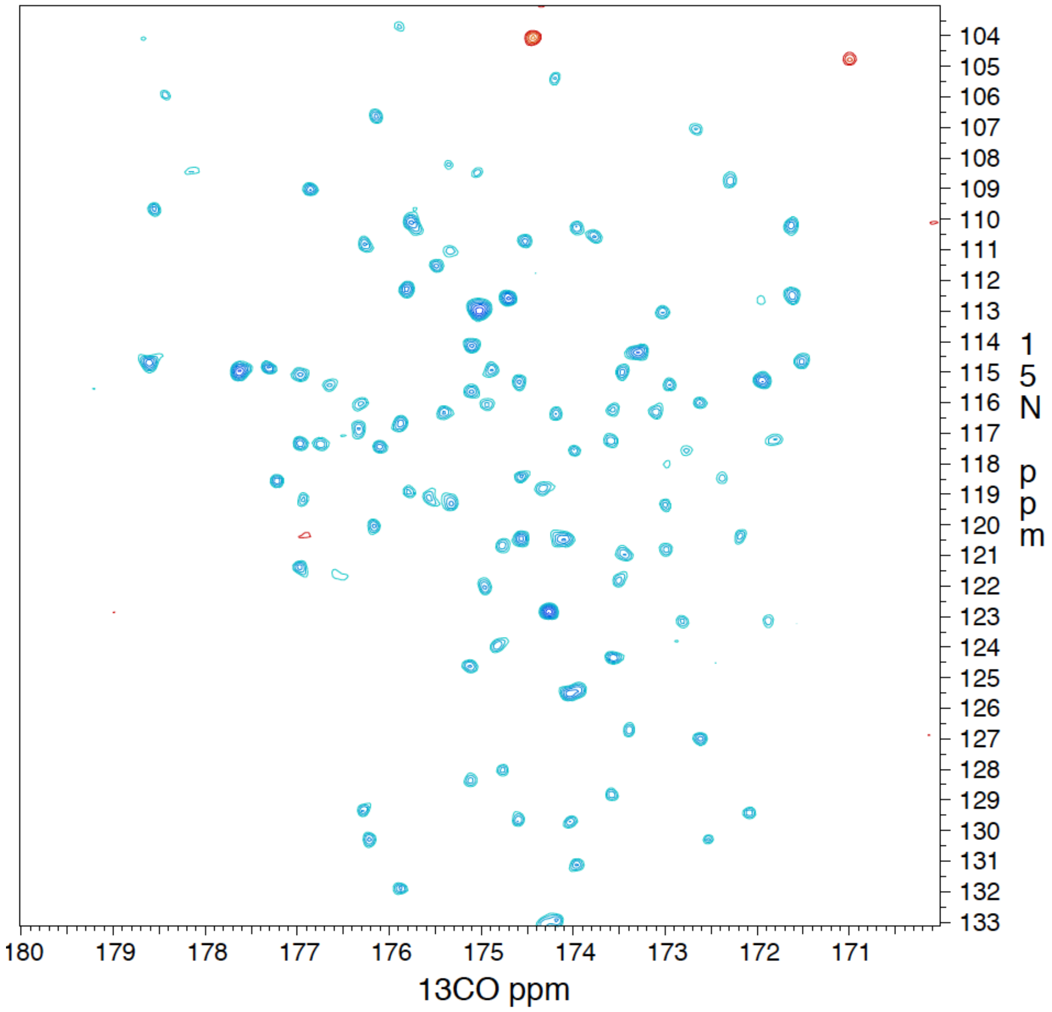




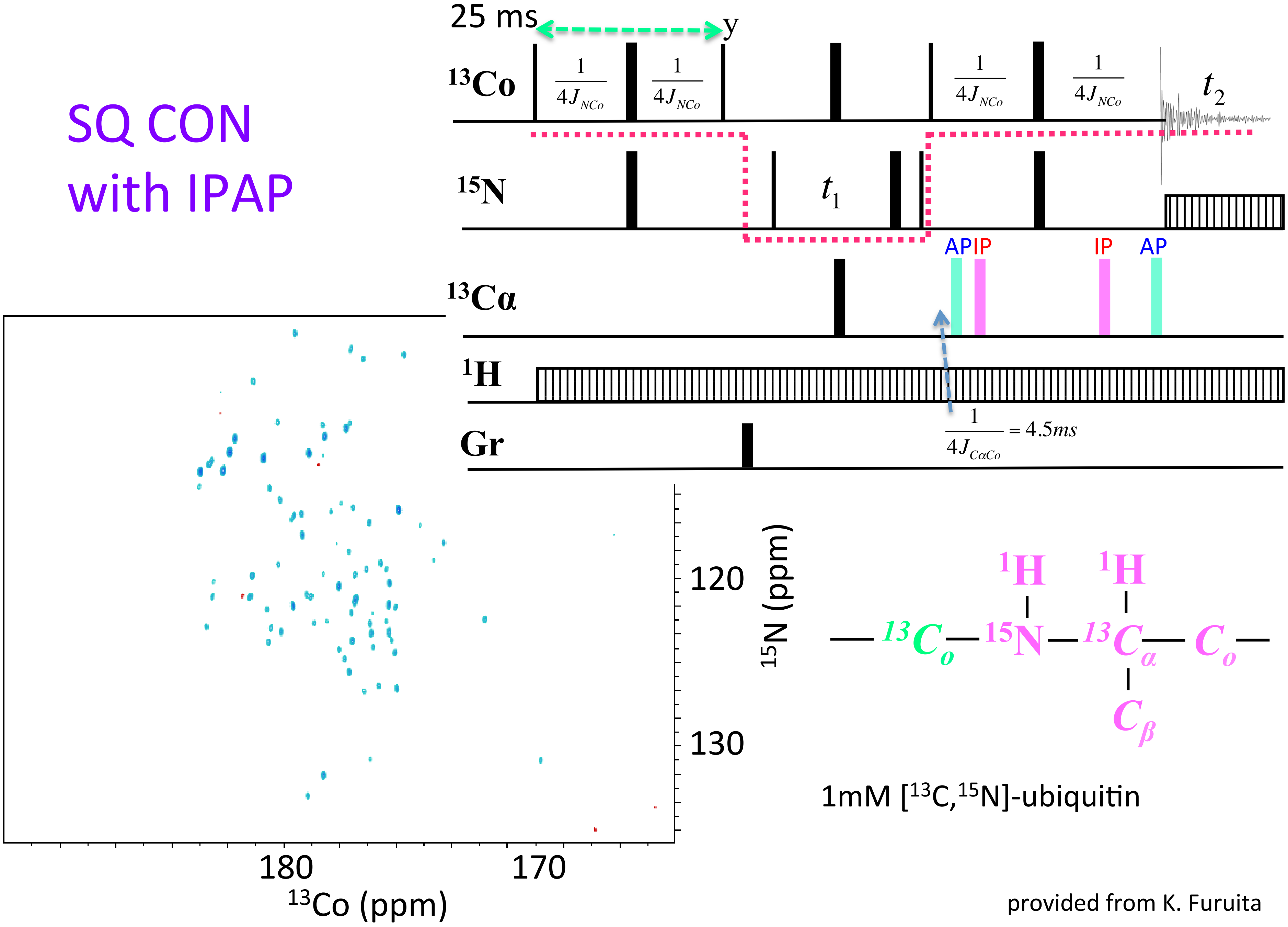
virtual decoupling



shifted by $\pm \frac{^{1}J_{CC}}{2}$



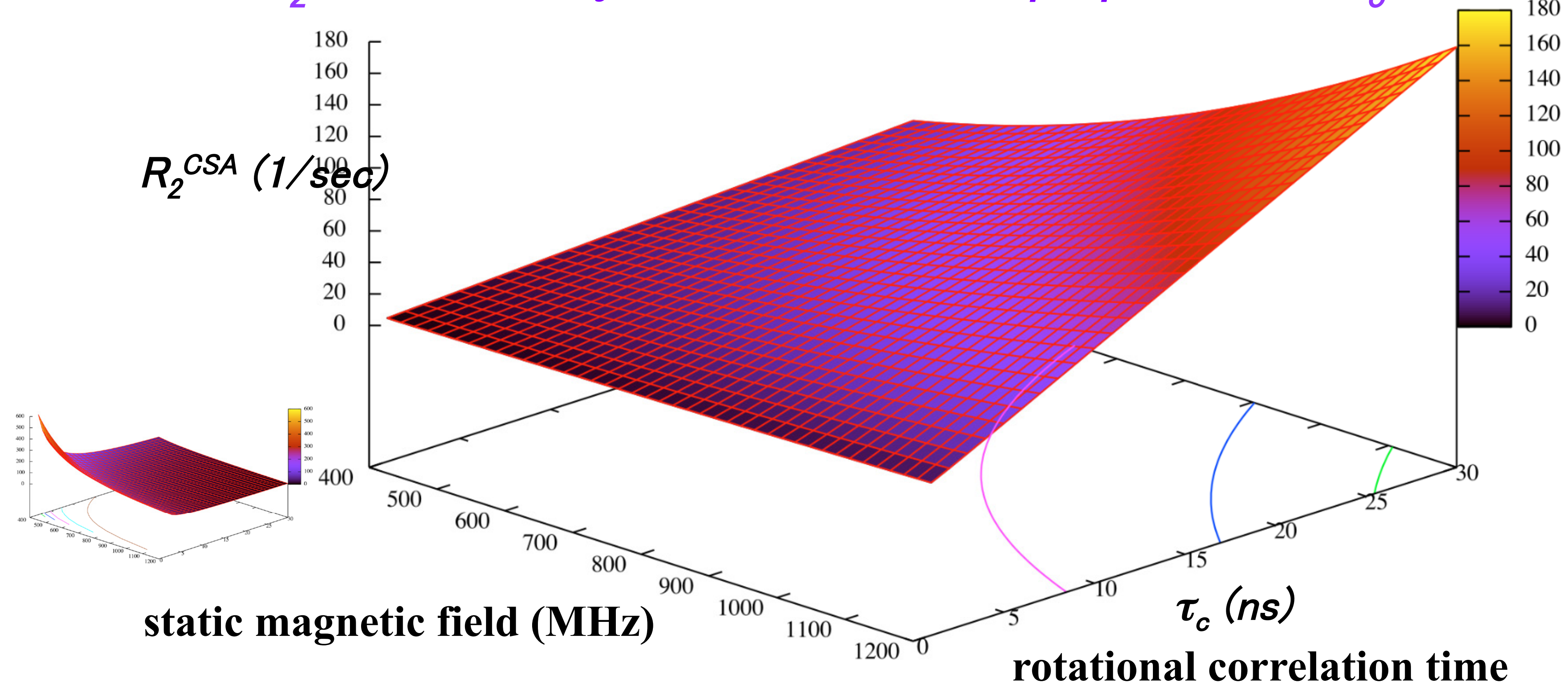
SQ CON with IPAP



1mM [^{13}C,^{15}N]-ubiquitin

provided from K. Furuita

The T_2 relaxation by CSA increases in proportion to B_o^2.



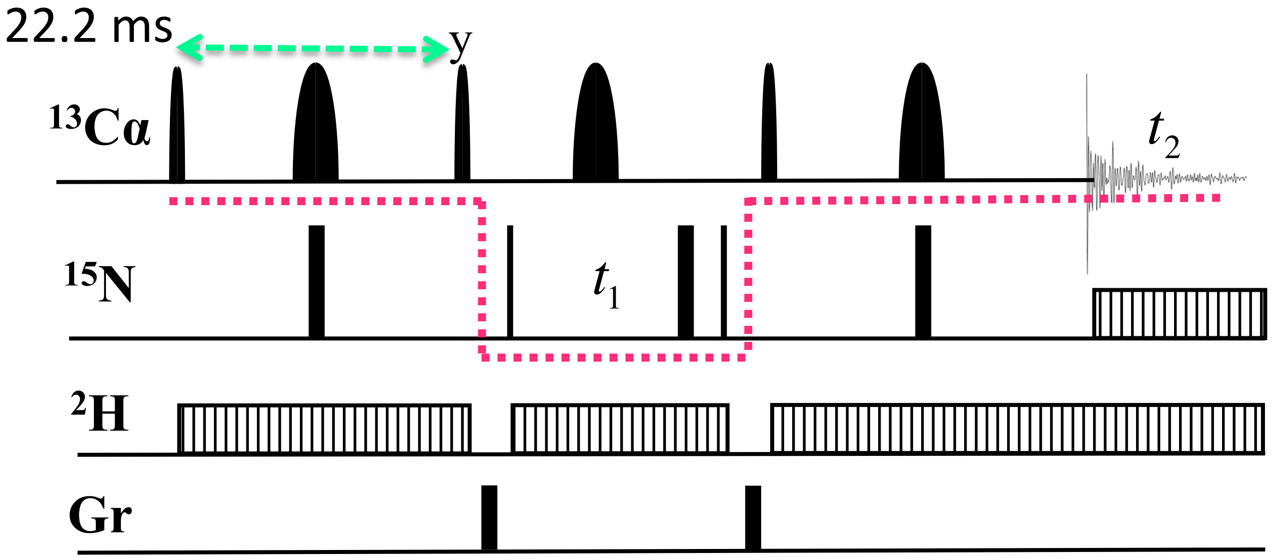
$$R_2^{CSA} = \frac{\left(\sigma_{II} - \sigma_{\perp}\right)^2 \gamma_I^2 B_0^2}{18}\left\{4J(0) + 3J(\omega_I)\right\}$$

$$J(\omega) = \frac{2}{5}\frac{\tau_c}{1+\omega^2\tau_c^2}$$

The CSA of ^{13}Co alone is considered.

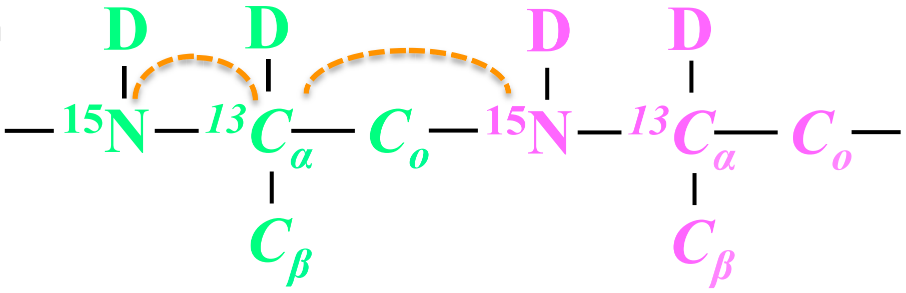
$$\delta_{xx} = -115.6\,ppm,\ \delta_{yy} = -48.6\,ppm,\ \delta_{zz} = 40.6\,ppm$$

2D SQ CAN



M9-minimum medium for *E.coli* expression

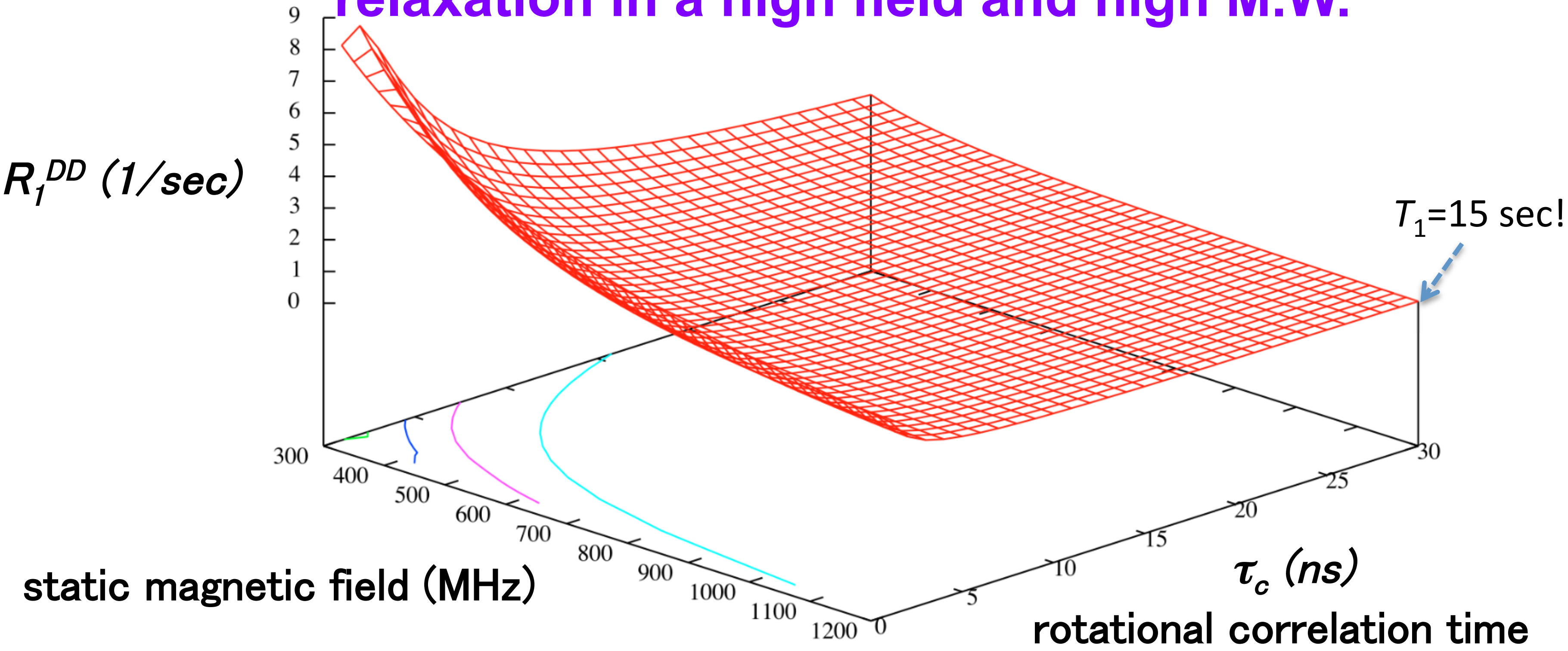
[2-^{13}C]-glycerol (or [1,3-^{13}C]-glycerol)
NaH^{13}CO$_3$
D$_2$O



Proline can be detected, which has no amide ^{1}H.
IPAP is not needed in ^{13}Cα-FID.
Sensitivity loss due to $^1J_{C\alpha C\beta}$ coupling does not occur.
The T_1 relaxation of ^{13}Cα can be enhanced by doping of paramagnetic metals.
The sequential assignment of main-chains is possible with 2D COCA.

Takeuchi, K. *et al.* (2008) *J.Am.Chem.Soc.* **130**, 17210.

A tiny contribution of *dd* (^{13}Cα-^{1}Hα) to the ^{13}Cα T_1 relaxation in a high field and high M.W.



$$R_1^{DD} = \frac{\hbar^2\gamma_I^2\gamma_S^2}{4r^6}\left(\frac{\mu_0}{4\pi}\right)^2\left\{J(\omega_I-\omega_S)+3J(\omega_I)+6J(\omega_I+\omega_S)\right\}$$

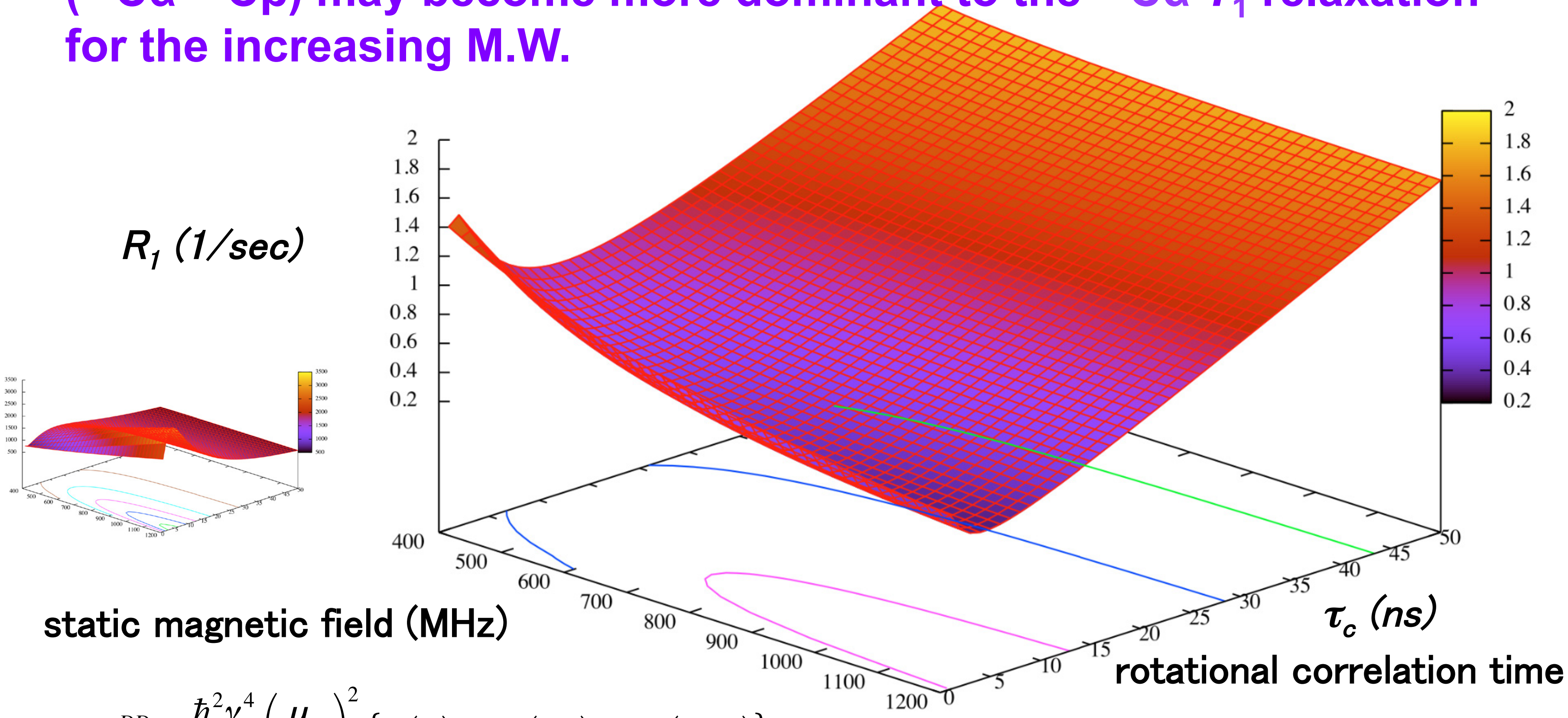
$$J(\omega)=\frac{2}{5}\frac{\tau_c}{1+\omega^2\tau_c^2}$$

^{13}Cα-^{1}Hα 2-spin system
DD alone is considered.

Is starting with ^{1}H, having faster T_1, better than starting with ^{13}C? However, samples must be protonated.

I (^{13}Cα) is detected.

The contribution from homonuclear *dd* (^{13}Cα-^{13}Co) and *dd* (^{13}Cα-^{13}Cβ) may become more dominant to the ^{13}Cα T_1 relaxation for the increasing M.W.



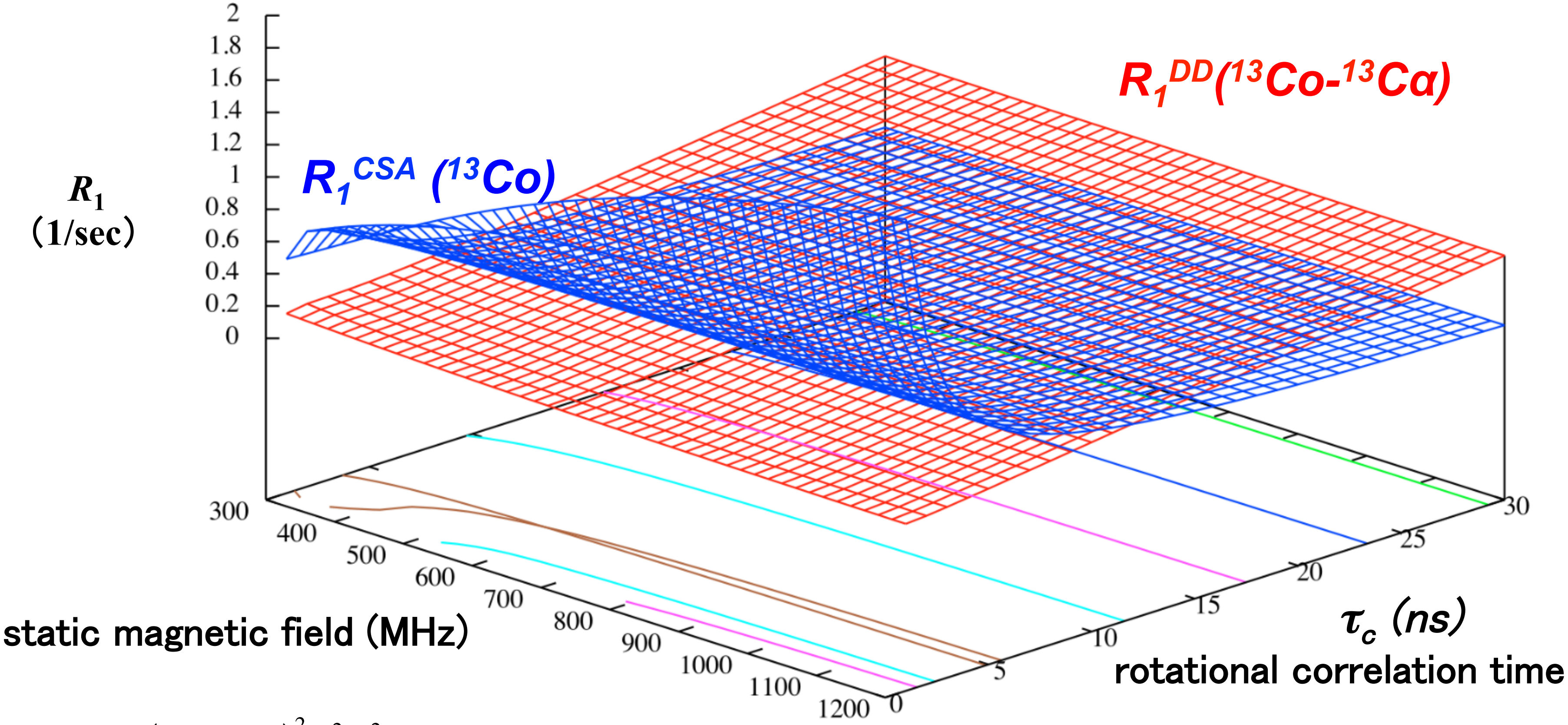
$$\rho_1^{DD} = \frac{\hbar^2\gamma_I^4}{4r^6}\left(\frac{\mu_0}{4\pi}\right)^2\left\{J(0)+3J(\omega_I)+6J(2\omega_I)\right\}$$

$$J(\omega)=\frac{2}{5}\frac{\tau_c}{1+\omega^2\tau_c^2}$$

Deuterated ^{13}Cα is detected.
Cross-relaxation is not considered as in ^{13}C SOFAST.

The flip-back of ^{13}Co and ^{13}Cβ to z would be a ^{13}C version of SOFAST.
The effect of deuteration is tiny (*dd* (^{13}Cα-^{1}Hα) accounts for ¼ of R_1).

Unlike T_2, T_1 relaxation by *CSA* does not so much depend on B_o.



$$R_1^{CSA} = \frac{(\sigma_{II} - \sigma_{\perp})^2 \gamma_I^2 B_0^2}{3} J(\omega_I)$$

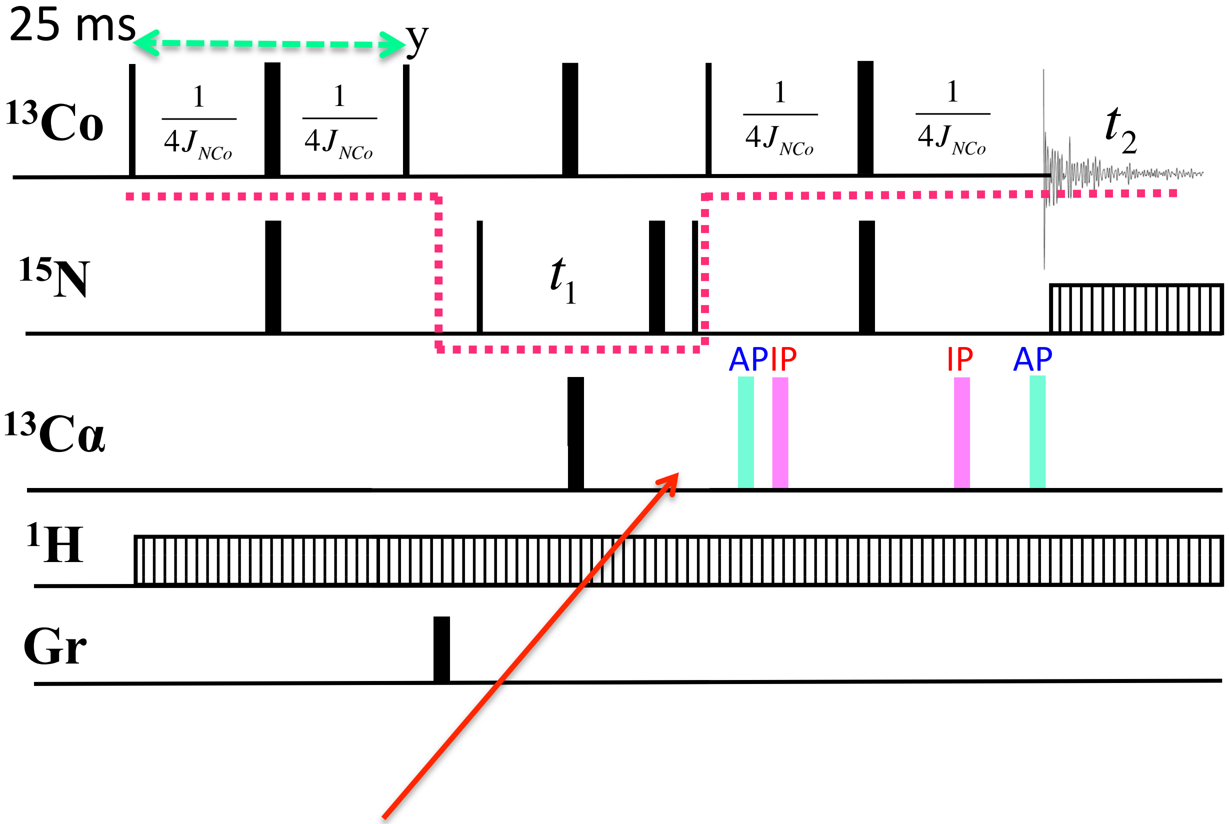
$$J(\omega) = \frac{2}{5}\frac{\tau_c}{1+\omega^2\tau_c^2}$$

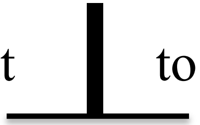
^{13}Co is detected.

If ^{13}Cα is flipped-back to z, *dd* (^{13}Cα-^{13}Co) becomes larger with the increasing M.W.

$$\delta_{xx} = -115.6\,ppm,\ \delta_{yy} = -48.6\,ppm,\ \delta_{zz} = 40.6\,ppm$$

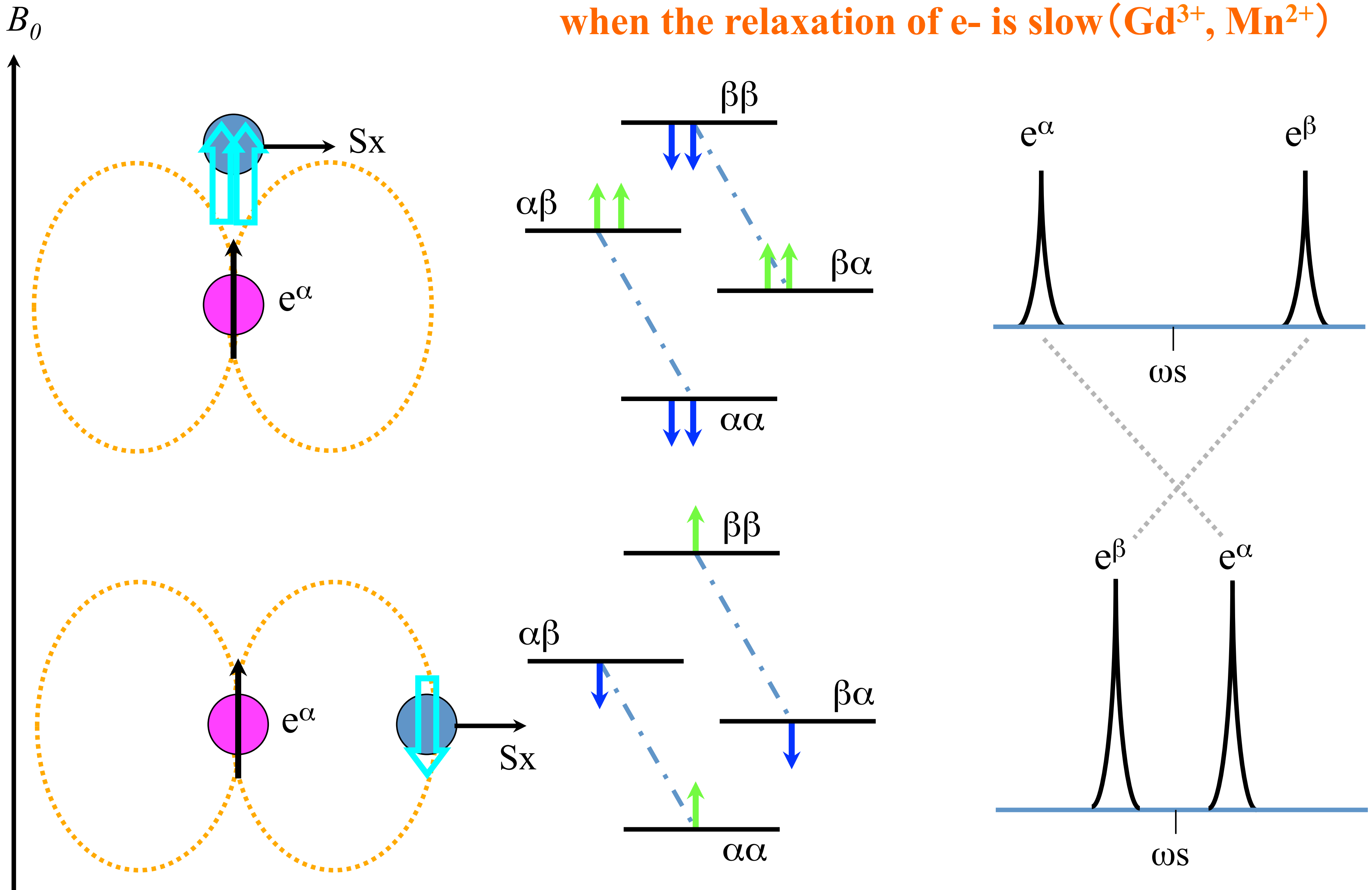
2D SOFAST CONCO ?



Should we put  to flip back ^{13}Cα to the z-direction?

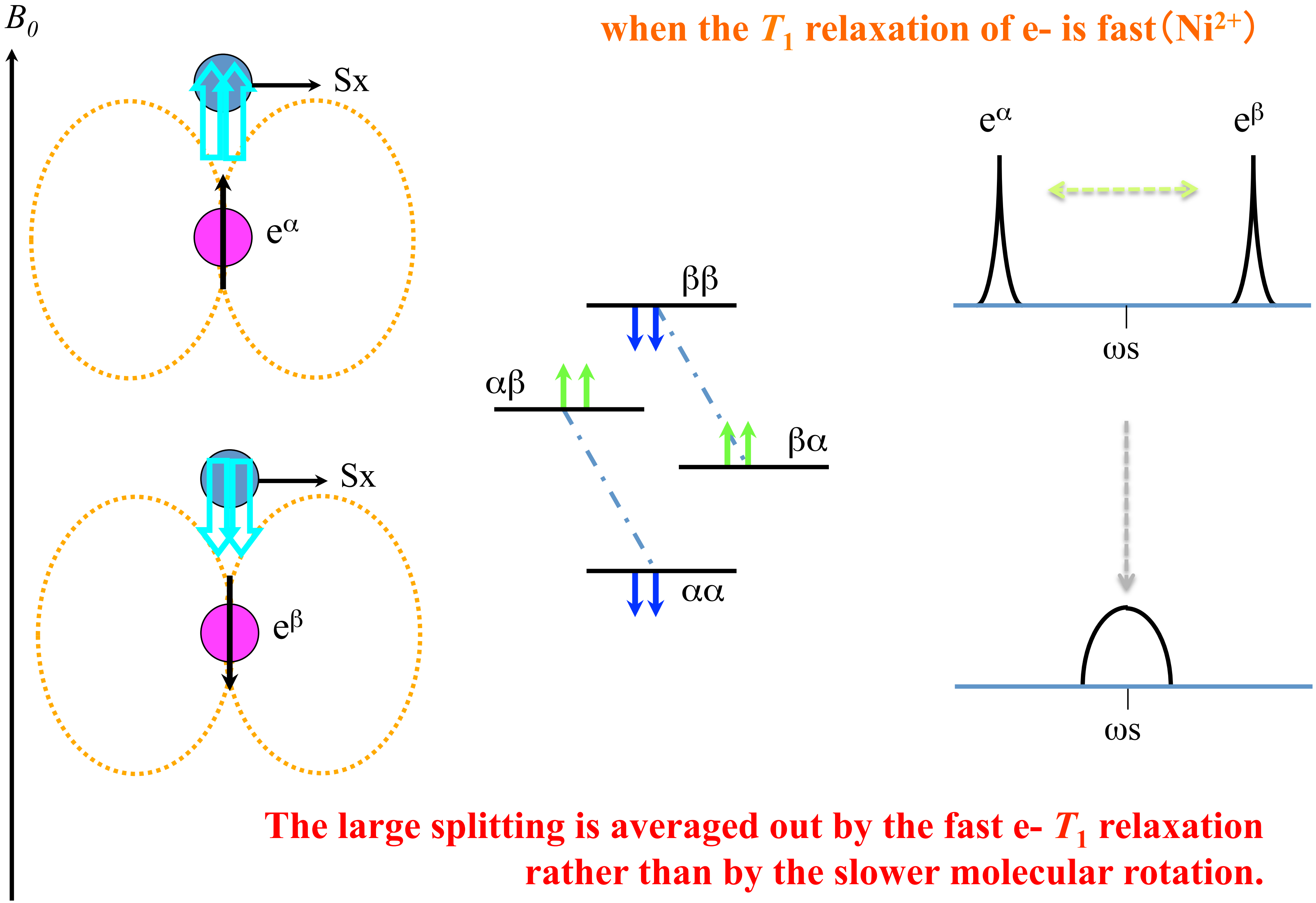
Dipole-dipole coupling interaction

when the relaxation of e- is slow（Gd^{3+}, Mn^{2+}）



The large splitting is averaged out by the slow molecular rotation.

Dipole-dipole coupling interaction



when the T_1 relaxation of e- is fast (Ni^{2+})

The large splitting is averaged out by the fast e- T_1 relaxation rather than by the slower molecular rotation.

What is more advantageous for ^{13}C–NMR over ^{1}H–NMR

◆ Since γ_{1H} is large, the *dd* relaxation is accordingly fast. By contrast, since γ_{13C} is small, the line width of ^{13}C is narrow enough to be detected even for high M.W. and metallo proteins.

◆ Useful information can be obtained even from deuterated proteins having less number of ^{1}H and from quaternary carbons.

◆ For unfolded proteins ^{13}C exhibits a wider distribution of its chemical shift than ^{1}H.

◆ Water suppression is not required. No artifact comes from water.

◆ There is no intensity loss due to exchange with water. Since ^{1}H$_N$ is labile, ^{1}H–^{15}N–HSQC shows weak signals for ^{1}H$_N$ that is exchanging with water fast.

◆ ^{13}C tends to be more tolerant to chemical and conformational exchanges than ^{1}H.

◆ A higher sensitivity and resolution can be obtained in the FID detection at a higher magnetic field, since the T_2 relaxation by the *dd* interaction does not so much depend on the static magnetic field.

◆ Signal loss due to high salt concentration is smaller?

Disadvantages in ^{13}C–NMR

- Low sensitivity due to the small γ_{13C}.
- $^{1}J_{13C-13C}$ should be removed in the direct dimension.
- Because of a long T_1 relaxation, a longer interscan–delay is required.

寒川作

Future prospects of ^{13}C–NMR

- Detection of ^{13}C is suitable for deuterated and large proteins under a high magnetic field. Since hydrogen is deuterated, the T_2 relaxation by *dd* is small, and thus the line width is narrower.
- Considering the current technology in terms of the sensitivity, however, the sequence starting with $^{1}H^{N}$ and ending with ^{13}C (FID) may be a compromise, which requires a shorter interscan–delay?