Emerging Trends in Solution NMR

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Limitations of solution NMR

Molecular size (< 100 kDa?)

Larger membrane proteins

Need deuteration for sharper lines

Express in *E.coli* with ^{15}N, ^{13}C, ^{2}H

^{2}H -> ^{1}H amide back exchange

Can ^{15}N detection help?

^{15}N detection is intrinsically much less sensitive than other nuclei
Are we stupid?

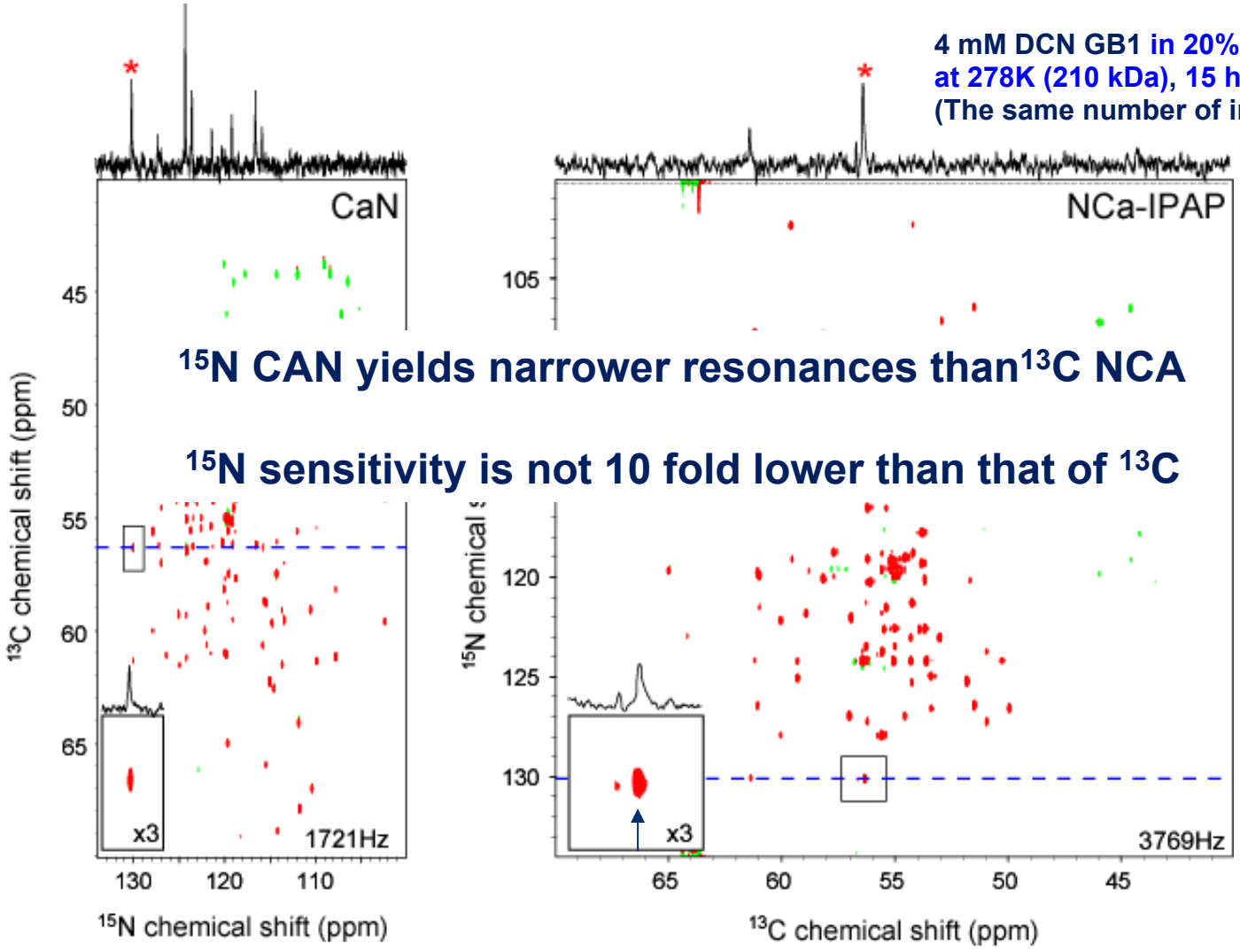
Intrinsic sensitivity $(S_0) \propto \gamma_e \cdot \gamma_d^{3/2} \cdot B_0^{3/2}$

Intrinsic sensitivity of ^{15}N against ^{1}H: 0.0032

Intrinsic sensitivity of ^{15}N against ^{13}C: 0.10

^{15}N CaN vs ^{13}C NCa

4 mM DCN GB1 in 20% glycerol/D_2O
at 278K (210 kDa), 15 hrs @500 MHz
(The same number of indirect points)



Why ^{15}N instead of ^{1}H detection

^{15}N has a much smaller gyromagnetic ratio and is of course much less sensitive (*I* = intensity ~ A = area)

$$I \propto A \propto \gamma_e \cdot \gamma_d^{3/2} \cdot B_0^{3/2}$$

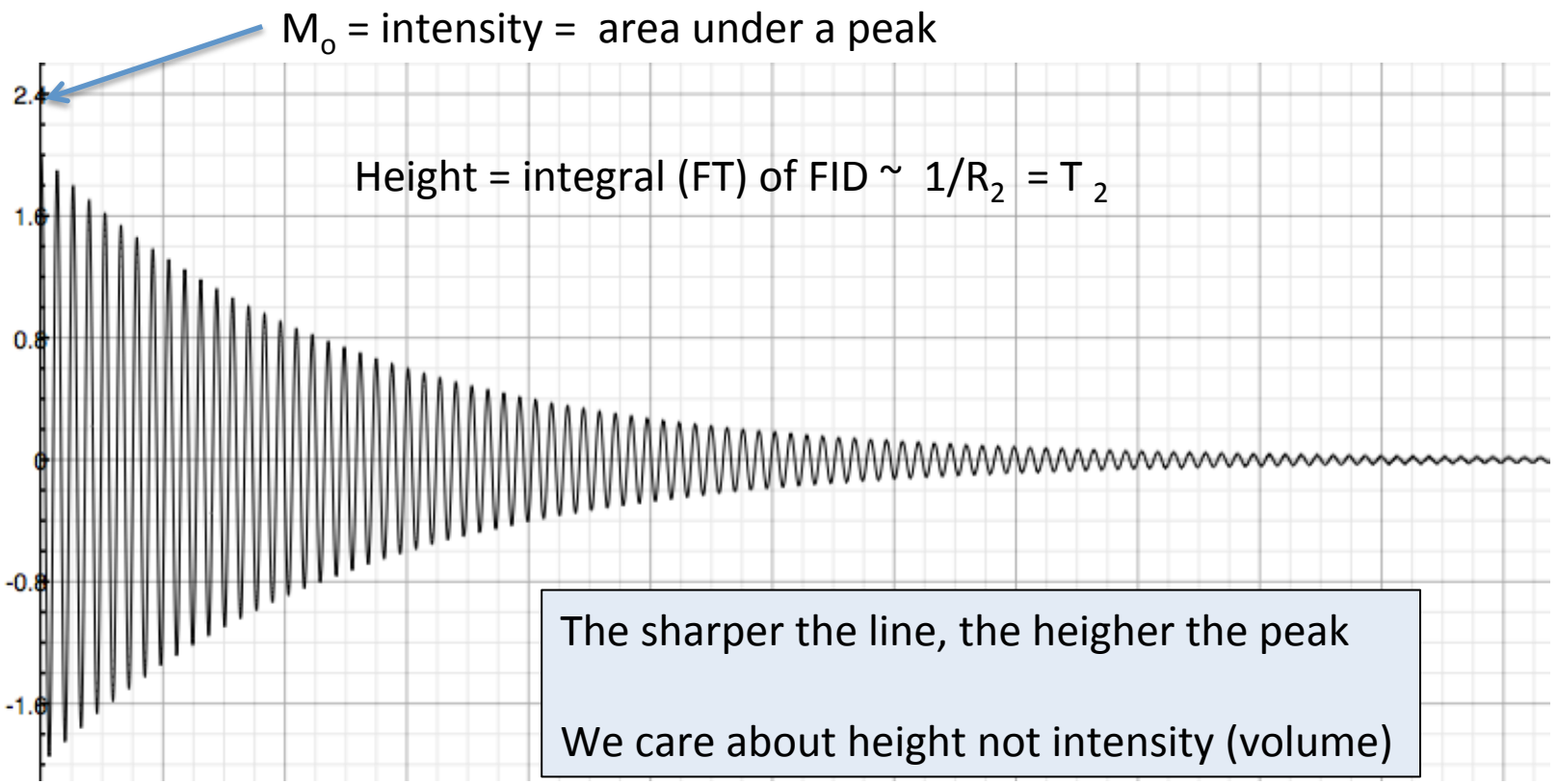
$$H \propto \gamma_e \cdot \gamma_d^{3/2} \cdot B_0^{3/2} / R_2$$

Peak height is proportional to integral (FT) over FID and is proportional to inverse of relaxation rate R_2

Koh Takeuchi & Hari Arthanari

Intensity and Peak height

$$S_c^c(\omega) = \int_0^\infty M_o^A \cos\omega_A t \cos\omega t \, e^{-t/T_2^A} \, dt = M_o^A \frac{T_2^A}{1 + 4\left(T_2^A\right)^2 (\omega - \omega_A)^2}$$



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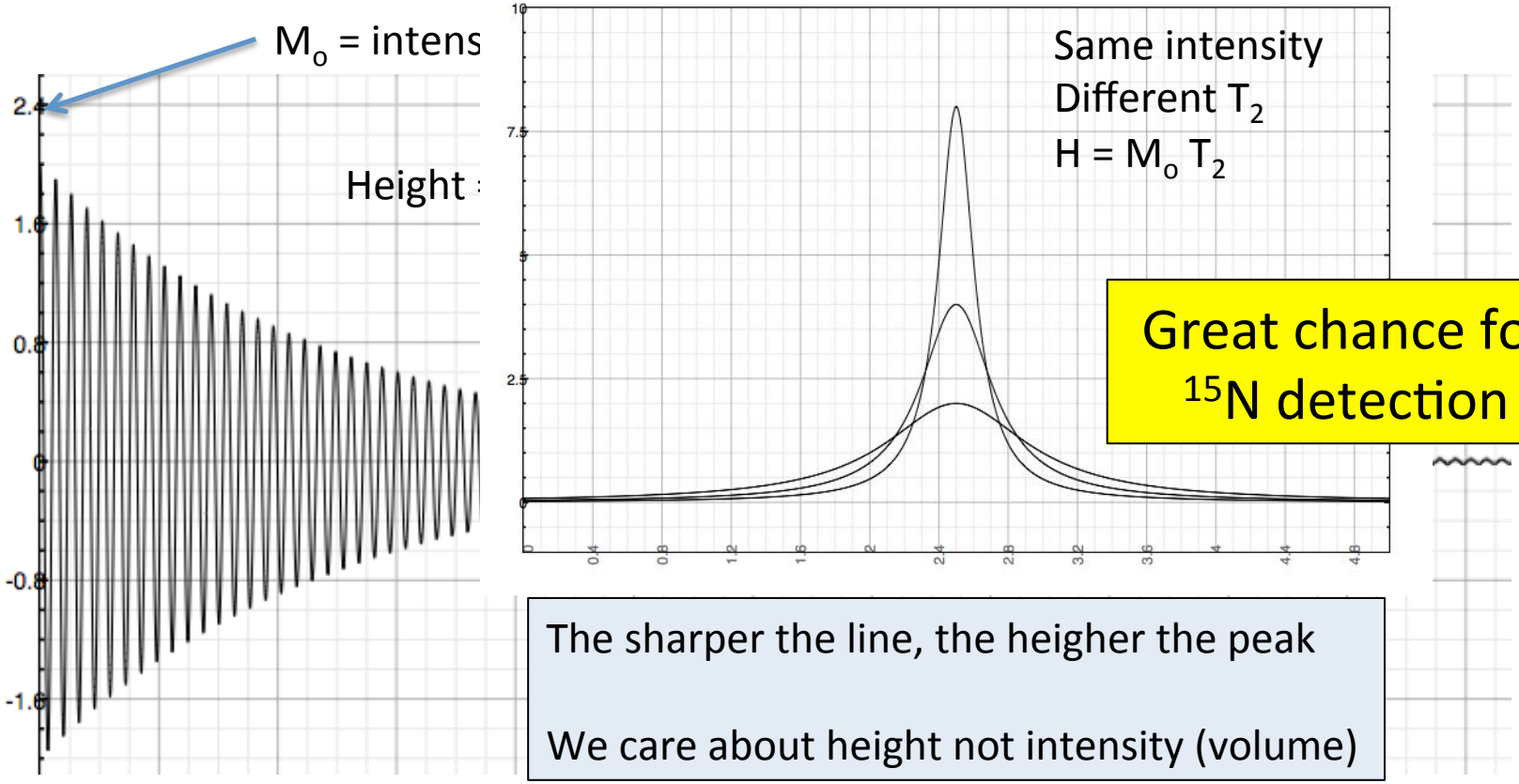
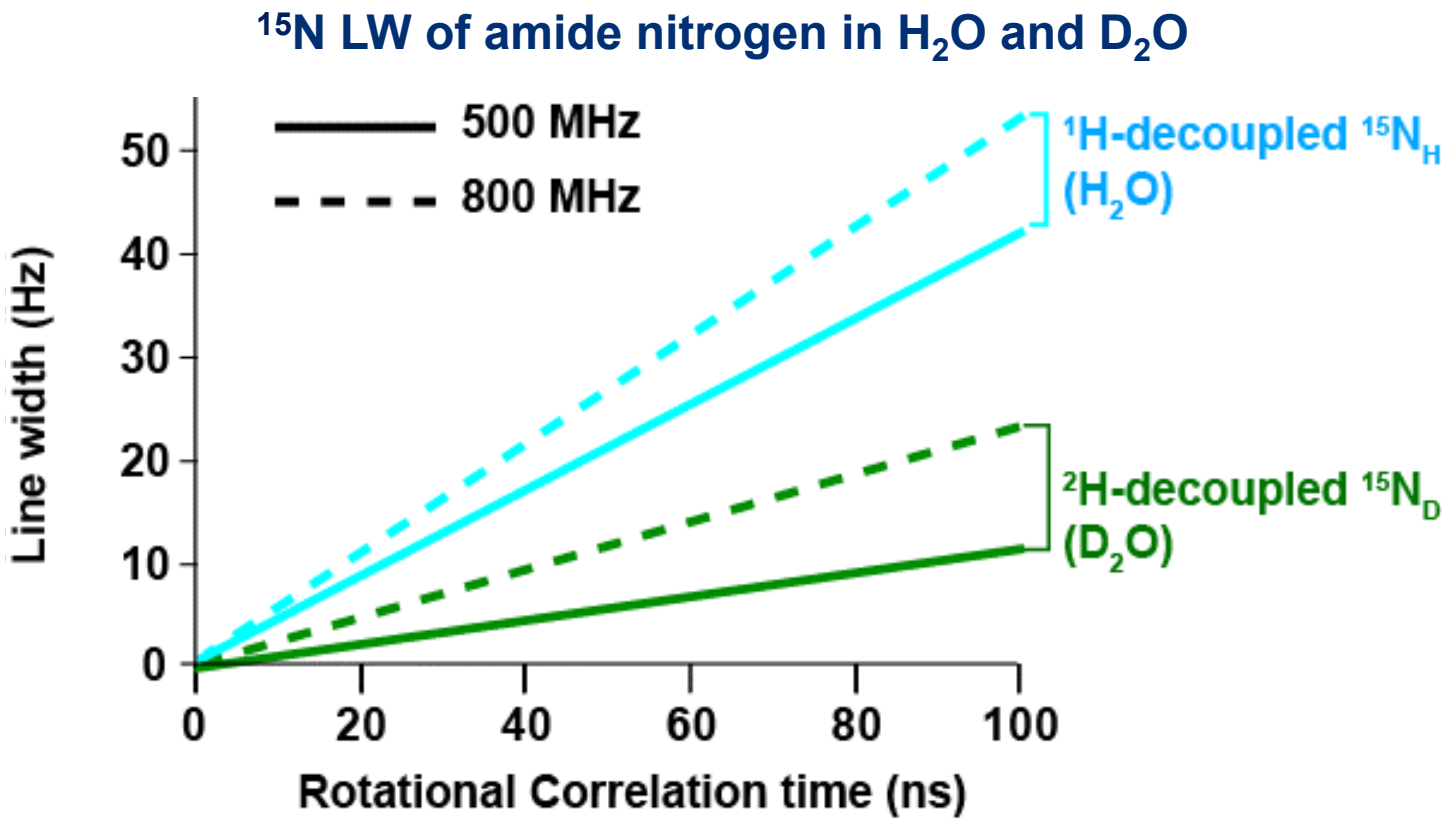


Very promising at Avance 500 ☺

cryoprobe for C,N-detection cold ^{15}N preamp

But no gain at Avance 800 ☹

Conventional ^{15}N detection uses deuterium-bound amides ($^{15}N_D$)



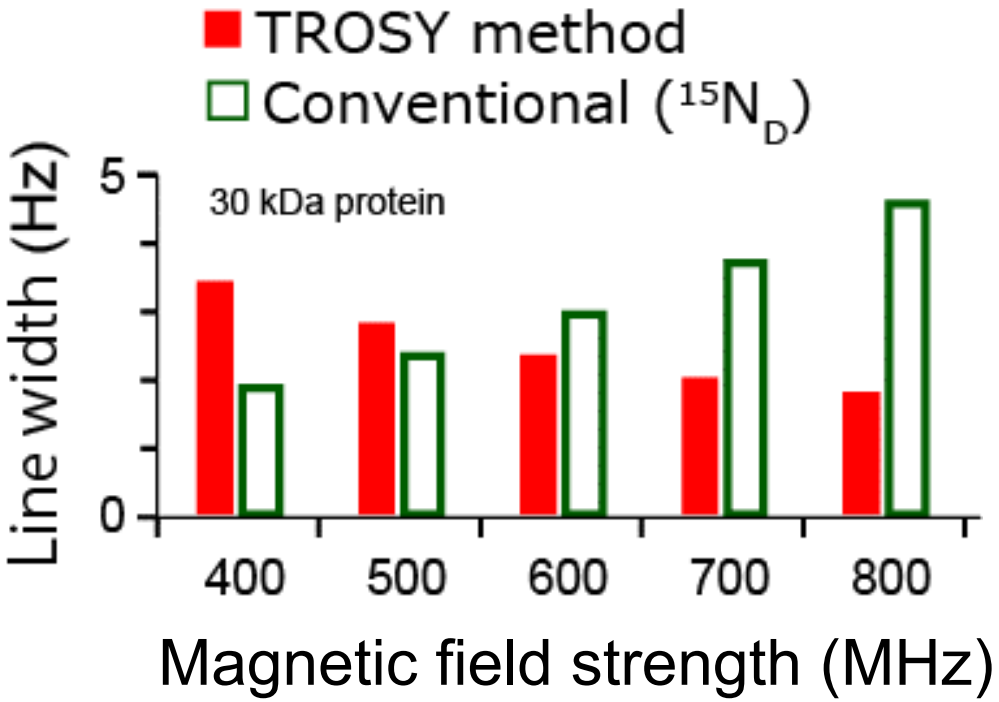
- ^{2}H-decoupled $^{15}N_D$ in D_2O is narrower than ^{1}H-decoupled $^{15}N_H$ in H_2O
- An expensive higher field magnet would simply broaden our resonance.

Reconsideration of detection strategy is needed to benefit from high-field.

$^{15}N_D$ line width increases with field strength

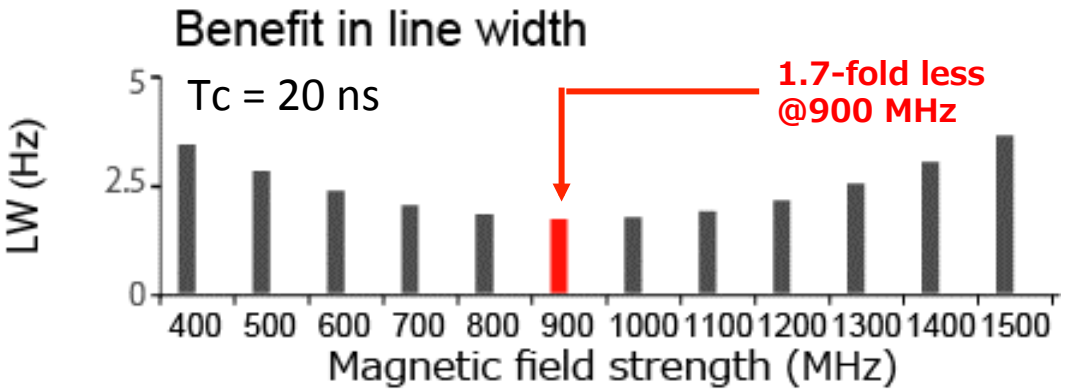
$^{15}N_H$ TROSY line width should decreases with field strength

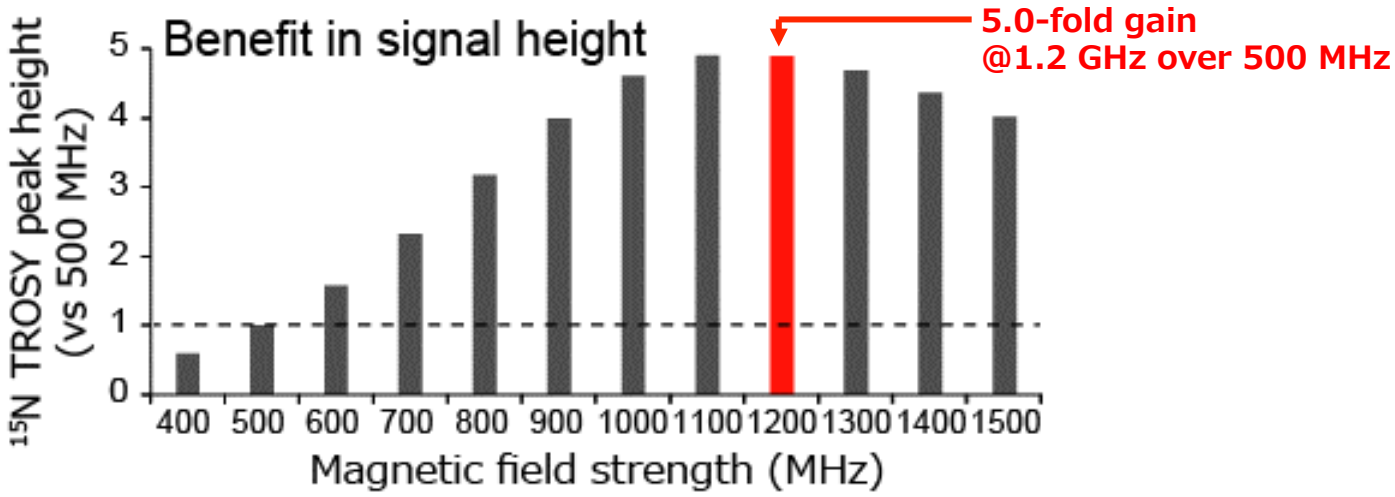
LW of conv. $^{15}N_D$ and TROSY $^{15}N_H$ resonances



TROSY will enhance resolution and sensitivity of ^{15}N detection at higher magnetic fields

^{15}N TROSY provides maximal benefits @900 MHz for resolution and @1.2 GHz for sensitivity



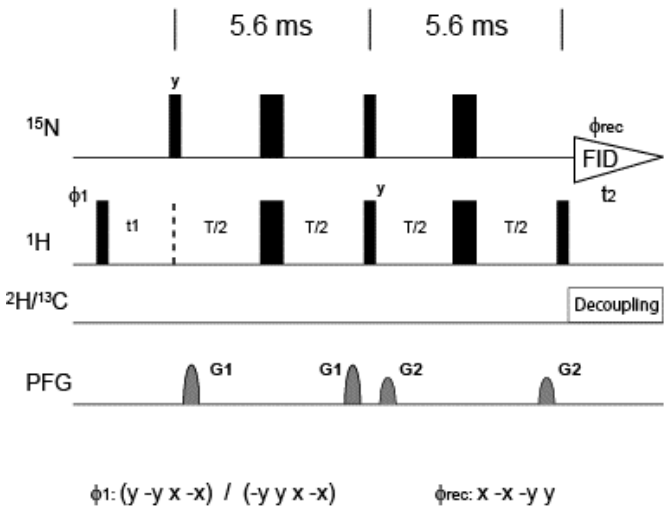


Combination of ^{15}N TROSY& higher magnetic fields will provide additional gains.

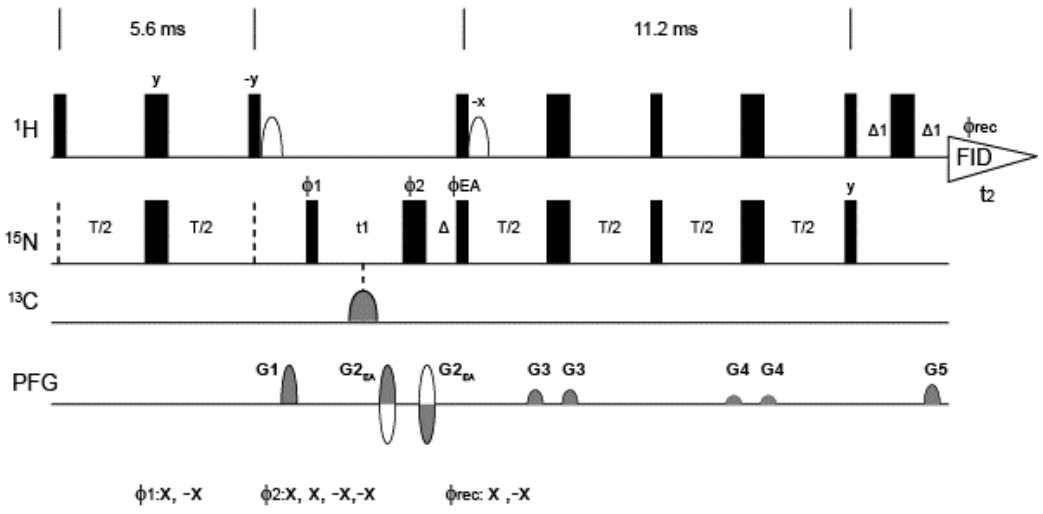
Takeuchi et al. JBNMR 2015

^{15}N-detected TROSY is shorter than the ^{1}H detected version

^{15}N-detected TROSY-HSQC

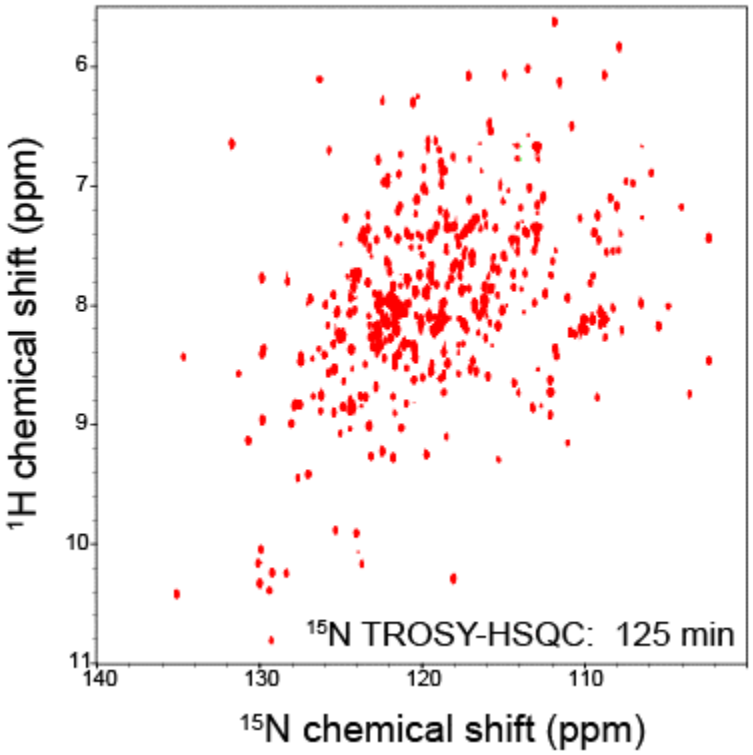


^{1}H-detected TROSY-HSQC

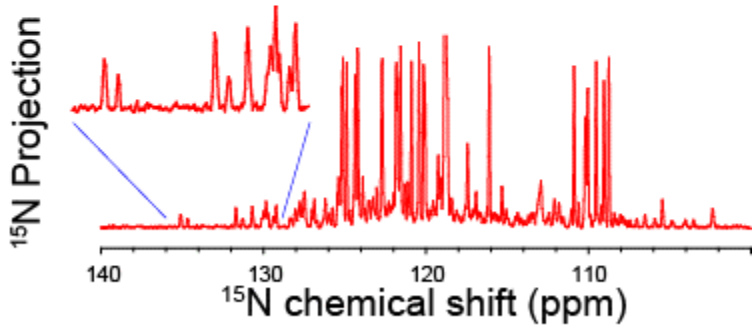


^{15}N-detected 2D TROSY experiment of 70 kDa protein

$^{2}H^{15}N$ MBP 1mM, 278K: Tc = 40 ns (~ 70K)

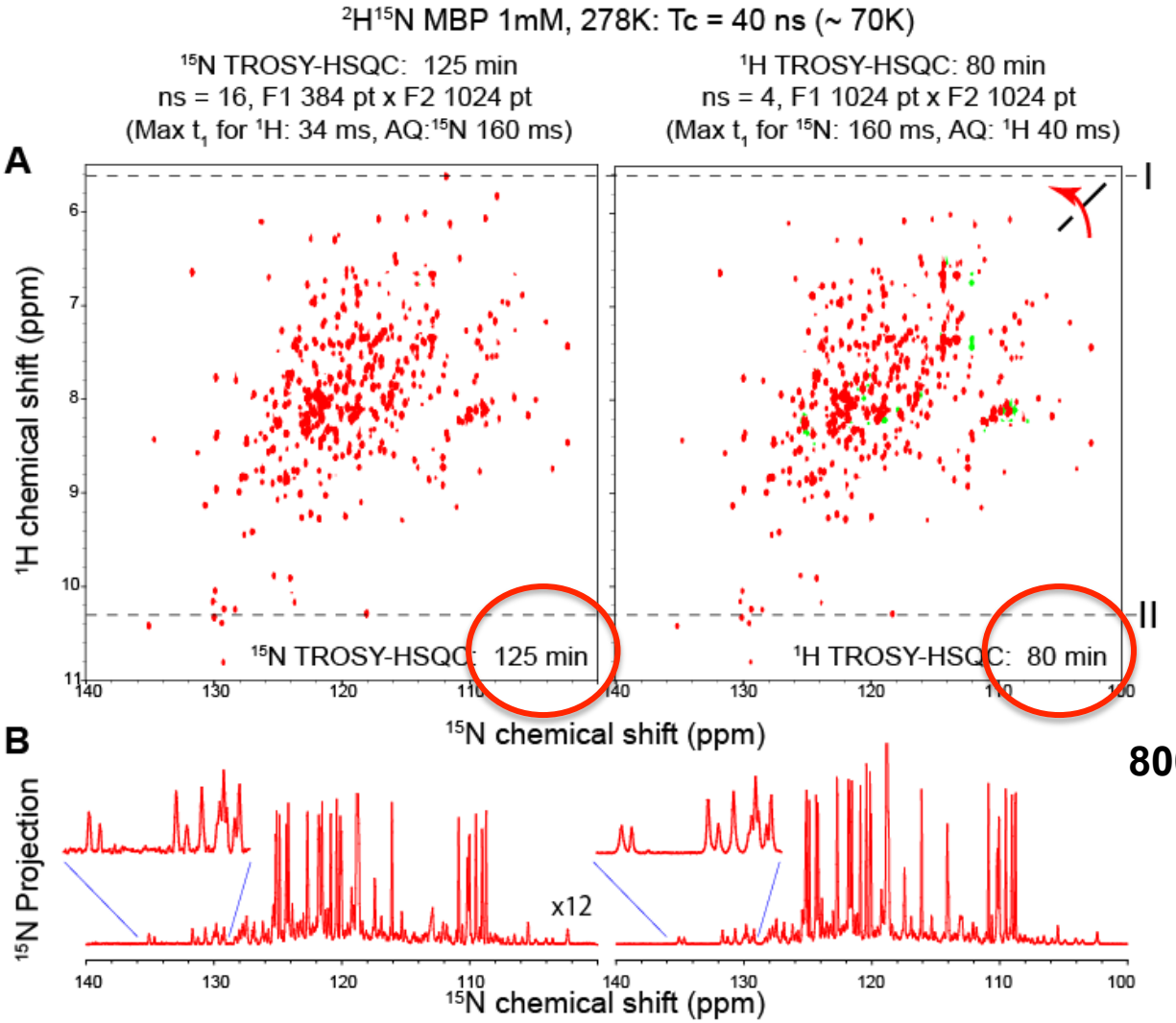


^{15}N TROSY-HSQC: 125 min
ns = 16, F1 384 pt x F2 1024 pt
(Max t_1 for ^{1}H: 34 ms, AQ:^{15}N 160 ms)
800 MHz magnet



^{15}N-detected TROSY-HSQC yield nice spectra with reasonable amount of time.

^{15}N TROSY-HSQC vs ^{1}H TROSY-HSQC



800 MHz magnet

$^{15}N_H$ TROSY-HSQC showed similar quality as $^{1}H_N$ TROSY-HSQC.

Do we need to deuterate?

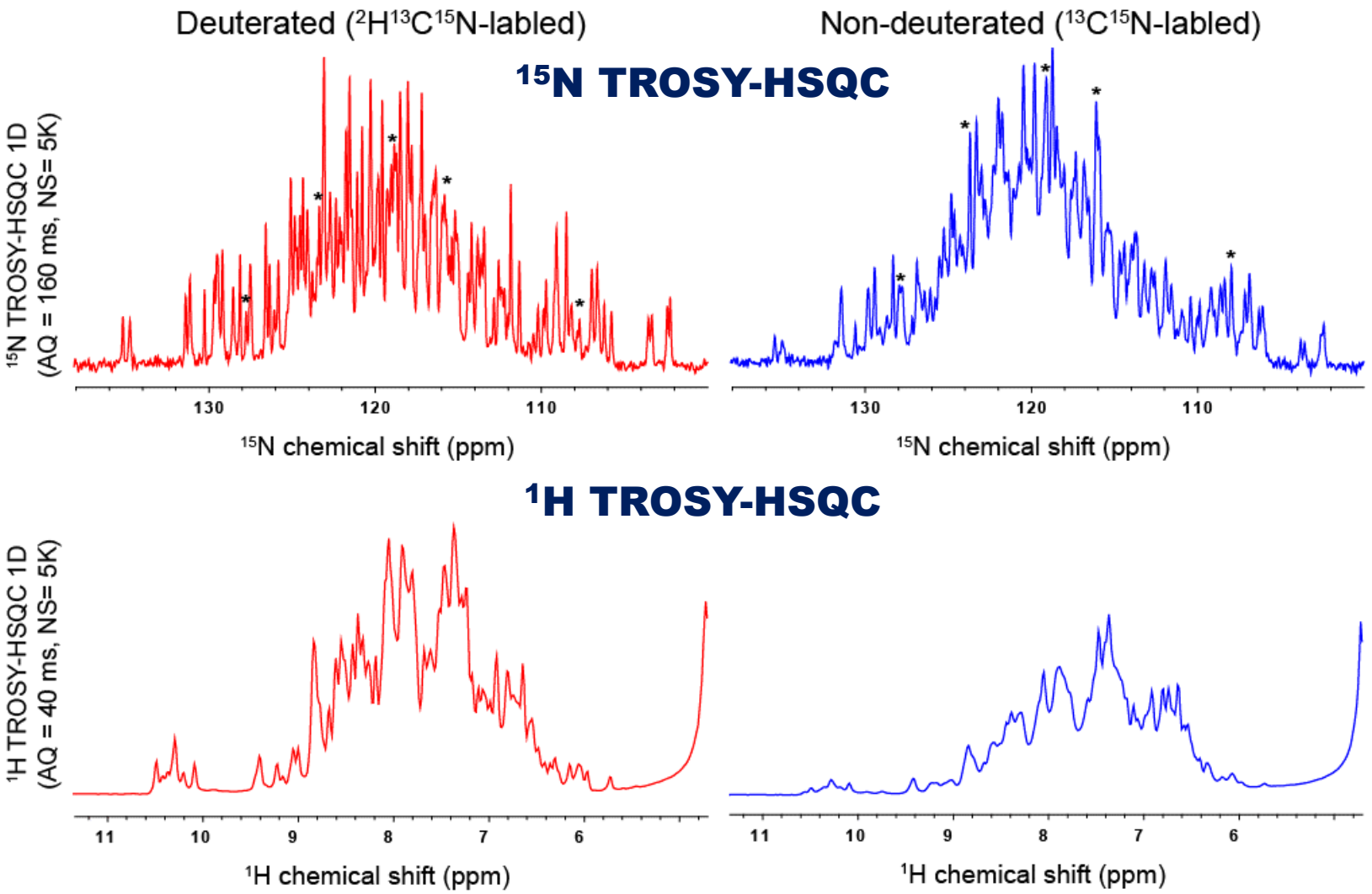
^{15}N has very weak dipolar interactions with nearby ^{1}H and shouldn't be much broadened

Would alleviate the need for amide back-exchange in deuterated proteins

Reduce cost of making deuterated samples!

Would not be limited to *E.coli* expression!

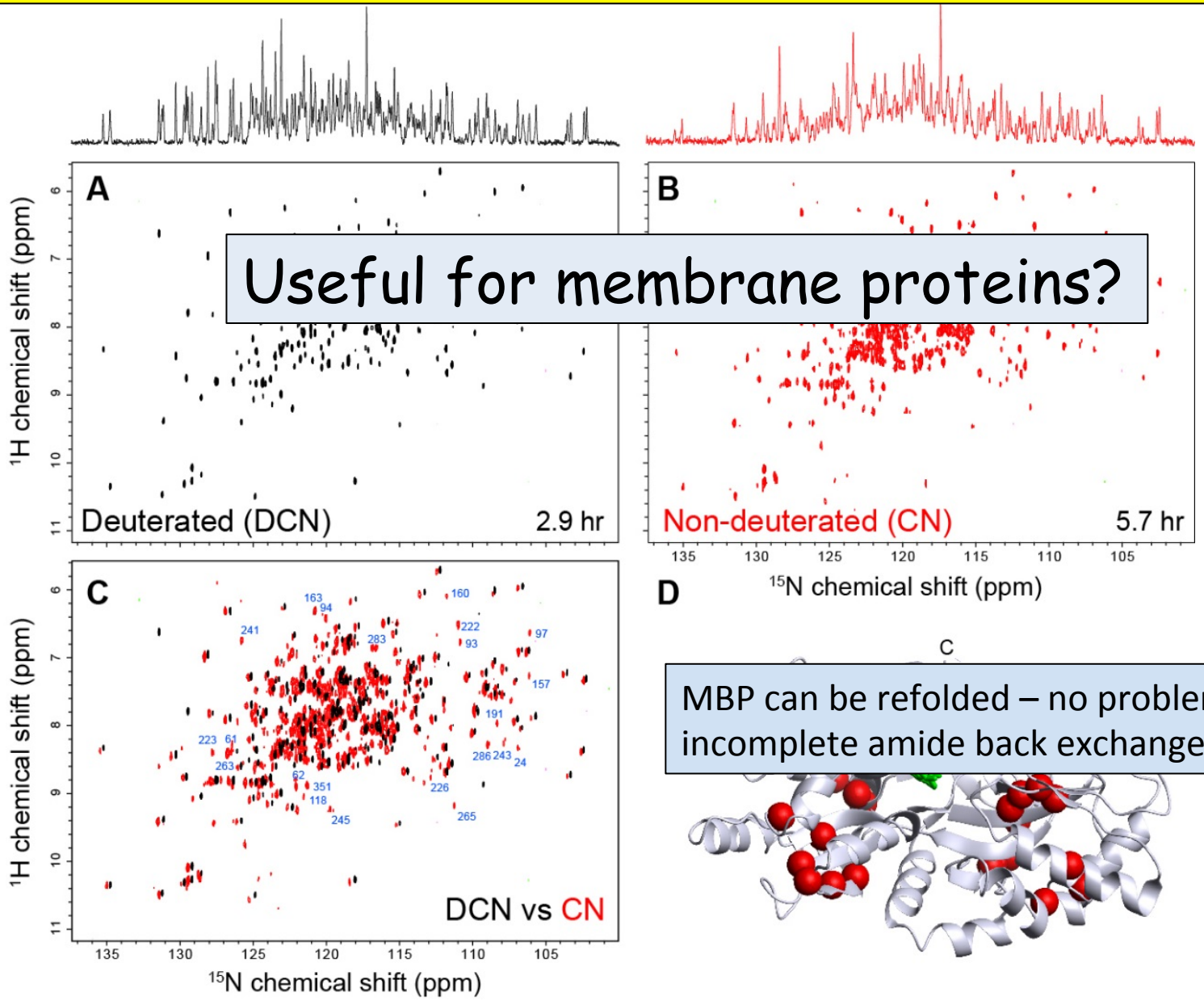
^{15}N and ^{1}H TROSY without deuteration



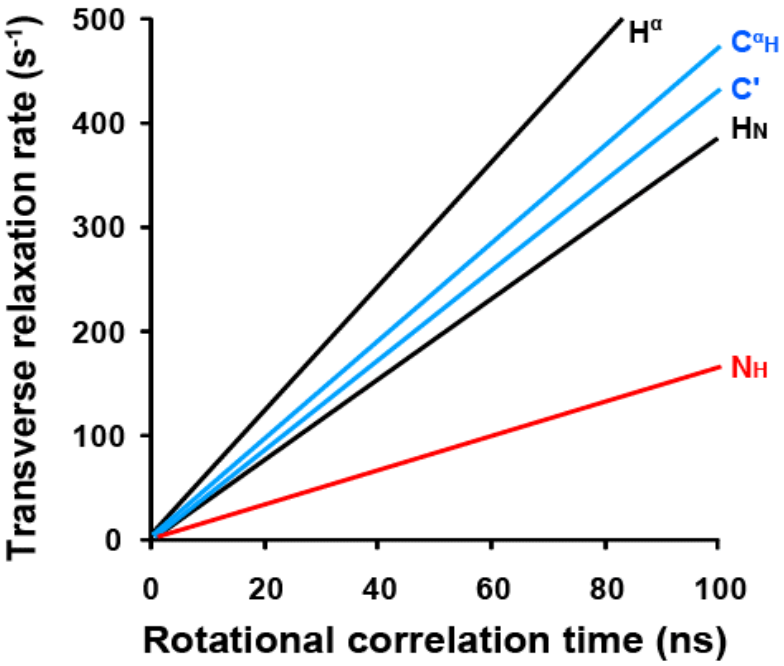
67K protein @800 MHz

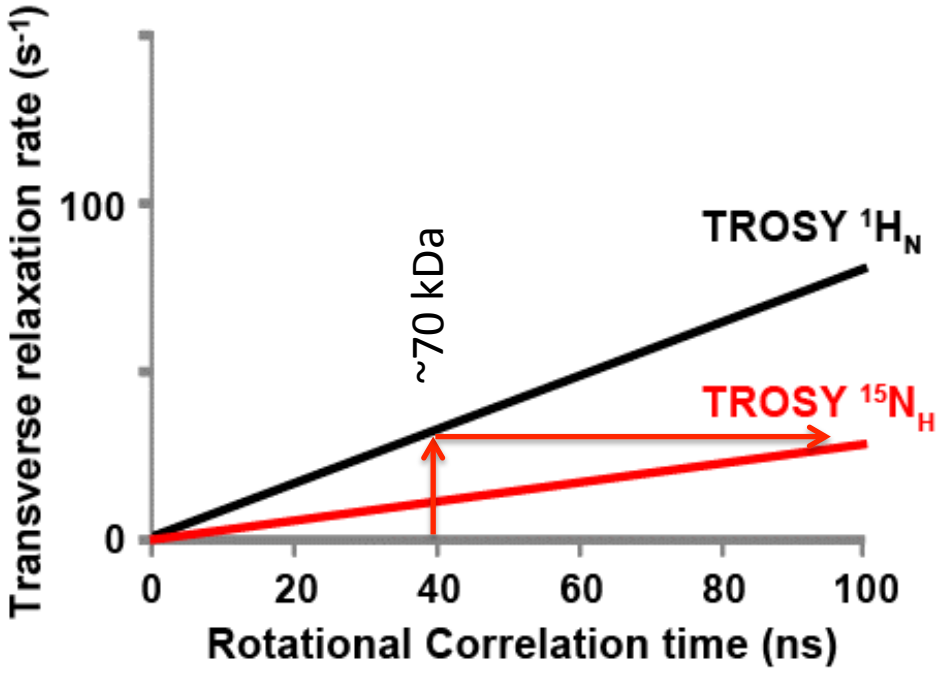
^{15}N TROSY would work as well for protonated proteins.

Comparison of $^{15}N_H$ detected TROSY using deuterated vs non-deuterated MBP



$^{15}N_H$-detected TROSY is least deteriorated when going to higher molecular weight





Summary

TROSY selection provides resolution and sensitivity of ^{15}N detection in high-magnetic field

$^{15}N_H$ TROSY-HSQC spectra can be recorded at only moderately lower sensitivity than $^{1}H_N$ TROSY HSQC

$^{15}N_H$ TROSY is least sensitive to molecular weight and promises to extend the size range of NMR to high-molecular weight systems

^{15}N TROSY would work as well for protonated proteins and will be beneficial for proteins that can be obtained only by eukaryotic expression

^{15}N TROSY with non-deuterated protein eliminates the incomplete-amide back-exchange problem

These results illustrate the potential of ^{15}N TROSY as a means to exploit the high resolution offered by high field magnets near and above 1 GHz

^{15}N detected TROSY will benefit assignments of crowded spectra of unstructures regulatory domains with prolines in phosphorylation sites and much more complex systems could be assigned at higher field

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