DNP enhanced frequency-selective TEDOR experiments in bacteriorhodopsin

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MR Seminar 2013

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Objectives

- To introduce a new approach (FS-TEDOR) to multiple $^{13}C-^{15}N$ distances measurements in uniformly labeled solids.
- To increase sensitivity by integrating high field DNP to the experiments.
- To demonstrate the method as a study tool for ^{15}N-^{13}C correlation spectroscopy in crystalline solids and membrane proteins
- To resolve correlation spectrum of Arg, $^{13}C_{\gamma}$-$^{15}N_{\varepsilon}$region in [U-^{13}C,^{15}N] -bacteriorhodopsin

Introduction

Heteronuclear distance measurements in **uniformly labeled sample** under MAS-NMR include complications:

- **Strong dipolar coupling**
  - The coupling dominates the spin dynamics and compromises sensitivity
  - The effect is crucial in protein with amino acids containing nitrogen side chain (Asp, Gln, Lys, Arg)

Solution: Frequency selective to solve N in backbone or sidechain

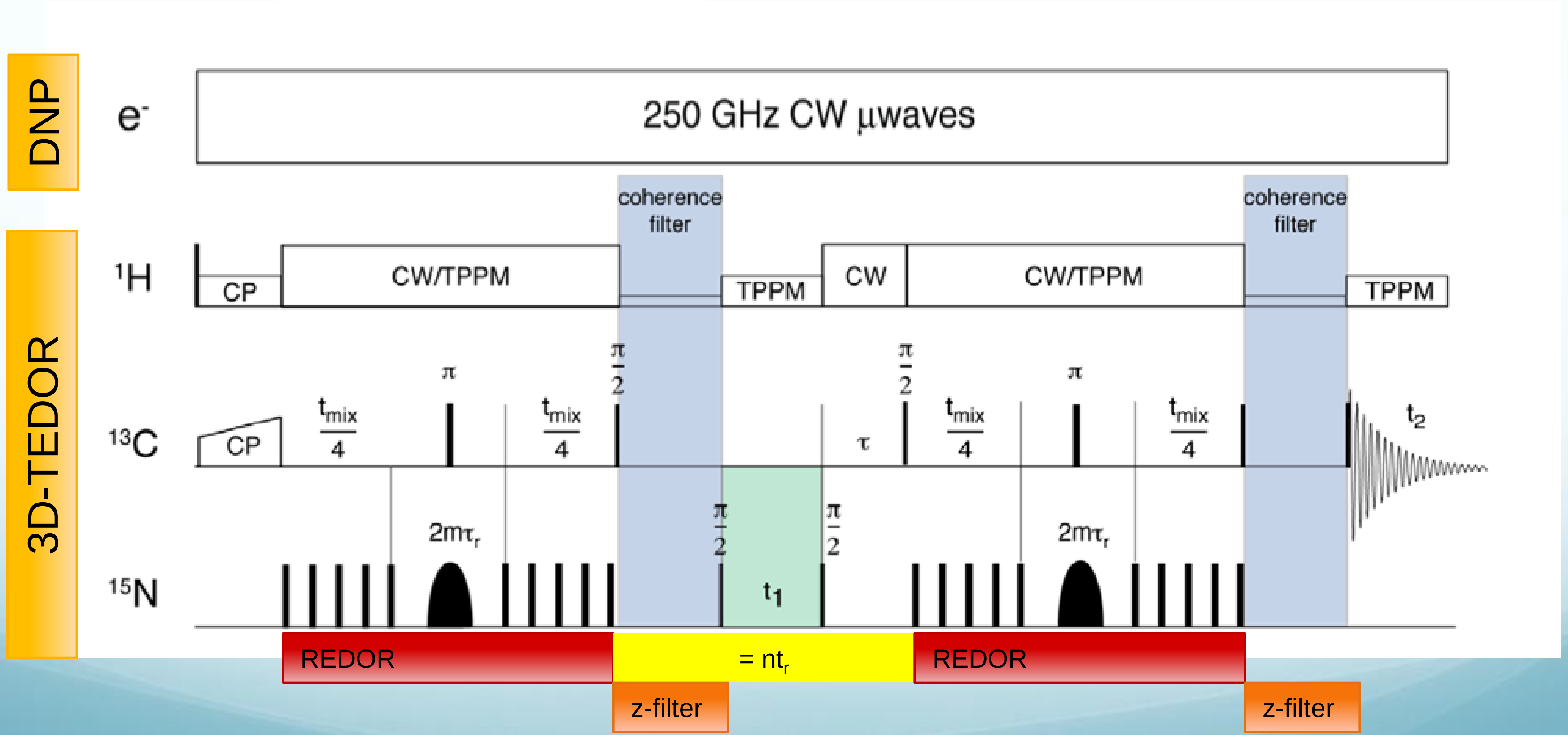
- **J-coupling (^{13}C-^{13}C)**
  - The J-coupling imposes dephasing during the recoupling period
  - The coupling can generate antiphase coherence

    Phase twisted lineshape

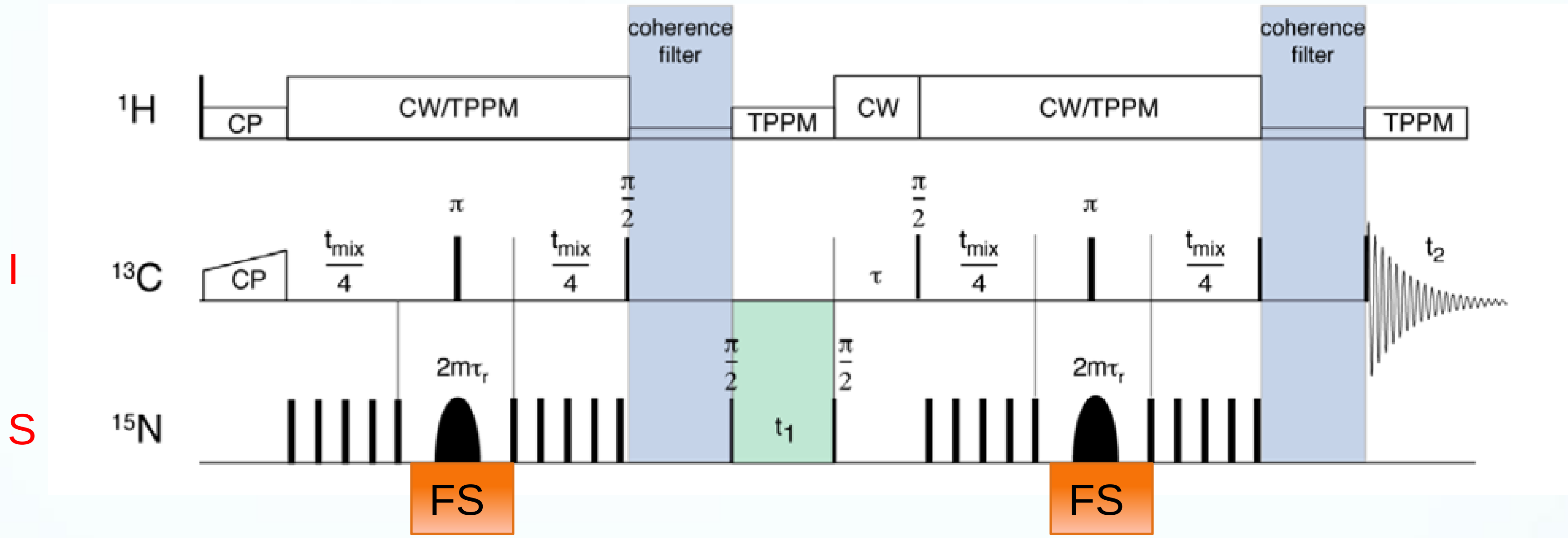
Solution: decoupling with coherance filter

Methods

- 3D-TEDOR (Transfer Echo DOuble Resonance)
- Frequency-Selective (FS)
- Coherence filter (z-filter)
- DNP



3D TEDOR pulse sequence



$$I_x \xrightarrow{REDOR} 2I_yS_z \sin(\omega t_{mix}/2)$$

$$\xrightarrow{90_x(I)90_y(S)} -2I_zS_y \sin(\omega t_{mix}/2)$$

$$\xrightarrow{t_1} -2I_zS_y \sin(\omega t_{mix}/2)e^{i\Omega_s t_1}$$

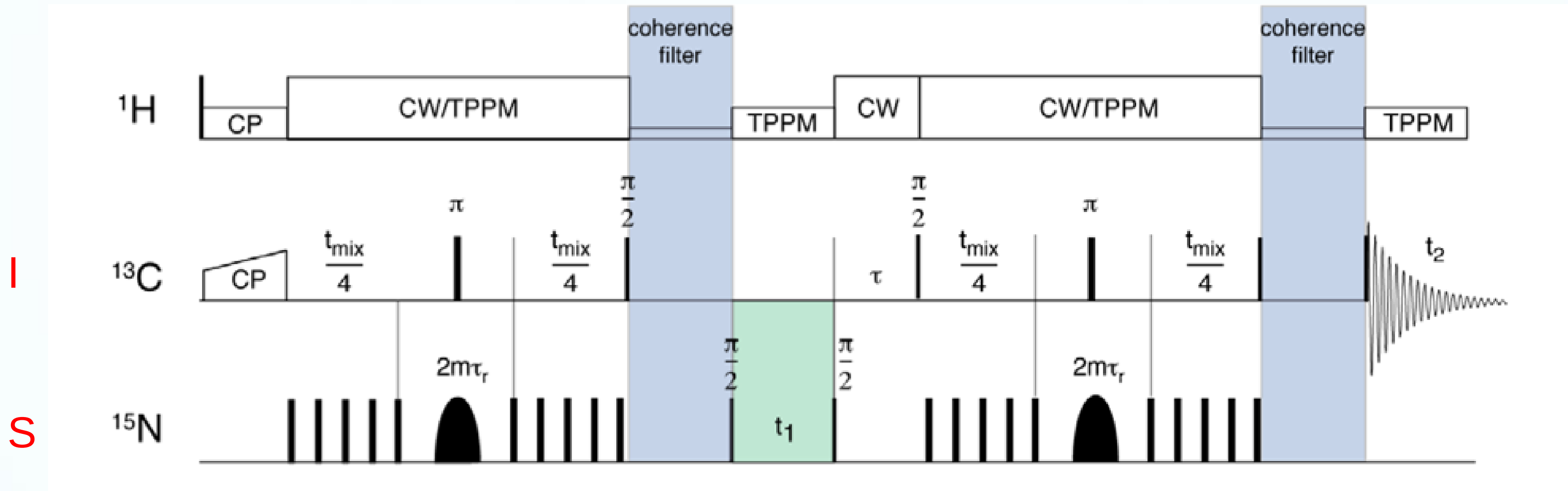
$$\xrightarrow{90_x(I)90_{xy}(S)} -2I_yS_z \sin(\omega t_{mix}/2)e^{i\Omega_s t_1}$$

$$\xrightarrow{REDOR} I_x \sin^2(\omega t_{mix}/2)e^{i\Omega_s t_1}e^{i\Omega_I t_2}$$

ω= effective dipolar coupling, Ω= isotropic CS

- Cross polarization
- 1st REDOR ($t_m/2$)
- 1st pair of π/2 pulses
- 2nd pair of π/2 pulses
- 2nd REDOR

3D TEDOR : Spin dynamics



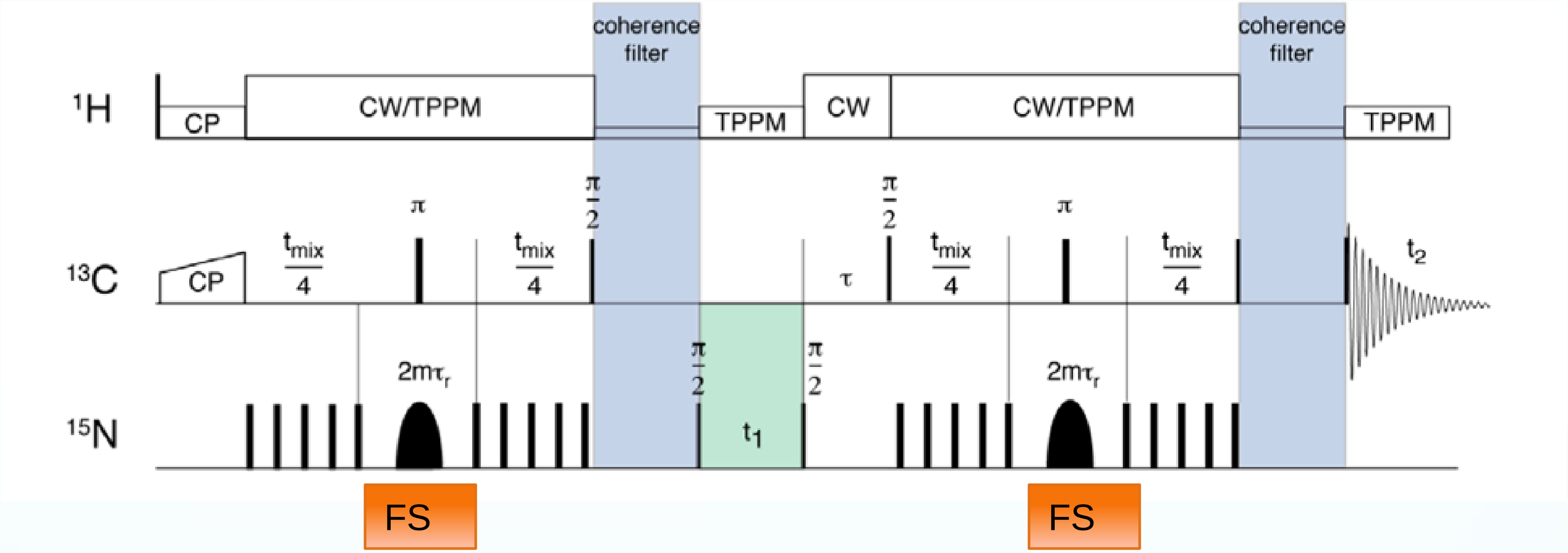
During evolution period (t_1 and t_2):

$$H = \sum_k \Omega_{Ik} I_{kz} + \sum_k \Omega_{Sk} S_{kz} + \sum_{j<k} \pi J_{jk} 2 I_{jz} I_{kz}$$

During mixing period (t_{mix}):

$$H = \sum_{j,k} \omega_{jk} 2 I_{jz} S_{kz} + \sum_{j<k} \pi J_{jk} 2 I_{jz} I_{kz}$$

FS-TEDOR



Applying frequency selective pulses centered in the REDOR period

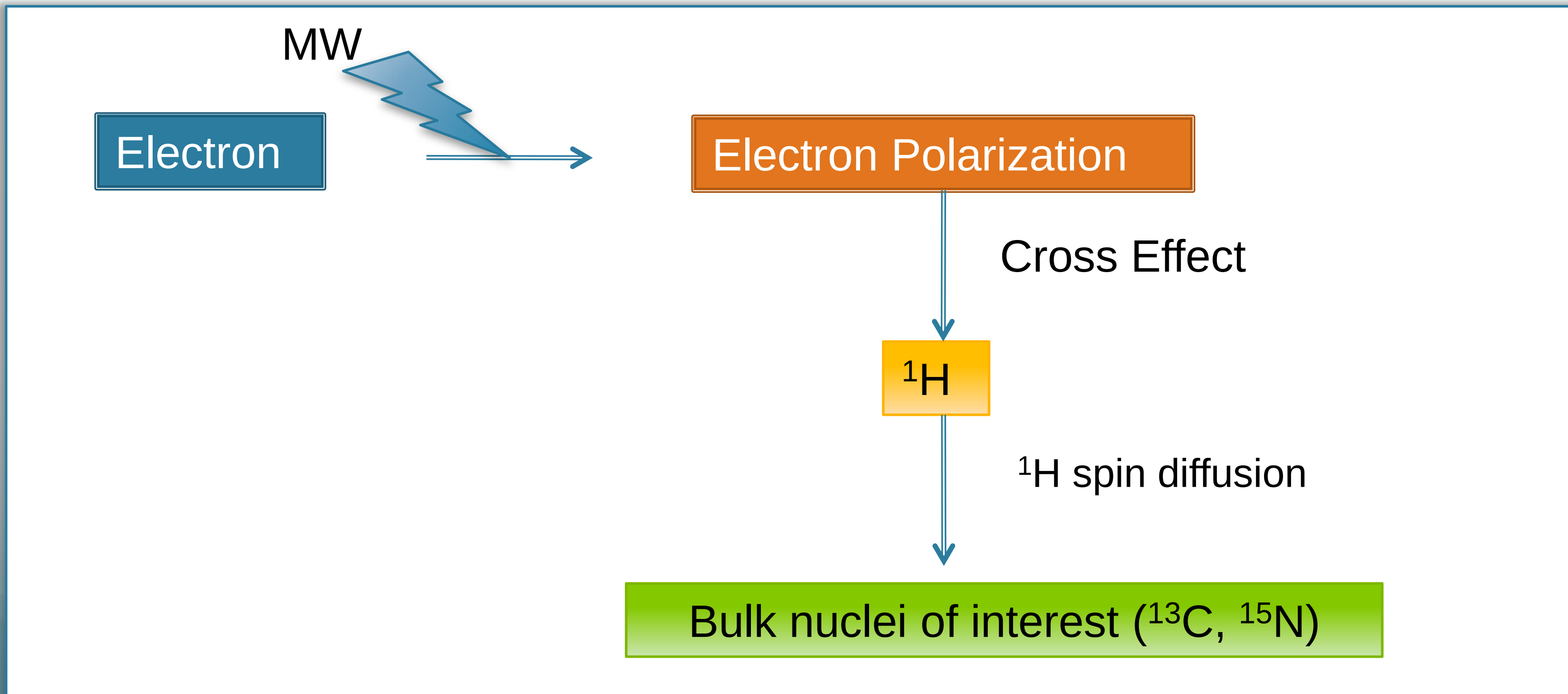
Only the nuclei within the bandwidth contribute to spin dynamics during REDOR mixing periods

High field DNP

DNP : Polarization transfer from electron to nuclei

Sensitivity Enhancement

$\gamma_e/\gamma_{1H} \approx 660$

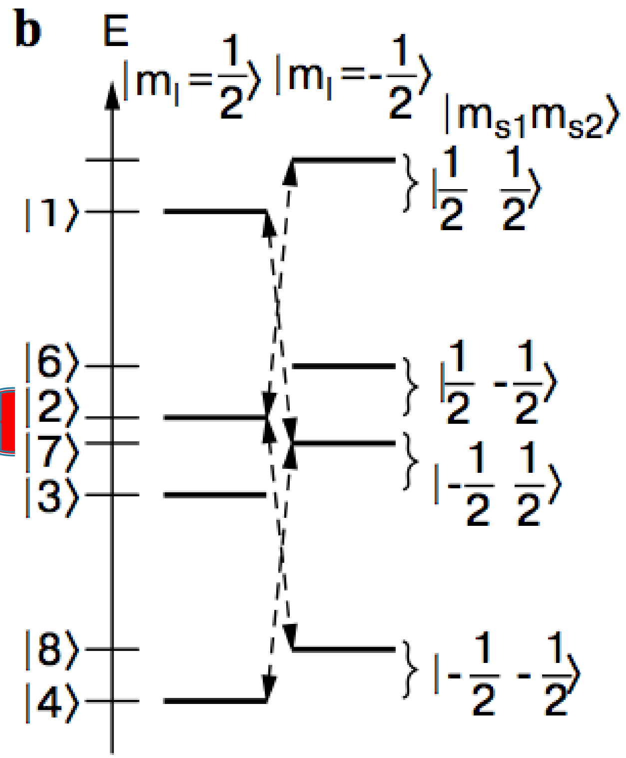


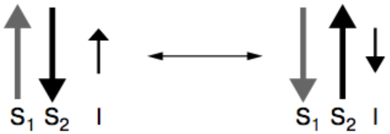
High field DNP: Mechanism

Cross Effect:

$\delta > \omega_n$

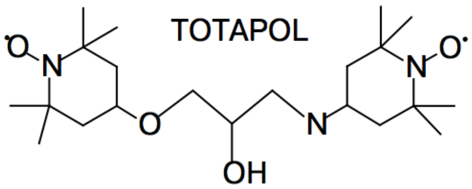
degenerate





energy-conerved flip flop process

Biradical:



glass forming solvent

deuterated Glycerol + D_2O + H_2O

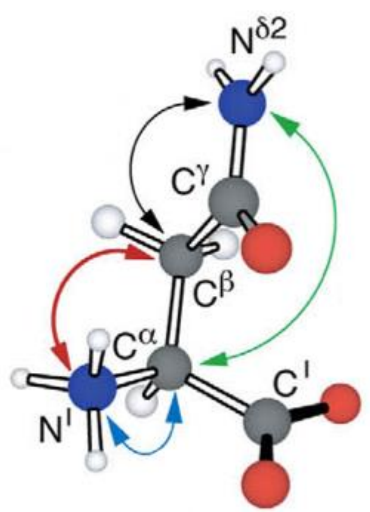
Ref [3]

Investigated system

FS-TEDOR

U-[^{13}C,^{15}N]-Asparagine

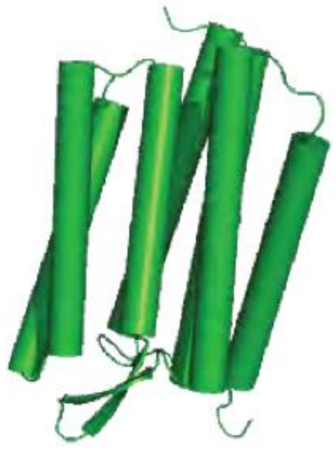
- Crystalline solid
- Uniformly labeled
- Two ^{15}N
- Four ^{13}C



FS-TEDOR + DNP

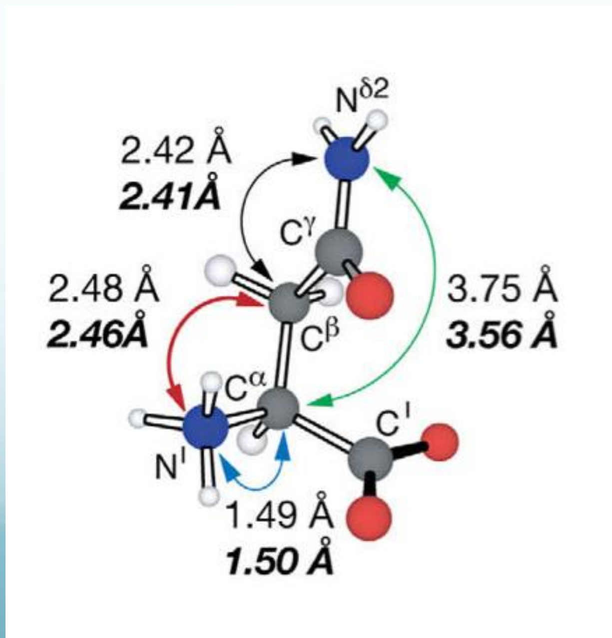
[U-^{13}C,^{15}N]-bacteriorhodopsin

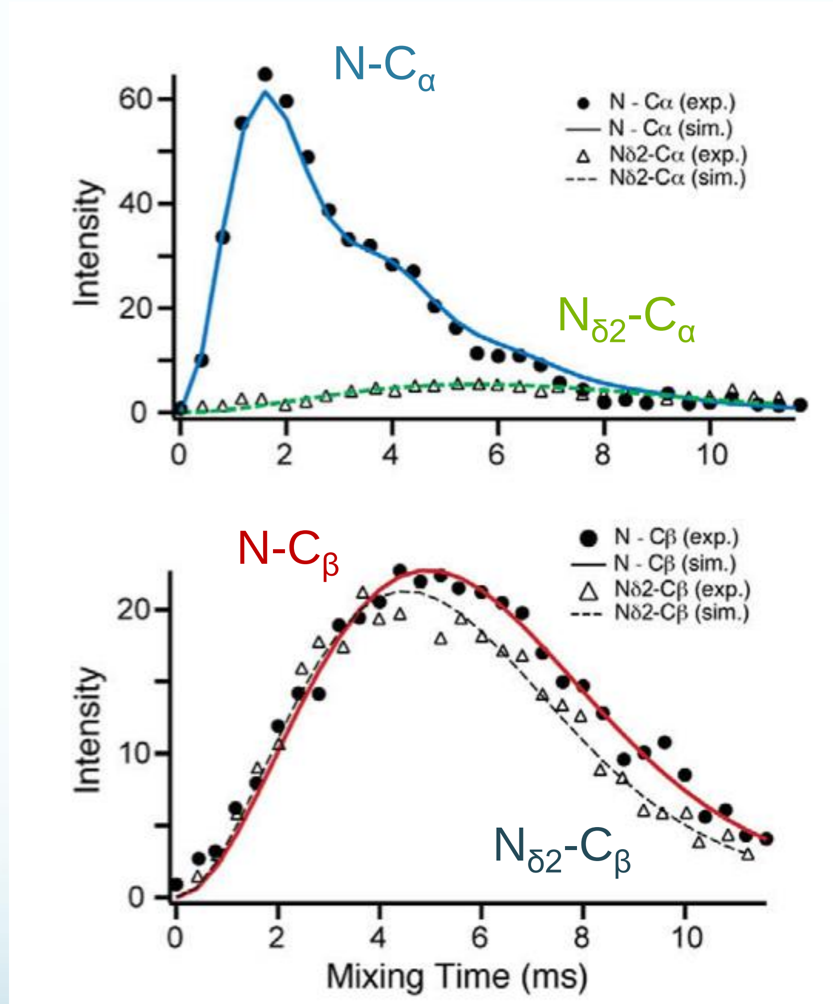
- Membrane protein
- produced by *Halobacterium Salinarum*
- Light-driven ion pump
- 26.6 kDa



FS-TEDOR on U-[^{13}C,^{15}N]-Asparagine

Experiment condition:
- 500 MHz(^{1}H)
- 100 kHz TPPM
- 1ms Gaussian refocusing pulse
    - Backbone
    - Side chain
- MAS 10 kHz
- 50 kHz REDOR pulses
- 10% diluted

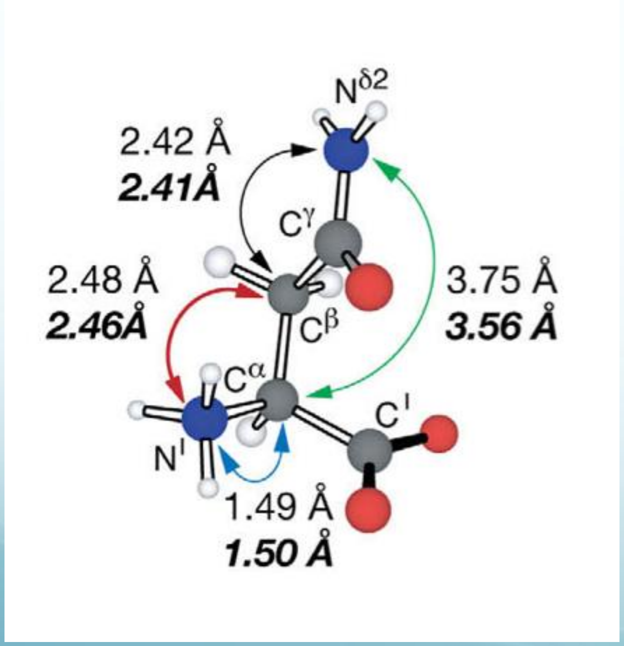




$$D = \left(\frac{\mu_0}{4\pi}\right)\frac{\gamma_I \gamma_S \bar{h}}{2\pi r^3}$$

Distance measurements by FS-TEDOR as compared to other techniques

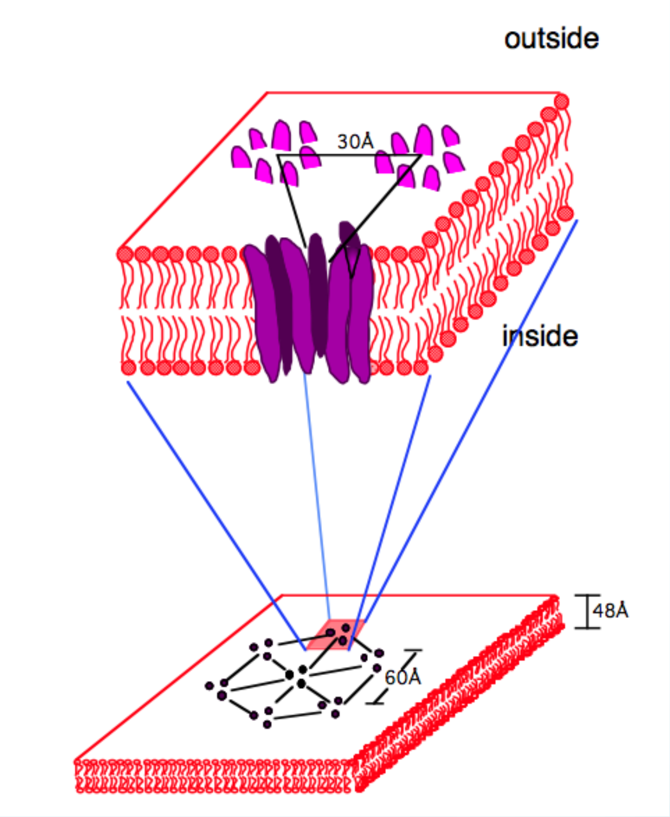
	FS-TEDOR (Å)	FS-REDOR (Å)	Neutron diffraction (Å)
$N–C^{\alpha}$	1.50	1.50	1.49
$N^{\delta 2}–C^{\alpha}$	3.56	3.58	3.75
$N–C^{\beta}$	2.46	2.49	2.48
$N^{\delta 2}–C^{\beta}$	2.41	2.44	2.42



The results are in good agreement with other methods

[U-^{13}C,^{15}N]-bacteriorhodopsin

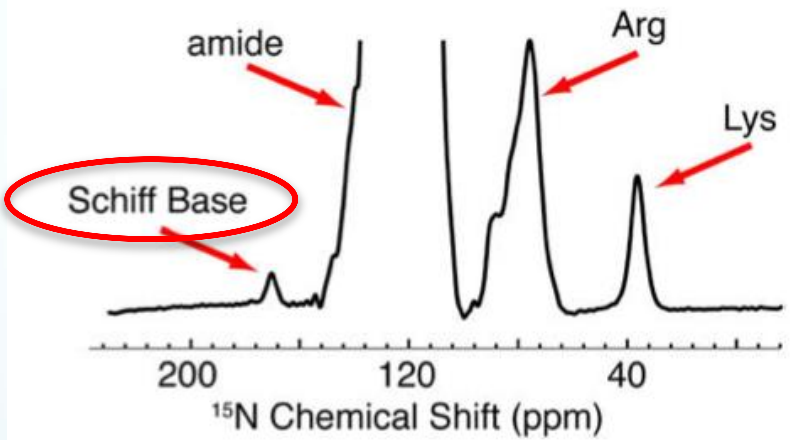
- Light-driven ion pump
- Seven transmembrane helixs
- Homotrimer
- Homotrimers aggregate to form a purple membarane
- The retinal chromophore is attached via a Shiff base linkage to Lys216
- Arg82 is part of the complex counterion



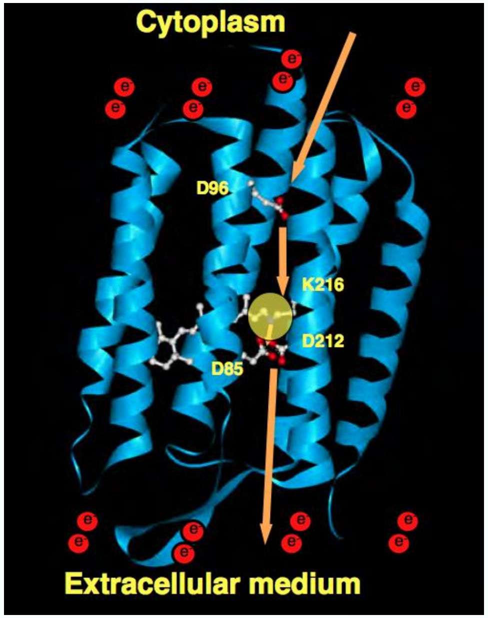
Ref [6]

Bacteriorhodopsin: 1D DNP enhanced ^{15}N spectrum

ε= 43 at 200 K
ε= 90 at 200 K



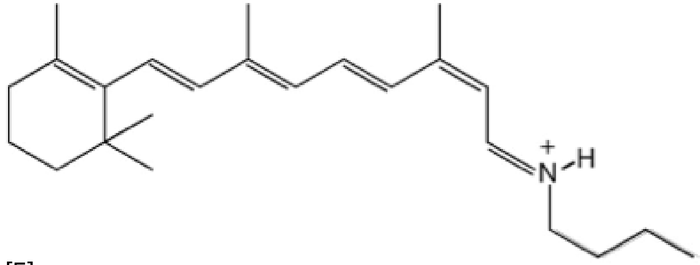
Excellent S/N!



Ref [6]

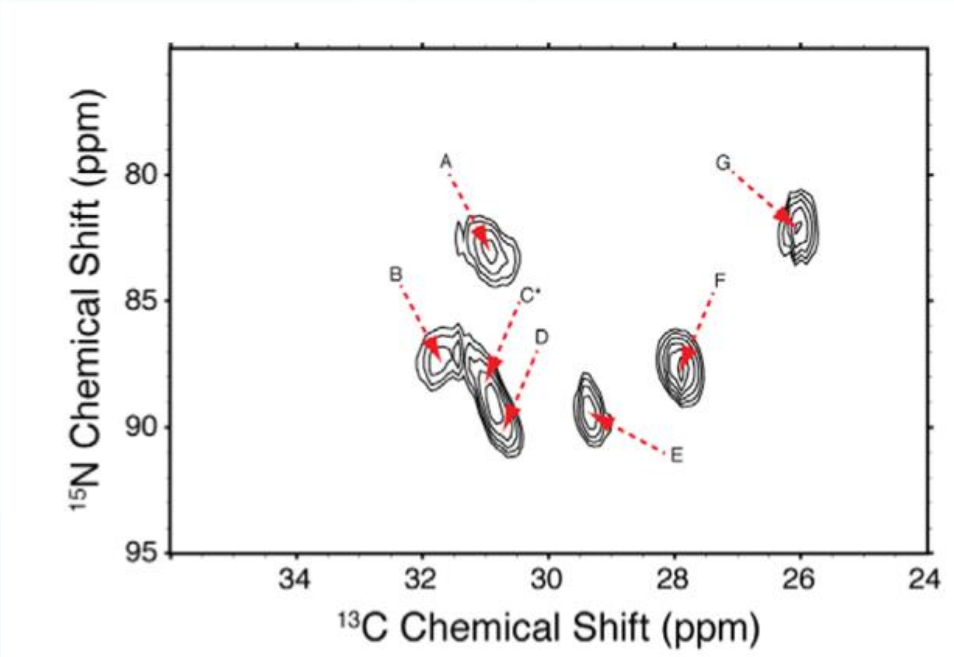
MAS-NMR
CW MW irradiation at 250GHz
at 90K
Polarizing agent: TOTAPOL

K,L (protonated)



Ref [5]

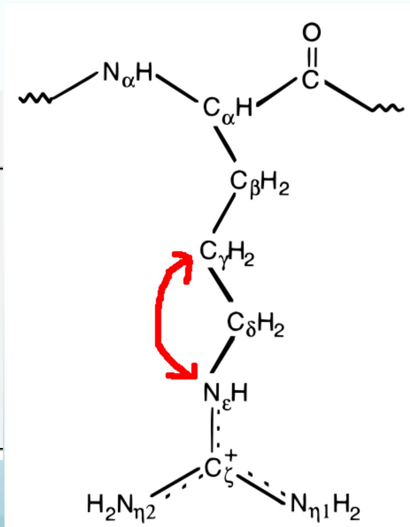
Bacteriorhodopsin: 2D $^{15}N_{\gamma}$-$^{13}C_{\varepsilon}$ selective correlation spectra



Peak	^{15}N chemical shift (ppm)	^{13}C chemical shift (ppm)	Peak volume (arbitrary units)
A	82.9	30.9	2.9
B	87.3	31.7	2.7
C[a]	87.8	31.0	2.7
D	89.9	30.7	3.1
E	89.4	29.3	3.0
F	87.6	27.9	3.5
G	82.1	26.0	3.1

7 Arg-$^{13}C_{\gamma}$-$^{15}N_{\varepsilon}$ of bR

- MAS 7.576 kHz, 380 MHz ^{1}H
- CW MW irradiation at 250GHz
- Temperature = 90K
- Polarizing agent: TOTAPOL
- 1.98 ms Gaussian refocusing pulse (selective pulse center at 90 ppm)
- REDOR mixing time 4 ms (favorable 2.4 -2.5 Å)



Arg$^{15}N_{\varepsilon}$: 90±19 ppm

Arg$^{15}N_{\eta 1}$: 75±14 ppm

Arg$^{15}N_{\eta 2}$: 75±16 ppm

Arg-$^{13}C_{\gamma}$: 27±2 ppm

Arg-$^{13}C_{\delta}$: 43.2±2 ppm

Conclusion

- FS-TEDOR can be used quantitataively and qualitatively for ^{15}N-^{13}C correlation spectroscopy in crystalline solids and membrane proteins.
- Six of seven Arg-$^{13}C_{\gamma}$-$^{15}N_{\varepsilon}$correlation spectra have been resolved using DNP.
- The method may be contemplated in lieu of specific isotopic labelling or suppression to simplify the spin dynamics.

References

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2. C. P. Jeroniec, C. Filip, R. G. Griffin, *J. Am. Chem. Soc.* **2002**, *124*, 10728-10742.

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4. A. T. Petkova, J. G. Hu, M. Bizounok, M. Simpson, R. G. Griffin, J. Herzfeld, *Biochemistry* **1999,** *38,* 1562-1572.

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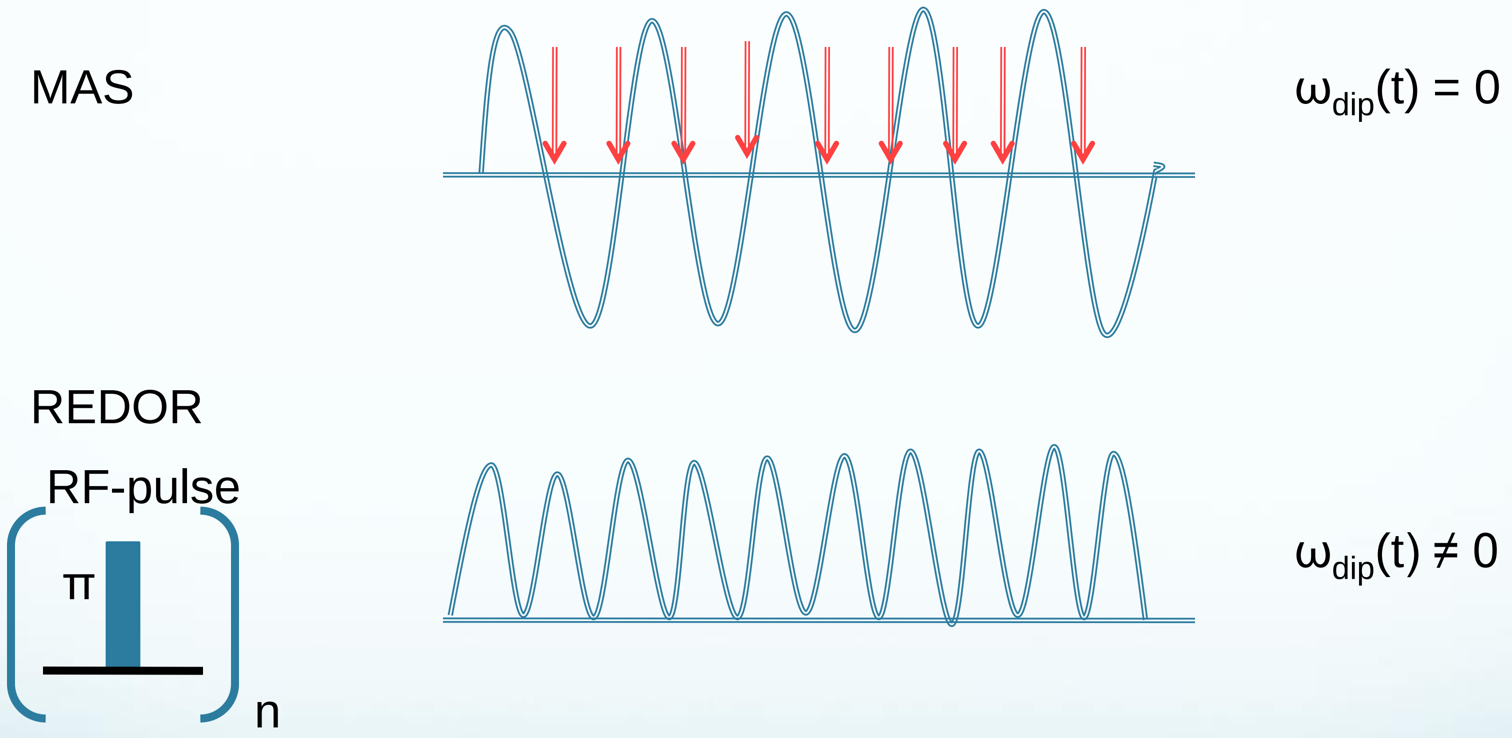
6. Lecture (Francis Bitter Magnet Laboratory and Department of Chemistry Massachusetts Institute of Technology), nmrwinterschool.com (accessed Sep, 17th, 2013)

Thank you very much

Supplements

REDOR

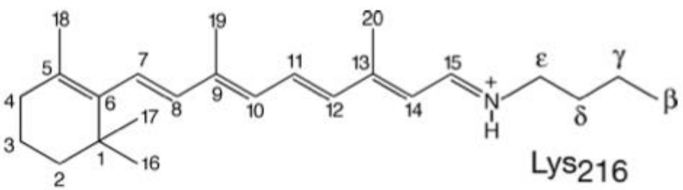


bR: Photocycle

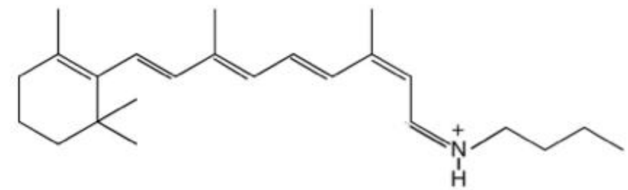
$$bR_{555} \rightleftarrows bR_{568} \xrightarrow{h\nu} J_{625} \rightarrow K_{590} \rightarrow L_{550} \xrightarrow{-H^+} M_{o\ 412}$$

$$\rightarrow M_{n\ 412} \xrightarrow{+H^+} N_{520} \rightarrow O_{640} \rightarrow bR_{568}$$

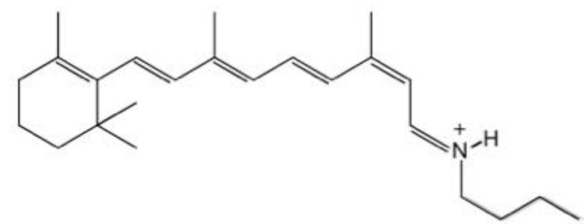
bR_{568}
13-*trans*, 15-*anti*
protonated



bR_{555}
13-*cis*, 15-*syn*
protonated



K, L
13-*cis*, 15-*anti*
protonated



M_o
13-*cis*, 15-*anti*
deprotonated