A BEGINNER'S PRIMER ON NOE MEASUREMENT

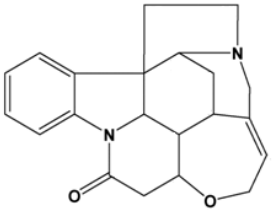
INSIGHTS INTO 3D STRUCTURE

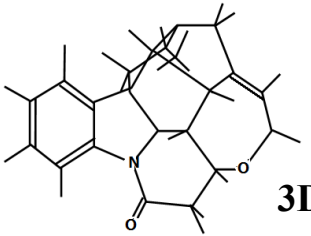
JEOL RESONANCE Inc.
(Translated by Pavlos Stampoulis)

Introduction

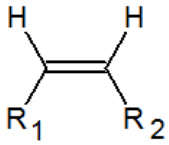
NOE : Nuclear Overhauser Effect

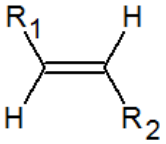
Because NOE can reveal 1H spins that are in proximity in 3D space, it is often used to assist research on the stereochemistry of organic compounds





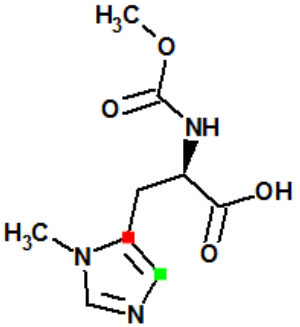
3D structure

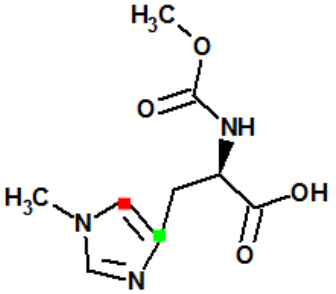




cis ? *trans ?*

Configuration of olefins





Position of substituent

This is a lecture on NOE as applied in small organic compounds

Contents

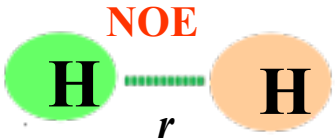
NOE as mentioned in a textbook ..

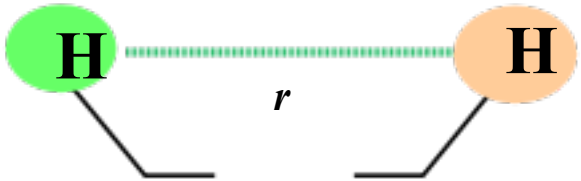
When a spin is saturated with a Radiofrequency pulse, the signal of that spin and the signal of the spins in spacial proximity to it , all change intensity. This NMR phenomenon is called NOE.

spin

(also known as cross-saturation)

NOE is a relaxation phenomenon caused by dipole-dipole interactions





(*r*: spin-spin distance)

For r < 6Å
NOE can be observed

NOE ∝ r^{-6}

NOE depends on the spin-spin distance and the tumbling of the molecule

The Difference NOE experiment

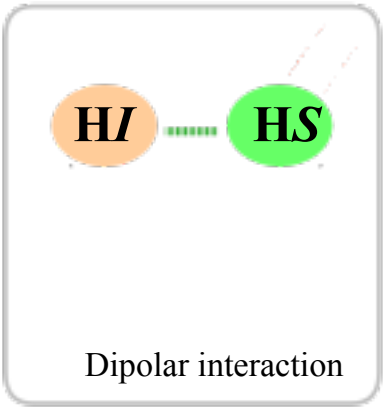
When a spin is saturated with a Radiofrequency pulse, the signal of that spin and the signal of the spins in spacial proximity to it , all change intensity. This NMR phenomenon is called NOE.

(A) With Saturation

NOE

(A) − (B)
difference
spectrum

正のNOE



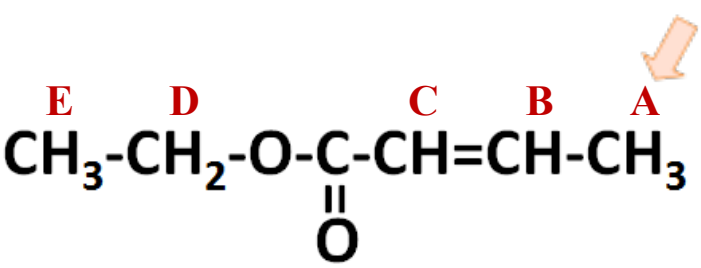
(B) Without Saturation

Only the saturated and the nearby signals are observed in the spectrum

The experiment records Steady state NOE

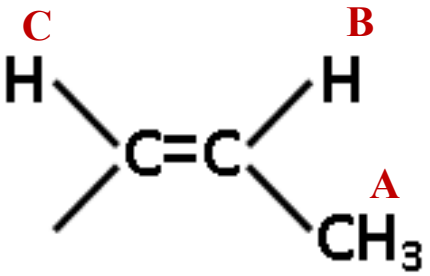
The Difference NOE experiment

Determination of double-bond stereoisomers
(*cis*, *trans* isomerism)

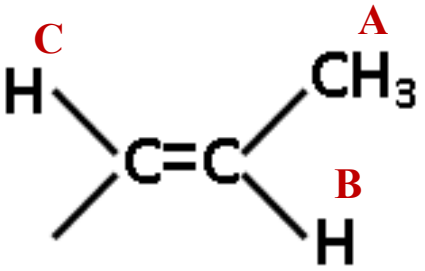


Ethyl crotonate
(MW 114.14)

NOE observation after irradiation of CH3 (A)



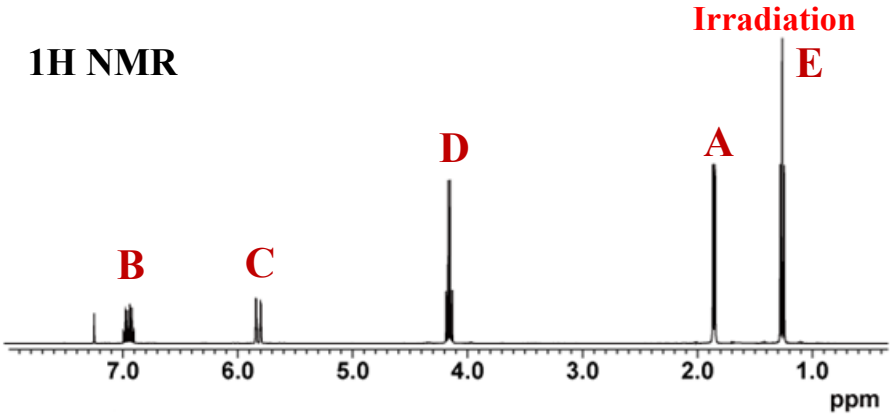
cis

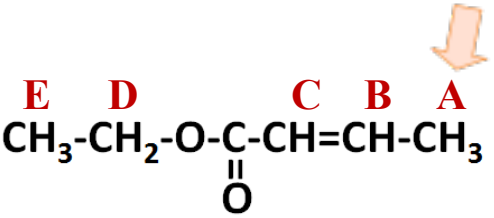


trans

The Difference NOE experiment

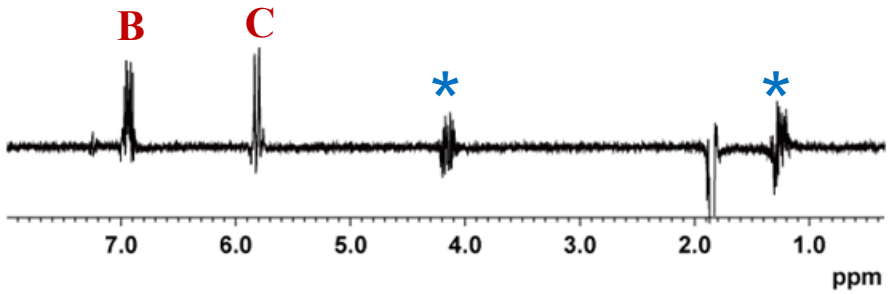
1H NMR





（10 mg/0.55 mL CDCl3 ）

Difference NOE spectrum

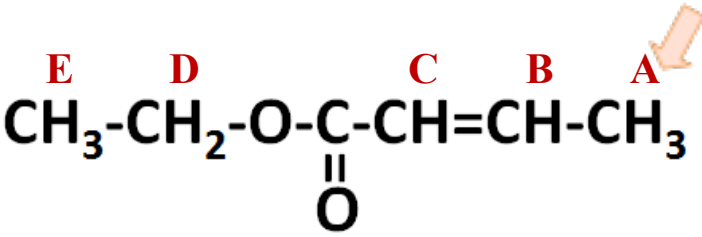


400 MHz
Saturation time 40sec
scans 16

Irradiation of CH3(A)⇒ Observation of positive NOE on B and C

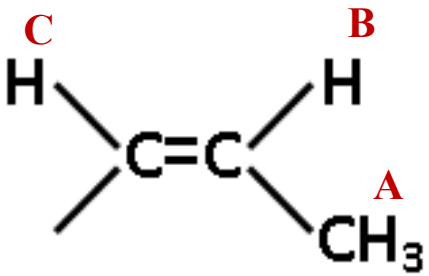
The Difference NOE experiment

Determination of double-bond stereoisomers
(*cis*, *trans* isomerism)

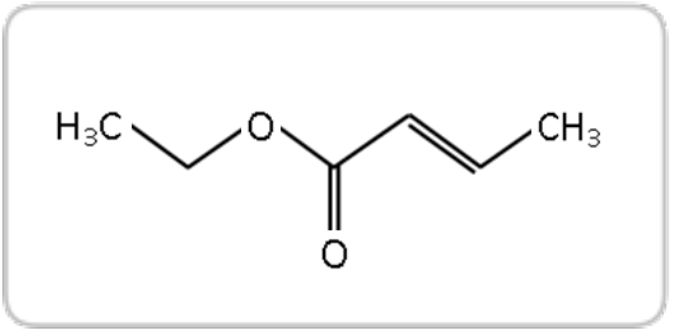


(MW 114.14)

Irradiation of CH3(A)⇒ Observation of positive NOE on B and C



cis



trans

Unfortunately, for the majority of cases ..

Troubleshooting with NOE

I cannot observe NOE!!

Check longitudinal relaxation （T1）！

In difference NOE: *set saturation time at 5 x T1*

In NOESY: set mixing time at about *T1*

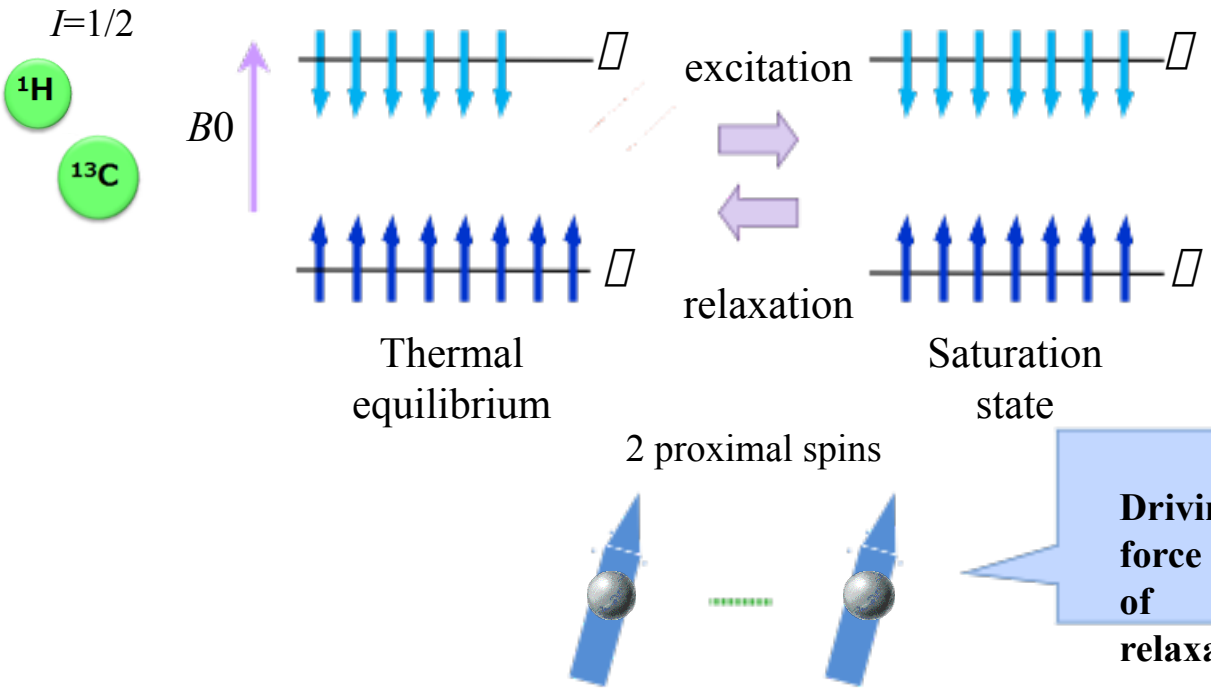
Why relaxation time? ?

NOE is a relaxation phenomenon caused by dipole-dipol

interactions

The Origin of NOE

NOE is a relaxation phenomenon caused by dipole-dipole interaction



In solution dipolar interaction is the main relaxation mechanism

Important for NOE

The NOE Effect

In the case of steady-state NOE:

Upon irradiation of signal S, the relative intensity increase for signal I due to NOE is

$$\eta_I\{S\} = 0.5$$

Dipole-dipole interaction

The maximum achievable increase is 50% → Increase of signal intensity 1.5 times (for fast tumbling molecule)

Be careful!!

This is valid for the case we consider only two spins within the molecule (2 spin system)..

In reality, additional relaxation pathways exist that cannot be ignored

The NOE Effect

In the case of steady-state NOE:

Upon irradiation of signal S, the relative intensity increase for signal I due to NOE is

$$\eta_I\{S\} = 0.5$$

Dipole-dipole interaction

The maximum achievable increase is 50% → Increase of signal intensity 1.5 times (for fast tumbling molecule)

Additional relaxation pathways

Intramolecular interactions (multi-spin system)

Intermolecular interactions

→ **diminished NOE!**

So in reality..

The maximum theoretical value 50% becomes → **0～25%**

Experimental methods for the Observation of NOE

Type of NOE	Method
steady state NOE	**Difference NOE**
transient NOE	**2D NOESY**
	1D NOESY (DPFGSE-NOE)

NOESY：Nuclear Overhauser Effect SpectroscopY

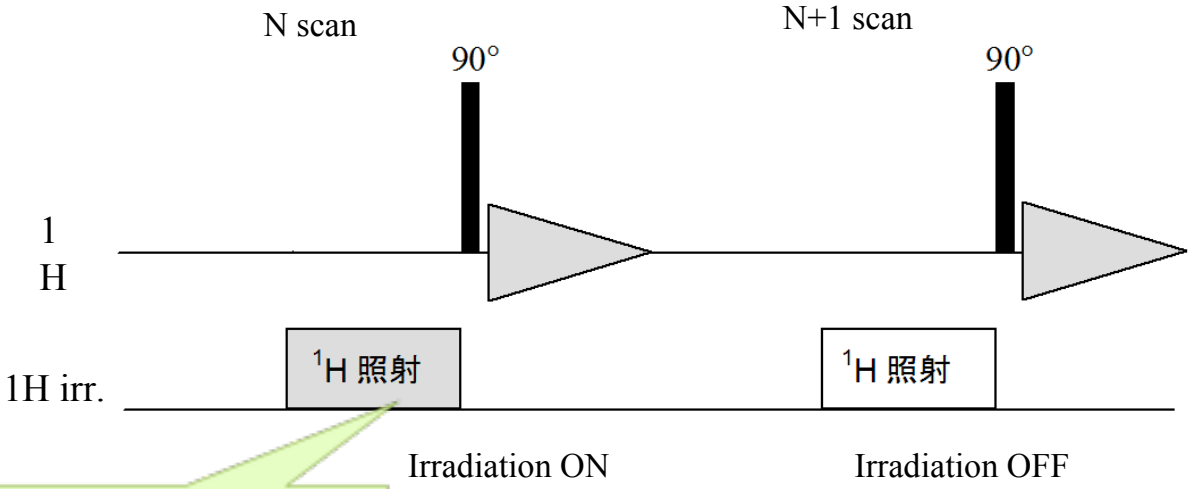
In difference NOE: *set saturation time at 5 x T*1

In NOESY: set mixing time at about *T*1

The Difference NOE Experiment

(steady state NOE)

Upon selective saturation of a 1H signal, the system relaxes to a new equilibrium state. The obtained spectrum in Difference NOE experiment reflects this new equilibrium state (steady state)



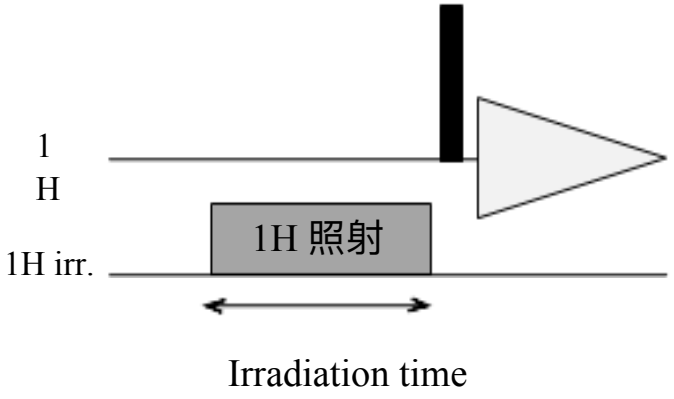
Irradiation time is the most important parameter

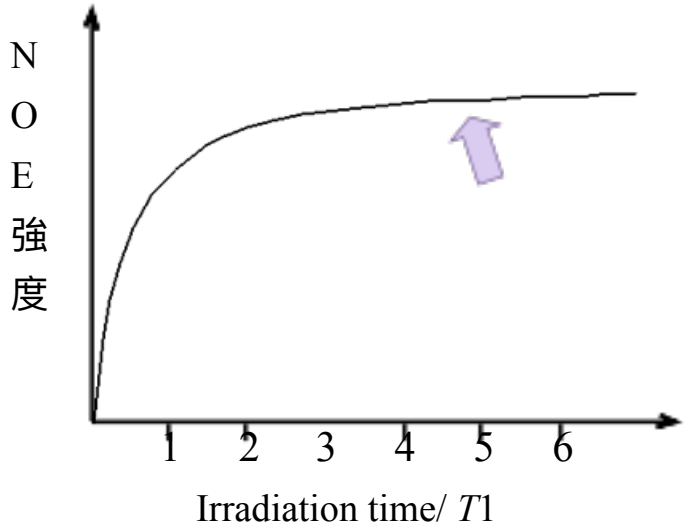
The Difference NOE pulse sequence

NOE Effect in Difference NOE Experiment

steady state NOE

Upon selective saturation of a 1H signal, the system relaxes to a new equilibrium state. The obtained spectrum in Difference NOE experiment reflects this new equilibrium state (steady state)





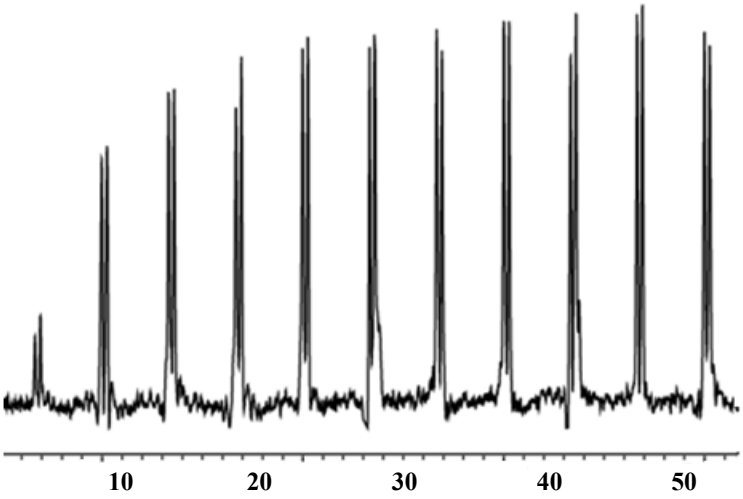
In Difference NOE: *set saturation time at 5 x T*1

→ allow for relaxation via dipolar interactions to complete

NOE Effect in Difference NOE Experiment

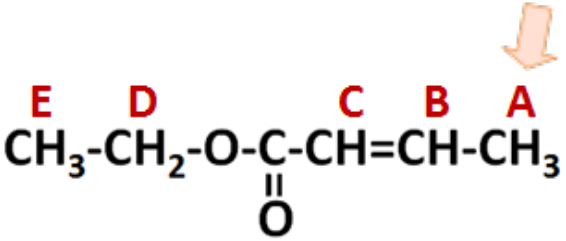
steady state NOE

Intensity change of C upon irradiation of A



Saturation time (s)

Irradiation



	*T*1 (s)
A	3.2
B	5.9
C	5.2
D	4.5
E	3.8

Estimate necessary irradiation from T1

5.2×5=26 (s)

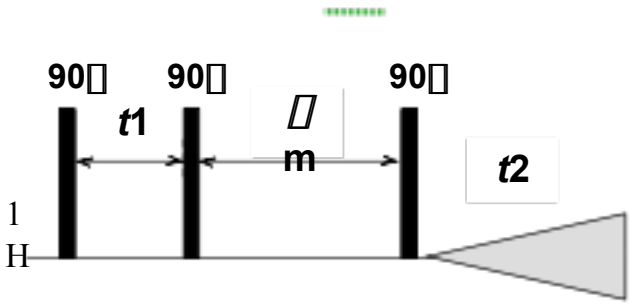
In difference NOE: *set saturation time at 5 x T1*

→ allow for relaxation via dipolar interactions to complete

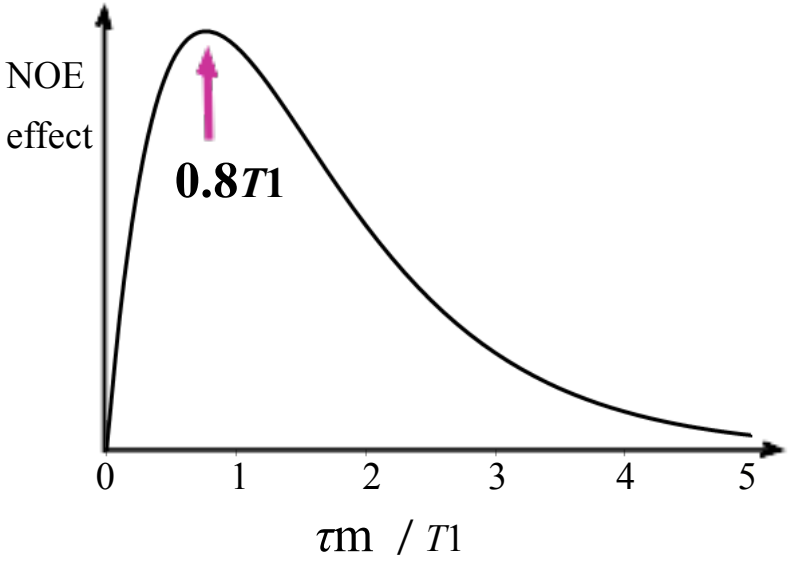
Signal Intensity in 2D NOESY

transient NOE

RF pulses cause perturbation. The 2D NOESY spectrum captures the NOE that develops during the subsequent mixing time



2D NOESY pulse sequence

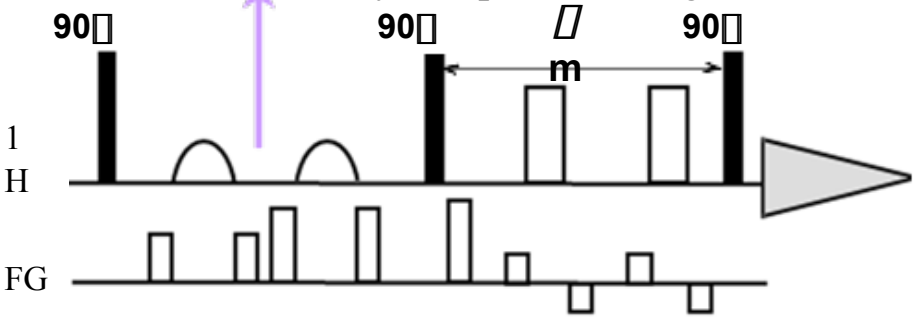


In NOESY: set mixing time at about *T*1

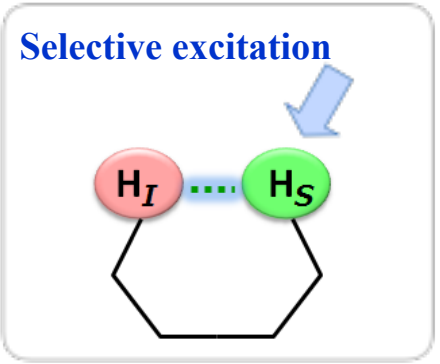
1D NOESY

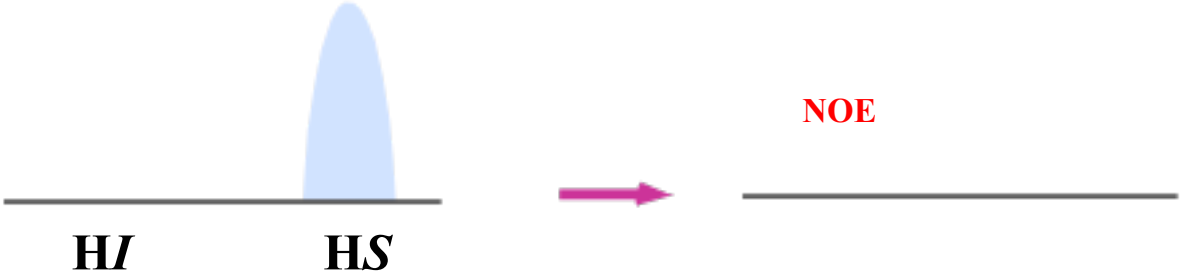
transient NOE

In 1D NOESY, a 1H signal is selectively perturbed and therefore the detected NOEs concerned only this particular signal



1D NOESY pulse sequence



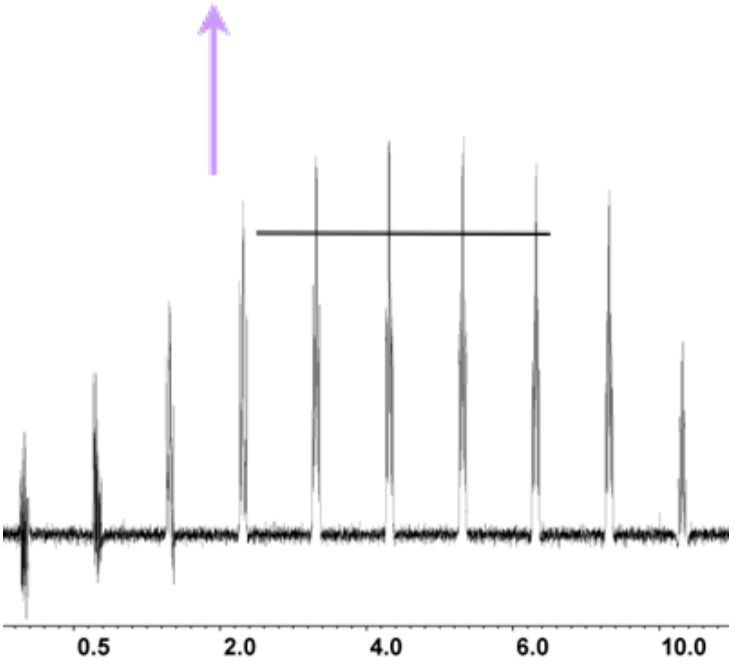


set mixing time at about *T*1

Signal Intensity in 1D NOESY

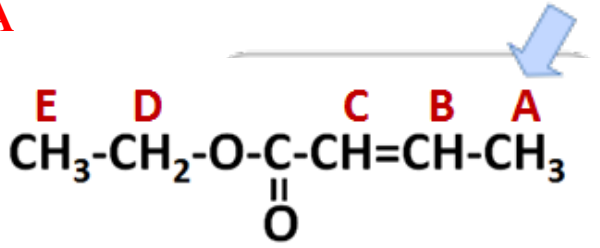
transient NOE

intensity change of B upon irradiation of A



Mixing time τm (s)

Selective excitation

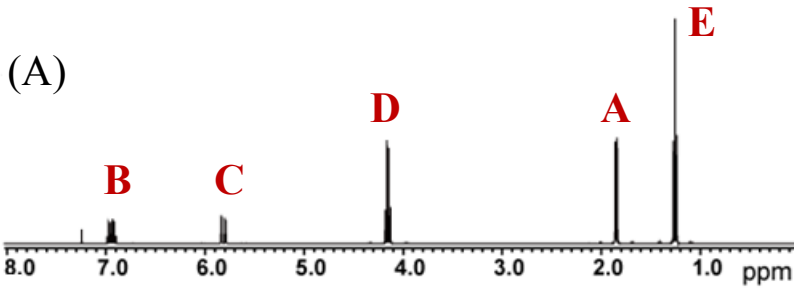


	*T*1 (s)
A	3.2
B	5.9
C	5.2
D	4.5
E	3.8

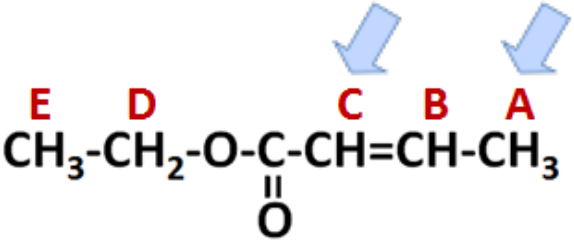
Estimate necessary irradiation time from T1

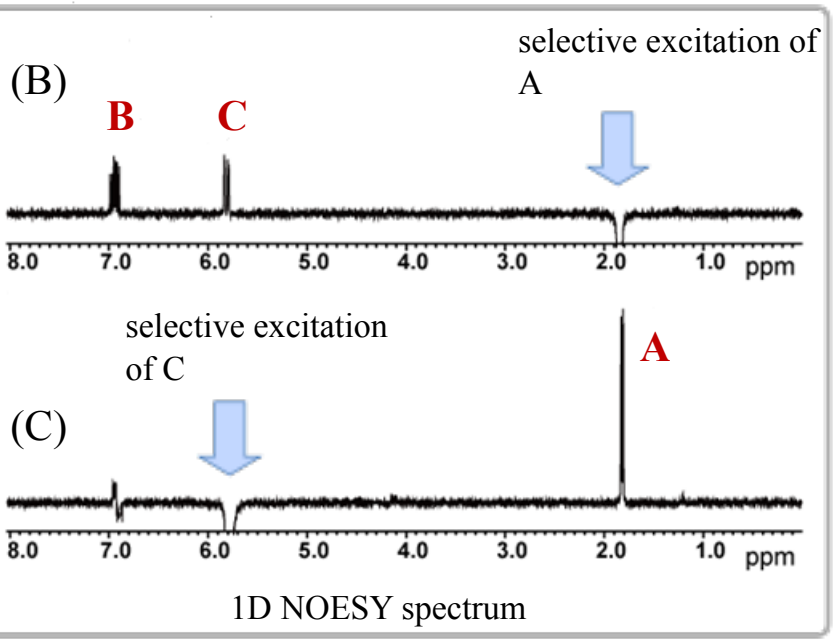
5.9×0.8= 4.7 (s)

1D NOESY

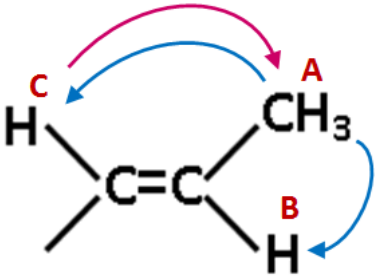


Selective excitation





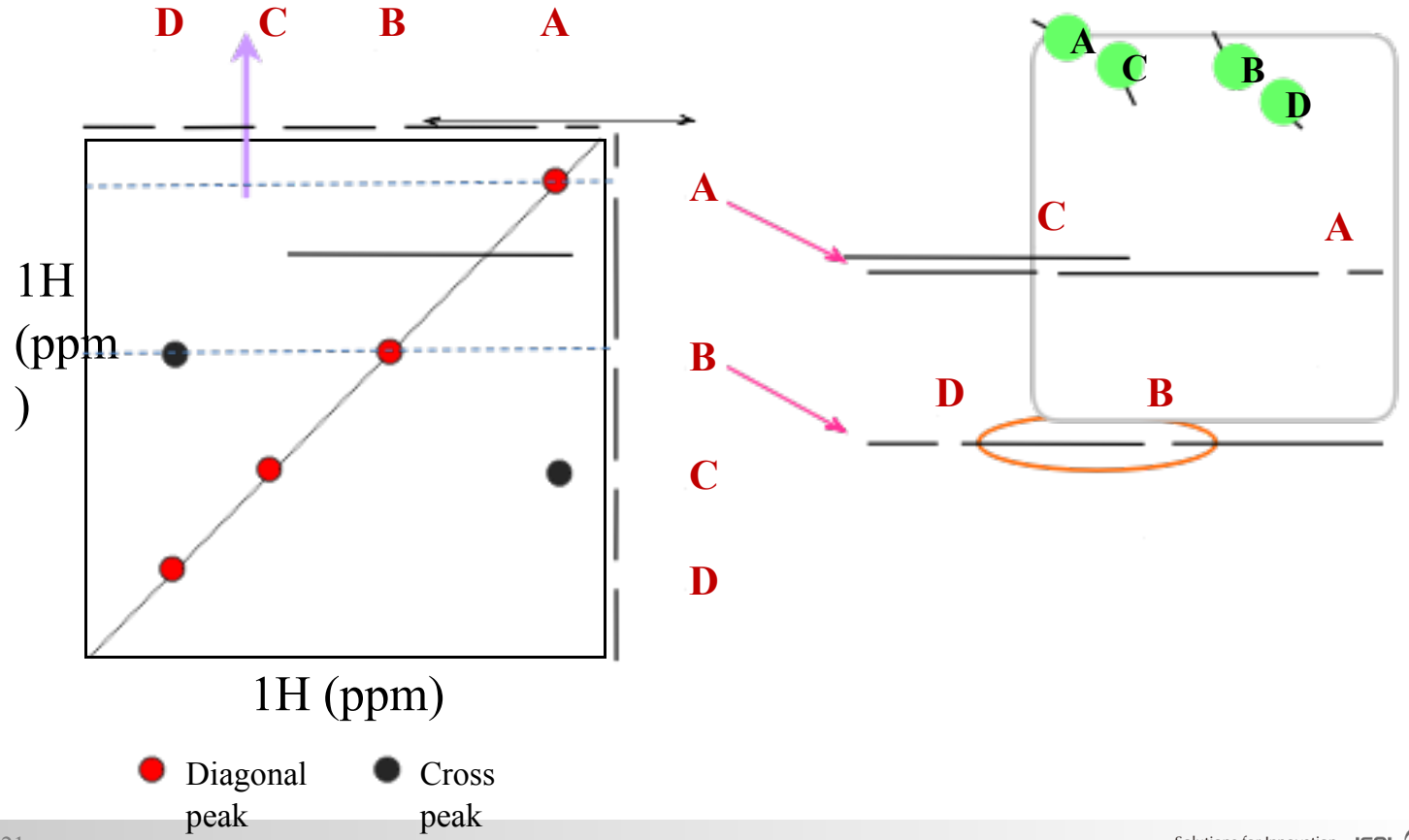
1D NOESY spectrum



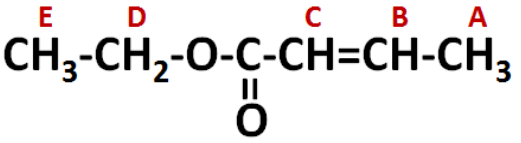
"read " the spectrum as you read the Difference NOE

2D NOESY

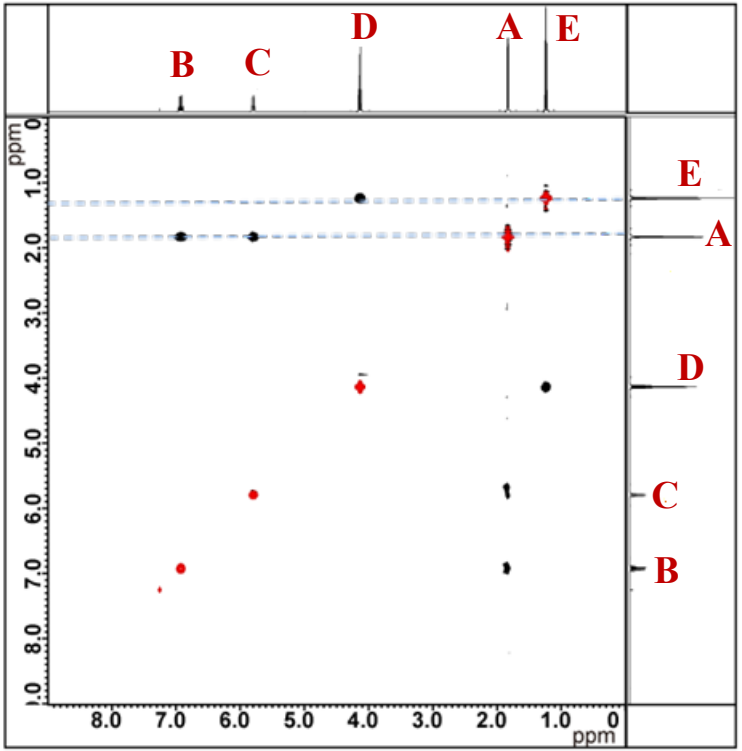
It reveals all the NOEs in the molecule

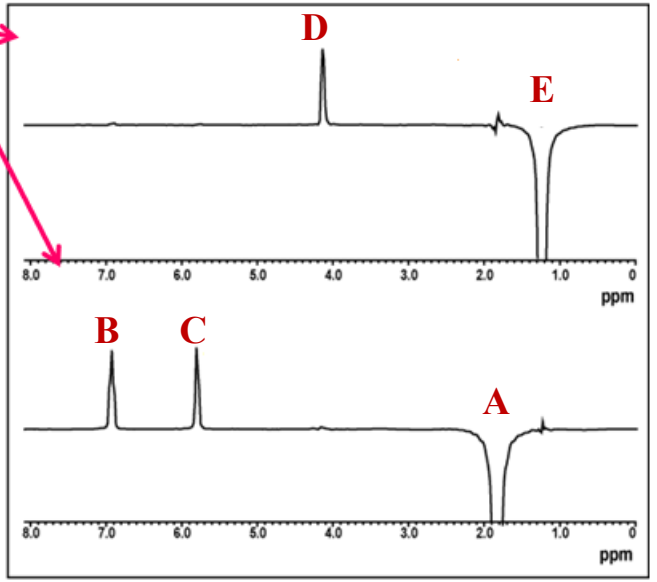


2D NOESY



Ethyl crotonate





Observation of NOE for the A··B, and A··C pairs

Pick up the right NOE experiment

- **Observation of all the NOEs in the molecule**

- **Assignment not established**

  2D NOESY

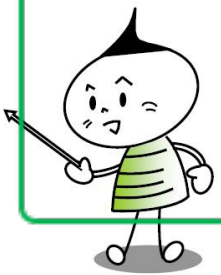
- **Observation of NOEs for a particular 1H**

  1D NOESY

  Difference NOE

Points that warrant your attention

- Caveats with sample preparation
- Dealing with existed NOEs that are not observed
- Caveats with spectrum analysis



Caveats in sample preparation

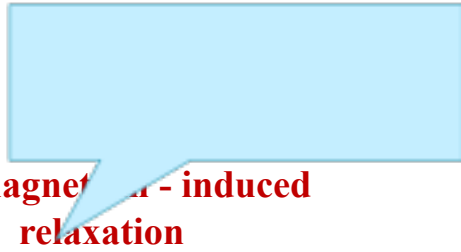
Intermolecular relaxation

① Concentration

avoid extremely high concentrations

② paramagnetic material

dissolved oxygen, metal ions



Paramagnetic - induced relaxation

weakened NOE effect

A simple and effective way to remove dissolved oxygen is to inject N2 or Argon gas into the sample through a capillary (degassing)

Points that warrant your attention

I see no NOE..

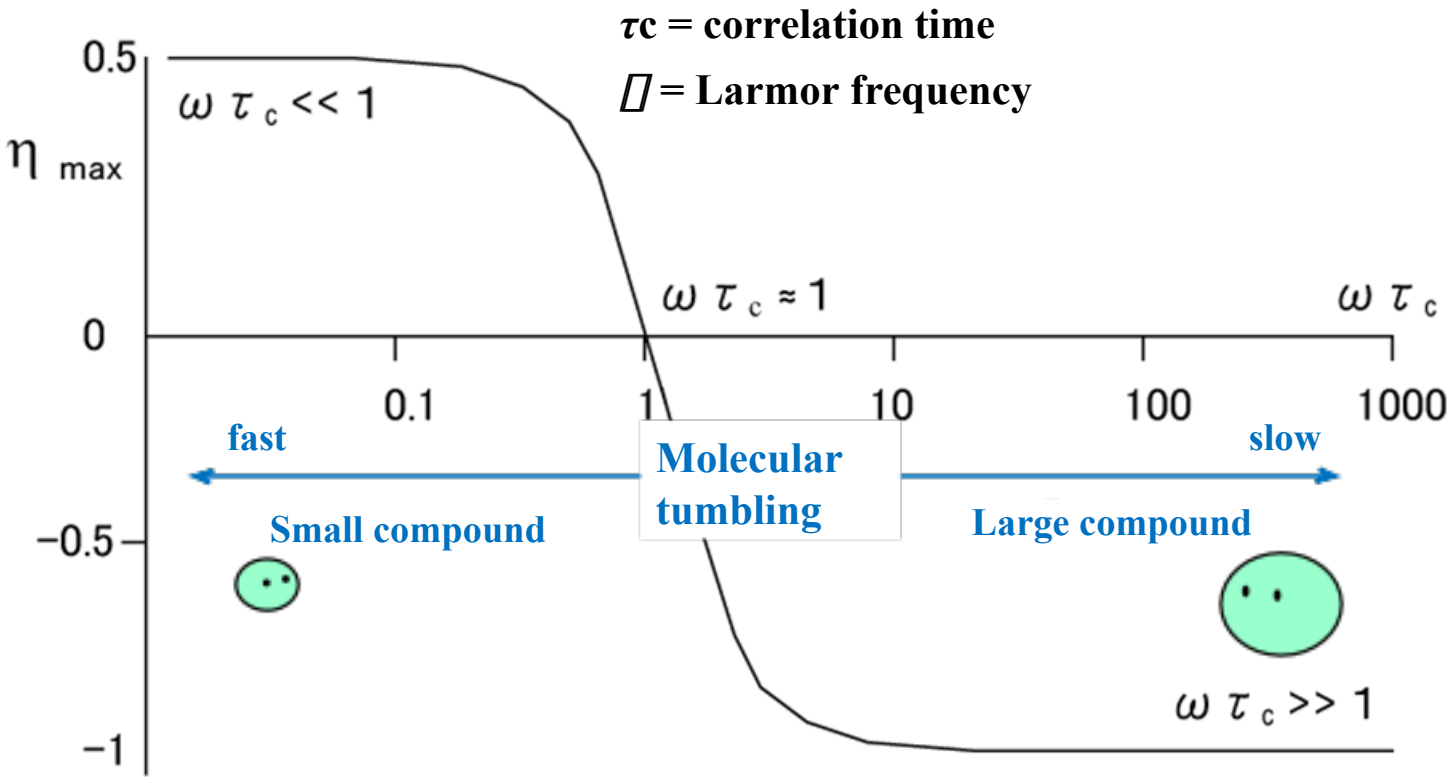
Parameters Setup: OK
Sample preparation: OK
But still..

A crucial point in NOE..

Is the relationship between dipolar interactions and molecular tumbling

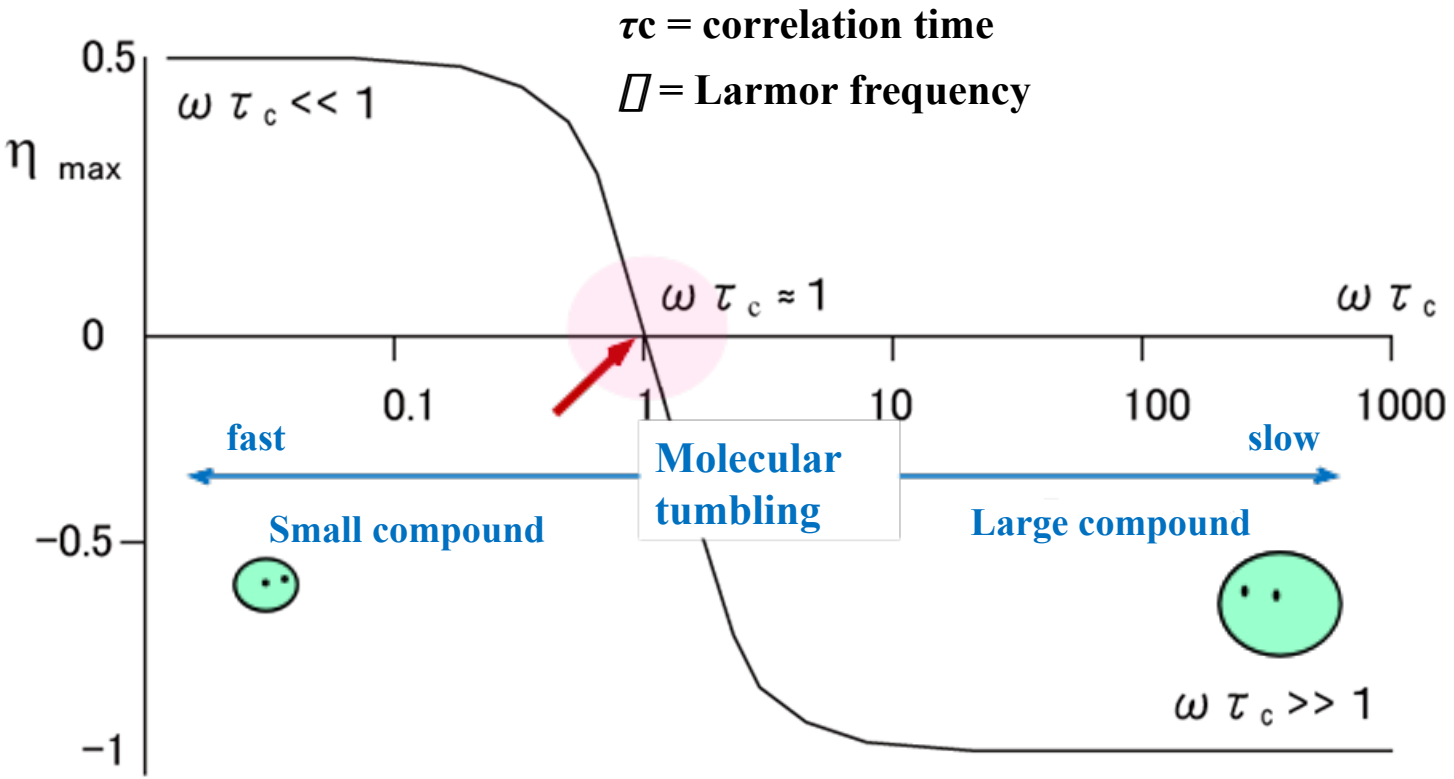
NOE effect depends on molecular tumbling

NOE intensity and molecular tumbling



Relation between NOE effect and the product $\omega\,\tau c$

NOE intensity and molecular motions



Relation between NOE effect and the product $\omega\,\tau c$

So..in case you cannot observe NOE

..despite optimal sample preparation and correct setup of experimental parameters

① change molecular motions（τc）

➡ Temperature, solvent (viscosity)

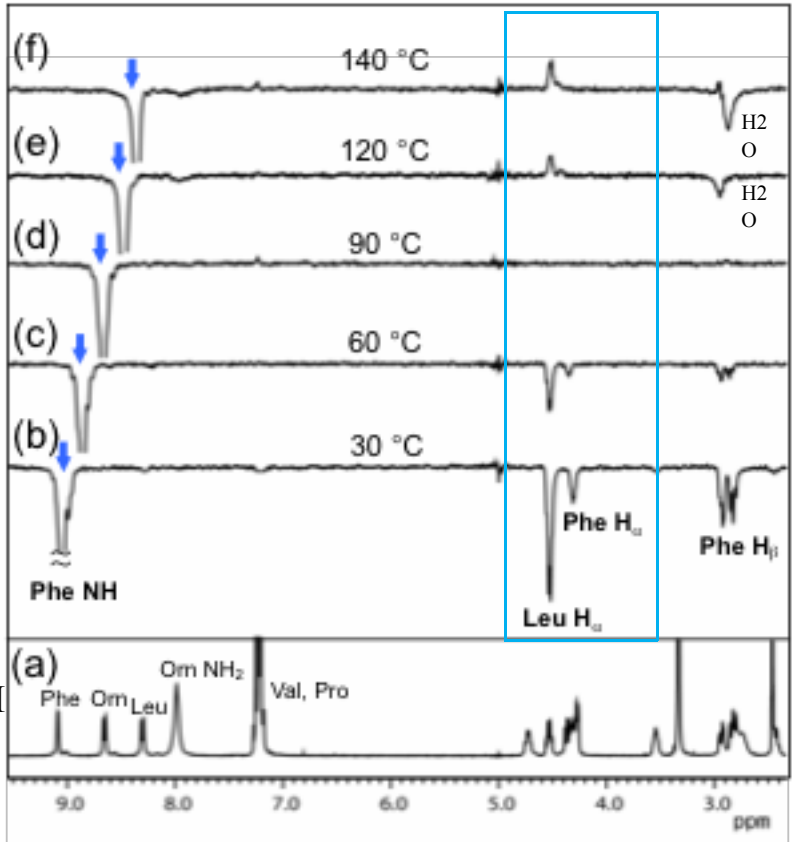
② change larmor frequency（ω）

➡ Measurement with a different magnet

③ change experiment

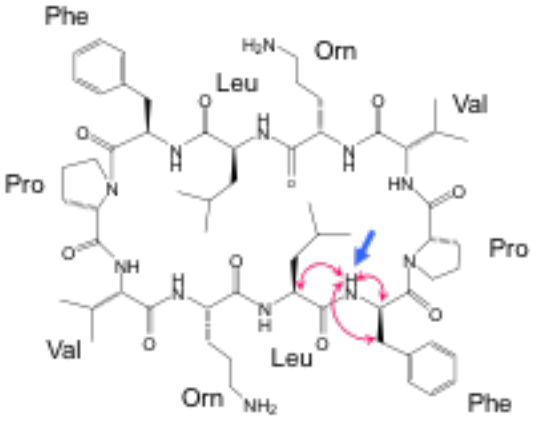
➡ ROESY

Change molecular motions （temperature）



1H

1D NOESY spectrum (400MHz)

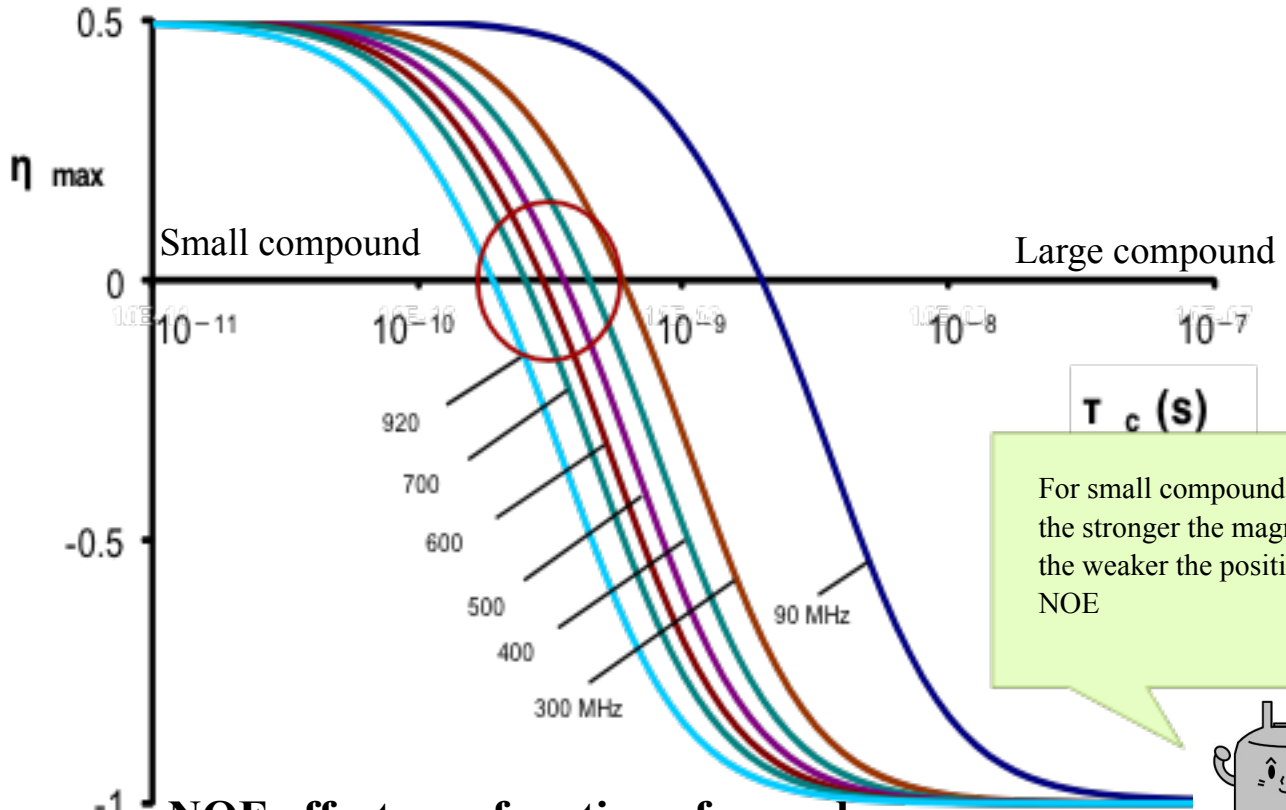


Gramicidin S

（MW = 1141）

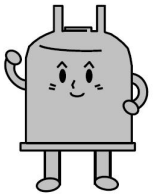
12mg / 0.6ml DMSO-d6

Change larmor frequency

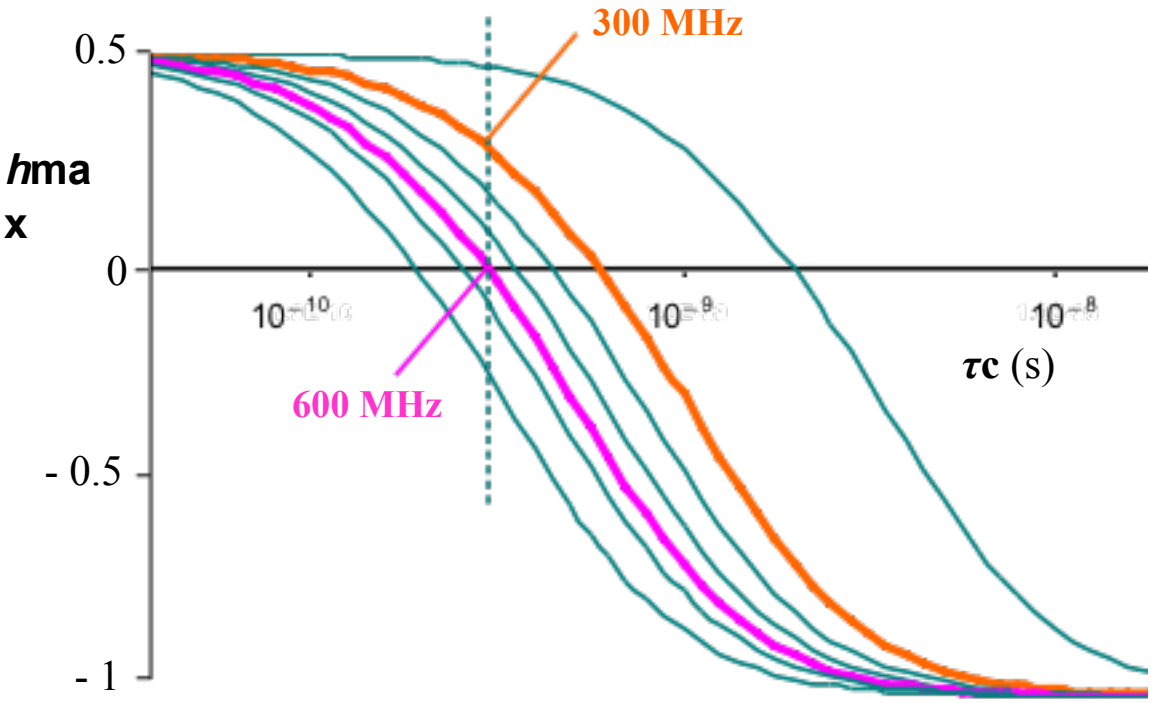


NOE effect as a function of τc and magnetic field

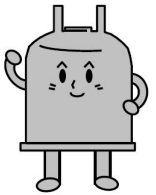
For small compounds, the stronger the magnet the weaker the positive NOE



Change larmor frequency

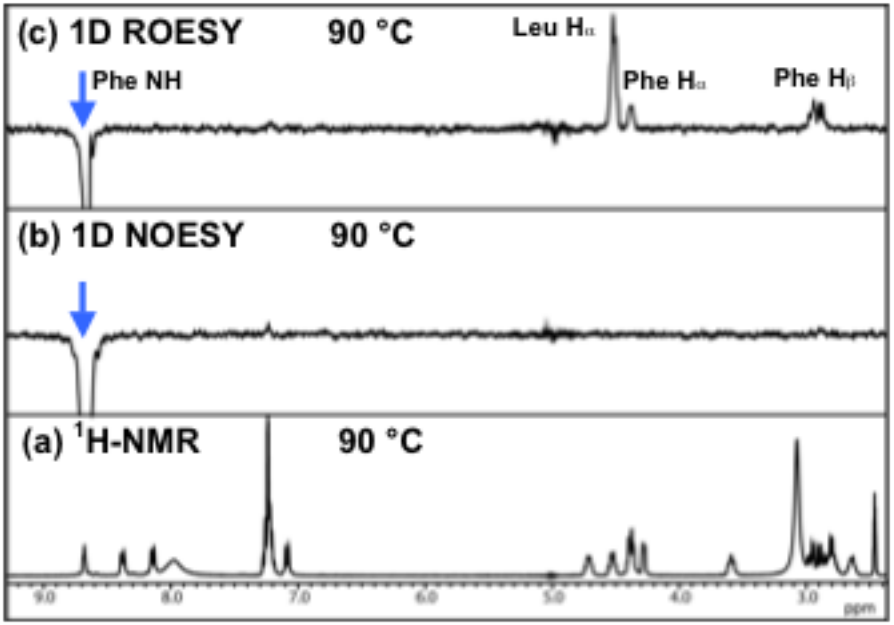


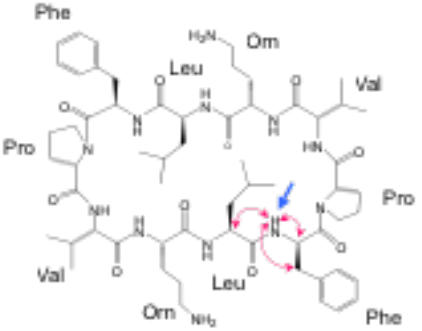
NOE effect as a function of τc and magnetic field

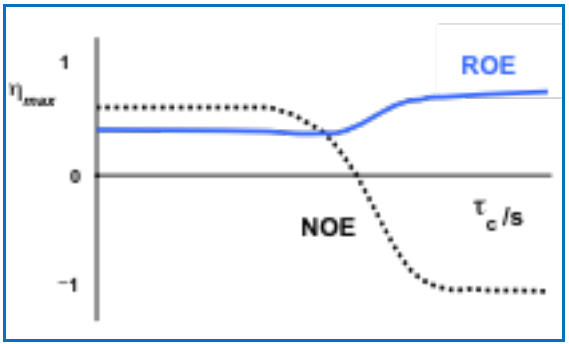


Try ROESY

Rotating frame nuclear Overhauser Effect SpectroscopY







★ NOE observed in ROESY is called ROE

Points that warrant your attention

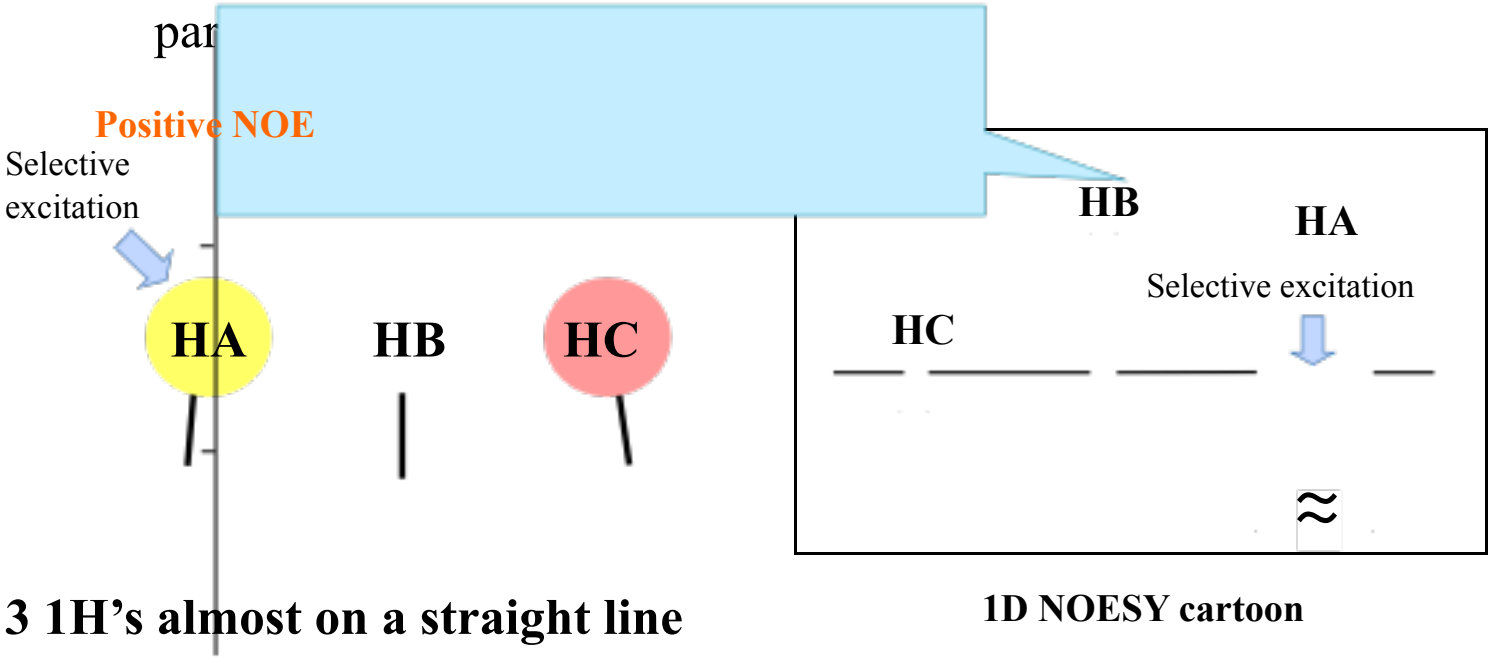
last but not least.....

NOE is not easy at all!

- **Small compounds can produce negative NOE?!**
- **So close in space and they don't give NOE..?!**

Q. Small compounds can produce negative NOE?!

A. It is known as Indirect NOE （three spin effect） and it is observed when protons in a small compound display a par

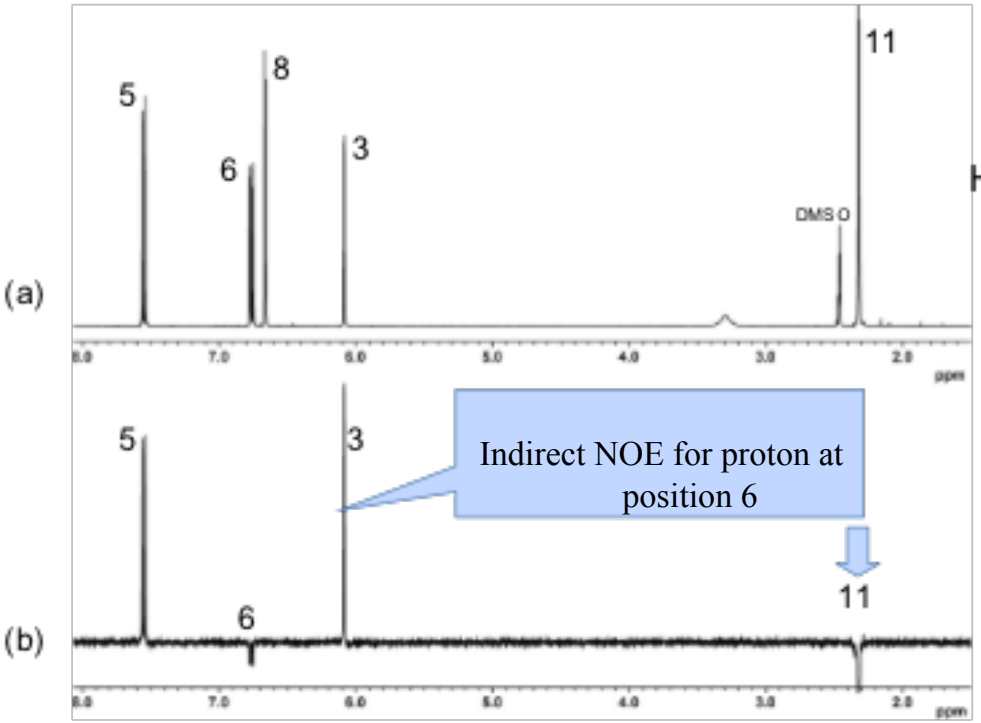


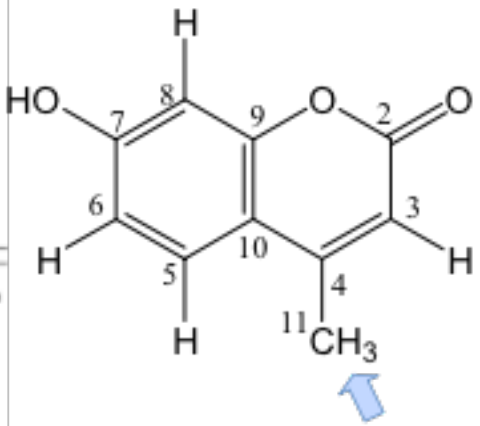
3 1H's almost on a straight line

1D NOESY cartoon

Three spin effect

Q. Small compounds can produce negative NOE?!



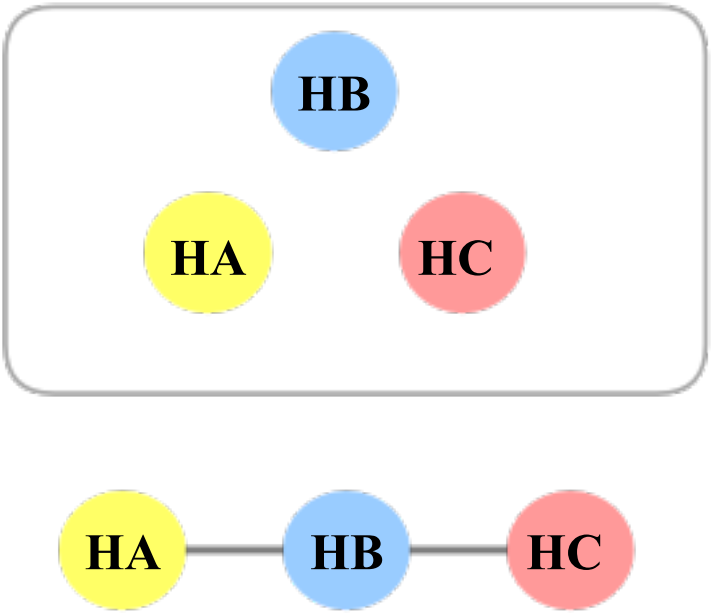


1D NOESY 4-methyl umbelliferone（10 mg / 0.6 ml DMSO-d6, 400 MHz）

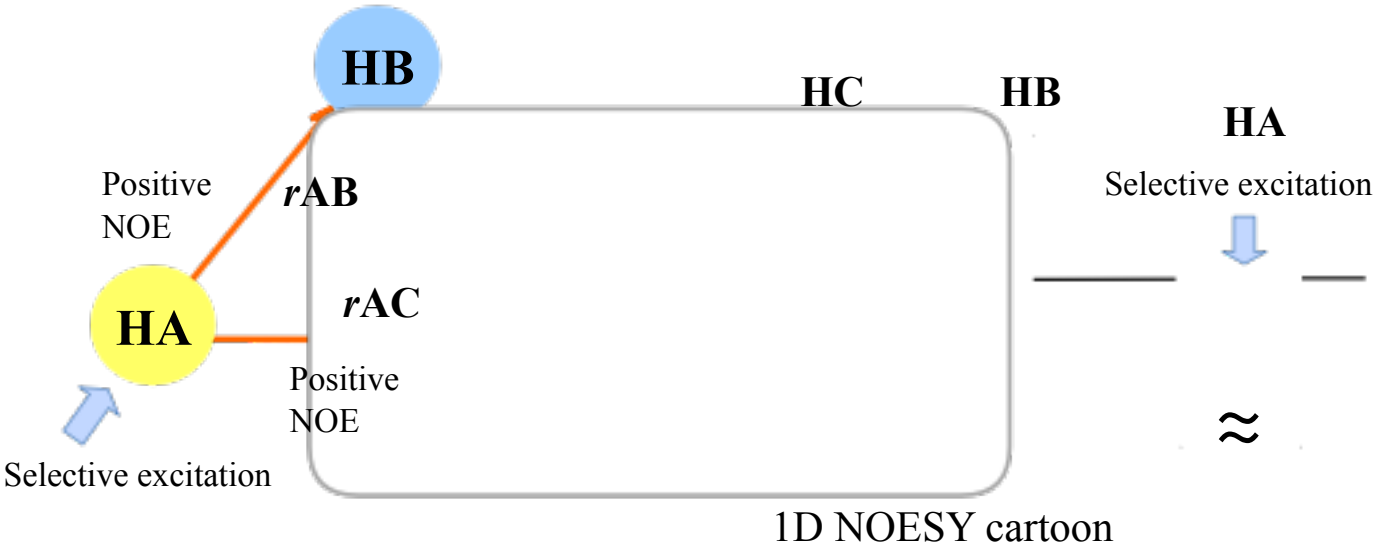
(a) Proton spectrum(b) 1D NOESY spectrum with selective excitation of 11-proton）

Q. So close in space but they don't give NOE..?!

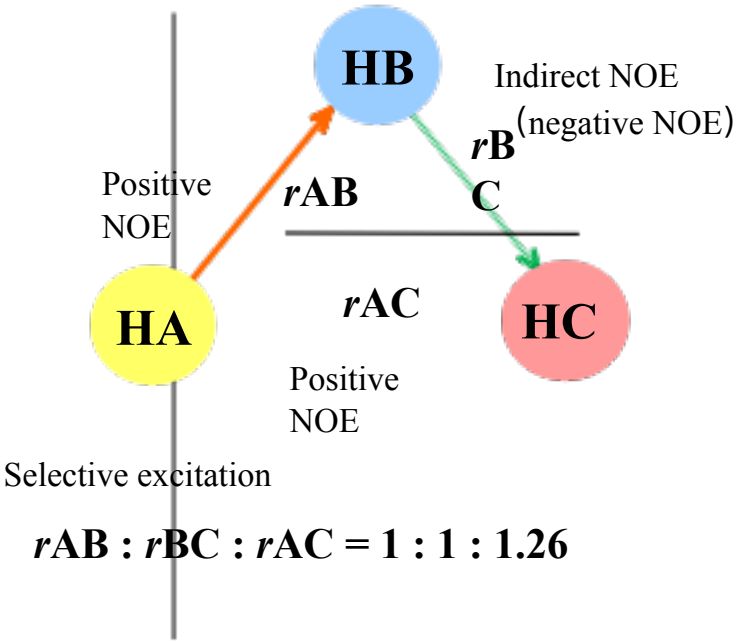
A. When three nearby protons take a particular configuration, then it is possible that you cannot observe NOE (triangle problem?)



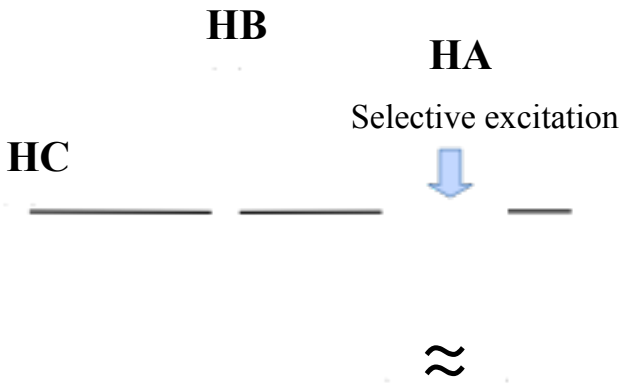
Q. So close in space and they don't give NOE..?!



Q. So close in space and they don't give NOE..?!



rAB : rBC : rAC = 1 : 1 : 1.26



1D NOESY cartoon

Sometimes despite the obvious proximity NOE, is not observed

Summary

1. On the experiment..

NOE is a relaxation phenomenon caused by dipolar interactions

***In NOE experiment it is important that you can manipulate this* dipolar interaction to your benefit**

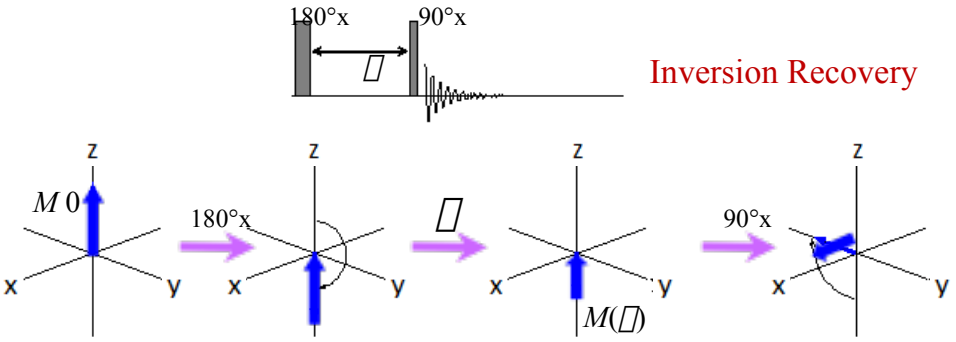
⇨ **Setup parameters according to *T*1**

In difference NOE: *set saturation time at 5 x T*1
In NOESY: set mixing time at about *T*1

2. On the results

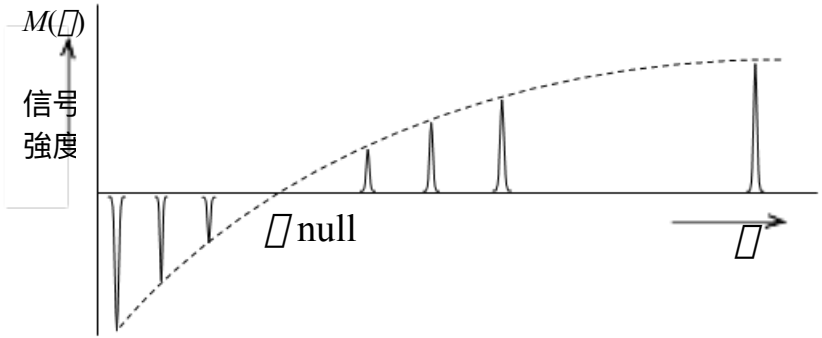
Observation of NOE indicates proximity in space but the opposite is not always true. Non observation of NOE does not necessarily mean that spins are far apart

Q. How I can estimate *T*1 ? .. a simple method



Changes in magnetization during the pulse sequence

relaxation/double_pulse.jxp



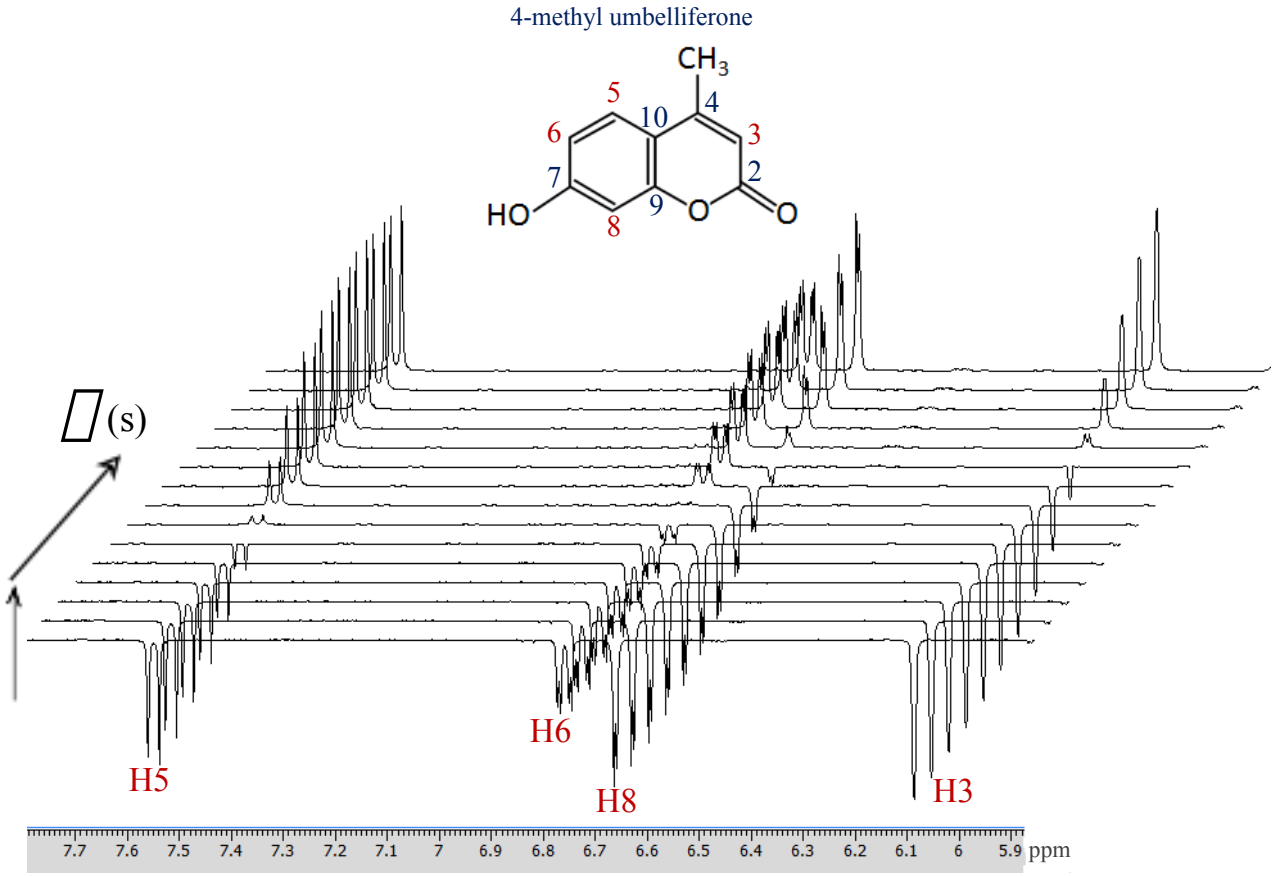
$$M(\tau) = M_0\left\{1 - 2\exp\left(-\frac{\tau}{T_1}\right)\right\}$$

$T1 \approx$ □null □ 1.44

An estimate of *T*1

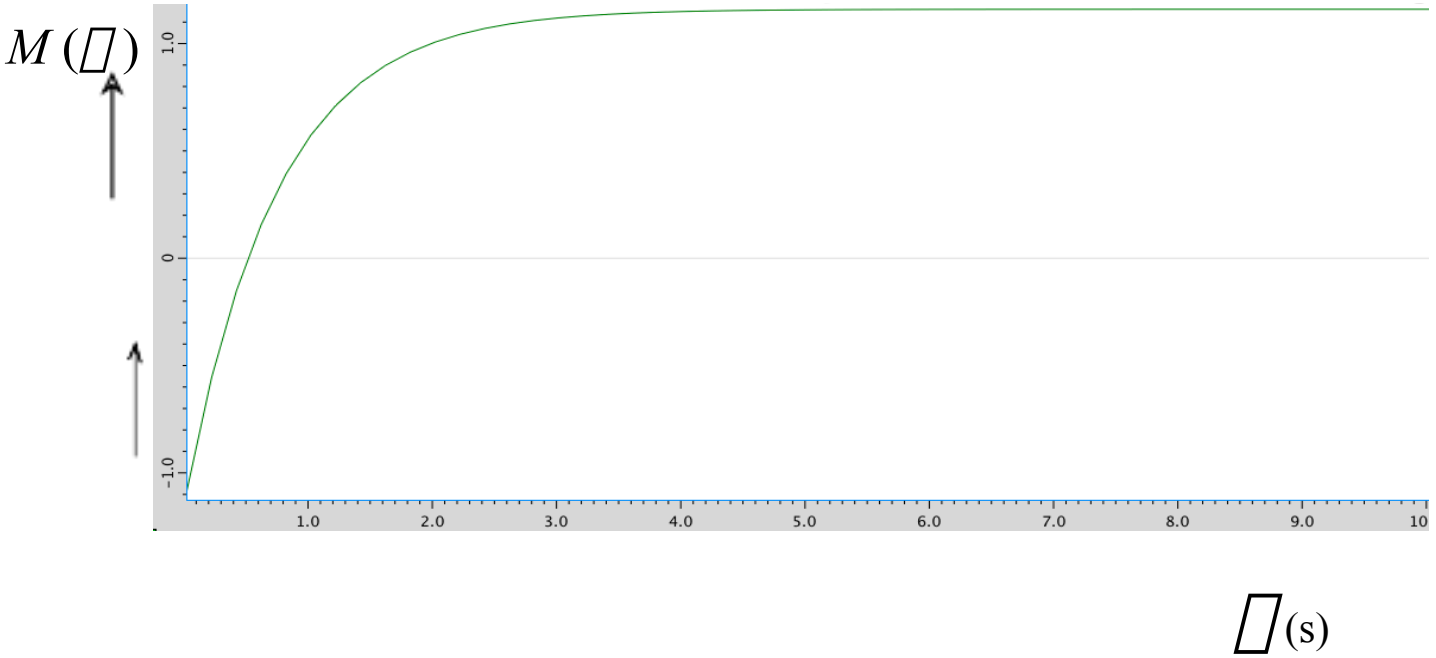
M(□) signal intensity as a function of □

Q. How I can estimate *T*1 ? .. a simple method



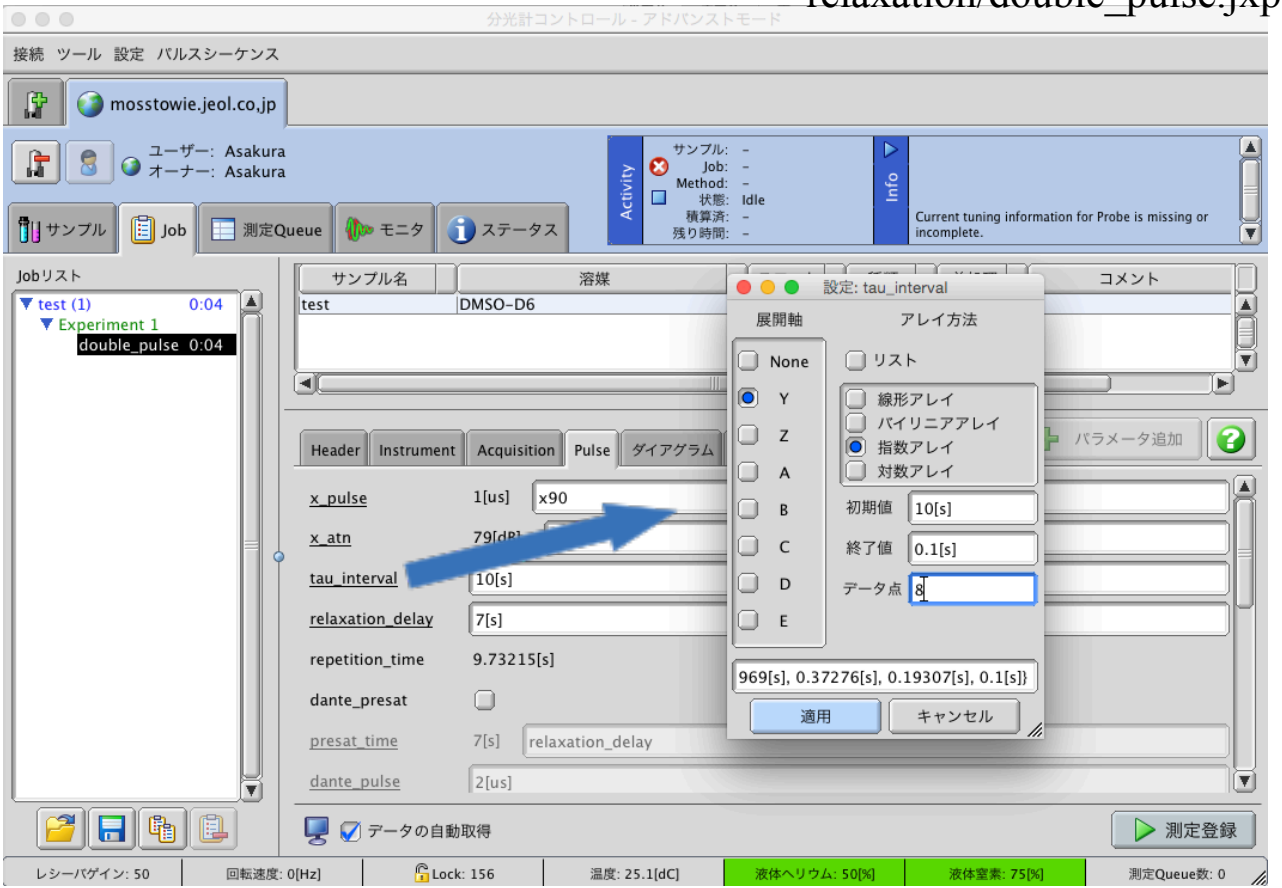
Q. How I can estimate *T*1 ? .. a simple method

$$M(\tau) = M_0\left\{1 - 2\exp\left(-\frac{\tau}{T_1}\right)\right\}$$



Q. How I can estimate *T*1 ? .. a simple method

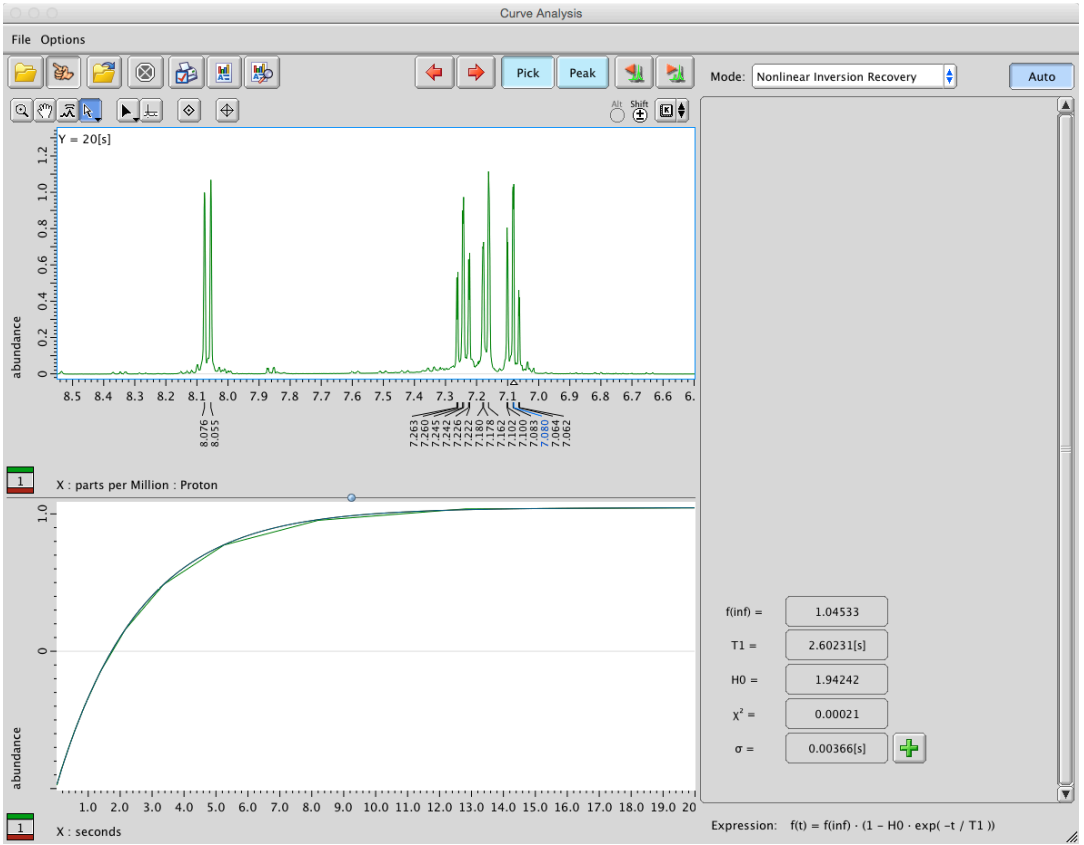
relaxation/double_pulse.jxp



(Delta V5.0)

Q. How I can estimate *T*1 ? .. a simple method

Curve Analysis Tool (Delta Ver. 5.0)




Thank You!