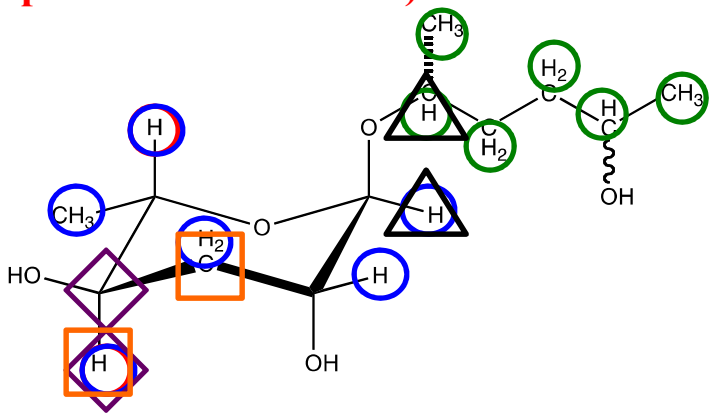
Information Content of Common 2D NMR Experiments

COSY (Correlation SpectroscopY): J coupling (generally up to 3 covalent bonds)

TOCSY (Total Correlation SpectroscopY):
J coupling along coupled networks

Blue is one TOCSY network, green is another

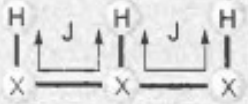


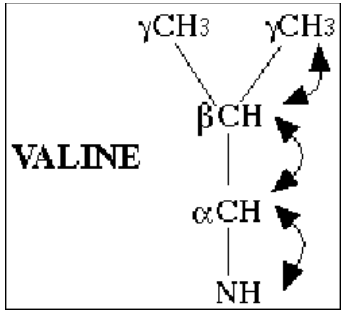
HSQC (Heteronuclear Single Quantum Correlation):
Directly bonded ^{13}C-^{1}H or ^{15}N-^{1}H

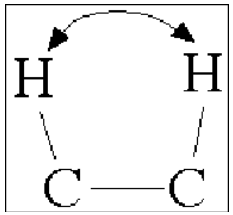
HMBC (Heteronuclear Multiple Bond Correlation):
2 or 3 bond ^{13}C---^{1}H or ^{15}N----^{1}H

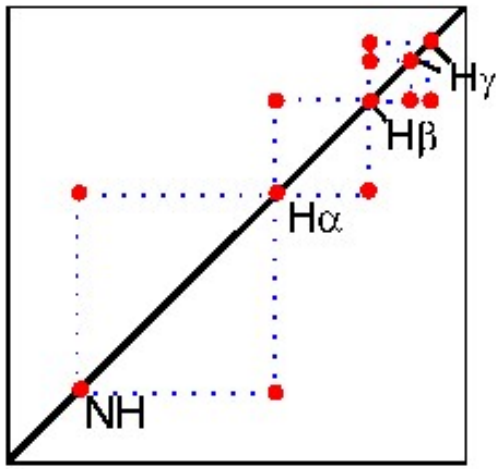
NOESY (Nuclear Overhauser Effect Spectroscopy)
Or ROESY (Rotating Frame Overhauser Effect Spectroscopy):
^{1}H to ^{1}H distances up to 5-6 Å

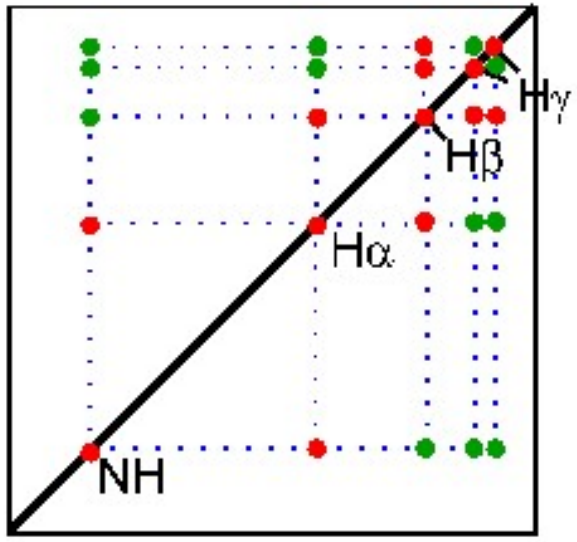
COSY & TOCSY

Correlation	Principal Technique(s)	Comments
	^1H–^1H COSY	Proton J coupling typically over 2 or 3 bonds.
	^1H–^1H TOCSY	Relayed proton J couplings within a coupled spin system. Remote protons may be correlated provided there is a continuous coupling network in between them.

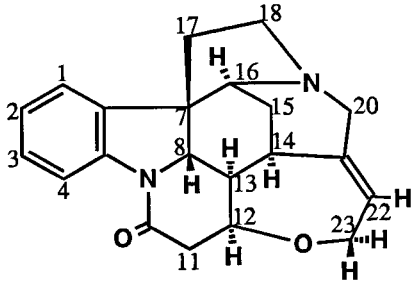


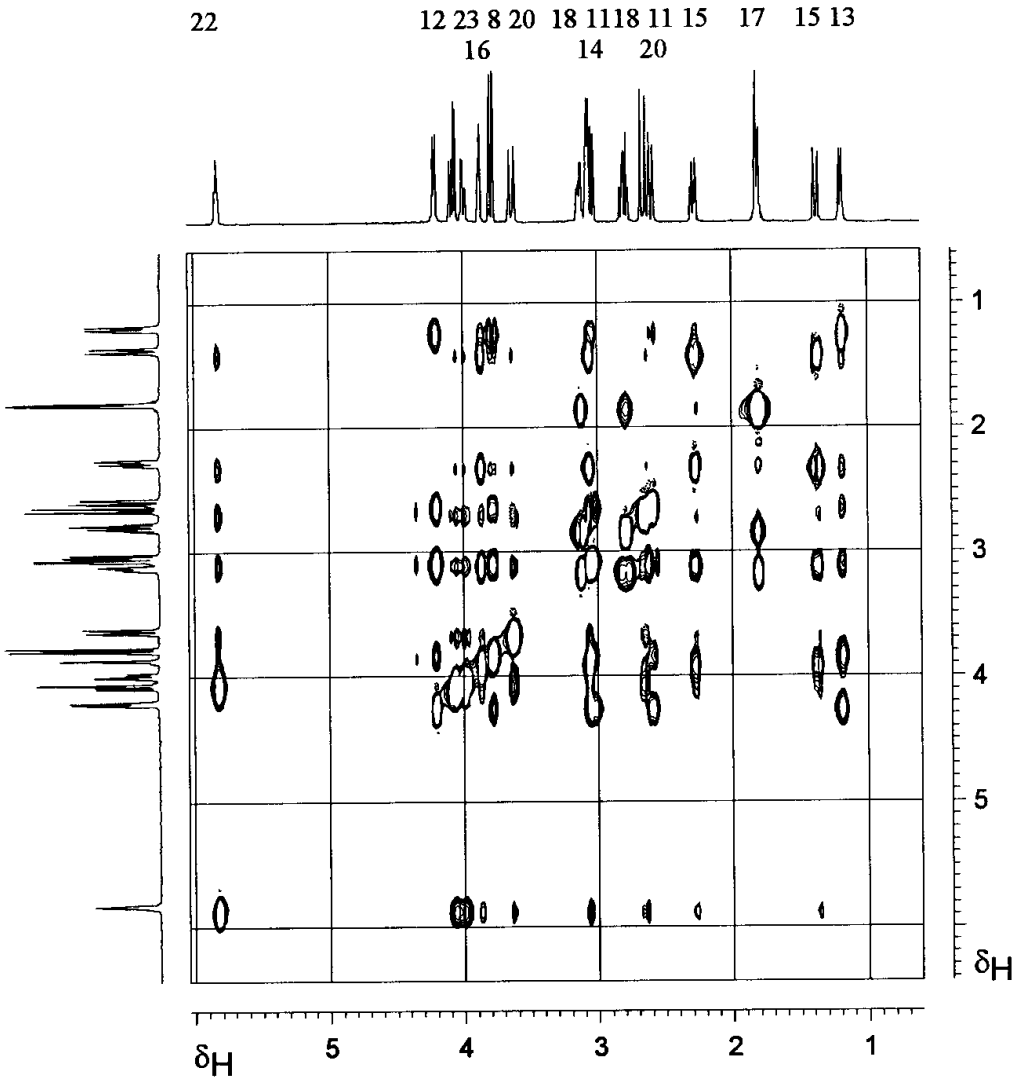




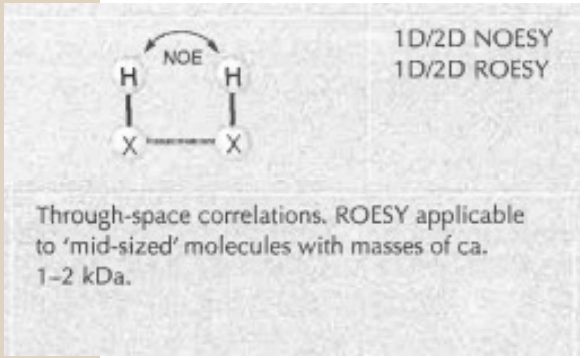


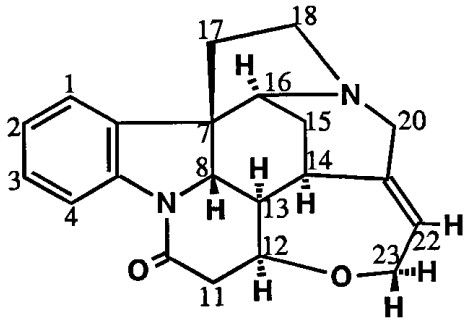
TOCSY of strychnine

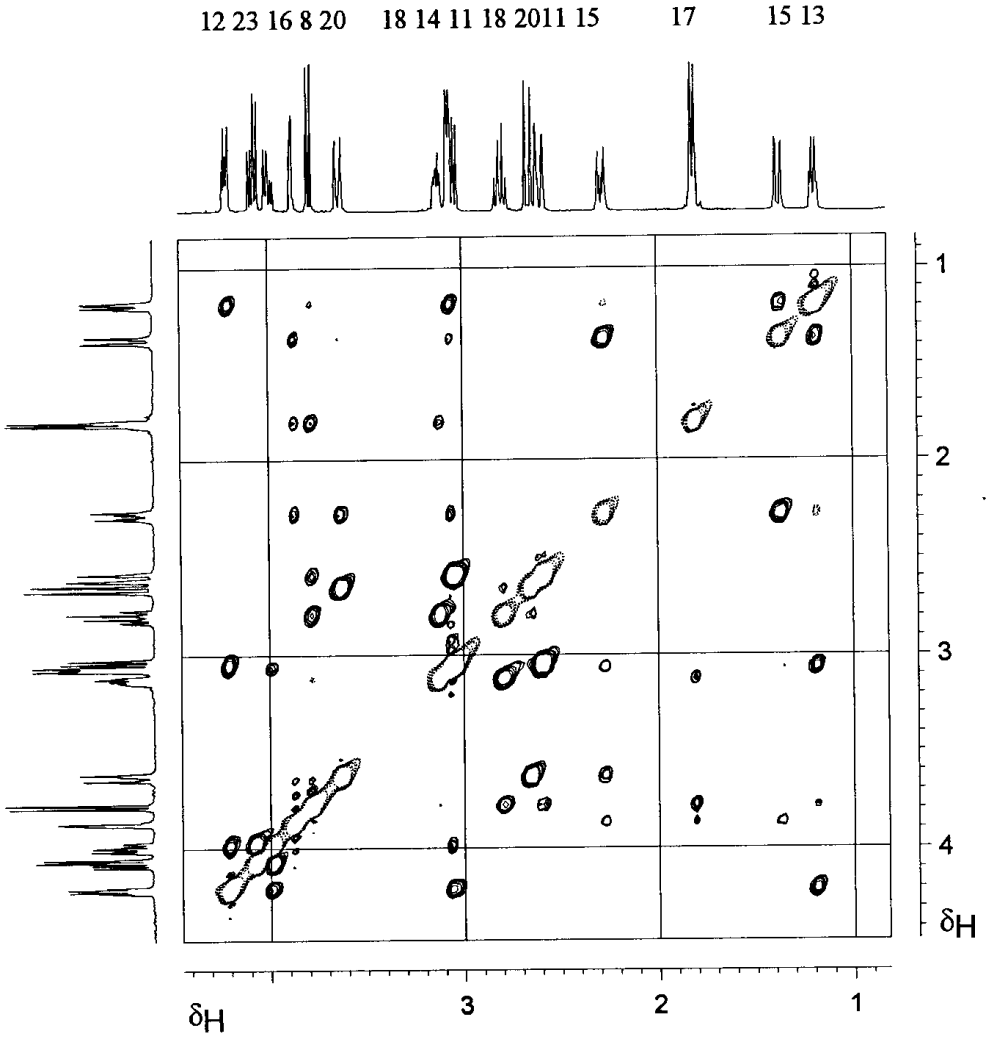




NOESY of strychnine





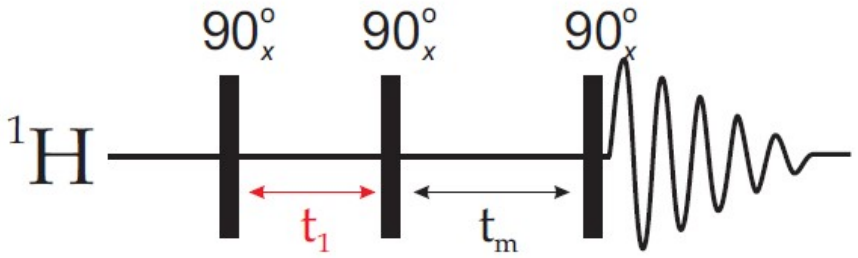


Nuclear Overhauser Enhancement/Effect SpectroscopY (NOESY)

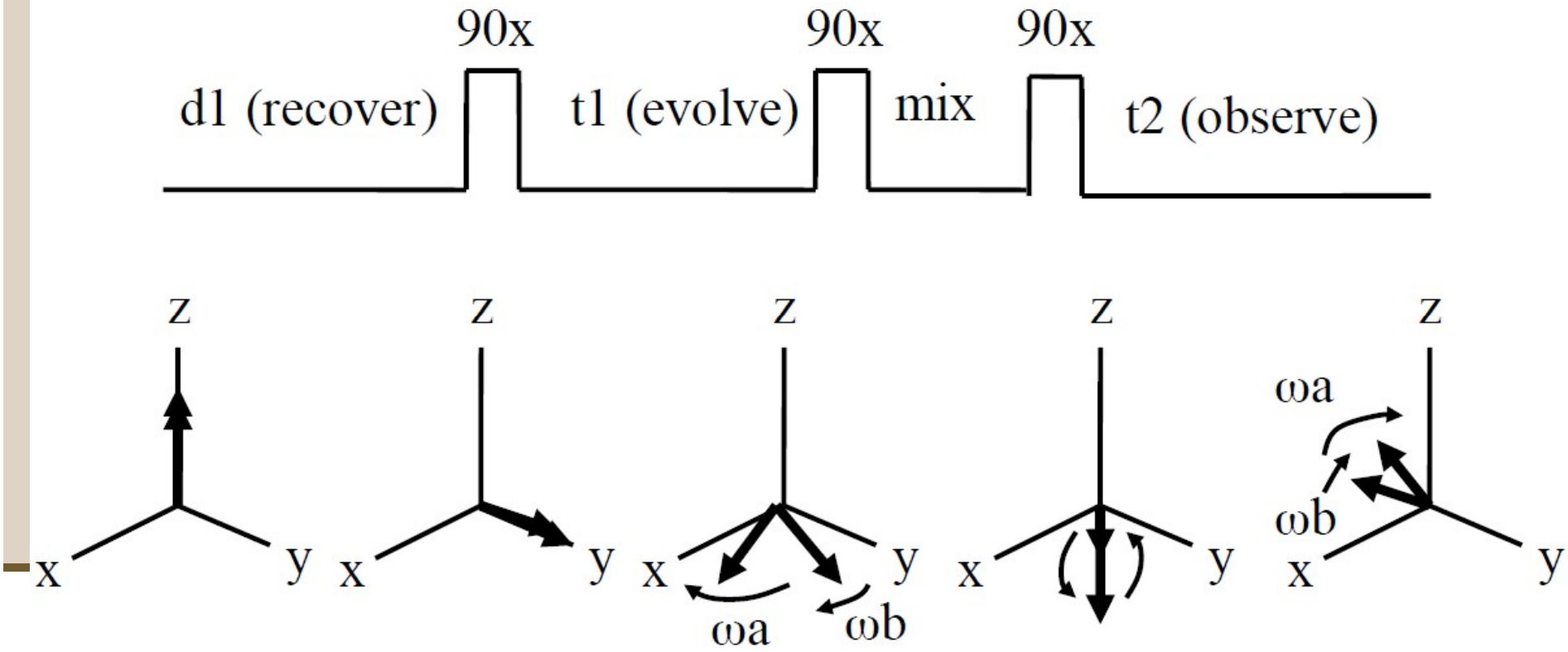
The 2D NOESY produces cross peaks between resonances that interact through space (dipolar coupled). Intensity of NOESY cross peaks can be used as a ***molecular ruler*** to measure distances between two atoms. This makes NOESY an important tool for the determination of 3D structures (bio-molecules).

Remember when we examined the COSY sequence we saw that the two pulses produced z-axis magnetization that was modulated by their chemical shifts. This z-axis magnetization can "transfer" to neighboring spins during the fixed ***mixing time*** (t_m) via the NOE mechanism (relaxation). The final pulse allows detection of the signals.

NOESY:



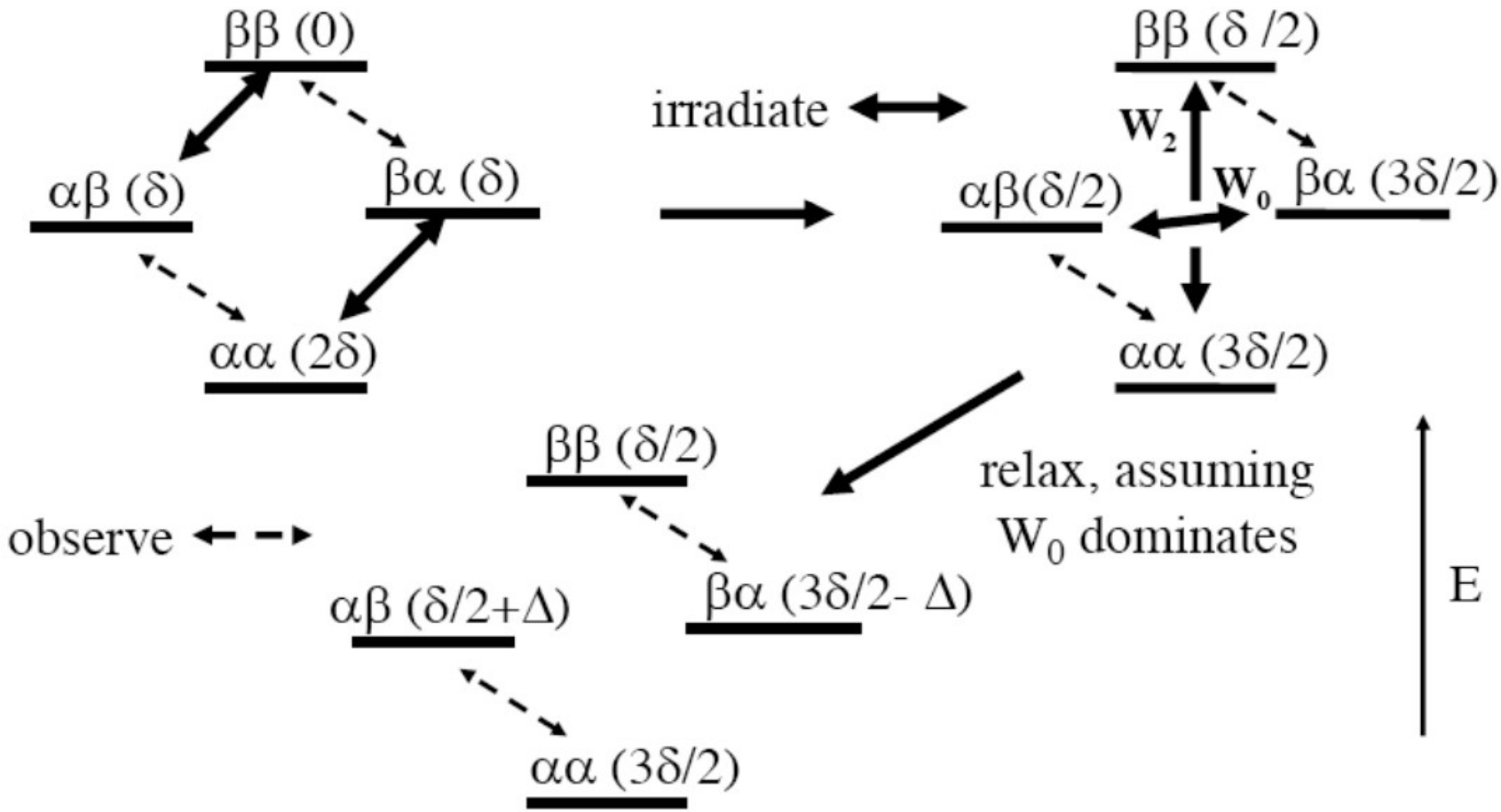
NOESY magnetization transfer



Magnetization precessing at ωa in t1 can precess at ωb in t2:
NOESY cross-peak

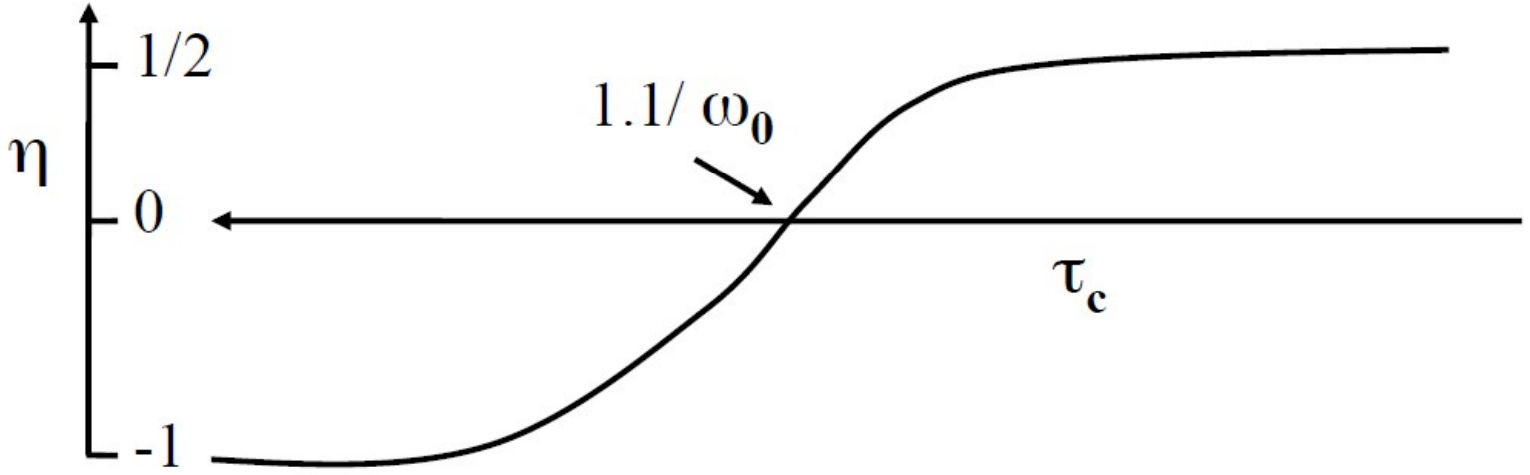
NOE signals rely on cross-polarization

-depends on competition between W_0 and W_2 processes:



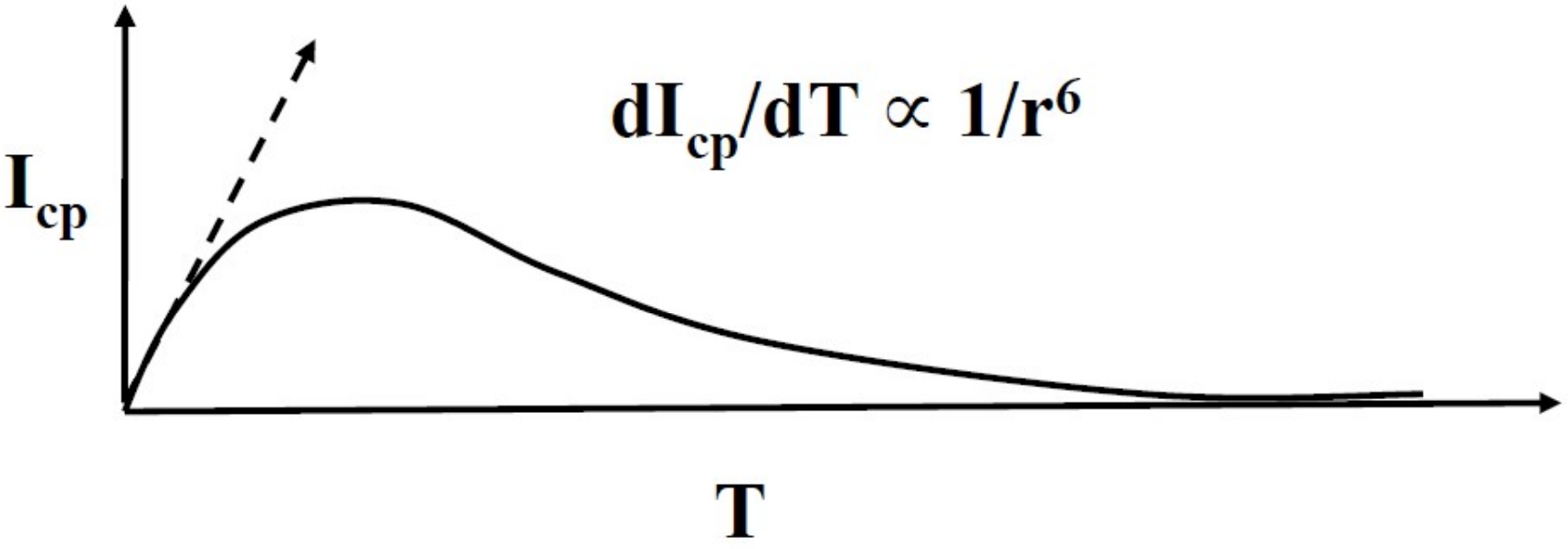
NOE signals are positive for small molecules and negative for large molecules

$$\eta = (-1 + 6/(1+4\omega_0^2\tau_c^2))/(1 + 3/(1+\omega_0^2\tau_c^2) + 6/(1+4\omega_0^2\tau_c^2))$$

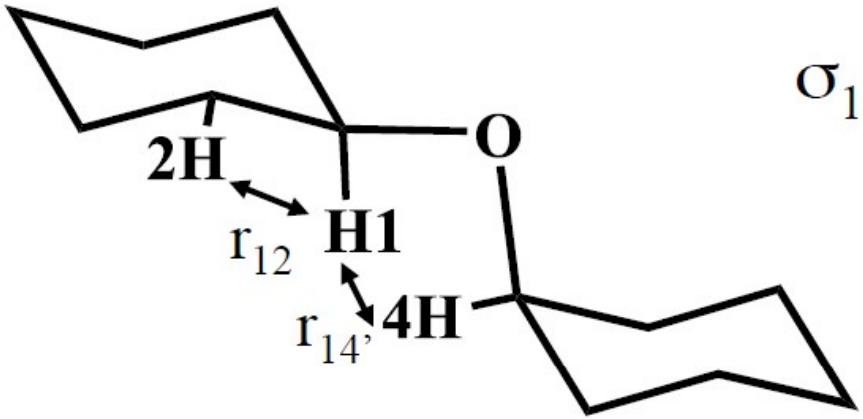


Note that enhancement can be 0. Occurs for ~2000 Da systems at 800 MHz. Rotating frame experiments (ROESY) avoid this.

In Practice Data May be Collected from Cross Peaks at a Series of Mixing Times



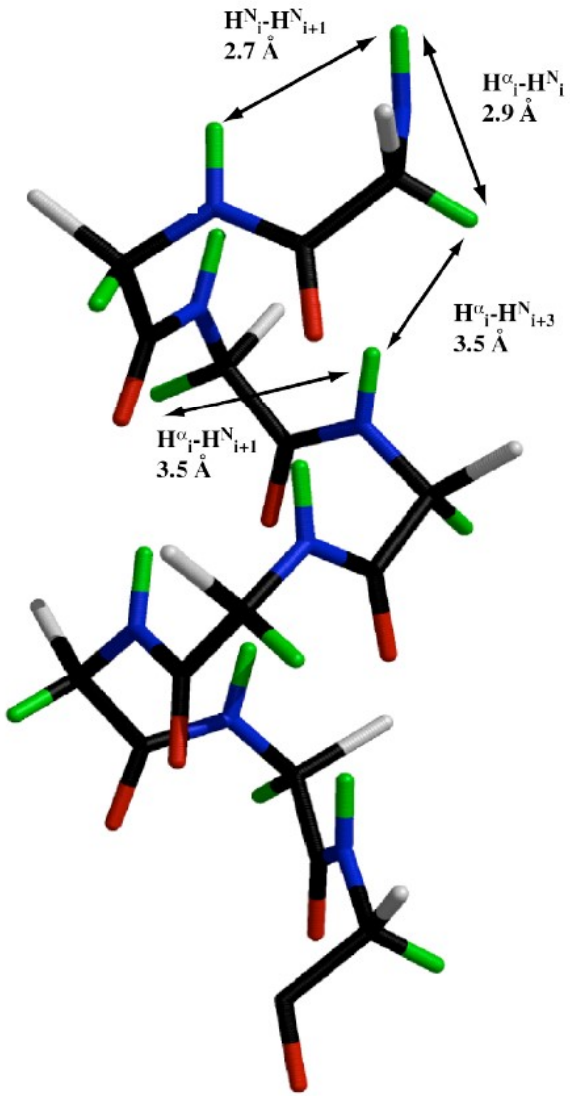
NOE signals give valuable Structural Information



$$\sigma_{14'} / \sigma_{12} = r_{12}^{6} / r_{14'}^{6}$$

$r_{12} = 2.5$ Å, $\sigma_{14'} / \sigma_{12} = 0.25$, implies: $r_{14'} = 3.15$ Å

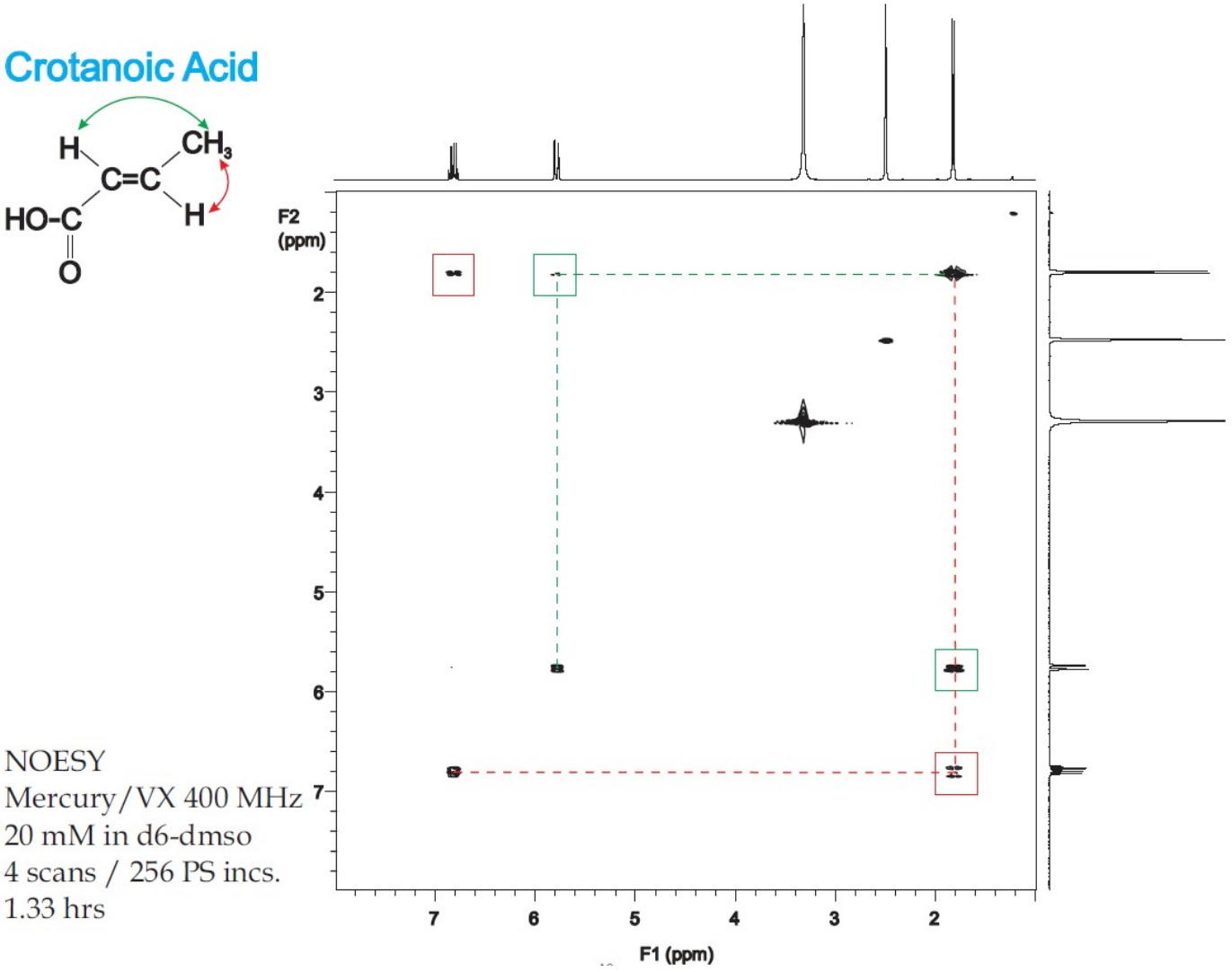
NOE signal pattern in an idelialized α-helix



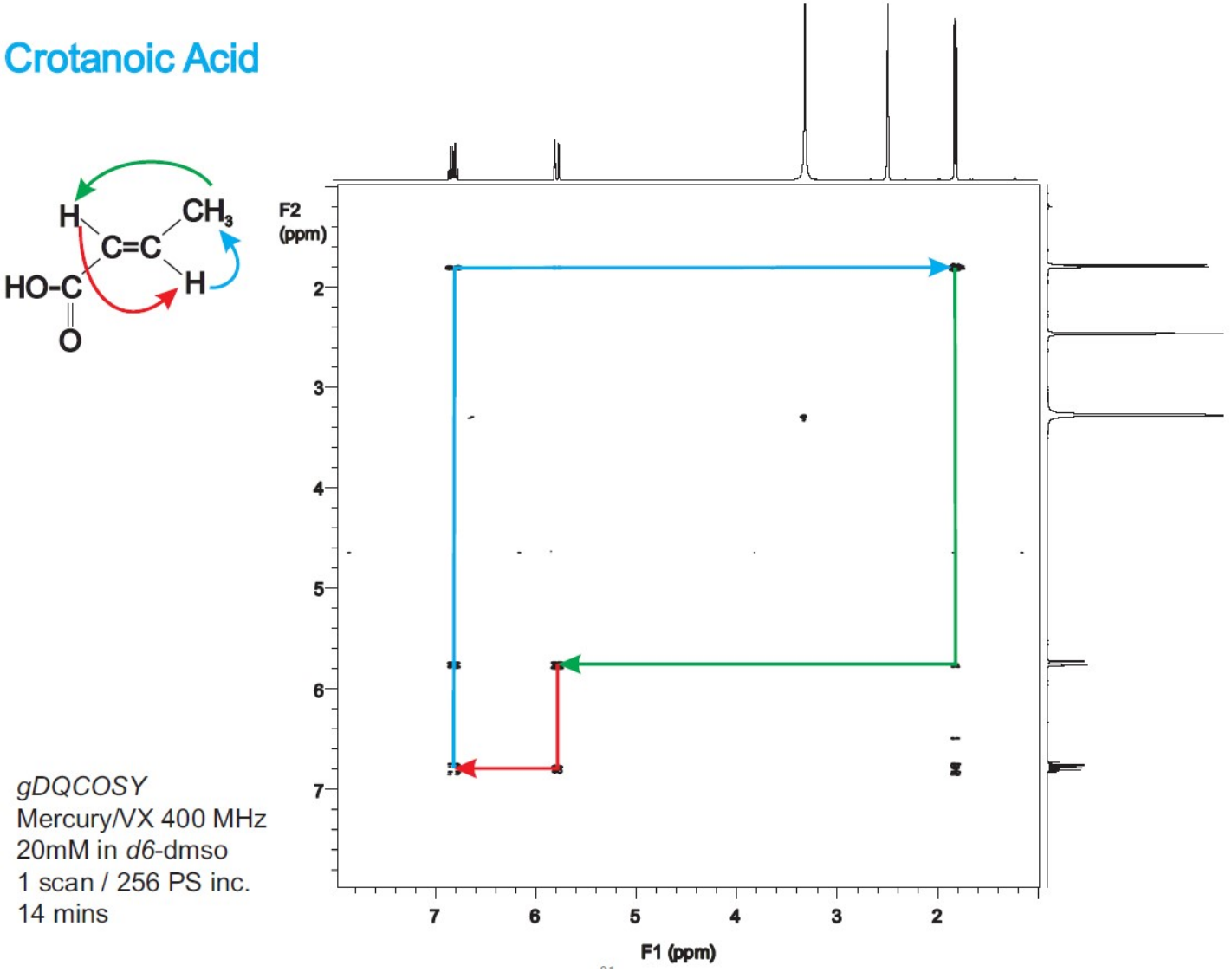
Potential NOE Interactions

In an Idealized α-Helix

NOESY of crotanoic acid

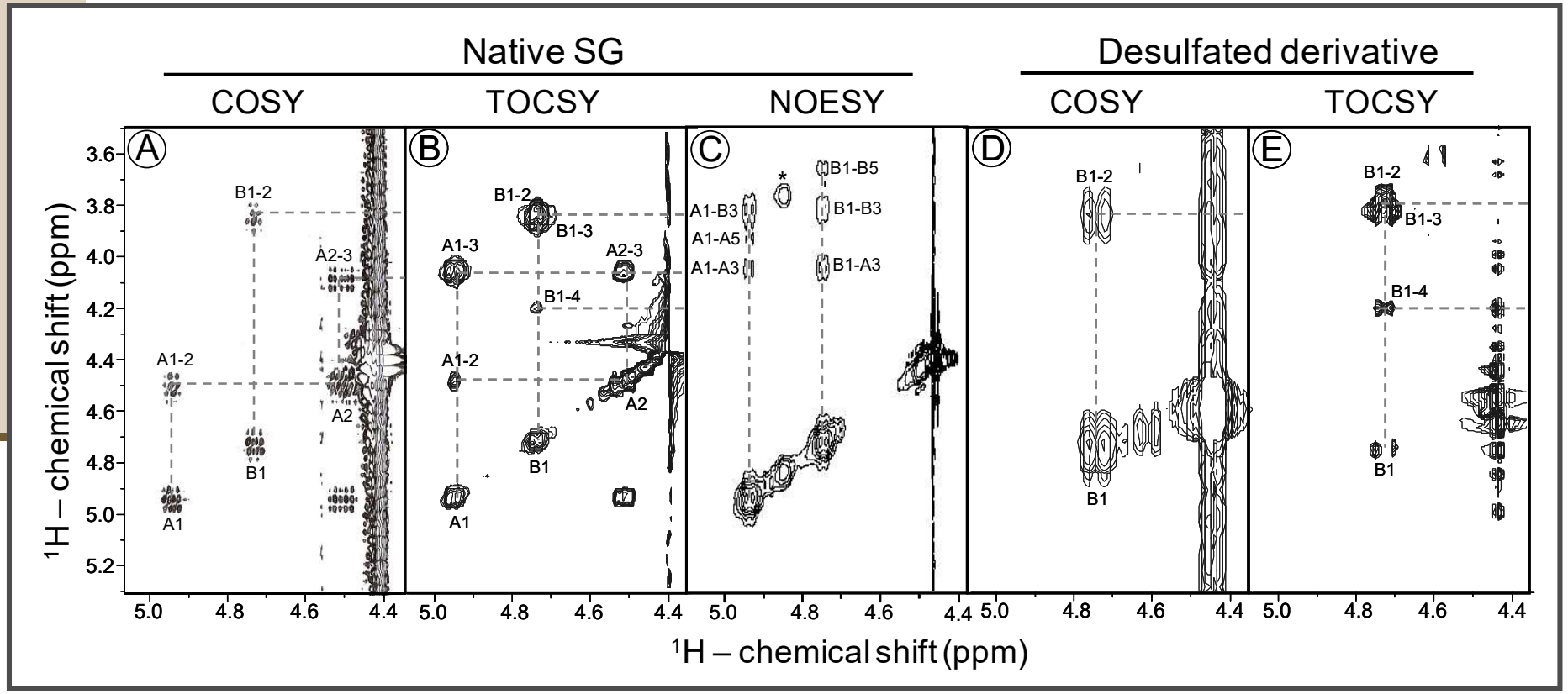


COSY of crotanoic acid



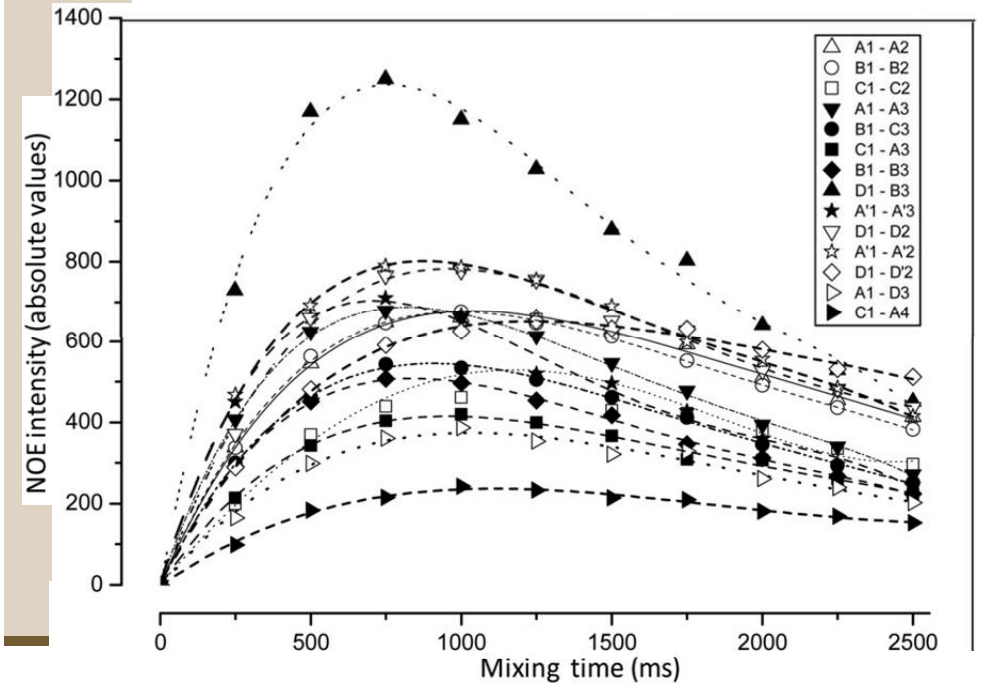
The combination of COSY, TOCSY and NOESY in structural determination

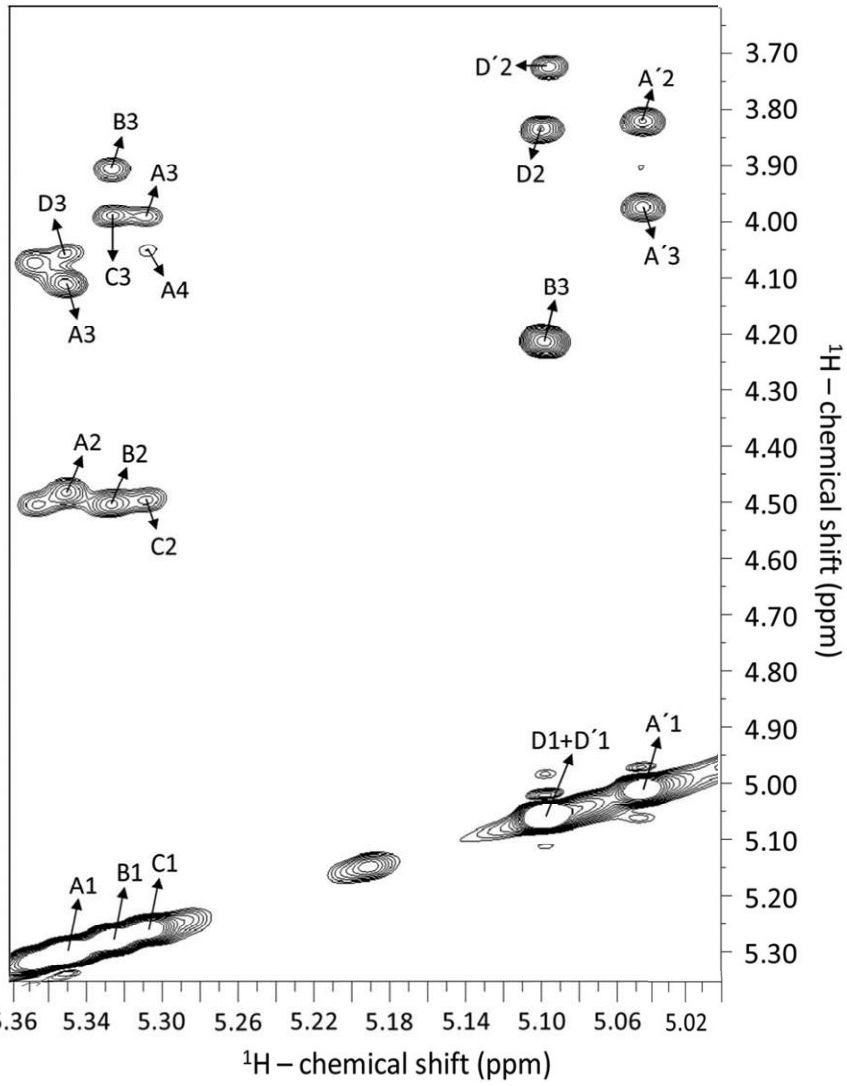
2D homonuclear ^{1}H-^{1}H NMR spectra at 400 MHz (anomeric region) of the (A-C) native β-SG from *G. crenularis* and (D and F) its desulfated derivative.



NOE intensity as function of mixing time

Conformation determined by through-space NOE contacts





NOE signals in 3D structure

Table II. Theoretical and calculated NOEs (intra- and inter-residues), chemical shifts, interproton distances and intensity ranges measured for the *Lv* I (octasaccharide, Structure 1), and the mixture of oligosaccharides from Structure 2

Type	Unit[a] and ^{1}H–^{1}H NOE[b]	F1 (ppm)	F2 (ppm)	Internuclear distance (Å)		Intensity[d]
				Experimental[c]	MD	
Lv I (octasaccharide, Structure 1)						
Intra	D'_{nr}H1–H2	3.84	5.10	2.4 ± 0.00	2.4 ± 0.1	Strong
	A_{nr} H1–H2	4.50	5.35	2.4 ± 0.03	2.4 ± 0.1	Strong
	B_r H1–H2	4.52	5.33	2.4 ± 0.03	2.4 ± 0.1	Strong
	B_{nr} H1–H2				2.4 ± 0.1	
	C_r H1–H2	4.52	5.31	2.6 ± 0.06	2.4 ± 0.1	Medium
	C_{nr} H1–H2				2.4 ± 0.1	
	A_{nr} H1–H3	4.12	5.35	3.4 ± 0.1	3.8 ± 0.1	Medium
	B_r H1–H3	3.90	5.33	3.5 ± 0.09	3.8 ± 0.1	Medium
	B_{nr} H1–H3				3.8 ± 0.1	
	A'_r H1–H3	3.98	5.05	3.4 ± 0.08	3.8 ± 0.1	Medium
	A'_r H1–H2	3.82	5.05	2.3 ± 0.03	2.4 ± 0.1	Strong
	D_r H1–H2	3.84	5.10	2.5 ± 0.05	2.4 ± 0.1	Strong
Inter	A_{nr} H1–D_r H3	4.07	5.35	2.6 ± 0.06	2.6 ± 0.4	Medium
	C_{nr} H1–A_{nr} H3	3.99	5.31	2.6 ± 0.04	2.6 ± 0.2	Medium
	C_{nr} H1–A_{nr} H4	4.06	5.31	2. 9 ± 0.05	2.3 ± 0.3	Medium
	B_r H1–C_r H3	3.99	5.33	2.5 ± 0.04	2.5 ± 0.3	Strong
	B_{nr} H1–C_r H3				2.6 ± 0.3	
	D_r H1–B_r H3	4.23	5.10	2.1 ± 0.02	2.5 ± 0.3	Strong

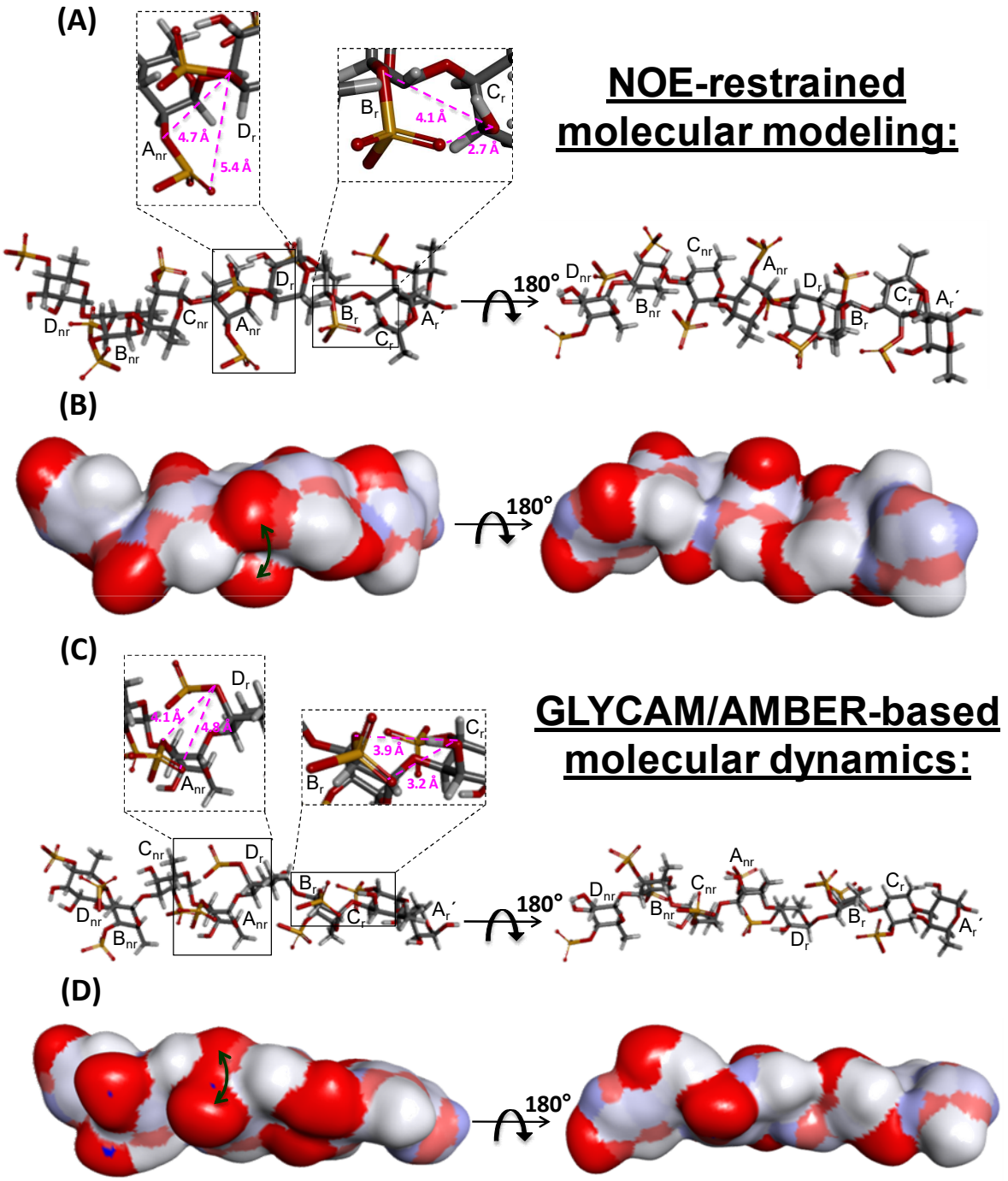
[a]Units labeled with subscript "nr" belong to the tetrasaccharide repeating units placed at the non-reducing end side, while those units labeled with subscript "r" belong to the tetrasaccharide repeating unit of the reducing end side.

[b]Connectivities are from NOESY spectra of Figure 5, supported by assignments from DQF-COSY spectrum (Supplementary data, Figure S3).

[c]The internuclear distances were obtained using the formula $NOE = NOE_{ref}(r_{ref}/r)^{1/6}$, in which r denotes the distances between the proton pairs, NOE is the intensity of the peaks and ref is the referential values used for normalization. Since the studied units are Fuc*p* at the exclusive $^1C_4$ ring configuration, the H1–H2 intra-residual NOE-based interproton distance must be 2.4 Å. The D'_{nr} H1–H2 distance of 2.4 Å was used for normalization. Ten NOESY spectra were collected under the same conditions for generating average and error values.

[d]NOE intensities were set as strong (≤2.5 Å) and medium (>2.5 and ≤3.7 Å).

NOE signals in 3D structure



Transferred NOE for identifying bound-state 3D structures of ligands in intermolecular complexes

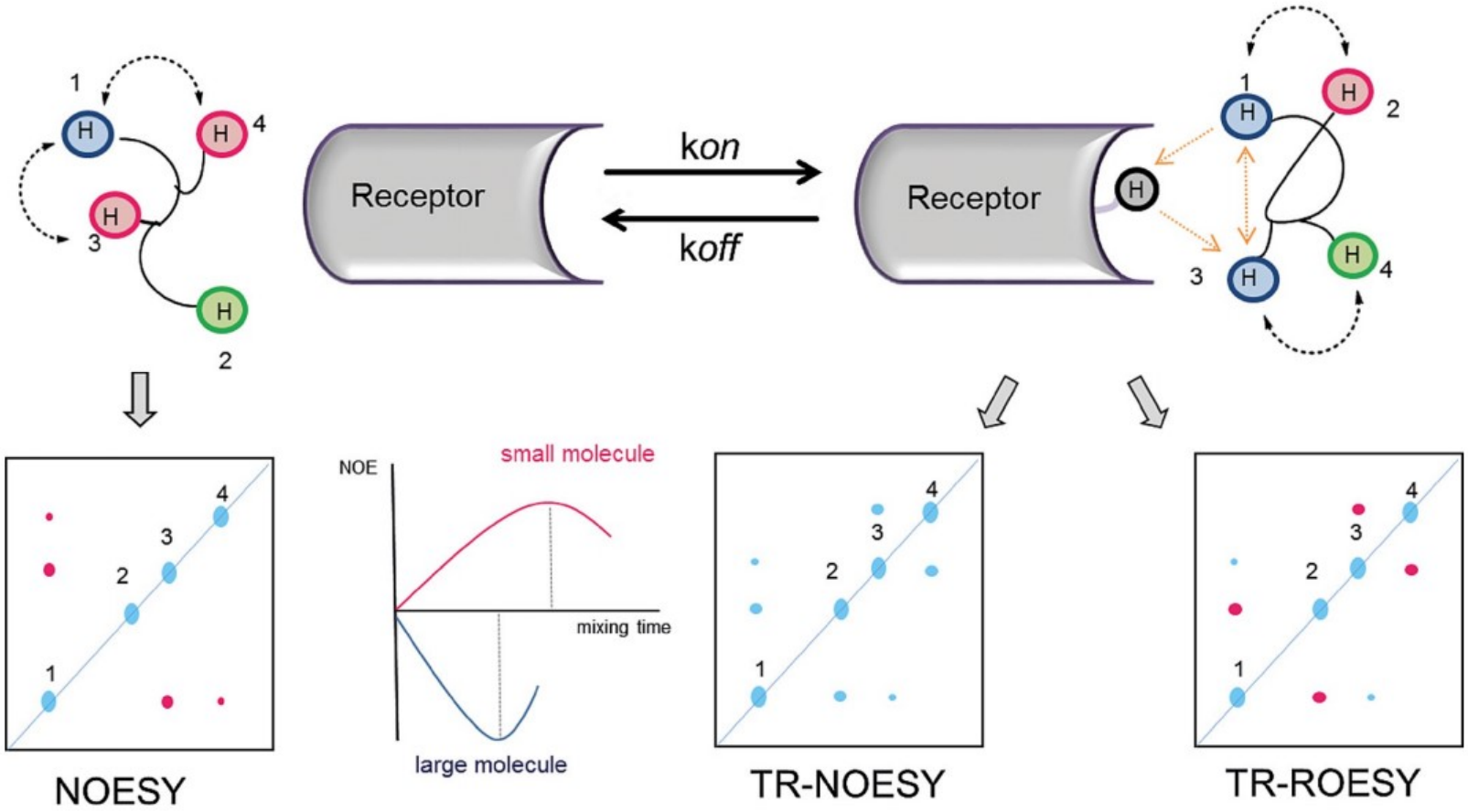


Figure 3. Schematic representation of a NOESY spectrum of a small ligand in the free state, which reaches the maximum of NOE intensity at longer mixing times; cross peaks and diagonal peaks have different signs (left). Schematic representation of tr-NOESY and tr-ROESY spectra recorded on the ligand in the bound state, characterized by faster build up rate (right). In the tr-NOESY spectrum, cross peaks and diagonal peaks show the same signs as expected for a large molecule, thus indicating binding to the protein. The relative sizes of the peaks and the appearance/disappearance of NOE contacts may be used to detect conformational variations. The tr-ROESY spin-diffusion cross peaks (H1/H3) and diagonal peaks display the same signs, whereas direct cross peaks (H1/H2; H3/H4) have a different sign to the diagonal peaks.[20]