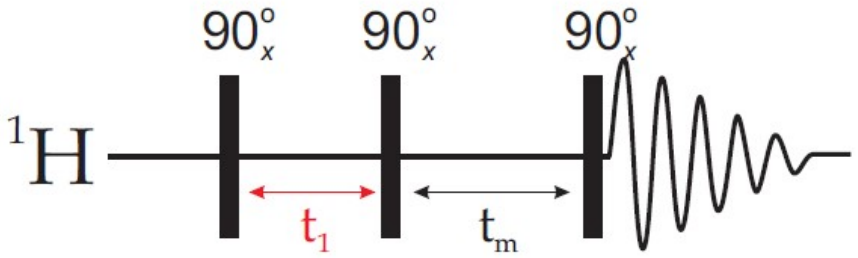
Nuclear Overhauser Enhancement/Effect SpectroscopY (NOESY)

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).

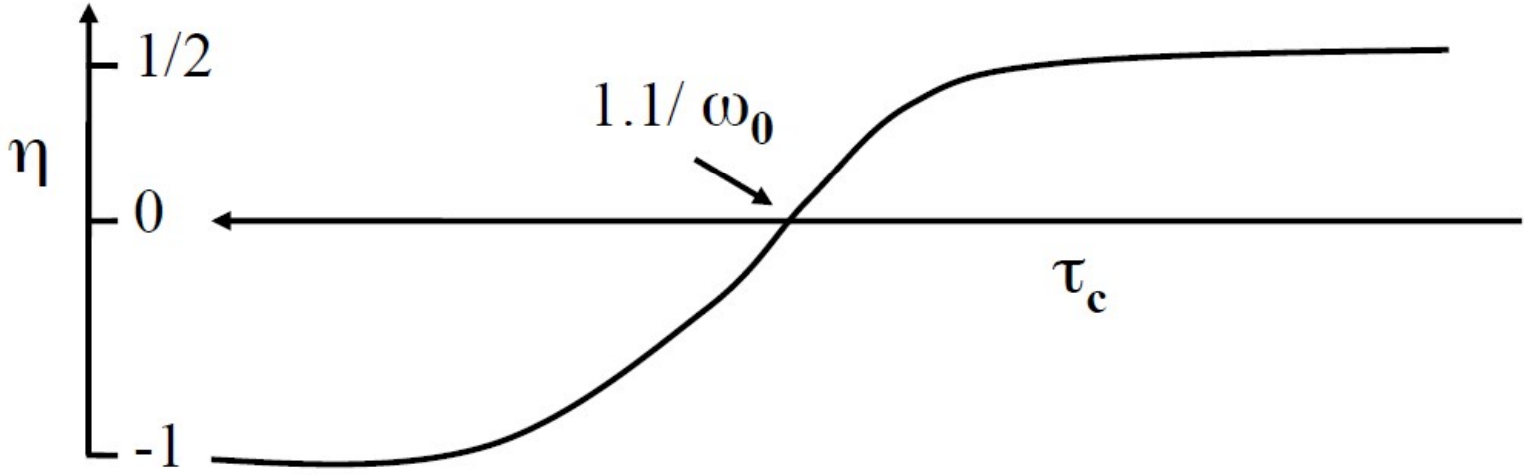
Remember when we examined the COSY sequence we saw that the two pulses produced z-axis magnetization that was modulated by their chemical shifts. This z-axis magnetization can "transfer" to neighboring spins during the fixed ***mixing time*** (t_m) via the NOE mechanism (relaxation). The final pulse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

NOESY:



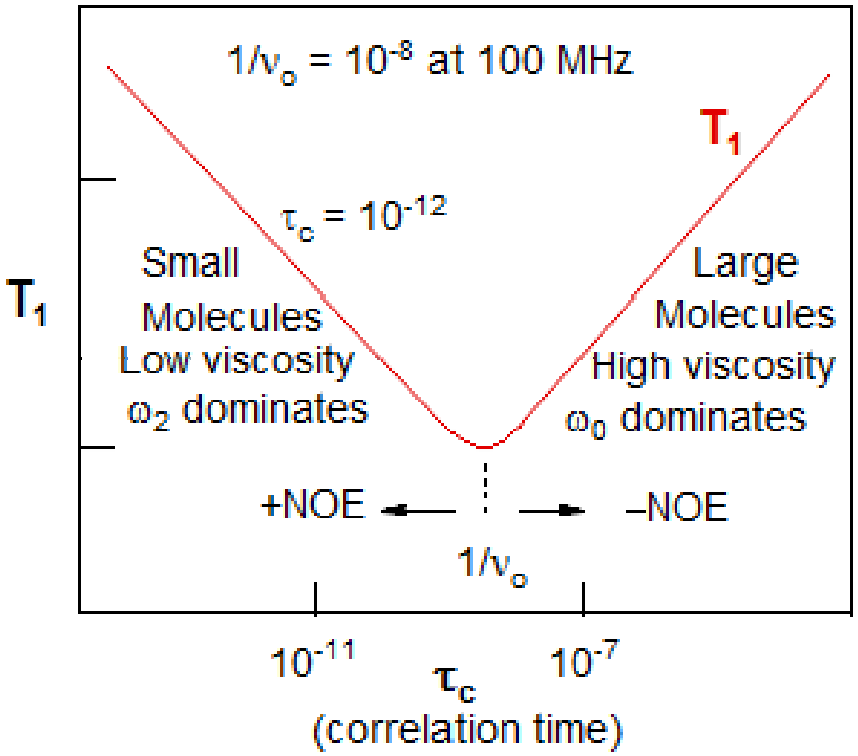
NOE signals are positive for small molecules and negative for large molecules

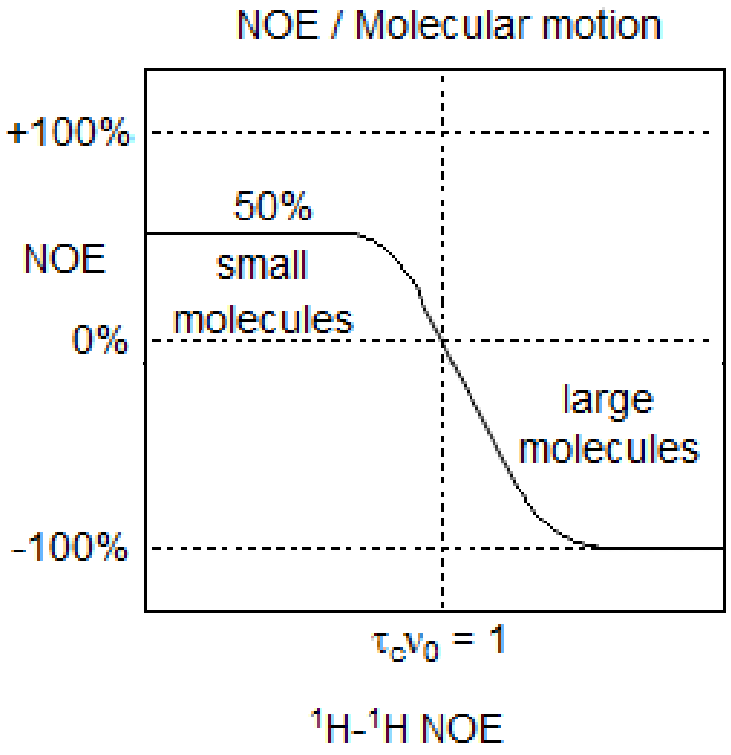
$$\eta = (-1 + 6/(1+4\omega_0^2\tau_c^2))/(1 + 3/(1+\omega_0^2\tau_c^2) + 6/(1+4\omega_0^2\tau_c^2))$$



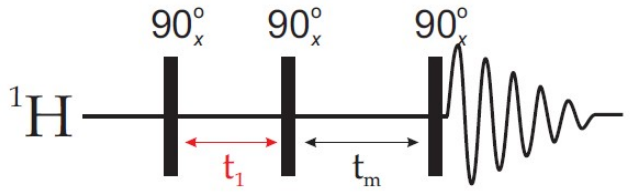
Note that enhancement can be 0. Occurs for ~2000 Da systems at 800 MHz. Rotating frame experiments (ROESY) avoid this.

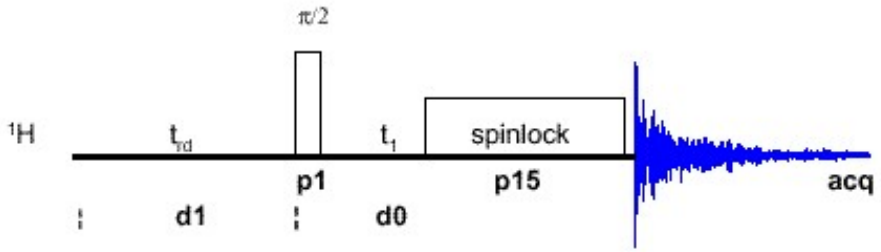
NOE parameters



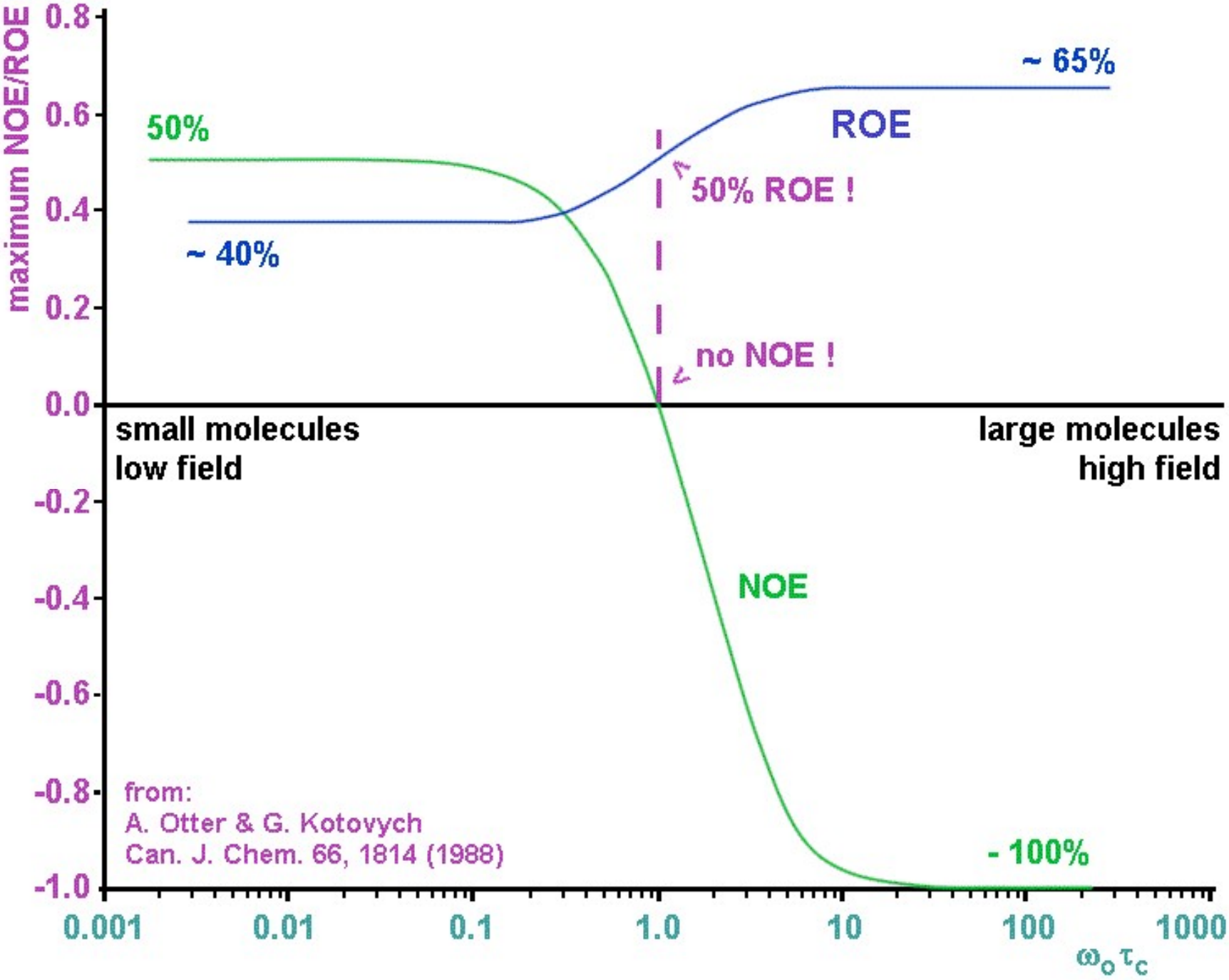


Rotating frame Overhause Effect SpectroscopY (ROESY)

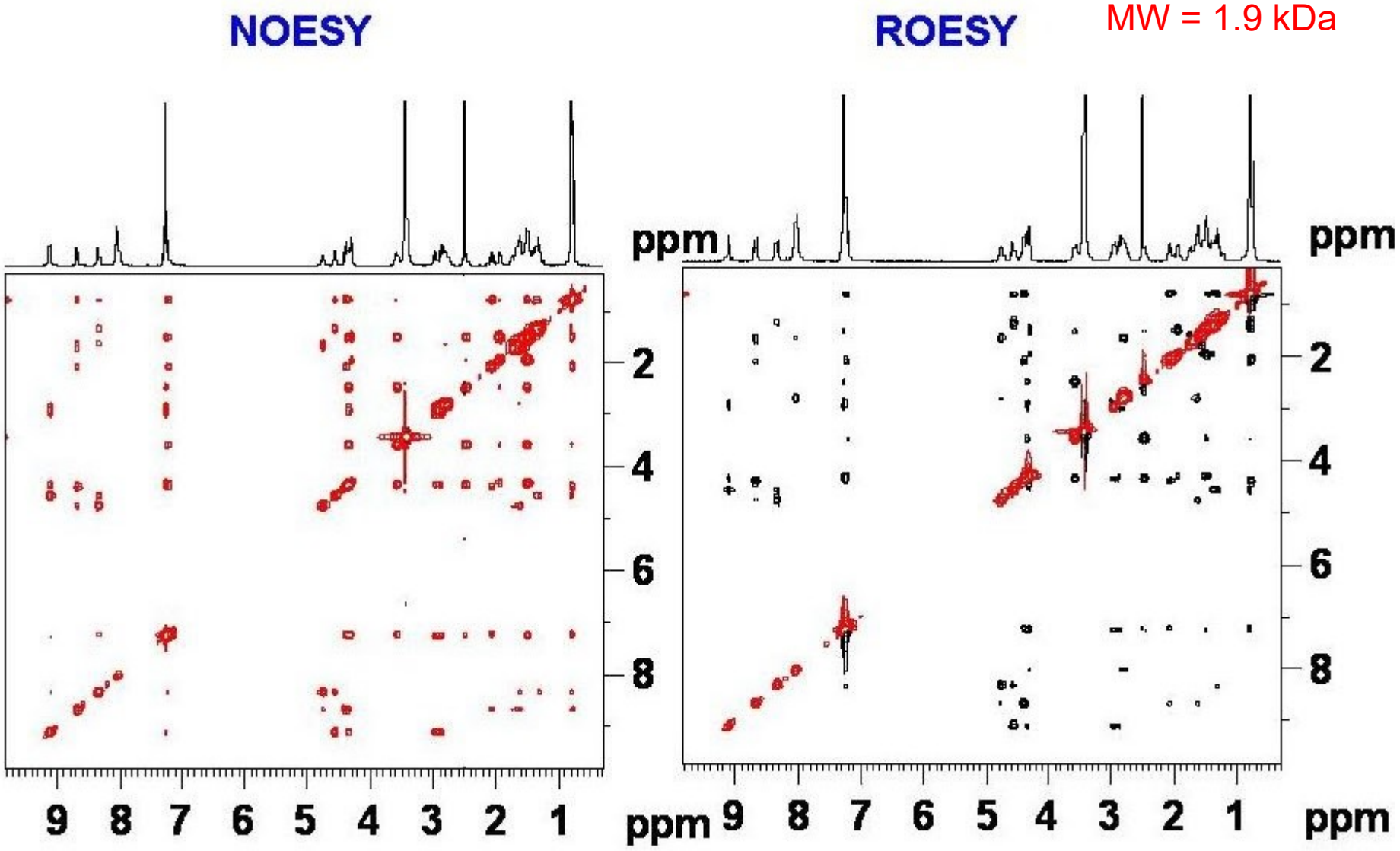




Parameter	NOESY	ROESY
Magnetization transfer	Longitudinal axis	Transverse axis
NOE Intensity	+0.38 to −1.00	+0.38 to +0.68
Cross peak buildup rate	Slow	Fast (double)
Usefulness: small molecules ($\omega\tau_c \ll 1$) Intermediate size ($\omega\tau_c \sim 1$) macro molecules ($\omega\tau_c \gg 1$	 Useful. Buildup rate is slow Not useful, zero NOE Preferred, Good NOEs	 Useful: buildup rate is fast Preferred, ROE is always +ve, Can be used with caution
Chemical exchage	Difficult to identify – diagonal and cross peaks – same sign	Easy identification – Diagonal and cross peaks – opposite sign
Spin diffusion	High probability	Very low probability – hence useful
Artifacts	Minimal (big advantage)	Problem (Probable TOCSY and offset effects)


maximum NOE/ROE
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
~ 65%
ROE
50%
50% ROE !
~ 40%
no NOE !
small molecules
low field
large molecules
high field
NOE
from:
A. Otter & G. Kotovych
Can. J. Chem. 66, 1814 (1988)
- 100%
0.001
0.01
0.1
1.0
10
100
1000
$\omega_o \tau_c$

NOESY vs ROESY for Gramicidin at 300 MHz



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

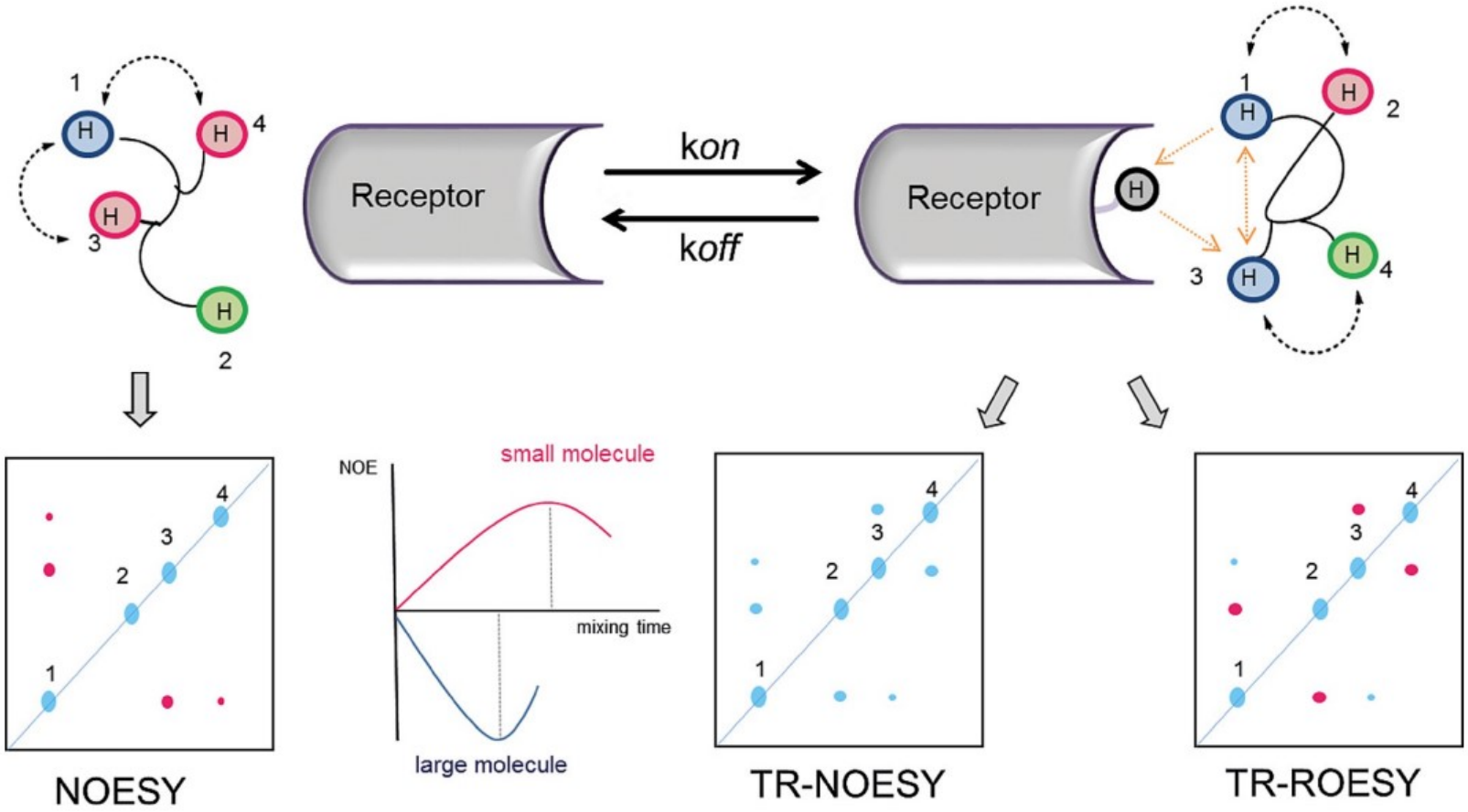


Figure 3. Schematic representation of a NOESY spectrum of a small ligand in the free state, which reaches the maximum of NOE intensity at longer mixing times; cross peaks and diagonal peaks have different signs (left). Schematic representation of tr-NOESY and tr-ROESY spectra recorded on the ligand in 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]

Heteronuclear Overhauser Enhancement/Effect SpectroscopY (HOESY)

• The **2D Heteronuclear NOESY (HOESY) experiment** allows to detect heteronuclear through-space NOE connectivies between nonbonded nuclei. This is the heteronuclear version of the NOESY experiment.

• The basic pulse sequence of the HOESY experiment is closely related to the conventional NOESY pulse sequence. After a 90º ^{1}H pulse, transverse magnetization evolves during the variable evolution period under heteronuclear decoupling. A second 90º ^{1}H pulse creates longitudinal magnetization and, during the mixing time, polarization transfer via dipolar coupling takes place. A final 90º pulse on the heteronucleus creates transverse magnetization which is detected under broadband proton decoupling.

Heteronuclear Overhauser Enhancement/Effect SpectroscopY (HOESY)