

C8953
NMR strukturní analýza
seminář
NOESY

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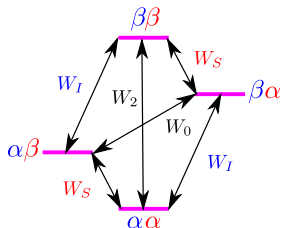
NOESY - úvod

Nukleární Overhauserův efekt

- ▶ dipól-dipólové interakce
- ▶ transfer magnetizace přes prostor způsobený cross-relaxací

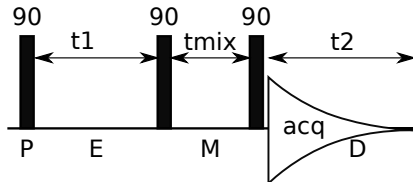
NOESY

- ▶ koreluje jádra vzdálená méně než cca 5 Å



$$\frac{d\Delta I_z}{dt} = -\rho_I(I_z - I_z^0) - \sigma_{IS}(S_z - S_z^0)$$

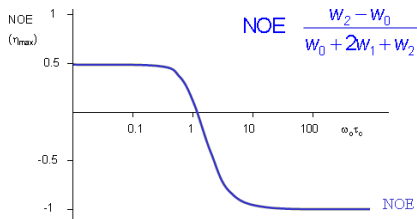
$$\sigma_{IS} = W_2 - W_0$$



NOE vs. velikost molekul

Korelační čas τ_c

- ▶ $\omega_0 \tau_c < 1$ (malé molekuly)
 - ▶ pozitivní NOE
 - ▶ crosspeaky s opačnou fází vůči diagonálním
- ▶ $\omega_0 \tau_c > 1$ (velké molekuly)
 - ▶ negativní NOE
 - ▶ crosspeaky se shodnou fází
- ▶ $\omega_0 \tau_c \approx 1$ (cca 1 kDa)
 - ▶ $\text{NOE} \approx 0$
 - ▶ žádné crosspeaky



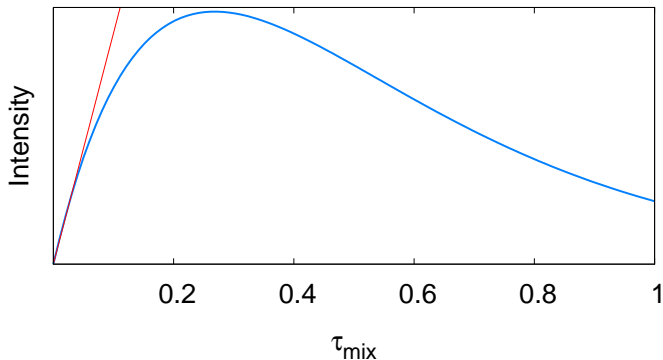
Využití NOESY

Směšovací čas τ_{mix}

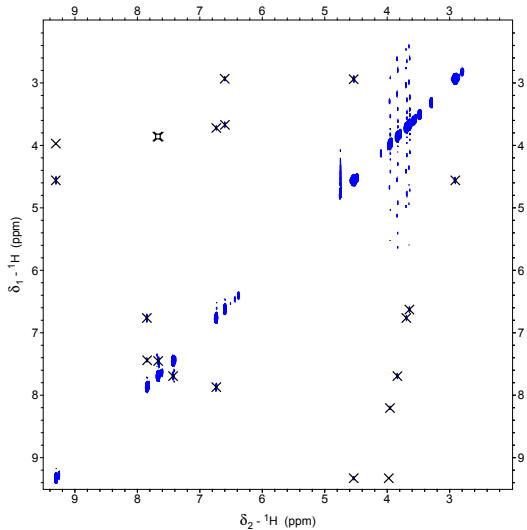
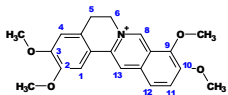
- ▶ malé molekuly $\tau_{\text{mix}} \approx 500 - 800 \text{ ms}$
- ▶ velké molekuly, biomolekuly $\tau_{\text{mix}} \approx 50 - 300 \text{ ms}$

odhad mezijaderných vzdáleností ($< 5 \text{ \AA}$)

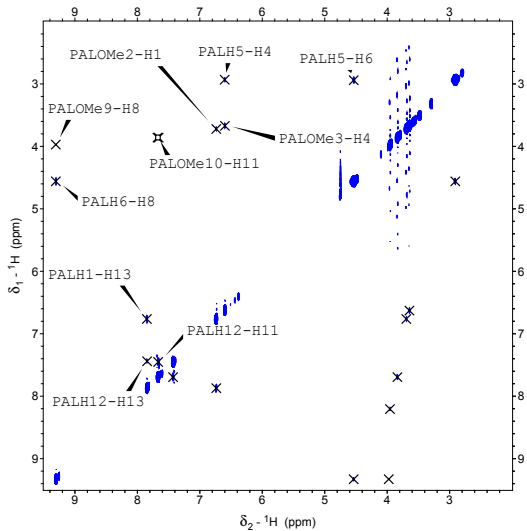
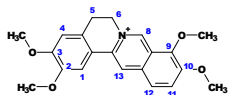
- ▶ při krátkém τ_{mix}
- ▶ $r_{ij} \approx A \times I_{ij}$



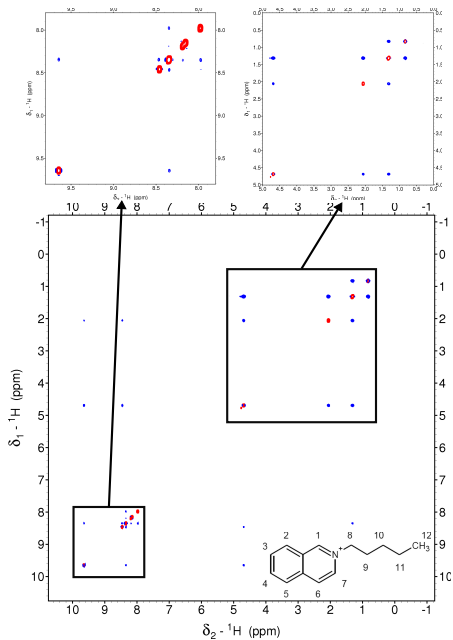
NOESY 1



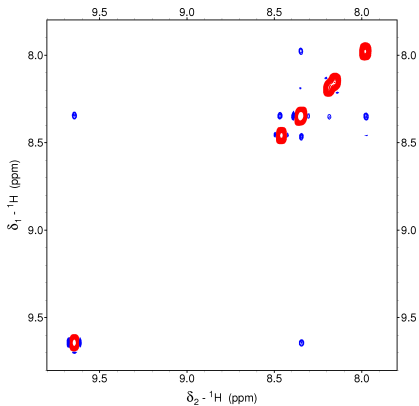
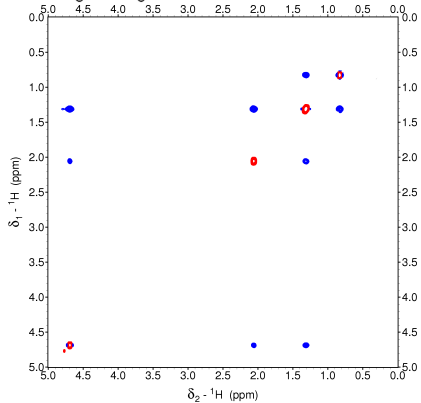
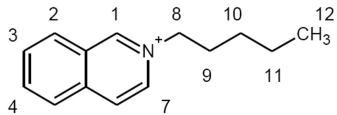
NOESY 1



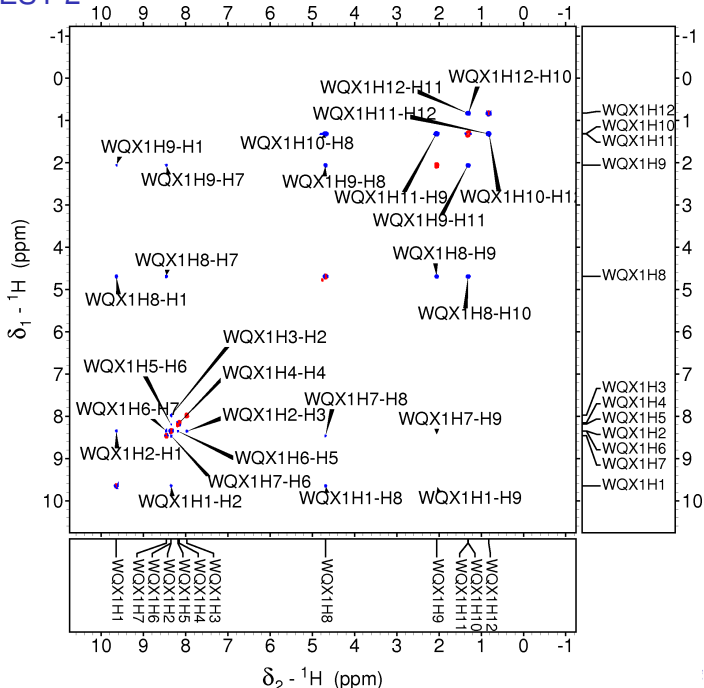
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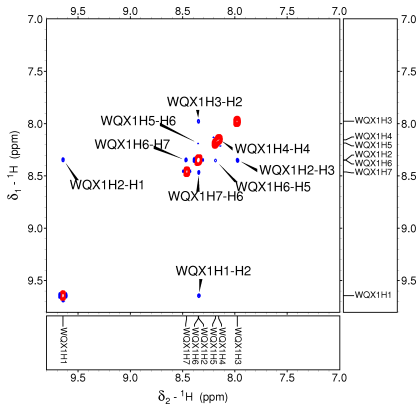
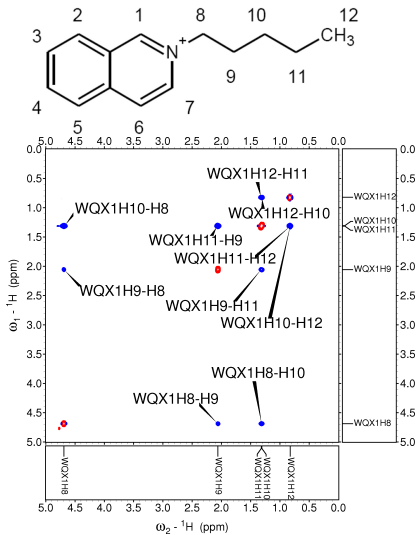
NOESY 2



NOESY 2



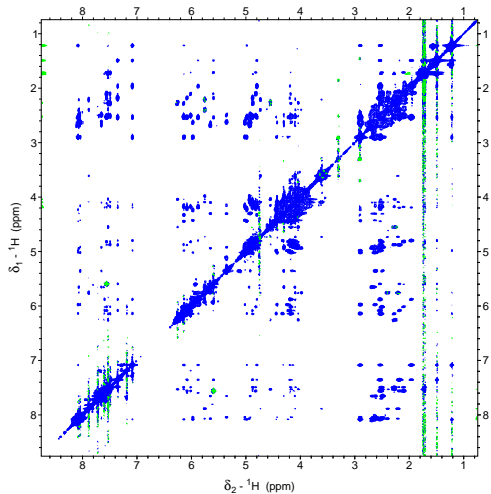
NOESY 2



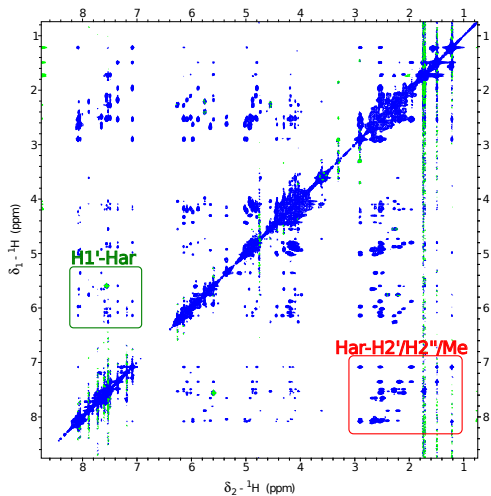
NOESY biomolekul

- ▶ konstituce a sekvence známa
- ▶ přibližné určení r_{HH}
- ▶ pro větší molekuly náročné přiřazení
 - ⇒ izotopové značení, 3D NOESY-HSQC...

Příklad: $d(\text{AAGAATTCTT})_2$, 100 ms NOESY v D_2O



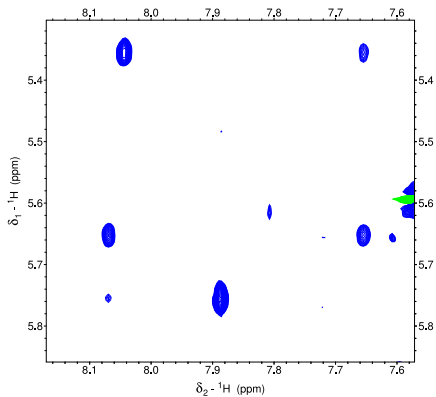
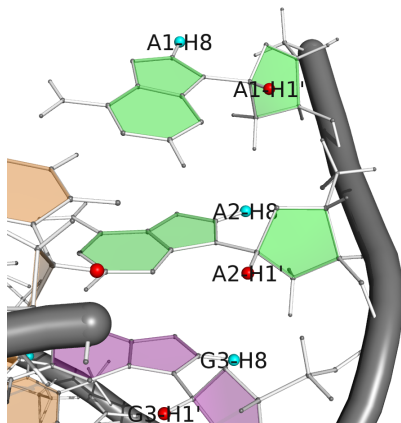
Příklad: $d(\text{AAGAATTCTT})_2$, 100 ms NOESY v D_2O



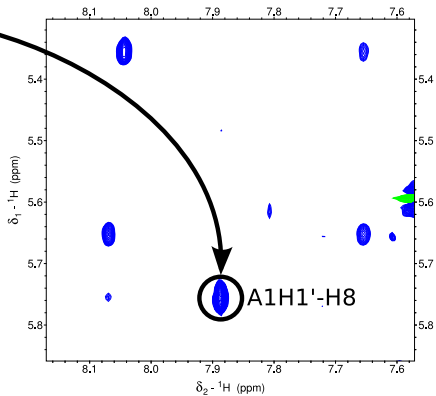
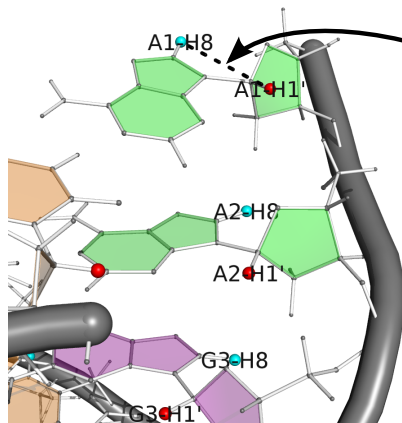
Sekvenční přiřazení rezonancí H1', H2'/2'', H(ar)

- ▶ intrareziduální NOE
- ▶ sekvenční NOE
- ▶ kromě H1'-H6/H8 vidíme i H1'-AdeH2, H1'-CytH5 atd.
- ▶ v B-DNA H2'-Har > H2''-H(ar),
 $\delta_{H2''} > \delta_{H2'}$

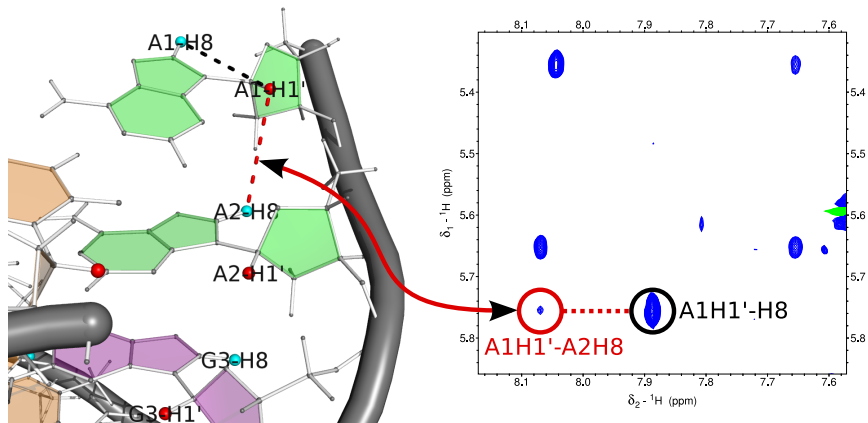
Postup při sekvenčním přiřazení v DNA



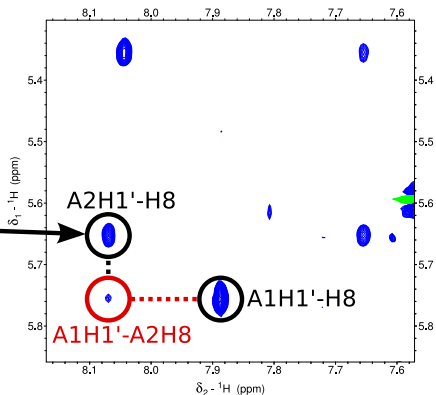
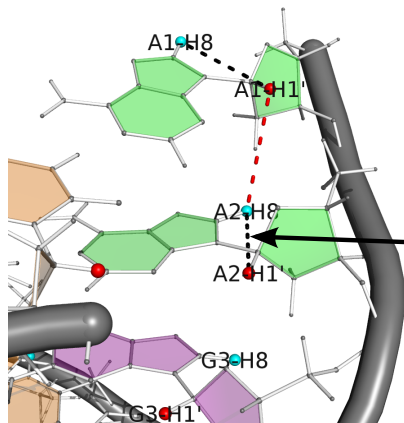
Postup při sekvenčním přiřazení v DNA



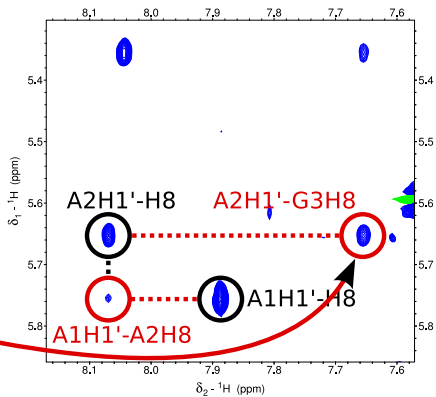
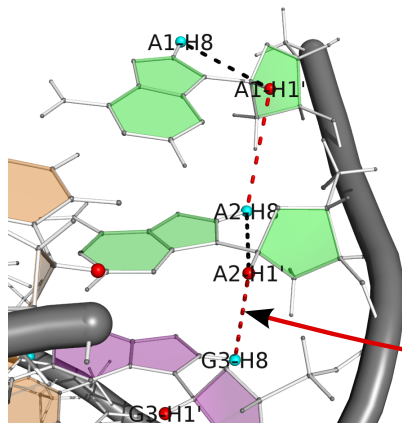
Postup při sekvenčním přiřazení v DNA



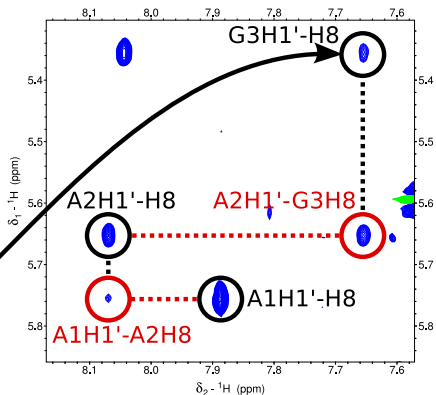
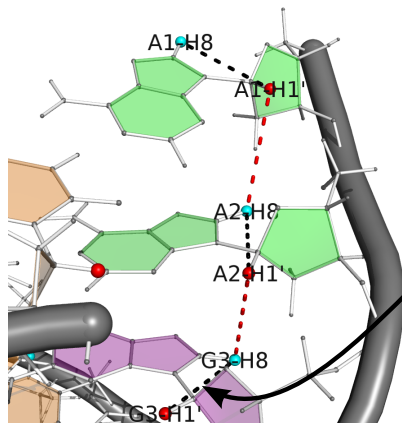
Postup při sekvenčním přiřazení v DNA



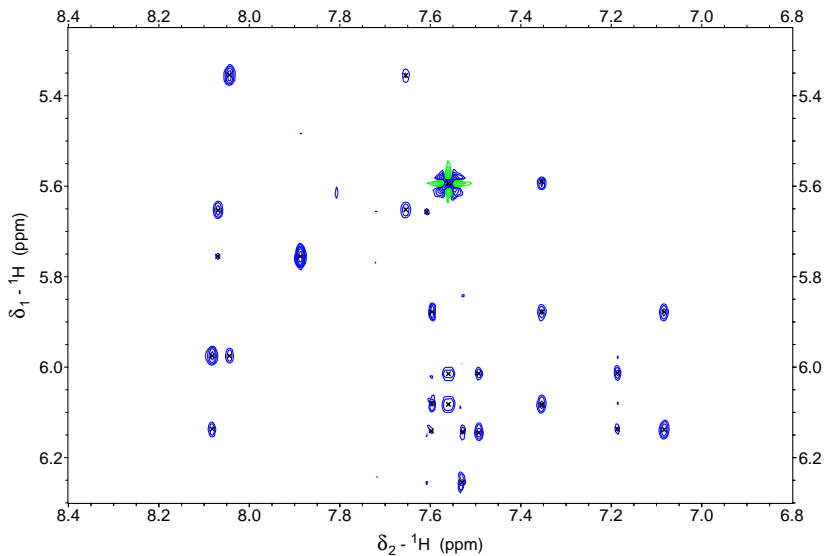
Postup při sekvenčním přiřazení v DNA



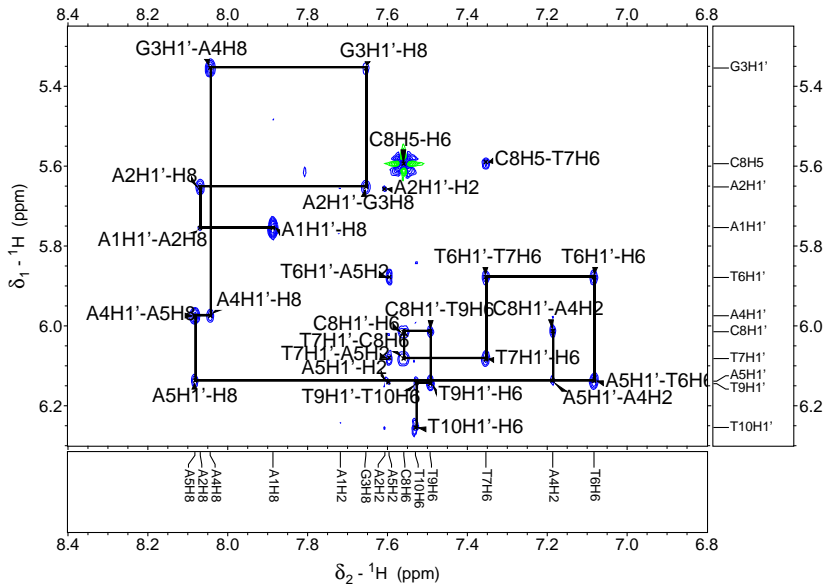
Postup při sekvenčním přiřazení v DNA



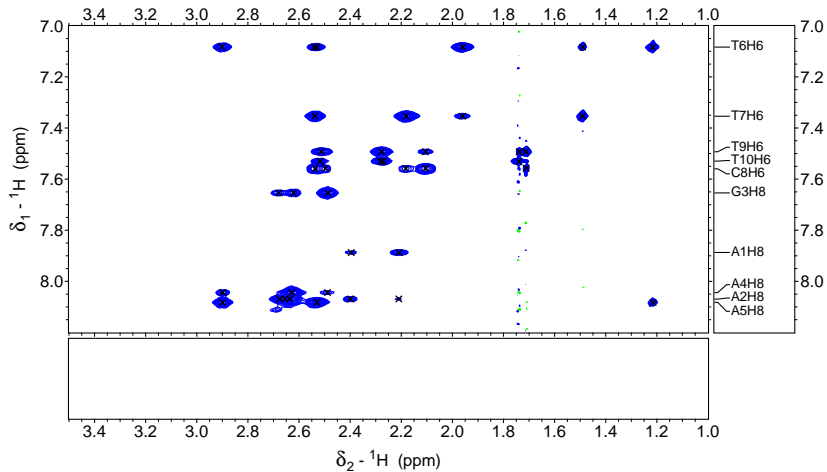
$d(\text{AAGAATTCTT})_2$, H1'-Har



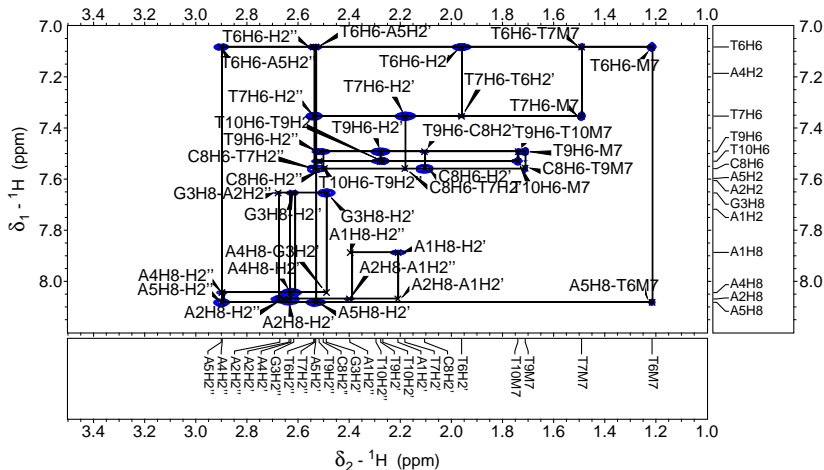
$d(\text{AAGAATTCTT})_2$, H1'-Har



$d(\text{AAGAATTCTT})_2$, Har-H2'/2"/Me

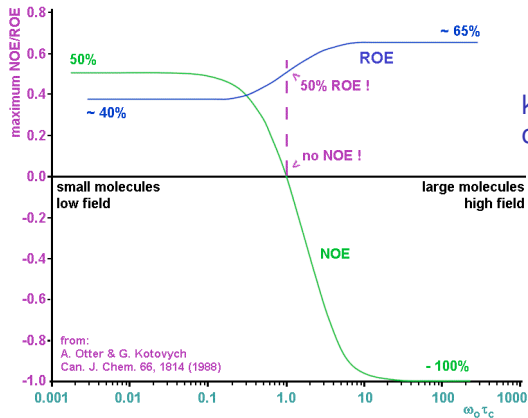


$d(\text{AAGAATTCTT})_2$, Har-H2'/2"/Me



ROESY-Rotating frame Overhauser Enhancement Spectroscopy

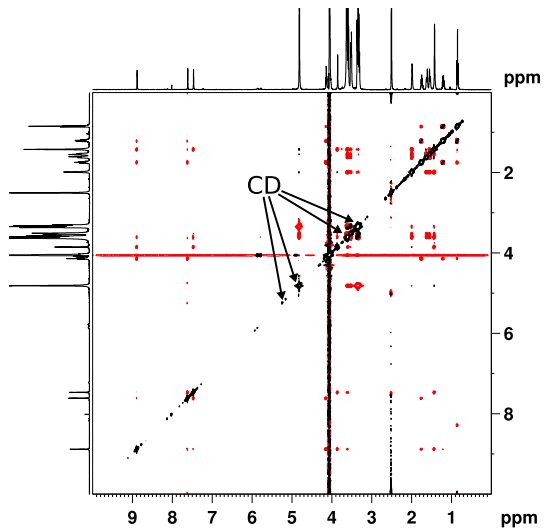
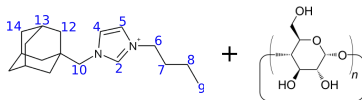
CAMELPSIN – Cross-relaxation Appropriate for Minimolecules Emulated by Locked SPINs



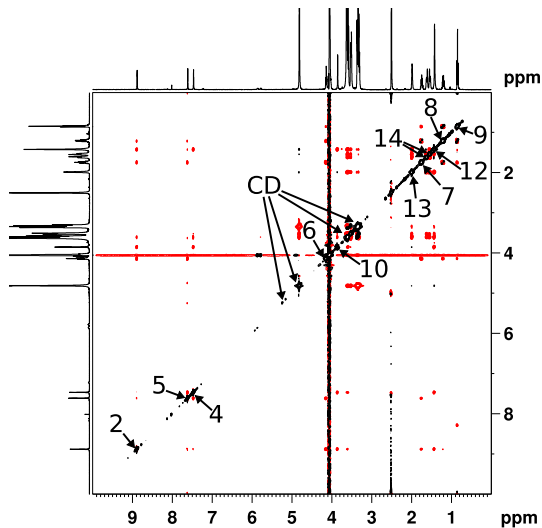
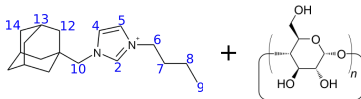
korelácia na základe
cross-relaxácie

- ▶ ROE vždy pozorovateľné
- ▶ intenzita závisí na r_{HH} a τ_{mix}
- ▶ opačná fáza ROE crosspeakov
- ▶ výmenné a TOCSY crosspeaky s rovnakou fázou

300 ms ROESY v D₂O:DMSO-d₆



300 ms ROESY v D₂O:DMSO-d₆



Příště:

? Heteronukleární korelace