

NMR - method suited to study biomolecules

Pavel Kadeřávek

1. NMR - introduction
2. Studies of interactions
3. Conformational exchange
4. Investigation ps-ns dynamics of structured proteins
5. High-resolution relaxometry - investigation of ps-ns dynamics of IDPs
6. High-resolution relaxometry - metabolomics
7. Two-field NMR
8. Dissolution dynamics nuclear polarisation (dDNP)

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7. Two-field NMR

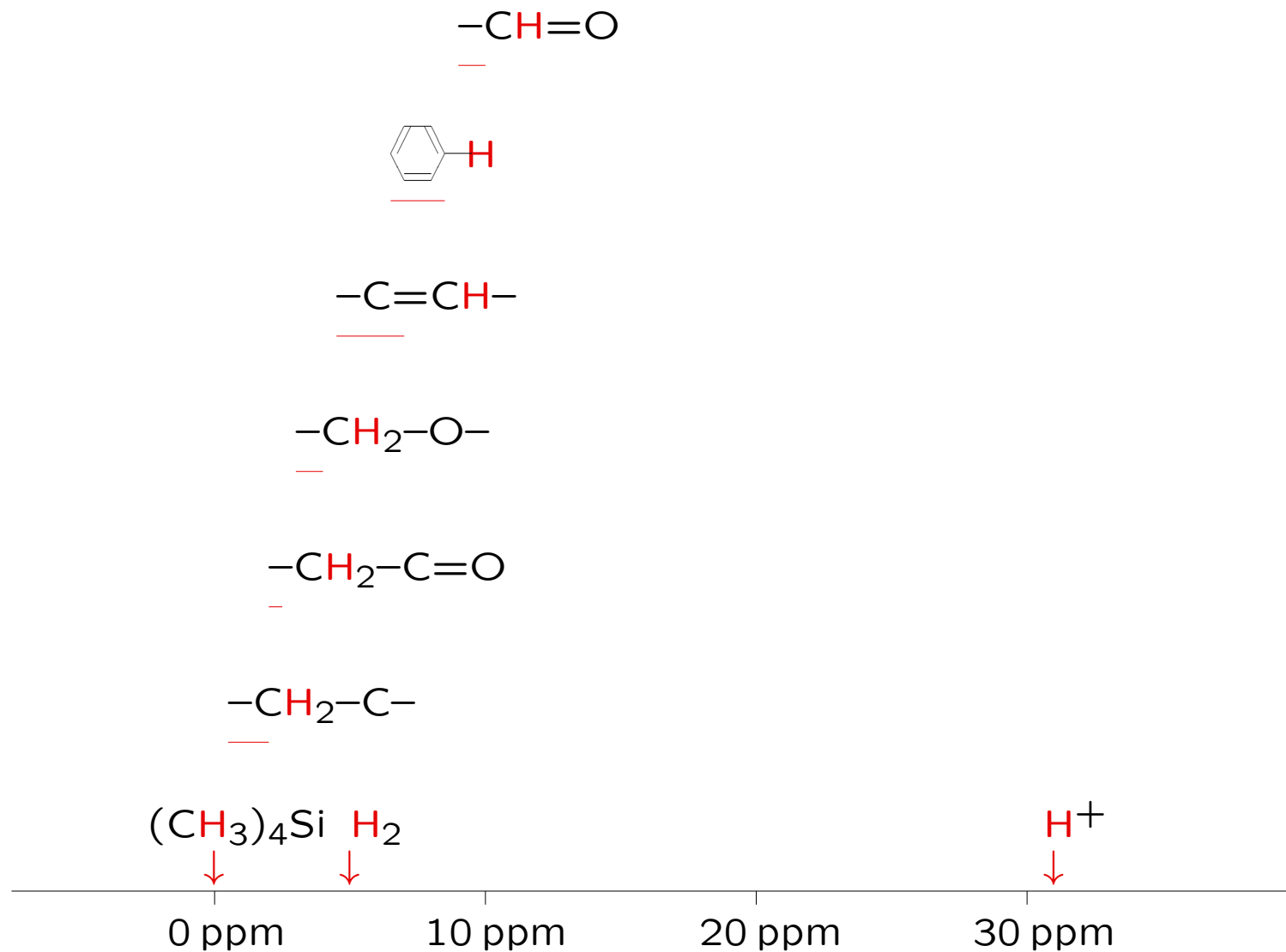
8. Dissolution dynamics nuclear polarisation (dDNP)

NMR introduction

- magnetic moments of nuclei (spin) - ^1H , ^{13}C , ^{15}N , ^{31}P
- Larmor frequencies $\omega = -\gamma B$ (we need strong B)
- slow relaxation \Rightarrow polarisation transfer

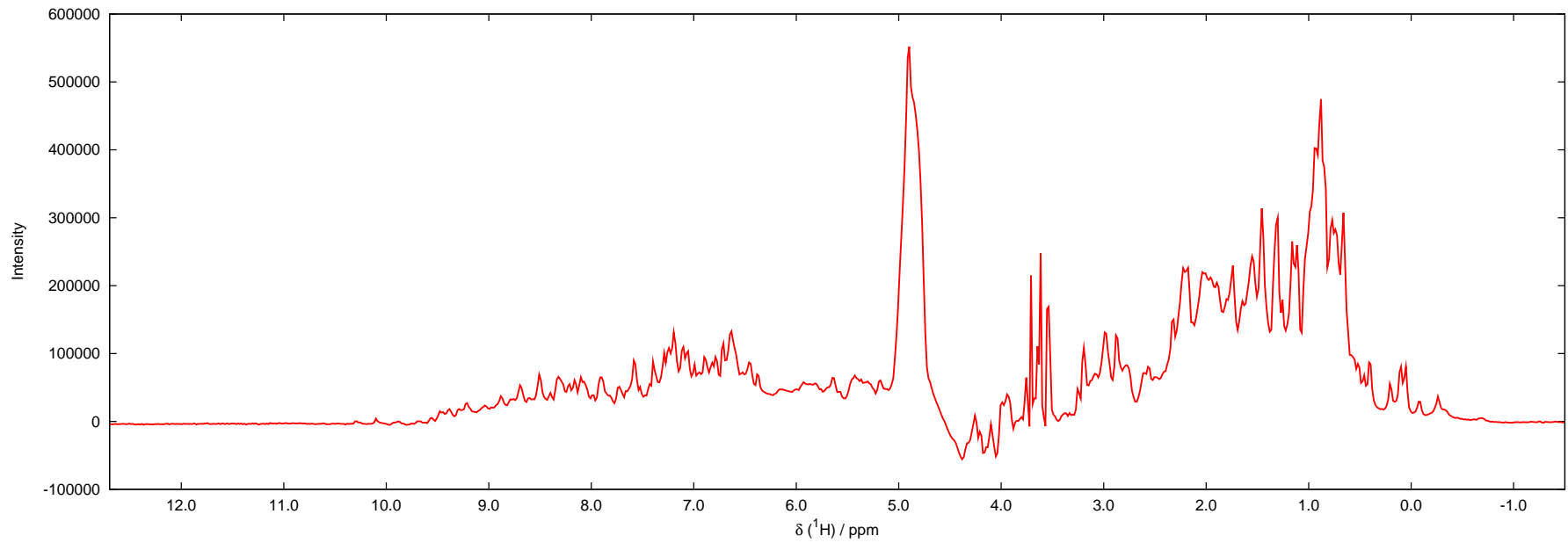


Effect of electron density



1950: Proctor, Yu

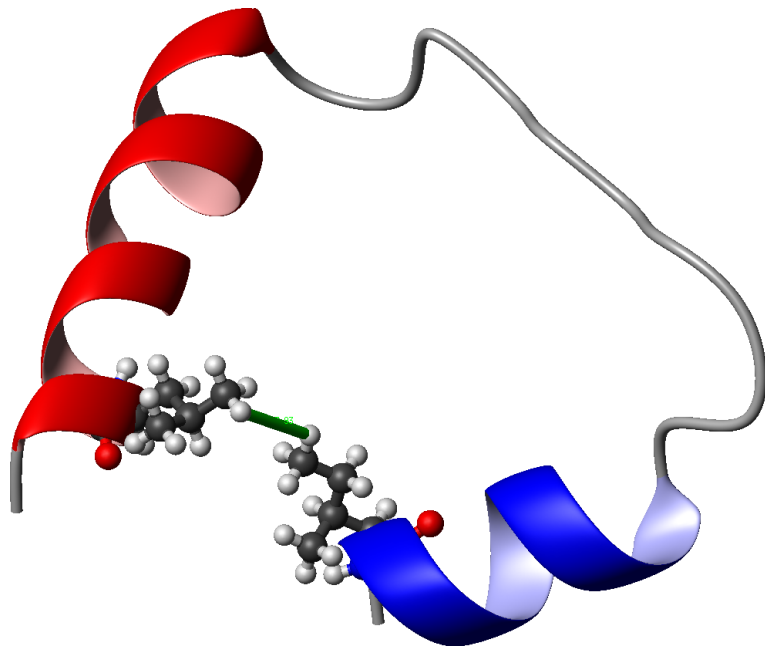
^1H NMR spectrum of a protein



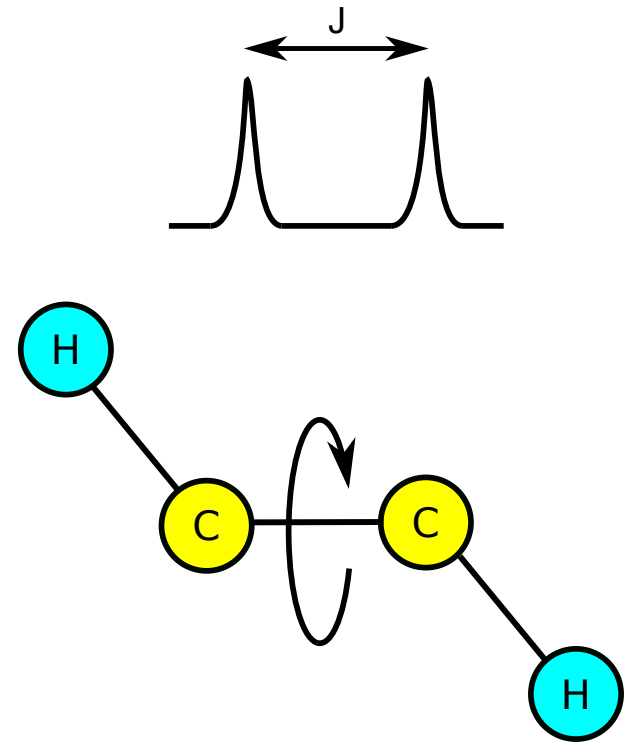
1956 - first NMR spectrum of a protein

Structure information

Nuclear Overhauser effect:
distance

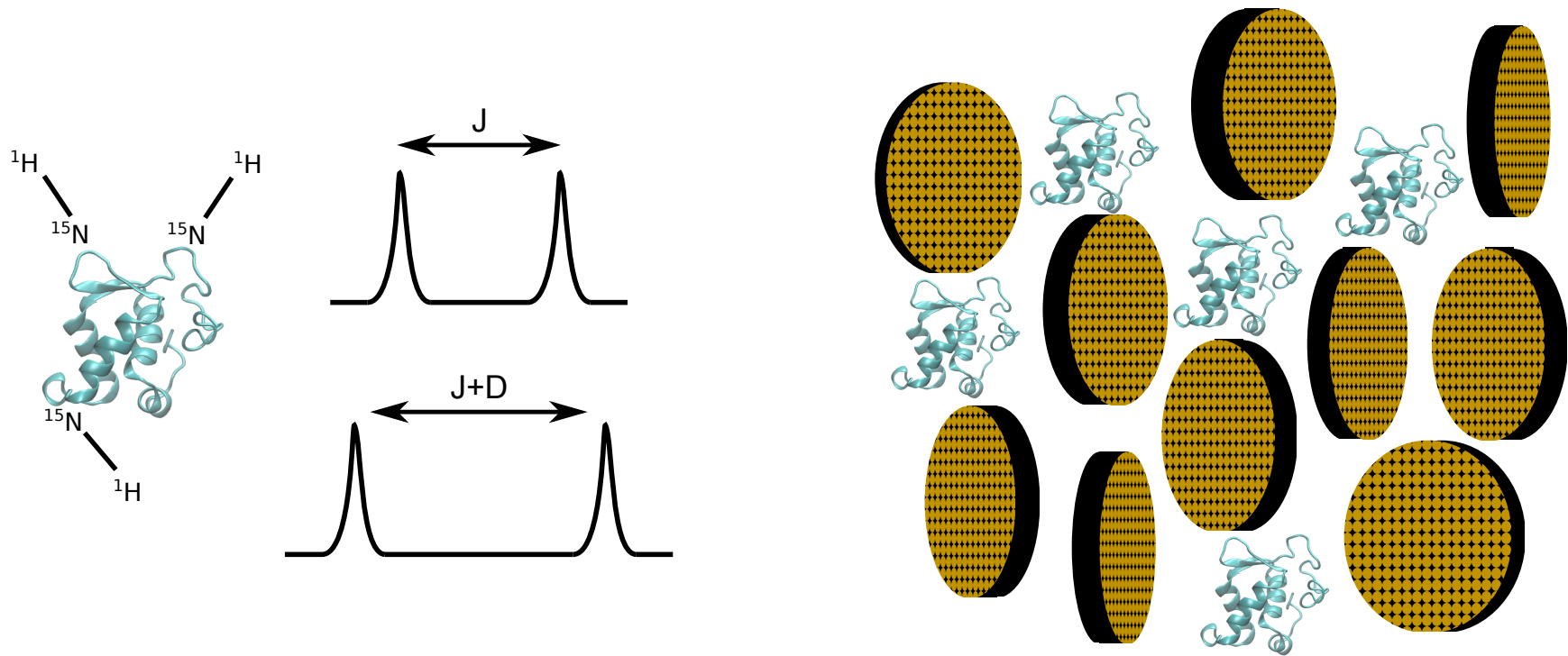


Spin-spin scalar coupling:
torsion angles



1985: first protein structure solved by NMR

Residual dipolar couplings - Structure information



1995: Prestegard J.H., 1996 Bax A.

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Interaction: titration

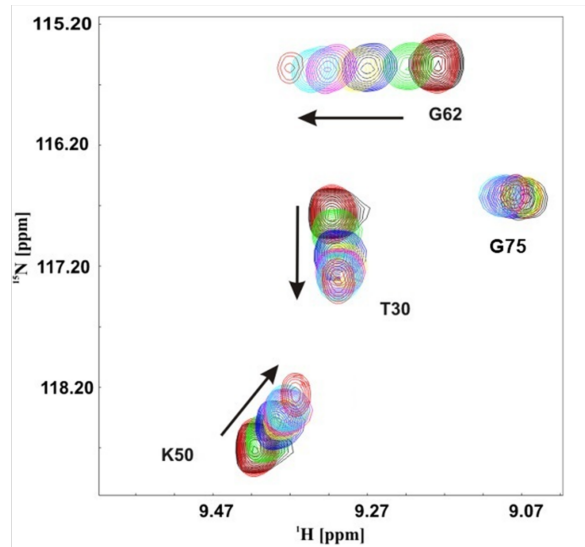


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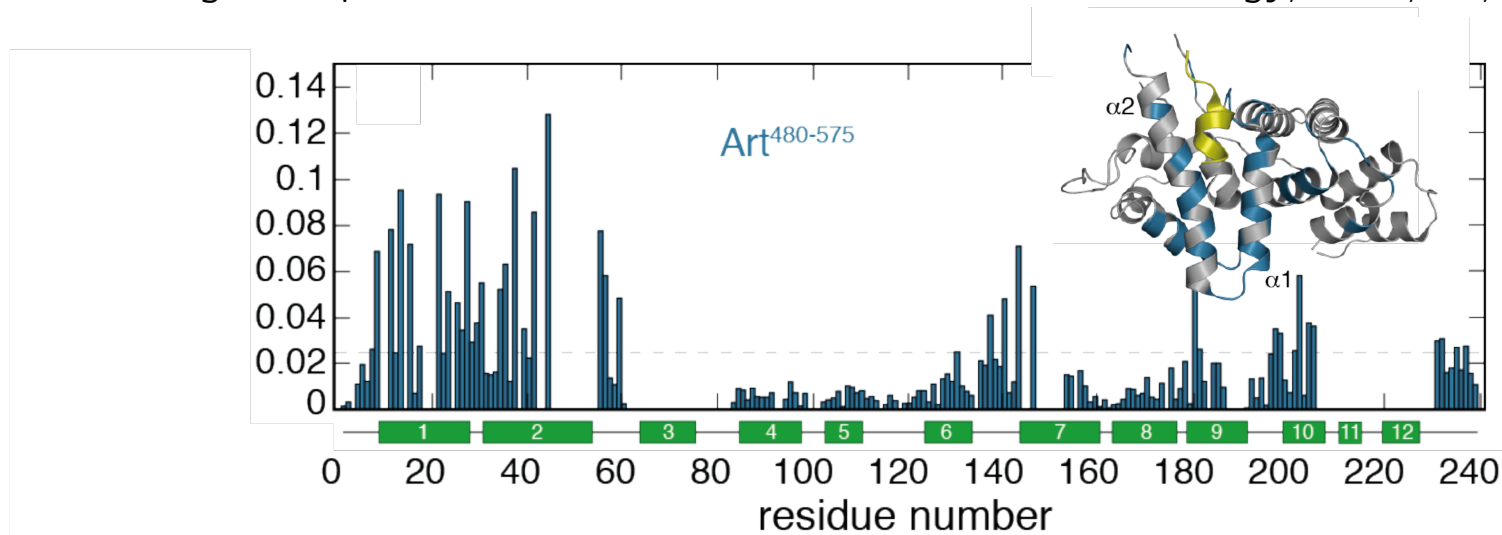
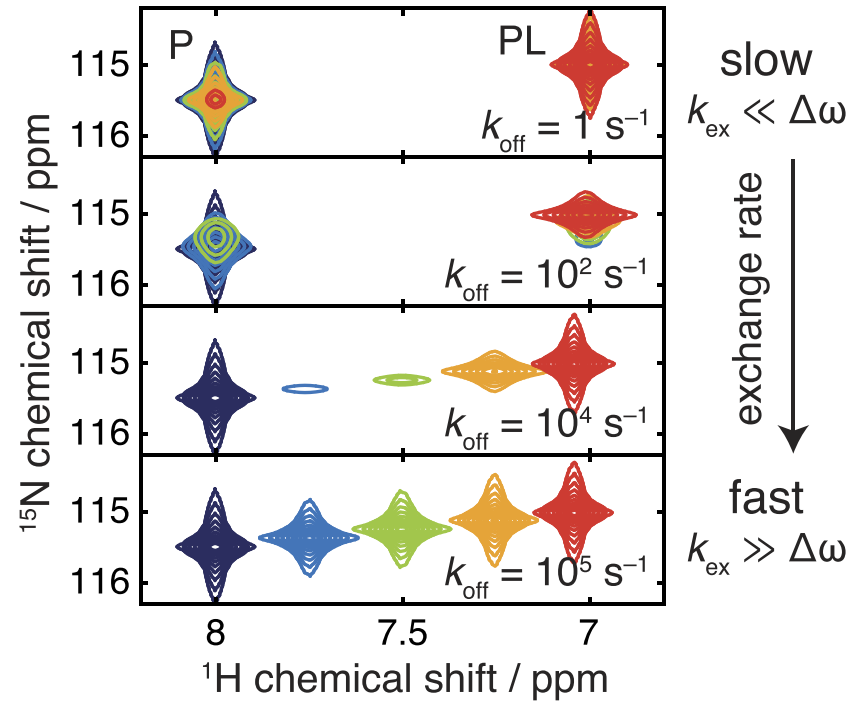
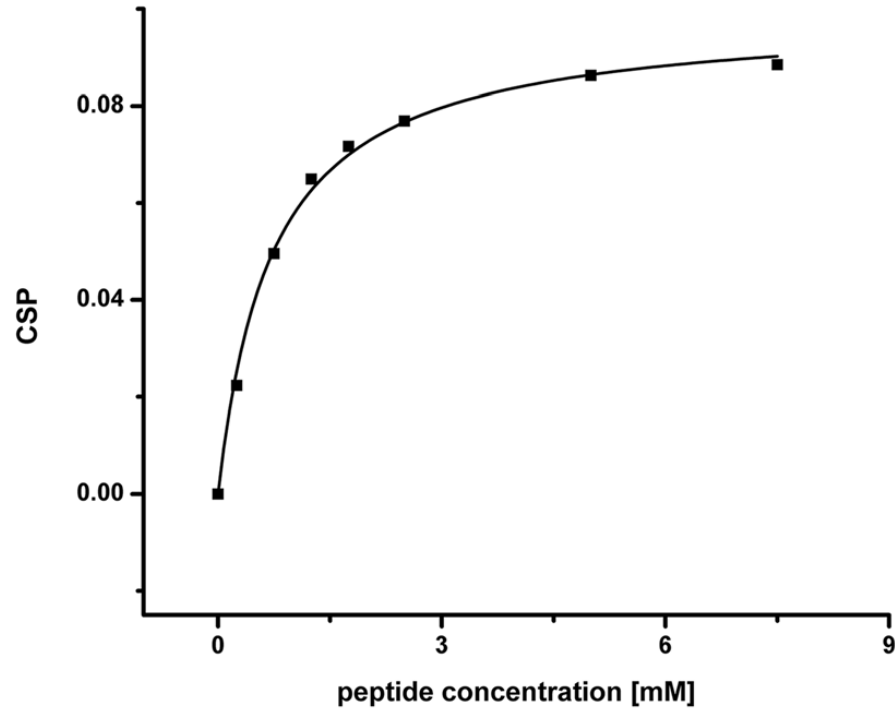


figure copied from Charlier C. et al., *J. Am. Chem. Soc.*, 2017, 139, 1219–12227

Interaction: titration

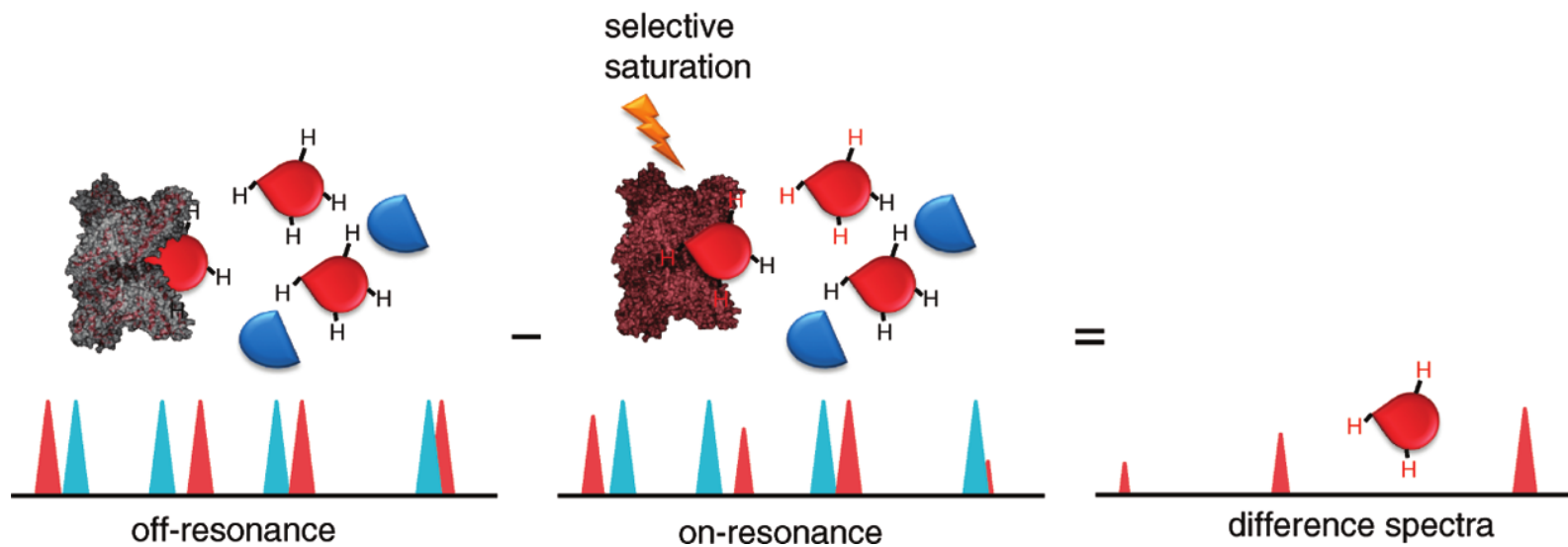
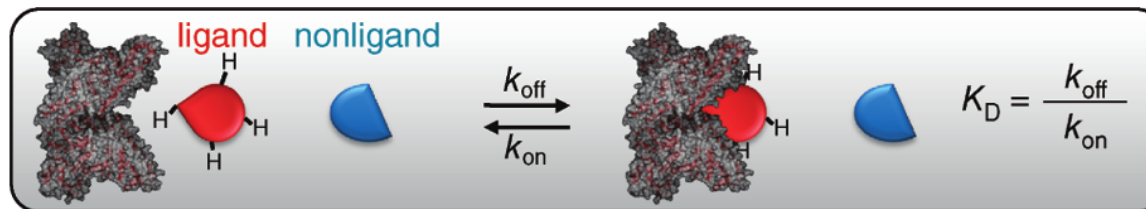


figures copied from publications:

Dicks et al., BMC Molecular and Cell Biology, 2019, 20: 23

Waudby et al., Scientific reports, 2016, 24826

Interaction of small molecule with a large molecule



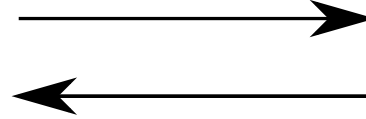
copied from publication: Addino Viegas et al., J. Chem. Educ., 2011, 88, 990-994

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Conformational Exchange = Structure Rearrangement



A

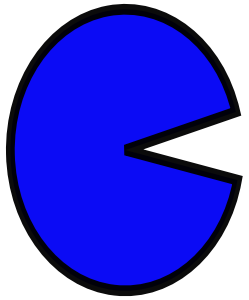


B

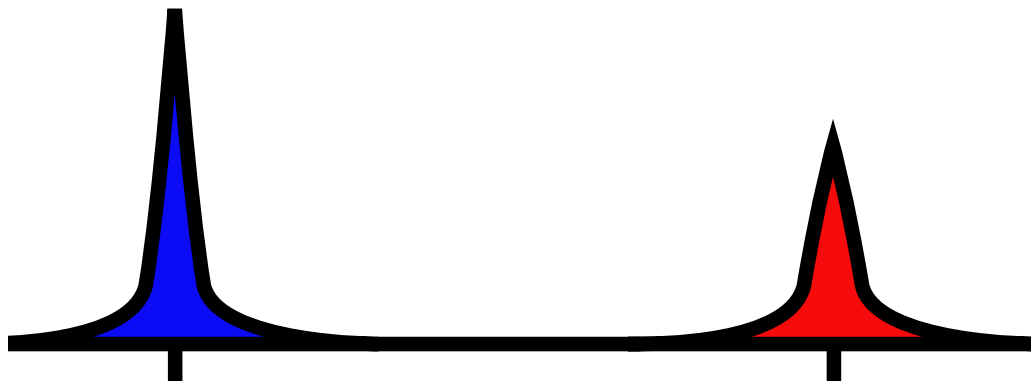
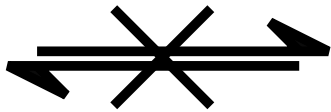
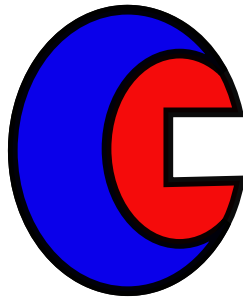
μs – ms motions

$$k_{\text{ex}} = 0$$

A

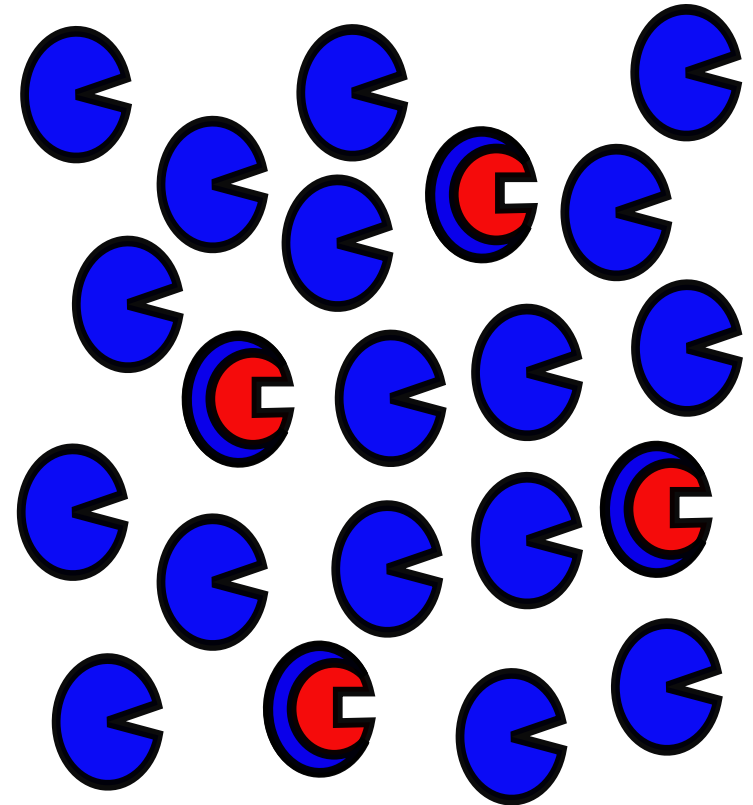


B



Ω_A

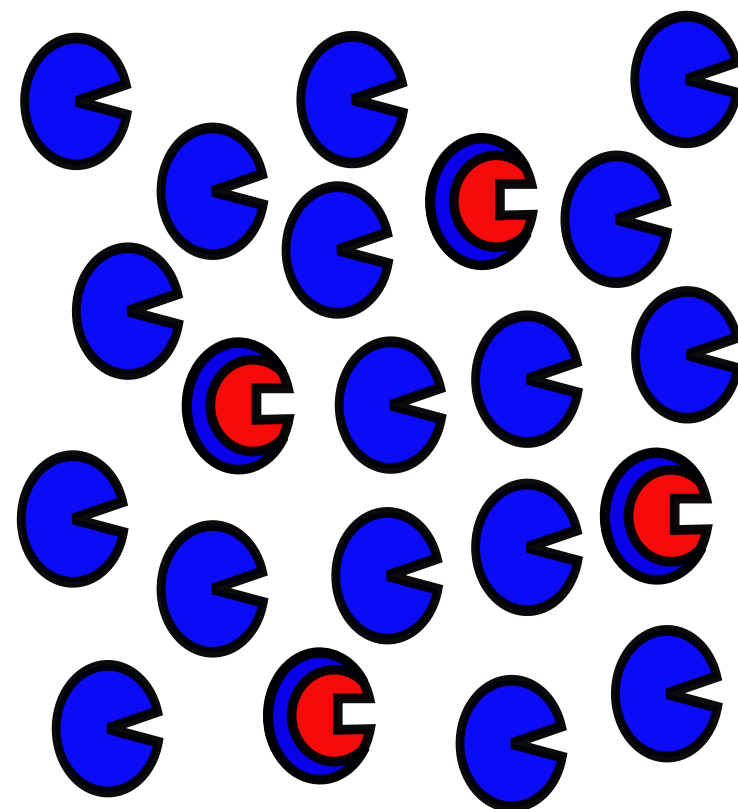
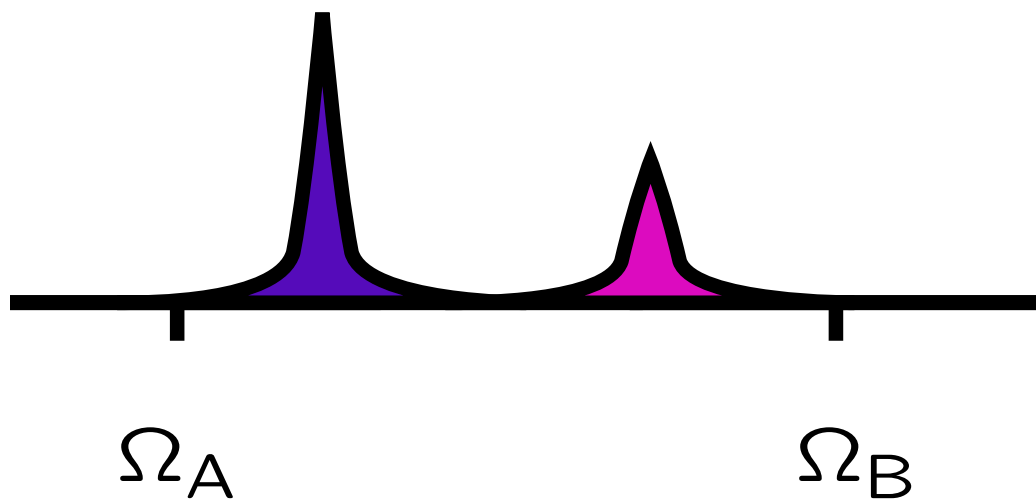
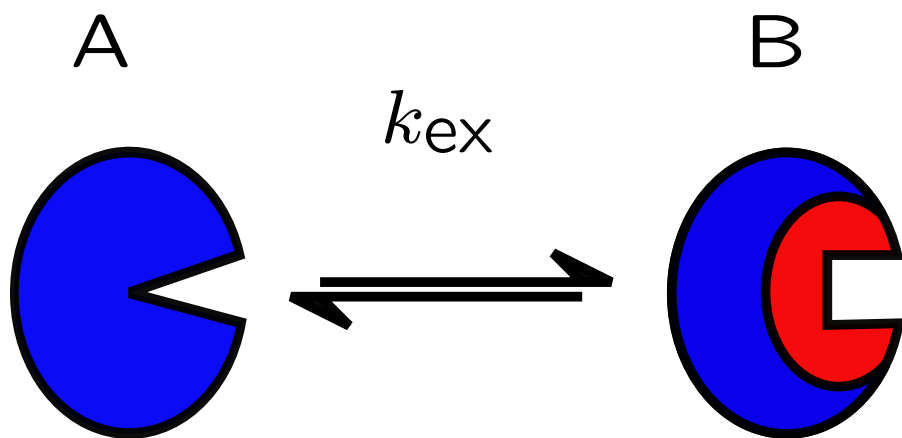
Ω_B



$$p_A, p_B = 1 - p_A$$

μs – ms motions

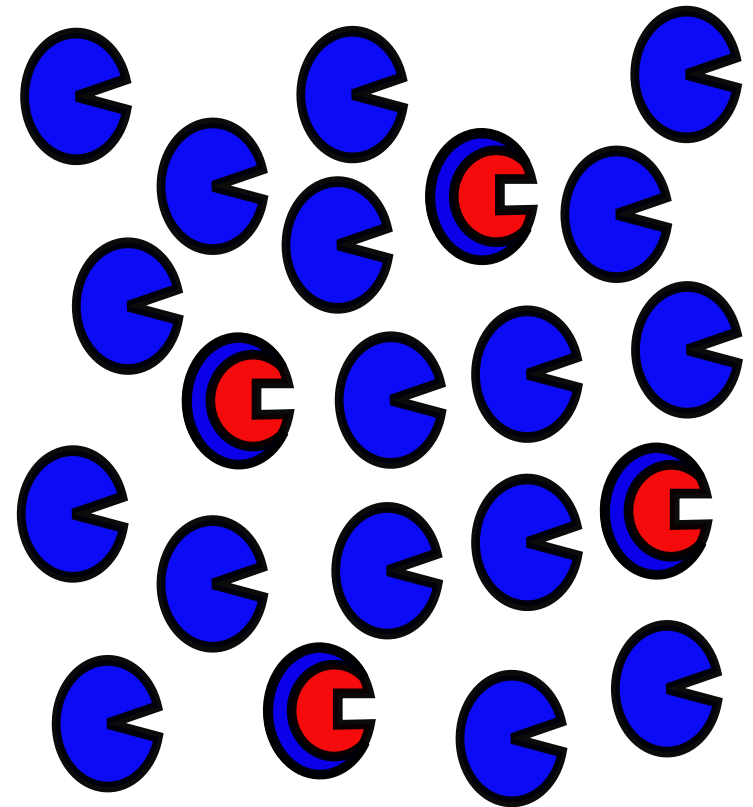
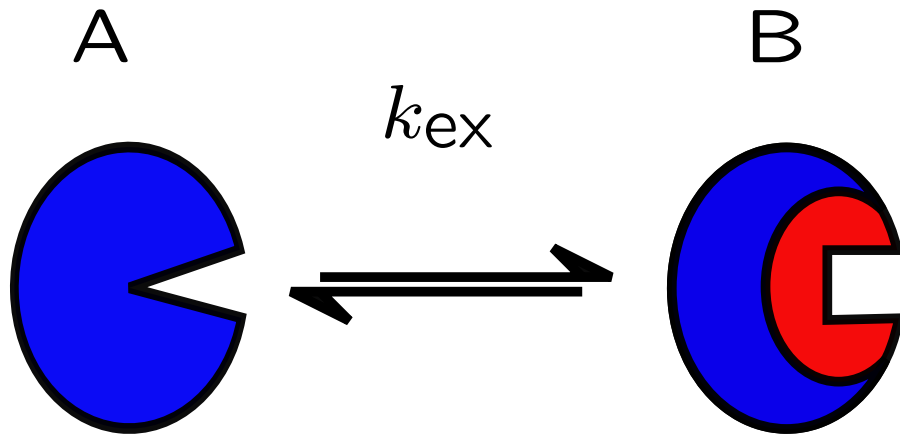
$$k_{\text{ex}} < |\Omega_A - \Omega_B| \Rightarrow R_{\text{ex}}(k_{\text{ex}}, \Omega_A, \Omega_B, p_B)$$



$$p_A, p_B = 1 - p_A$$

μs – ms motions

$$k_{\text{ex}} \approx |\Omega_A - \Omega_B|$$

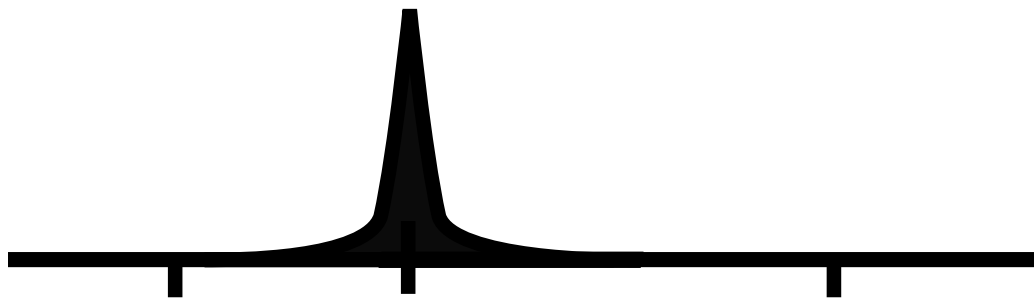
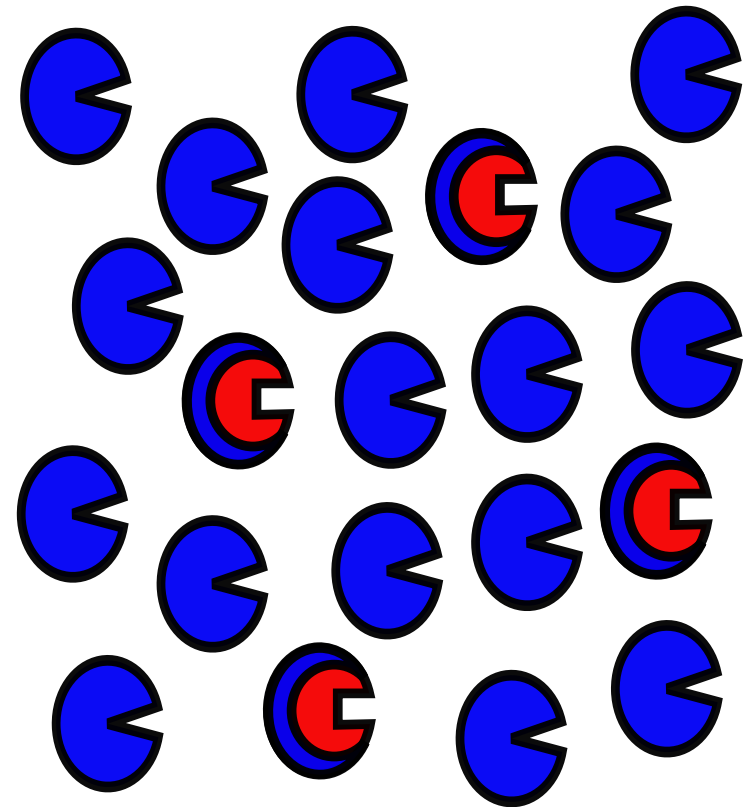
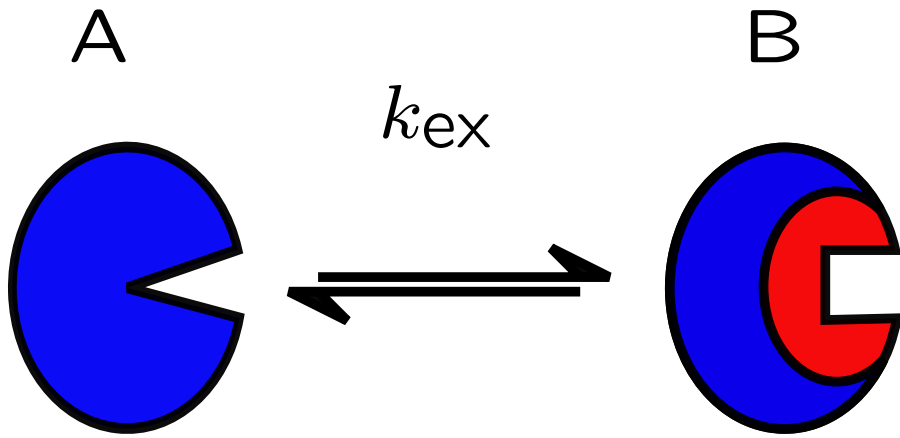


Ω_A Ω_{obs} Ω_B

$p_A, p_B = 1 - p_A$

μs – ms motions

$$k_{\text{ex}} > |\Omega_A - \Omega_B| \Rightarrow R_{\text{ex}}(k_{\text{ex}}, \Omega_A, \Omega_B, p_B)$$



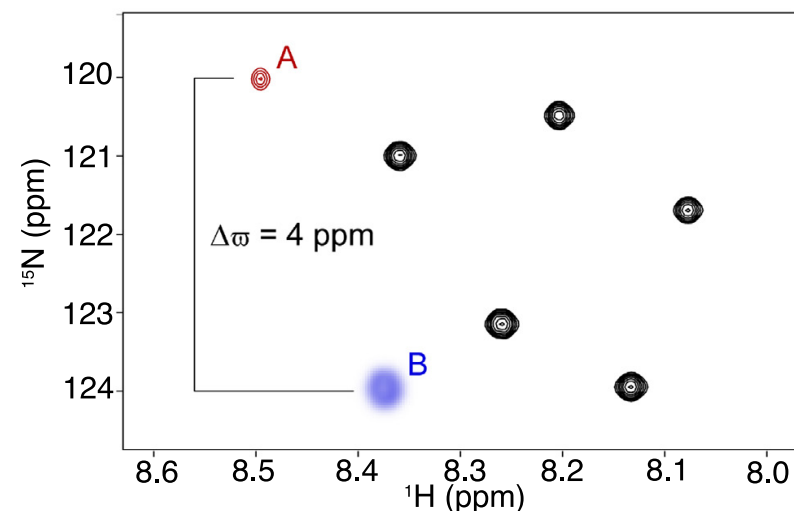
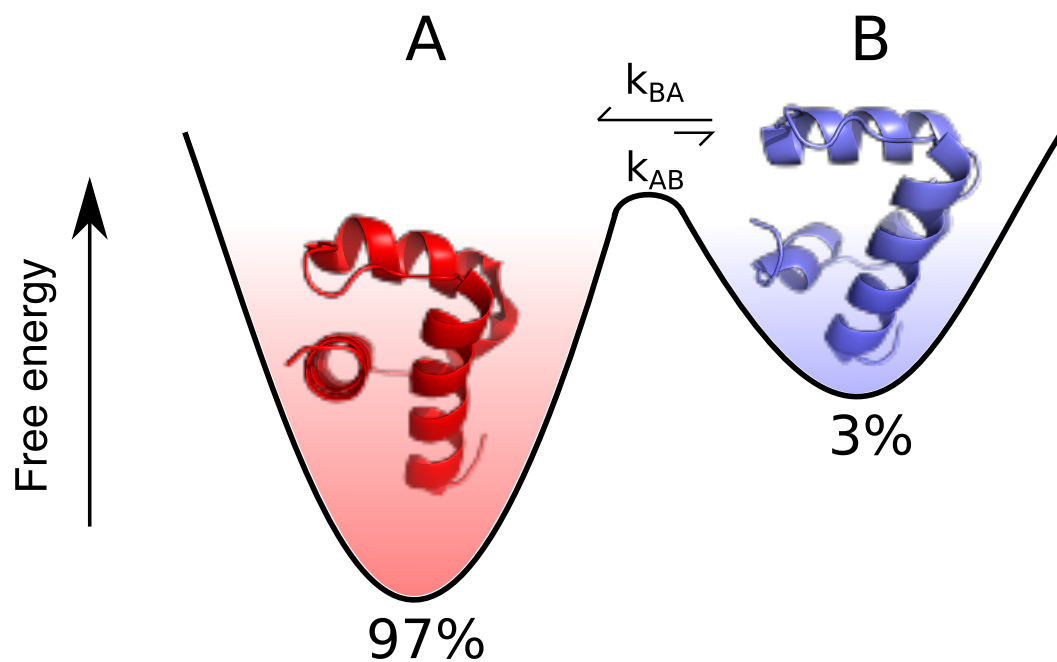
Ω_A

Ω_{obs}

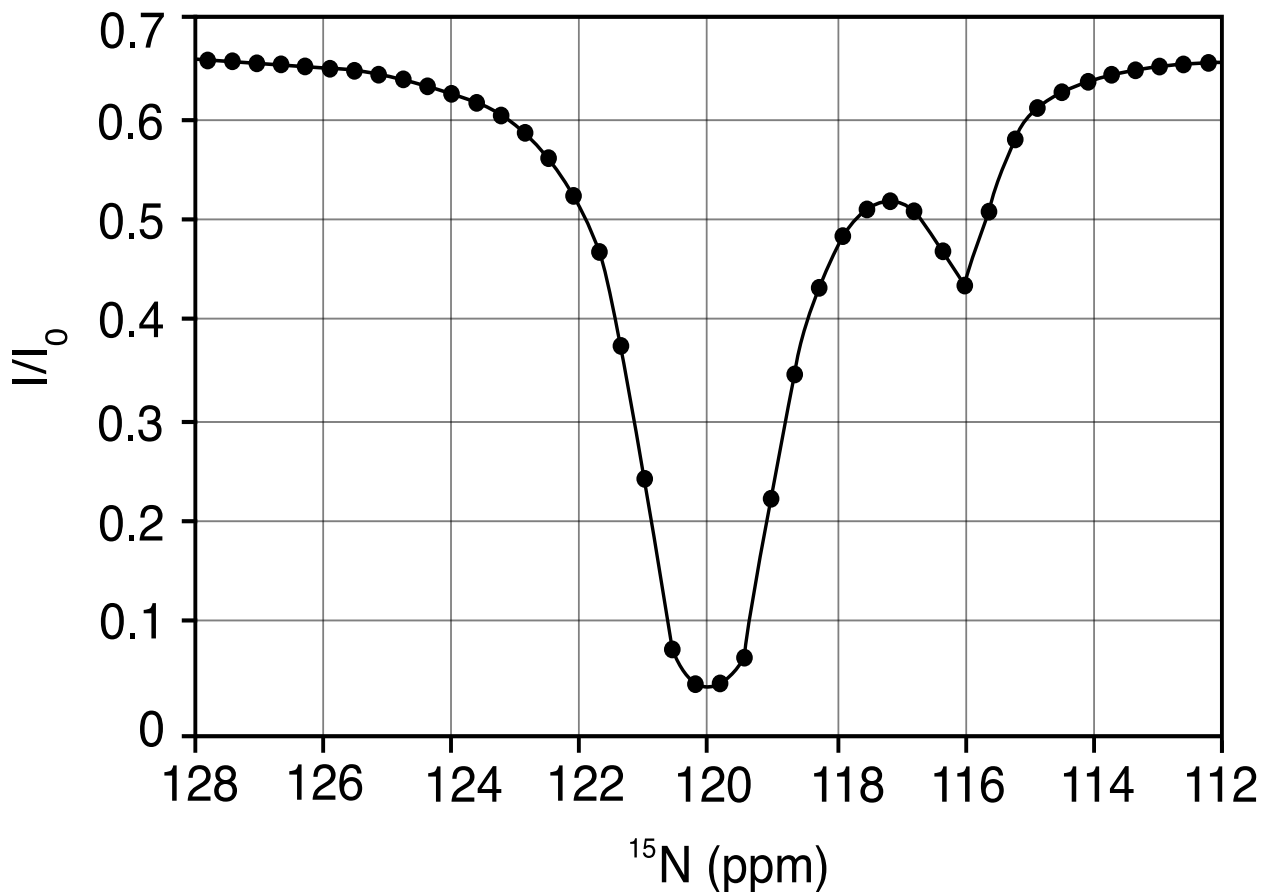
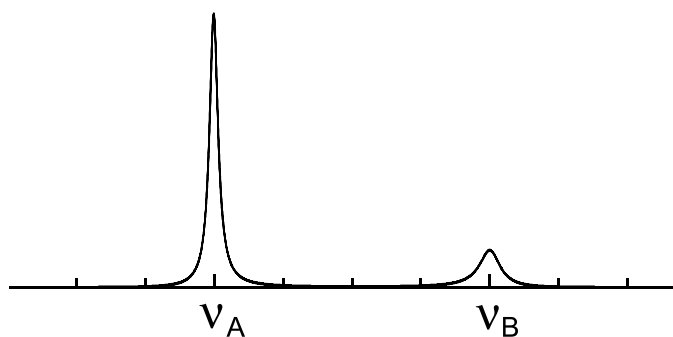
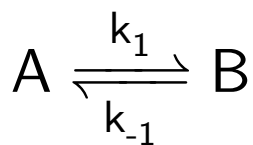
Ω_B

$$p_A, p_B = 1 - p_A$$

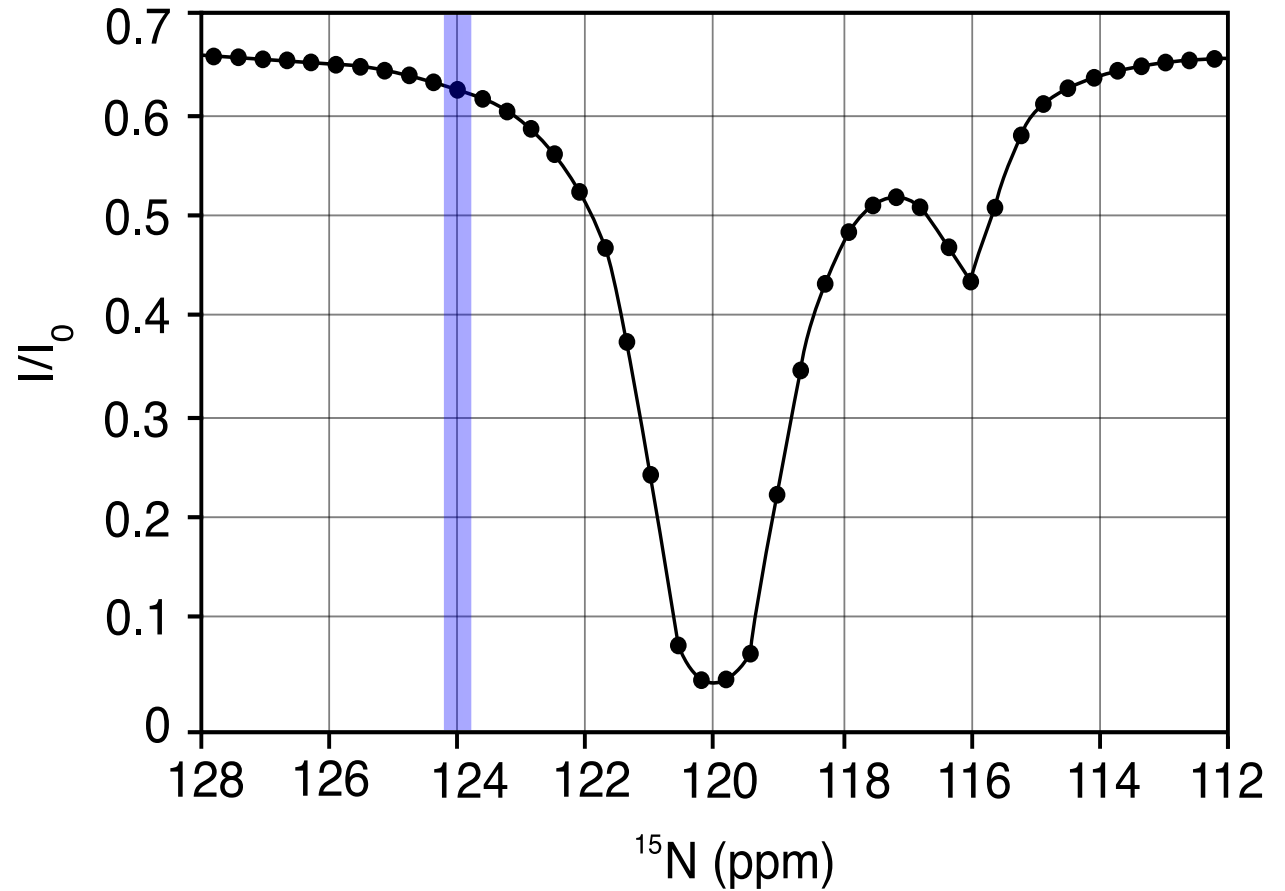
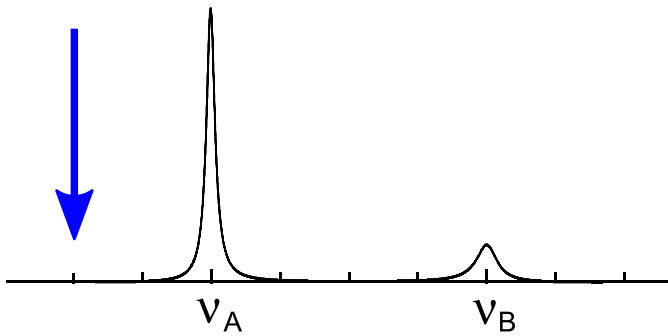
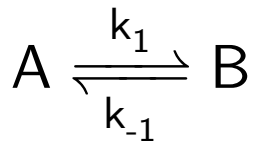
Conformational Exchange = Structure Rearrangement



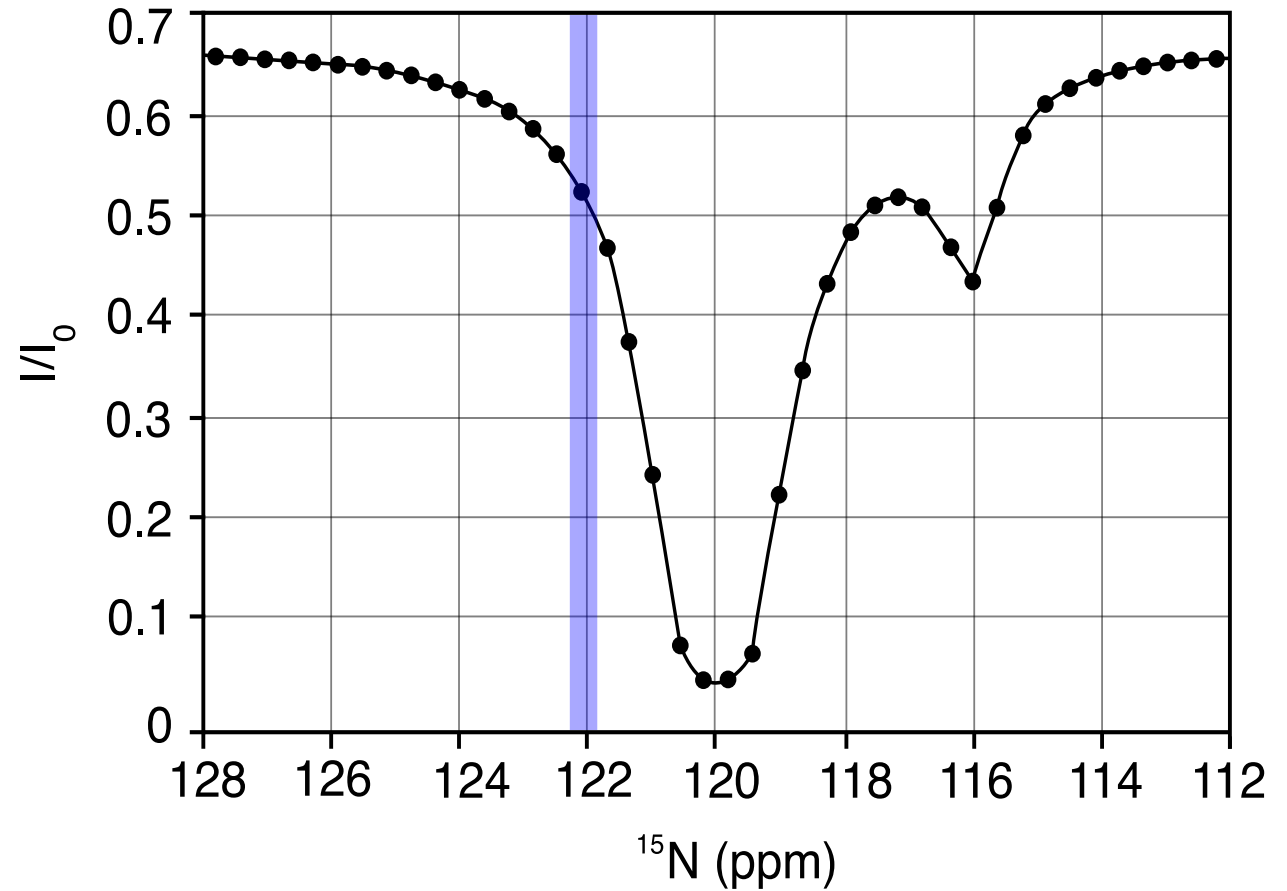
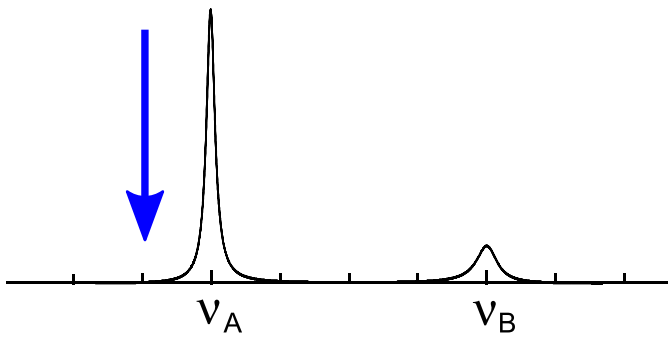
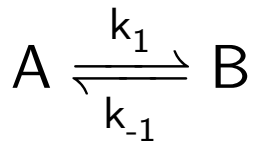
Chemical Exchange Saturation Transfer



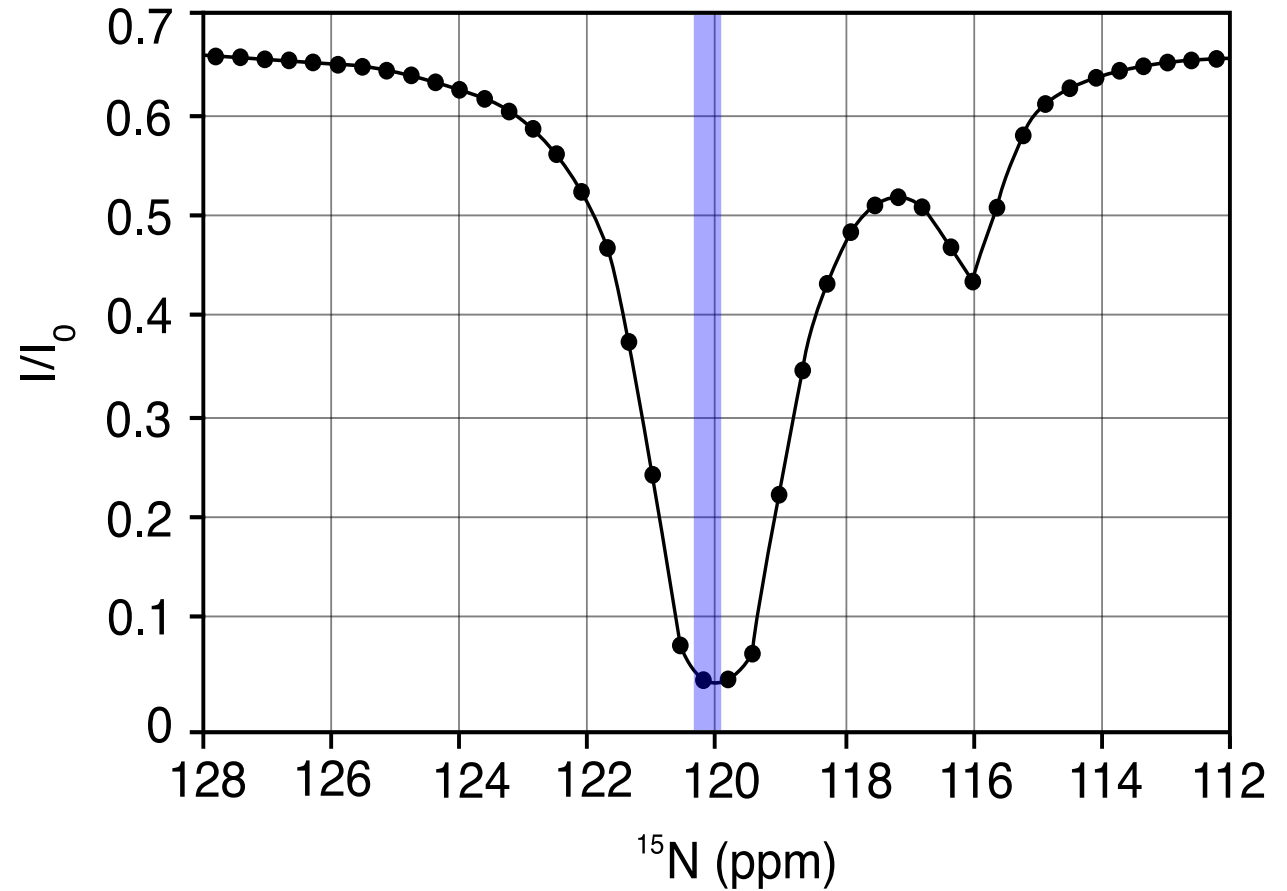
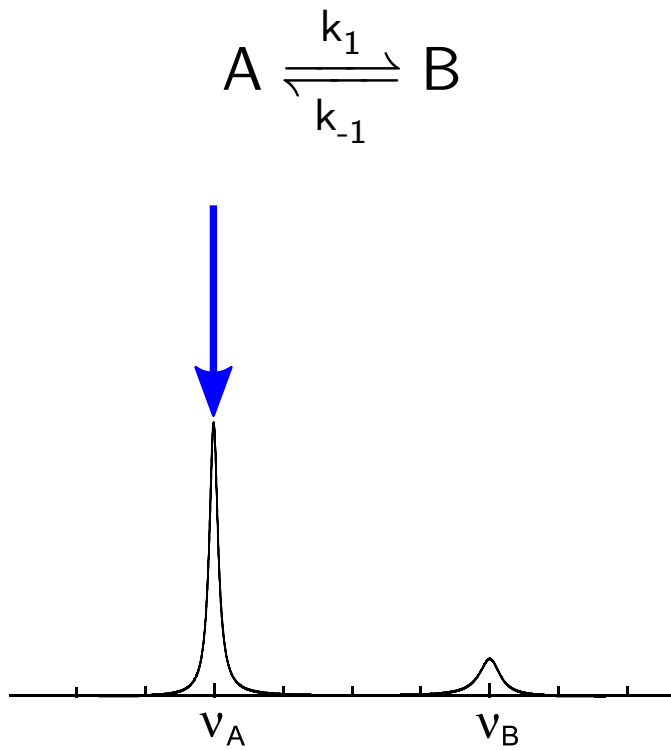
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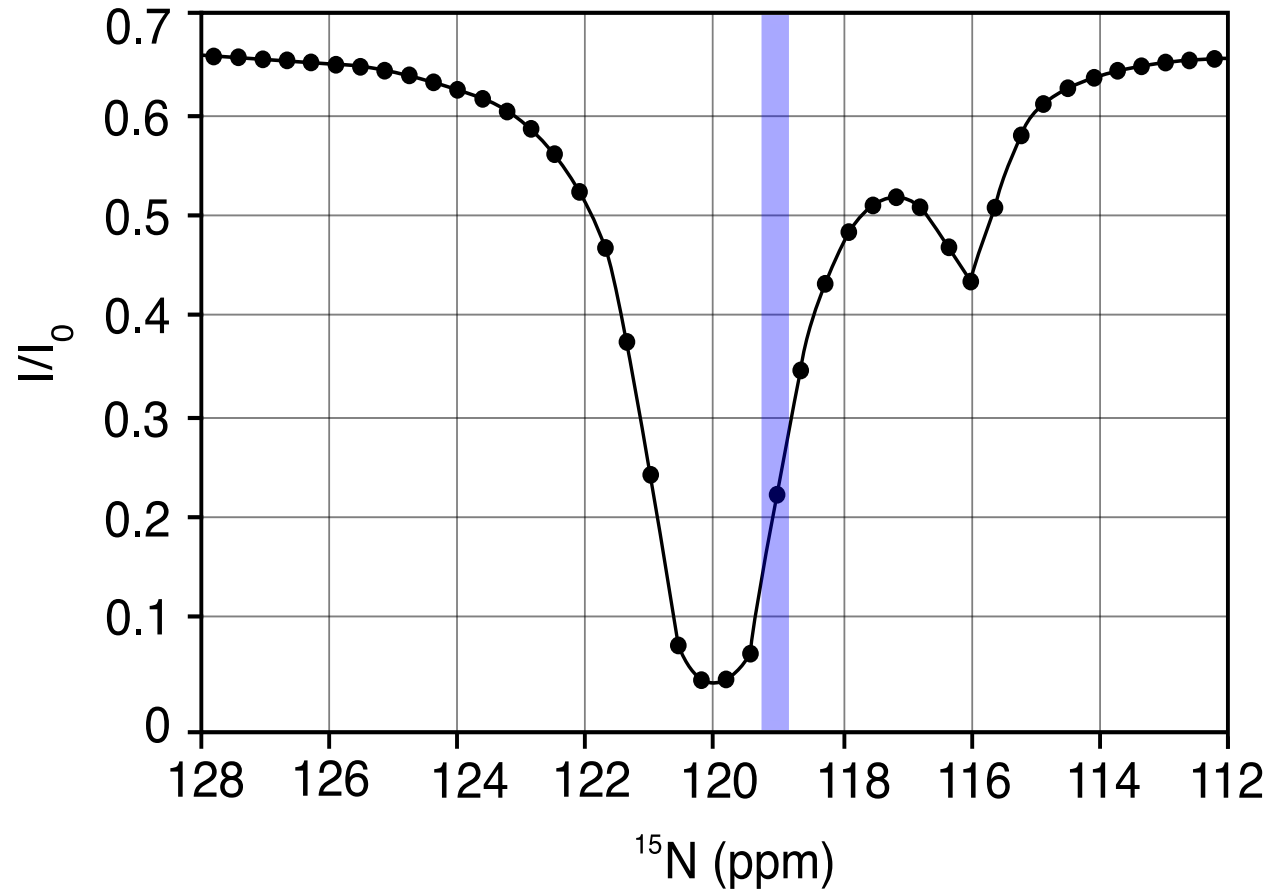
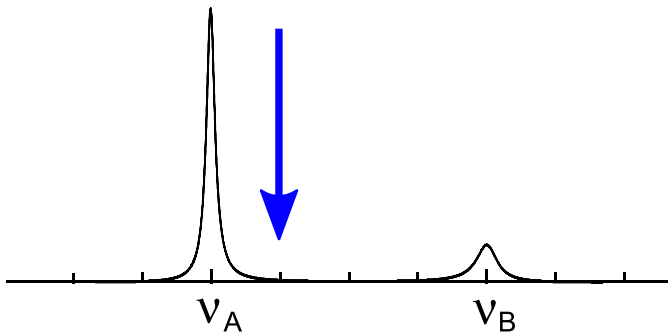
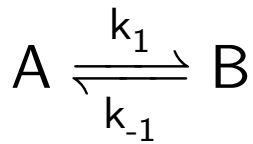
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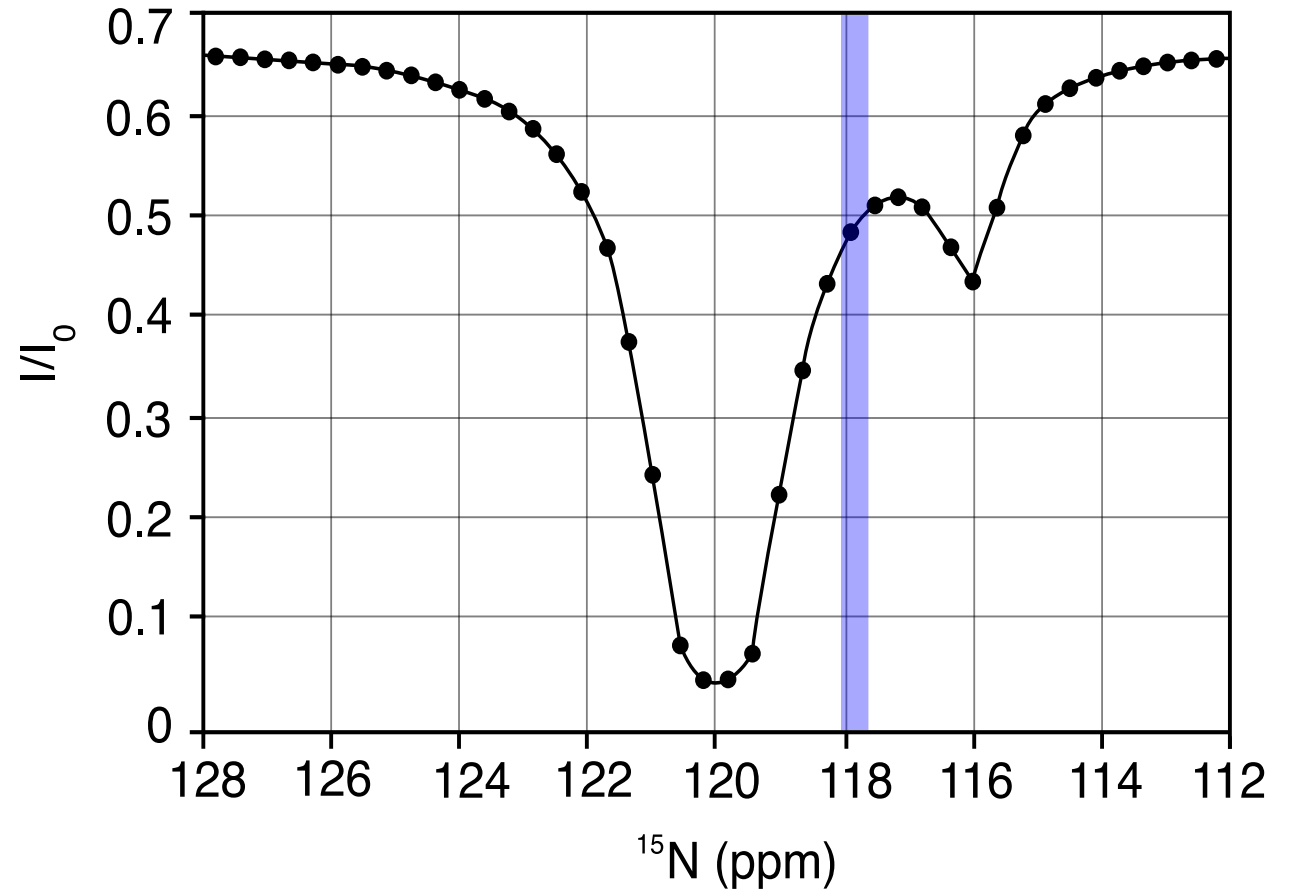
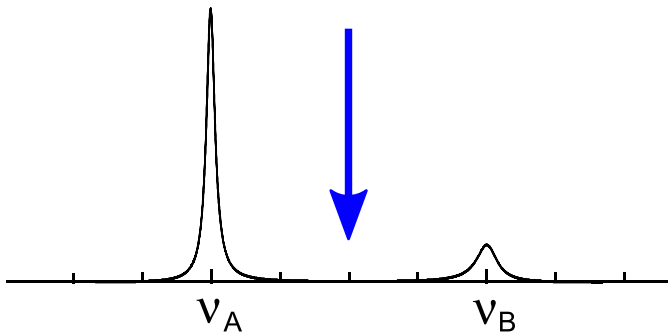
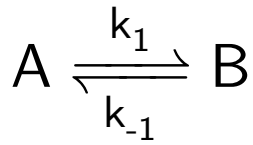
Chemical Exchange Saturation Transfer



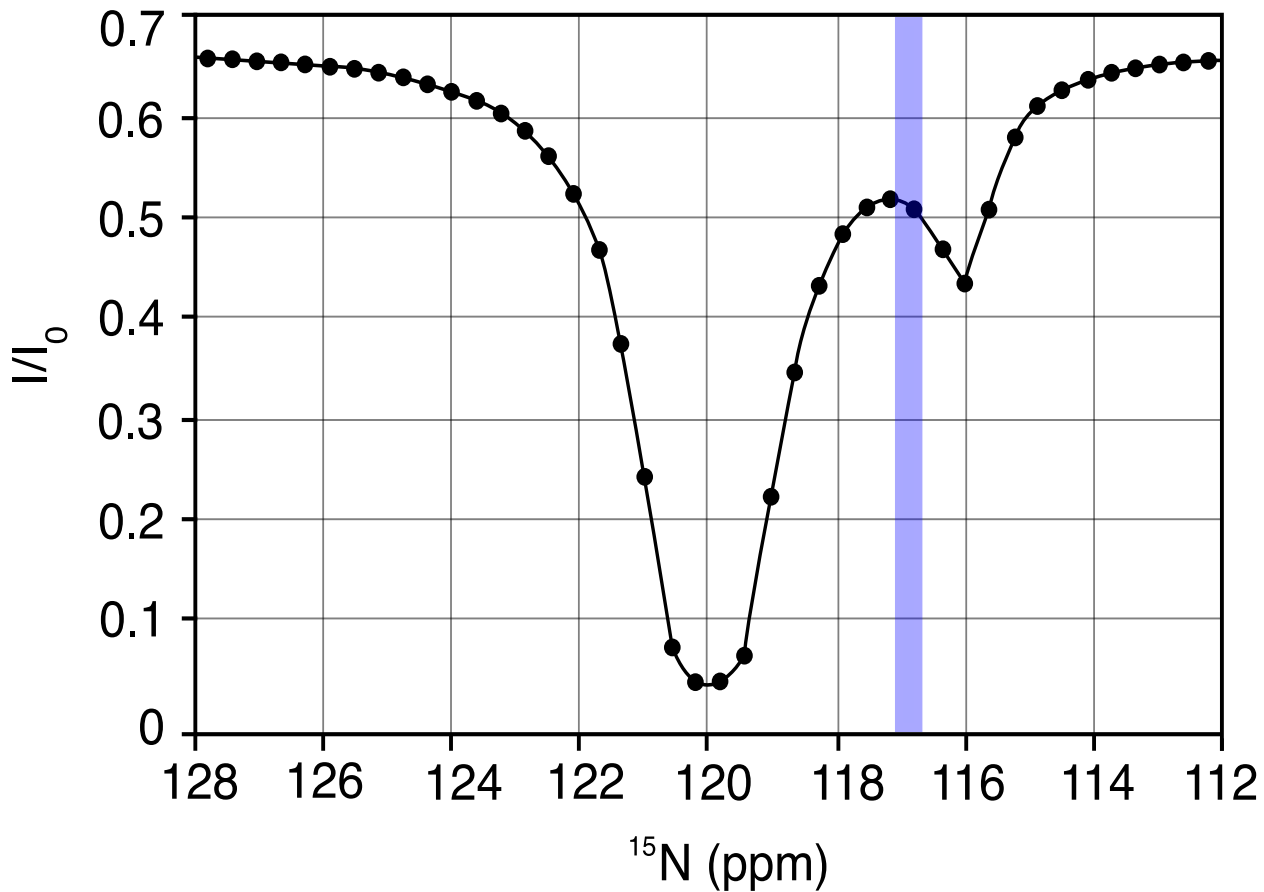
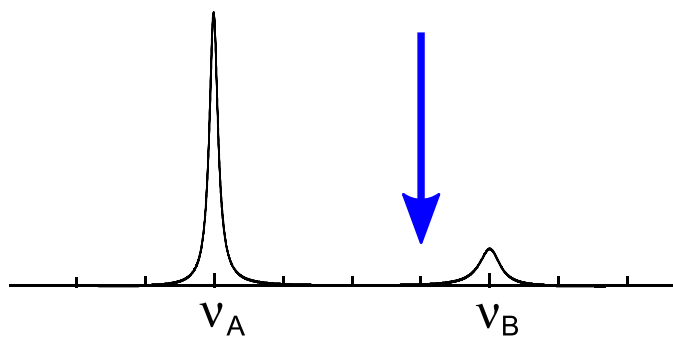
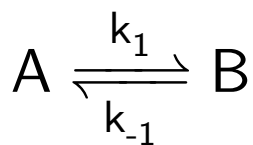
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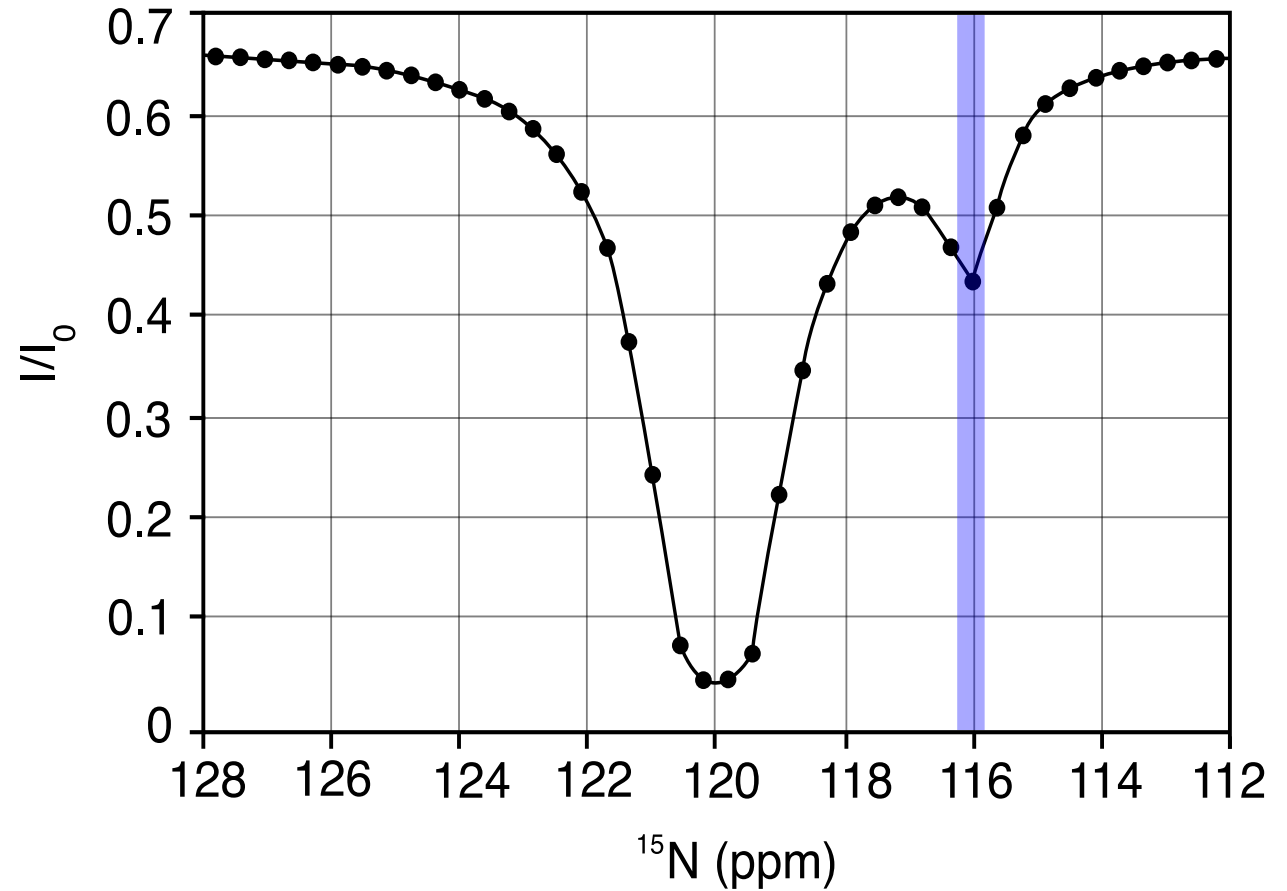
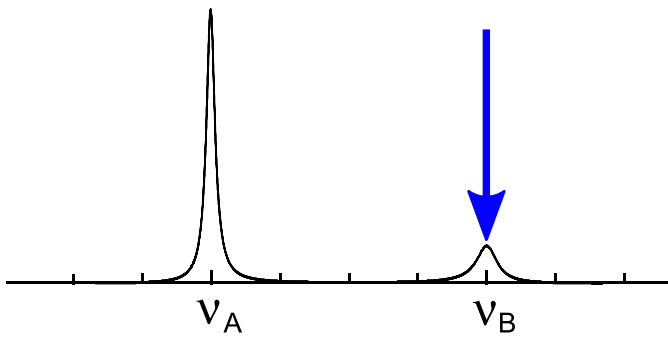
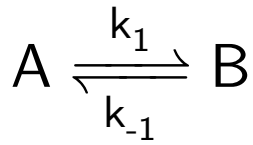
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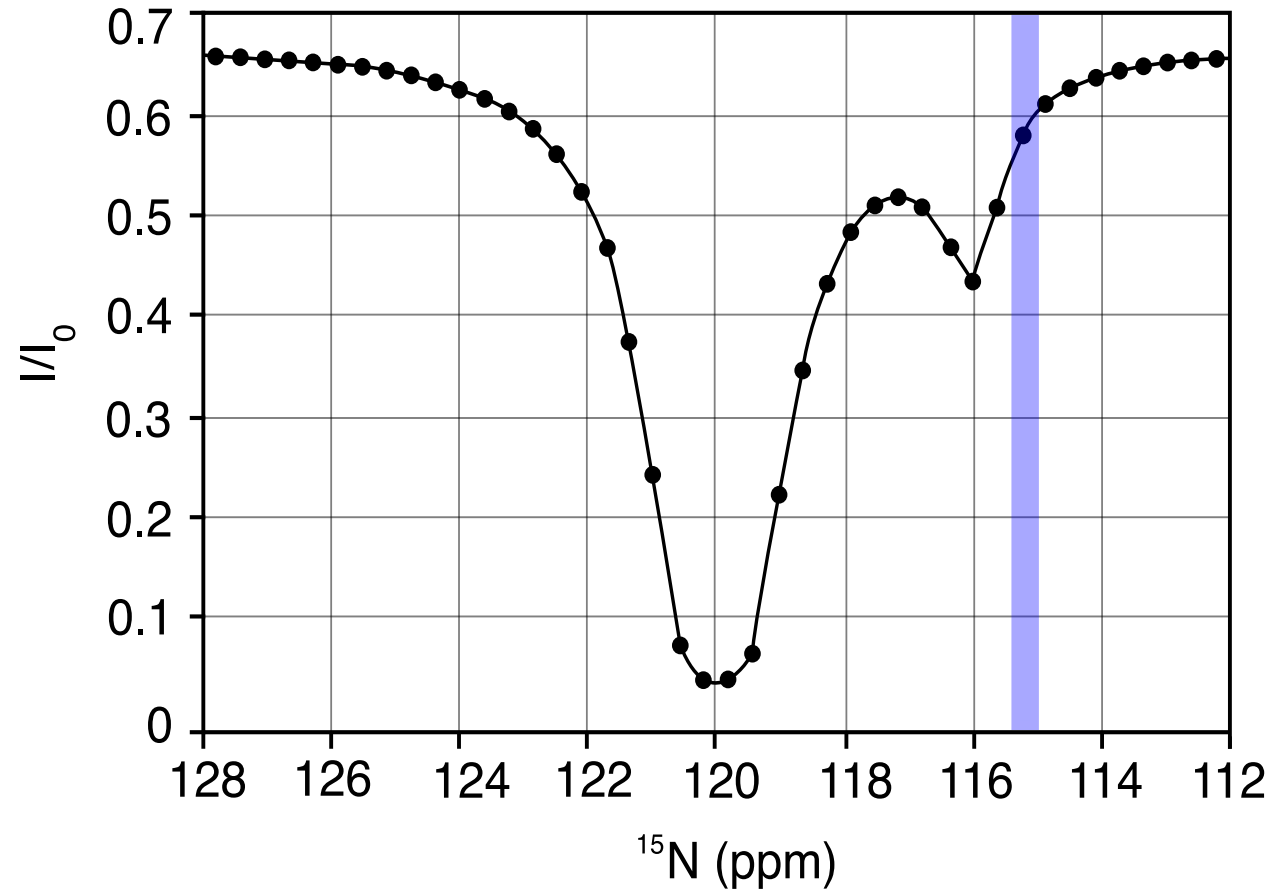
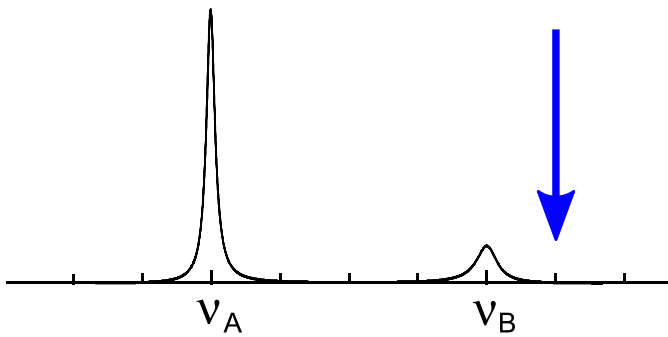
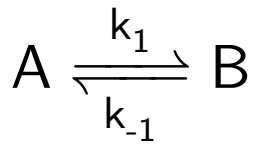
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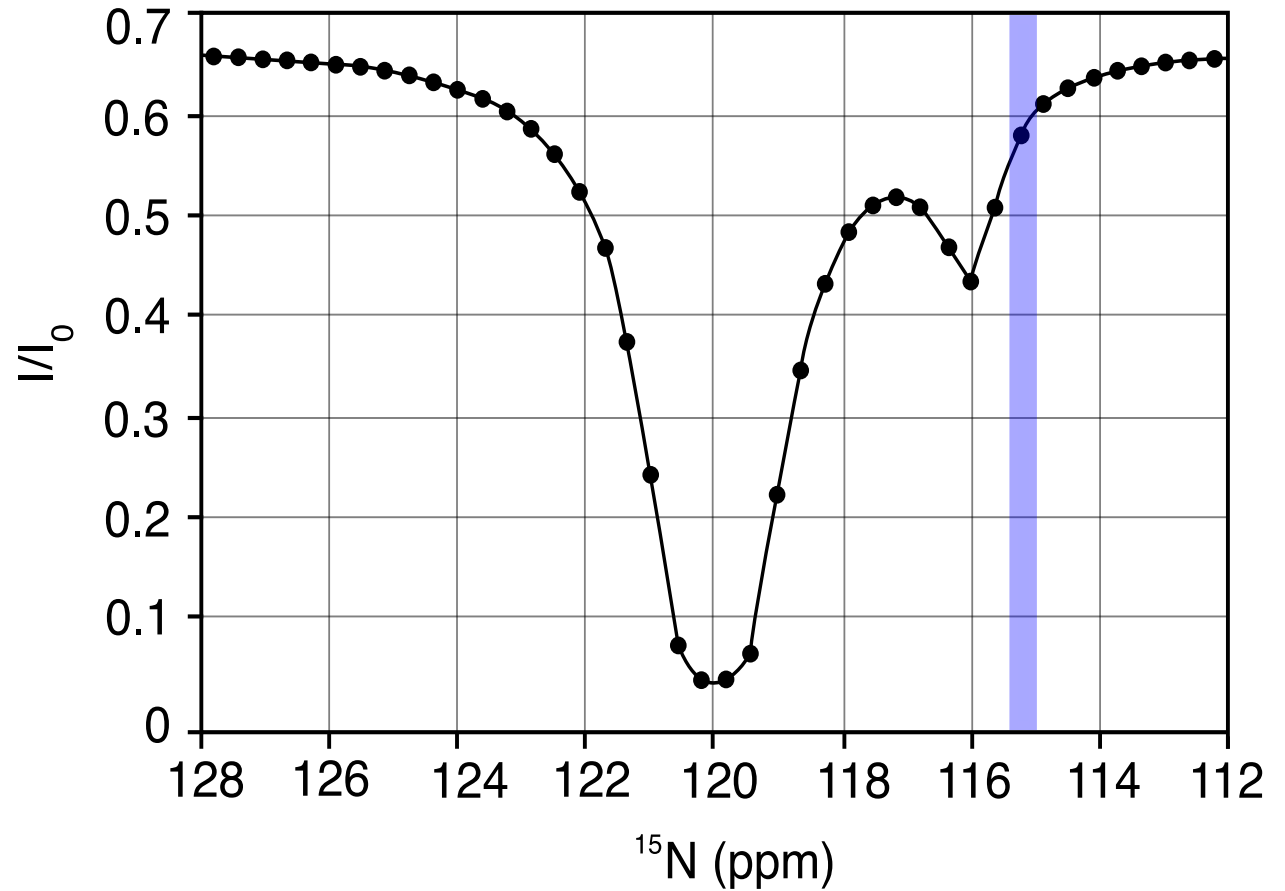
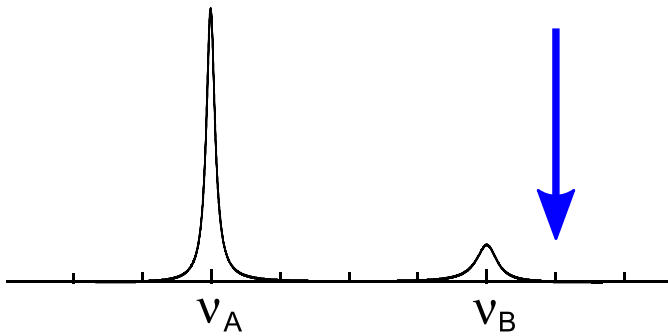
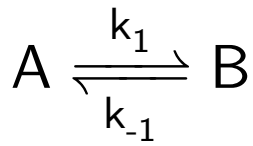
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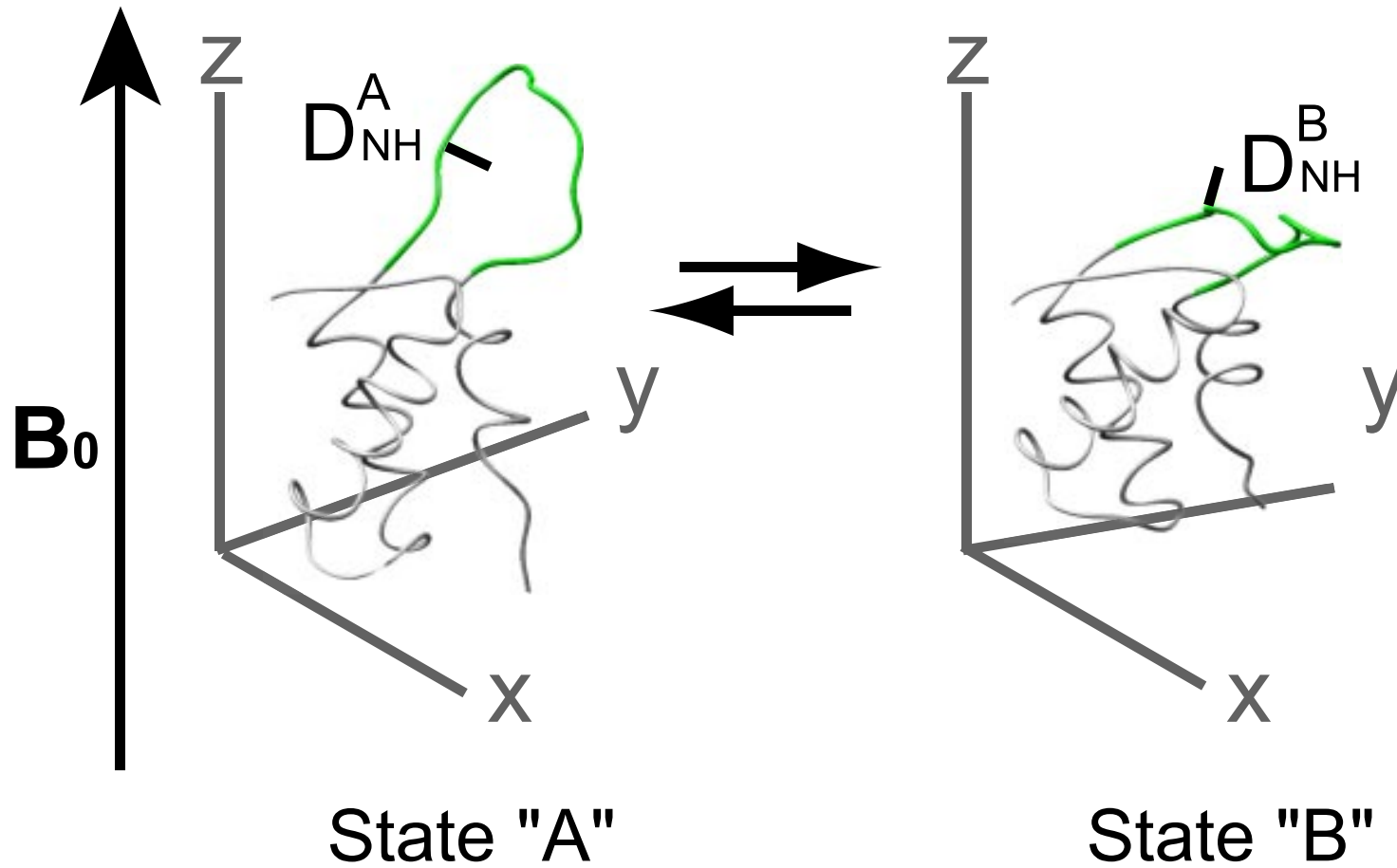
Chemical Exchange Saturation Transfer



Chemical Exchange Saturation Transfer



Residual Dipolar Coupling



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RNA polymerase

DNA

Transcription

RNA polymerase



RNA

Translation

Ribosom



PROTEIN

- Gram positive bacteria

- *Bacillus subtilis*

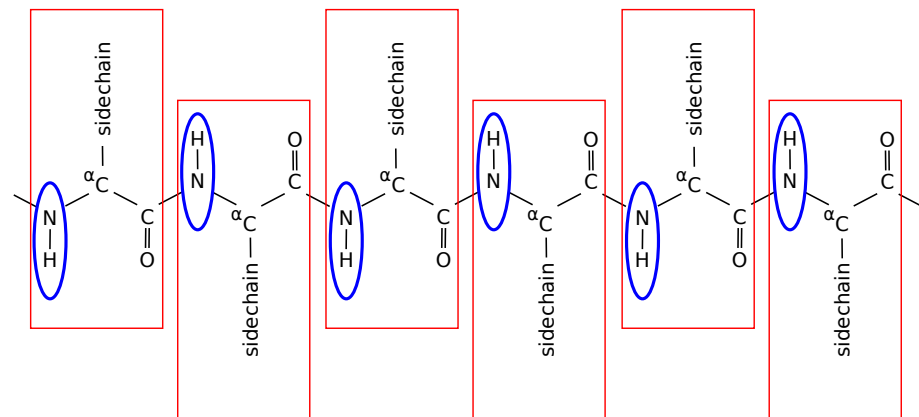
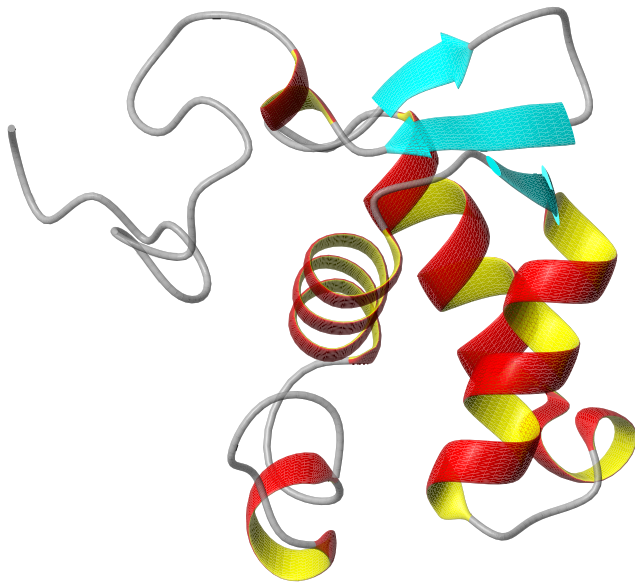
- RNA polymerase core:

α α β β' ω δ ϵ

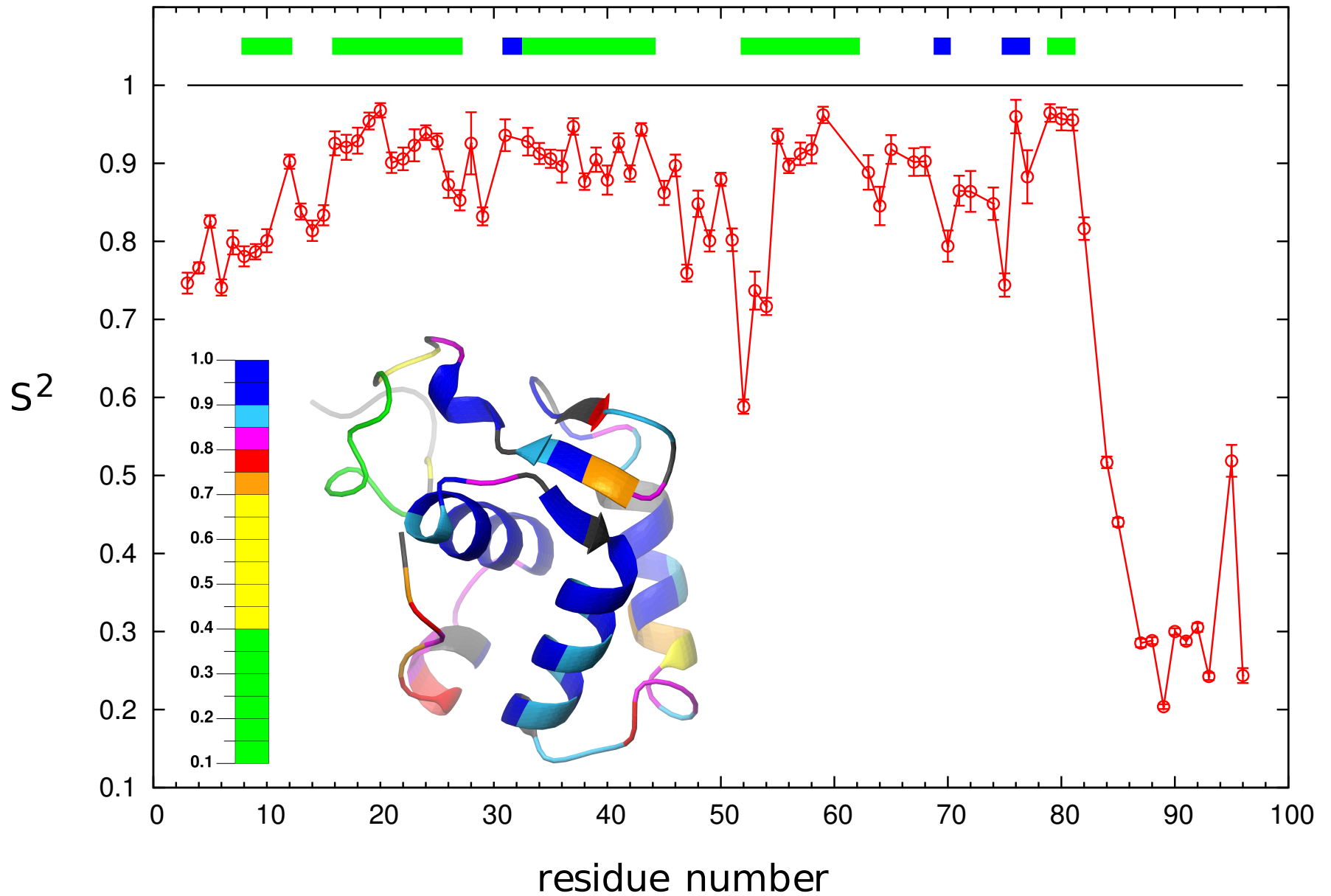
δ subunit

- important for virulency
- 2 domain protein:
 - N-terminal domain: structured
 - C-terminal domain: disordered

MGIKQYSQEE LKEMALVEIA HELFEEHKKP VPFQELLNEI
ASLLGVKKEE LGDRIAQFYT DLNIDGRFLA LSDQTWGLRS
WYPYDQLDEE TQPTVKAKKK KAKKAVEEDL DLDEFEEIDE
DDLDEVEE ELDLEADDFD EEDLDEDDDD LEIEEDIIDE
DDEDYDDEEE EIK



Dynamics (ps-ns)



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Intrinsically disordered protein (IDP)

- intrinsically disordered protein (IDP)
 - lack of stable 3D structure
 - high flexibility
 - structural adaptability
 - polyfunctionality

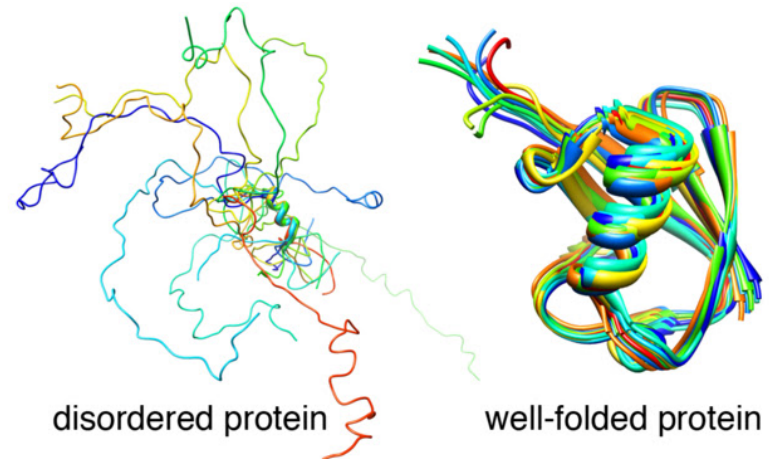


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softsimu.blogspot.fr/2013/preformed-structural-elements-in-long.html

Intrinsically disordered protein (IDP) - challenges

Free energy landscape

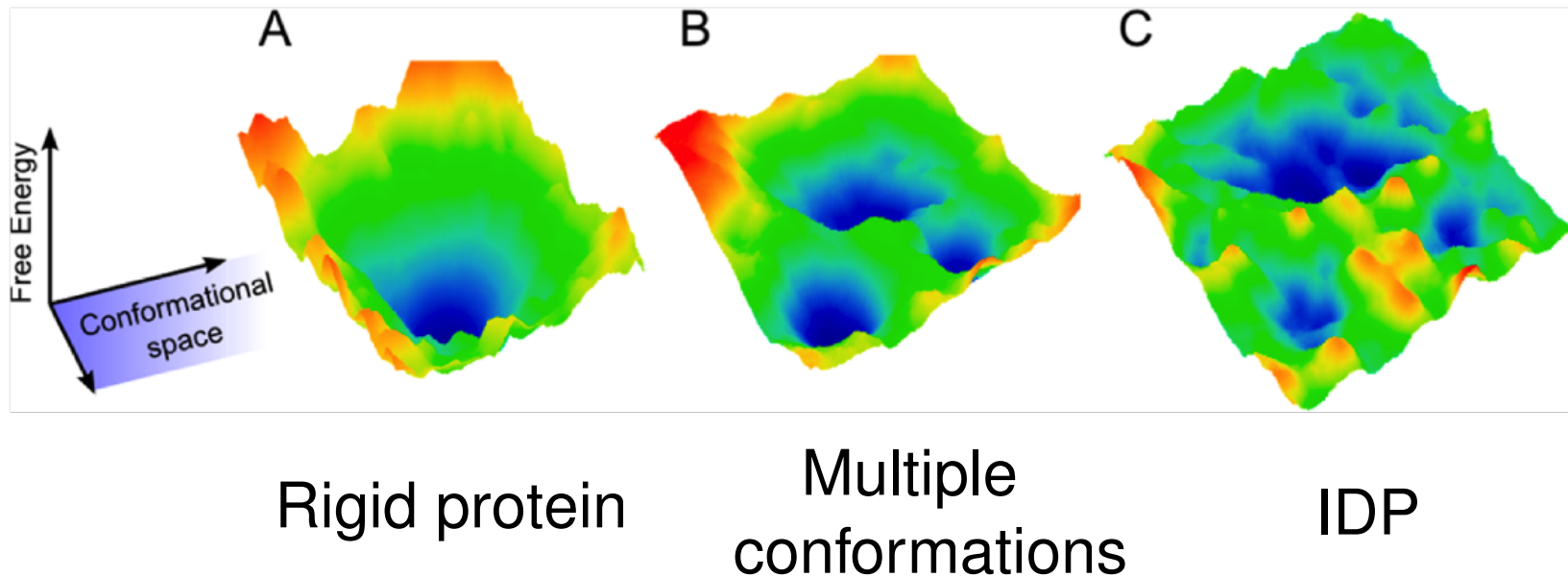
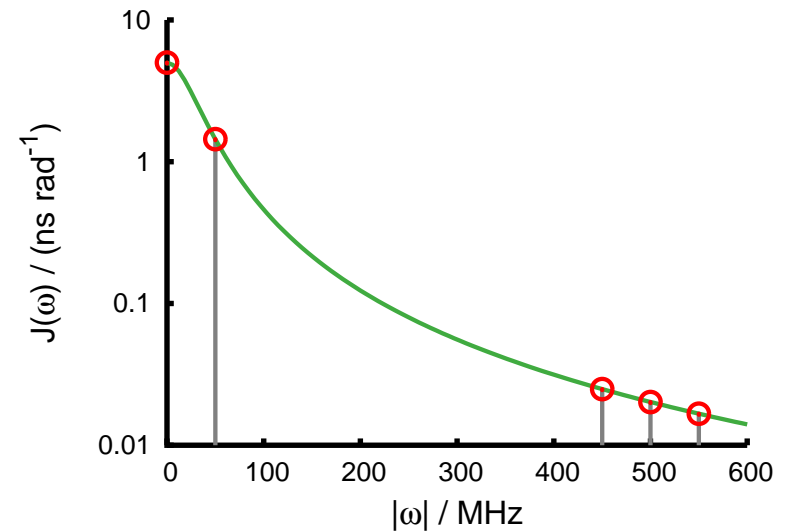
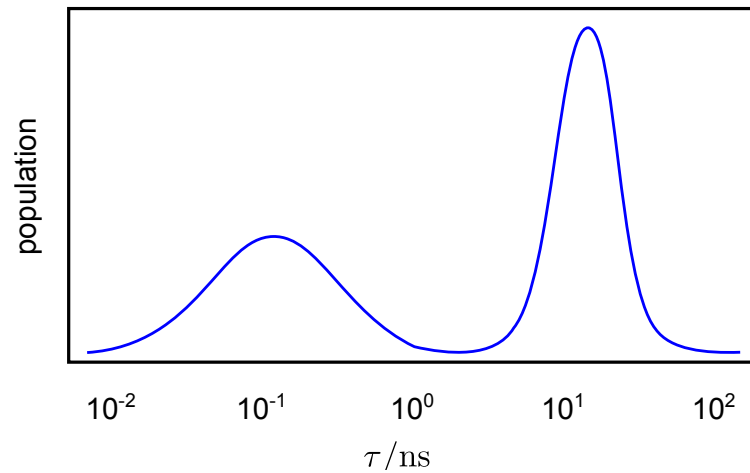


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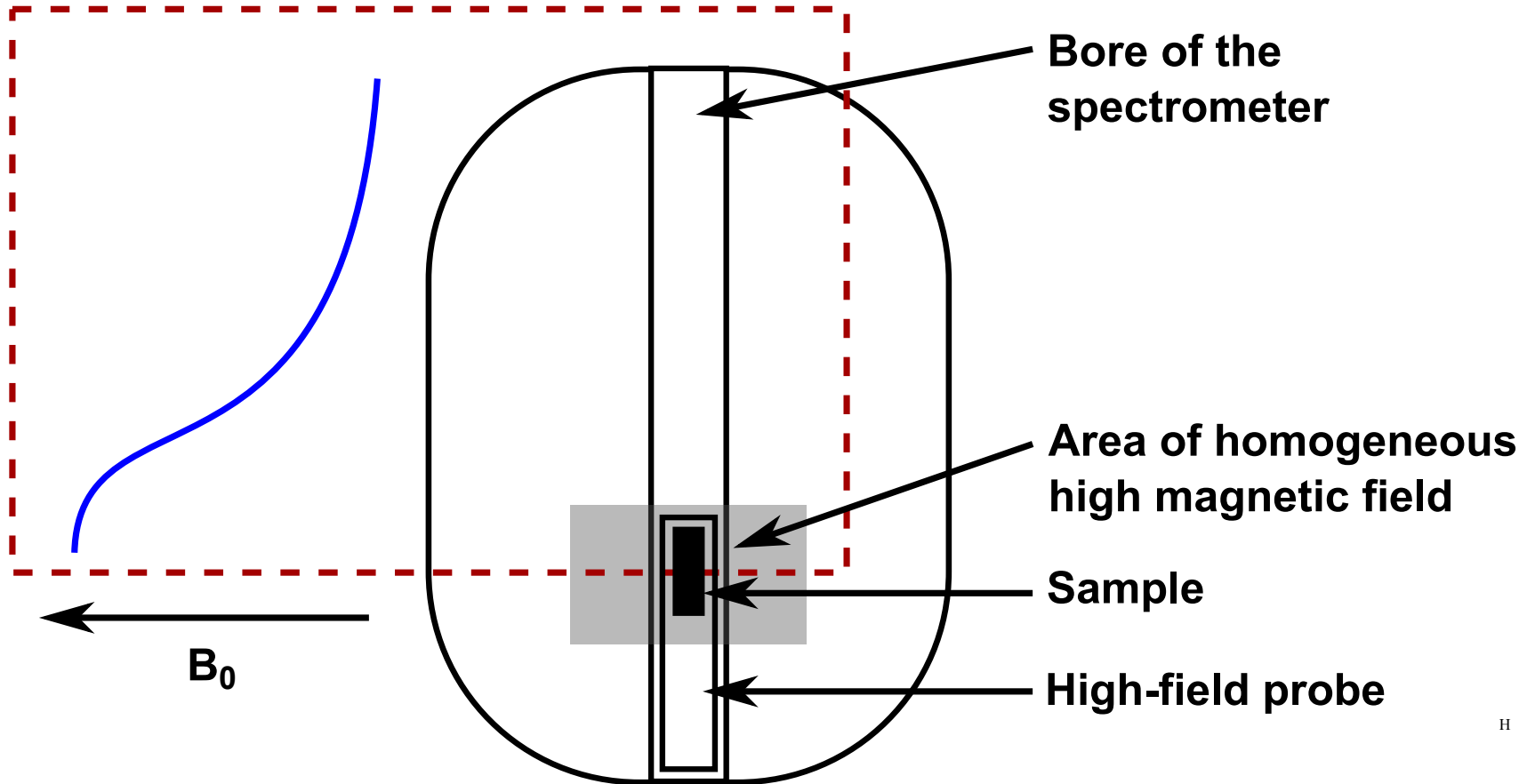
M. Bruscale, B. Schuler, B. Samori, *Chem. Rev.*, **2014**, vol. 114, 3281

Intrinsically disordered protein (IDP) - challenges

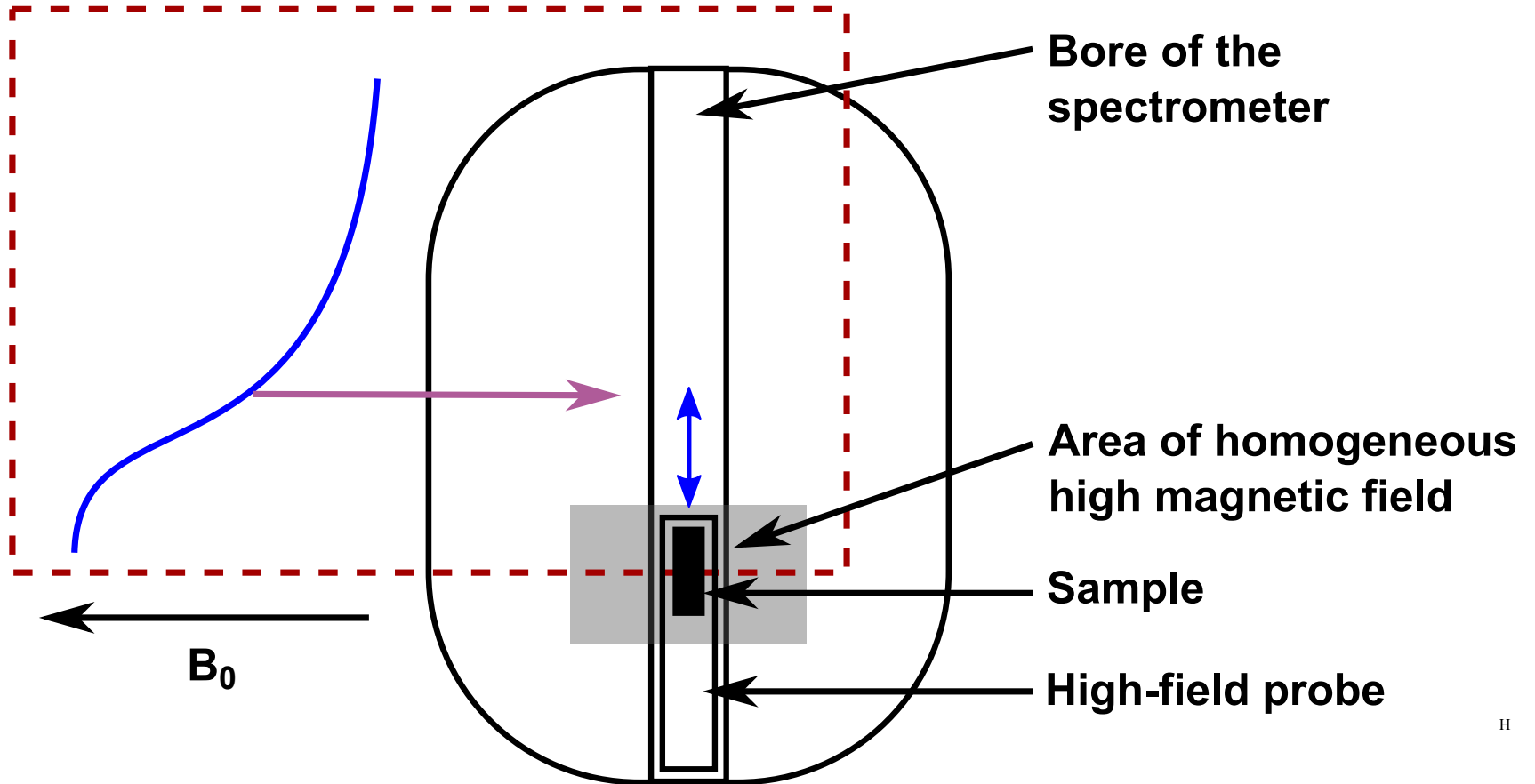
- complex motion - distribution of timescales
- NMR relaxation at different magnetic fields - sensitive to various frequencies
- low frequencies \Rightarrow low $B_0 =$ low resolution



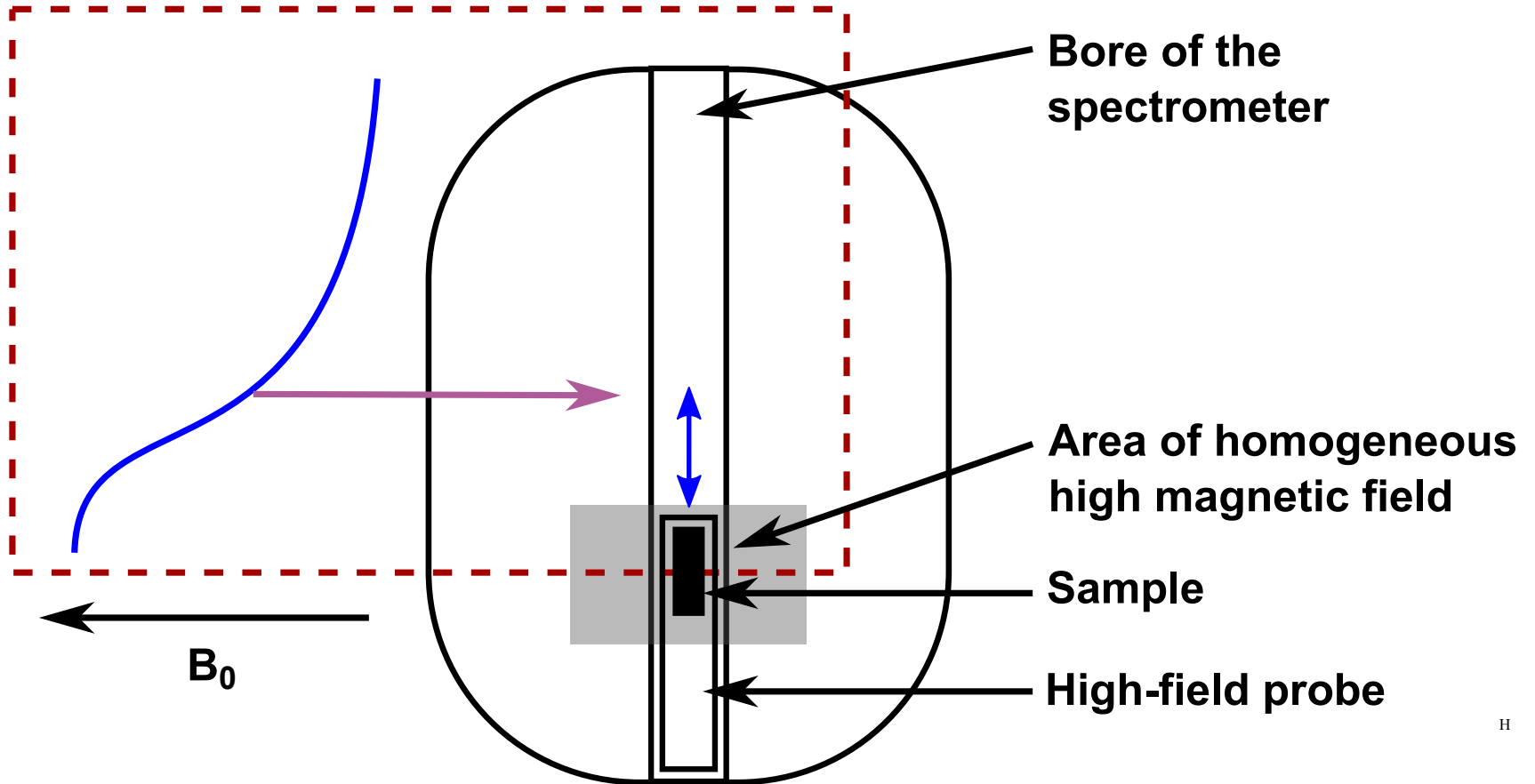
High-resolution relaxometry



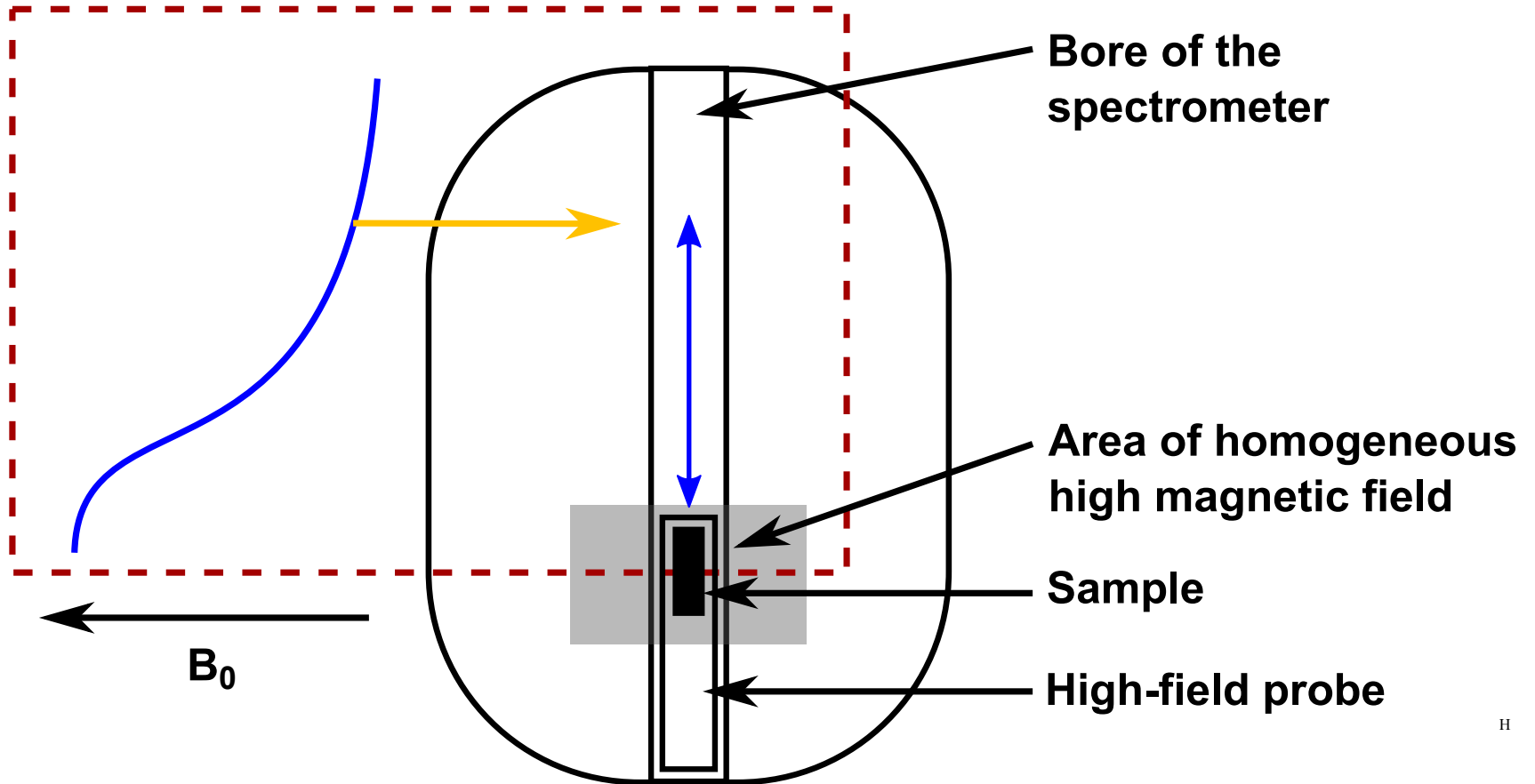
High-resolution relaxometry



High-resolution relaxometry

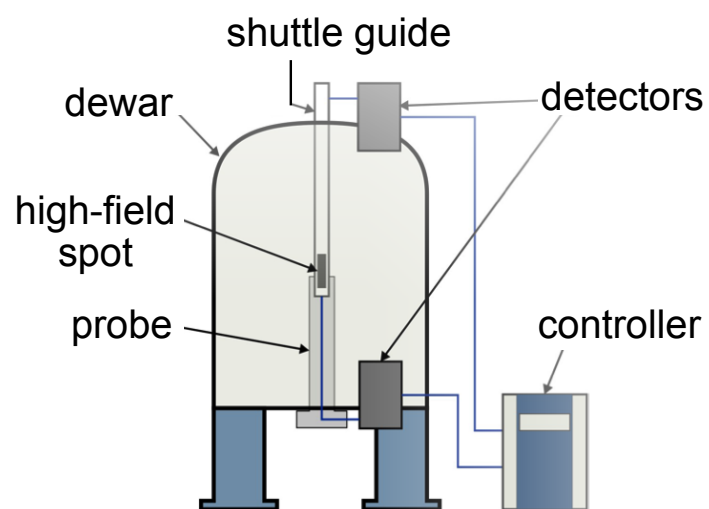


High-resolution relaxometry



High-resolution relaxometry - device

NMR spectrometer



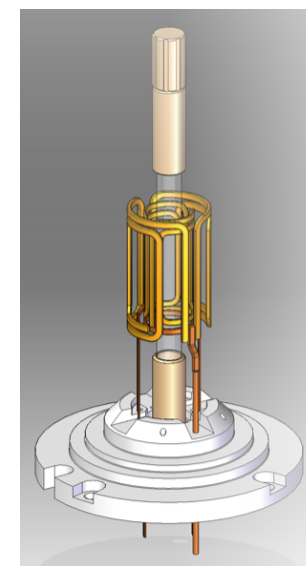
Sample shuttle tube

bubble trap

active volume
~ 0.12 ml



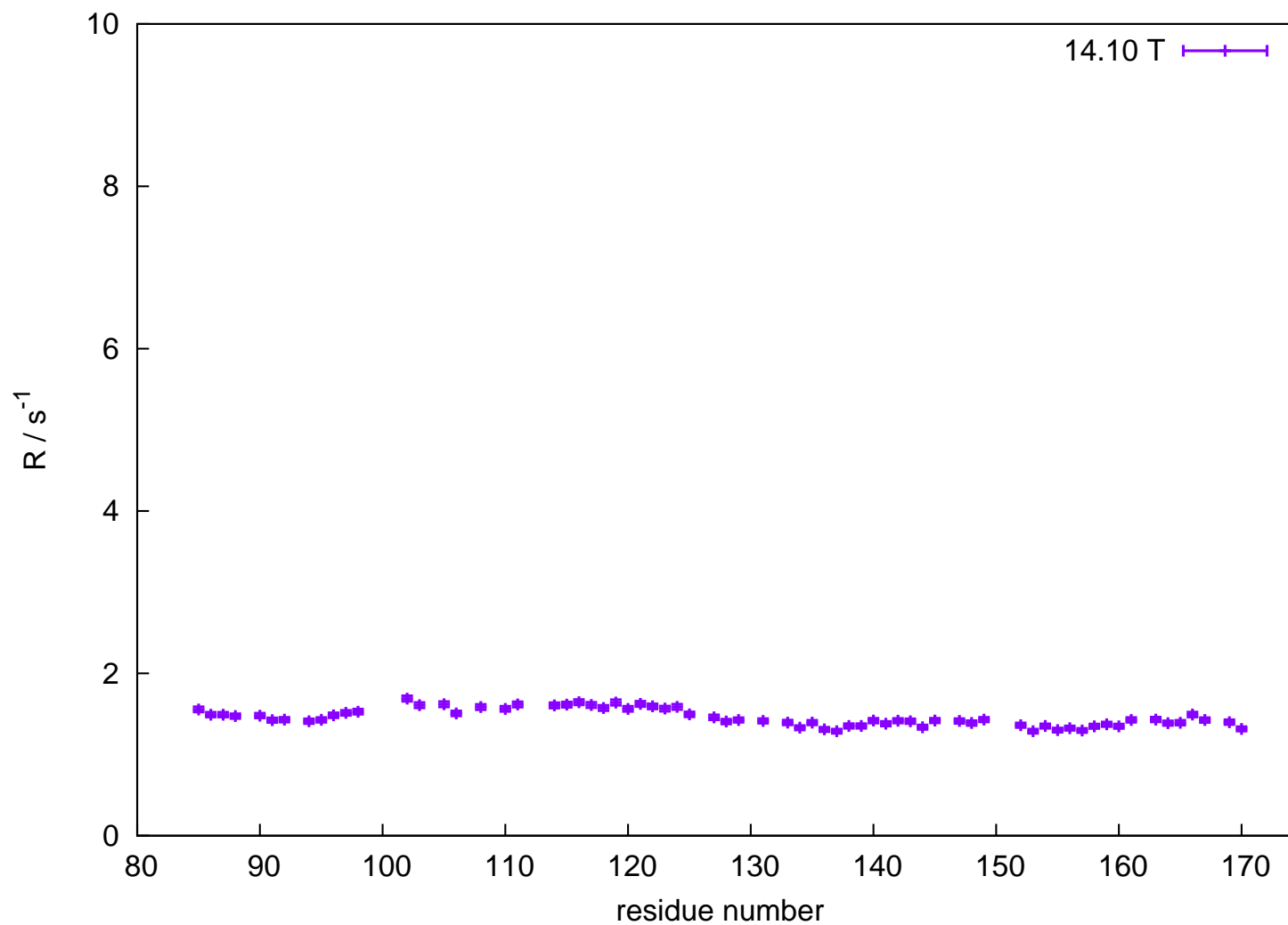
Triple resonance probe



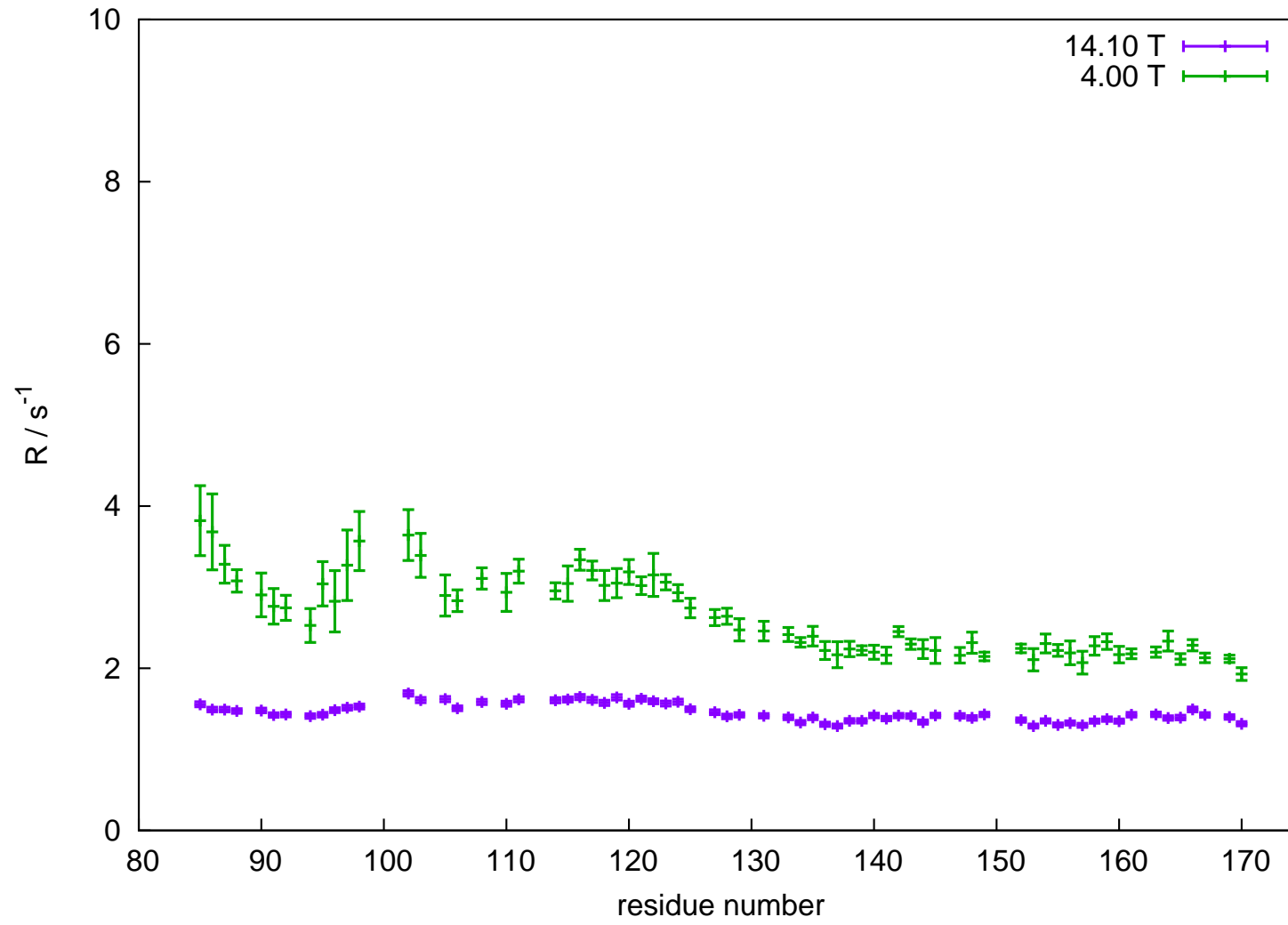
pneumatic shuttling: 0.5 m in ≈ 120 ms

Charlier C. et al., *J. Am. Chem. Soc.*, 2013, 135 (49), 18665–18672

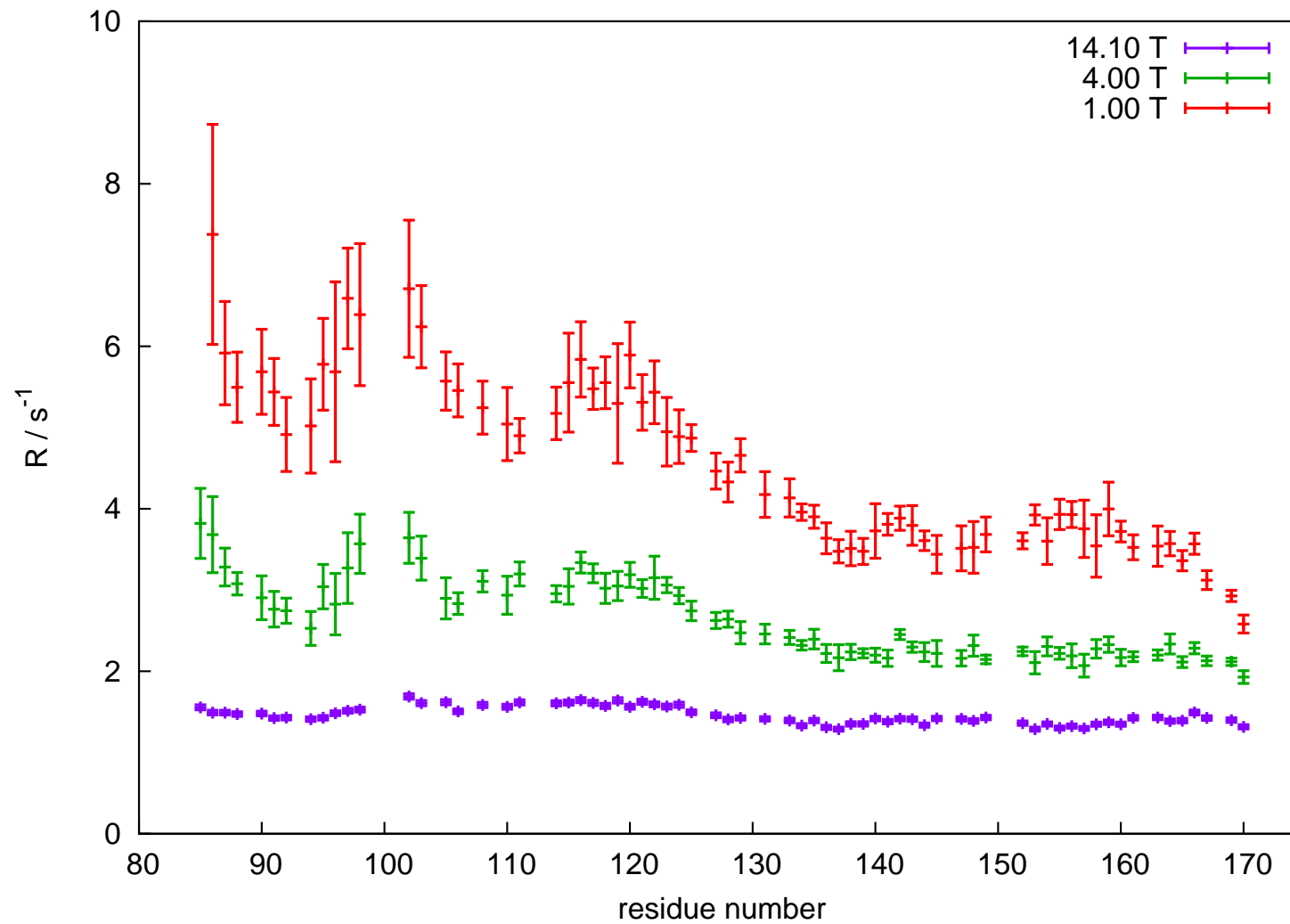
delta subunit: longitudinal relaxation rate



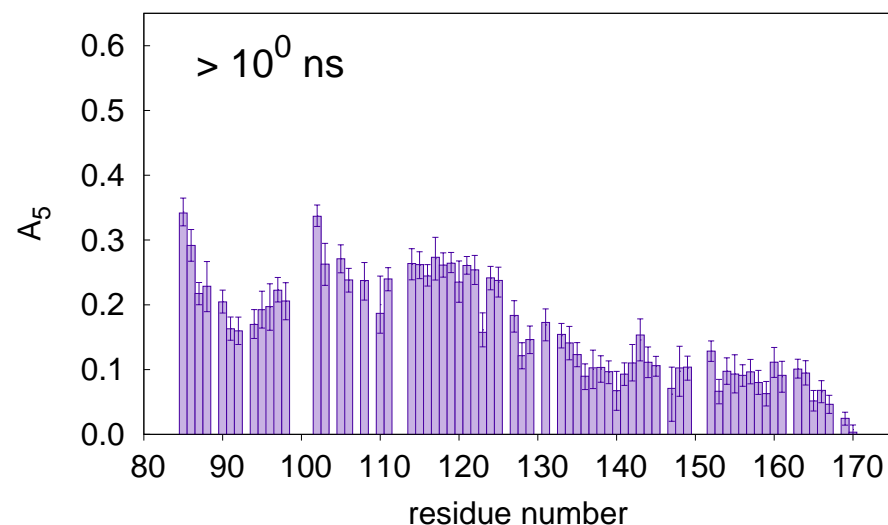
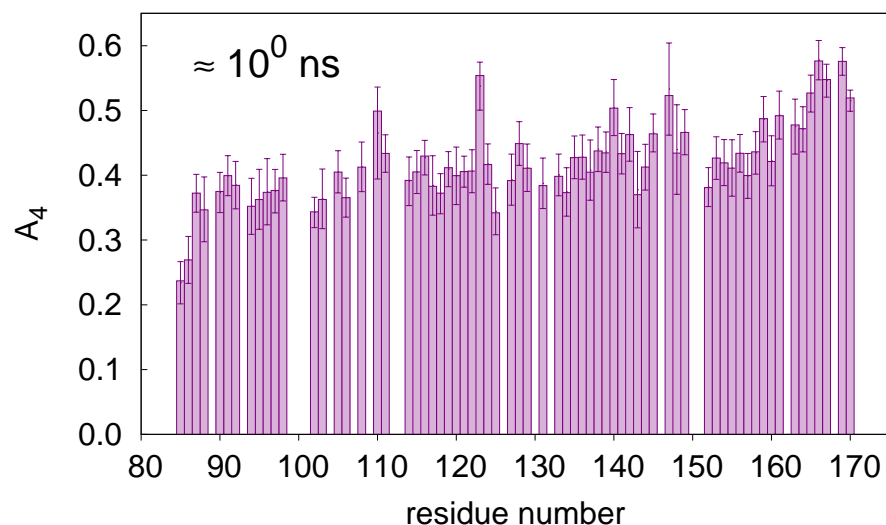
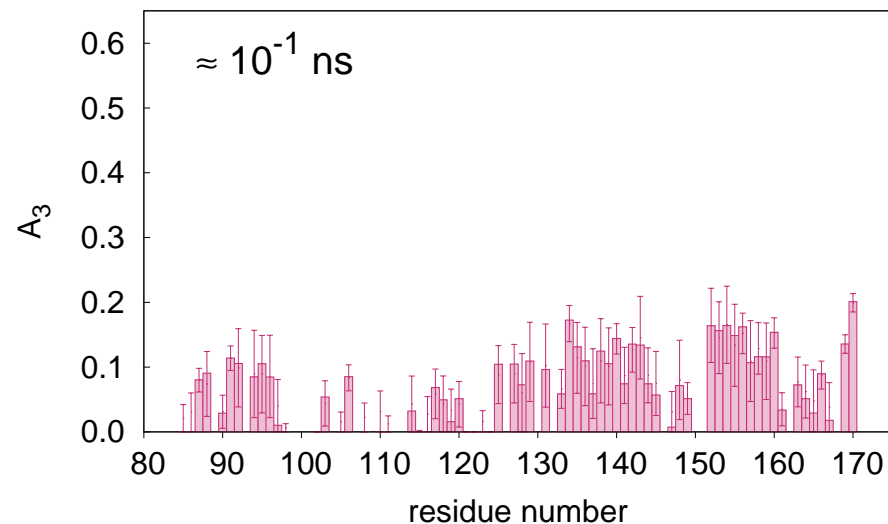
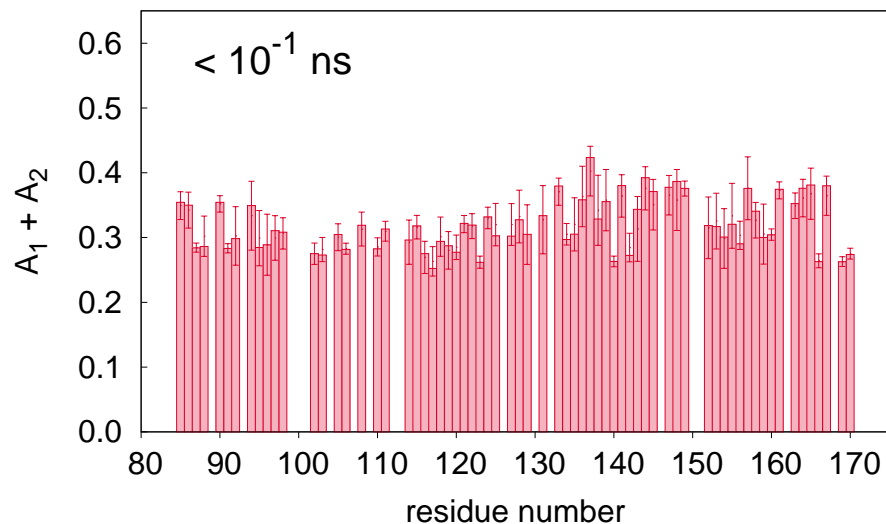
delta subunit: relaxometry relaxation rate



delta subunit: relaxometry relaxation rate



delta subunit: distribution of motions

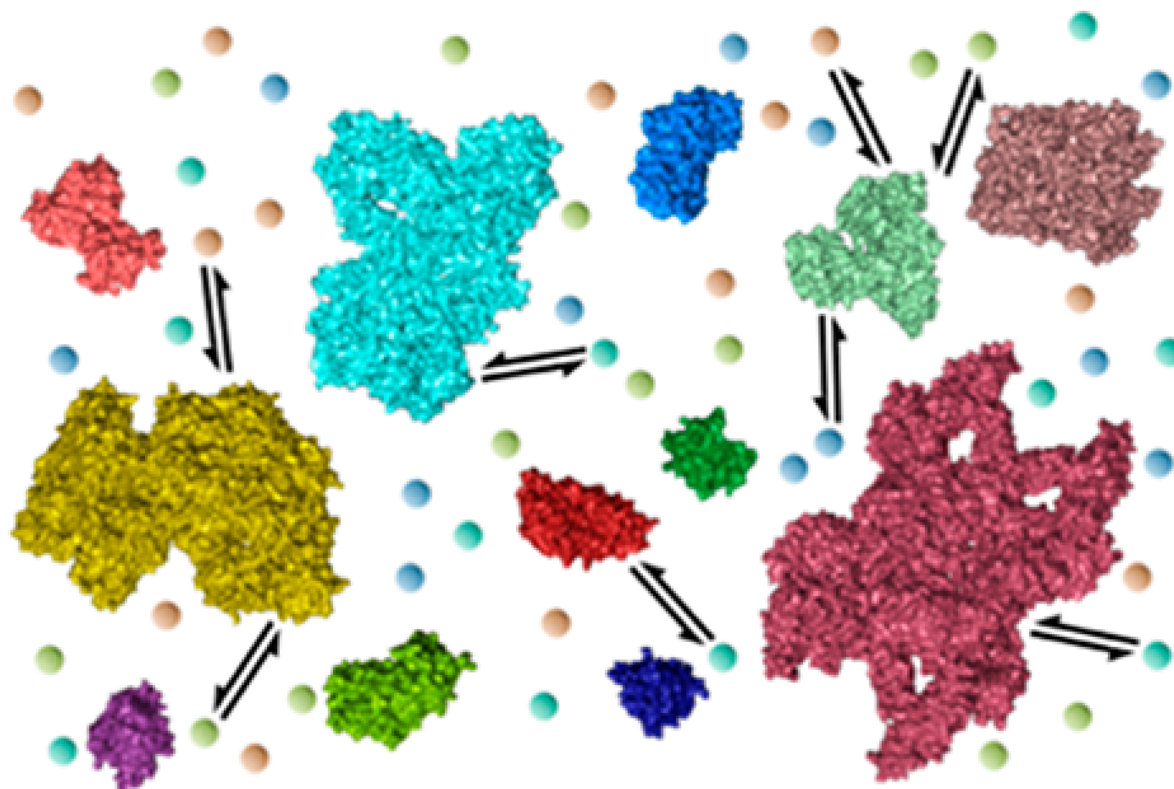


81 . . . DQLDEE TQPTVKAKKK KAKKAVEEDL DLDEFEEIDE DDLDLDEVVEE
 ELDLEADDFD EEDLDEDDDD LEIEEDIIDE DDEDYDDEEE EIK

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2. Studies of interactions
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5. High-resolution relaxometry - investigation of ps-ns dynamics of IDPs
6. **High-resolution relaxometry - metabolomics**
7. Two-field NMR
8. Dissolution dynamics nuclear polarisation (dDNP)

Metabolomics

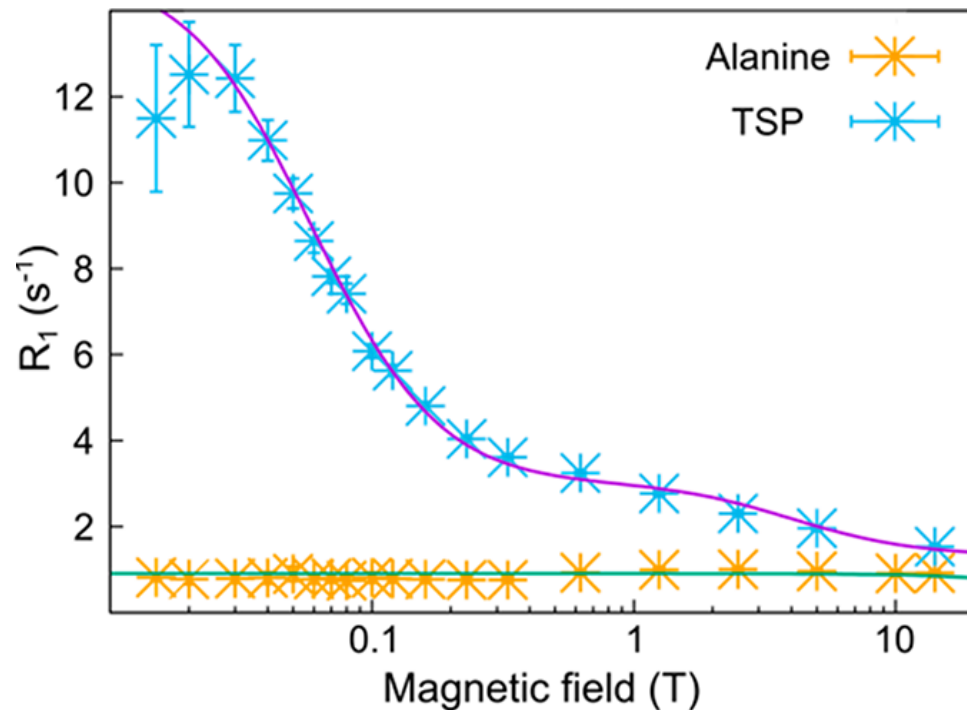
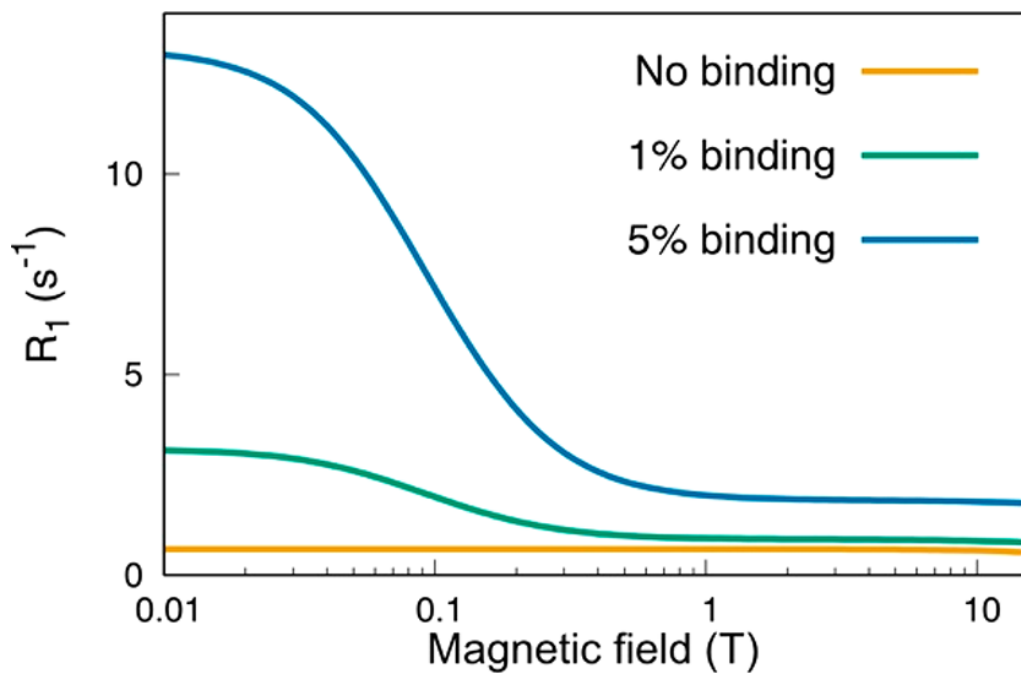
- investigation of metabolites in biological fluids
- substrates and products of enzymatic reactions
- cofactors or regulators



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Metabolomics

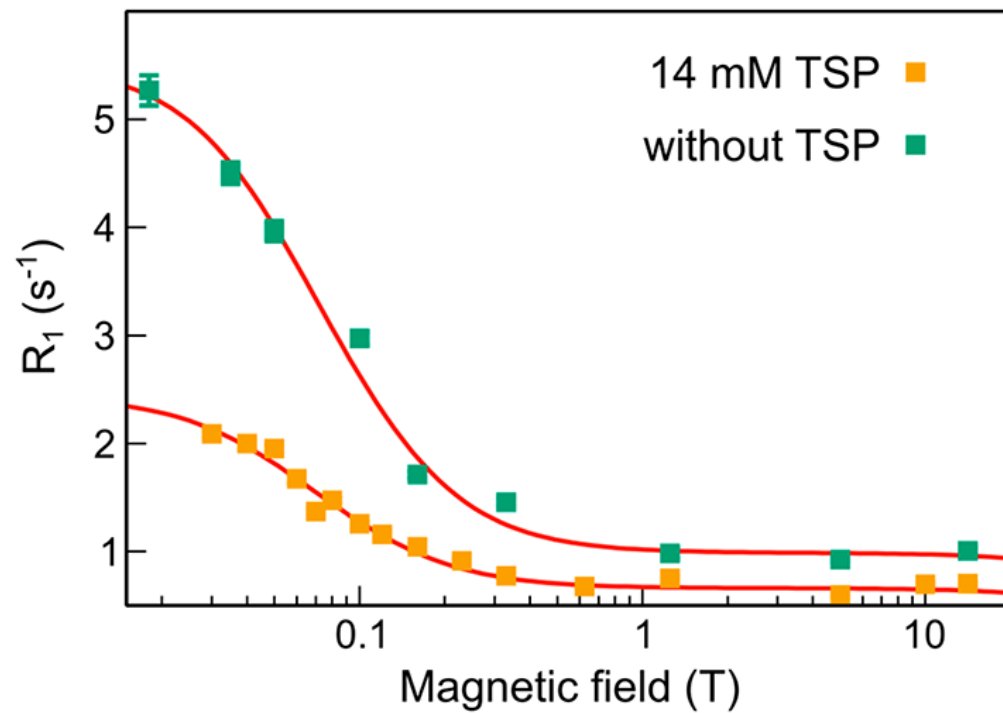
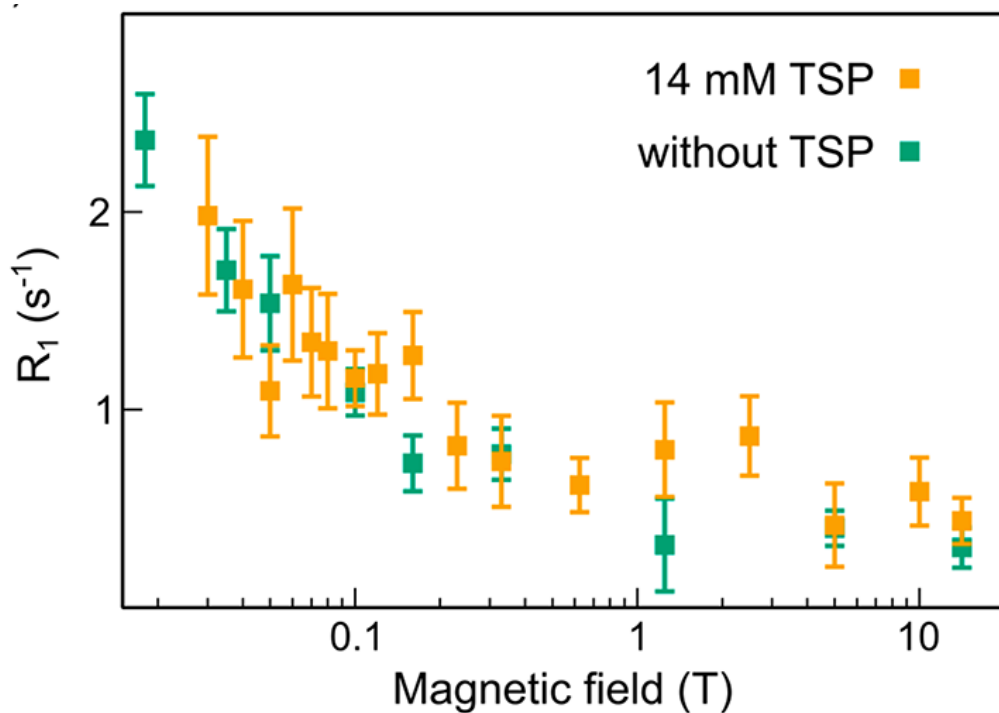
- dependence of relaxation rate on rotational diffusion
- Stokes law \Rightarrow size of the molecule \Rightarrow free \times bound



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Metabolomics

- competition between ligands



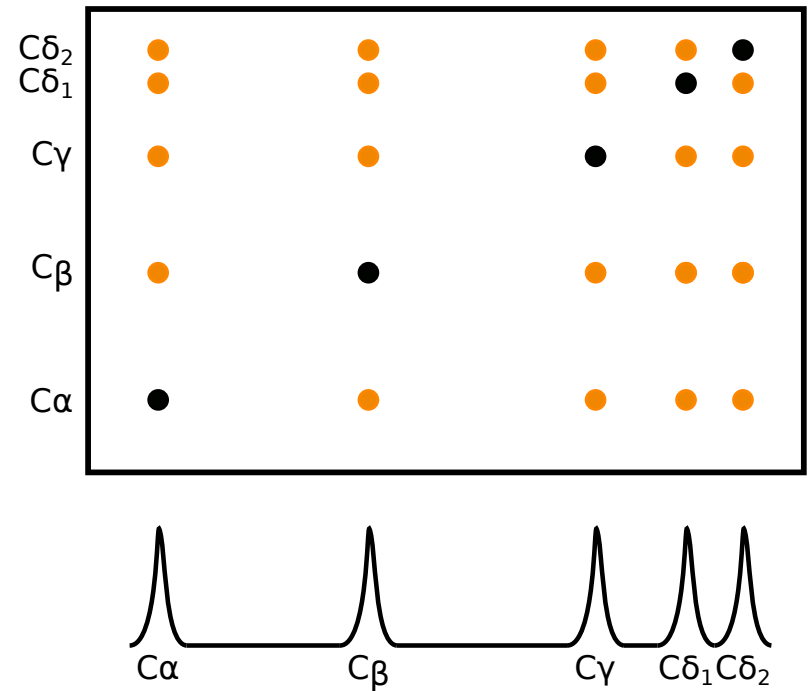
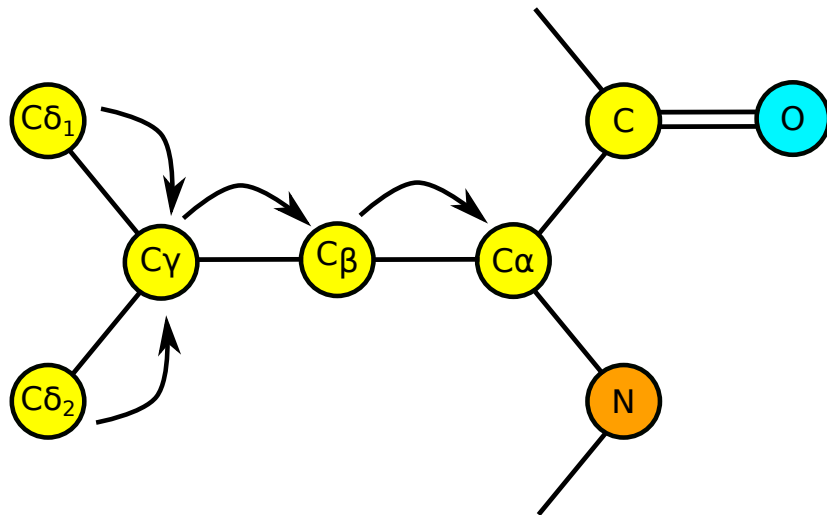
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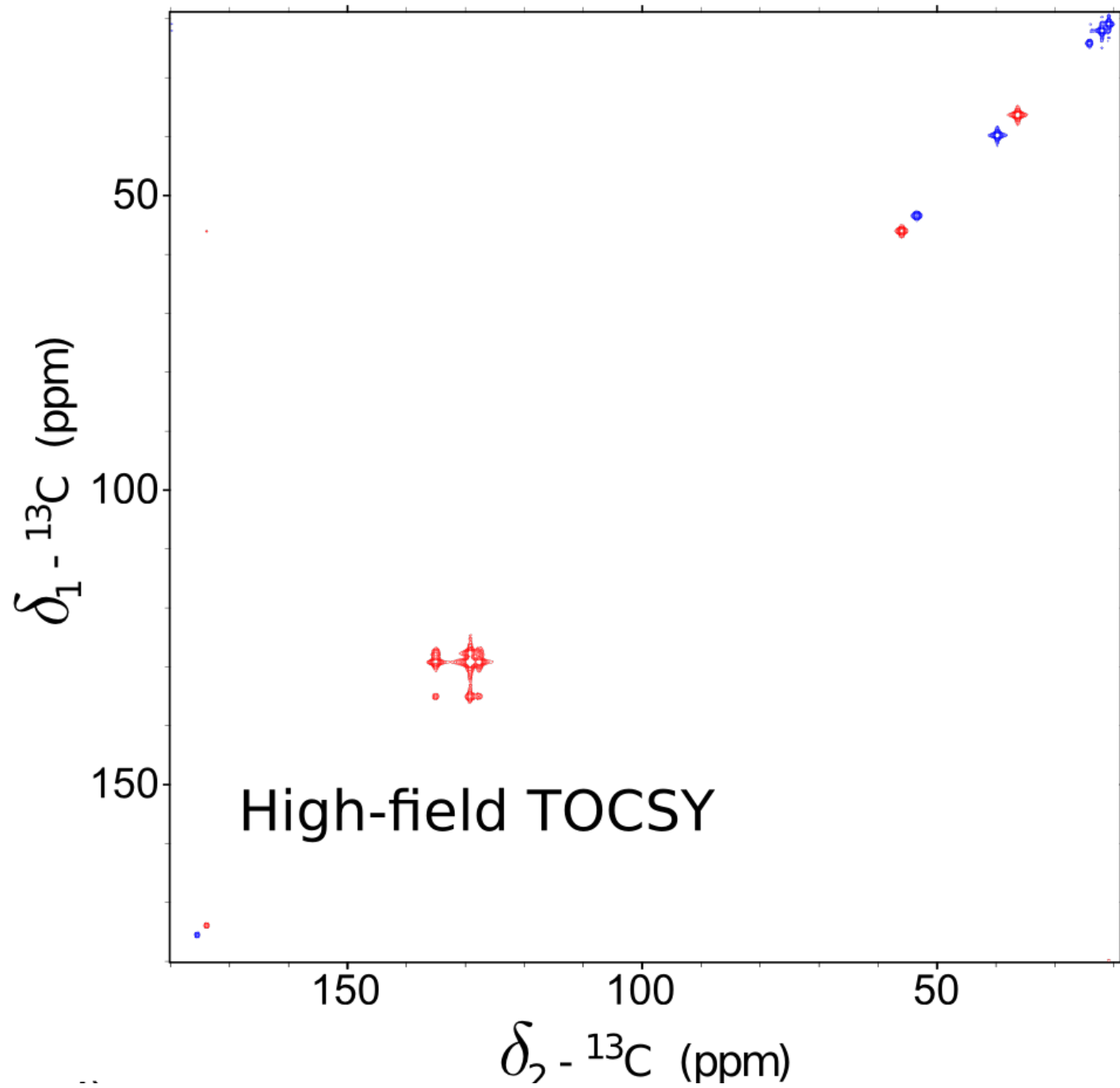
TOCSY

TOCSY = total correlation spectroscopy:

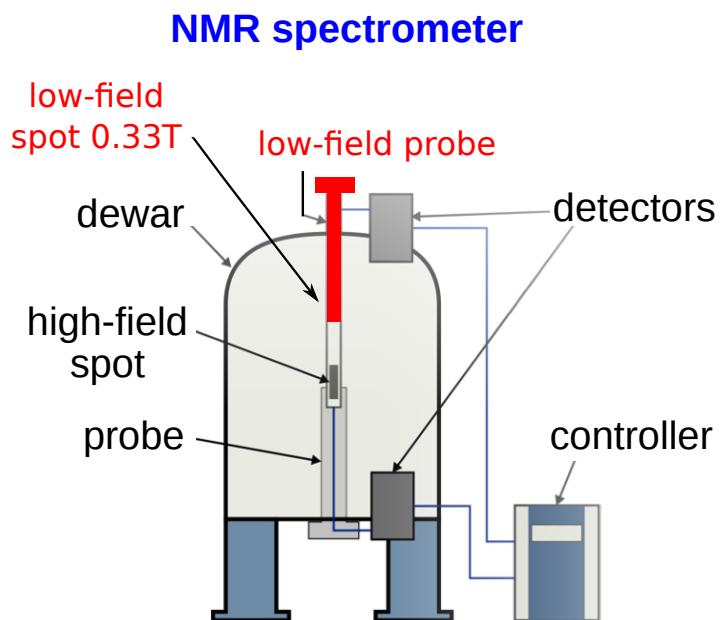
- information about bonds
- requires high irradiation suppressing effects of chemical shift
- stronger magnetic field = stronger irradiation



TOCSY



Two-field NMR spectrometer



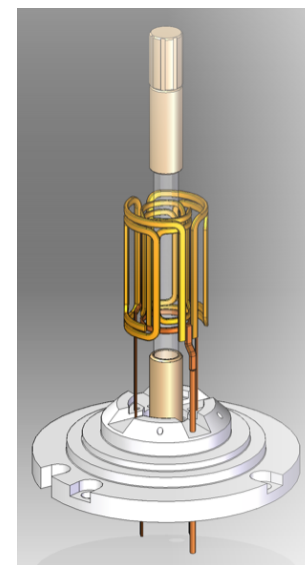
Sample shuttle tube

bubble trap

active volume
~ 0.12 ml



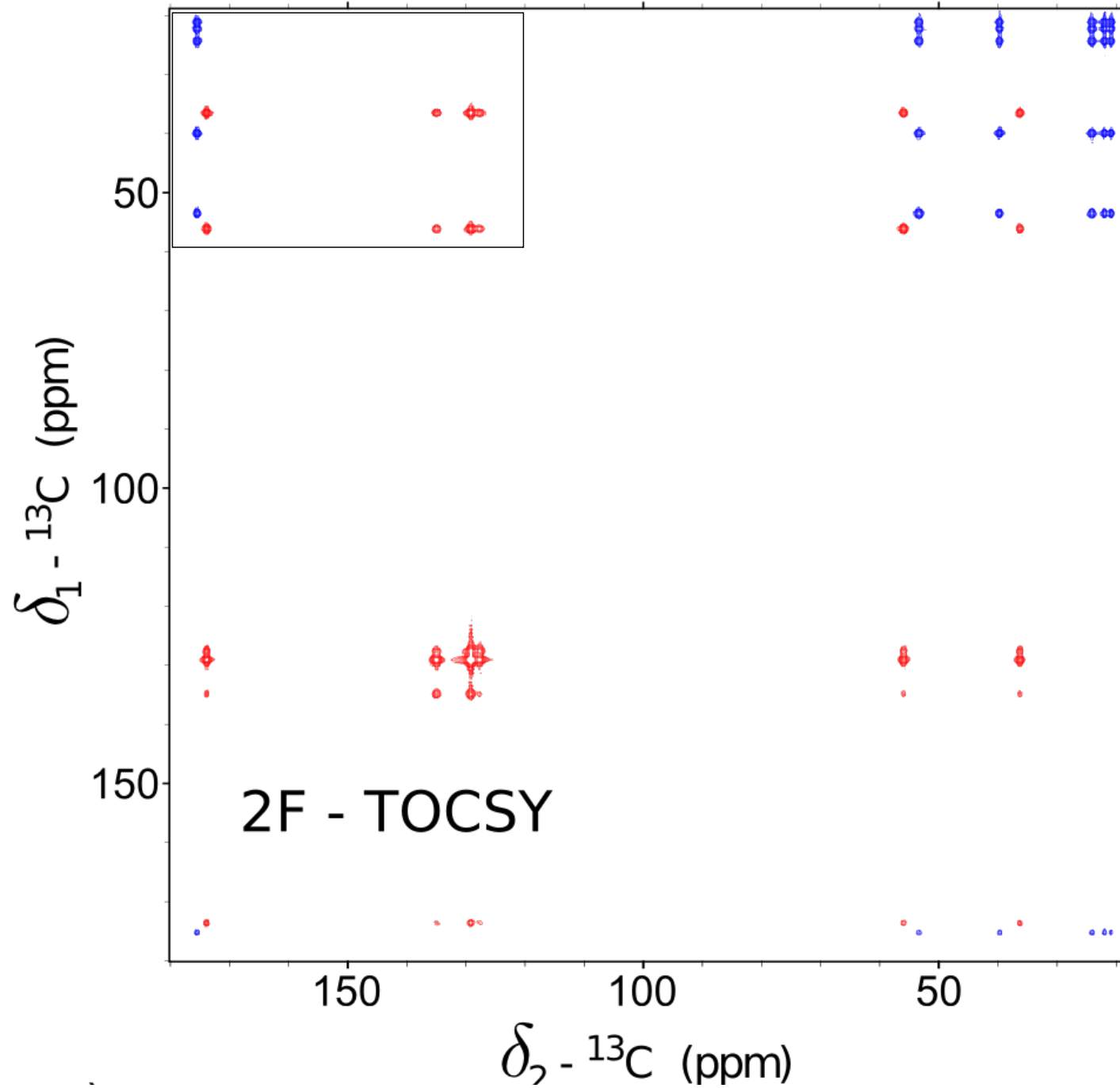
Triple resonance probe



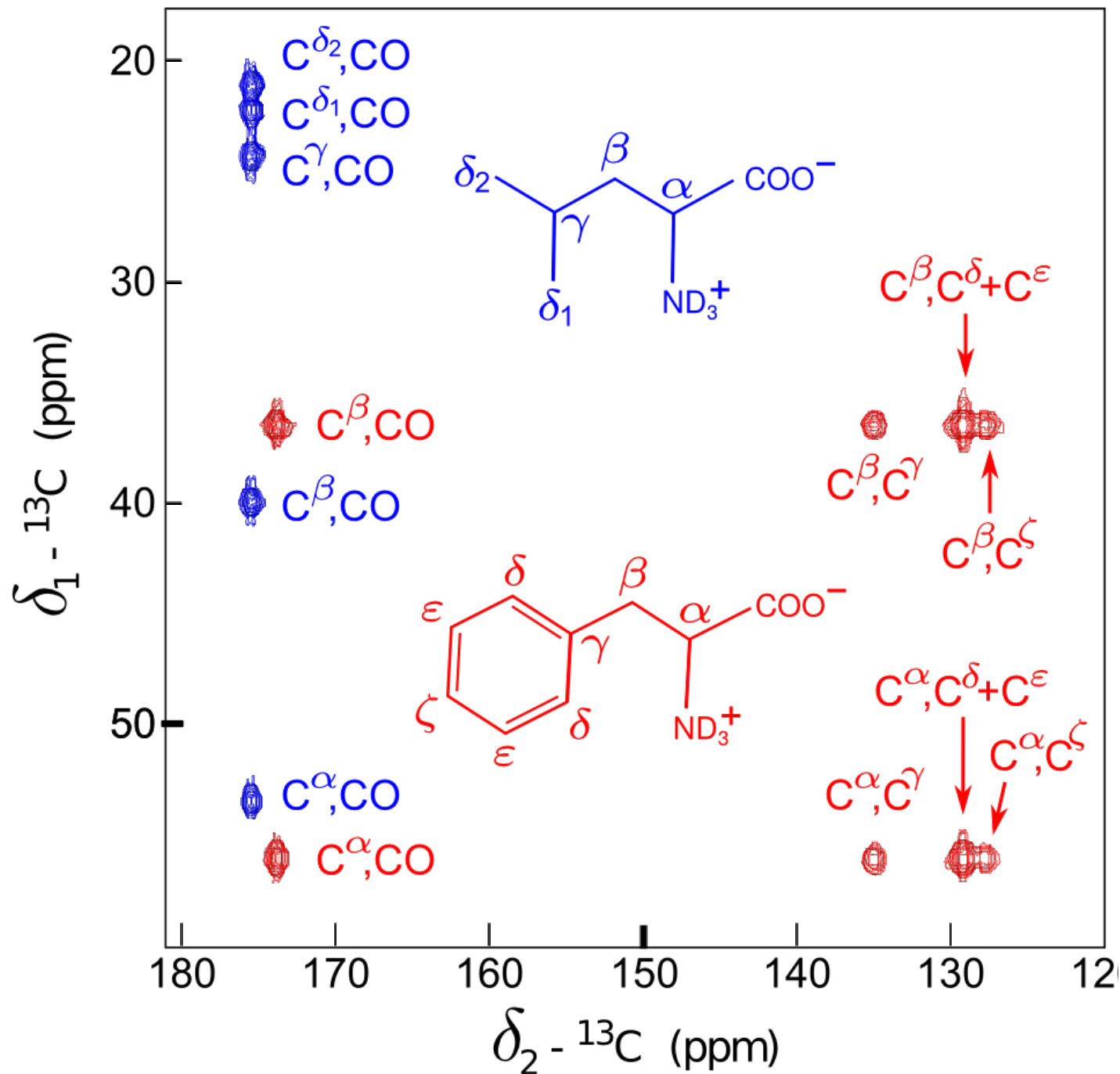
pneumatic shuttling: 0.5 m in ≈ 120 ms

Cousin S.F. et al., *Phys Chem Chem Phys*, 2016, 18 (48), 33187–33194

TOCSY at two-fields

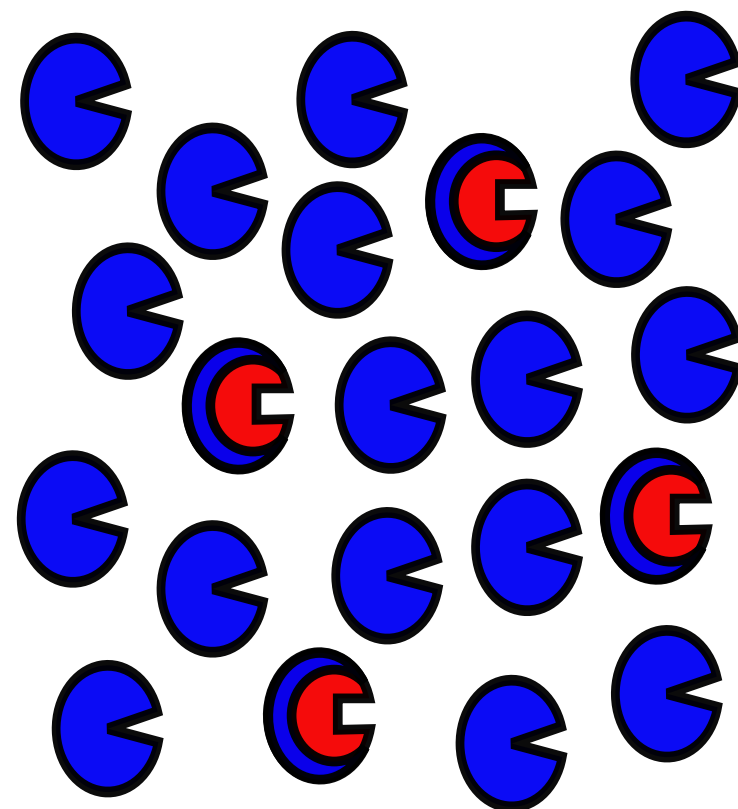
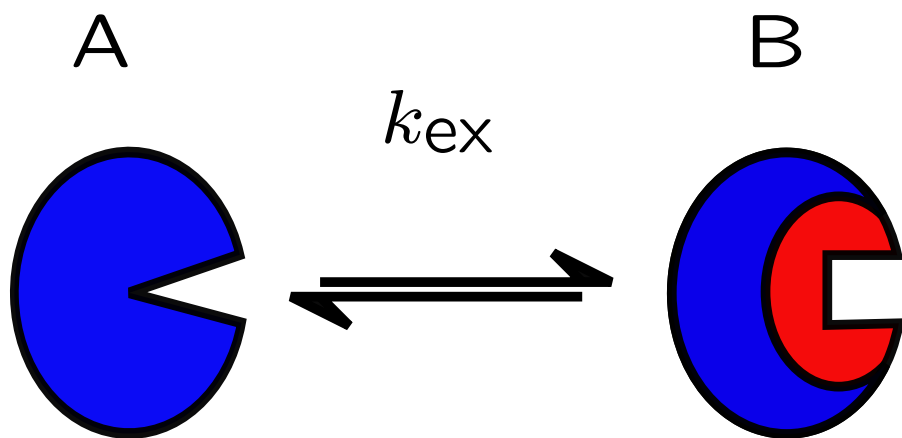


TOCSY at two-fields



μs – ms motions

$$k_{\text{ex}} \approx |\Omega_A - \Omega_B|$$



Ω_A

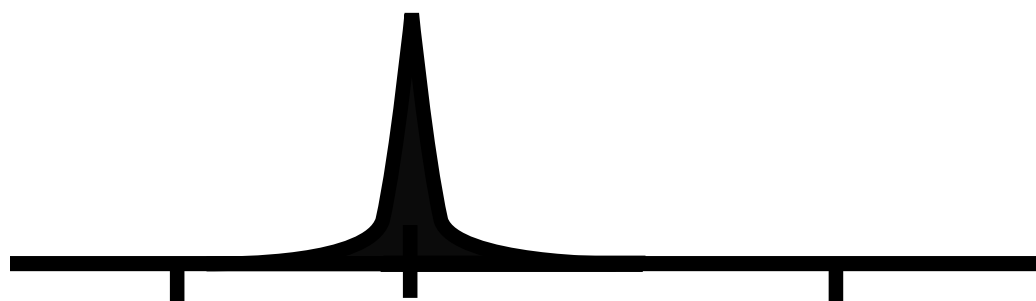
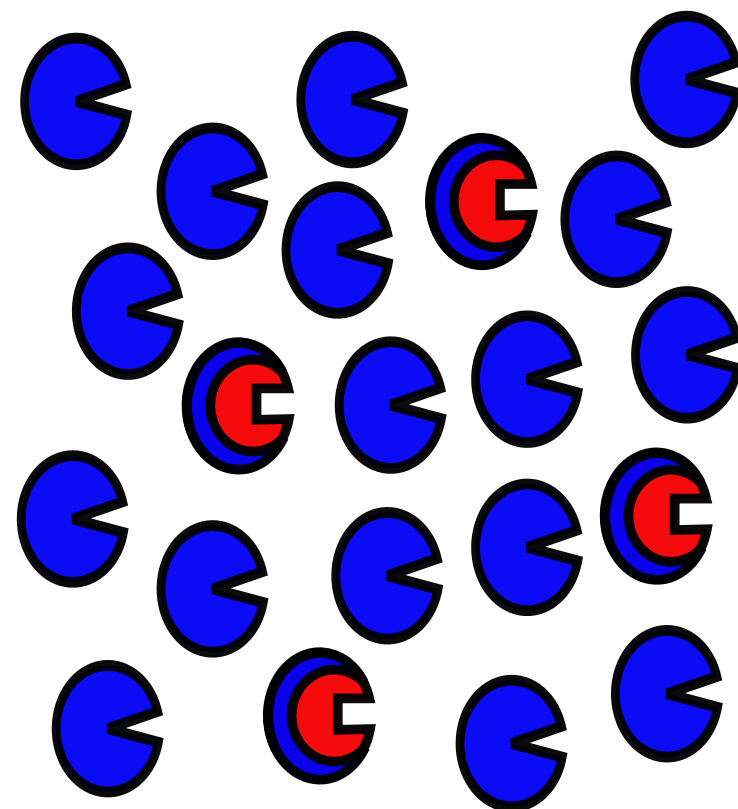
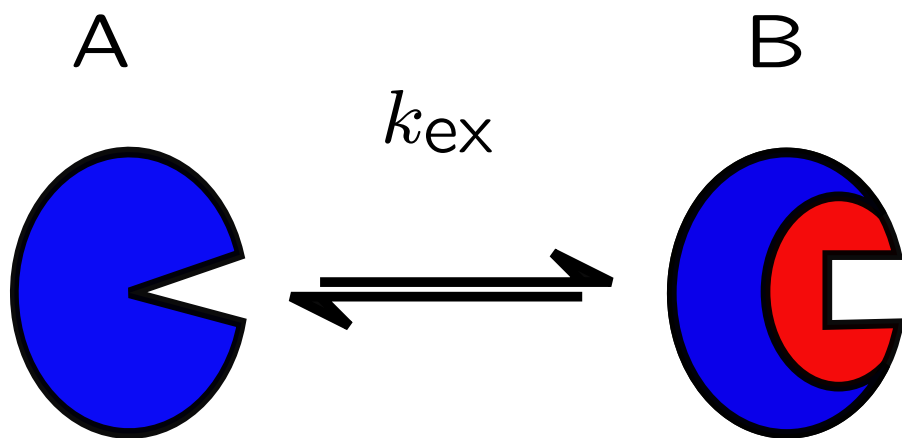
Ω_{obs}

Ω_B

$$p_A, p_B = 1 - p_A$$

μs – ms motions

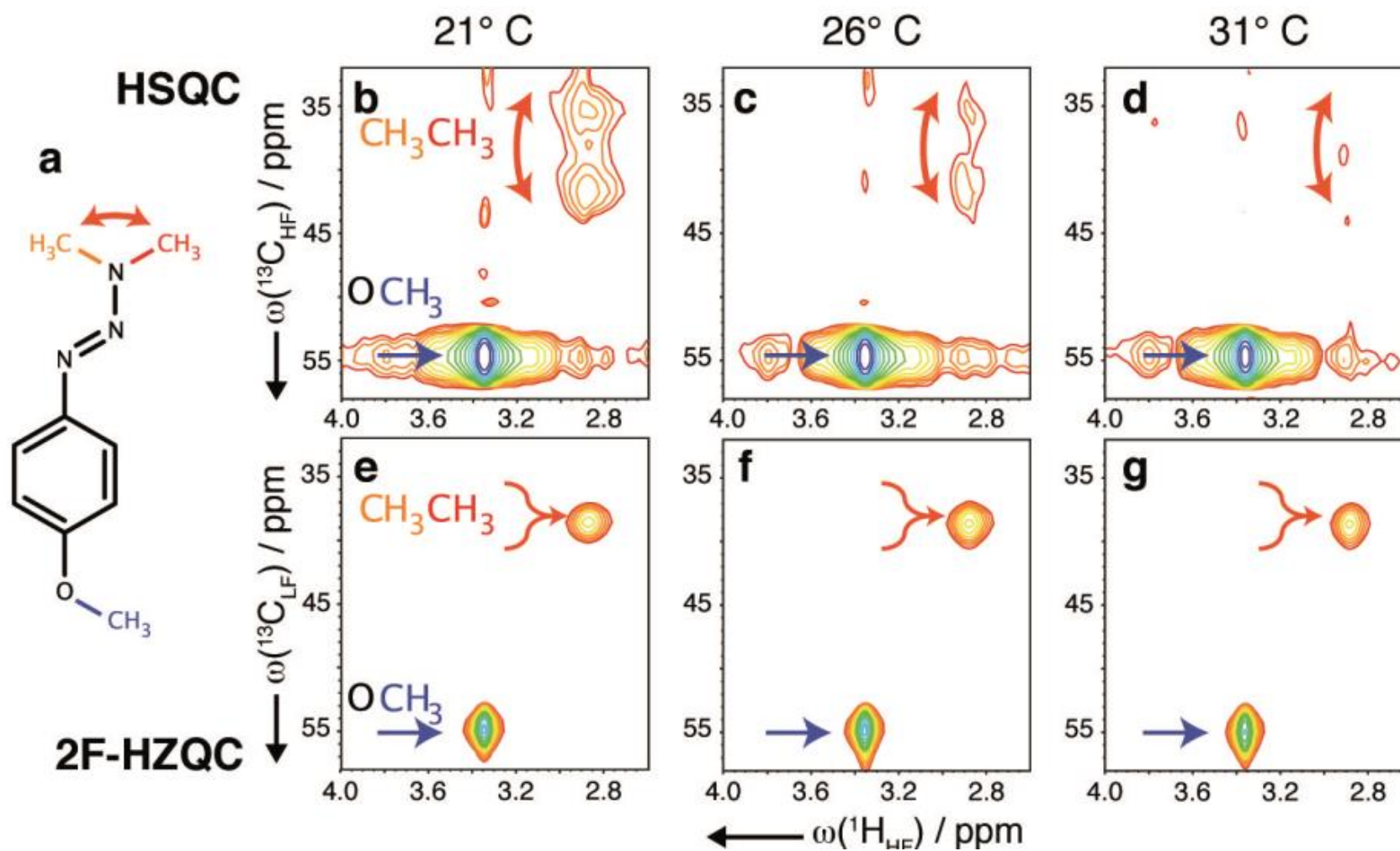
$$k_{\text{ex}} > |\Omega_A - \Omega_B| \Rightarrow R_{\text{ex}}(k_{\text{ex}}, \Omega_A, \Omega_B, p_B)$$



Ω_A Ω_{obs} Ω_B

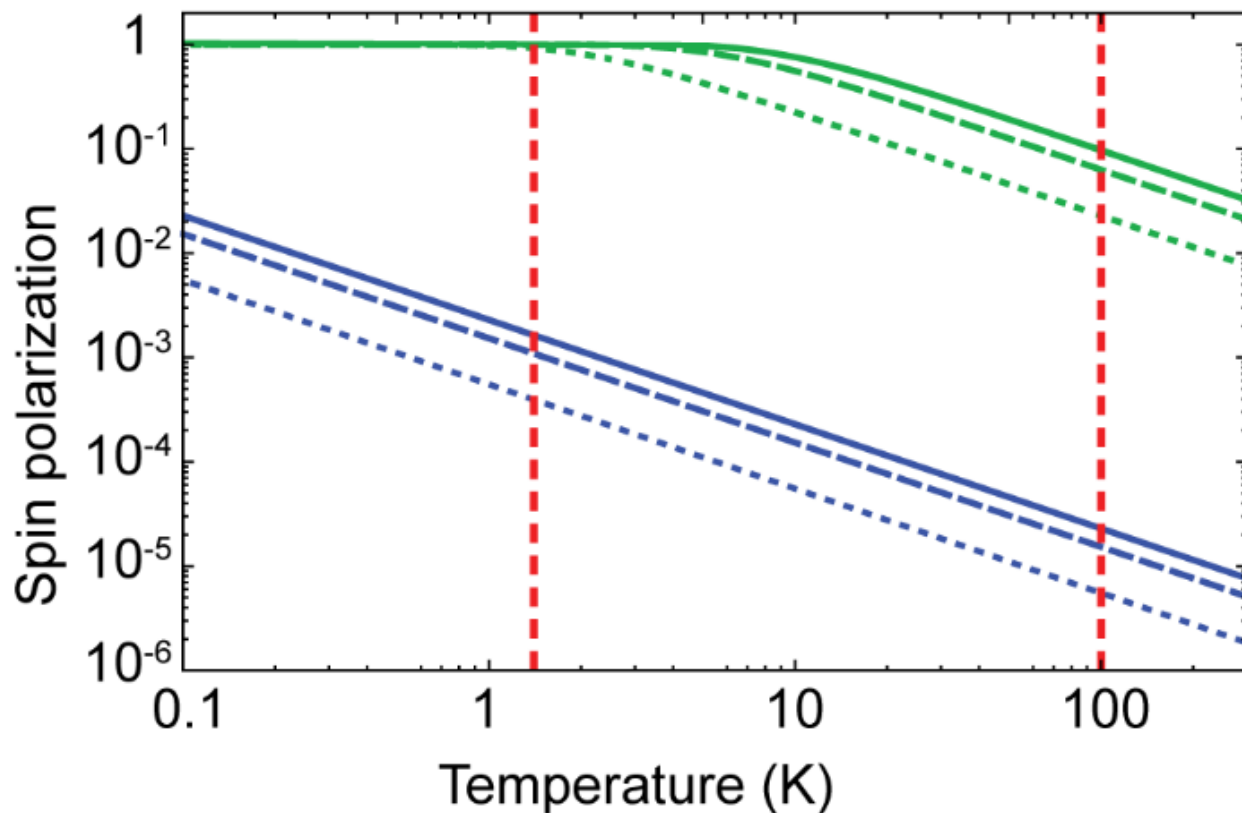
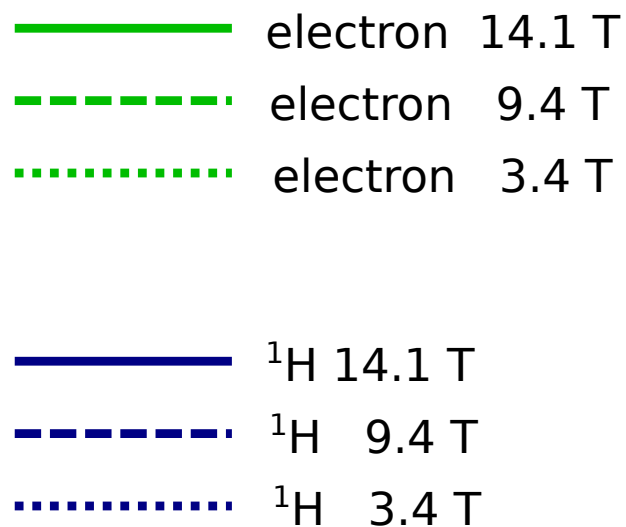
$p_A, p_B = 1 - p_A$

Exchange effects suppression at two-fields

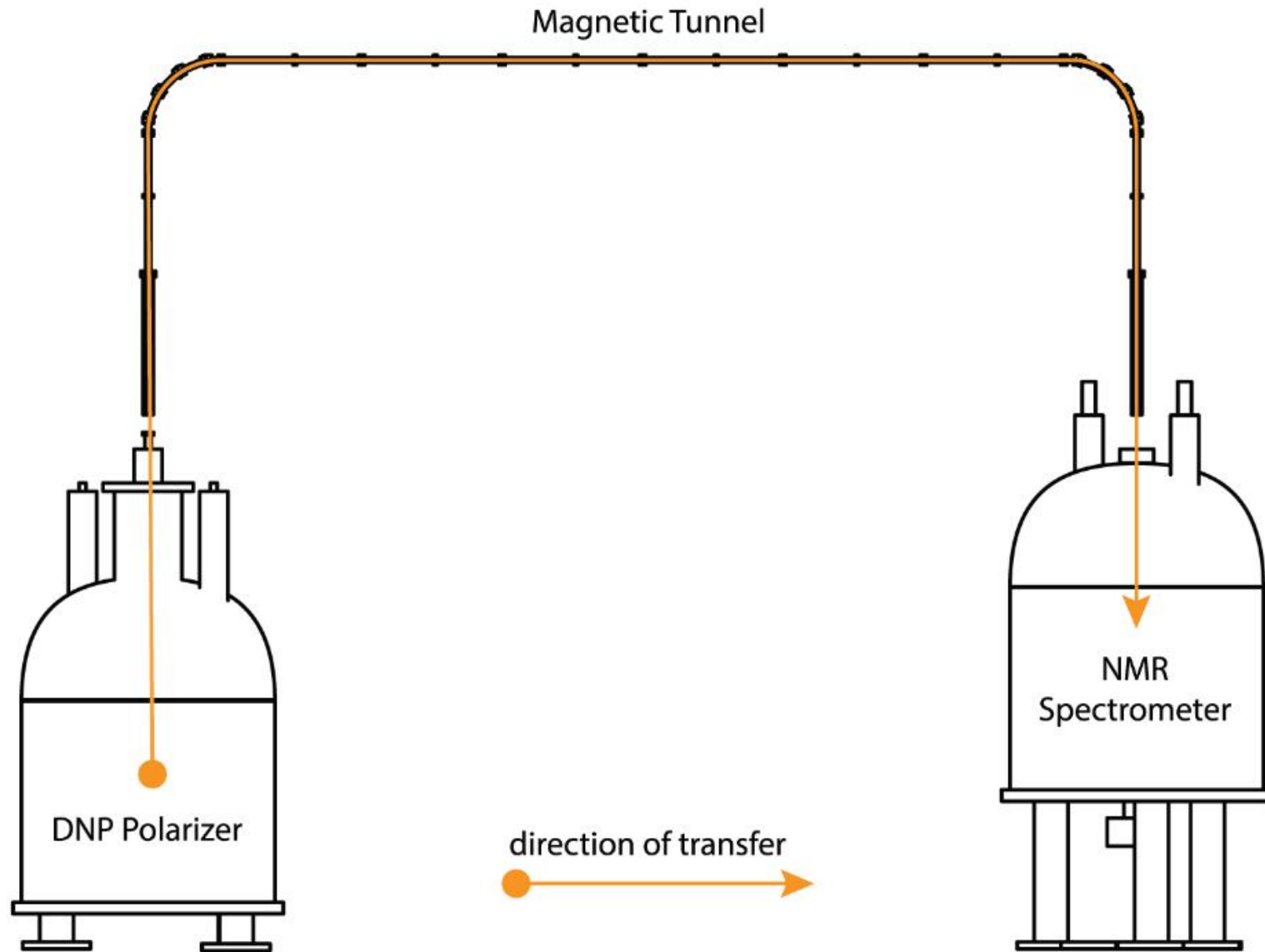


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