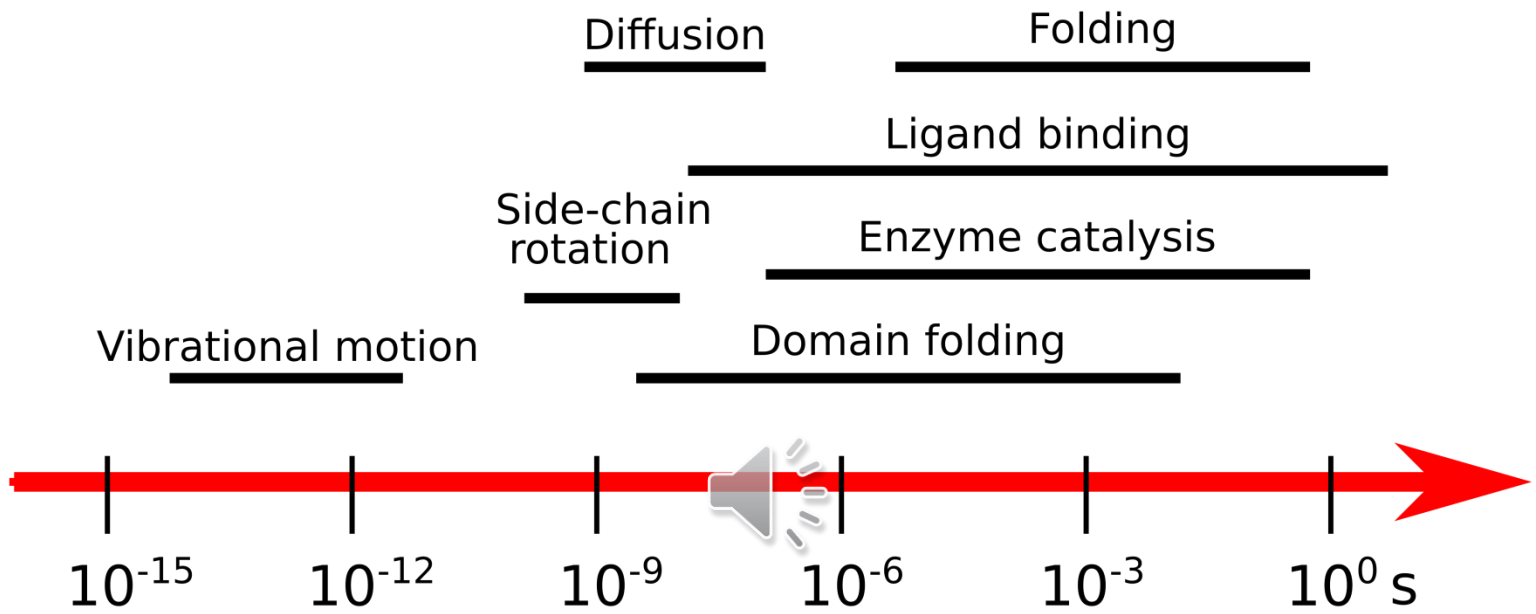
Dynamics, interactions and transient states

For Application to Protein Characterisation

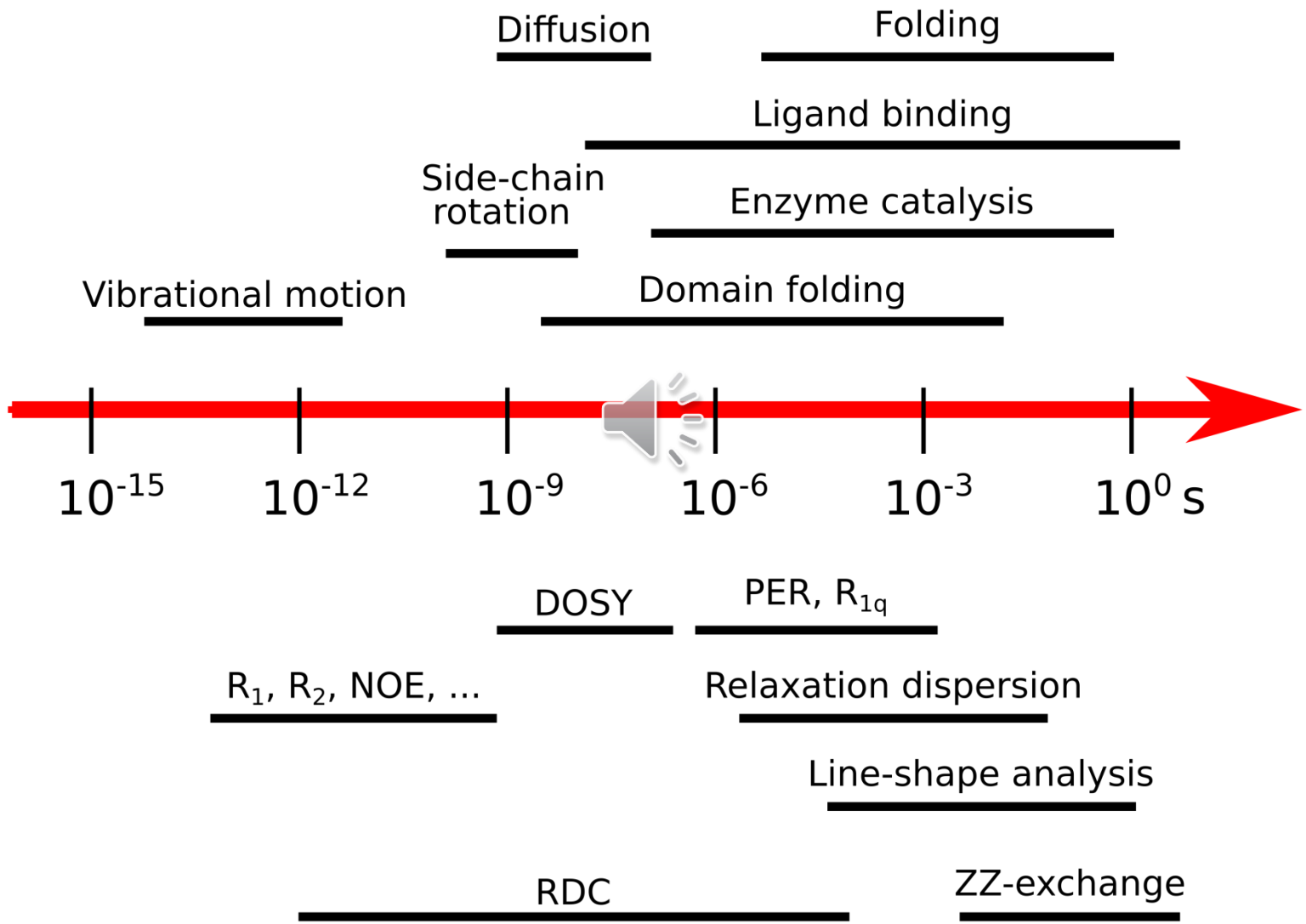
by
Radovan Fiala, Karel Kubíček, and Pavel Kadeřávek
CEITEC, Masaryk University

This project received funding by the European Commission under the GA n° 2019-1-IT02-KA203-063391

Motions in proteins



Motions in proteins & suitable NMR experiments

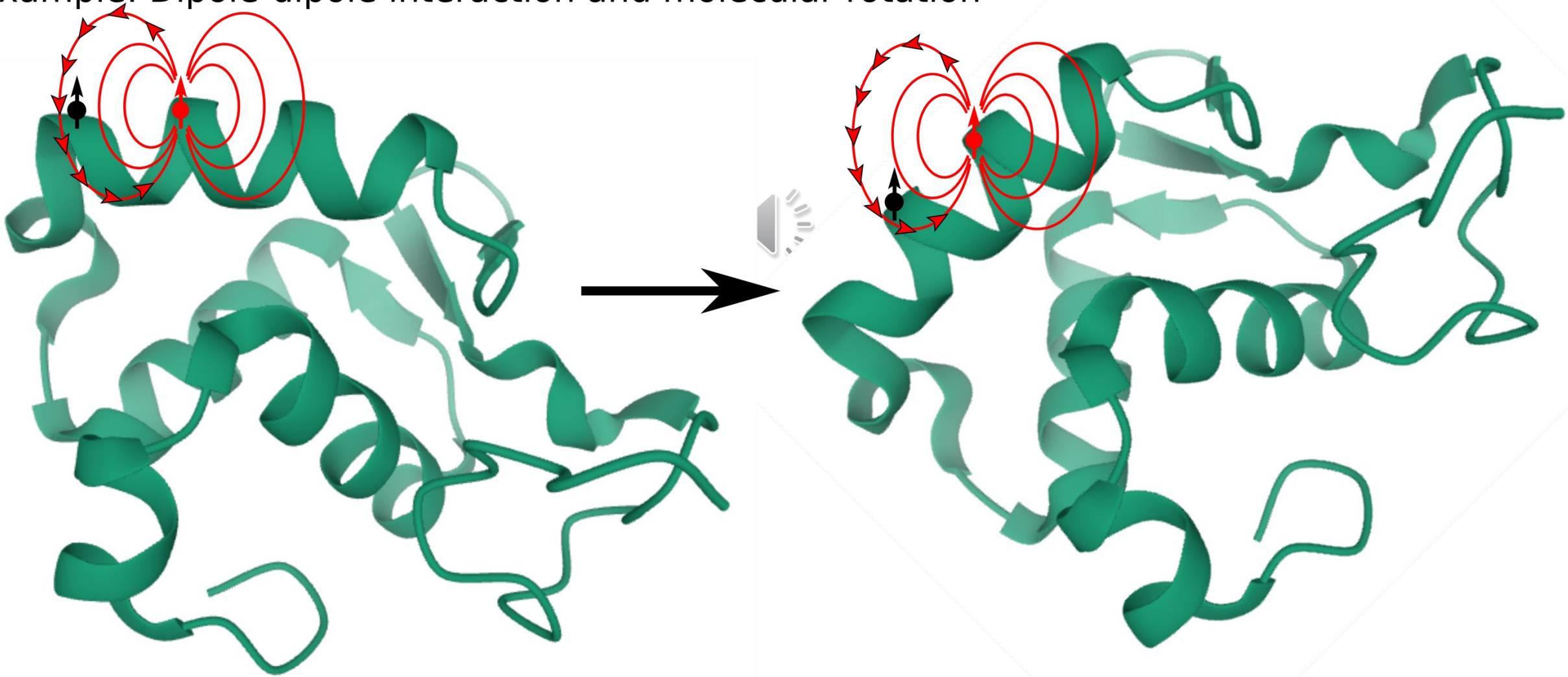


Motions in proteins & relaxations

Motion of molecule (overall rotation, internal motions) alternate effects of local interactions

Fluctuation of the magnetic field can cause a return of a spin state to equilibrium = relaxation

Example: Dipole-dipole interaction and molecular rotation



Measurement of relaxation

preparation
of the targeted spin state

↓

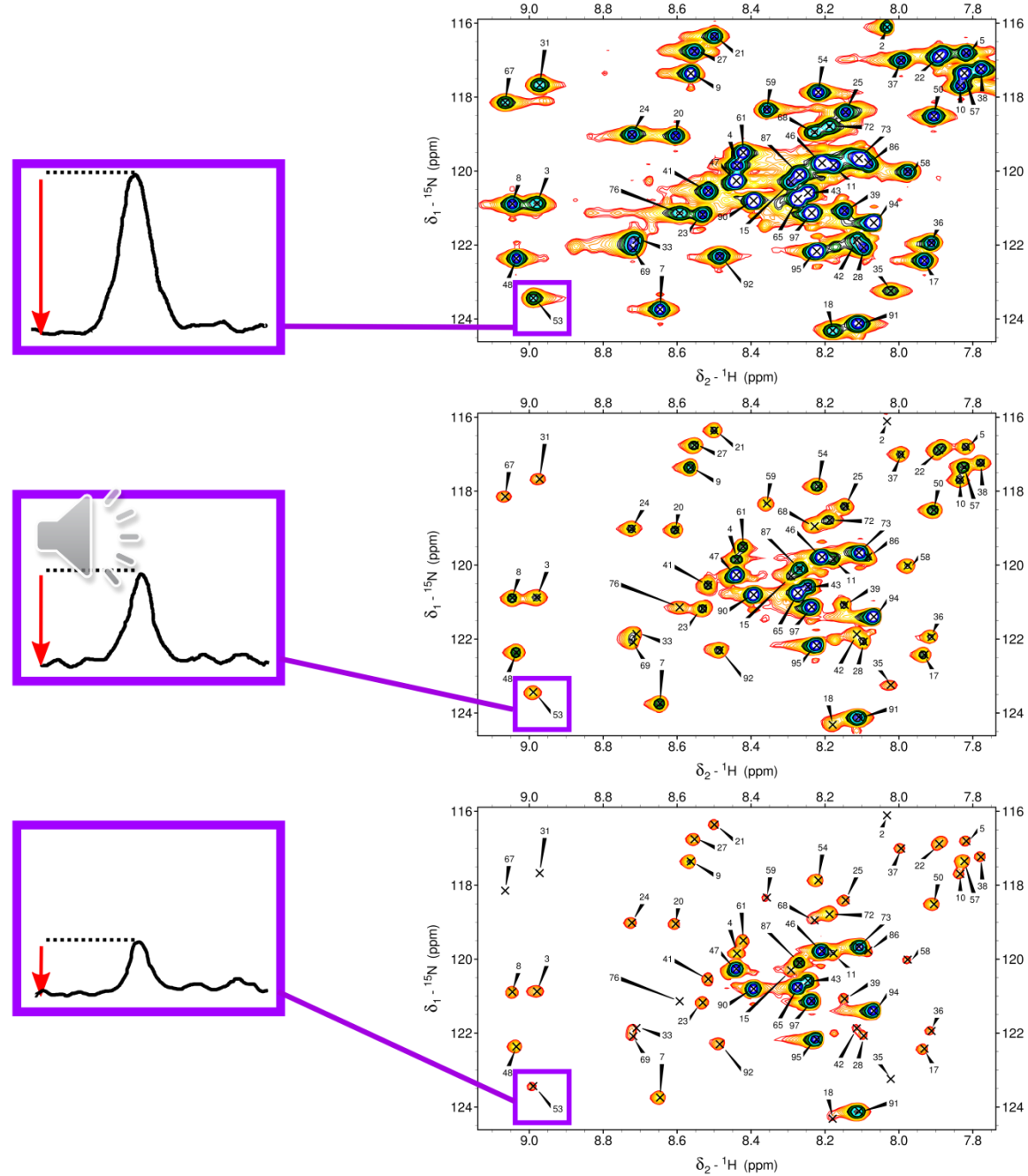
variable relaxation delay

↓

transformation of the spin state
to an observable signal

↓

detection



ps-ns motions

Experiments

most common - backbone dynamics - ^{15}N enriched sample

R_1 longitudinal relaxation rate

R_2 transverse relaxation rate (effect of slow motions)

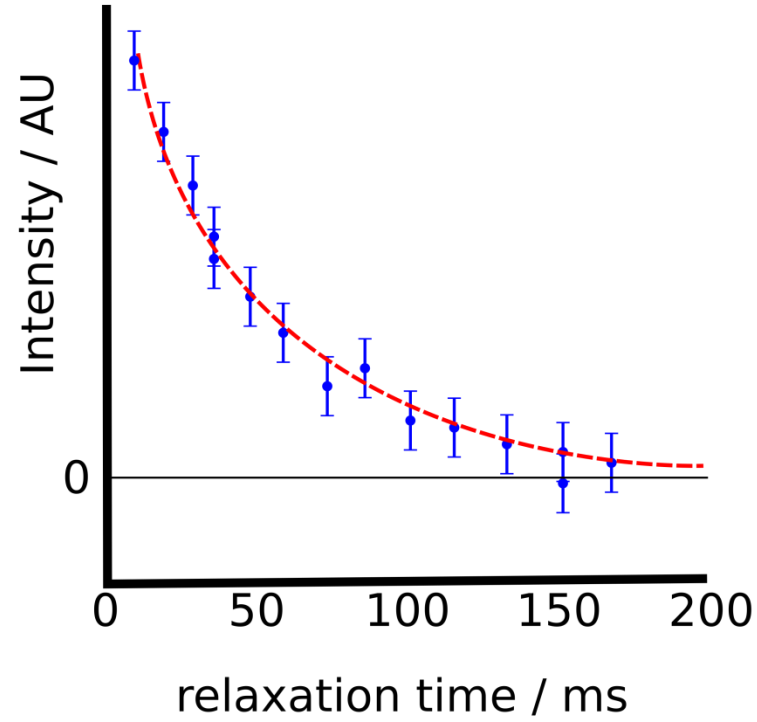
R_1 and R_2 - measurement of relaxation series

- exponential decay (peak intensities/volumes)

ssNOE - steady state Nuclear Overhauser Effect

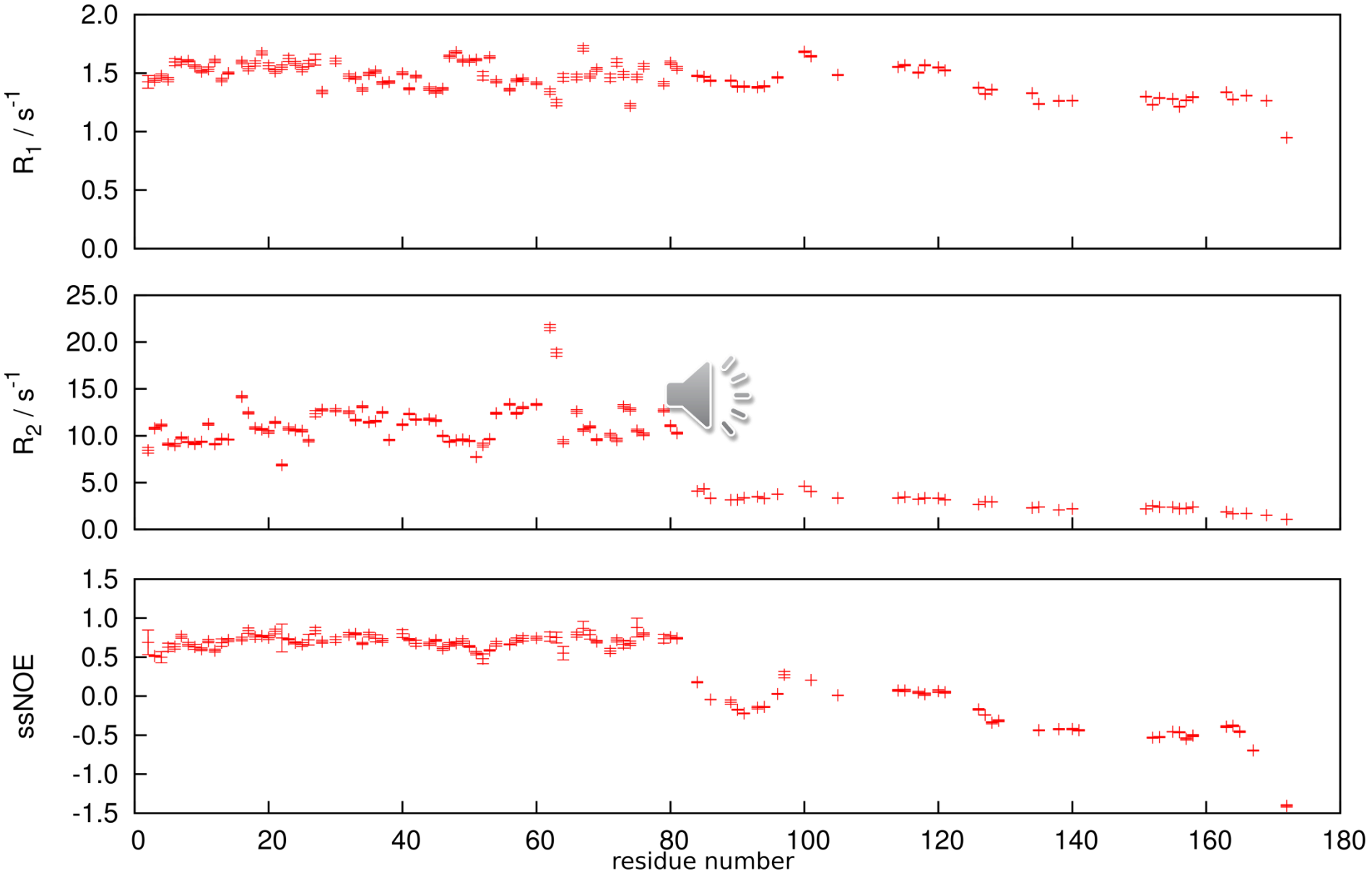
- measurement 2 spectra: reference and with saturation

(cross-correlated cross-relaxation rates ...)



ps-ns motion

Experiments



ps-ns motions

Interpretation - basic methods

Spectral density mapping

- fast
- robust
- straightforward
- data acquired at single field
- interpretation not obvious

Reduced spectral density mapping

- most common
- requires only R_1, R_2, ssNOE
  (assumptions introduced - justified
  for backbone ^{15}N in proteins)

Model-Free approach

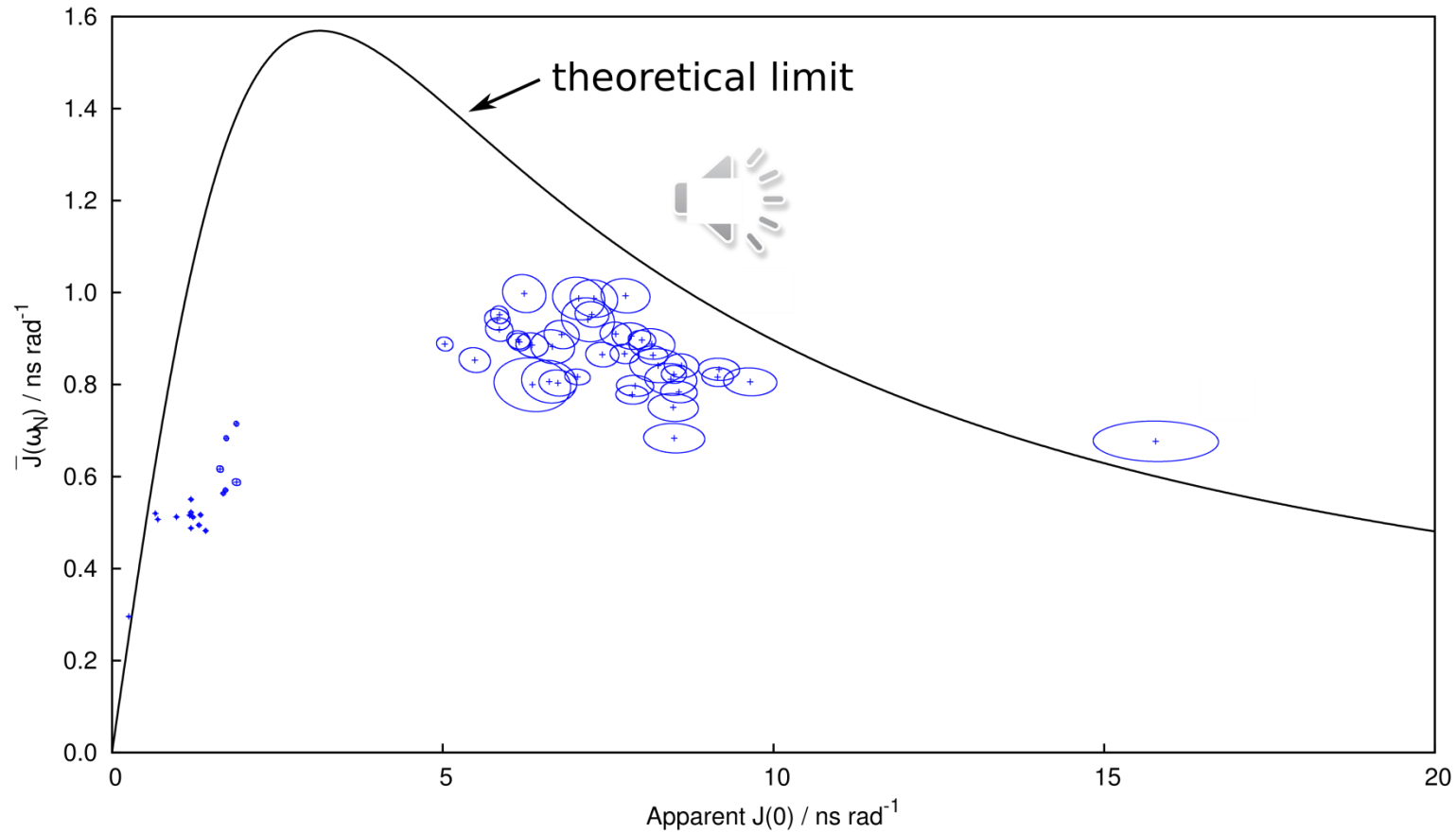
- includes fits
- assumption of independency
  of different motions
- applicable only to folded proteins
- clear interpretation

Model-dependent interpretation ...

ps-ns motions

Interpretation - spectral density mapping

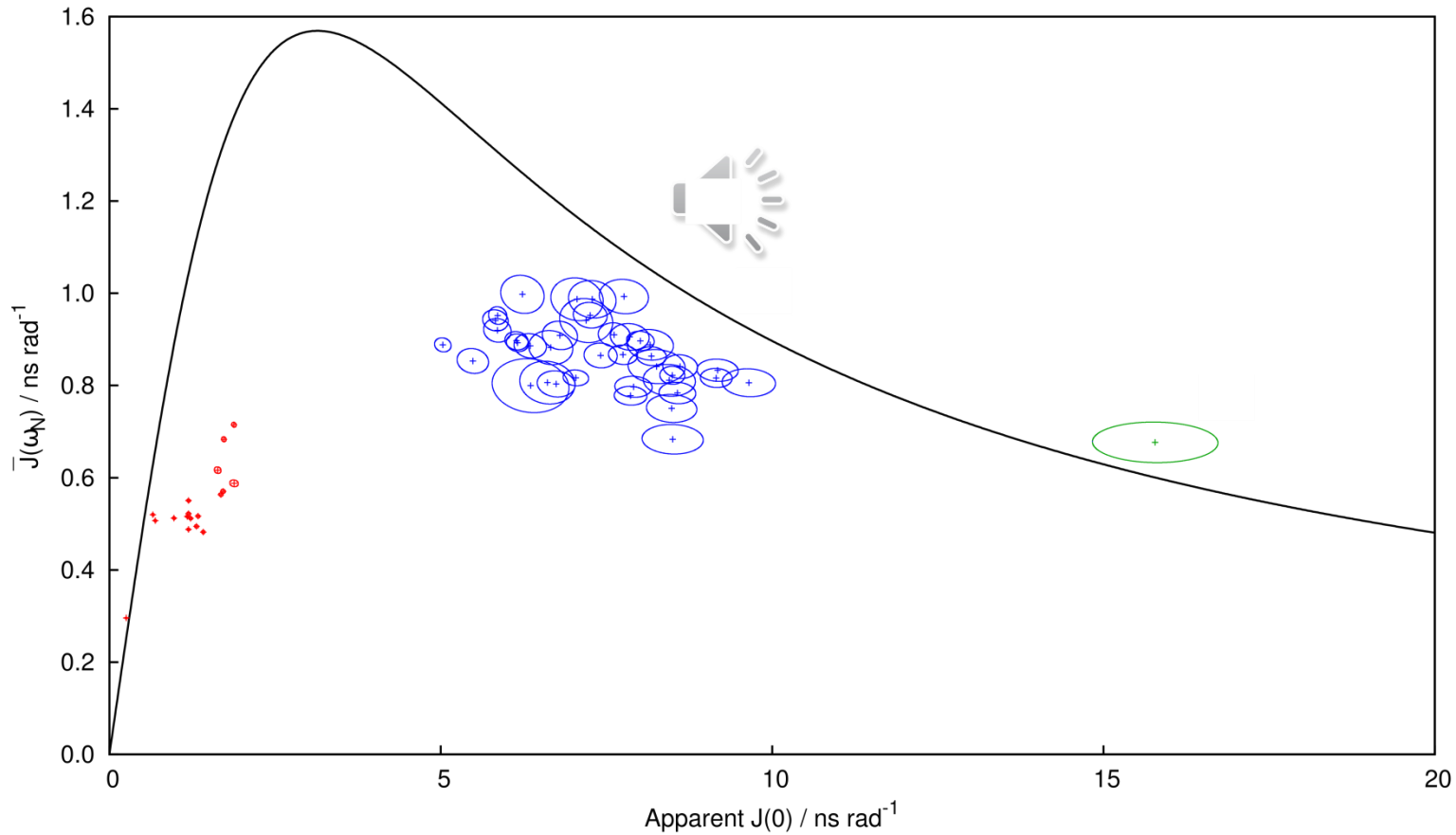
- spectral density function J(ω) - description of probability of finding motion at frequency ω
- relaxation rates are linear combination of discrete values J(ω) at specific frequencies
- spectral desnsity mapping: linear combination of relaxation rates to extract J(ω) values



ps-ns motions

Interpretation - Spectral density mapping

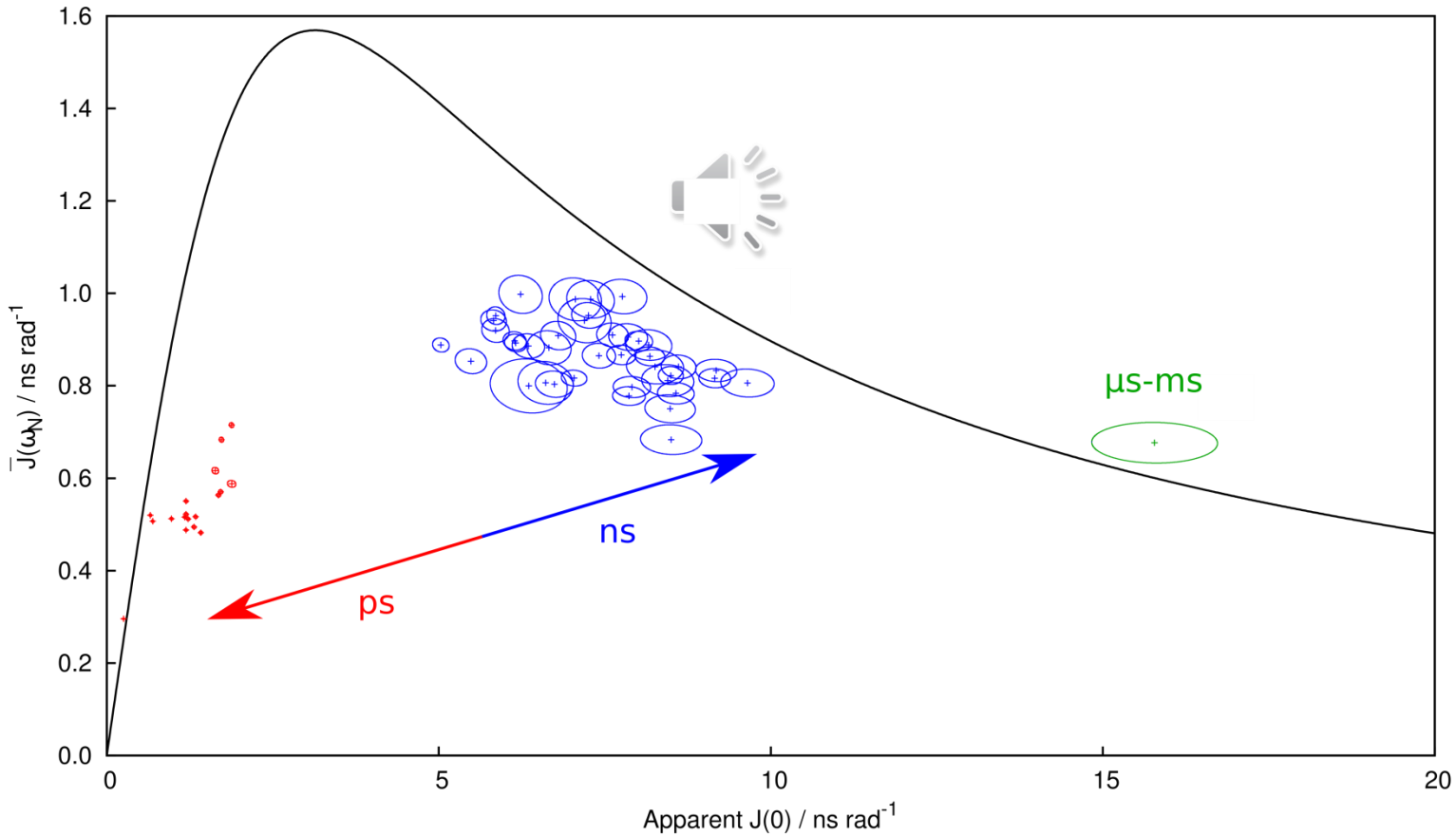
- data clustering



ps-ns motions

Interpretation -Spectral density mapping

- qualitative evaluation



ps-ns motions

Interpretation - Model Free

- Assumption of statistically independent motional modes = assumption on the form of the spectral density function, but not the type of motion
- Typically - slow overall tumbling of protein in solution + fast internal motion
- Extended Model-free - several internal independent motions

- Fitted parameters:

  Each motion is characterized by its timescale and order parameter S^2

  S^2 measures restrains of the motional mode: $S^2 = 0$ unrestrained motion

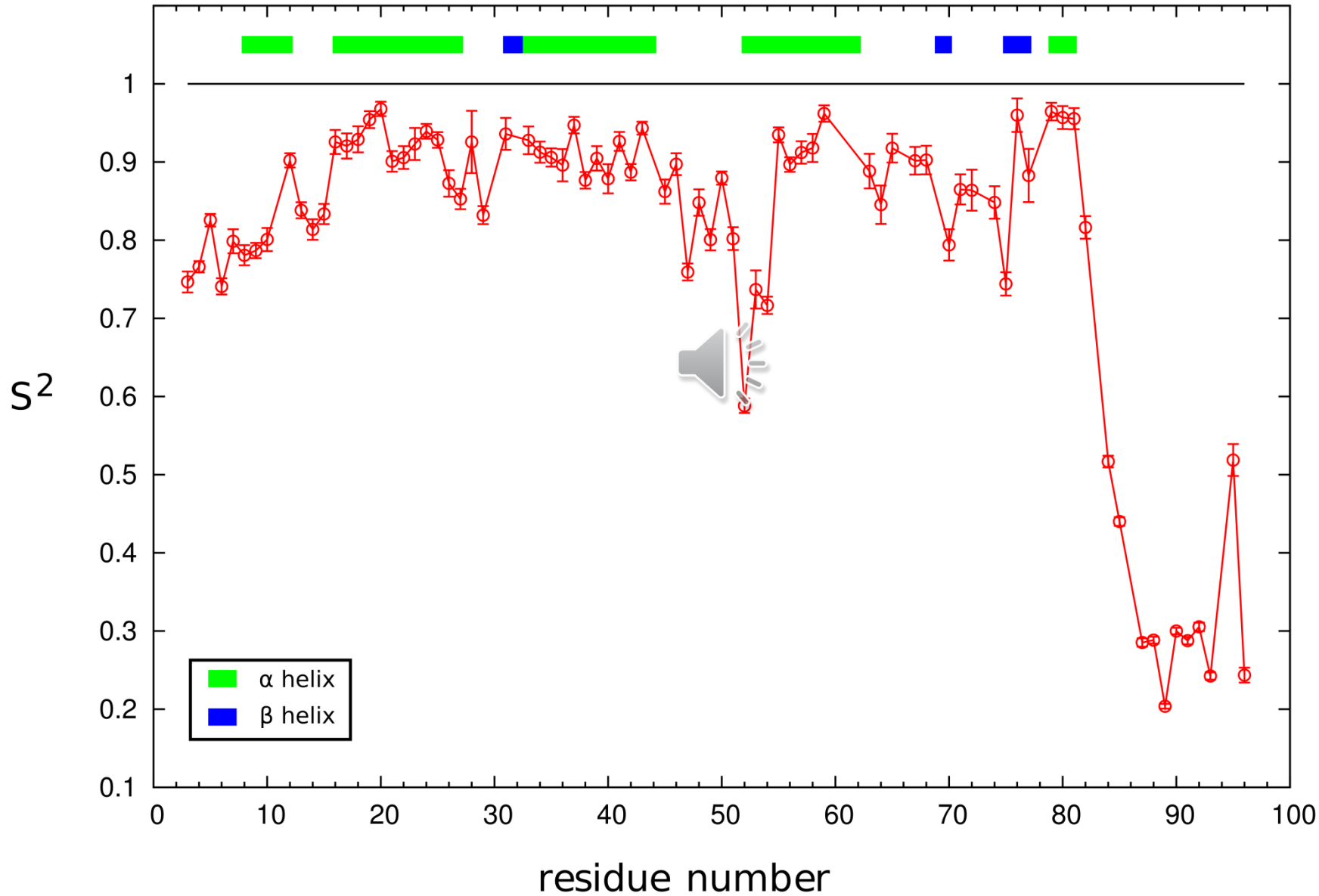
  $S^2 = 1$ rigidity

  (additional parameter - separation of contribution of μs-ms motion to R_2)

- Global motion - common to all residues in the molecule
- Iterative optimization of global and internal motions

ps-ns motions

Interpretation -Model Free



Extension towards µs motions

Residual dipolar coupling - RDC

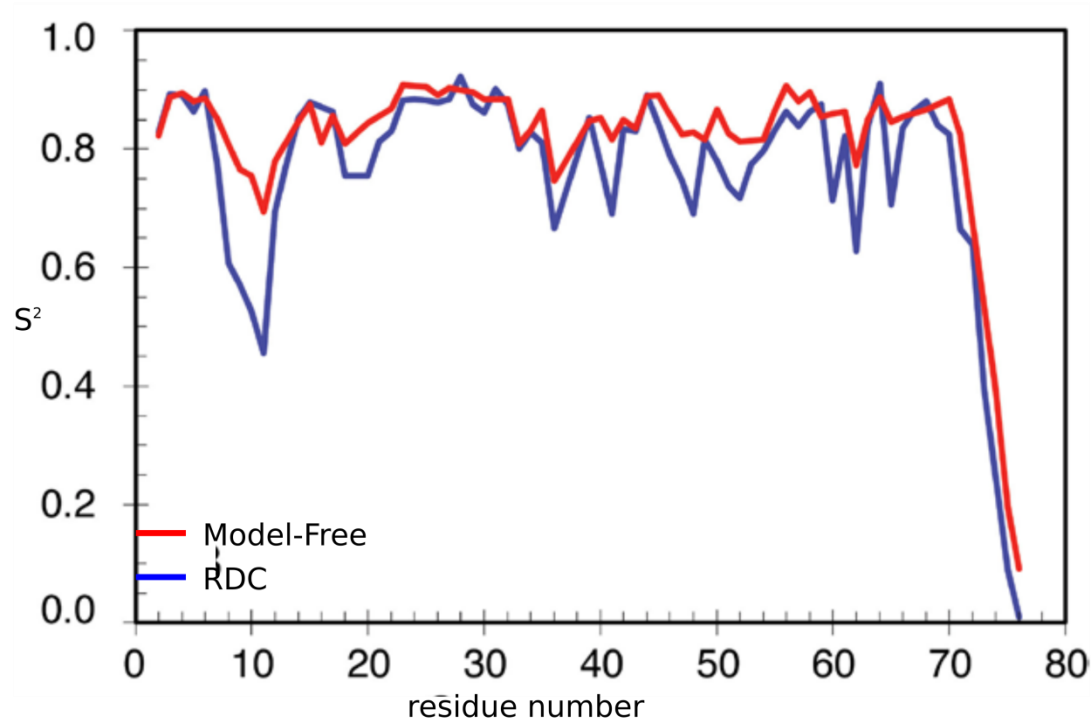
requirement - several NMR samples in various media, which causes partial protein orientation with respect to the external magnetic field (+ spin labeling is required, i.e. ^{15}N for study of protein backbone motions)

partial orientation media: philamentous phage, bicelles, DNA, ...

provides S^2 but not the timescale of the motion

typically obtained lower S^2 than from Model-free

it is sensitive to more motions

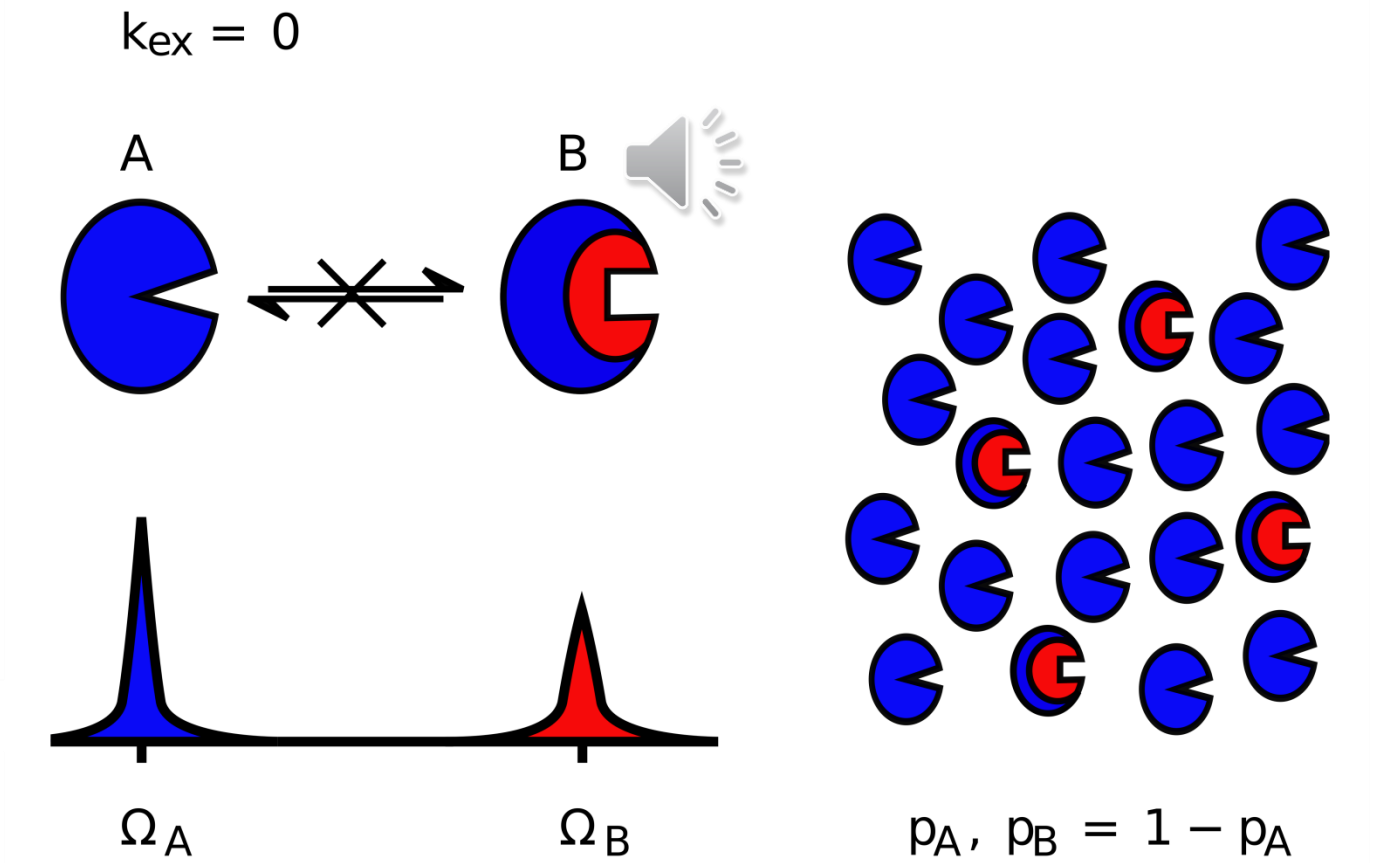


Picture coped from Loïc Salmon and Martin Blackledge 2015 Rep. Prog. Phys.**78** 126601

µs-ms motions

- possibility to study low populated states of proteins (few percent)
- important relation between timescale of exchange and difference between the chemical shifts of interexchanging states - effect on NMR spectrum:

Case 1: no exchange (two peaks detected, no broadening)

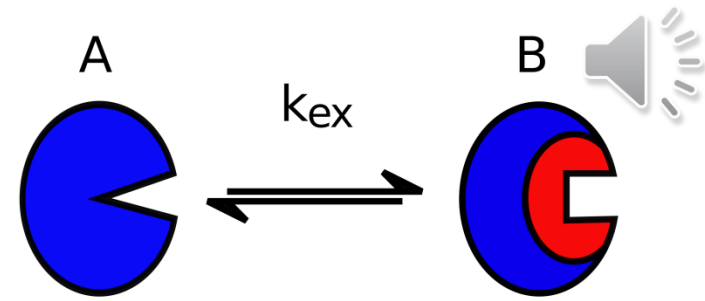


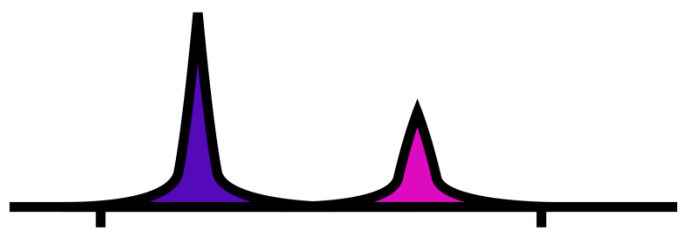
µs-ms motions

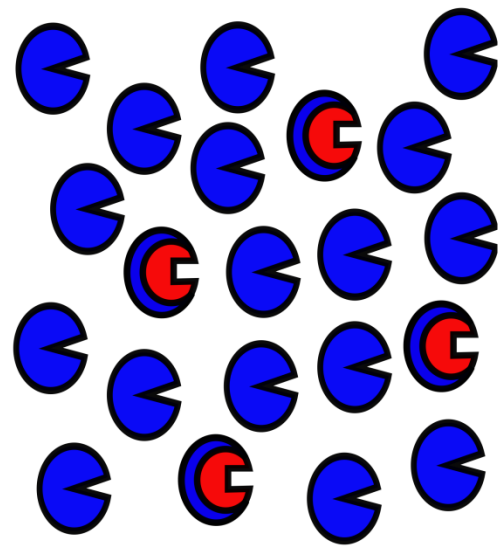
- possibility to study low populated states of proteins (few percent)
- important relation between timescale of exchange and difference between the chemical shifts of interexchanging states - effect on NMR spectrum:

Case 2: slow exchange (two peaks detected, broadening)

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







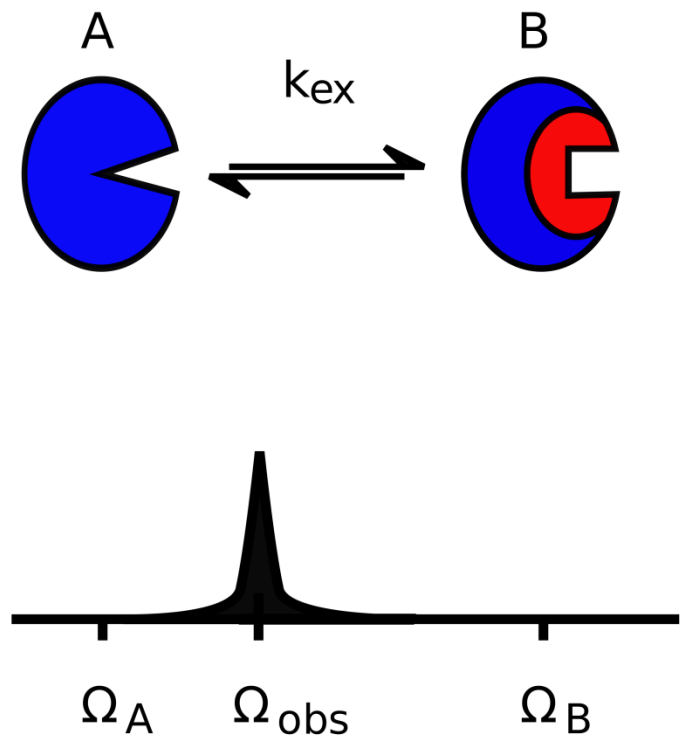
$p_A,\ p_B = 1 - p_A$

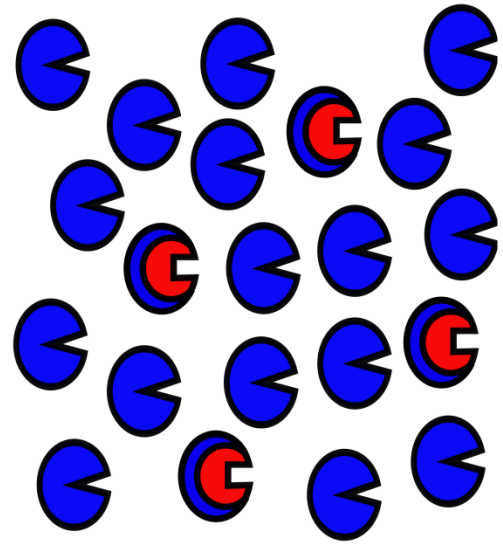
µs-ms motions

- possibility to study low populated states of proteins (few percent)
- important relation between timescale of exchange and difference between the chemical shifts of interexchanging states - effect on NMR spectrum:

Case 3: fast exchange (single peak detected, broadening)

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



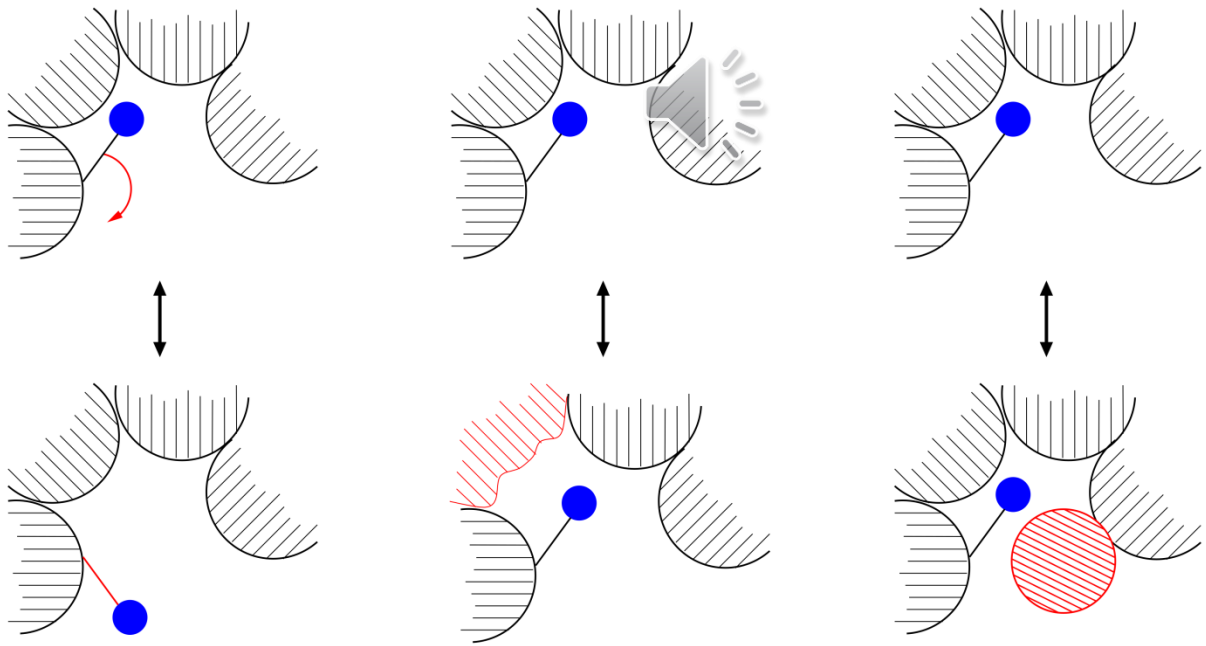


$p_A,\ p_B = 1 - p_A$

µs-ms motions

Relaxation of detected peak depends on:

1) population of states (thermodynamic)
2) exchange rate (kinetics)
3) chemical shifts of exchanging states (structural information), various events causing change of chemical shift:



motion

structural change in neighbourhood

binding

µs-ms motions

Experiments

- measurement of various relaxation delays (similar to R_2)
- modification of rf-irradiation during relaxation delay
- sample ^{15}N (^{13}C,^{2}H) enriched

methods:

CPMG - modification of overall intensity of irradiation composed from separate refocusing pulses

$T_{1\rho}$ - modification of intensity (kHz) of continuous irradiation (spin lock) and its carrier frequency

CEST - very weak continuous irradiation (typically up to 50 Hz), modification especially its carrier frequency

µs-ms motions

Experiments

CPMG - decay indicate a dynamics

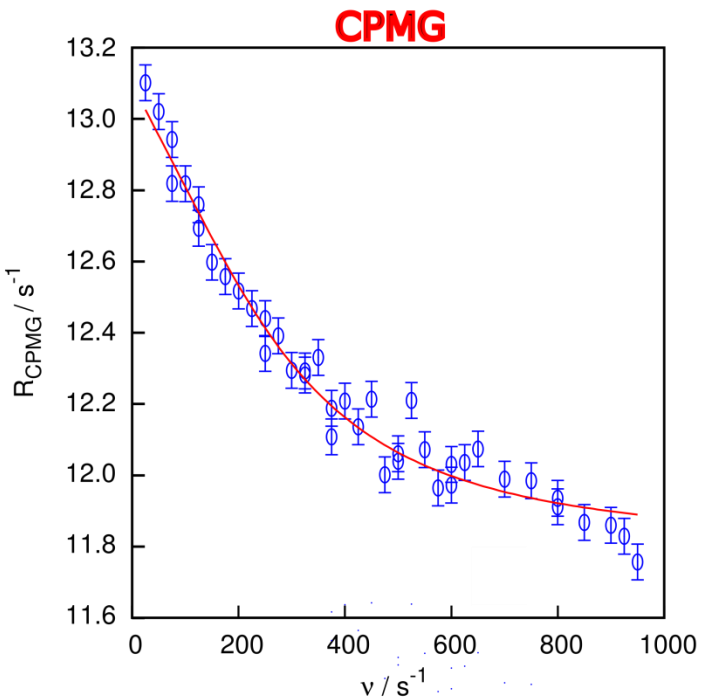
- fit of data acquired preferentially at multiple magnetic fields

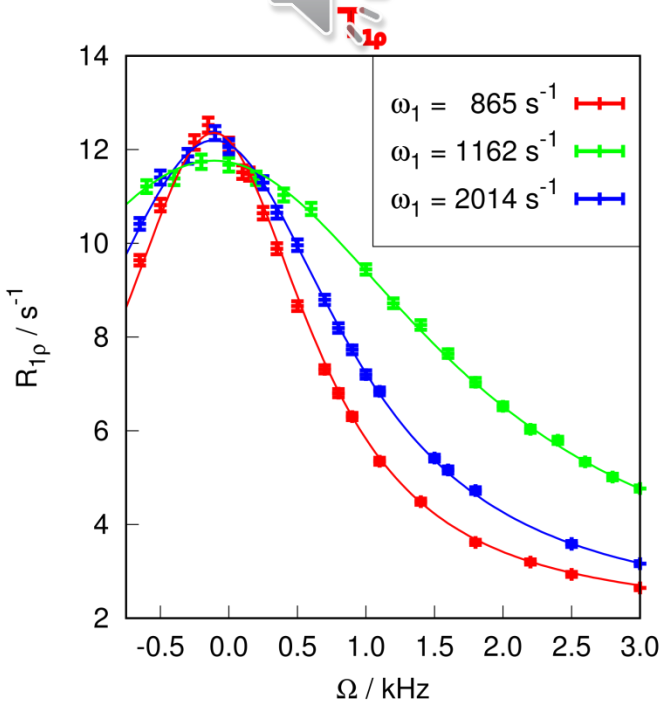
$T_{1\rho}$ - dynamic indication: difference of rates on-resonance with the observed frequency

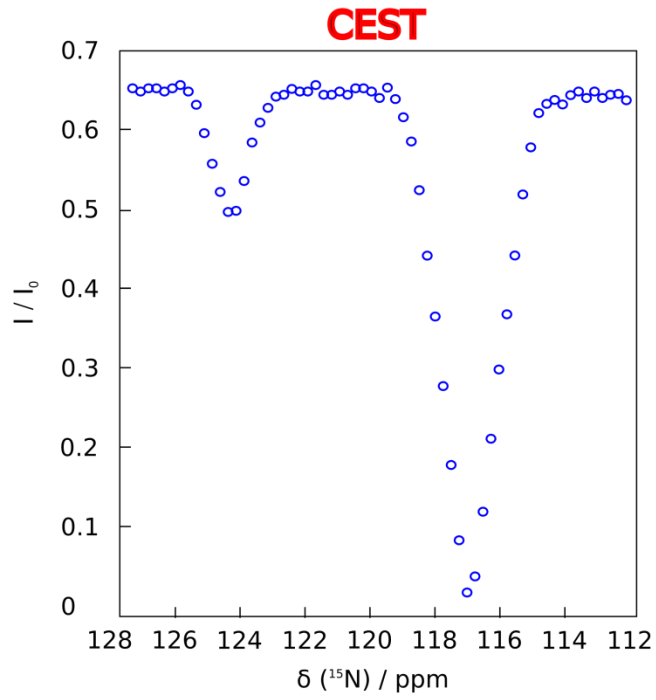
- fit of a series of at least two spin lock intensity

CEST - two (multiple) signal intensity decreases detected in the series

- direct reading of chemical shifts of the exchanging states



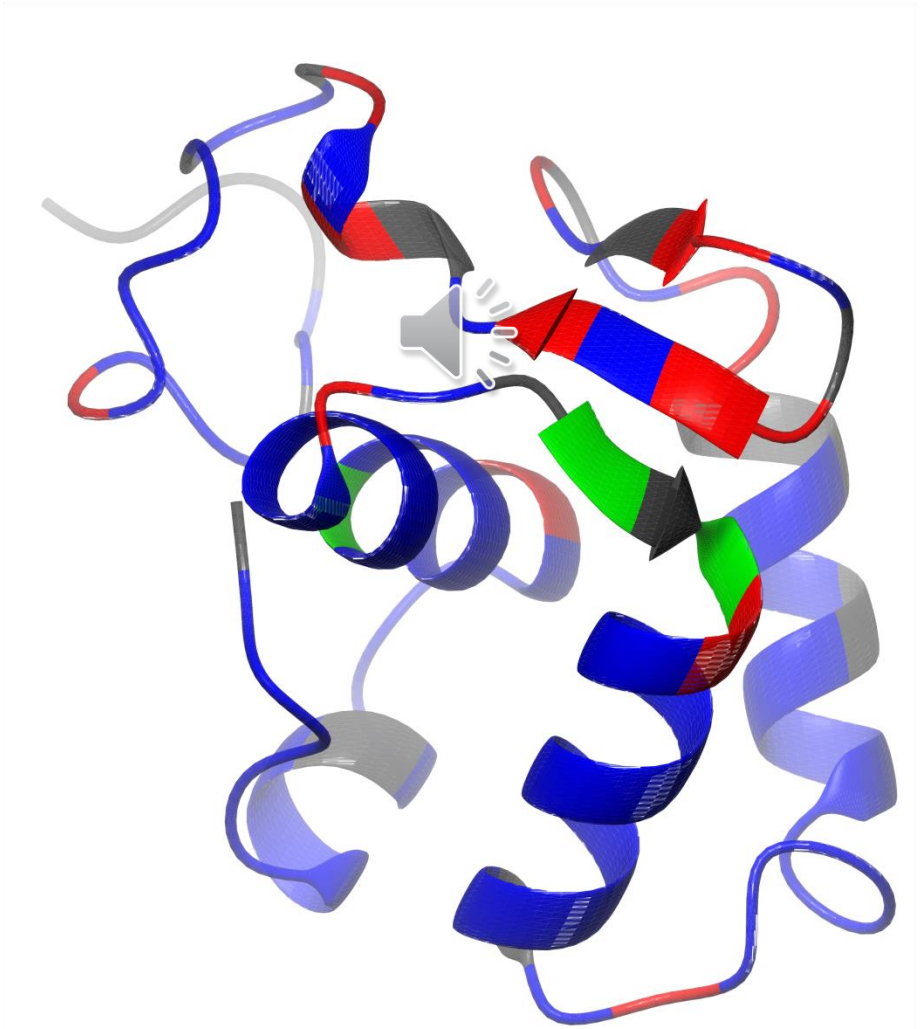




µs-ms motions

Results

- mapping of dynamical residues
- clustering of residues with same dynamics (population of states and kinetics)

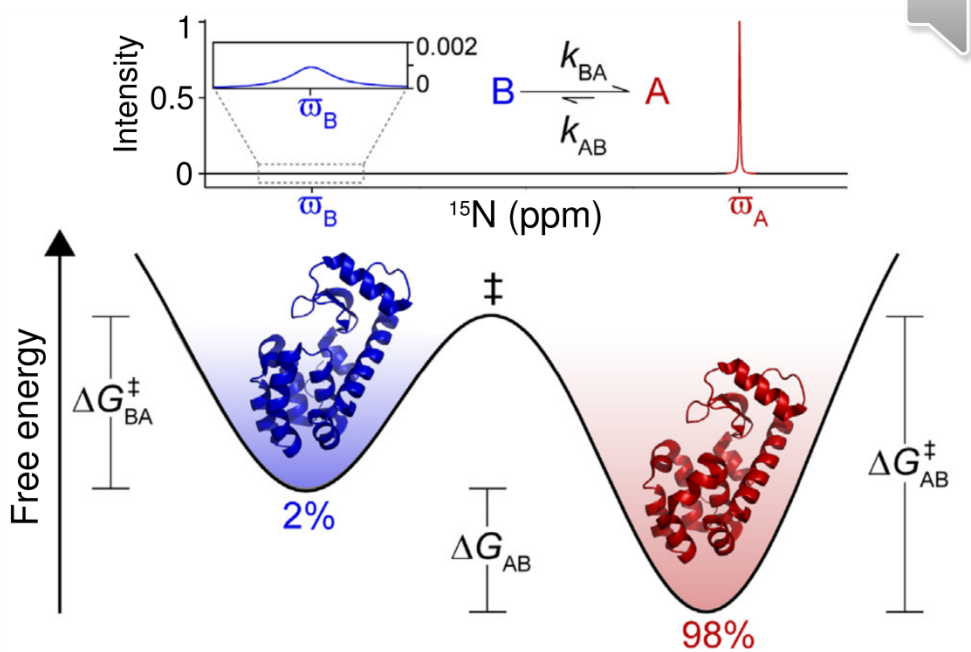


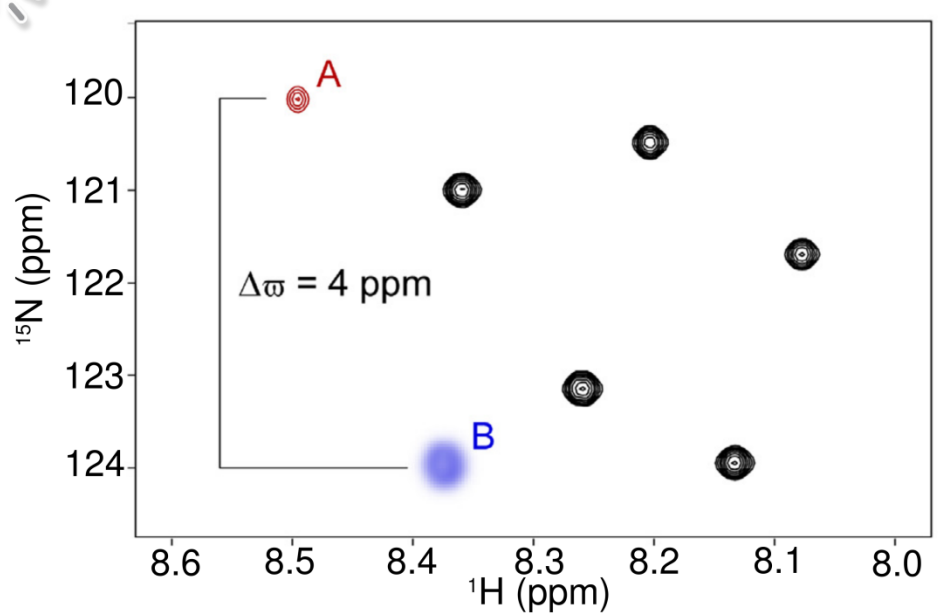
µs-ms motions

Invisible states

- determination of chemical shift of excited state (indirect structural information)
- measurement of bond orientation in the excited state by measurement of relaxation dispersion experiments in alignment media

=> possibility to reveal structure of the state whose signals are not visible in the NMR spectra





picture copied from Anderson T.R., Kay L.E.Curr Opinion in Struct Biol, 2020, 60: 39-49

Interactions and very slow kinetics

Real time NMR

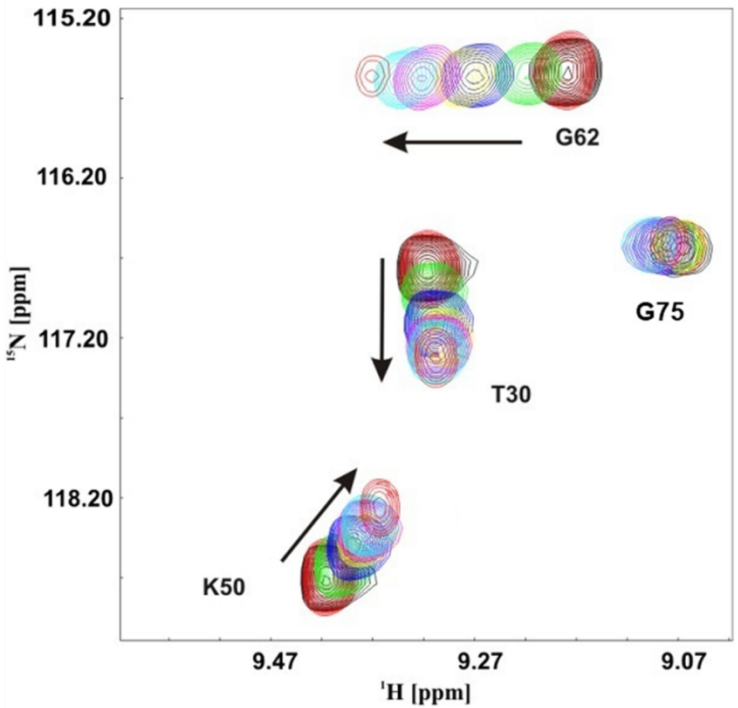
- repetition of experiments (1D, 2D) and tracking the changes in spectra

Titration

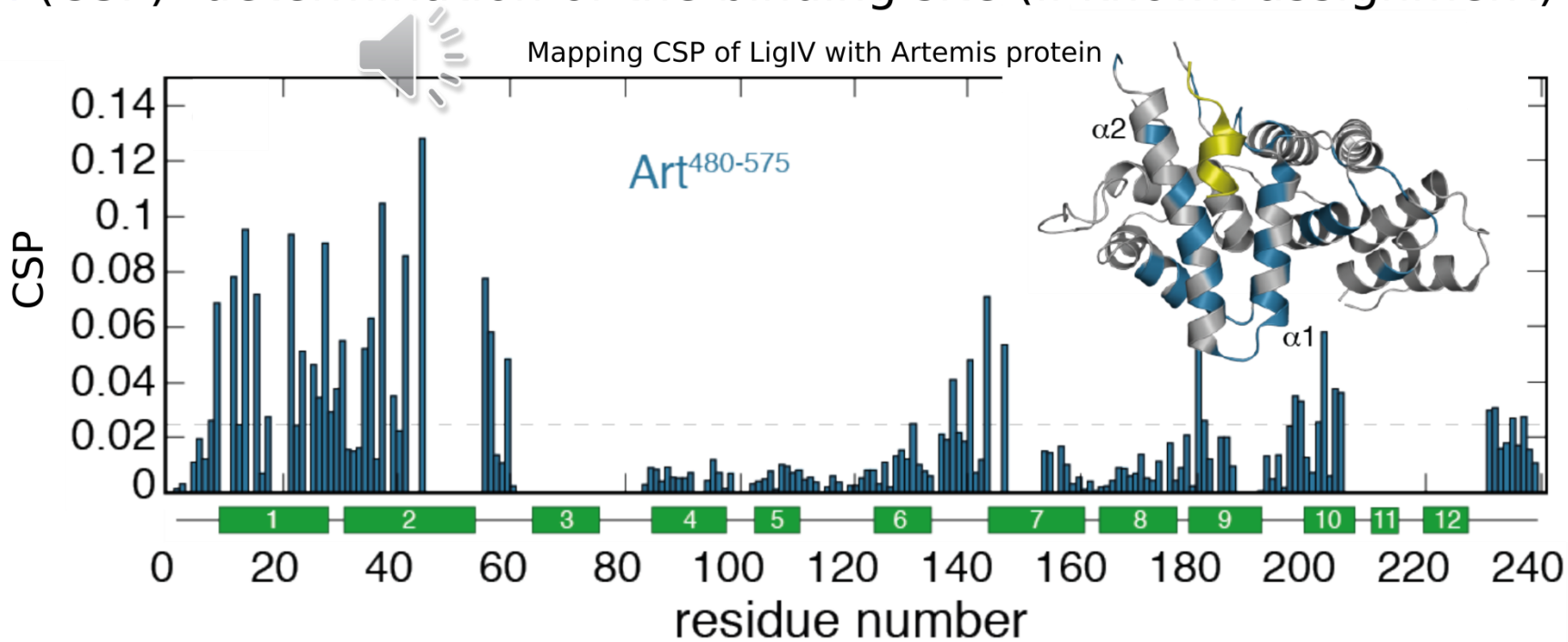
-mapping changes in spectra of protein (typically ^{15}N-^{1}H HSQC) upon titration with its ligand, binding partner (drug, inhibitor, cofactor, ...)

- effective confirmation of an interaction

- chemical shift perturbation (CSP)- determination of the binding site (if known assignment)



picture copied from Dicks et al. BMC Molecular and Cell Biology (2019) 20:23



picture copied from Charlier C. et al., J. Am. Chem. Soc., 2017, 139 (5), 1219-12227

Interactions and very slow kinetics

Real time NMR

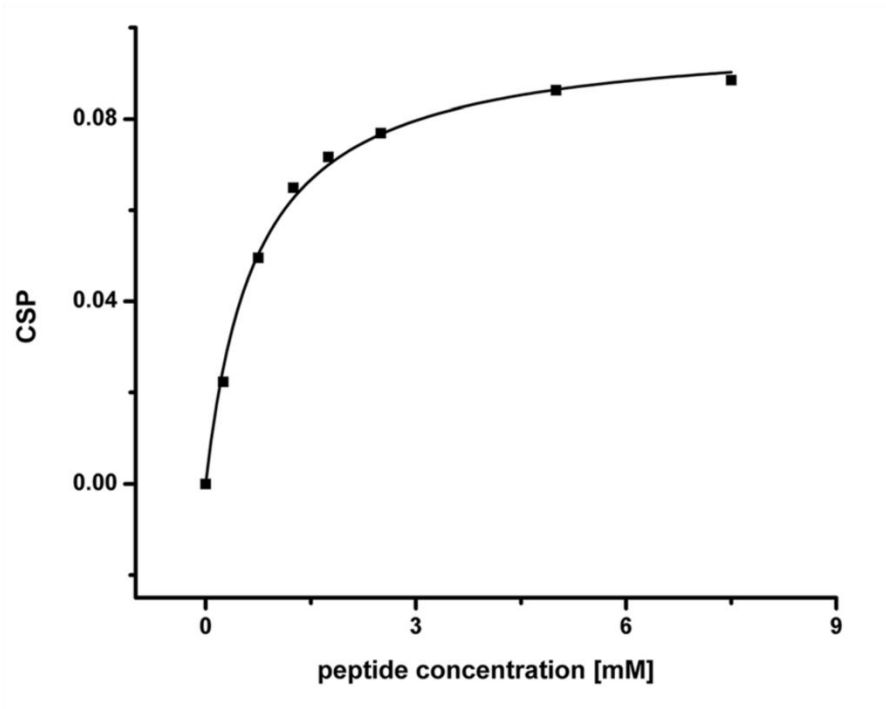
- repetition of experiments (1D, 2D) and tracking the changes in spectra

Titration

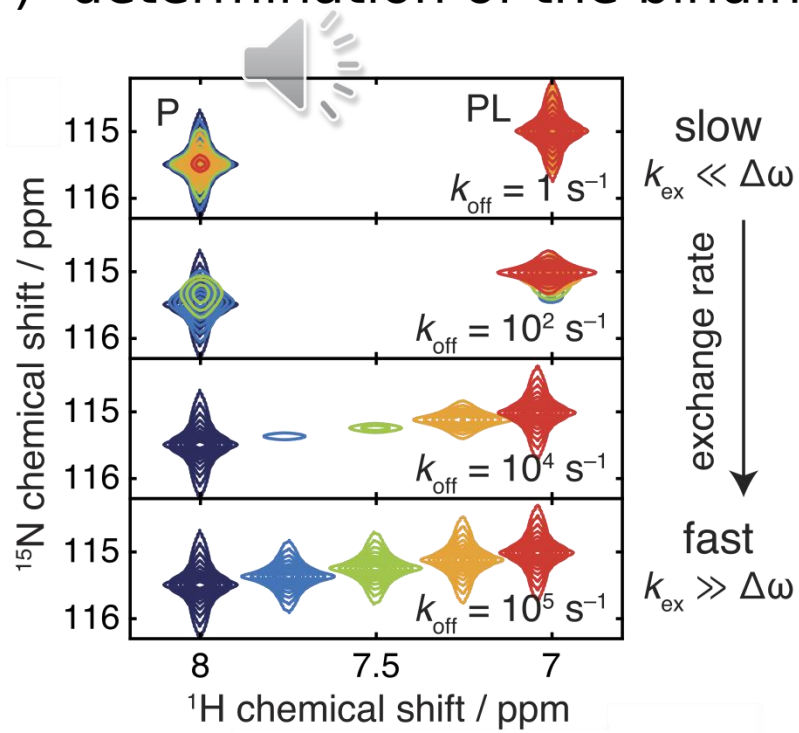
-mapping changes in spectra of protein (typically ^{15}N-^{1}H HSQC) upon titration with its ligand, binding partner (drug, inhibitor, cofactor, ...)

- effective confirmation of an interaction

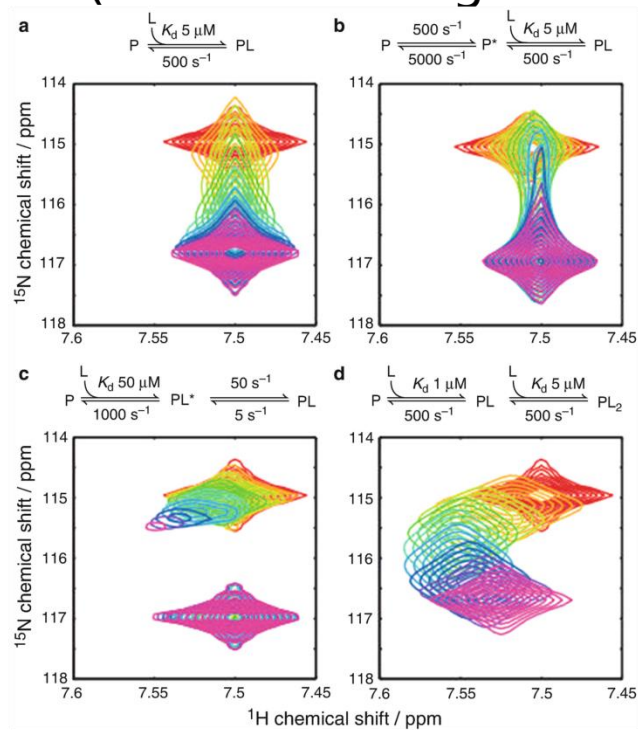
- chemical shift perturbation (CSP)- determination of the binding site (if known assignment)



picture copied from Dicks et al. BMC Molecular and Cell Biology (2019) 20:23



picture copied from Waudby et al. Scientific reports (2016) 24826



Picture copied from Williamson M.P. 2018, Modern Magnetic Resonance, Springer

Interactions

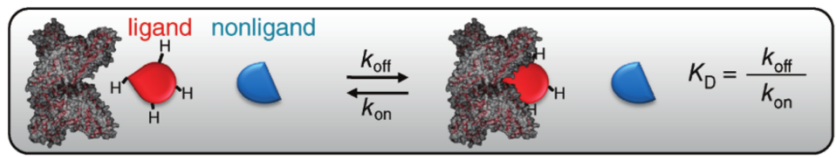
Saturation transfer difference (STD) experiment

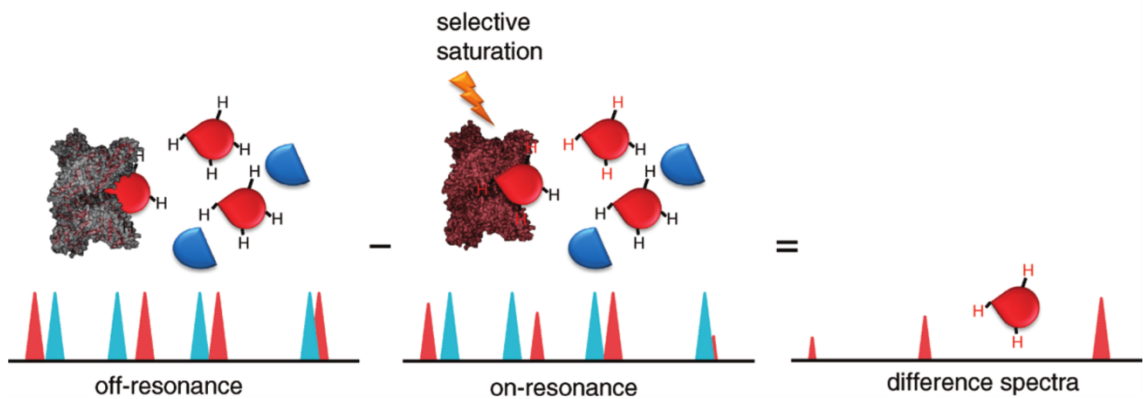
- popular in pharmaceutical industry - drug design
- determination of binding between a protein and small molecules
- no isotope labeling needed, low protein concentration ~10μM

Two spectra acquired:

- selective irradiation at resonances specific for protein (no ligand signals)
- far off-resonance reference spectra

Difference reveals signal of protons of ligand(s)





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

Interactions

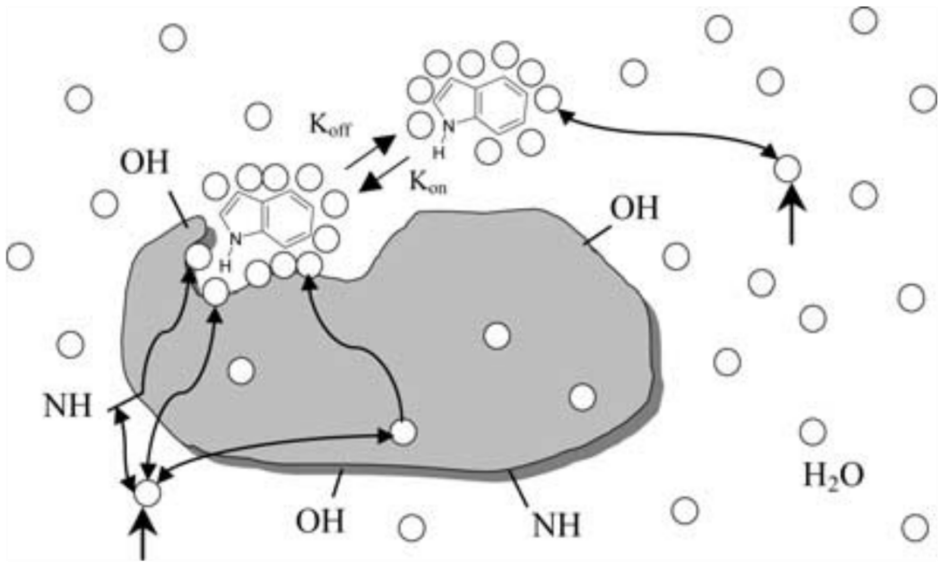
WaterLOGSY

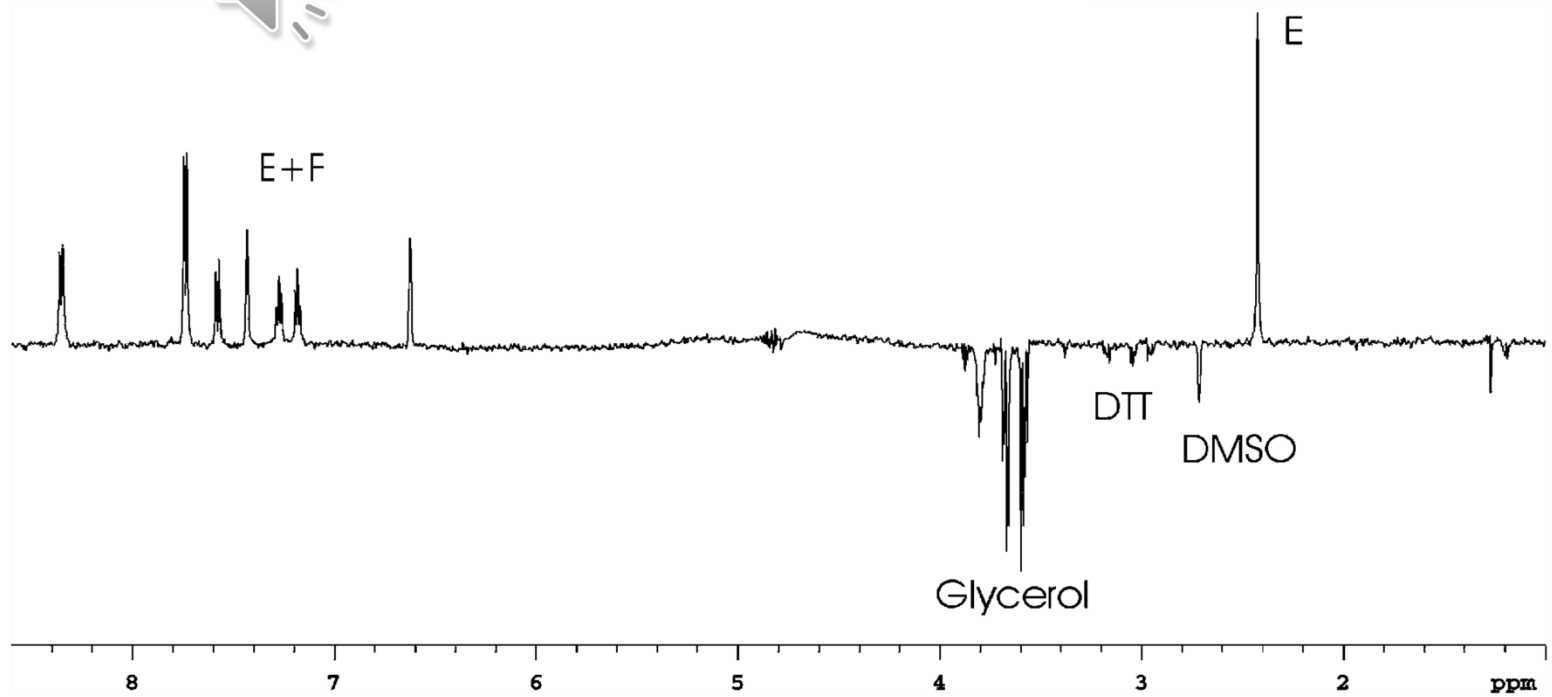
- determination of binding between a protein and small molecules
- no isotope labeling is needed
- low protein concentration ~10µM

Transfer of strong water signal to protein-ligand signal

Signal detected for small molecules after relesing complex:

interacting and non-binding molecules have signals with opposite sign in spectra





pictures copied from Claudio Dalvit et al,
J. Biomol. NMR 2001, 21, 349-359

Further reading

ps-ns dynamics
D.M. Korzhnev, M. Billeter, A.S. Arseniev, V.Y.Orekhov, 2001, Prog. NMR Spec., **38**, 197/266, DOI: 10.1016/S0079-6565(00)00028-5

Residual dipolar couplings (RDC)
L. Salmon and M. Blackledge 2015 Rep. Prog. Phys.**78**, 126601, DOI: 10.1088/0034-4885/78/12/126601

µs-ms dynamics
A.G. Palmer, C.D. Kroenke, J.P. Loria, 2001, Methods in Enzymology, Academic Press, London, **339**, 204-238, DOI: 10.1016/S0076-6879(01)39315-1

P. Vallurupaldi, G. Bouvignies, L.E. Kay, 2012, J. Am. Chem. Soc., **134**, 8148-8161, DOI: 10.1021/ja3001419

Invisible states
T.R. Anderson, and L.E. Kay, 2020, Curr Opinion in Struct Biol, **60**, 39-49, DOI: 10.1016/j.sbi.2019.10.008

Lineshape analysis (titrations)
C.A. Waudby, A. Ramos, L.D. Cabrita, and J. Christodoulou, 2016, Scientific reports , **6**: 24826, DOI: 10.1038/srep24826

Saturation transfer difference (STD)
A. Viegas, J. Manso, F.L. Nobrega, and Eurico J. Cabrita, 2011 , J. Chem. Educ. **88**, 990-994, DOI: 10.1021/ed101169t

WaterLOGSY
C. Dalvit, G. Fogliatto, A. Stewart, M. Veronesi, and B. Stockman, 2001, J. Biomol. NMR, **21**, 349-359, DOI: 10.1023/A:1013302231549