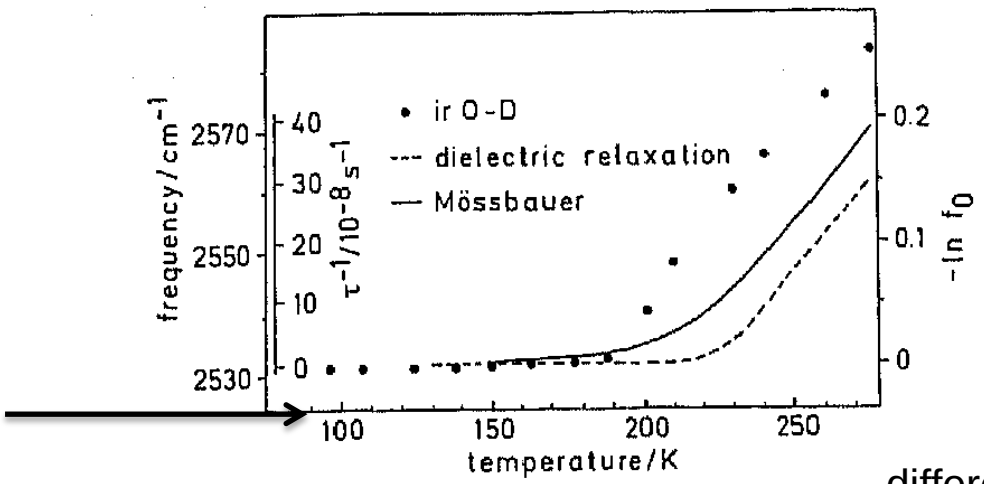
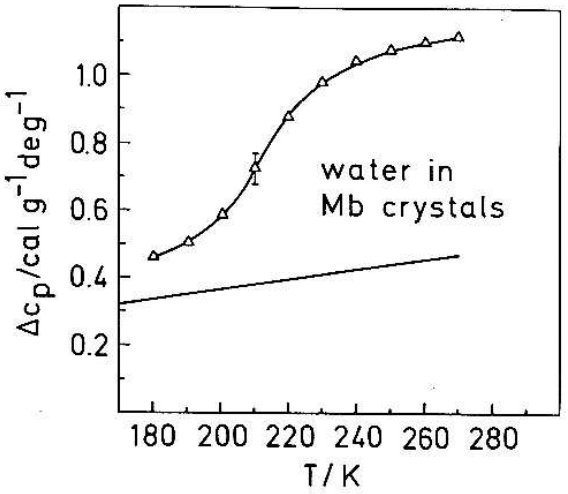
50 years of Neutron Backscattering Spectroscopy applied to molecular biology: 30 years „Protein Dynamical Transition“ (1986)

W. Doster
Glass Transition of protein hydration water and structural flexibility of myoglobin
in Structure, Dynamics and Function of Biomolecules (EBSA, Stockholm 1985)
Springer Series in Biophysics Vol. 1 (1986) and

Wolfgang Doster, Anton Bachleitner, Rainer Dunau, Manfred Hiebl and
Edgar Lüscher, Physics Department E 13, TUM
Thermal Properties of Water in Myoglobin Crystals and Solutions
at Subzero Temperatures, Biophysical Journal, 50, 213 (1986)

„glass“ transition of hydration water from the T-dependence of ir-OD, LMF and DR and specific heat around 200 K:

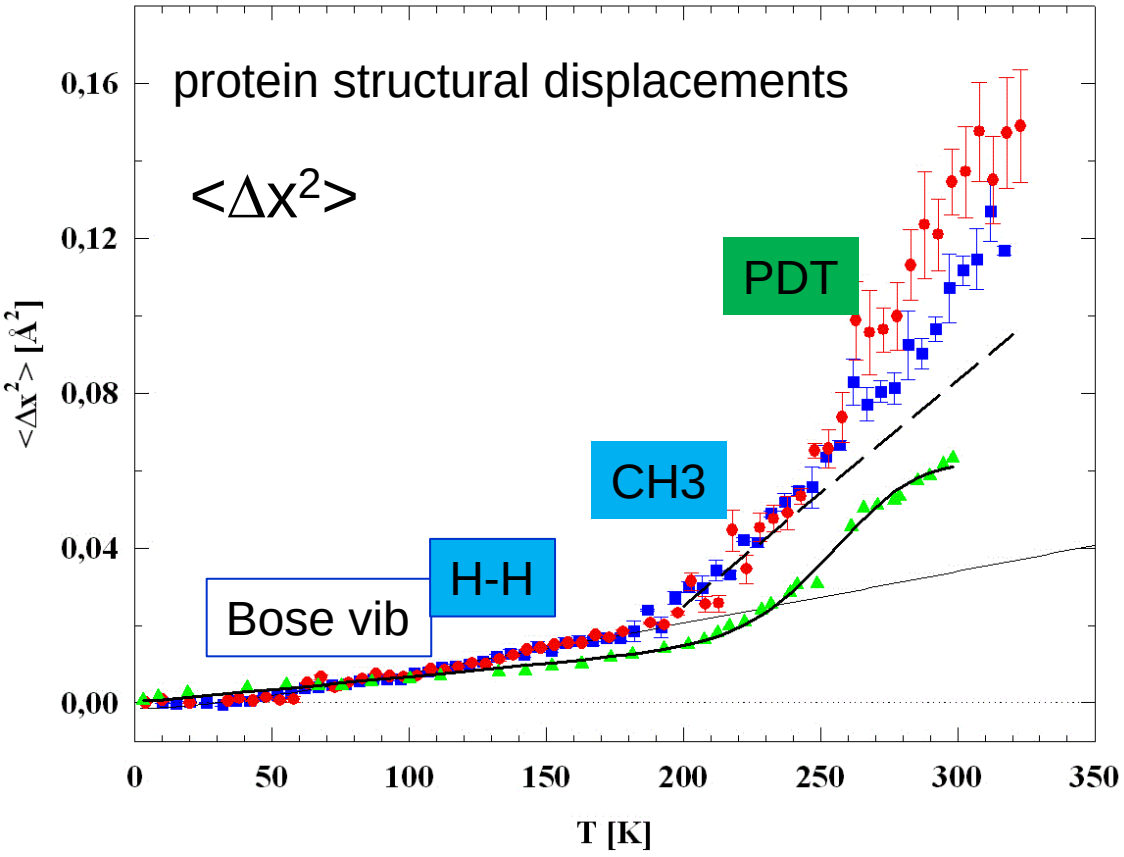




different onset temperatures
interpreted by different instrument resolution

50 years of Neutron Backscattering Spectroscopy:
A Protein Dynamical Transition (PDT) from Elastic Back-Scattering Displacements (1989) W. Doster

IN 13: D_2O-hydrated myoglobin, less water, PDT Fit



Water Coupled Protein Motions

why elastic scattering?

low cross section
200 – 600 mg sample

simple analysis:
like Guinier SANS
$I_{el} = \exp -(1/3Q^2 R^2)$

however:
several transitions!

Is Protein Dynamics Relevant to Enzyme Catalyis? MD Simulations

Structural Fluctuations from Combination of QE Neutron Scattering and MD Simulation (since 1988)

Physica B 156 & 157 (1989) 437-443
North-Holland, Amsterdam

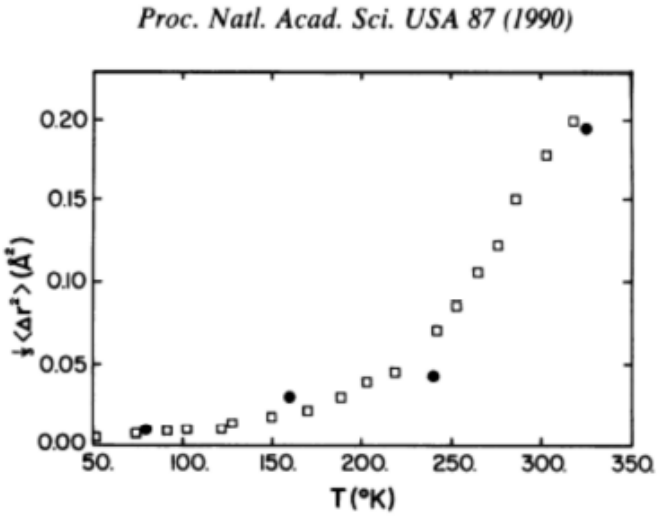
CHAPTER 11 MACROMOLECULES AND BIOLOGICAL SYSTEMS

INTERNAL DYNAMICS OF GLOBULAR PROTEINS: COMPARISON OF NEUTRON SCATTERING MEASUREMENTS AND THEORETICAL MODELS

Jeremy SMITH', Krzysztof KUCZERA',*, Bruce TIDOR', Wolfgang DOSTER', Stephen CUSACK and Martin KARPLUS'

'Chemistry Department, Harvard University, 12 Oxford St., Cambridge, MA 02138, USA
**Technische Universitiit Miinchen, Physik-Department E13, D-8046 Garching, Fed. Rep. Germany*

3E. M. B. L., cl01 *ILL, Avenue des Martyrs, 156X, 38042 Grenoble Cedex, France*
invited paper



nature 337 Feb. 1989

Dynamical transition of myoglobin revealed by inelastic neutron scattering

Wolfgang Doster*, Stephen Cusack† & Winfried Petry‡

* Physik Department E13, Technischen Universität München, D-8046 Garching, FRG
† EMBL Grenoble Outstation, Institut Laue-Langevin, 156X, 38042 Grenoble Cedex, France
‡ Institut Laue-Langevin, 156X, 38042 Grenoble Cedex, France

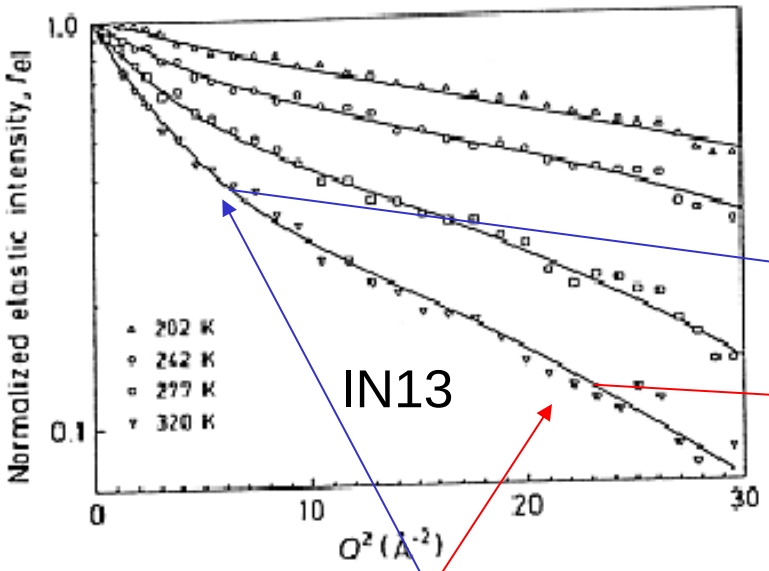
ca 1000 citations

four main novel features of bio-NS:

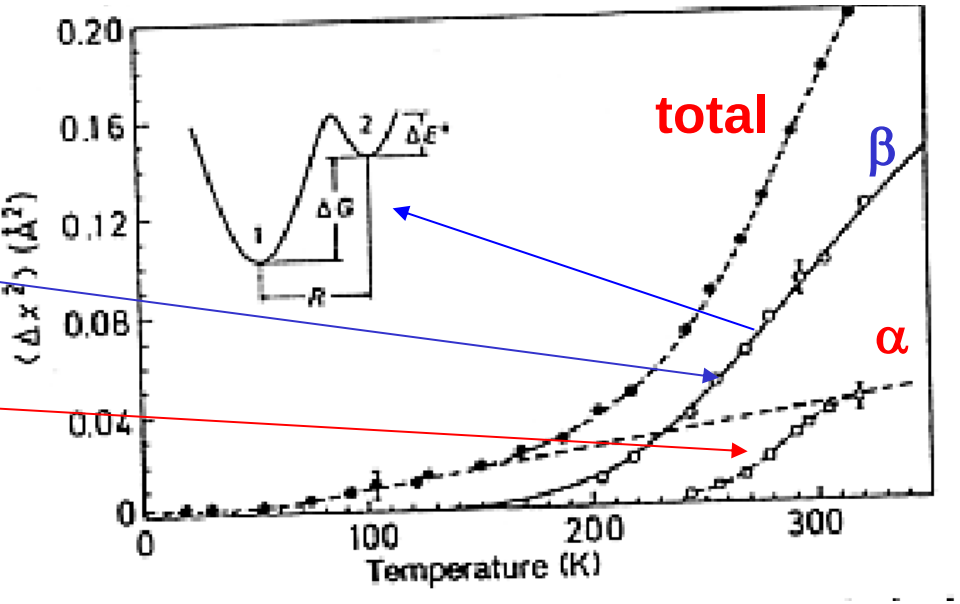
- study D_2O-hydrated proteins not solutions: reduce solvent scattering and protein diffusion, low T
- combination of BS and TOF,
- elastic and inelastic analysis: Dynamical Transition
- very wide temperature, Q and frequency range

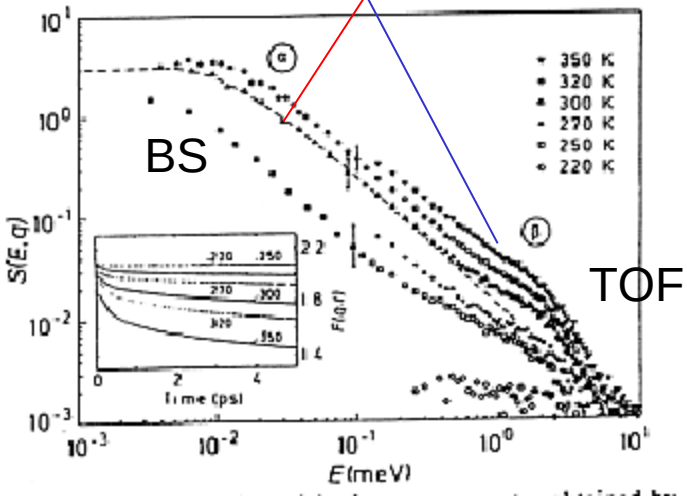
Protein Dynamical Transition: Neutrons Scattering

Nature (**1989**) with S. Cusack and W. Petry



IN13





BS

TOF

1) EISF(Q) is not Gaussian
2) types of molecular displacements:
a) fast torsional jumps (β): A(T)
b) slow Gaussian diffusive motions, water coupled (α): τ(T)
3) 2-component non-Lorentzian spectra combination of TOF and BS

Elastic Scattering Analysis
Effect of Environment: dry, glassy, hydrated: IN 13

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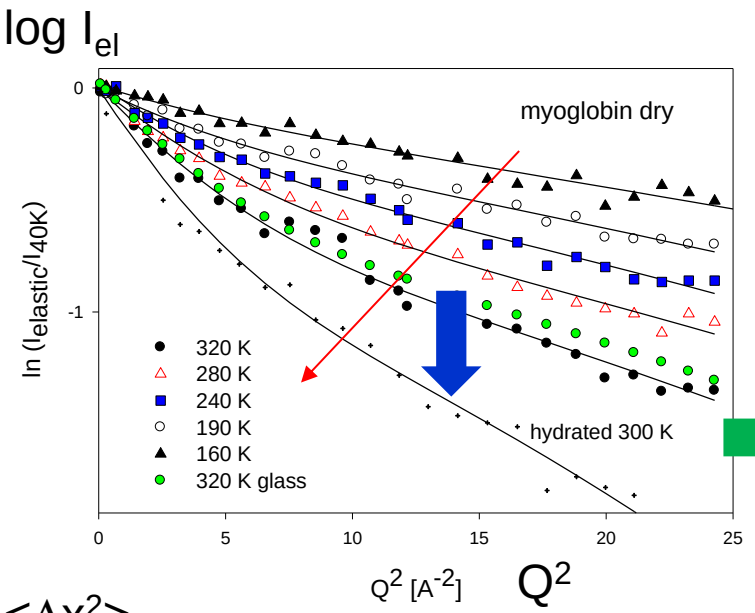
myoglobin: two processes



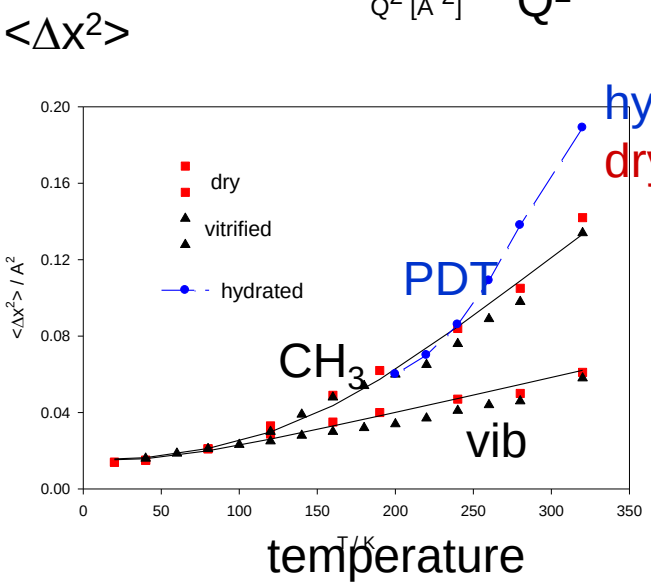
Elastic incoherent structure factor:

two dynamic components:

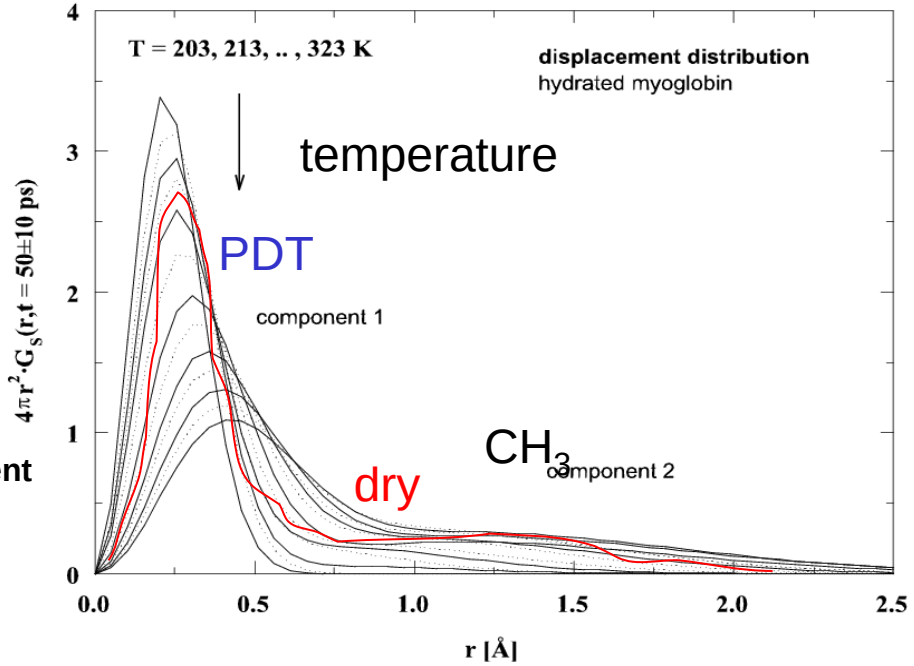
-internal rotational jumps

- water coupled small scale translations (PDT)

Displacement Distribution Function

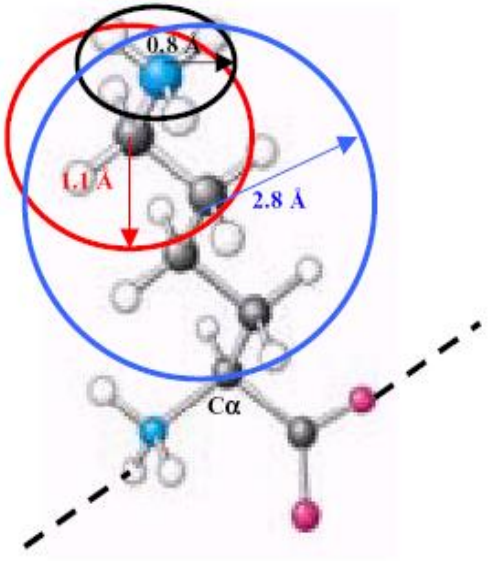


second moment



protein dynamics and function: 2 major components I, S

Doster et al. Nature 1989
Doster, Settles BBA 2005
Doster in Dynamics of Soft Matter 2011

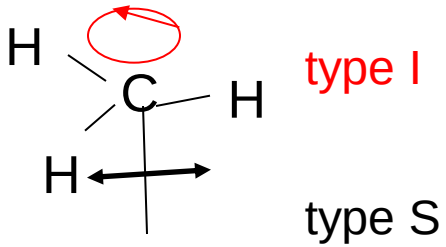


1) internal: independent of solvent environment

non-Gaussian, discontinuous jumps,
torsional jumps of side chains, methyl groups
and main chain dihedral transitions

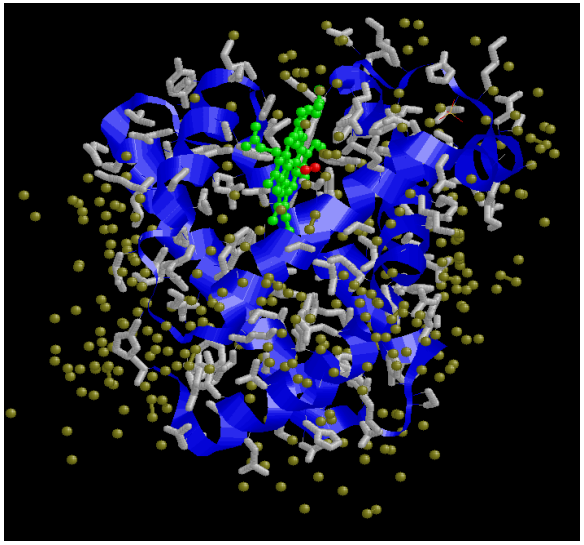
2) surface coupled motions: solvent viscosity

Gaussian, water-assisted, small scale
continuous translational displacements
α- relaxation of hydration water
responsible for **PDT**

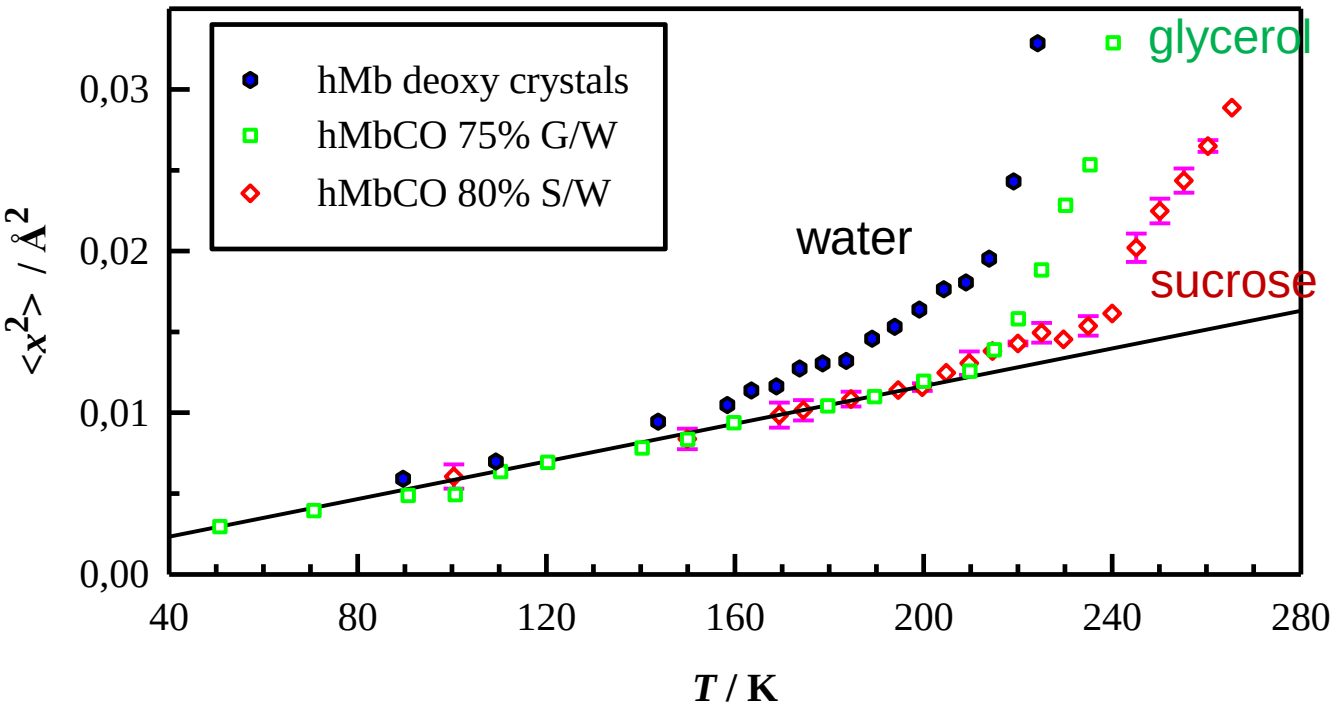


Heme displacements (Mössbauer)

Lichtenegger, Doster, Vogel et al. 1999 Biophys. J. 76,414
Effect of solvent: water, 75% glycerol/water, 80 % sucrose water



hydrated myoglobin



Heme PDT: onset temperature increases with solvent viscosity

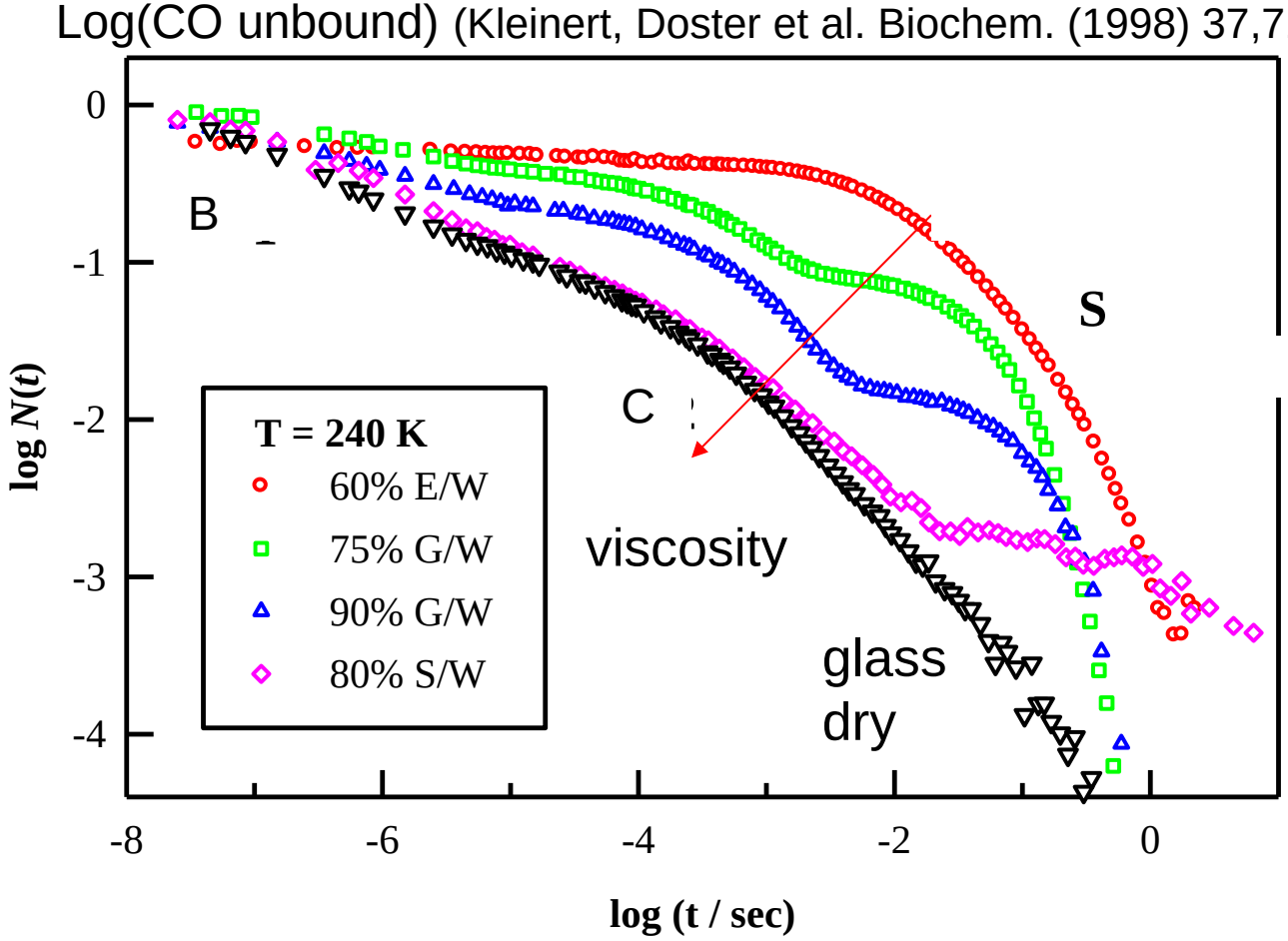
heme displacements are dominated by solvent motions near surface, less by internal protein dynamics!!

Onset temperature: solvent $\tau_c \cong$ 200 ns

Protein Function

Kinetics of CO- binding to myoglobin

different solvents

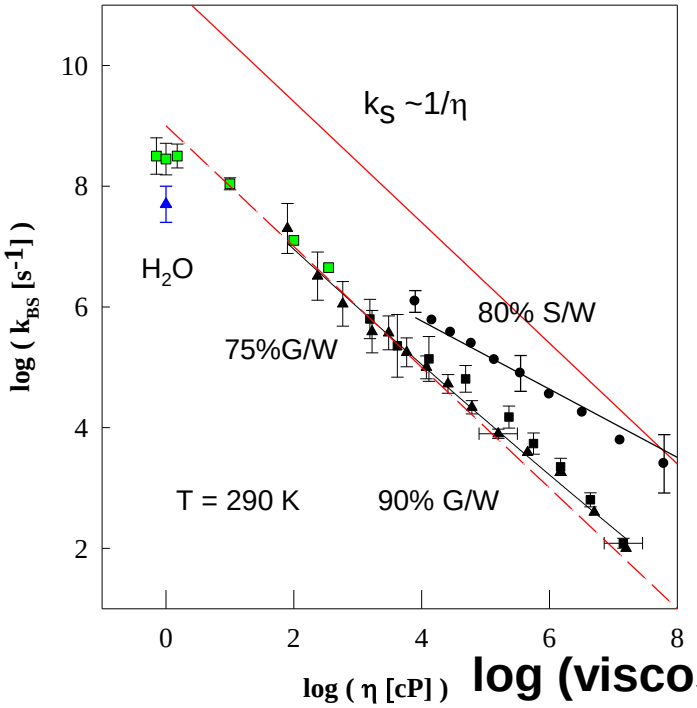


multiple binding steps: B,C (internal) S (from solvent)

Two kinds of elementary reaction rates

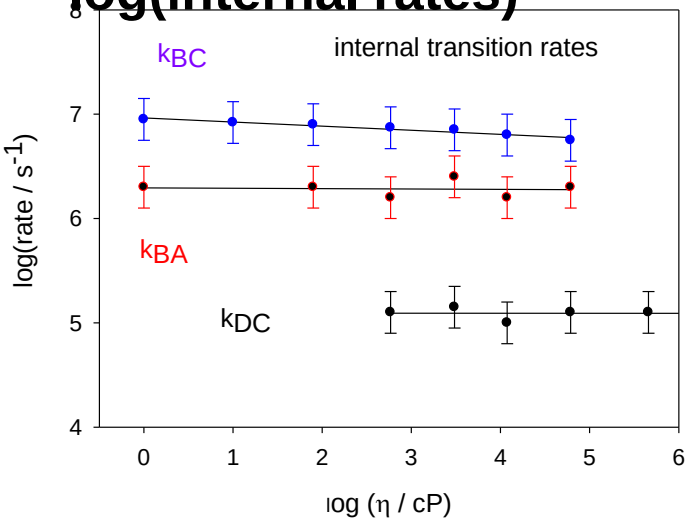
(Kleinert, Doster et al, Biochem. 1998), Doster Longeville in Dynamics of Soft Matter, Neutron Applications, Ch. 8 (2012) Springer

log (ligand exit rate B->S)



log (viscosity)

log(internal rates)



Log (viscosity)

Results:

Two types **of functional motions**

Type I: internal,decoupled from solvent viscosity, hydration

Type S: coupled to protein surface viscosity, hydration

30 years of PDT

What is a Protein Dynamical Transition? GT and PT

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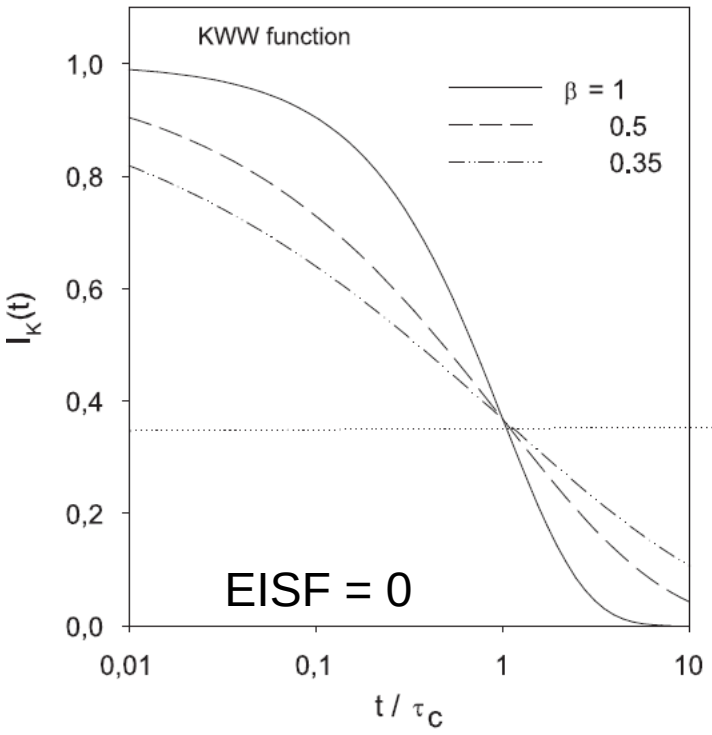
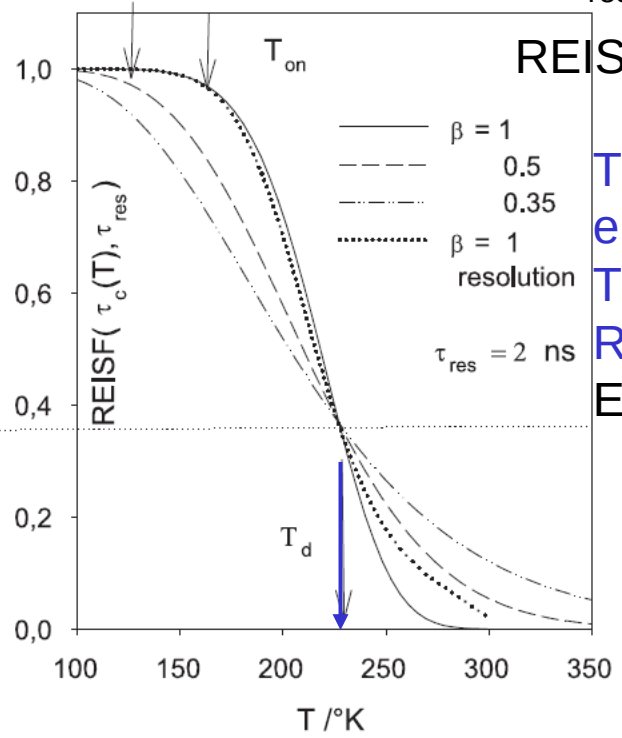
NS: Incoherent intermediate scattering function I(Q,t,T, τ_c)

versus time, T,Q fixed



versus temperature, τ_{res}, Q fixed



REISF(τ_c(T)/ τ_{res})

Transition of elastic intensity, T_D defined by REISF (T_D) = 1/e

EISF = 0

PDT (1)transition of the elastic intensity versus T at fixed resolution

(2) plus: molecular process couples to solvent viscosity

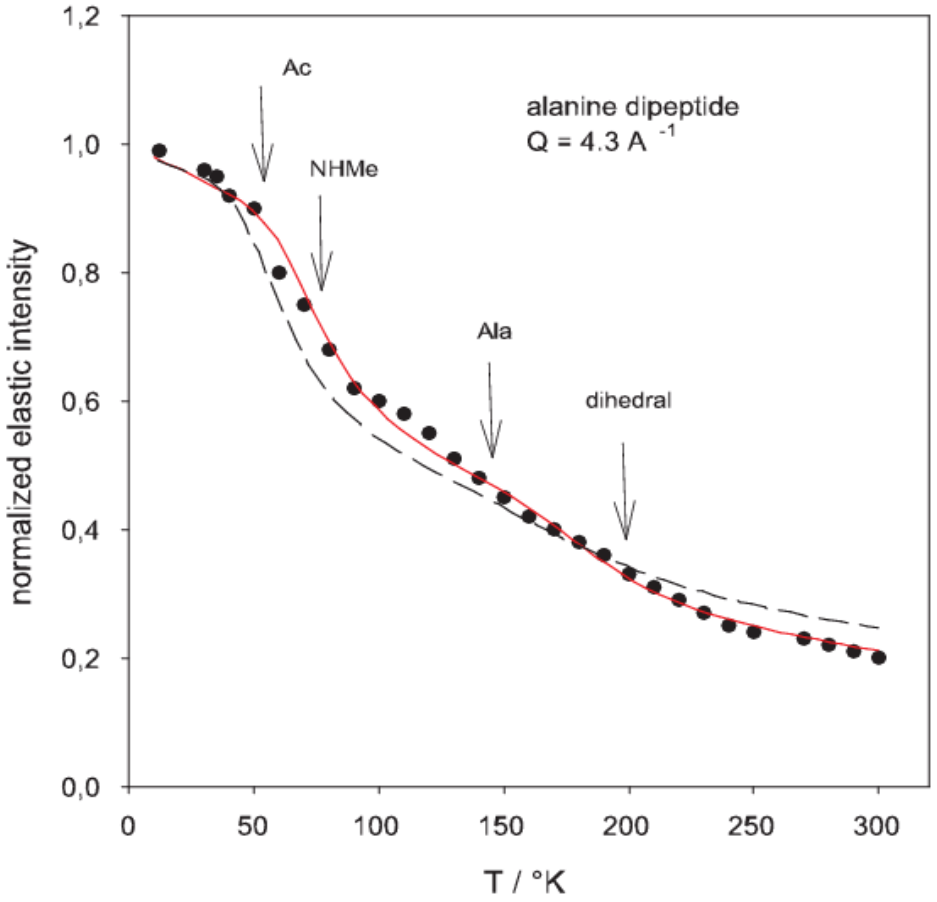
(3) no structural transition at T_D like the FST

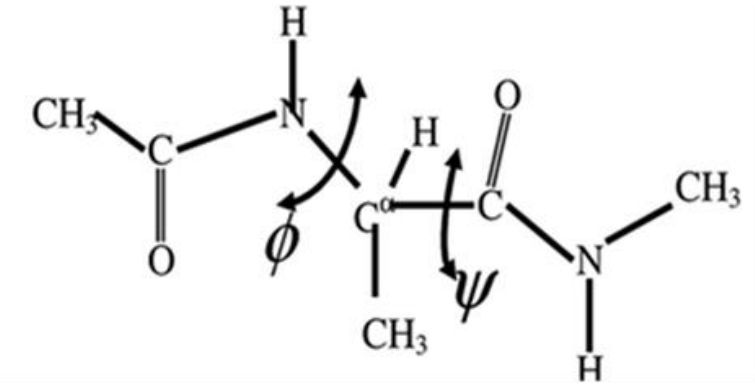
„Dynamical“ Transition in the Alanine Dipeptide in Crystal, Q, τ

an elastic transition occurs for each molecular process

Elastic Intensity (IN 13) and Model Fit with 3 methyls and dihedrals at Q = 4,3 A

Smith, Doster et al. JCP1996
Doster et al. JCP 2013





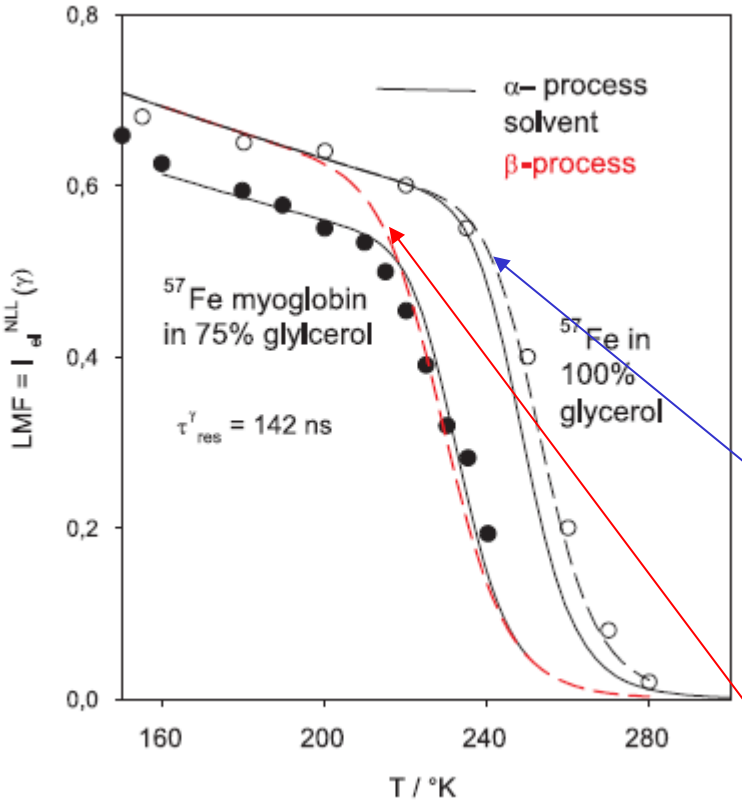
AcMe dihedr AlaMe NHMe

for each methyl group a step is observed in the elastic scattering, this is not a PDT because it is unrelated to solvent and viscosity

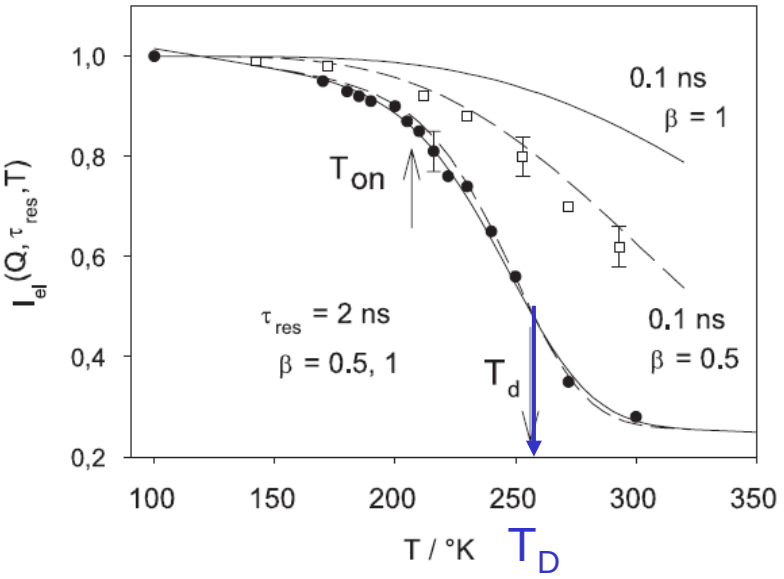
PDT α or β relaxation?

Mößbauer Effect :
LMF, Fe in glycerol and myoglobin
Doster et al. JCP 2013, Champeney, Woodhams, Nagel 1986

NBS: SPHERES, IN13:
protein hydration water H_2O
perdeuterated PC



045105-10 Doster, Nakagawa, and Appavou

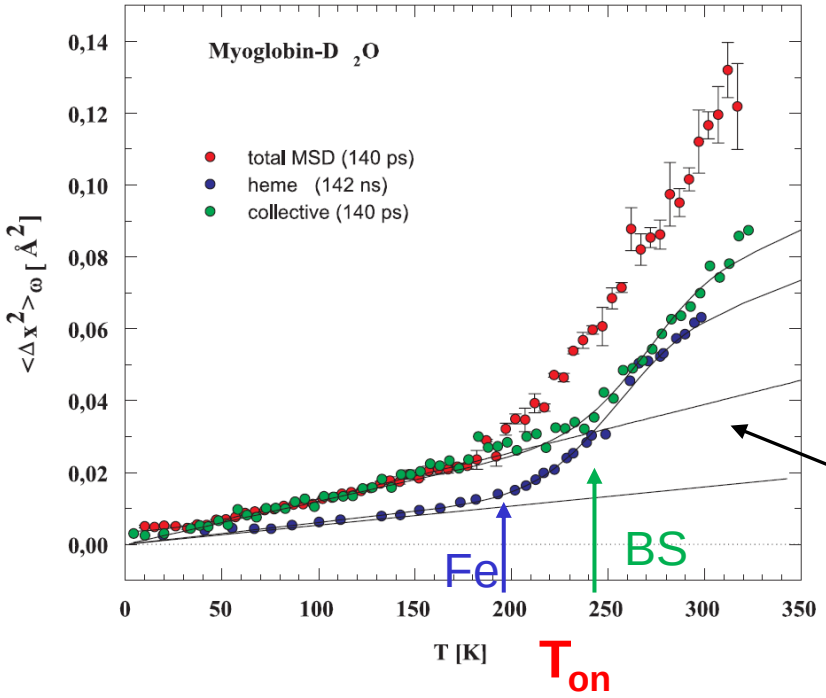


PDT is related to solvent α-relaxation and viscosity
β-relaxation irrelevant

LMF = QEISF(Q) / (1 + $\tau_c(T)/\tau_{res}$) +EISF(Q)

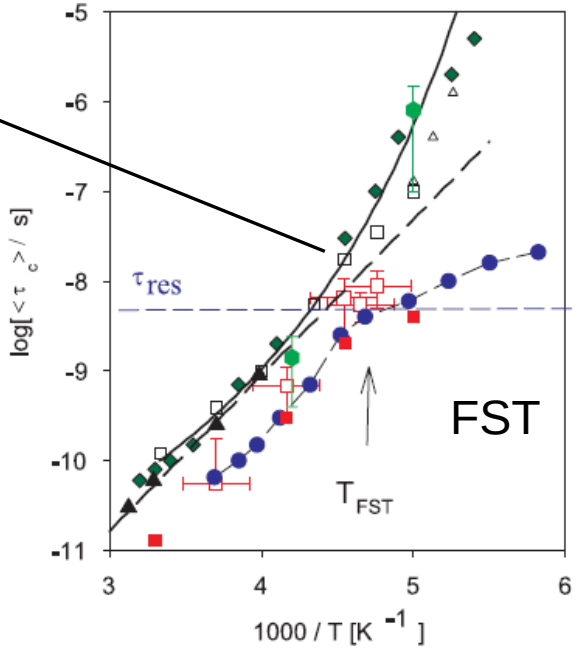
τ_{res} = 142 ns input: $\tau_c(T)$, DR, NBS

Comparison of heme and structural displacements of myoglobin



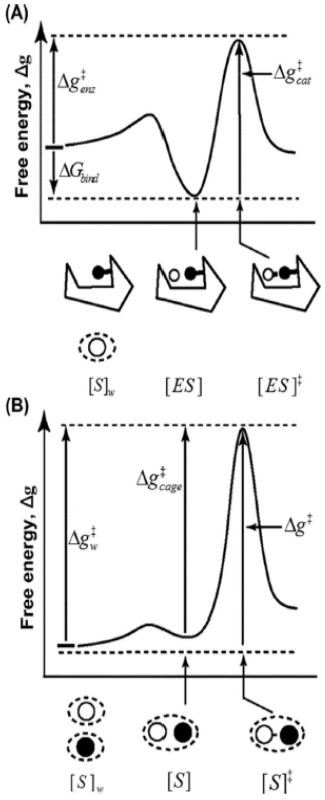
heme Mössbauer 200 K (140 ns)
protein BS IN13 240 K (100 ps)

input fit: hydration water $\tau_C(T)$
SPHERES, Doster et al. PRL 104(2010) 98101, JCP 139, 45105 (2013)



PDT(Fe, H) reflects collective motions coupled to hydration water, observed at different time scales.
major heme motions are induced by solvent consistent with MD (Karplus et al.)

Is Protein Dynamics Essential to Enzyme Catalysis?



enzyme catalysed reaction

reaction in solution

Arieh Warshel and R.P. Bora
Defining and quantifying the role of dynamics in enzyme catalysis
J.Chem. Phys. 144, 180901 **(2016)**

Arieh Warshel: Nobel Prize in Chemistry 2013, MD simulations of protein function

„The complex energy landscape is not the reason of the catalytic power of proteins! flexibility is interfering with rate acceleration, catalysis requires rigid stereo-chemical structures“

however:
this applies only to the catalytic step

the entrance and exit of ligands into enzymes require structural flexibility!!

Summary and Main Results of NBSS of Proteins

- Two kinds of molecular processes in proteins identified:

1) internal rotational transitions of side chains, CH_3
independent of protein environment and viscosity
protein internal ligand displacements and reactions

2) solvent viscosity dependent displacements
coupled to translational diffusion of hydration water
ligand exchange between solvent and active site

- PDT of heme iron and H $<\Delta x^2(T)>$ explained consistently:
common cause: α-relaxation of solvent at different resolution
- no β- relaxation in the hydration shell above 200 K
- no need to invoke a Frauenfelder energy landscape
to explain the back-scattering spectra of protein dynamics and water!!

Collaborators

M. Settles	Physik E 13 TU-München
M. Diehl	
F. Post	
Th. Kleinert	
X. Cai	
S. Busch	JNC Garching FRM II
J. Wuttke	
M.S. Appavou	
A. Gaspar	European Patent Office
H. Nakagawa	JAEA, Tokai
S. Longeville	LLB CEA Paris

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