Hydrophobic Effects on Water

(Hydrophobic and Hydrophilic interactions in water-methanol and lysozyme solutions evidenced by NMR experiments)

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1 - Water-Methanol relaxation times

2 - Water-Lysozyme magnetization intensities

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Erice-Water 16

1 - Water-Methanol relaxation times

The NMR spin-lattice (T_1) and spin-spin relaxation times (T_2).

Actual and literature data.

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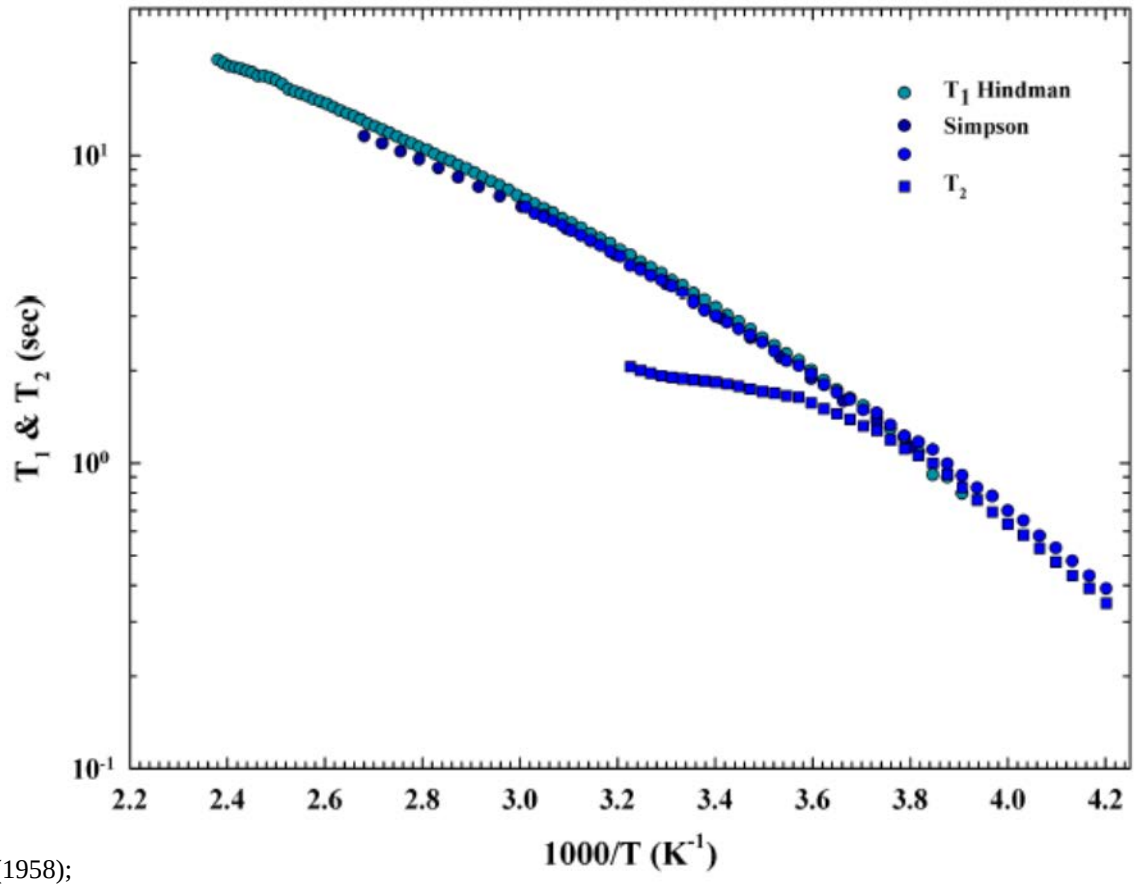

J.C. Hindman, J. Chem. Phys . 60, 4488 (1974);
J.H. Simpson and H.Y. Carr, Phys. Rev. 111, 1201 (1958);

These relaxation times can be explained at nuclear level by two approaches developed by Bluembergen, Purcell and Pound (BPP) and Kubo, Tomita and Hubbard (KTH) that allows the calculation of correlation times, coming from the effects of the thermal motion of the magnetic nuclei upon the spin-spin interaction.

N. Bluembergen, E.M. Purcell and R.V. Pound, Phys. Rev., 73, 679 (1948). R. Kubo and K. Tomita, J. of the Physical Soc. of Japan, 9, 888 (1954). P.S. Hubbard, Phys. Rev. 131, 275 (1963).

In the BPP approach to NMR spectroscopy, the system exposure to the perturbing radiation tends to upset the original equilibrium state, by equalizing the population of the various quantum levels.

The effect of the nuclear motion on the dipolar broadening, can be treated as a sort of random modulation of dipolar field owing to the Brownian motion of the atomic nuclei.

The spin-lattice relaxation time, T_1, represents essentially how long one must wait, after the application of the constant field H_0, for the establishment of thermal equilibrium. It is :

$$(1/(T_1))=(3/2)\gamma^4\hbar^2 I(I+1)\sum_i J_i(i\nu_0)$$

where γ is the gyromagnetic ratio of proton, $2\pi\hbar$ is the Planck constant, I the spins energetic levels, $\omega_0/2\nu=\nu_0$ is the Larmor frequency, and J_i the spectral components.

By assuming that the intermolecular effects are neglegible and the system is dominated only by the dipole-dipole interaction, T_1 is:

$$(1/(T_1))=(3/2)\ \gamma^4\hbar^2 I(I+1)[J_1(\nu_0)+(1/2)J_2(2\nu_0)] \qquad (1)$$

On looking to the latter equation $1/T_1$ can be divided into two parts $(1/T_1=(1/T_1)_{\nu_0}+(1/T_1)_{2\nu_0})$ corresponding to the single spin- and double spin-inversion processes, each part is expressed by the corresponding J_N.

In the water case by considering only the effect on a given proton of its nearest neighbor (the other proton in the H_20 molecule) and by assuming:

i) **the molecule as rigid**
ii) **that the orientation of the vector connecting the two protons varies randomly, without preferred directions,**

BBP proposes that the measured ^1H T_1 is determined by interaction fluctuations induced by molecular motions characterized by only a correlation time τ_c, coming from the effects of the thermal motion of the magnetic nuclei upon the spin-spin interaction (the local Brownian motion closely related to the characteristic time of the Debye's theory of polar liquids). Hence:

$$1/T_1 = (3\gamma^4 \hbar^2/10r^6)(J_1 + J_2) \qquad (2a)$$

with

$$J_1 = (\tau_c/(1 + (\omega_0 \tau_c)^2)) \quad \text{and} \quad J_2 = (4\tau_c/(1 + 4(\omega_0 \tau_c)^2)) \qquad (2b)$$

where r represents is the inter-proton distance.
There is also a relation between τ_c and the nuclear Larmor frequency if this latter quantity is much less than $1/T_1$ the perturbations caused by the local field nearly average out, and the width of the resonance line, in frequency will be $\sim \tau_c$.

Lately, the NMR absorption has been reconsidered by Kubo&Tomita for a general expression for the ω-dependent susceptibility $\chi_H(\omega)$, in terms of a quantum-statistical method based on the theory of irreversible process by considering the system Hamiltonian as the sum of a "secular" terms that commutes with the unperturbed terms plus a "non-secular" part of the perturbation.

Secular and non-secular contributions lead to different spectral functions J_i with different relaxation times τ_i . Only in the case of an exponential system relaxation there is a single relaxation time τ_c.

In the ideal case of isotropic nuclear arrangements, the two kinds of spin inversion processes have different weights for the spin-lattice relaxation: the secular one is represented by the Eq. 1, whereas the non-secular broadening effect is

$$1/T'_1 = \gamma^4 \hbar^2 I(I+1)[15/4 J_1(\nu_0) + 3/8 J_2(2\nu_0)] \quad (3)$$

Generally, $T_1 \neq T'_1$.

The model give also a proper relation for the spin-spin relaxation time T_2 ($1/T_2 = \gamma^4\hbar^2 I(I+1)\tau_0 + 1/T'_1$) that in the ideal case in which all the relaxation times corresponding to the different spectral functions are identical to τ_c, is:

$$1/T_2 = (3\gamma^4 \hbar^2/20r^6)(3\,\tau_c + 5J_1 + J_2/2) \quad (4)$$

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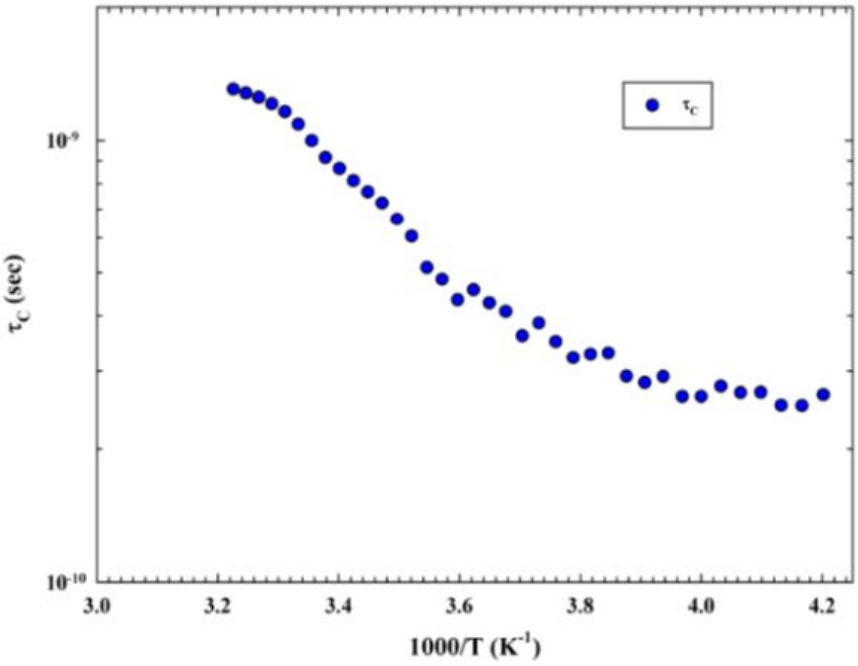
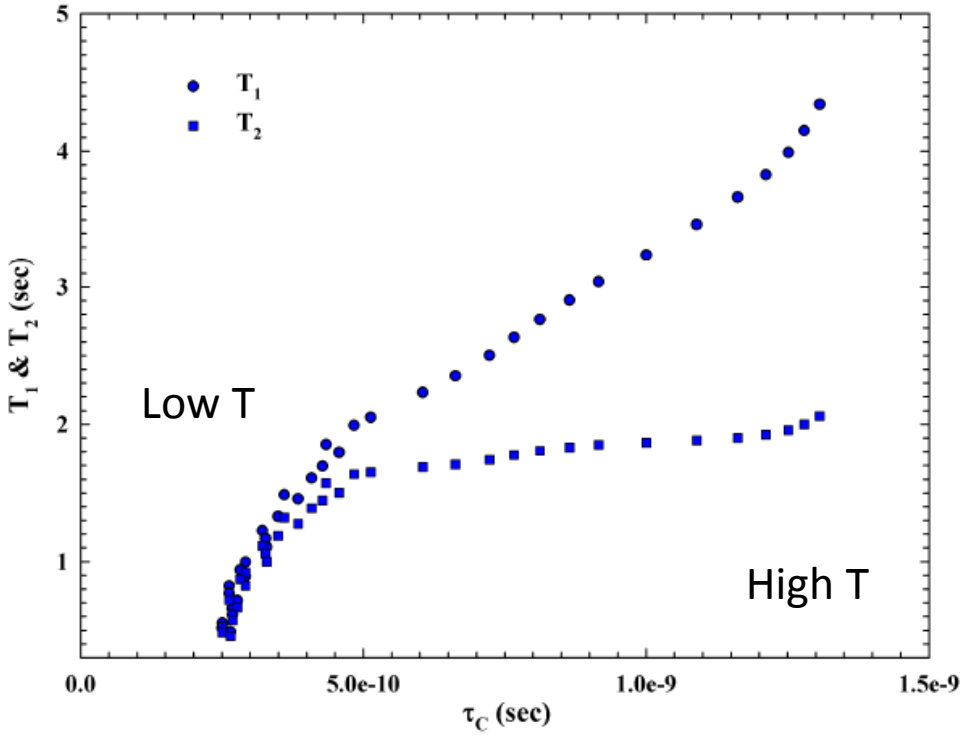


The Arrhenius plot of τ_c evaluated by means of the Eq. 2 and 4



The Bluembergen, Purcell and Pound analisys of T1 and T2 vs τ_c

The KT approach have suggested a new way to treat the T_1 data* by assuming that to the observed protons relaxation contribute three terms: two dipolar (intermolecular (D-inter), and intramolecular (D-Intra)) and the nuclear spin-rotation interactions (SR):

$$1/T_1 = 1/T_1^{D\text{-}inter} + 1/T_1^{D\text{-}intra} + 1/T_1^{SR}$$

The rotational nuclear if compared with the dipolar one is smaller; for water case may be evaluated only for $T \geq 350$ K (strongly contributes to the system dynamics in the region of the critical point) **. Therefore:

$$1/T_1^{D} = 1/T_1^{inter} + 1/T_1^{intra}$$

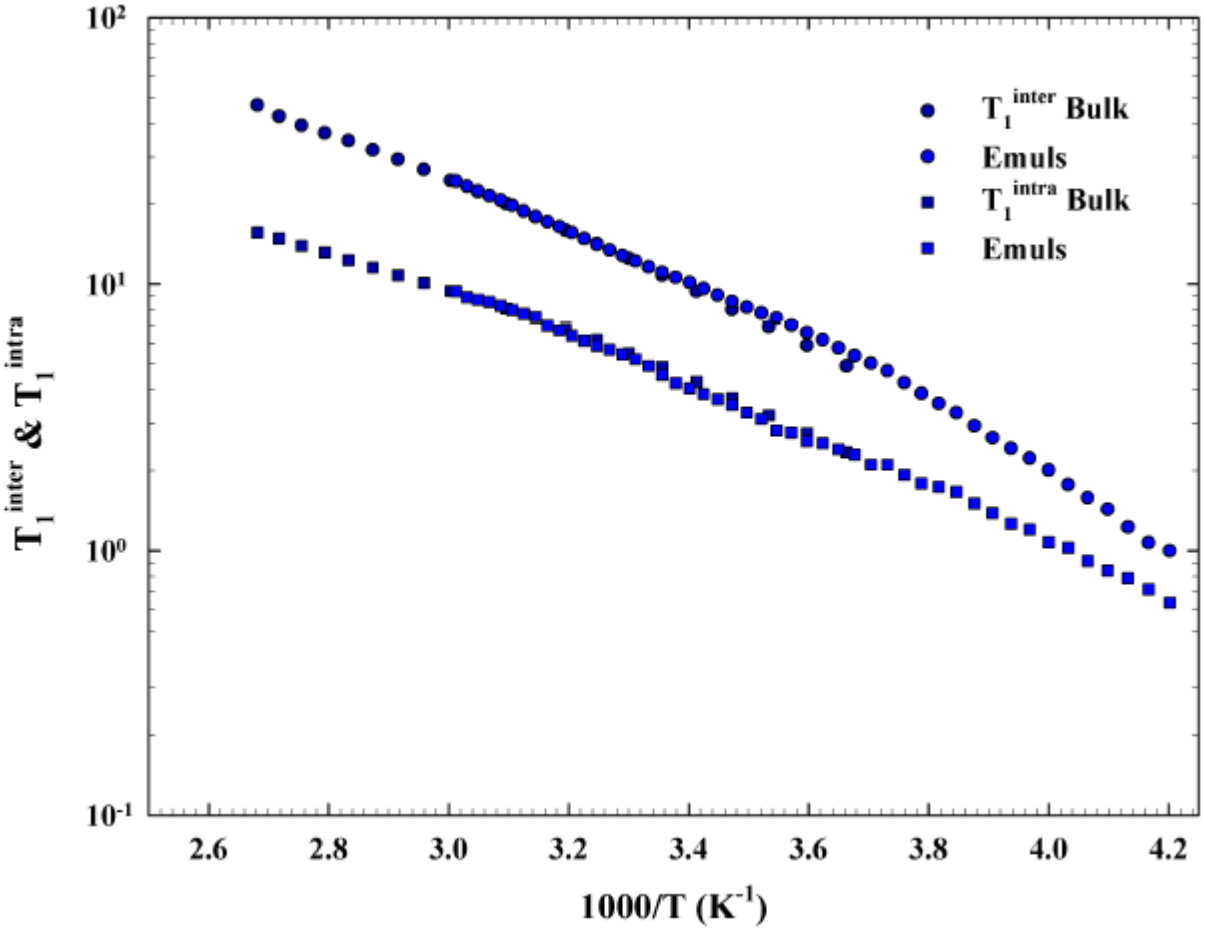
On these bases Hubbard proposed that the intermolecular dipolar contribution is strongly related with the molecular self diffusion coefficient D_S :

$$1/T_1^{inter} = N\pi\gamma^4\hbar^2/(5aD_S))[[1+0.233((b/a))^2+0.15((b/a))^4+ \ldots]$$

Thus having D_S such a contribution can be evaluated and from the measured T_1 we can have $T_1^{D\text{-intra}}$, that under the condition $1/\omega_0 \gg \tau_i$, can be written *** in terms of the system viscosity , or as a molecular rotational time τ_θ :

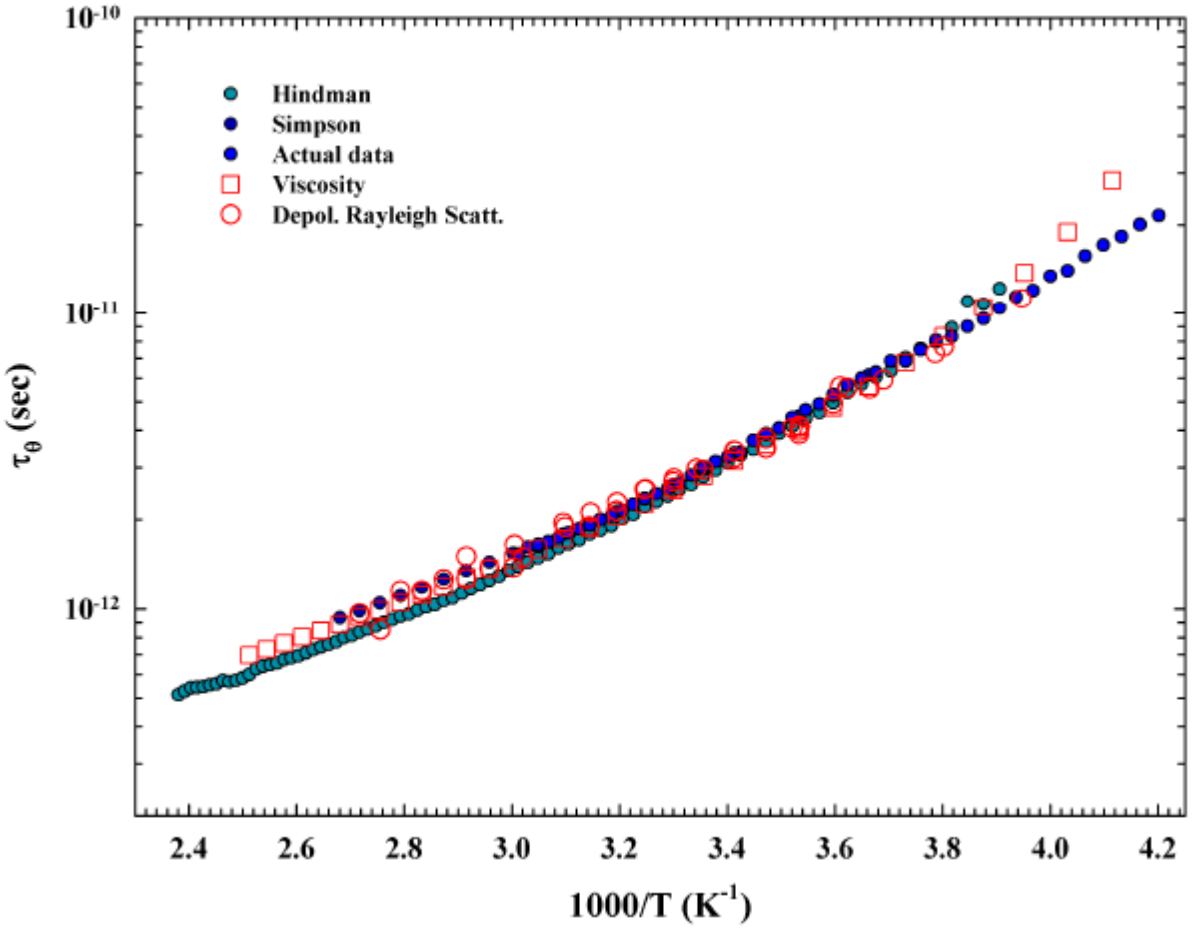
$$1/T_1^{D\text{-}intra} = (3\gamma^4\hbar^2/r^6)\,\tau_\theta$$

*D.W.G. Smith and J.G. Powles, J. Mol. Phys. 10, 451 (1966); P.S. Hubbard, Phys. Rev. 131, 275 (1963); **T. DeFries and J. Jonas, J. Chem. Phys. 66, 896 (1977); *** E. Lang and H.D. Ludemann, J. Chem. Phys, 67, 718 (1977).

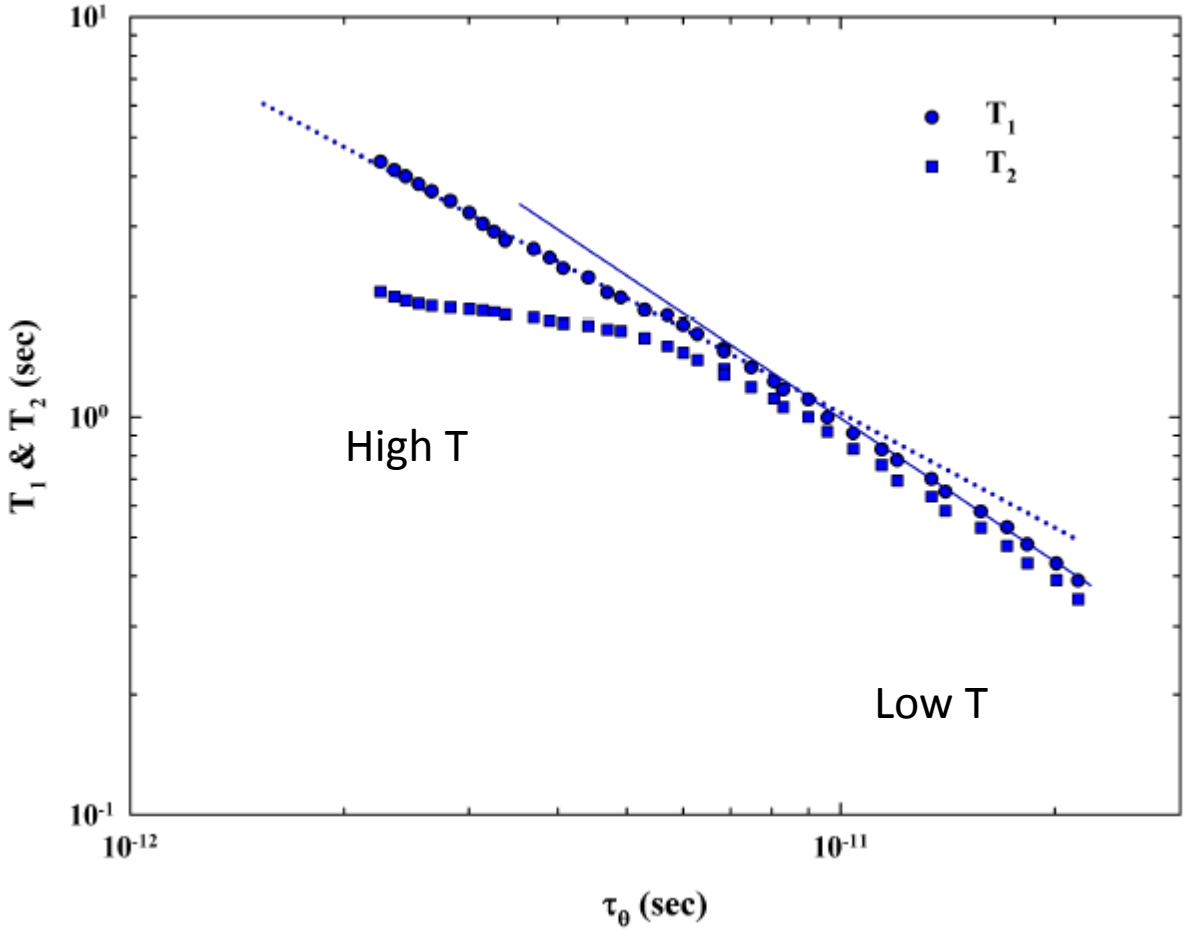


The T_1^{inter} and T_1^{intra} of bulk and emulsioned water.

The bulk and emulsioned water τ_θ coming from the present experiment and literature data in an Arrhenius plot; are also reported (properly scaled) the viscosity and the relaxation times coming from depolarized Rayleigh Scattering experiments.



J.C. Hindman, J. Chem. Phys . 60, 4488 (1974); J.H. Simpson and H.Y. Carr, Phys. Rev. 111, 1201 (1958); C. H: Cho et al, J.Phys. Chem. B 103, 1991 (1999; V. Mazzacurati et al. J. Chem. Phys. 93, 7767 (1990); M. Paolantoni et al. J. Chem. Phys. 127, 024504 (2007).



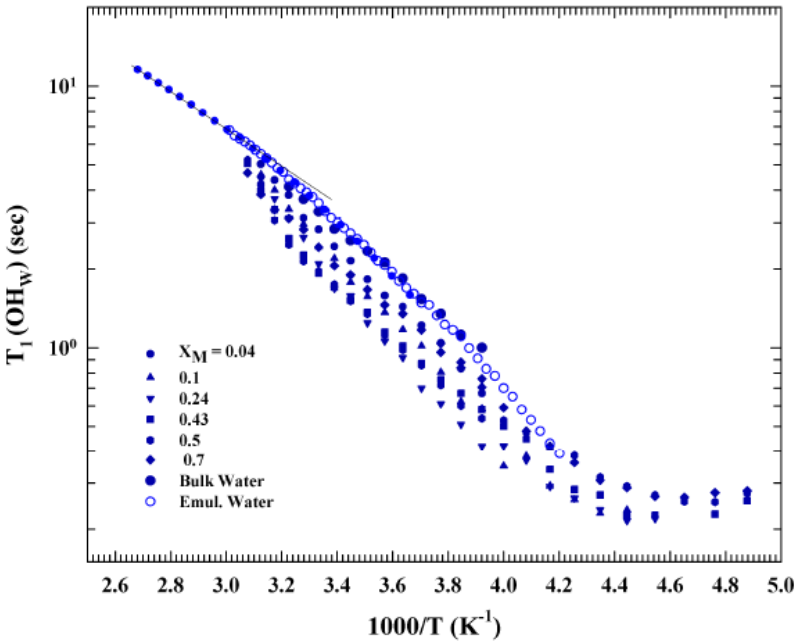
Full correlation in the low temperature regime dominate by the OH network, a well different behavior for T ≈ 270 where the HB life-time is of the order of picoseconds.

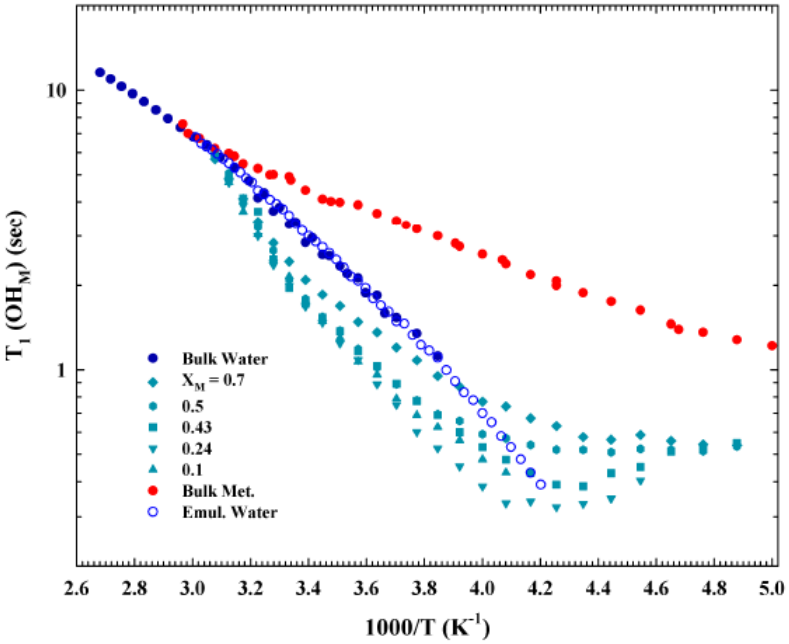
Water and Methanol

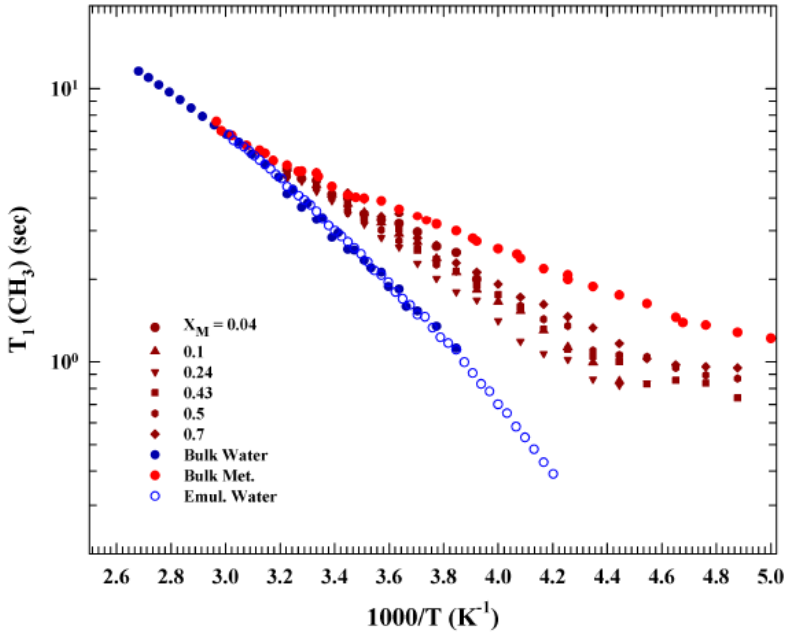
NMR allow to study separately the behaviors of the hydrophilic (hydroxyl) and hydrophobic parts of the same molecule (methyl):

OH water
OH methanol
CH3 methanol.

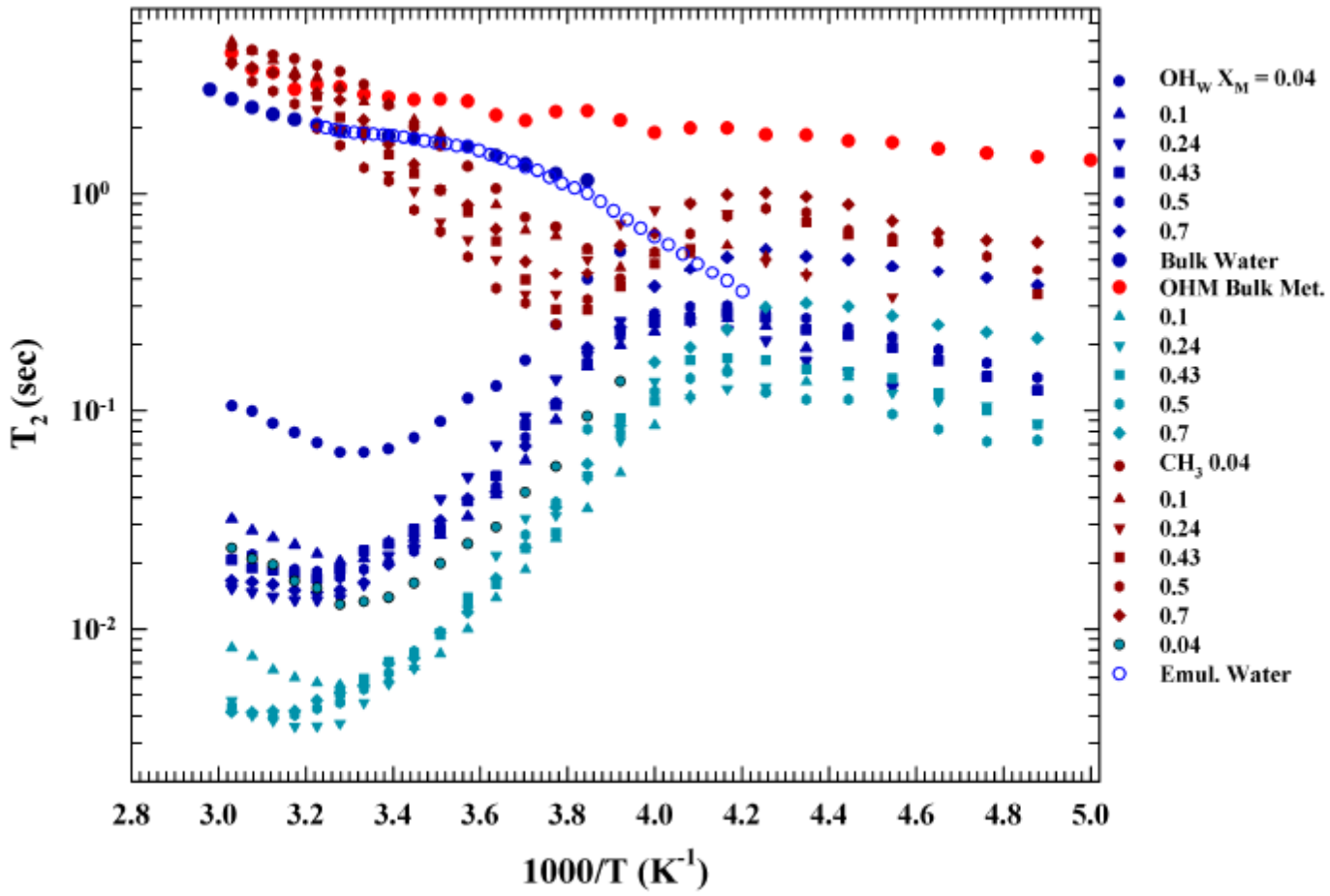
Here we report their evolutions in water-methanol mixtures as a function of the concentrations (methanol molar fraction X_M) and temperature.

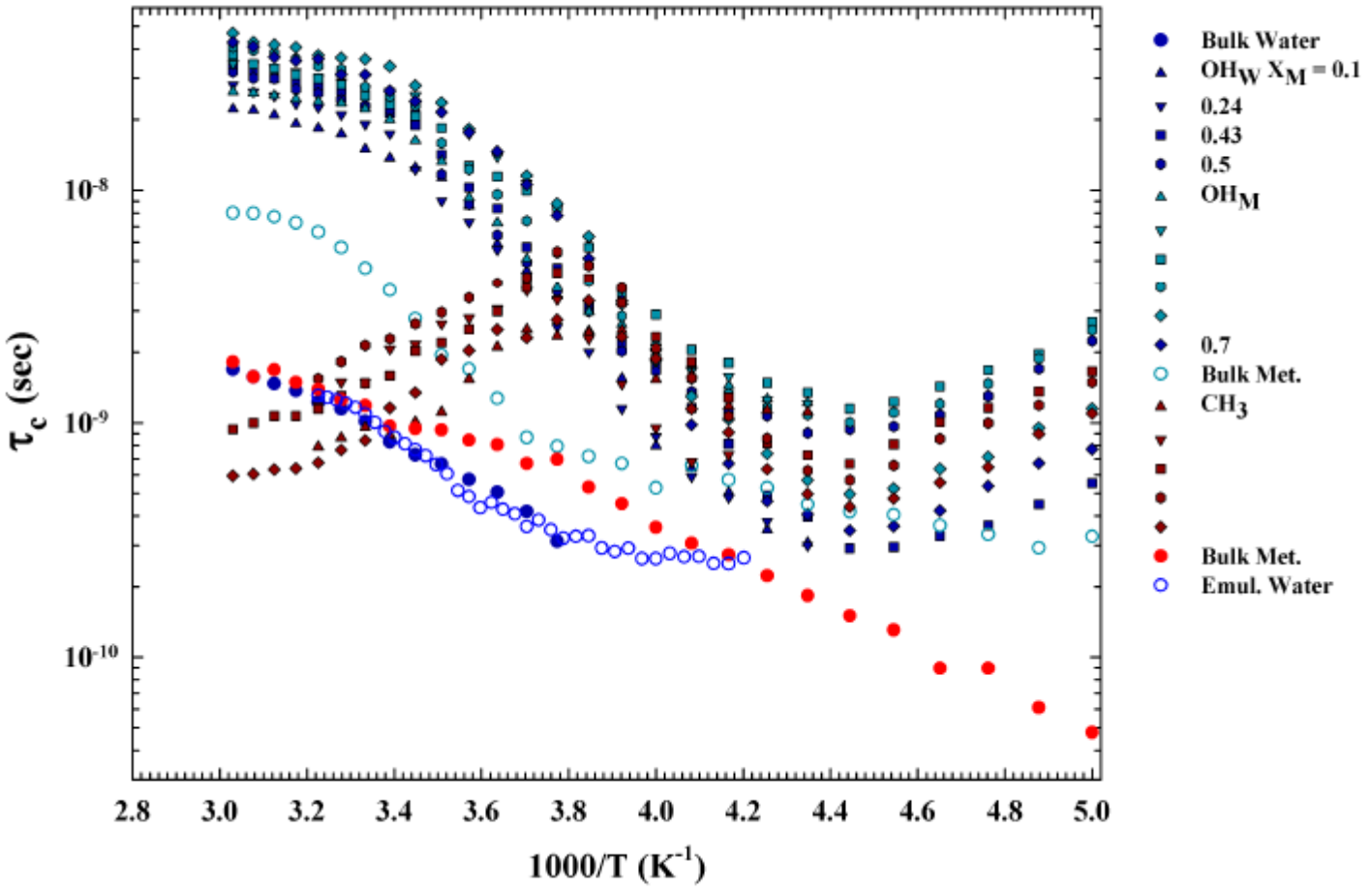




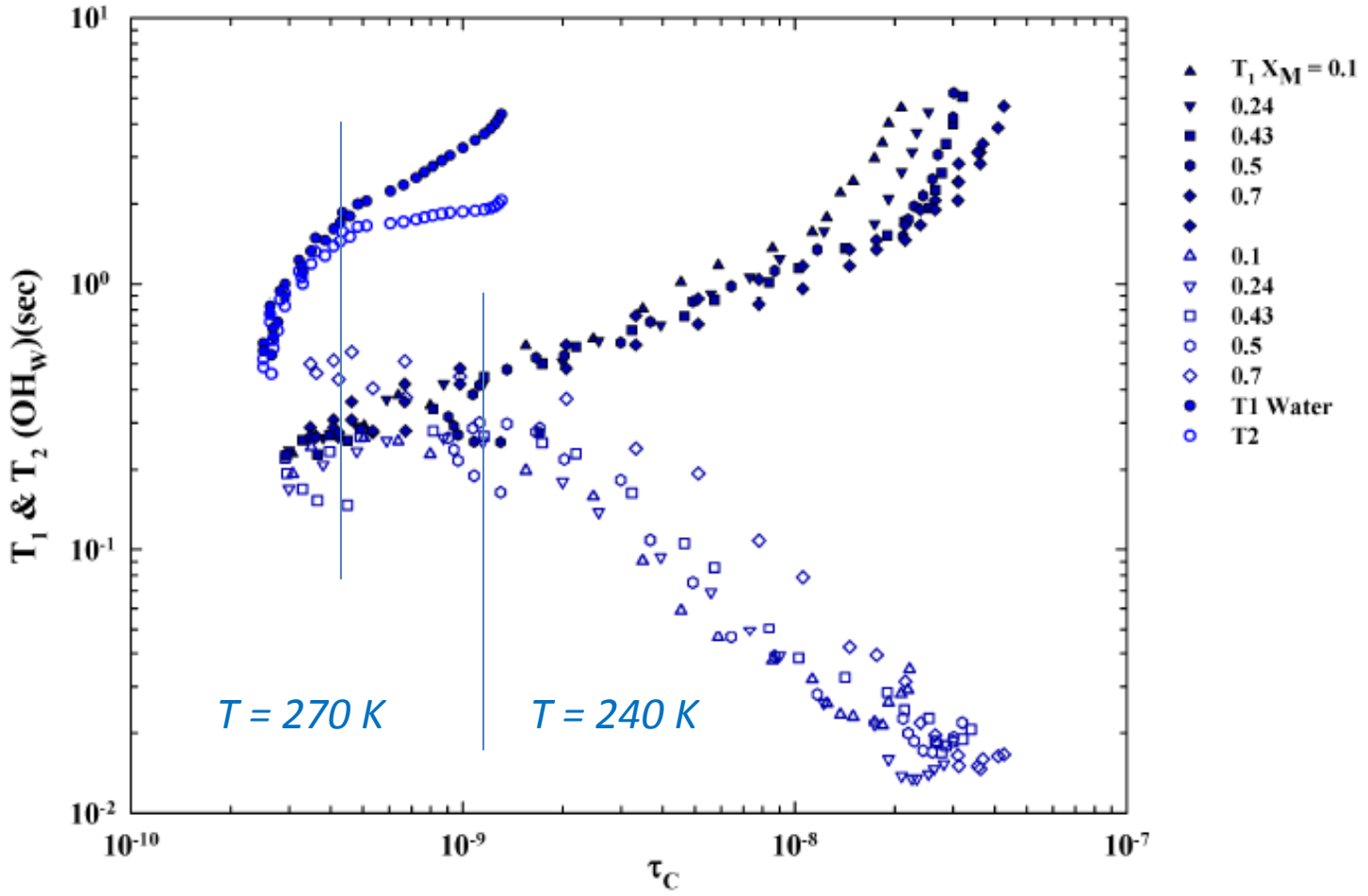


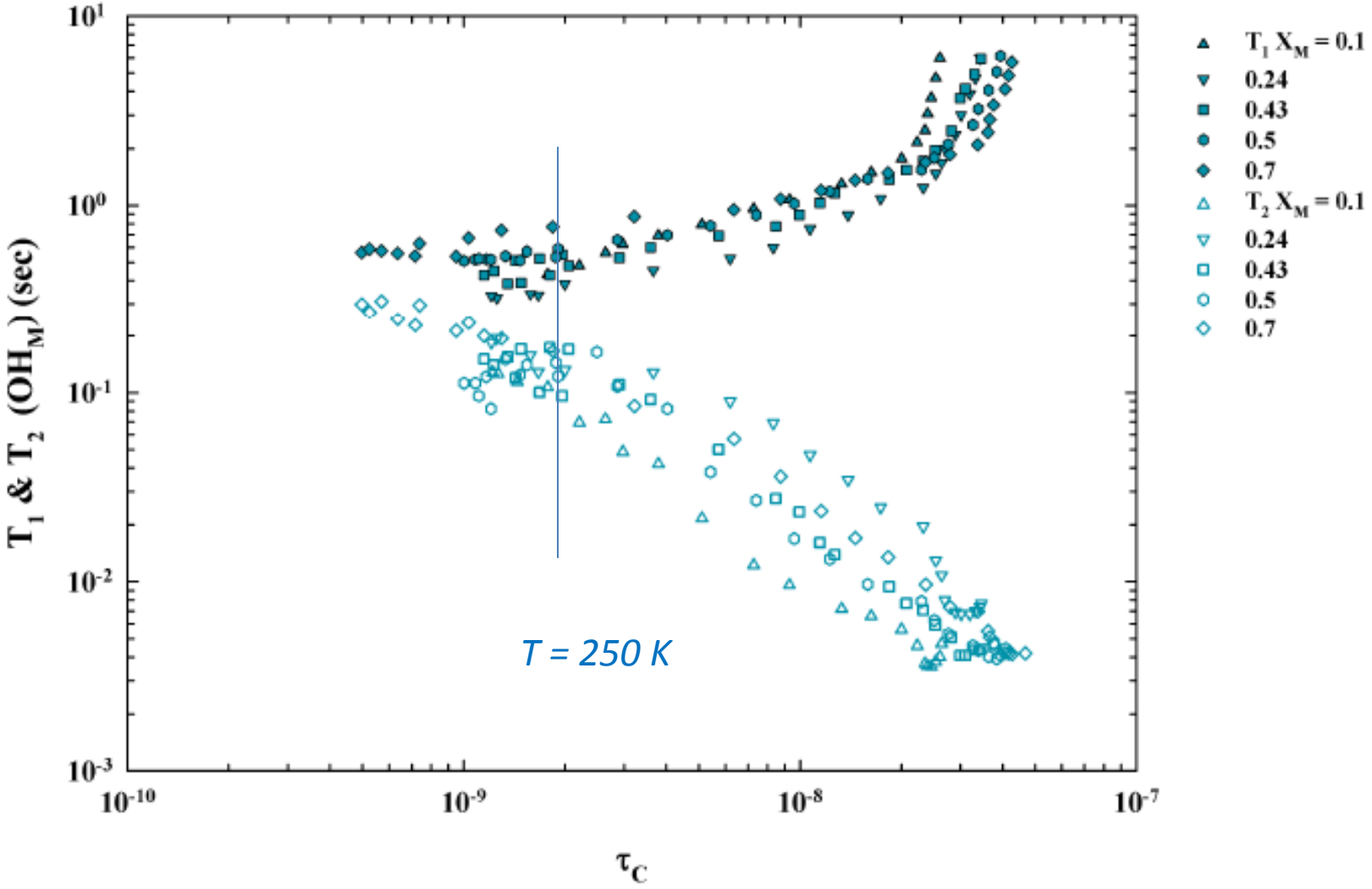
Erice-Water 16


T_2 (sec)
10^0
10^{-1}
10^{-2}
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
1000/T (K^{-1})
OH_W X_M = 0.04
0.1
0.24
0.43
0.5
0.7
Bulk Water
OHM Bulk Met.
0.1
0.24
0.43
0.5
0.7
CH_3 0.04
0.1
0.24
0.43
0.5
0.7
0.04
Emul. Water



Erice-Water 16


10^{1}
10^{0}
10^{-1}
10^{-2}
T_1 & T_2 (OH_W)(sec)
10^{-10}
10^{-9}
10^{-8}
10^{-7}
τ_C
T = 270 K
T = 240 K
T_1 X_M = 0.1
0.24
0.43
0.5
0.7
0.1
0.24
0.43
0.5
0.7
T1 Water
T2



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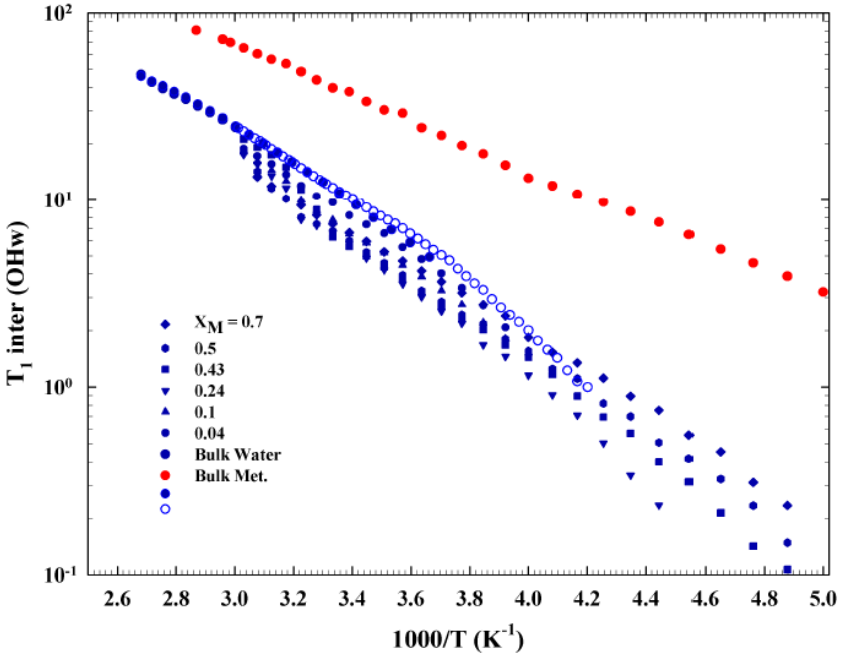

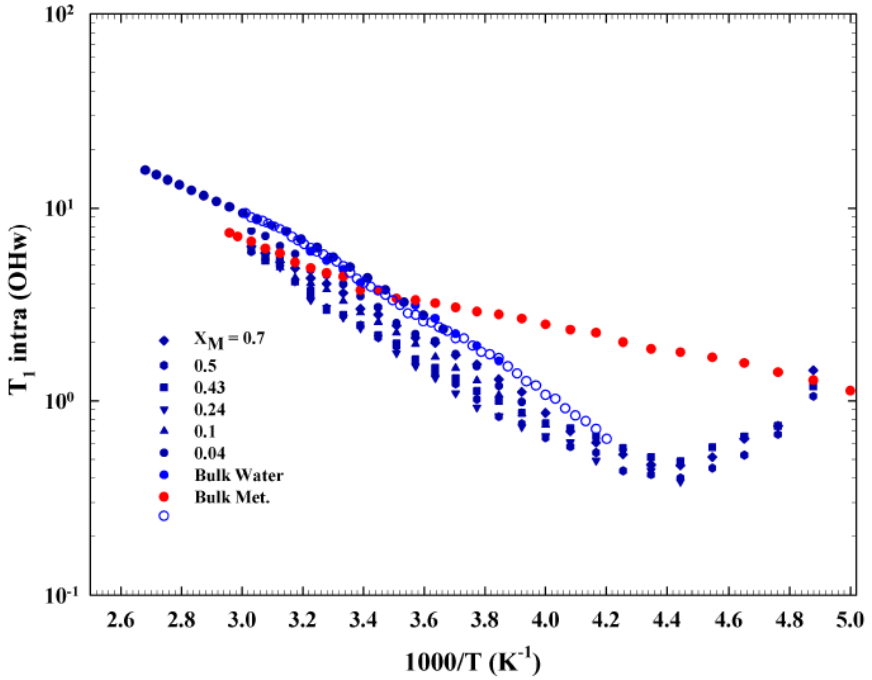

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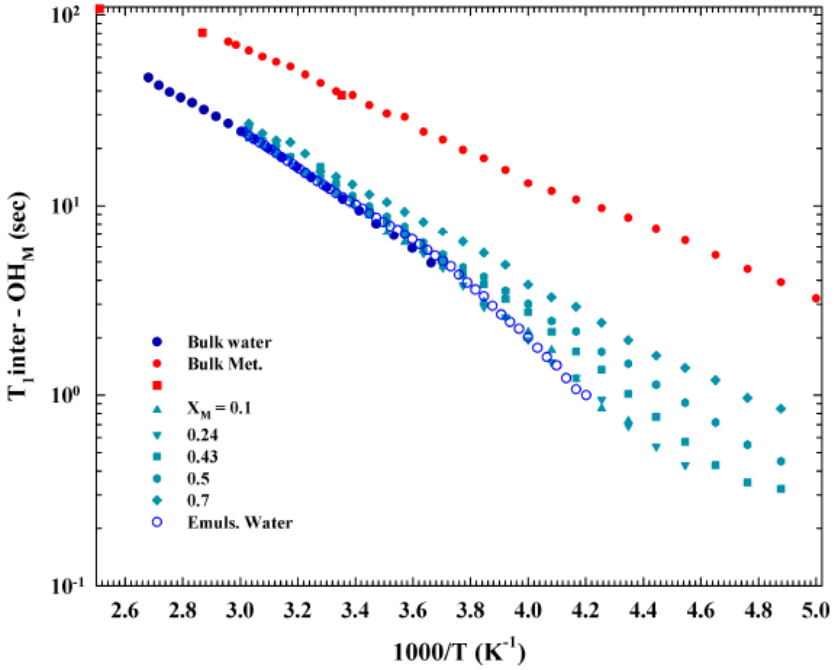

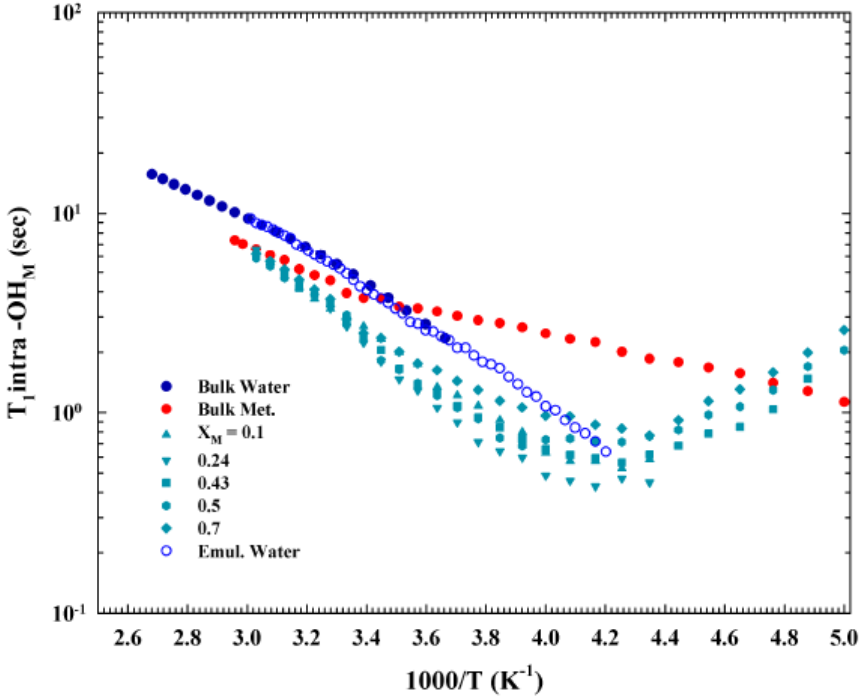

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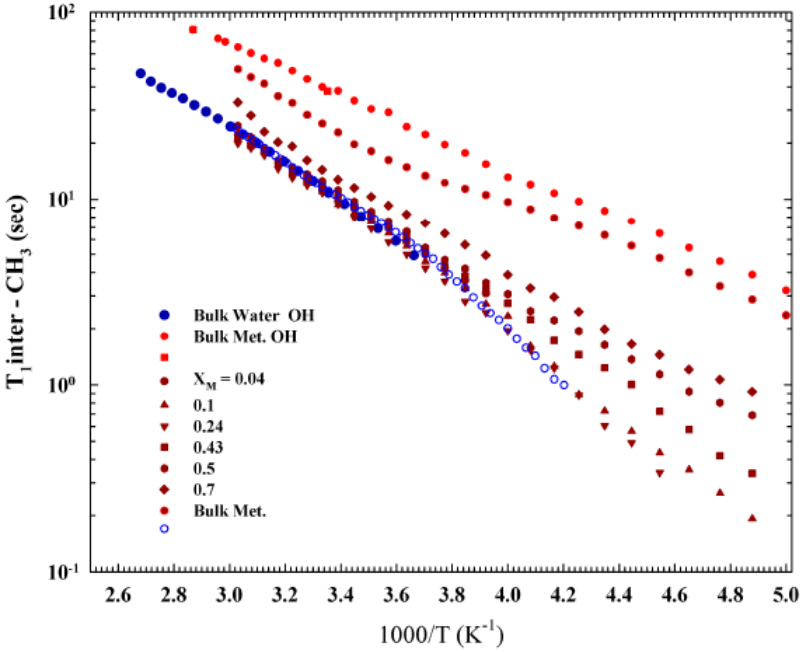

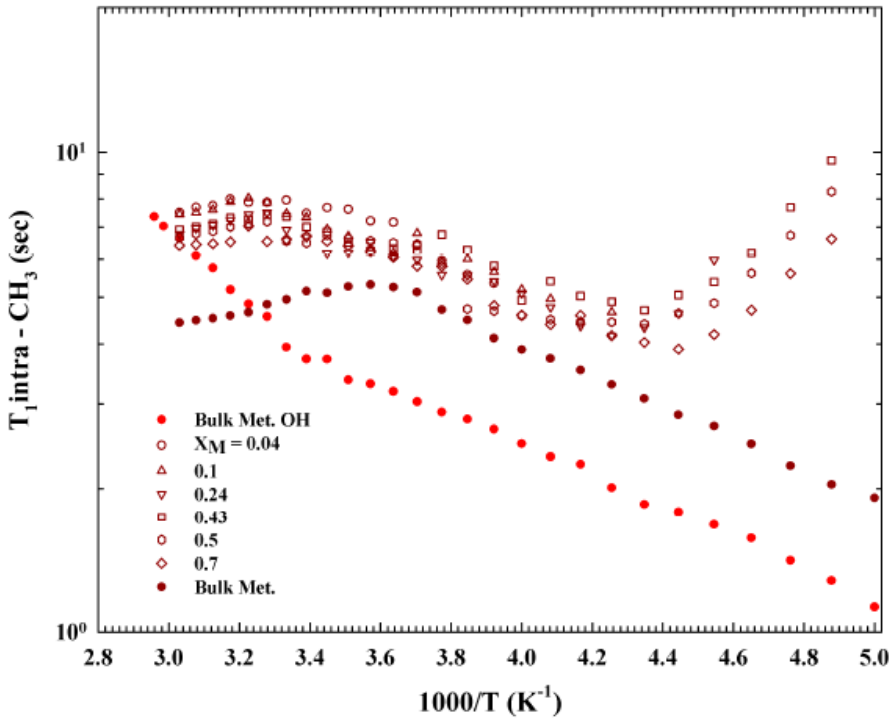

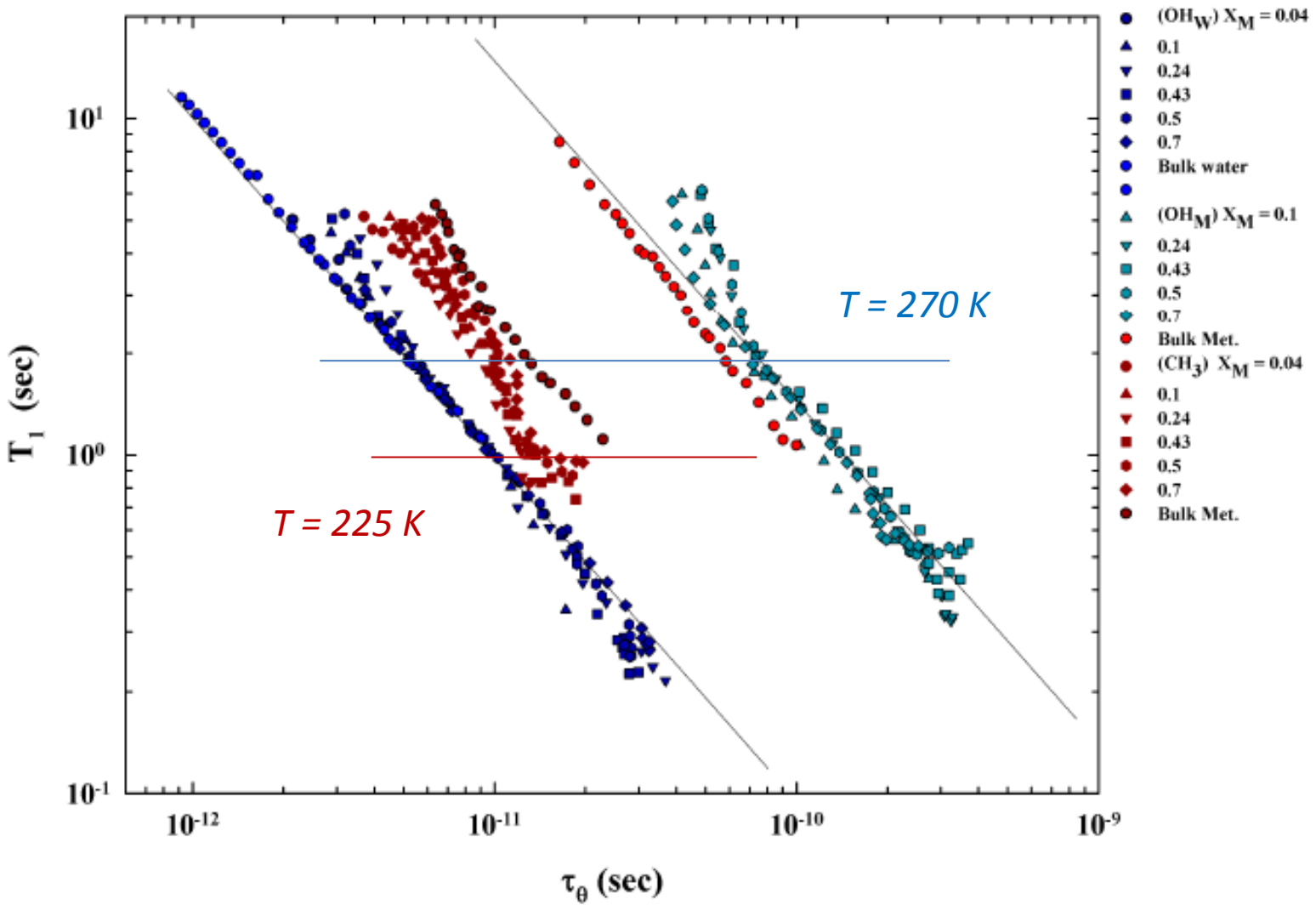

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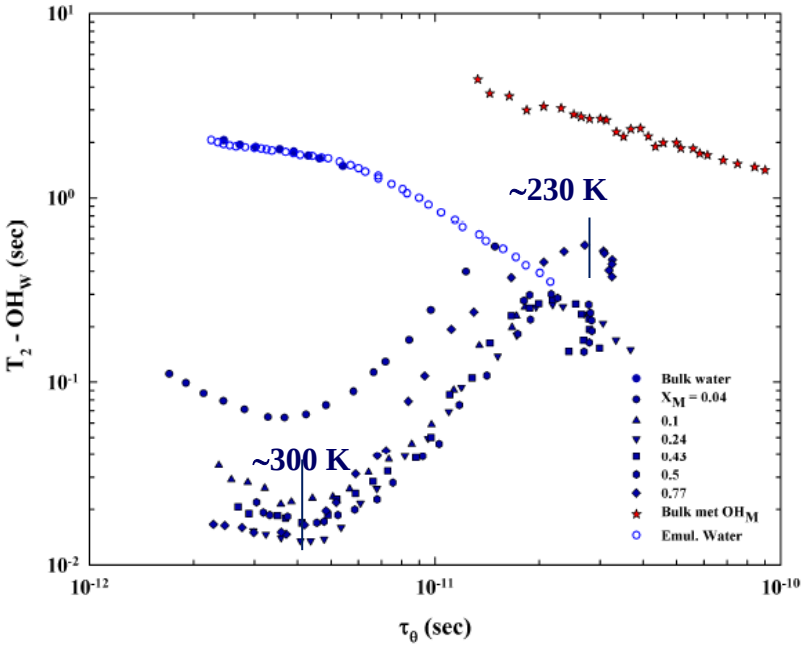
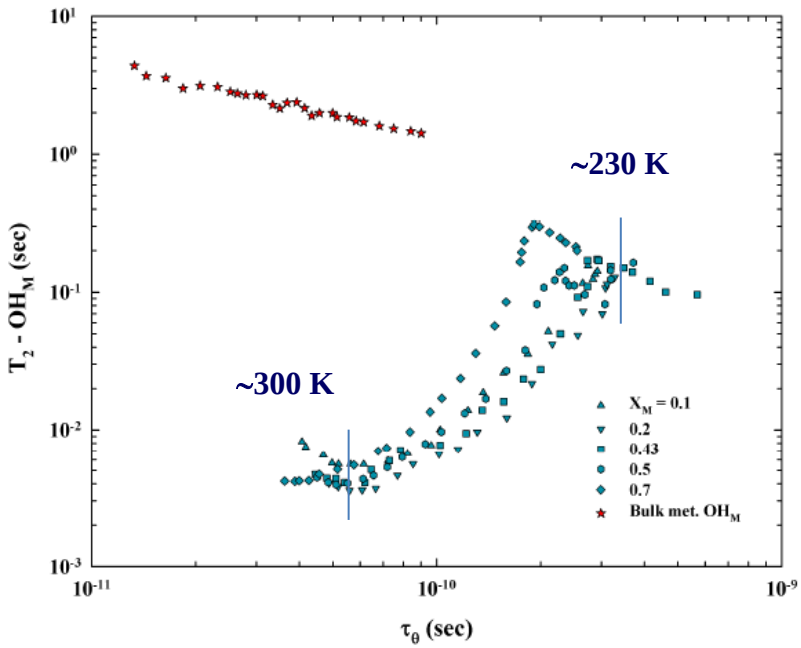
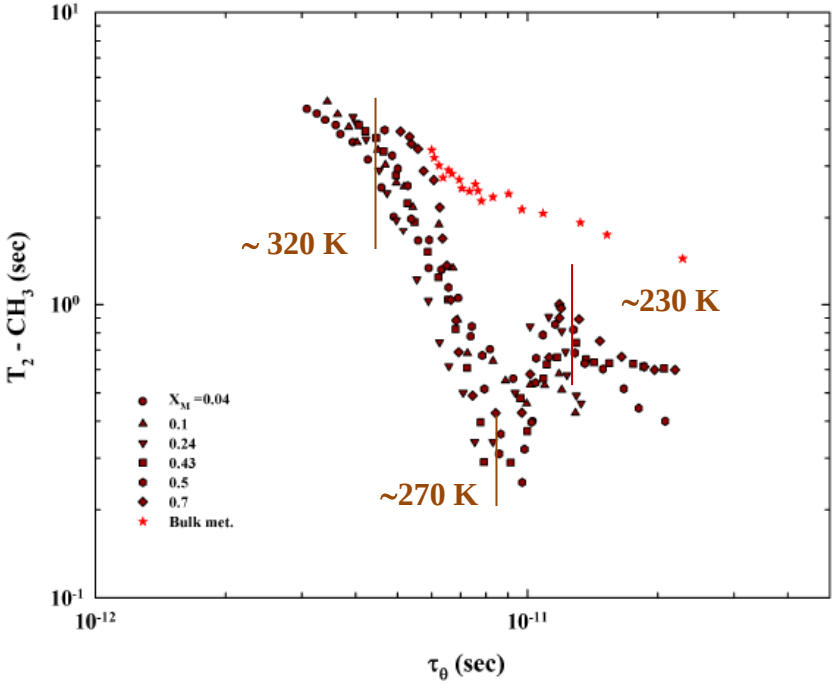






Erice-Water 16

2 - Water-Lysozyme magnetization intensities

The Amide-Water HB Interactions and the protein stability

HB stabilizes the amide functional groups and the configuration stability for hydrated peptides comes out by the Amide-Water HB strength*.

Stable configuration has two HBs, a water proton donor bond to the carbonyl oxygen (C-O) and an amide N-H proton donor bond to the water oxygen.

The peptide can be hydrated, either externally by a water molecule HB to the C = O backbone, or internally by forming a HB bridge between this group and the amide group.

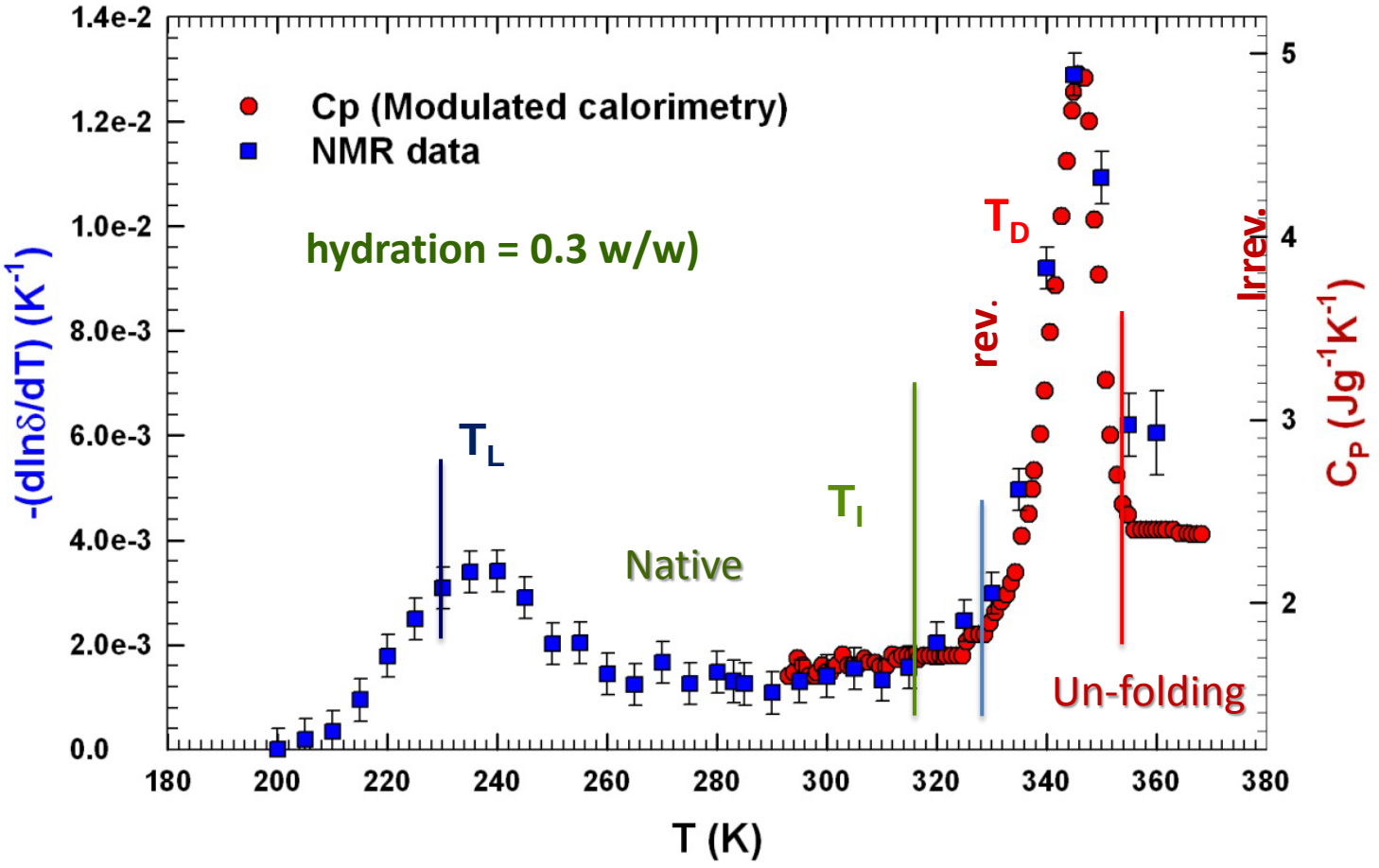
Water acts as an HB *glue* between the carbonylic and amidic groups.

Water can be bonded at either the same or different peptides.

The hydrophobic sides ?

*DA. Dixon et al, J. Phys. Chem. 98, 13435, 1994. **T. Blundell et al, Nature 306, 281 1983.
** M. Sundaralingam & YC. Sekharudu, Science 244, 1333 1989.

The hydrated protein (lysozyme) behavior due to HB - hydrophobicity competition.



The specific heat evidences a significant changes in the system configurational energy.

Erice-Water 16

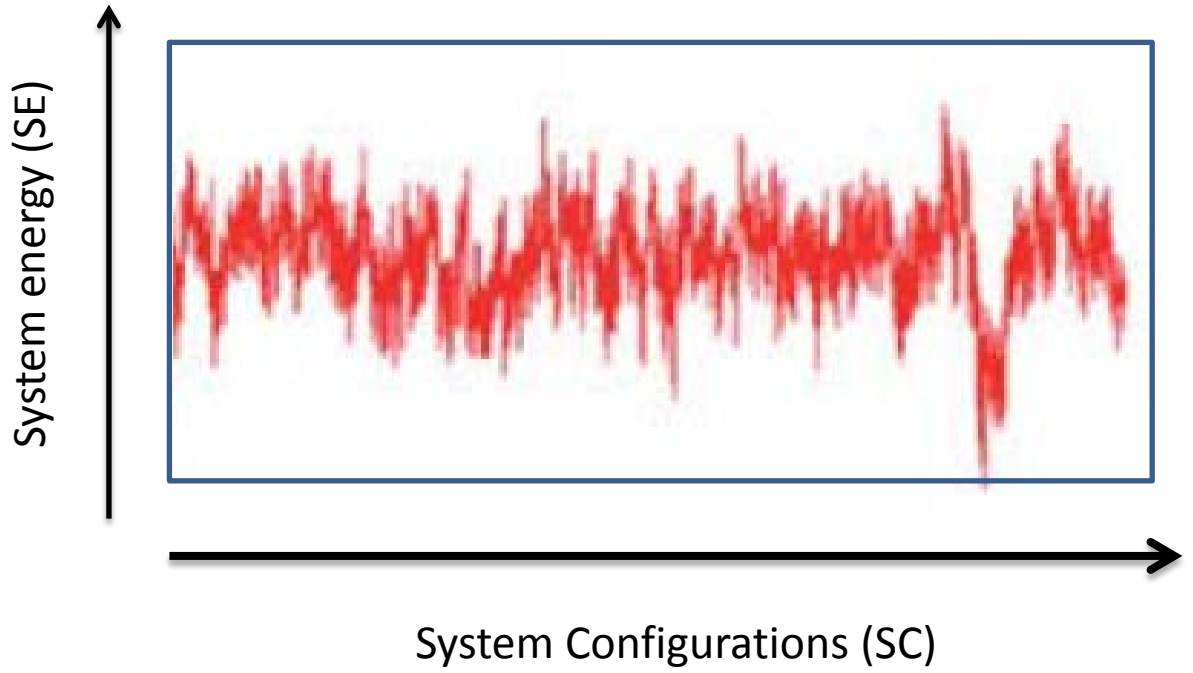
The protein unfolding reversible/irreversible detected by the high resolution NMR technique.

The study of the protein metabolites (hydrophilic and hydrophobic) in a thermal cycle.

The system magnetization is studied as a function of the temperature.

The aim is an experimental proof of the folding funnel (an energy landscape)

A conceptual sketch of the energy basins landscape



Being the system in a metastable, out of the equilibrium, state the SE, SC and corresponding kinetics (and relaxations) are determined by the thermodynamical conditions.

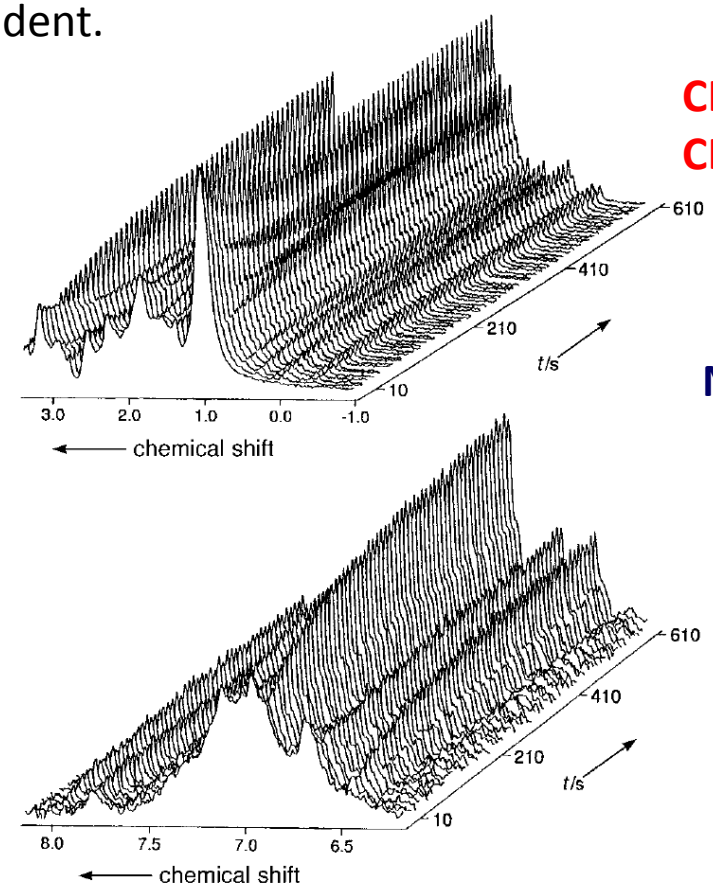
- **In the folding-unfolding reactions the effective system energy (SE) contains all contributions to the total free energy (e.g., the hydrophobic effect, solvation of polar groups, hydrogen bonding that involve both van der Waals and electrostatic interactions and the configurational entropy).**

- **The configurational entropy, is characteristic of the denaturation process and arises from the conformation space available to the polypeptide main chain and side chains (more restricted in the native state than in the denatured state).**

- **The SE nonpolar groups favor the folded state owing to the attractive van der Waals interactions in the packed native structure and the hydrophobic effect, which favors the burial of nonpolar groups. In particular, solvent molecules internal of the resulting "solid like structure" take part to the entire process .**

- **The polar groups (the peptide groups and the polar and charged side chains), instead, make a much smaller contribution to the stability of the native state owing to a balance of the interactions in the interior of the protein and those with the solvent.**

A way suggested to study the complex SE situation of the folding-unfolding process is the use of NMR spectroscopy*: the protein magnetization spectra (M(t,ν)) are sensitive of the process (refolding case of α-lactalbumin) and thus can properly describe it. From the figure, the spectral chagnes in time during the refolding kinetics of the protein main chemical contributions (namely methyl, methylene, aromatic and amidic groups) are evident.



CH

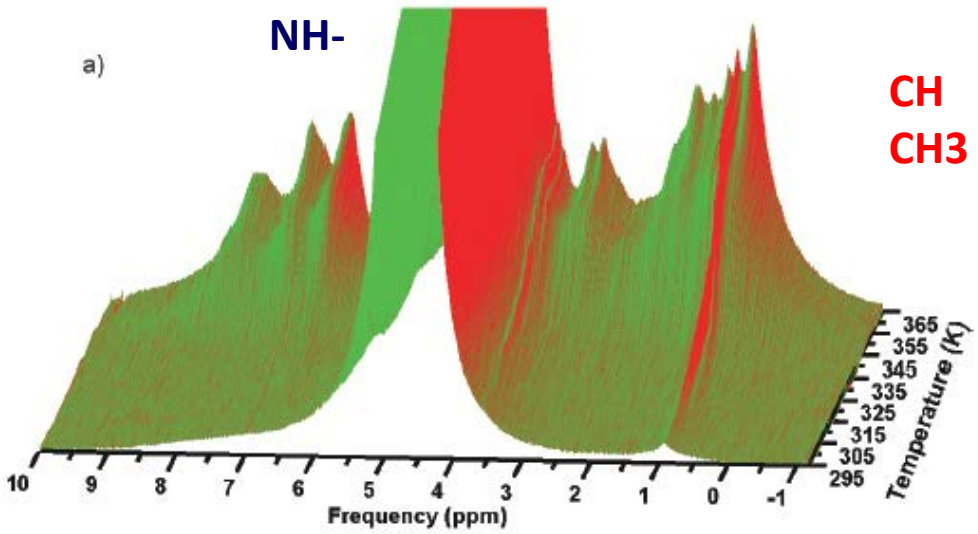
CH3

NH-

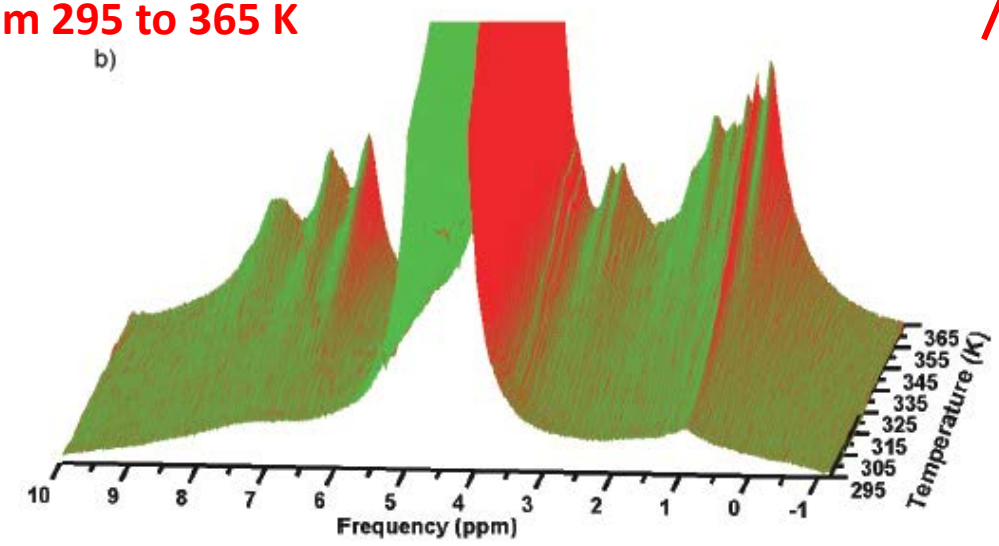
*Dobson CM, Sali A and Karplus M. Angew. Chem. Int. Ed. 37, 868-893 (1999).

This is the motivation of our study, with the aim to detail SE situation in the thermal denaturation of the hydrated lysozyne by looking at the evolution of some individual chemical groups (CH, CH3 and NH-).

The thermal evolution of the NMR magnetization M(T,ν) spectra in a thermal cycle.



a) T increases from 295 to 365 K



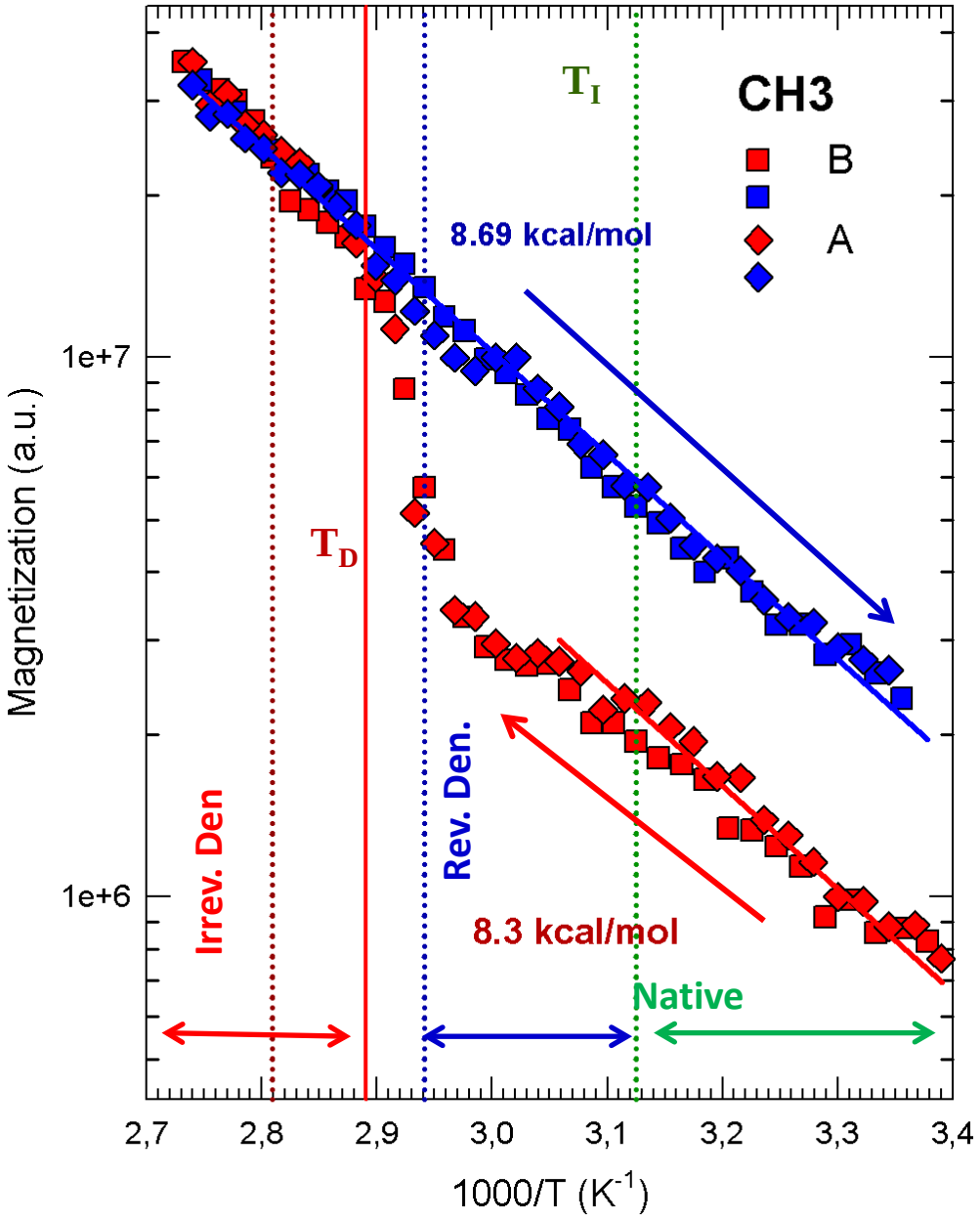
b) T decreases from 365 to 295 K

Spectral deconvolution (a)

Hydrophobic Groups (CH_3)

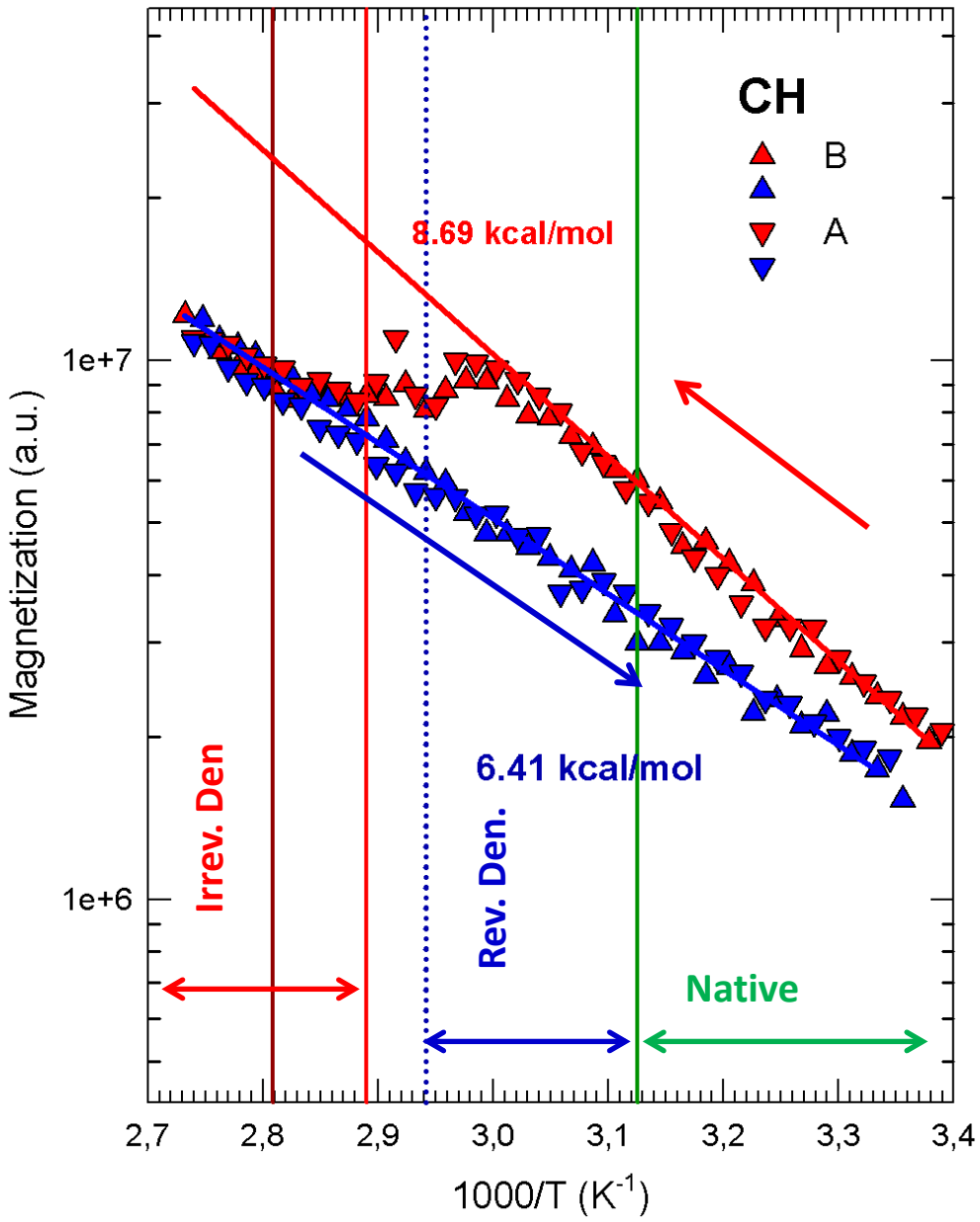
In between the two Arrhenius behaviors there is a transition in which the system explores the energy landscape!

The irreversible unfolding process takes place during the heating phase if the threshold temperature T_D is surpassed. Whereas the onset of the process is at T_I.



Spectral deconvolution (b)

Hydrophobic Groups (CH)

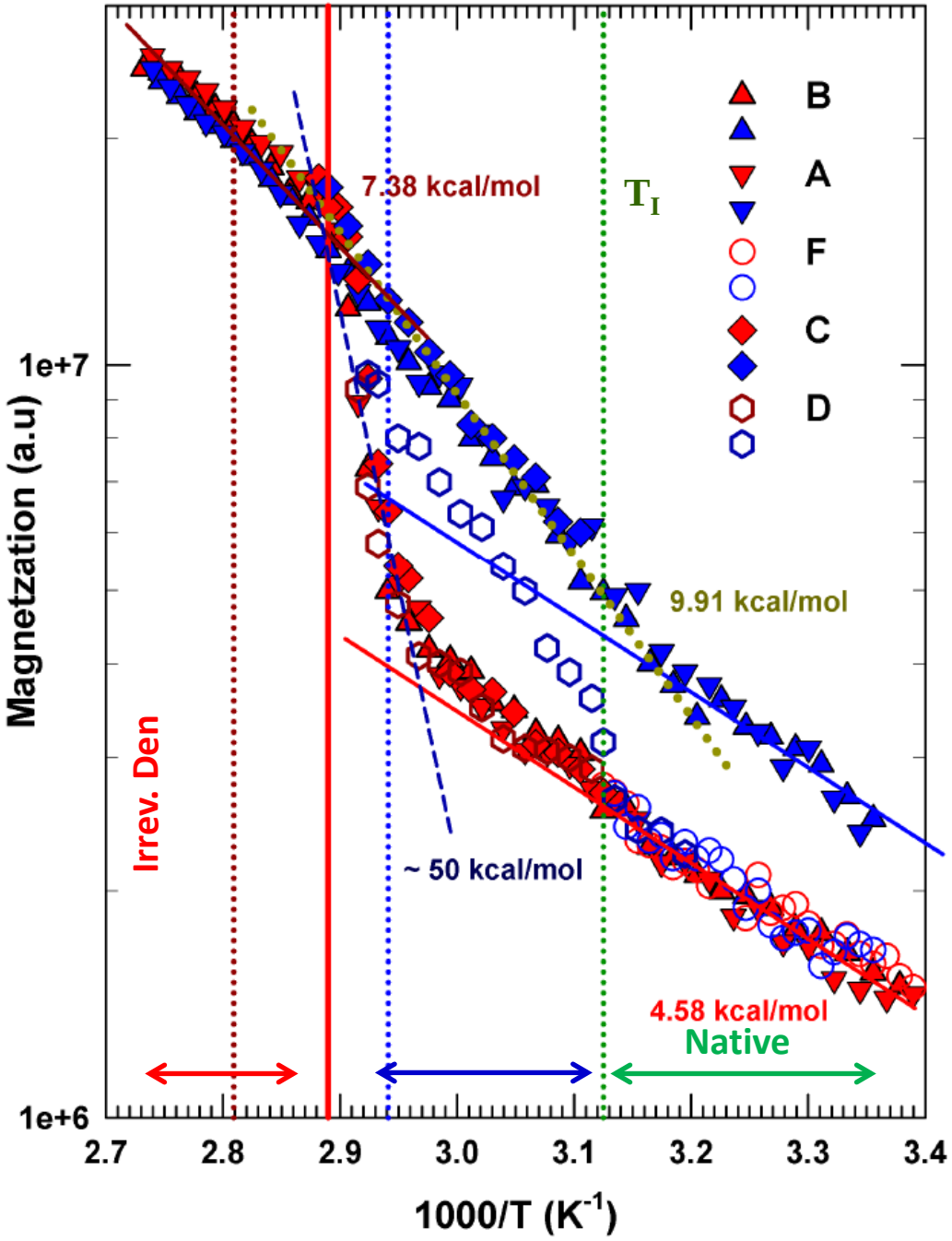


Spectral deconvolution (c)

Hydrophilic Groups (NH)

The cycle D indicates that the reversibility limit is T_D.

The dark blue dashed line gives an estimation of the unfolding energy.



- For lysozyme in water, simulations estimate that all the nonpolar groups contribute 450 kcal/mol and the polar groups contribute 87 kcal/mol at 25 °C to the free energy of denaturation.

- The stabilization of the native state due to all the SE terms (about 537 kcal/mol) is counterbalanced by a configurational entropy contribution of about 523 kcal/mol at 25 °C.

- This yields a net free energy of unfolding of 14 kcal/mol (on the order of 0.1 kcal/mol per residue), which is a typical value for globular proteins.

- In contrast, the energy or enthalpy difference between the native and unfolded state can be significantly larger; for lysozyme at 25 °C, the unfolding enthalpy is 58 kcal/mol. A value in agreement with our experiment.

In between the two Arrhenius behaviors there is a transition region in which the system explores the energy landscape!

A situation typical of critical processes and complex materials like polymers and strong interacting fluids!

Systems characterized by a multi scale structure and dynamical properties well represented by scaling behaviors and power laws.

E.g. situations typical of physical systems with a self-organized criticality like large polymers and polyelectrolytes at the percolation threshold (or at the Sol-Gel transitions).

A possible explanation

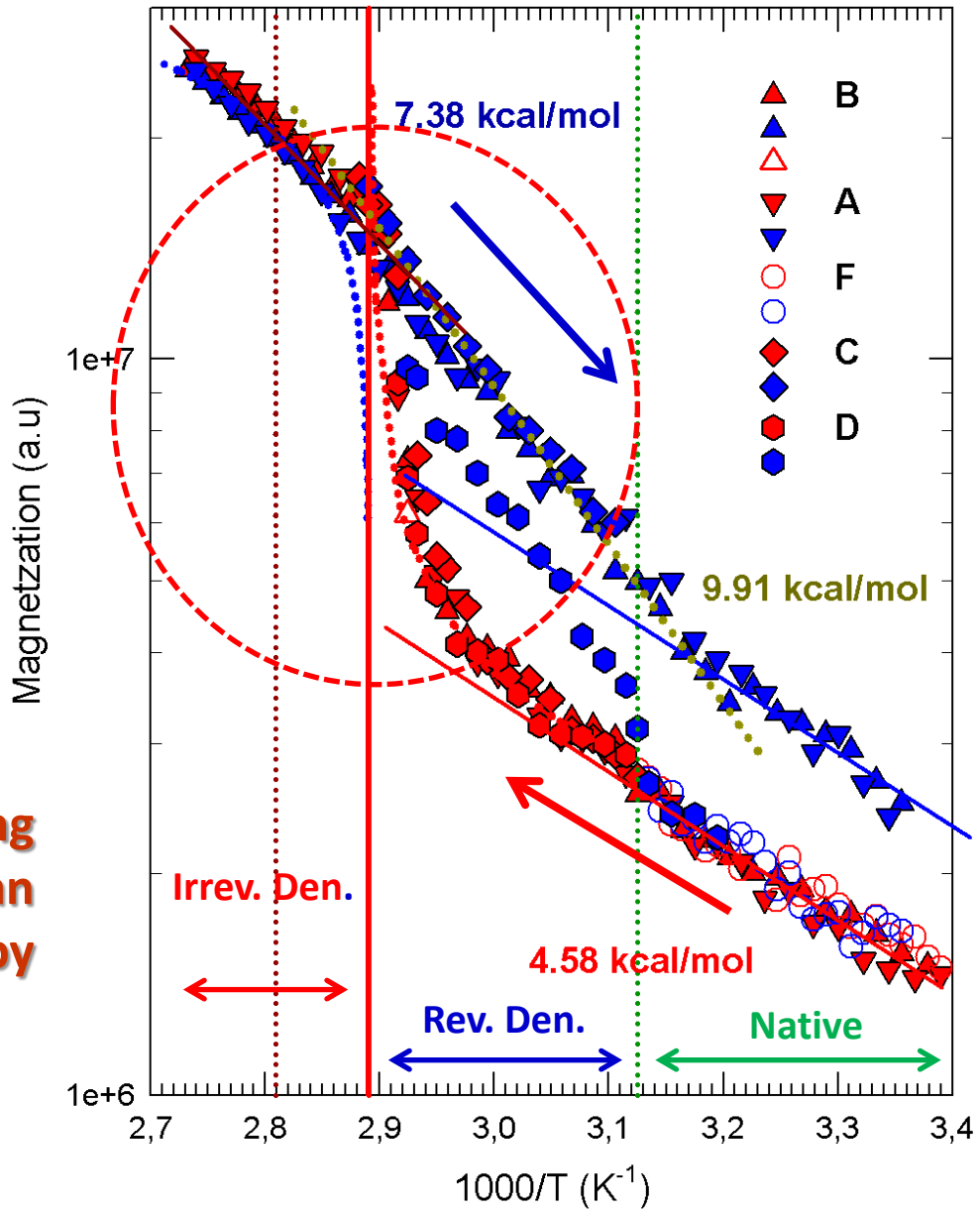
a) Hydrophilic Groups (NH)

$$M \approx |(T - T_D)|^{-\gamma}$$

$T > T_D$; $T_D \approx 345.7$ K; $\gamma \approx 0.517$

$T < T_D$; $T_D \approx 345.9$ K; $\gamma \approx 0.37$

A power law analysis suggesting a *SOL-GEL transition* in an infinite polymer cross-linked by water



CONCLUSIONS

I) water-methanol

i) To study hydrophobicity we studied these solutions by using both the Bluembergen, Purcell and Pound (BPP) and Kubo, Tomita and Hubbard (KTH) approaches.

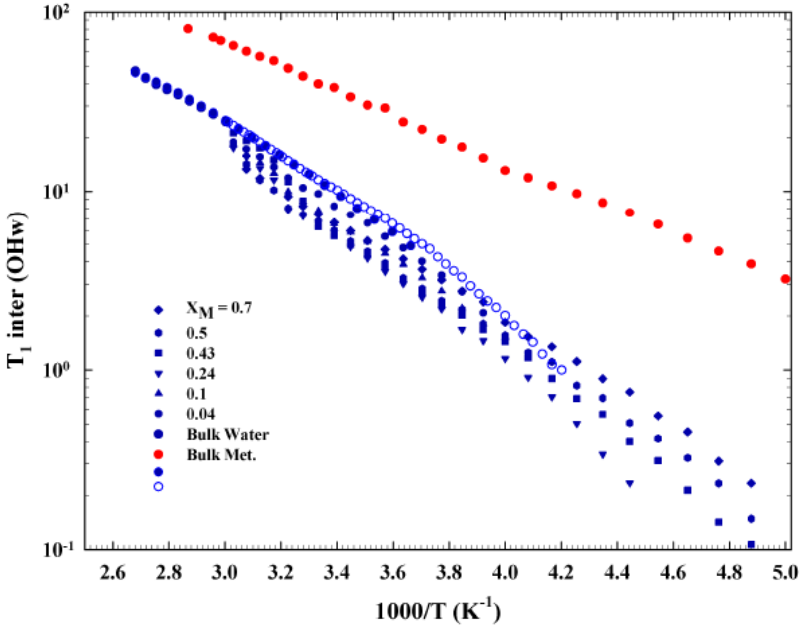
ii) The reported NMR results give evidence , especially from the thermal evolution of the CH3 relaxation times, that the hydrophobicity becomes significant for the solution properties inside the water stable liquid phase (T > 273 K).

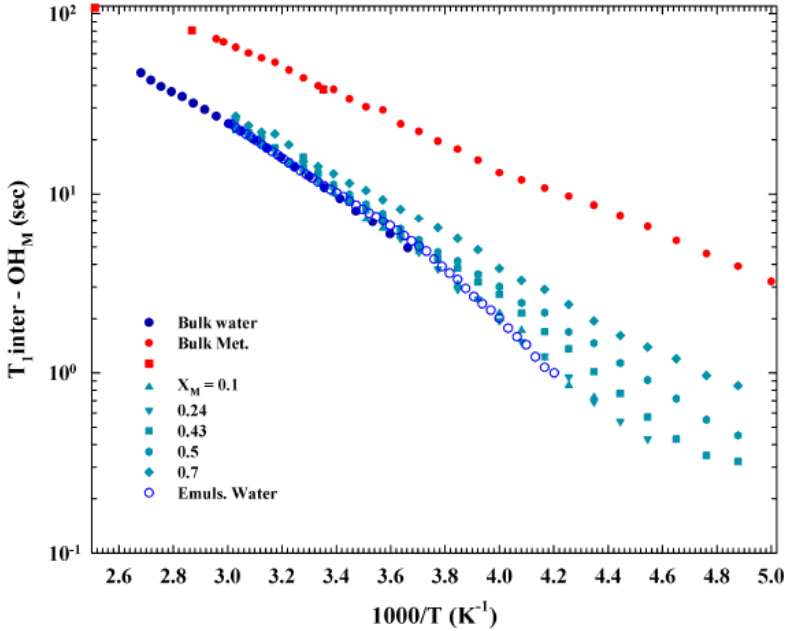
iii) The KTH relaxation time τ_θ represents a true rotational time with the same T-behavior of the depolarized Rayleigh one.

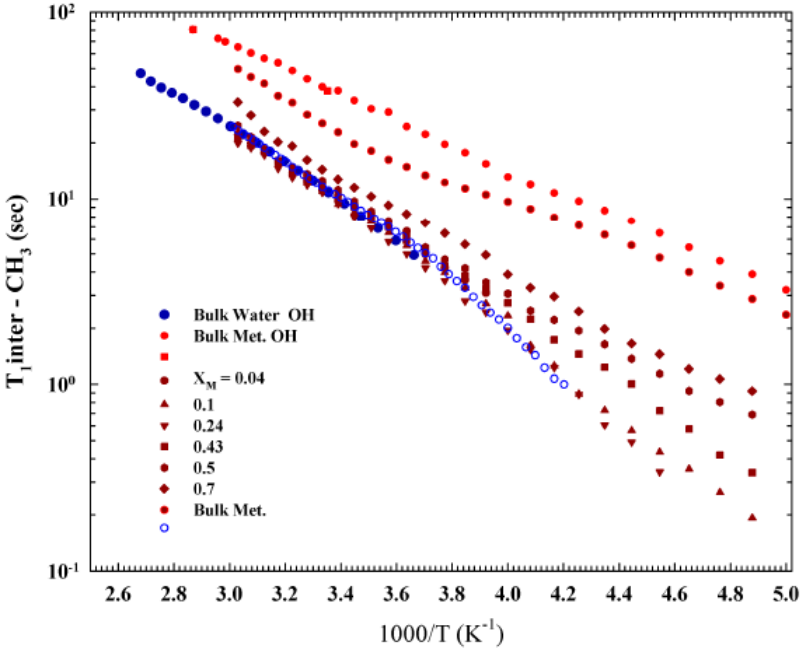
II) water-lysozyme

The NMR magnetization spectra suggests a proper way to study from the energetic point of view the reversible/irreversible protein denaturation process evidencing the difference in the behavior of hydrophilic and hidrophobic metabolites.

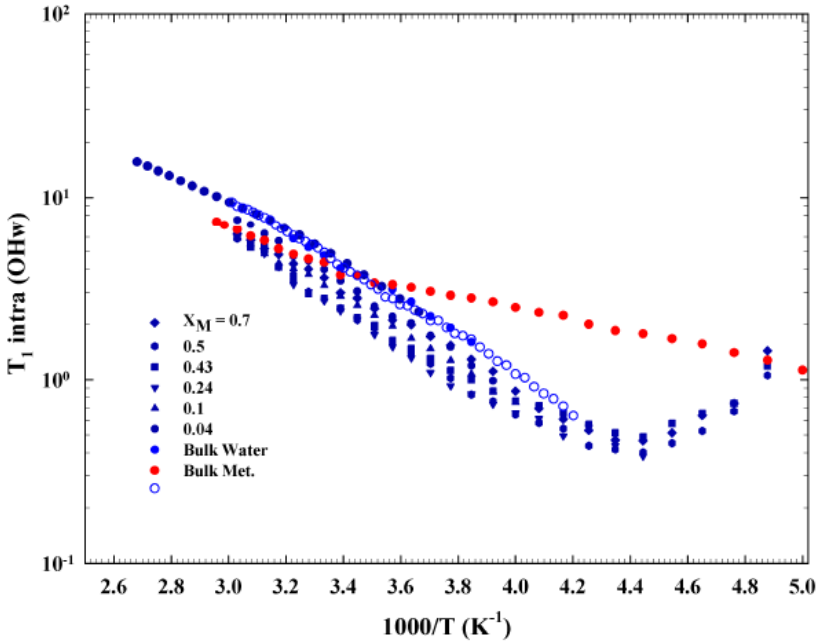
A lot of Thanks

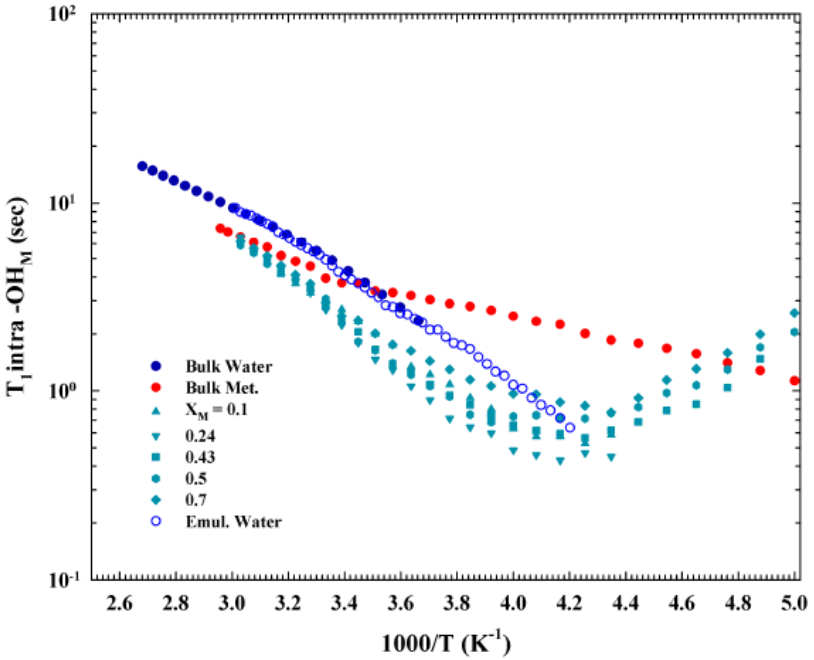


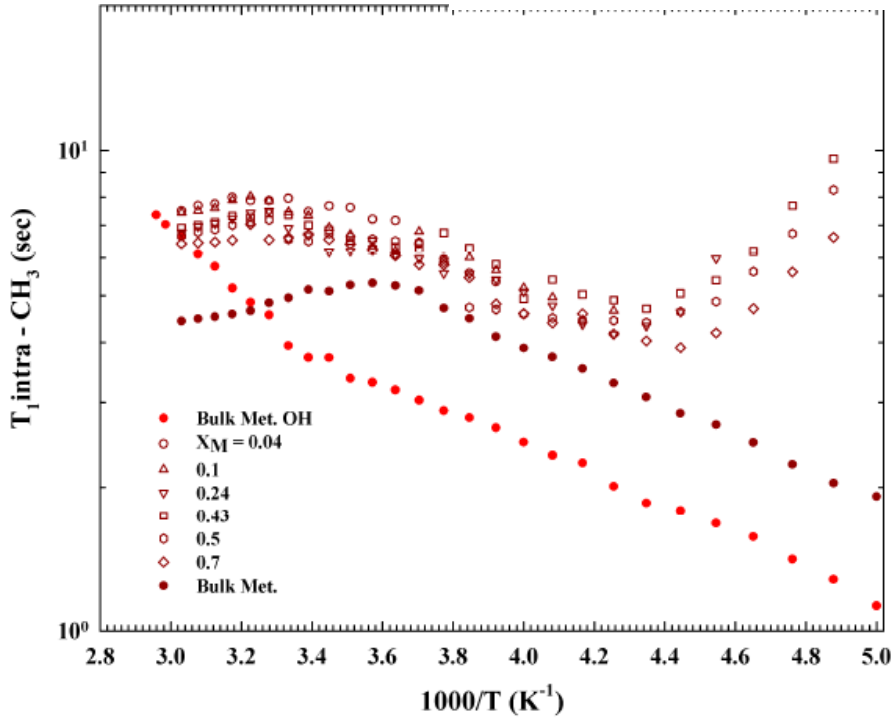




Erice-Water 16







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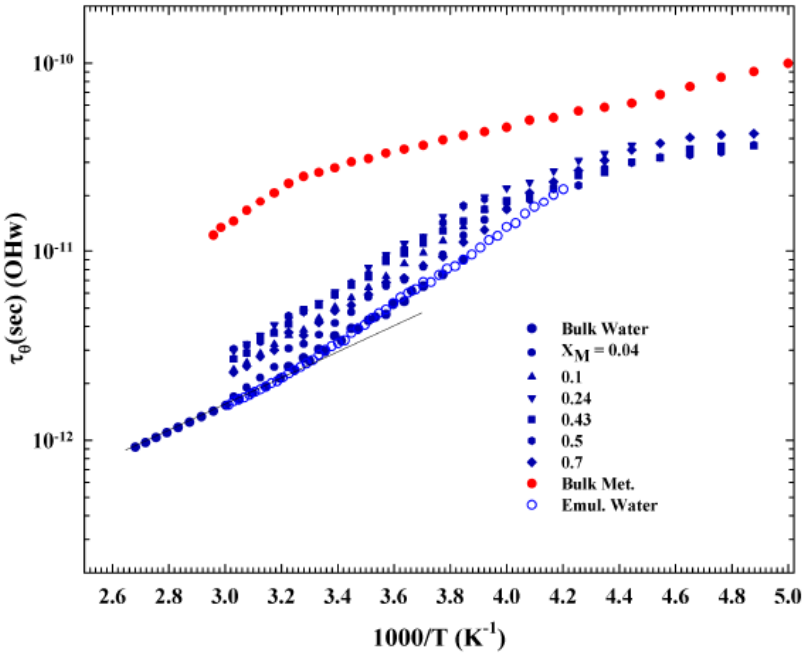

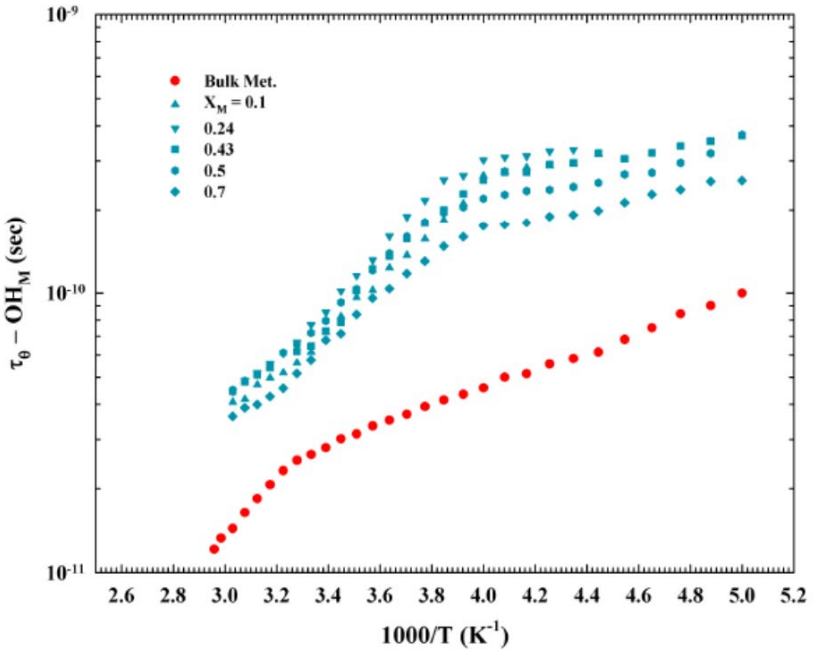

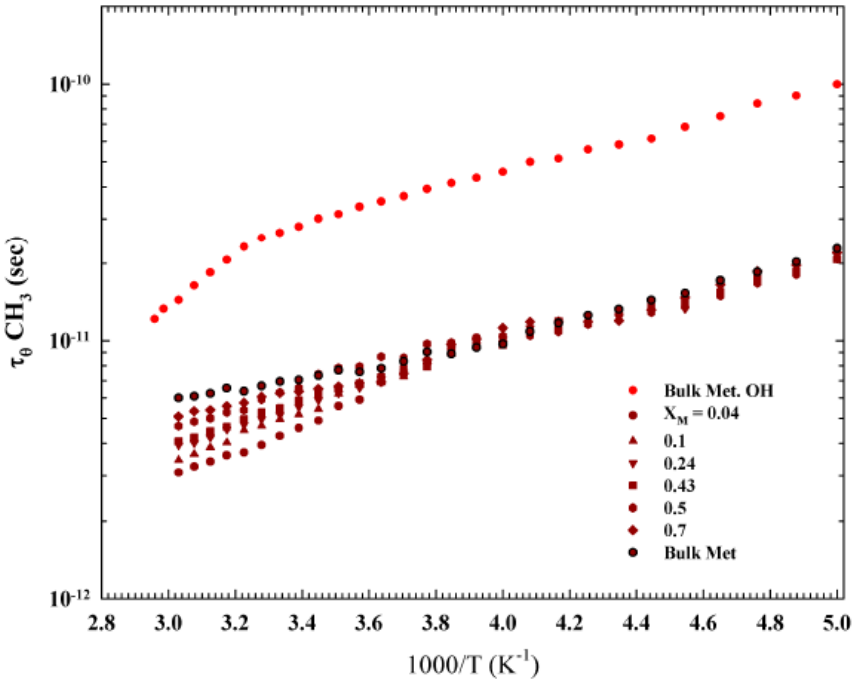

Erice-Water 16