Water in halophile Dead Sea organisms: neutron scattering studies of adaptation to extreme environments

Zaccai G., Jasnin M., Tehei M.

Institut Laue Langevin and Institut de Biologie Structurale, Grenoble, France

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The living Cell

An open thermodynamic system
in constant activity, even when it is not dividing

Membrane proteins: differences in chemical potential
DNA repair mechanisms
RNA synthesis and processing
Protein synthesis and processing
Internal membrane traffic
Cytoskeleton dynamics

and and and . . .

All working in a coordinated synergistic way !

Cells in a human being turn over ~ 20 kg of ATP molecules per day

. . .

The Extremophile living Cell

All the above

under extreme conditions of

salt concentration

temperature

pressure

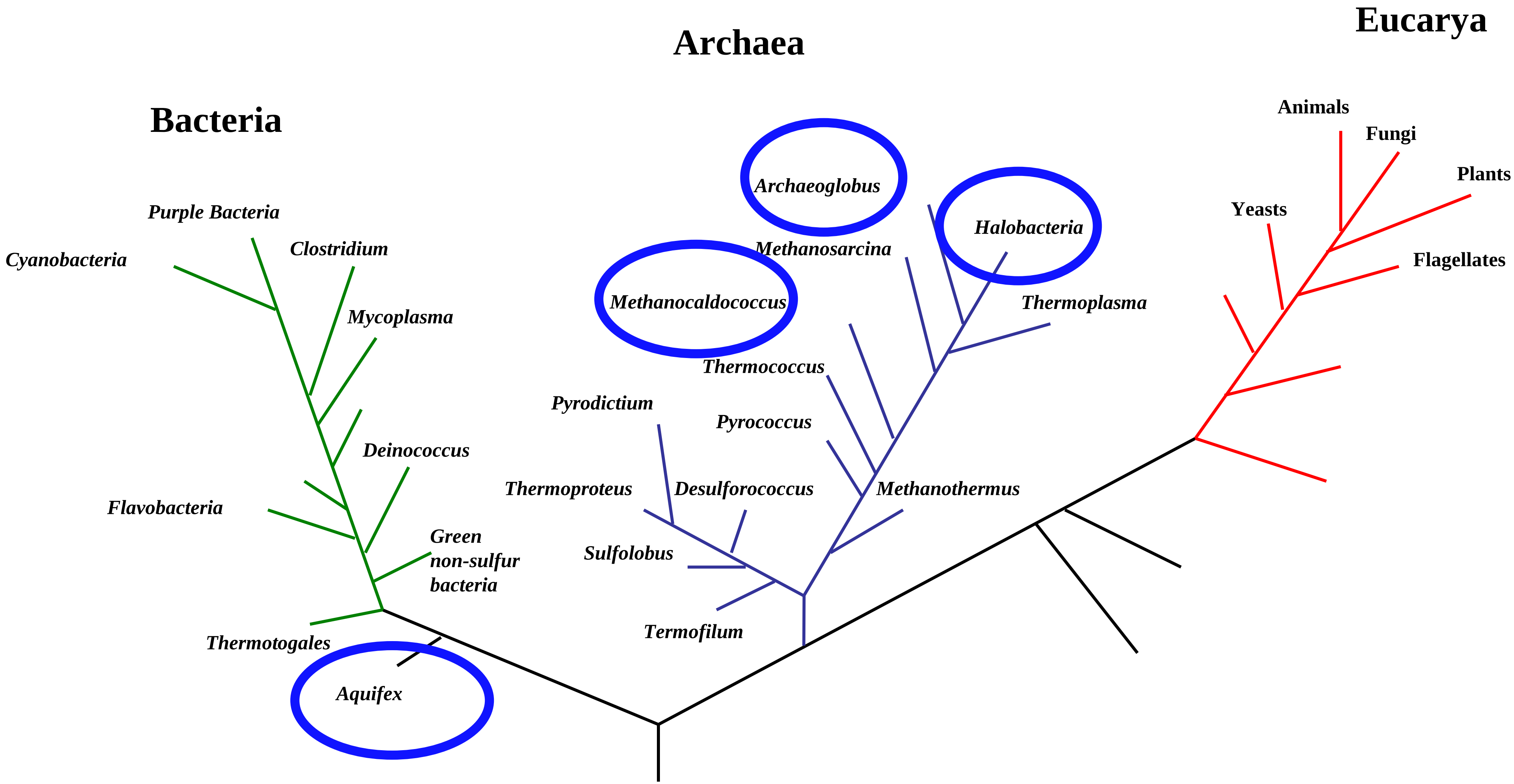
pH

. . .

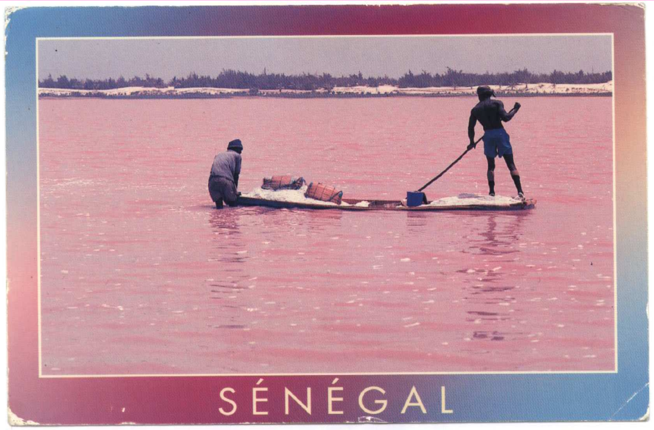
and . . .

is water behaviour also

extreme ?



Eucarya
Archaea
Bacteria
Animals
Fungi
Plants
Yeasts
Flagellates
Archaeoglobus
Halobacteria
Methanosarcina
Methanocaldococcus
Thermoplasma
Thermococcus
Pyrodictium
Pyrococcus
Thermoproteus
Desulforococcus
Methanothermus
Sulfolobus
Termofilum
Purple Bacteria
Cyanobacteria
Clostridium
Mycoplasma
Deinococcus
Flavobacteria
Green
non-sulfur
bacteria
Thermotogales
Aquifex



The coloured
Life of Salt

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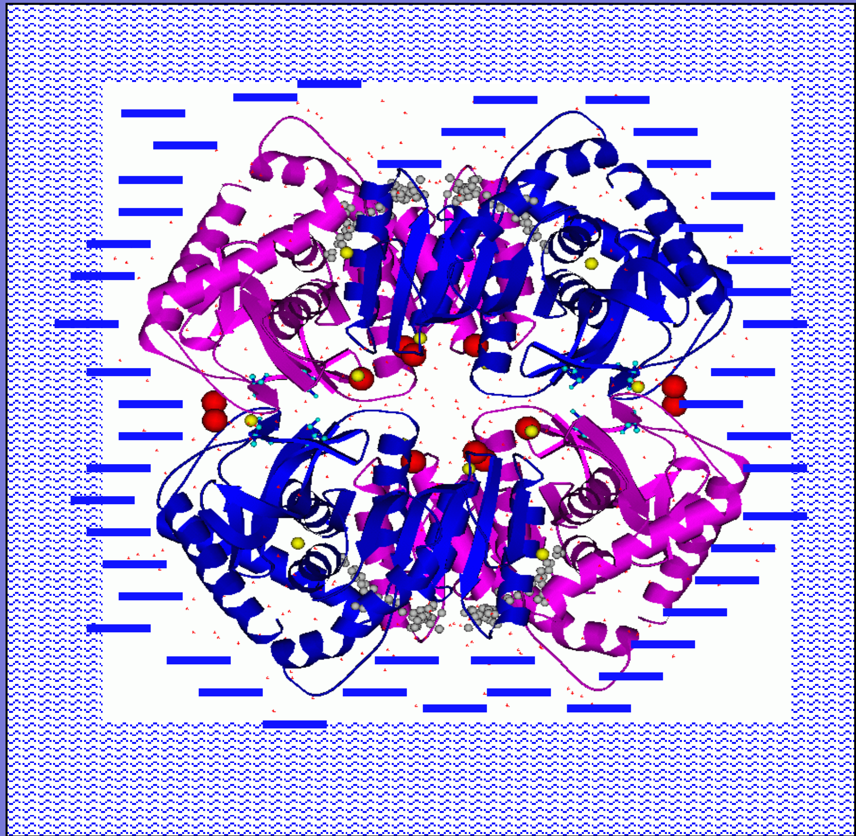
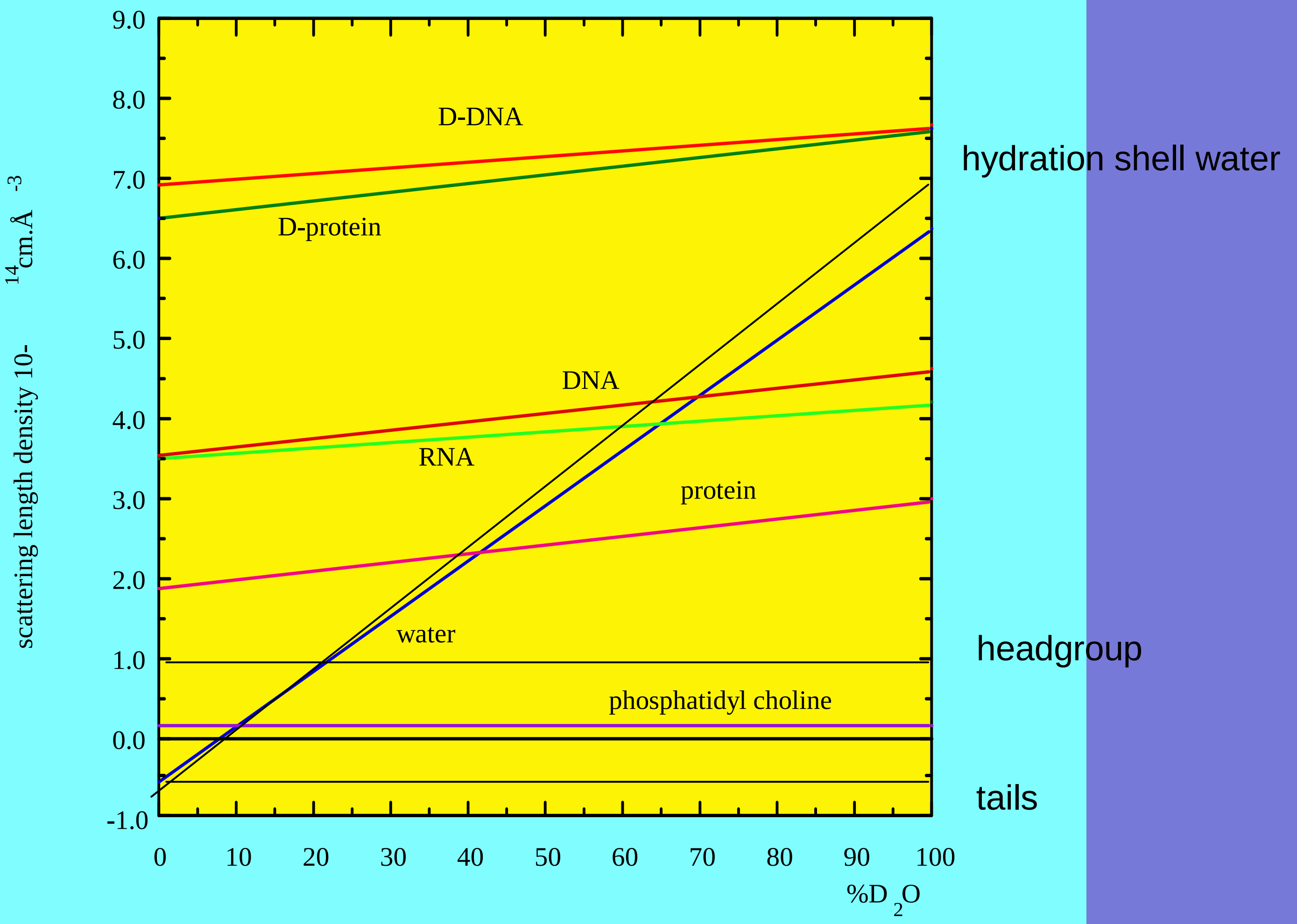
…Parts of the lake seen from a short distance appeared of a reddish colour, and this perhaps was owing to some infusorial animalcula …How surprising it is that any creatures should be able to exist in brine, and that they should be crawling among crystals of sulphate of soda and lime! …Thus we have a little living world within itself, adapted to these inland lakes of brine …

(Darwin, *Voyage of H.M.S. Beagle*)



Contrast Variation



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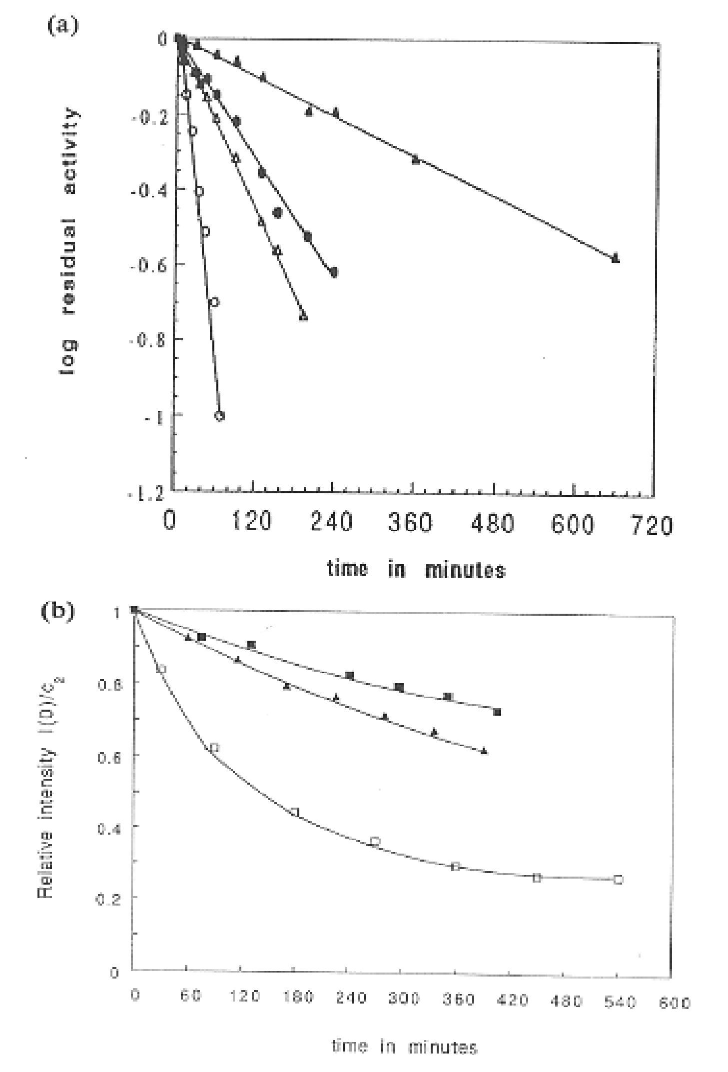
“Proteins unwind when exposed to heat and they do the same when exposed to salt.”

(from *Salt, a world history, part one: A discourse on salt, cadavers and pungent sauces,* **by Mark Kurlanski)**

Stability

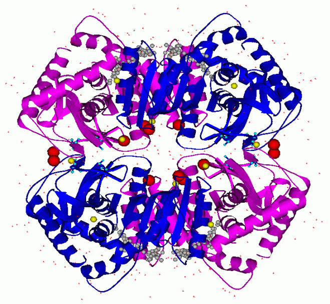


1M NaCl D_2O
0.8M NaCl D_2O

1M NaCl H_2O
0.8M NaCl H_2O

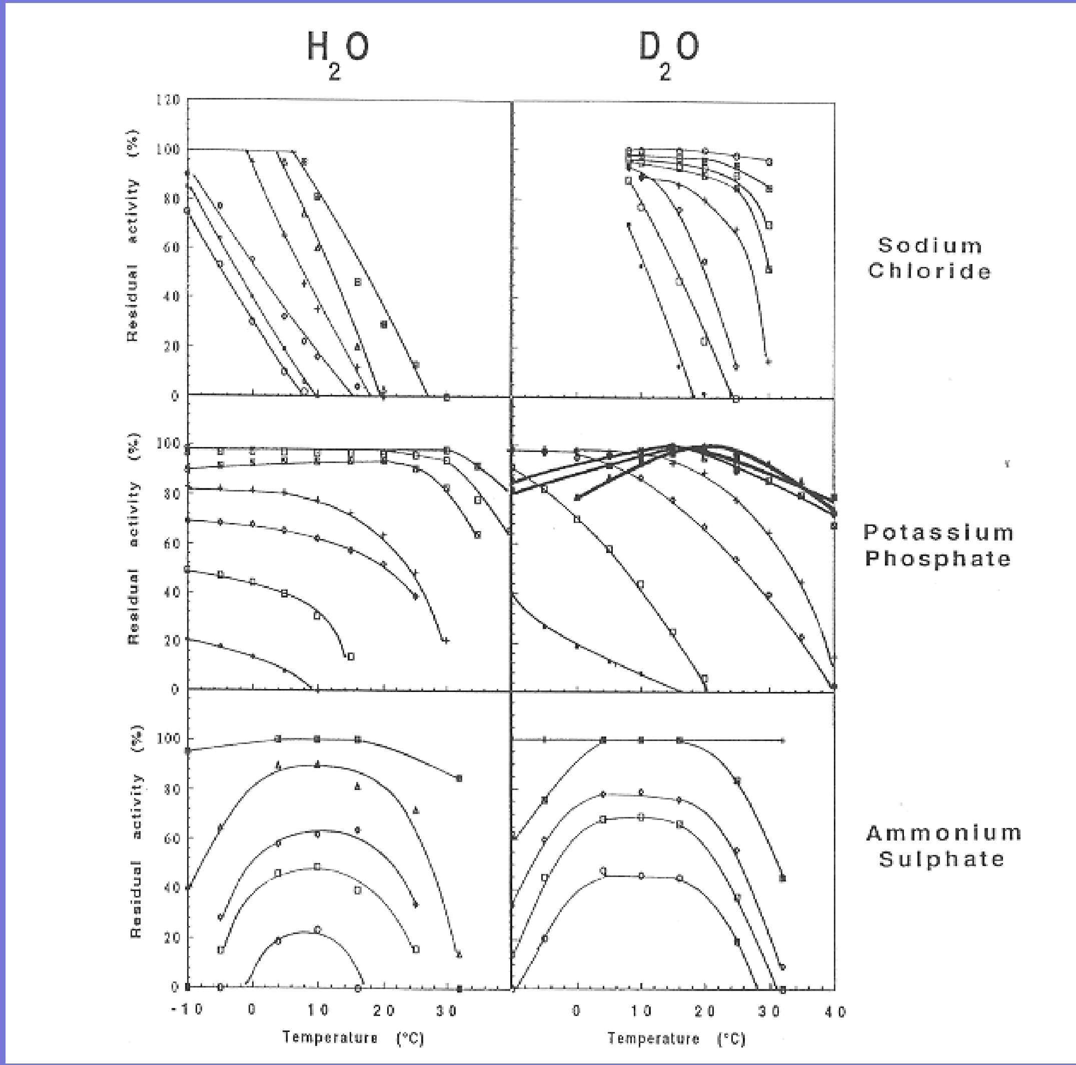
0.5M NaCl D_2O
0.5M KCl D_2O

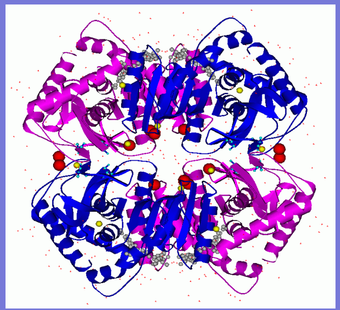
1M NaCl H_2O



Bonneté et al. (1994)

Stability



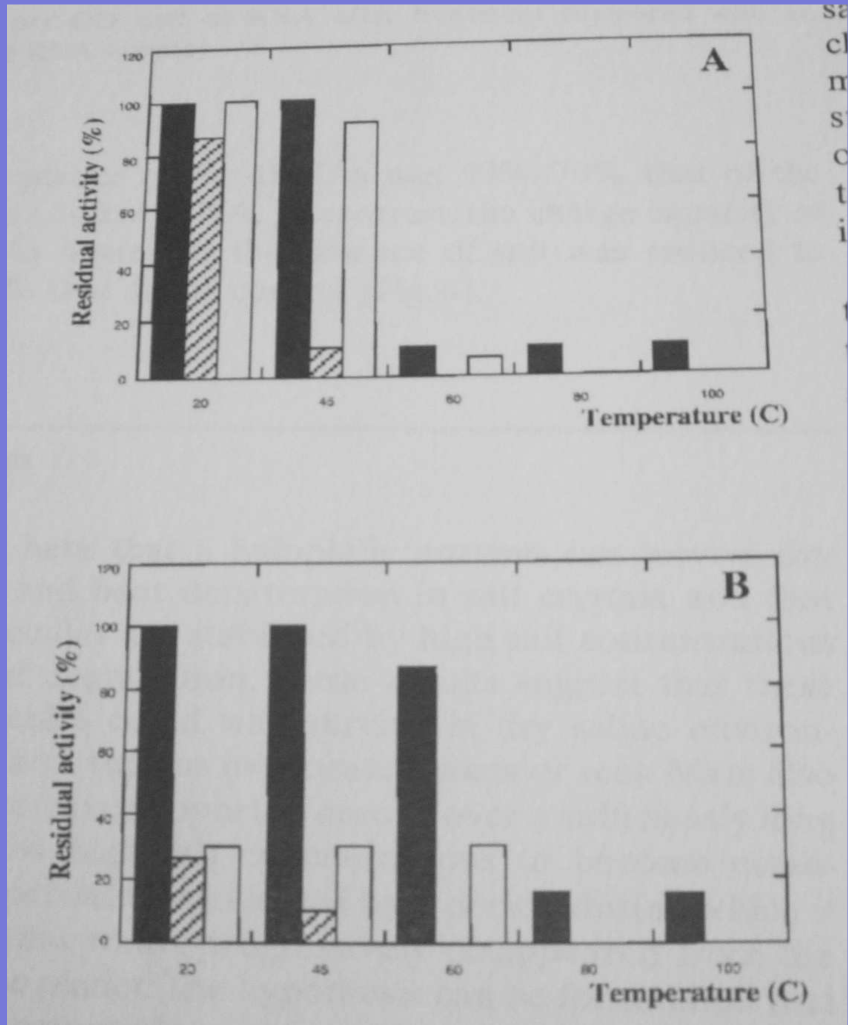


Bonneté et al. (1994)

Looking for traces of Life : protection by salt

wet

‘dry’



halo + salt

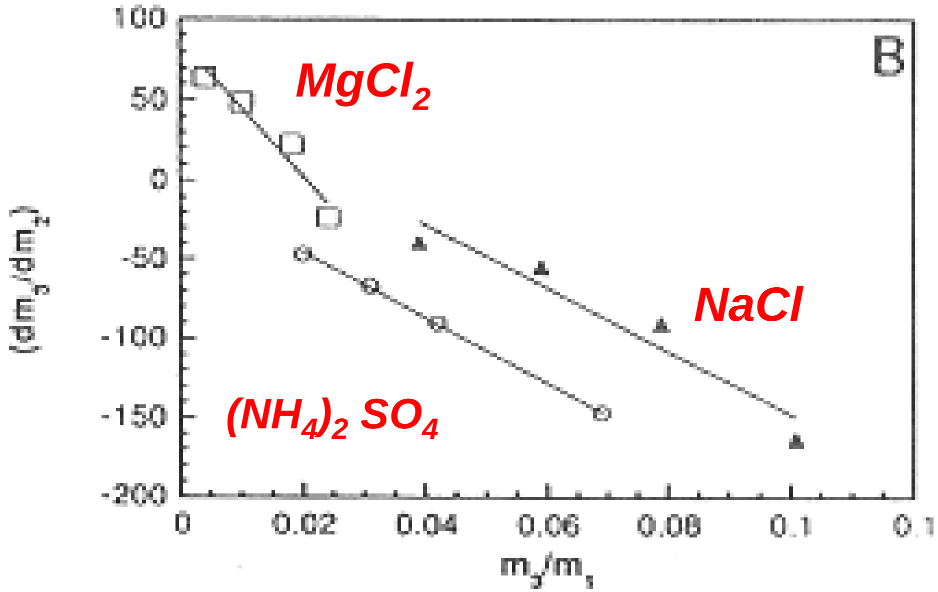
meso +salt

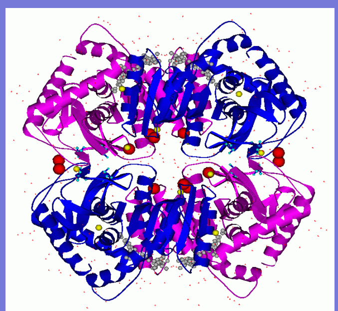
meso - salt

salt = 4M NaCl

Tehei et al (2000)

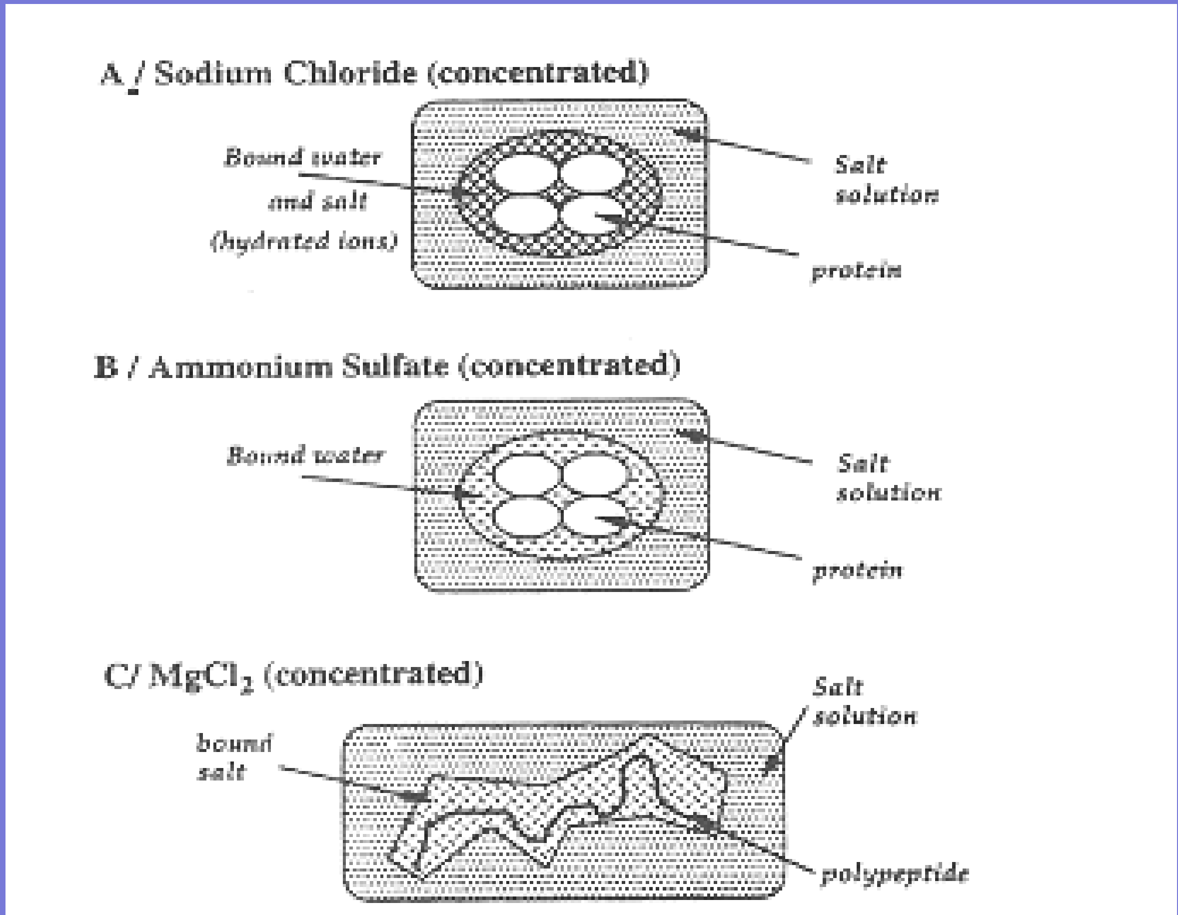
Stability and Solvation

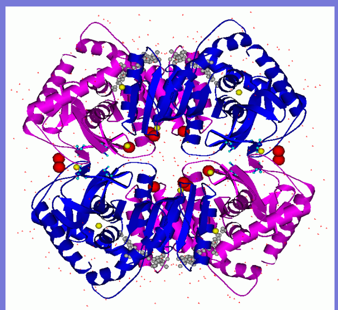




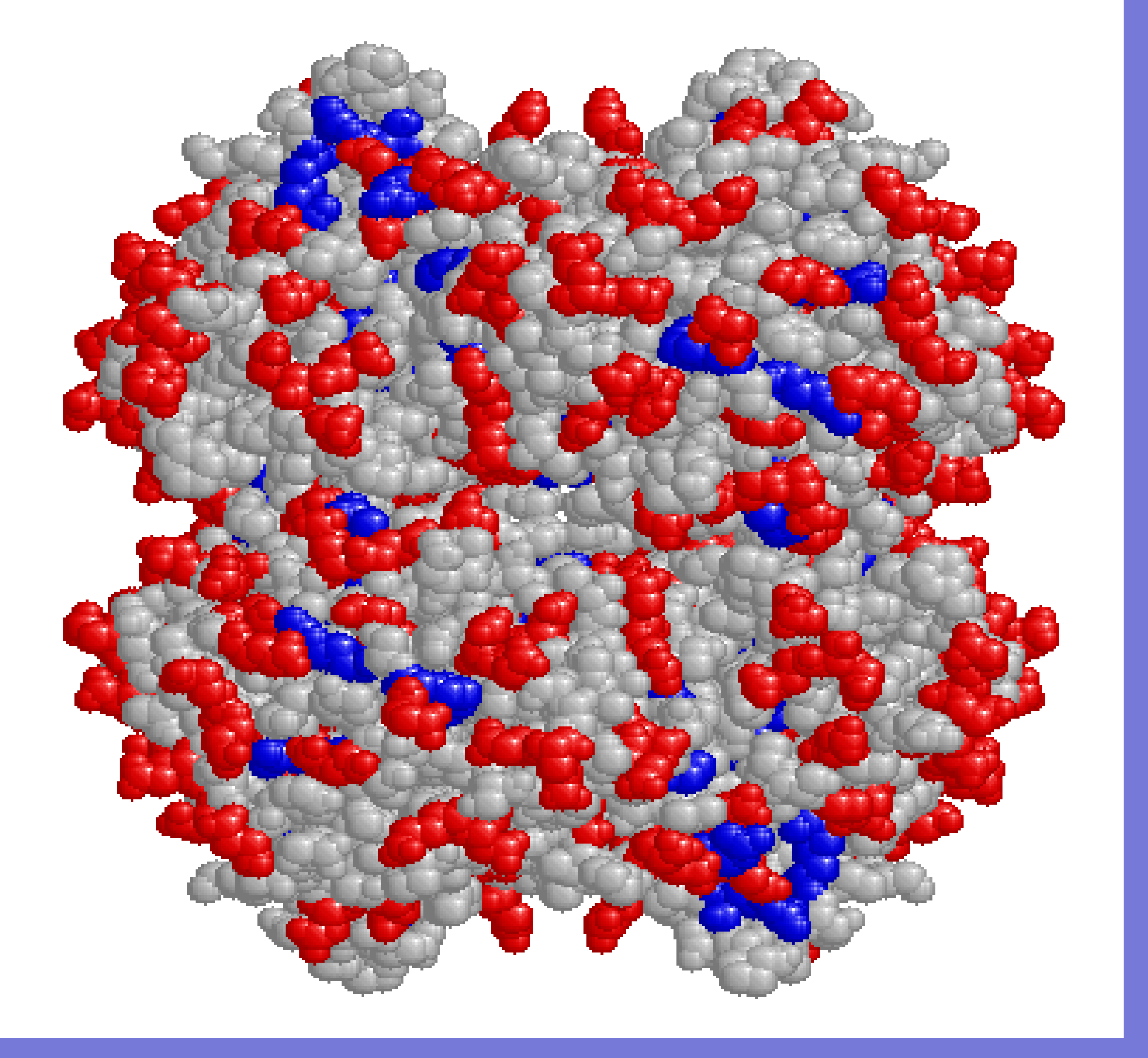
Quantities of water and salt associated with the protein under various conditions can be weighed and located experimentally by using
SANS, AUC, densimetry in a complementary approach

Solvation
salt and water 'binding'

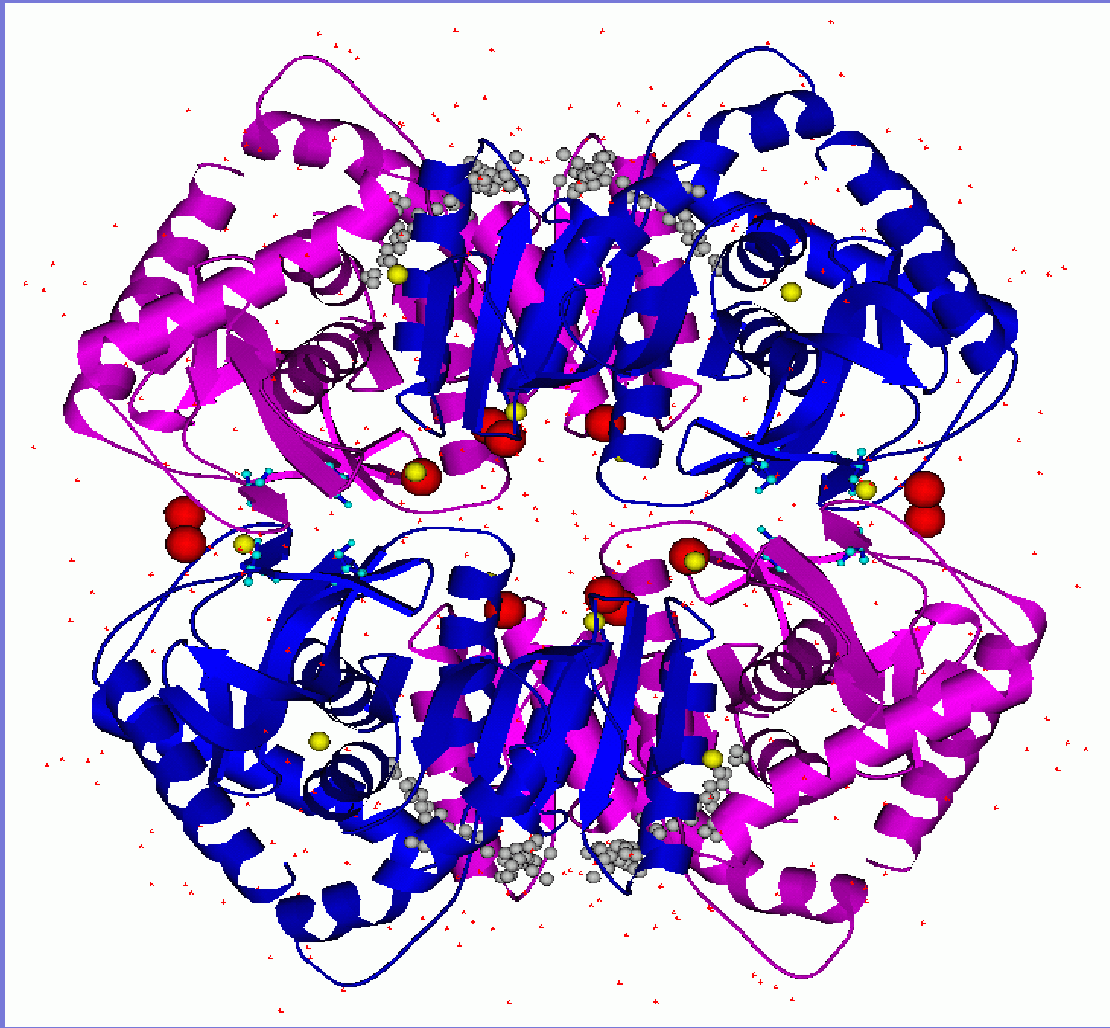




Surface acidic (red) and basic (blue) residues

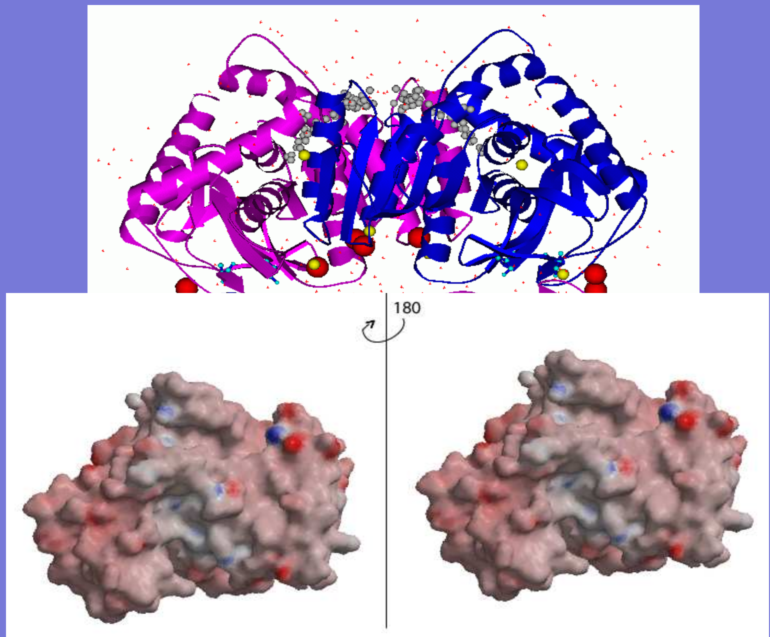


Halophilic malate dehydrogenase is stabilised by its solvation shell

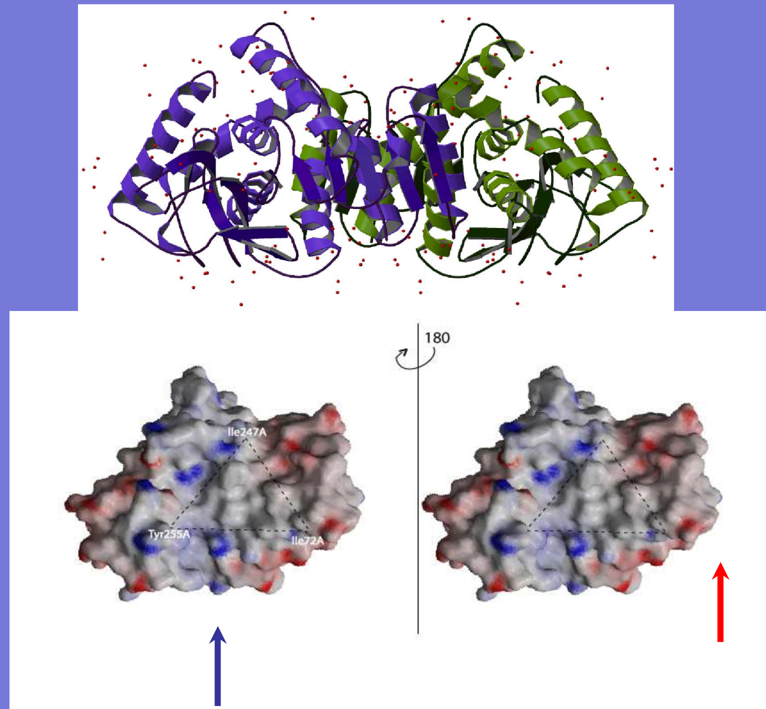


Ebel et al. (2002)

Malate dehydrogenase



Haloarcula marismortui



Archaeoglobus fulgidus

Irimia et al., 2004

Underlying protein structure,

the forces that stabilise it :

Dynamics !

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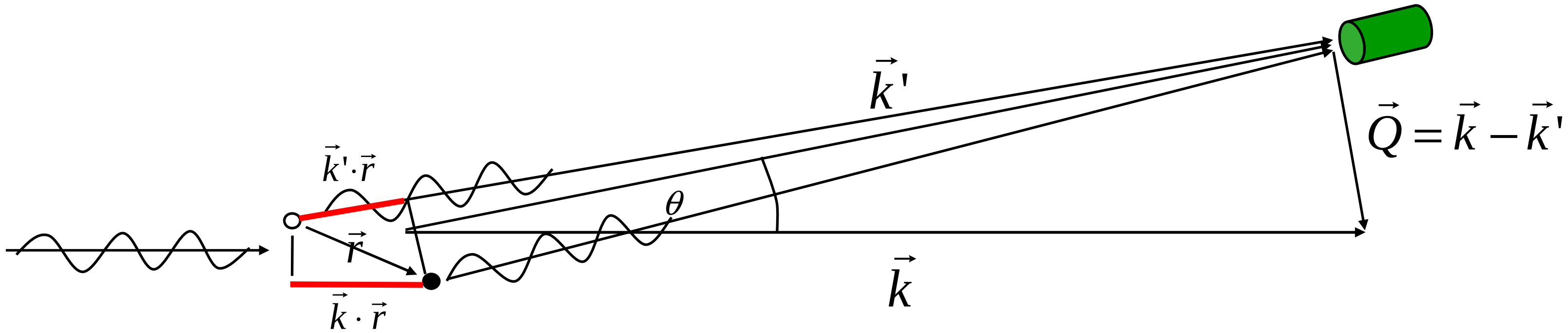
Thermal and cold neutrons: The perfect radiation to study protein dynamics

wavelengths < > fluctuation amplitudes

energies < > fluctuation energies

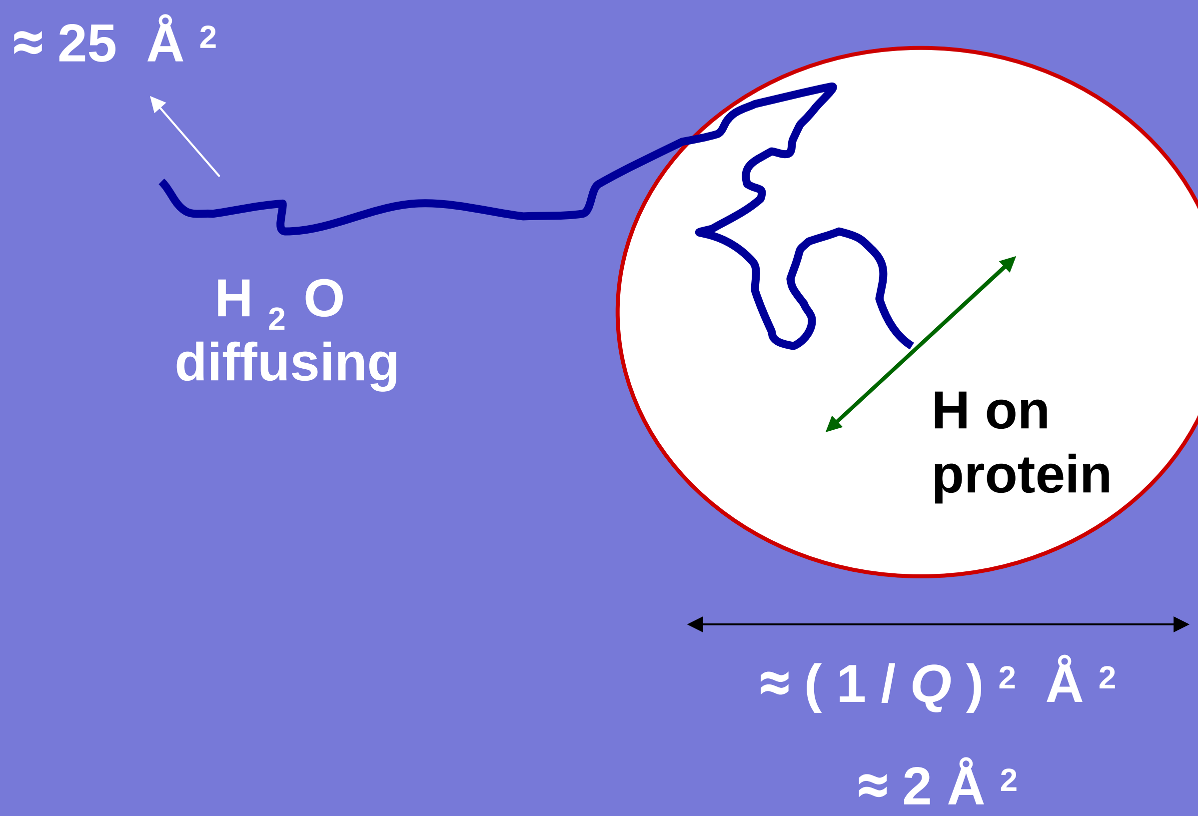
Neutron incoherent scattering



$$S(\mathbf{Q},\omega)=\frac{\mathbf{1}}{\mathbf{2}\pi}\int I(\mathbf{Q},t)\exp(-i\omega t)\mathrm{d}t$$

$$I(\mathbf{Q},t)=\frac{1}{N}\sum_{k,j}<\mathrm{e}^{\mathrm{i}\mathbf{Q}.\mathbf{r}_k(t)}\mathrm{e}^{-\mathrm{i}\mathbf{Q}.\mathbf{r}_j(0)}>$$

A window in space - time

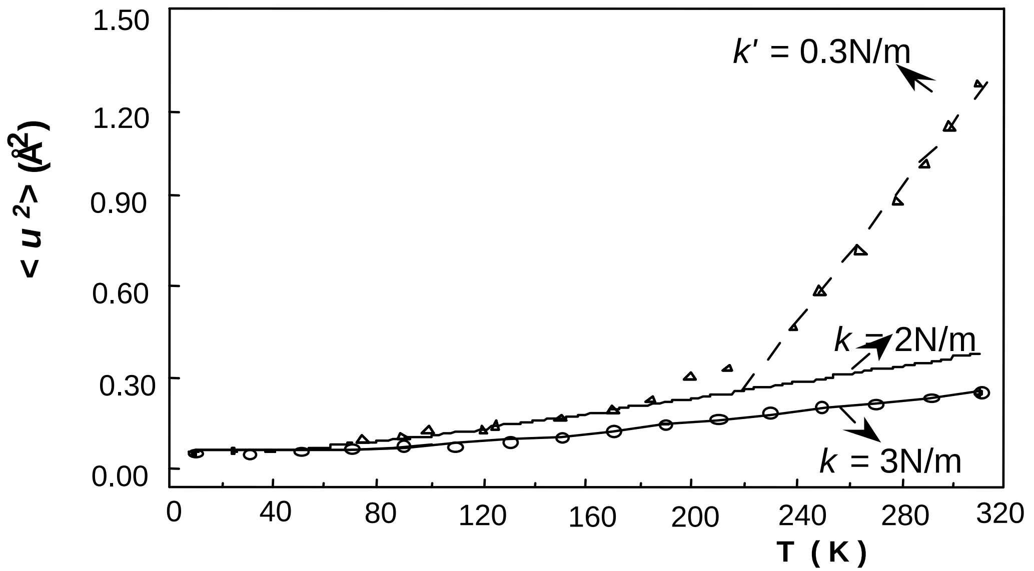


time ≈ 100 ps
on IN13

on IN13 at ILL

Fluctuations and force constants

Myoglobin



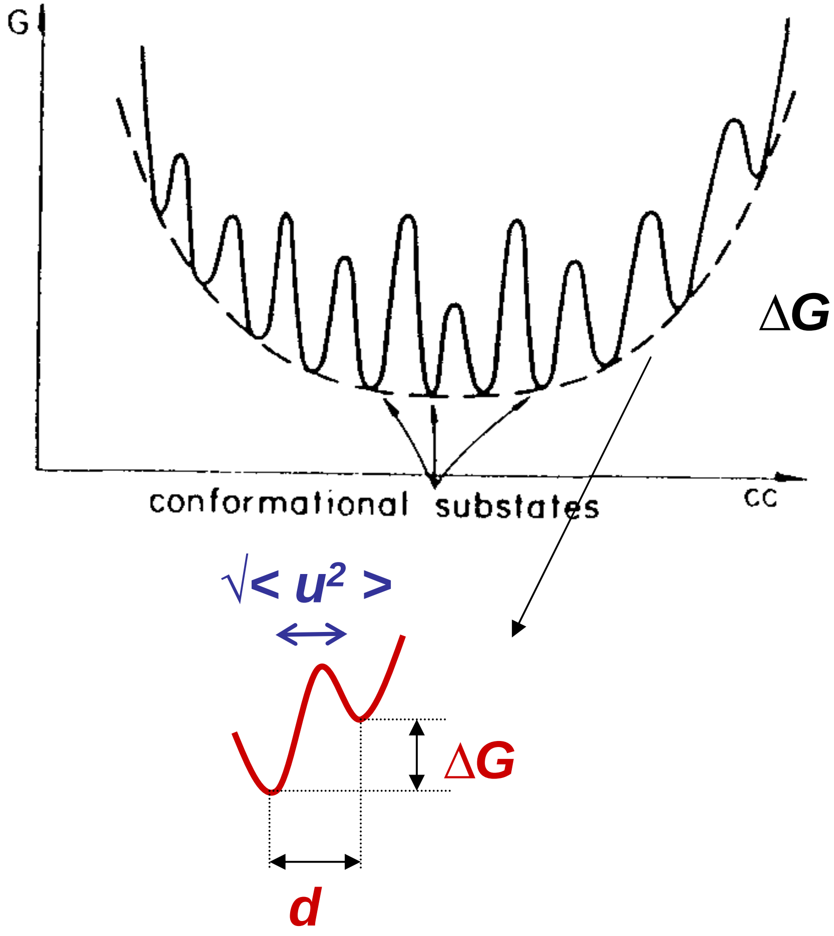
hydrated

in a trehalose glass

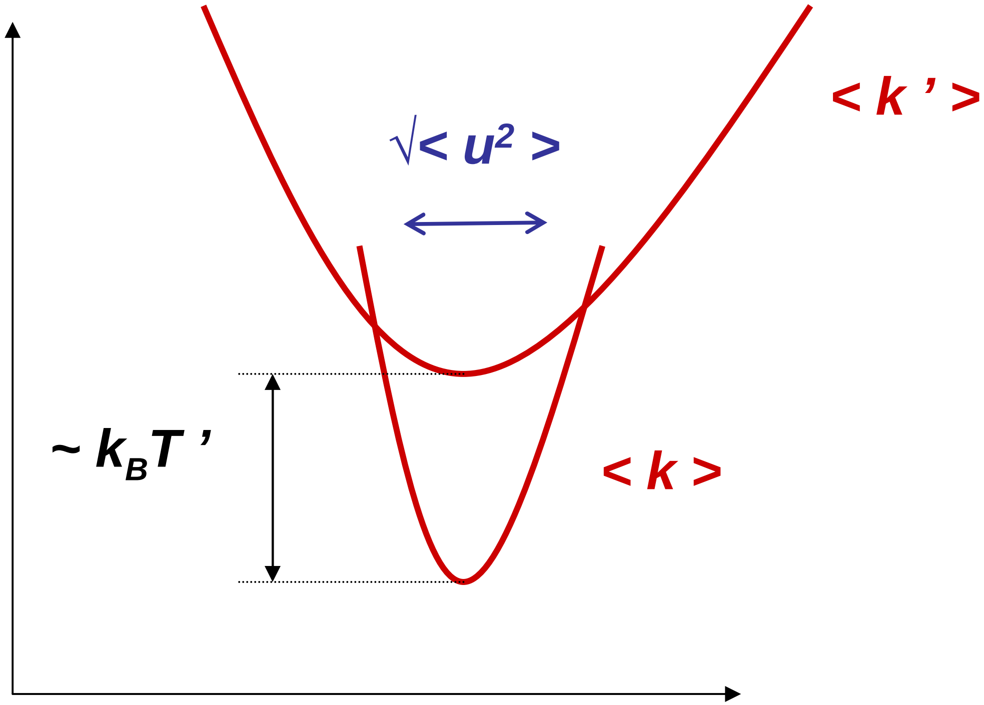
< *k* ' > ~ 1 / (d< u^2 > /d*T*)

Doster et al. (1989), Cordone et al. (2000), Zaccai (2000)

Model for protein dynamics



$$\langle k' \rangle \sim \Delta G / 2d^2$$



quasi-harmonic approximaion

(Frauenfelder et al., 1988)

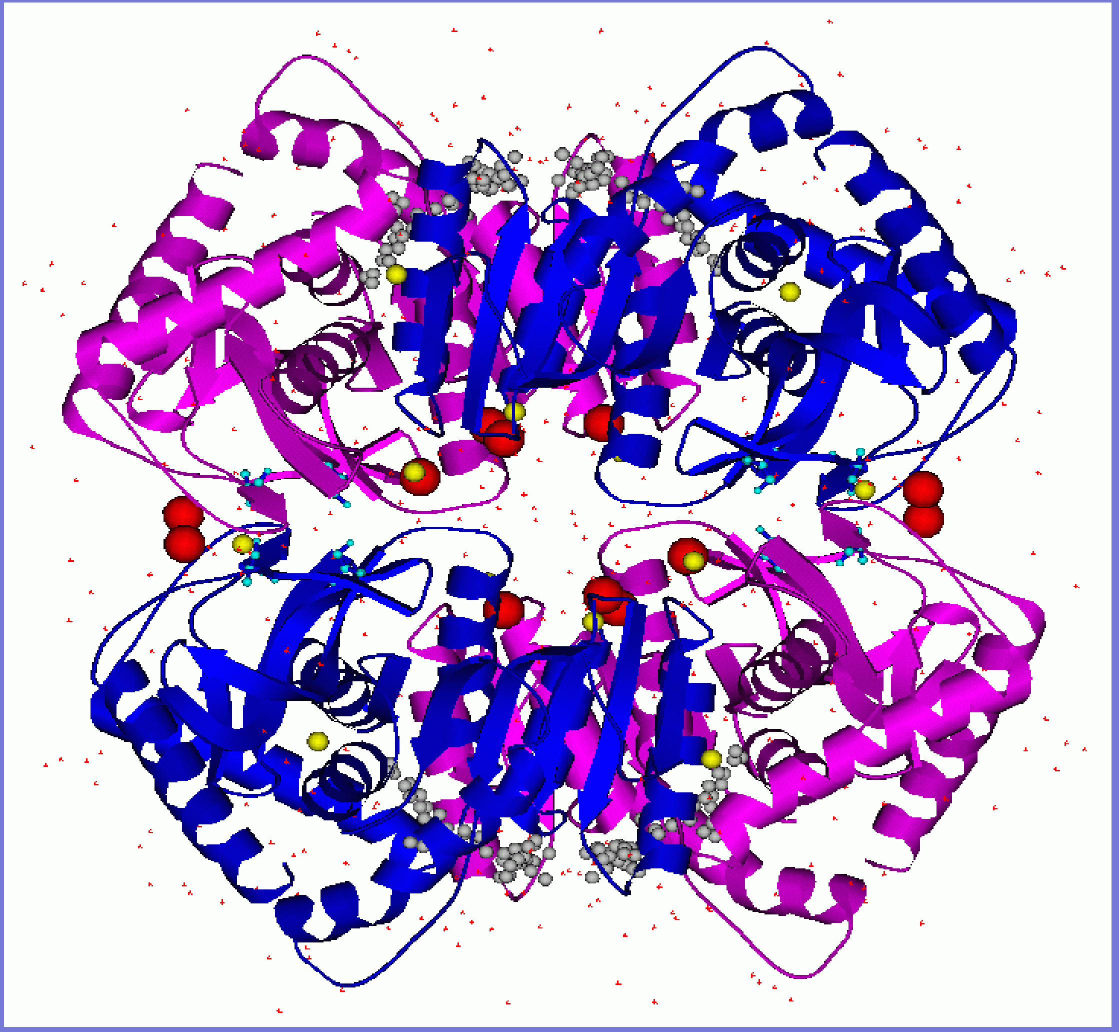
Held by forces, atoms fluctuate about their positions in the structure

neutron scattering >>>

1) Fluctuation amplitude 2) Effective force constant

what biologists call :

1) Flexibility 2) Resilience



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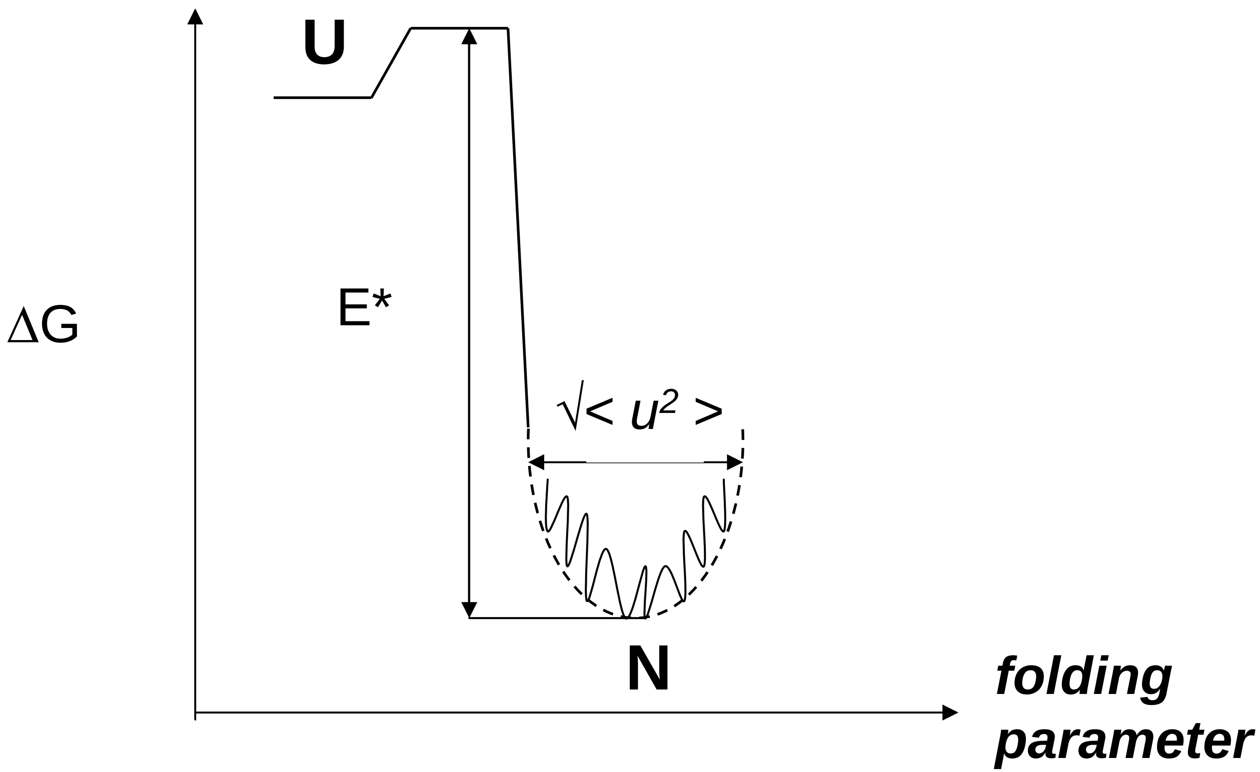
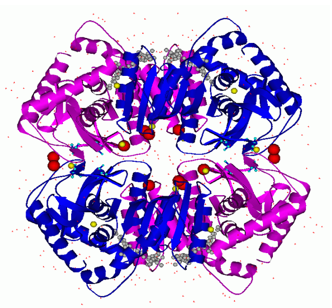
Correlation

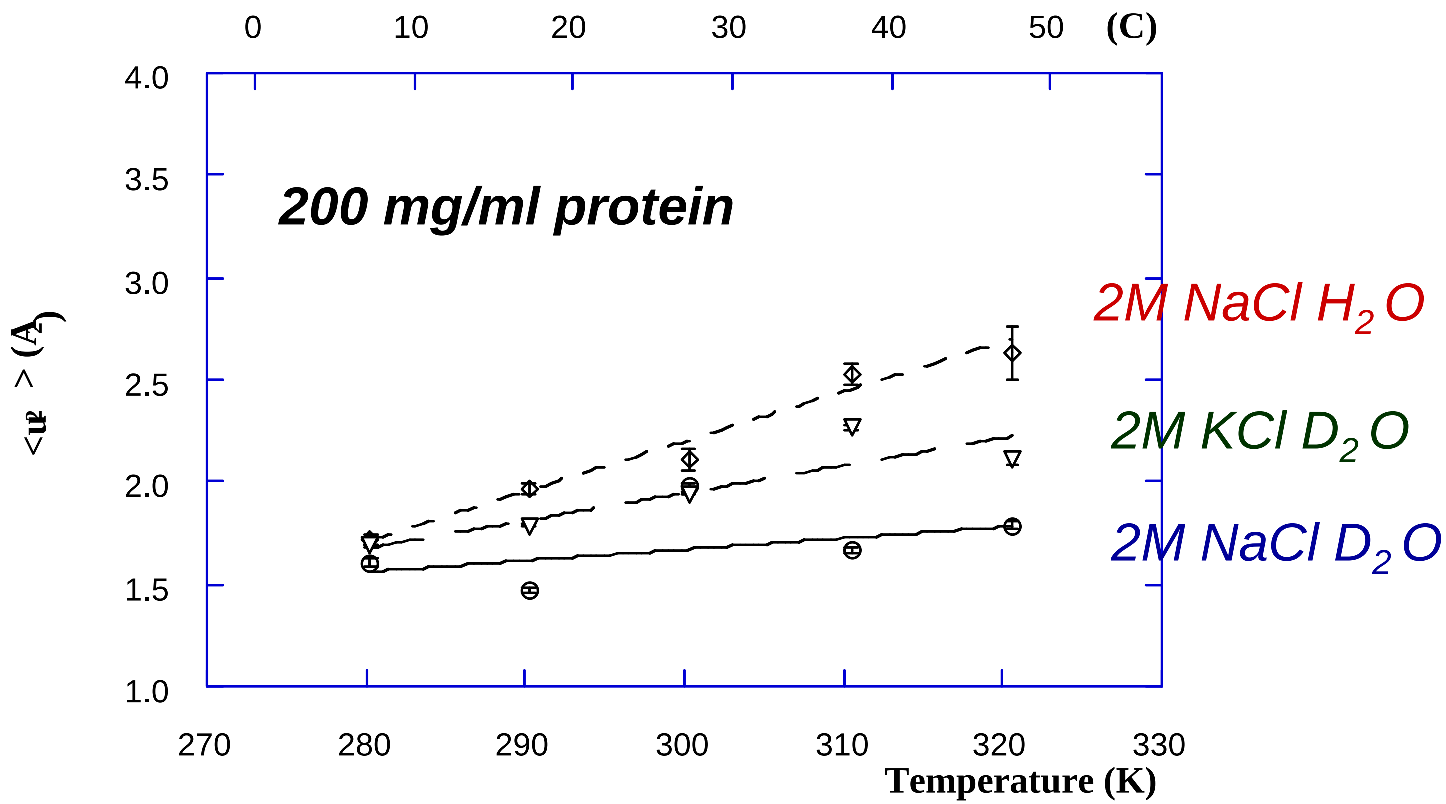
dynamics of the native structure < > stability ?

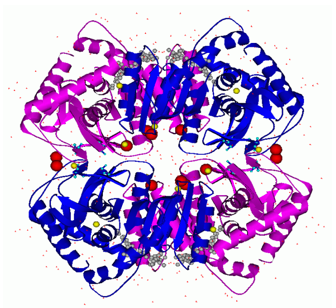




Tehei et al.(2001)

Fluctuations and force constants depend on the solvent





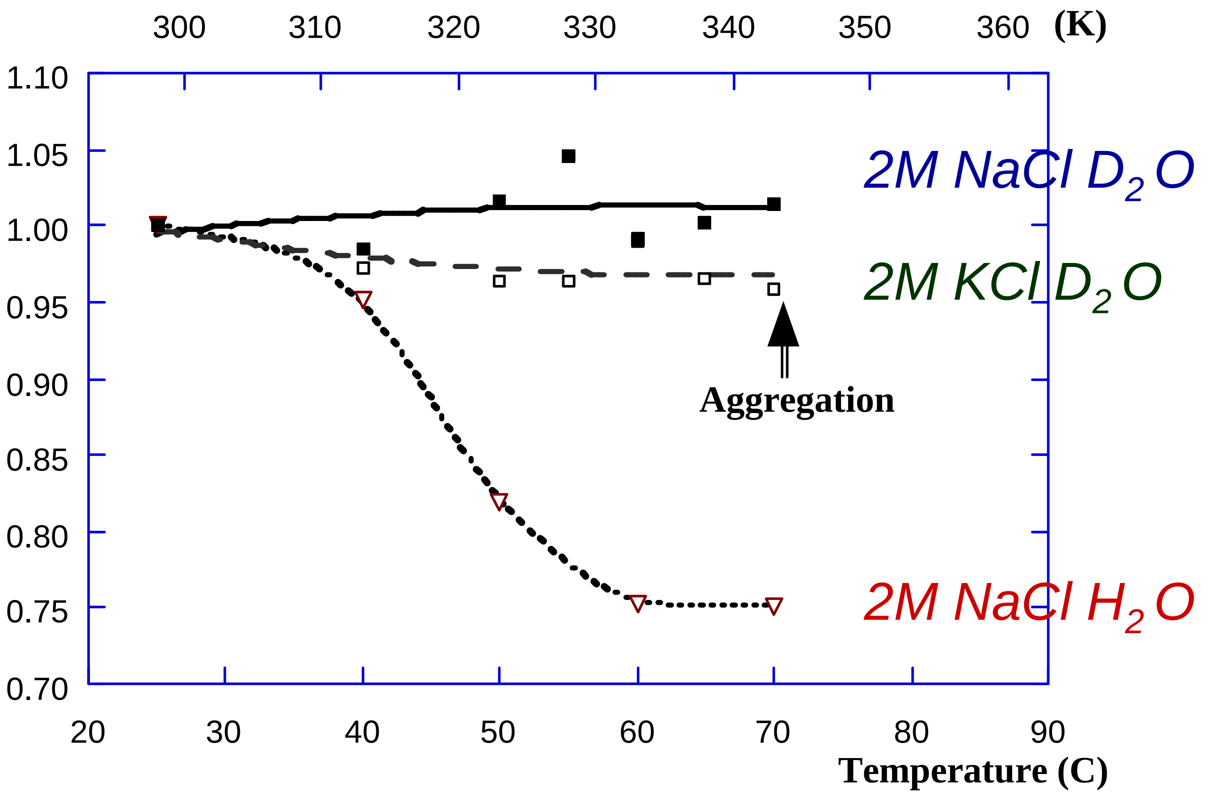
< k' > = 0.1 Newtons/metre

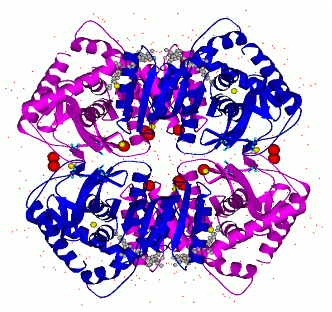
< k' > = 0.2 N/m

< k' > = 0.5 N/m

Tehei et al.(2001)

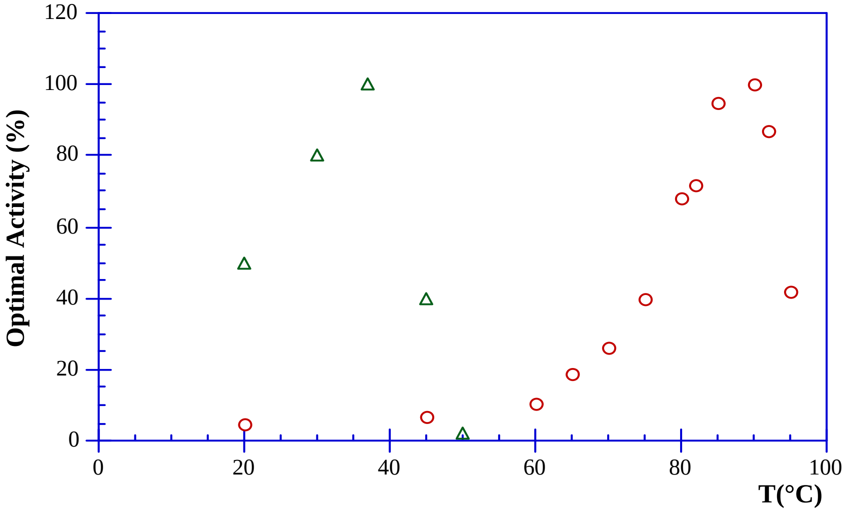
Stability depends on the solvent





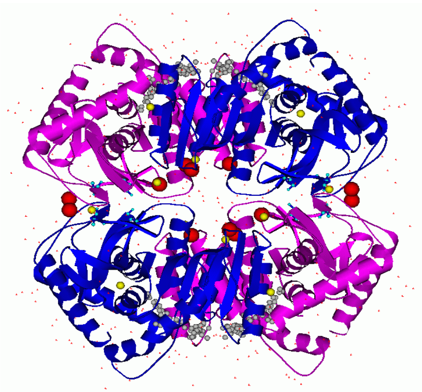
Tehei et al.(2001)

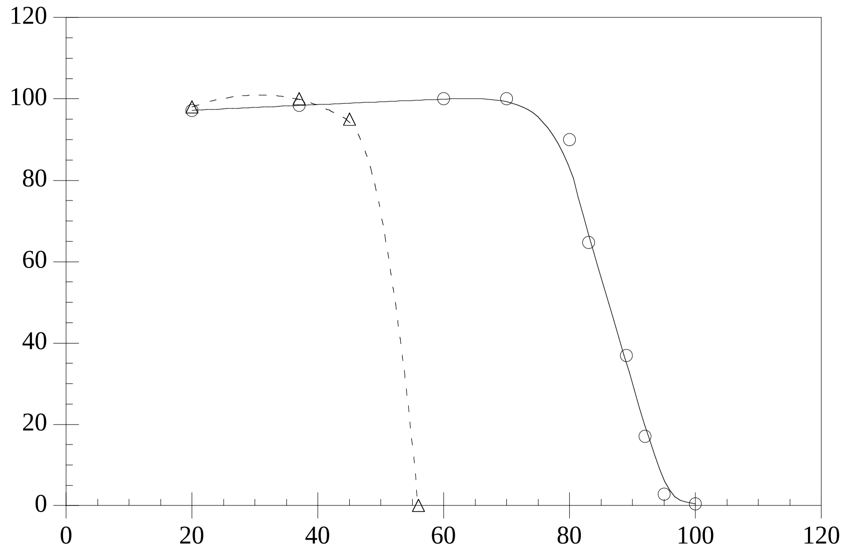
Adaptation to heat



Rabbit LDH,

Methanocaldococcus jannaschii MDH



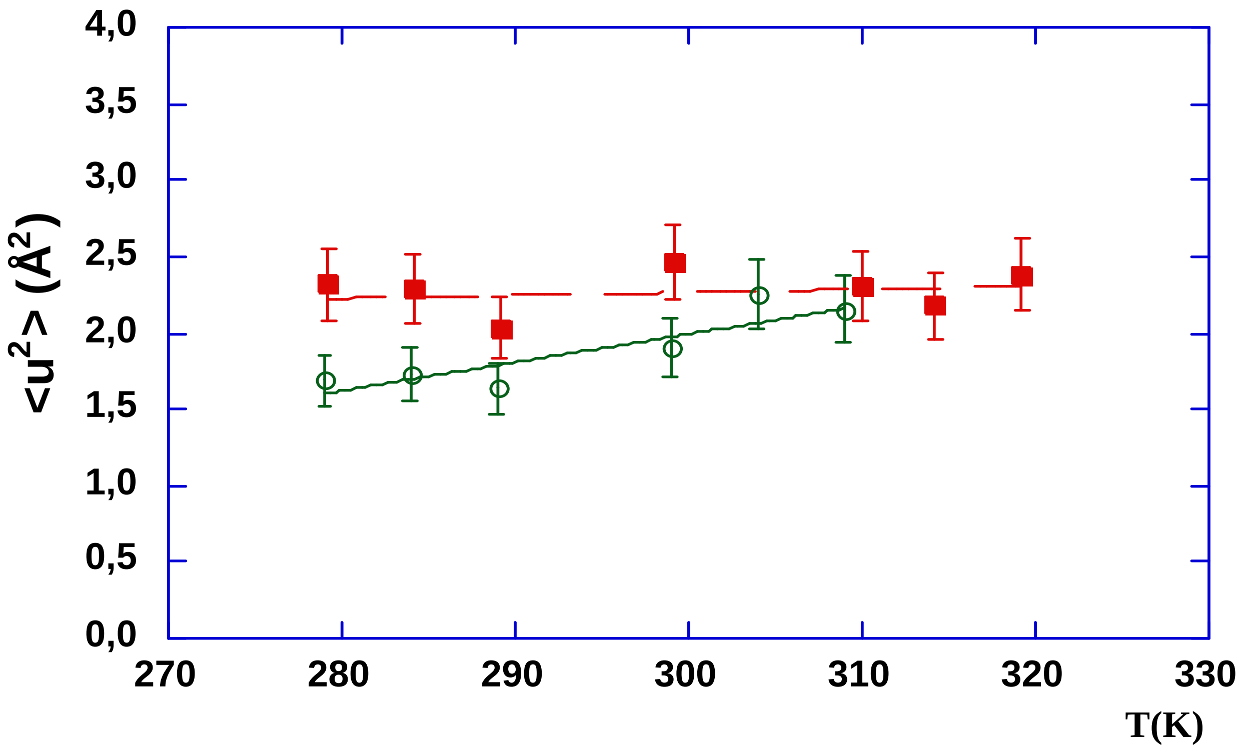


Tehei, M., D. Madern, B. Franzetti & G. Zaccai (2005)

Rabbit muscle MalDH *Methanocaldococcus jannaschii MalDH*

Resilient hyperthermophile

Softer mesophile



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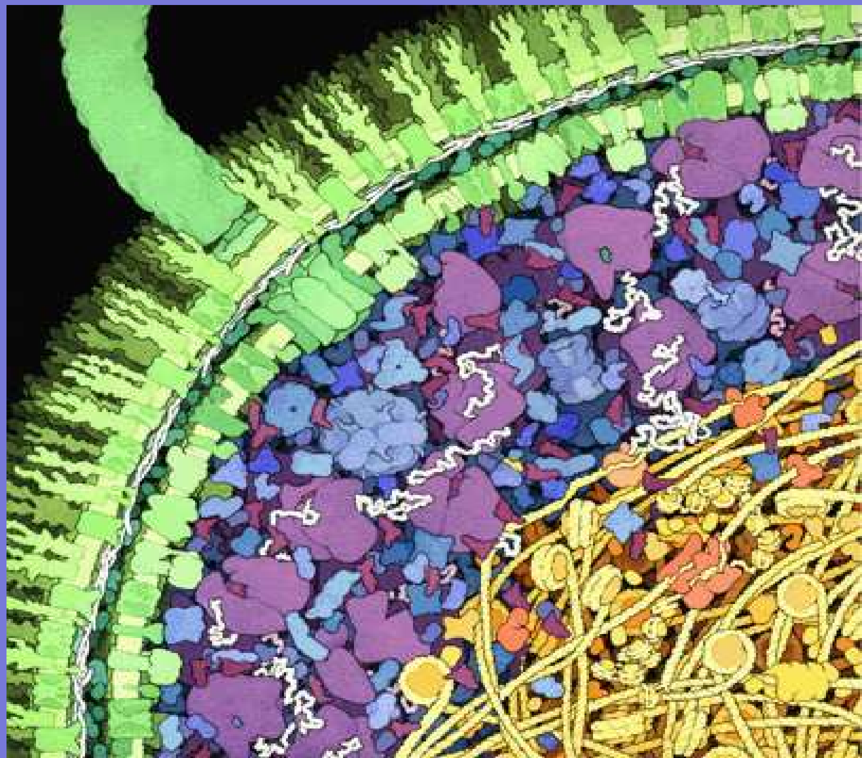
Mapping intracellular molecular dynamics

Aquaspirillum arcticum
psychrophile 4°C



Proteus mirabilis
mesophile 37°C

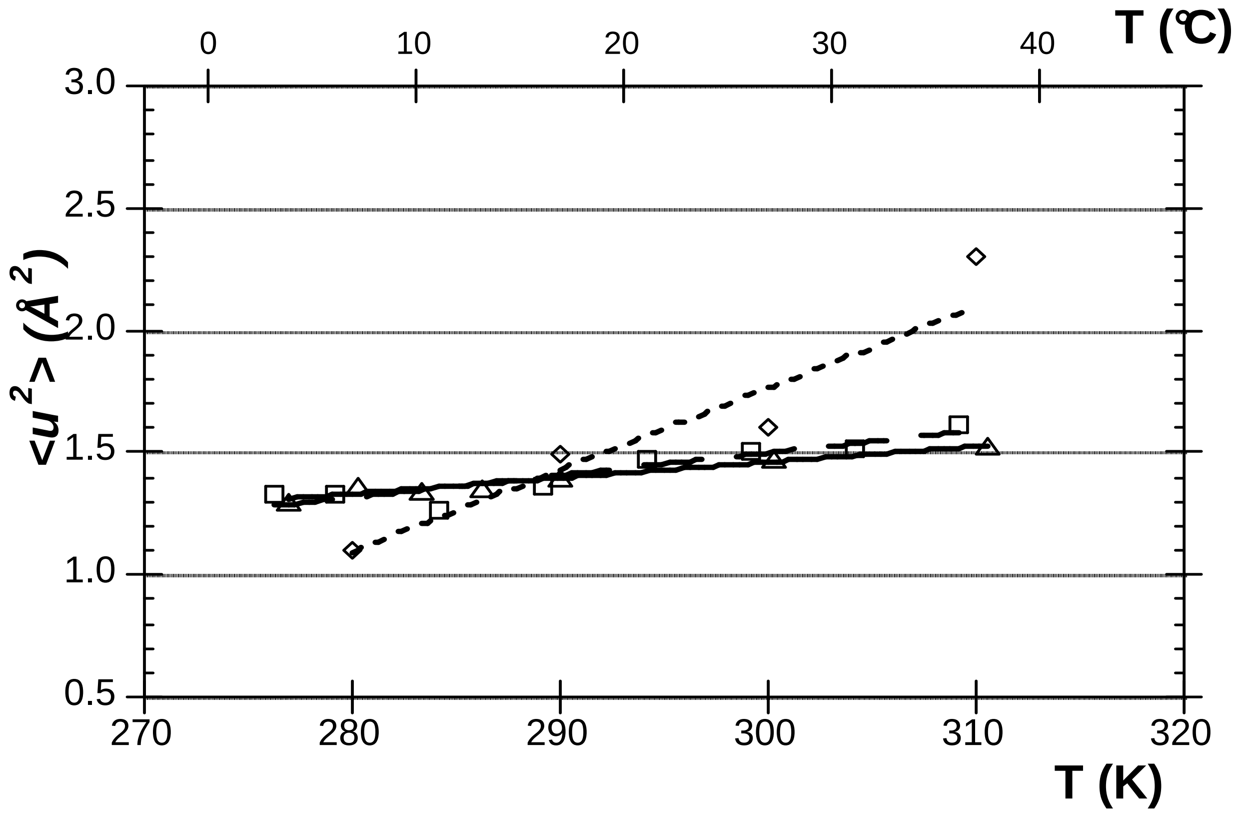
Escherichia coli
mesophile 37°C

Thermus thermophilus
thermophile 65°C

Aquifex pyrofilus
hyperthermophile 85°C

Tehei et al. (2004)

E. coli : alive and cooked



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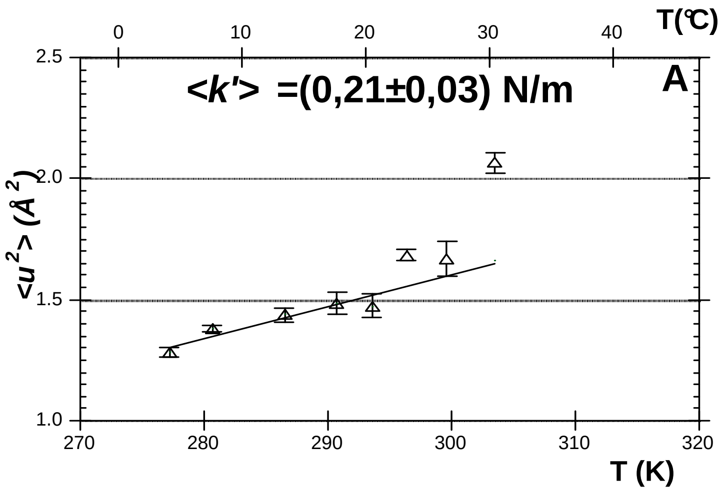
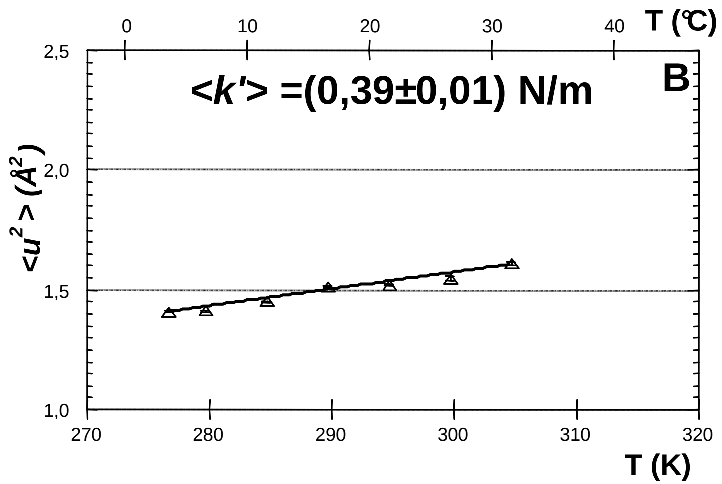
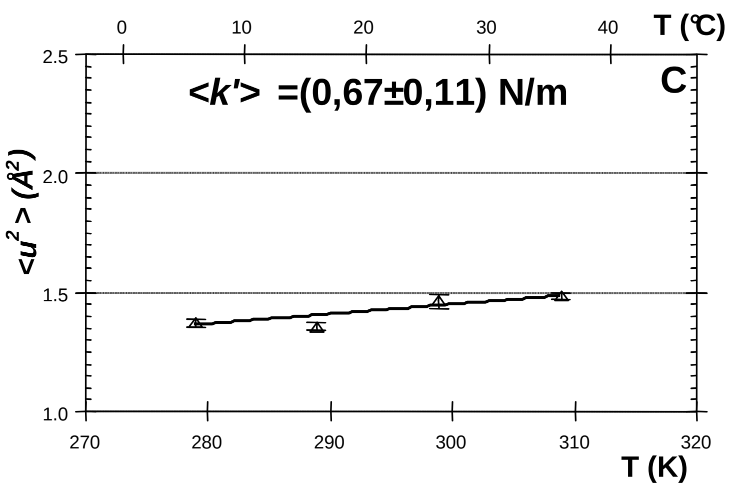
A. arcticum



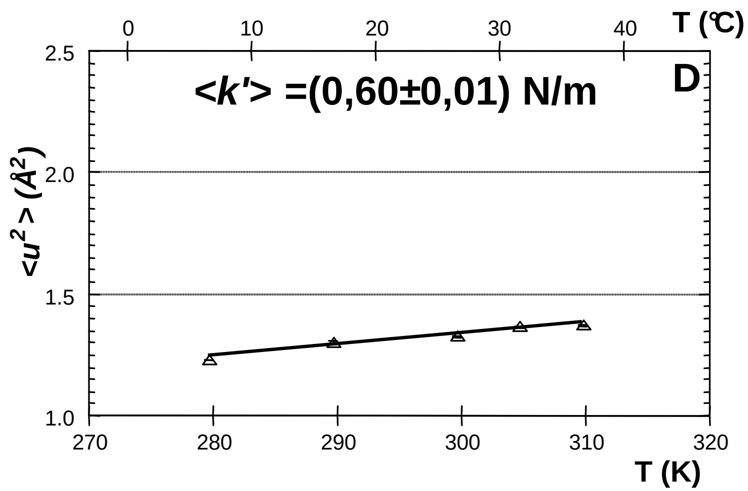
P. mirabilis



T. thermophilus

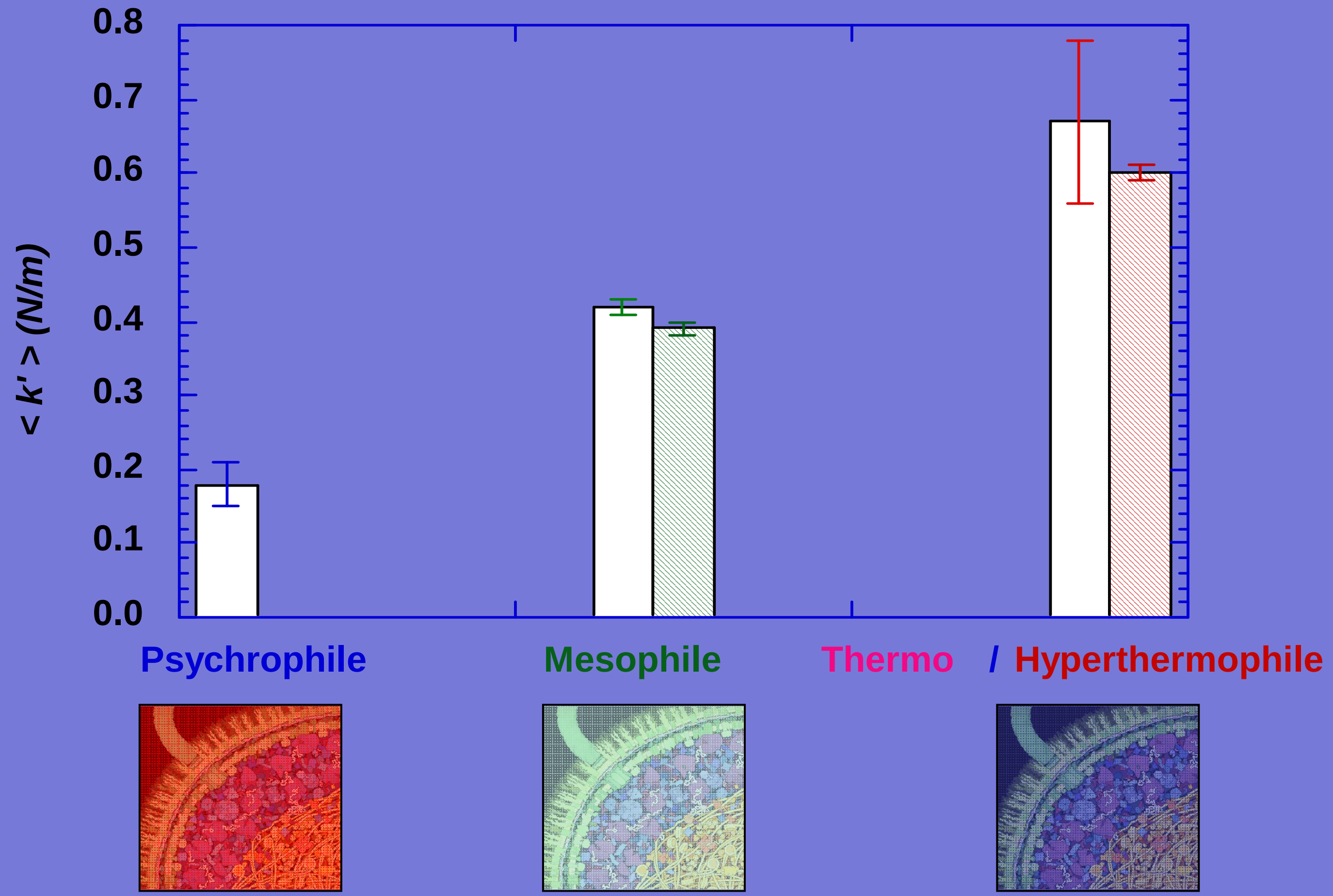


A. pyrofilus



Forces stabilise protein structures and allow functional motions

The mean effective force constant
is adapted to maintain
stability and a similar rms fluctuation amplitude
at
physiological temperature



The mean force force constant is lowest for the psychro. bacteria (softest) and highest (stiffest) for the thermoph.

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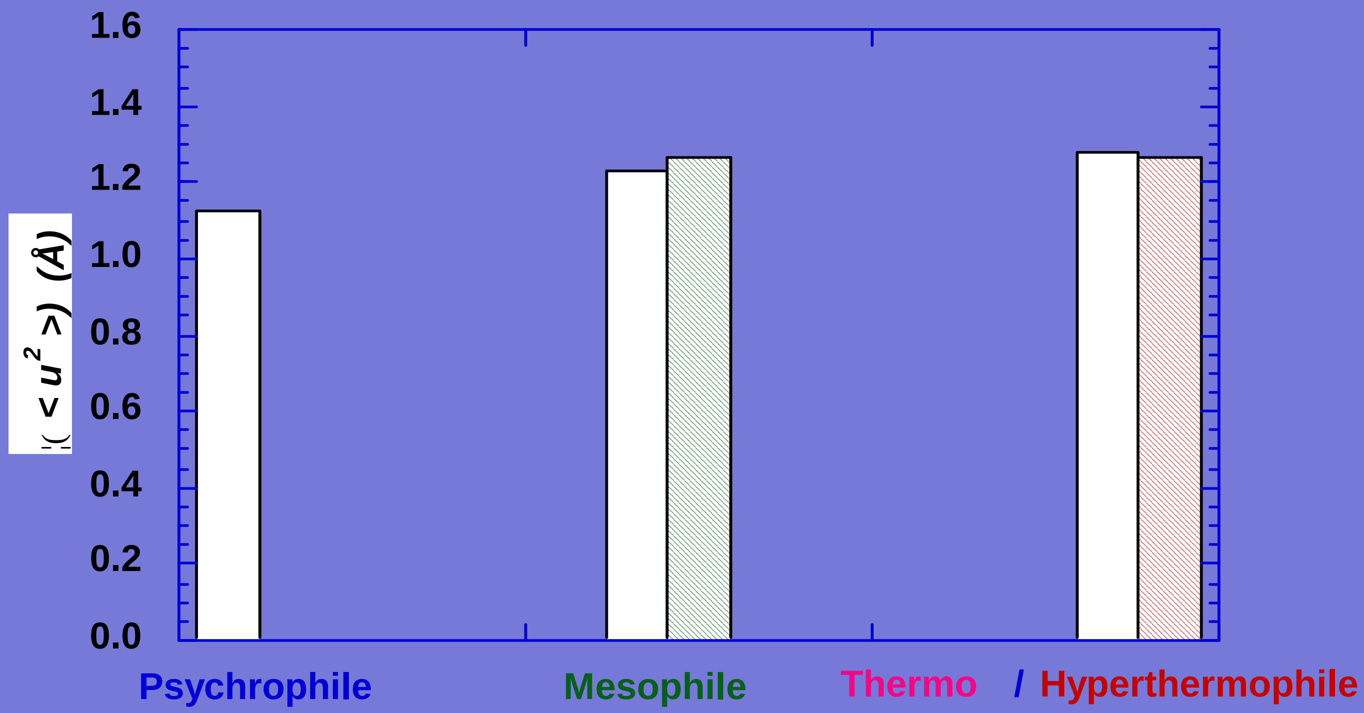
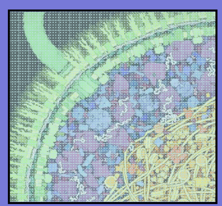
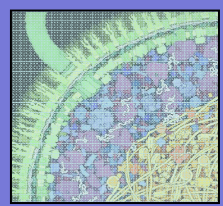
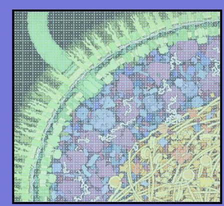








Similar rms fluctuation amplitudes at respective physiol. temp.,

EVOLUTION

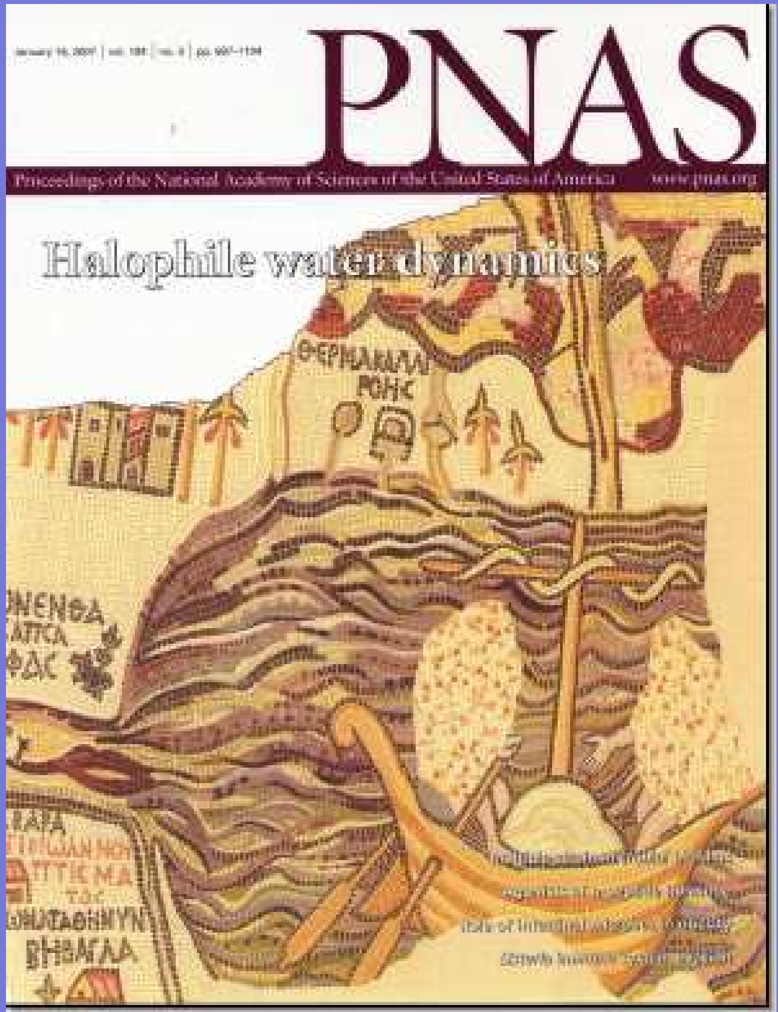
selects

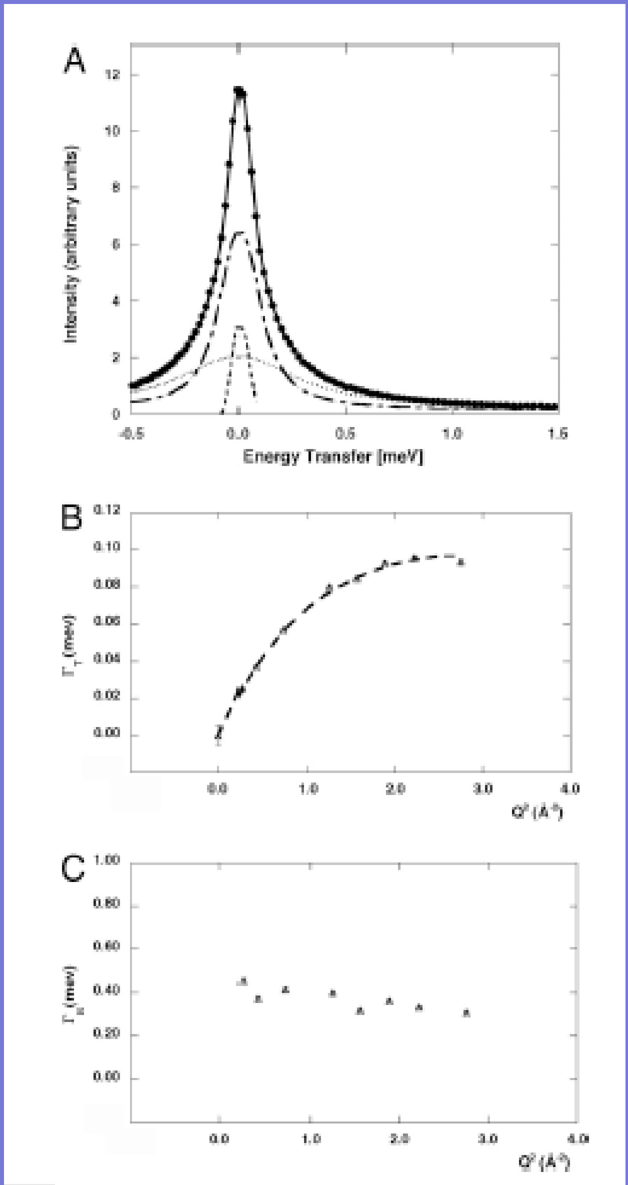
DYNAMICS

Forces that define
structure stability AND motions

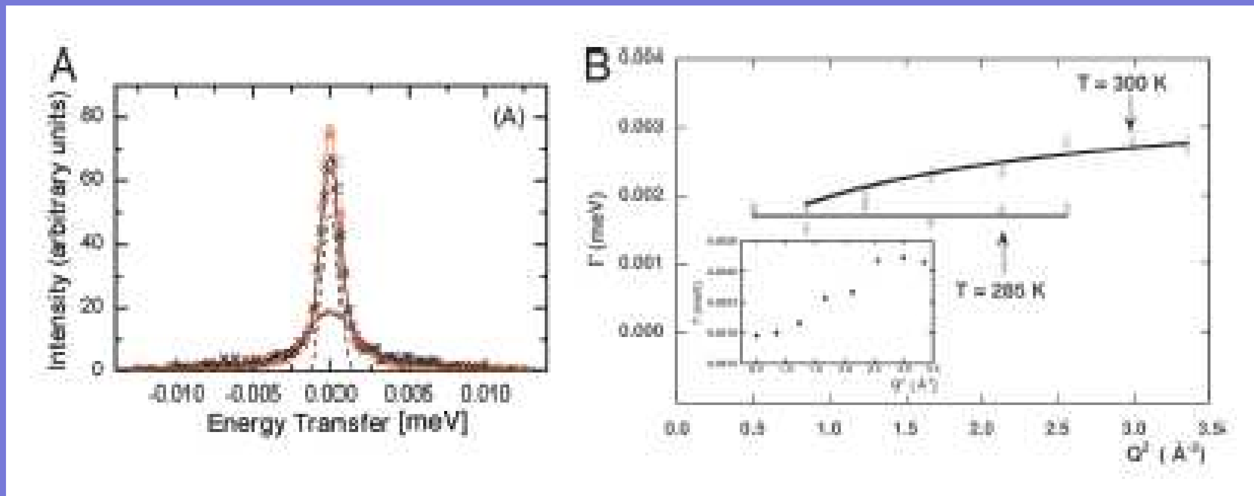
Neutron scattering reveals extremely slow cell water in a Dead Sea organism

Moeava Tehei[*,†], Bruno Franzetti[*], Kathleen Wood[‡,§], Frank Gabel[*], Elisa Fabiani[*,‡], Marion Jasnin[*], Michaela Zamponi[¶], Dieter Oesterhelt[§], Giuseppe Zaccai[*,‡,||], Margaret Ginzburg[**], and Ben-Zion Ginzburg[**]



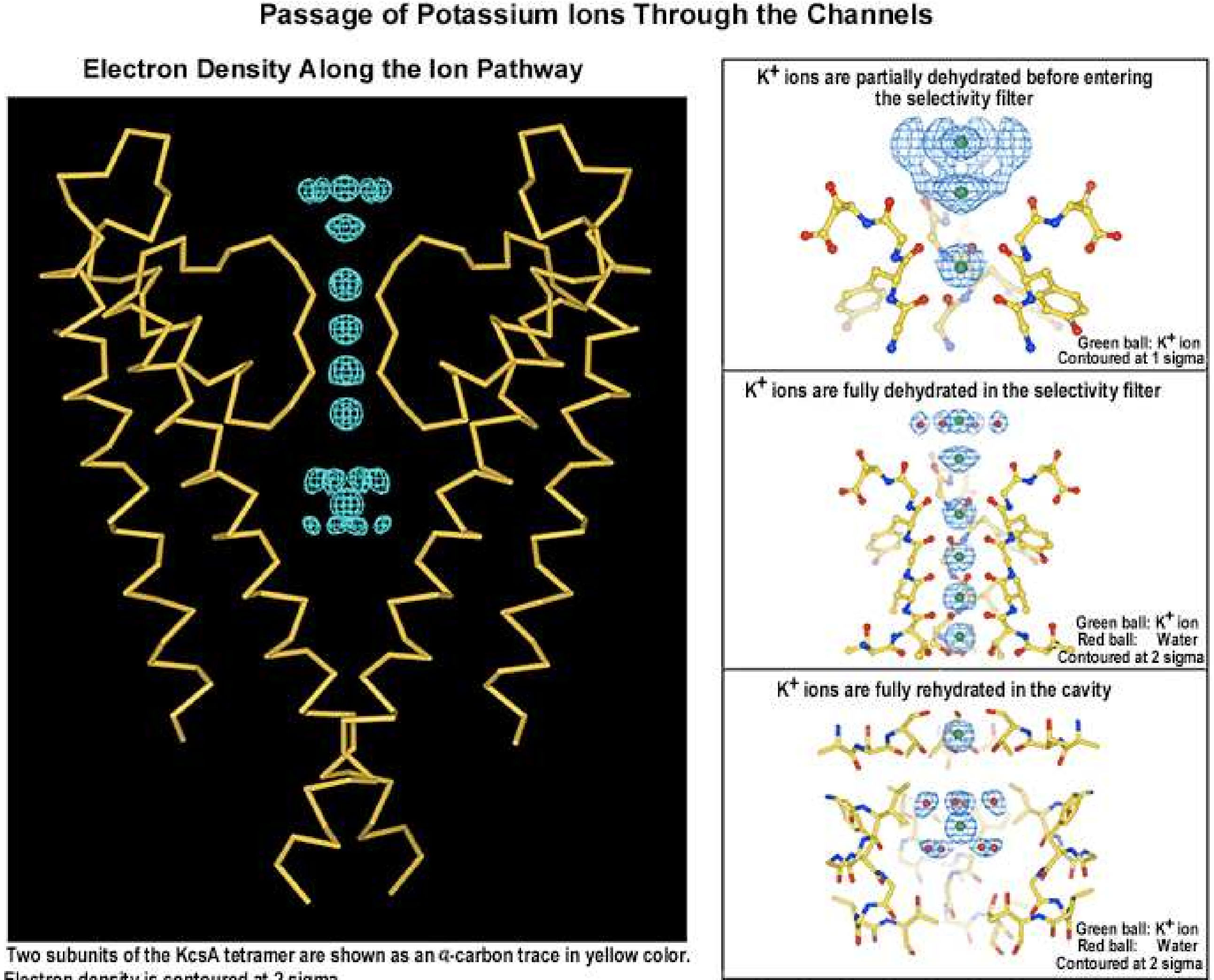


IN6 10ps time-scale
Dt ~ Dt of H2O in 3.5M NaCl
τR ~ 2ps



IN16 1ns time-scale
Dt ~ 1/250 Dt of bulk H2O

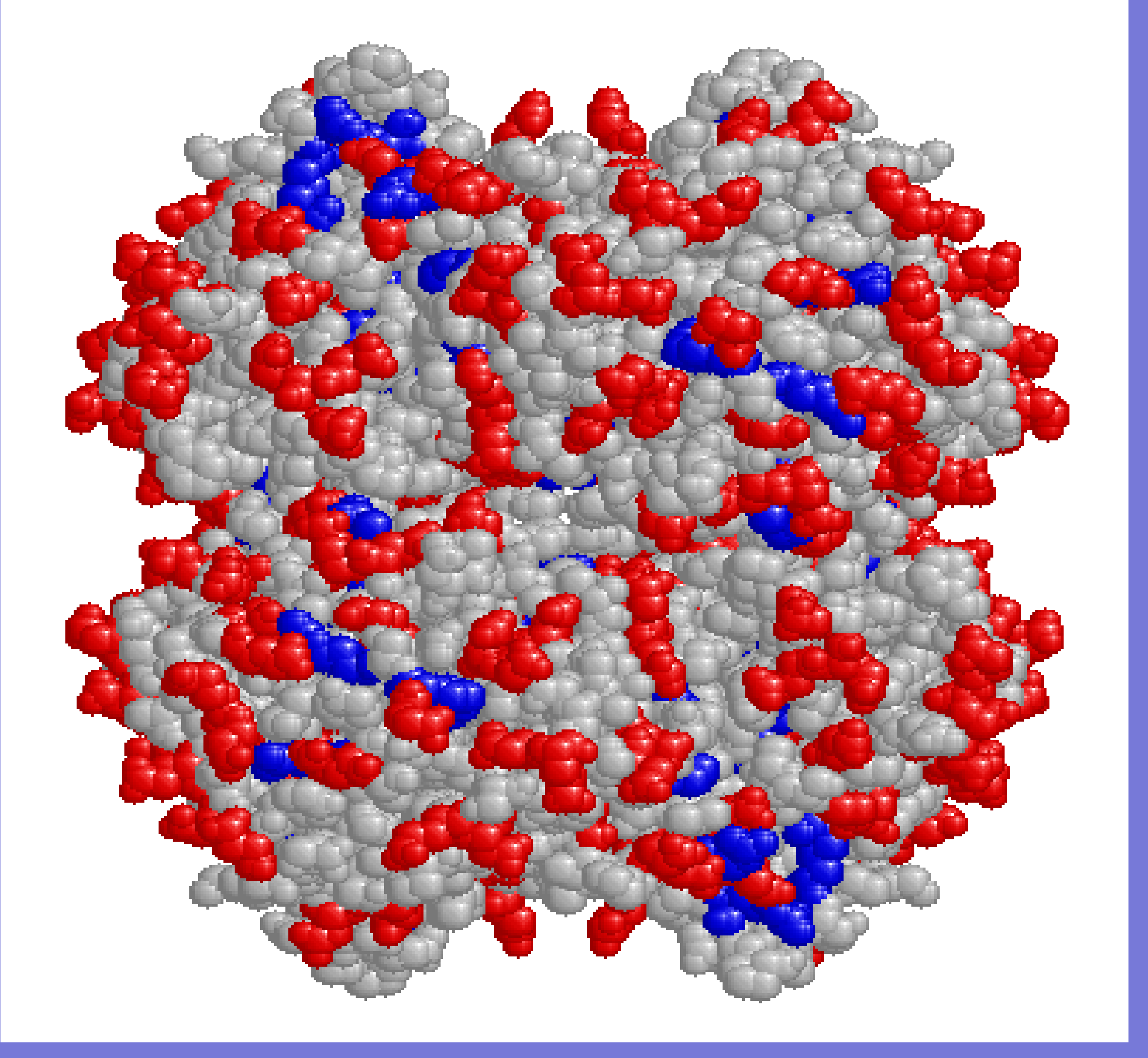
Passage of potassium ions through the channels...



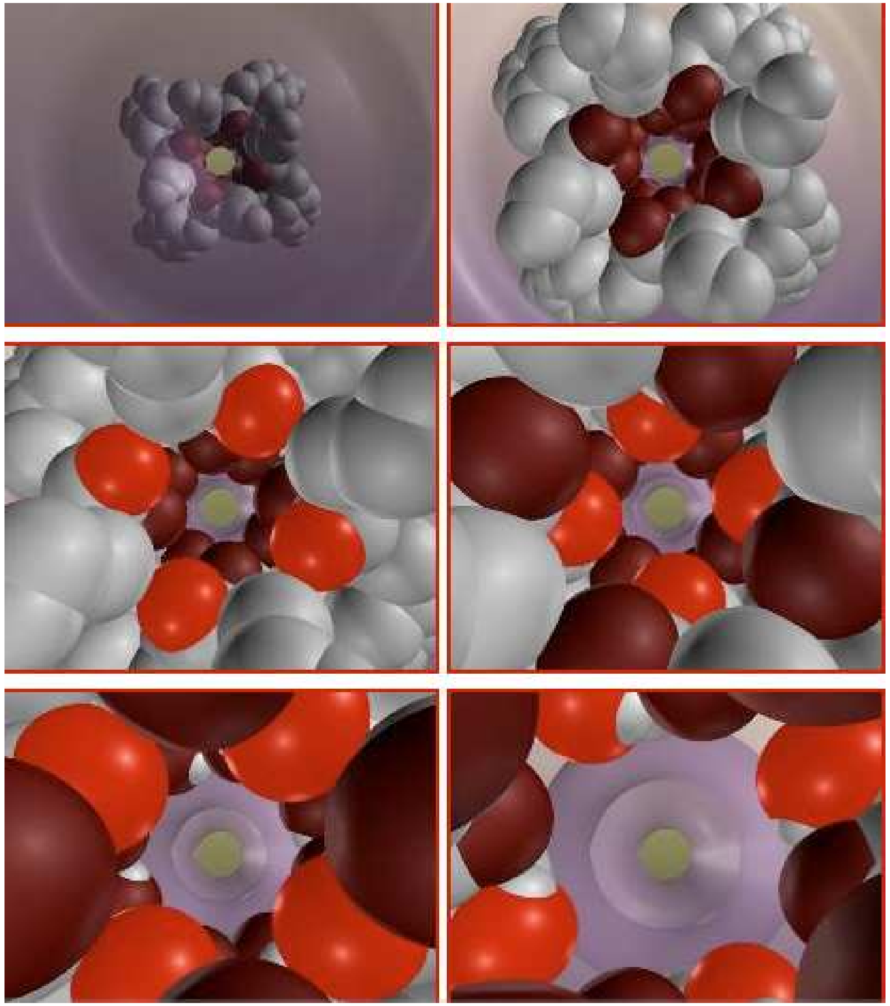
Two subunits of the KcsA tetramer are shown as an α-carbon trace in yellow color. Electron density is contoured at 2 sigma.

R. McKinnon at www.hhmi.org

Surface acidic (red) and basic (blue) residues



Potassium Selectivity Channel



Shin-ho H Chung at anusf.anu.edu.au

Thank you

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