The role of environmental fluctuations in the function of proton pumps

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Salts at moderate and high concentrations (above 50-100 mM) control the aggregation state of proteins, as well as their structure and dynamics in a correlated manner ("Hofmeister-effects").

The effects are dominated by anions.

Hofmeister (1888) ordered the anions in a series according to their effectiveness in precipitating serum albumin:

$$SO_4^{--} > F^- > CH_3COO^- > Cl^- > Br^- > I^- > ClO_4^-, SCN^-$$

kosmotropes	chaotropes
salting out	salting in
increased aggregation	increased solubility

Effect on protein structure: **kosmotropes - stabilization**
chaotropes - destabilization

What is the underlying mechanism?

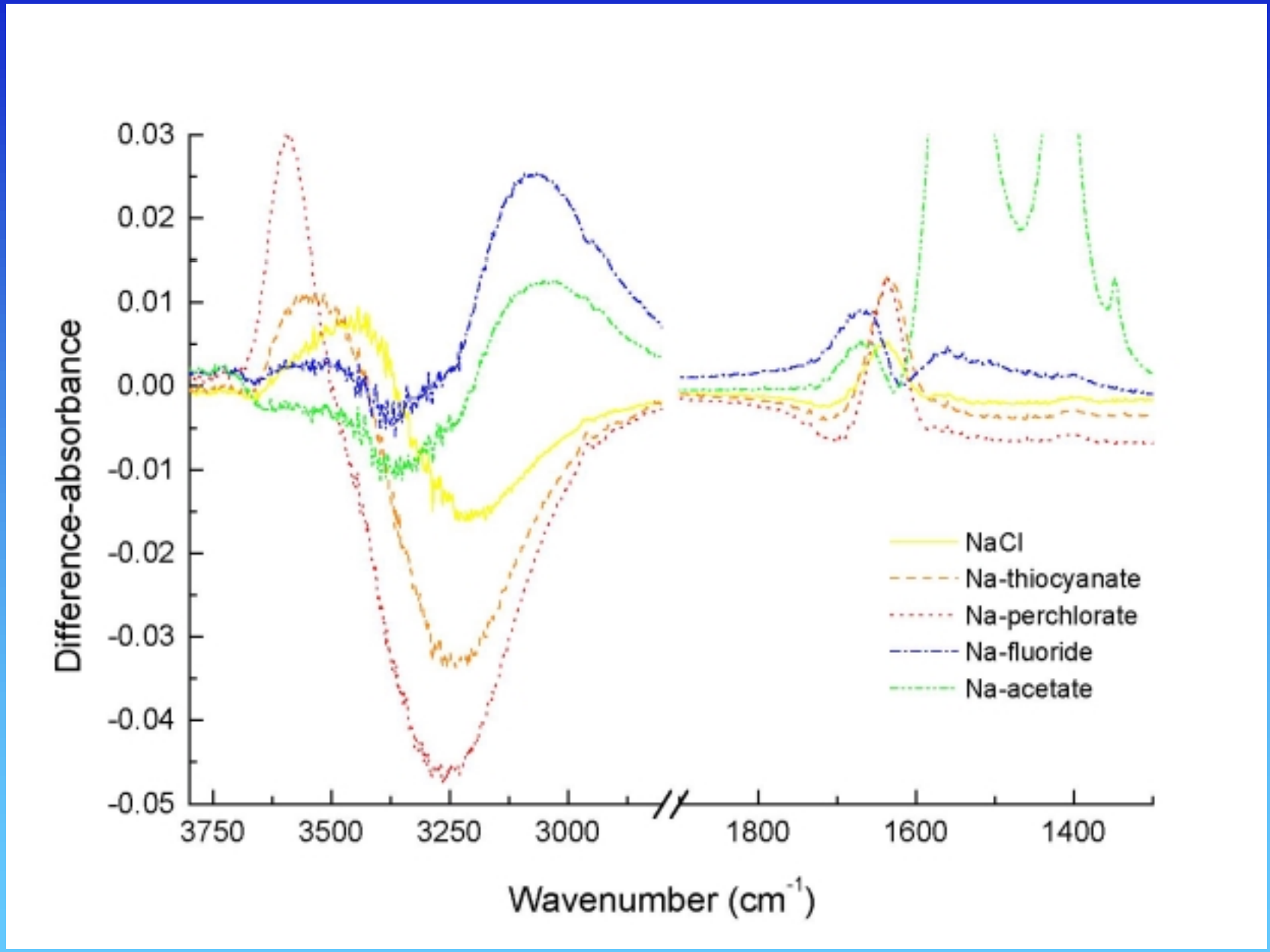
Possible explanations:

1. Chaotropic anions bind directly to protein surfaces and loosen H-bonds
2. Anions exert their effect indirectly, via changing the water structure

Experimental evidences:

n-diffraction and NMR experiments on water – rotational mobility changes of water molecules upon addition of salts [Leberman & Soper, 1995, Nature, Müller & Hertz, 1996, J.Phys. Chem.]

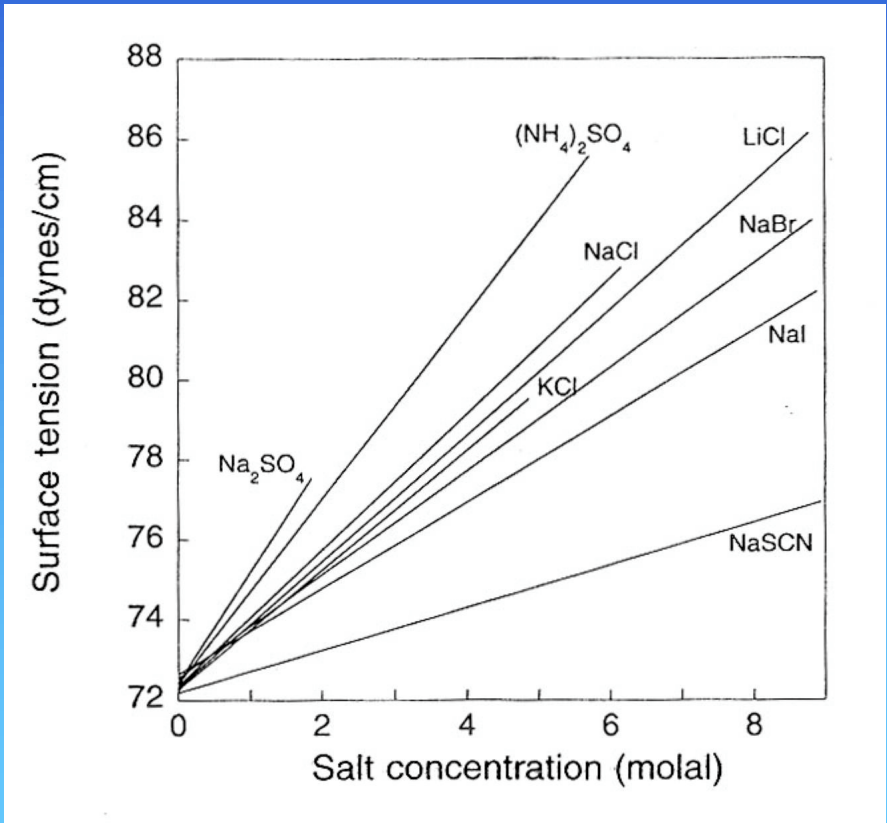
Our FTIR experiments



Conclusion:

kosmotropes strengthen H-bonds (“water structure makers”)
chaotropes loosen them (“water structure breakers”)

Surface tension measurements [Jarvis and Scheiman, 1968]



Again the same Hofmeister-series!

Cavity model (Melander, Horvath, 1977, ABB, modified by Baldwin, 1996, Biophys J.):

$$\Delta G = \Delta G_{cav} + \Delta G_{solv}$$

$\Delta G_{cav} = \gamma \Delta A$ (explains "salting-out"), where γ is the surface tension of water, and ΔA is the area change. The second term stands for "salting-in".

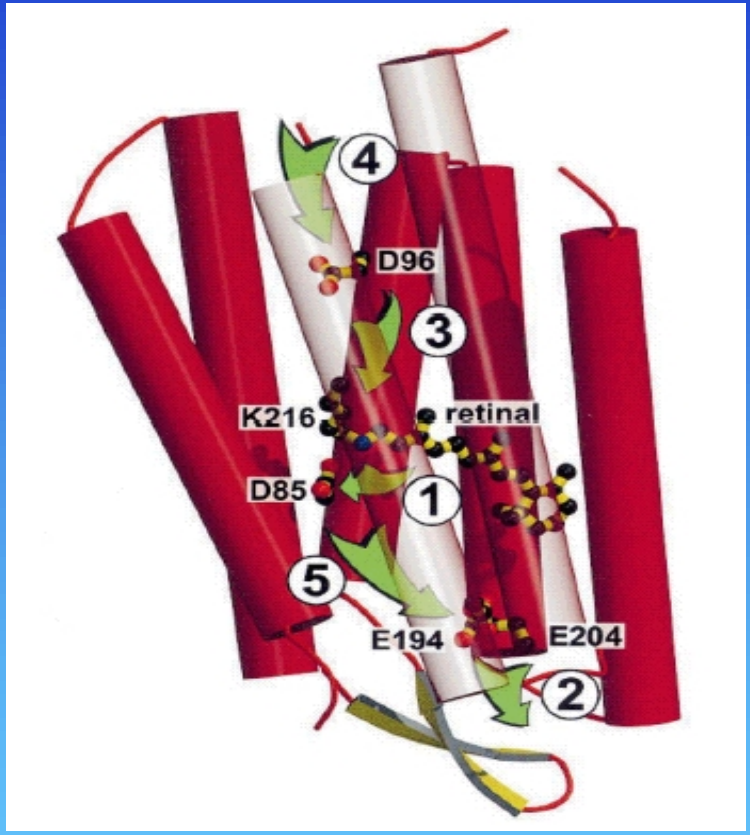
Criticism of the theory:

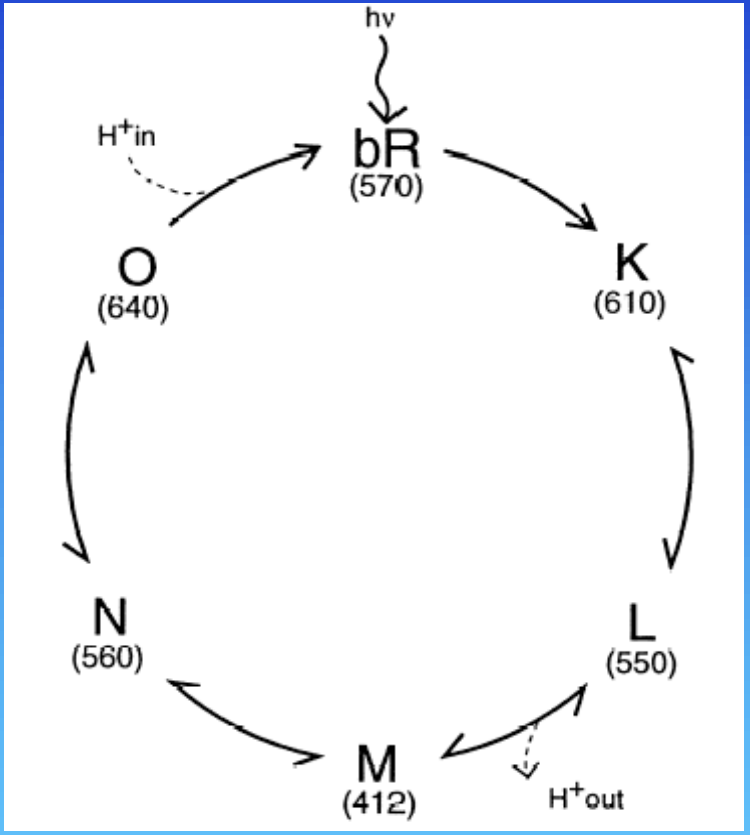
1. Qualitative and phenomenological; In molecular dimensions the surface tension is not defined.
2. It could not readily forecast dynamic phenomena.

Not too many data on dynamic systems, but normally kosmotropes increase enzyme activity

Is it possible that chaotropes increase enzyme activity?

Bacteriorhodopsin: the simplest proton pump in biological systems: a paradigm for ion pumps and the 7-helix receptor family (similar to visual rhodopsin)



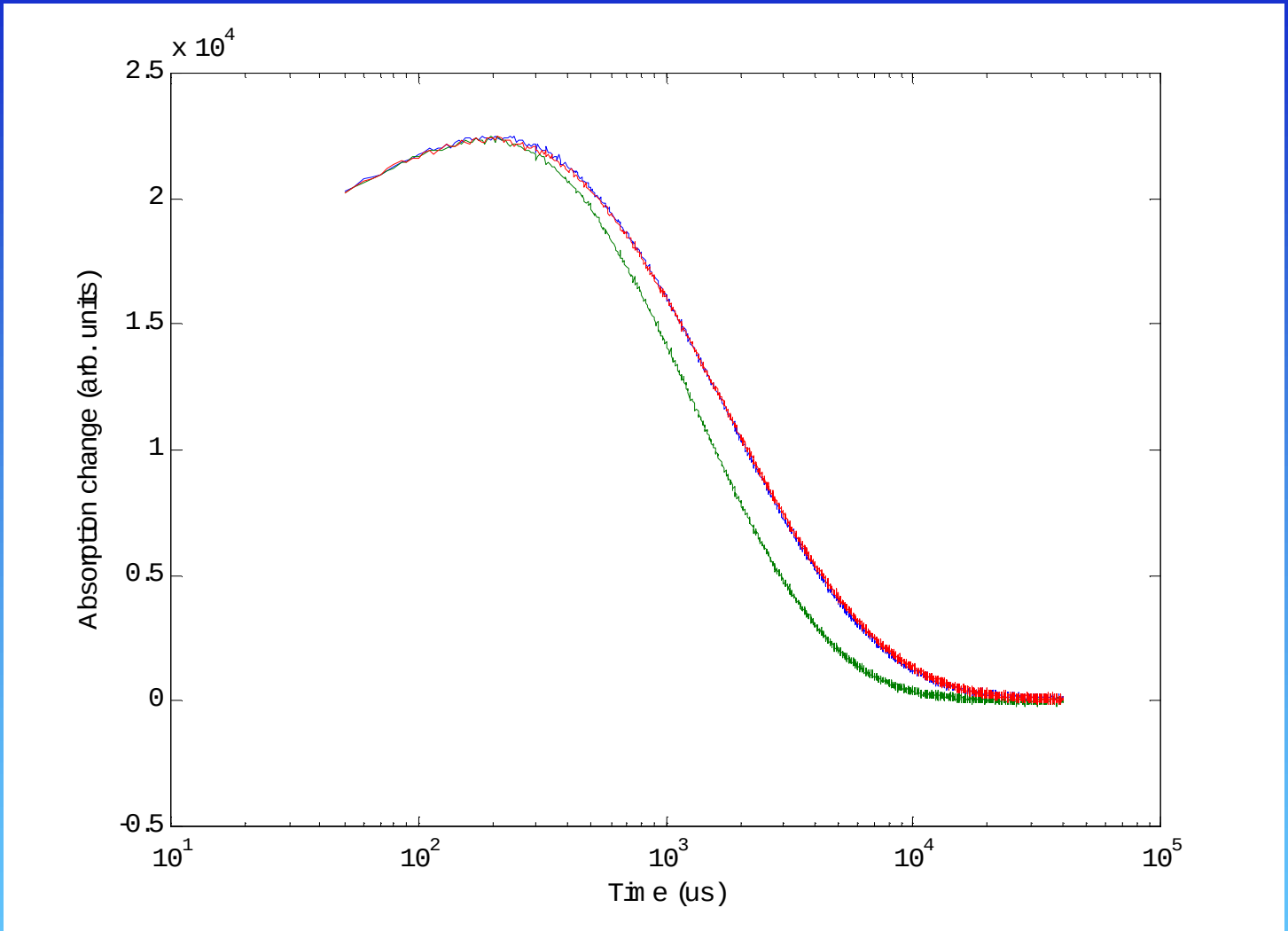


Luecke, BBA 1460, 133-156, 2000

Photocycle

Under normal conditions the "M"-decay is rate-limiting.

We measured the kinetics of “M” in the presence of different (monovalent) salts (Dér and Ramsden, Naturwissenschaft.1988)



Phenomenological interpretation: chaotropes loosened the protein structure (they increased flexibility)

Common sense: Proteins need some flexibility to function (but too much flexibility is already destructive).

Proteins work as thermal engines: They get “energized” by a substrate (in this case light) and dissipate this energy via thermal fluctuations in a controlled way (defined by the protein structure).

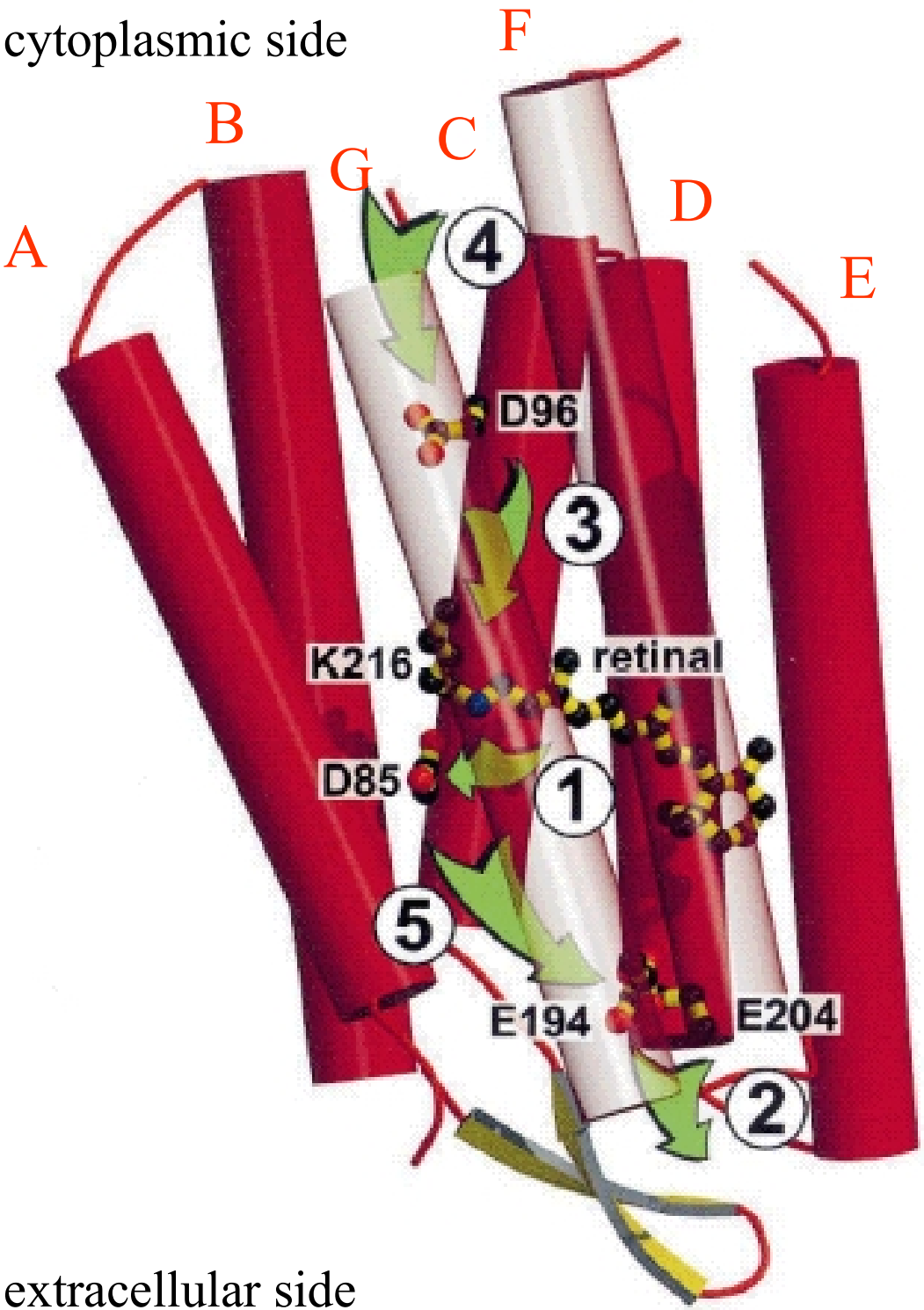
Fluctuation-dissipation theorem: higher protein flexibility – higher structural fluctuations.

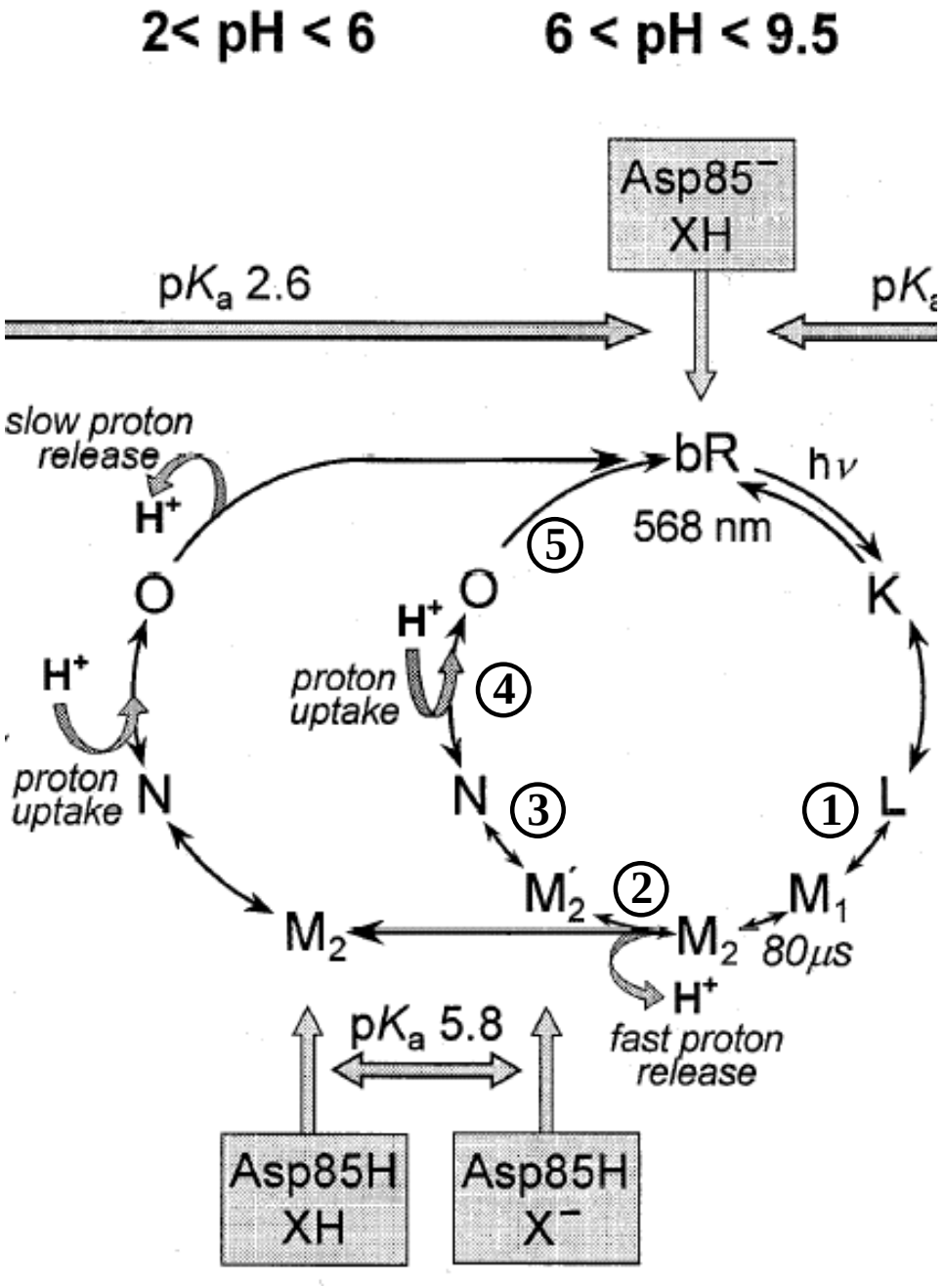
Under optimal conditions (if originally flexibility was set to optimum) increasing flexibility is destructive, but if the protein was too rigid, it is expected to help. (In case of bR we suspect this to be the case.)

But why do salts affect only the late part of the photocycle?

Summary of the proton transport steps

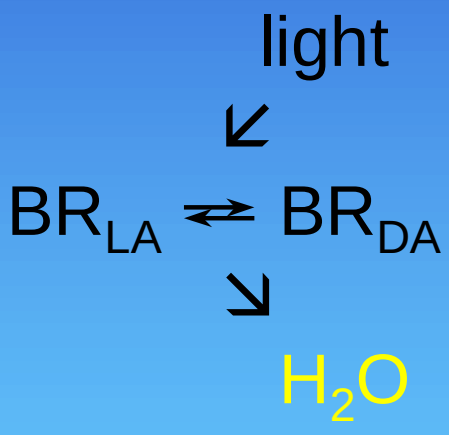
Balashov, BBA 1460, 75-94, 2000



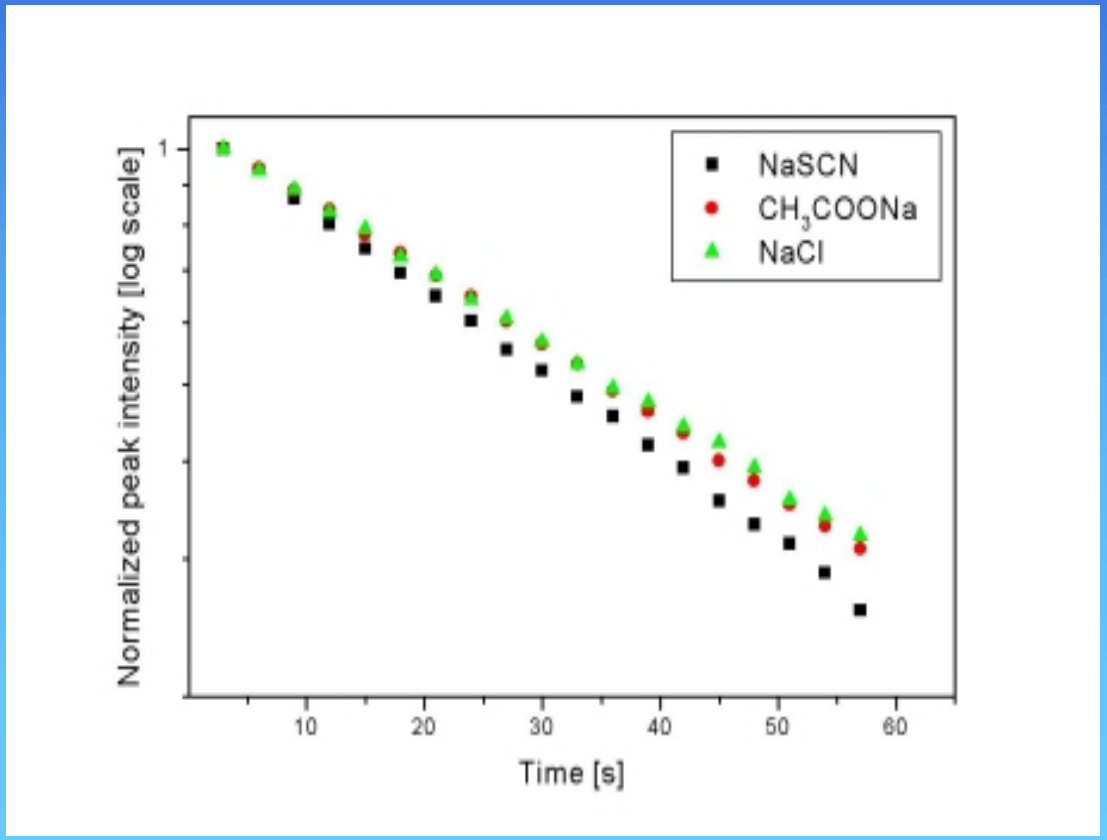


During M-decay (affected by Hofmeister-ions): Conformational changes and proton (H_3O^+) uptake by diffusion – **changes of protein surface exposed to water**

Another reaction of bR where Hofmeister-ions affect: "dark-adaptation"



Again water!

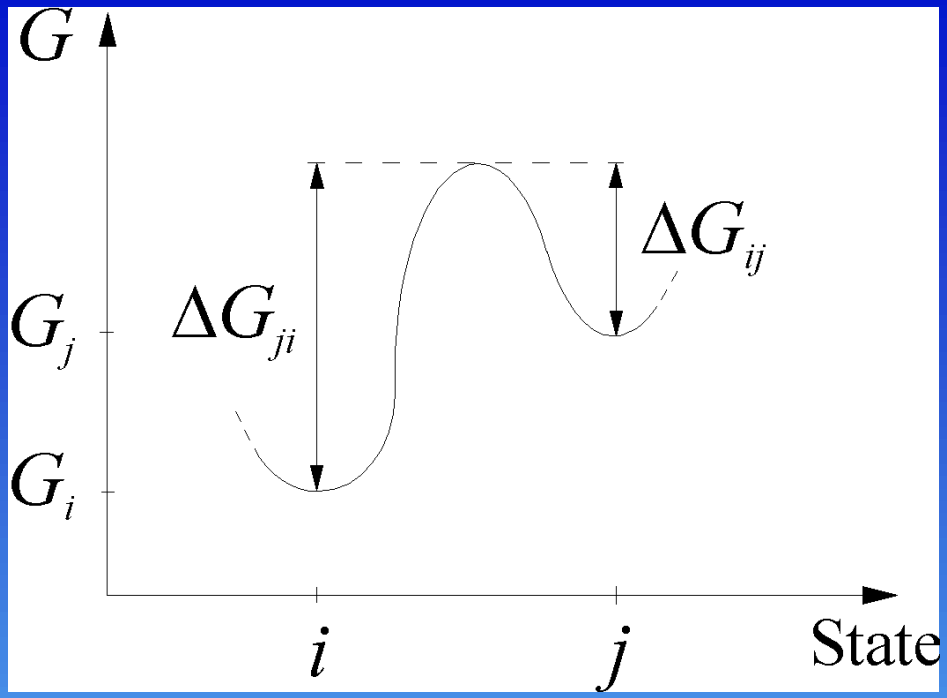


Water is essential for the operation of bR. If we extract fluid water (moderately dehydrated samples: 30-80 % relative humidity), the photocycle stops at M_1 [Váró,1983]. At the same time, molecular fluctuations drastically decrease [Dencher,1997].

Water controls fluctuations, anions control water structure.

Hypothesis: Anion-controlled Hofmeister-effects can be explained on the basis of altered levels of fluctuations.

Assumption: Large-scale structural fluctuations imply barrier fluctuations for such transitions of the photocycle that involve major conformational changes and water transfer (Neagu, Neagu, Dér, 2001).



The fluctuations were implemented in the form of a Markovian symmetric dichotomous noise added to the barriers with magnitudes proportional to the salt concentration.

$$\xi(t) \in \{-1,1\}$$

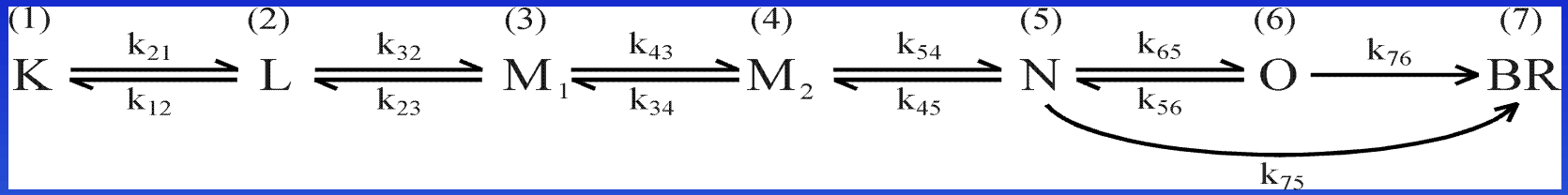
$$<\xi(t)>= 0$$

$$<\xi(t)\xi(t')>= \exp\left(-\lambda|t-t'|\right)$$

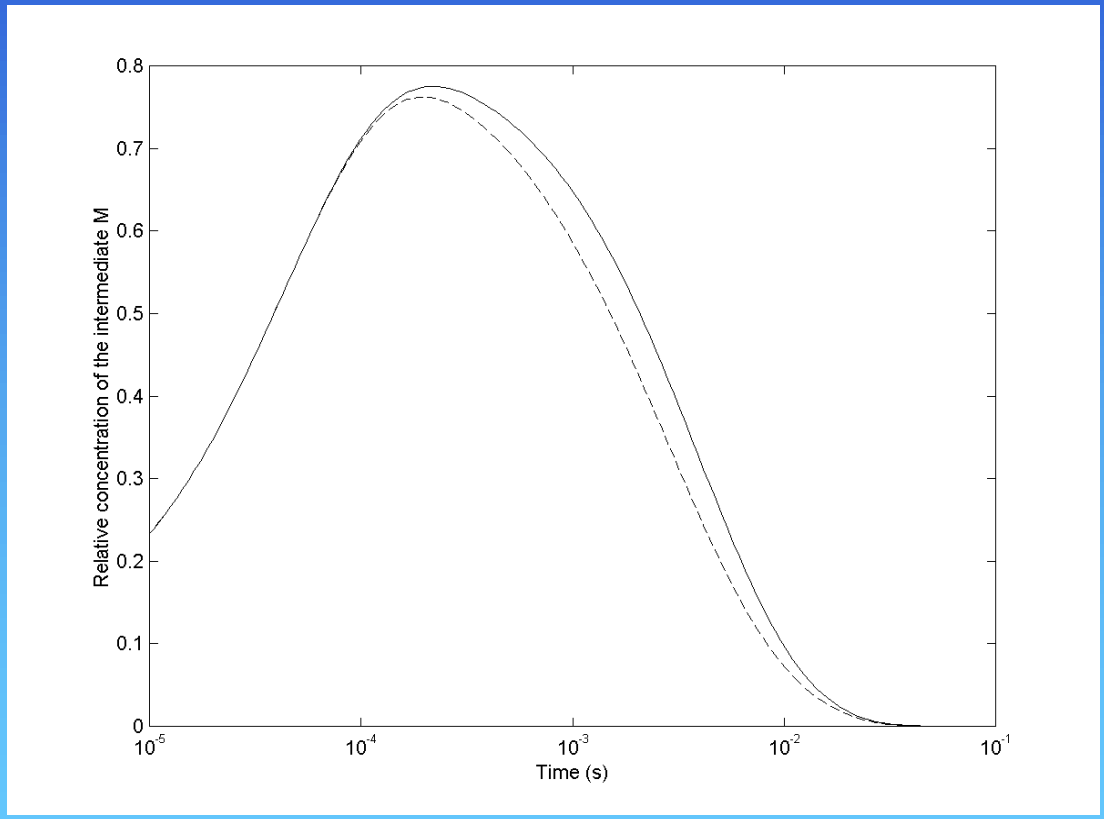
<…> stands for the mean value, λ for the noise correlation parameter, which is the average frequency of jumps of the random function, $\xi(t)$, from one value to the other (λ^{-1} is the noise correlation time)

$$\tilde{k}_{ij} = k_{ij} \exp\left(-\frac{a_{ij} c_s}{RT}\xi(t)\right)$$

For the bR photocycle, we used the following scheme:



The kinetics of the intermediate M (the sum of the relative concentrations of M_1 and M_2) with and without fluctuations added to the N? O transition.



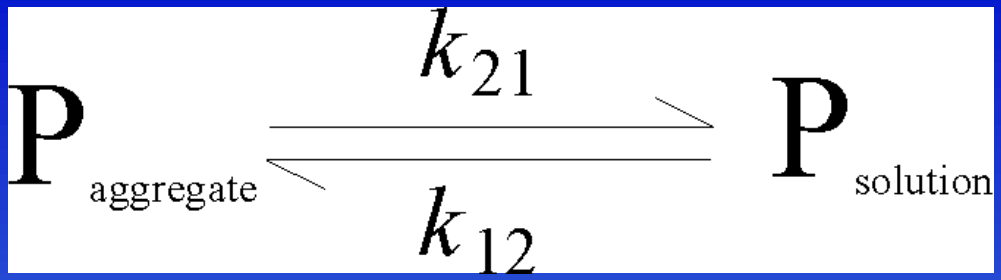
Note that transition is affected the most where proton (H_3O^+) uptake occurs together with conformational changes.

Can the fluctuation hypothesis be generalized so as to explain other Hofmeister phenomena?

We applied the concept to the classical problem of solubility [Neagu, Neagu, Dér, 2001b]. Here the so-called Setschenow's law, an empirical relationship, qualitatively describes the salt-dependent phenomena [Setschenow, 1889]:

$$\log \frac{S_o}{S} = K_s c_s$$

where K_s is a phenomenological solubility constant, which is a measure of the Hofmeister effect exerted by a given salt. Salting-in salts have negative Setschenow constants, while salting-out agents correspond to positive . This is the reason why it is also known as salting-out constant.



$G_1{}^{\sim}=G_1+c_s*a_1*\xi_1(t)$ (solution)

$G_2{}^{\sim t}=G_2+c_s*a_2*\xi_2(t)$ (aggregate)

$G_{ac}{}^{\sim}=G_1+c_s*a_{ac}*\xi_{ac}(t)$ (activation barrier)

For protein solubility:

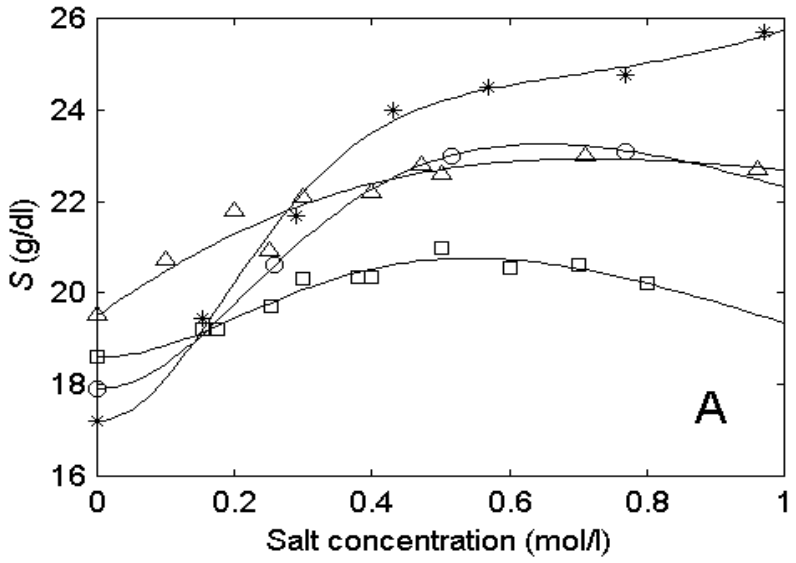
$$S = S_o \frac{\cosh\left[\frac{(a_{21}-a_{12})c_s}{RT}\right] + r\cdot\cosh\left(\frac{a_{21}c_s}{RT}\right)}{1 + r\cdot\cosh\left(\frac{a_{12}c_s}{RT}\right)}$$

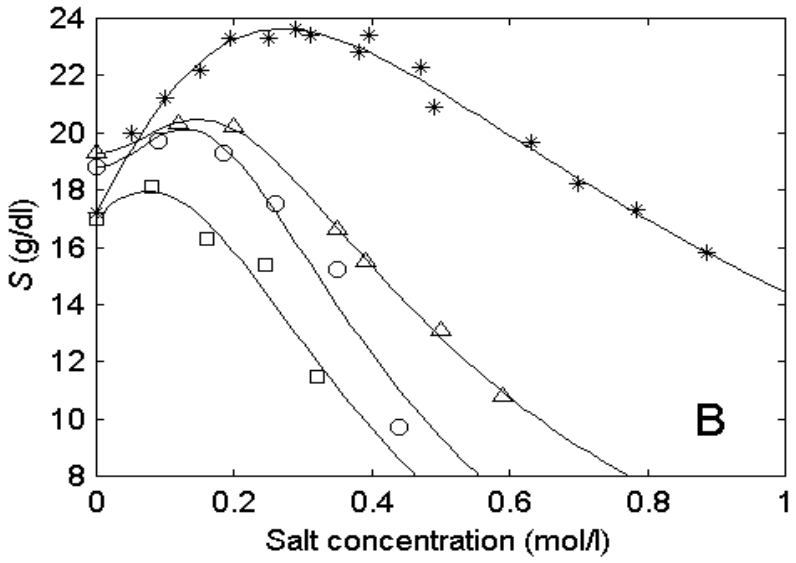
where $r = \lambda/k_{12}$, and S_o is the protein solubility in the salt-free case.

In the limit of low noise correlation time, it turns into the Setschenow equation if c_s is high enough:

$$S \cong S_o \exp\left[-\frac{(a_{12}-a_{21})c_s}{RT}\right]$$

When analyzing experimental solubility data available in the literature [e.g. for deoxigenated sickle hemoglobin, Poillon and Bertles, 1979, J. Biol Chem.], we got excellent fits even at lower salt concentrations:





How is our fluctuation model related to previous macroscopic models?

Improved cavity model:

$\Delta G = \Delta G_{cav} + \Delta G_{solv}$,
where $\Delta G_{cav} = \gamma \Delta A$.

If instead of γ (the surface tension of water related to air) we consider γ_{wp} (surface tension of water at the solute inteface), we can explain both salting out and salting in.

The definition of **surface tension**: $\gamma = \Delta E / \Delta A$
The probability of **fluctuations**: $P(\Delta A) \propto \exp(-\Delta E / kT)$

If γ is smaller, the probability of interfacial area fluctuations is higher.

(Fluctuation-dissipation theorem)

Conclusions:

Our fluctuation theory is in concert with the cavity model, and represents its natural extension. At the same time it suggests a deeper interpretation for the salt-controlled free-energy fluctuations.

Salts can modify interfacial surface properties by changing cohesion forces between water molecules.

Water structure at protein interfaces is of primary importance in determining protein dynamics via controlling its fluctuations.

Question:

How water molecules (inhomogeneously distributed in proteins, giving rise to an anisotropy of fluctuations) control energy dissipation in a working enzyme?

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