

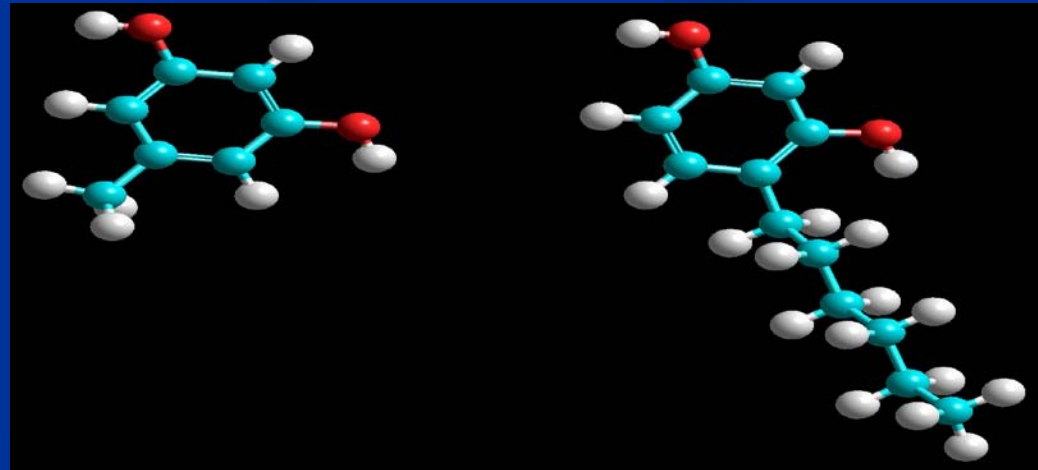
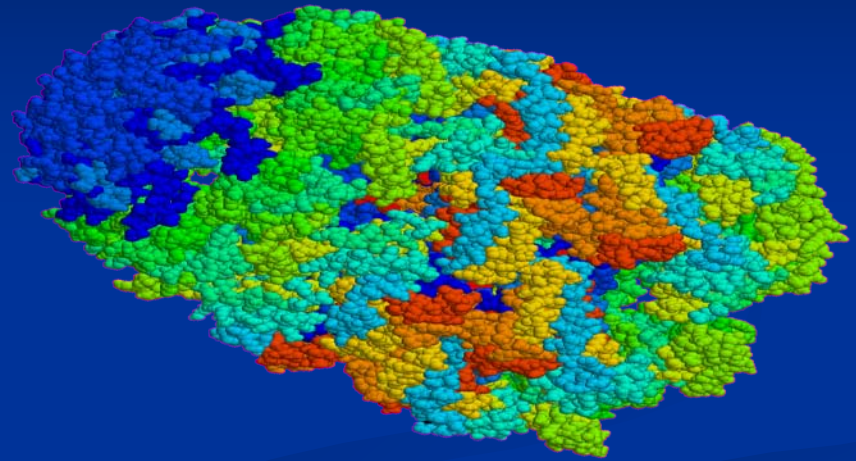
**Influence of Chemical Chaperone
Hexylresorcinol on Lysozyme.
Changes of Structure, Dynamics and
Functional Activity.**

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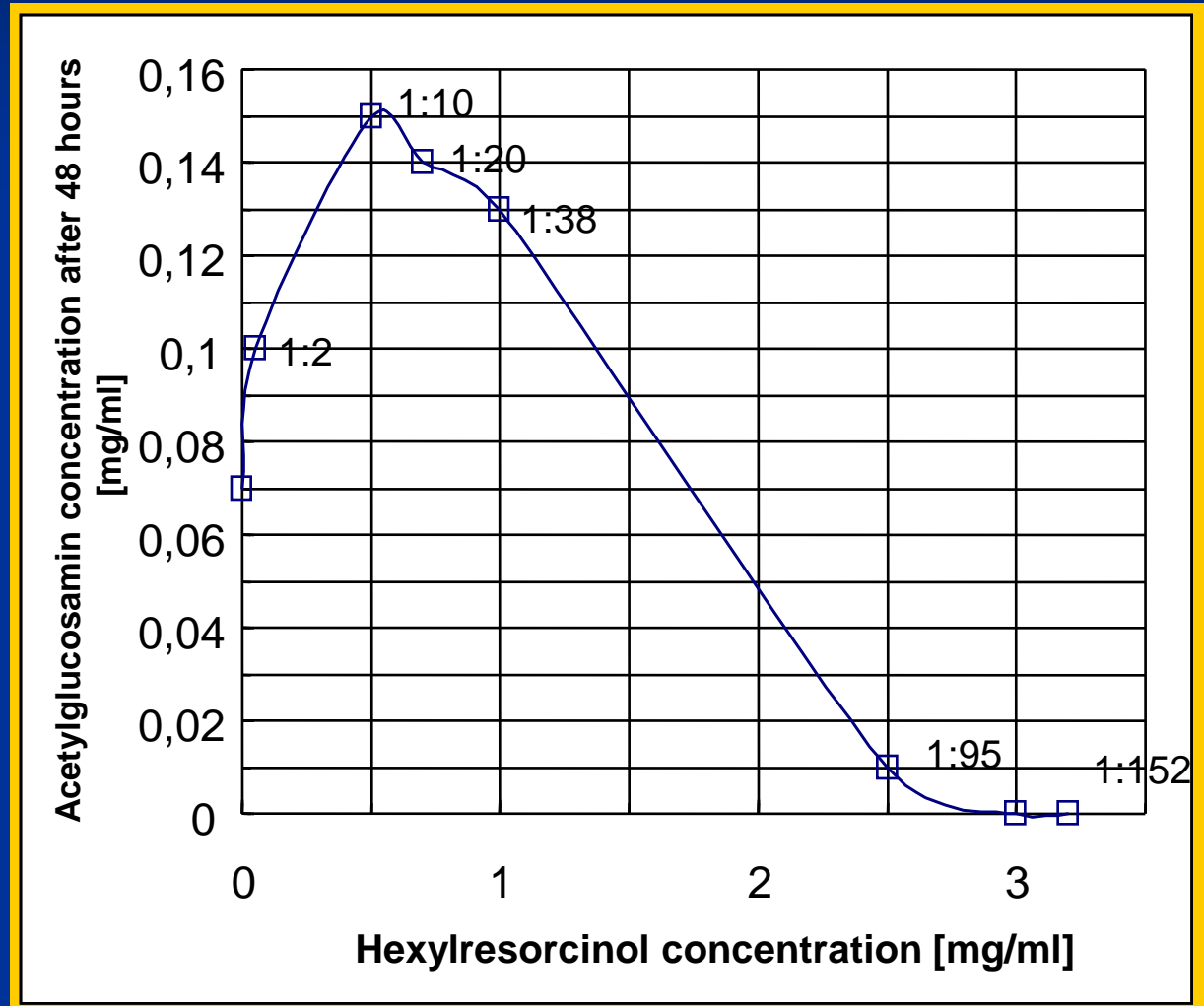
Chemical Chaperones

- Protein - misfolding deceases:
Alzheimer,
Parkinson, diabetes,
cancer, prion
deceases et al.
- Molecular chaperones prevent misfolding.
- Chemical chaperones have functions close to those of molecular chaperones.



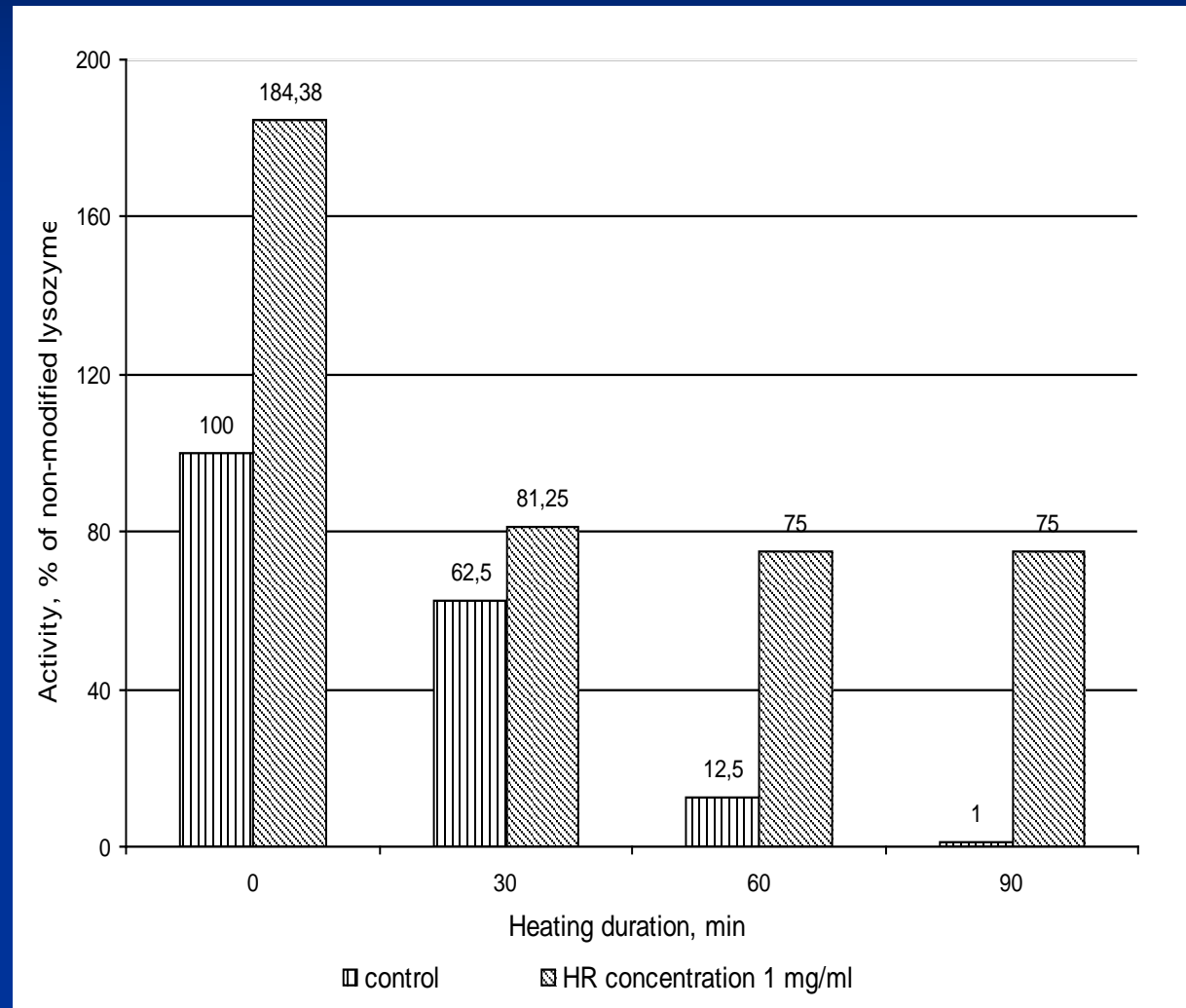
Regulation of enzyme activity of Lysozyme

Regulatory factor → hexylresorcinol concentration. Here regulatory mechanism similar to regulation at change of pH or temperature is observed but differs from those to allosteric enzymes. Maximum activity at 0.5mg/ml of hexylresorcinol (T = 55 C, pH = 7.4).

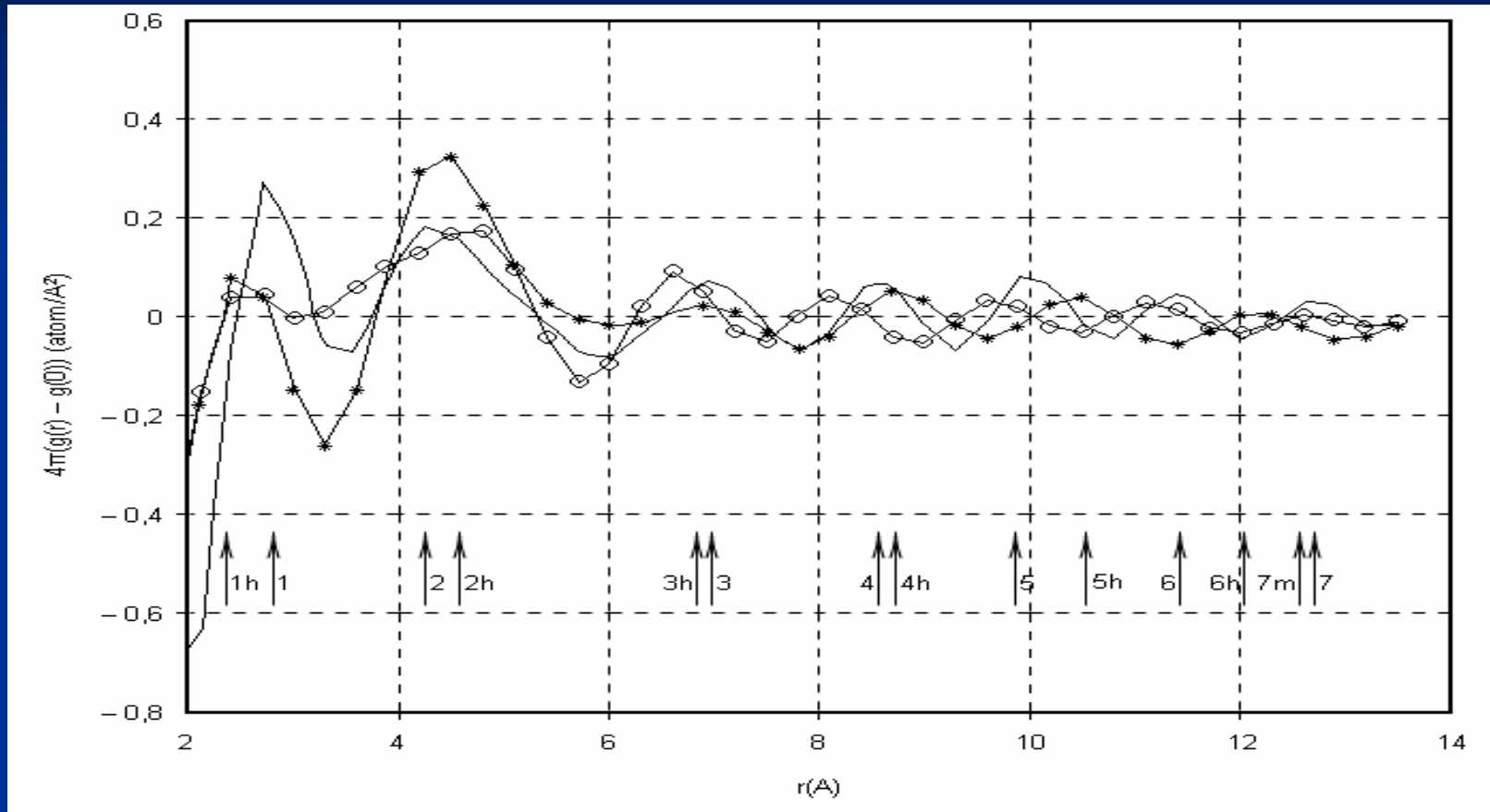


Thermostability of Lysozyme

- Hexylresorcinol concentration - 1 mg/ml (corresponds to activation). Heating up to 70 C. Hexylresorcinol allows to save enzyme activity 75 times more (at 90 min. heating) than those for native Lys.

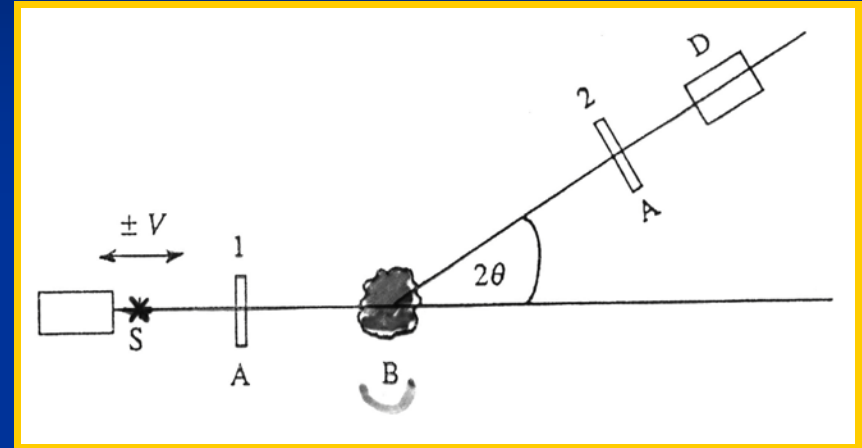


Radial distribution functions



- 40 hexylresorcinol molecules increase gyration radius. Some free volume appears.

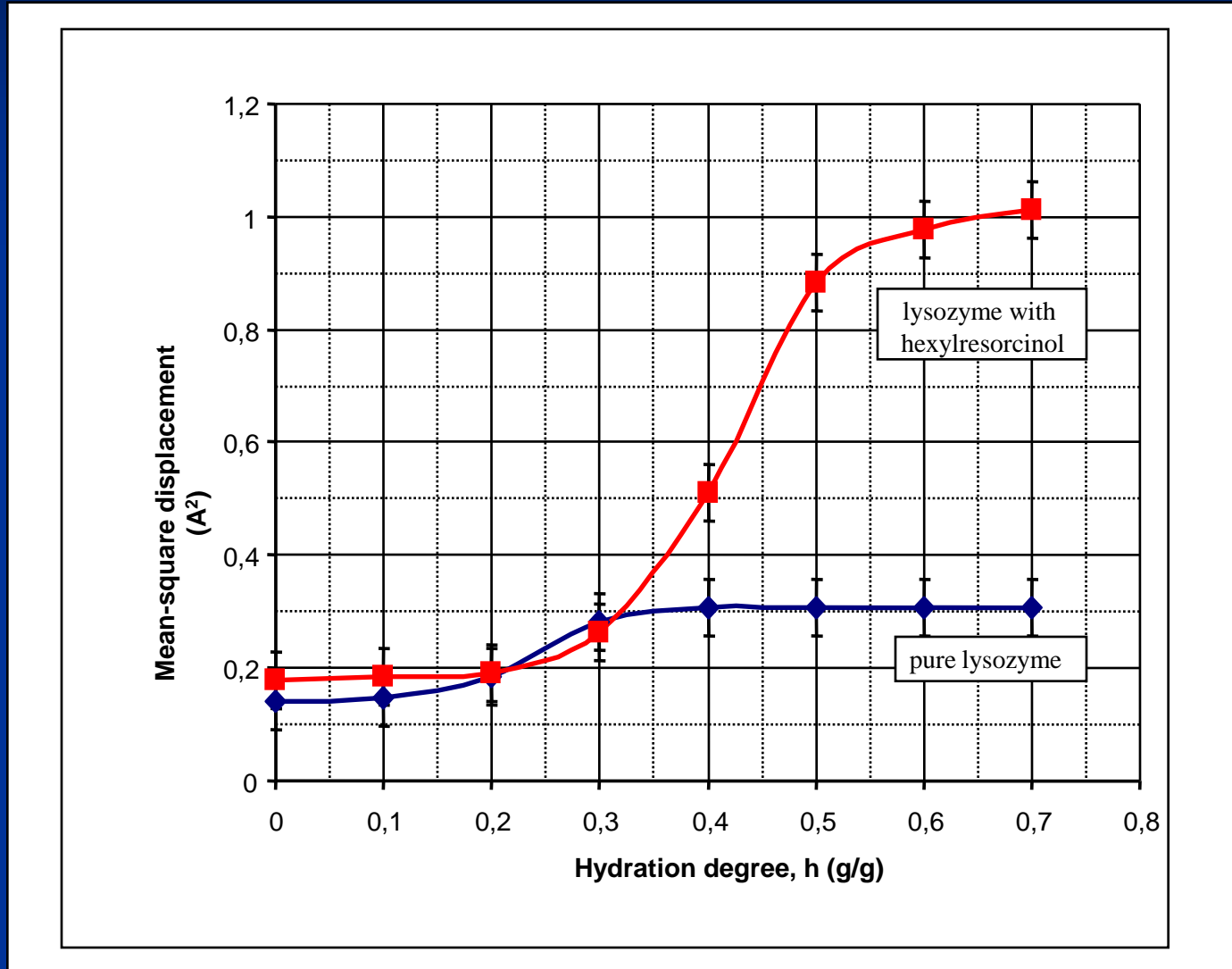
Rayleigh scattering of Moessbauer radiation technique(RSMR).



- **Combination of MR and MA $\Rightarrow \Delta\varepsilon \sim 10$ nano eV**
- **$n \sim 10 \mu\text{ eV}$;
 $SR \sim \text{milli eV}$**
- **Iron not necessary**
- **Background for biological application**

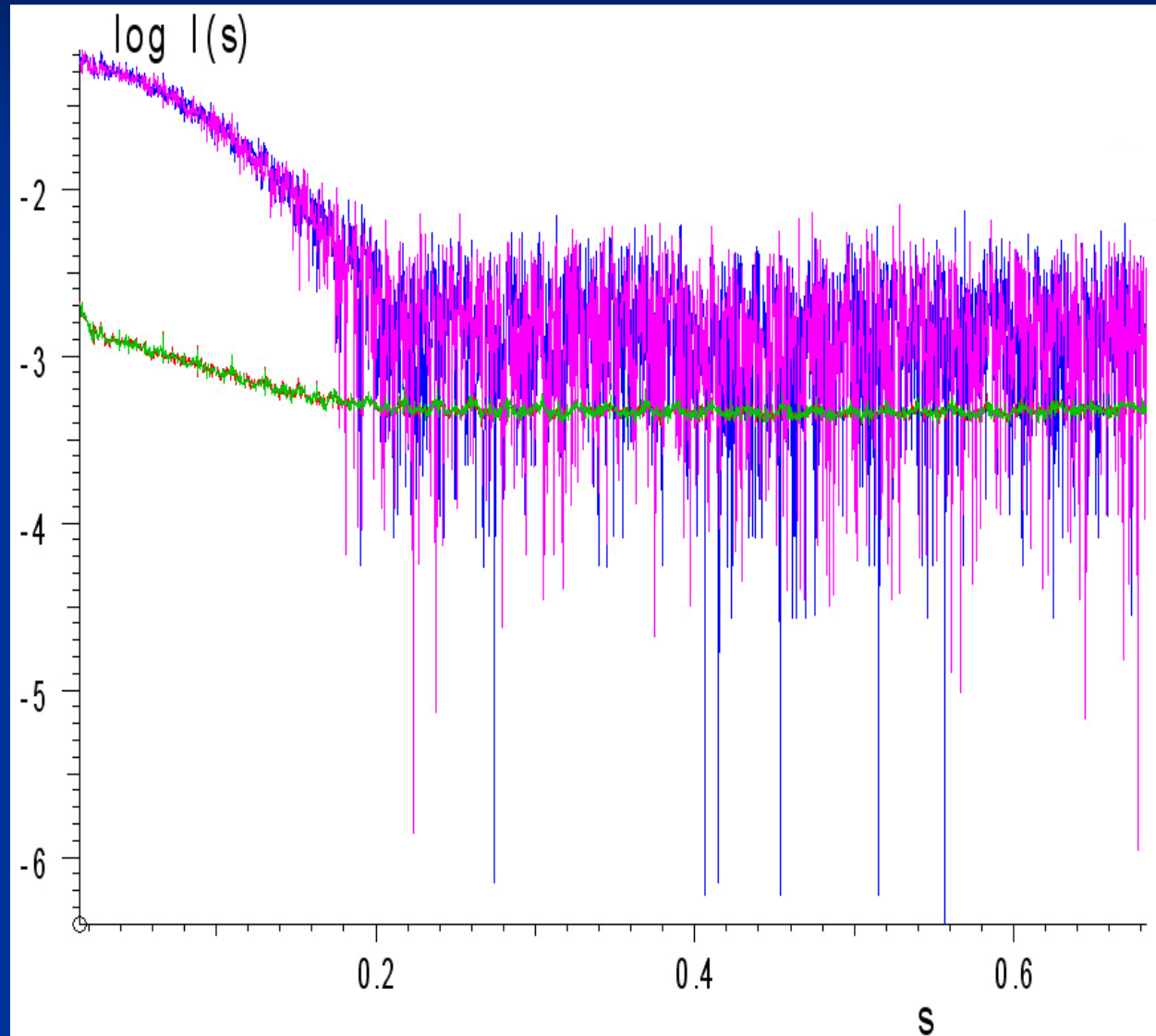
$\langle x^2 \rangle$ for Lys and Lys + 15 Hexylresorcinols

■ $\langle x^2 \rangle$
is
much
higher
for
Lysoz.
with
Hexyl-
resorc.



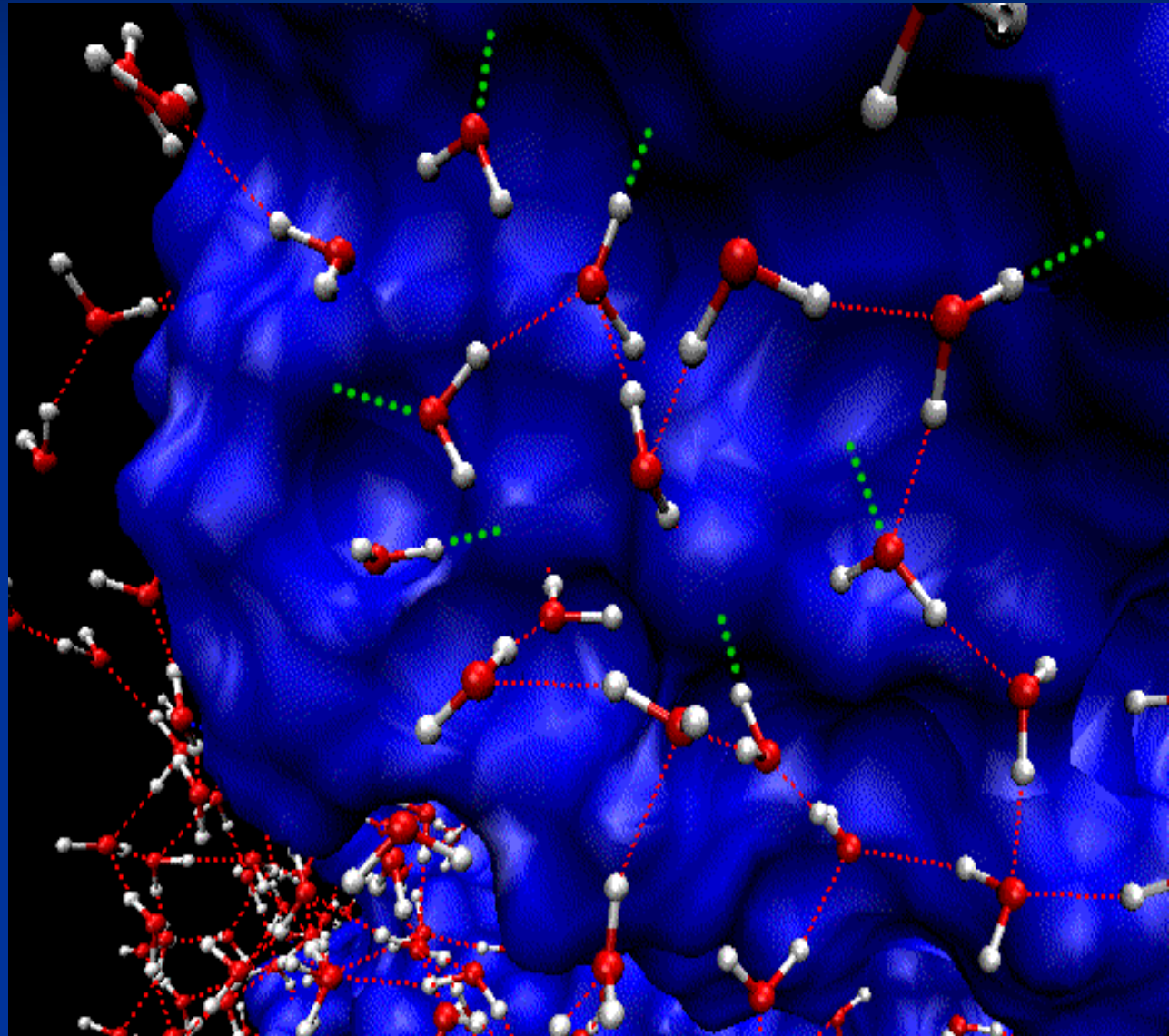
SAXS DATA

- Gyration radiuses are nearly identical.
- This means that we are far from denaturing conditions



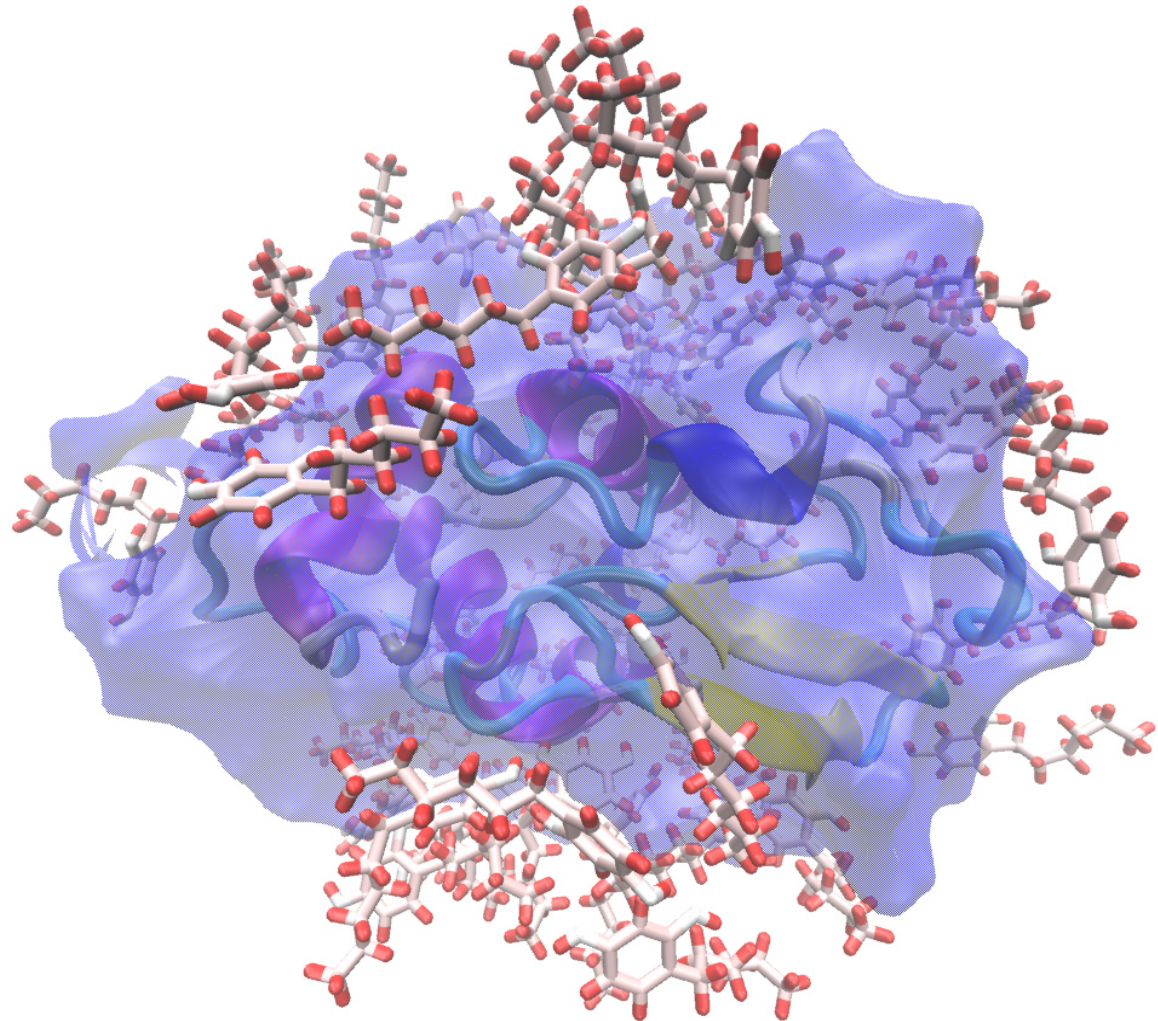
Hydrated proteins

- Protein works only in hydrated state.
- Hydrated waters arrange the net of H-bonds between water molecules and surface amino acid groups.
- H-bonds are green and red



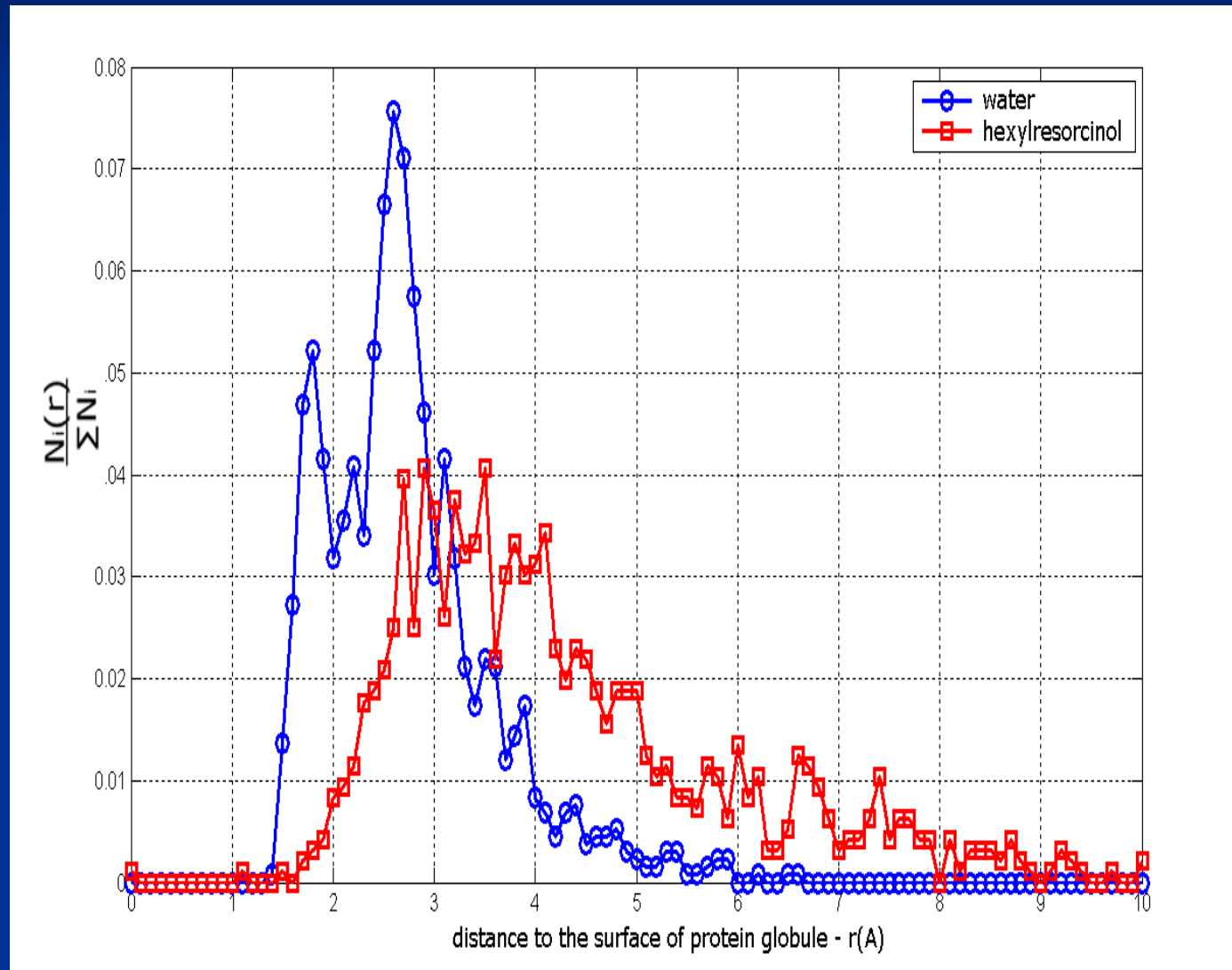
COMPUTER MODELLING

- 49 molecules of hexylresorcinol
- 284 water molecules
- $t \sim 2$ nanoseconds.
- Hydration water - transparent lilac cloud
- One may notice preferential hydration of lysozyme.
- Hexylresorcinol begin to arrange micelles of low density.



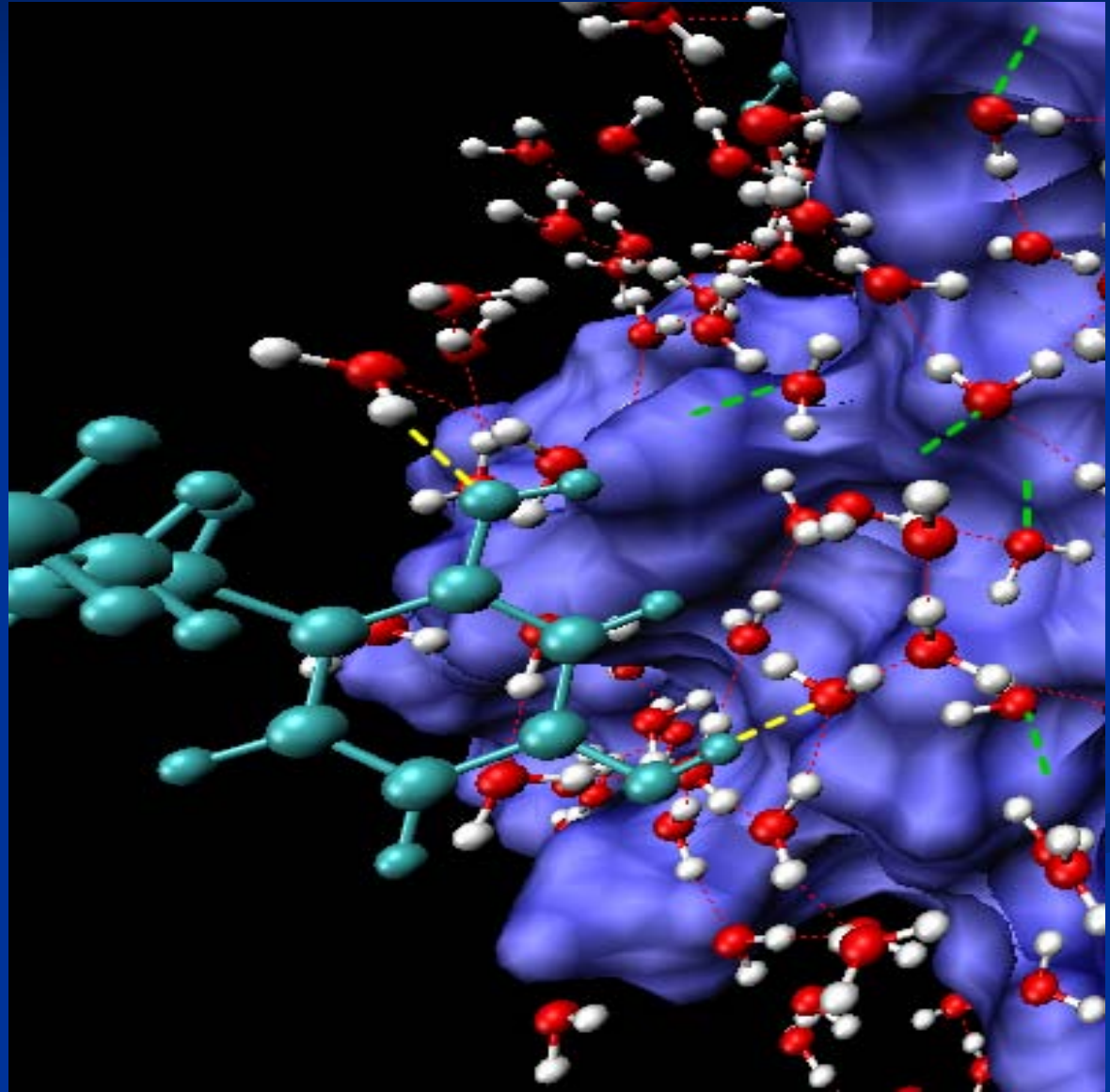
DISTRIBUTION OF WATERS AND HEXYLRESORCINOL AROUND LYSOZYME

- Quantitative confirmation of preferential hydration of Lysozyme.
- Waters are closer to the surface of protein than HR.



POSSIBLE HEXYLRESORCINOL BINDING TO HYDRATED LYSOZYME

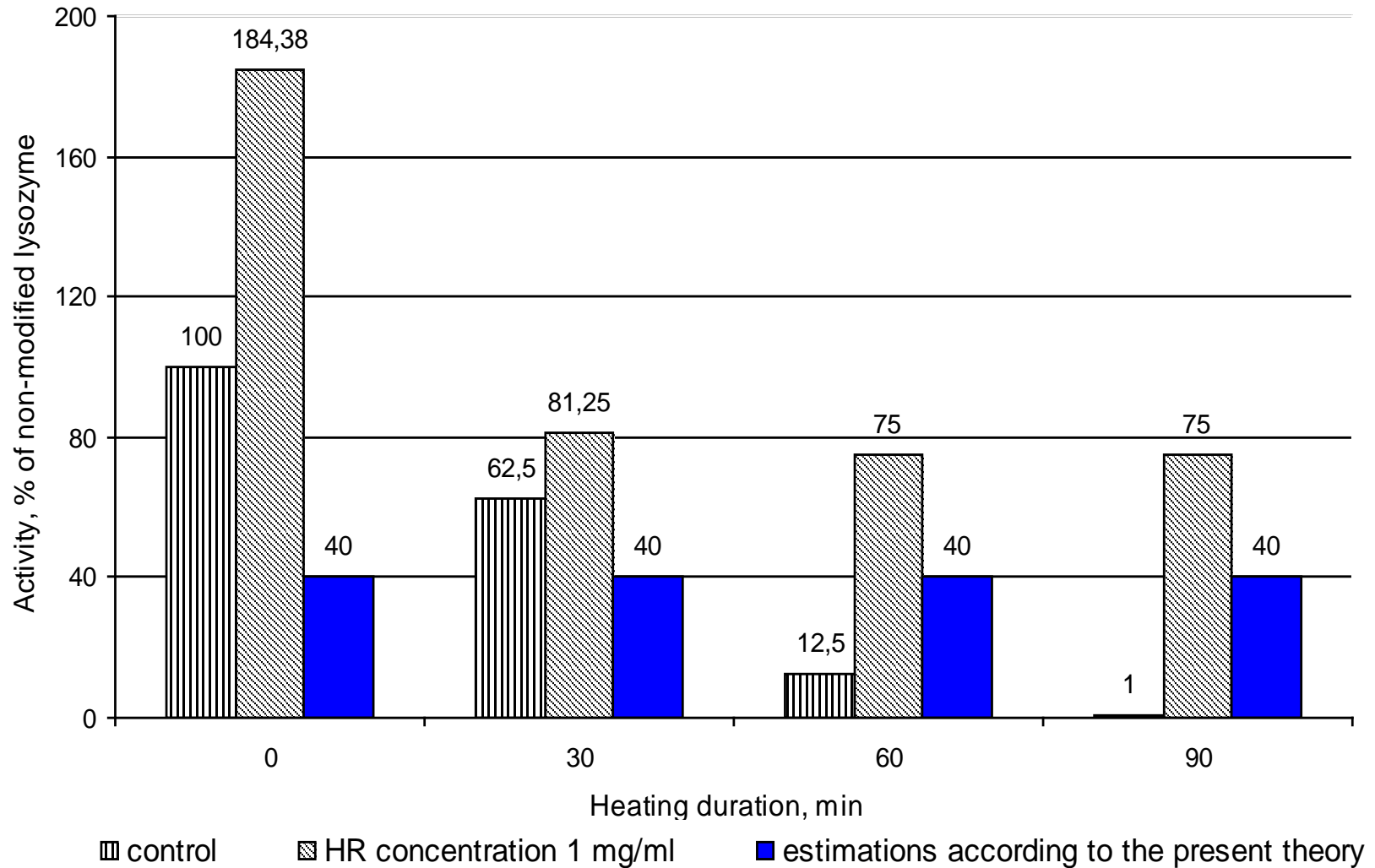
- Water atoms:
 - O – red
 - H – white
- HEXYLRESORCINOL
 - green
- Lysozyme – blue
- Hydrogen bonds:
 1. between waters – red
 2. between water and Lys - green
 3. between HEXYLRESORCINOL and waters - yellow



STABILIZER OR IMITATOR?

- Hexylresorcinols help to save 75% of the activity after 90-minutes heating
- In previous work we always consider that activity (A) of enzyme is $\sim \langle x^2 \rangle$ (Standard model.)
Here the rise of $\langle x^2 \rangle$ is correlate with the increase of A
- The measure of stability – temperature of denature (T_d) is inverse proportional to $\langle x^2 \rangle$, ($T_d \uparrow \rightarrow \langle x^2 \rangle \downarrow$)
- Consider that hexylresorcinol works as stabilizer. Then $T_d \uparrow$, $\langle x^2 \rangle$ and A should \downarrow for lysozyme. If Lys + HR does not denature due to $T_d \uparrow$, one may estimate that about 40% of molecules does not denatured at 90 min. heating \rightarrow blue columns on the next slide. It means that 40% of molecules does not denatured at all durations of heating.
- Evident contradiction with experiment !!!! See next slide.
- Hexylresorcinols – not stabilizer but rather imitator of the action of molecular chaperones.

STABILIZER OR IMITATOR?



Conclusions I

- New example of regulation of enzyme activity is observed for the enzyme - lysozyme.
- Low concentrations: Hexylresorcinols interact via hydrogen bond net and lead to a tiny swell of a globule, increase of $\langle r^2 \rangle$ and to the rise of A of lysozyme. Hexylresorcinols save 75% of the activity of macromolecules after its 90-minutes heating. Pure lysozyme lost its activity completely at the same conditions. Hexylresorcinols do not stabilize the protein but rather imitate the action of molecular chaperons. Hexylresorcinols arrange micelles of low density, create steric hindrances in conformation space for extensive unfolding of Lys and assist in refolding when initial temperature return.
(Either our standard models are wrong!!)
- High concentrations: The activity of lysozyme is completely inhibited. Effect is due to preferential hydration of protein and to the arrangement of high density micelles, completely hindering the substrate to pass to the active site of lysozyme.

Conclusions II

- RSMR, in principle, excellent and relatively cheap technique to achieve extremely high energetic resolution \sim nano eV
- From elastic fraction, RSMR spectra and angle dependences of RSMR intensities reach dynamic information may be obtained .
- Only one but big disadvantage – small source brilliance. It strongly reduces all the abilities of 1 and 2 points.
- Future of RSMR is in the use of SR with high energetic resolution (See Gopal Shenoy talk).

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