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RAMAN SCATTERING AS A TOOL TO INVESTIGATE PROTEIN HYDRATION SHELL

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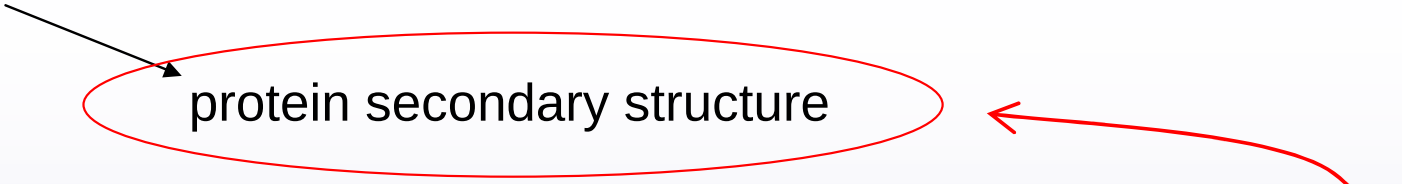
CONTENTS

OUTLINE

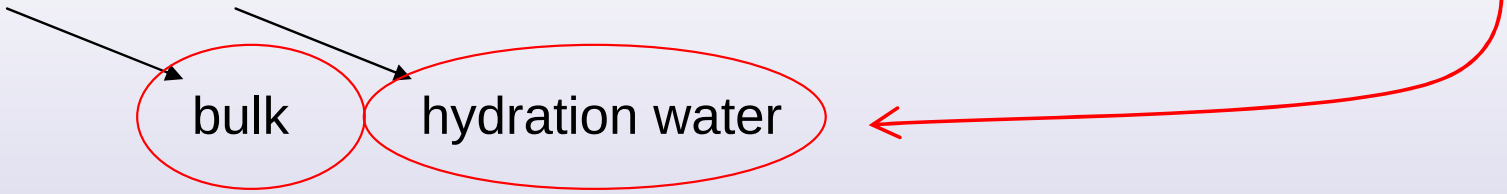
- the H/D isotopic exchange as a tool to get information about the solvent-accessible surface area (SASA) and the protein stability

RAMAN INVESTIGATION

- the Amide I region (1500-1800 cm^{-1})



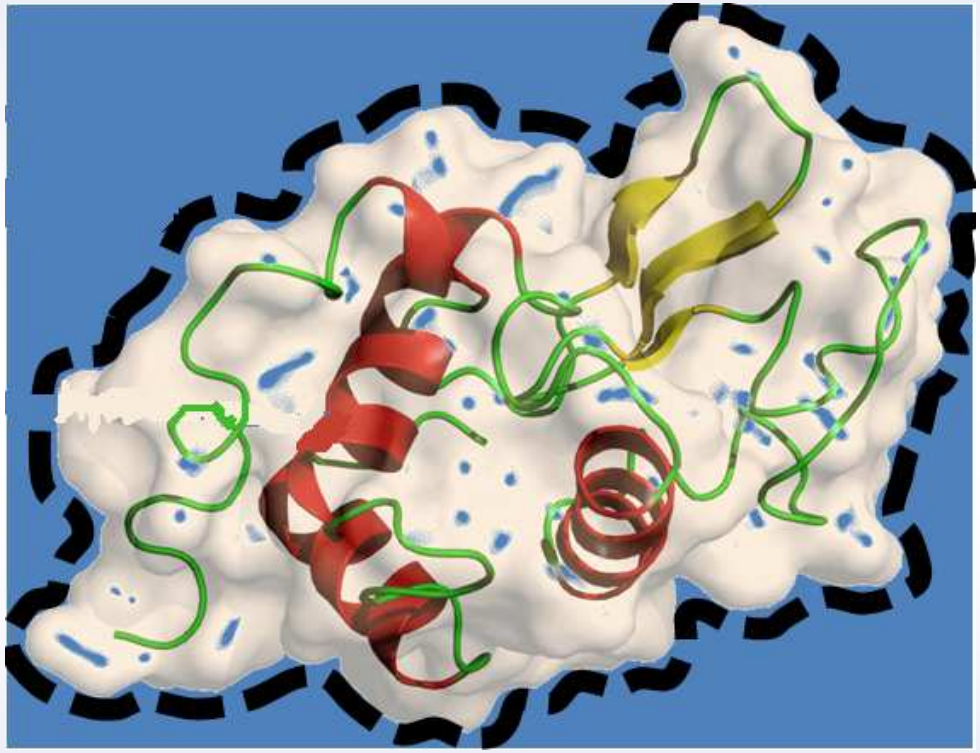
- the O-D and O-H stretching region (2000-3800 cm^{-1})



CONCLUSIONS

Outline

Proteins are a fundamental constituent for life



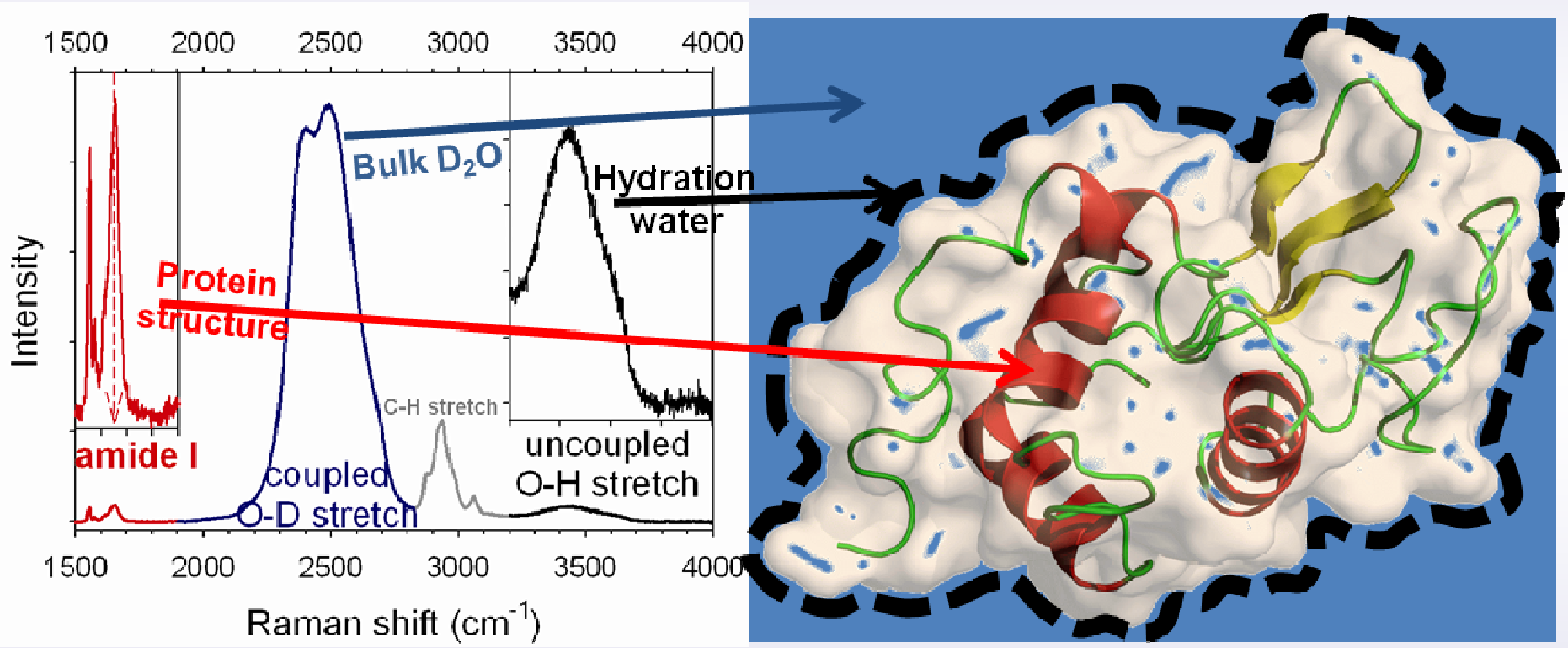
function, structure, dynamics

stability

hydration shell

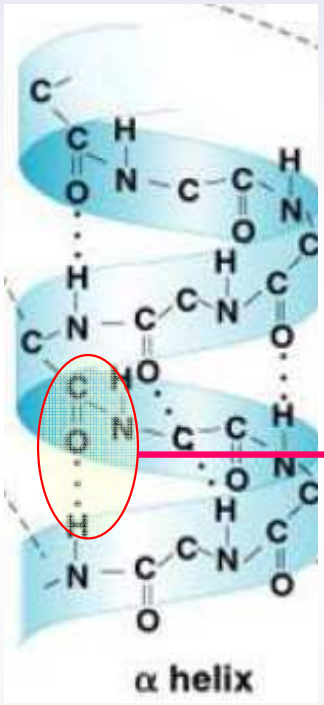
D_2O allows to investigate the solvent accessible surface area (SASA) since isotopic exchange between the protein and the bulk occurs

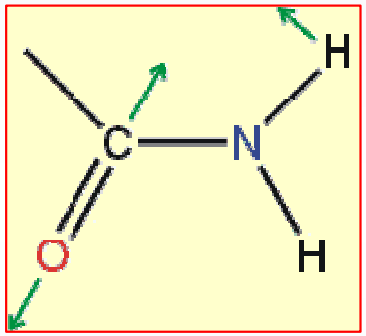
Raman scattering is able to distinguish among the protein structure and the D_2O bulk, and it can give information on the hydration water

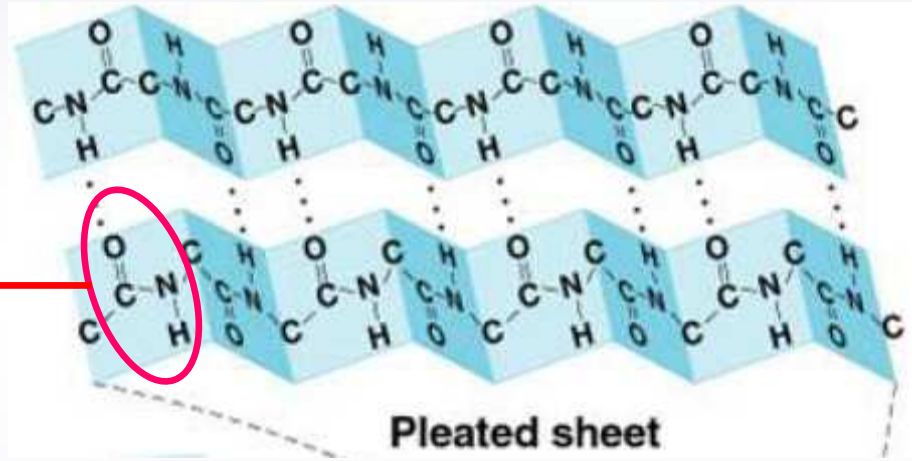


By adding biopreservers in the solution, such as trehalose, this technique can be used to investigate their effect on the protein.

Amide I band (~ 1650 cm^{-1}) → protein secondary structure





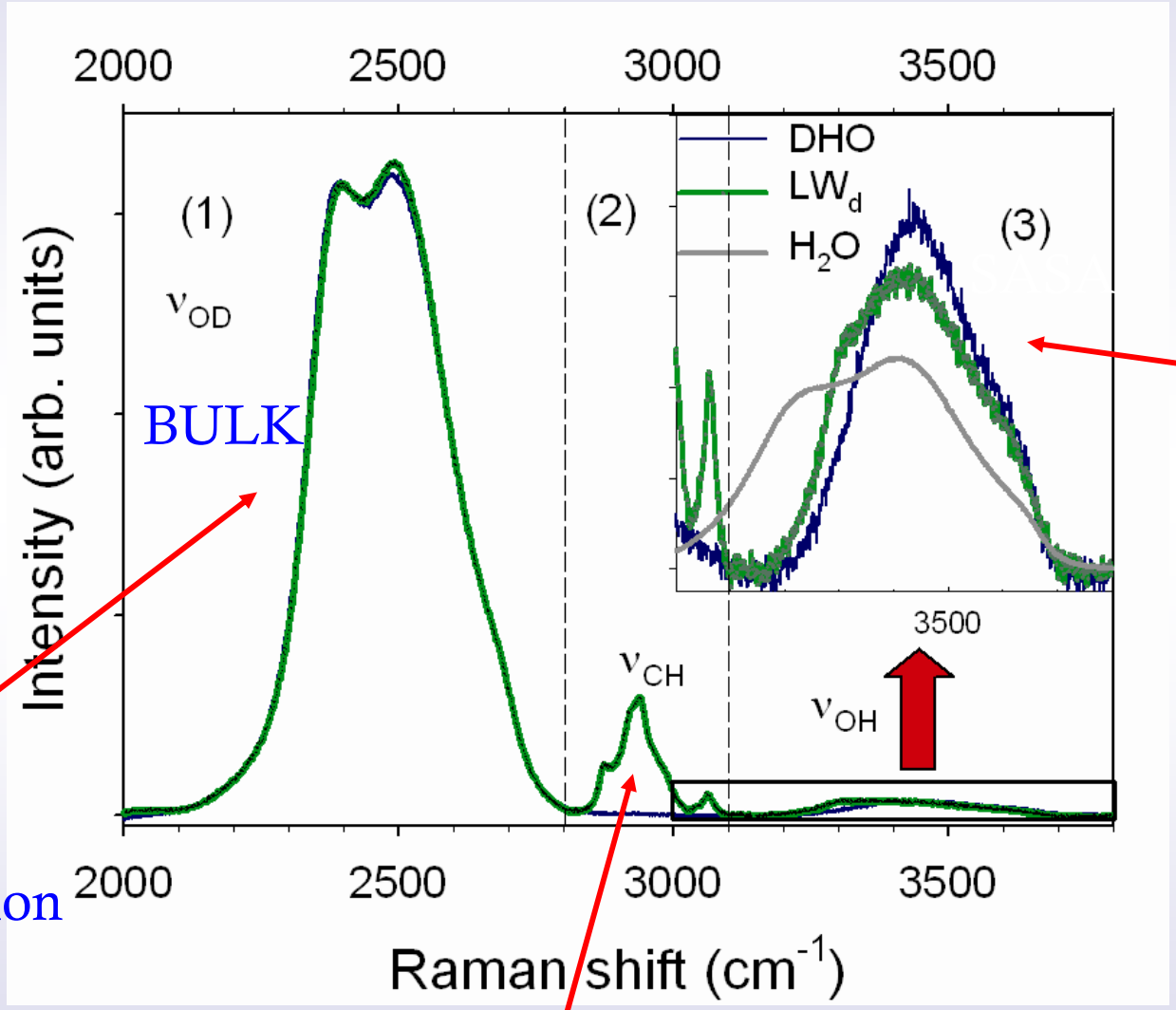


$\nu_{C=O} + \delta_{NH}$

Sensitivity to protein conformation

Sensitivity to N-deuteration of the backbone

intramolecular stretching vibrations



BULK

O–D stretching coupling vibration

O–H stretching vibration

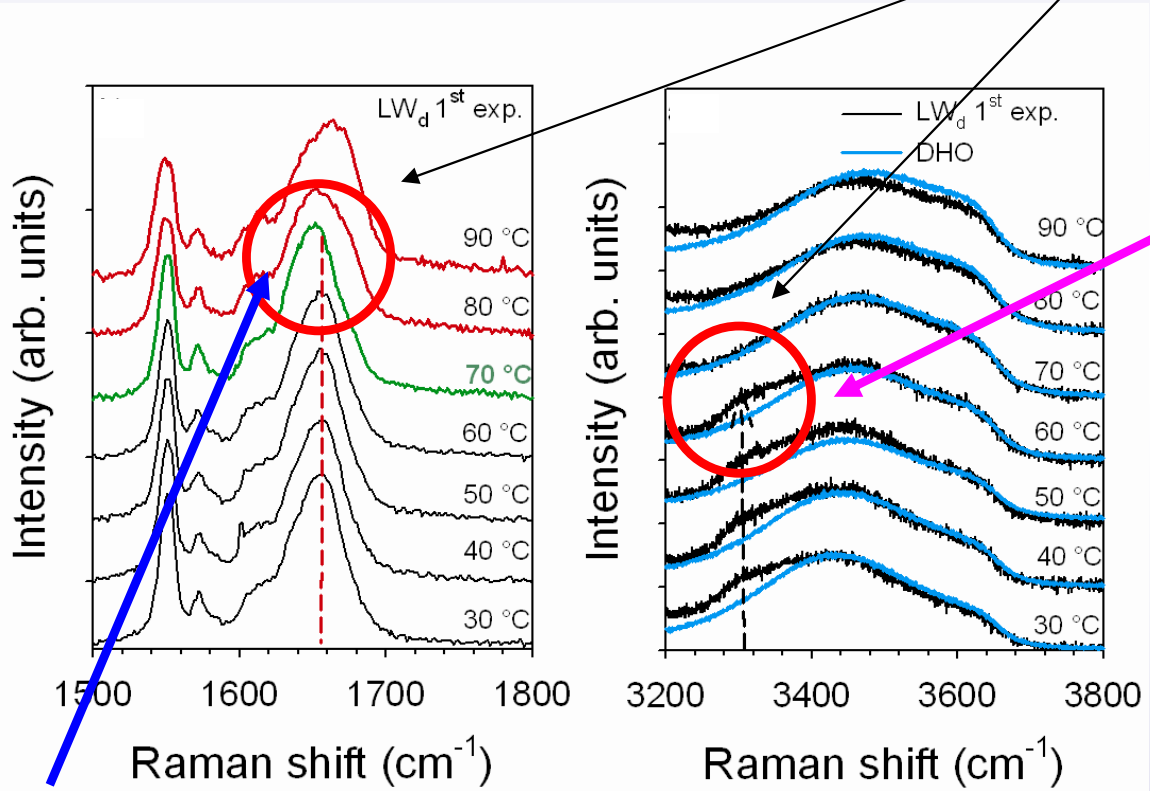
no contribution from intermolecular coupling vibrations

C–H stretching Lysozyme

Raman Investigation

1st exp:
heating from 30°C up to 90°C

sensitive to protein denaturation process

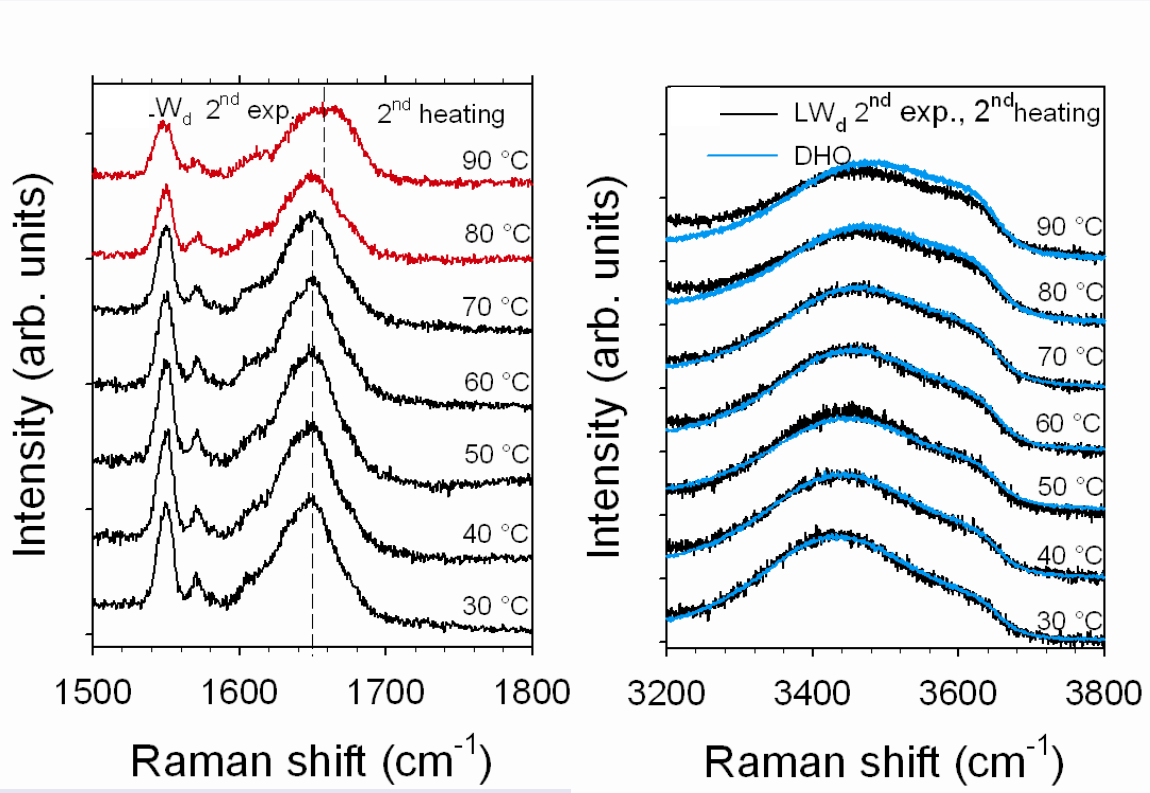


O–H stretching in Lysozyme absent for $T \geq 70°C$

Penetration of the solvent

downshift => NH/ND exchange: opening of the protein and exposure of the inner residues

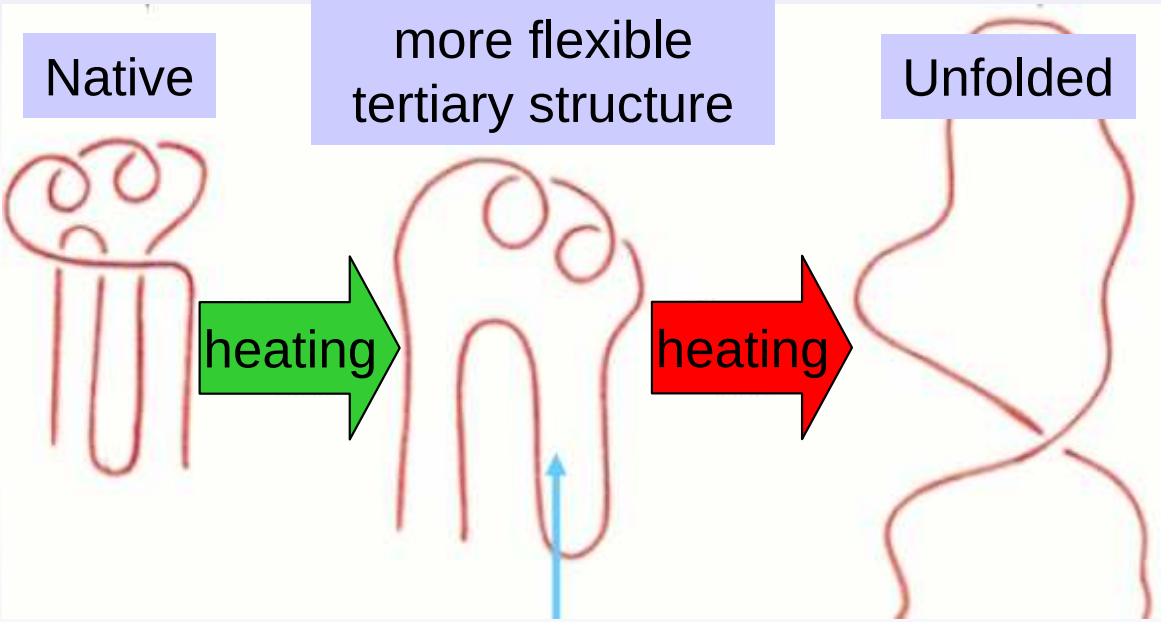
2nd exp:
heating from 30°C up to 70°C,
then cooling to 30°C and re–heating up to 90°C

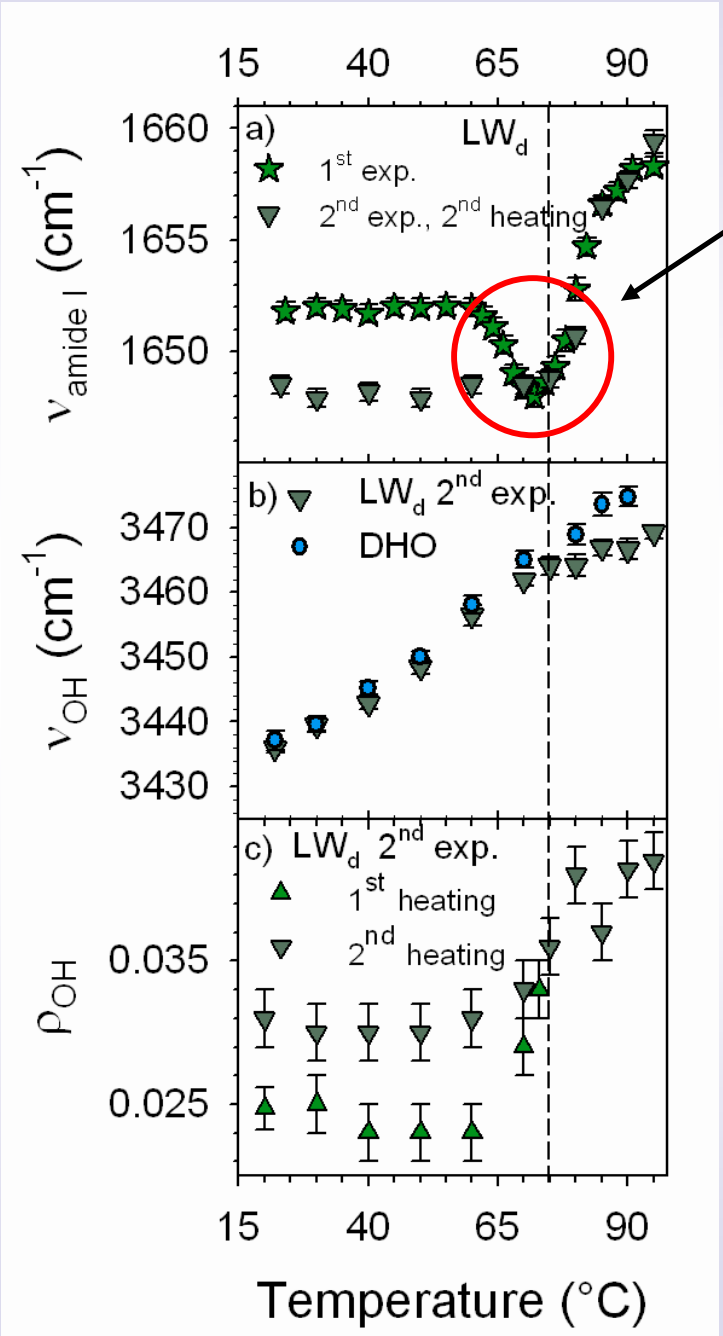


no Amide I band downshift
no 3310 cm^{-1} band

the unfolding is a two-step process in which the H/D exchange is irreversible

Molten Globule:
more flexible
tertiary structure





penetration of the solvent,
molten globule
evidenced only in D_2O

ν_{OH} increases by heating:
intermolecular OH stretching harder
$\Rightarrow$H–bonds softer

For T<73°C the protein does not alter the water H-bond network
For T>73°C the unfolded protein makes the water H-bonds network stronger than in the bulk

$\rho_{OH}=I_{OH}/I_{OD}= 0.0246\pm0.0015$ @ T_{room}
$\Rightarrow$ 158±8 water molecules in the SASA
@ 73°C: SASA is 1.34 greater (n_w= 212)
@ 90°C: SASA is 1.75 greater (n_w= 276)

CONCLUSIONS

the H/D isotopic exchange in Raman scattering

allows to obtain a detailed information on the

protein hydration water

the number of water molecules in the hydration shell

can be evaluated from I_{OH}/I_{OD} during the whole

denaturation process

THANK YOU
FOR YOUR ATTENTION