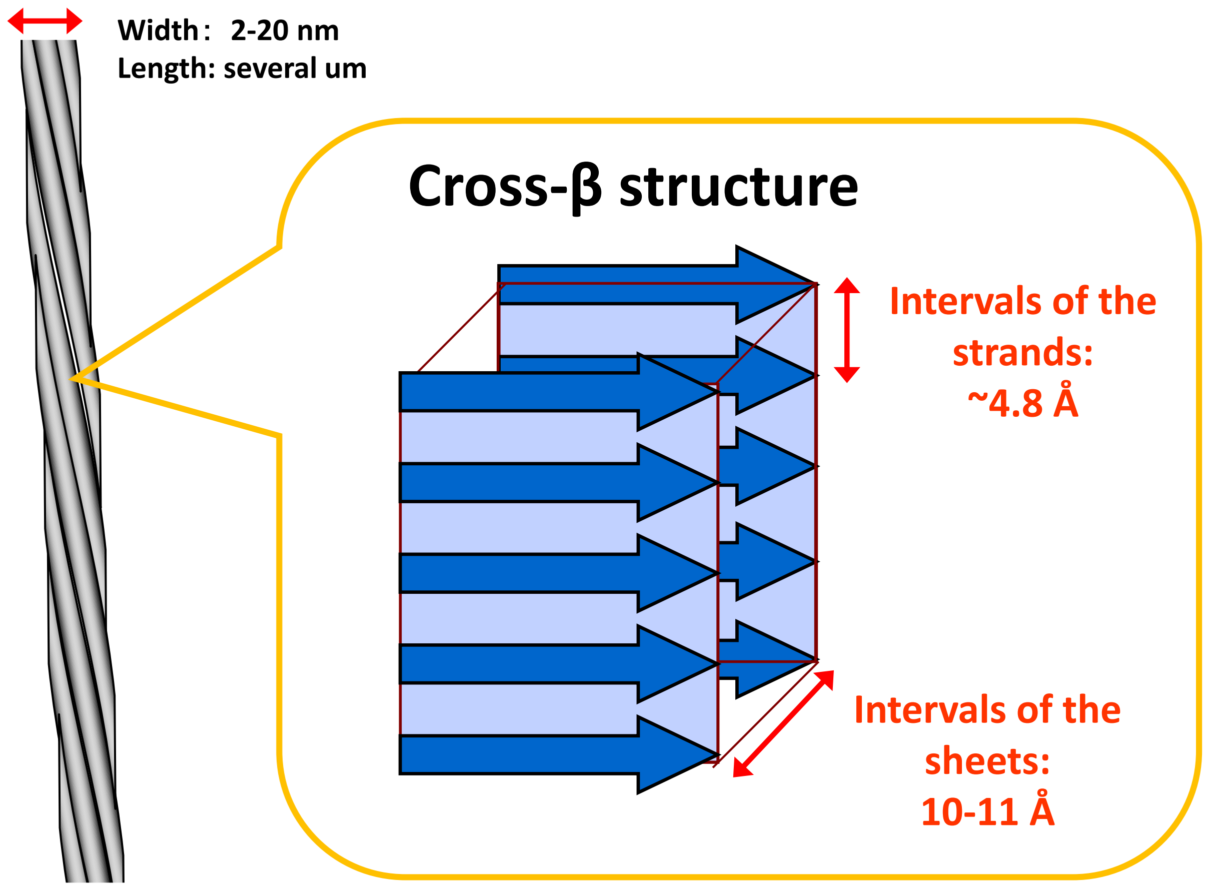
Investigation of Water Molecular System Dynamics in the Early Stages of Amyloid Formation

Eri Chatani[1], Takato Hiramatsu[1], Yukari Itakura[2], and Roumiana Tsenkova[2]

[1]*Graduate School of Science, Kobe University*

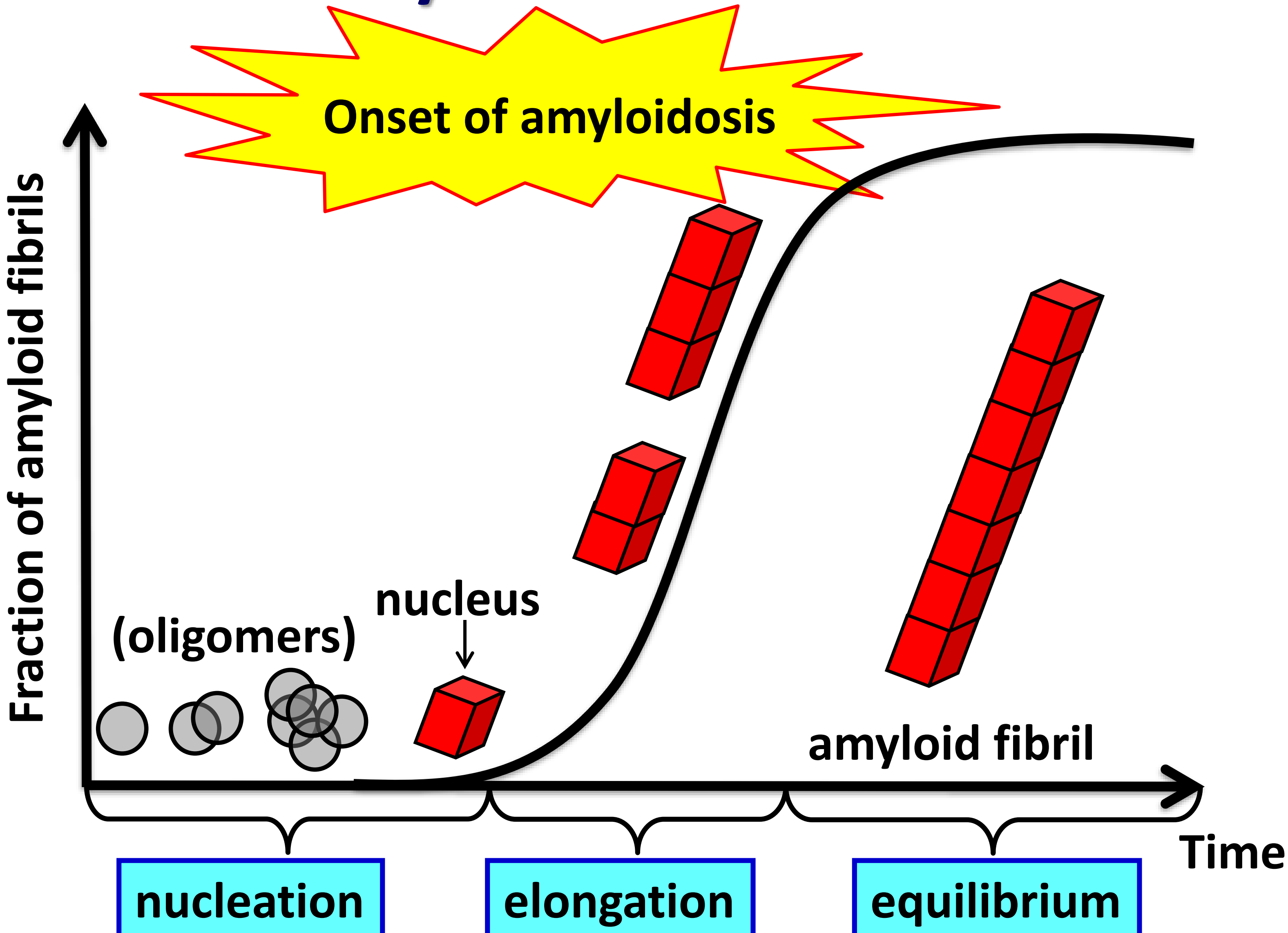
[2]*Graduate School of Agricultural Science, Kobe University*

Amyloid fibrils

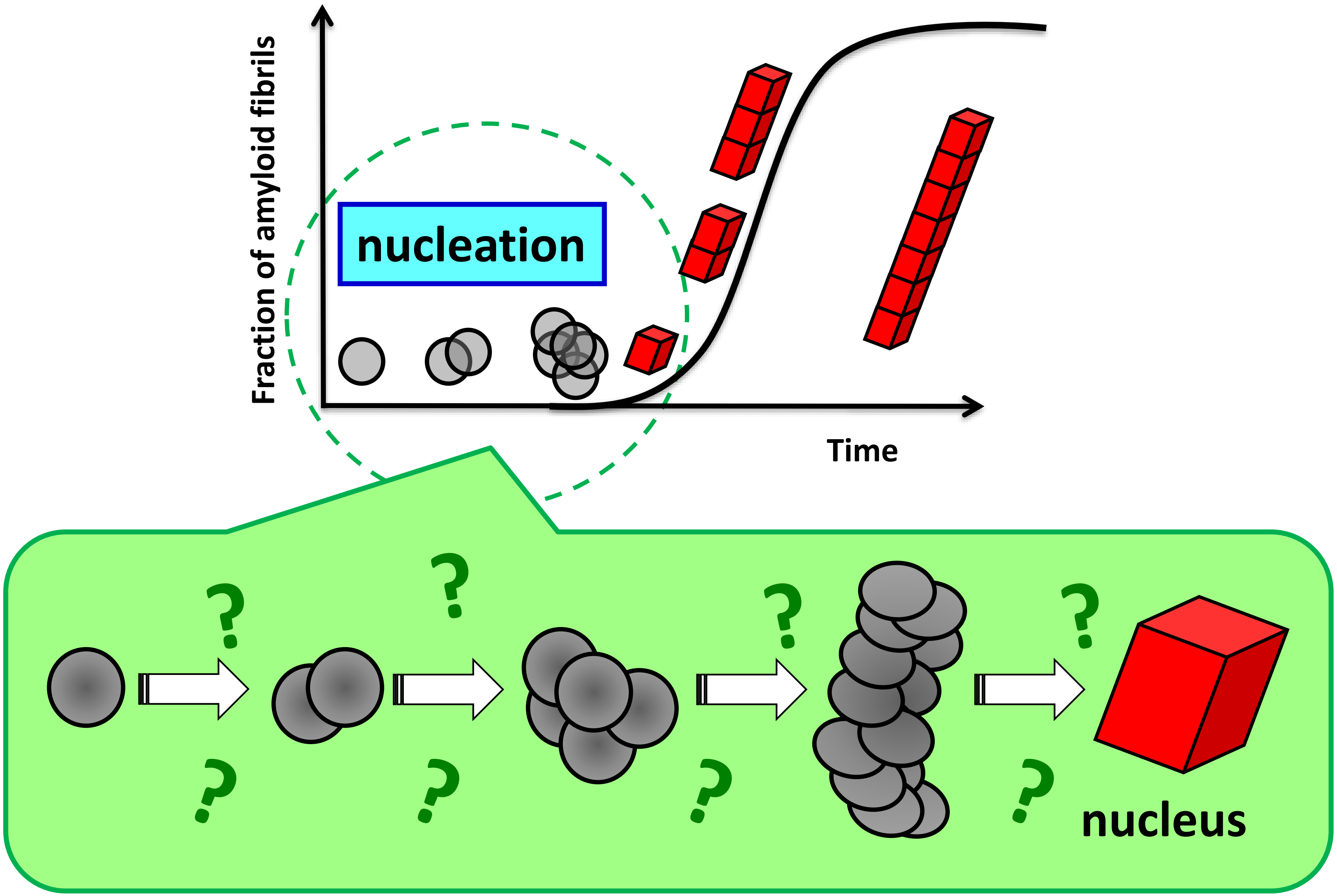


Amyloid fibrils

Nucleation-dependent mechanism of amyloid formation

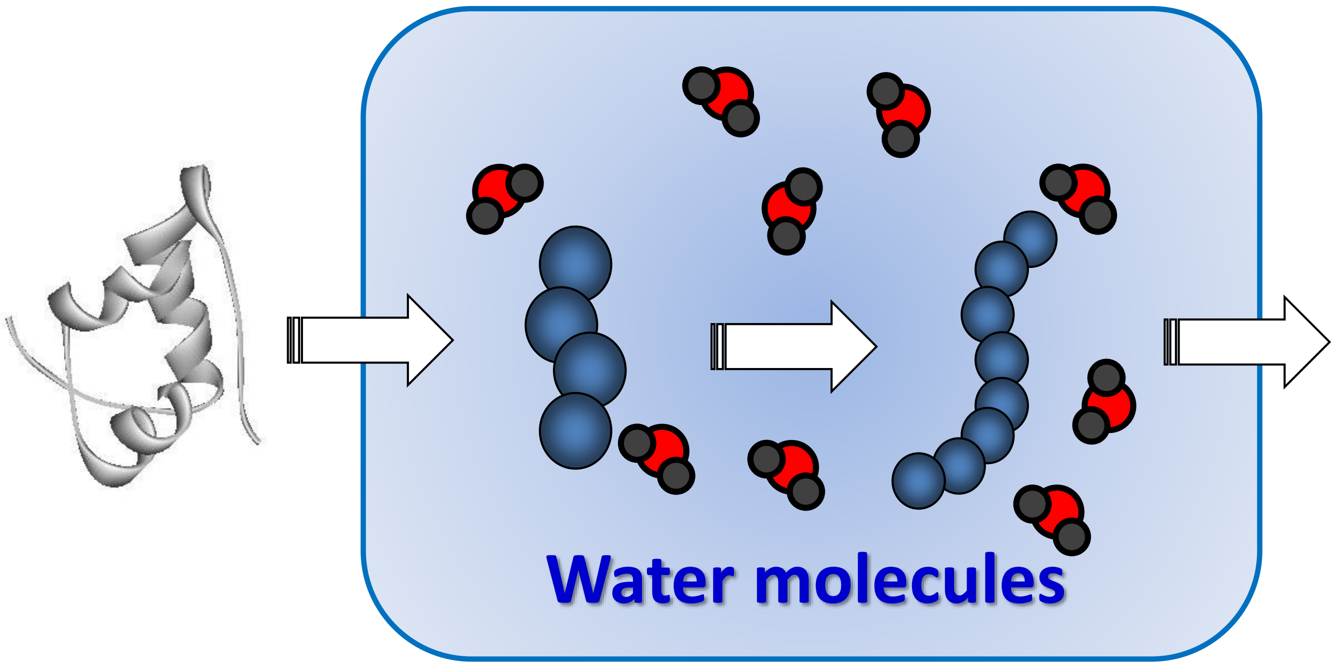


What is crucial for nucleation?



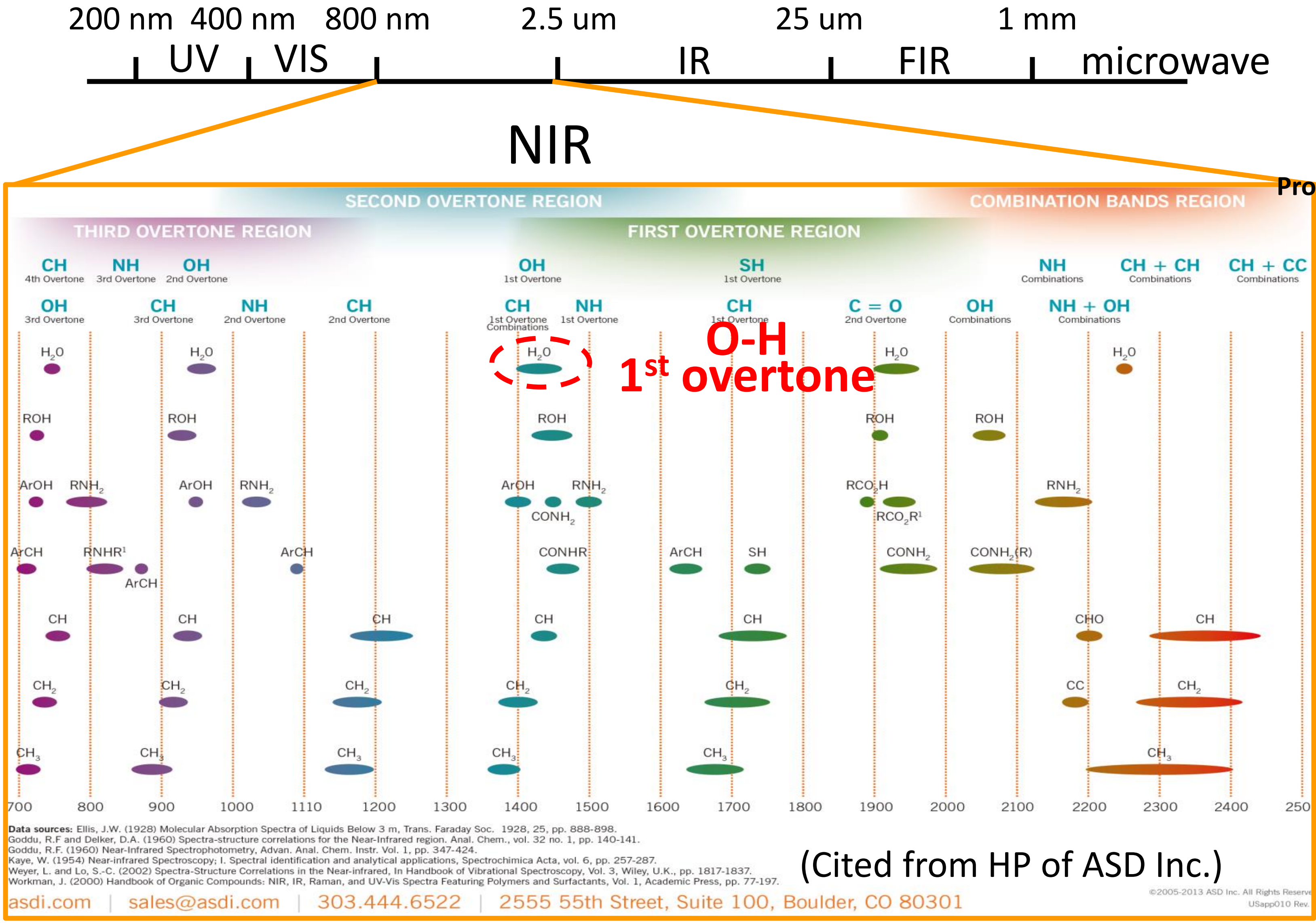
Molecular mechanisms that dictate the nucleation are poorly understood

Can we find any spectroscopic signals that tell us the formation of amyloid fibrils?



We performed near infrared spectroscopic analysis to monitor water molecular system dynamics during the amyloid fibril formation reaction

Near Infrared Spectroscopy



(Cited from HP of ASD Inc.)

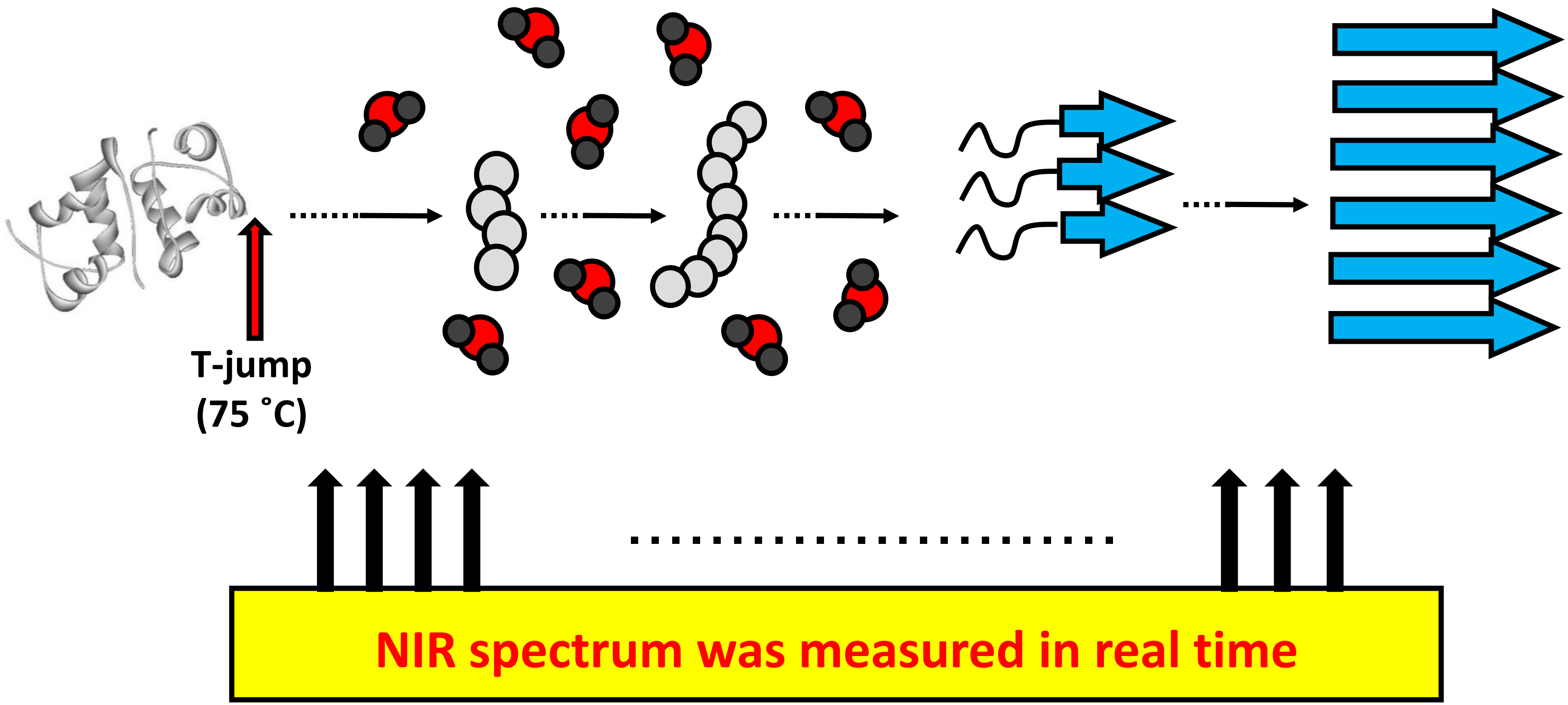
Prof. Roumiana Tsenkov
Kobe Univ.

NIR spectroscopy is expected to be powerful analytical technique for investigating water structures

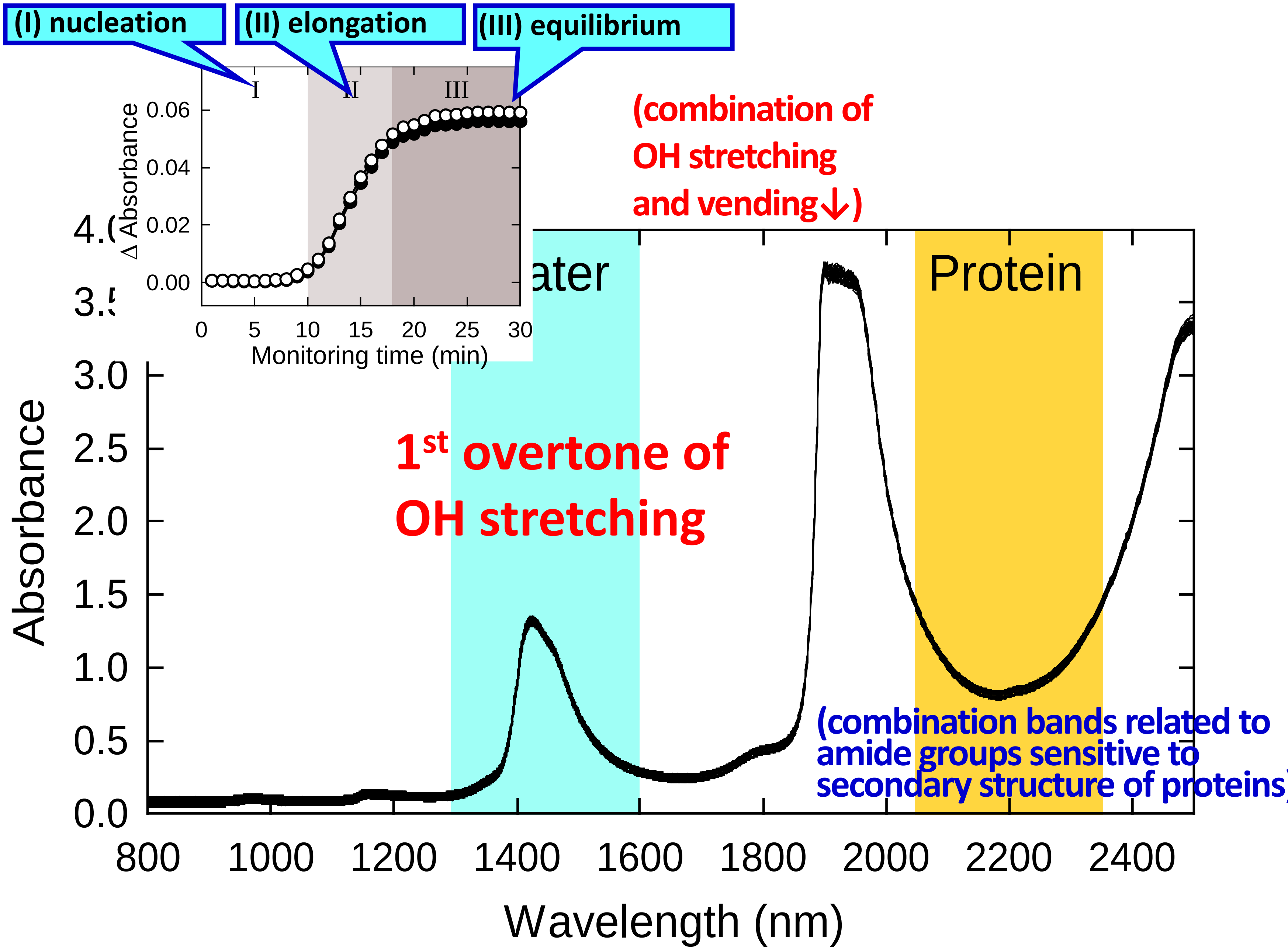
Experimental procedure

(Chatani et al., *PLoS One* 9, e101997 (2014))

Sample : 3 mg/ml human insulin in 25 mM HCl, 100 mM NaCl

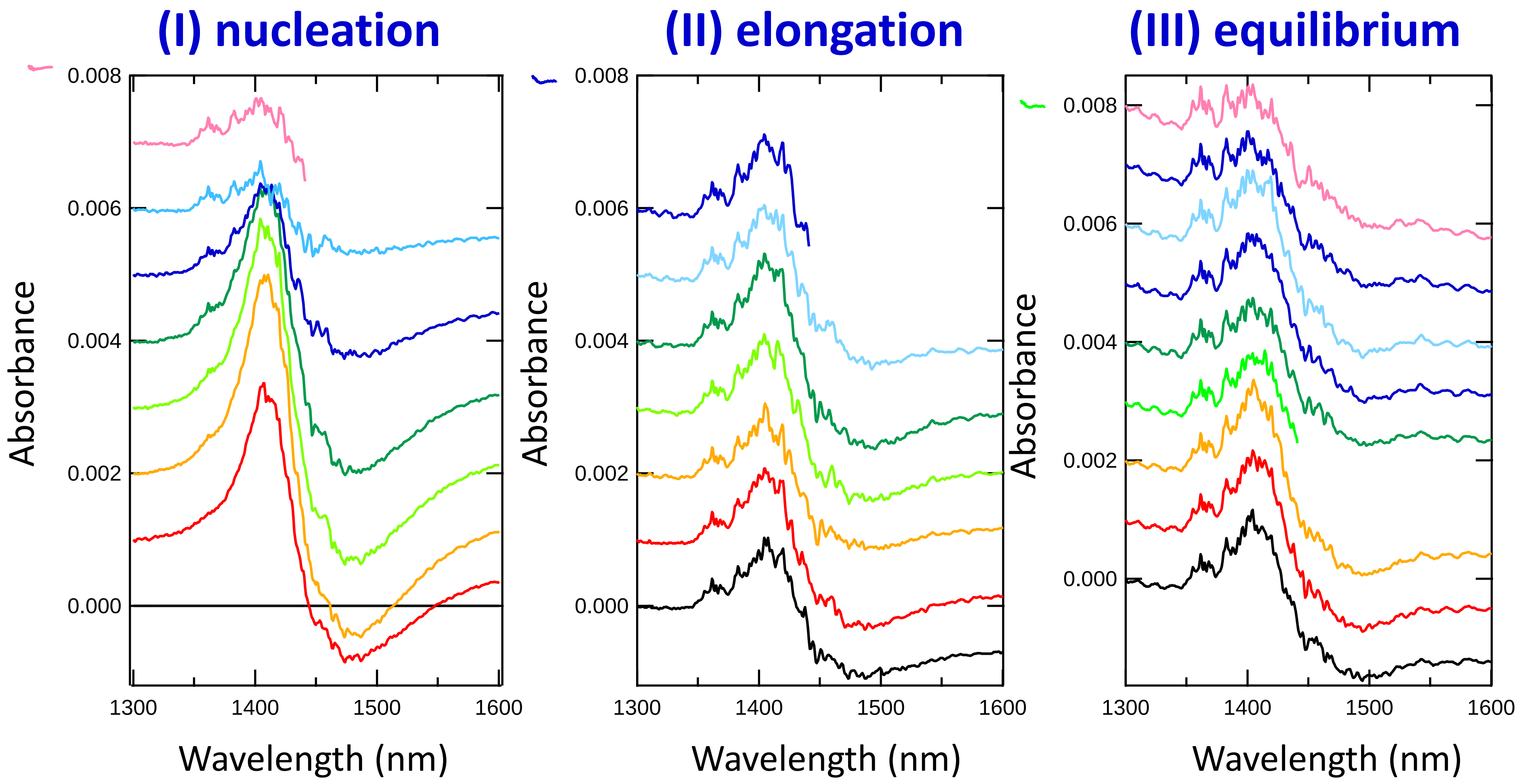


Overview of NIR spectra of fibrillation sample



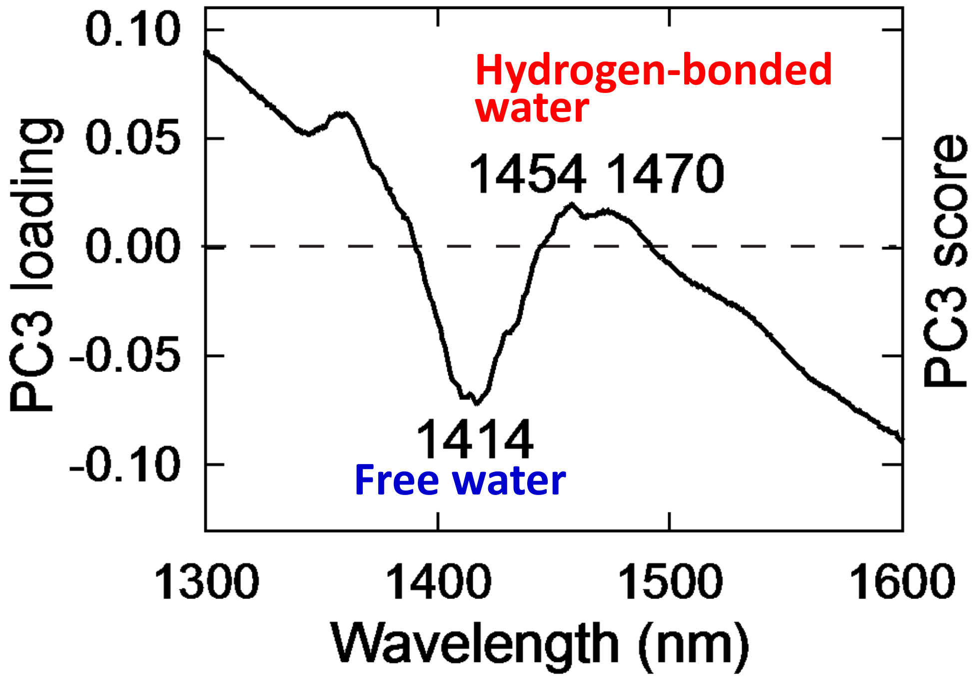
Spectral changes in water 1st overtone

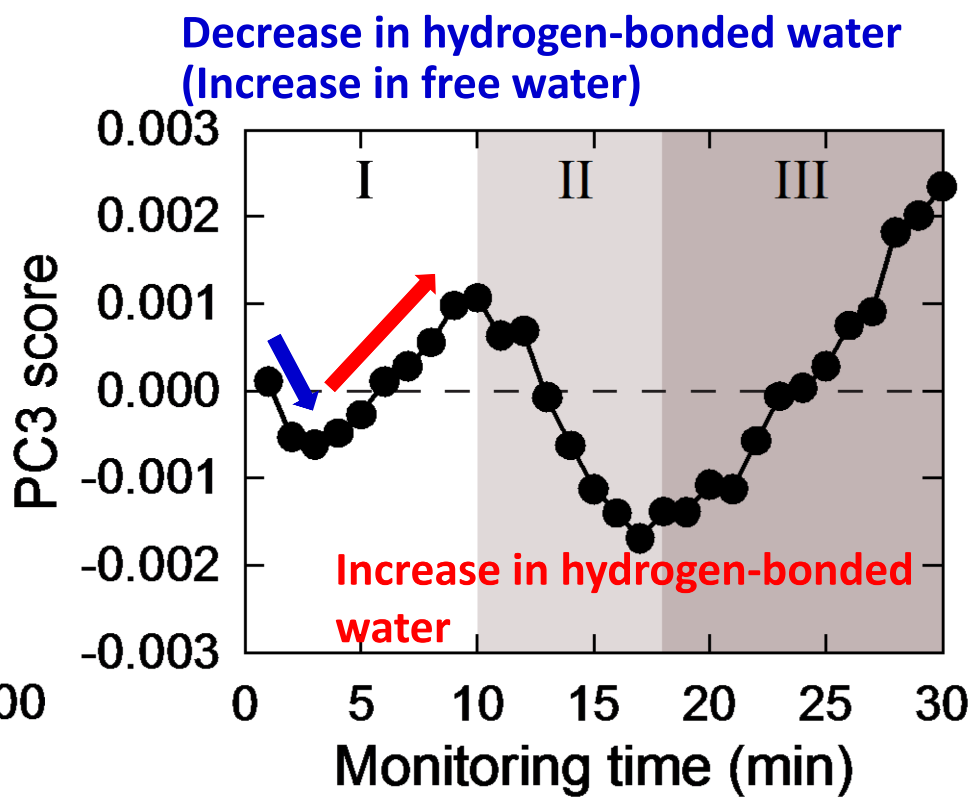
(Difference spectra obtained by Subtraction of the spectrum at 3 min)



PCA was applied to extract spectral peaks changing simultaneously with the fibrillation reaction

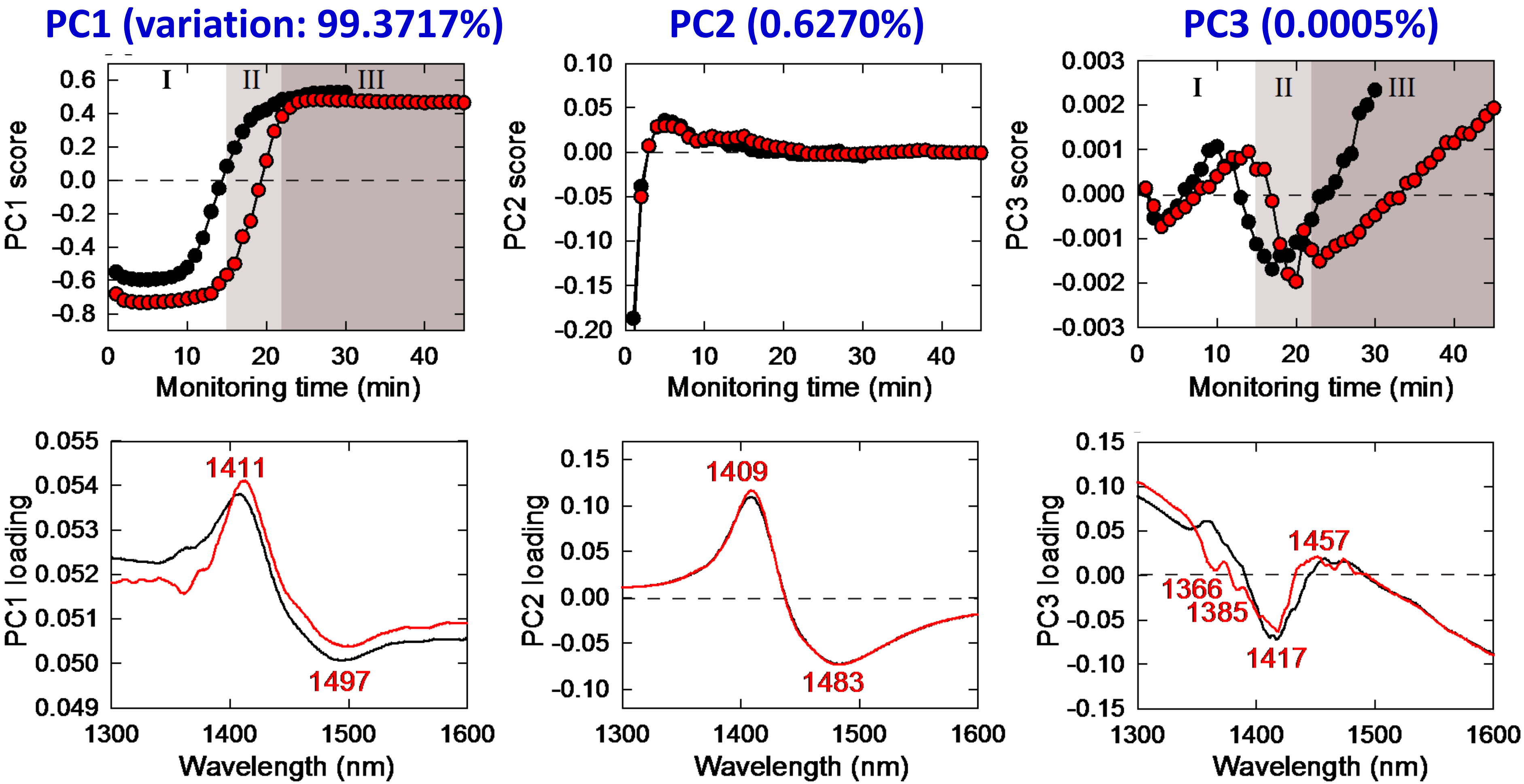
PCA for water structure change
– 3rd principal component (PC3) –





PC3 suggested that free water is generated transiently, and afterwards, hydrogen-bonded water increased

Reproducibility of PCA results
– 1300-1600 nm–



Repeated measurements of the same reaction verified that the spectral changes were reproducible

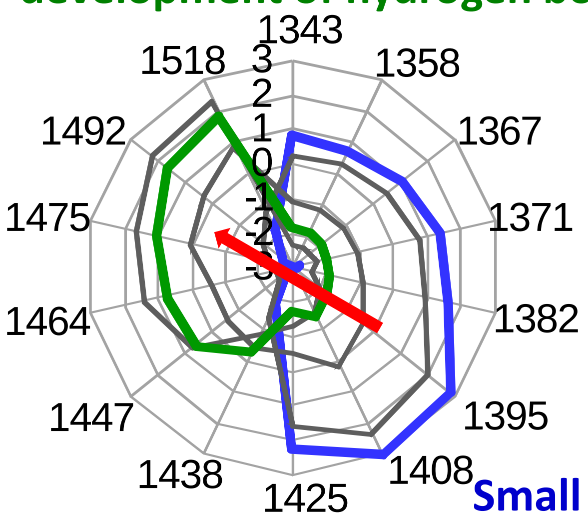
Construction of water absorbance pattern, Aquagram

$$A'_\lambda = \frac{A_\lambda - \mu_\lambda}{\sigma_\lambda}$$

A': value of Aquagram
A: absorbance after MSC applied on 1st overtone
μ: mean of all spectra
σ: SD of all spectra
λ: wavelentgh

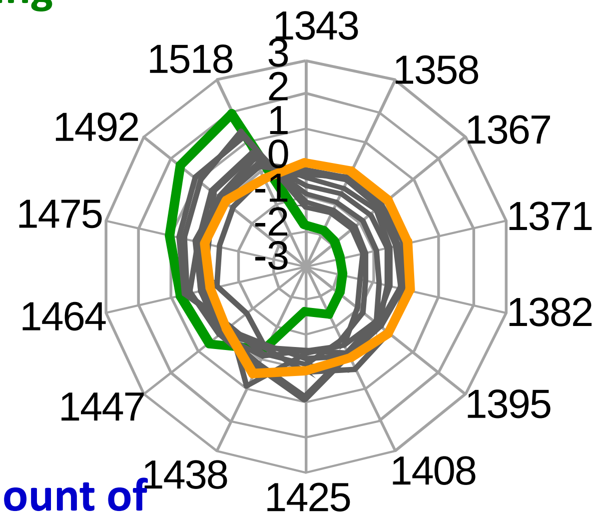
(I) nucleation

development of hydrogen bonding

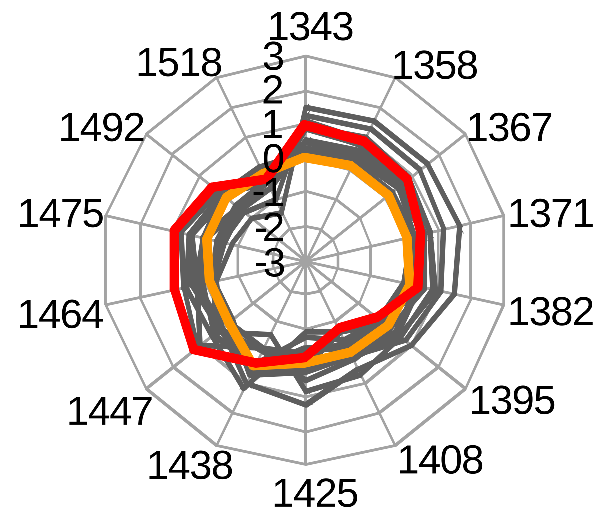


Small amount of hydrogen bonding

(II) elongation

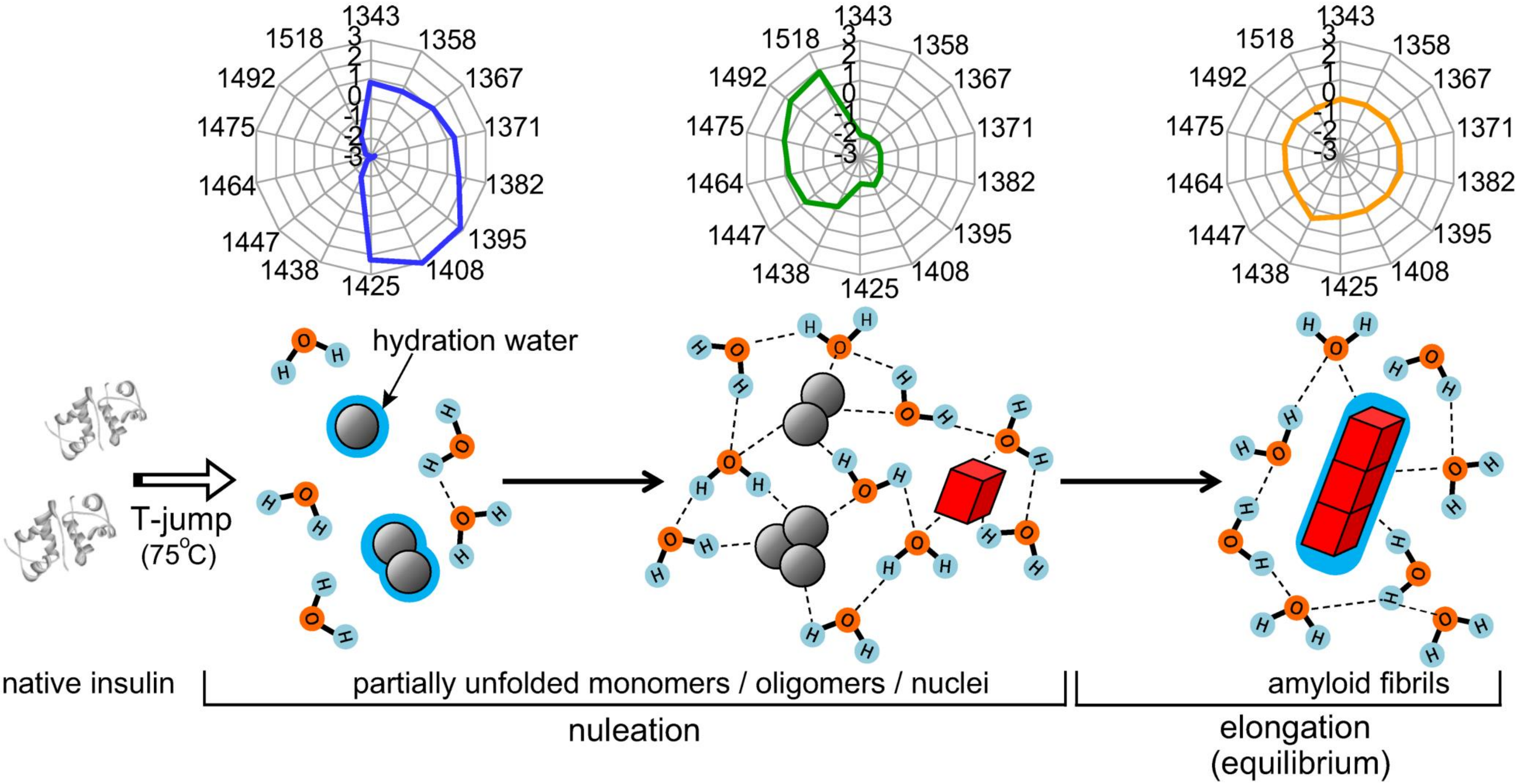


(III) equilibrium



Aquagram supported transient dissociation and subsequent development of hydrogen-bonded water networks in the nucleation phase

Schematic model for transformation of water structures

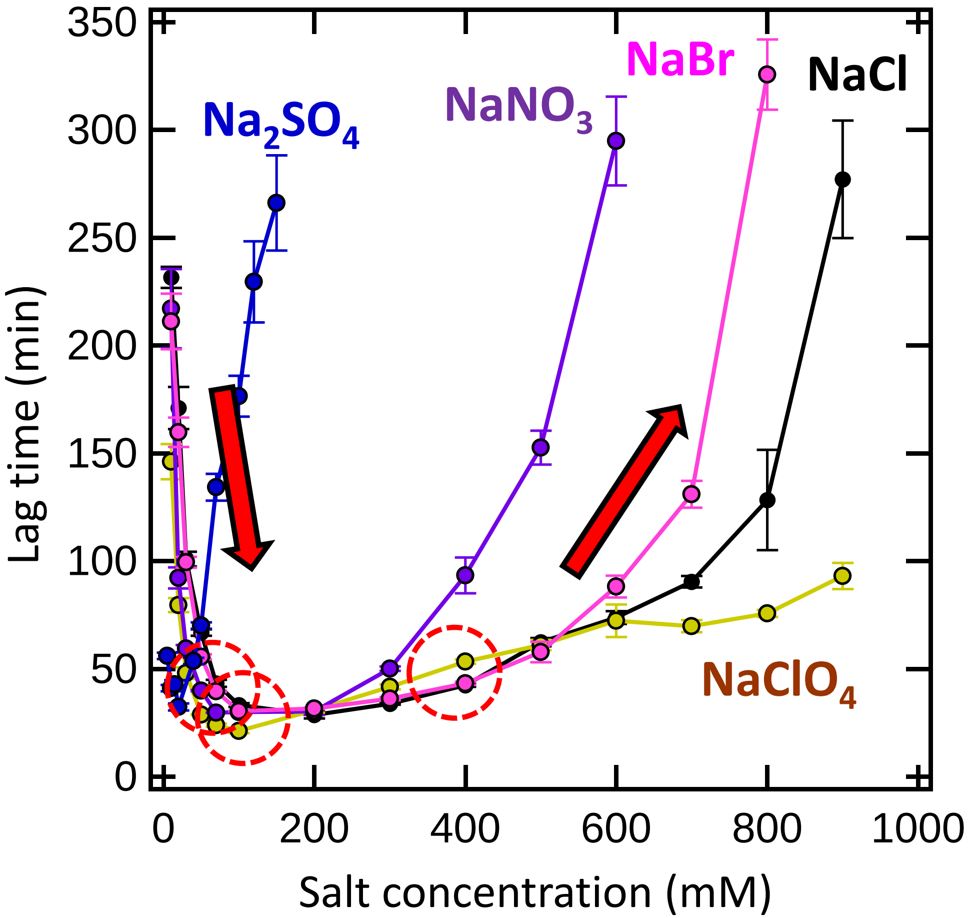


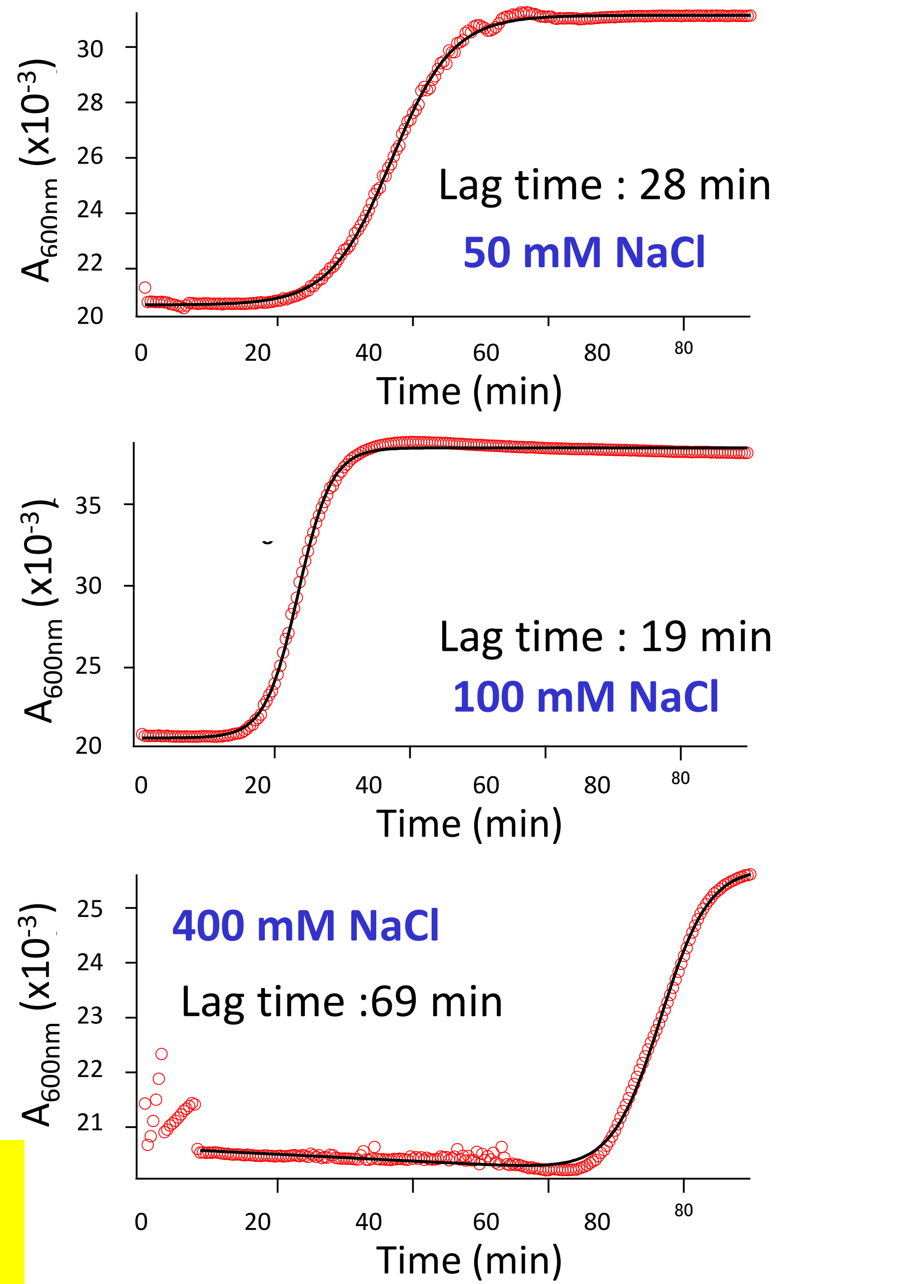
Organization of nuclei may be mediated by water molecules?

NIR measurement at different NaCl concentrations

The length of lag time is dependent of salt concentration

(Masuda et al., manuscript in preparation)





NIR spectrum was monitored at three different conc. NaCl

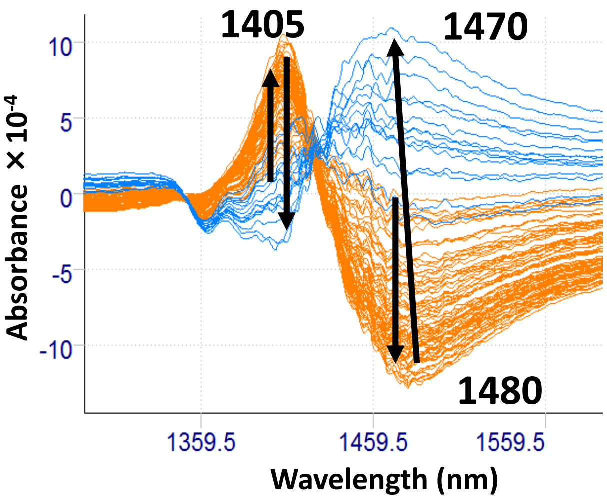
Biphasic spectral changes in the nucleation phase

Hiramatsu et al.: poster No. 12!!

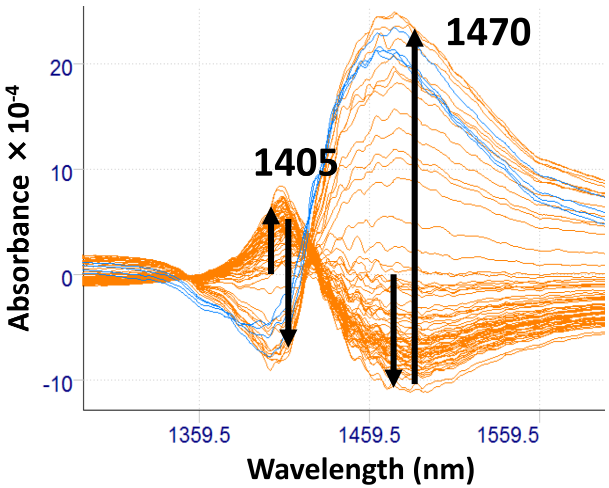
nucleation   elongation

The top of the sample liquid was covered by mineral oil

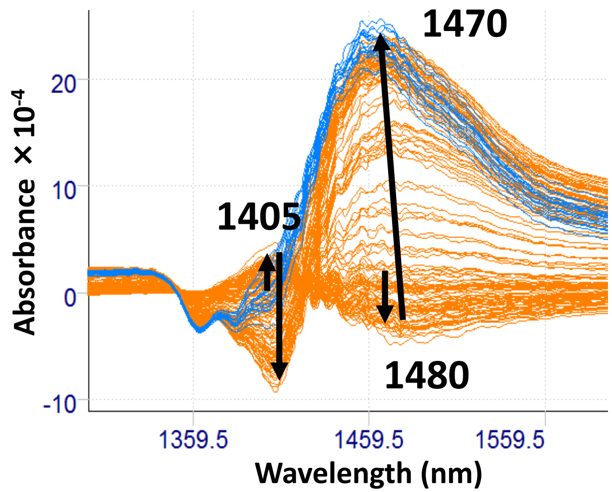
50 mM



100 mM



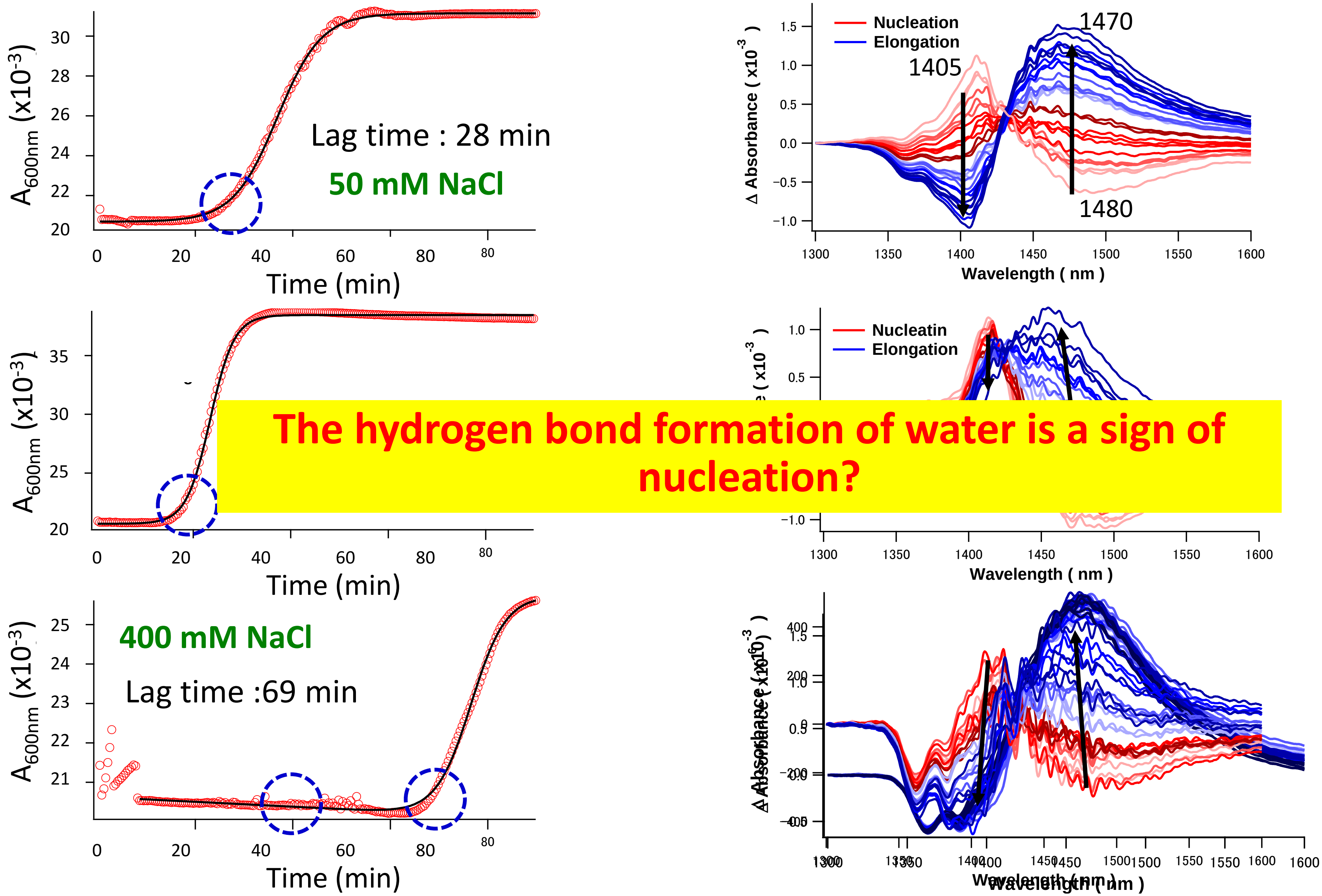
400 mM



Difference spectra obtained by subtraction of the spectrum at 5 min (the baseline was corrected)

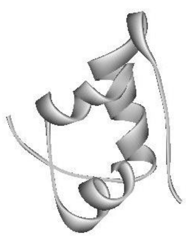
For all conditions, free water is initially formed, and afterwards, hydrogen-bonded water is formed

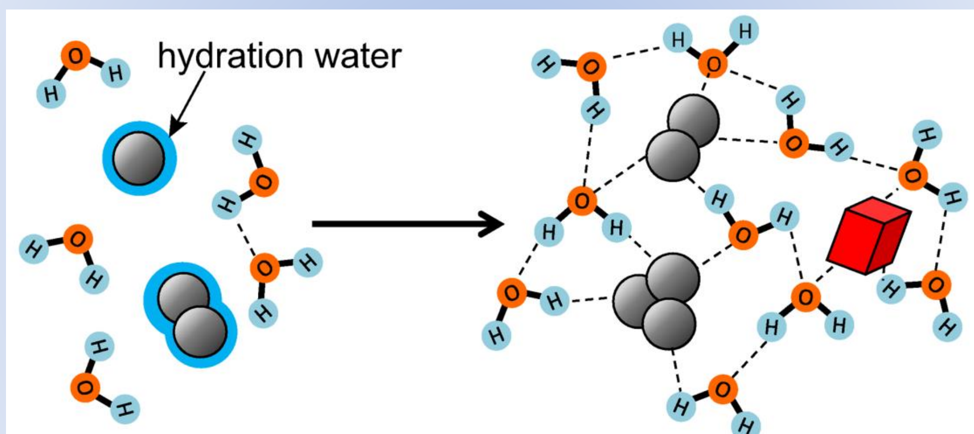
The timing of the water structural changes



The hydrogen bond formation of water is a sign of nucleation?

Summary and future perspectives





NIR measurements shed light on the transformation of water structures in the fibril nucleation. Water may be used as a new biomarker for early non-invasive diagnosis of amyloid-related diseases.

Jimenez et al., *PNAS*, 99, 9196 (2002)