Exploring water molecular system dynamics in the formation of amyloid fibrils using time-resolved NIR spectroscopy and Aquaphotomics

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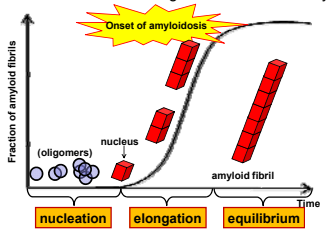
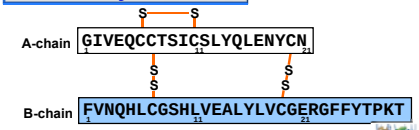
Introduction

Amyloid fibrils are protein aggregates associated with numerous serious diseases. The formation of amyloid fibrils generally proceeds through nucleation and elongation phases. The nucleation is one of the most important phases controlling the overall kinetics and final structure of amyloid fibrils formed. However, little is known about the details of how proteins and their surrounding water molecules vary.

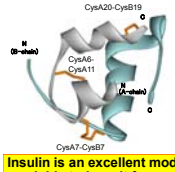


In this study, we have performed *in-situ* near infrared (NIR) spectral monitoring of the heat-induced spontaneous fibrillation reaction of human insulin.

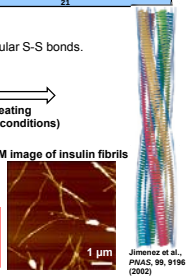
Insulin amyloid fibrils



- Hormone protein
- 51 residues in two polypeptide chains
- One intramolecular and two intermolecular S-S bonds.

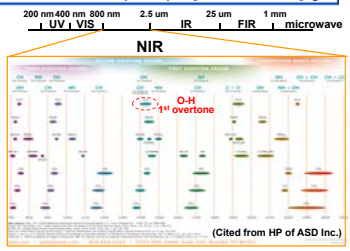


AFM image of insulin fibrils



Insulin is an excellent model in the amyloid study as it forms amyloid fibrils readily upon heating under the acidic conditions.

Near Infrared (NIR) spectroscopy



NIR spectroscopy is based on molecular overtone and combination vibrations

- Permeable
- Non-invasive

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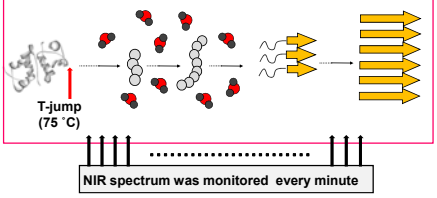
Application NIR for quality assessment

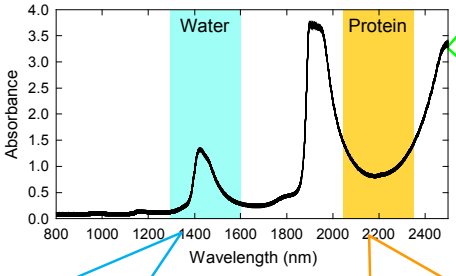
In-situ NIR monitoring

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

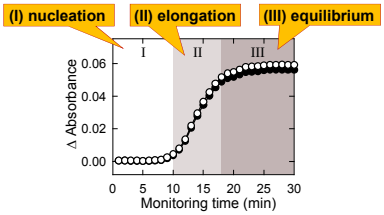


- Spectrophotometer: MPA (Bluker optics Co., Ltd.)
- Optical length: 1 mm
- Mode: transmittance

Overview of NIR spectra



Baseline analysis



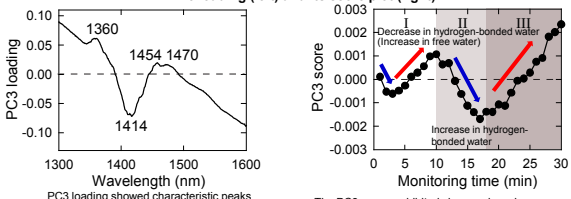
When increment of absorbance baseline was plotted against incubation time, sigmoidal shape was observed, verifying the spontaneous formation of amyloid fibrils.

Based on the absorbance baseline increment, the fibrillation time course was categorized into three phases, i.e., nucleation (I), elongation (II), and equilibrium (III).

Principal component analysis (PCA)

Water structure change – water 1st overtone region –

PC3 loading (left) and its score plot (right)



PC3 loading showed characteristic peaks at 1360, 1414, 1454, and 1470 nm.

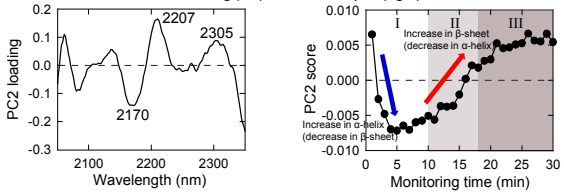
The PC3 score exhibited changes in a zig-zag manner, and the time course of each change coincided well with the proposed nucleation, elongation, and equilibrium phases.

Peak assignment:
1360 nm: hydration shell
1414 nm: free water
1454 nm: intermolecularly hydrogen-bonded hydroxyls
1470 nm: water with three hydrogen bonds (S_3)

→ Characteristic transformations of water structure during the fibrillatioin have been depicted as PC3 .

Protein tructure change – amide I region –

PC2 loading (left) and its score plot (right)



PC2 loading showed characteristic peaks at 2170, 2207, and 2305 nm.

The shape of PC3 score plot seemed fundamentally similar to that observed for the baseline change.

Peak assignment:
2170 nm: α-helix
2207 nm, 2305 nm: β-sheet

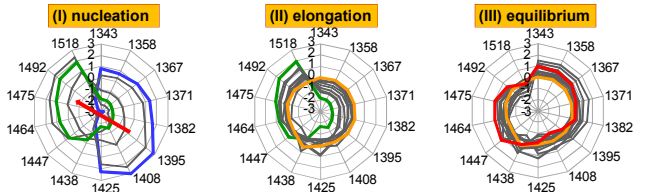
→ The time dependency of PC2 successfully tracks the cross- β formation

Construction of Aquagram

For 14 wavelengths, mean-centering and normalization of dispersion was conducted to construct Aquagram, a radar chart displaying time-dependency of normalized absorbance at these water.

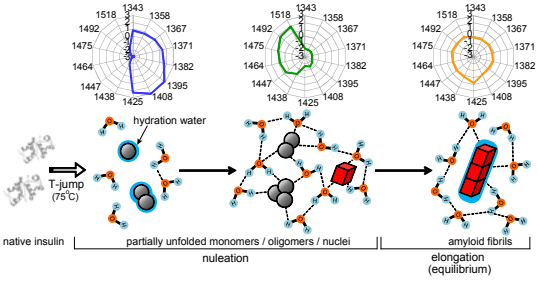
$$A'_\lambda = \frac{A_\lambda - \mu_\lambda}{\sigma_\lambda}$$

A': value of Aquagram
A: absorbance after MSC applied on 1st overtone
μ: mean of all spectra
σ: SD of all spectra
λ: 14 wavelentghs related to water molecular conformation



Additional evaluation of water absorbance pattern (Aquagram) also proposed unique water molecular system dynamics, i.e., transient dissociation and subsequent development of hydrogen-bonded water networks during the nucleation phase of fibrillation.

Schematic model for the transformation of water structures



Conclusion

- **We could obtain information about unique changes of water structure by performing time-resolved NIR spectra.**
  - **Time course of fibrillation reaction was determined by baseline shift of solution spectra**
  - **PCA result and Aquagram suggested two-step transformation of water structure in the nucleation phase.**
- **The specific transformations of water spectral pattern shed light on the role of water molecules in the formation of amyloid fibrils, and furthermore, could be used as a new biomarker for early non-invasive diagnosis of amyloid-related diseases.**