1

Label-free detection of amyloid fibrils based on their intrinsic fluorescence properties

Manuela Grelich-Mucha*, Joanna Olesiak-Bańska

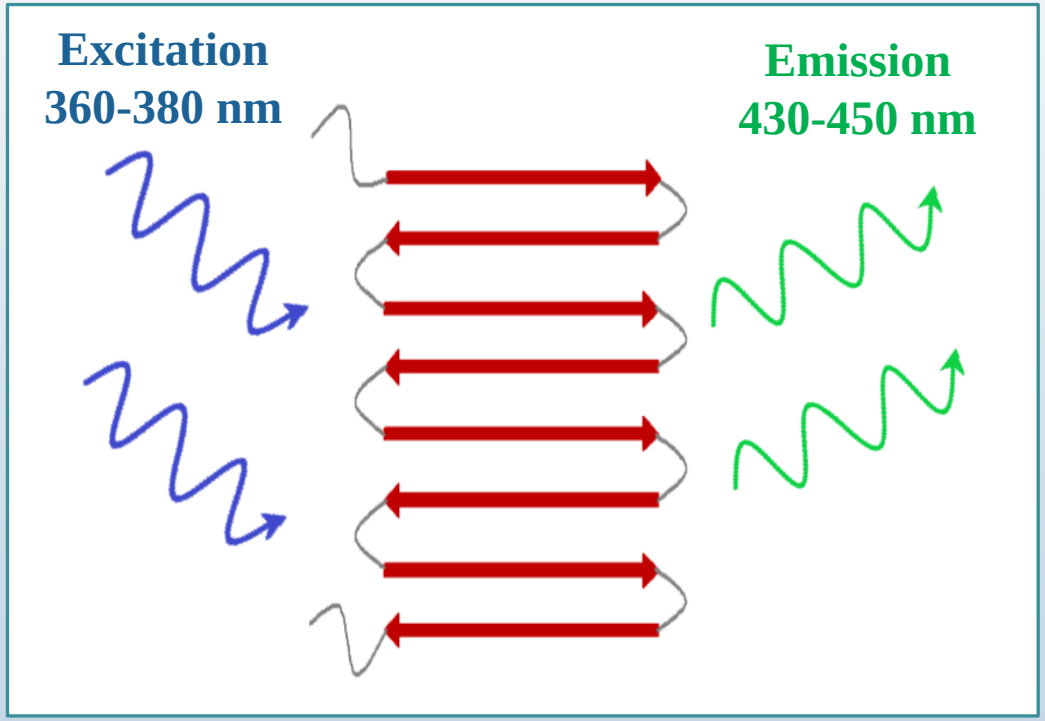
Advanced Materials Engineering and Modelling Group, Wroclaw University of Science and Technology, Wroclaw, Poland

* manuela.grelich@pwr.edu.pl

Many peptides and proteins undergo misfolding into amyloid fibrils.

The most commonly-known method to detect amyloid fibrils is **Thioflavin T** (ThT) binding assay. Incorporation of the dye induces characteristic fluorescence – upon excitation at ~450 nm fluorescence emission occurs at ~480 nm.[1]

However, a promising alternative for fibrils detection constitutes their **intrinsic fluorescence** assay. Fibrils, when excited at **~360 nm** emit in the visible range, with a maximum at **~450 nm**.[2-3]



1. Biancalana, M.; *et al.*, *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 2010, *1804* (7), 1405-1412.

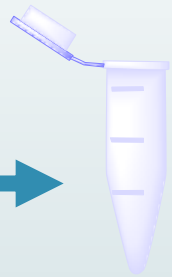
2. Pinotsi, D.; *et al.*, *Journal of the American Chemical Society* 2016, *138* (9), 3046-3057.

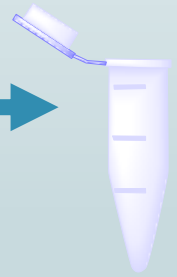
3. Joseph, S. K.; *et al.*, *The Journal of Physical Chemistry A* 2019, *123* (9), 1758-1765.

Methods

Bovine insulin

pH=1.4
2 mg/ml

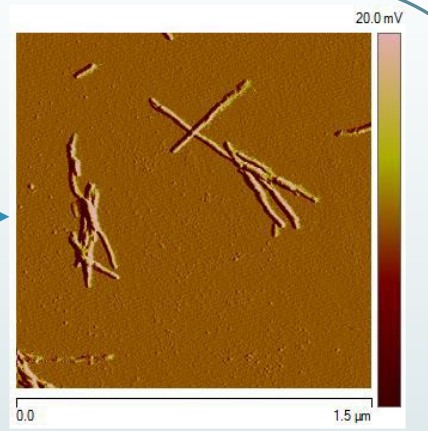


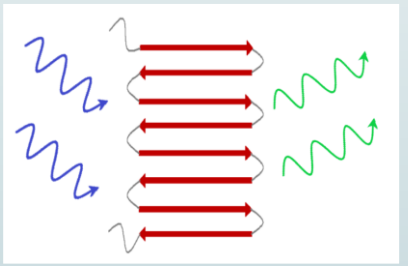


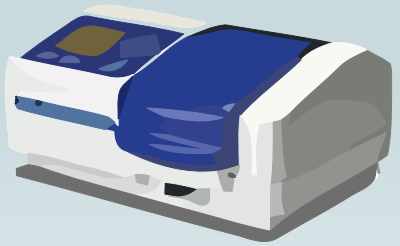
ThT
20 µM

Incubation

43.5 h, 700 rpm, 45°C





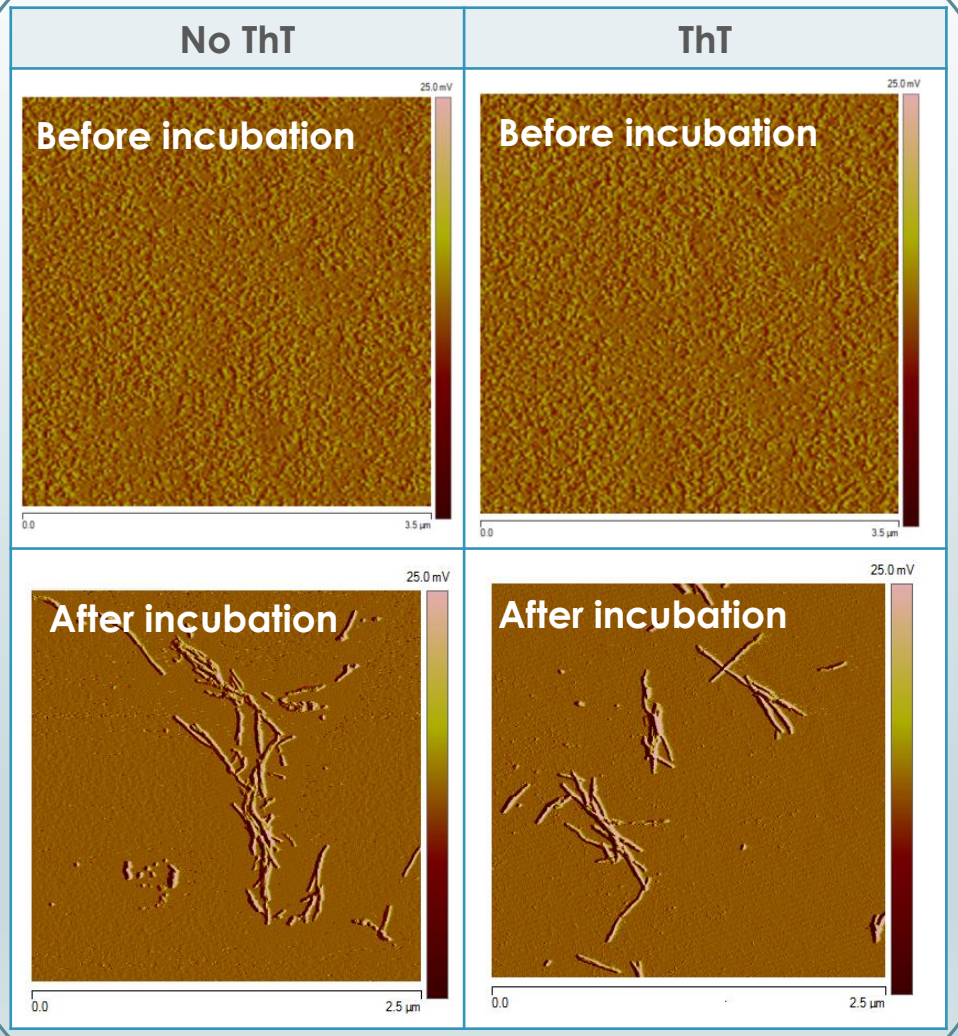


Simplified scheme presenting preparation method and investigation tools used to study the samples.

Results

3

AFM imaging



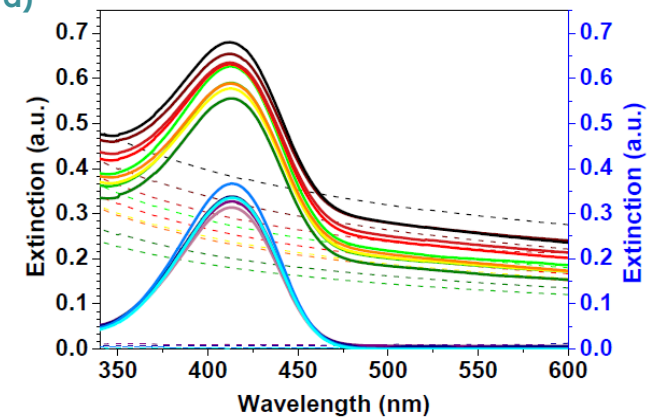
Label	Width (nm)	SD	Height (nm)	SD	Length (nm)	SD
No ThT	41.3	7.48	7.49	1.38	603	164.85
ThT	42.3	3.27	6.37	1.87	626	221.48

Dimensions of the fibrils

Results

Extinction (a) and fluorescence spectra (b, c)

a)

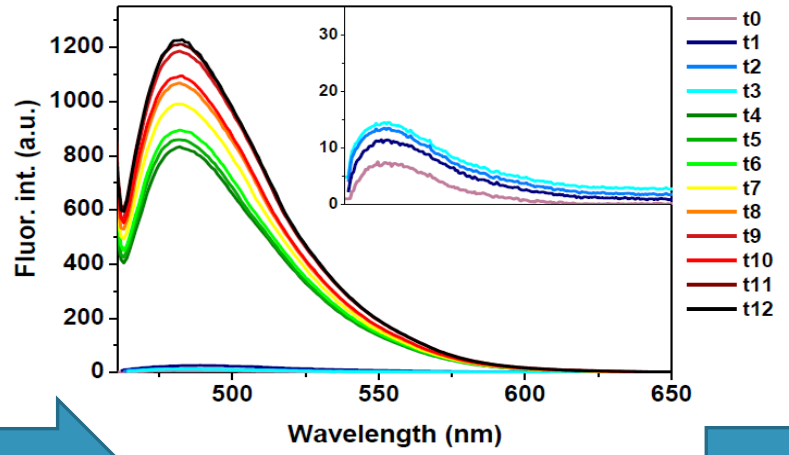


t0 – 0 h
t1 – 1 h 30 min
t2 – 3 h 40 min
t3 – 5 h 20 min
t4 – 17 h 40 min
t5 – 20 h 40 min
t6 – 21 h 52 min
t7 – 22 h 46 min
t8 – 24 h 53 min
t9 – 27 h 17 min
t10 – 28 h 20 min
t11 – 40 h 30 min
t12 – 43 h 30 min

Extinction spectra of bovine insulin incubated in the absence (dotted lines) and presence (straight lines) of ThT. Measurements were taken at given time points. The total time of incubation was 43.5 h.

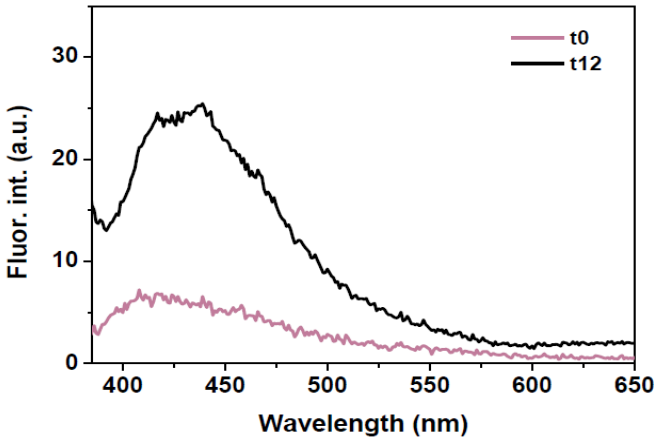
b)

Insulin with ThT; λ_{exc} = 444 nm.



c)

Insulin; λ_{exc} = 360 nm.



Max emission at λ = **483 nm**.

The final fluorescence emission intensity **increased ~112x** during the incubation.

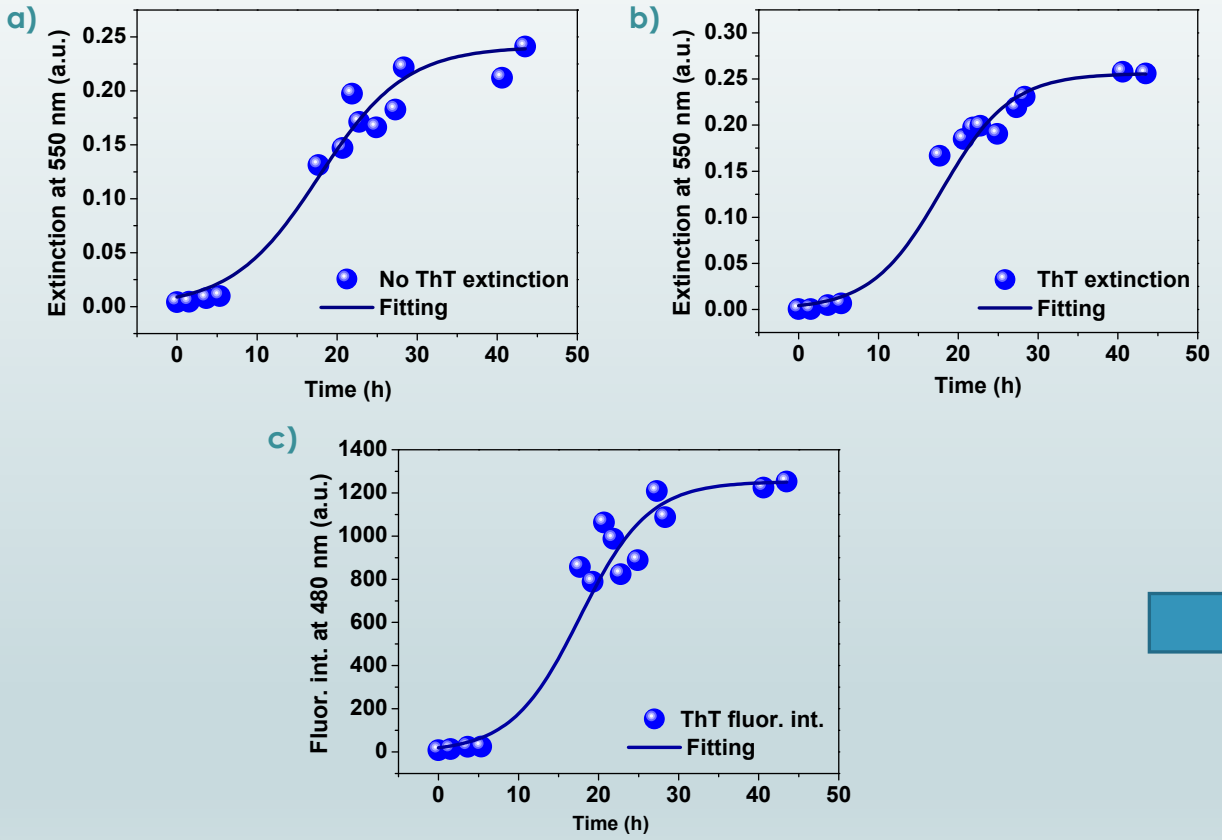
Max emission in the range **400-500 nm** (**Vis range**).

Confirmation of intrinsic fluorescence properties.

Fluorescence int. **increased ~ 4x**.

Results

Fibrillation kinetics based on extinction (a, b) and fluorescence intensities (c)



Sigmoidal growth kinetics assays observed for (a, b and c).

Dye-free samples characterized by **shorter** lag time (6.96 h) and **faster** k_{app} (0.19 1/h).

ThT has an influence on the kinetics of amyloid fibrils formation.

Sample	R^2	Lag time (h)	k_{app} (1/h)
Extinction at 550 nm no ThT	0.962	6.96	0.19
Extinction at 550 nm ThT	0.952	9.13	0.23
Fluor. intensity at 480 nm ThT	0.945	9.17	0.23

Conclusions

- We confirmed autofluorescence properties of amyloid fibrils.
- Kinetics studies evidence that **ThT has impact on the rate of fibrils formation**. In the presence of the dye, the lag phase is longer and the growth is slower compared to conditions without ThT.
- These results strongly recommend to develop label-free studies of fibrillization.

Acknowledgement

NOVA
Nonlinear optics, nanoparticles and amyloids
FNP FIRST TEAM

FNP Fundacja na rzecz Nauki Polskiej

Wrocław University of Science and Technology