107° CONGRESSO NAZIONALE
della SOCIETÀ ITALIANA DI FISICA

Comparison of protein conformational properties *in solution* and *in the crystalline state* by Fourier transform infrared spectroscopy

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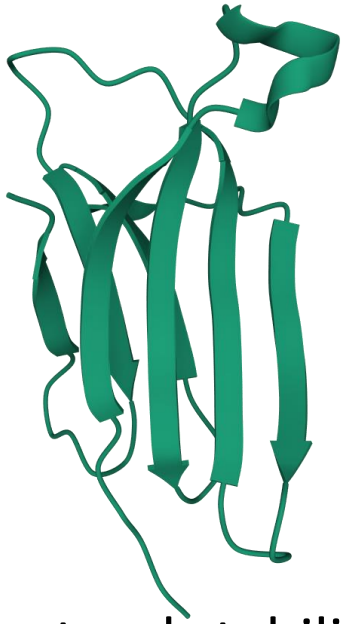
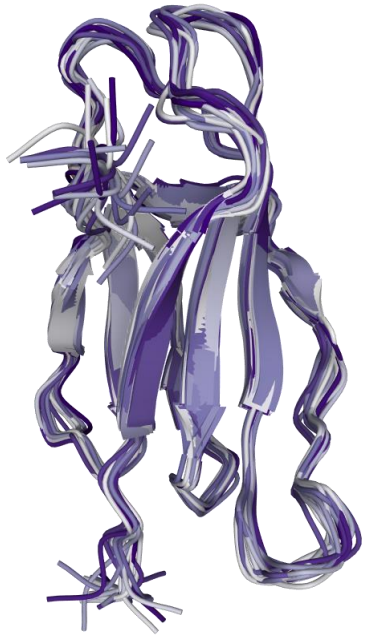
Proteins in solution and in the crystalline state

- Detailed 3D structures can be obtained both in solution and in the crystalline state

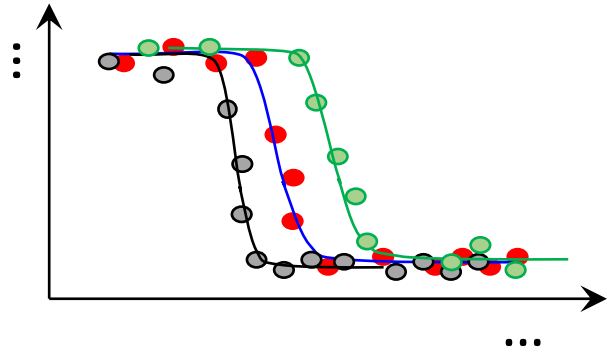
1LDS
Crystal Structure of monomeric human beta-2-microglobulin
DOI: 10.2210/pdb1LDS/pdb

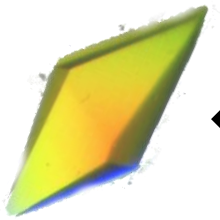




1JNJ
NMR solution structure of the human beta2-microglobulin
DOI: 10.2210/pdb1JNJ/pdb

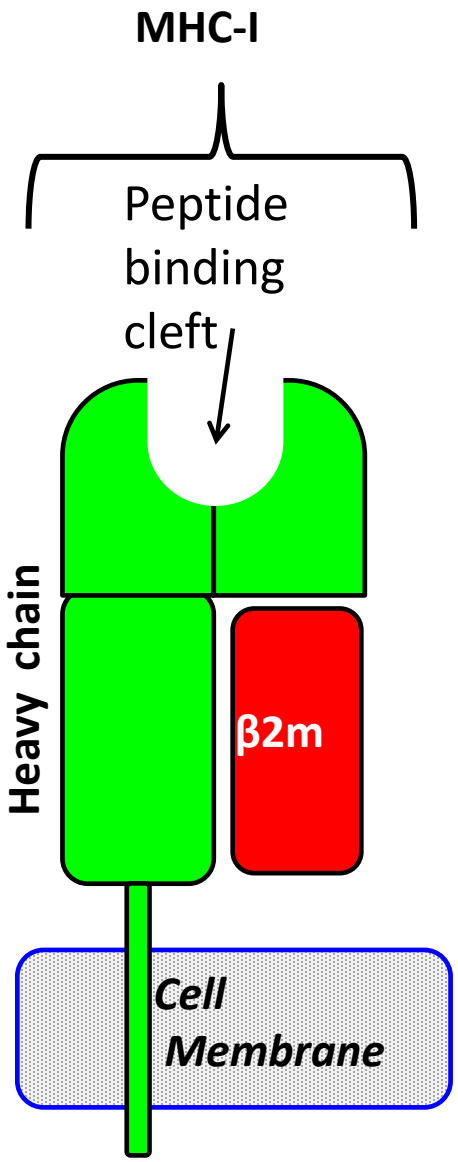
- Protein conformational properties (i.e. structural stability and aggregation propensity) are typically studied in solution





- Are these “in solution” properties detectable in the protein crystals?
- How we can study these properties in the protein crystals?

The model system: β-2microglobulin



→β-2microglobulin (β2m) is the light chain of class I major histocompatibility complex (MHC-I).

→In vivo, β2m is degraded and excreted by kidneys.

→As a consequence of renal failure, the plasma concentration of β2m increases up to 60-fold. This leads to the accumulation of β2m around bones and joints in form of amyloid deposits, resulting in the development of the dialysis-related amyloidosis (DRA).

→ The natural occurring variant of β2m D76N causes an autosomal dominant, hereditary systemic amyloidosis with extensive visceral amyloid deposits*.

*Valleix et al. 2012 N Engl J Med 366:2276-2283

β2-microglobulin is the causing agent of two types of systemic amyloidosis:

Dialysis Related Amyloidosis		D76N β_2m Amyloidosis
Acquired	←Type of amyloidosis→	Hereditary
After 10 years of Dialysis	←Onset→	After 50-60 years of life
50-70 mg/L	←β_2m plasma concentration→	2 mg/L
Osteotendinuos tissue	←Site of deposition→	Systemic
Full length WT β2m + ΔN6β2m 70% 30%	←Fibril composition→	Full-length D76N β2m

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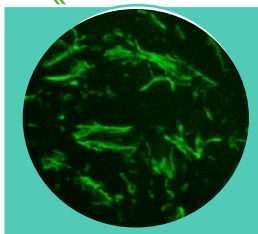
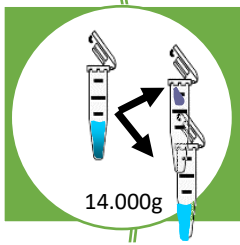
Time course of aggregation studied by biophysical and biochemical approaches: PBS pH 7.4, 37°C, stirring.

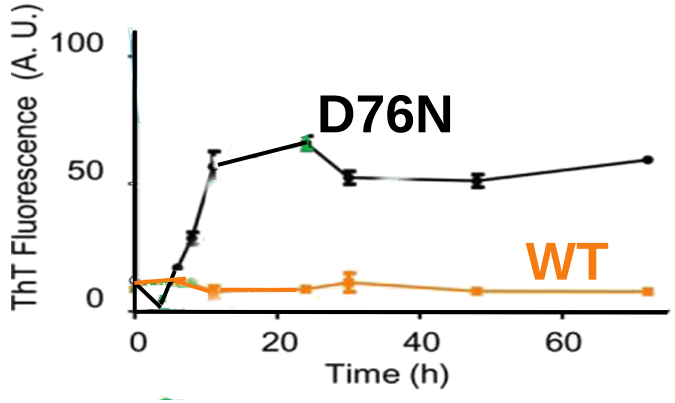


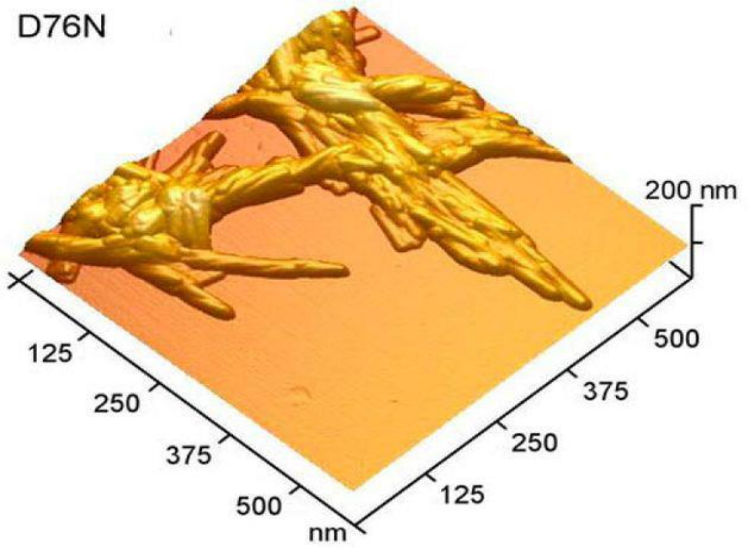
ThT assay: ThT binding is a common property of amyloid fibrils obtained from different precursor proteins



SDS-Page

Microscopy: aggregate morphology at the nm scale





Natalello et al. 2016 J. Biol. Chem. 291:9678-9689

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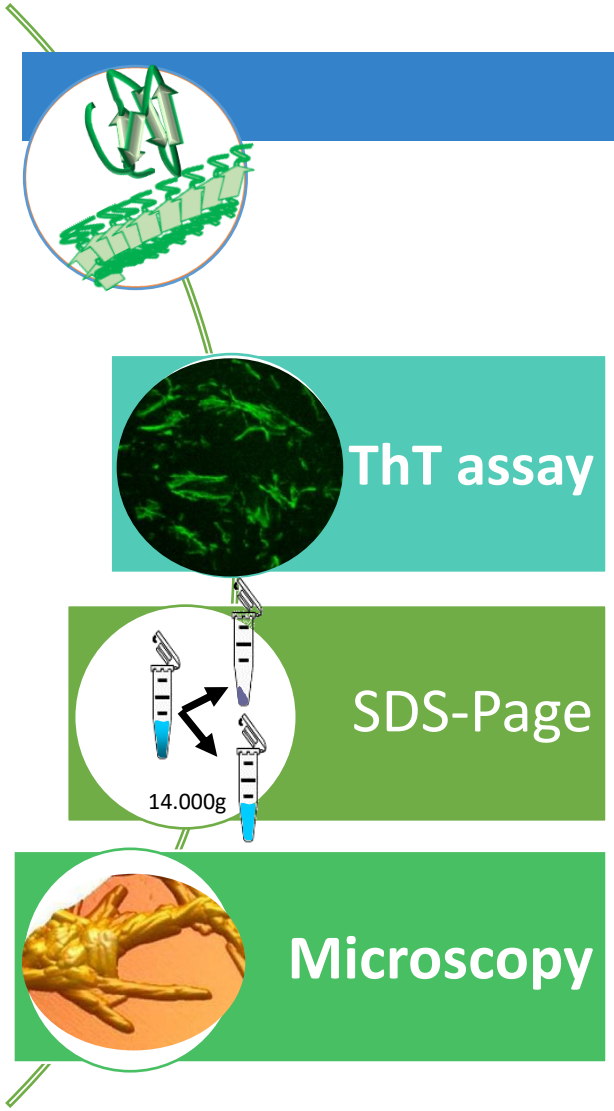
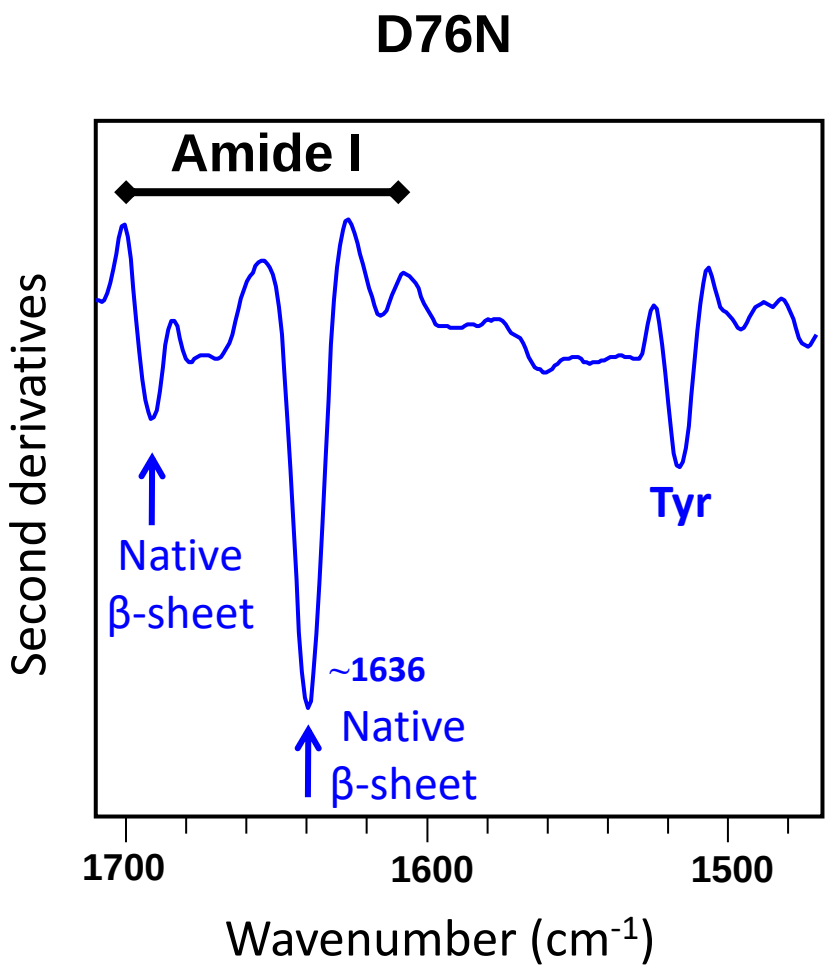


FTIR spectroscopy

- It allows to study complex samples in different physical states (from soluble/insoluble proteins to intact cells and tissues)
- It allows discriminating native intramolecular β-sheets from intermolecular β-sheets



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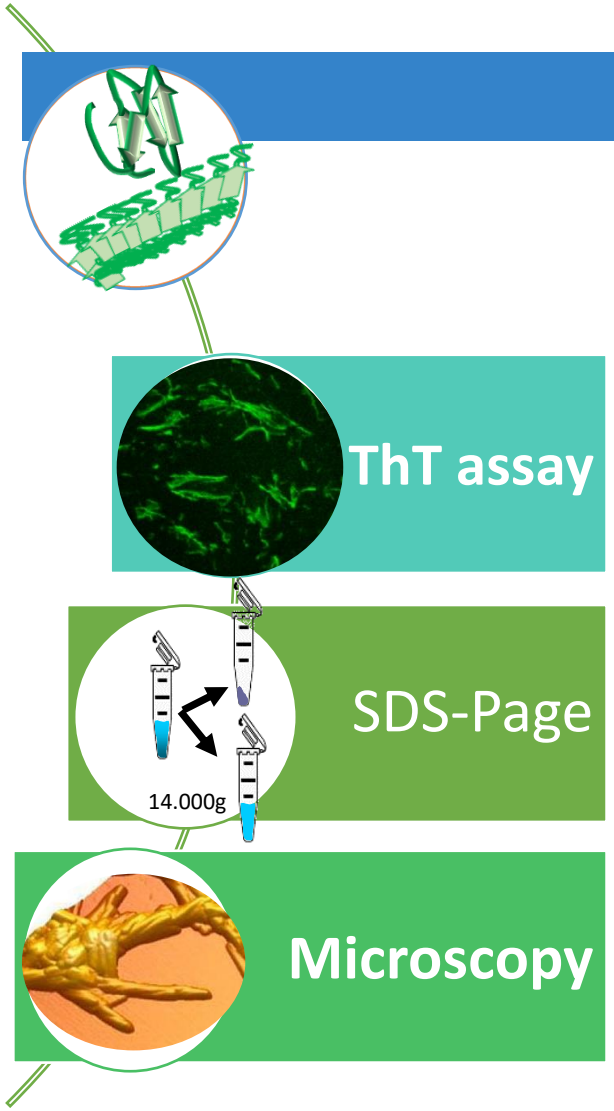

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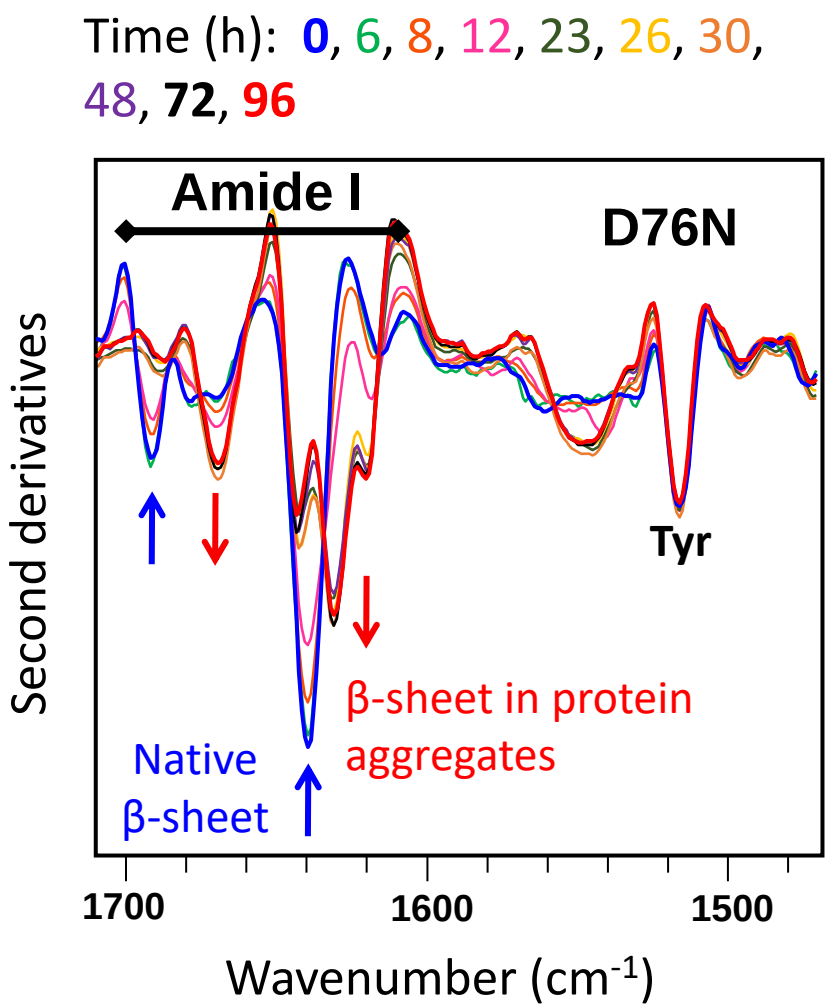

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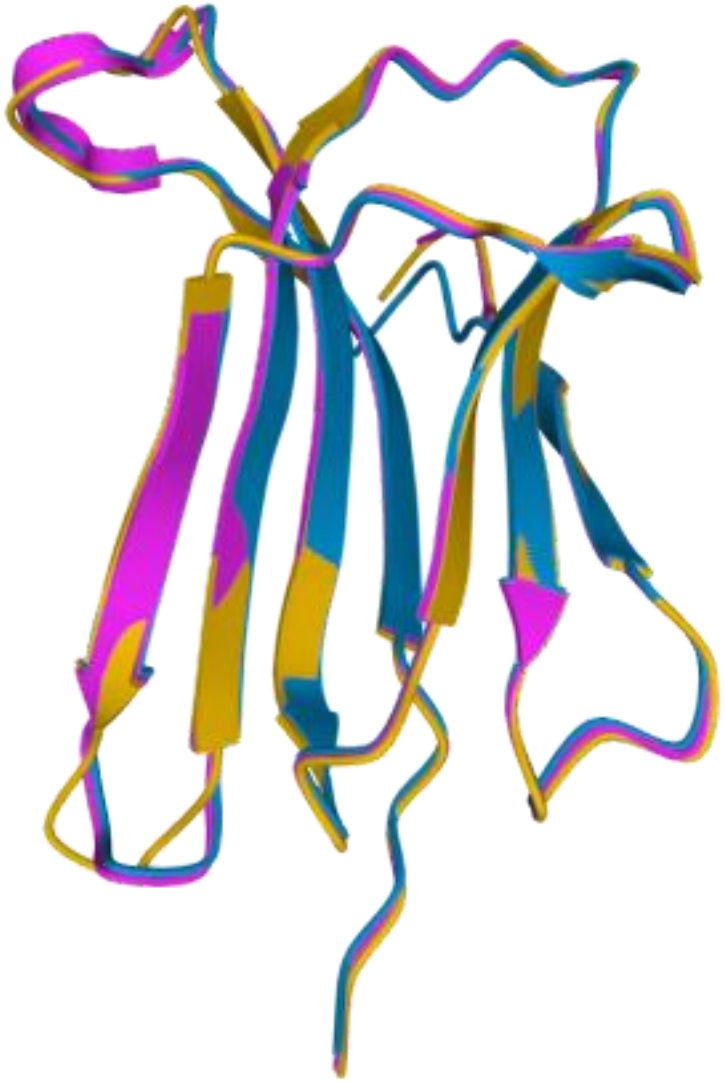
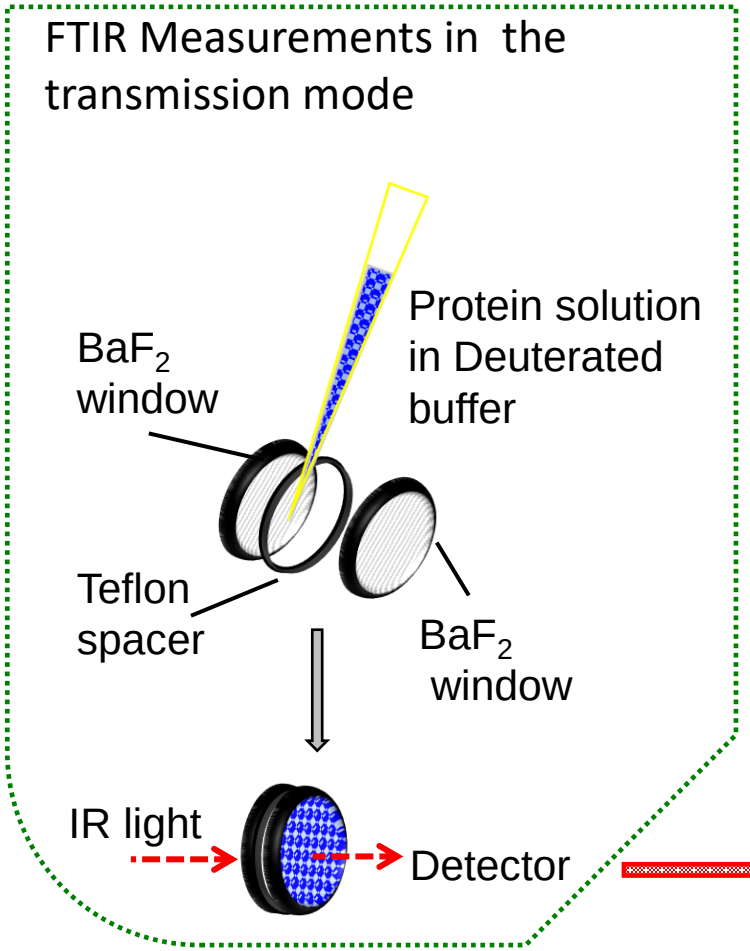
The model system: β-2microglobulin *variants*

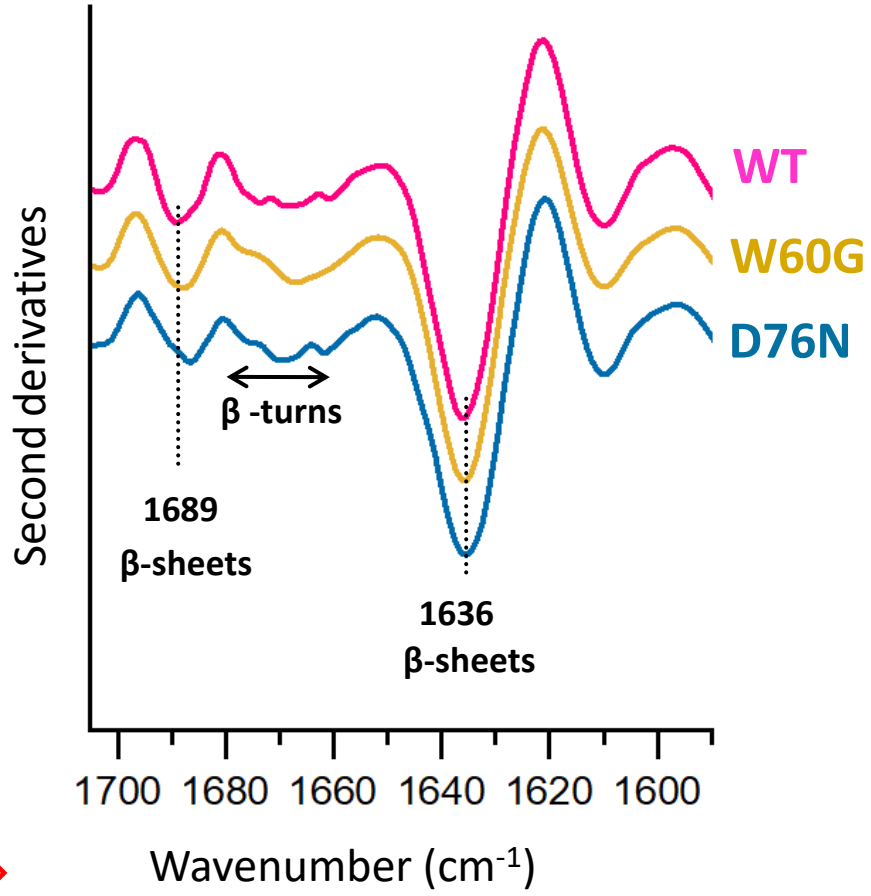


Superposition of the crystal structure of human **WT (PDB: 2YXF)** β2m, **W60G (PDB: 2Z9T)** and **(D76N 4FXL)** mutants

- In addition to the Amyloid-prone mutant (**D76N**), a stable variant was also considered for comparison: the **W60G** protein
- The structures of the **W60G** and **D76N** mutants are very similar to the structure of the **WT** protein.
- The three variants crystallize under identical conditions and form the intermolecular interactions in identical crystal packing.

Native β2m variants displayed identical secondary structures *in solution* as observed by FTIR spectroscopy

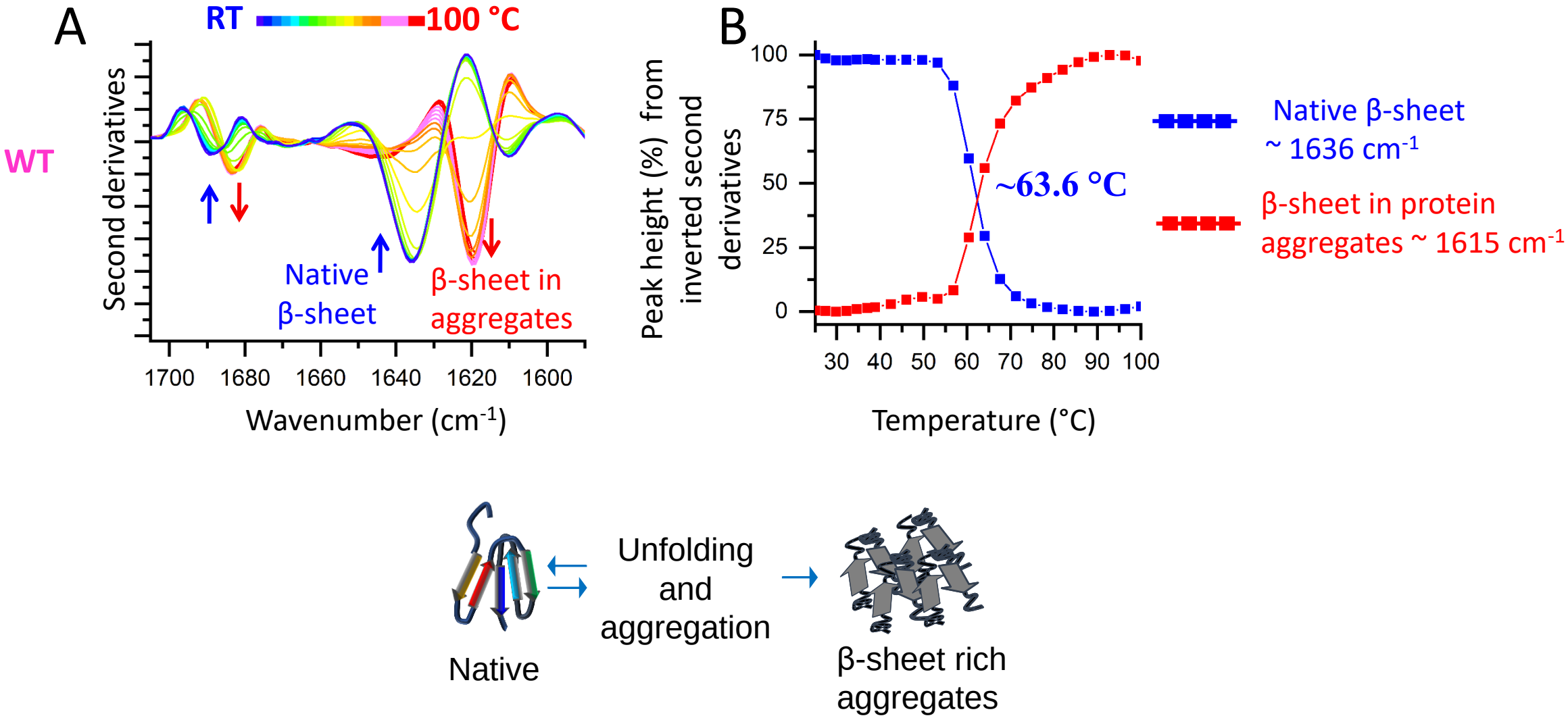




❖ **The same secondary structures in solution**

Ami et al. 2012 Biophys J 102:1676-1684
Sala et al. 2020 Biophys J. 119:978-988

Thermal stability and aggregation of β2m variants *in solution* by FTIR spectroscopy



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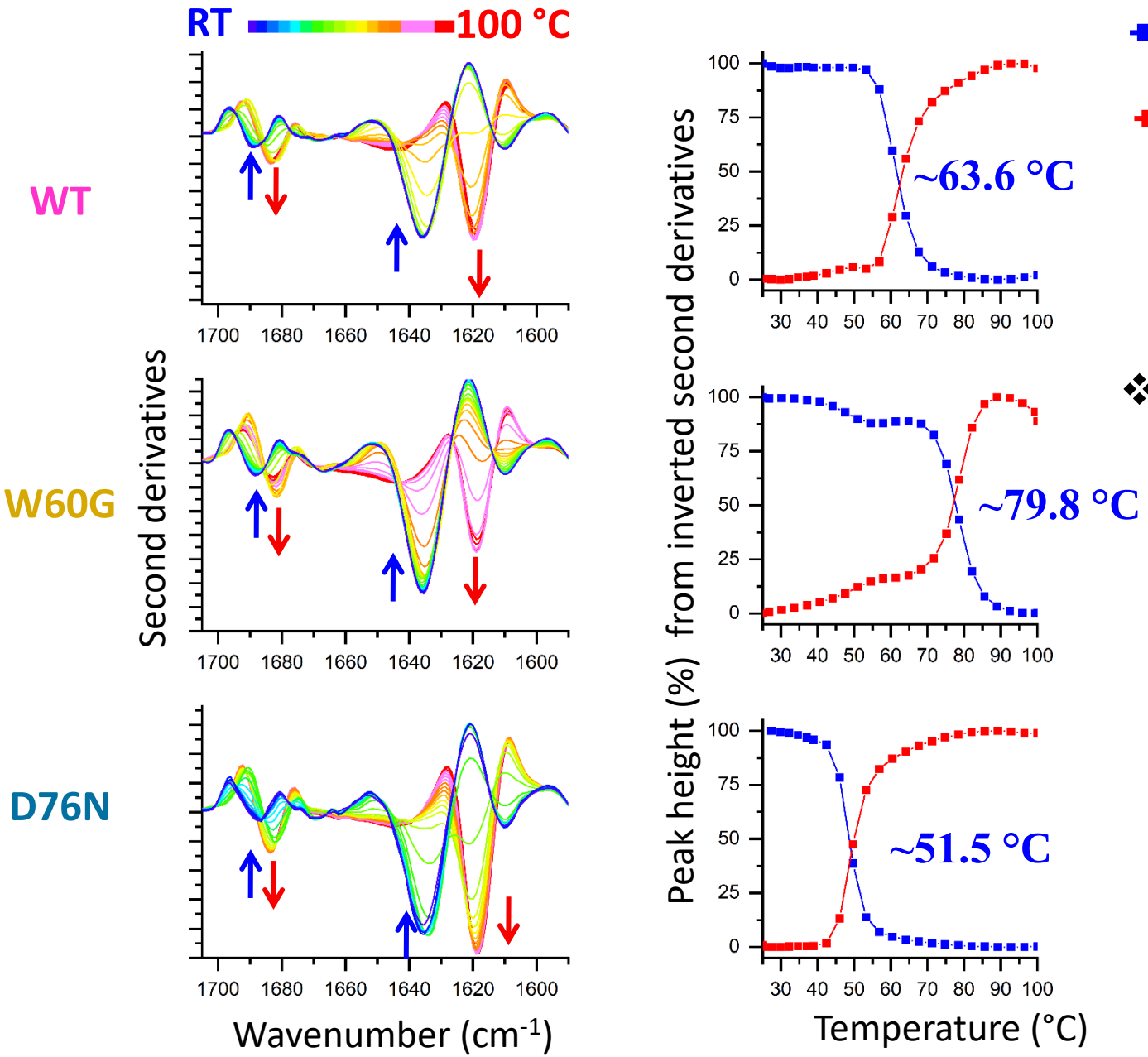

Native β-sheet ~ 1636 cm^{-1}

β-sheet in protein aggregates ~ 1615 cm^{-1}

❖ **The same secondary structures in solution**

❖ **Different thermal stability in solution**

T_{mp}:

W60G > WT > D76N

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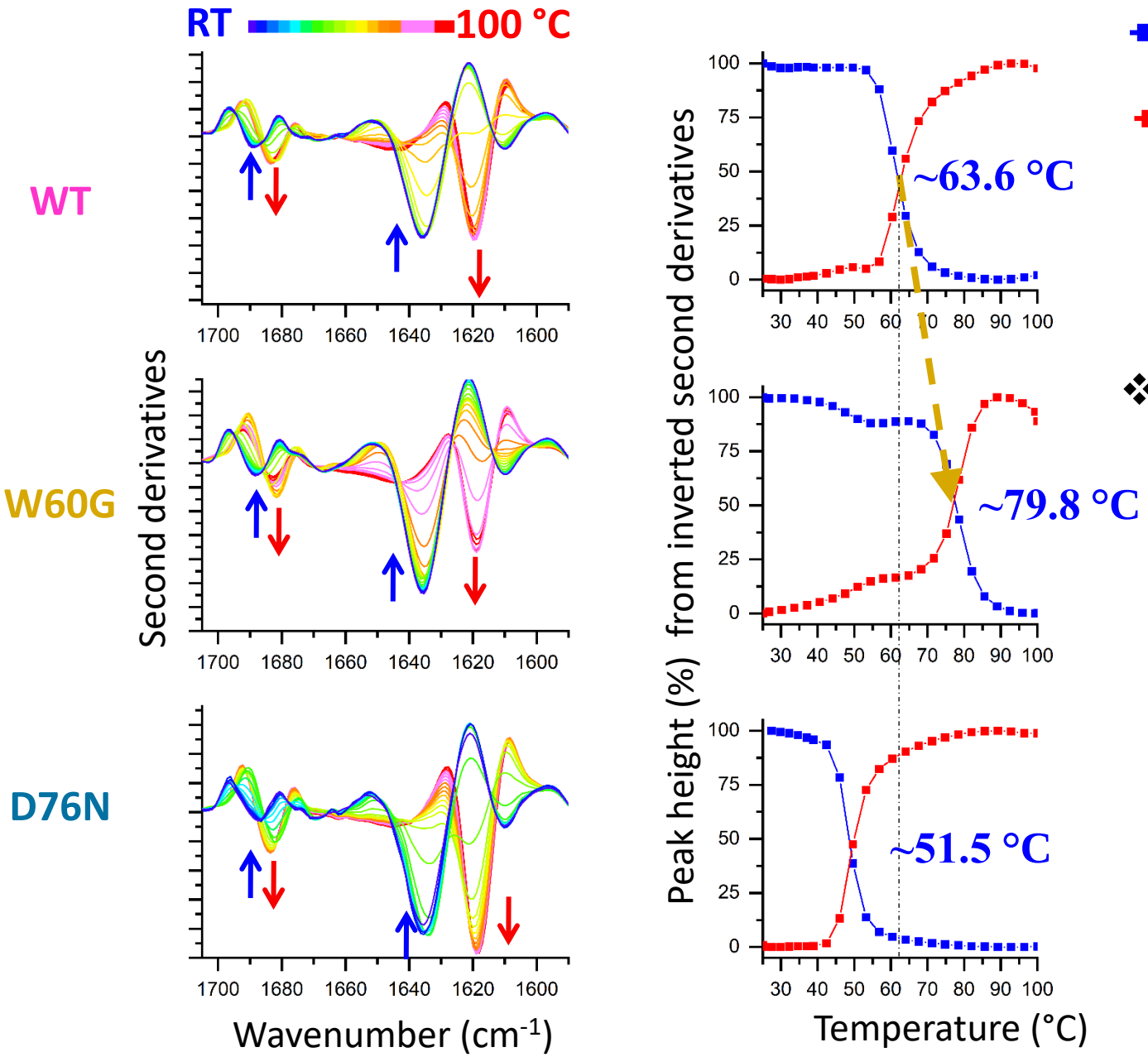

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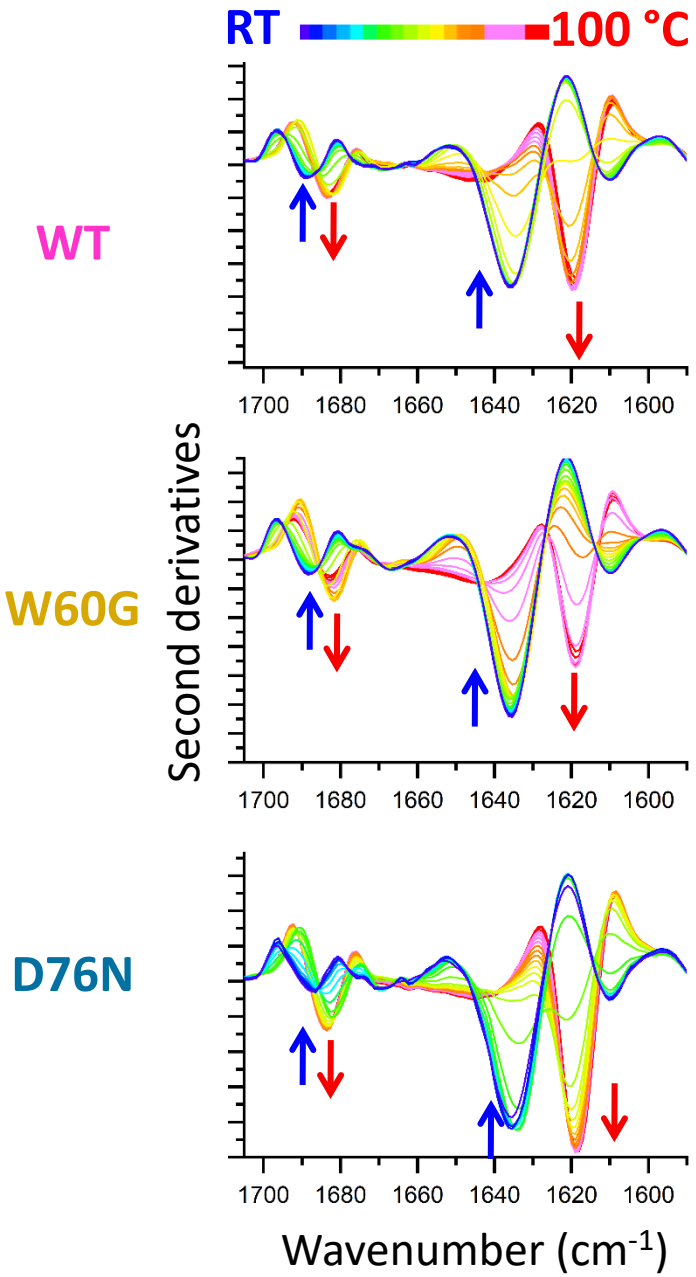

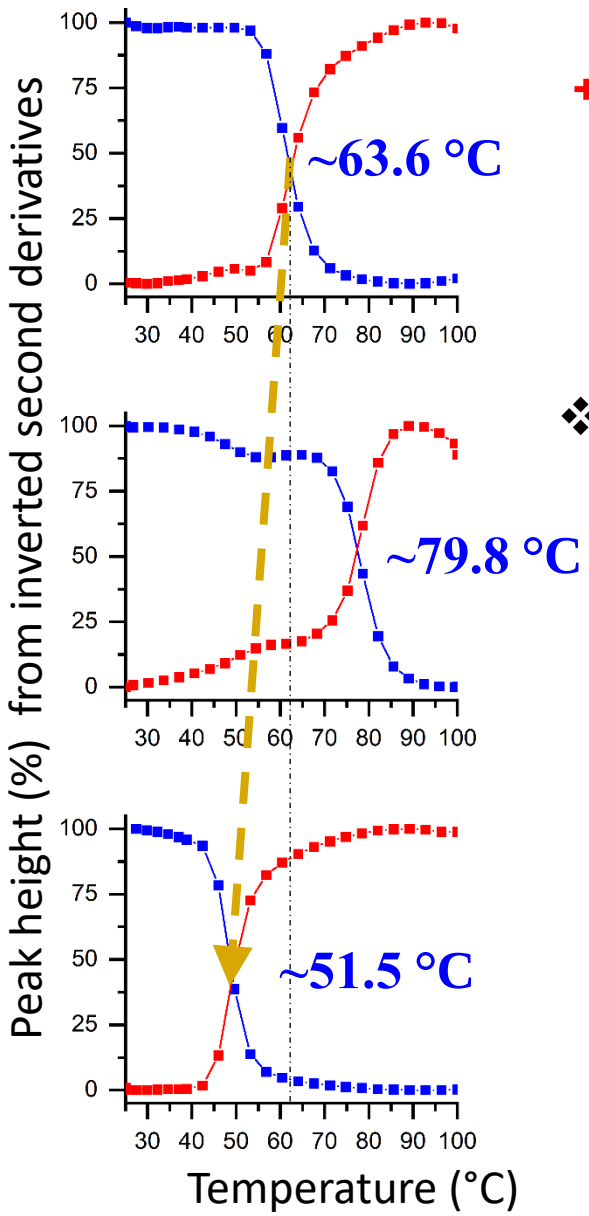

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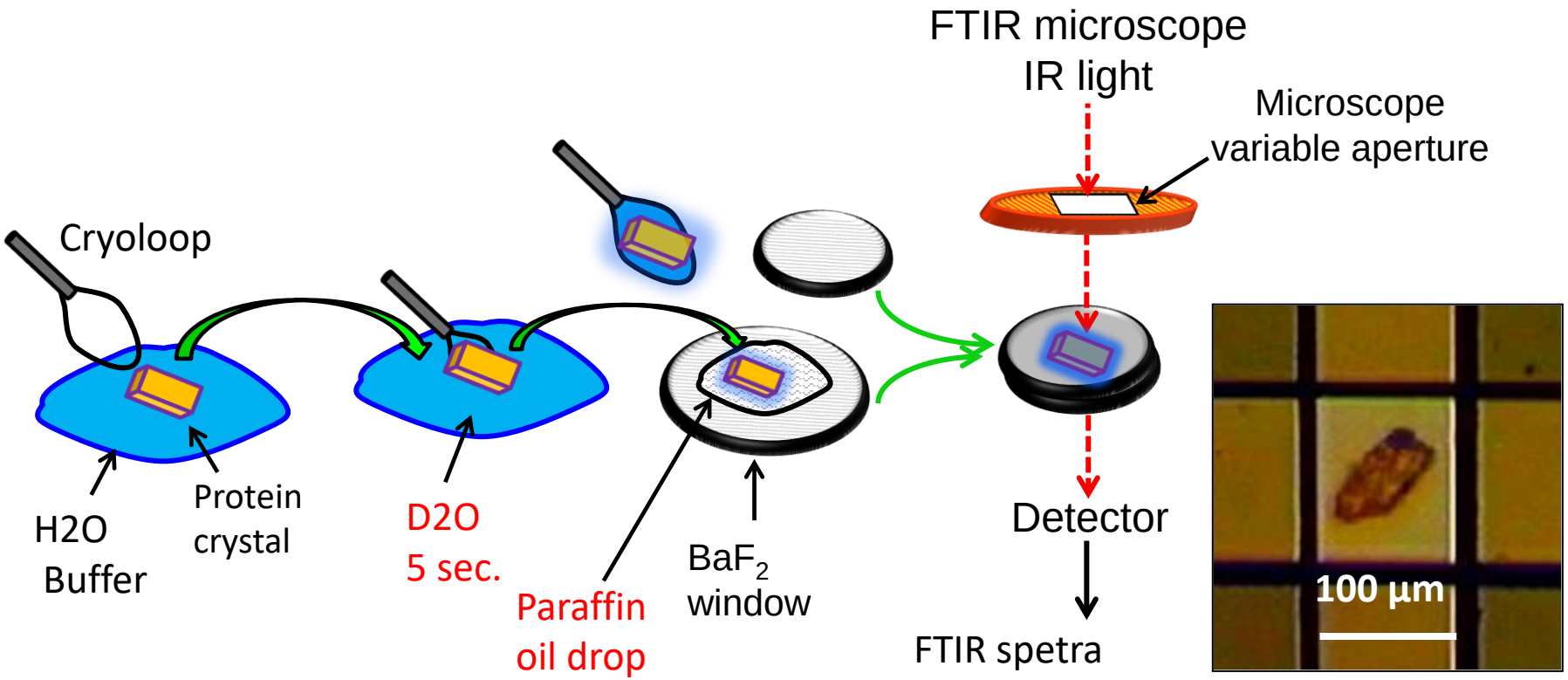
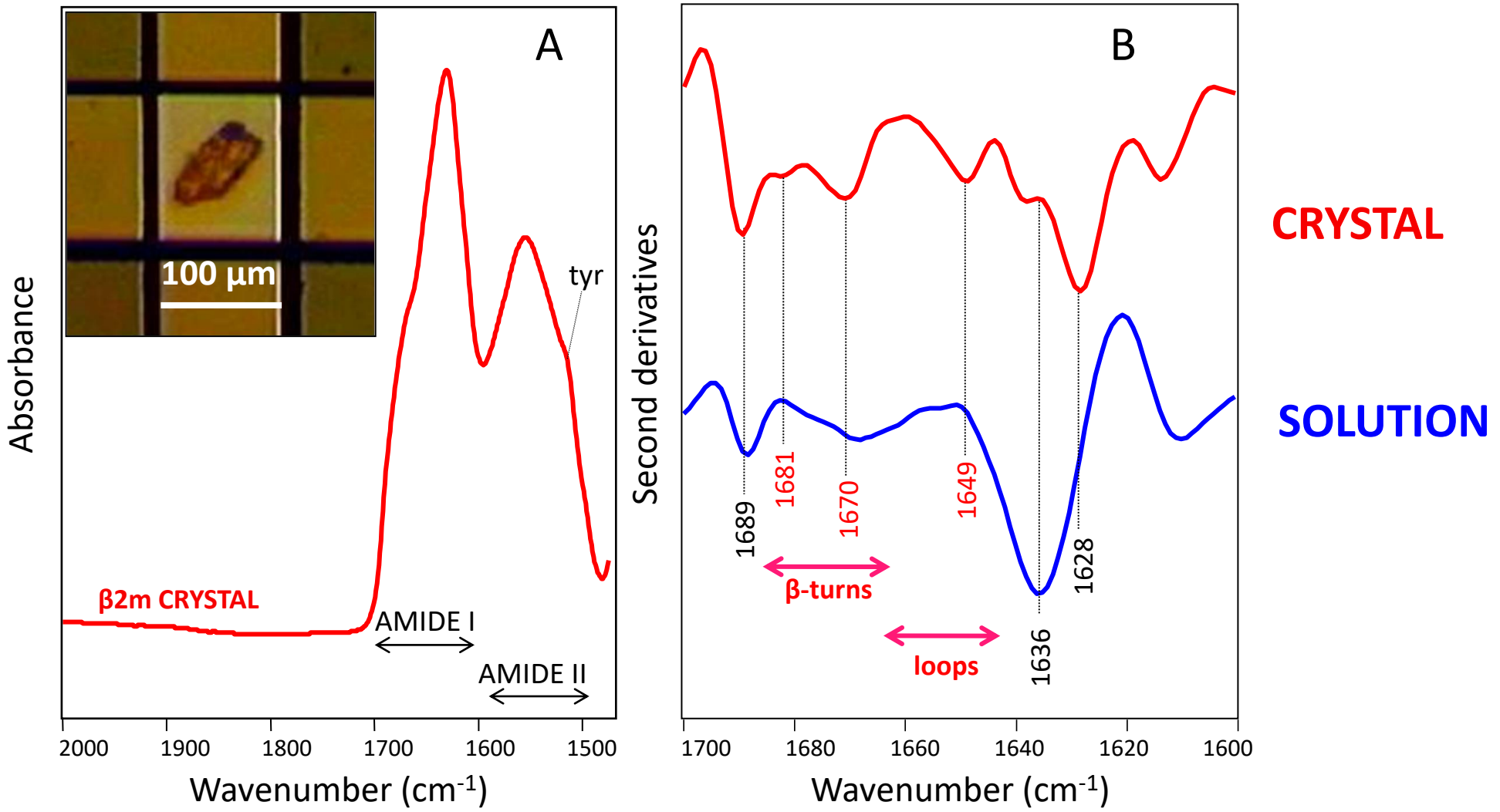
FTIR microspectroscopy of β2m single crystals



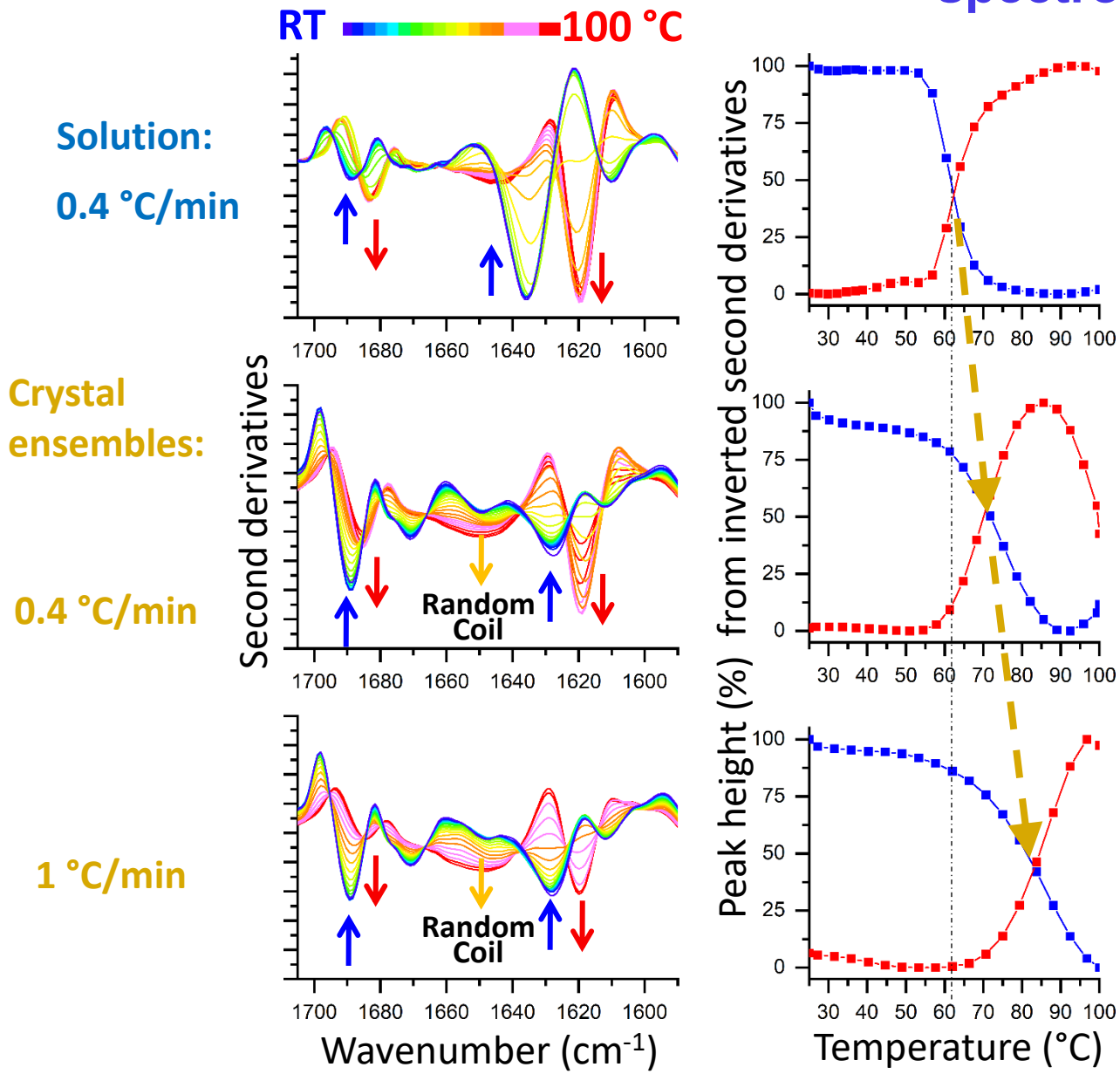
Ami et al. 2012 Biophys J 102:1676-1684

FTIR microspectroscopy of wt β2m single crystals



Ami et al. 2012 Biophys J 102:1676-1684

Thermal stability and aggregation of WT β2m *in solution* and *in crystallo* by FTIR spectroscopy



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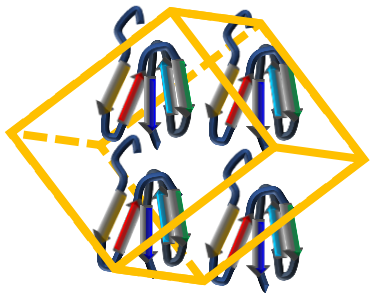

Crystal melting/protein unfolding and aggregation

β-sheet rich aggregates

- **Increased protein thermal stability *in crystallo* compared to *in solution***
- **Heating rate affects T_{mp}**

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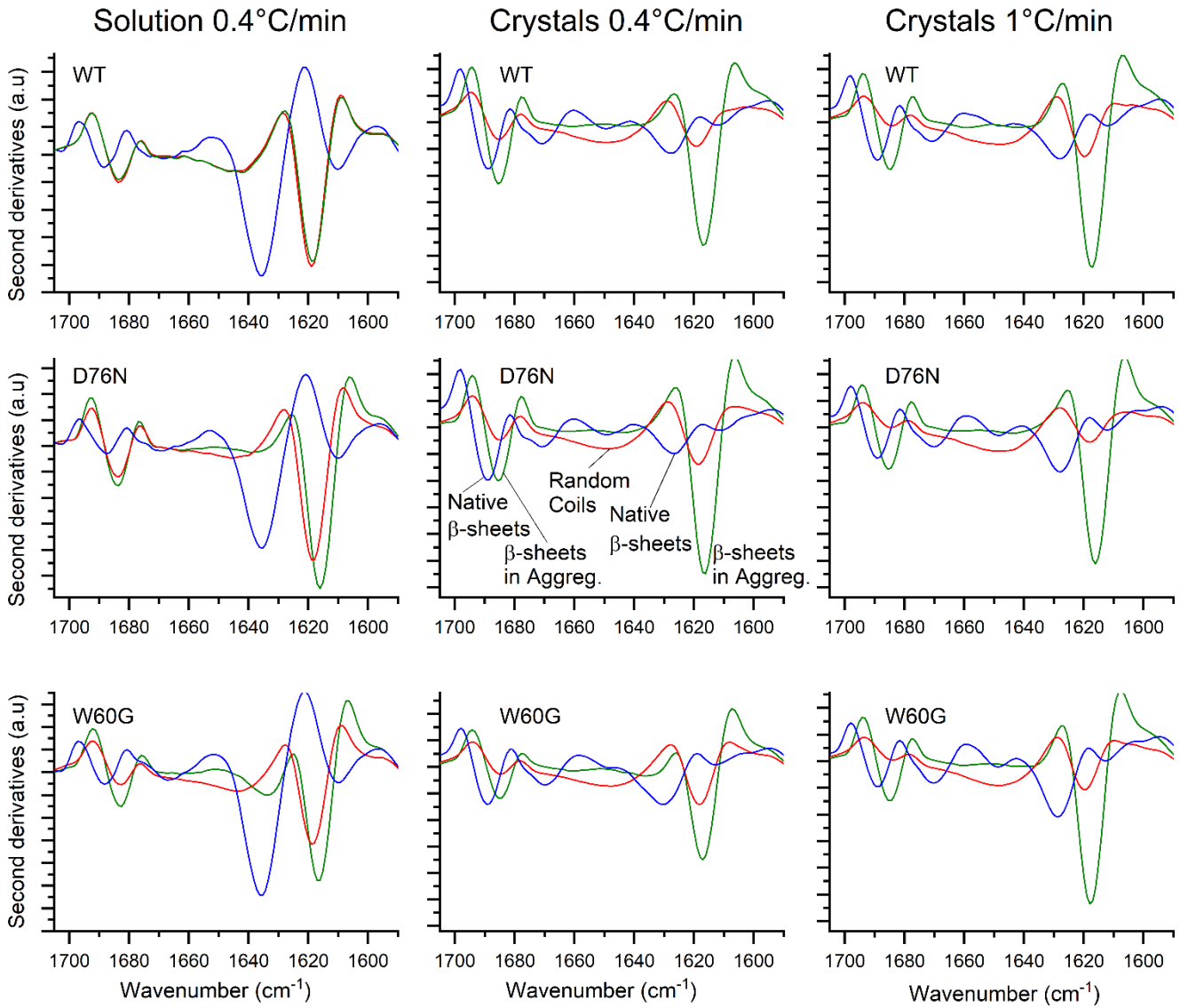
Aggregation of the β2m variants induced by thermal treatment

Room temperature ➔100°C➔ Room temperature



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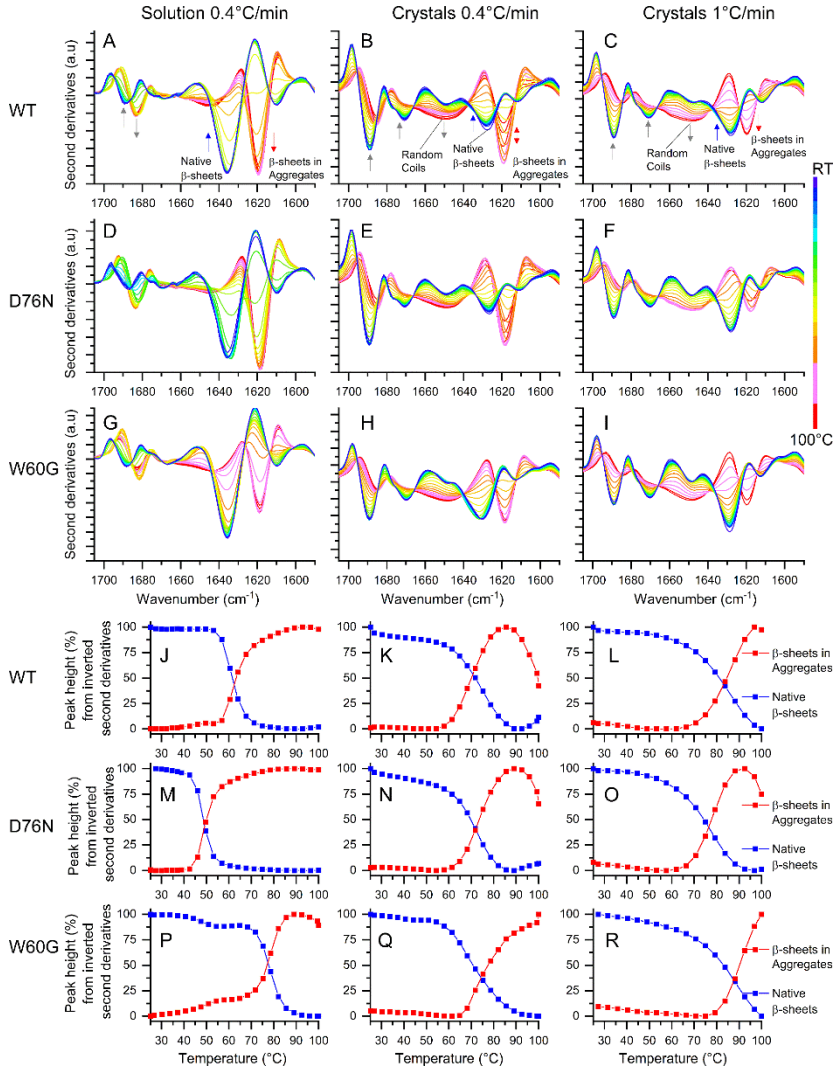
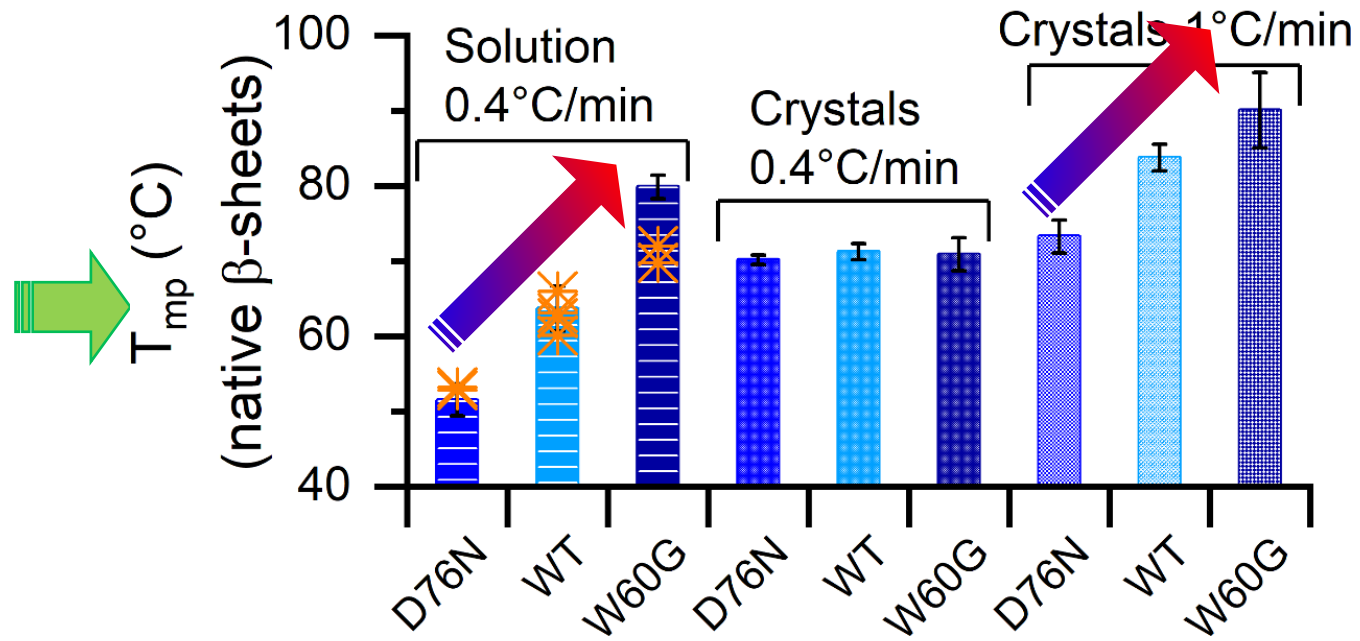
Thermal stability of β2m variants assessed by FTIR spectroscopy





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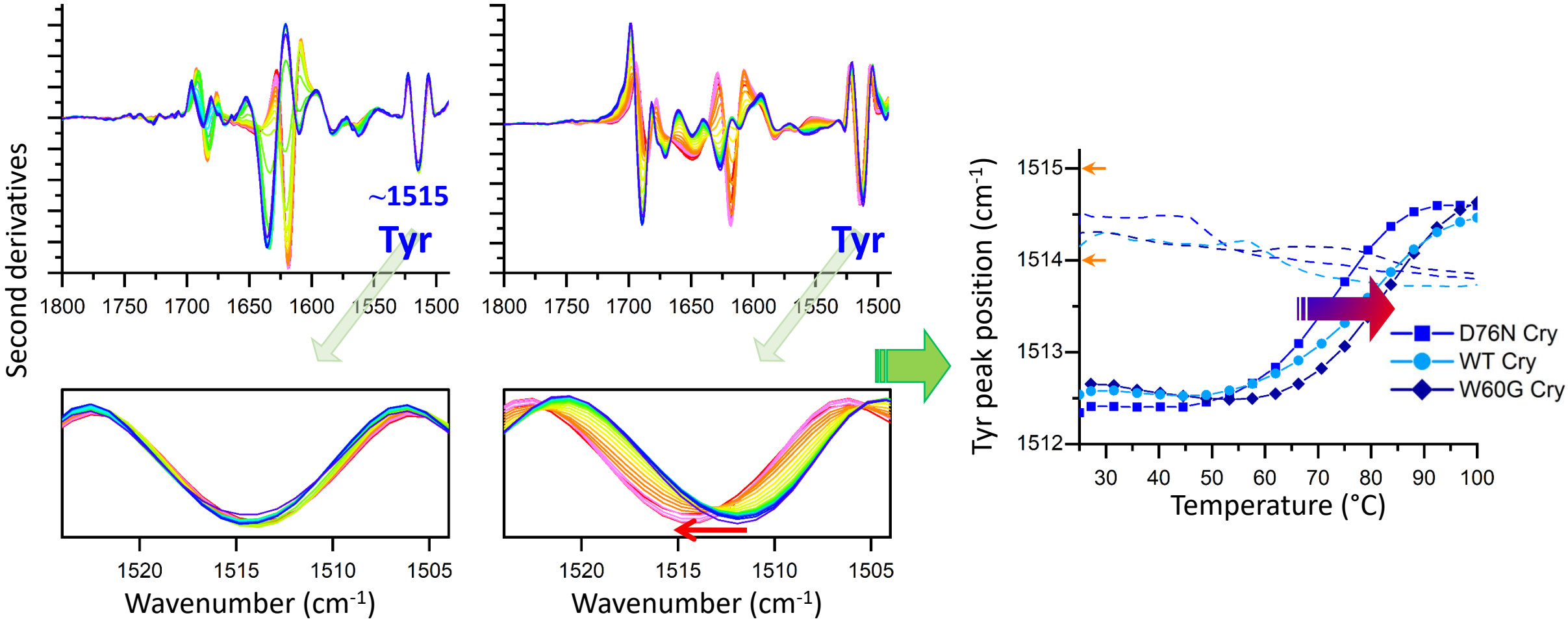
Temperature dependence of the tyrosine peak positions (ring ν(CC) mode) of β2m variants in solution and in the crystalline states



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Conclusions

- Our data indicate that FTIR (micro-)spectroscopy allows
  - to study the secondary and tertiary structures of individual protein crystals and of crystal ensembles
  - to assess crystal stability by monitoring secondary and tertiary structure unfolding and protein aggregation simultaneously.

- FTIR spectra of crystals provide in general more information compared with the spectra measured in solution because of a higher number of well resolved Amide I components.

- The detailed comparison of protein stability using crystalline and solution samples indicates
  - a higher stability of the protein in the crystalline state compared to the solution state
  - the same stability trend for the protein in the crystalline state compared to the solution state.

- FTIR (micro-)spectroscopy, being a non-invasive approach, could be applied to the study of reactions, interactions, and protein conformational rearrangements within crystals, representing a promising tool to obtain structural information on protein crystals complementary to crystallographic analyses.

Acknowledgements

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Thank you !