Protein fibrillation followed by SAXS

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bioSAXS

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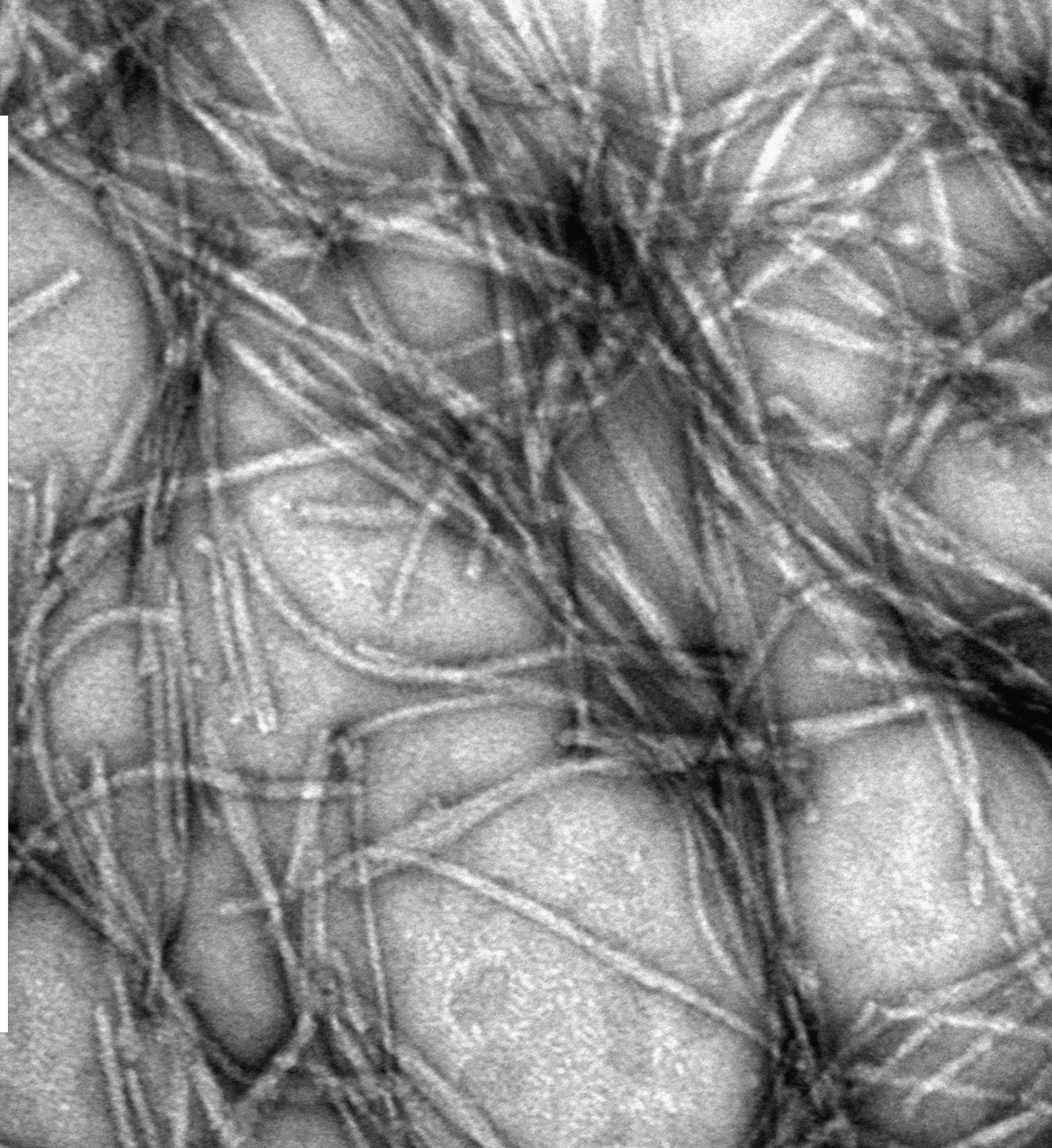
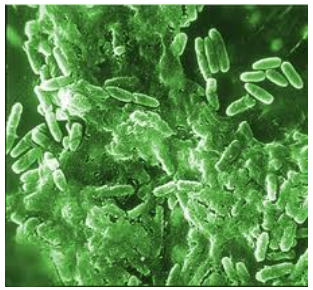
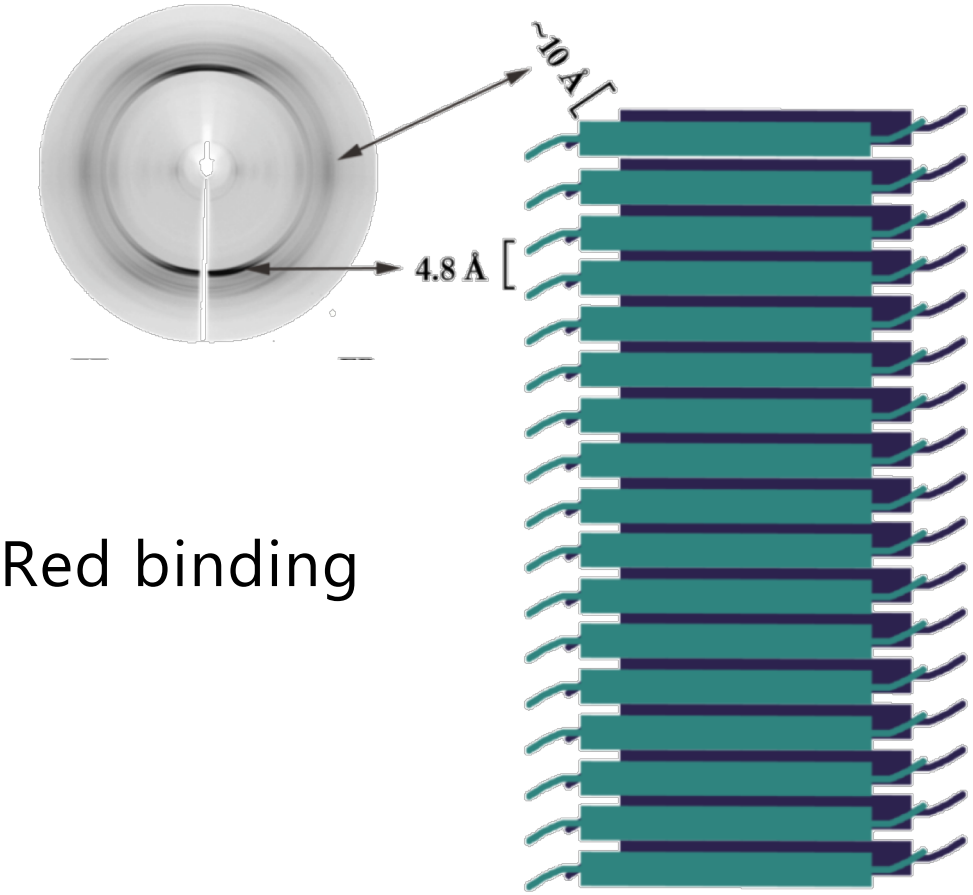


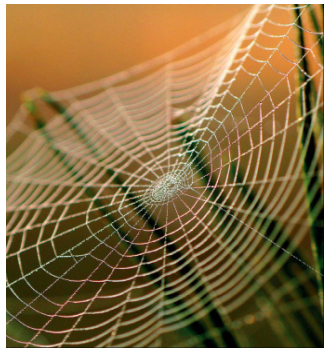
Amyloid fibrils

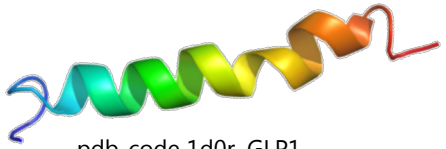
- Disease Related
- Unbranched
- Extracellular
- *In vivo*
- Green birefringance upon Congo Red binding
- Cross-β pattern (fiber diffraction)
- … and lots of amyloid-like fibrils



E. coli Biofilm AJC1/Flickr







pdb-code 1d0r, GLP1

www.alzheimersinfo.info

Parkinson's Disease and α-synuclein

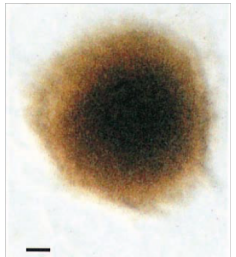
PD

- The 2nd most common neurodegenerative disorder – 10 mio. world wide
- Symptoms include tremor, motor impairment, cognitive impairment
- Idiopathic (most common, >60 years of age)
- Early-onset/familial (before 50 years of age)

Neuropathological characterization

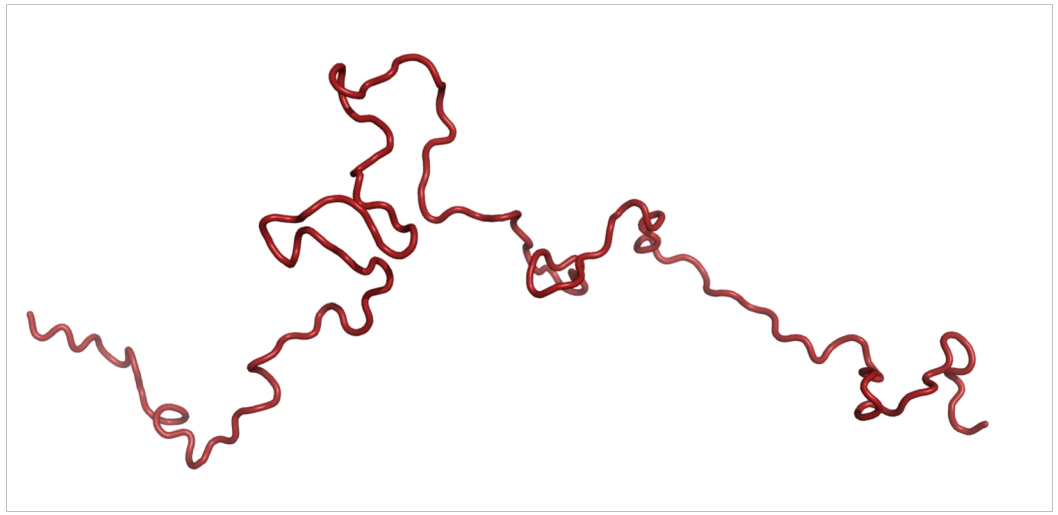
- Degradation of dopaminergic neurons
- Lewy bodies, **amyloid fibrillar α-synuclein**

Spillantini *et al*, Nature, 1997



α-synuclein

- 140 aa, 14.5 kD, intrinsically disordered



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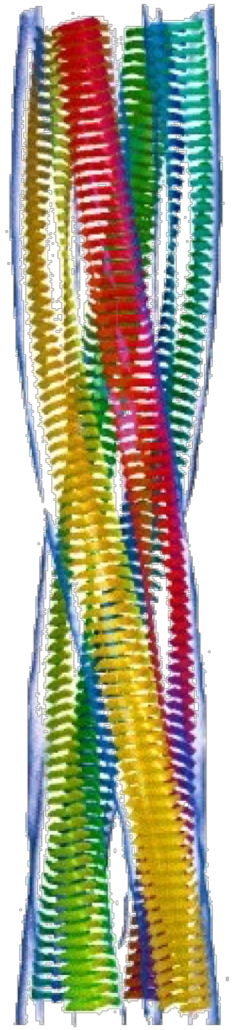
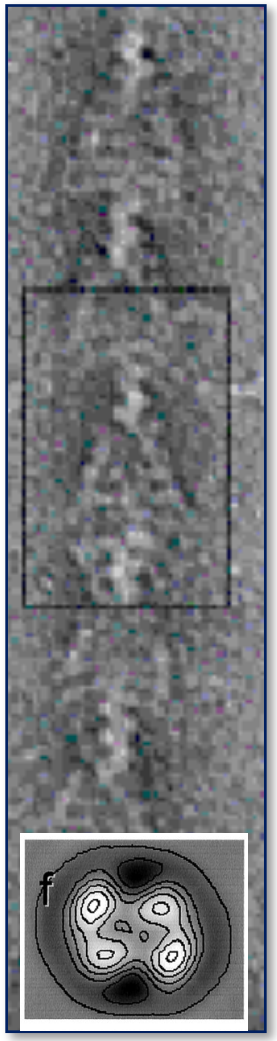
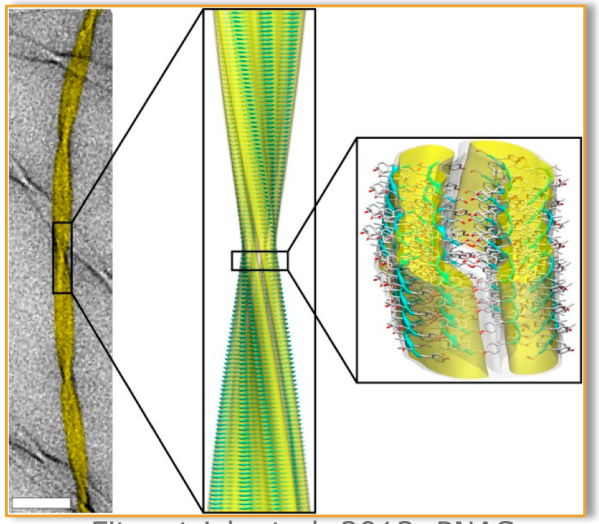
Fibril Structure

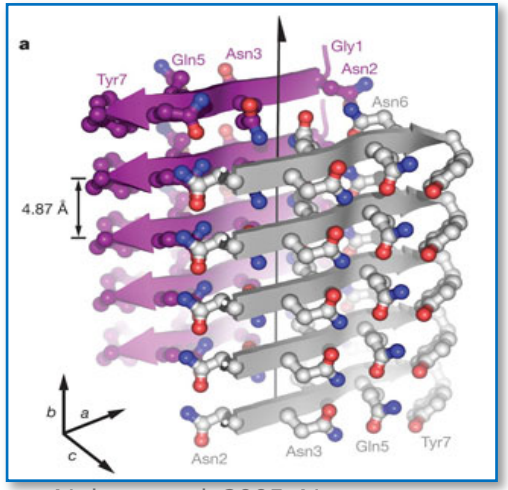




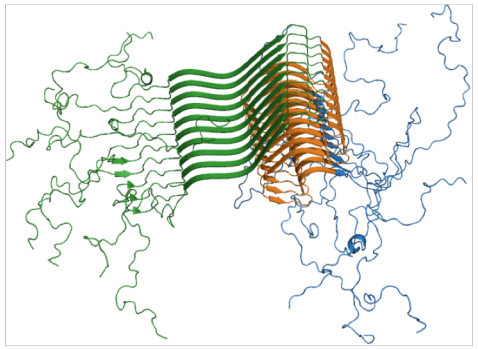
Jimenez et al, 2002, PNAS
Human Insulin



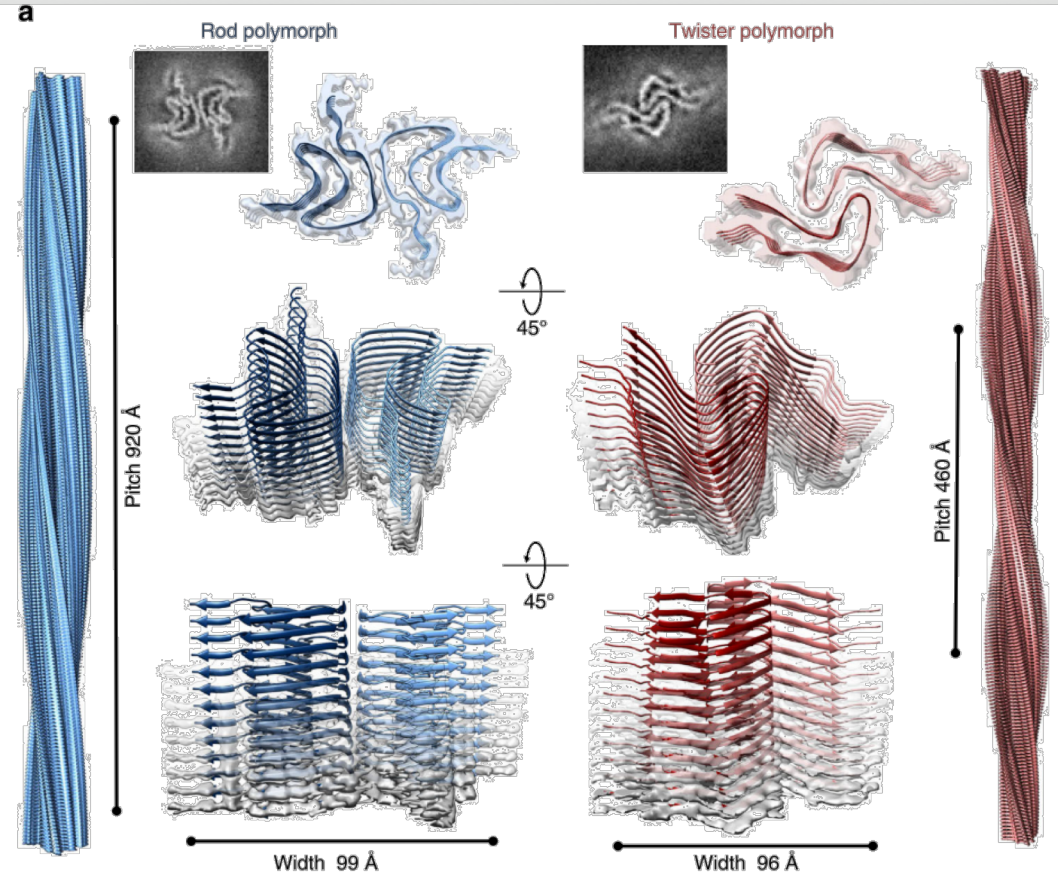
Fitzpatrick et al, 2013, PNAS
TTR 105-115



Nelson et al, 2005, Nature
Peptide/yeast prion protein

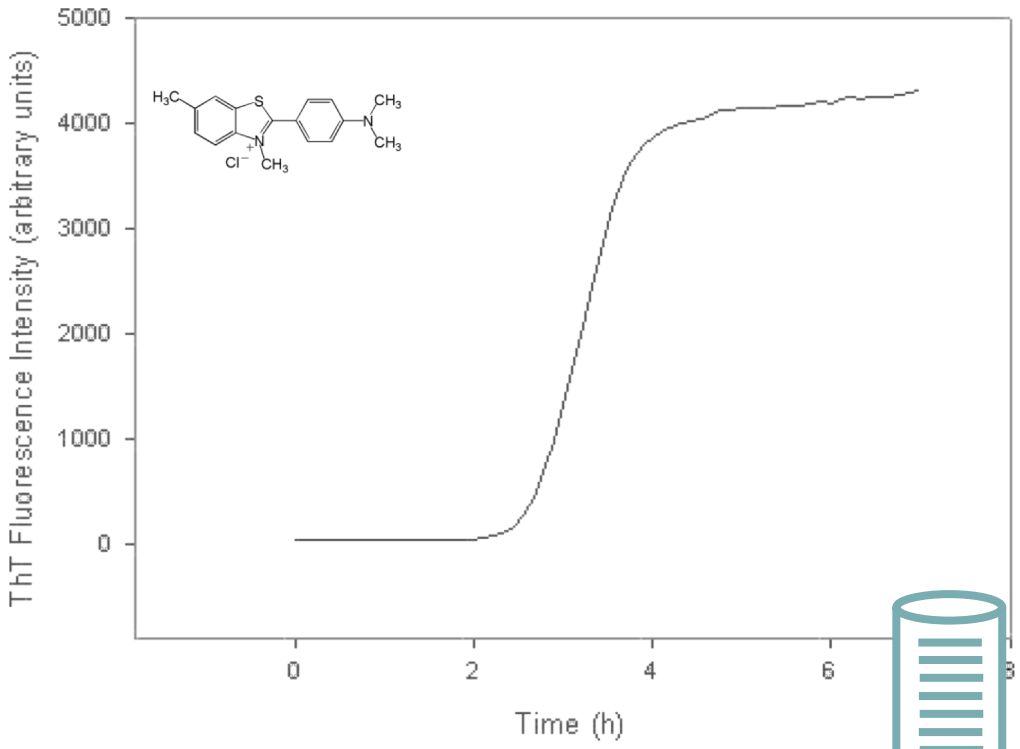


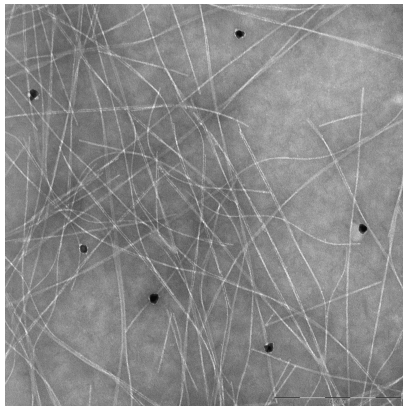
Tuttle *et al*, Nature Struc.Mol.Bio., 2016

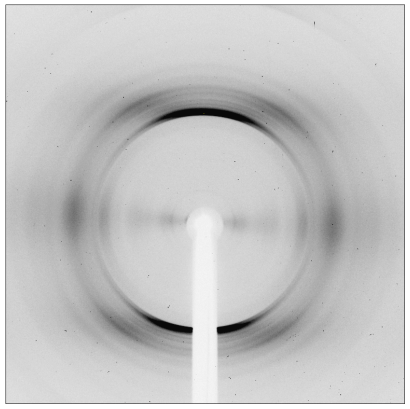


from: Li et al. (2018) *Nature Communications*, **9**, 3609.

An evolving process with multiple states







Native interactions

Partially (un)folded (membrane vicinity)

Native unfolded monomer

Amorphous aggregate

Amorphous aggregate

Fibrillation related off-pathway oligomer

Fibrillation prone conformation

Nucleus On-pathway oligomer

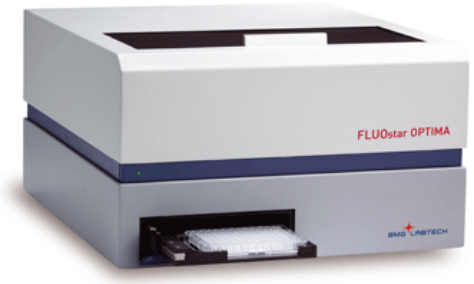
FIBRIL strain 1

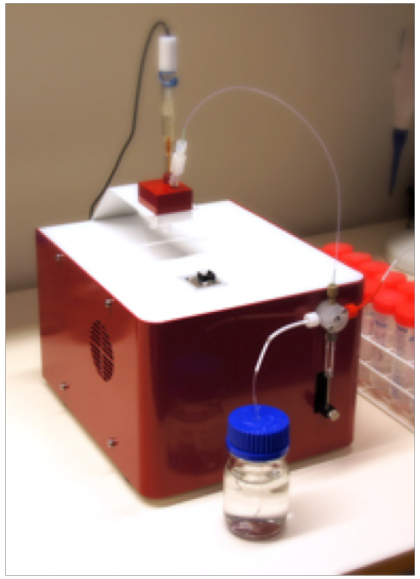
Alternative fibrillation pathways

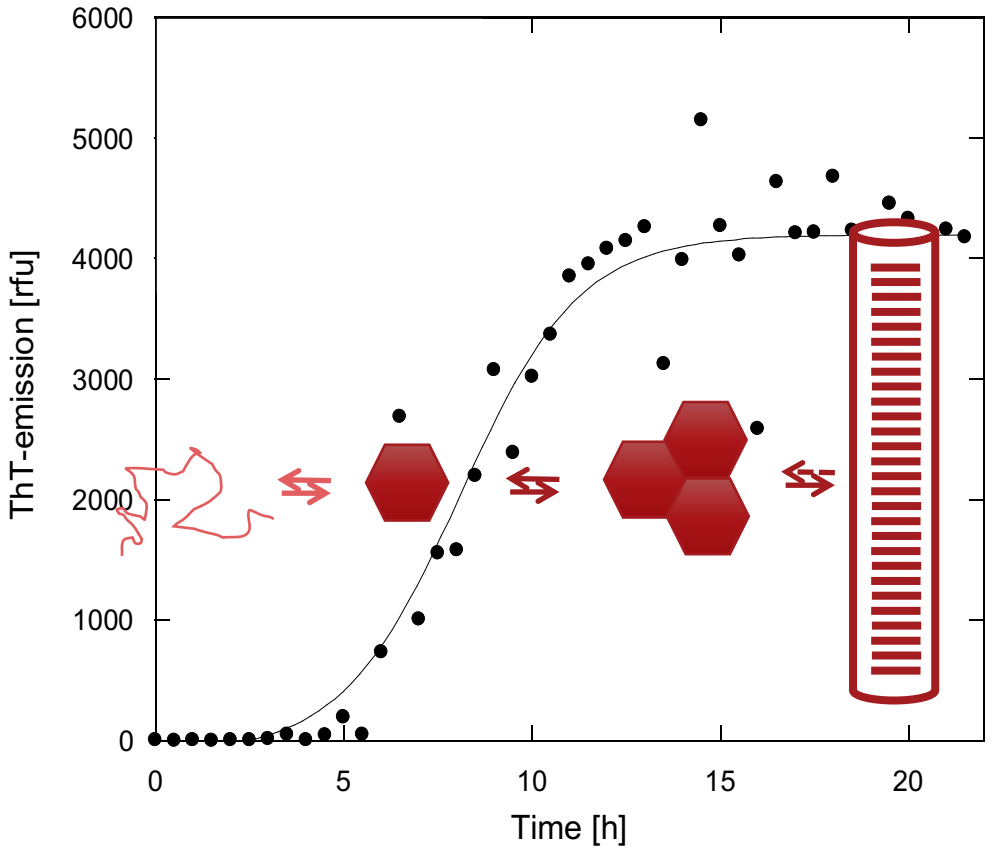
FIBRIL strain 2

bio saxs

'Time resolved' SAXS during fibrillation (α-synuclein)







Vestergaard B, et al(2007) PLoS Biol, 5, e134
Giehm L, Svergun DI, Otzen DE, Vestergaard B (2011) PNAS 108, 3246-3251
Langkilde AE, Herranz-Trillo F, Bernadó P, Vestergaard B. (2018) Meth.Mol. Biol. 1779, 209-239

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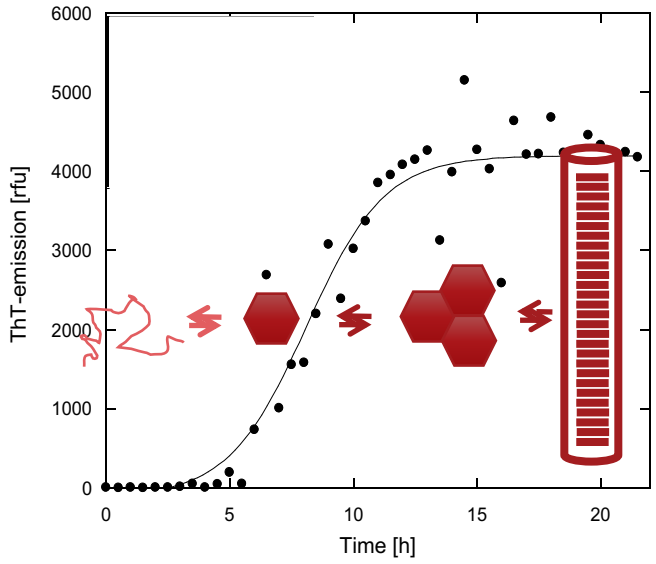
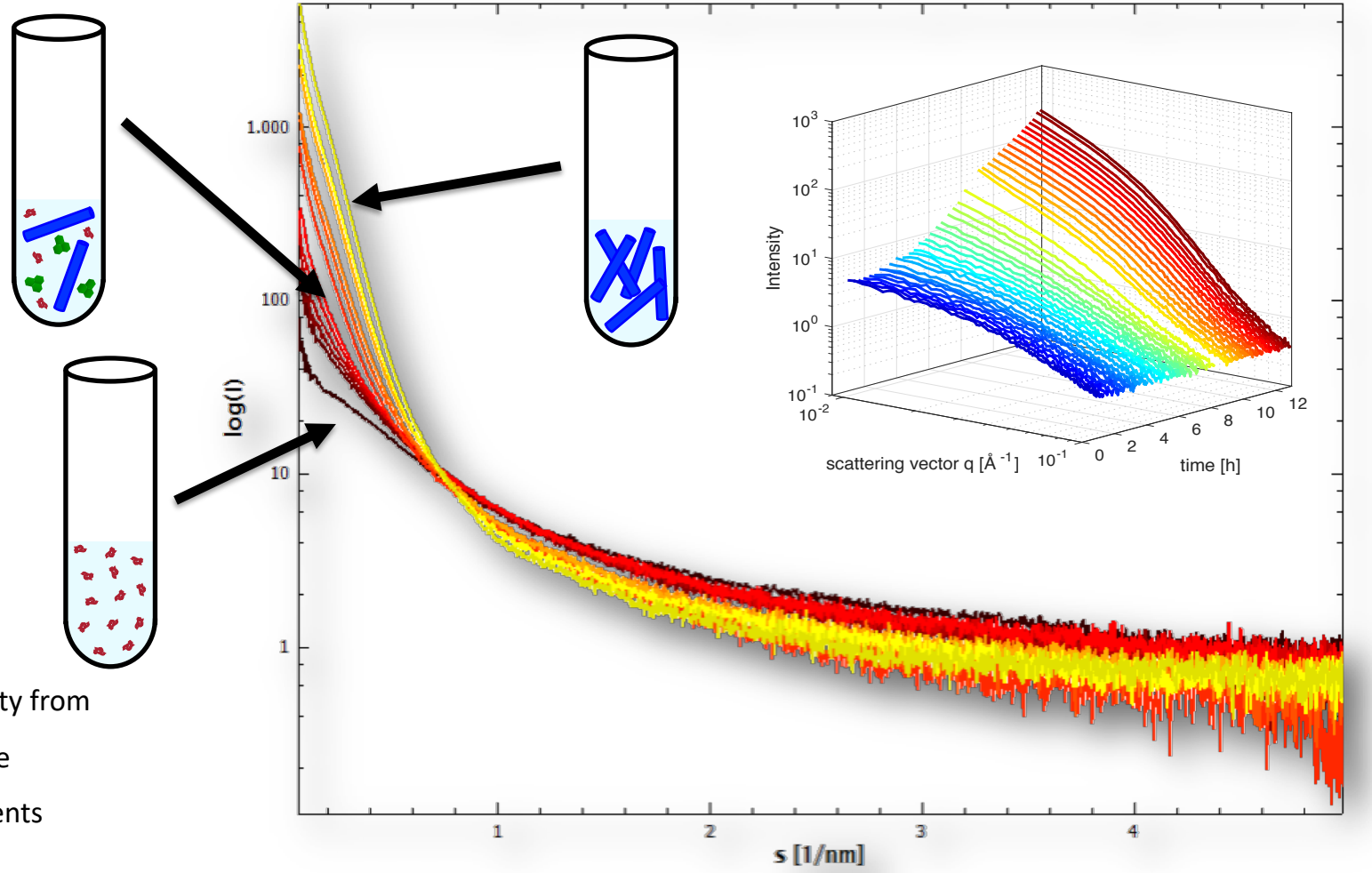
'Time resolved' SAXS during fibrillation (α-synuclein)





$$I(q)=\sum_{k=1}^{K} n_k I_k(q)$$

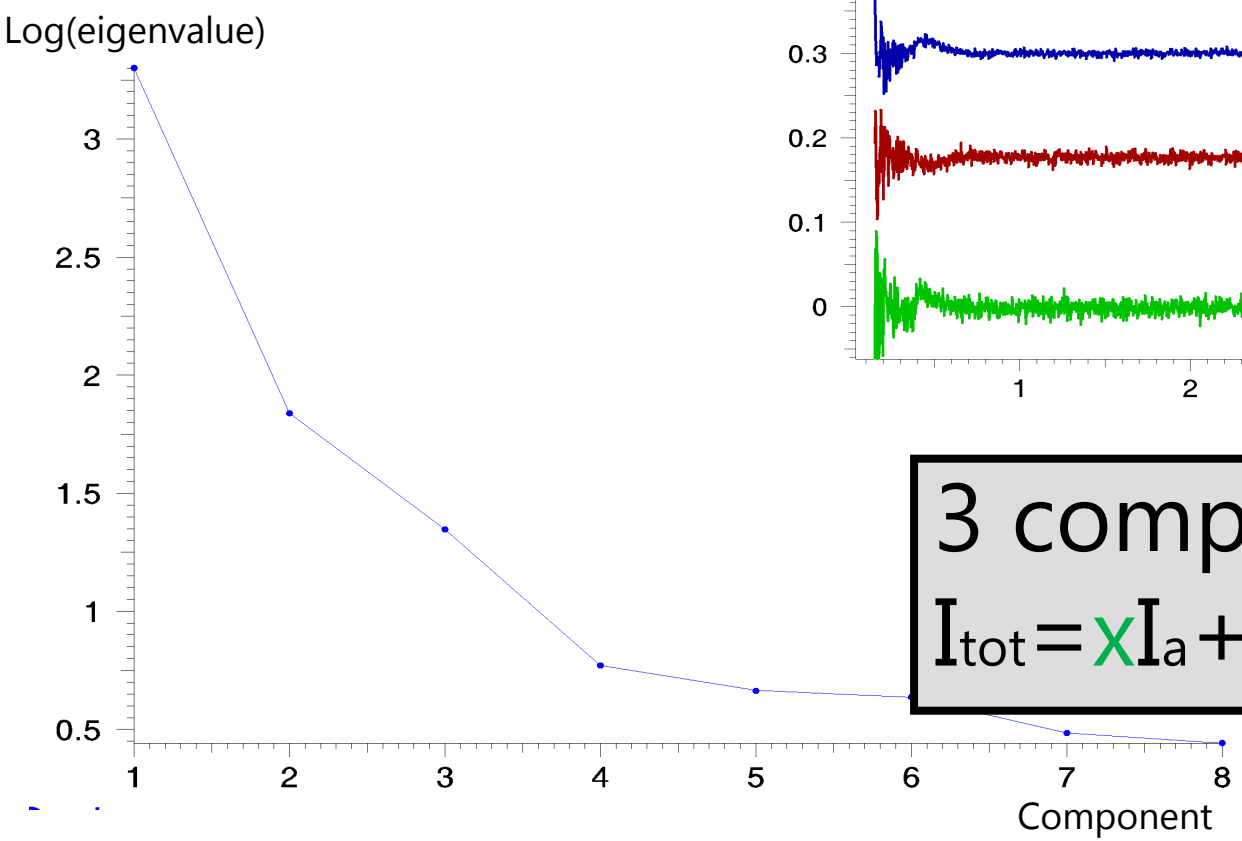
n_k: volume fraction

$I_k(q)$: scattering intensity from the k-th type of particle

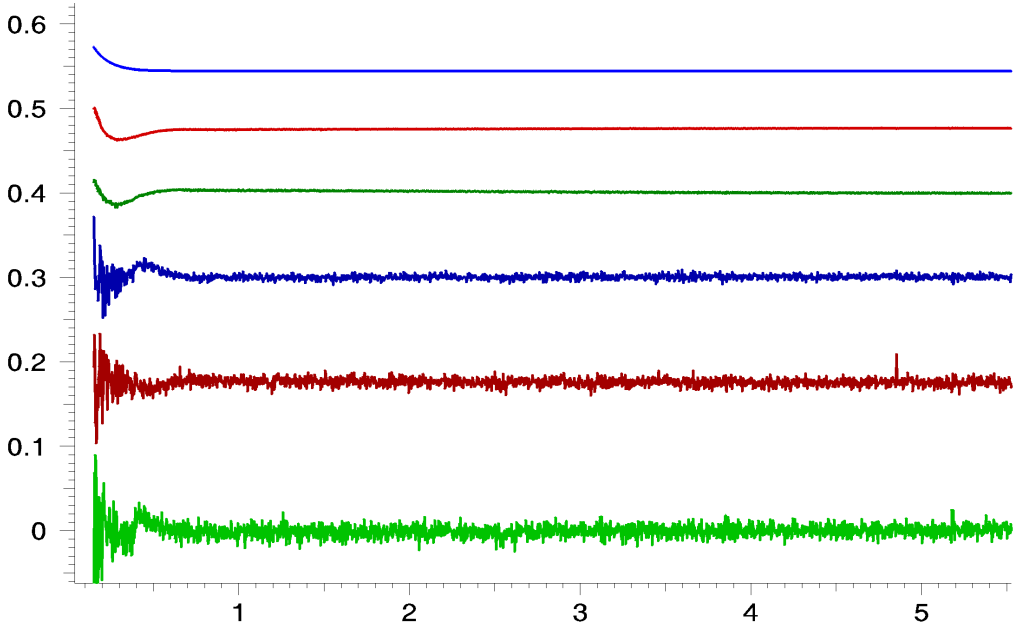
K: number of components

How many species?

Singular Value Decomposition (SVDplot)



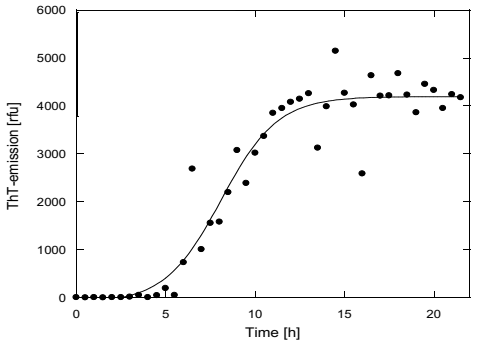
Normalized eigenvectors



3 components:

$I_{tot} = xI_a + yI_b + zI_c$

Isolating the scattering curves - using OLIGOMER



3 components:

$$I_{tot} = xI_{native} + yI_{???} + zI_{fibril}$$

1

$$I_{tot} = xI_{native} + zI_{fibril}$$

Get residuals, as first estimate

$$I_{tot} = xI_{native} + yI_{unknown} + zI_{fibril}$$

Get **x, y** and **z** estimates
Recalculate and refine using residuals

2

$$I_{tot} = xI_{native} + yI_{model} + zI_{fibril}$$

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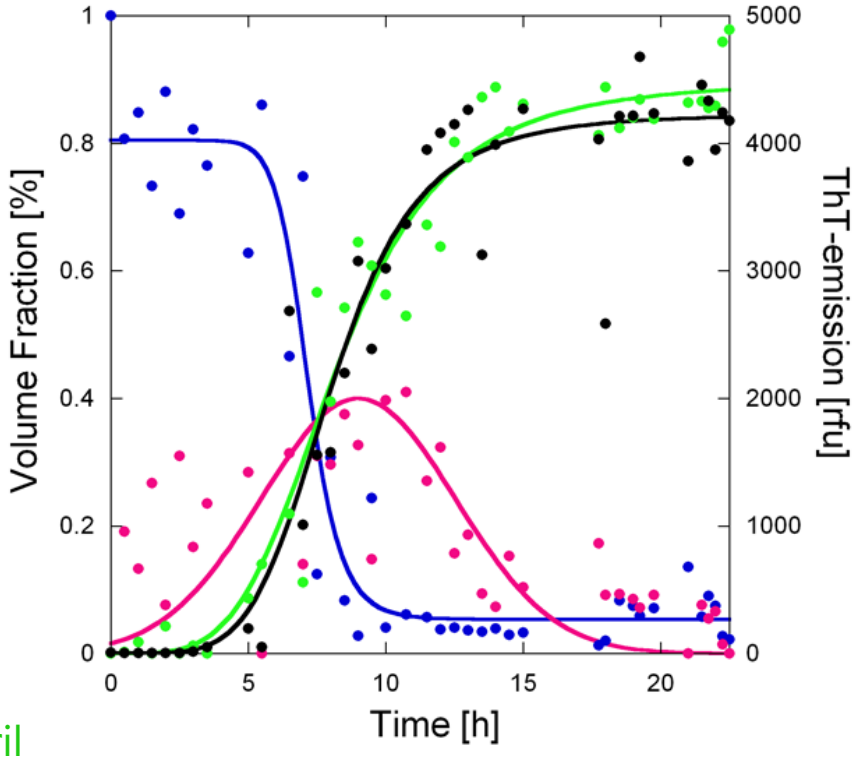
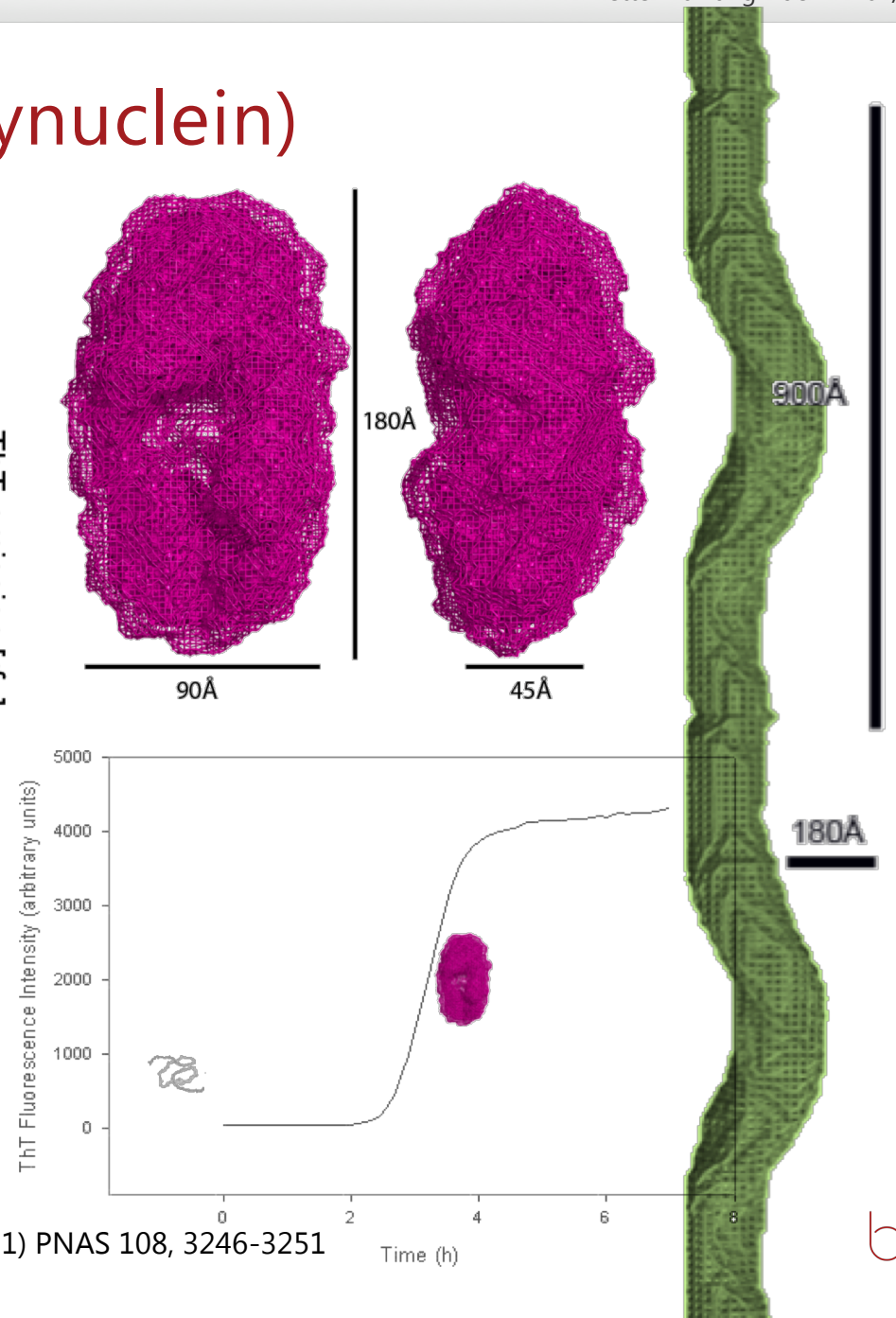
Characterization of species (α-synuclein)

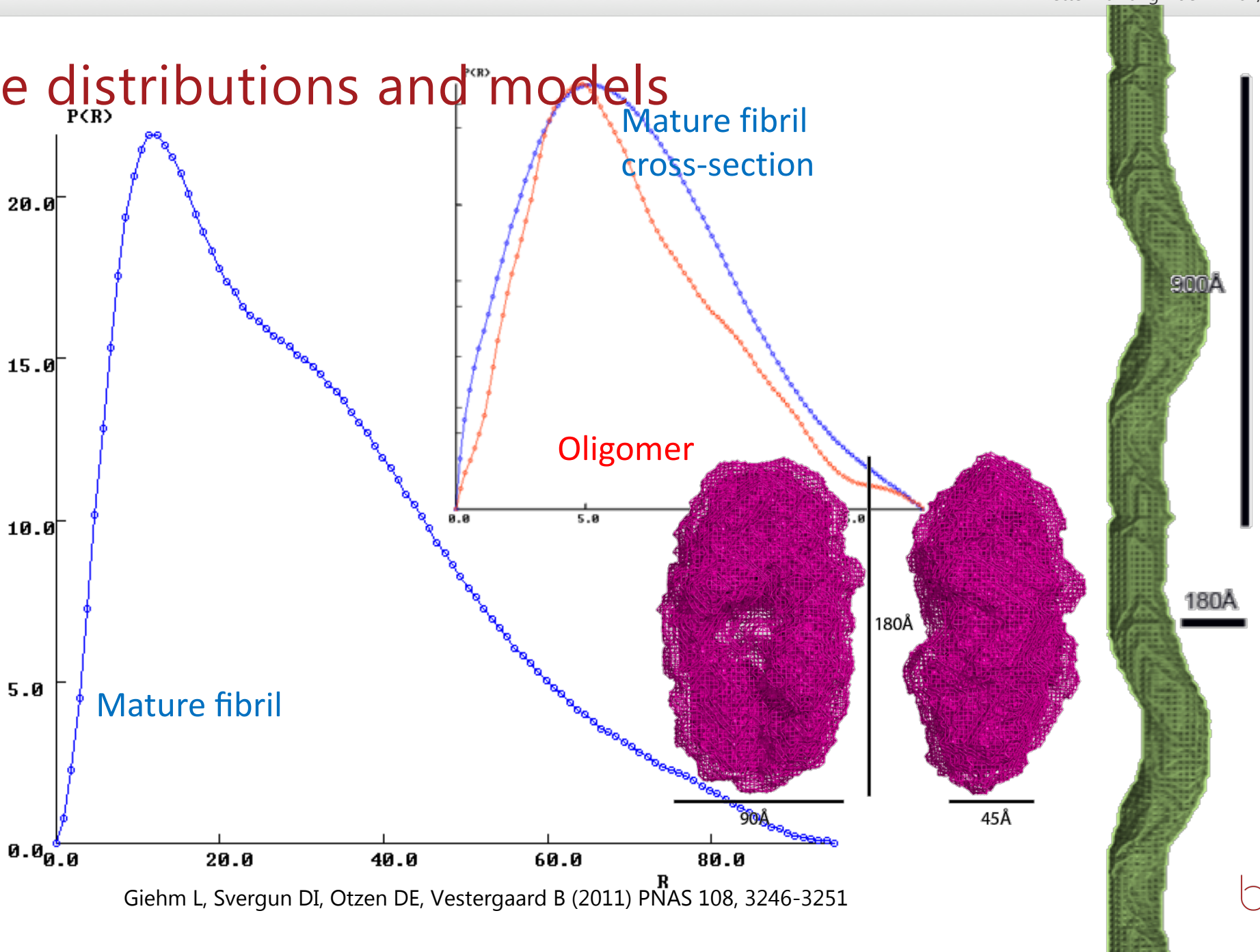


Fibril
Monomer/dimer
Oligomer
ThT



Giehm L, Svergun DI, Otzen DE, Vestergaard B (2011) PNAS 108, 3246-3251

Distance distributions and models



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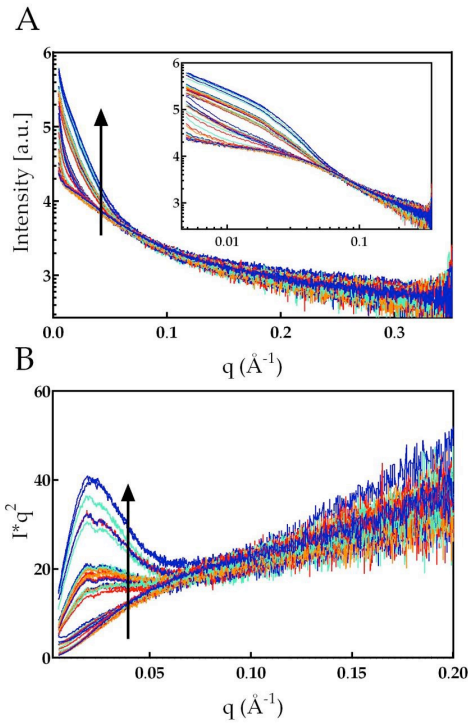
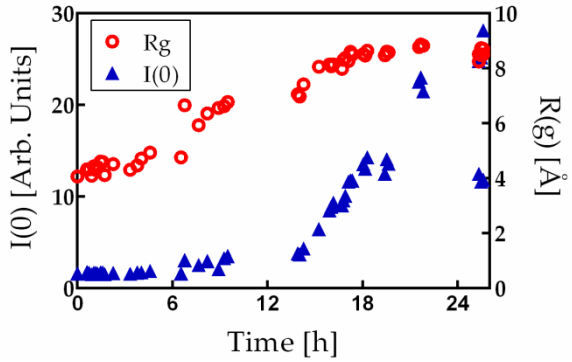
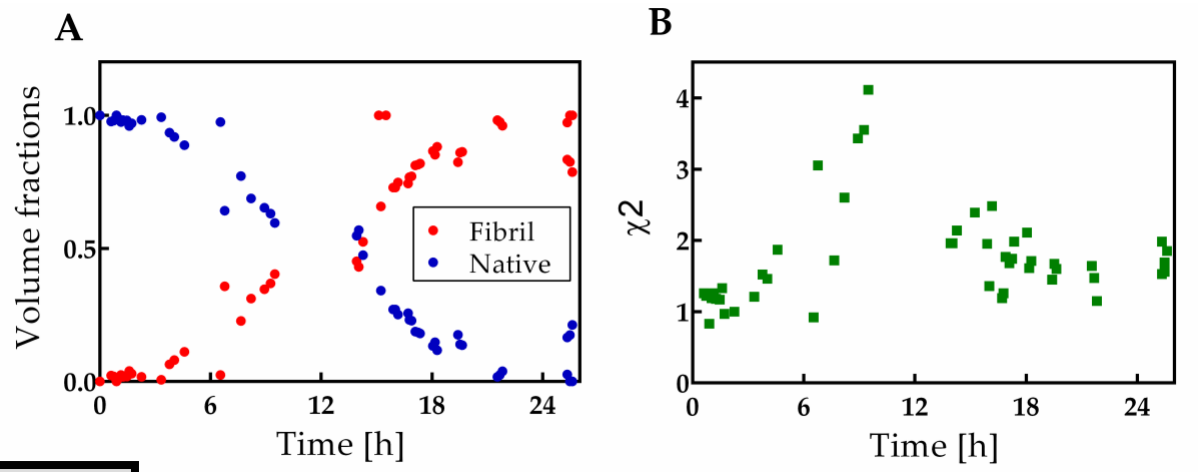
αSN E46K







$$I_{tot} = xI_{native} + zI_{fibril}$$

Get residuals, as first estimate

$$I_{tot} = xI_{native} + yI_{unknown} + zI_{fibril}$$

Get **x**, **y** and **z** estimates
Recalculate and refine using residuals

A. Van Maarschalkerweert, PhD thesis

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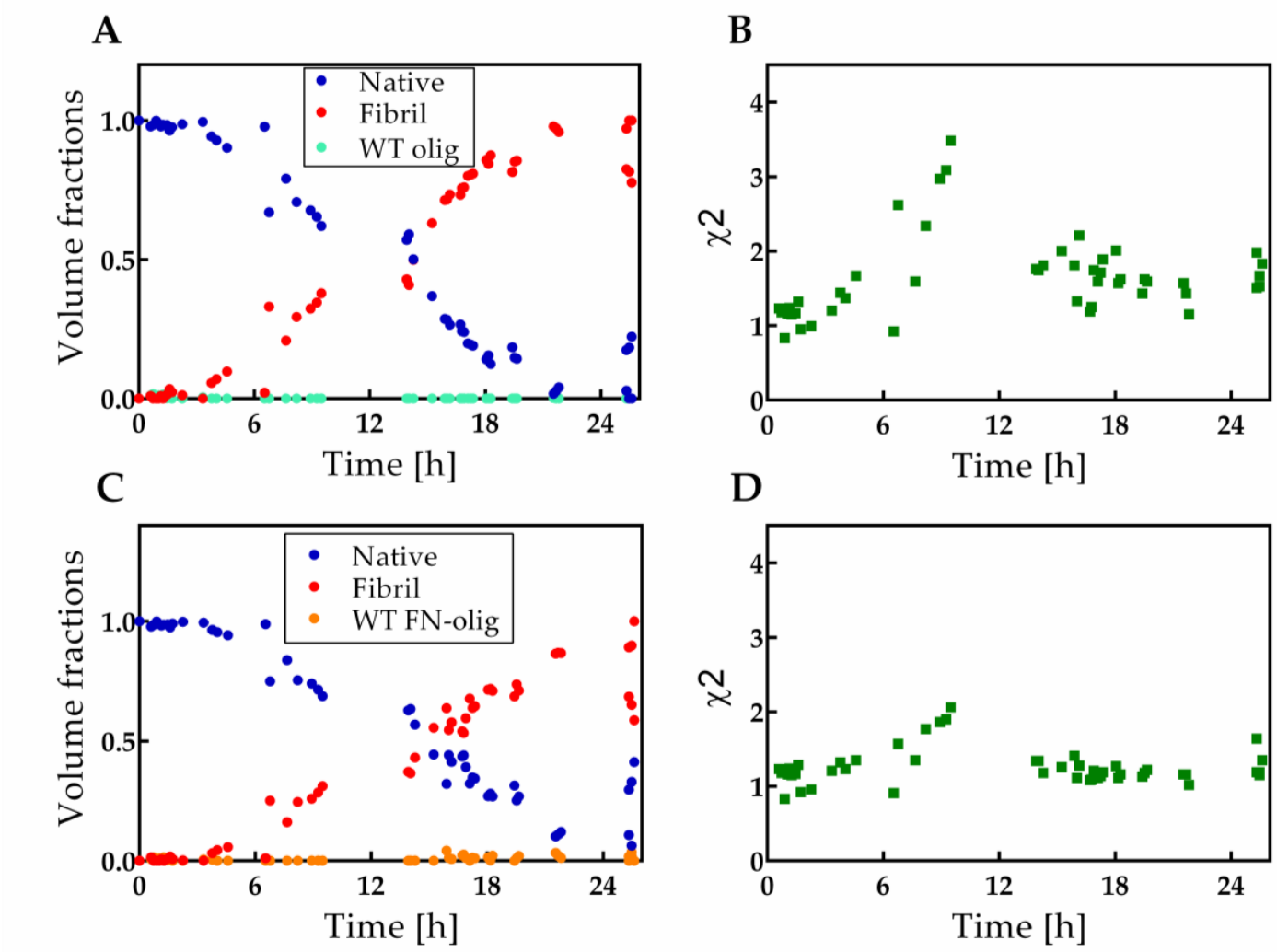
αSN E46K



A. Van Maarschalkerweert, PhD thesis

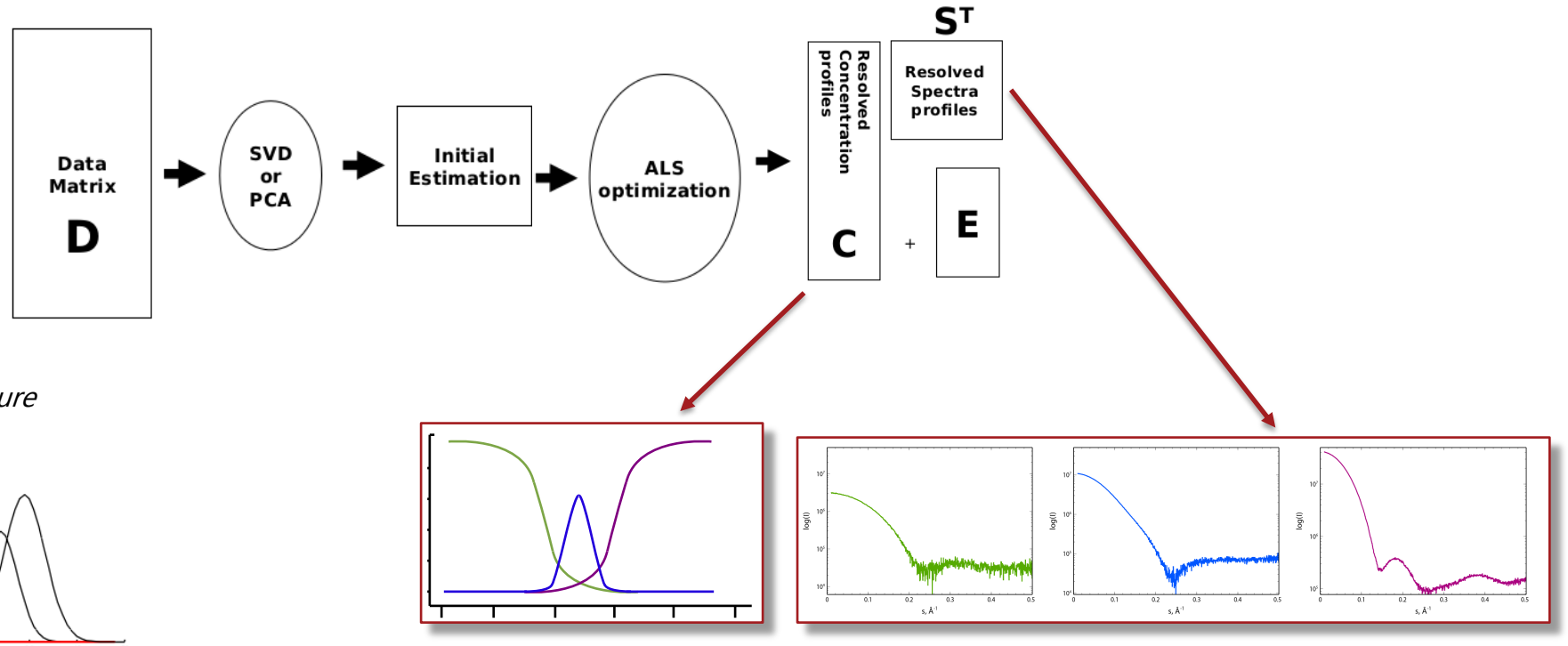
Decomposition using COSMiCS

Herranz-Trillo, F.; Groenning, M.; Maarschalkerweerd, A. van; Tauler, R.; Vestergaard, B.; Bernadó, P. Structural Analysis of Multi-Component Amyloid Systems by Chemometric SAXS Data Decomposition. *Structure* **2017**, *25*, 5–15.

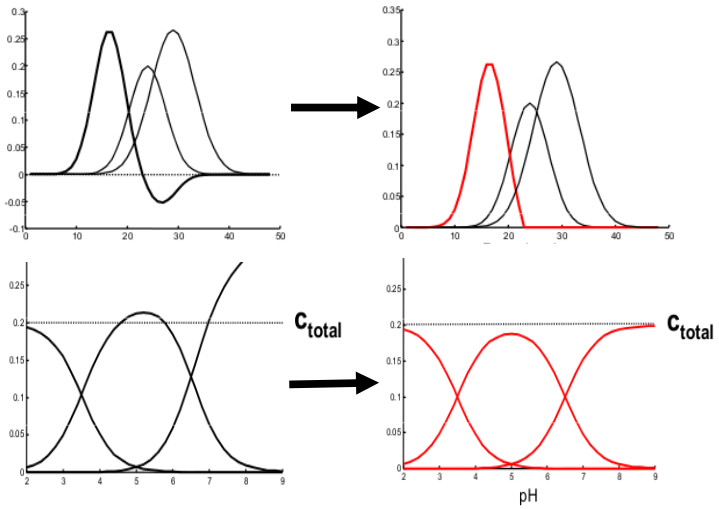
Chemometrics-inspired approach based on Multivariate Curve Resolution Alternating Least Squares (MCR-ALS)

Reduce ambiguities:

Use of multiple data matrices



Constrains: non-negativity and closure



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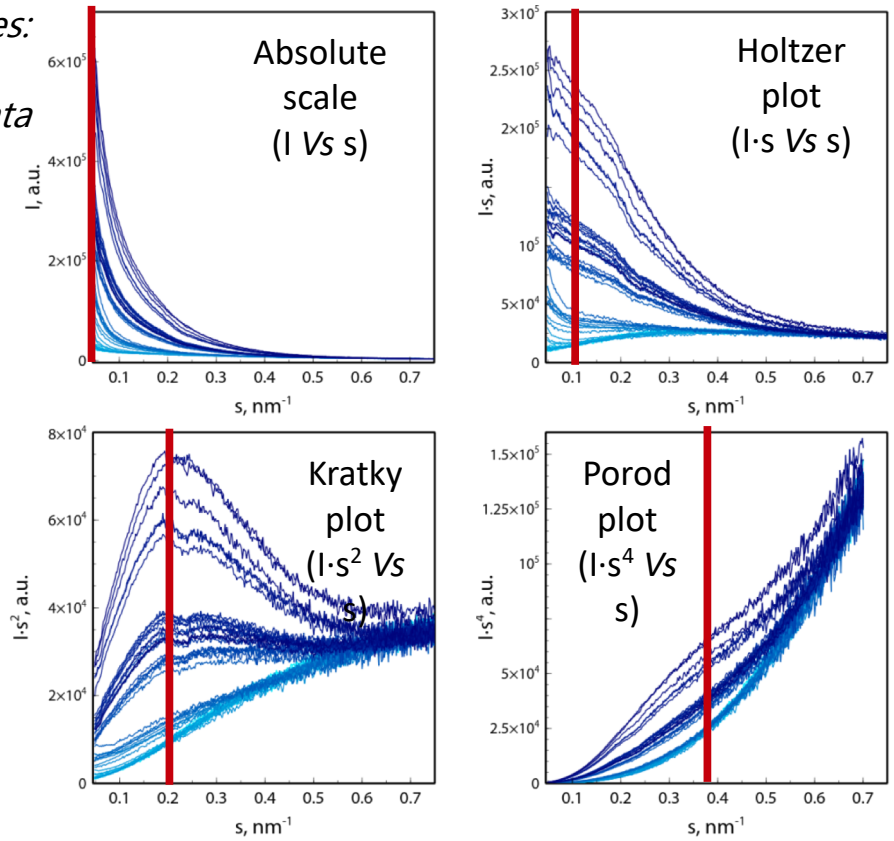
Reduce ambiguities:

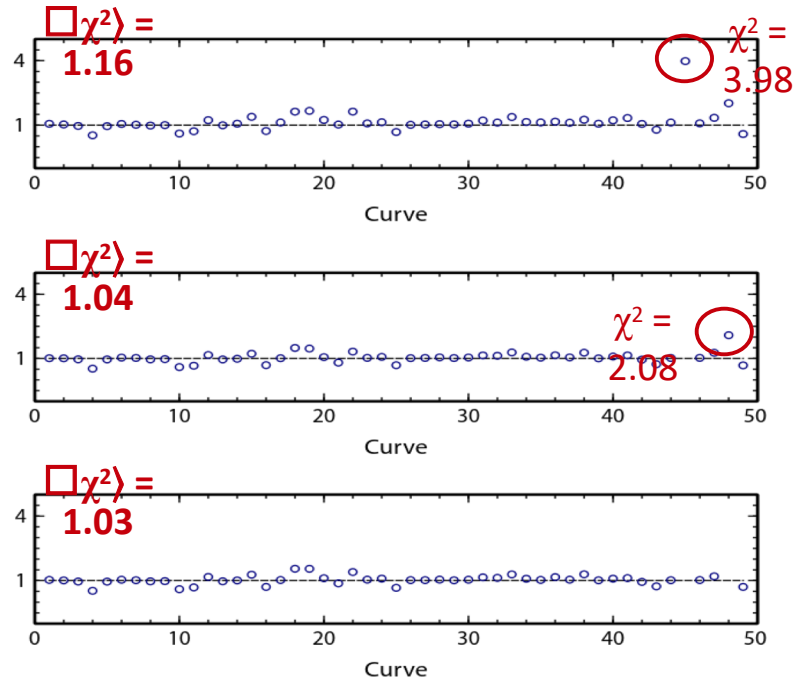
Use of multiple data matrices

Data Matrix

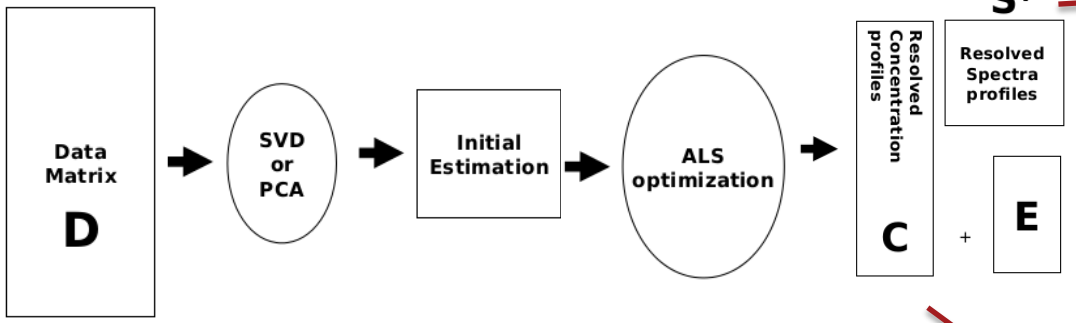
D

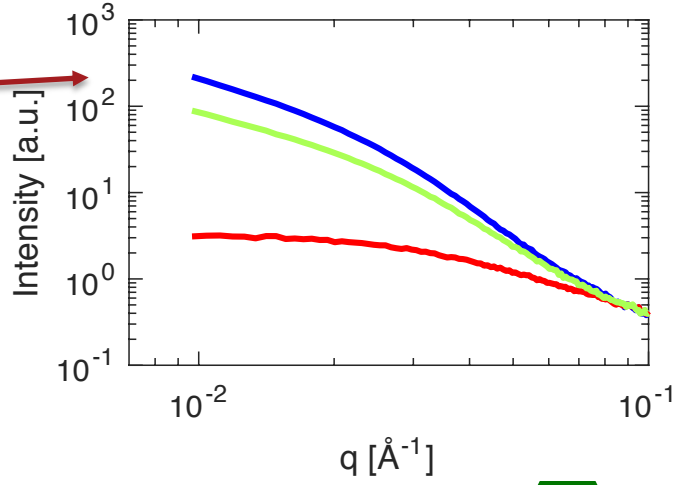


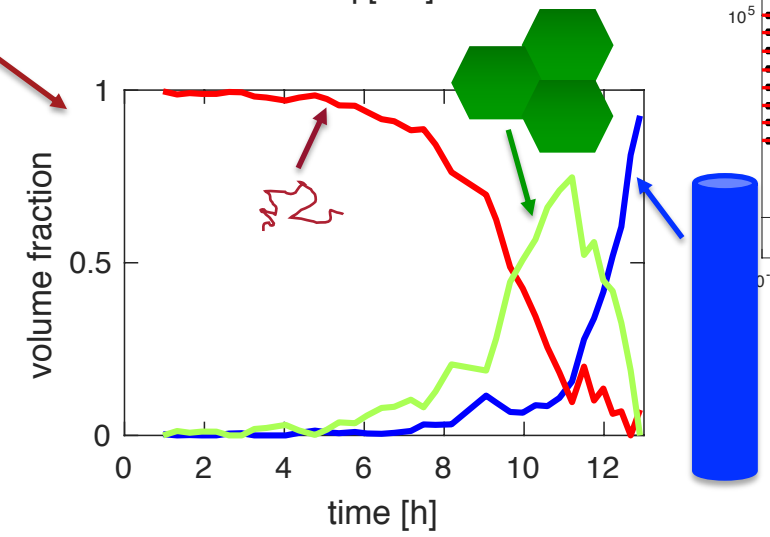
I	I · s	I · s²	I · s⁴	⟨χ²⟩
✗				2.62
✗		✗		1.18
✗	✗			1.22
✗			✗	1.33
✗	✗	✗		1.16
✗	✗		✗	1.24
✗		✗	✗	1.16
✗	✗	✗	✗	1.16

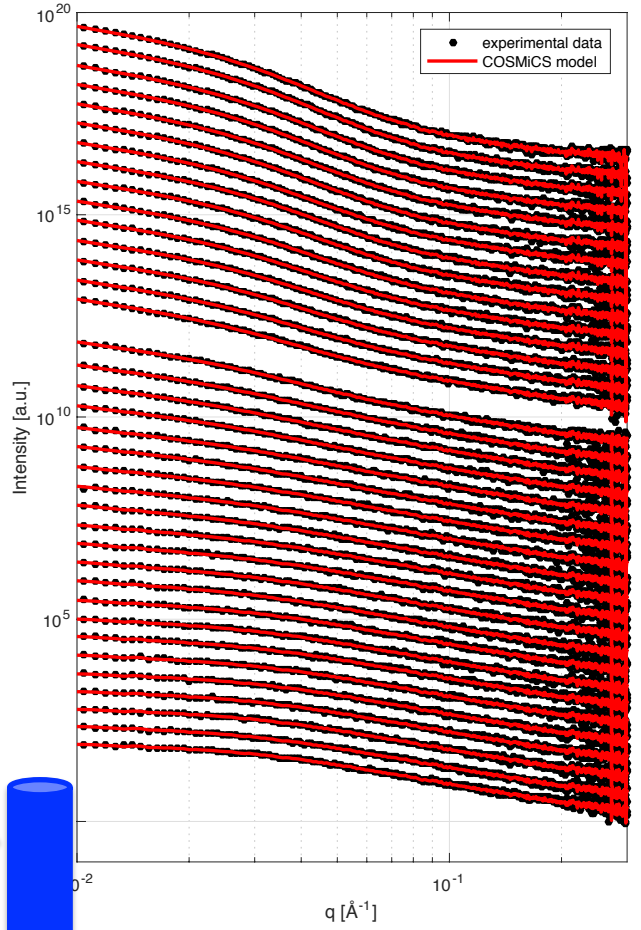


Decomposition using COSMiCS









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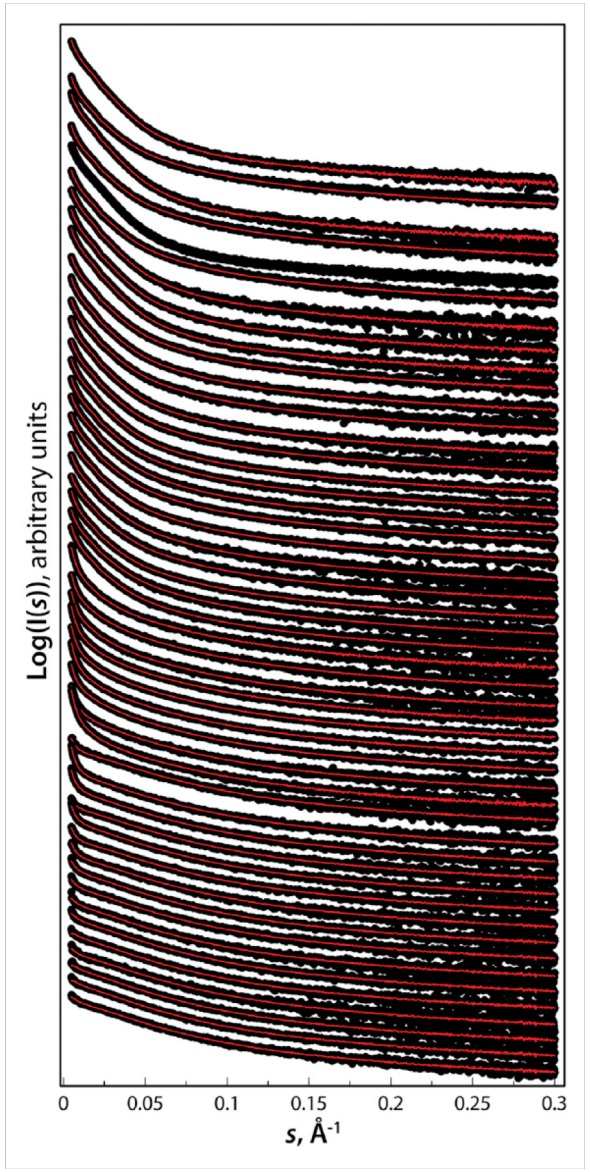
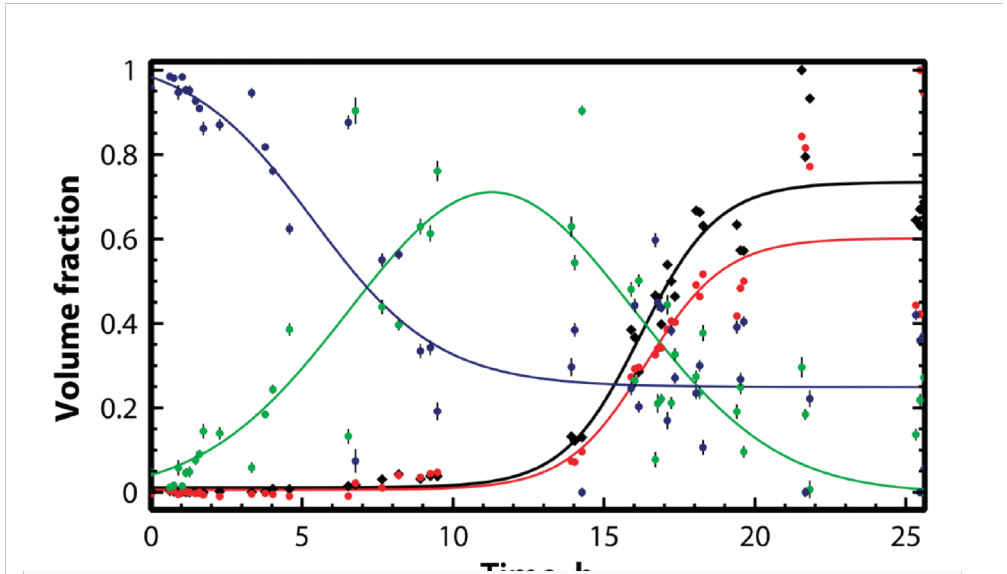
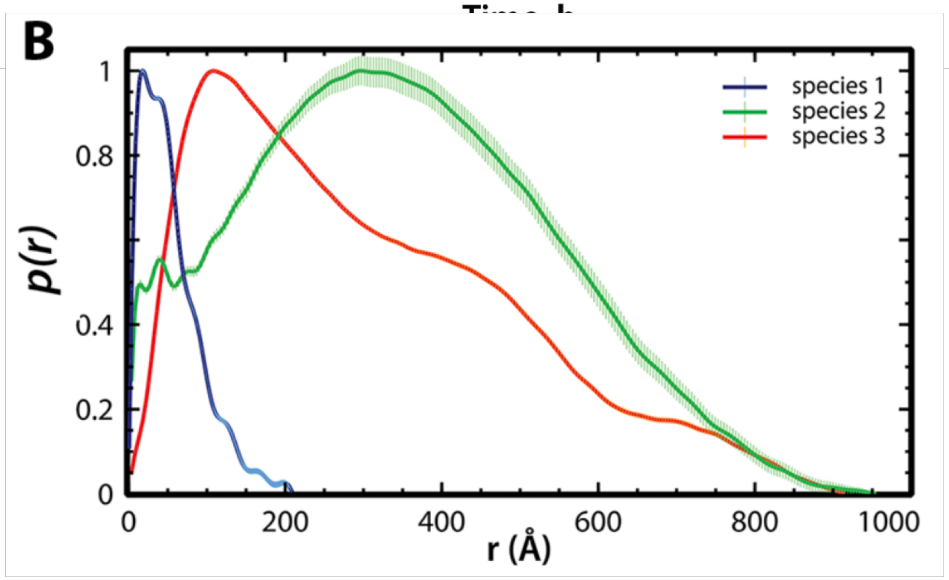
E46K α-synuclein revisited

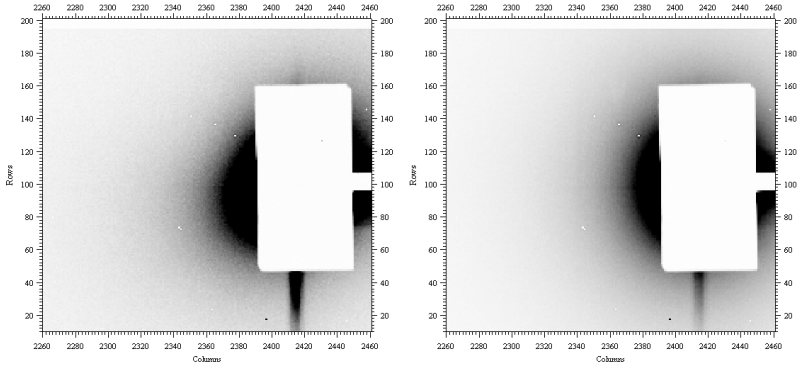






Some practical aspects

- Test your system, find optimal conditions
- Know your system
  - complementary methods, e.g. TEM and FD
  - Consider beamline stability, time frames, additional equipment etc



- Check 2D images
- Check buffers, basic parameters
- …and double check!
- Test different inputs, parameters, number of species
- Decompose using different methods

Langkilde AE, Herranz-Trillo F, Bernadó P, Vestergaard B. (2018) Meth.Mol. Biol. 1779, 209-239

Planning your beamtime

- Samples
- Stability
- Preparation (upconc, dilute, sec…)
- Temperature
- Radiation sensitivity
- Mixing? Incubation time?
- Prioritize. Need to have or nice to have
- Go enough people

- Sample volume
- Turnover time
- Lab access for sample prep
- Special equipment
- Injections? Top-up?

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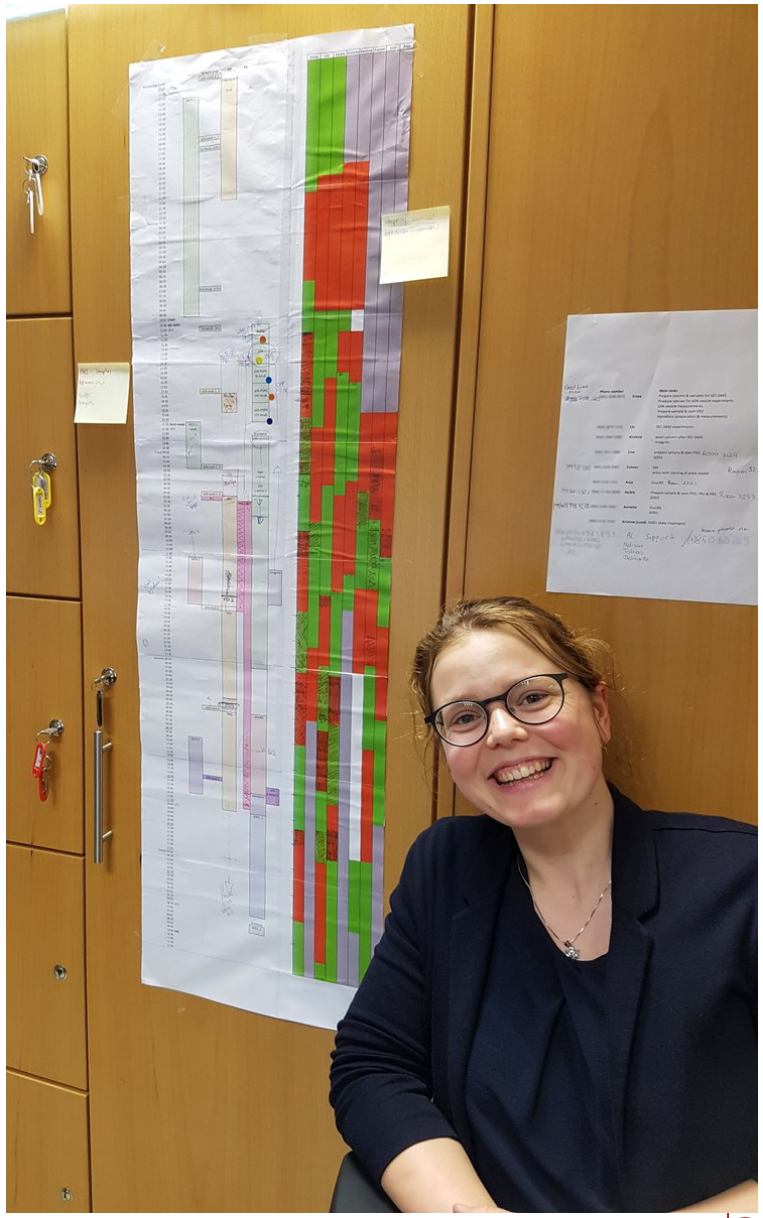
Planning your beamtime

- Keep log book
- Talk to your colleagues
- Evaluate

☺ Have fun and collect great data!



Acknowledgements

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- **Fatima Herranz-Trillo**, University of Copenhagen

- **Pau Bernadó**, University of Montpellier
- **Roma Tauler**, CSIC, Barcelona

- **Beamline staff** DESY/EMBL, ESRF, MAX-IV
- **SAXSlab/Xenocs**

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