Structural studies of amyloidogenic peptides and proteins

Annette Eva Langkilde
Dept. Of Drug Design and Pharmacology / Dept. Of Chemistry
University of Copenhagen, Denmark
Amyloid fibrils

- Disease Related
- Unbranched
- Extracellular
- In vivo
- Green birefringance upon Congo Red binding
- Cross-β fiber diffraction pattern
- ... and lots of amyloid-like fibrils
Fibril Structure

Jimenez et al, 2002, PNAS
Human Insulin

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

Fitzpatrick et al, 2013, PNAS
TTR 105-115

Jimenez et al, 2002, PNAS
Human Insulin
The fibrillation process

Stationary phase (maturation)

Growth phase (elongation)

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

Lashuel et al. JMB
a-synuclein

Grønning, Vestergaard
Human Insulin

Jimenez et al, 2002, PNAS
Human Insulin


Hua & Weiss (2004)
Insulin, pH~1

Langkilde, Vestergaard
Peptide/yeast prion protein

Nelson et al, 2005, Nature
Peptide/yeast prion protein
Method? SAXS!

Native oligomer ↔ Native folded monomer ↔ Partially (un)folding (membrane vicinity) ↔ Fibrillation prone conformation ↔ Fibrillation related off-pathway oligomer ↔ Nucleus? On-pathway oligomer ↔ Amorphous aggregate

Native interactions ↔ Native unfolded monomer ↔ Amorphous aggregate

Alternative fibrillation pathways → FIBRIL strain 1

FIBRIL strain 2
Time resolved SAXS during fibrillation (α-synuclein)

SAXS data – additive

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

- \( n_k \): 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)

Log(eigenvalue)

Normalized eigenvectors

3 components:

\[ I_{\text{tot}} = x I_a + y I_b + z I_c \]
Isolating the scattering curves - OLIGOMER

3 components:

\[ I_{\text{tot}} = x I_{\text{native}} + y I_{\text{fibril}} \]

Get residuals, as first estimate

\[ I_{\text{tot}} = x I_{\text{native}} + z I_{\text{fibril}} \]

Get \( x, y \) and \( z \) estimates

Recalculate and refine using residuals

\[ I_{\text{tot}} = x I_{\text{native}} + y I_{\text{unknown}} + z I_{\text{fibril}} \]
Decomposition of species (α-synuclein)

Compare typical distances

Mature fibril cross-section

Mature fibril

Oligomer

Ab initio models of αSynuclein fibrillar species

Corresponding sizes...
Do the oligomers build the fibrils?

When it doesn’t work....
\( \alpha \text{SN E46K} \)

\[ I_{\text{tot}} = x I_{\text{native}} + y I_{\text{unknown}} + z I_{\text{fibril}} \]

Get residuals, as first estimate

\[ I_{\text{tot}} = x I_{\text{native}} + y I_{\text{unknown}} + z I_{\text{fibril}} \]

Get \( x, y \) and \( z \) estimates

Recalculate and refine using residuals

αSN E46K

Development of an OBJECTIVE ROUTINE for the decomposition

Chemometric method: Multivariate Curve Resolution using Alternating Least Squares (MCR-ALS)

Fátima Herranz-Trillo & Prof. Pau Bernado, U. Montpellier;
Prof. Roma Tauler, U. Barcelona Bente Vestergaard, U. Copenhagen

Reduce Ambiguities: Use of multiple data matrices

χ² = 1.16
χ² = 3.98
χ² = 2.08
Insulin fibrils

~ 5-6 monomers

1 monomer

1240 monomers

360 Å

160 Å

200 Å

690 Å

Insulin & E46 revisited

Coming soon to a journal near you...

Structural Analysis of Multicomponent Amyloid Systems by Chemometric SAXS Data Decomposition

Herranz-Trillo F, Groenning M, van Maarschalkerweerd A, Tauler R, Vestergaard B and Bernadó P

Structure (2016) Accepted
**Full-length proteins vs peptide fragments**

GNNQQNY

- No lag-phase
- No oligomer build-up
- Monomeric starting state
- Ribbon like fibrils
- Bragg peak (late fibril state)
Fibre diffraction

SAXS vs FD - complementarity

SAXS data recorded at ID14-EH3, ESRF
Detector distance: 2.34 m
Wavelength: 0.931 Å
Resolution range: ~ 1000 - 10 Å

FD recorded at 911:2, MAXlab
Detector distance: 0.22 m
Wavelength: 1.04 Å
Resolution range: ~ 50 - 3 Å

A. E. Langkilde
Small-angle X-ray scattering (in solution)

SAXS data recorded at ID14-EH3, ESRF
Detector distance: 2.34 m
Wavelength: 0.931 Å
Resolution range: ~ 1000 - 10 Å
Combining SAXS, FD, and TEM

Packing model

Fiber diffraction simulation - CLEARER

Experimental diffraction image

Simulated diffraction image

Transthyretin (TTR)

- Native state: 55 kDa Homotetramer
- TTR misfolding and aggregation → amyloid diseases
- Wildtype ATTR amyloidosis (senile systemic amyloidosis)
- Familial amyloid polyneuropathy
- Familial amyloid cardiomyopathy

![Diagram showing the transition from native tetramer to protofibrils/fibrils](image)
SAXS on TTR fibrillation

- Pre-ini MW ≈ 3.3 monomers
- Pre-ini ≠ xtal
- Pre-ini ≠ xtal+dimer/monomer
- Pre-ini = 85% xtal + 15% unfolded
Prefibrillar transthyretin oligomers and cold stored native tetrameric transthyretin are cytotoxic in cell culture

Karin Sörgjerd, Therése Klingstedt, Mikael Lindgren, Katarina Kågedal, Per Hammarström

IFM- Department of Chemistry, Linköping University, SE-581 83 Linköping, Sweden
Division of Experimental Pathology, Faculty of Health Sciences, Linköping University, S-581 85 Linköping, Sweden
Department of Physics, Norwegian University of Science and Technology, 7491 Trondheim, Norway
The unfolded state
Some practical aspects

• Test your system, find optimal conditions
• Know your system
  • complementary methods, e.g. TEM and FD (“SAXS and ...” talks tomorrow)
• Consider beamline stability, time frames, additional equipment etc

• Check 2D images
• Check buffers, basic parameters
• ...and double check!
• Test different inputs and parameters (consistent solutions)
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