Analysis of Heterogeneous Systems

- an attempt to provide a few pedagogical examples

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Homogeneous/Heterogeneous?

1: Impurities

Sample preparation of utmost importance! Stay in the lab, don’t do SAXS (yet!).

Is NOT fixed by the beamlines providing in-line FPLC. Optimize sample preparations in all possible ways!

'Not atypical' Lab Gel
Courtesy of Mads Gravers Jeppesen
Homogeneous/Heterogeneous?

A: No fixed position of domain(s)

B: More than one distinct orientation of domain(s) (different functional states?)

C: (Partial) Complex formation (e.g., dimerisation)

D: (Partial) Complex formation AND domain movements

E: D+ significant secondary/tertiary structural changes

A challenge – yes!!!! – but an excellent reason to do SAXS

SEVERAL words of caution are relevant!
Termination of Bacterial Protein Synthesis

AAA UGA
    P A

RF2

AAA UGA
    P A

Recycling

AAA UGA
    P A

RF2

AAA UGA
    P A

RF3
Termination of Bacterial Protein Synthesis

Contact to Peptidyl Transferase Center:

GGQ
(Universal)

Decoding:
(PXT or) SPF

RF1
RF2

eRF1
aRF1

Decoding:
(PXT or) SPF

Contact to Peptidyl Transferase Center:

GGQ
(Universal)
Crystal Structure of RF2

- 2-loped comma-shaped structure
- Show in orientation with SPF downwards
- GGQ and SPF too close for distance on ribosome
- Later RF1 same (show, mention that it is non-chronological)

Vestergaard et al. (2001), Molecular Cell
Cryo-EM structures of *in situ* RF2

Bruno Klaholz et al. (2002) *Nature*

Urmila B. Rawat et al. (2002) *Nature*
Mechanism for RF proposed

Rather controversial....

Stop codon recognition
Crystal Structures of RF1 and RF2

Shin et al. (2004) JMB
Suitable for SAXS?

But what about flexibility???

\[ R_g(\text{cryo}) \sim 30 \text{ Å} \]
\[ R_g(\text{cryst}) \sim 25 \text{ Å} \]
Check flexibility of individual domains
Check flexibility of individual domains

- CRYSTAL
- EOM
Complementary Rigid Body Analysis - SASREF

Simultaneous rigid body modelling against data from truncated and full-length protein
Both present in solution?

OLIGOMER

7%:93% MARGINAL IMPROVAL OF FIT
What do xtals represent?

L. Moras et al. & R. Buckingham
But....

Thermophilus Closed Xtal
Open Ribosome Mix 78:22

Full-length, no truncation analysis....*sigh*
Part II: ANALYSIS OF THE OLIGOMERISATION PATHWAY OF A NOVEL INSULIN ANALOGUE

Malene Hillerup Jensen
- now @ Novo Nordisk A/S
Modern Insulin Therapy Mimicks Natural Insulin Levels

ωchl

\[ \text{Lys}^{829}_{\text{N}-\omega\text{-carboxyheptadecanoyl}-\text{des(B30)}} \text{ Human Insulin} \]

**Suitable for SAXS?**

- Long acting insulin

**Duration of Action**

- **Long acting**

**Quaternary state**

- Monomer
- Dimer
- Hexamer
- Multihexamer

**Serum Insulin concentration (pmol/L)**

- **Breakfast**
- **Lunch**
- **Dinner**
- **Supper**

**Blood Glucose Concentration (mmol/L)**
Working hypothesis prior to project

Question 1: What is the solution state in formulations?

Question 2: What is the solution state upon injection?

Question 3: What is the process?
SAXS on Phenol Formulations – prior to self-association

Example: 3 Zn/6 Ins

- OLIGOMER analysis: Equilibrium between dimers, $R_6$ hexamers, and dihexamers
- Indicative of an RTTR dodecamer, but not conclusive

SAXS on Phenol Formulations – prior to self-association

- SEC/SAXS on mixture at SWING beamline
- Dodecameric peak analysed

Conclusion I – formulation of $\omega$chl
Pre-selfassociation state (including phenol)

- In formulation $\omega$chl exists in an equilibrium state between hexamer and dodecamer
- The purified dihexamer was found to be in the $R_3T_3T_3R_3$ state
SAXS on the Self-Associated State

OLIGOMER

CRYSOL

24 hour data were OLIGOMER fitted with a combination of multihexamers (2, 8, 10, 14, and 30 hexamers)

\( T_6 \)

\( R_6 \)

50 Å

33.9±0.2 Å

\( T_3R_3 \)

37.2±0.8 Å

DAMMIN *ab initio* models of the Self-Associated State

*Ab initio* model

Overall dimensions fits with model of $T_6$-multihexamer

24 hour data were OLIGOMER fitted with a combination of multihexamers (2, 8, 10, 14, and 30 hexamers)

Conclusion II – Self-associated State

• The mature self-associates are long rod-like multihexamers.
• The hexamer is the smallest repeating unit in the multihexamer.
• The distance between the hexamers are 35.1 Å.
• The hexamers are in the T₆-state.
Investigation of the self association process
Time Resolved-Circular Dichroism

- Upon removal of phenol the allosteric state shifts immediately from the R to the T state

- Addition of 0.6 mM phenol slows down the self-association process

Investigation of the self-association process @SWING
3 concentrations (3 starting volumes)
Low (0.6 mM) phenol concentration

Size/length
Depends on concentration, time
Zn/hex and presence of phenol

Time Resolved - Dynamic Light Scattering

- Final size of the multihexamers are dependent on the initial concentration
- The initial self-associating rate is linearly proportional with the concentration
Conclusions III
During Self-Association

- Upon removal of phenol the allosteric state of the hexamer shifts from the R to the T state
- Addition of 0.6 mM phenol slows down the process
- The final size is dependent on the initial concentration > distributions of sizes
- The smallest building block is the $T_6T_6$-dihexamer
Conclusion

- Proposed Mechanism for Self-Association of ωchI

INITIATION

Phenol removal
causes
conformational
change

ELONGATION

\[ R_6 + R_3T_3T_3R_3 \rightarrow [T_6T_6] \rightarrow T_6 \]

Hexamer
Dihexamer
Dihexamer
Rod-like multihexamers

Part III: Structural characterisation of prefibrillar intermediates and amyloid-like fibrils

Annette Eva Langkilde
Amyloid(-like) fibrils

- Amyloid definition:
- Disease Related
- Unbranched
- Extracellular
- *In vivo*
- Green birefringance upon Congo Red binding
- **Cross-β fiber diffraction pattern**
The fibrillation process
"Time resolved" SAXS during fibrillation (α-synuclein)

Decomposition of species (α-synuclein)

- Fibril
- Monomer/dimer
- Oligomer
- ThT

Full-length proteins vs peptide fragments


Mature peptide fibrils

Cross-section analysis

Dias 41
Combining SAXS, FD, and TEM

Fibre diffraction of aligned fibrils

\[ a = 31 \text{ Å} \]
\[ b = 4.7 \text{ Å} \]
\[ c = 48 \text{ Å} \]
Fibrils vs crystals

Aligned fibrils

Aligned needle shaped crystals

PDBid 1yjp

PDBid 2omm

a=31 Å

b=4.7 Å

c=48 Å
Fiber diffraction simulation

Experimental diffraction image

Simulated diffraction image
Quasi-atomic models

Log(I) vs. q [Å⁻¹]
Conclusions

- Ribbon
- Filaments/laminar structure
- Unit cell determined
- Mass per unit cell determined
- Plausible packing model (including ssNMR; TEM; SAXS; FD; xtal) -> quasi-atomic resolution models
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