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Chaperones, protein folding and unexpected accidents

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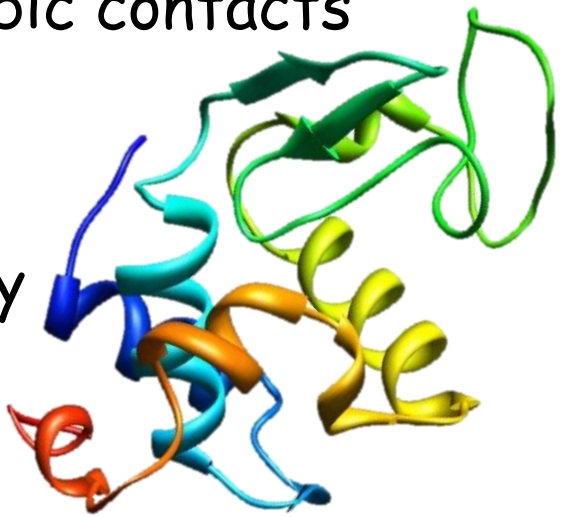
Colloqui interdisciplinari sulla biologia,
Università di Roma Tor Vergata, 15 marzo 2017

OUTLOOK

- Protein Folding: why it is so appealing for physicists
- Misfolding and Amyloid fibrils
- Chaperons and Chaperonins
- Chaperonins toward amyoid fibril aggregation

PROTEIN FOLDING

Native structure is compact and stabilized by multiple hydrophobic contacts and hydrogen bonds

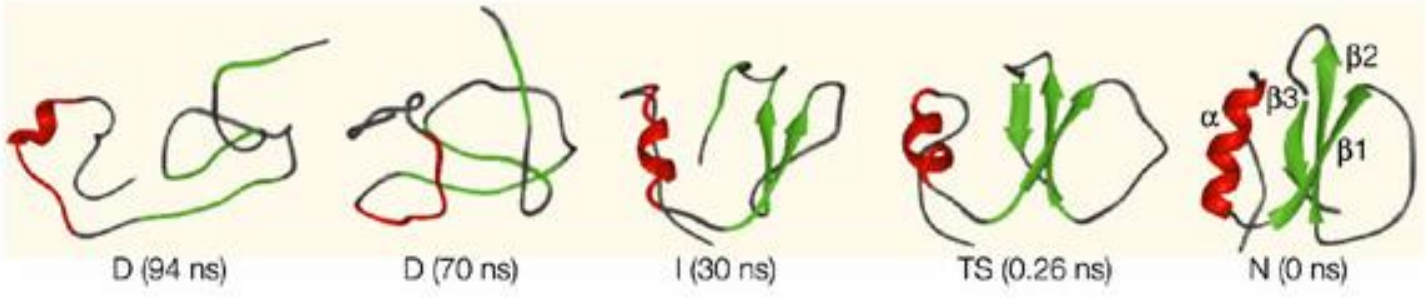


In a folding reaction, the native state has the lowest free energy

protein folding is spontaneous *in principle*

in practice, impractically slow

Folding intermediates are flexible, less compact, with exposed hydrophobicity



Model of folding reaction
Daggett and Fersht

PROTEIN FOLDING

Anfinsen's Dogma

- Native structure is the thermodynamically most stable (favored) state for most proteins.

- Native tertiary structure is determined by the primary structure (amino acid sequence) of a protein

The three-dimensional structure of a native protein in its normal physiological milieu (solvent, pH, ionic strength, presence of other components such as metal ions or prosthetic groups, temperature, etc.) is the one in which the Gibbs free energy of the whole system is lowest; that is, that the native conformation is determined by the totality of interatomic interactions and hence by the amino acid sequence, in a given environment.

PROTEIN FOLDING

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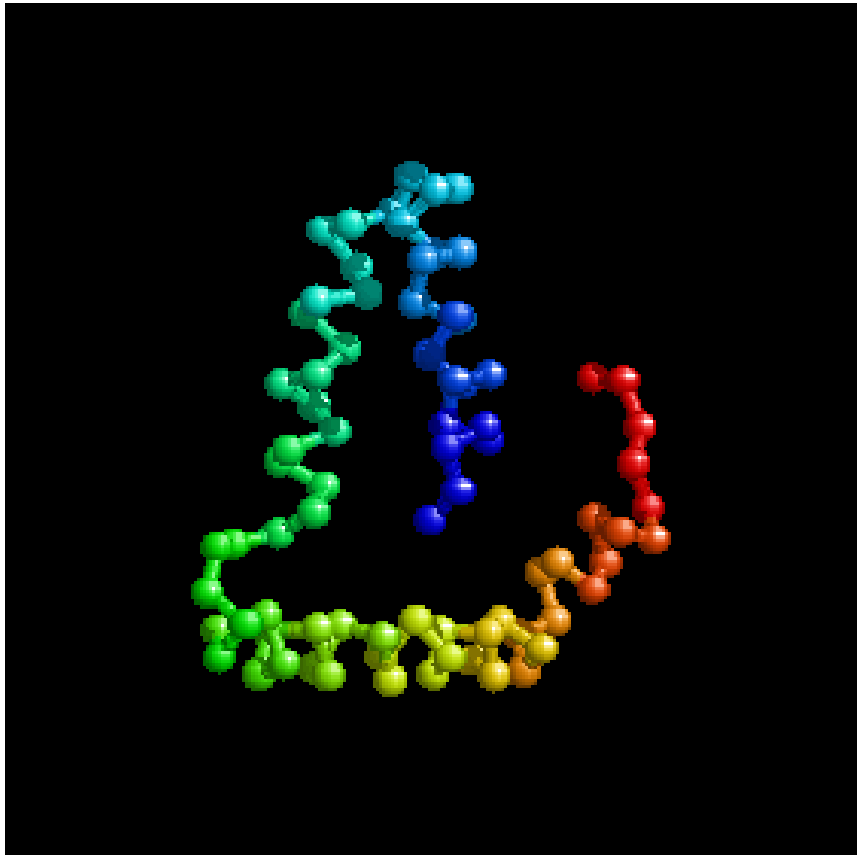

porcine NK-lysin

Synthetic folding predicted using FRAGFOLD software. The basic idea of fragment assembly is to take fragments of already known protein structures and to recombine them randomly to create possible new protein folds

Each frame of the animation was generated by linearly interpolating the coordinates from "snapshots" taken during the protein folding simulation, and so is not intended to be physically realistic.

Jones, D.T. (1997) Successful ab initio prediction of the tertiary structure of NK-Lysin using multiple sequences and recognized supersecondary structural motifs. PROTEINS.1, 185-191.

PROTEIN FOLDING

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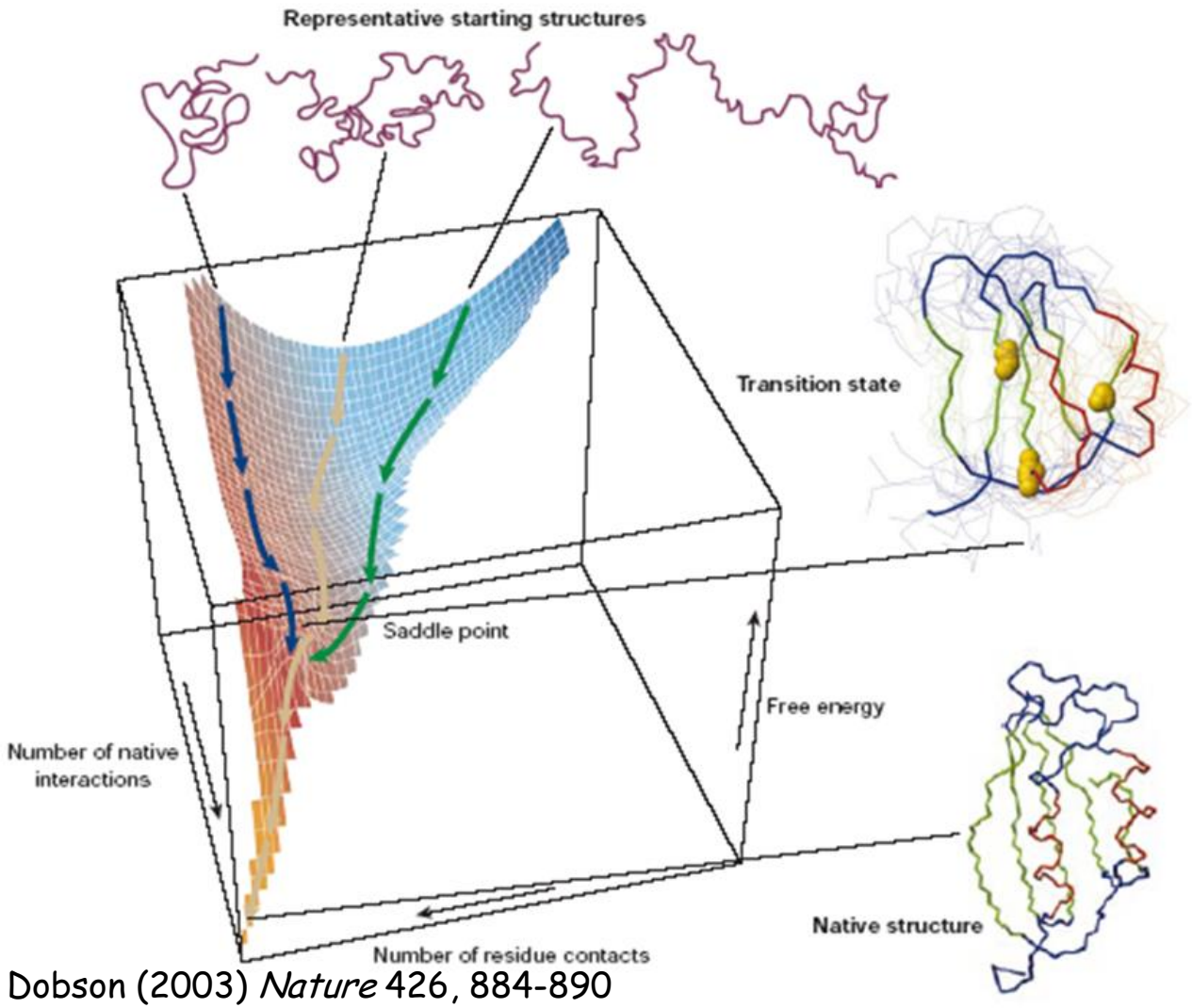
Synthetic folding trajectory for a protein of unknown function from T. thermophiles which in this case incorporates a small beta-sheet. This protein was predicted using FRAGFOLD3 -able to model all of the details of the protein structure, including all of the side chain atom positions- and the prediction had an RMSD of only 2.4 Å from the experimental structure.

By looking at such simulations we can make some educated guesses as to how proteins might fold in nature.

Jones D.T., Bryson K., Coleman A., McGuffin L.J., Sadowski M.I., Sodhi J.S., Ward J.J. (2005) Prediction of novel and analogous folds using fragment assembly and fold recognition. Proteins. 61 Suppl 7:143-51.

PROTEIN FOLDING

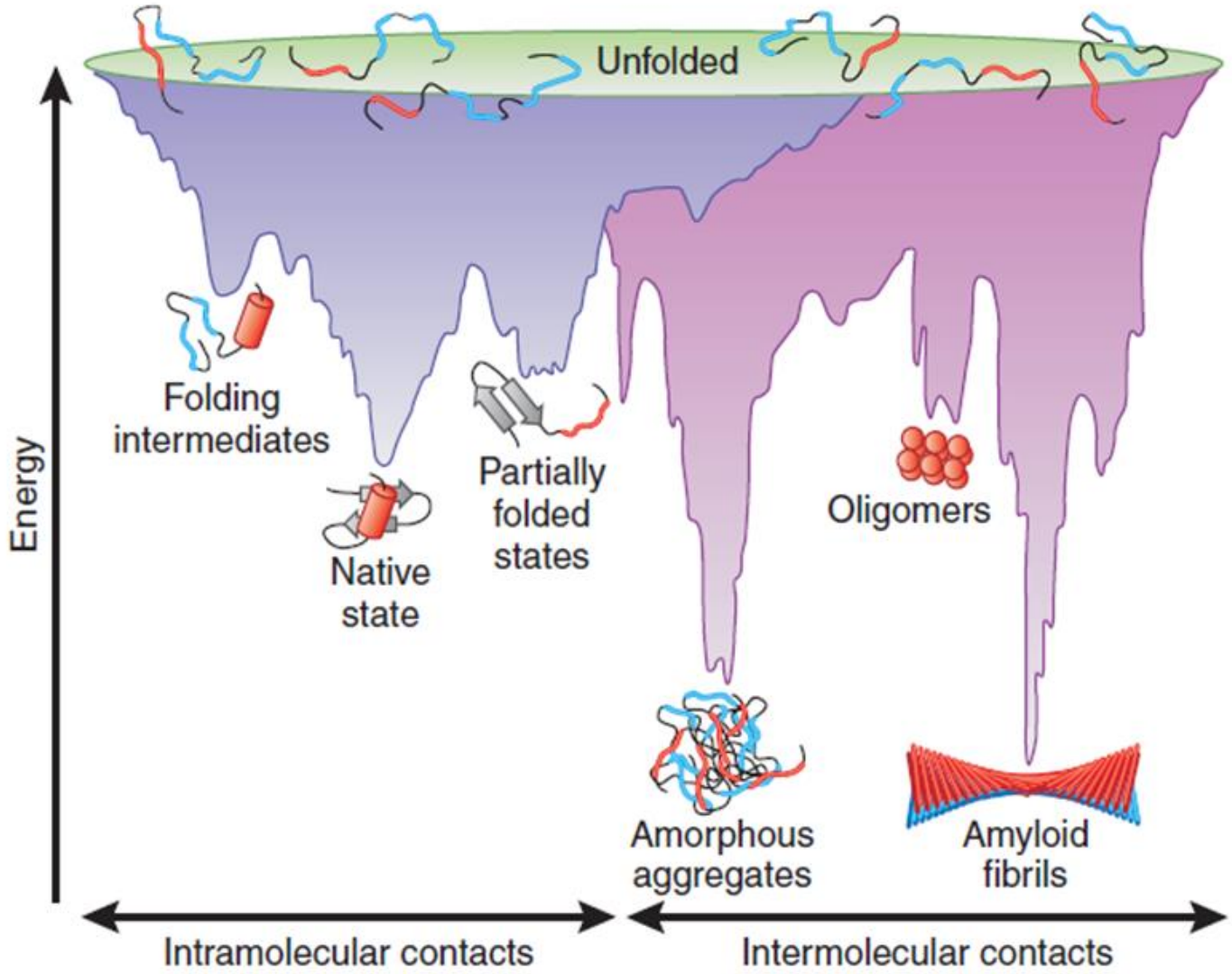


Dobson (2003) *Nature* 426, 884-890

A protein's native structure is determined solely by the protein's amino acid sequence components and the environmental conditions in which the folding occurs, such that the native structure is a unique, stable, and kinetically accessible state corresponding to a Gibbs free energy minimum.

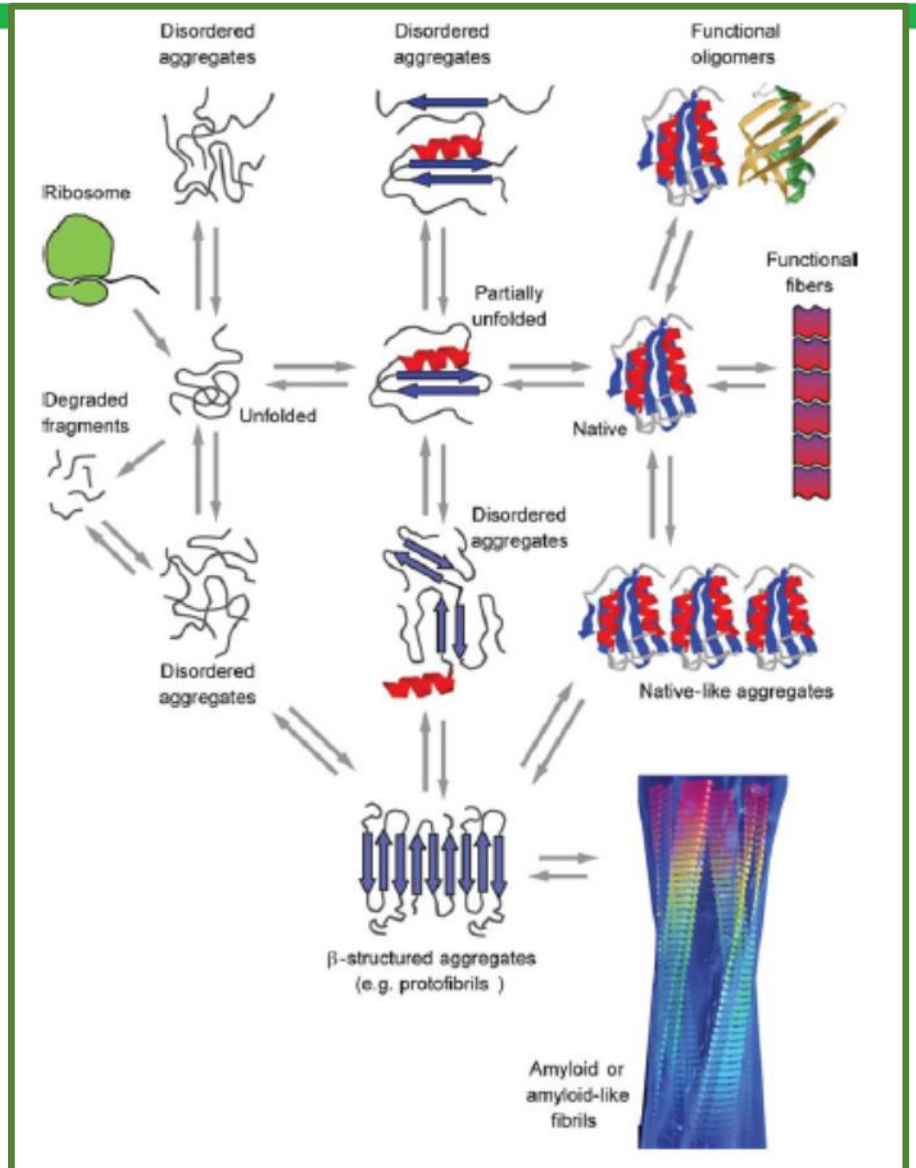
Many different unfolded and partially folded states different folding pathways lead to one native state intermediates can persist in local free energy minima "kinetically trapped", requires energy to escape minimum

PROTEIN FOLDING



- Multiple intermolecular contacts in aggregates can make them more stable than individual native state
- Amyloid aggregates are the most stable

AMYLOID AGGREGATION



"The amyloid state is more like the default state of a protein, and in the absence of specific protective mechanisms, many of our proteins could fall into it. "

Christopher Dobson, Annu. Rev. Biochemistry, 2006

"effectively all complex proteins have these short segments that, if exposed and flexible enough, are capable of triggering amyloid formation"

David Eisenberg, PNAS, 2010

AMYLOID AGGREGATION

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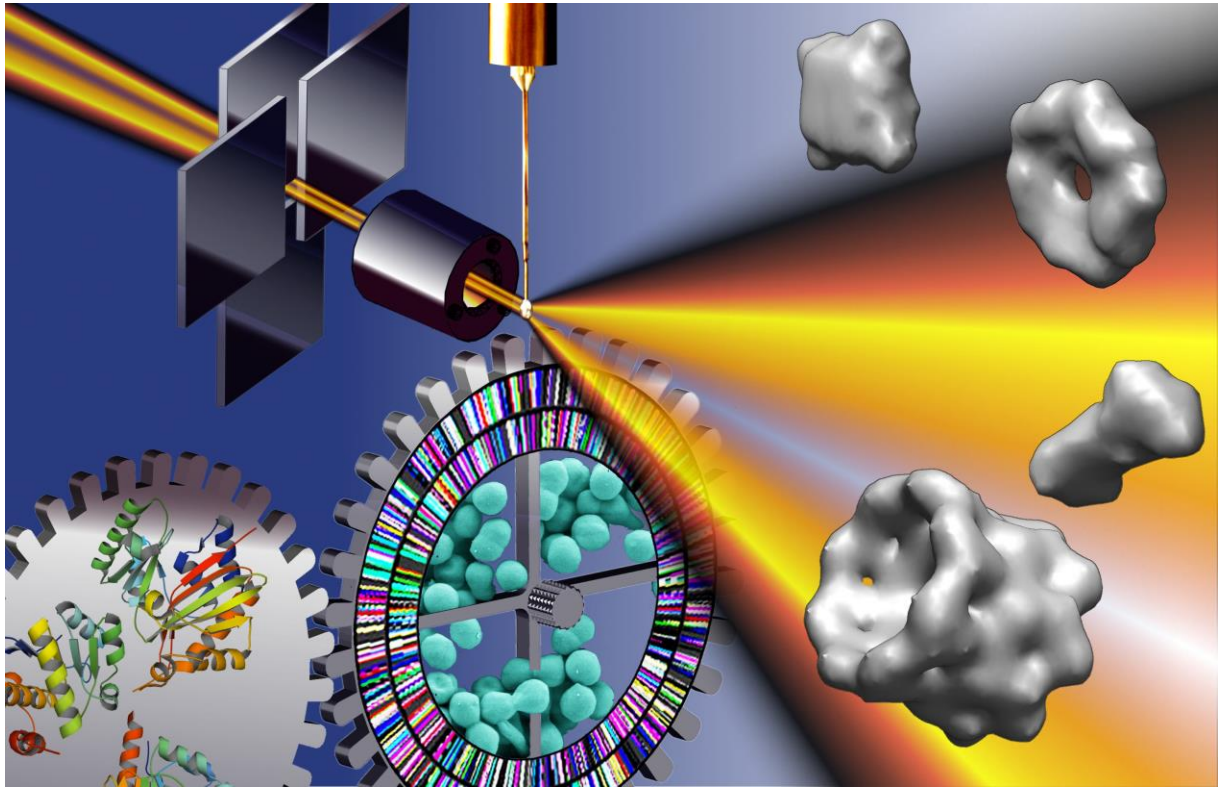
Small Angle X-ray Scattering (SAXS)



*From "Course on SAXS from Biological Material" at SYBILS beamline, Lawrence Berkeley Laboratory

AMYLOID AGGREGATION

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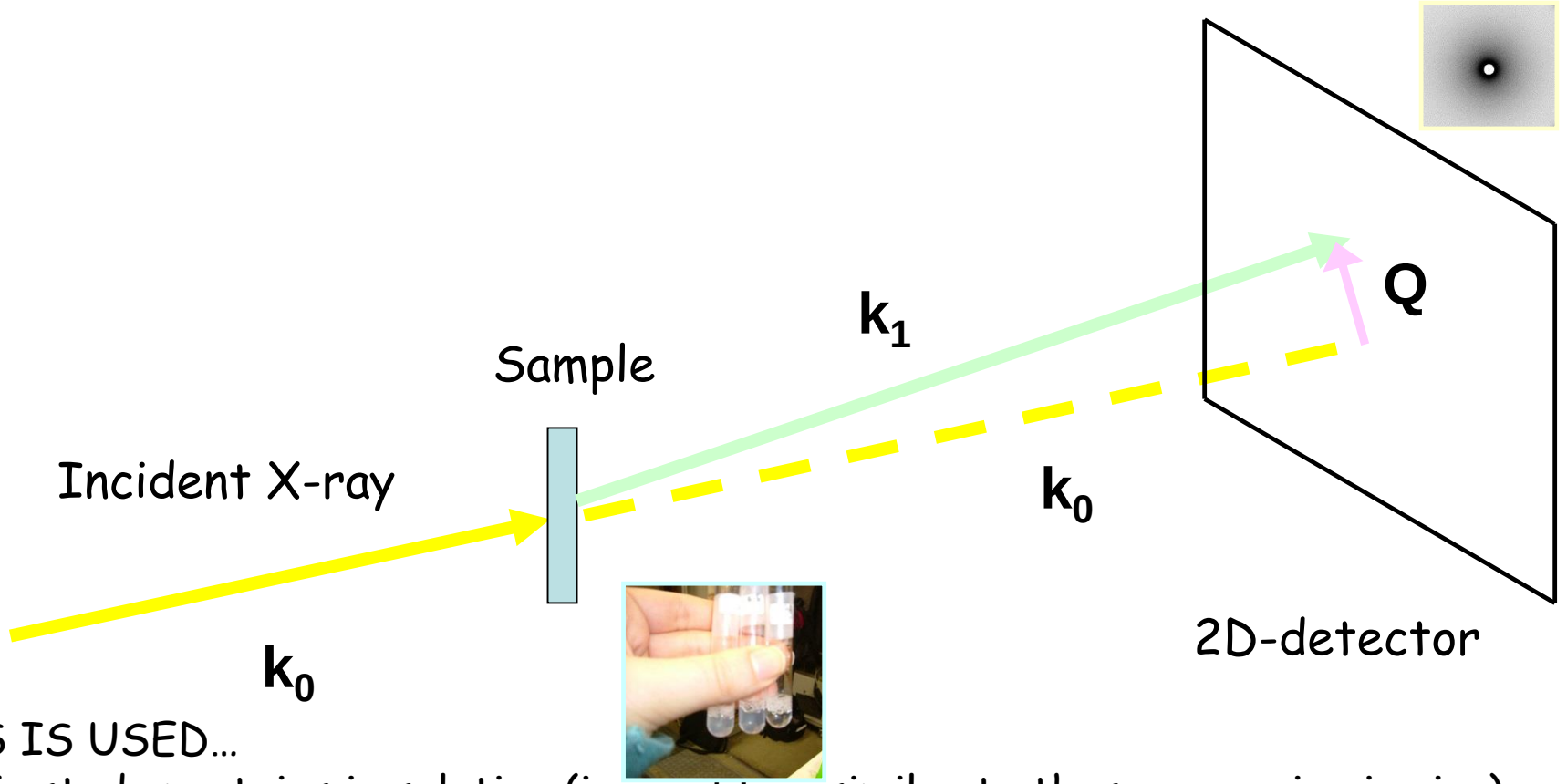

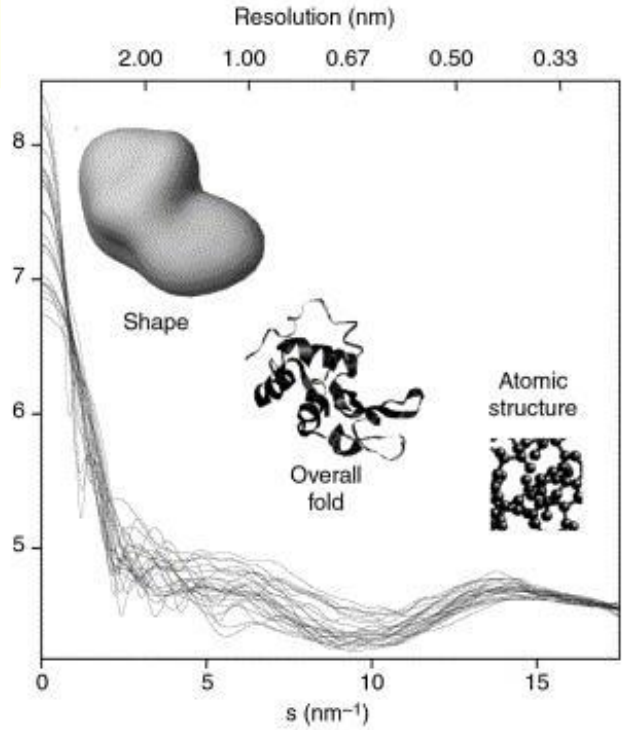

SAXS IS USED…

- To study proteins in solution (in condition similar to those occurring in vivo)
- To be able to measure just low amount of sample
- To provide information on a wide dimension range
- To simultaneously describe structure and interaction

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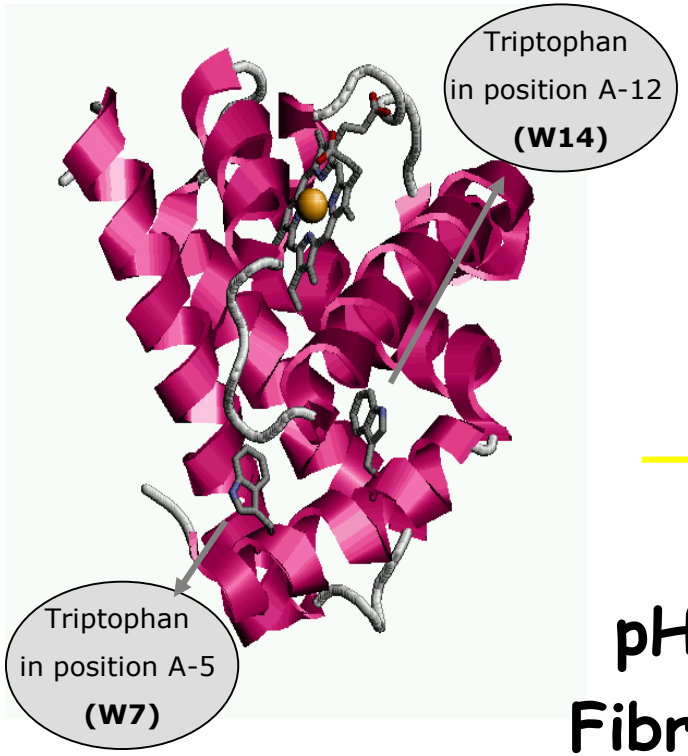
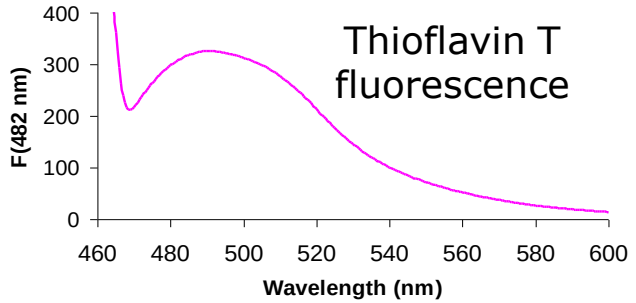
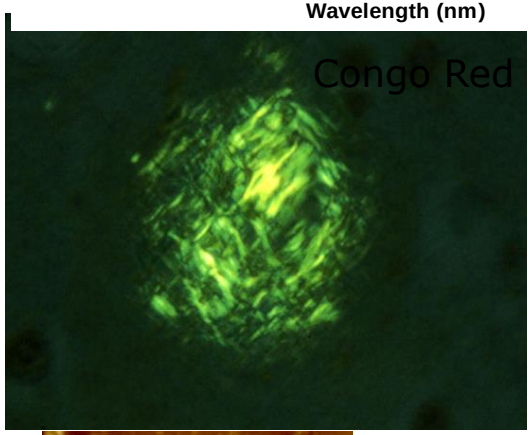
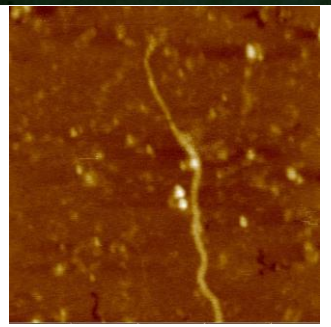


W7FW14F
amyloid fibrils

pH 4 → 7
Fibril growth







Sirangelo, I. et al., *J. Biol. Chem.* (2002) 48:45887-45891.

Sirangelo, I. et al., *J. Biol. Chem.* (2004) 279:13183-13189.

AMYLOID AGGREGATION

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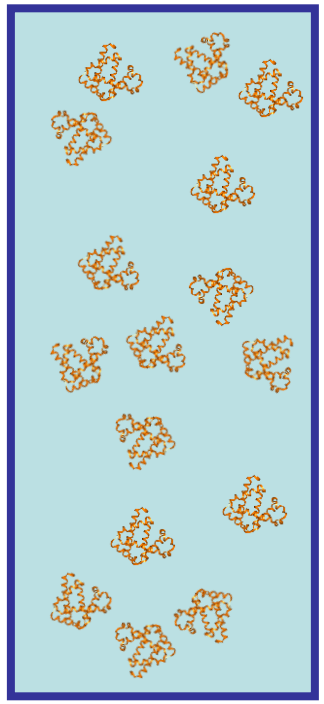
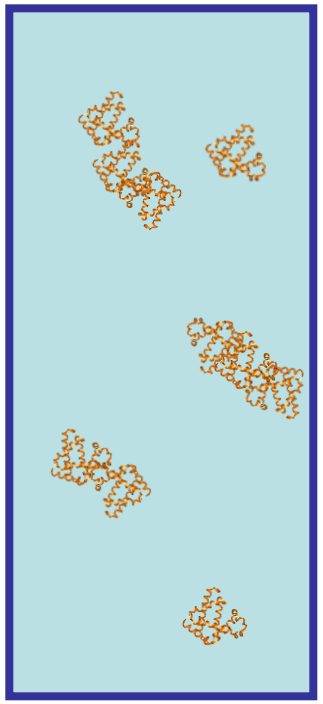


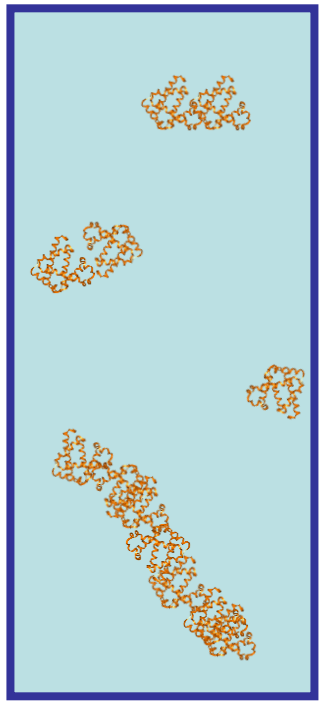
W7FW14F
4 g l^{-1}
pH 4.0

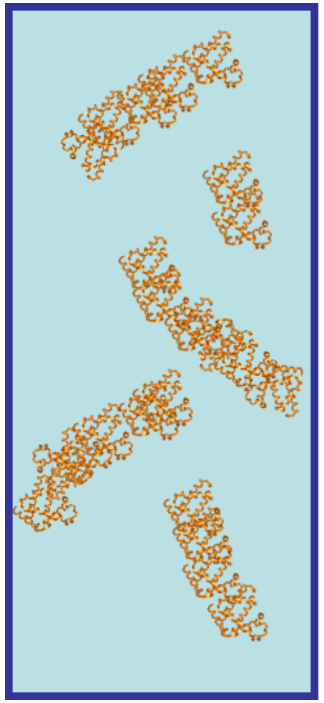
pH jump
from 4.0 to 7.0

Buffer
Solution
pH 7.0



W7FW14F
2 g l^{-1}
pH 7.0





As time goes on…

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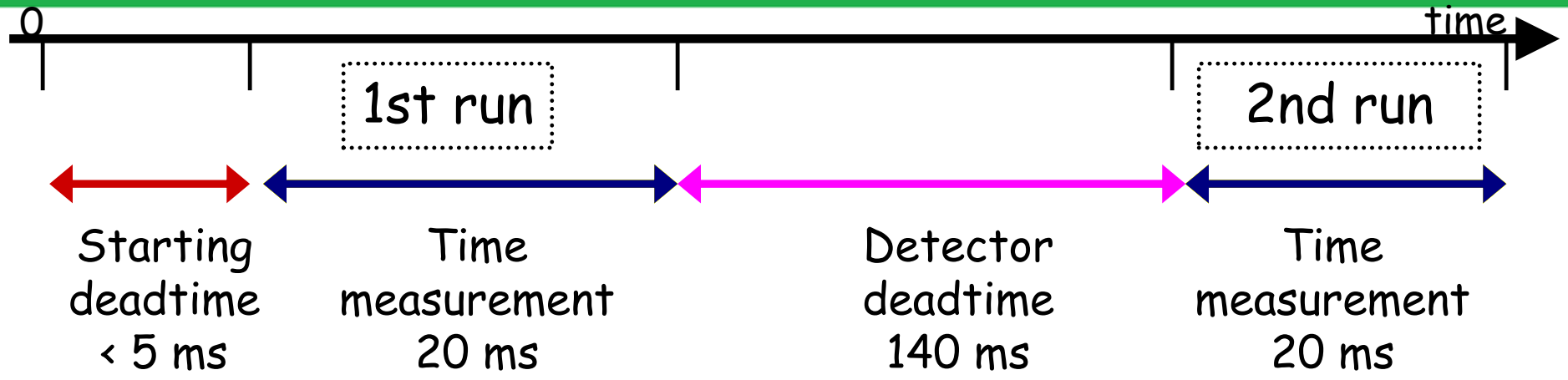
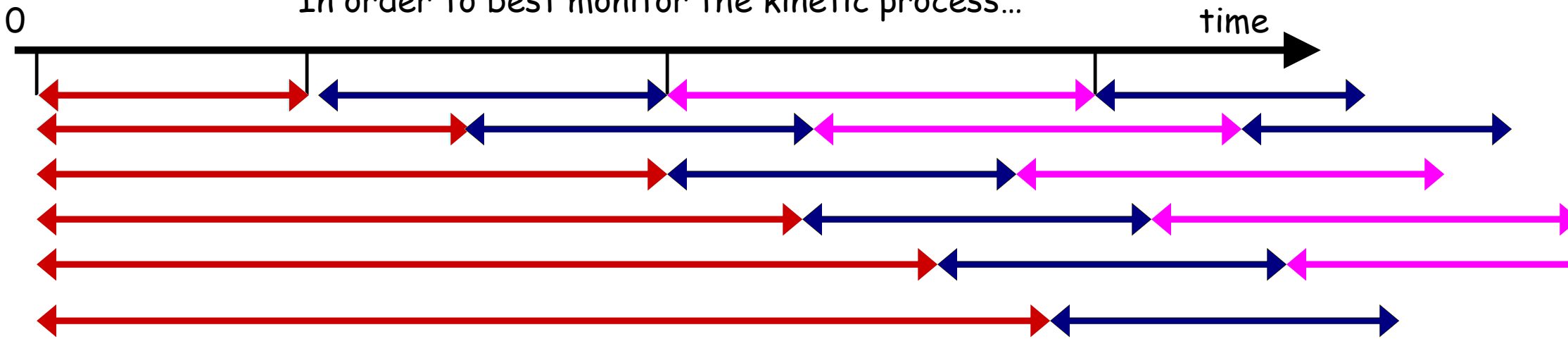


In order to best monitor the kinetic process…



AMYLOID AGGREGATION

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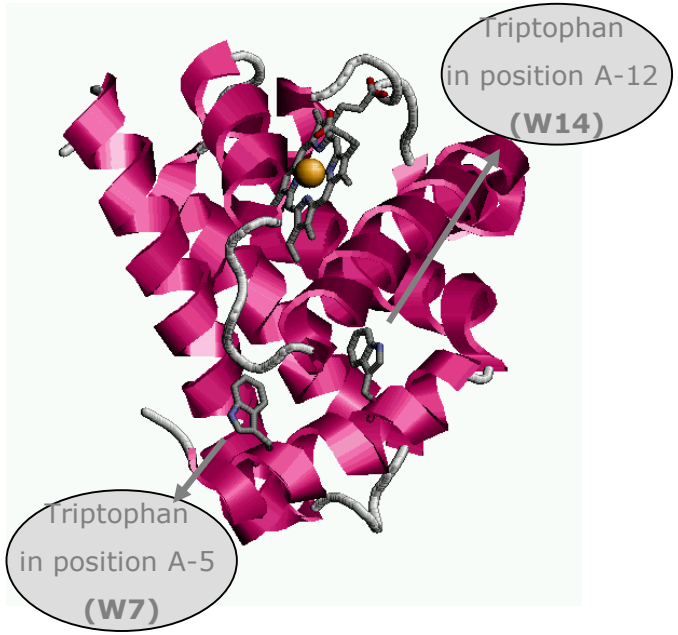
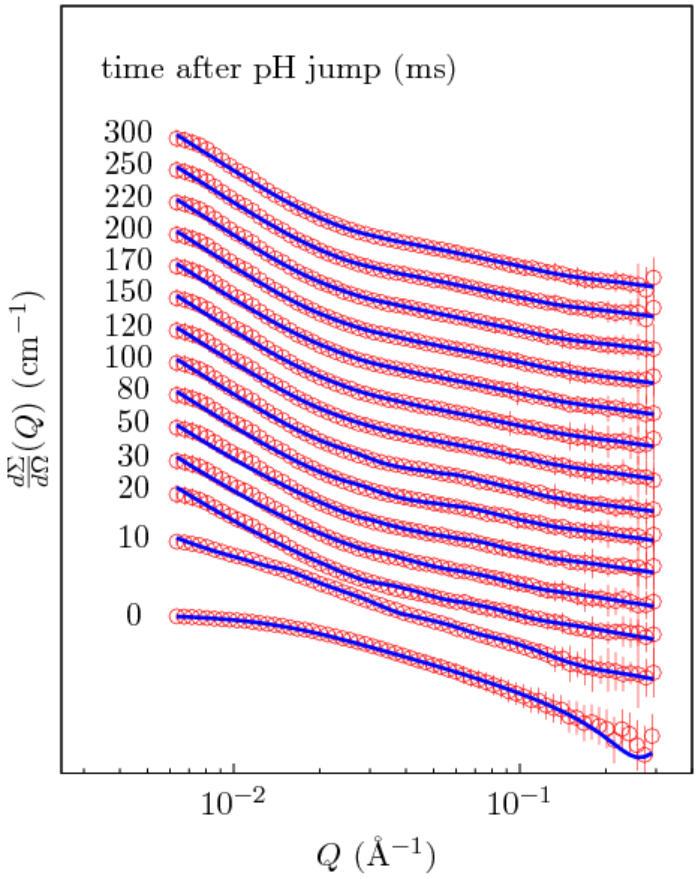
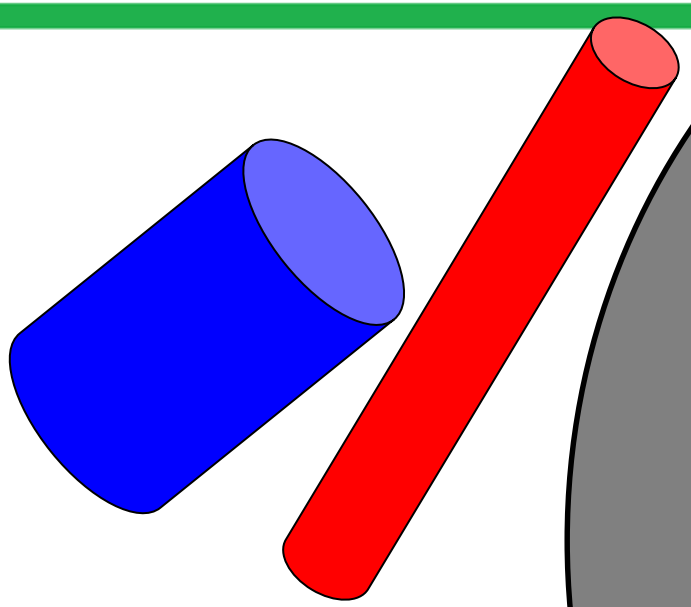
W7FW14F apomyoglobin

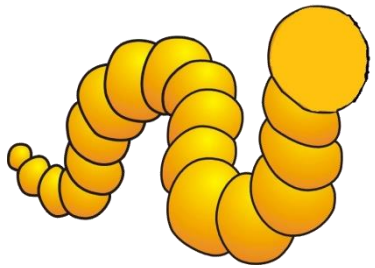


pH 4 → 7

Fibril growth







$$\frac{d\Sigma}{d\Omega}(Q) = x_{\mathrm{cyl},1}\left.\frac{d\Sigma}{d\Omega}\right|_{\mathrm{cyl},1}(Q) + x_{\mathrm{cyl},2}\left.\frac{d\Sigma}{d\Omega}\right|_{\mathrm{cyl},2}(Q) + x_{\mathrm{wlk}}\left.\frac{d\Sigma}{d\Omega}\right|_{\mathrm{wlk}}(Q) + x_{\mathrm{por}}\left.\frac{d\Sigma}{d\Omega}\right|_{\mathrm{por}}(Q)$$

AMYLOID AGGREGATION

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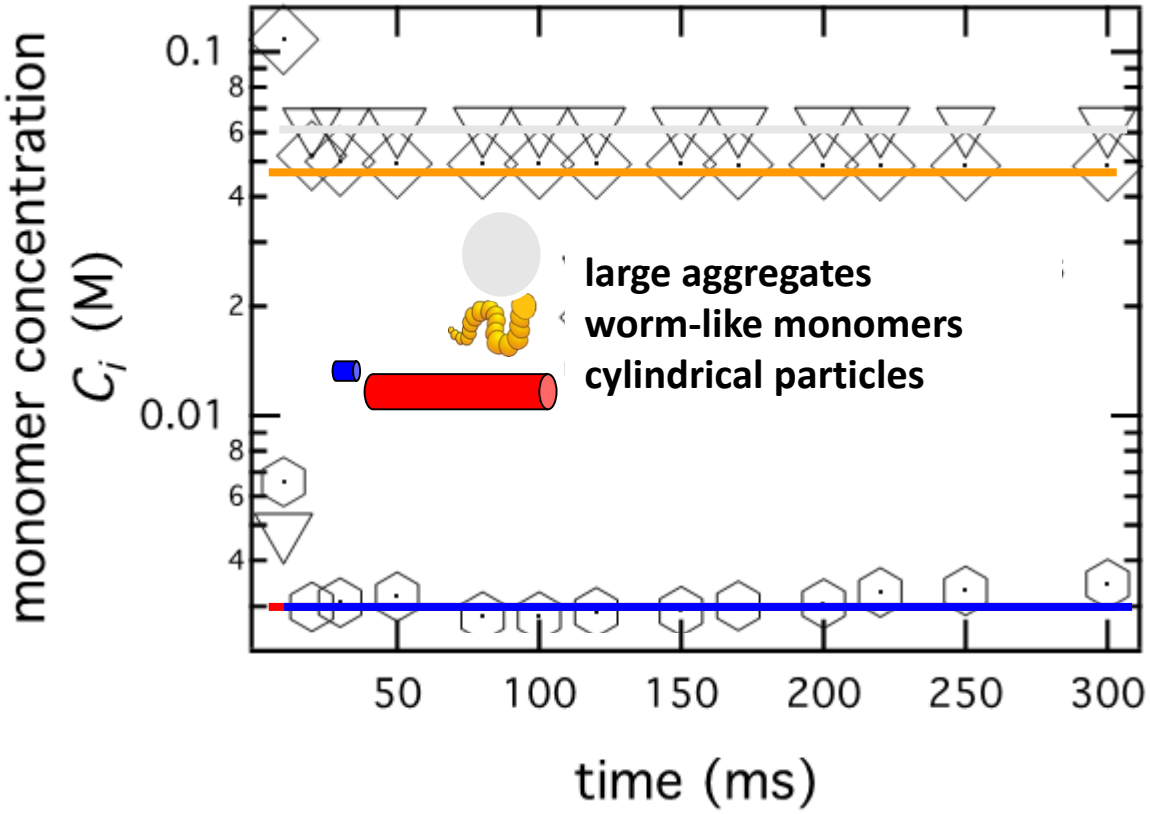
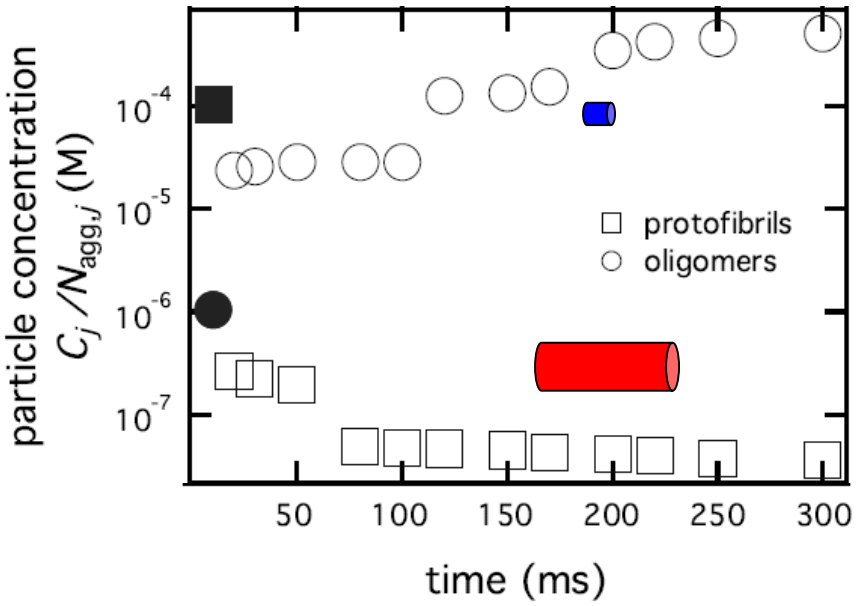


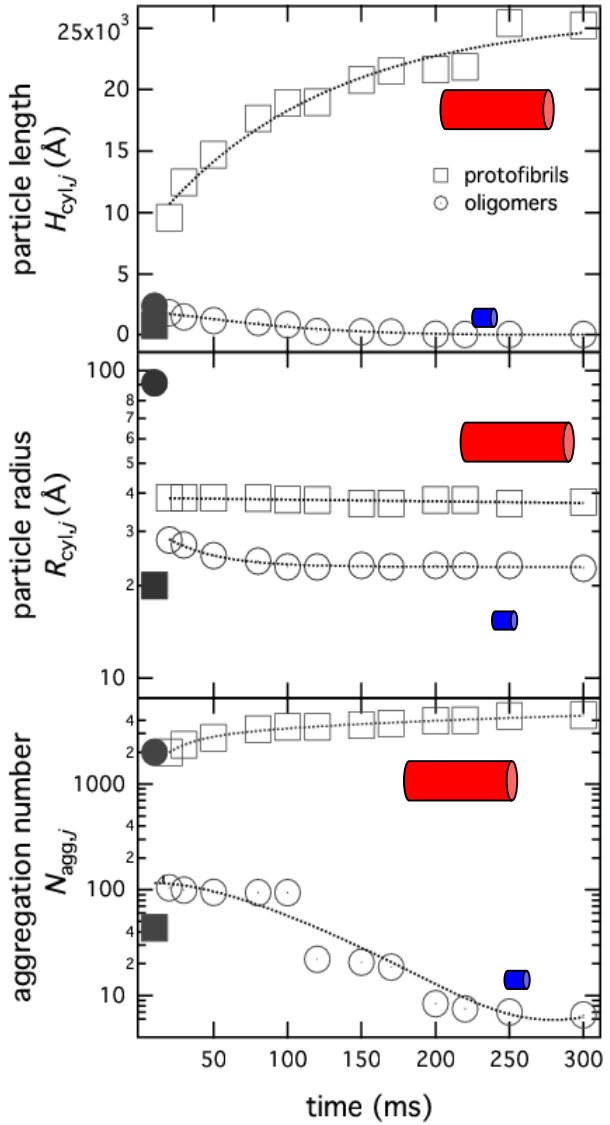
$$C_i = x_i c/(N_A M_{apo})$$

Amount of monomers in the particles

1. After a few ten of ms, ≈ 50% of W7FW14F are in the large aggregates, whose concentration remains rather costant with time.
2. The first aggregates are cylindrically shaped
3. A large amount of monomeric, worm-like protein is still present in solution.

AMYLOID AGGREGATION





MG. Ortore et al. Phys Rev E 84, 061904 (2011)

AMYLOID AGGREGATION

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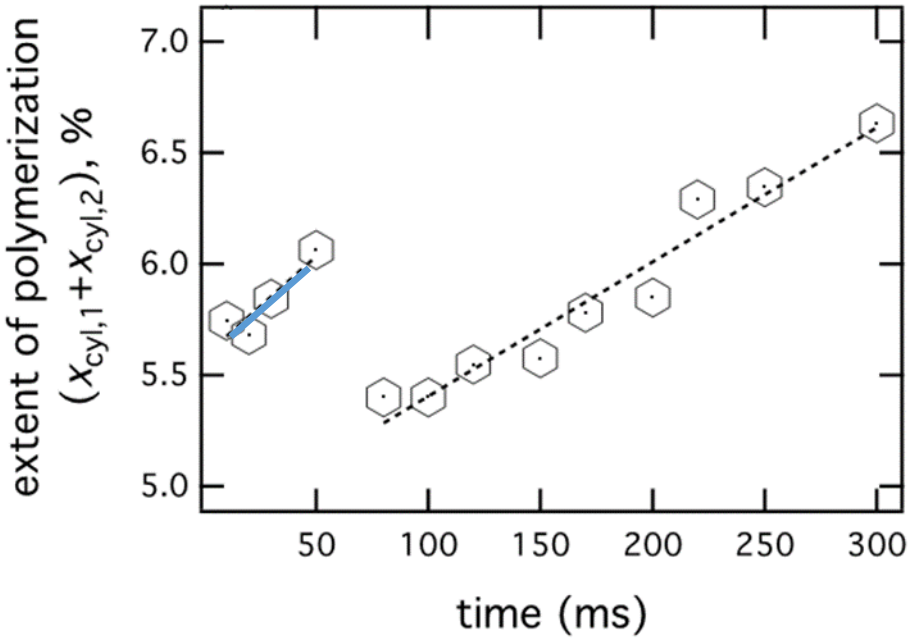
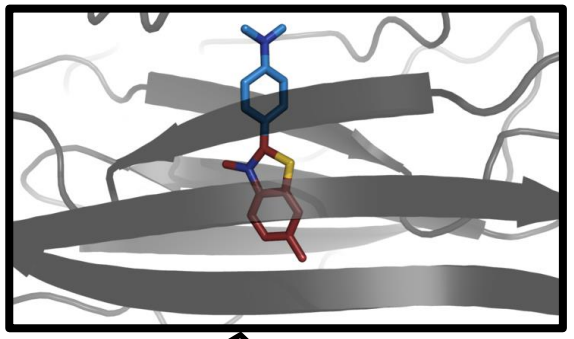


Complex nucleation-elongation mechanism: particles grow instantaneously, then the process stops, resets, and restarts.

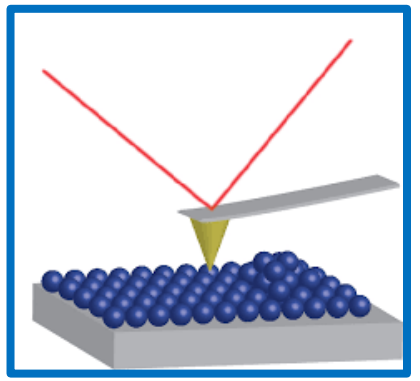
MG. Ortore et al. Phys Rev E 84, 061904 (2011)

The initial acceleration is probably due to the addition of monomers on the seeds rapidly formed after the pH jump.

The polymerization reaction gives rise to two different aggregates (at least!), the protofibrils, rather compact and fast growing, and the soluble oligomers, loosely packed and rather unstable.



There is a long lag phase before fibrils appear. My name is ThT.



Fibrils are homogeneous. My name is AFM.

AMYLOID AGGREGATION

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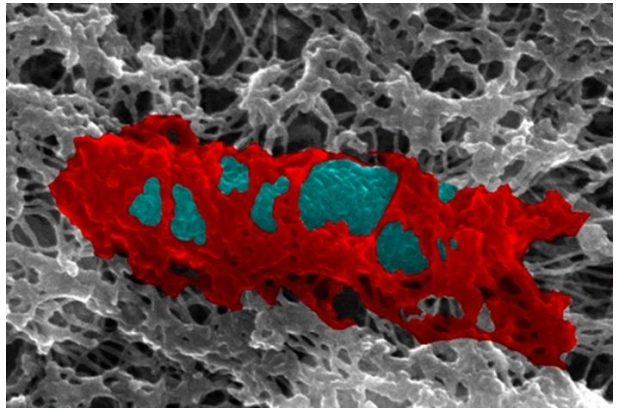
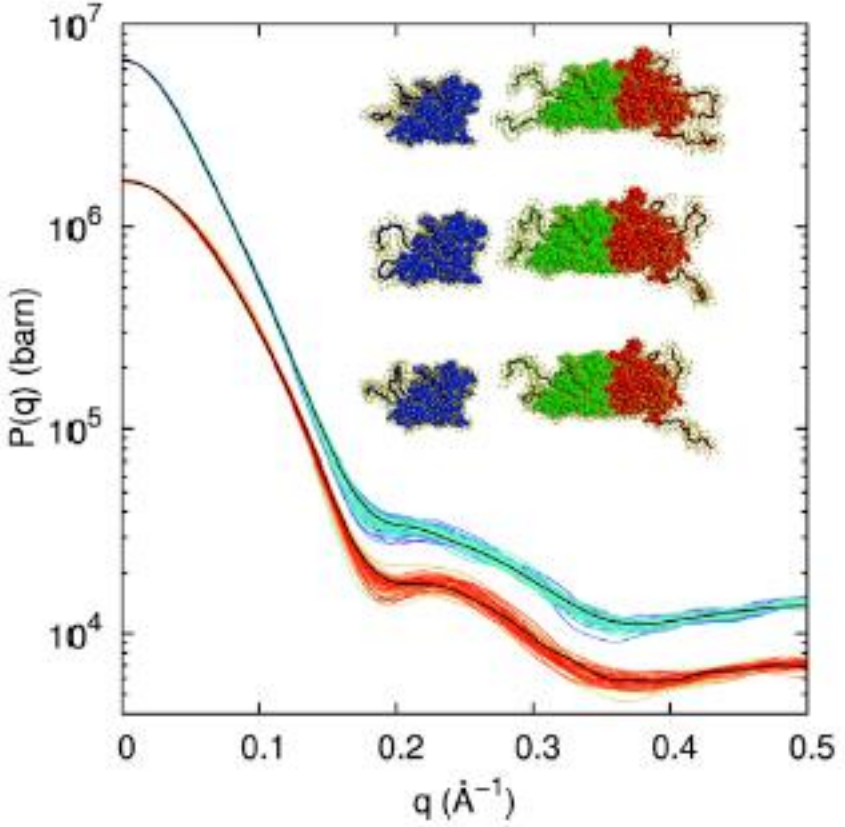


Mostowy, S. & Cossart, P. Autophagy 7, 780–782 (2011).

Septins build 'cages' around bacterial pathogens, immobilizing the harmful microbes and preventing them from invading other healthy cells.
Septins are GTP binding proteins and are involved in leukaemia, colon cancer, Parkinson's disease and Alzheimer's disease.

Human Septin 3-*GC*
Form factors of SEPT3-*GC*1 monomers (red curves) and SEPT3-*GC*2 dimers (cyan curves) corresponding to 100 conformations of each species obtained by using the 3SOP crystallographic structure as template, including the construct primary amino-acid sequence (59–350 aa), the HisTag linked with the N-terminal domain and other missing residues.
Three representative conformations (blue, green, and red) of monomers and dimers are shown.



AMYLOID AGGREGATION

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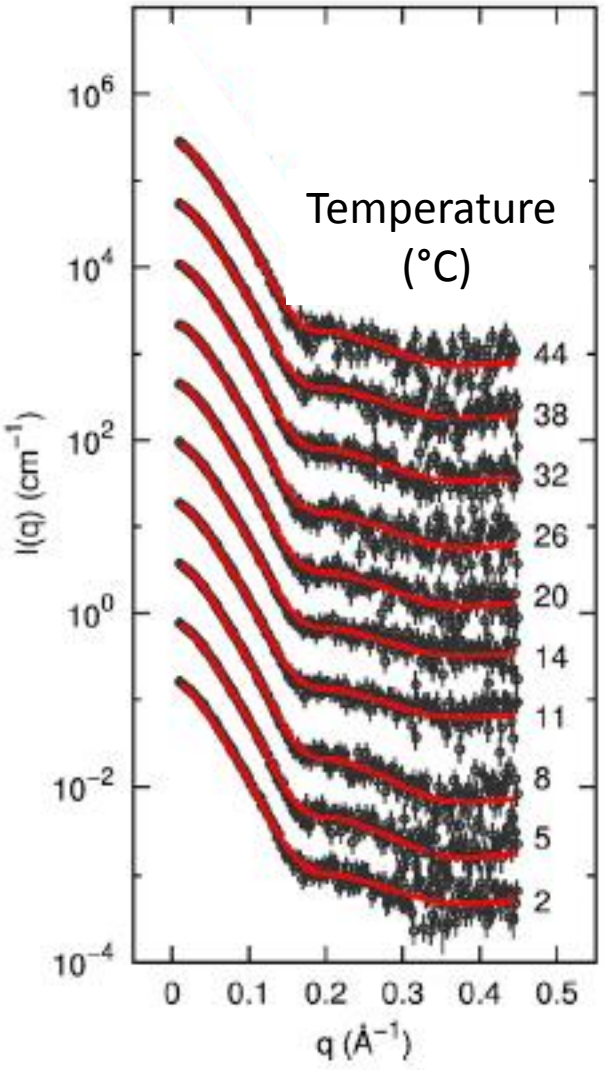
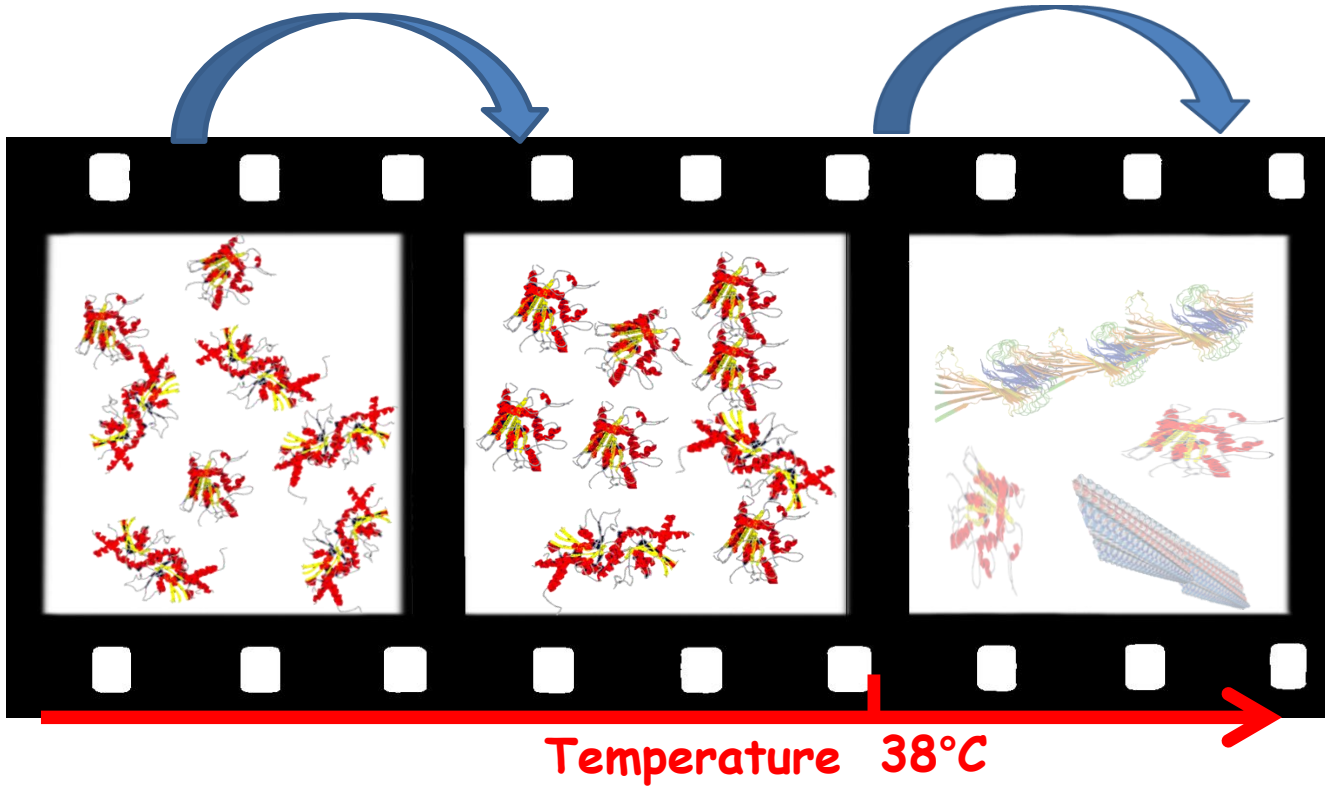


SEPT3-*GC* 5.4 g l^{-1}
pH 7.8



MG. Ortore et al. Biophysical J. 108, 2896. (2015)

AMYLOID AGGREGATION

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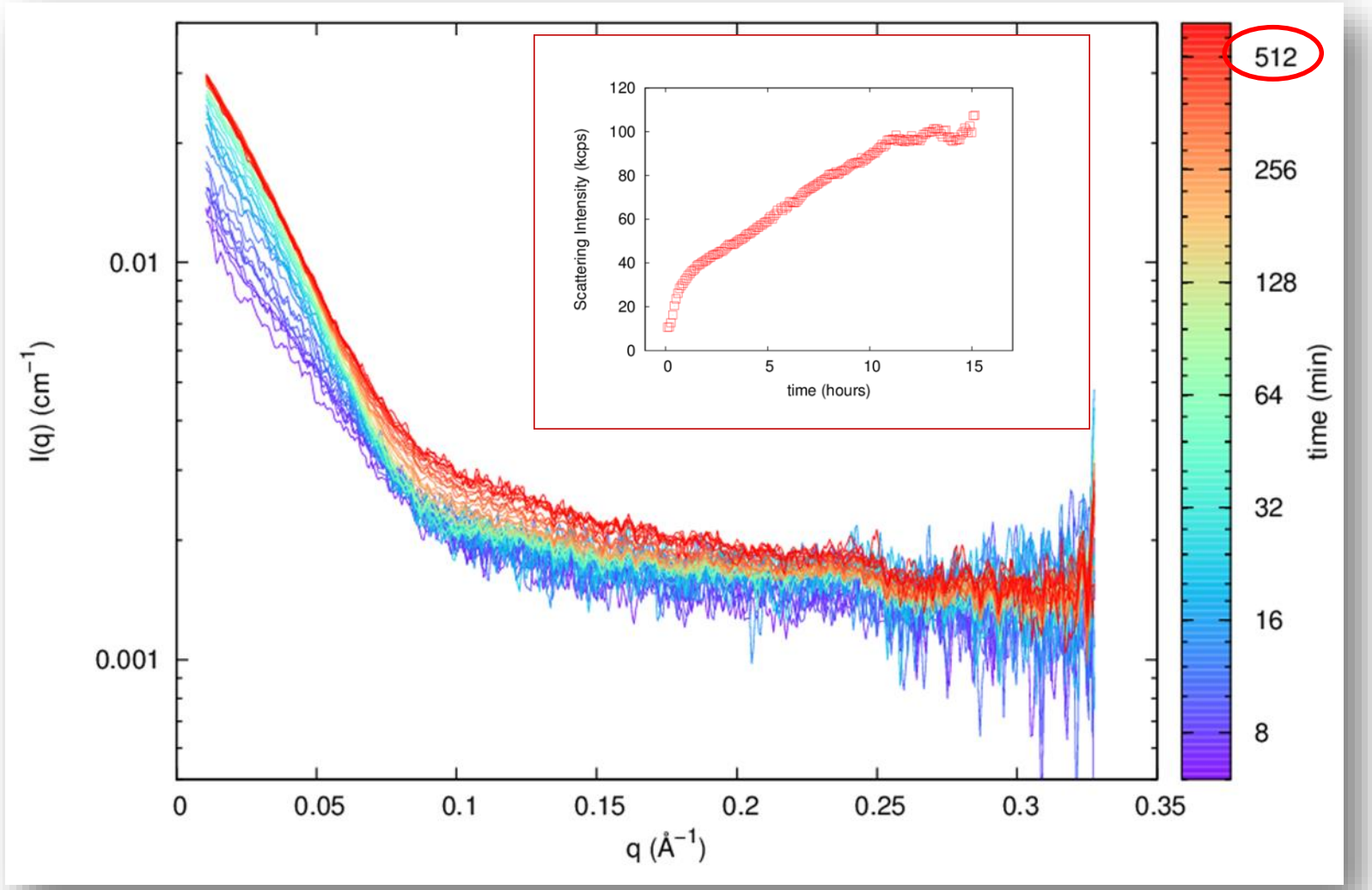

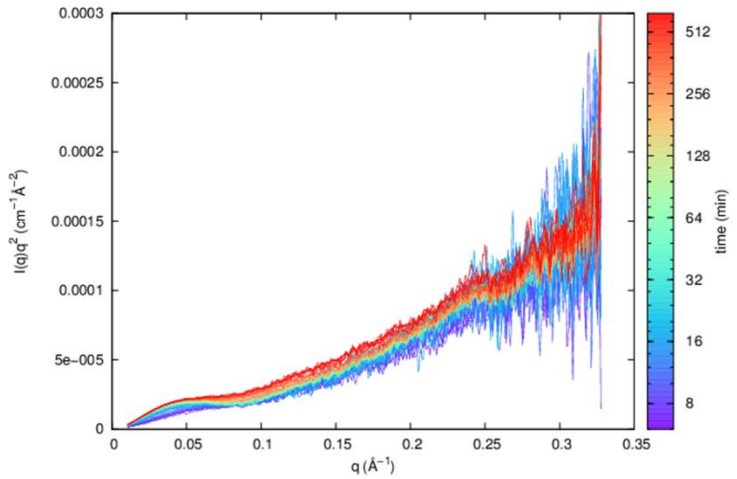

Amyloid β peptide ($A\beta^{42}$) experiment performed at ID2, ESRF, Grenoble, France

AMYLOID AGGREGATION

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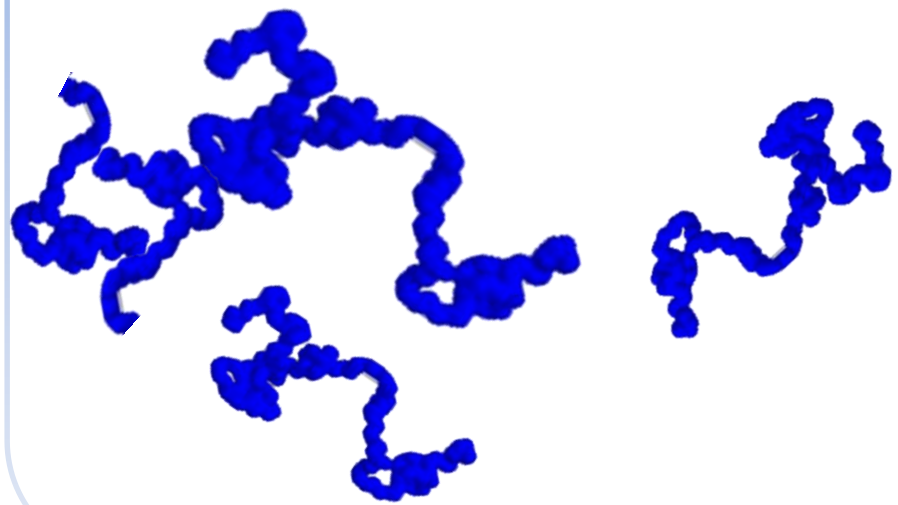
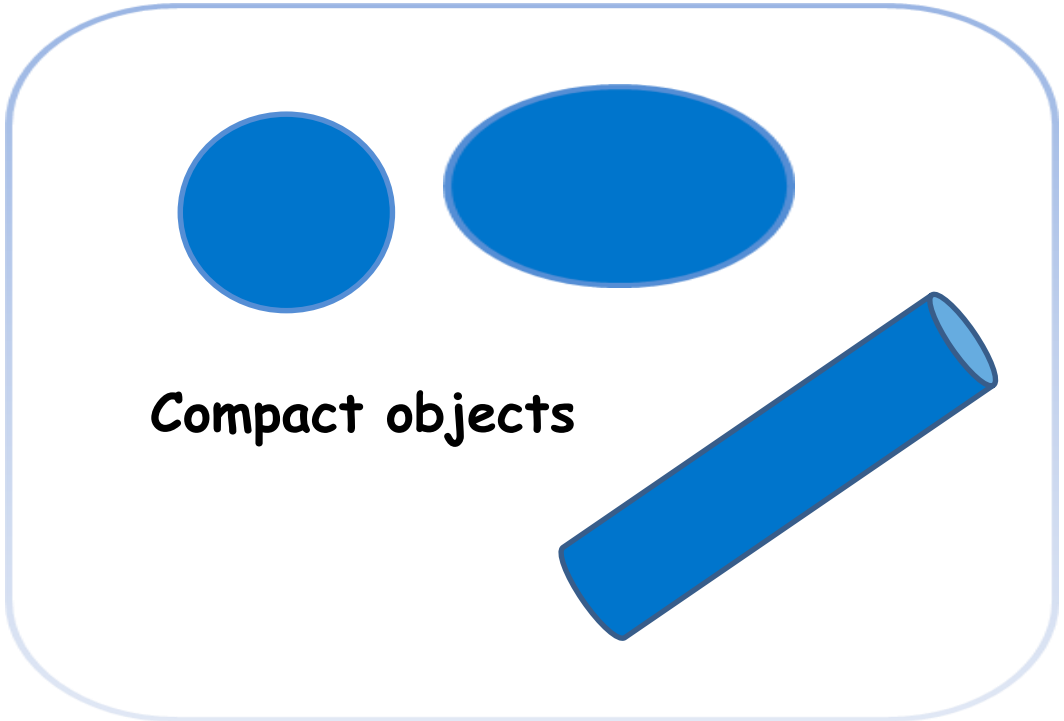
The simplest model…to have the minimum parameters number

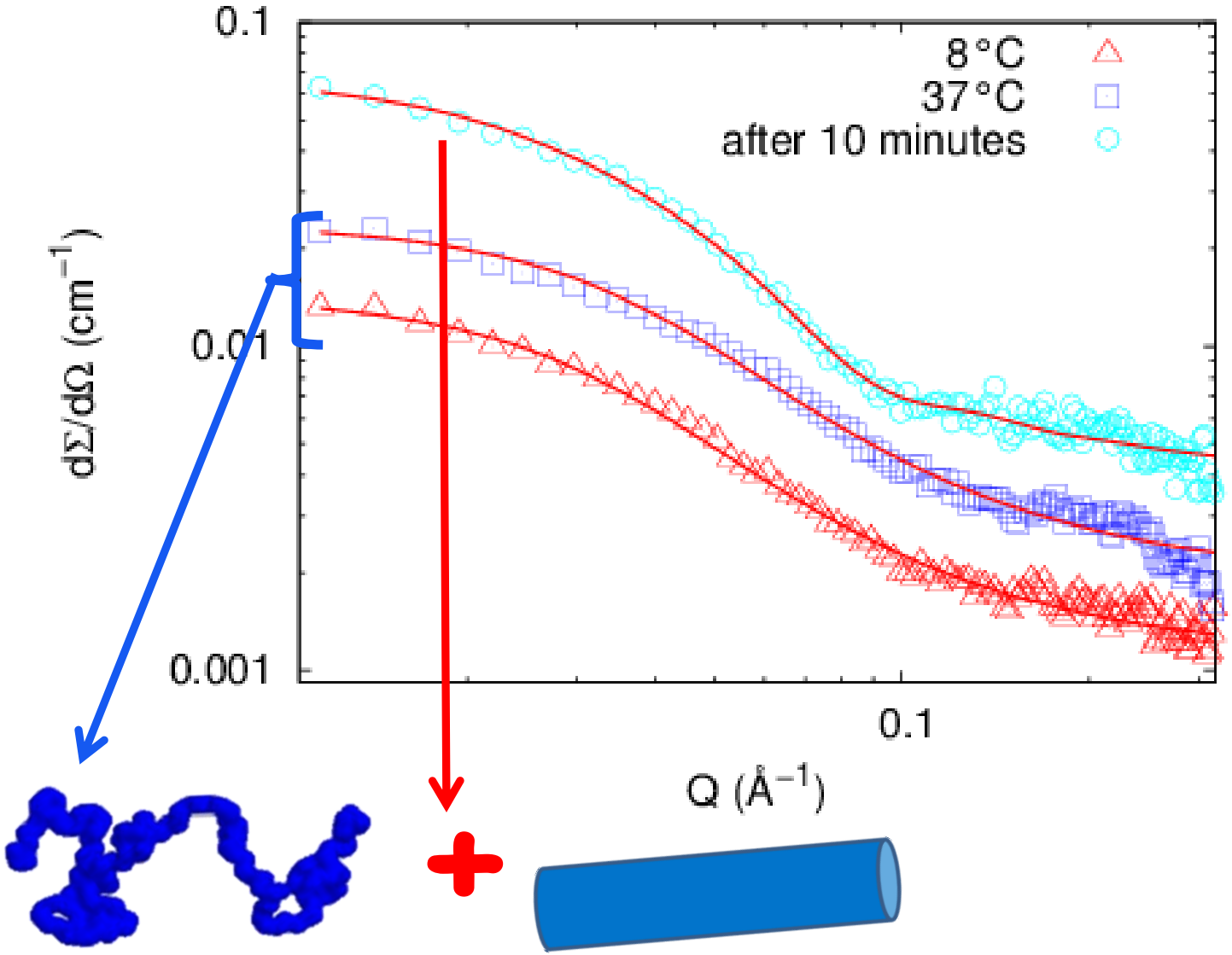
Worm-like

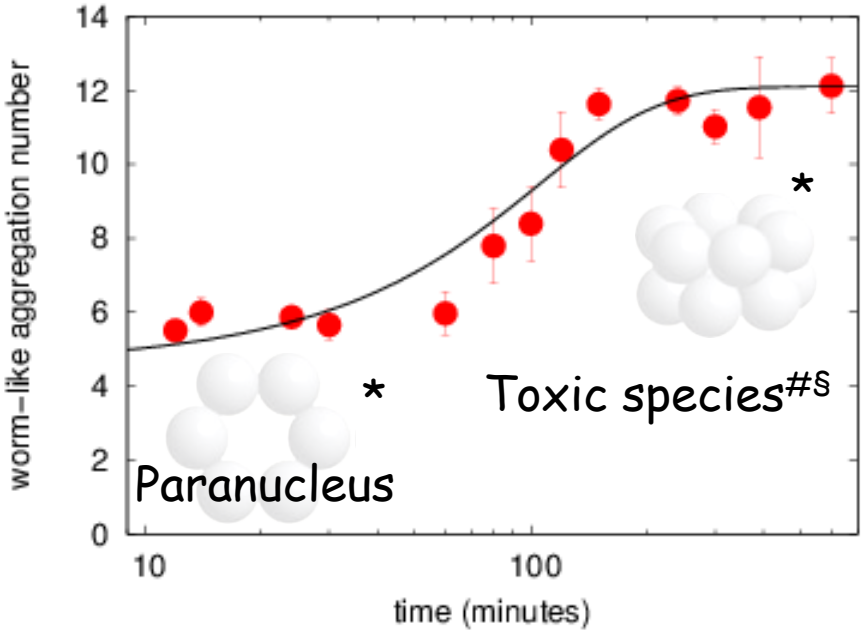




$$\frac{d\Sigma}{d\Omega}(Q) = x_{wl}\frac{d\Sigma}{d\Omega}(Q)\Big|_{wl} + x_{cyl}\frac{d\Sigma}{d\Omega}(Q)\Big|_{cyl}$$

AMYLOID AGGREGATION





*S.L. Bernestein et al. 2009, 10.1038/NCHEM.247

#S. Lesné et al. Nature 2006, 440:352-357

§MN Reed et alNeurobiol Aging 2009

BACK to PROTEIN FOLDING

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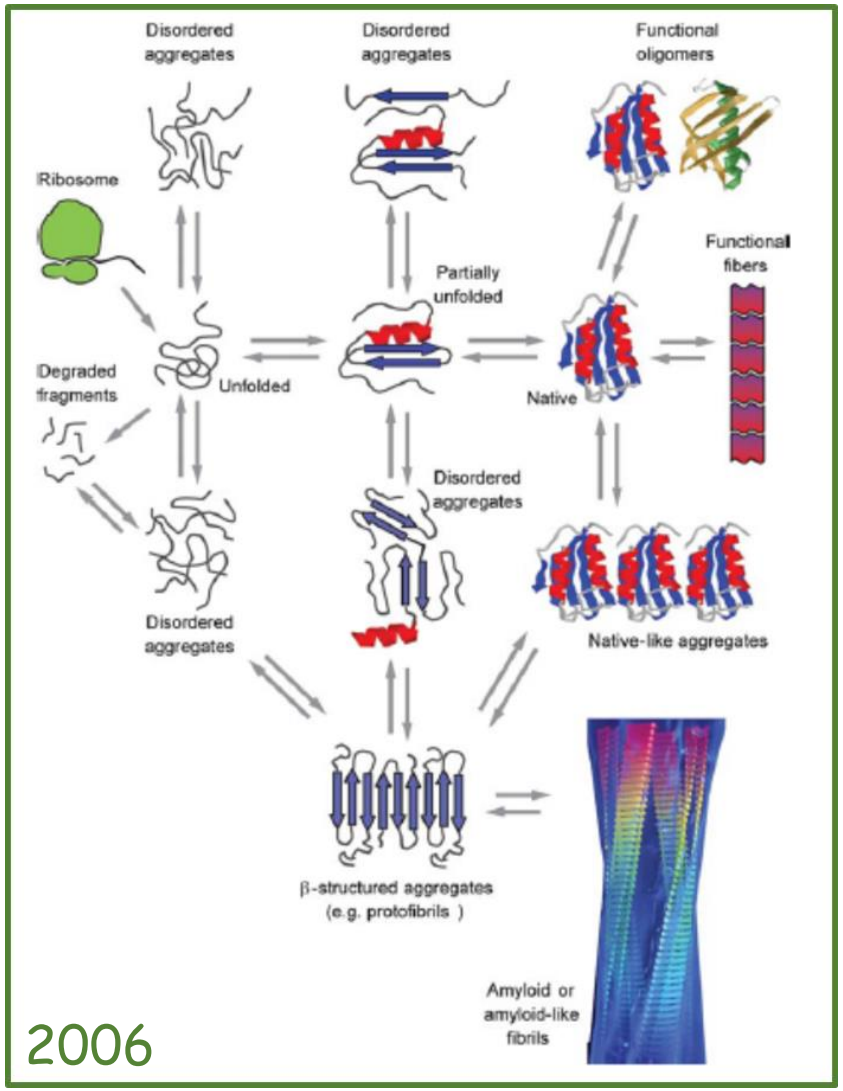
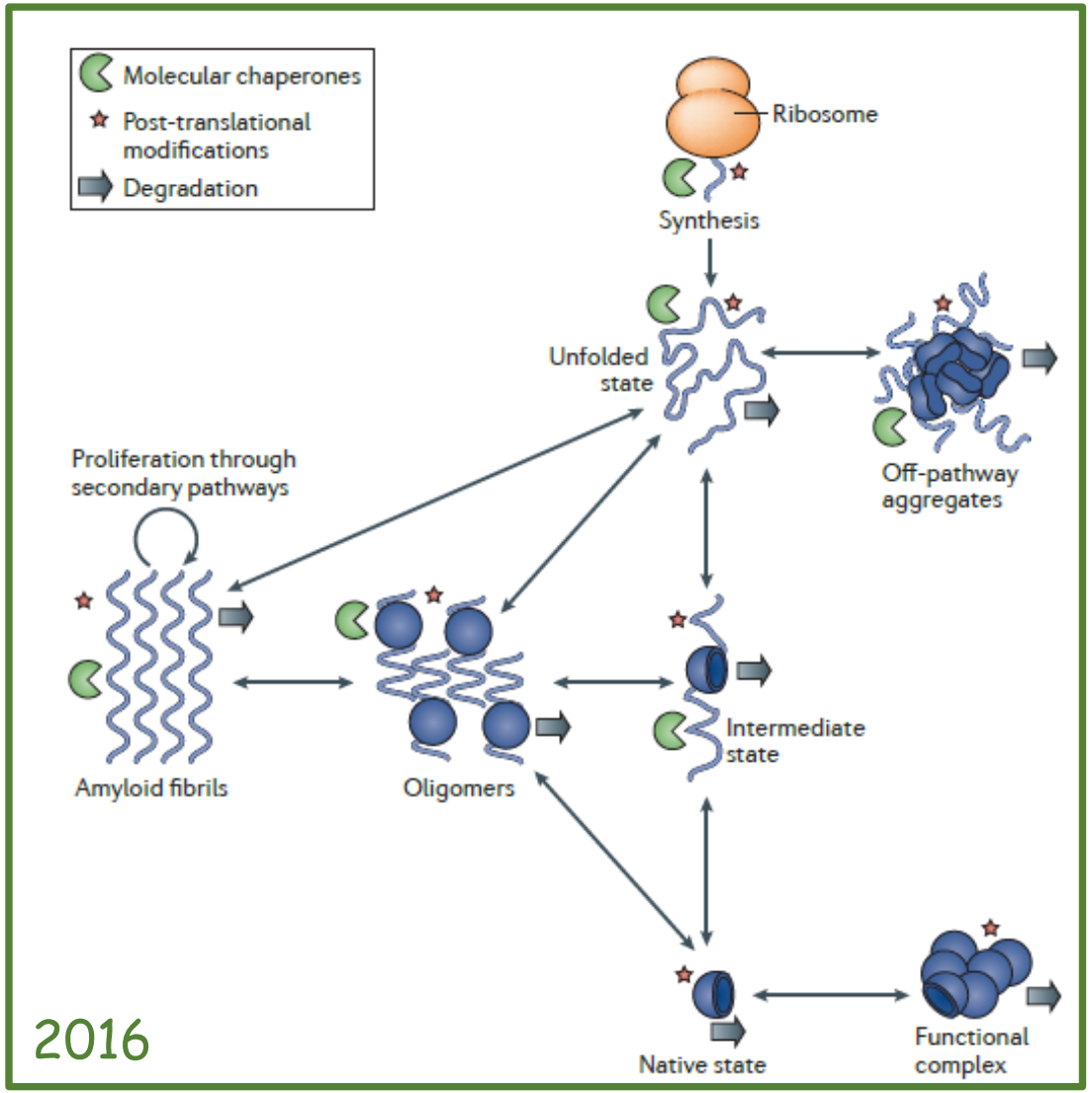
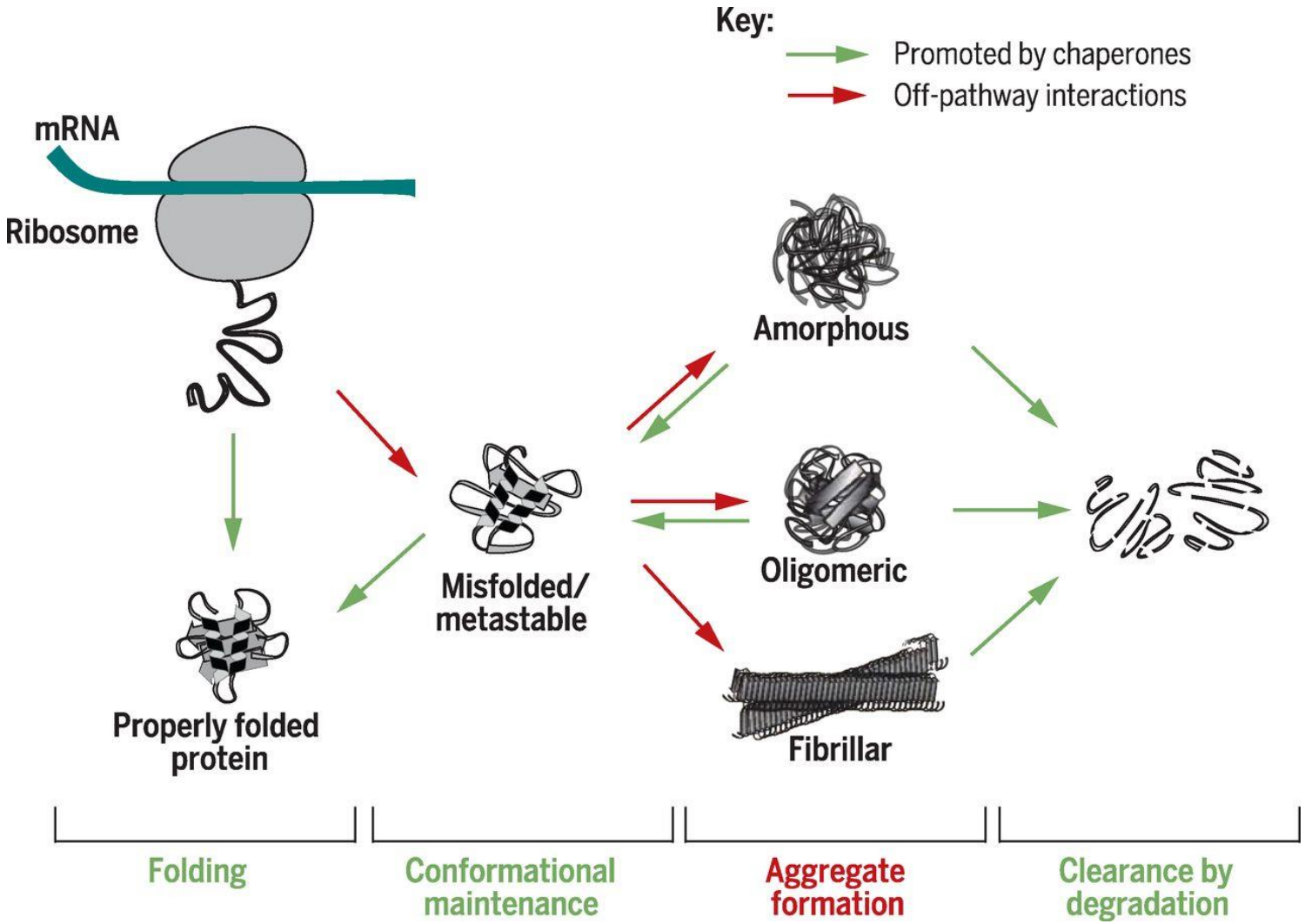




CHAPERONES



MOLECULAR CHAPERONES

- promote the folding of newly synthesized proteins,
- function in conformational maintenance,
- prevent potentially toxic off-pathway aggregation,
- cooperate with other components of the proteostasis network, such as the proteasome system and autophagy, in the removal of terminally misfolded and aggregated proteins through proteolytic degradation.

In vivo aspects of protein folding and quality control. D. Balchin, M. Hayer-Hartl, F. Ulrich Hartl. Science 2016: Vol. 353, 6294.

CHAPERONINES

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- Chaperonines are a class of chaperones that assist in folding of (largely) newly synthesized proteins with the help of ATP

(all chaperonines can be referred to as chaperones, but all chaperones need not be chaperonins)

- Chaperonines have a more specific function.
- They are usually barrel shaped and have a hydrophobic chamber to facilitate folding without the effects of crowding.

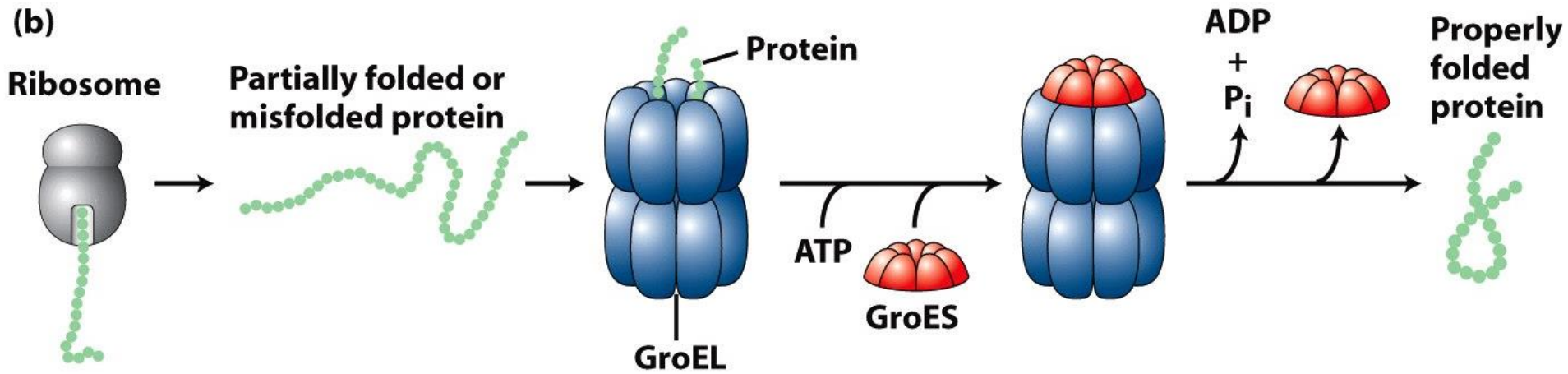
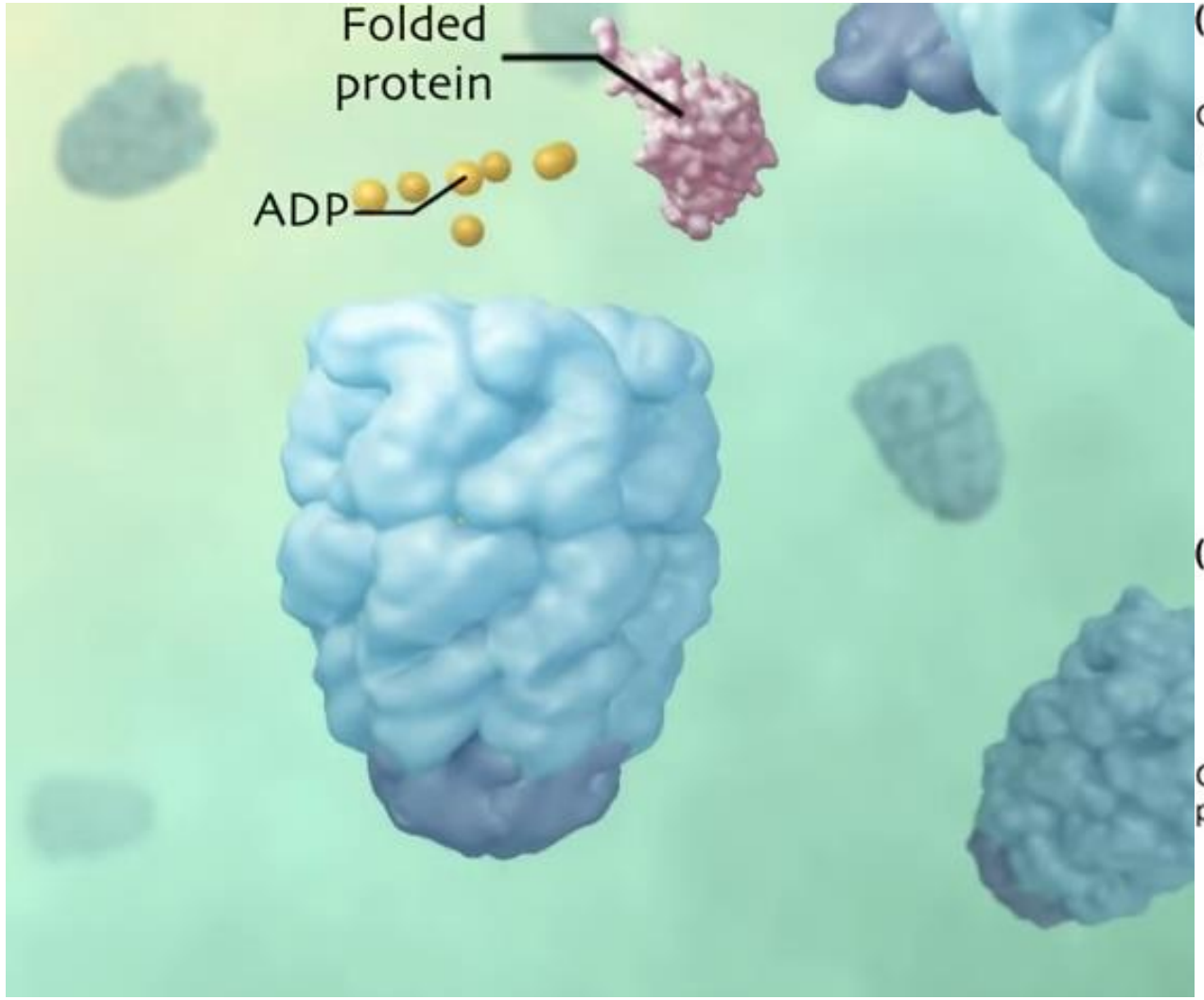
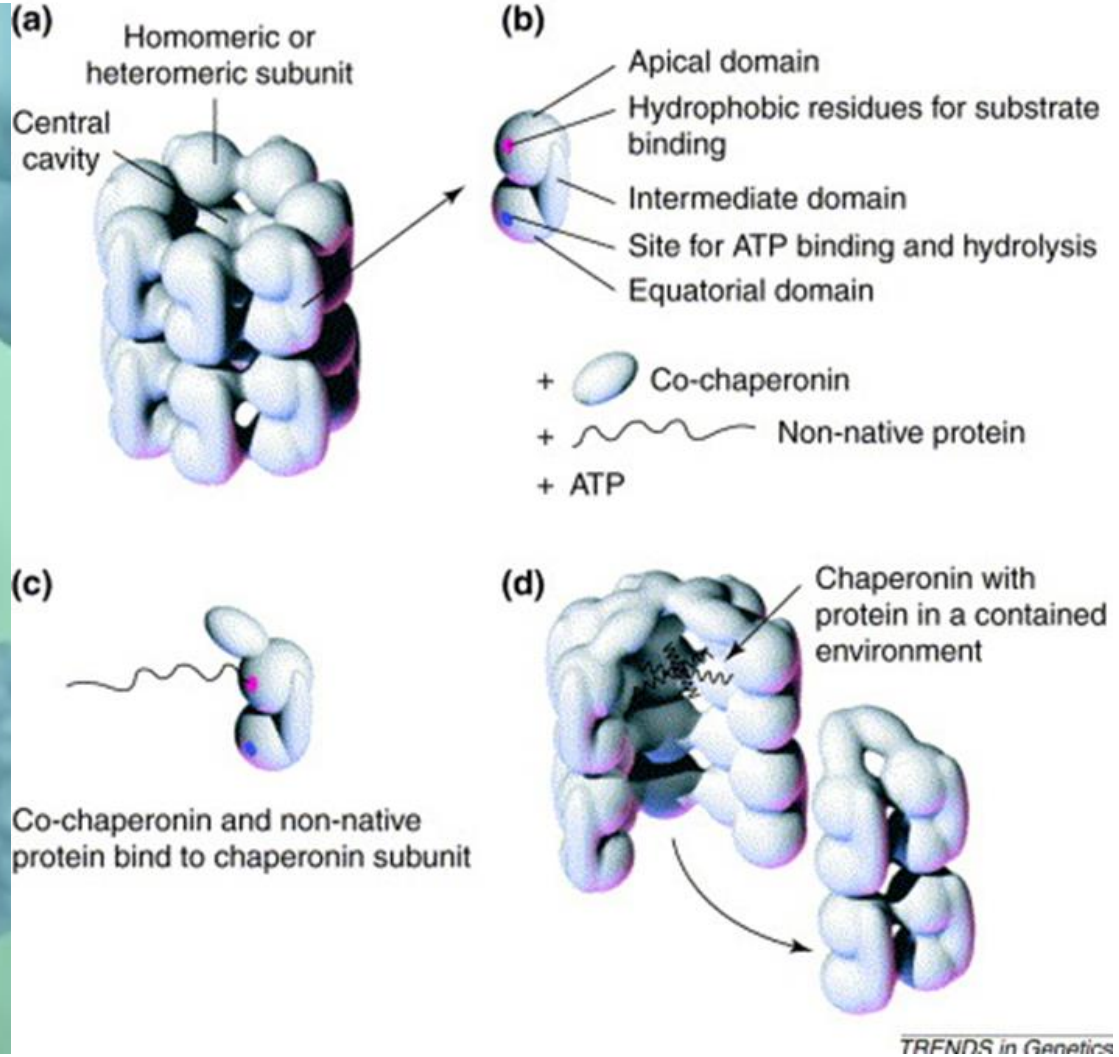


Figure 3-17
Molecular Cell Biology, Sixth Edition

CHAPERONINES





CHAPERONINES

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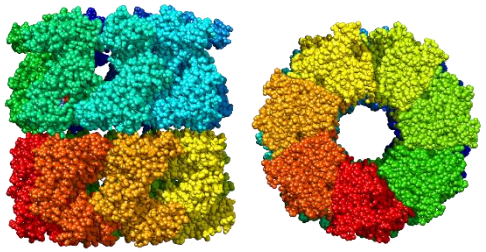
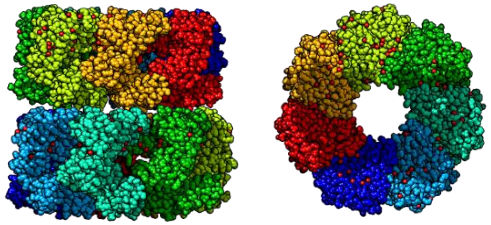
GroEL (MW=840,000)

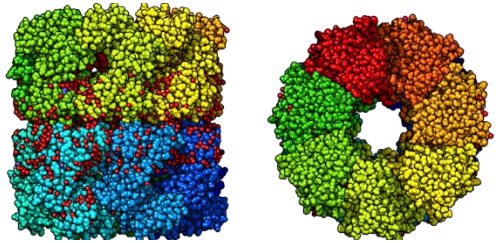
- is a bacterial chaperonine
- assists client protein's folding by forming a tetradecameric structure with a barrel shape obtained by two heptameric rings
- is one of the most deeply and widely studied Heat shock proteins



1KP8
Method: ELECTRON MICROSCOPY
Resolution: 8.0 Å

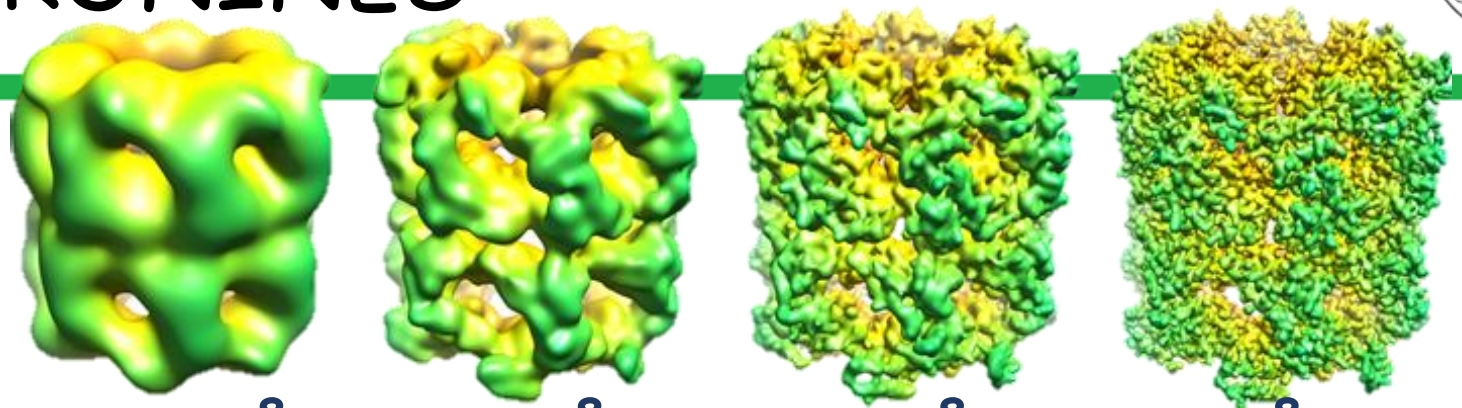


1OEL
Method: X-RAY DIFFRACTION
Resolution: 2.8 Å

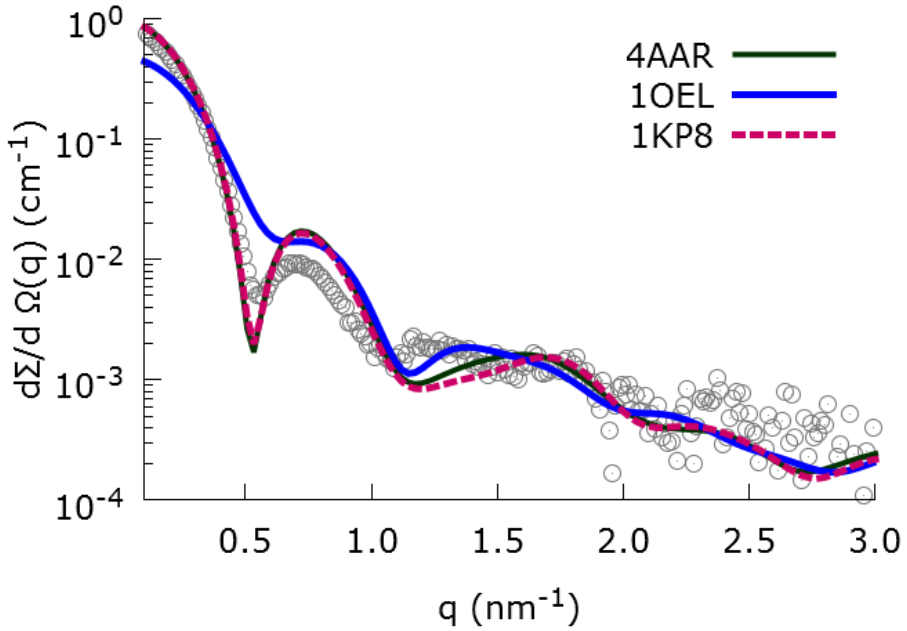


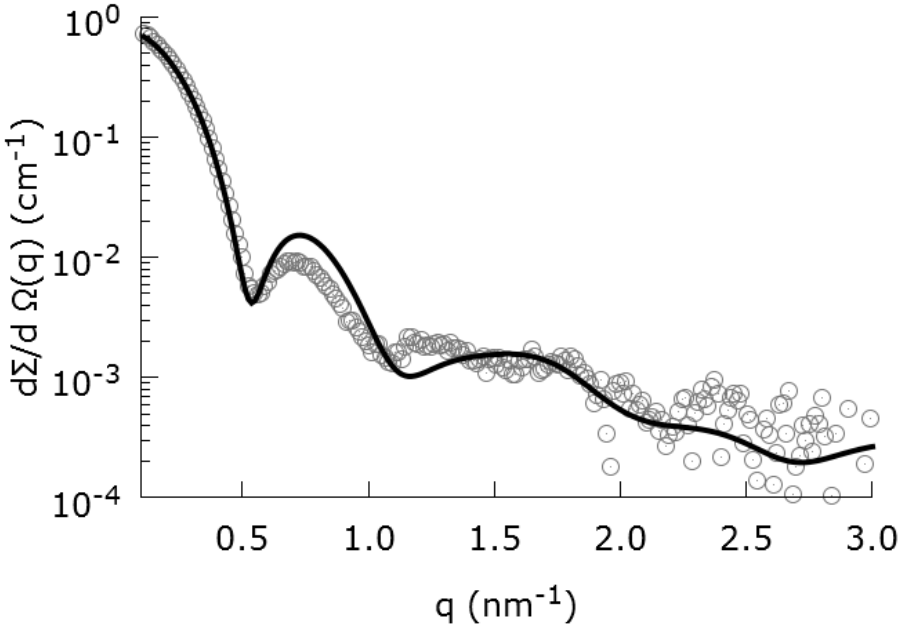
1KP8
Method: X-RAY DIFFRACTION
Resolution: 2.0 Å

CHAPERONINES



Resolution: 32 Å **16 Å** **8 Å** **4 Å**





SAXS experimental profiles of GroEL (c=3 gL−1) with tentative fitting curves obtained from the crystallographic structures indicated in the legend (left), and from a combination (right) of tetradecamers and heptamers

CHAPERONINES

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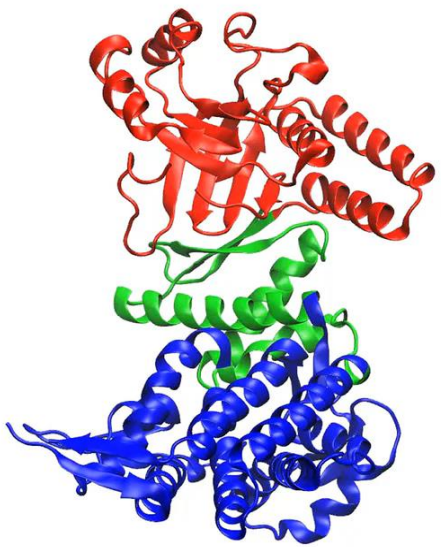
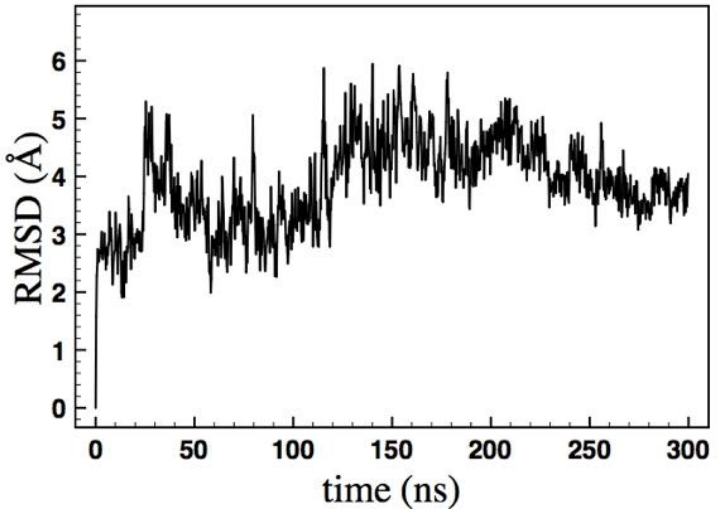
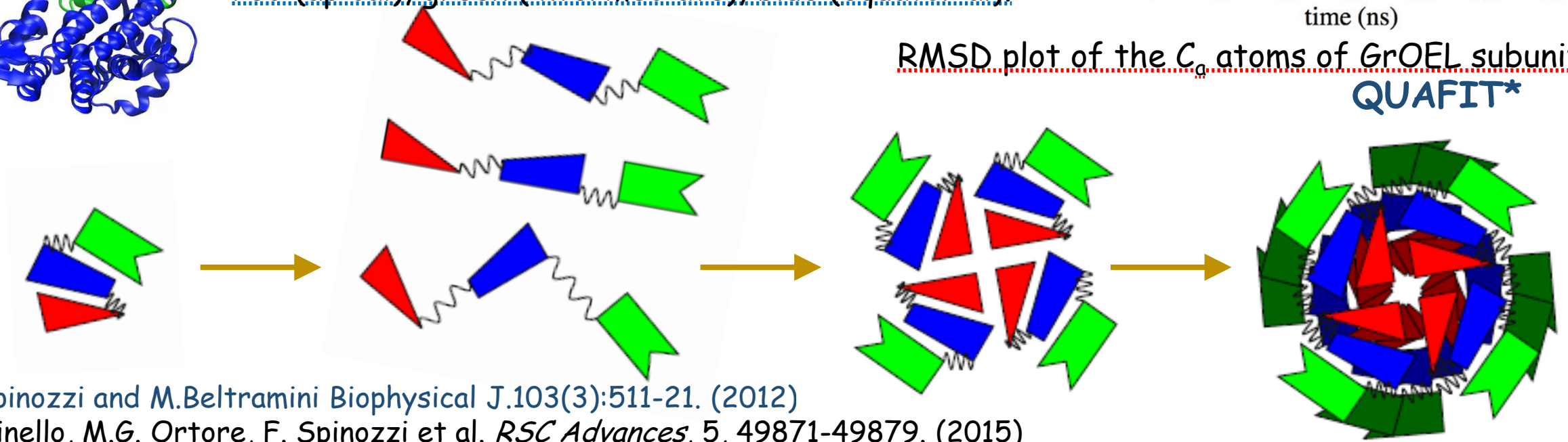


MD simulation performed by GROMACS 4.6.5 with Amber ff99SB-ILDN force field on GroEL monomer extracted from the ring T of 4AAR PDB entry. Principal component analysis (PCA) has revealed the presence of three main bending modes. Representative snapshot with the 3 domains shown in red (apical), green (intermediate), blue (equatorial).



RMSD plot of the C_α atoms of GrOEL subunit.

QUAFIT*



*F. Spinozzi and M.Beltramini Biophysical J.103(3):511-21. (2012)
A. Spinello, M.G. Ortore, F. Spinozzi et al. *RSC Advances*, 5, 49871-49879. (2015)

CHAPERONINES

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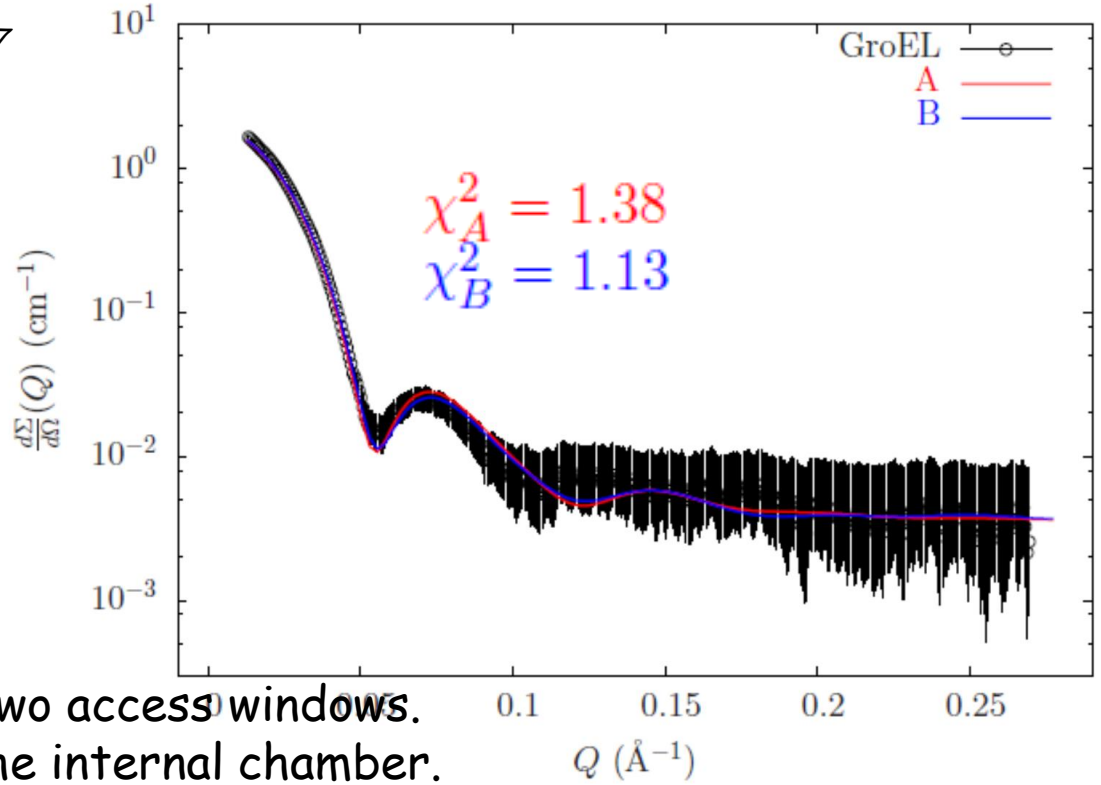
Point group symmetry D_7

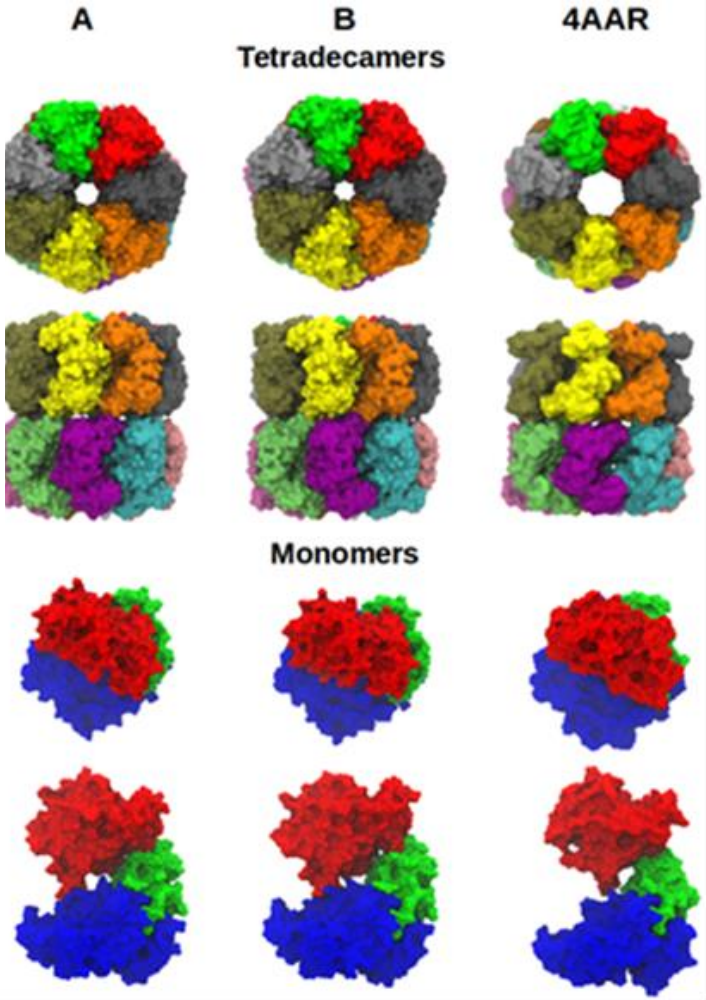
Rigid Domains:

- 1-187 and 377-524
- 192-372

Flexible Linkers:

- 188-191
- 373-376





- ✓ The lower size of the two access windows.
- ✓ The smaller shape of the internal chamber.
- ✓ The conformational changes of the monomer are close to the bending mode of the first eigenvector evidenced by PCA.
- ✓ New intra-ring and inter-ring hydrogen bonds and salt bridges.

A. Spinello, M.G. Ortore, F. Spinozzi et al. *RSC Advances*, 5, 49871-49879 (2015)

CHAPERONINES

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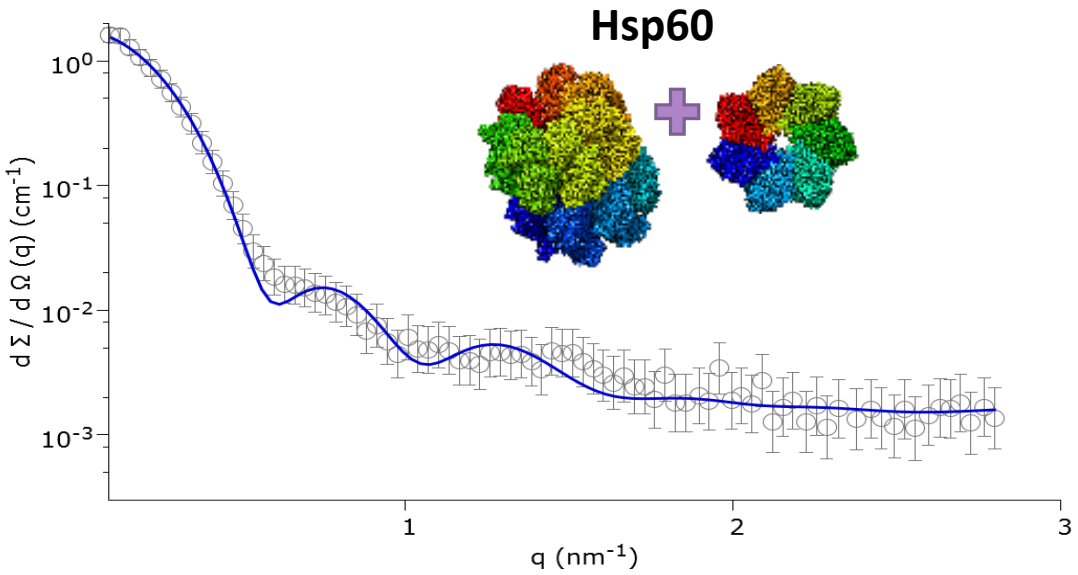
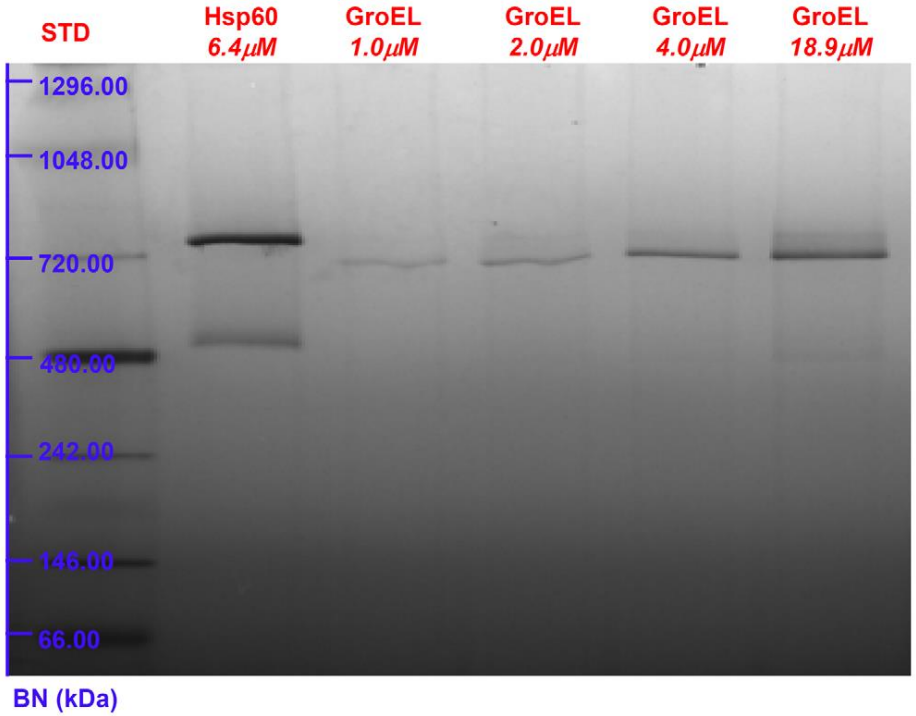


Human homolog Hsp60 was structurally resolved…

…confirming previous experimental evidences*



*S. Vilasi et al. PLoS One 9(5): e97657 (2014)

A. Spinello, M.G. Ortore, F. Spinozzi et al. *RSC Advances*, 5, 49871-49879 (2015)

CHAPERONINES and AMYLOIDS

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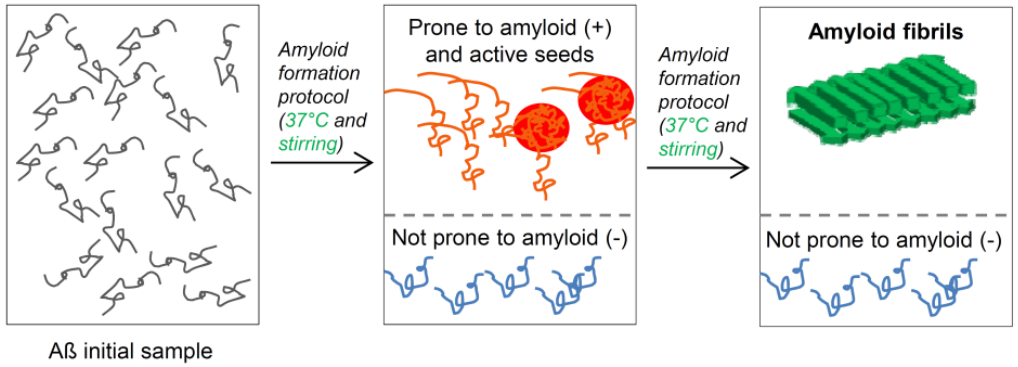
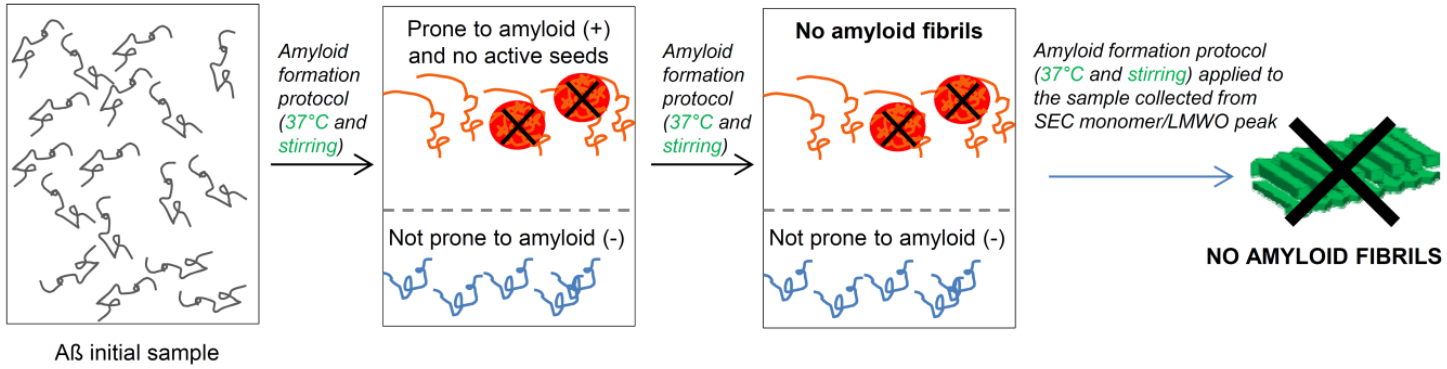
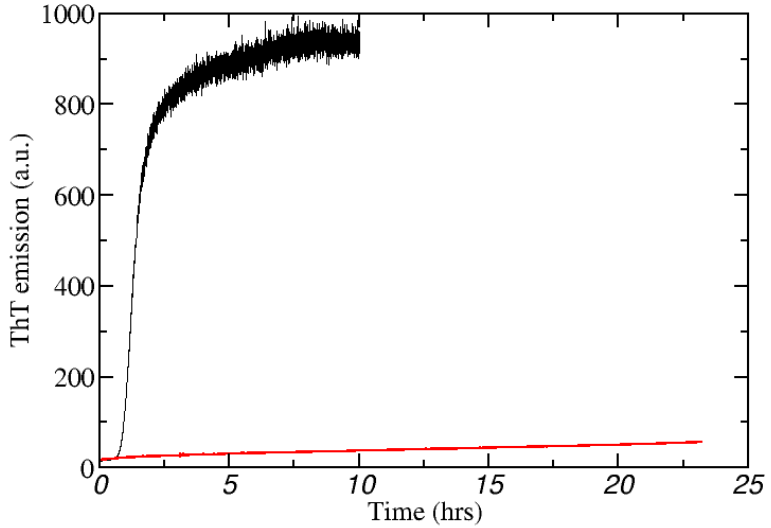
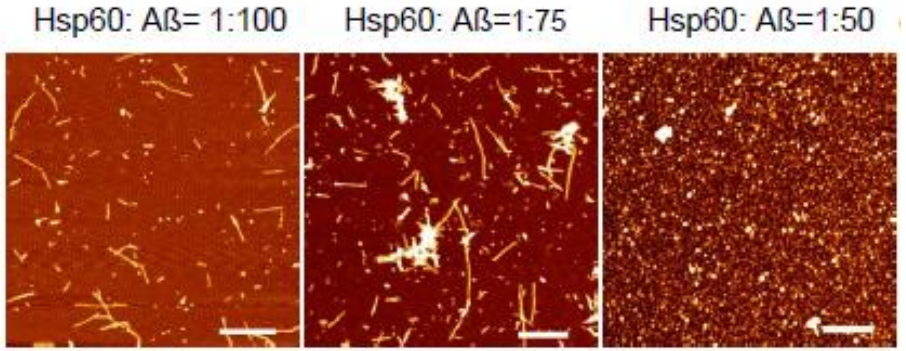
IN THE ABSENCE OF HSP60



IN THE PRESENCE OF HSP60







M. R. Mangione, S. Vilasi et al. BBA Gen. Subject, 2016.

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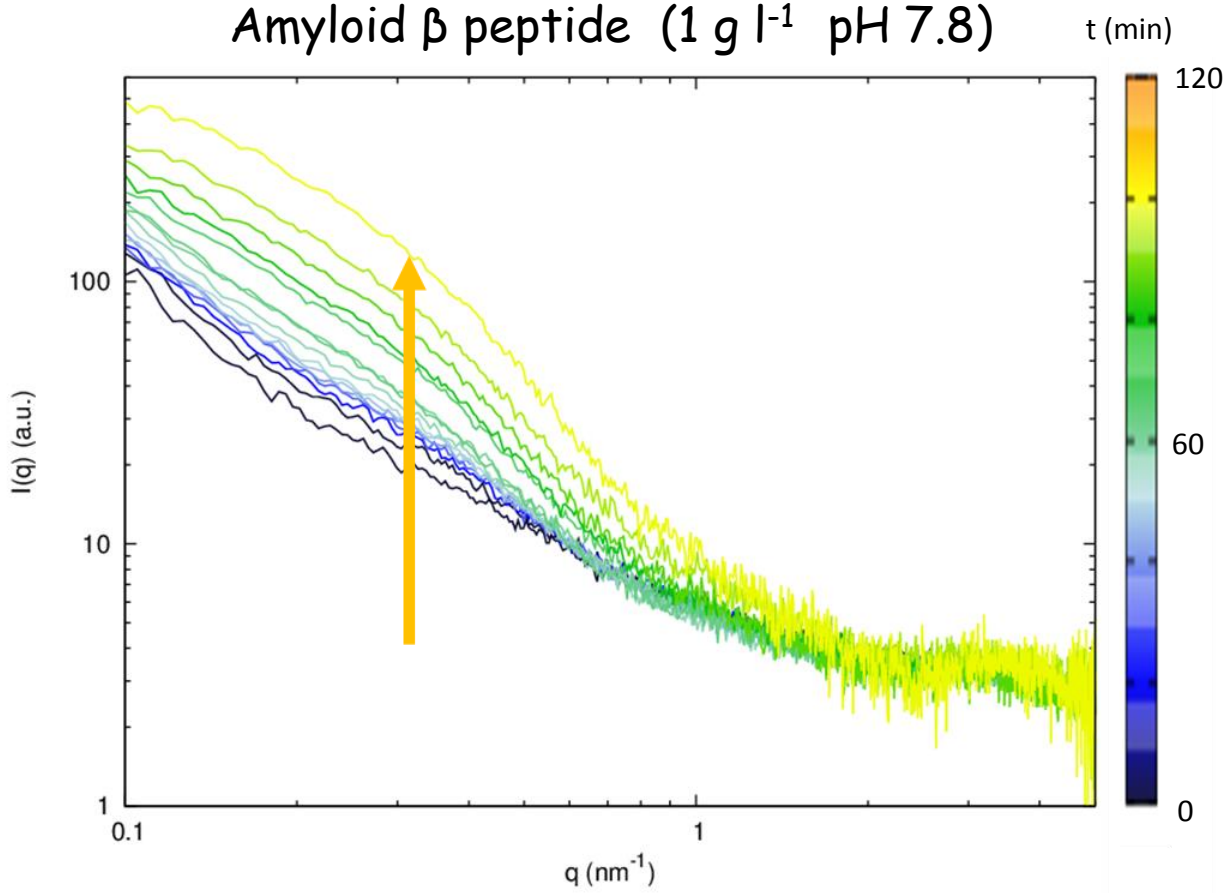
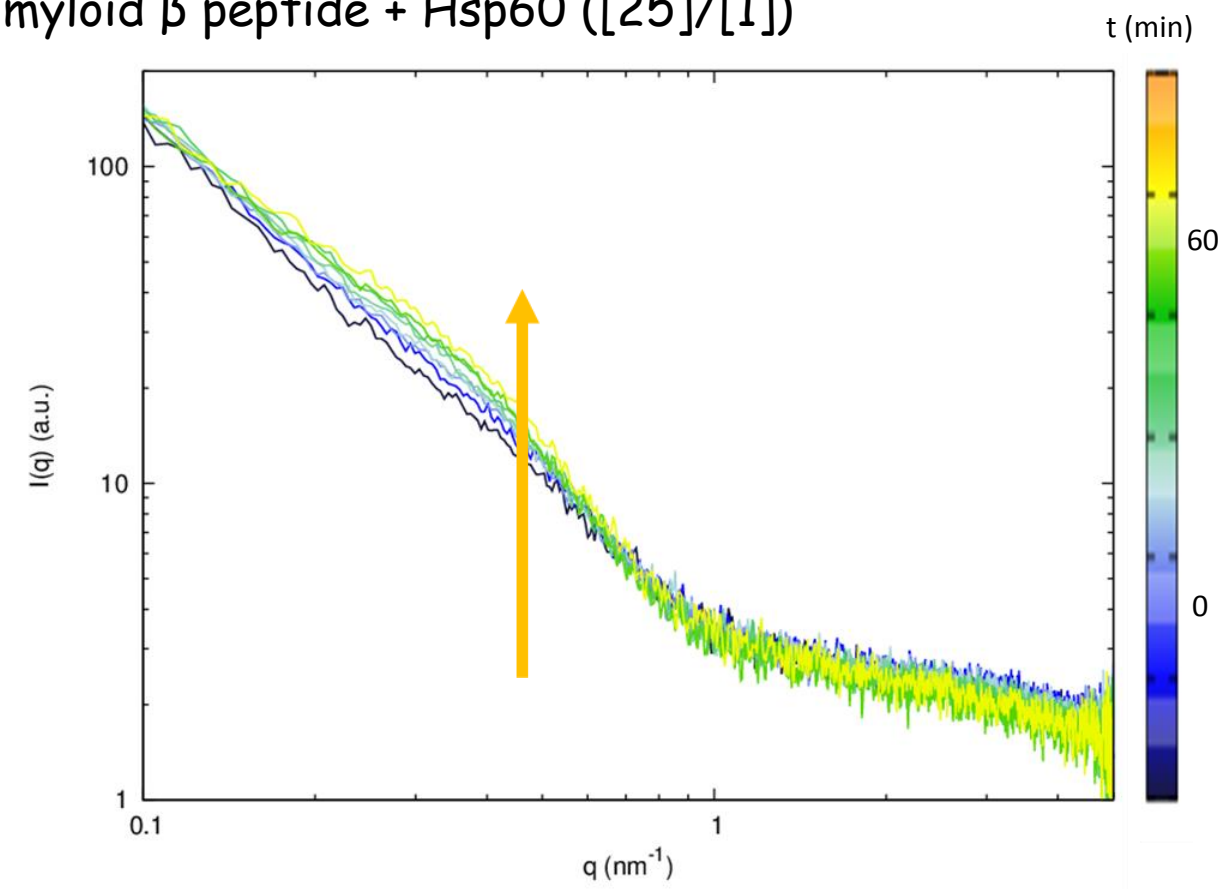
Amyloid β peptide (1 g l^{-1} pH 7.8)



Amyloid β peptide + Hsp60 ([25]/[1])



CHAPERONINES and AMYLOIDS

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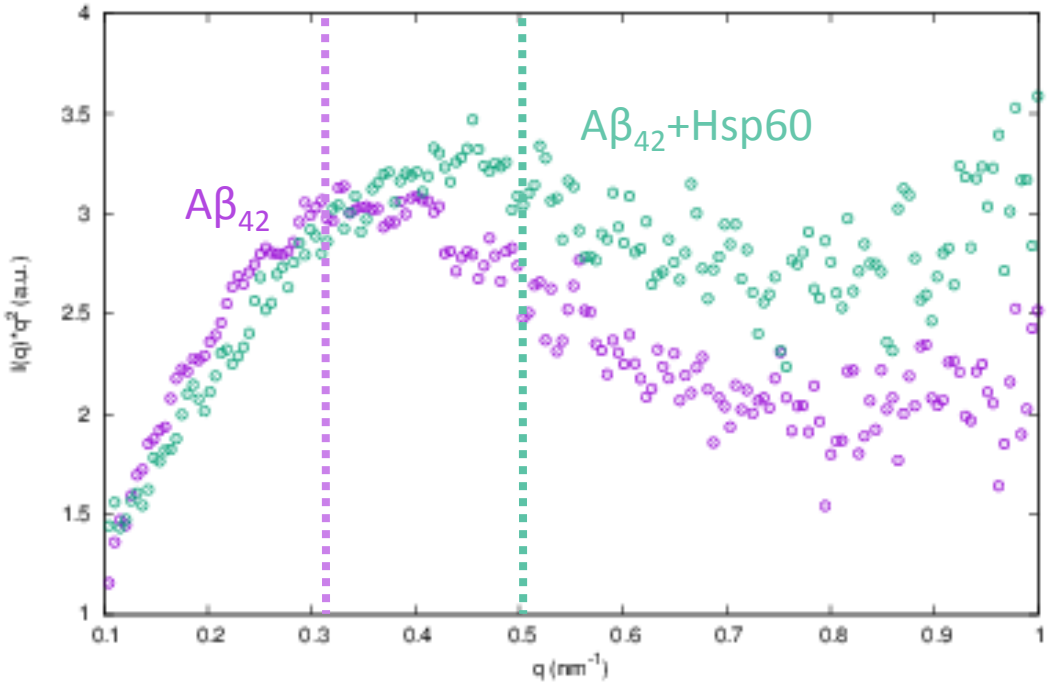
Kratky plots evidences that both with and without Hsp60 the final product of aggregation is mainly compact.
The average dimension of the final aggregation is ≈30% greater in absence of Hsp60

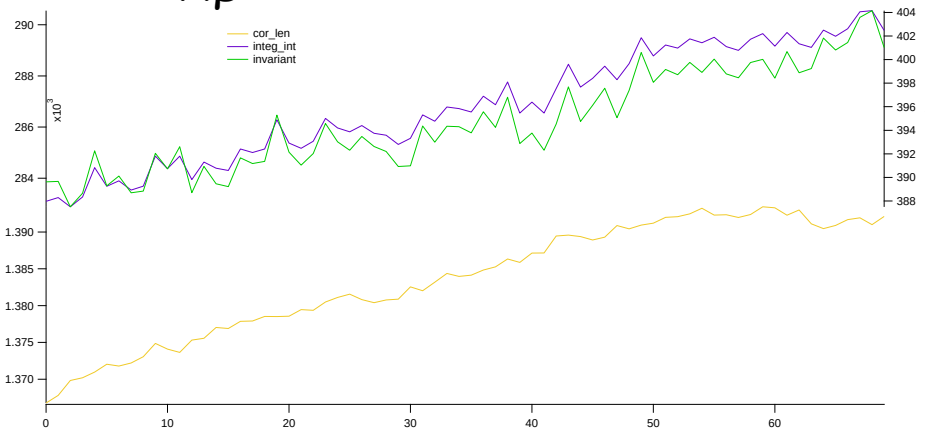


Invariant

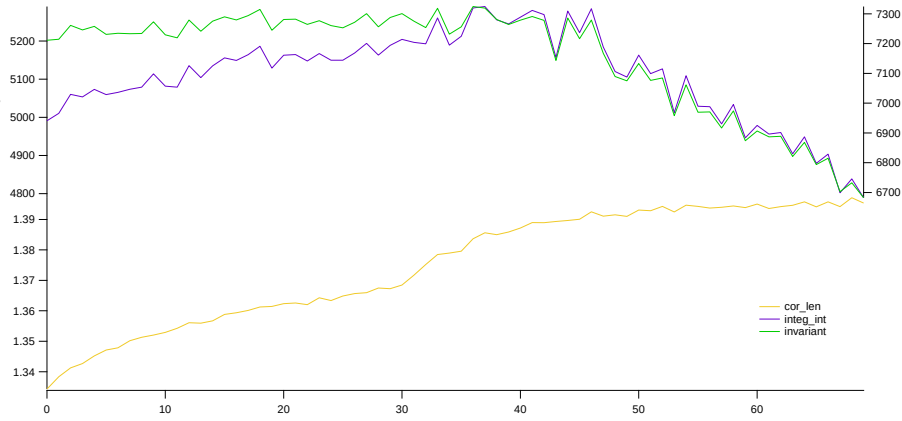
Integrated Intensity

Correlation lengths

Aβ



Aβ + HSP60 [25]:[1]



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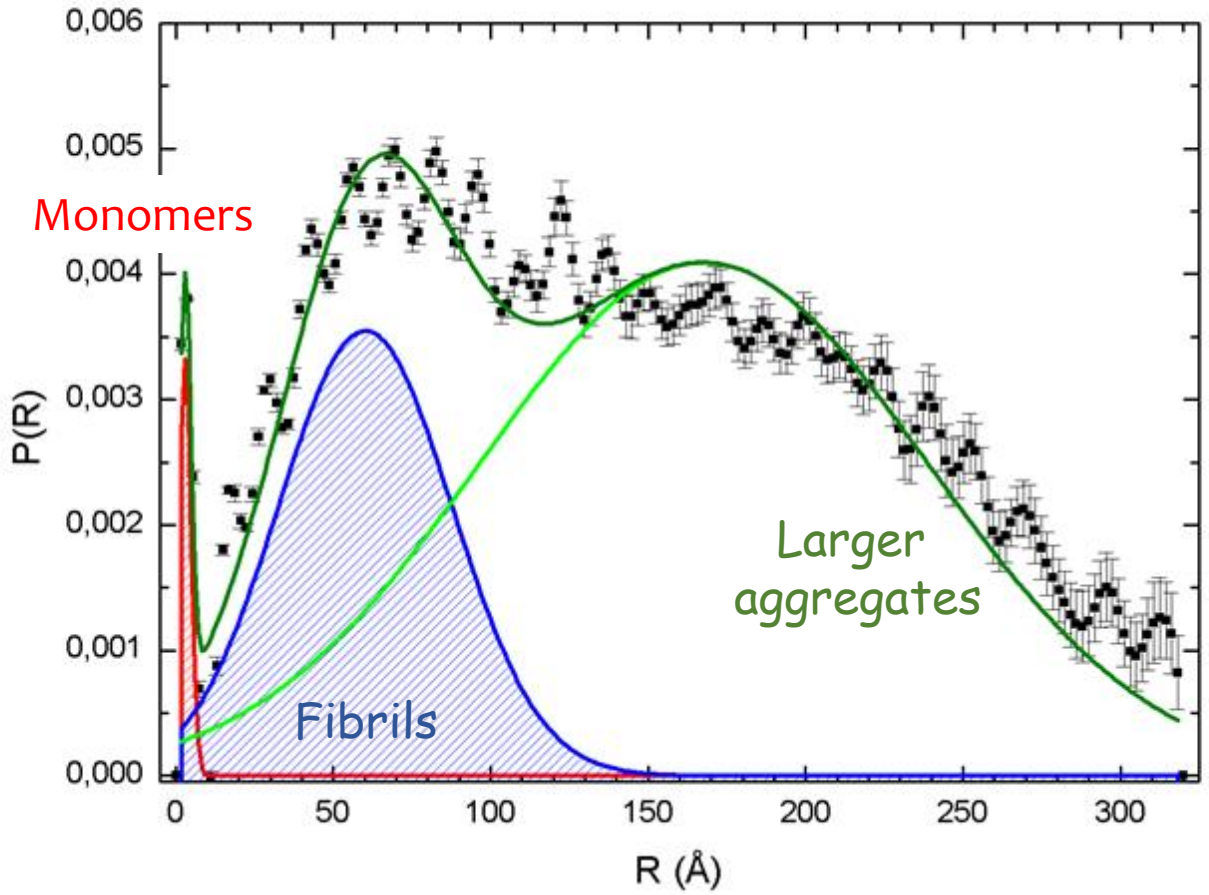

CHAPERONINES and AMYLOIDS

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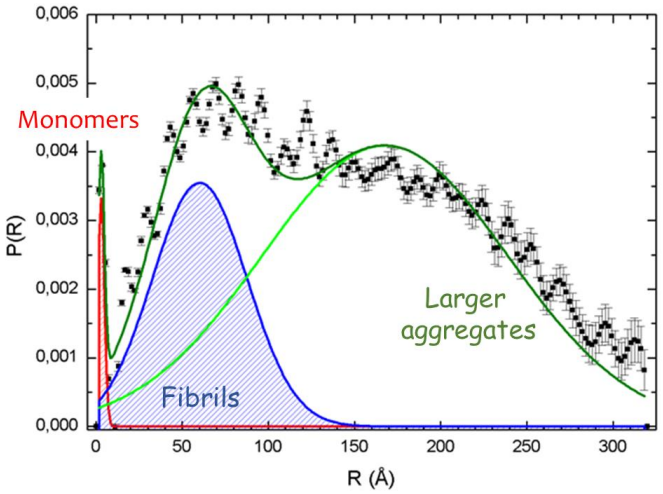

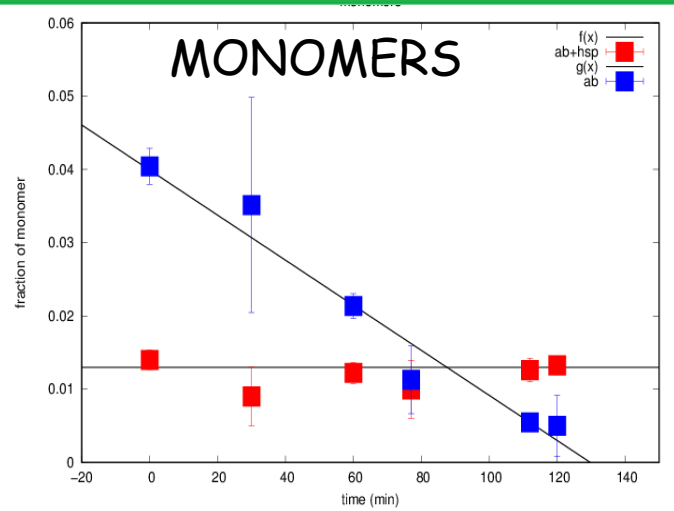

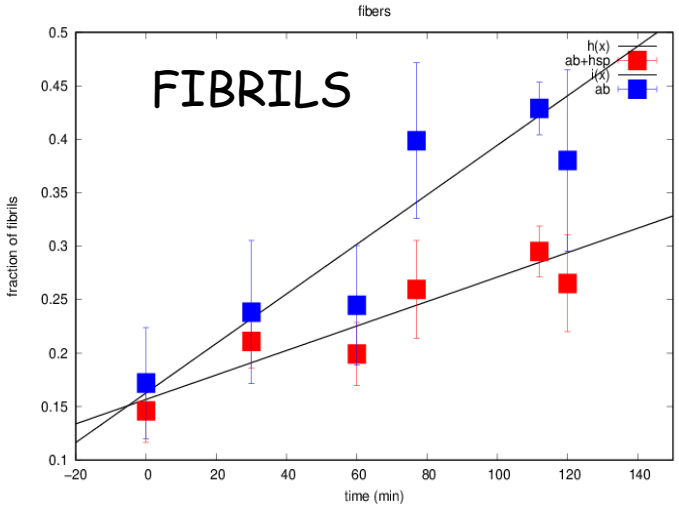

$A\beta^{42}$ 200 μM

$A\beta^{42}$ 200 μM + Hsp60 8 μM

Hsp60 seems to interact with monomers, seizing them and inhibiting their aggregation, slowing the fibril formation.

CHAPERONINES and AMYLOIDS

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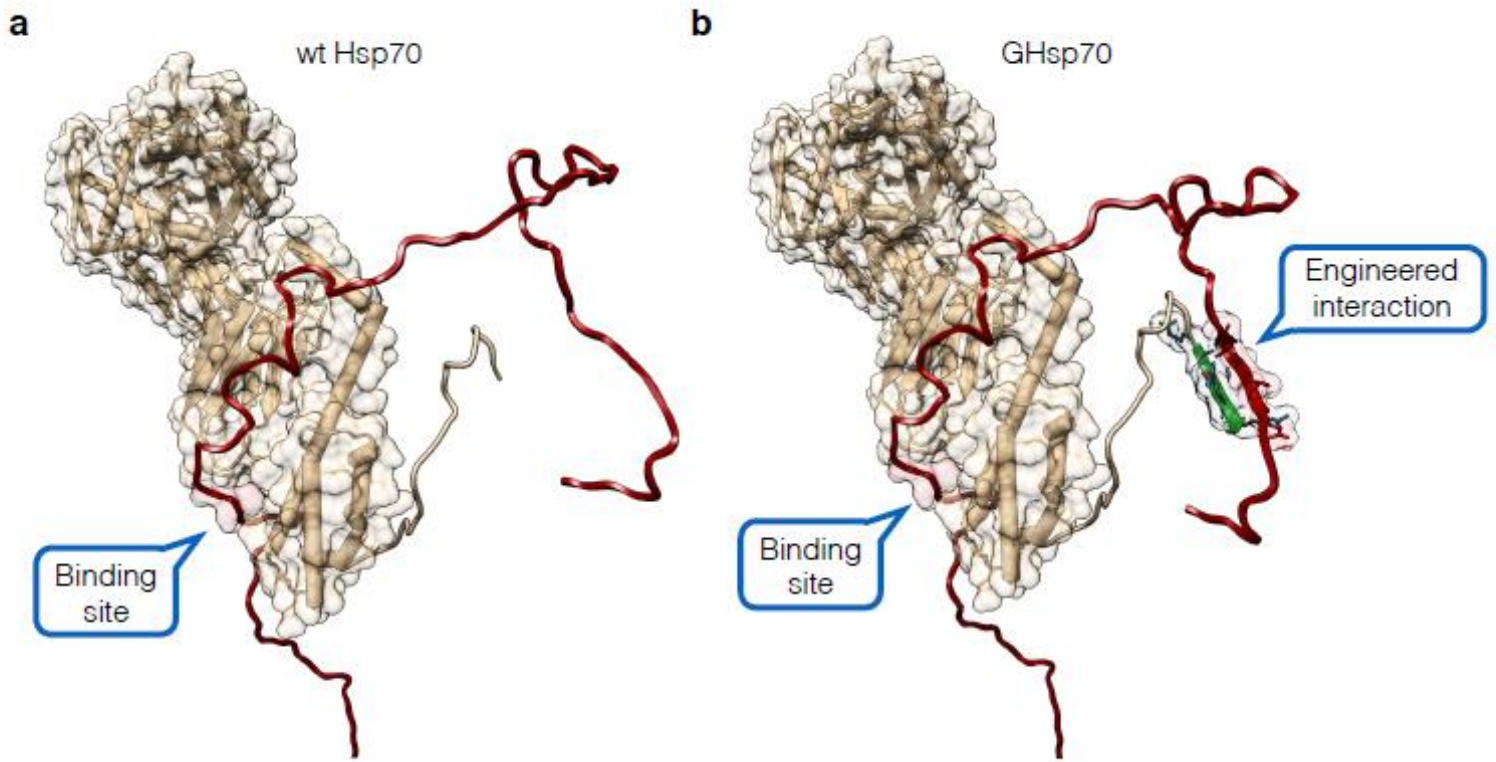
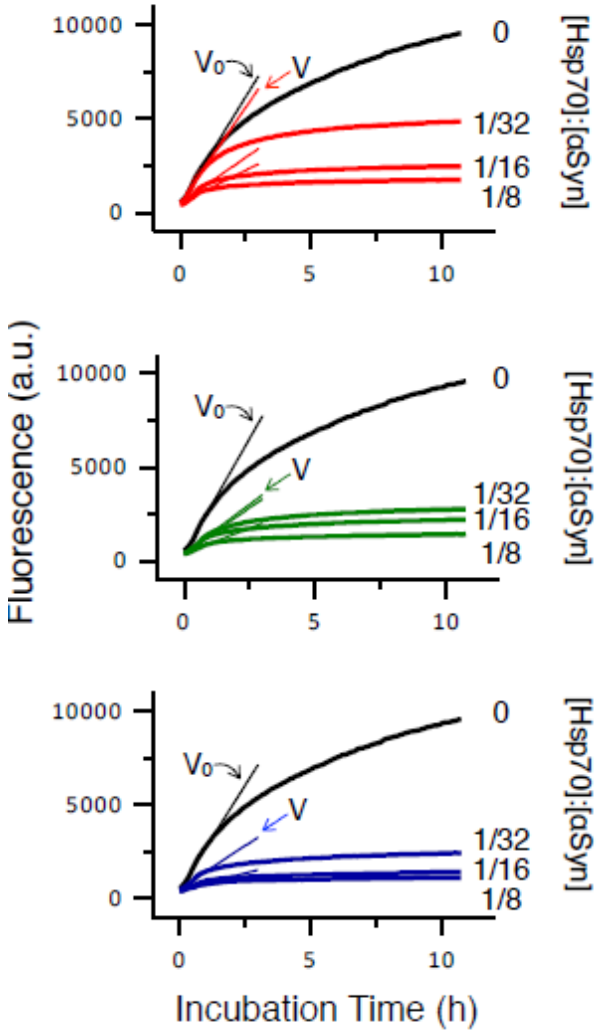
Hsp70 + α-synuclein





F. A. Aprile, P. Sormanni, M. Vendruscolo. Biochemistry 2015

IN PROGRESS…

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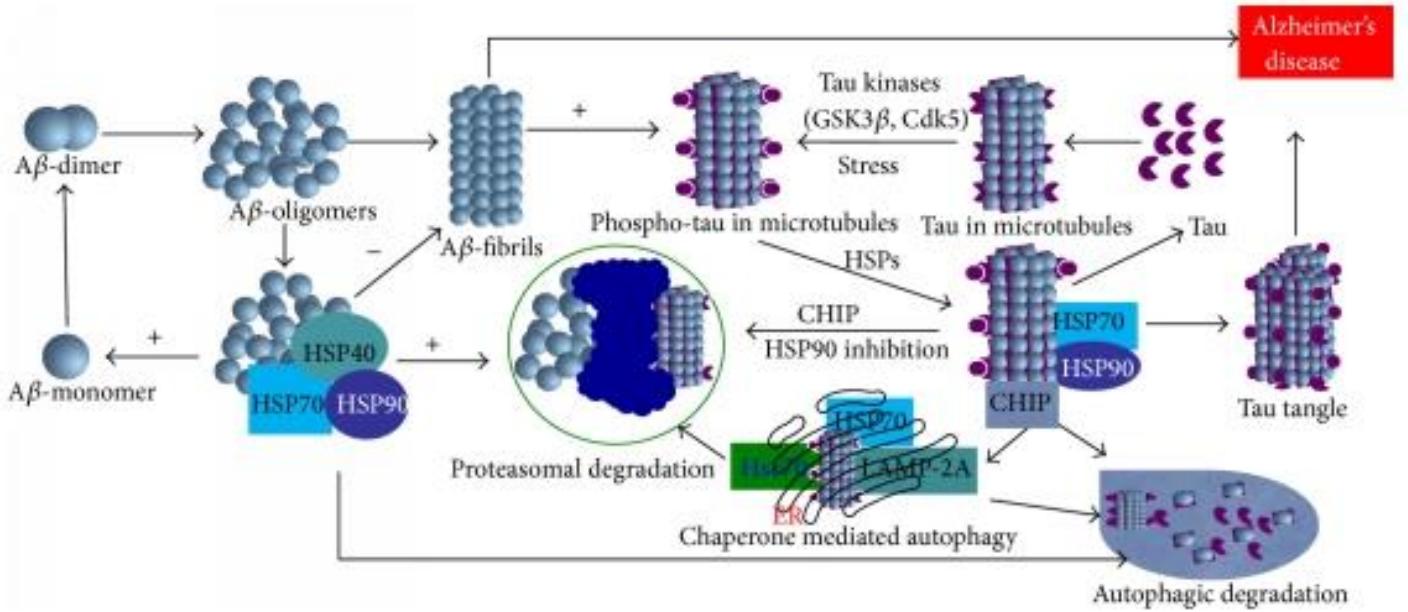
SEVERAL PARAMETERS NEED TO BE FURTHER INVESTIGATED

- Crowding
- Co-aggregation phenomena
- Ionic strenght and salts specificity
- Specific elements influence (Cu!)

AND MANY OTHERS

The Scientist must set in order. Science is built up with facts, as a house is with stones. But a collection of facts is no more a science than a heap of stones is a house.
Henri Poincaré



BioMed Research International 2014(1):495091 DOI: 10.1155/2014/495091 ·

Thank you for paying attention!

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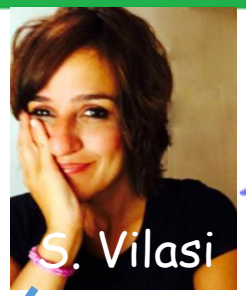
P. Moretti


C. Ricci


P. Mariani


F. Spinozzi


S. Vilasi

ibf


J. Teixeira

Orphée Laboratoire Léon Brillouin


R. Sinibaldi

FUTURO IN RICERCA

ESRF The European Synchrotron


C. Ferrero


H. Amenitsch


R. Itri

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