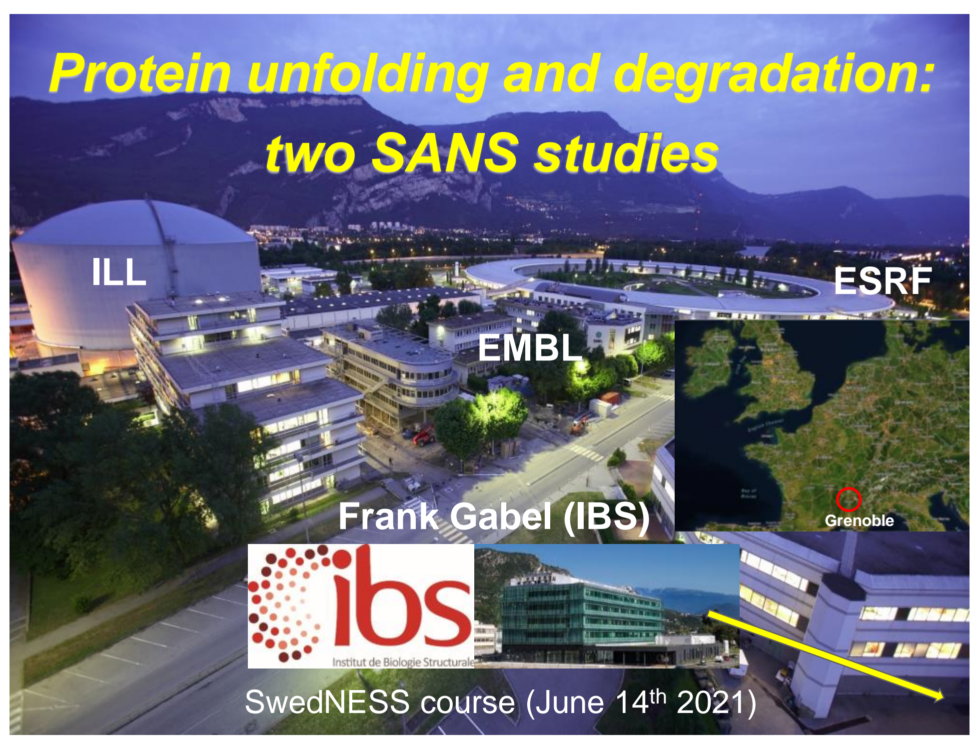
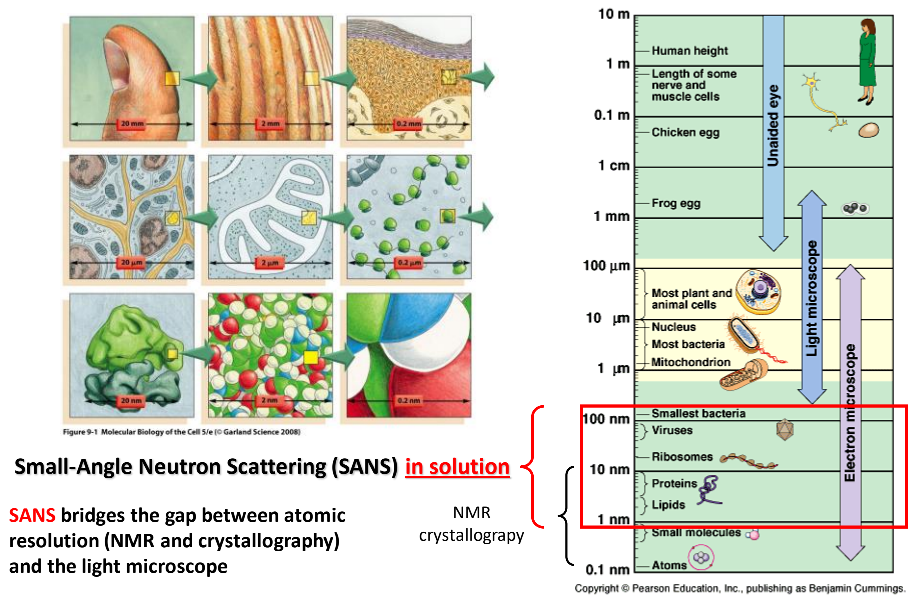


PART 1:

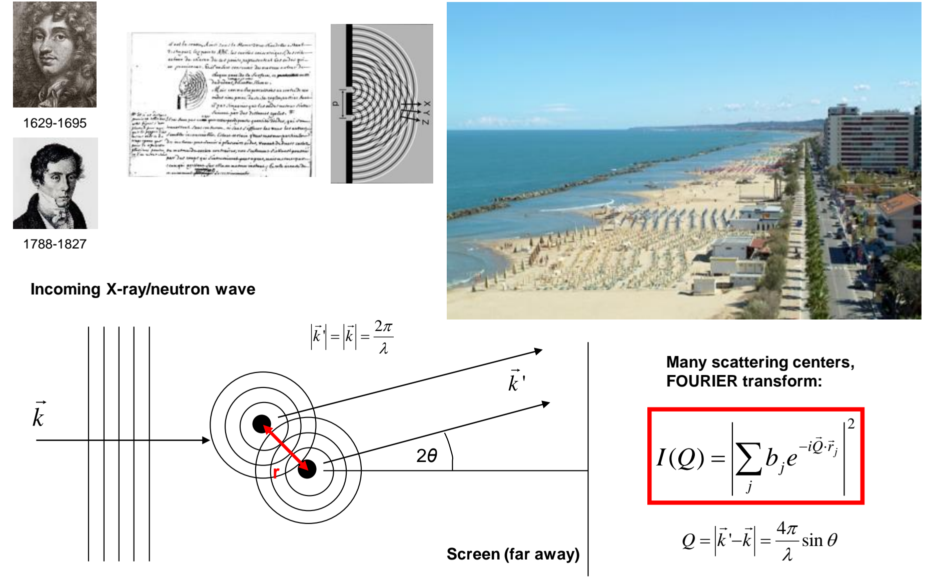
A brief introduction to SANS

See Susana Texeira's lecture and practical tomorrow for more details!

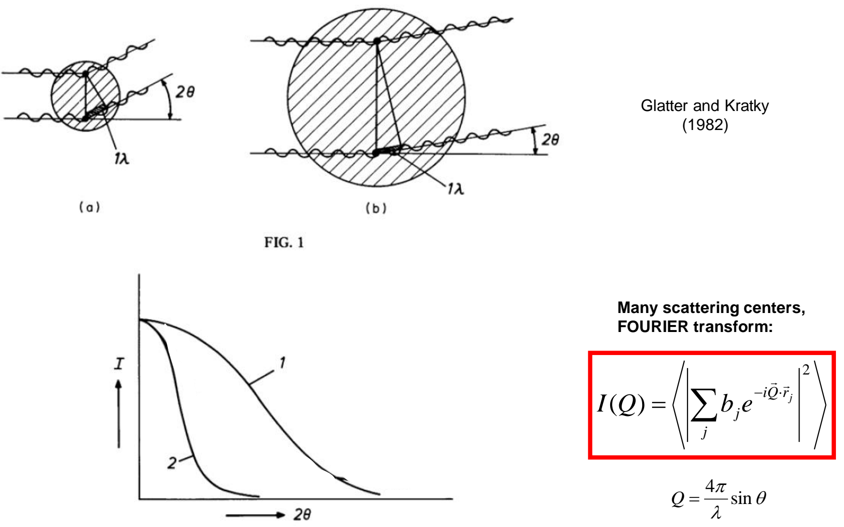
Typical lengthscales



Scattering basics: Huygens-Fresnel principle



Reciprocal relationship between real space and the diffraction pattern

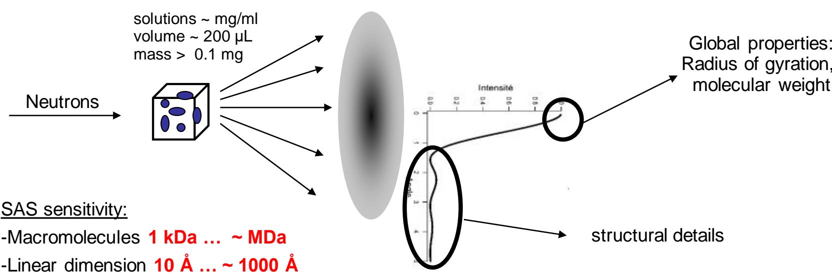


$$I(Q) = \left\langle \left| \sum_j b_j e^{-i\vec{Q}\cdot\vec{r}_j} \right|^2 \right\rangle$$

$$Q = \frac{4\pi}{\lambda}\sin\theta$$

SANS sample conditions and information obtained

Neutrons



SAS sensitivity:

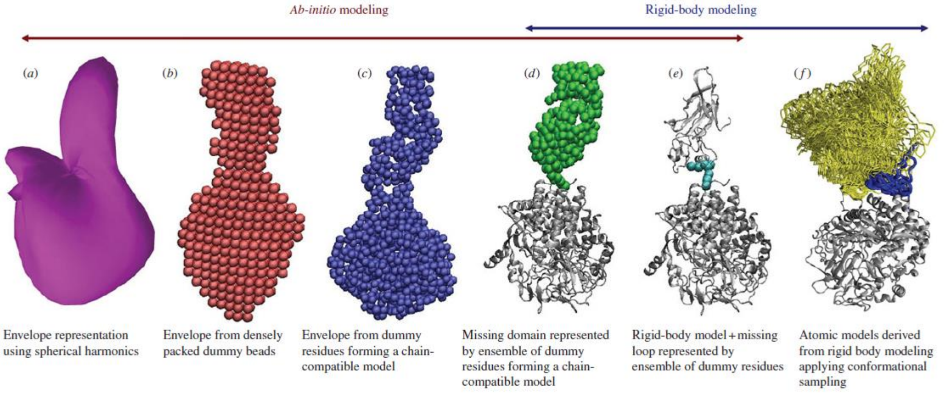
-Macromolecules **1 kDa … ~ MDa**

-Linear dimension **10 Å … ~ 1000 Å**

Information obtained by SANS:

1) Oligomeric state of macromolecules
2) Shape or conformation (globular, stick etc…)
3) Interaction of different macromolecules
4) Variation of points (1)-(3) as a function of pH, salt, ligands, T, p, ...
5) **Contrast variation**: visualisation of individual sub-units *in situ*

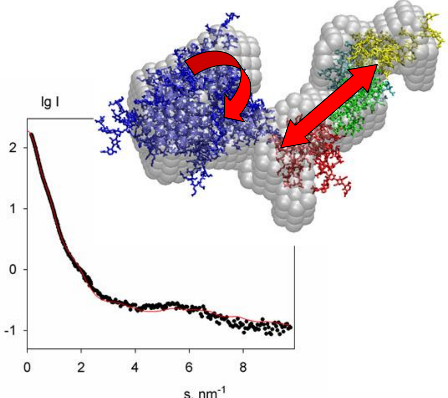
Modeling techniques using SAS in structural biology



Putnam et al. (2007) *Q. Rev. Biophys.* **40**(3), 191-285

What can SANS provide that is different from SAXS?

SANS allows to go beyond the global shape and study internal structure!



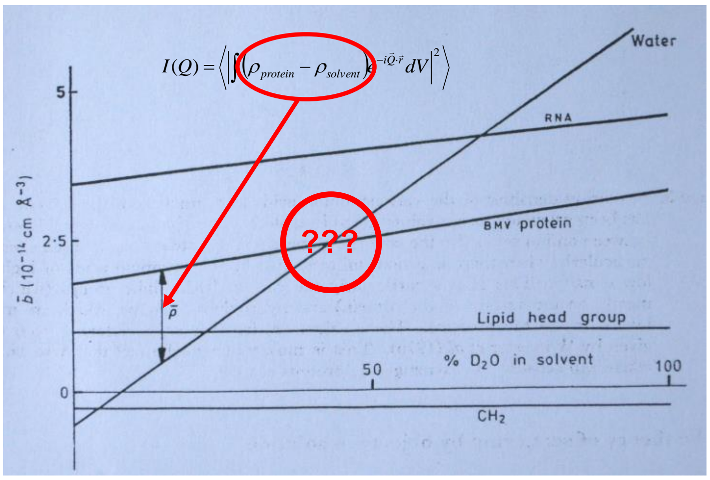
Often problematic to position/orient subunits in a larger complex using SAXS alone…

Internal structure: contrast variation and SANS!

Idea of contrast variation

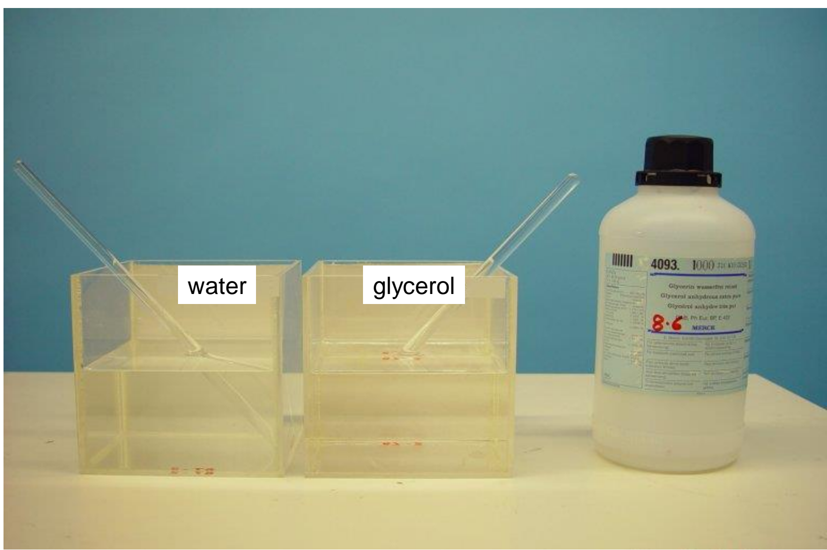
Vary scattering behaviour of (parts of) solutes with respect to solvent

Natural Contrast

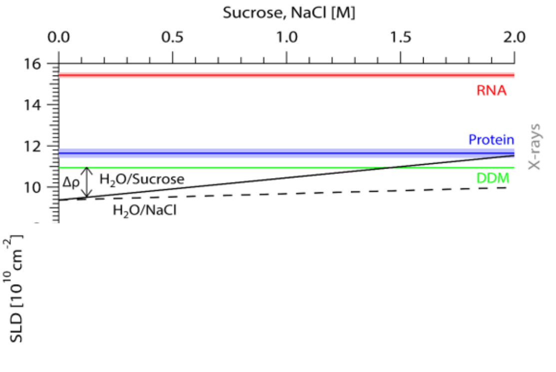


In practice, all biomacromolecules can be matched in SANS, i.e. made invisible!!!
Not so easy with SAXS…

An analogon in optics: refractive index



SAXS and contrast variation?

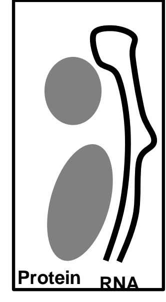


- **Accessible range of solvent electron densities is limited**
- **Contrast agents (salt, sugar…) need to be added at high molarities and may not be inert to biomolecules**
- **Electron density of biomolecules cannot be modified globally**

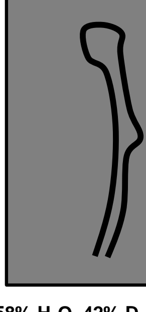
Mahieu, E. & Gabel, F. (2018). *Acta Cryst.* ***D74****(Pt 8), 715-726.*
Gabel, F. et al. (2019) *IUCrJ* ***6****(Pt 4):521-525.*

Contrast variation in SANS: natural contrast

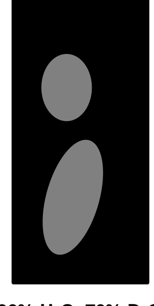
Proteins and RNAs have different proton densities



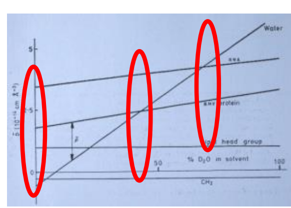
100% H_2O



58% H_2O, 42% D_2O

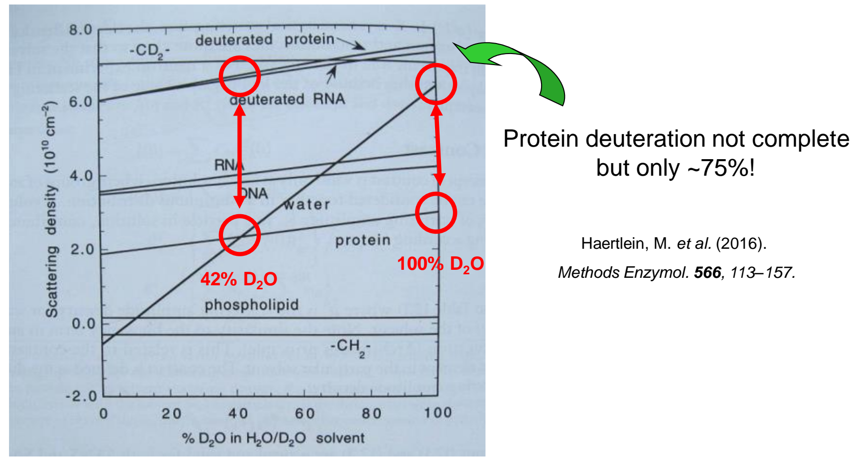


30% H_2O, 70% D_2O



Also possible for protein-protein complexes (deuteration)!

Artificial contrast using deuteration

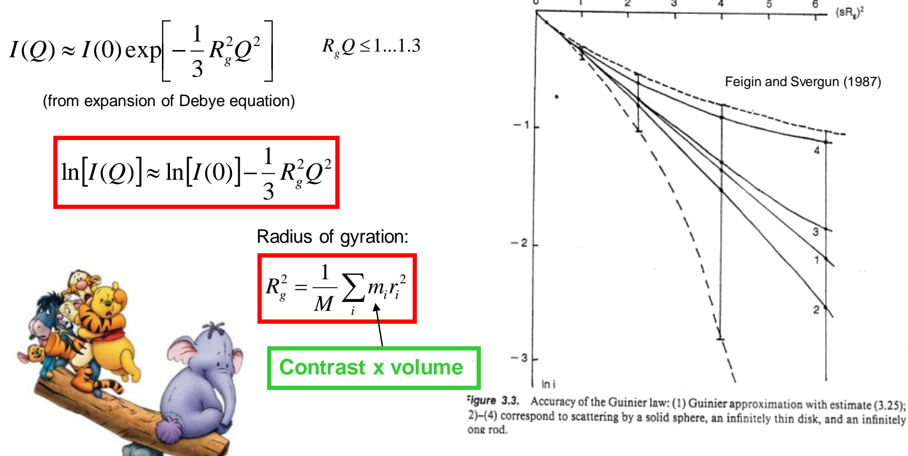


Protein deuteration not complete but only ~75%!

Haertlein, M. *et al.* (2016). *Methods Enzymol.* ***566**, 113–157.*

Careful at **high D_2O** levels in the solvent: favours **oligomerisation/aggregation**!

Guinier approximation and radius of gyration



“See-saw analogy”

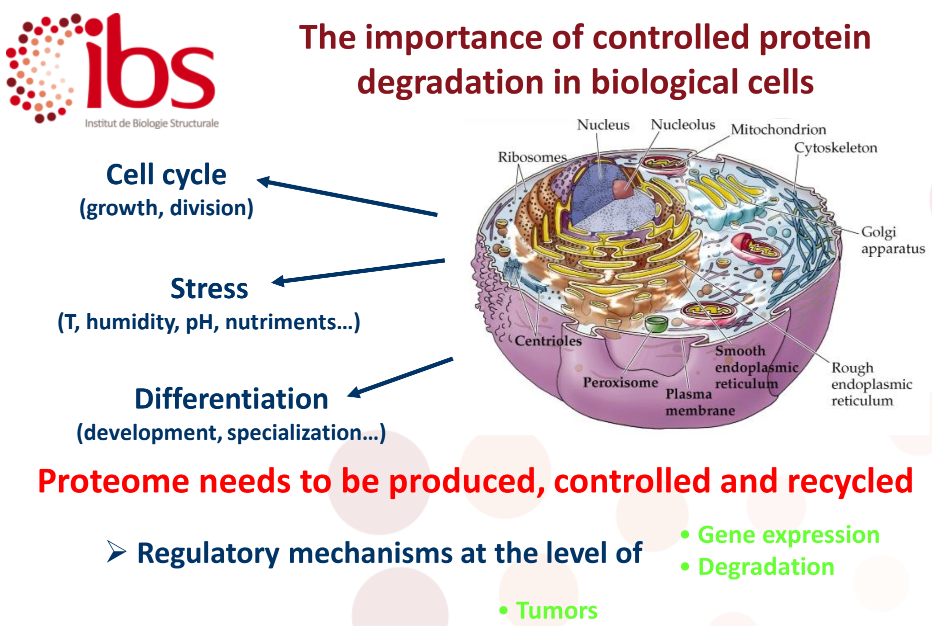
For a given molecular weight, a sphere has the smallest R_g, *i.e.* it is the most compact object

PART 2:

a static and a time-resolved (TR) SANS study on protein degradation

What unique insight can neutrons provide?

The importance of controlled protein degradation in biological cells



Cell cycle
(growth, division)

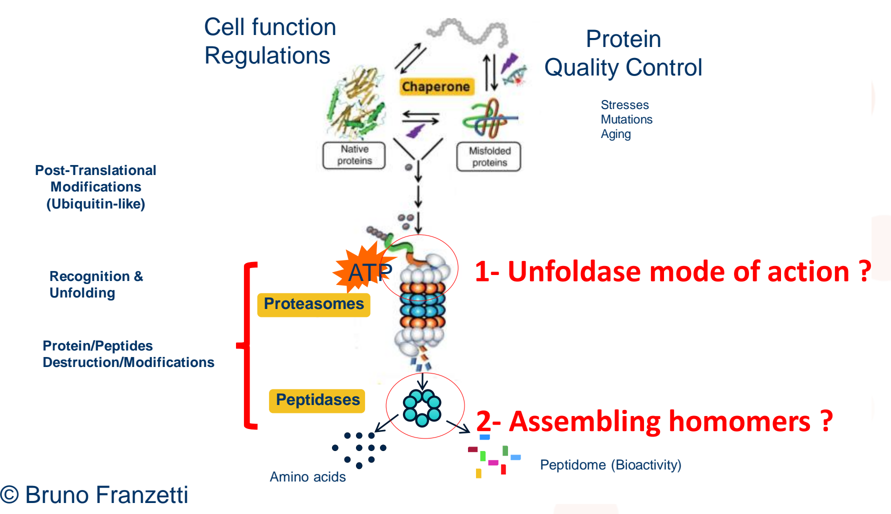
Stress
(T, humidity, pH, nutriments…)

Differentiation
(development, specialization…)

Proteome needs to be produced, controlled and recycled

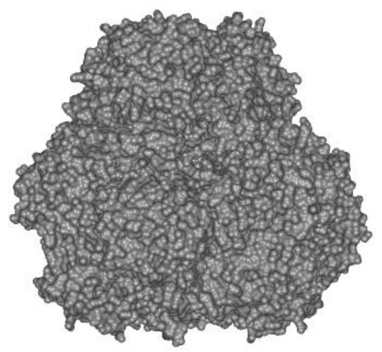
- **Regulatory mechanisms at the level of**
  - Gene expression
  - Degradation
- **Dysfunction can result in**
  - Tumors
  - Neurodegenerative diseases
  - Ageing

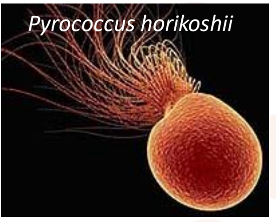
Intracellular proteostasis

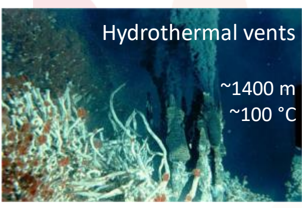


© Bruno Franzetti

Example 1: the TET peptidase

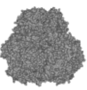




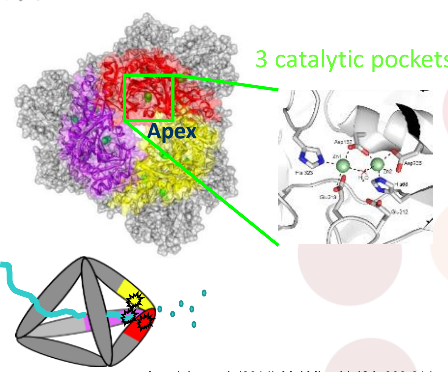


Appolaire A, Girard E, Colombo M, Durá MA, Moulin M, Härtlein M, Franzetti B, Gabel F. (2014). Small-angle neutron scattering reveals the assembly mode and oligomeric architecture of TET, a large, dodecameric aminopeptidase. ***Acta Cryst. D*** D70(Pt 11), 2983-2993.

TET peptidase functions

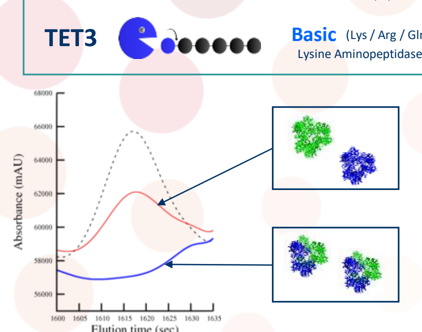


- Large (500 kDa) self-compartmentalized dodecamers — Franzetti *et al*. 2002. *EMBO. J*
- Discovered as key players in low salt stress — Schoehn *et al*. 2006. *J. Biol. Chem*
- 4 copies with **different substrate specificities** (TET1/**TET2**/**TET3**/TET4) — Durà *et al*. 2005. *Biochemistry*



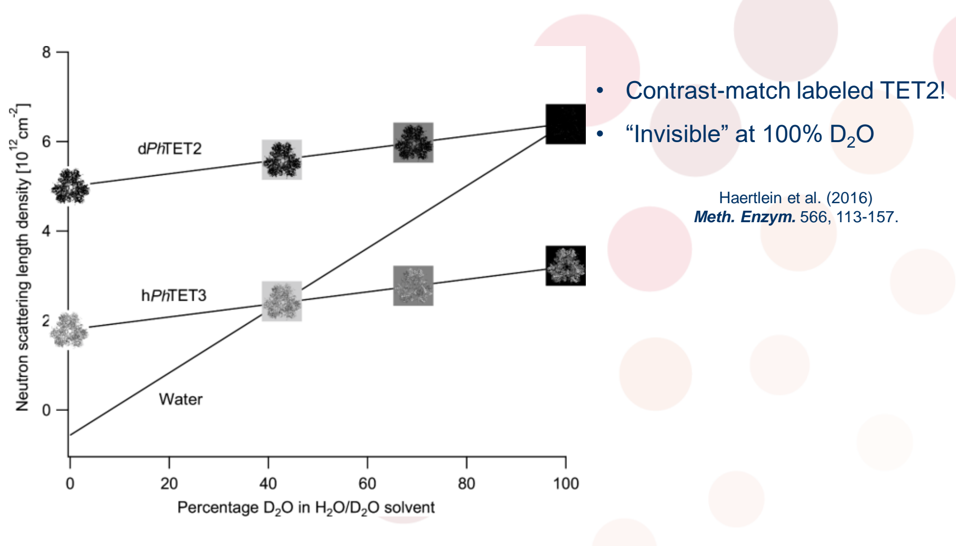
Appolaire et al. (2014) *Mol Microbiol* **94**: 803-814.

TET2	Neutral (Leu / Ile / Met)	Serine aminopeptidase
TET3	Basic (Lys / Arg / Gln)	Lysine Aminopeptidase



- **How do such complex biological nanoparticles self-assemble?**
- **Why is hetero-oligomer more efficient than mixture of homo-oligomers?**

A very specific SANS labeling strategy

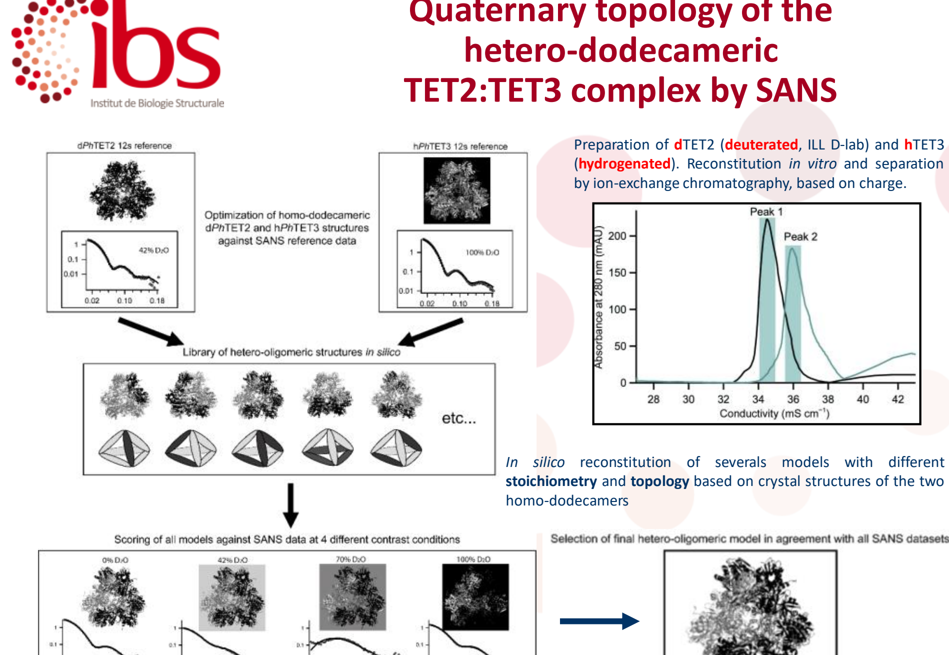


- Contrast-match labeled TET2!
- "Invisible" at 100% D_2O

Haertlein et al. (2016)
Meth. Enzym. 566, 113-157.

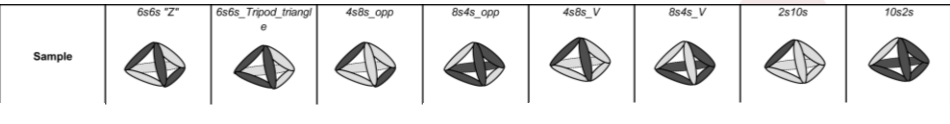
Quaternary topology of the hetero-dodecameric TET2:TET3 complex by SANS

Preparation of **d**TET2 (**deuterated**, ILL D-lab) and **h**TET3 (**hydrogenated**). Reconstitution *in vitro* and separation by ion-exchange chromatography, based on charge.



In silico reconstitution of severals models with different **stoichiometry** and **topology** based on crystal structures of the two homo-dodecamers

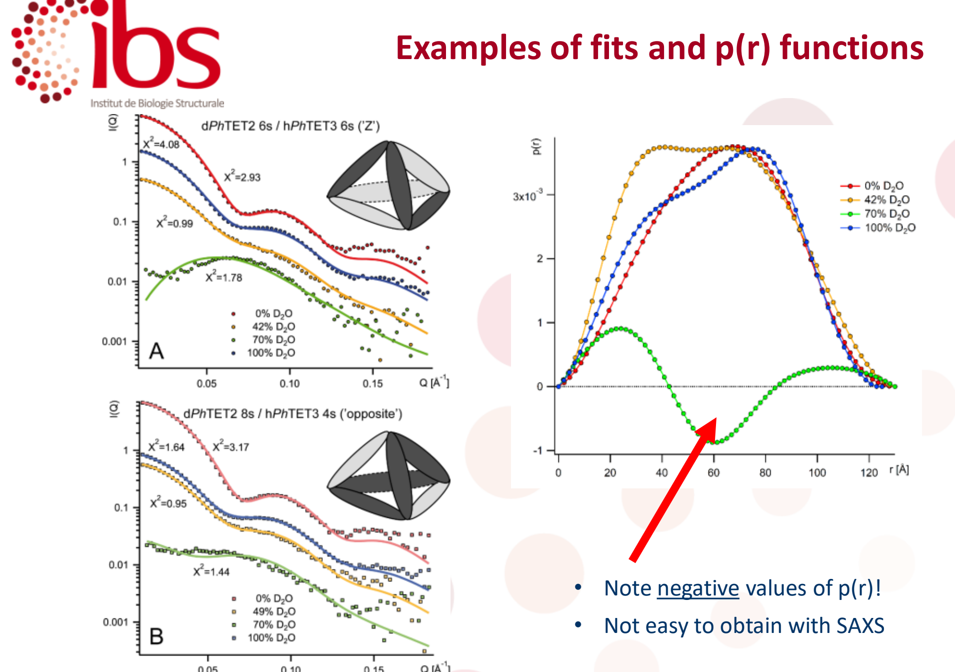
A Herculian SANS study…



Sample	6s6s "Z"	6s6s_Tripod_triangle	4s8s_opp	8s4s_opp	4s8s_V	8s4s_V	2s10s	10s2s

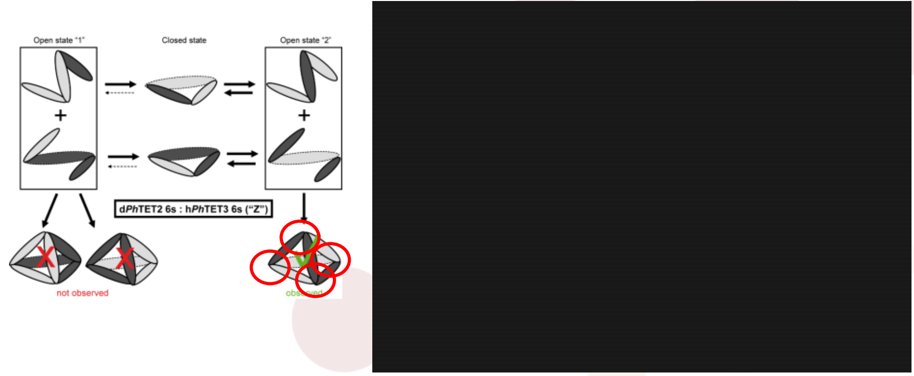
D22 (ILL)

Examples of fits and p(r) functions



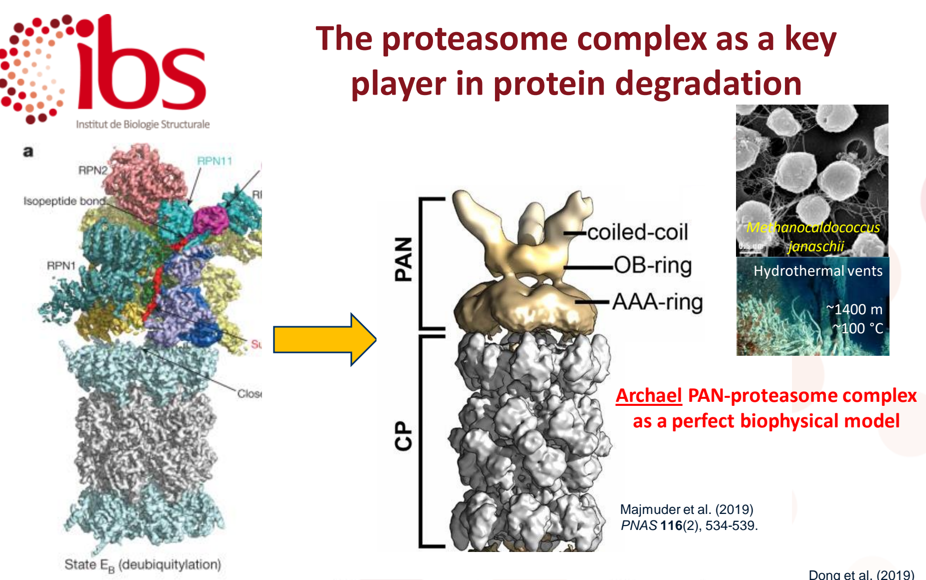
- Note negative values of p(r)!
- Not easy to obtain with SAXS

Models for the assembling pathways: the ‘Z’-shaped particle



Combined SANS, EM and crystallography study:
Exclusively hetero-catalytic pockets increase the catalytic efficiency towards substrates!

The proteasome complex as a key player in protein degradation



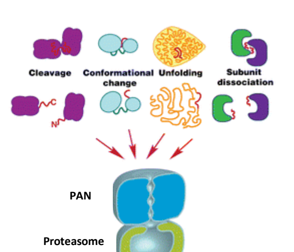
Dong et al. (2019) *Nature* **565**, 49-55.

- Many exciting static “snapshots” after Cryo-EM revolution
- Lack of dynamic data of the substrate processing

Eukaryotic systems too complex for biophysical studies!

Specificity in protein recognition: the proteasome activating complex PAN

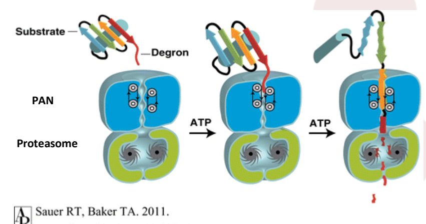
(Proteasome Activating Nucleotidase)



Protein degradation needs to be specific

PAN is a molecular nanomachine that unfolds/directs proteins for the proteases

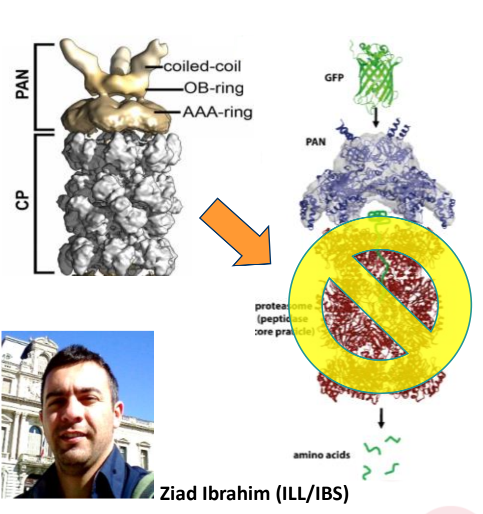
- Mode of action / substrate processing?
- Conformational changes involved?

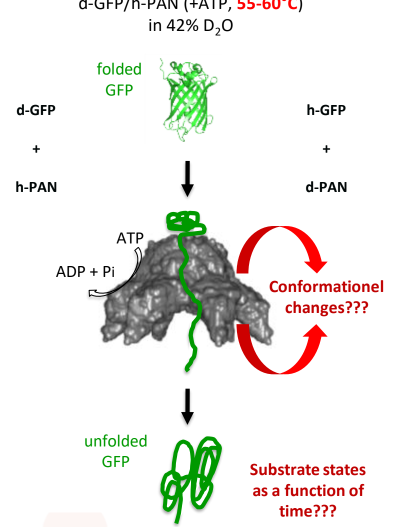


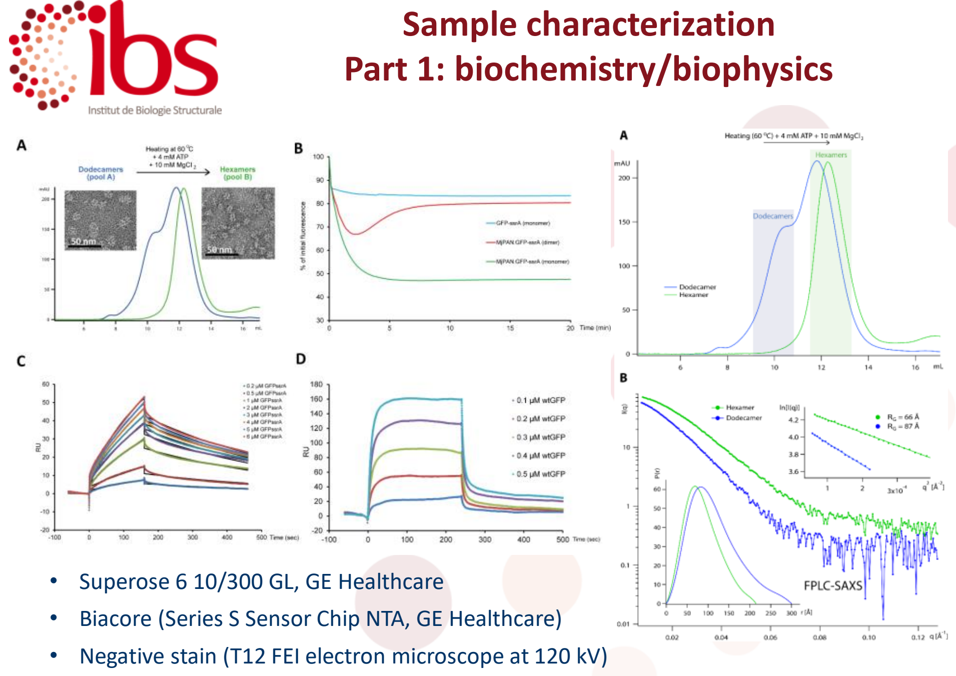
PAN
CP
coiled-coil
OB-ring
AAA-ring
1 MDa

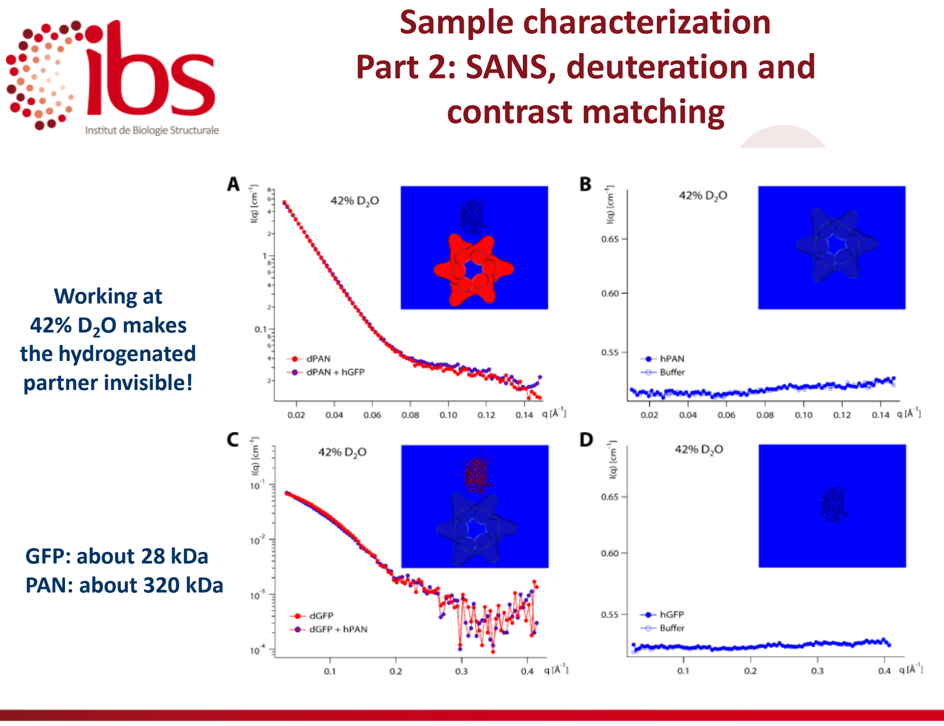
➢ Time-resolved SANS!

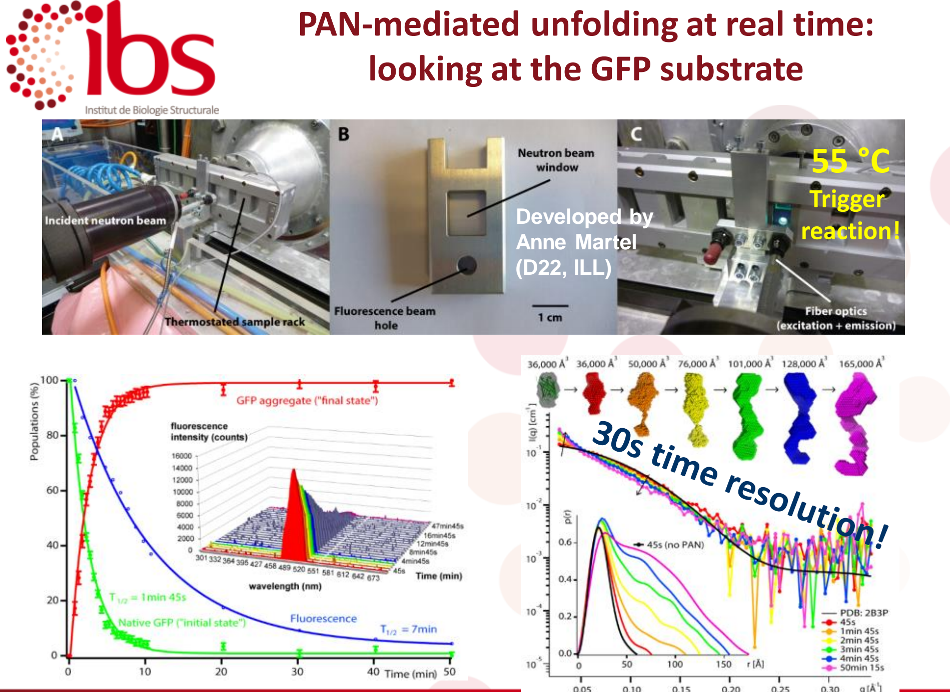
TR-SANS, part 1: see the PAN-substrate complex "at work"



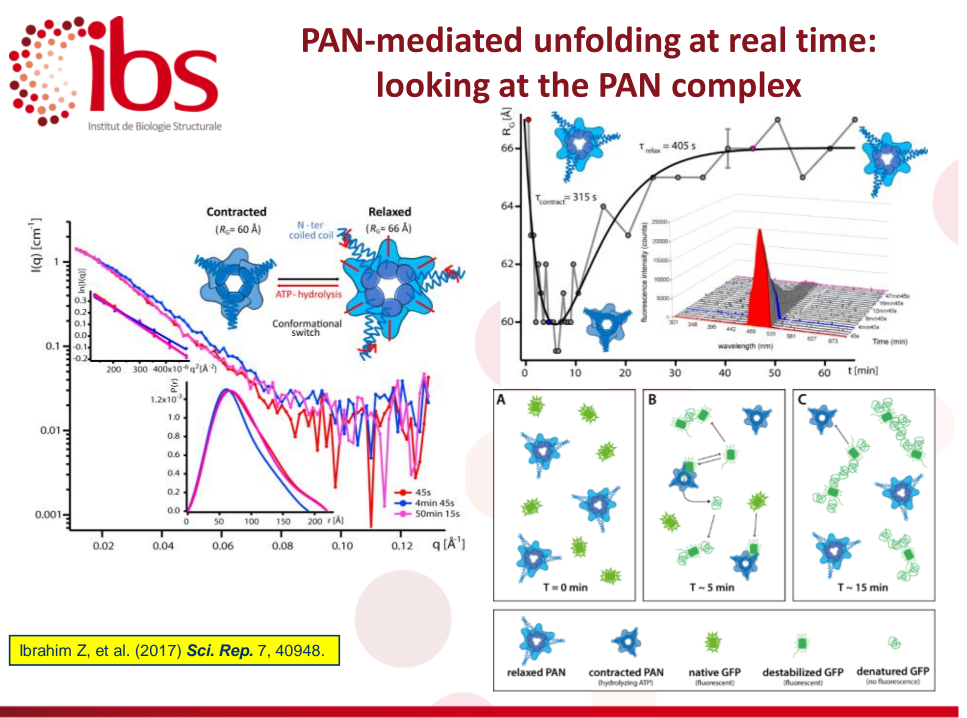



ibs
Institut de Biologie Structurale
Sample characterization
Part 1: biochemistry/biophysics
Dodecamers (pool A)
Hexamers (pool B)
50 nm
Dodecamer
Hexamer
Dodecamers
Hexamers
FPLC-SAXS
• Superose 6 10/300 GL, GE Healthcare
• Biacore (Series S Sensor Chip NTA, GE Healthcare)
• Negative stain (T12 FEI electron microscope at 120 kV)

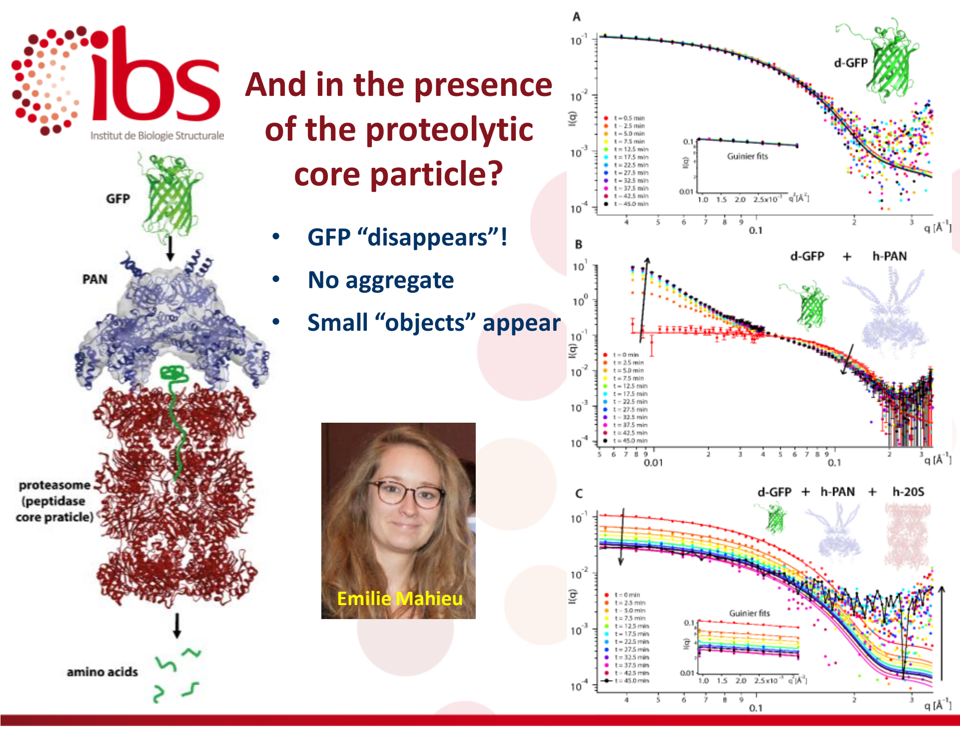

ibs
Institut de Biologie Structurale
Sample characterization
Part 2: SANS, deuteration and contrast matching
Working at
42% D2O makes
the hydrogenated
partner invisible!
GFP: about 28 kDa
PAN: about 320 kDa
42% D2O
dPAN
dPAN + hGFP
hPAN
Buffer
dGFP
dGFP + hPAN
hGFP
Buffer



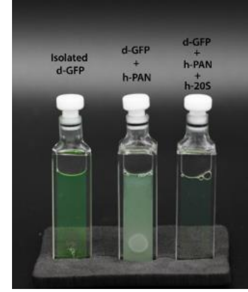
PAN-mediated unfolding at real time: looking at the GFP substrate
Institut de Biologie Structurale
Incident neutron beam
Thermostated sample rack
Neutron beam window
Developed by Anne Martel (D22, ILL)
Fluorescence beam hole
1 cm
55 °C
Trigger reaction!
Fiber optics (excitation + emission)
GFP aggregate ("final state")
fluorescence intensity (counts)
wavelength (nm)
Time (min)
Native GFP ("initial state")
Fluorescence
30s time resolution!
PDB: 2B3P

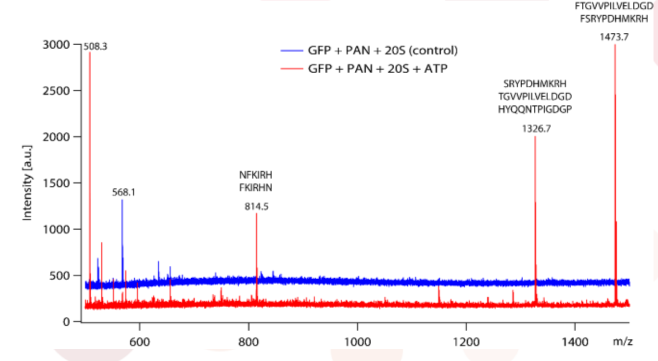


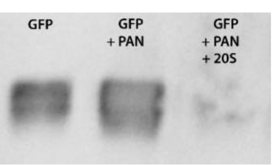
PAN-mediated unfolding at real time: looking at the PAN complex
Institut de Biologie Structurale
Contracted
Relaxed
ATP- hydrolysis
Conformational switch
relaxed PAN
contracted PAN
native GFP
destabilized GFP
denatured GFP
Ibrahim Z, et al. (2017) Sci. Rep. 7, 40948.



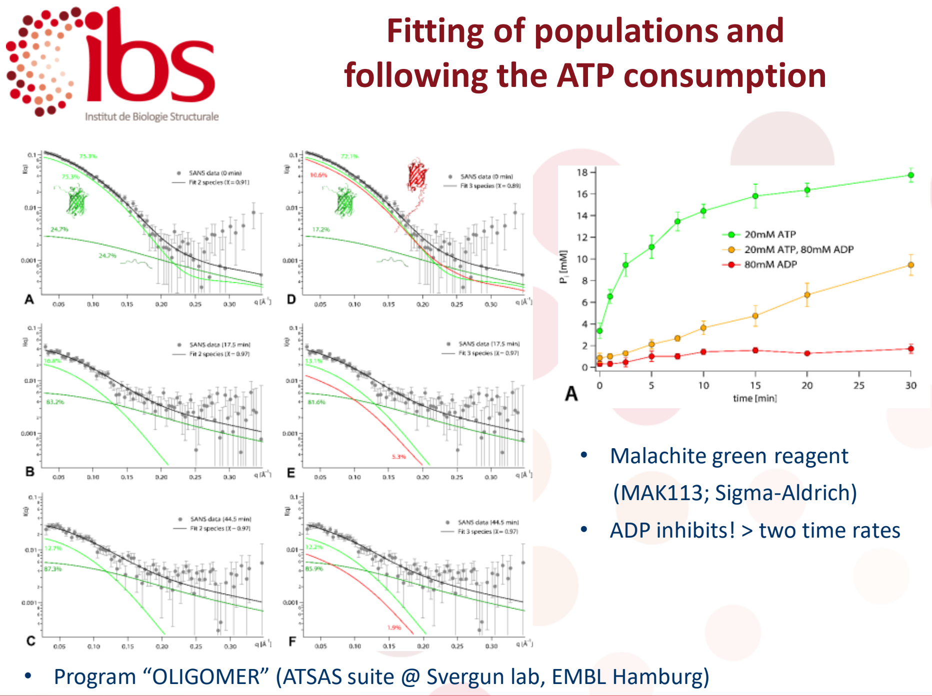
Supporting information for interpretation of the SANS results







- Inspection of samples "by eye" after experiment
- Mass spectrometry of products (LC/electrospray ionization-TOF)
- Western blot of folded GFP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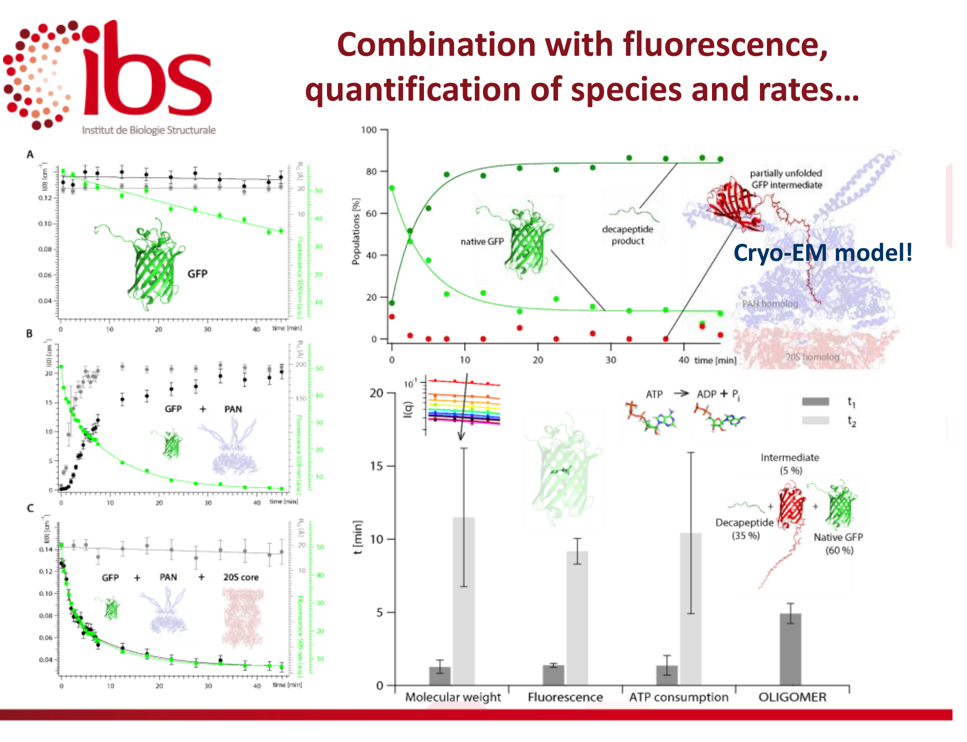
Fitting of populations and following the ATP consumption
20mM ATP
20mM ATP, 80mM ADP
80mM ADP
time [min]
Malachite green reagent (MAK113; Sigma-Aldrich)
ADP inhibits! > two time rates
Program "OLIGOMER" (ATSAS suite @ Svergun lab, EMBL Hamburg)


ibs
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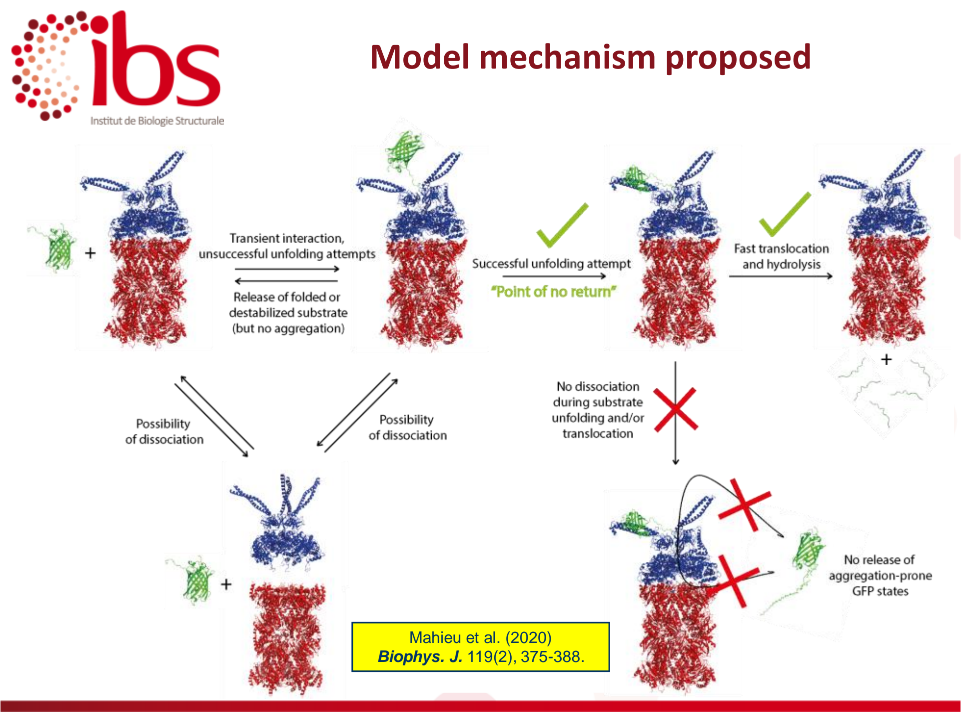
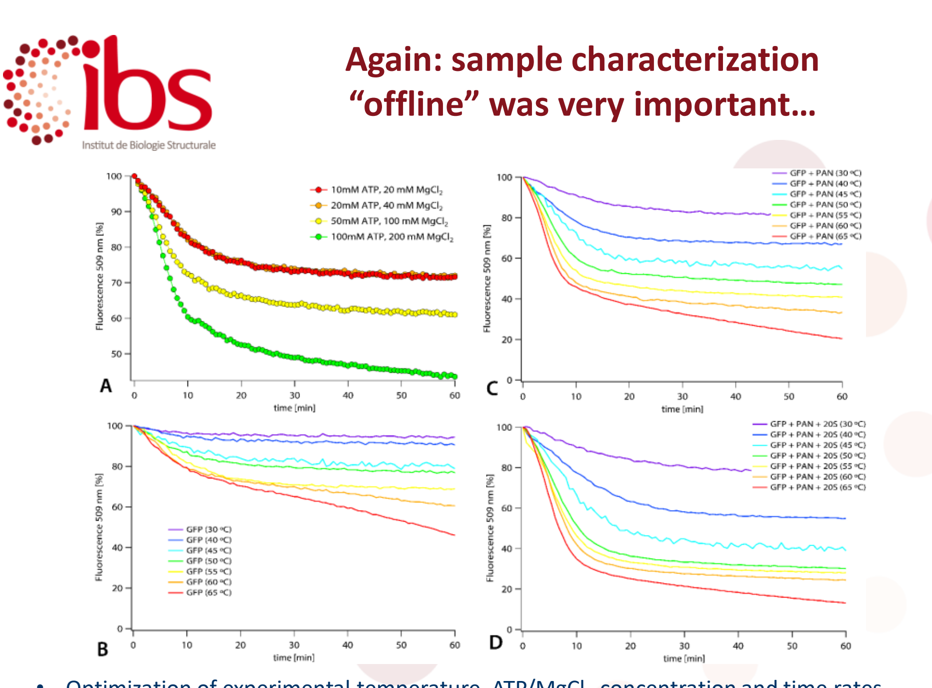
Combination with fluorescence, quantification of species and rates…
GFP
GFP + PAN
GFP + PAN + 20S core
Populations [%]
native GFP
decapeptide product
partially unfolded GFP intermediate
Cryo-EM model!
PAN homolog
20S homolog
time [min]
ATP → ADP + P_i
t_1
t_2
Intermediate (5 %)
Decapeptide (35 %)
Native GFP (60 %)
t [min]
Molecular weight
Fluorescence
ATP consumption
OLIGOMER

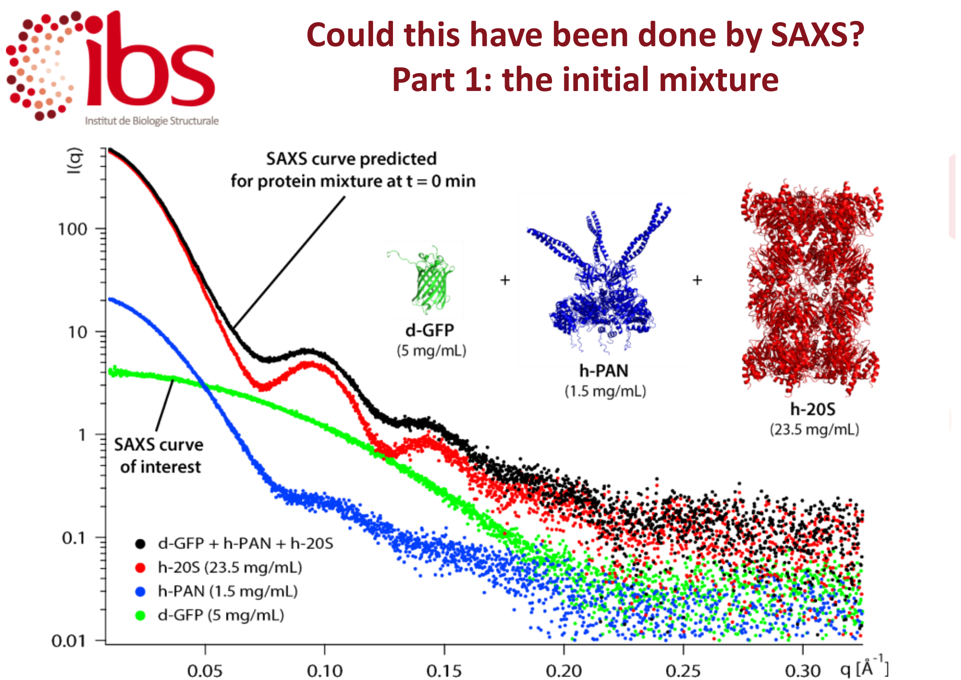
Model mechanism proposed

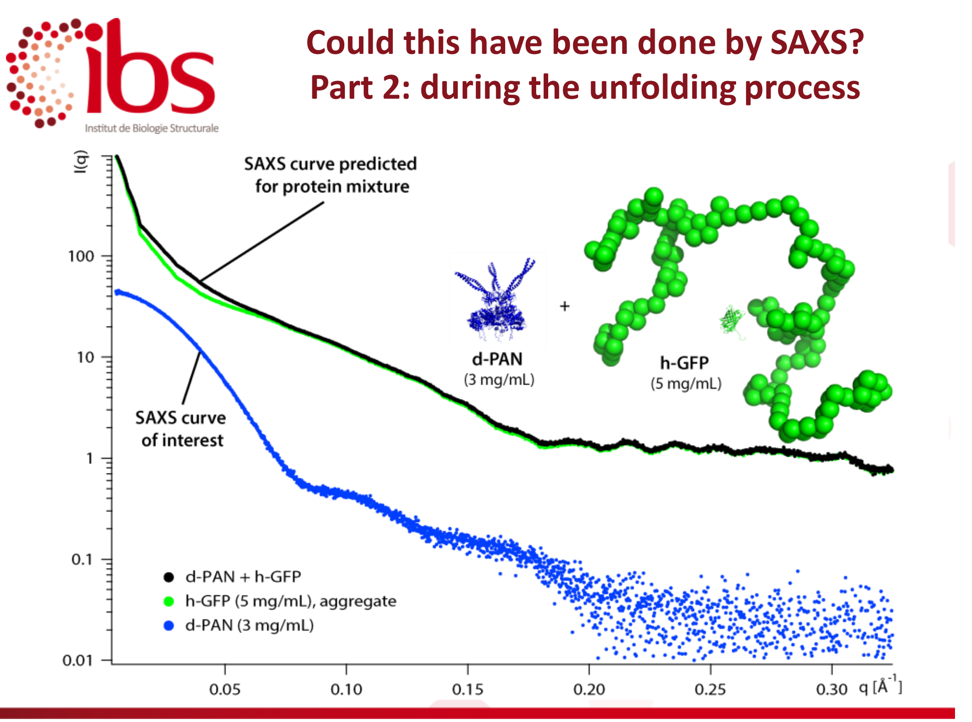


Again: sample characterization "offline" was very important…

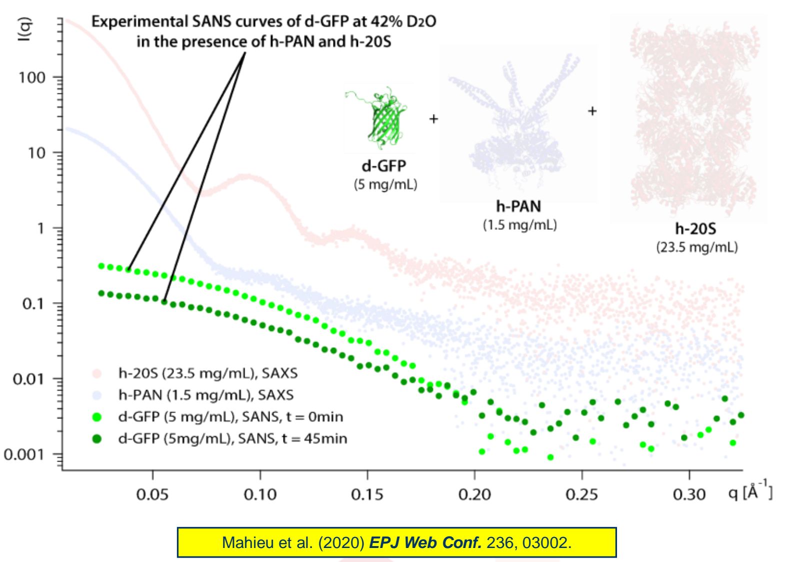


- Optimization of experimental temperature, ATP/$MgCl_2$ concentration and time rates


ibs
Institut de Biologie Structurale
Could this have been done by SAXS?
Part 1: the initial mixture
SAXS curve predicted
for protein mixture at t = 0 min
d-GFP
(5 mg/mL)
h-PAN
(1.5 mg/mL)
h-20S
(23.5 mg/mL)
SAXS curve
of interest
d-GFP + h-PAN + h-20S
h-20S (23.5 mg/mL)
h-PAN (1.5 mg/mL)
d-GFP (5 mg/mL)
I(q)
q [Å^{-1}]


ibs
Institut de Biologie Structurale
Could this have been done by SAXS?
Part 2: during the unfolding process
SAXS curve predicted
for protein mixture
d-PAN
(3 mg/mL)
h-GFP
(5 mg/mL)
SAXS curve
of interest
d-PAN + h-GFP
h-GFP (5 mg/mL), aggregate
d-PAN (3 mg/mL)
I(q)
q [Å^{-1}]

Only SANS allows to see small objects in the presence of (very) big ones!



Mahieu et al. (2020) ***EPJ Web Conf.*** 236, 03002.

Conclusions

- **TR-SANS allows to reach sub-minute time-resolution (5 sec!)**
- **Combinations with optical spectroscopy possible**
- **Insight into dynamic processes of important biological systems**
- **Complementary to “static” techniques (Cryo-EM, crystallography)**
- **Importance of selecting an “adapted” biological system**
- **Importance of accurate deuteration labeling**

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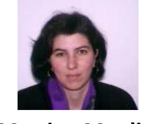

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