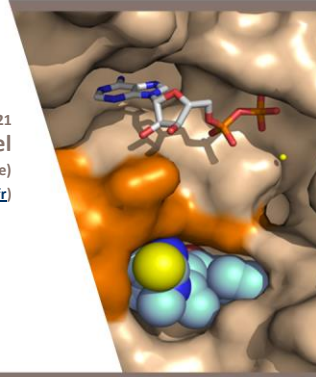




# SANS for structural and dynamical studies of bio-macromolecular complexes in solution

Neutrons in Life Sciences, January 19<sup>th</sup> 2021  
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F. Gabel, SANS lecture, Jan. 19th 2021, Grenoble

## Typical lengthscales

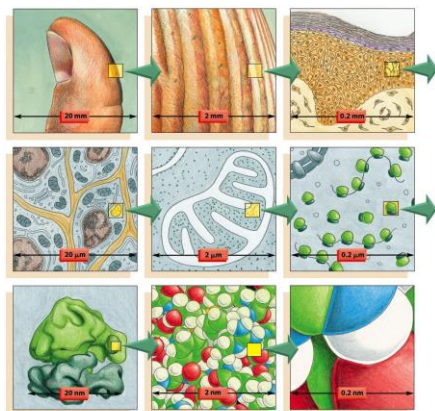
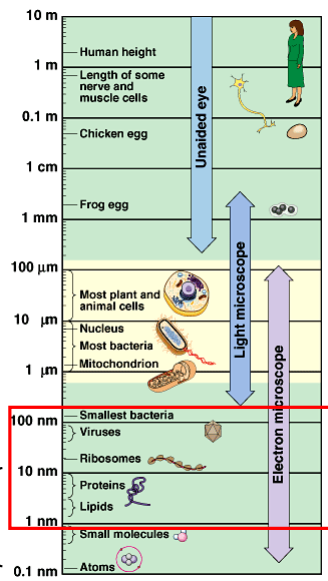


Figure 9-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Small-Angle Neutron Scattering (SANS) **in solution**

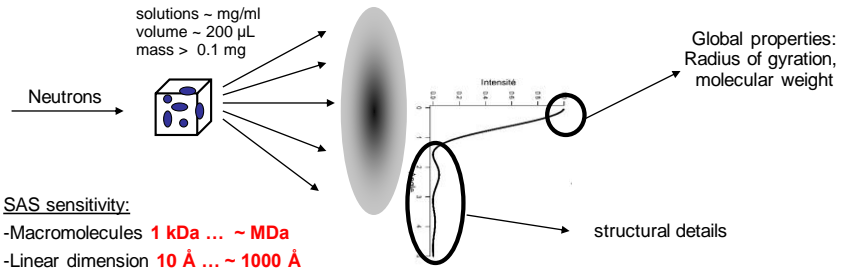
SANS bridges the gap between atomic resolution (NMR and crystallography) and the light microscope

NMR  
crystallography



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# SANS sample conditions and information obtained

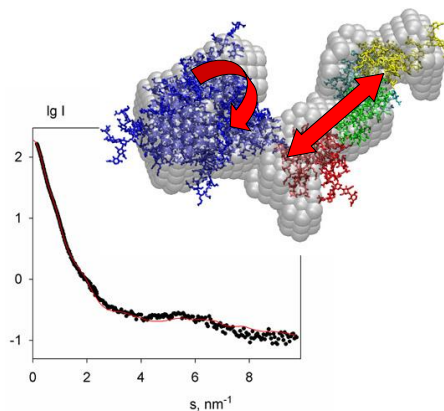


## Information obtained by SANS:

- 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*

**Difference with SAXS!**

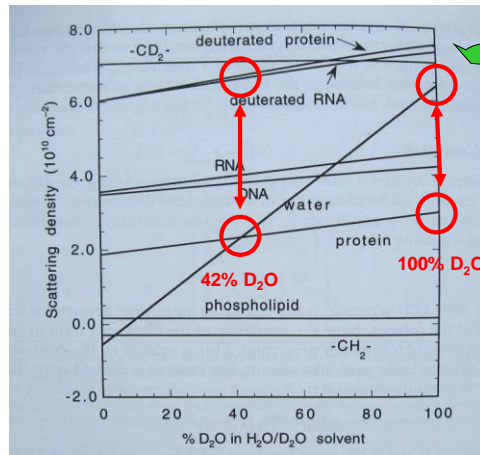
# SANS allows to go beyond the global shape and study internal structure!



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

**→ Internal structure: contrast variation (and deuteration)!**

## Natural and artificial contrast (deuteration!)

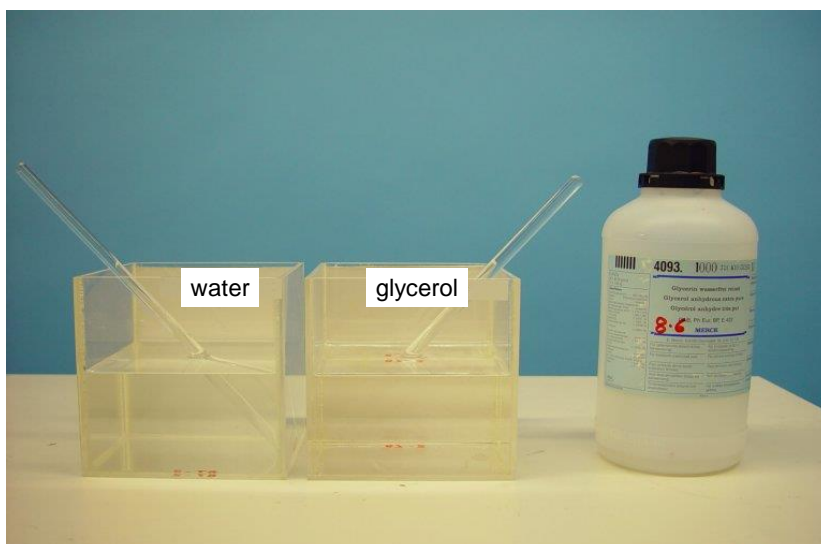


Protein deuteration not complete but only ~75!

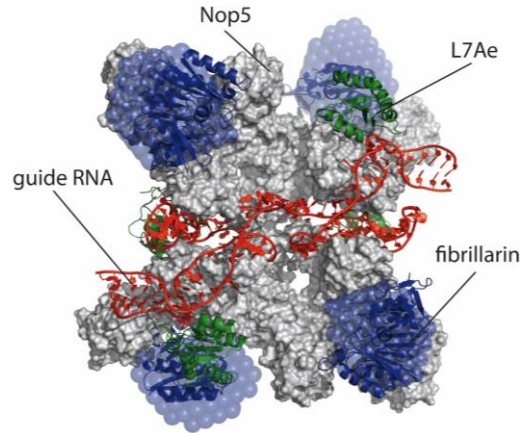
D-labs at neutron facilities!

Careful at **high  $\text{D}_2\text{O}$**  levels in the solvent: favours **oligomerisation/aggregation!**

## An analogon in optics: refractive index



# Example 1: atomic model of a big (~400 kDa) complex by combining SANS (SAXS) and NMR



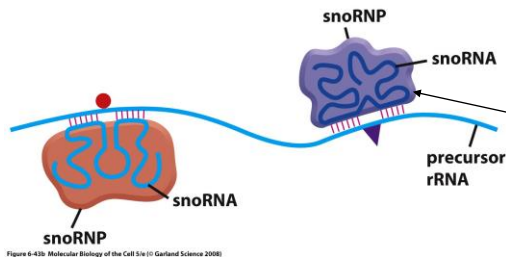
Lapinaite, A., Simon, B., Skjaerven, L., Rakwalska-Bange, M., Gabel, F. and Carlomagno T. (2013) *Nature* 502(7472), 519-523.

## RNA modifications: snoRNPs, snoRNAs and box C/D

snoRNP = "Small nucleolar Ribonucleo-Protein"

Only in archaea and eukaryotes,  
**not** in bacteria

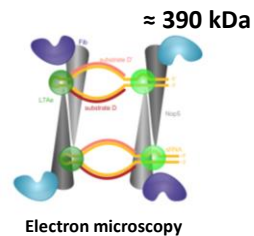
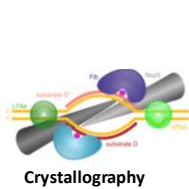
snoRNP = sRNP in archaea



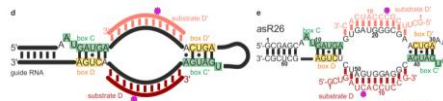
"Guide RNA"  
(> 100 in humans!)

Figure 6-43b Molecular Biology of the Cell 5/e (© Garland Science 2008)

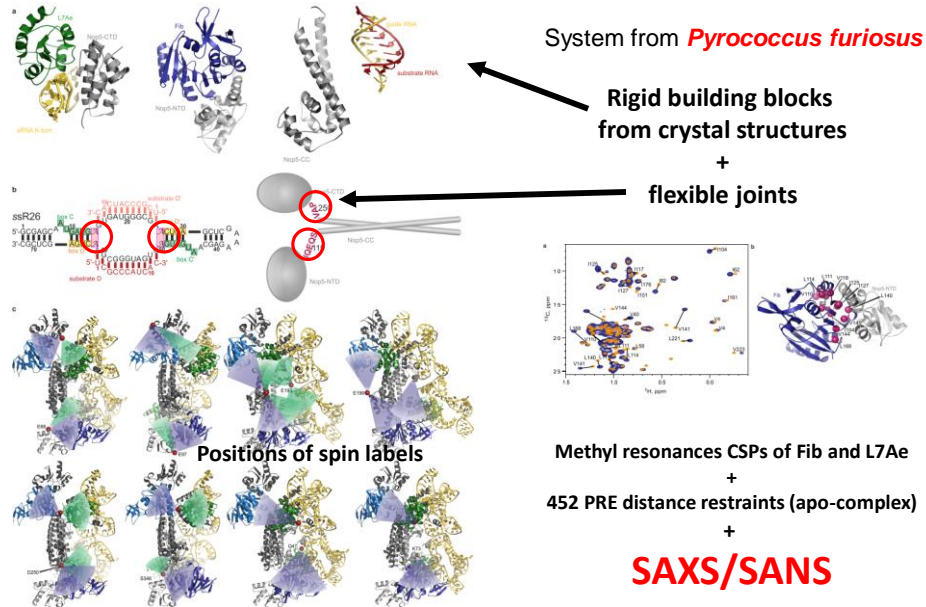
Two different architectures  
have been proposed:



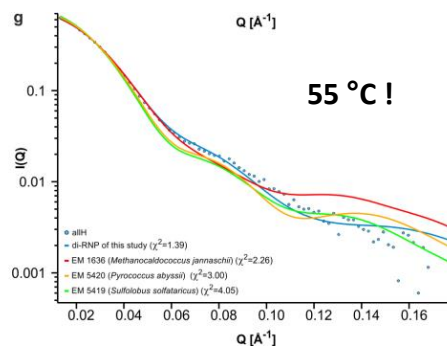
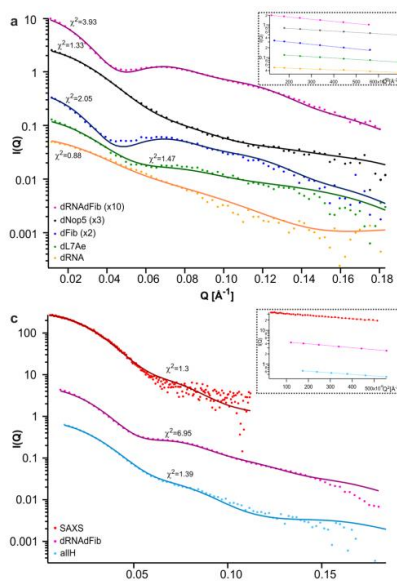
- Where is the sRNA situated?
- What is mechanism of methylation?
- Why two asymmetric methylation sites?



## Structural refinement strategy



## SANS (D22) and SAXS (BM29) data



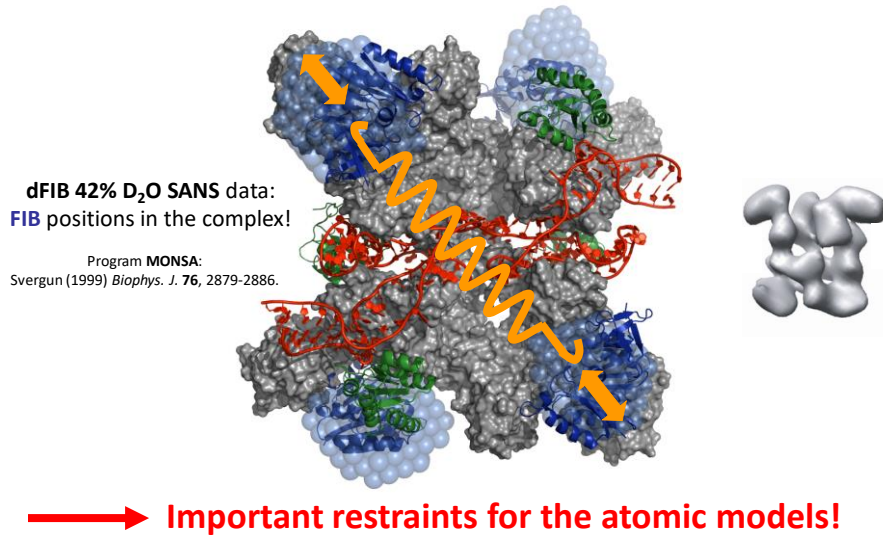
A total of 26 SANS samples:  
dFIB, dL7e, dRNA, dNOP5, dRNAdFIB  
0, 42, 70% D<sub>2</sub>O

D22 (ILL) Local contact: Anne Martel

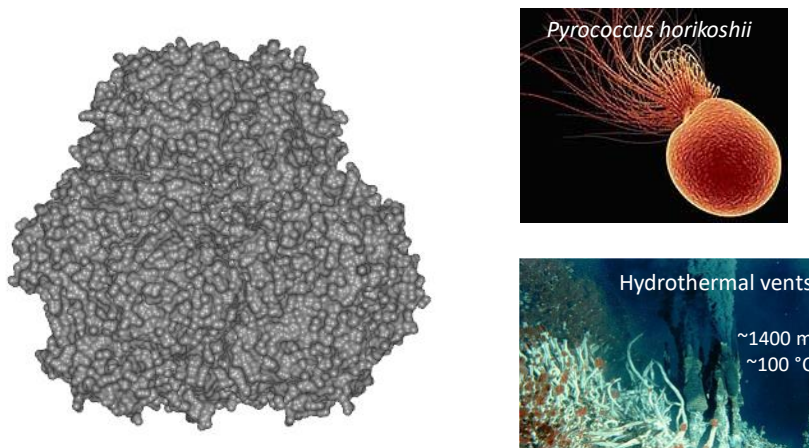
BM29 (ESRF) Local contact: Petra Pernot



## Relative positions of FIB proteins within the complex from SANS data



## Example 2: internal topology of a dodecameric protein complex



Appolaire A, Girard E, Colombo M, Durá MA, Moulin M, Härtle M, Franzetti B,  
Gabel F. (2014). *Acta Cryst. D* D70(Pt 11), 2983-2993.

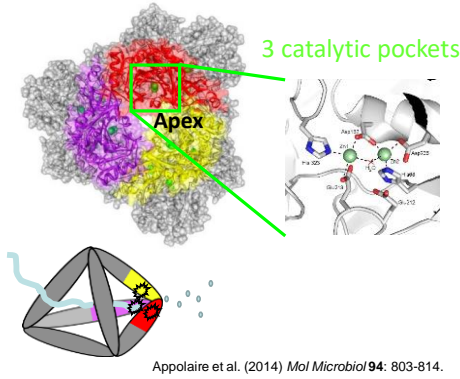
# TET protease functions



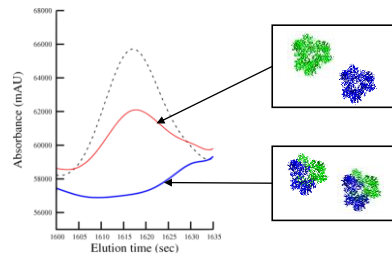
- Large (500 kDa) self-compartmentalized dodecamers
- Discovered as key players in low salt stress
- 4 copies with **different substrate specificities** (TET1/TET2/TET3/TET4) Durà et al. 2005. *Biochemistry*

Franzetti et al. 2002. *EMBO. J*

Schoehn et al. 2006. *J. Biol. Chem*

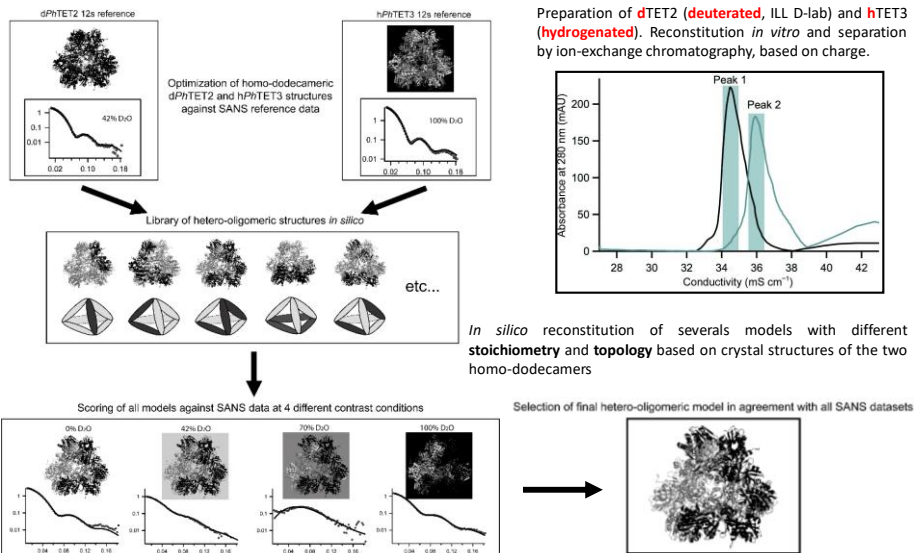


<b>TET2</b>		<b>Neutral</b> (Leu / Ile / Met) Serine aminopeptidase
<b>TET3</b>		<b>Basic</b> (Lys / Arg / Gln) Lysine Aminopeptidase

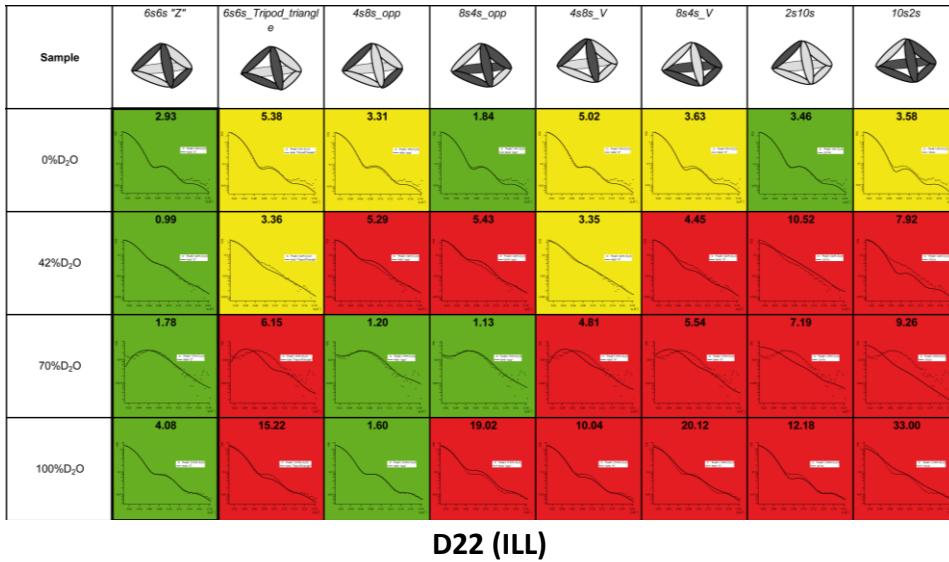


- **How do such complex biological nanoparticles self-assemble?**
- **Why is hetero-oligomer more efficient than mixture of homo-oligomers?**

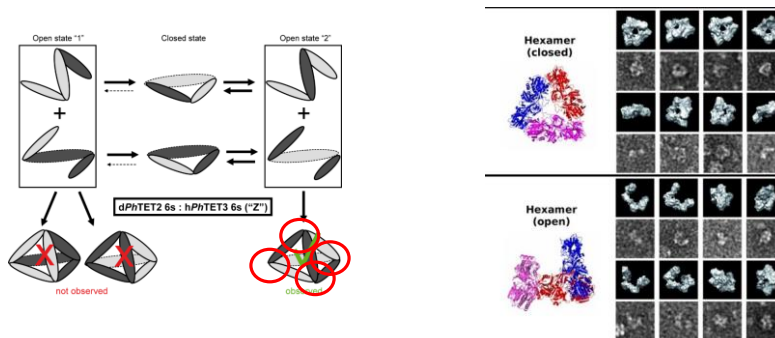
# Quaternary topology of a hetero-dodecameric TET2:TET3 complex by SANS



## An extensive SANS study...



## Models for the assembling pathways: the 'Z'-shaped particle

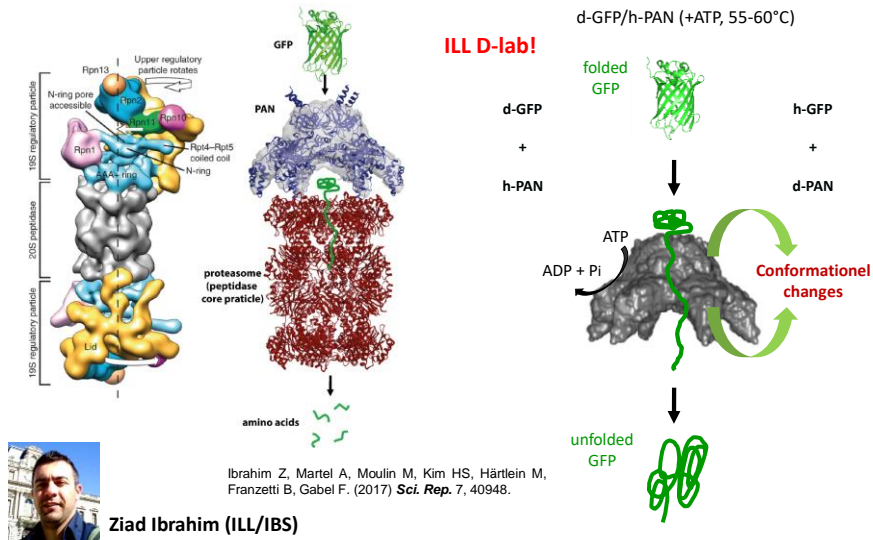


Appolaire (2013) *J. Biol. Chem.* 288(31), 22542-22554

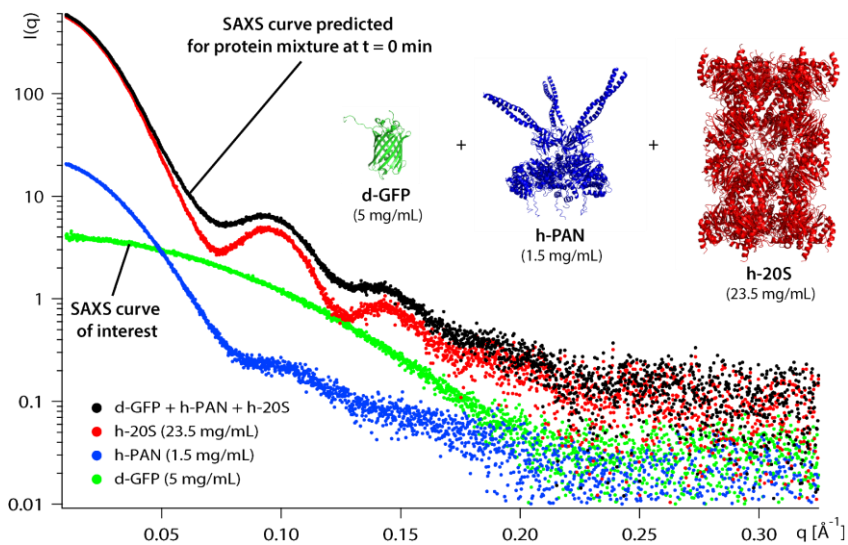
**Combined SANS, EM and crystallography study:**  
**Exclusively hetero-catalytic pockets increase**  
**the catalytic efficiency towards substrates!**



## Example 3: time-resolved SANS experiments see complex macromolecules “at work”

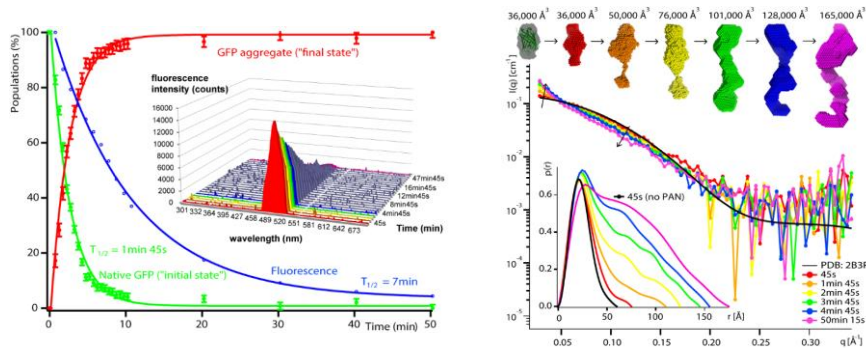
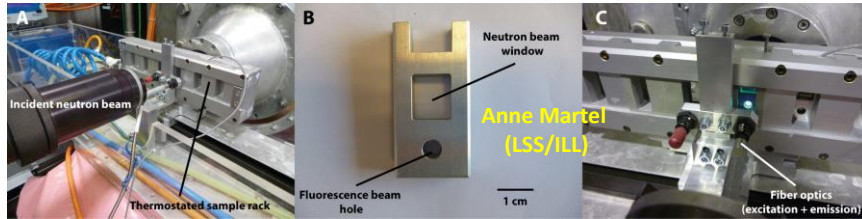


## Possible with SAXS? **No!**

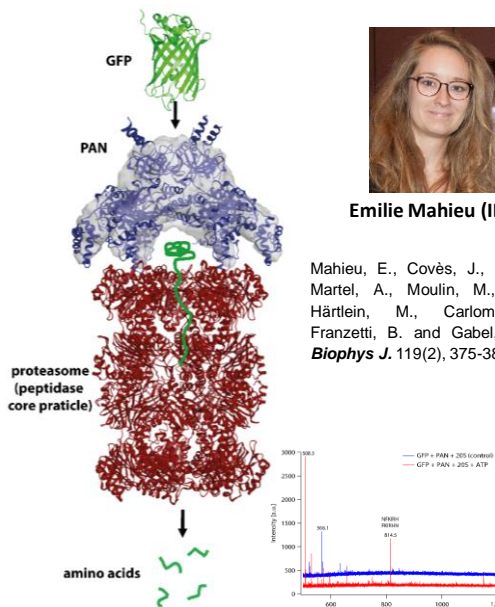


Mahieu, E., Ibrahim, Z., Moulin, M., Härtlein, M., Franzetti, B., Martel, A. and Gabel, F. (2020) *EPJ Web Conf.* 236, 03002.

# PAN-mediated unfolding of GFP at real-time

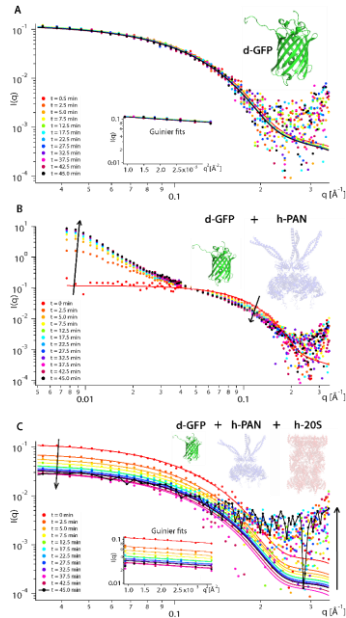


# And in the presence of the proteasome?



Emilie Mahieu (IBS)

Mahieu, E., Covès, J., Krüger, G., Martel, A., Moulin, M., Carl, N., Härtlein, M., Carlomagno, T., Franzetti, B. and Gabel, F. (2020) *Biophys J.* 119(2), 375-388.



# Possibility to propose degradation mechanism in solution!

