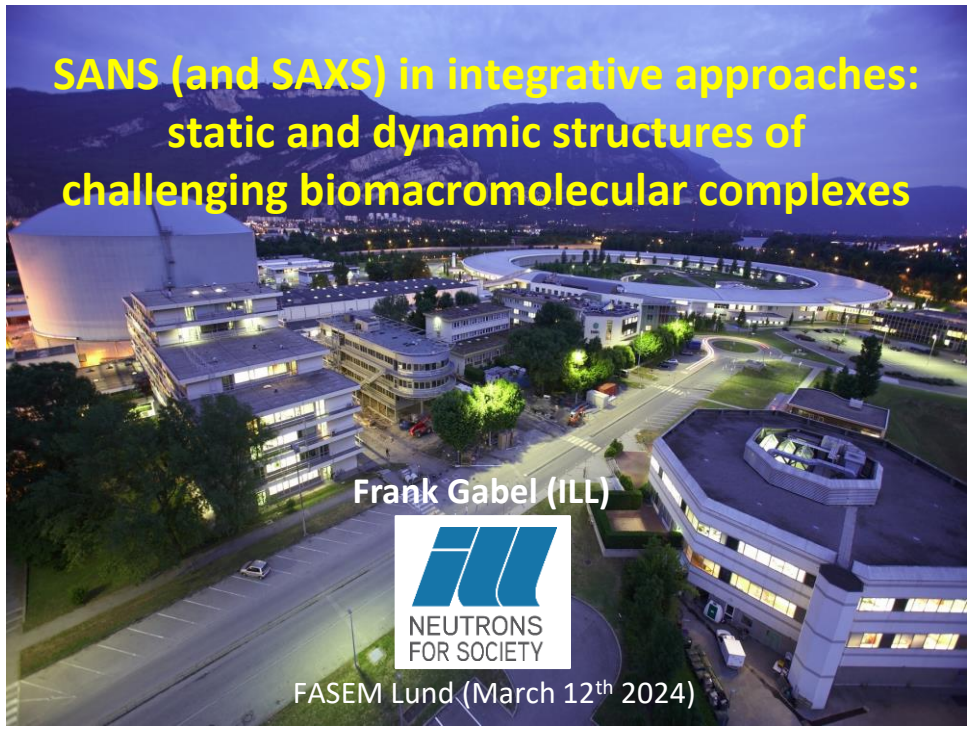
SANS (and SAXS) in integrative approaches: static and dynamic structures of challenging biomacromolecular complexes



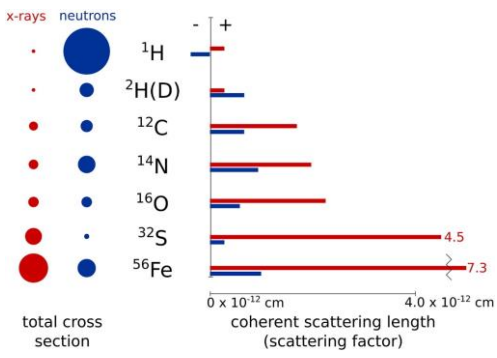
Frank Gabel (ILL)

NEUTRONS FOR SOCIETY

FASEM Lund (March 12th 2024)

Neutrons and X-rays "see" matter differently

- Neutrons have spin and are **isotope-sensitive (H^1 vs H^2=D)**
- Neutrons induce **no radiation damage** and **penetrate bulk matter**
- Low energy of neutrons allows to monitor **thermal motions in samples**
- **Structure** and **dynamics** can be probed (**coherent** vs **incoherent** scattering)



Castellanos et al. (2017) ***Compt. Struct. Biol. J.*** 15, 117-130

LENS consortium of neutron centers in Europe

X-ray vs neutron scattering lengths

Atom	Nucleus	b_{coh} (10^{-12} cm)	$f_{X\text{-}ray}$ ($\theta = 0°$) (10^{-12} cm)
Hydrogen	^{1}H	−0·3742	0·28
Deuterium	^{2}H (D)	0·6671	0·28
Carbon	^{12}C	0·6651	1·69
Nitrogen	^{14}N	0·940	1·97
Oxygen	^{16}O	0·5804	2·25
Phosphorus	^{31}P	0·517	4·23
Sulphur	Mostly ^{32}S	0·2847	4·5

(a) Water

	Σb	$\bar{b}$ (10^{-12} cm Å^{-3})
H_2O	−0·168	−0·00562
D_2O	1·915	+0·06404

(b) Amino acids and proteins

Amino acid	Chemical composition	H (ex)	b_{tot} (H_2O) (10^{-12} cm)	b_{tot} (D_2O) (10^{-12} cm)	b_{tot} (deuterated) (10^{-12} cm)	V (Å^3)
Glycine	C_2NOH_3	1	1·728	2·769	4·85	66·4
Alanine	C_3NOH_5	1	1·645	2·686	6·852	91·5
Valine	C_5NOH_9	1	1·479	2·520	10·854	141·7
Leucine	C_6NOH_{11}	1	1·396	2·437	12·850	167·9
Isoleucine	C_6NOH_{11}	1	1·396	2·437	12·850	168·8
Phenylalanine	C_9NOH_9	1	4·139	5·180	13·51	203·4
Tyrosine	$C_9NO_2H_9$	2	4·719	6·802	14·09	203·6
Tryptophan	$C_{11}N_2OH_{10}$	2	6·035	8·118	16·45	237·6
Aspartic acid	$C_4NO_3H_4$	1	3·845	4·886	8·010	113·6
Glutamic acid	$C_5NO_3H_6$	1	3·762	4·803	10·01	140·6
Serine	$C_3NO_2H_5$	2	2·225	4·308	7·432	99·1
Threonine	$C_4NO_2H_7$	2	2·142	4·224	9·431	122·1
Asparagine	$C_4N_2O_3H_6$	3	3·456	6·580	9·704	135·2
Glutamine	$C_5N_2O_3H_8$	3	3·373	6·497	11·70	161·1
Lysine	$C_6N_2OH_{13}$	4	1·586	5·752	15·12	176·2
Arginine	$C_6N_4OH_{13}$	6	3·466	9·714	17·00	180·8
Histidine	$C_6N_3OH_{6.5}$	1·5	4·959	6·521	11·73	167·3
Methionine	C_5NOSH_9	1	1·764	2·805	11·14	170·8
Cysteine	C_3NOSH_5	2	1·930	4·013	7·137	105·6
Proline	C_5NOH_7	0	2·227	2·227	9·516	129·3

(c) Nucleotides and nucleic acid

Base		Chemical composition	H (ex)	b_{tot} (H_2O) (10^{-12} cm)	b_{tot} (D_2O) (10^{-12} cm)	b (deuterated) (10^{-12} cm)
Adenine	RNA	$PN_5C_{10}O_6H_{11}$	3	11·23	14·35	22·68
	DNA	$PN_5C_{10}O_5H_{11}$	2	10·65	12·73	22·10
Guanine	RNA	$PN_5C_{10}O_7H_{11}$	4	11·81	15·98	23·26
	DNA	$PN_5C_{10}O_6H_{11}$	3	11·23	14·35	22·68
Cytosine	RNA	$PN_3C_9O_7H_{11}$	3	9·26	12·39	20·72
	DNA	$PN_3C_9O_6H_{11}$	2	8·68	10·77	20·14
Uracil	RNA	$PN_2C_9O_8H_{10}$	2	9·28	11·36	19·69
Thyminine	DNA	$PN_2C_{10}O_7H_{12}$	1	8·61	9·65	21·11

$$\bar{b} = \rho_{protein} = \sum_j \frac{b_j}{V}$$

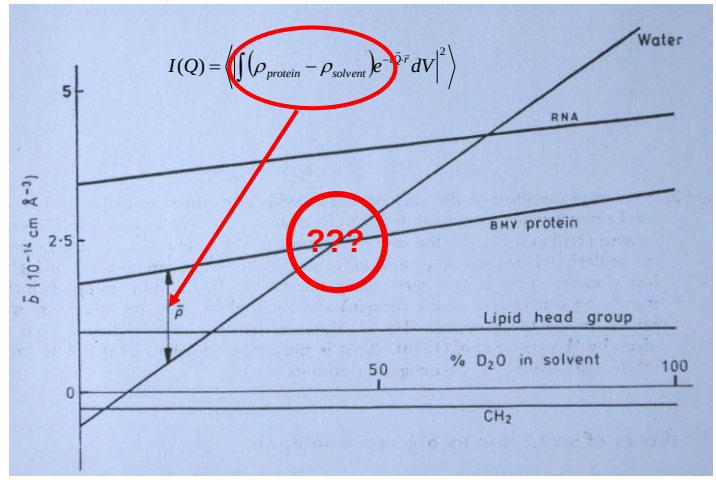
Example glycine in H_2O:

$\rho = [2*0.67+0.94+0.58+3*(-0.37)]10^{-12}$cm/66.4Å^3

$= 2.68*10^{-14}$ cm/Å^3 = $2.68*10^{10}$ cm^{-2}

Jacrot, B. (1976) *Rep. Prog. Phys.* **39**, 911-953.

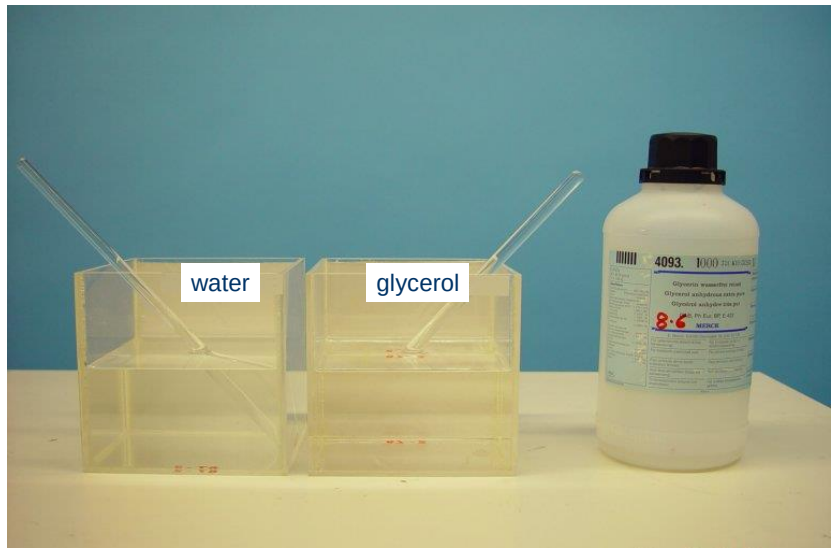
Contrast in H_2O/D_2O buffers



In practice, all biomacromolecules can be matched in SANS, i.e. made invisible!!!

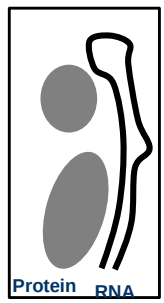
Not so easy with SAXS…

An analogon in optics: refractive index

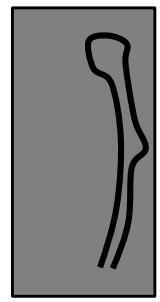


Contrast variation in SANS: natural contrast

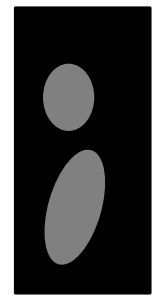
Proteins and RNAs have different proton densities



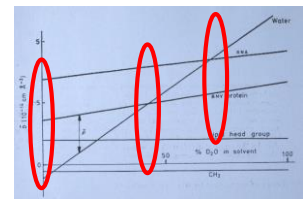
100% H_2O



58% H_2O, 42% D_2O

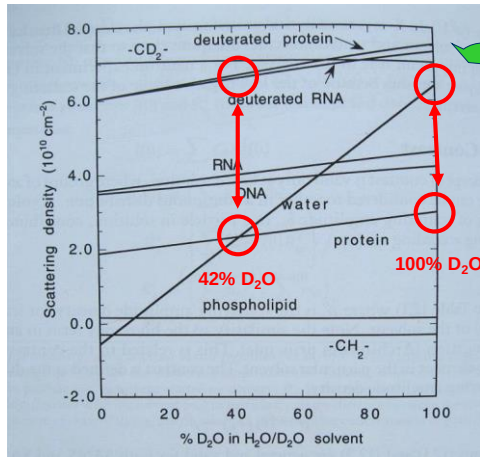


30% H_2O, 70% D_2O



Also possible for protein-protein complexes (deuteration)!

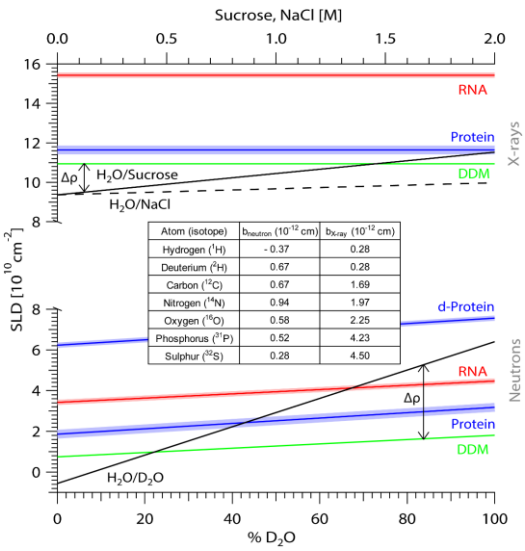
Artificial contrast using deuteration



Protein deuteration not complete but only ~75%!

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

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**

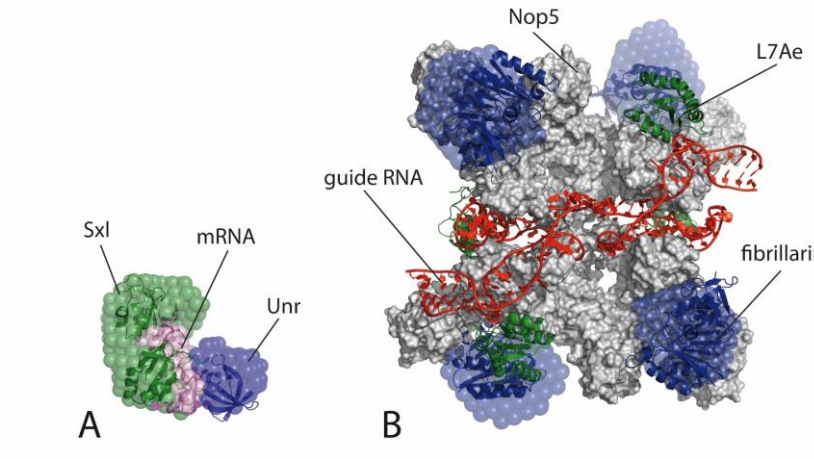
Mahieu & Gabel (2018). *Acta Cryst.* **D74**(Pt 8), 715-726

Gabel et al. (2019). *IUCrJ* **6**(4), 521-525

Gabel et al. (2022). *Acta Cryst.* **D78** (9), 1120-1130

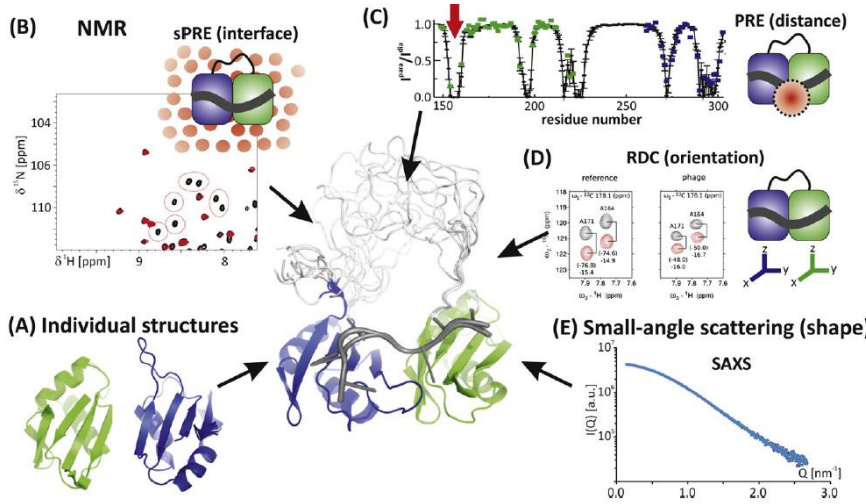
Sophisticated approaches using SANS (SAXS) and NMR:

two "static" examples



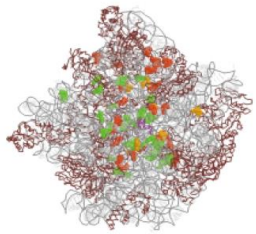
Gabel (2015) Small-angle neutron scattering for structural biology of protein-RNA complexes. *Methods in Enzymology* **558**, 391-415.

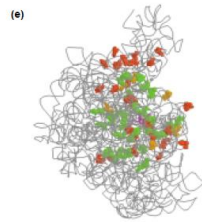
Combination of SANS and (solution) NMR: two complementary techniques



Madl, T., Gabel, F. and Sattler, M. (2011) *J. Struct. Biol.* **173**, 472-482

rRNA modifications and function

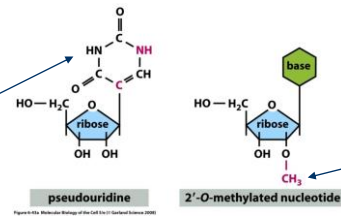




Dozens of modifications in structurally and functionally important (and conserved) regions; their number increases with "complexity" of organism.

Single mutations can be tolerated, absence of all modifications is lethal.

Decatur, W.A. and Fournier, M.J. (2002) rRNA modifications and ribosome function *TIBS* **27**(7), 344-351.



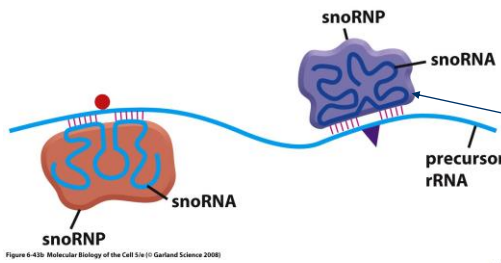
H/ACA complex

Box C/D Complex
(archaea and eukarya)

Number of modifications: bacteria < archaea < eukarya

RNA modifications: snoRNPs, snoRNAs and box C/D

snoRNP = "Small nucleolar Ribonucleo-Protein"

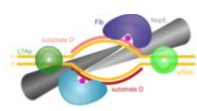


Only in archaea and eukaryotes, ***not*** in bacteria

snoRNP = sRNP in archaea

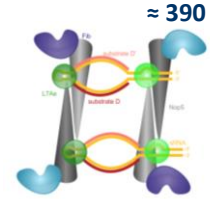
"Guide RNA"
(> 100 in humans!)

Two different architectures have been proposed:



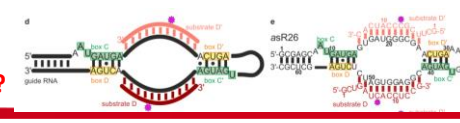
Crystallography

≈ 390 kDa

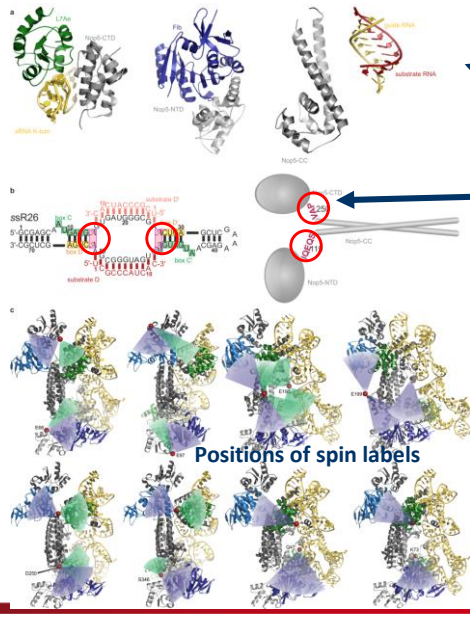


Electron microscopy

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



Structural refinement strategy



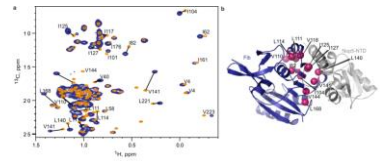
Positions of spin labels

System from ***Pyrococcus furiosus***

Rigid building blocks from crystal structures

+

flexible joints



Methyl resonances CSPs of Fib and L7Ae

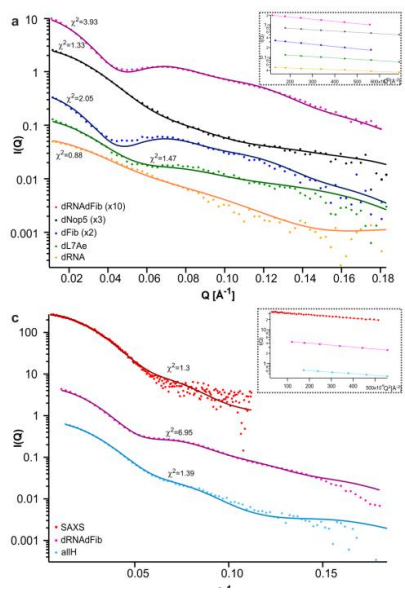
+

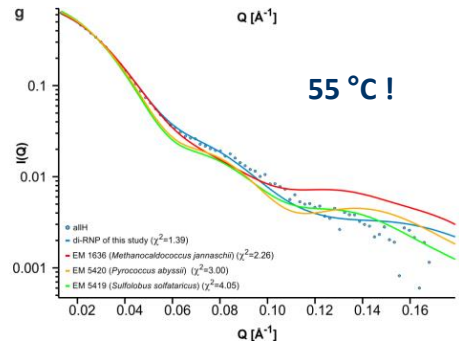
452 PRE distance restraints (apo-complex)

+

SAXS/SANS

SANS (D22) and SAXS (BM29) data





A total of 26 SANS samples:
dFIB, dL7e, dRNA, dNOP5, dRNAdFIB
0, 42, 70% D_2O

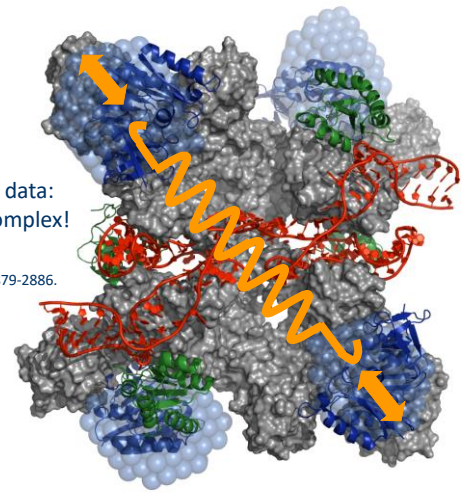
D22 (ILL) Local contact: **Anne Martel**

BM29 (ESRF) Local contact: **Petra Pernot**

Relative positions of FIB proteins within the complex from SANS data

dFIB 42% D_2O SANS data:
FIB positions in the complex!

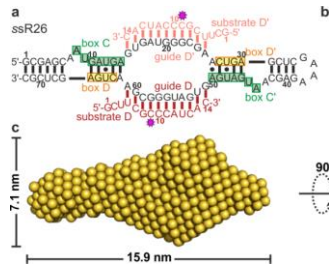
Program **MONSA**:
Svergun (1999) *Biophys. J.* **76**, 2879-2886.

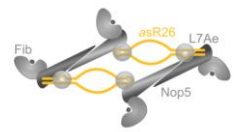


→ **Important restraints for the atomic models!**

Frank Gabel: FASEM Lund March 2024

RNA shape within the complex from SANS data

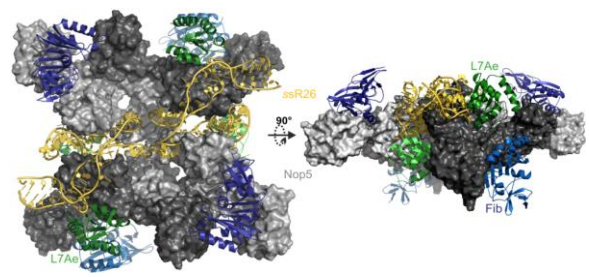




Continuous shape of both **RNAs** in the apo-complex, in contrast to EM models

dRNA SANS data at 42%D_2O

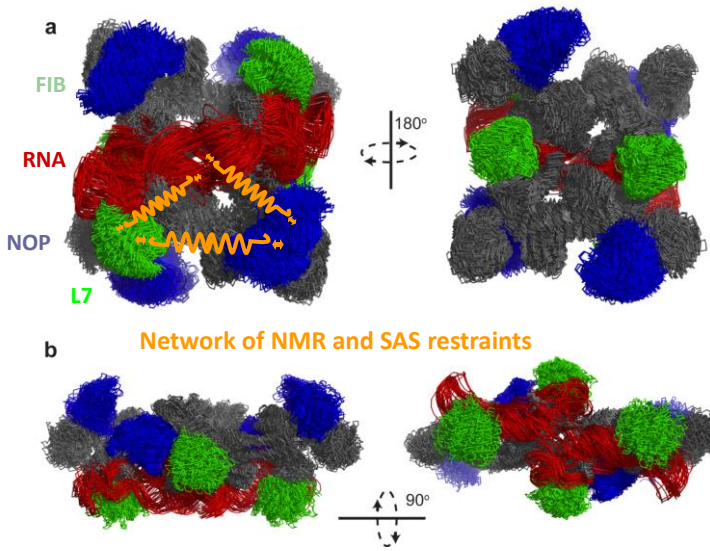
FIBs are not in contact with the **guide RNA** (in an "off" position); only 2 FIB copies can reach RNA



Lapinaite, A., Simon, B., Skjaerven, L., Rakwalska-Bange, M., Gabel, F. and Carlomagno T. (2013)
The structure of the box C/D enzyme reveals regulation of RNA methylation. *Nature* **502**(7472), 519-523.

Frank Gabel: FASEM Lund March 2024

Family of refined structures



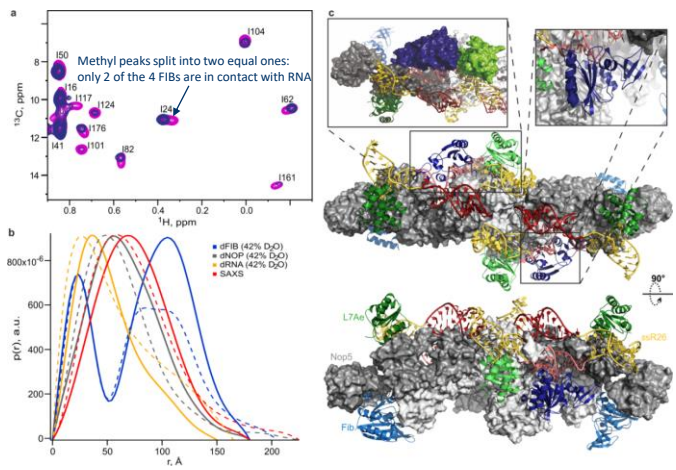
Apo
(no rRNA substrate)

Holo
(with rRNA substrate)

Large conformational change upon substrate binding!

Frank Gabel: FASEM Lund March 2024

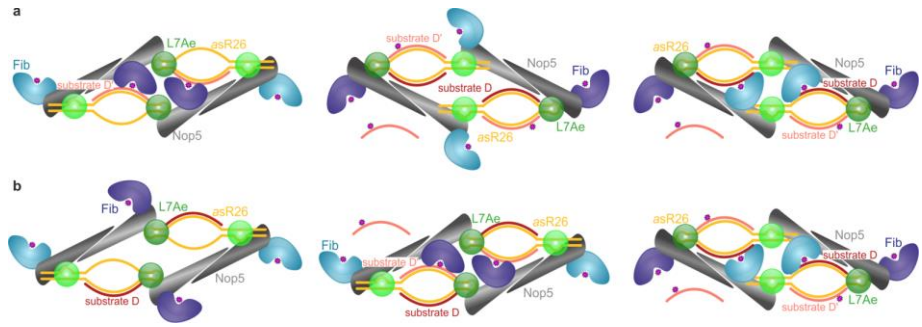
The holo complex



RNA/**RNA**-binding pulls two **NOP** dimers apart, **FIBs** are in contact with **RNA**

Large conformational change upon substrate (RNA) binding to an elongated form (SAXS/SANS+ 257 PRE distance restraints)

Proposed model for the sequential methylation and conformational changes



The structural model of the holo-enzyme, together with the NMR assays, suggests that methylation at the two sites occurs in a

sequential, well-defined order!

→ **Implications on folding pathways for ribosome…**

Acknowledgements


Audrone Lapinaite


Bernd Simon


Teresa Carlomagno

EMBL

ibs Institut de Biologie Structurale

NEUTRONS FOR SCIENCE

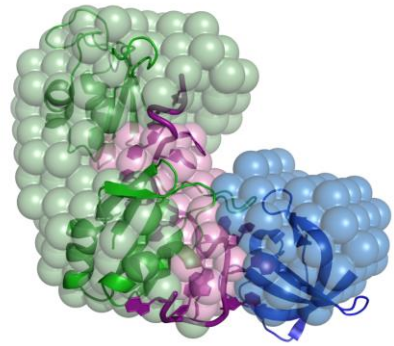
D22 (ILL) Local contact: **Anne Martel**

BM29 (ESRF) Local contact: **Petra Pernot**

ILL and ESRF for beamtime

Financial support by DFG grant CA294/3-1, EU FP7 ITN project RNPnet (contract number 289007) and by the EMBL.

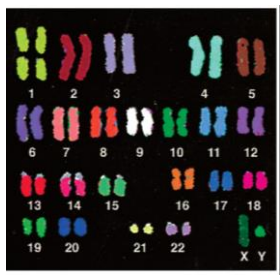
A novel mechanism for translational regulation in *Drosophila melanogaster*



Hennig J, Militti C, Popowicz G, Wang I, Sonntag M, Geerlof A, Gabel F, Gebauer F, and Sattler M (2014). Structural basis for the assembly of the SXL-UNR translation regulatory complex. ***Nature*** 515(7526), 287-290.

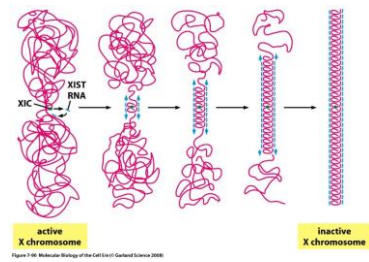
Dosage compensation

Human (male) karyotype



Females: XX

XIST gene silencing system in female mammals



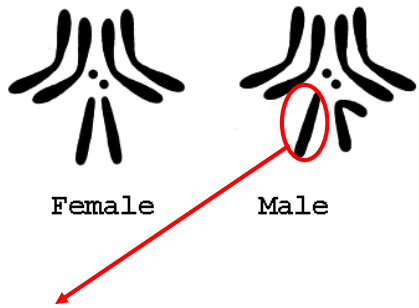
Unequal proteins amounts from XX and XY pairs: needs compensation mechanisms

(Klinefelder syndrome in humans: XXY)

Calico cat

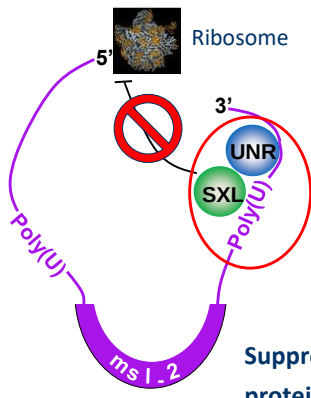
Dosage compensation in *D. melanogaster*

Drosophila **(fruit fly) karyotypes**

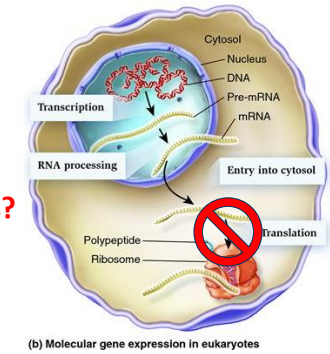


- **Up-regulated by " DCC" (Dosage compensation complex) constituted of 5 proteins and 2 non-coding RNAs**
- **Female-specific protein "SXL" (sex-lethal) silences the expression of a protein of the DCC complex in females by binding to its mRNA transcript and inhibiting its interaction with the ribosome**

Translational repression in *D. melanogaster* dosage compensation



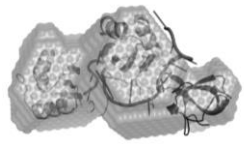
Structural details?



Suppression of the translation of the mRNA of one of the protein partners of the dosage compensation complex in female flies!

→ **Combined NMR/SAXS/SANS structural study**

SANS-specific information

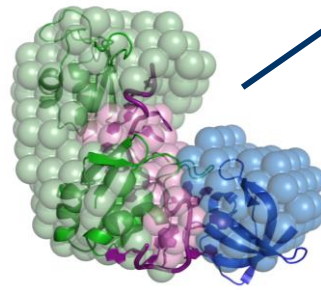


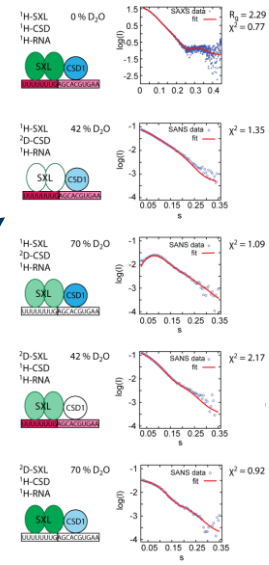
SAXS: overall envelope

RNA sandwiched between proteins!?

SXL

SANS: Internal information





Program **MONSA**:
Svergun (1999) *Biophys. J.* **76**, 2879-2886.

Acknowledgements 'SXL'

Janosch Hennig, Iren Wang, Miriam Sonntag, Grzegorz Popowicz, Arie Geerlof, **Michael Sattler** (TUM/Helmholtz NMR Group)

Cristina Militti, **Fátima Gebauer** (Centre for Genomic Regulation, Barcelona)

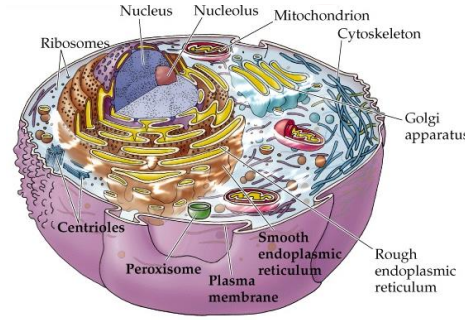
Anne Martel (D22, ILL, BAG system)
Louiza Zerrad (BM29, ESRF)

The importance of controlled protein degradation in biological cells

Cell cycle
(growth, division)

Stress
(T, salt, pH, nutriments, infections…)

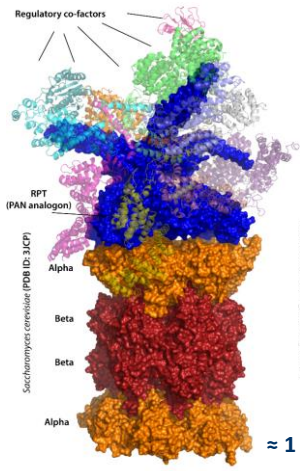
Differentiation
(development, specialization…)



Proteome needs to be produced, controlled and recycled

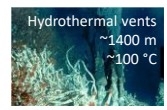
- **Regulatory mechanisms at the level of**
  - Gene expression
  - Degradation
- **Dysfunction can result in**
  - Tumors
  - Neurodegenerative diseases
  - (accelerated) ageing

The proteasome as a key player in protein degradation



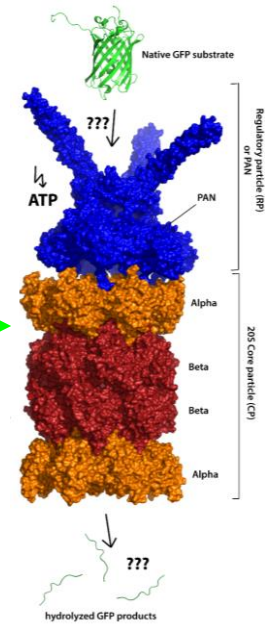
≈ 1 MDa

Pyrococcus horikoshii



Archaeal PAN-proteasome complex

as a biophysical model system

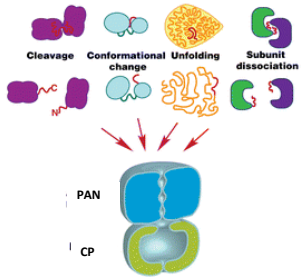


- Exciting static "snapshots" after Cryo-EM revolution
- Lack of dynamic data of substrate processing

Eukaryotic systems too complex for biophysical studies!

Questions related to protein degradation by the PAN - catalytic particle (CP) complex

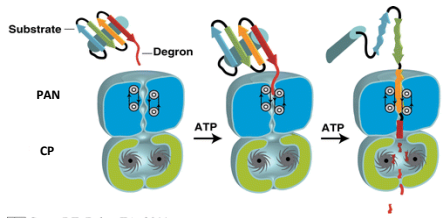
(Proteasome Activating Nucleotidase)



Protein degradation needs to be specific

PAN is a molecular nanomachine that unfolds/directs proteins to the CP

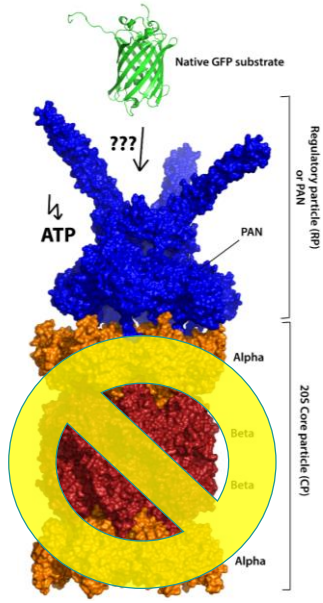
- **Mode of action / substrate processing?**
- **Conformational changes involved?**
- **Evolution of substrate states over time?**



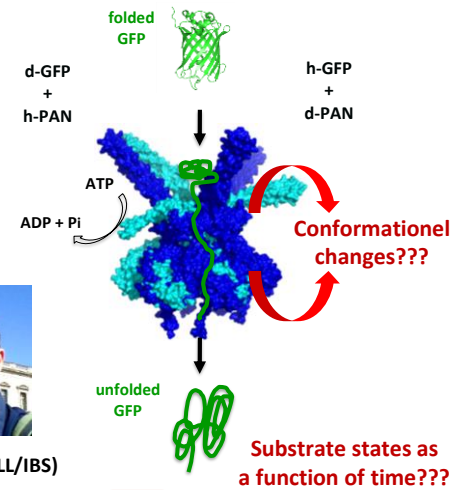
➢ **Time-resolved SANS + specific deuteration!**

Sauer RT, Baker TA. 2011. Annu. Rev. Biochem. 80:587–612

Part 1: looking at the PAN unfoldase "at work"



GFP + PAN (+ATP, 55-60°C) in 42% D_2O

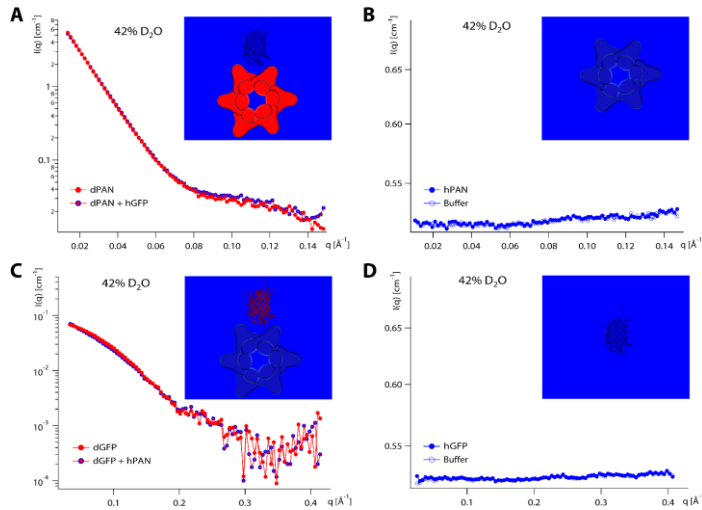


Ziad Ibrahim (ILL/IBS)

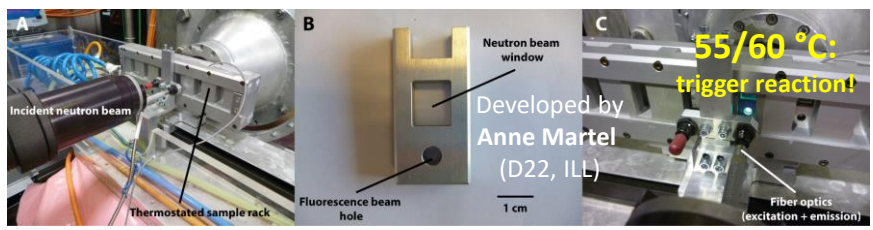
SANS, deuteration and contrast matching

Working at **42% D_2O** makes the **hydrogenated partner invisible!**

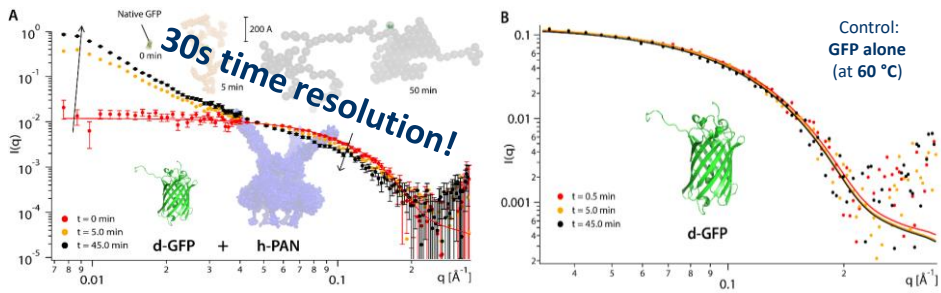
GFP ≈ 28 kDa
PAN ≈ 320 kDa



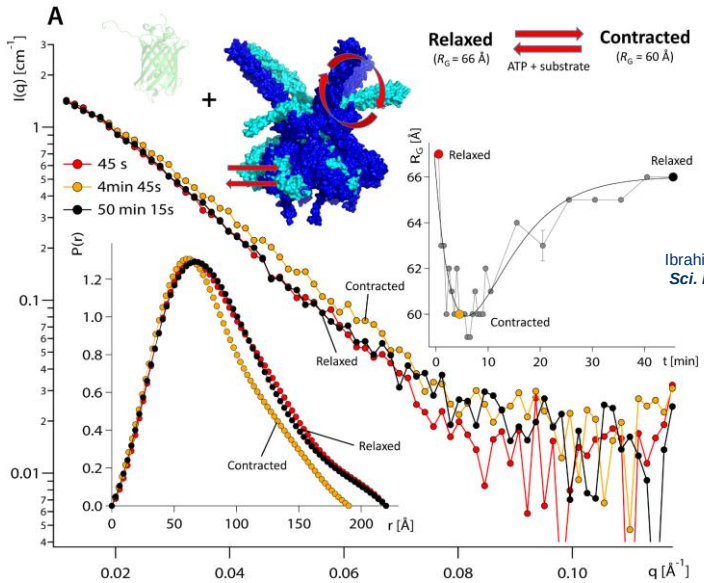
TR-SANS: looking at the GFP substrate



Developed by **Anne Martel** (D22, ILL)

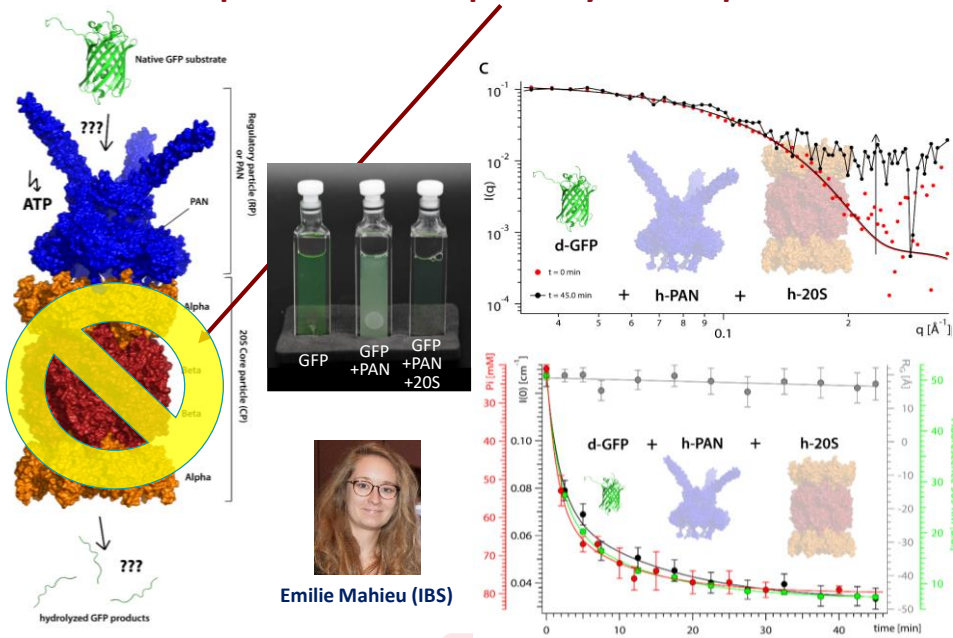


TR-SANS: looking at the PAN complex



Ibrahim et al. (2017)
Sci. Rep. 7, 40948

And in the presence of the proteolytic core particle 20S?

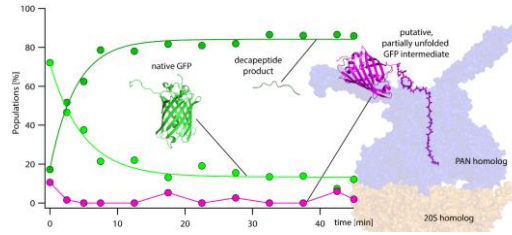


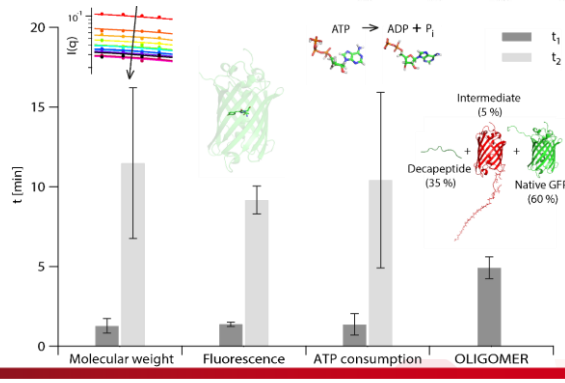
Emilie Mahieu (IBS)

Native GFP, oligopeptide products and a putative intermediate state

Oligopeptide products
detected by **mass spec**

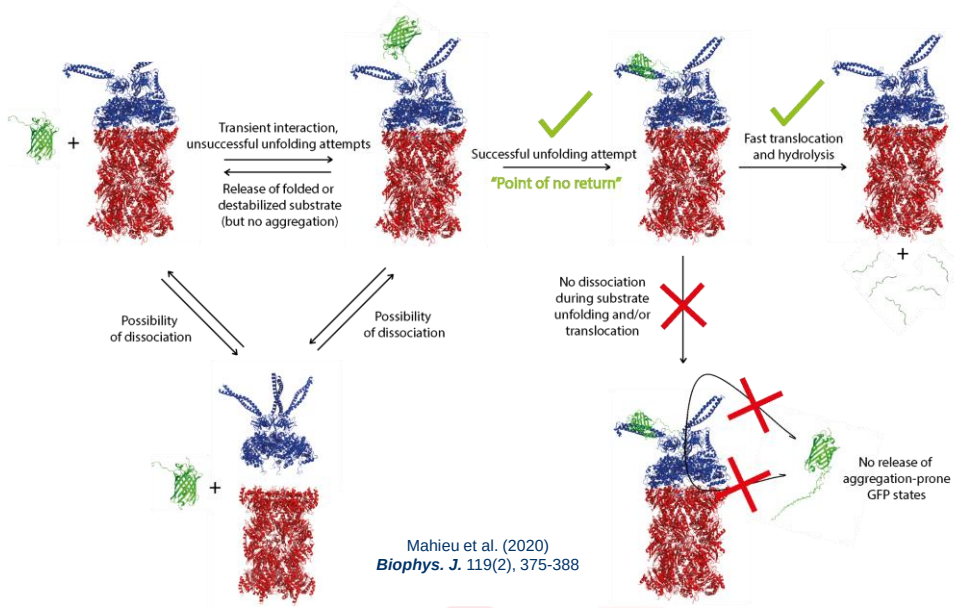
Partially unfolded
intermediate state
observed by **Cryo-EM**





Global information:
- I(0) molecular mass
- Fluoresence
- ATP assay
- Fitting of species

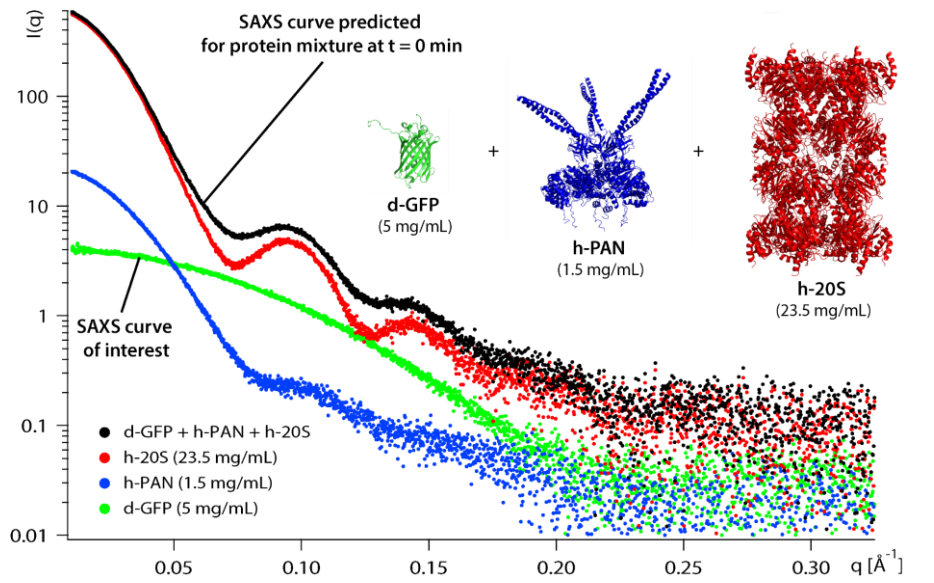
Model mechanism proposed



Mahieu et al. (2020)
Biophys. J. 119(2), 375-388

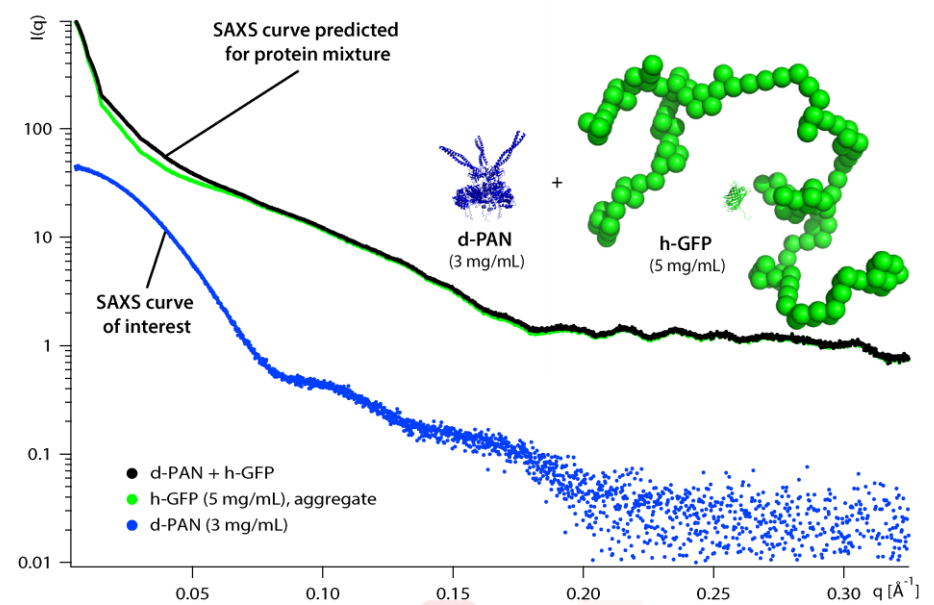
Could this have been done by SAXS?

(Part 1: **GFP** in the **initial mixture**)

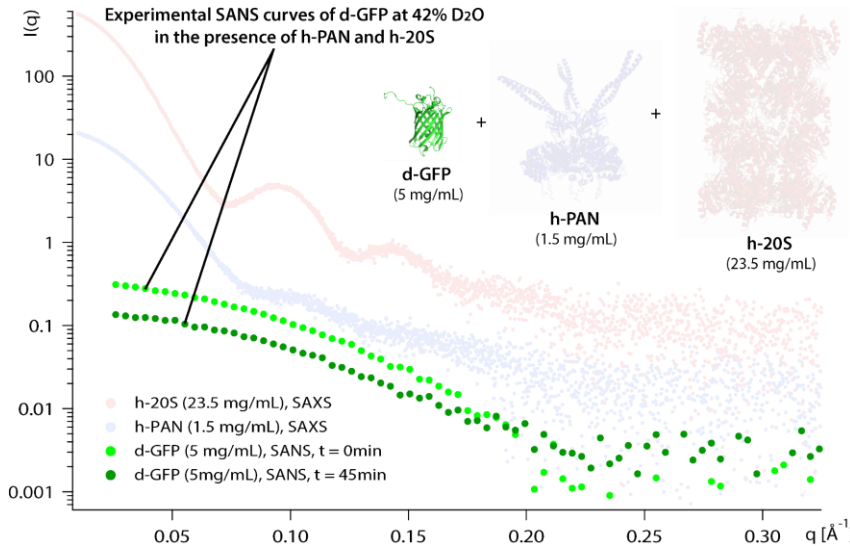


Could this have been done by SAXS?

(Part 2: **PAN** during the **unfolding process**)



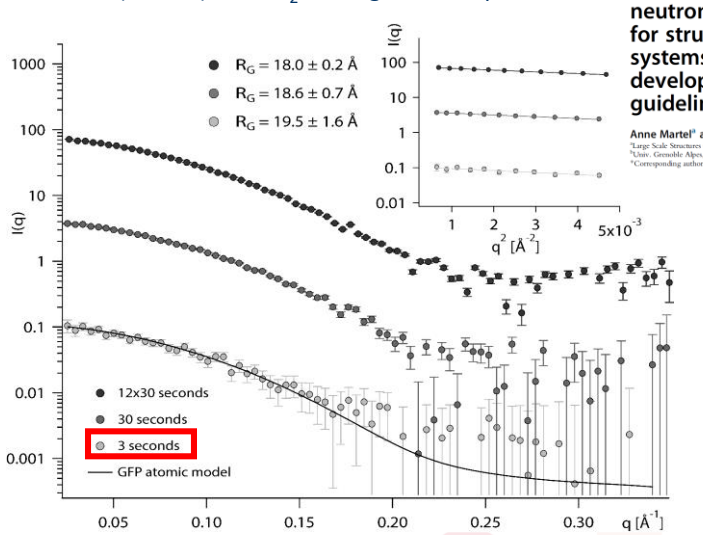
Only SANS allows to focus on small objects in the presence of (very) big ones!



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

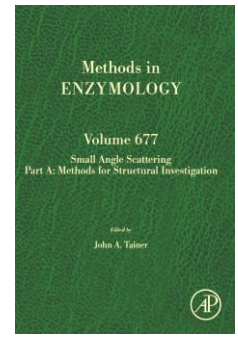
What time-resolution can be achieved on a high-flux SANS instrument?

dGFP (**28** kDa), 42% D_2O, **2** mg/mL, 150 µL, **D22**



Time-resolved small-angle neutron scattering (TR-SANS) for structural biology of dynamic systems: Principles, recent developments, and practical guidelines

Anne Martel[a] and Frank Gabel[b,*]
[a]Large Scale Structures Group, Institut Laue-Langevin, Grenoble, France
[b]Univ. Grenoble Alpes, CEA, CNRS, IBS, Grenoble, France
*Corresponding author: e-mail address: frank.gabel@ibs.fr



Conclusions

- TR-SANS allows to reach **sub-minute** time-resolution
- **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 ("**trigger**")
- Importance of **(per-)deuteration** labeling

Acknowledgements

LSS group (ILL): **Anne Martel**, Susana Teixeira, Nico Carl, Lionel Porcar

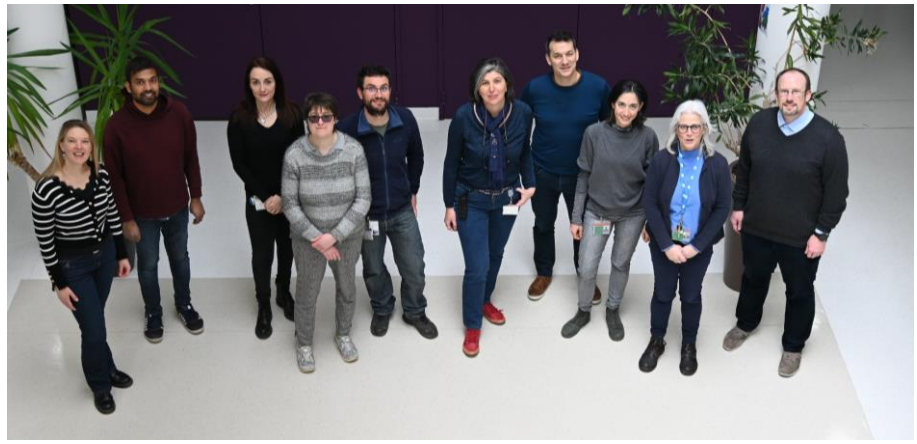
D-lab (ILL): **Martine Moulin**, Michael Härtlein

Univ. Hanover/Helmholtz Braunschweig: Georg Krüger, Teresa Carlomagno: **NMR!** (just accepted in *JMB*)

Anne Martel

Martine Moulin

The new « Biology, Deuteration, Chemistry and Soft Matter » (BDCS) group at ILL



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Different locations on the EPN campus: come and visit us!

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Sandrine Verdon

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Leonardo Chiappisi

Joint staff with PSB partners (CIBB building)

Florent Bernaudat

Jennyfer Gauthier

Lipid lab (Science Building)

Krishna Chaithanya Batchu

Deuteration lab (CIBB Building)

Martine Moulin

Juliette Devos

Valérie Laux

Thank you

for your attention!

https://meetings.embo.org/event/24-sax