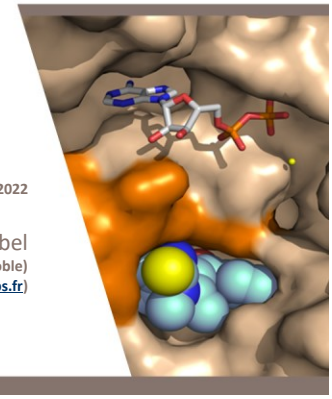
Small-angle neutron scattering (for biology)



EMBO SAXS/SANS practical course, June 20th 2022

Frank Gabel
Institut de Biologie Structurale (Grenoble)
(frank.gabel@ibs.fr)

Typical lengthscales

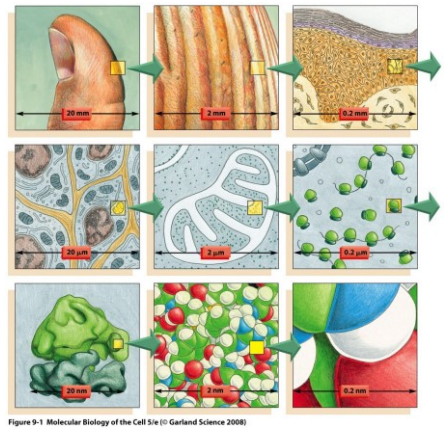
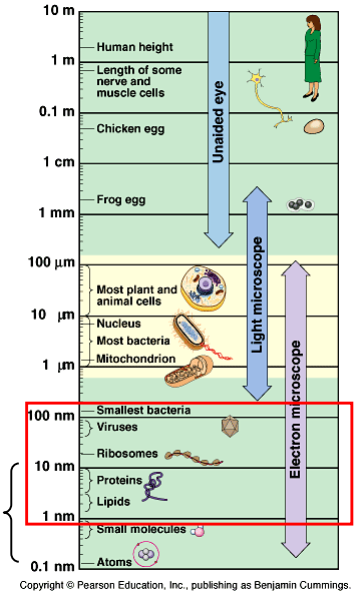


Figure 9-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)



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Small-Angle Neutron Scattering (SANS) in solution

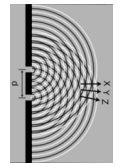
SANS bridges the gap between atomic resolution (NMR and crystallography) and the light microscope

NMR
crystallograpy

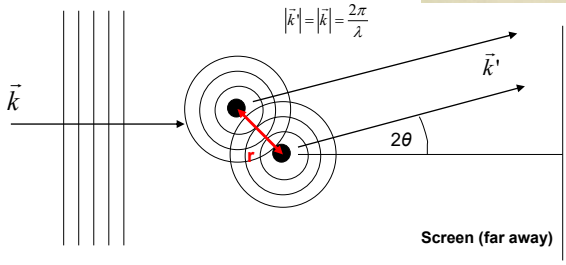
Scattering basics: Huygens-Fresnel principle

1629-1695

1788-1827



Incoming X-ray/neutron wave



Many scattering centers, FOURIER transform:

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

$$Q = |\vec{k}' - \vec{k}| = \frac{4\pi}{\lambda} \sin\theta$$

Reciprocal relationship between real space and the diffraction pattern

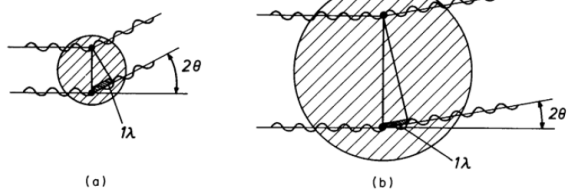
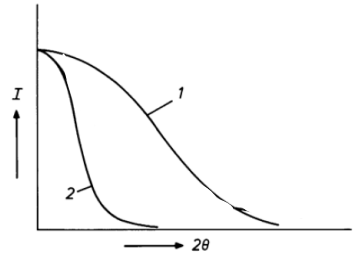


FIG. 1

Glatter and Kratky (1982)



Many scattering centers, FOURIER transform:

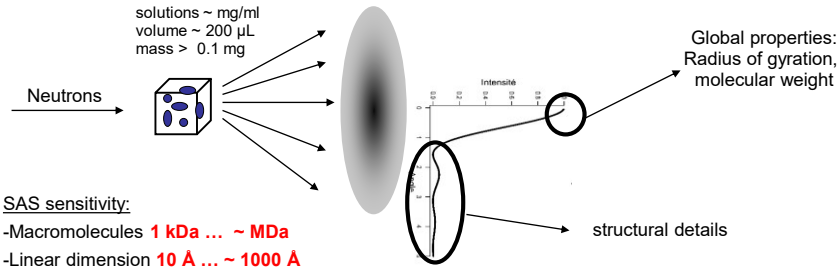
$$I(Q) = \left\langle \left| \sum_j b_j e^{-i\vec{Q}\cdot\vec{r}_j} \right|^2 \right\rangle$$

Orientational averaging

$$I(Q) = \sum_{i,j} b_i b_j \frac{\sin Q(r_i - r_j)}{Q(r_i - r_j)}$$

Debye equation (1915)

SANS sample conditions and information obtained



SAS sensitivity:

-Macromolecules **1 kDa … ~ MDa**

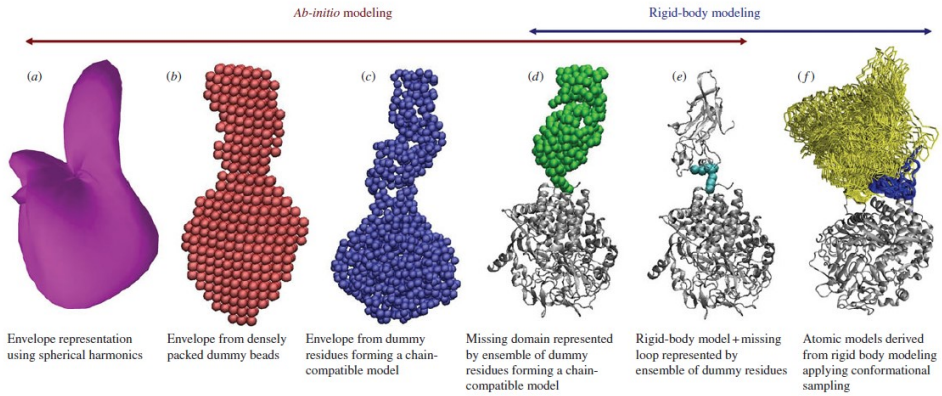
-Linear dimension **10 Å … ~ 1000 Å**

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

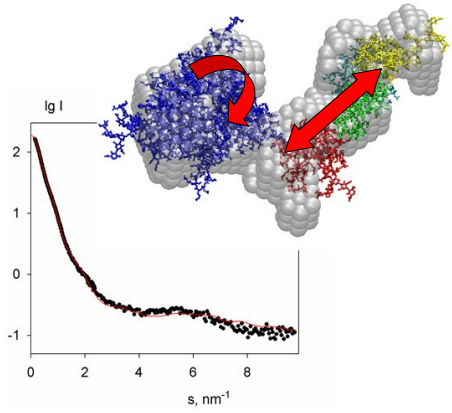
(SAXS lectures, SASquest tutorials,…)



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!**

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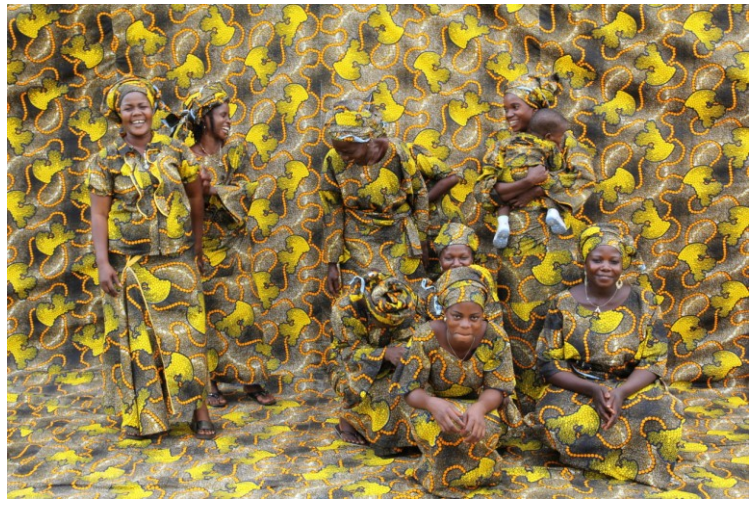
Idea of contrast variation



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

Concept of scattering density and contrast

In vacuo:

$$I(Q)=\left|\sum_j b_j e^{-i\vec{Q}\cdot\vec{r}_j}\right|^2 \qquad I(Q)=\left|\int \frac{b_j}{V_j} e^{-i\vec{Q}\cdot\vec{r}_j} dV\right|^2 \approx \left|\int \rho_{protein} e^{-i\vec{Q}\cdot\vec{r}} dV\right|^2 \qquad I(Q)=N\left\langle\left|\int \rho_{protein} e^{-i\vec{Q}\cdot\vec{r}} dV\right|^2\right\rangle$$

In solution: $$I(Q)=\left\langle\left|\int\left(\rho_{protein}-\rho_{solvent}\right)e^{-i\vec{Q}\cdot\vec{r}}dV\right|^2\right\rangle$$

Continuum approximation: $$\frac{b_j}{V_j}\approx\rho_{protein}=const$$

-How are scattering densities calculated?

-Under which conditions is the approximation valid?

Ideal solutions: no inter-particle effects, only form-factors

Scattering densities in biological samples

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	$C_4N_2O_2H_6$	3	3·456	6·580	9·704	135·2
Glutamine	$C_5N_2O_2H_8$	3	3·373	6·497	11·70	161·1
Lysine	$C_6N_2OH_{12}$	4	1·586	5·752	15·12	176·2
Arginine	$C_6N_4OH_{12}$	6	3·466	9·714	17·00	180·8
Histidine	$C_6N_3OH_{6\cdot5}$	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

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

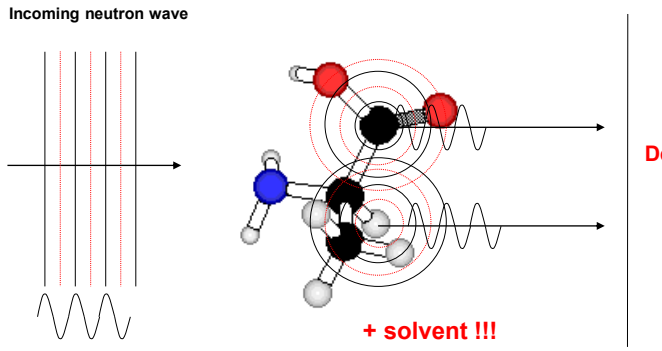
Example glycine in H_2O:

ρ = [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.

Destructive interference in SANS



Destructive interference!
Signal gets weaker!

Atom	Nucleus	b_{coh} (10^{-12} cm)
Hydrogen	^{1}H	−0·3742
Deuterium	^{2}H (D)	0·6671
Carbon	^{12}C	0·6651

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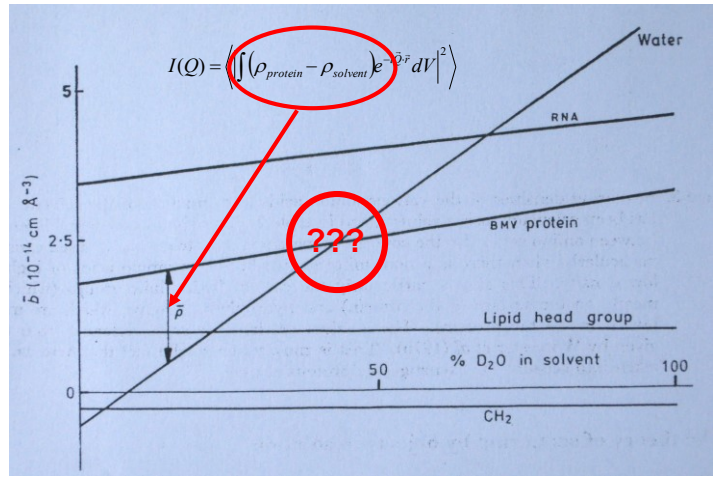
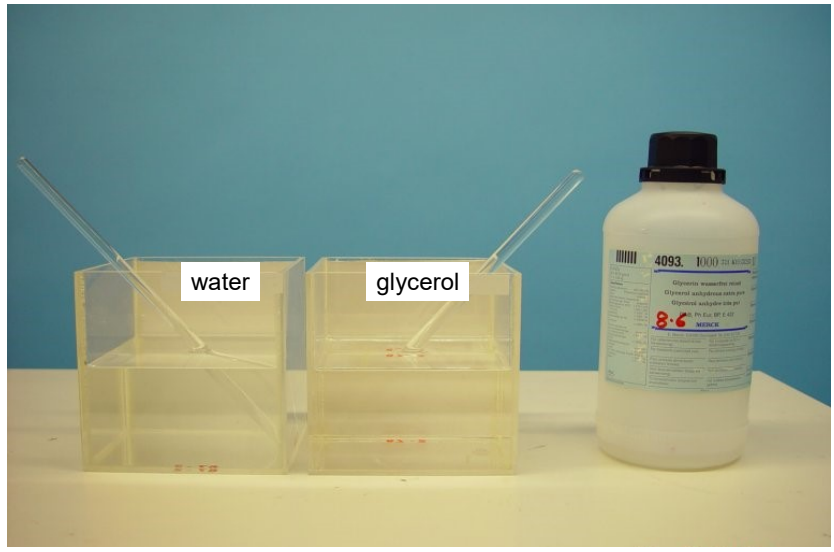
Natural Contrast



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

An analogon in optics: refractive index



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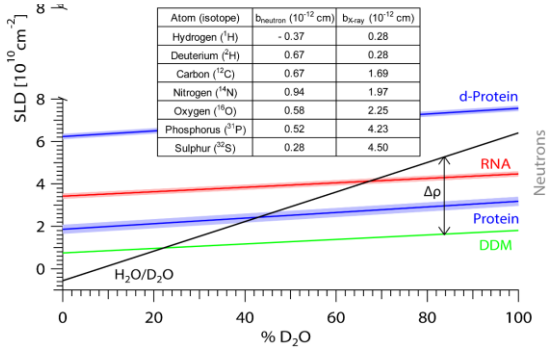
SAXS and contrast variation?

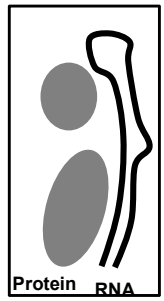


- **Accessible range of solvent electron densities is limited**
- **Contrast agents (salt, sugar…) need to be added at high molarities and may not be inert to biomolecules**
- **Electron density of biomolecules cannot be modified globally**

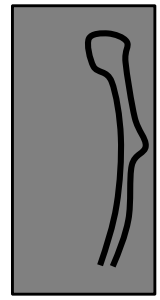
Mahieu, E. & Gabel, F. (2018). *Acta Cryst.* ***D74****(Pt 8), 715-726.*

Contrast variation in SANS: natural contrast

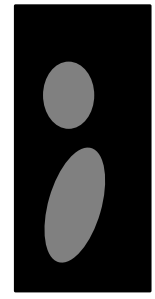
Proteins and RNAs have different proton densities



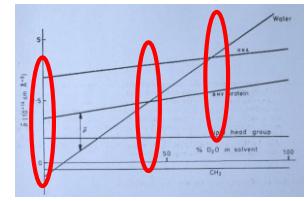
100% H_2O



58% H_2O, 42% D_2O



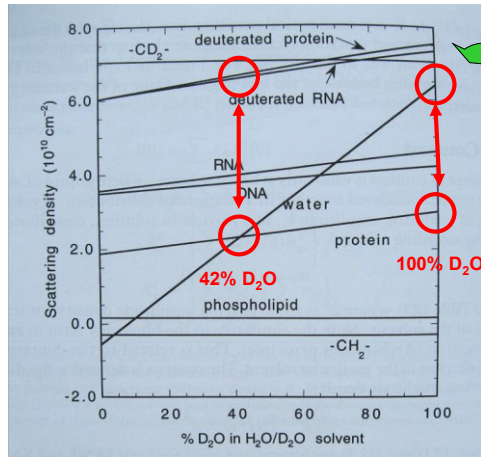
30% H_2O, 70% D_2O



Also possible for protein-protein complexes (deuteration)!

Michael Haertlein's talk

Artificial contrast using deuteration



Protein deuteration not complete but only ~75%!

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

Experimental determination of the zero contrast point (“contrast match point”)

$$I(Q) = \left\langle \left| \int (\rho_{protein} - \rho_{solvent}) e^{-i\vec{Q}\vec{r}} dV \right|^2 \right\rangle$$

$$\rho_{protein} = \frac{\sum_i b_i}{V_{prot}}$$

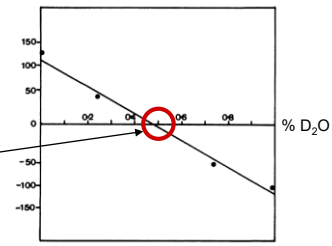
$$\xrightarrow{Q=0} \quad I(0) \sim C \cdot \left[\left(\frac{\sum_i b_i}{V_{prot}} - \rho_{solvent} \right) V_{prot} \right]^2$$

Contrast match-point: $I(0) = 0$

Measure $I(0)$ experimentally (Guinier!) and trace the following expression as a function of H_2O/D_2O:

$$\sqrt{\frac{I(0)}{C}} \sim (\rho_{prot} - \rho_{solvent}) V_{prot}$$

“Contrast match-point”



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Guinier approximation and radius of gyration

$$I(Q) \approx I(0) \exp\left[-\frac{1}{3} R_g^2 Q^2 \right] \qquad R_g Q \leq 1 \ldots 1.3$$

(from expansion of Debye equation)

$$\ln[I(Q)] \approx \ln[I(0)] - \frac{1}{3} R_g^2 Q^2$$

Radius of gyration:

$$R_g^2 = \frac{1}{M} \sum_i m_i r_i^2$$

Contrast x volume

“See-saw analogy”

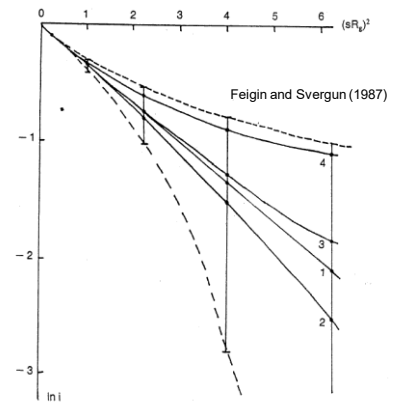
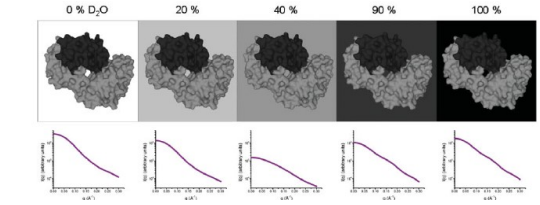


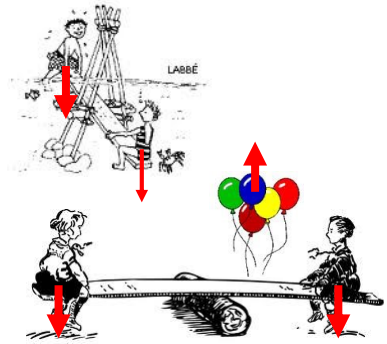
Figure 3.3. Accuracy of the Guinier law: (1) Guinier approximation with estimate (3.25); 2)–(4) correspond to scattering by a solid sphere, an infinitely thin disk, and an infinitely one rod.

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

Protein-protein complexes



Jacques and Trewhella (2010)
Prot. Sci. **19**, 642-657



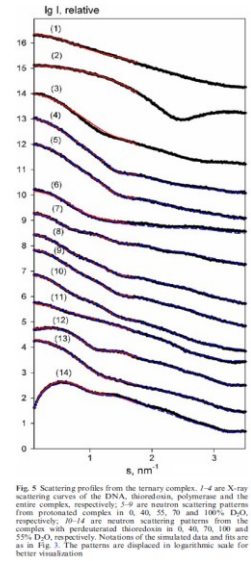


Fig. 5 Scattering profiles from the ternary complex. *1–4* are X-ray scattering curves of the DNA, thioredoxin, polymerase and the entire complex, respectively. *5–9* are neutron scattering patterns from protonated complex in 0, 40, 55, 70 and 100% D_2O, respectively. *10–14* are neutron scattering patterns from the complex with perdeuterated thioredoxin in 0, 40, 70, 100 and 55% D_2O, respectively. Notations of the simulated data and fits are as in Fig. 3. The patterns are displaced in logarithmic scale for better visualization

Petoukhov and Svergun (2006)
Eur. Biophys. J. **35**, 567-576

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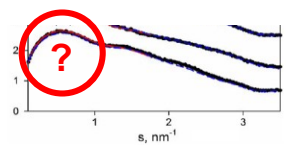
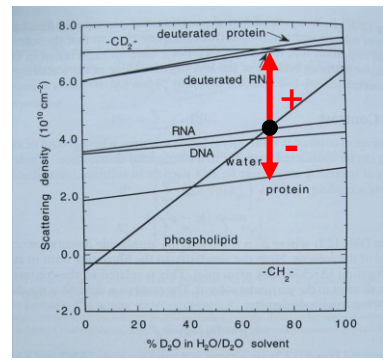
Negative radii of gyration!?

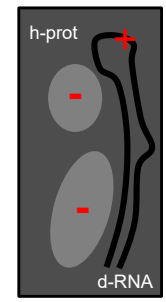


$$\ln[I(Q)] \approx \ln[I(0)] - \frac{1}{3} R_g^2 Q^2$$

$$R_g^2 = \frac{1}{M}\sum_i m_i r_i^2 = \frac{1}{M}\sum_i \Delta\rho_i V_i r_i^2$$

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

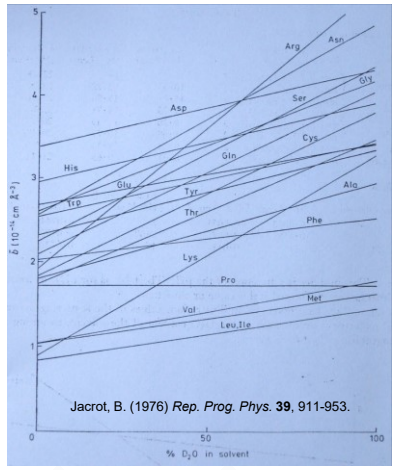




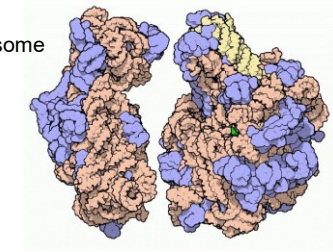
~ 70% D_2O

- Scattering in forward direction, *I*(0), can be weak (or zero)
- Scattering can **get stronger going to higher angles**
- Result: “apparent” negative radius of gyration

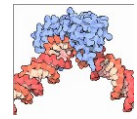
Can proteins be considered as homogeneous particles?

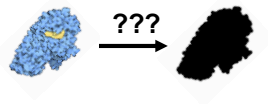


Ribosome



Transcription factor





Depends on the context and on the lengthscale studied!

Summary: homogeneity of biomacromolecules in SANS experiments

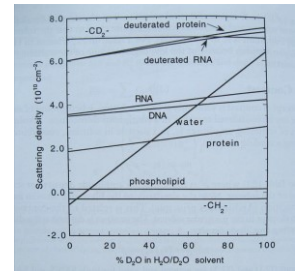
- Proteins can be considered as homogeneous if the scattering density fluctuations are **not ordered** (*e.g.* core vs. outer shell) and/or if they occur on a **length-scale much smaller** than the overall protein dimension and/or the molecules of interest to be studied in a complex

- RNA and DNA are **more homogeneous** than proteins regarding the scattering density fluctuations

- Lipids are in general **heterogeneous** (head *vs.* tail) but can be made homogeneous using **deuteration**

- The approximation of homogeneous particles improves at **smaller angles**

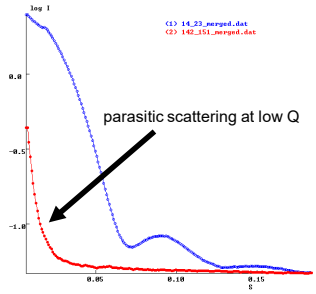
- **Careful** with *ab initio* techniques in SANS!



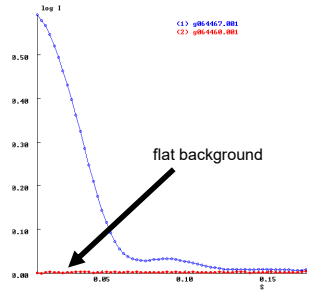
Practical aspects

SAXS *vs* SANS: some practical aspects

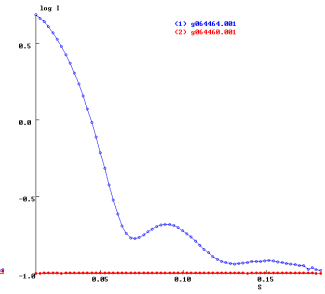
	sample amount	flux	contrast
SAXS	~15 µl @ 1-10 mg/ml	high	weak
SANS	~150µl @ 1-10 mg/ml	low	high (D_2O)



SAXS, ID02, 10s exposure time

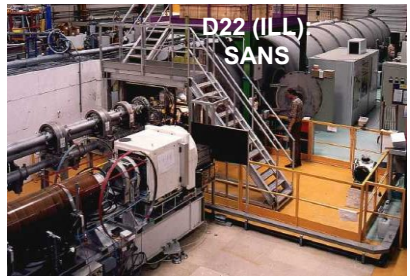


SANS, D22, 20min exposure time (H_2O)



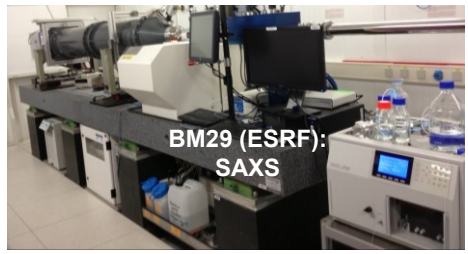
(D_2O)

SANS *vs* SAXS instruments



Quartz cuvette (SANS)

- easy manipulation with pipettes
- multi-sample holder
- no radiation damage!



Exposure times:
~ 5-20 minutes (D22)
~ 1-10 seconds (BM29)

Including sample change:
~ 5-20 minutes (D22)
~ 2-3 minutes (BM29)

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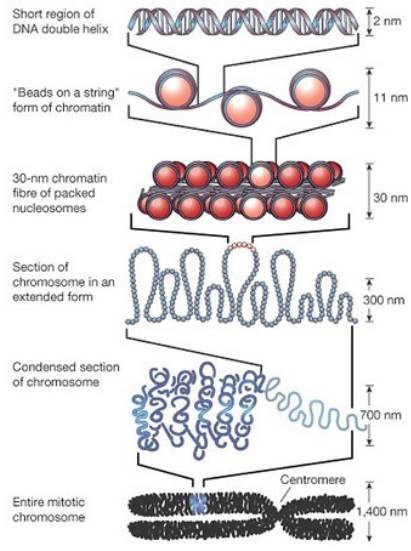
Historical example 1:
Chromatin

The chromatin structure



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Contrast variation: relative arrangement of DNA and protein

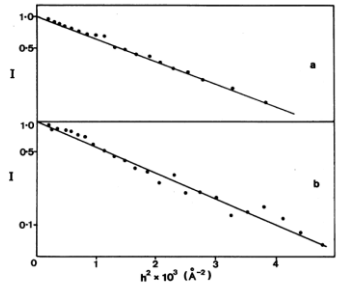

Figure 2: Examples of Guinier plots obtained from chromatin particles (a) Particles in D_2O (b) in H_2O. Semi-log plot of intensity (I) versus h^2, where $h = 4\pi \sin\theta/\lambda$ and 2θ is the angle of scattering. Each data set is fitted with a straight line by a variance-weighted least squares procedure. The data are arbitrarily scaled with respect to I for convenience in plotting them on the same graph.

Relative topology of DNA and protein at low resolution **before** availability of high-resolution models!

Pardon et al. (1975) *Nucl. Acids Res.* **2**(11) 2163-2176

TABLE 1

Percentage D_2O in Buffer	Particle Concentration mg/ml	Radius of Gyration (not corrected) Å	Radius of Gyration (corrected for slit smearing) Å
100	11	38.4 ± 0.3	39.1 ± 0.3
100	5	38.2 ± 0.3	
100	2.5	35.9 ± 1.1	
75	11	36.3 ± 0.9	36.8 ± 0.9
25	11	45.3 ± 6.0	49.9 ± 5.5
0	11	41.8 ± 0.7	43.0 ± 0.7

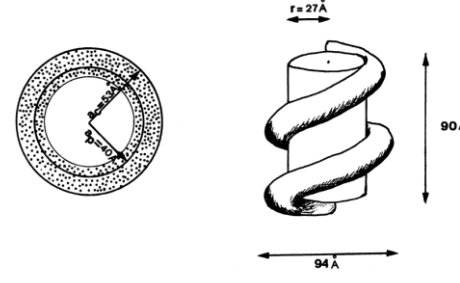

Figure 5: Two possible kinds of structure for the chromatin particle containing 140 base pairs of DNA and eight histones. (a) A spherical particle with overall diameter a_C = 53Å, derived from R_T = 41.1, in which the inner protein core has radius a_P = 40 from the experimental R_P = 30.6Å. The region occupying the DNA is shaded. (b) A cylindrical model in which two turns of helix with pitch 45Å and radius 37Å wound on an inner protein core of radius 27Å.

Historical example 2:
The ribosome

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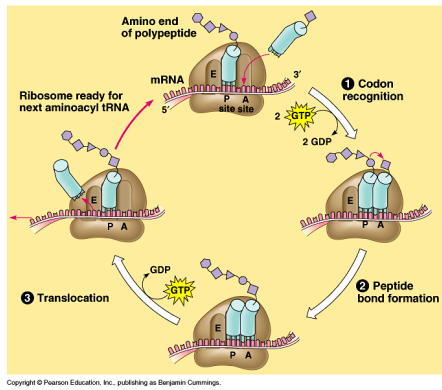
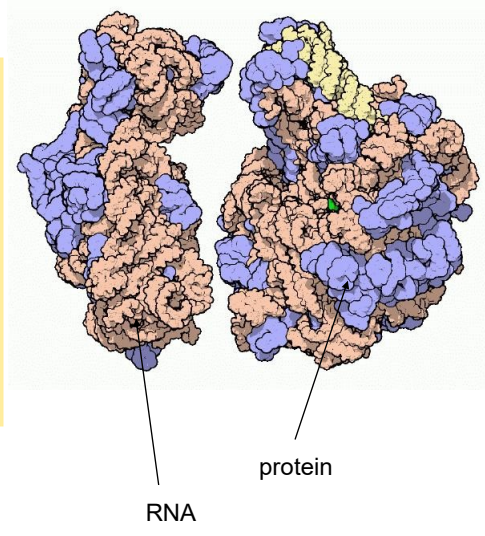
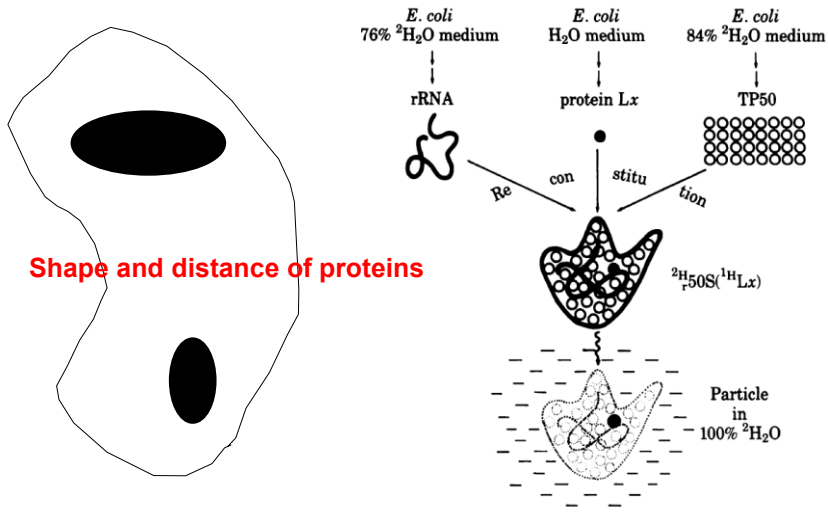
The ribosome structure





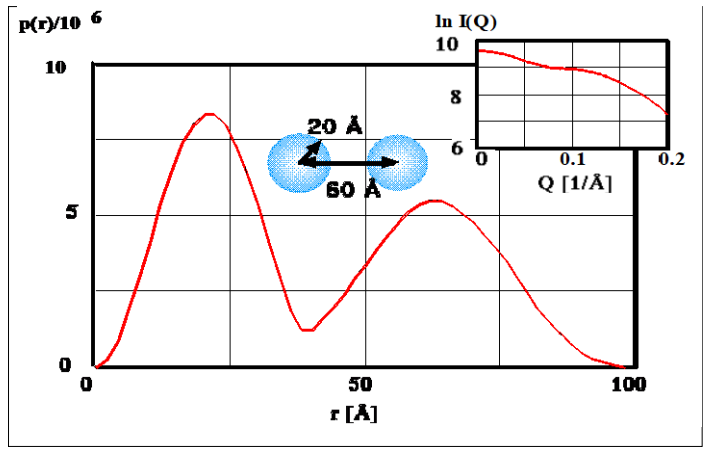
The “glassy” ribosome



Shape and distance of proteins

Nierhaus et al. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2889-2893

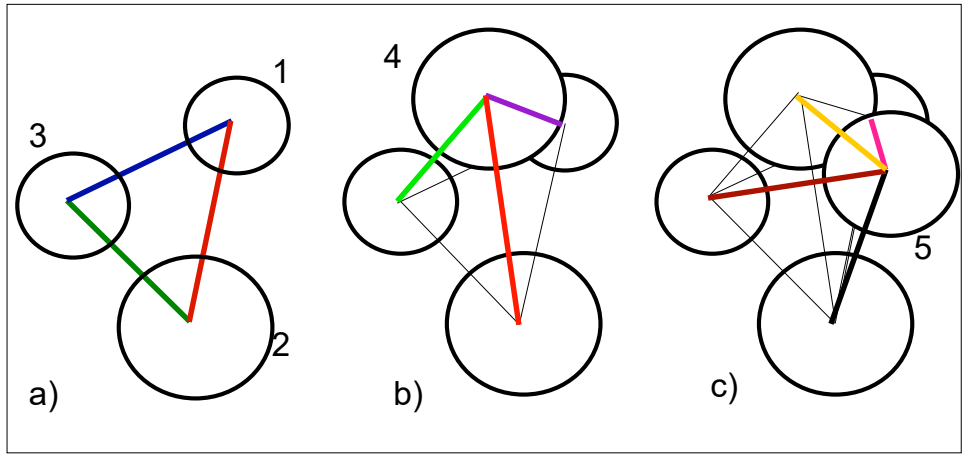
Triangulation



The distance between two components of a complex can be extracted from the scattering curve

courtesy Roland May

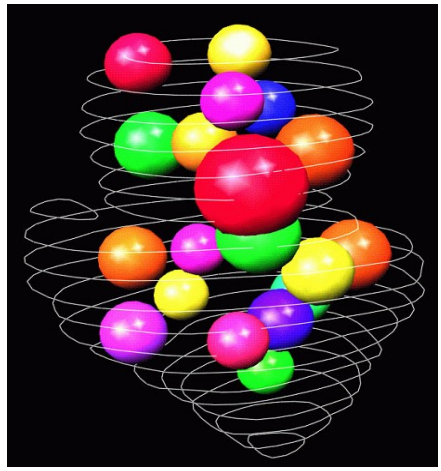
Triangulation



3 distances define a triangle (a), another 3 a tetrahedron of undefined handedness (b). Each further 4 distances add another component in space (c).

courtesy Roland May

Ribosome at low resolution



M.S. Capel, D.M. Engelman, B.R. Freeborn, M. Kjeldgaard, J.A. Langer, V. Ramakrishnan, D.G. Schindler, D.K. Schneider, B.P. Schoenborn, I.-Y. Sillers, S. Yabuki, P.B. Moore (1987) *Science* **238**, 1403-1406

Map of the 30S ribosomal subunit from E. coli. Each protein is represented by a sphere whose volume is the same as that of the protein. The maximum linear dimension of the array is about 190 Å.

courtesy Roland May

EXAMPLE 3:
Membrane proteins

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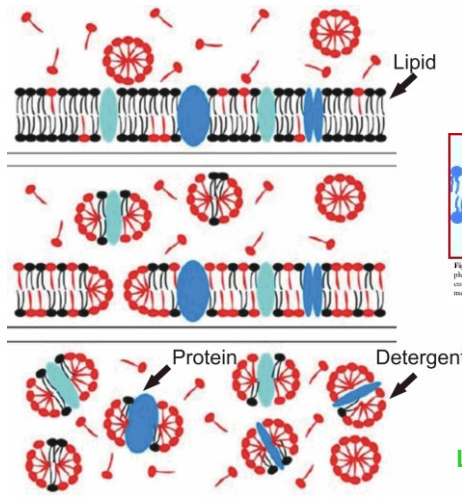
Membrane proteins and lipids/detergents



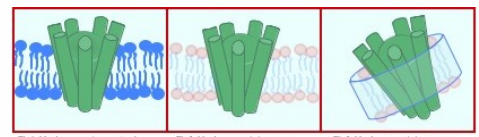


Fig 1: Membrane protein (green) and phospholipid bilayer (blue) in the standard contrast situation. Both bilayer and membrane protein are clearly visible.

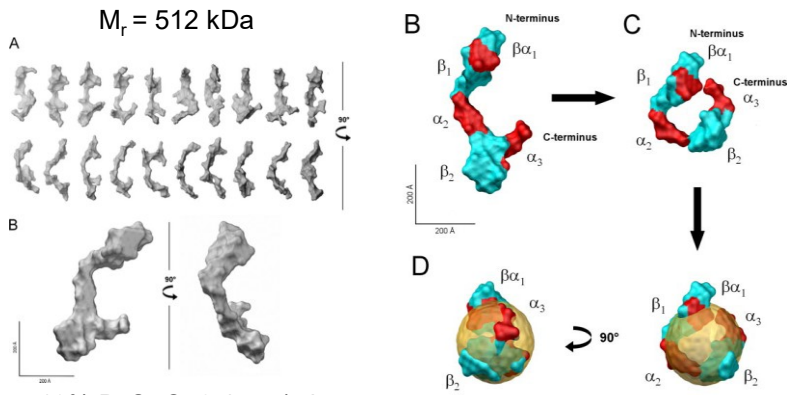
Fig 2: Membrane protein in contrast-matched phospholipid bilayer. Only the membrane protein is visible, but it is confined to a 2D membrane geometry

Fig 3: Membrane protein in contrast-matched Nanodisc. Only the membrane protein is visible and it is floating free in solution

Nanodiscs

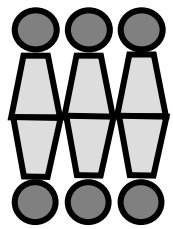
Lise Arleth's talk

Modeling of a membrane protein: apolipoprotein B-100

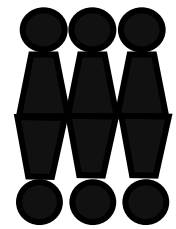


Johs et al. (2006) *J. Biol. Chem.* **281**, 19732-19739

Scattering contrast of lipids: heterogeneity



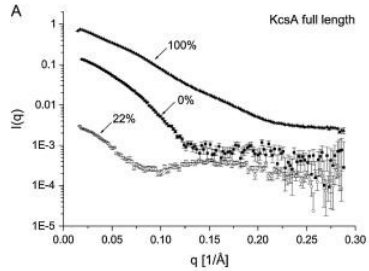
H-lipid headgroup ‘denser’

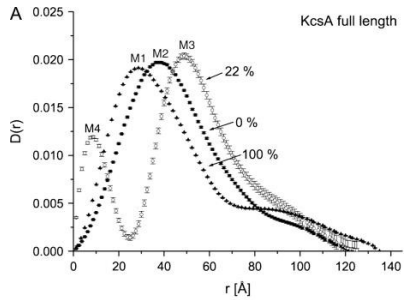


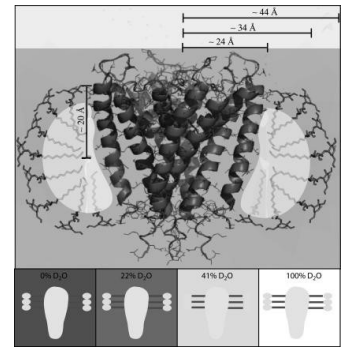
D-lipid

(*d*) *Lipids*				
Phosphyrylcholine	b (10^{-12} cm)	$\bar{b}$ (10^{-12} cm Å^{-3})	b (deuterated) (10^{-12} cm)	$\bar{b}$ (10^{-12} cm Å^{-3})
CH_2	−0·0833	−0·0031	2	0·0744
CH_3	−0·458	−0·0085	2·67	0·0495
$C_5H_{13}NPO_4$	2·24	0·011	15·76	0·072

pH-induced transition in KcsA







concentration. On the basis of published detergent scattering match points, we estimated the detergent scattering match point for decyl-β-D-maltopyranoside ($C_{22}H_{42}O_{11}$) at ~22% D_2O solvent concentration (24), thereby minimizing the contribution of the solubilizing detergent micelle to the observed neutron scattering. However, the fluctuation of scattering length of the DM detergent molecule must be stressed. This is due to the individual scattering match points of the polar maltoside headgroup of ~46% vs. ~3% D_2O solvent concentration for the hydrophobic decyl side chain. The overall match point at 22% D_2O solvent concentration, mini-

Zimmer et al. (2006) *Biophys. J.* **90**, 1752-1766

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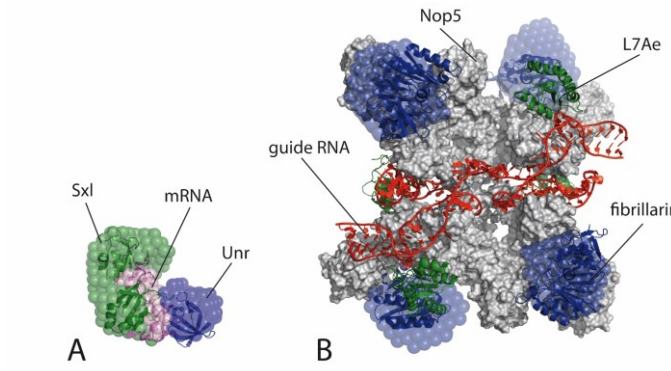
EXAMPLE 4:

Sophisticated approaches using SANS (SAXS) and NMR:

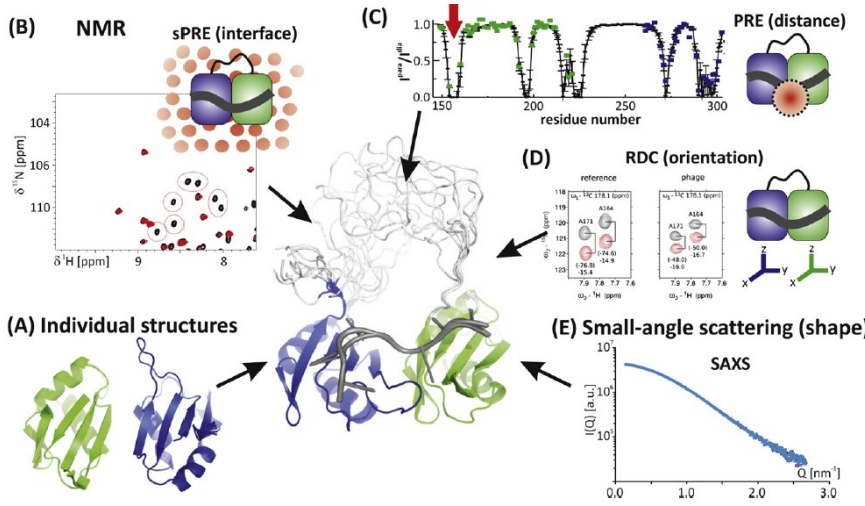
a « tour d'horizon » using a recent example: the BOX C/D complex



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

Bernd Simon's talk

Combining NMR with SAS



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

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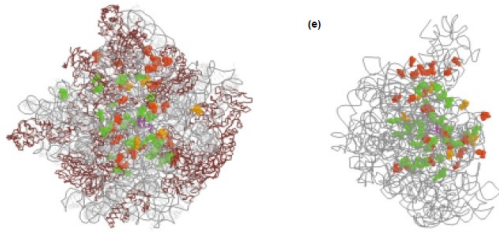
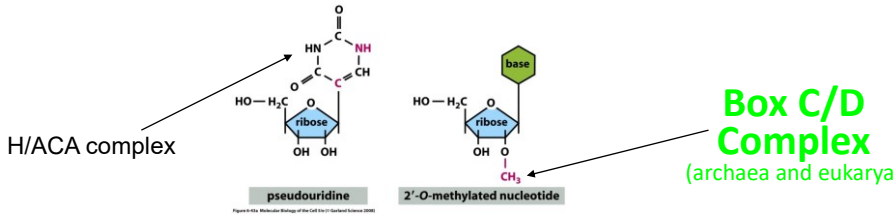
rRNA modifications and function



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.



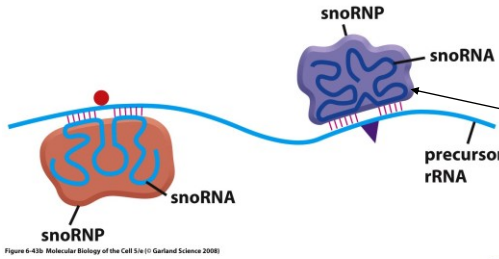
Number of modifications: bacteria < archaea < eukarya

RNA modifications: snoRNPs, snoRNAs and box C/D

snoRNP = “Small nucleolar Ribonucleo-Protein”

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

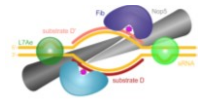
snoRNP = sRNP in archaea



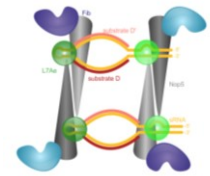
“Guide RNA” (> 100 in humans!)

≈ 390 kDa

Two different architectures have been proposed:

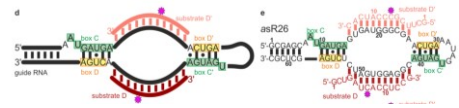


Crystallography

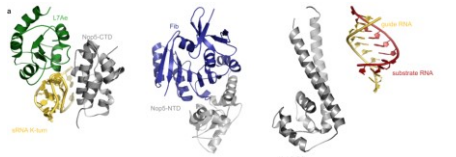


Electron microscopy

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



Structural refinement strategy

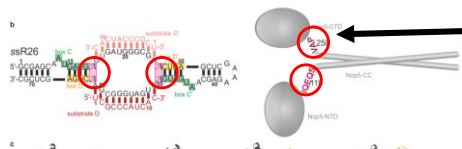


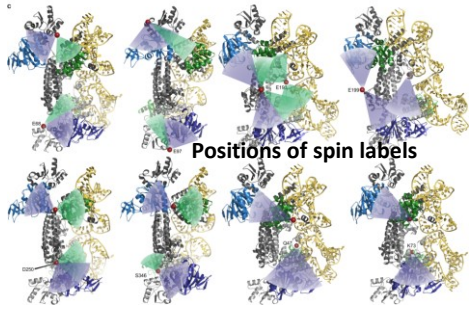
System from ***Pyrococcus furiosus***

Rigid building blocks from crystal structures

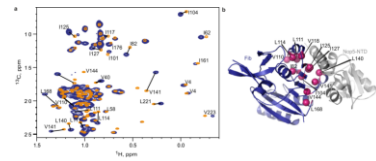
+

flexible joints





Positions of spin labels



Methyl resonances CSPs of Fib and L7Ae

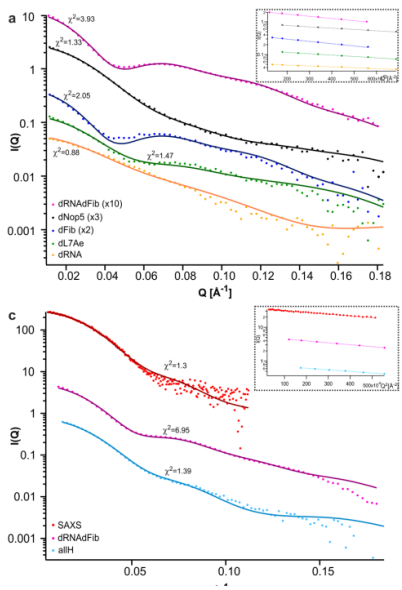
+

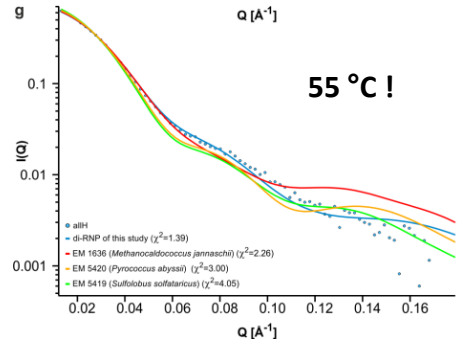
452 PRE distance restraints (apo-complex)

+

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**

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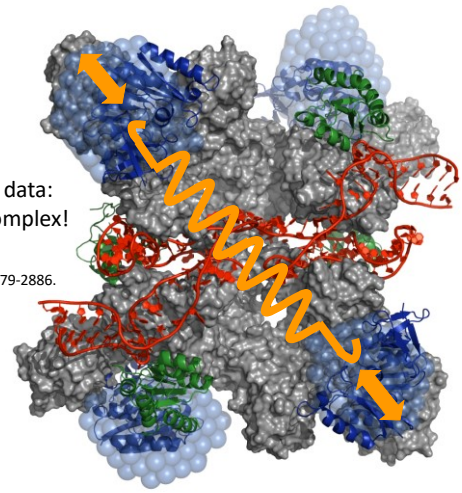
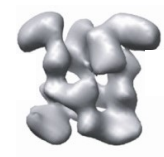
Relative positions of FIB proteins within the complex from SANS data

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

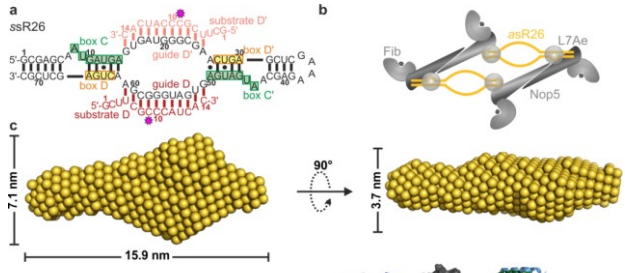
Program **MONSA**:
Svergun (1999) *Biophys. J.* **76**, 2879-2886.





Important restraints for the atomic models!

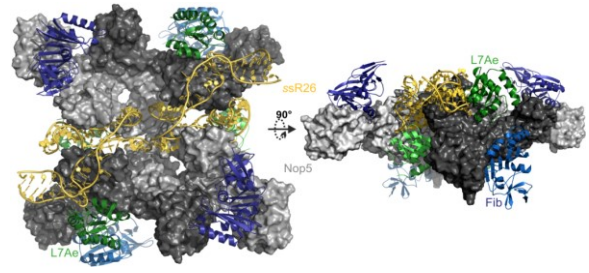
RNA shape within the complex from SANS data



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.

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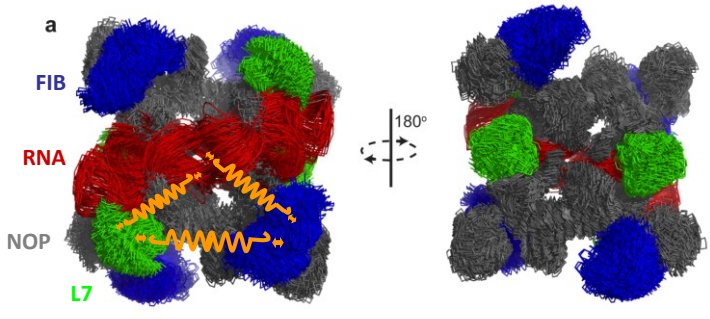
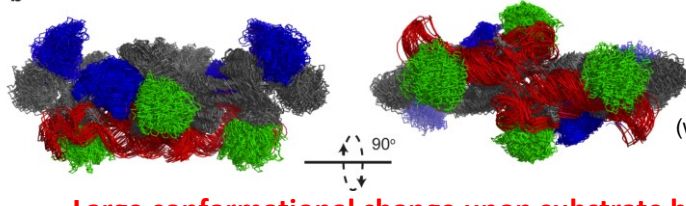
Family of refined structures



Apo
(no rRNA substrate)

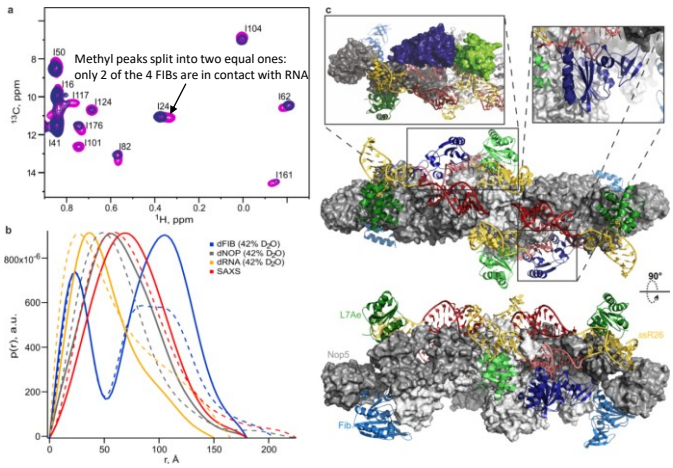
Network of NMR and SAS restraints



Holo
(with rRNA substrate)

Large conformational change upon substrate binding!

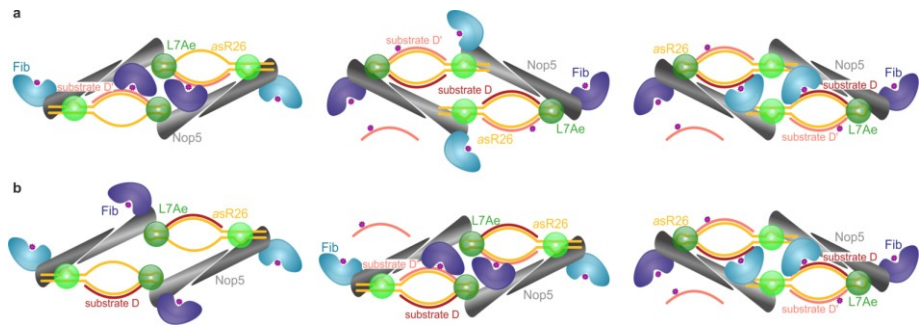
The holo complex



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

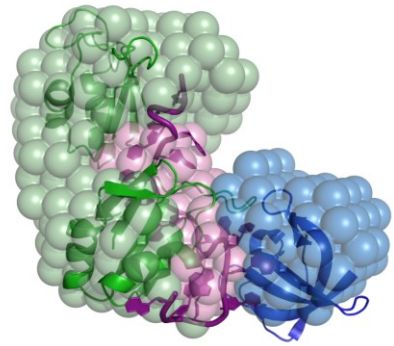


The structural model of the holo-enzyme, together with the NMR assays, suggests that methylation at the two sites occurs in a

sequential, well-defined order!

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

A novel mechanism for translational regulation in *Drosophila melanogaster*



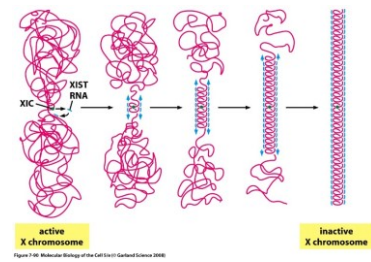
Hennig J, Militti C, Popowicz G, Wang I, Sonntag M, Geerlof A, Gabel F, Gebauer F, and Sattler M (2014). Structural basis for the assembly of the SXL-UNR translation regulatory complex. ***Nature*** 515(7526), 287-290.

Dosage compensation

Human (male) karyotype

Females: XX

XIST gene silencing system in female mammals



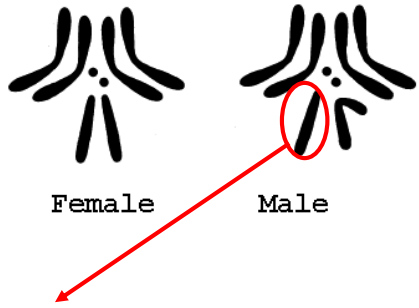
Unequal proteins amounts from XX and XY pairs: needs compensation mechanisms

(Klinefelder syndrome in humans: XXY)

Calico cat

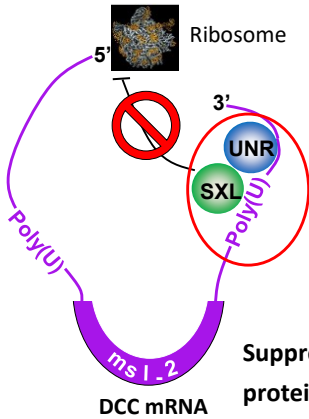
Dosage compensation in *D. melanogaster*

Drosophila **(fruit fly) karyotypes**

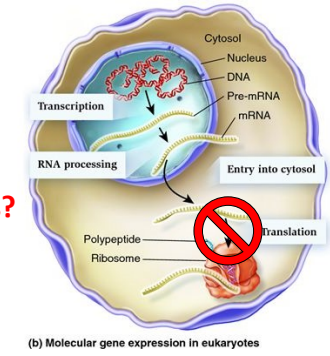


- **Up-regulated by " DCC" (Dosage compensation complex) constituted of 5 proteins and 2 non-coding RNAs**
- **Female-specific protein "SXL" (sex-lethal) silences the expression of a protein of the DCC complex in females by binding to its mRNA transcript and inhibiting its interaction with the ribosome**

Translational repression in *D. melanogaster* dosage compensation



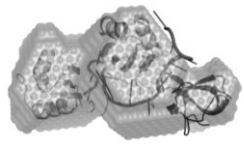
Structural details?



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**

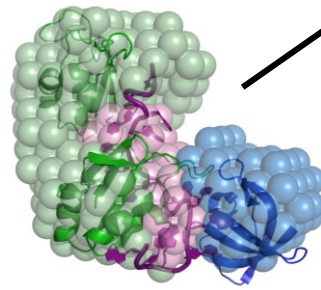
SANS-specific information



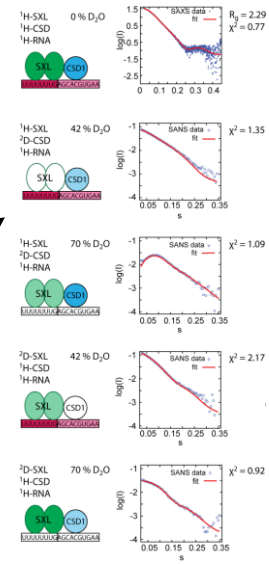
SAXS: overall envelope

RNA sandwiched between proteins!?

SXL



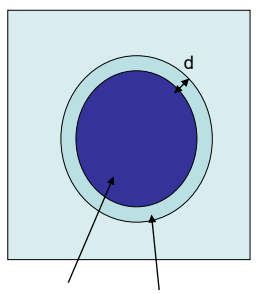
SANS: Internal information

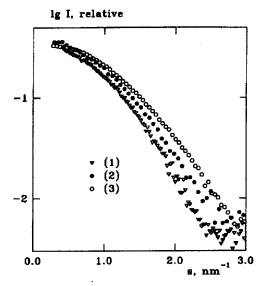


RNA UNR/CSD1

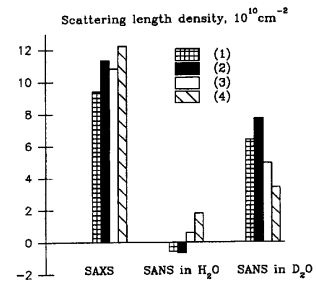
Program **MONSA**:
Svergun (1999) *Biophys. J.* **76**, 2879-2886.

Example 5: SANS provides more than shapes!





(1) X-rays
(2) neutrons in 100% H_2O
(3) neutrons in 100% D_2O



(1) bulk solvent
(2) hydration shell 20% denser
(3) flexible side chains
(4) protein

$$I(Q) = \left\langle \left| A_a(\vec{Q}) - \rho_s A_s(\vec{Q}) + \delta\rho_H A_H(\vec{Q}) \right|^2 \right\rangle_\Omega$$

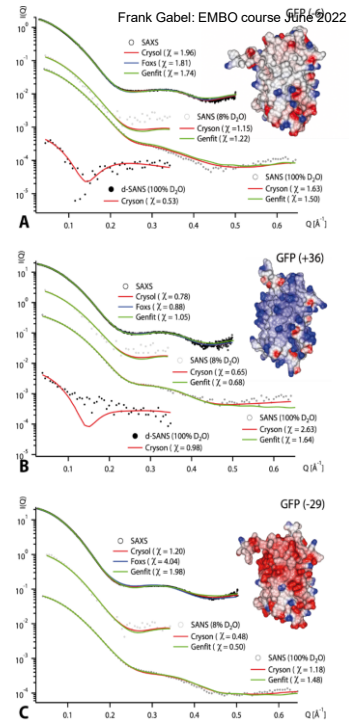
$$\delta\rho_H \approx 0.1\rho_s$$

Svergun DI, Richard S, Koch MH, Sayers Z, Kuprin S, Zaccai G. (1998) Protein hydration in solution: experimental observation by x-ray and neutron scattering. *Proc Natl Acad Sci U S A.* **95**(5):2267-2272.

Jochen Hub's talk

Influence of surface charge?

	GFP(-6)	GFP(-29)	GFP(+36)
SAXS			
HS $\Delta\rho / \rho_{bulk}$ **(%)**	5.00 ± 0.57	8.80 ± 0.62	4.13 ± 0.31
SANS (100 %)			
HS $\Delta\rho / \rho_{bulk}$ **(%)**	0.00 ± 0.00	2.65 ± 0.60	0.27 ± 0.47
SANS (8 %)			
HS $\Delta\rho / \rho_{bulk}$ **(%)**	(0.00±0.00)	(0.00±0.00)	(0.00±0.00)
d-SANS (100 %)			
HS $\Delta\rho / \rho_{bulk}$ **(%)**	0.95 ± 0.35	N.D.	0.00 ± 0.00



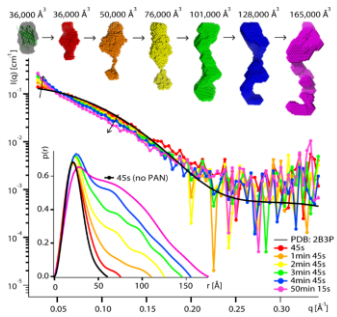
Residue-specific densities!

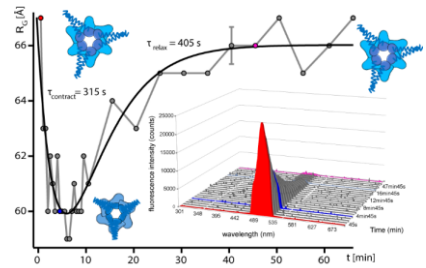
Denser around acidic residues

Kim, H.S., Martel, A., Girard, E., Moulin, M., Härtlein, M., Madern, D., Blackledge, M., Franzetti· B. and Gabel· F. (2016) Solution scattering of X-rays and neutrons on supercharged proteins reveals residue-specific modifications of the hydration shell. ***Biophys. J.*** 110(10), 2185-2194.

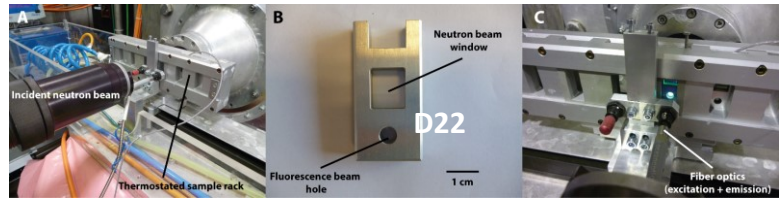
Frank Gabel: EMBO course June 2022

Example 6: time-resolved SANS



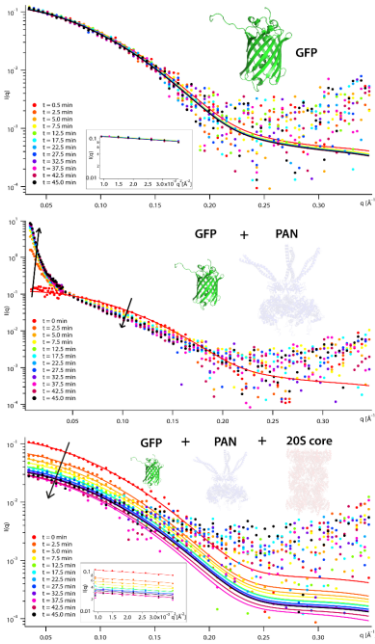


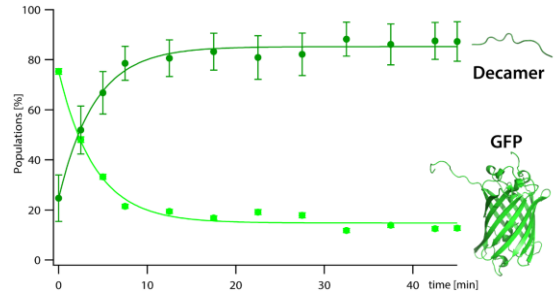
30 second frames!



Ibrahim, Z., Martel, A., Moulin, M., Kim, H.S., Härtlein, M., Franzetti, B. and Gabel, F. (2017) *Sci. Rep.* **7**, 40948.

And in the presence of the proteasome?

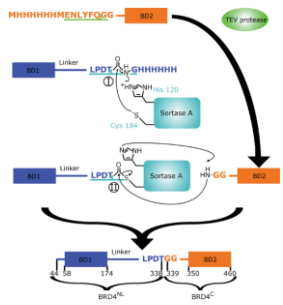




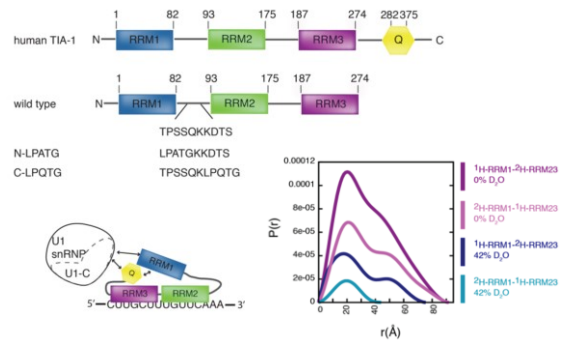
- **No** aggregates
- GFP **disappears**
- Products **appear**

Mahieu et al. *Biophys. J.* (2020)

Example 7: segmental labeling



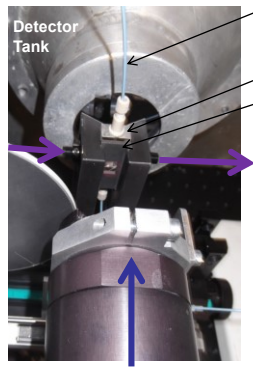
Williams et al. (2016) ***PLoS ONE*** 11(4):e0154607

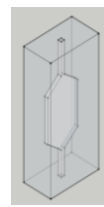


Sonntag et al. (2017) ***Angew. Chem. Intl. Ed.*** 56(32):9322-9325

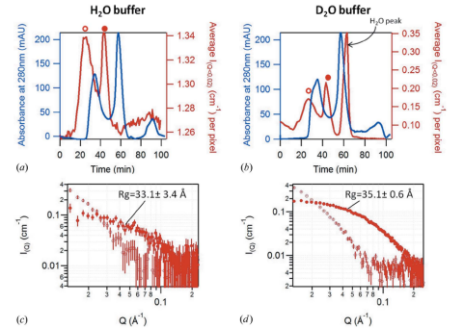
More detailed insight into multi-domain proteins

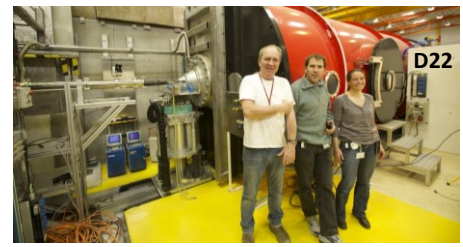
Example 8: SEC-SANS





Cell: 8mm suprasil quartz + 1mm sample (T=82%)





Courtesy Anne Martel (ILL D22)

Frank Gabel: EMBO course June 2022

Practical aspects: doing SANS experiments

- use SAXS for **homogeneous** systems composed of a **single** body
- neutrons only possible at **large facilities** (no “home sources” for the moment!)
- request for measurement time is generally *via* an **electronic proposal system**
- deadlines are usually twice a year, beamtime is attributed about **6 months** later
- BAG (“Block allocation group”) systems allow more flexible access
- for continuation proposals, **reports** need to be submitted regularly
- **experiments need to be prepared with great care** (i.e. isotopic effect of D_2O)**!!**
- “local contacts”, often beamline responsibles, **assist** during experiments
- access (for non-industrial use) is in general **free**
- **no maintenance**, user friendly (software etc…)

Summary

1) **Low-resolution** information in solution

2) **Non-destructive** technique, easy to use

3) Possibility to use **contrast** and focus on **subsystems** within complexes

4) Special applications: protein/DNA-RNA complexes, membrane systems

5) Doing biochemistry on samples ***in situ*** during measurement

6) **Complementary** information to SAXS

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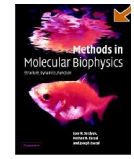
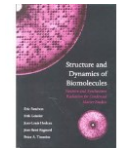
Literature





Basics (scattering, quantum mechanics):
- The Feynman lectures on Physics, Volume 3: Quantum mechanics (Addison Wesley, 2006)
- Cohen-Tannoudji et al.: Mécanique Quantique, Vol. 2. Chapter on diffusion. (Hermann, 1997)

General books on neutron scattering:
- Lovesey: Theory of Neutron Scattering from Condensed Matter (Clarendon, 1986)
- Geissler et al.: Structure and dynamics of biomolecules (Oxford University Press, 2000)

Books on small angle (neutron) scattering:
- Svergun: Structure Analysis by Small-Angle X-Ray and Neutron Scattering (Plenum, 1987)
- Guinier/Fournet: Small angle scattering of X-rays (John Wiley & Sons, 1955)
- Serdyuk, Zaccai, Zaccai: Methods in molecular biophysics (Cambridge University Press, 2007)

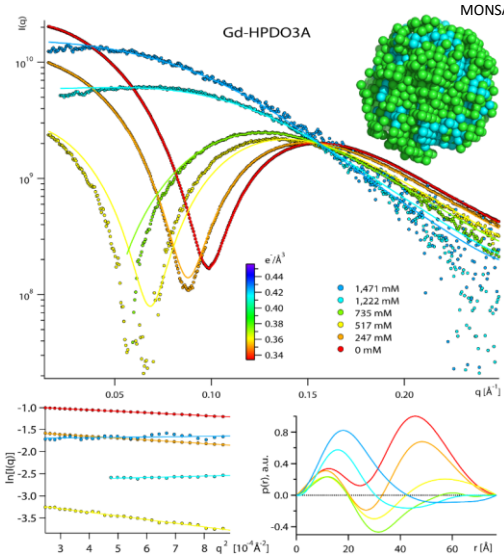
Reviews on SAXS/SANS:
- Jacrot, B. (1976) The Study of biological structures by neutron scattering from solution. *Rep. Prog. Phys.* **39**, 911-953.
- Putnam et al. (2007) X-ray solution scattering (SAXS) combined with crystallography and computation: defining accurate macromolecular structures, conformations and assemblies in solution. *Q. Rev. Biophys.* **40**(3):191-285.

SAXS contrast variation project: Postdoc position available!

“DeepSAXS” Grant

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

MONSA



water

ρ ≈ 0.34 e⁻/Å³

tail

ρ ≈ 0.29 e⁻/Å³

head

ρ ≈ 0.52 e⁻/Å³