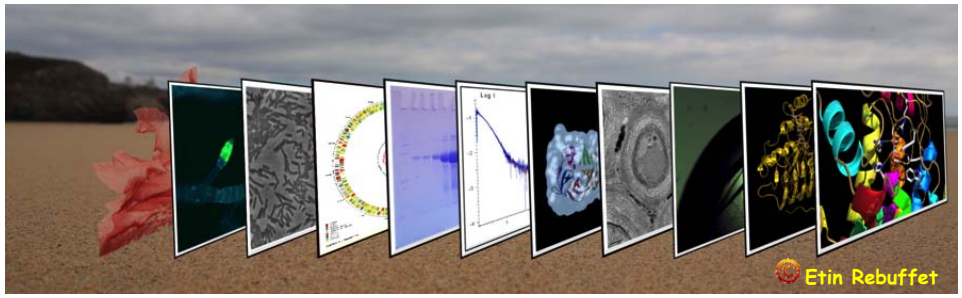
Mirjam Czjzek Research Director of CNRS

Station Biologique de Roscoff
(CNRS-UPMC Paris VI)

czjzek@sb-roscoff.fr

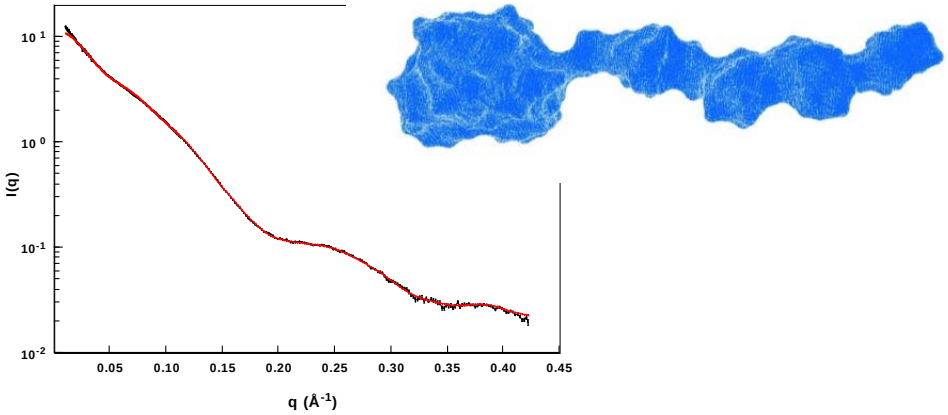
Marine Glycobiology

From genomes to structure/function relationship:
the metabolism of macro-algal polysaccharides



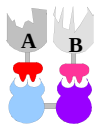
The basis of Small Angle X-ray Scattering and comparison to X-ray crystallography

advantages and limits

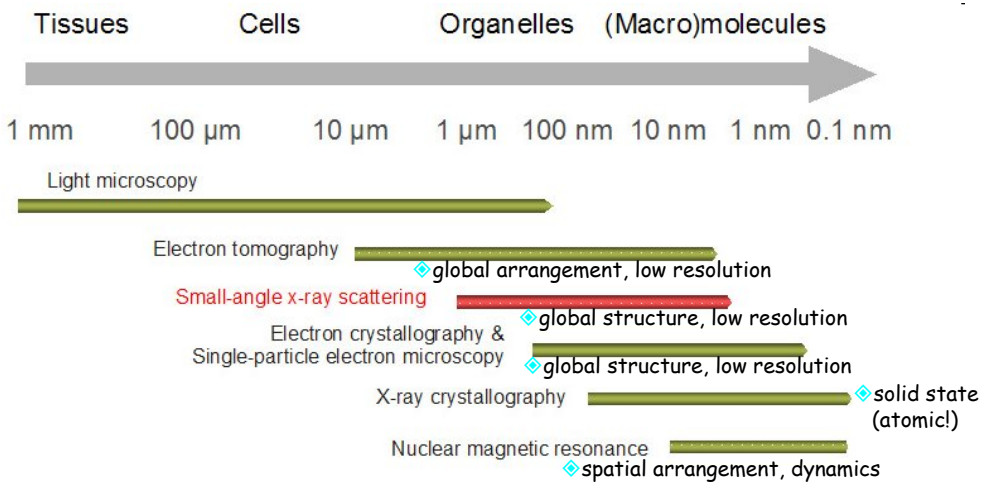


Plan of presentation

- Position of SAXS with respect to other structural methods
- Data acquisition and experimental setup
- Brief theory and principles of small angle X-ray scattering
- What do we measure?
- Data interpretation : modeling structures into envelopes
- comparison (and complementarity) to crystallography quality control, advantages and limits

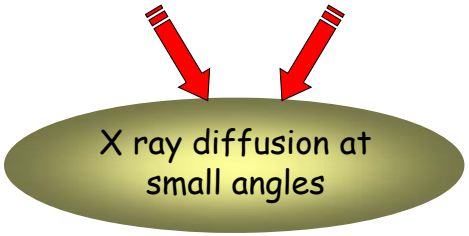


Techniques for structure determination of macromolecules



X ray diffusion at small angles

- Structural heterogeneity
- Flexibility of polypeptide chain
- low compactness
- non recombinant protein, or too large for RMN and too small for EM



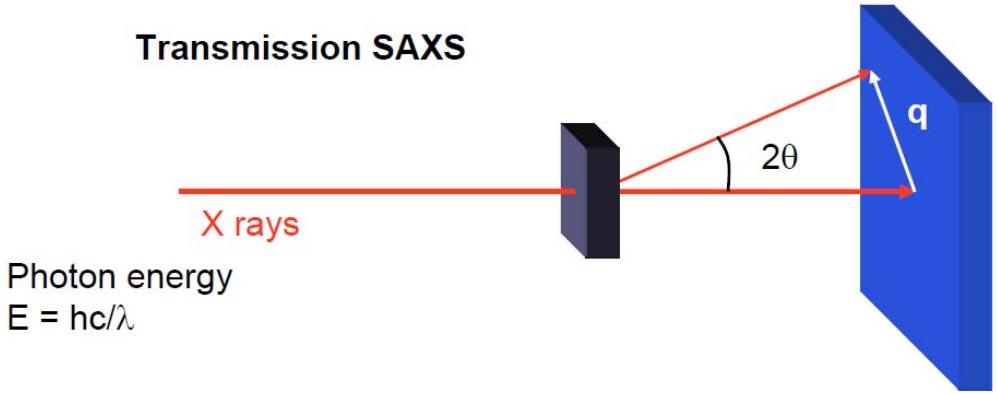
➡ "low resolution structure" of macromolecules in solution

BUT

high-precision information with respect to size and shape

➡ Very powerful when combined with X-ray crystallography

SAS in transmission mode with 2D detector



Origin of diffusion

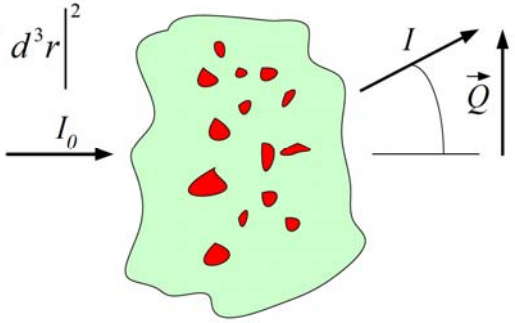
the diffusion arises from heterogeneity of density
of scattering lengths between macromolecules and the surrounding solvent

While the diffusing material and its solvent are homogenous

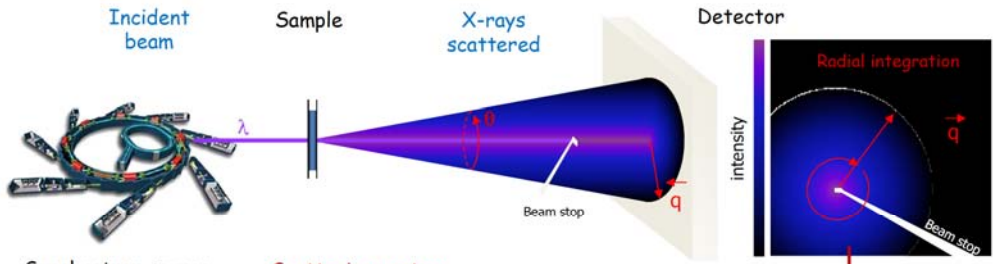
The waves are scattered once or not at all (no multiple diffusion)

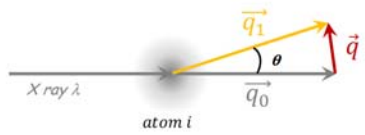
$$I(Q) \propto \frac{d\Sigma}{d\Omega}(Q) = \left| V_p^{-1} \int_{V_p^{-1}} \rho(r)\, e^{-Q\cdot r}\, d^3r \right|^2$$

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



The principle of X-ray diffusion





$\vec{q} = \vec{q_1} - \vec{q_0}$

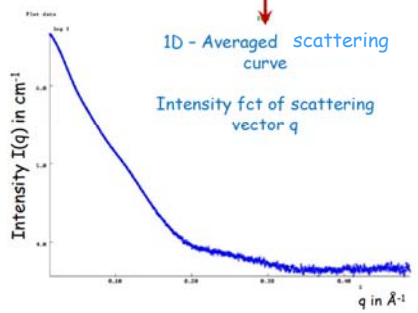
q = 4π sinθ / λ

s = 2sinθ / λ

$\vec{q}$ scattering vector

$\vec{q_0}$ vector of incident wave

$\vec{q_1}$ vector of scattered wave

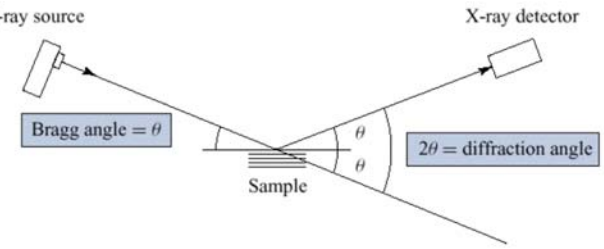


What are we measuring?

Sizes measured

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

$$d = \frac{2\pi}{Q}$$

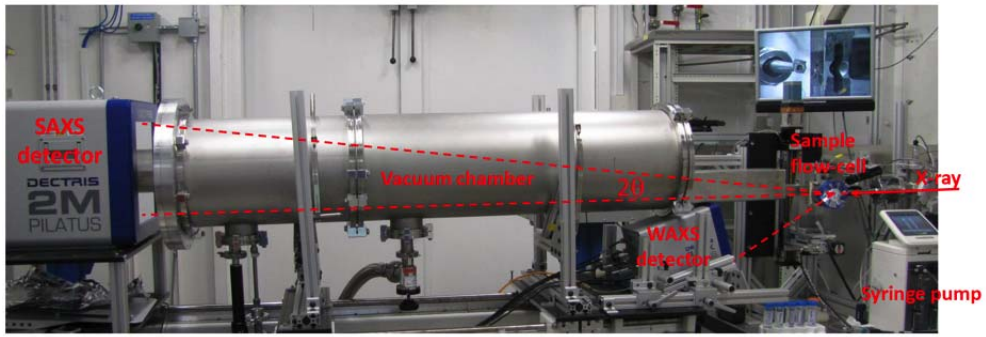


Q [A^{-1}]	D [nm]	2Θ [deg] 12keV
1	0.6	10
0.1	6	1
0.01	60	0.1
0.001	600	0.01
0.0002	3000 (3 um)	0.001

Detector dynamic range is important – Intensity ~ q^{-4}

Experimental setup Argon National Lab USA

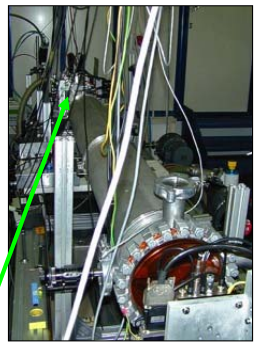
SAXS/WAXS setup at 12ID-B at APS



Important parameters to set up an experience

- minimum 5 concentrations ⇒ 2-10 mg of protein
- Dialysis buffers + radio protectant (DTT (TCDE), glycerol, etc.)
- Cell : stopped-flow : exposure time 30 x 500ms
  10 min for acquisition or HPLC

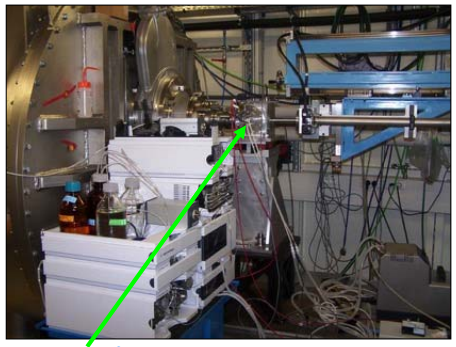
Elettra Italie



Detector

4m

SWING Soleil



Sample

Experimental conditions

SAXS

Crystallography

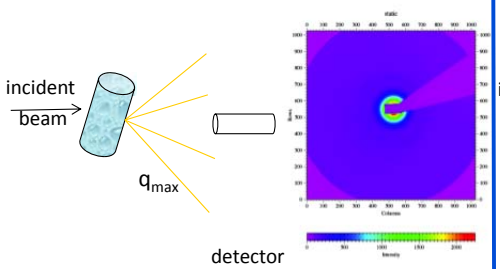
Gamme d'énergie	Between ~5 and 17 keV
Résolution en énergie	~2 eV
Source	In-vacuum U20 undulator. Source Size (sigma, µm): 348 (H) x 8.1 (V) Source Divergence (sigma, µrad): 14.5 (H) x 4.6 (V)
Optiques	Diaphragm at 11.7 m (1x0.5 mm²) Fixed exit DCM Si_{111} at 20 m Fixed incidence focusing KB at 22.5 m Sample position : 30 - 32 m Detector / Sample Distance : 0.5 - 8 m
Environnements d'échantillon	X / Z precision table Stopped flow device for chemistry Online HPLC for proteins in solution (SAXIER project) High throughput sampler for proteins in solution (SAXIER project) Couette Cell (collaboration with LPS, Orsay) GISAXS chamber Automatic sample changer (50 samples, thermostated) Linkam heating stage THMS600
Taille du faisceau	450x20 µm² FWHM dans la cabane expériences
Flux sur l'échantillon	8.10^{12} ph/s @7keV, 8.10^{11} ph/s @16keV (à 400 mA de courant anneau)
Détecteurs	SAXS : PCCD170170 (AVIEX), Gain > 3ADU/ph, Noise=2ADU WAXS (2014) : Détecteur à pixels hybrides
Chambre de détection	Vide primaire, positions du détecteur SAXS : - 0.20 / + 0. 20 m (horiz), -0.20 / +0.20 (vert), 0.5 m / +6 m (le long du faisceau).

Données techniques	
Energy range	Between 5 KeV and 15 KeV
Energy Resolution	~0.000075 (Si 311) - ~0.0002 (Si 111)
Source	U20 In-vacuum undulator
Flux @ first optical element	White beam – depends on undulator settings
Optics	Kirkpatrick-Baez pair of bi-morph mirrors plus channel cut cryogenically cc monochromator crystal
Sample Environment	3 circle κ goniostat (10 µm sphere of confusion) 6 axis robot sample changer (MSC/Rigaku ACTOR) Oxford Cryosystems cryostream Si drift diode energy dispersive detector plus MCA for fluoresc measurements
Beam size at sample	Variable between 100x100 µm² to 250x250 µm²
Flux on sample	> 2.0 e+12 Phot/s/0.02 bw for 500 mA stored current.
Detectors	ADSC Q315r
Polarization	Linear

Comparison of methods

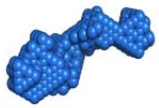
SAXS

average of conformation in solution



data treatment

Analyses
ab initio
molecular modeling

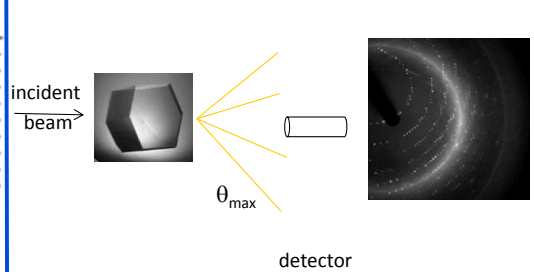


envelope, shape

Limiting factors : aggregation, heterogeneity

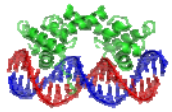
crystallography

average of atomic crystal structure



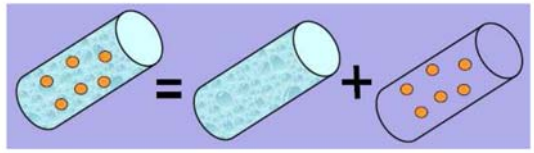
data treatment

Phasing
molecular replacement
MAD, SAD, MIR...



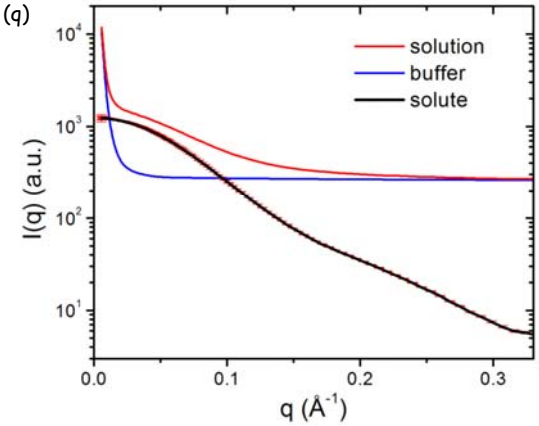
3D atomic structure

Limiting factors : diffracting crystals, phasing signal



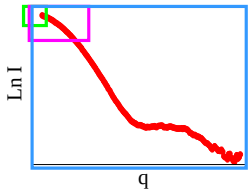
1D SAXS profiles

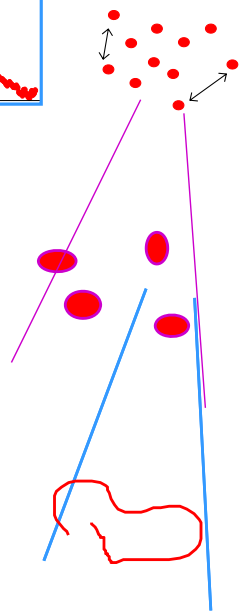
$I_{solution}(q) = I_{buffer}(q) + I_{solute}(q)$



The measurement of diffusion often contains a lot of 'noise' t.

the diffusion vector q :
reciprocal space, inverse of a distance





***q*=0 (determination of I(0))**

molecular weight (Mw), oligomerisation state and intermolecular interactions in solution

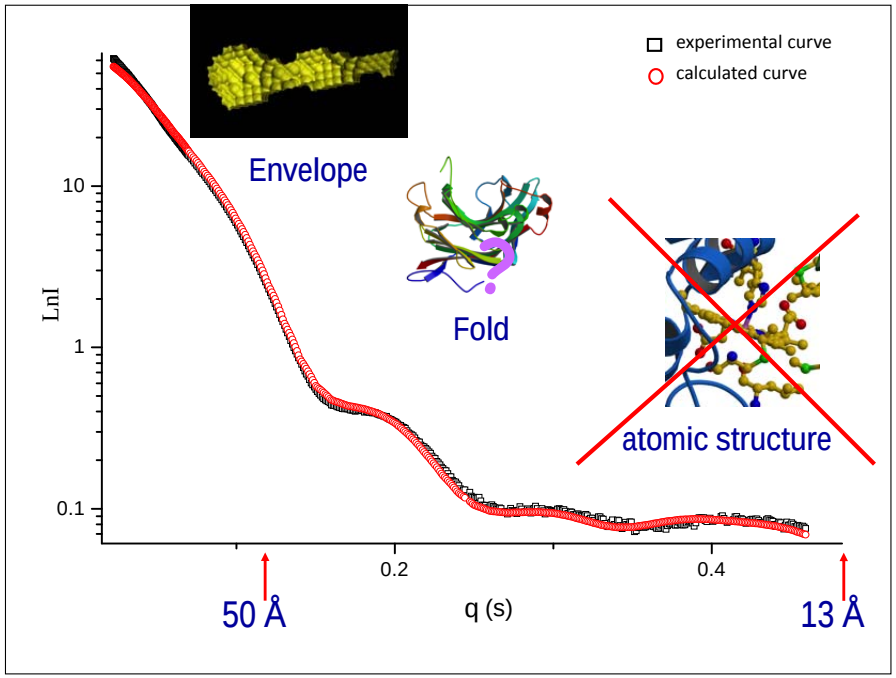
Region of small *q*

Particle dimensions (Radius of giration)

Region of larger *q*

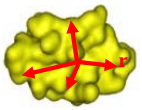
Particle overall shapes
(polypeptide chain conformations in solution)

Information from small angle scattering



The radius of gyration

$$R_g^2 = \frac{\int_V \rho(\vec{r})\, r^2 \quad d^3\vec{r}}{\int_V \rho(\vec{r}) \quad d^3\vec{r}}$$

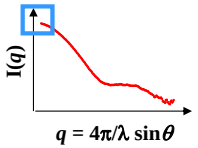


⇒ **Mean square of atomic distances from center of gravity,**
(weighted by electron density ρ(r))

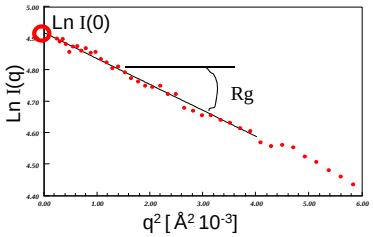
Guinier Law:

$$I(q) = I(0) \exp\left(-\frac{R_g^2 q^2}{3}\right)$$

$qRg \leq 1.0$



$\ln I(q) = \ln I(0) - q^2 R_g^2 / 3$



⇒ Determination of *average dimension* of the particle

⇒ Determination of its *molecular masse*

$I(0) \propto c.M_w/N$ (Cste)

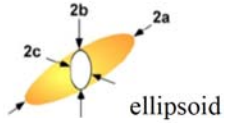
R_g : Mean square of atomic distances from center of gravity, (weighted by electron density ρ(r))

- In polymer physics, ıe radius of gyration is pı distance between tl.. parts of the object:

$$R_g^2 = \frac{\int r^2 \rho(r) dr}{\int \rho(r) dr}$$

Gaussian chain

$$\langle R_g^2 \rangle = N\frac{l^2}{6}$$



ellipsoid

$$R_g^2 = \frac{1}{5}(a^2 + b^2 + c^2)$$

Sphere

$$R_g^2 = \frac{3}{5}R^2$$

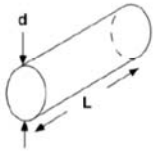
Thin rod

$$R_g^2 = \frac{L^2}{12}$$

Thin disc

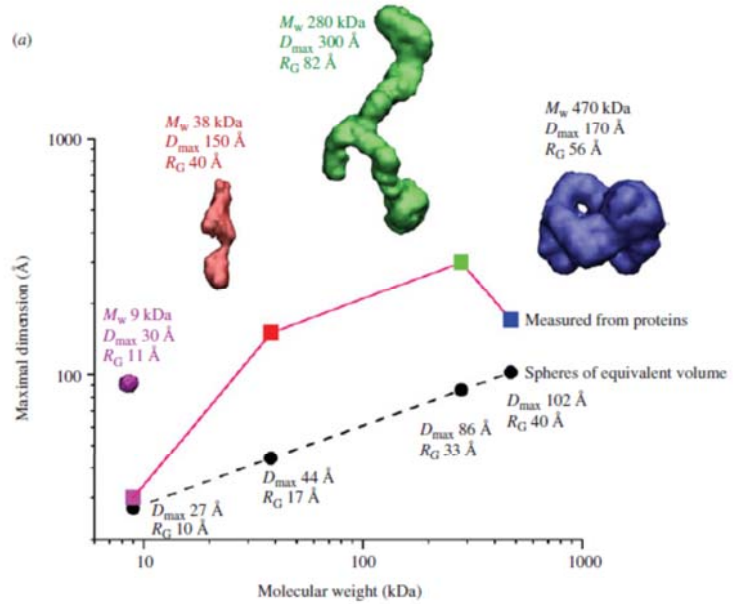
$$R_g^2 = \frac{R^2}{2}$$

Cylinder

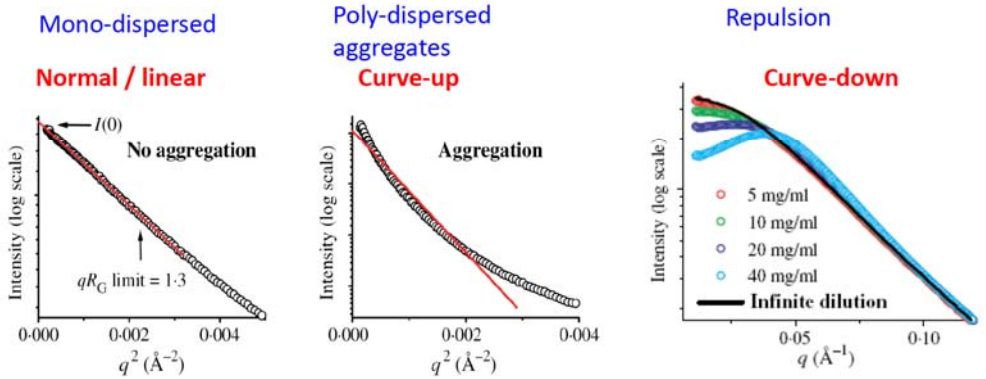


$$R_g^2 = \frac{R^2}{2} + \frac{L^2}{12}$$

Relation of R_G to molecular weight (Mw) – roughly linear ONLY for spherical proteins



Guinier Plot: interactions & sample condition



Figures from: Putnam, D., et al. (2007) Quart. Rev. Biophys. 40, 191-285.

Forward scattering I(0) measures molecular weight

$$MW = \frac{N_A I(0)/c}{(\Delta\rho_M)^2}$$

I(0) at absolute scale; *c* mg/ml
Scattering length density diff: $\Delta\rho_M = \rho_M - \rho_s$
$N_A = 6.02*10^{23}$

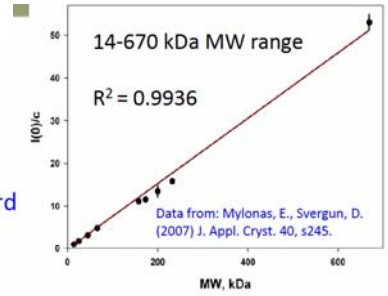
- Absolute scale: calibrate with water or other standard
  - Water: 1.632×10^{-2} cm^{-1} at 293K

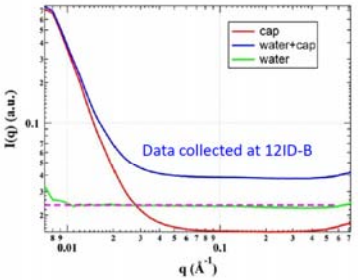
  For protein on average: $\Delta\rho_M = 2.086*10^{10}$ cm^{-2}

$$MW = 1385 \times I(0)/c$$

MW in kDa; *I(0)* in cm^{-1}, *c* in mg/ml

Orthaber, Bergmann and Glatter, J. Appl. Cryst. (2000). 33, 218





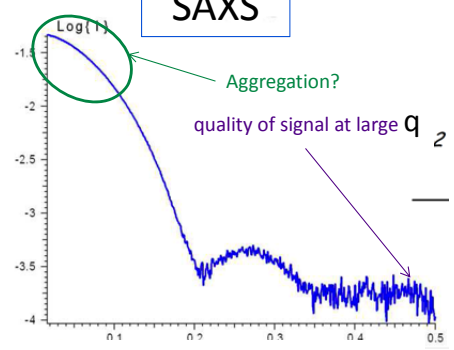
- Relative scale: secondary standard
  - Lysozyme, BSA, etc

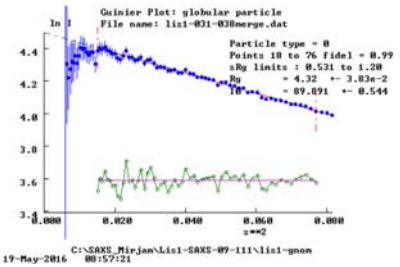
$$MW_p = I(0)_p / C_p \frac{MW_{st}}{I(0)_{st}/C_{st}}$$

➢ Standard should has **same nature** of the molecules to determine, and with close MW. Using multiple standards is suggested.

Direct information from scattering

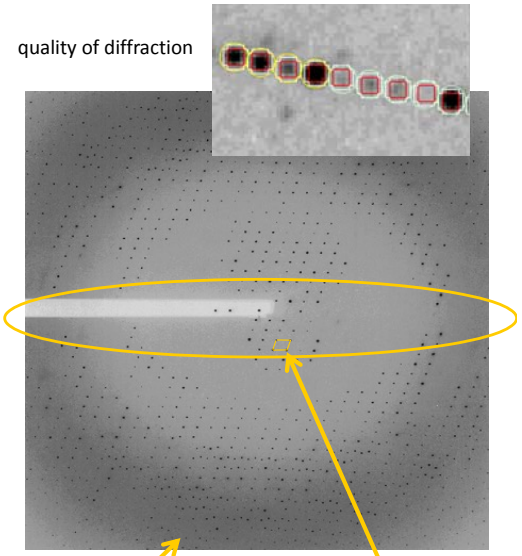
SAXS



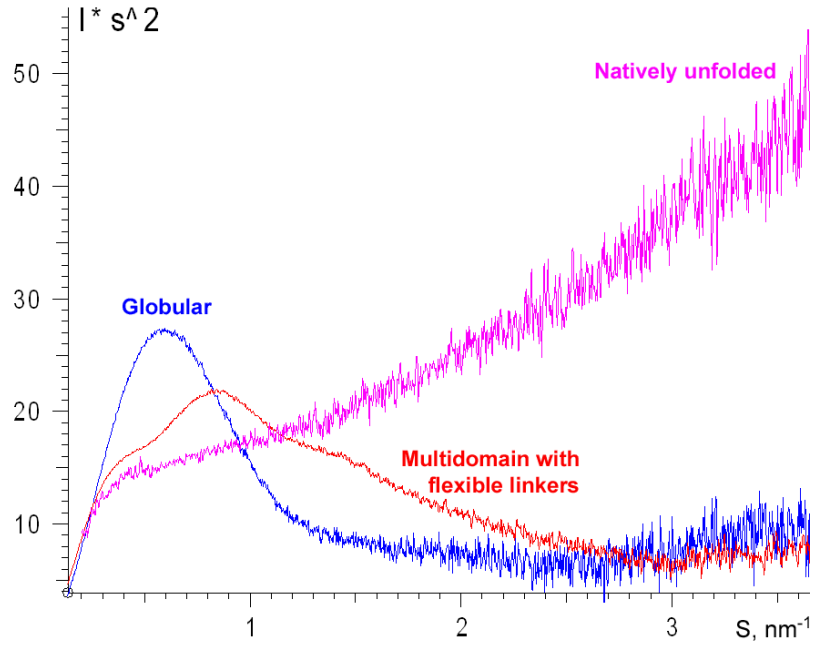


Guinier plot : R_g and M_w

crystallography



Kratky plot



Porod law

Hypothesis : the particle has a well defined interface with the surrounding buffer and a uniform electron density

$$\mathbf{Q} = \int_0^\infty I(q) \cdot q^2 dq = 2\pi^2 \phi \cdot (1-\phi) \cdot r_e^2 (\Delta\rho)^2$$

Porod invariant — Protein volumic concentration

$$\phi = \frac{N \cdot V_{obj}}{V}$$

Number of proteins — Protein volume — Solution volume

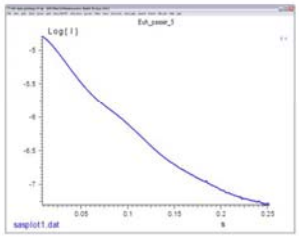
Does not depend on shape, only on contrast

The Porod Invariant is the integral of this curve

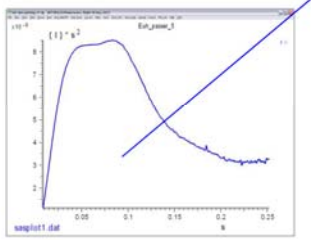
$$\mathbf{Q} = \int_0^\infty I(q) \cdot q^2 dq$$

$$V_{obj} = \frac{2\pi^2 \cdot I(0)}{\mathbf{Q}}$$

Representation : Log I(Q) vs Q



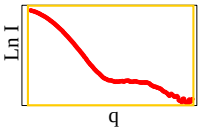
Kratky representation : I(Q)·Q² vs Q



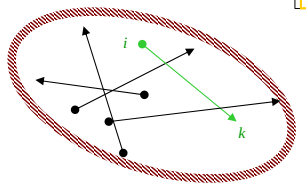
- Valid for diluted systems
- Does not require absolute units
- Not valid for unfolded objects
- Not precise

Back to real space: distance distribution function

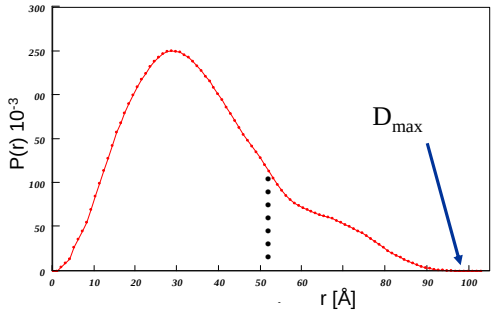
=> Transformation into real space with a Fourrier transform F (I(q)) :



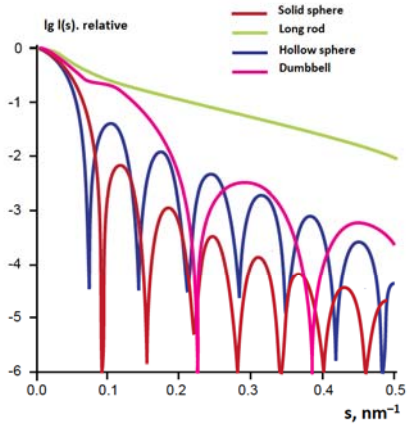
$$P(r) = \mathcal{F}(I(q)) = \int I(q) e^{-i\vec{q}\vec{r}} dq$$

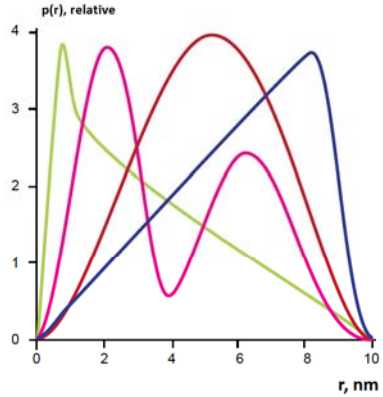


- **Function of distribution of distances between all atomes.**
- **Histogram of all existing intramolecular distances**



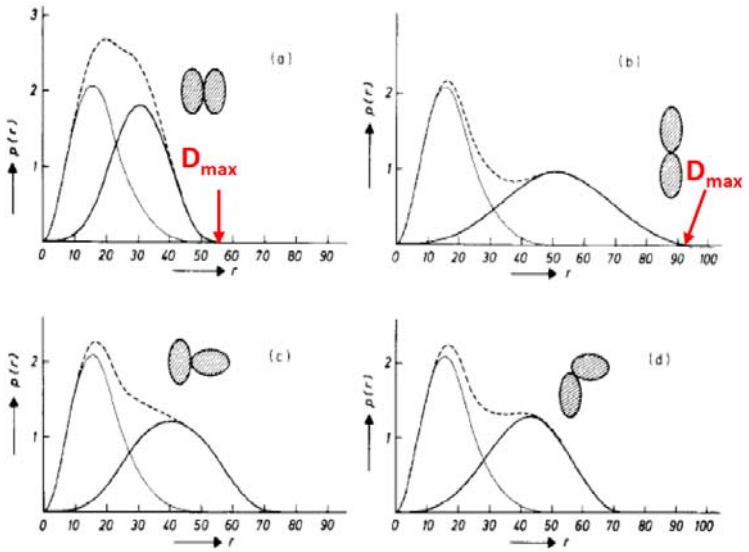
SAXS and P(r) of different forms





Adapted from: Svergun, D., Koch, M. (2003) Rep. Prog. Phys. 66, 1735-1782.

P(r) functions for different 'options' of a dimer



As well the form and the value of D_{max} vary for different options

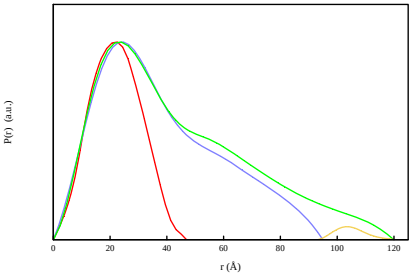
Humicola insolens EGV native and truncated

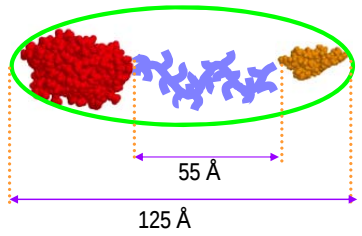
Mw = 38 kDa N = 210+36+38 aa

	Rg (Å)	D_{max} (Å)
Catalytic domain	17.3 ± 0.3	45 ± 5
EGV without CBD	30.0 ± 0.4	100 ± 10
EGV full lenfth	33.5 ± 0.5	125 ± 5
CBD	9.2	31

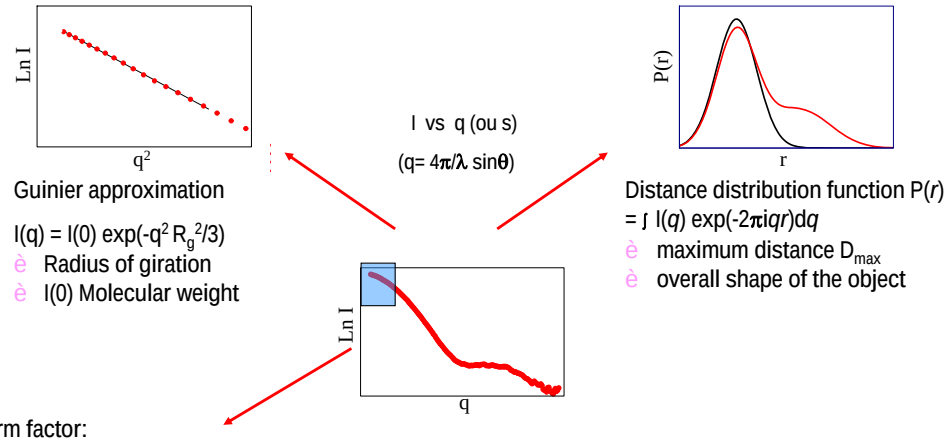
Linker : ≈ 0.7 residues/Å

Distance distribution function





Small angle scattering



Guinier approximation

$I(q) = I(0) \exp(-q^2 R_g^2/3)$

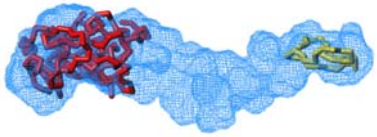
- è Radius of giration
- è I(0) Molecular weight

Distance distribution function $P(r) = \int I(q) \exp(-2\pi i q r) dq$

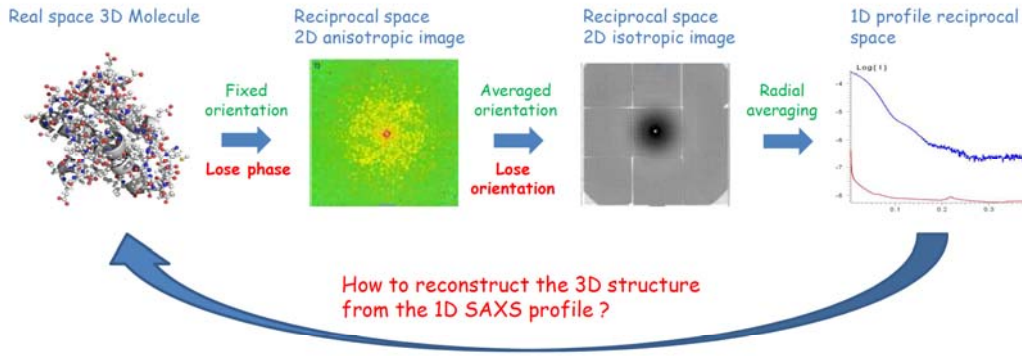
- è maximum distance D_{max}
- è overall shape of the object

Form factor:

- è Comparaison with crystal structure
- è conformational modeling
- è Calculation of envelopes or volumes



Reconstruction of the 3D structure from 1D SAXS is an ill-conditioned problem

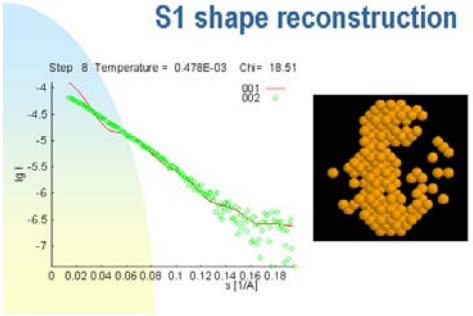


Ambiguity of the solution :

One 3D structure → One SAXS curve	BUT	One SAXS curve → Many 3D structures

ab initio calculation of overall shape

➡ Fill envelope with dummy-atoms with a given diameter (3.8 to 10Å)

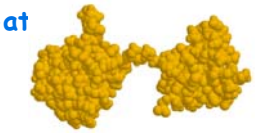


- ♦ **Global shape of macromolecule in solution**
- ♦ **Respective position of sub domains (known 3D structure)**

Programs DAMMIF, GASBOR (D. Svergun), DALAI_GA (F. Diaz)

Shape calculations

➡ **Calculation of an envelope filled with pseudo-at of variable diameter (3.8 to 10Å)**



- **DAMMIN/DAMMIF : Simulated annealing with constraints to minimize the surface**
- **GASBOR : Simulated annealing with distance constraints of close neighbors Ø=3.8Å**

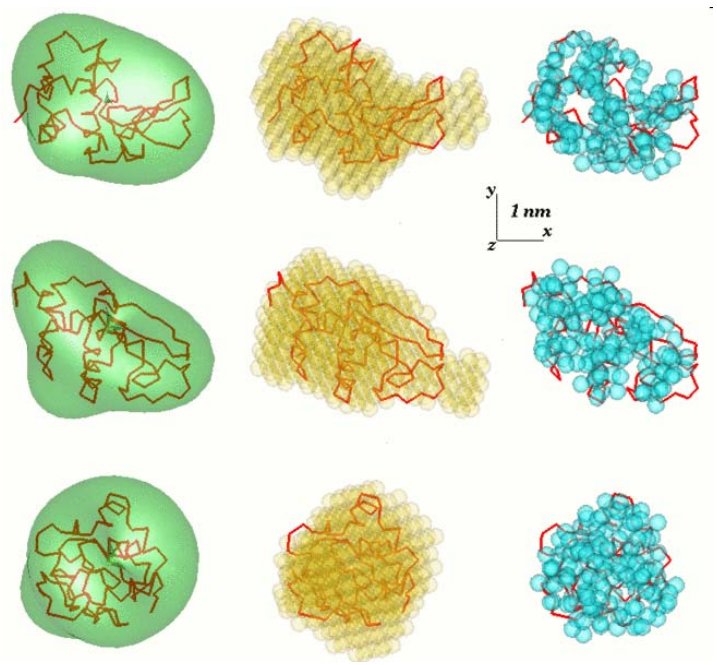
(D. Svergun)

- **DALAI_GA : Genetic Algorithm without external constraints Ø depends on resolution**

(F. Diaz)

http://www.embl-hambourg.de/ExternalInfo/Research/Sax/software.html

DALAI_GA DAMMIN GASBOR



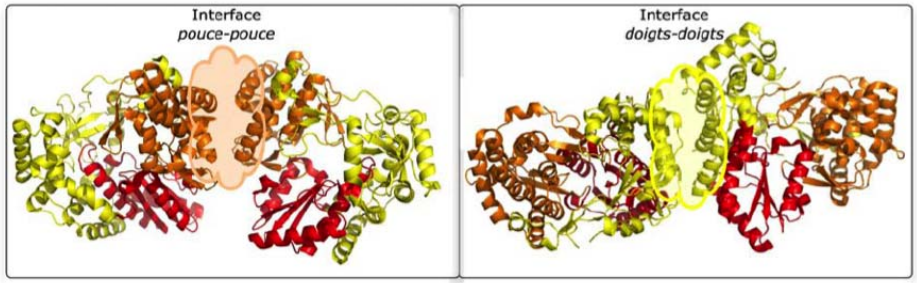
quaternary structure in solution and complexes

If 3D crystal structure of each sub-unit is known

⇨ Find the respective position of each sub-unit

- **SASREF : Rigid Body Modelling**

(D. Svergun)

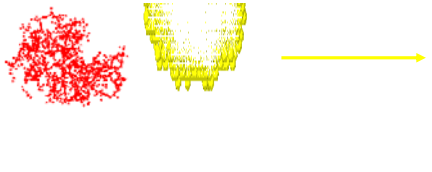


conformational flexibility

⇨ **Comparaison of solution structure to crystal structure**

- **CRYSOL: Calculate theoretical diffusion curve of a macromolecule taking in account the hydratation shell**

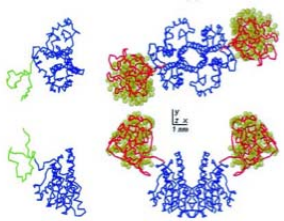
(D. Svergun)



⇨ **Calculate the conformation of a missing loop**

- **CREDO & BUNCH : position of C_α of a missing domain or portion of overall structure**

(D. Svergun)



⇨ **Comparaison of solution structure to crystal structure**

.....more programs

Svergun D, Barberato C, and Koch M.H.J. (1995) **CRYSOL** - a program to evaluate x-ray solution scattering of biological macromolecules from atomic coordinates.
J. Appl. Cryst. 28, 768
Most popular for BioSAXS, stand-alone program, fit model to data, fast computational algorithm
http://www.embl-hamburg.de/biosaxs/atsas-online/crysol.php

Grishaev A, Guo L, Irving T, Bax A. (2010) **AXES** Improved Fitting of Solution X-ray Scattering Data to Macromolecular Structures and Structural Ensembles by Explicit Water Modeling. J. Am. Chem. Soc. 132, 15484-6.
Use explicit water modeling solvation layer, robust fitting approach
http://spin.niddk.nih.gov/bax/nmrserver/saxs1/

J. Bardhan, S. Park and L. Makowski (2009) **SoftWAXS**: a computational tool for modeling wide-angle X-ray solution scattering from biomolecules J. Appl. Cryst. 42, 932-943
A program to compute WAXS,
Upon request

Schneidman-Duhovny D, Hammel M, Sali A. (2010) **FoXS**: a web server for rapid computation and fitting of SAXS profiles. Nucleic Acids Res. 38 Suppl:W540-4.
Debye-like computation, web server based
http://modbase.compbio.ucsf.edu/foxs/

Zuo X, Zhang R, Tiede DM. **SolX**: A computer program for solution molecular x-ray scattering simulations . Photosynth Res. 2009 Nov-Dec; 102(2-3): 267-279.
Debye-like computation, Windows-based, can handle non-standard atoms/residues, for biomolecules and supramolecules
Upon request zuox@anl.gov, tiede@anl.gov

Group of Dmitri Svergun:

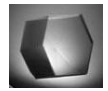
EMBL HAMBURG — Biological Small Angle Scattering

http://www.embl-hamburg.de/biosaxs/

SAXS Forum:

http://www.saxier.org/

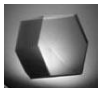
Comparison of methodological limits

SAXS	crystallography
	
✓ provides envelope or overall shape average 3D conformations in solution	✓ provides an atomic (average) 3D structure of the crystallized molecule
✓ needs pure samples	✓ needs pure samples
✓ large quantities	✓ large quantities
✓ supports irradiation with time	✓ crystallizes
✓ does not form aggregates	✓ produces Bragg diffraction
✓ "low resolution" – no atomic information	✓ no information of conformation in solution
✓ average of particles in solution	✓ (almost) no information on dynamics

Complementarity of the methods

SAXS

crystallography



- mean global conformation in solution
- possibility to constrain modelisation of flexibility
- allows to verify which oligomerisation is compatible with exp. SAXS curve
- allows to analyze (estimate) the flexibility domain conformations in solution

- solid state atomic 3D structure
- Ambiguity about oligomeric state
- Ambiguity about domain (or loop) organisation/conformations

SASBDB

Conclusions