Solution X-ray Scattering from Biological Macromolecules

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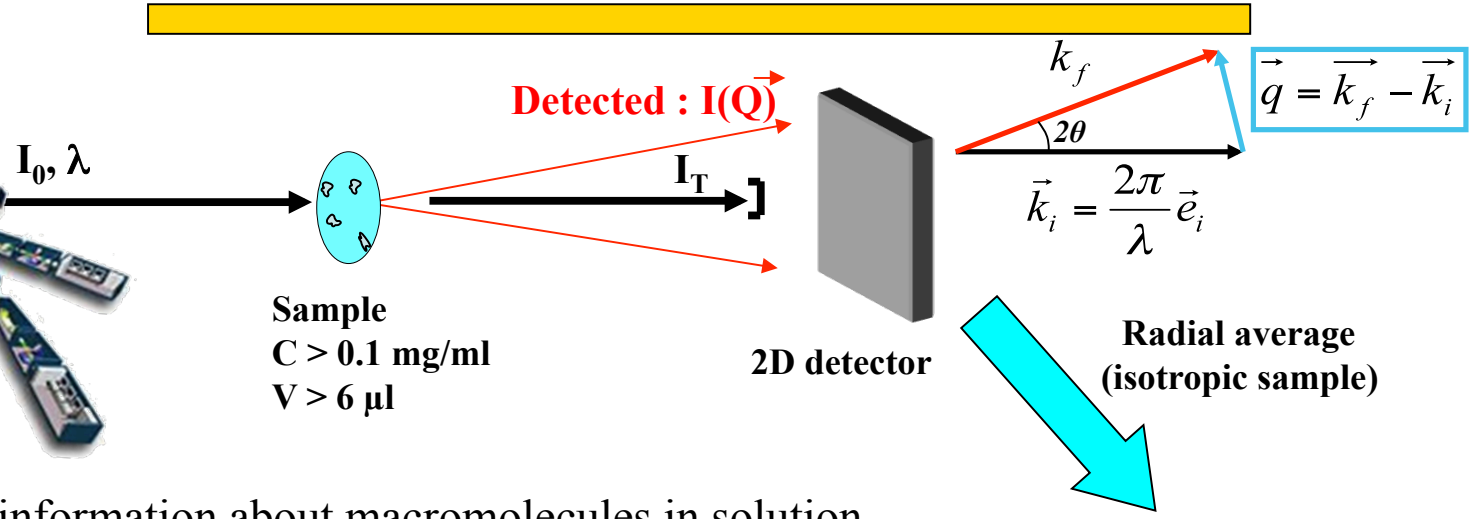
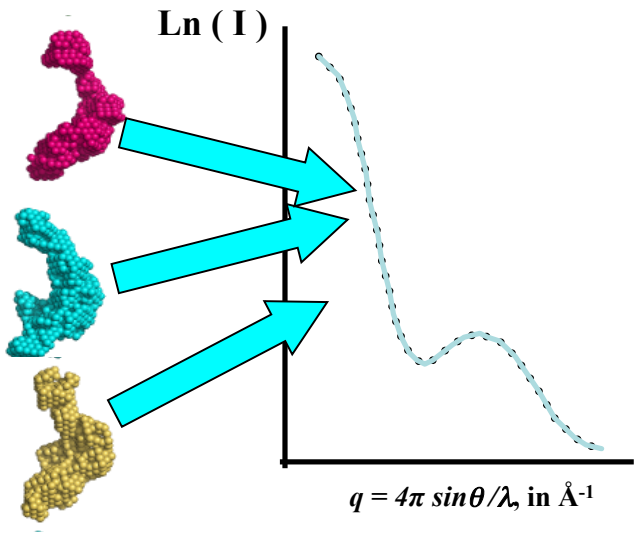
INTRODUCTION

Principles of Small Angle X-ray Scattering in solution



SAXS provides structural information about macromolecules in solution

- **Limits**
  - **spherically averaged information → low resolution**
  - **non-unicity of the solution**
  - **does not distinguish elements in a mixture**
- **Advantages**
  - **solution: no need to grow crystals**
  - **relatively easy to carry experiments**
  - **can be used to test atomic models**



SAXS is best used in combination with complementary (structural) information

Principles of Small Angle X-ray Scattering in solution

Structural information obtained from a scattering curve

- biophysical parameters (size and shape type)
- molecular mass, oligomerization state and volume.

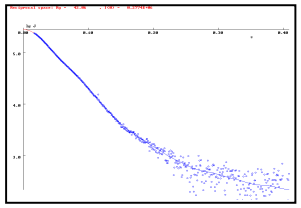
Biophysical informations derived directly from the SAXS curve

- possible low resolution molecular shape (ab initio methods)
- direct comparison with high resolution model
- possible model of (un)structured missing parts
- rigid body of complex

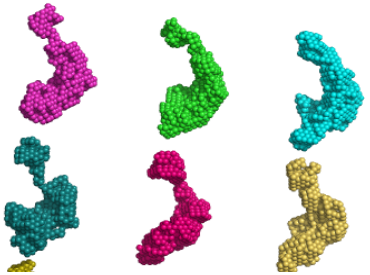
3D structural modeling
→ models compatible with SAXS data
NOT a unique model,
NO electron density map.

Structural information about macromolecules in solution

Nothing known (except the curve)

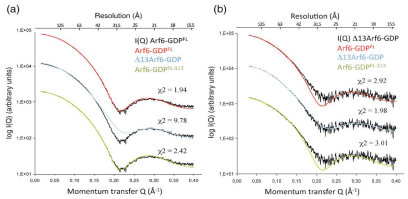


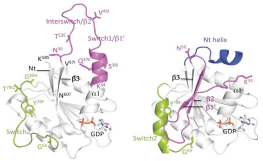
Shape determination



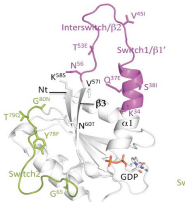
DAMMIN
DAMMIF
DENFERT

Known or hypothetical all-atom models



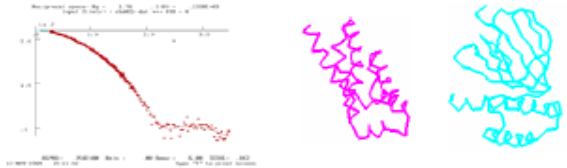


Model validation / elimination

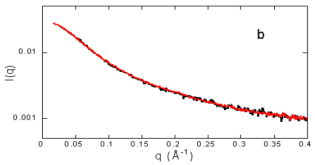


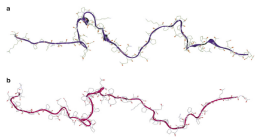
CRYSOL
FOXS

Structure of subunits available

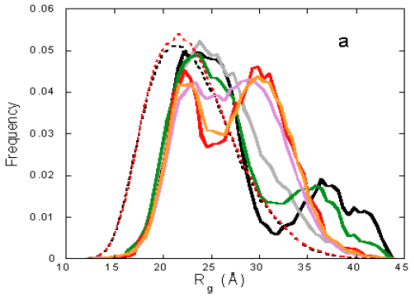


Zones of supposed high flexibility





Selection within an Ensemble of Random Conformations



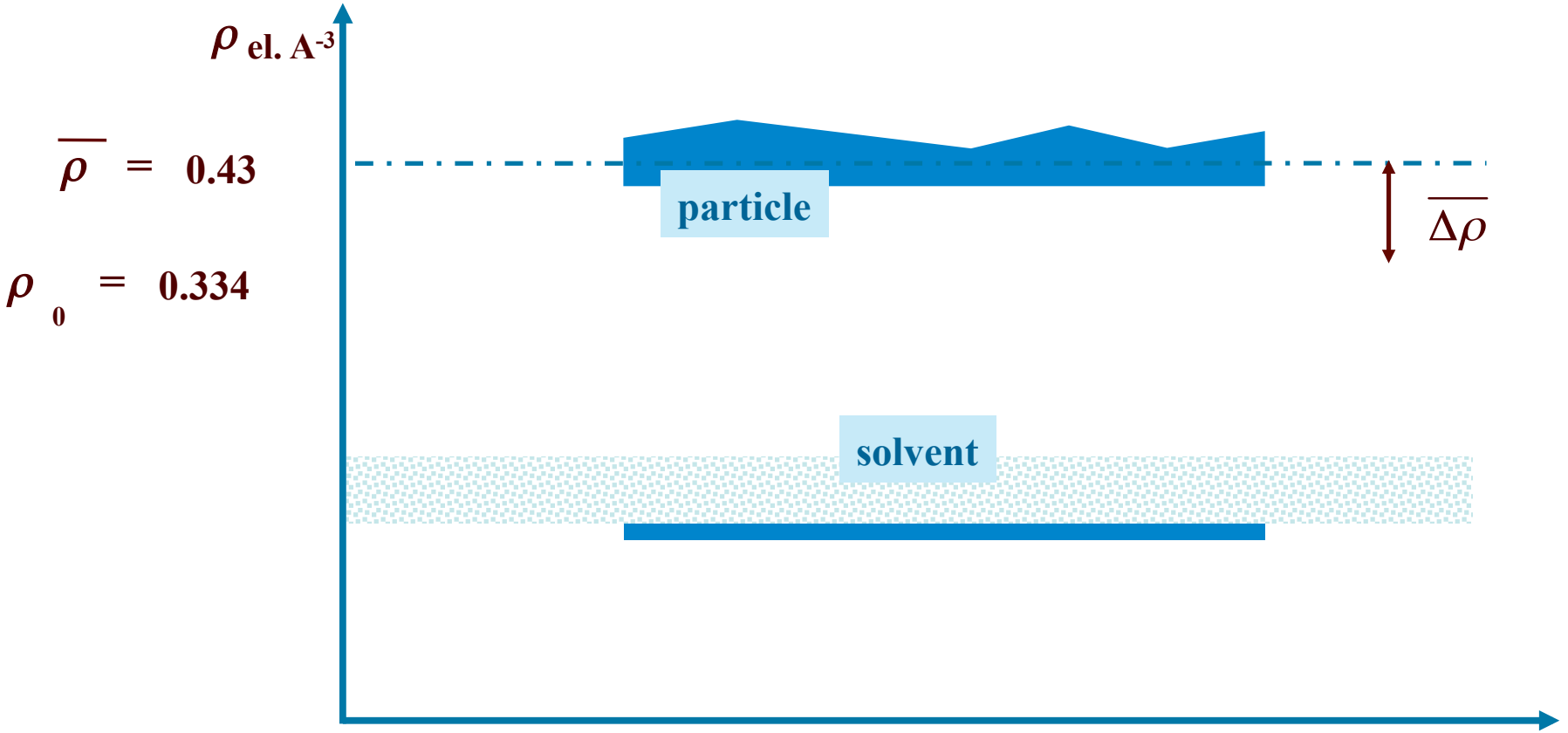
EOM

MES

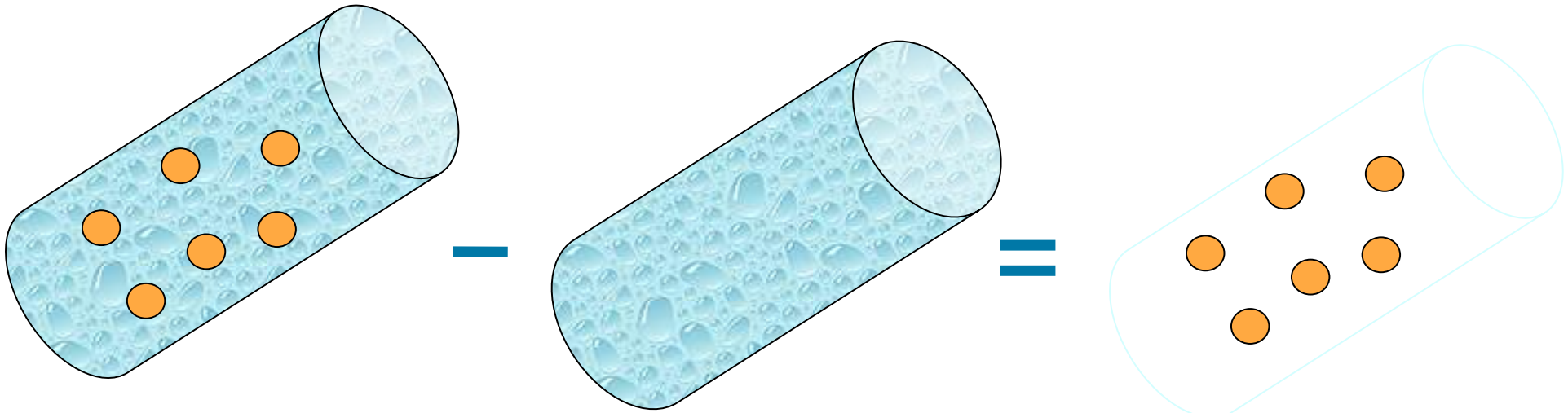
SAXS BASICS

Particles in solution

- A particle is described by the associated electron density distribution $\rho_p(r)$.
- In solution, what contributes to scattering is the *contrast* of electron density between the particle and the matrix $\Delta\rho(r) = \rho_p(r) - \rho_0$ that may be very small for biological samples.



SAXS: a pair of measurements



$I_{sample}(q)$ $I_{buffer}(q)$ $I_{particle}(q)$

- **To obtain scattering from the particles, buffer scattering must be subtracted, which also permits to significantly reduce contribution from parasitic background (slits, sample holder etc) which should be reduced to a minimum.**

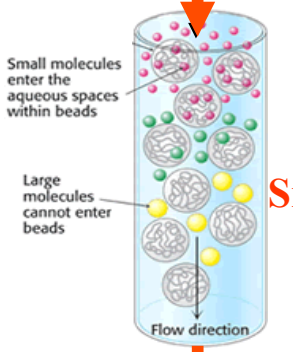
SE-HPLC / Solution Sampler

SAXS - WAXS - GISAXS

SWING

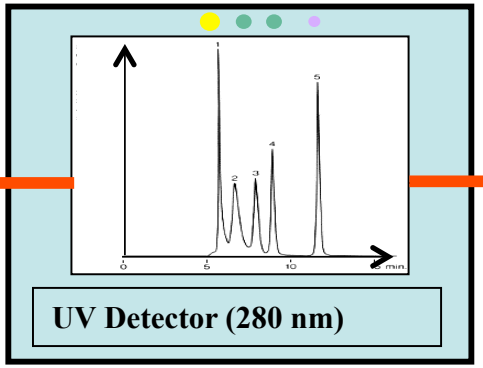
Flow rate 200 µl/min

- Monodispersed solution
- Aggregation is eliminated
- Oligomeric conformations can be distinguished
- Equilibrium states can be transiently separated
- Perfect background subtraction
- Automatic concentration series



Size Exclusion

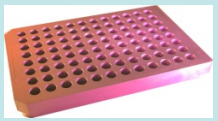
Incident X-ray



UV-Vis in

Pump

Injection-mixing



Flow rate 5-50 µl/min

Pure sample

SAXS Cell

UV-Vis out

Detector

- Small volumes (~ 10 µl)
- No dilution
- High rate (a few minutes)

DATA ANALYSIS

Data Analysis

- **Guinier Analysis**
- **Kratky plot : why is it so interesting ?**
- **Real-space : Distance distribution function P(r)**

Behaviour at small angles : Guinier law

The scattering intensity of a particle can be described by a Gaussian curve in the vicinity of the origin.
The validity domain actually depends on the shape of the particle and is around Q*Rg < 1.2 for a globular shape.

Prof. André Guinier
1911-2000
Orsay, France

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

Extrapolated intensity at origin

Radius of gyration

Guinier law, in log form :

$$\ln[I(Q)] = \ln[I(0)] - \frac{Q^2 Rg^2}{3}$$

$$R_g^2 = \frac{\int_{V_r} \Delta\rho(\mathbf{r}) r^2 dV_r}{\int_{V_r} \Delta\rho(\mathbf{r}) dV_r}$$

ln(I(Q)) vs Q^2 : linear variation (Guinier plot).
Linear regression on the experimental Guinier plot directly provides Rg and I(0).

Mass retrieval from Guinier analysis

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

Absolute Unit : cm^{-1}

Classical electron radius

$$I(0) = \frac{c \cdot M \cdot r_0^2}{N_A} \cdot \left[v_p\left(\rho_{prot} - \rho_{buf}\right)\right]^2$$

Mass concentration

Electronic density contrast

Protein specific volume

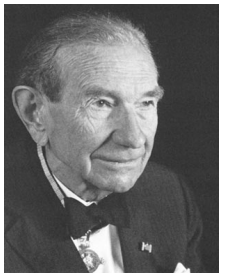
An estimate of the molar mass of the molecule can be derived from the value of I(0)/c.

Typically : M (kDa) = 1500 * I(0) (cm^{-1}) / C (mg/ml)

Data Analysis

- Guinier Analysis
- **Kratky plot : why is it so interesting ?**
- Real-space : Distance distribution function P(r)

Kratky Plot

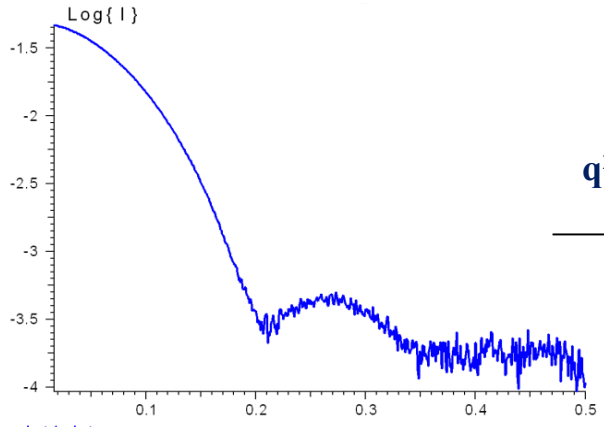


Prof. Otto Kratky
1902-1995
Graz, Austria

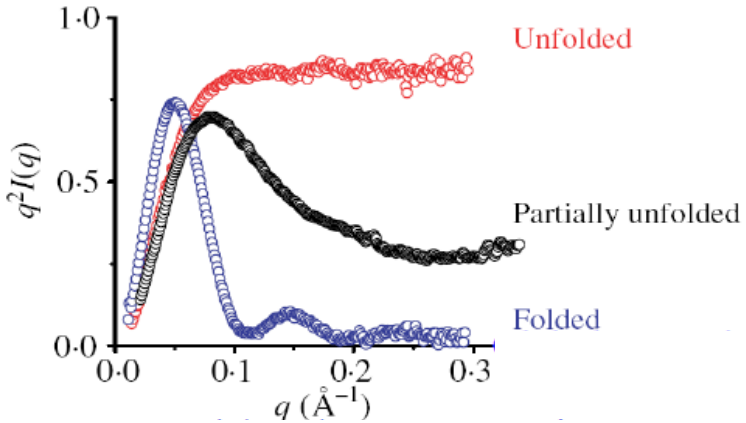
SAXS provides a sensitive means to evaluate *the degree of compactness* of a protein:

- **To determine whether a protein is globular, extended or unfolded**
- **To monitor the folding or unfolding transition of a protein**

This is most conveniently represented using the so-called Kratky plot:



q^2 I(q) *versus* q



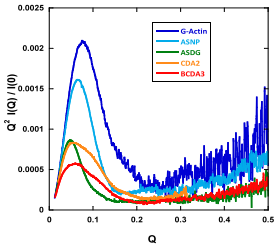
Putnam, D., et al. (2007) Quart. Rev. Biophys. 40, 191-285.

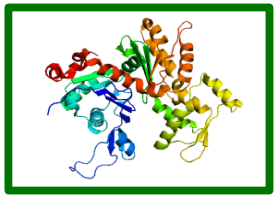
Folded particle : ***bell-shaped curve*** (asymptotic behaviour $I(Q) \sim Q^{-4}$)

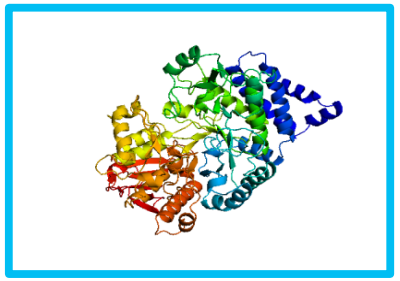
Random polymer chain : ***plateau*** at large q-values (asymptotic behaviour in $I(Q) \sim Q^{-2}$)

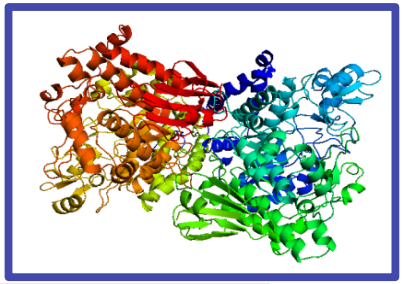
Extended polymer chain : ***increase*** at large q-values (asymptotic behaviour in $I(Q) \sim Q^{-1.x}$)

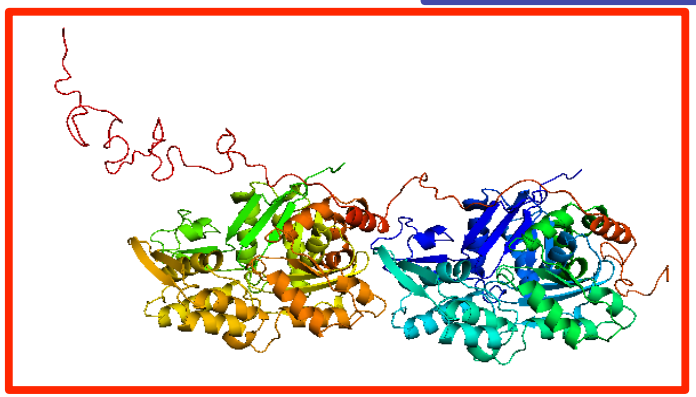
Kratky Plots of folded proteins

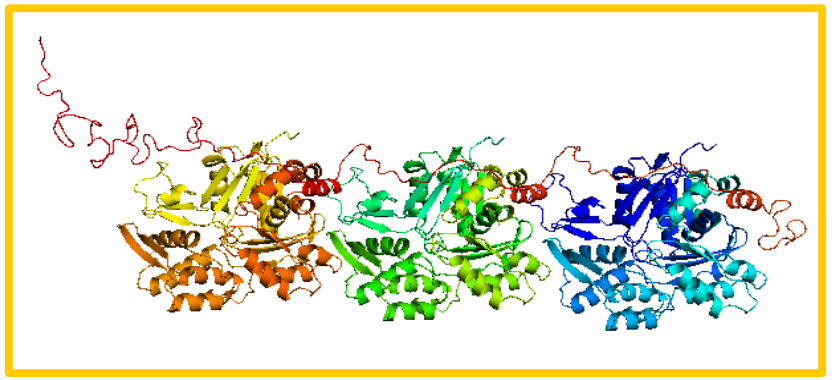








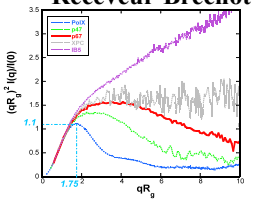


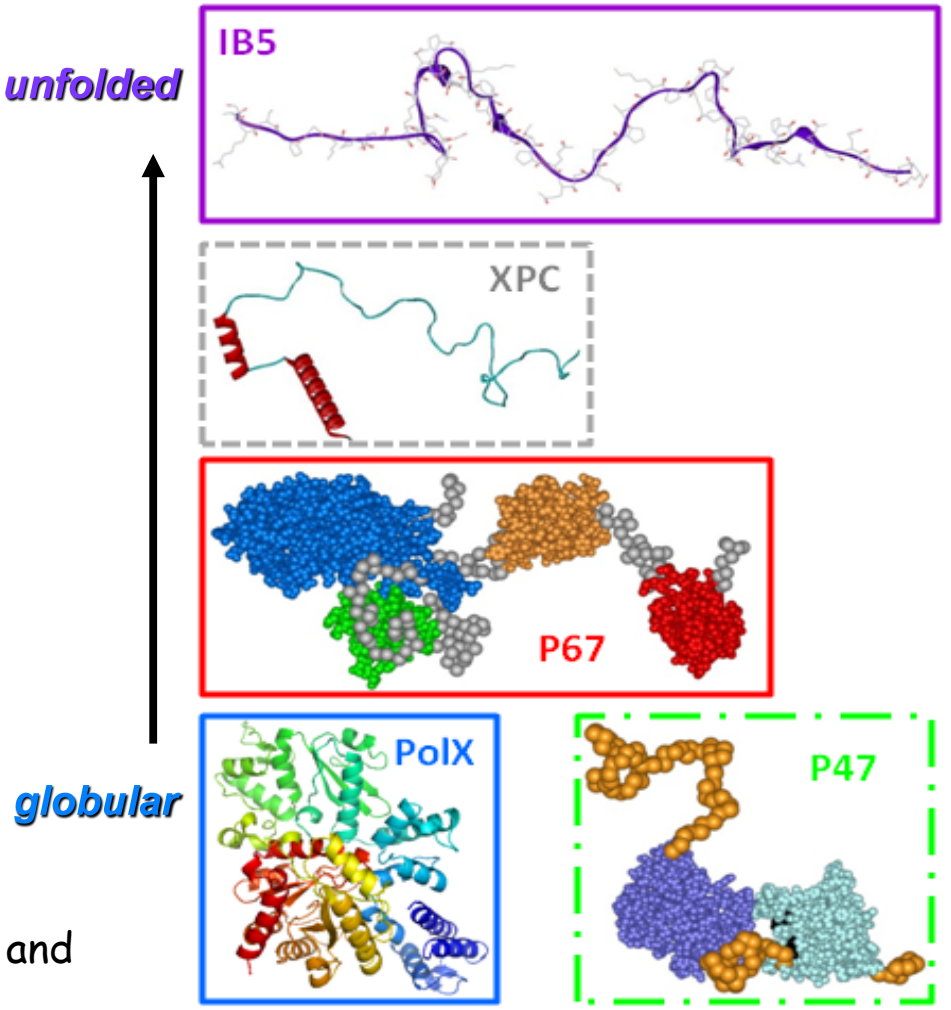


Folded proteins display a bell shape. Can we go further?

Dimensionless Kratky Plots of (partially) unfolded proteins

Receveur-Bréchot V. and Durand D (2012), Curr. Protein Pept. Sci., 13:55-75.





The bell shape vanishes as folded domains disappear and flexibility increases.

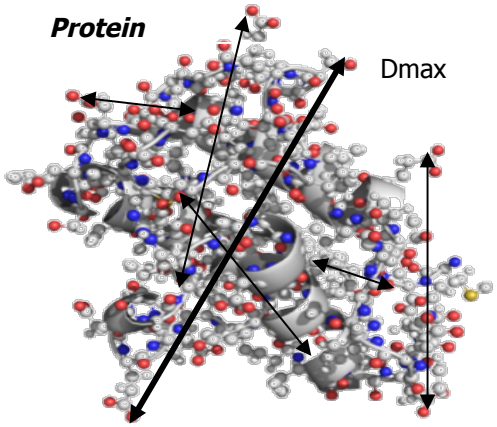
The curve increases at large Q as the structure extends.

Data Analysis

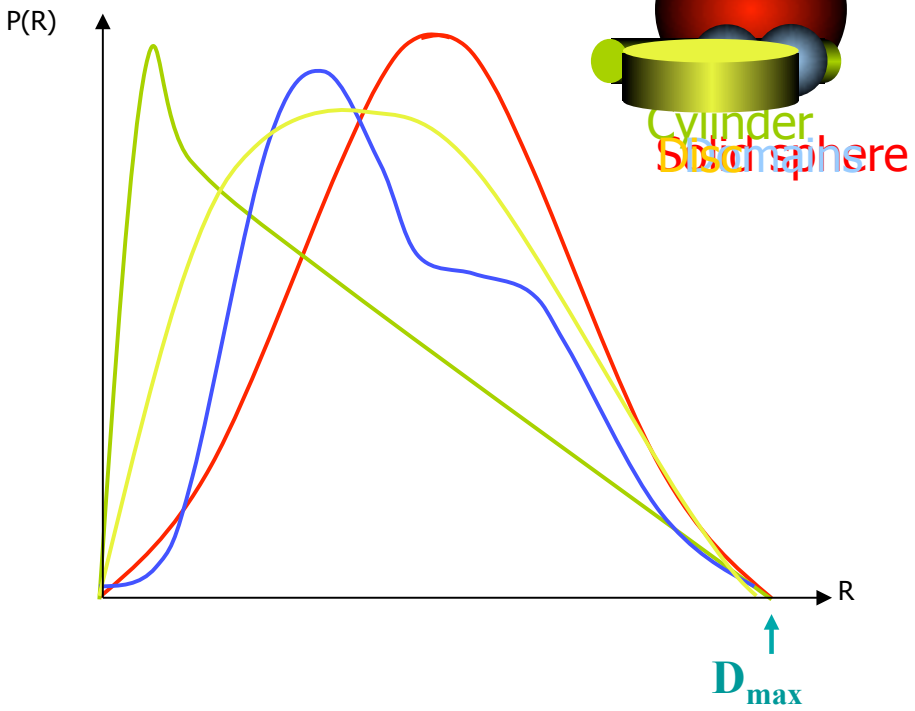
- Guinier Analysis
- Kratky plot : why is it so interesting ?
- **Real-space : Distance distribution function P(r)**

Distance Distribution Function p(r)

p(r) is obtained by histogramming the distances between any pair of scattering elements within the particle.



p(r) vanishes at r = D_{max}



The distance distribution function characterises the shape of the particle **in real space**

CONCLUSIONS

- **A scattering pattern can be calculated from atomic coordinates, thereby providing a link between crystal and solution work.**

- **Using SAXS patterns, a*b initio* methods can determine the shape of a molecule**

- **Rigid-body modeling allows one to propose models for complexes best fitting the data.**

- **Useful though limited structural information about flexible systems can be derived from SAXS data.**

CONCLUSIONS

- ✓ **The information content is limited and the method is best used in combination with other structural (cristallography, NMR, EM), dynamic (NMR, fluorescence, MALS), biochemical (e.g. cross-link + MS) and/or computational (data-driven docking, molecular dynamics) approaches.**

- ✓ **3D modeling requires a monodispersed and ideal solution, which has to be checked independently.**

- ✓ **Otherwise :**

IN **SAXS**

OUT