AB INITIO MODELING FOR SAS HANDS-ON

FASEM 2024

ALEXANDROS KOUTSIOUMPAS

JÜLICH CENTRE FOR NEUTRON SCIENCE (JCNS)
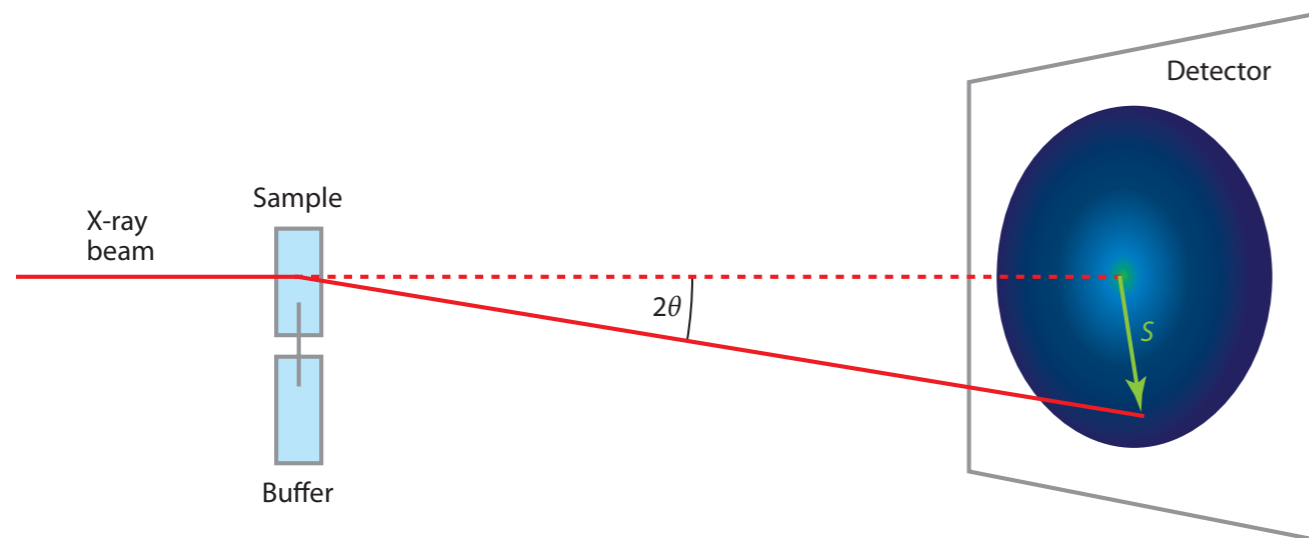
AT MAIER LEIBNITZ ZENTRUM (MLZ)

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SCATTERING AT SMALL ANGLES

Probing more "mesoscopic" structure in the presence of solvent
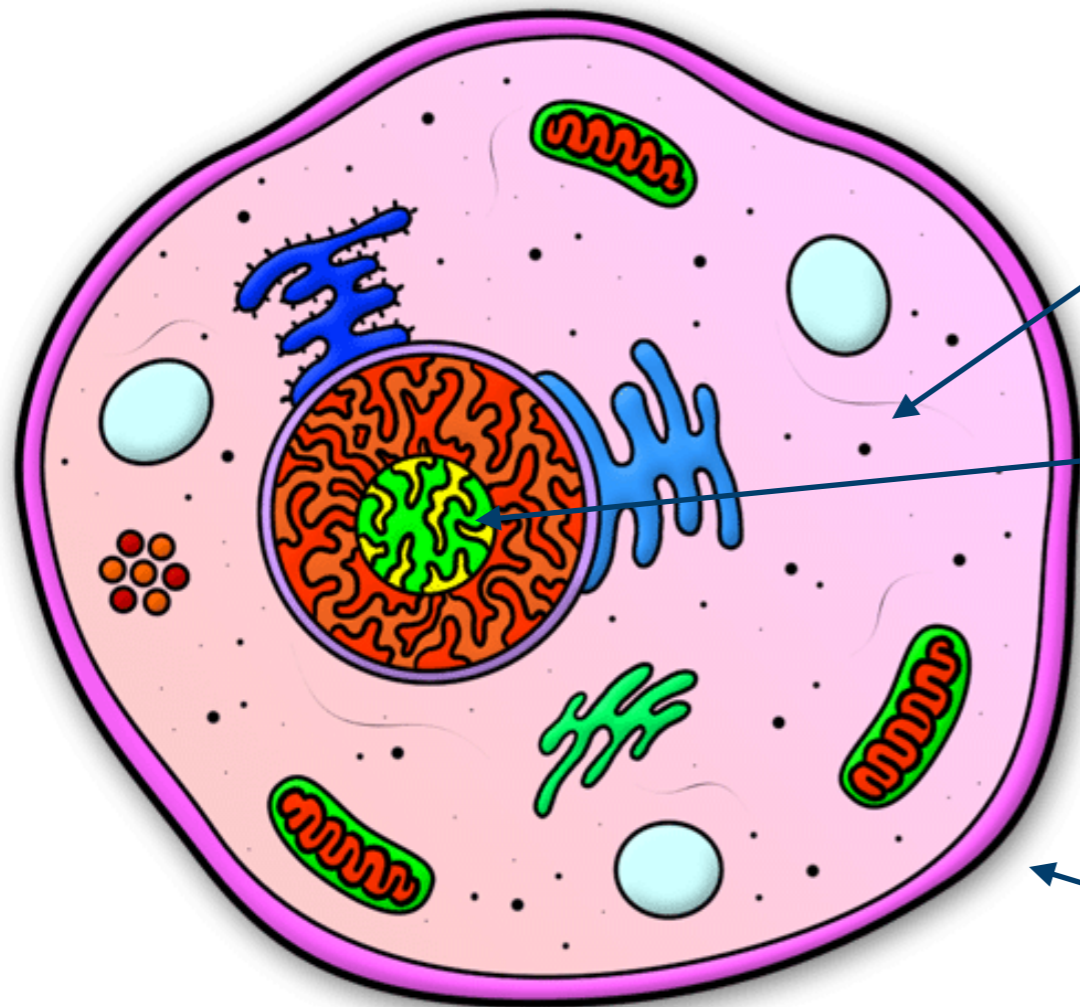
Small-angle scattering

Exploiting information at the inverse space at low-Q (large-D)
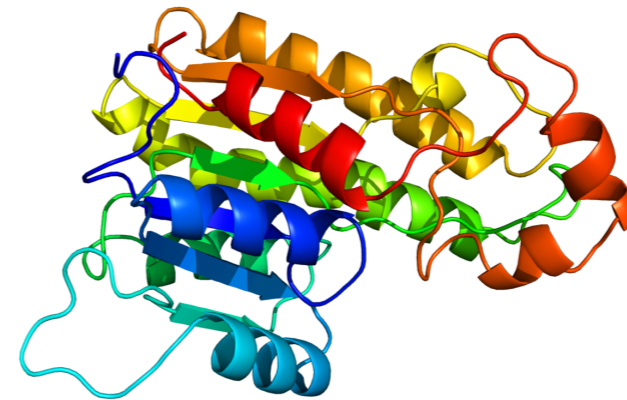
$$Q \sim 0.01 - 0.5 \mathring{A}^{-1}$$
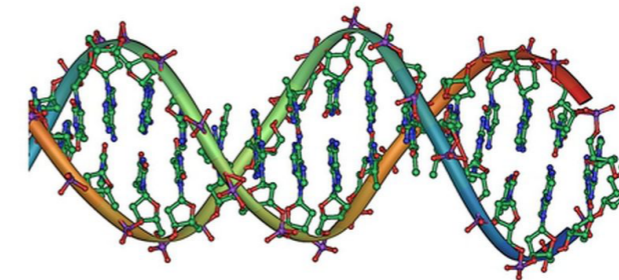
$$D \sim 10 - 1000 \mathring{A}$$
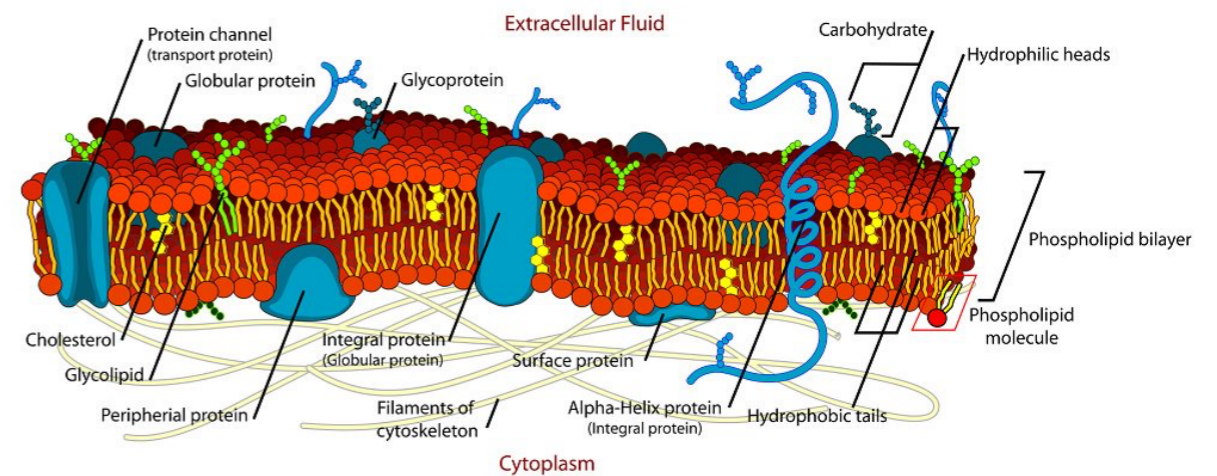
INTRODUCTION

The usual suspects

proteins

nucleic acids

membranes

Extracellular Fluid

Protein channel (transport protein)
Globular protein
Glycoprotein
Carbohydrate
Hydrophilic heads
Cholesterol
Glycolipid
Integral protein (Globular protein)
Surface protein
Phospholipid bilayer
Phospholipid molecule
Peripherial protein
Filaments of cytoskeleton
Alpha-Helix protein (Integral protein)
Hydrophobic tails
Cytoplasm

living cell

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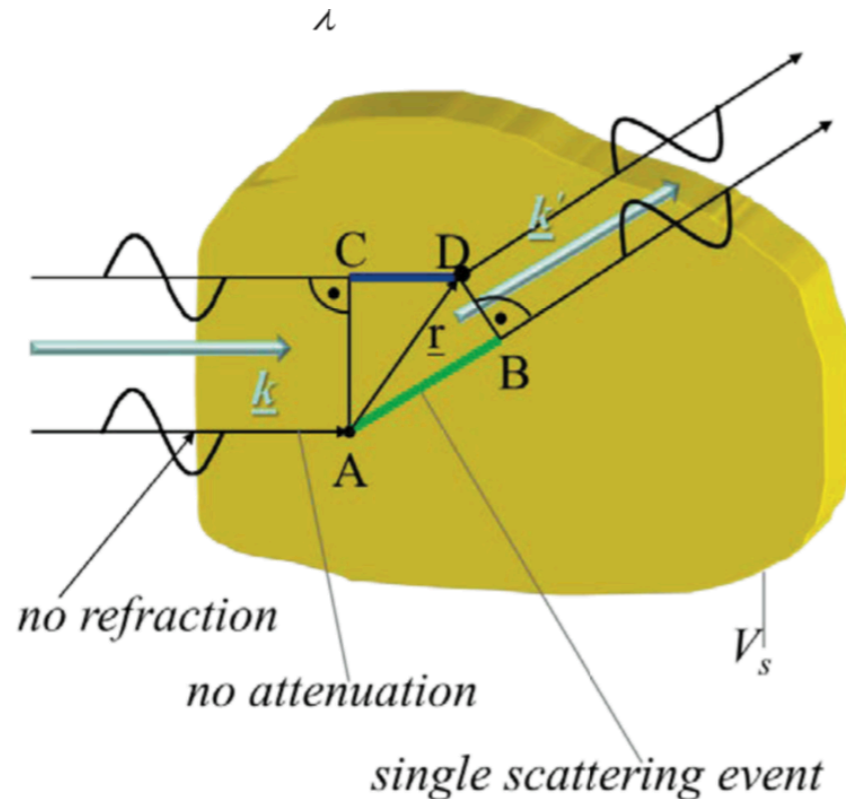
SCATTERING

Elastic scattering

$$\Delta\Phi = 2\pi \frac{\vec{AB} - \vec{CD}}{\lambda} = \vec{k'}\vec{r} - \vec{k}\vec{r} = \vec{Q}\vec{r}$$

total scattering amplitude is given by the superposition of scattered waves [modulated by the potential V(r)] with a phase difference ΔΦ

$$A(\vec{Q}) \sim \int V(\vec{r}) e^{i\vec{Q}\vec{r}} d^3 r$$

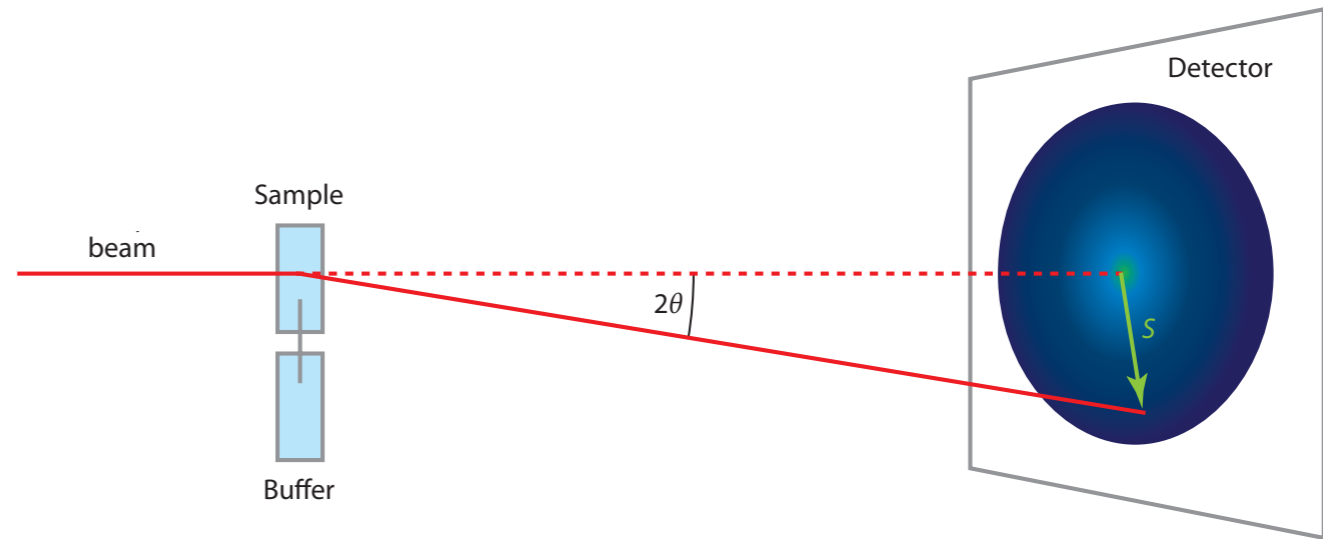JÜLICH
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Generalities of solution SAS

scattering from an assembly of atoms

$$A(\vec{Q}) = \sum_j f_j \exp(i\vec{Q}\vec{r}_j)$$

valid for a collection of atoms in vacuum

$$A(\vec{Q}) = \sum_j (f_j - \rho_w v_j) \exp(i\vec{Q}\vec{r}_j)$$

valid for a collection of atoms in solvent

contrast

dilute solution

$$I_N(\vec{Q}) = \sum_{n=1}^{N} |\vec{A}_n(\vec{Q})|^2 \xrightarrow{\text{rotational averaging}} I_N(Q) = N < |\vec{A}_n(\vec{Q})|^2 >$$

$$< exp(i\vec{Q}\vec{r}) > = \sin Qr / Qr \longrightarrow I_N(Q) = N \sum_j \sum_k (f_j - \rho_w v_j)(f_k - \rho_w v_k) \frac{\sin Qr_{jk}}{Qr_{jk}}$$

using this classic formula
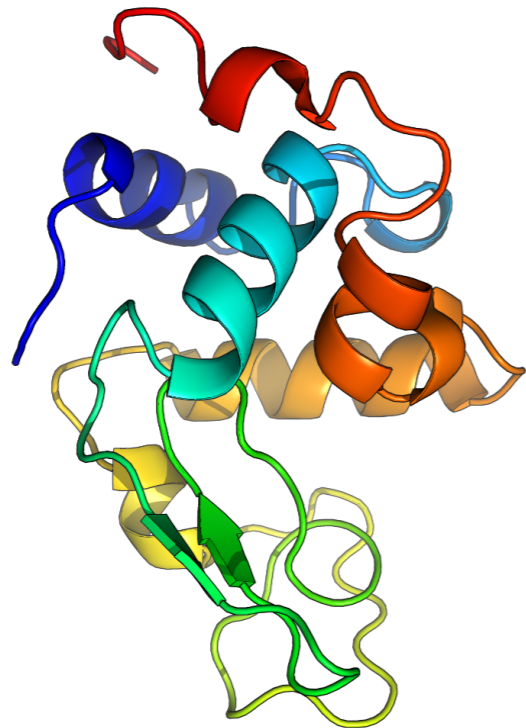
Debye equation

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The forward problem
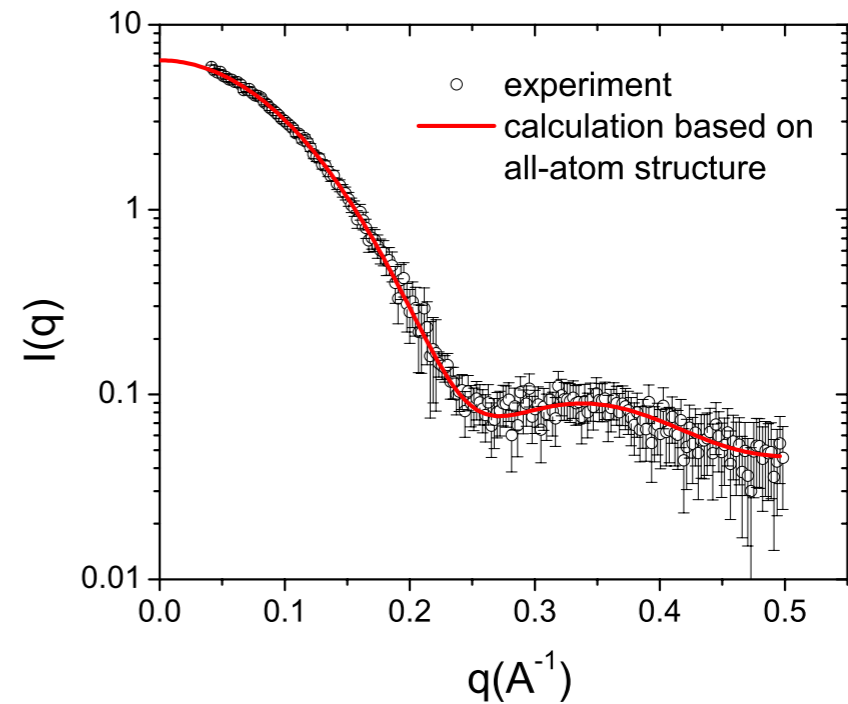
Debye equation

$$I_N(Q) = N \sum_j \sum_k (f_j - \rho_w v_j)(f_k - \rho_w v_k) \frac{\sin Q r_{jk}}{Q r_{jk}}$$

protein data bank (6lyz)

```
ATOM      1  N   LYS A   1       3.287  10.092  10.329  1.00  5.89           N
ATOM      2  CA  LYS A   1       2.445  10.457   9.182  1.00  8.16           C
ATOM      3  C   LYS A   1       2.500  11.978   9.038  1.00  8.04           C
ATOM      4  O   LYS A   1       2.588  12.719  10.041  1.00  7.07           O
ATOM      5  CB  LYS A   1       1.006   9.995   9.385  1.00  3.88           C
ATOM      6  CG  LYS A   1       0.016  10.546   8.377  1.00  3.81           C
```
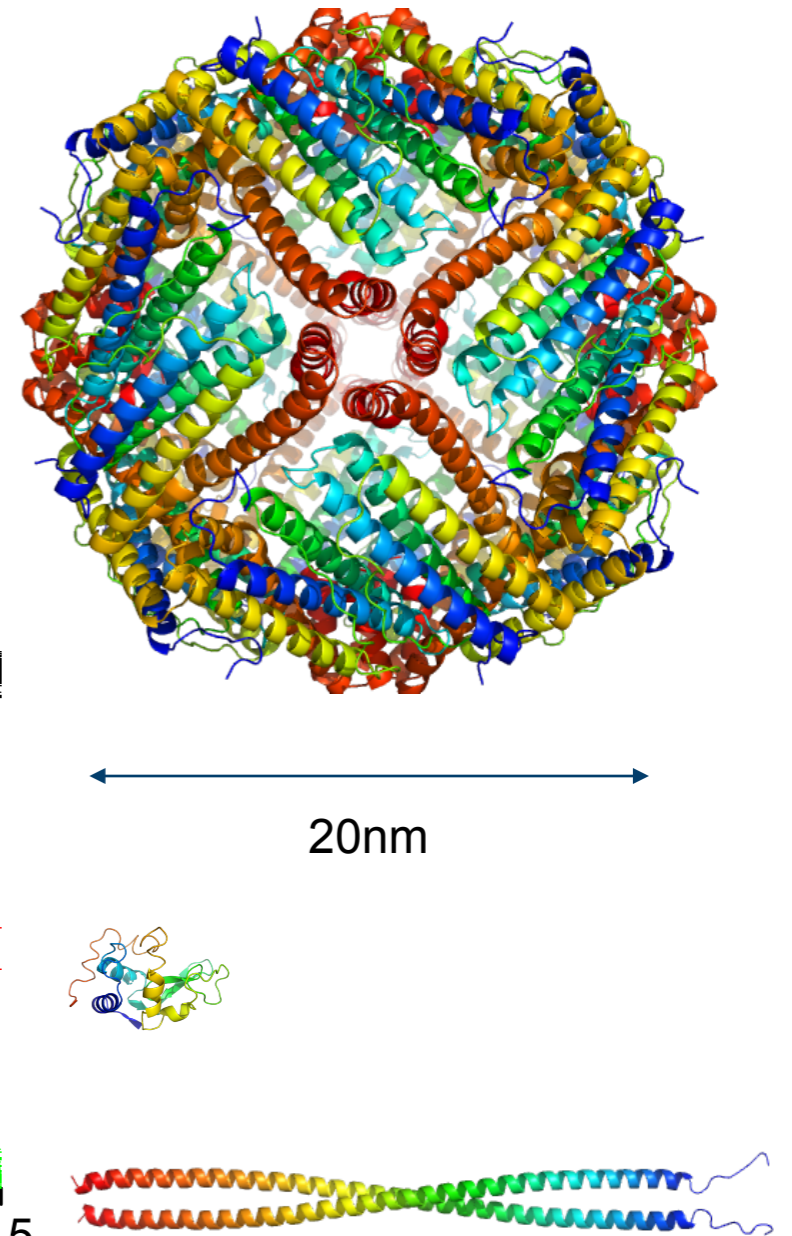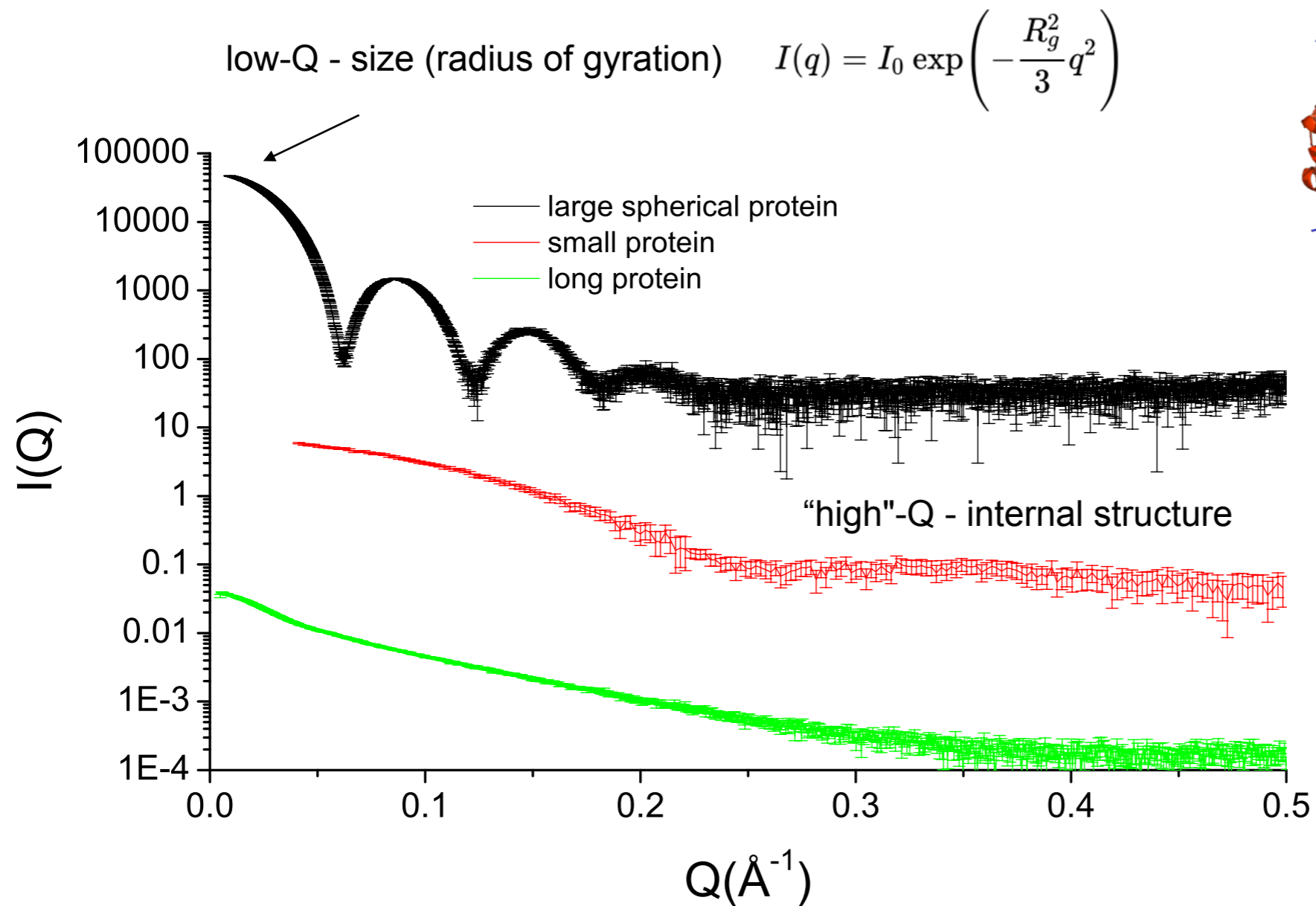
Debye equation

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Scattering curve features

low-Q - size (radius of gyration)

$$I(q) = I_0 \exp\left(-\frac{R_g^2}{3}q^2\right)$$

large spherical protein
small protein
long protein

"high"-Q - internal structure

I(Q)

Q(Å^{-1})

20nm

progressively looking at smaller length scales

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The inverse problem

exploiting information contained in the low-q part of the scattering curve where I(q) is dominated by the overall shape of the particles in solution
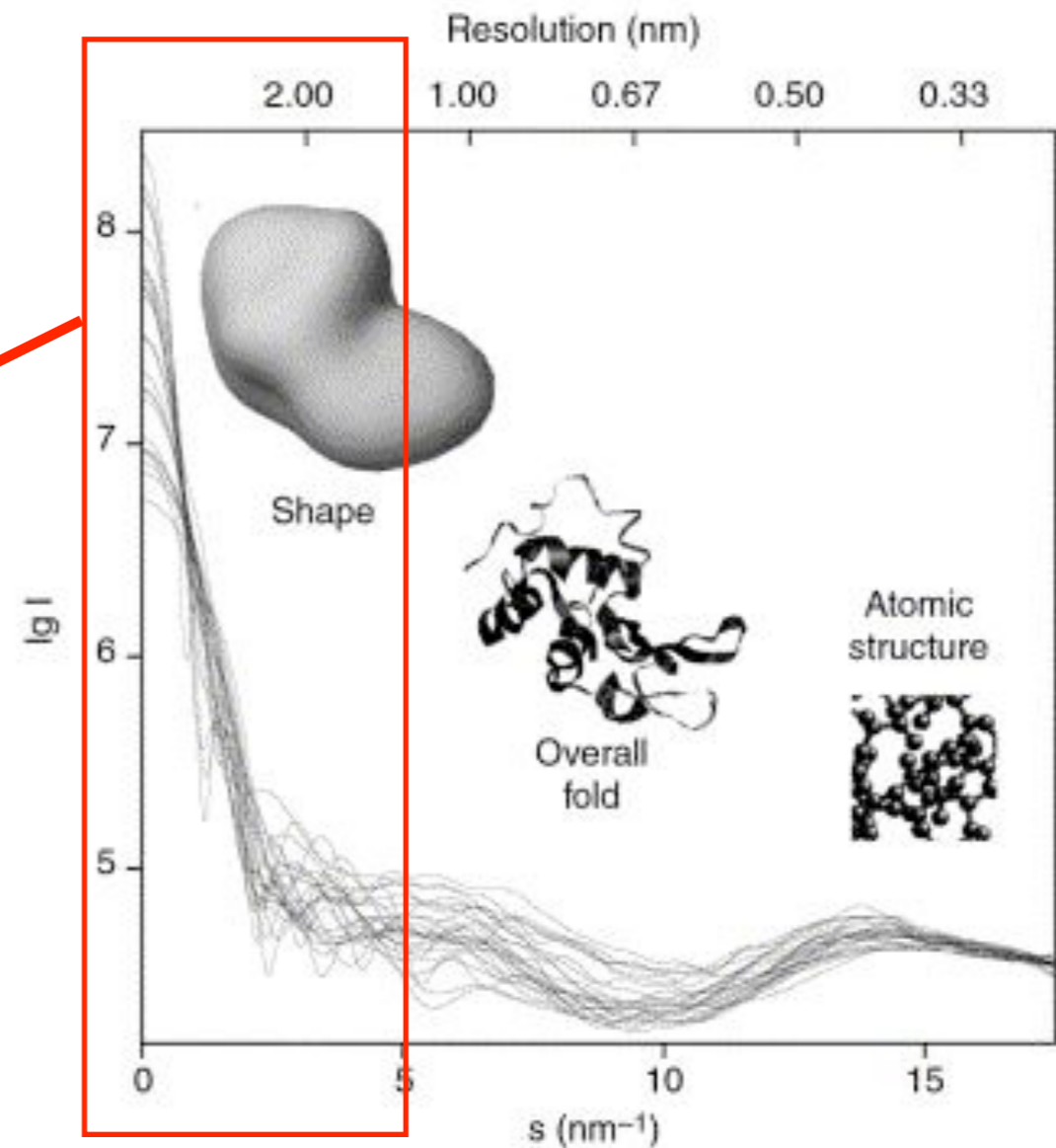
Svergun, *et al.* (2001). *Biophys. J.*, **80**, 2946.

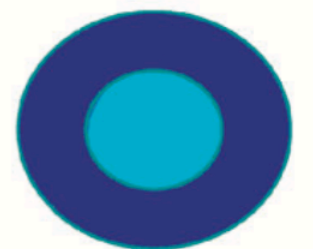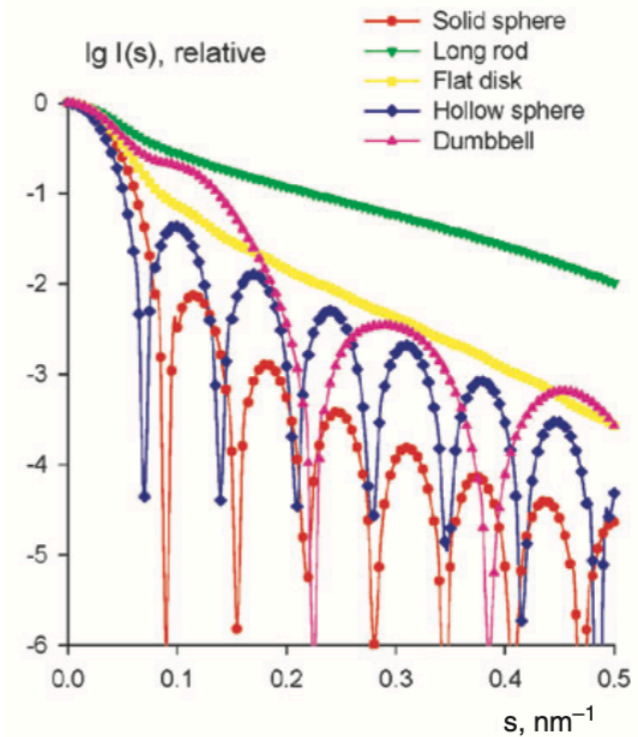JÜLICH
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Pair Distribution Function

$$I(q) = 4\pi \int_0^{D_{max}} r^2 \gamma(r) \frac{\sin qr}{qr} dr$$

$$\gamma(r) = \left\langle \int \Delta\rho(\vec{u})\Delta\rho(\vec{u}+\vec{r}) \right\rangle_\omega$$

spherically averaged autocorrelation function of the excess scattering density

$$p(r) = \frac{r^2}{2\pi^2} \int_0^\infty q^2 I(q) \frac{\sin qr}{qr} dr$$

distribution of distances between volume elements inside the particle

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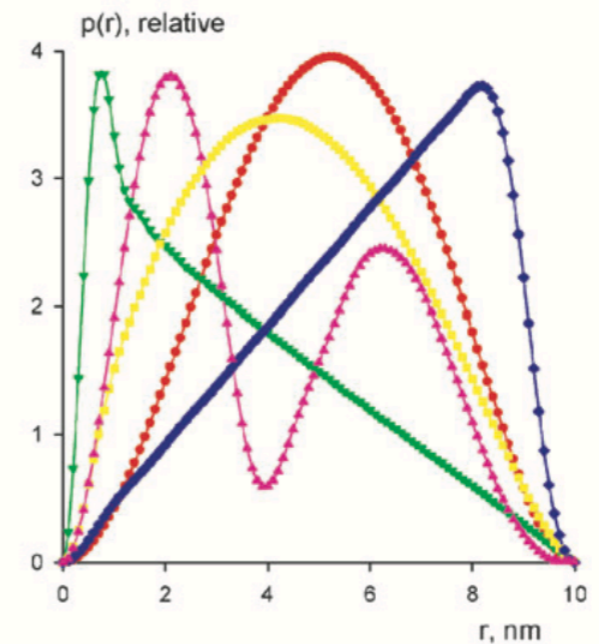
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Ab-initio modelling

What we know about proteins and nucleic acids:
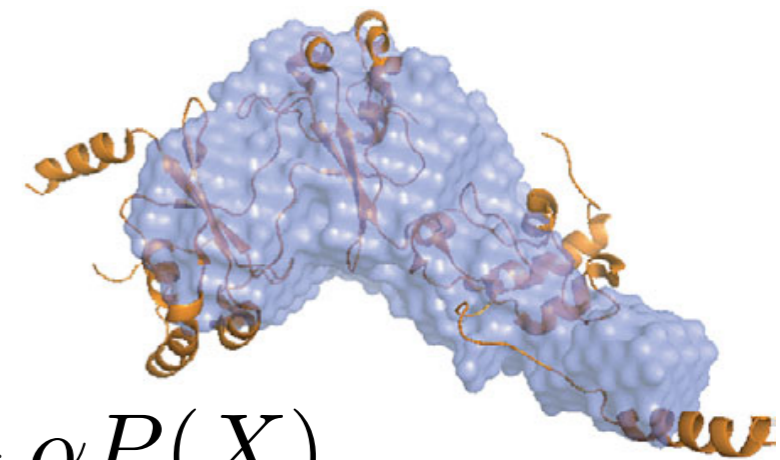In general <u>compact</u> & <u>interconnected</u> structures

Simulated annealing   Svergun, *et al.* (1999). *Biophys. J.*, **76**, 2879.

▸ Search for a spatial arrangement of "beads" (*X*) that reproduces the scattering curve

▸ No unique convergence unless constrained

$$f(X) = \chi^2[I_{exp}(q), I_{model}(q, X)] + \alpha P(X)$$
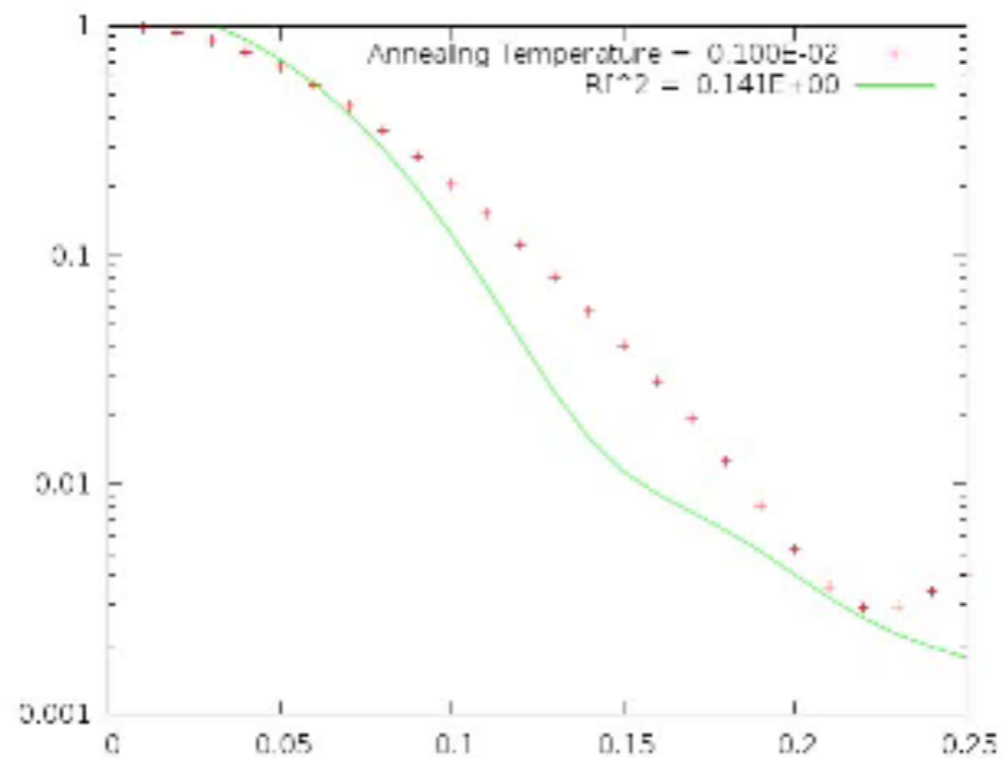
experimental data - model discrepancy

connectivity and compactness penalty

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Ab-initio modelling

evolution of the fit   evolution of the shape

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Ab-initio modelling

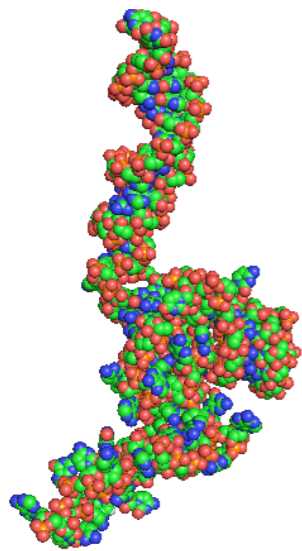evolution of the fit   evolution of the shape

Annealing Temperature = 0.100E-02
RF^2 = 0.141E+00
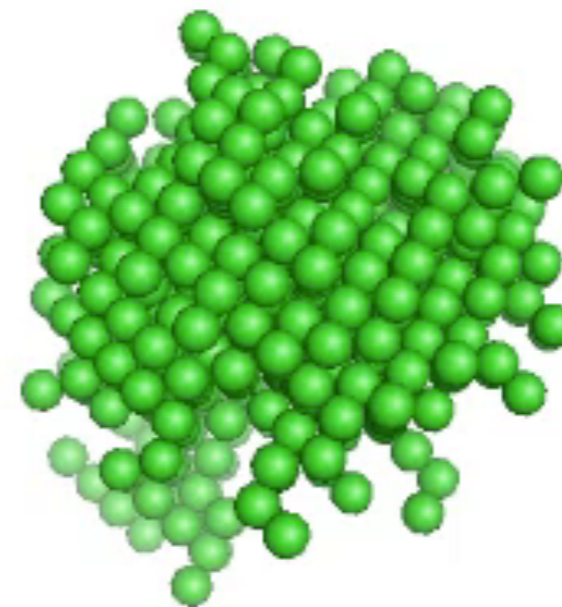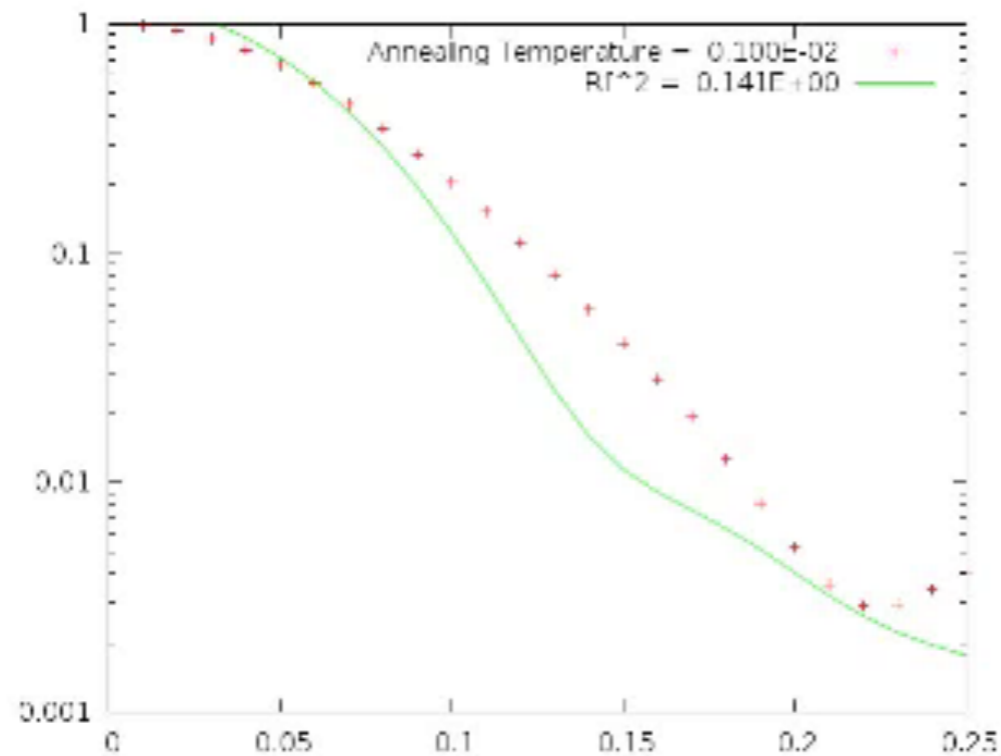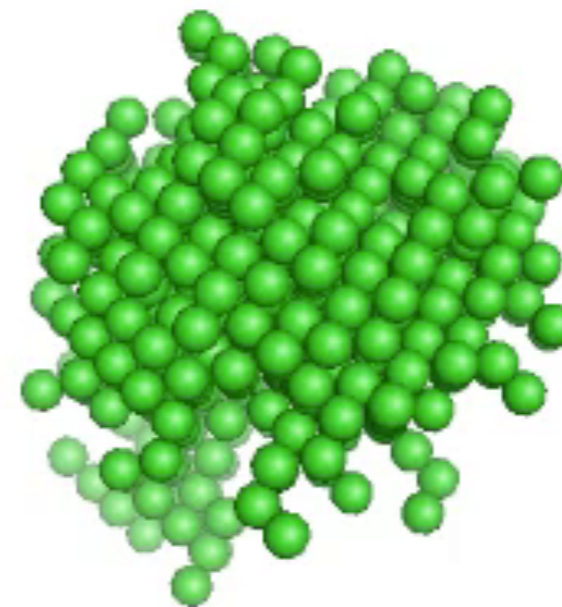
at the end compact, interconnected structure
and a nice fit of the experimental data

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Uniqueness

5S RNA   Different Ab-initio runs
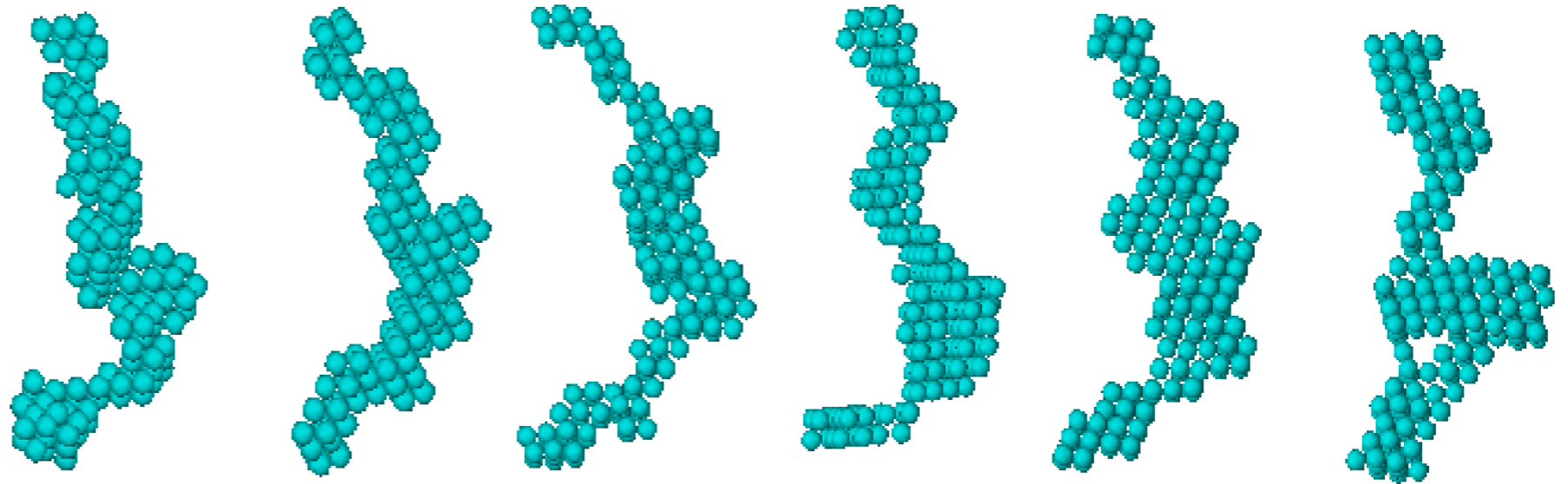
No unique solution! But an ensemble of shapes that approximate the real molecular envelope

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The programs

ATSAS Suite

- GNOM (IFT)

- DAMMIN, DAMMIF (dummy bead modelling)

- GASBOR (dummy residue modelling)

- MONSA (multi-phase modelling)

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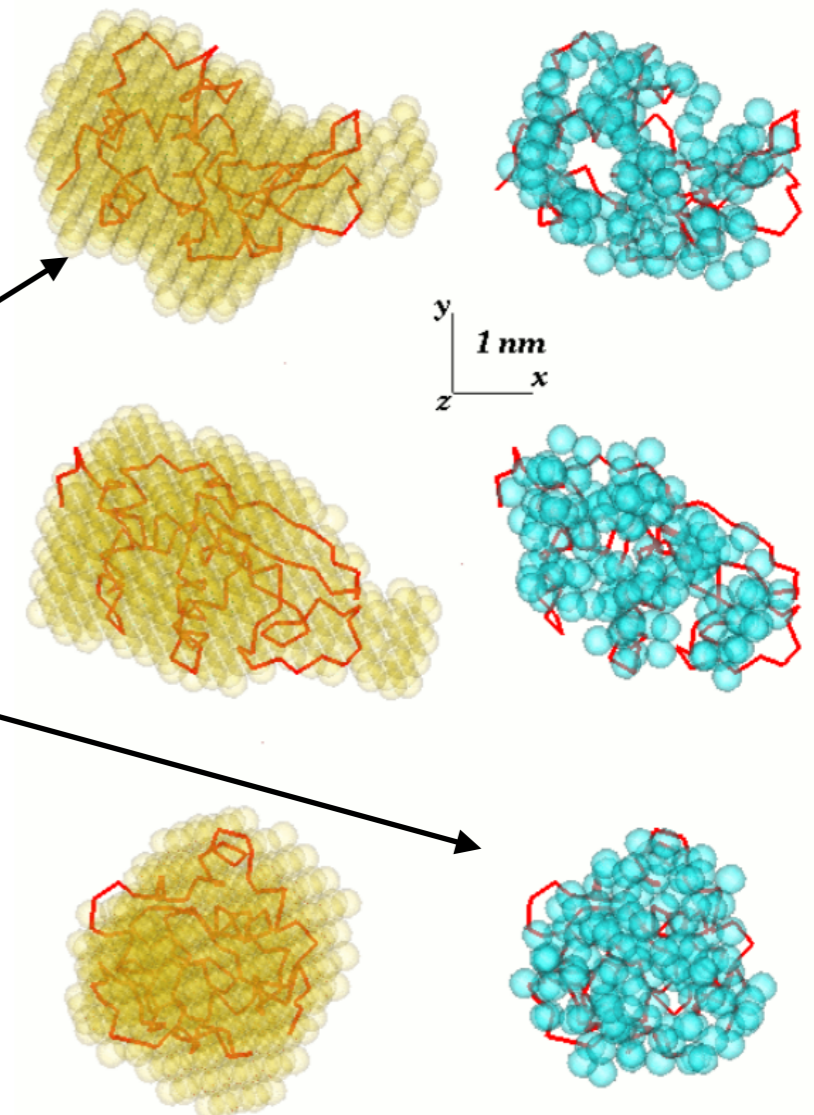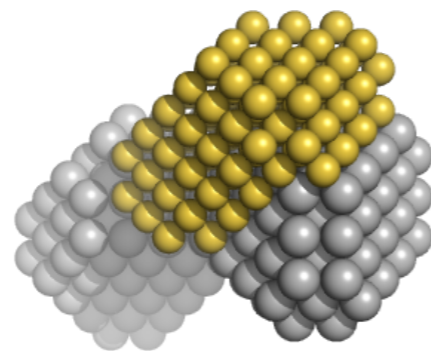
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General workflow

- pre-characterization (complementary techniques)

- Scattering experiment Neutrons or X-rays

- Guinier plot

- IFT - Dmax - distance distribution

- ab-initio run (multiple)

- consensus model

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es de la diffusion des rayons X
s angles (SAXS)
avier Pérez

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The program we will use today

DENFERT (taking into account hydration layer)

Cours M2, 6 décem

atter and O. Kratky (1982), Academic Press Inc.,

 n and O. Kratky hydration layer Denss atom' *ab initio* structural modelling of biological macromolecules

Alexandros Koutsioubas, Javier Pérez
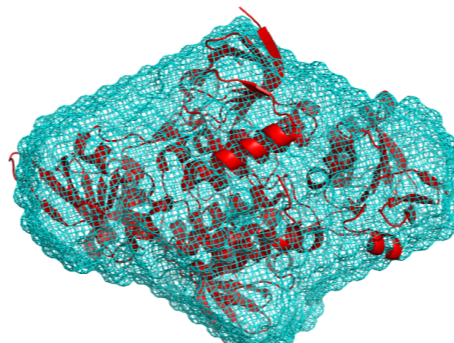
First published: 10 November 2013 | https://doi.org/10.1107/S0021889813025387 | Citations: 1

✉ Javier Pérez, e-mail: perez@synchrotron-soleil.fr
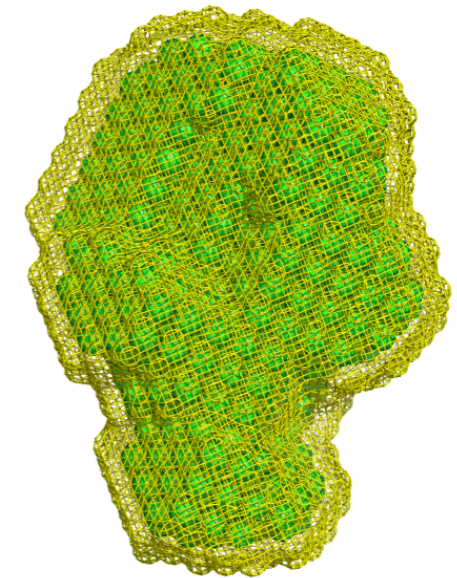
reconstruction for hexokinase

Cours M2, 6 déc
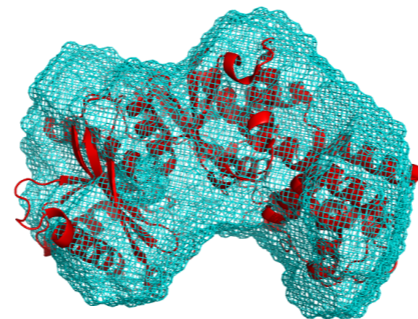
reconstruction for thioredoxin reductase
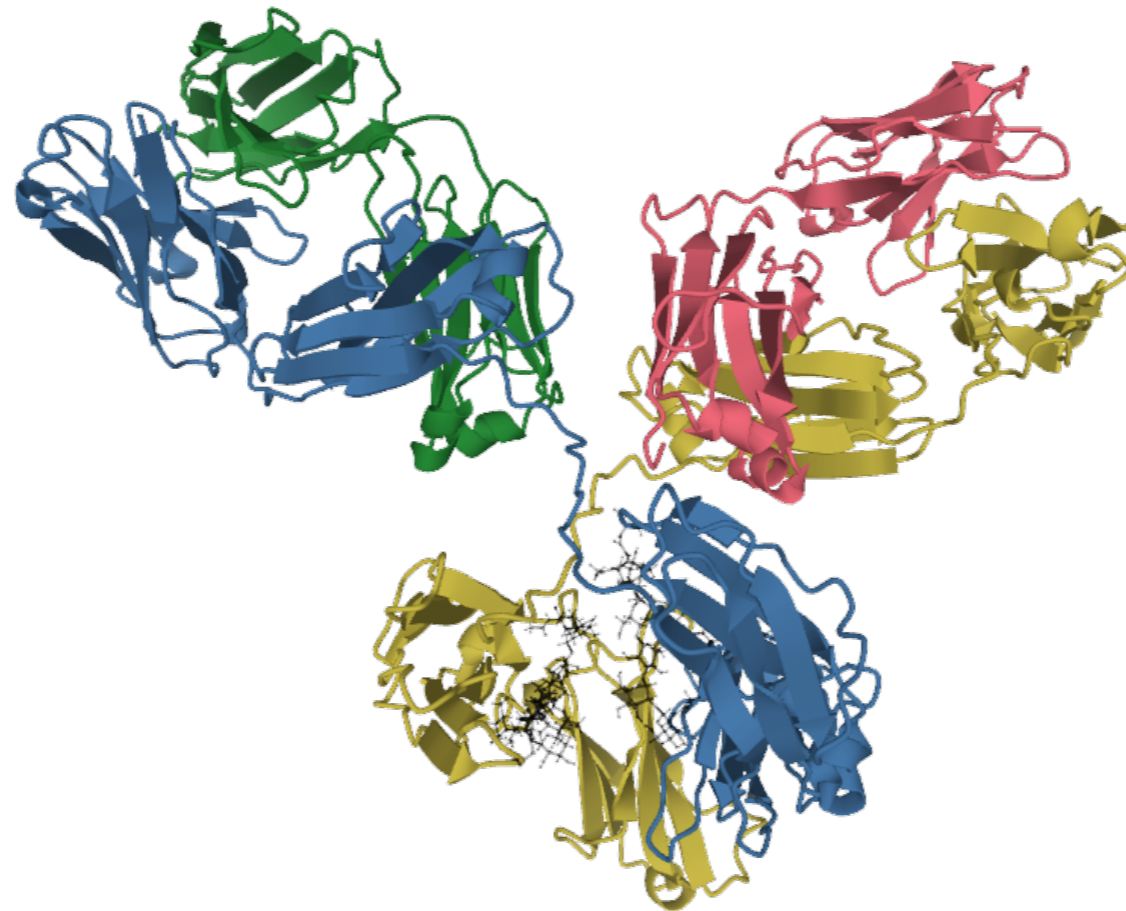
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Let's run some ab initio reconstructions

Lysozyme

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Let's run some ab initio reconstructions

Immunoglobulin

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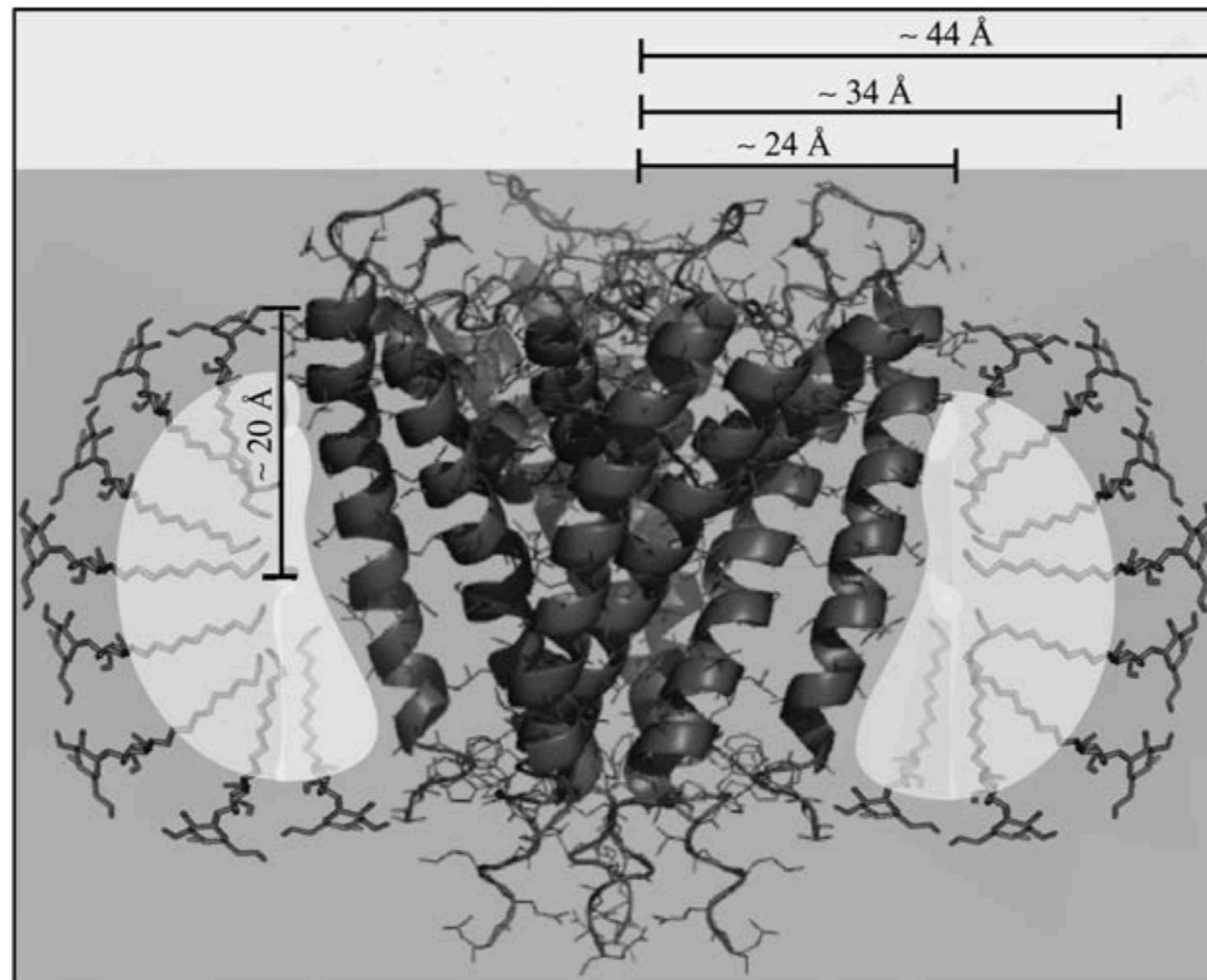**Your turn!**

https://www.sasbdb.org

https://alexandros-koutsioumpas.github.io/Denfert/
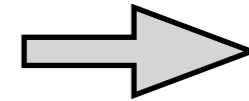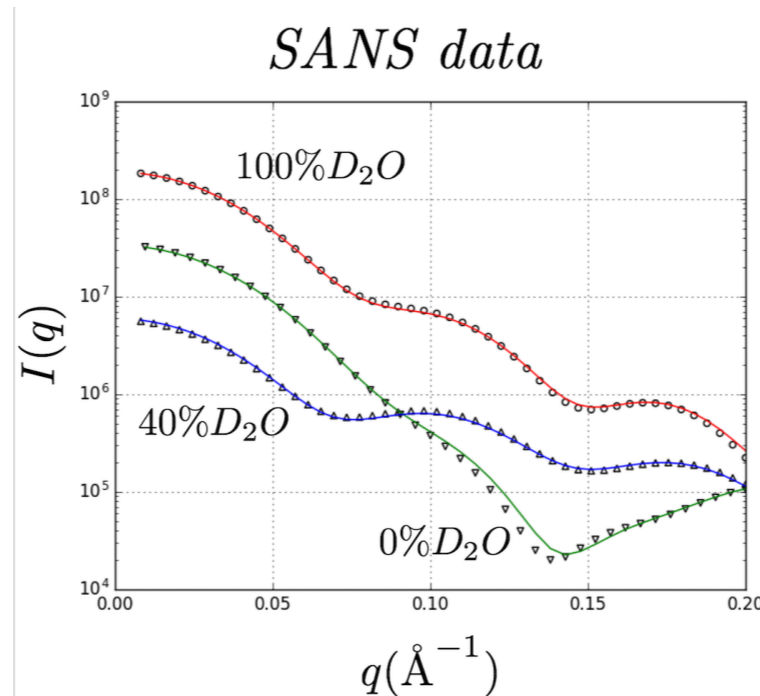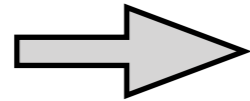
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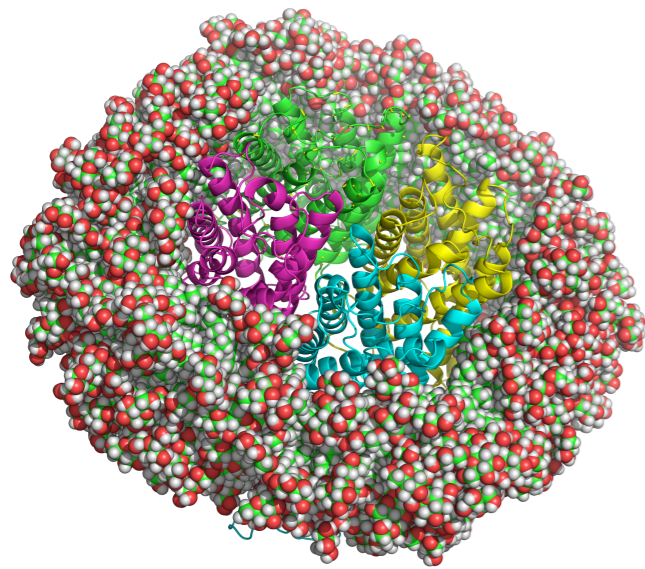
Special case

membrane protein / detergent complex

Zimmer et al. Biophysical Journal (2006) 90,1752

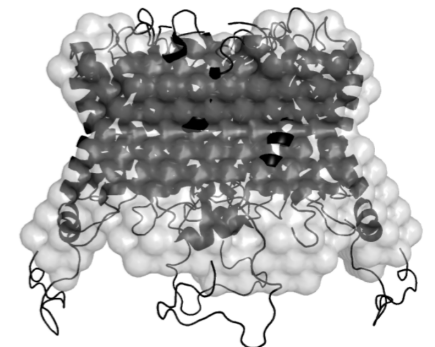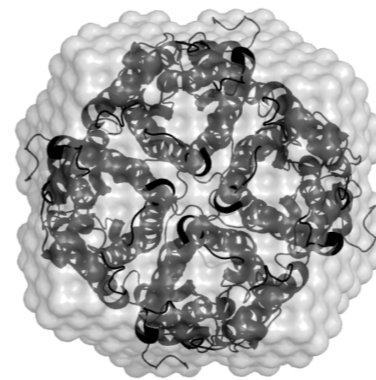# SMALL ANGLE SCATTERING

Multiple phase ab-initio modelling for membrane proteins
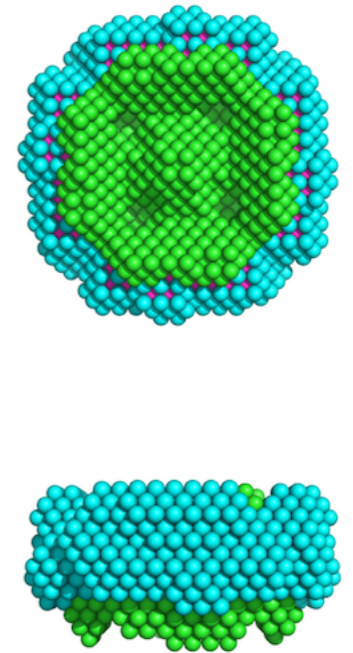
Comparison of reconstructed envelope
with the crystallographic structure

low-resolution
model

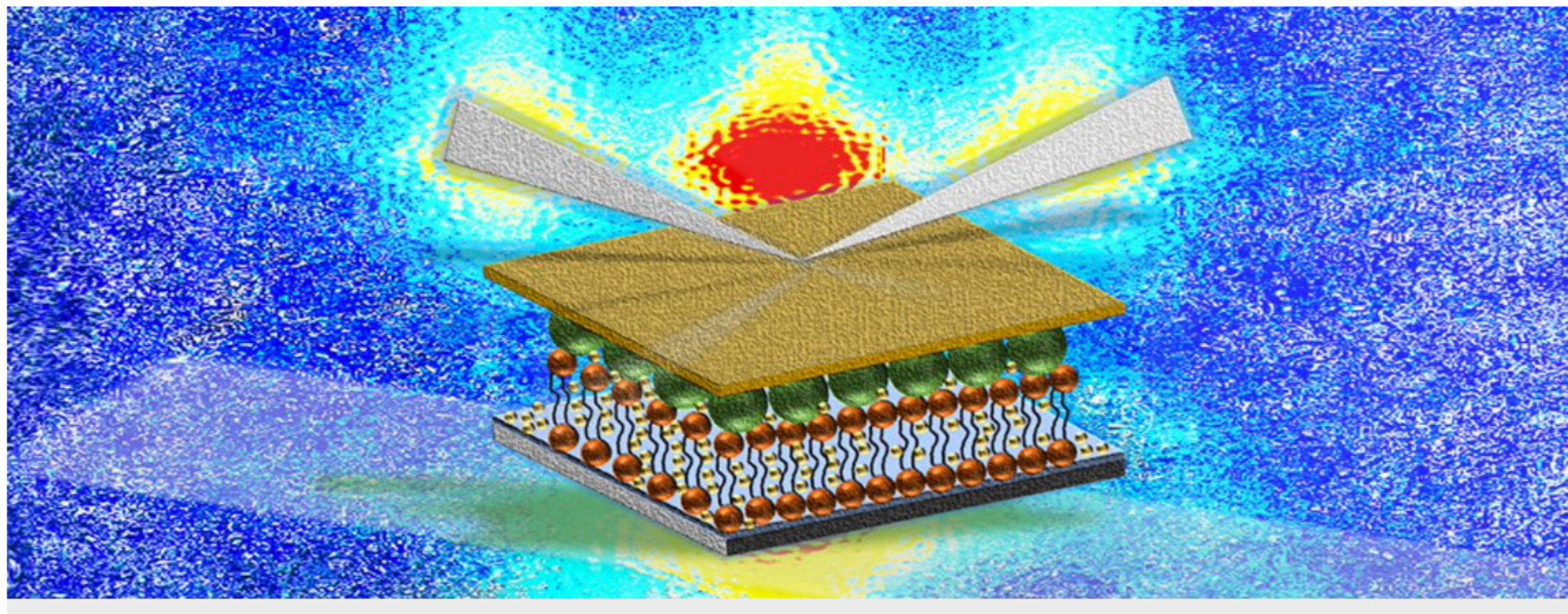**Biophysical Journal** (2017) 113, 2373–2382

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Small advertisement

JCNS Workshop 2024

Trends and Perspectives in Neutron Scattering: Functional Interfaces

08 – 11 October 2024, Tutzing, Germany

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Happy to answer your questions

a.koutsioumpas@fz-juelich.de

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