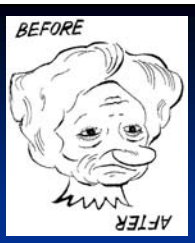
Ab initio methods: how/why do they work

D.Svergun

EMBL

A word of caution or Main problem of SAXS



- Sample preparation
- Experiment
- Data processing
- Unambiguous interpretation
- Changing conditions
- Relation to function

Scattering from dilute macromolecular solutions (monodisperse systems)

$$I(s) = 4\pi \int_0^D p(r) \frac{\sin sr}{sr} dr$$

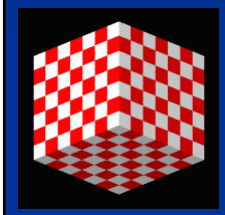
The scattering is proportional to that of a single particle averaged over all orientations, which allows one to determine size, shape and internal structure of the particle at low (*1-10 nm*) resolution.

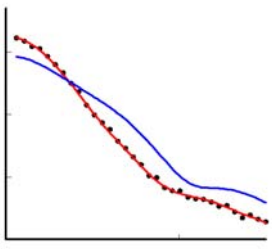
Shape determination: how?

3D search model
M parameters

Trial-and-erro

Non-line
search





Lack of 3D information inevitably leads to ambiguous interpretation, and additional information is always required

Ab initio methods

Advanced methods of SAS data analysis employ spherical harmonics (Stuhrmann, 1970) instead of Fourier transformations

The use of spherical harmonics

SAS intensity is $I(s) = \langle I(\mathbf{s})\rangle_\Omega = \langle\{F[\rho(\mathbf{r})]\}^2\rangle_\Omega$, where F denotes the Fourier transform, $\langle\rangle_\Omega$ stands for the spherical average, and $s=(\mathbf{s}, \Omega)$ is the scattering vector. Expanding $\rho(\mathbf{r})$ in spherical harmonics

$$\rho(\mathbf{r}) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \rho_{lm}(r) Y_{lm}(\omega)$$

the scattering intensity is expressed as

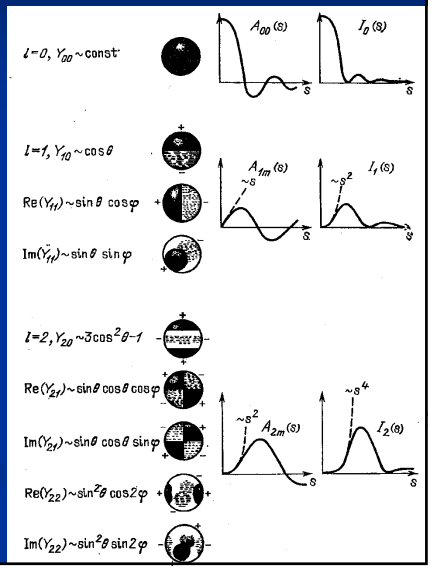
$$I(s) = 2\pi^2 \sum_{l=0}^{\infty} \sum_{m=-l}^{l} |A_{lm}(s)|^2$$

where the partial amplitudes $A_{lm}(s)$ are the Hankel transforms from the radial functions

$$A_{lm}(s) = i^l \sqrt{2/\pi} \int_0^{\infty} \rho_{lm}(r) j_l(sr) r^2 dr$$

and $j_l(sr)$ are the spherical Bessel functions.

Stuhrmann, H.B. Acta Cryst., **A26** (1970) 297.



Structure of bacterial virus T7

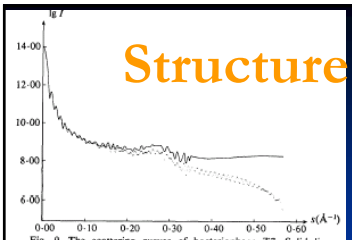


Fig. 9. The scattering curves of bacteriophage T7. Solid line: experimental curve. Dotted line: the intensity of the restored structure.

SAXS, 1982

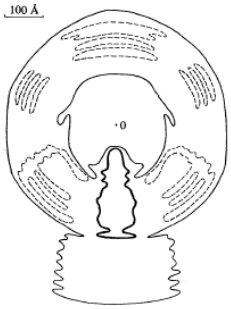
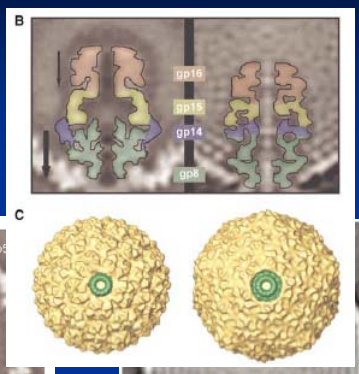
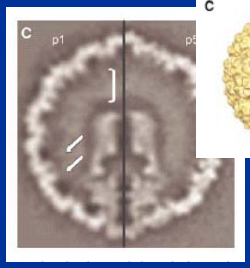


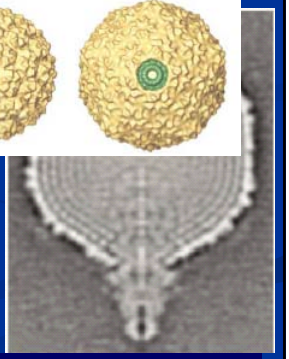
Fig. 10. The electron density map in bacteriophage T7. The cross section containing the axis of rotation (indicated by an arrow) is shown. Solid line: level 0·38 Å⁻³ (hydrated protein); dashed line:

Svergun, D.I., Feigin, L.A. & Schedrin, B.M. (1982) *Acta Cryst.* **A38**, 827

Cryo-EM, 2005







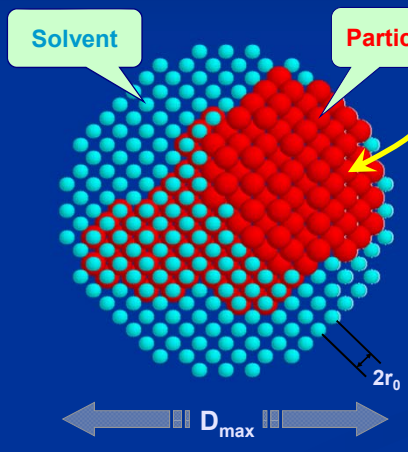
Pro-head

Mature virus

Agirrezabala, J. M. *et al.* & Carrascosa J.L. (2005) *EMBO J.* **24**, 3820

Bead (dummy atoms) model

A sphere of radius D_{max} is filled by densely packed beads of radius $r_0 << D_{max}$



Vector of model parameters:

$$Position\ (j) = X(j) = \begin{cases} 1 & if\ particle \\ 0 & if\ solvent \end{cases}$$

(phase assignments)

Number of model parameters $M \approx (D_{max} / r_0)^3 \approx 10^3$ is too big for conventional minimization methods – Monte-Carlo like approaches are to be used

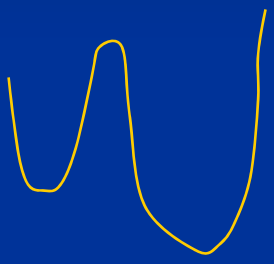
But: This model is able to describe rather complex shapes

Chacón, P. *et al.* (1998) *Biophys. J.* 74, 2760-2775.

Svergun, D.I. (1999) *Biophys. J.* 76, 2879-2886

Finding a global minimum

Pure Monte Carlo runs in a danger to be trapped into a local minimum



Solution: use a global minimization method like simulated annealing or genetic algorithm

Local and global search on the Great Wall

Local search always goes to a better point and can thus be trapped in a local minimum

Pure Monte-Carlo search always goes to the closest local minimum (nature: rapid quenching and vitreous ice formation)

To get out of local minima, global search must be able to (sometimes) go to a worse point

Slower annealing allows to search for a global minimum (nature: normal, e.g. slow freezing of water and ice formation)

Simulated annealing

Aim: find a vector of *M* variables *{x}* minimizing a function *f(x)*

1. **Start from a random configuration *x* at a "high" temperature *T*.**
2. **Make a small step (random modification of the configuration) $x \rightarrow x'$ and compute the difference $\Delta = f(x') - f(x)$.**
3. ***If* $\Delta < 0$, accept the step; if $\Delta > 0$, accept it with a probability $e^{-\Delta/T}$**
4. ***Make another step from the old (if the previous step has been rejected) or from the new (if the step has been accepted) configuration.***
5. ***Anneal the system at this temperature, i.e.* repeat steps *2-4* "many" *(say, 100M* tries or *10M* successful tries, whichever comes first) times, then decrease the temperature *(T' = cT, c<1).***
6. ***Continue cooling the system until no improvement in f(x)* is observed.**

Shape determination: M≈ 10^3 variables (e.g. 0 or 1 bead assignments in DAMMIN

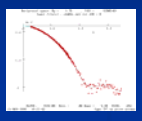
Rigid body methods: M≈ 10^1 variables (positional and rotational parameters of the subunits)

f(x) is always (Discrepancy + Penalty)

Ab initio program DAMMIN

Using simulated annealing, finds a compact dummy atoms configuration X that fits the scattering data by minimizing

$$f(X) = \chi^2[I_{\exp}(s), I(s, X)] + \alpha P(X)$$



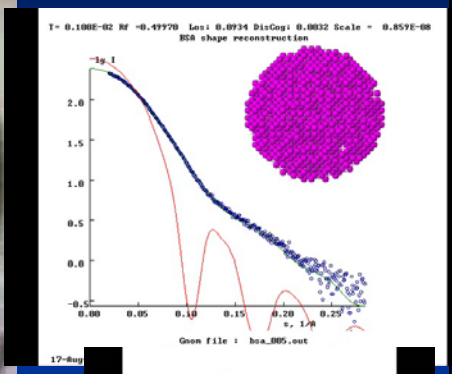
where χ is the discrepancy between the experimental and calculated curves, *P(X)* is the penalty to ensure compactness and connectivity, $\alpha > 0$ its weight.

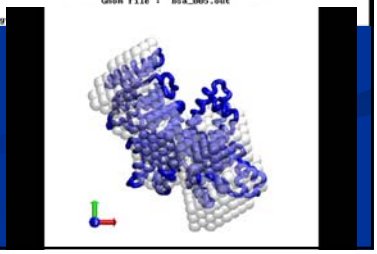
compact

loose

disconnected

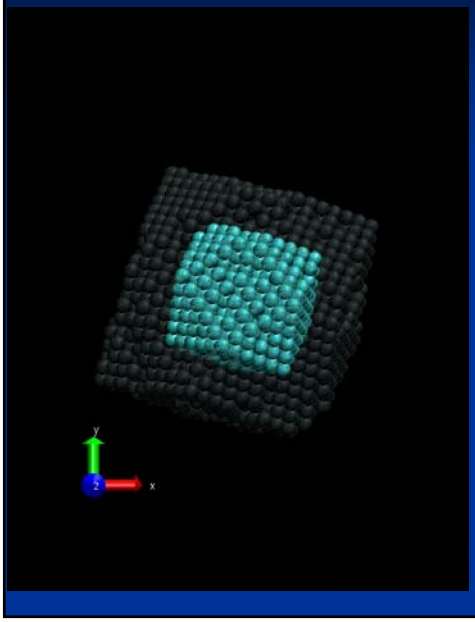
Why/how do *ab initio* methods work





The 3D model is required not only to fit the data but also to fulfill (often stringent) physical and/or biochemical constrains

DAMMIF, a fast DAMMIN



DAMMIF is a completely reimplemented DAMMIN written in object-oriented code

- **About 25-40 times faster than DAMMIN (in fast mode, takes about 1-2 min on a PC)**
- **Employs adaptive search volume**
- **Makes use of multiple CPUs**

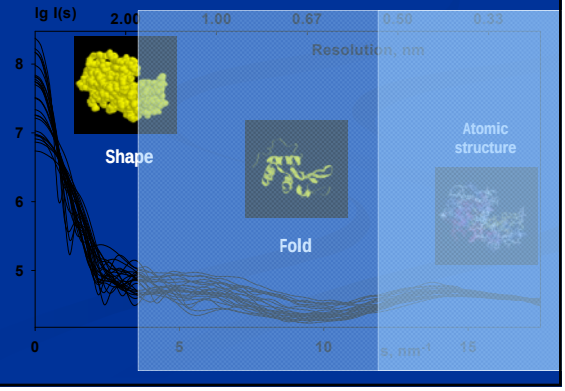
Franke, D. & Svergun, D. I. (2009)
J. Appl. Cryst. **42**, 342–346

Limitations of bead modelling

- Very low resolution
- Ambiguity of the models

Accounts for a restricted portion of the data

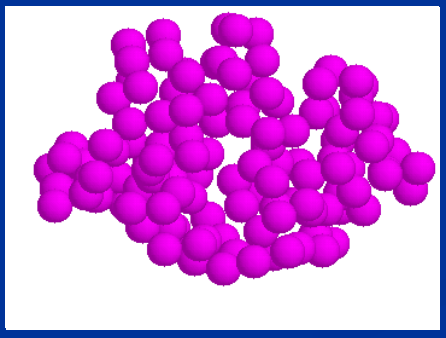
How to construct *ab initio* models accounting for higher resolution data?



Ab initio dummy residues model

- Proteins typically consist of folded polypeptide chains composed of amino acid residues

At a resolution of 0.5 nm a protein can be represented by an ensemble of K dummy residues centered at the Cα positions with coordinates $\{\boldsymbol{r}_j\}$

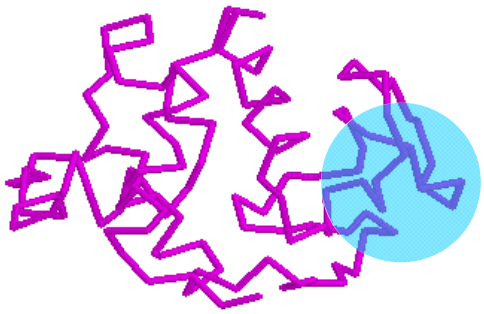


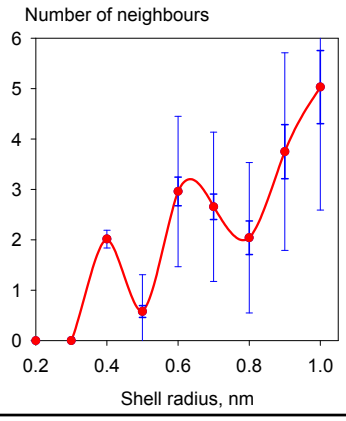
Scattering from such a model is computed using the Debye (1915) formula.

Starting from a random model, simulated annealing is employed similar to DAMMIN

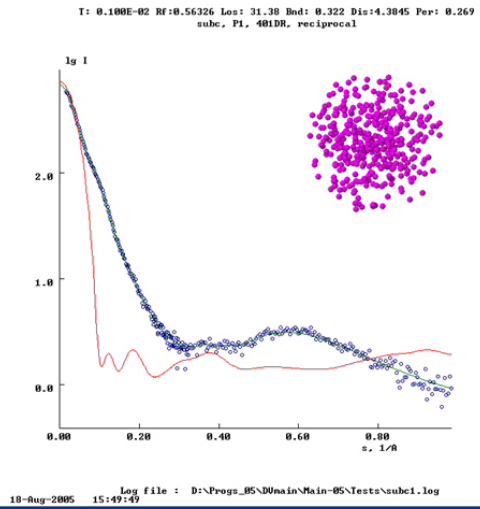
Distribution of neighbors

Excluded volume effects and local interactions lead to a characteristic distribution of nearest neighbors around a given residue in a polypeptide chain





GASBOR run on C subunit of V-ATPase



Starting from a random "gas" of 401 dummy residues, fits the data by a locally chain-compatible model

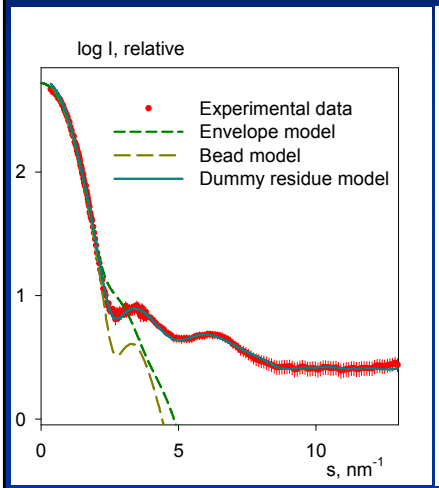
GASBOR run on C subunit of V-ATPase

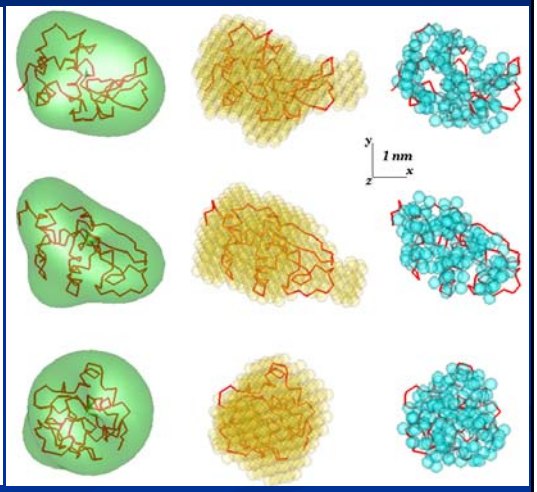
Beads: Ambruster *et al.* (2004, June) *FEBS Lett.* **570,** 119

C_α trace: Drory *et al.* (2004, November), *EMBO reports,* **5**, 1148

Benchmarking *ab initio* methods

Envelope ***Bead model*** ***Dummy residues***

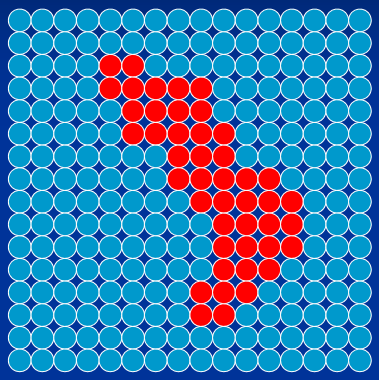


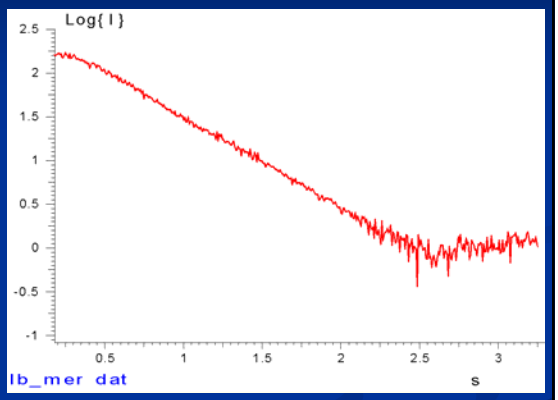


Comparison with the crystal structure of lysozyme

SASHA 1996 ***DAMMIN 1999*** ***GASBOR 2001***

Shape analysis for multi-component systems: principle

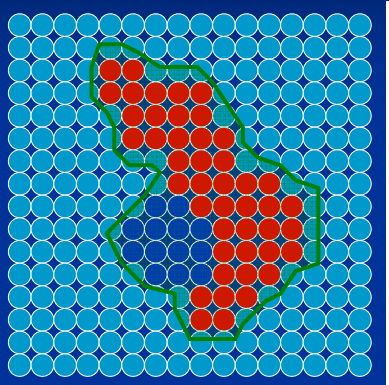


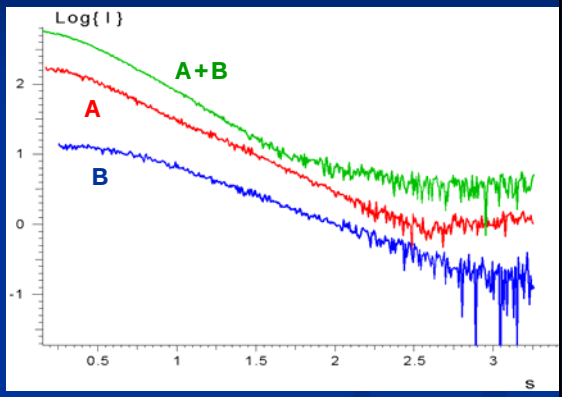


One component, one scattering pattern: “normal” shape determination

Chacón, P. et al. (1998) Biophys. J. 74, 2760-2775
Svergun, D.I. (1999) Biophys. J. **76**, 2879-2886

Shape analysis for multi-component systems: principle

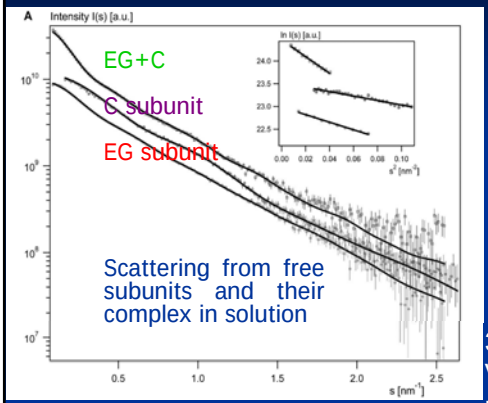


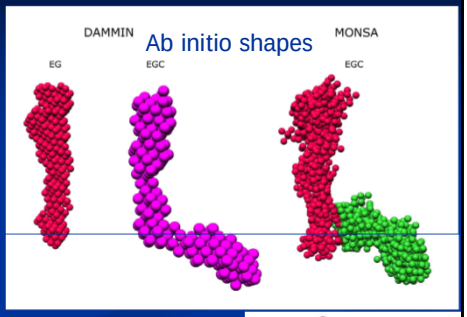


Many components, many scattering patterns: shape and internal structure

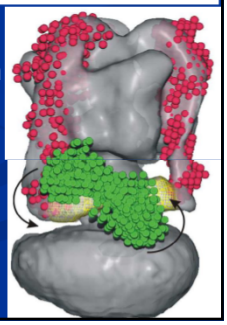
Svergun, D.I. (1999) Biophys. J. **76**, 2879-2886
Svergun, D.I. & Nierhaus, K.H. (2000) J. Biol. Chem. 275, 14432-14439

EGC stator sub-complex of V-ATPase





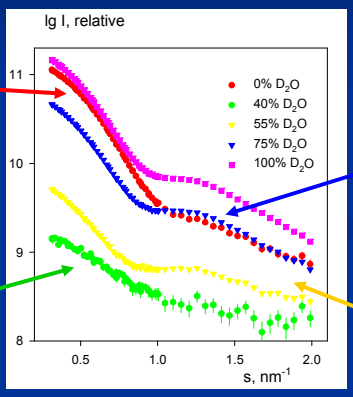
3D map of the yeast V-ATPase by electron microscopy.



In solution, EG makes an L-shaped assembly with subunit-C. This model is supported by the EM showing three copies of EG, two of them linked by C. The data further indicate a conformational change of EGC during regulatory assembly/disassembly.

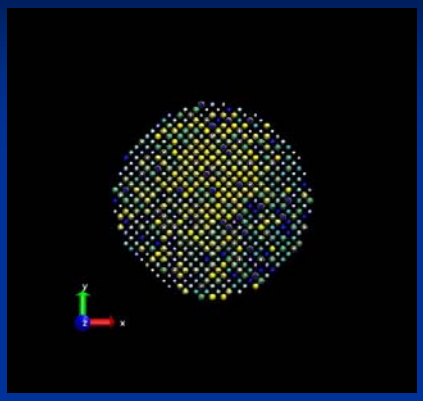
Diepholz, M. *et al.* (2008) Structure **16**, 1789-1798

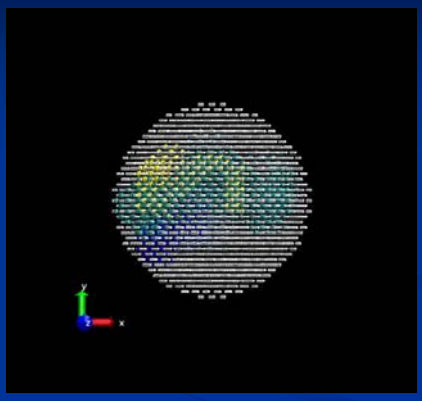
Scattering from a multiphase particle: power of neurtons



$$I_m(s) = \sum_j (\Delta\rho_j^m)^2 I_j(s) + 2\sum_{j>k} \Delta\rho_j^m \Delta\rho_i^m I_{jk}(s)$$

Ab initio multiphase modelling



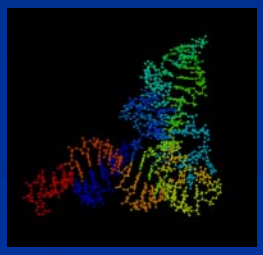


Start: random phase assignments within the search volume, no fit to the experimental data

Finish: condensed multiphase model with minimum interfacial area fitting multiple data sets

Program MONSA, Svergun, D.I. (1999) *Biophys. J.* **76**, 2879;
Petoukhov, M.V. & Svergun, D. I. (2006) *Eur. Biophys. J.* **35**, 567.

Ternary complex: Exportin-t/Ran/tRNA



Ran (structure known)

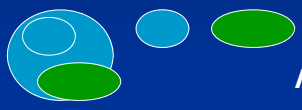
Exportin-t
(tentative homology model)

t-RNA (structure known)

Scattering data from Exportin-t/Ran/tRNA

X-ray scattering

- From Exportin-t, Ran, tRNA 3 curves



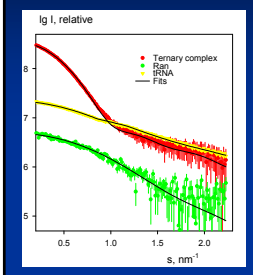
Neutron scattering

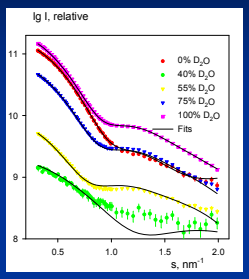
- Ternary complex with protonated Ran
  in 0, 40, 55, 75, 100% D_2O 5 curves

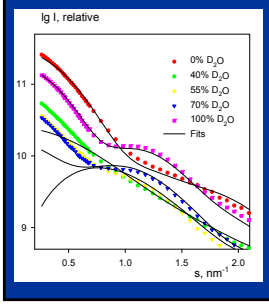
- Ternary complex with deuterated Ran
  in 0, 40, 55, 70, 100% D_2O 5 curves

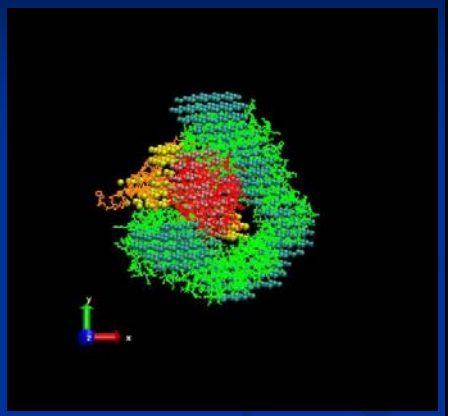
TOTAL ***13 curves***

Ternary complex: Exportin-t/Ran/tRNA







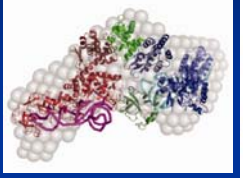


High resolution models of the components docked into the three-phase ab initio model of the complex based on X-ray and neutron scattering from selectively deuterated particles

Shapes from recent projects at EMBL-HH

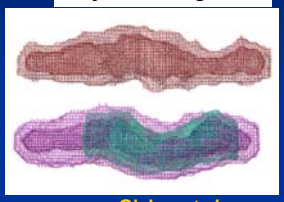
Complexes and assemblies

Aminoacyl-tRNA synthetases complex



Koehler *et al* NAR (2013)

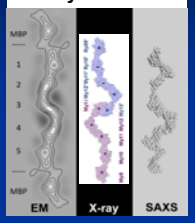
α-synuclein oligomers



Giehm *et al* PNAS USA (2011)

Domain and quaternary structure

Myomesin



Pinotsis *et al* Plos Biol (2012)

Toxin B

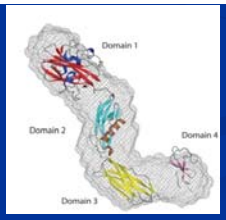
Albesa-Jové *et al* JMB (2010)

Flexible/transient systems

Complement factor H

Morgan *et al* NSMB (2011)

Colonization factor GbpA



Wong *et al* , Plos Pathog (2012)

Nanocomposites

Shtykova *et al* JPC (2012)

Ab initio programs for SAS

- Genetic algorithm DALAI_GA (Chacon et al., 1998, 2000)
- 'Give-n-take' procedure SAXS3D (Bada et al., 2000)
- Spheres modeling program GA_STRUCT (Heller et al., 2002)
- Envelope models: SASHA[(1)] (Svergun et al., 1996)
- Dummy atoms: DAMMIN[(1,4)] & MONSA[(1,2)] (Svergun, 1999)
- Dummy residues: GASBOR[(1,3)] (Petoukhov et al., 2001)

[(1)] Able to impose symmetry and anisometry constrains

[(2)] Multiphase inhomogeneous models

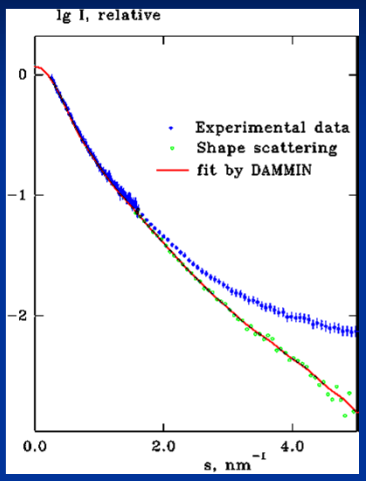
[(3)] Accounts for higher resolution data

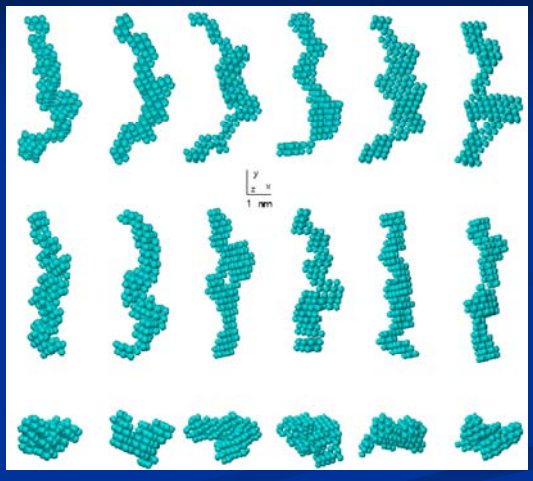
[(4)] DAMMIF is 30 times faster (D.Franke & D.Svergun, 2009)

Some words of caution

Or Always remember about ambiguity!

Shape determination of 5S RNA: a variety of DAMMIN models yielding identical fits

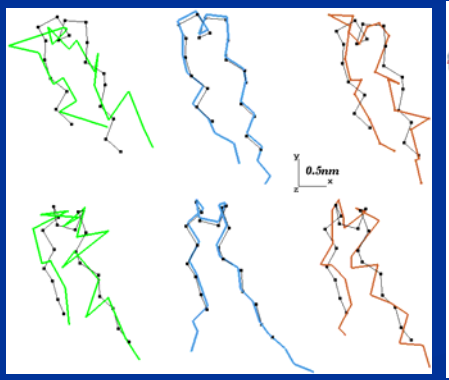


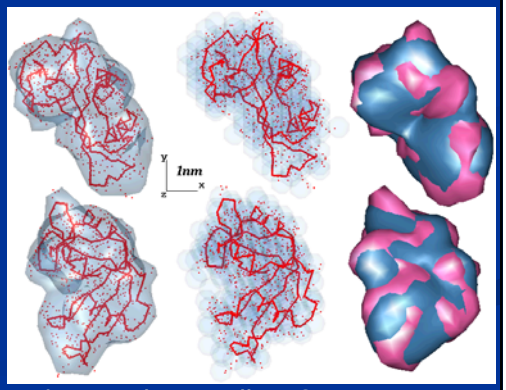


Funari, S., Rapp, G., Perbandt, M., Dierks, K., Vallazza, M., Betzel, Ch., Erdmann, V. A. & Svergun, D. I. (2000) *J. Biol. Chem.* **275**, 31283-31288.

Program SUPCOMB – a tool to align and conquer

- Aligns heterogeneous high- and low-resolution models and provides a dissimilarity measure (NSD)
- For shape determination, allows one to find common features in a series of independent reconstructions





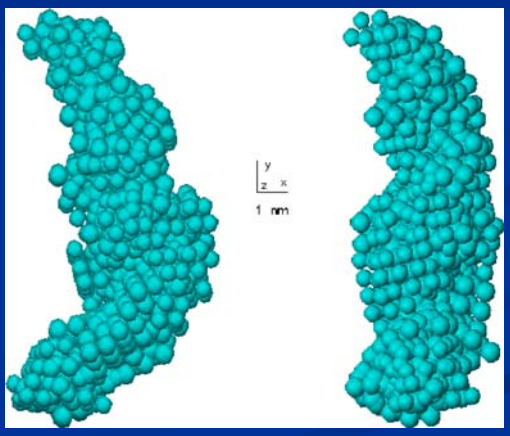
Kozin, M.B. & Svergun, D.I. (2001) *J. Appl. Crystallogr.* **34,** 33-41

Automated analysis of multiple models

1. **Find a set of solutions starting from random initial models and superimpose all pairs of models with SUPCOMB.**
2. **Find the most probable model (which is on average least different from all the others) and align all the other models with this reference one.**
3. **Remap all models onto a common grid to obtain the solution spread region and compute the spatial occupancy density of the grid points.**
4. **Reduce the spread region by rejecting knots with lowest occupancy to find the most populated volume**
5. **These steps are automatically done by a package called DAMAVER if you just put all multiple solutions in one directory**

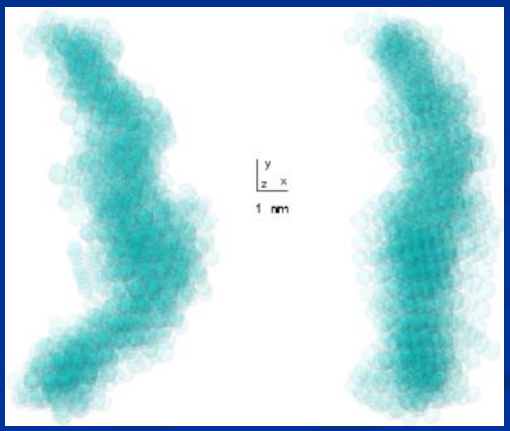
Program DAMAVER, Volkov & Svergun (2003) *J. Appl. Crystallogr*. 36, 860

5S RNA: ten shapes superimposed



Solution spread region

5S RNA: ten shapes superimposed



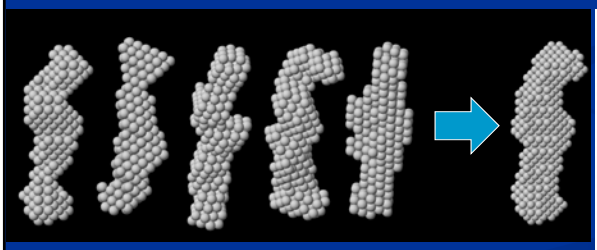
Most populated volume

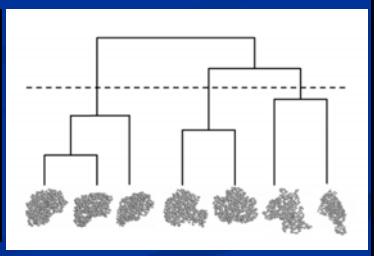
Damaver and Damclust

Damaver superposes multiple *ab initio* models, computes deviations between them using a normalized spatial discrepancy (NSD), finds the most probable and an averaged model.

Outliers, if any, are discarded

Damclust superposes multiple *ab initio* or rigid body models, computes deviations between them using NSD or RMSD, and attributes the models to distinct clusters



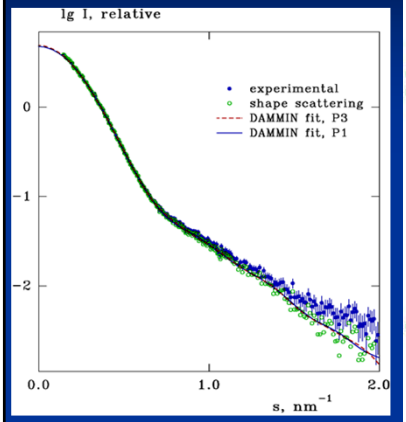


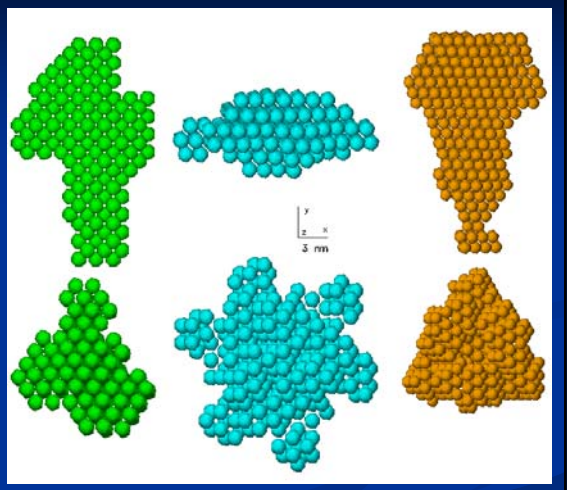
Result:

Most probable model
Single averaged model

Representatives of clusters
Distances between clusters

Use of symmetry: powerful but needs extra caution



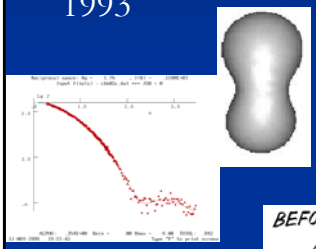


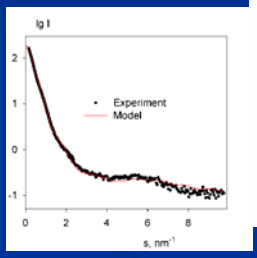
P1 P3 P3, prolate

Svergun, D.I., Konrad, S., Huss, M., Koch, M.H.J., Wieczorek, H., Altendorf, K.-H., Volkov, V.V. & Grueber, G. (1998) Biochemistry 37, 17659-17663.

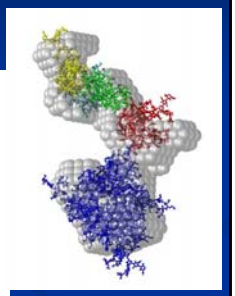
Progress in *ab initio* methods

1993

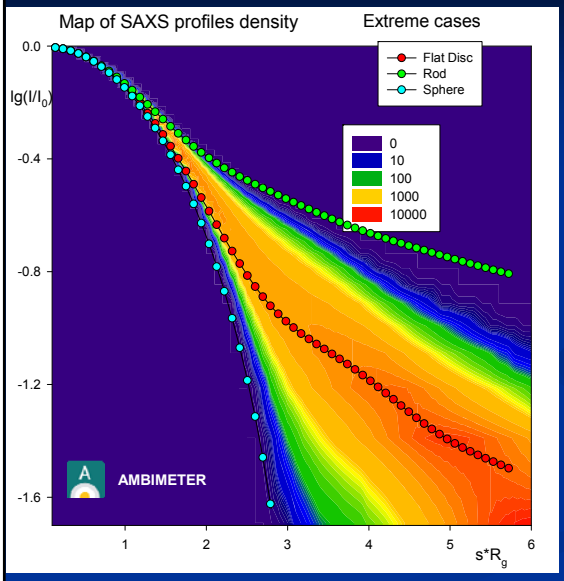




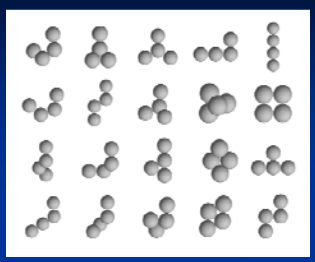
2014



Quantifying Inherent Ambiguity of SAS data

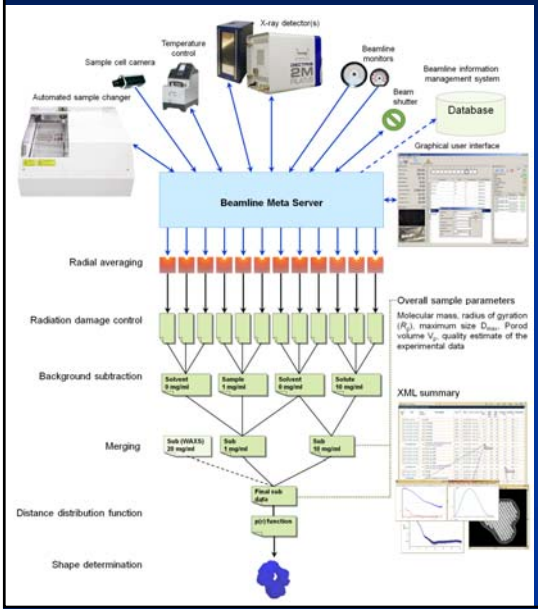


Petoukhov & Svergun, submitted



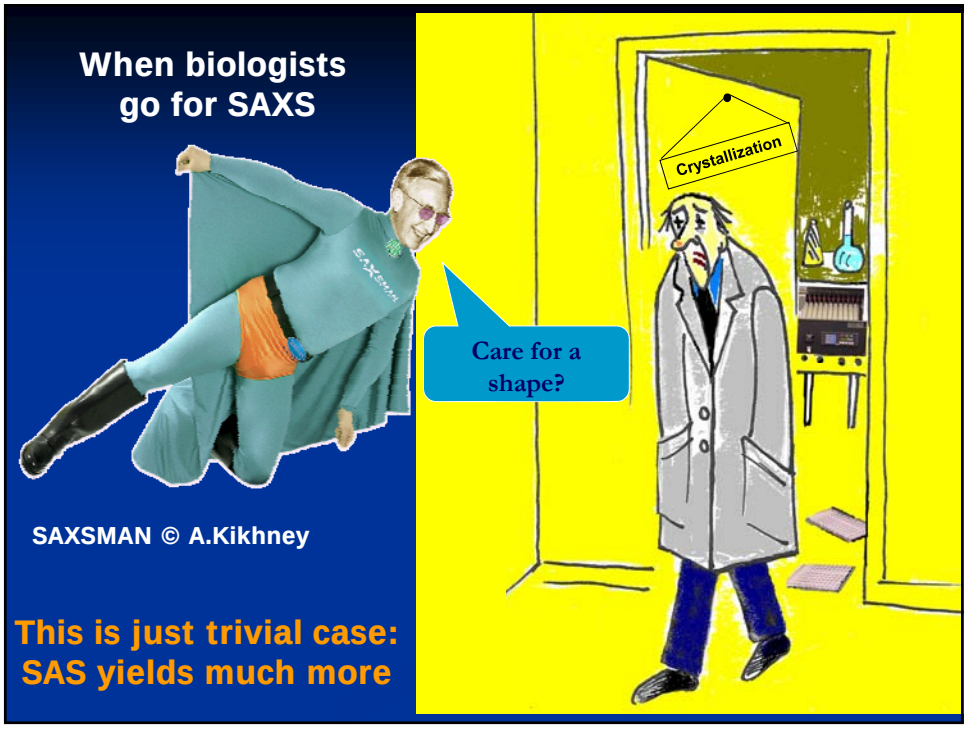
Exhaustive calculations of scattering by all (14112) possible skeletons represented by up to seven densely packed interconnected beads on a grid provided a map of ambiguity, i.e. the propensity that a given scattering pattern yields an ambiguous shape reconstruction

Automation of experiment and analysis



A new generation of the experiment control system allows for interactive, unattended and remote data collection

The data is handed over to an analysis pipeline for large scale on-line data processing and analysis without user intervention

The results including the computed parameters and 3D models are stored in convenient human-and machine-readable formats **(A.Kikhney, D.Franke)**

BioStruct-X