



**THE UNIVERSITY *of* TEXAS**

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SCHOOL OF HEALTH INFORMATION  
SCIENCES AT HOUSTON

# Small-Angle Scattering from Biomolecular Solutions

For students of HI 6001-125

“Computational Structural Biology”

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EMBL, Hamburg Outstation

<http://biomachina.org/courses/structures/05.html>

# Small-angle scattering experiment

Monochromatic beam



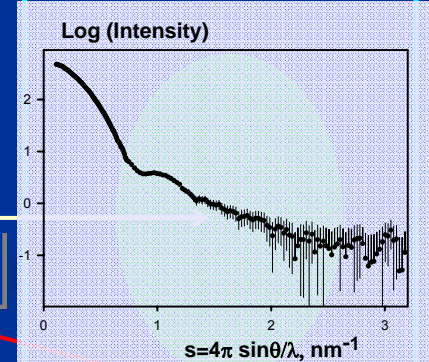
Wave vector  $k$ ,  $k=2\pi/\lambda$



Sample

$2\theta$

Detector



$k_1$

Radiation sources:

- X-ray generator ( $\lambda = 0.1 - 0.2 \text{ nm}$ )
- Synchrotron ( $\lambda = 0.03 - 0.35 \text{ nm}$ )
- Thermal neutrons ( $\lambda = 0.2 - 1 \text{ nm}$ )

Scattering vector  $s=k_1-k$ ,  
 $s=4\pi \sin\theta/\lambda$

# 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.

# X-rays

# versus

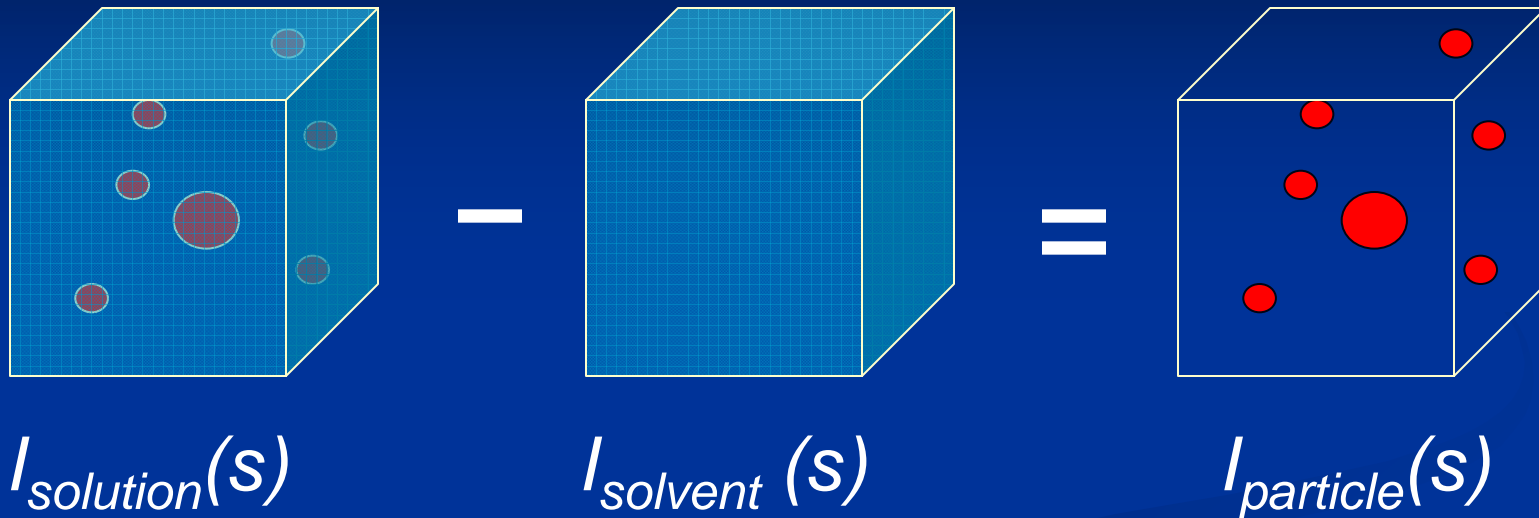


# Neutrons

- **X-rays:** scattering factor increases with atomic number, no difference between H and D
- **Neutrons:** scattering factor is irregular, may be negative, huge difference between H and D

Element	H	D	C	N	O	P	S	Au
At. Weight	1	2	12	14	16	30	32	197
N electrons	1	1	6	7	8	15	16	79
$b_X, 10^{-12}$ cm	0.282	0.282	1.69	1.97	2.16	3.23	4.51	22.3
$b_N, 10^{-12}$ cm	-0.374	0.667	0.665	0.940	0.580	0.510	0.280	0.760

# Solvent scattering and contrast

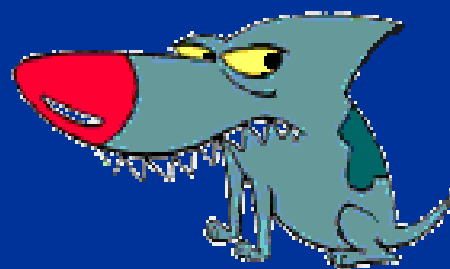


- ◆ To obtain scattering from the particles, solvent scattering must be subtracted
- ◆ **Contrast**  $\Delta\rho = \langle\rho(r) - \rho_s\rangle$ , where  $\rho_s$  is the scattering density of the solvent (is usually very small for biological samples)

# Contrast variation



- Changing solvent density separates information about shape and internal structure



- Selective labelling permits to visualize specific structural fragments

Stuhrmann, H.B. & Kirste, R.G. (1965) *Z. Phys. Chem.* **46**, 247

# X-rays



# Neutrons

*Addition of sucrose or salts*

RNA, 550 e/nm<sup>3</sup>

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60% sucrose, 430 e/nm<sup>3</sup>

Protein, 410 e/nm<sup>3</sup>

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H<sub>2</sub>O, 344 e/nm<sup>3</sup>

*Isotopic H/D substitution*

D-Protein, 130% D<sub>2</sub>O

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D-RNA, 120% D<sub>2</sub>O

D<sub>2</sub>O, 6.38×10<sup>10</sup> cm<sup>-2</sup>

H-RNA, 70% D<sub>2</sub>O

---

H-Protein, 40% D<sub>2</sub>O

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H<sub>2</sub>O, -0.59×10<sup>10</sup> cm<sup>-2</sup>

# Major applications of solution scattering



*Ab initio* low resolution structure analysis

Addition of missing fragments to high resolution models



Rigid body refinement of complexes

Quantitative characterization of mixtures



Contrast variation on multicomponent particles

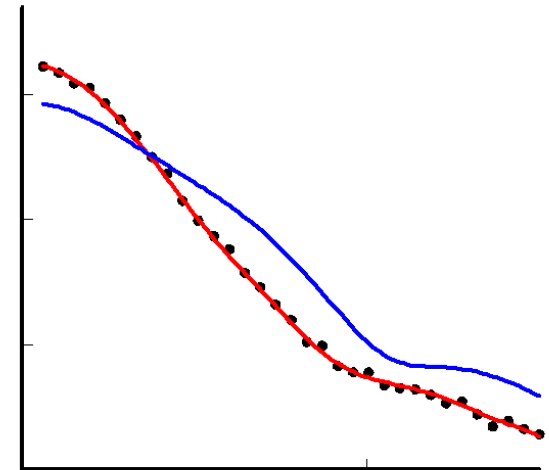


# Major problem of scattering data analysis

3D search model  
M parameters

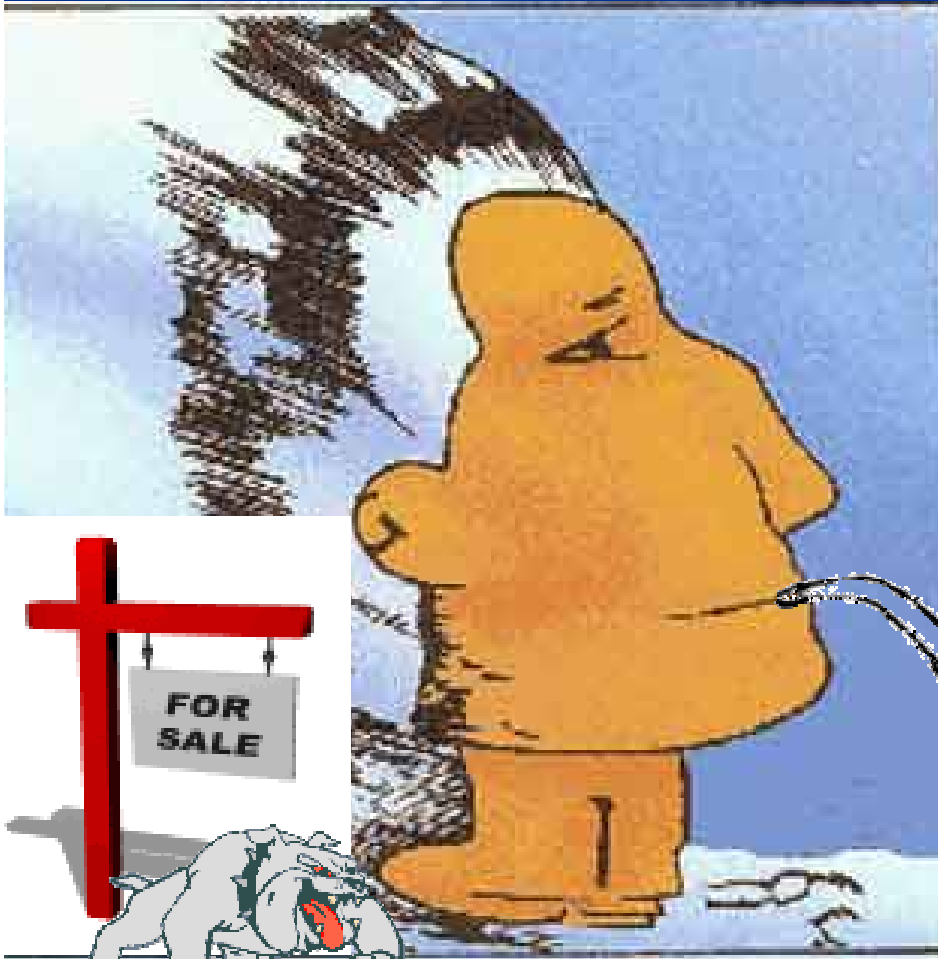
Trial-and-error

Non-linear  
search



Additional information

**ALWAYS** required to  
resolve or reduce  
ambiguity of  
interpretation



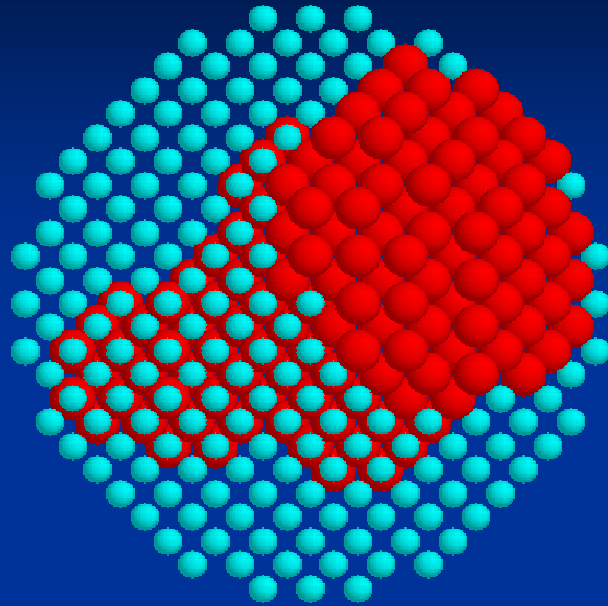
# *Ab initio* methods



Envelope function

Stuhrmann, H. B. (1970) *Z. Physik. Chem. N.F.* **72**, 177

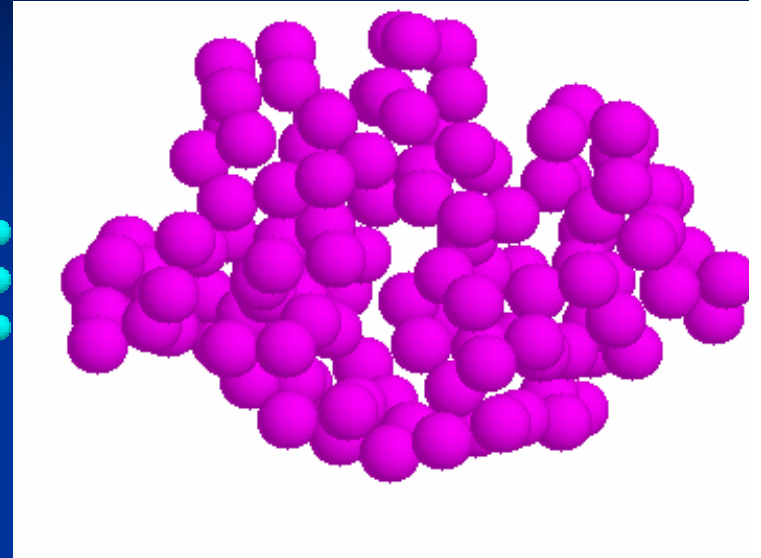
Svergun, D.I. *et al.* (1996) *Acta Crystallogr.* **A52**, 419



Bead models

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

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

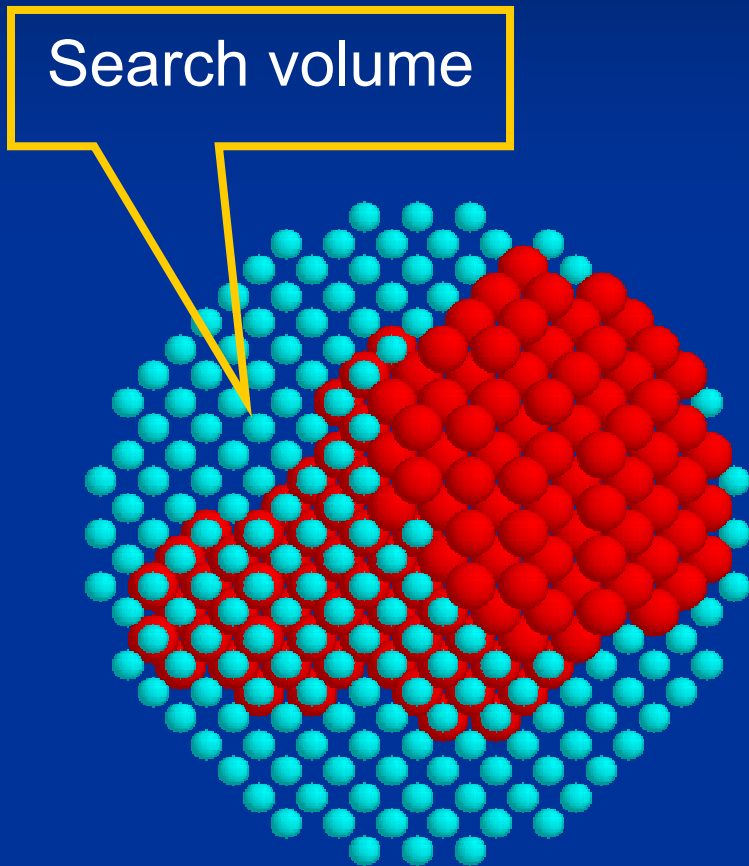


Dummy residues model

Svergun, D.I., Petoukhov, M.V. & Koch, M.H.J. (2001) *Biophys. J.* **80**, 2946-2953.

All the methods minimize  $\text{Discrepancy}[\text{Data}] + \text{Penalty}[\text{Additional info}]$

# Bead (dummy atoms) models



- ◆ The particle is represented as an ensemble of  $\approx 10^3 - 10^4$  small densely packed volume elements (beads) in the search volume (e.g. a sphere with diameter  $D_{\max}$ )
- ◆ The structure is described by phase assignments of each of these positions (e.g. for shape determination 1 = particle, 0 = solvent)
- ◆ A Monte-Carlo type search is employed to build a model fitting the scattering data

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

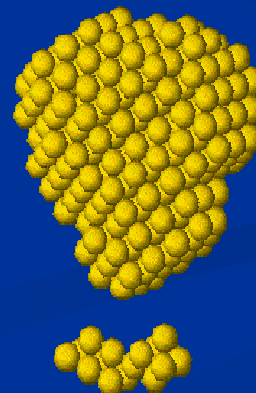
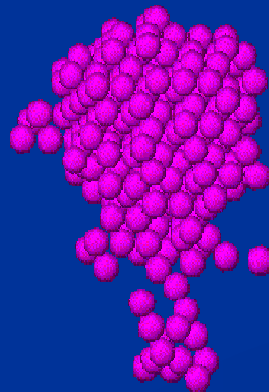
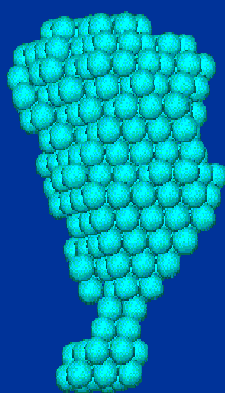
Svergun, D.I. (1999) *Biophys. J.* **76**, 2879-2886

# *Ab initio* program DAMMIN

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

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

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



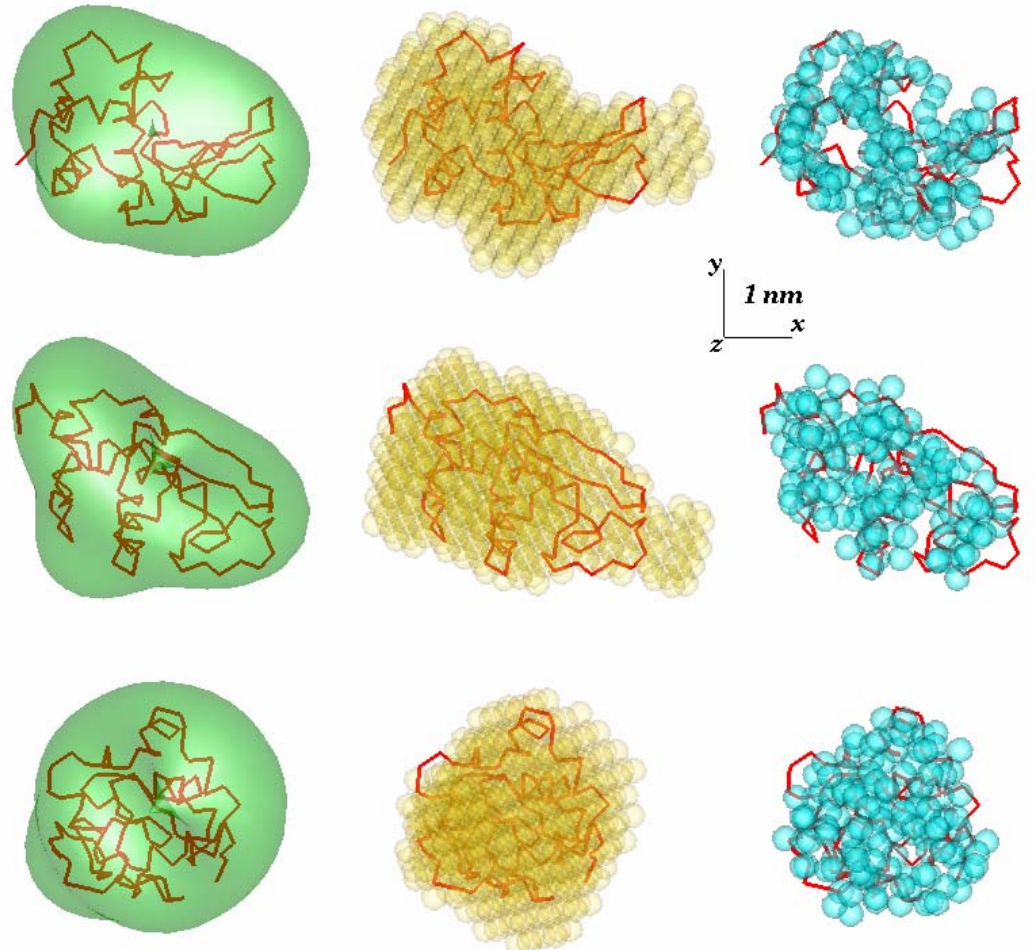
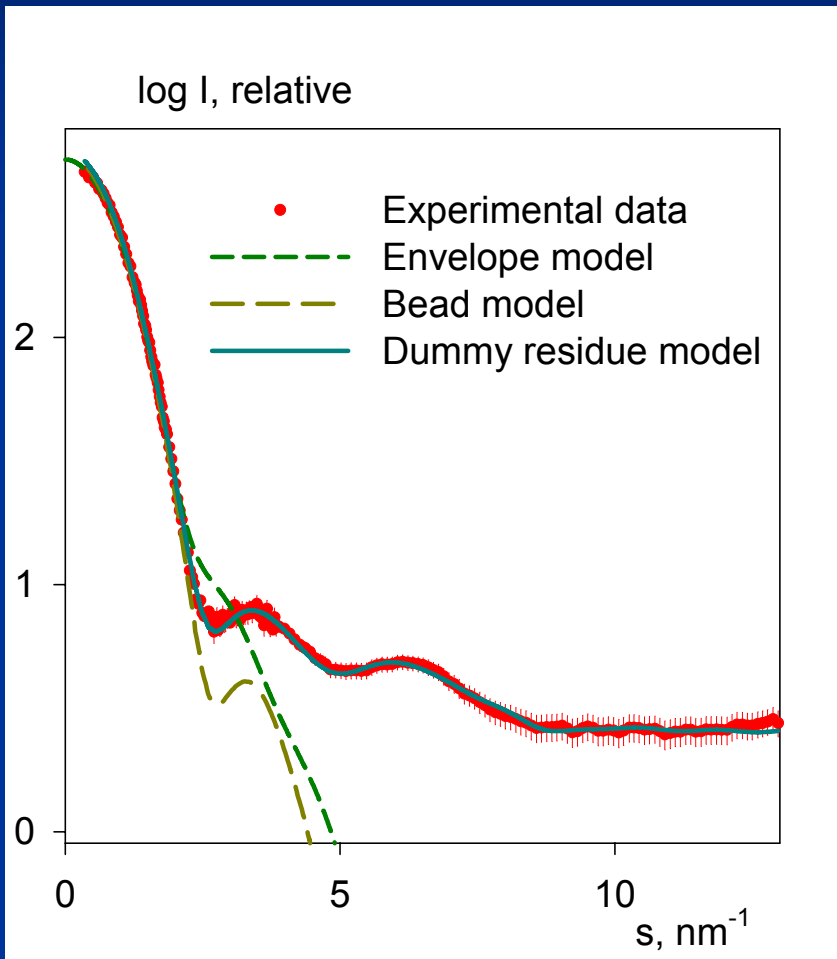
*compact*

*loose*

*disconnected*

# Benchmarking *ab initio* methods

*Envelope* *Bead model* *Dummy residues*



*Comparison with the crystal structure of lysozyme*

*SASHA*  
1996

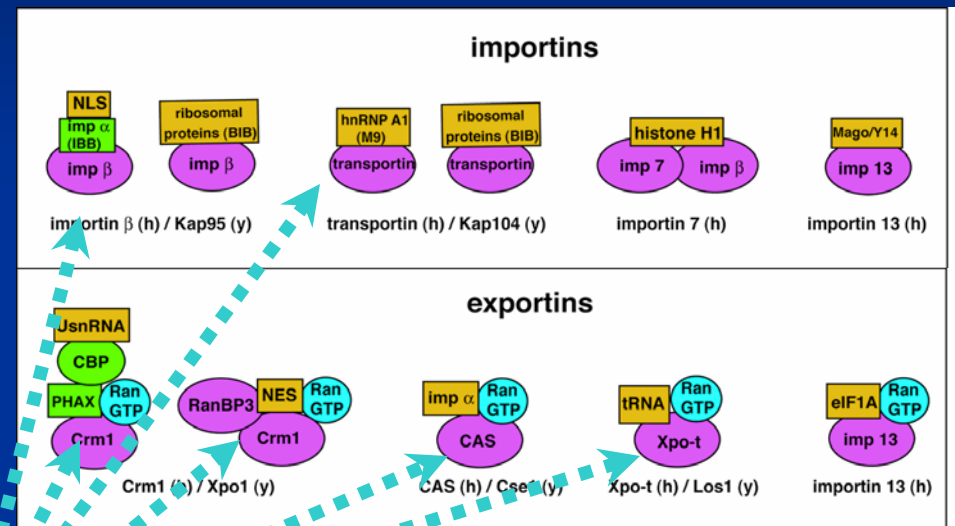
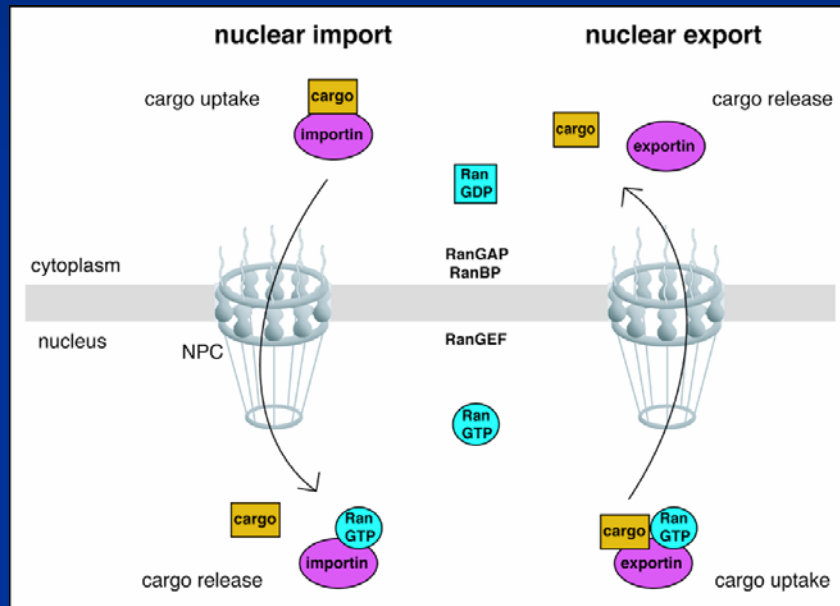
*DAMMIN*  
1999

*GASBOR*  
2001

# Study of nuclear exportins and importins

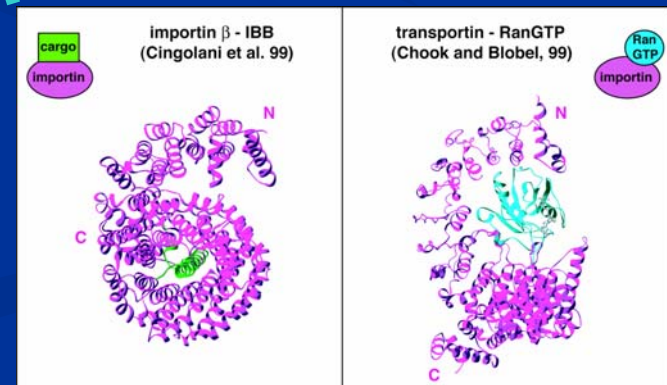
Importin/exportin-mediated nuclear transport

Examples of cargo recognition



Targets for small-angle scattering study

Two available crystal structures of bound importins built by stacks of HEAT repeats

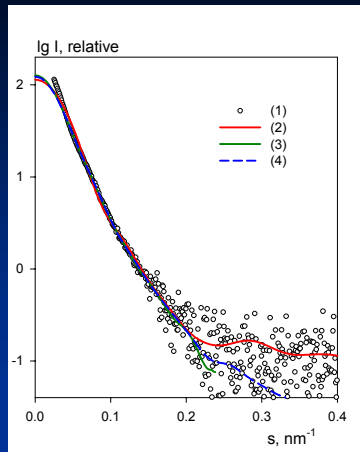


Snail-like importin  $\beta$

Z-like transportin

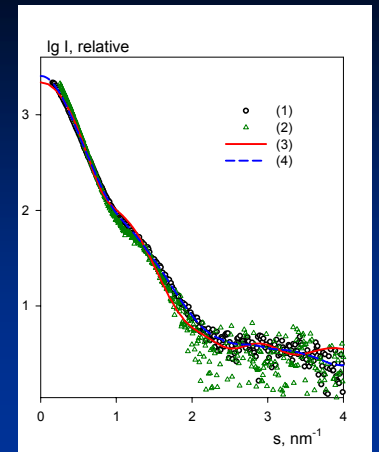
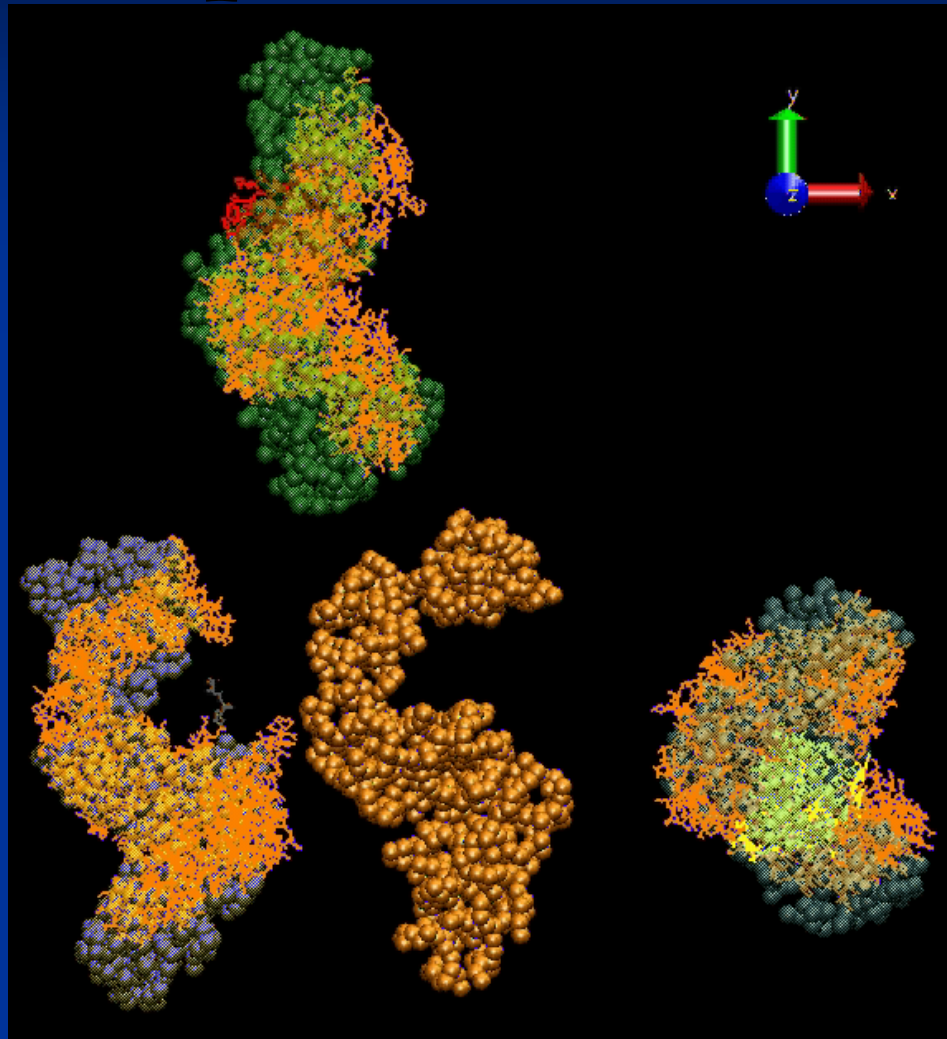
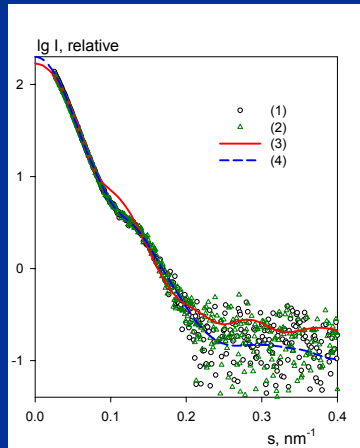


# Importins *ab initio*



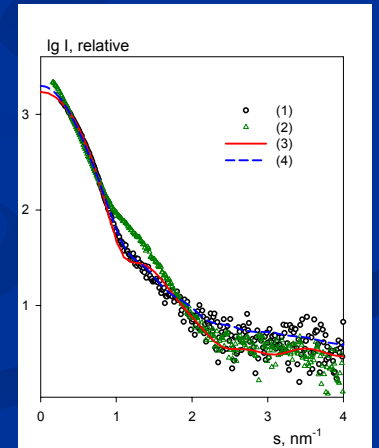
Transportin-  
RanGTP: Z-like

Free transportin:  
Z-like

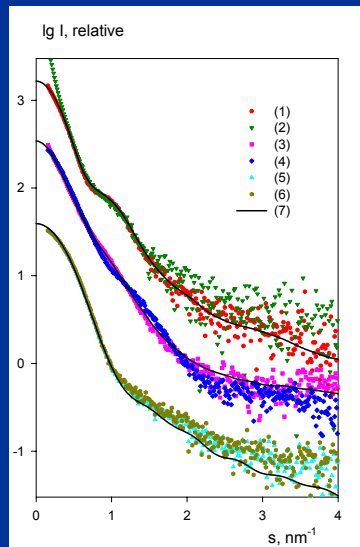
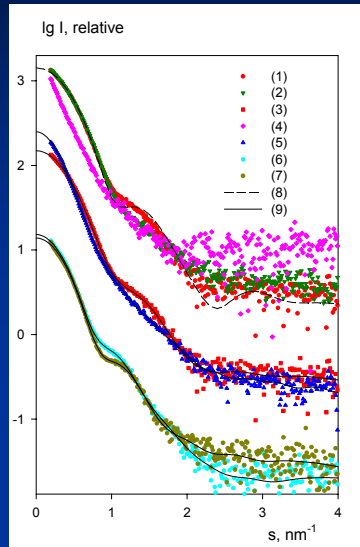


Free importin  $\beta$ :  
Z-like

Bound importin  $\beta$ :  
snail-like



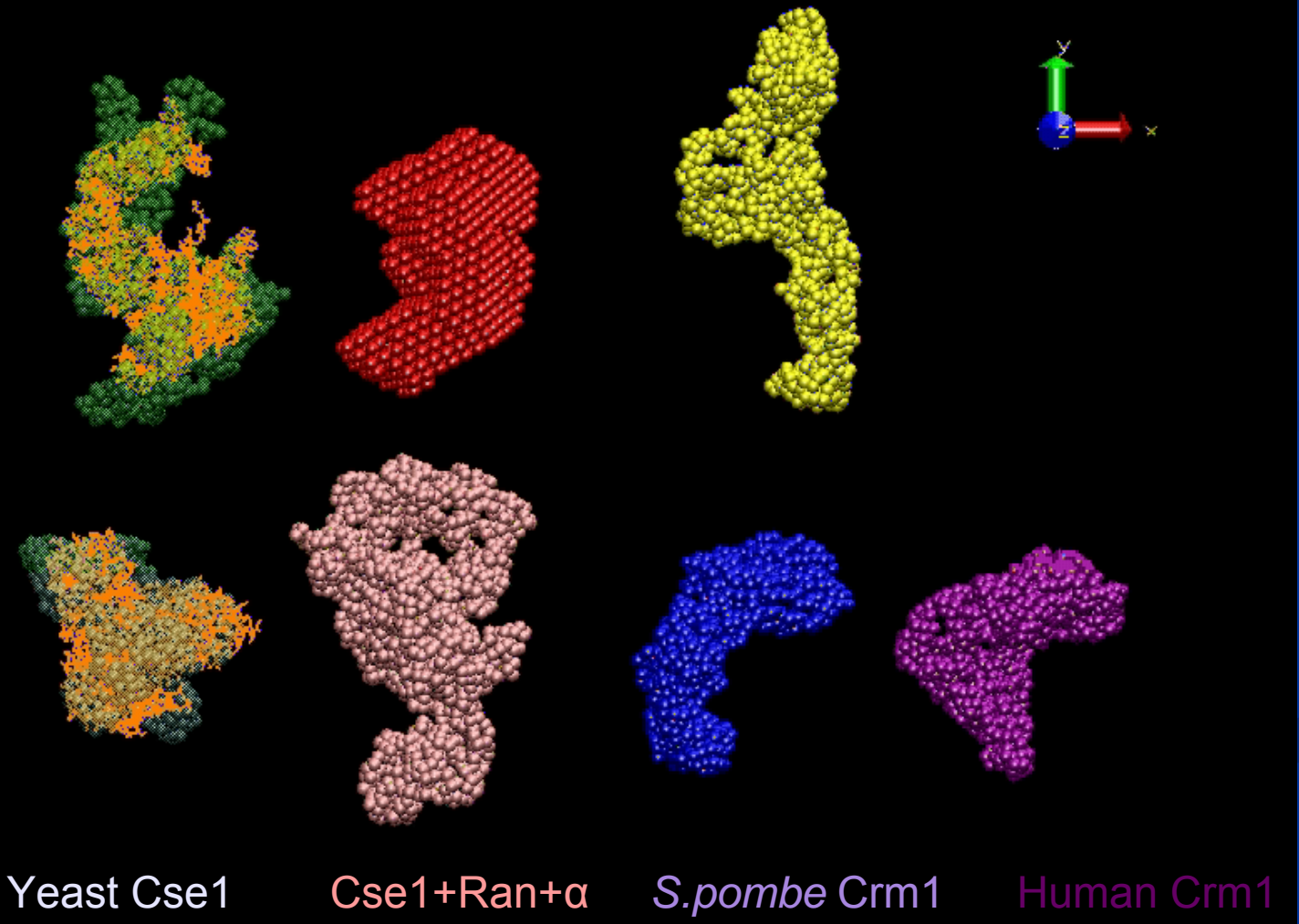
# Exportins *ab initio*



*S.pombe* Xpot

Xpot+Ran+tRNA

Yeast Los1



Yeast Cse1

Cse1+Ran+α

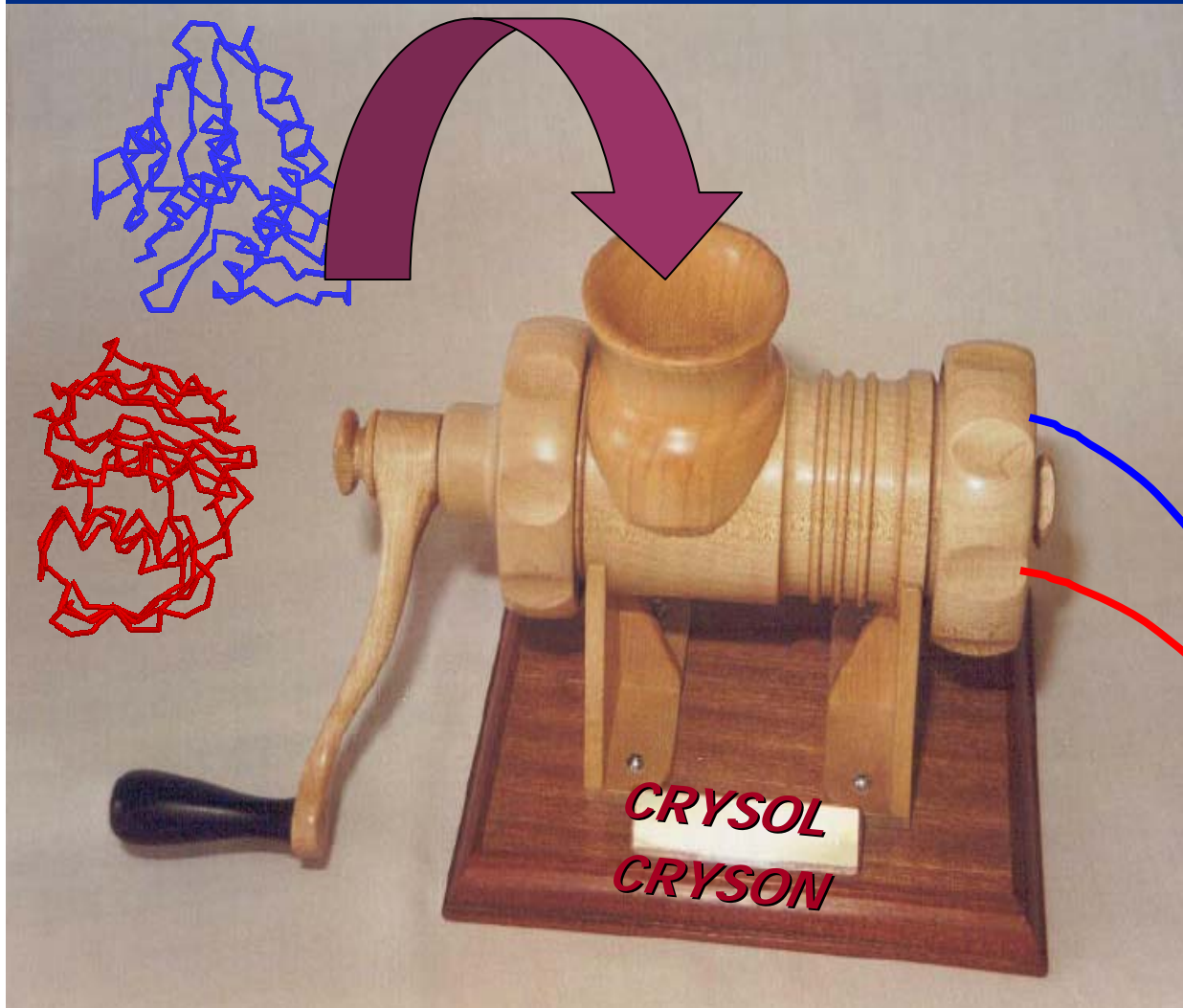
*S.pombe* Crm1

Human Crm1



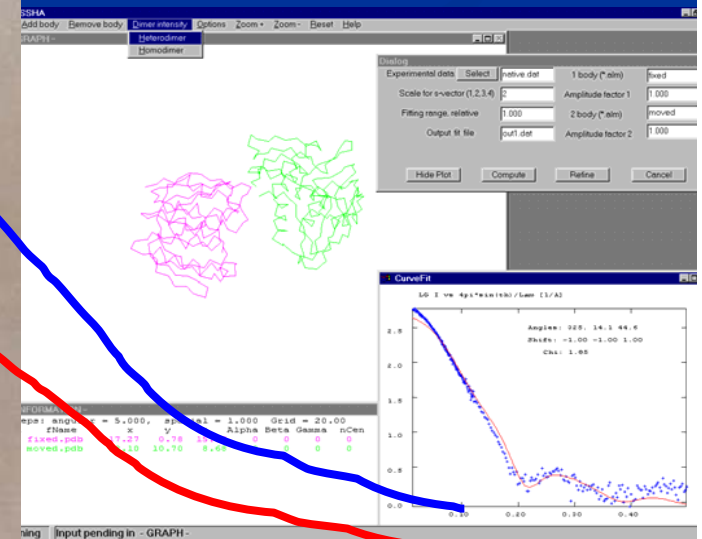
# Rigid body modelling

Scattering amplitudes of the subunits are pre-computed and positional parameters are refined to fit the scattering from the complex



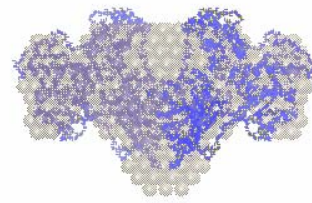
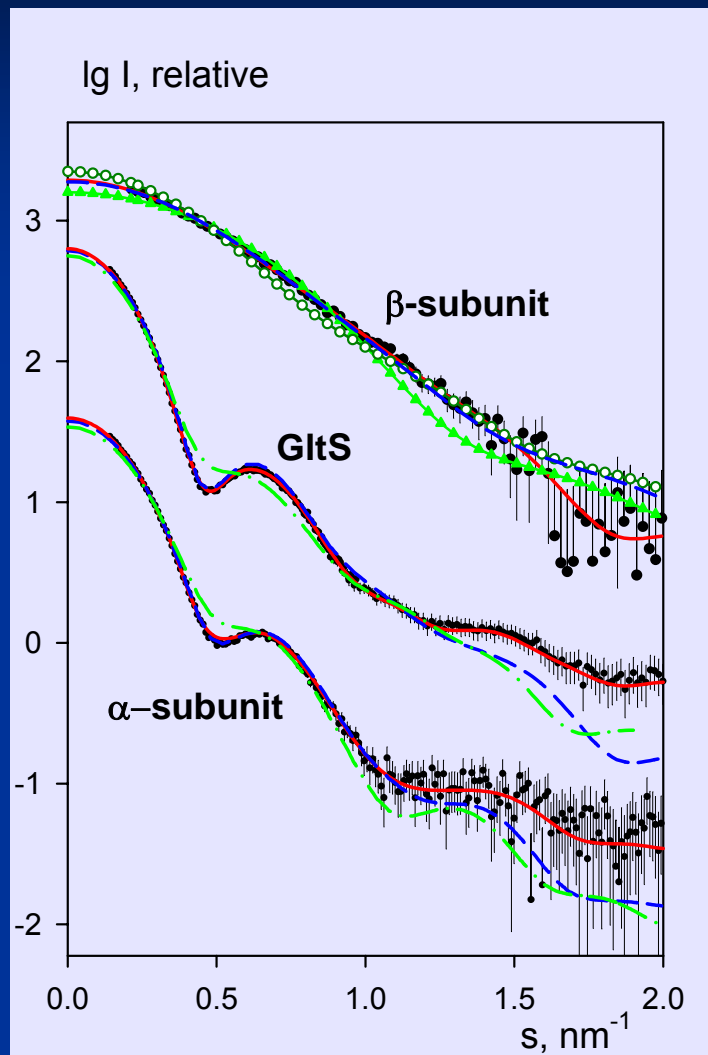
Kozin & Svergun (2000). *J. Appl. Cryst.* **33**, 775-777

Konarev, Petoukhov & Svergun (2001). *J. Appl. Cryst.* **34**, 527-532

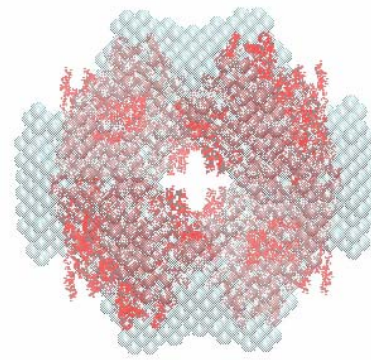


- ◆ **MASSHA** (Windows PC)
- ◆ **ASSA** (SUN/SGI/DEC)

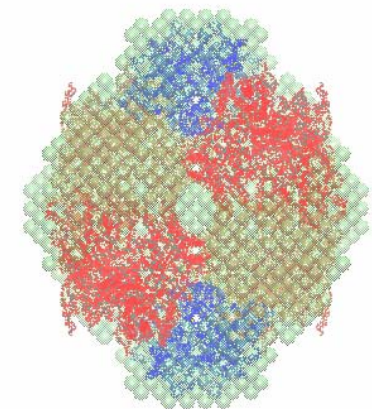
# Quaternary structure of glutamate synthase



$\beta$ -dimer



$\alpha$ -tetramer



$\alpha\beta$ -tetramer

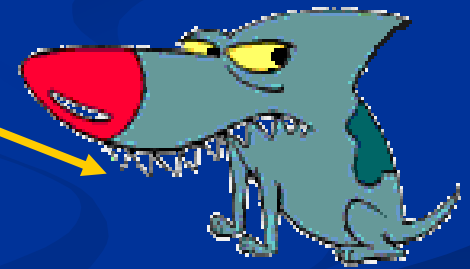
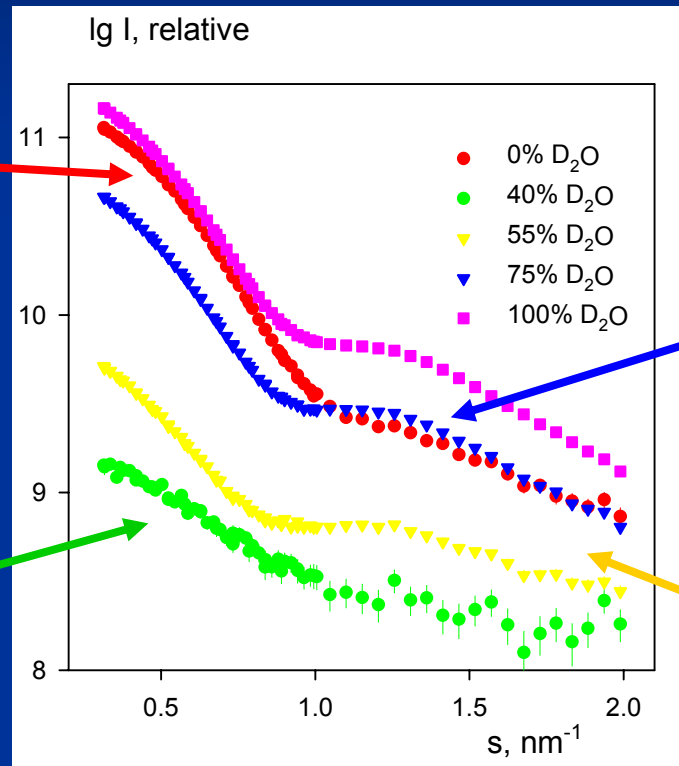
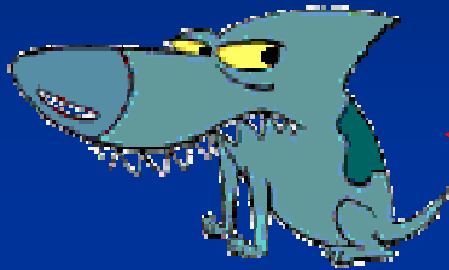
Catalytically active  $\alpha\beta$  holoenzyme of the iron-sulfur flavoprotein GltS, contains

$\alpha$  subunit (162 kDa)

$\beta$  subunit (52 kDa)

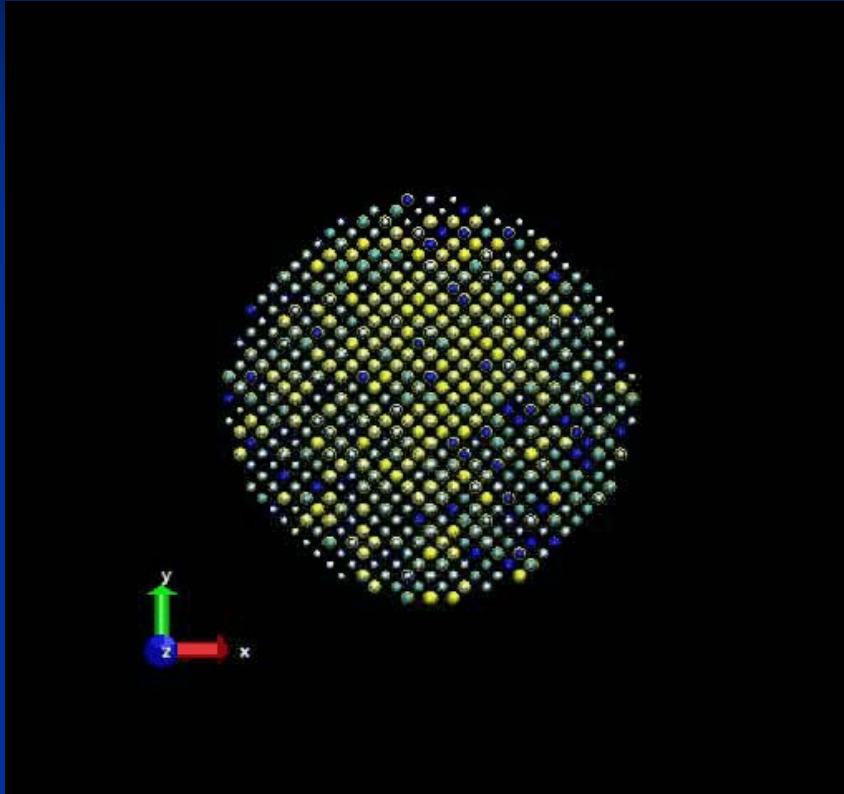
M.V. Petoukhov, D.I. Svergun, P.V. Konarev, S. Ravasio, R.H.H. van den Heuvel, B. Curti & M.A. Vanoni (2003). *J. Biol. Chem.*, **278**, 29933

# Scattering from a multiphase particle

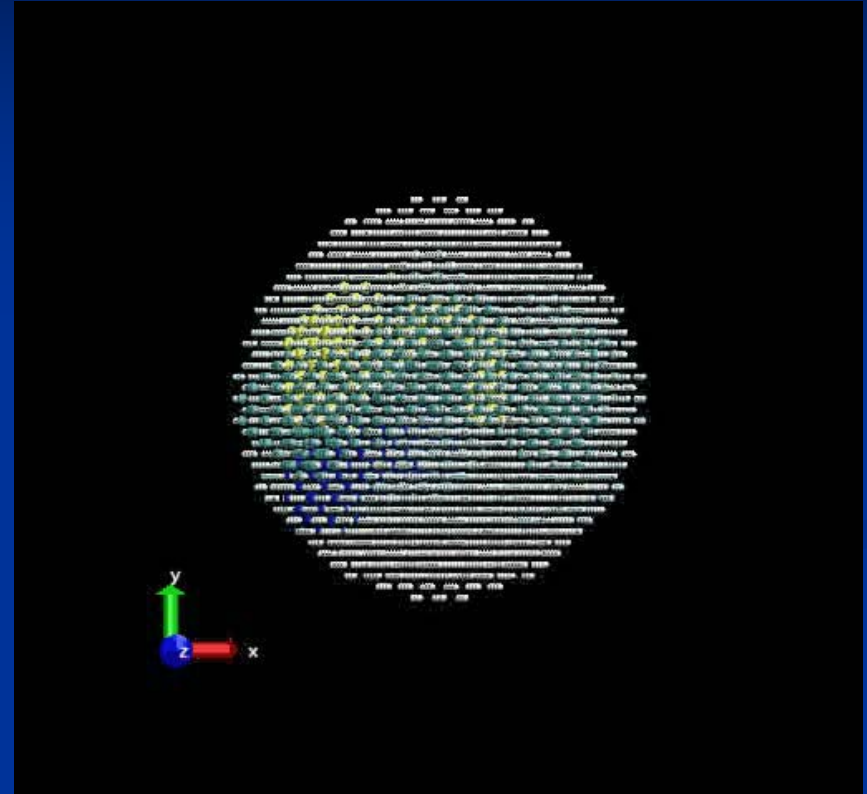


$$I_m(s) = \sum_j (\Delta\rho_j^m)^2 I_j(s) + 2 \sum_{j>k} \Delta\rho_j^m \Delta\rho_k^m I_{jk}(s)$$

# *Ab initio* multiphase modelling



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

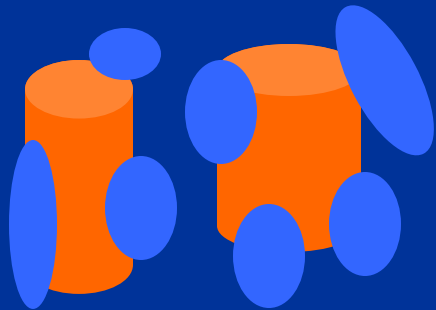


Finish: condensed multiphase model with minimum interfacial area fitting multiple data sets

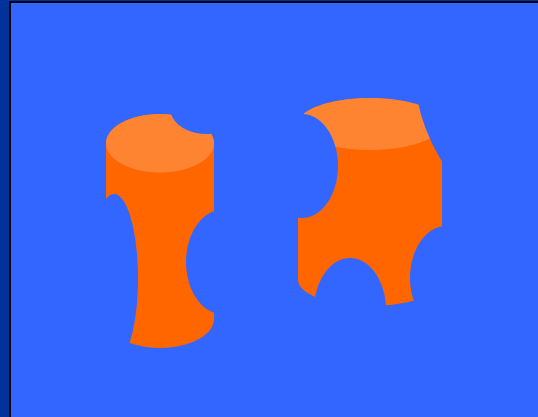
Program MONSA, Svergun, D.I. (1999) *Biophys. J.* **76**, 2879

# Contrast variation on hybrid ribosomes

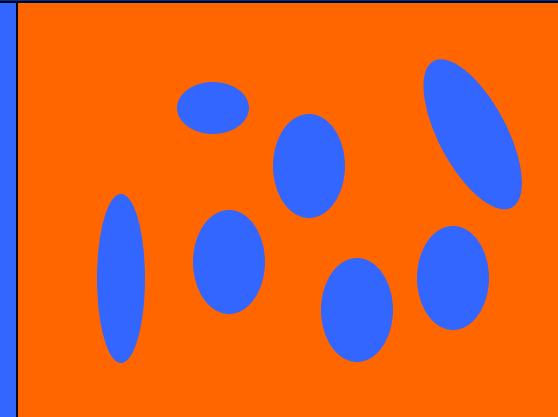
0% D<sub>2</sub>O



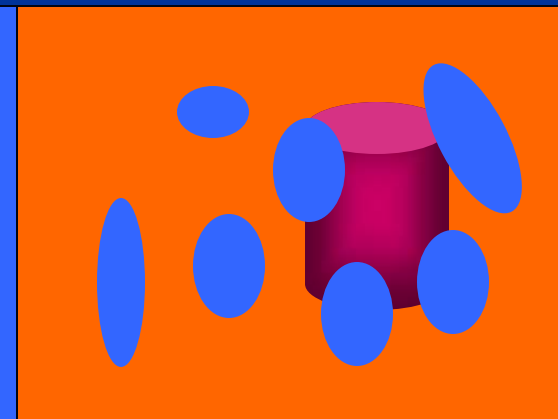
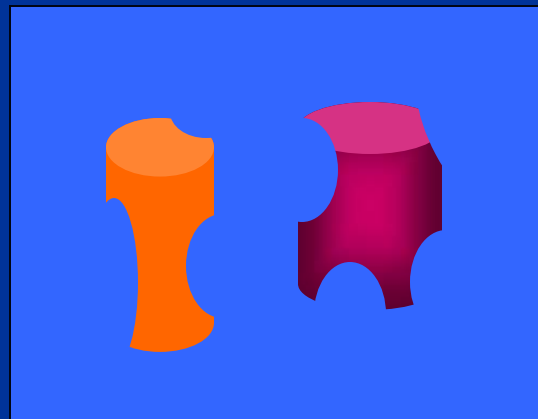
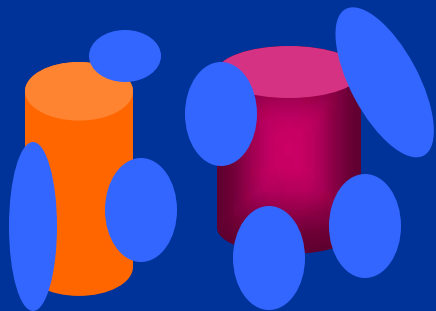
40% D<sub>2</sub>O



70% D<sub>2</sub>O



Protonated 70S ribosome, HH30+HH50



Hybrid 70S with 23S RNA deuterated, HH30+HD50

# Scattering data from hybrid ribosomes

## *Contrast variation by solvent exchange*

- HH30+HH50 DD30+HH50 DH30+HH50  
in 0, 35, 50, 75, 100% D<sub>2</sub>O 15 curves
- HH30+DD50 in 0, 35, 50, 75% D<sub>2</sub>O 4 curves
- DH30+DD50 and HH30+DH50  
in 0, 40, 60, 100% D<sub>2</sub>O 8 curves
- HH30 and HH50 in 0, 100% D<sub>2</sub>O 4 curves
- DD30 and DD50 in 0% D<sub>2</sub>O 2 curves

## *Spin-dependent contrast variation data*

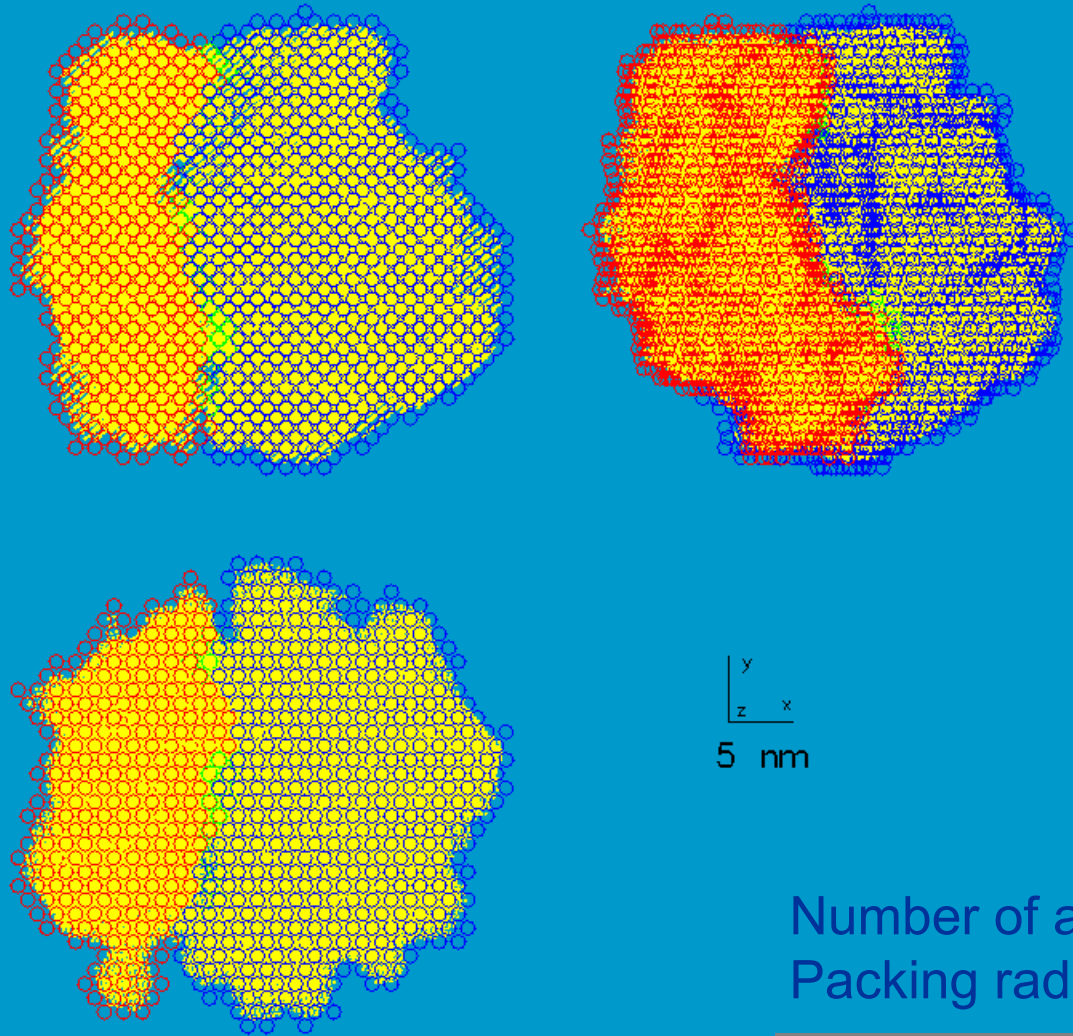
- HH30+DD50, DD30+HH50, DH30+DH50  
Polarization = 0 and 1 6 curves
- *X-ray scattering curves* from 70S, 30S and 50S 3 curves

***TOTAL***

***42 curves***



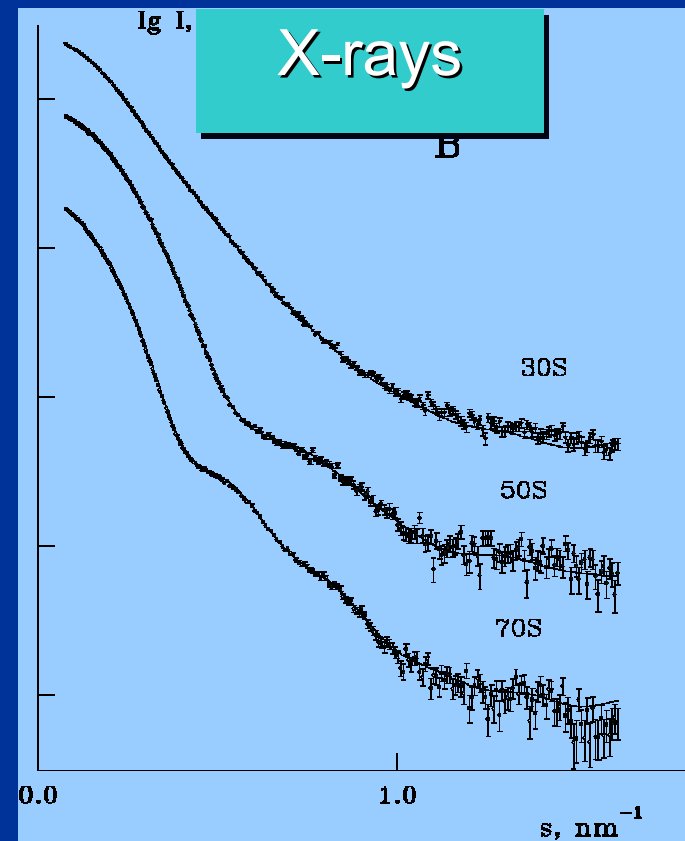
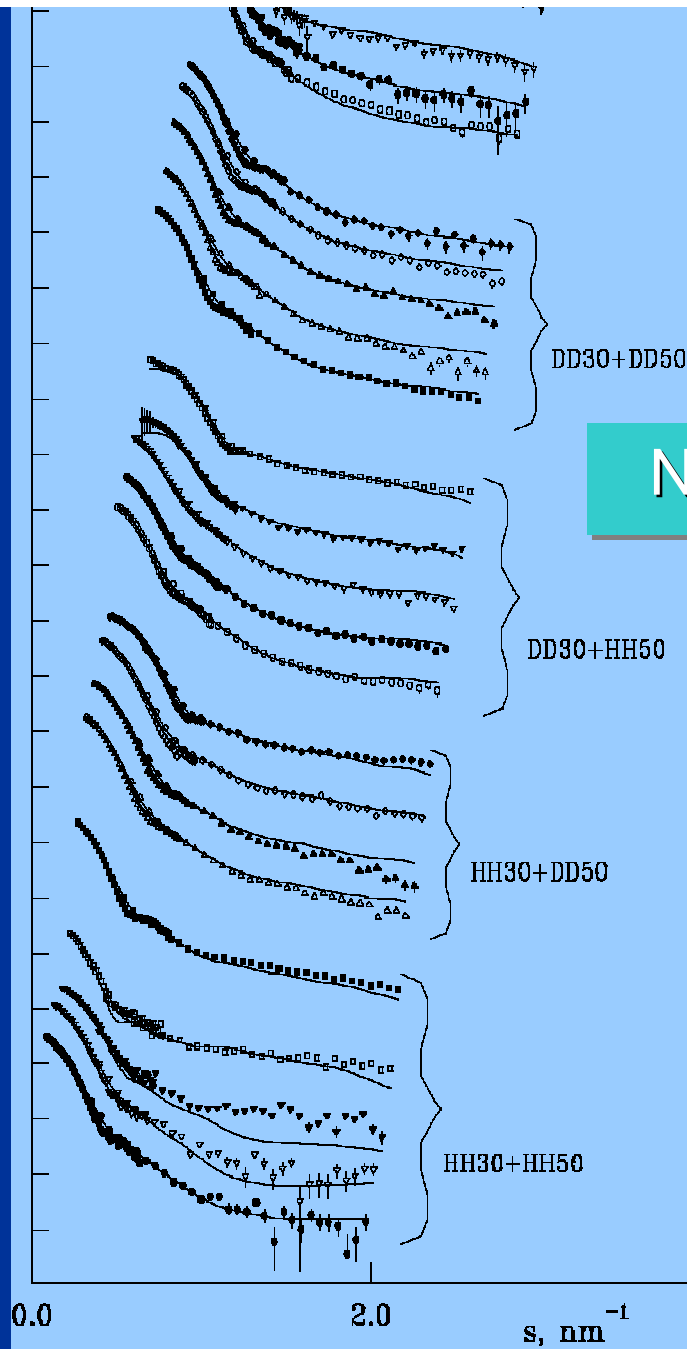
# Search volume for the 70S ribosome



- Yellow pixels: cryo-EM model of Frank *et al.* (1995)
- Red and blue circles: dummy atoms belonging to the 30S and 50S subunits, respectively

Number of atoms  $M=7860$   
Packing radius  $r_0=0.5 \text{ nm}$

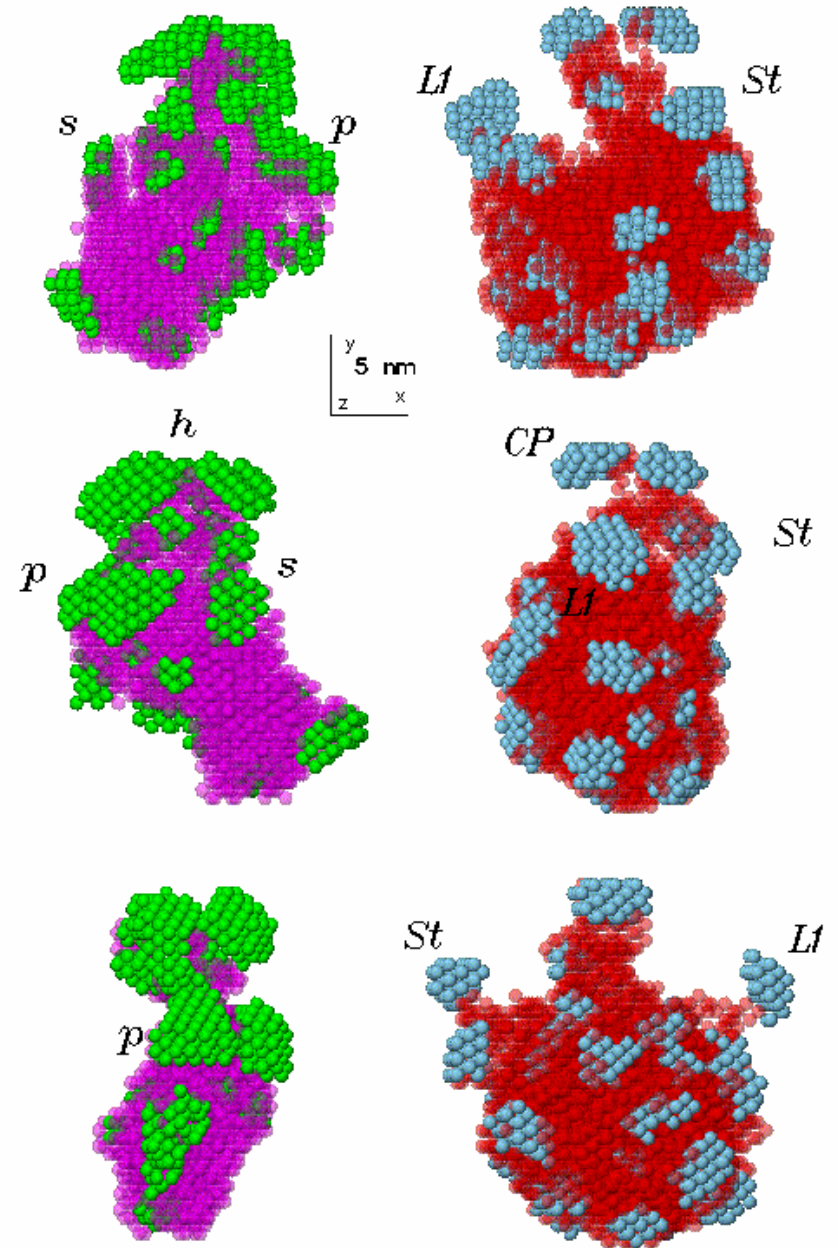
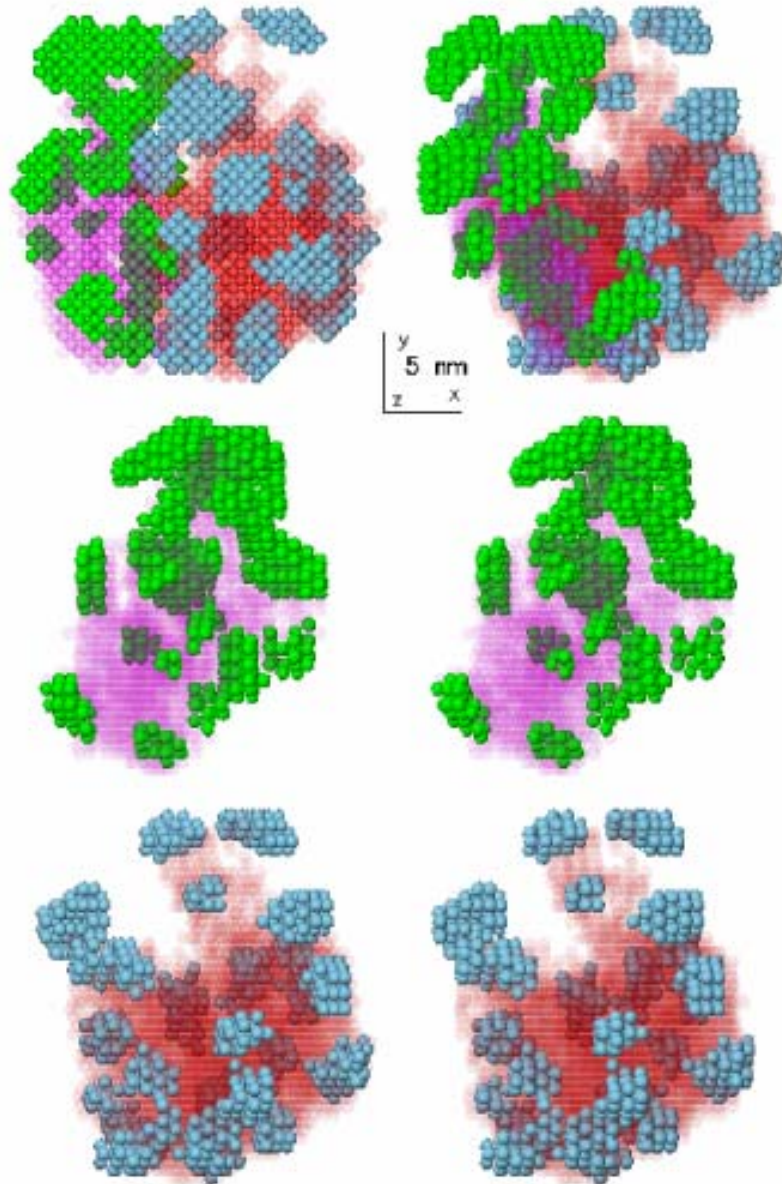
# Ribosomal data fitted



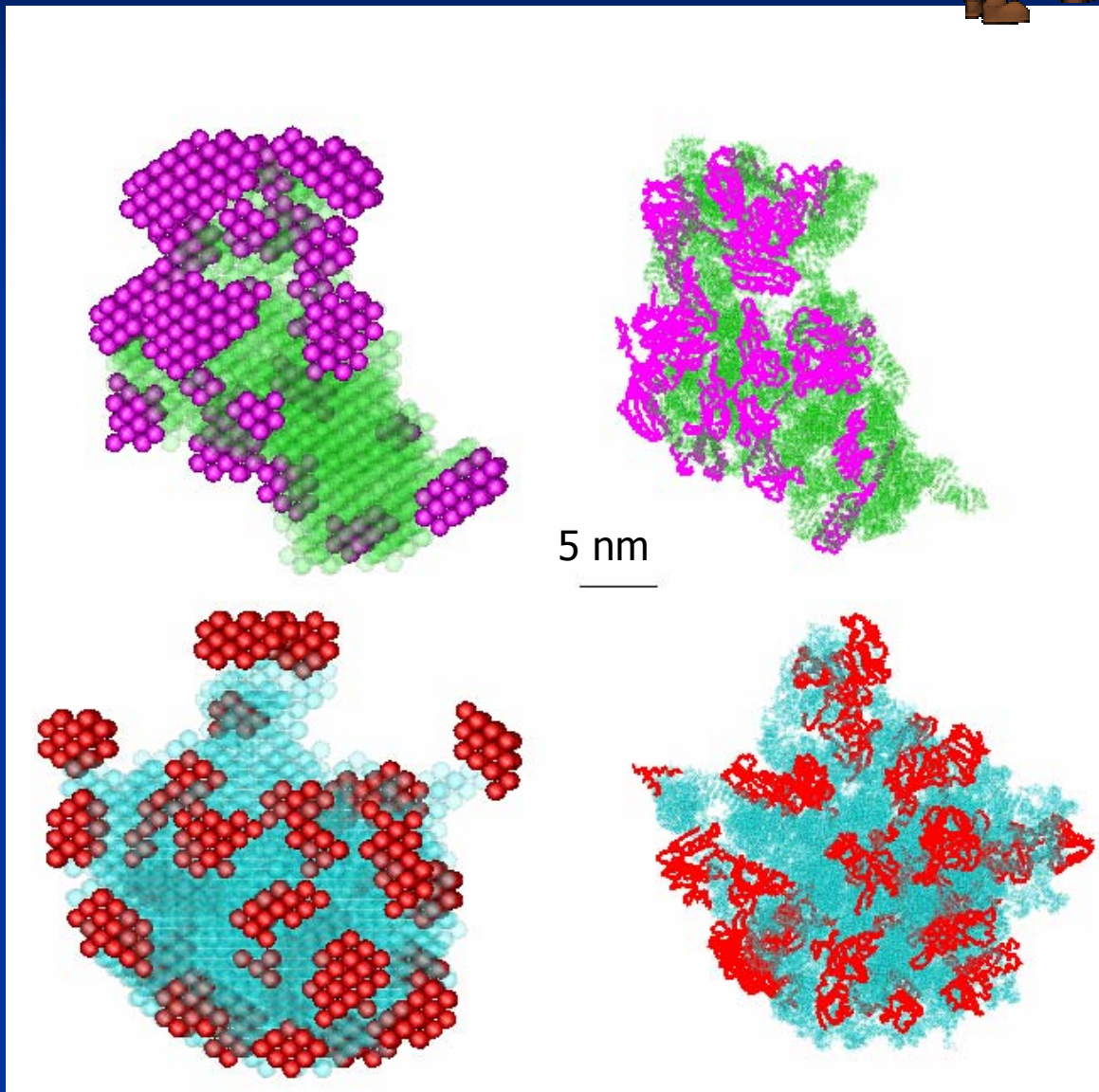
Svergun, D.I. & Nierhaus, K.H. (2000)  
*J. Biol. Chem.* **275**, 14432-14439



# A protein-RNA map in the 70S ribosome *E.coli*



# Solution <sup>versus</sup> crystal



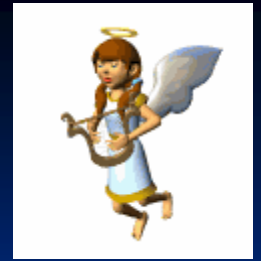
X-ray and neutron scattering map of protein-RNA distribution in the 70S ribosome *E. coli* (left, resolution 3 nm) compared with later crystallographic models (right).

Top, 30S subunit from *Th. thermophilus*, resolution 0.33 nm (Schluenzen, F, et al, & Yonath, A. (2000) *Cell*, **10**, 615).

Bottom, 50S subunit from *H. marismortui*, resolution 0.24 nm (Ban, N., Nissen, P., Hansen, J., Moore, P.B. & Steitz, T.A. (2000) *Science*, **289**, 905).



# Acknowledgments



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A.V.Sokolova (Institute of Crystallography, Moscow)

<http://www.embl-hamburg.de/ExternalInfo/Research/Sax>

- **GItS:** M.A.Vanoni (Milan University)
- **Ribosome:** K.H.Nierhaus (MPIMG, Berlin), H.B.Stuhrmann (GKSS, Geesthacht), J.Frank (Wadsworth Center, Albany), J. Skov Pedersen (Aarhus University)
- **Importins/Exportins:** N.Fukuhara (IBS, Grenoble), E.Conti (EMBL, Heidelberg), P.Timmins (ILL, Grenoble)

**Animation:** <http://www.animfactory.net/>