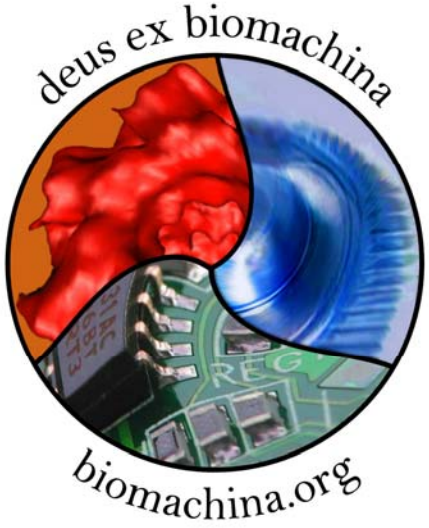
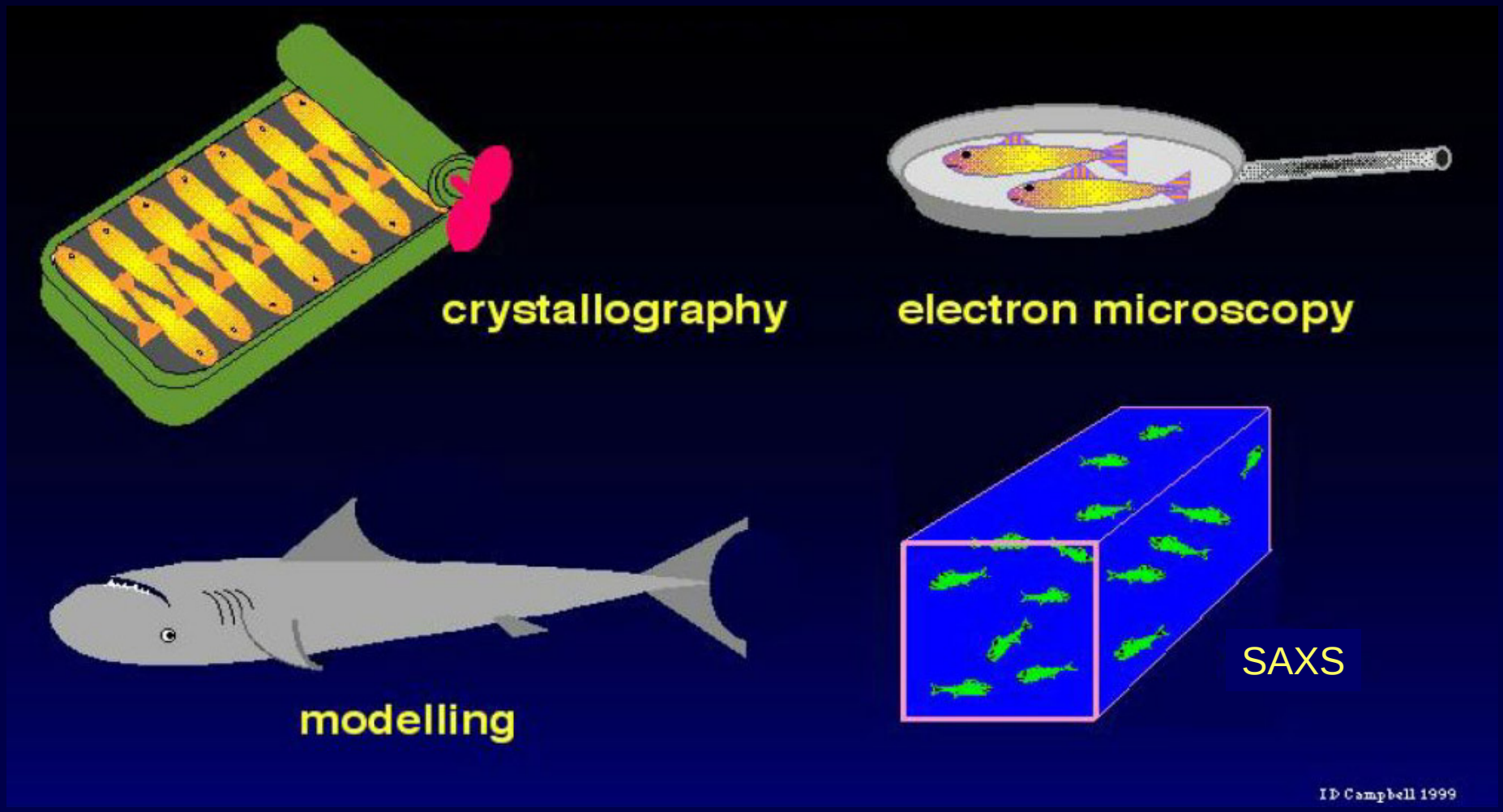


SAXS Refinement

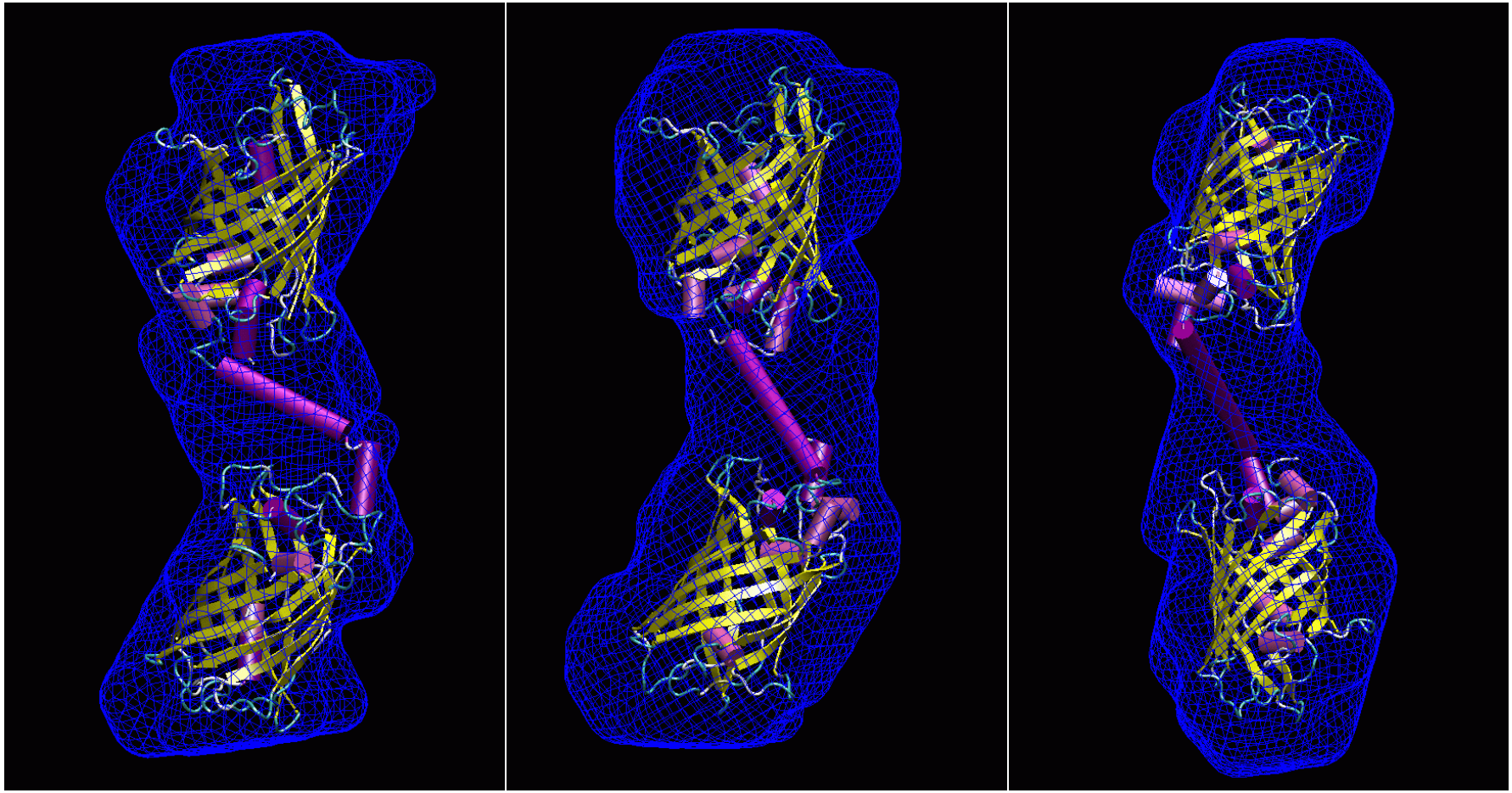
Willy Wriggers
School of Health Information Sciences &
Institute of Molecular Medicine
University of Texas – Houston

Structural Biology



BL45 SAXS Station

EBFP-linker-EGFP fusion proteins
(Tetsuro Fujisawa)



LAEAAAKEAAAKEAAAKAAA (20)

LAEAAAKEAAAKEAAAKEAAAKAAA (25)

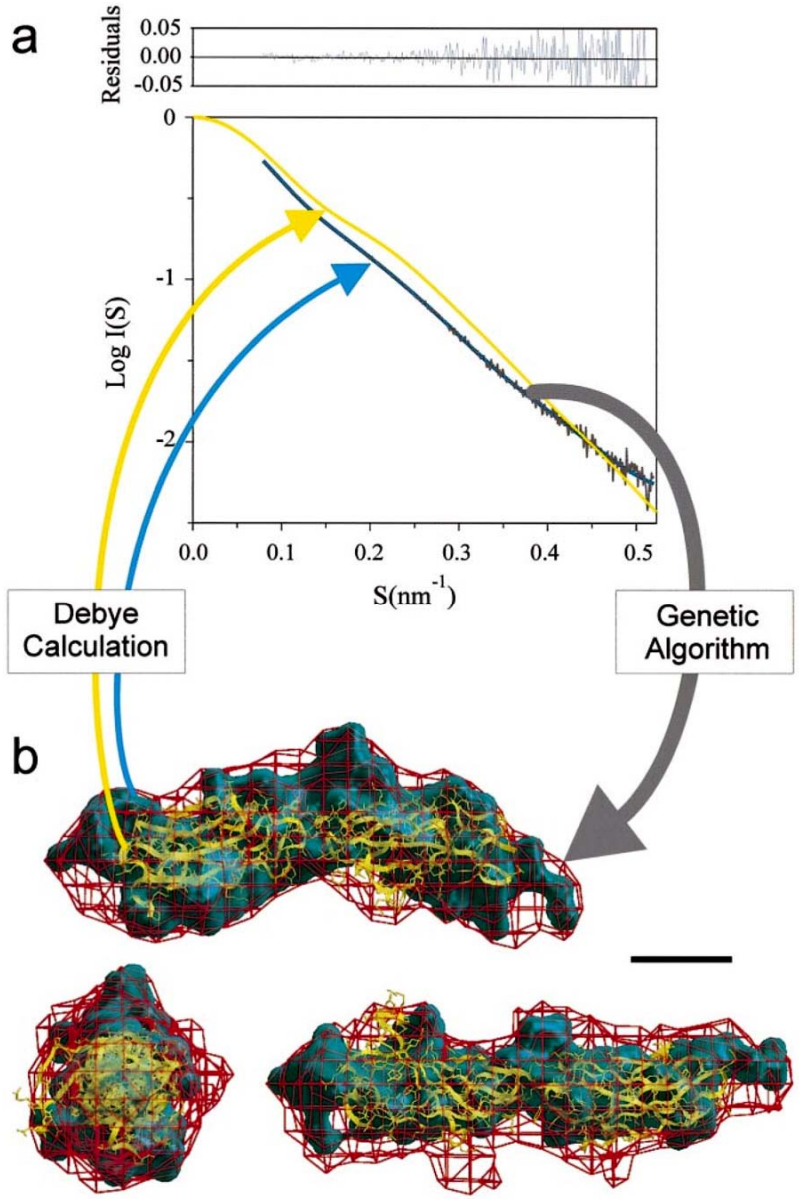
LAEAAAKEAAAKEAAAKEAAAKEAAAKAAA (30)

Generating 3D Structures from 1D SAXS Data

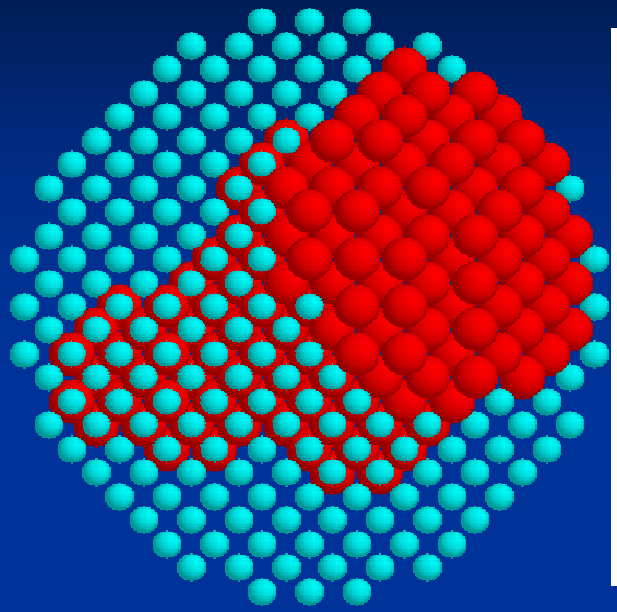
Low-resolution
3D shapes from
1D scattering profiles!

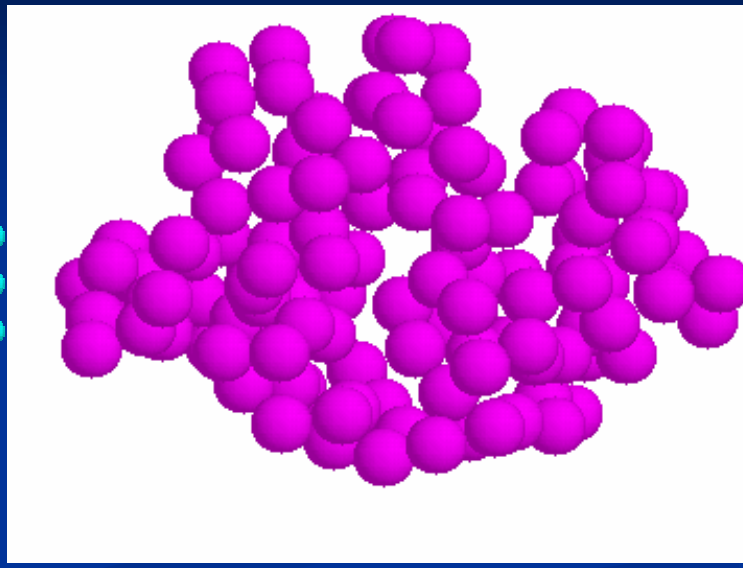
Small molecules OK!

Chacón et al., JMB (2000) 299:1289



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

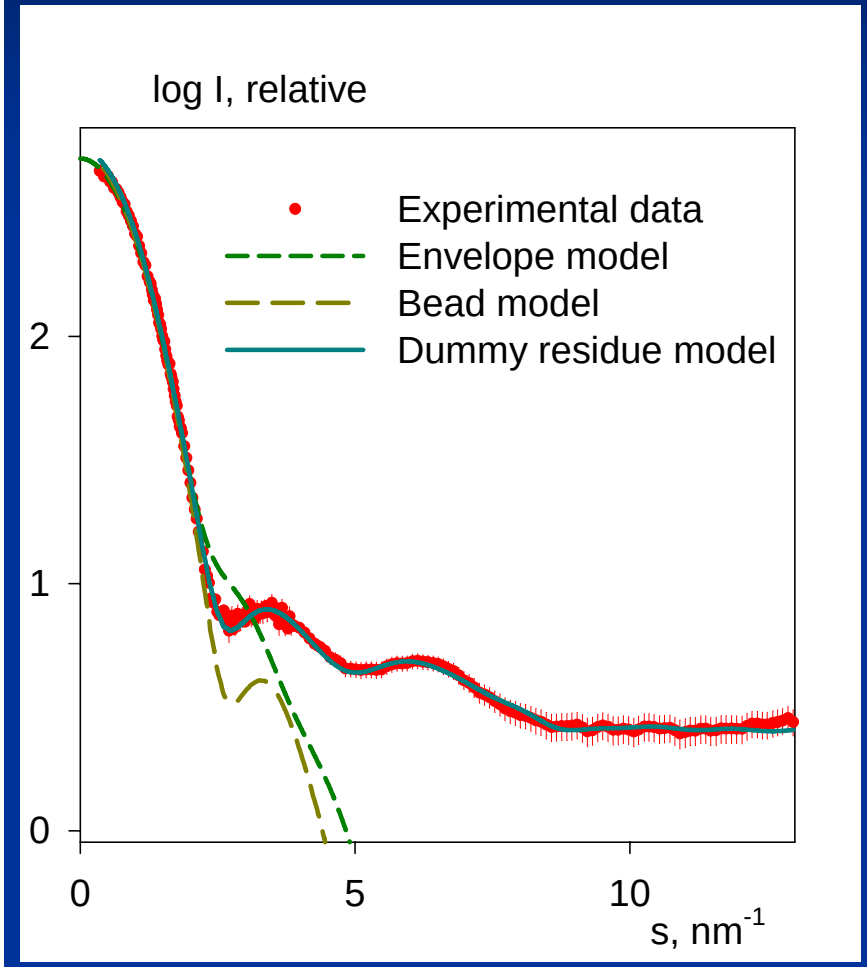
Dummy residues model

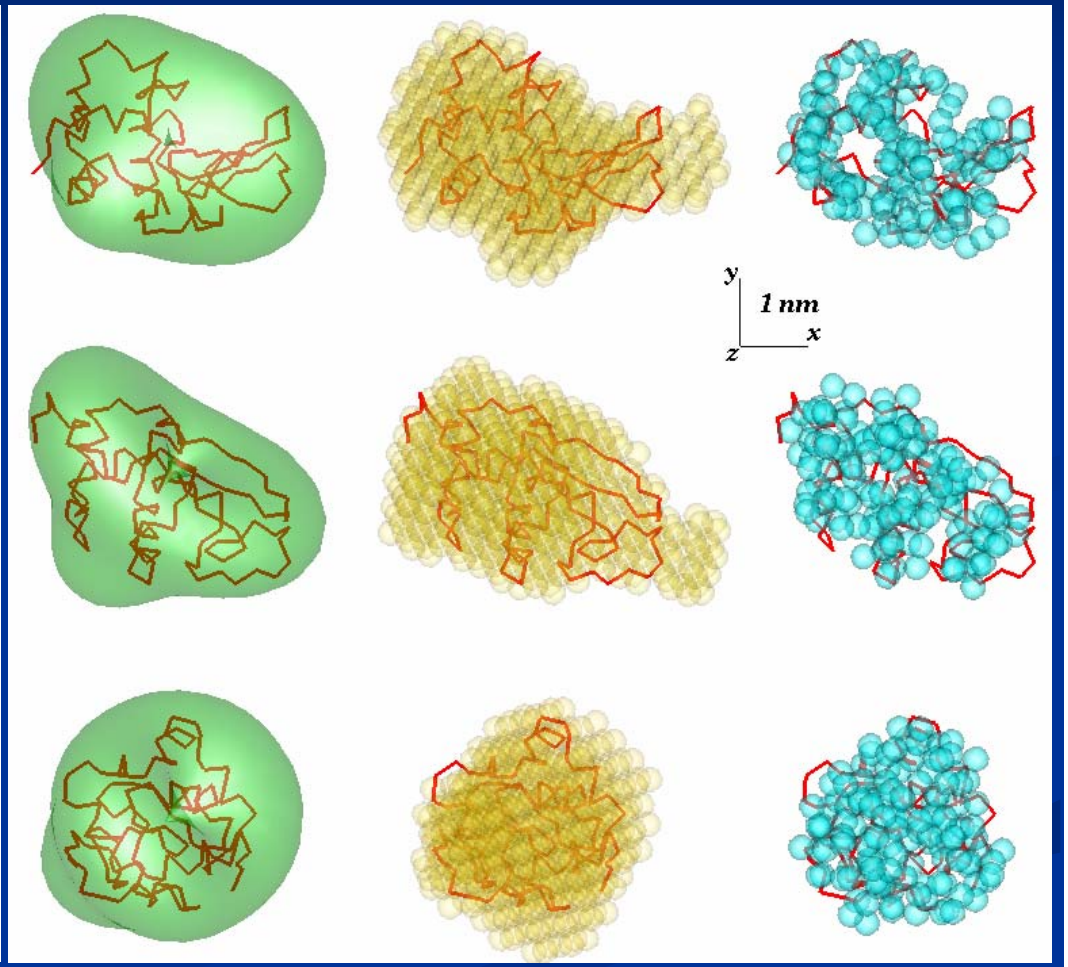
Svergun, D.I., Petoukhov, M.V. & Koch, M.H.J. (2001) *Biophys. J.* **80**, 2946-2953.

All the methods minimize Discrepancy[Data] + Penalty[Additional info]

Benchmarking *ab initio* Methods

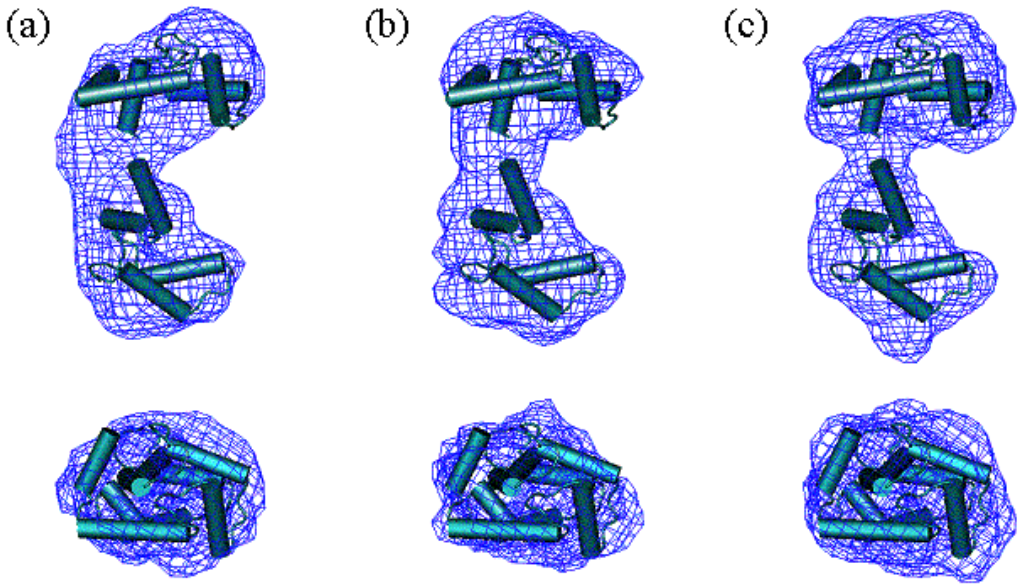
Envelope Bead model Dummy residues





Comparison with the crystal structure of lysozyme

How to Validate SAXS Bead Modeling?



Takahashi, Y., Nishikawa, Y., Fujisawa, T. Evaluation of three algorithms for ab initio determination of three-dimensional shape from one-dimensional solution scattering profiles, (2003) *J Appl Cryst*, **36**, 549-552.

Ab initio Shape Determination using a Single Phase Dummy Atom Model

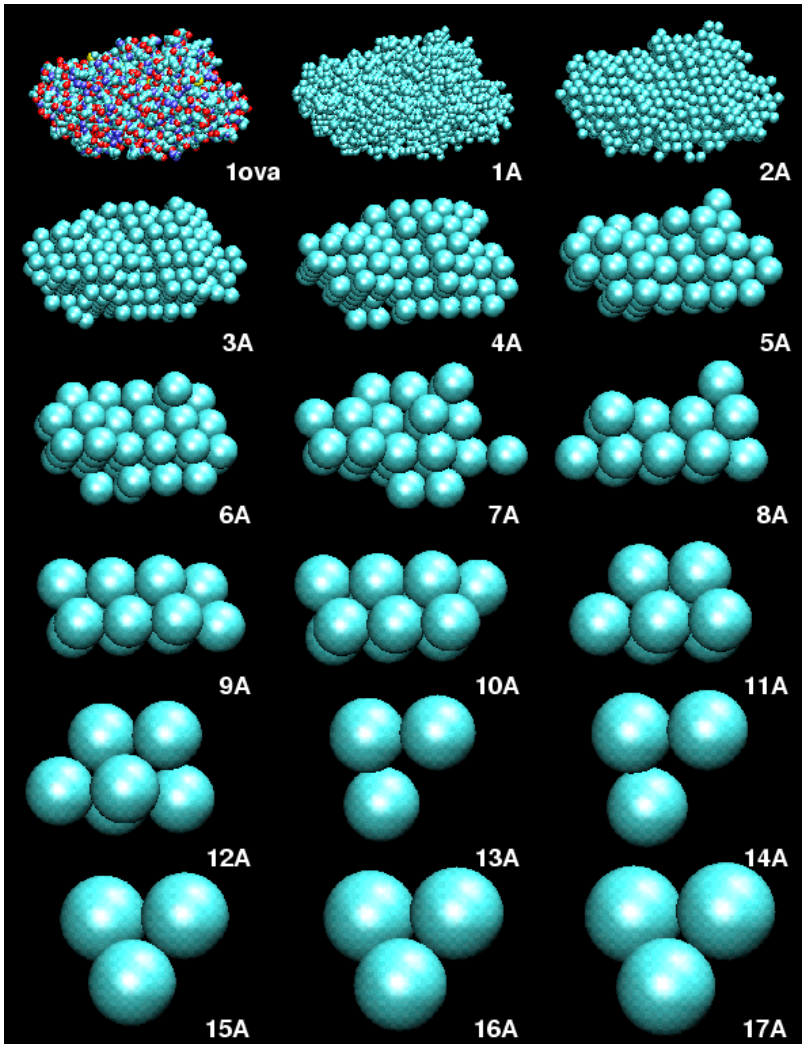
by Simulated Annealing (**DAMMIN**,Svergun)

by Genetic Algorithm (**DALAI_GA**, Chacon)

by Monte Calro style give 'n' take algorithm (**SAXS3D**, Walther)

Independent of algorithms, bead models converged to the average structure.

Simulated Bead Models for Validation

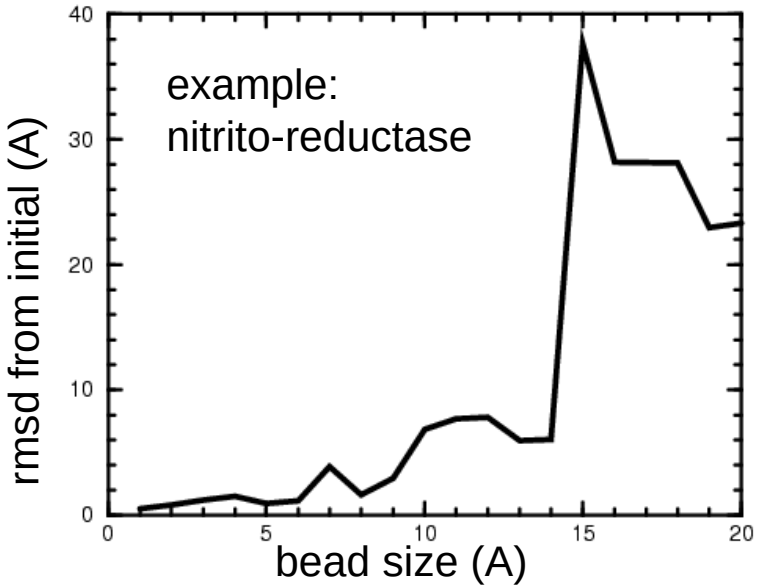


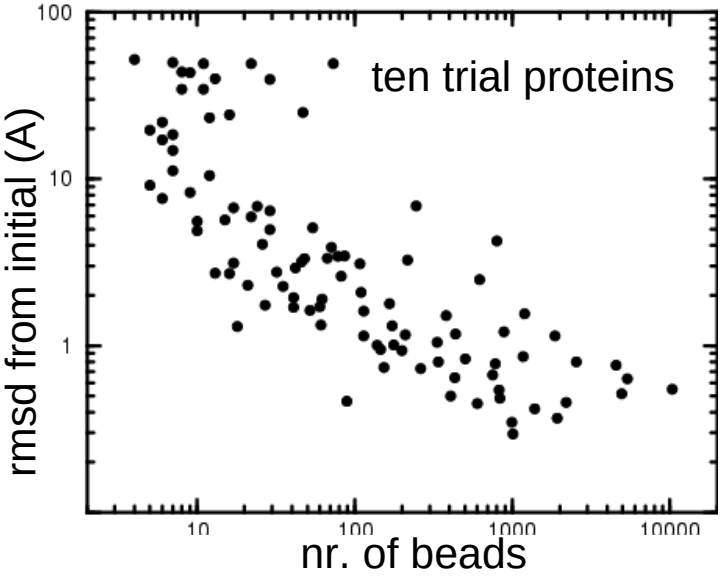
simulated bead models on a Hexagonal Close-Packed (HCP) lattice: “pdb2sax”

Example: Ovalbumin

(Wriggers & Chacon, J. Appl. Cryst., 34:773 (2001)

Accuracy of SAXS Rigid-Body Fitting





- rmsd is stable in lower single digits, until break-down occurs
- ten trial proteins (7cat,1qg3,2cga,1mbn,2nrd,1ova,1xso,1spp,1top,1tub):
  - correct match missed: 0 times
  - unique fit: 7 times (2 times symmetry-related)
  - ambiguous fit (degenerate set of highest-scoring fits): 3 times
  - it turns out that these 3 cases are proteins of sphericity > 0.5.

Accuracy of SAXS Rigid-Body Fitting

Table 1. Characteristics of simulated SAXS bead models

Protein used for generating bead model	PDB entry	Critical bead size (Å)*	Docking precision (Å)†	Oligomeric symmetry	Degeneracy of best fit‡	Sphericity ς
Catalase	7cat	16	0.8	4×	4×	0.57
β-4-integrin	1qg3	4	0.8	1×	1×	0.16
Chymotrypsinogen A	2cga	5	2.4	1×	2×	0.66
Myoglobin	1mbn	8	0.8	1×	2×	0.56
Nitrito-reductase	2nrd	15	0.8	3×	6×	0.61
Ovalbumin	1ova	7	0.6	1×	1×	0.48
Spermadhesin	1spp	8	0.8	1×	1×	0.48
Superoxide dismutase	1xso	9	2.7	2×	2×	0.46
Troponin C	1top	9	0.7	1×	1×	0.18
$\alpha\beta$-tubulin	1tub	11	1.8	1×	1×	0.48

* The value given is the smallest bead radius for which the rms deviation of the docked§ to the target structure exceeded 10 Å.

† The stated value is the rms deviation of the docked§ to the target structure averaged for bead radii 1, 2, and 3 Å.

‡ The degeneracy is the number of optimum fits (at sub-critical bead size) that were empirically found to cluster within a narrow numeric range of the optimum score, due to symmetry effects or due to ambiguity of matching.

§ For rmsd evaluations, the fit with the lowest rmsd among any degenerate fits was selected.

Accuracy of SAXS Rigid-Body Fitting

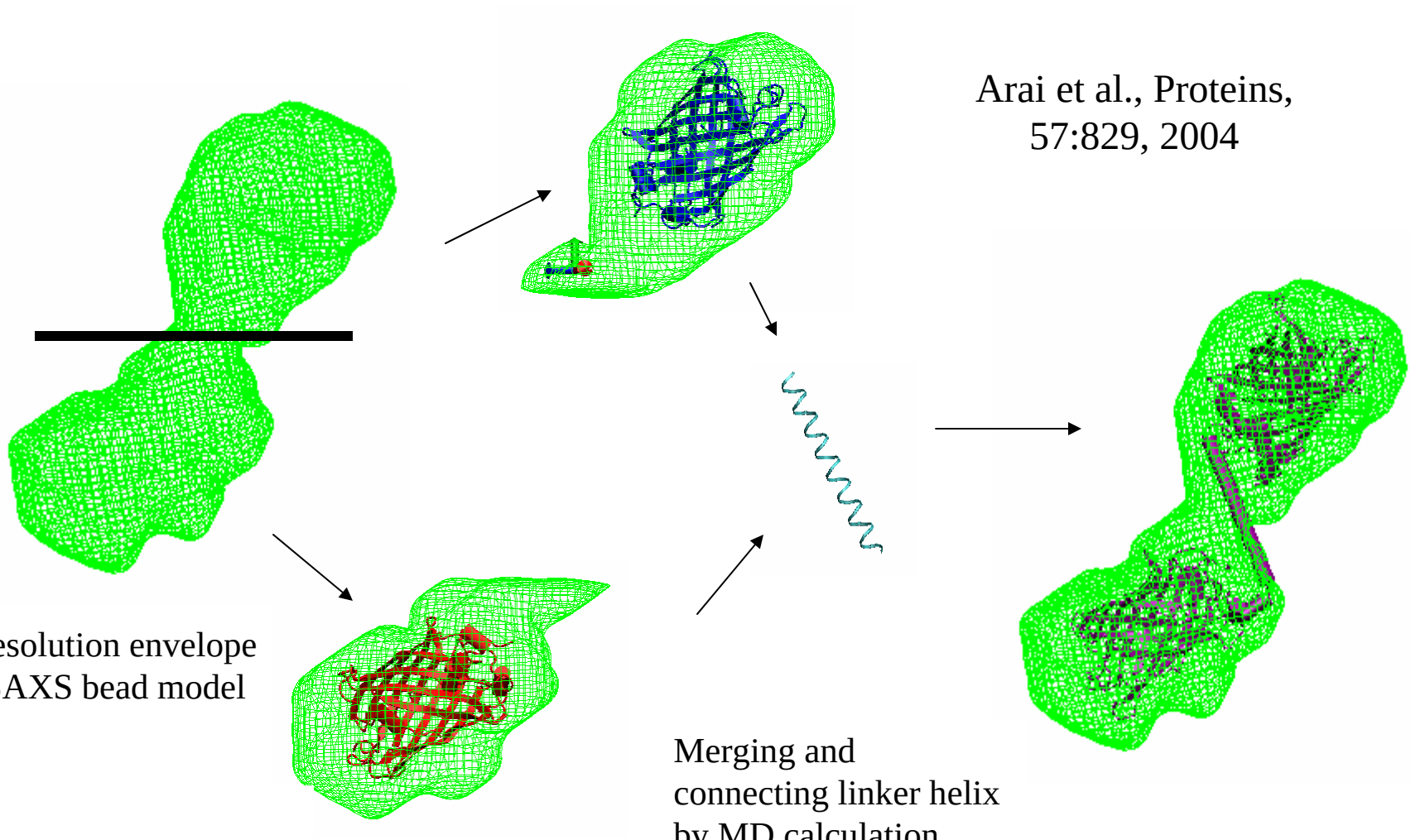
Based on the simulated SAXS trial runs, we can expect that fitting

- does not miss the correct match,

- but on occasion (for globular proteins), the correct match may be part of a number of degenerate highest-scoring fits (need more information).

The accuracy of the docking is on the order of 1A (or better) for more than 100 beads.

Large Systems: Connecting the Domains



Arai et al., Proteins, 57:829, 2004

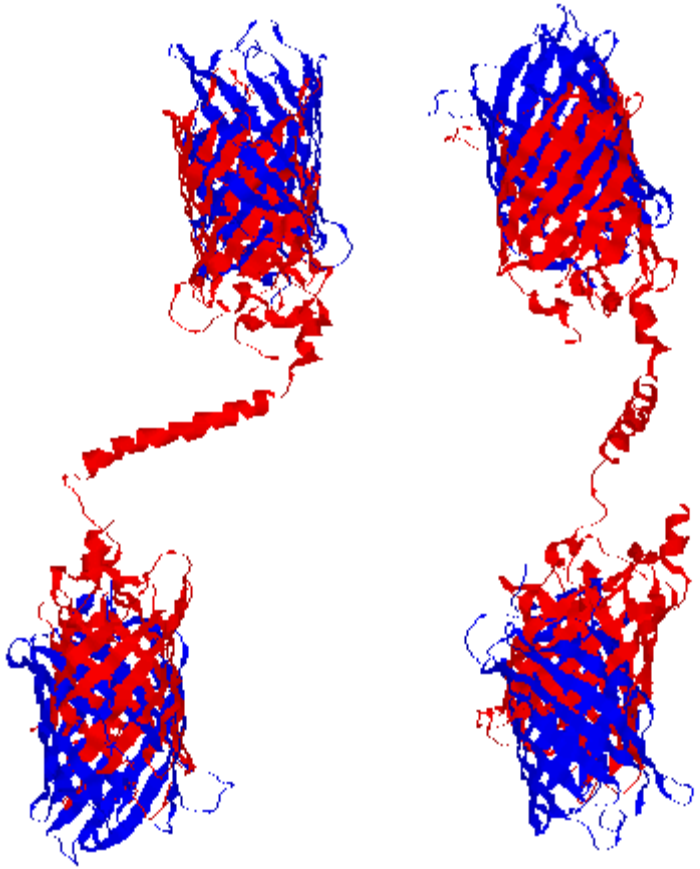
Low resolution envelope from SAXS bead model

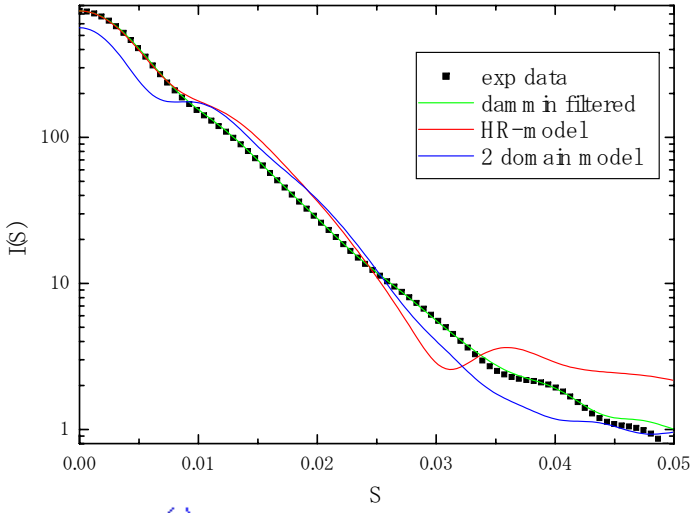
Fitting each domain to each envelope

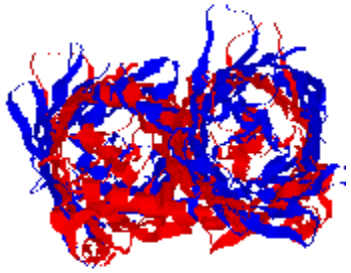
Merging and connecting linker helix by MD calculation

S-tag and His-tag are still missing

Real vs. Reciprocal Space Refinement







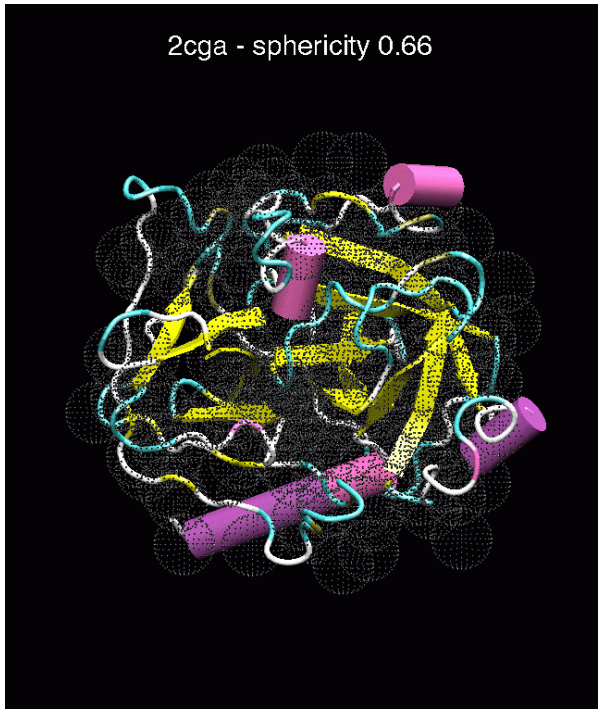
2 domain fitting with Masha

High resolution model with Situs

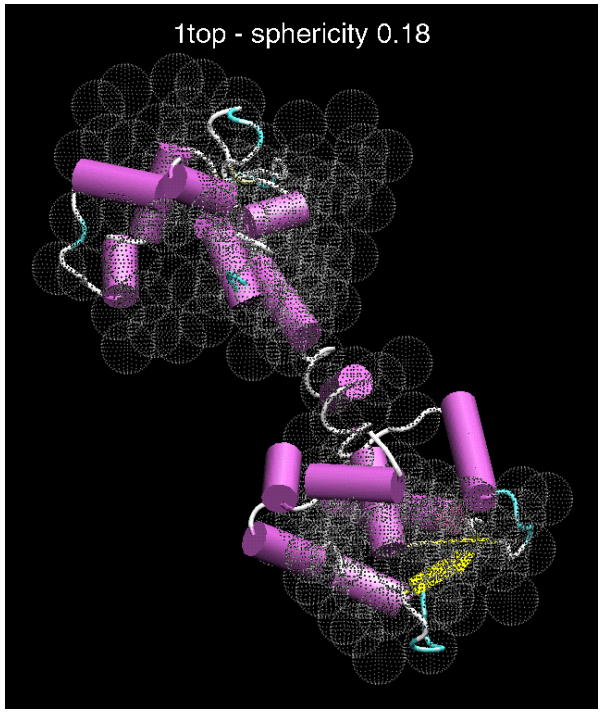
The domain orientation is robust

2001: First SAXS Application of Situs

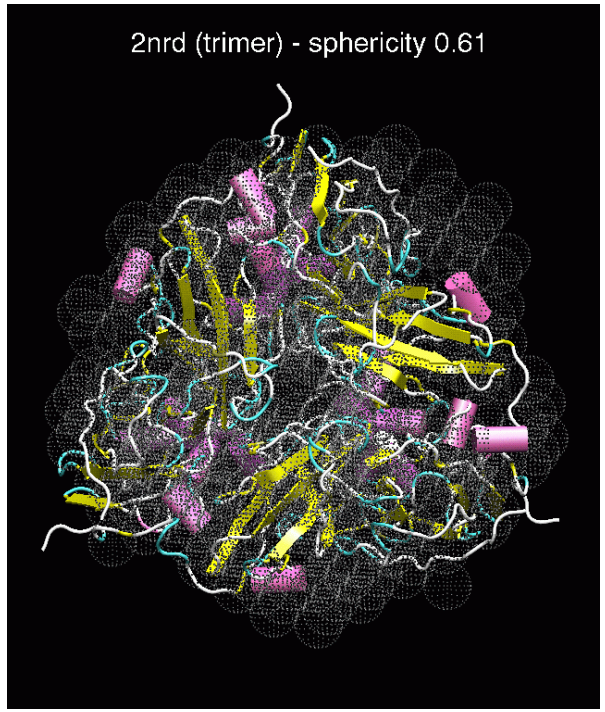
(Wriggers & Chacon, J. Appl. Cryst., 34:773 (2001)



Chymotrypsinogen A

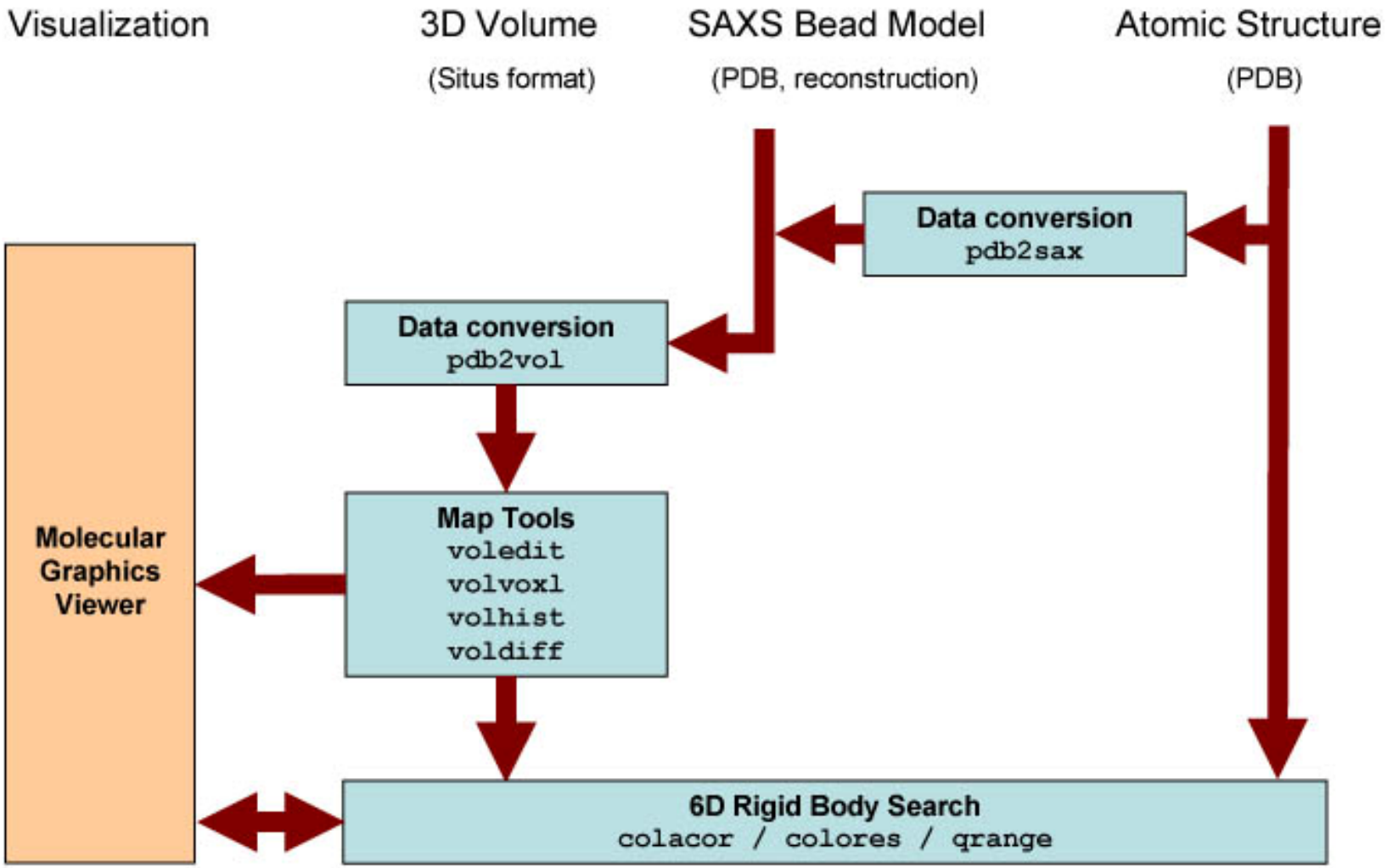


Troponin C

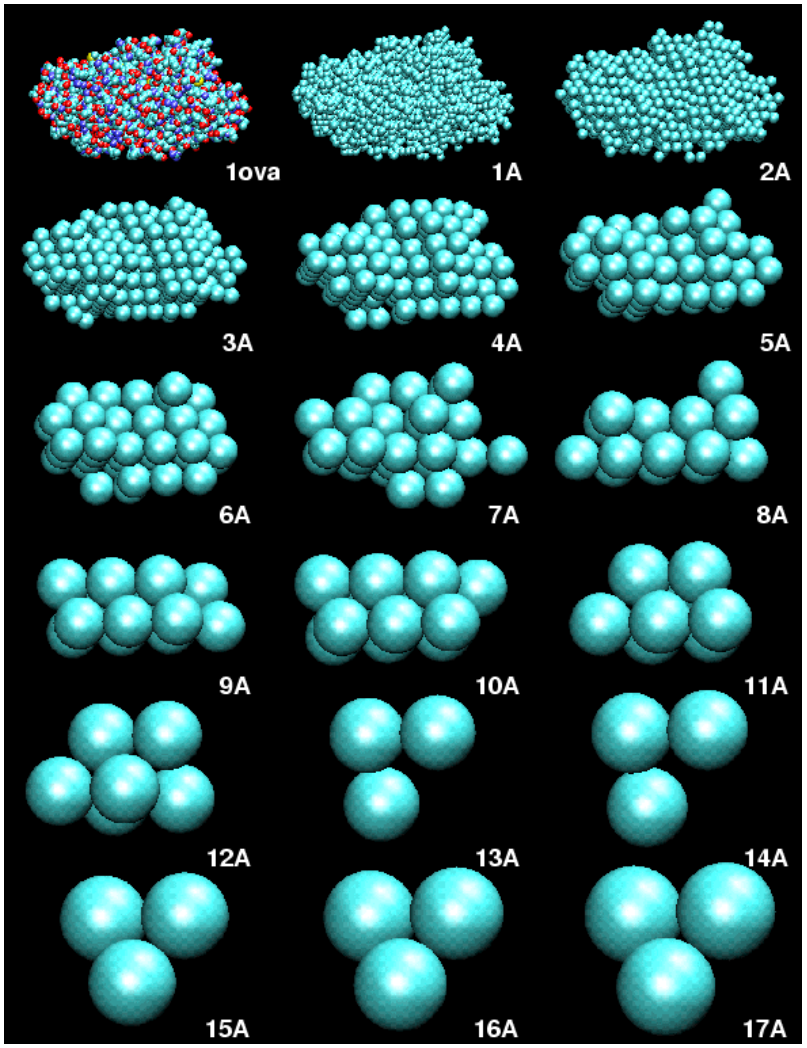


Nitrito-Reductase

Design (SAXS)


Visualization
3D Volume
(Situs format)
SAXS Bead Model
(PDB, reconstruction)
Atomic Structure
(PDB)
Data conversion
pdb2sax
Data conversion
pdb2vol
Map Tools
voledit
volvoxl
volhist
voldiff
Molecular
Graphics
Viewer
6D Rigid Body Search
colacor / colores / qrange

pdb2sax



simulated bead models on a Hexagonal Close-Packed (HCP) lattice

Example: Ovalbumin

pdb2vol

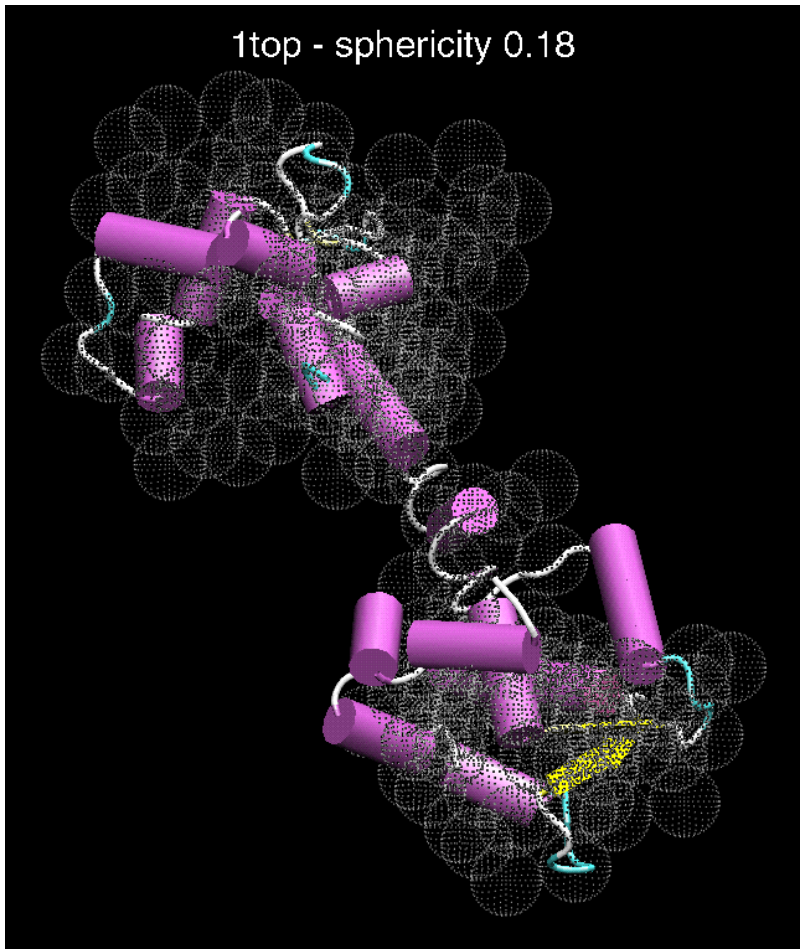
Convolution with various kernels:
Gaussian, hard sphere, cone, etc

2 uses:

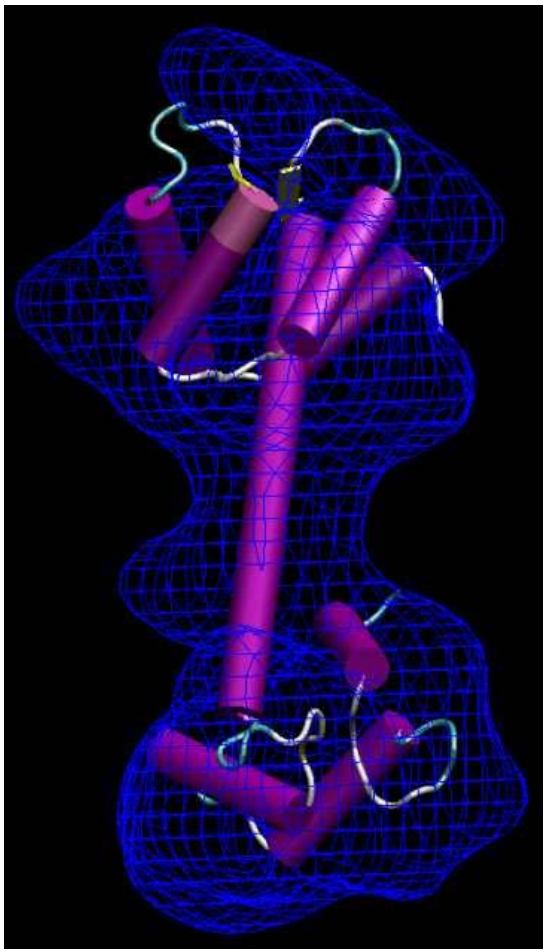
• take SAXS bead model (centers of beads) and create EM style 3D volume map (hard sphere)

• create smooth envelope surrounding beads for visualization (Gaussian)

Visualization



old style: transparent beads



new style: isocontour

Fitting/Refinement

- colores: 6D FFT-based rigid-body (slow, but parallelized)

- colacor: rigid-body refinement of manual fit (gradient ascent of cross correlation)

- qrange: fast rigid-body (based on simulated markers, reduced search space)

- qpdb + qvol + MD refinement (X-PLOR): flexible fitting

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http://biomachina.org

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