Synchrotron Small-Angle X-Ray Scattering as Universal Instrument of Structural Analysis of Bio and Nanosystems

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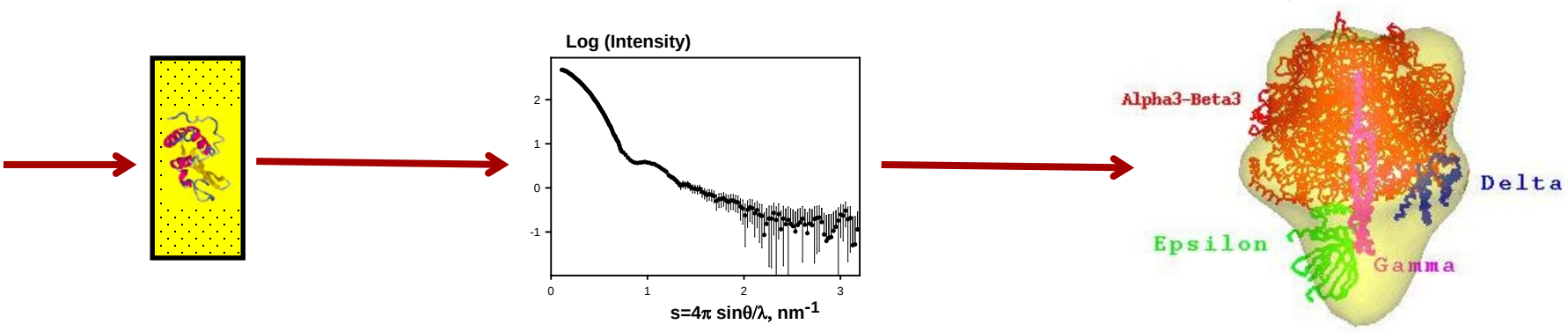
Motivation for Small-angle X-ray scattering in structural study

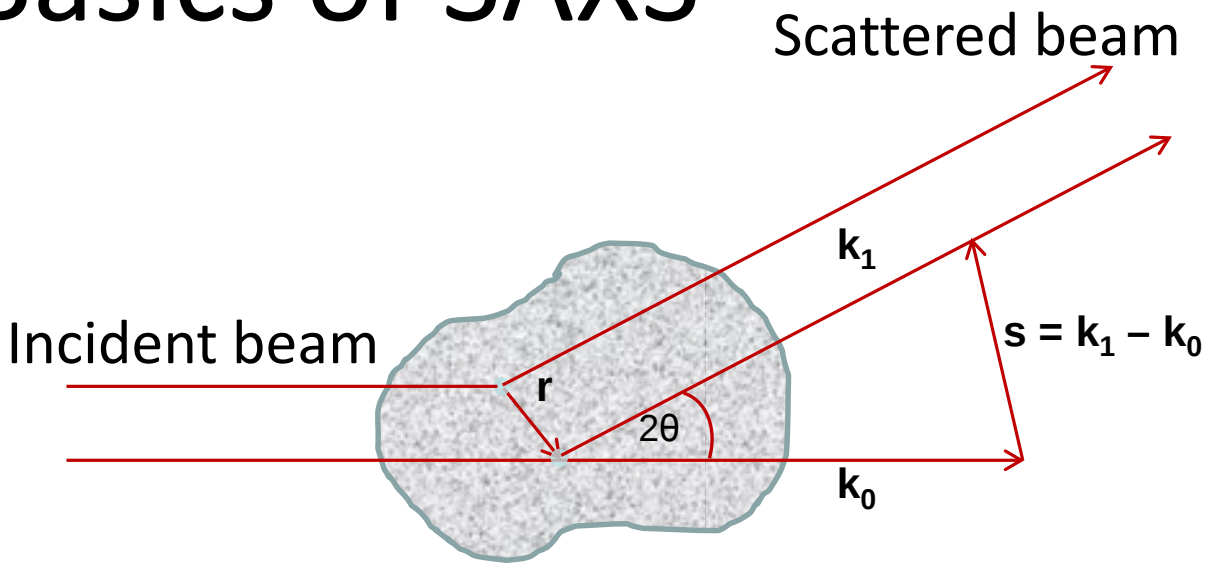
SAXS is universal low resolution method for structural analysis



- is applied to solutions, polydisperse systems, gels, fractal systems, multilayered structures, supramolecular structures, nanocomposites, biological complexes, *etc*
- requires neither crystals nor special sample preparation
- is applicable under nearly physiological conditions
- yields complementary information to other structural methods like crystallography, NMR, EM, AFM, *etc*
- permits quantitative analysis of complex systems and kinetic processes
- allows to study structural transitions and conformational changes

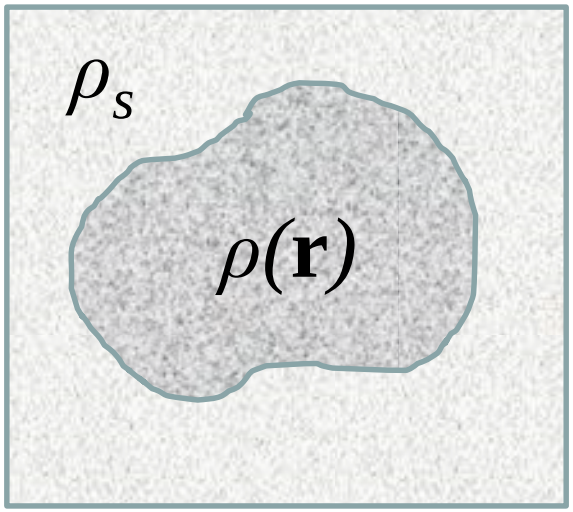
Basics of SAXS

X-ray beam is scattered by the bound electrons of the sample



Concept of contrast

$$\Delta\rho(\mathbf{r}) = \rho(\mathbf{r}) - \rho_s$$



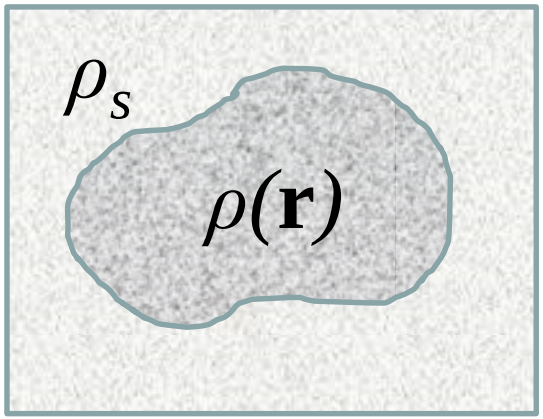
ρ_s - electron density of the medium

$\rho(\mathbf{r})$ - electron density of the particle

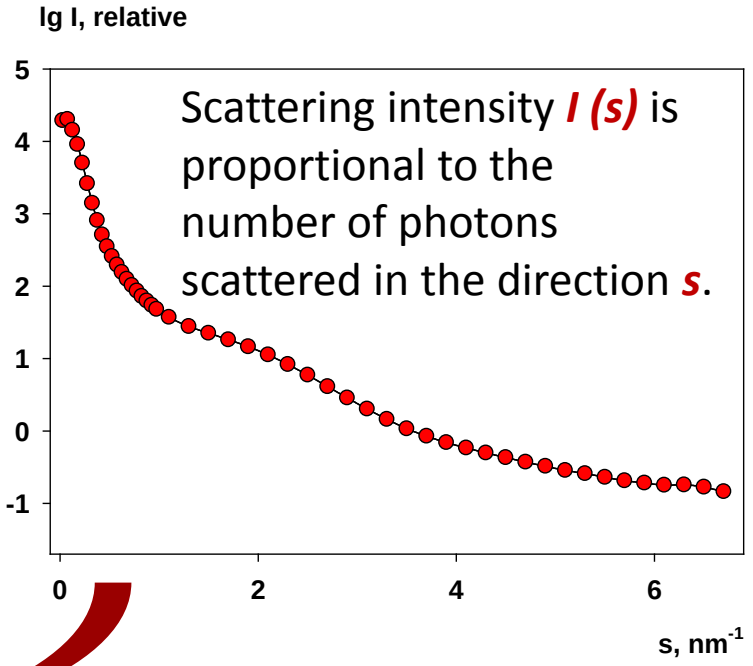
$$A(\mathbf{s}) = \Im[\rho(\mathbf{r})] = \int_V \Delta\rho(\mathbf{r}) \exp(i\mathbf{sr}) d\mathbf{r}$$

Problems

Amplitude ***A (s)*** is not measured in the experiment!



Scattering intensity ***I(s) = [A(s)]²*** is measured.



To extract information about the structure of the objects under study one needs to solve the reciprocal task: using **1D scattering curve *I (s)*** to restore **3D structure**.

In general, the solution of the reciprocal tasks is ambiguous.

Problems

3D search model

Trial-and-error

1D scattering data

Additional information is ALWAYS required to resolve or reduce ambiguity of interpretation

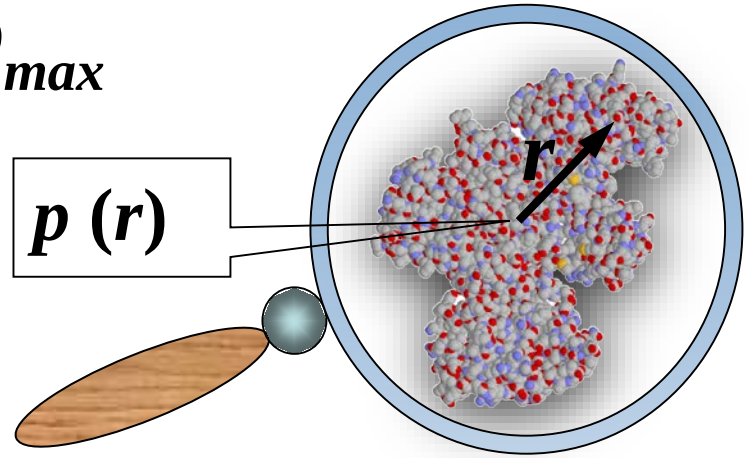
Distance distribution function *p(r)*

$$I(s) = \int_0^{D_{\max}} p(r)\frac{\sin(sr)}{sr}dr$$

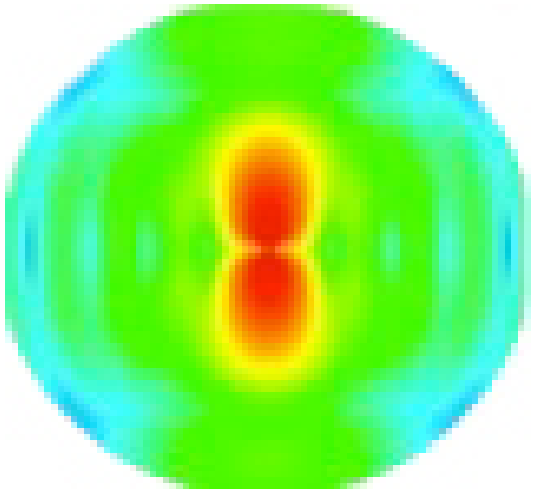
Using distance distribution function one can determine size, shape and internal structure of the particle at low (*1-10 nm*) resolution

$$p(r) = \frac{r^2}{2\pi^2}\int_0^{D_{\max}} s^2 I(s)\frac{\sin sr}{sr}ds$$

$$p(r) = 0 \text{ at } r > D_{max}$$



Many different programs requared for SAXS data interpretation are placed in this portal

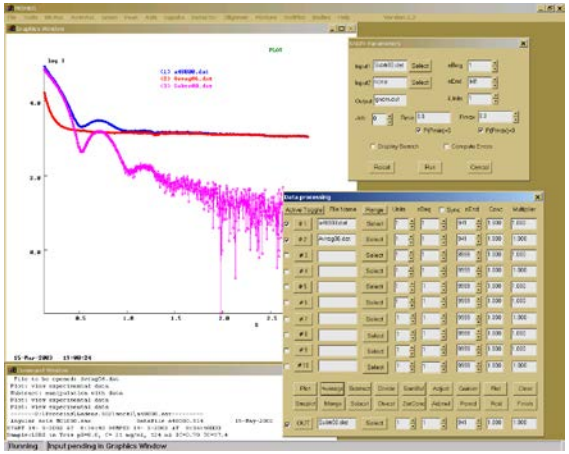


SAS Portal

Software

http://smallangle.org/

Data Processing and Analysis



PRIMUS
(preliminary data processing)

Calibration and normalization
Raw data processing
Data manipulations
Merging and splicing
Concentration series analysis
Computation of invariants
Indirect Fourier transformation
Simple bodies modelling
Analysis of mixtures
Peak analysis

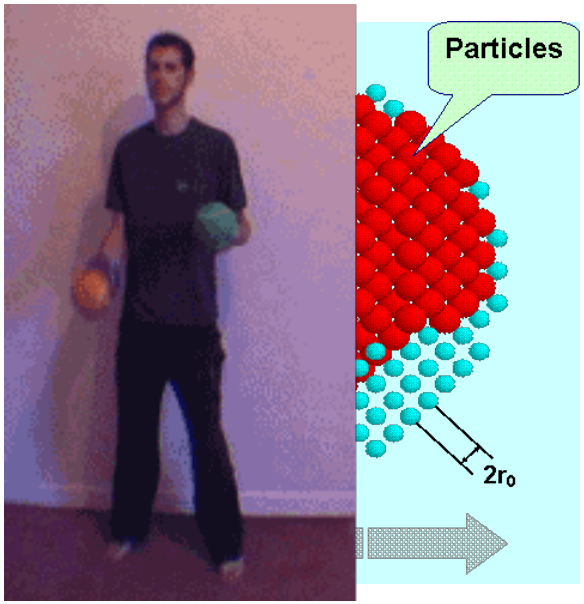
A program suite
ATSAS
All That SAS

It allows data reduction, processing and structure analysis using 3 main approaches to the data interpretation:

1. *Ab initio* shape reconstruction;
2. Rigid body modeling (method of molecular tectonics);
3. Hybrid methods

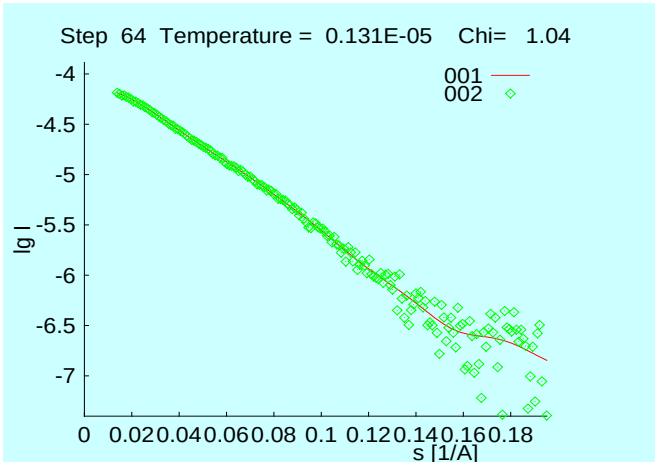
Major programs running on a PC under Win9x/NT and/or on UNIX workstations are documented and available at:
http://www.embl-hamburg.de/ExternalInfo/Research/Sax

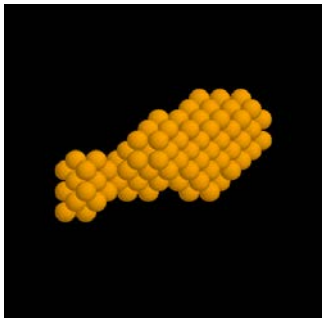
Ab initio shape restoration



Principle of *ab initio* method of simulated annealing

DAMMIN, GASBOR

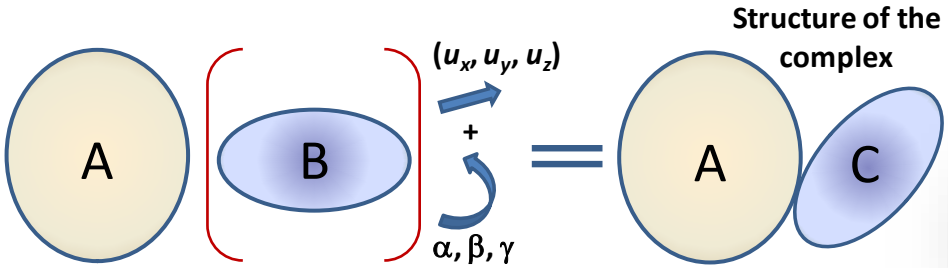


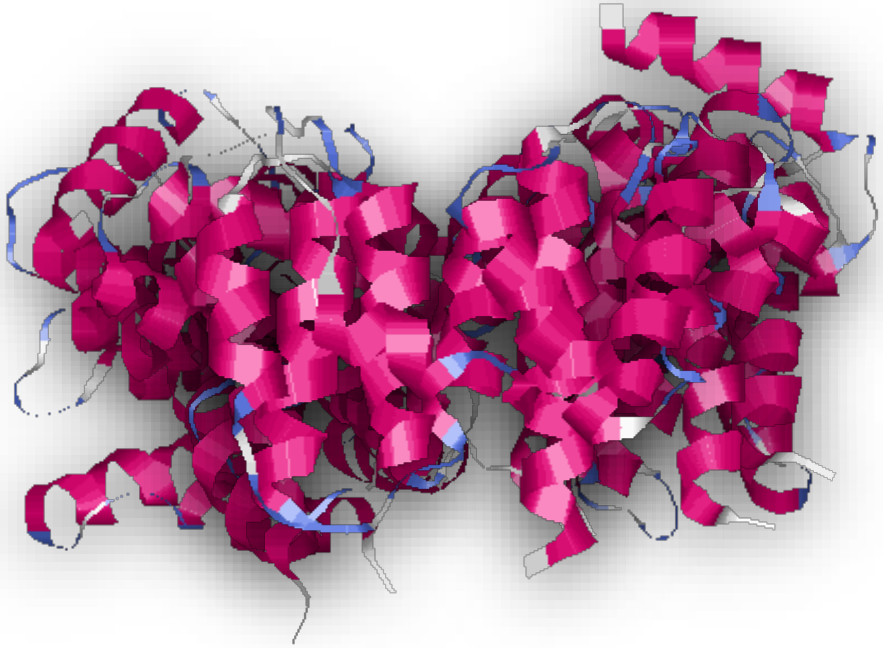


Svergun, D. I. Biophys. J. 1999, 76, 2879.

The maximum size D_{max} is the largest size of the scattering object. It determines the diameter of the search volume, and it is calculated using the size distribution analysis, *i.e.* from the *p(r)* function by the program GNOM (Svergun, D. I. *J. Appl. Crystallogr.* 1992, *25*, 495.)

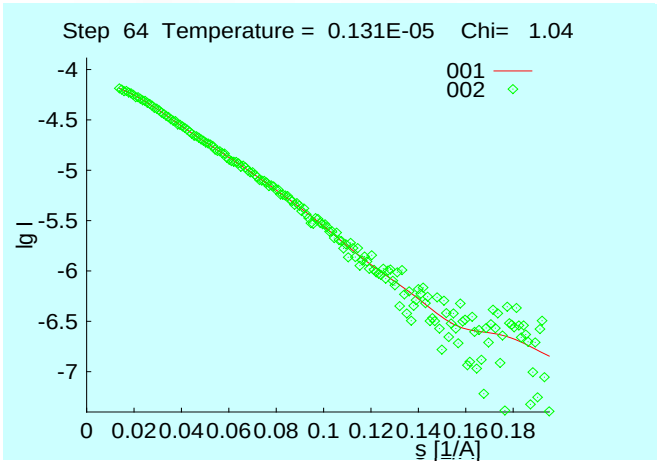
Rigid body refinement (method of molecular tectonics)





- The structures of two subunits are known.
- Arbitrary complex can be constructed by moving and rotating the subunits.
- This operation depends on three Euler rotation angles and three Cartesian shifts.

The process of rotation and moving stops when model curve fits the experimental data.

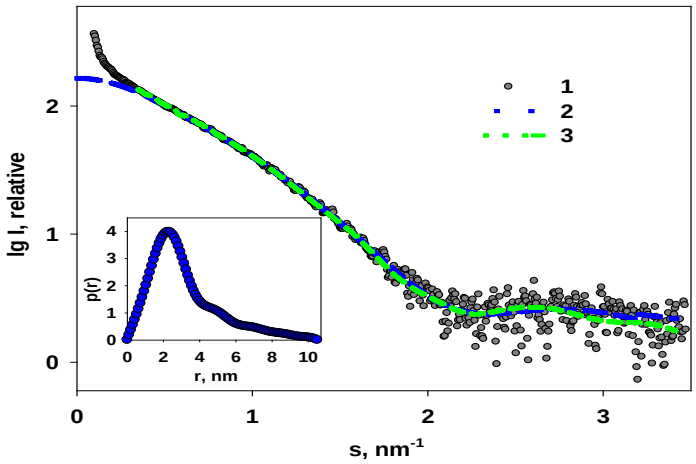


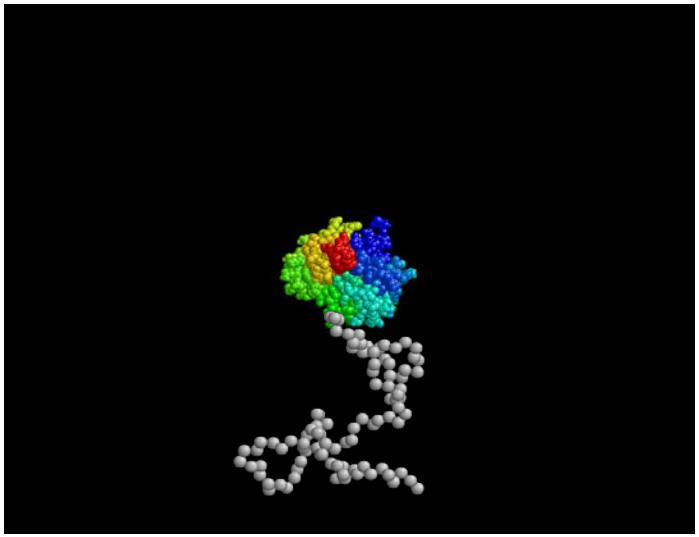
Petoukhov MV, Svergun DI. Global rigid body modelling of macromolecular complexes against small-angle scattering data. Biophys J. 2005; 89: 1237-1250

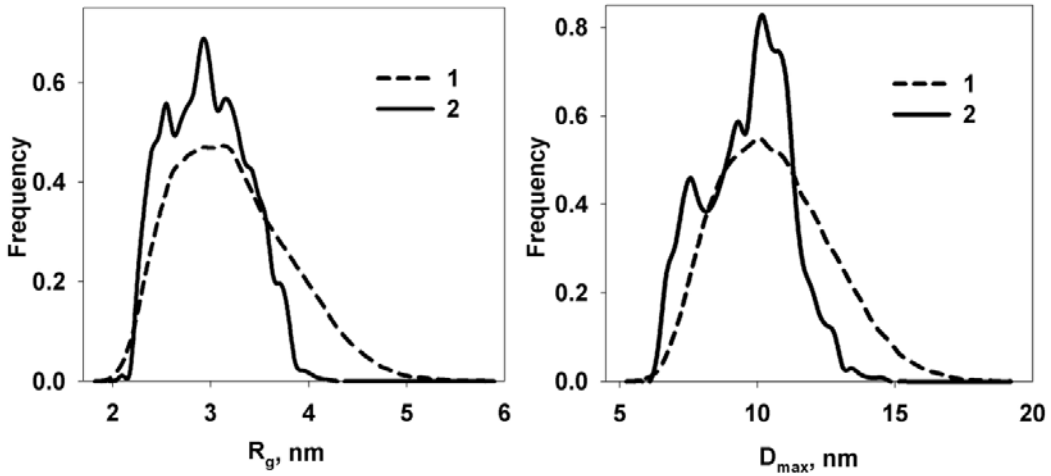
Hybrid methods:

Combination of ***ab initio*** **reconstruction and molecular tectonics**

Analysis of Flexibility of Multidomain Macromolecules







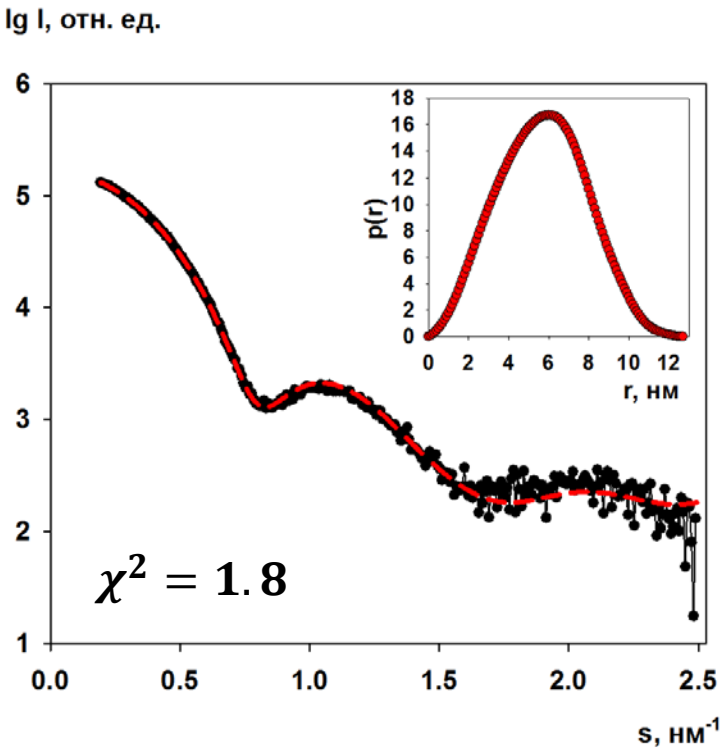
R_g и D_{max} distributions:
1 – random pool,
2 – selected ensemble

Bernado, P., Mylonas, E., Petoukhov, M.V., Blackledge, M., Svergun, D.I. (2007) Structural Characterization of Flexible Proteins Using Small-Angle X-ray Scattering. *J. Am. Chem. Soc.* **129(17)**, 5656-5664

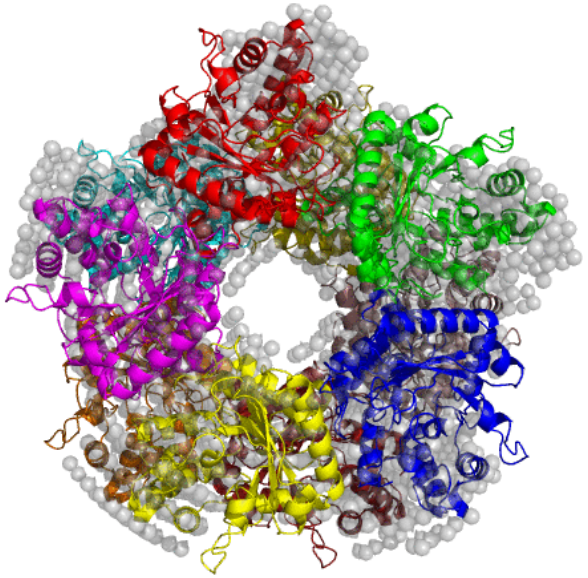
Some examples of SAXS application to biological samples:

4 important enzymes involved in metabolic processes in living cells

Class I fructose-1,6-bisphosphate aldolase (FbaB)



SASBDB: SASDBZ2



Comparison of models obtained by *ab initio* and hybrid methods

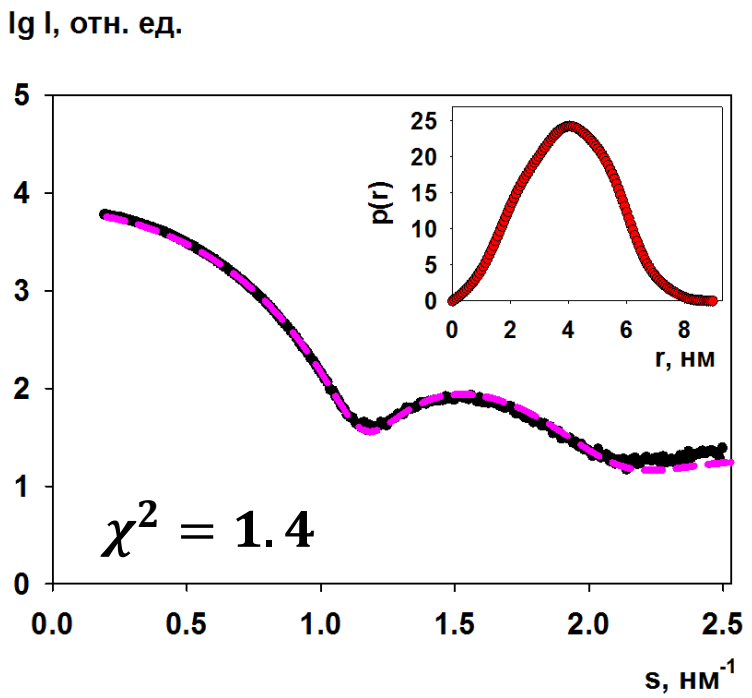
Synchrotron radiation

Sample	R_g, nm	D_{max}, nm	$MM_{I(0)}$, kDa	MM_{Porod}, Kda	$R_{g\ cryst}$*, nm	MM_{aa}, kDa
FbaB	4.4±0.1	12.7±0.6	340±20	335±15	4.4	339.14

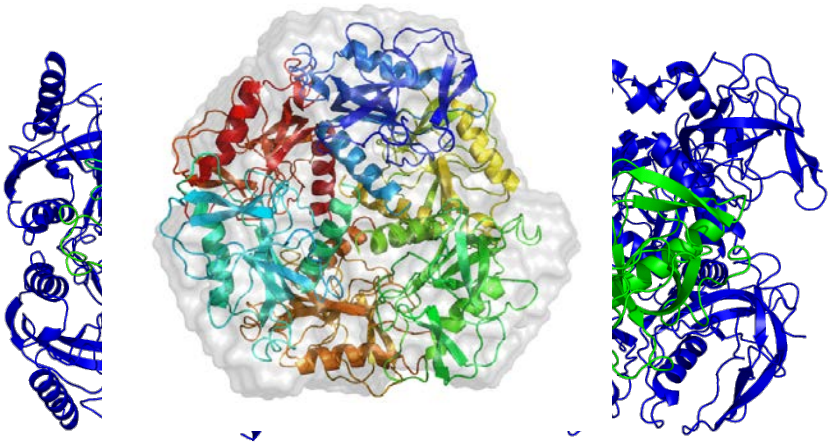
*$R_{g\ cryst}$ for decamer of archaea FbaB class I from *Thermoproteus tenax* (homologue) (PDB ID: 1OJX)

FbaB – an enzyme with a previously unknown structure – can associate into decamers where each individual protomer has a core TIM-barrel fold.

Inorganic pyrophosphatase (PPase)



SASBDB: SASDBY2



Crystal structure

Comparison of models obtained by *ab initio* method and method of molecular tectonics

Ab initio modeling

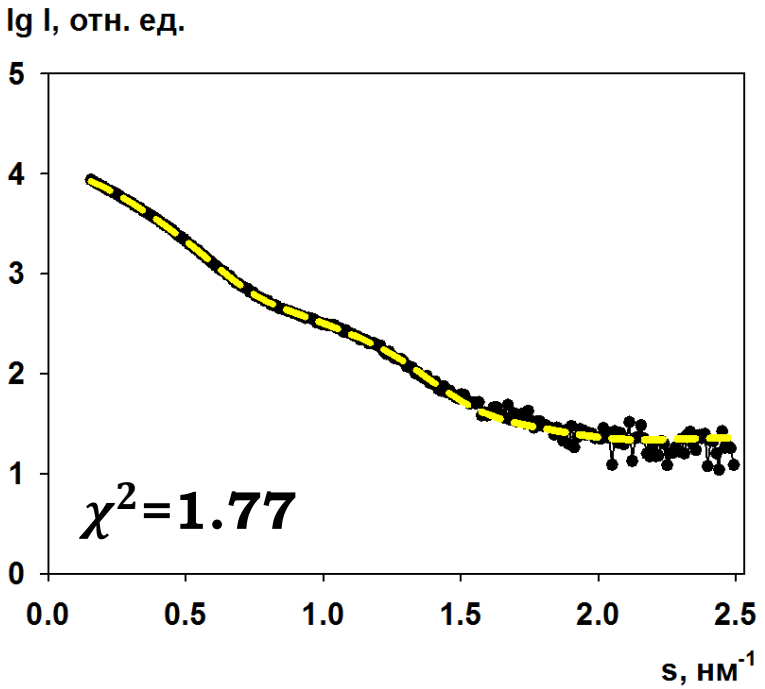
Rigid body reconstruction

Symmetry P 32

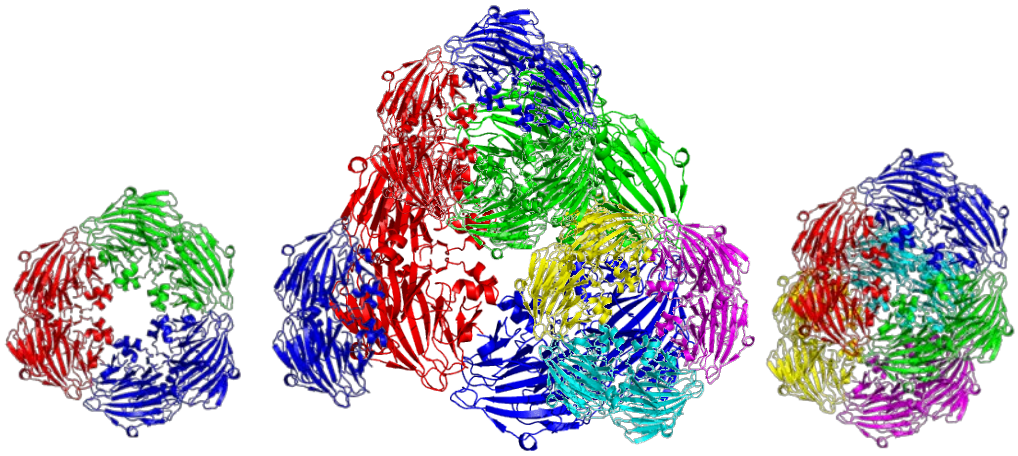
Sample	R_g, nm	D_{max}, nm	$MM_{I(0)}$, kDa	MM_{Porod}, kDa	$R_{g\ cryst}$, nm	MM_{aa}, kDa
PPase	2.8±0.1	7.7 ±0.5	130±10	116±10	2.9	117.28

The main difference between the crystal and solution conformations appears to be a rotational shift in the orientation of the individual PPase subunits. The overall low-resolution shape fits the experimental curve very well and are spatially superimposable with the rigid body model

5-keto-4-deoxyuronate isomerase (KduI)



SASBDB: SASDB23



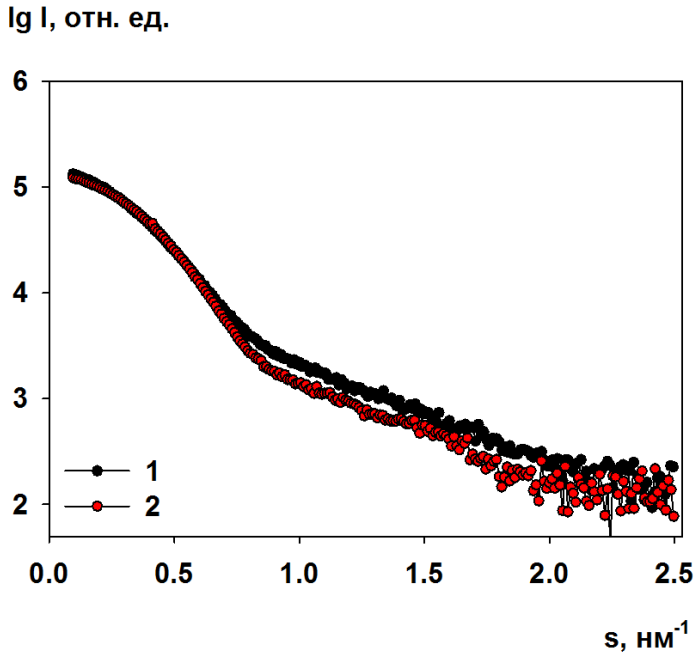
v_i = 0.31 v_i = 0.28 v_i = 0.24 v_i = 0.17

PDB: 1XRU

Sample	R_g, nm	D_{max}, nm	$MM_{I(0)}$, kDa	MM_{Porod}, kDa	$R_{g\ cryst}$, nm	MM_{aa}, kDa
KduI	6.2±0.1 4.5±0.1	-	183±10	182±10	3.9	187.35

The ability of KduI to form mixtures of different oligomeric species is an important property of the protein that contributes to regulating enzymatic activity. The different oligomeric forms differ in their catalytic efficiency, as it has been established for a number of other allosteric enzymes

Glutamate decarboxylase (GadA)



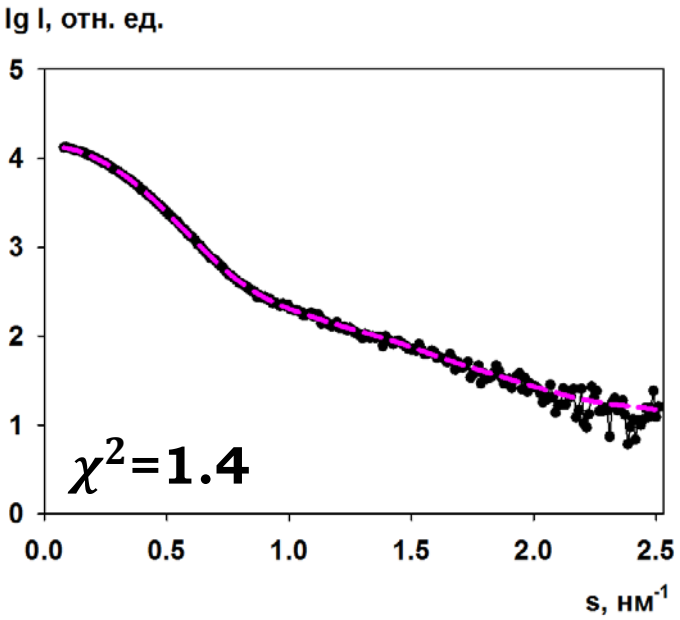
Condition of the sample preparation

The composition of the buffer:

1. 100 mM Na-acetate, **10 mM NaCl**, 1 mM DTT, pH 4.6.
2. 100 мМ Na-acetate, 1 mM DTT, pH 4.6.

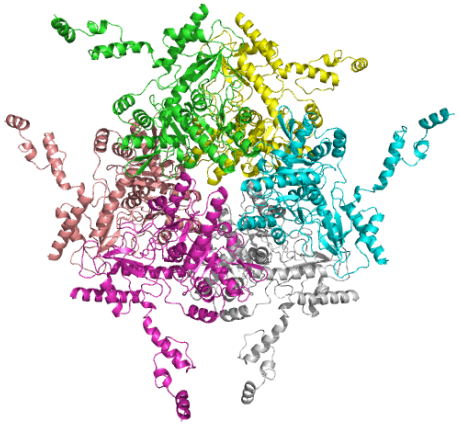
Sample	R_g, nm	$MM_{I(0)}$, kDa	MM_{Porod}, kDa	$R_{g\ cryst}$, nm	MM_{aa}, kDa
GadA	4.8±0.1	249±15	252±15	4.2	316
GadA, low salt	4.4±0.1	260±15	265±15	4.2	316

Glutamate decarboxylase (GadA)

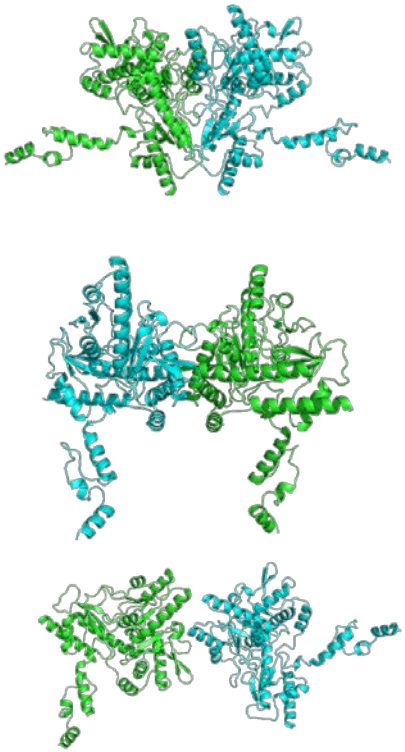


PDB:1XEY

SASBDB: SASDB33

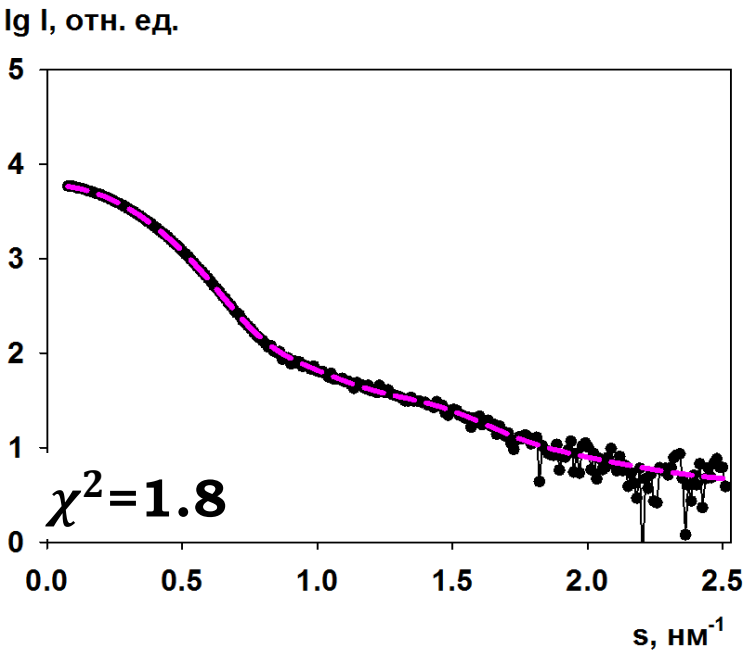


Volume fraction of hexamers 60%

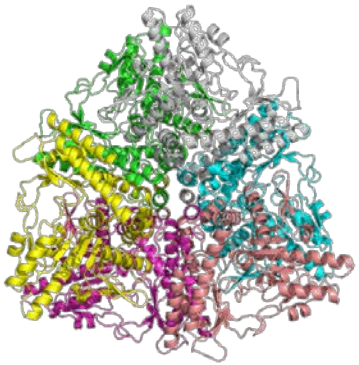


Volume faction of dimers 40%

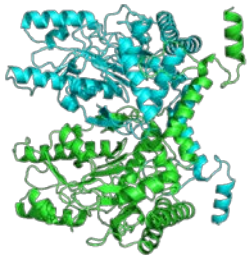
Glutamate decarboxylase (GadA)



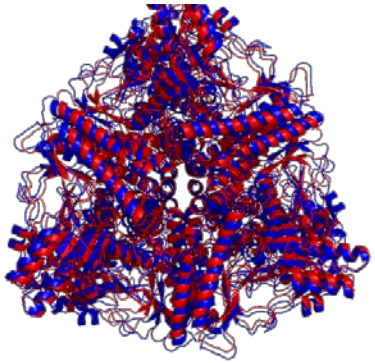
SASBDB: SASDBS4



Volume fraction of hexamers 80%



Volume fraction of dimers 20%



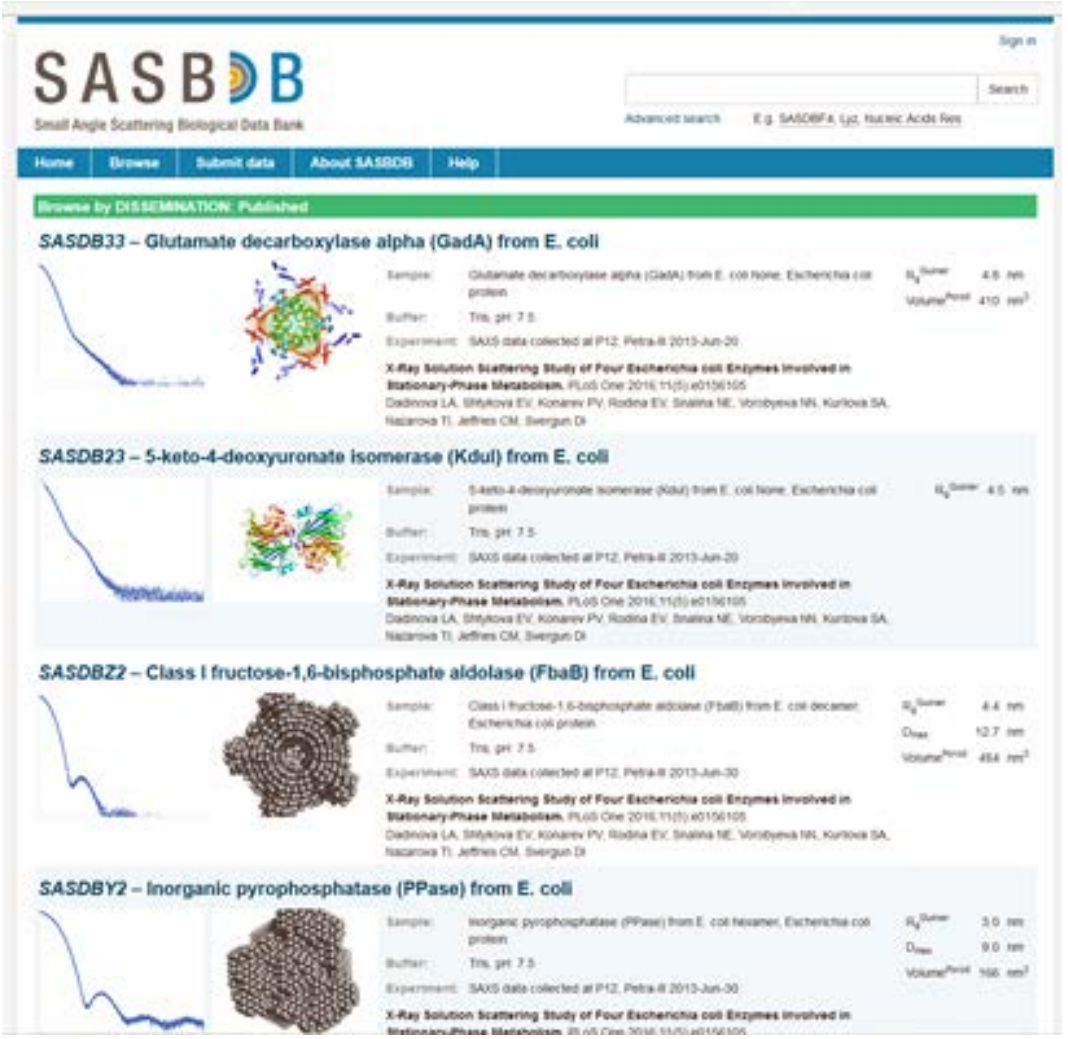
Comparison of hexamer structures: **crystal structure**, and **modeled by program SASREFMX**

IMPORTANT: All structural rearrangements in solution observed by us could be crucial for revealing new binding sites that form additional protein-protein interfaces for modulating enzyme activity within cells.

Small-Angle Scattering Biological Data Bank

http://www.sasbdb.org/browse-dissemination/

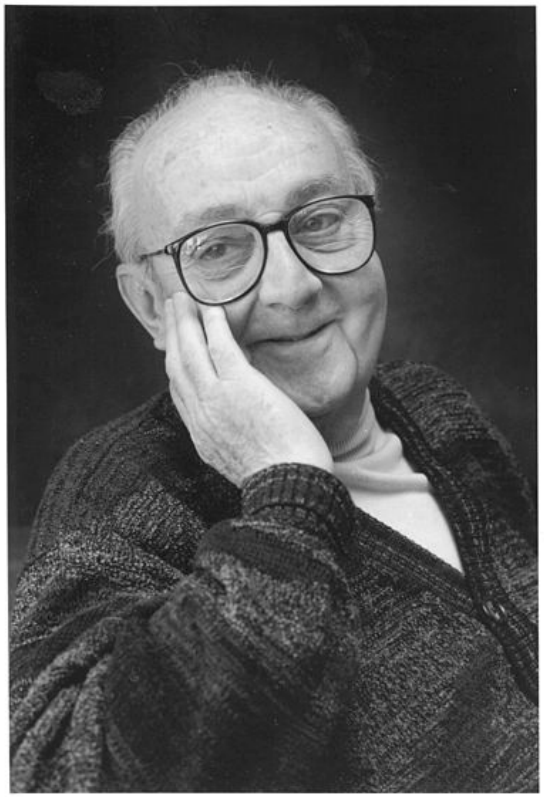
All obtained structures were placed in SASBDB



Investigations were mostly performed by L. Dadinova (postgraduate student):

Liubov A. Dadinova, Eleonora V. Shtykova, Petr V. Konarev, et al. X-Ray Solution Scattering Study of Four Escherichia coli Enzymes Involved in Stationary-Phase Metabolism. *PLoS One*, 2016, 11(5): e0156105. doi:10.1371/journal.pone.0156105

About the benefits of modeling



"Essentially, all models are wrong, but some are useful”

George Edward Pelham Box,
British statistician

I invite you to see more examples of SAXS application during the poster session today:
posters 18, 20, 21, 22 and 47

The main conclusion

SAXS and advanced SAXS data analysis methods can be employed to systematically characterize structure of different complicated nanosized systems which can be used in biology and medicine.

Thanks for your attention!