

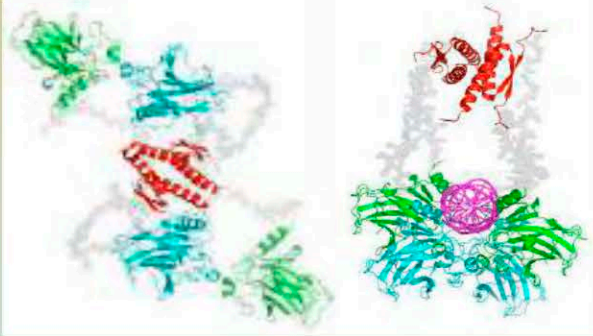


Некоторые примеры решения задач определения формы (EMBL, станция X33)

Complexes and assemblies

Domain and quaternary structure

Tumor suppressor p53 and its complex with DNA



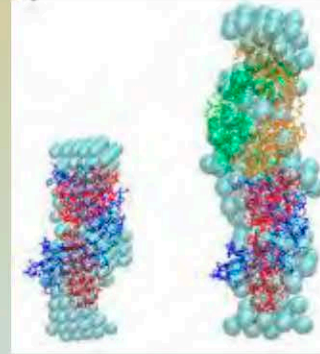
Tidow *et al*
PNAS USA (2007)

Insulin fibrillation



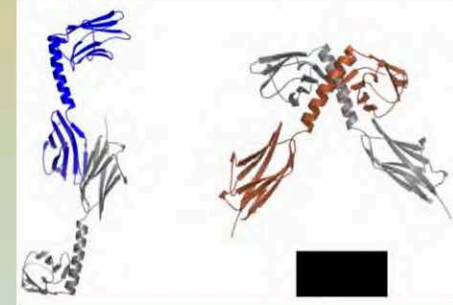
Vestergaard *et al*
PLoS Biol (2007)

Fab-dye interactions



Hillig *et al*
JMB (2008)

Myomesin-1 dimer

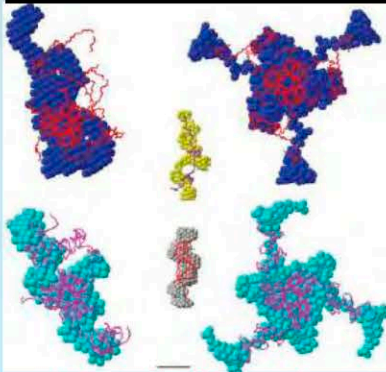


Pinotsis *et al*
EMBO J (2008)

Flexible loops and domains

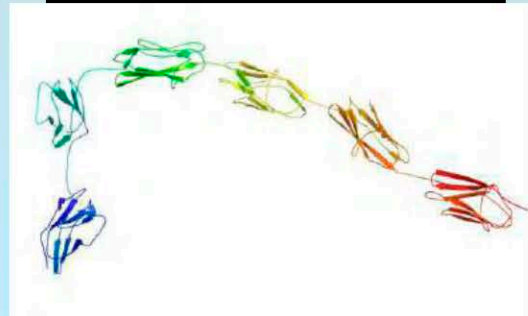
Structural transitions

(NC)-dUTPase



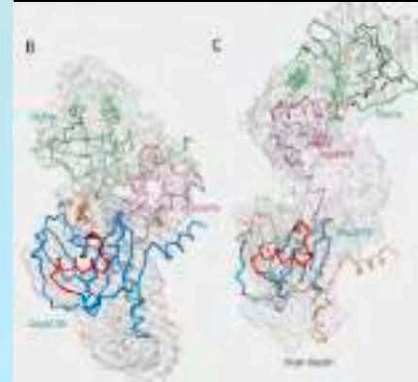
Németh-Pongrácz
et al NAR (2007)

Ig super-motifs in titin



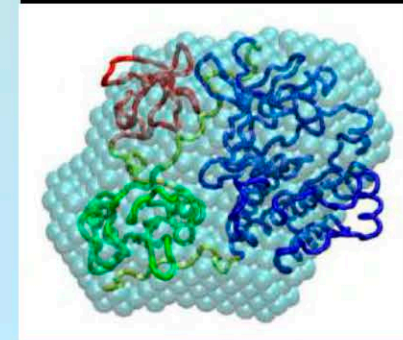
von Castelmur *et al*
PNAS (2008)

Dcp1/Dcp2 complex



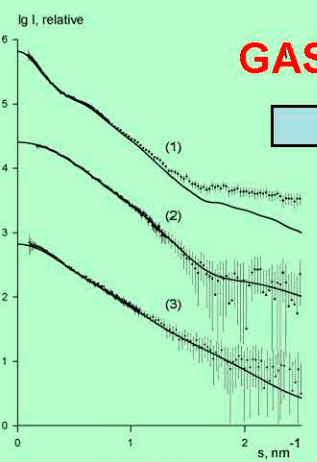
She *et al*, Mol Cell (2008)

Src kinase

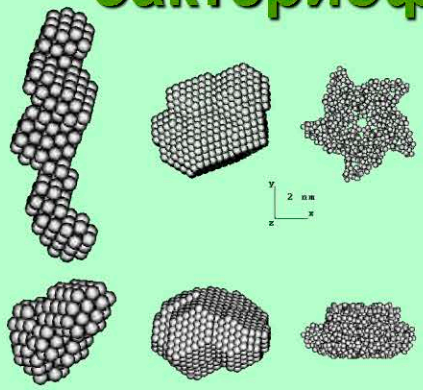
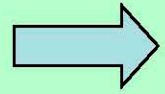


Bernado *et al*
JMB (2008)

Сочетание различных методов определения формы: структура вертексного комплекса бактериофага PRD1

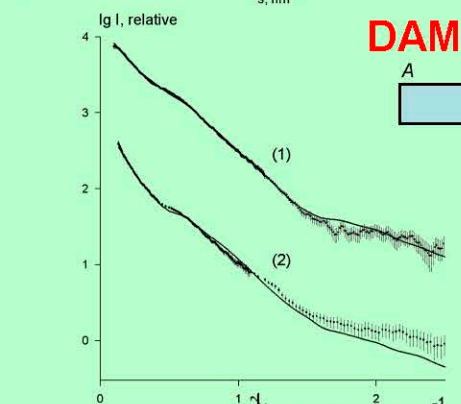


GASBOR

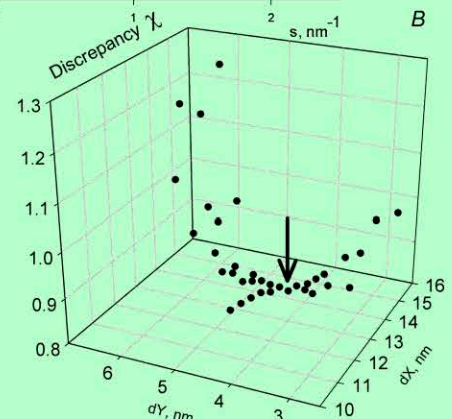
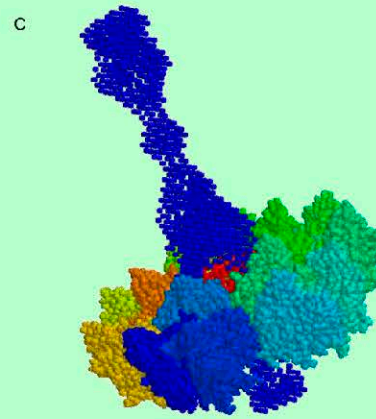
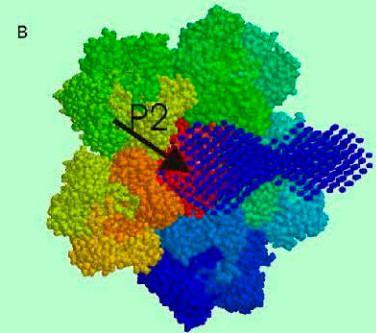
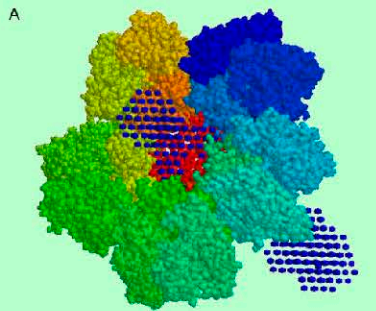
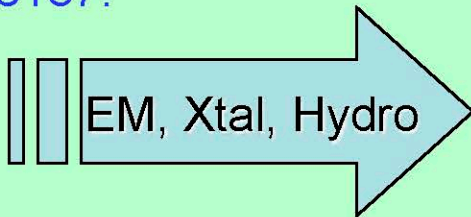
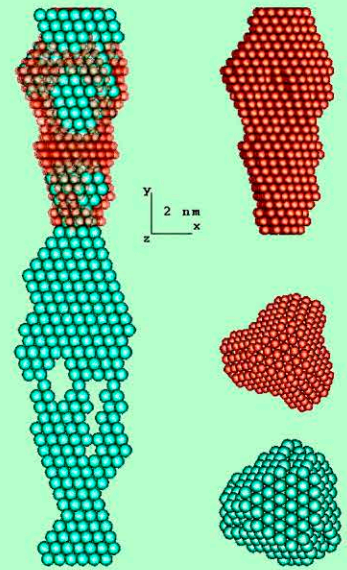
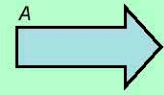


PRD1 has an icosahedral capsid similar to that of adenovirus

Sokolova, A. et al. (2001) *J. Biol. Chem.* **276**, 46187.



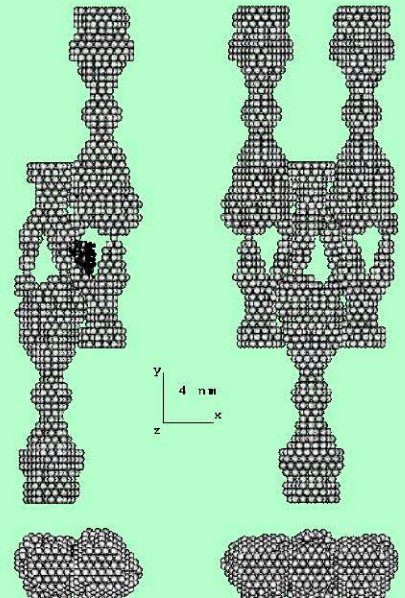
DAMMIN



MASSHA



OLIGOMER



**Нейтронное
рассеяние:
вариация контраста**

Варьирование контраста: рентгеновское рассеяние и рассеяние нейтронов

Рентгеновские лучи:
влияние сахарозы или
соли

РНК, 550 e/nm^3

60% сахарозы, 430 e/nm^3

белок, 410 e/nm^3

H_2O , 344 e/nm^3

Нейтроны:
изотопное замещение H/D

D-белки, "130%" D_2O

D-РНК, "120%" D_2O

D_2O , $6.38 \times 10^{10} \text{ cm}^{-2}$

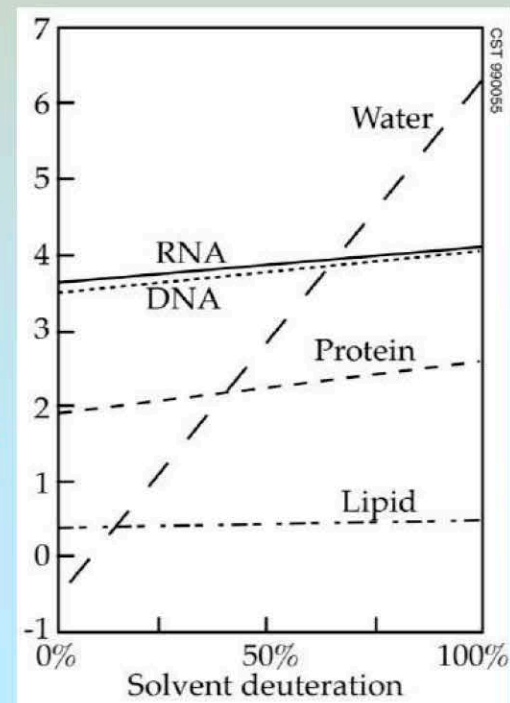
H-РНК, 70% D_2O

H-белки, 40% D_2O

H_2O , $-0.59 \times 10^{10} \text{ cm}^{-2}$

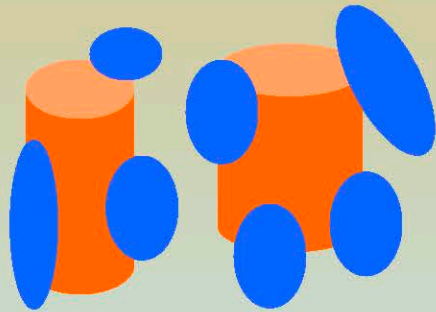
По вертикали
показана
рассеивающая
способность
в отн. ед.

Mean scattering length density (10^{10} cm^{-2})

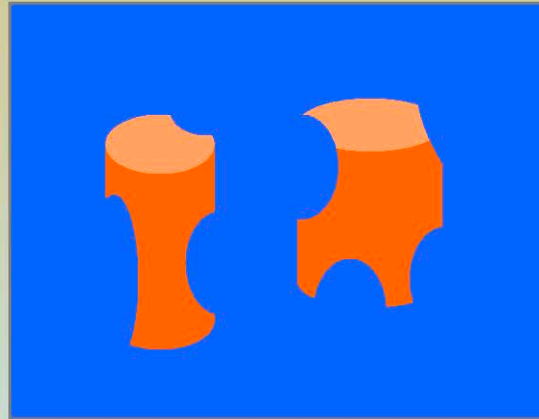


Нейтронное рассеяние от растворов рибосомы 70S: вариация контраста путем частичного дейтерирования

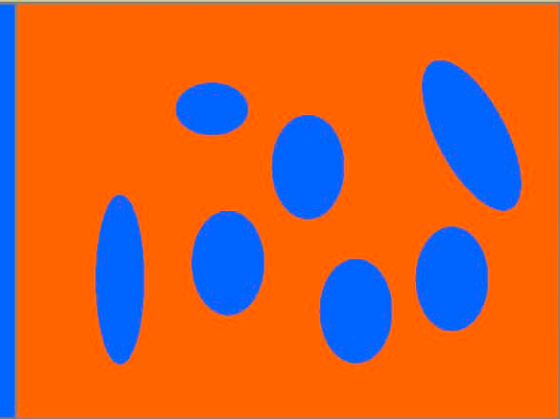
0% D₂O



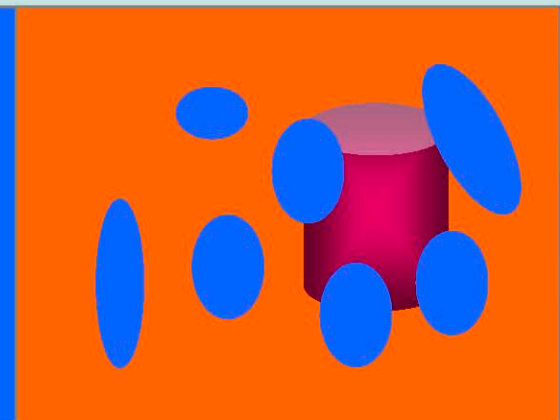
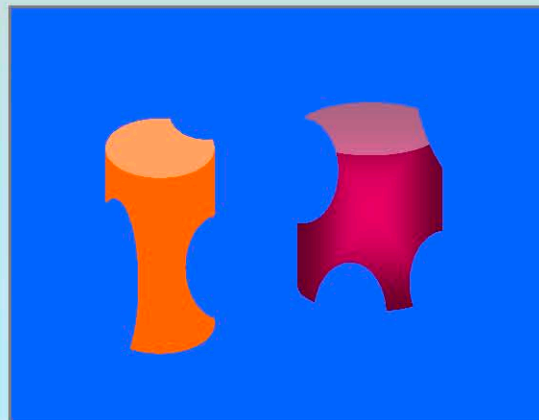
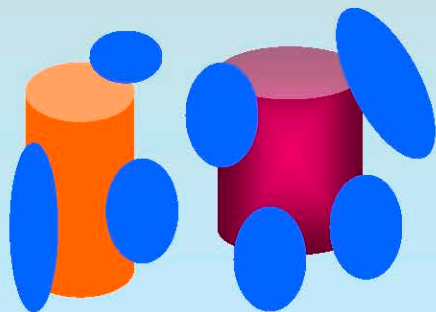
40% D₂O



70% D₂O



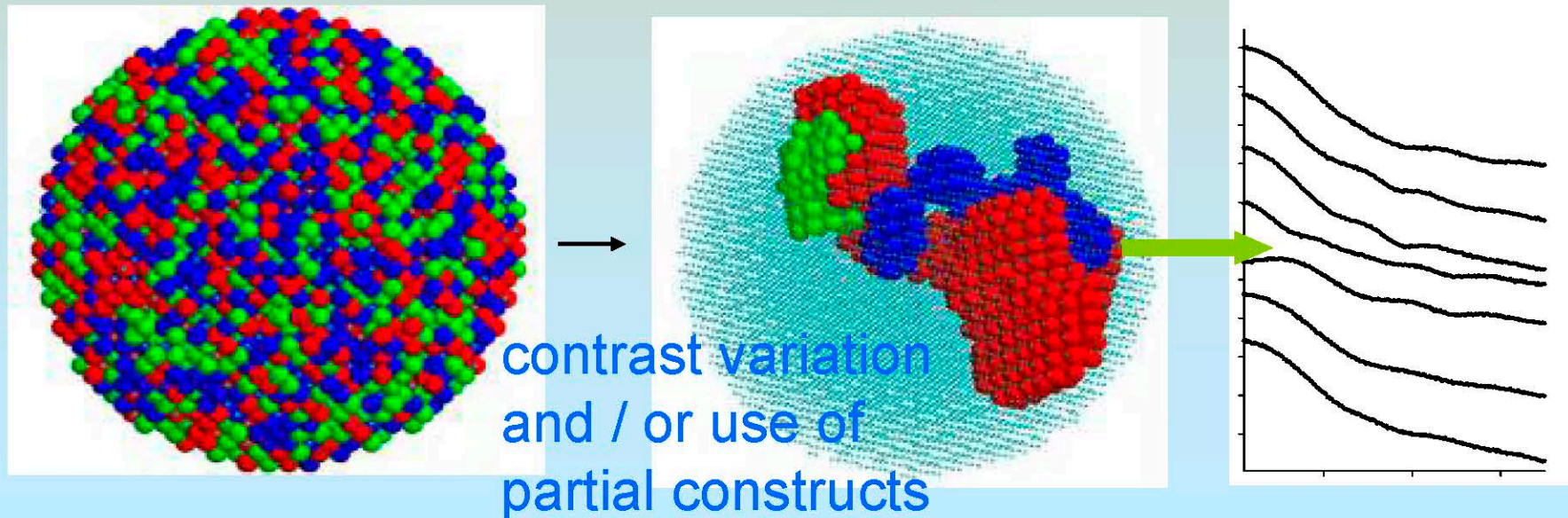
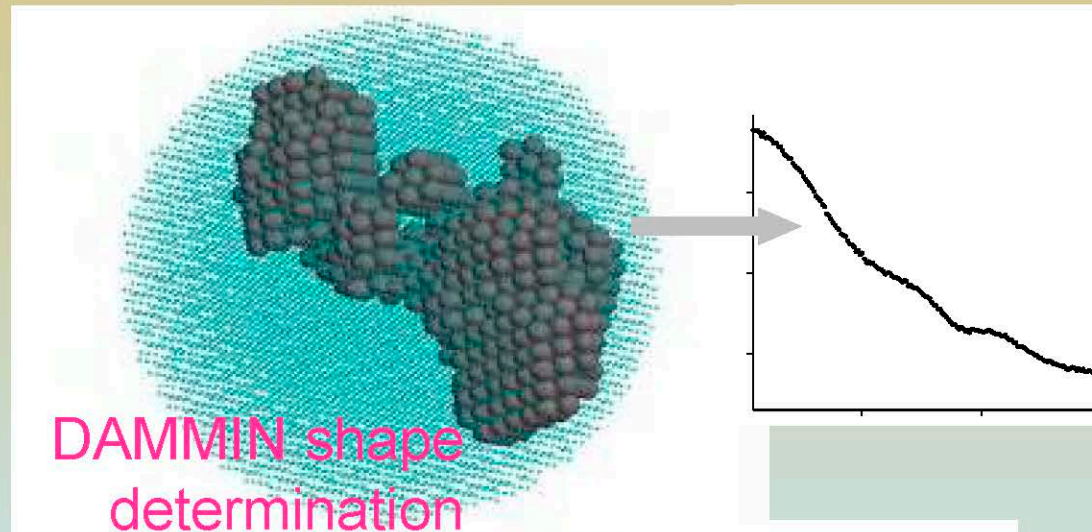
Protonated 70S ribosome, HH30+HH50



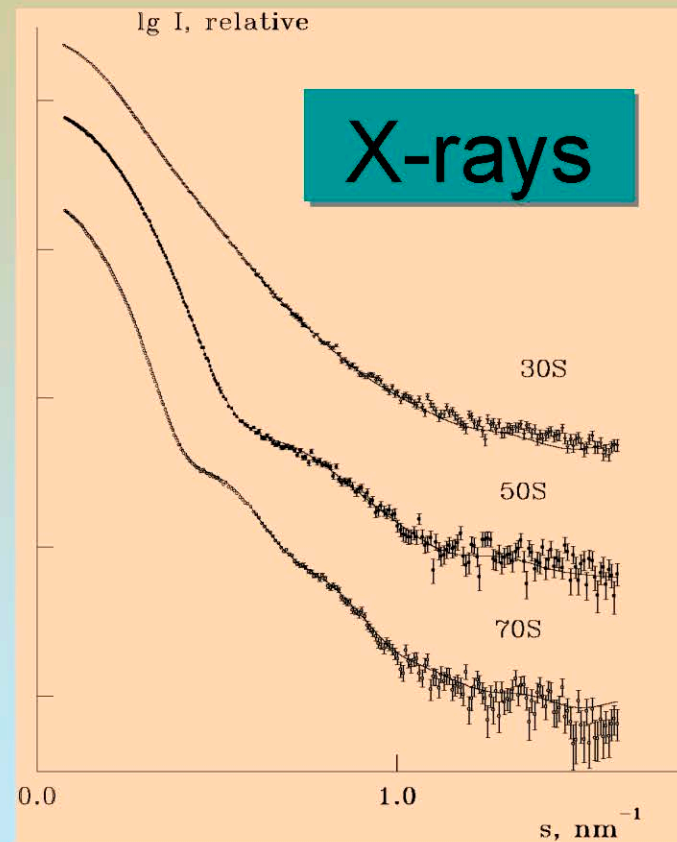
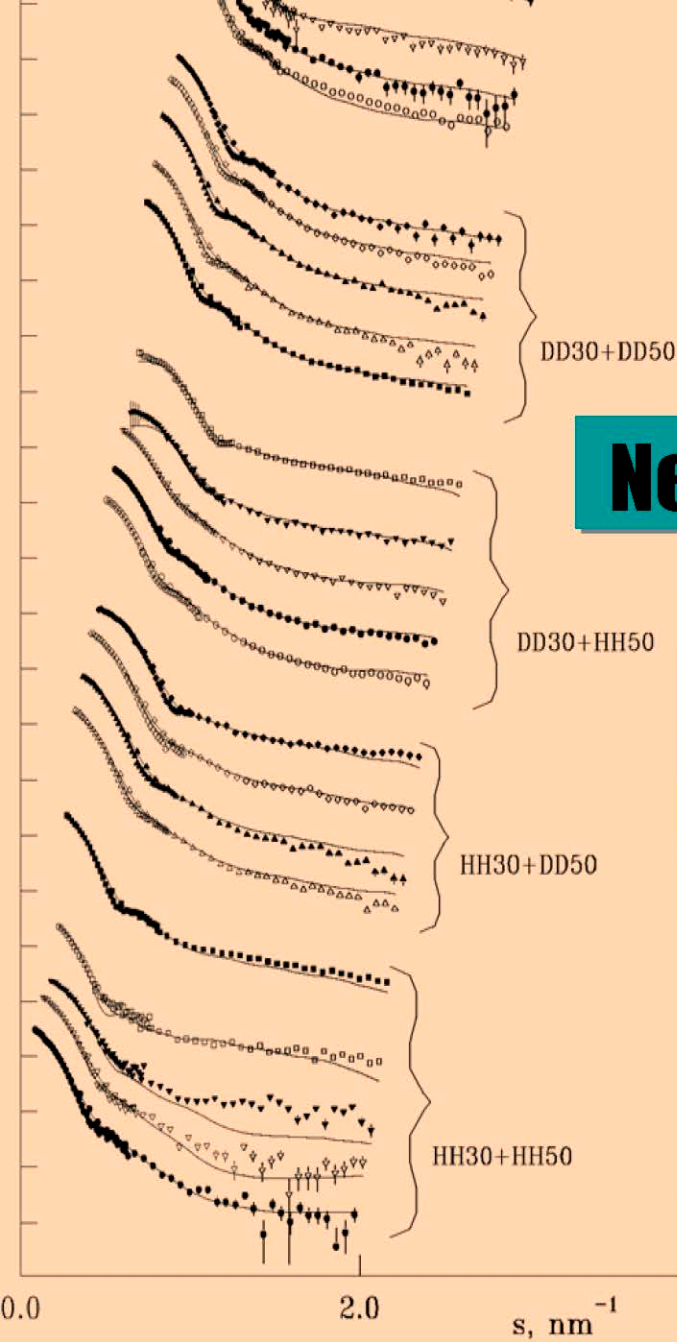
Hybrid 70S with 23S RNA deuterated, HH30+HD50

MONSA (многофазное моделирование)

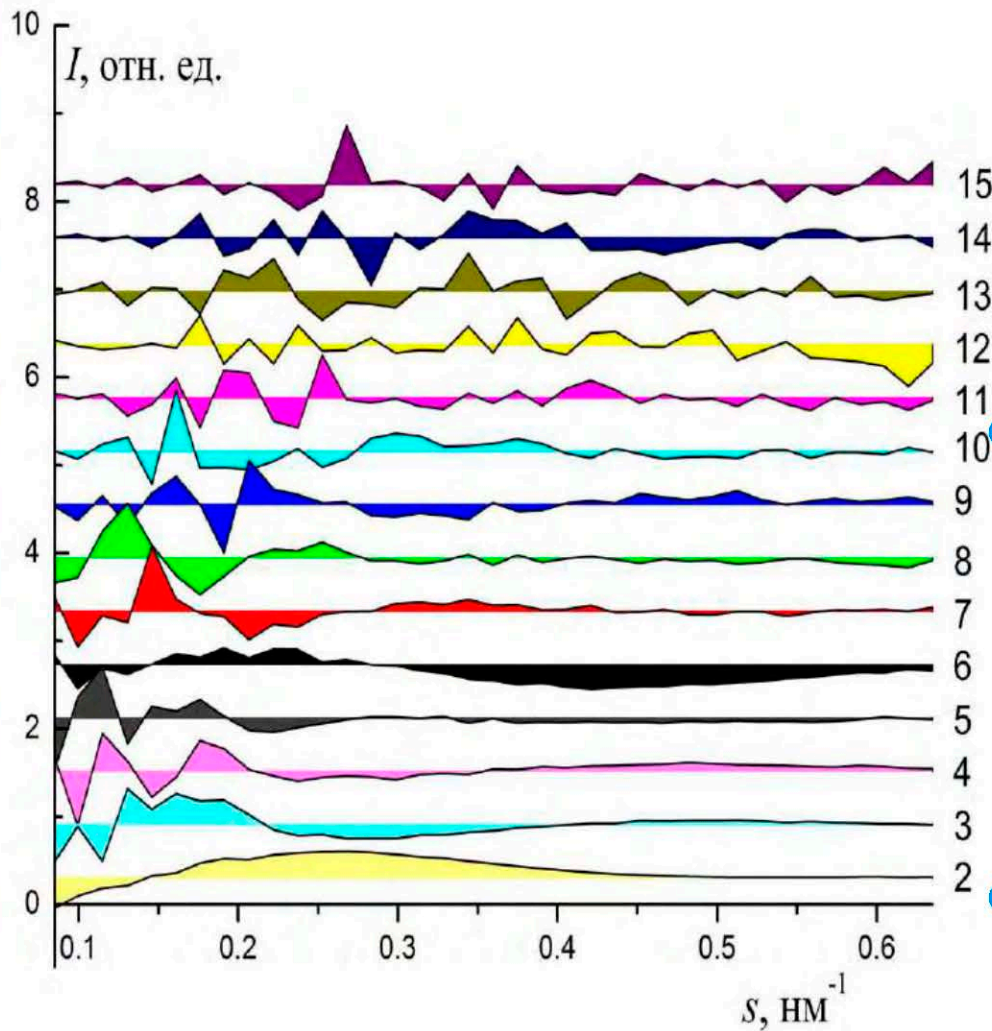
- 1 phase = 1 component of a complex particle
- For each phase, R_g , V / and or its scattering curve can be given
- For each curve, contrast of each phase are specified



Данные малоуглового рассеяния от образцов рибосомы 70S *E.coli*



Оценка числа компонентов в матрице данных рассеяния по левым сингулярным векторам



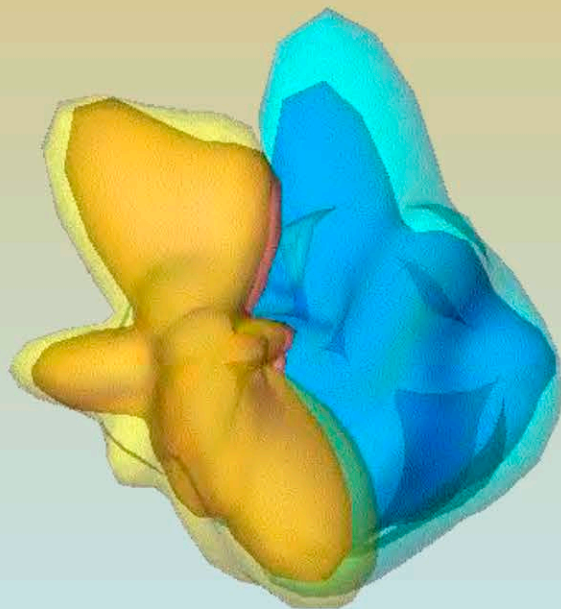
Первые 15 сингулярных векторов матрицы данных нейтронного рассеяния, представленные в виде контуров относительно вектора рассеяния.

Из рисунка видно, что систематическое поведение контуров заметно до 10 вектора включительно.

Критерий Дарбина-Ватсона наличия автокорреляции в матрице остатков F^0 после учета первых 10 компонентов в кривых рассеяния был $1.7 > 1.5$, что говорит о приемлемости оценки числа базисных функций и о числе компонентов рибосомы = 4

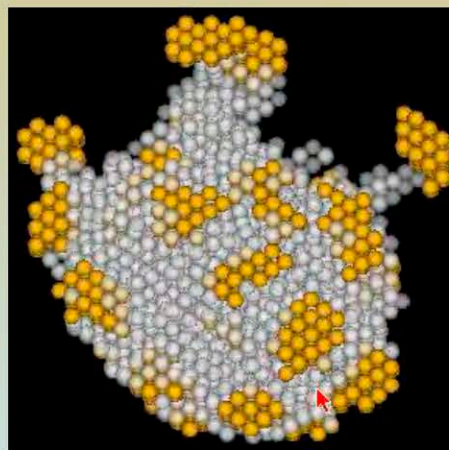
$$F^0 = D - D^0, \quad D^0 = U_{j=1,..K} \cdot \Lambda \cdot V_{j=1,..K}^T$$

Структура рибосомы 70S *E.coli* в растворе по данным рентгеновского и нейтронного малоуглового рассеяния

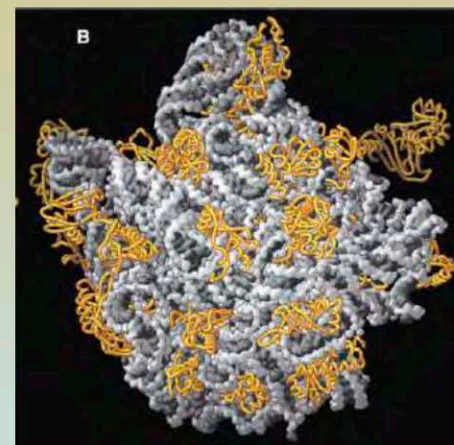


10 nm

D.I.Svergun, N.Burkhardt,
J.Skov Pedersen, M.H.J.
Koch, V.V.Volkov,
M.B.Kozin, et al. J. Mol.
Biol. (1997),. 271, 588-601



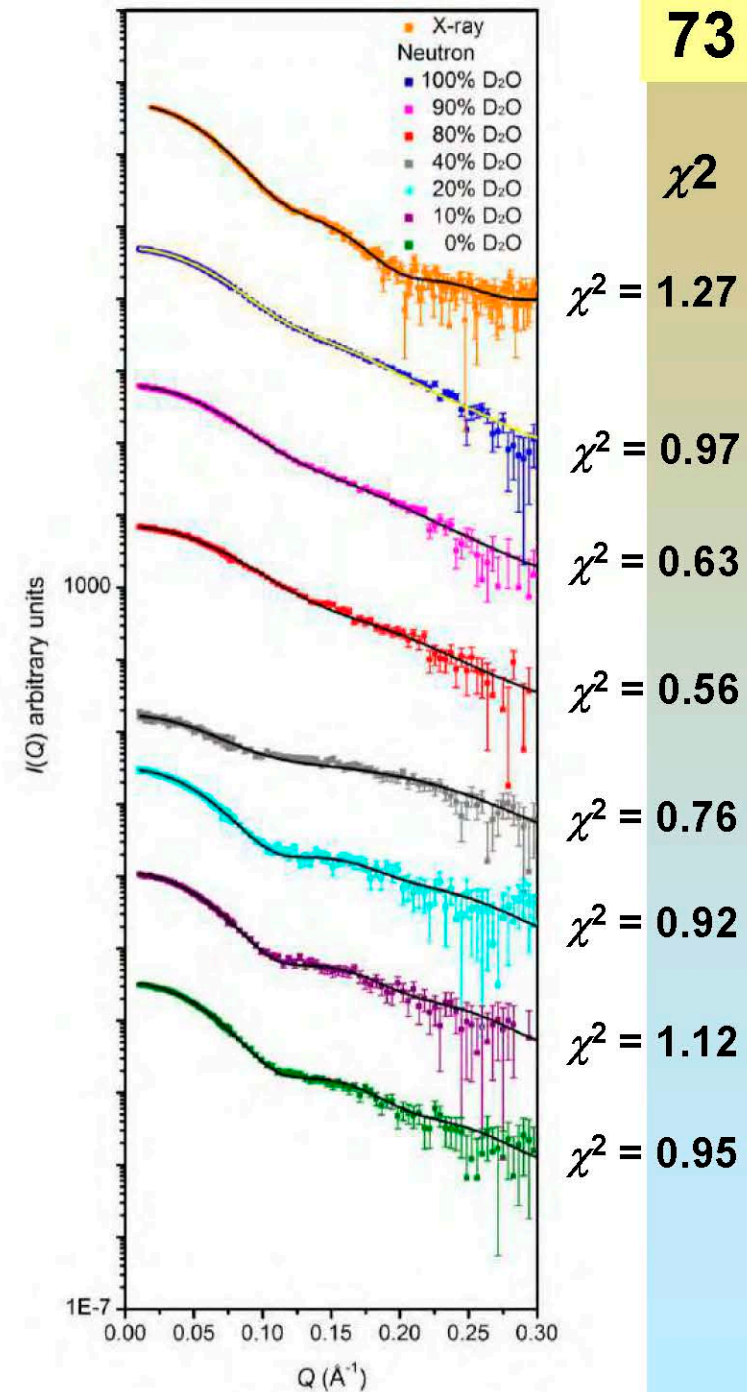
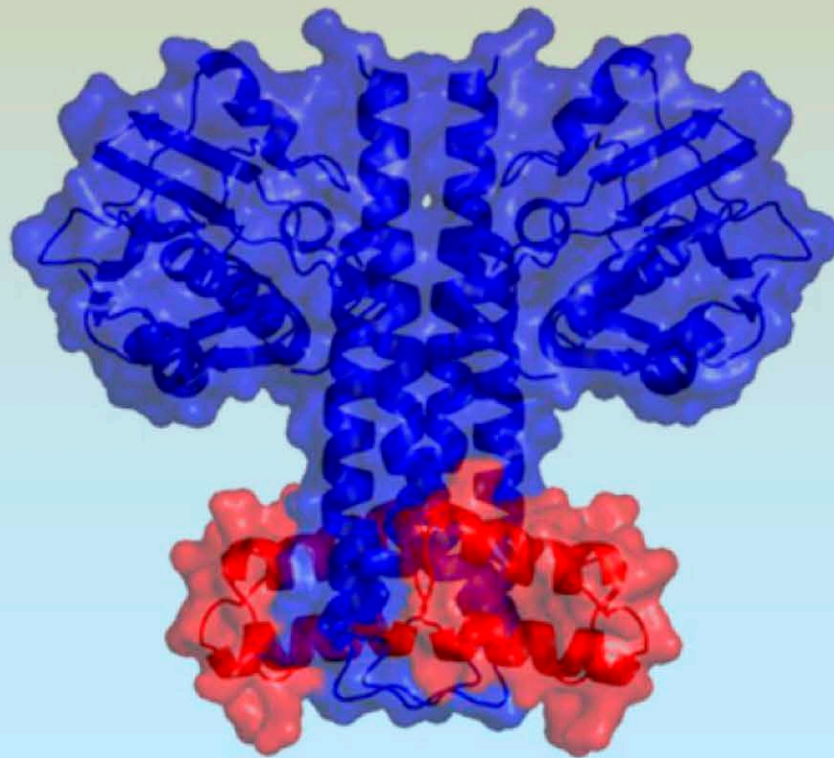
Шариковая модель
субъединицы 50S
рибосомы 70S *E.coli*
с разрешением 1 nm
по данным МУР
(Svergun & Nierhaus,
May 2000)



Для сравнения:
кристаллическая
модель
субъединицы 50S
H.marismortui
(Steitz group,
August 2000)



SASREF7: «rigid body refinement» набора компонентов с известной кристаллической структурой: нейтронное рассеяние + дейтерирование.



Data analysis software ATSAS 2.6.0

<http://www.embl-hamburg.de/biosaxs/software.html>

A program suite for small-angle scattering data analysis from biological macromolecules

Data processing

- PRIMUS - manipulations with experimental 1D SAS data
 GNOM - indirect transform program that evaluates the particle distance distribution function $p(r)$
 Data manipulation and analysis tools - AUTORG, ALMERGE, DATGNOM, DATPOROD etc.

Ab initio methods

- DAMMIN - ab initio shape determination using a dummy atom model
 DAMMIF - rapid shape determination
 GASBOR - reconstruction of a protein structure by a chain-like ensemble of dummy residues
 MONSA - shape determination using a multiphase dummy atom model

Rigid body modelling

- SASREF - modelling of multisubunit complexes
 BUNCH - modelling of multidomain proteins against multiple data sets
 CORAL - modelling of multidomain protein complexes against multiple data sets
 MASSHA - interactive modelling of atomic structures and shape analysis
 GLOBSYMM - rigid body modelling of symmetric oligomers

Mixtures and flexible systems

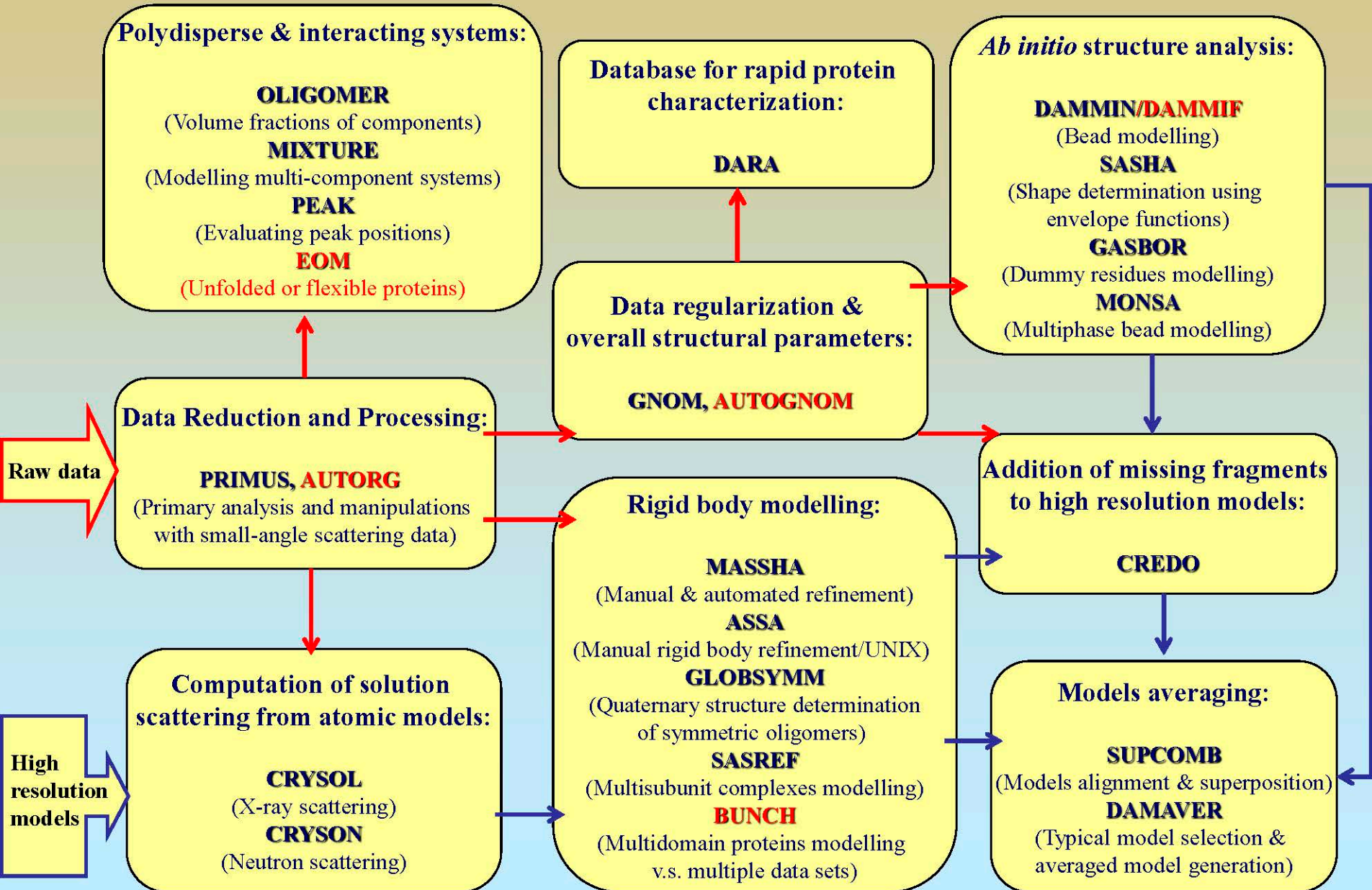
- OLIGOMER - volume fractions of mixtures with known scattering intensities from the components
 MIXTURE - modelling of multicomponent systems
 BUNCH - modelling of multidomain proteins against multiple data sets
 EOM - Ensemble Optimization Method for flexible proteins

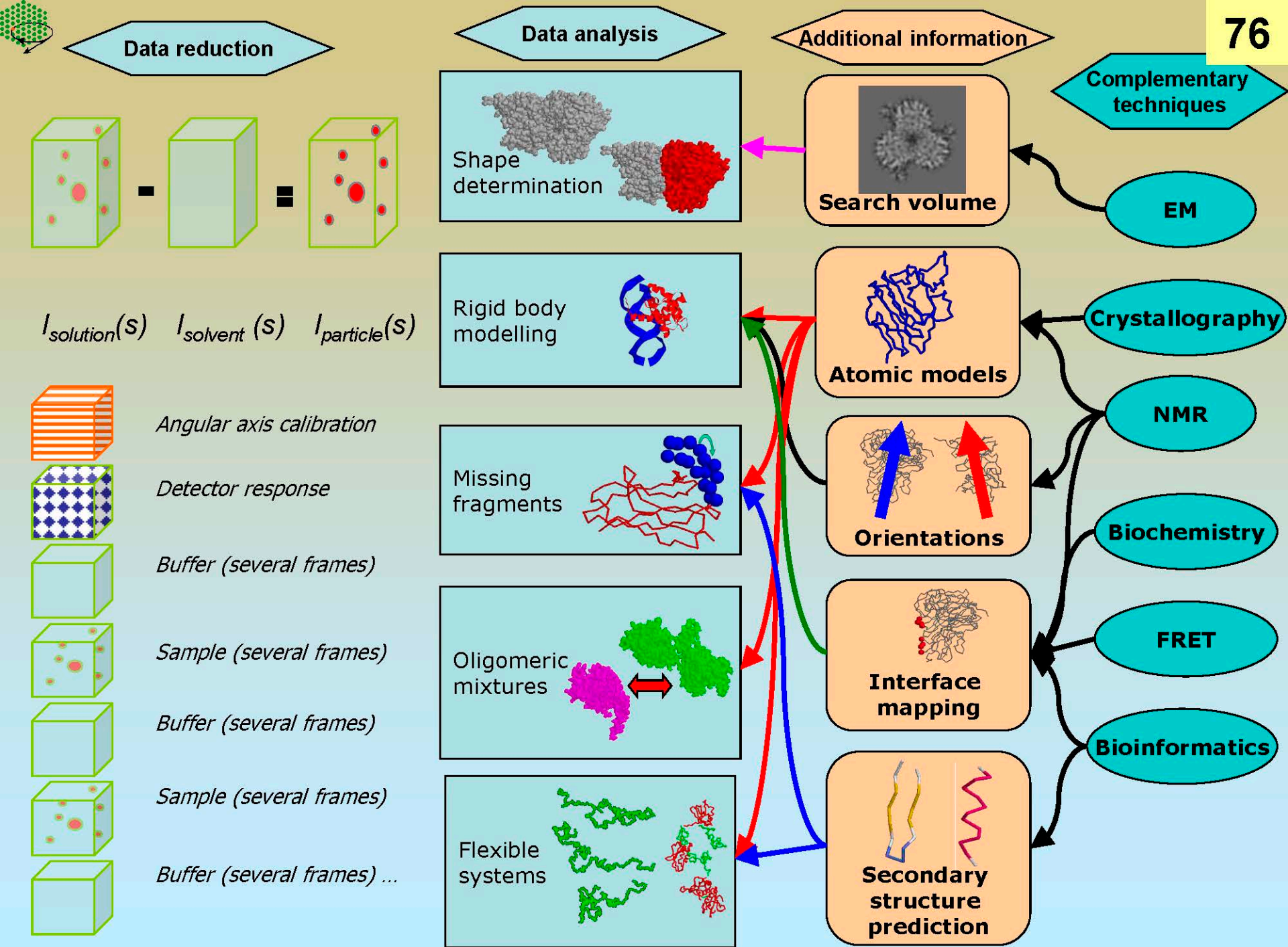
PDB oriented tools

- CRYSOL - X-ray scattering patterns from known hi-res structures
 CRYSON - neutron scattering patterns from known hi-res structures
 SUPCOMB - superimposes one 3D structure onto another
 DAMAVER - align ab initio models, select the most typical one



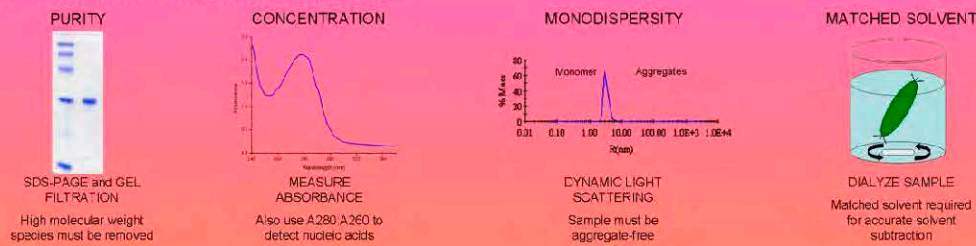
Outline of ATSAS



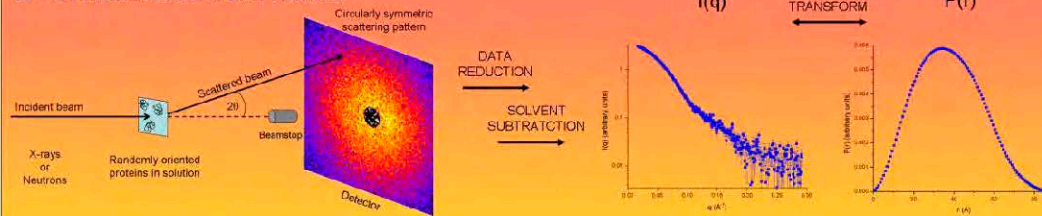


Общая схема интерпретации данных малоуглового эксперимента от растворов белковых структур

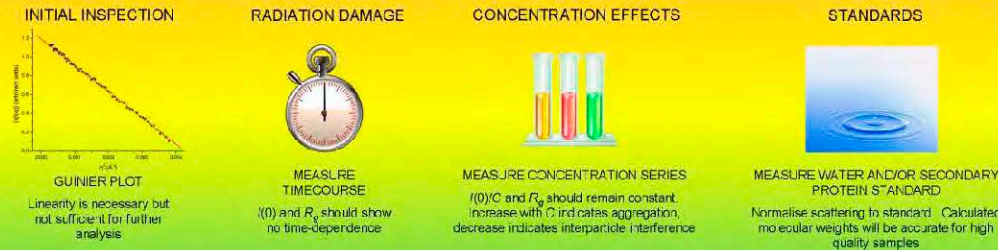
A PRELIMINARY CHARACTERISATION



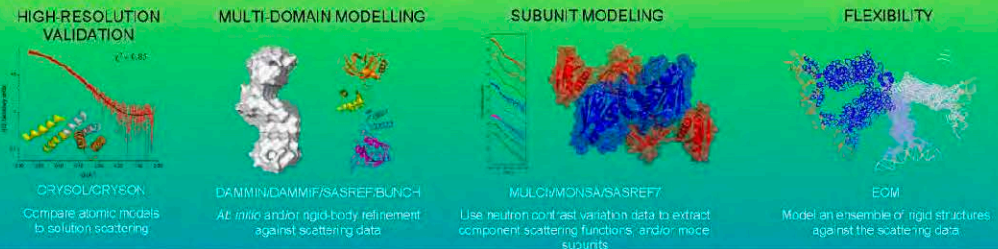
B SMALL-ANGLE SCATTERING



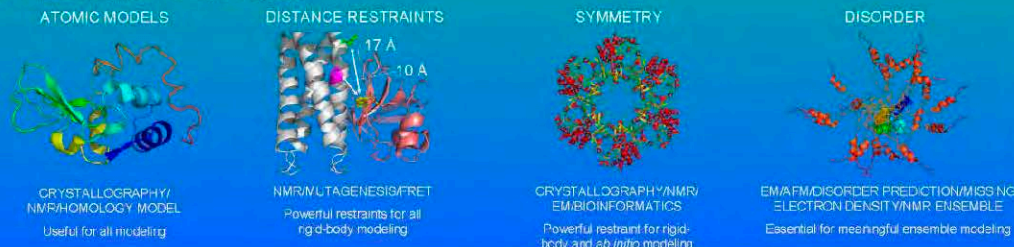
C DATA VALIDATION



D MODELING



E ADDITIONAL INFORMATION



Jacques & Trewhella (2010)
 “Small-angle Scattering for Structural Biology; Expanding the Frontier While Avoiding the Pitfalls,”
Protein Science 19, 642-657

Метод малоуглового рассеяния наиболее эффективен в сочетании с другими методами исследования структуры и обладает уникальными возможностями

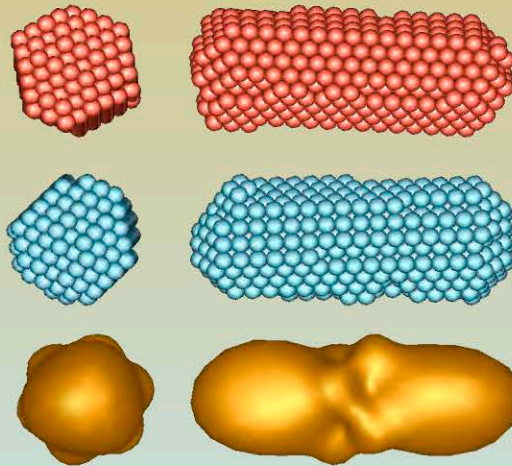
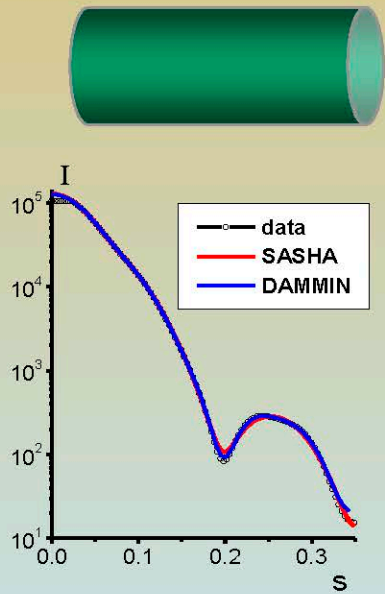


*Слайды с пометкой  взяты и адаптированы из презентаций сотрудников группы Д.И. Свергуна (EMBL c/o DESY, Гамбург)

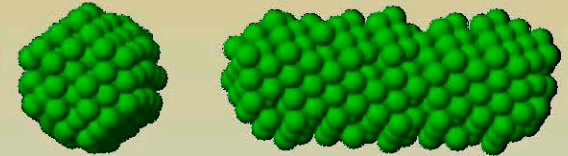
Благодарю за внимание!

Stable solutions

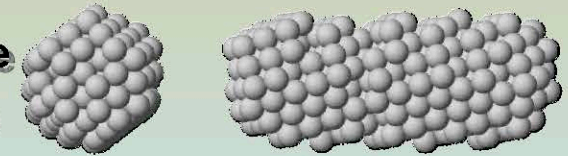
cylinder 2:5



Spread region

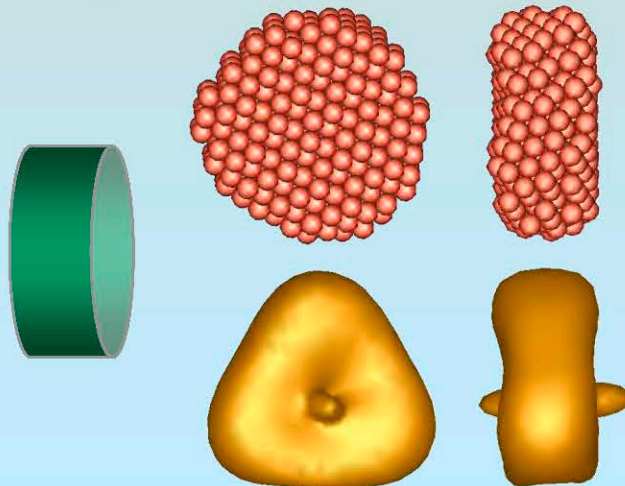
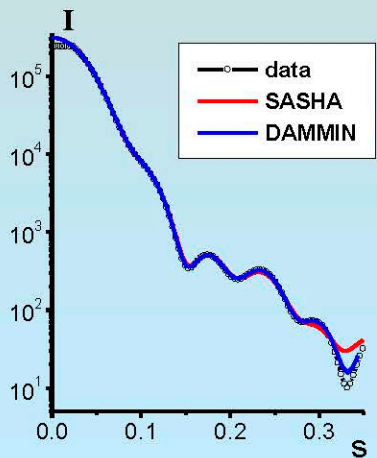


Most probable volume

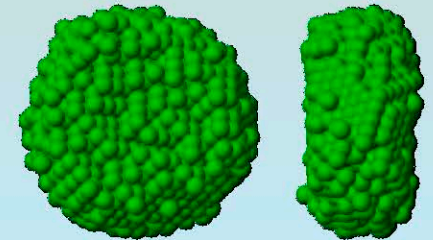


Average NSD ≈ 0.5

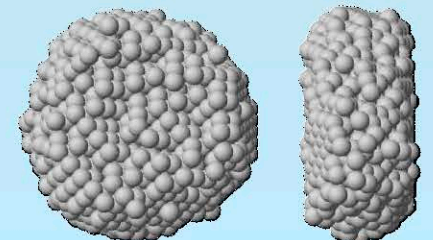
cylinder 5:2



Spread region

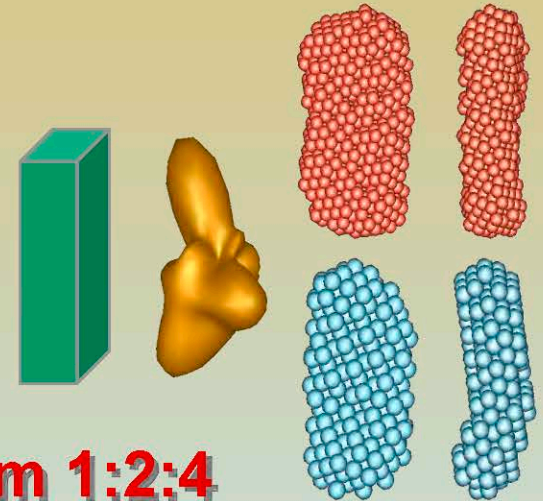
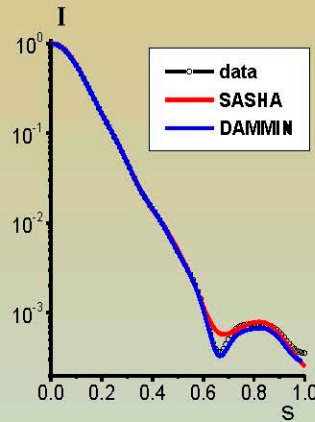
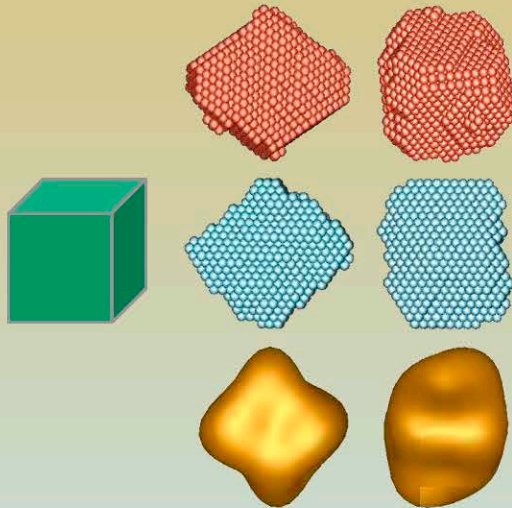
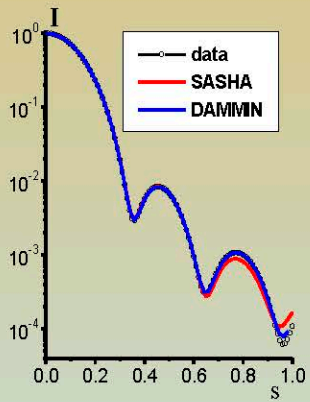


Most probable volume



Stable solutions

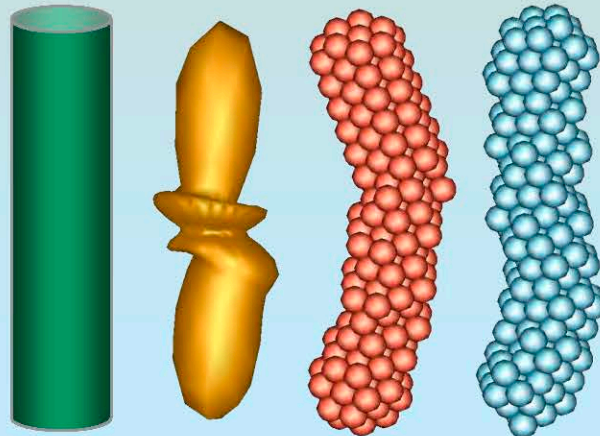
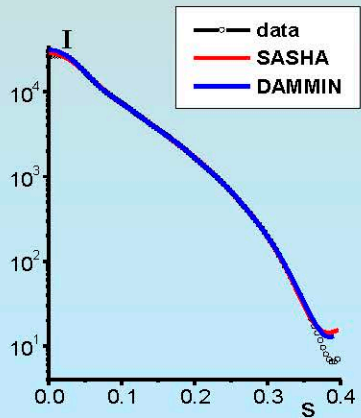
cube



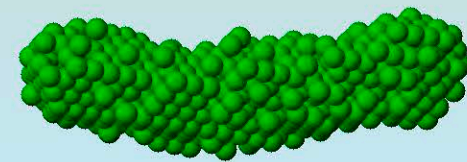
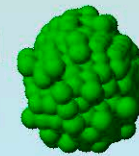
Prism 1:2:4

cylinder 2:5

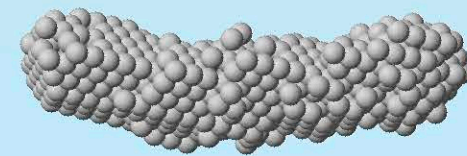
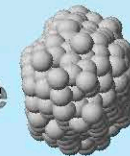
Average NSD ≈ 0.5



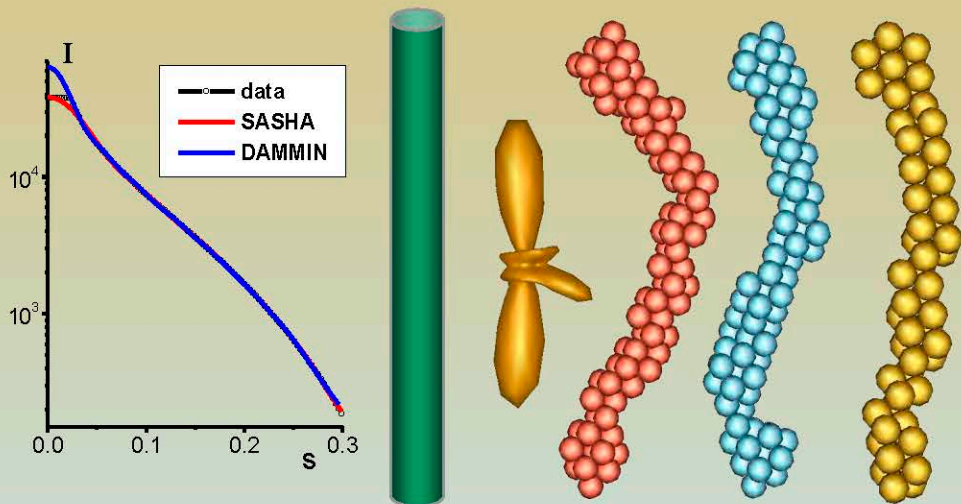
Spread
region



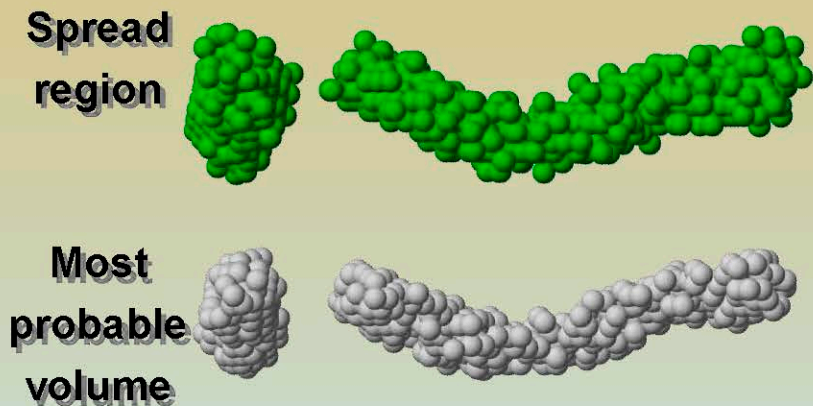
Most
probable
volume



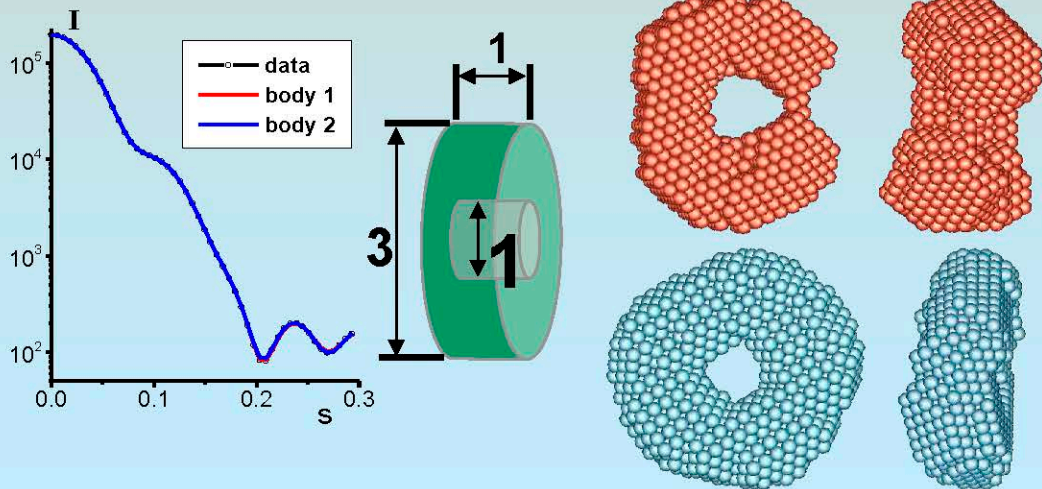
cylinder 1:10



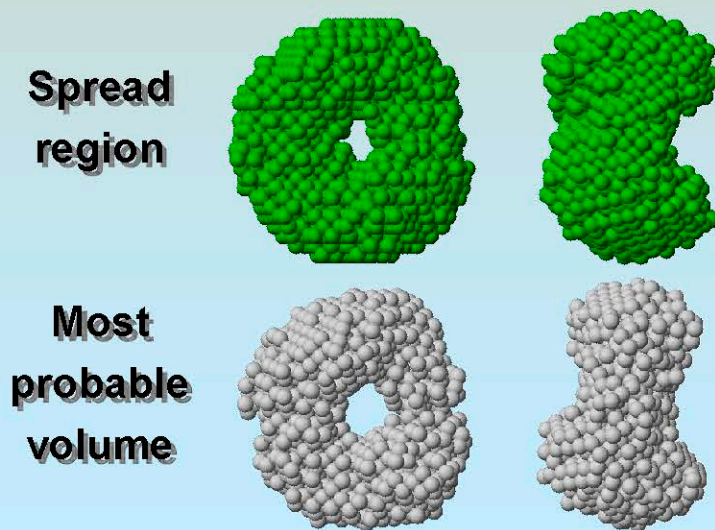
Fair stability



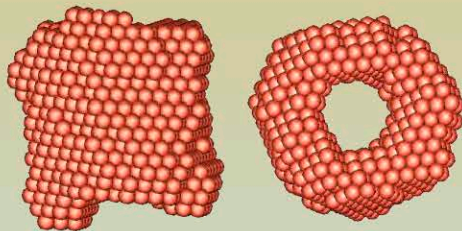
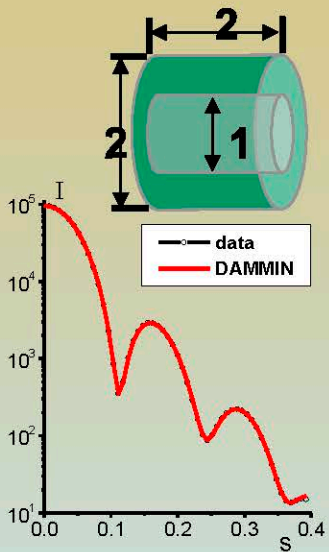
Ring 1:3:1



Average NSD ≈ 0.9

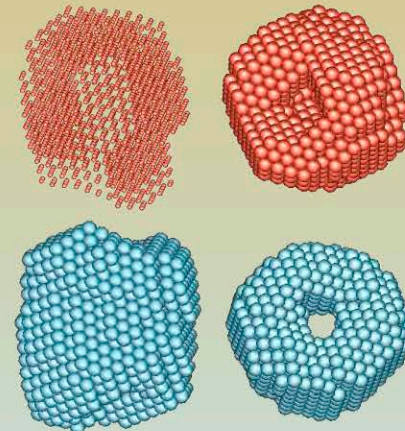
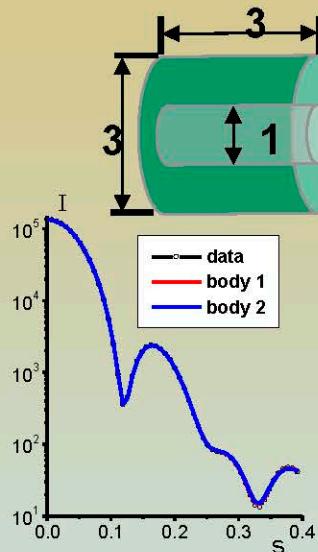


tube 1:2:2

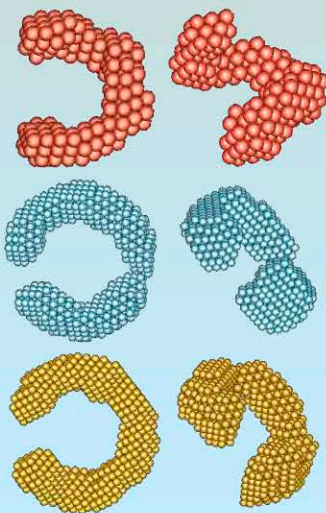
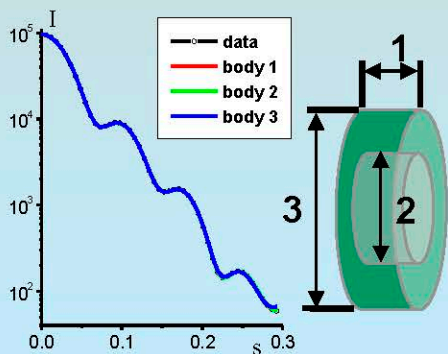


Fair stability

Tube 1:3:3

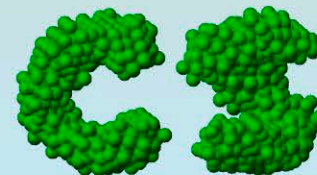


ring 1:3:2

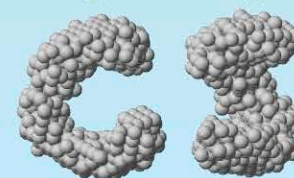


Average NSD ≈ 1.0

Spread region

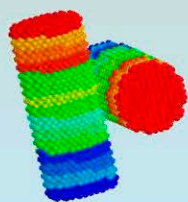
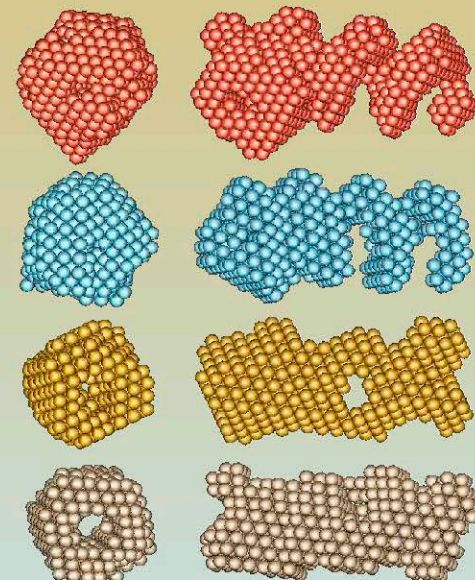
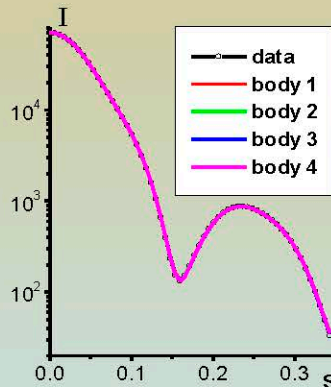
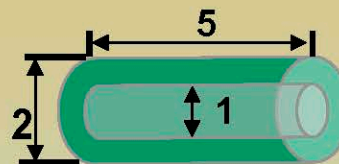
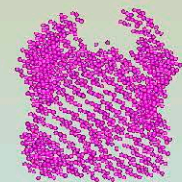
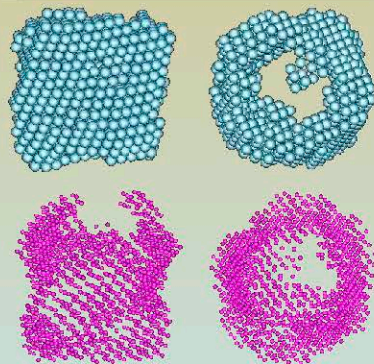
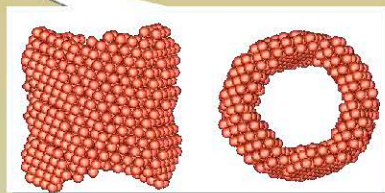
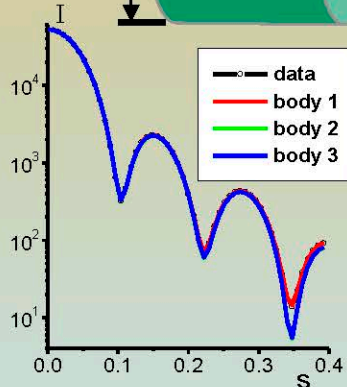
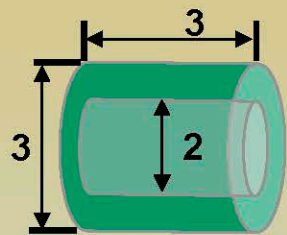


Most probable volume

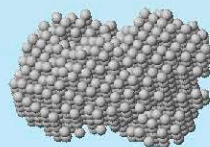
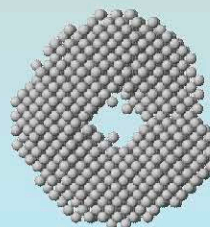
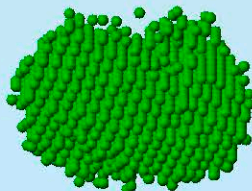
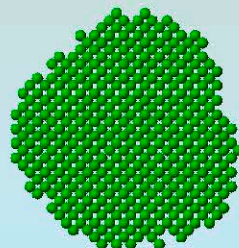
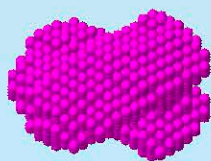
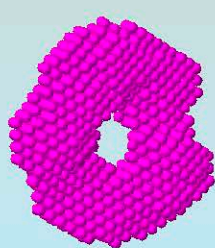


Very long search may provide more accurate model

Fair stability



This structure can not be restored without use of additional information

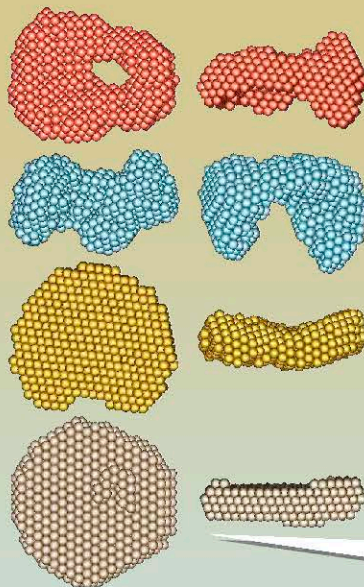
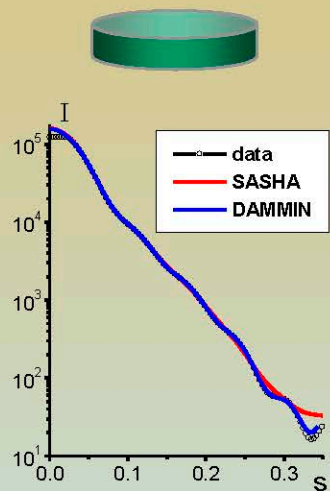


Average NSD ≈ 1.0

Spread region

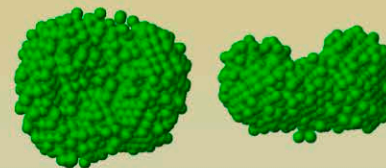
Most probable volume

Disk 5:1

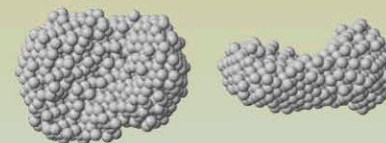


Poor stability

Spread region

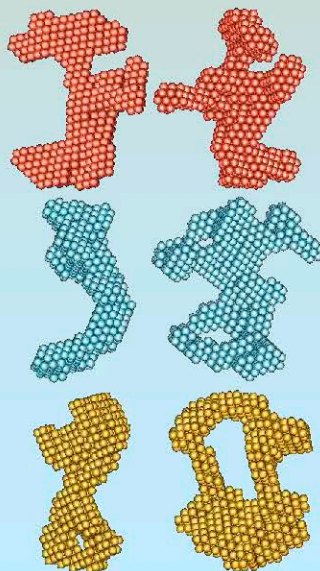
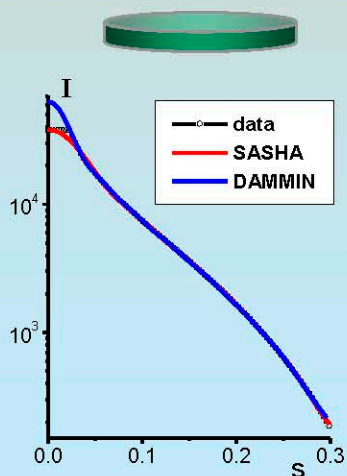


Most probable volume

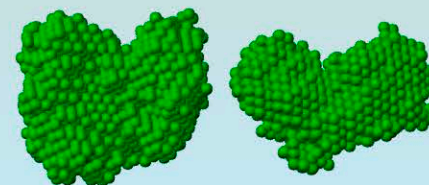


Very long search may provide more accurate model

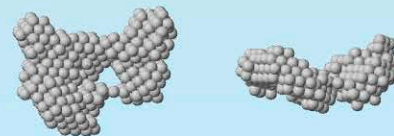
Disk 10:1



Spread region



Most probable volume

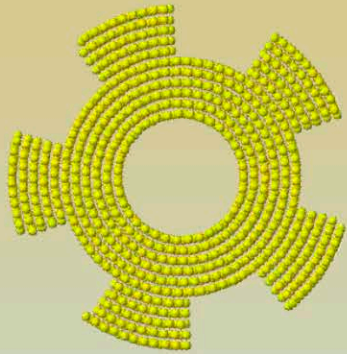


Average NSD >1.0

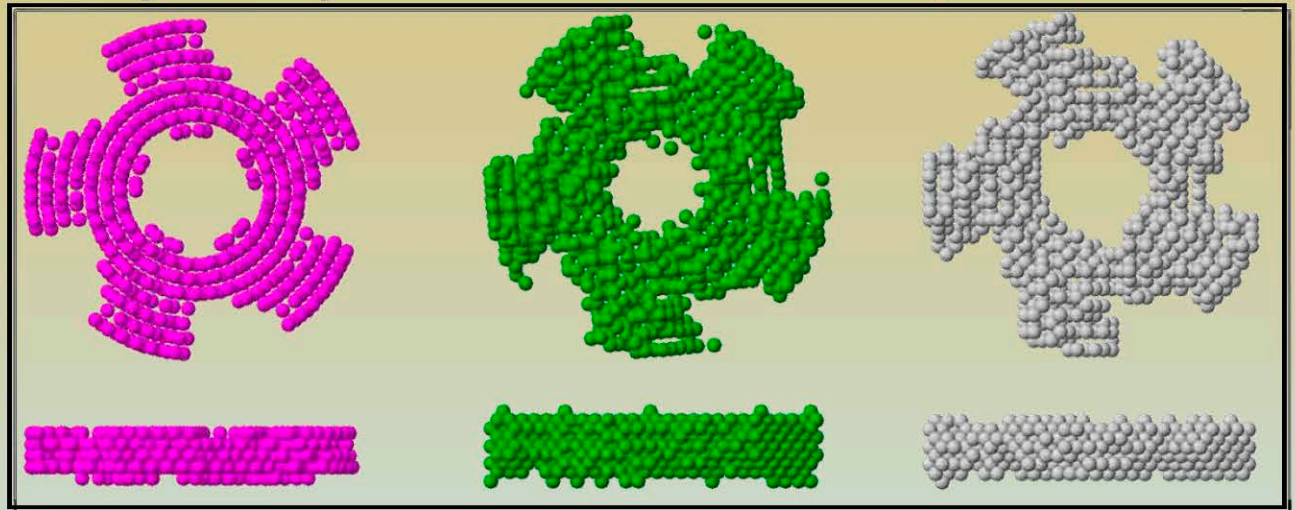
This structure can not be restored without use of additional information

Typical solution with
P5 symmetry

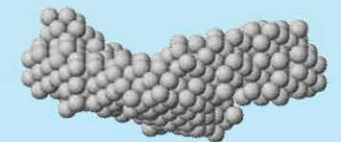
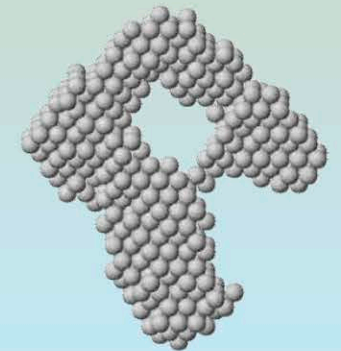
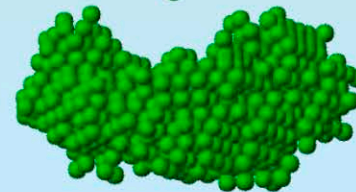
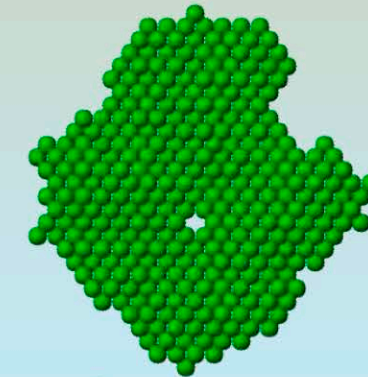
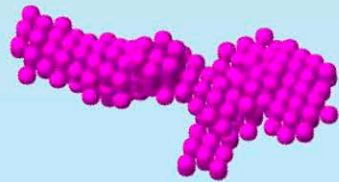
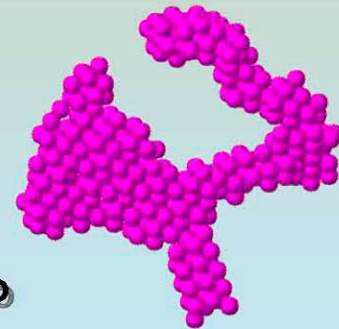
Use of symmetry



Original body



Typical solution with no
symmetry

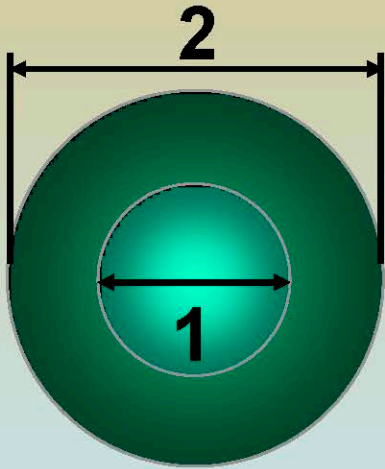


Spread region

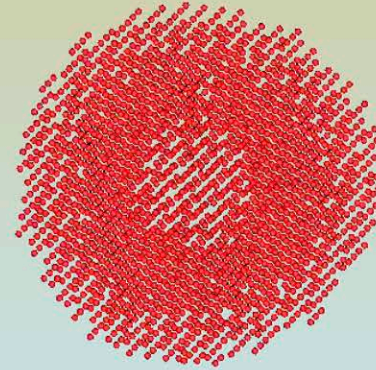
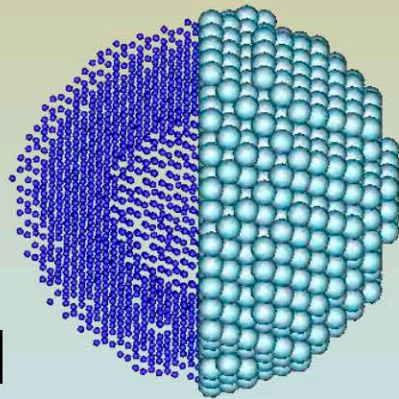
Most probable volume

Hollow spheres

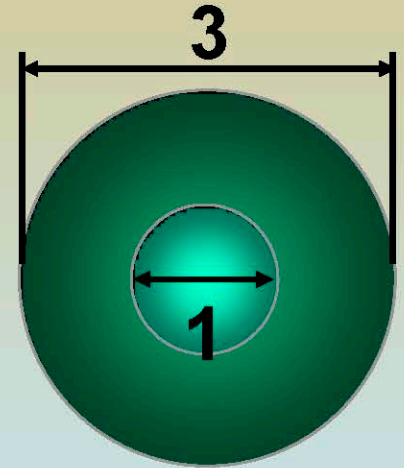
Coaxial cavities are restored with more stability than the shifted ones...



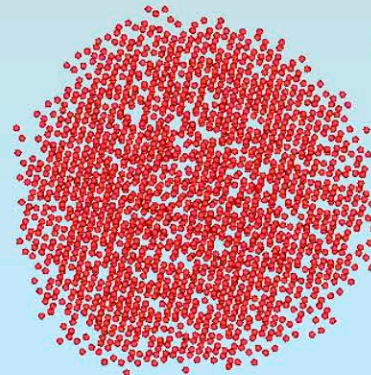
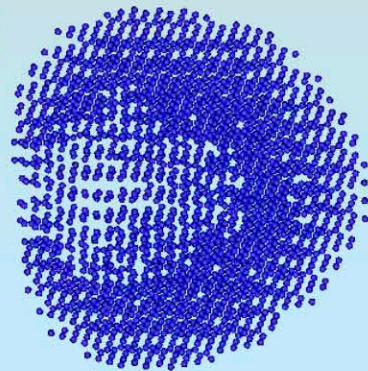
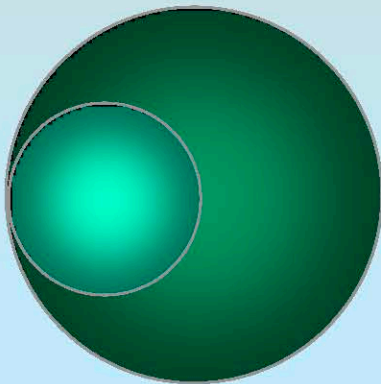
1



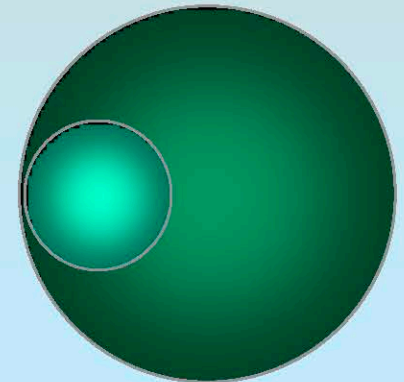
3



2



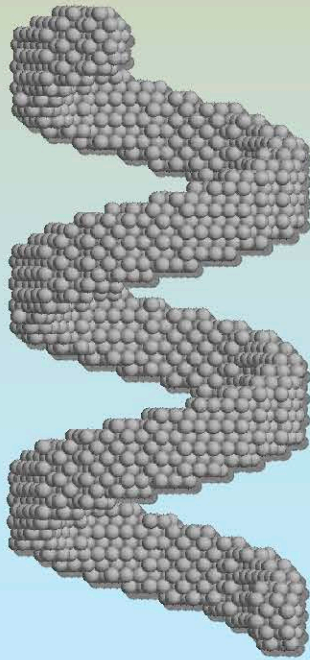
4



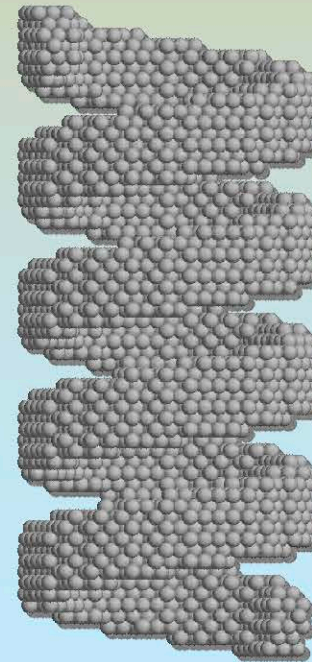
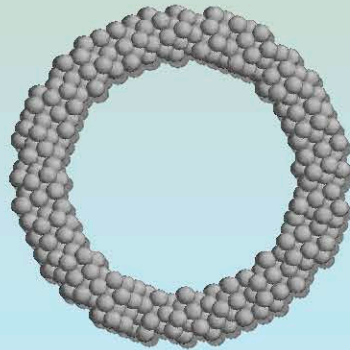
Now consider *ab initio* restoration of complex structures.

We will try to restore helix particles from simulated SAS data calculated on different angular ranges from two helicoidal bodies of the same length and diameter:

Here, the inner structure seems to be more pronounced than for the 4.5-turns model on the right



2.5-turns



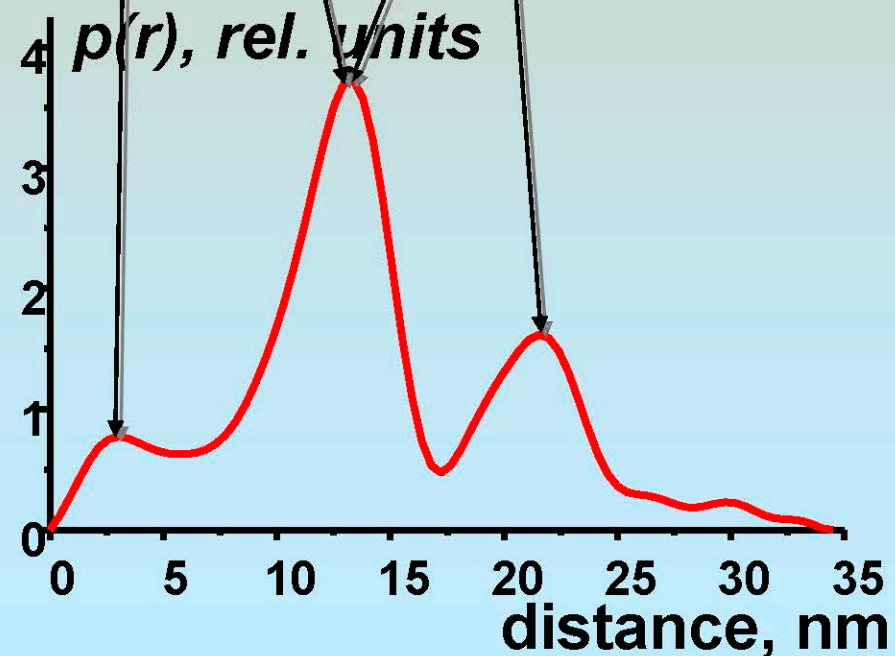
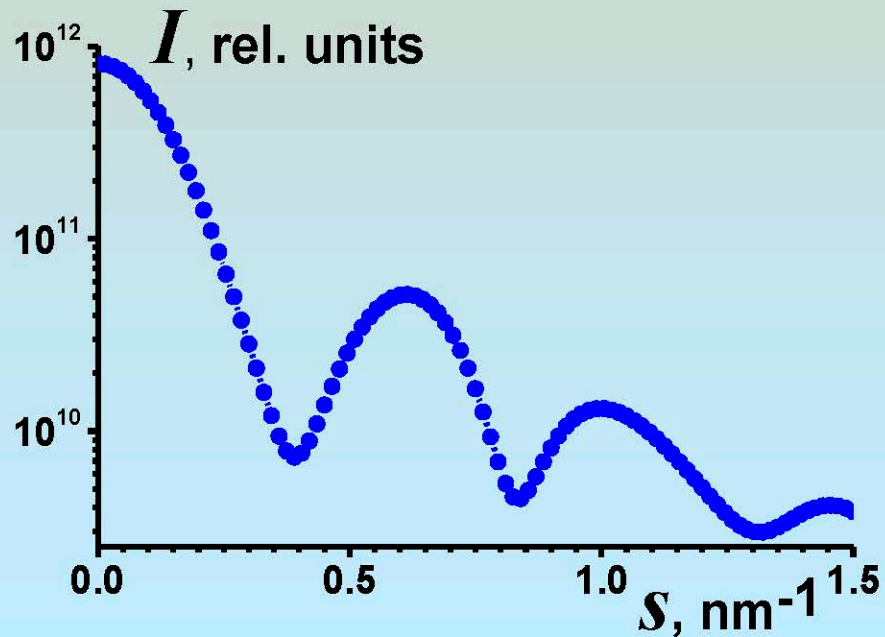
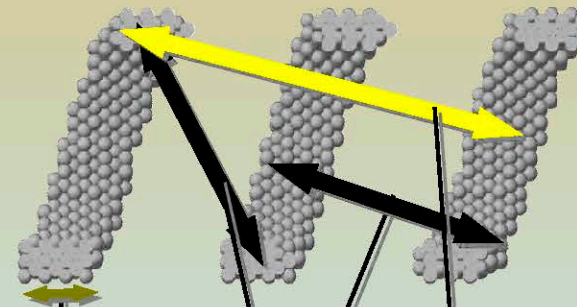
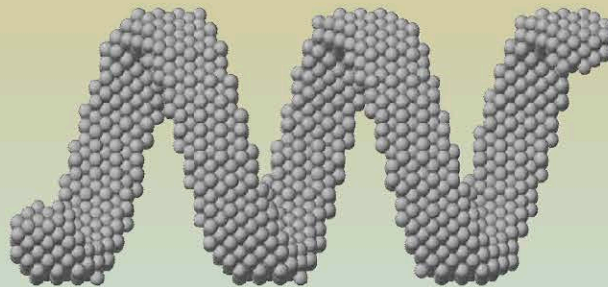
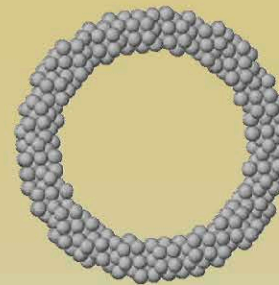
4.5-turns

This model is expected to be too complex to be restored...

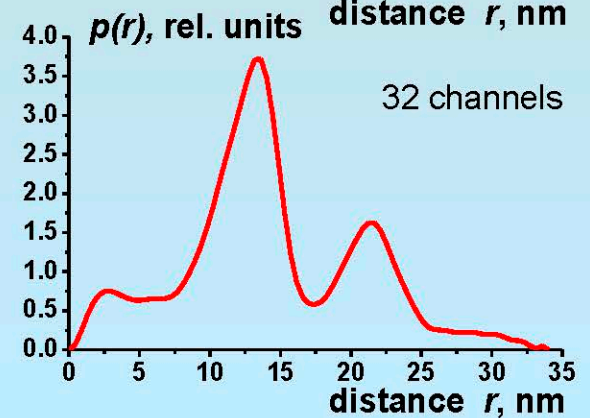
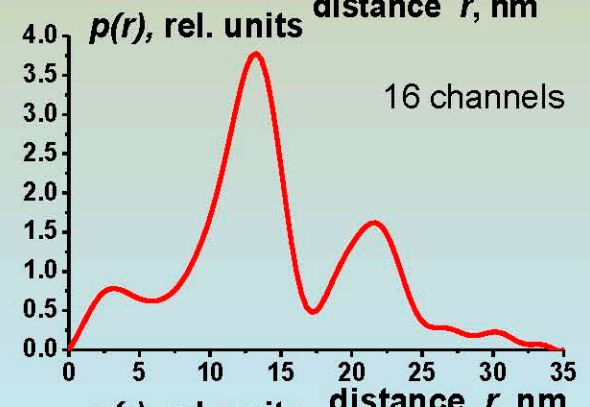
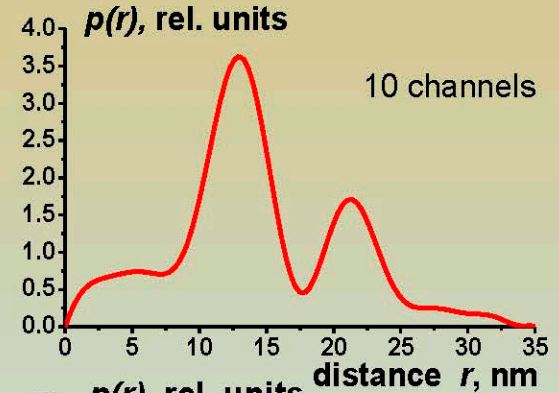
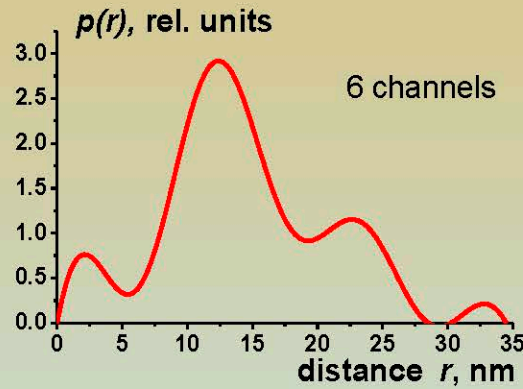
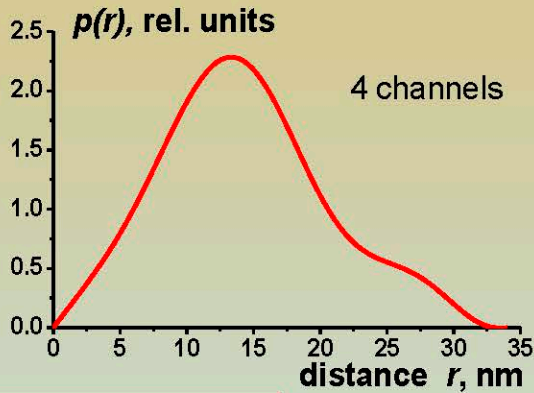
OK, we will try and see what happen

Complex particle: 2.5-turns helix

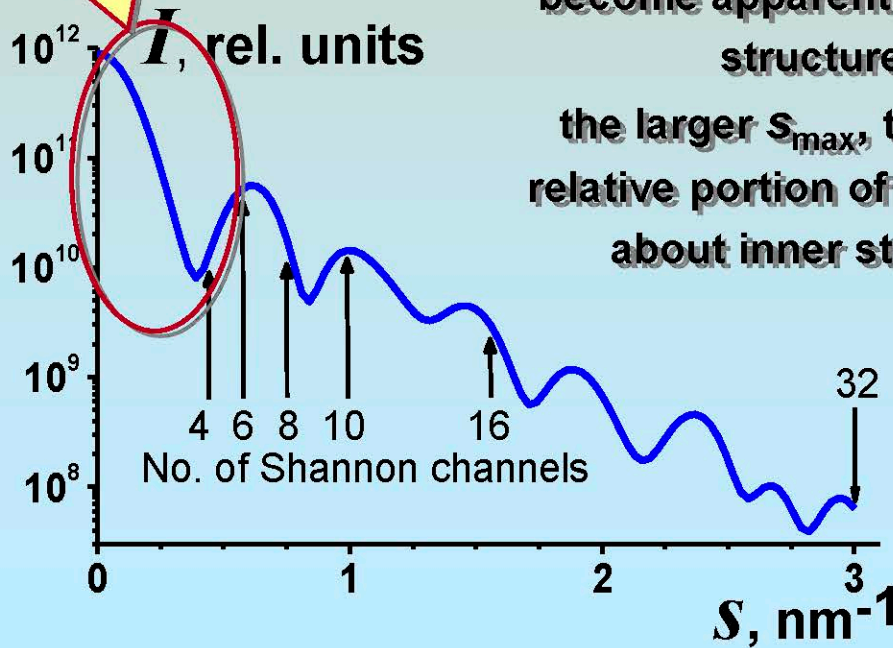
How to see the frequently occurring distances



Dependence of $p(r)$ calculated from SAS patterns from s_{\max}



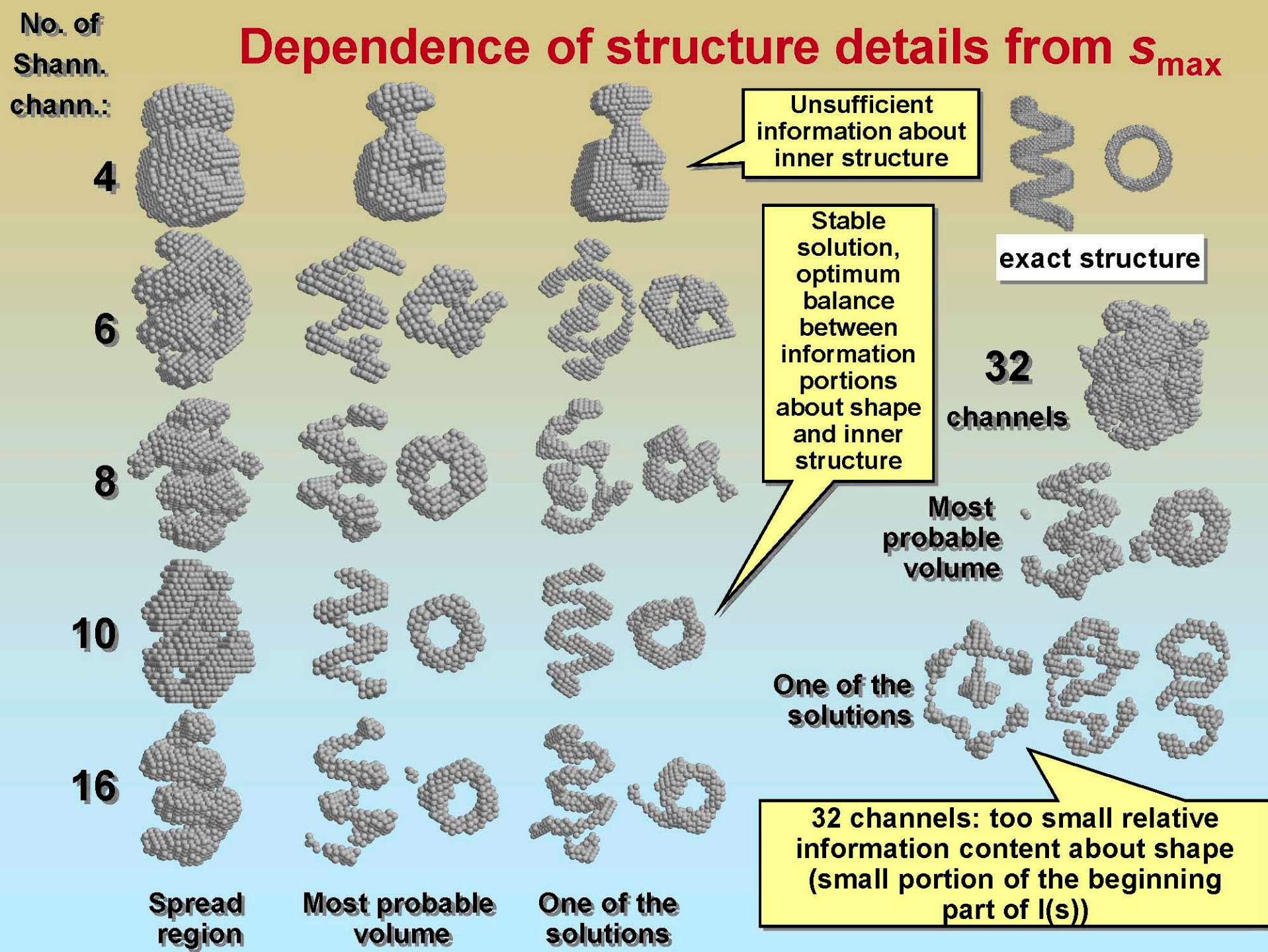
Scattering from particle shape



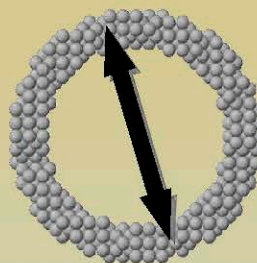
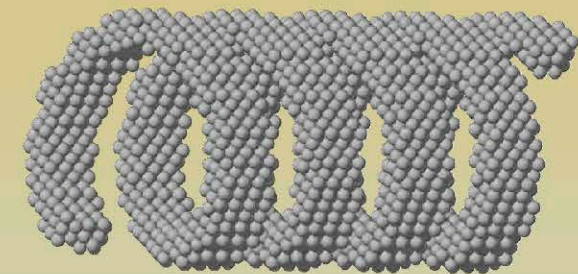
The difference in $p(r)$ should become apparent in restored structures:

the larger s_{\max} , the greater relative portion of information about inner structure.

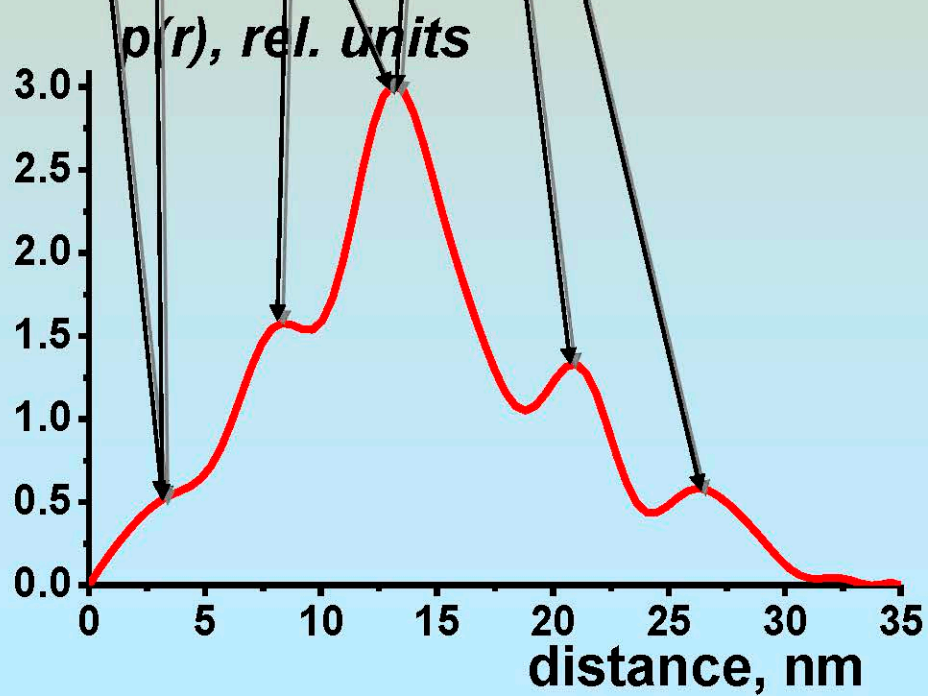
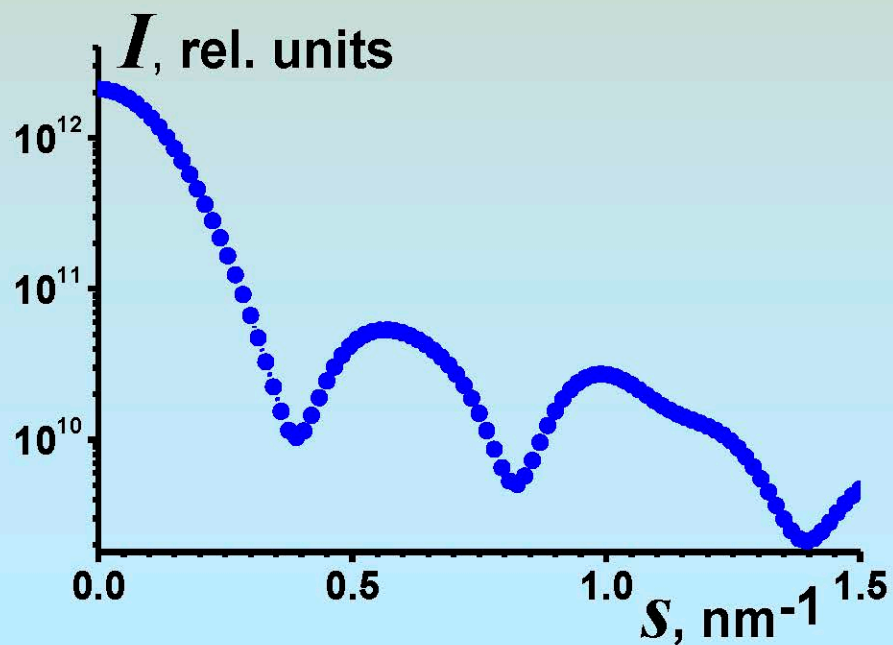
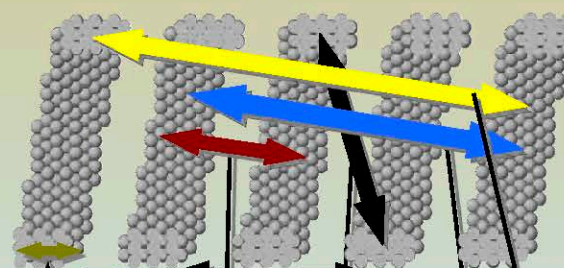
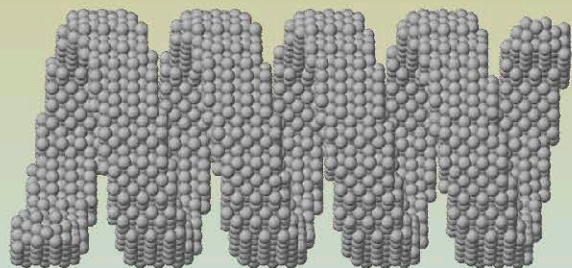
Dependence of structure details from S_{max}



Complex particle: 4.5-turns helix



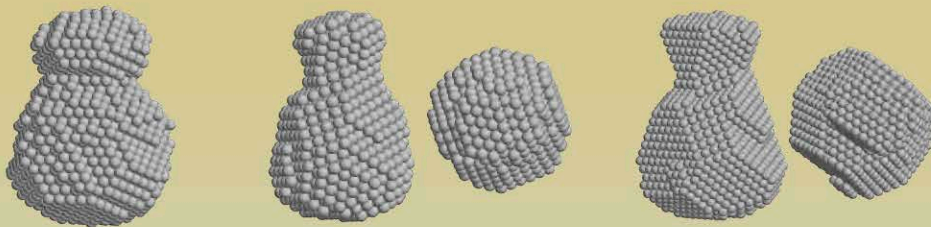
See the frequently occurring distances



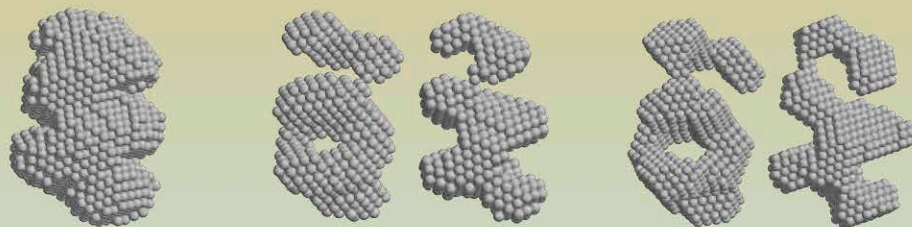
No. of
Shann.
chann.:

Dependence of structure details from s_{\max} , 4.5 turn helix

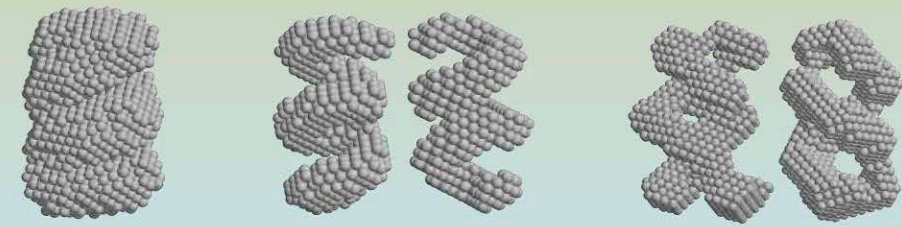
4



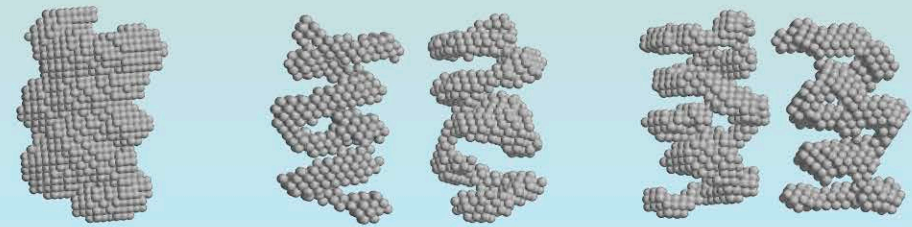
6



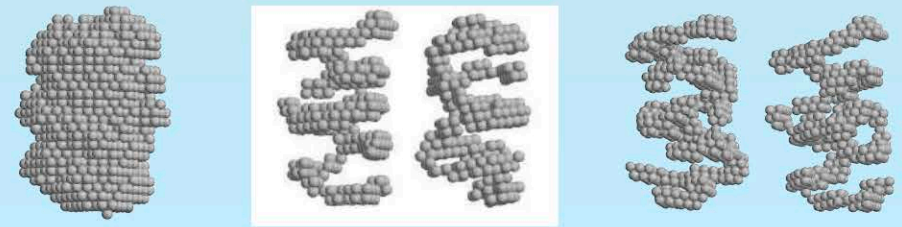
8



10



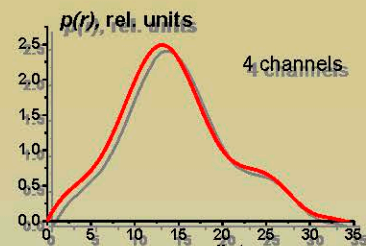
16



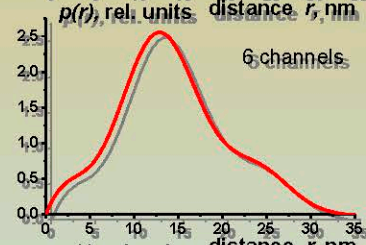
Spread region

Most probable volume

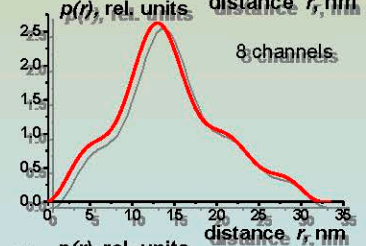
One of the solutions



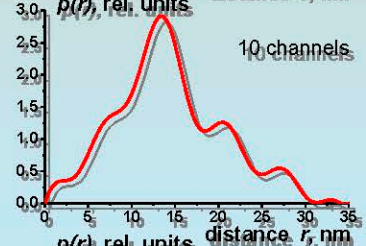
Unsufficient information about inner details



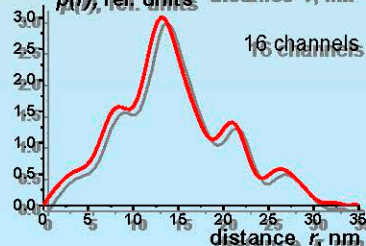
Still unsufficient information about inner details



Tries to restore helix but with wrong number of turns



The structure seems to be too difficult for rectoration, but its feature is clear.



Increasing data interval has no effect

Distance distribution