

# Small-angle neutron scattering: a high resolution, non-destructive probe of biomacromolecular structure

Olwyn Byron

Institute of Biomedical & Life Sciences

University of Glasgow



# In this talk

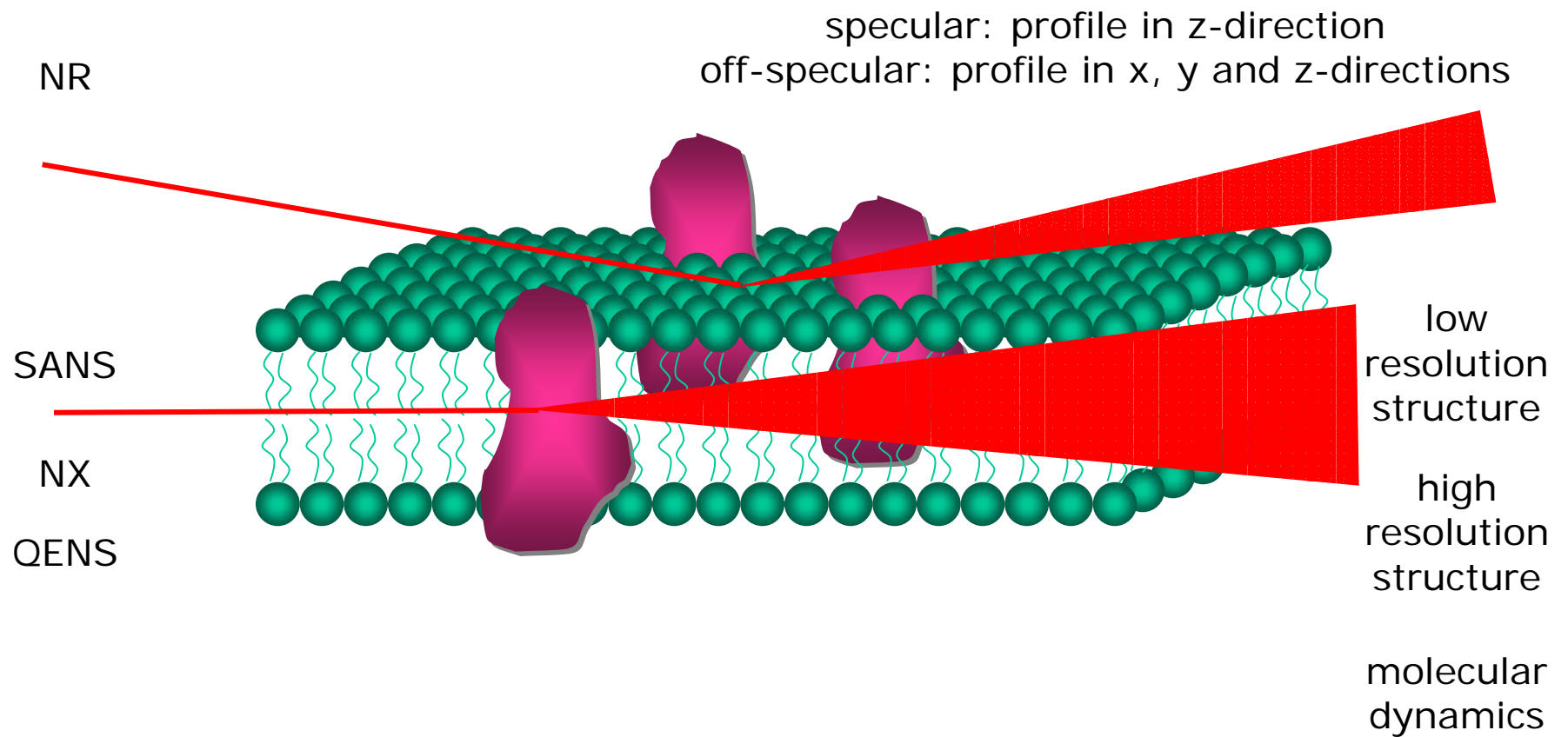
- Brief introduction to biomacromolecular SANS
- *Ab initio* structure restoration
  - Examples
    - Ribosome
    - SecA
    - *Taq* polymerase
- Rigid body modelling
  - Example
    - Tn3 resolvase

nmi3



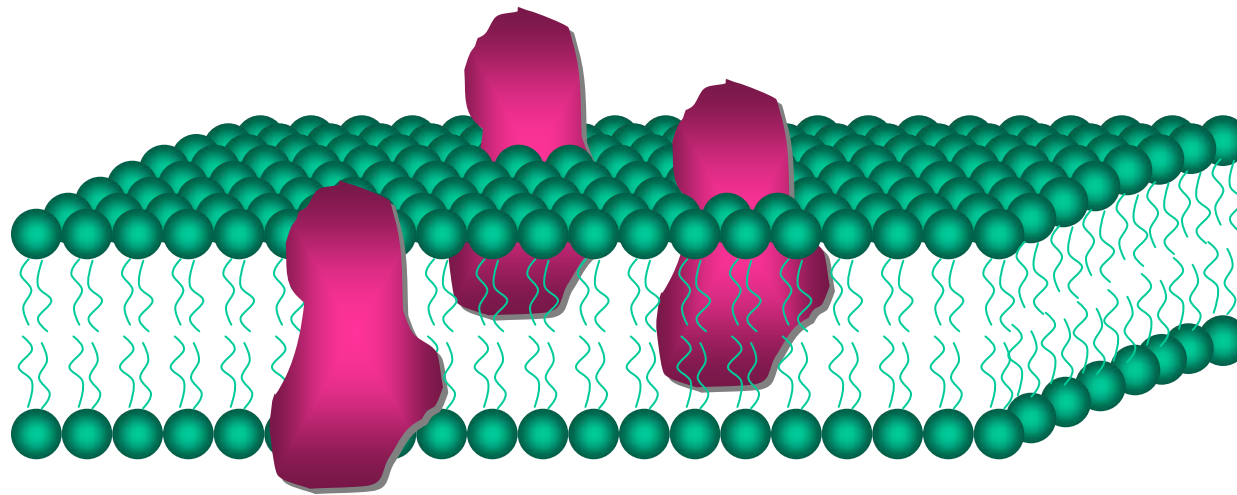
wellcome<sup>trust</sup>

# Neutrons reveal the structure....



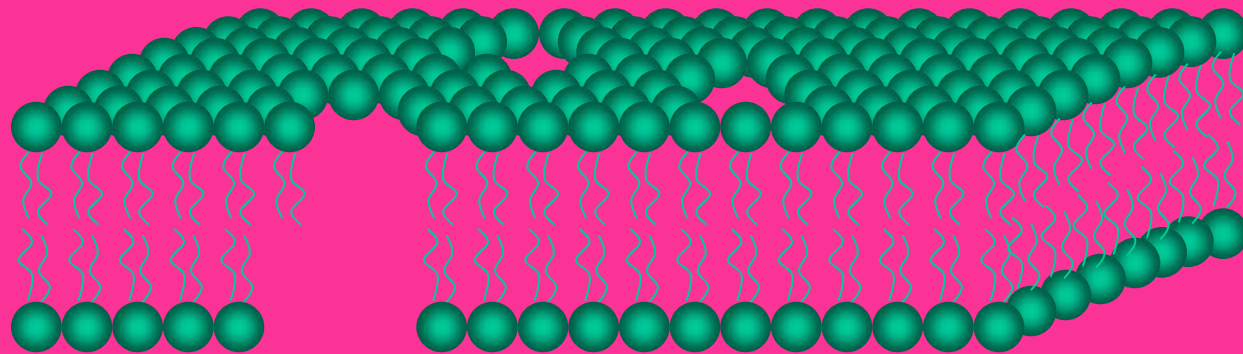
...of the whole system...

100% D<sub>2</sub>O



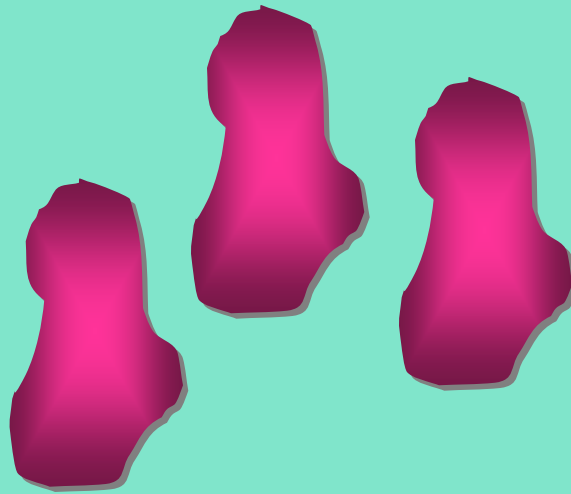
...or selected constituents...

43% D<sub>2</sub>O

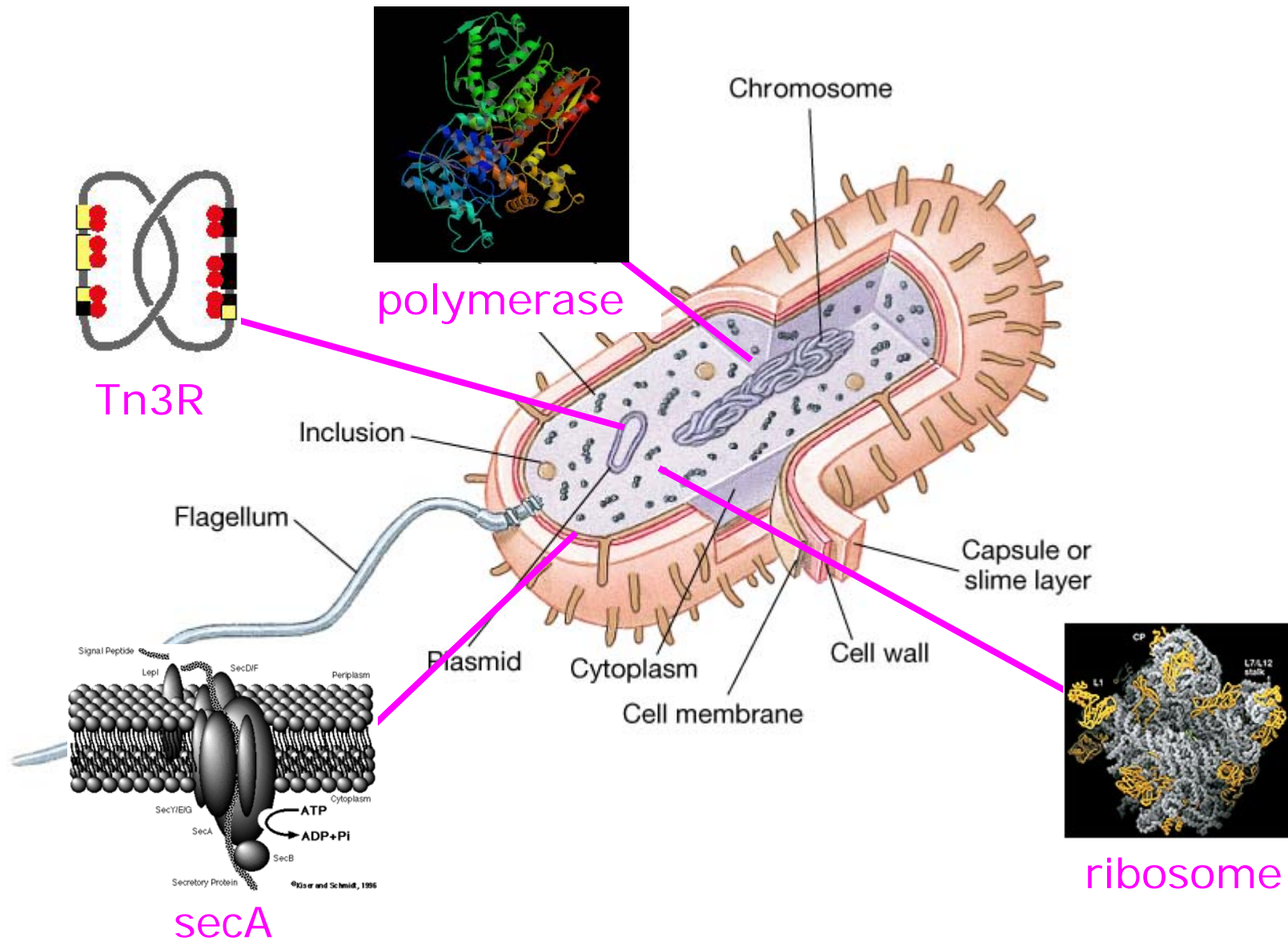


...via contrast matching

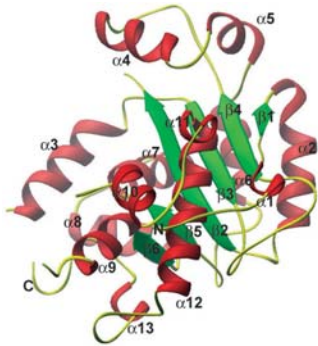
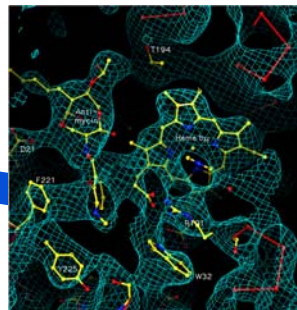
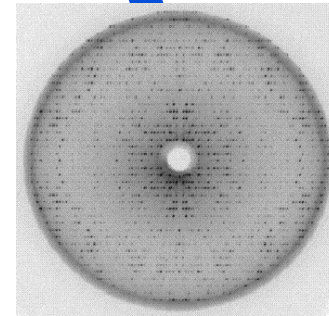
13% D<sub>2</sub>O



# Dissection of the bacterial cell

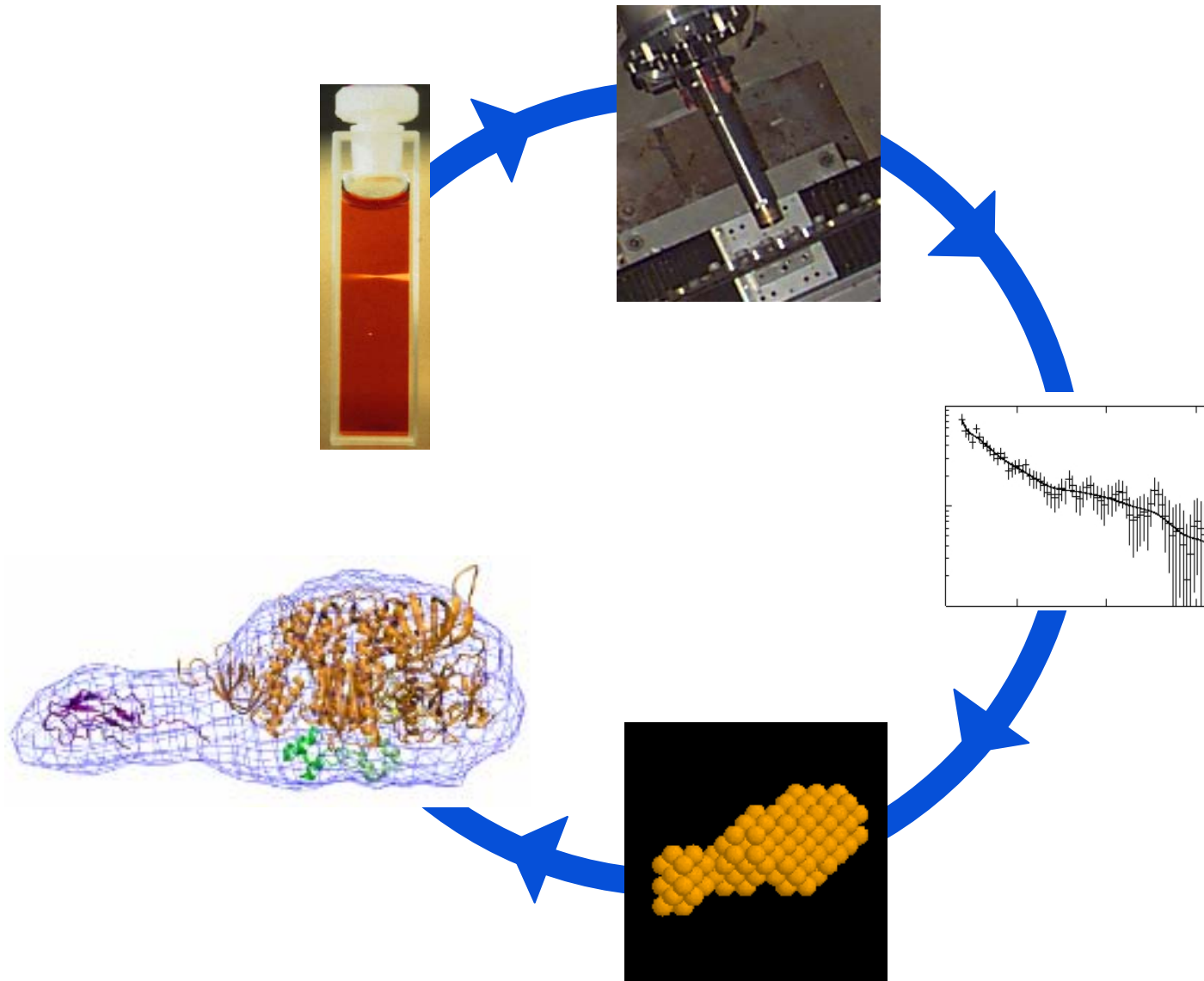


# X-ray crystallography

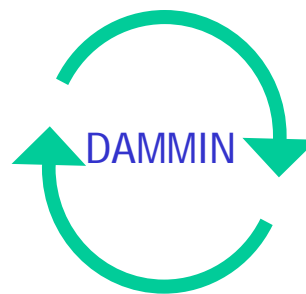
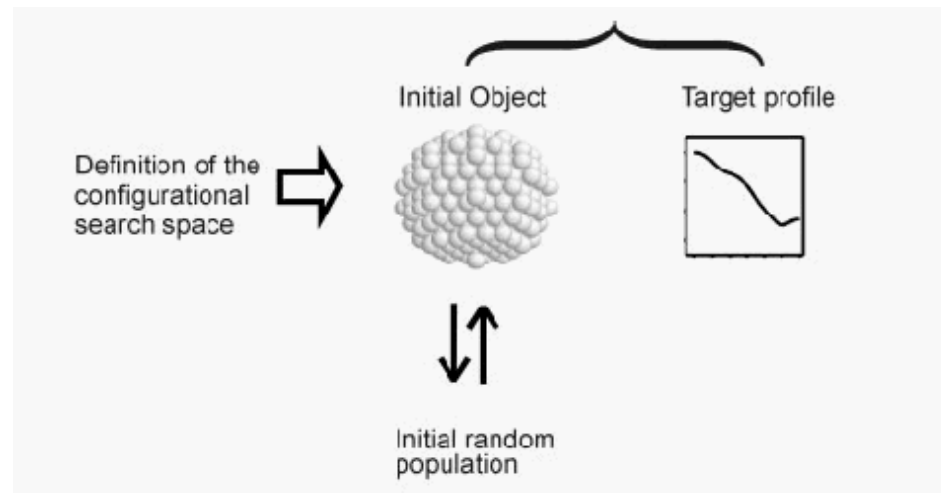




# Small angle neutron scattering



# *Ab initio* structure restoration



# S1 shape reconstruction

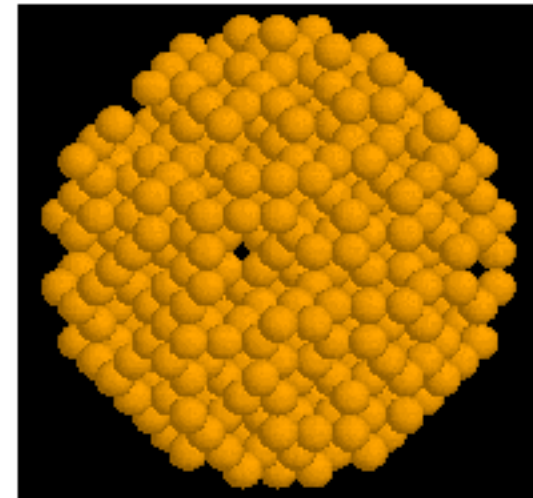
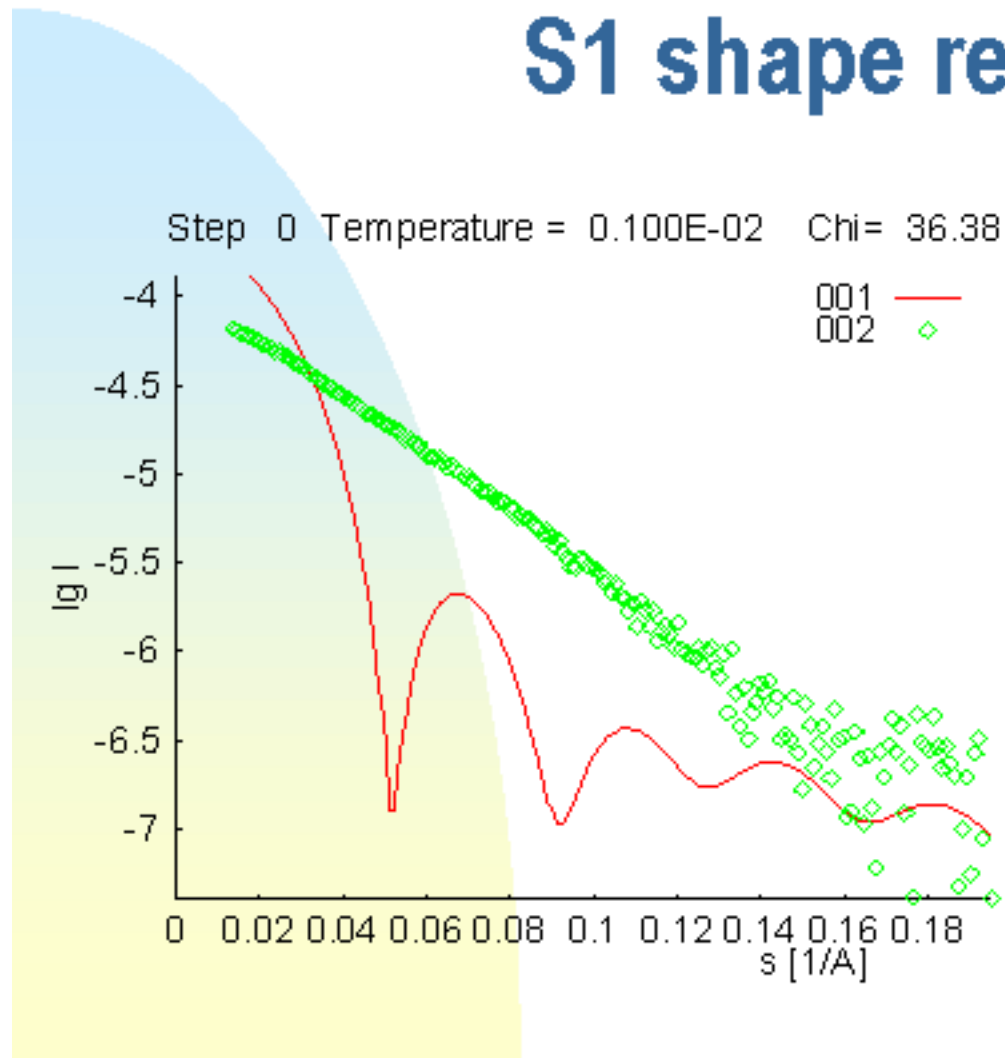


Image from Svergun website

# S1 shape reconstruction

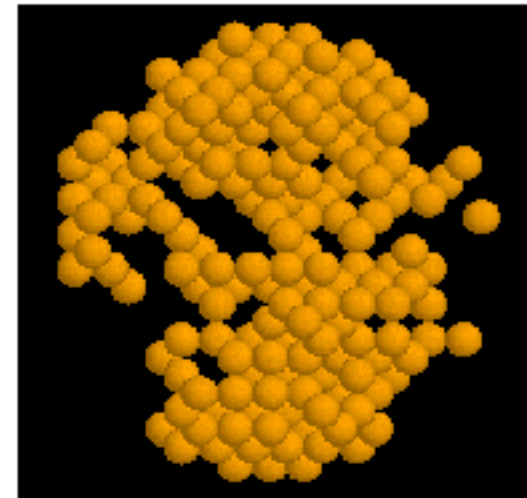
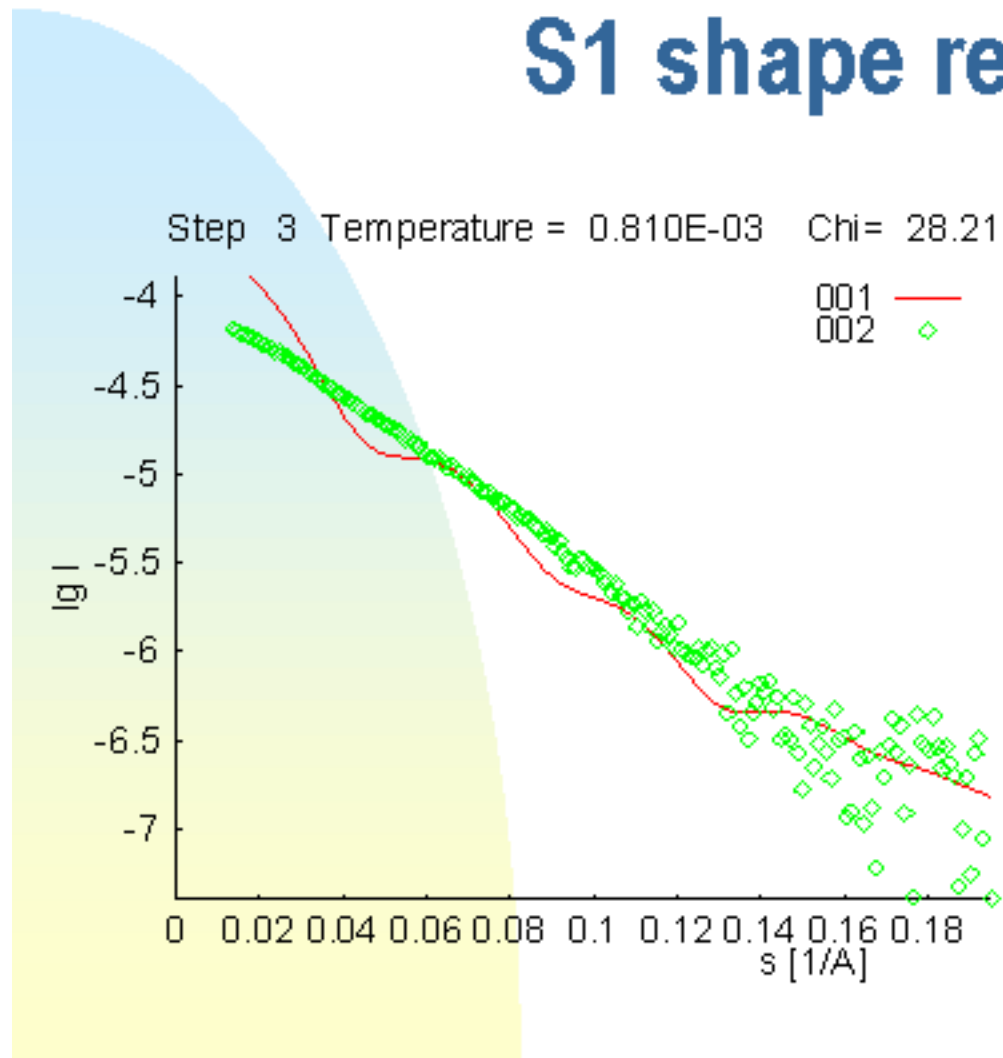


Image from Svergun website

# S1 shape reconstruction

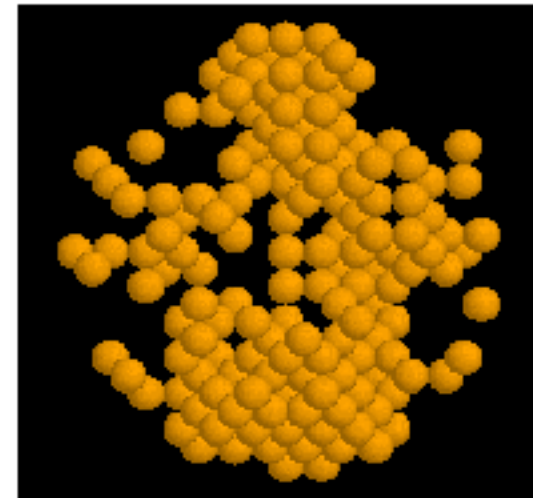
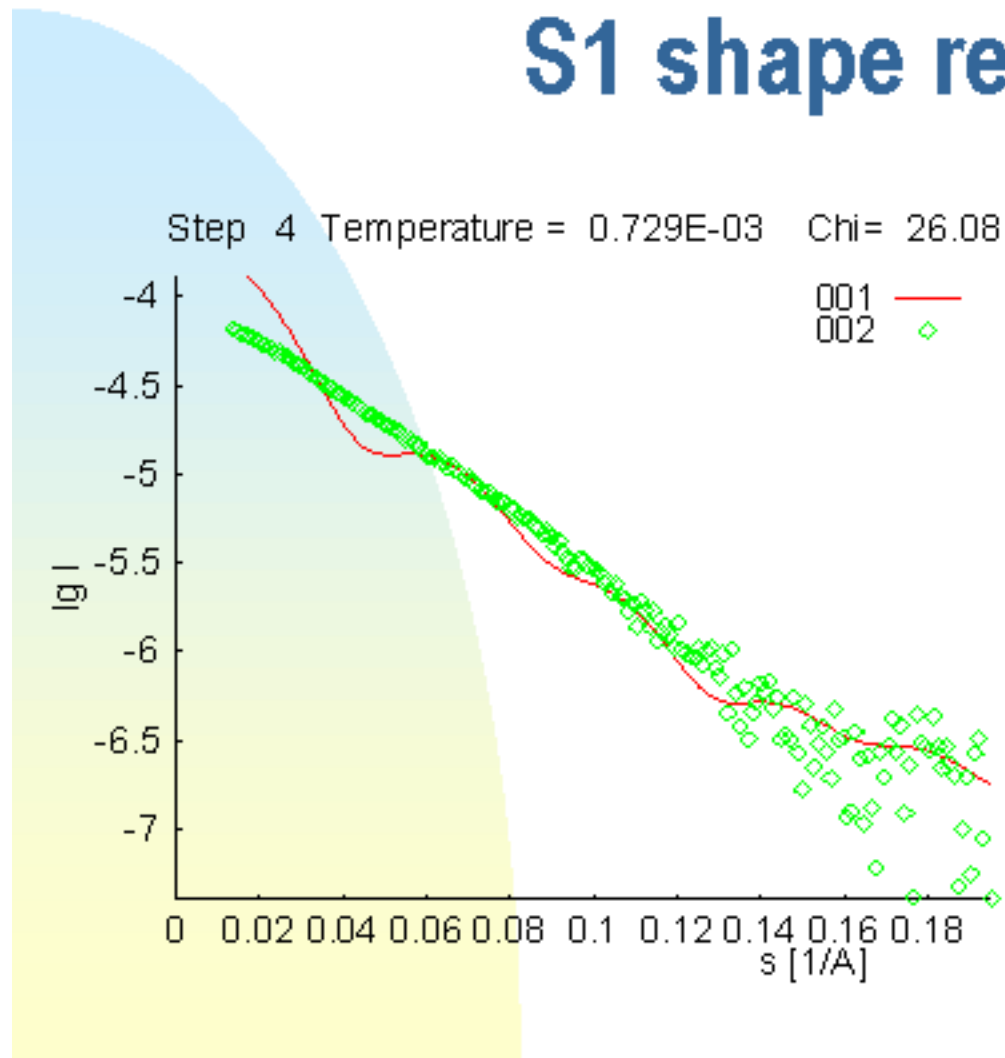


Image from Svergun website

# S1 shape reconstruction

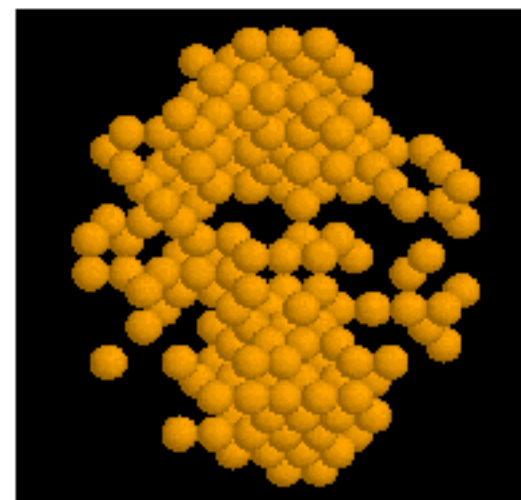
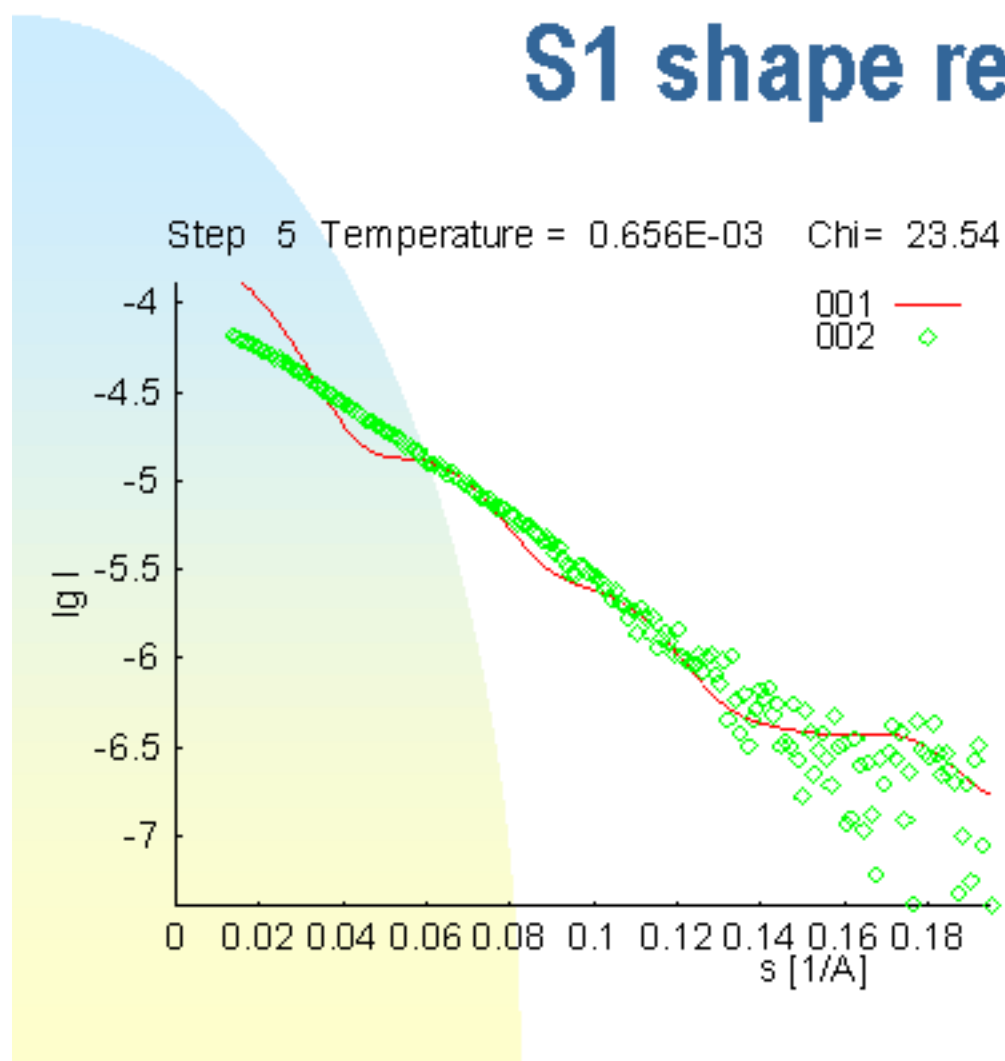


Image from Svergun website

# S1 shape reconstruction

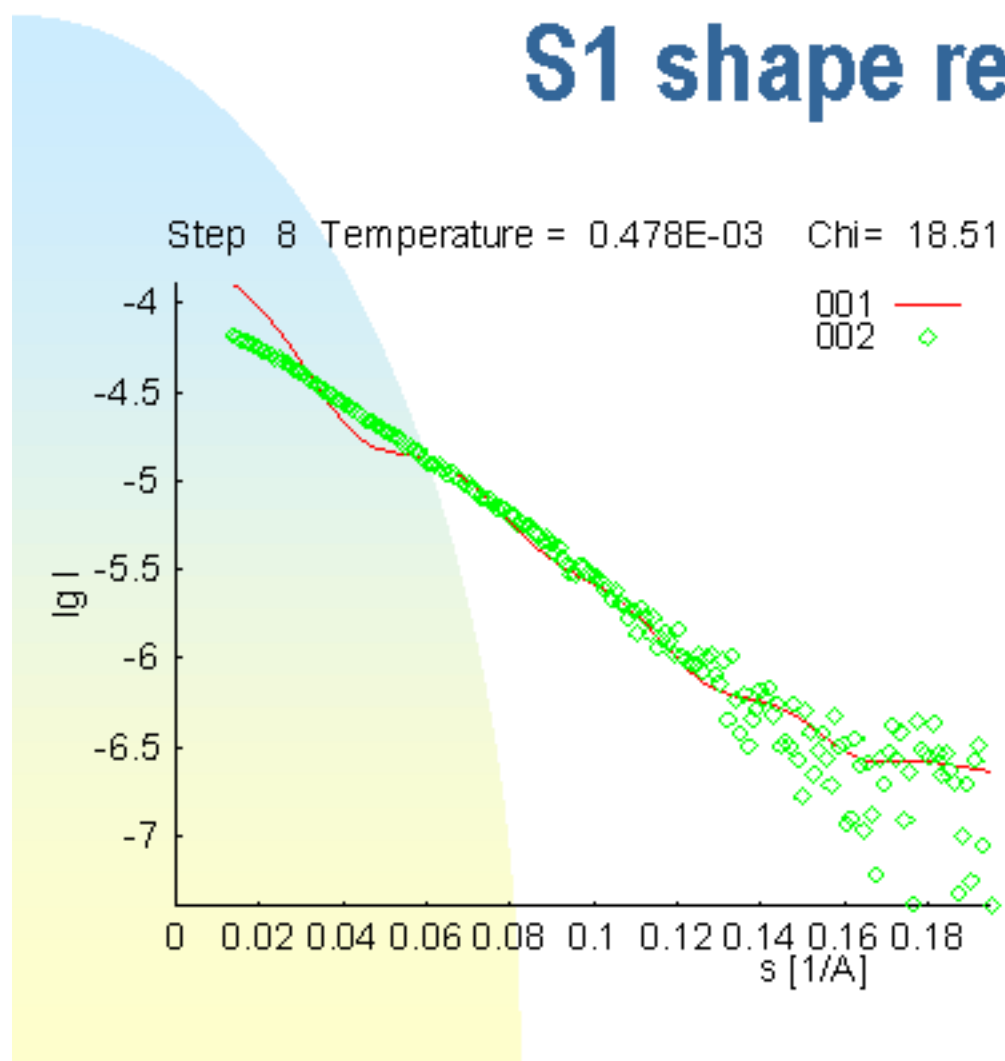


Image from Svergun website

# S1 shape reconstruction

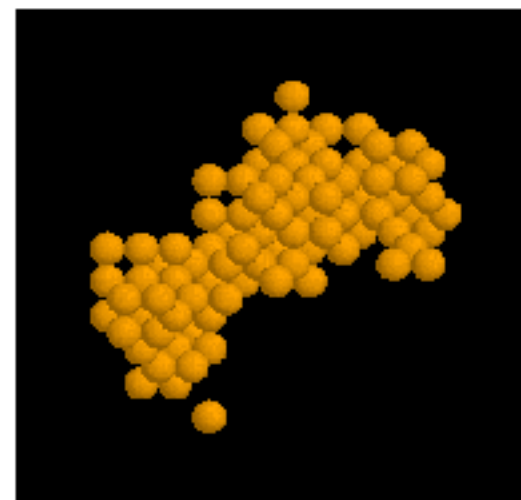
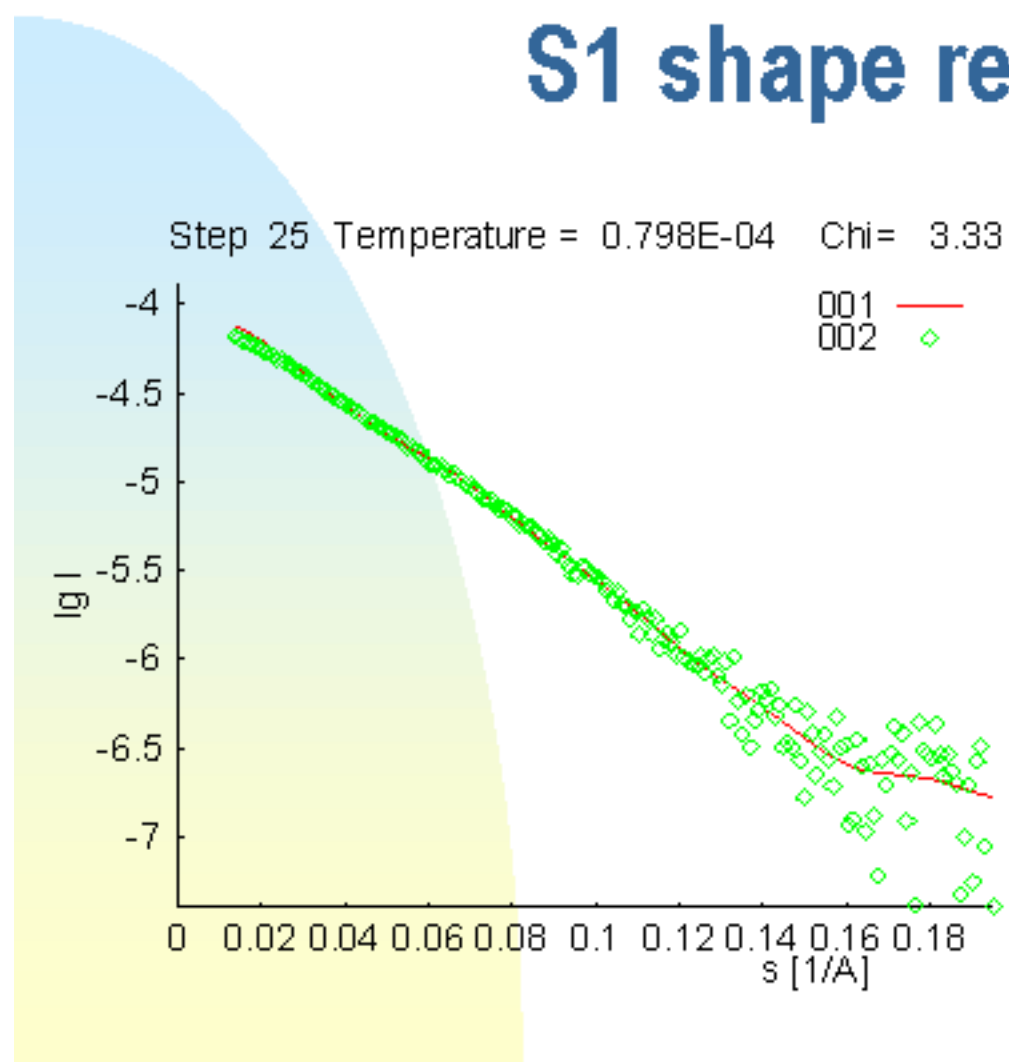


Image from Svergun website



# S1 shape reconstruction

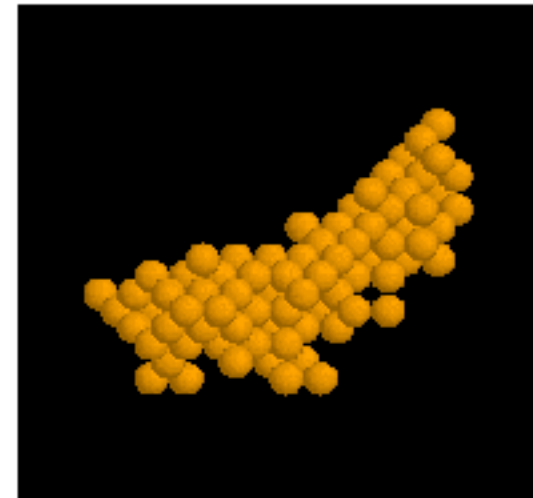
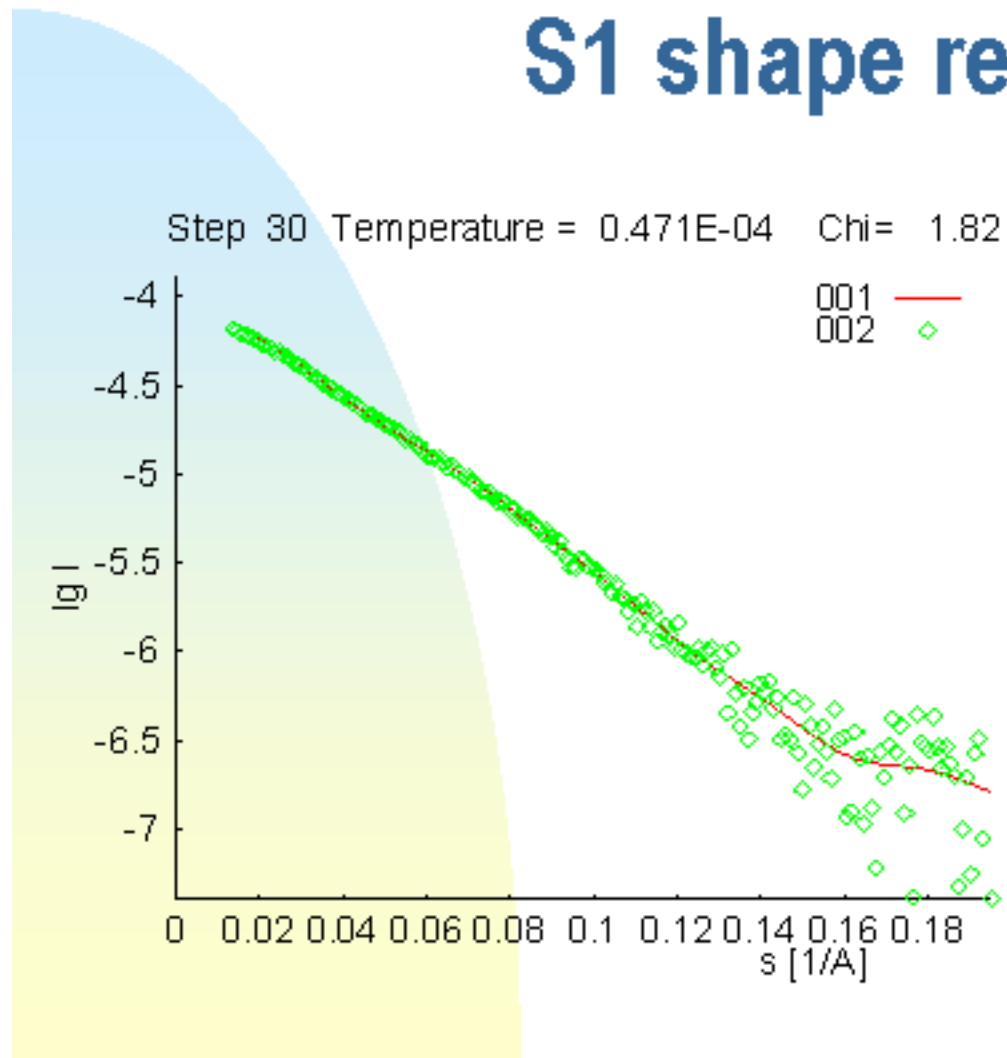


Image from Svergun website

# S1 shape reconstruction

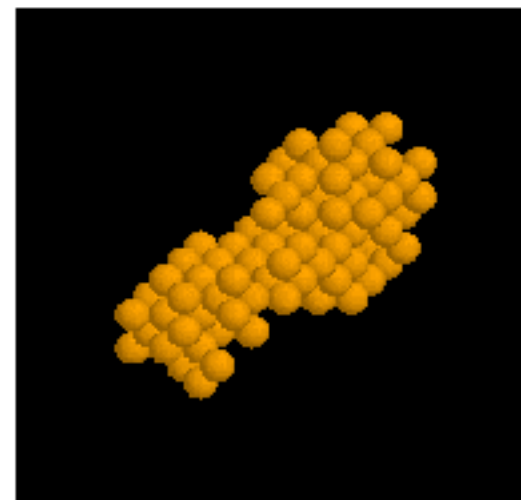
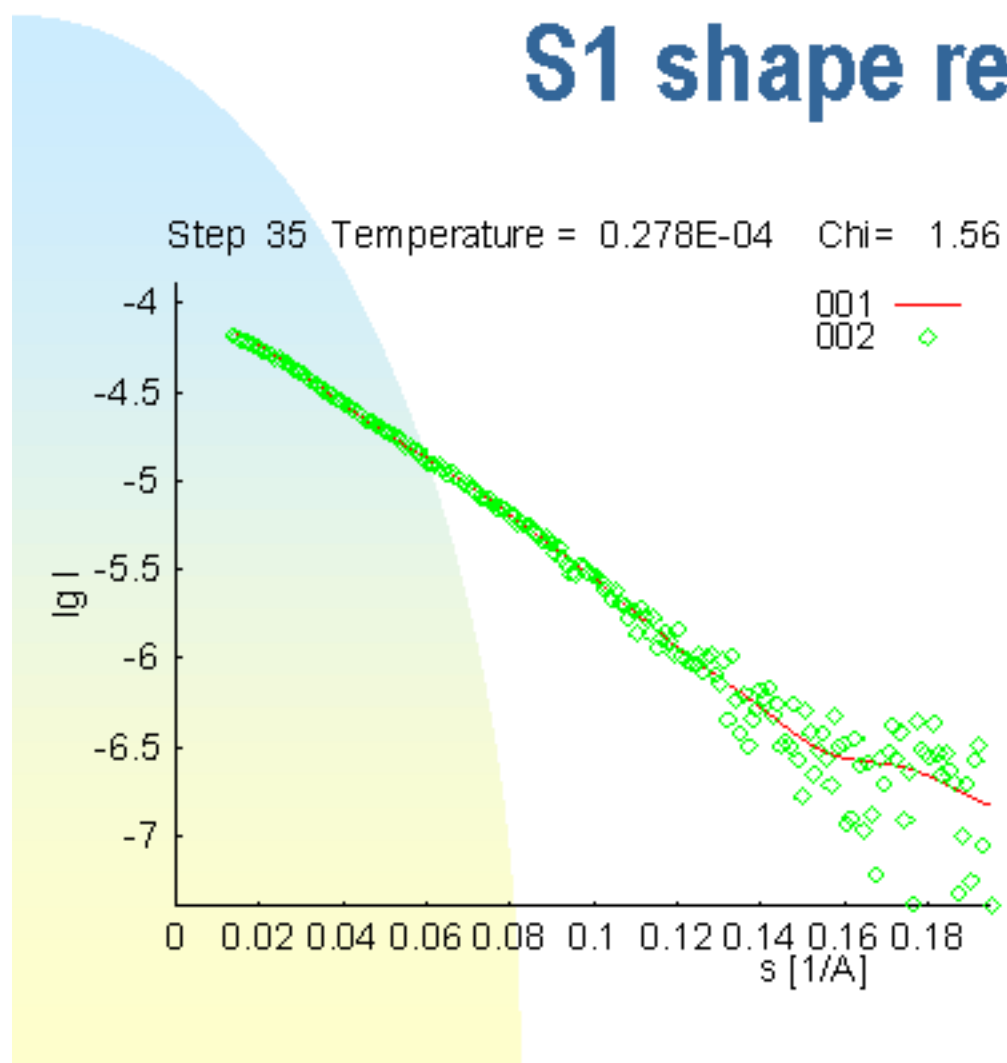


Image from Svergun website

# S1 shape reconstruction

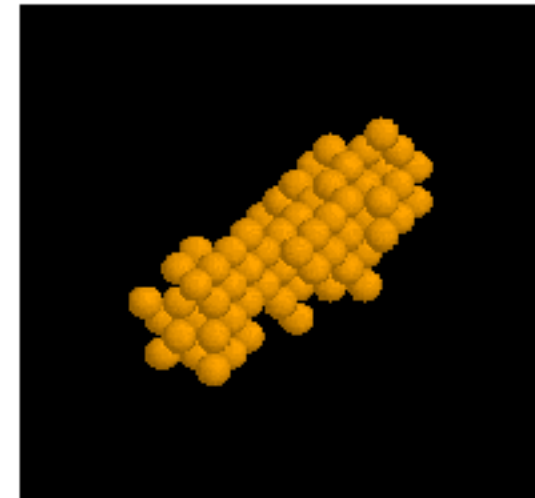
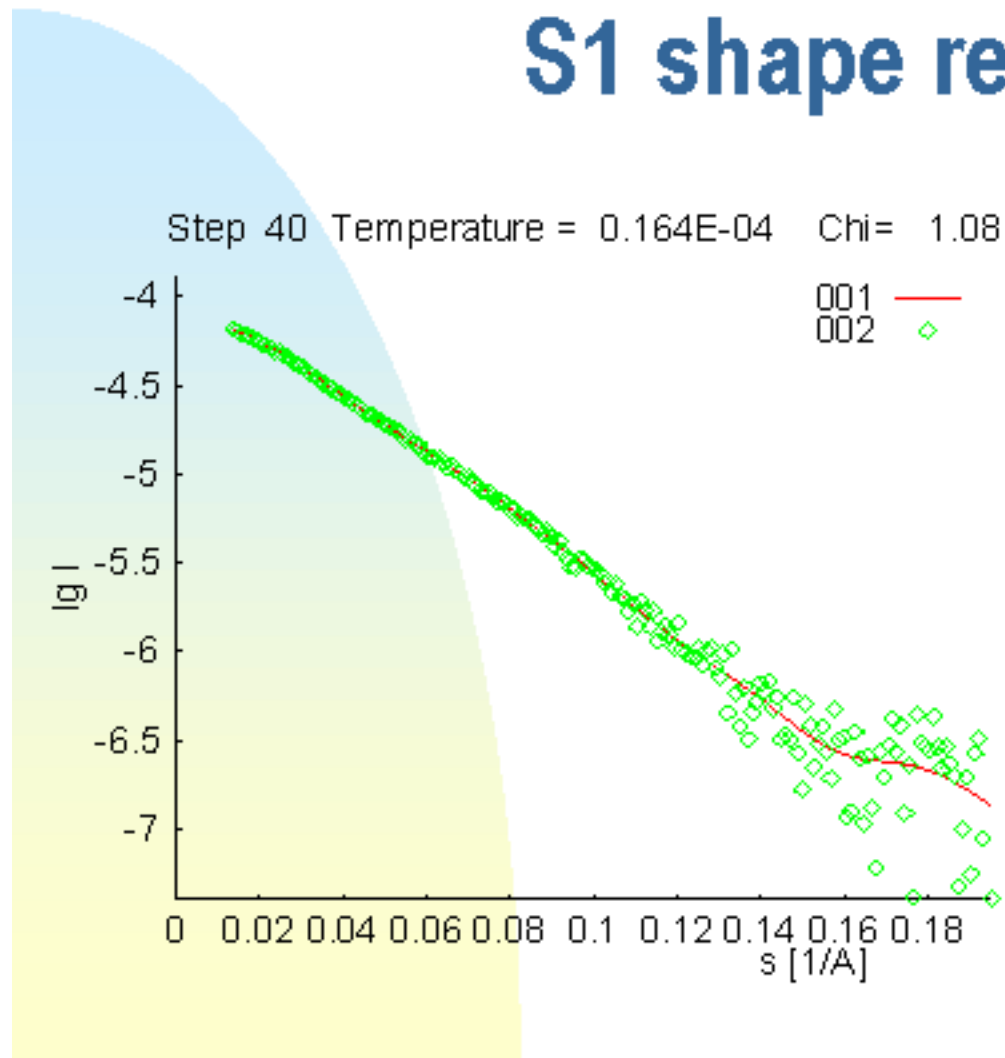


Image from Svergun website

# S1 shape reconstruction

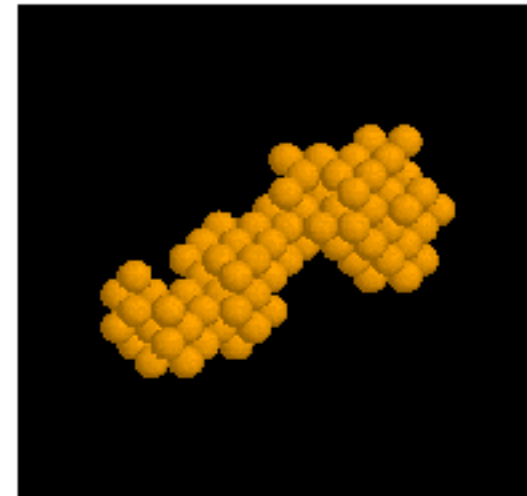
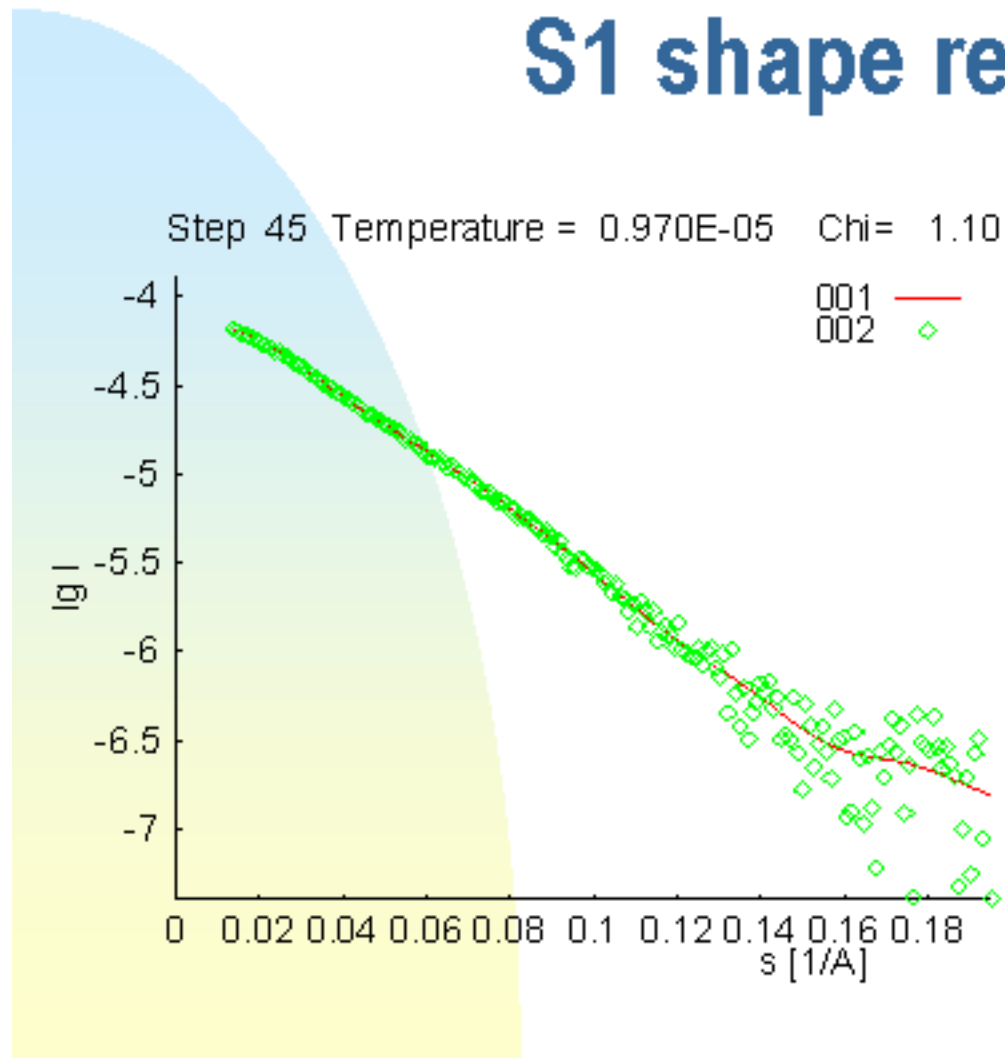


Image from Svergun website

# S1 shape reconstruction

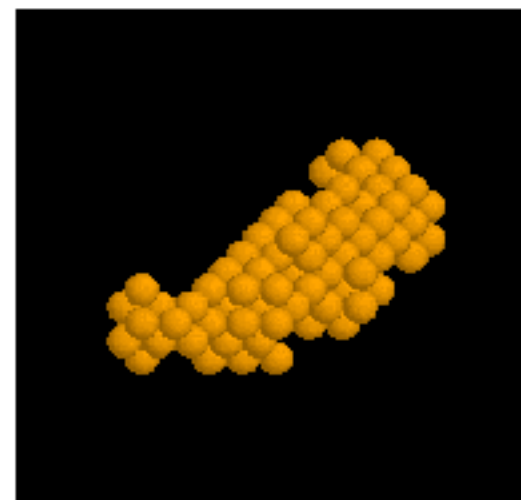
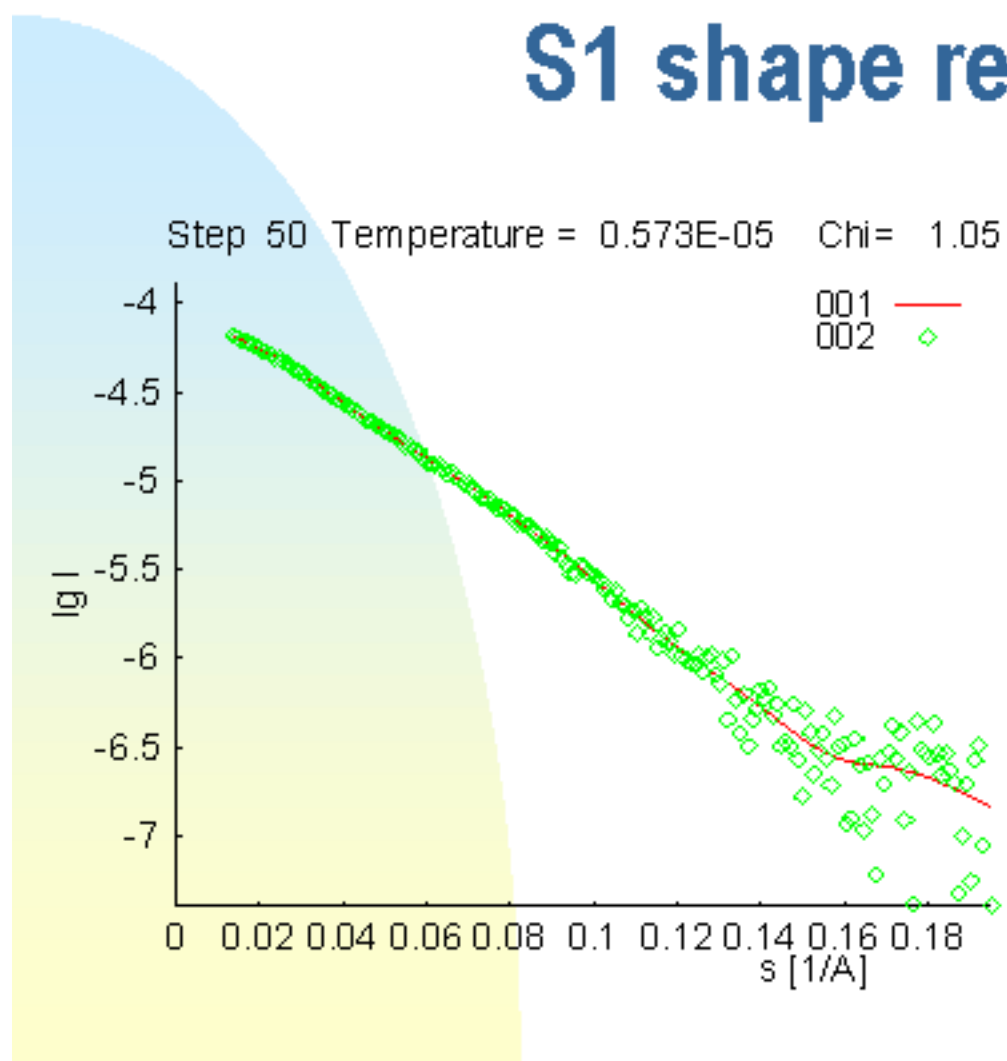


Image from Svergun website

# S1 shape reconstruction

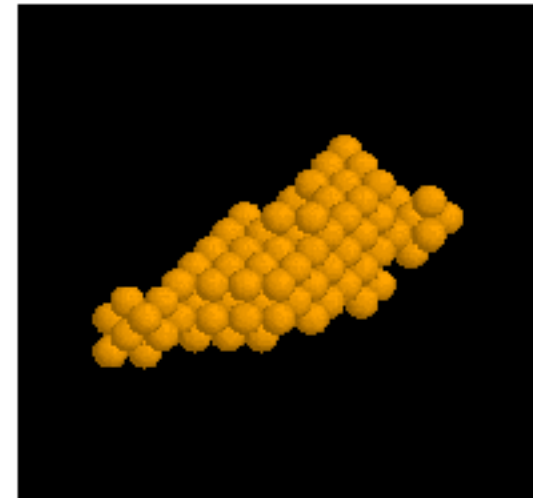
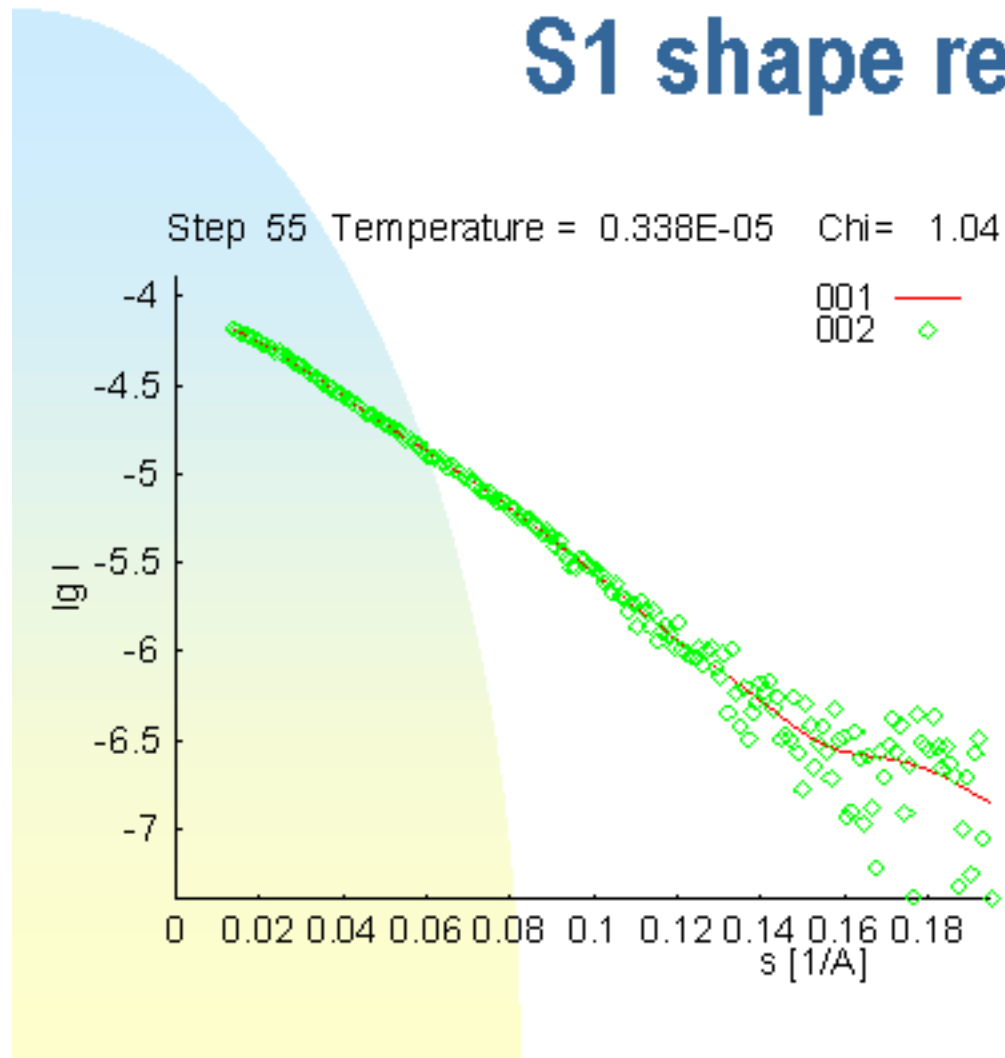


Image from Svergun website

# S1 shape reconstruction

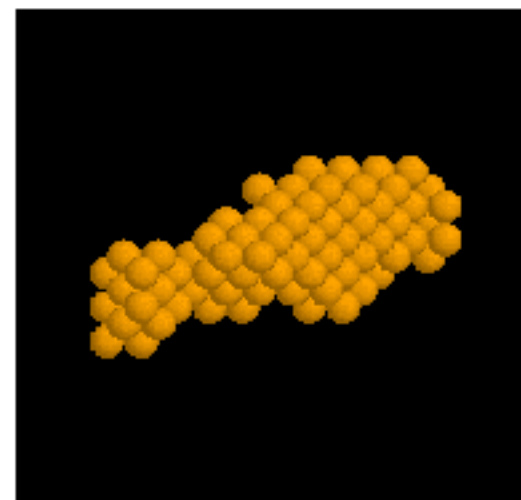
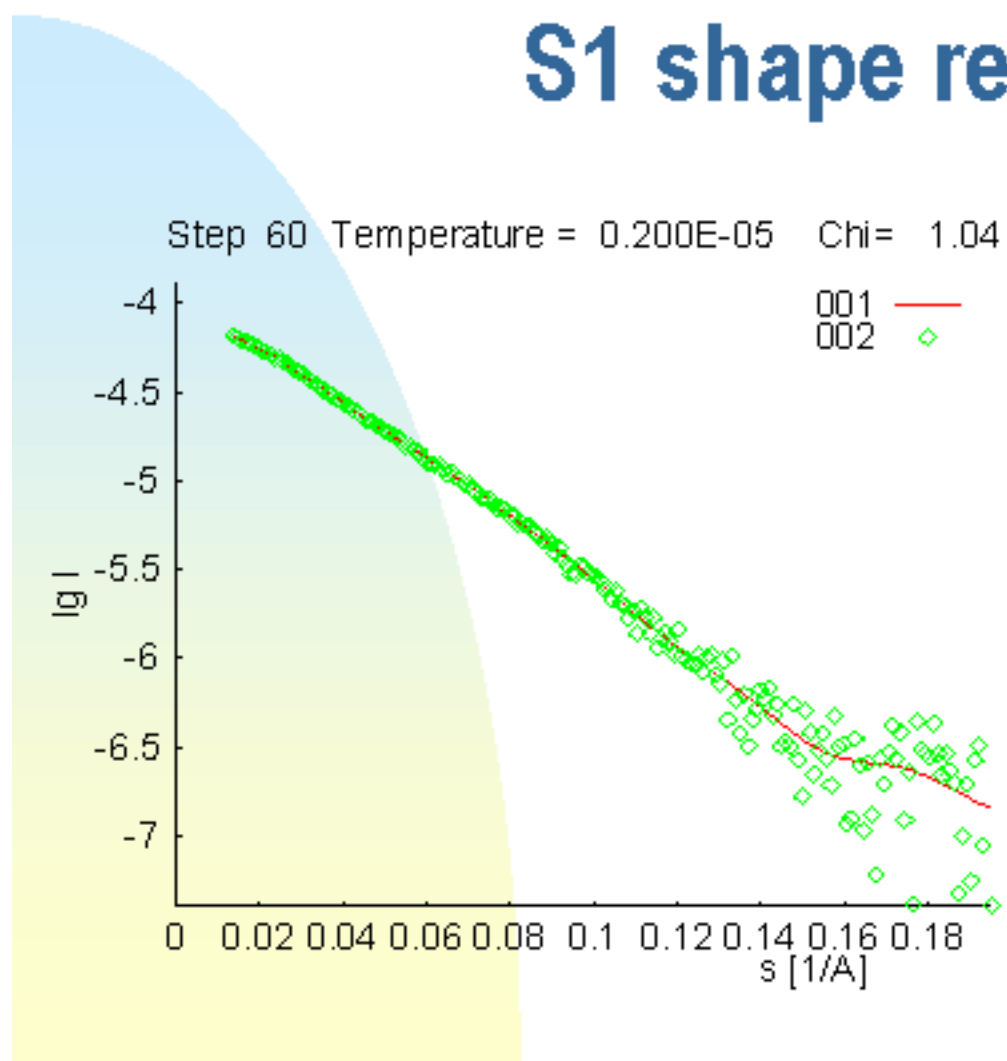


Image from Svergun website

# S1 shape reconstruction

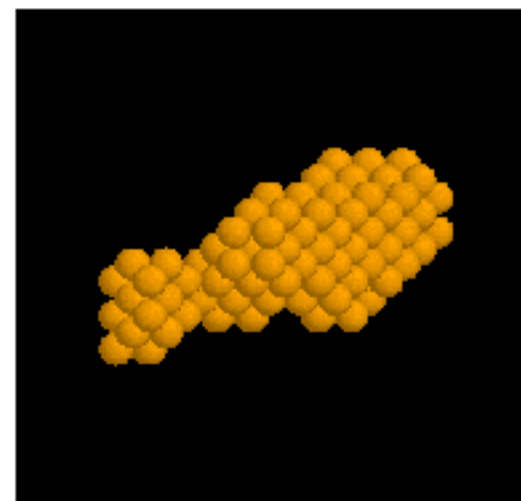
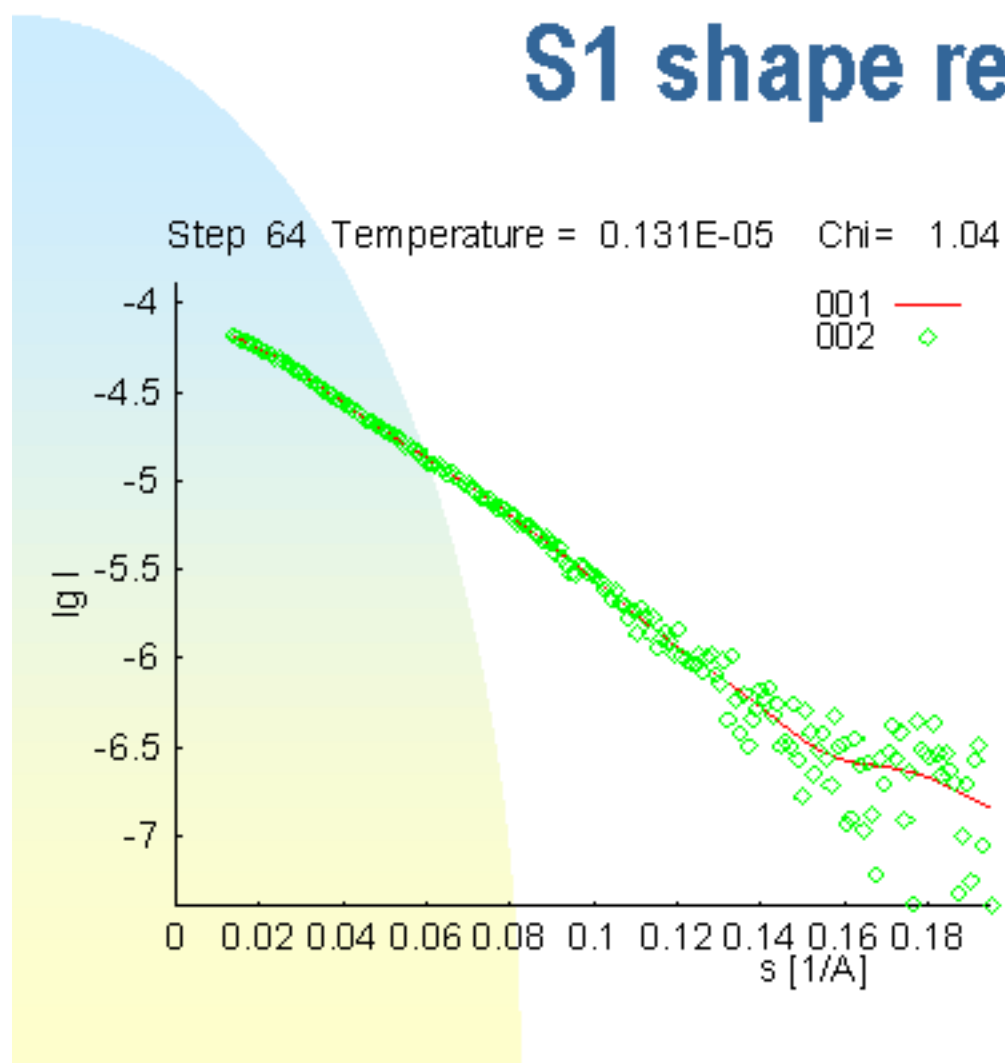
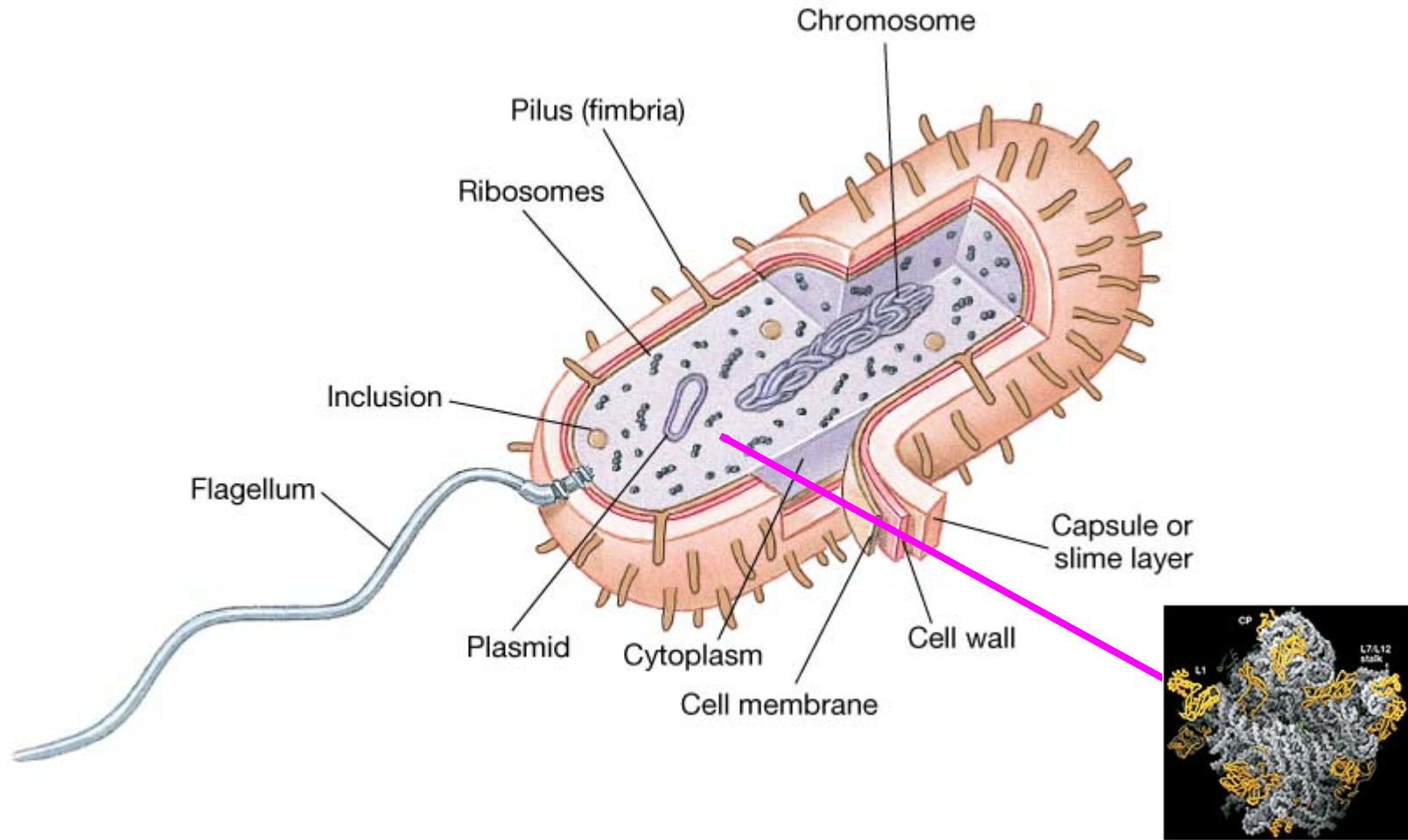


Image from Svergun website



# Dissection of the bacterial cell

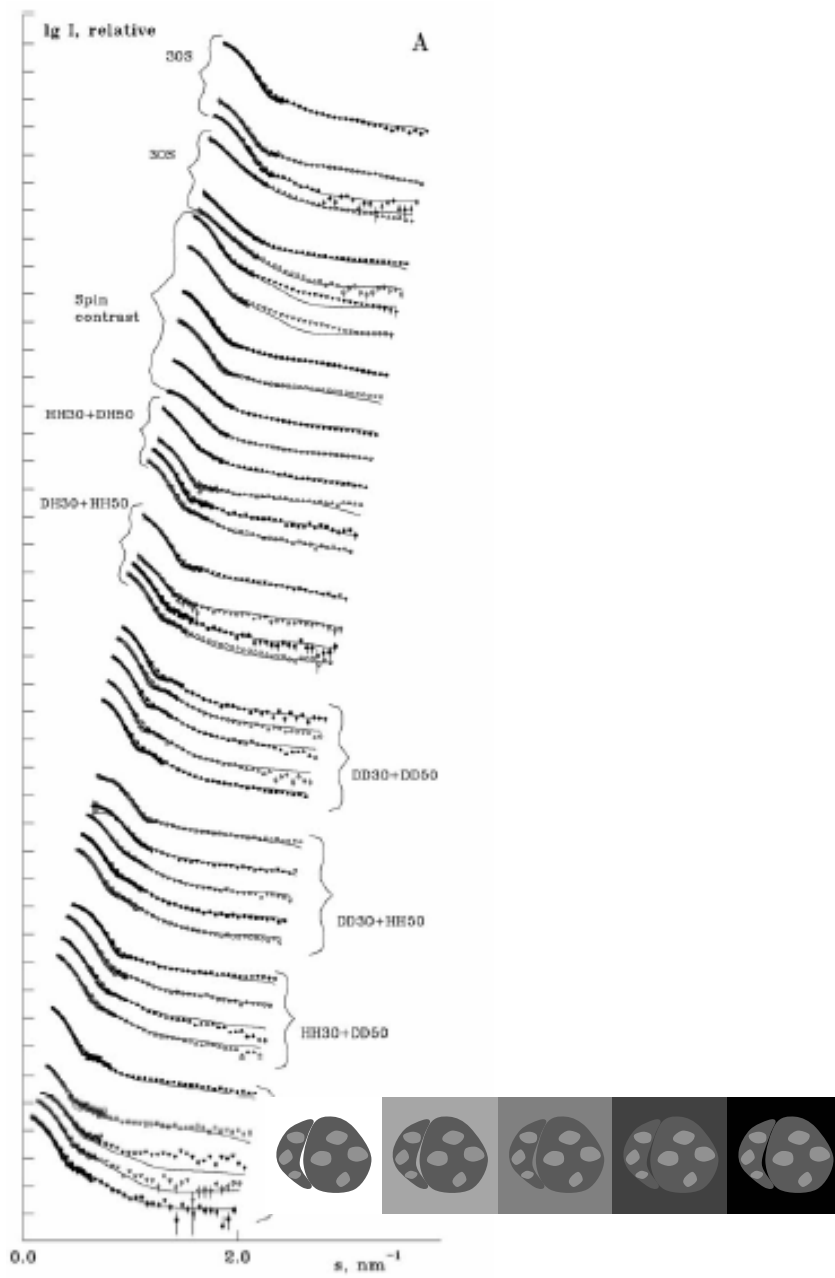


## A Map of Protein-rRNA Distribution in the 70 S *Escherichia coli* Ribosome\*

Received for publication, November 10, 1999, and in revised form, January 10, 2000

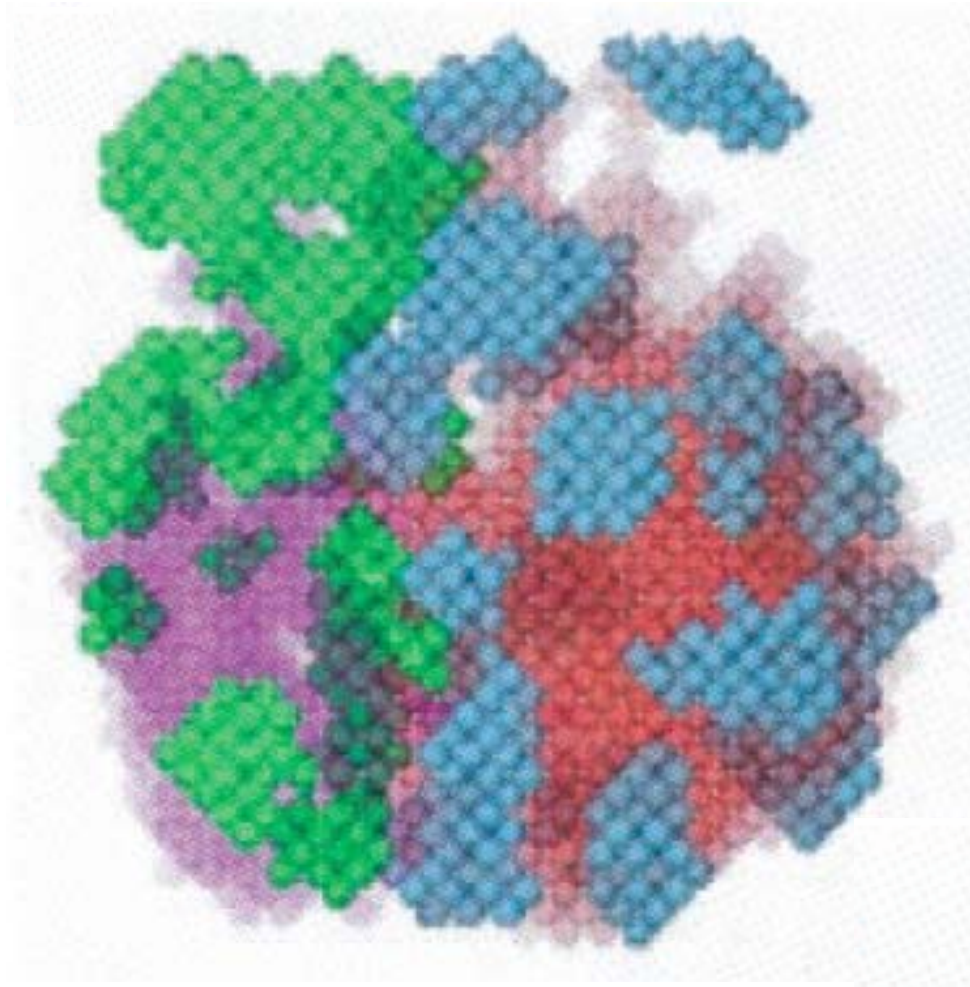
**Dmitri I. Svergun**<sup>‡§¶</sup> and **Knud H. Nierhaus**<sup>||</sup>

*From the ‡European Molecular Biology Laboratory, EMBL c/o DESY, Notkestraße 85, D-22603 Hamburg, Germany, the §Institute of Crystallography, Russian Academy of Sciences, Leninsky pr. 59, 117333 Moscow, Russia, and the ||Max Planck Institut für Molekulare Genetik, AG Ribosomen, Ihnestr. 73, 14195 Berlin, Germany*



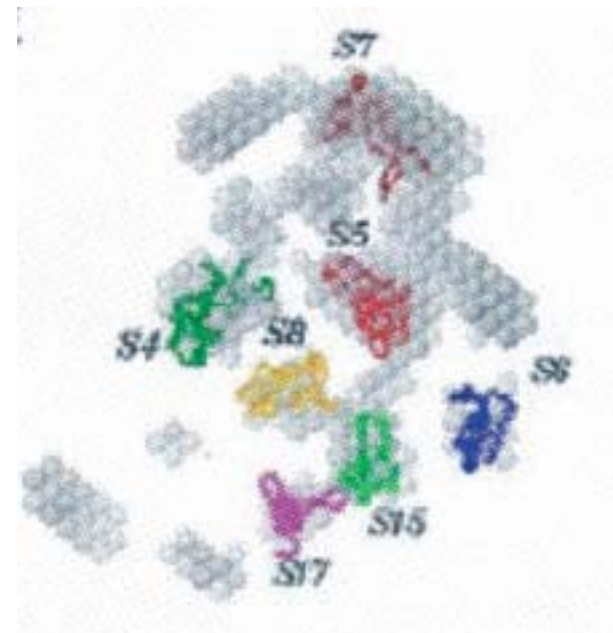
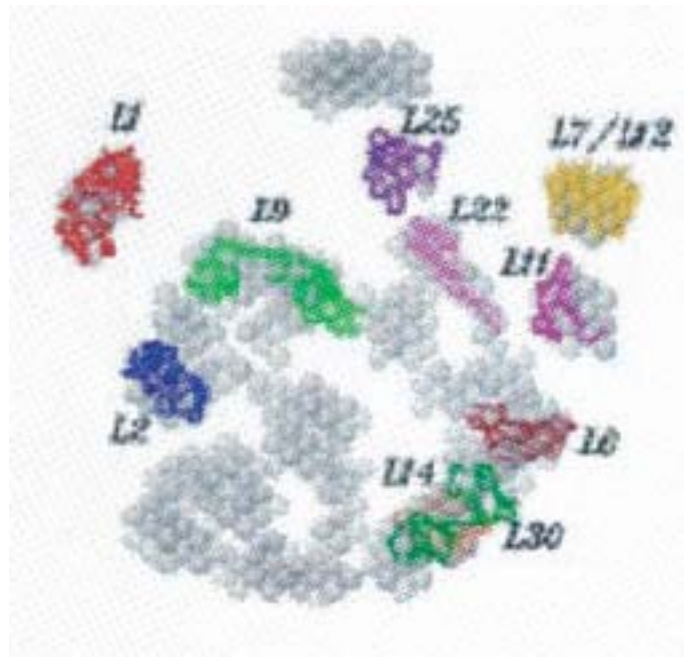
Svergun & Nierhaus J. Biol. Chem. (2000) 275, 14432-14439

# Protein-rRNA distribution in the 70 S ribosome

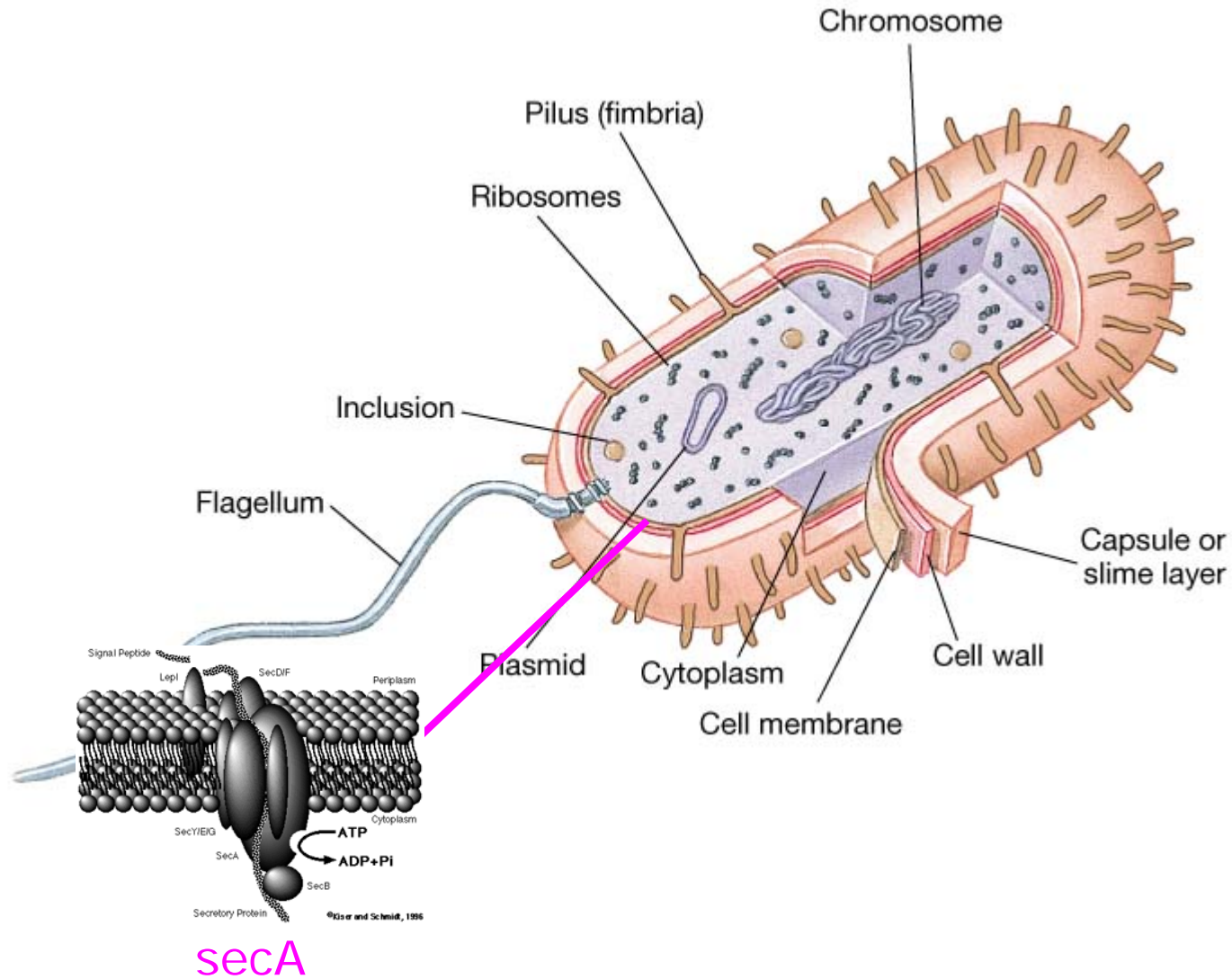


Svergun & Nierhaus J. Biol. Chem. (2000) 275, 14432-14439

# Modelling-in of protein crystal structures



# Dissection of the bacterial cell



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COMMUNICATION

# **Nucleotide Binding Induces Changes in the Oligomeric State and Conformation of Sec A in a Lipid Environment: A Small-angle Neutron-scattering Study**

**Zimei Bu, Ligong Wang and Debra A. Kendall\***

*Department of Molecular  
and Cell Biology  
University of Connecticut  
91 N. Eagleville Road  
Storrs, CT 06269-3125  
USA*



# Sec translocase exports proteins through membrane to non-cytoplasmic environment

- SecA is motor part of translocase
  - Membrane bound and cytoplasmic
  - Powered by ATP hydrolysis
- How does SecA translate chemical energy from ATP hydrolysis into mechanical energy for pre-protein movement?

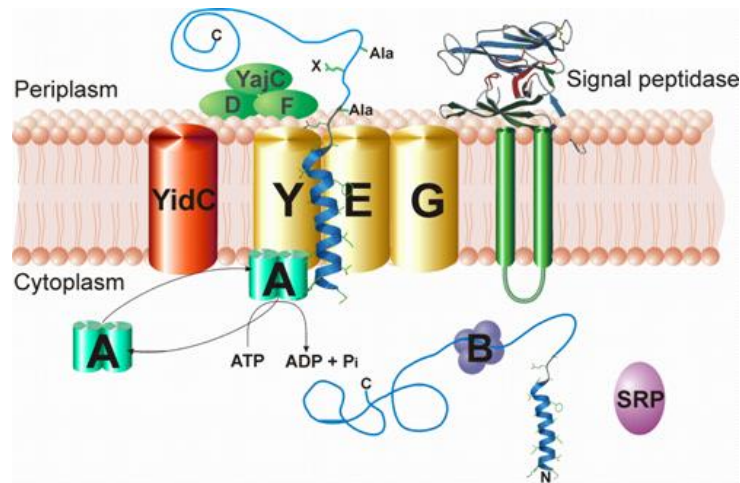
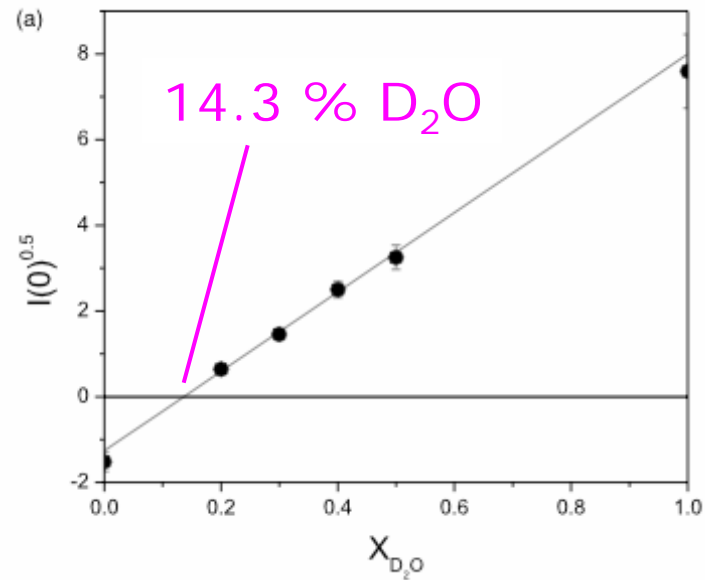


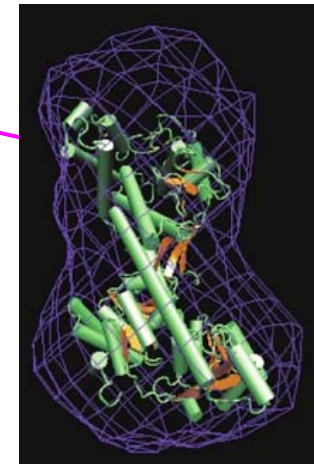
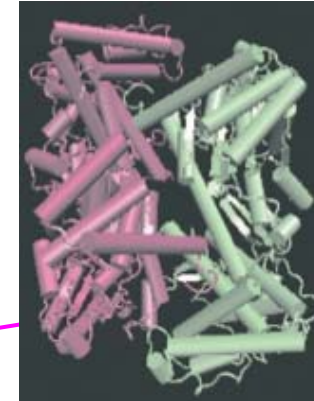
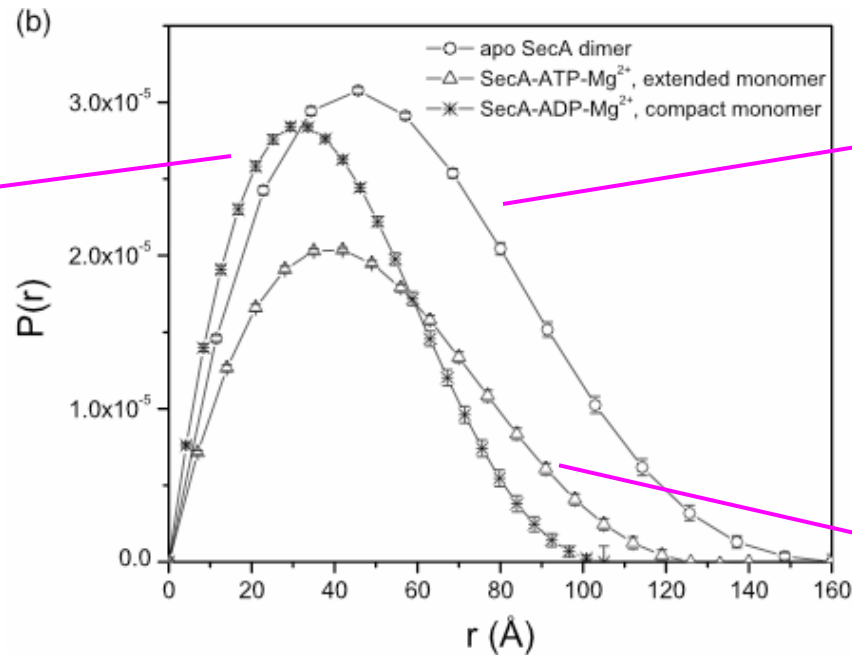
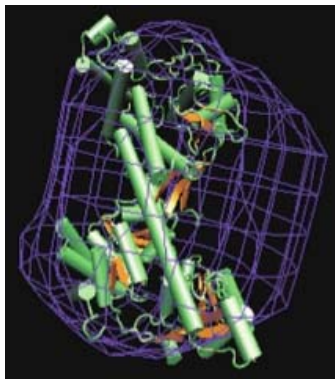
Image from [http://www.sfu.ca/mbb/mbb/faculty/paetzel/Mark\\_Paetzel\\_Page\\_files/IMAGE002.JPG](http://www.sfu.ca/mbb/mbb/faculty/paetzel/Mark_Paetzel_Page_files/IMAGE002.JPG)



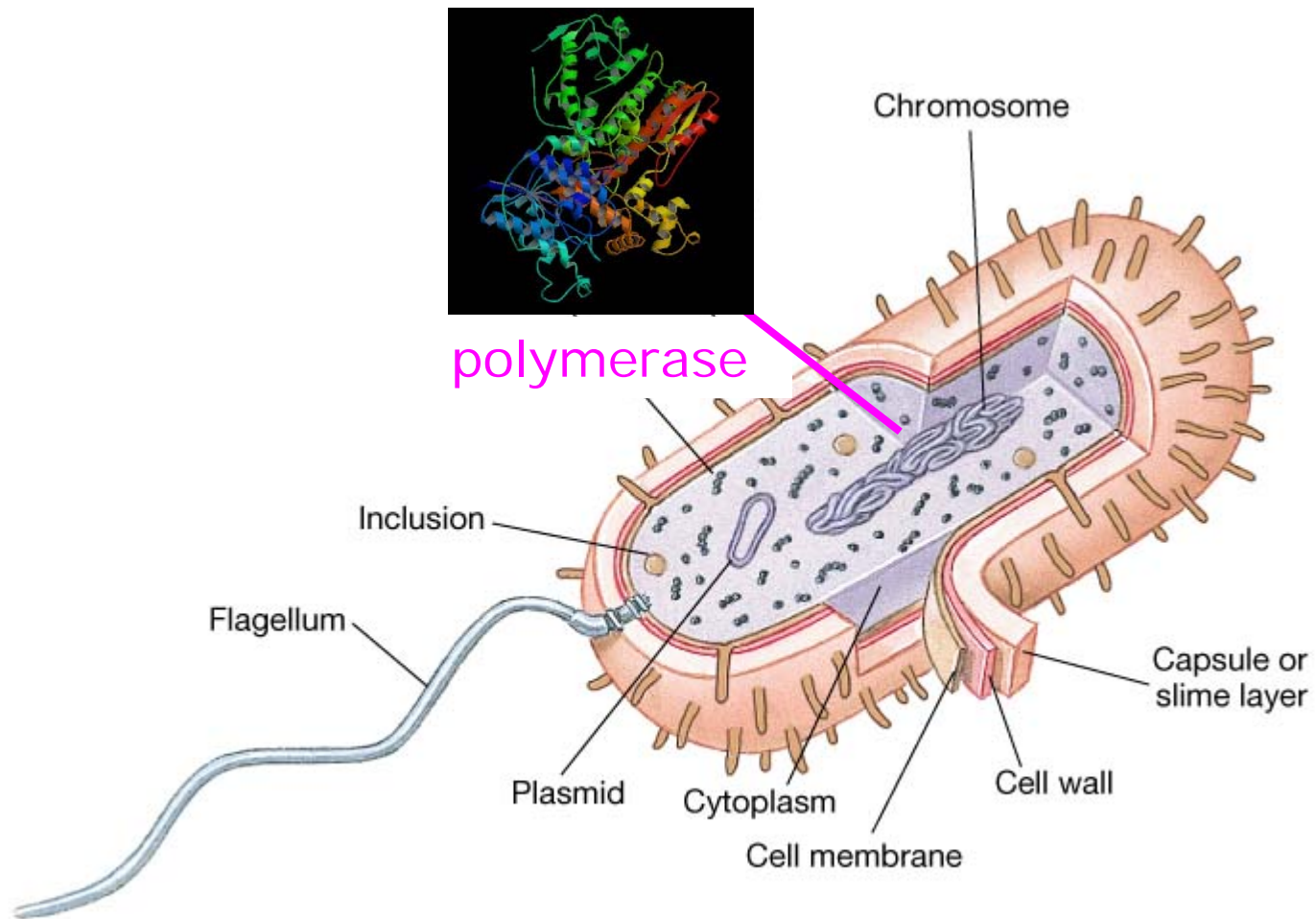
# Lipid vesicles are matched out at 14.3 % $D_2O$



# Nucleotide binding disrupts SecA dimer in lipid vesicles



# Dissection of the bacterial cell



## Structure-specific DNA-induced Conformational Changes in *Taq* Polymerase Revealed by Small Angle Neutron Scattering\*

Received for publication, April 26, 2004, and in revised form, June 30, 2004  
Published, JBC Papers in Press, July 7, 2004, DOI 10.1074/jbc.M404565200

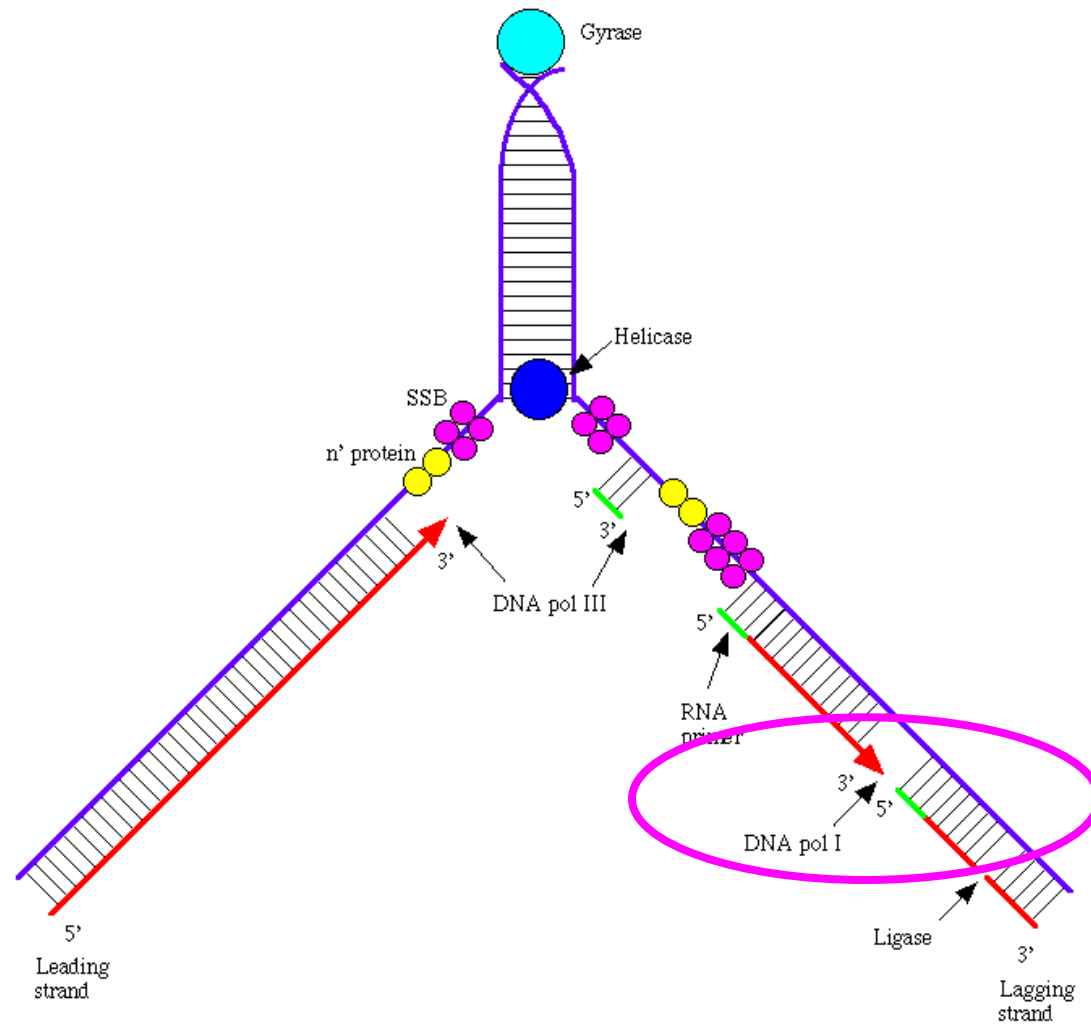
**Derek L. Ho<sup>‡</sup>, W. Malcolm Byrnes<sup>§</sup>, Wu-po Ma<sup>¶</sup>, Yuan Shi<sup>||</sup>, David J. E. Callaway<sup>||\*\*</sup>,  
and Zimei Bu<sup>‡‡§§</sup>**

*From the <sup>‡</sup>National Institute of Standards and Technology, Gaithersburg, Maryland 20898, the <sup>§</sup>Department of Biochemistry and Molecular Biology, Howard University College of Medicine, Washington, D. C. 20059, <sup>¶</sup>Third Wave Technologies, Inc., Madison, Wisconsin 53719, <sup>||</sup>North Shore/LIJ Research Institute, New York University School of Medicine, Manhasset, New York 11030, and the <sup>‡‡</sup>Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111*

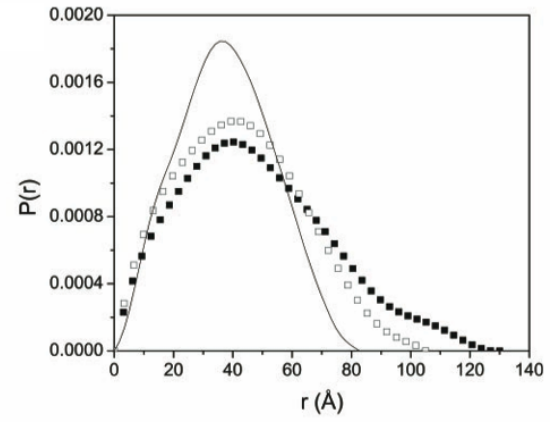
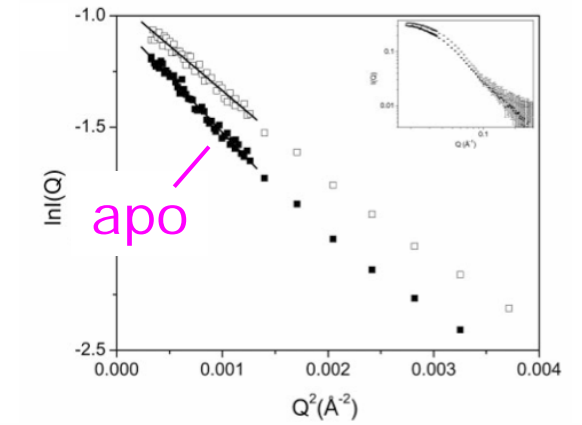
# *Taq* DNA pol I

- ↳ Performs lagging strand DNA synthesis and repair
- ↳ Comprises
  - ↳ Polymerase domain for making new DNA (Klentaq)
  - ↳ 5' nuclease domain for cleaving RNA primers or damaged DNA
- ↳ Puzzle: not apparent from crystal structure how *taq* polymerase coordinates 2 activities to leave only a “nick” and not a gap

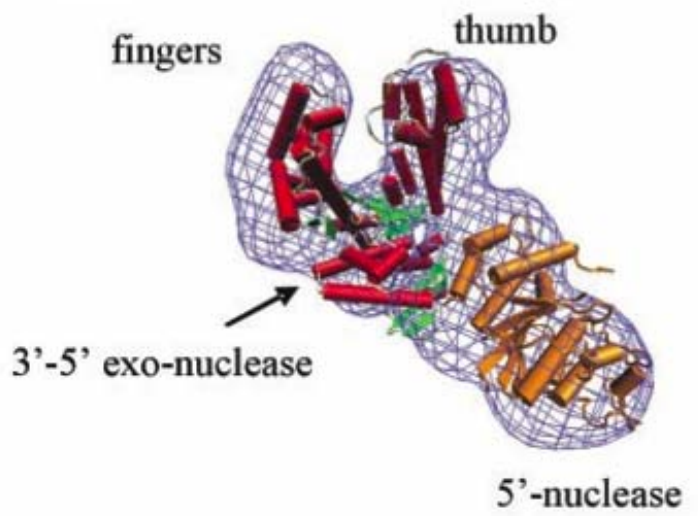
# Pol I is part of a replication machine



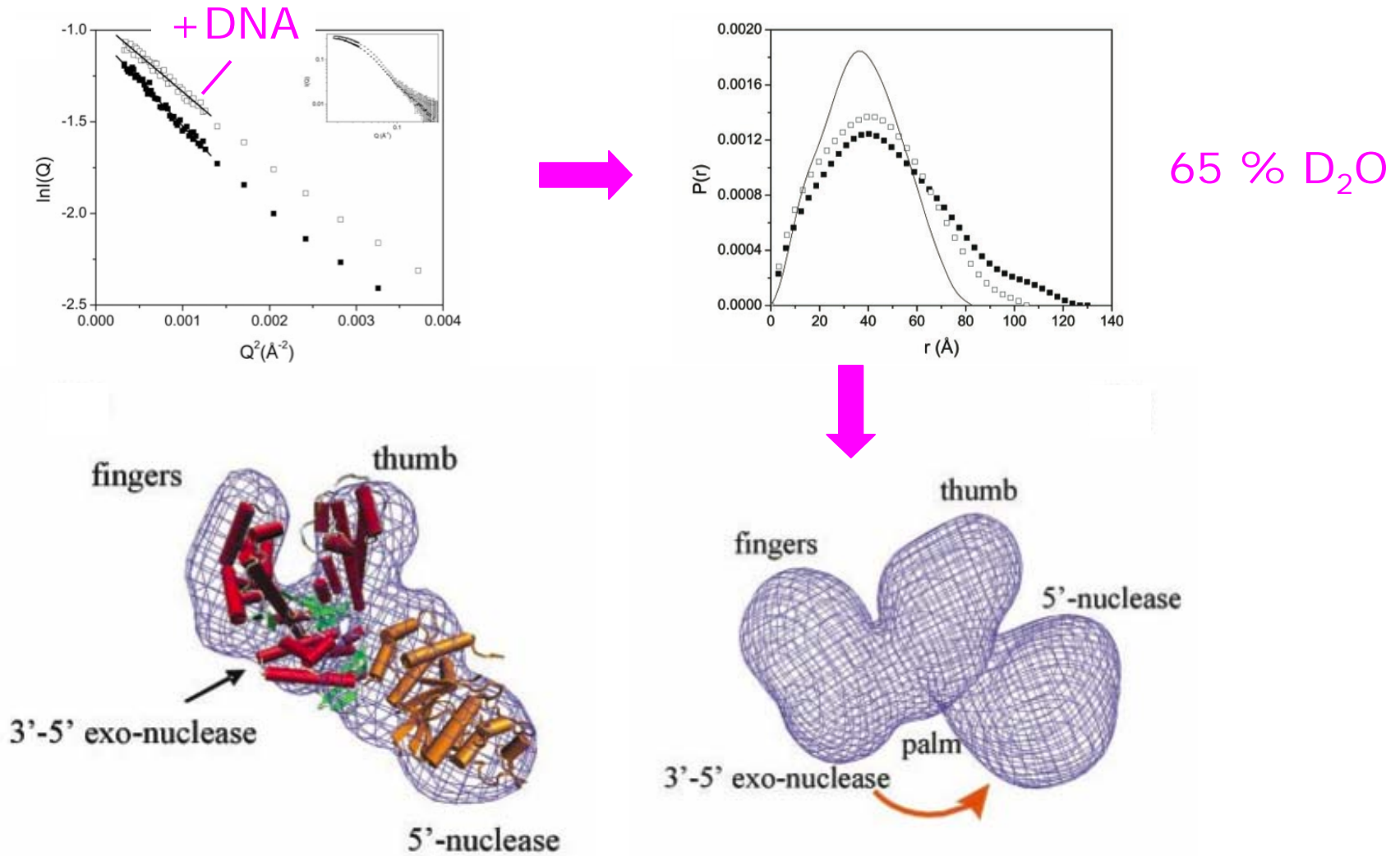
# Apo *taq* pol solution structure is similar to crystal structure...



90 % D<sub>2</sub>O

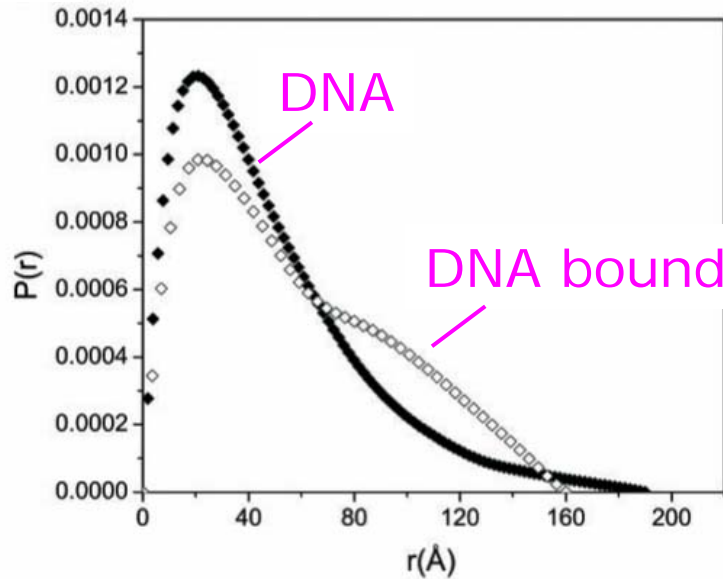


# ..but DNA induces 5' nuclease domain rearrangement

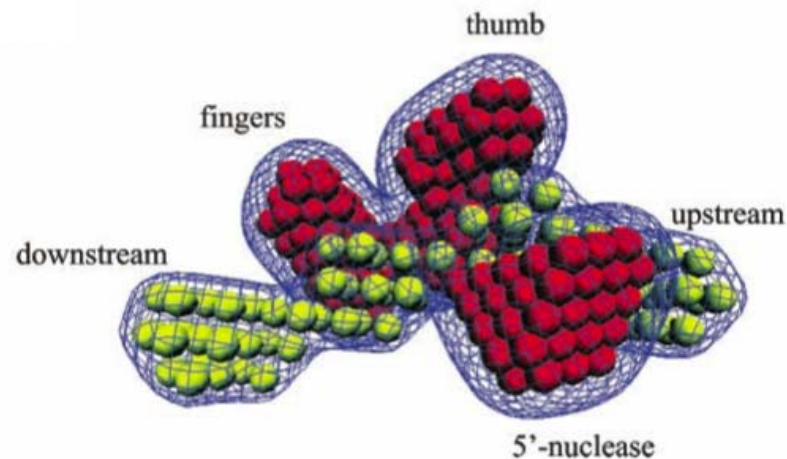
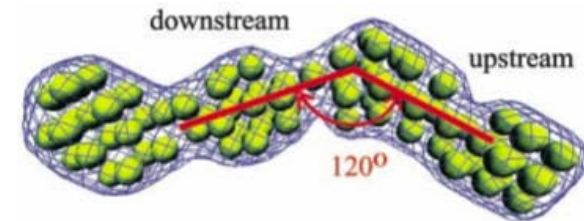




# DNA is bent by $120^\circ$ when bound to 5' nuclease or *taq* polymerase



45 %  $\text{D}_2\text{O}$



## **A global multi-technique approach to study low-resolution solution structures**

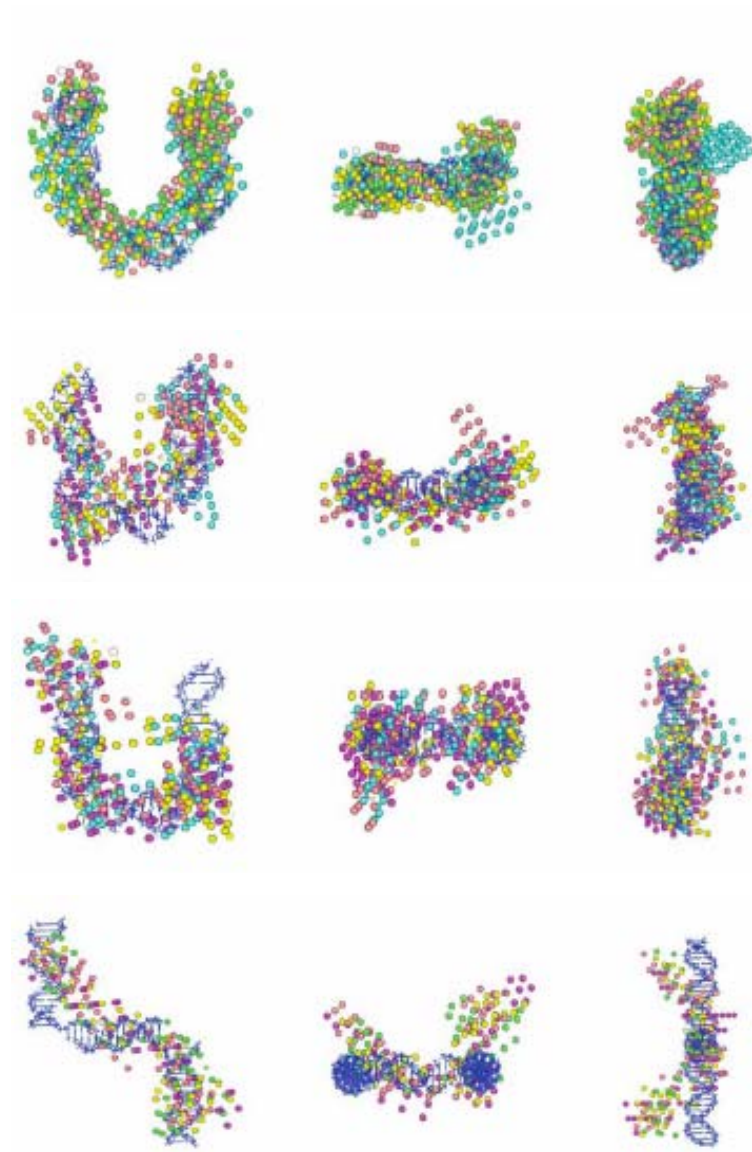
**Marcelo Nöllmann,<sup>a,b\*</sup> W. Marshall Stark<sup>a</sup> and Olwyn Byron<sup>b</sup>**

<sup>a</sup>Division of Molecular Genetics, IBLS, University of Glasgow, Glasgow G12 8QQ, Scotland, and

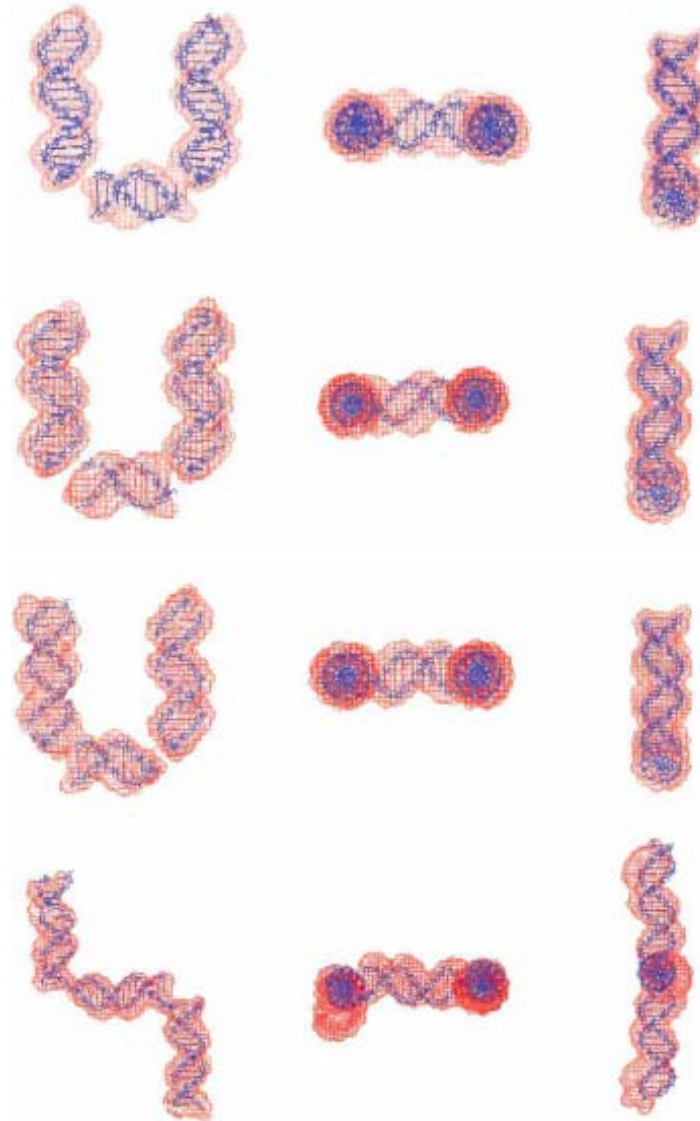
<sup>b</sup>Division of Infection and Immunity, IBLS, University of Glasgow, Glasgow G12 8QQ, Scotland.

Correspondence e-mail: [marcnol@berkeley.edu](mailto:marcnol@berkeley.edu)

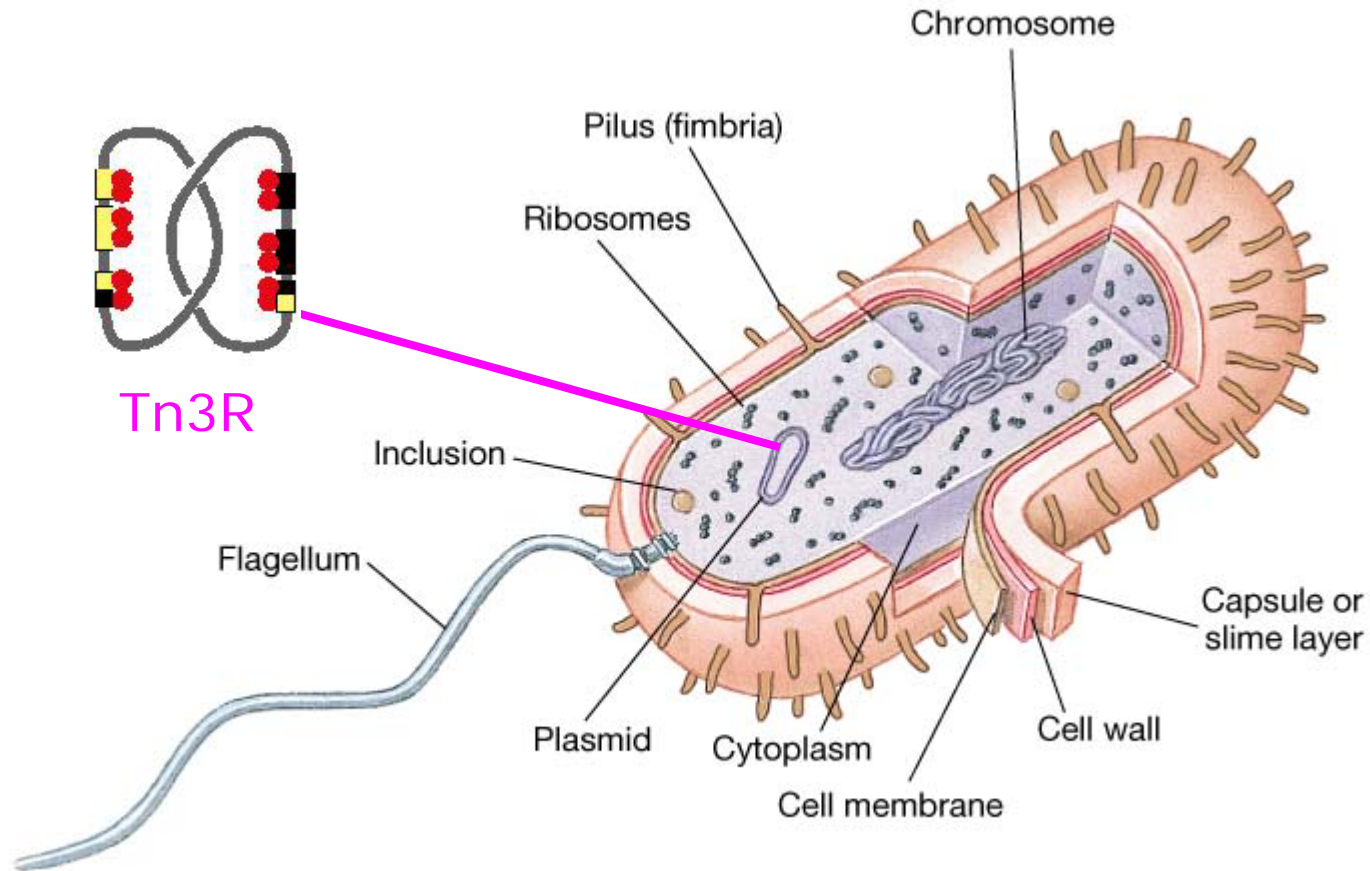
When *ab initio* modelling fails...



...rigid body modelling offers a solution



# Dissection of the bacterial cell



Molecular Cell, Vol. 16, 127–137, October 8, 2004, Copyright ©2004 by Cell Press

# **Solution Structure of the Tn3 Resolvase-Crossover Site Synaptic Complex**

**Marcelo Nöllmann,<sup>1,2</sup> Jiuya He,<sup>1</sup> Olwyn Byron,<sup>2</sup>  
and W. Marshall Stark<sup>1,\*</sup>**

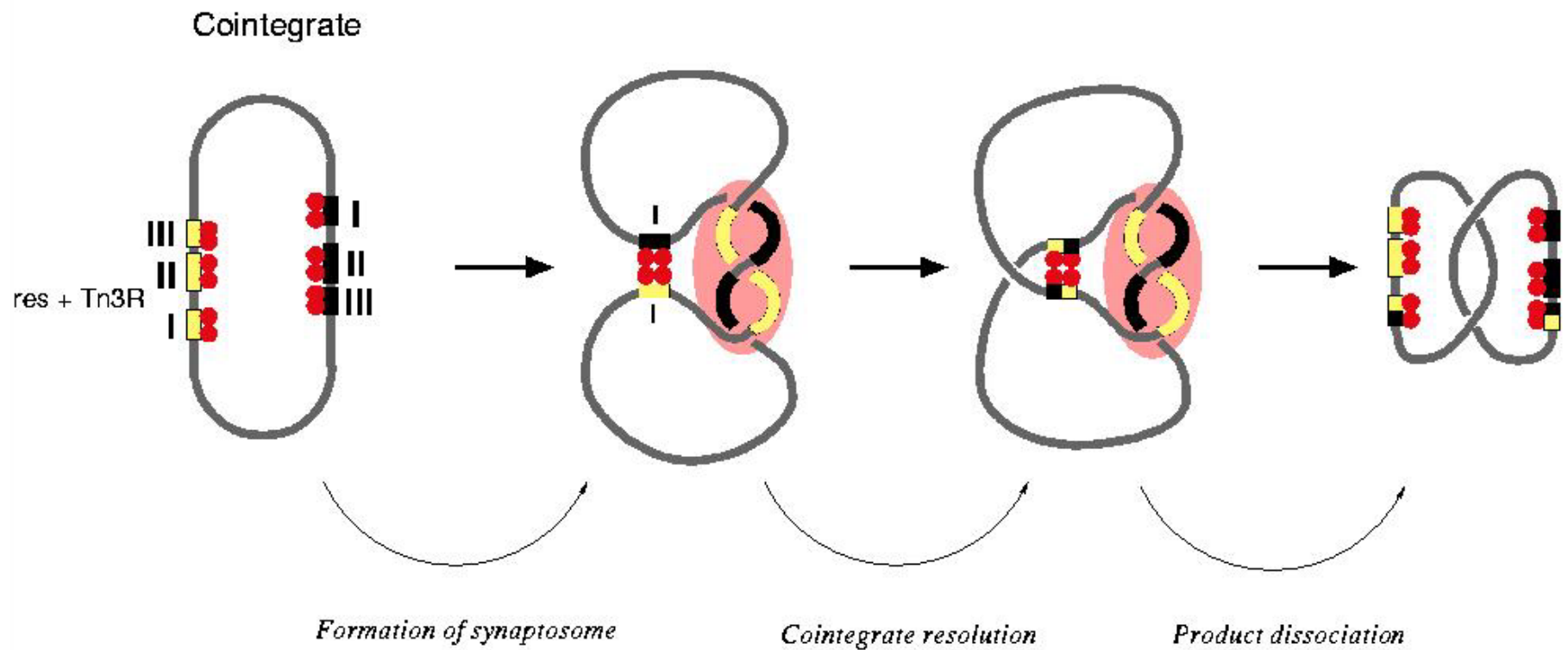
<sup>1</sup>Division of Molecular Genetics  
Institute of Biomedical and Life Sciences  
University of Glasgow  
Glasgow G11 6NU  
Scotland  
United Kingdom

<sup>2</sup>Division of Infection & Immunity  
Institute of Biomedical & Life Sciences  
University of Glasgow  
Glasgow G12 8QQ  
Scotland  
United Kingdom

# Tn3 resolvase

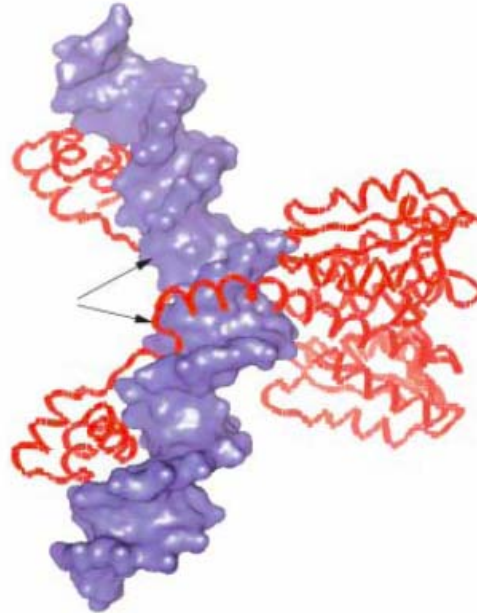
- 👉 Protein
- 👉 Brings about rearrangement of bacterial DNA by catalysing cleavage and re-joining of DNA strands
- 👉 Forms a large DNA-protein complex = synaptosome

# Architecture of synaptosome is unknown

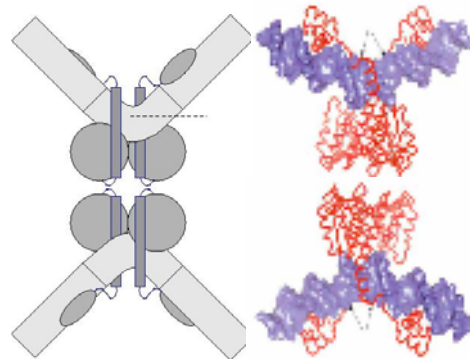
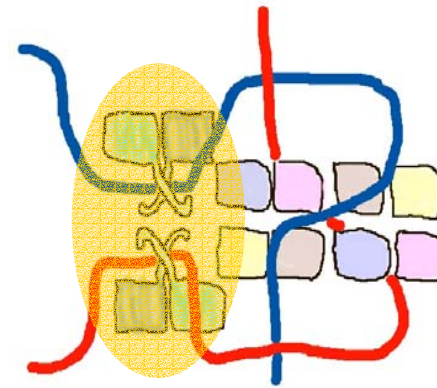
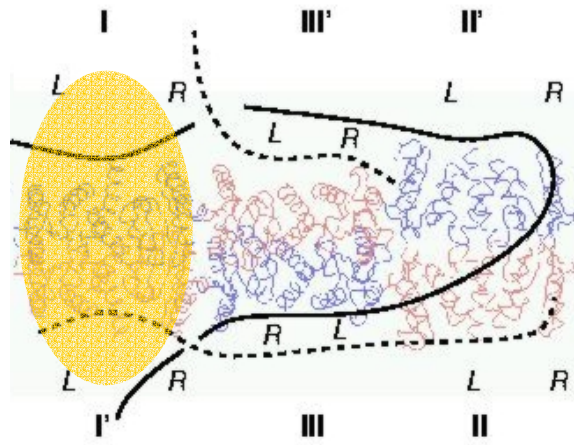




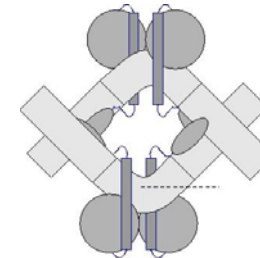
# High resolution structure of one-sixth of synaptosome ( $\gamma\delta$ resolvase)



# Models for synaptosome: DNA in or out?

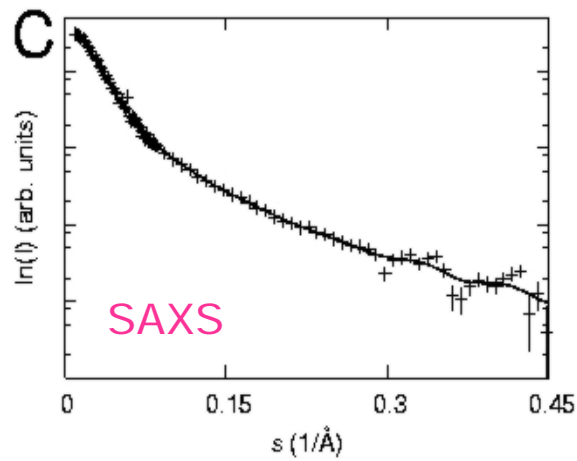
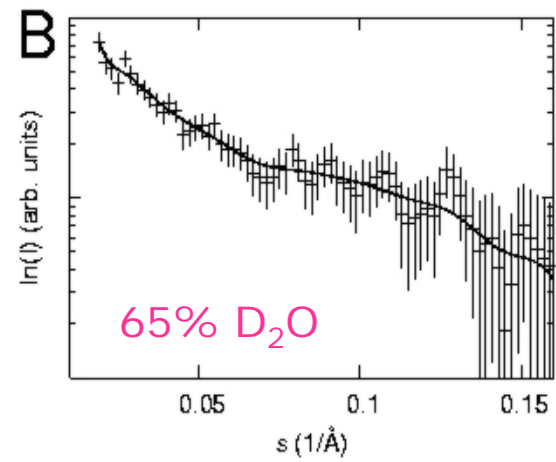
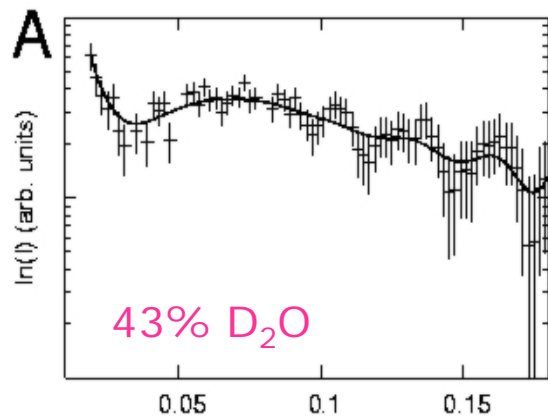


DNA-out<sup>BB</sup>  
(Grindley, 2001)

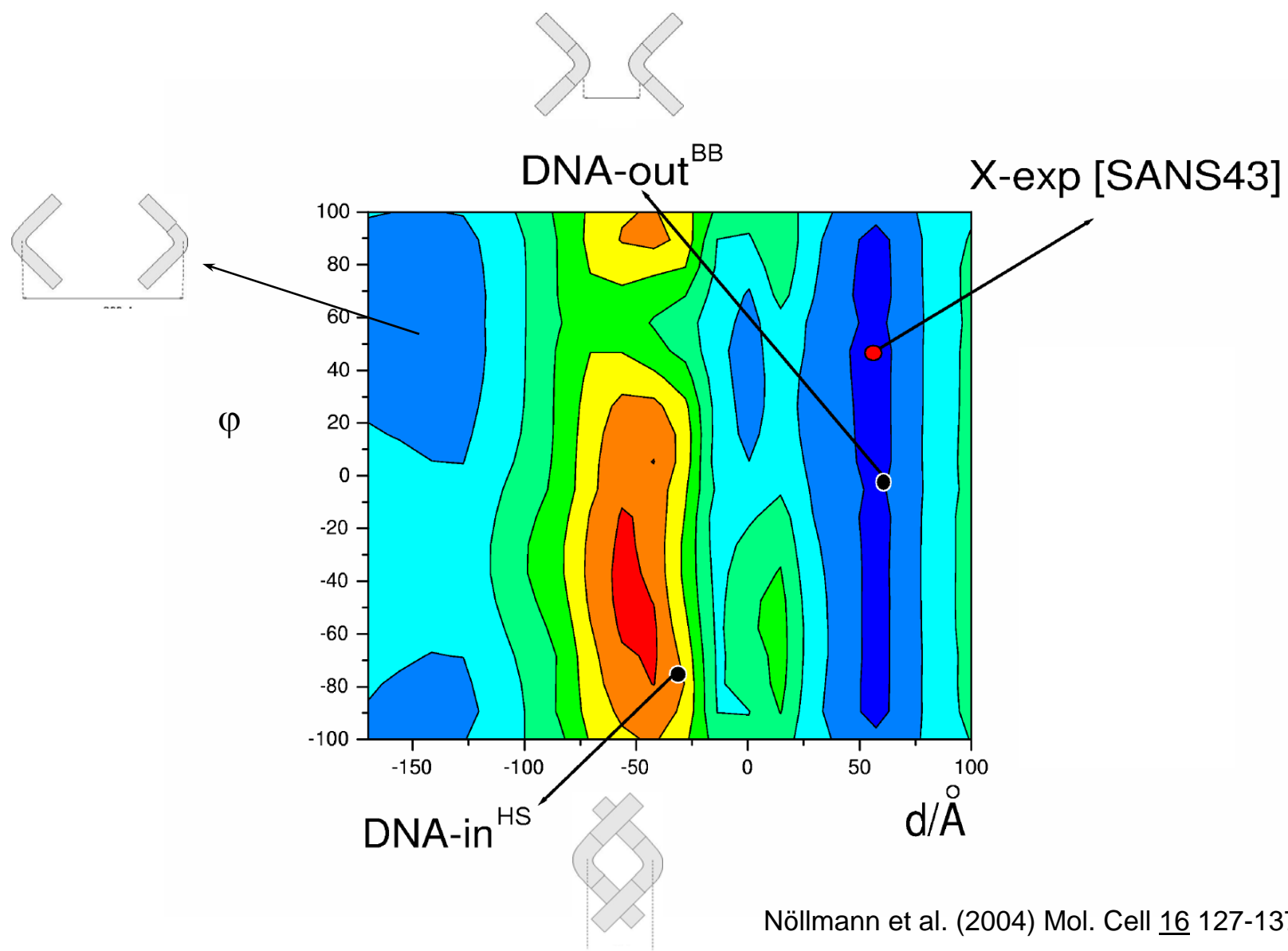


DNA-in<sup>HS</sup>  
(Rice & Steitz, 1994)

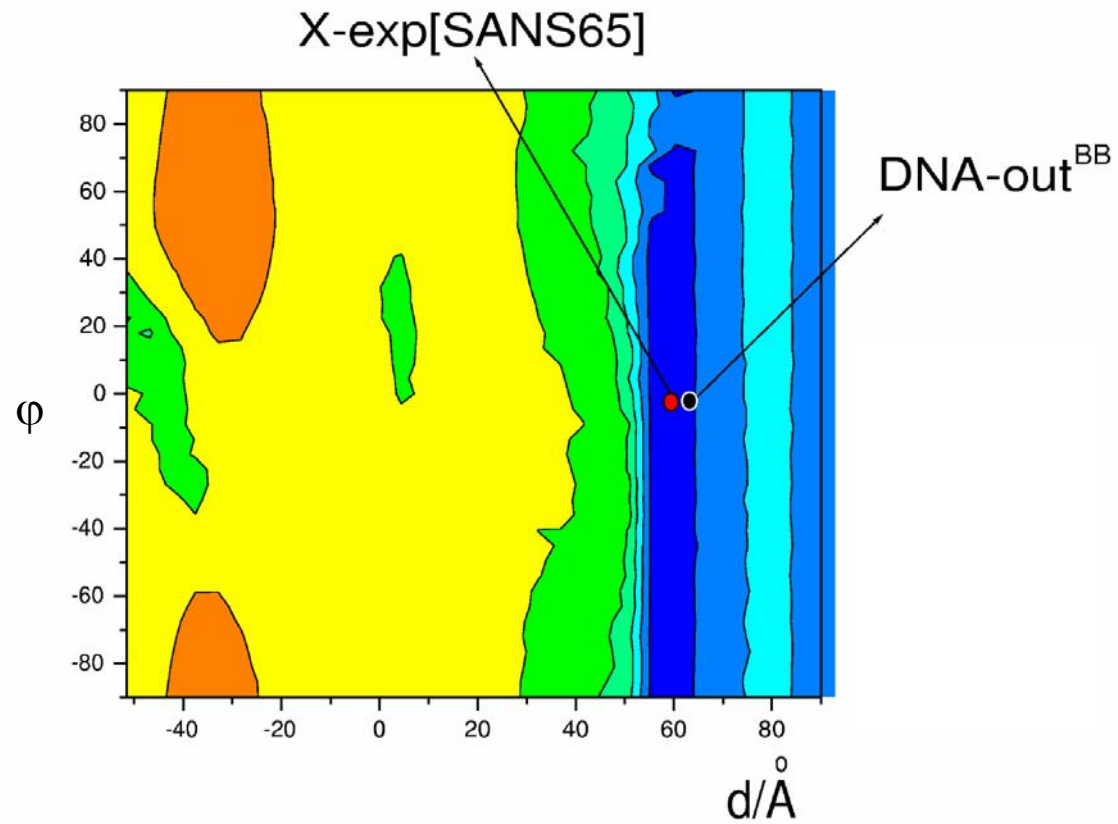
# SAXS & SANS studies



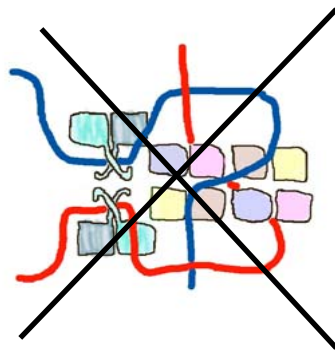
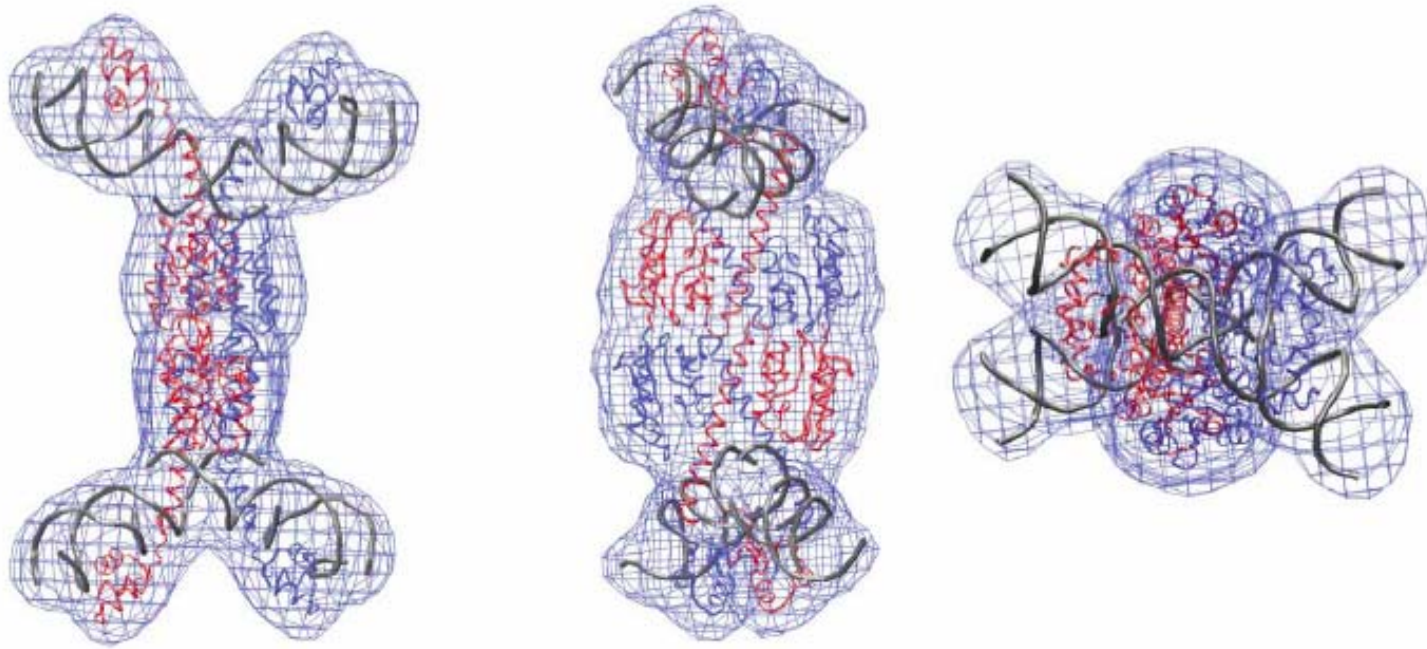
# Protein contrast matched: shape of DNA?



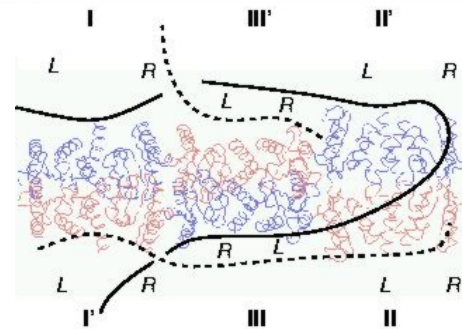
# DNA contrast matched: shape of protein?



# Final model: DNA out



[Rice & Steitz, 1995]



(Sarkis & Grindley, 2001)

Nöllmann et al. (2004) *Mol. Cell* **16** 127-137

Thank you!