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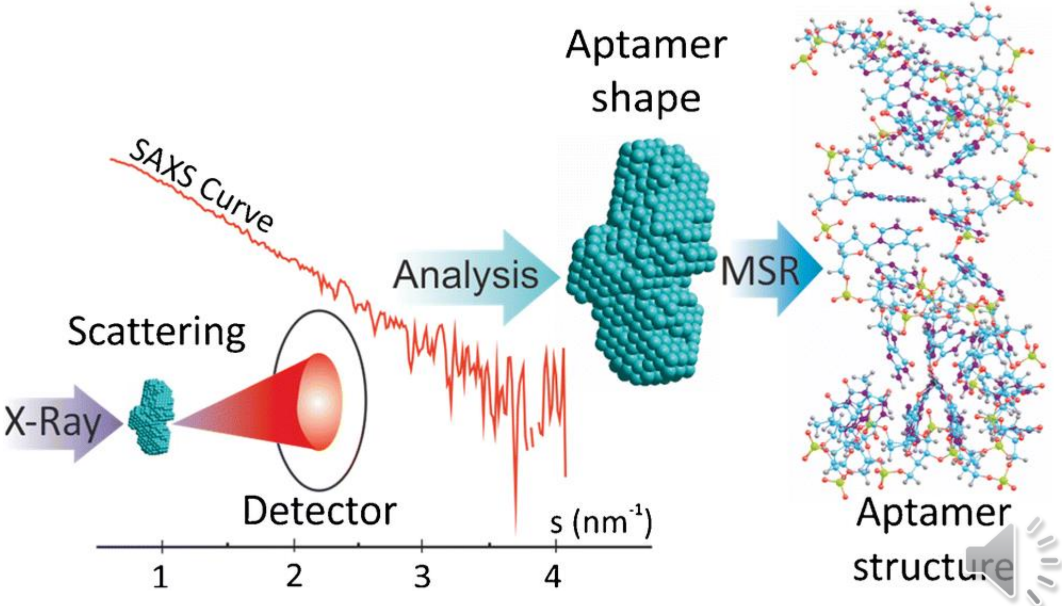
Small-Angle scattering applications to the analysis of aptamer structure and conformational changes

Moryachkov Roman
Zabluda V.N., Spiridonova V.A., Artyushenko P.V., Shchugoreva I.A., Berlina A.N.,
Peters G.S., Kichkailo A.S. and Sokolov A.E.
Kirensky Institute of Physics
14.07.2020

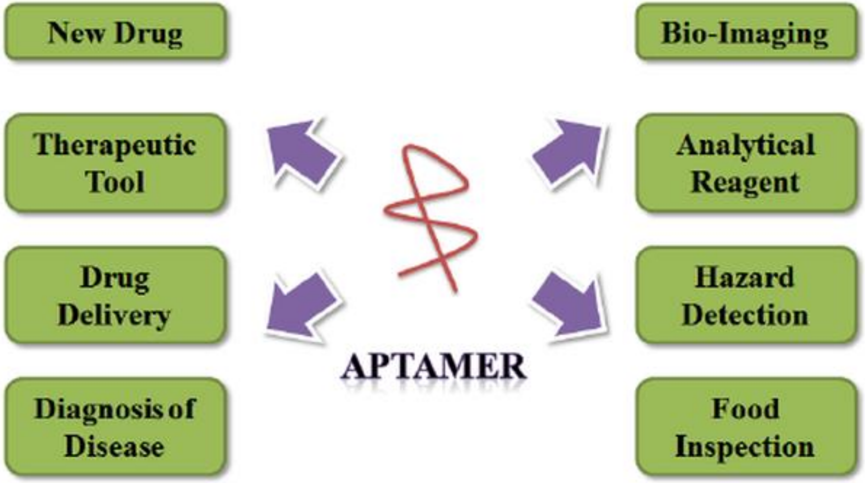
Laboratory for Digital
Controlled Drugs
& Theranostics

Outline

- Introduction
- SAXS experiment description
- SAXS applications
- Some results
- Conclusion



Aptamers are short DNA, RNA, or peptide structures that have **enormous therapeutic potential**, because they possess the ability to target molecules and proteins for which antibodies are not well suited with equally **high specificity and affinity.**


New Drug

Therapeutic Tool

Drug Delivery

Diagnosis of Disease

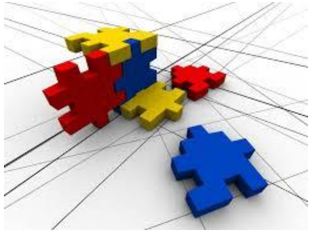
APTAMER

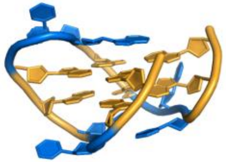
Bio-Imaging

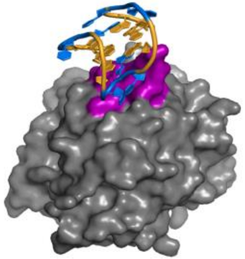
Analytical Reagent

Hazard Detection

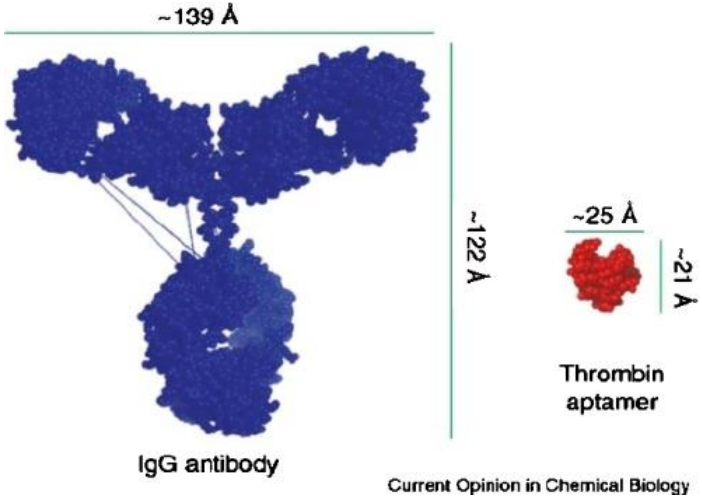
Food Inspection







“aptus” (Latin) - fit, suitable

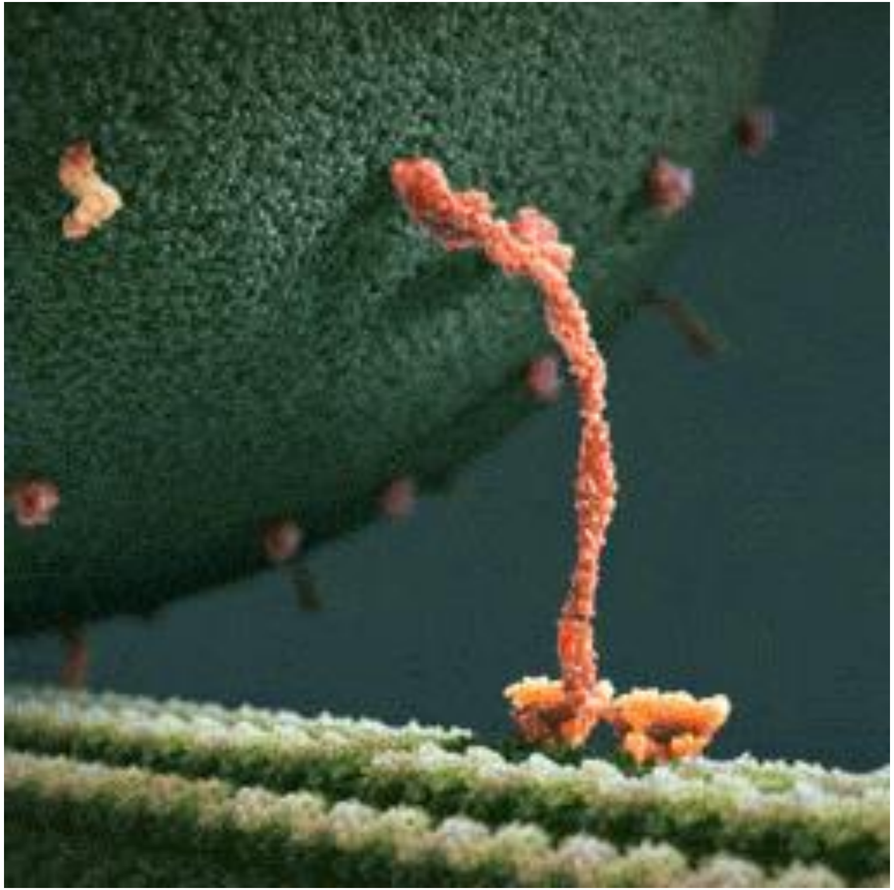


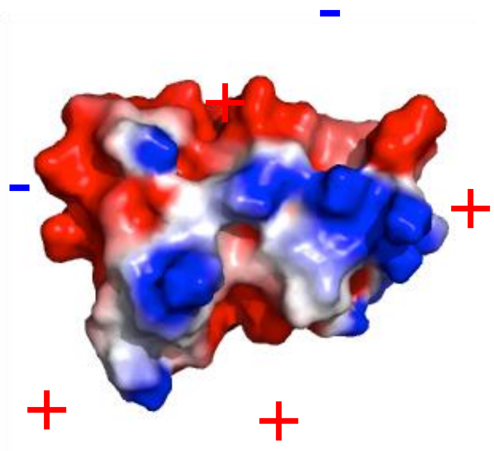
Current Opinion in Chemical Biology

Aptamers:

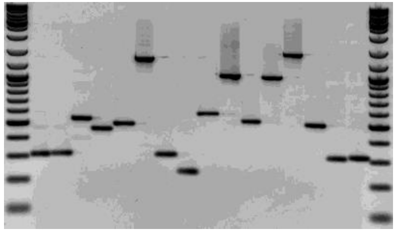
- small, compact
- cost effective
- do not require refrigeration
- do not evoke a negative immune response
- can bind to smaller targets that antibodies cannot reach
- can be targeted against toxins and other molecules that do not cause strong immune responses

The structure provides the function



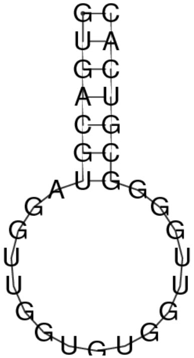


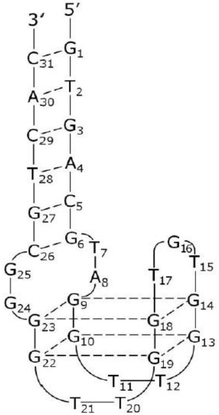
Primary structure is known:
5′-GTGACGTAGGTTGGTGTGGTTGGGGCGTCAC-3′

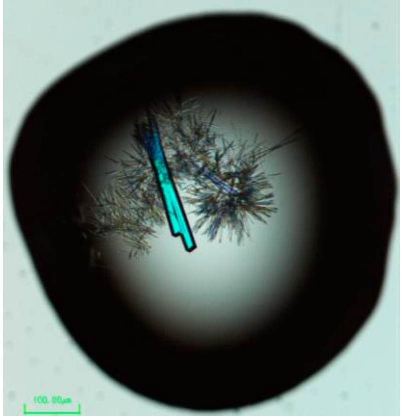


DNA/RNA sequencing

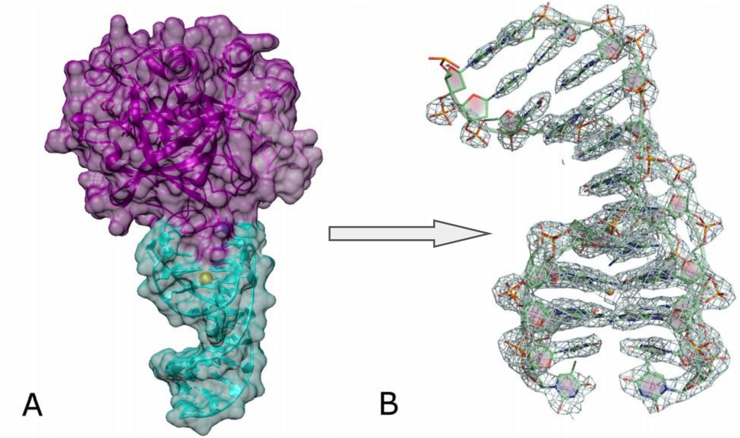
Secondary structure modeling







crystal of the protein-aptamer complex



X-ray diffraction structure

Electron density map of the aptamer RE31 to thrombi

Single-stranded nucleic acids are mostly not crystallized

Except several protein-aptamer complexes

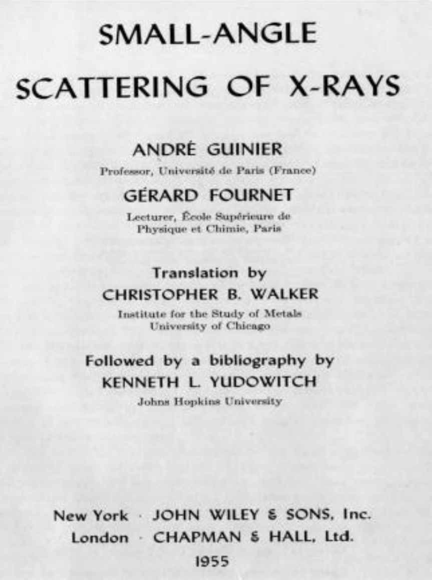
Russo Krauss et al. *Acta Crystallographica Section D: Biological Crystallography* (2013)

X-ray scattering power of a protein solution

A 1 mg/ml solution of a globular protein 15kDa molecular mass

such as lysozyme or myoglobin will scatter in the order of

1 photon in 10^6 incident photons



SMALL-ANGLE
SCATTERING OF X-RAYS

ANDRÉ GUINIER
Professor, Université de Paris (France)
GÉRARD FOURNET
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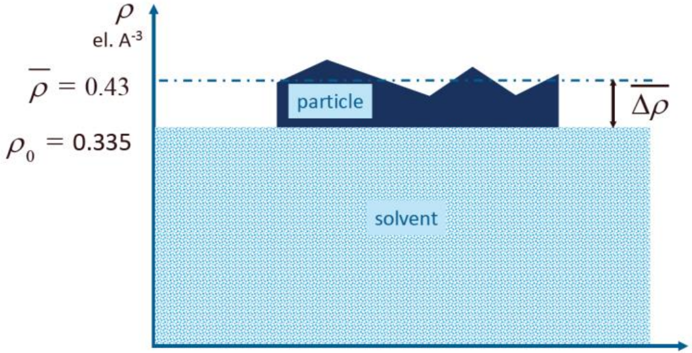
University of Chicago

Followed by a bibliography by
KENNETH L. YUDOWITCH
Johns Hopkins University

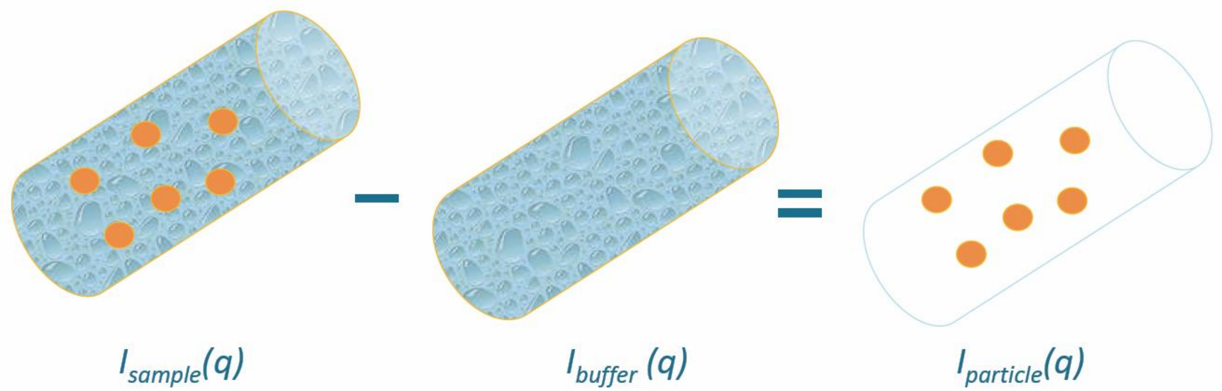
New York · JOHN WILEY & SONS, Inc.
London · CHAPMAN & HALL, Ltd.
1955

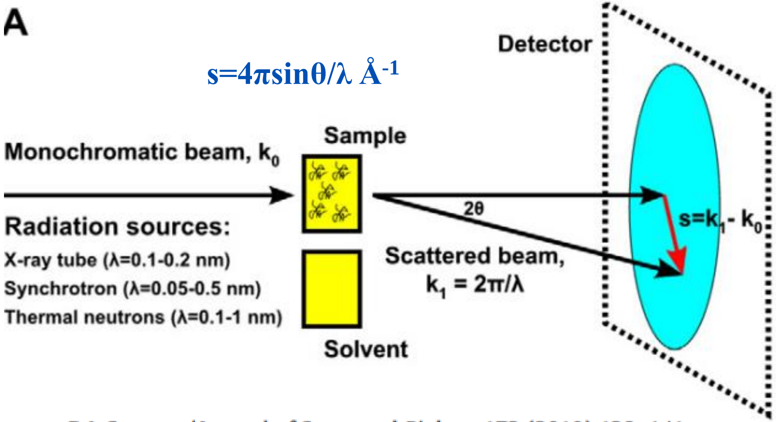
from H.B. Stuhrmann
Synchrotron Radiation Research
H. Winick, S. Doniach Eds. (1980)



Contrast

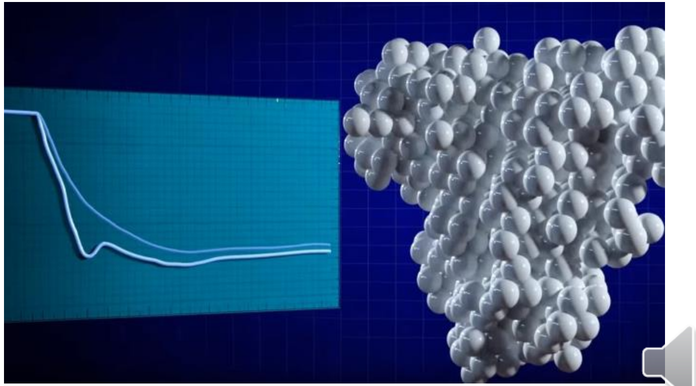
Small-Angle X-Ray Scattering (SAXS)





$s=4\pi\sin\theta/\lambda$ Å^{-1}

H.D.T. Mertens, D.I. Svergun / Journal of Structural Biology 172 (2010) 128–141



DIKSI station
Wavelength - 0,162 nm (7,65 keV)
Beam size - 0.3x0.2 mm
Detector - Dectris Pilatus 1M

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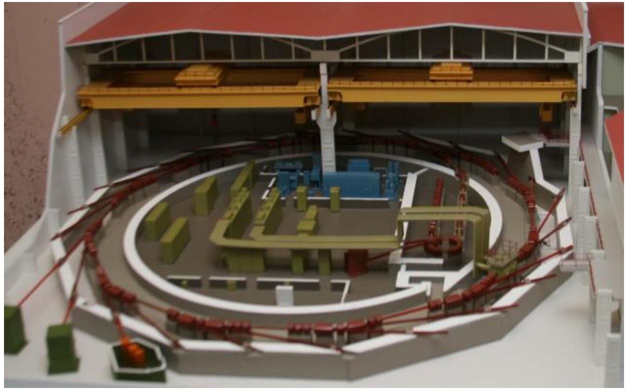
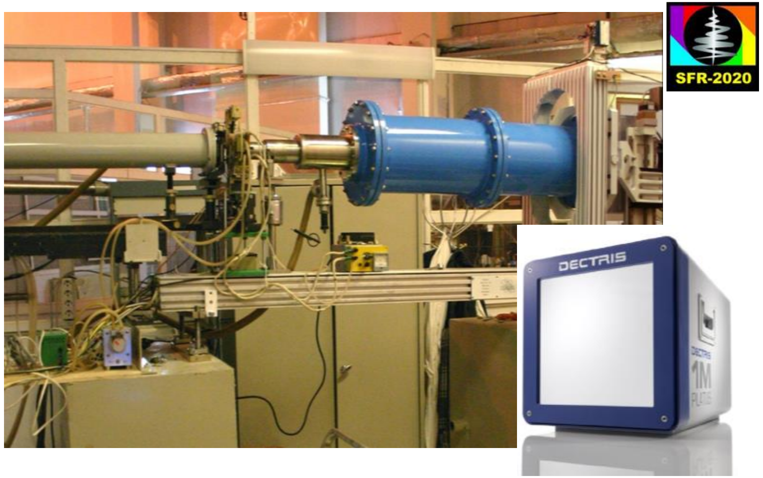
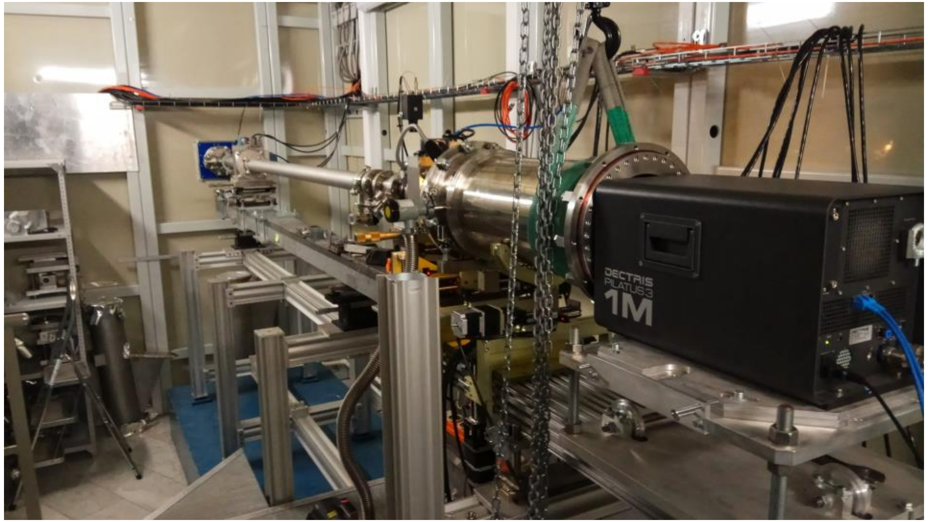




BioSAXS station (from Dec. 2017)

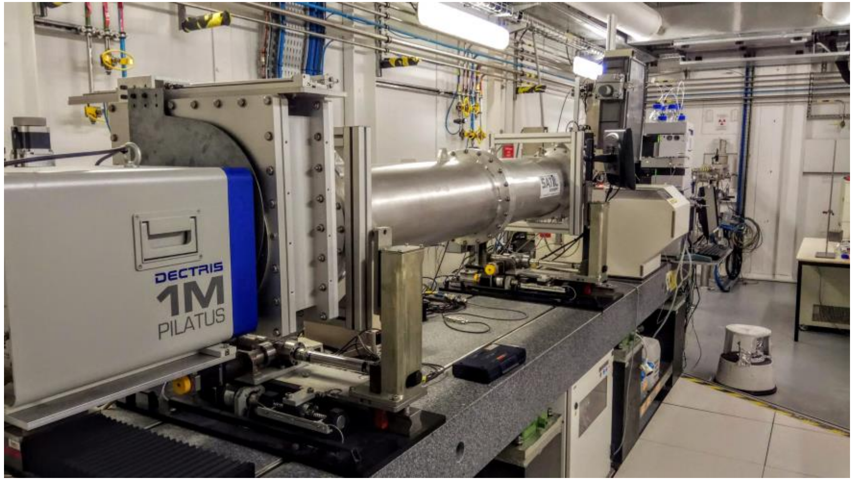


European Synchrotron Radiation Facility

BioSAXS beamline BM29

ESRF
The European Synchrotron

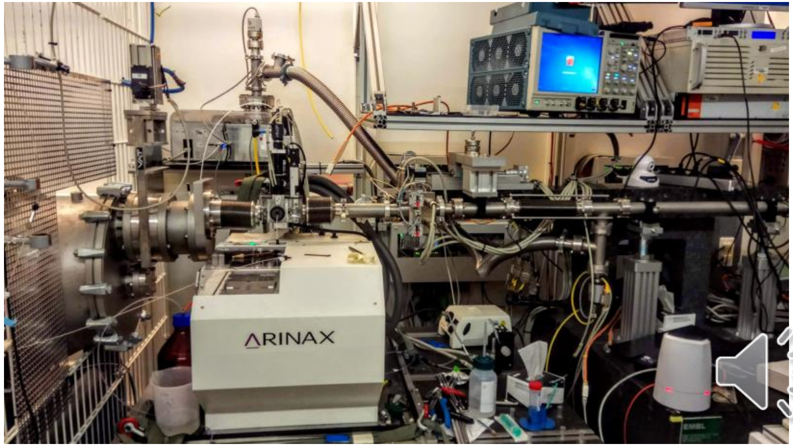
DESY.

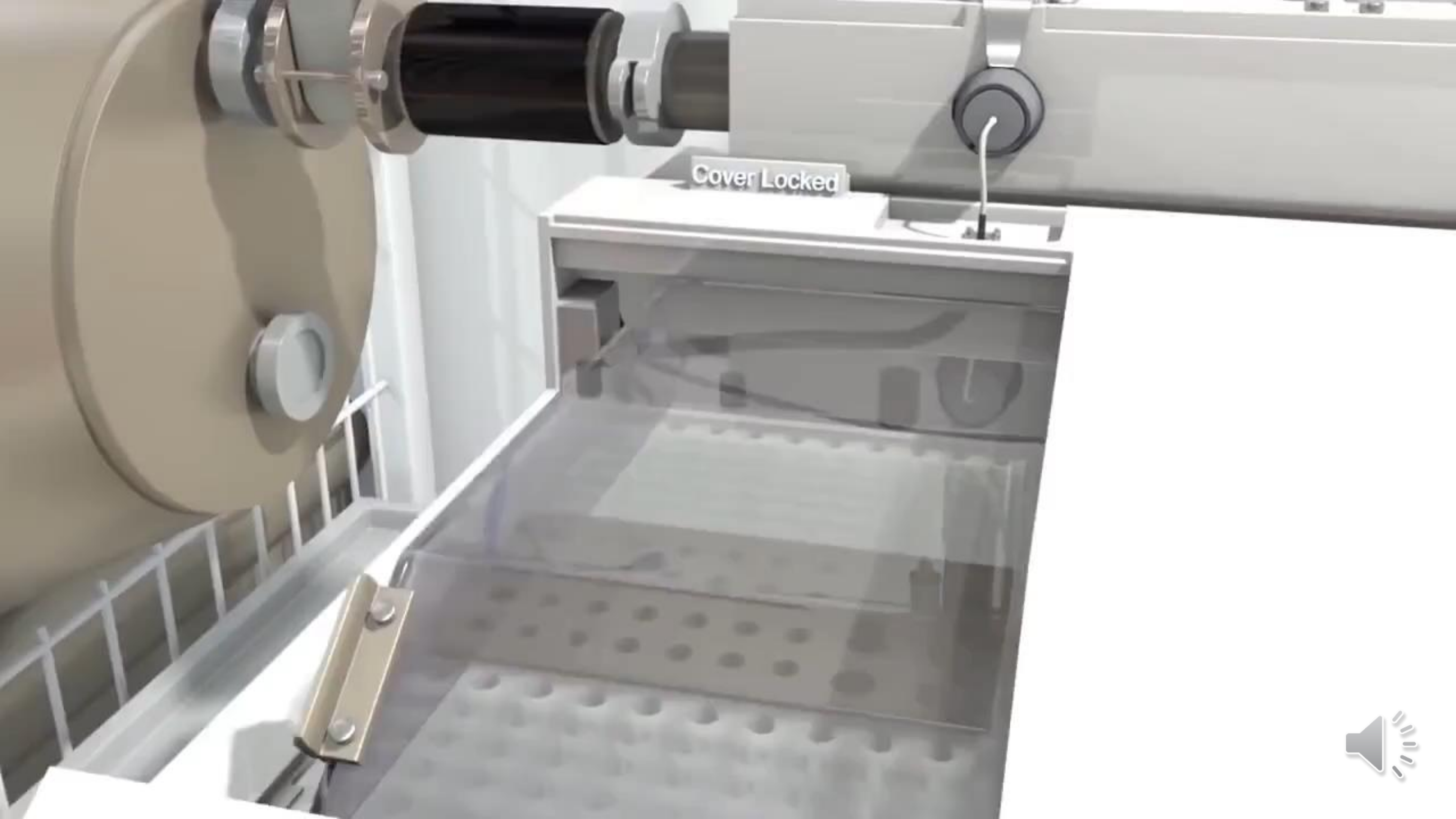


Deutsches Elektronen-Synchrotron (DESY)

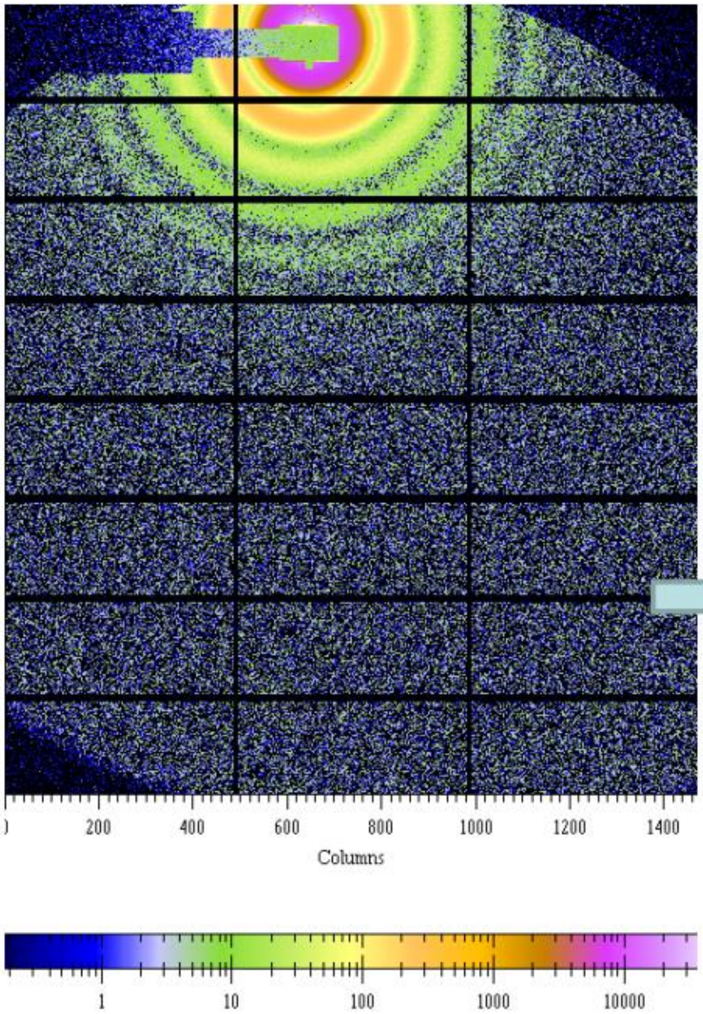
BioSAXS beamline P12

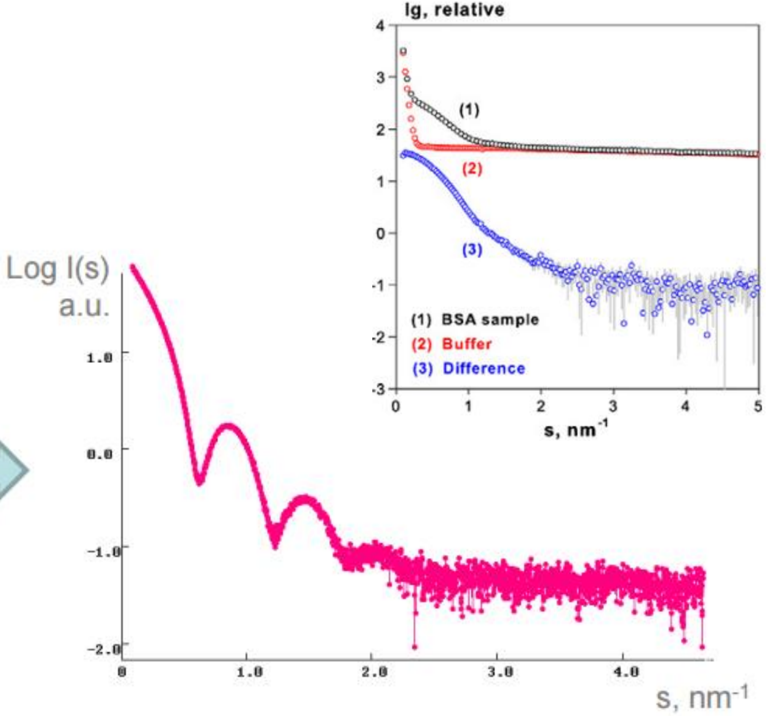
EMBL




Cover Locked

Data acquisition, integration

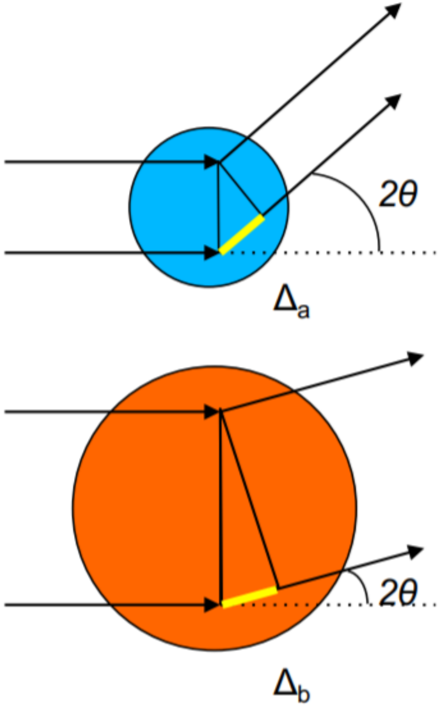


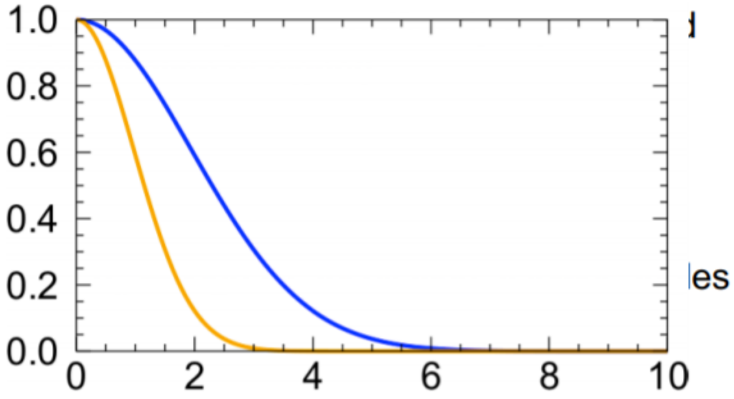


$$I(s) = \langle \mathbf{I}(\mathbf{s}) \rangle_{\Omega} = \langle \mathbf{A}(\mathbf{s})\mathbf{A}^{*}(\mathbf{s}) \rangle_{\Omega}$$

Big vs small objects and the scattering angle!

- Intensity drops off more rapidly for larger particles!

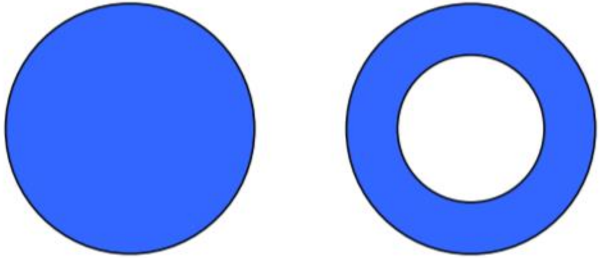




Adapted from: Kratky, O. (1963) *Prog. Biophys. Mol. Biol.* 13.

Radius of gyration, R_g

- **A SAXS parameter for "size"**
  - Distribution of components around an axis (or center of mass)
  - "the root-mean-square distance of all elemental scattering volumes from their center of mass weighted by their scattering densities"



$$R_g^2 = \frac{\int \Delta\rho(r_1) r_i^2 dV_i}{\int \Delta\rho(r_i) dV_i}$$

R_g solid sphere < R_g hollow sphere

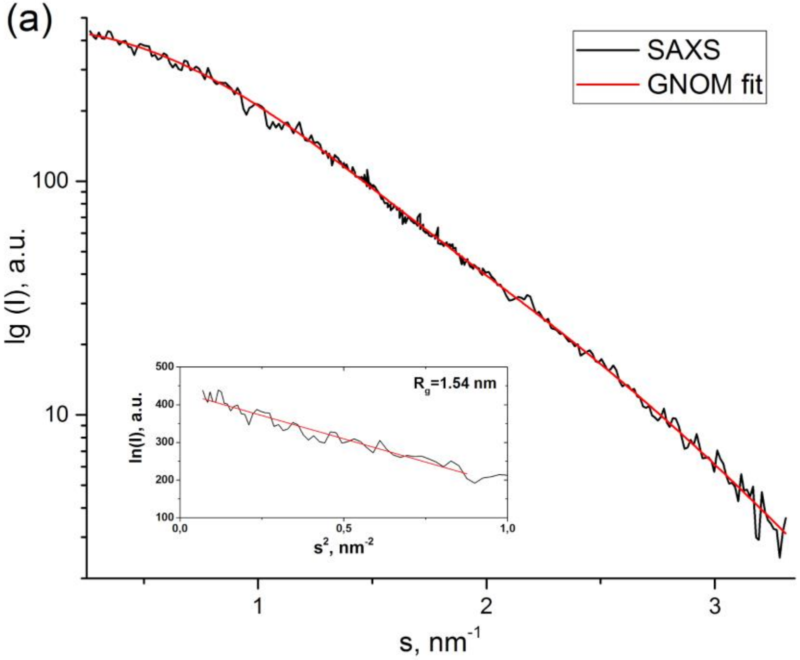
Andre Guinier
1930s

Guinier analysis

Guinier equation for globula:

$$I(s) = I(0)\exp\left(-\frac{1}{3}R_g^2 s^2\right)$$

Guinier plot ($\ln[I(s)]$vs s^2) - approximation at small angles

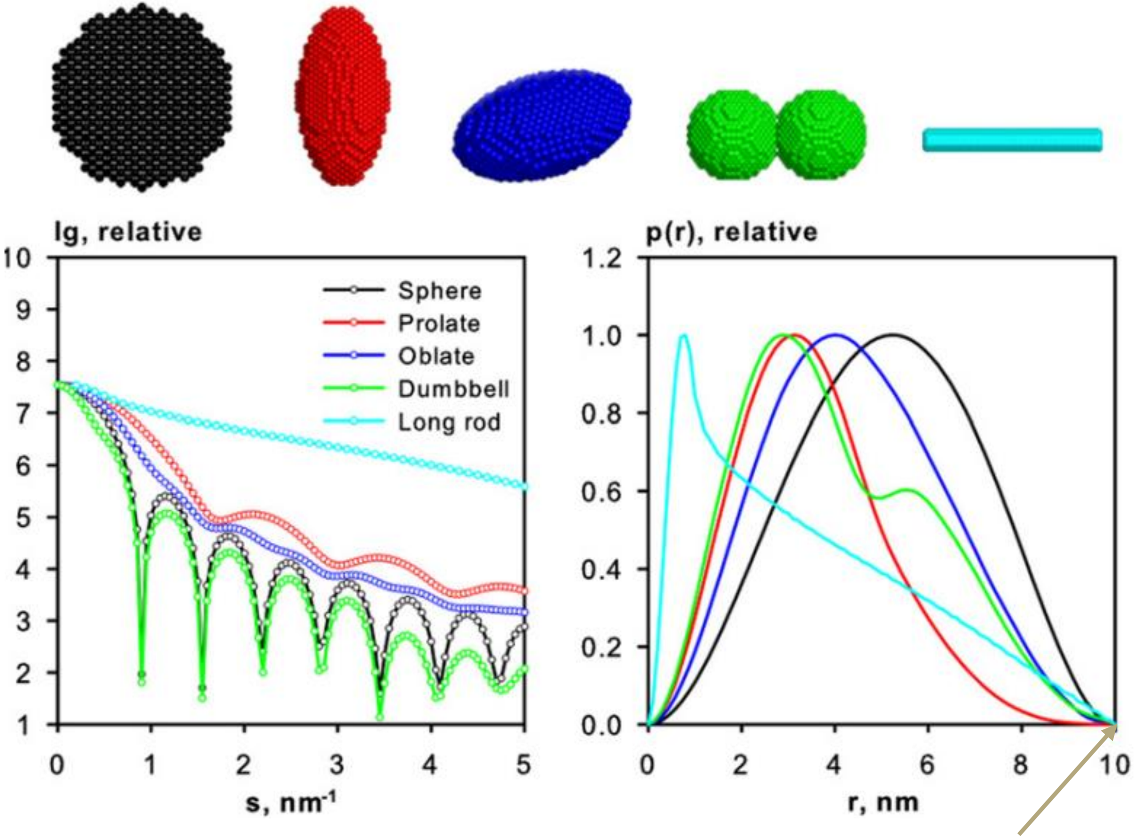


SAXS and Guinier plots for RE31 aptamer

Tomilin, F.N., Moryachkov, R., Shchugoreva, I. et al. Anal Bioanal Chem (2019). https://doi.org/10.1007/s00216-019-02045-0

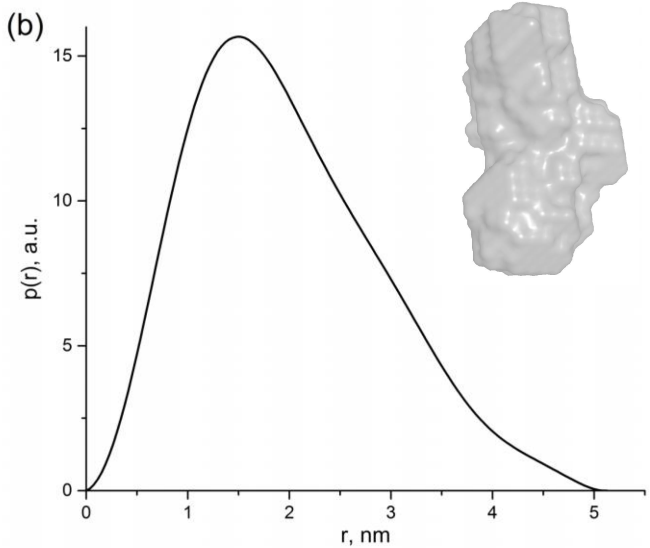
Indirect Fourier transformation

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



D_{max}

H.D.T. Mertens, D.I. Svergun / Journal of Structural Biology 172 (2010) 128–141

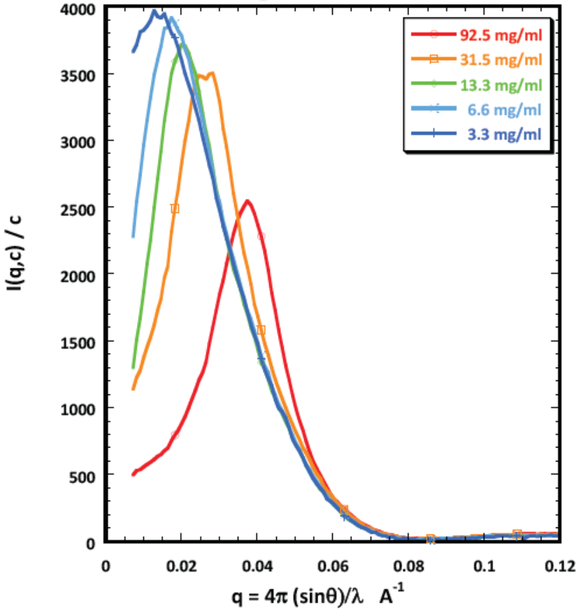


Distance distribution function plot for RE31 aptamer

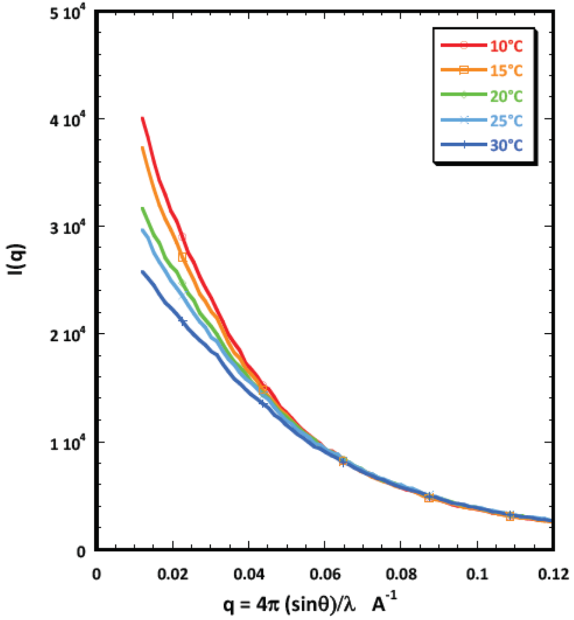
Tomilin, F.N., Moryachkov, R., Shchugoreva, I. et al. Anal Bioanal Chem (2019). https://doi.org/10.1007/s00216-019-02045-0

Non ideality: interactions

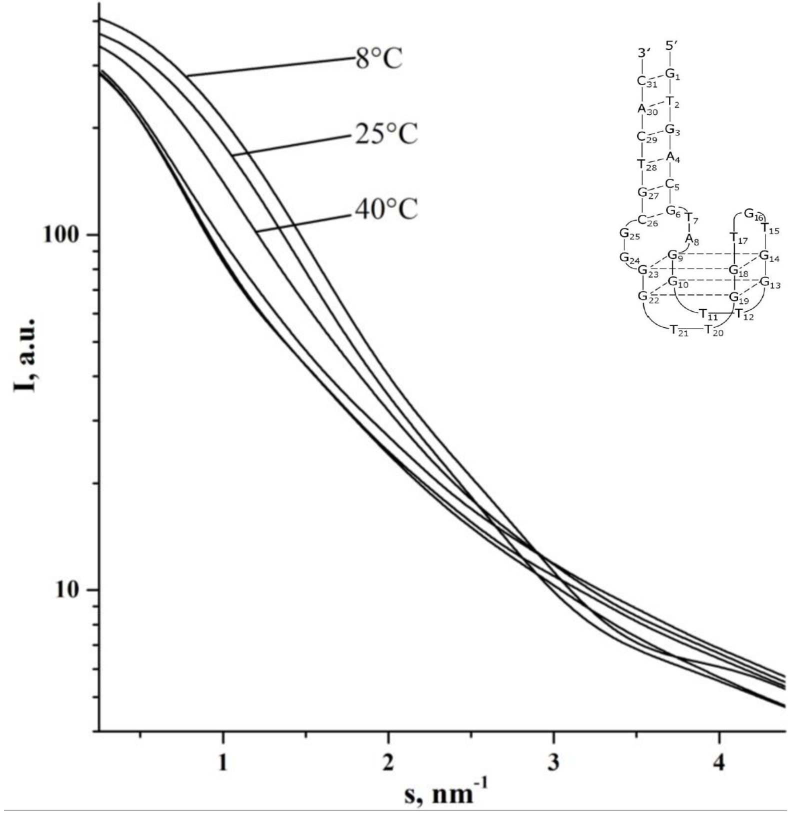
repulsion



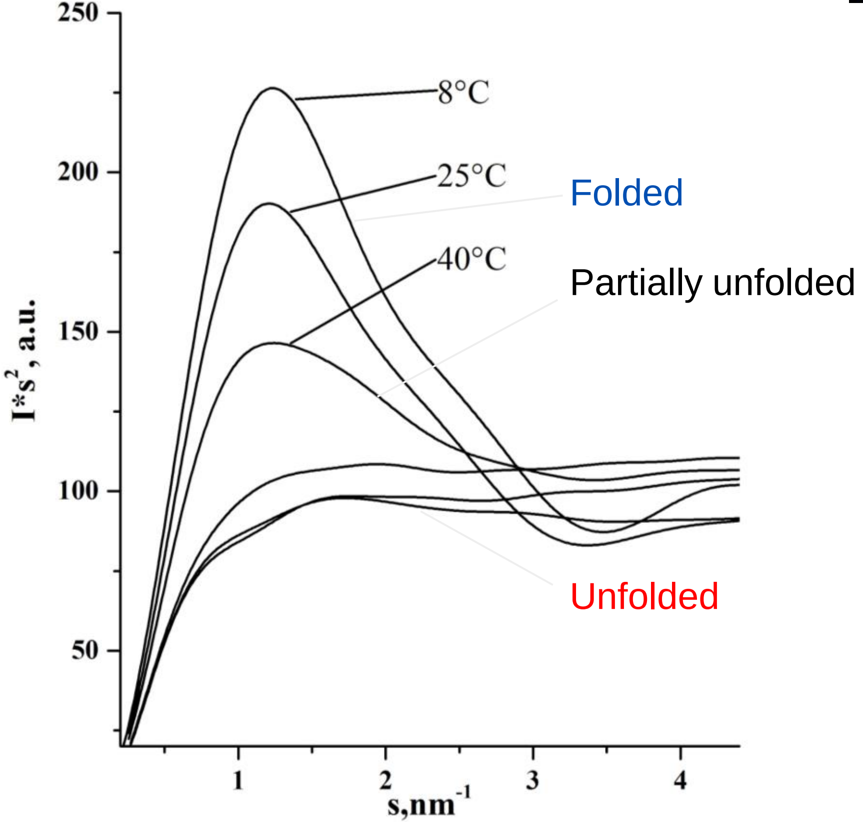
attraction



Kratky analysis



SAXS curves for aptamer RE31



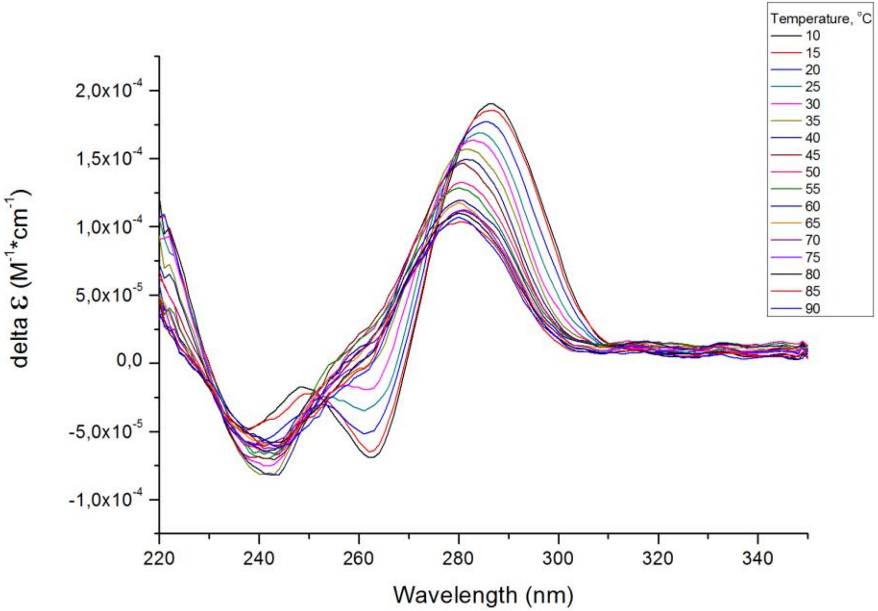
Folded

Partially unfolded

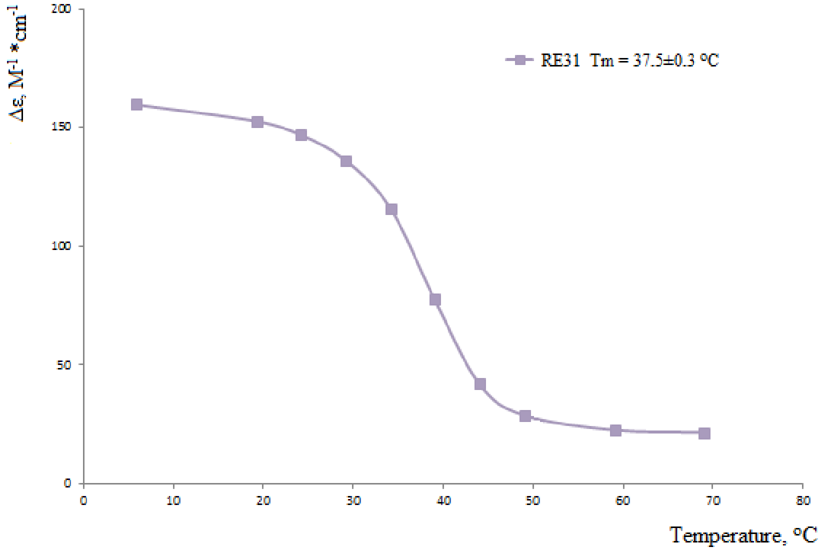
Unfolded

Kratky plots for RE31

Tomilin, F.N., Moryachkov, R., Shchugoreva, I. et al. Anal Bioanal Chem (2019). https://doi.org/10.1007/s00216-019-02045-0



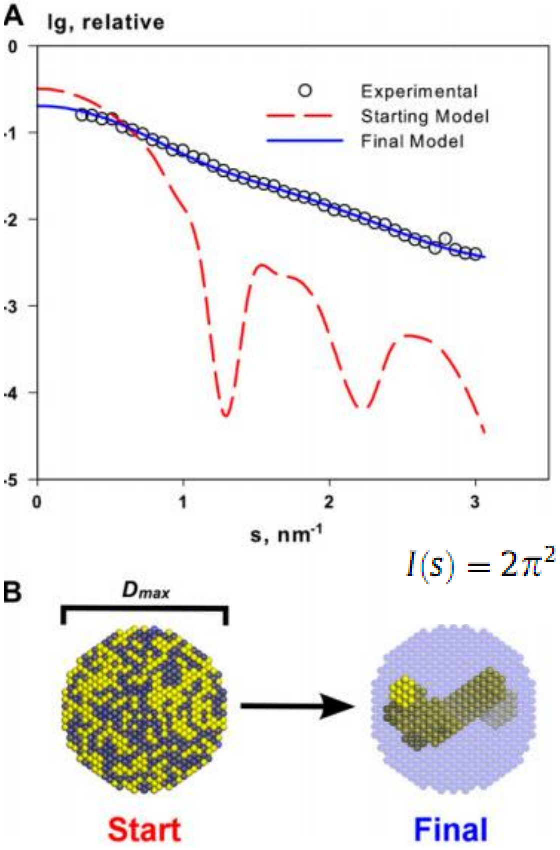
Temperature correlation of CD spectra of aptamer RE31



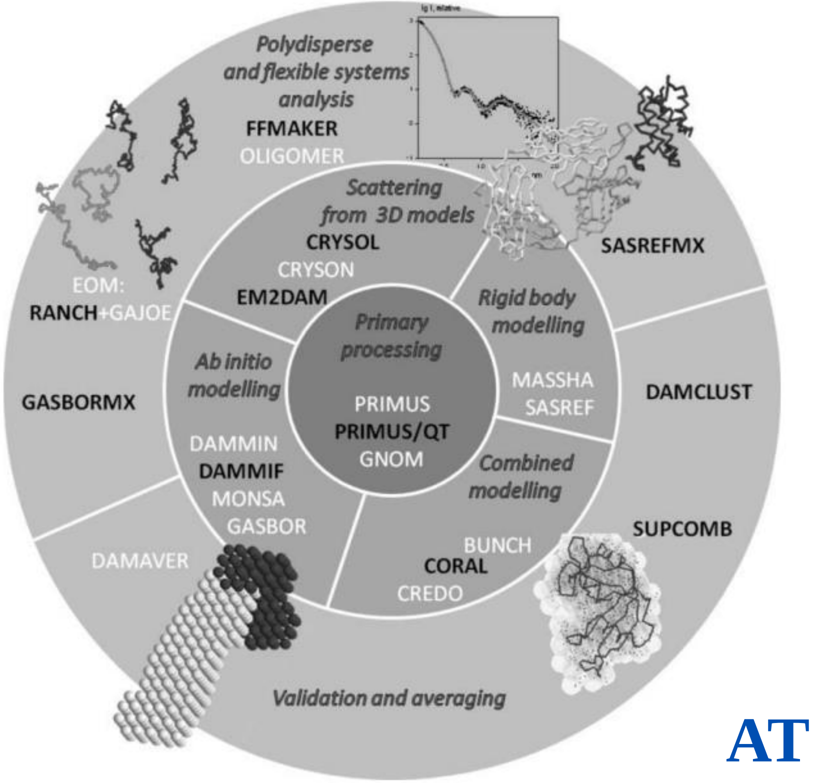
Melting curve of aptamer RE31

Tomilin, F.N., Moryachkov, R., Shchugoreva, I. et al. Anal Bioanal Chem (2019).
https://doi.org/10.1007/s00216-019-02045-0

Simulated annealing, modeling



$$I(s) = 2\pi^2 \sum_{l=0}^{\infty} \sum_{m=-l}^{l} |A_{lm}(s)|^2$$



Polydisperse and flexible systems analysis

FFMAKER

OLIGOMER

EOM:

RANCH+GAJOE

Scattering from 3D models

CRYSOL

CRYSON

EM2DAM

SASREFMX

Primary processing

PRIMUS

PRIMUS/QT

GNOM

Rigid body modelling

MASSHA

SASREF

DAMCLUST

Ab initio modelling

DAMMIN

DAMMIF

MONSA

GASBOR

GASBORMX

Combined modelling

BUNCH

CORAL

CREDO

SUPCOMB

DAMAVER

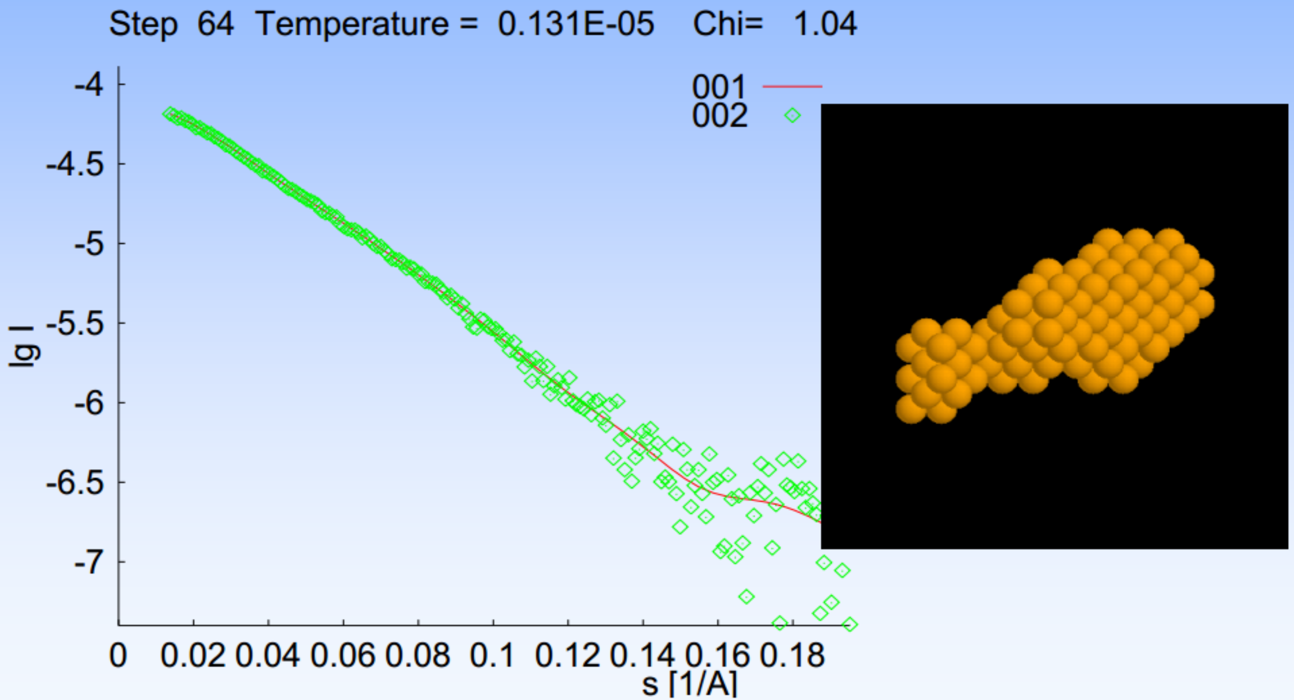
Validation and averaging

ATSAS

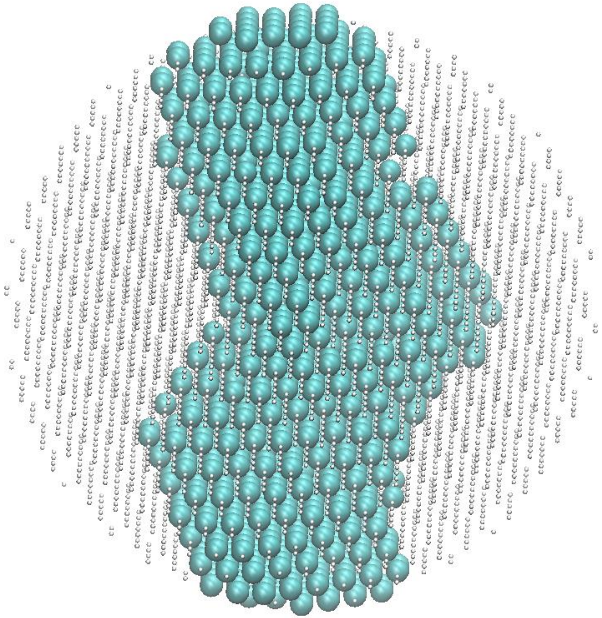
Data analysis software

H.D.T. Mertens, D.I. Svergun / Journal of Structural Biology 172 (2010) 128–141

Case study: determination of structure of the myosin head S1

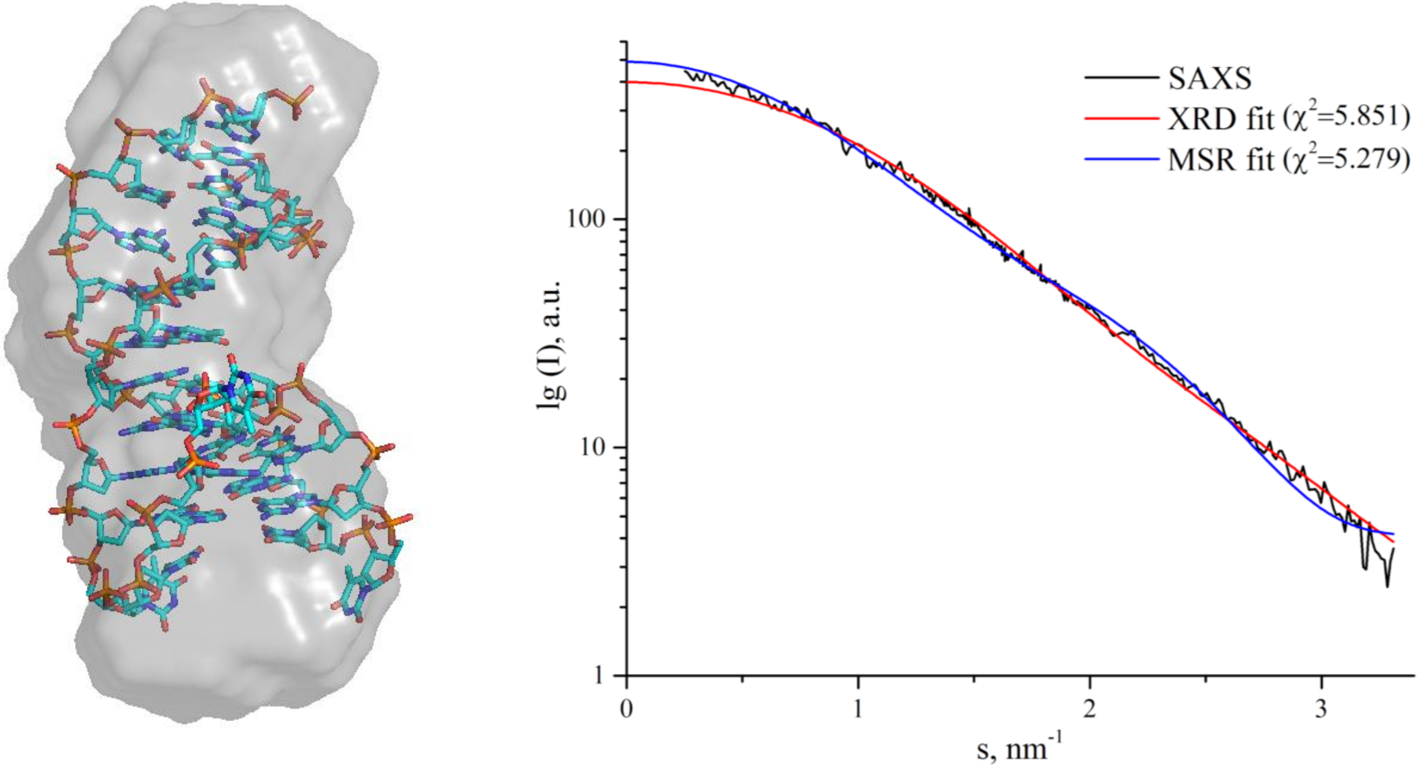

Step 64 Temperature = 0.131E-05 Chi= 1.04

RE31 aptamer



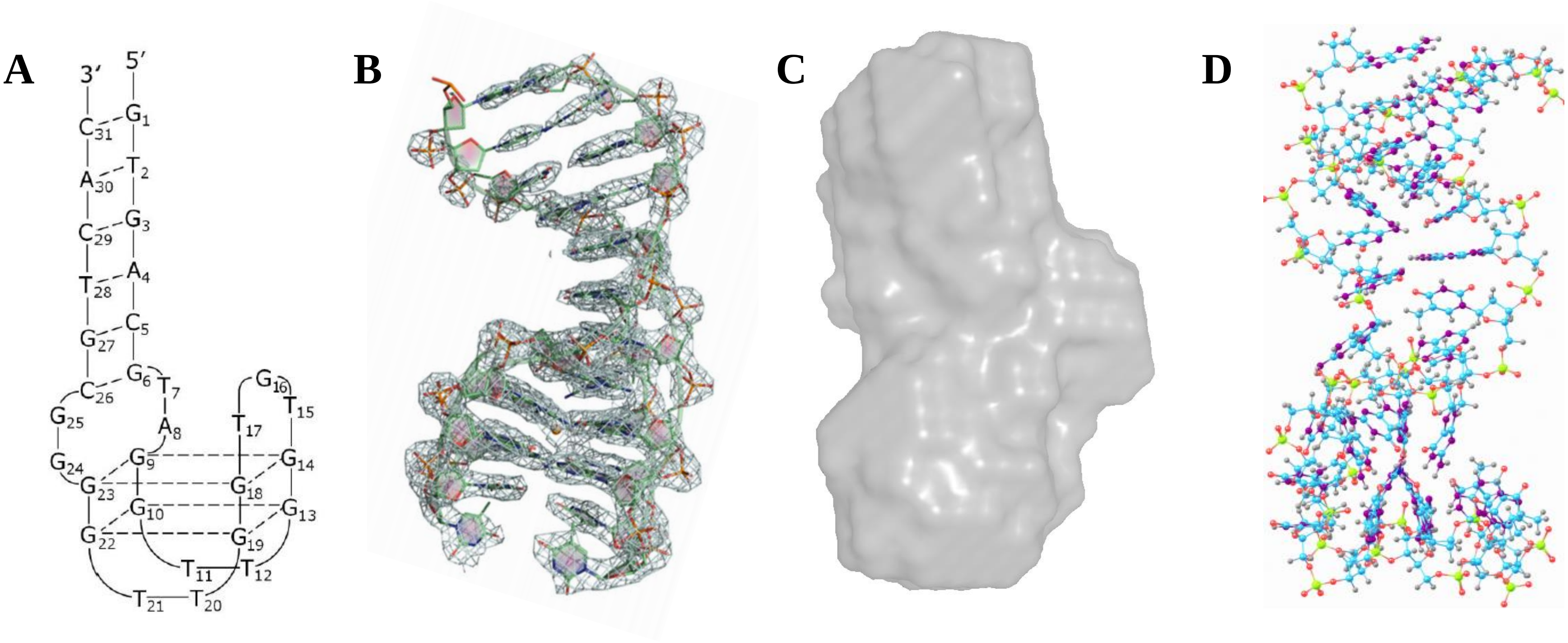
SAXS 3D model

Tomilin, F.N., Moryachkov, R., Shchugoreva, I. et al. Anal Bioanal Chem (2019). https://doi.org/10.1007/s00216-019-02045-0

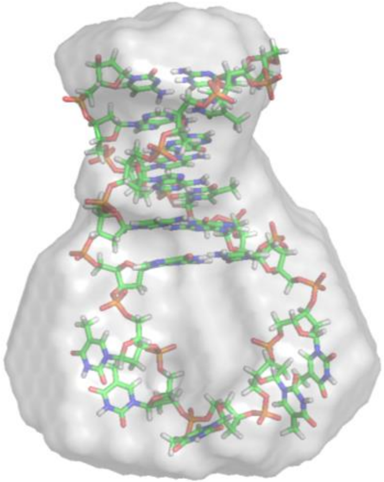


Fit of the bead model of RE31 with the structure obtained from X-Ray diffraction (XRD) analysis (left) and comparison of calculated scattering curves from SAXS, XRD and Molecular Structure Restoration (MSR) performed in CRYSOL software

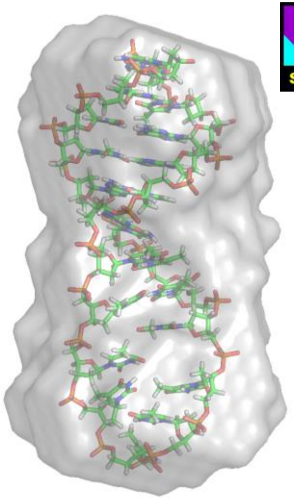
Tomilin, F.N., Moryachkov, R., Shchugoreva, I. et al. Anal Bioanal Chem (2019). https://doi.org/10.1007/s00216-019-02045-0

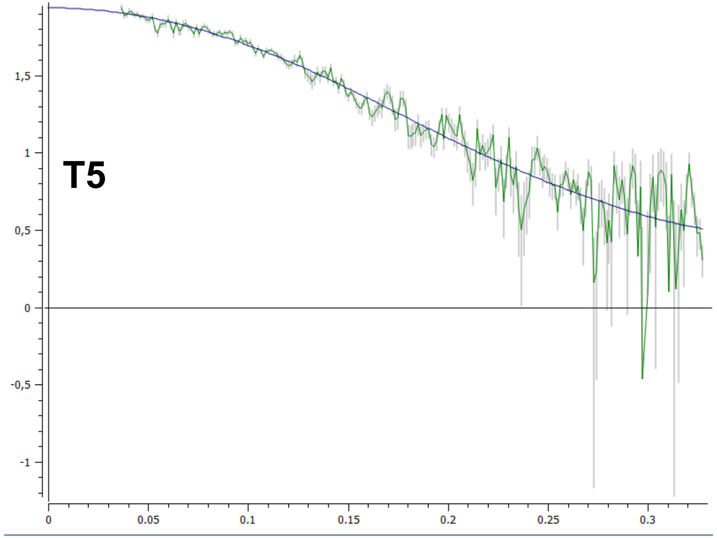


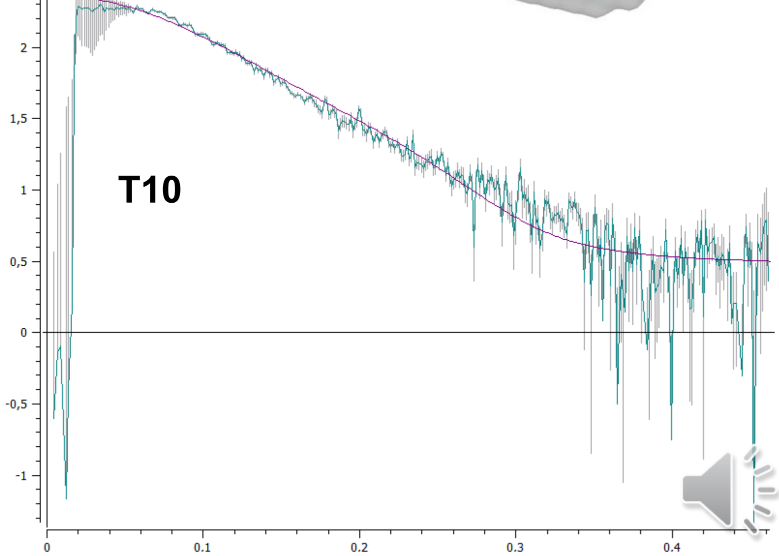
The secondary structure of the DNA aptamer RE31 (A), electron density distribution based on X-Ray diffraction (B), Small-Angle X-Ray scattering (C) and molecular modeling (D).

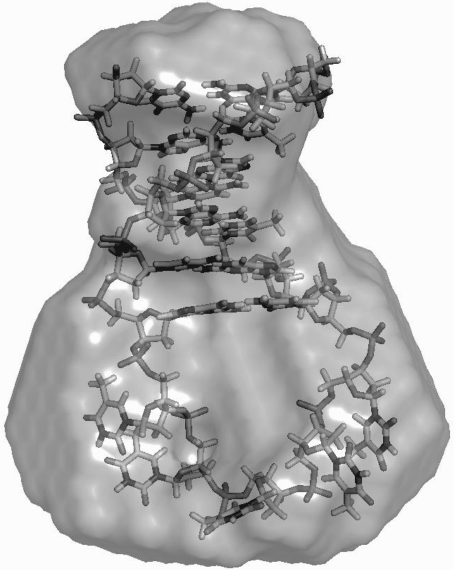


Oligonucleotide (sequence)	Gyration radius R_g, nm	Maximal dimension D_{max}, nm
T5 (GTGACGTATTTTTGGCGTCAC)	1.30	3.91
T10 (GTGACGTATTTTTTTTTTGGCGTCAC)	1.42	4.23
T15 (GTGACGTATTTTTTTTTTTTTTTGGCGTCAC)	1.58	4.38

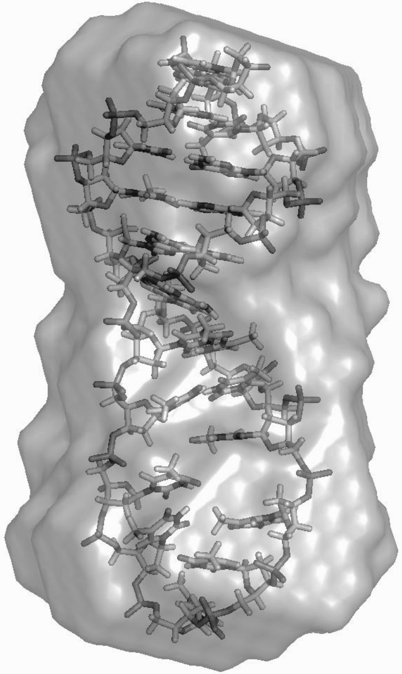




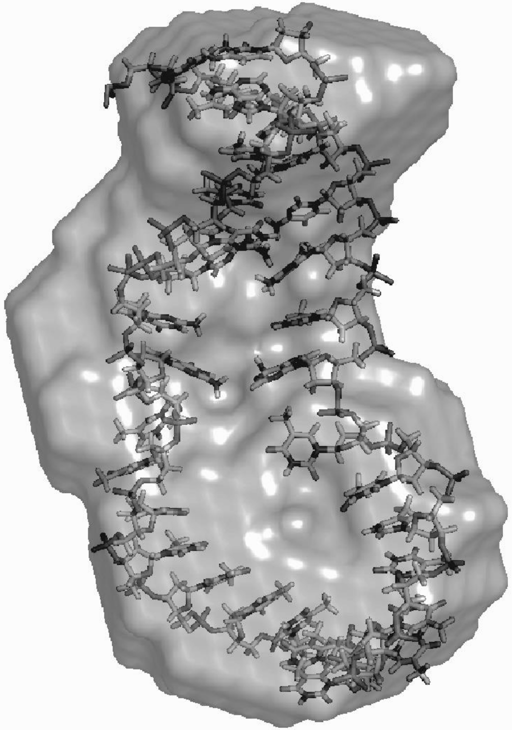




T5



T10



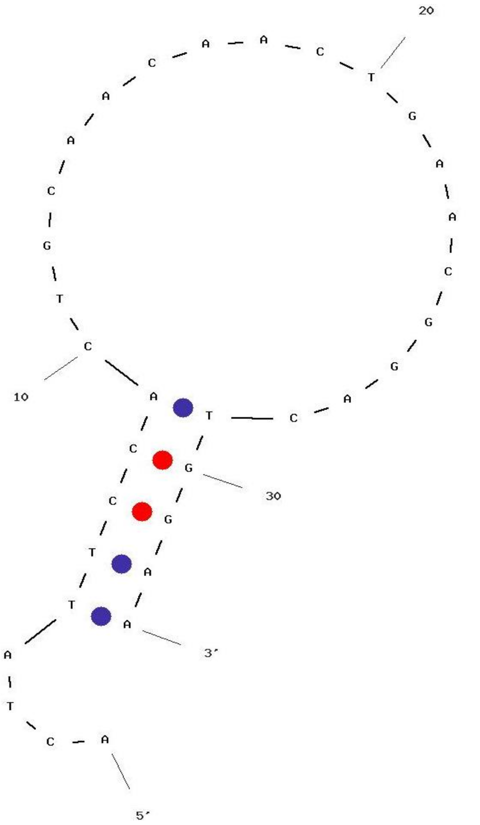
T15

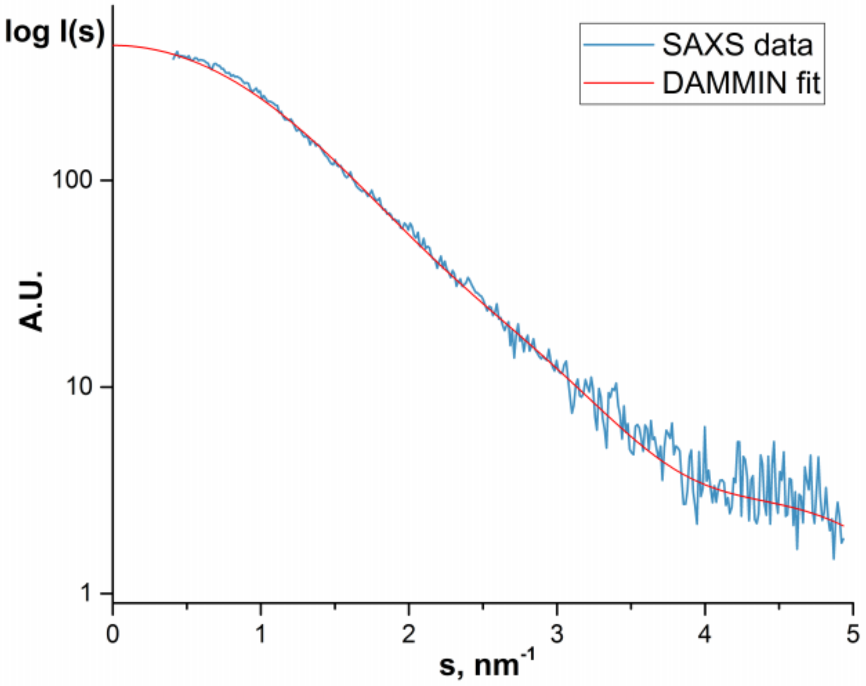
DNA aptamer Gli-233

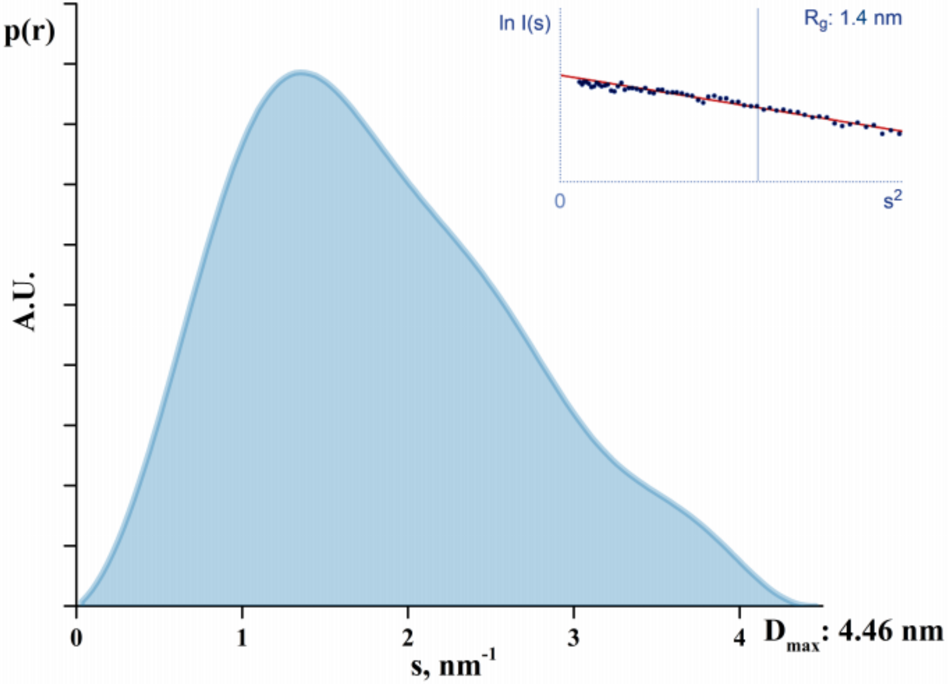
Primary structure:

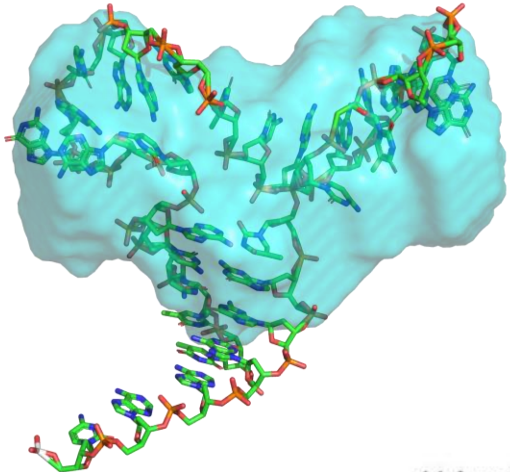
5’-ACTATTCCAC TGCAACAACT GAACGGACTG GAA-3’

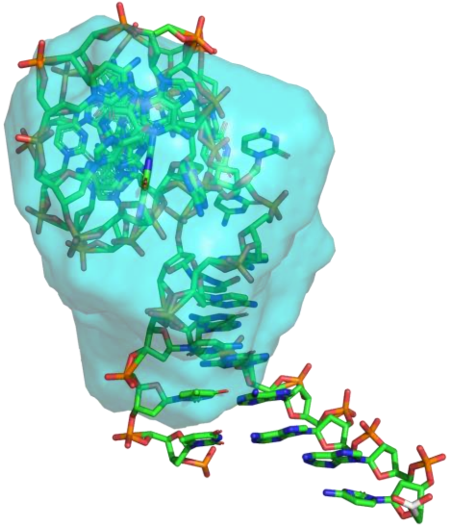
Secondary structure:

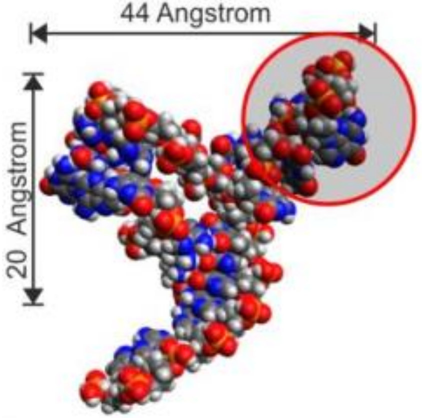



log I(s)
A.U.
SAXS data
DAMMIN fit
100
10
1
0
1
2
3
4
5
s, nm^{-1}


p(r)
A.U.
ln I(s)
R$_g$: 1.4 nm
0
s^2
0
1
2
3
4
s, nm^{-1}
D$_{max}$: 4.46 nm







Gli-233 aptamer

SAXS-model
of the spatial structure
fitted with the molecular model

Parameters:

R_g = 1.4 nm
D_{max} = 4.46 nm
V_p = 11.05 nm^3
MW = 12.44 kDa

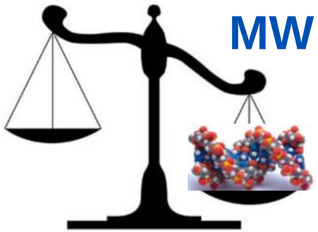
Conclusions

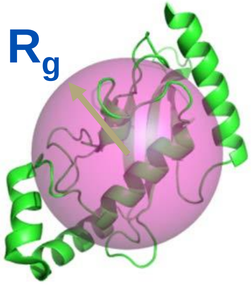
Structural parameters:

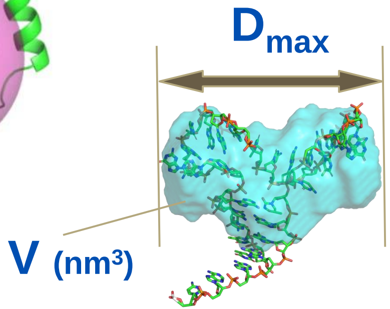
- Maximum dimension
- Radius of gyration
- Molecular Weight
- Volume of the molecule

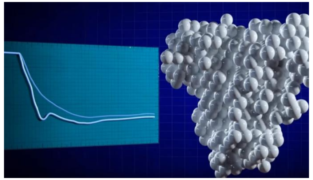
Possibilities:

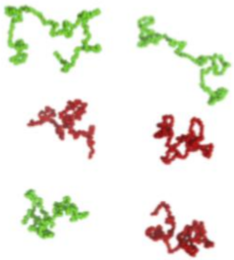
- ab initio shape reconstruction
- atomic modeling validation
- missing fragments
- refinement
- conformational transitions
- rigid-body modeling

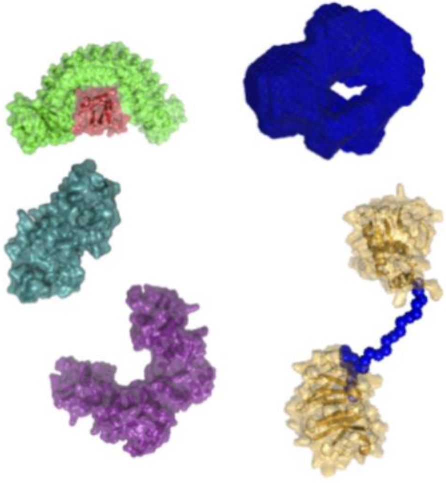












Thank you for attention!

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