Лекция 4+5

Другие методы исследования структуры белков (SAXS/SANS, Cryo-EM, Cryo-electrotomography, NMR, native-MS, crosslinking MS, HDX-MS). Интегральный подход и моделирование белков по гомологии (iTasser). Примеры.

Случанко Н. Н.

Small-angle X-ray scattering (SAXS)

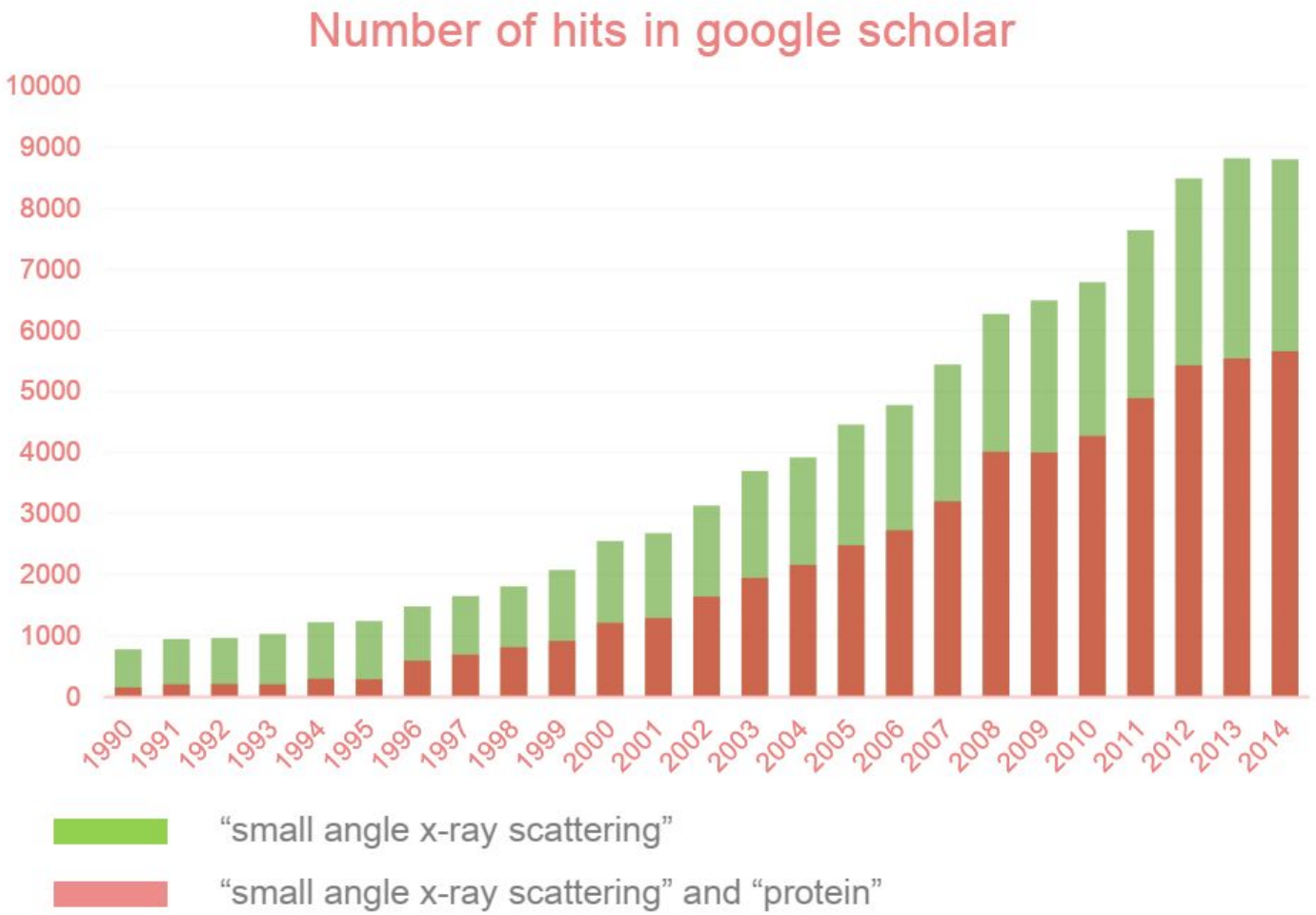
+

Small-angle neutron scattering (SANS)

||

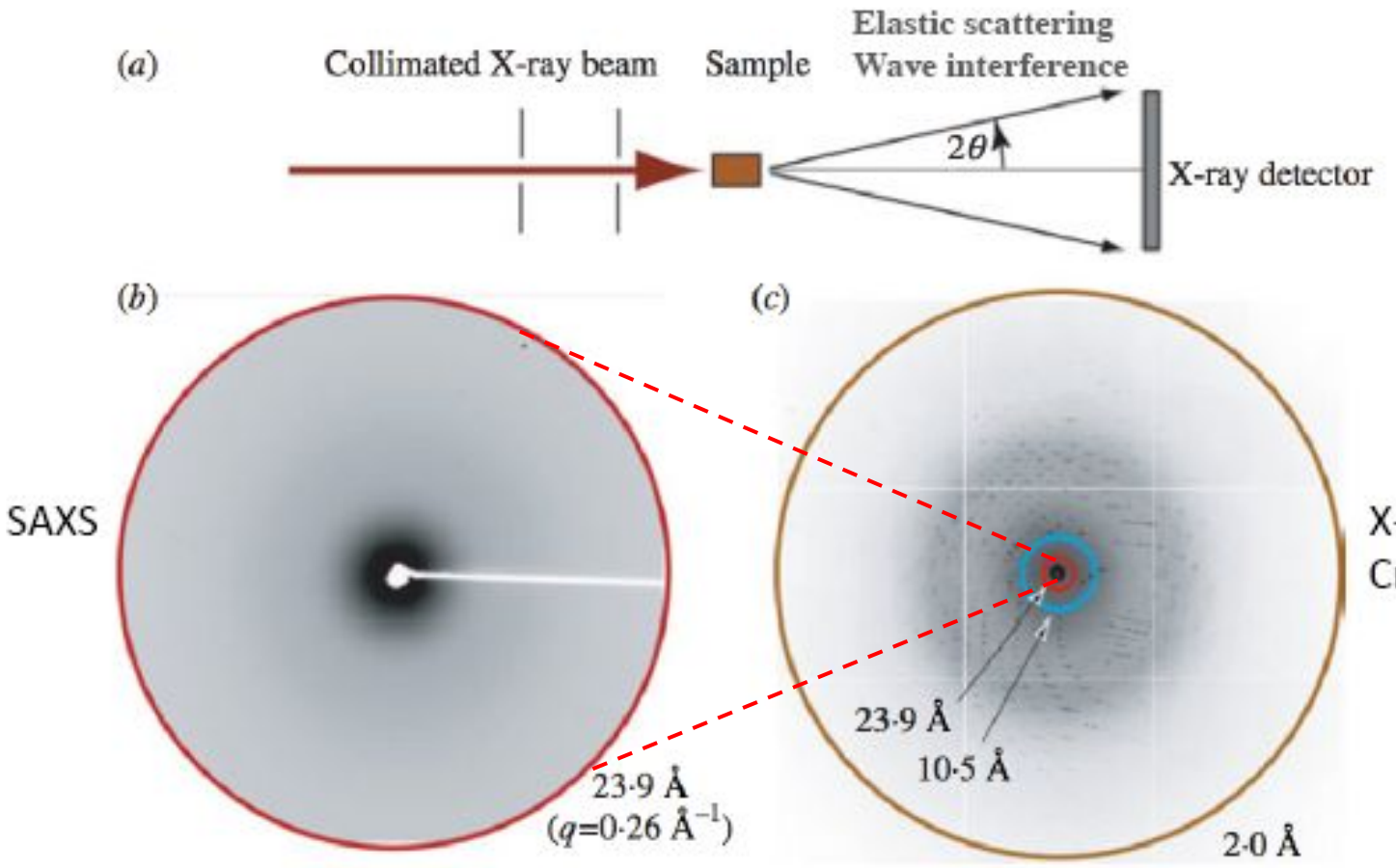
Small-angle scattering (SAS)

SAXS popularity



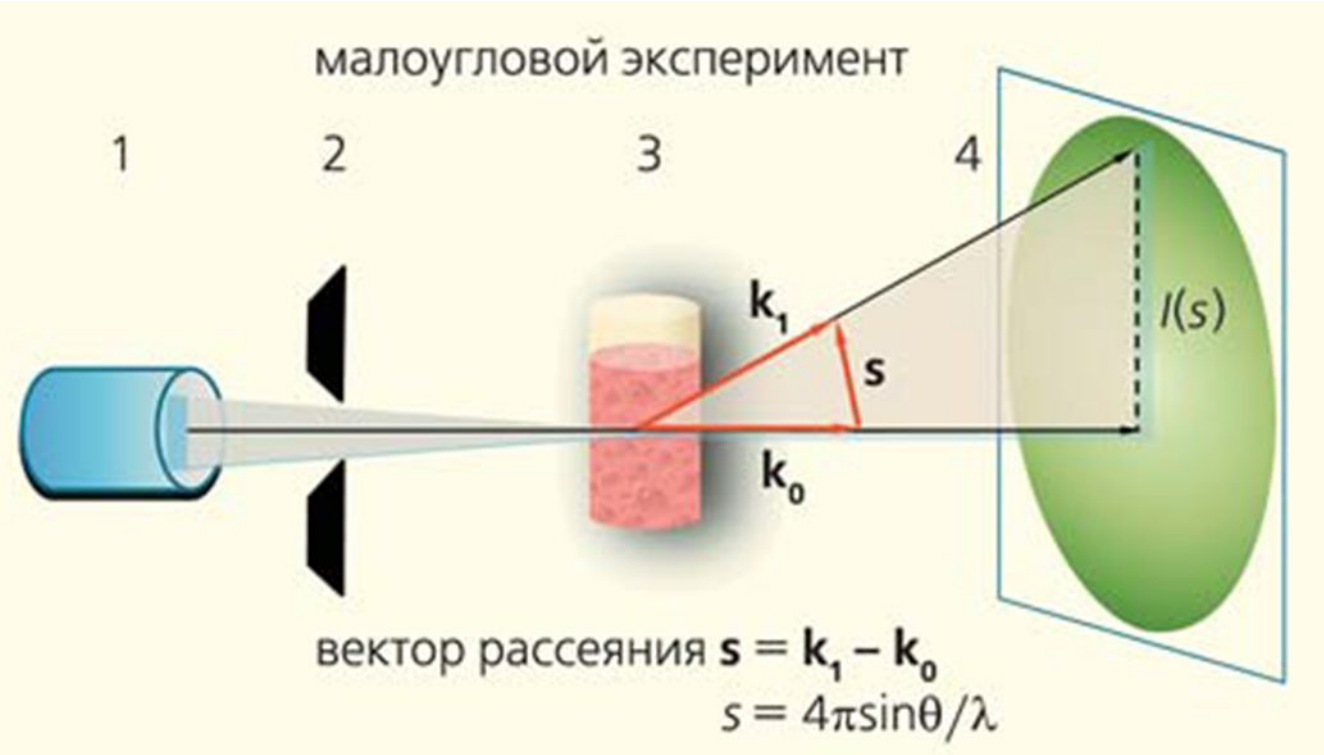
Blanchet C. (c)

Основы SAS



~1 photon in 10^6 incident photons

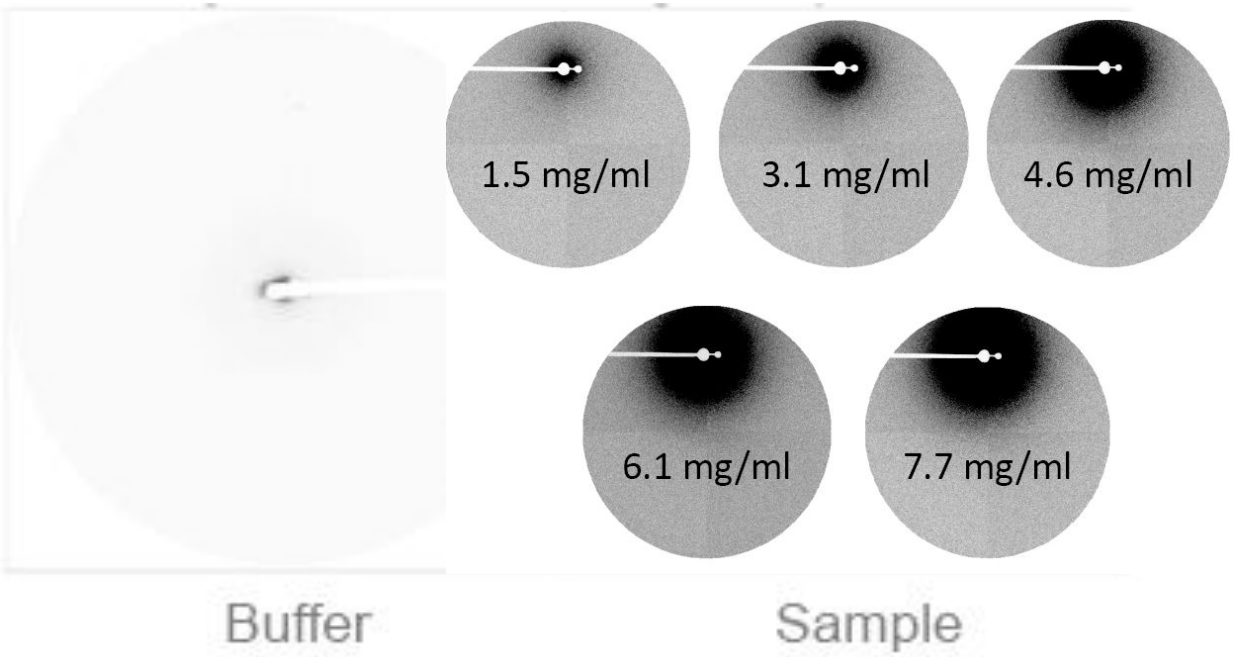
Основы SAS



$$d = 2\pi/s$$ ~ 10-20 Å

s and q are just alternative designations of the scattering vector, usually from 0 to 0.5 Å^{-1}

Contrast and careful buffer subtraction

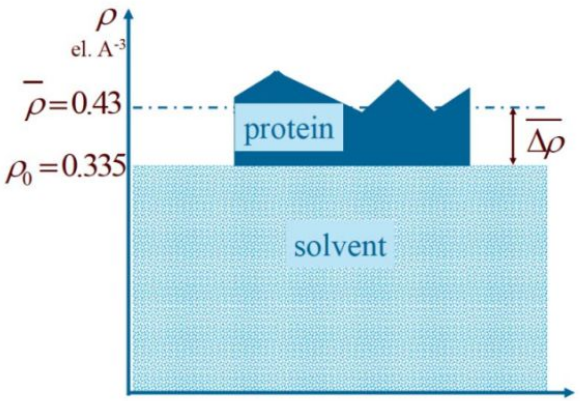


Measured in the same cell, buffer exactly matches

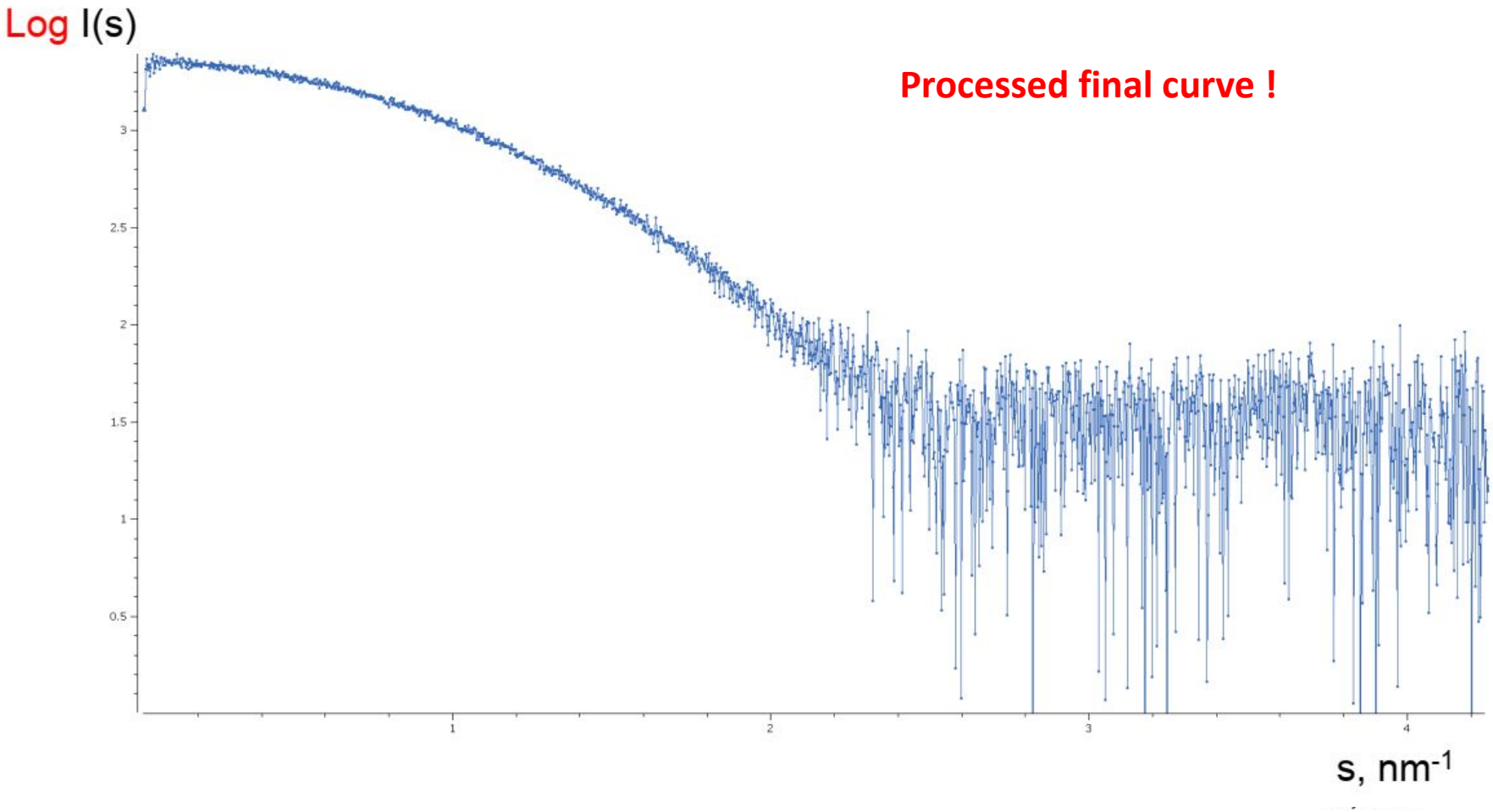
SAXS is a **contrast method** where the scattering signal is derived from the difference in the average electron density, ρr(r), of the protein molecules of interest, ρ(r), and the bulk solvent ρs (buffer)

$$\Delta\rho(r) = \rho(r) - \rho s$$

Difference in the scattering density (contrast):



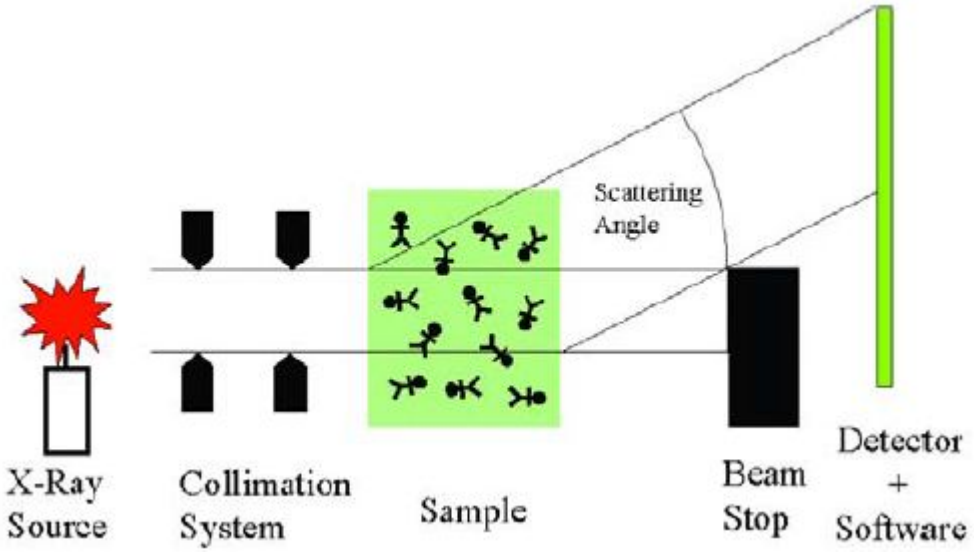
Sample and buffer



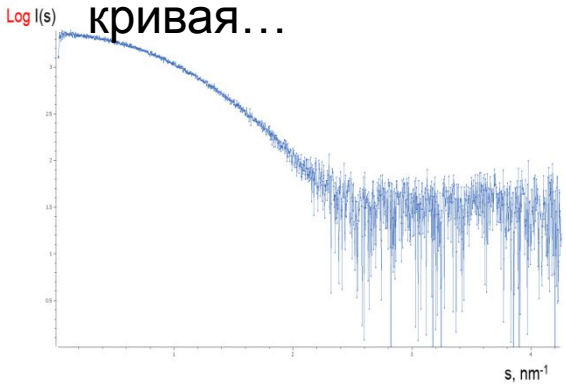
Kikhney A (c)

Особенности

- Макромолекулы **свободно** вращаются, не ориентированы строго при падающем пучке X-ray
- Может быть **несколько конформаций** одновременно
- В результате наблюдаемое рассеяние это сферическое **усреднение** (изотропное) и усреднение по времени
- **Теряется 3D информация**
- Данные при радиальном усреднении дают 1D кривую распределения I(q) с небольшим числом параметров

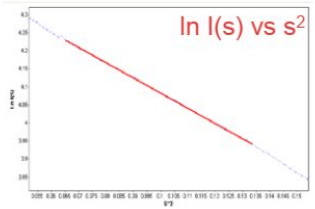


Данные – 1D кривая…

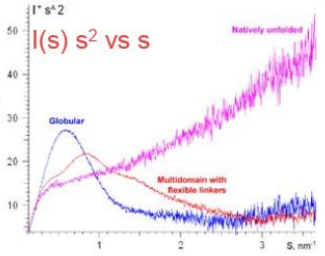


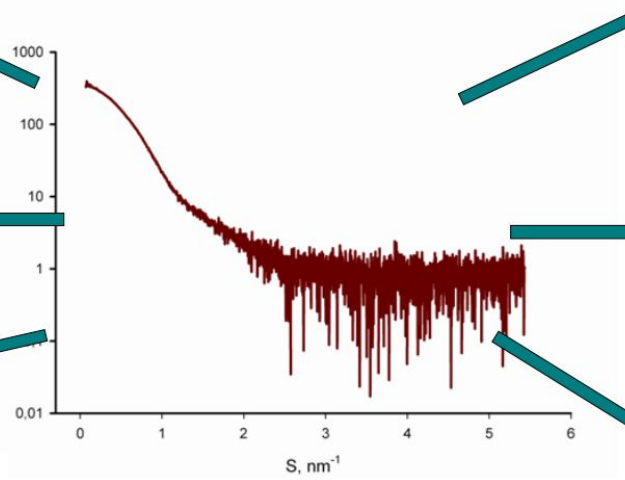
Information directly obtainable from the data

Radius of gyration (Guinier)



Flexibility (Kratky)



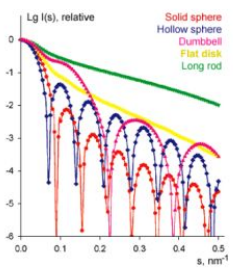


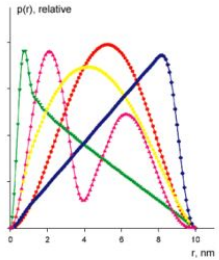
Molecular weight (forward scattering)

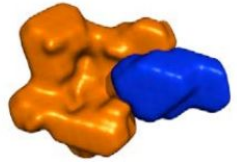
Volume (Porod Invariant)

Distance distribution function

bead models





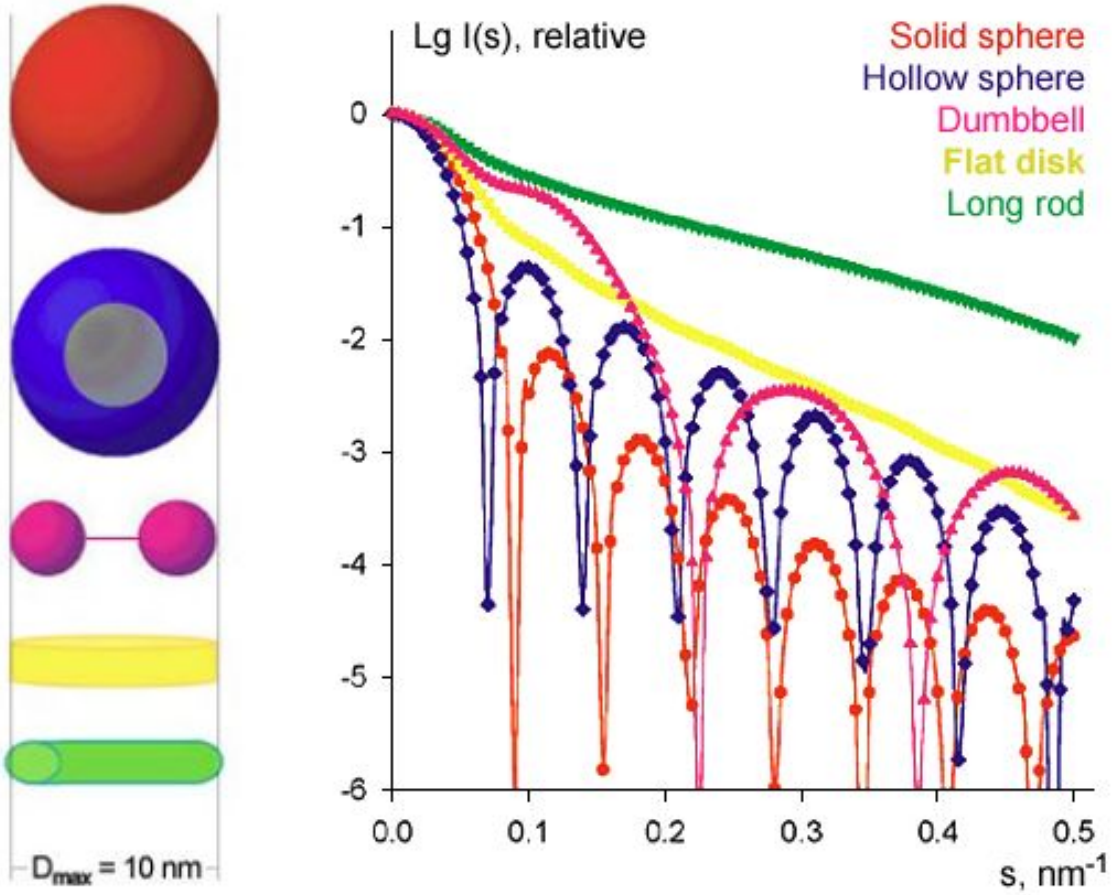


Fitting software

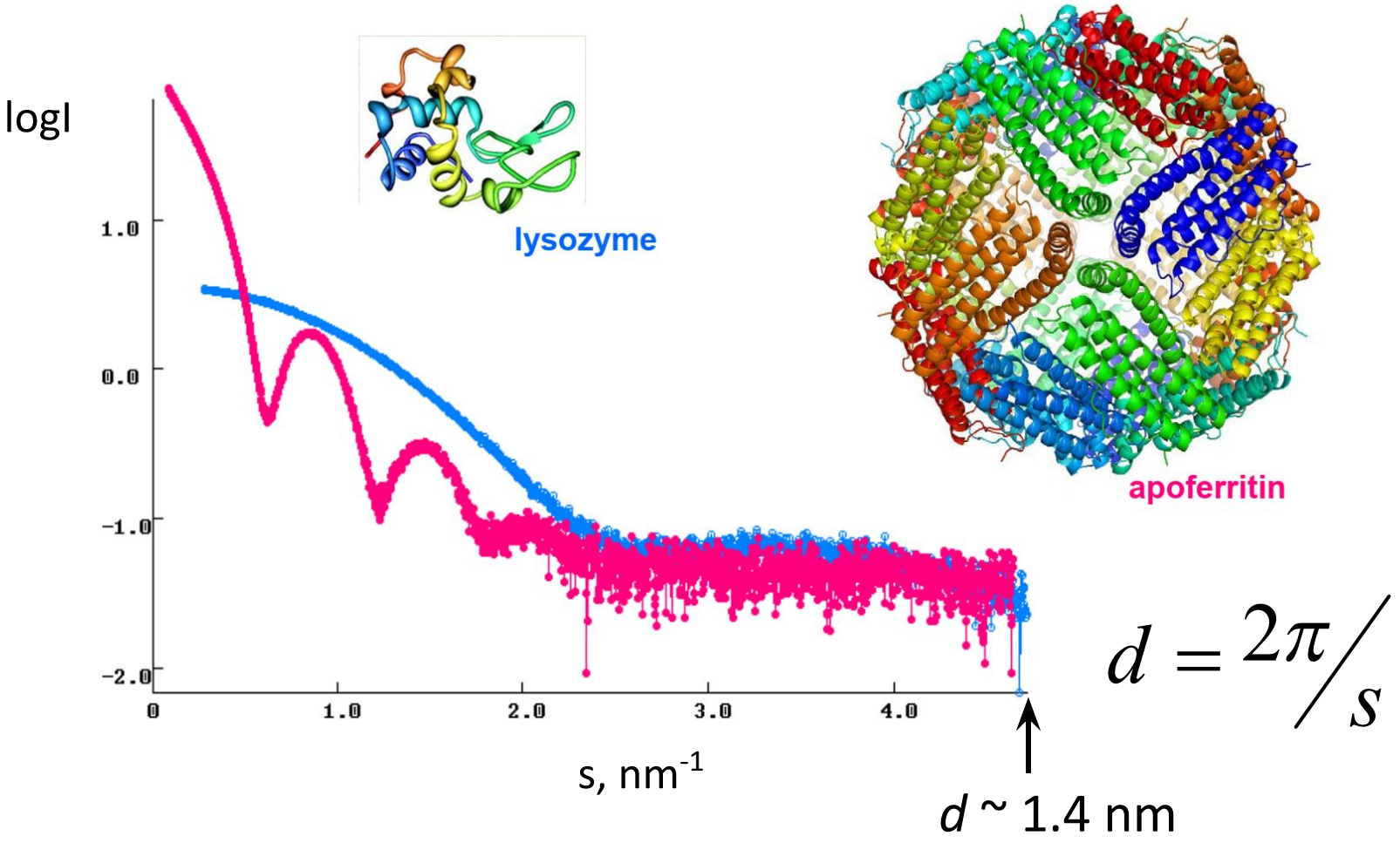
- **SAXSFit**
  - Ideal for beginners
  - Hard spheres, interactions according to local monodisperse approximation (Pedersen 1994), 1 or 2 populations
  - http://www.irl.cri.nz/SAXSfiles
- **Irena**
  - Based on Igor (demo version available, 30 days)
  - Wide-ranging functionality - Various models available: Unified fit (Beaucage: Guinier + Porod), hard sphere models with various form factors and structure factors, multiple populations
  - http://usaxs.xor.aps.anl.gov/staff/ilavsky/irena.html
- **ATSAS**
  - Biological molecules
  - http://www.embl-hamburg.de/biosaxs/software.html
- **FISH**
  - SANS (including ToF)
  - http://www.small-angle.ac.uk/small-angle/Software/FISH.html
- **SASFit**
  - SANS
  - http://kur.web.psi.ch/sans1/SANSSoft/sasfit.html

https://www.embl-hamburg.de/biosaxs/software.html

Форма кривой SAXS сильно зависит от размера и формы частиц



What does the curve already tell us about the size of the particles? What is the resolution?

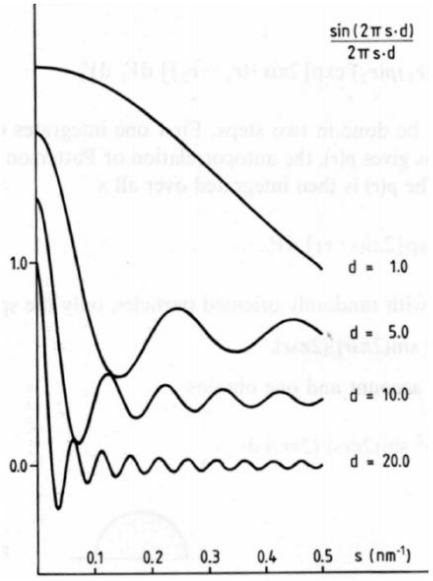


$$d = 2\pi / s$$

Pairwise distance distribution function p(r)

FFT

$$I(s) = 4\pi \int_0^{D_{max}} p(r) \frac{\sin(sr)}{sr} dr \qquad p(r) = \frac{r^2}{2\pi} \int_0^{\infty} s^2 I(s) \frac{\sin(sr)}{sr} ds$$



In isotropic systems, each distance d = r_{ij} contributes a *sin(x)/x* –like term to the intensity.

Large distances correspond to high frequencies and only contribute at **low angles** (i.e. at low resolution, where particle shape is seen)

Short distances correspond to low frequencies and contribute over a large angular range. Clearly at **high angles** their contribution dominates the scattering pattern.

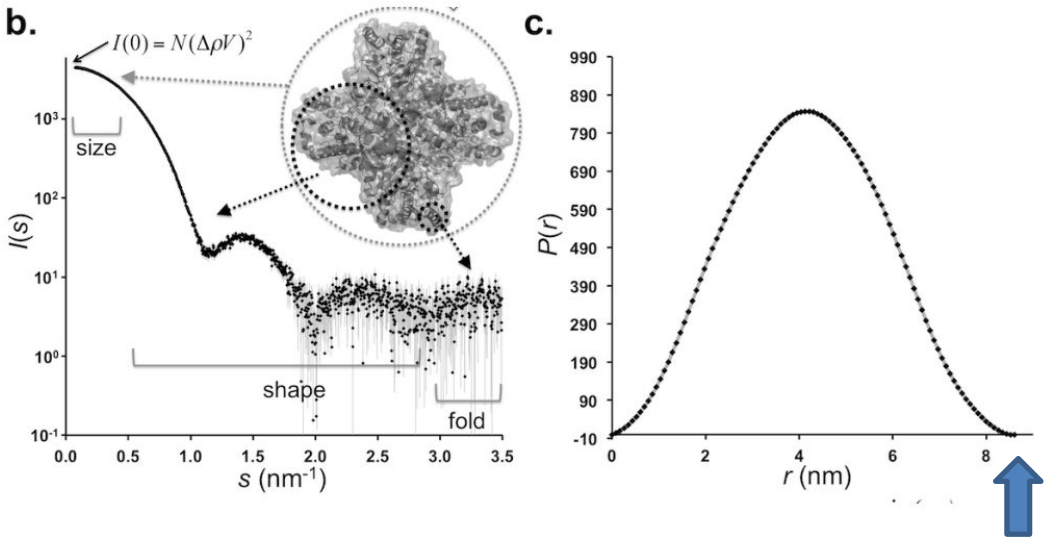
Blanchet C. (c)

Pairwise distance distribution function p(r)

FFT

$$I(s) = 4\pi \int_0^{D_{max}} p(r) \frac{\sin(sr)}{sr} dr \qquad p(r) = \frac{r^2}{2\pi} \int_0^{\infty} s^2 I(s) \frac{\sin(sr)}{sr} ds$$

Reciprocal space intensity

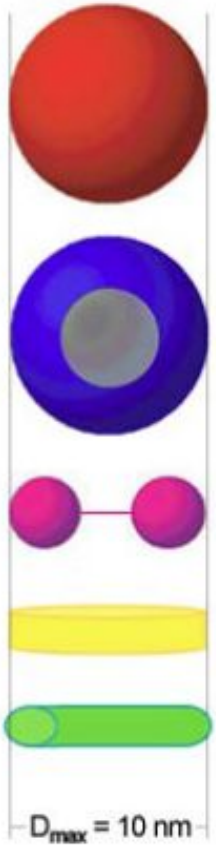


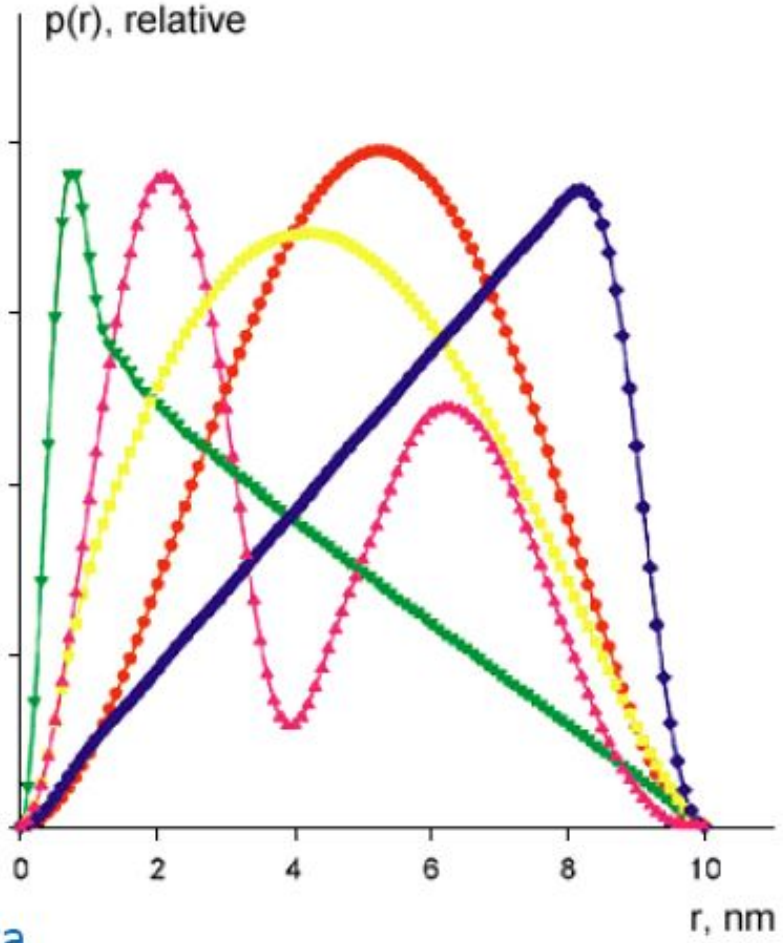
Real space (probable) distance frequencies

Dmax
maximum intra-particle distance

Blanchet C. (c)

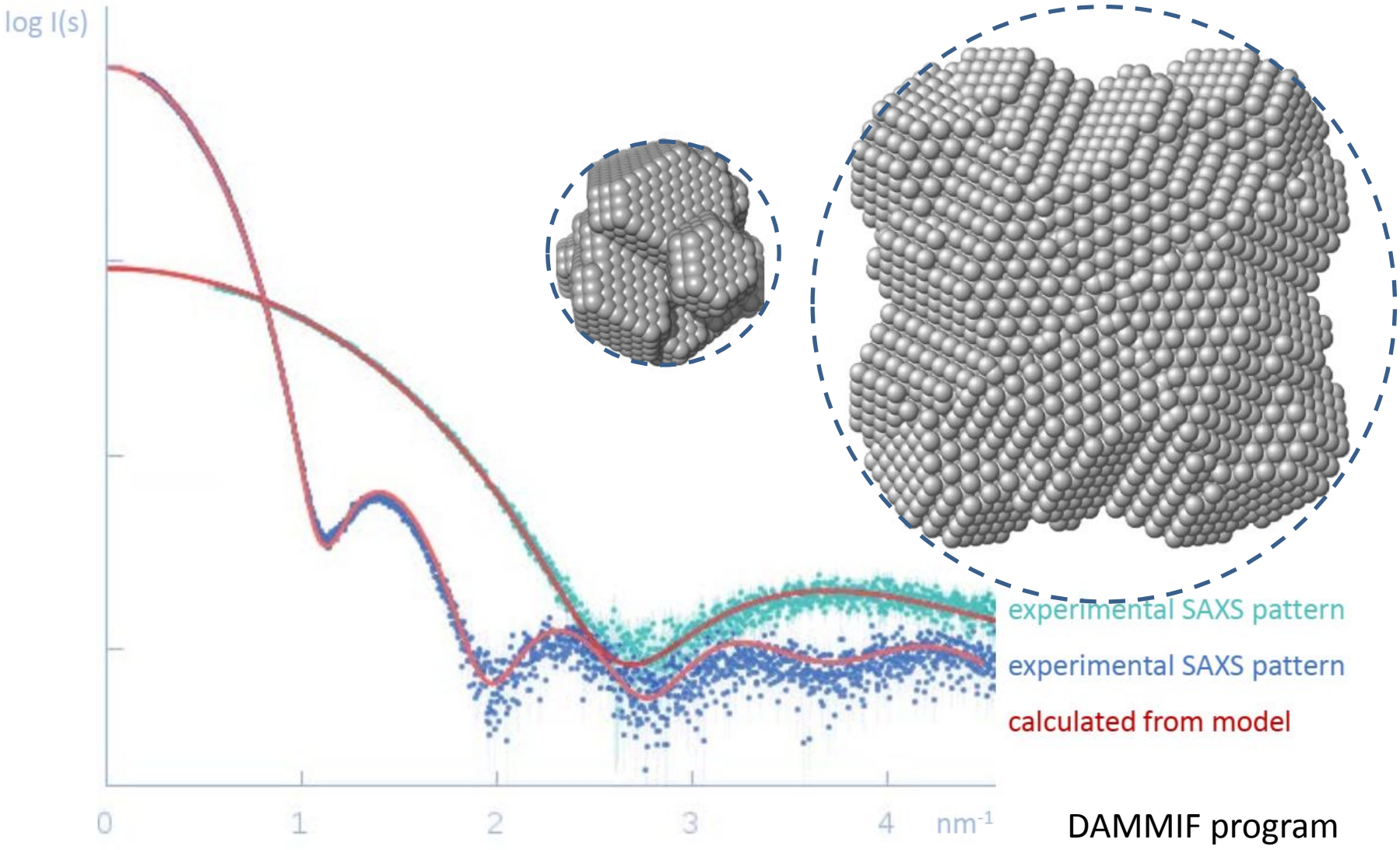
Pair distribution function





Fourier transform of data.

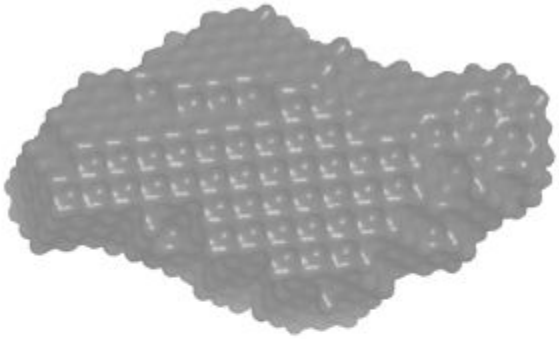
Ab initio shape reconstruction: dummy atom modelling



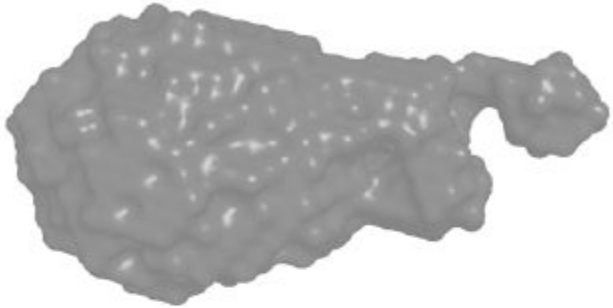
Kikhney A (c)

DAMMIF program
https://www.embl-hamburg.de/biosaxs/dammif.html

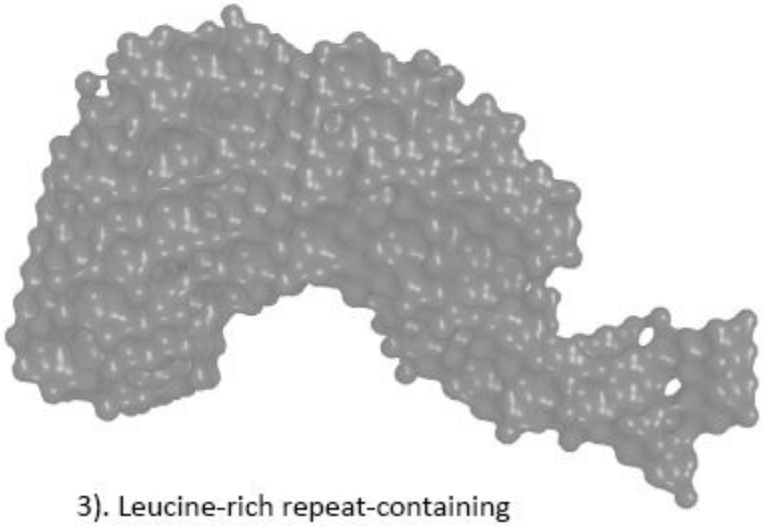
Ab intio envelopes



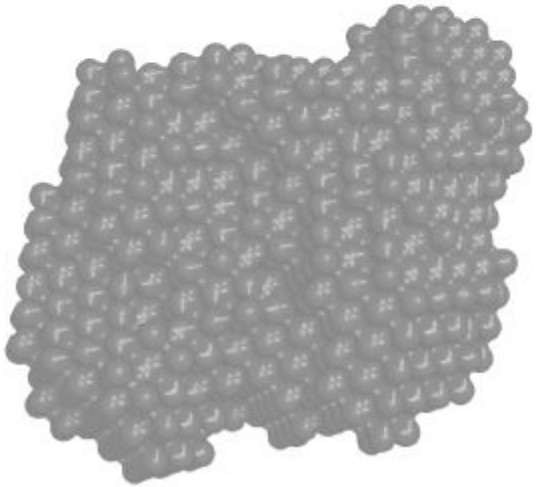
1). alr0221 protein from Nostoc (18.6 kDa)



2). C-terminal domain of a chitobiase (17.9 kDa)

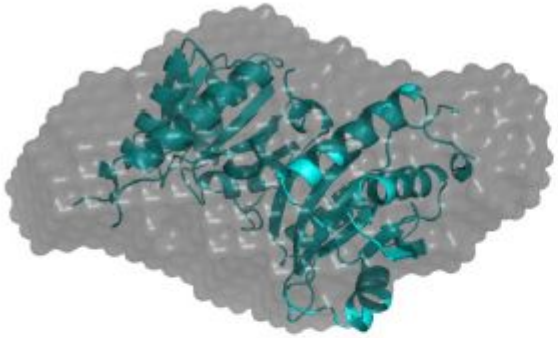


3). Leucine-rich repeat-containing protein LegL7 (39 kDa)



4). *E. Coli.* Cystine desulfurase activator complex (170 kDa)

Overlaid with subsequent X-ray structures



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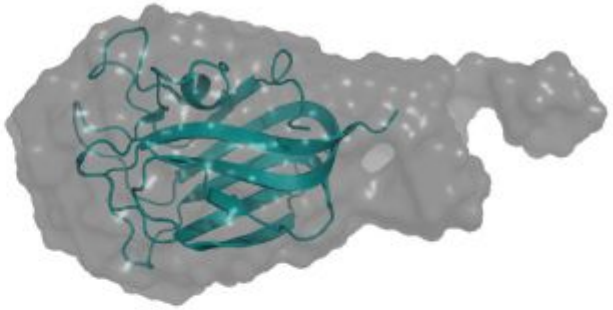

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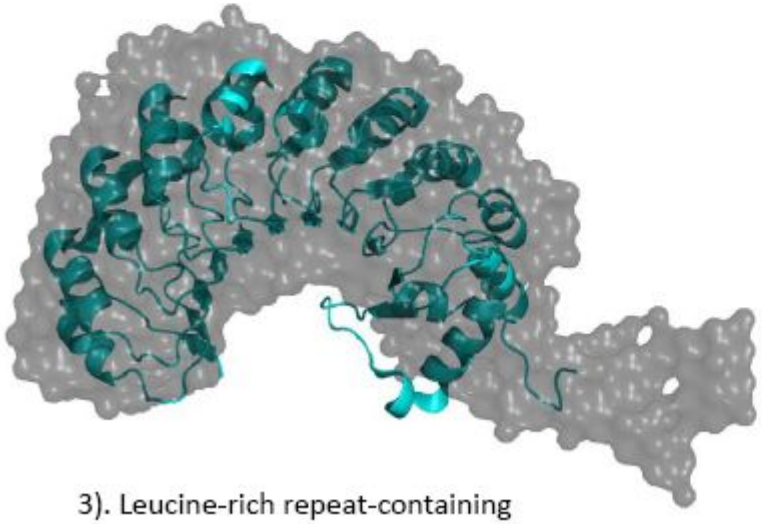
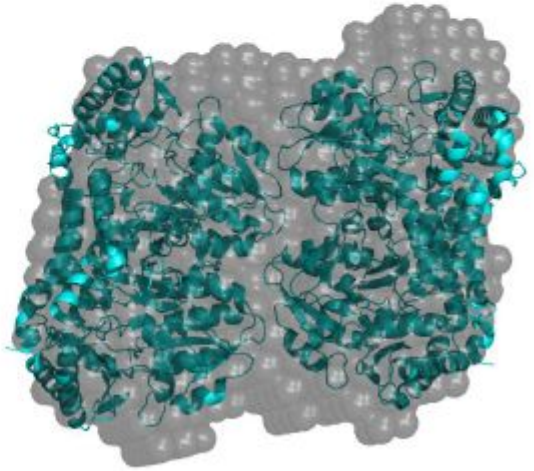
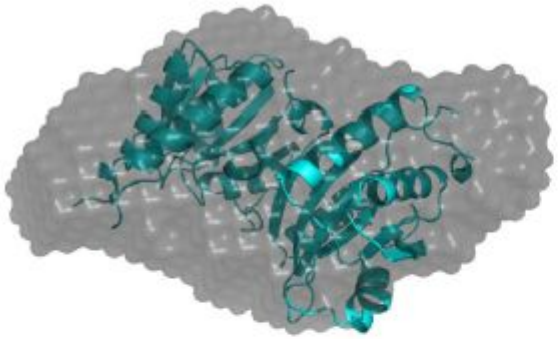


3). Leucine-rich repeat-containing protein LegL7 (39 kDa)



4). *E. Coli.* Cystine desulfurase activator complex (170 kDa)

And data on what was missing …



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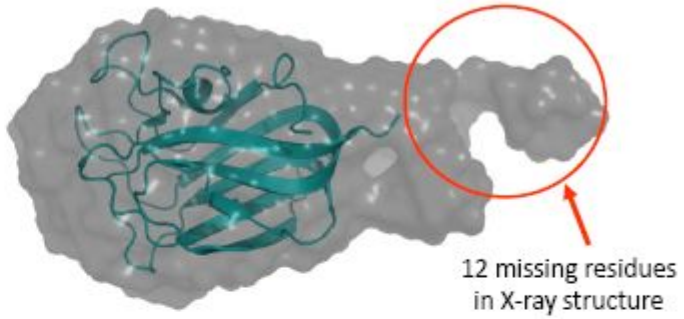

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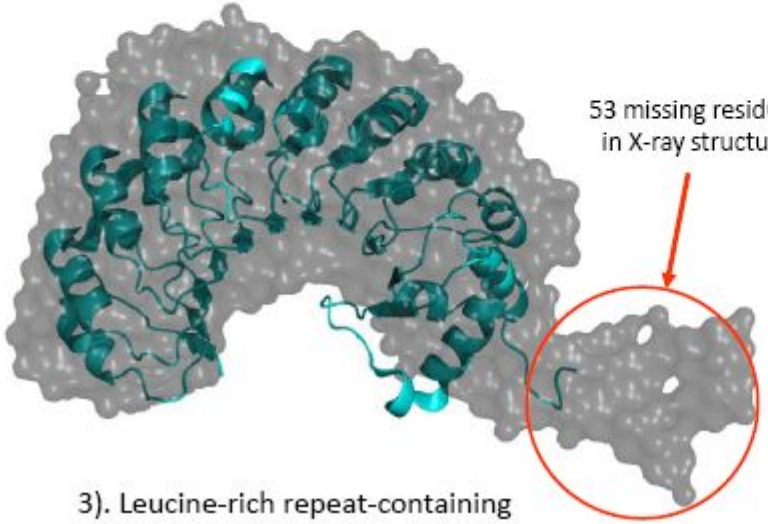

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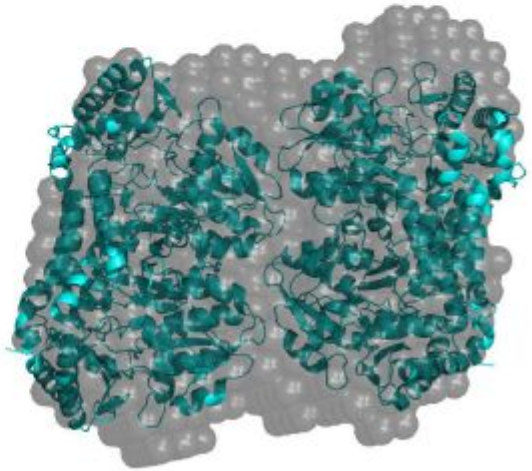
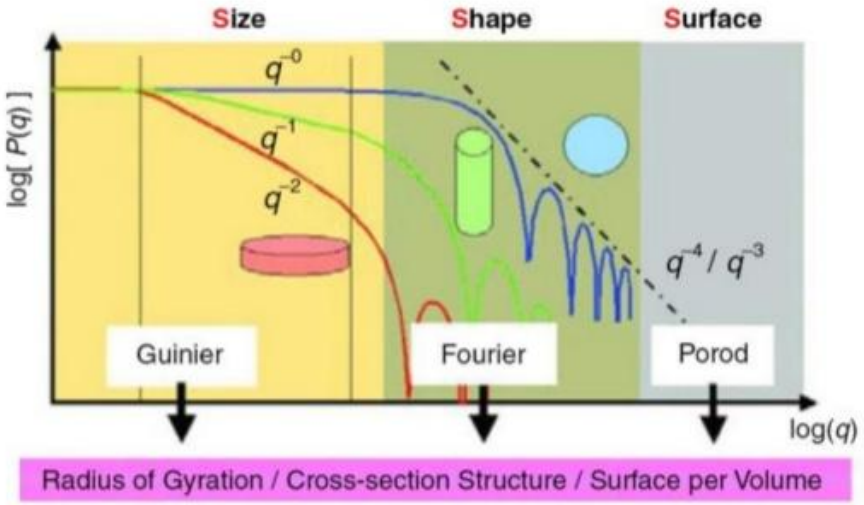


4). *E. Coli.* Cystine desulfurase activator complex (170 kDa)

Analysis of SAXS curves

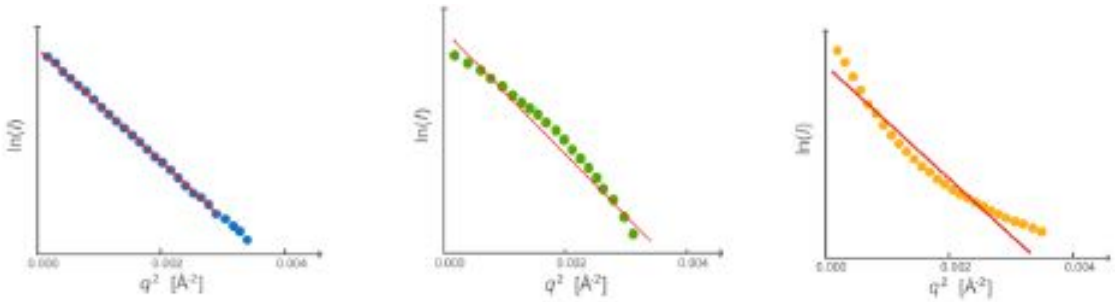


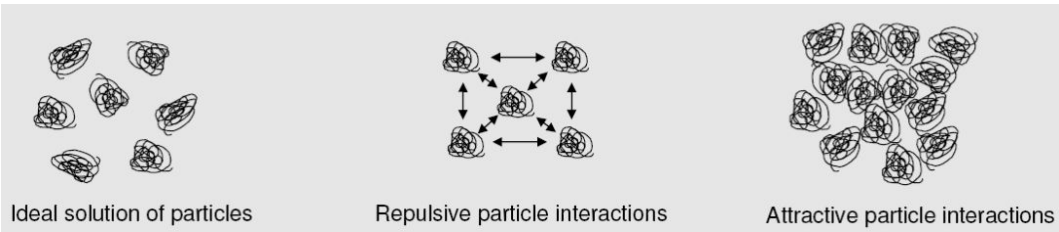
Linear ≠ monodisperse
(also for mixed systems)

$$I_{total} = x_1 I_1 + x_2 I_2 + x_n I_n$$

x_n – molar fraction of component n

I_1 – scattering intensity of component n





Guinier plot and Rg

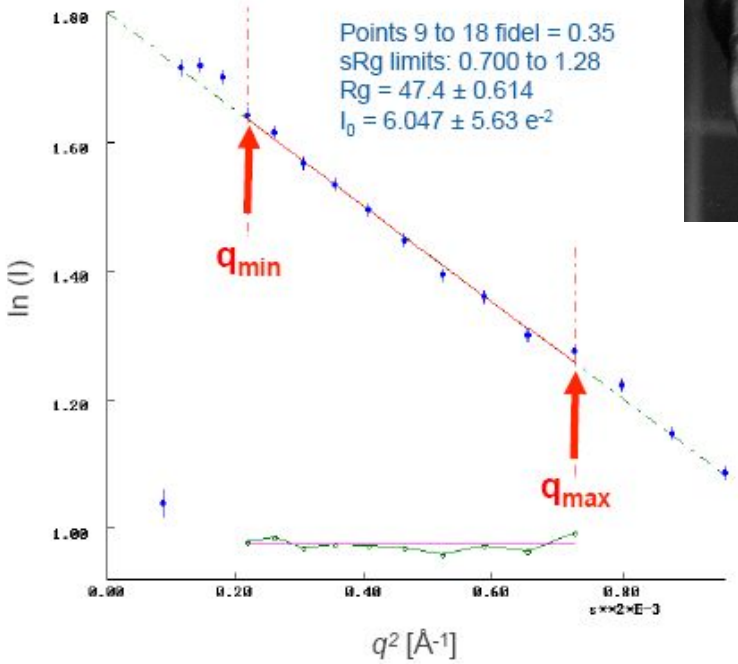
A. Guinier

- Guinier Law

$$\ln\left[I(q)\right] \cong -\frac{q^2 R_G^2}{3} + \ln\left[I(0)\right]$$

R_g – *radius of gyration*
I(0) – forward scattering

- Plot ln *I* vs. q^2
  - *q*-min < *q* < $1.3R_G$[1]
  - Slope α R_g
  - Check for linearity

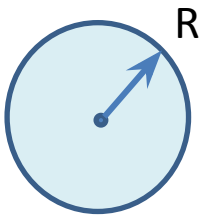


$$R_g^2 = \frac{\sum\left(R_i - R_0\right)^2}{N}$$

Average of square center-of-mass distances in the molecule

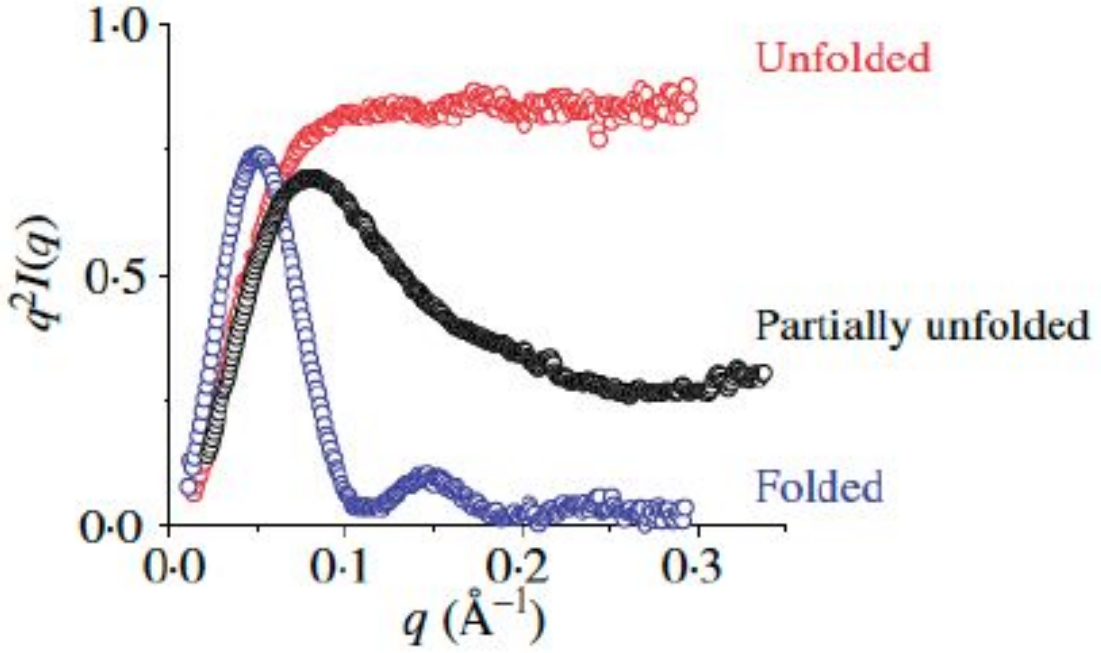
Measure of the overall size of the molecule

$$R_g = \sqrt{\frac{3}{5}}R$$



Kratky plot and flexibility

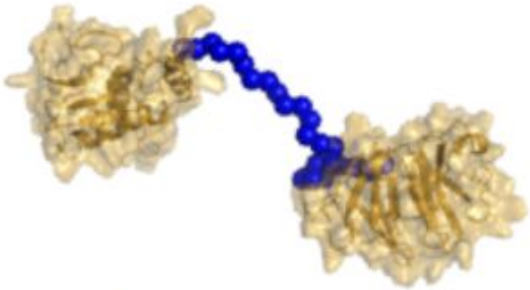
- Identification of unfolded samples
- Globular proteins have bell-shaped curves (parabola)

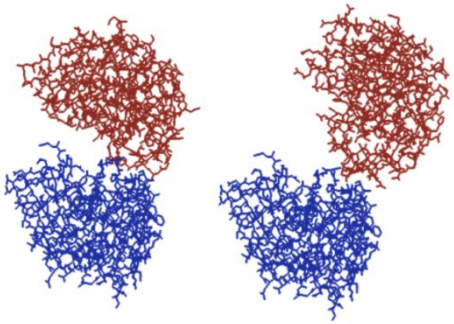


If X-ray structures are available…

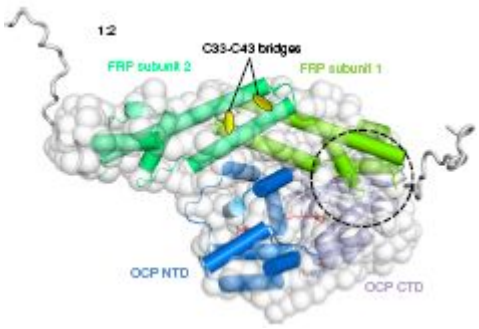
Atomistic modeling:

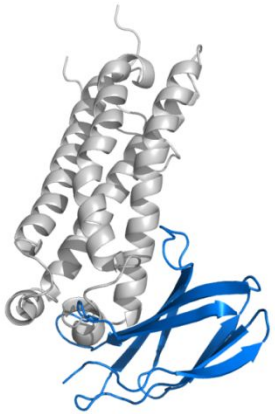
- Validation of the crystal structure against solution situation
- Rigid-body fitting
- Missing fragments (loops)
- Conformational transitions

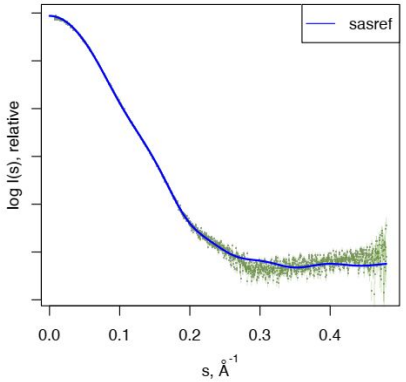




Theoretical SAXS profile can be calculated by CRYSOL program, necessary for fitting







Validation of the crystal structure in solution situation

1.75A

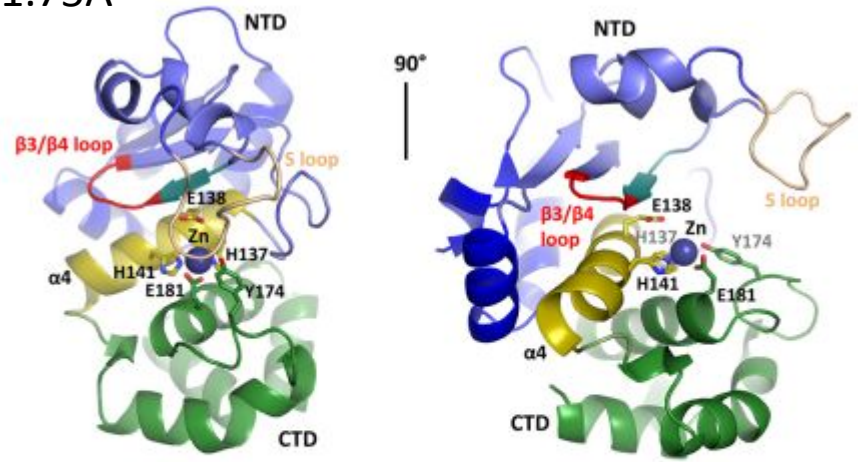
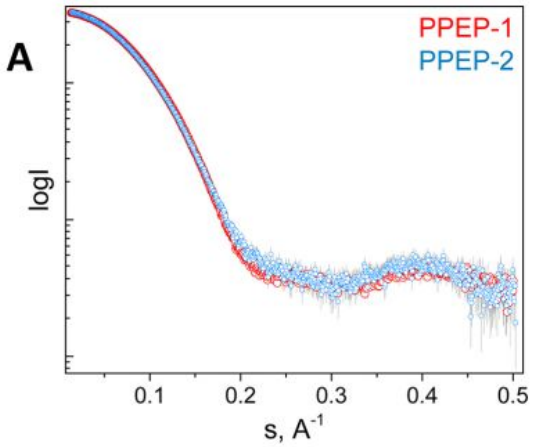
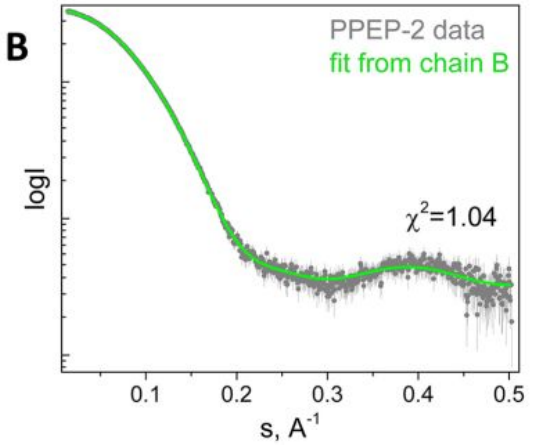


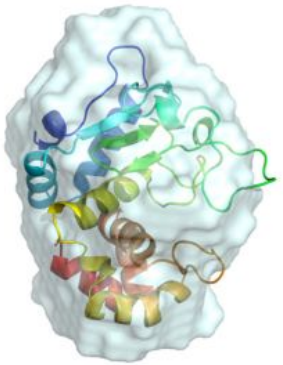
Figure 3. Atomic structure of PPEP-2. Atomic structure of chain A is in cartoon representation. Definition of the domains is the same as for PPEP-1: N-terminal domain (*NTD*), *blue*; active site (helix α4), *yellow*; C-terminal domain (*CTD*), *green*. Zinc-coordinating residues and residues involved in catalysis are shown as *sticks*. Zinc ion is shown as a *sphere*. For more details on the crystal unit, see Table 1 and the supporting information.

J. Biol. Chem. (2018) 293(28) 11154–11165

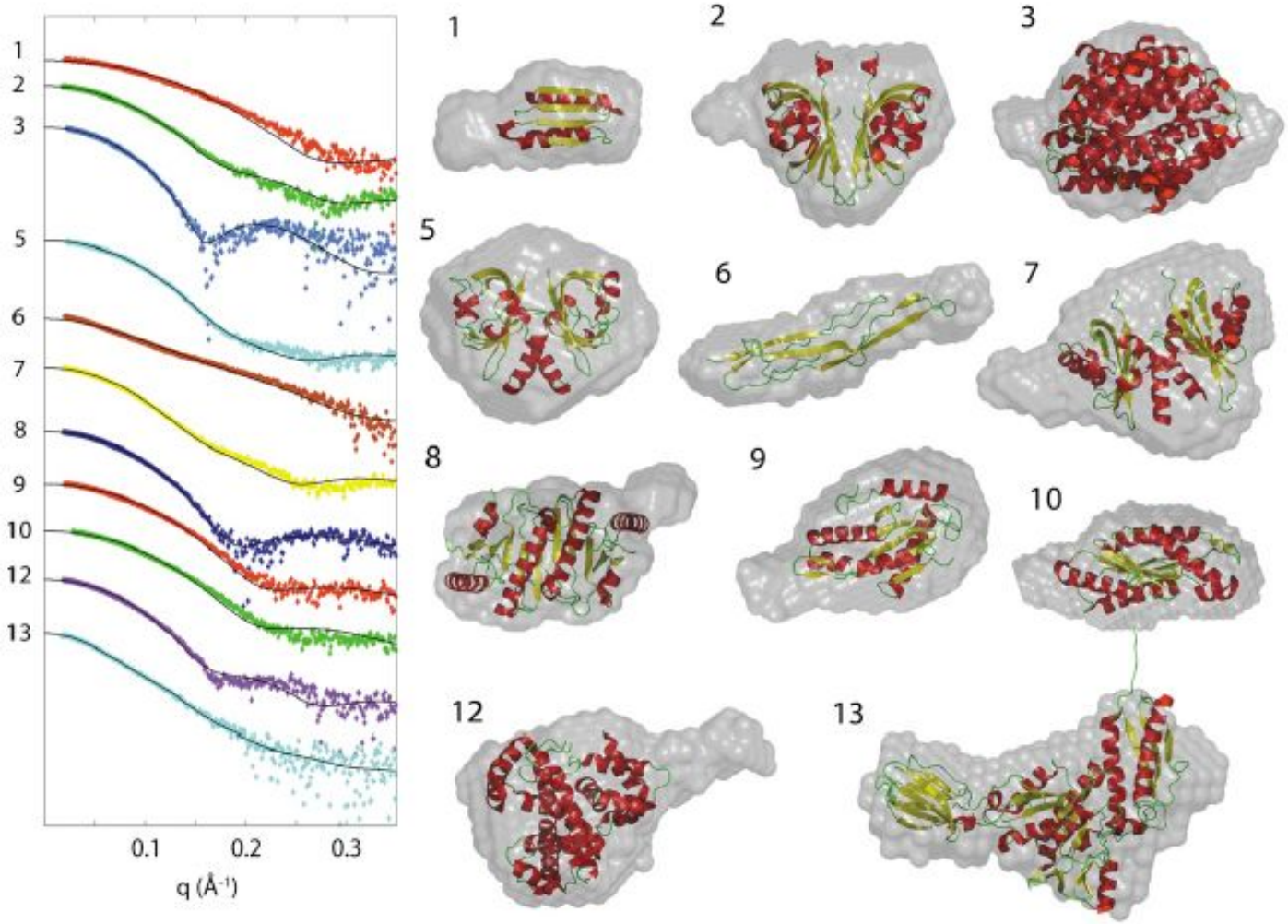




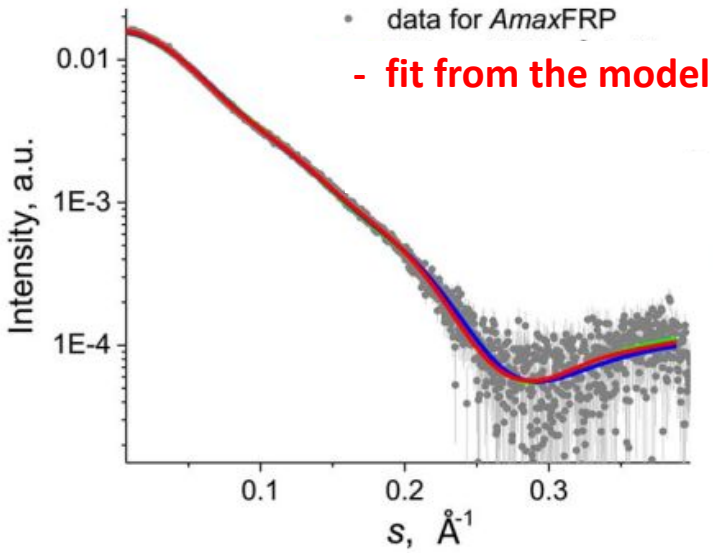
C

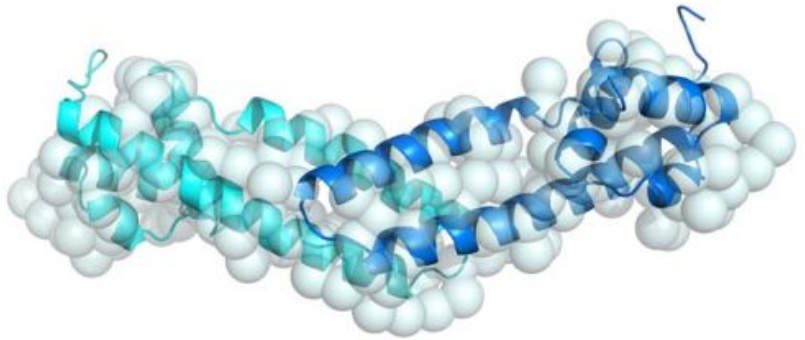


Comparison of the crystal structures and *ab initio* envelopes



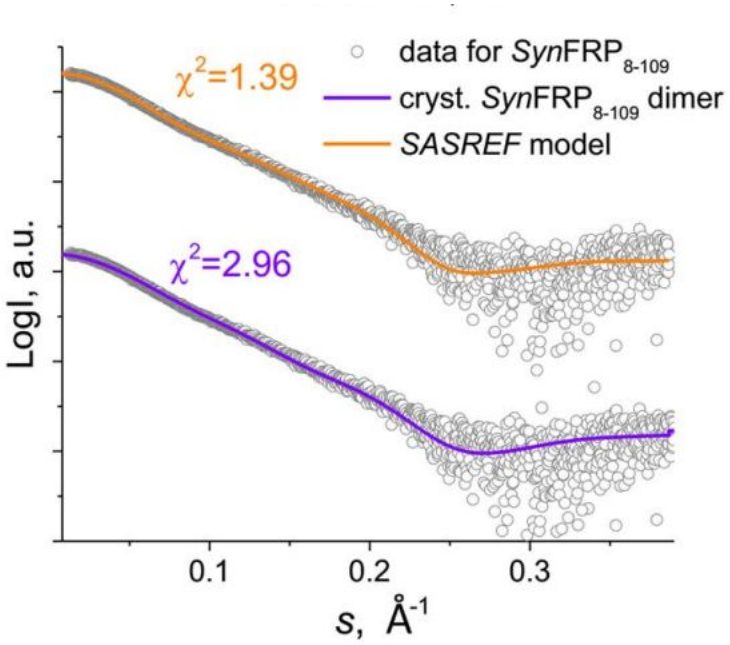
Conformational change



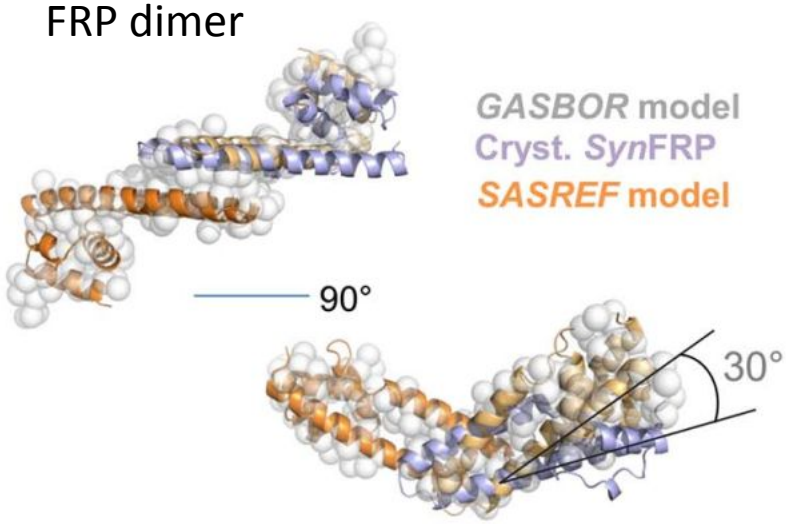


BBA - Bioenergetics 1859 (2018) 382–393

Conformational change

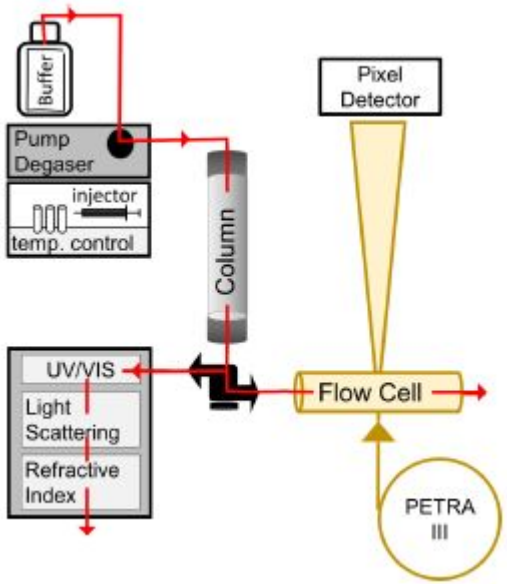


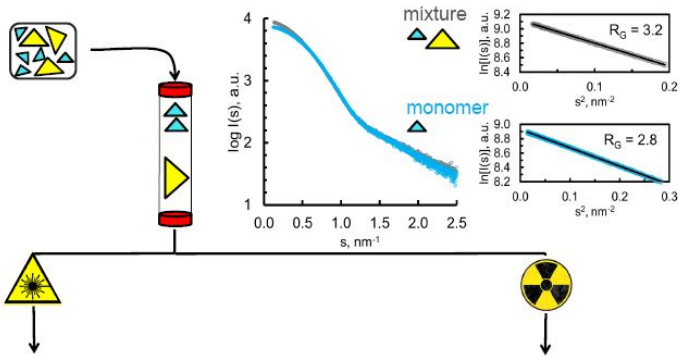
FRP dimer

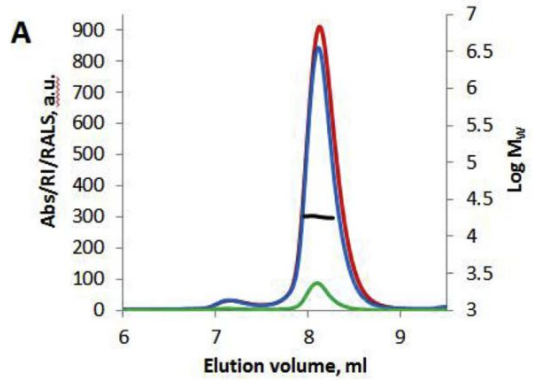


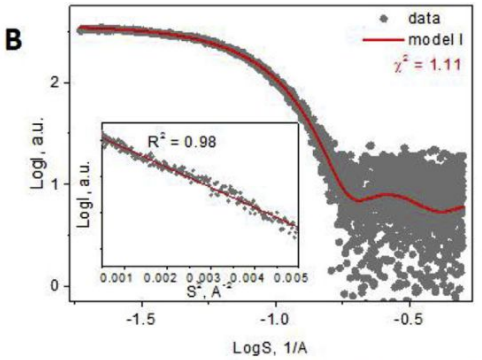
BBA - Bioenergetics 1859 (2018) 382–393

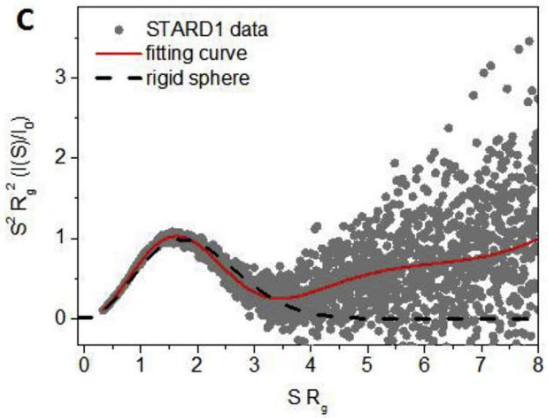
SEC-SAXS for contaminated samples

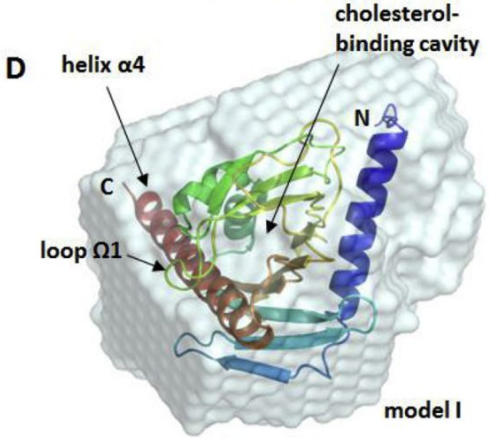










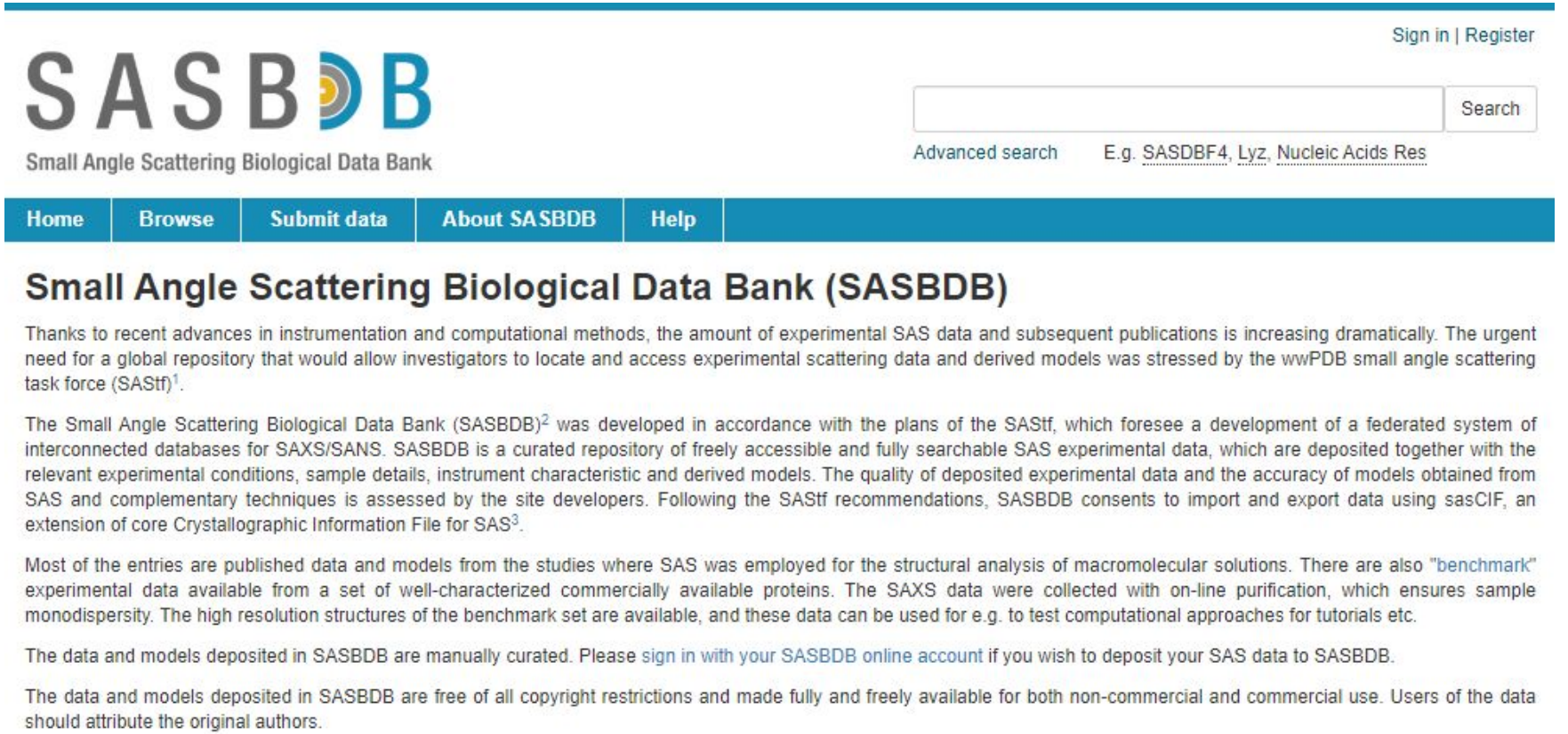


M. Graewert (c)

Biochemical and Biophysical Research Communications 489 (2017) 445–450

SASBDB

https://www.sasbdb.org/aboutSASBDB/



Трезвый взгляд на SAXS

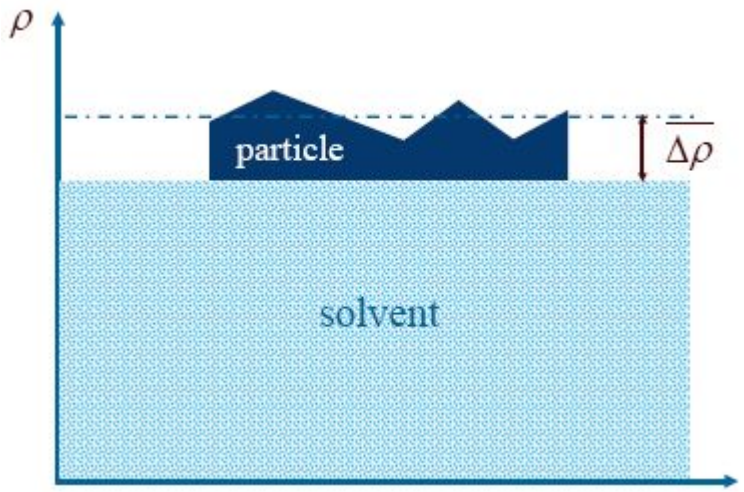
- Дает хорошую информацию о гидродинамических свойствах частиц (структурных свойствах) в растворе
- Хорош для тестирования гипотез о структуре, форме, комплексе и т.п.
- Вспомогательный метод структурной биологии
- Необходимо сверяться с как можно большим количеством экспериментальных данных (стехиометрия, олигомерное состояние, размеры, масса, радиус, пространственные ограничения, знания об интерфейсах, топологии субъединиц и т.п.)
- В одиночку SAXS не стоит использовать для структурной биологии (ambiguity)

SANS

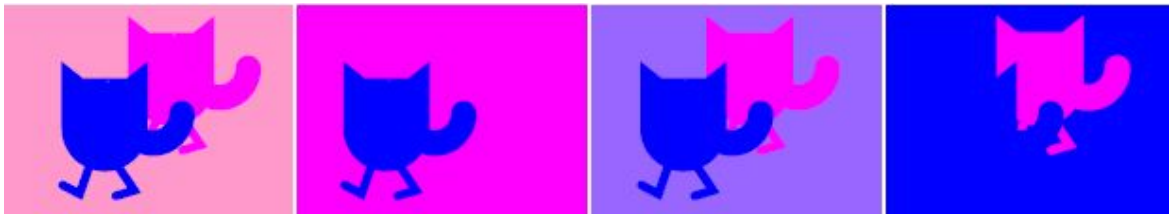
Features:

- Neutron source (rare)
- Non-ionizing radiation
- Coherent scattering (=elastic)
- Incoherent scattering (^{1}H affects)
- Contrast is very different in H_2O and D_2O
- SAXS and SANS are complementary!

Difference in the scattering density (contrast)



Contrast variation by increasing D_2O content:



Study of conformational changes of selected proteins within the complexes !!!

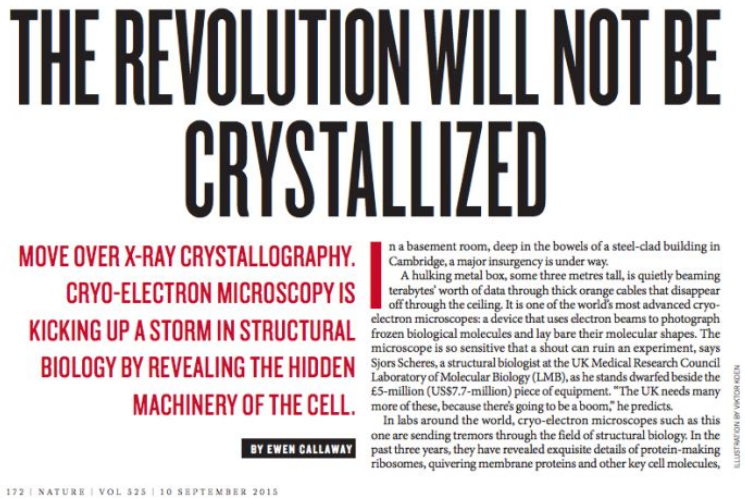
Samples for SAXS and SANS

- Protein concentration: 0.1-10 mg/ml
- Volume: 5-50 microliter (SAXS), 200-300 microliter (SANS)
- Time:
  - lab source: 5-60 min
  - Synchrotron: seconds
  - Neutrons: 30 minutes - hours

CryoEM

https://www.youtube.com/watch?v=aHhmnxD6RCI

Jacques Dubochet | Joachim Frank | Richard Henderson



THE REVOLUTION WILL NOT BE CRYSTALLIZED

MOVE OVER X-RAY CRYSTALLOGRAPHY. CRYO-ELECTRON MICROSCOPY IS KICKING UP A STORM IN STRUCTURAL BIOLOGY BY REVEALING THE HIDDEN MACHINERY OF THE CELL.

BY EWEN CALLAWAY

In a basement room, deep in the bowels of a steel-clad building in Cambridge, a major insurgency is under way.

A hulking metal box, some three metres tall, is quietly beaming terabytes' worth of data through thick orange cables that disappear off through the ceiling. It is one of the world's most advanced cryo-electron microscopes: a device that uses electron beams to photograph frozen biological molecules and lay bare their molecular shapes. The microscope is so sensitive that a shout can ruin an experiment, says Sjors Scheres, a structural biologist at the UK Medical Research Council Laboratory of Molecular Biology (LMB), as he stands dwarfed beside the £5-million (US$7.7-million) piece of equipment. "The UK needs many more of these, because there's going to be a boom," he predicts.

In labs around the world, cryo-electron microscopes such as this one are sending tremors through the field of structural biology. In the past three years, they have revealed exquisite details of protein-making ribosomes, quivering membrane proteins and other key cell molecules,

ILLUSTRATION BY VIKTOR KOEN

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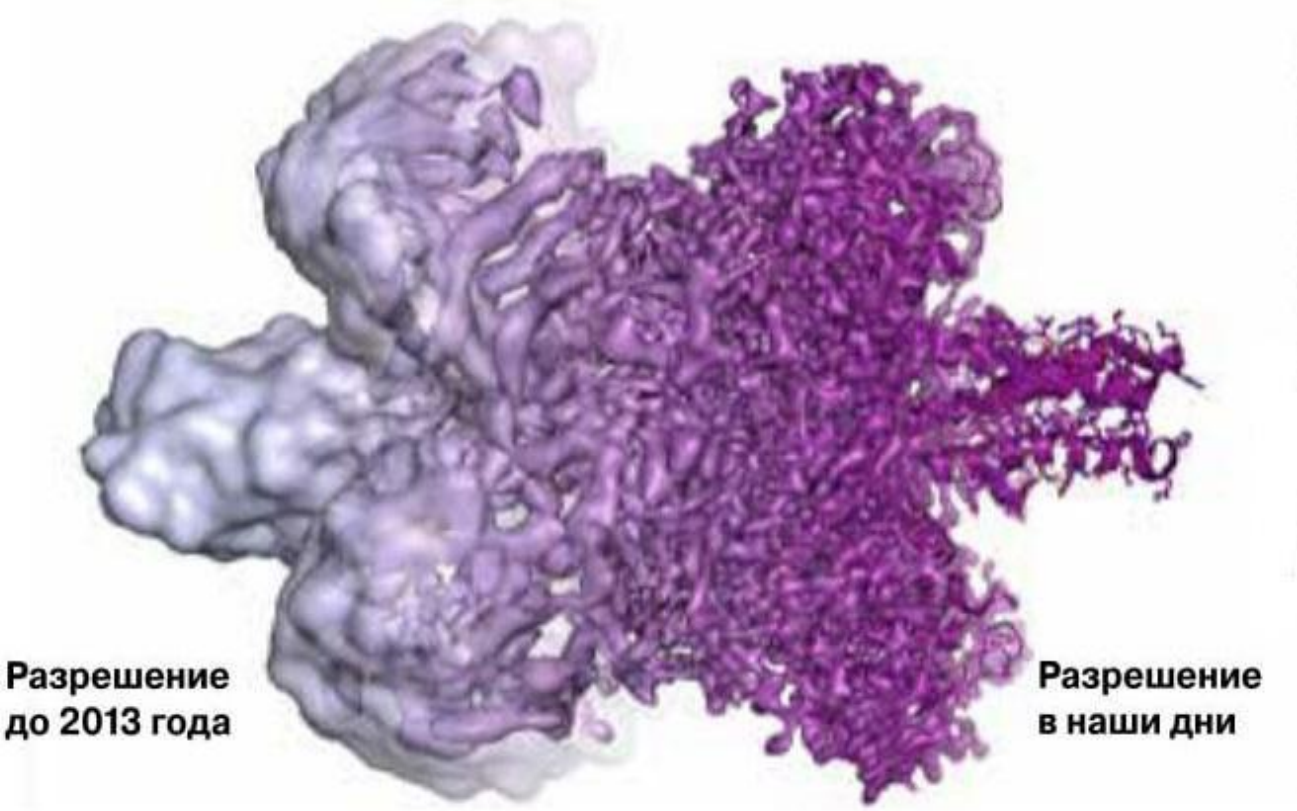
https://www.nature.com/news/the-revolution-will-not-be-crystallized-a-new-method-sweeps-through-structural-biology-1.18335

Resolution revolution



- появление прямых детекторов электронов
- развитие софта для обработки огромного количества картинок
- совершенствование микроскопов, адаптация к криоусловиям

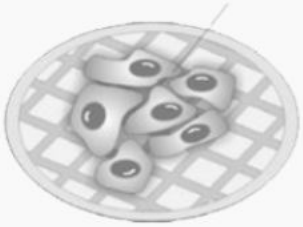
Purified Protein

Sample Preparation

Imaging Processing

Model Building

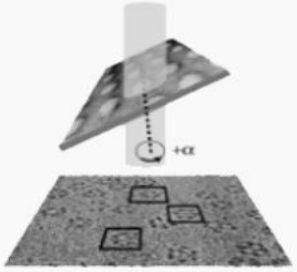
The recipe includes



Sample preparation

After protein purification, these samples will be treated with cryo-fixation. In this method, protein samples are placed on a specially treated EM grid consisting of tiny holes in a film supported by a metal frame. The grid is then plunged into liquid ethane to flash-freeze it, resulting in the protein samples being embedded in a thin layer of vitreous ice. Once the frozen-hydrated grid is prepared, it is placed in the electron microscope and kept at approximately -180 K throughout the experiment.

https://www.youtube.com/watch?v=BJKkC0W-6Qk



EM imaging and data processing

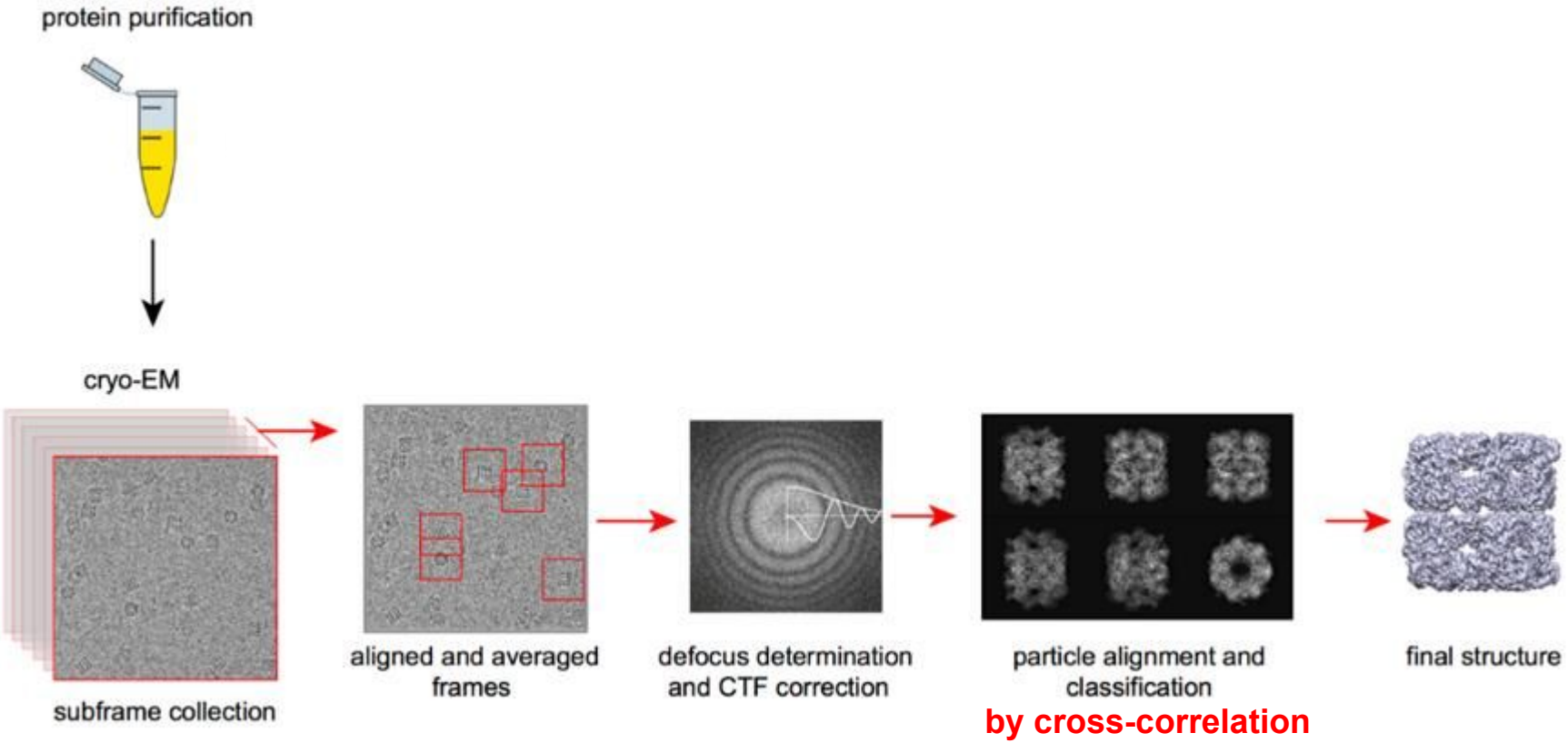
- Data collection;
- Initial 3D model calculation;
- Beam-induced motion correction;
- Micrograph screening;
- Automatic particle picking and normalization;
- 2D, 3D classification and refinement.

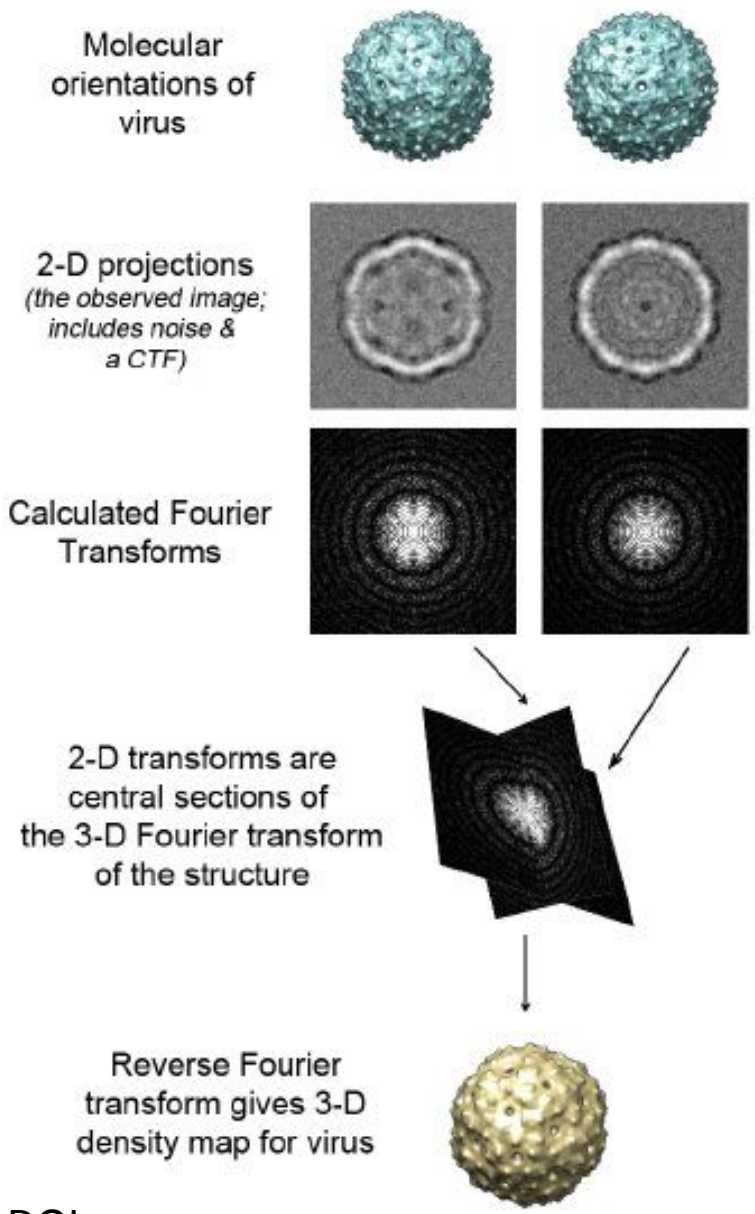
Unstained, in vitreous ice

Model building and refinement

Using our high-performance computers and software packages, we are able to interpret EM maps and reconstruct them into three-dimensional structural models that satisfy principles of physics and stereochemistry. (Our scientists are also very experienced with challenging targets such as membrane proteins by manual intervention to improve the initial fit or even build *de novo* models.) After an initial model is built, refinement is performed to maximize the agreement between the model and experimentally observed data by adjusting atomic coordinates, B factors, and other parameters.

The process of Cryo-EM single particle analysis technique





Features, 2D->3D

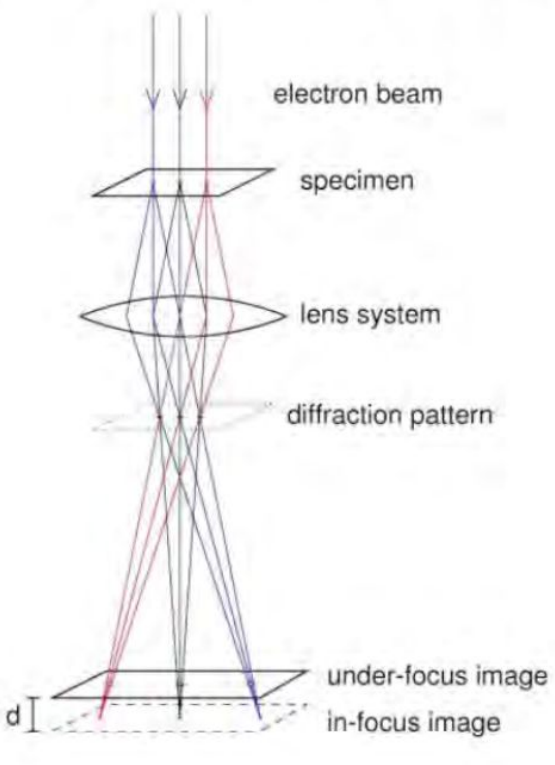
- Biological samples – low doses and dehydration (high vacuum)
- Freezing allows to avoid these, but the images have a very low contrast
- Each picture - 2D projection of a 3D object
- Multiple 2D projections can be used to reconstruct the 3D object

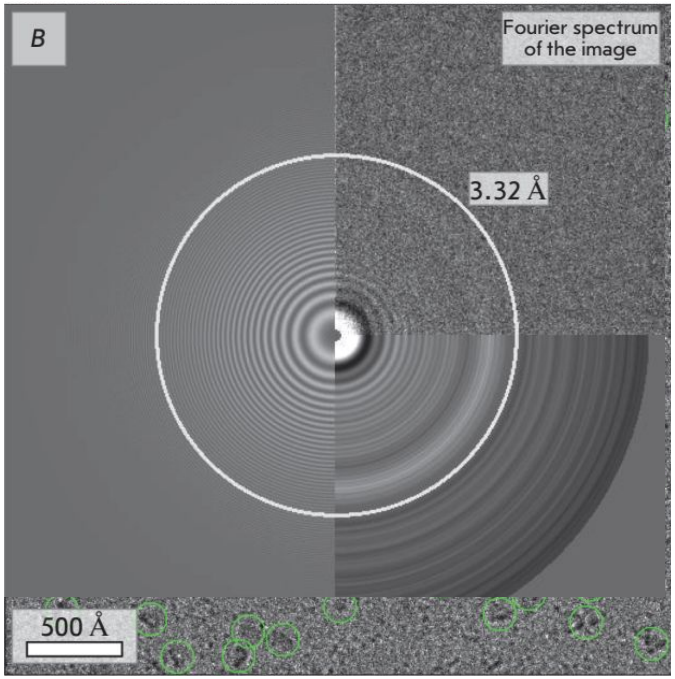
DOI:
10.1142/9781848164666_0001

http://www.ejectamenta.com/Imaging-Experiments/fourierimagefiltering.html

Contrast transfer function and defocus

- At perfect focus, biological specimens produce little contrast in vitreous ice.
- To produce phase contrast, pictures are taken underfocus, at the expense of systematic alteration of the image data (not all waves are well transferred -> CTF)
- Each picture is undergoing FT to see Thon rings (~resolution rings in Xtallography) – contrast transfer function (CTF)
- Some waves are lost but can be CTF-corrected upon changing defocus (d below)





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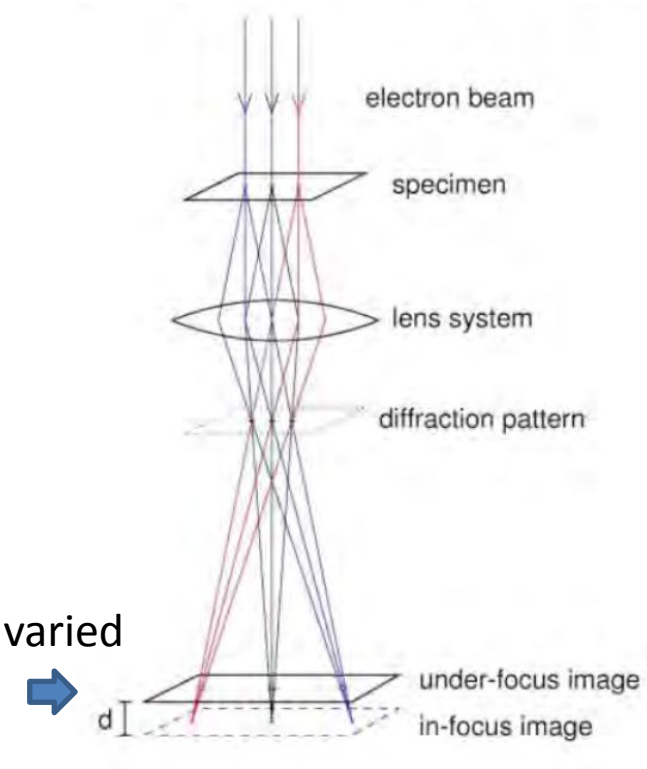
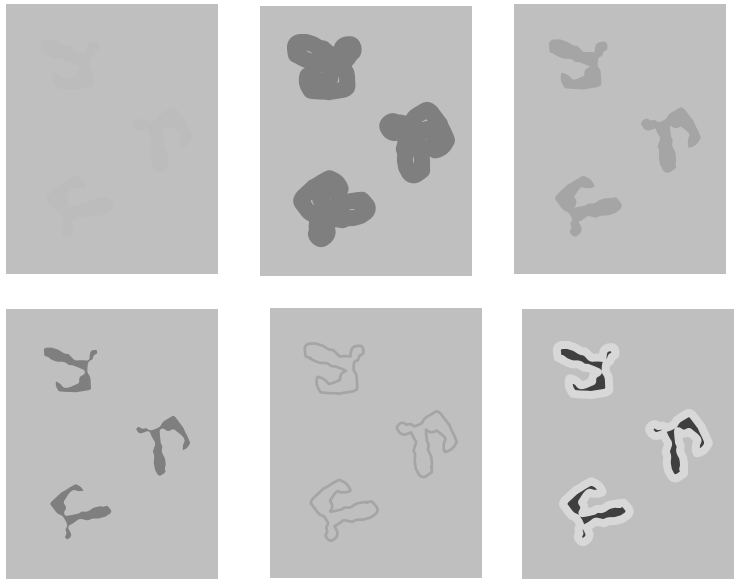
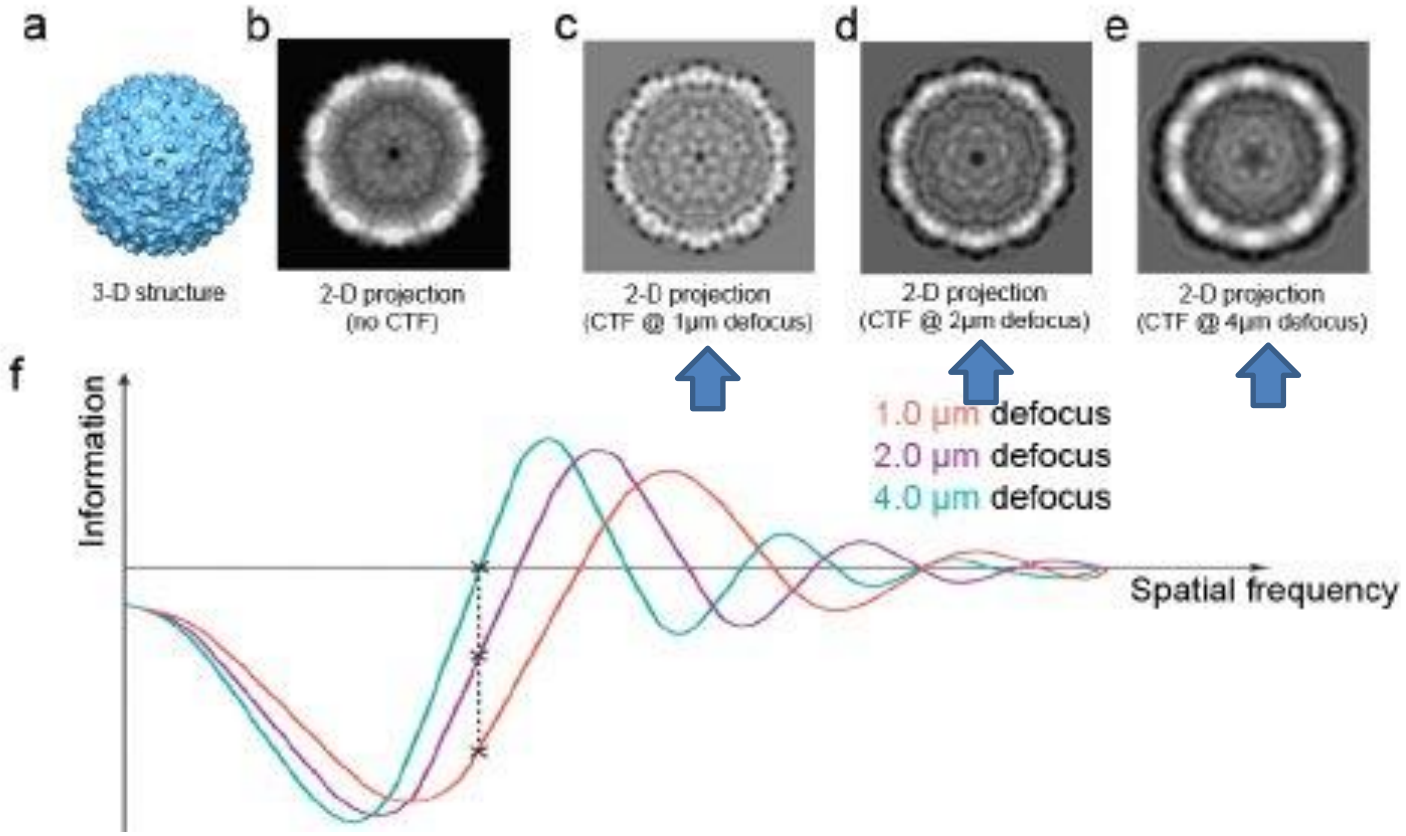


d is varied



Contrast transfer function and defocus



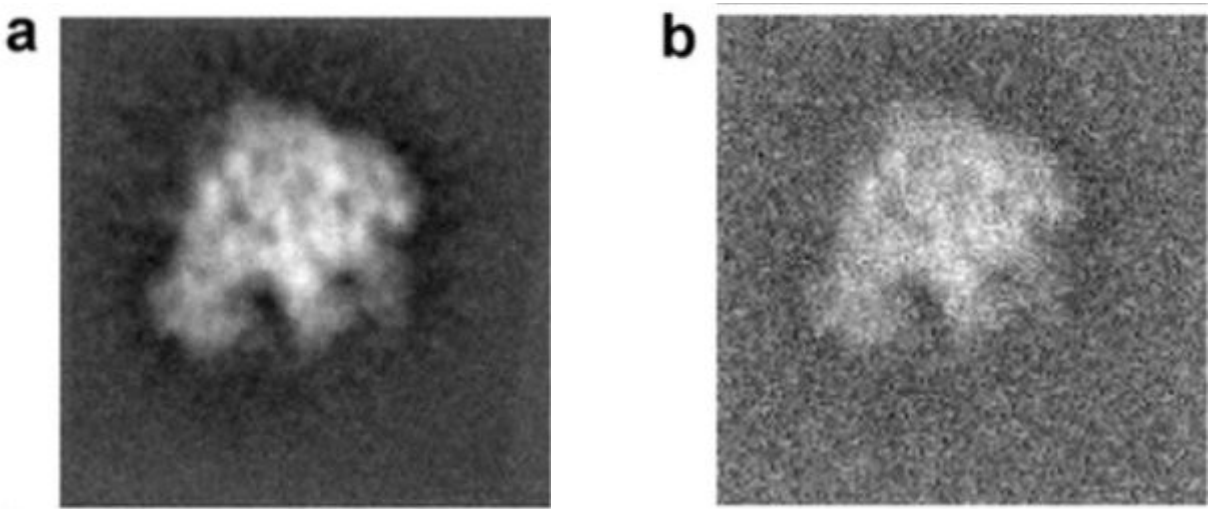
DOI: 10.1142/9781848164666_0001

Single particle cryoEM requires tons of images

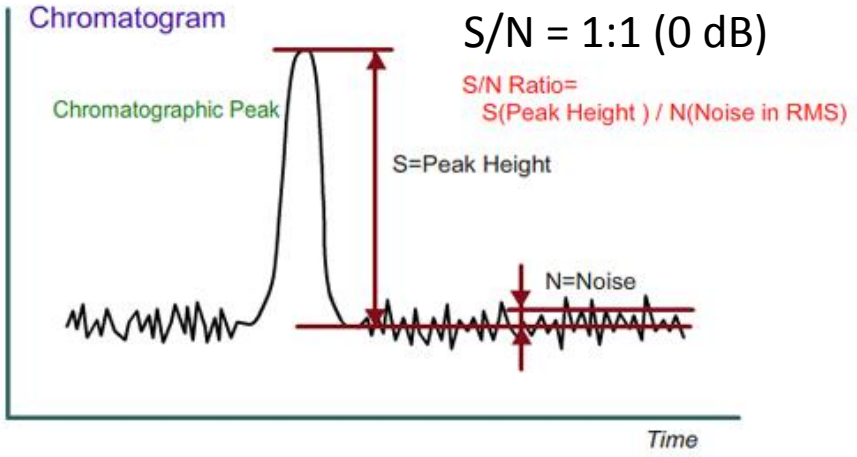
- Particle orientations are classified by cross-correlation
- Each class should be represented by thousands of images
- Also, at different defocus values
- Some images are discarded

Signal and noise

50S ribosome projection



5:1 1:1



S/N = 1:1 (0 dB)

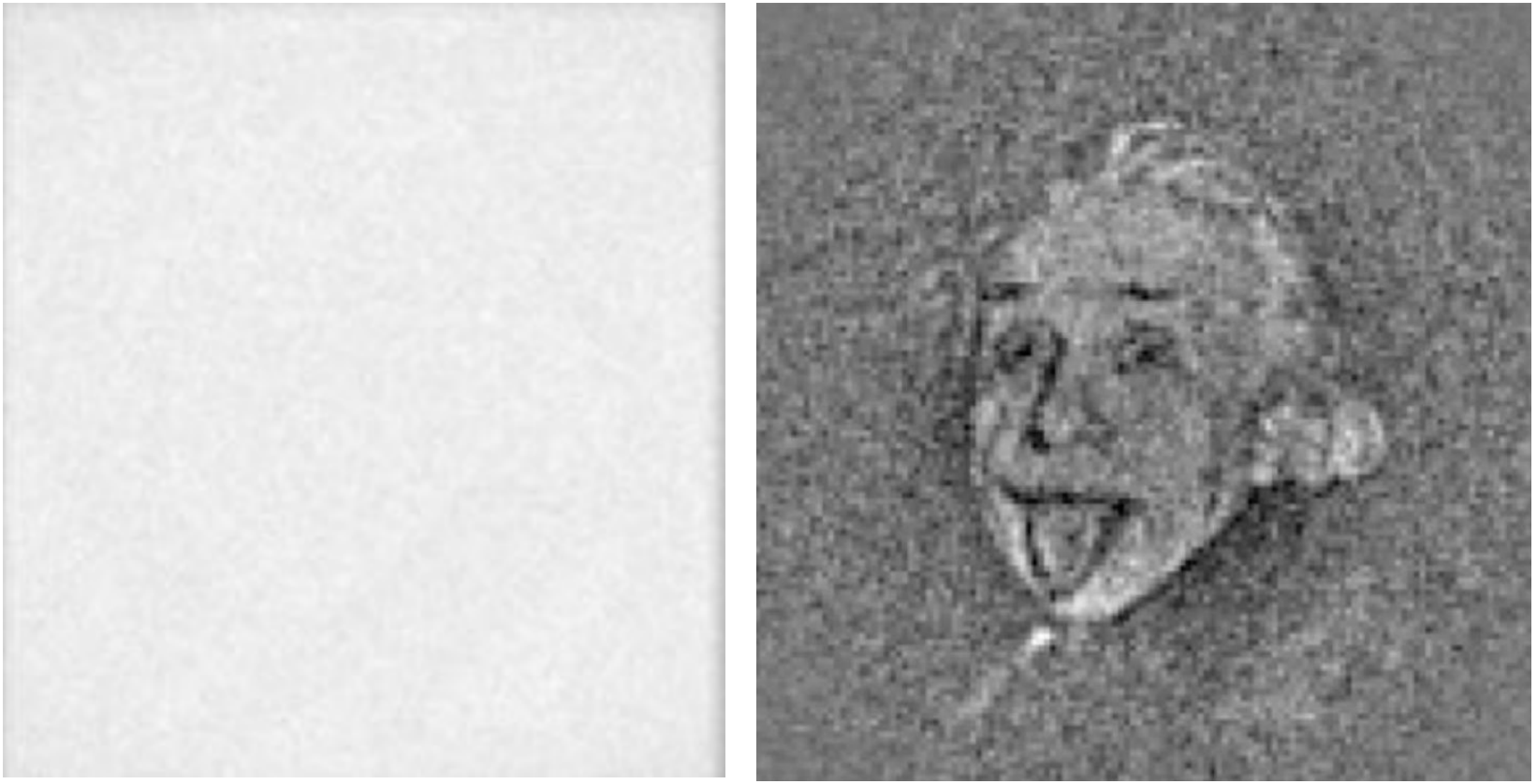
Improving S/N by repetition and averaging

$$\mathrm{SNR}_N = \sqrt{N}\,\mathrm{SNR}$$

4 measurements = 2 *S/N

Accurate alignment and the target model are important

Einstein from noise



An image of Einstein appears from averaged 1000 images of pure white noise by using a normalized cross-correlation function and the photo as a model.

doi: 10.1016/j.jsb.2008.12.008

Обучение крио ЭМ

- https://ru.coursera.org/learn/cryo-em
- https://em-learning.com

Grant J. Jensen

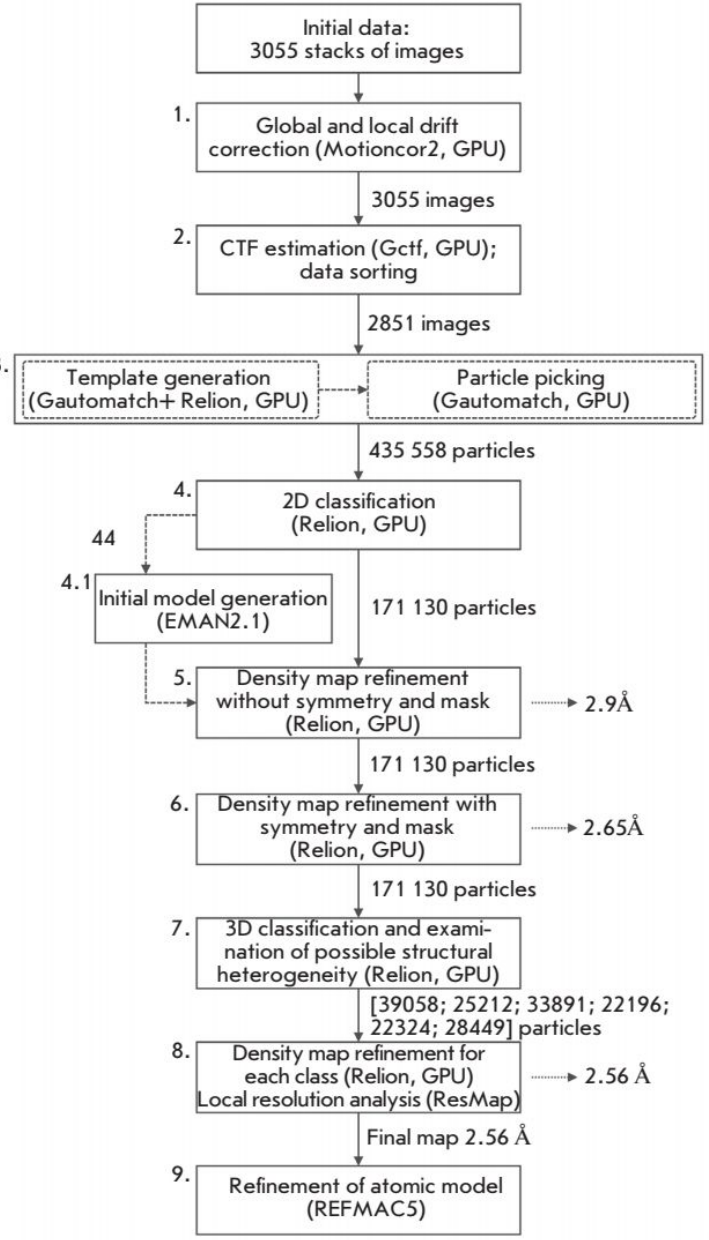
http://www.jensenlab.caltech.edu/

Professor of Biophysics and Biology; Investigator, Howard Hughes Medical Institute

Caltech

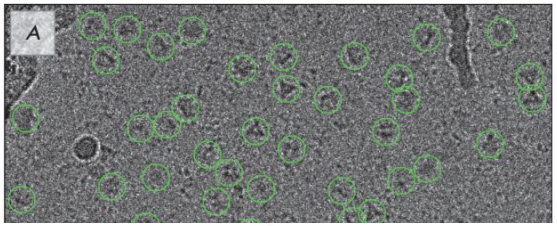
Prof. Yifan Cheng

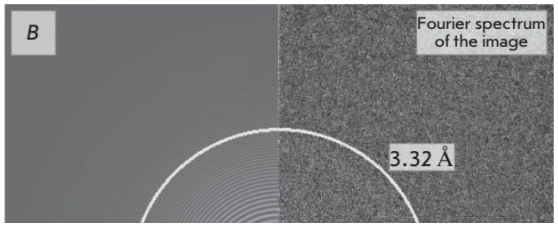
https://www.youtube.com/watch?v=Bk5lBvwSe-s

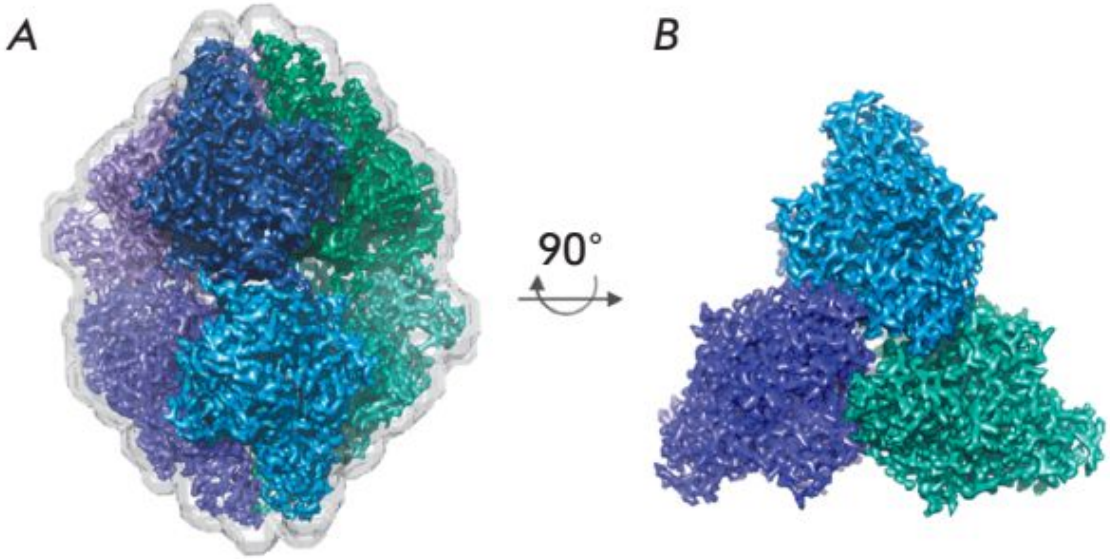


Three-Dimensional Structure of Cytochrome *c* Nitrite Reductase As Determined by Cryo-Electron Microscopy

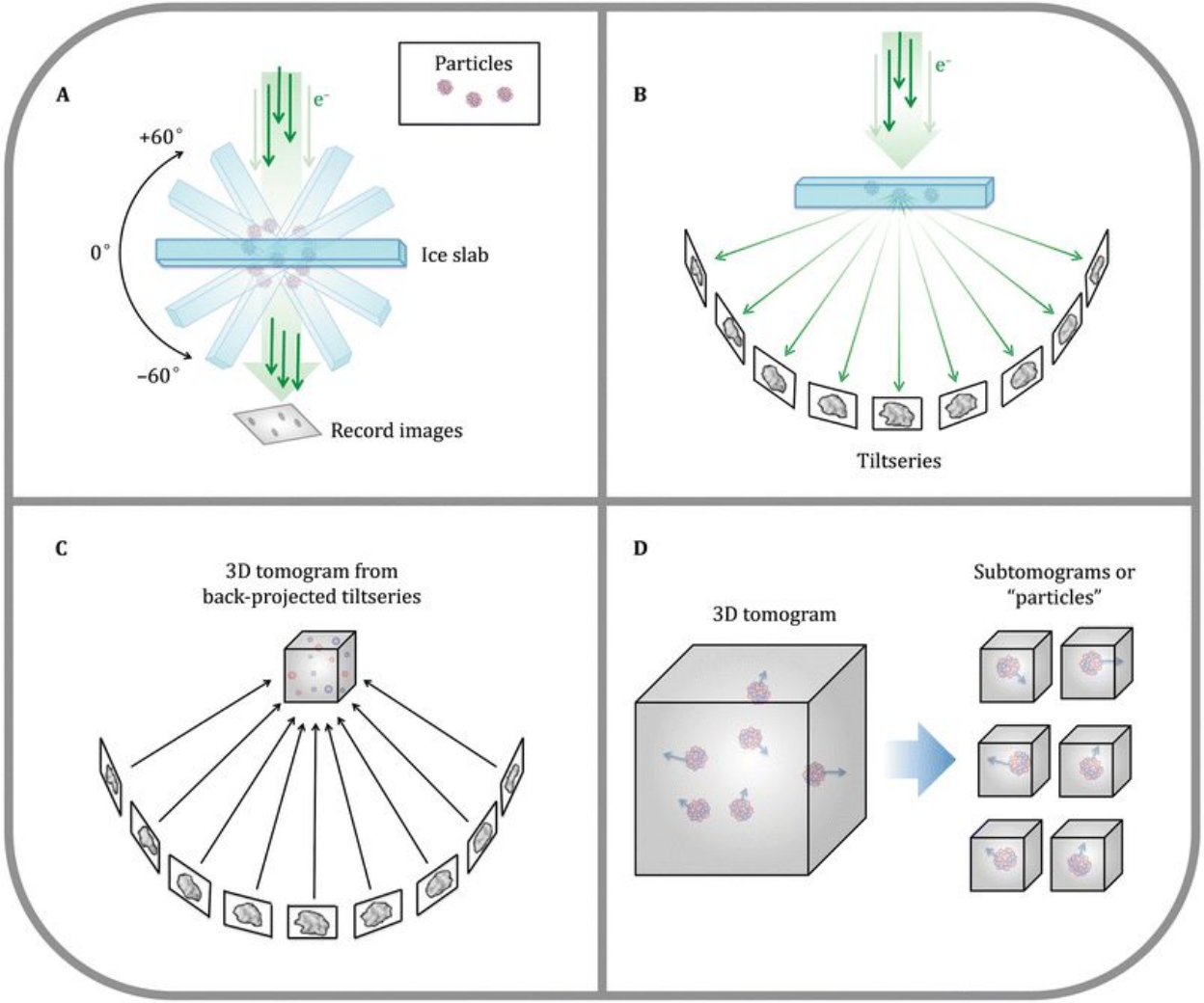
T. N. Baymukhametov[1], Y. M. Chesnokov[1], E. B. Pichkur[1], K. M. Boyko[1,2], T. V. Tikhonova[2], A. G. Myasnikov[3,4,5], A. L. Vasiliev[1,6], A. V. Lipkin[1], V. O. Popov[1,2*], M. V. Kovalchuk[1,6]



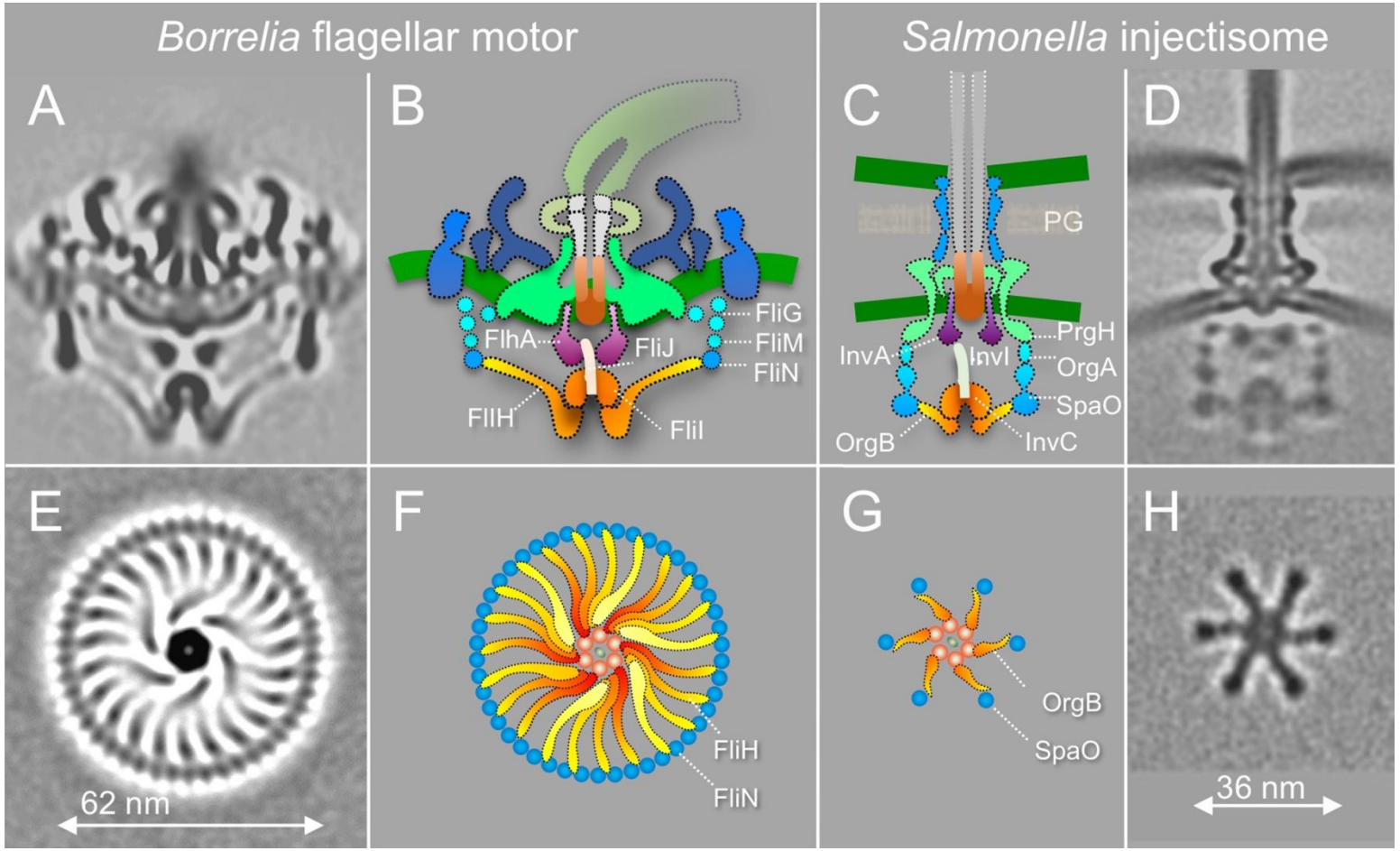




Cryo-electrotomography (Cryo-ET)

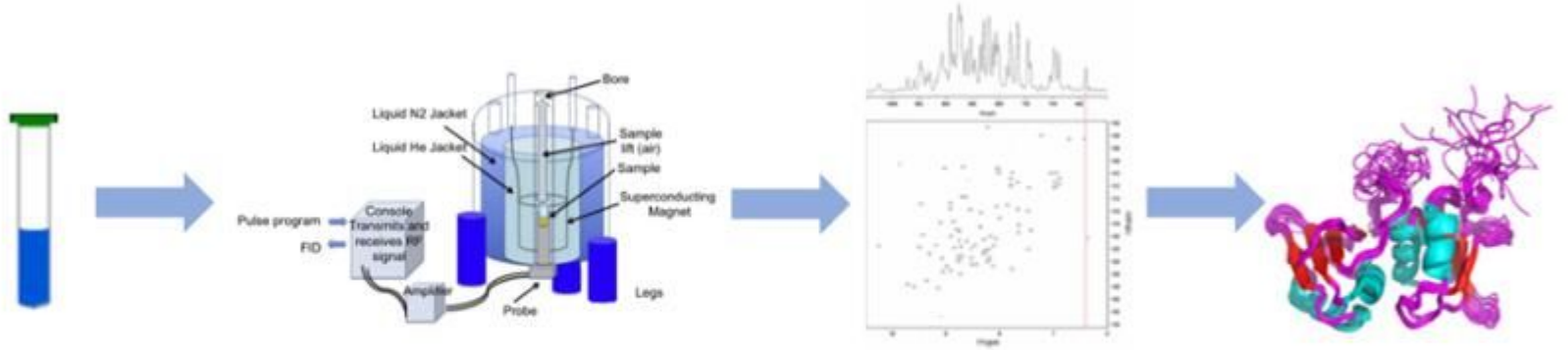


Cryo-electrotomography (Cryo-ET)



https://doi.org/10.1371/journal.pbio.3000050

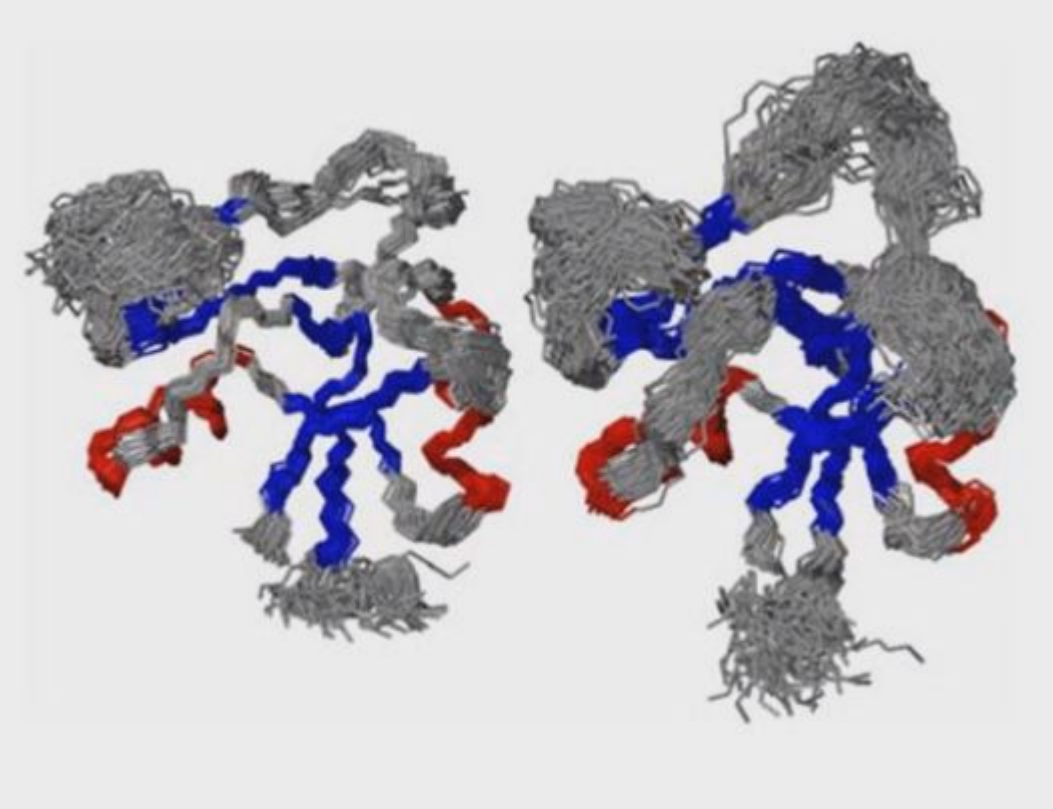
NMR – nuclear magnetic resonance



Sample preparation | Data acquisition | Spectral processing | Structural analysis

NMR made super easy: https://www.youtube.com/watch?v=0s7Cbl8bZLM
https://www.youtube.com/watch?v=eY0NyE0SQjE ☺

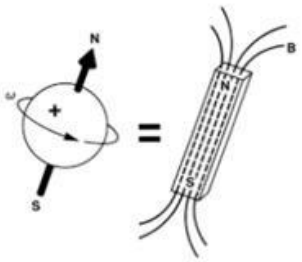
The output of the (successful) multidimensional NMR experiment

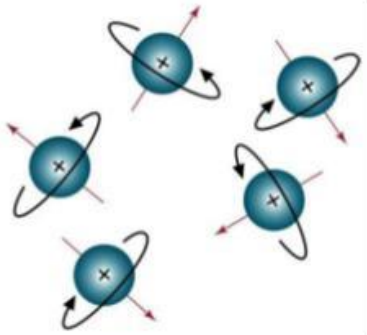


A set of structural models that satisfy the experimental constraints but also obey the chemistry rules

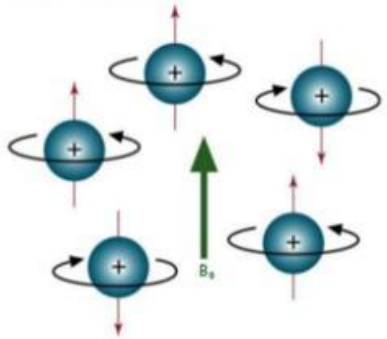
NMR

Spin up
Or
Spin down





No external magnetic field



Apply external magnetic field B_0

- Only nuclei with **non-zero spin quantum** number are "*magnets*"
- Commonly used spins are spin ½ nuclei: ^{1}H, ^{13}C, ^{15}N, ^{31}P *etc.*

https://www.youtube.com/watch?v=PmYwYUQw-Rw

Properties of some nuclei

Nucleus	I	γ (rad . T^{-1} . s^{-1})	Natural abundance (%)
^{1}H	$\frac{1}{2}$	2.6752×10^{8}	99.98
^{2}H	1	4.107×10^{7}	0.02
^{13}C	$\frac{1}{2}$	6.728×10^{7}	1.11
^{14}N	1	1.934×10^{7}	99.64
^{15}N	$\frac{1}{2}$	-2.712×10^{7}	0.36
^{17}O	$\frac{5}{2}$	-3.628×10^{7}	0.04
^{19}F	$\frac{1}{2}$	2.5181×10^{8}	100.00
^{23}Na	$\frac{3}{2}$	7.080×10^{7}	100.00
^{31}P	$\frac{1}{2}$	1.0841×10^{8}	100.00
^{113}Cd	$\frac{1}{2}$	5.934×10^{7}	12.26

[a] The angular momentum quantum number, I, and the gyromagnetic ratio, γ, and natural isotopic abundance for nuclei of particular importance in biological NMR spectroscopy are shown.

Bonvin A (c)

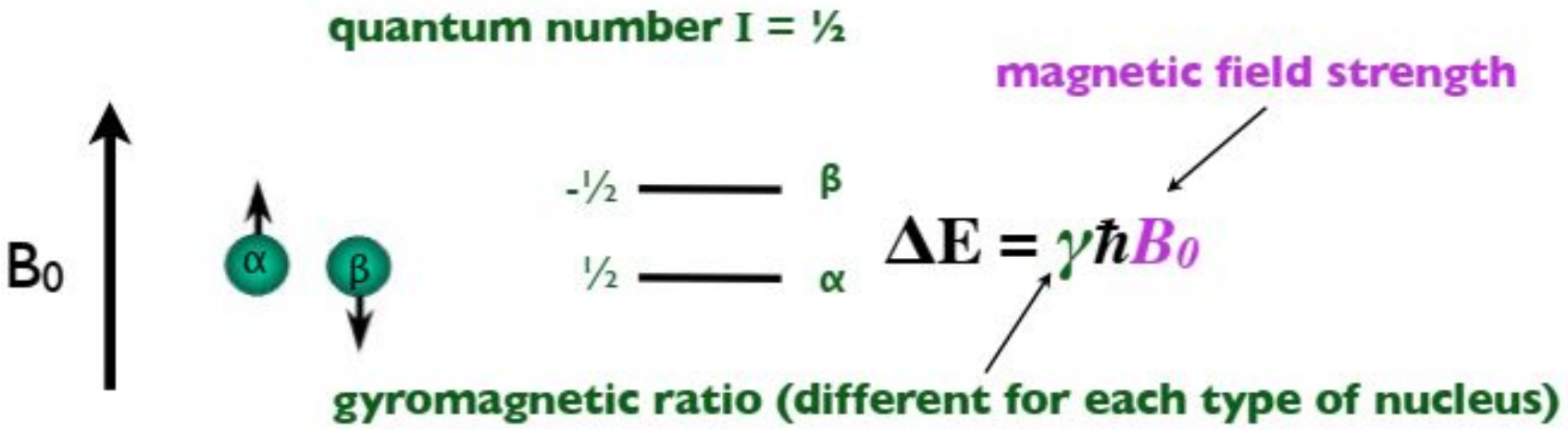
NMR sample

- **Isotope labeling**
  - ^{15}N, ^{13}C, ^{2}H
  - selective labeling (e.g. only methyl groups)
  - recombinant expression in *E.coli*

- **Sample**
  - pure, stable and high concentration
    - 500 uL of 0.5 mM solution -> ~ 5 mg per sample
  - preferably low salt, low pH
  - no additives

Bonvin A (c)

Nuclear spin



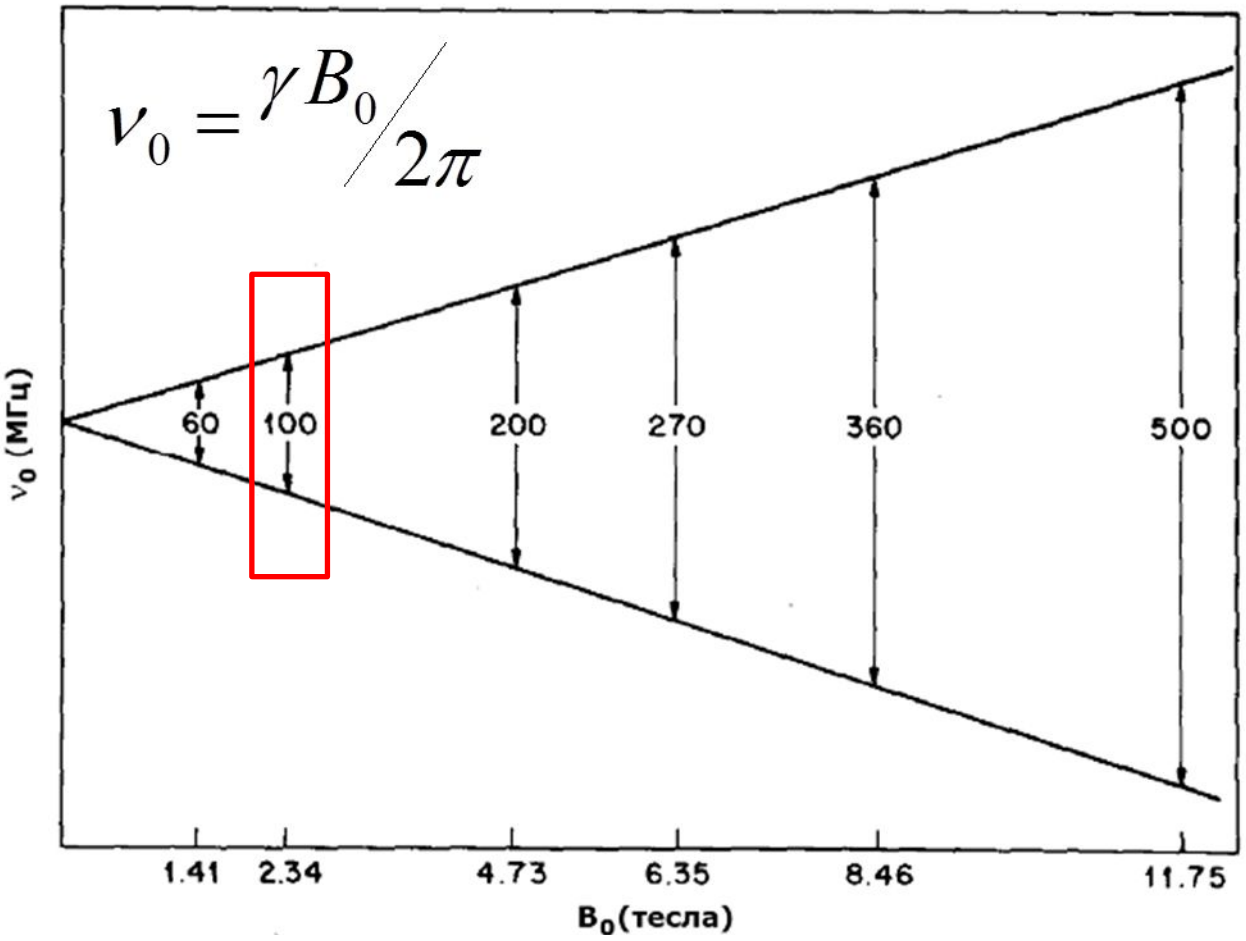
$$\nu_0 = \gamma B_0 / 2\pi$$

Частота прецессии (Ларморова частота)

Bonvin A (c)

Energy between α (+1/2) and β (-1/2) levels

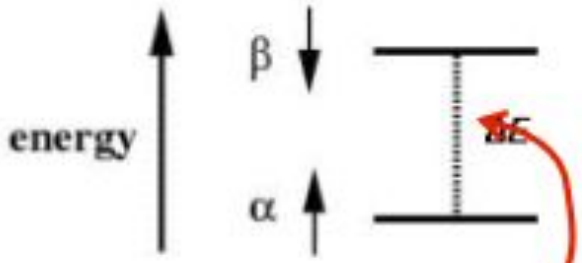
^{1}H

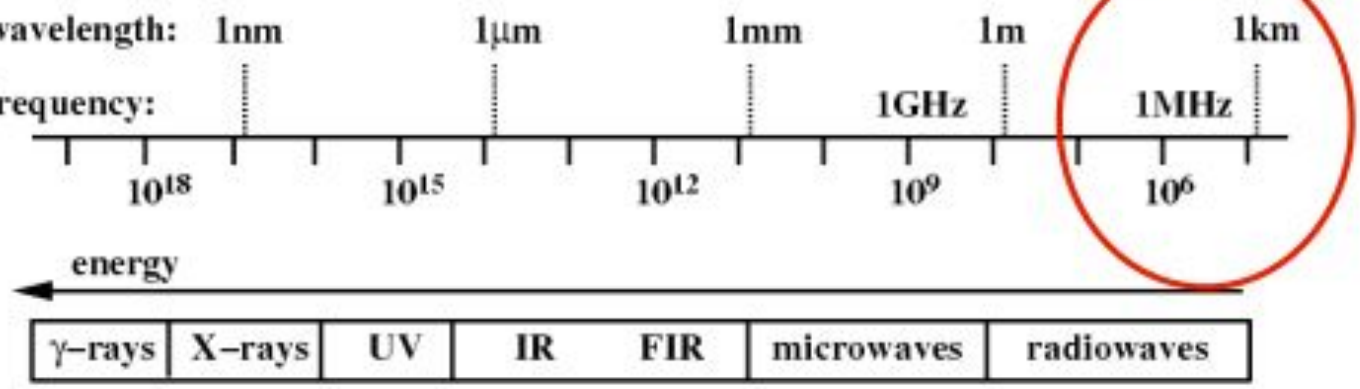


Атом	Резонансная частота при 2.34 T
^{1}H	100 МГц
^{13}C	25.1504 МГц
^{15}N	10.1398 МГц
^{17}O	13.5613 МГц
^{31}P	40.5178 МГц

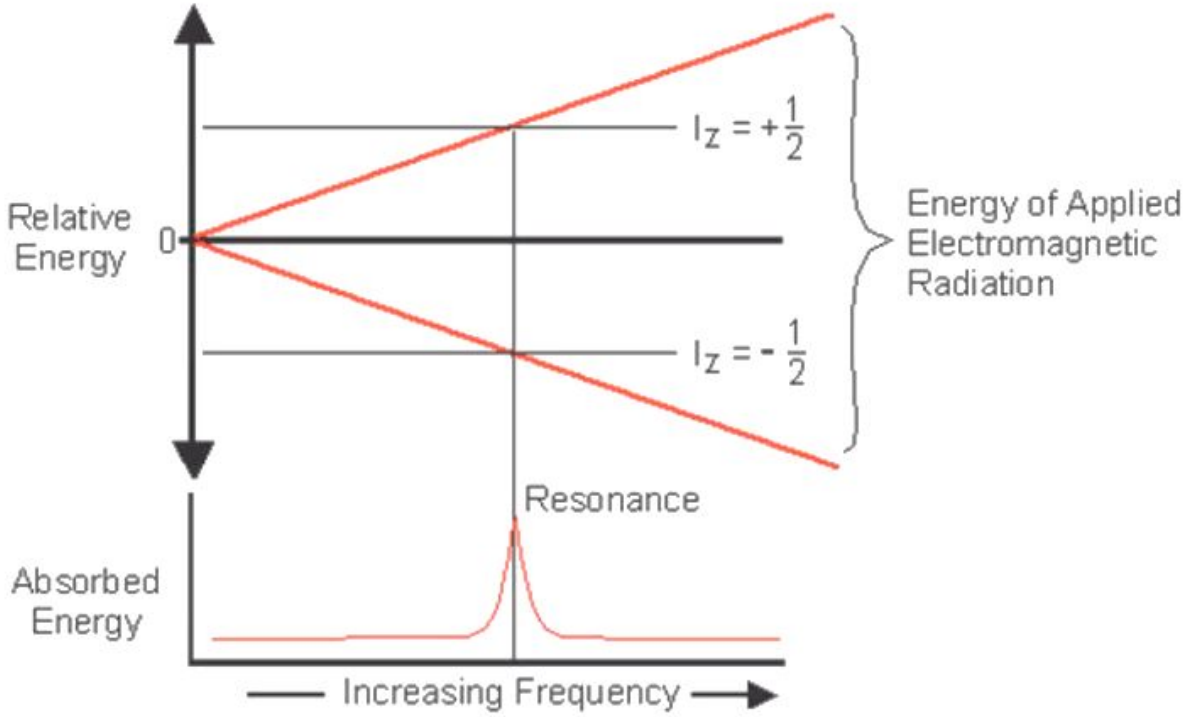
Nuclear spin & radiowaves

- **NMR a non invasive technique**
  - **Low energy radiowaves**
- **Transitions between levels are possible**





NMR, a spectroscopy technique



In a magnetic field *magnetic* nuclei will resonate with a specific frequency

Pulse

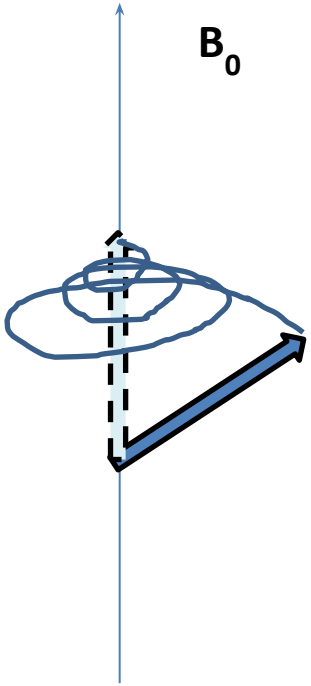
- **Radio frequency pulses**
  - **Turn on an amplifier for a certain amount of time & certain amount of power (B_1 field)**

2π

only rotation around B_1 is observed

rotating frame: observe with frequency ν_0

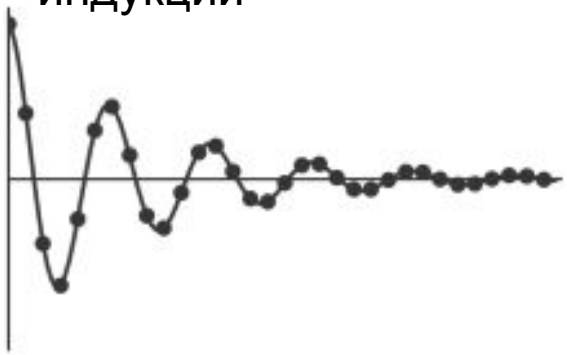
Magnetization (M) gets back to the B_0-oriented position after being affected by external field



Exponential decay

Free induction decay (FID)

=спад свободной индукции



Relaxation

Chemical shift due to the local environment changing frequency of the nuclei

shielding constant

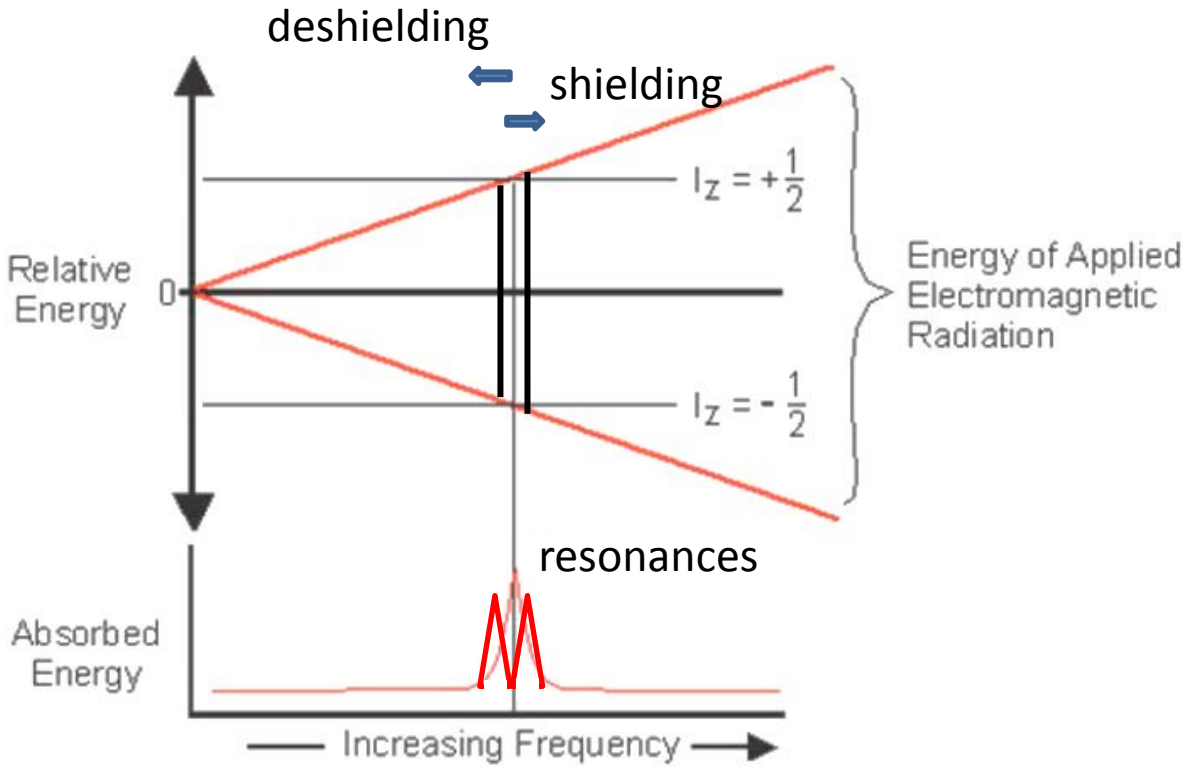
$$\nu = \frac{\gamma B_0}{2\pi}(1-\sigma)$$

Expressed as part per million (ppm) by comparison to the reference frequency:

(may also be presented in Hz)

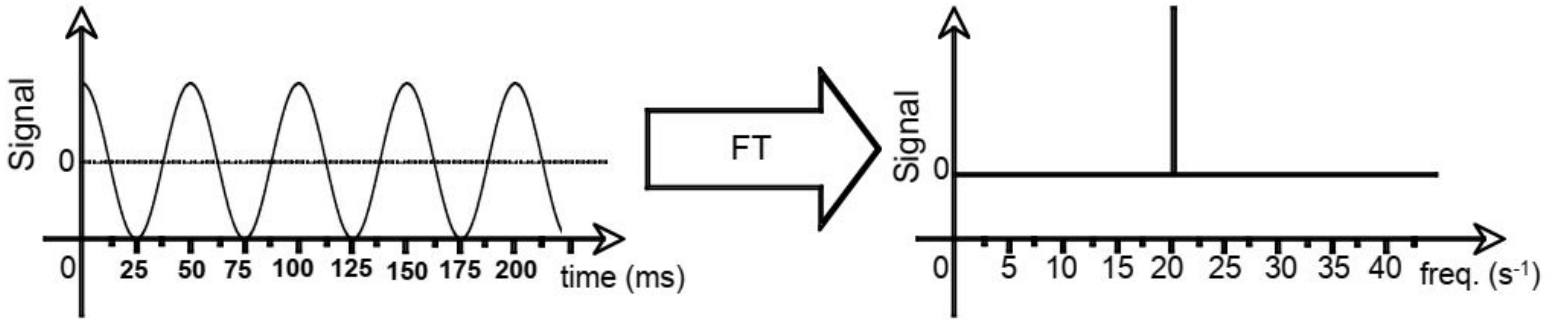
$$\delta = 10^6 \frac{\upsilon - \upsilon_{ref}}{\upsilon_{ref}}$$

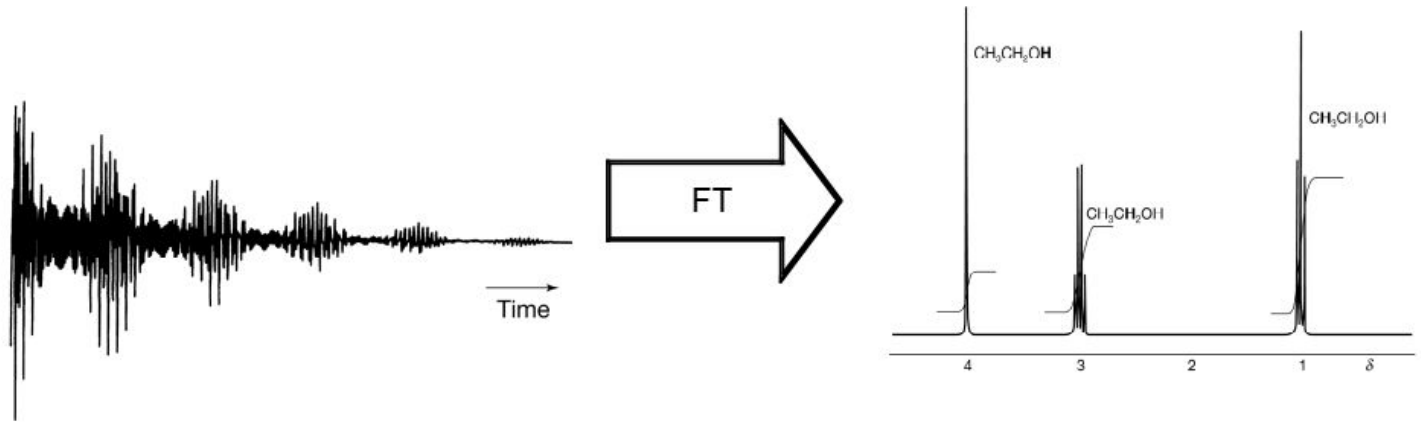
The local electronic environment of the nucleus may change the frequency: shielding effect



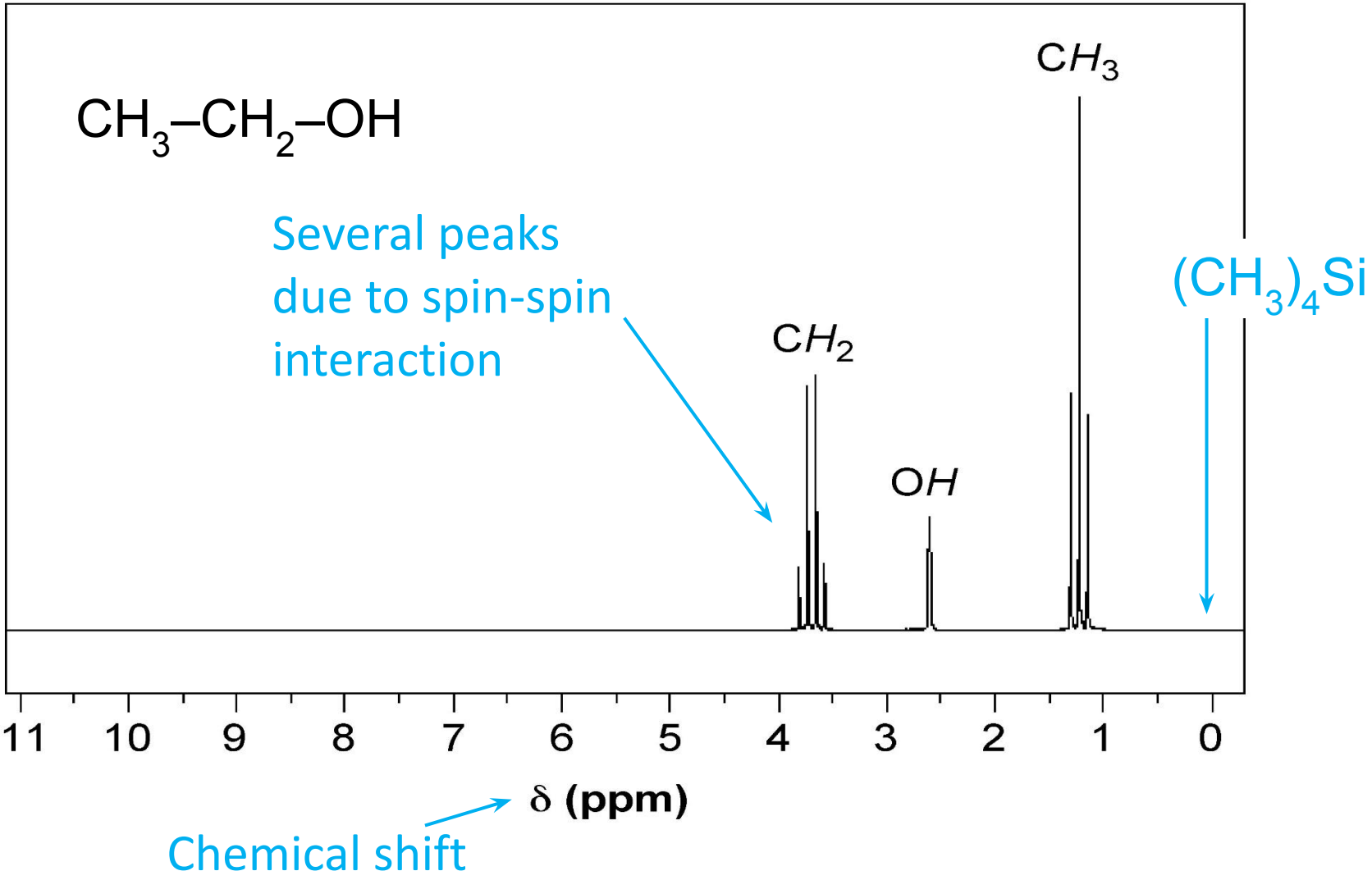
Pulse method to deliver a set of ν and then do

… Good old Fourier !

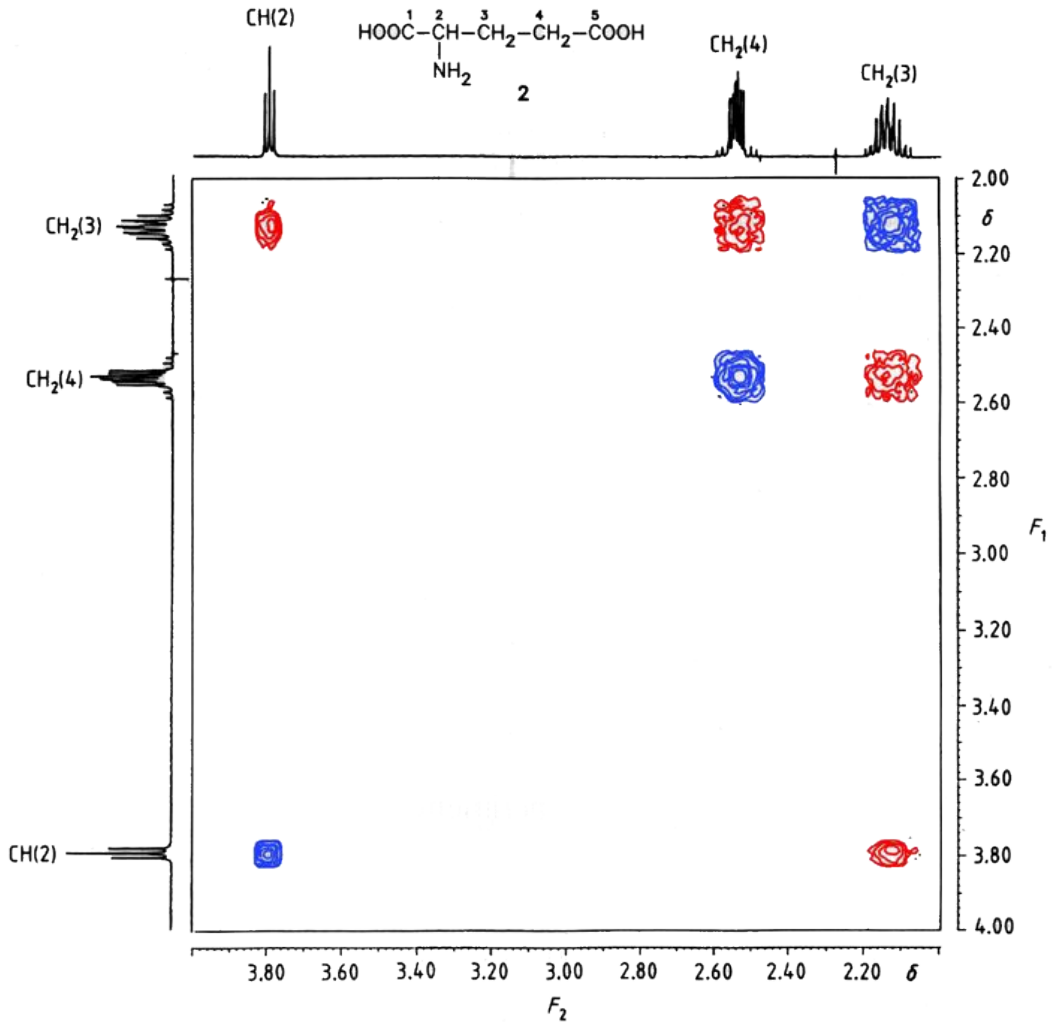




1D ^{1}H-spectrum of ethanol



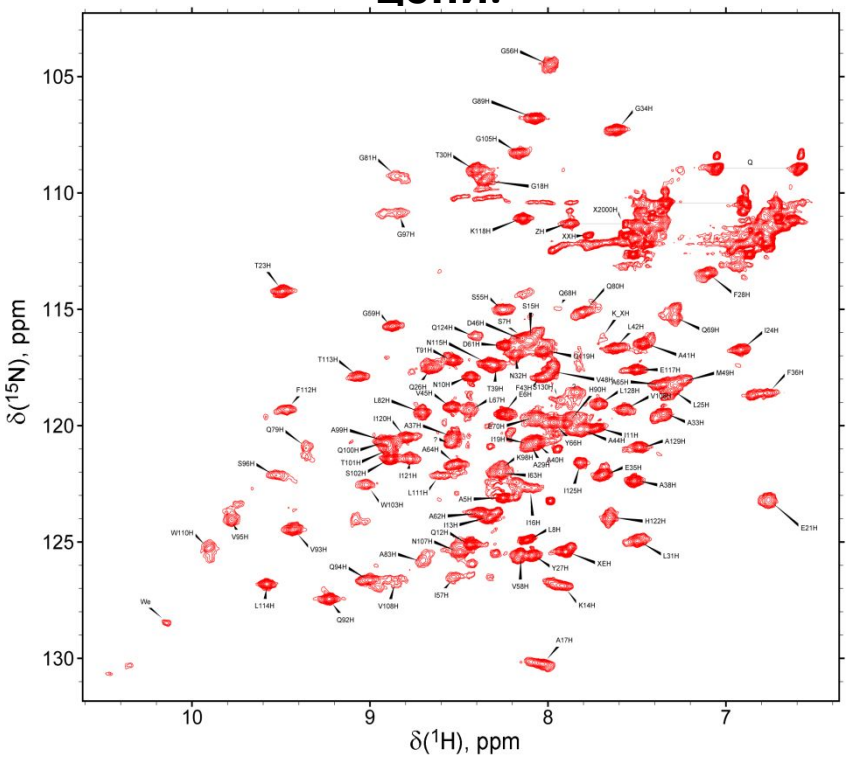
2D spectra



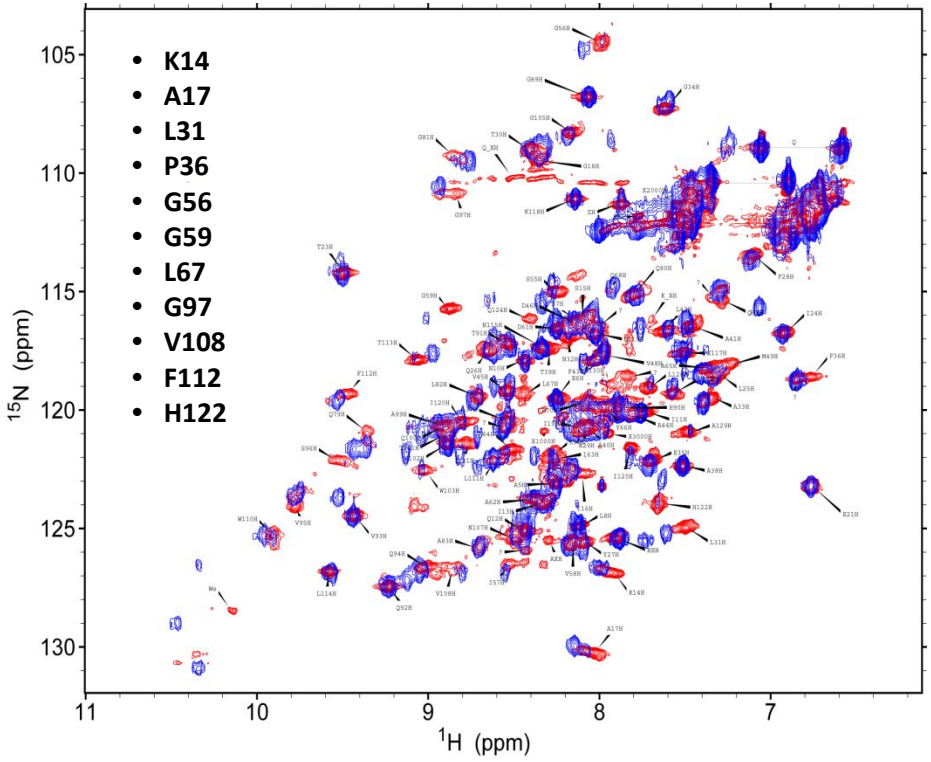
- Series of pulses to cause transitions
- 2D Fourier transformation

Proximal functional groups affect the magnetization of a particular nucleus in the structure

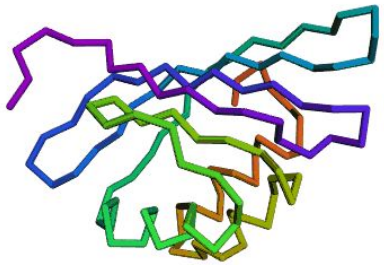
Спектр ^{15}N-^{1}H HSQC apo-CTDH (0.5 mM), при 800 MHz и 35°C. Отнесены сигналы амидных групп белковой цепи.

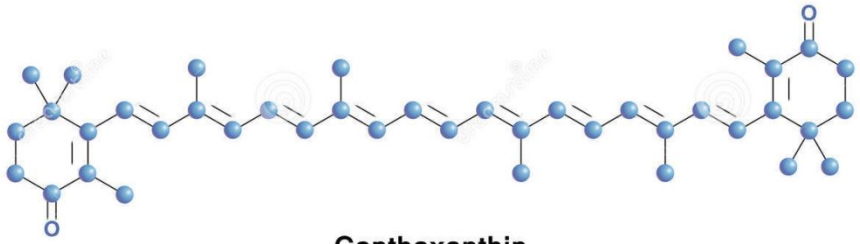


Наложение спектров ^{15}N-^{1}H HSQC apo-CTDH (красные) и CTDH-Canthaxanthin (синие)



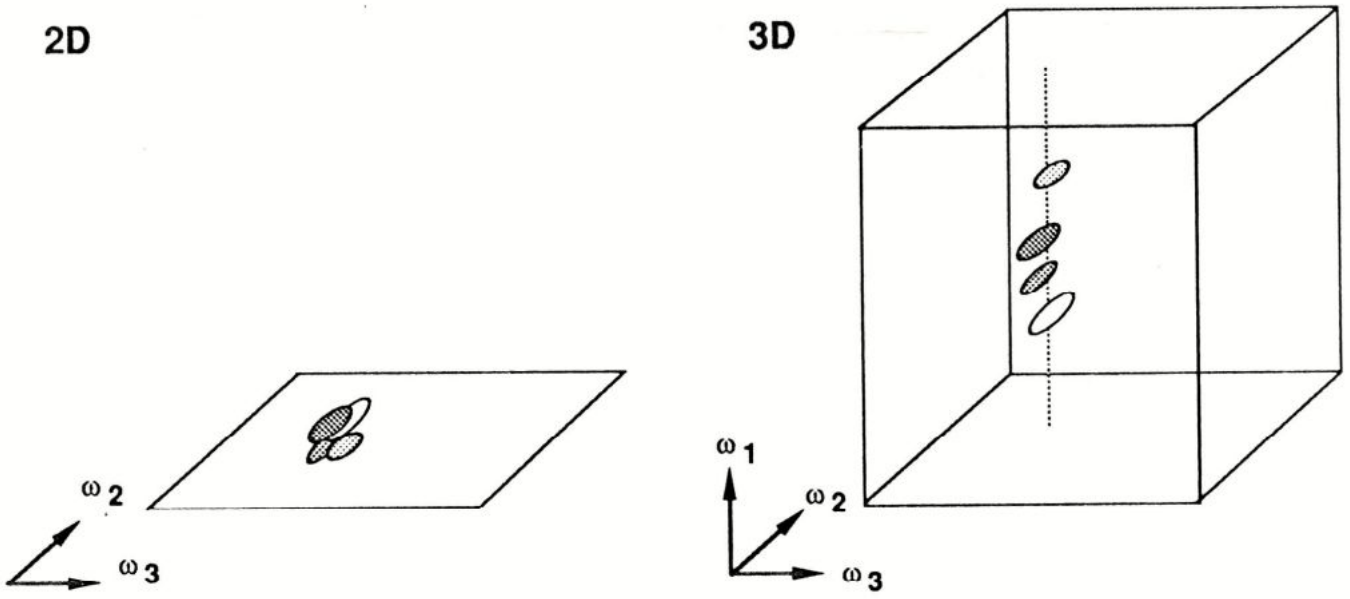
ApoCTDH
6FEJ.pdb





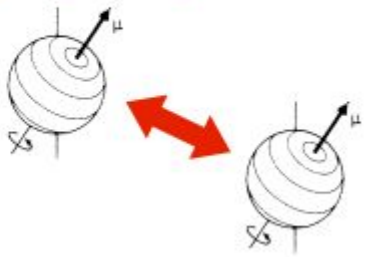
http://pdbflex.org/index.html

Resolution of the peaks is increased upon increasing dimensionality

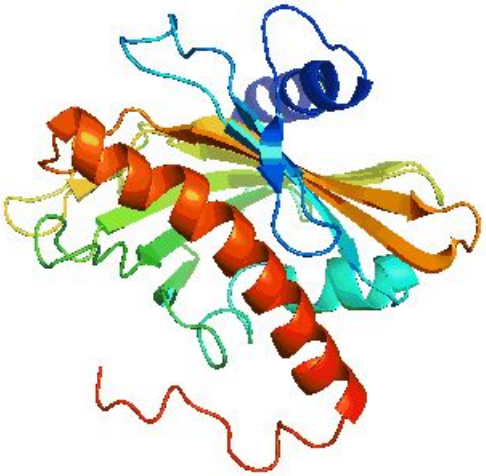


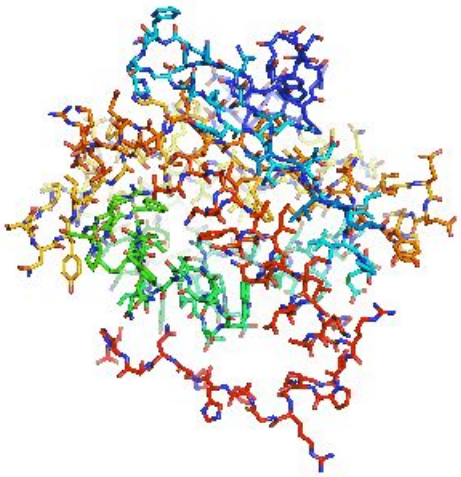
Structural models of small proteins

- **Magnetic dipole interaction (NOE)** dipole-dipole interaction
  - **Nuclear Overhauser Effect**
  - **through space**
  - **distance dependent (1/r6)**
  - **NOESY -> distance restraints**
    - **Distances between neighboring atoms**
    - **Angles ψ and φ of the polypeptide chain**

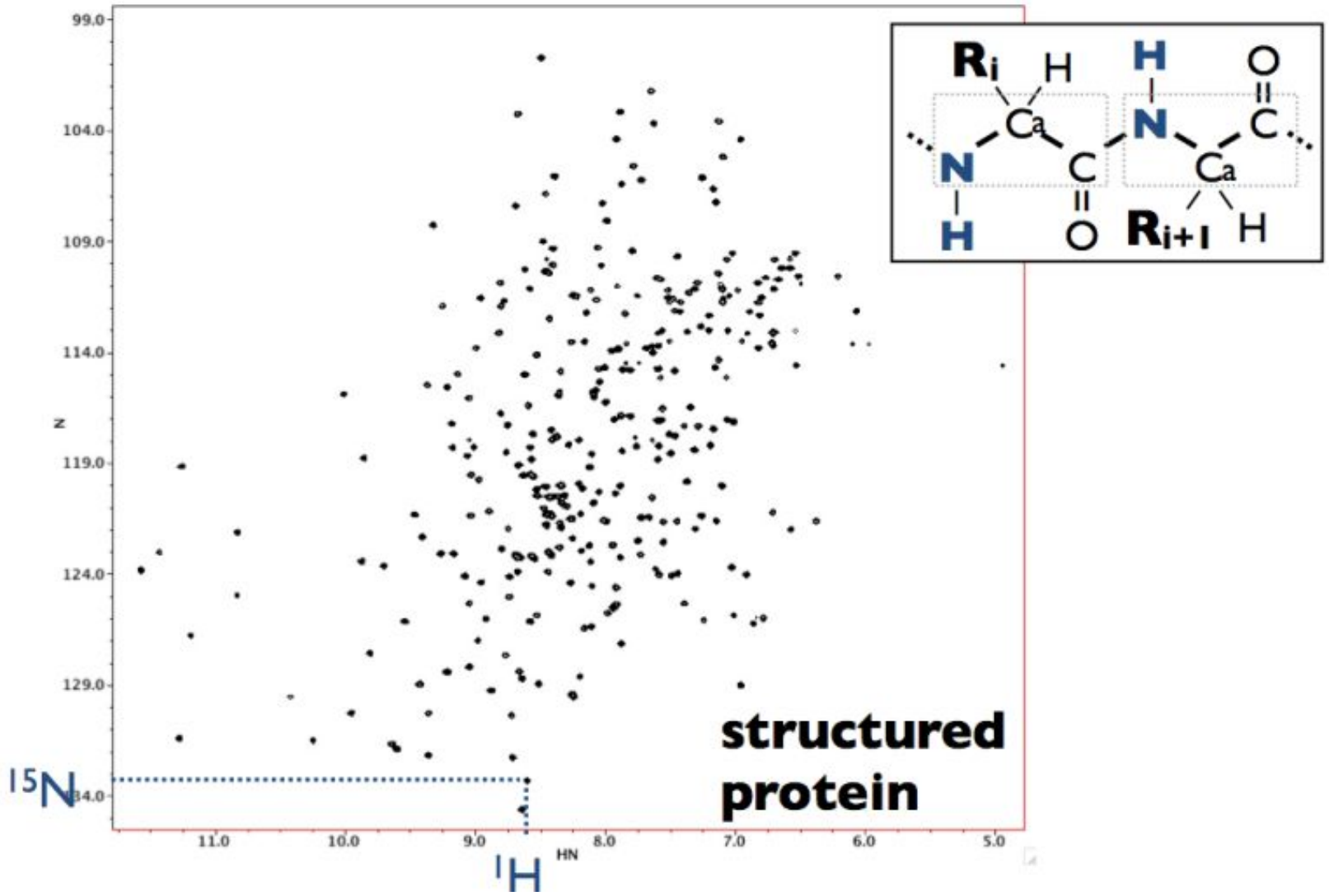


2MOU.pdb
STARD6
20 structures

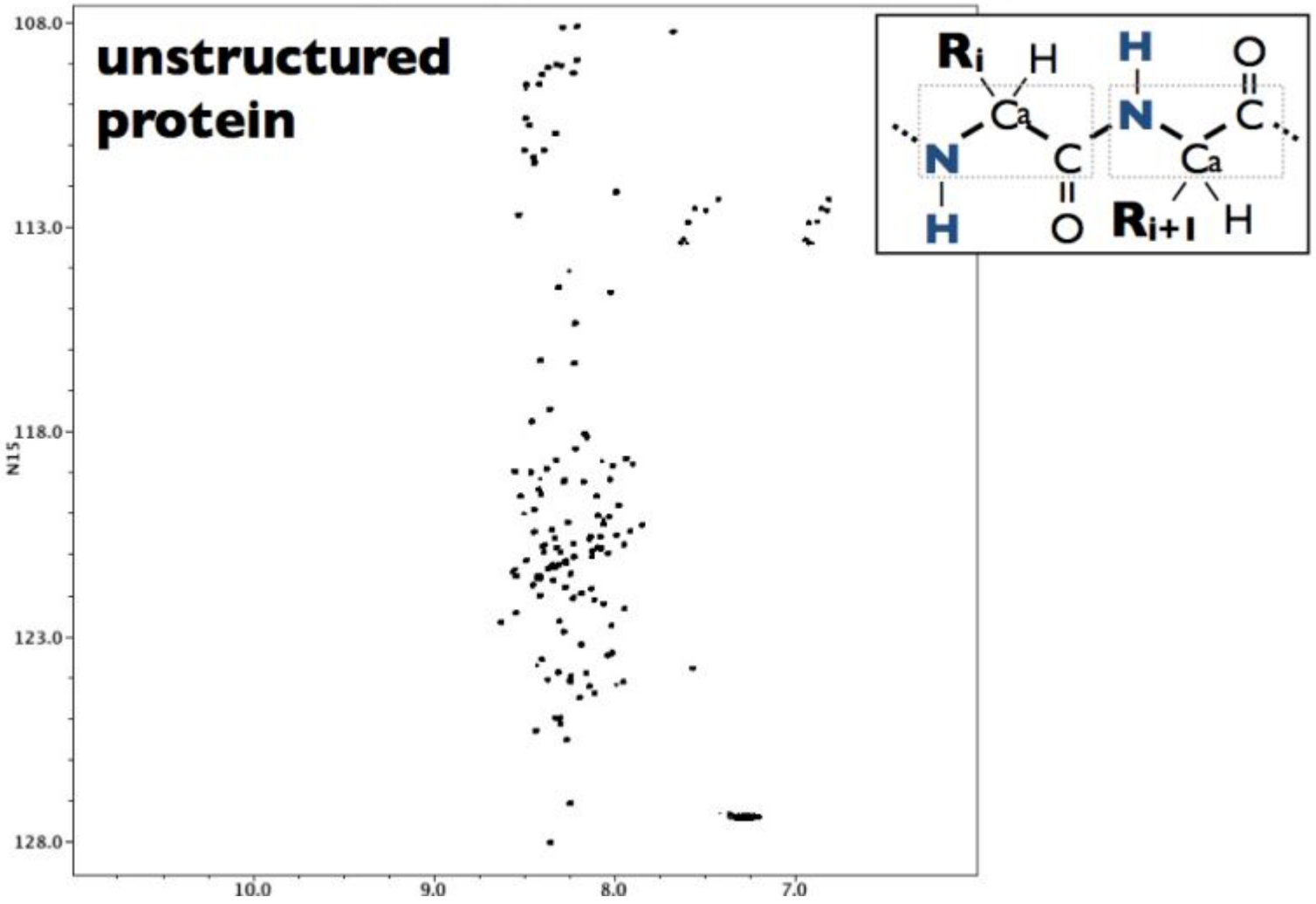




NMR tackles both structured proteins and IDPs



NMR tackles both structured proteins and IDPs



i-Tasser. Protein structure prediction

Zhang Lab

University of Michigan

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Online Services
- I-TASSER
- QUARK
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- COFACTOR
- MetaGO
- MUSTER
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- FG-MD
- ModRefiner
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- SPRING
- COTH
- BSpred
- SVMSEQ
- ANGLOR
- BSP-SLIM
- SAXSTER
- ThreaDom
- ThreaDomEx
- EvoDesign
- GPCR-I-TASSER
- BindProf
- BindProfX
- ResQ
- IonCom
- STRUM
- DAMpred
- TM-score

I-TASSER
Protein Structure & Function Predictions

(The server completed predictions for 464695 proteins submitted by 110805 users from 144 countries)
(The template library was updated on 2019/05/03)

I-TASSER (Iterative Threading ASSEmbly Refinement) is a hierarchical approach to protein structure and function prediction. It first identifies structural templates from the PDB by multiple threading approach LOMETS, with full-length atomic models constructed by iterative template-based fragment assembly simulations. Function insights of the target are then derived by threading the 3D models through protein function database BioLiP. I-TASSER (as 'Zhang-Server') was ranked as the No 1 server for protein structure prediction in recent community-wide CASP7, CASP8, CASP9, CASP10, CASP11, CASP12, and CASP13 experiments. It was also ranked as the best for function prediction in CASP9. The server is in active development with the goal to provide the most accurate structural and function predictions using state-of-the-art algorithms. Please report problems and questions at I-TASSER message board and our developers will study and answer the questions accordingly. (>> More about the server ...)

[Queue] [Forum] [Download] [Search] [Registration] [Statistics] [Remove] [Potential] [Decoys] [News] [Annotation] [About] [FAQ]

I-TASSER On-line Server (View an example of I-TASSER output):

Copy and paste your sequence below ([10, 1500] residues in FASTA format). Click here for a sample input:

FASTA format of sequence

Or upload the sequence from your local computer:
Выберите файл Файл не выбран

Email: (mandatory, where results will be sent to)
nikolai.sluchanko@mail.ru

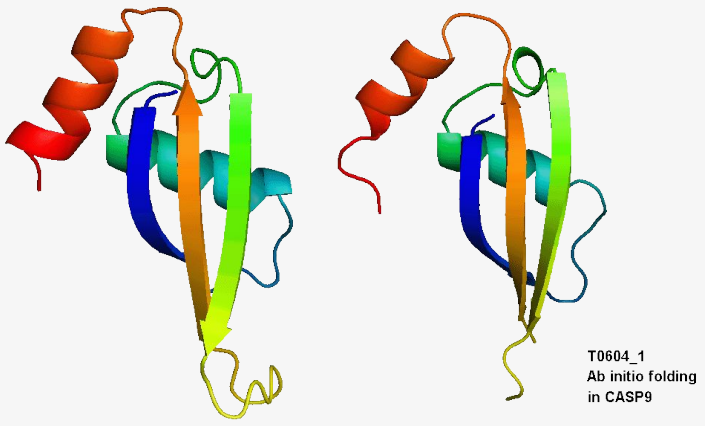
Password: (mandatory, please click here if you do not have a password)
••••••••

ID: (optional, your given name of the protein)

Option I: Assign additional restraints & templates to guide I-TASSER modeling.

Option II: Exclude some templates from I-TASSER template library.

Option III: Specify secondary structure for specific residues.



Predicted model (2.66Å) X-ray structure

https://zhanglab.ccmb.med.umich.edu/I-TASSER/

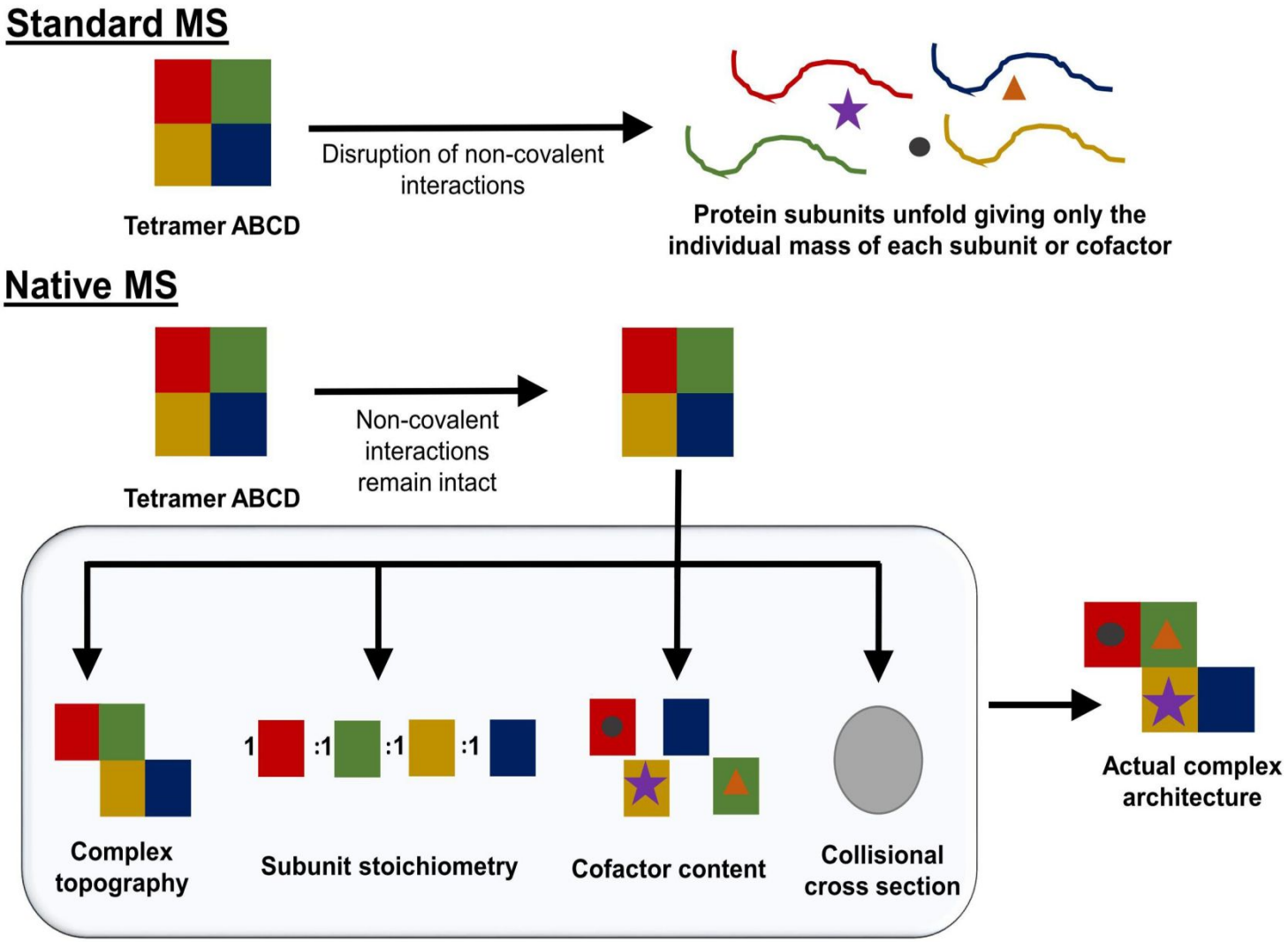
Comparison of different structural techniques

Method	Advantages	Disadvantages	Objects	Resolution
X-ray crystallography	High resolution, Well-developed, Any size, Now accessible, Software available	Crystallization is a challenge, diffraction is not promised, static crystalline state structure	Crystallizable samples, high purity and concentration achievable, almost any size	high
Solution NMR	High reso, 3D structure in solution (native state?), Good for dynamic studies, Non-crystallizable proteins, IDPs	Highest purity of the sample, isotope labeling, rather small proteins, interpretation of data is very challenging	Mw <40-50 kDa, water soluble, soluble at high concentration, must be very stable (days-weeks!). Isotopes 15N, 13C and 2H	high
Single particle Cryo-EM	Easy sample preparation, small sample consumption, structure in the frozen native state, different conformations	Relatively low resolution, only high Mw samples, highly dependent on EM facilities and operators, costly equipment, not readily accessible	Proteins and their complexes >150 kDa	Low-Moderate-High
SAXS	In solution, moderate sample consumption, complexes and conformational heterogeneity, IDPs	Low resolution, complementary structural method only, high ambiguity of the models requires additional data	Protein samples and their complexes of almost any size (not aggregated). Purity and monodispersity determine the quality of the data	Low

Integrated approaches in structural biology

- X-ray crystallography
- SAXS
- NMR
- CryoEM
- Auxillary techniques: fluorescence resonanse energy transfer (FRET), limited proteolysis, native-MS, crosslinking, HDX, molecular dynamics and computational biology

Native-MS

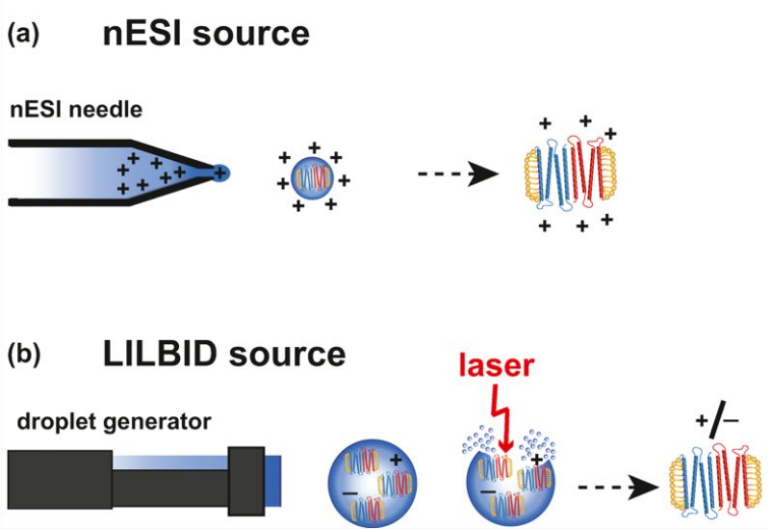


https://doi.org/10.3389/fmicb.2018.01397

Native-MS

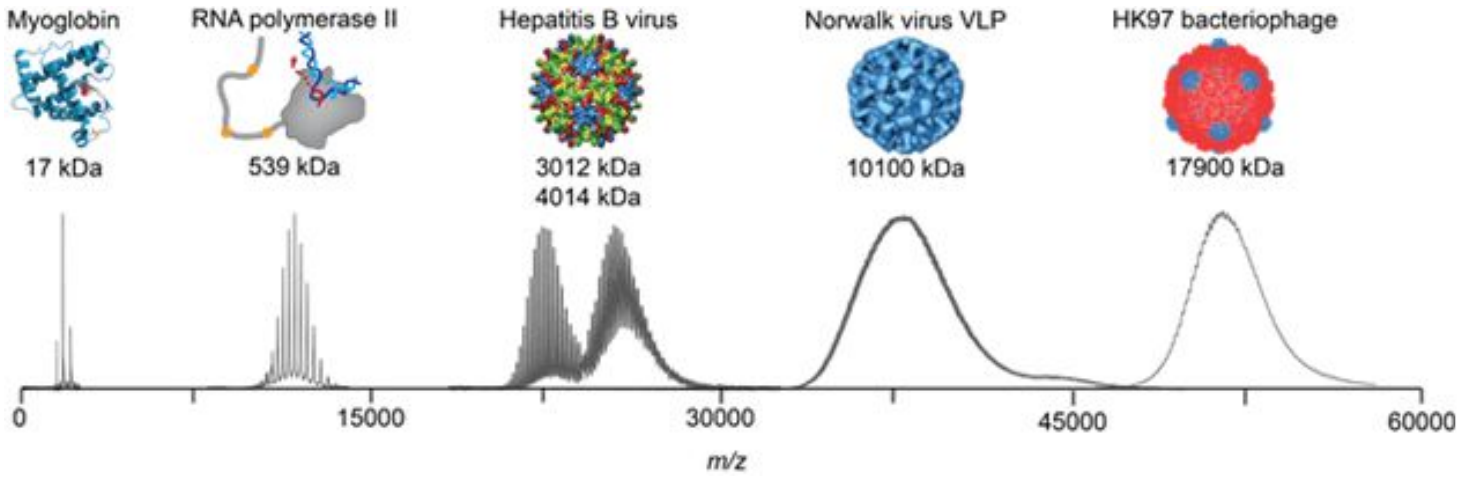
https://doi.org/10.1007/s13361-018-2061-4
https://www.nature.com/articles/nmeth.1265

https://www.pnas.org/content/116/4/1116
DOI: 10.1007/978-1-4939-7151-0_11



highly charged complexes

no additional charges



Hydrogen/deuterium exchange mass-spectrometry

https://doi.org/10.1016/j.sbi.2019.06.007

https://onlinelibrary.wiley.com/doi/abs/10.1002/pro.3790

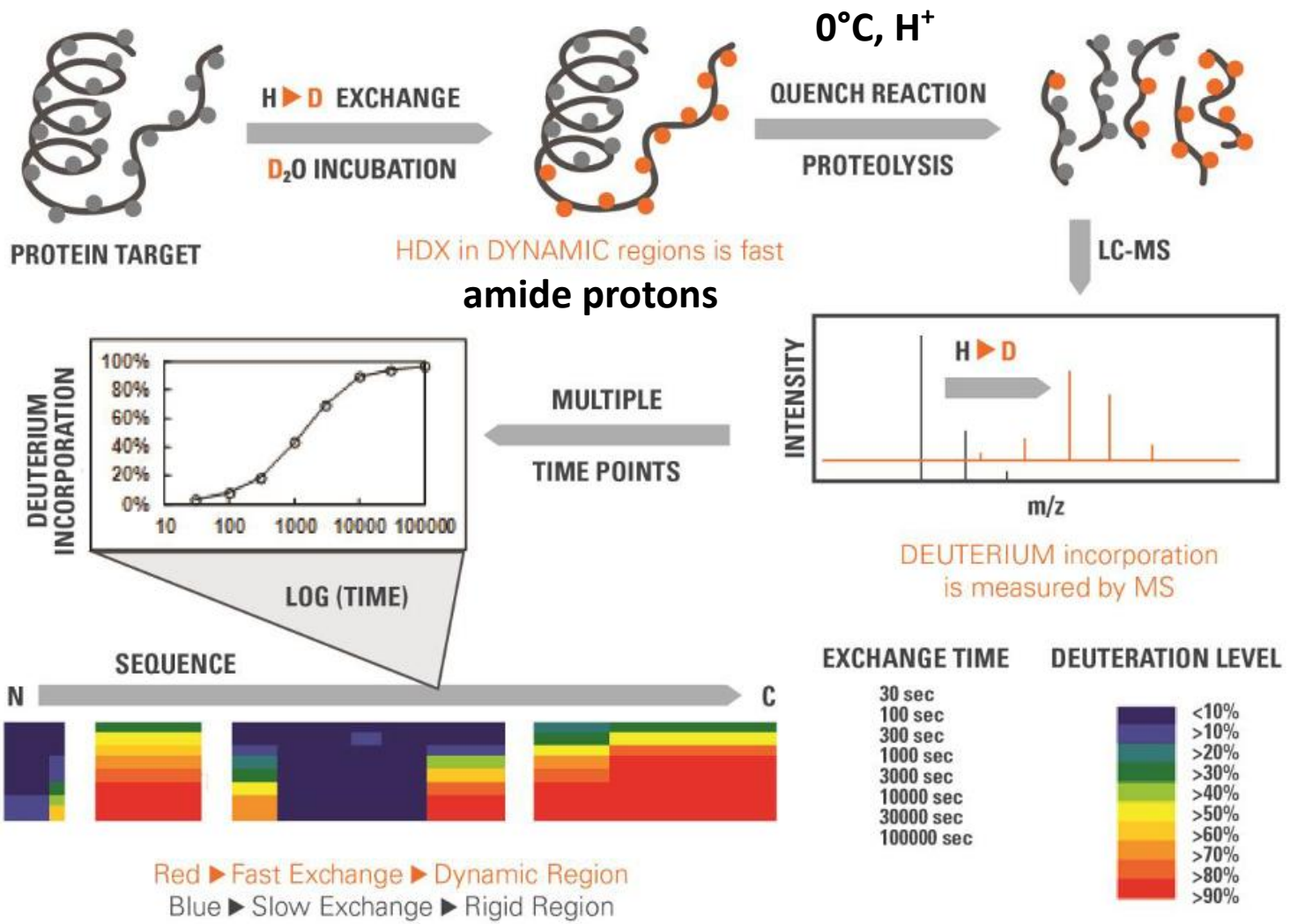


Figure 1 – HDX-MS method overview.

Yoshitomo Hamuro ©

nature structural & molecular biology

ARTICLES

https://doi.org/10.1038/s41594-019-0332-9

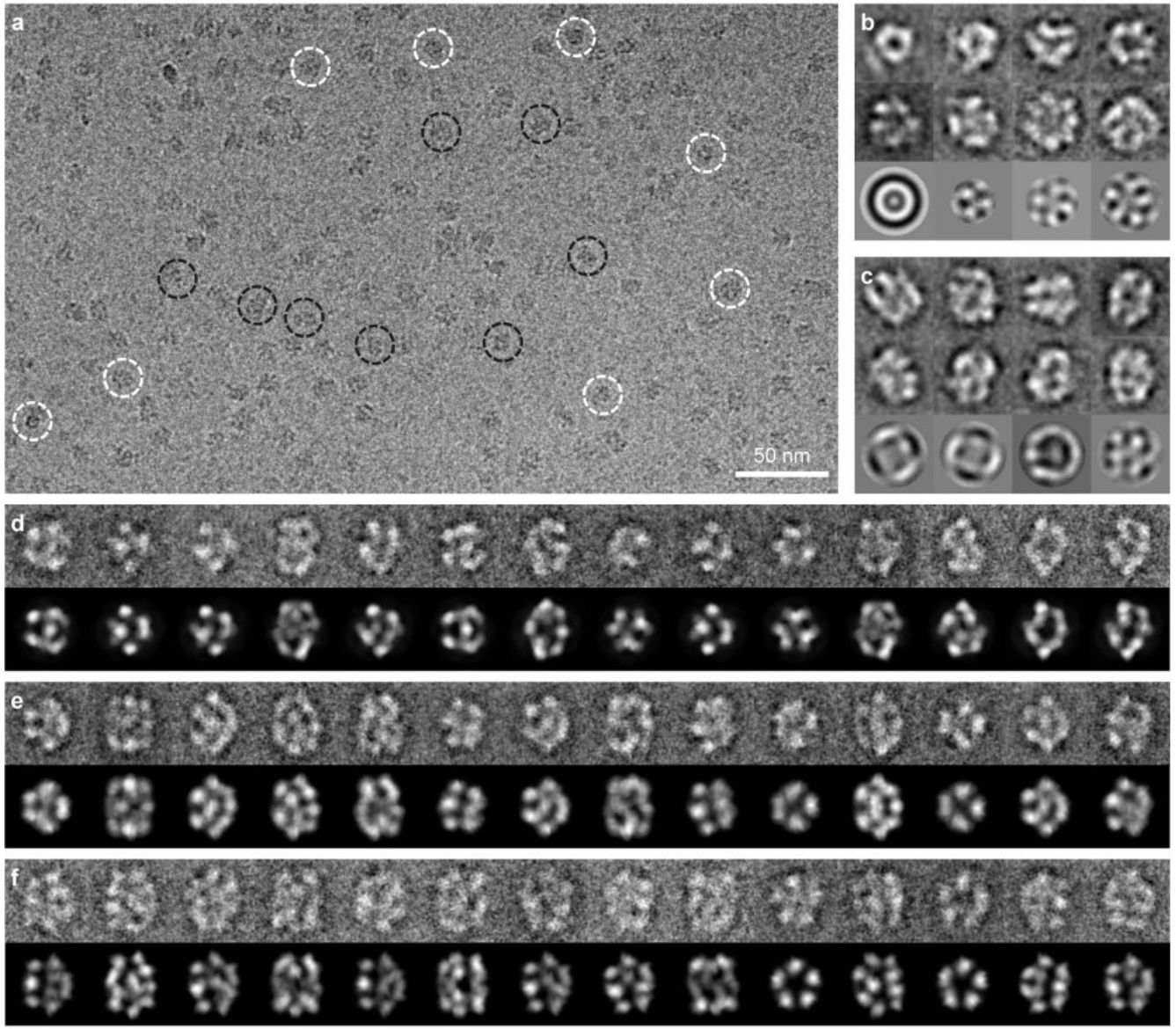
The structure and oxidation of the eye lens chaperone αA-crystallin

Christoph J. O. Kaiser[1,7], Carsten Peters[1,7], Philipp W. N. Schmid[1], Maria Stavropoulou[1,2], Juan Zou[3], Vinay Dahiya[1], Evgeny V. Mymrikov[1,6], Beate Rockel[1], Sam Asami[1,2], Martin Haslbeck[1], Juri Rappsilber[3,4], Bernd Reif[1,2], Martin Zacharias[5], Johannes Buchner[1*] and Sevil Weinkauf[1*]

Pseudoatomic models built by a combination of:

- Single particle Cryo-EM
- Crosslinking MS
- HDX MS
- Modelling

Cryo-EM micrograph of human alphaA-crystallin



12-mer

16-mer

20-mer

Cryo-EM 3D reconstructions of human αA-crystallin (reduced) oligomers

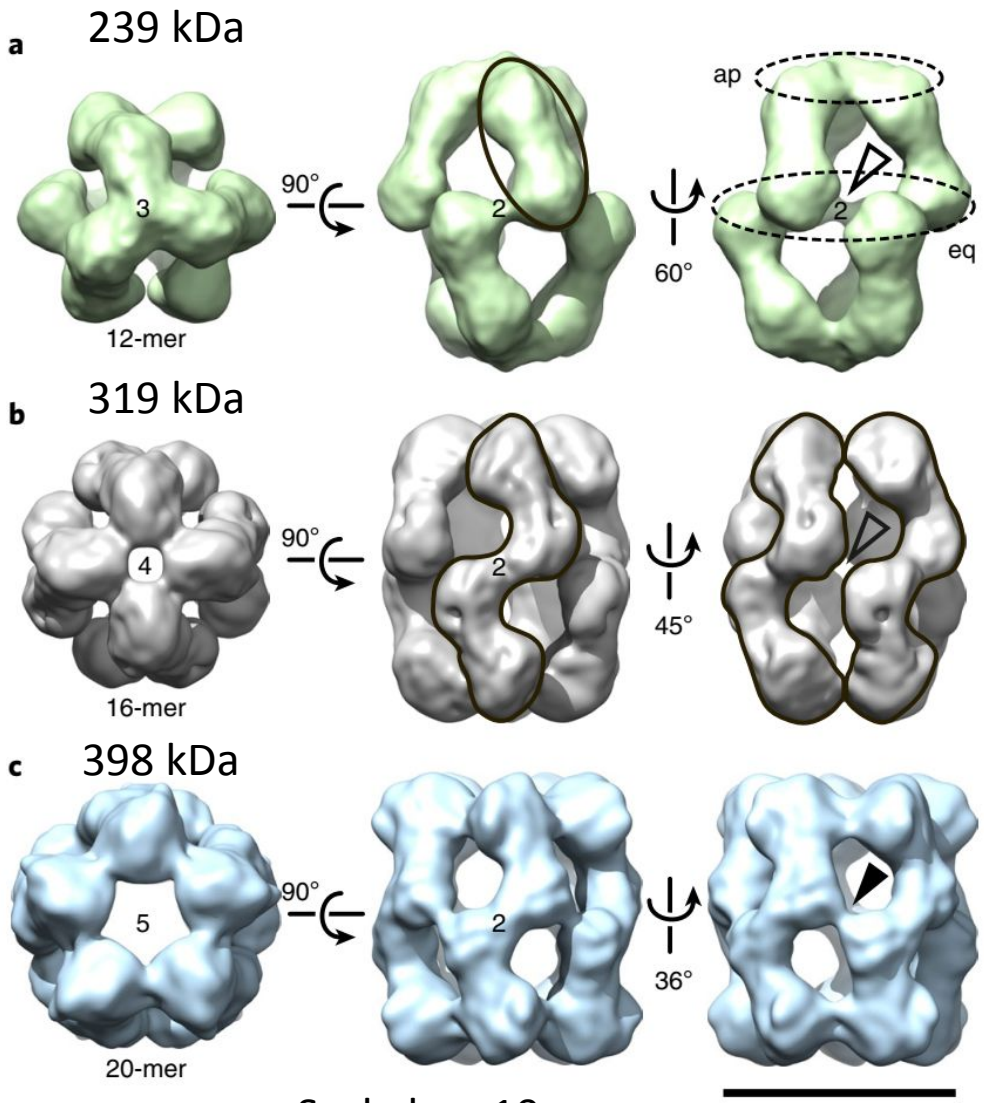


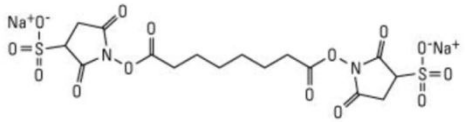
Table 1 | Cryo-EM data collection and validation statistics for αA-crystallin oligomer reconstructions

	12-mer (*D3*) (EMD-4895)	16-mer (*D4*) (EMD-4894, PDB 6T1R)	20-mer (*D5*) (EMD-4896)
Data collection and processing			
Molecular mass (kDa)	238.9	318.5	398.2
Magnification	37,000	37,000	37,000
Voltage (kV)	300	300	300
Electron exposure (e^{-} Å^{-2})	30	30	30
Defocus range (μm)	1.2–2.5	1.2–2.5	1.2–2.5
Pixel size (Å)	1.35	1.35	1.35
Symmetry imposed	*D3*	*D4*	*D5*
Initial particle images (no.)	74,068	74,068	74,068
Final particle images (no.)	26,596	19,783	14,336
Relative abundance (%)[a]	35.9	26.7	19.4
Map resolution (Å)	9.2	9.8	9.0
FSC threshold	0.143	0.143	0.143
Dimensions (width × height, in Å)	10.8 × 13.6	10.9 × 13.8	12.0 × 13.7
Validation			
MolProbity score	-	2.23	-
Clashscore	-	17	-
Poor rotamers (%)	-	0	-
Ramachandran plot			
Favored (%)	-	92	-
Allowed (%)	-	8	-
Disallowed (%)	-	1	-

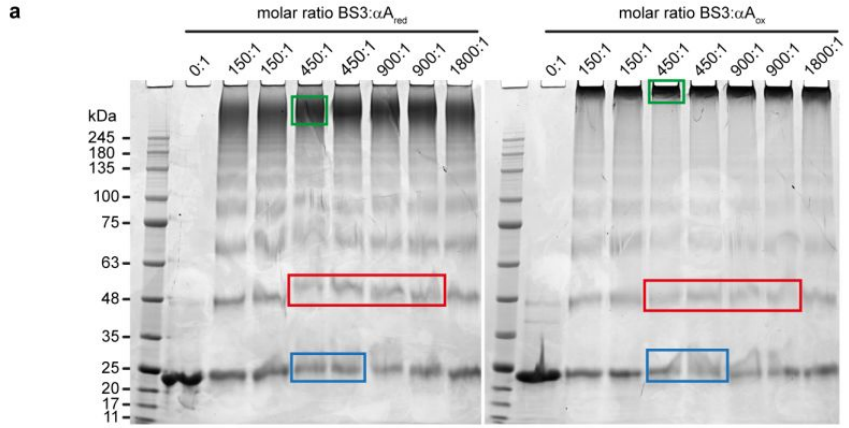
[a]Relative abundance with respect to the total number of images in the initial cryo-EM dataset.

Crosslinking by BS3 and MS

bis(sulfosuccinimidyl)suberate (BS3)



11.4A

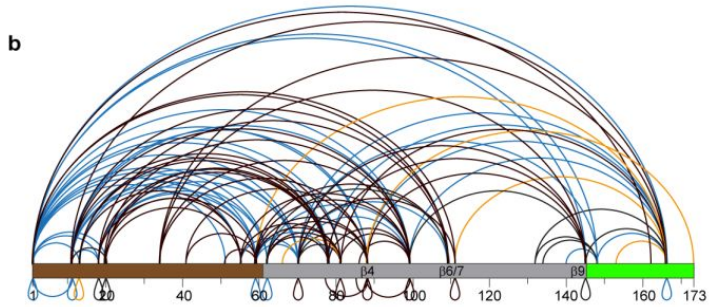


a

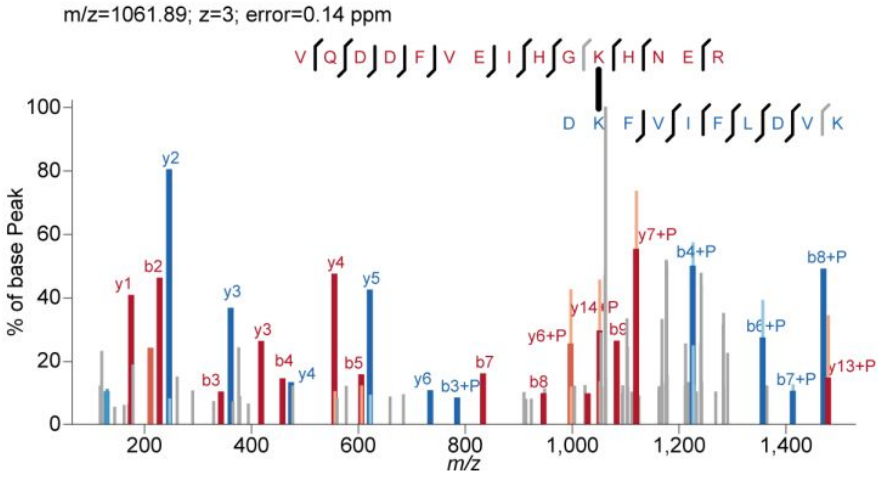
MDVTIQHPWF KRTLGPFYPS RLFDQFFGEG LFEYDLLPFL SSTISPYYRQ SLFRTVLDSG ISEVRSDRDK FVIFLDVKHF

SPEDLTVKVQ DDFVEIHGKH NERQDDHGYI SREFHRRYRL PSNVDQSALS CSLSADGMLT FCGPKIQTGL DATHAERAIP

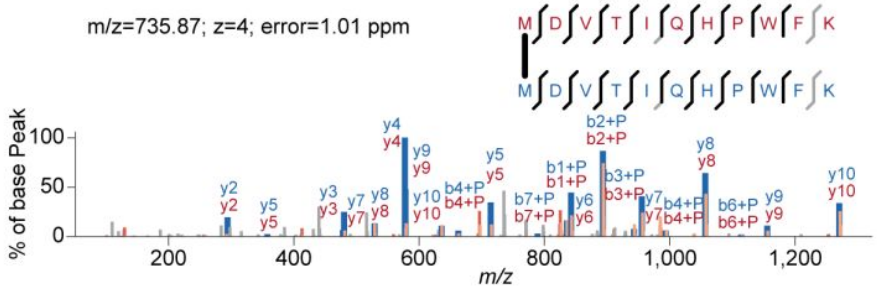
VSREEKPTSA PSS



Fragmentation spectrum of a cross-linked peptide with an intramolecular link between K70 and K99



Fragmentation spectrum of a cross-linked peptide with an intermolecular cross-link between M1 and M1



Pseudoatomic model of the 16-mer

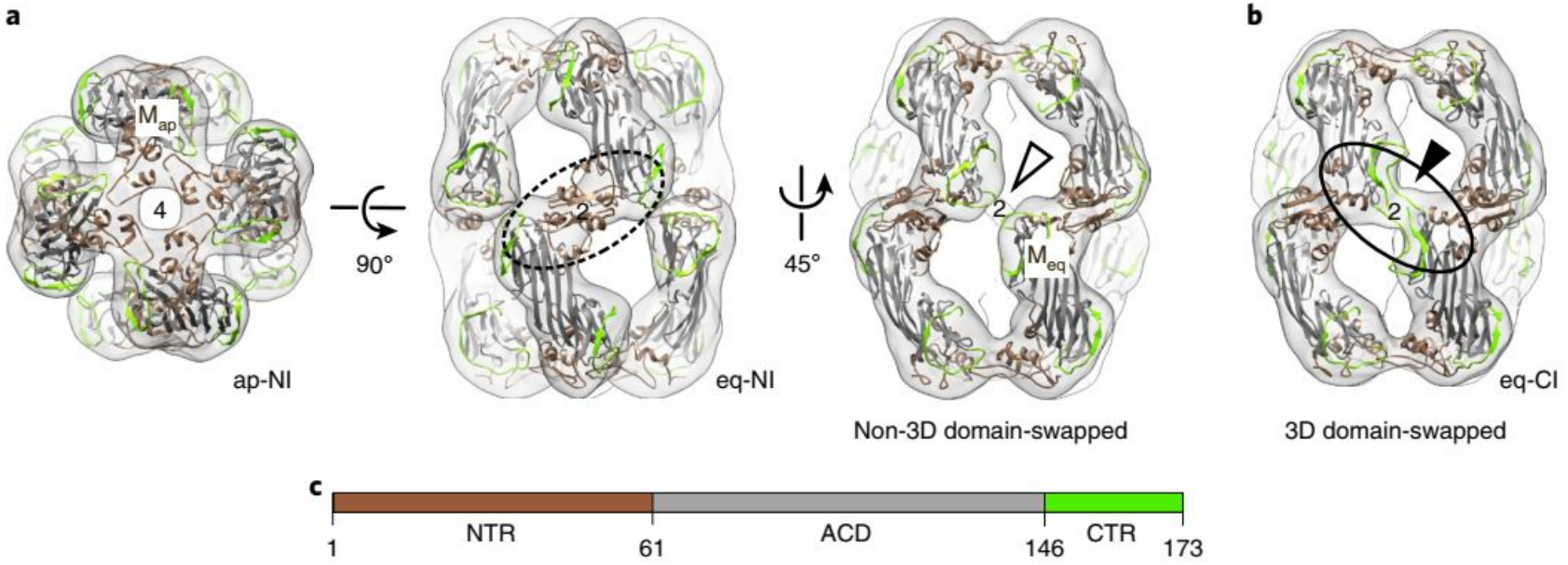
Modelling by molecular dynamics flexible fitting was based on:

-shape, symmetry and low-resolution features from 9-10 Å resolution Cryo-EM maps

-crystal structures of truncated versions (domains)

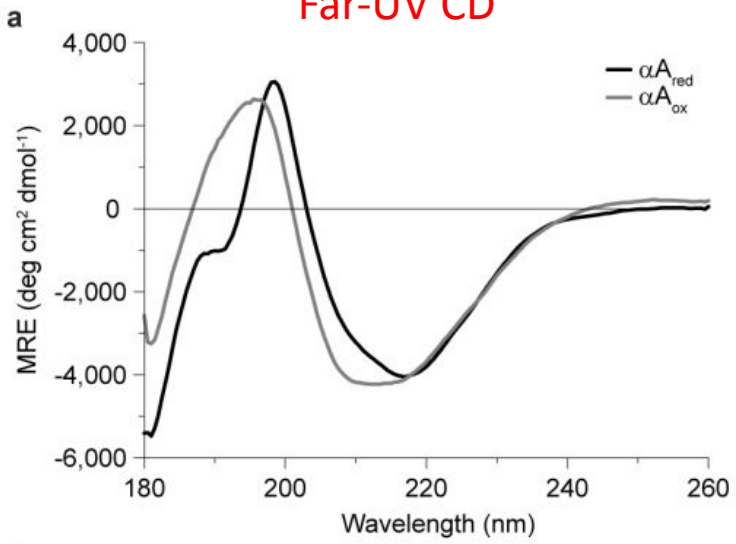
-crosslinking MS data (pairs of residues located within certain distance)

-stereochemistry restraints

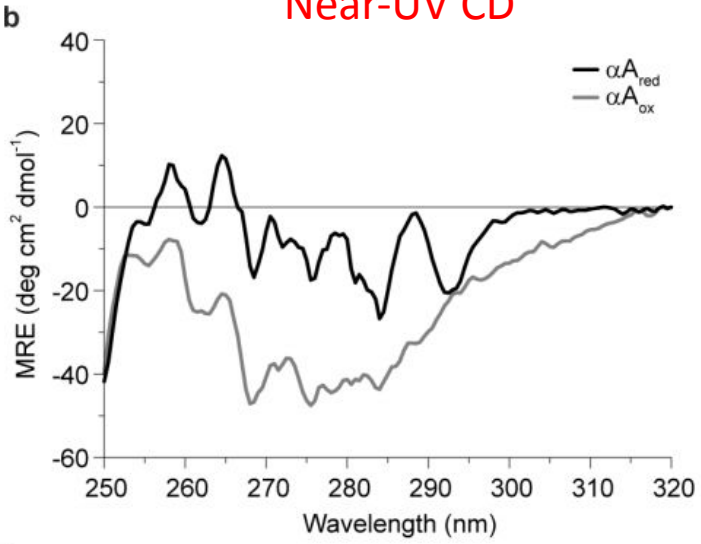


Effect of alphaA-crystallin oxidation

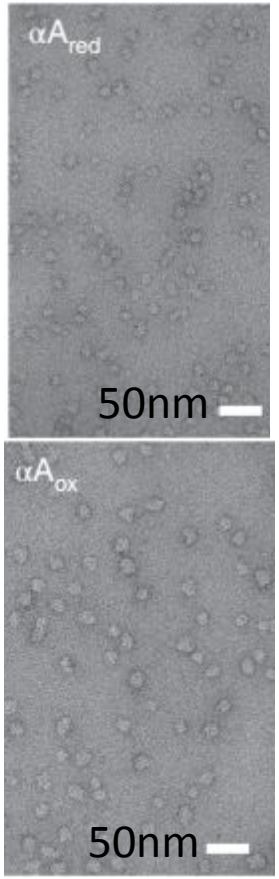
Far-UV CD

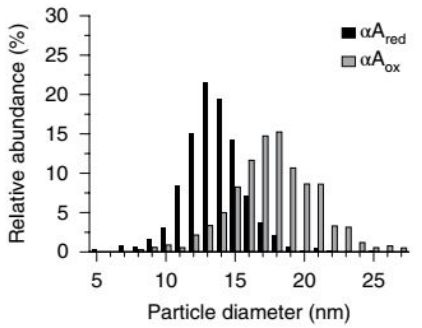


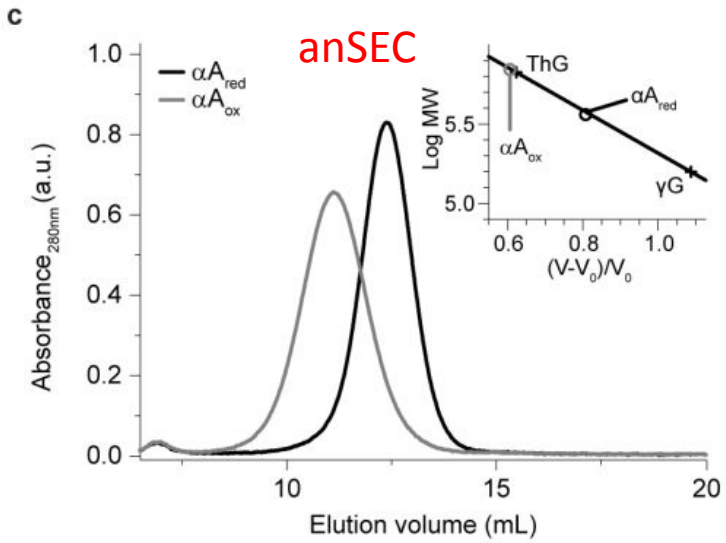
Near-UV CD

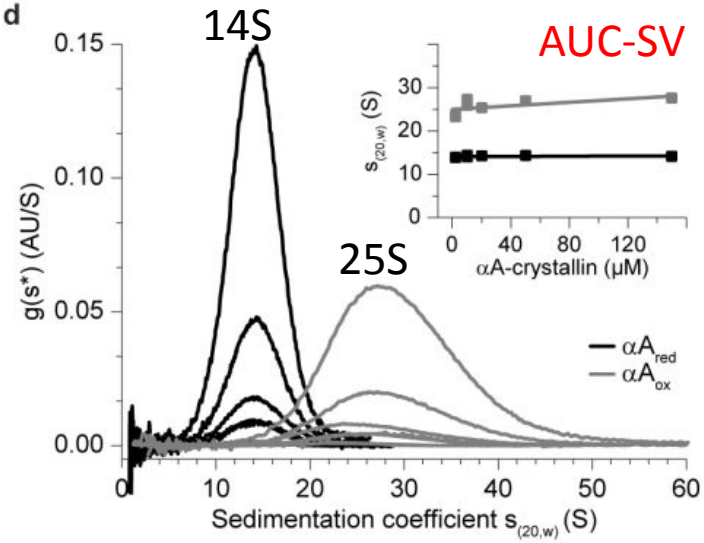


Negative stain TEM



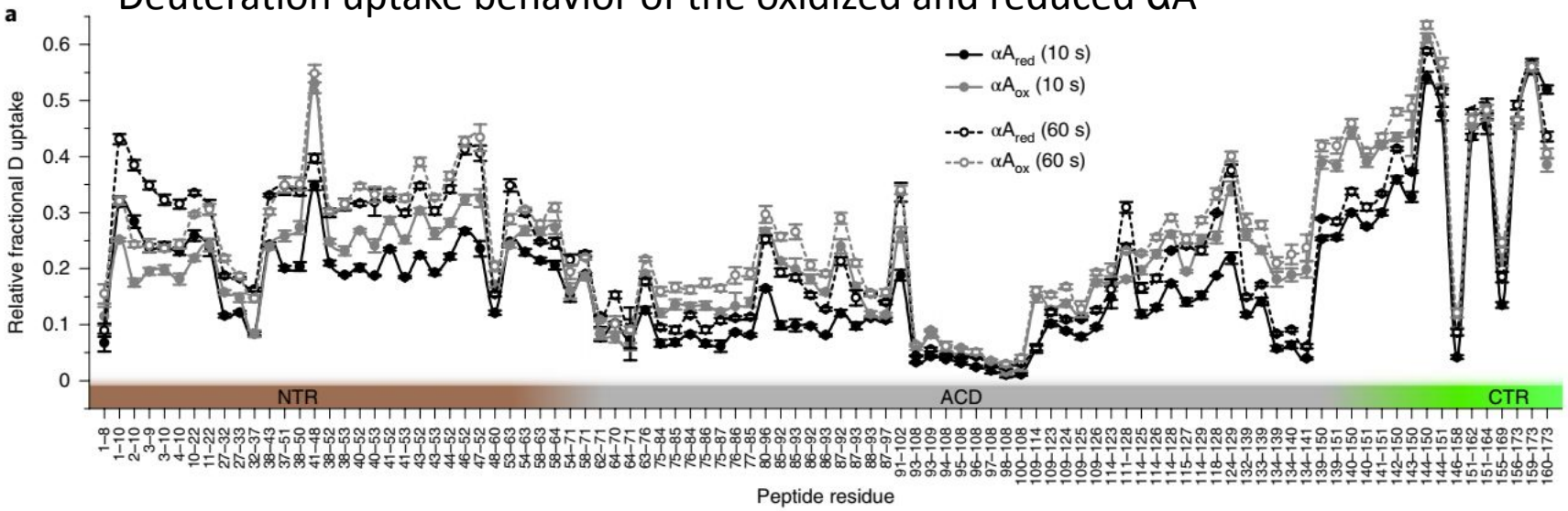






HDX-MS shows incresed local structural dynamics of alphaA-crystallin

Deuteration uptake behavior of the oxidized and reduced αA



Difference in local relative deuterium uptake (ΔD uptake αAox − αAred)