

Small angle scattering for large problems: how to fight diseases with X-Rays

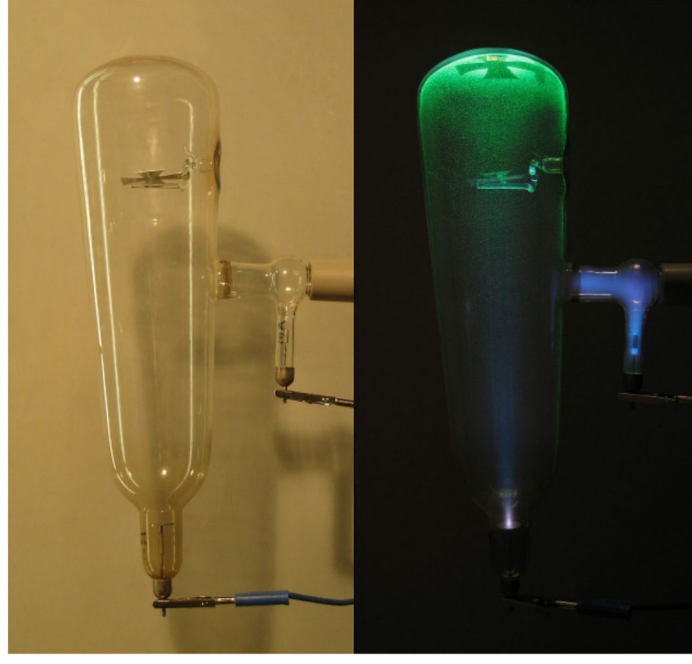
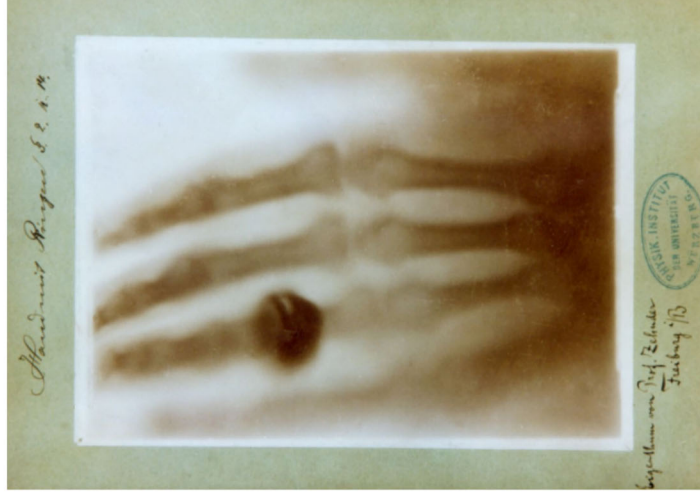
Andrey Gruzinov

7th EIROforum School on instrumentation
7-11 June 2021



X-Rays – The Beginning

W.C. Röntgen

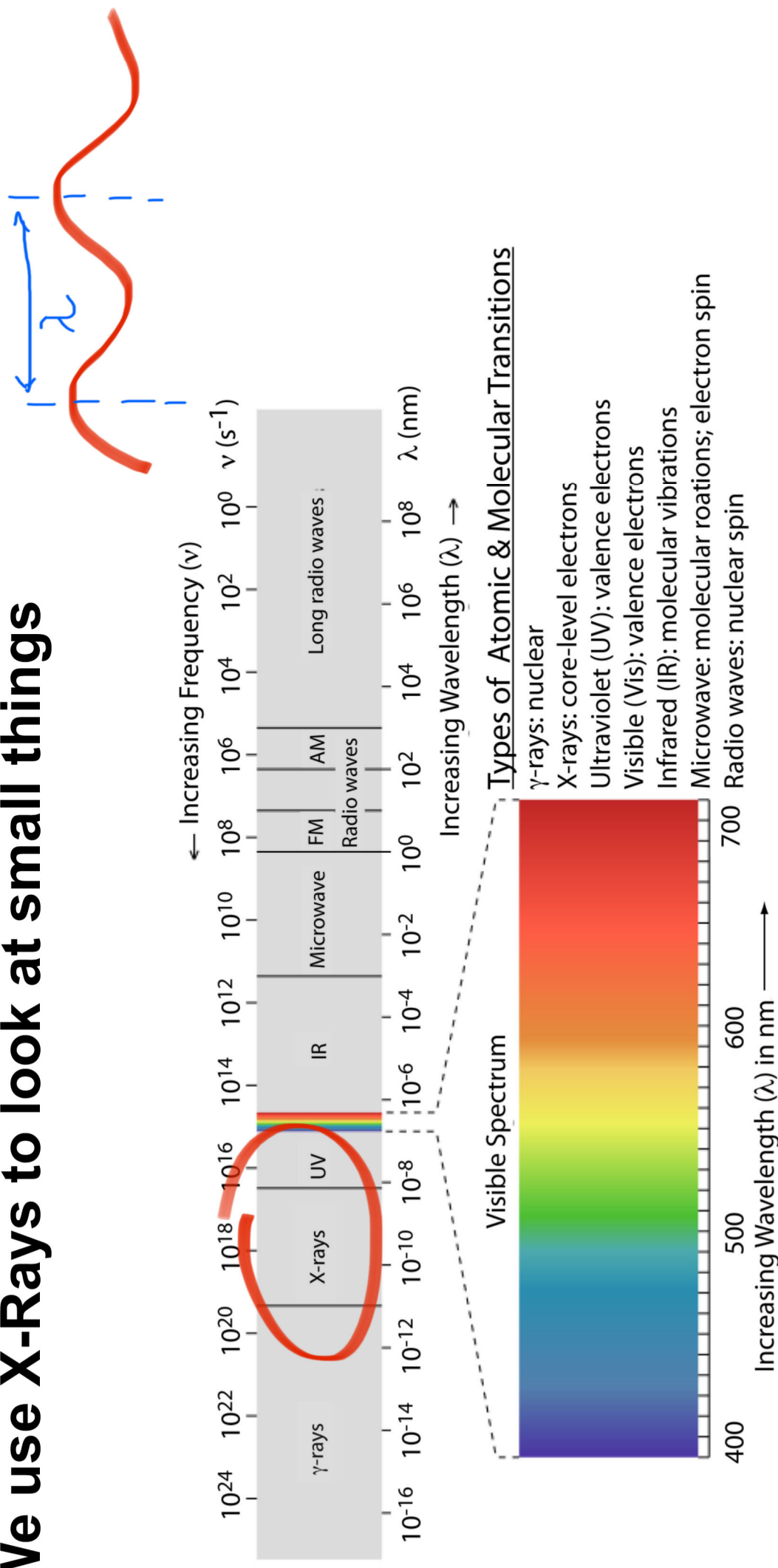


Anna Röntgen's left hand 1895-1896

This Photo by Unknown Author is licensed under CC BY-SA

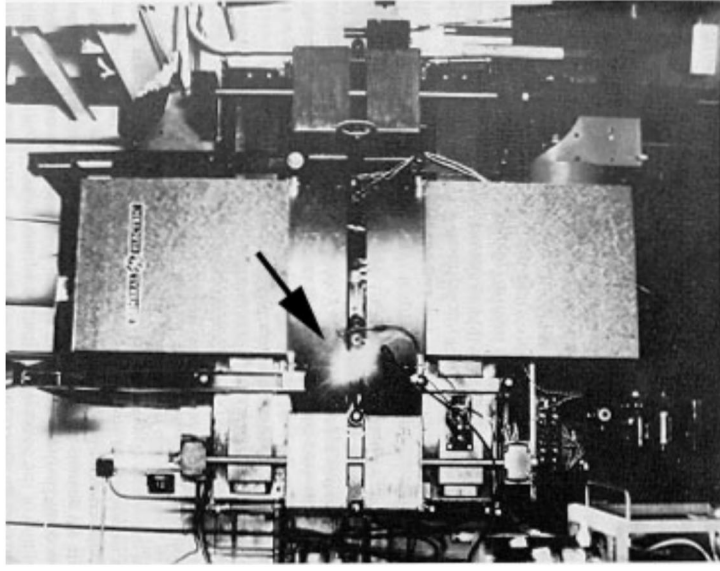
<https://www.spektrum.de/news/entdeckung-der-roentgenstrahlen/1715798>

We use X-Rays to look at small things



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X-Rays as a by-product of Accelerator physics



11
12/12
13/12
14/12

1957



PETRA III



PETRA III





Part one: Structural Biology

The mantra of structural biologists

STRUCTURE



FUNCTION

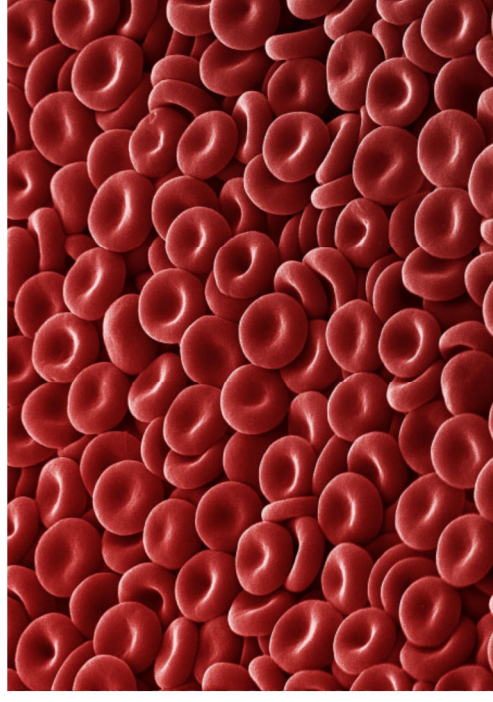


Flight feather

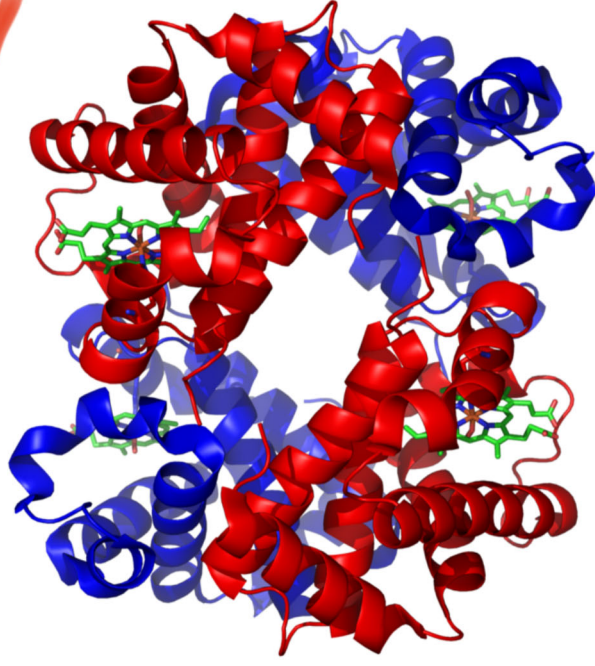


Down feather

The mantra of structural biologists



STRUCTURE

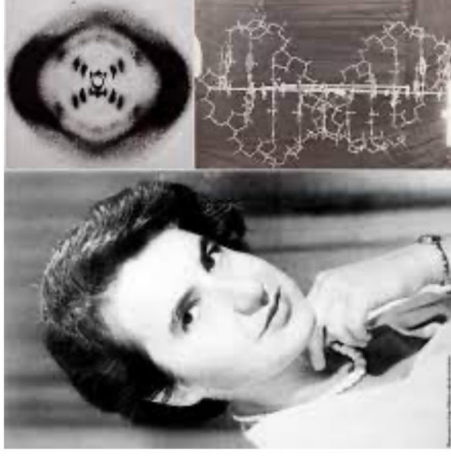


FUNCTION

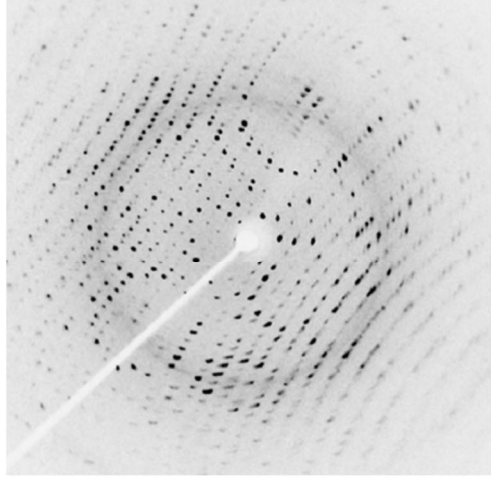


X-Rays – A bit further along the road

The structure of DNA



The structure of proteins



PDB 1GZX

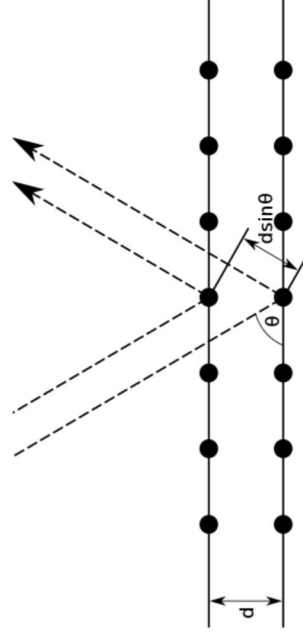
X-Rays – Crystallography



1912

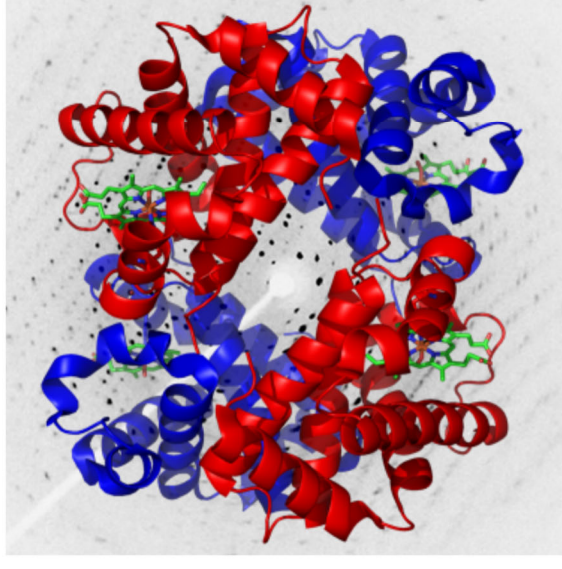
Bragg's Law

$$2d \sin \theta = n\lambda$$



This I

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X-Rays – (Biological) Small-angle scattering

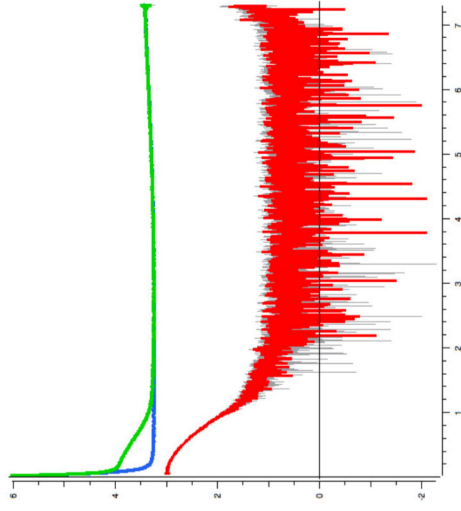


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However: amorphous solids, alloys can be also studied with SAXS.

Small-angle scattering experiment





<https://biosaxs.com/technique.html>

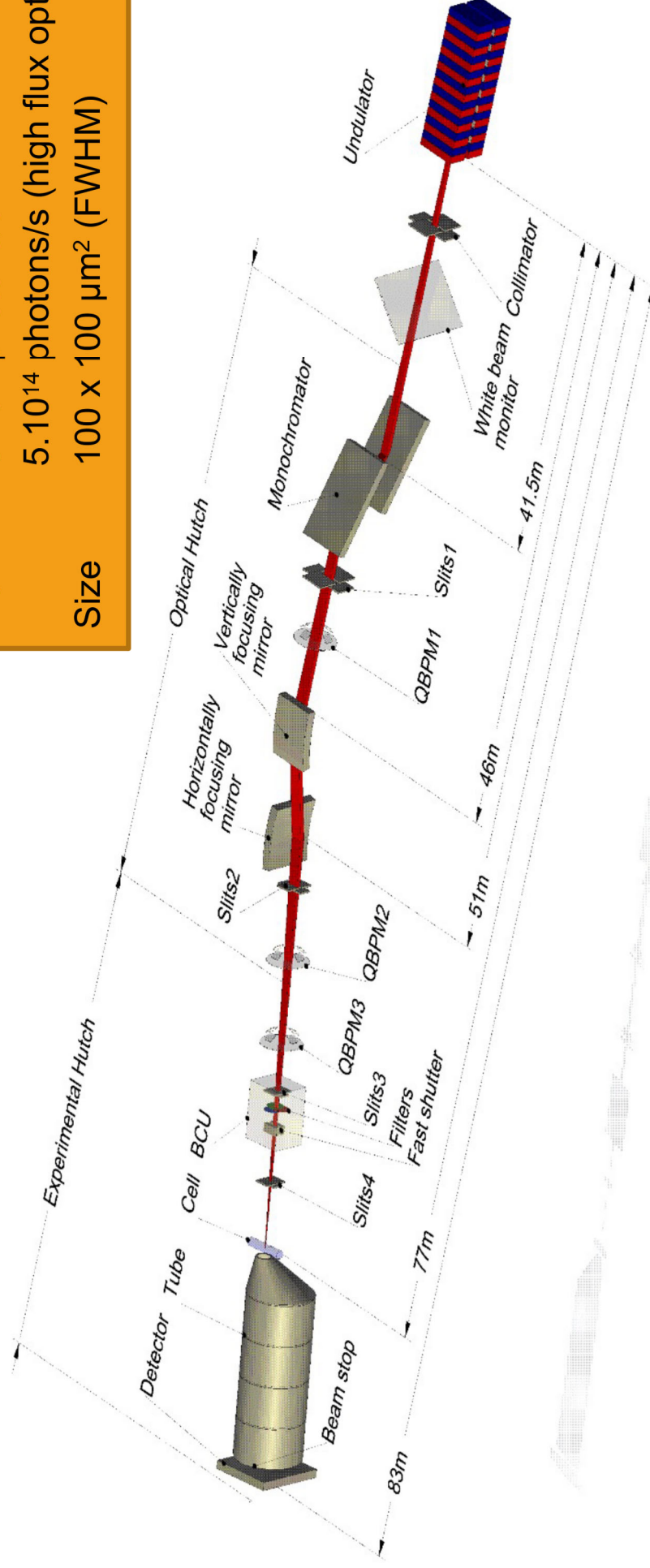
BioSAXS

- Weakly scattering samples
- Radiation damage
- Careful sample preparation and handling
- Well characterized before SAXS
- Low amount of sample available



P12 BioSAXS beamline overview

Energy	4 (6) - 20 keV
Flux	$5 \cdot 10^{12}$ photons/s
	$5 \cdot 10^{14}$ photons/s (high flux option)
Size	$100 \times 100 \mu\text{m}^2$ (FWHM)



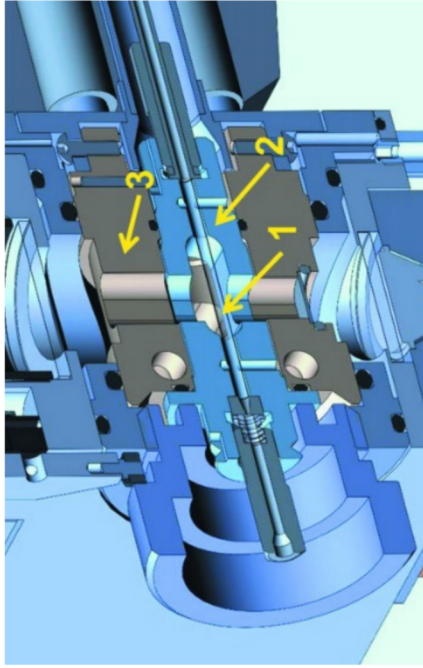
Beamline P12



Data collection on weakly scattering sample

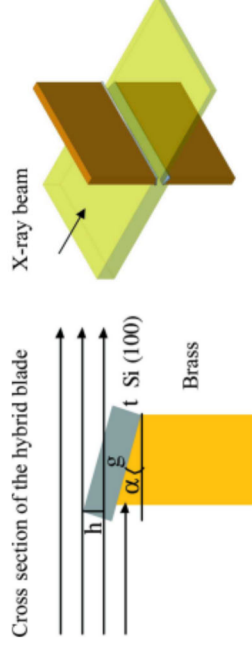
1. In-vacuum flow through capillary

Instrumental background can be minimized using in-vacuum sample environment and scatterless slits

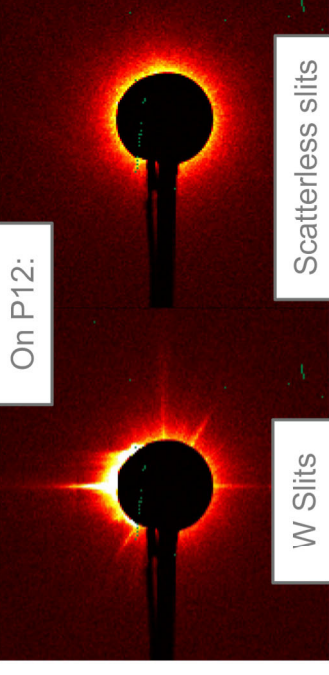


Round, et al.. *J. Appl. Cryst* (2008). 41(5), 913-917.

2. Scatterless slits

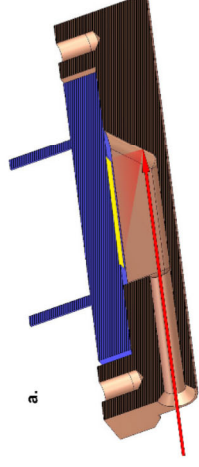


Li et al. *J. Appl. Cryst.* (2008). 41, 1134-1139



3. Small Active beamstop

Scattering curves collected on the sample and buffer needs to be accurately scaled to the beam intensity



Blanchet et al. *J. Synchrotron rad.* (2015). 22, 461-464

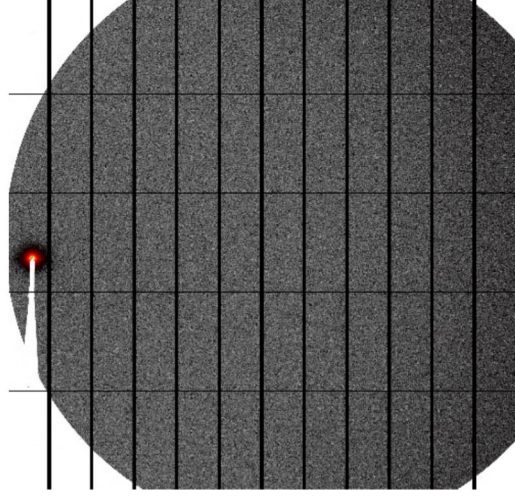
Data collection on weakly scattering sample

Photon counting detector

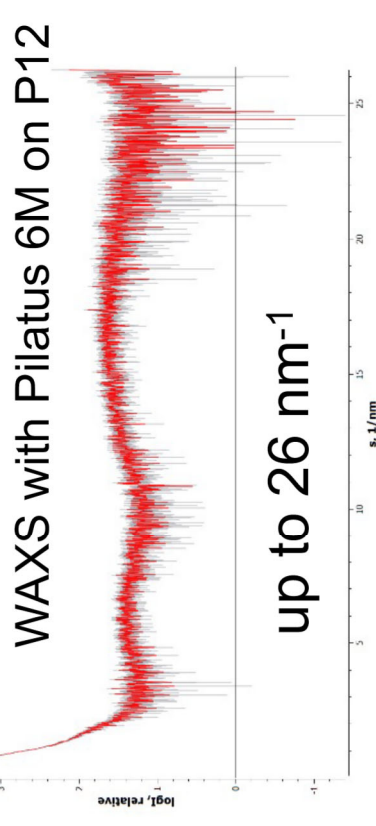


Ideal detector for BioSAXS:

- Have a large dynamic range
- Have a high count rate
- Have no intrinsic noise
- Have a high readout speed (for high throughput and kinetic measurements)



WAXS, Bovine Serum Albumin



Sample handling

Sample changer

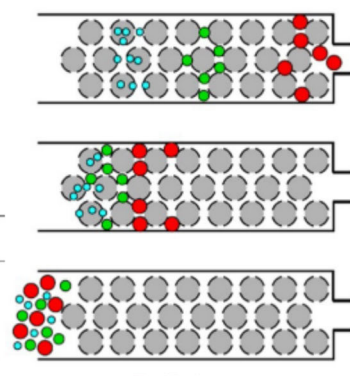
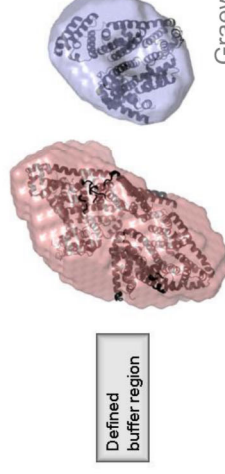
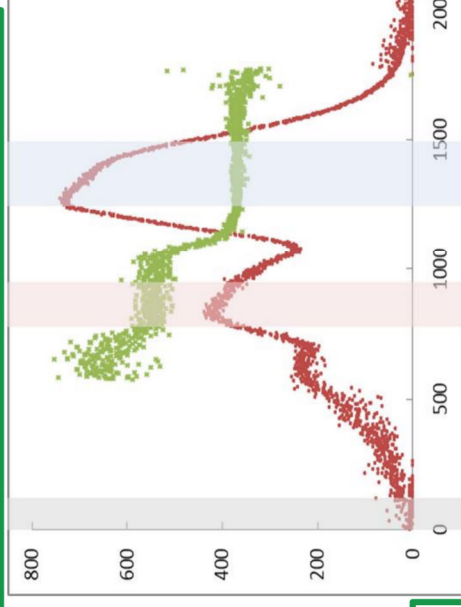


- Large capacity more than 250 samples
- Full cycle time $\approx$ 1min
- Sample volume: 10 – 30 μ l
- Flow measurement
- Rapid and efficient cell cleaning



SEC-SAXS

- Online purification
- Online spectroscopic characterization:
 - UV/Vis absorption
 - MALS
 - Refractive index



Experiment



SASFLOW pipeline



BEamline Control: QUEue and RELax (BECQUEREL)

... and data analysis

Hajizadeh, N. R., Franke, D. & Svergun, D. I. (2018). J. Synchrotron Rad. 25, 906-914.

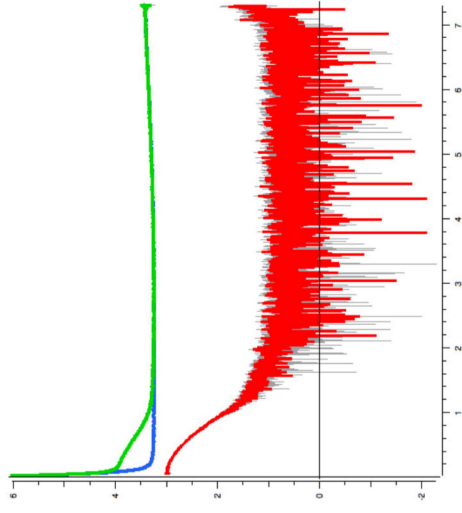
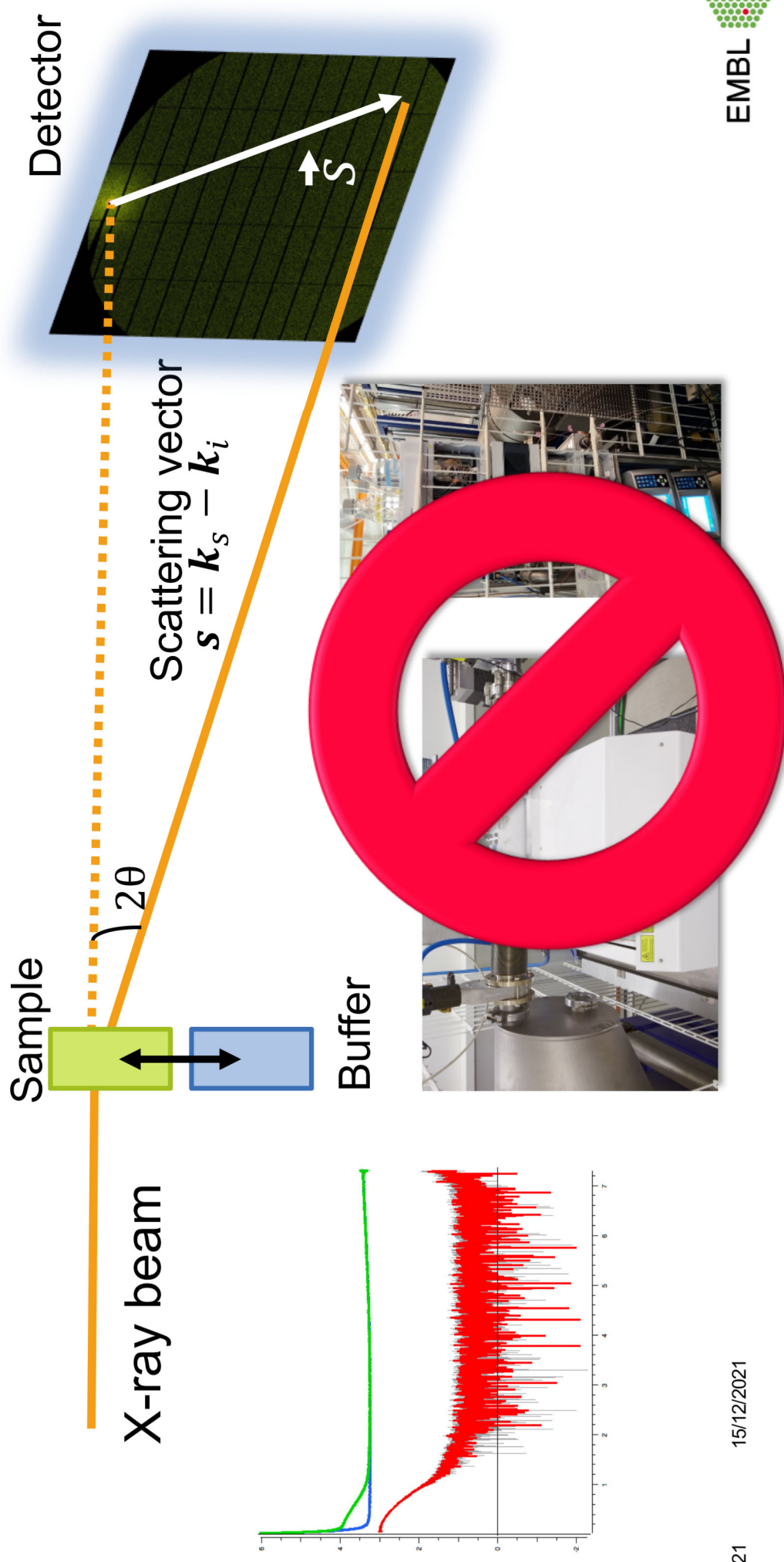
20 15/12/2021

SAXSWAXS/GISAXS User Workshop @ DESY 2020

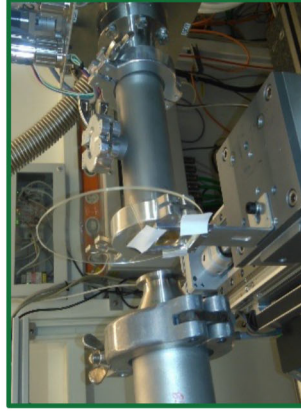
EMBL



“Non-standard” experiment



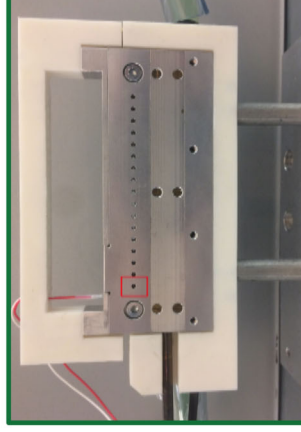
“Non-standard” experiments



Microfluidic sample environments



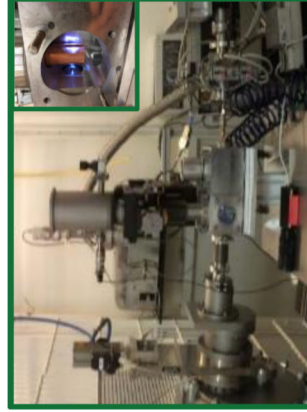
Microfluidics cell for THz illumination and SAXS measurements



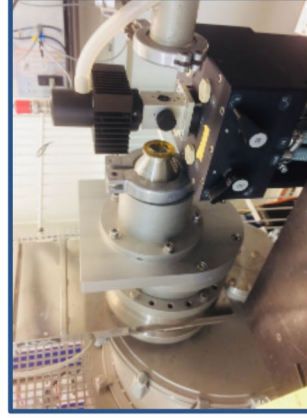
Thermostated capillary holder



Scanning-SAXS



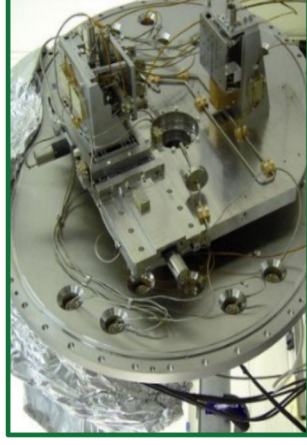
CryoSAXS



Stopped-flow



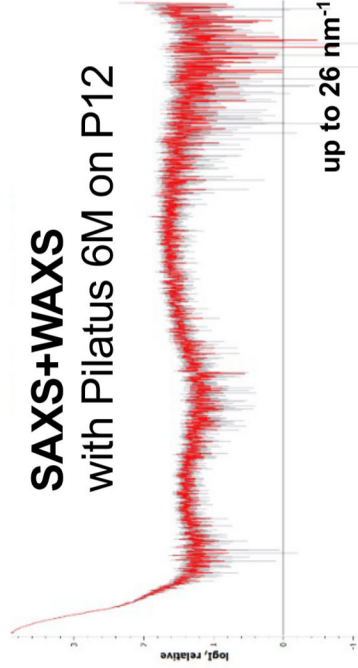
Laser setup



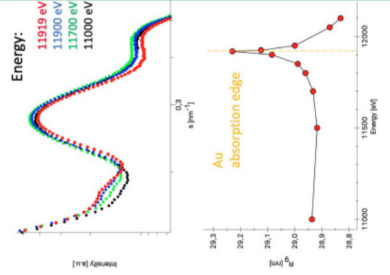
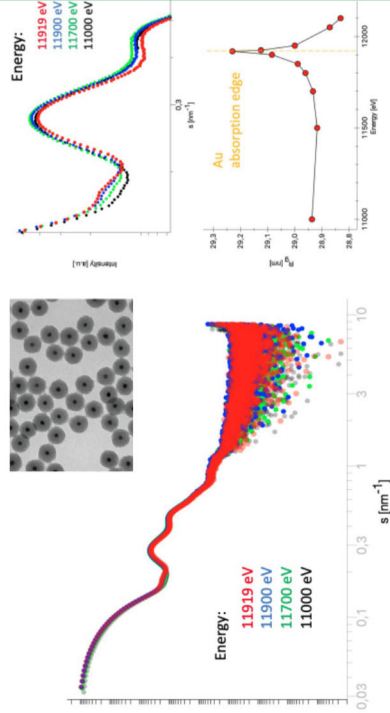
Energy change

Advanced BioSAXS

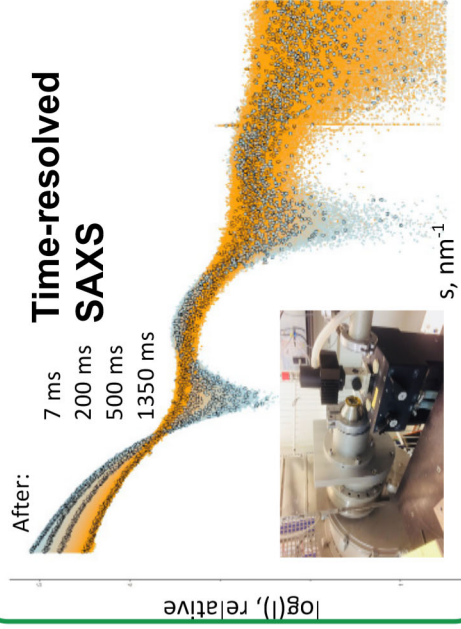
SAXS+WAXS
with Pilatus 6M on P12



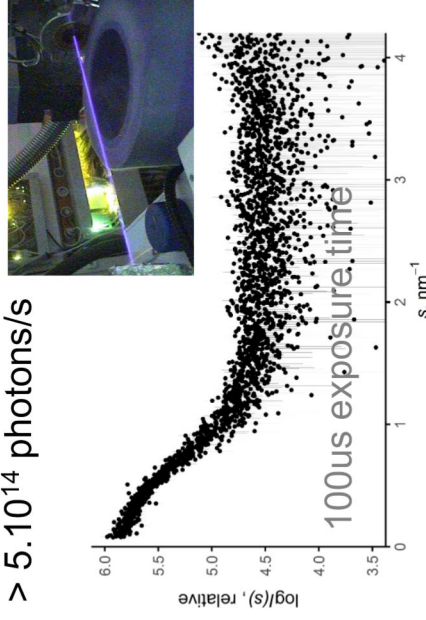
ASAXS Silica coated AuNP



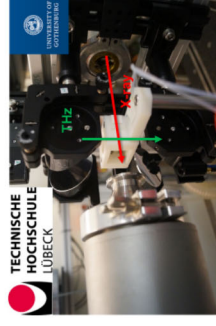
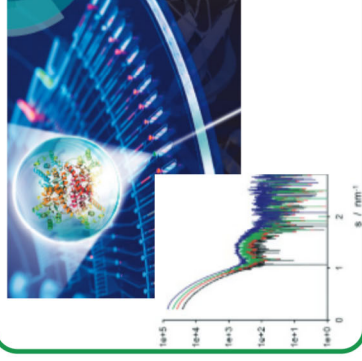
Time-resolved SAXS



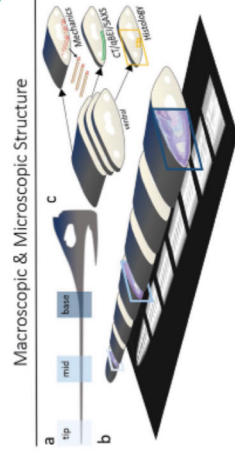
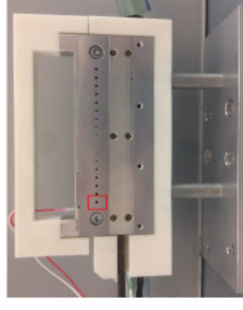
High Flux option
> $5 \cdot 10^{14}$ photons/s



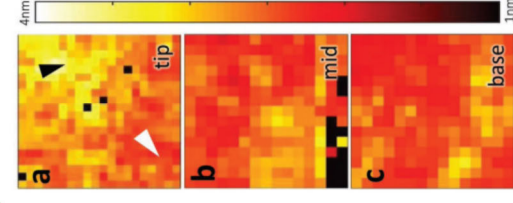
Microfluidics



High temperature and aggressive chemical



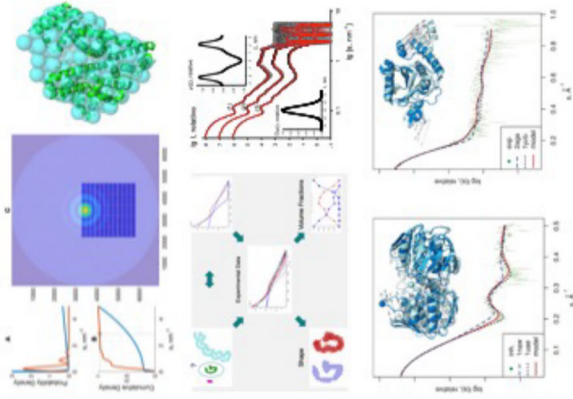
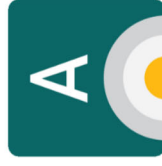
Scanning SAXS



Schmidt et al. Adv. Sci. 2019, 1900287.



ATSAS 3.0.0 package update



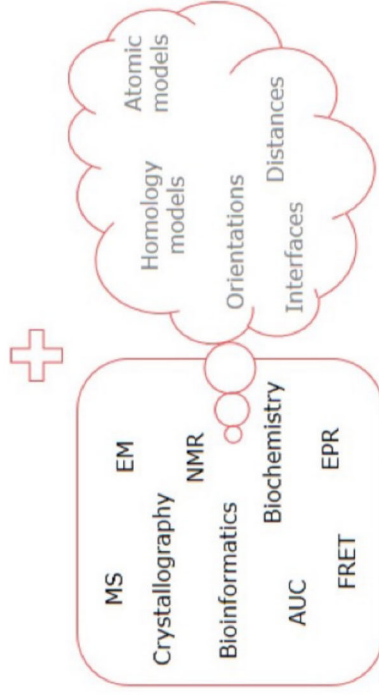
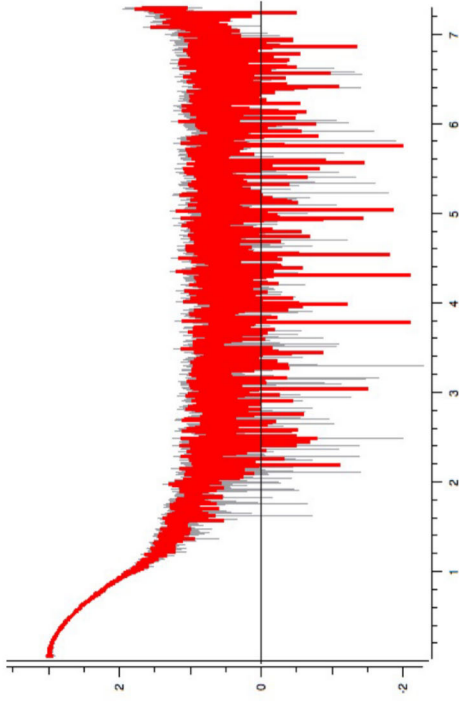
<https://www.embl-hamburg.de/biosaxs/download.html>

A database of SAS patterns/models

[illegible]

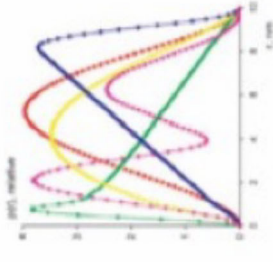
<https://www.sasbdb.org>

BioSAXS

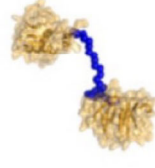
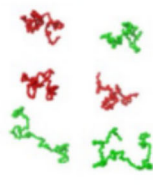


Complementary techniques → additional information

Pair distribution functions

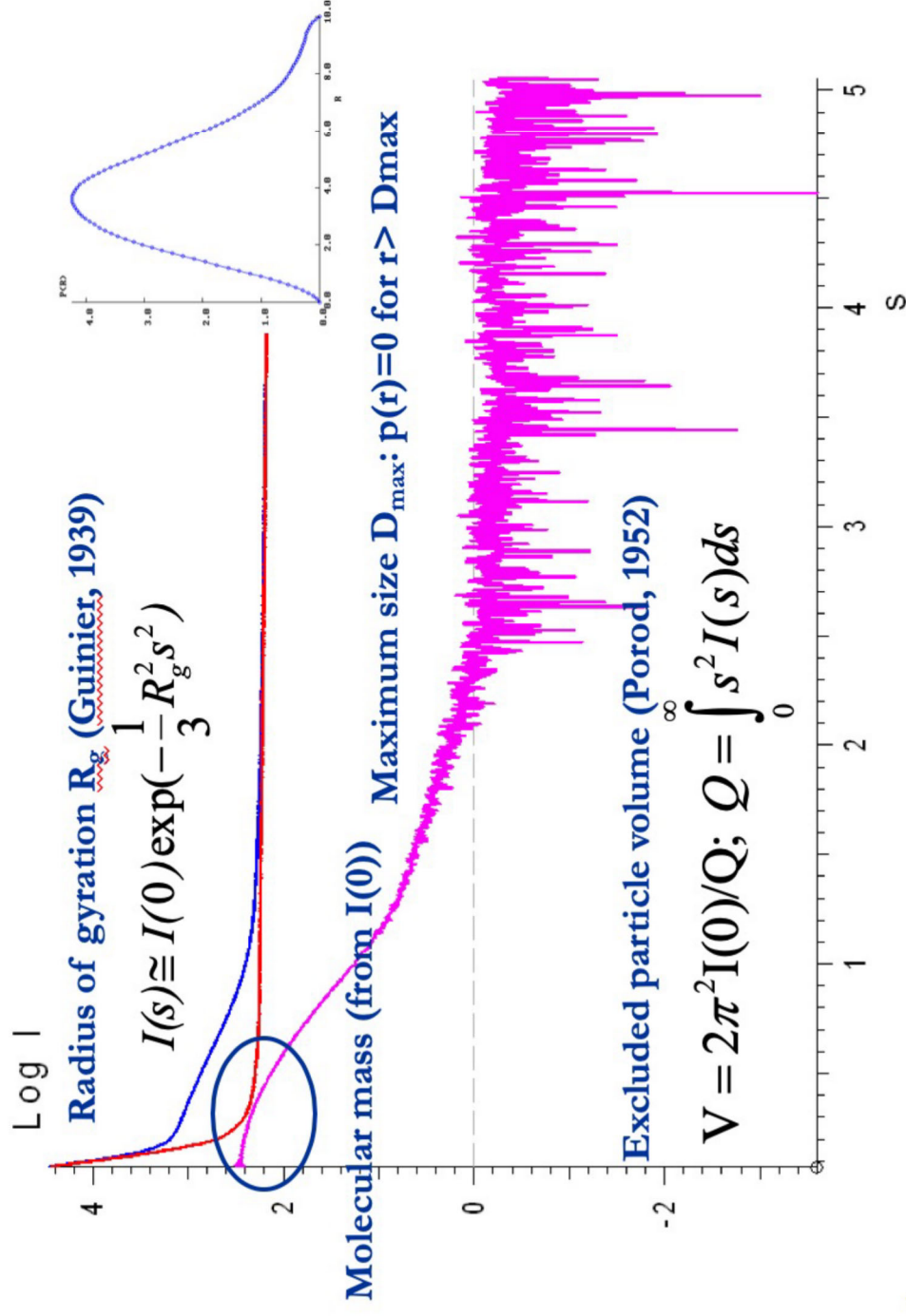


- Radius of gyration
- Volume
- Flexibility
- Distance distribution function
- Shape determination

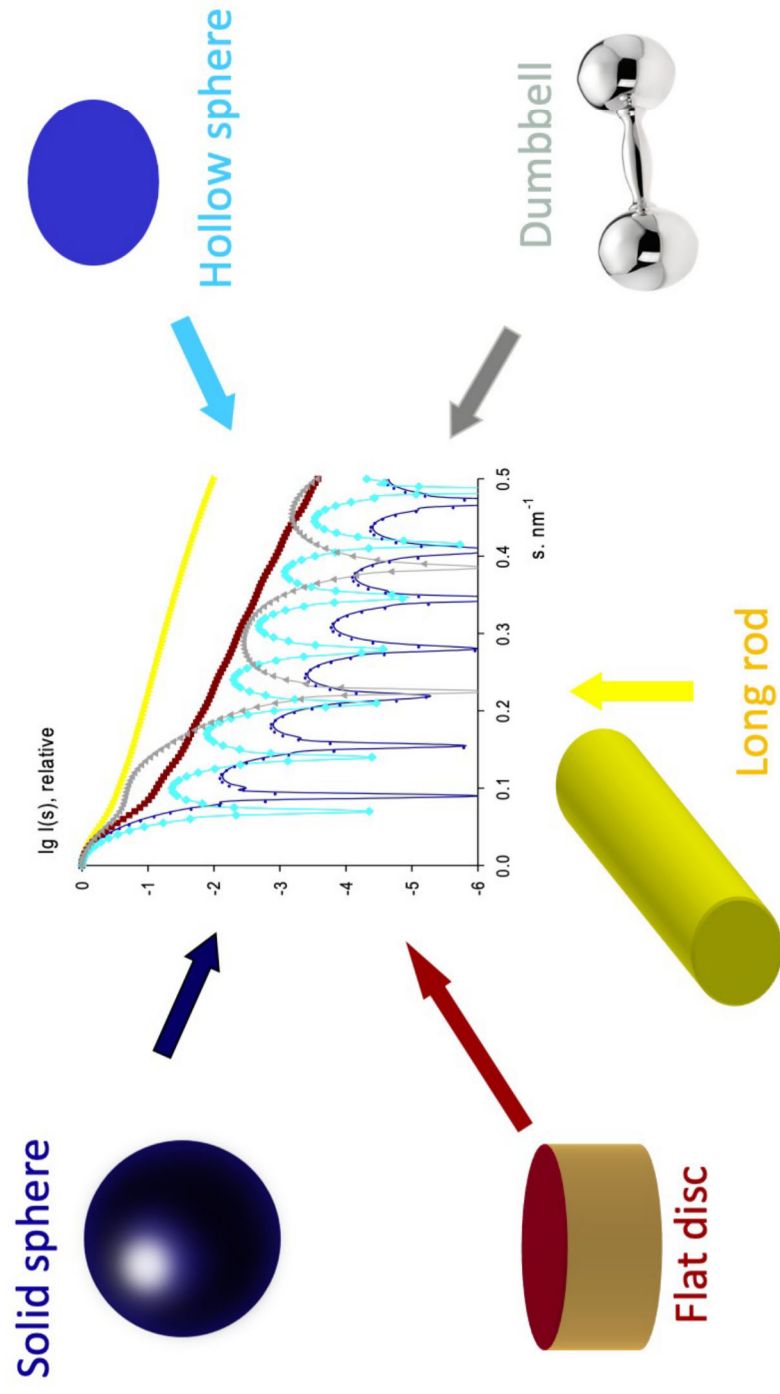


- Hybrid methods
- Rigid body modelling
 - Missing fragments
 - Oligomeric mixtures
 - Flexible systems

Overall parameters from SAXS



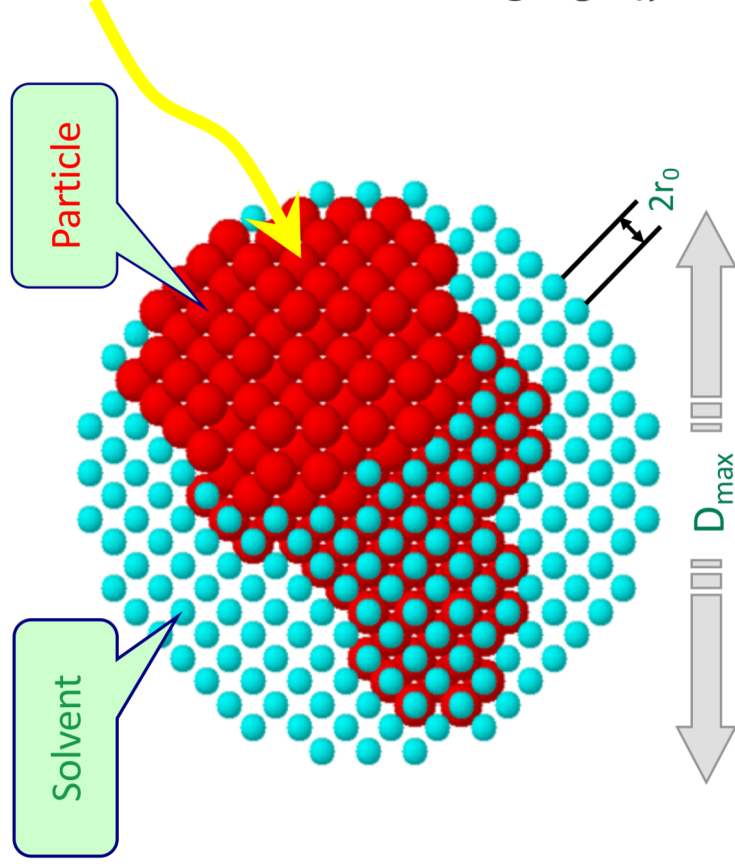
Overall parameters from SAXS



Bead (dummy atoms) model

A sphere of radius $D_{\max}$ is filled by densely packed beads of radius

$$r_0 \ll D_{\max}$$



Vector of model parameters:

$$\text{Position } (j) = X(j) = \begin{cases} 1 & \text{if particle} \\ 0 & \text{if solvent} \end{cases}$$

(phase assignments)

Number of model parameters

$$M \approx (D_{\max} / r_0)^3 \approx 10^3$$

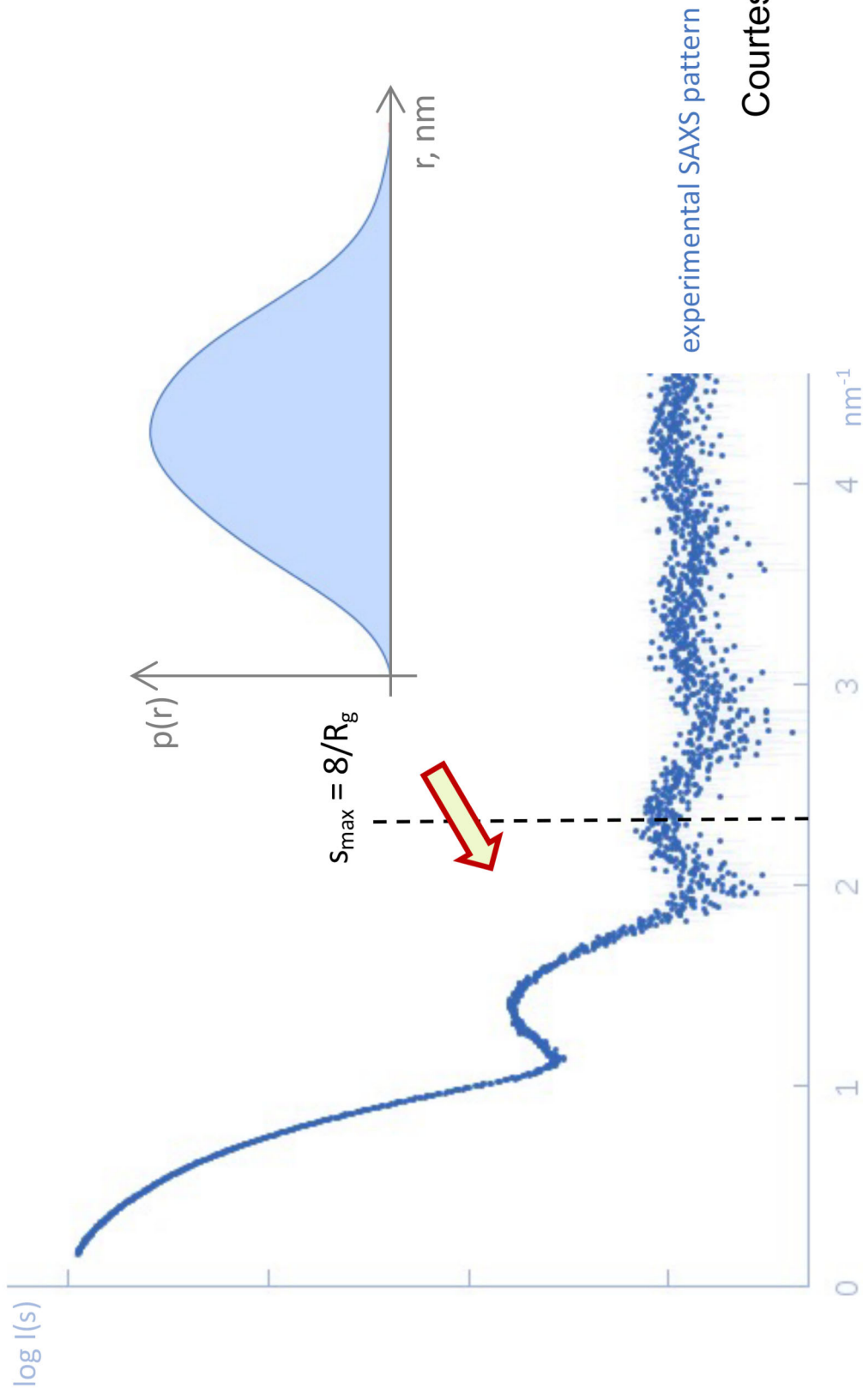
is too big for conventional minimization methods – Monte-Carlo like approaches are to be used

But: This model is able to describe rather complex shapes

Chacón, P. et al. (1998) *Biophys. J.* 74, 2760-2775.

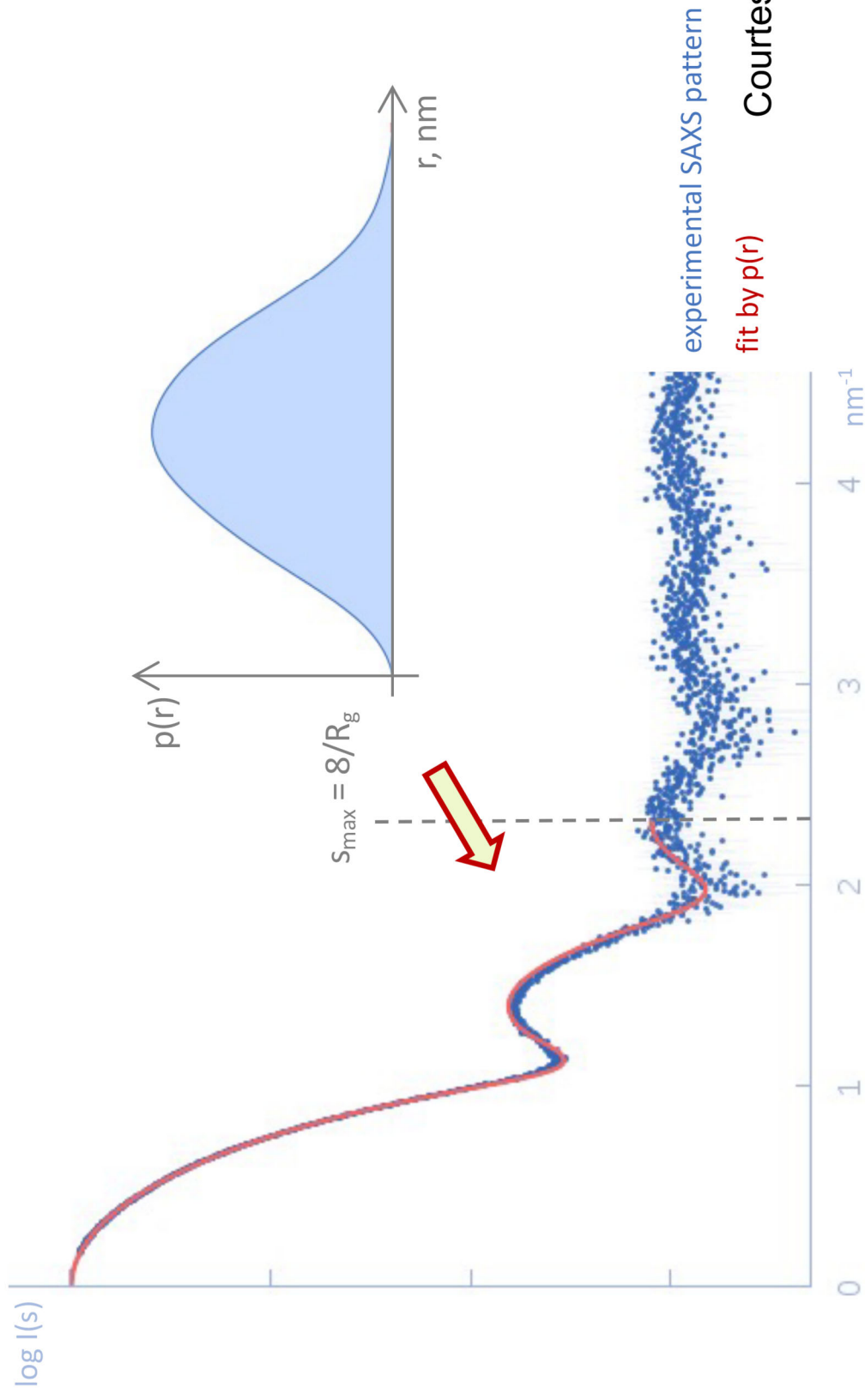
Svergun, D.I. (1999) *Biophys. J.* 76, 2879-2886

Example: *Ab initio* DAM shape reconstruction



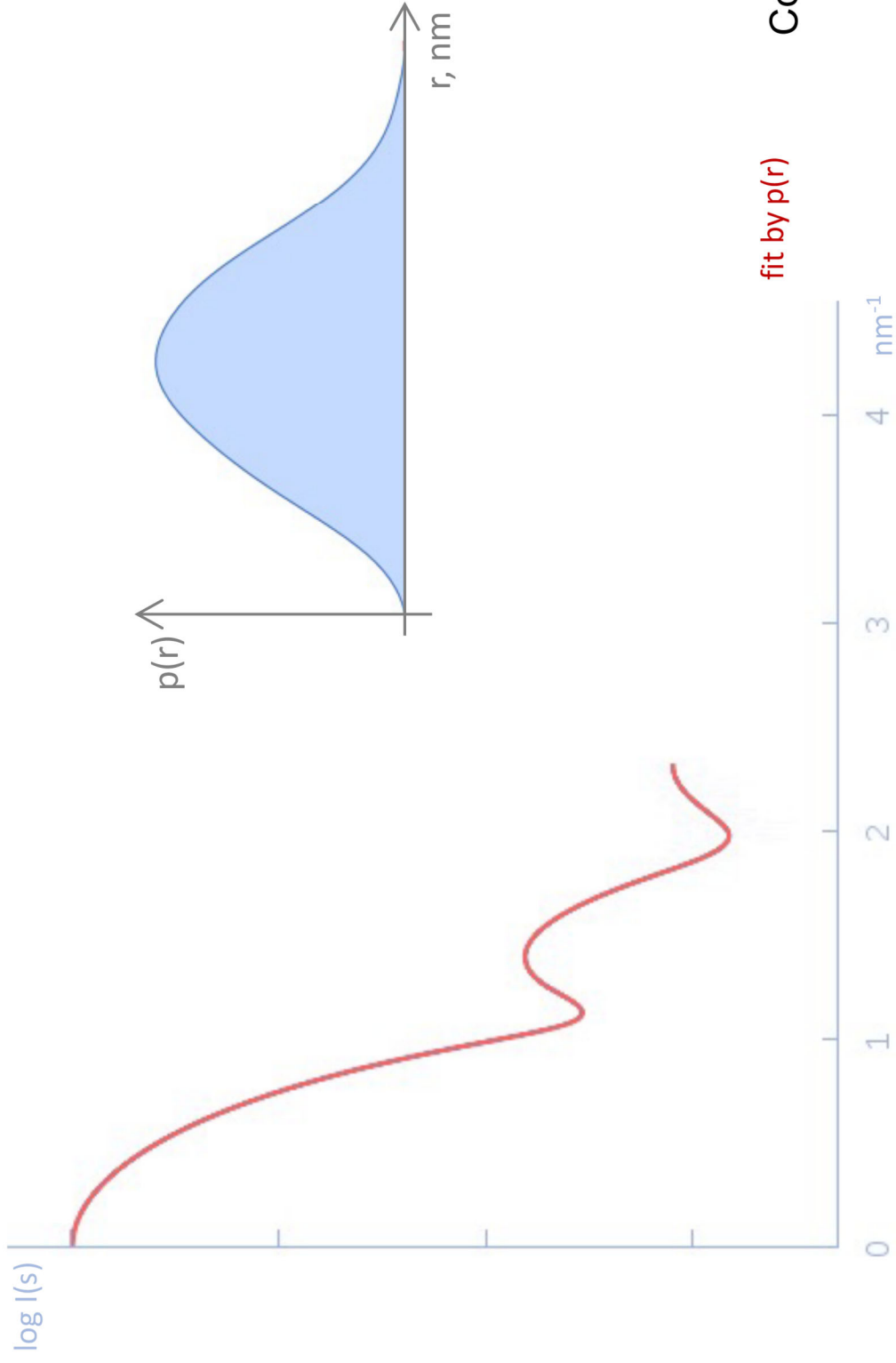
Courtesy of Al Kikhney

Example: *Ab initio* DAM shape reconstruction



Courtesy of AI Kikhney

Example: *Ab initio* DAM shape reconstruction

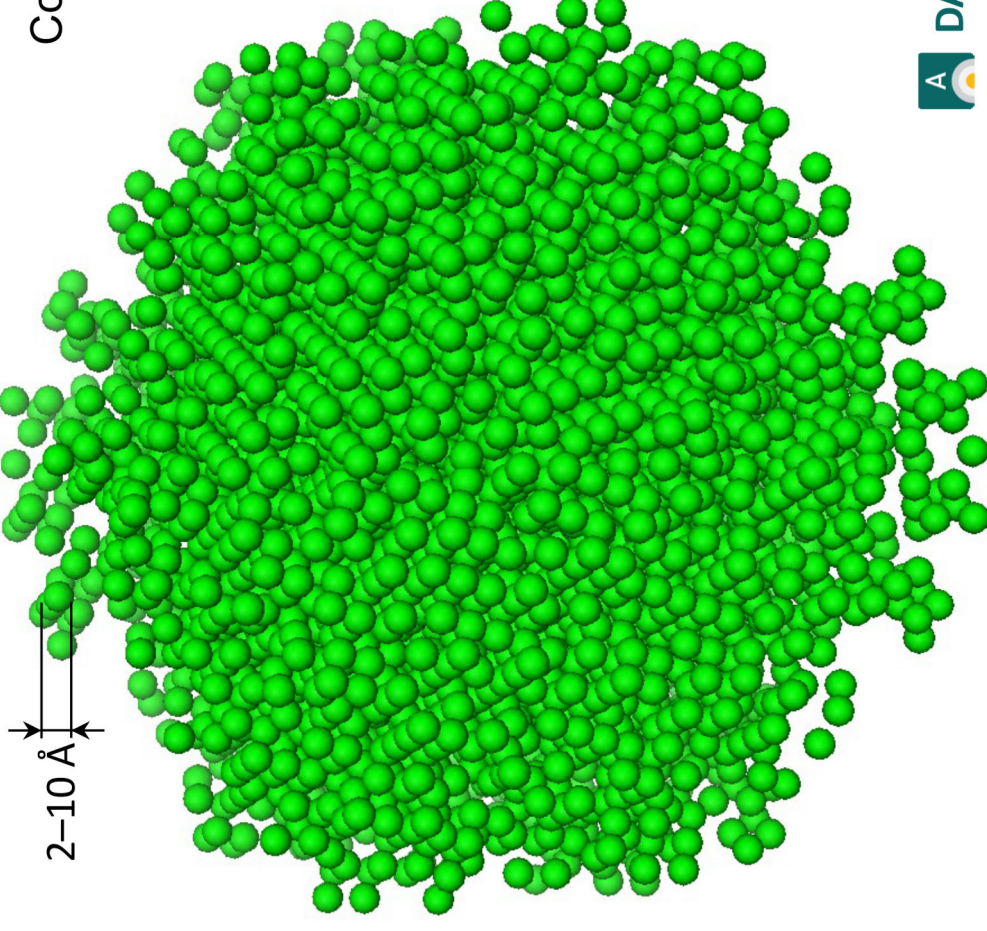


fit by $p(r)$

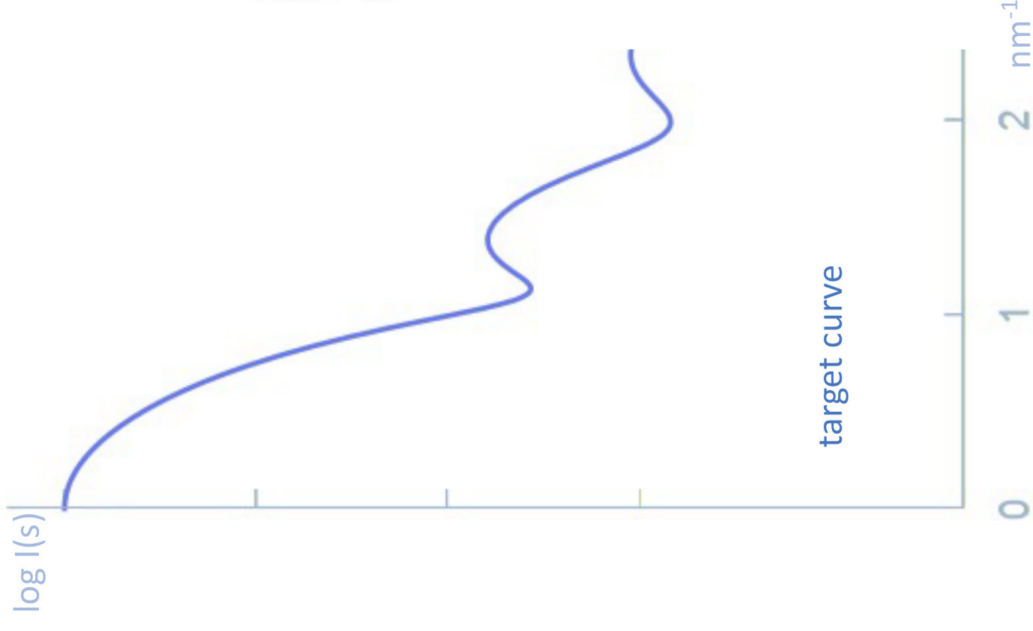
Courtesy of Al Kikhney

Example: DAMMIF

≈2000–10000 “dummy atoms”



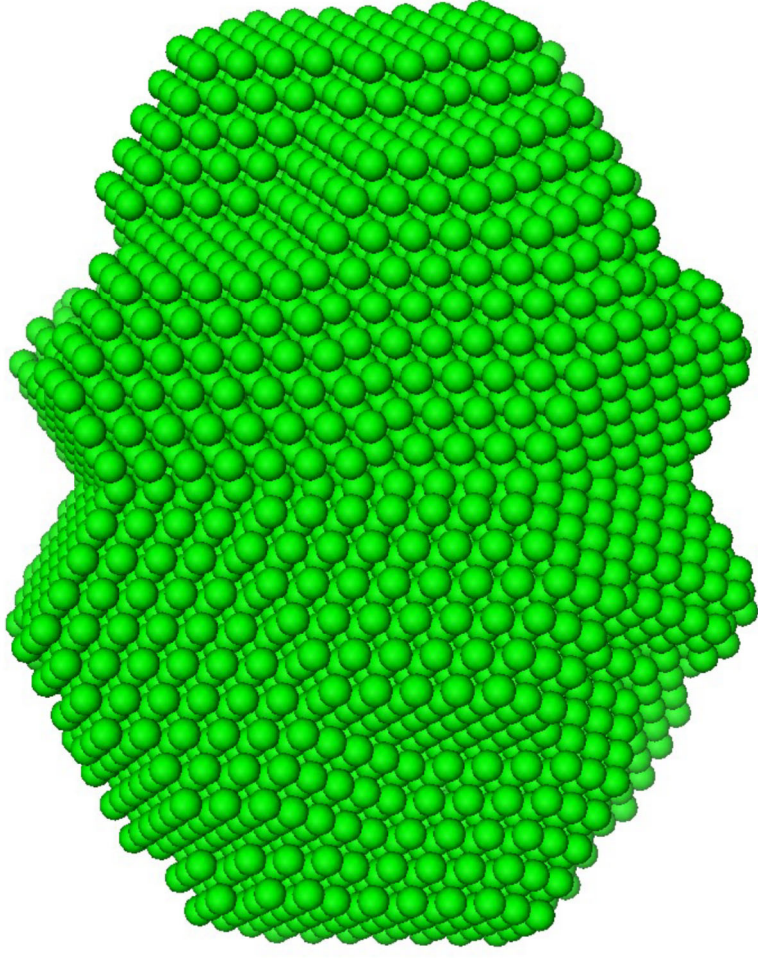
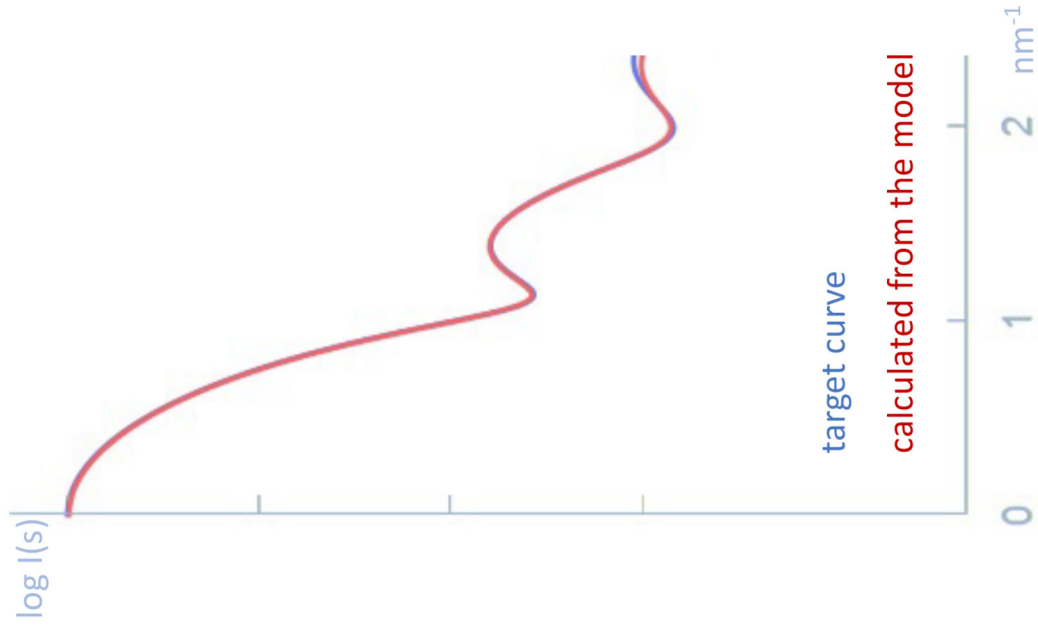
Courtesy of Al Kikhney



Franke, D. and Svergun, D.I. (2009)

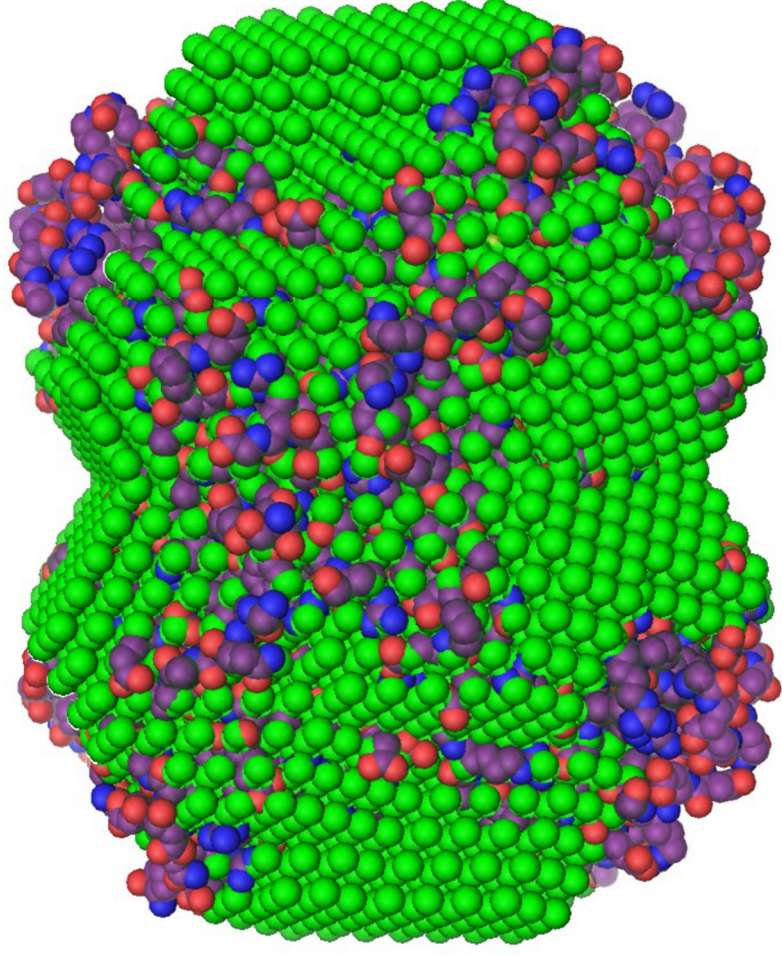
J Appl Cryst 42, 342–346.

Example: DAMMIF



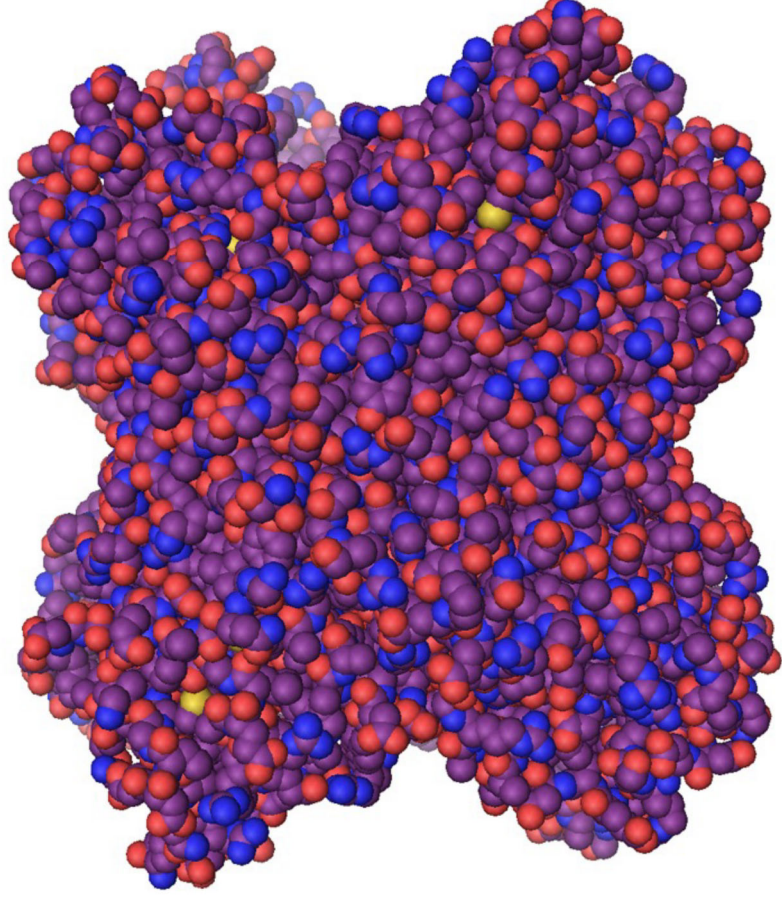
Courtesy of Al Kikhney

Example: DAMMIF



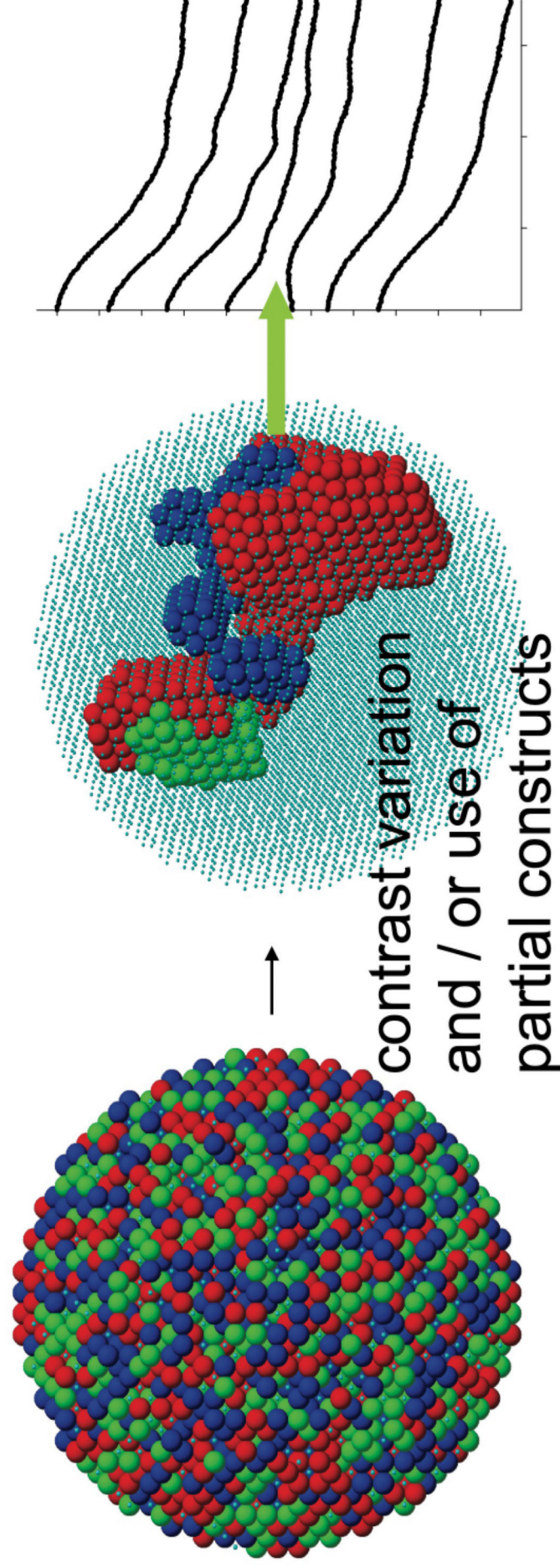
Courtesy of Al Kikhney

Example: DAMMIF



Courtesy of Al Kikhney

Multiphase approach: MONSA



When the protein crystal structure is known

CRY SOL:

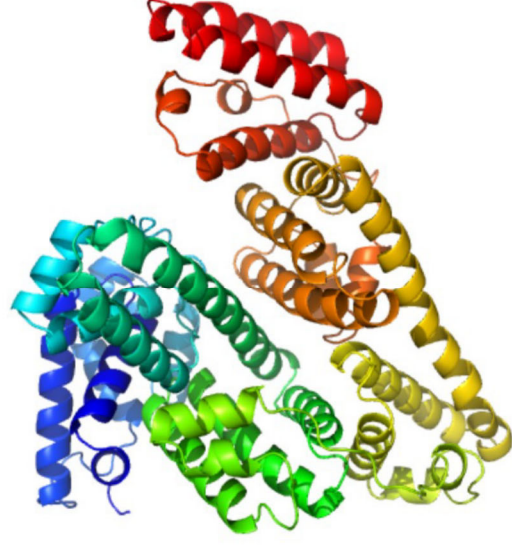
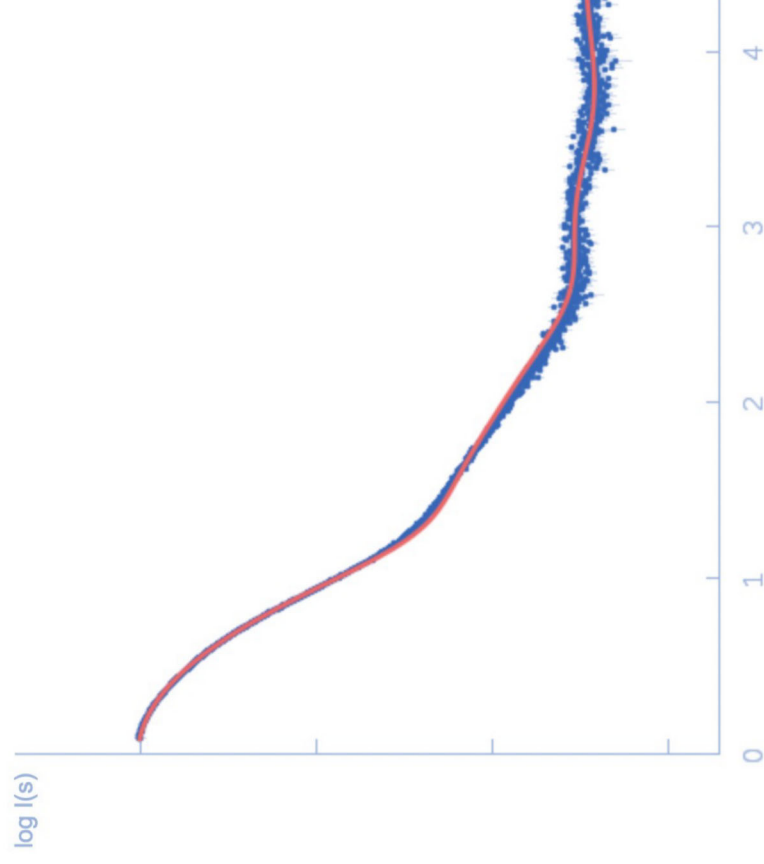
$$I(\mathbf{s}) = \left\langle |A(\mathbf{s})|^2 \right\rangle_{\Omega} = \left\langle |A_a(\mathbf{s}) - \rho_s A_s(\mathbf{s}) + \delta \rho_b A_b(\mathbf{s})|^2 \right\rangle_{\Omega}$$



- $A_a(\mathbf{s})$: atomic scattering in vacuum
- $A_s(\mathbf{s})$: scattering from the excluded volume
- $A_b(\mathbf{s})$: scattering from the hydration shell

When the protein crystal structure is known

CRY SOL:

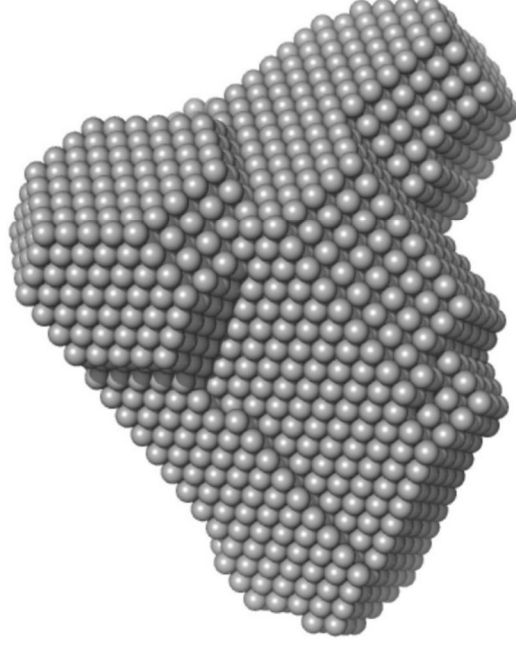
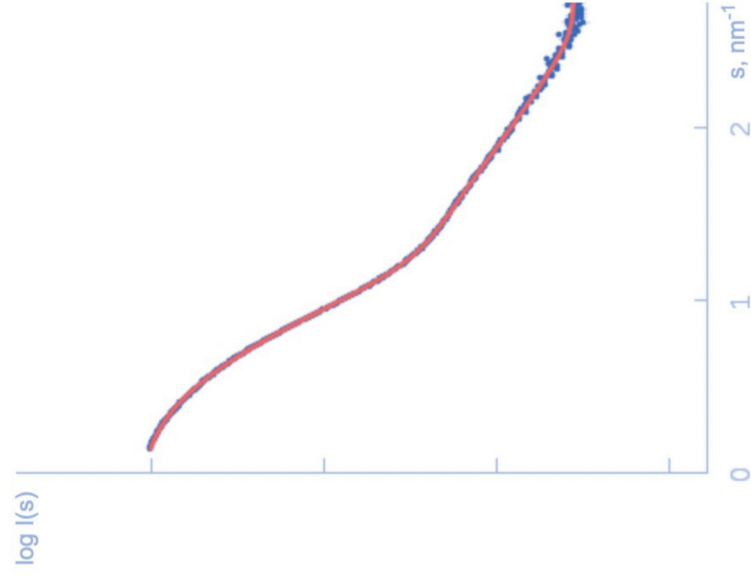


PDB 3V03

Svergun et al. (1995). J. Appl. Cryst. 28, 768

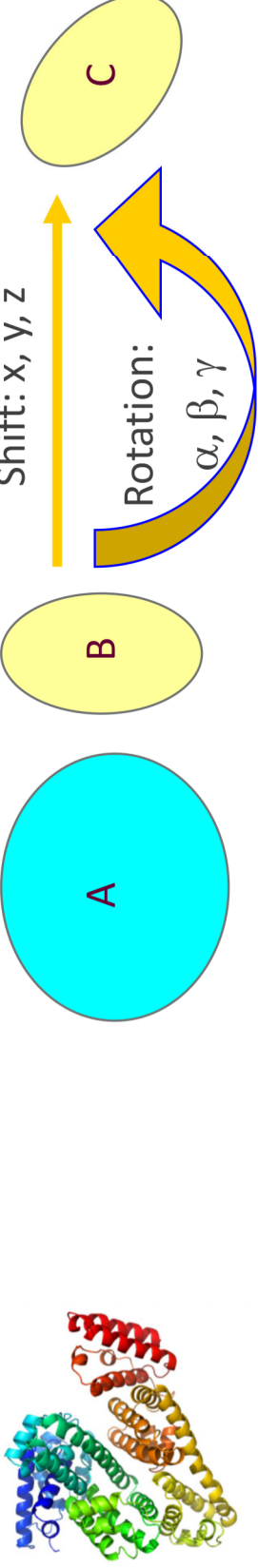
When the protein crystal structure is known

Compare with *Ab-initio* modeling:



Svergun et al. (1995). J. Appl. Cryst. 28, 768

Rigid body modelling

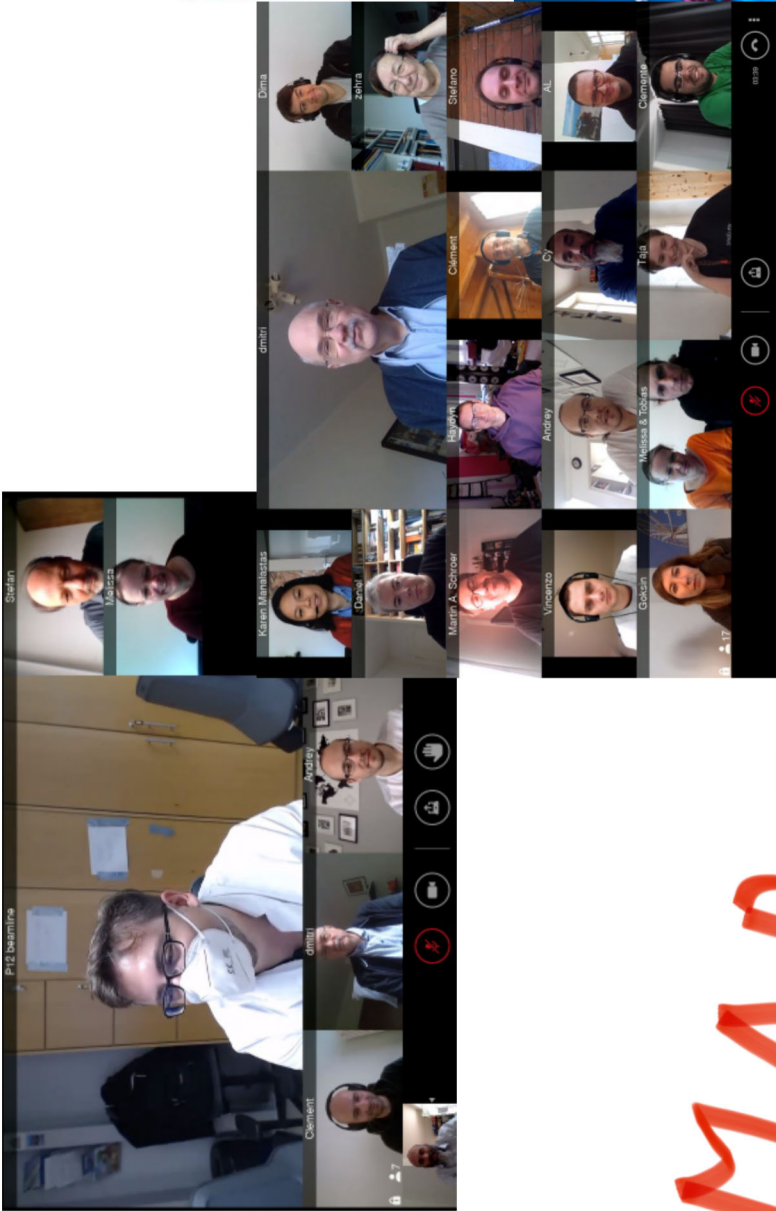
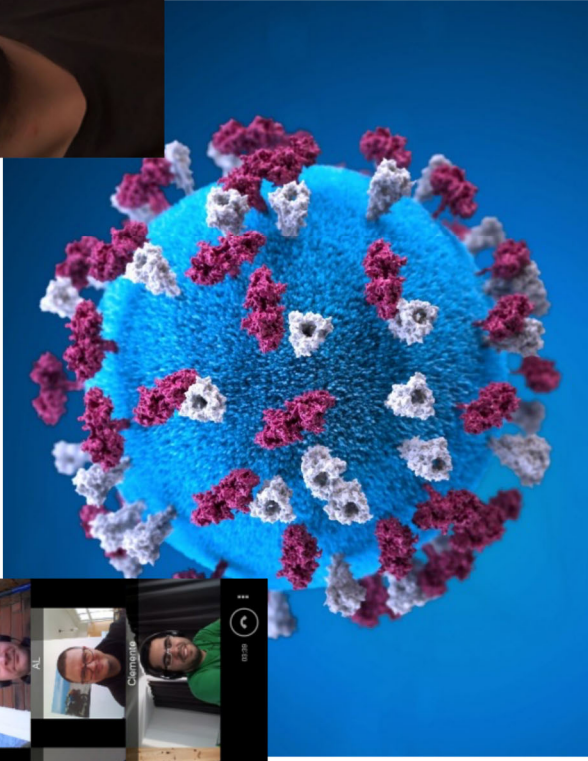


Using spherical harmonics, the amplitude(s) of arbitrarily rotated and displaced subunit(s) are analytically expressed *via* the initial amplitude and the six positional parameters: $C_{lm}(s) = C_{lm}(B_{lm}, \alpha, \beta, \gamma, x, y, z)$.

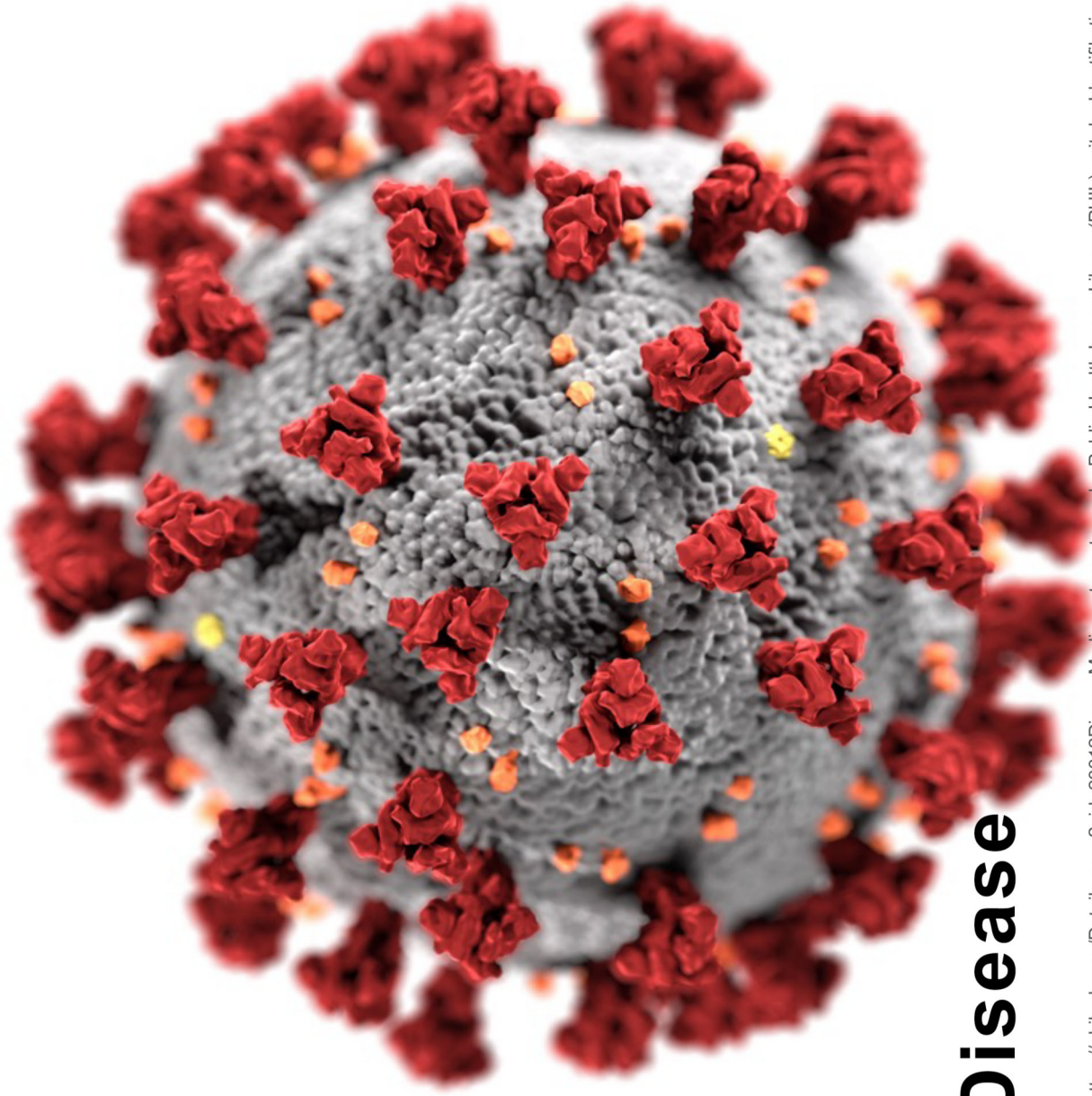
The scattering from the complex is then rapidly calculated as

$$I(s) = I_A(s) + I_B(s) + 4\pi^2 \sum_{l=0}^{\infty} \sum_{-l}^l \text{Re}[A_{lm}(s)C_{lm}^*(s)]$$

Svergun, D.I. (1991). *J. Appl. Cryst.* 24, 485-492



MARCH 2020...



Part two: Disease

Von CDC/ Alissa Eckert, MS; Dan Higgins, MAM - <https://phil.cdc.gov/Details.aspx?pid=23312> Dieses Medium stammt aus der Public Health Image Library (PHIL), mit der Identifikationsnummer #23312 der Centers for Disease Control and Prevention. <https://commons.wikimedia.org/w/index.php?curid=86425232>

Neutralize SARS-CoV-2 with sybodies

Collaboration with C.Loew (EMBL-HH) and M.Hallberg (Karolinska, Sweden)



ARTICLE

<https://doi.org/10.1038/s41467-020-19204-y>

OPEN



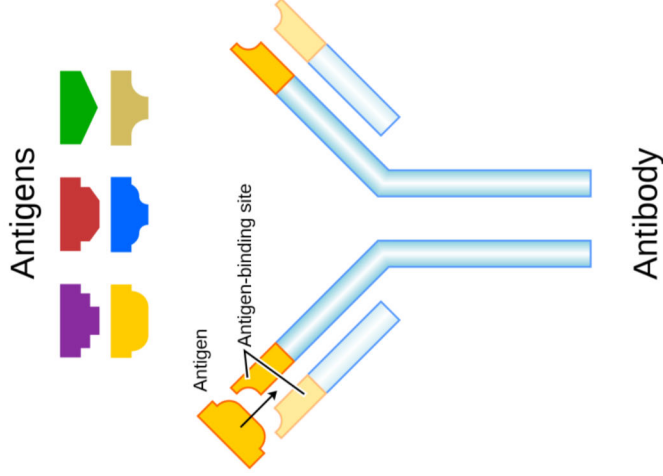
Selection, biophysical and structural analysis of synthetic nanobodies that effectively neutralize SARS-CoV-2

Tânia F. Custódio¹, Hrishikesh Das², Daniel J. Sheward^{3,4}, Leo Hanke³, Samuel Pazicky¹, Joanna Pieprzyk¹, Michèle Sorgenfrei⁵, Martin A. Schroer⁶, Andrey Yu. Gruzinov⁶, Cy M. Jeffries⁶, Melissa A. Graewert⁶, Dmitri I. Svergun⁶, Nikolay Dobrev⁷, Kim Remans⁷, Markus A. Seeger⁵, Gerald M. McInerney³, Ben Murrell³, B. Martin Hällberg^{2,8} & Christian Löw¹✉

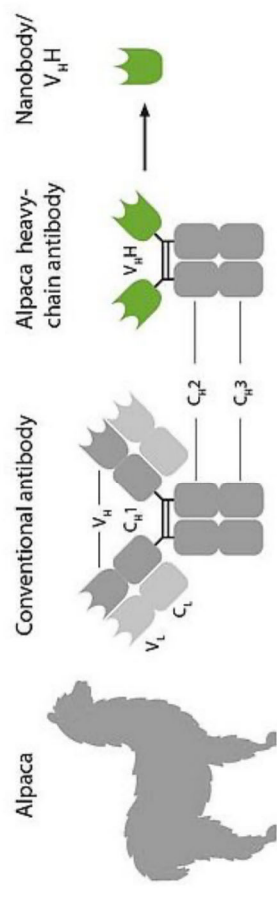
Antibodies vs Nanobodies (Sybodies)

Antibody – A Y-shaped protein used in the body's immune response to identify and remove pathogens such as bacteria or viruses. The **variable region** of each antibody recognizes and binds to a specific 'antigen'.

Key for the adaptive immune system

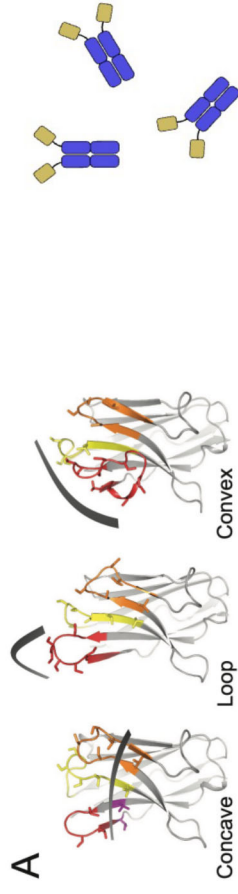


Nanobody - is an antibody fragment consisting of a single monomeric variable antibody domain.



=> Synthetic library

Can be produced in bacteria!

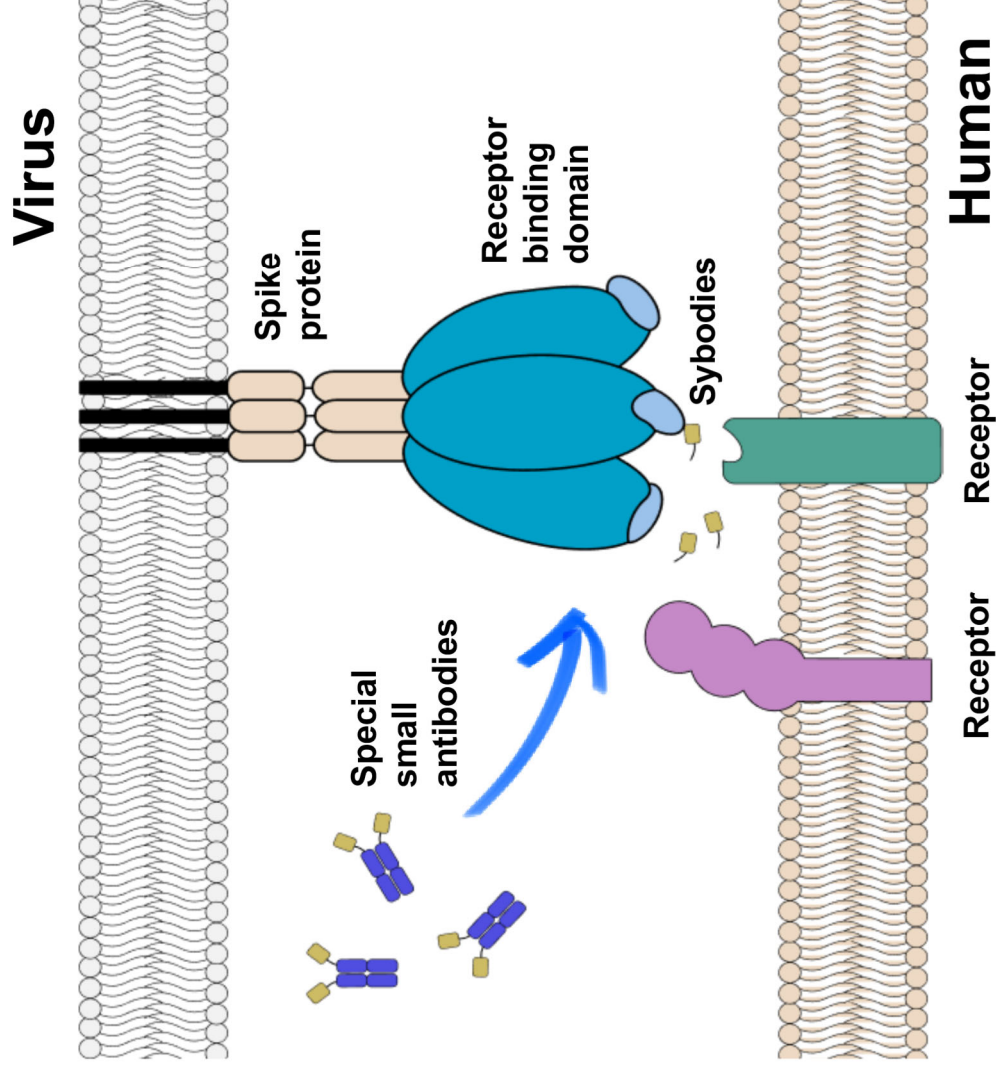


Zimmermann, Iwan, et al. "Synthetic single domain antibodies for the conformational trapping of membrane proteins." *Elife* 7 (2018): e34317.

<https://www.chromotek.com/technology/discovery-of-nanobodies/>

Neutralize SARS-CoV-2 with sybodies

Collaboration with C.Loew (EMBL-HH) and M.Hallberg (Karolinska, Sweden)



How to find out fast which sybodies is the best?

SAXS for fast sceening experiments

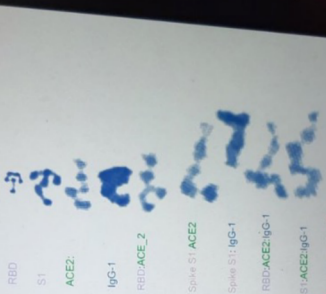
 **Svergun Group** @svergungroup · May 8, 2020

Looking at first #COVID19 related #SAXS data from @embl's P12 beamline during today's virtual group meeting. #EMBLatHOME

High throughput screening of antibodies to SARS-CoV-2 spike protein using small-angle X-ray scattering.



+ MANY OTHERS
socially distant but scientifically present



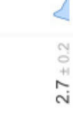
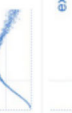
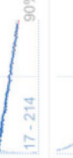
RBD
S1
ACE2
IgG-1
REDUCE_2
Spike S1 ACE2
Spike S1 IgG-1
RBD ACE2 IgG-1
S1 ACE2 IgG-1

Melissa Graewert

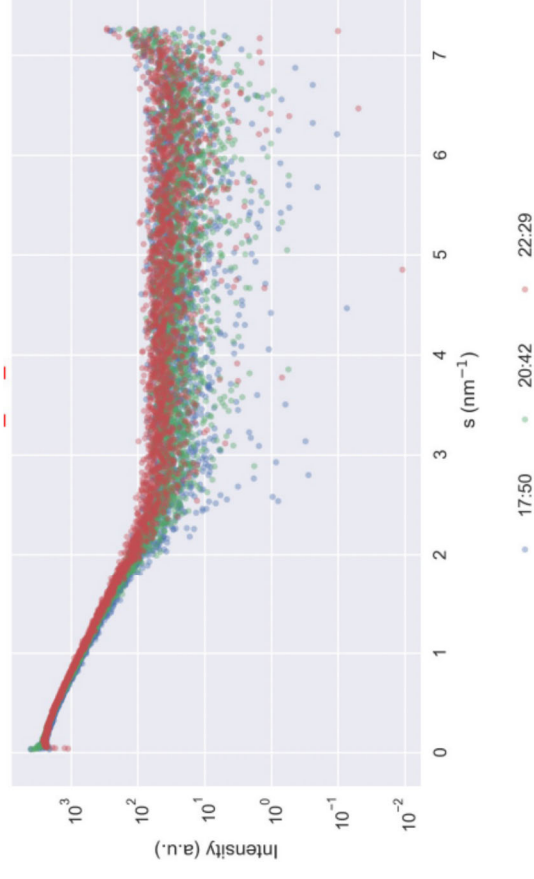
6 39

How to find out fast which sybodies is the best?

Large library:

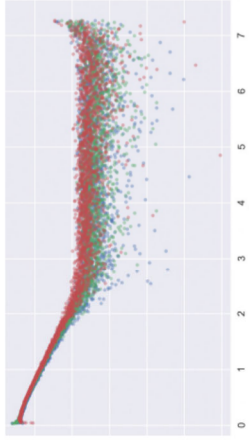
2.2				2.7 ± 0.2		9.7	27	17	20^{+0}_{-3}
0.6				1.9 ± 0.0		5.4	20	14	7^{+2}_{-1}
0.7				2.0 ± 0.1		5.7	18	16	9^{+1}_{-2}
1.4				2.2 ± 0.1		6.3	20	15	11^{+1}_{-1}
1.2				2.7 ± 0.1		8.7	27	20	15^{+3}_{-1}
0.5				2.0 ± 0.1		5.9	19	16	7^{+1}_{-1}
1.0				2.1 ± 0.1		5.9	18	14	10^{+0}_{-2}
2.3				2.5 ± 0.1		8.6	25	17	15^{+2}_{-1}
1.7				2.1 ± 0.0		6.1	21	14	13^{+1}_{-0}
1.4				2.1 ± 0.2		5.8	21	14	12^{+2}_{-1}
1.2				2.2 ± 0.0		6.6	23	17	13^{+1}_{-3}

Several repetitions:



How to find out fast which sybodies is the best?

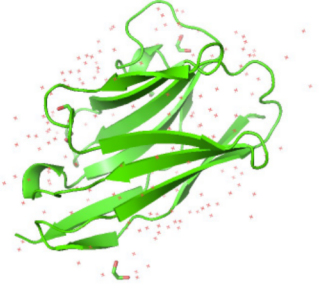
Scattering profile:



Validation



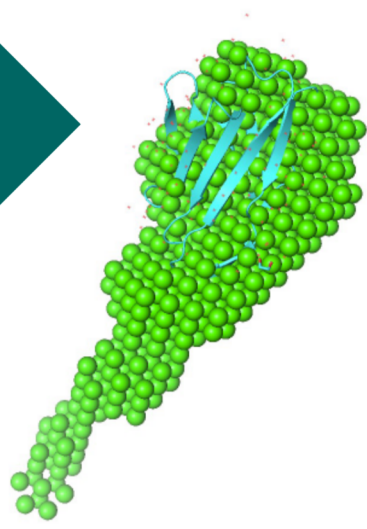
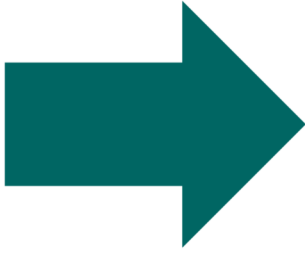
Crystal structure:



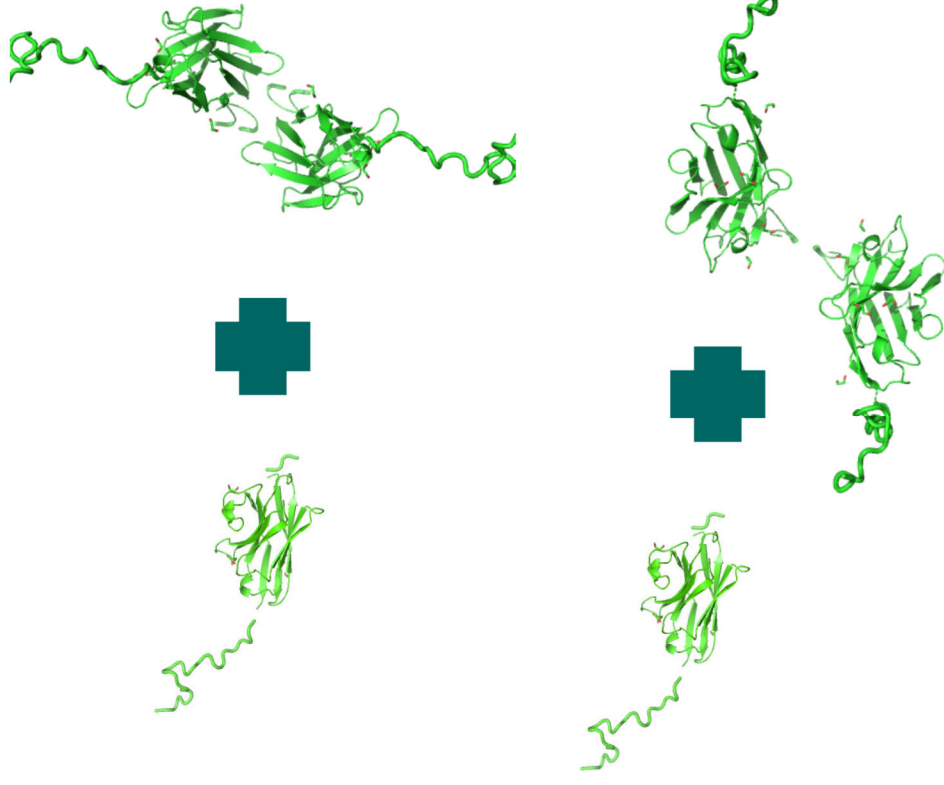
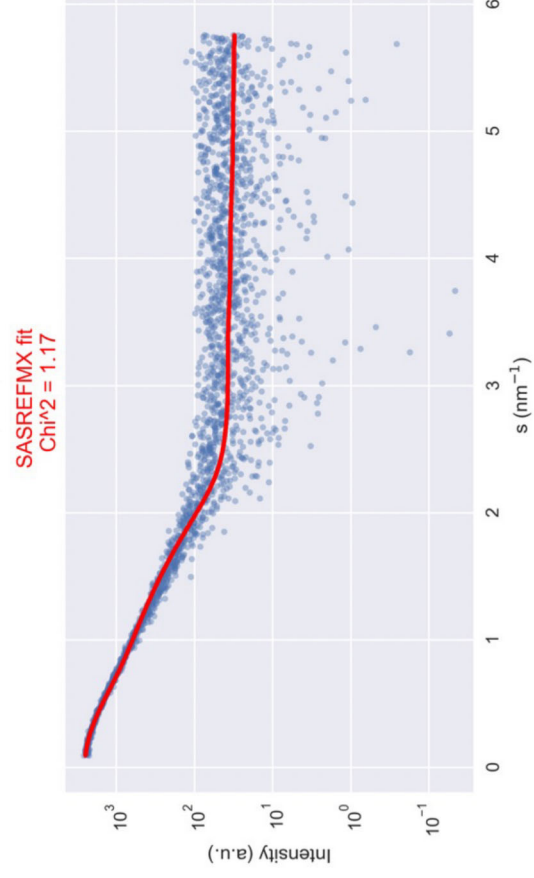
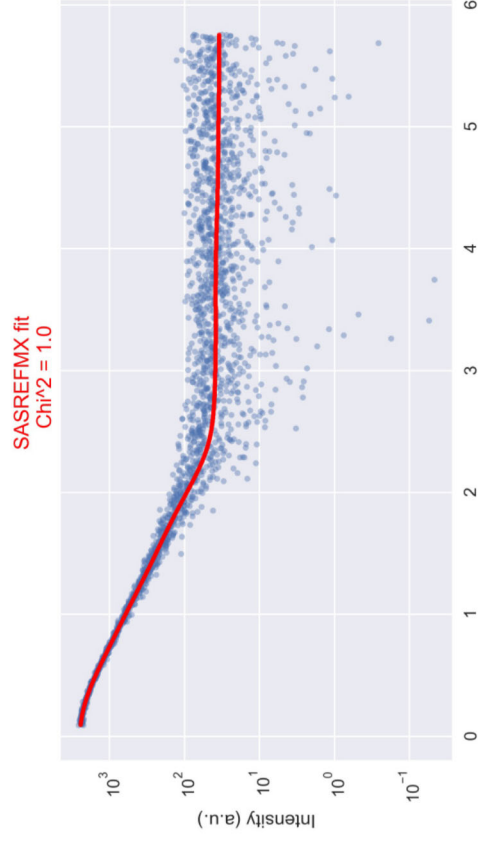
Refined model
(fragment reconstruction)



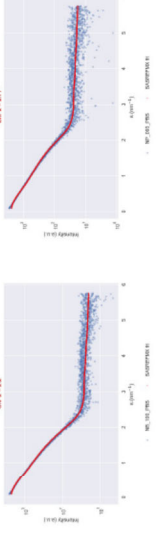
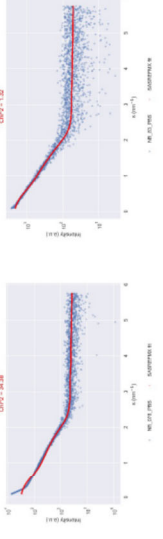
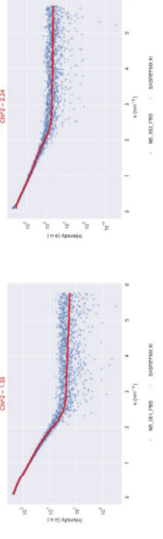
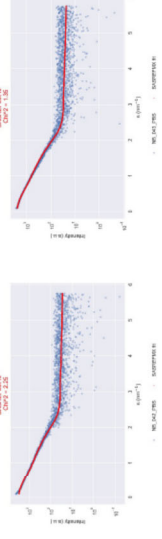
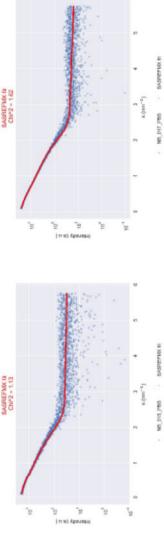
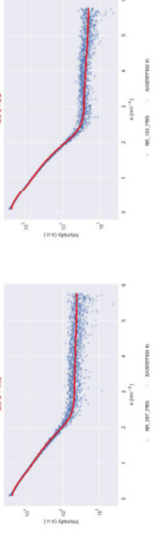
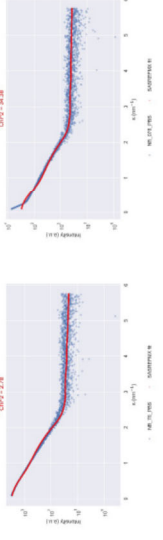
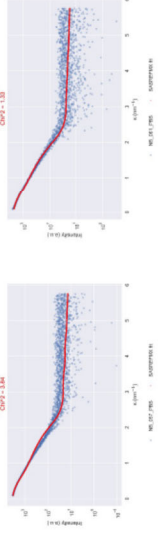
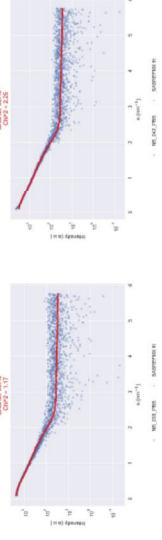
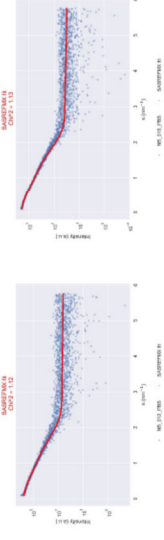
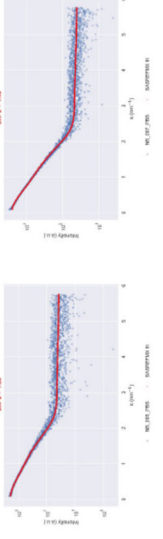
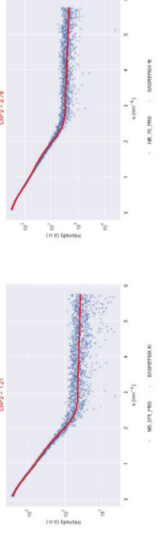
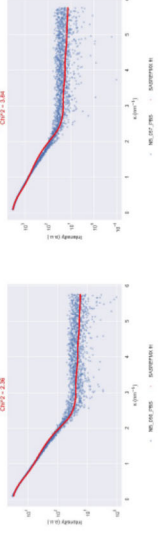
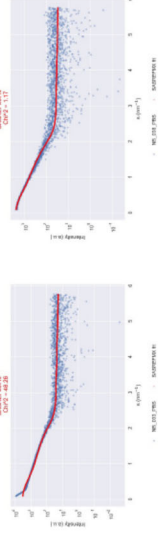
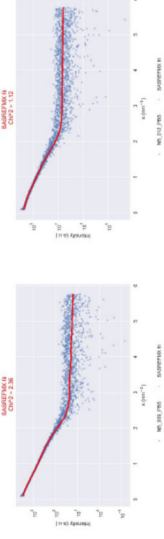
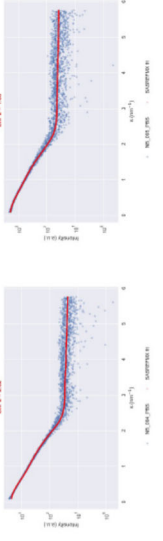
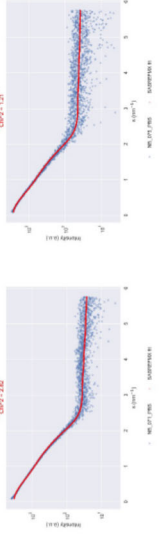
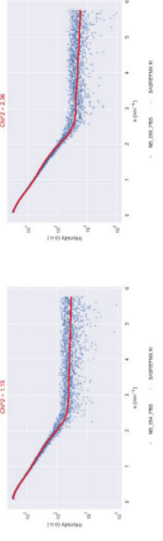
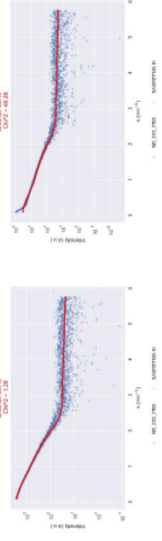
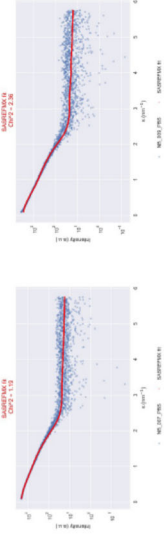
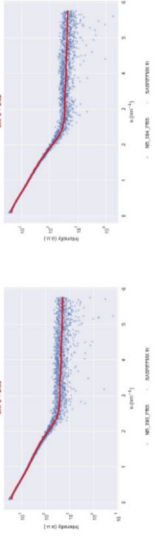
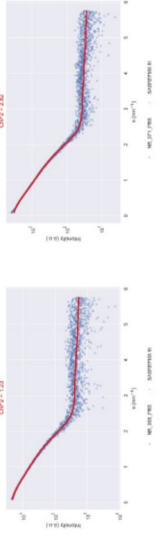
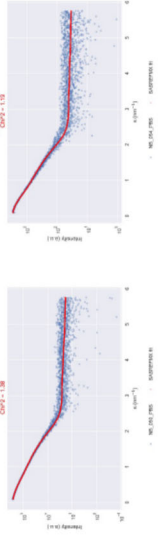
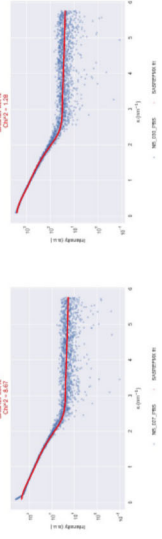
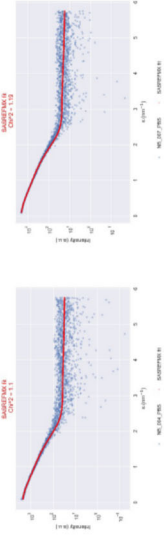
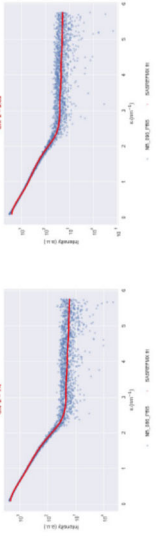
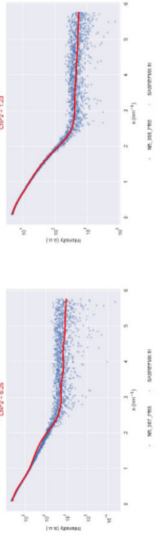
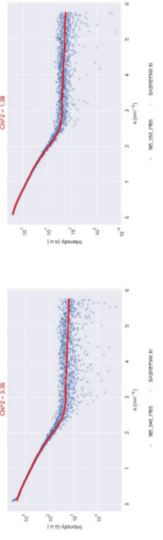
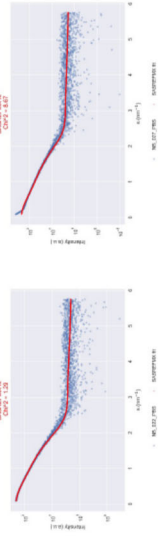
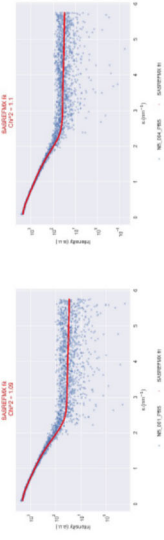
Ab-initio model



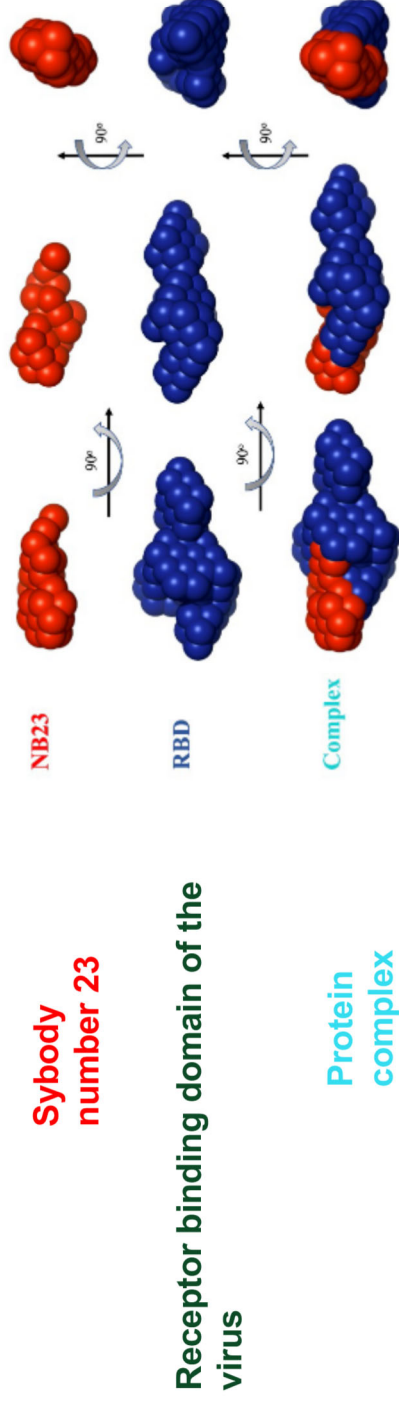
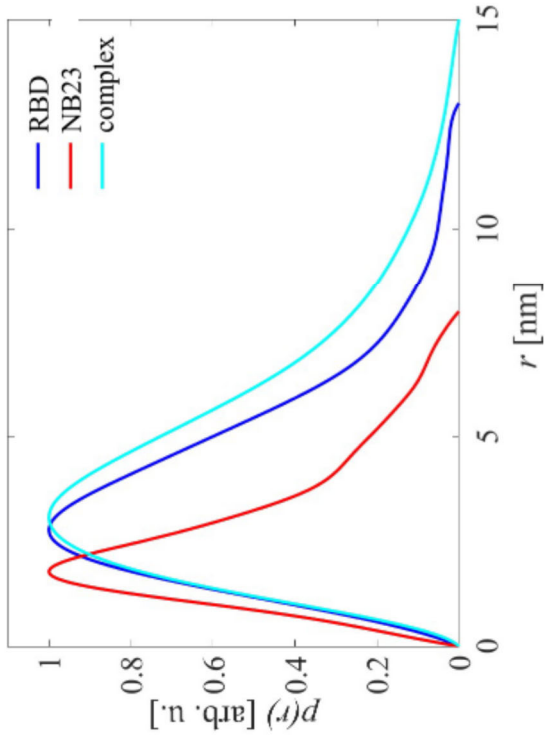
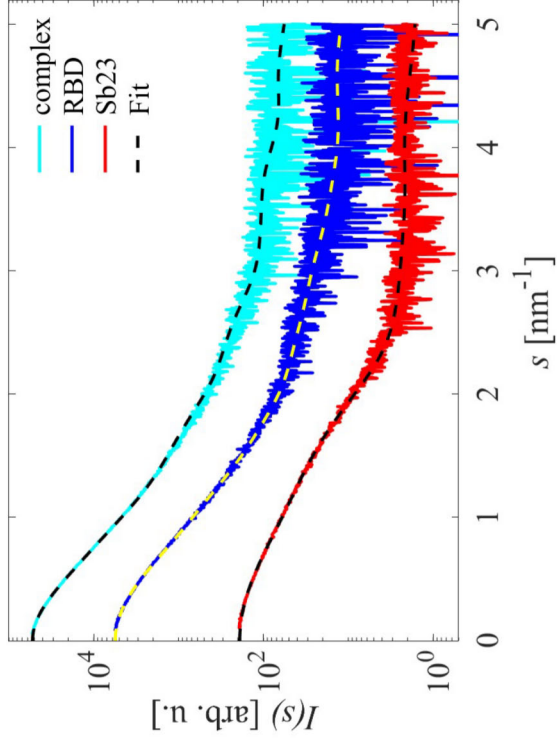
Quality control



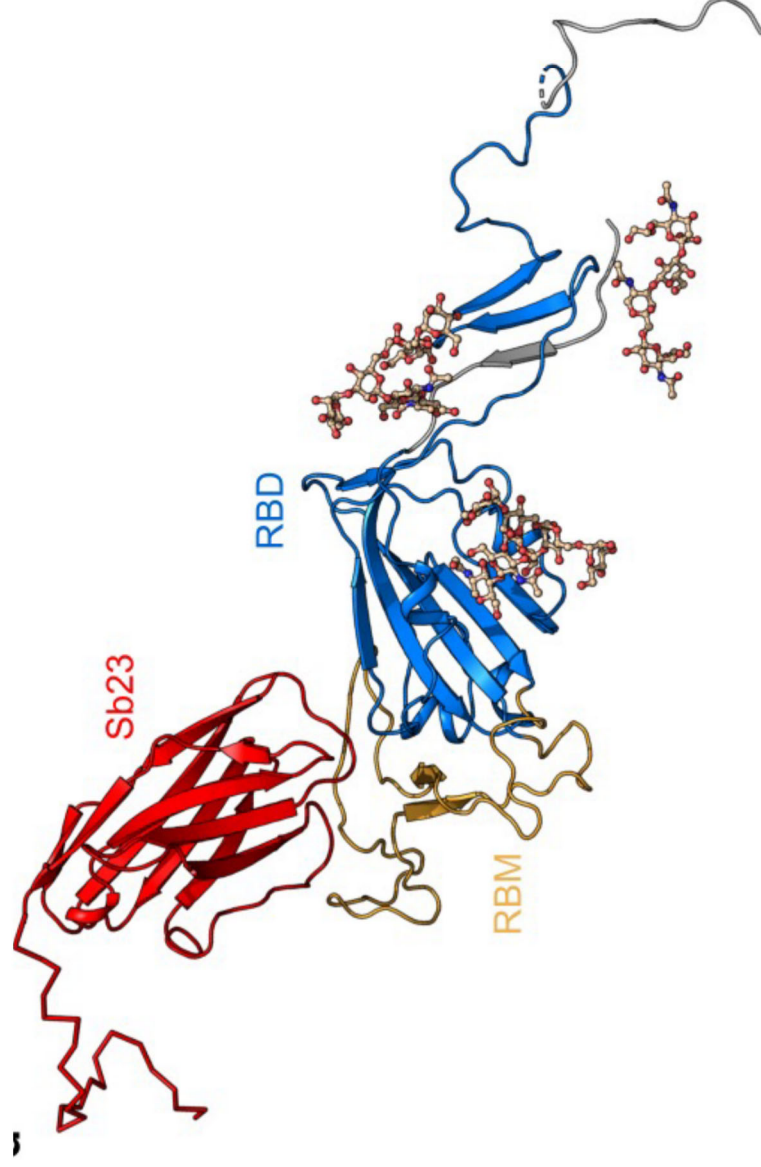
Quality control



Does sybody 23 bind tightly to the virus receptor?

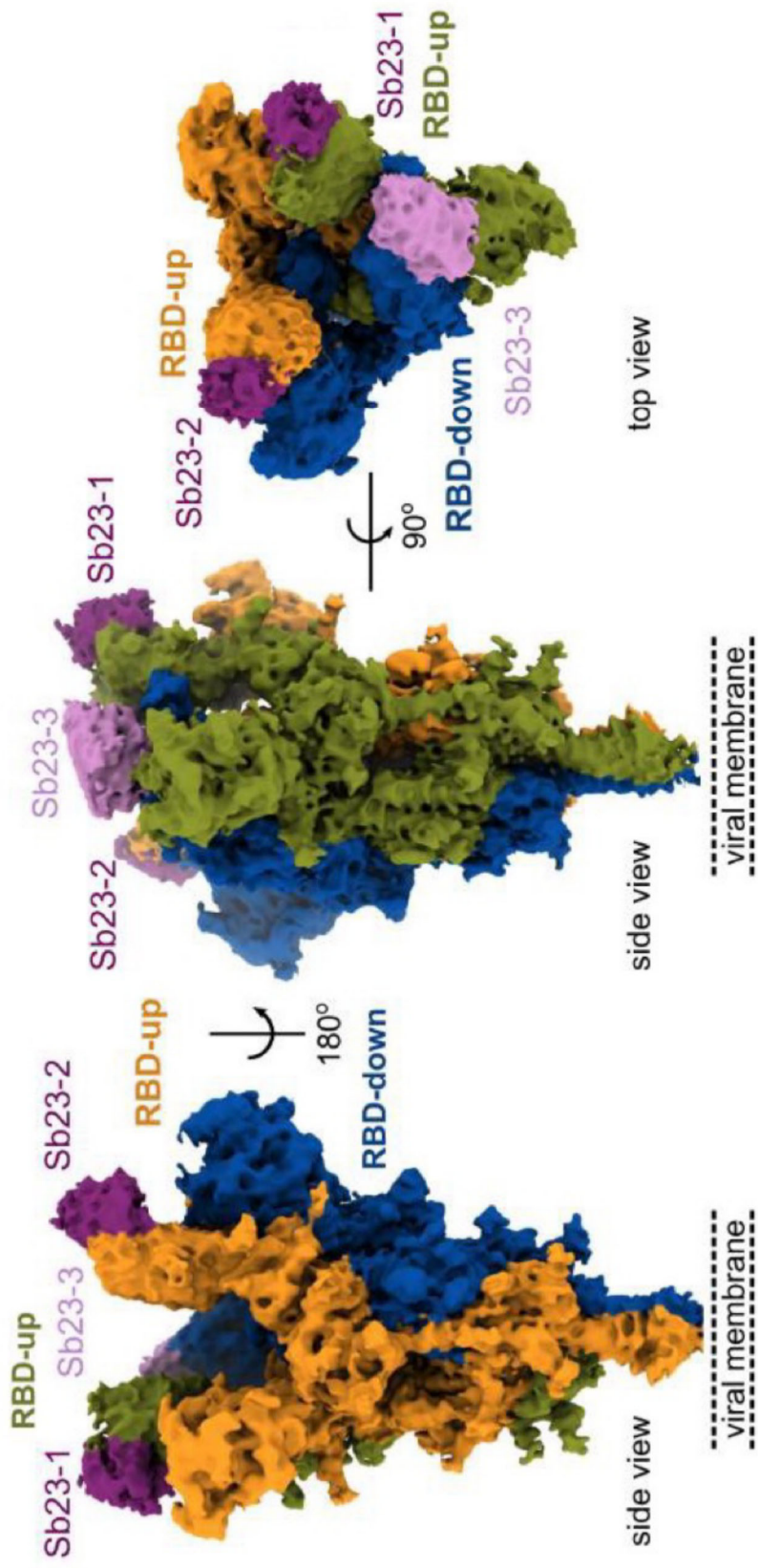


A hybrid SAXS model of RBP bound to Sb23



Sb23 is placed next to the ACE2 binding site and binds sidewise to the RBD as expected for a binder from the concave designed library

Does sybody 23 prevent the virus to bind to human cells?



Sb23 binds in the inner edge of the ACE2 interaction interface of the RBD effectively hindering ACE2 binding