

The role of BioSAXS in the fight against Covid-19

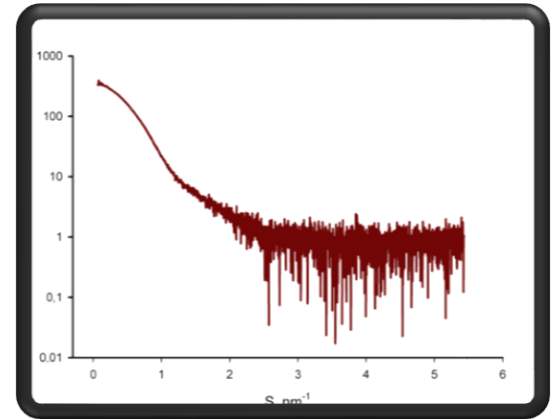
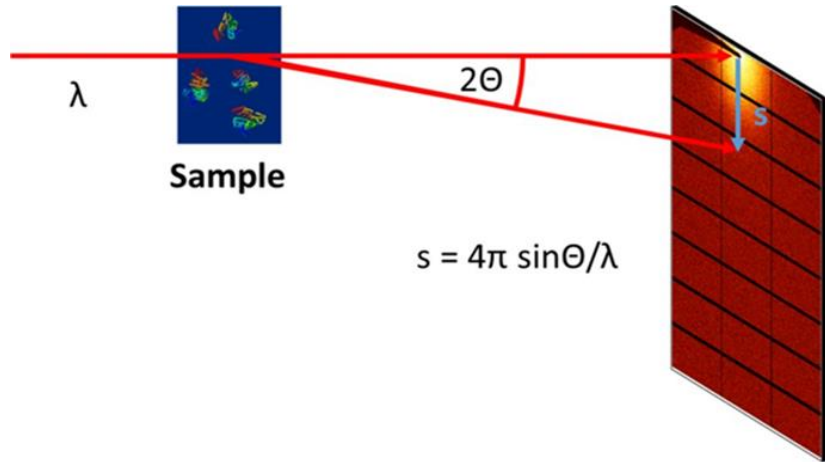
Halos Symposium – 31.01.2022

Clement Blanchet – EMBL Hamburg

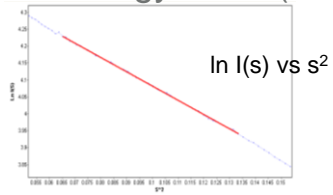


BioSAXS

SAXS on biological molecules in solution

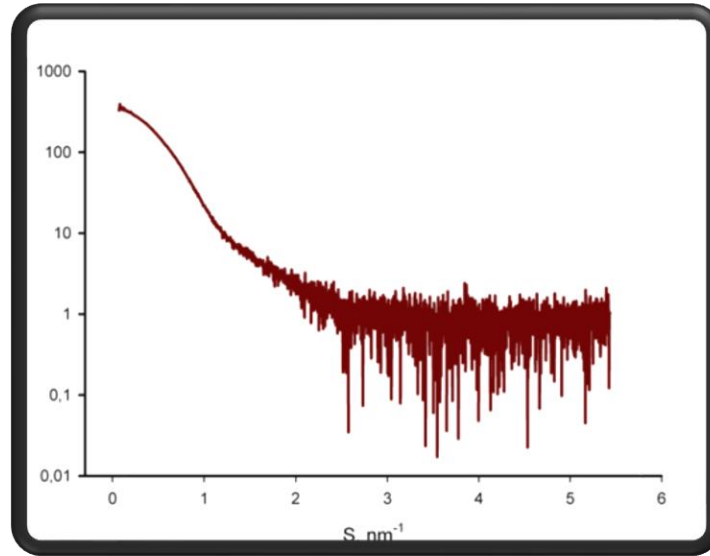


Radius of gyration (Guinier)

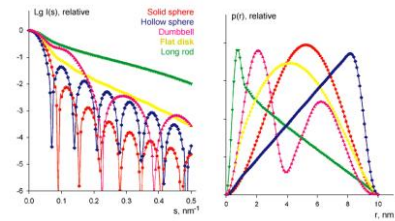


BIOSAXS

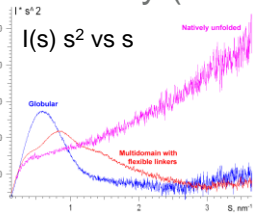
Molecular weight, volume



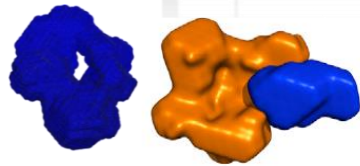
Distance distribution function



Flexibility (Kratky)

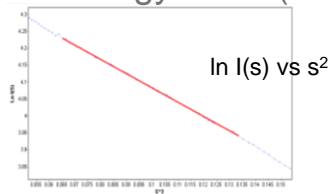


bead models

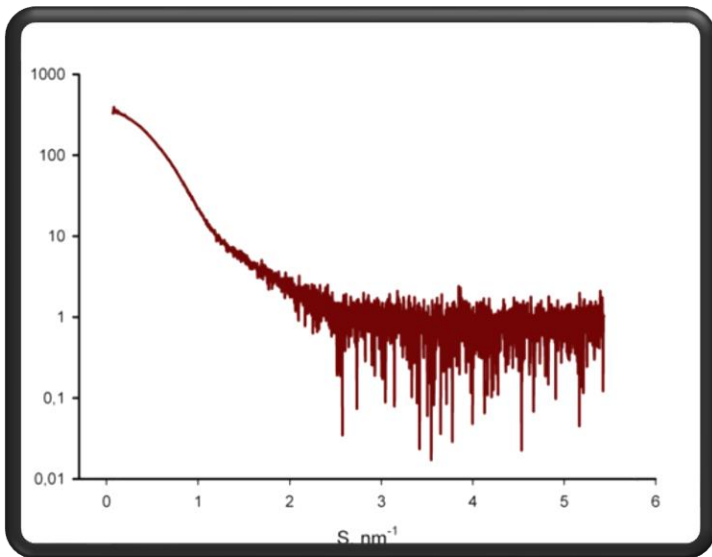


Radius of gyration (Guinier)

BIOSAXS



Molecular weight, volume



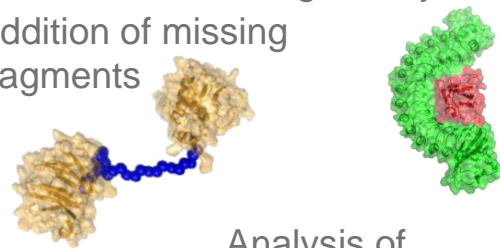
Complementary methods

MS	EM
Crystallography	NMR
Bioinformatics	Biochemistry
AUC	EPR
FRET	



Rigid body modelling

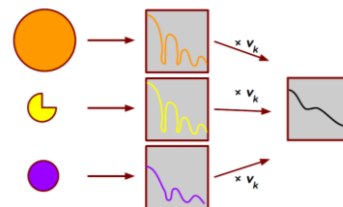
Addition of missing fragments



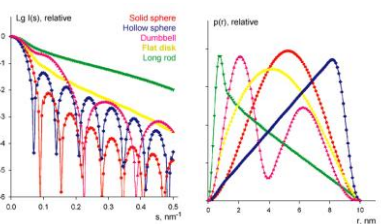
Analysis of flexible systems



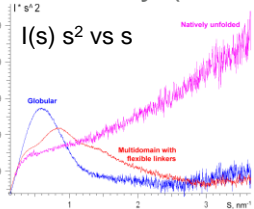
Study of mixtures



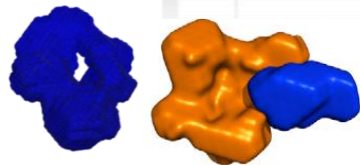
Distance distribution function



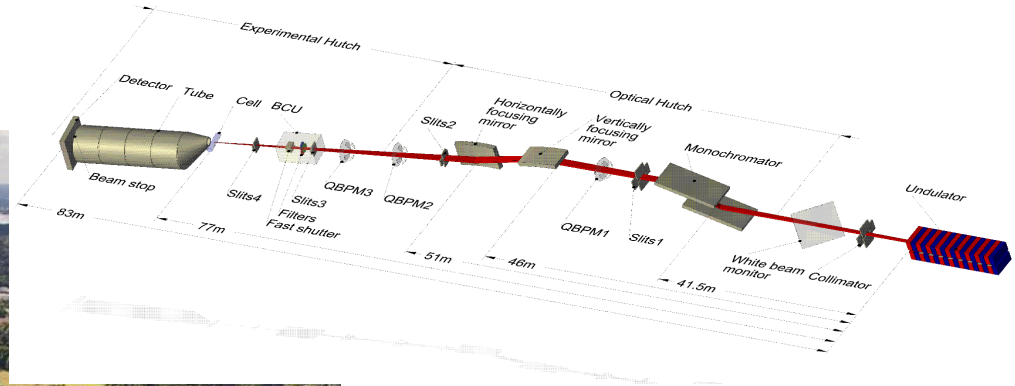
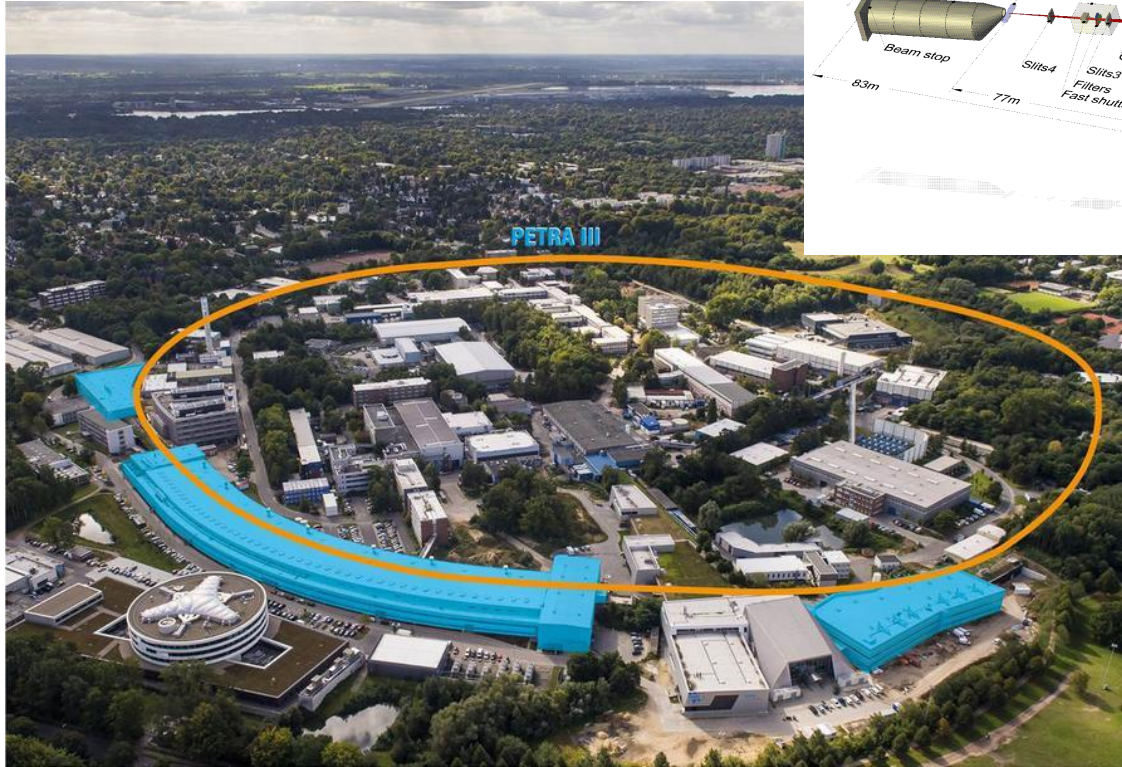
Flexibility (Kratky)



bead models



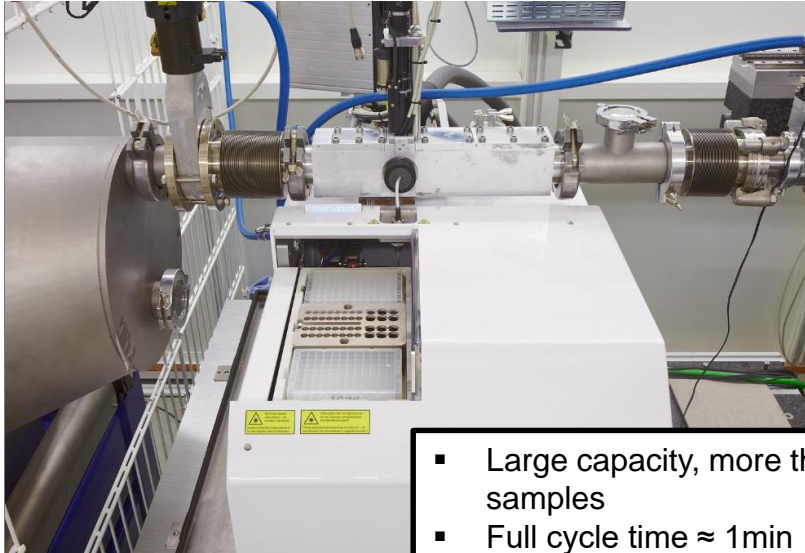
P12 beamline on PETRAIII



- One of the three EMBL beamline on PETRAIII
- Dedicated to BioSAXS

P12 sample environment

Sample changer



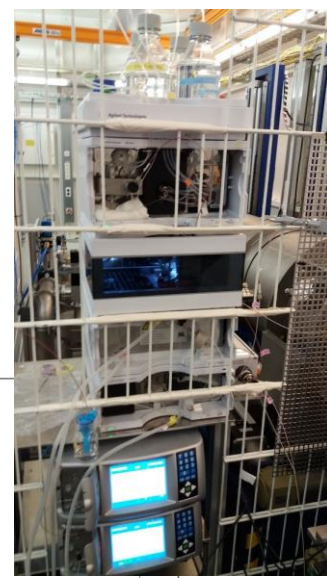
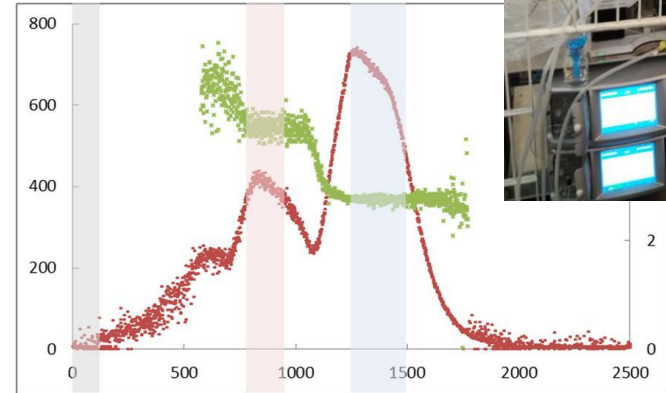
- Large capacity, more than 250 samples
- Full cycle time \approx 1min
- Sample volume: 10 – 30 μ l
- Flow measurement
- Rapid and efficient cell cleaning

EMBL

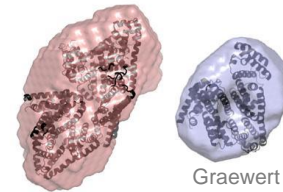


SEC-SAXS

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

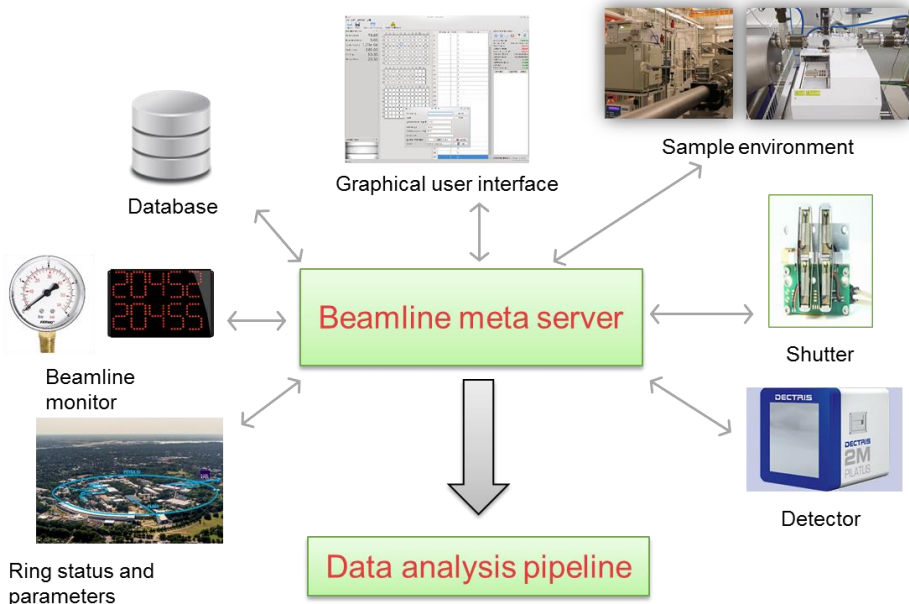


Defined
buffer region



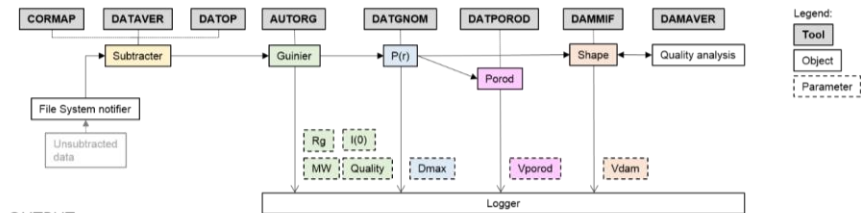
Graewert et al. *Scientific Reports* 5 (2015) 10734.

Automation



Automated data collection...

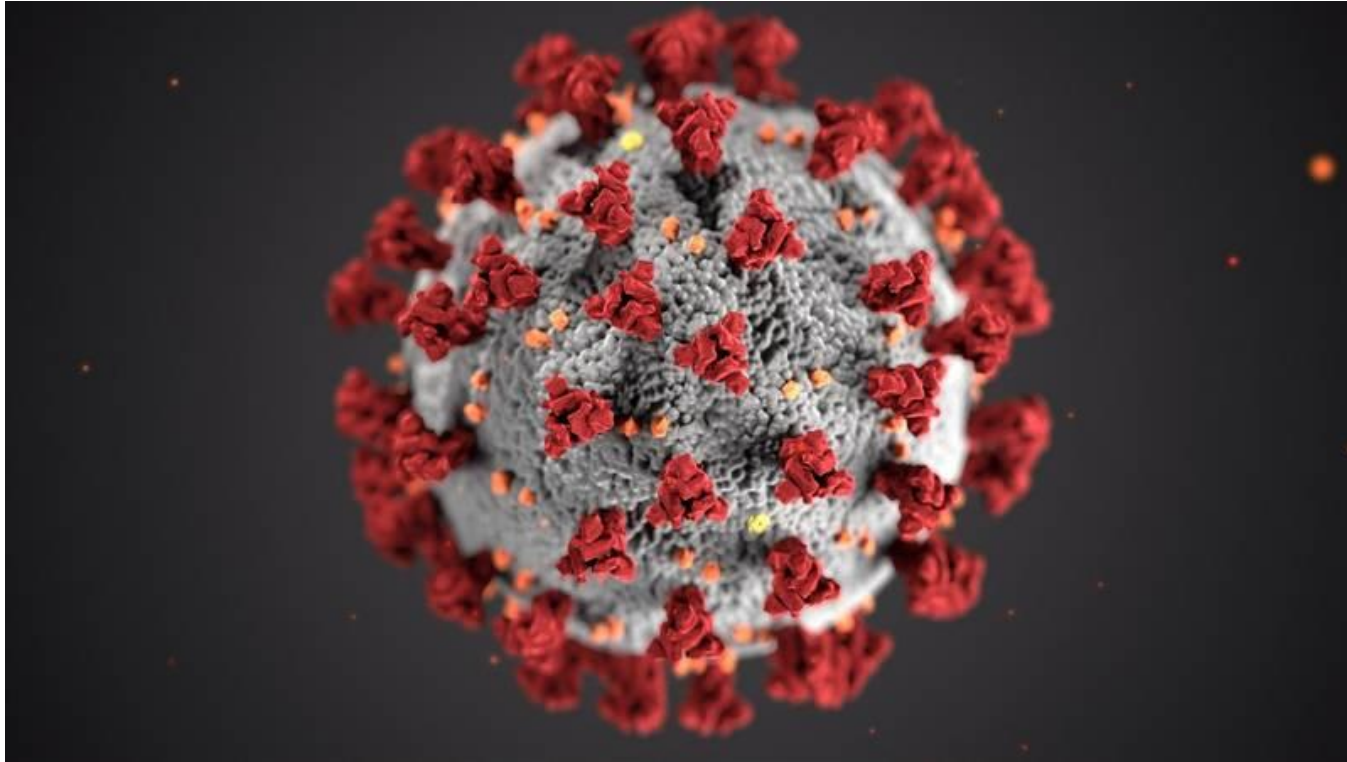
... and data analysis



OUTPUT:

Run#	Description	Code	Conc.	Log plot	Kratky plot	Guinier plot	Quality	R_g	$p(r)$	D_{max}	V_{porod}	V_{dam}	MW_w	MW_z	$MW_{z,w}$	Comments
2	database	cat	15.0				72%	3.9		17.2	306	282	62	105	191	30 out of 100 buffer frames used, 43 out of 50 sample frames used
4	database	cat	7.5				72%	3.9		12.0	312	362	63	72	195	89 out of 100 buffer frames used, 44 out of 50 sample frames used
6	database	cat	3.7				81%	4.4		12.8	325	382	65	80	203	92 out of 100 buffer frames used, 46 out of 50 sample frames used
9	ovalbumin	ova	15.0				69%	2.8		12.7	68	65	32	38	43	92 out of 100 buffer frames used, 44 out of 50 sample frames used
11	ovalbumin	ova	7.5				69%	2.8		8.2	88	82	33	33	55	33 out of 100 buffer frames used, 43 out of 50 sample frames used

Covid-related scientific project

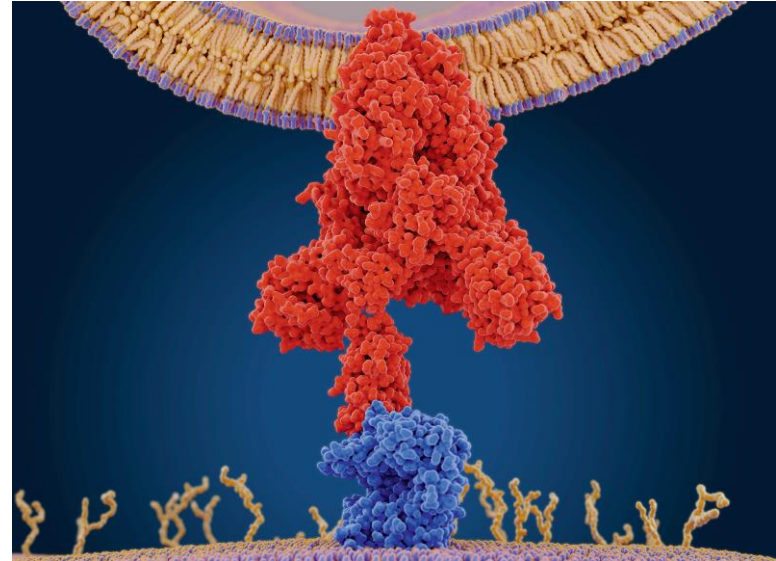


Selection, biophysical and structural analysis of synthetic nanobodies that effectively neutralize SARS-CoV-2

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

Nanobodies from the synthetic library, sybodies (Sb), to target the RBD of the SARS-CoV-2 spike protein are rapidly selected and characterized.

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



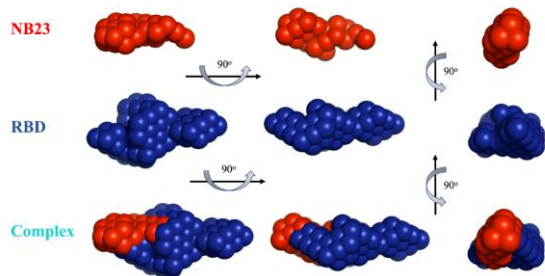
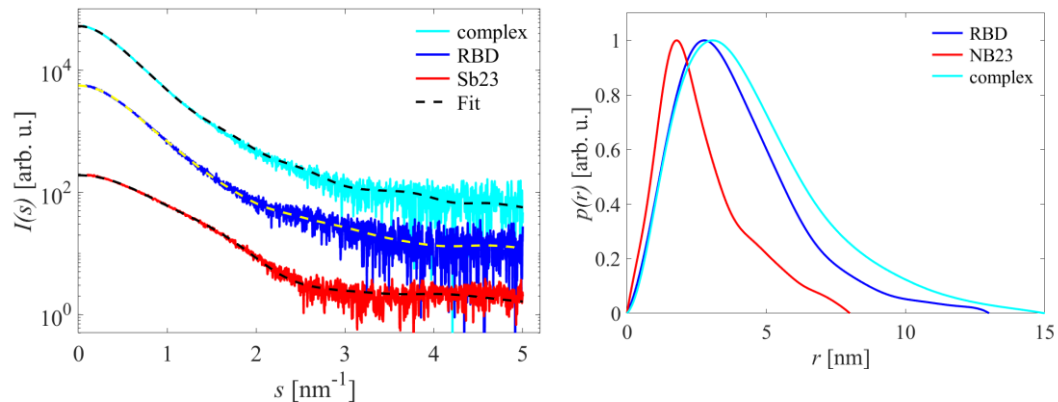
Custódio TF, Das H, Sheward DJ, Hanke L, Pazicky S, Pieprzyk J, Sorgenfrei M, Schroer MA, Gruzinov AY, Jeffries CM, Graewert MA, Svergun DI, Dobrev N, Remans K, Seeger MA, McInerney GM, Murrell B, Hällberg BM, Löw C. *Nat Commun.* 2020 Nov 4; 11(1):5588.

SAXS screening of synthetic nanobodies



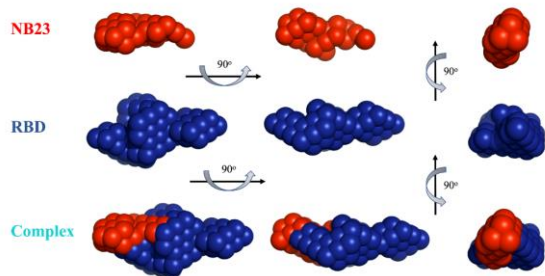
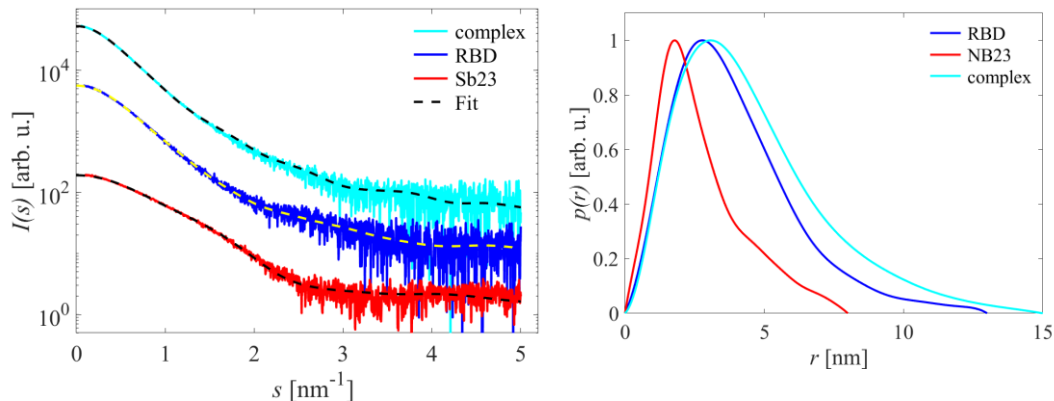
Nr.	MW, Da	c, mg/ml	R _g , nm	MW _{SAXS}	Vfr (mon/dim)	χ ²	Probable state/comment
1	15563.04	0.624170832	2.1	13	0.87/0.13	1.09	Largely monomeric
4	15740.29	0.726381875	2.2	15	0.8/0.2	1.1	Largely monomeric
7	15642.19	1.383658425	2.2	17	0.8/0.2	1.19	Largely monomeric
9	15928.57	1.238969189	2.9	23	0.48/0.52	2.36	Mon/Dim plus long (unspecific) aggregates
12	15966.57	0.481692335	2.1	10	0.84/0.16	1.12	Largely monomeric
15	15708.31	1.007458545	2.2	11	0.85/0.15	1.13	Largely monomeric
17	15584.28	2.310170221	2.5	20	0.61/0.39	1.62	Monomer/dimer equilibrium
22	15584.28	1.663551226	2.1	15	0.85/0.15	1.29	Largely monomeric
23	15748.32	1.833179385	2.2	16	0.81/0.19	2.3	Largely monomeric
27	15652.36	1.433618484	9.9	86	0.65/0.35	8.67	Mon/Dim plus large (specific) aggregates
30	15854.49	1.17029019	2.4	18	0.77/0.3	1.28	Monomer/dimer equilibrium
33	15633.32	1.178573185	10.4	479	0.21/0.79	48.26	Mon/Dim plus large (specific) aggregates
38	16532.16	0.615295516	2.4	16	0.59/0.41	1.17	Monomer/dimer equilibrium
42	16772.42	0.895659391	2.7	22	0.52/0.48	2.25	Mon/Dim plus long (unspecific) aggregates
43	16594.19	1.091047267	2.4	19	0.64/0.36	1.35	Monomer/dimer equilibrium
46	16437.19	1.653220266	2.2	17	0.69/0.31	3.35	Mon/Dim plus long (unspecific) aggregates
50	16718.38	1.810420691	2.3	18	0.72/0.28	1.38	Monomer/dimer equilibrium
54	16678.26	0.878722995	2.6	20	0.55/0.45	1.19	Monomer/dimer equilibrium
56	16579.33	1.856029685	2.8	25	0.39/0.61	2.36	Monomer/dimer equilibrium
57	16490.13	1.352340812	2.6	24	0.42/0.58	3.84	Monomer/dimer equilibrium
61	16783.4	1.097412818	2.4	19	0.67/0.33	1.33	Monomer/dimer equilibrium
62	16670.24	0.536295805	2.8	16	0.31/0.69	2.24	Mon/Dim plus long (unspecific) aggregates
67	16529.22	1.478203966	3.4	35	0.03/0.97	6.25	Mon/Dim plus long (unspecific) aggregates
68	16662.35	2.096041201	2.2	15	0.79/0.21	1.23	Largely monomeric
71	16744.4	2.033805247	2.4	17	0.64/0.36	2.82	Mon/Dim plus long (unspecific) aggregates
75	16813.56	2.903799277	2.6	15	0.6/0.4	1.21	Monomer/dimer equilibrium
76	17034.67	1.025215834	2.5	20	0.45/0.55	2.78	Mon/Dim plus long (unspecific) aggregates
78	16811.58	1.398091412	10.3	53	0.17/0.83	34.38	Mon/Dim plus large (specific) aggregates
83	16740.55	0.636167451	2.6	12	0.55/0.45	1.32	Mon/Dim plus long (unspecific) aggregates
85	16646.34	2.138284755	2.5	20	0.62/0.38	1.4	Monomer/dimer equilibrium
90	16847.6	1.767520997	3.2	24	0.36/0.64	2.89	Mon/Dim plus long (unspecific) aggregates
93	16750.46	2.194328018	2.6	19	0.53/0.47	2.77	Mon/Dim plus long (unspecific) aggregates
94	16822.52	1.595226357	2.7	15	0.55/0.45	2.02	Mon/Dim plus long (unspecific) aggregates
95	16626.35	0.838997398	2.1	13	0.82/0.18	1.06	Largely monomeric
97	16921.6	1.044573482	2.4	12	0.67/0.33	1.46	Mon/Dim plus long (unspecific) aggregates
100	16740.35	2.350144374	2.3	15	0.77/0.3	2.2	Mon/Dim plus long (unspecific) aggregates

Sb23 – Spike RBD complex



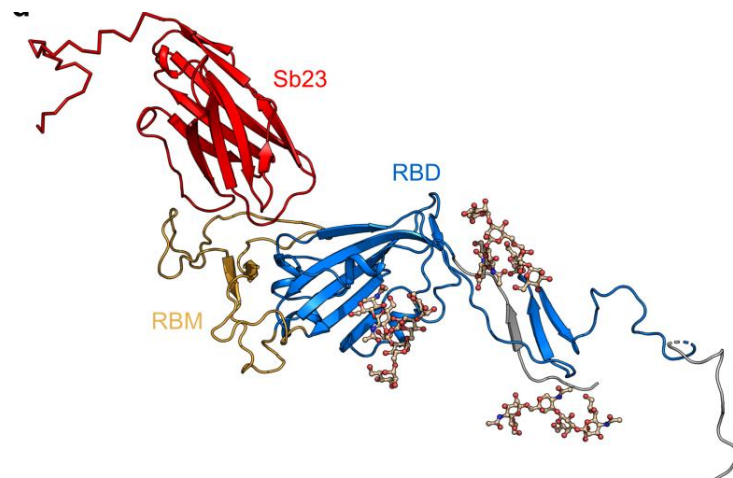
Ab initio multiphase models built from SAXS curves of Sb23, RBD and the complex

Sb23 – Spike RBD complex

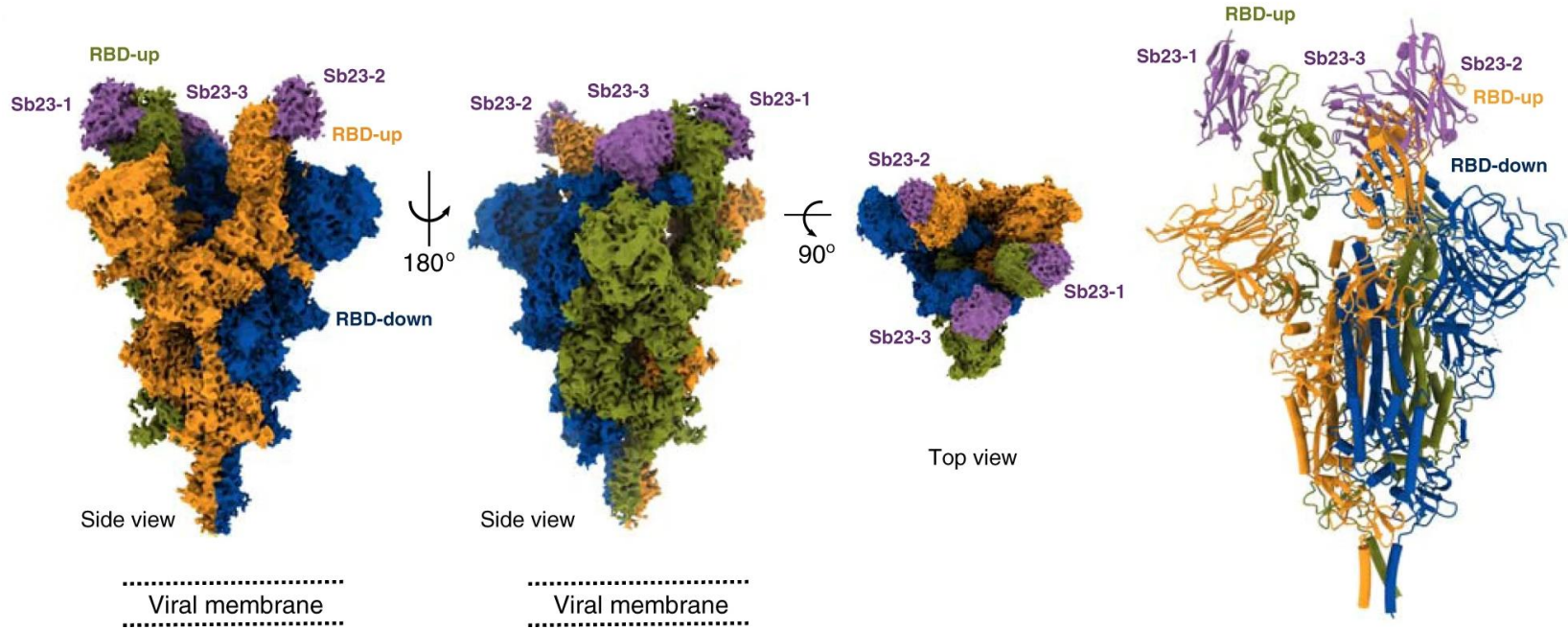


Ab initio multiphase models built from SAXS curves of Sb23, RBD and the complex

Rigid body model from the complex built using SAXS collected on the complex and available high resolution structure of RBD and Sb23

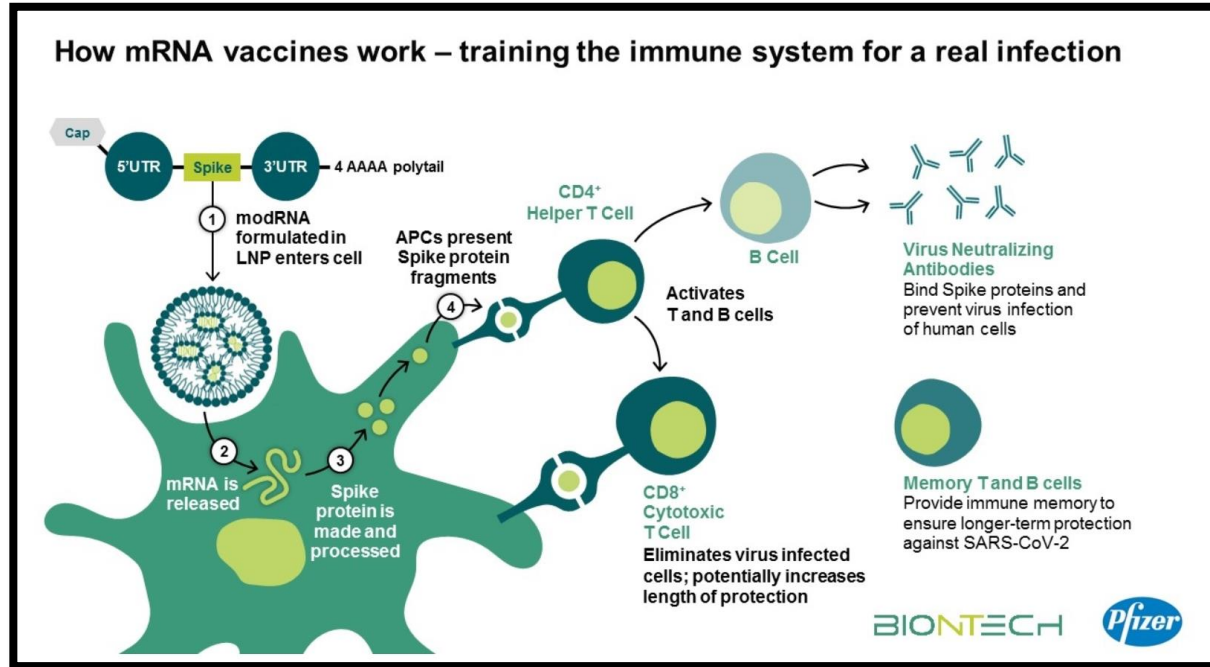


Sybodies



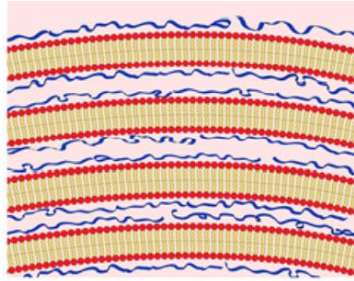
mRNA based vaccine

Idea: mRNA injected into the cell, translated into spike protein → train immune system

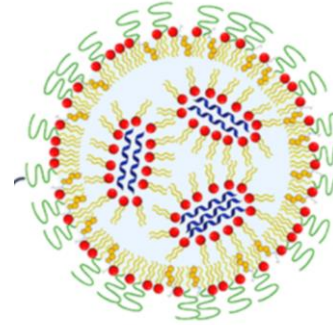
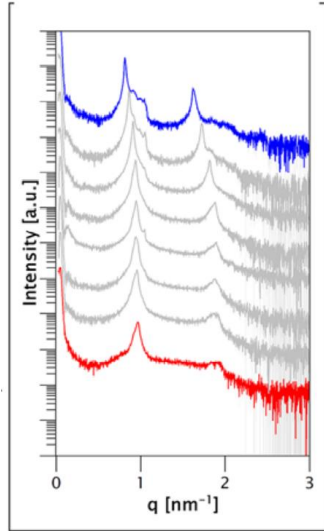


Pb: RNA is very fragile and easily degraded → needs to be protected in lipid nanoparticles

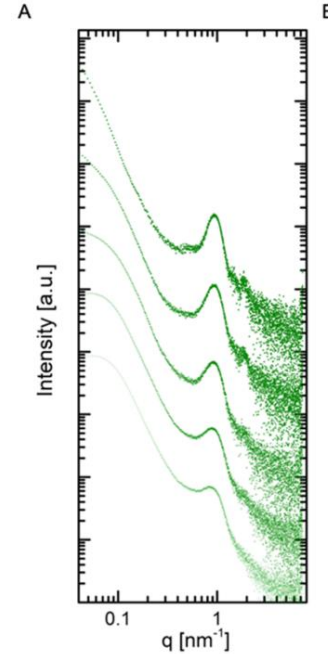
SAXS on lipid-RNA construct



Lipoplex

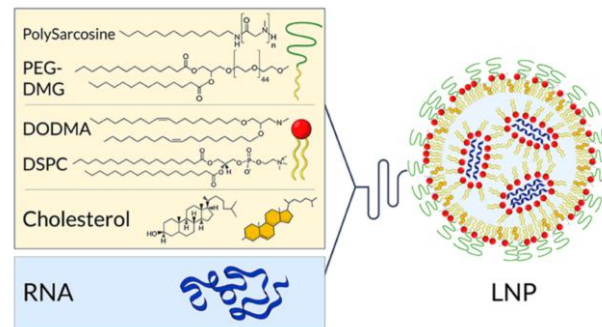
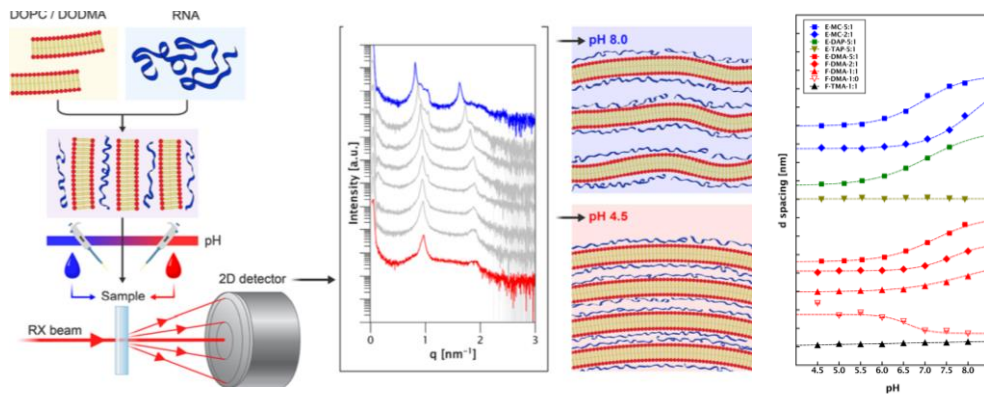


LNP



Positions of the peaks inform on the distances, width on the order.
Fractal dimension computed from small angles

Exploring different pH, compositions, preparations

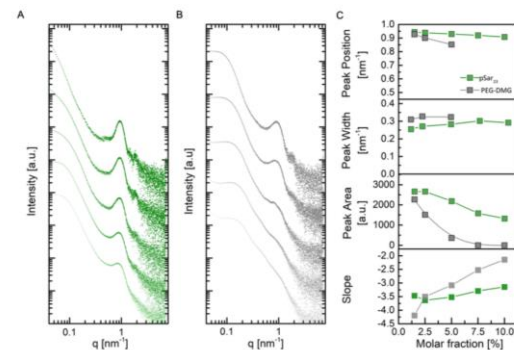


'Protamine Core'	'Protamine Shell'	'Mixed'
Protamine/RNA core	DOTAP/RNA core	Self assembly before mRNA addition
 RNA Protamine + PC _{high} (0.70/1) + DOTAP → PC _{high} (1.70/3)	 RNA DOTAP + PS _{high} (0.8/1) + Protamine → PS _{high} (1.70/3)	 Protamine DOTAP + RNA → MP _{high} (0.70/3)
 RNA Protamine + PC _{low} (0.80/1) + DOTAP → PC _{low} (0.8/1)	 RNA DOTAP + PS _{low} (0.8/1) + Protamine → PS _{low} (0.8/1)	 Protamine DOTAP + RNA → MP _{low} (0.8/1)
 RNA Protamine + PC _{low} (0.3/1) + DOTAP → PC _{low} (0.3/1)	 RNA DOTAP + PS _{low} (0.3/1) + Protamine → PS _{low} (0.3/1)	 Protamine DOTAP + RNA → MP _{low} (0.3/1)
Sequential assembly		Single-step assembly

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BIONTECH

C. D. Siewert, et al. Cells 2020, 9(9), 2034
 S. S. Nogueira et al. ACS Appl. Nano Mater. 2020
 L. Uebbing et al. Langmuir 2020 36 (44), 13331-13341

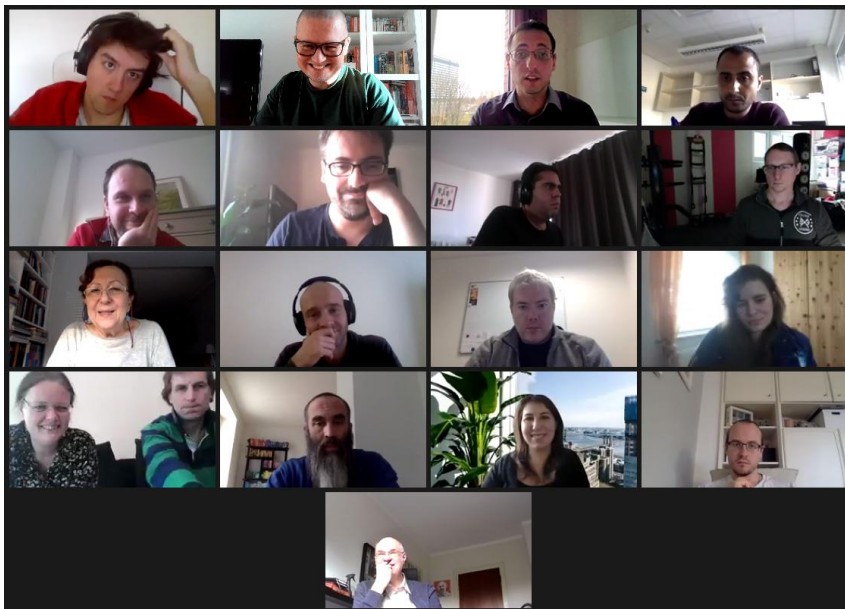


Conclusion

- SAXS can rapidly provide structural information on biological molecules and construct in solution.
- BioSAXS plays a role in the current fight against coronavirus (basic description but also development of therapeutics and vaccine).
- SAXS is not only limited to protein and nucleic acid and can be used to study larger construct, in particular lipid-RNA construct.

Acknowledgements

SAXS Group
EMBL Hamburg
Dmitri Svergun
Clemente Borges
Taja Cheremnykh
Stefano Da Vela
Daniel Franke
Melissa Gräwert
Tobias Gräwert
Andrey Gruzinov
Cy Jeffries
Al Kikhney
Petar Markov
Haydyn Mertens
Ahmed Mohammed
Dima Molodenskiy
Martin Schroer



BIONTECH

Heinrich Haas



Peter Langguth
Lukas Uebbing



Christian Loew and
collaborators



