

BioSAXS on 4th generation synchrotron source

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BioSAXS @ EMBL Hamburg

SAXS Group

EMBL Hamburg

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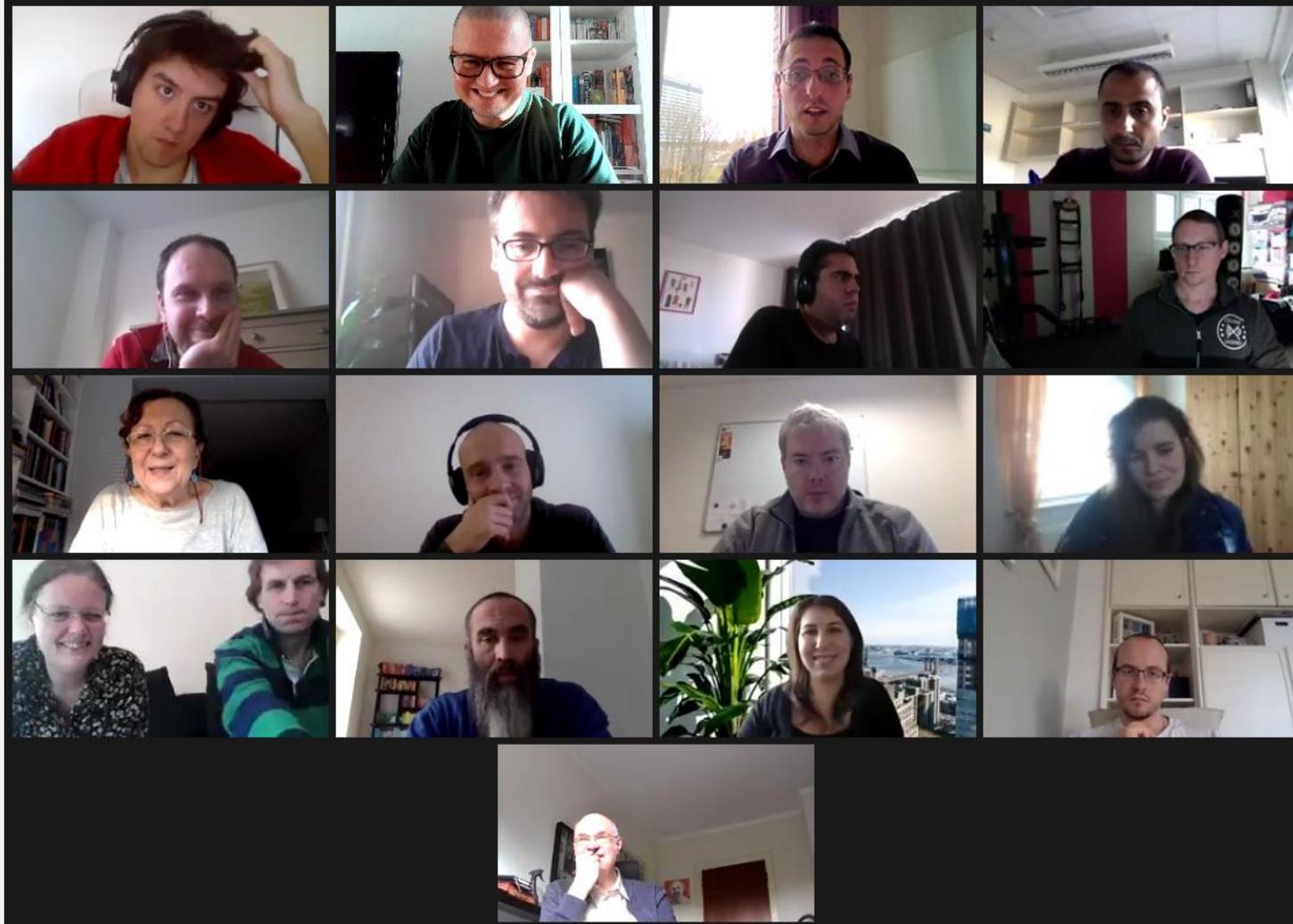
Petar Markov

Haydyn Mertens

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Dima Molodenskiy

Martin Schroer



Instrumentation team

EMBL Hamburg

Stefan Fiedler

Elias Ben Boehme

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Jochen Meyer

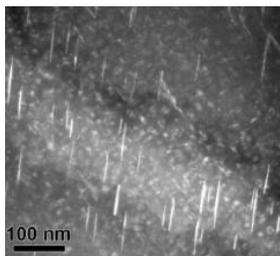
Marina Nikolova

Vamsee Krishna Palnati

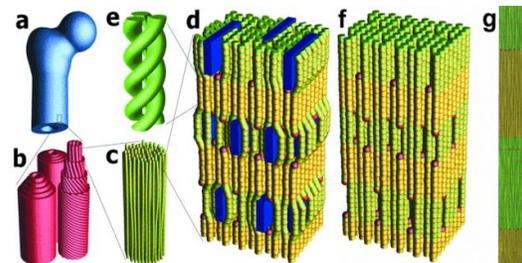
Uwe Ristau

Small Angle X-ray scattering

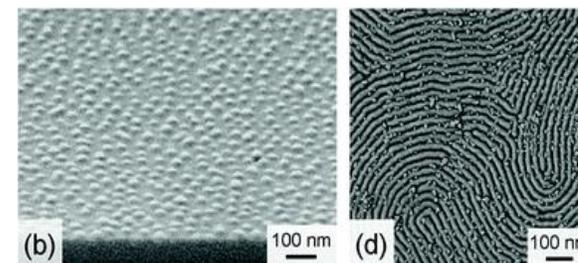
Metal alloys



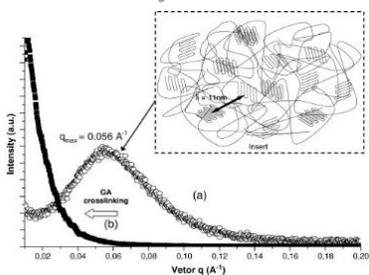
Tissues



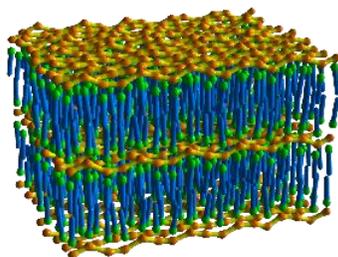
Nanomagnetic materials



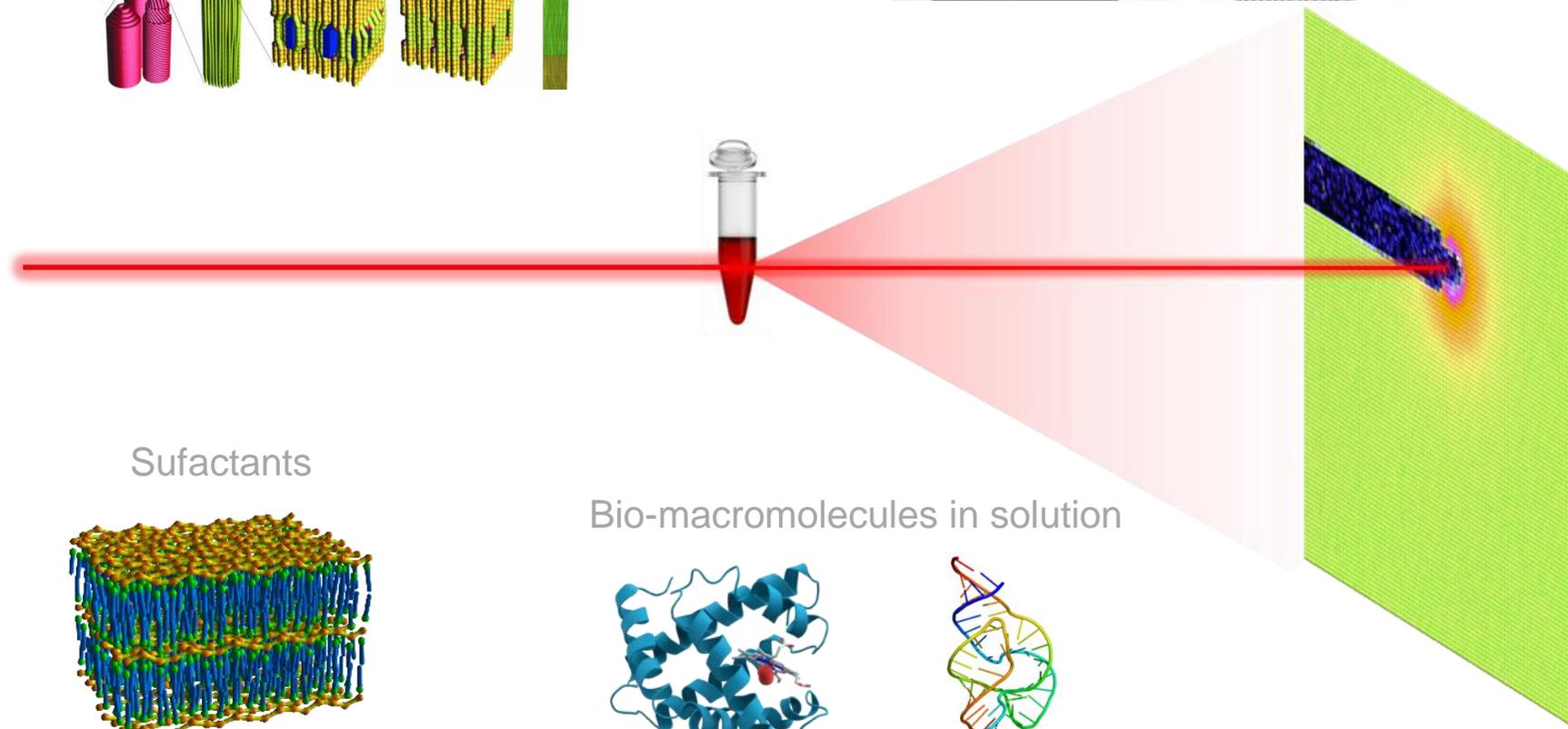
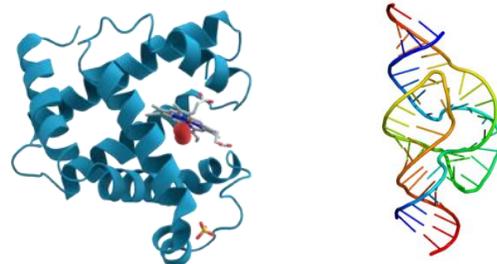
Polymers



Surfactants



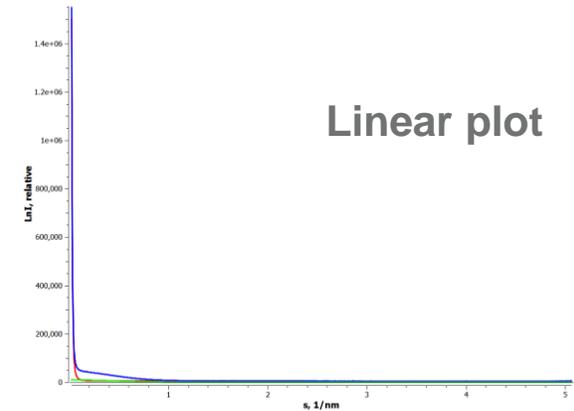
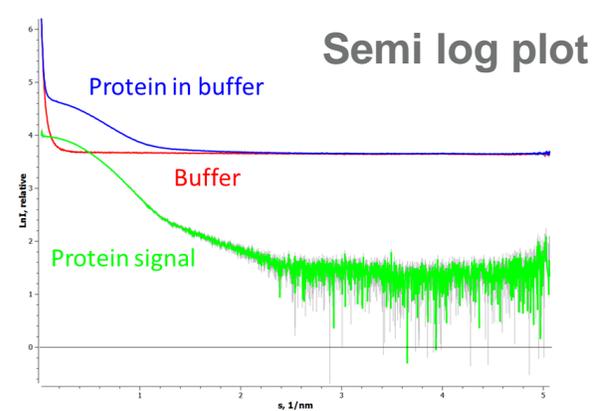
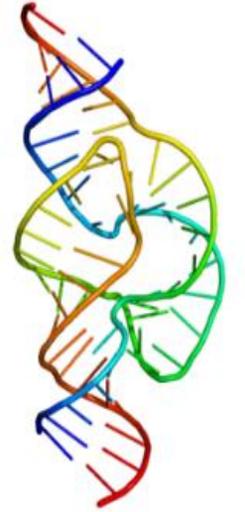
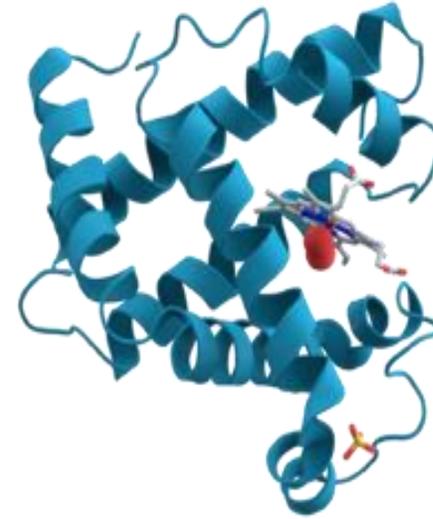
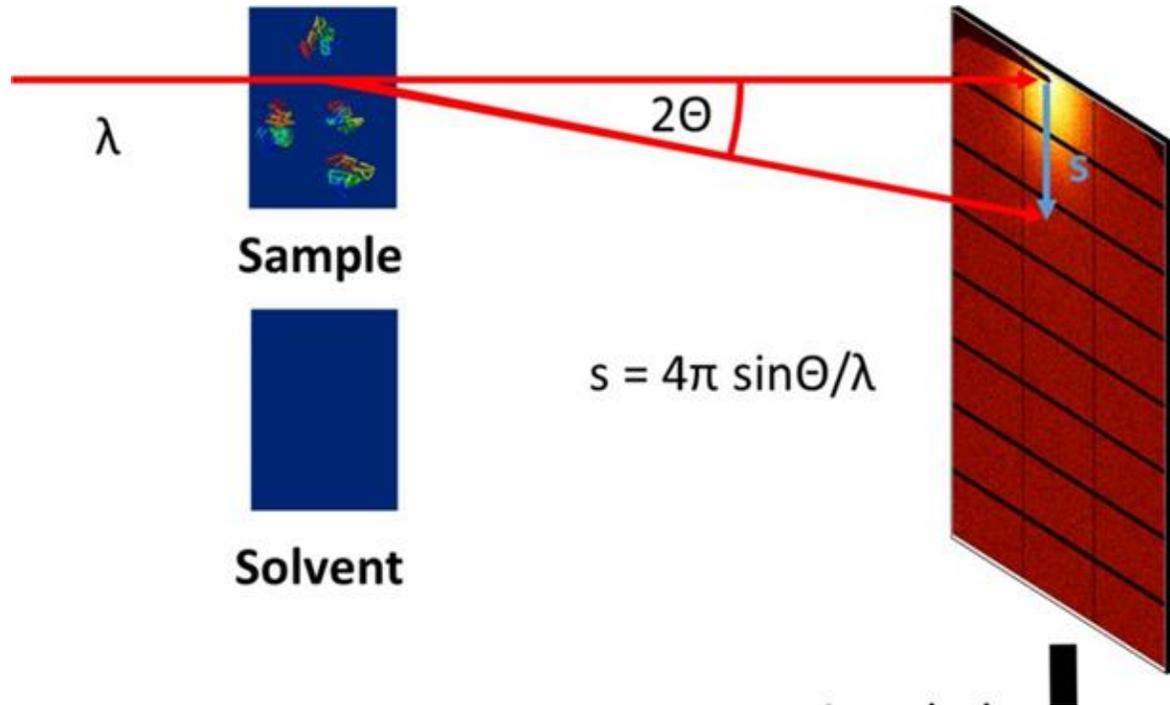
Bio-macromolecules in solution



BioSAXS

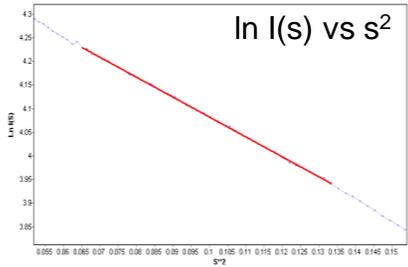
SAXS on biological molecules in solution

- Weakly scattering
- Fragile, limited sample volume

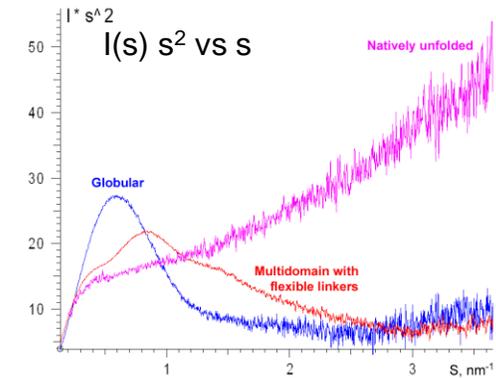


Information directly obtainable from the data

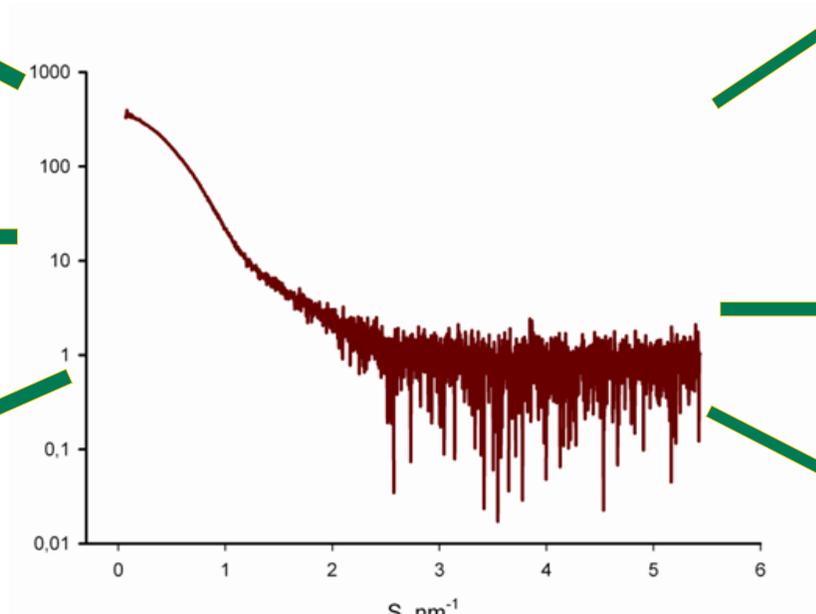
Radius of gyration (Guinier)



Flexibility (Kratky)

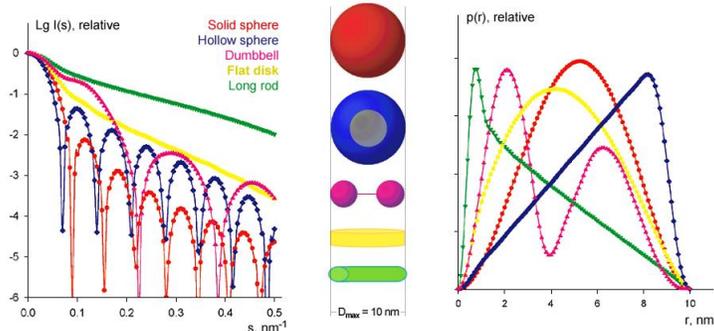


Molecular weight (forward scattering)

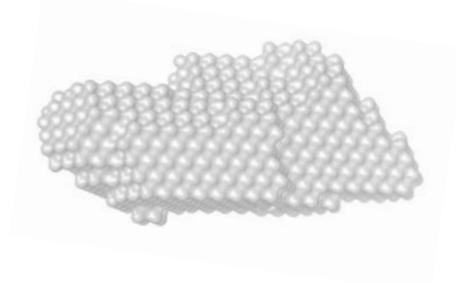


Volume (Porod Invariant)

Distance distribution function

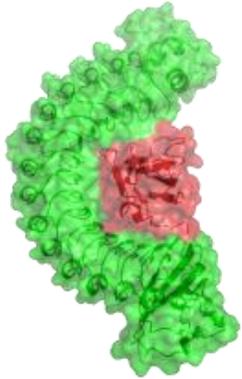


Bead model

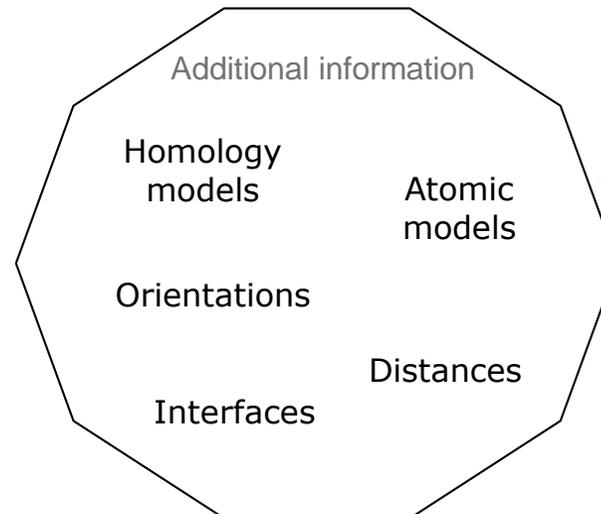
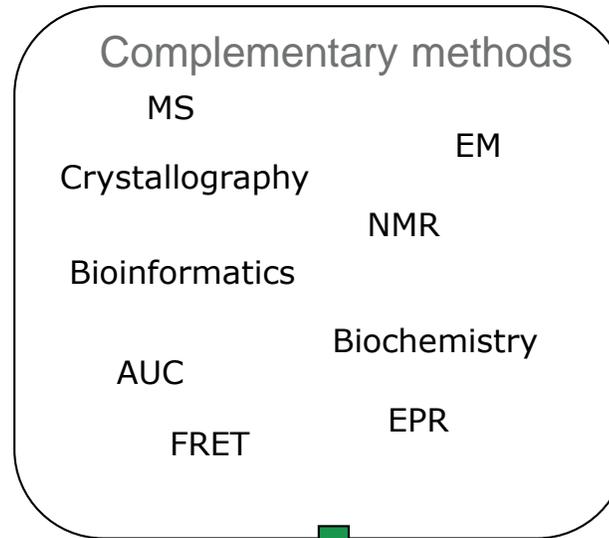
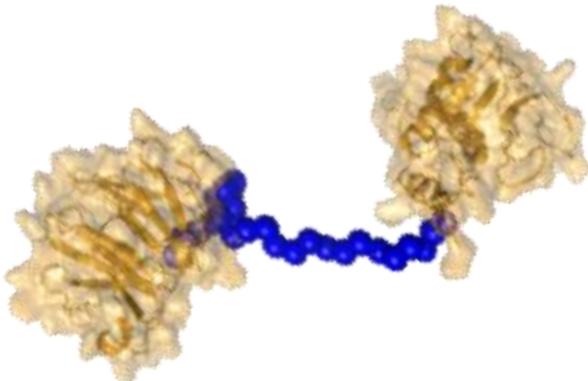


Modelling using complementary methods

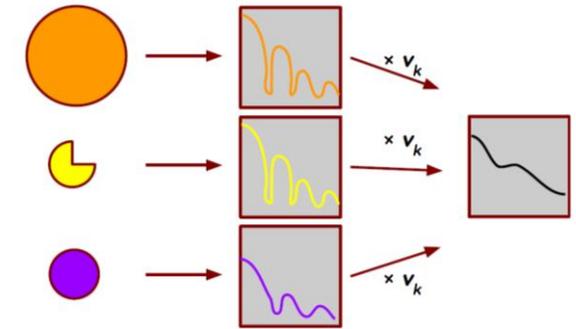
Rigid body modelling



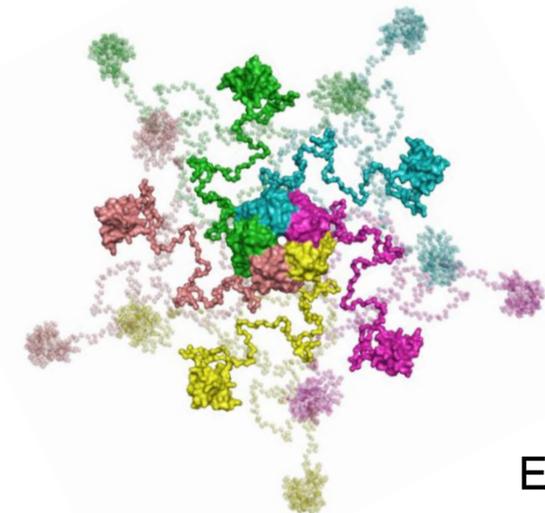
Addition of missing fragments



Study of mixtures

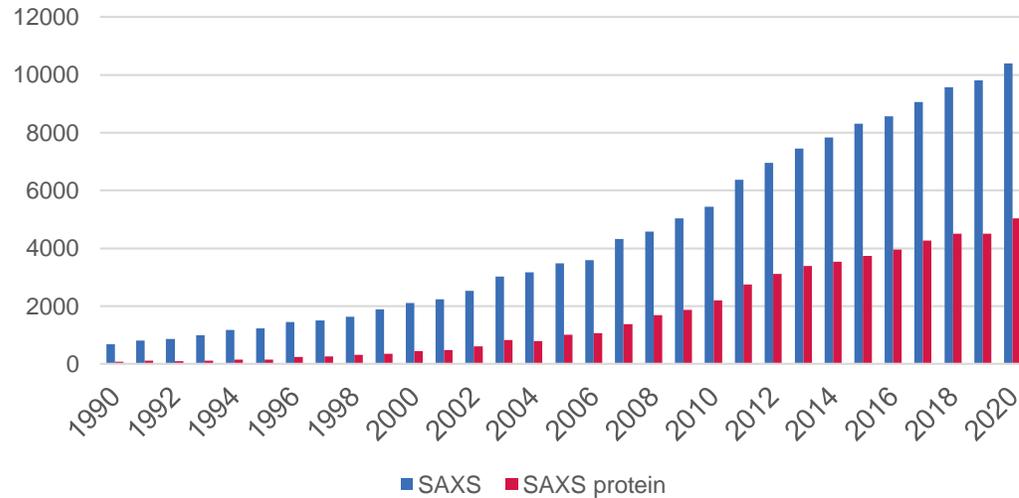


Analysis of flexible systems



An active BioSAXS user community

Number of results per year for "SAXS" and "SAXS protein" on google scholar



Publication guidelines for structural modelling of small-angle scattering data from biomolecules in solution

David A. Jacques,^a J. Mitchell Guss,^{a*} Dmitri I. Svergun,^b and Jill Trewhella^a

2017 publication guidelines for structural modelling of small-angle scattering data from biomolecules in solution: an update

Jill Trewhella,^{a*} Anthony P. Duff,^b Dominique Durand,^c Frank Gabel,^d J. Mitchell Guss,^a Wayne A. Hendrickson,^e Greg L. Hura,^f David A. Jacques,^g Nigel M. Kirby,^h Ann H. Kwan,^a Javier Pérez,ⁱ Lois Pollack,^j Timothy M. Ryan,^h Andrej Sali,^k Dina Schneidman-Duhovny,^l Torsten Schwede,^m Dmitri I. Svergun,ⁿ Masaaki Sugiyama,^o John A. Tainer,^p Patrice Vachette,^c John Westbrook^q and Andrew E. Whitten^b

Graewert, M. A., & Svergun, D. I. (2013). *Current opinion in structural biology*, 23(5), 748-754.

(c)

- (1) BioCAT at APS (Argonne National Laboratory) <http://small-angle.aps.anl.gov/>
- (2) SIBYLS beam line (BL123.1) at Advanced Light Source http://bl1231.als.lbl.gov/saxs_protocols/
- (3) G1- BioSAXS at MacCHESS <http://www.macchess.cornell.edu/MacCHESS/biosaxs.html>
- (4) SSRL Beam Line 4-2 at SLAC-National Accelerator Laboratory <http://ssrl.slac.stanford.edu/~saxs/index.htm>
- (5) Beamline X9 at National Synchrotron Light Source <http://beamlines.ps.bnl.gov/beamline.aspx?blid=X9>
- (6) SAXS 1/2 beamline at Brazilian Synchrotron Light Laboratory (LNLS) <http://lnls.cnpm.br/saxs/>
- (7) European Synchrotron Radiation Facility: ID2 <http://www.esrf.eu/UsersAndScience/Experiments/SoftMatter/ID02>
- (8) BL45XU-SAXS at Spring8 <http://www.spring8.or.jp/wknl/>
- (9) SAXS/WAXS beamline at The Australian Synchrotron <http://www.synchrotron.org.au/index.php/aussyncbeamlines/saxswaxs>
- (10) Beamline P12, EMBL-Hamburg, Petra III (DESY synchrotron) <http://www.embl-hamburg.de/biosaxs/p12>
- (11) I22 at Diamond Light Source <http://www.diamond.ac.uk/Home/Beamlines/small-angle/I22.html>
- (12) SWING beamline at SOLEIL synchrotron <http://www.synchrotron-soleil.fr/portal/page/portal/Recherche/LignesLumiere/SWING>

SASBDB
Small Angle Scattering Biological Data Bank

Home Browse Submit data About SASBDB Help

Advanced search E.g. P00921, BSA, Nucleic Acids Res

Curated repository for small angle scattering data and models

Small angle scattering (SAS) of X-ray and neutrons provides structural information on biological macromolecules in solution at a resolution of 1-2 nm. SASBDB is a fully searchable curated repository of freely accessible and downloadable experimental data, which are deposited together with the relevant experimental conditions, sample details, derived models and their fits to the data.

SASBDB currently contains:
2063 experimental data sets
3098 models
532 experimental data sets on hold
561 models on hold

Recent depositions:

SASD JL6 – Sarcomeric F-actin crosslinking protein α -actinin-2 (spectrin repeat rod domain, rod- α -actinin-2)

Sample: Rod domain of α -actinin-2 dimer, 112 kDa *Homo sapiens* protein R_g Guinier 6.7 nm

Buffer: 50 mM Tris-HCl 150 mM NaCl, pH: 7.5 D_{max} 27.2 nm

Experiment: SAXS data collected at EMBL P12, PETRA III on 2017 Dec 5 Volume Porod 214 nm³

Order from disorder in the sarcomere: FATZ forms a fuzzy but tight complex and phase-separated condensates with α -actinin. *Sci Adv* 7(22) (2021)

Sponga A, Arolas JL, Schwarz TC, Jeffries CM, Rodriguez Chamorro A, Kostan J, Ghisleni A, Drepper F, Polyansky A, De Almeida Ribeiro E, Pedron M, Zawadzka-Kazmierczuk A, Mlynek G, Peterbauer T, Doto P, Schreiner C, Holler E, Mateos B, Geist L, Faulkner G, Kozminski W, Svergun DI, Warscheid B, Zagrovic B, Gautel M, Konrat R, Djinovic-Carugo K

The truncated RTX domain of *S. pneumoniae* *J Biol Chem* :100833 (2021 May 26)

SAXS data for the complex of *S. pneumoniae* *Scientific Reports* 11(1) (2021 May 26)

Full length SRP Alu RNA from *S. pneumoniae* *Commun Biol* 4(1):600 (2021 May 26)

Probable S-adenosyl-L-methionine *Acta Crystallographica Section D* 17(1) (2021 May 26)

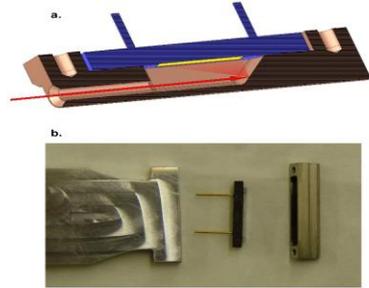
HIV-1 Primer Binding Site (PDB: 1H97) *Nucleic Acids Res* (2021 May 12)

P12 BioSAXS beamline on PETRAIII

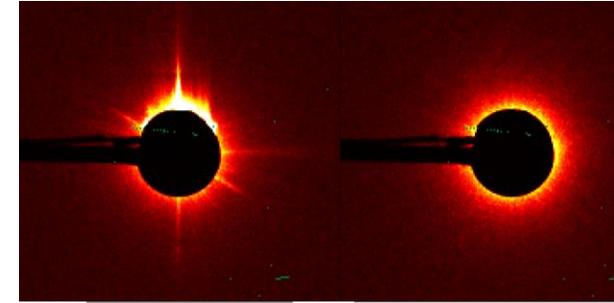
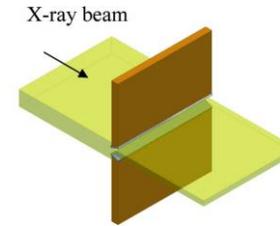
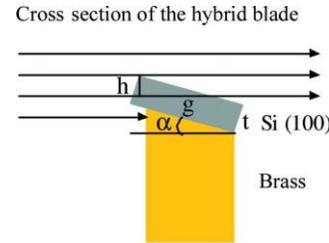
Photon counting detector



Active beamstop

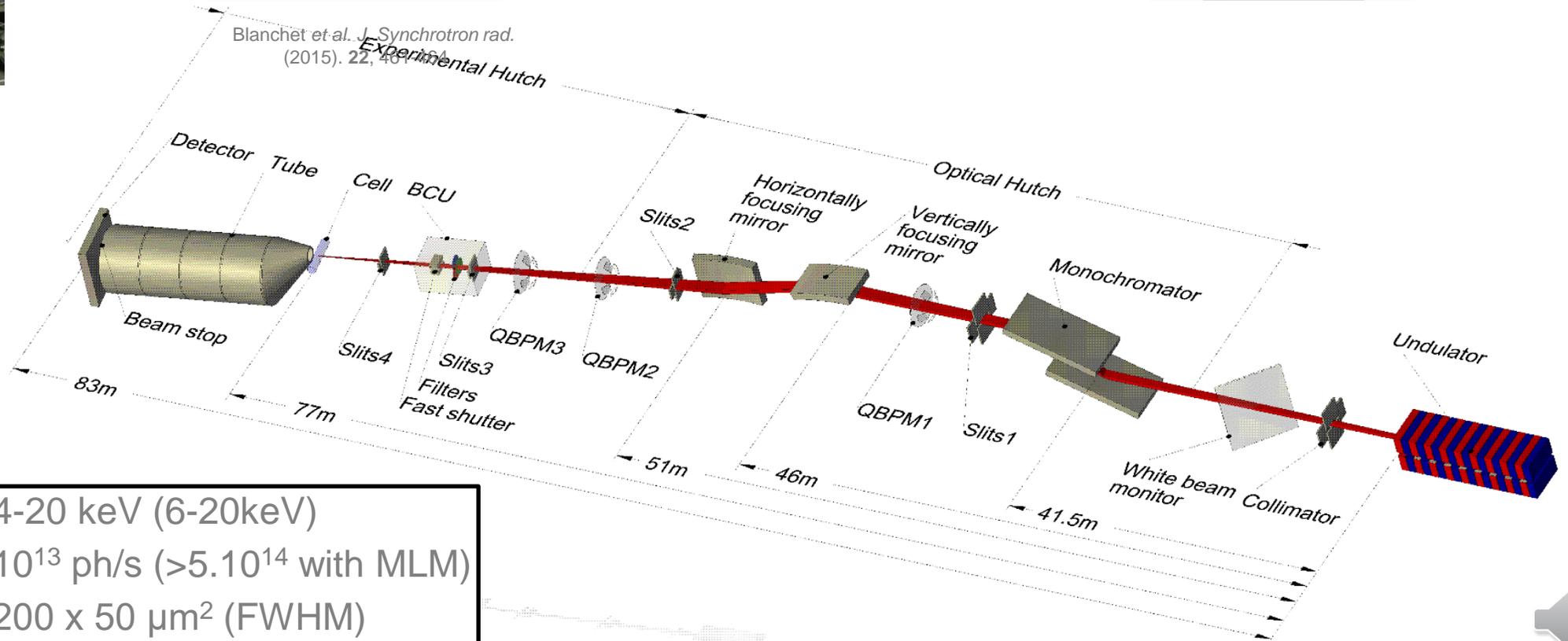


Scatterless slits



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

Blanchet et al. J. Synchrotron rad. (2015). 22, 401-404

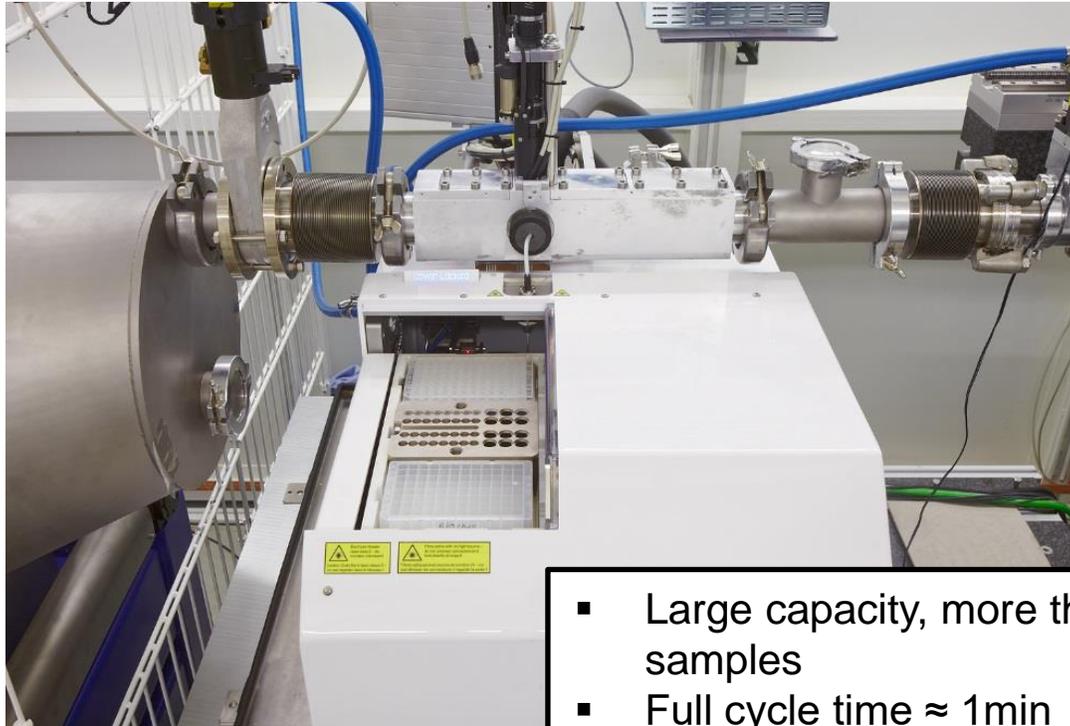


Energy	4-20 keV (6-20keV)
Flux	10^{13} ph/s ($>5 \cdot 10^{14}$ with MLM)
Beam Size	$200 \times 50 \mu\text{m}^2$ (FWHM)

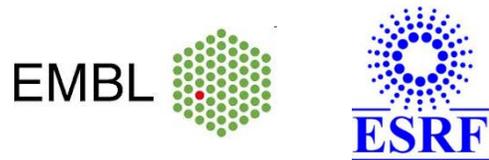


P12 sample environment

Sample changer

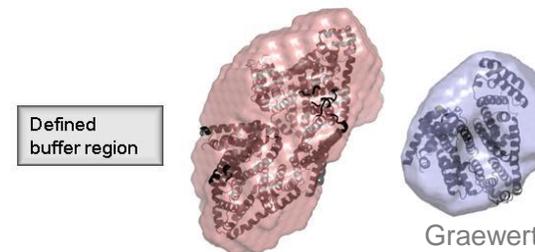
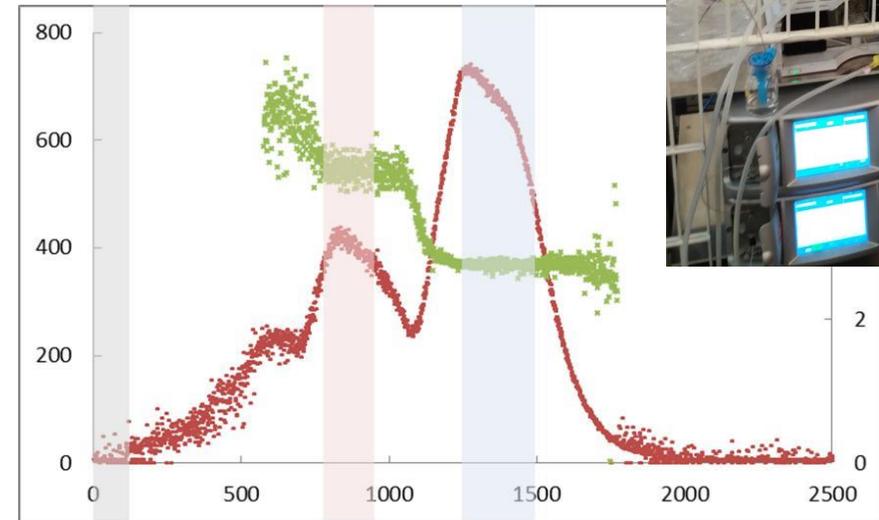


- Large capacity, more than 250 samples
- Full cycle time \approx 1min
- Sample volume: 10 – 30 μ l
- Flow measurement
- Rapid and efficient cell cleaning



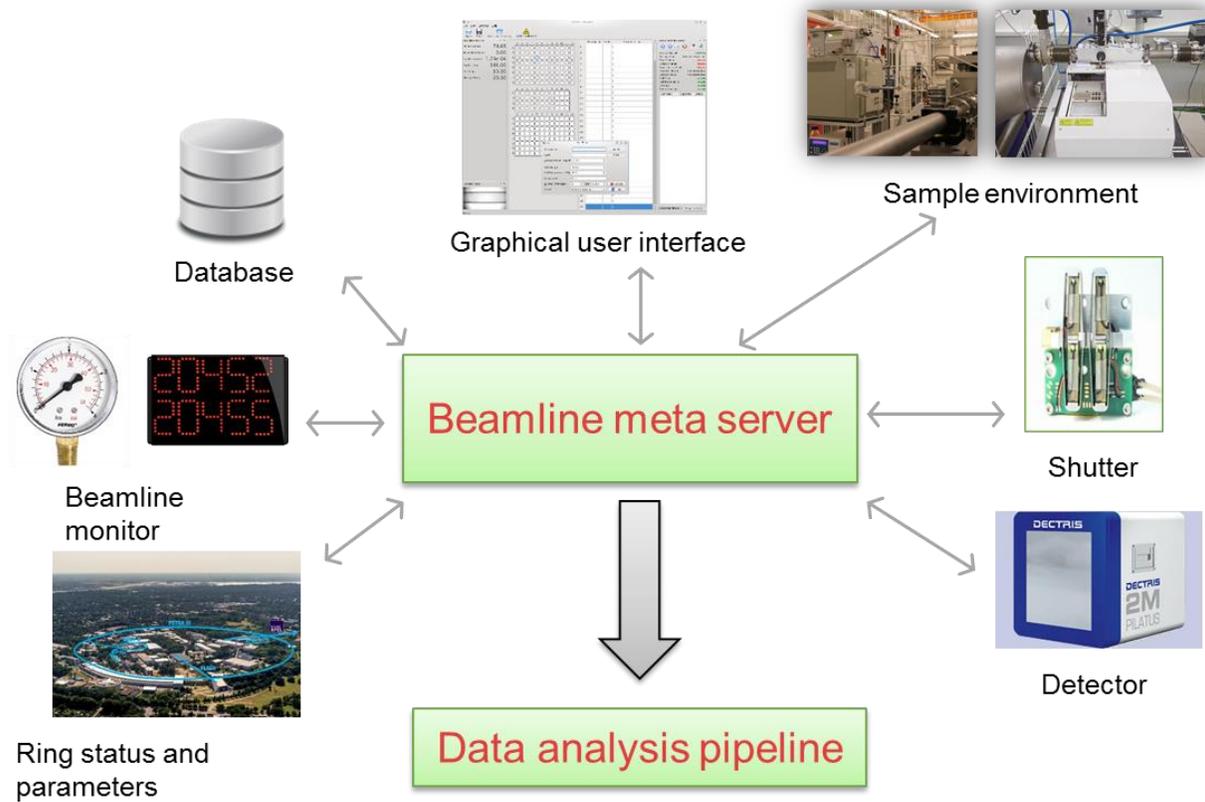
SEC-SAXS

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



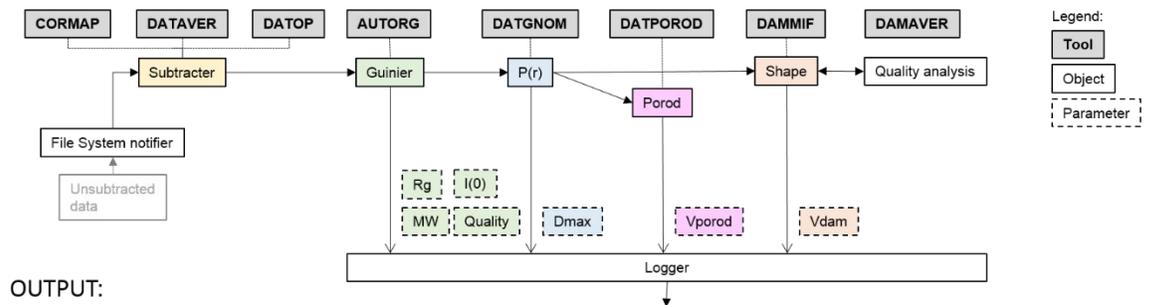
Graewert et al. *Scientific Reports* 5 (2015) 10734.

Automation



Automated data collection...

... and data analysis

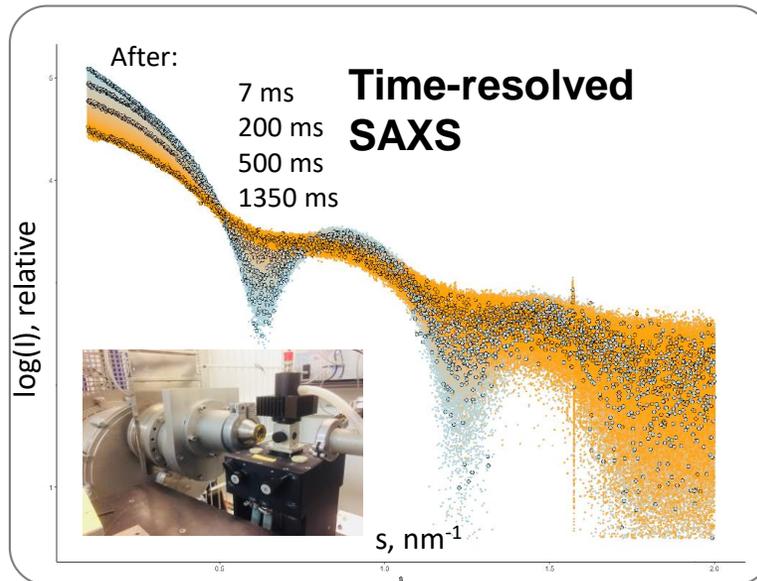
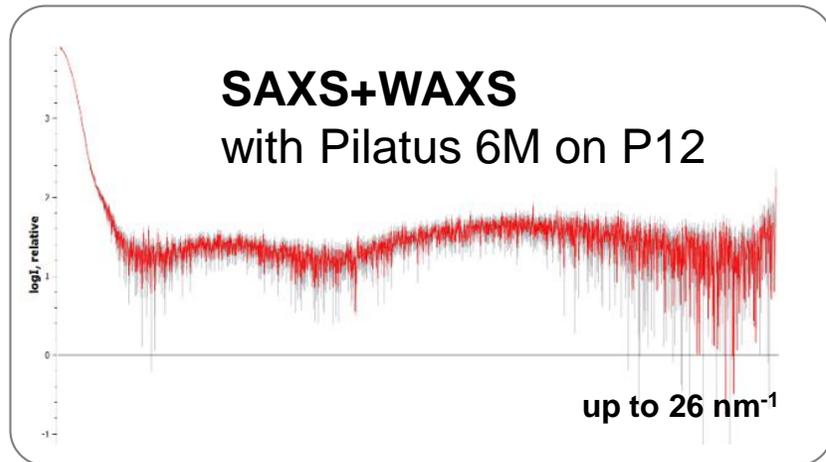


OUTPUT:

The screenshot shows the **vader** web interface. The table below summarizes the data analysis results for various samples.

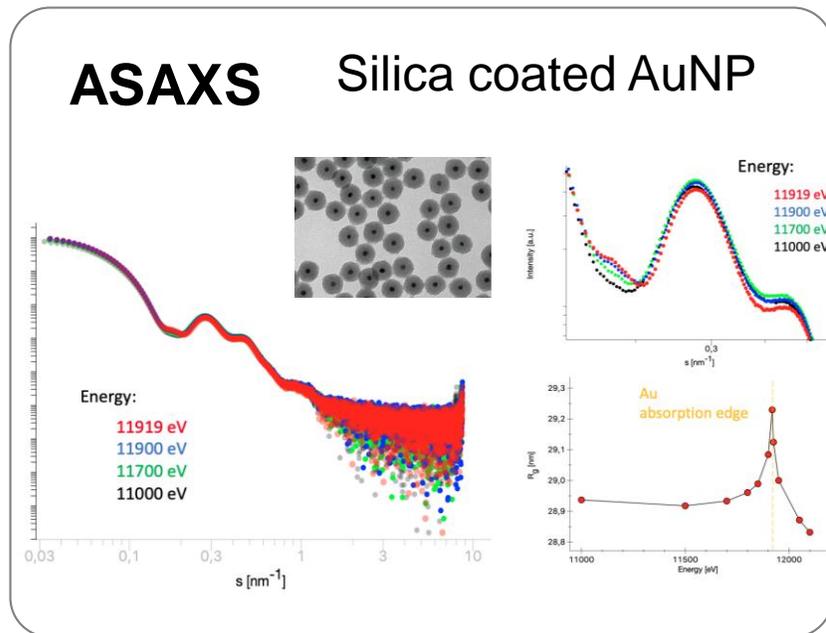
Run#	Description	Code	Conc. (mg/ml)	Log plot	Kratky plot	Guinier plot	Quality	R _g (nm)	p(r)	D _{max} (nm)	V _{porod} (nm ³)	V _{DAM} (nm ³)	MW _{vc} (kDa)	MW _{vc} (kDa)	MW _{porod} (kDa)	MW _{DAM} (kDa)	Comments
2	catalase	cat	15.0				72%	3.9 ± 0.5		17.2	306	282	62	105	191	141	92 out of 100 buffer frames used, 42 out of 50 sample frames used
4	catalase	cat	7.5				72%	3.9 ± 0.5		12.0	312	362	63	72	195	181	88 out of 100 buffer frames used, 44 out of 50 sample frames used
6	catalase	cat	3.7				81%	4.4 ± 0.4		12.8	325	382	65	80	203	191	92 out of 100 buffer frames used, 46 out of 50 sample frames used
9	ovalbumin	ova	15.0				69%	2.6 ± 0.5		12.7	68	65	32	38	43	33	92 out of 100 buffer frames used, 44 out of 50 sample frames used
11	ovalbumin	ova	7.5				69%	2.8 ± 0.5		8.2	88	82	33	33	55	41	92 out of 100 buffer frames used, 43 out of 50 sample frames used

Advanced BioSAXS

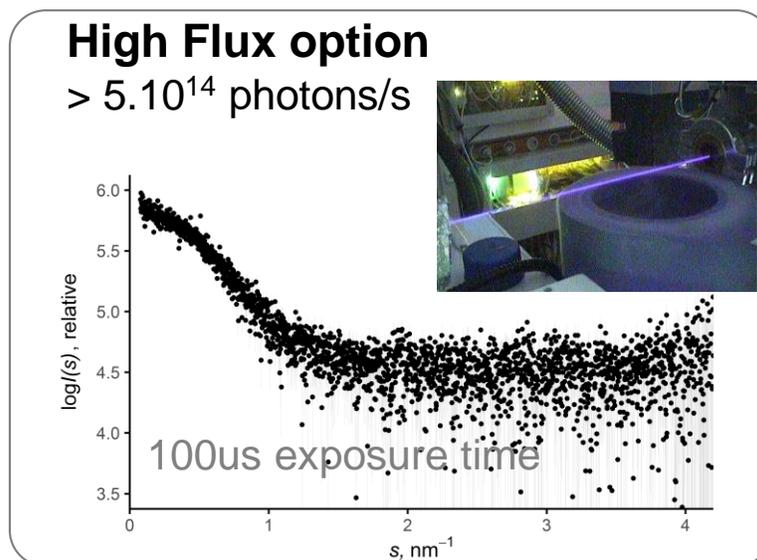


Microfluidics

TECHNISCHE HOCHSCHULE LÜBECK
UNIVERSITY OF GÖTTINGEN



High temperature and aggressive chemical



Scanning SAXS

Macroscopic & Microscopic Structure

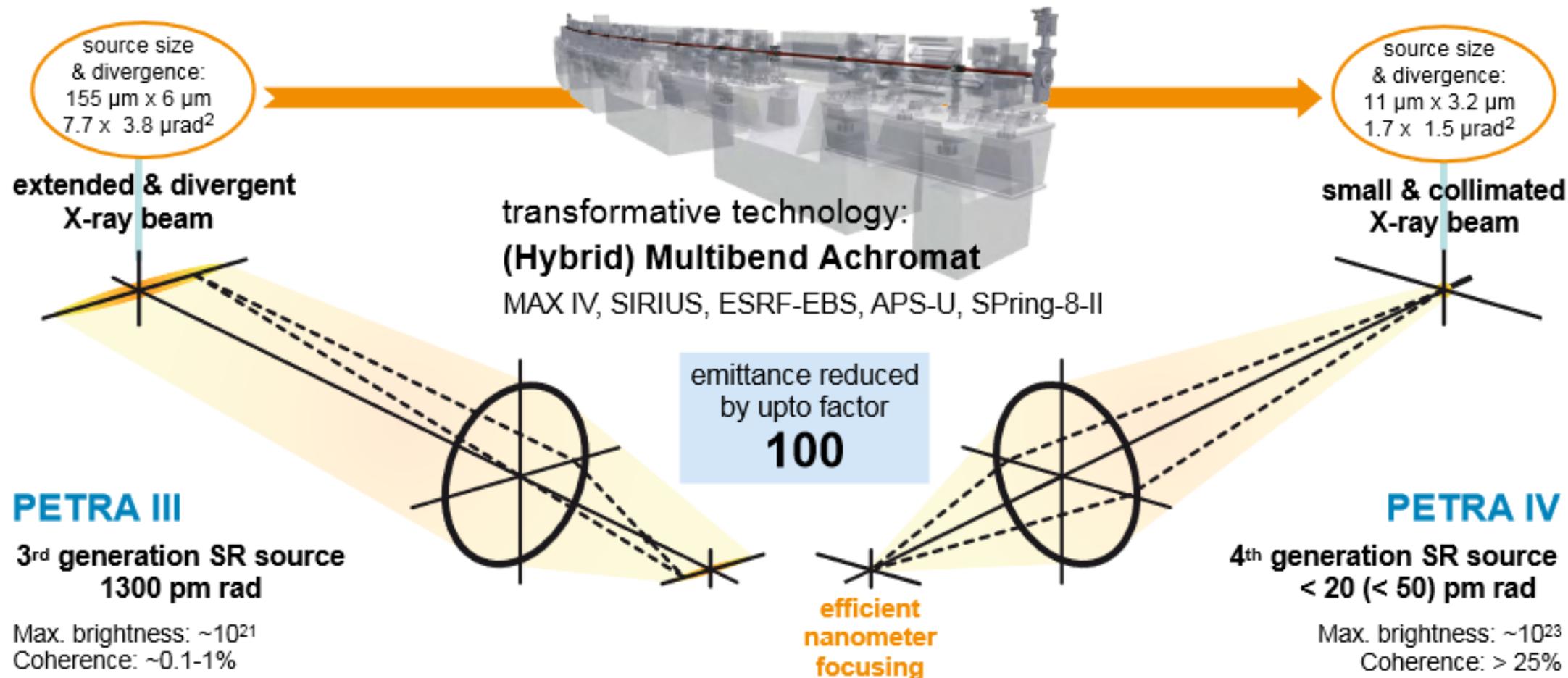
a tip mid base
b
c
tip
mid
base

Mechanics
CT/AFM/SAXS
Histology

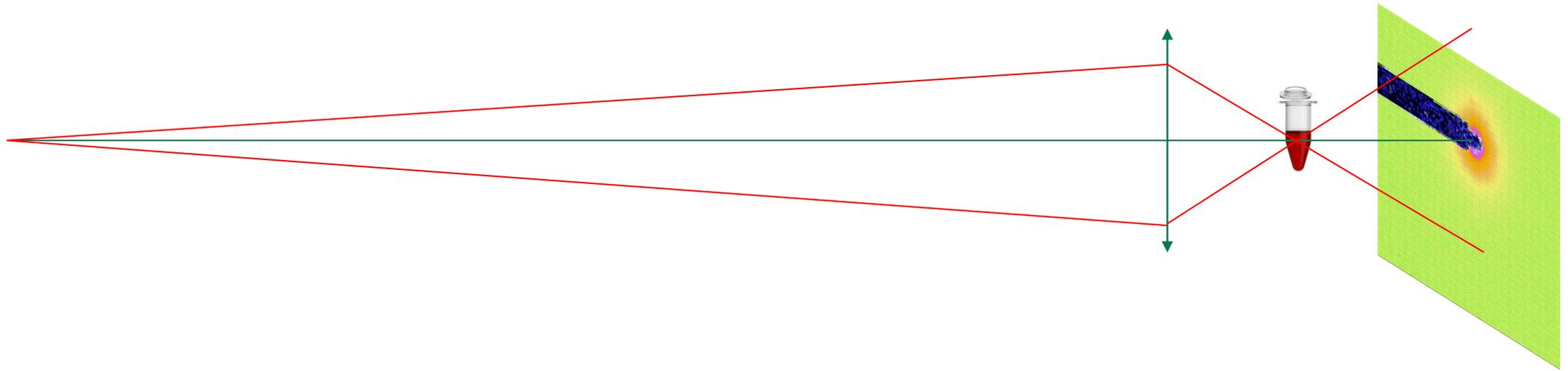
Schmidt *et al. Adv. Sci.* 2019, 1900287.

EMBL

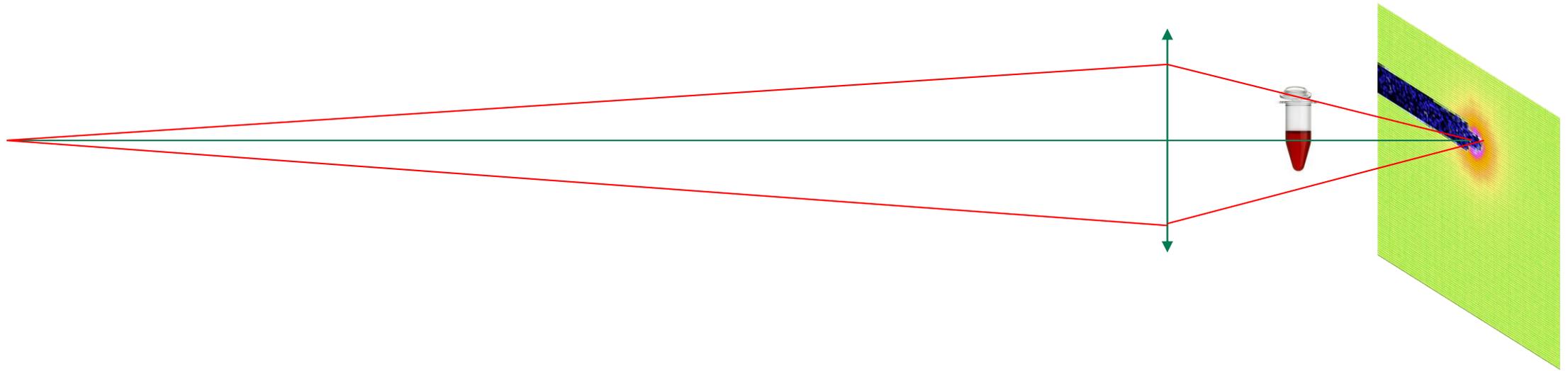
PETRA IV



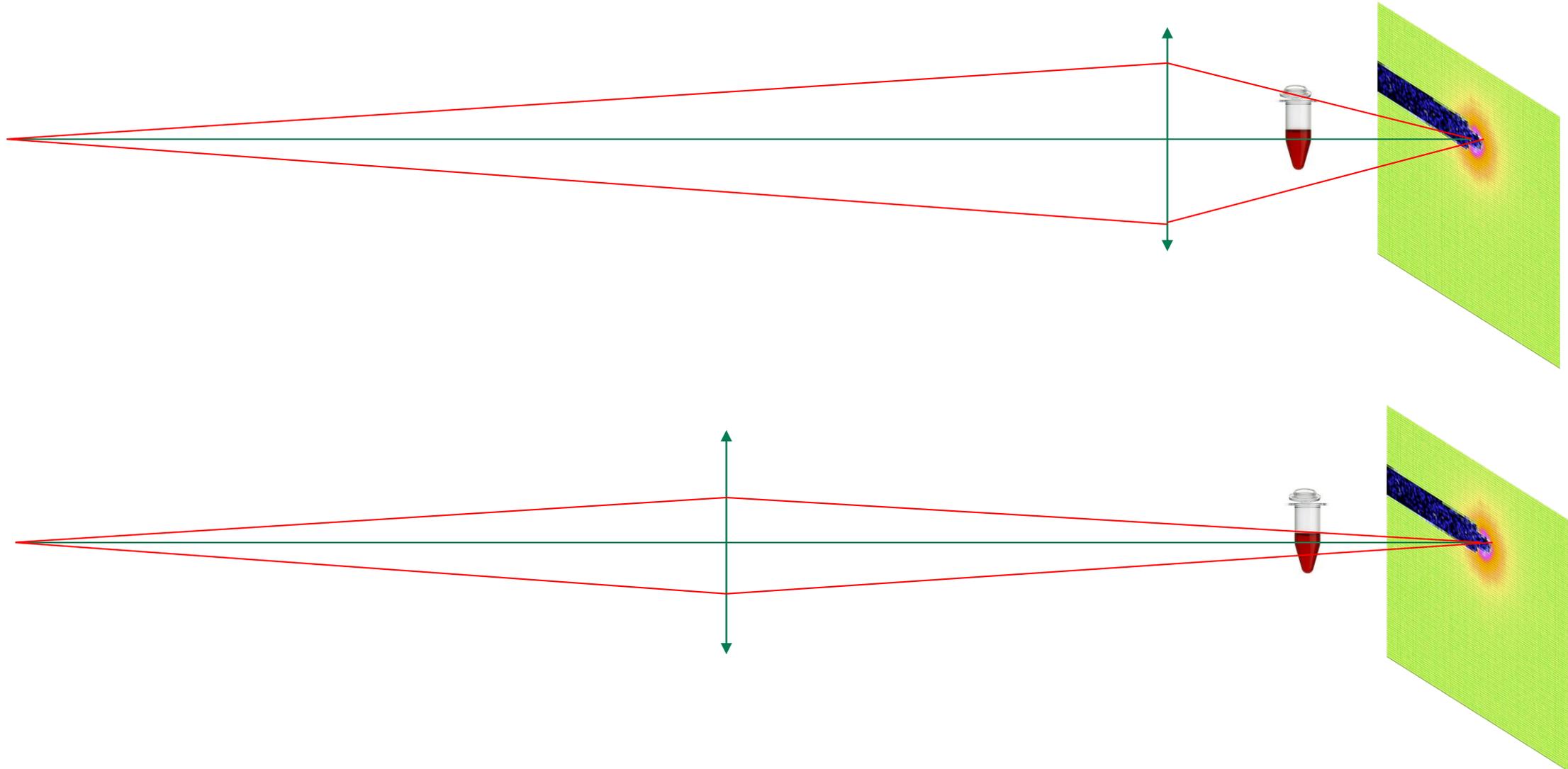
Small beam for SAXS



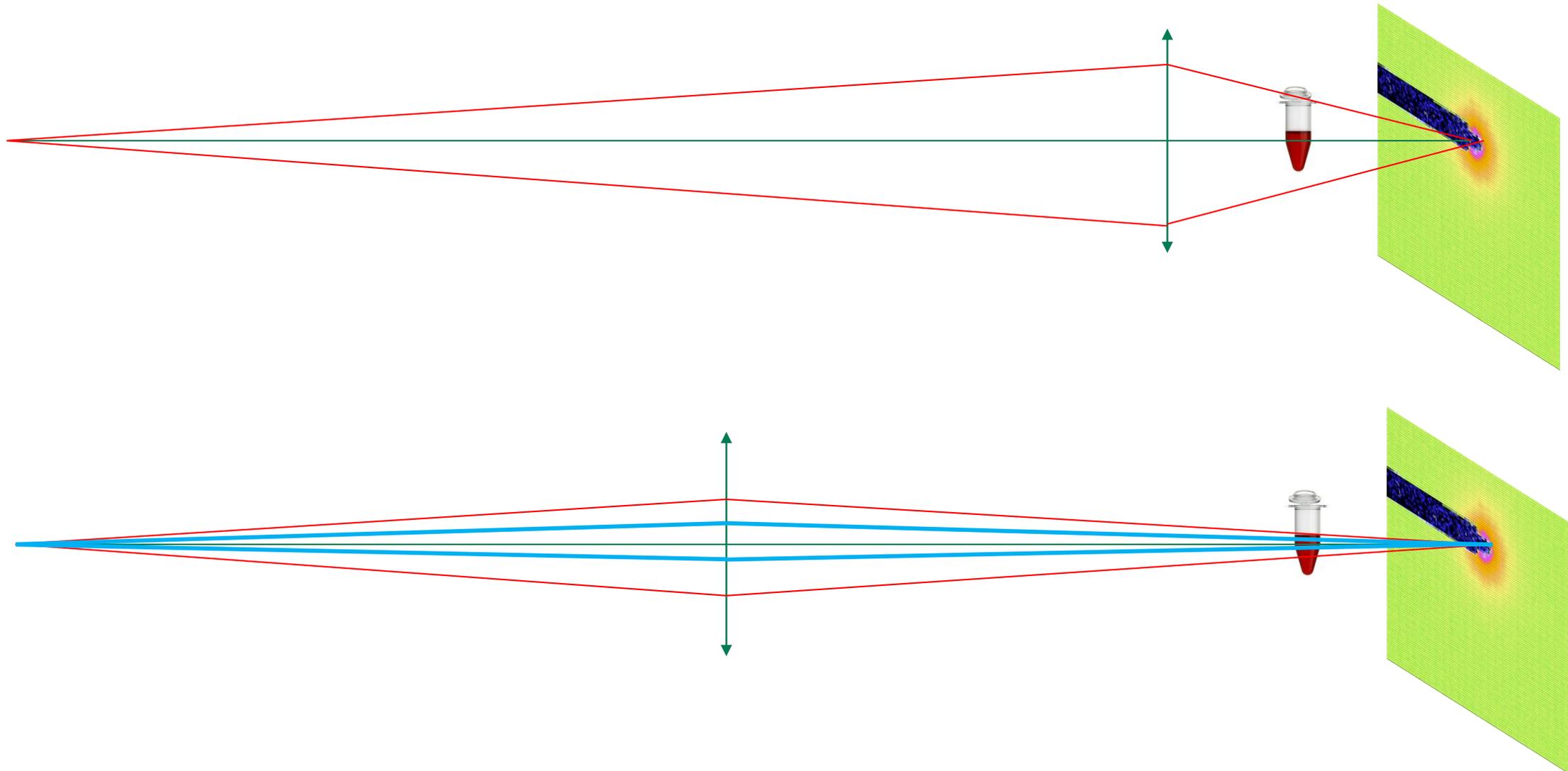
Small beam for SAXS



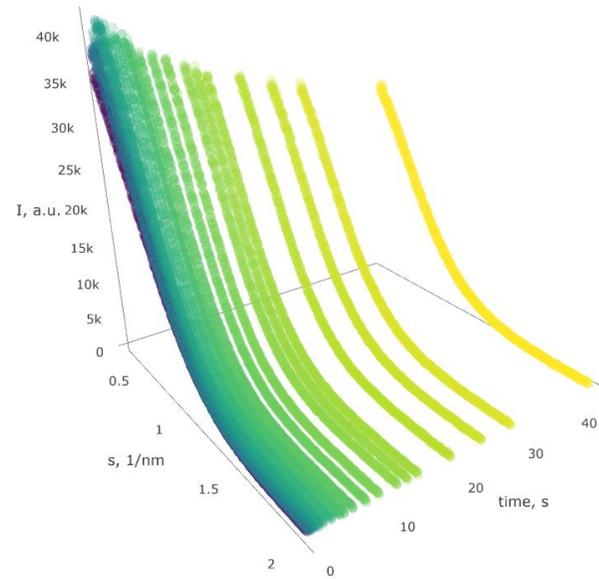
Small beam for SAXS (at sample and detector position)



Small beam for SAXS (at sample and detector position)



4th generation synchrotron beam for advanced BioSAXS



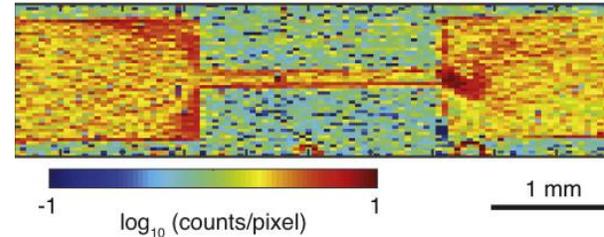
Time resolved SAXS

Smaller beamsize → shorter dead time:

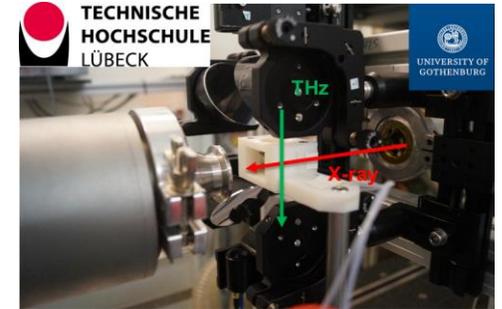
Smaller volume:

- faster mixing
- faster P-jump
- faster T-jump
- higher laser intensity for light trigger reaction

In-situ SAXS

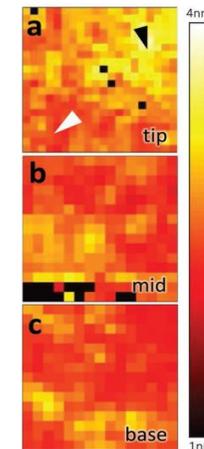
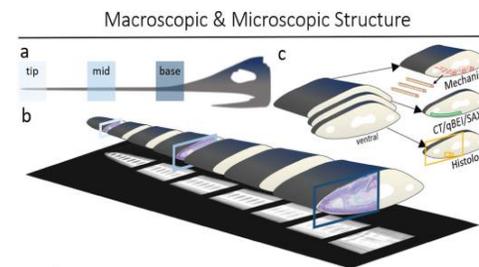


Buscema, M. *et al.* (2019). *Materials Today Bio*, 1, 100003.



Scanning SAXS

bridge the gap between information in the real and reciprocal space

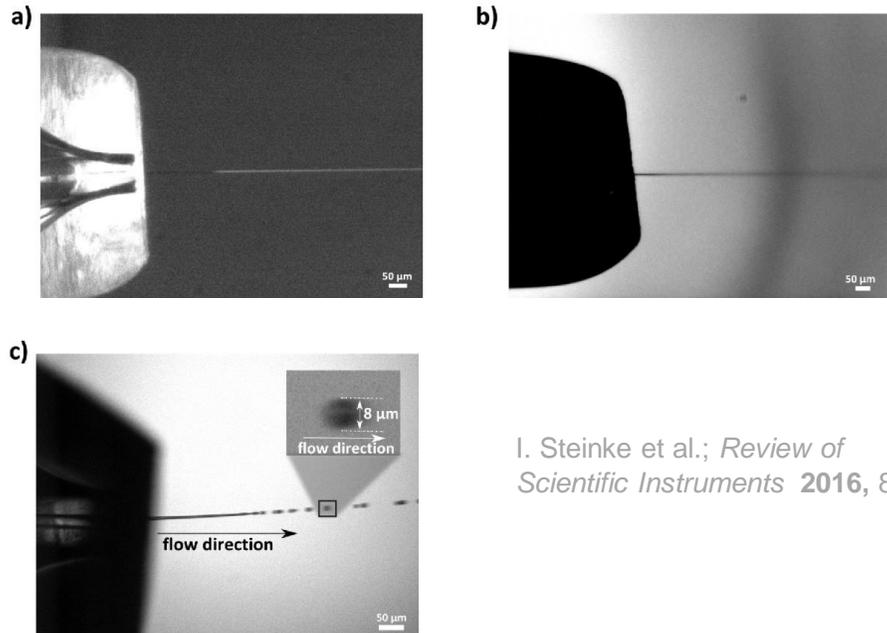


Schmidt *et al.* *Adv. Sci.* 2019, 1900287.

Further improve the sensitivity of the technique

Windowless setup

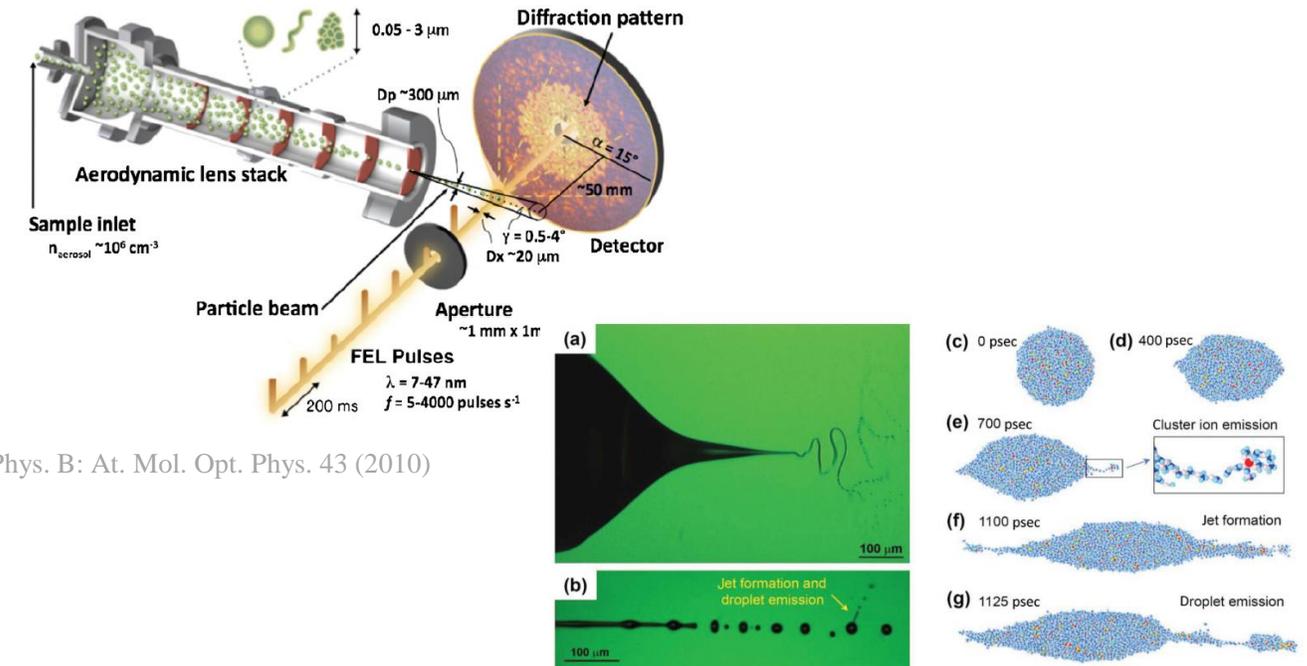
P12 instrumental background comes mostly from the capillary scattering.
Can one measure without capillary?



I. Steinke et al.; *Review of Scientific Instruments* **2016**, 87

Protein in vacuum?

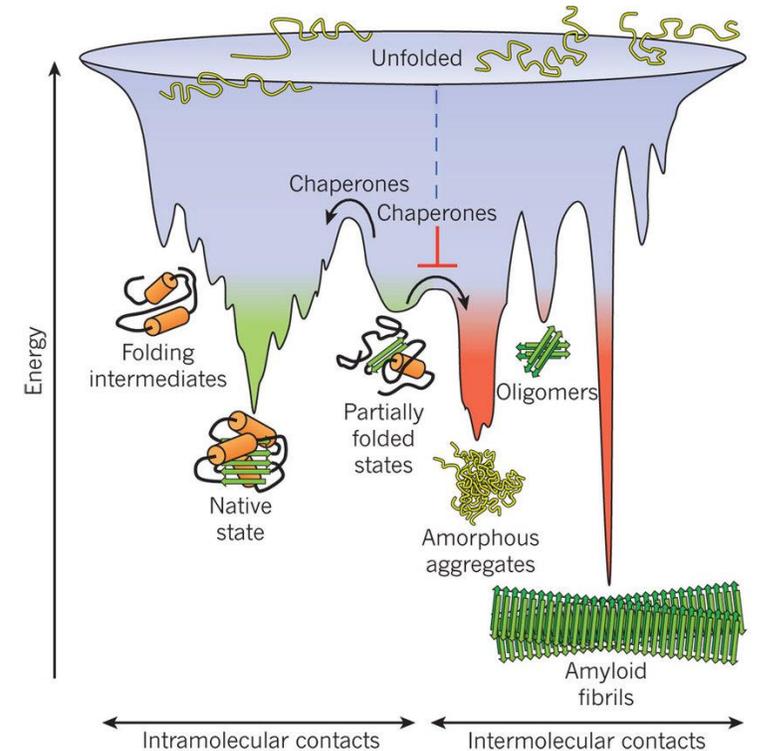
Most photons are scattered by the buffer, not the biomolecules in solution → proteins in vacuum
Aerosol, electrospray.



J. Phys. B: At. Mol. Opt. Phys. 43 (2010)

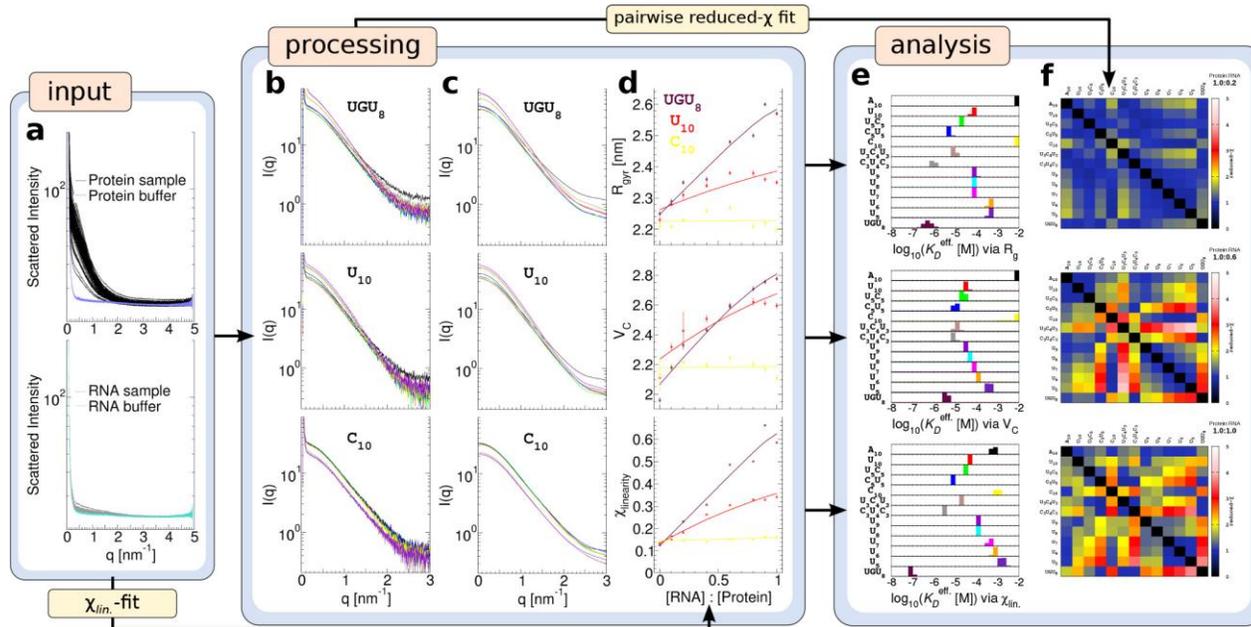
BioSAXS from a structural biologist perspective

- Structure? High resolution structure obtained by MX, CryoEM, or computationally. But obtained on frozen, crystallized sample.
- BioSAXS: molecules in solution.
 - Protein in its native state
 - The pH, the concentration of protein, binding partner, salt, can be easily modified to explore the conformational space of the molecule
- Size range from a few kDa to several Mda
- Polydispersity:
 - analysis of mixture: oligomeric state, complexes formation
 - Conformational polydispersity: intrinsically disordered proteins



→ Well suited for high throughput screening, titration method

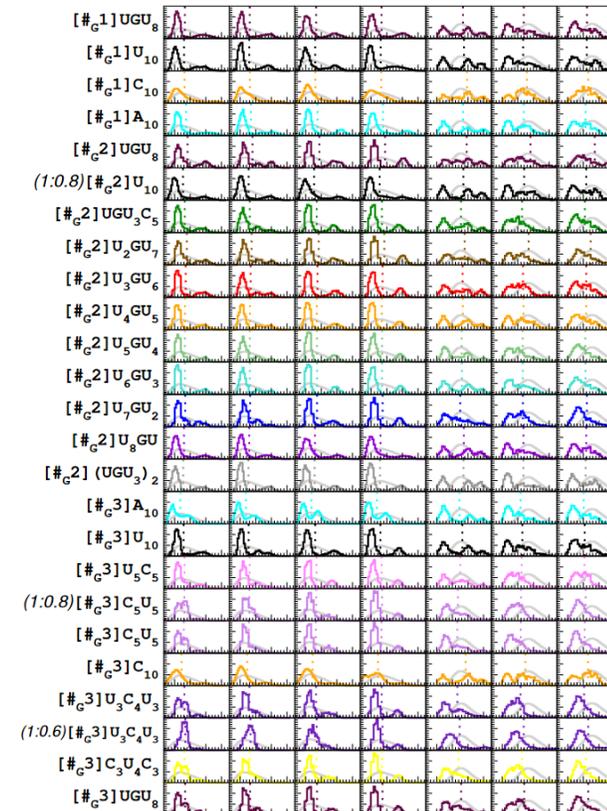
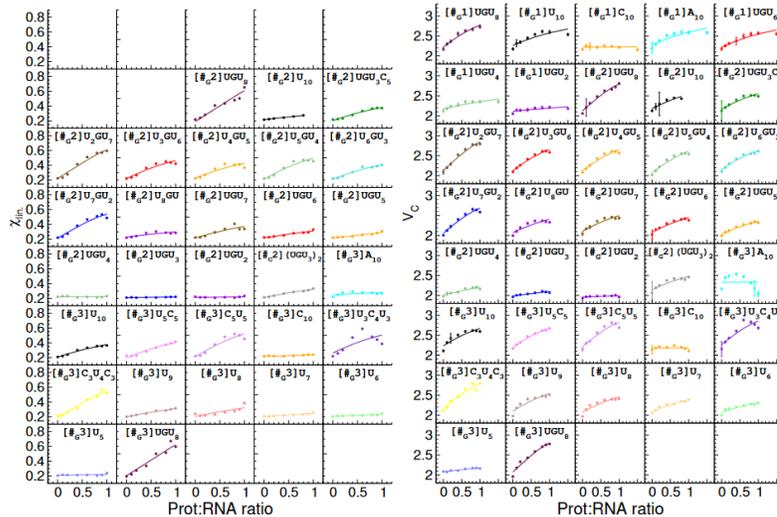
Structure based screening using SAXS



Screening of RNA protein interaction by SAXS

Affinity profile and binding mechanisms of the two RRM-domain SxL protein.

Screening against 35 RNA motifs



Chen, Po-chia, et al. "A general small-angle x-ray scattering-based screening protocol validated for protein-RNA interactions." *ACS combinatorial science* 20.4 (2018): 197-202

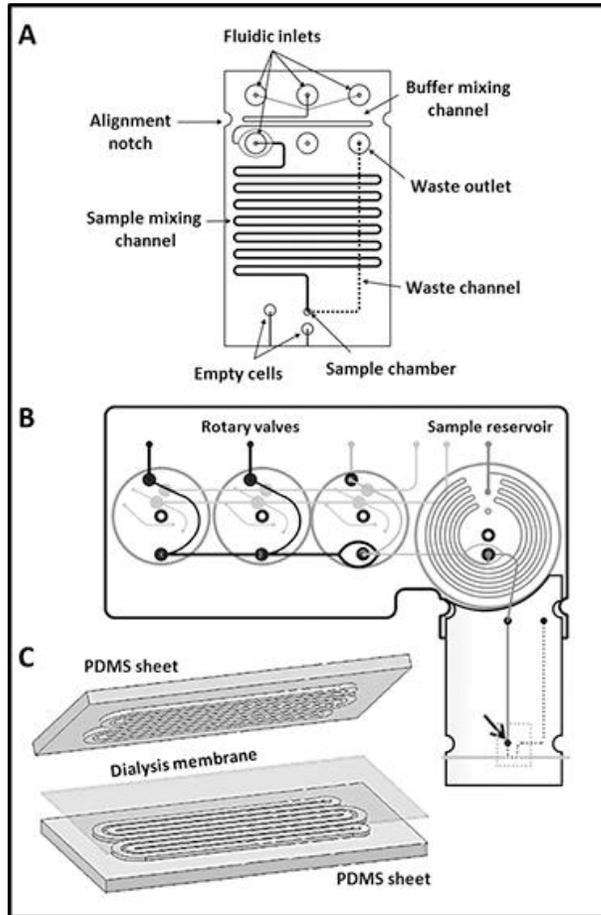
Cell volume estimation



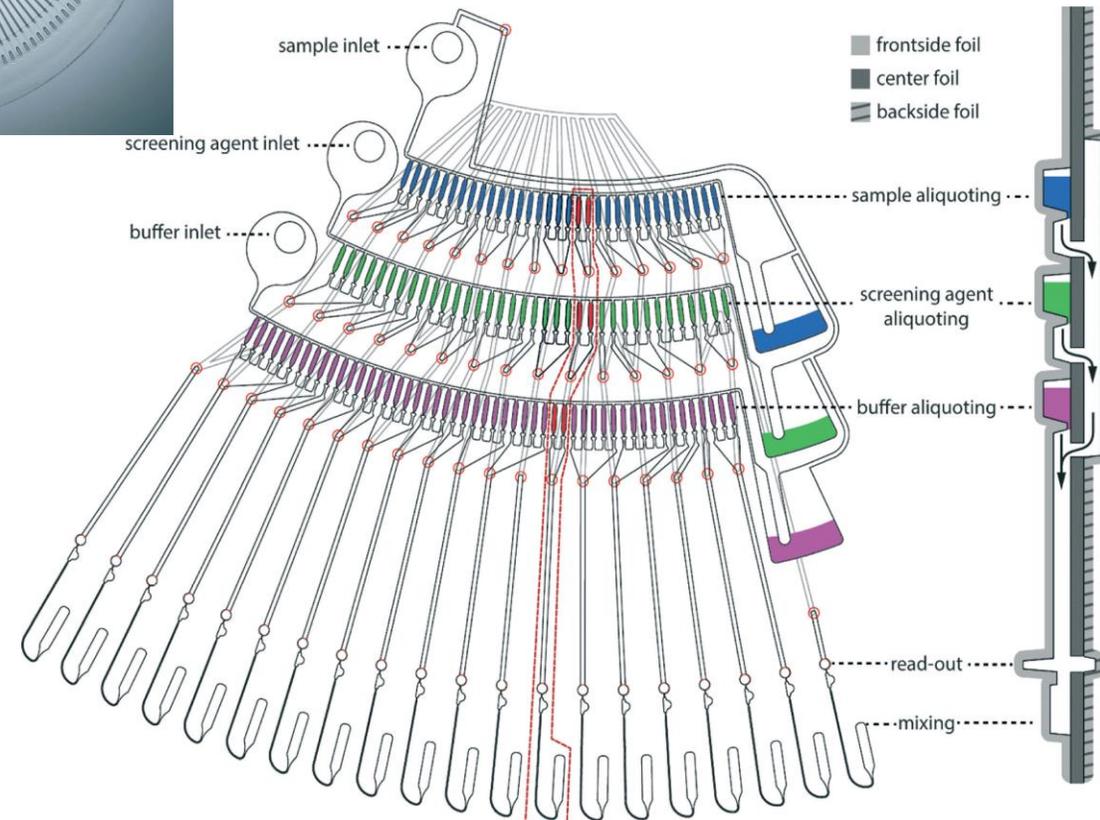
	PETRAIII conservative	PETRAIV conservative	PETRAIV “ambitious”
diameter	500 μm	200 μm	50 μm
height	1000 μm	500 μm	200 μm
volume	<200 nl	<20 nl	<0.4 nl
Sample density	100 samples/cm ²	600 samples/cm ²	10000 samples/cm ²
throughput	1 to 10 measurements / s		

Microfluidics

BioXTAS chips



SAXS disc



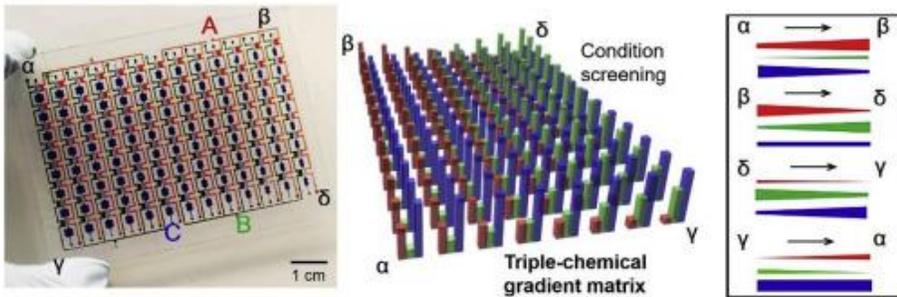
Toft, K. Nørgaard, et al. *Analytical Chemistry* 80.10 (2008): 3648-3654.
 Skou, Magda, et al. *Journal of applied crystallography* 47.4 (2014): 1355-1366.
 Lafleur, Josiane P., et al. *Journal of Applied Crystallography* 44.5 (2011): 1090-1099.

Schwemmer, Frank, et al. *Lab on a Chip* 16.7 (2016): 1161-1170.

Sample preparation

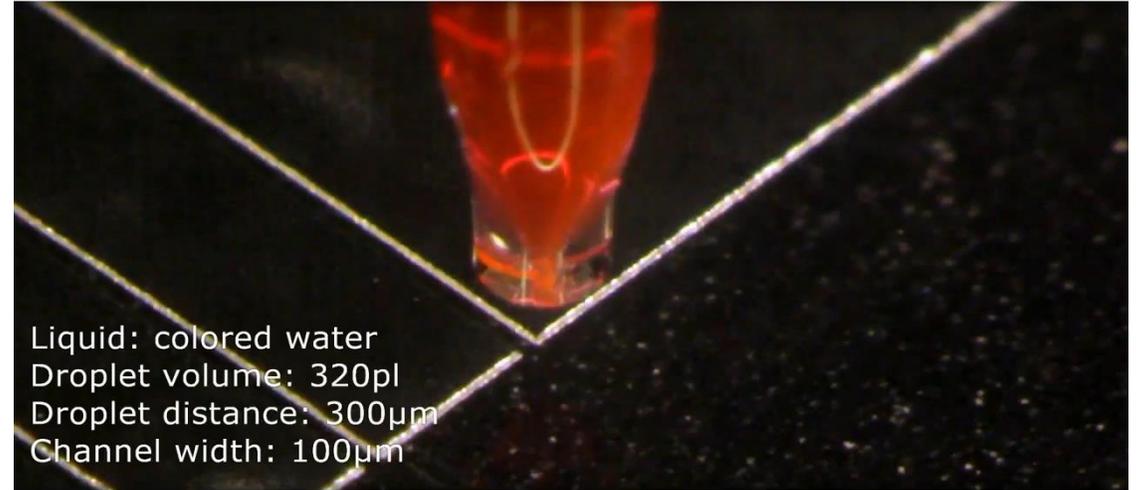
Offline sample preparation: parallelizable, no in-beam time lost due to sample preparation.

Lab on a chip? Preparation of screening/concentration series and transfer to the measurement chambers in a single chip.

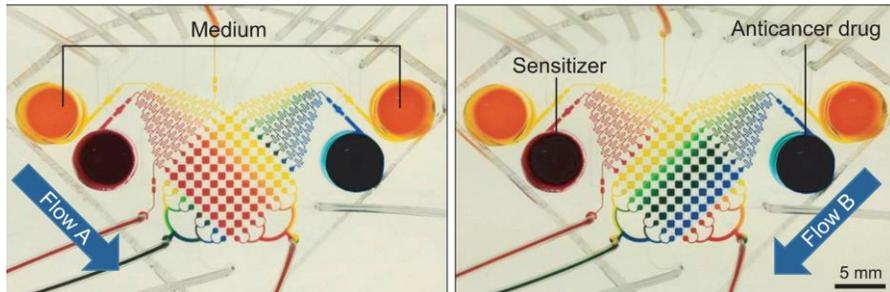


Off-chip sample preparation (more flexibility) pipetting robot, droplet dispenser.

microdrop
TECHNOLOGIES



Advancing the Art of Microdispensing



Conclusion

Protein sequence



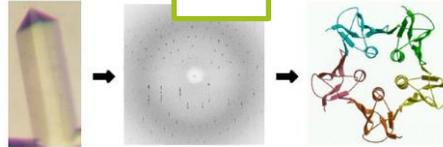
3D structure



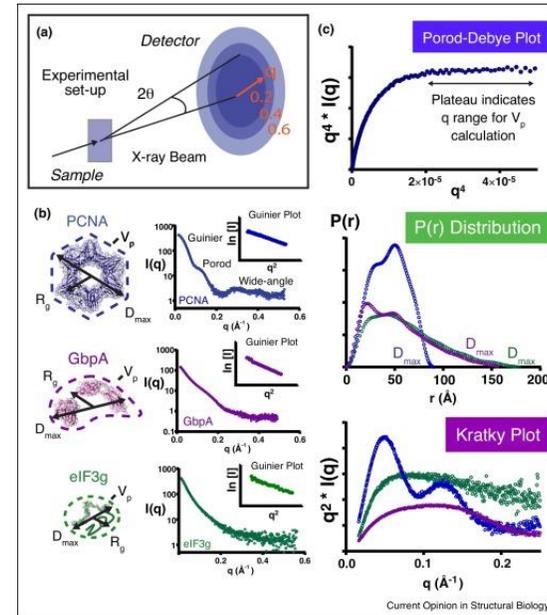
Protein function

WORLDWIDE
PDB
PROTEIN DATA BANK

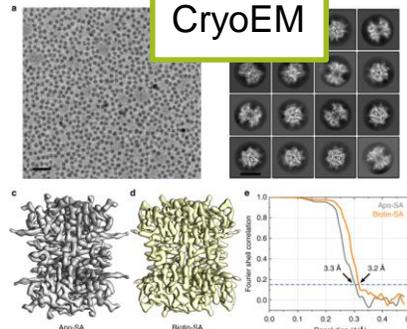
MX



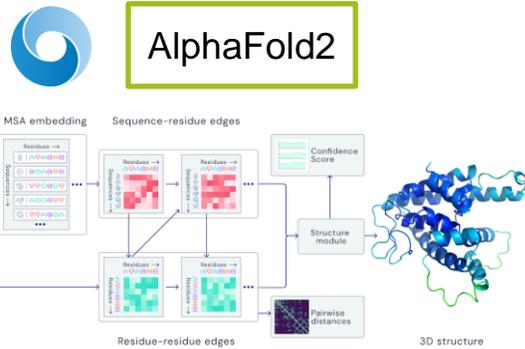
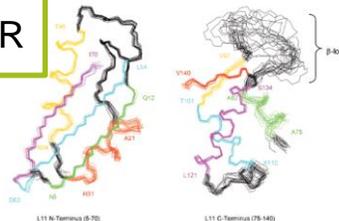
SAXS



CryoEM



NMR



AlphaFold2



Summary

4th generation sources with their low divergence is very attractive for BioSAXS.

The improved beam property open new experimental possibilities and advanced bioSAXS experiments (TR SAXS, scanning SAXS, in situ SAXS, etc.).

The beam delivered by fourth generation sources coupled with adapted sample environments, would allow to further increase the **sensitivity** and **throughput** of the methods, makings it useable for **large scale screening/titration study**.

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