Small-angle Scattering from Biological Solutions: potential of the ERL/USR Sources

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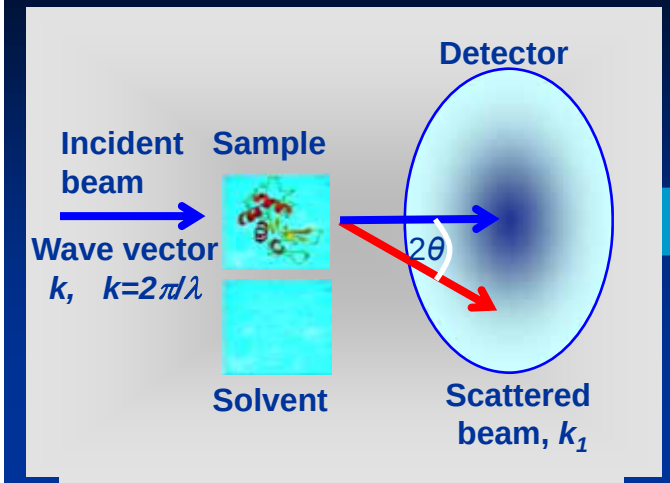
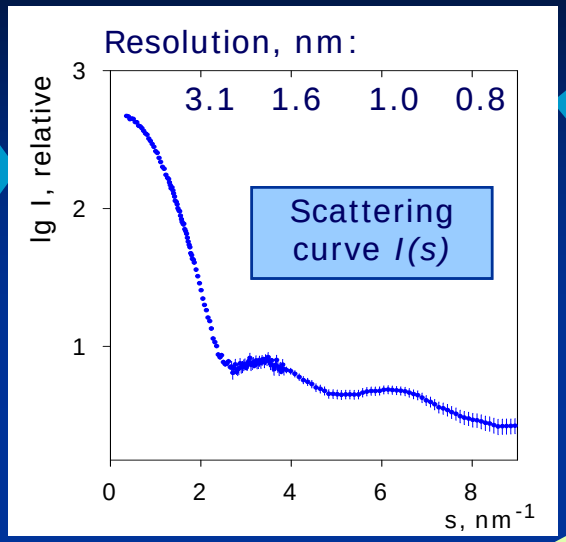
EMBL

SAXS in structural biology



Radiation sources:

X-ray tube	*(λ = 0.1 - 0.2 nm)*
Synchrotron	*(λ = 0.05 - 0.5 nm)*
Thermal neutrons	*(λ = 0.1 - 1 nm)*



Data analysis

- **Shape determination**
- **Rigid body modelling**
- **Missing fragments**
- **Oligomeric mixtures**
- **Hierarchical systems**
- **Flexible systems**

Complementary techniques

MS, EM, Crystallography, NMR, Bioinformatics, Biochemistry, AUC, FRET, EPR

Additional information

Homology models, Atomic models, Distances, Orientations, Interfaces

The Major Problem of biological solution SAXS

- Sample preparation
- Experiment
- Data processing
- Unambiguous interpretation
- Changing conditions
- Relation to function

ATSAS (All That SAS)

a program suite for small-angle scattering data analysis from isotropic systems

Consists of over 25 programs developed at the EMBL, Hamburg Outstation, since 1991

1999: program executables available from the Web
2003: copyright protected by EMBLEM
2007: on-line access to selected programs
2010: release 2.4 with numerous new features

Analogous public program complexes: none
Number of users (laboratories): over 4000 (1400)
Over 50% biological SAS publications worldwide use and cite ATSAS programs

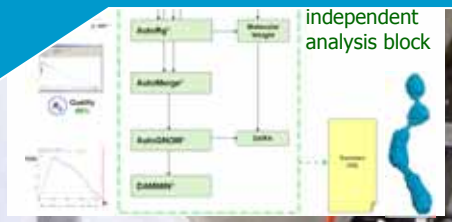
X33 SAXS beamline of the EMBL

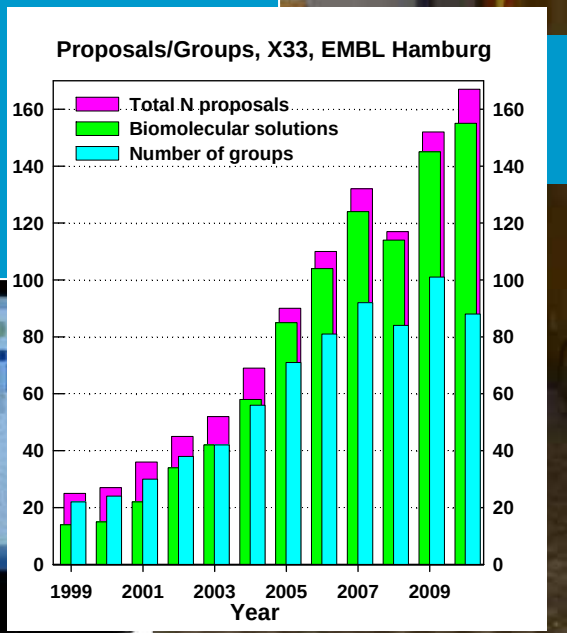
First automated SAXS sample changer for solutions (2007)

First multi-module Pilatus detector outside SLS (2007)

First automated SAXS data analysis pipeline (2008)

First remote SAXS experiment (2009)





Automated data take and analysis pipeline

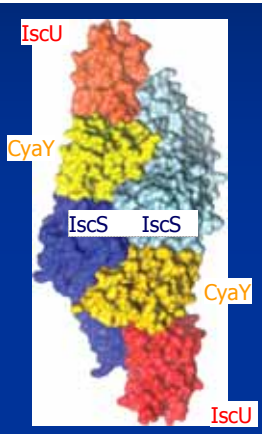
Recent EMBL-HH collaborative projects

Complexes and assemblies

S-layer proteins

Fagan *et al* Mol. Microbiol (2009)

Frataxin complexes



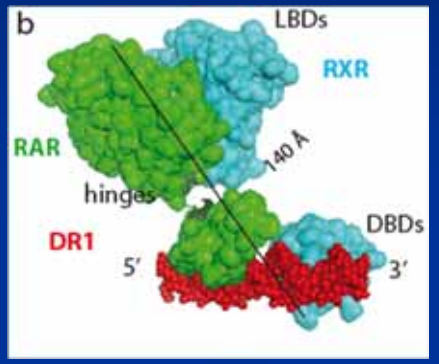
Prischi *et al* *Nat Commun* (2010)

Domain/quaternary structure

Toxin B

Albesa-Jové *et al* JMB (2010)

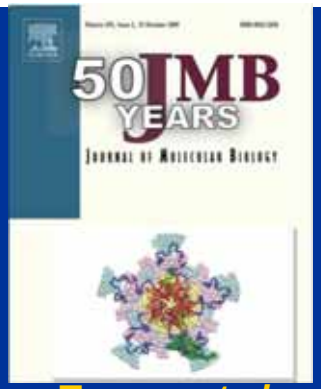
Nuclear receptors



Rochel *et al* NSMB (2011)

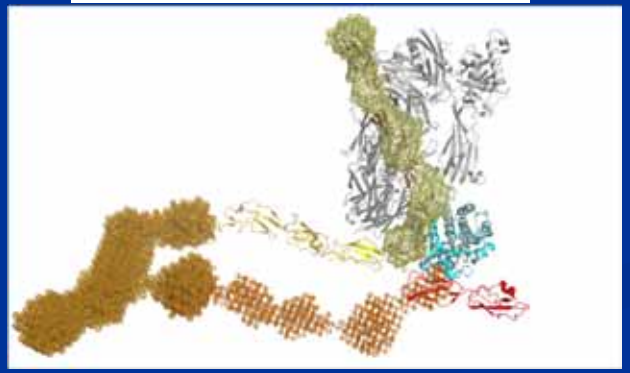
Flexible macromolecules

Nucleoplasmin/histone



Taneva *et al* JMB (2009)

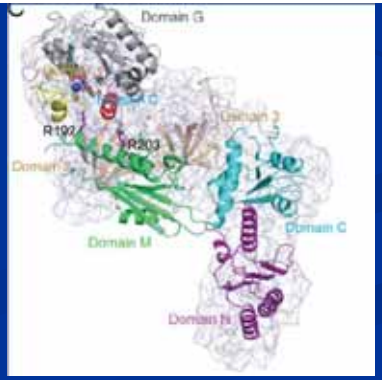
Complement factor H



Morgan *et al* NSMB (2011)

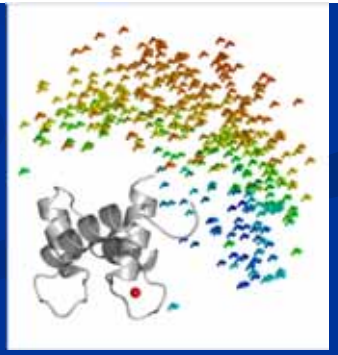
Structural transitions

eRF3/eFR1 interactions



Cheng *et al* Genes Dev (2009)

Calmodulin



Bertini *et al* JACS (2010)

X33 shows that one may be faster even with a less fancy hardware

Biological cryoSAXS

- PROS of flash-freezing:
  - Reduction of the radiation damage
  - Downscaling the sample size
  - Possibility of scanning the specimen and potentially observing a “few particle” scattering
  - Reaching higher temporal resolution by stalling the biological reactions
- CONS of flash-freezing :
  - Cumbersome sample handling procedures
  - Complicated specimen holders
  - Higher background (especially ice crystal formation)

Biological cryoSAXS: sample preparation

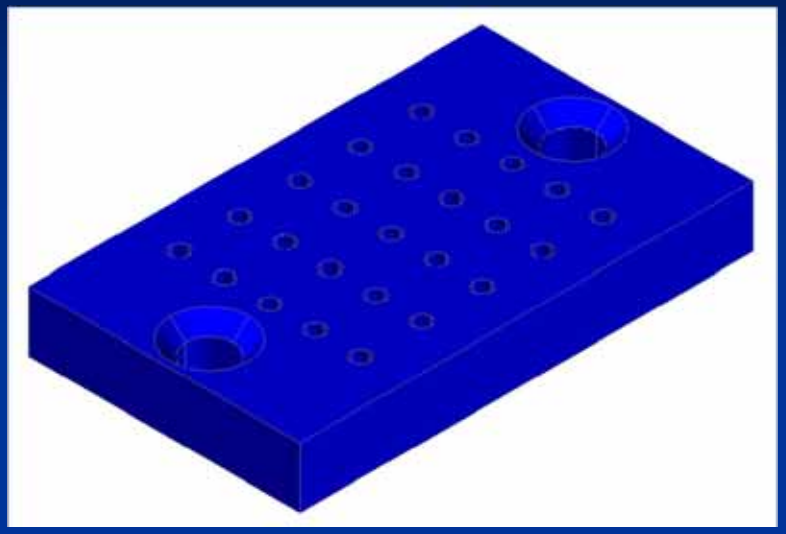
- The scattering intensity is proportional to $t*exp(-\mu t)$. For water at λ=0.15 nm, optimum t is about 1 mm.

Leica EM-PACT2 high pressure freezing machine (2.5 kbar)

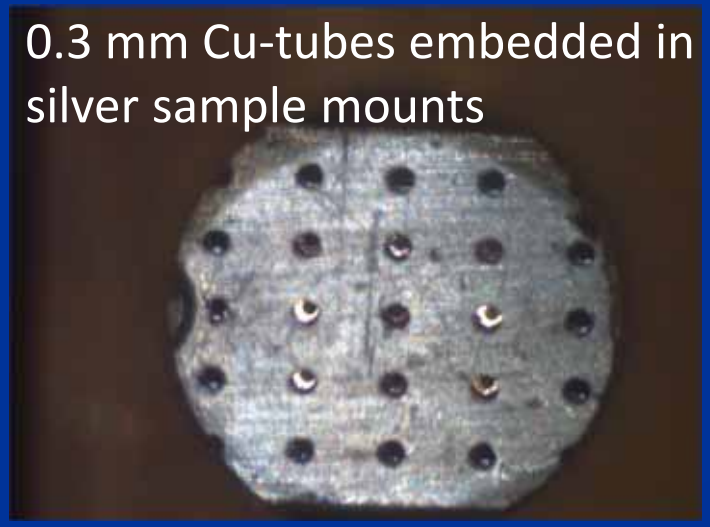
Leica Ultracut ultra-cryomicrotome. Cryo-protectant 33 v/v% ethylene glycol

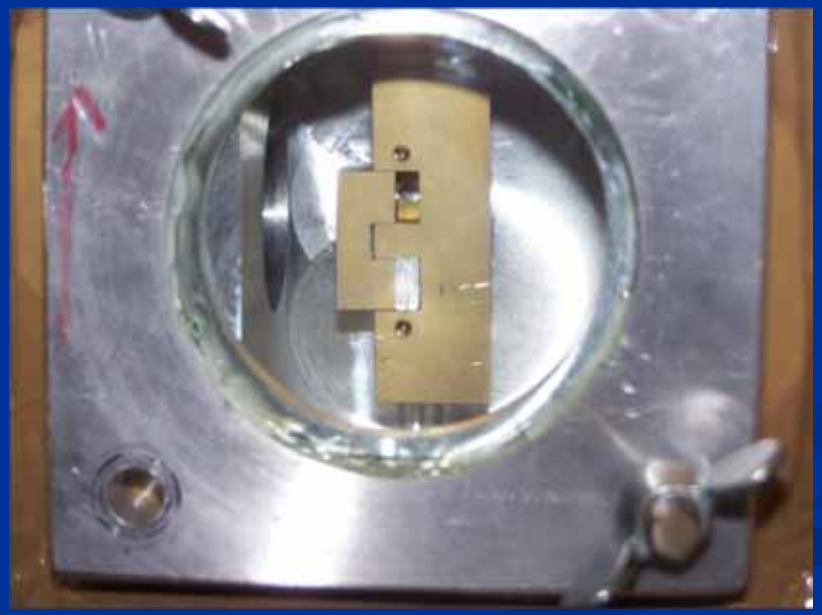
Custom specimen holder

Biological cryoSAXS: sample mounts



High precision sample mount
25 sample positions on silver plate
Cryo-Sample in **1 mm x 0.3 mm** Cu tube
~150 nl sample volume

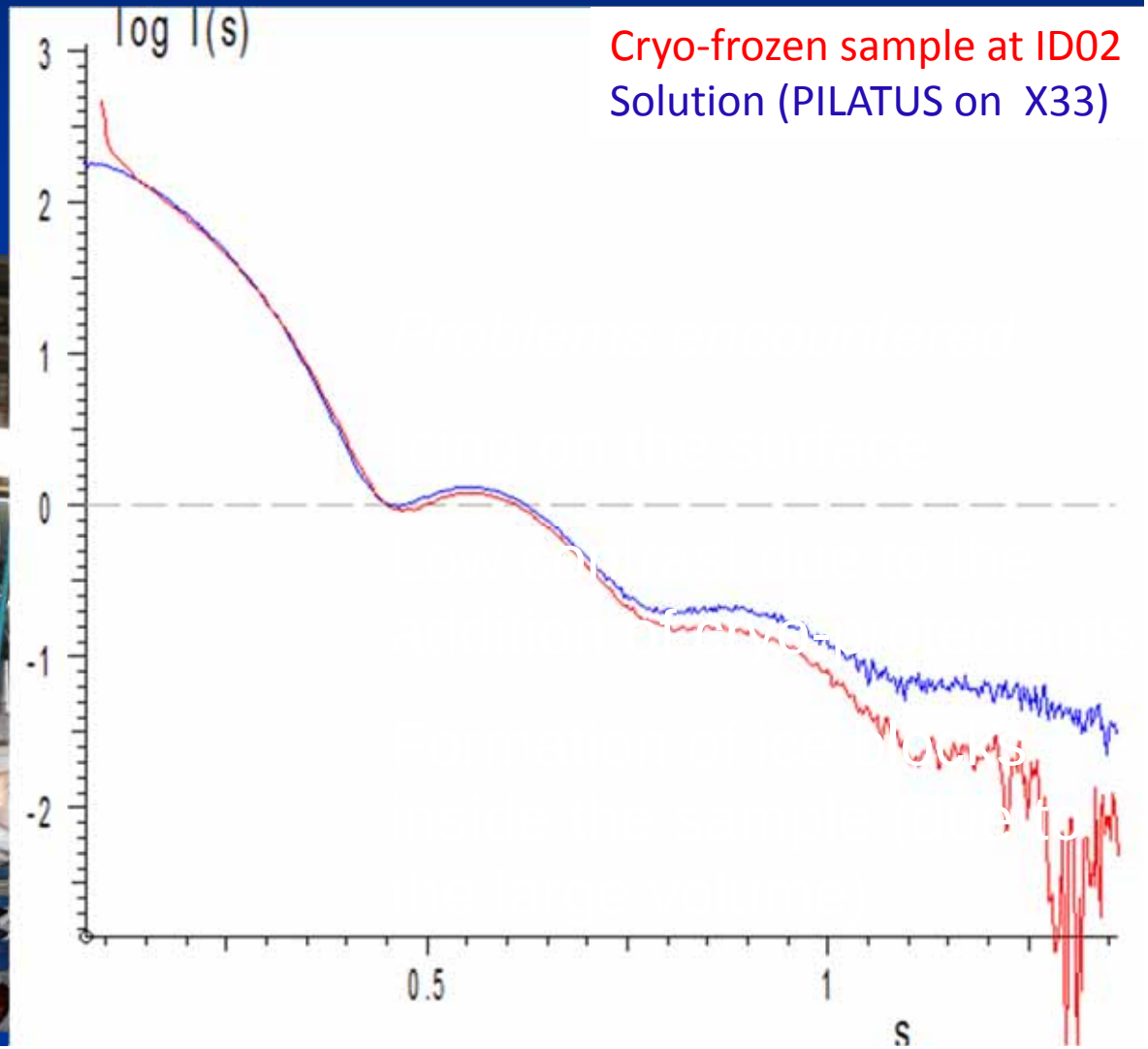




Easy mountable on the cryo-finger
Massive brass block as “cold trap”

Biological cryoSAXS: setup and tests

Scattering from 20 nm gold nanoparticles

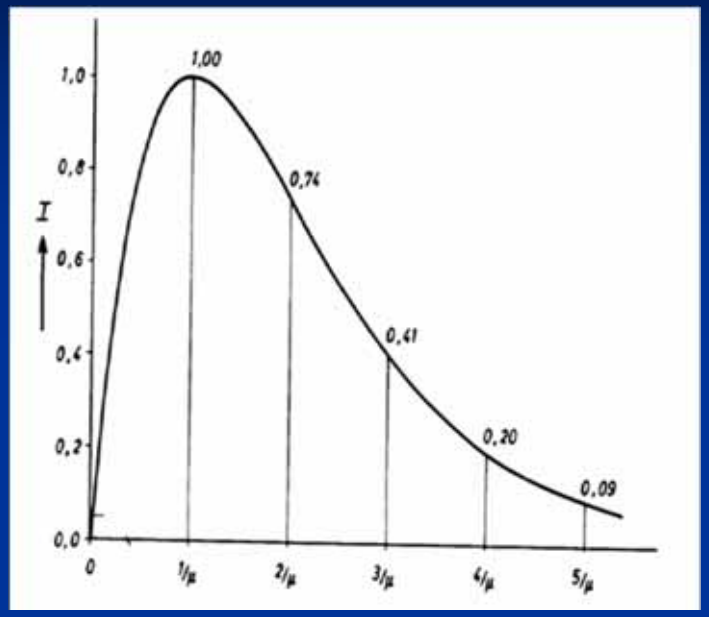


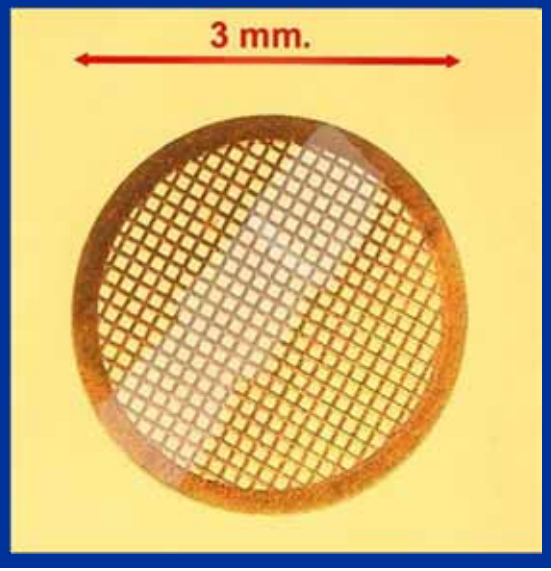
Cryo-frozen sample at ID02
Solution (PILATUS on X33)

Experiments done at:

- X33 EMBL Hamburg
- ID02 ESRF Grenoble
- ID13 ESRF Grenoble
- SWING Soleil Paris

Biological cryoSAXS: ERL/USR potential



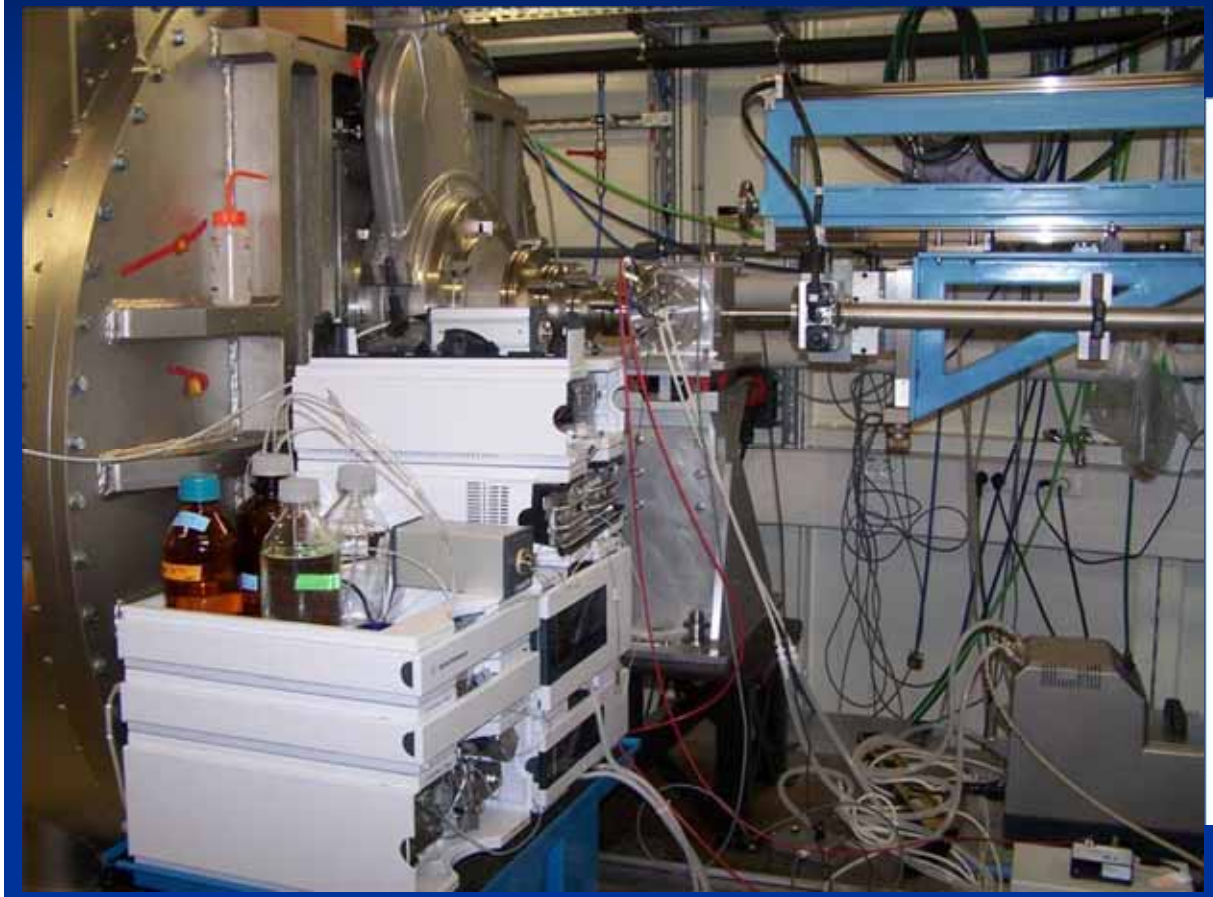


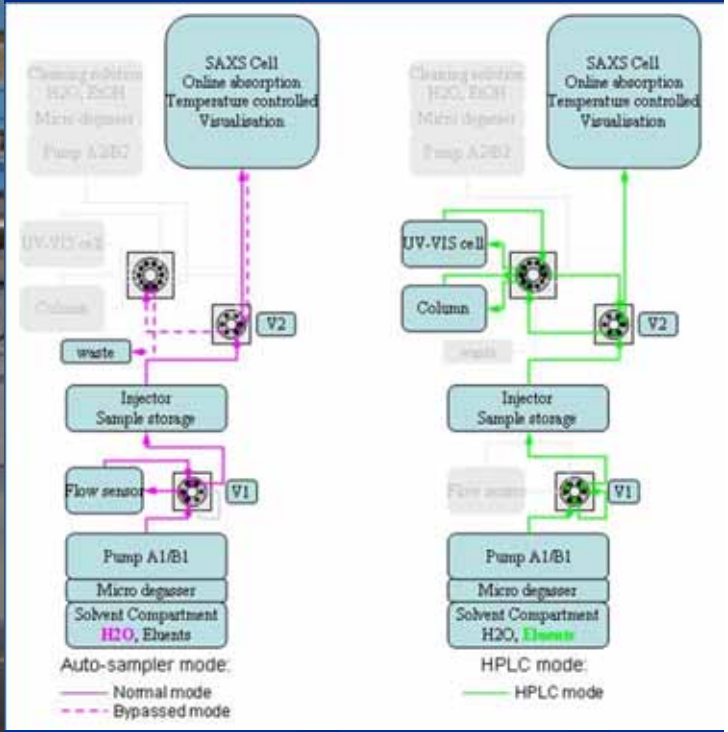
- Thinner samples requiring less volume and lower percentage of cryo-protectant are a must
- On an EM grid, t is about 100 nm, leading to about 10^5 decrease in the intensity compared to the optimum sample thickness
- This can be compensated by the much higher brilliance of the ERL/USR

On-line SEC with SAXS

- PROS of on-line size exclusion chromatograpy:
  - Clean monodisperse solutions
  - Separation into biologically relevant species
  - Detection of transient and short-lived complexes
  - Possibility of parallel biophysical characterization
- CONS of on-line size exclusion chromatograpy:
  - Relatively long experiment times
  - Significant dilution of the samples
  - Larger amounts of material required

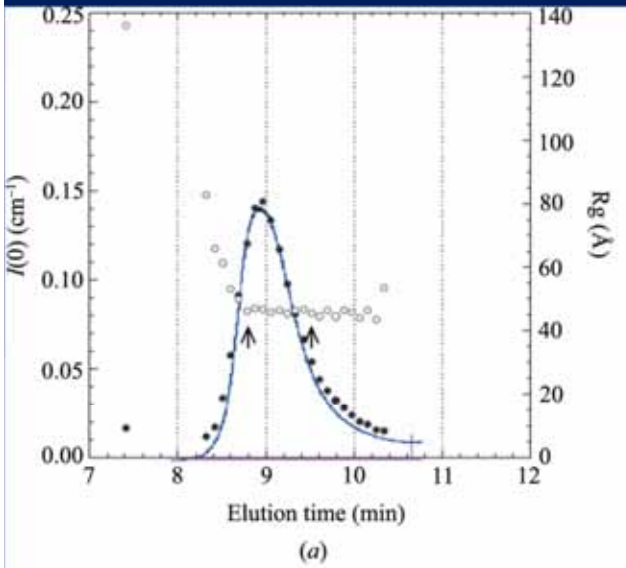
On-line SEC with SAXS

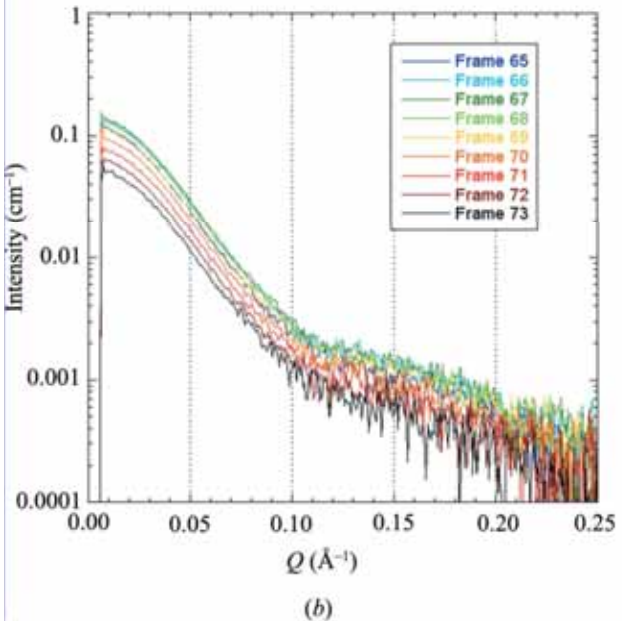


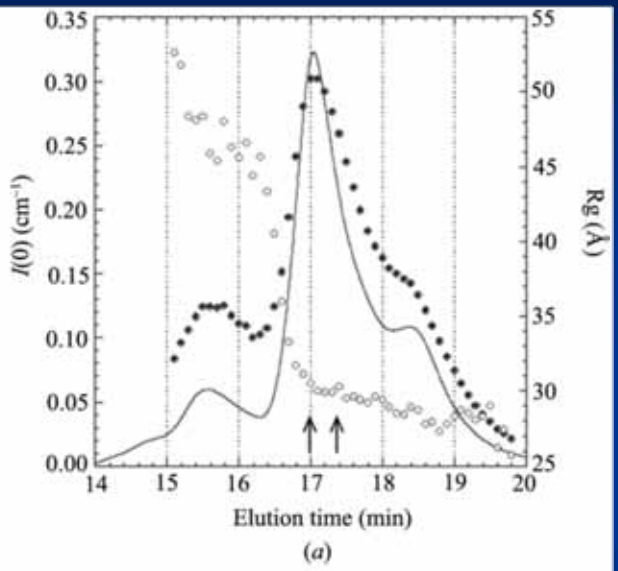


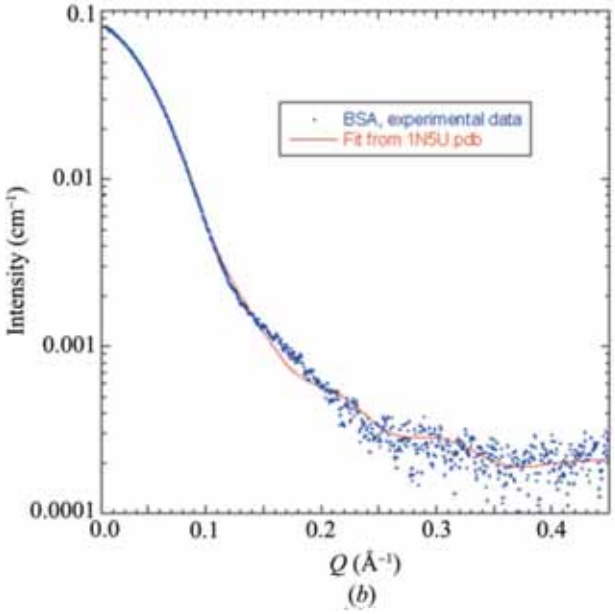
Agilent on-line HPLC-SAXS system in user operation at the SWING beamline (Soleil)

On-line SEC with SAXS









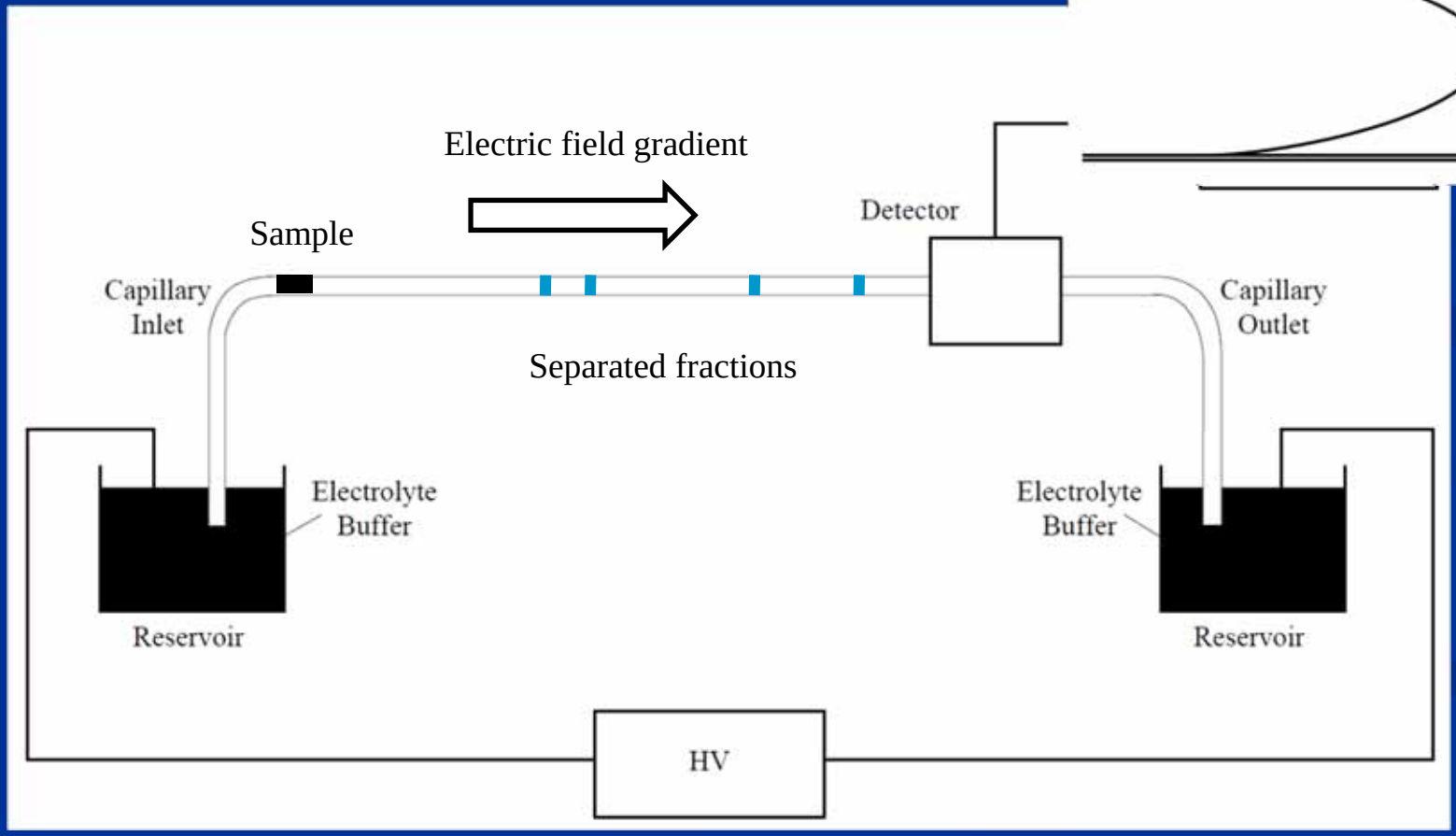
A proof of principle:

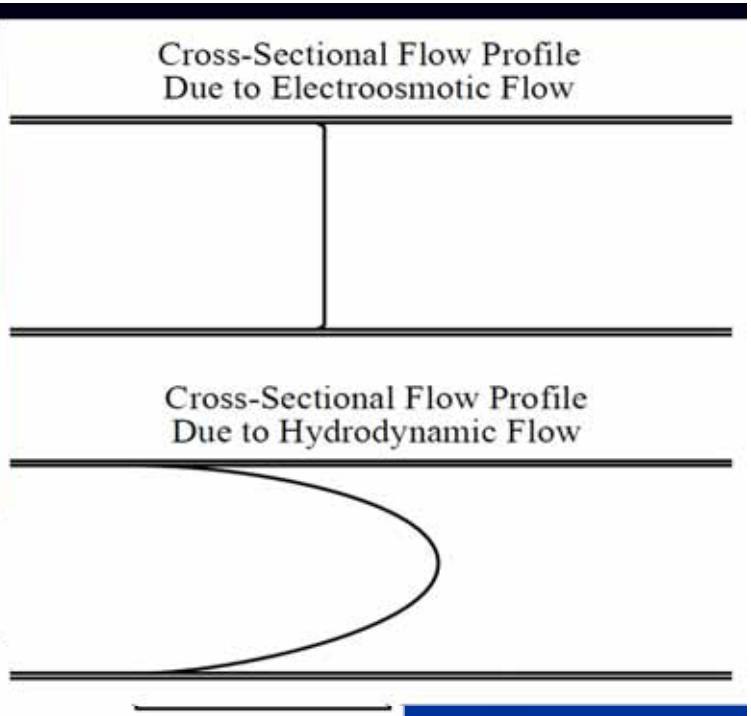
Left, near monodisperse protein solution

Right, commercial solution of BSA

David G. and Perez J. (2009) *J. Appl. Cryst.* 42, 892–900

On-line SEC with SAXS: capillary electrophoresis



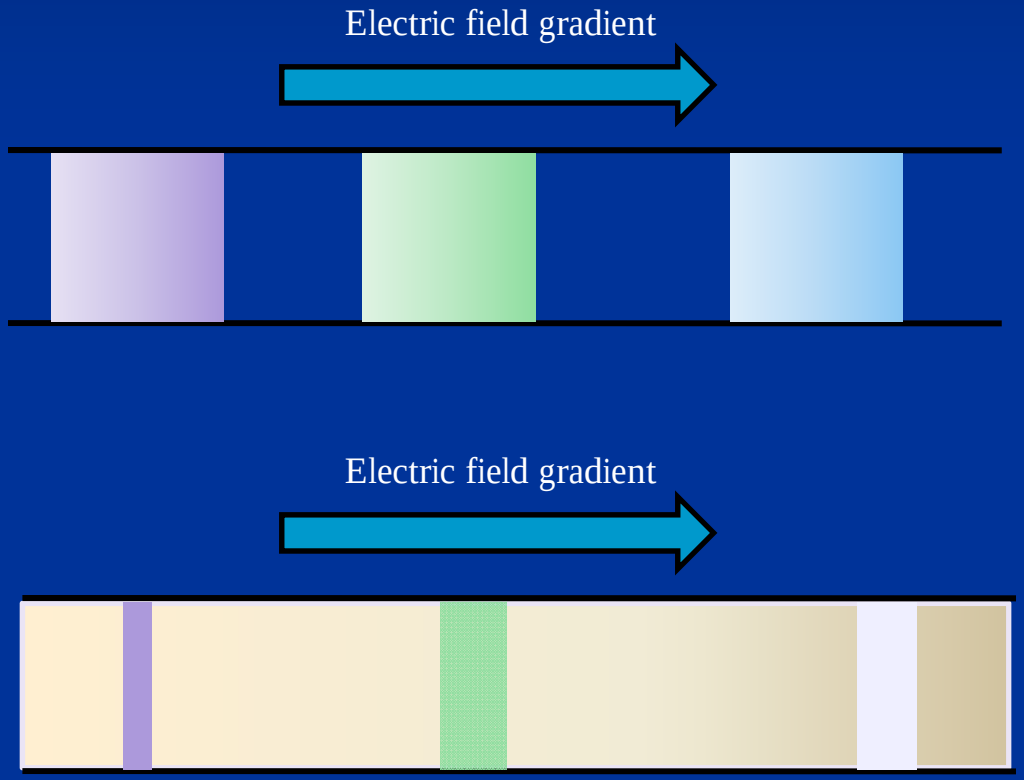


On-line SEC with SAXS: capillary electrophoresis

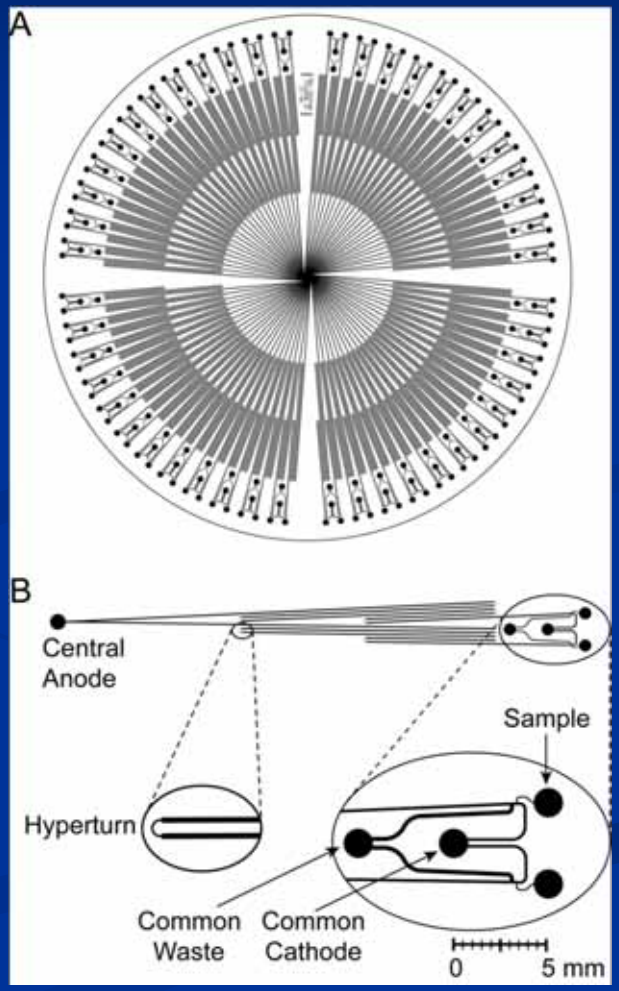
- Diameter of the capillary 25 – 75 μm
- Separation by electro osmotic forces based on charge, hydrodynamic radius and friction
- Good resolution power
- Faster than classical separation methods
- Sharp separation border, less dilution
- Re-concentration by isoelectric focusing of the separated protein in the capillary
- Adaptable on a micro lab-on-a-chip

On-line SEC : capillary electrophoresis

Isoelectric focussing



Lab-on-a-chip system

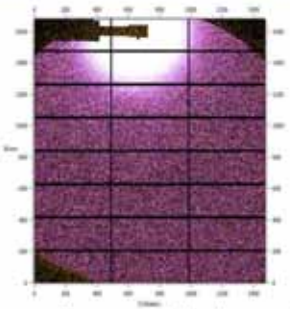


Yeung et al (2006) J Forensic Sci, **51**, 740

BioSAXS beamline (Petra-3, undulator)

Second generation sample changer (collaboration with EMBL-GR, prototypes operate at ID14-3 and X33 since 2009; installed at Petra-3 in March 2011)

BioSAXS P12 beamline fully utilizes the automation developed for X33

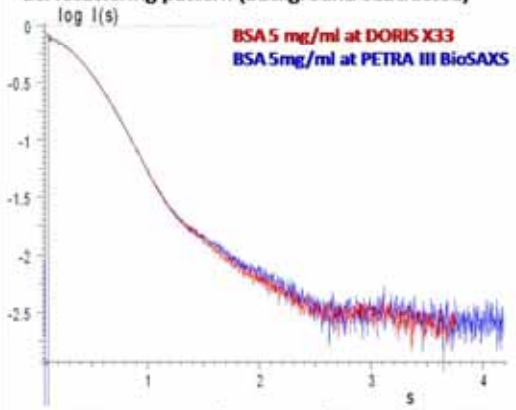


The scattering of BSA was recorded on **Thursday 9.06.2011.**

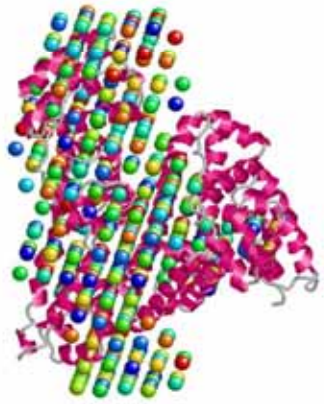
The protein solution of 5mg/ml was loaded to the sample cell using the robotic BioSAXS sample changer.
The inhouse developed software (BMS) controlled the loading and data analysis in a completely automated way.

The results are comparable with data collected at X33 which is still serving as benchmark.

BSA scattering pattern (background subtracted)



BSA scattering functions of X33 and BioSAXS



Fast ab initio bead model of BSA based on SAXS data, compared with the crystal structure of HSA (human serum albuminum).

Pilot projects for BioSAXS@PETRA3

Integrated sample characterization and online quality control

High throughput microfluidics

Large scale expression
Purification
Crystallisation

Auto-sampler

QC **HPLC**

UV, RI, SLS, DLS

SAXS

SAXS BioDisk, requires *nl* sample volumes (with Freiburg University)

What does SAXS tell about biological macromolecules

- Nothing known: *ab initio* low resolution structure
- Incomplete hi
  struct
  SAXS
- Mix
  ts
- Fl
  configurational ensembles

Not just high throughput: future of SAXS is also in the analysis of complicated systems (time and space resolution, transient complexes, flexible objects etc).
The brilliance of ERL/USRs does provide means for HTP and also for novel SAXS experiments, which are difficult on third generation sources

Biological SAXS @ EMBL-HH

Group leader: **D. Svergun**

Petra-3 project leader: **M. Roessle**

Staff scientist: **M. Petoukhov**

Senior technical officers:

C.Blanchet, P. Konarev, D.Franke

Postdocs: **H.Mertens, W.Shang, M.Gajda, C.Gorba, A. Kikhney**

Predocs: **A.Shkumatov, G.Tria, I.Danciu**