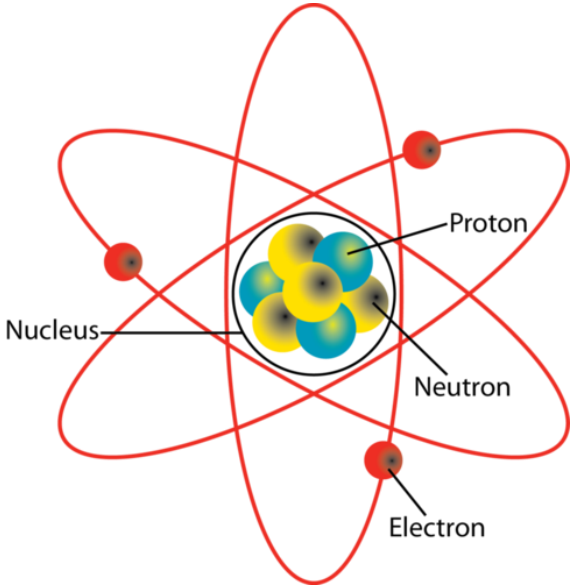
SANS instrument and Sample environment

Anne MARTEL, D22, LSS group

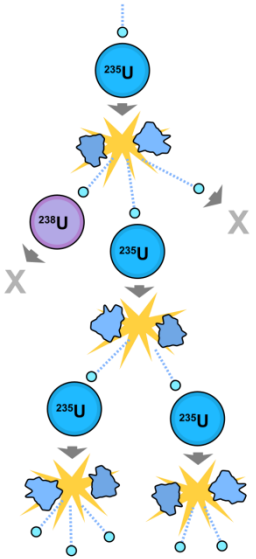
Introduction:

Neutrons:

Neutrons are part of the nucleus of any atom.

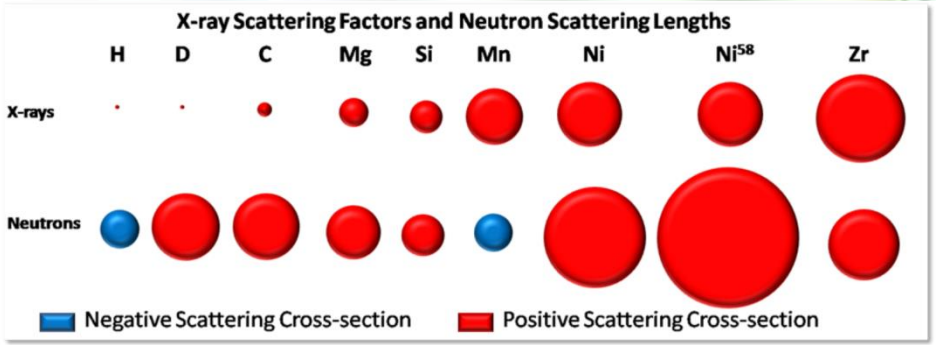


ILL, the European neutron source, produces neutrons by nuclear reaction

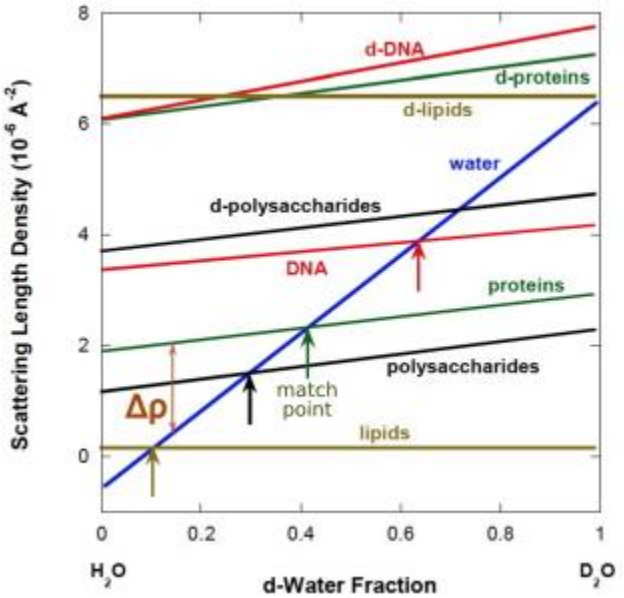


Main strength: Neutrons “see” hydrogens.

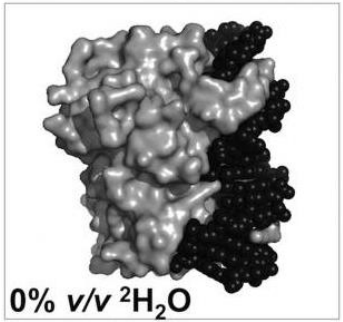
Neutrons vs X-rays: Contrast Variation



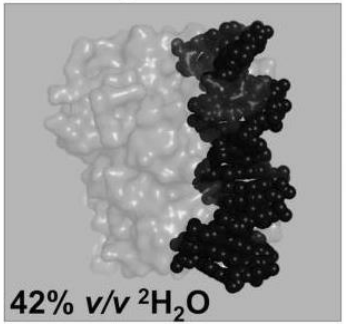
http://www.ncnr.nist.gov/resources/n-lengths/
http://www.isis.rl.ac.uk/ISISPublic/reference/Xray_scatfac.htm



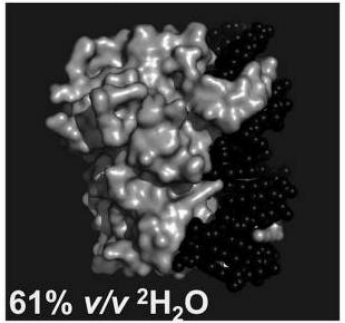
Protein/DNA Complex



Protein match point



DNA match point

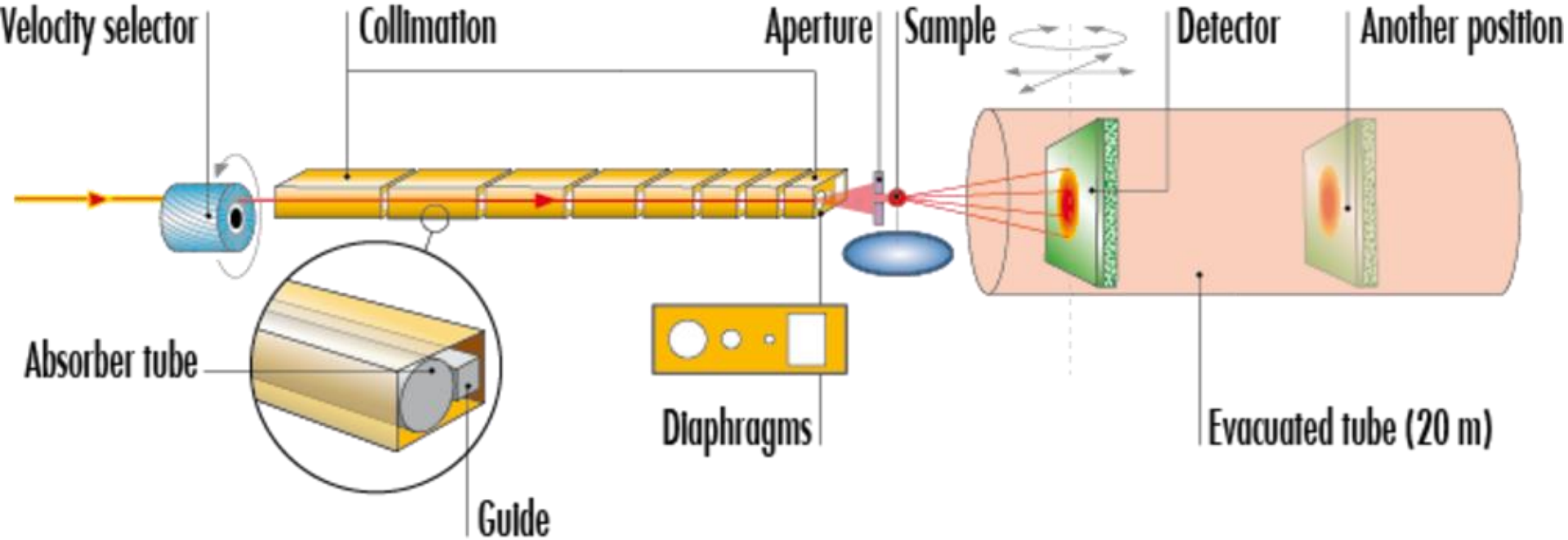


Castellanos et al., Computational and Structural Biotechnology Journal (2016)
Jeffries *et al.*, Nature Protocols volume 11, pages 2122–2153 (2016)

$$Q = 4\,\pi.\sin\Theta / \lambda$$

From LLB

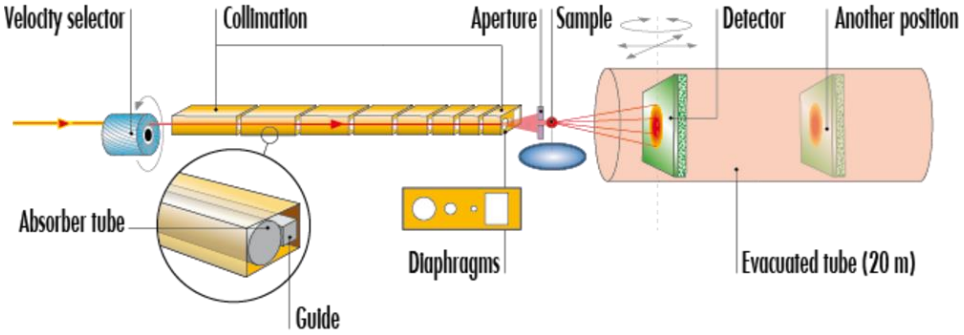
D22, a monochromatic SANS instrument

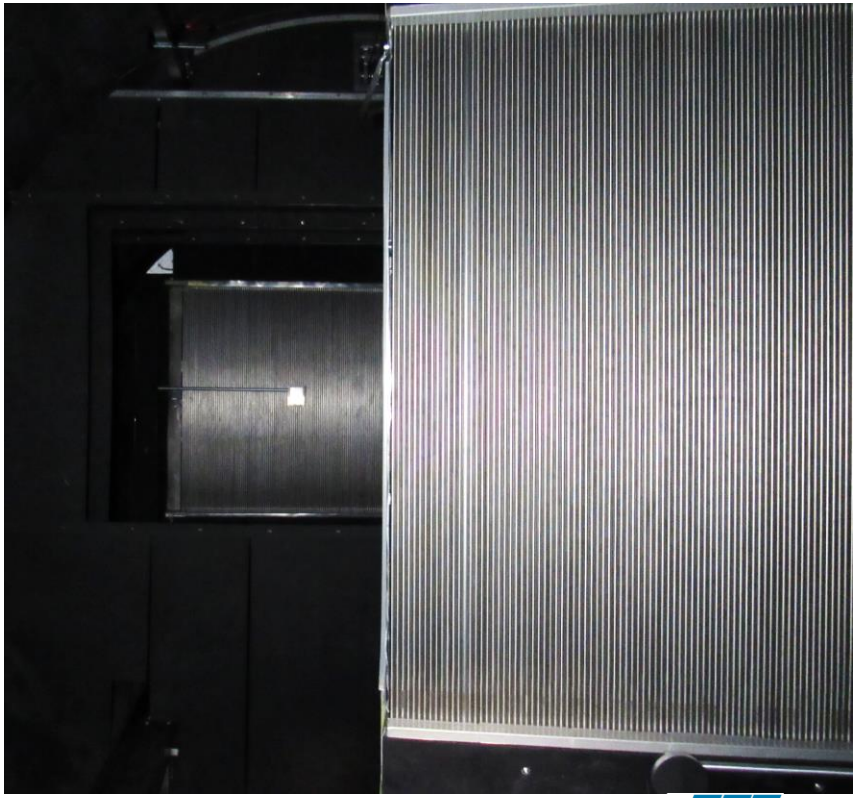


D11 description:
https://www.ill.eu/fileadmin/user_upload/ILL/3_Users/Instruments/Instruments_list/00_-_LARGE_SCALE_STRUCTURES/D11/html5/D11-principle/D11.html

A. Filhol

D22 ++





2nd detector > whole Q-range in one set up

Semitransparent beamstop

L. Porcar, D. Barkats, E. Ruiz, C. Cocho

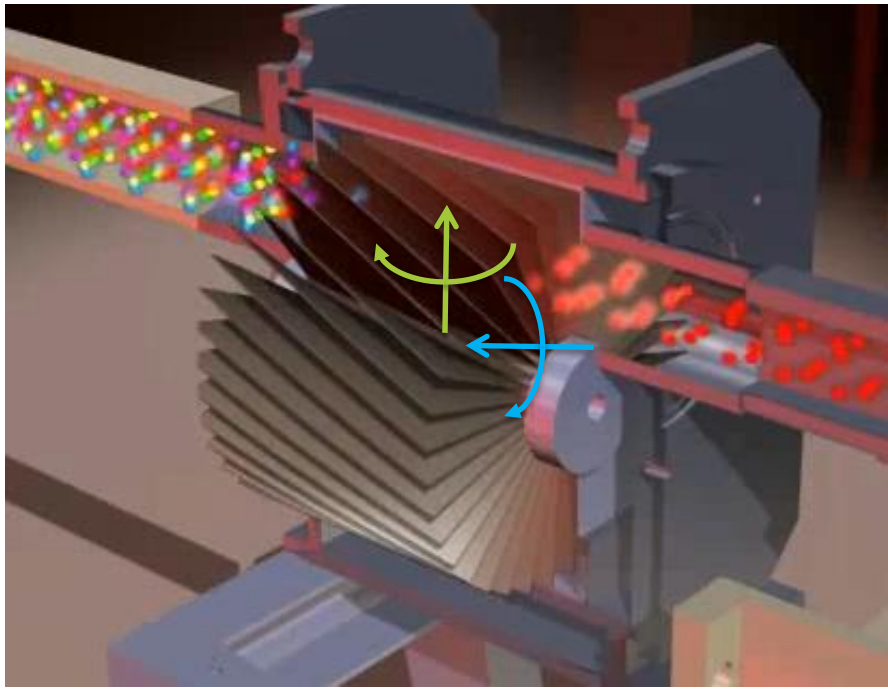
Velocity selector

Wavelength and Smearing

Selector Speed → Nominal Wavelength (6 - 12 Å)

Selector Tilt → Wavelength Spread

Wavelength Spread
Beam size
Sample size
pixel size
} Smearing or Q-resolution (4^{th} column in data files)



$Q = 4 \pi.\sin\Theta / \lambda$

Refraction based monochromators also exist for neutrons (D16). Lower flux!

Basic requirements

- Large enough sample quantity: one cuvette = 200uL at C ≥ 1mg/mL of non-matched out part of sample.

- Monodispersity and absence of interactions (according to analysis, as for SAXS)

- Optimized contrast:

  Intensity proportional to contrast^2.
  Background proportional to H_2O buffer content (from 0.05 to 1 cm^{-1}).

>> The ideal sample is an hydrogenated molecule in D_2O buffer. Matched out partners should be deuterated to have the same SLD as D_2O.

Compared to SAXS

Clear weaknesses:

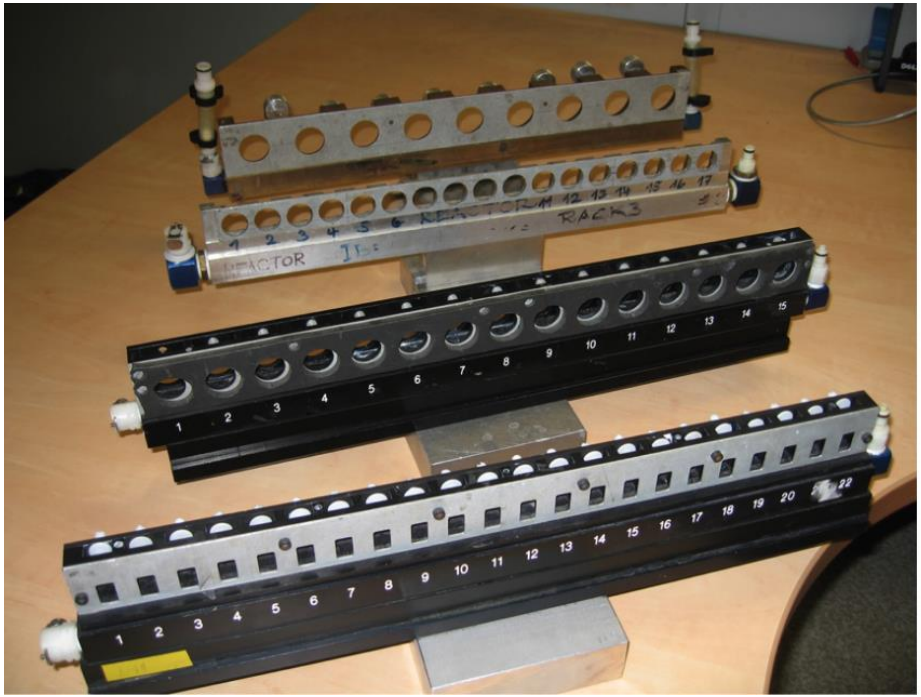
- Low flux: big samples, long measurement times (stability, time resolution)
- Broad wavelength: resolution smearing along Q
- Potential sample activation

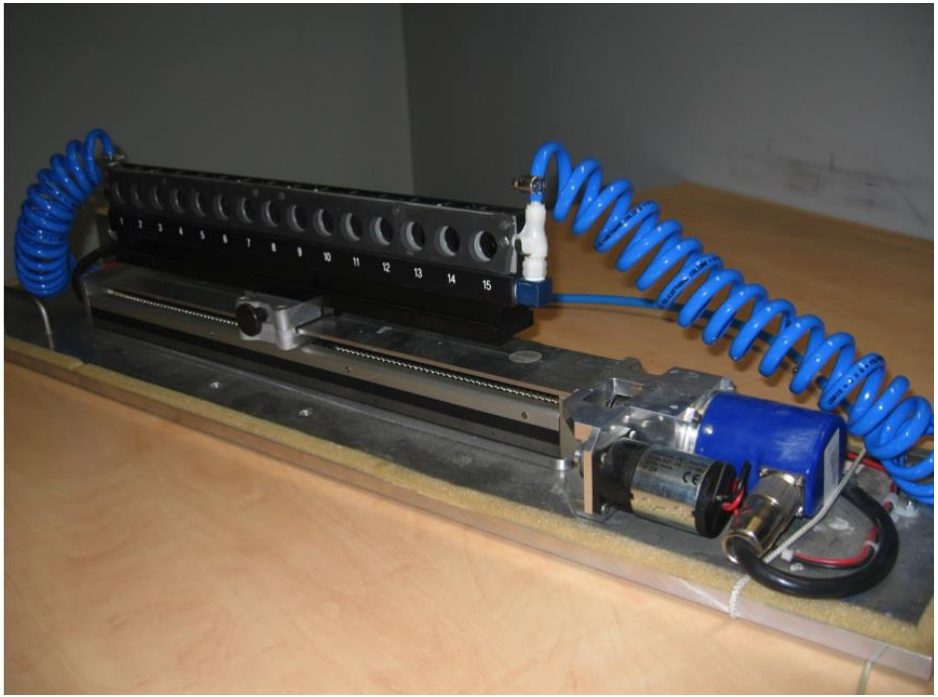
But, a few advantages:

- No radiation damage
- Contrast variation
- Large air gap to accommodate sample environment

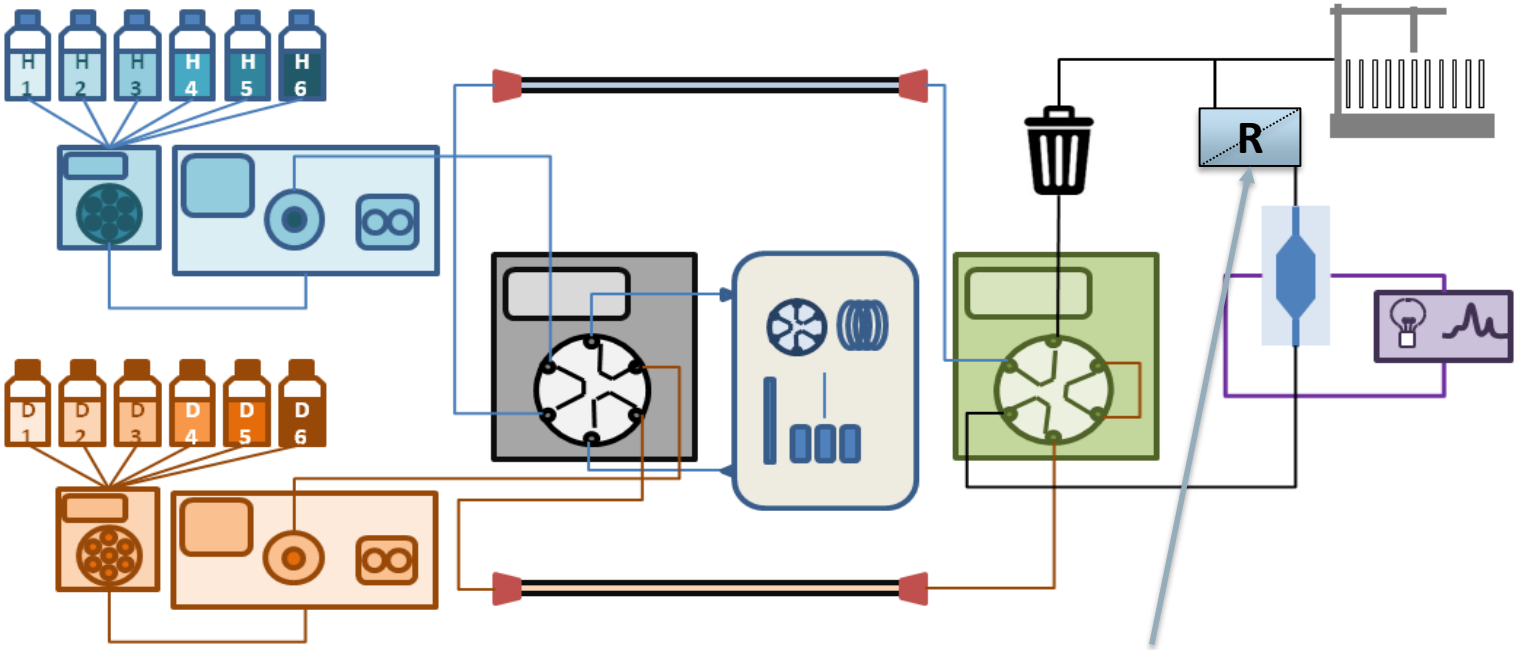
Sample changers

200uL or 300uL sample





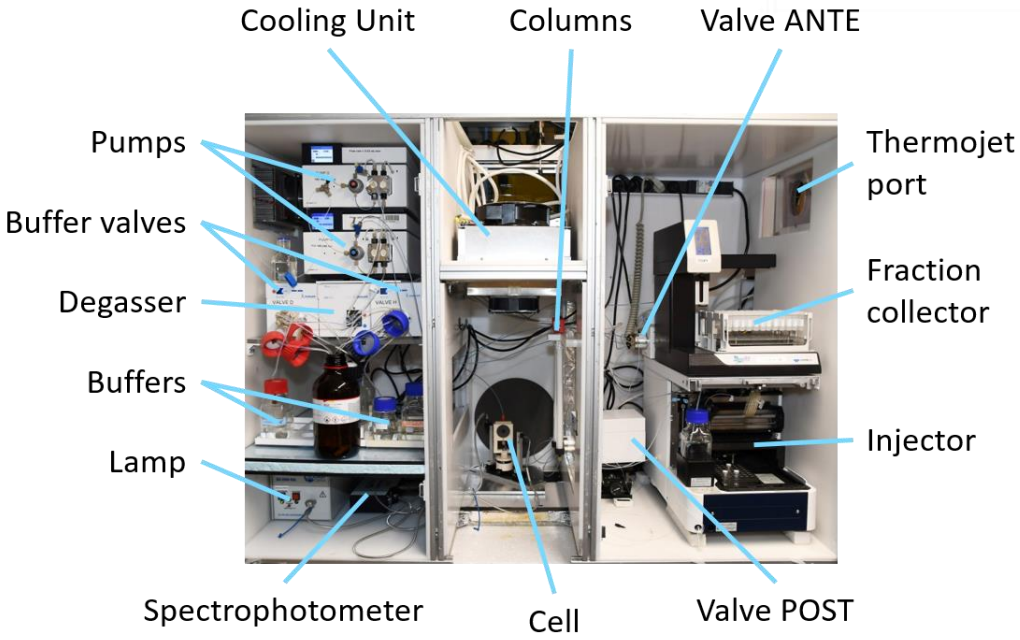
In situ SEC:

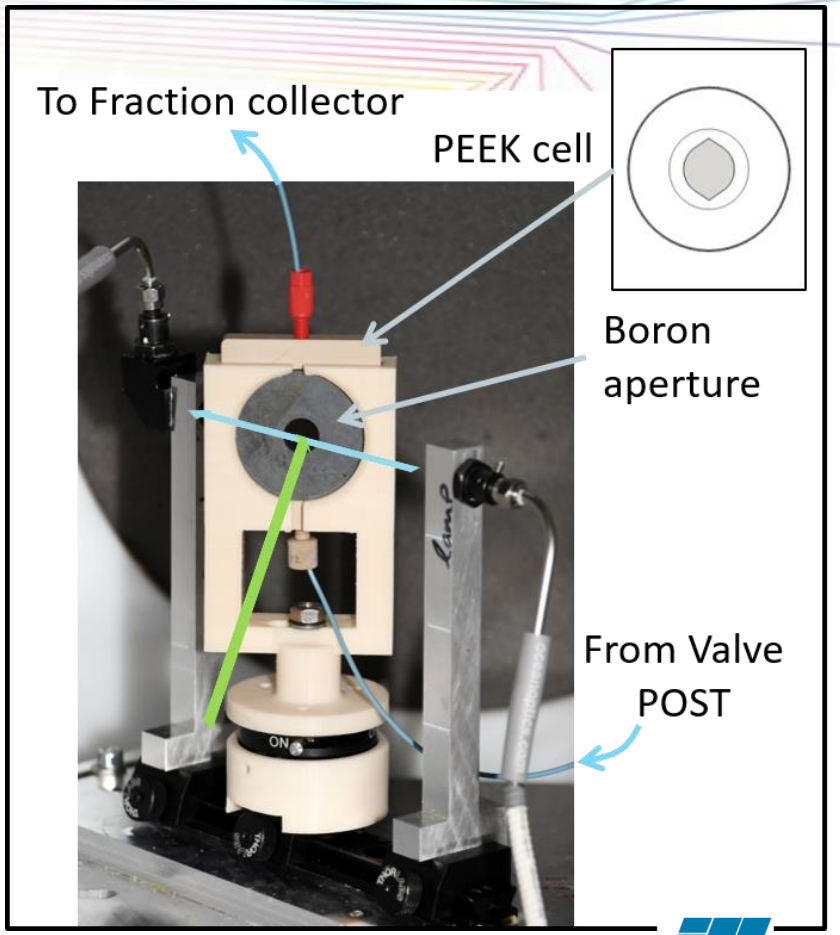


Refractive index detector coming soon (S. Combet, F. Bonnete)

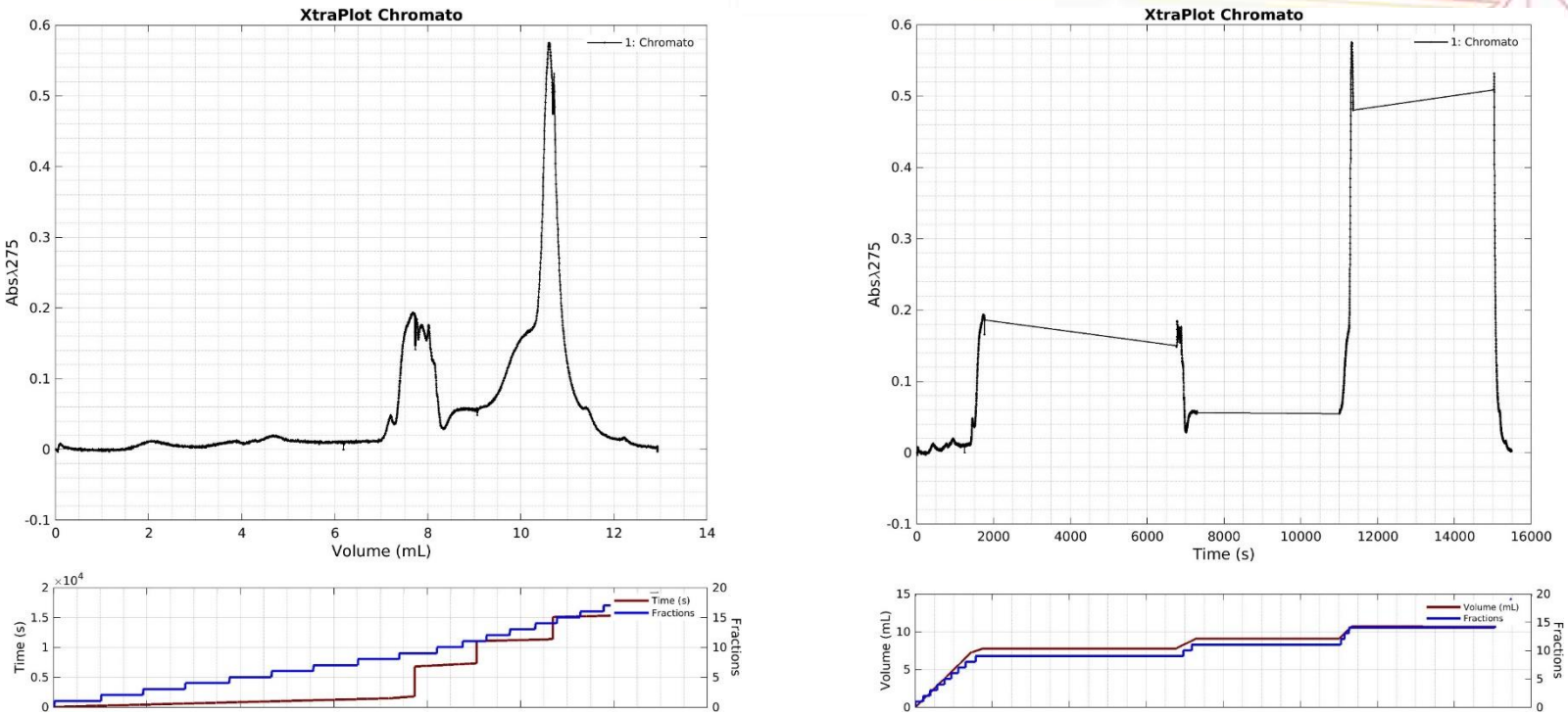
In situ purification > improved sample quality. Solvent and detergent exchange.

SEC-SANS





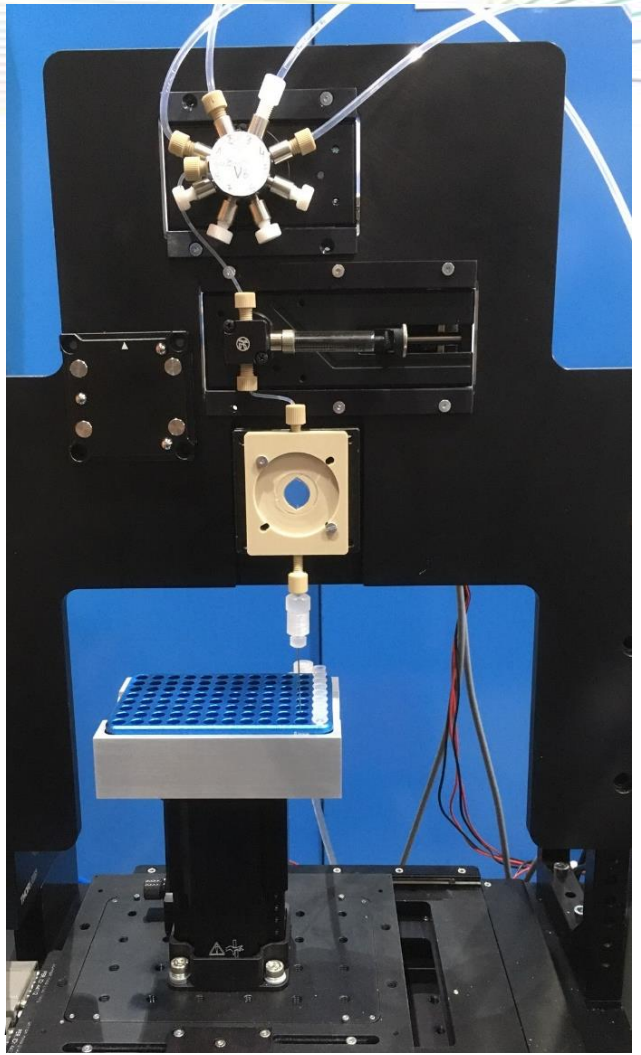
Buffer and protein measured in the same cell
> improved buffer substraction



Rg and I(0)/Abs on each SANS profile. Still under development by C. Dewhurst…

Autosampler

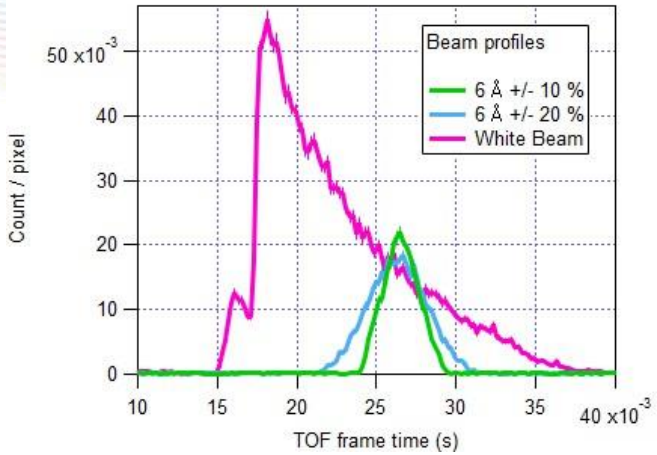
- Up to 96 samples, in PCR tube strips (like SAXS)
- Volume 200uL
- Up to 8 cleaning/rinsing/drying fluids
- Temperature controlled by thermojet

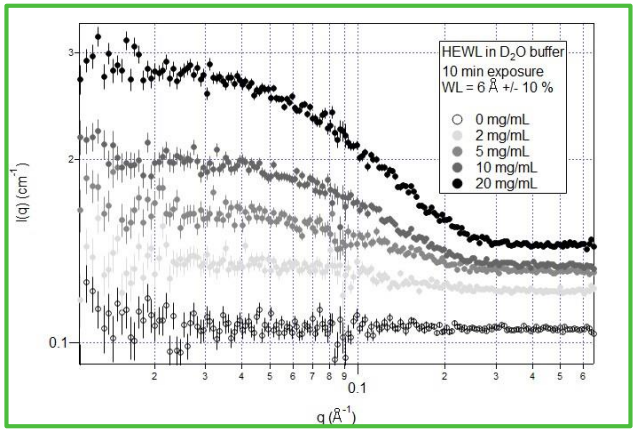


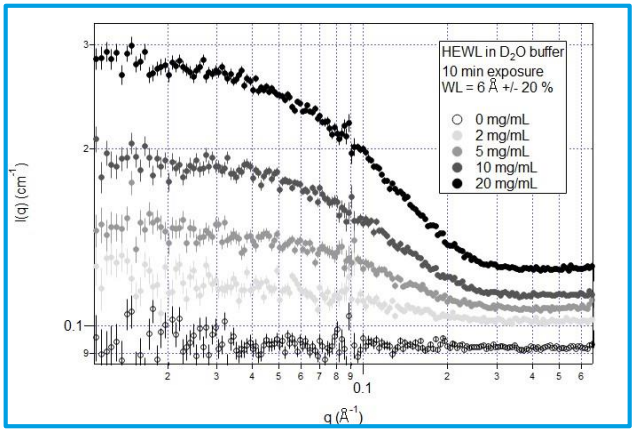
Commissioning using 3 beam profiles

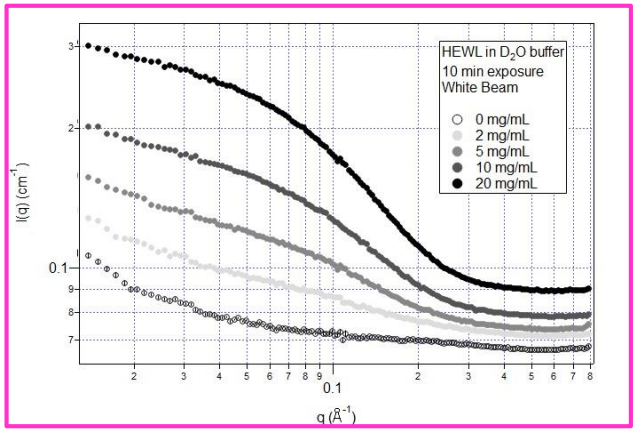
HEWL concentration series at :

- WL = 6 Å ± 10 %
- WL = 6 Å ± 20 %
- White beam option



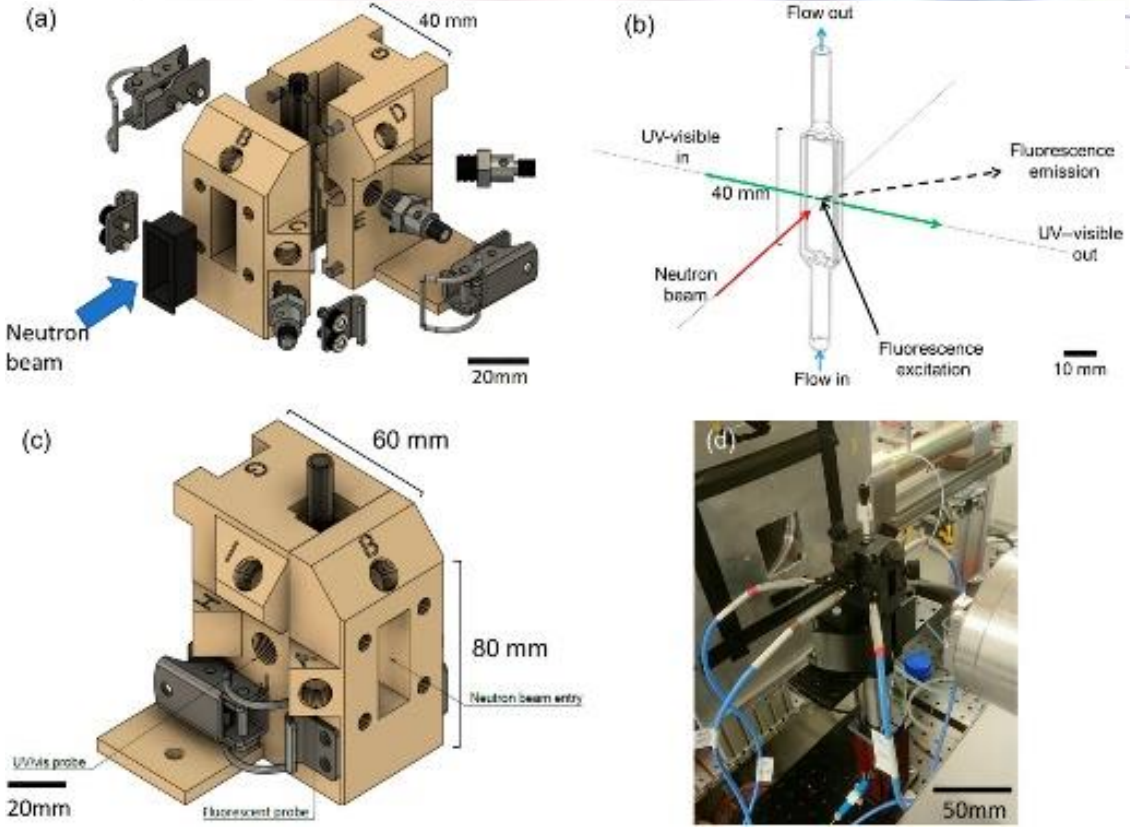






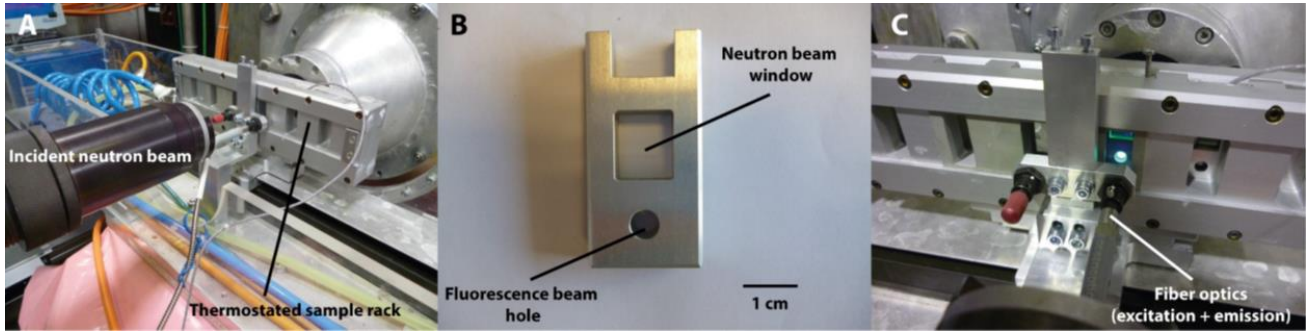
NURF

NUrF—Optimization of *in situ* UV–vis and fluorescence and autonomous characterization techniques with small-angle neutron scattering instrumentation C. Dicko et al., Review of Scientific Instruments **91**, 075111 (2020)

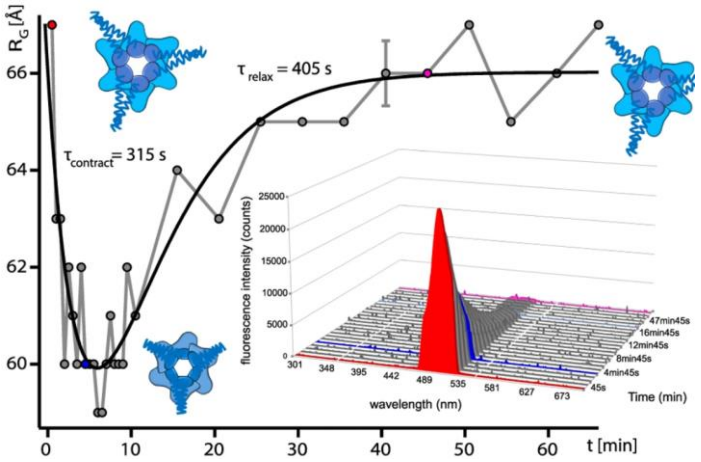


cedric.dicko@tbiokem.lth.se

In situ Fluo



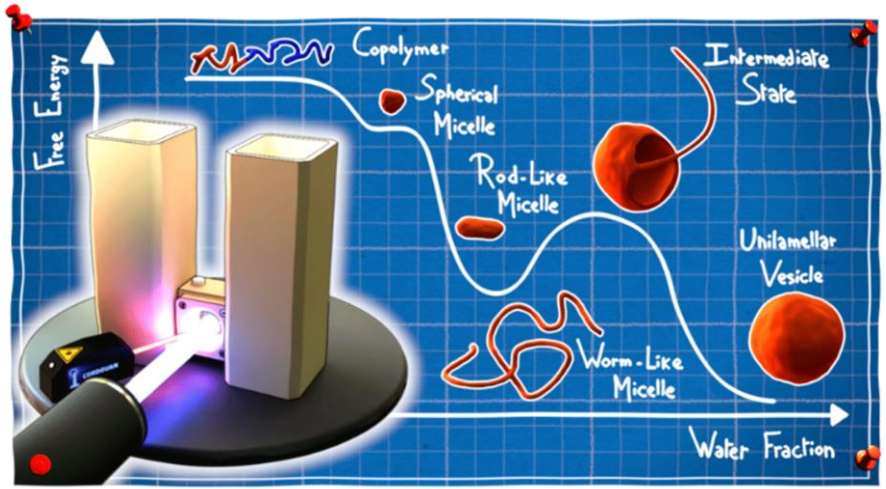
Ibrahim Z. *et al.* Time-resolved neutron scattering provides new insight into protein substrate processing by a AAA+ unfoldase. *Sci Rep* **7,** 40948 (2017).

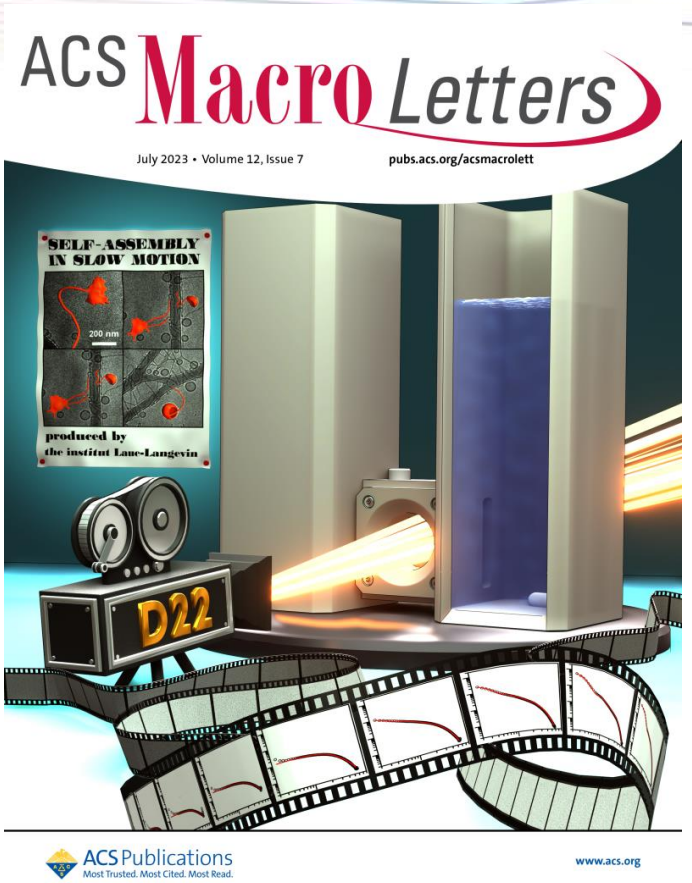


Dialysis Cell

In situ monitoring of block copolymer self-assembly via solvent ex-change through controlled dialysis with light and neutron scattering detection

Martin Fauquignon,* Lionel Porcar,* Annie Brûlet, Jean-François Le Meins, Olivier Sandre, Jean-Paul Chapel, Marc Schmutz, and Christophe Schatz*

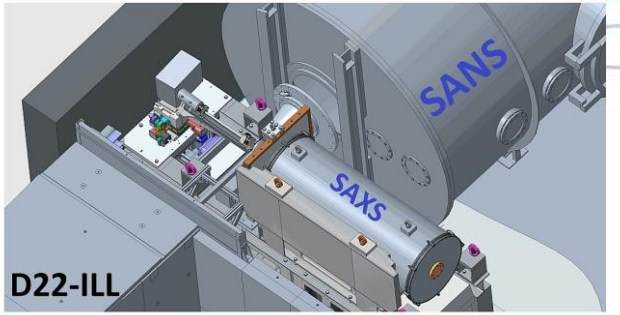


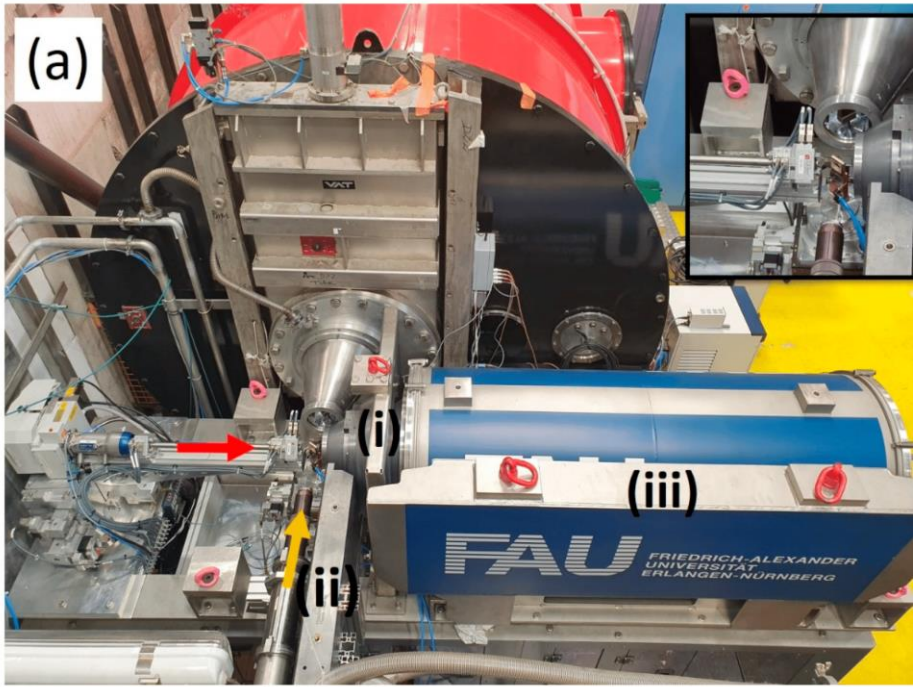


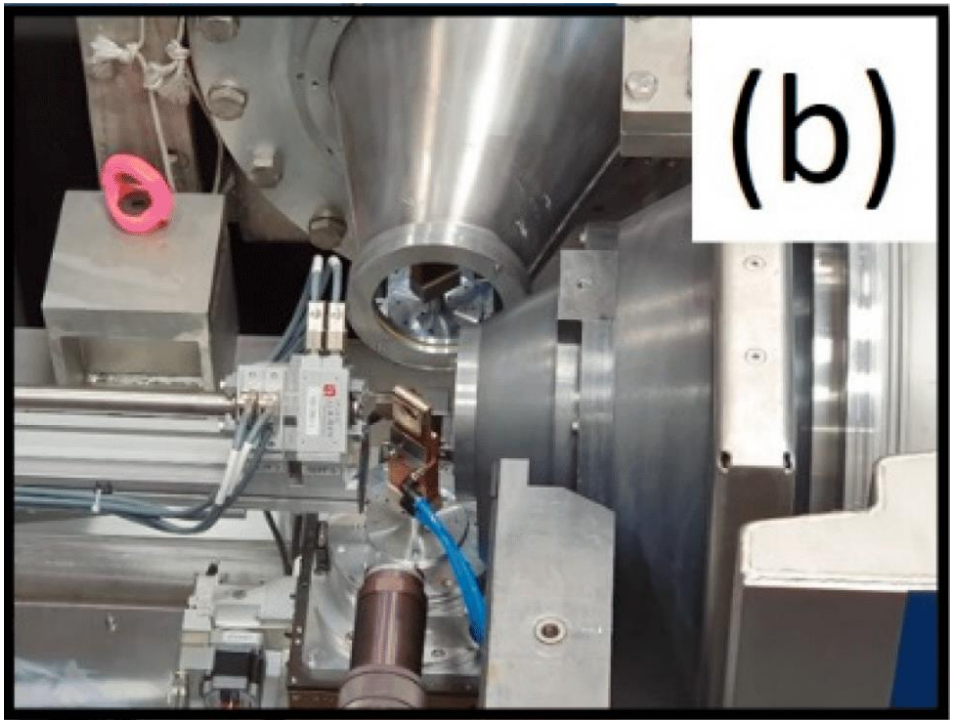
SAXS-SANS

Simultaneous SAXS/SANS Method at D22 of ILL: Instrument Upgrade

Ezzeldin Metwalli, Klaus Götz, Tobias Zech, Christian Bär, Isabel Schuldes, Anne Martel, Lionel Porcar and Tobias Unruh

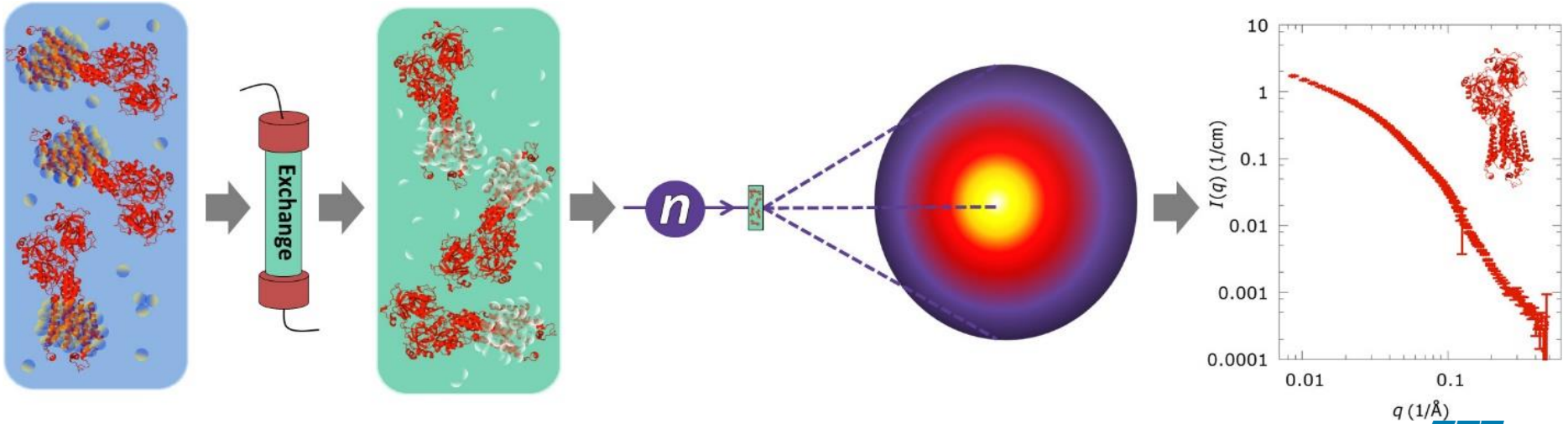






Examples of deuteration for membrane protein studies:

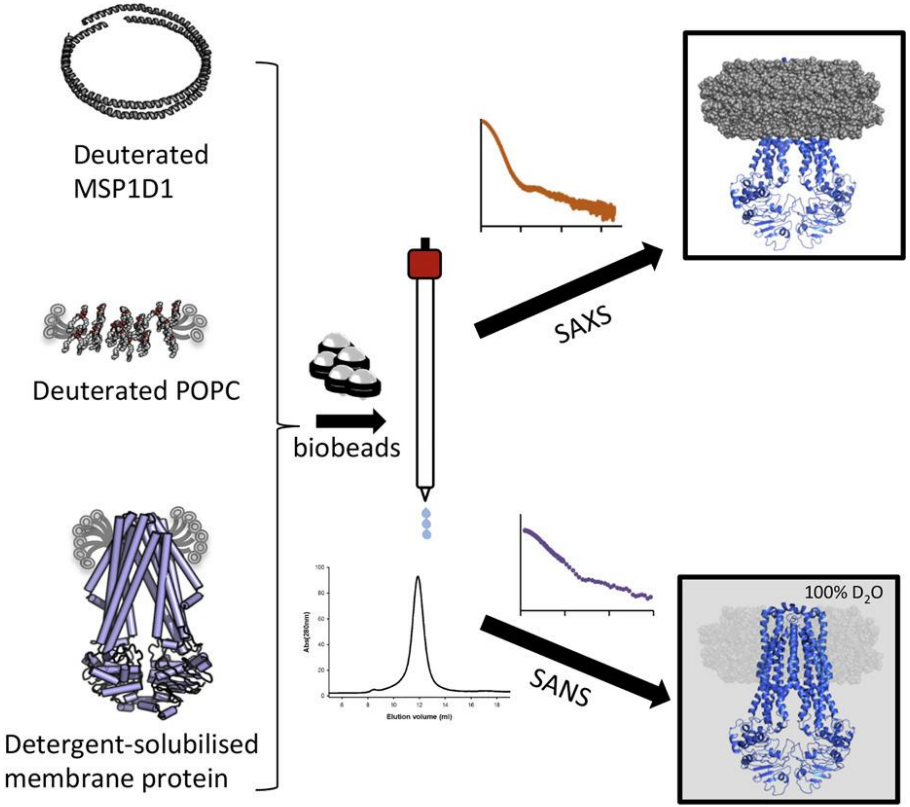
Invisible detergents:
Mitgaard et al., 2018
doi: 10.1111/febs.14345. Epub 2017 Dec 30.

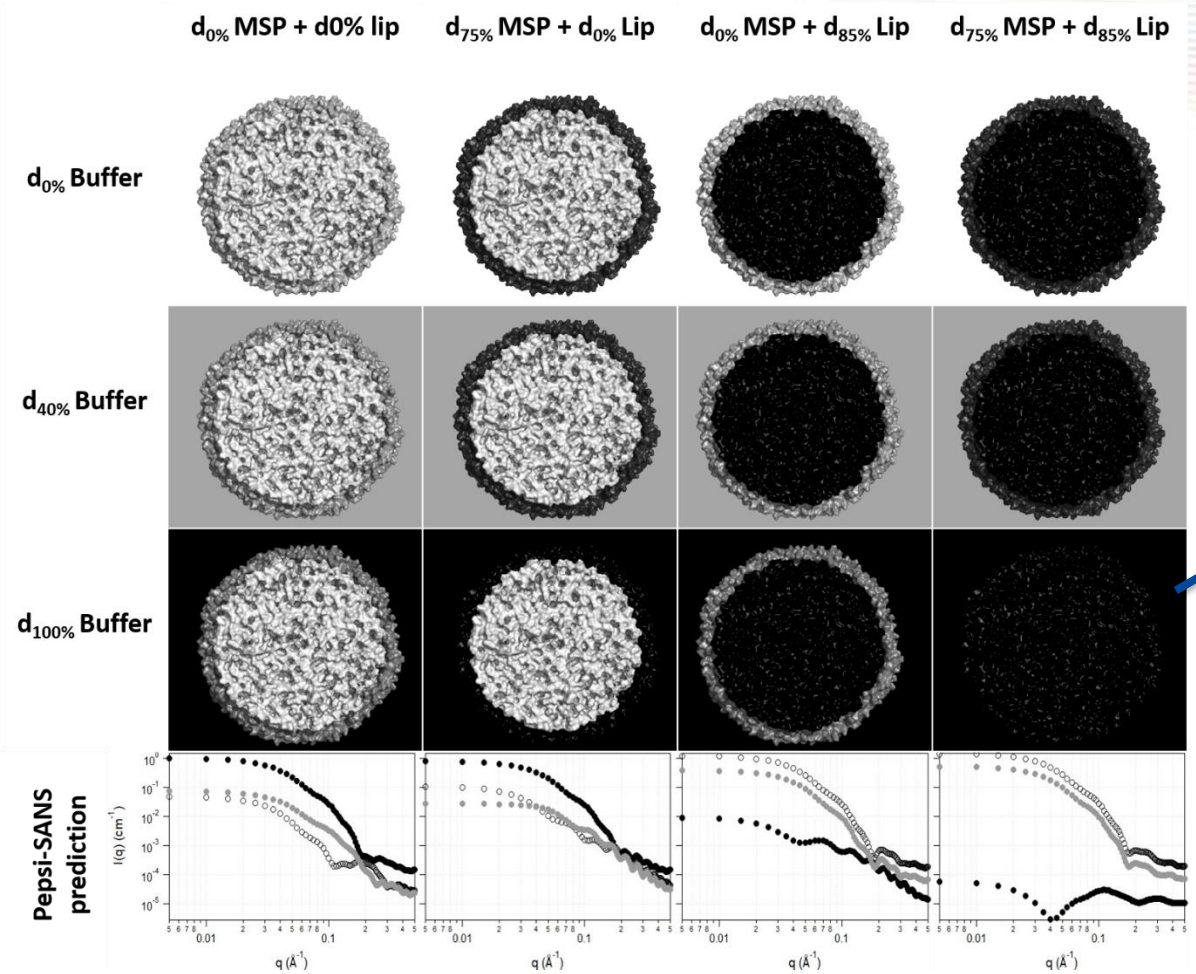


Invisible Nanodiscs

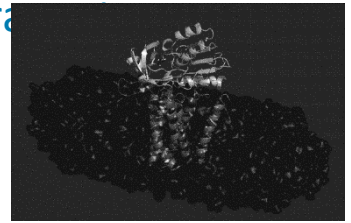
Josts et al., 2018
https://doi.org/10.1016/j.str.2018.05.007

If the deuterated molecule you need is not commercially available, contact DeuNET: proteins, peptides, natural and synthetic lipids, detergents…





Study of protein in their natural lipid environment: Full polar lipids from *E. coli* or *P. Pastoris*, ILL L-lab, K. Batchu and G. Fra



Study of specific lipid-protein interactions: addition of a specific hydrogenated lipid.

Study of ND formation or protein insertion mechanism…

SAXS-SANS plateform

ESRF/BM29 and ILL/D22-D11-D33

Sample requirements for SAXS, for SANS

Beamtime application


Thank you!
Questions, Suggestions…?