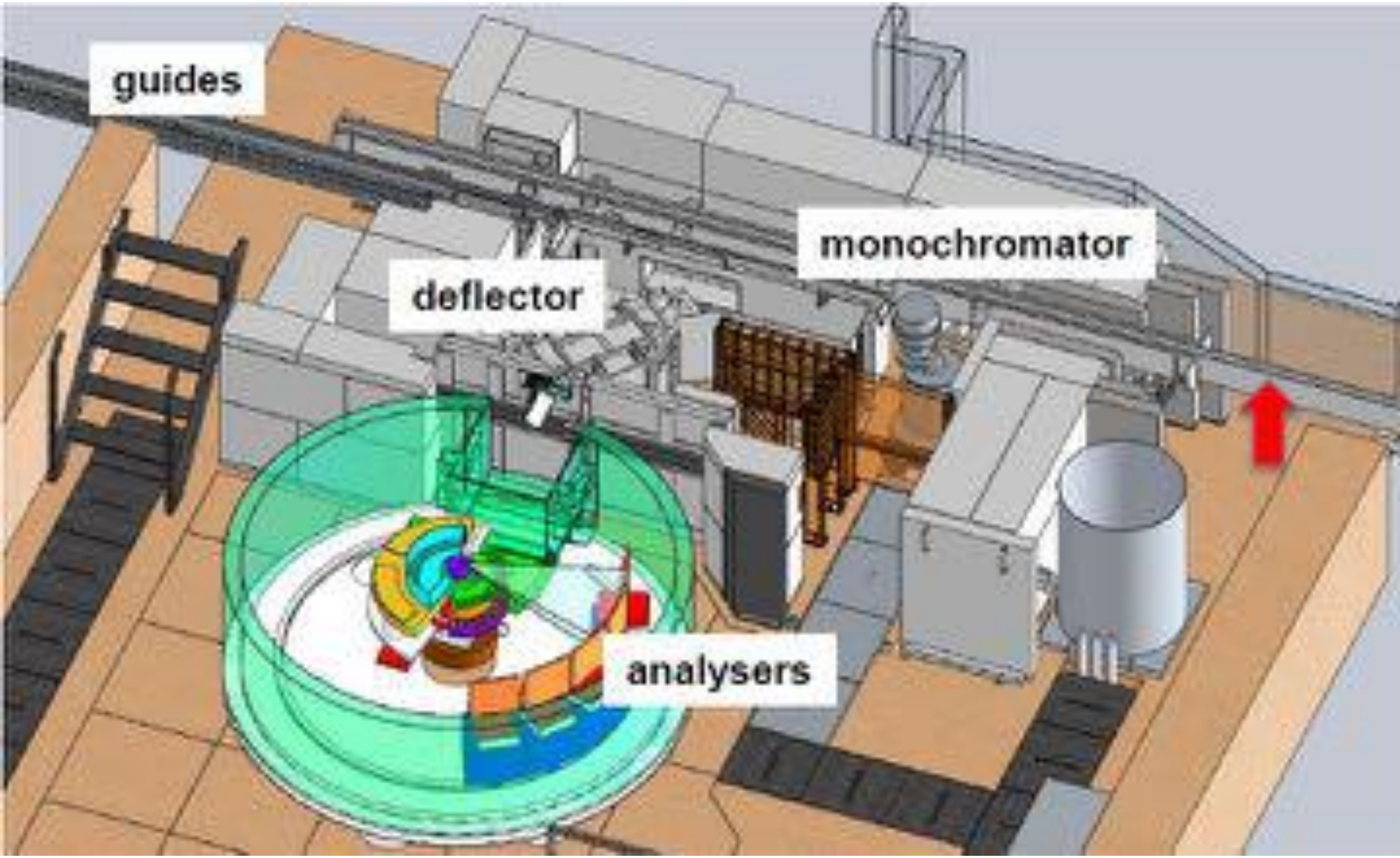
Upgrade of IN13 and high pressure applications

Judith Peters (UGA, LiPhy and ILL, Grenoble)

Francesca Natali (CNR and ILL, Grenoble)

IN13+ Endurance program



IN13+ Endurance program: contributions by the INP and the Federation Neutronique (40 kEuro)

The deflector crystals have to be renewed as well as their mount.

IN13+ Endurance program: contributions by the INP and the Federation Neutronique (40 kEuro)

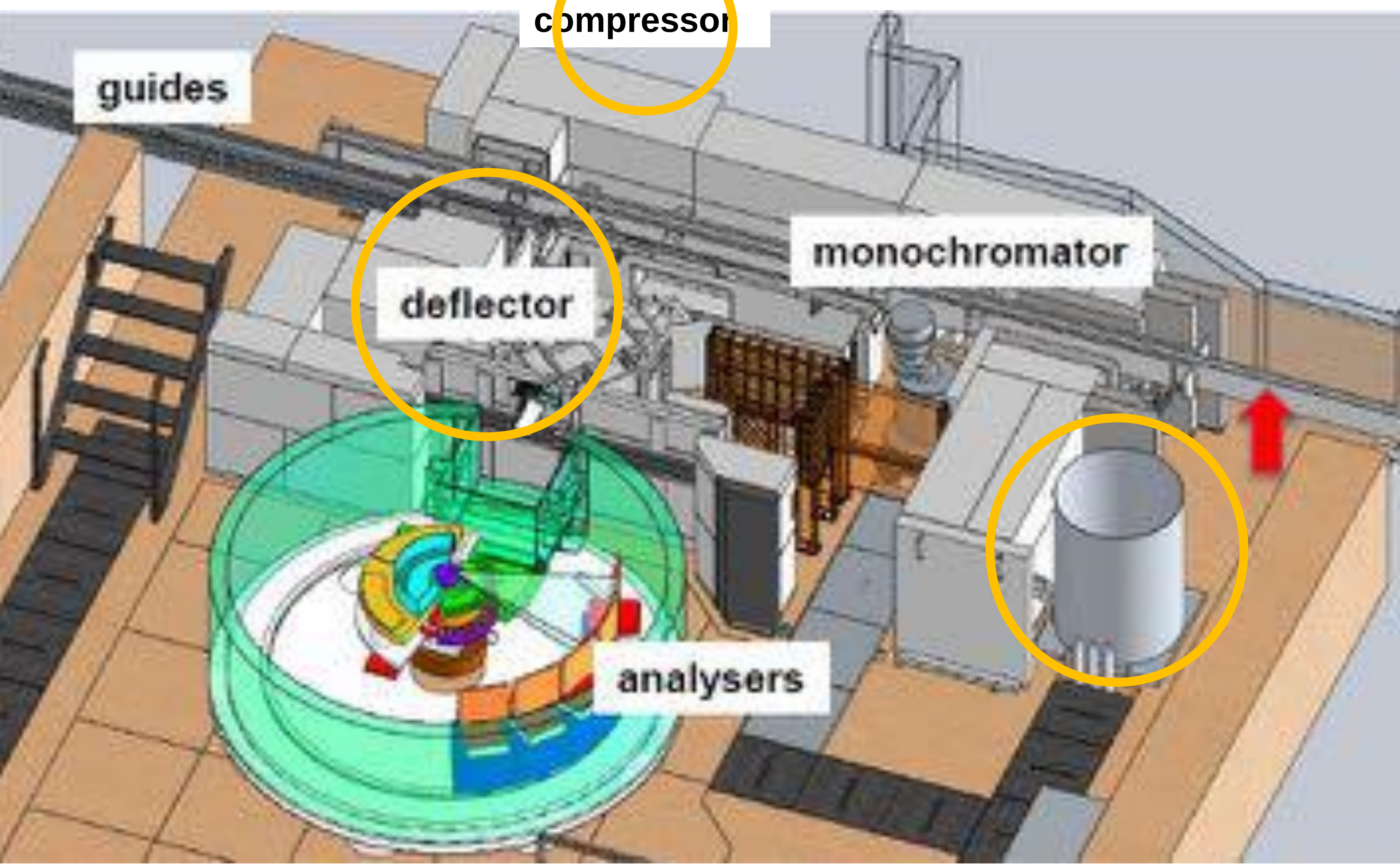
- Nitrogen vessel TPV 400L built by Wessington Cryogenics including the valve and the regulation of the levels, 5380 Euros

- 9 graphite crystals HOPG for the deflector of IN13 (50*15*2 mm^3), 15350 Euros

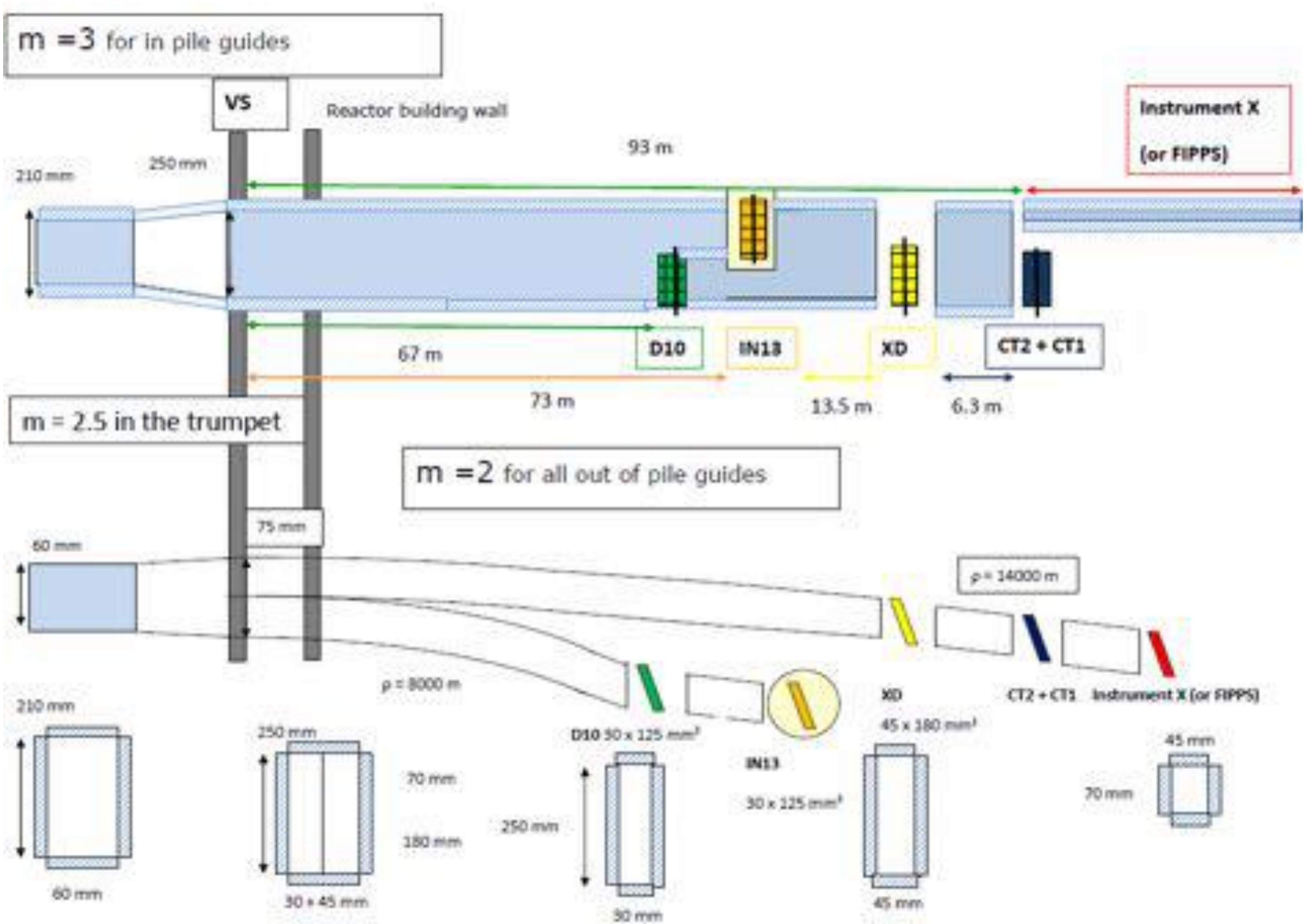
- Scheme of the deflector mount containing the new crystals HOPG, developed by the engineering office of the ILL, 8190 Euros

- Cryocooler compressor Sumitomo F-50H, 10062 Euros

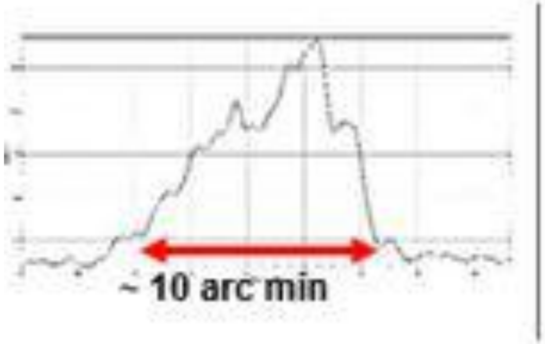
IN13+ Endurance program: contributions by the INP and the Federation Neutronique (40 kEuro)



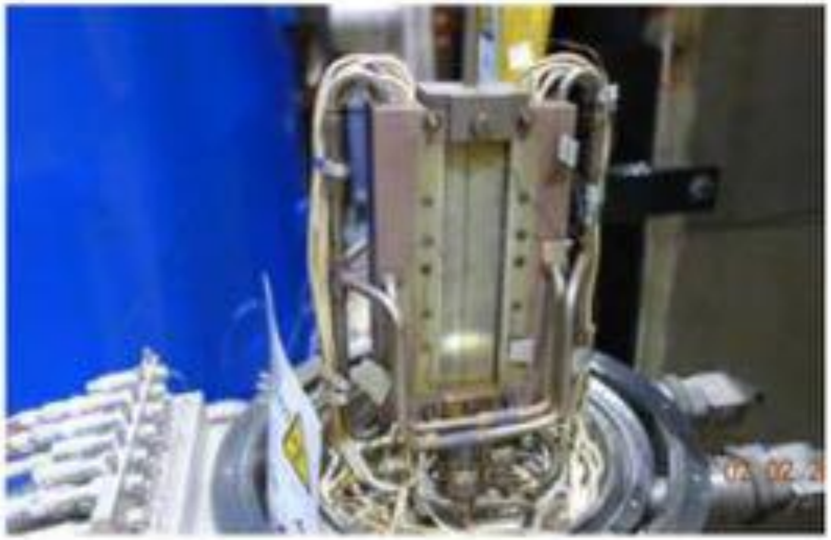
IN13+ Endurance program: renewal of the guide H24. IN13+ will get an end position.

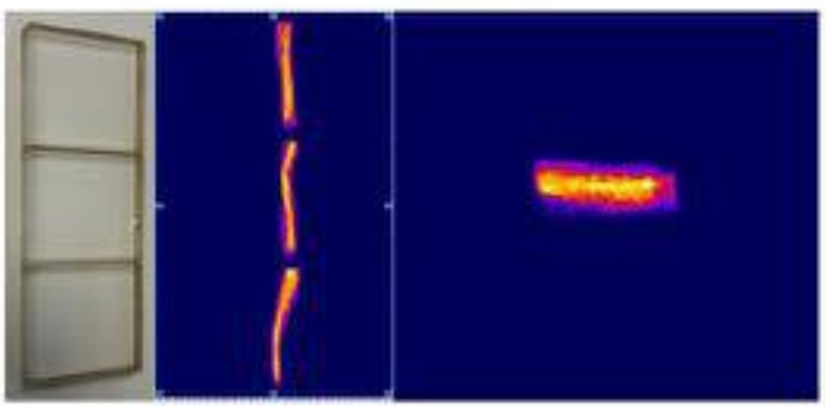


IN13+ Endurance program: Broken analyser crystals will be replaced and the surface better covered.



IN13+ Endurance program: Temperature gradient monochromator and mosaic crystals



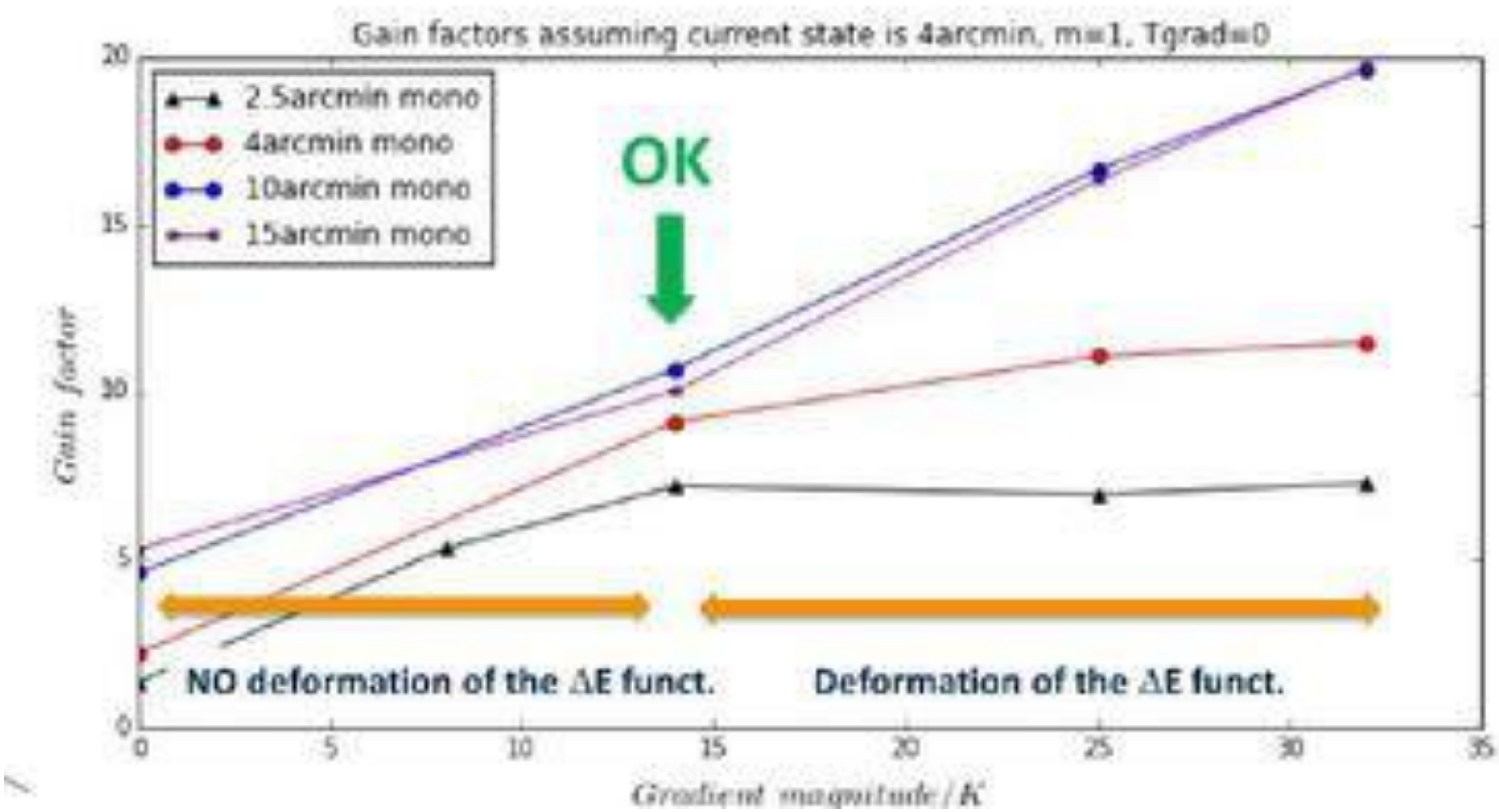


Wanted situation:
- Guide m=2
- Crystal mosaicity: 10 arcmin
- 14 C T gradient

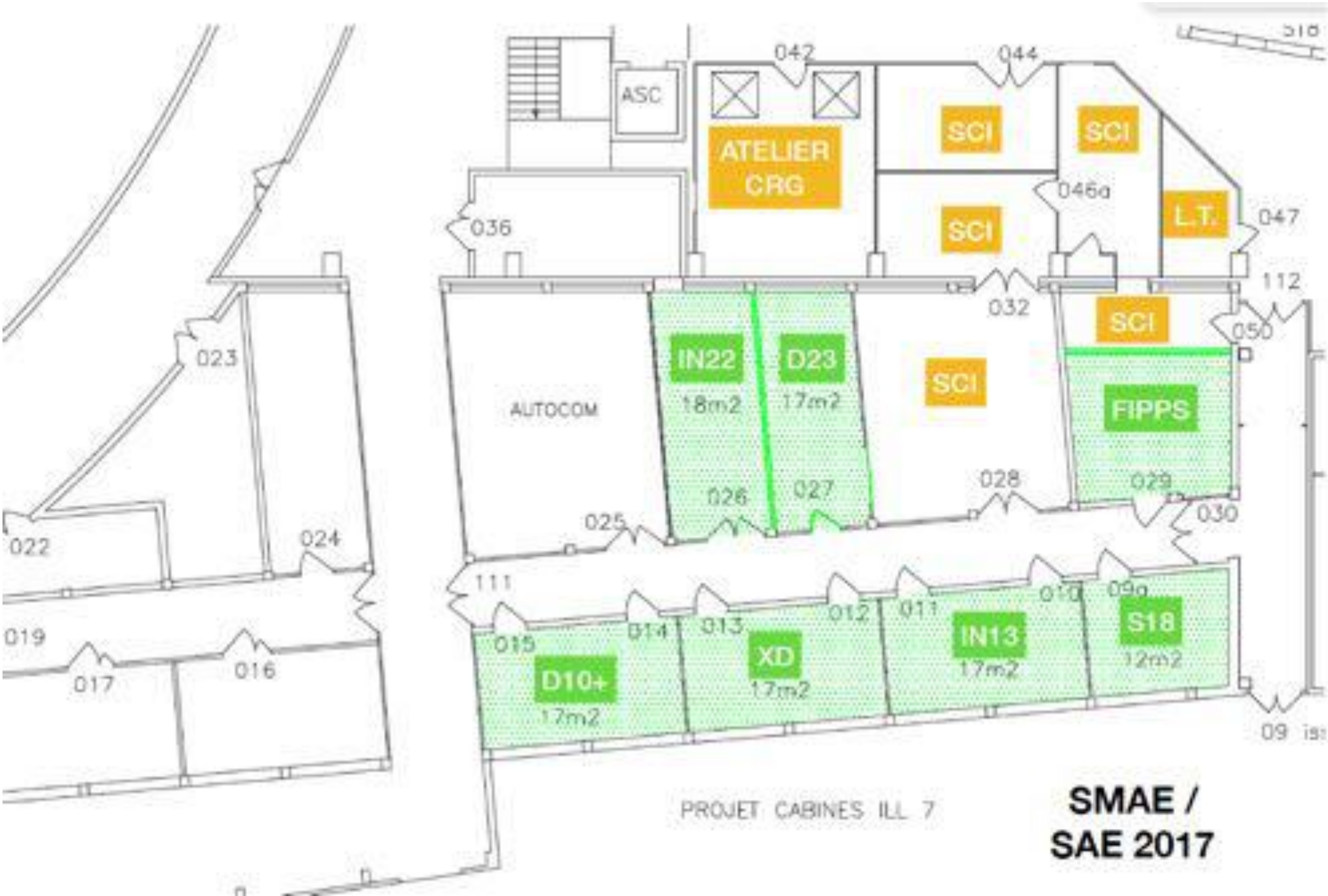
Current situation:
- Guide m=1 and old
- Crystal mosaicity: 2-4 arcmin
- No T gradient

IN13+ Endurance program: Expected gain in flux

- From the guide: x 2 – 4
- From the monochromator: x 5 - 10



IN13+ will get a new cabin and a remote control point close to IN13+



How to study biological systems under high pressure ?

Incoherent neutron scattering

IN13: Backscattering spectrometer at ILL

Incoherent diffusion

of H atoms dominates

+

Homogeneous distribution

of H atoms within the proteins

=

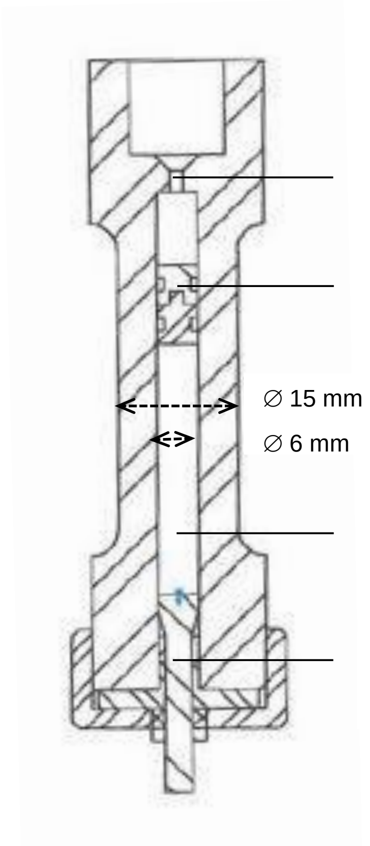
Incoherent diffusion of neutrons

probes the global dynamics of

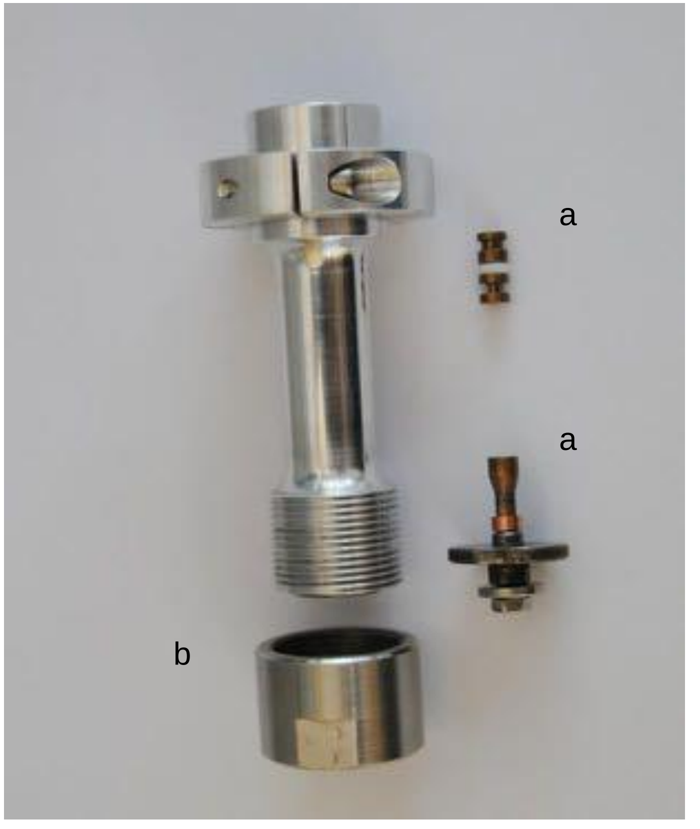
proteins

(time scale: ns-ps)

High pressure equipment (adapted from a design of M.-C. Bellissent-Funel's group) :



Cylindrical cell (with insert) of Aluminum Al 7049T6 of 9 mm thickness



a: Cu-Be

b: stain-less steel

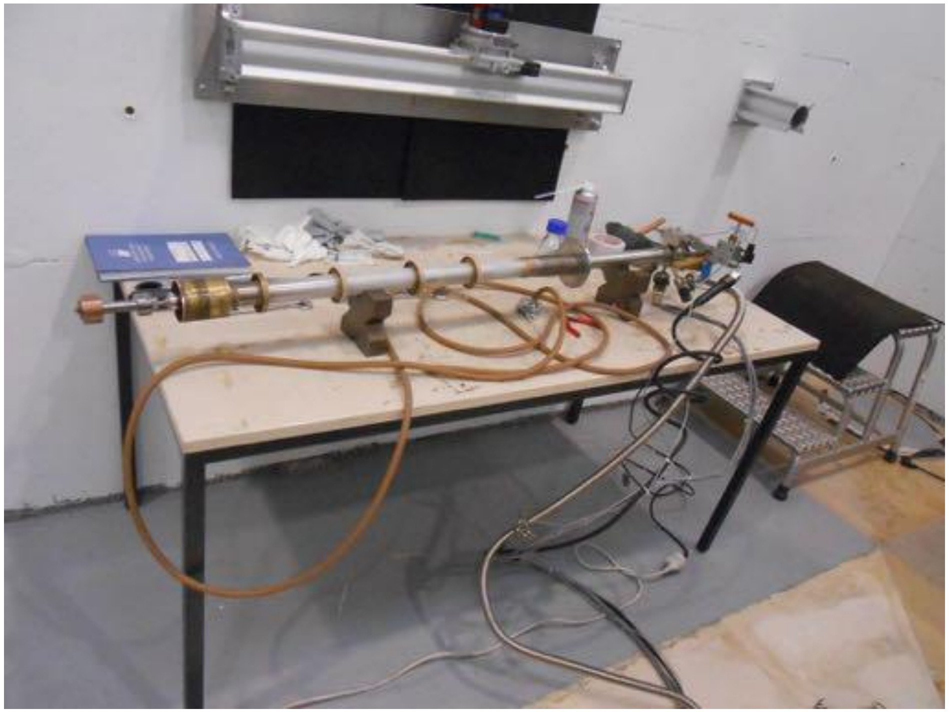
Measured transmission: ≈ 81 %

Sample in solution, volume ≈ 1 ml

ILL high pressure controller by Sitec

Lecture of pressure values by NOMAD $\Rightarrow$ Control of stability over time

ILL high pressure equipment: Sample stick



- up to 6000 bar and 15 – 370 K.

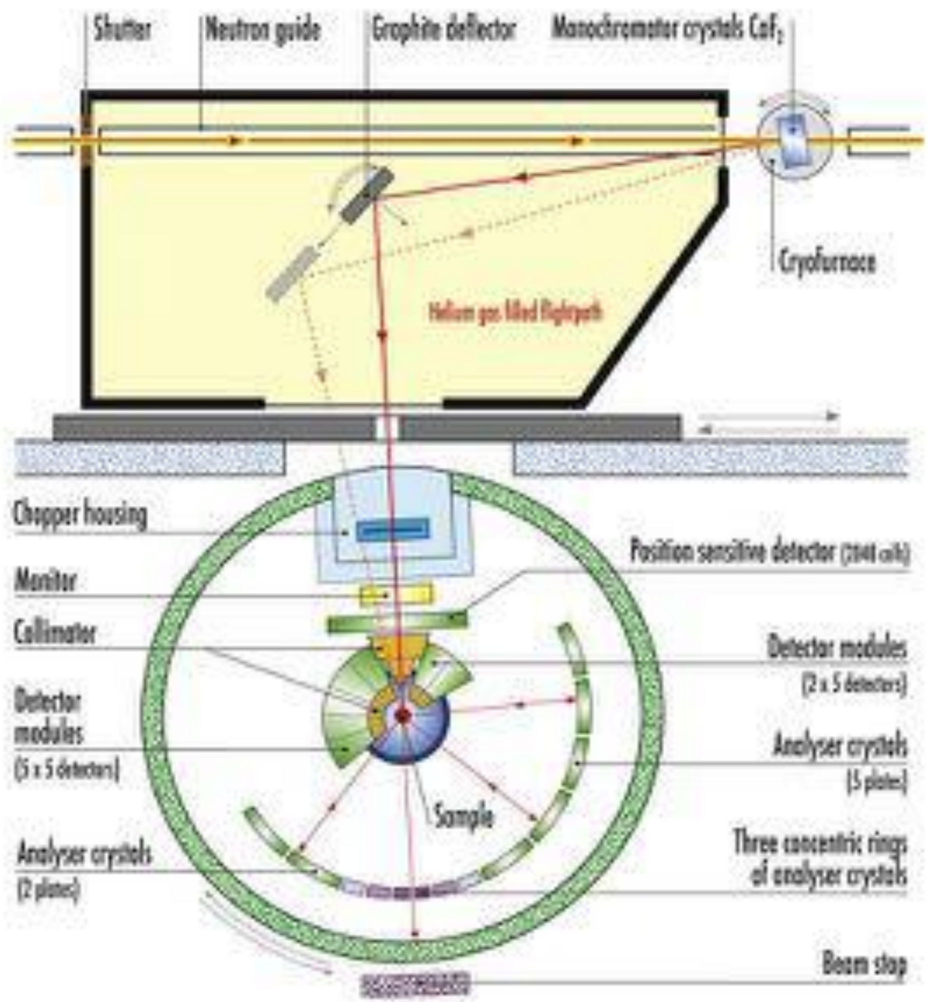
- whole upper part of the sample stick and the capillaries are under secondary vacuum

- a linear heating element consisting of a 1 mm diameter stainless steel Thermocoax wire is introduced inside each capillary

- a RhFe temperature sensor is placed close to the coldest point of the cryostat, which is at the temperature of liquid nitrogen (77 K), to check at this critical point the temperature of the pressure transmitting liquid.

E. Lelièvre-Berna et al., Journal of Neutron Research 19 (2017) 77–84

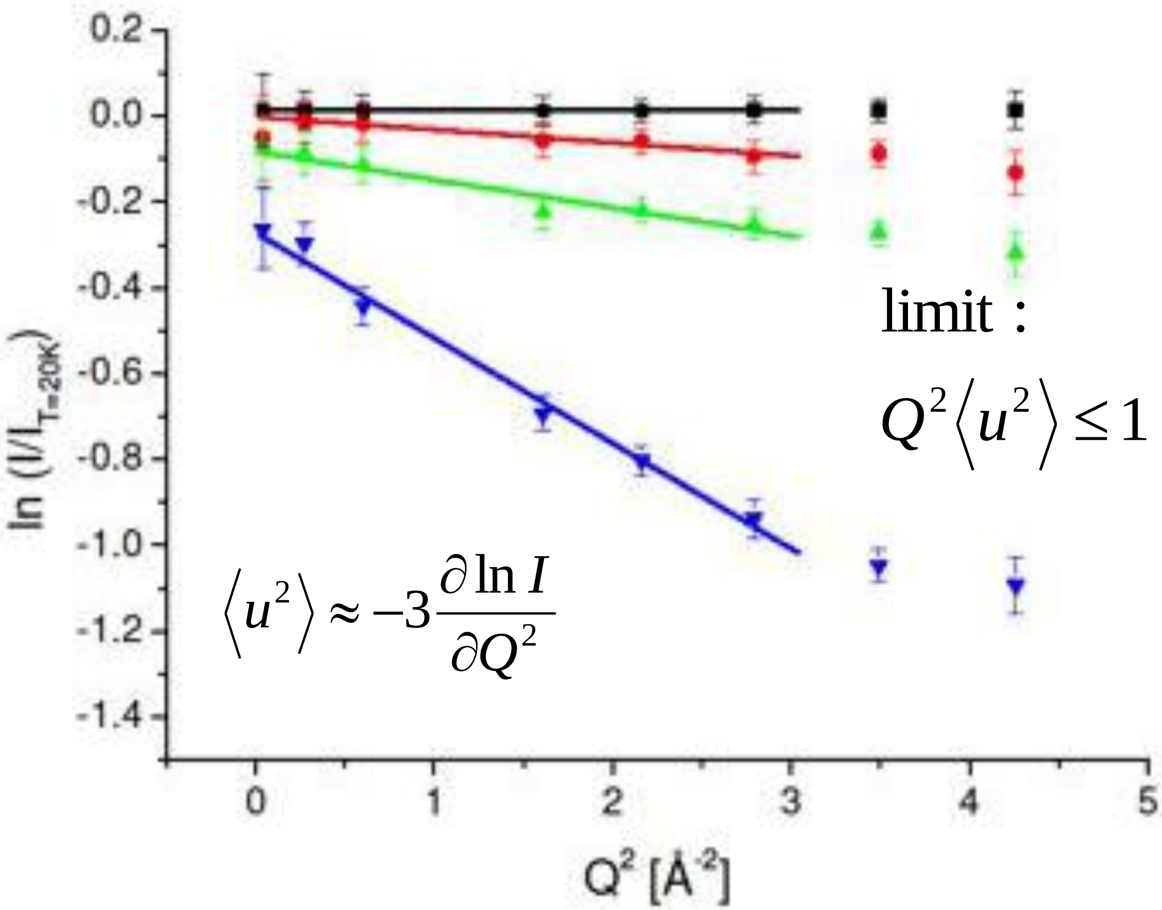
Schematic layout of IN13



- Thermal backscattering spectrometer
- Elastic energy resolution ≈ 10μeV
- Q-range: 0.2 – 4.9 Å^{-1}

$$Q = \frac{4\pi}{\lambda}\sin\vartheta$$

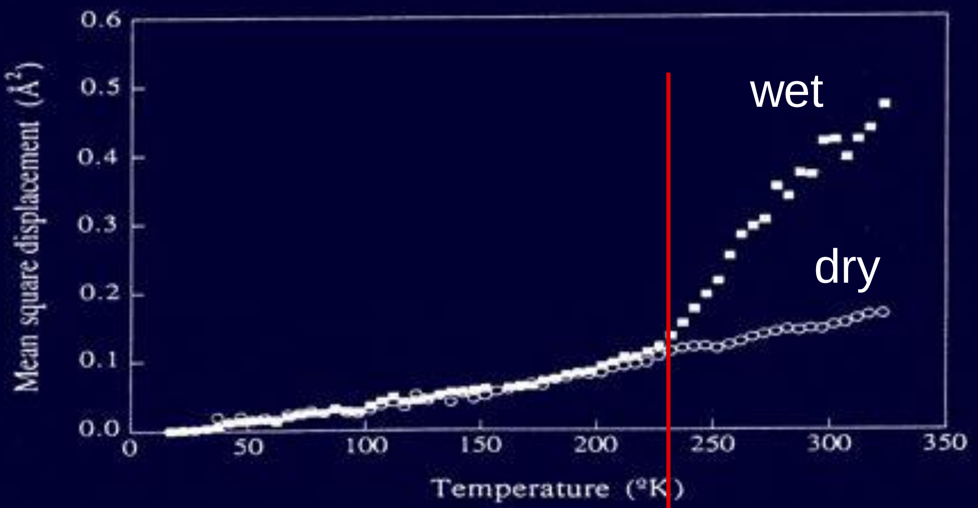
Example: <u²> obtained from IN13 data



$I(Q, \Delta\omega = 0) = \text{const.} \times \exp((1/3)(-\langle u^2\rangle Q^2))$

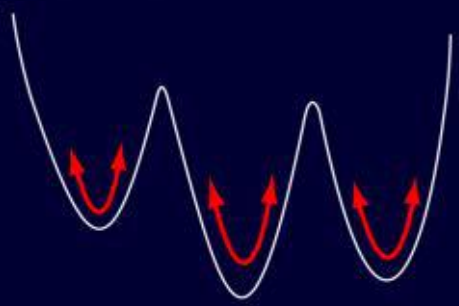
Dynamical transitions in proteins

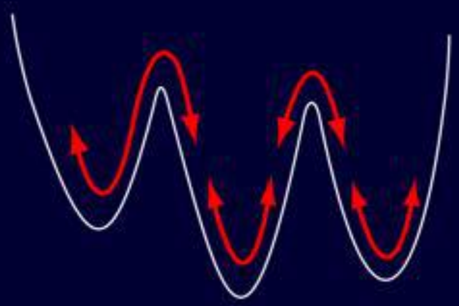
ns - ps motions - purple membrane



Mössbauer spectroscopy,
Neutron scattering,
MD simulation

Ferrand *et al*. (1993) *PNAS* **90**, 9668



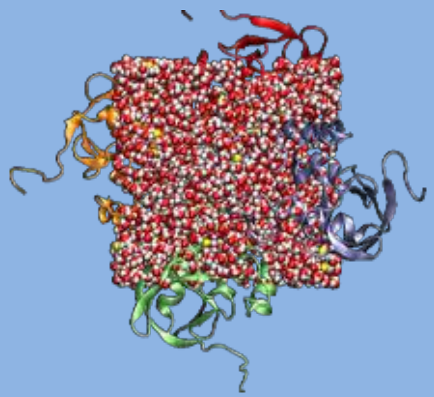


- harmonic motions
- substates frozen out

- non-harmonic motions
- transitions between substates

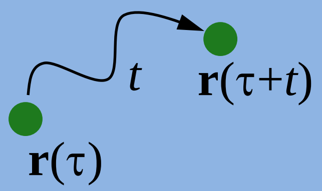
Frauenfelder (1998) *Nat. Struct. Biol*. **5**, 757

Dynamical transition investigations as function of pressure : molecular dynamics simulations

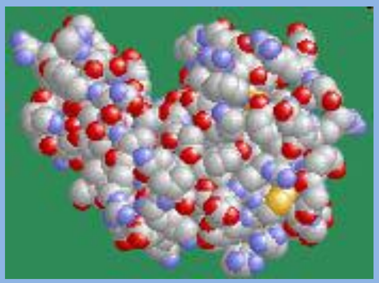


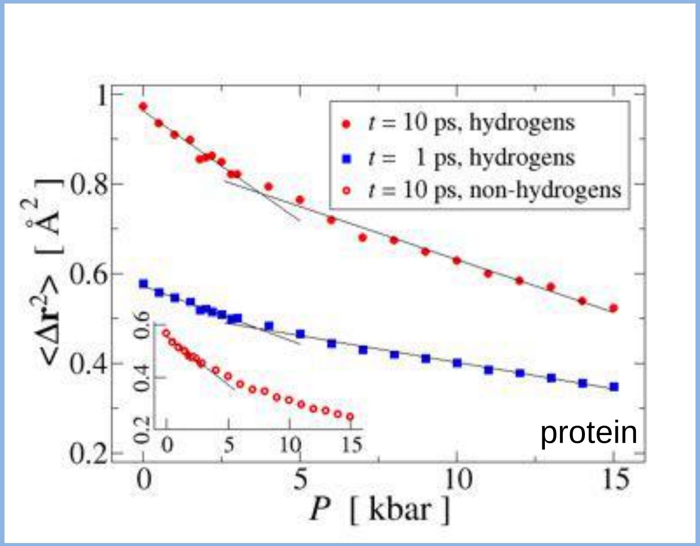
Staphylococcal nuclease

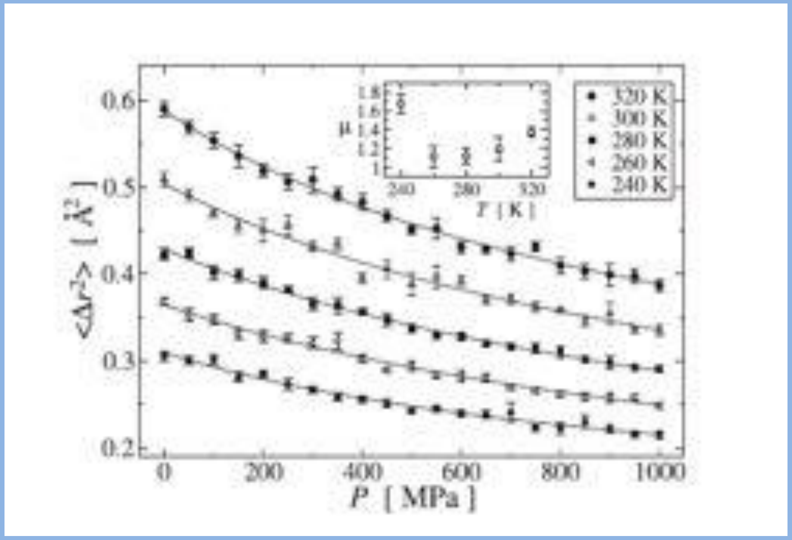
MSD: $\left\langle \Delta \mathbf{r}^2 \right\rangle(t) = \left\langle \left[\mathbf{r}_k(\tau) - \mathbf{r}_k(\tau + t) \right]^2 \right\rangle$



Lysozyme





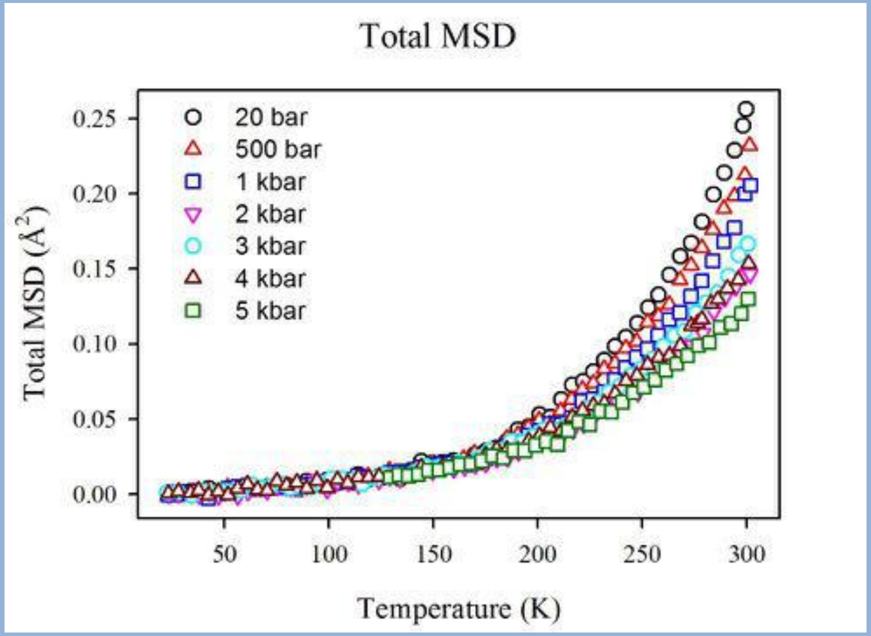


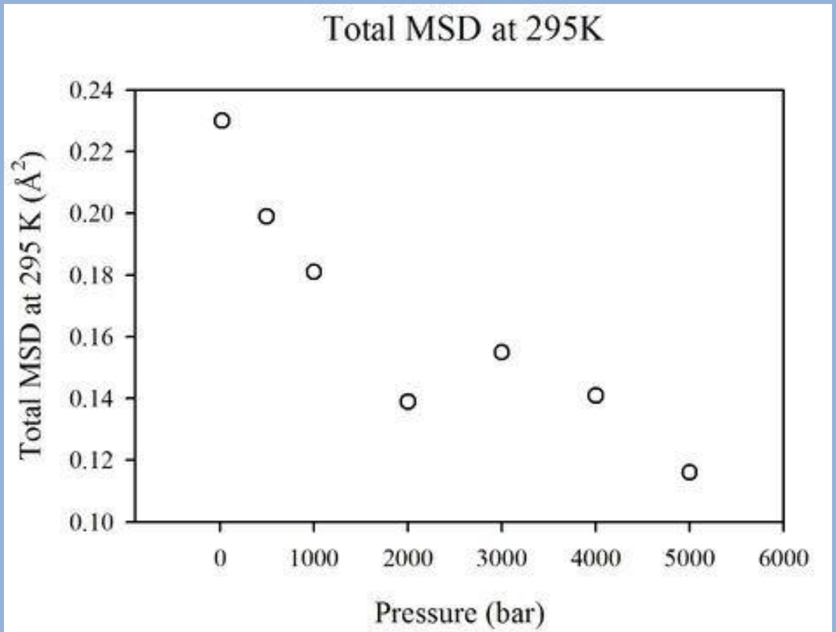
Meinhold, Smith. PRE **72**:061908 (2005)

Meinhold, Smith, Kitao, Zewail, PNAS 104, 17261 (2007)

The effects of pressure on the energy landscape of myoglobin

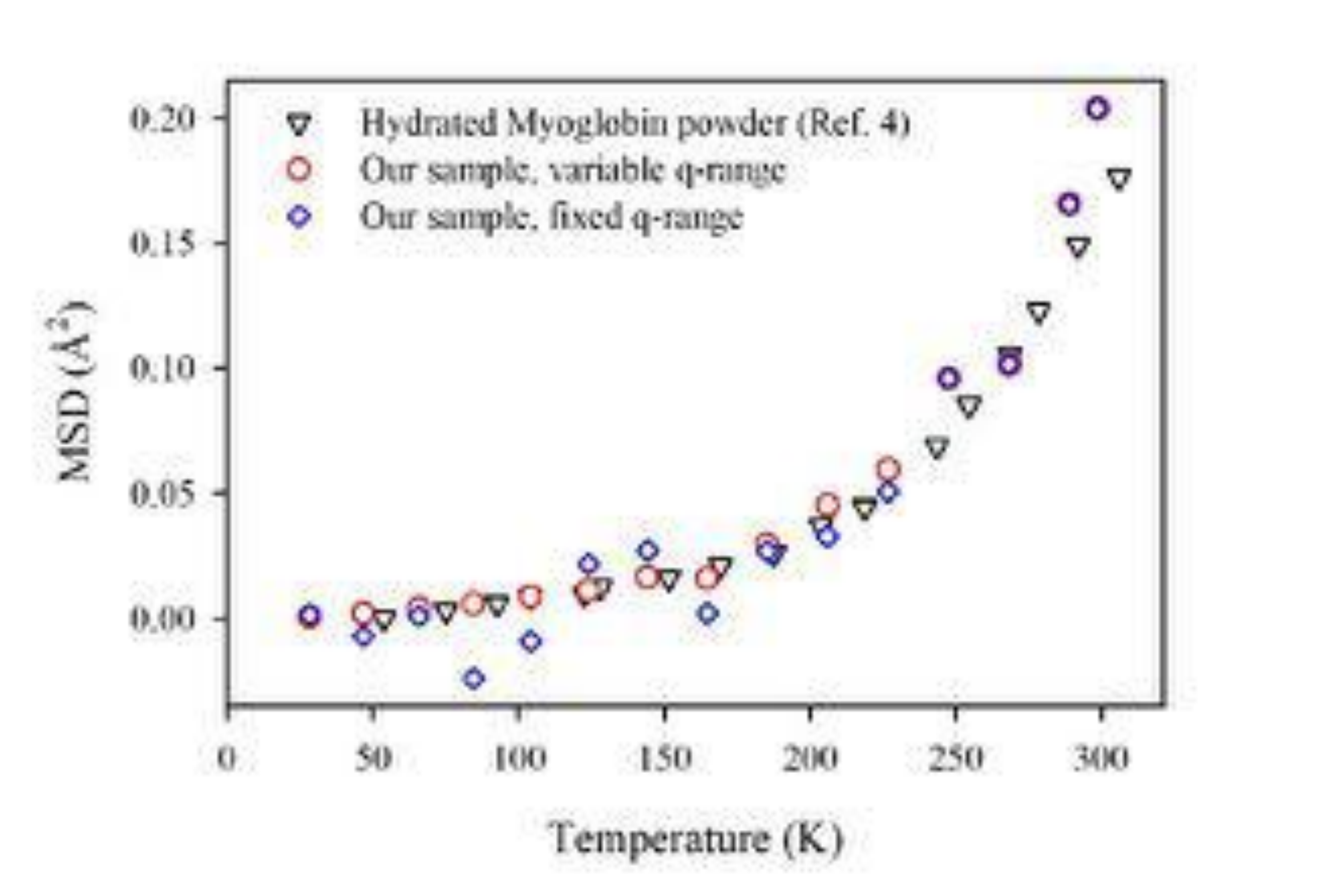
Sample: ultraviscous mixture of Myoglobin (50% Mb, 33% D8-Glycerol, 17% D2O). T: 20–300 K and p: 20–5000 bar to avoid freezing at cryogenic temperatures, while keeping the protein in a liquid-like environment, suitable for pressure transmission.





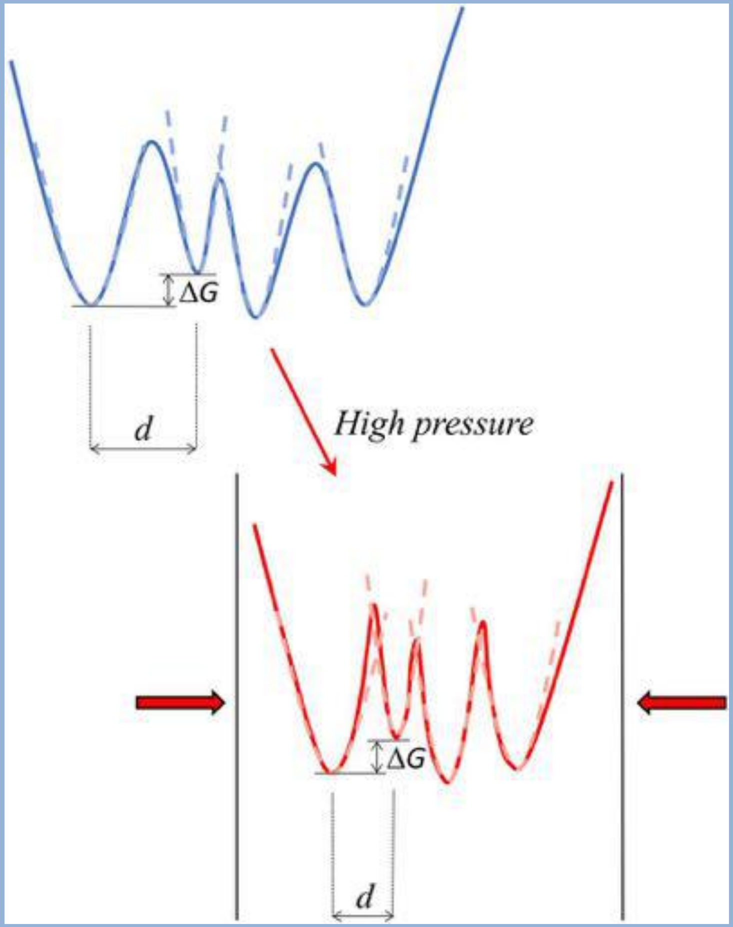
Librizzi, Carrotta, Peters, Cupane, Sc.Rep. 2018

The effects of pressure on the energy landscape of myoglobin



Doster, Cusack, Petry, *Nature* **337**, 754–756 (1989)

The effects of pressure on the energy landscape of myoglobin



Analysis through double-well potential model:

$$I(q) = e^{-\langle \Delta x^2 \rangle_0 q^2} \left\{ 1 - 2p_1 p_2 \left(1 - \frac{\sin(qd)}{qd} \right) \right\}$$

High pressure reduces protein motions, but does not affect the onset temperature for the Protein Dynamical Transition, indicating that the energy differences and barriers among conformational substates do not change with pressure. Instead, high pressure values strongly reduce the average structural differences between the accessible conformational substates, thus increasing the roughness of the free energy landscape of the system.

Acknowledgements

- ILL for allocation of beam time
- Myoglobin project: A. Cupane, F. Librizzi, R. Carrotta, A. Calio
- Financial support by the ANR (ANR; project number ANR 2010 BLAN 1725 01 Living deep).
- Financial support by the CNRS (INP) and the Federation Neutronique