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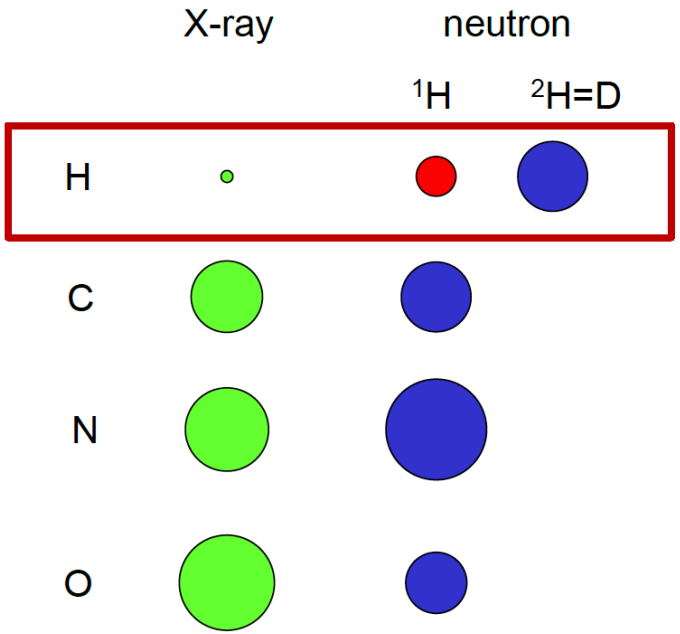
In-situ macroseeding apparatus for protein crystal growth

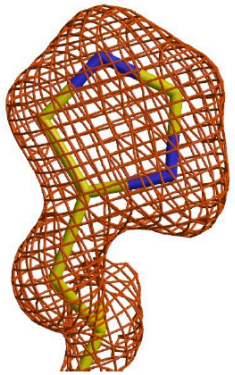
17 September 2018 | Longo Marialucia, Tobias Schrader

Why do we use neutron crystallography?

Powerful complement to X-ray Crystallography: → Location of Hydrogen atoms in Biological Macromolecules

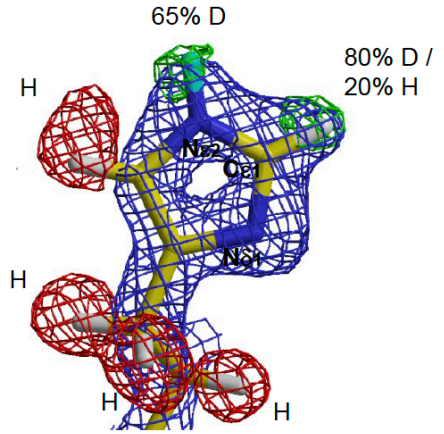


X-ray d_{min}= 1.5Å:



2Fo-Fc map; +1.5σ

neutrons d_{min}= 1.5Å:

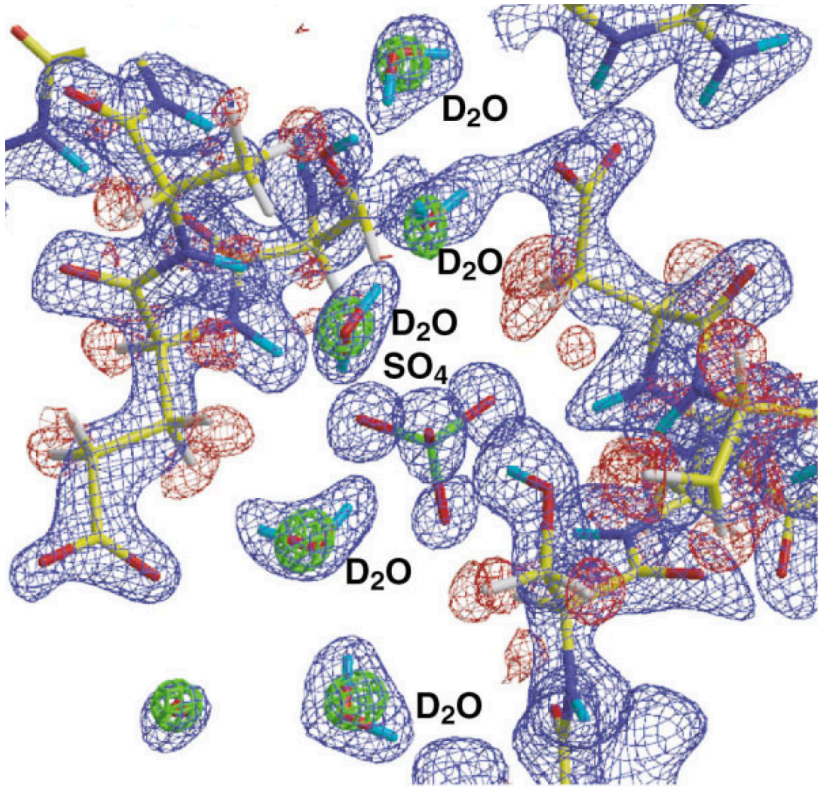


2Fo-Fc map; +1.5σ
Fo-Fc omit-map; -3.0σ
Fo-Fc omit-map; +3.0σ

Hydrogen/Deuterium atoms can be resolved even at a resolution of d_{min}=2.5Å

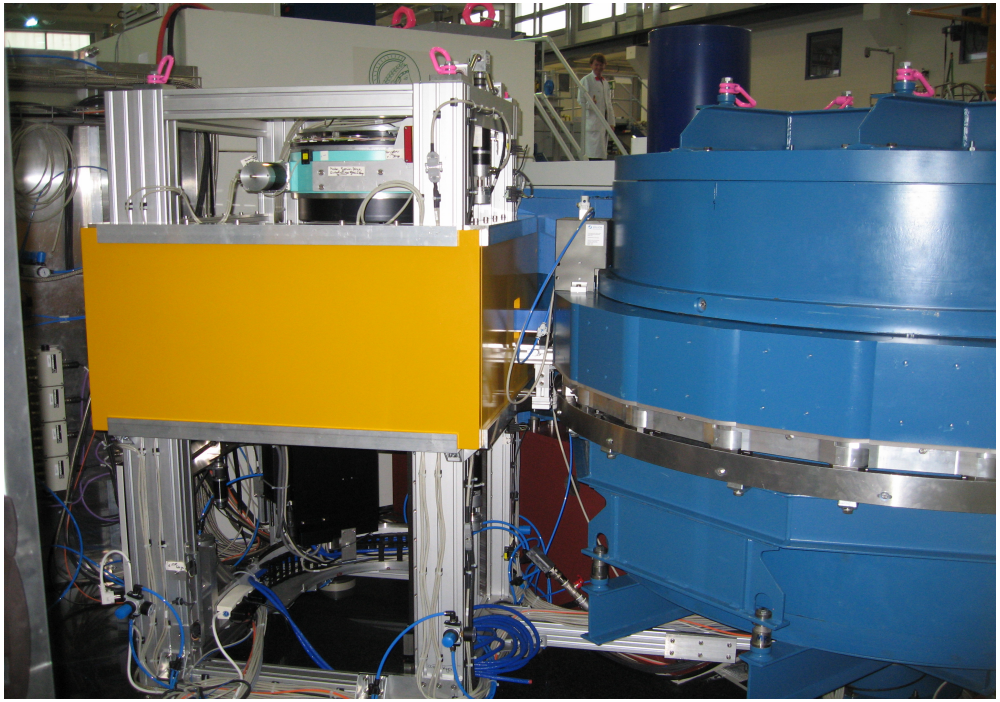
Why are we interested in H atoms?

- Direct determination of the protonation states of amino acid side chain and ligands
- Geometry of hydrogen bonds
- Orientation of H atoms in the solvent structure (enzymatic reactions, molecular recognition and protein folding)
- Deuterium exchange as a measure of flexibility and accessibility
- No radiation damage



Protein-protein contact in Myoglobine:
2Fo-Fc map (blue): 1.5σ
2Fo-Fc map (red): -2σ
2Fo-Fc map (green) : X-ray map

BioDiff @ FRM II



Monochromatic single crystal diffractometer for large unit cells.

(Instrument scientist: Tobias Schrader, Andreas Ostermann)

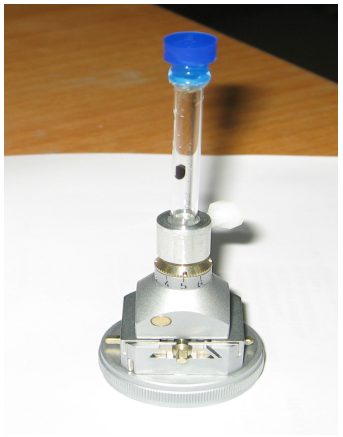
Neutron crystallography for biological macromolecules!

Disadvantages of neutron crystallography

- Phase problem: No neutron structure without X-ray structure!
- Deuteration desired (σ^{H}_{inch}=80 barn, σ^{D}_{inch}=7.6 barn)

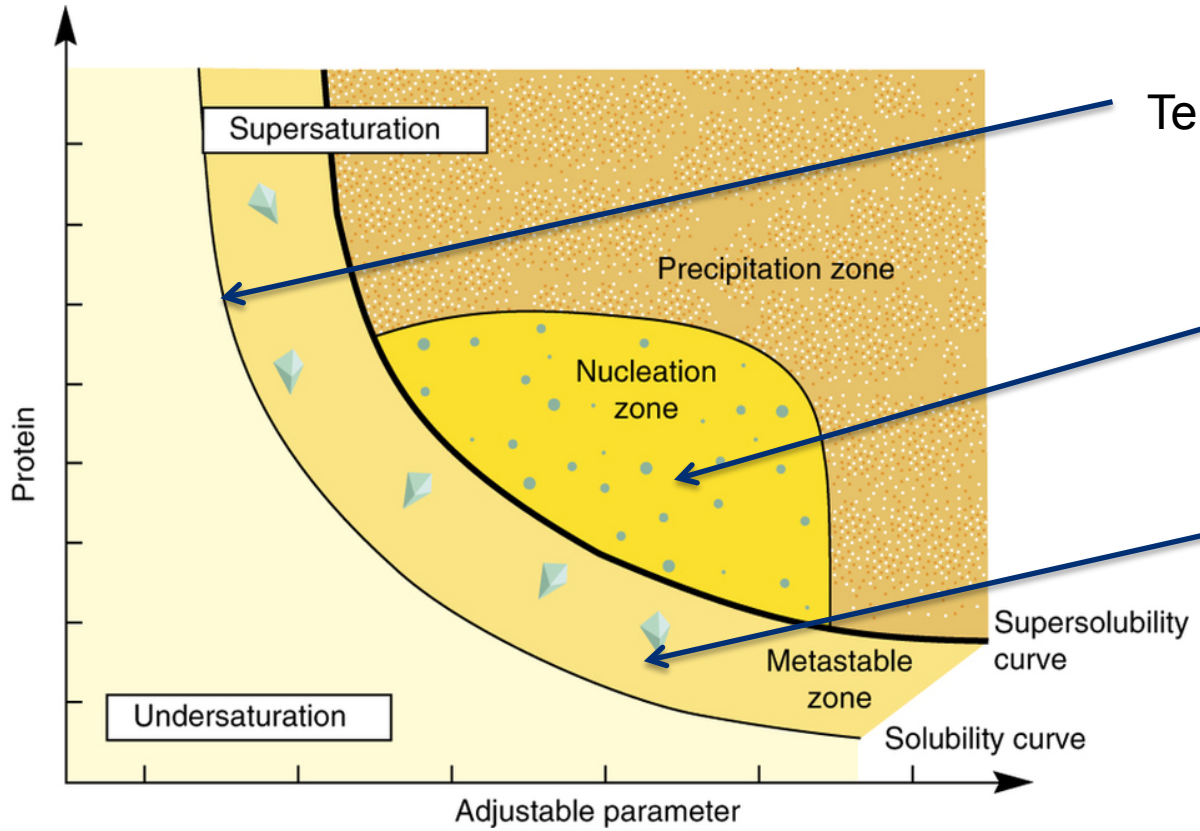
$$I \propto tI_0 \varepsilon \frac{V_{crystal}}{V_{cell}^2} \lambda^2 T_{DW} \left\langle \left| F_{hkl} \right|^2 \right\rangle$$

- **Large protein crystals needed!**



Minimum crystals size: ~ 0.5 mm^3

How can we improve the production of large protein crystals?



Temperature dependent

Nucleation

Crystal growth

Seed a crystal and stay in the metastable zone as much as possible

In-situ macroseeding apparatus

GOAL: change the crystallization condition without moving the crystal

Controlled parameters:

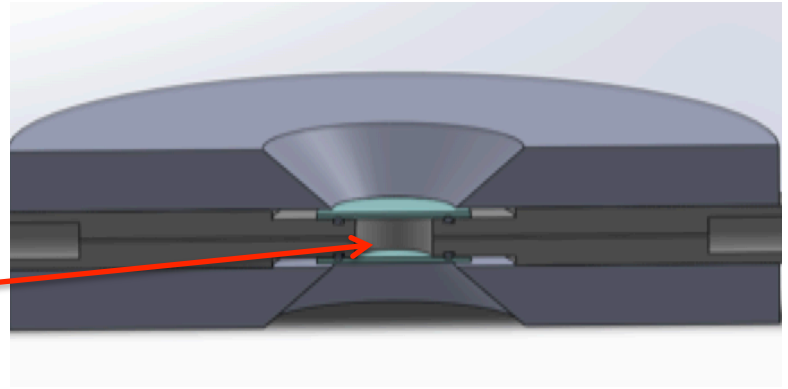
- Precipitant and protein concentration
- Temperature

Flexibility due to different configuration:

- Batch crystallization
- Vapour diffusion (sitting drop configuration)

Batch crystallization configuration

How can we define the crystallization chamber?

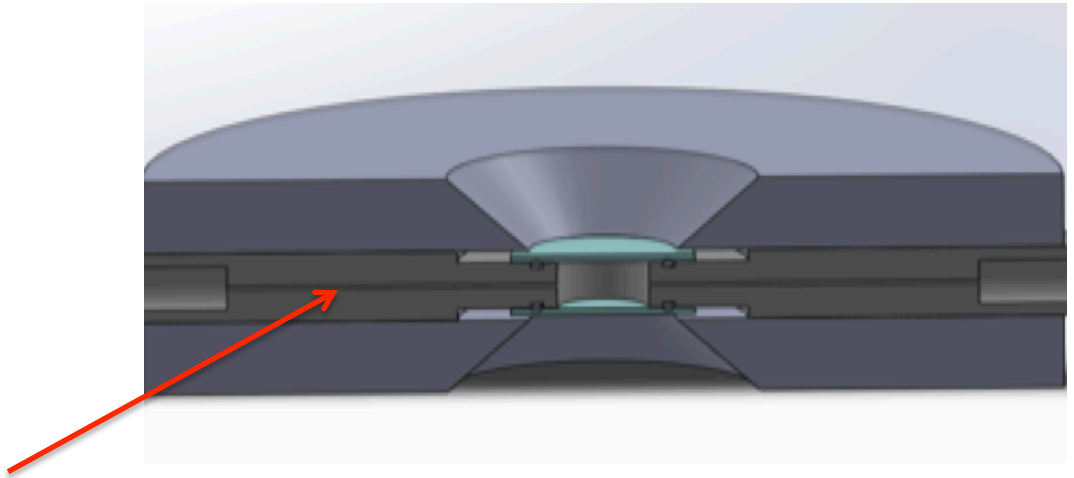


Crystallization Chamber
(internal volume ~ 0.5ml)

- Round flat glass windows (20 mm diameter and 1 mm thickness)
- O-ring (11 mm internal diameter)
- Internal spacer built by a 3-D printer (circular symmetry)
- Stainless steel external holders (circular symmetry)

Batch crystallization configuration

How can we change the mother liquor around the crystal?

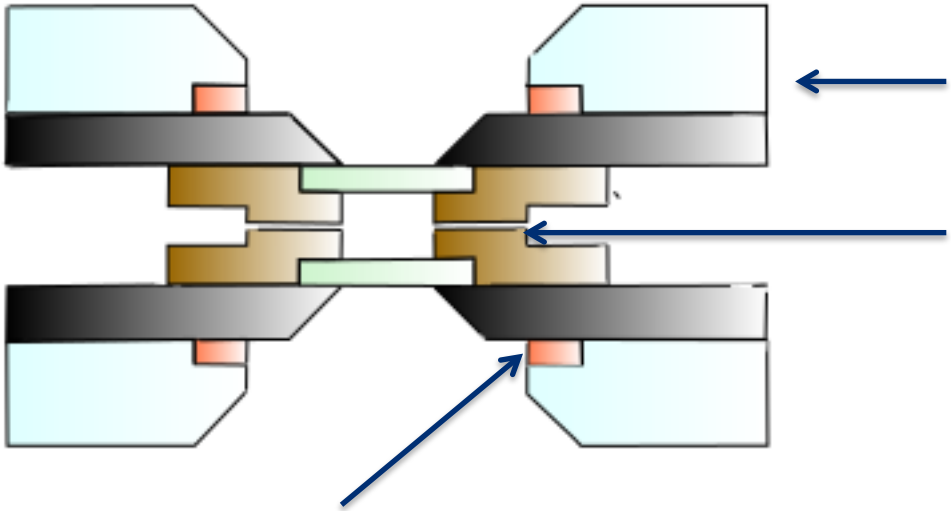


Two capillarity built in the spacer (0.5 mm diameter)

Osmotic Shock? → Continuous variation from a solution 1 to a solution 2 with a slow gradient

Batch crystallization configuration

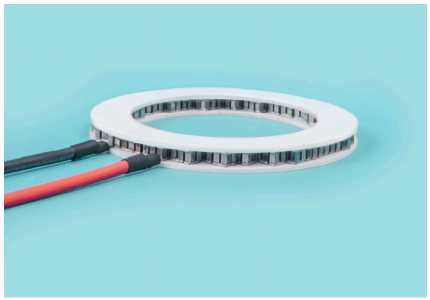
How can we control the Temperature in an uniform way?



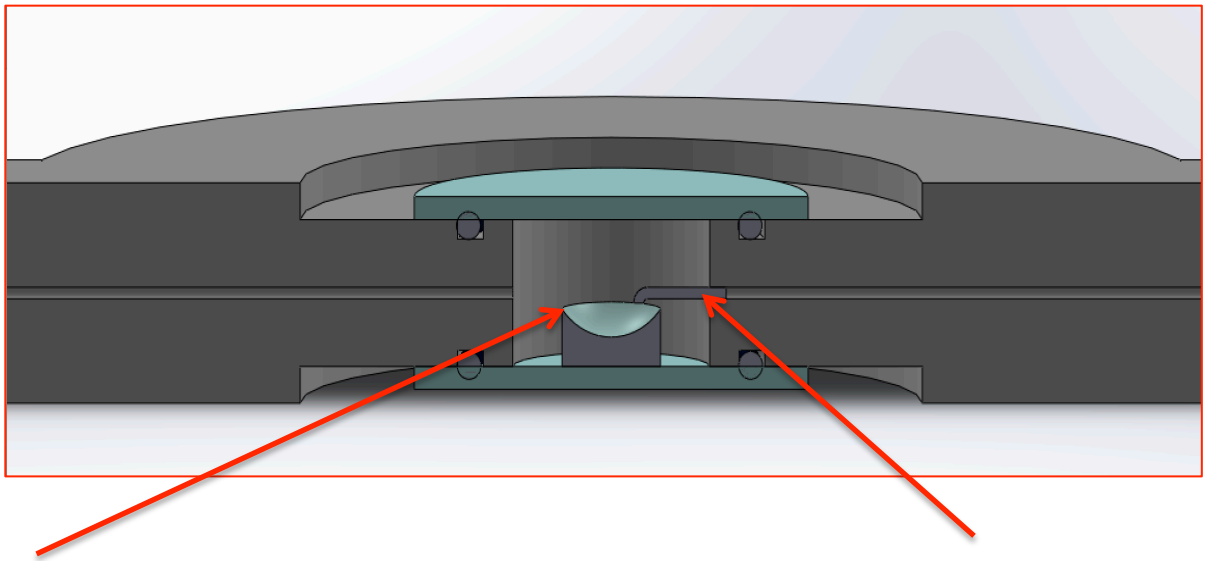
Water cooling element (circular symmetry) T~25 ºC

PT100 temperature sensor (cylindrical shape)

Peltier Element
(Circular symmetry)



Vapour diffusion configuration

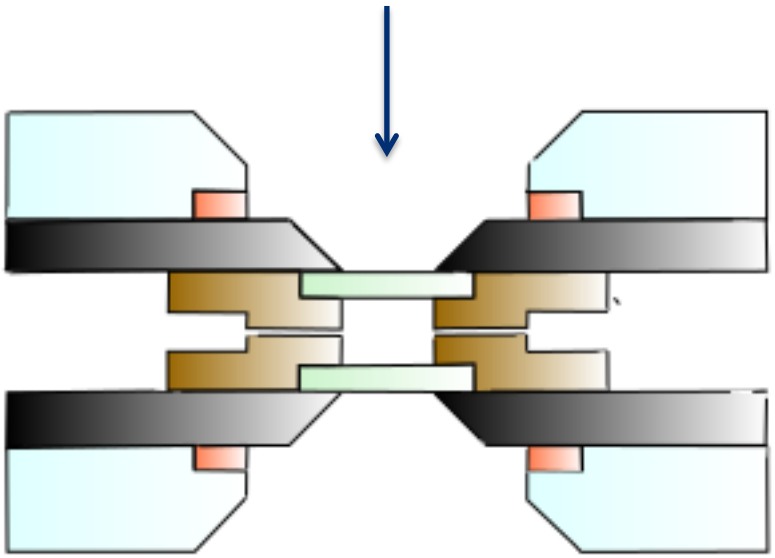


Sitting drop bridge in the crystallization chamber

Micro-pipe to change the drop Crystallization condition e.g. more protein (3D built)

Applicable techniques:

Inverted microscope: to visualize the crystal during the crystal growth



DLS: to monitor new crystallites or aggregates from the mother liquor solution around the crystal

ATR-UV spectroscopy: to measure the protein concentration during the crystallization process

Summary :

- Introduction to large protein crystal problem in neutron crystallography
- Design of the in-situ crystallization apparatus
- Flexibility of the crystallization methods by means of the selected 3D printed internal spacer
- Availability of the crystallization operator to users

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Thank you for the attention!