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Visiting Scientist at the ESRF

Chemistry and Biology of Metals laboratory

MX BAG Meeting, Monday 7th February 2022

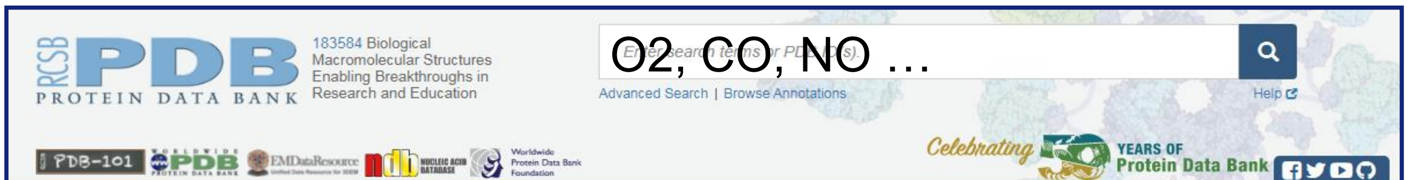
The High Pressure Freezing Laboratory for Macromolecular Crystallography (HPMX)

- Overview: Aim of the service, methods, instruments
- Examples of use
- General information

- The High Pressure Freezing Laboratory for Macromolecular Crystallography (HPMX)

is a service proposed in complement to diffraction experiments on MX beamlines (ESRF).
Protein crystals are frozen in pure pressurized gas atmospheres.
Gas derivatization of bio-crystals allows answering specific questions in structural biology, or is used in methodological applications.

- Importance of gas containing structures in the PDB (~ **1454 structures**)



Gas	Number PDB	Proteins
O2 (OXY, PER)	543	Oxidoreductases: oxygenases, oxidases
CO (CMO)	404	Hemoproteins
NO	171	
CO2	117	Carbonic anhydrases, carboxylases, CO2 reductases
N2O	17	Nitrous oxide reductases
Xenon	142	Chemically inert but with many biological & medical properties: analgesia, anesthesia, neuroprotection… Used in methodological applications
Krypton	29 (16 HPMX)	
Argon	23 (19 HPMX)	

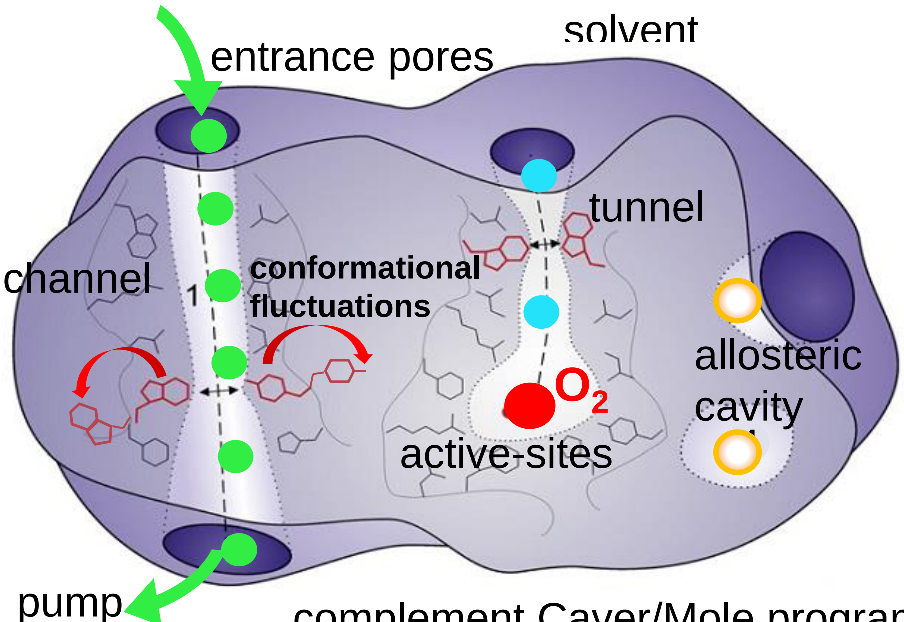
Many proteins crystallized without their gas ligands

- HPMX service allows for the preparation of protein gas derivatives

45 structures in the PDB of Ar, Kr and O2 derivative crystals prepared in the HPMX lab

USE OF PRESSURIZED GASES IN STRUCTURAL BIOLOGY

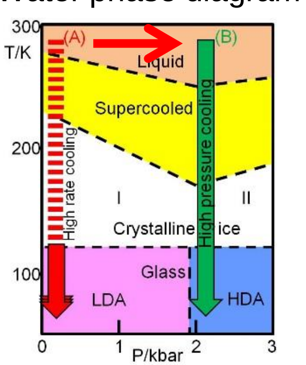
- **Studies of internal architectures of proteins using pressurized O2/Kr/Ar/He**

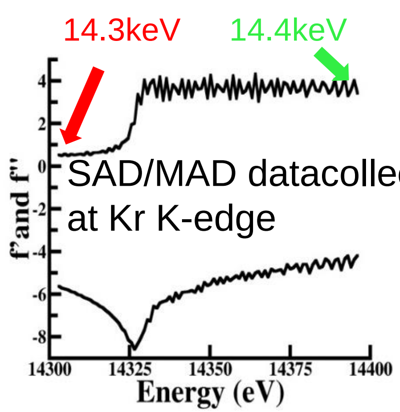


complement Caver/Mole programs

- **Methology**

Water phase diagram





Applications:

-1- Trace functional channels through proteins (transport proteins)

-2- Map functional tunnels, pathways for substrates and products from solvent to active-sites.

-3- Label surface cavities/excavations (potentially functional, allosteric effect)

-4- Reveal “binding sites” of gas substrates or products (O2, CO2, NO). Studies of enzymatic mechanisms.

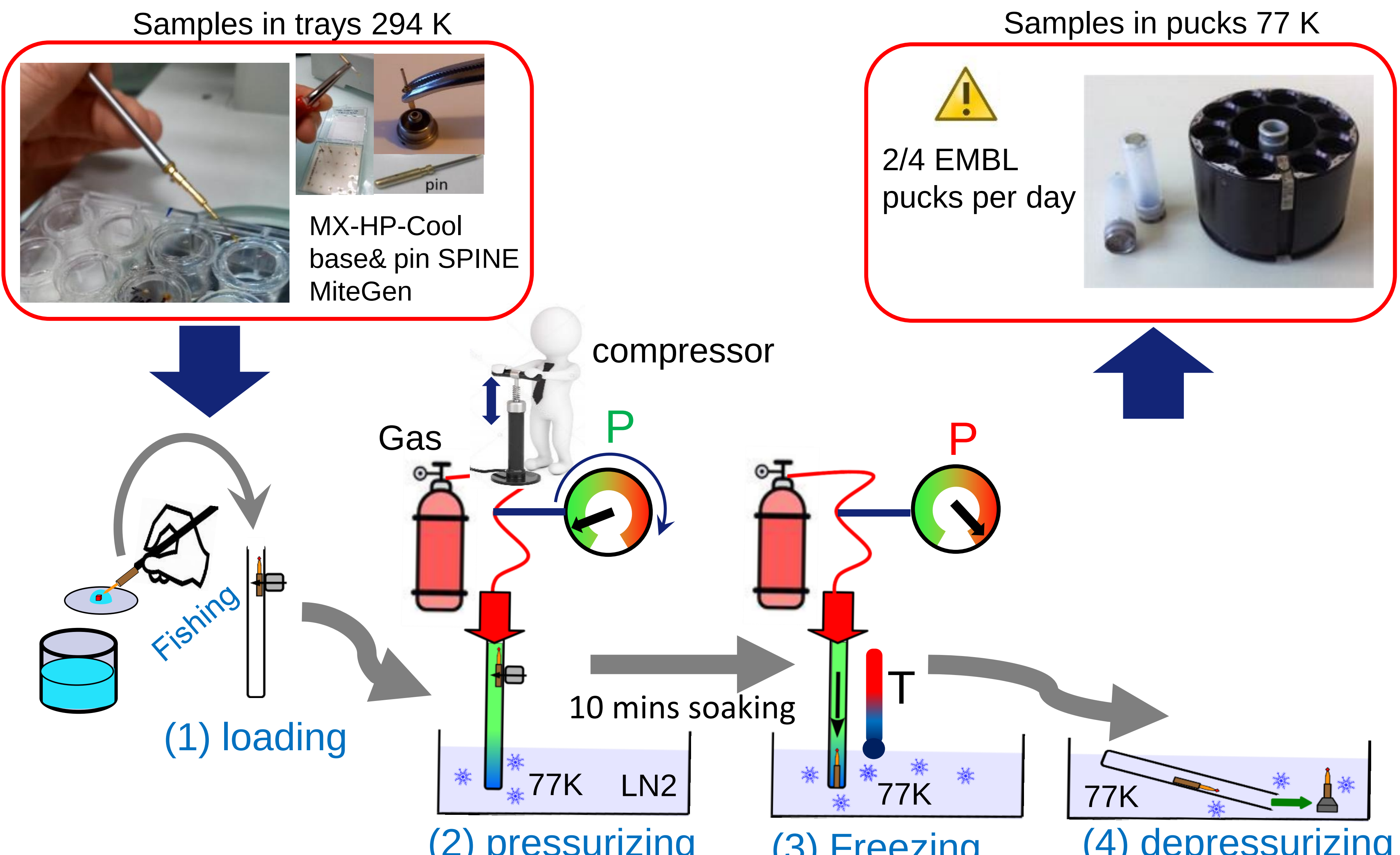
-5- Pressure (He/Ar 2000 bar) induce local structural modifications, exploration of conformational fluctuations

-6- Crystals freezing at High pressure (He 2000 bar) without cryopretection.

-7- Use noble gases (Kr, Xe) heavy atoms for protein structures determination

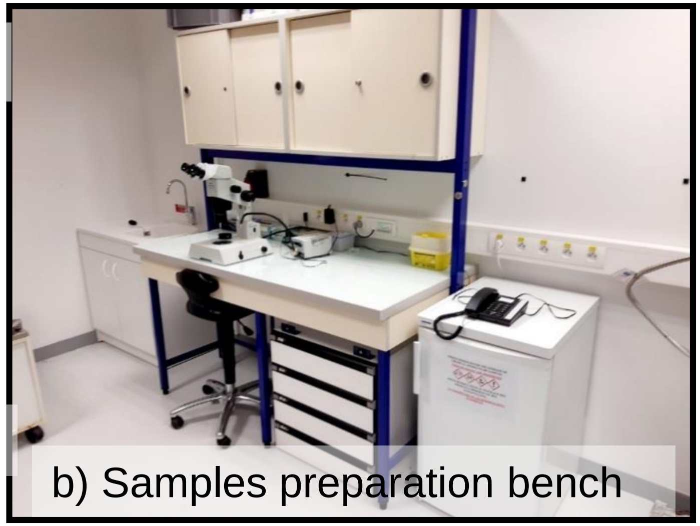
- Others …

“SOAK AND FREEZE” METHOD, FREEZING CRYSTAL UNDER HIGH PRESSURE (HE, AR, KR, XE, O2 …)



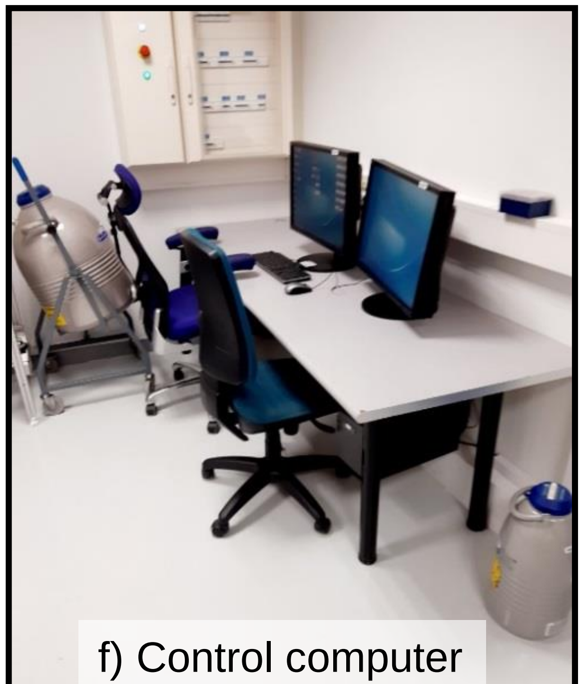
B. Lafumat *et al*, J. Applied Crys. (2016)

a) Location


b) Samples preparation bench


c) Fume hood


e) Different pressure cells


f) Control computer


d) Glove-bag
(100 to 500 ppm O2)

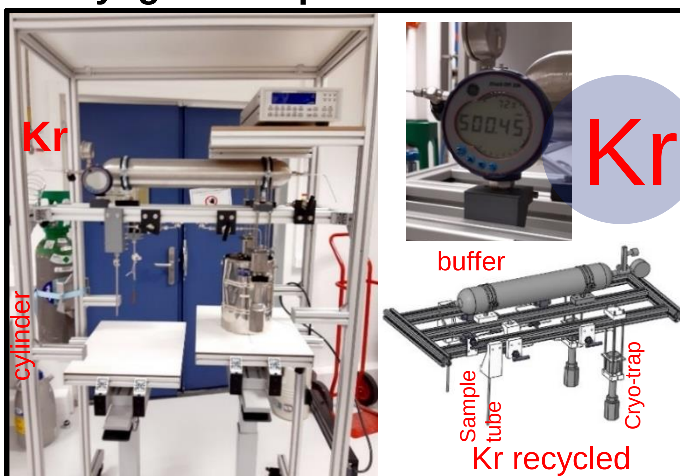
THE DIFFERENT CRYOGENIC PRESSURE CELLS

Physical properties of gases	He	Ar	Kr	Xe	O2	CO2	N2
vdW rad Å	1.4	1.5	2.0	2.2	1.6/2 .1	1.7/2.7	1.6/2.1
Solubility mM/bar	0.4	1.4	2.5	4.3	1.3	35	0.6
Pressure	2000	2000	50/500	-	70	50	-
Icing T (K)	0.9	84	116	161	54	195	63

- **Cryogenic high pressure bench He or Ar 2000 bar,**

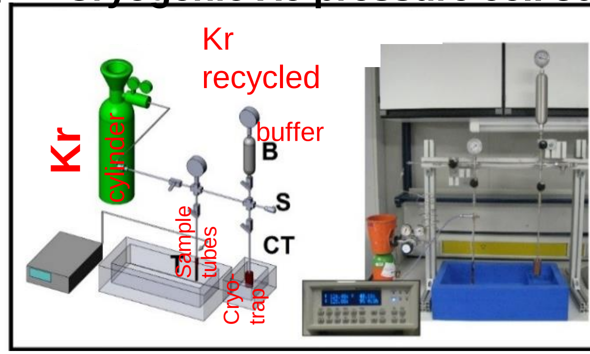
He
Ar

- **Cryogenic Kr pressure cell 500 bar**



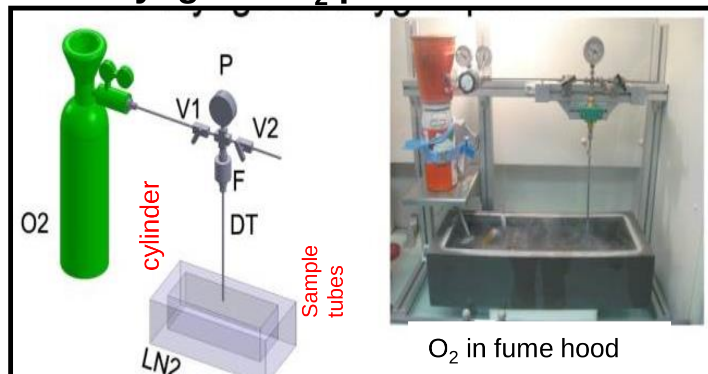
Kr

- **Cryogenic Kr pressure cell 150 bar**
- **Cryogenic Xe pressure cell 30 bar ?**



Kr
Xe?

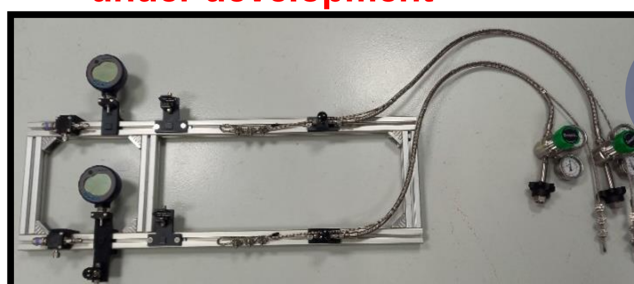
- **Cryogenic O_2 pressure cell 70 bar**



O_2 in fume hood

O_2

- **Cryogenic CO_2 pressure cell 50 bar, under development**

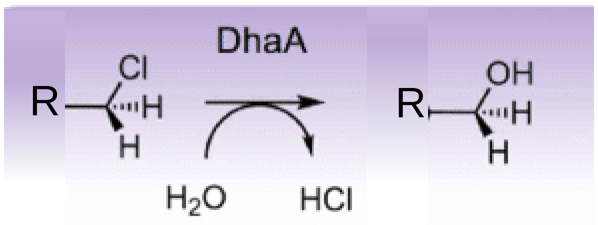


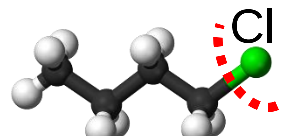
CO_2

USING KRYPTON TO STUDY AN IMPROVED HALOALKANE DEHALOGENASE

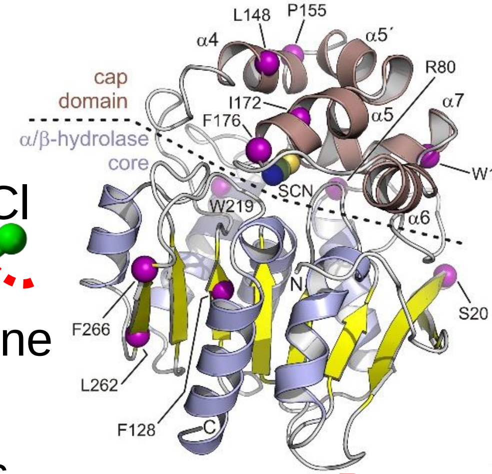
Haloalkane dehalogenases HLD (hydrolases) catalyze the hydrolysis of halogenated compounds by cleavage of the carbon-halogen bond. (Bioremediation, pollutants decontamination)

M. Marek and K. Markova, Masaryk University, Brno, Czech Republic





1-chlorobutane



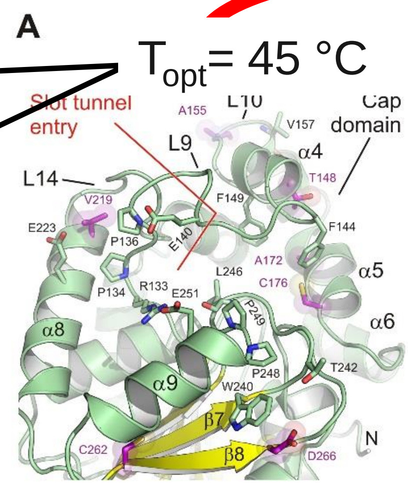
DhaA from *Rhodococcus rhodochrous*

- canonical α/β-hydrolase core
- cap domain
- buried catalytic center

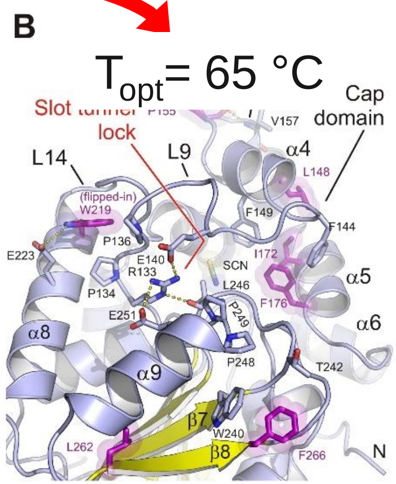
Thermostable enzymes ➡ Applications

FireProt computational pipeline (energy- and evolution-based approaches), design of an outstanding thermostable mutant:
- Native DhaA (T_{opt}= 45 °C)
- Mutant DhaA115 (T_{opt}= 65 °C)

DhaA **Program FireProt** DhaA115



T_{opt}= 45 °C

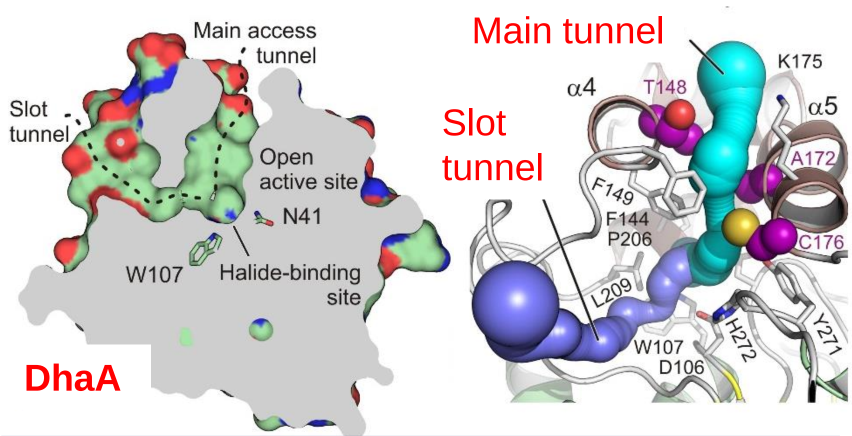


T_{opt}= 65 °C

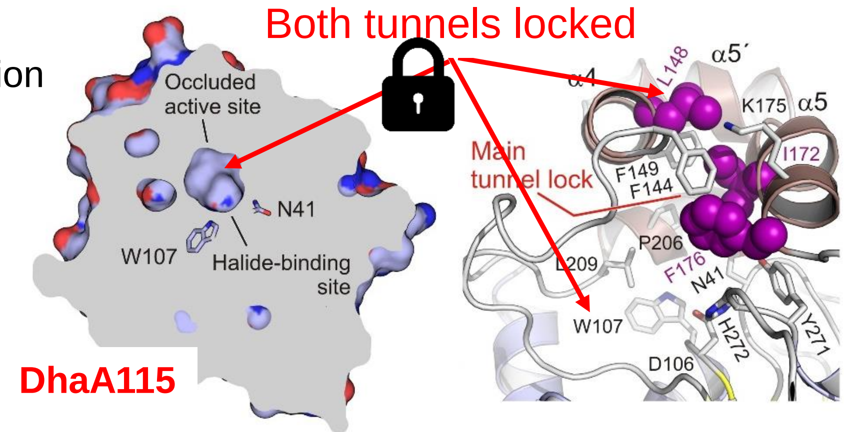
Understand the structural basis of this hyper-stabilization

11 mutations C128F, T148L, A172I, C176F, D198W, V219W, C262L, D266F, E20S, F80R and A155P

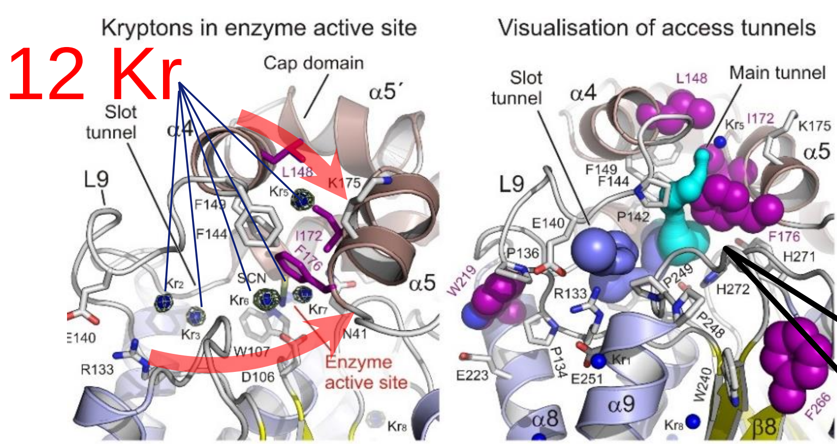
STUDY OF TUNNELS IN AN IMPROVED HALOALKANE DEHALOGENASE



Tunnels computation CAVER



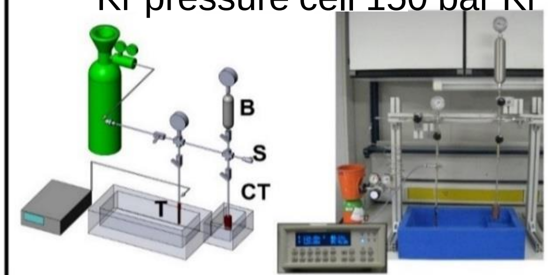
Buried active site connected with the solvent via a main and a slot tunnel (crucial for catalytic activity and substrate selectivity). The mutations provoke the apparent occlusion of the active site.



DhaA115 derivatives, crystals frozen under 150 bar Kr
12 Kr sites in anomalous map 4σ

K Markova et al. Chemical science 2020

Kr pressure cell 150 bar Kr



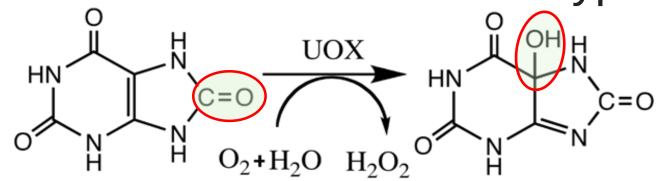
Mapping of DhaA115 tunnels by krypton shows that the substrate is still transported to the active site. Tunnels permeability increase with temperature (pressure) Topt = 65 °C. This provides the structural basis of thermo-stabilization to design new thermostable protein catalysts.

USING ARGON TO STUDY URATE OXIDASE

T. Prangé, Paris, Faculté de Pharmacie

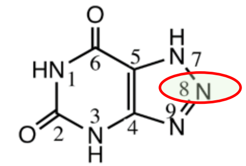
Ar croygenic pressure cell

UOX



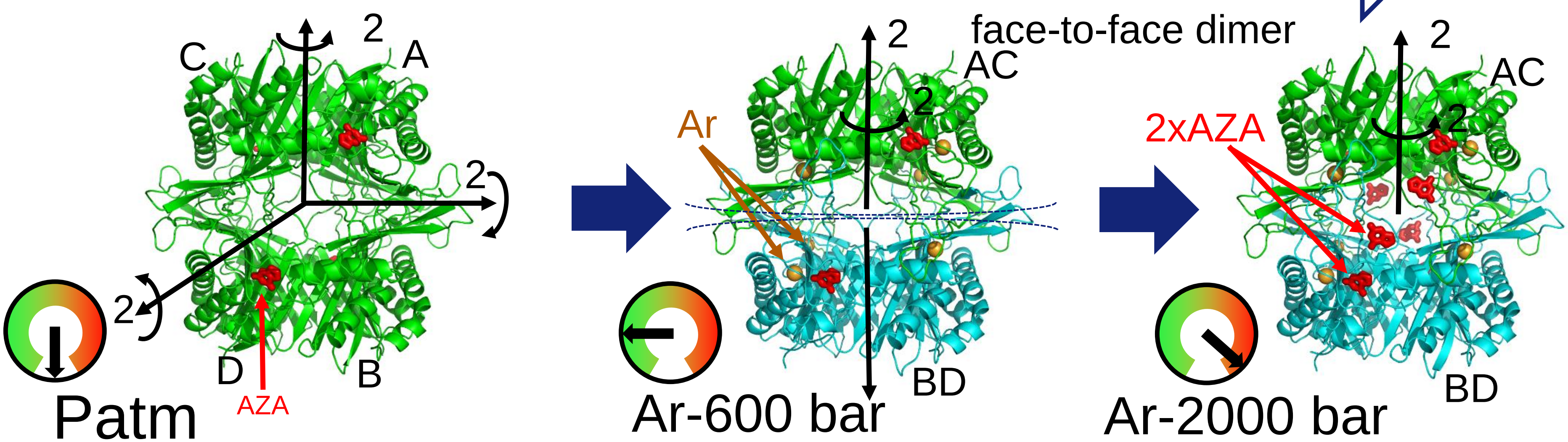
oxidation of uric acid
to 5-hydroxy-isourate.

Drug treatment of
hyper-uricemia



Inhibitor 8-azaxanthine
Crystals UOX-AZA

7 pressures: atm, 120, 220, 600, 1000, 1500 and 2000 bar

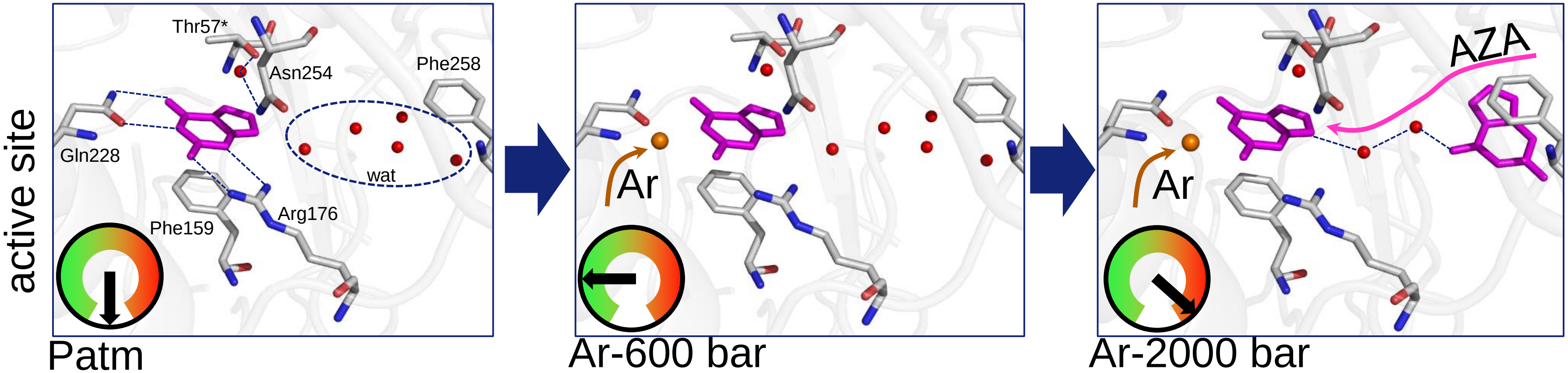


Homo-tetramer α_4
space group I222

2homo-dimers $(\alpha\beta)_2$
space group $P2_12_12$

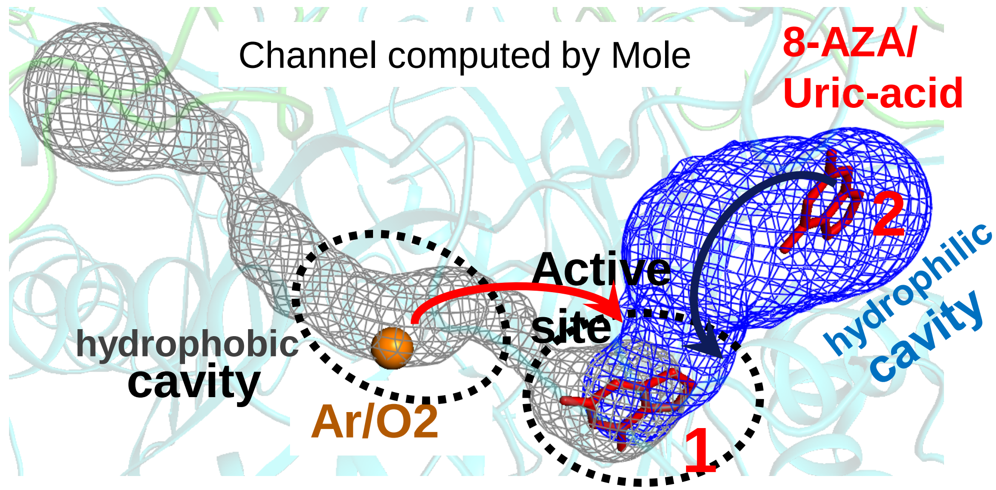
2homo-dimers $(\alpha\beta)_2$
space group $P2_12_12$

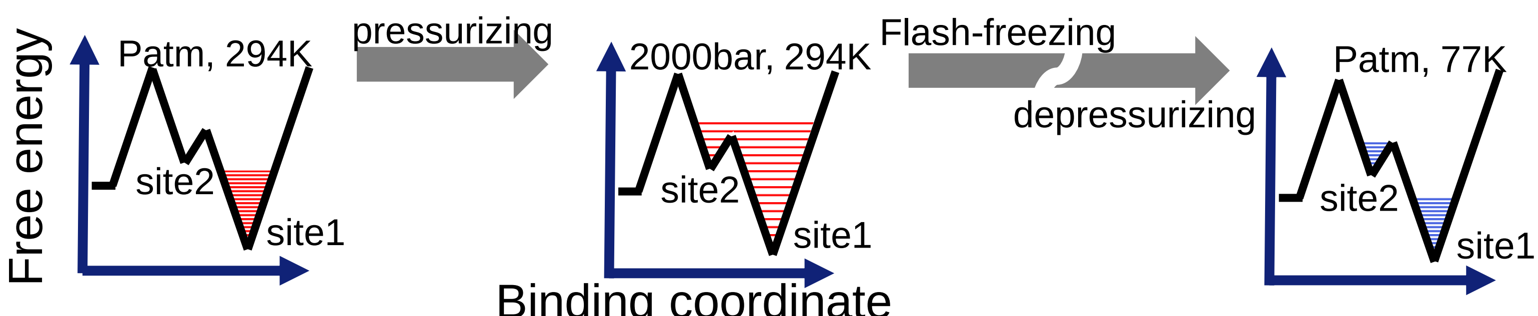
USING ARGON TO STUDY URATE OXIDASE



- Pressurized Ar labels a hydrophobic cavity next to the active site, O2 transient storage site
- Pressure can populate substrates "secondary" binding sites. Here, 8-aza 2nd site in the channel tracing the pathway for the substrate (uric acid)

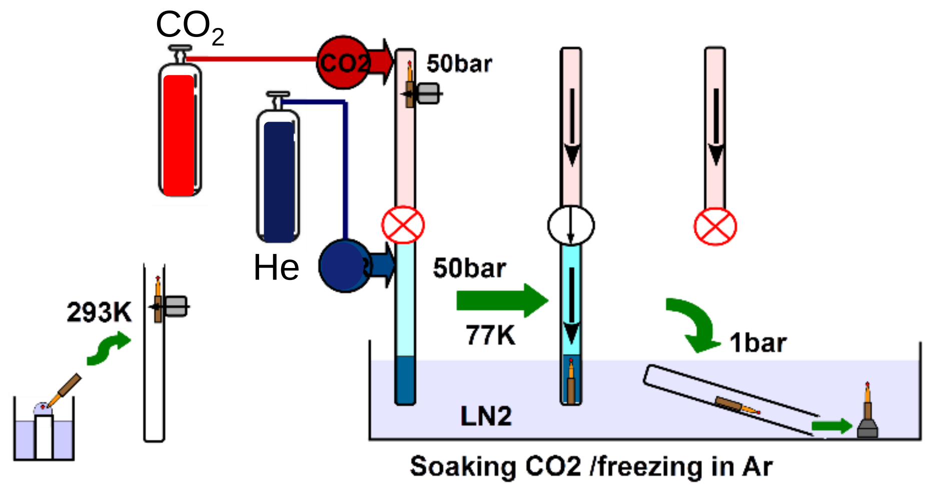
T. Prangé *et al.*, Acta D (2022)

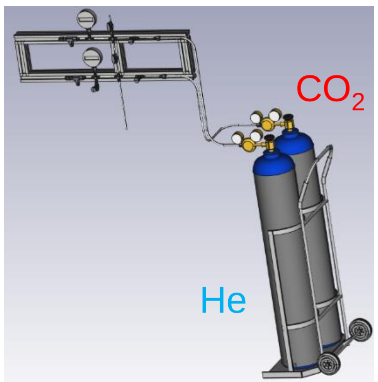


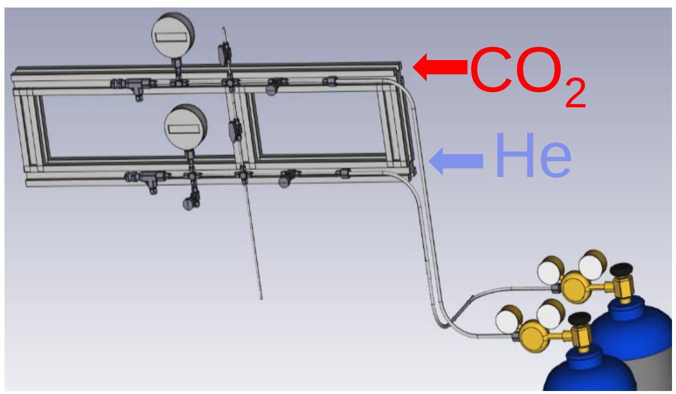


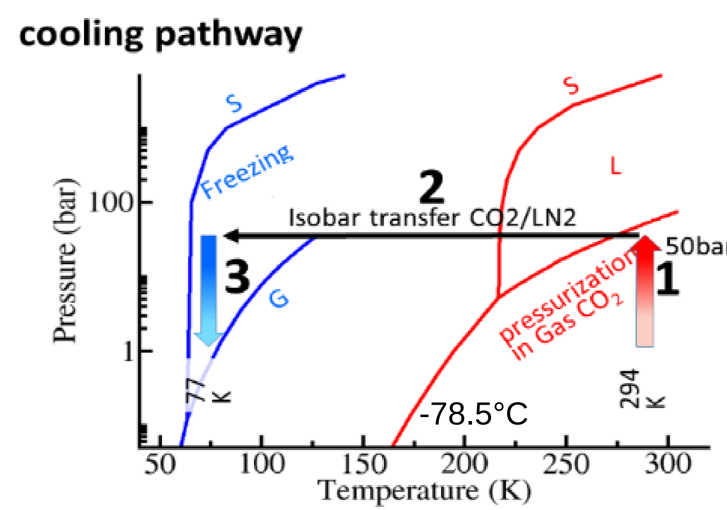
ON GOING DEVELOPMENT: CO_2 CRYOGENIC PRESSURE CELL

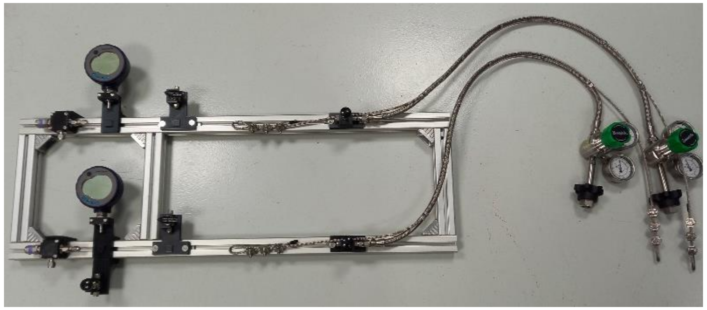
researches of new sustainable resources Importance of studying proteins that process, capture or transform green house gases or pollutants possibly into valuable molecules : carbon dioxide **(CO_2)**, carbon monoxide **(CO)**, methane **(CH_4)**, nitric oxide **(NO)** and nitrogen dioxide **(NO_2)**.











2 stages to avoid CO_2 icing at 194.7 K (−78.5 °C)

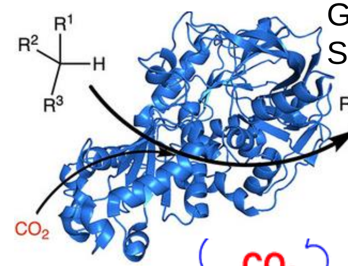
- Pressurizing in CO_2 at 50bar, 294 K
- Freezing in He at 50bar, 77 K

CO_2 CRYOGENIC PRESSURE CELL: POSSIBLE USE

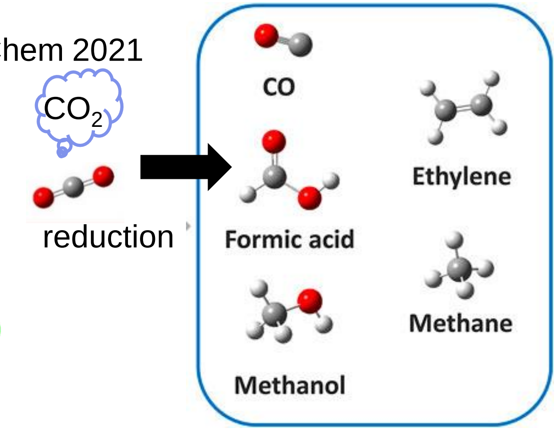
Already 108 PDB in the literature , examples:

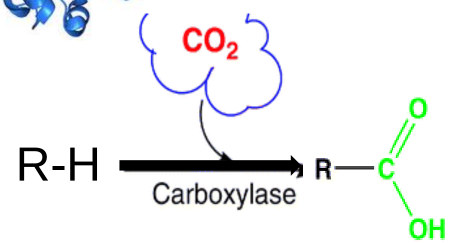
CO_2-converting enzymes

- ✓ Carboxylases, capture and conversion of CO2
- ✓ Reduction of CO2 into methane, methanol, …
- ✓ Others …

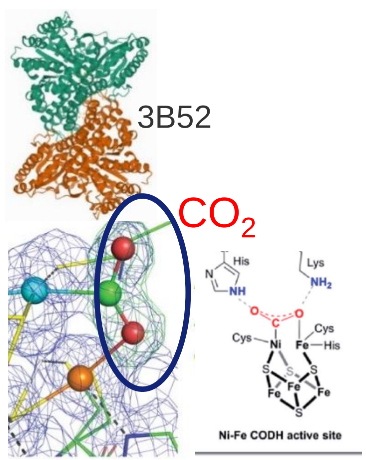


Godwin A.
ShemSusChem 2021



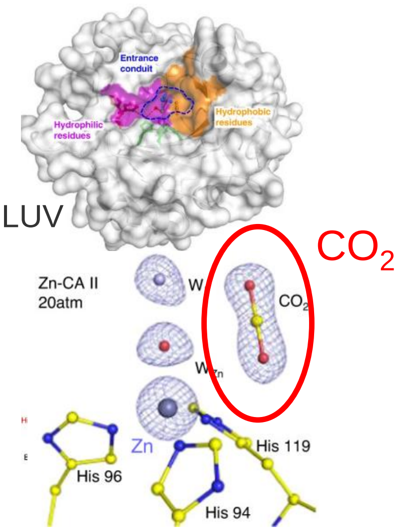


Carbon monoxide deshydrogenase:
CO_2 +2e⁻ +2H⁺ ⟷ CO + H_2O



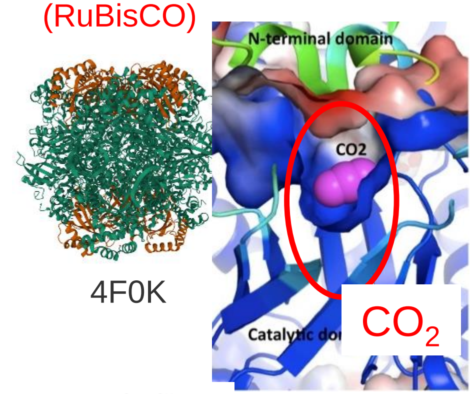
Jeoung, J.H. et al. (2007) Science

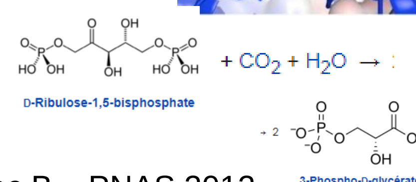
carbonic anhydrase:
$CO_2 + H_2O \longleftrightarrow HCO_3^- + H^+$.



Kim, C.U.et al.
Nat Commun 2020

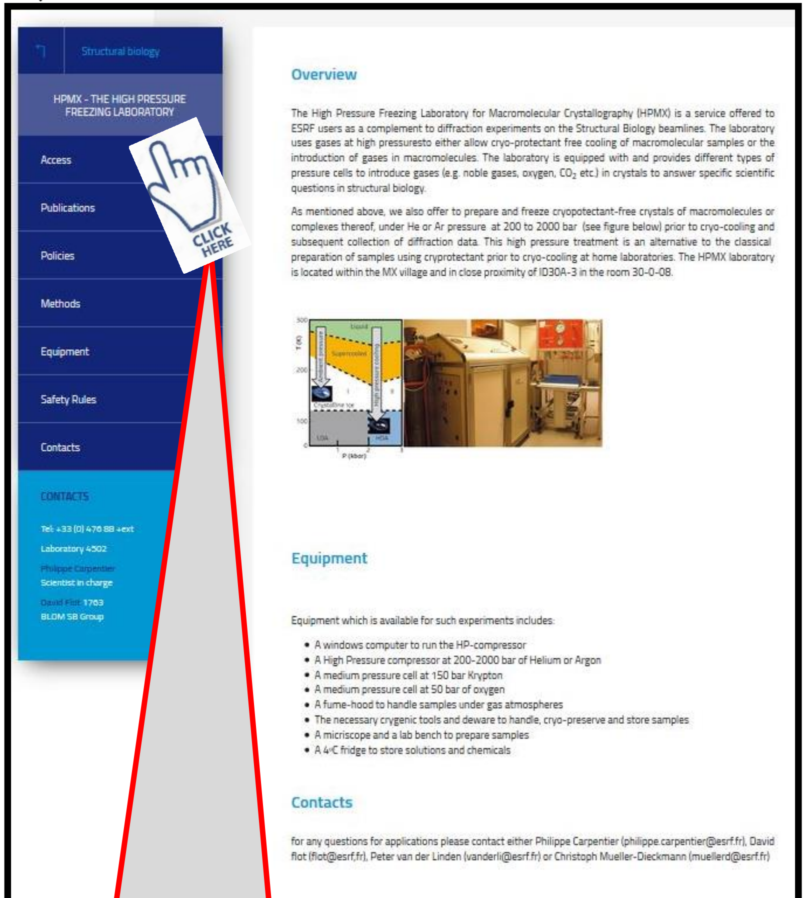
Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO)





Stec B. , PNAS 2012

https://www.esrf.eu/home/UsersAndScience/Experiments/MX/HPMX.html


Structural biology

HPMX - THE HIGH PRESSURE FREEZING LABORATORY

- Access
- Publications
- Policies
- Methods
- Equipment
- Safety Rules
- Contacts

CONTACTS

Overview

The High Pressure Freezing Laboratory for Macromolecular Crystallography (HPMX) is a service offered to ESRF users as a complement to diffraction experiments on the Structural Biology beamlines. The laboratory uses gases at high pressuresto either allow cryo-protectant free cooling of macromolecular samples or the introduction of gases in macromolecules. The laboratory is equipped with and provides different types of pressure cells to introduce gases (e.g. noble gases, oxygen, CO_2 etc.) in crystals to answer specific scientific questions in structural biology.

As mentioned above, we also offer to prepare and freeze cryopotectant-free crystals of macromolecules or complexes thereof, under He or Ar pressure at 200 to 2000 bar (see figure below) prior to cryo-cooling and subsequent collection of diffraction data. This high pressure treatment is an alternative to the classical preparation of samples using cryprotectant prior to cryo-cooling at home laboratories. The HPMX laboratory is located within the MX village and in close proximity of ID30A-3 in the room 30-0-08.

Equipment

Equipment which is available for such experiments includes:

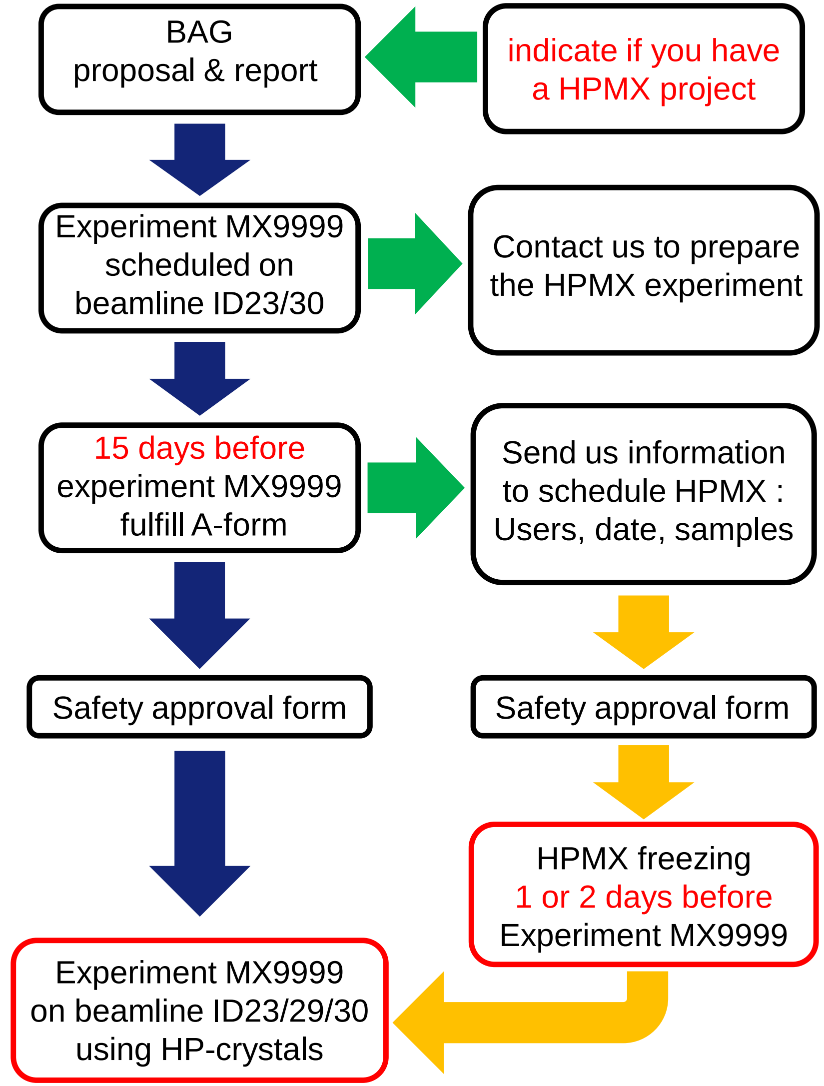
- A windows computer to run the HP-compressor
- A High Pressure compressor at 200-2000 bar of Helium or Argon
- A medium pressure cell at 150 bar Krypton
- A medium pressure cell at 50 bar of oxygen
- A fume-hood to handle samples under gas atmospheres
- The necessary crygenic tools and deware to handle, cryo-preserve and store samples
- A micriscope and a lab bench to prepare samples
- A 4°C fridge to store solutions and chemicals

Contacts

for any questions for applications please contact either Philippe Carpentier (philippe.carpentier@esrf.fr), David flot (flot@esrf,fr), Peter van der Linden (vanderli@esrf.fr) or Christoph Mueller-Dieckmann (muellerd@esrf.fr)

More information about the HPMX lab
- Description of the service
- Examples of use
- Contacts

Users access to the HPMX service



- **HPMX reference papers:**

- Towards a high-throughput system for high-pressure cooling…, P. Linden *et al*, J. Applied Crystallography 47 (2), 584 (2014)
- Gas-sensitive biological crystals processed in pressurized…, B. Lafumat *et al,* J. Applied Crystallography 49 (5), 1478 (2016)

- **Technical support:**

- Fabien Dobias
- Thierry Giraud
- Jonathan Gigmes
- Hugo Caserotto

- **Contacts:**

For any questions or projects of application please contact (15 days before the HP experiment for safety and preparation)

- Philippe Carpentier (philippe.carpentier@esrf.fr),
- David flot (flot@esrf,fr),
- Peter van der Linden (vanderli@esrf.fr)
- Christoph Mueller-Dieckmann (muellerd@esrf.fr)