Philippe Carpentier

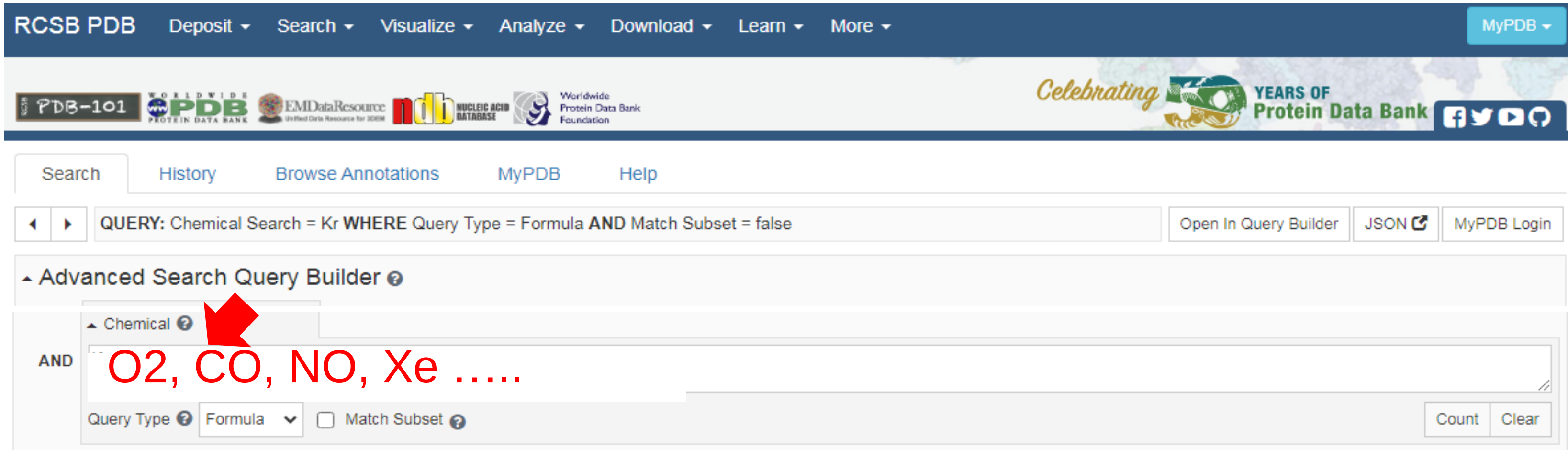
Chemistry and Biology of Metals laboratory
and Visiting Scientist at the ESRF

MX BAG Meeting, Monday 8th February 2021

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 gas atmospheres at high pressures to :
- to introduce gases in macromolecules,
- to avoidance cryo-protection of crystals.

Gas derivatization of bio-crystals allows to answer specific scientific questions in structural biology, or is used in methodological applications.

LIGHT GASES LIGANDS IN PDB STRUCTURES



- 498 PDB structures with O2 (OXY, PER) → <u>Oxidoreductases: oxygenases, oxidases</u>
- 386 PDB structure with CO (CMO) → <u>Hemoproteins</u>
- 166 PDB structures with NO
- 86 PDB structures with CO2 → <u>Carbonic anhydrases, carboxylases</u>
- 17 PDB structures with N2O → Nitrous oxide reductases
- 8 PDB structures with N2 (HDZ) → Nitrogenases

- 138 PDB structures with Xenon
- 28 PDB structures with Krypton
- 20 PDB structures with Argon

chemically inert but with many biological & medical properties : analgesia, anaesthesia, neuroprotection… Used in methodological appliactions

- ➢ Many structures crystallized with ligands
- ➢ HP service allows to produce the derivatives

Xe/Kr/Ar labelling + Software tools (Caver/Mole)

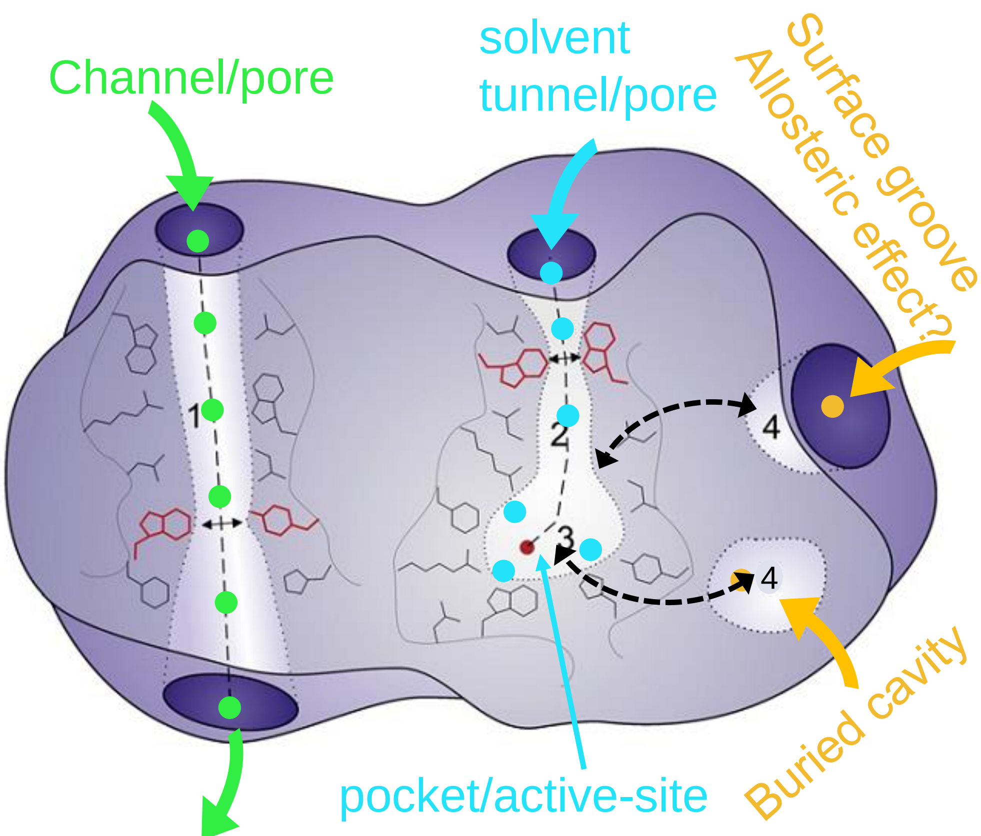


Figure from, J. Brezovsky et al., Biotech. Adv. 31 (2013) 38

-1- Reveal surface entrance pores and detect functional channels through protein (e.g. transport proteins pumps)

-2- Reveal functional tunnels, pathways for substrates and products between active-sites and solvent.

-3- Map pockets of active sites

-4- highlight buried cavities and surface grooves/excavations (potentially functional, allosteric effect)

THE USE OF GAS DERIVATIVES IN STRUCTURAL BIOLOGY

He HP-Freezing

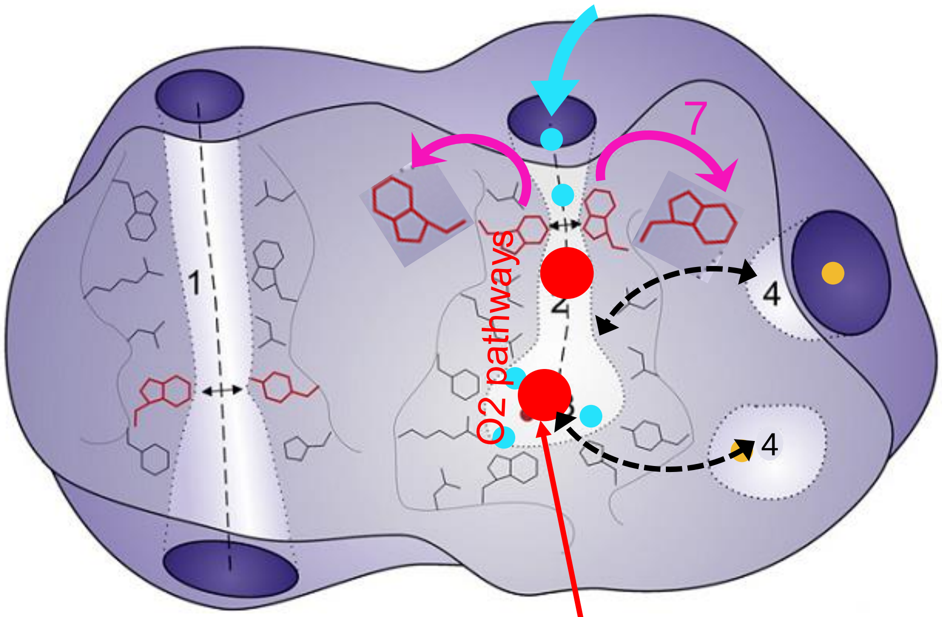


Figure from, J. Brezovsky et al., Biotech. Adv. 31 (2013) 38

-1- Reveal surface entrance pores and detect functional channels through protein (e.g. transport proteins pumps)

-2- Reveal functional tunnels, pathways for substrates and products between active-sites and solvent.

-3- Map pockets of active sites

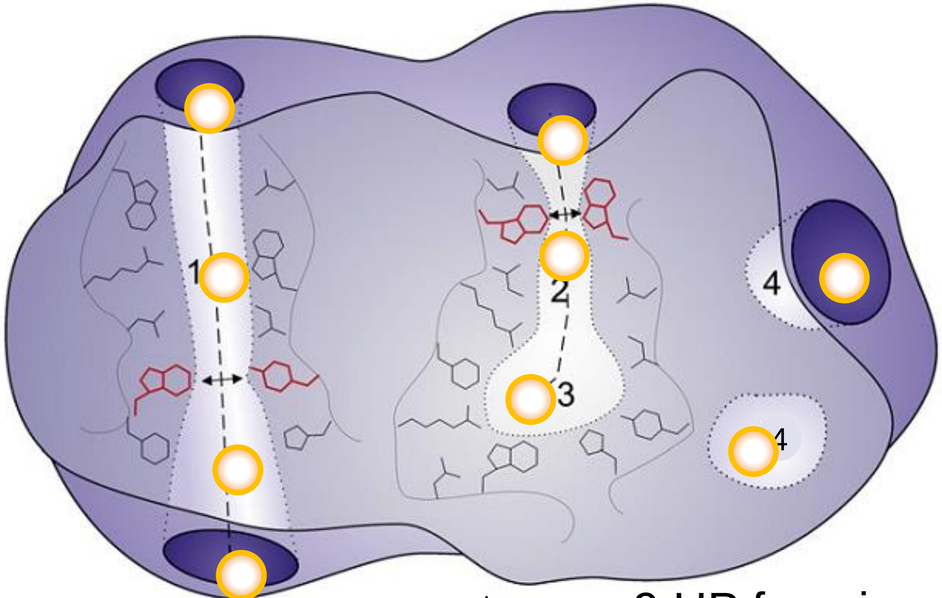
-4- Label buried cavities and surface grooves/excavations (potentially functional, allosteric effect)

-6- Reveal “binding sites” of gas ligands, substrates or products (e.g. O_2, CO_2, NO, CO), O_2 pathways, reactivity studies (intermediates, enzymatic mechanisms)

-7- Pressure (He 2000 bar) induce local structural modifications, exploration functional conformational fluctuations. Limited

THE USE OF GAS DERIVATIVES IN STRUCTURAL BIOLOGY

Methods Xe/Kr/Ar/He HP-Freezing



-1- Reveal surface entrance pores and detect functional channels through protein (e.g. transport proteins pumps)

-2- Reveal functional tunnels, pathways for substrates and products between active-sites and solvent.

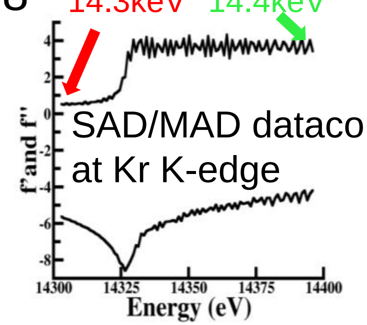
-3- Map pockets of active sites

-4- Label buried cavities and surface grooves/excavations (potentially functional, allosteric effect)

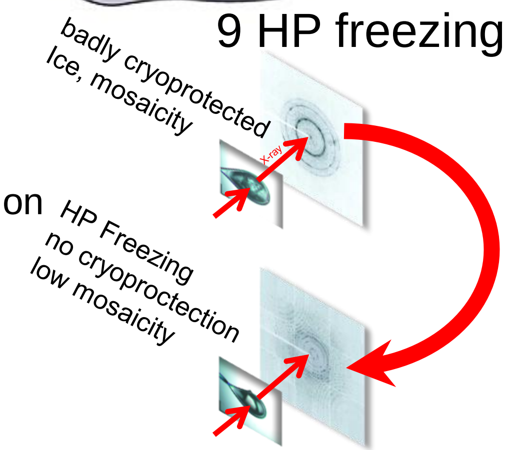
-6- Reveal “binding sites” of gas ligands, substrates or products (e.g. O2, CO2, NO, CO), O2 pathways, reactivity studies (intermediates, enzymatic mechanisms)

-7- Pressure (He 2000 bar) induce local structural modifications, exploration functional conformational fluctuations. Limited

8



9 HP freezing



-8- Use noble gases (Xe, Kr, Ar) as heavy atoms for protein structures determination

-9- Crystals freezing at High pressure (He 2000 bar) without cryopretection, solving cryoprotection issues: ice, broken crystals, high mosaicity

PROPERTIES OF LIGHT AND NOBLE GASES FOR STRUCTURALE BIOLOGY

Noble gases	He	Ne	Ar	Kr	Xe
vdW radius Å	1.40	1.54	1.88	2.02	2.16
Polarizability Å^3	0.21	0.40	1.64	2.48	4.04
Solubility H[P](mM/bar)	0.39	0.46	1.40	2.51	4.34
Highest pressure (bar)	2000	NA	2000	150 (->500)	NA (55 RT)
Collect X-ray λ(Å)	NA	NA	1.77 SAD	0.87 MAD	1.77 SAD
Anomalous f''(e)	NA	NA	1.1	3.8	9.2

Probe size vdW radius
Tunnel size

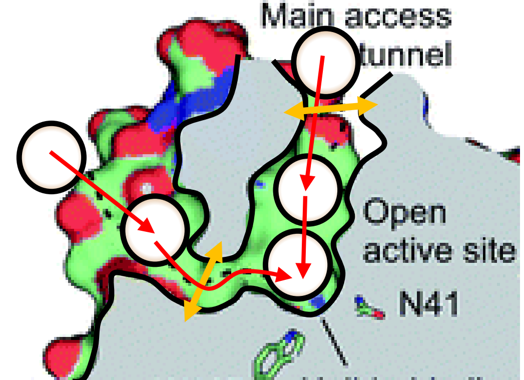
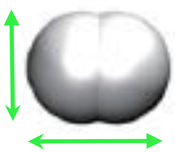


Figure from K. Markova et al., Chem Sci (2020)

H_2

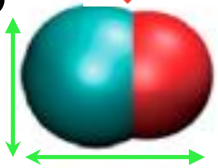


1.2/1.57Å

CO_2

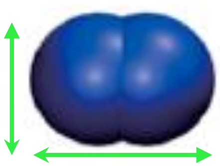
1.7/2.68Å
H:35 mM/bar

CO
NO



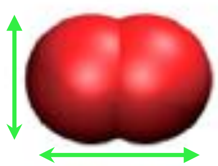
1.7/2.18Å

N_2



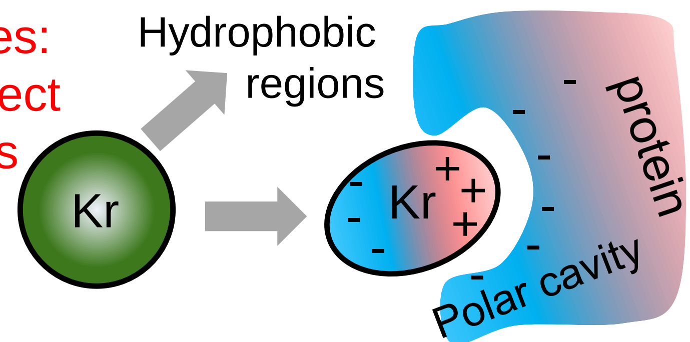
1.55/2.1Å

O_2



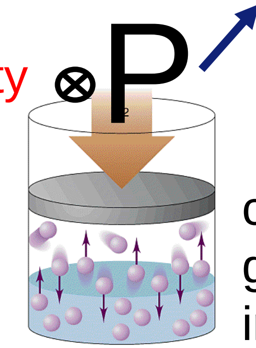
1.52/2.12Å
H:1.3 mM/bar

Interaction forces:
hydrophobic effect
& London forces



Polarizability → transient dipole

Gas solubility ⊗



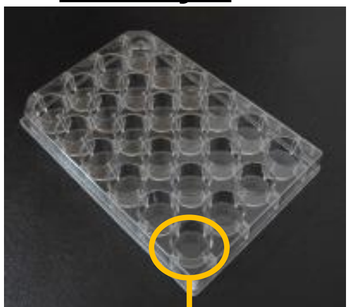
favor
Xtal soaking

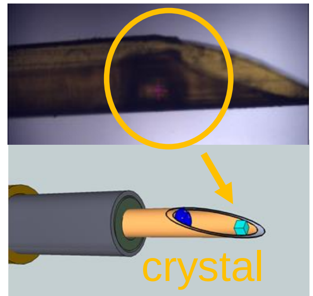
concentration of
gas dissolved
in solvent

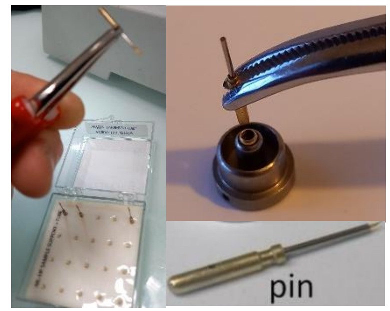
"SOAK AND FREEZE" METHOD, FREEZING CRYSTAL UNDER HIGH PRESSURE

<u>Users bring their samples in trays</u>

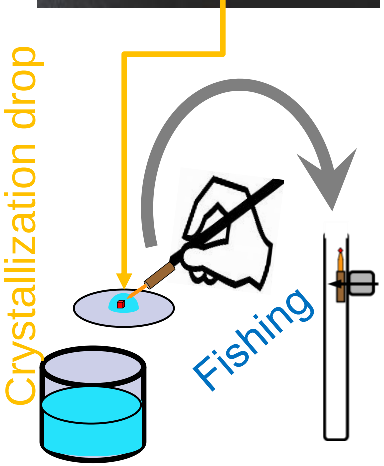
Light gases of bio interest
O_2, He, Ar, Kr, …



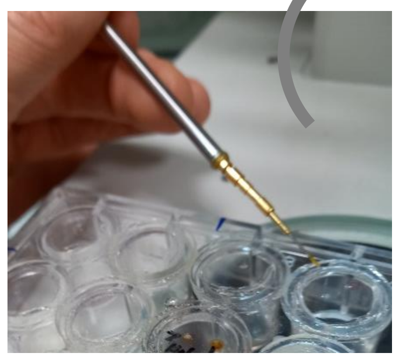




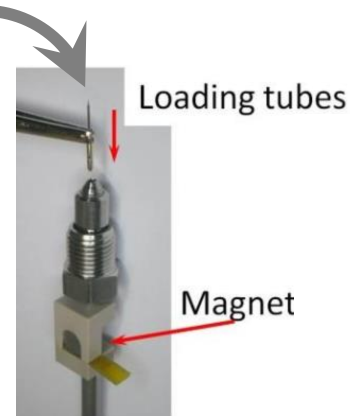
<u>MX-HP-Cool base& Support-pin from MiteGen</u>



(1) loading



Fishing tool

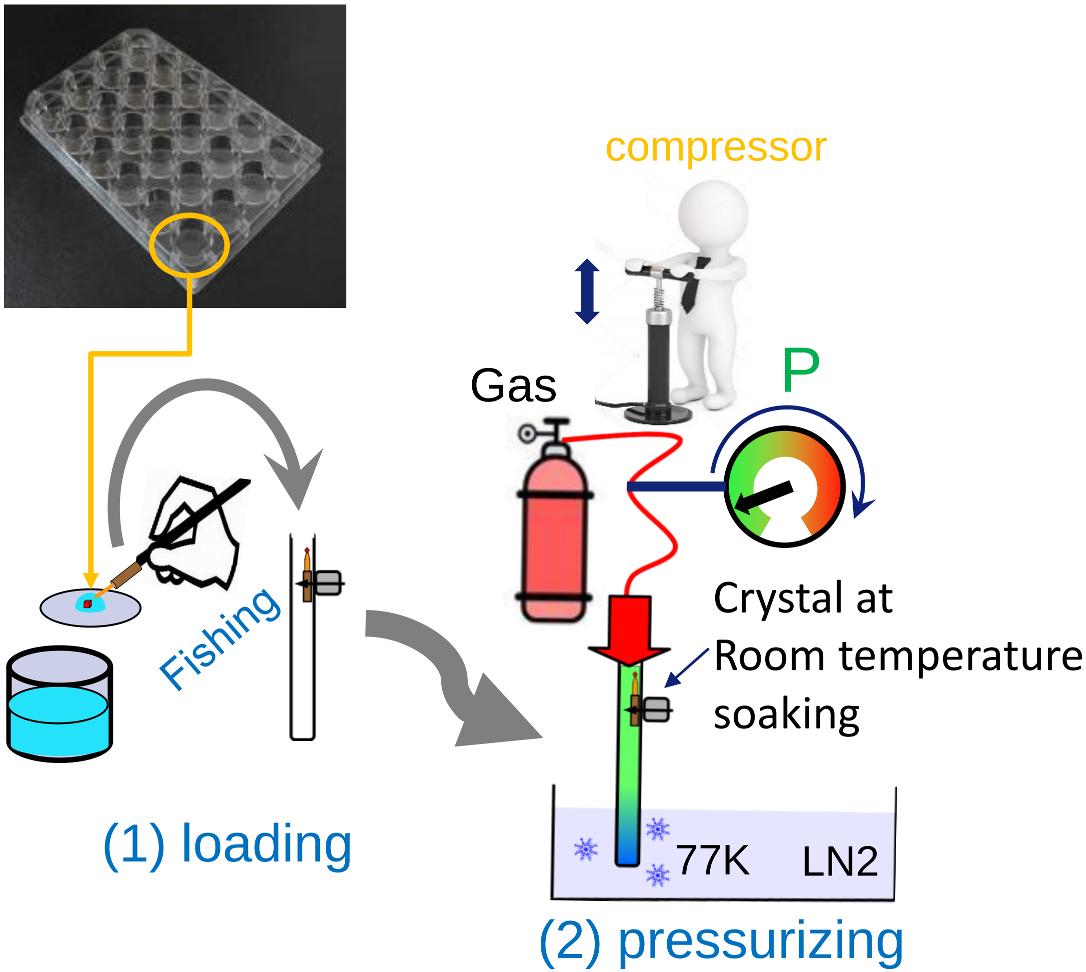


Pressure tube

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

“SOAK AND FREEZE” METHOD, FREEZING CRYSTAL UNDER HIGH PRESSURE

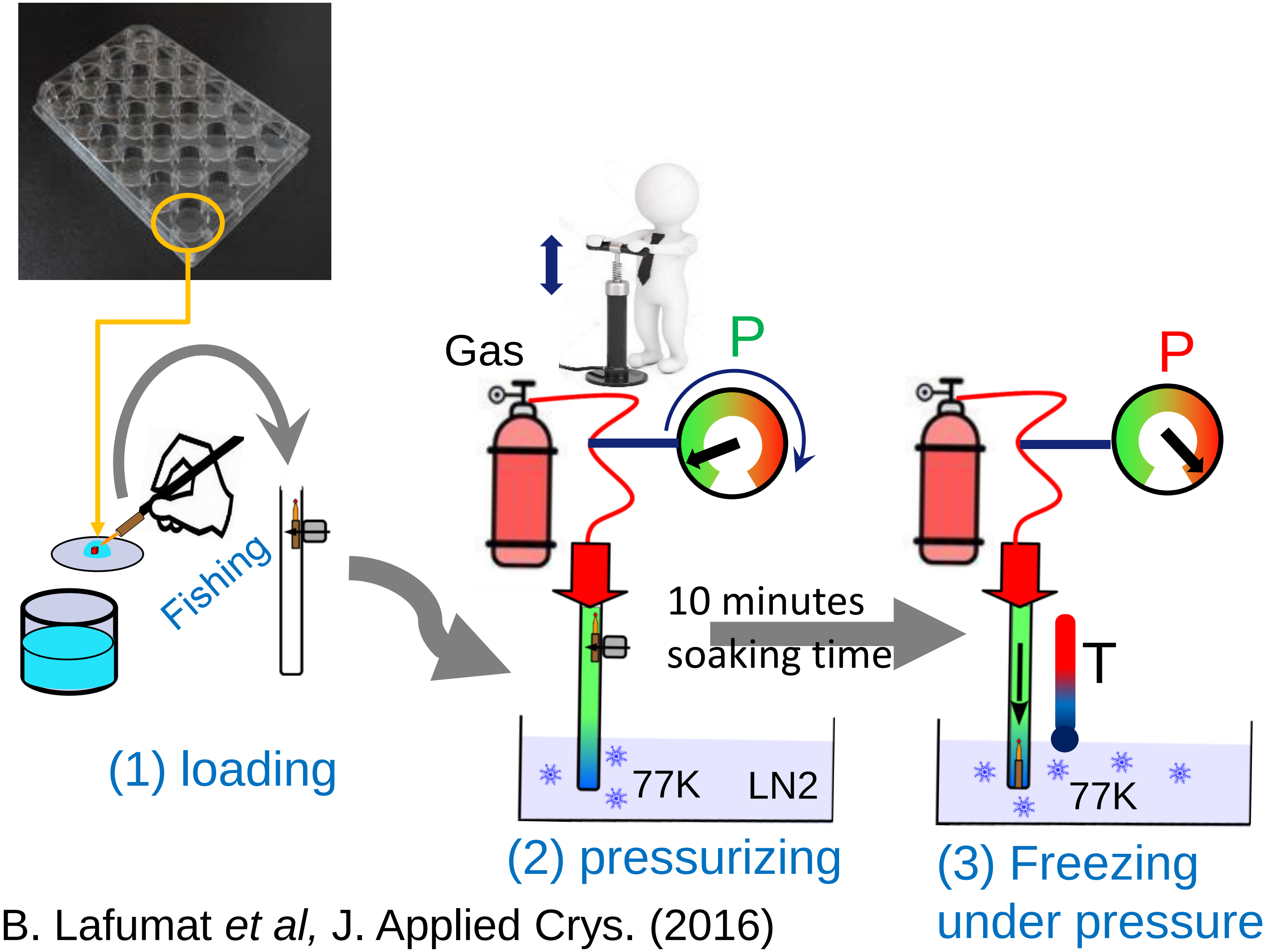
Light gases of bio interest
O_2, He, Ar, Kr, …



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

"SOAK AND FREEZE" METHOD, FREEZING CRYSTAL UNDER HIGH PRESSURE

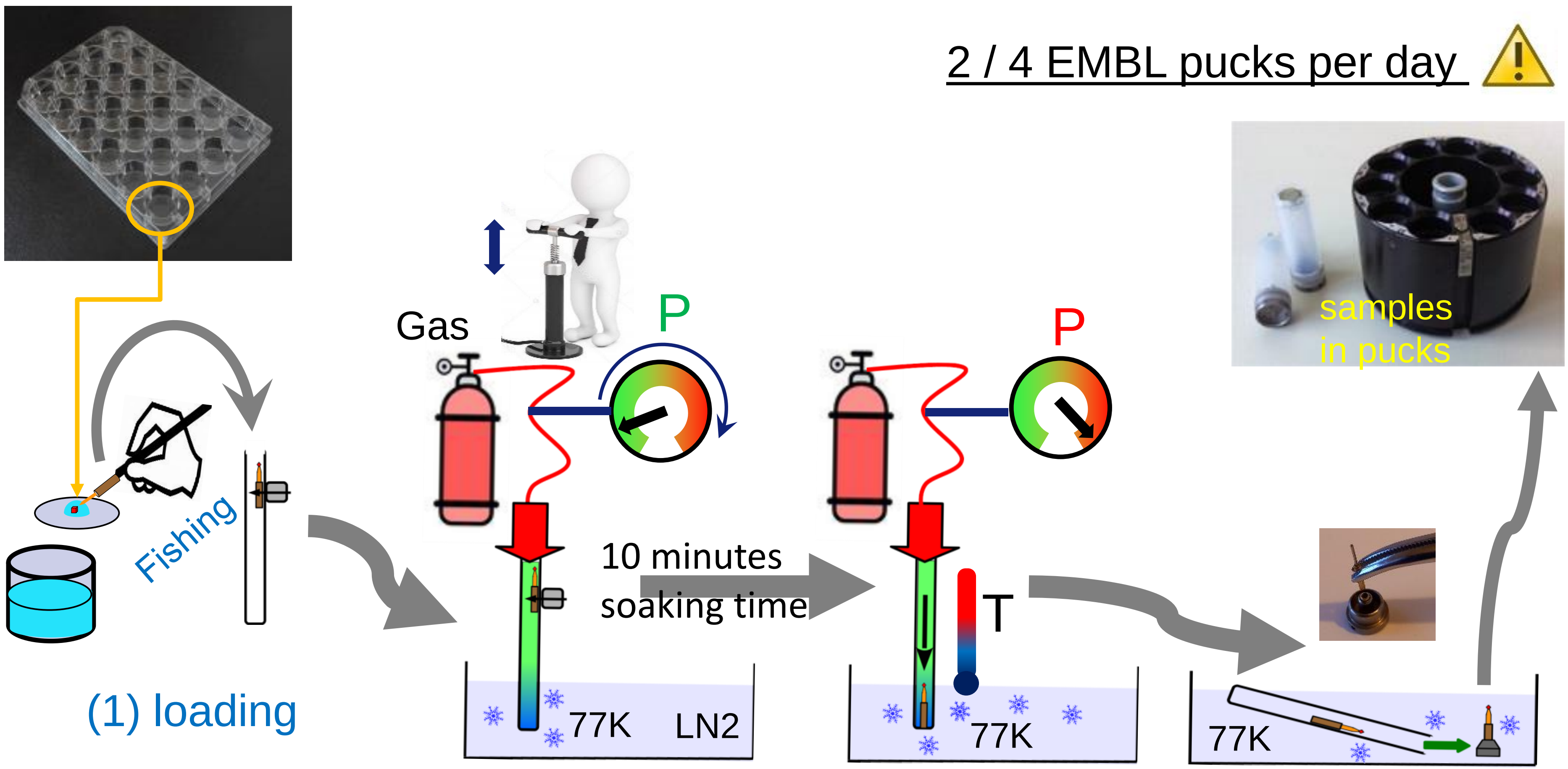
Light gases of bio interest
O_2, He, Ar, Kr, …



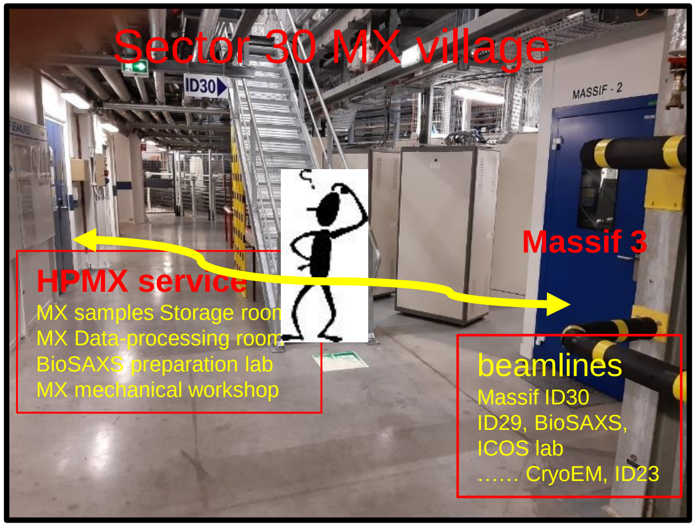
B. Lafumat *et al,* J. Applied Crys. (2016)

"SOAK AND FREEZE" METHOD, FREEZING CRYSTAL UNDER HIGH PRESSURE

Light gases of bio interest
O_2, He, Ar, Kr, …

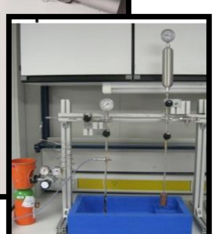


B. Lafumat *et al,* J. Applied Crys. (2016)



The HPMX lab is

- located the the MX village next to ID30A-3, room 30-0-08
- Equipped with different types of pressure cells to introduce light gases in protein crystals
- Equipped with a samples preparation benches and tools



THE DIFFERENT TYPES OF CRYOGENIC PRESSURE CELLS

1

- Cryogenic high pressure bench He 2000 bar, crystals without cyoprotection.
- or Ar 2000 bar, crystals with reduced cryo

He
Ar

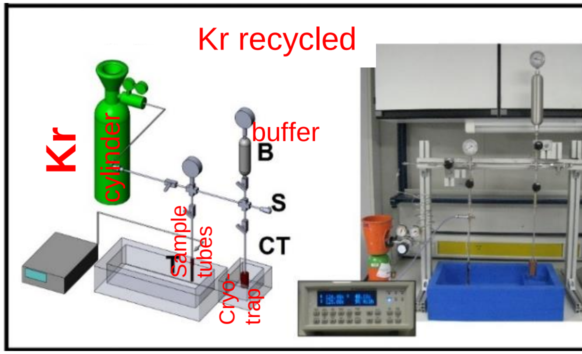
2014
2019

2

Cryogenic Kr pressure cell 150 bar
Cryogenic Xe pressure cell 100 bar ?

Kr
Xe?

2015

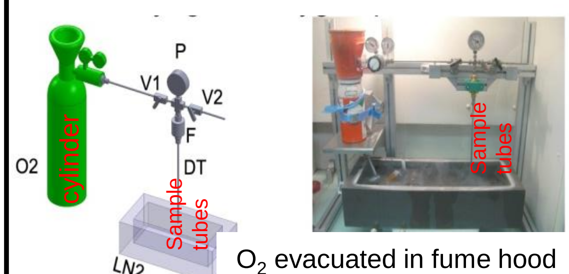


3

Cryogenic O_2 pressure cell 70 bar

O_2

2016



O_2 evacuated in fume hood

4

Cryogenic Kr pressure cell 500 bar

soon

2021

Kr

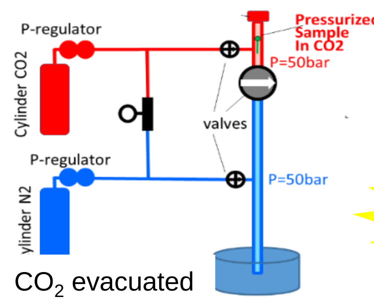
5

Cryogenic CO_2 pressure cell 50 bar

CO_2

Project disgn

2022



CO_2 evacuated

6

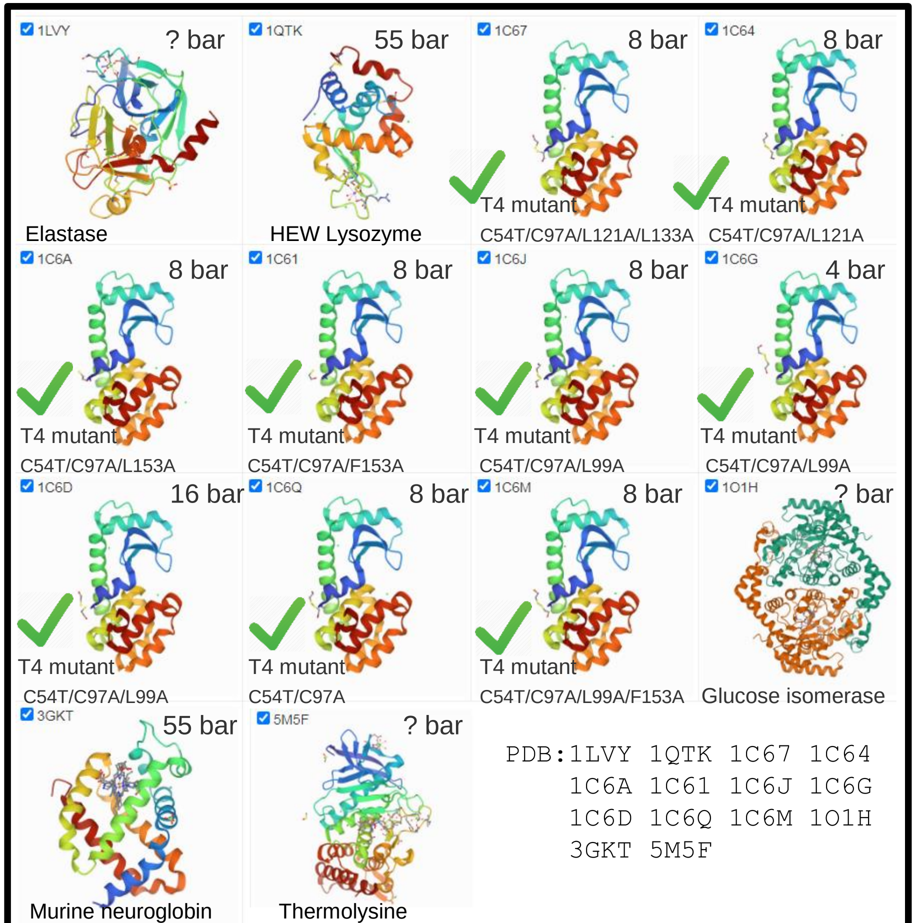
Room temperature cell
I. Melnikov
beamline

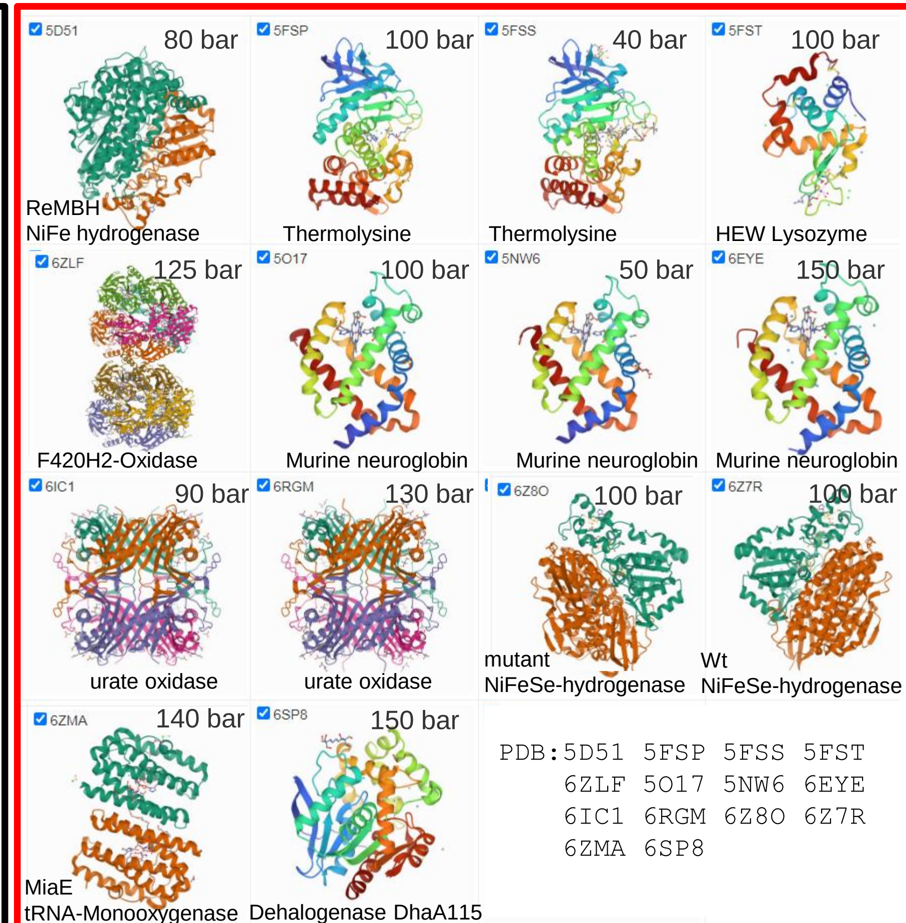
O2/Kr

PDB OF KRYPTON DERIVATIVE CRYSTALS

1995/2017 PDB standard

2016/2020 PDB at the HPMX





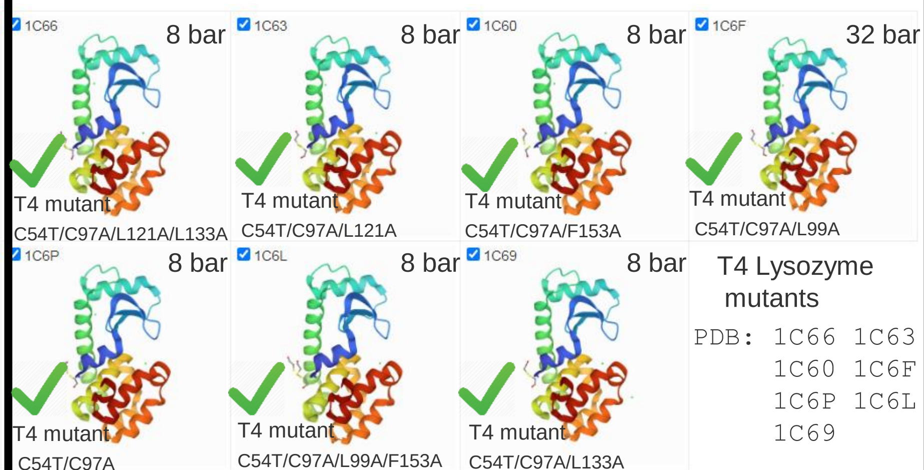
Krypton volatile, classical cell pressures too low

Soak end freeze: higher pressures

- 6 Hydrolases EC3
- 6 Oxidoreductases EC1
- 1 Ligase EC6
- 1 O_2 storage protein

PDB OF ARGON DERIVATIVE CRYSTALS

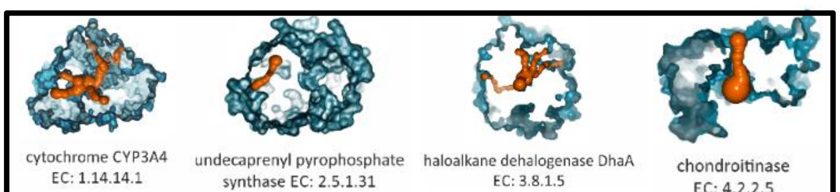
2000/2015 PDB standard

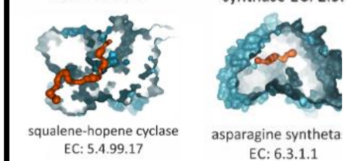


T4 Lysozyme mutants

PDB: 1C66 1C63 1C60 1C6F 1C6P 1C6L 1C69

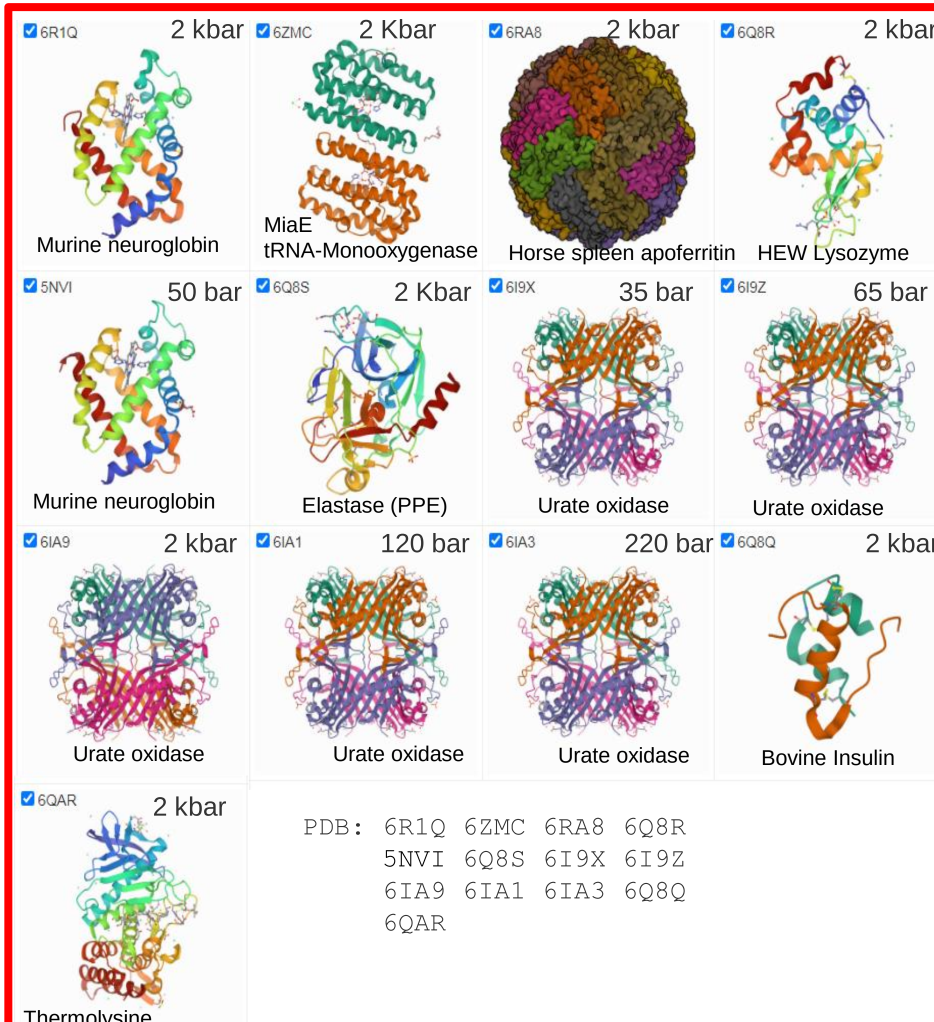
- 4 Hydrolases EC3
- 2 Oxidoreductases EC1
- 1 Fe transport protein
- 1 O_2 storage protein
- 1 Hormone





Tunnels in six representatives of all enzyme classes.
CAVER web site: www.caver.cz

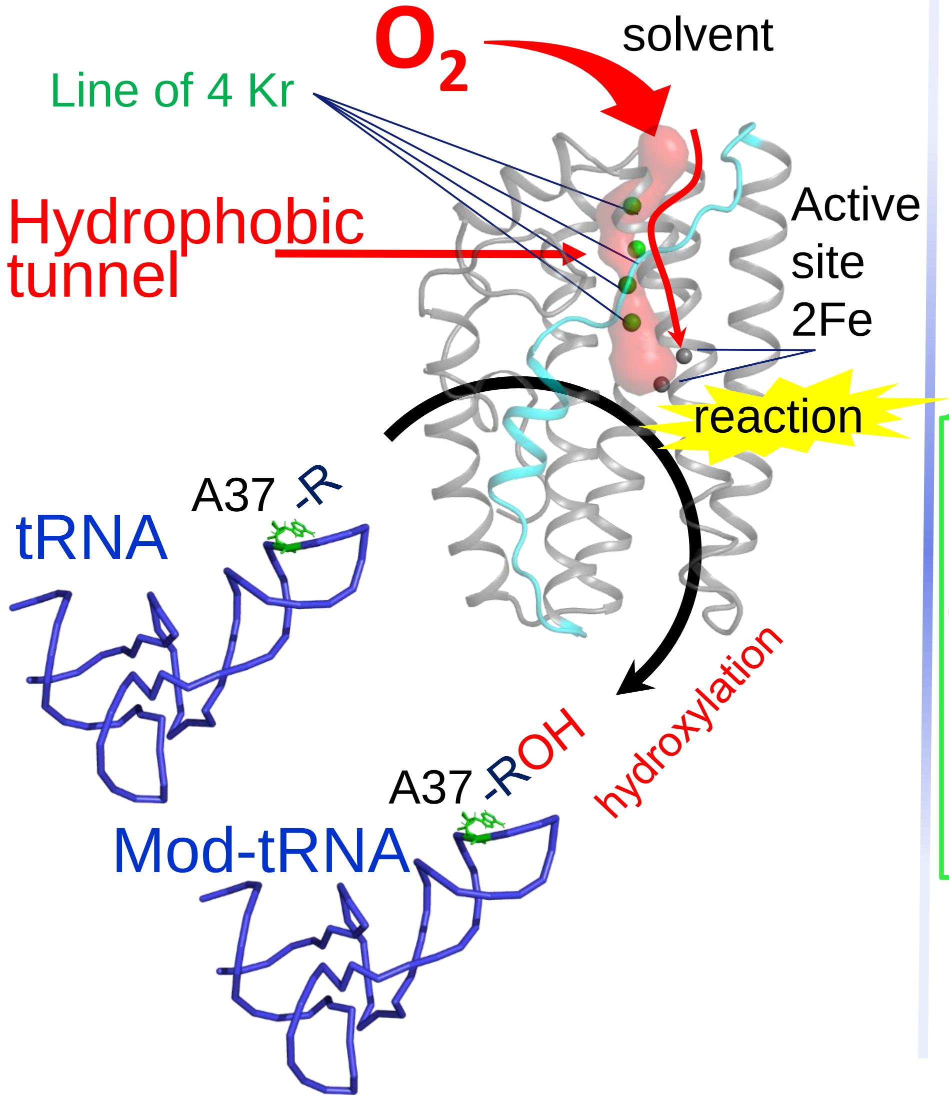
2019/2020 PDB at the HPMX



PDB: 6R1Q 6ZMC 6RA8 6Q8R 5NVI 6Q8S 6I9X 6I9Z 6IA9 6IA1 6IA3 6Q8Q 6QAR

Effective Ar derivatives HP 2000 bar

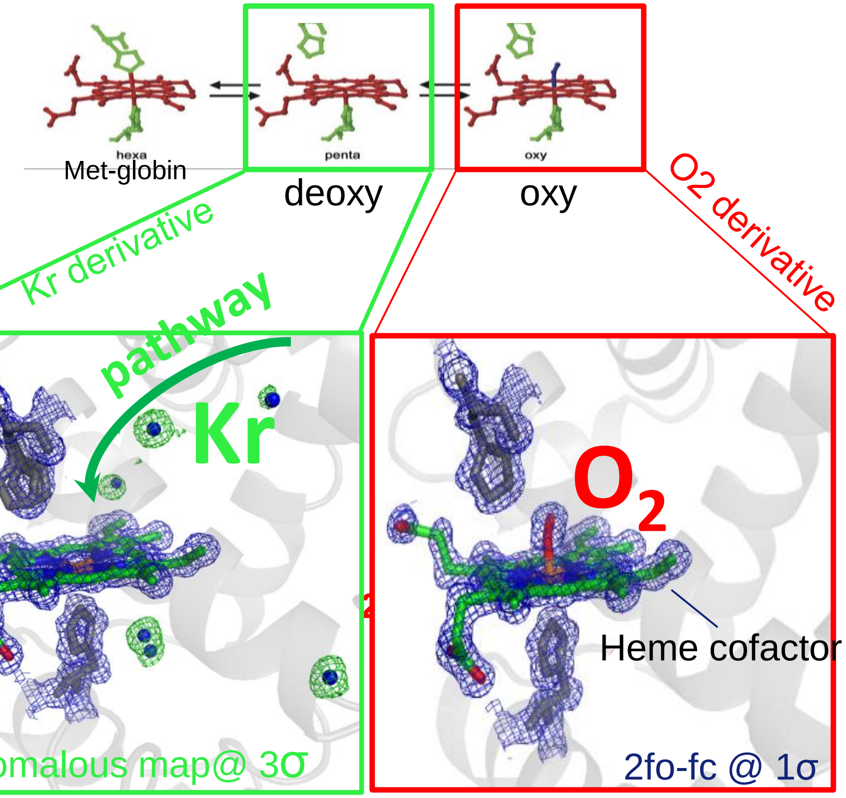
Kr probes the O2 tunnel in the redox diiron tRNA modifying enzyme MiaE (using Kr at 140 bar)



P. Carpentier *et al,* Nucleic acids research (2020)

Kr probes tunnels Krypton at 140bar) and O_2 binds Hemes in globins (oxygen at 65bar)

Figure from M. Brunori *et al.* , Cell. Mol. Life Sci. 2007



Crystal structure of O2 and Kr derivatives of myoglobin crystals
S. Popov & G katchalova published soon

EXAMPLES OF USE

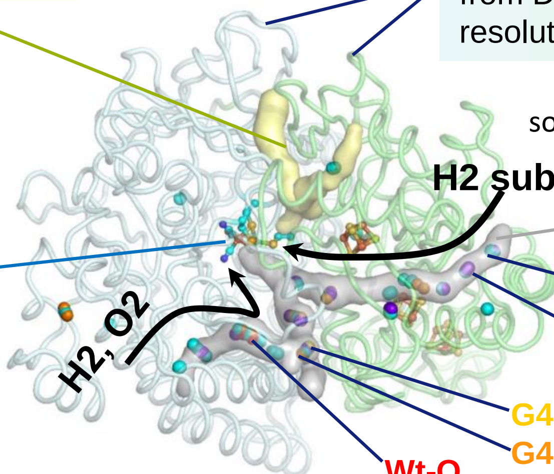
Study of Gas Access Routes in a [NiFeSe] Hydrogenase using Kr at 100 bar and O2 at 75 bar

$H_2 \rightleftarrows 2H^+ + 2e^-$

hydrophilic channel

Structure of [NiFeSe] hydrogenase from Desulfovibrio vulgaris at 0.95 Å resolution (PDB 5JSK)

Hydrophobic channel
CAVER computation
0.9Å probe radius



active site
O2 inactivation

Wt-Kr

G491A-Kr

G491S-O_2^{LD},

G491S-O_2^{HD}

Wt-O_2

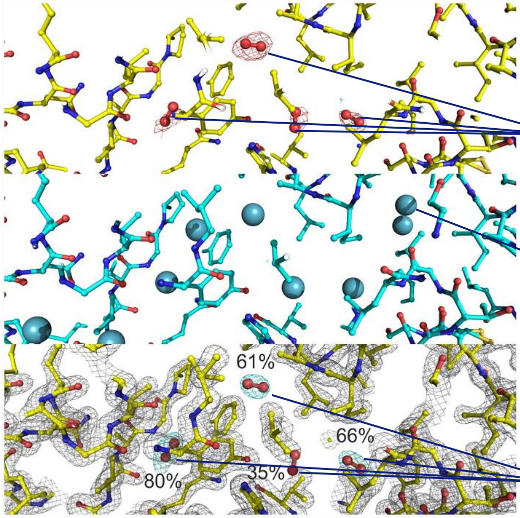
Map the "classic" hydrophobic channel access

O_2 in channels are highly sensitive to X-rays

G491A-O_2

Wt-Kr

G491A-O_2



O_2

Kr

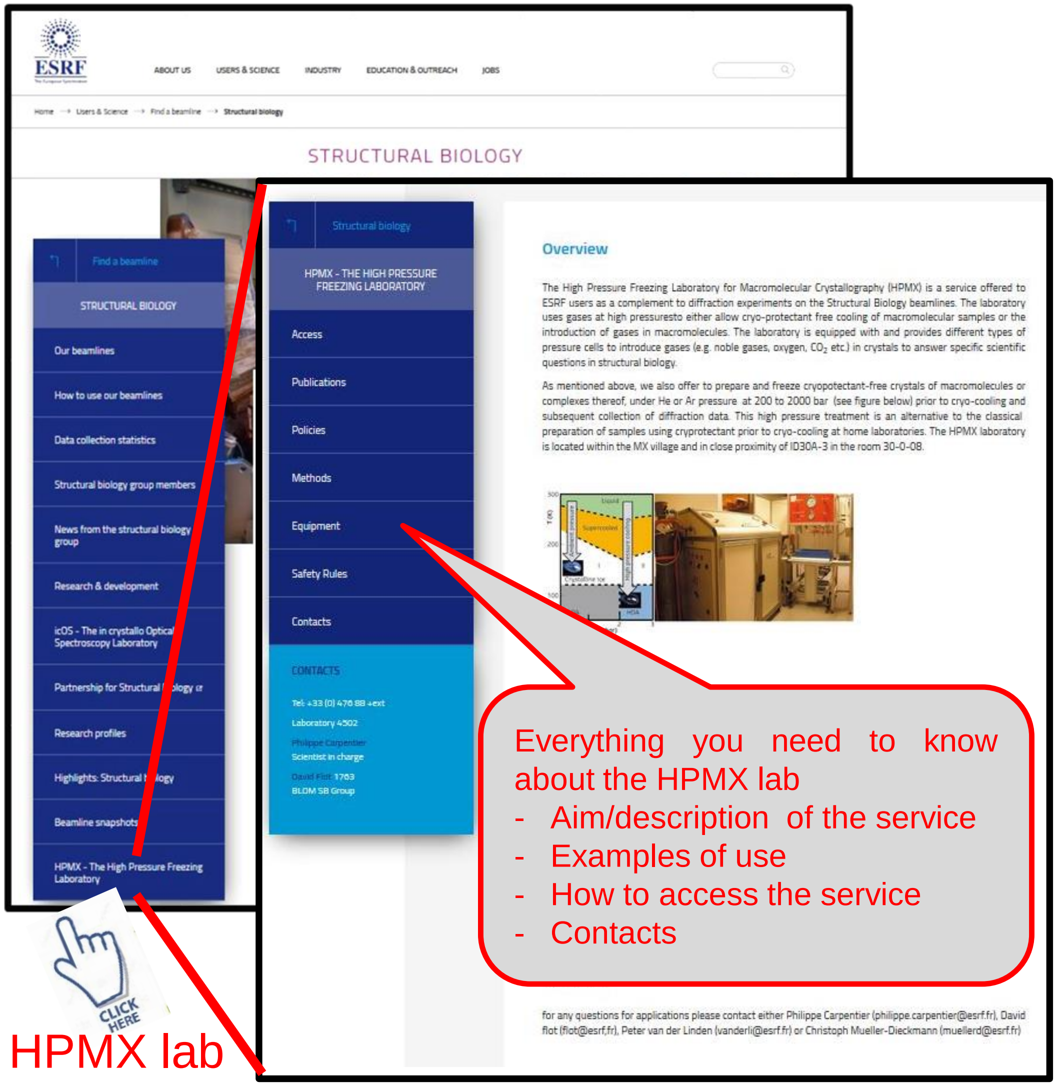
O_2

Tracking O_2 in
High dose 1.35MG
– low dose 0.12MG
$|Fo^{hd}|-|Fo,^{ld}|$
difference map 3σ

Refinement
2|Fo,ld|-|Fo,ld|

P. Matias Lab ITQB Portugal

S Zacarias *et al*, Journal of Biological Inorganic Chemistry (2020) https://www.esrf.eu/home/UsersAndScience/Experiments/MX/HPMX.html



Users access to the HPMX service

- BAG proposal & report ← indicate if you have a HPMX project
- ↓
- Experiment MX9999 scheduled on beamline ID23/29/30 → Contact us to prepare the HPMX experiment
- ↓
- 15 days before experiment MX9999 fulfill A-form → Send us information to schedule HPMX : Users, date, samples
- ↓ Safety approval form | ↓ Safety approval form
- ↓ HPMX freezing 1 or 2 days before Experiment MX9999
- ↓ Experiment MX9999 on beamline ID23/29/30 using HP-crystals

HPMX REFERENCES, CONTACTS

Web pages:

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

Selected Papers:

- **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)

- **Paper using HP krypton**

- Decoding the intricate network…, K. Markova *et al*, Chemical Science 11 (41), 11162 (2020)
- Krypton-derivatization highlights…, S. Engilberge *et al*, Chemical Communications 56 (74), 10863 (2020)

- **Paper using HP Krypton and Argon**

- P. Carpentier *et al,* Nucleic acids research 48 (17), 9918 (2020)

- **Papers using HP Krypton and Oxygen**

- Tracking the route of molecular oxygen…, J. Kalms *et al*, PNAS, 115 (10), E2229 (2018)
- Exploring the gas access routes…, S Zacarias *et al*, JBIC Journal of Biological Inorganic Chemistry 25 (6), 863 (2020)

Technical support:

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

Contacts:

for any questions or for any 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)