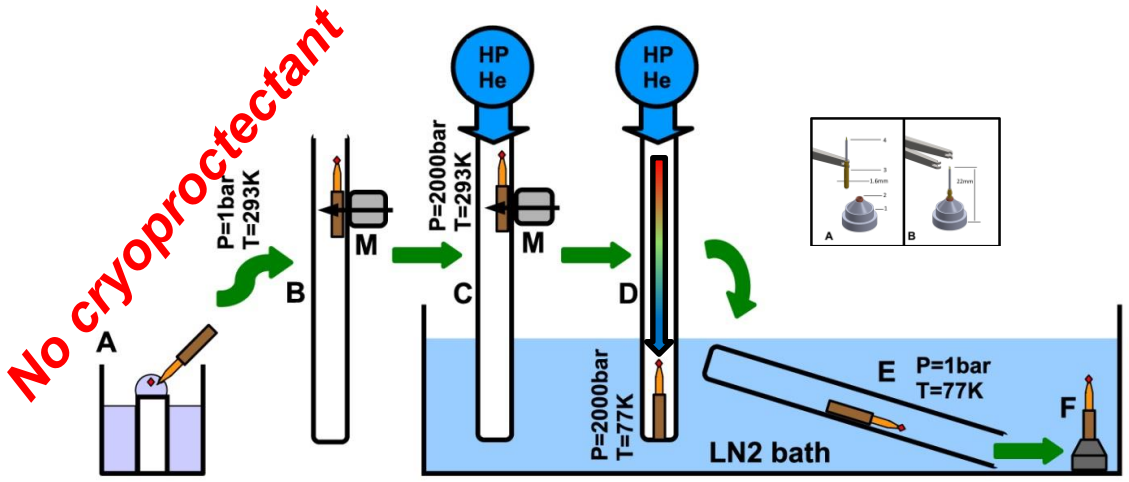
High Pressure Freezing

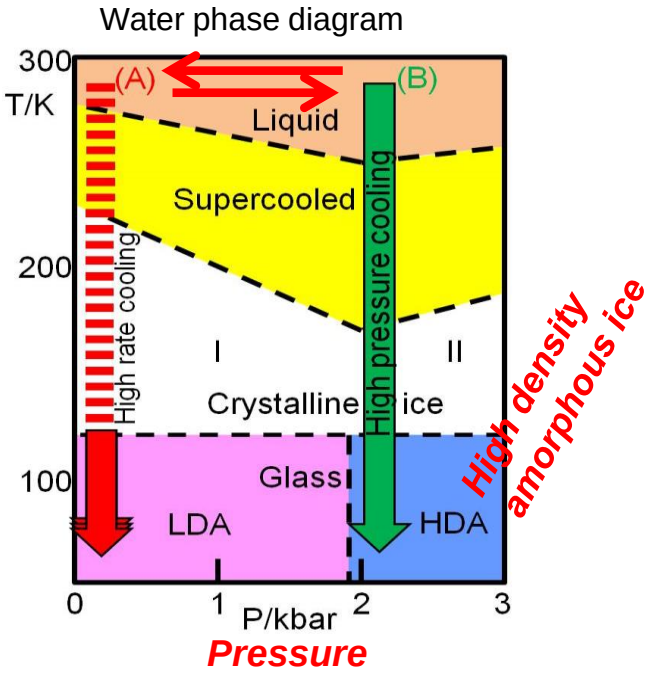
Philippe Carpentier,

MX BAG Meeting, Monday February 8th 2016

-1- High pressure freezing of crystals without cryo-protectant.
-2- Recent developments of oxygen and noble gas cryo-cells.
-3- Perspectives for “very” high pressure freezing and room temperature pressure cell.

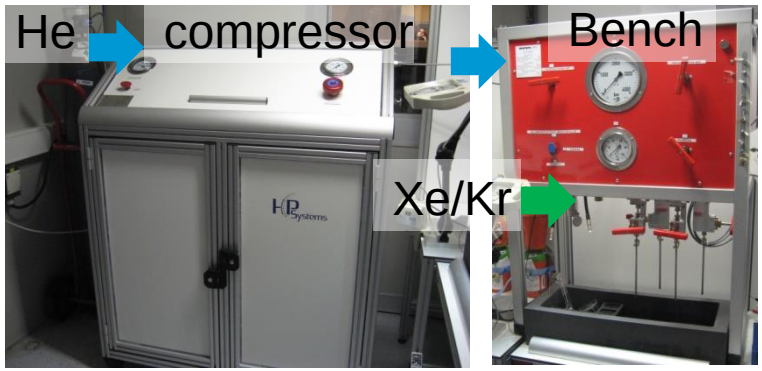
HIGH PRESSURE FREEZING SYSTEM AND METHOD (P. VAN DER LINDEN)



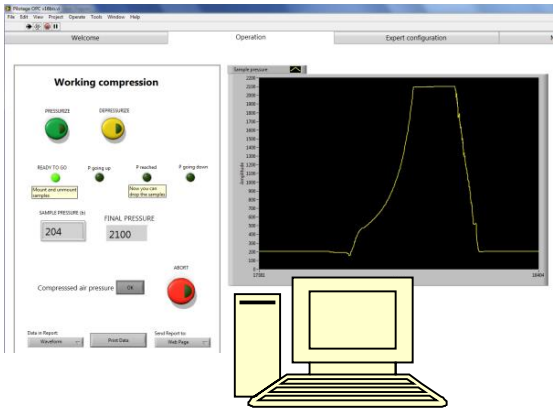


HP-cooling process:

(A) Crystal fishing ((293K, 1bar),
(B) Loading in drop tubes, (293K, 200bar)
(C) Pressurization (293K, 2000 bar),
(D) Cooling under HP (77K, 2000bar),
(E and F) Pins/bases assembly in LN2 @ 77K, 1bar



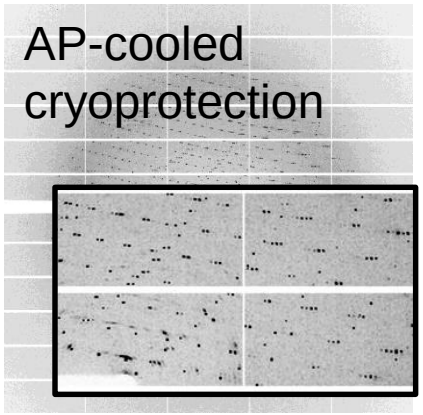
Control software (automated)



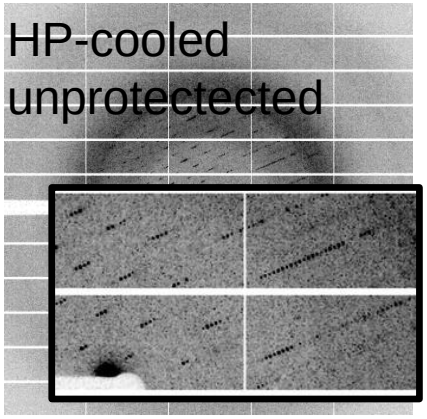
User mode since June 2014

HIGH PRESSURE COOLING, APPLICATIONS

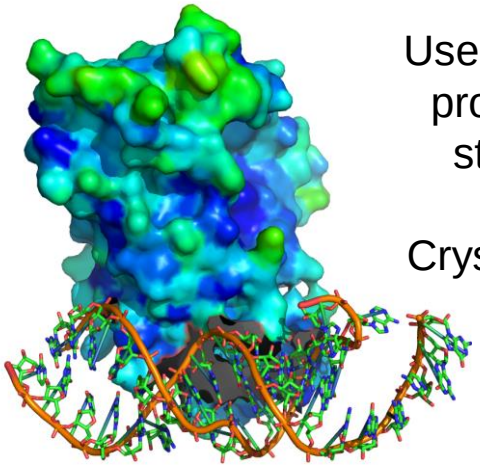
(1) Cryoprotection-free, improvement of crystal quality



- space group $P2_1$?
- Resolution ~ 3Å
- Mosaic/broken, twin

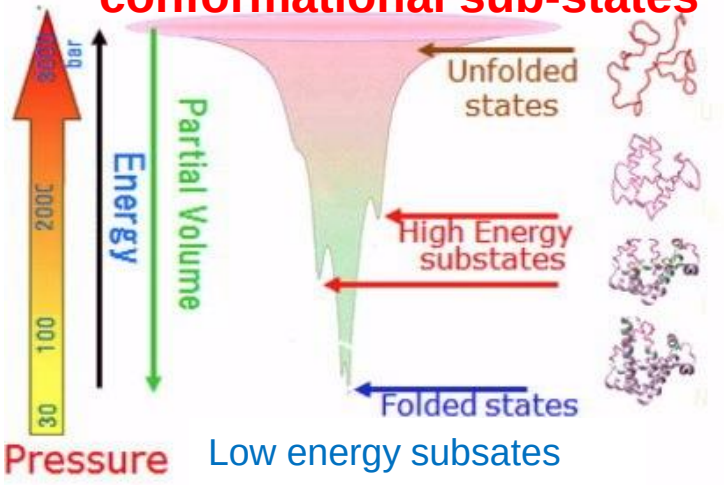


- space group $P2_12_12_1$,
- Resolution ~ 2.5Å
- Lower mosaicity

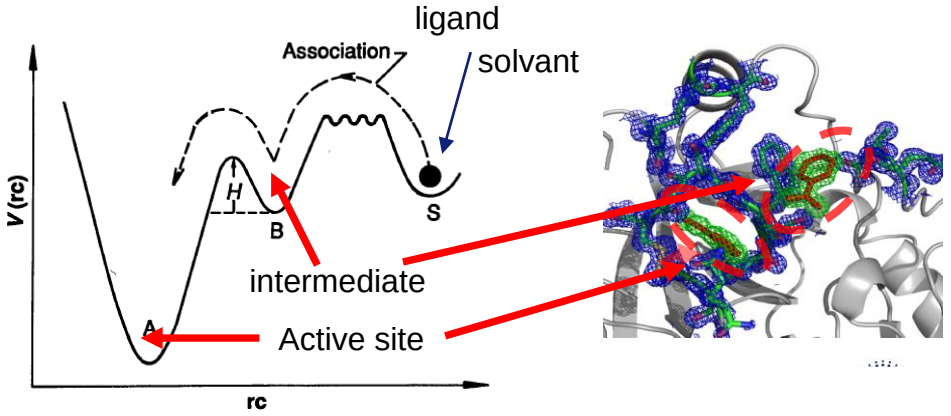


User challenging project protein-RNA complex structure solved by High Pressure Crystallography @ 2.5Å

(2) Exploration of conformational sub-states



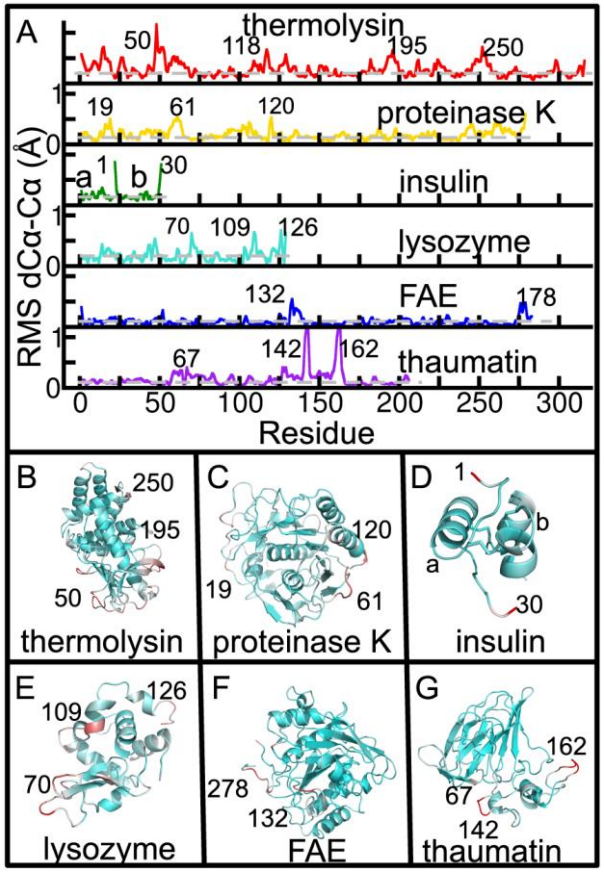
(3) Studies of reaction, ligand binding intermediates



ASSESSMENT OF THE SYSTEM WITH TEST CRYSTALS

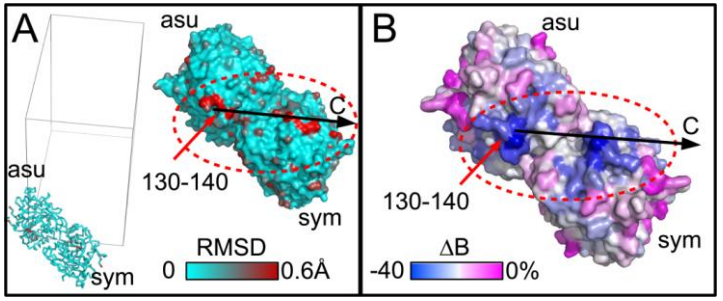
Gallery of HP-cooled protein crystals.

Cα backbone & 3d displacements representation



Phase transition in FAE

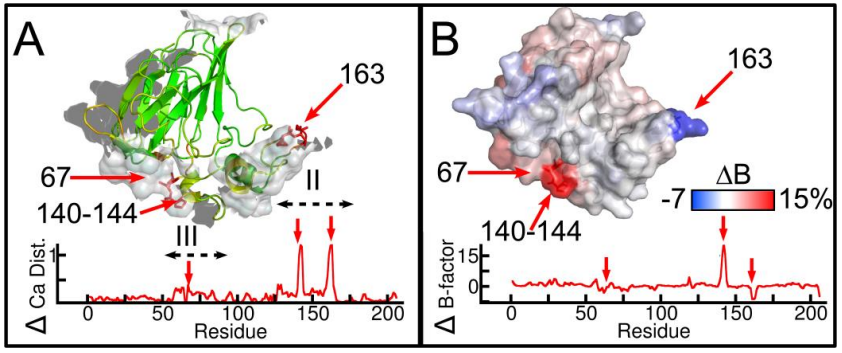
Flash freezing 1bar	2000bar
Space group $P2_12_12_1$	$P4_12_12$
Bfact (Å²): 14.2	19.6



short loop 130–140 r.m.s. displacement< 0.6Å

Improvement of crystalline quality (few cases)

flexible domains in thaumatin



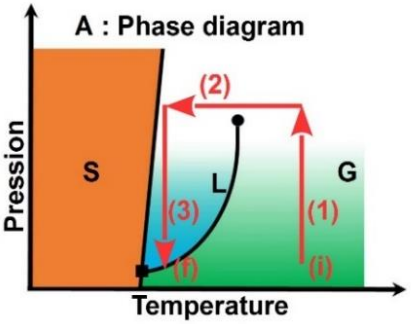
Pro141 buried in crystal contacts, Lys163 exposed to the solvent

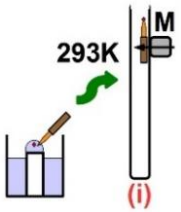
Exploration of conformation sub-states (some case)

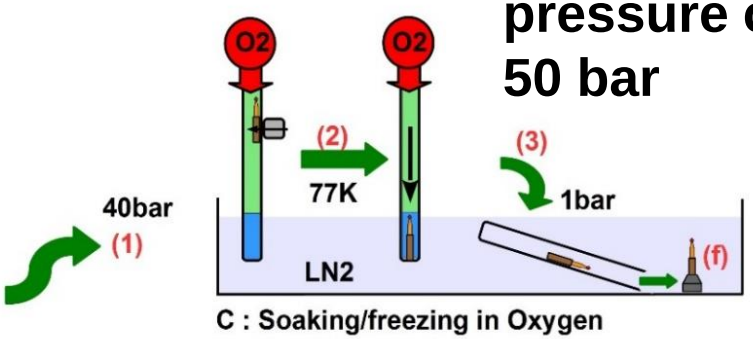
- Structural changes few and localized (surface)
- Structures HP-freezing isomorphous with AP -
- Method applicable to all projects
- Avoid search of cryoprotection conditions

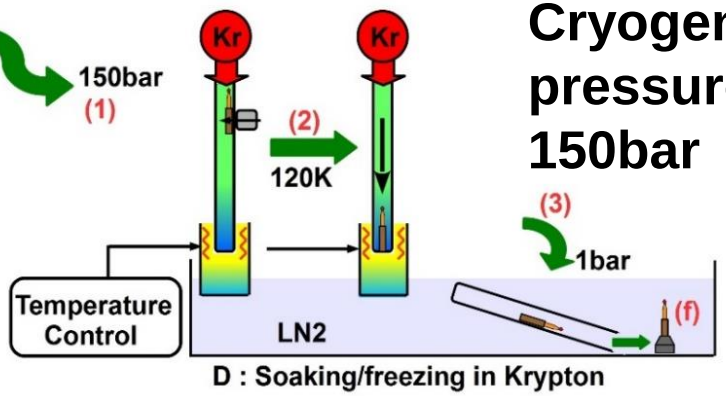
P. van der Linden *et al.*, Journal of Applied Crystallography 2014

FREEZE BIO-XTALS IN PRESSURIZED O_2 AND KR GASES









Cryogenic O_2 pressure cell 50 bar

Cryogenic Kr pressure cell 150bar

(I) cryogenic oxygen pressure cell (P. van der Linden and A. Royant)

- System dedicated to proteins requiring O2 as a cofactor or substrate
Myoglobin, Hemoglobin, Cytochrome P450, oxidase , photosensitizers ....
- Reveal Oxygen sites of affinity in oxygen sensitive proteins

(II) cryogenic noble gas pressure cell (P. van der Linden)

- Search of pores, channels and cavities in proteins
- Production of efficient derivative of crystals for phasing

HIGHER PRESSURES BY CRYO-PUMPING

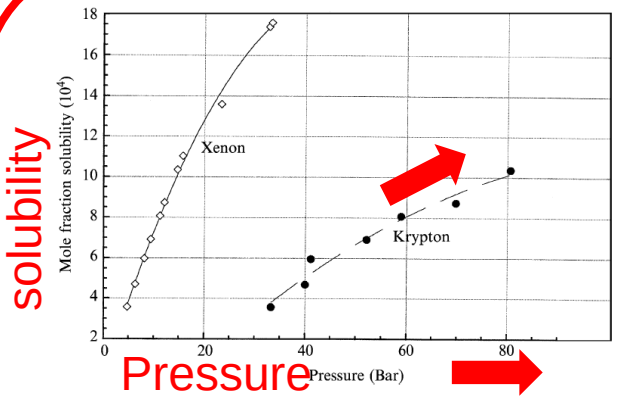


FIG. 3. Solubilities of noble gases. The plot represents the concentration (in mole fraction) of xenon and krypton in pure water as a function of their partial pressure. Data computed from Ref. 61.

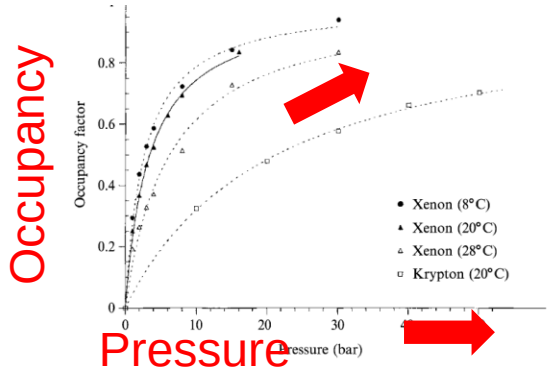
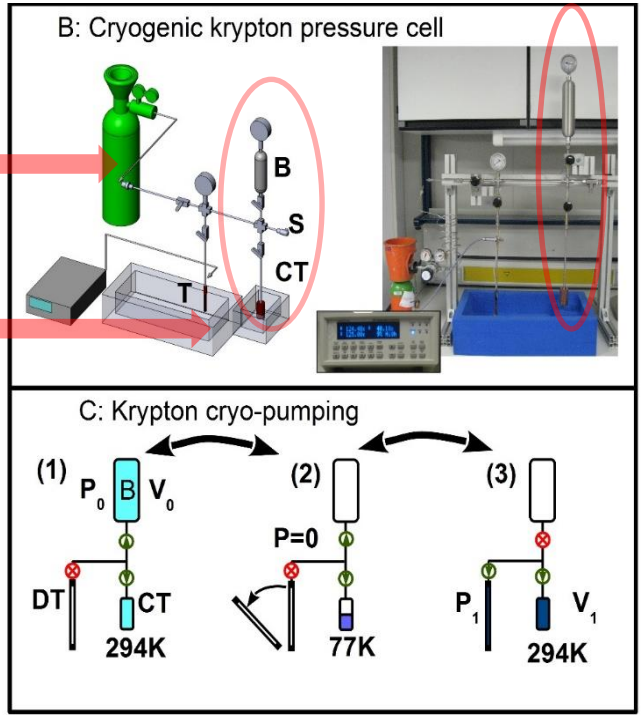


FIG. 11. Isotherms of xenon and krypton binding to porcine pancreatic elastase at temperatures of 281.15 K (8°), 293.15 K (20°), and 301.15 K (28°). The occupancies of the noble gas atoms at the various pressure points were determined from X-ray diffraction experiments. Langmuir isotherms were fitted to the experimental data points by a least-squares procedure.

Schiltz M. *et al.*, Methods Enz. 2003

Cylinder 30bar

Sample 150bar



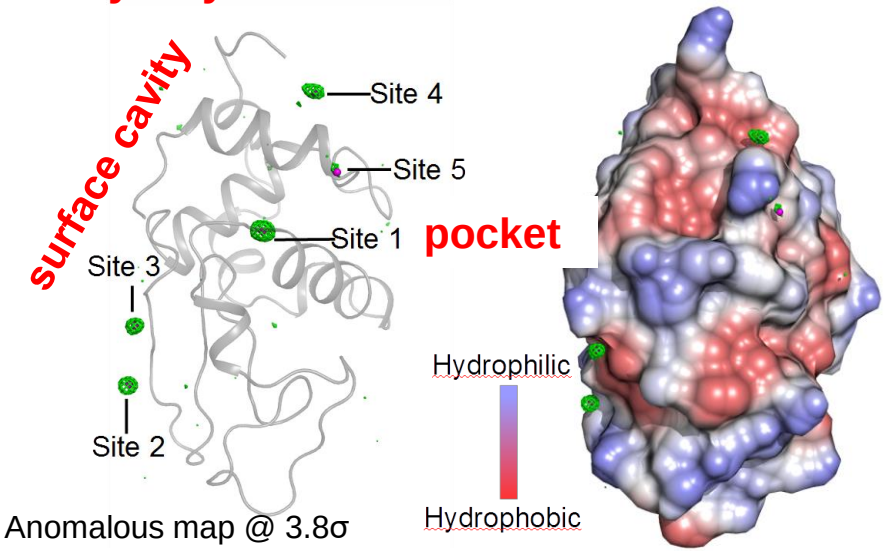
Possible gases of biological interest

Gas	T point T(K)	K_{henry}(mM/bar)
O_2	54.4	1.3
CO	68.2	0.9
Ar	83.8	1.4
CH_4	90.5	1.3
C_2H_4	103.2	4.9
NO	109.5	9.3
Kr	115.8	2.5

Higher pressures, higher occupancies, sample frozen under pressure at the equilibrium

ASSESSMENT OF THE CRYOGENIC KR-CELL USING TEST CRYSTALS

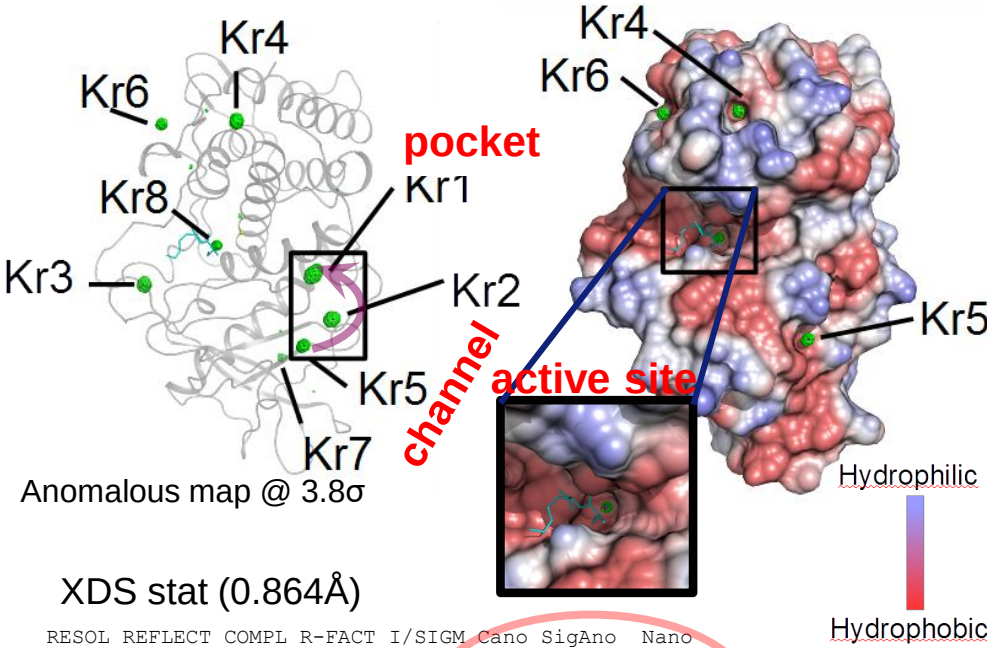
- **Lysozyme 100bar of Kr**



XDS stat (0.864Å)

RESOL	REFLECT	COMPL	R-FACT	I/SIGMA	CAno	SigAno	Nano
3.59	36495	99.9%	3.5%	79.29	**82* 2.258**		**1079**
1.20	154904	99.4%	53.9%	4.65	0	0.766	5388
total	964801	99.9%	5.9%	24.66	**21* 1.066**		**32827**

- **Thermolysin 100bar of Kr**



XDS stat (0.864Å)

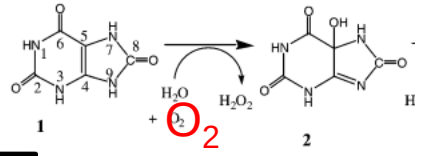
RESOL	REFLECT	COMPL	R-FACT	I/SIGM	Cano	SigAno	Nano
5.06	45271	99.8%	4.4%	56.24	**82* 2.514**		**1029**
1.70	182315	98.1%	64.5%	5.82	21*	0.920	5144
total	1189266	99.7%	10.9%	24.26	**32* 1.158**		**31878**

	thermolysin ID23-1	lysozyme ID29
Space group	$P6_122$	$P4_32_12$
Resol (Å)	1.70	1.20
λ (Å)	0.864/0.866	0.864/0.866
U cell (Å/°)	93.0 93.0 129.1 90 90 120	79.0 79.0 37.1 90 90 90
R/Rfree(%)	15.9/18.0	12.9/15.3
Nb sites Occ	**8 Kr** 1.00, 0.55, 0.34, 0.20, 0.28, 0.24, 0.22, 0.23	**5 Kr** 0.33, 0.25, 0.25, 0.20, 0.25
Lit, PDB	**1QTK: 1 Kr, 1C10 : 2 Xe**	**3LS7: 1 Xe**

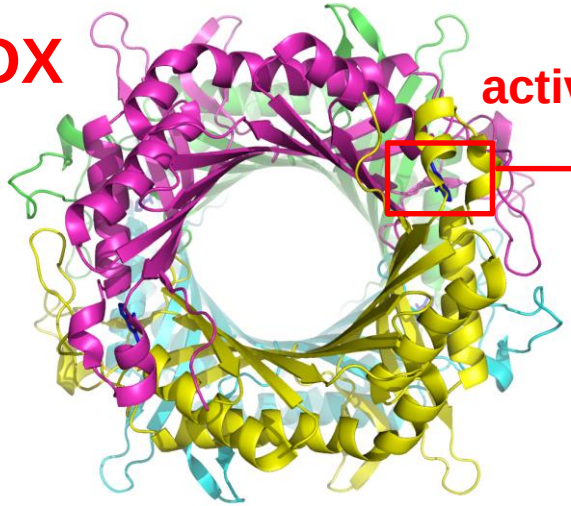
- **Phasing, kr anomalous signal @ k-edge**
- **Revealing pores and channels**
- **Labelling active sites**
- **Probing hydrophobicity (surface cavities)**

ASSESSMENT OF THE CRYOGENIC O2-CELL USING TEST CRYSTALS

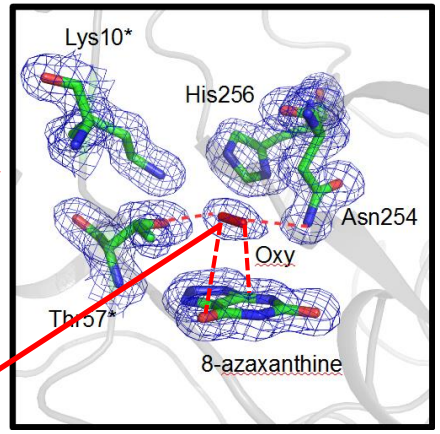
N. Colloc'h at al. Biophys. J. 2008
UOX catalyzes oxidation of uric acid to 5-hydroxyisourate



UOX



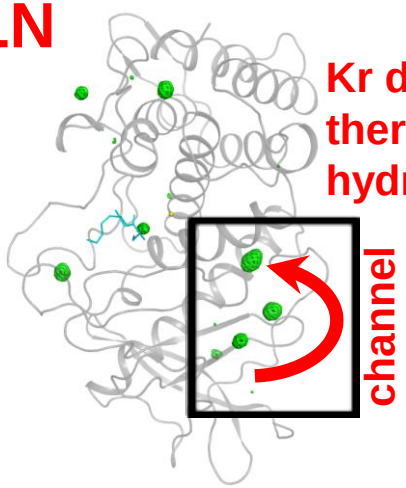
active site



O2 , 42bar
Omit map @ 1σ
Resol. 1.4 Å

O2 location, elements of UOX, mechanism

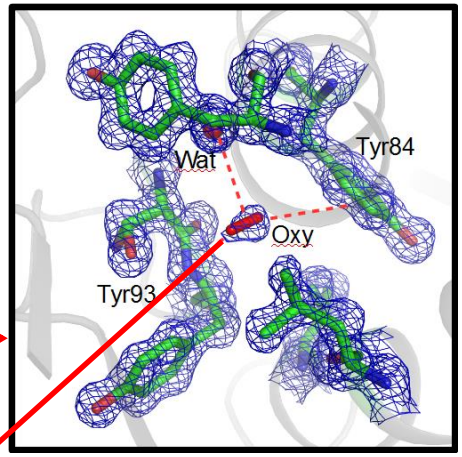
TLN



Kr derivative thermolysin internal hydrophobic pocket

channel

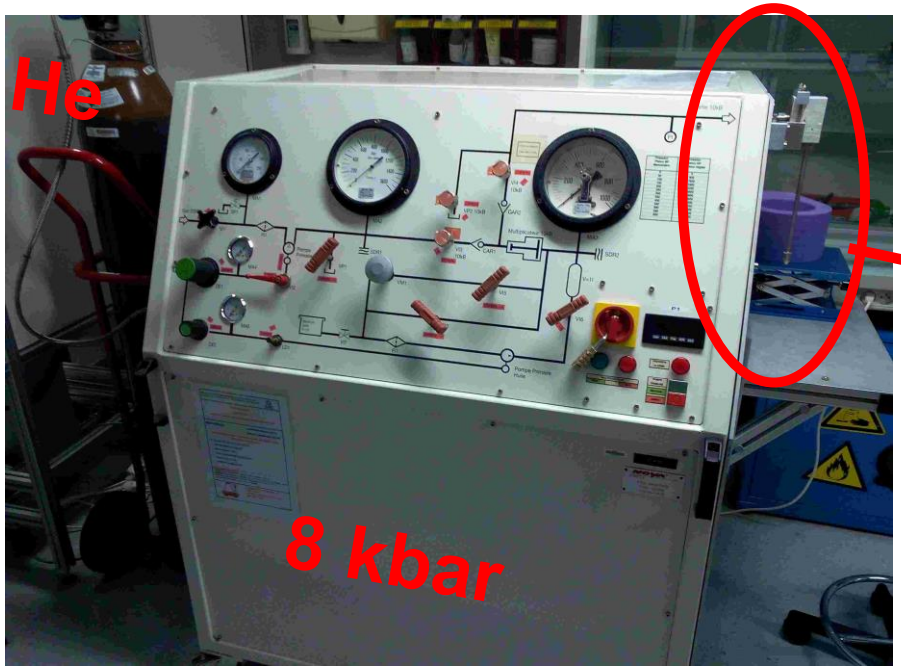
TLN frozen
42 bar O_2

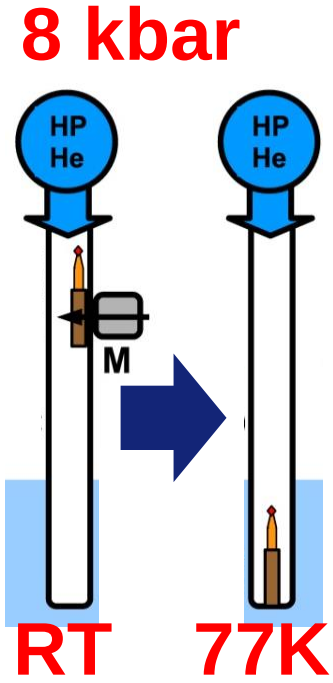


O2 , 42bar
Omit map @ 1σ
Resol. 1.2 Å

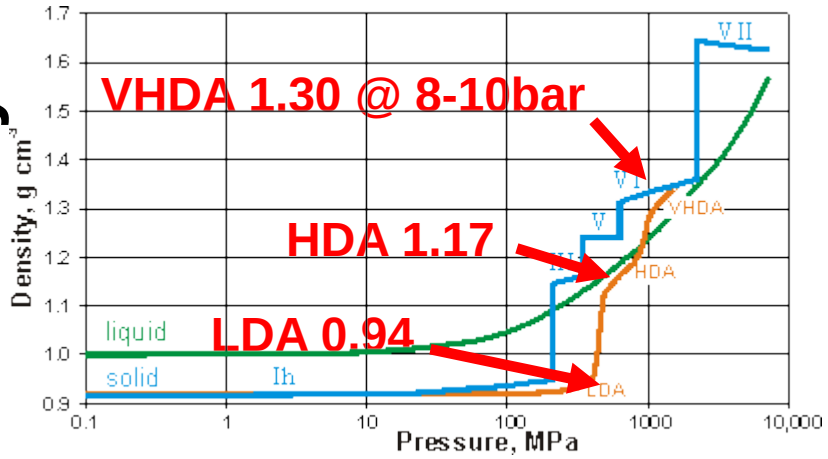
O_2 bind in the TLN internal hydrophobic pocket
(small molecules as phenol, isopropanol, acetone, function?)

“VERY” HIGH PRESSURE FREEZING



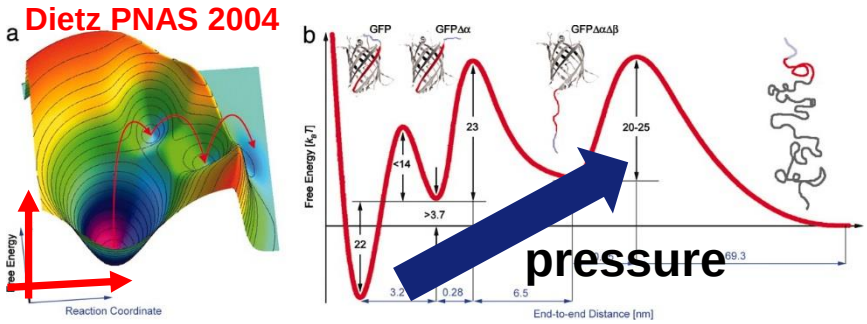


density



Pressure

Dietz PNAS 2004

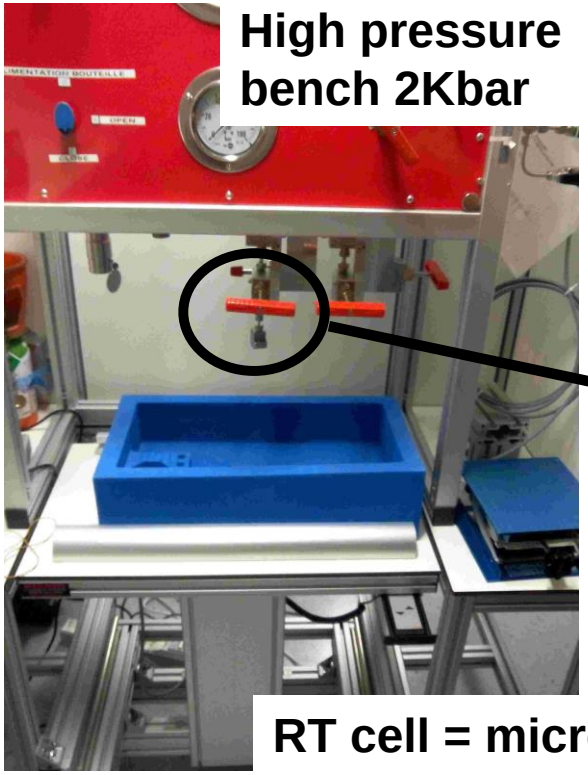


“Very” pressure cooling @ 8 kbar:

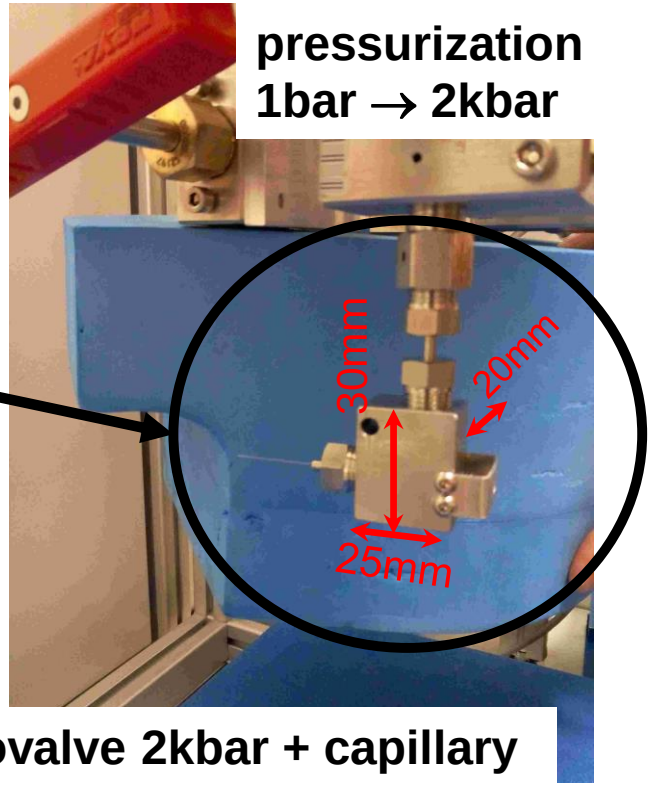
- Protein frozen in VHDA ice matrix ?
- Larger exploration of energy landscapes
- Protein unfolding ?

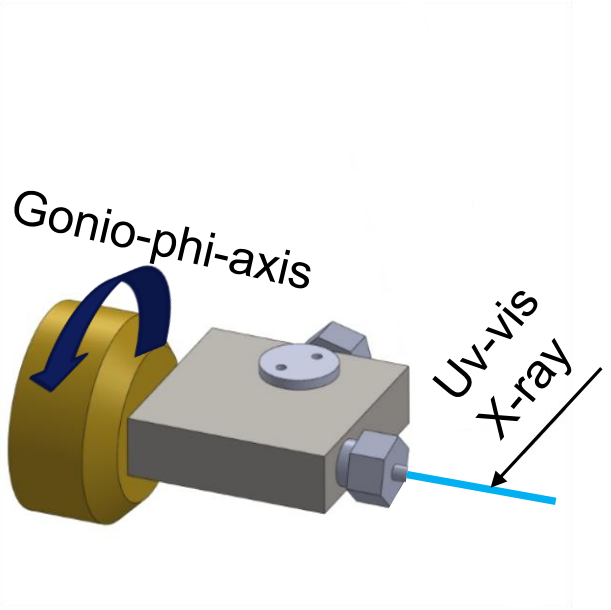
RT PRESSURE CELL FOR MX EXPERIMENTS

High pressure bench 2Kbar



pressurization 1bar → 2kbar





RT cell = microvalve 2kbar + capillary

RT pressure cell :

- **Biological solutions/crystals**
- **High pressure (Cte ~ 2kbar), Room temperature**
- **X-ray diffraction**
- **UV/vis spectroscopy**

- **HP-Freezing in the laboratory of ID23 in User mode**
- **Freezing session for a mx experiment (2 weeks before mail @ Local contact or @ D. Flot)**

Acknowledgements

- **HP-Freezing:** van der Linden P., Dobias F., Vitoux H., Kapp U., Jacobs J., Mueller-Dieckmann C., Leonard G.

- **Oxygen/krypton cryo-cell**: Mueller-Dieckmann C., Leonard G., Giraud T., Dobias F., Royant A., van der Linden P.

- **PhD Thesis:** Lafumat B.

- **All the SB-Group**

ESRF
The European Synchrotron

And thanks to the audience for its attention !