

High Pressure Freezing

Philippe Carpentier,



- The system
- The method
- Some typical examples

ESRF Users Meeting 2015: Meeting of MX BAG Representatives and Beamline Staff,
9th February 2015

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INTRODUCTION

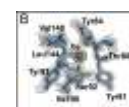
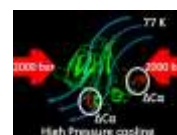


P. van der Linden
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Based on the original concept demonstrated by
S. M. Gruner's group (CHESS, Ithaca, USA)
many references, Kim C.U. *et al Acta Cryst. D* (2005).

High pressure cryo-cooling of biological crystals under 2000 bar He:

- Cooling protein crystals avoiding cryo-protection
- Improvement of crystalline quality (HDA-ice matrix)
- Exploration of protein conformational sub-states
- Production of noble gas derivatives
- "user-friendly" system, Spine standard and sample changer compatibility

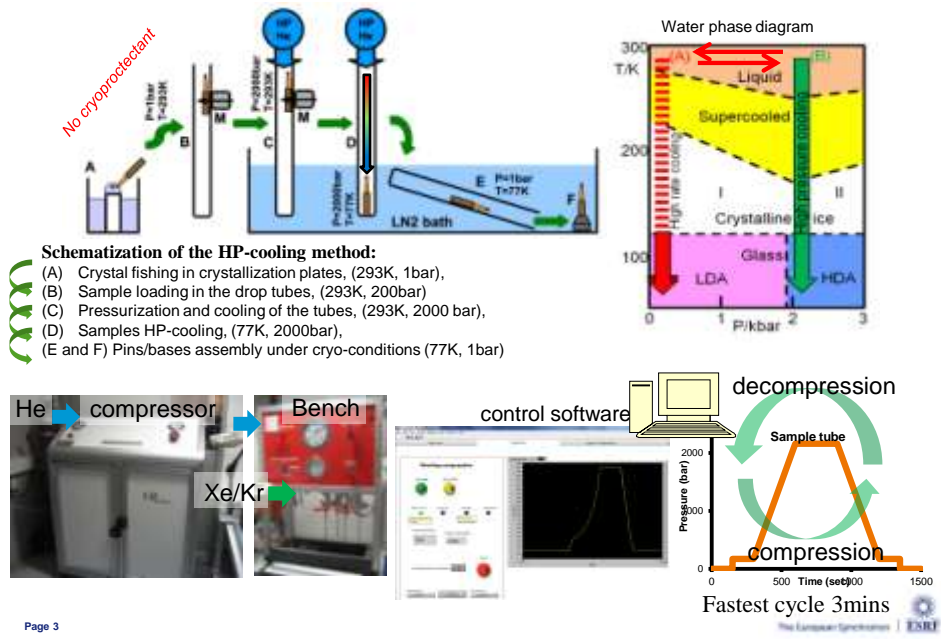


HP-freezing service is offered to users in combination with MX experiments since April 2014 (20 sessions > 1000 samples cooled).

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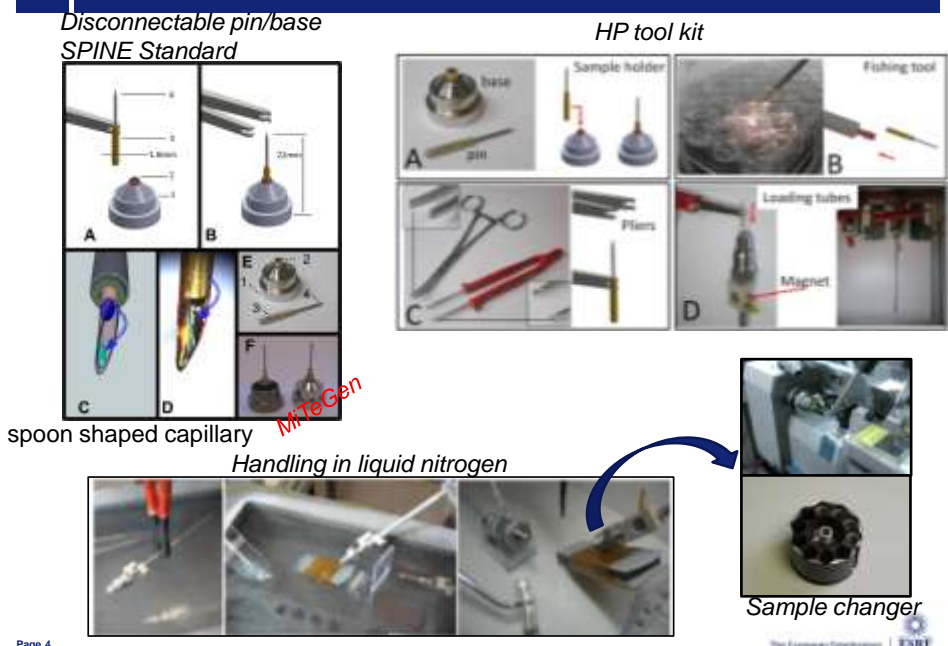


HIGH PRESSURE COOLING SYSTEM AND METHOD



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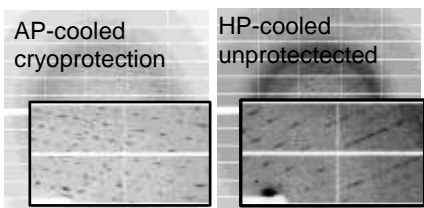
SAMPLE HOLDER AND HANDLING TOOLS



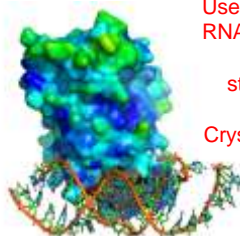
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HIGH PRESSURE COOLING, APPLICATIONS

(1) Cryoprotection problem, improvement of crystal quality

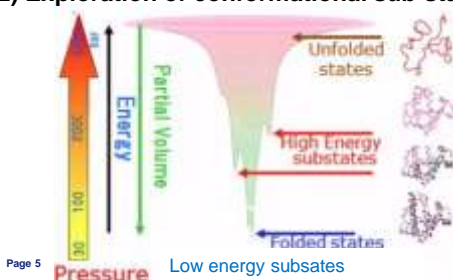


- space group $P2_1?$
- Resolution ~ 3Å
- Mosaic/broken, twin
- space group $P2_12_12_1$,
- Resolution ~ 2.5Å
- Lower mosaicity



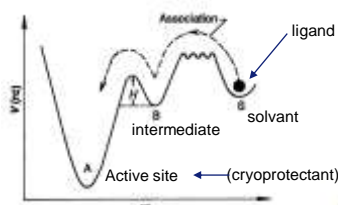
User challenging project
 RNA-methyltransferase:
 RNA complex
 structure solved by
 High Pressure
 Crystallography @ 2.5Å

(2) Exploration of conformational sub-states



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(3) Studies of reaction, ligand binding intermediates

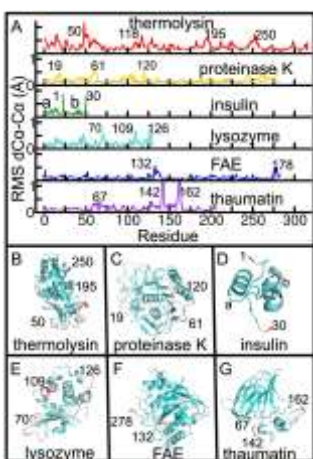


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ASSESSMENT OF THE SYSTEM WITH TEST CRYSTALS

Gallery of HP-cooled protein crystals.

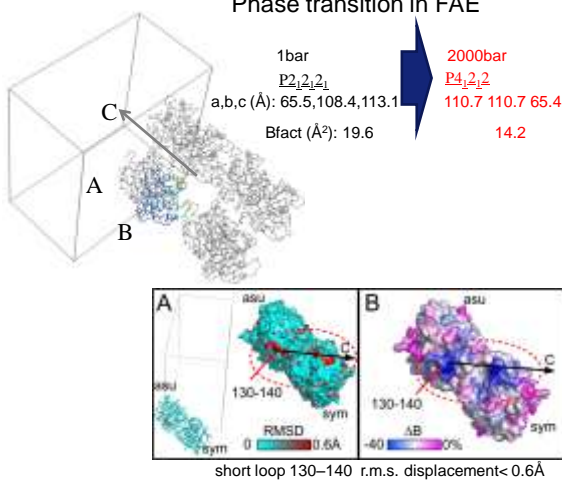
C α backbone displacements
 and 3d representation of structural changes



Structural changes are few and localized
 in loops at the surface, structured parts are stable

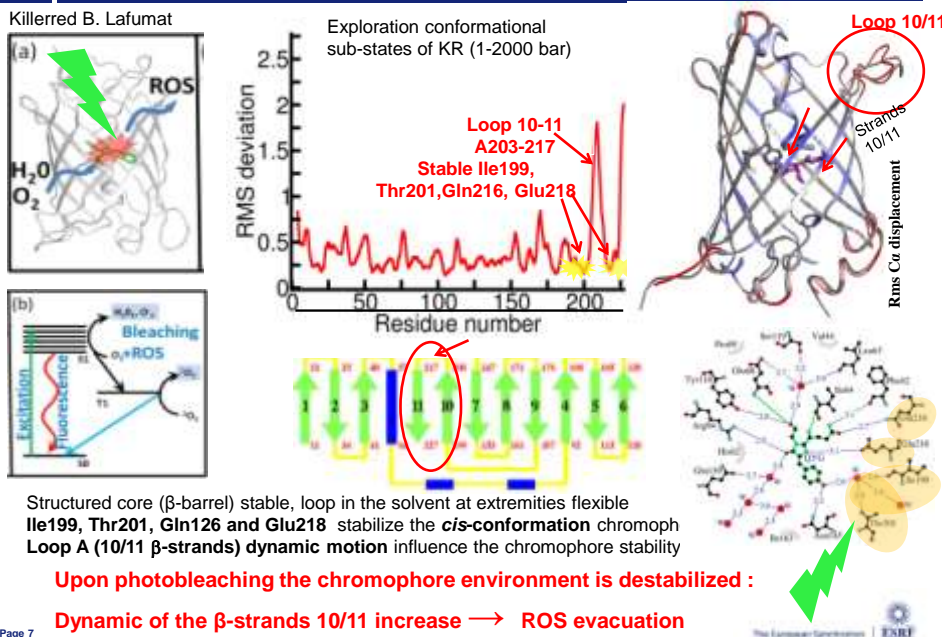
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Phase transition in FAE



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HP INDUCED STRUCTURAL MODIFICATIONS IN KILLERED

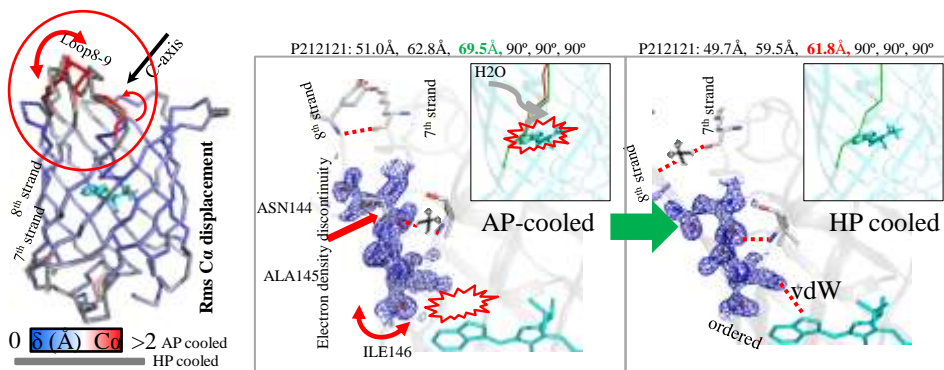


HIGH PRESSURE CONFORMATION TRANSITION STATE IN THE B-BARREL OF FP CERULEAN



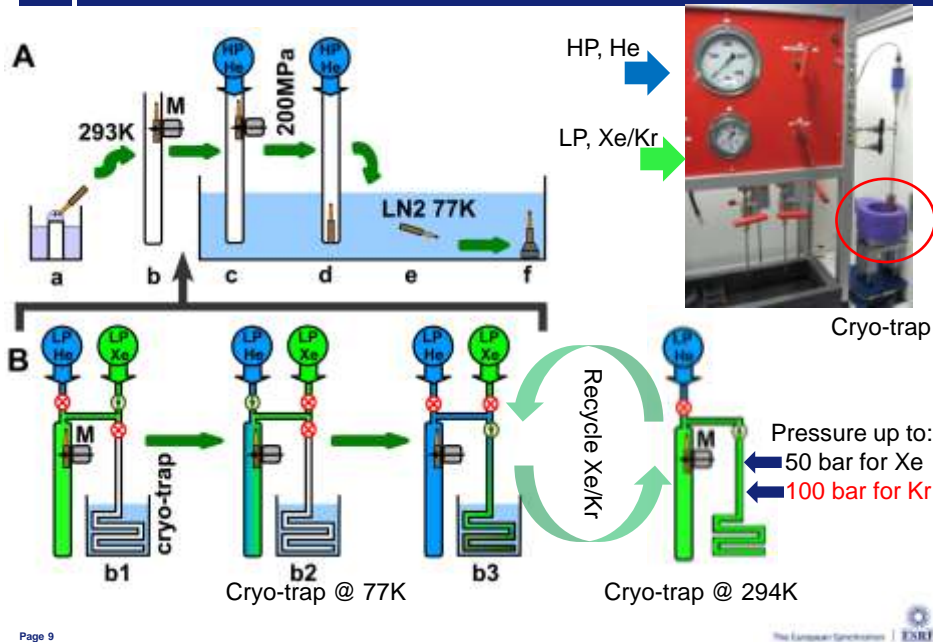
G. Gotthard
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IBS, ESRF

Cerulean, a bright cyan fluorescent protein, 11 β -strands with Trp-base chromophore, used for FRET studies in couple with YFP.



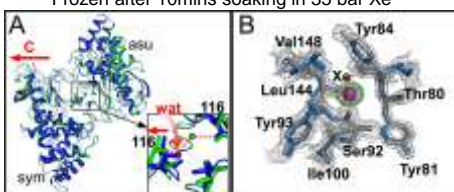
- 7th strand disorder in cerulean quenches the fluorescence (QY=44%)
- Pressure allows studying the order/disorder state and flexibility of the strand 7
- Stabilization by the mutation H148D in the following generation of super bright Cyan FPs as mTurquoise (QY=93%) ...

SOAK AND FREEZE CRYSTALS IN PRESSURIZED Kr/Xe, METHOD



THERMOLYSIN AND LYSOZYME NOBLE GAS DERIVATIVES

Assessment of the system with Xe in Thermolysin
 Frozen after 10mins soaking in 35 bar Xe



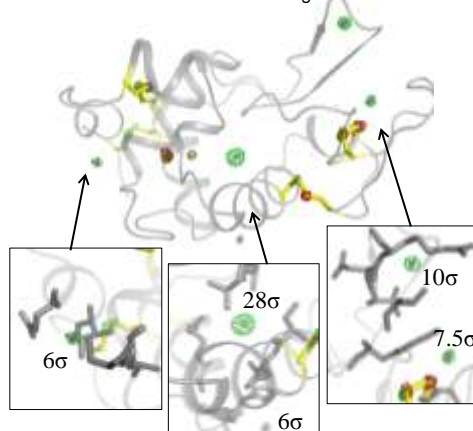
2Fo-Fc Map 1.2σ ILE100
 anomalous map +/-4σ, max 34σ (Xe 5.1e- @ 10keV)

comparison HP/AP-cooled

Statistics	Thermolysin Xe HP	Thermolysin Xe AP 3LS7
Source (Å)	ESRF ID14-4 (1.24Å)	Rot Anode (1.54Å)
Resolution (Å)	1.75	1.98
Unit cell (Å ³)	93.7 93.7 141.6	93.1 93.1 130.2
P6(1)22	90.0 90.0 120.0	90.0 90.0 120.0
Solvent (%)	51.3%	46.3%
Xe non occ.	0.8	0.8
Rms HP/AP		0.284

- Reduce number of manipulations to produce the Xe derivative and preserve the quality of the crystal

with Kr in Lysozyme
 Mizuno N, et al. J Synchrotron Radiat 2013 (4 Xenon sites)
 Frozen after 10mins soaking in 60 bar Kr



anomalous map 4σ (Kr 4e- @ 14.4keV)
 anomalous map 4σ (Kr 0.4e- @ 14.3keV)

- 1 main site confirmed in the internal hydrophobic cavity
 - 4 secondary sites of low affinity in the fold at the surface

CONCLUSION, CURRENT STATUS

- System, method and reliability have been validated using test crystals
van der Linden et al. J Appl. Cryst. 2014
- User Mode since April 2014 with an automatic compressor (>1000 samples cooled)
- No cryoprotectantion
- Possible improvement of crystalline quality for problematic cryoprotection
- Exploration of conformational sub-states
- Possibility to produce efficient noble gas derivatives
- Limitation for very small crystals in capillaries (< 30µm)
- **HP-freezing service open to users in combination with MX experiments for dedicated projects, possibility of cooling 30 samples, contact: philippe.carpentier@esrf.fr**

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ACKNOWLEDGEMENTS



**Pressure High pressure protein crystallography
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Mueller-Dieckmann C. ESRF-SB



Leonard G. ESRF-SB

And thank to the audience for its attention !

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