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Pressure and high-pressure macromolecular crystallography at the ESRF

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Almost all diffraction experiments in structural biology are done at cryogenic temperature to mitigate radiation damage with cryoprotected crystals to avoid solvent crystallization and its harmful consequences: formation of ice rings, loss of diffraction and exaggeration of mosaicities. Crystallographers often protect crystals with glycerol rather than with genuine conditions whose search is time consuming. Moreover, cryo-protection has detrimental consequences: Soaking somewhat destabilizes crystals, mosaicity expend upon cooling, cryo-agents may interact with macromolecules and in extreme cases, cryo-conditions are destructive. High pressure (HP) cooling is an alternative method which consists of flash-cooling cryoprotectant-free samples under 200 MPa of Helium [1]. The solvent is directly turned into high density amorphous (HDA) ice avoiding water crystallization and preserving/improving the sample quality owing to the absence of cryo-agents and to HAD-ice properties. We have developed a HP-cooling system and its associated methodology paying attention to user-friendliness and throughput aspects through a new pluggable Spine compatible base-pin and to its handling-toolkit [2]. The machine allows vitrifying solution with 5% glycerol for cooling diluted bio-objects, but importantly, it is very effective for cryoprotectant-free crystals since the sole mother liquor components act as anti-freeze agents. Its capability was demonstrated with test crystals and resulting structures appear isomorphous to those deposited in the PDB since the structural changes are limited to flexible loops. Nevertheless, pressure is a key thermodynamic variable which produces structural modifications associated with different conformational protein substates or reaction coordinates [3], and we provide examples of HP structures which address biological questions. On the basis of the HP-cooling technique, we have designed a novel of pressure cell for gases of biological interest. The method takes advantage of thermodynamic properties which allow liquefying pressurized gases; crystals are soaked in the gas phase prior to be flash-frozen in the liquid phase without pressure relaxation. This technique has been applied to noble gas derivatives and oxygen sensitive macromolecules.

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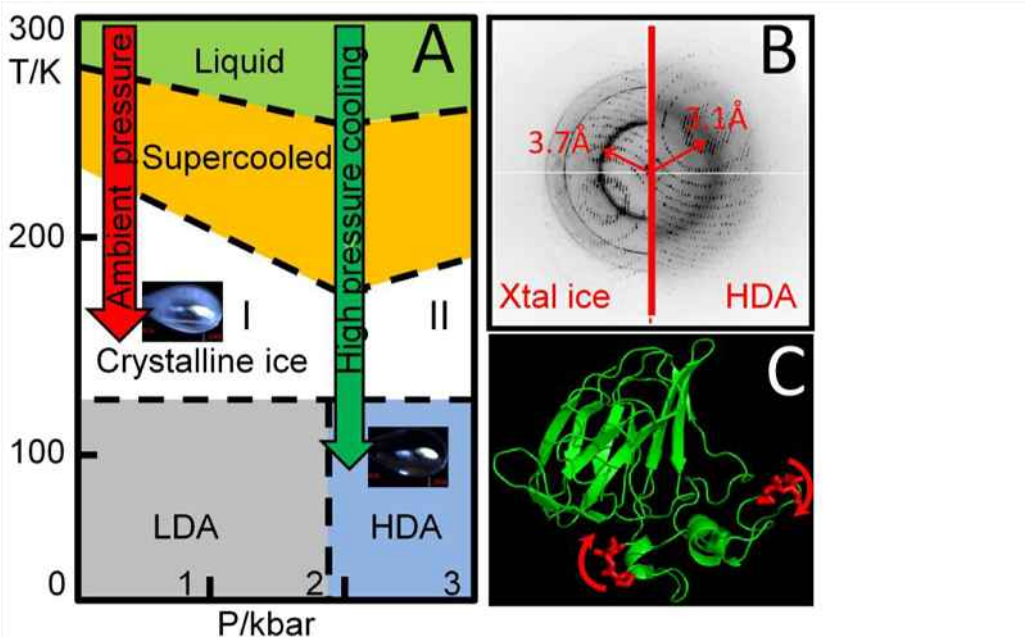

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