From past to future: FIP's contribution to the use of lanthanide complexes and high pressure in macromolecular crystallography.

E. Girard[1], N. Colloc'h[2] et O. Maury[3]

[1]IBS, Univ. Grenoble Alpes CEA CNRS, Grenoble, France
[2]ISTCT CNRS Univ. Caen-Normandie, Caen, France
[3]Univ. Lyon, ENS de Lyon, CNRS UMR 5182, Laboratoire de Chimie, France.
Courriel : eric.girard@ibs.fr

In this talk, I will first recall briefly BM30A/BM07's long-standing contribution to the development of the use of lanthanide complexes (Girard, Stelter, Vicat *et al.*, 2003; Girard, Stelter, Anelli *et al.*, 2003), both for the exploitation of anomalous scattering to solve the phase problem (before the advent of artificial intelligence-based tools) and for protein crystallization thanks to the crystallophore, a unique tool combining phasing, nucleating and imaging properties (Engilberge *et al.*, 2017, 2019; Sauter *et al.*, 2024).
Then I will show the interest to use pressure perturbation to derive structural information on biological molecules. Indeed, the effect of high hydrostatic pressure (HP) on a system is directly linked to volume changes of the considered system. In the case of proteins, changes in volume are associated with biological activity. As a consequence pressure is an ideal tool to explore the conformational landscape of proteins, since it allows to increase the population of high-energy states functionally relevant, but rare at ambient pressure. Combined to Macromolecular Crystallography (HPMX), pressure perturbation is an ideal tool to study with a high precision excited states of proteins (Fourme *et al.*, 2012; Dhaussy & Girard, 2015; Colloc'h *et al.*, 2023). I will illustrate this with the example of the Ras protein. Ras in an oncogenic protein involved in a large number of cancers. However, the development of efficient inhibitors of Ras is still challenging, since Ras proteins possess multiple conformational states. Using HPMX, we have been able to induce an in-crystallo phase transition, allowing a precise description of the different segments of Ras which adopt transient intermediates states corresponding to conformers interacting with different regulators or effectors (Girard *et al.*, 2022). Moreover, high pressure has driven Ras toward an excited state where an inhibitor targeted for this rare but functionally important state can bind, allowing for a precise description of its binding site (Girard *et al.*, 2024). Finally, I will present future developments planned on BM07 to democratize the use of HPMX.

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