

**FRAGMENT-BASED DRUG DISCOVERY ON LACTATE DEHYDROGENASE  
CRYSTALS GROWN IN PRESENCE OF CRYSTALLOPHORE.**

A.Y. ROBIN <sup>1,2</sup>, D. MADERN <sup>2</sup>, A. ROUX <sup>3,4</sup>, C. CHAPELLE <sup>4</sup>, G. COSTE <sup>5</sup>, D. ROCHE <sup>5</sup>, F. RIOBE <sup>3</sup>, O. MAURY <sup>3</sup>, J.A. MARQUEZ <sup>1,6</sup>, E. GIRARD <sup>2</sup>

<sup>1</sup>EMBL - Grenoble (France), <sup>2</sup>IBS - Grenoble (France), <sup>3</sup>ENS - Lyon (France), <sup>4</sup>Polyvalan - Lyon (France), <sup>5</sup>EDELIRIS - Lyon (France), <sup>6</sup>ALPX - Grenoble (France)

Fragment-based drug design discovery by X-ray crystallography requires to produce large numbers of crystals for testing libraries of up to 1.000 different fragments. Robust crystallization systems can be laborious to establish with poor crystallization rate in a given crystallization condition.

Crystallophore molecules (lanthanide complexes from Polyvalan, [www.crystallophore.fr](http://www.crystallophore.fr)) are all-in-one nucleating and phasing agents. Furthermore, they increase the crystallization space of a given crystallization condition with, for instance, crystallization over a wider precipitant concentration range. [1] The latest property can be an advantage to build a robust crystallization system. We thus tested the reproducibility of protein crystals obtained from co-crystallization with crystallophore molecules at the HTX laboratory (High-Throughput Crystallization, EMBL, Grenoble), a highly automated crystallization and crystallography facility essential to perform large-scale fragment screening. [2]

We then performed a complete fragment-based drug discovery project to find allosteric inhibitors to a lactate dehydrogenase of unknown structure. After identification of crystallization hits in the presence of crystallophore molecules and crystal growth optimization, the novel structure of the lactate dehydrogenase could be phased using the anomalous signal from the lanthanide complex. A reproducible crystallization system was subsequently established and validated before the phase of fragment screening. The robustness of the crystallophore induced-crystallization condition allows us to successfully run a 500 fragments screening campaign.

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[1] Engilberge et al, Crystallophore: a versatile lanthanide complex for protein crystallography combining nucleating effects, phasing properties, and luminescence, *Chem.Sci.*, 2017, 8, 5909.

[2] Cornaciu et al, The automated crystallography pipelines at the EMBL HTX Facility in Grenoble, *J. Vis. Exp.*, 2021, 172, e62491.