Posters - Automation in Crystallization

P4-1

CRYSTALLOGRAPHY PLATFORM AT THE PASTEUR INSTITUTE

P. Weber, C. Pissis, R. Navaza, F. Saul and A. Haouz

Institut Pasteur, CNRS-UMR 3528, 25-28, rue du Dr Roux 75724 Paris, France
pf6@pasteur.fr

The goal of the crystallography platform is to provide research teams working in the field of macromolecular crystallography at Institut Pasteur with the expertise and technology for high throughput crystallization, X-ray diffraction measurements, and crystallographic computing as a core facility. Our second mission is to offer expertise in bio-crystallography, from crystallization of selected targets to resolution of 3D crystal structures by participating as a partner in research projects involving structural studies of single or protein complexes. These projects arise from direct collaboration with research groups at Institut Pasteur and outside organisations.

Depending on the expertise of the users, three options can be offered: service provision, instrument allocation, and scientific collaboration. Service provision, which corresponds to the automated crystallization experiments performed in standard conditions, is the option used by the crystallographers. If initial crystallization trials are successful, the platform assists users to reproduce and optimize the crystallization conditions in order to obtain suitable crystals for X-ray data collection.

Since 2010, the platform has been involved in more than 24 scientific collaborations in association with 14 research units from 8 scientific departments of the Institut Pasteur and 6 laboratories from other institutions (French or foreign), leading to our co-authorship of 32 peer-reviewed publications. These projects cover many disciplines related to infectious diseases and human health, including defence mechanisms against pathogens, antibiotic resistance, regulation pathways, genetic disorders and drug design.

In our poster, we present the instrumentation and robotics available in the platform and a summary of results obtained during the last five years.

P4-2

MICROSEED MATRIX SCREENING: MORE HITS IN LESS TIME

Moroz O.V.[1], Blagova E.B.[1], Friis E.P. [2], Davies G.J.[1] and Wilson K.S.[1]

[1]Department of Chemistry, York Structural Biology Laboratory, University of York, York, UK
[2]Novozymes A/S, Bagsvaerd, Denmark;

Microseed matrix screening (MMS) is an approach in macromolecular crystallisation where crushed crystals from one hit, often poor, are used as nucleation points in conditions different from the initial ones. The technique was introduced by Ireton & Stoddard in 2004 [1] and subsequently automated by D'Arcy *et al.* [2]. The rationale behind this approach is in separating nucleation from crystal growth, leading to new hits and better diffracting crystals. A number of groups have reported success in using the technique, with results not only from self-seeding (with crushed crystals of the same protein), but also cross seeding with a homologous protein, as well as seeding of a complex with crystals of one of the components [3,4,5]. The approach was recently extended to crystallisation of membrane proteins, using lipid cubic phase seed stock [6].

We present here examples of successful application of MMS in crystallisation of a number of industrially important enzymes: proteases, lipases and amylases. In our experience, MMS often increases the number of hits, improves the quality of crystals and shortens the time required for growth of diffraction quality crystals.

1. Ireton G.C.& Stoddard B.L. *Acta Cryst*. **D**60, 601-5 (2004).
2. D'Arcy A, Villard F, & Marsh M. *Acta Cryst*. **D**63, 550-4 (2007).
3. Obmolova G, et al., *Acta Cryst.* **D**66, 927-33 (2010).
4. Shaw Stewart P.D., et al., *Cryst. Growth Des*. **11**, 3432–3441 (2011).
5. D'Arcy A. et al., *Acta Cryst.* **F**70, 1117-1126 (2014).
6. Kolek S.A. et al., *Acta Cryst.* **F**72, 307-312 (2016).