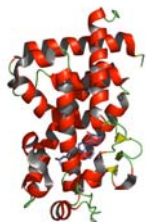


Structural Genomics of human targets: Crystallization strategies at SBGP

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Human VDR bound to the vitamin D⁽¹⁾

The Structural Biology and Genomics Department is involved in a structural genomics program whose goal is solving crystal structure of protein families members, related to human health, alone or in complex with ligands and protein partners. With this aim, the Department has implemented a Platform (Structural Biology and Genomics Platform – SBGP) consisting of different interconnected modules (cloning/mini-expression, production and crystallization). We have developed automated methods, emphasising their flexibility, in order to adapt each step to project requirements. Here we present a global view of procedures we have established to tackle crystallization of protein samples.

Pure protein sample

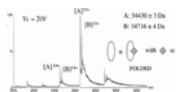


Characterization :

Mass spectrometry

DLS

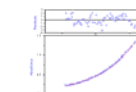
Analytical ultracentrifugation



Nuclear receptor domain



Nuclear receptor



General transcription factor

Preliminary tests : Optimum Solubility Screen⁽²⁾ and Pre-Crystallization Test

to determine the suitable buffer and protein concentration for crystallization



Vapour Diffusion (sitting drops)

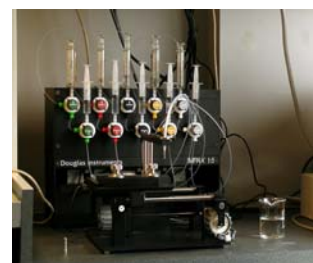


Tecan Genesis Workstation

Screening

9 Commercial screens
3 Home-made screens
Total of more than 1000 conditions

Micro-batch



Douglas Impax I-5

Optimization

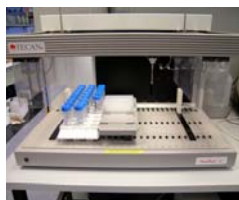
Screen refinement

From a collection of over 100 stock solutions (regularly checked)



On the robot (sitting drops):

- Different plates and conditions
- Additives screens
- Crystallization in gels



Tecan Miniprep

Micro-batch (IMPAX):

- 2D interpolation (phase diagram determination)

The **Leica Visualization System** for image capture of crystallization trials using SBS plates

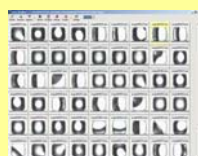


Image gallery



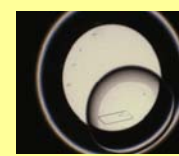
Sitting drop



Crystallization in gels



Hanging drop



Micro-batch

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(1) Rochel et al. (2000) *Mol Cell.* 5 : 173-9. (2) Jancarik et al. (2004) *Acta Cryst.* D60 : 1670-3.