Robotic chain for the 2D crystallization of membrane proteins (Cracam)

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1. INTRODUCTION

To understand protein functions at a molecular level, high-resolution structures are an invaluable tool for soluble as well as for membrane proteins. Although many structures can be obtained using X-ray diffraction, electron crystallography has recently proven to yield high quality structures in the field of membrane proteins. The two techniques are similar in some aspects, but the information that they provide are complementary, since the conformation of the protein in 3D and 2D crystals is the result of different environments. The importance of electron crystallography for elucidating the structure of membrane proteins arises from the fact that the environment of the 2D crystals is more similar to the native environment of the protein and, thus, the structure determined by means of 2D crystallography is thought to be closer to the native one.
Obtaining good quality crystals, large enough to yield high-resolution information is a bottleneck in the structural determination by electron microscopy on 2D crystals. Whereas 3D crystallization robots are very popular and widely used, automatic systems available to perform 2D crystallization trials are much rarer. Usually, a large number of parameters have to be tested to find conditions in which the protein forms 2D ordered arrays and to optimize them. We are designing a robotic chain which allows to test many different conditions for the 2D crystallisation of membrane proteins.

2. RESULTS

2.1 Experimental conditions

We are designing a robotic chain for the 2D crystallization of membrane proteins (Cracam). This device will allow the 2D Crystallisation of membrane proteins in the bulk of the solution; the 2D crystallisation of soluble and membrane proteins at the air-water interface on a lipid monolayer; the 2D Crystallisation on a solid surface and in addition, the adsorption of single particles on a lipid monolayer for single particle image analysis

Starting with the compact robotic workstation from Hamilton: MICROLAB® STAR Line liquid handling platform, we are customizing this workstation so it will be used with success for our purpose.

2.2 Results

We have designed the crystallization wells and developed a protocol to avoid the evaporation of the liquid from the crystallization solution. We have also set up a device to mix the solution and a optical device in order to monitor in real time the growth of the 2D crystallisation.
Using tour fully automated method implemented on a Hamilton workstation, we have already obtained our first 2D crystals of protein crystallised on a lipid monolayer.

In order to complete this robotic chain, we are in the process of developing a device which will transfer automatically the 2D crystals from the crystallization solution to a electron microscope grid.
In addition, we are hoping to identify automatically the presence of 2D crystals transferred onto an electron microscope grid using a software specially implemented for this purpose.

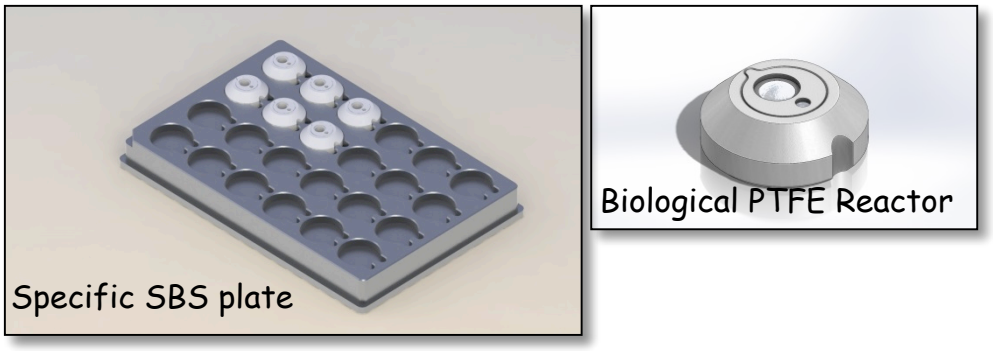

Figure 1. Crystallization through used for the 2D crystallisation at the air-water interface

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