Microdialysis on-chip crystallization of soluble and membrane proteins with the MicroCrys platform and *in situ* X-ray diffraction case studies

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The work presented summarizes the endeavour to develop an automated platform, named MicroCrys, based on two different macro-scale pipelines [1,2] previously designed and operating in our laboratory for the rational optimization of crystal growth by mapping temperature and/or precipitant concentration crystallization phase diagrams. MicroCrys is a miniaturization of these instruments and constitutes the third-generation platform exploiting the advantages of microfluidic technology in combination with precise flow and thermal regulation.
The design and operation of the MicroCrys platform in automating the on-chip dialysis crystallization process via chemical composition and temperature control, facilitating the dynamic screening of crystallization conditions and the exploration of protein phase diagrams will be demonstrated. A custom-built computer software displaying a user-friendly graphical interface has been designed to manipulate all the separate functionalities of the MicroCrys platform including visualization, image acquisition and recording, as well as the fluidic components integrated for mixing, circulating and dynamically exchanging the crystallization solutions in the dialysis chip and a customized metallic support for thermal regulation via the Peltier effect.
Architecture and critical proprieties of microfluidic chips for on-chip protein crystallization with the microdialysis method developed for this purpose [3,4] will also be detailed. Two model soluble proteins, Hen Egg White Lysozyme (HEWL) and Thaumatin from *Thaumatococcus danielli*, were used to exemplify the competence of MicroCrys for growing high-quality protein crystals for *in situ* X-ray diffraction experiments at room temperature. HEWL was also used to validate the compatibility of the dialysis chips with the lipid cubic phase (LCP) crystallization method and the microcrystals were used for room temperature *in situ* synchrotron serial X-ray crystallography (SSX). Finally, SERCA from *Oryctolagus cuniculus* has been used as a case study to demonstrate the feasibility of on-chip microdialysis crystallization of membrane proteins.

References

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