

Microdialysis device to set up kinetic crystallography experiments

A. Bonnardel¹, P. Jacquet², B. Franzetti¹, A. Royant², J. Dupuy², E. Girard^{1*}

¹Institut de Biologie structurale, 71 avenue des martyrs, Grenoble, France (CNRS-UGA-CEA) ²Institut de Biologie Structurale/European Synchrotron Radiation Facility, 71 avenue des martyrs, Grenoble, France (CNRS-UGA-CEA)

Anna.bonnardel@ibs.fr, eric.girard@ibs.fr

The application of time-resolved crystallography to enzyme catalytic processes raises a number of issues in the preparation of experiments [1, 2, 3, 4]. In addition to crystallogenesis, it is necessary to verify the preservation of the enzyme activity in the crystalline form as well as adjusting a number of parameters controlling the reaction kinetics. Indeed, substrate diffusion times within the crystal matrix must be determined as well as the concentrations required to ensure that the different reaction states are captured. The possibility to pre-calibrate most of these parameters at home-laboratory would increase the efficiency of the foreseen kinetic experiments and the access to synchrotron (XFEL) instruments as well as dedicated platforms such as the icOS platform in Grenoble. For this reason, we have developed a 3D-printed support to allow microdialysis with a reduced external volume.

Microdialysis also allows us to check the enzyme activity within the crystal compared to the soluble protein under the same conditions. This tool makes it possible to prepare kinetic crystallography experiments by determining the substrate concentrations and the diffusion time required for the experiment and by taking into account the relationship between the diffusion time and the density of crystallisation solutions. Microdialysis by reducing the required volume is cost effective in particular for precious protein samples and/or expensive substrates. It also allows to multiply the measurement points.

The exploitation of this new tool will be illustrated through several examples such as substrate diffusion for two different enzymes but also pH changes for a fluorescent protein. Ultimately, we will also show that the tool can be exploited to study the activity of a membrane protein complex system that require the physical separation of the different key players in the reaction.

[1] Caramello N. & Royant A. (2024), *Acta Cryst.* **D80**, 60-79.

[2] Henkel A. & Oberthür D. (2024), *Acta Cryst.* **D80**, 563-679

[3] Shoeman R. L., Hartmann E. & Schlichting I. (2023), *Nat Protoc.* **18**, 854-882

[4] Jose M. Martin-Garcia (2021), *Crystals*, **11(5)**, 521

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