Protein-Crystal Multimodal Imaging (ProXMI)

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As the crystallization behavior of a target protein is not usually known beforehand, a broad screening exercise with hundreds of different solution conditions is necessary. To facilitate the process of manually identifying crystals among so many unsuccessful experiments, automatic crystal detection algorithms have been developed[1]. Such methods usually rely on edge detection or contrast to identify crystals. However, amorphous precipitates or the drying skin of crystallization drops can also present apparent straight edges, leading to false positives. Protein-crystal identification is routinely carried out as an essential step in protein structure determination. Many high-throughput instruments are available. They are based on optical microscopy techniques at low magnification over relatively large fields of view to maximize the probability of detecting diffuse microcrystals.

ProXMI is a Passport Project, (call for research and valorization projects of LAPHIA 2014-Round 2), for one year, whose aim is to validate the potential use of multimodal imaging techniques (linear and nonlinear optic combined microscopies) to follow and detect protein crystals growth. Indeed, Second Order Nonlinear optical Imaging of Chiral Crystals (SONICC) is an emerging technique for crystals imaging, characterization and automatic detection based on the fact that Second Harmonic Generation (SHG) signals are generated only by certain ordered assemblies and only in highly intense fields. This project is based additionally on the original combination of SHG/Raman microscopies developed at the "Institut des Sciences Moléculaires" (ISM).[2] The proof of concept is performed on Hen Egg-White Lysozyme (HEWL) which is one of the most study enzyme in structural biology to date (this was the first enzyme to have its three-dimensional structure determined).

This project is a collaboration between two academic labs (ISM and IECB) and the company Explora Nova. The tasks are divided as follow between the different partners:

i. The crystallization process is fully mastered by IECB. Crystals can be obtained in a week using a crystallization conditions based on salt (NaCl) as a precipitant agent.

ii. The detection of the protein crystals thanks to the SHG/Raman microscopies will be performed at the ISM and exploited at Explora Nova. The multimodal images will be interpreted and evaluated by the three partners.

iii. Finally, if SHG/Raman multimodal imaging proves to be helpful in scoring the crystallization screening, a transfer of technology will be processed involving "Aquitaine Science Transfer" (AST). The multimodal imaging should allow Explora Nova to improve his actual system of protein crystal experiment design, imaging & analysis: ***Xtal Focus***.

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[1] Bern, M., Goldberg, D., Stevens, R. C. & Kuhn, P., J. Appl. Cryst. 2004, 37, 279-287; Cumbaa, C. A., Lauricella, A., Fehrman, N., Veatch, C., Collins, R., Luft, J., DeTitta, G. & Jurisica, I., Acta Cryst. 2003, D59, 1619-1627; Rupp, B.; Acc. Chem. Res. 2003, 36, 173-181.

[2] See e.g. V. Rodriguez et al., Chem. Phys. Lett., 2006, 431, 190, M. Dussauze et al., J. Phys. Chem. C 2010, 114 (29), 12754; H. Vigouroux et al., Adv.Func.Mat. 2012, 22, 3985.