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Monitoring the preparation of protein derivative crystals via Raman-microscopy. Alessandro Vergara. *Dept. Chemistry, University of Naples "Federico II".* E-mail: avergara@unina.it

Raman microscopy is having increasing applications into molecular biology. Primary structure features can be analyzed, such as Se-Met labeled protein crystals to be used for MAD crystallography [1] or to check heavy metal incorporation in isomorphous derivative crystals. Secondary structure modification can be clearly identified according to relationships between amide band frequencies and Ramachandran angles [2]. Ligand binding [3] and protein dynamics/reactivity can also be efficiently followed.
The combined Raman and X-ray crystallography approach reinforces the interpretation of biophysical data. In particular, reactivity and dynamics of metal proteins have been investigated. This approach is particularly informative and well developed for hemoproteins [4, 5].

[1] A. Vergara, A. Merlino, E. Pizzo, G. D'Alessio, L. Mazzarella, *Acta Cryst. D.* (2008) D64, 167-171. [2] A. Merlino, F. Sica, A. Zagari, L. Mazzarella, A. Vergara, *Biophys. Chem.* (2008), 137, 24-27. [3] P. R. Carey, *Ann. Rev. Phys. Chem.* 2006, 57, 527-554. [4] L. Vitagliano, A. Vergara, G. Bonomi, A. Merlino, G. Smulevich, B. Howes, G. di Prisco, C. Verde, L. Mazzarella, *J. Am. Chem. Soc.* (2008) 130, 10527-10535. [5] A. Merlino, L. Vitagliano, B. Howes, C. Verde, G. di Prisco, G. Smulevich, F. Sica, A. Vergara, *Biopolymers* (2009), 91(12), 1117-1125.

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Practical variations on the Microseed Matrix Screening (MMS) method in protein crystallisation. Patrick Shaw Stewart, Peter F.M. Baldock, Richard A. Briggs, Stefan A. Kolek. *Opticryst, see http://www.opticryst.org.* E-mail: patrick@douglas.co.uk

Traditionally, microseeding has been used as an optimization step, i.e. seed crystals are dispensed or transferred into variations of the original crystallization hit solution [e.g. 1, 2]. A novel, systematic approach, referred to as the Microseed Matrix Screening (MMS) method, was introduced by Ireton and Stoddard [3]. This method was automated and further improved by D'Arcy et al.[4], who first used seeding with commercial random screening kits. Experience has shown that MMS used in this way not only produces more hits, it also generates better-diffracting crystals because crystals are more likely to grow in the metastable zone [5]. Systematic users of the method report that it gives a useful improvement in about 75% of cases [6].
Douglas Instruments - as a member of the Opticryst consortium - has investigated several variations in the method. In real-life projects, new crystallization conditions are often sought in an effort to reduce twinning, improve diffraction resolution, find crystals with new space groups etc. Moreover, it is desirable to reduce the number of salt crystals that typically arise from combining the hit solution with all of the various solutions in a screen. We therefore investigated suspending the seed crystals in alternative solutions (i.e. replacing of the original hit solution, which D'Arcy et al. used). Solutions investigated included protein solution ("preseeding the protein stock"), ammonium sulphate, PEG, NaCl and ethanol. The original hit solution was found to be slightly more effective, but several other solutions also worked well. Note that these alternative solutions reduce the likelihood of crystallization by an additive effect, so they can be used when the original conditions give poor diffraction (especially PEG or NaCl, which are less likely to give salt crystals). Throughout the project, the statistical significance of experiments carried out was increased by focusing on "pregnant" conditions, which were defined as conditions that reliably gave crystals when seeds were present, but which otherwise did not support crystallization.
The stability of seeds in various solutions at room temperature was also investigated. When using the original hit solution (see above), up to 75% of seeds were lost after 3 hours, and 99% after 24 hours.
Obviously the MMS method has the disadvantage that it cannot be used until at least one hit has been obtained. We therefore investigated nucleation with micro-porous glass [7] and zeolites [8]. These materials were less effective than microseeding, but may nevertheless be useful - because they can of course be used in the absence of previous hits.

[1] Terese Bergfors. *Journal of Structural Biology*, 142 (2003), 66-76. [2] Enrico A. Stura (1999). 'Protein Crystallization', 139-153. [3] Gregory Ireton and Barry Stoddard. *Acta Crystallographica section D60 (2004), 601–605*. [4] Allan D'Arcy, Frederic Villarda, May Marsh. *Acta Crystallographica* section D63 (2007), 550–554. [5] See http://www.douglas.co.uk/mms.htm. [6] Personal communication, Paris Ward, Thomas Malia, Galina Obmolova, Allan D'Arcy and others. [7 N.E. Chayen; E. Saridakis; R.P. Sear. *P NATL ACAD SCI USA*. 103:597-601. [8] M. Sugahara, Y. Asada, Y. Morikawa, Y. Kageyama and N. Kunishima. *Acta Cryst*. (2008). D64, 686-695.

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Single Crystal X-ray Diffraction in Laser-Heated Diamond Anvil Cells. L. Dubrovinsky[a], N. Dubrovinskaia[b,c], K. Glazyrin[a] M. Merlini[d], M. Hanfland[e], T. Pippenger[b]. [a]*Bayerisches Geoinstitut, Universität Bayreuth, Germany.* [b]*Mineralphysik, Institut für Geowissenschaften, Universität Heidelberg, Germany.* [c]*Lehrstuhl für Kristallographie, Physikalisches Institut, Universität Bayreuth, Germany.* [d]*ESRF, Grenoble, France.* [e]*Dipartimento di Scienze della Terra, Universita degli Studi di Milano Via Botticelli, Italy.*
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Diamond anvil cell technique is the most successful method of pressure generation capable for working in the multimegabar pressure range. However, there are still a number of problems related to high-temperature experiments in DACs. Laser heating techniques cover a wide P-T field: P>200 GPa, T=1300-5000 K. A sample preparation for laser-heating experiments is relatively easy and there is practically no risk for the diamonds due to heating. However, so far all existing DAC laser-heating systems are stationary, they are linked either to certain equipment (an optical or Raman spectrometer, for example) or a beam-line, and do not allow rotating a cell