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New Approaches to Cryo- and Room-Temperature Crystallography

Robert E. Thorne[a,b], Viatcheslav Berejnov,[a] Matthew Warkentin,[a] Y. Kalinin,[a] Naji Husseini,[c] Osama Alsaeid,[d]

*[a]Physics Department, Cornell University, Ithaca NY USA; [b]Mitegen LLC, Ithaca, NY USA; [c]Department of Applied and Engineering Physics, Cornell University, Ithaca, NY USA; [d]Weill Cornell Medical College, Doha. Qatar. *Email: ret6@cornell.edu*

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Despite the enormous success of cryocrystallographic techniques, there has been surprisingly little understanding of the fundamental mechanisms relevant to their successful application. I will describe a variety of experiments designed to provide a quantitative, rational basis for cryoprotection and flash cooling [1]. These experiments have resulted in a new approach to cryopreserving protein crystals. Both the cryoprotectant concentrations required to inhibit hexagonal ice formation and the mosaicities of frozen crystals are dramatically reduced over current best practices, simplifying cryoprotectant screening and yielding higher resolution structural information.

At the same time, there are many excellent reasons to collect diffraction data at room temperature. Room temperature screening can diagnose problems in as-grown crystal order and in cryoprotective procedures, allowing problem crystals to be weeded out early in the diffraction pipeline. Room temperature may also yield higher resolution structures that more faithfully represent the biologically relevant conformation. I will describe a simple technology [2] that makes room temperature data collection as easy as at low temperatures, and that can be implemented in a high-throughput environment.

[1] Berejnov, V., *et al.*, *Acta Cryst. D* 2006, 62 (in press); Warkentin, M., Berejnov, V., and Thorne, R. E. (in preparation).
[2] Kalinin, Y. *et al*., *J. Appl. Cryst.* 2005, 38, 333; Thorne, R. E., Chew, G., and Sochor, M. (in preparation).

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A new lanthanide complex for protein structure determination using anomalous diffraction

Guillaume Pompidor[a], Olivier Maury[b], Jean Vicat[a] and Richard Kahn[a]

[a]Institut de Biologie Structurale, CEA-CNRS-UJF, UMR 5075, Grenoble, France. [b]Laboratoire de Chimie, UMR 5182, ENS-Lyon, France.

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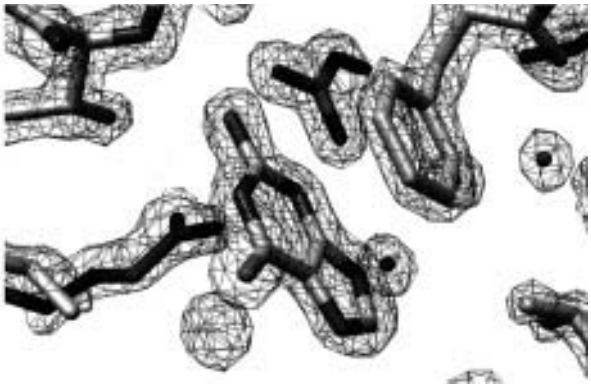
$Na_3.[Ln(DPA)_3]$ complex, where Ln and DPA stand for lanthanides and pyridine-2,6 dicarboxylate respectively, was used to prepare derivative crystals of three proteins: HEWL, thaumatin from *Thaumatoccus daniellii* and urate oxidase from *Aspergillus flavus*.

Hen egg-white lysozyme derivative crystals were obtained by cocrystallization in 50 mM solution of the Eu complex leading to a new crystal form belonging to space group C2. The new structure was solved by the SAD method using data collected with a rotating anode generator.

Thaumatin and urate oxidase derivative crystals were obtained by soaking native crystals in 100 mM $Eu(DPA)_3^{3-}$ and 150 mM $Lu(DPA)_3^{3-}$ solutions respectively. Data were collected at the corresponding Ln L_{III} absorption edge and at a remote wavelength around 1.0 Å. Phases were determined by the MAD method.

In the three cases, experimental phases led to easily interpretable electron-density maps. Protein substracts, if any, and water molecules were clearly identifiable in the experimental maps.



Experimental electron density map at 1 sigma level of urate oxidase in the region of its substact azaxanthine at 1.7 Å resolution.

The electron densities corresponding to the complexes in the most occupied sites were clearly visible. The structures were refined in order to study the fixation mode of the complex. Lanthanides complex molecules are bound to the proteins through interactions between DPA ligands and positively charged amino-acids such as arginine, lysine and histidine residues.