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Structure-based drug design / Structure-based protein engineering / Applications of synchrotron radiation to the pharmaceutical industry / Protein crystallography in the pharmaceutical industry

Protein Crystallography is Dead: Long Live Protein Crystallography!

I ventured into the field of protein crystallography in the early seventies, a few years after my undergraduate degree in physics from the Spanish educational system. This was a system where trespassing the boundaries of traditional disciplines was not encouraged. I was interested in biophysics as a general area of scientific enquiry and I needed to find a venue for my scientific interest and curiosity. Three years of independent work at the University of Salamanca, Spain, reading textbooks, papers and attending lectures in biochemistry, microbiology and genetics gave me enough background to move on. However, I needed to have a more formal training in the field. Complementing my scientific studies with studying English allowed me to come to the U.S. with a Fulbright Scholarship in 1972 to pursue graduate studies in biophysics at the University of Texas at Austin.

There, I encountered protein crystallography as a worthwhile field of endeavor for a Ph. D. work, mentored by Profs. Marvin L. Hackert, Hugo Steinfink and Larry Fox. Six extraordinary years at Purdue University with Prof. Michael G. Rossmann completed my training, and I ended up using protein crystallography as a tool to design drugs in the pharmaceutical industry. A practical use for protein crystallography was an amazing, and certainly unforeseen, professional and technical development.

In those years, the annual meetings of the ACA were vibrant with methodology sessions related to protein crystallography. All the different facets of protein crystallography were presented and discussed: the chemical trickery of growing crystals and preparing multiple isomorphous derivatives; methods and strategies of data collection and processing; the difficulties of extracting anomalous signal; the subtlety of using molecular replacement techniques to solve structurally related proteins; and discussions on the best pathway to a successful refinement. Of course, there were also sessions on novel structures, but these were relatively few.

Protein Crystallography has become the unexpected victim of the ingenuity, inspiration and hard work of protein crystallographers themselves. Years of crystallographic

and mathematical expertise are now canned in effective, rapid and user-friendly software packages, covering all the aspects of the process: from Patterson function solution and heavy atom location to phase calculation and model refinement. Rapid computers with practically unlimited disk space and memory drive all this crystallographic wizardry.

Protein crystallography, as a discipline of further technological development appears to be dead, or is it?

The icons of the structural revolution in the biological sciences propelled by the success of protein crystallography can be seen in those myriads of molecular images in textbooks and scientific journals in areas far removed from crystallography. Novel protein structures are unveiled all over the world at the rate of two-to-three per day and the number of homologous proteins structures and protein:inhibitor complexes analyzed daily runs probably in the hundreds. The Protein Data Bank now holds over sixteen thousand protein (or protein-nucleic) acid entries, just prior to the structural genomics revolution.

Thus, is it worth teaching crystallography? Is it a legitimate field of study? Yes, but in a different context. Protein crystallography shares with other methods of structural study (i.e. electron microscopy, image reconstruction, fiber diffraction, NMR, etc.) concepts and mathematical tools. In view of the power and effectiveness of the crystallographic software, the teaching of macromolecular crystallography should be included within the framework of other diffraction and image reconstruction methods as hands-on classes. Courses emphasizing not the grinding details of all the structural methods, but their strengths, limitations and future extensions to other experimental setups should be planned. The amazing possibilities of routine access to synchrotron sources of tunable, intense X-rays for all kinds of structural analysis should be emphasized to inspire the new generations to devise novel experimental designs.

Indeed, there is much that remains to be done, both in quantity and quality. The number of protein and macromolecular structures that remain to be uncovered by crystallographic or diffraction methods is staggering. It is worth mentioning that the first draft of the human genome is estimated to contain a minimum of approximately 40,000 proteins, with several thousands or several tens of thousands having been sequenced in organisms ranging from bacteria to the fruit fly. The numbers *per se* should keep protein crystallographers busy for a few decades still, even after developing high throughput methods, so popular now in the first years of the twenty first century.

It should not be forgotten, that in spite of how streamlined the process of protein structure determination is, 'direct methods', analogous to the ones used in the small molecule crystallography domain, are not yet routine in protein crystallography. Mathematical and computational crystallographers are still trying to cut open a clear path in this realm of protein crystallography. This goal appears closer and even reachable; much more so than only a few years ago. Achieving this goal would certainly be a precious feather in the cap of methods development and mathematical crystallography.

A well-known fact should also be mentioned. The re-

finement R-factors in most of the refined proteins ($\sim$20%) do not reach the level achieved by small molecule crystallography ($<$10%). Possibly, our refined protein structures do not fully account for all the atomic intricacies (i.e. motions, conformational variations, solvent structure among others). Whether this is an inherent limitation of protein crystallography or just restricted by our current tools is an open question.

It is the novel results and future experimental developments that intrigue me the most, and where I would suggest the most spectacular and amazing functional discoveries will be made. The physico-chemical dissipative processes that are responsible for life are dynamic and far from thermodynamic equilibrium. Currently, protein crystallography is unveiling the structures of pieces in a detached, context-free environment. Processes such as the functioning of biological clocks, signal transduction and organismic development are dynamic processes. The complexity and subtlety of these processes demand that macromolecular crystallographers fine-tune their tools and experimental methods to get closer to the dynamic reality where macromolecules 'play their act'. In spite of known successes, time-resolved diffraction studies of macromolecues are still in their infancy. In addition, would it ever be possible to examine the spatial and temporal order of dissipative structures using scattering or diffraction methods combined with synchrotron radiation sources?

After learning its methods and seeing the amazing development that has followed, I can say that protein crystallography is not dead and will never be; on the contrary. It will live forever ingrained within the fabric and texture of biochemistry, structural and molecular biology, cell biology and so many other fields. Its practitioners are now disguised as prominent molecular and cell biologists, biochemists, protein and materials engineers, drug designers, and many other biophysical and biomedical researchers. It is subjacent in all the branches of scientific enquiry that attempt to understand life processes at the molecular and structural level.

Acknowledgments. Comments and suggestions from Drs. J. Greer, K. Longenecker, G. Stamper and V. Stoll are greatly appreciated.