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SYNCHROTRON RADIATION FOR HIGH QUALITY DATA IN PROTEIN CRYSTALOGRAPHY

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Abstract content

Protein crystallography is the unsurpassable technique to obtain atomic resolution of biological entities. Those entities can have molecular masses from 6 kDa like the Immunoglobulin-binding domain B1 of streptococcal protein G (GB1) to MDa like the ribosome. In the last 15 years, a number of specific technologies like crystal freezing, SAD and MAD phasing using metals or selenium containing recombinant protein crystals and the growing amount of structures to be used as scaffold for molecular replacement makes feasible that biochemists and molecular biologists with out little or no training in protein crystallography can efficiently solve crystal structures. Today protein crystallography has two bottle necks: 1) Obtain a protein crystal 2) Obtain high quality X-ray data suitable for phasing. The problem of growth protein crystals is usually tackled by a combination of molecular biology and biochemical approaches (i.e. site directed mutagenesis, partial proteolysis, etc). The problem of high quality data is tackled by an intense beam (to increase the signal to noise ratio) with the potential of tunable wavelength (to obtain phasing information). Although the development of in house X-ray sources, the only way to have tunable wavelength is with synchrotron radiation. The typical users in protein crystallography go to the synchrotron to improve data resolution of to obtain phases. We present examples of data sets take in "house" versus synchrotron data. The main result is the improvement of the maps. For instance in our research we need a resolution better of 2.2 Å to assure the effect of specific changes in protein folding. Current crystal problems have been solved by molecular replacement methodologies; we present examples of proteins in which no homologous protein has been crystallized and will no be solved by molecular replacement. The need to use SAD or MAD approaches is critical in those cases.

Summary

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