X-ray crystallography of protein kinase structure research

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X-RAY CRYSTALLOGRAPHY OF PROTEIN KINASE STRUCTURE

X-ray crystallography allows researchers to get a closer look at the three-dimensional architecture of a protein, atom by atom. In the protein kinase studies, published in today's issue of Science, researchers at the University of California, San Diego describe the three dimensional structure of cyclic-AMPdependent protein kinase bound to a specific inhibitor.

The kinase studies illustrate how X-ray crystallography, combined with advances in computational science and visualization, allow complex structures such as the protein kinase to be mapped.

The first essential step, accomplished by UCSD researchers Janusz Sowadski, Jianhua Zheng and Daniel Knighton, was to obtain a high-quality crystal of the catalyic subunit. Crystals produce characteristic diffraction or scatter patterns when placed in the path of an X-ray beam.

A device called the Multiwire X-ray Area Detector -invented by UCSD researcher Nguyen-huu Xuong -- was used to convert the diffraction patterns into pulses of electricity that could be displayed directly on a computer screen. Diffraction patterns can be used to calculate a map of the electron density in the crystals. After obtaining an initial pattern, some heavy atoms (usually metals) can be introduced into the crystal to produce known small changes in the structure. These changes, and corresponding changes in the new diffraction pattern, yield the information that allows electron density maps to be interpreted. They allow electron density maps to be interpreted. In this case, Sowadski, Knighton and Zheng spent two years searching for suitable heavy atom derivatives.

The next stage was to build a model of the molecule to fit the electron-density map.

"We also had to be faithful to the known sequence of amino acids and to the known chemistry of the protein," said Knighton. The model building required many passes and adjustments and much computation on SDSC's giant CRAY Y-MP supercomputer.

"Only after Daniel and Jianhua had fitted all the amino acid side chaings to the density map did we sit down together to determine whether the structure was consistent with the chemistry," said Susan Taylor, professor of chemistry at UCSD.

"For this final step, the three-dimensional visualization facilities at SDSC were invaluable," she added.

The graphics equipment at the Advanced Scientific Visualization Laboratory (VisLab) at SDSC offered the researchers an opportunity to graphically display their structure in three dimensions and color, on a large screen.

"This allowed us to all to look together simultaneously, which helped a great deal," said Taylor.

"Only at this point were we able to fully merge our collective knowledge of macromolecular structure, mutagenesis and chemical modifications," she added. "That first joint session was a remarkable experience for all of us because each chemical clue, one after the other, suddenly was interpreted before our eyes. It immediately became apparent that the structure we were all seeing for the first time was the 'Rosetta Stone' for the entire protein kinase family."

The solution of the structure of the catalytic subunit- inhibitor complex was aided by data collection facilities at the Center for Macromolecular Structure.

"It is those facilities that made the final stages of solving the structure relatively quick once the slow and painstaking crystallography work was done," said Sowadski.

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