

Seeing the unseeable: X-ray crystallography

The reverse transcriptase protein, part of the machinery involved in the replication of AIDS, is much too small to be seen under powerful microscopes. To design drugs that latch onto the protein and block its operation, scientists must use an elaborate process that allows them to discern the protein's structure.

This technique, called X-ray crystallography, allows scientists to study a substance that is virtually invisible.

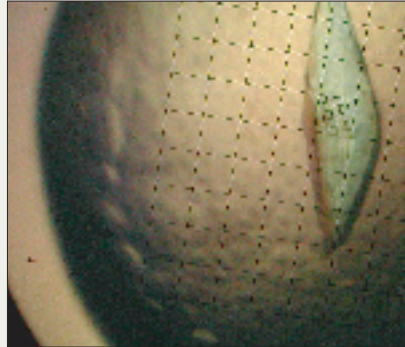


Image shows a magnified view of a single crystal of RT with the DAPY compound TMC-125.

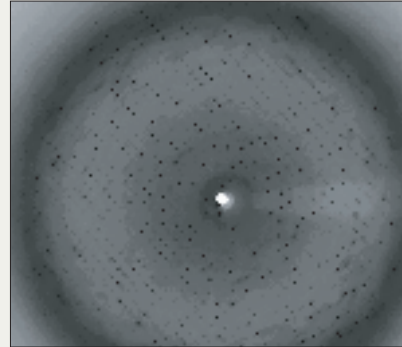


Image shows diffraction pattern of the crystal when it is bombarded with X-ray beams.

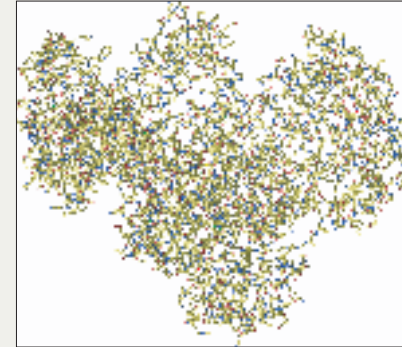


Image shows the mesh structure of RT created by computer analysis of the diffraction pattern.



Image shows a simplified ribbon representation of the whole RT molecule built from the mesh.

Making the crystal

First, scientists take samples of RT and the RT inhibitor and combine them in a crystal. A single crystal is composed of thousands of inhibitor-bound RT molecules arranged in an orderly pattern and is an excellent subject for atom-by-atom analysis of the protein molecule.

To make a crystal, scientists take samples of the protein in solution and place small droplets of the sample solution on a glass plate called a cover slip. Each droplet is mixed with

a precipitating solution containing salt or an organic solvent like alcohol. Finally, the cover slip is placed over a glass well containing a higher concentration of precipitation solution at the bottom. If conditions permit, the protein molecules gradually assemble to form a crystal in the hanging droplet that is smaller than a teardrop. Often the crystals are grown in a cold room and then fished out of the droplet and frozen in liquid nitrogen.

X-ray vision

To get at the structure of the materials, scientists blast the frozen crystals with highly intense X-ray beams. The beam ricochets off particles in the crystal. The scattered X-rays are recorded on a CCD or charge-coupled-device detector which registers images; each image contains thousands of dots. The resulting pattern reveals the atomic content and molecular structure of the crystal.

The 3D picture

Through extensive computation based on data gathered by X-ray diffraction, scientists can extrapolate, constructing a three-dimensional image of the protein and interlocking drug. The image, which provides information on atom-to-atom interactions between the protein and the drug, can be rotated and flipped and enlarged for closer examination.

Ribbon diagram

The analysis produces what is known as a ribbon diagram of the molecule, an idealized version of the protein and the drug showing the key structural units. In the diagram above, the double helix structure of DNA can be seen in purple in the middle. The RT is captured in the process of creating DNA.

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SOURCE: Center for Advanced Biotechnology and Medicine, Rutgers University and UMDNJ