

Bombarding the AIDS virus Reverse Transcriptase with synchrotron radiation at CHESS

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When the research article "Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with double-stranded DNA at 3.0 Å resolution shows bent DNA" was published in the *Proc. Natl. Acad. Sci. USA* 90 (1993), the entire research group working on RT at CABM and Rutgers University could no longer contain their excitement and joyfulness; not only because this article represented a major breakthrough in the understanding of the structure and function of a very important enzyme, but, also, it symbolized that their persistence and hard work over the past six years would finally be acknowledged.

For six years, the RT group led by Dr. Eddy Arnold, an Associate Professor of Chemistry at Rutgers, had worked very hard to visualize and solve the crystal structure of human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT). HIV-1 RT is the enzyme which is responsible for the catalytic transformation of the AIDS virus RNA genome into a double-stranded DNA which can be permanently integrated into host cell chromosomes and cause the deadly disease, acquired immunodeficiency syndrome (AIDS).

Looking back over the six years, every member in the group had countless sleepless nights; we had frustration and encouragement, pain and joy, we had failures and eventually successes. Not many of us can really tell the entire story. However, people can never forget the synchrotron trips to CHESS, in Ithaca, New York. Without the synchrotron facility at CHESS we could not have achieved these accomplishments. Also, every single day we spent at CHESS was colorful and full of different kinds of stories.

The dark night in the winter

In 1987, after having worked in the laboratory of Dr. Michael Rossmann at Purdue University for five years to complete the structure determination and refinement of human rhinovirus 14, one of the common cold viruses, Dr. Eddy Arnold moved on to establish his own macromolecular crystallography laboratory at CABM and Rutgers University. Though it might have seemed to be too ambitious and risky for a young scientist, Dr. Arnold had no hesitation in choosing to investigate the molecular structure of HIV-1 RT as the first major project in his lab.

AIDS, caused by HIV, continues to be one of the world's most serious health problems, and current protocols are not adequate for either prevention or successful long-term treatment of the disease. HIV-1 RT is a potential therapeutic target of many inhibitors against AIDS. Indeed, nucleoside analog inhibitors of RT, such as AZT, ddI, and ddC, are clinically effective drugs for treating HIV-1 infection. However, their effectiveness is limited by toxicity, which may reflect inhibition of cellular polymerases and/or alteration of nucleoside pools, and the mechanism of RT inhibition by these compounds is also unknown. It is anticipated that the three-dimensional structure of HIV-1 RT will provide a structural basis for understanding the inhibition mechanism and the mechanism of antiviral resistance to RT, which may lead to the development of improved inhibitors for the treatment of AIDS.

In the first three years, our laboratory struggled to crystallize RT crystals that could diffract X-rays. More than two years efforts were in vain and we were frustrated. However, we did not give up and continually used a variety of approaches to

determine the proper crystallization conditions. Finally, after unprecedented efforts particularly by Dr. Alfredo Jacobo-Molina and Art Clark, our group emerged as the one of a few groups able to successfully crystallize RT crystals. We had succeeded in obtaining crystals in several morphological types with size of up to 1.0 mm × 1.0 mm × 0.5 mm. This was really a breakthrough in our work. Unfortunately, as we mounted the crystals on the Xuong-Hamlin Mark II area detector in our home X-ray laboratory, the crystals refused to diffract X-rays; there were no spots on the image at all.

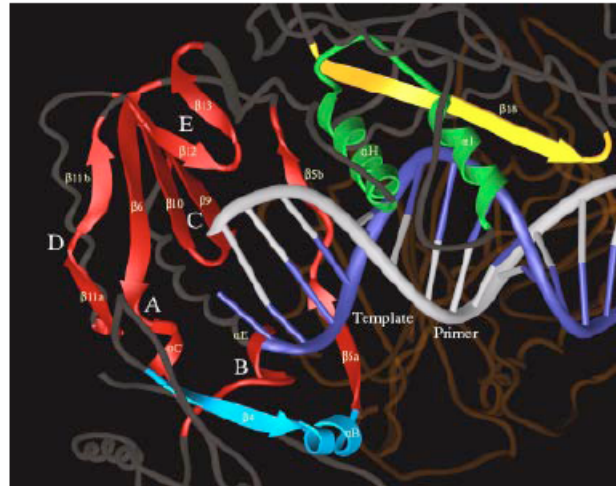
Our next hope was to bring the crystals to CHESS where the group was already collecting data successfully from virus crystals. CHESS is equipped with one of the most unique synchrotron facilities in the world; the synchrotron radiation there is extremely bright with short wavelengths which can extend crystal lifetime via reduced radiation damage. Even though the crystals of RT gave a no-spot diffraction pattern at the old A1 station with wavelength $\lambda=1.55\text{Å}$, they did diffract X-rays to 6 Å resolution at the F1 beam line with wavelength $\lambda=0.91\text{Å}$. This success initiated more frequent synchrotron trips to CHESS for the purpose of RT data collection.

A big event in the laboratory

Almost everyone in our lab has experience working in the synchrotron at CHESS. Synchrotron trips have become a big event in our entire lab, not only for the RT group. Usually months before a scheduled synchrotron trip, the preparations are in progress; crystallization, crystal searching to distinguish different kinds of crystal quality and size, heavy atom soaking, and ordering of

materials and equipment needed at CHESS. One member of the group whom we were always joking about because of his organizational tendencies was Dr. Raymond Nanri. He was such a careful and meticulous person that he prepared boxes with corresponding lists for each workstation at CHESS. No single item could be missing or be misplaced in the boxes. Of course, this effort proved to be very helpful and we are still benefiting from following his lists after he left this lab. Nevertheless, mistakes are still unavoidable. On a trip during winter, the synchrotron team drove a van full of boxes and equipment to Ithaca. As they prepared to shoot the crystal, they realized that they had brought everything they needed *except* the crystals. They had to call back to the lab and ask someone to drive overnight to Ithaca with the crystals.

In order to make our task at CHESS as painless as possible, we organized our team personnel into workstation subgroups; camera (taking photographic diffraction data), cold-room (mounting crystals), dark-room (developing photographic films), and scanner (scanning phosphor image plates). For best use of the precious beam time allocated for synchrotron data collection, the whole team is divided into two shifts and we work around the clock. For refreshment, the lounge room and the refrigerator were full of food, fruits, and drinks purchased from the local supermarket. Although everybody complained about these arrangements, no one wanted to spend an hour at a restaurant to have a good meal because people realized that sleeping is more crucial than eating. Especially for the night-shift people, after dancing with the RT crystals for more than twelve hours, they could simply fall asleep sitting on a chair in the early morning during the CESR injection. However, in celebrating the collection of a number of new datasets after five days and nights of work, everyone still looked very energetic and enthusiastic.



Hope sprouts in the spring

As long as we saw the diffraction spots on the diffracting image of RT crystals, even if only to about 5 Å resolution at F1 station, we knew that a bright future was ahead. The winter was over, spring was just ahead. The low resolution diffraction of RT crystals greatly inspired us and fueled our enthusiasm. We tried various approaches to further improve and optimize the crystallization of HIV-1 RT. These included protein engineering aimed at changing specific amino acids on the surface, making complexes with antibody fragments, making complexes with synthetic nucleic acids that mimic template-primer substrates, and soaking the crystals in different conditions. Through these efforts, the quality of the crystals was significantly improved and especially for the crystals of the ternary complex of RT/dsDNA/Fab28, they diffracted X-rays increasingly better.

With these crystals, we were able to collect entire diffraction datasets to about 6 Å resolution with a single crystal using the Xuong-Hamlin Mark II area detector system at our home X-ray laboratory. Although the analyses of these results

Structural elements near the HIV-1 RT polymerase active site that make potential contacts with the DNA; those corresponding to conserved motifs are shown and the secondary structural units are labeled.

greatly facilitated the interpretation of heavy atom derivatives and initial structure determination of RT at 7 Å resolution, the resolution limit of the diffraction data could not permit us to solve the structure of RT in atomic detail. At the same time, the experiments carried out at CHESS made great progress: the diffraction of ternary complex RT/dsDNA/Fab28 crystals advanced from 5 Å to 4 Å, 3.5 Å, 3 Å, and finally to 2.8 Å resolution at -15°C using the beam at the F1 station of CHESS. Using the same crystallization and soaking conditions, we have been able to routinely collect a more complete native dataset to 2.8 Å resolution, more than twelve heavy atom derivative datasets in the resolution range of 3.0-4.0 Å for the RT/dsDNA/Fab28 crystals, and a number of datasets with comparable resolution for other RT complexes. This represented the beginning of a new era in the structure determination.

Summer makes people crazy

In the spring of 1992, after bring-

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ing back to our lab over a few thousand photographic films and a few hundred 8-mm magnetic tapes which recorded the digitized diffraction images the crystallographers in the group began to work more than 15 hours a day at the computer trying every effort possible to index and process every film and image plate using a modified version of the Purdue oscillation film processing package. With a few months of effort, the digitized images were transformed into a number of datasets. One typical dataset was usually merged together from more than sixty films and/or image plates with 0.7-1.0° oscillation collected from twenty to forty crystals. For RT/dsDNA/Fab28 complex, the native dataset was merged from 186 films and image plates exposed from 36 crystals and has 76,903 unique reflections ($I > 3\sigma$) to 2.8 Å resolution with an R_{merge} (I) factor of 0.13 and completeness of 88%.

As the diffraction data were be-

ing processed, the initial phase problem was solved using the multiple isomorphous replacement method and solvent flattening techniques, and improved by successively incorporating more heavy atom derivatives information (in a total of twenty one derivatives used, eleven datasets came from CHESS). The MIR phased maps very clearly showed the polypeptide chains of the protein and the backbone of the bound DNA.

The people here went crazy, working fiercely day and night for many weeks to finish the backbone tracing along the whole course of polypeptide chains in the RT p66/p51 heterodimer, the antibody fragment Fab28, and bound 19/18 template-primer dsDNA. At this time, the structure of HIV-1 RT complexed with a small molecule, the non-nucleoside inhibitor nevirapine at 3.5 Å resolution, was reported by Dr. Thomas Steitz's laboratory in Science. The availability of the folding diagrams from the RT/nevirapine structure further facilitated the assign-

ment of some connection regions in our RT/dsDNA/Fab28 structure. Further phase improvement, by combining partial structure information with MIR phases and structure refinement, was carried out using the program XPLOR on the CRAY supercomputer at NCI, NIH (another key facility that enabled us to solve the RT/dsDNA/Fab28 structure at high resolution). This significantly improved the quality and reliability of the structure of RT and permitted us to visualize three-dimensionally the pernicious enzyme in atomic detail.

Autumn is the harvest season

Now the crystal structure of HIV-1 RT/dsDNA/Fab28 was unveiled some very important and intriguing results ensued. Though the two peptides of p66 and p51 subunits share the identical amino acid sequence in the N-terminal polymerase domain, the spatial arrangement of their secondary structural elements are dramatically different. As the first structure of a polymerase bound