

## This One Goes to Eleven (hundred)- Data Collection on Crystals with a very Long Unit Cell

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In intracellular traffic mediated by clathrin-coated vesicles, loading of membrane-anchored cargo requires one or more “adaptor” proteins. These adaptors associate with clathrin, while at the same time recognizing specific, cargo-displayed sorting signals. One important subset of clathrin adaptors is a family of heterotetrameric adaptor protein complexes, or APs, which direct traffic to distinct intracellular compartments.

The structure of the compact 200kDa globular core of one clathrin adaptor complex, AP2, which is involved in sorting between plasma membrane and endosomes, was determined previously [1]. Although different APs share a high degree of homology, they function at distinct locations in the cell and recognize different cargo. We were eager to determine how differences in structure could explain why different adaptors function at different locations. I focused my efforts on the clathrin adaptor complex AP1, which participates in traffic from the trans-Golgi network (TGN) to endosomes and lysosomes. After extensive crystallization trials using AP1 core, I obtained small ( $80 \times 80 \times 20 \mu\text{m}^3$ ) but sharp-edged crystals resembling thick hexagonal plates. Although I found freezing conditions that preserved the integrity of crystals, the diffraction at the home source yielded only a few low resolution spots,  $< 15 \text{ \AA}$ , which precluded any further characterization. Fortunately, a scheduled trip to CHESS was coming up and I was eager to test my crystals there.

Seeing the first diffraction images from a new crystal can be exhilarating and sometimes disappointing. But nothing I had seen prepared me for the mess of diffraction from the AP1 crystals: long rows of smeared spots that were reminiscent of fused beads on strings. Having initially suspected that the crystals were affected by some splitting or cracking disorder, I soon realized that the spots were, in fact, evenly spaced. Subsequent indexing revealed that the crystals belonged to a trigonal space group with the unit cell dimensions  $a = b = 180 \text{ \AA}$ ,  $c = 1100 \text{ \AA}$ ,  $\alpha = \beta = 90^\circ$ , and  $\gamma = 120^\circ$ . On some diffraction images, one could even discern a systematic pattern of absences with only every 3<sup>rd</sup> reflection present, indicating a 3-fold screw along the c cell edge. The unit cell dimensions of over  $1000 \text{ \AA}$  have been previously seen for crystals of viruses [2], but how could relatively small globular molecules of 200kDa pack to form crystals with such a long unit cell?

Data collection on crystals with a very long unit cell is notoriously challenging due to severe spot overlap. Nevertheless, I decided to attempt solving the structure of this crystal form of AP1 because AP1 failed to crystallize under any other conditions.

A series of quick exposures made it clear that even with a tightly focused, 0.1 mm beam, to achieve spot separation of even 1-2 pixels, the detector would have

to be positioned  $\sim 900 \text{ mm}$  away from the crystal. Any closer and the spots would run into one another. With the detector distance at 900 mm, the spots were no longer in smeared lines and were sufficiently separated (Figure 1), but the highest resolution at the edge of the detector was now only  $7.5 \text{ \AA}$ .

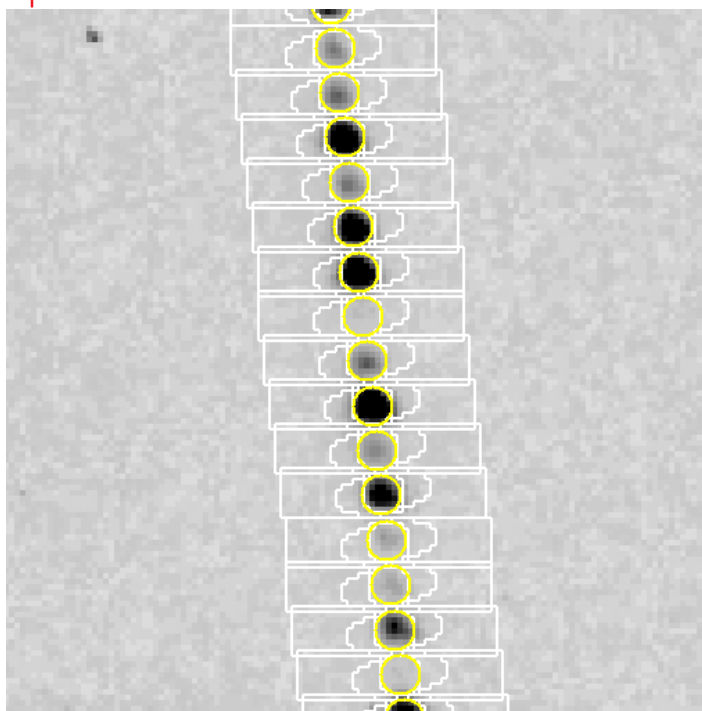


Fig 1: A close-up view of diffraction from an AP1 crystal.

Such low resolution was insufficient even to attempt solving the structure by molecular replacement, let alone to see atomic-level differences between AP1 and AP2 molecules. Thus, we needed to find a way to record higher-resolution diffraction.

Fortunately, I was able to take advantage of the double detector installation at CHESS F1 beamline. Arranged vertically, the 2X2 CCD detectors are essentially equivalent to a 2X4 detector, except that the images recorded on the top and the bottom detectors must be processed separately. Using the double detector, I could now record diffraction beyond 4.0 Å (Figure 2).

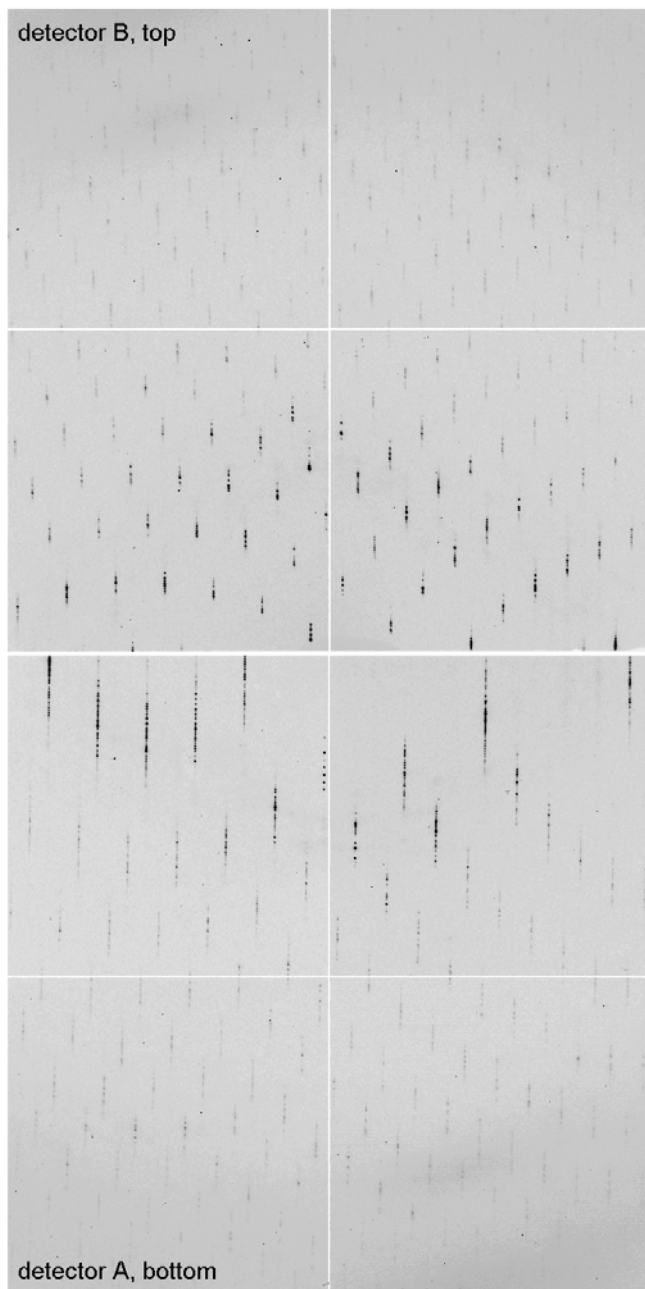


Fig 2: Diffraction on the double detector at CHESS F1 beamline.

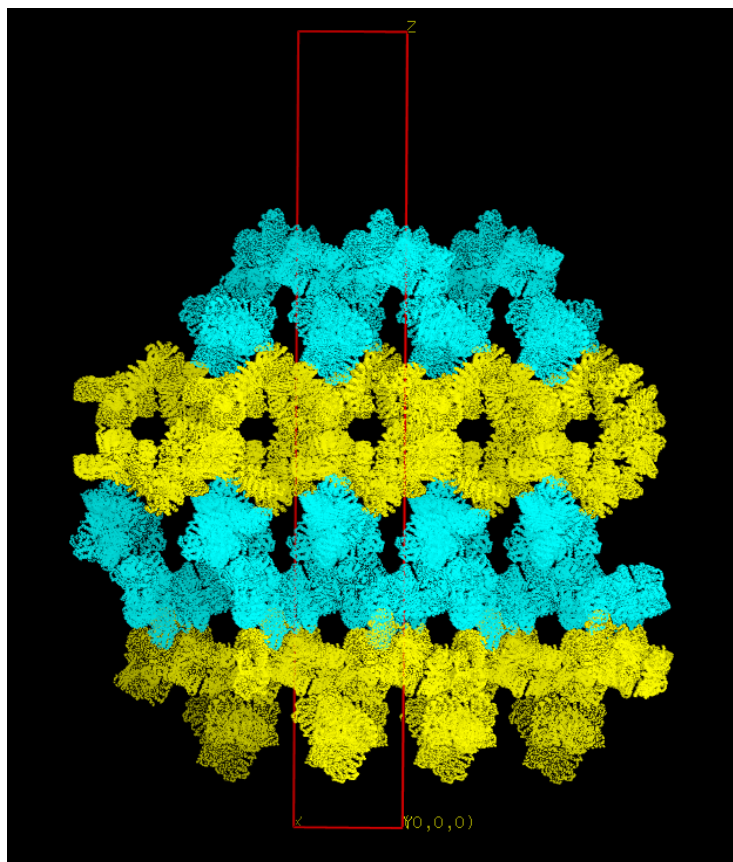
One problem with using double detector was that the beam center was between the two detectors, and thus its position, crucial for indexing and scaling, could not be determined directly. To obtain a rough estimate of the position of the beam center, I had to record powder diffraction of ice and, from the known positions of ice diffraction rings, calculate the beam center coordinates. This was accomplished using DPS software with the help of MacCHESS Staff Scientist Marian Szebenyi. Even such an estimate was still quite far from the true beam center. To determine the precise position, I had to process the data while systematically varying the beam position and then scaling these high-resolution data with a low-resolution data set, for which the accurate beam center was already known.

The long c cell dimension dictated the data collection strategy. To minimize overlaps, one would prefer to orient the crystals with the longest cell dimension parallel to the spindle. That, however, was quite hard to achieve with my plate-shaped crystals and so instead, I resorted to collecting data in thin oscillation frames. For proper indexing, each crystal was oriented such that its longest cell edge was perpendicular to the beam (see Figure 1 and Figure 2, bottom) and a 0.4° oscillation frame was collected. From this orientation, the first 25° segment of data could be collected in 0.4° wedges but the subsequent 40-50° segment had to be collected in 0.2° wedges to reduce overlaps. Rapid crystal decay, due to radiation sensitivity, necessitated collecting data from several crystals. Furthermore the majority of the crystals grew as thick plates, many of which would warp during freezing, rendering only approximately one third of the crystals suitable for data collection. Ultimately, 2 low-resolution (40-7.5) data sets collected using a single 2X2 CCD detector and 10 high-resolution (20-4.0) data sets collected using a double 2X2 CCD detector, or 1500 frames, were merged into the final dataset, 80% complete to 4.0 Å. The data were processed with Denzo and scaled with Scalepack [3].

The structure was solved by the molecular replacement method using Amore [4] with the entire AP2 core as a search model. After some tweaking of the search parameters, the rotation search yielded 3 top solutions that were correct, despite being barely above the background noise level. In the translation search, both  $P3_212$  and  $P3_112$  space groups were tested but only  $P3_212$  yielded the correct solution. Each rotation solution yielded 2 translation solutions, thus locating 6 molecules in the asymmetric unit.

The molecular replacement solution finally revealed how a relatively small, globular protein could yield crystals with a very long unit cell. In the crystals, the AP1 molecules form helical stacks, with three molecules per helical turn.

These stacks traverse the crystal from one unit cell to the next, perpendicular to the longest cell dimension and to the crystallographic 3-fold screw axis (Figure 3). There are two crystallographically independent stacks of this kind (Figure 3, shown in yellow and cyan), which cross at an angle of  $110^\circ$ . Such packing results in a dimer of trimers, or 6 molecules, in the asymmetric unit where each trimer forms one turn of a helical stack. There is no known physiological role for such stacking, and the complexes were homogenous heterotetramers in solution.



**Fig 3:** Molecular packing in the crystals of AP1. Crystallographically independent stacks are shown in yellow and cyan. The boundaries of the unit cell are shown in red.

To conclude, as crystallographers attempt to crystallize large complexes, the incidence of crystals with large unit cell will become more frequent. Hopefully, the work described here demonstrates that collecting data on crystals with a very long unit cell is not so daunting as it may seem and can be accomplished by taking advantage of large CCD detectors and a small, tightly focused beam.

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**References:**

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