

Structure Determination of ChrR Mutant Enzymes used for Bioremediation



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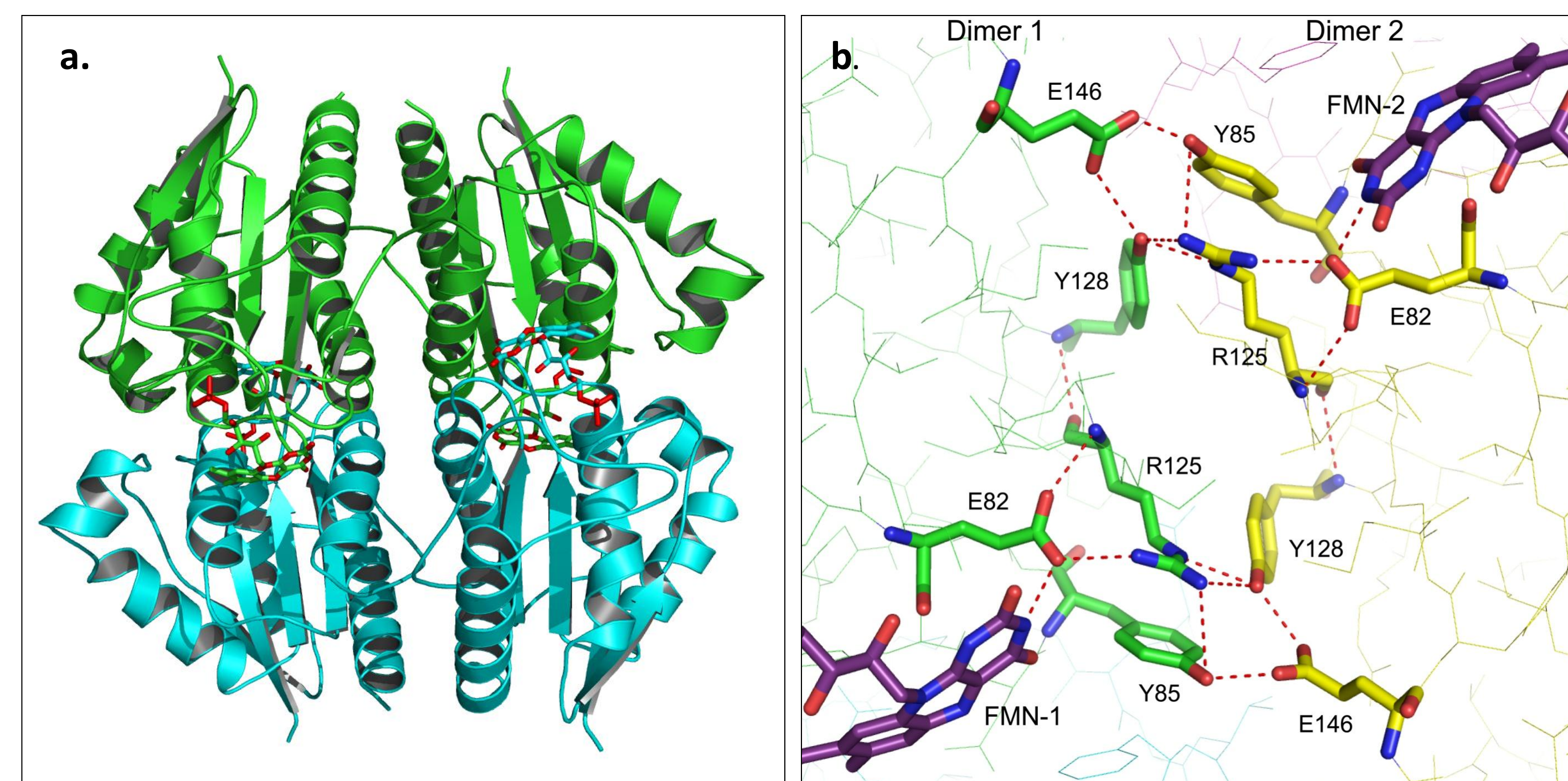
Abstract

Escherichia coli ChrR is a quinone reductase enzyme which has many useful applications, such as chromate bioremediation, and the activation of cancer prodrugs through oxidation-reduction reactions. The crystal structure of the wild type ChrR enzyme showed tetramer association, and networks of hydrogen bonds observed between the residues Tyr128, Glu146, Arg125, and Tyr85. Mutation studies show that these residues play a critical role in the activity of the enzyme. Structural analysis of these mutants is important to determine the mutant's roles in chromate reduction and activation of cancer prodrugs. Therefore, ChrR mutants were transformed, expressed, purified, and crystallized. Data from two mutant crystals were collected at the NSLS for structural analysis. Structure was determined for ChrR Y85N mutant.

Keywords: *E. coli* ChrR enzyme, chromate bioremediation, structural analysis

Introduction

The ChrR enzyme from *Escherichia coli* is an obligatory quinone reductase that is successful in a safe means of chromate reduction for bioremediation, as well as the activation of cancer prodrugs [1,2]. Understanding ChrR mutant's role in chromate reduction is crucial because chromate is a heavy-metal contaminant found in groundwater, soil, and sediments, which has become a serious environmental pollutant due to the wide use of chromium compounds in industries such as tanning, corrosion control, plating, pigment manufacture, and nuclear weapons production [3]. The crystal structure of the wild type ChrR enzyme has been determined [1], showing hydrogen bond networks between residues Tyr128, Glu146, Arg125, and Tyr85 of the tetramer associated protein (Figure 1). Biochemical assays have shown that ChrR mutants tend to increase the chromate reductase activity of the enzyme, therefore structural analysis is necessary to confirm these results [2].



Figures 1. Structure of Native ChrR enzyme. (a) Demonstrates the tetrameric association, and (b) shows the hydrogen-bond formations at the tetramer interface.

Materials and Methods

- pET-28a(+) Plasmid with ChrR mutants (Y128N, E146T, R125T, Y85N) were expressed in BL21(DE3) RIPL cells using auto-induction method.
- Proteins were purified using Nickel column procedures, and size exclusion chromatography (Figs 2).
- Purified protein was dialyzed in order to exchange buffers prior to crystallization.
- Mutants were crystallized using sitting drop vapor diffusion technique (Figs 3), and taken to the NSLS for x-ray analysis.
- Data was collected (Figure 4, Table 1), and the crystal structure was determined for Y85N mutant using molecular replacement.

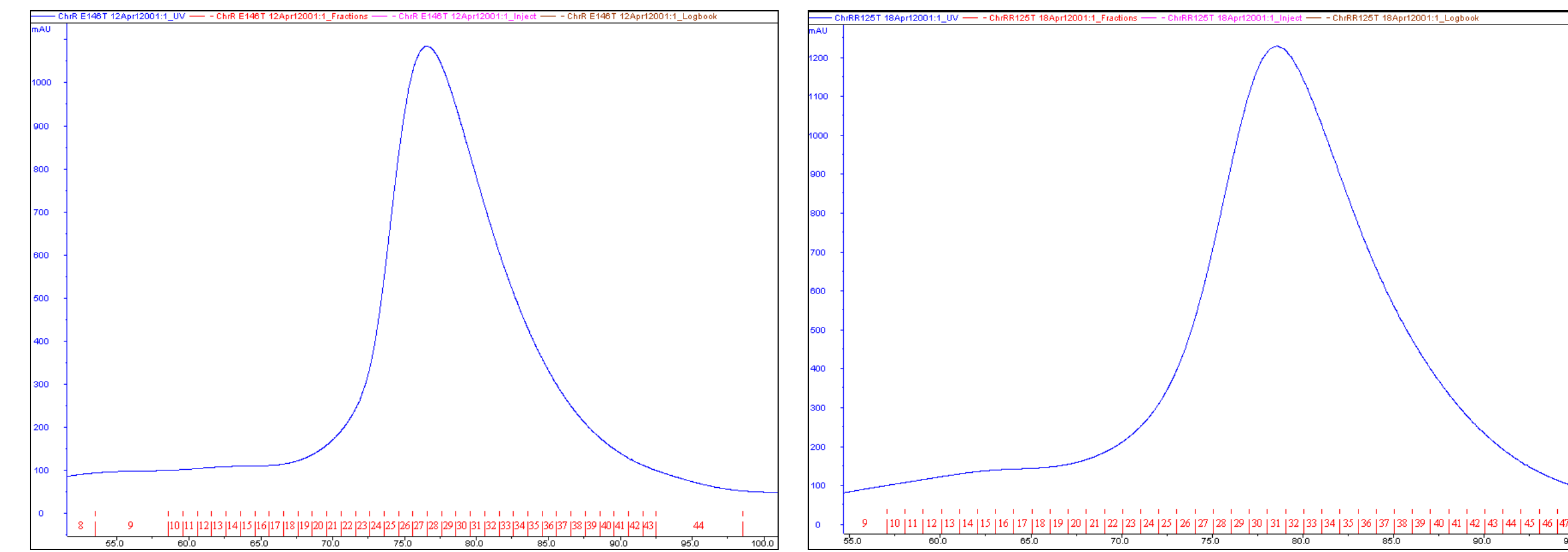


Figure 2a. ChrR E146T mutant

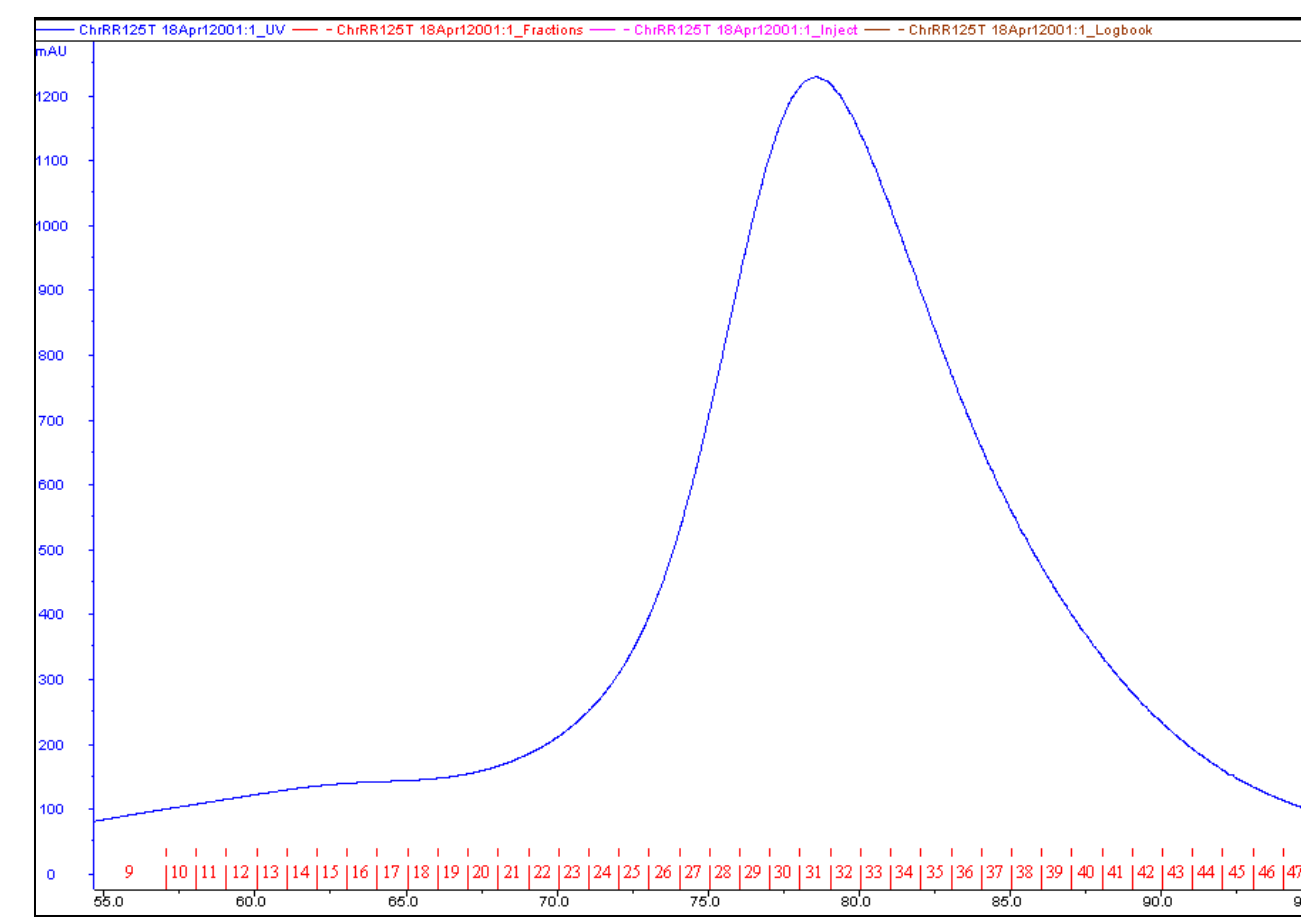


Figure 2b. ChrR R125T mutant

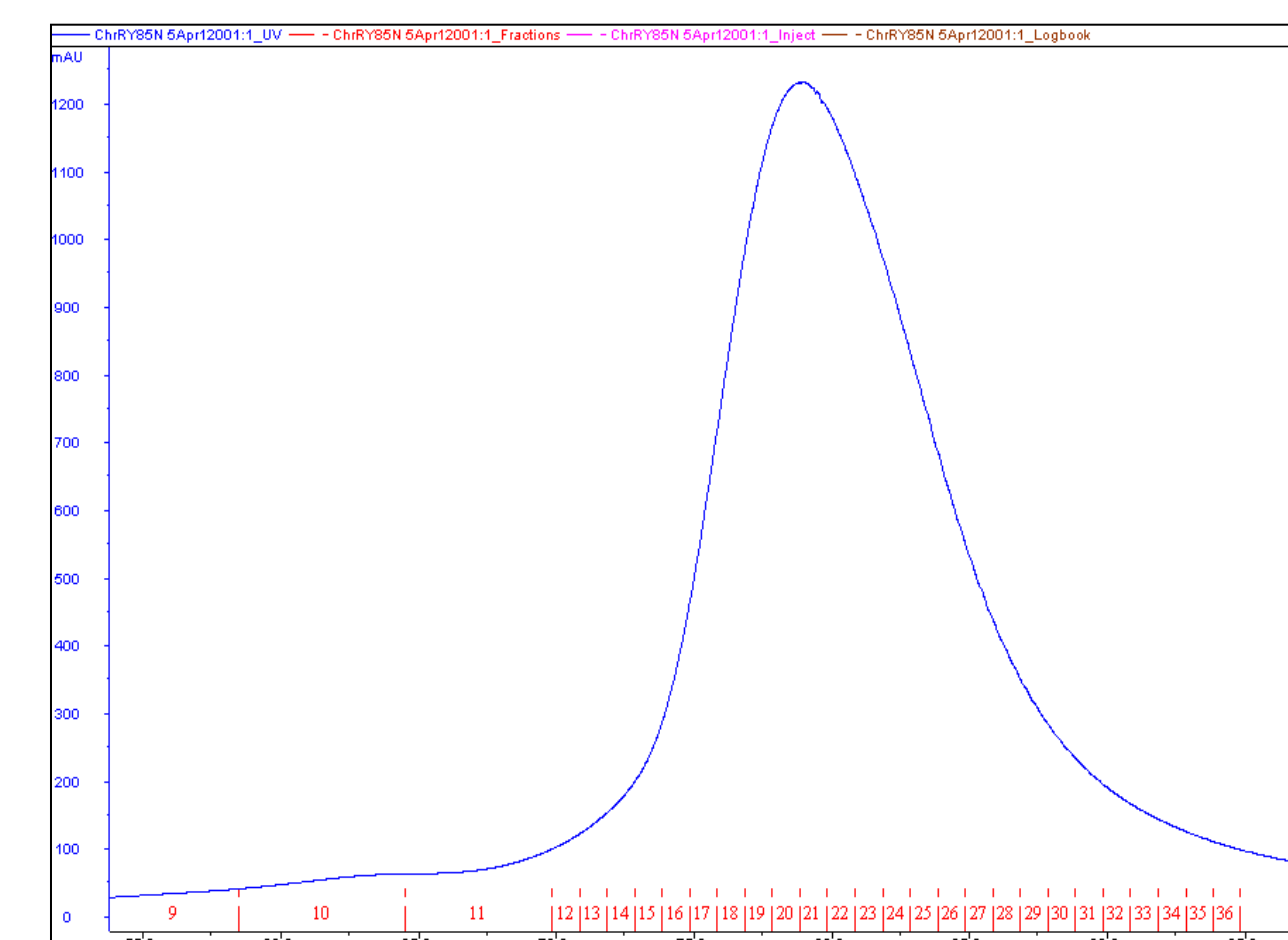


Figure 2c. ChrR Y85N mutant

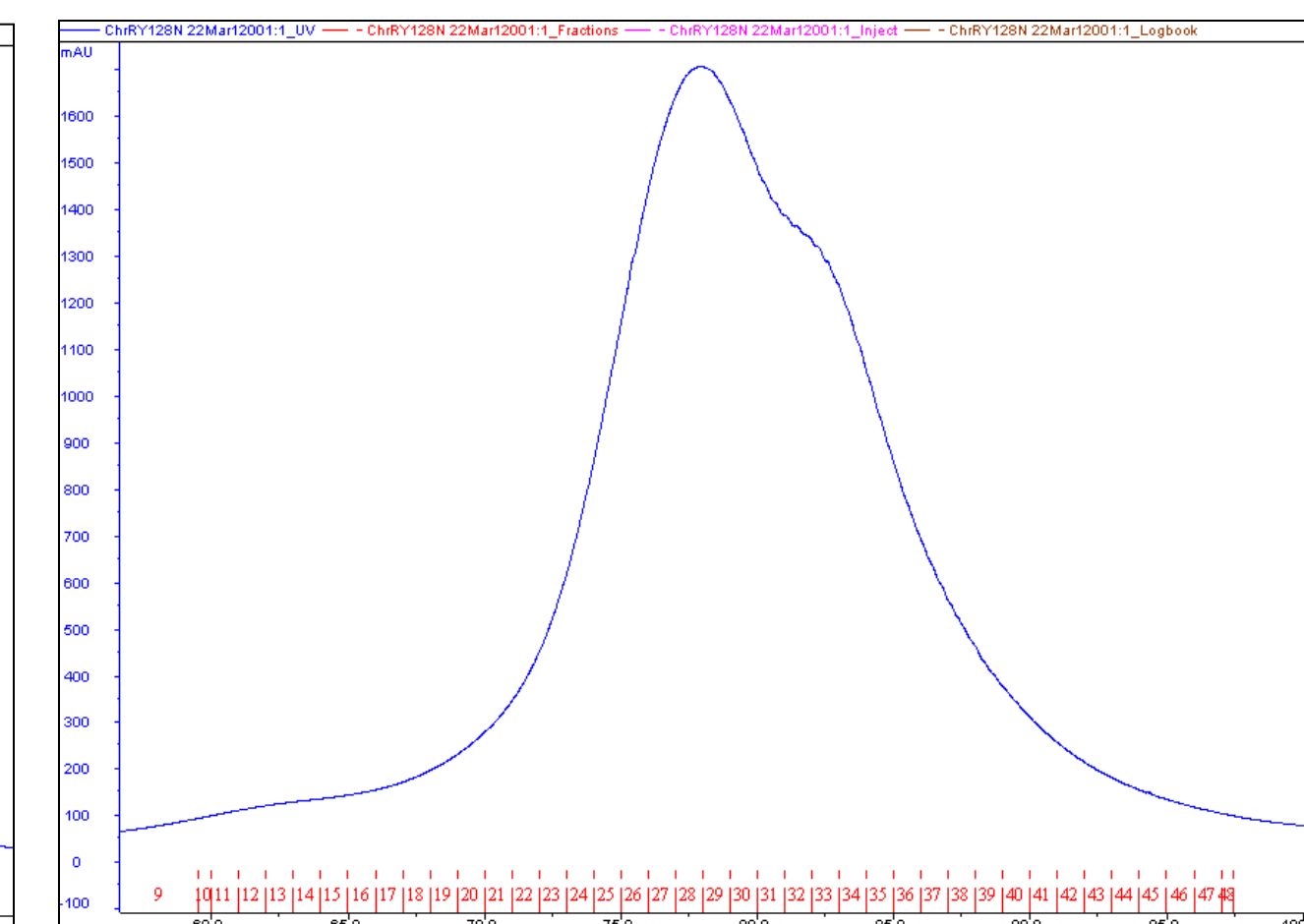


Figure 2d. ChrR Y128N mutant

Figures 2. Demonstrates the dual peaks (tetramer and dimer) of the ChrR mutant enzymes displayed after size exclusion purification.

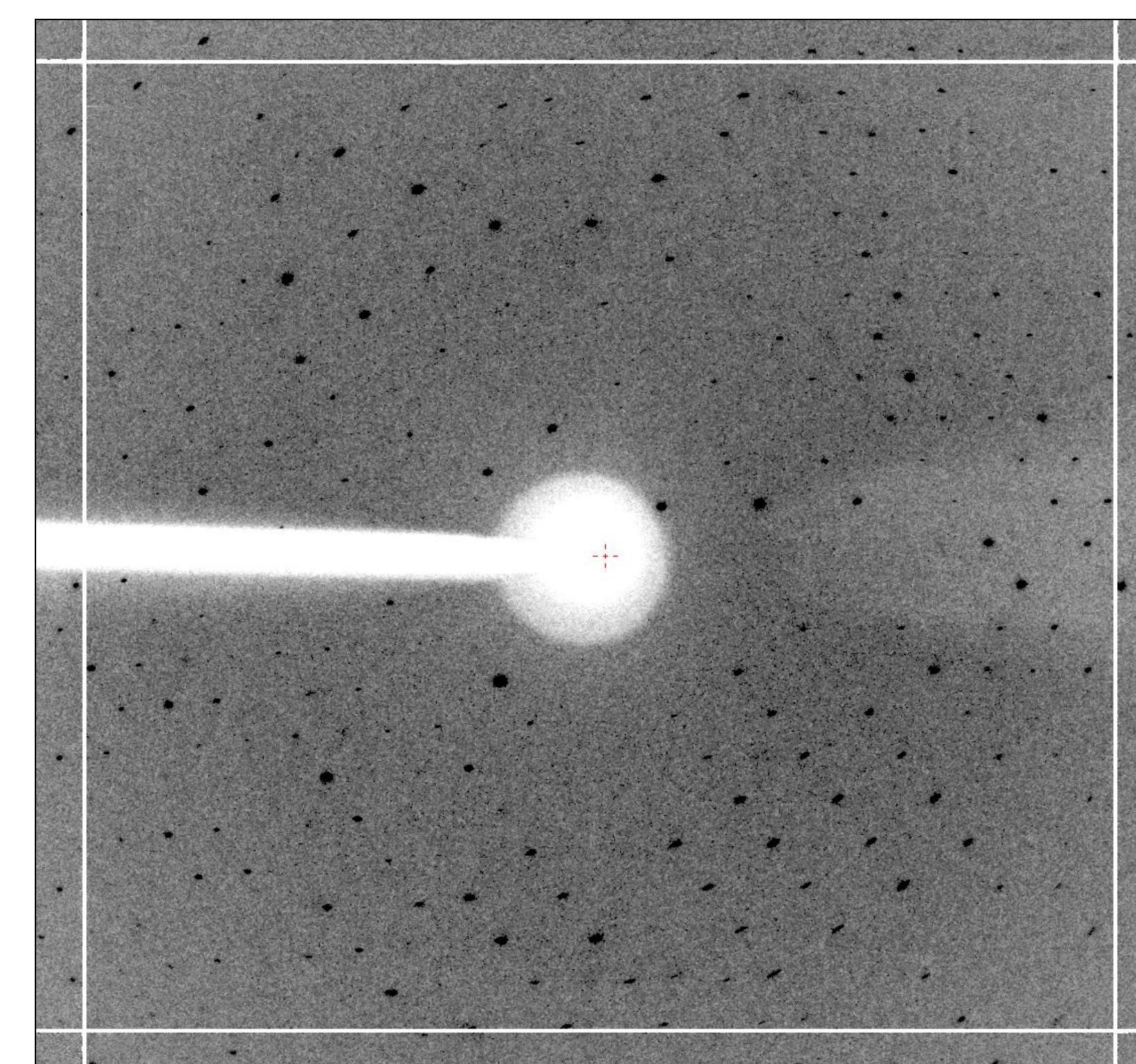
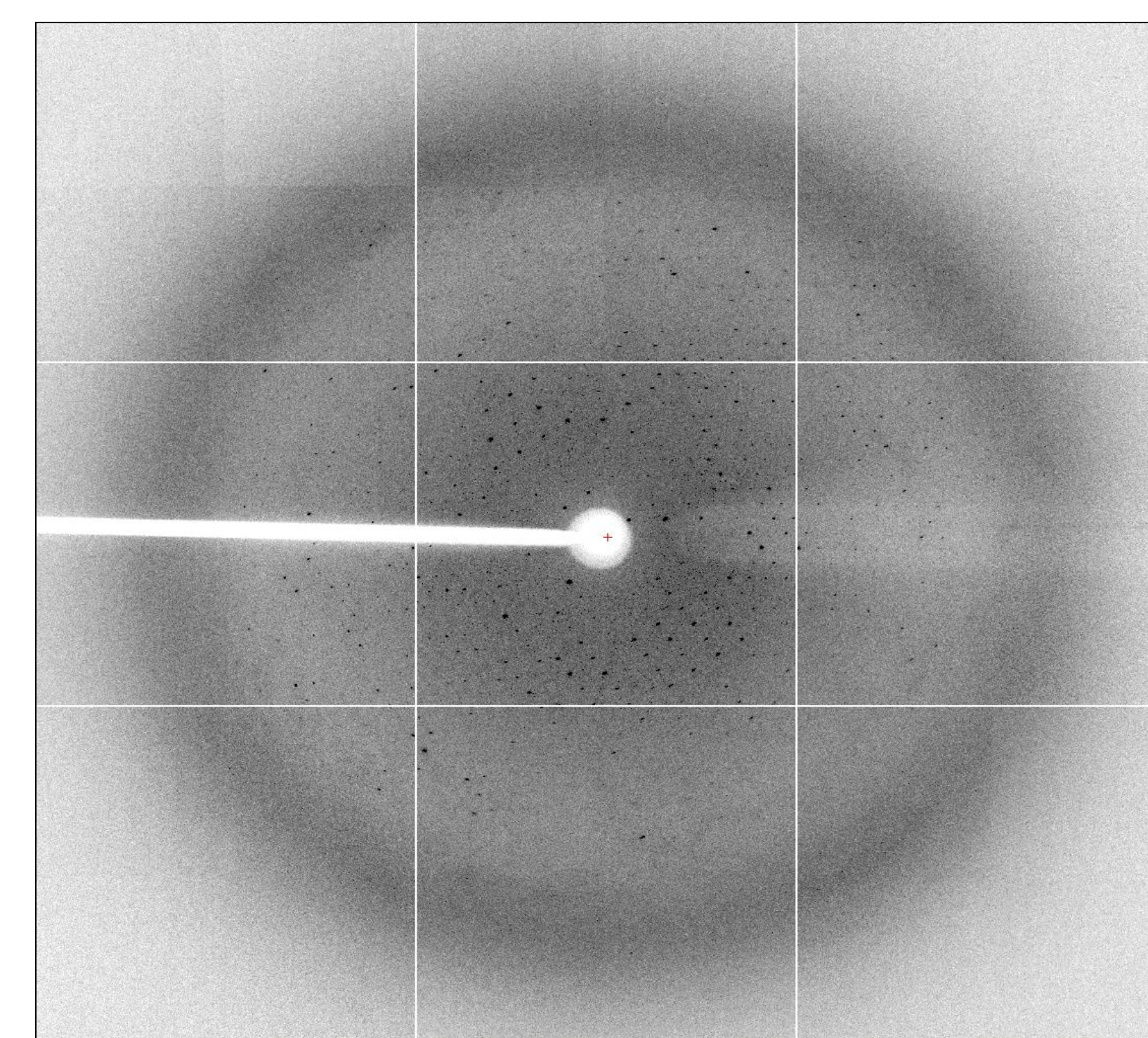
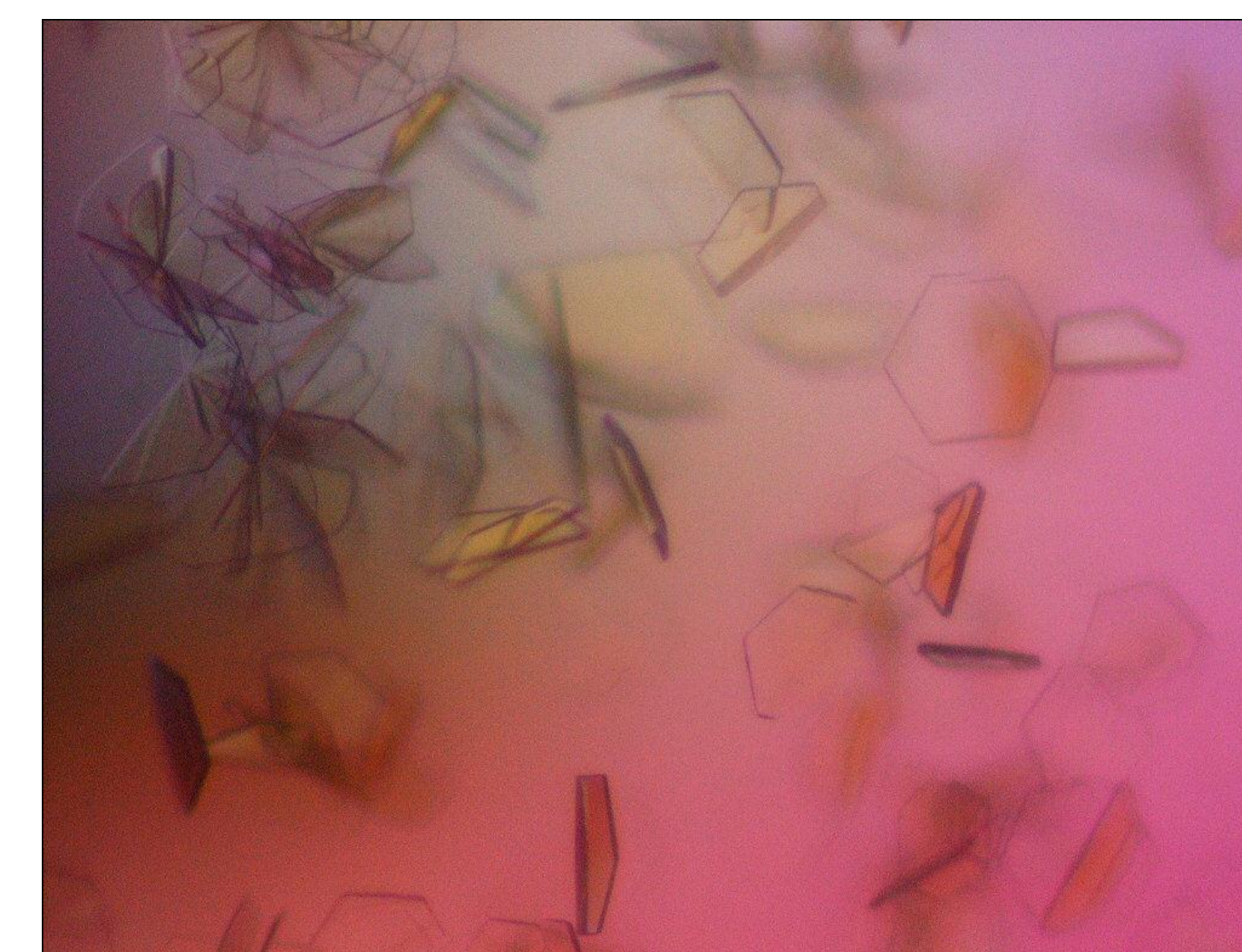


Figure 4. Diffraction pattern of the ChrR Y128N mutant crystal.



Figures 3. ChrR Y128N mutant crystals observed after 2 weeks.

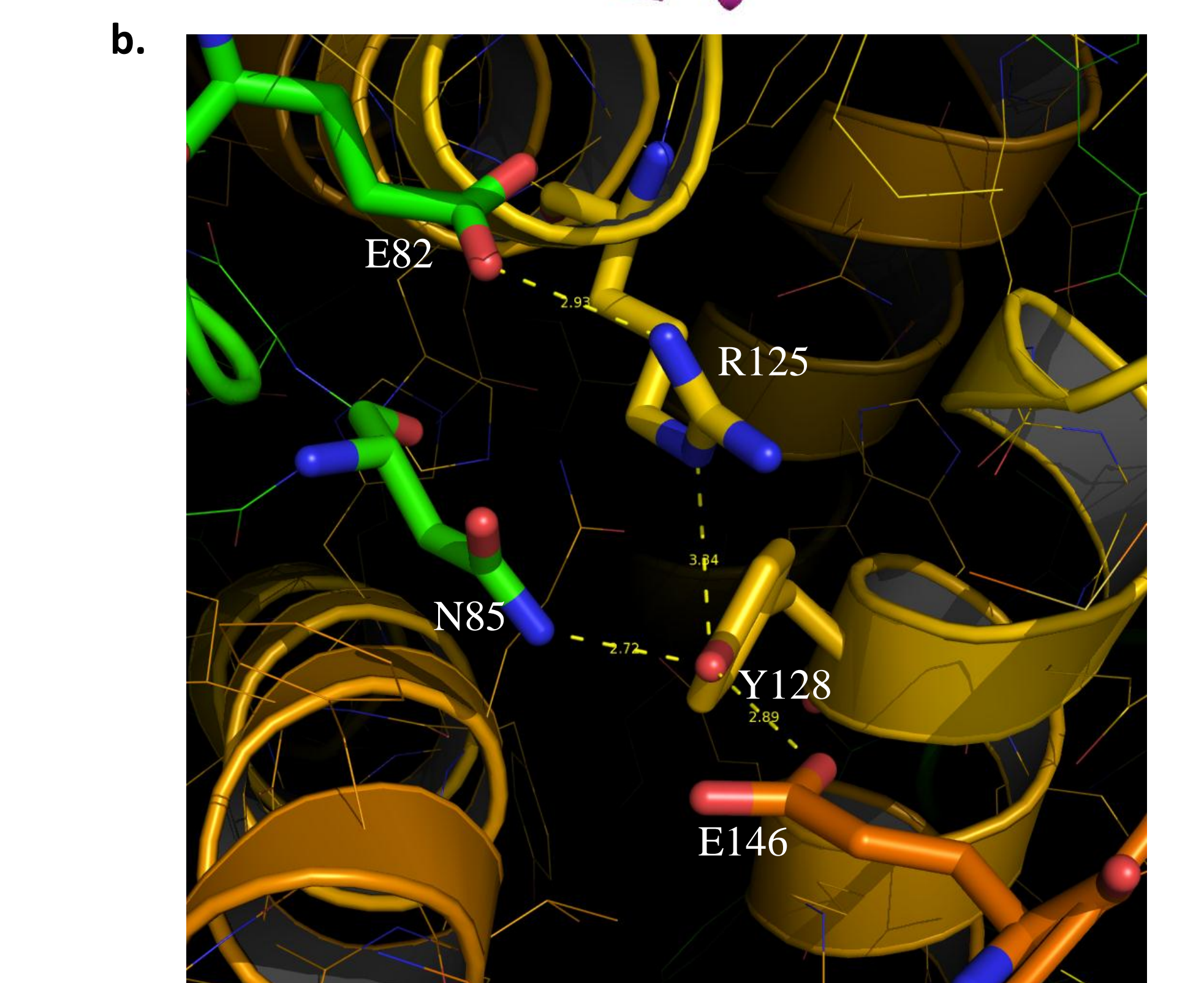
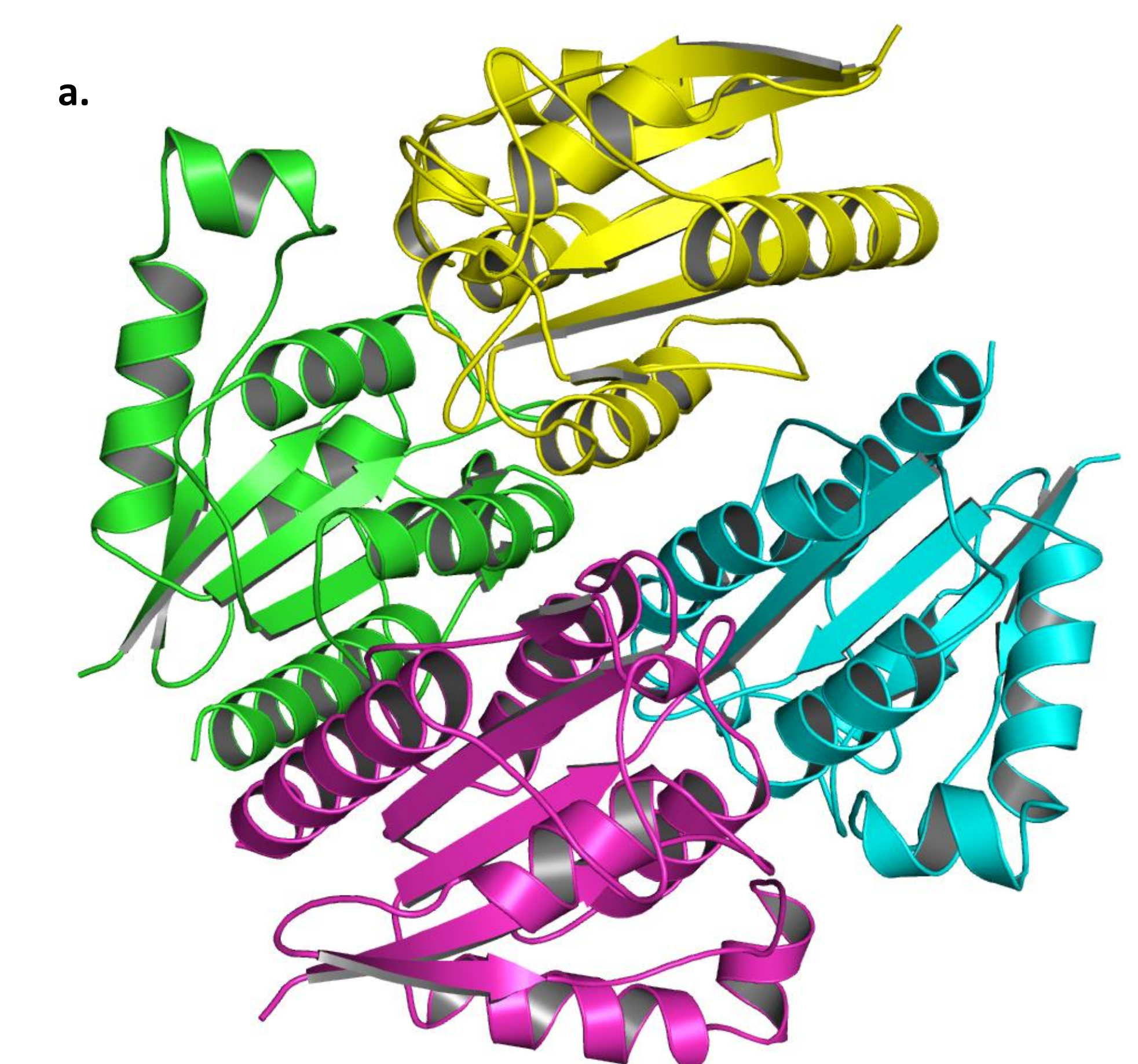
Data	Y128N mutant	Y85N mutant
Detector distance	430mm	300mm
Exposure rate	1°oscillation/ 1 sec	1°oscillation/ 1 sec
Images collected	360	360
Space group	P6 ₃ 22	P622
Unit cell Parameters	a= b= 103.33 Å c= 134.08 Å α= β= 90.0° γ= 120.0°	a= b= 103.776 Å c= 66.037 Å α= β= 90.0° γ= 120.0°
Resolution	3.8 Å	2.7 Å
Indexing	Good	Good
Mosaicity	1.27	0.476
Wavelength	0.9791 Å	1.075 Å
R-merge	0.33	0.08

Table 1. Data collection measurements from Y128N and Y85N mutant crystals.



Results and Discussion

All four mutant proteins were obtained in pure form, and successfully crystallized. Crystals from Y128N, and Y85N mutants were large enough to mount for x-ray diffraction analysis (Figs 3). Crystals from Y128N diffracted to approximately 4.0 Å resolution. Mutant Y85N crystals diffracted to 2.7 Å resolution. Using molecular replacement method, the crystal structure was solved for Y85N mutant (Figs 5). Structure was refined as a rigid body, and further refinement is in progress. Though the hydrogen bond formations at the tetramer interface varied slightly from the native structure, the overall tetramer interface is not changed by the mutation. The structure determined that all four residues in study Y128, E146, R125 and Y85N (Y85 replaced by N85) were in good contact, and the enzyme's activity is expected to function at the same capacity as the wild type enzyme.



Figures 5. Structure of ChrR mutant Y85N. (a) Demonstrates the tetrameric association, and (b) shows the hydrogen-bond formations at the tetramer interface.

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References

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