1.82 Å Membrane Protein Structure

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First Cryo-EM Structure of the Membrane Protein GLRA3 at 1.82 Å Resolution with Clear Side Chain Densities and ~500 Water Molecules

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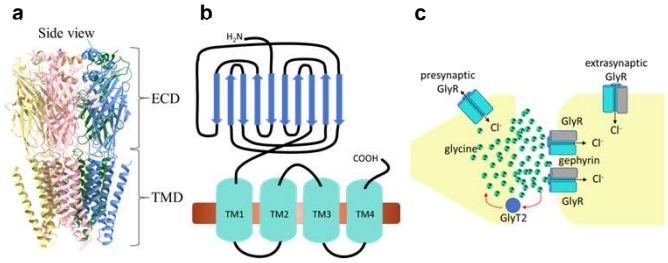
Abstract

GLRA3 (Glycine Receptor Alpha 3), a ligand-gated ion channel subtype, is predominantly found in the central nervous system, with high expression in the spinal cord and brainstem. It is crucial for regulating neurotransmission and neural excitability and is implicated in several neurological diseases such as chronic pain, epilepsy, and autism spectrum disorder. Dysfunction in the spinal cord GLRA3 may cause hyperalgesia, while its deficiency can contribute to epilepsy and disrupt neural network balance in autism. Currently, drugs targeting GLRA3 are in early-stage research and preclinical trials, with no extensive clinical use yet. When we start the project, there was only X-ray crystal structures GLRA3 have been reported with limited resolutions, and no cryo-EM structures were reported. In this study, we purified GLRA3 protein in detergent, prepared cryo-EM grids, collected cryo-EM data, and determined the cryo-EM structure at 1.82 Å high resolution. The 1.82 Å map provides detailed electron density for most amino acid side chains, including approximately 500 water molecules. This research lays a structural foundation for developing GLRA3-targeted therapeutics, aiding in the design of precise compounds or antibodies targeting its active sites.

Introduction

Fig.1 Background of GLRs



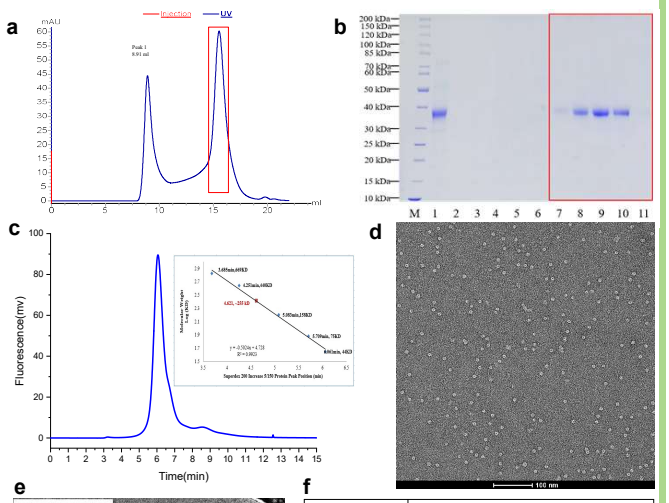
a. Pentameric ligand-gated ion channels that mediate fast inhibitory synaptic transmission. In vivo, GLRA3 can exist as homopentamers containing only α-subunits or hetero pentamers comprising both α- and β-subunits.
b. Two subfamilies: GLRA, expressed in the spinal cord and brain stem; GLRB, expressed in the retina, a monomer of 46 kDa, it has 4 transmembrane domains.
c. Upon binding of the neurotransmitter glycine to the extracellular domain (ECD), GLRs undergo conformational changes that allow the transmembrane domain (TMD) to selectively open to permeant anions such as chloride.

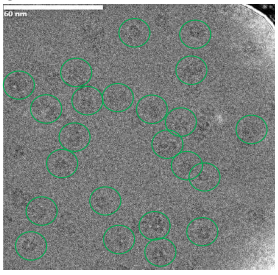
Aim of The Study

- Determine the first cryo-EM structure of GLRA3.
- Optimize the sample protein production, cryo-EM sample preparation, cryo-EM data collection, image processing, and model building of GLRA3.
- Push the resolution of GLRA3 better than 2.0 Å.

Results

Fig.2 GLRA3 Protein Production, QCs, and Cryo-EM Data Collection





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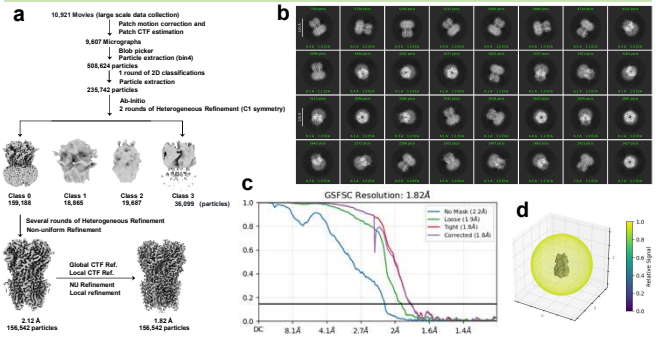
Sample	GLRA3
Concentration	1.97 mg/ml
Buffer	20 mM Tris-Cl (pH 8.0), 150 mM NaCl, and 0.005% GDN
EM	Titan Krios G4 at Biortus
Camera	Falcon 4i
Voltage	300 kV
Pixel Size	0.580 Å/pix
Movie	10921
Magnification	215 K
Movie format	EER format
Total dose	60 e⁻/Å²
defocus range	-1.0 ~ -1.8 um
Mode	Counting mode bin 1
Motion correction	Patch motion correction

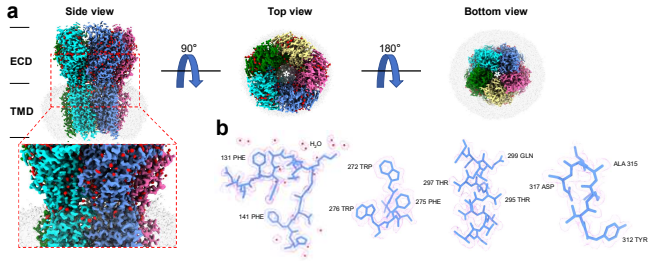
a, b. Size-exclusion chromatography and SDS-PAGE; **c.** QC1: Analytical SEC; **d.** QC2: Negative Staining; **e.** QC3: Representative cryo-EM micrographs; **f.** Cryo-EM data collection parameters

Fig.3 Image Processing of GLRA3 at 1.82 Å Using CryoSPARC



a. Flowchart for cryo-EM image processing. **b.** Cryo-EM 2D Classes of GLRA3. **c.** Gold-standard Fourier shell correlation (GSFSC) curve for GLRA3 map generated using cryoSPARC 4.7. **d.** Euler angle distribution of the classified particles used for the final three-dimensional refinement of the overall map.

Fig.4 Clear Side Chain Densities and ~500 Water Molecules in GLRA3

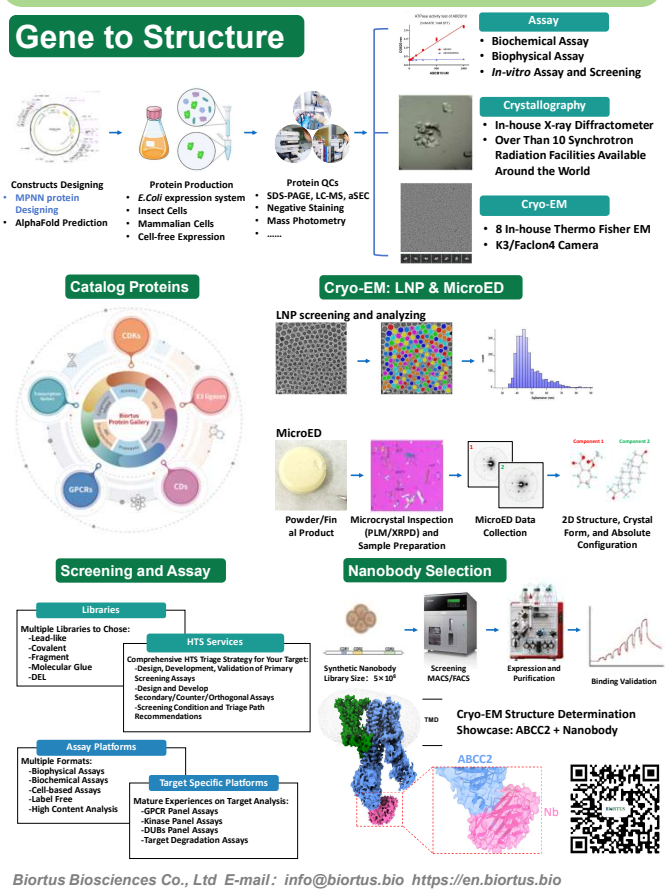


a. The final cryo-EM map has side views, top views, bottom views, and a zoomed-in side view showing water molecules. **b.** Representative amino acid and water molecules density.

Conclusions

- We expressed and purified of 2.4 L Hi-5 insect cells, and finally obtained 0.23 mg of high-quality GLRA3 protein for cryo-EM sample preparation. We optimized the sample protein production, cryo-EM sample preparation, cryo-EM data collection, image processing, and model building.
- We initially obtained a map at 1.94 Å resolution and submitted to EMDB (EMD-61518), then using the same dataset, we pushed the resolution to 1.82 Å. Most side chains of the amino acids are very clear. There are ~500 water molecules resolved.
- In the next, we plan to add ligand/s and try to resolve the high-resolution cryo-EM structure of the complex.

Gene to Structure



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