

Figure 1 Protein Prep: Top left, membrane and cell lysate extracts show expression of hetero-GlyR subunits (ZF GlyR $\alpha$ , GlyR $\beta$ b) as well as the co-expressed GlyR binding domain of gephyrin (Geph-DE). Each subunit contains a distinct tag as shown. Western blots were stained separately but imaged together. Top right, the gel filtration profile following purification, the protein peak used for imaging is shown with an arrow. Below, a representative micrograph and the resulting 2D classes from data collected of hetero-GlyR in the presence of 1 mM glycine.

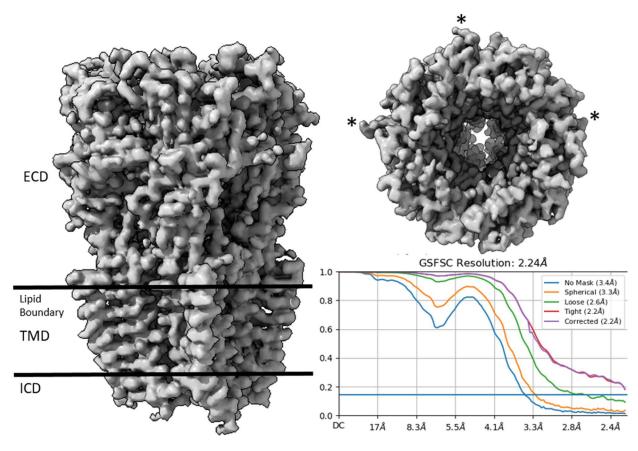


Figure 2: The resulting structure of hetero-GlyR in the presence of 1 mM glycine shown from the side and looking down the pore axis. Data came from about 10,000 movies and 200K particles collected at Case Western Reserve University. We believe there is a  $3\alpha:2\beta$  ratio based on subunit specific glycosylation patterns as shown with asterisks in the top right image.

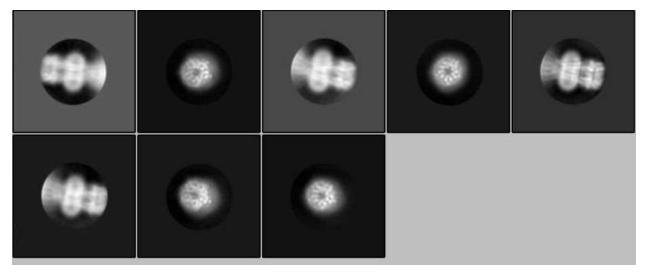


Figure 3: Preliminary 2D classes of hetero-GlyR collected in the presence of 1 mM glycine and 1  $\mu$ M ivermectin. These are from about 11,000 movies and about 180K particles in these classes from data recently collected at NYSBC.