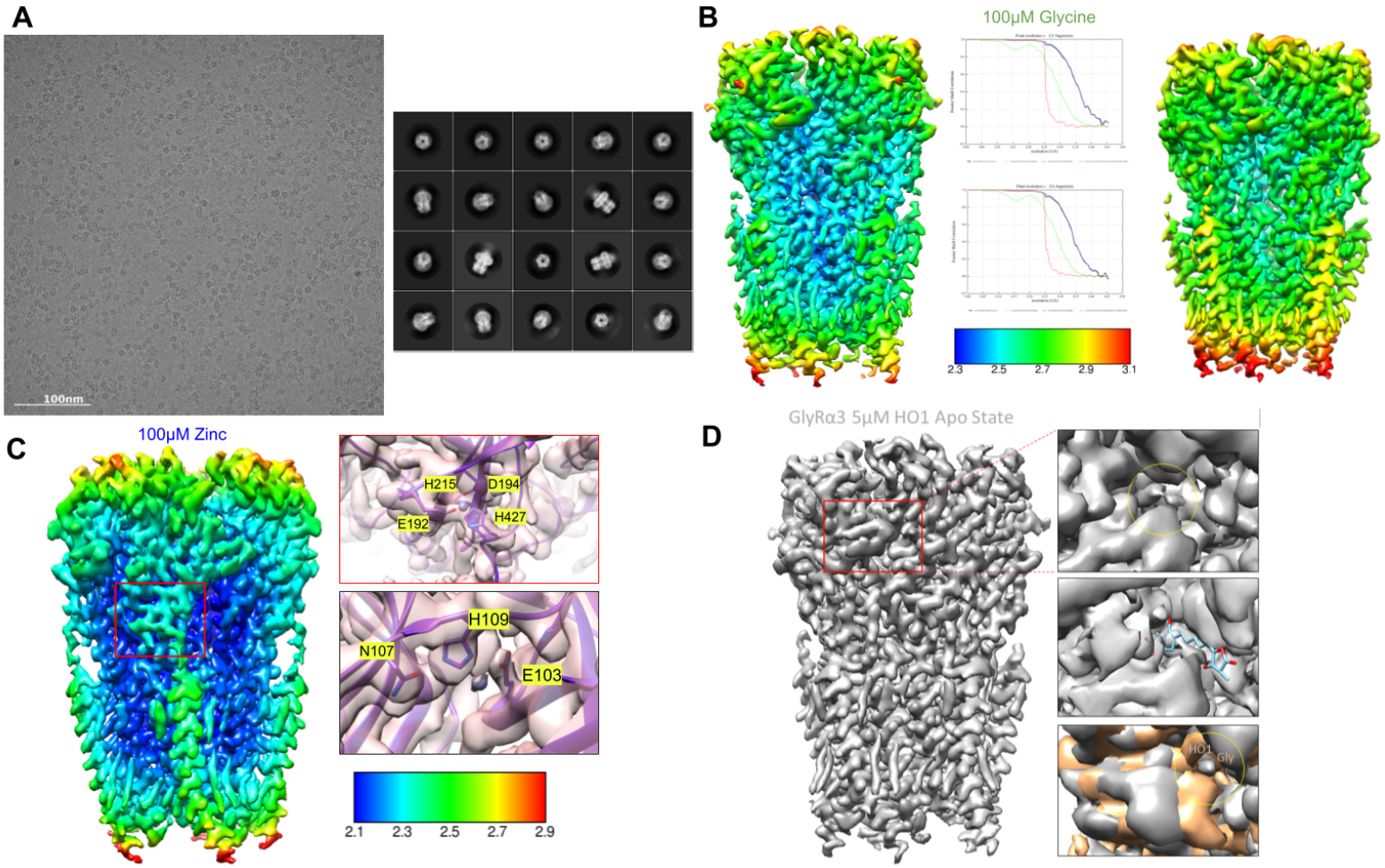

Figure 1. Structural characterization of GlyRα3 modulation. A) (Left) A sample micrograph of GlyRα3 with 1mM glycine on a Quantifoil Cu 1.2/1.3 grid. Image collected on Glacios 1 at NCCAT. **(Right)** 2D classes of the 1mM glycine sample after data collection on Krios 6 at NCCAT. Processed in Relion, pixel size of 1.069Å/px, ~1.1million particles total in all classes. **B)** Local resolution maps and FSC curves of GlyRα3 solved with 100μM glycine. The map on the left is the resting/apo state (2.5Å resolution), and the map on the right is the desensitized state (2.6Å resolution) that occurs after glycine binding. Data was processed with C5 symmetry, collected at CCMSB with a pixel size of 1.1Å/px. **C)** Local resolution map of GlyRα3 with 100μM zinc and 100μM glycine in the resting/apo state. The red box encloses one of two zinc binding sites on the receptor, which is expanded in the top inset on the right. The zinc ion is coordinated by two histidines and two negatively-charged residues. The bottom inset shows the second zinc binding site, not visible in the map on the left. Data was processed with C5 symmetry, collected at CCMSB with a pixel size of 0.84Å/px. **D)** Map of GlyRα3 in the apo state with a positive modulator. The red box encloses the glycine binding site, which is expanded in the right inset on the top. The middle box shows the rough placement of the ligand model in the binding site for size comparison. The bottom box shows an overlay of this map with a glycine-bound state, illustrating that the modulator binds close to the glycine binding site but the two densities do not overlap. Data was processed with C1 symmetry, 2.9Å resolution, collected on Krios 6 at NCCAT with a pixel size of 1.069Å/px.