

Figure 1. Western Blot Analysis of FLAG-tagged Components of the Signaling Complex.

293T cells (2 ml) were seeded in a 6-well plate and infected the following day with 75 μ l of P2 baculovirus. Sodium butyrate was added 8 hours later to a final concentration of 10 mM. Cells were harvested 72 hours post-infection, lysed via sonication, and centrifuged at 13,000 rpm for 10 minutes. The resulting supernatant was subjected to Western blot analysis using antibodies against the FLAG tag. The expected molecular weights of the FLAG-tagged components are as follows: *C. elegans* G protein β subunit, 38.4 kDa; *C. elegans* hormone α 2 chain, 11.5 kDa; truncated CeLGR31_712 (80 kDa, with the last 200 amino acids truncated due to disorder and native signal peptide replaced with an HA signal peptide for enhanced solubility). All components were observed at their expected molecular weights and were soluble. *C. elegans* G protein α subunit, γ subunit, and hormone β subunit do not have a tag, thus cannot be tested with WB.

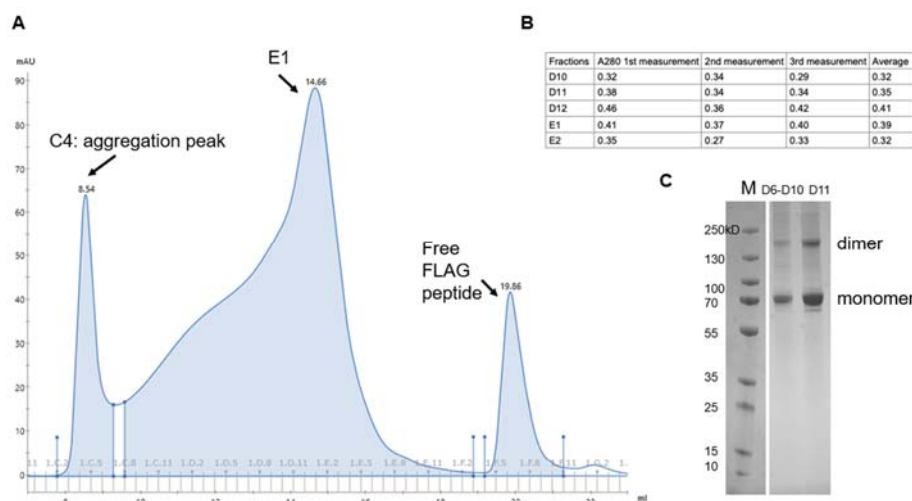


Figure 2. Size Exclusion Chromatography of CeLGR31_712.

(A) Size exclusion chromatogram of CeLGR31_712. (B) A280 absorbance measurements of eluted fractions. (C) SDS-PAGE analysis of the peak fraction and adjacent fractions.

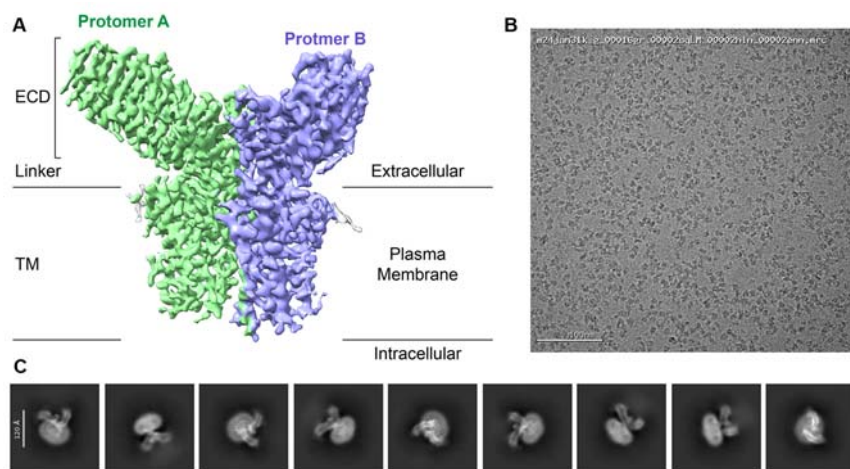


Figure 3. Density Map of CeLGR Receptor Dimer and Representative Micrograph and 2D Classes.

(A) Density map of CeLGR receptor dimer. (B) Representative micrograph for receptor sample, acquired using a Glacios microscope. (C) Representative 2D classes of the receptor. The dataset was obtained with a Titan Krios microscope.