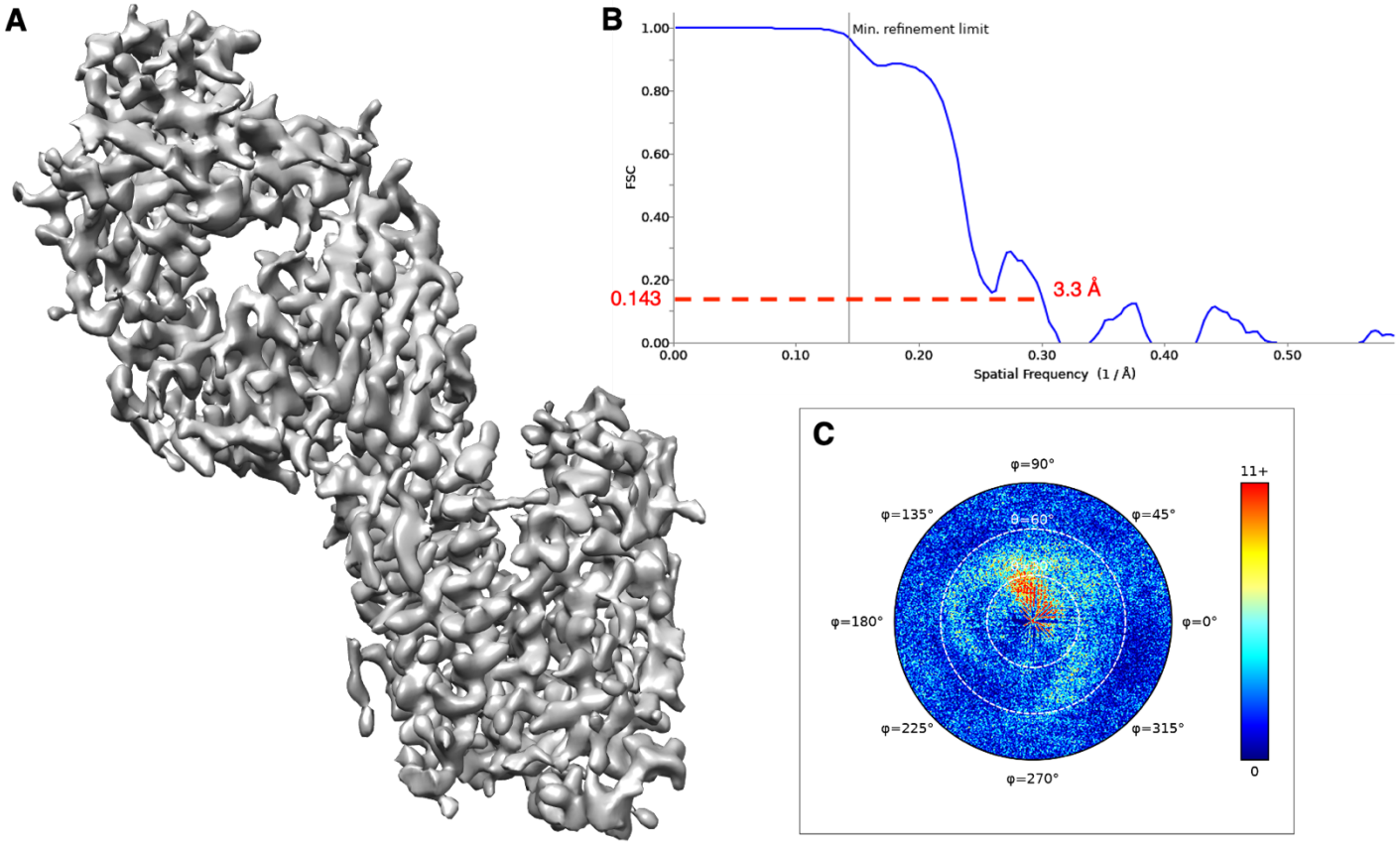


**Figure 1. Structure of the apo VGLUT-Fab complex.** Current cryo-EM reconstruction of VGLUT-Fab complex without ligand **(A)** is determined to 3.3 Å resolution **(B)** but with preferred top-view **(C)**.

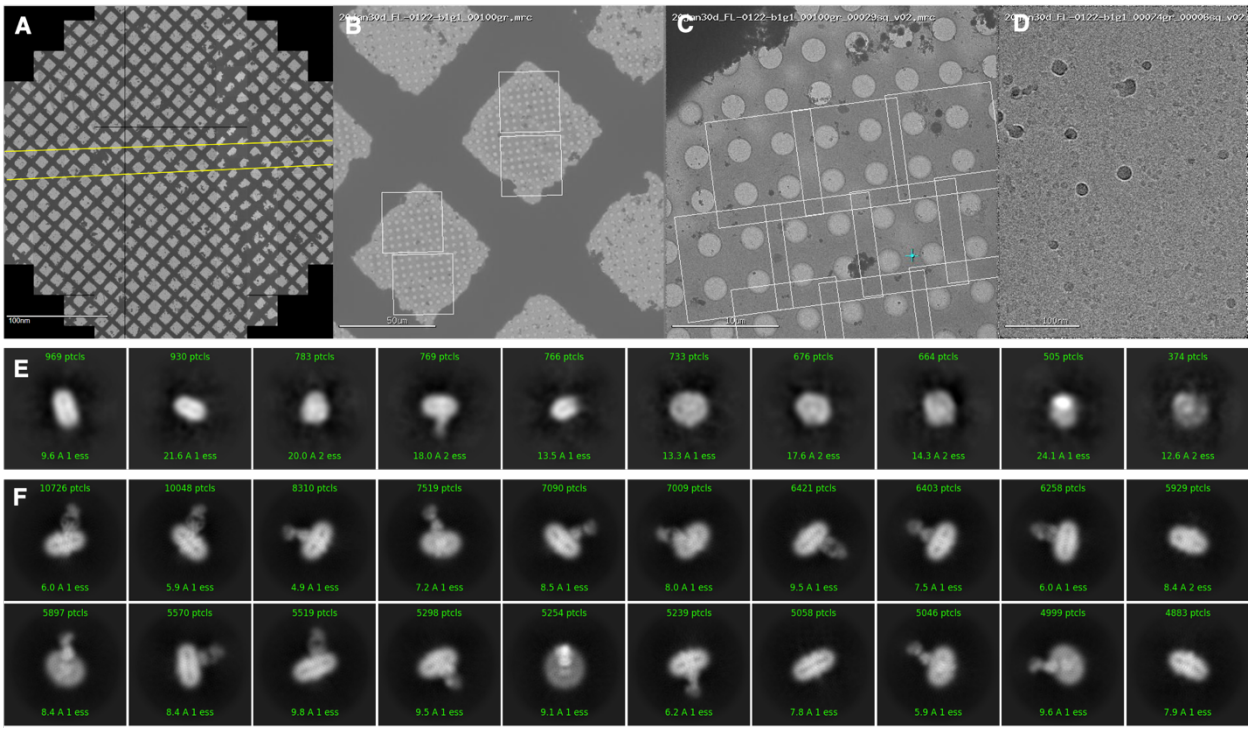


**Figure 2. Preliminary data collected from grid frozen by Chameleon.** Preliminary screening result of grid frozen with chameleon shows that sufficient area of the grid is covered with thin ice (between yellow lines in **(A)**), and with squares **(B)** and holes **(C)** suitable for automated data collection. **(D)** A representative micrograph collected from grid shown in **(A)** shows that particles are evenly distributed and with sufficient concentration. **(E)** Preliminary 2D class averages showing that particles obtained from the current grid are comparable with previous dataset collected for the apo protein (**F**).

**Project Name** * Structural Basis of Quantal Release

**Abstract**.
Communication between neurons relies on the quantal release of classical neurotransmitters by exocytosis, a process that requires their packaging inside synaptic vesicles. The amount of transmitter released per vesicle in turn dictates the extent of receptor activation and which receptors are activated, *e.g.,* low versus high affinity and synaptic versus extrasynaptic, and hence downstream signaling. Transport of neurotransmitter into synaptic vesicles is driven by a $H^+$ electrochemical gradient ($\Delta\mu_{H+}=\Delta pH+\Delta\psi$), created by the vacuolar-type $H^+$-ATPase (V-ATPase) that pumps $H^+$ into synaptic vesicles. As a result, the activity of the V-ATPase as well as other factors influencing the balance between the chemical gradient ($\Delta$pH) and membrane potential ($\Delta\psi$) regulate vesicular neurotransmitter transport. Despite its fundamental importance, the mechanism and regulation of neurotransmitter packaging remain poorly understood. Vesicular glutamate transporters (VGLUTs) are located on the synaptic vesicle membrane of glutamatergic neurons and transport the major excitatory neurotransmitter glutamate into synaptic vesicles, thus playing a critical role in synaptic transmission. They predominantly use membrane potential ($\Delta\psi$) as driving force and are also allosterically regulated by $H^+$ and $Cl^-$ and exhibit an associated $Cl^-$ conductance. However, the mechanism and physiological significance of these unusual but highly conserved properties remain unknown. VGLUTs also exhibit incredible high specificity toward the substrate; the very similar aspartate that is often recognized by other glutamate transporters and receptors is not transported by VGLUTs. In this project, we seek to define the mechanisms for substrate recognition and allosteric regulation of VGLUTs by determining high-resolution structures of a VGLUT in different pH and $Cl^-$ conditions with and without substrate analog using single particle cryo-electron microscopy (cryo-EM).

**Scientific Impact** *
The proposed studies will provide new insight into the regulation of quantal release. The high-resolution cryo-EM structures of a VGLUT at different states determined will provide the opportunity for me to investigate VGLUT function with molecular details. To achieve the proposed goals, I will develop strategies to overcome current limitations to allow high-resolution structure determination of small membrane proteins by single particle cryo-EM. As integral membrane proteins of ~60 kDa, VGLUTs serve as excellent platforms for me to develop new strategies that will make cryo-EM accessible to thousands of membrane proteins currently not tractable using this powerful methodology. As a transporter, cycling between multiple conformations is required for function but presents a challenge for structure determination. Cryo-EM could be extremely useful in such situations as it allows a certain degree of conformational heterogeneity and also provides the possibility of capturing multiple conformations, exactly what is required to understand the transport mechanism. However, cryo-EM methodology needs to be optimized for proteins such as VGLUTs. In particular, while the theoretical lower size limitation for cryo-EM is 38 kDa[26], proteins around 100 kDa are currently still challenging. Detergent micelles present additional challenges for data processing. I will develop several key strategies that will be highly applicable to other similar proteins and systems.

**Scientific Feasibility** *
Cryo-EM has becoming a powerful tool for protein structure determination. However, it remains challenging to determine structure of proteins below 100 kDa, especially membrane proteins as they are embedded in the detergent micelles. Given that the majority of membrane proteins are in fact smaller than 100 kDa, VGLUTs (~60 kDa) serve as excellent tests for developing new strategies that would allow cryo-EM to be used with a large number of membrane proteins not currently accessible to the technology. Using a high affinity Fab against VGLUT, I have determined an apo structure of VGLUT to ~3.3 Å resolution (Fig. 1). Structure of VGLUT at multiple states during the transport cycle with and without substrate analog will elucidate their transport mechanisms and regulation with molecular details.

**Technical Feasibility** *
Through the GUP2 program at NCCAT, Dr. Wei has optimized the sample freezing condition of the VGLUT-Fab complex with a substrate analog ACPD using Chameleon. He has prepared 4 grids with sufficient area with suitable ice for automated data collection (Fig. 2A-E). Preliminary 2D class average shows that this sample contains particles compared to the VGLUT-Fab complex without ligand (Fig. 2F). Previous data collected with the apo protein sample yielded a reconstruction at ~3.3 Å resolution using around 1.3 million total particles from ~3500 micrographs (Fig. 1).

**Resources Requested** *
To obtained around 1-2 million particles, I expect to require 2 days of microscope time on a Titan Krios.

**Geographic/Demographics** *
UCSF has 1 titan Krios available to my project. However, time on this instrument is mostly allocated to the core EM labs (Cheng lab, Aragd lab, and Frost lab). The Stroud and Edwards lab both belong to the “other user” group, which include all labs at UCSF who wishes to use this microscope. Currently, 4 days/months is allocated to the other users and shared among multiple labs. It is thus extremely hard to get enough time to collect enough data for my project.