General User Proposals

Example GUP3 review for reference

Project ID: NCCAT-GUP3-XG211223
Project name: Structural Basis of CFTR Dysfunction and Pharmacology
Primary username: Xiaolong Gao
eRA Commons username: 0000-0001-8933-9286
Institution: University of Missouri-Columbia
Submission date: 12/23/2021

Averaged URC scores:
(i) scientific impact: **1.3**
(ii) technical feasibility: **1**
(iii) resources requested: **2.7**
(iv) user EM background and history: **2**
(v) geographical demographics or need: **1.3**
Raw average score: 1.7

Comments:

Reviewer 1:

The applicant proposed to capture CFTR in different conformations and in complex of drugs by cryo-EM. The preliminary data showed good protein purification results and promising 2D class averages for structural determination. However, the proposed goals (determine CFTR structures in different conformations and ligand-bound states) and most of the proposed experiments (use half-day of each 2-day session for grid screening) is beyond the scope of GUP3 proposals which are dedicated for freezing and grid screening.

Reviewer 2:

This proposal consists of two parts, examine the CFTR chloride channel in different conformations and to identify drug binding sites. In total they are requesting 7 x 2 days on a Krios, and 5 x ½ days on a screening scope prior to each Krios session. For the first part, the number and description of the multiple conformations should be indicated.

Project ID: NCCAT-GUP3-XG211223
Project name: Structural Basis of CFTR Dysfunction and Pharmacology
Primary username: Xiaolong Gao
eRA Commons username: 0000-0001-8933-9286
Institution: University of Missouri-Columbia
Submission date: 12/23/2021

Project Abstract:
Malfunction of the CFTR chloride channel causes cystic fibrosis (CF), the most common fetal genetic disease among Caucasians. Decades of basic research have provided significant insights into CFTR's working mechanism, and pharmaceutical development in the past ten years have delivered several clinically-effective drugs to remedy the dysfunction of disease-associated CFTR. However, a comprehensive understanding of CFTR's function remains unattainable due to a lack of enough structural information of the protein. Meanwhile, with more and more CF drugs are being developed, the structural interactions between CFTR and different compounds become highly relevant for designing even better CF drugs. To tackle these issues, we will take advantage of the accumulated fundamental insights from three decades of intensive research on CFTR to design proper cryo-EM experiments to resolve the conformational changes of CFTR during its gating cycle and to illuminate the mechanism of action for CFTR modulators.

Aims & Impact:
Aim 1: To obtain the open channel and physiologically relevant intermediate channel structures of CFTR. These structures will help construct a more complete conformational change pathway in CFTR's gating cycle. Moreover, we would also determine the structures of several badly-needed disease-associated CFTR variants. Such structural information will shed light on the pathogenesis of each CF genotype and serve as a template guiding accurate therapy prescription in the era of personalized medicine.
Aim 2: To identify the binding sites on CFTR for clinically approved drugs [redacted] and compounds with known pharmacological effects on CFTR gating. Structural studies using these drugs will reveal the specific interactions between the CFTR protein and these compounds. It is anticipated that insights derived from this line of research will be valuable for designing and optimizing CF drugs to achieve better clinical outcomes.

Feasibility & Data:
Human CFTR has a molecular weight of ~130 kDa (deglycosylated). We can readily purify the CFTR protein in large quantity in a mammalian expression system. The protein is functional when reconstituted in lipid bilayers, stable in buffer and can be concentrated to 6 or 7 mg/ml for cryo-grids freezing. Initially, we plan to image the protein in detergent, but we will consider to reconstitute it into nanodiscs if a lipid environment may be needed to permit multiple conformations.
As demonstrated in Figure 1 (Preliminary Results), the size exclusion chromatography (SEC) profile shows that the protein is eluted homogeneously at about 15 ml position on Superose 6 Increase 10/300 column and the monomeric protein between the two red lines is collected for single particle study (Figure 1A). The SDS-PAGE gel reveals the right molecular weight of the CFTR protein off the column (Figure 1B). With the protein in detergent, our negative staining images (Figure 1C and D) show

that the protein molecules are separated well from each other, denoting great mono-dispersity. We have initially imaged the cryo-EM grids frozen with CFTR in detergent (Figure 1E). The resulting cryo-EM images show uniformly distributed particles with clear contours of CFTR proteins in detergent as published previously. Our initial data processing in cryoSPARC also generated very nice 2D classes of CFTR protein (Figure 1F). We are thus optimistic that our purified CFTR proteins are proper samples for cryo-EM study.

Proposed Experiments:

For Aim 1, we first will determine the structure of CFTR bound with [redacted]. An alternative strategy to capture the open state is to image CFTR in the buffer [redacted] as this [redacted] can bind at the narrow region and lead to a constantly open channel. For the disease-associated CFTR mutants, we plan to determine the structure of G551D-CFTR, G1349D-CFTR and R117H-CFTR. We expect to obtain different conformations of CFTR and disease-associated CFTR variants.

For Aim 2, we would solve the structure of CFTR bound with CF drug [redacted] We expect to see high resolution density of both the protein and the compound to identify specific amino acid residues in CFTR that interact with the drug.

Goals & Expectations:

The goals of this project are two folds: Firstly, to obtain multiple conformations of CFTR chloride channel (including those of disease-associated CFTR variants). Secondly, to identify drug binding site(s) on CFTR. For the first goal, we will need at least five 2-day Krios sessions each led by a half-day screening session on Glacios or equivalent for our grids. At our home institute, we are able to freeze the cryo-grids with Vitrobot Mark IV, but we lack the microscopes for grids screening and data collection. For the second goal, we initially request two 2-day Krios sessions each led by a half-day screening session on Glacios or equivalent. Besides, we need the center to clip all cryo-grids for us as our home institute does not have a clipping station yet.

Expertise & Resources:

The spokesperson (Xiaolong Gao) was trained for five years on cryo-EM at Weill Cornell Medicine and is an active user of NYSBC facility (both under previous project registered with Cornell and under GUP1 program now at NCCAT). Xiaolong is proficient in sample vitrification with Vitrobot and was trained on JOEL JEM-1400 TEM and Thermo Fisher Glacios with SerialEM. He is familiar with data collection work flow on Arctica/Glacios/Krios. He has solved multiple cryo-EM structures of ion channel proteins and is equipped with a full pipeline of knowledge from sample preparation to final model building. Xiaolong has collected cryo-EM data many times at NYSBC on site. The principal investigator (TC Hwang) has been studying CFTR for almost three decades and is known for his work on structural and functional relationship of the CFTR protein.

The University of Missouri-Columbia provides enough space for data storage as well as a cluster with many GPU and CPU cores for data analysis. The EM core at MU will also provide access to workstations and technical assistance at all times.

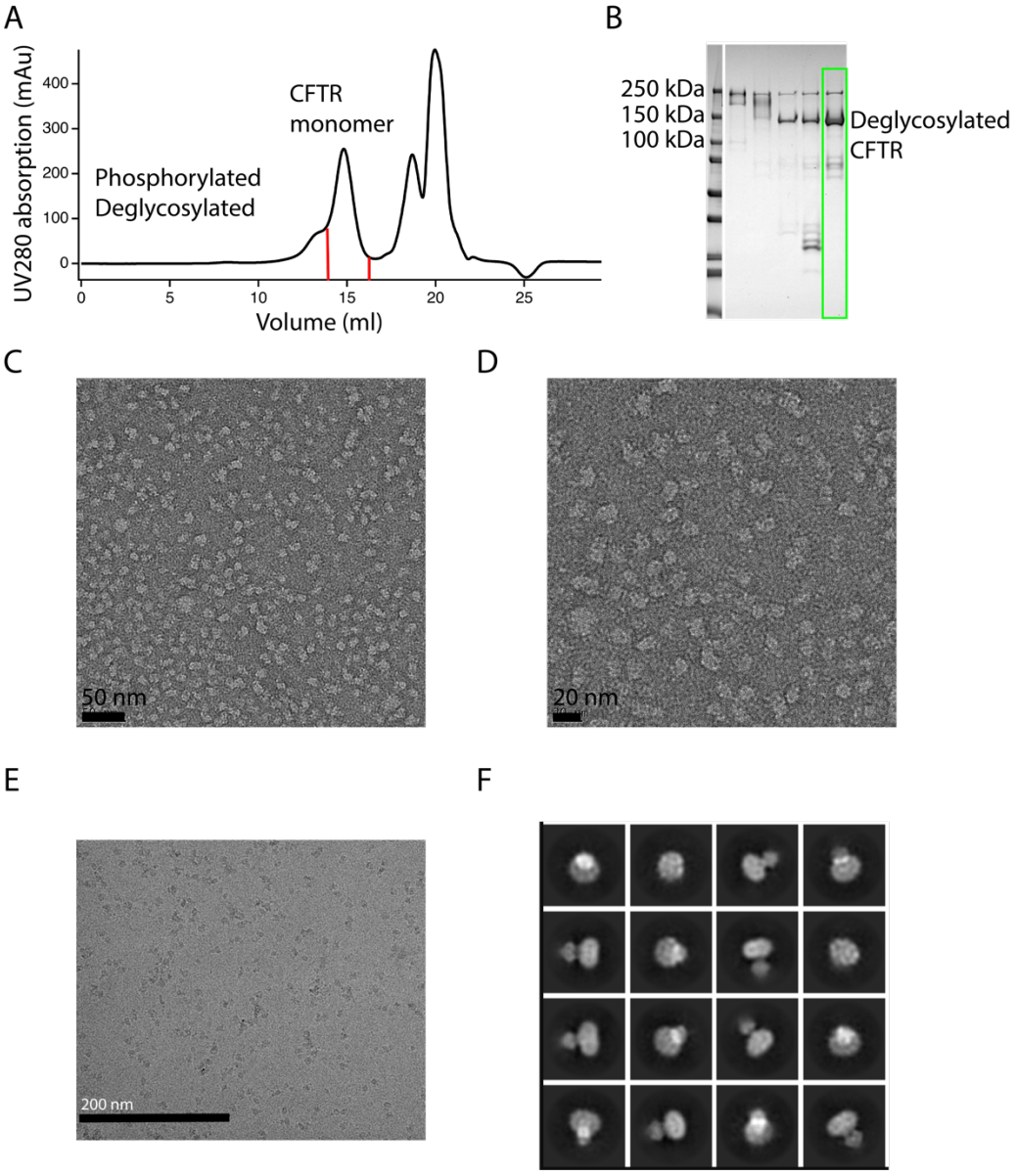

Figure 1. Protein purification and initial EM data of CFTR chloride channel. A. Size exclusion chromatography profile showing CFTR monomer can be readily purified in detergent. **B**. SDS-PAGE gel indicating the deglycosylated CFTR has a molecular weight at around 130 kDa. **C and D**. Negative staining images under different magnifications showing CFTR molecules separate well when in detergent. Different scale bars at the bottom of the image also indicate the right size of the protein. **E**. cryo-EM images of CFTR (magnification: 81000) showing the CFTR particles uniformly distribute in the vitrified ice. **F**. Initial 2D classes of CFTR generated in cryoSPARC showing multiple orientations of the molecule in the cryo-EM images.