Your Name: Richard Walsh
Your PI's Name: Li
Your collaborator Name(s):
Collaborator PI's Name(s):
Project ID: CEMC0000

Describe project progress and why you need more time:

This project is focused on the α4β2 subtype of the nicotinic acetylcholine receptor. We have already collected data on the Krios on this receptor target with agonist bound (proposal from August 2016) and propose collecting data with inhibitor in an attempt to elucidate a different conformation of this receptor. As with our two successful (published) projects on α4β2 nicotinic receptor and the GABA-A receptor, we used Fab fragments to break the 5-fold pseudo symmetry in particle alignment. We prepared EM grids of the receptor-Fab complex bound to an inhibitor and collected a small dataset on the Talos from 4/14-4/17. Data collection parameters and statistics are summarized in Table 1 and preliminary Data are shown in Figure 1. We propose to collect data on this receptor bound to inhibitor and therefore request an additional allotment of time on the Titan Krios to collect a dataset that we hope will allow us to obtain a 3-4 Å reconstruction. For this proposal, we request a total of 48 hr of microscope time.

Specimen molecular weight: ~250 kDa.
Specimen dimensions: 100 x 180Å
Current status

Microscope used for Collection	Talos Arctica
Pixel Size	1.47 Å/pix.
Number of Images	1150
Starting particle number:	373,340
Final particle number	126,164
Current Resolution	5.9 Å
Symmetry	C1

Preliminary Data:

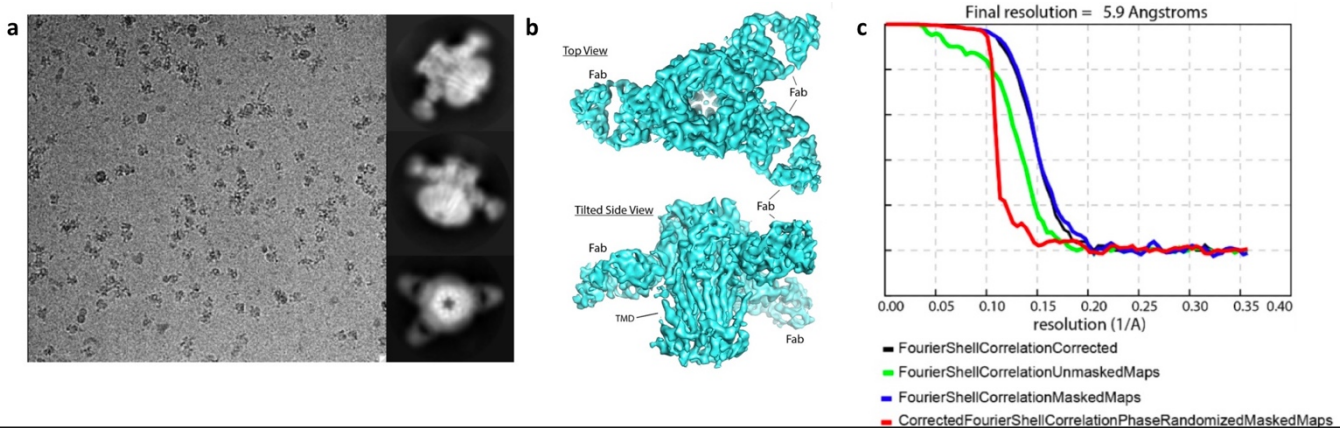

Figure 1: Preliminary data of α4β2:inhibitor complex. **a**, representative micrograph and subset of 2D class averages. **b**, Top and side view of 3D reconstruction. **c**, FSC curve estimating current resolution. Image processing and refinement were carried out using RELION 3.0.

Requested number of sessions on the Titan Krios (up to 4):
Phase Plate Required (Y/N): No