Pushing the performance limits of cryo-EM for membrane receptors

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Electron cryo-microscopy (cryo-EM) saw great advances in recent years. It became a method of choice for many researchers working on high-resolution structural studies of proteins and complexes. There are a few remaining challenges,[1] such as sample preparation, but overall cryo-EM has already entered the mainstream and is generating a constant flow of outstanding results.

In our projects, we use cryo-EM to investigate the structure-function relationships of G-protein-coupled receptors (GPCRs). Their relatively small size and lack of symmetry make them a challenging target for cryo-EM. To overcome the potential performance limitations, in our earlier work we used phase plates that greatly increase the contrast of images. Our recent results, however, show that phase plates are not necessary for obtaining high-resolution structures and only complicate the experiment. For the last two years, we have been collecting data exclusively without phase plates and were able to obtain multiple GPCR structures at better than 2.5 Å resolution (Figure 1). Furthermore, we observe protein dynamics that exemplify thermal motions as the main performance-limiting factor in present cryo-EM studies of GPCRs.

There is still room for improvement of the technique and many parameters can be optimized further. Recently, we quantified the effects of energy filtering, phase plates, objective lens aperture and defocus. Overall, the Volta phase plate does not provide practical benefits and noticeably reduces the achievable resolution. Zero-loss energy filtering and lower defocus have a positive effect on the performance, while the aperture does not seem to make a difference (Figure 2).[2]

In my talk, I will present several recent GPCR structures and discuss the practical aspects of present-day cryo-EM of membrane proteins. We currently use a multi-shot beam-image shift automated data acquisition approach that allows collection of more than 7000 cryo-EM moves per day. This allows the determination of the 3D structure of a properly optimized sample with just one day of data collection. The current bottleneck in terms of project time is data processing. It usually takes one to several weeks to process the data for each sample. Further developments in image processing methods based on deep learning and floating-point calculations code optimization would hopefully alleviate this issue.

1) R. Danev, H. Yanagisawa, M. Kikkawa, *Trends Biochem Sci.* **2019**, *44*, 837. 2) R. Danev, M. Belousoff, Y.-L. Liang, X. Zhang, D. Wootten, P.-M. Sexton, *bioRxiv* **2020**, 2020.08.21.260851.

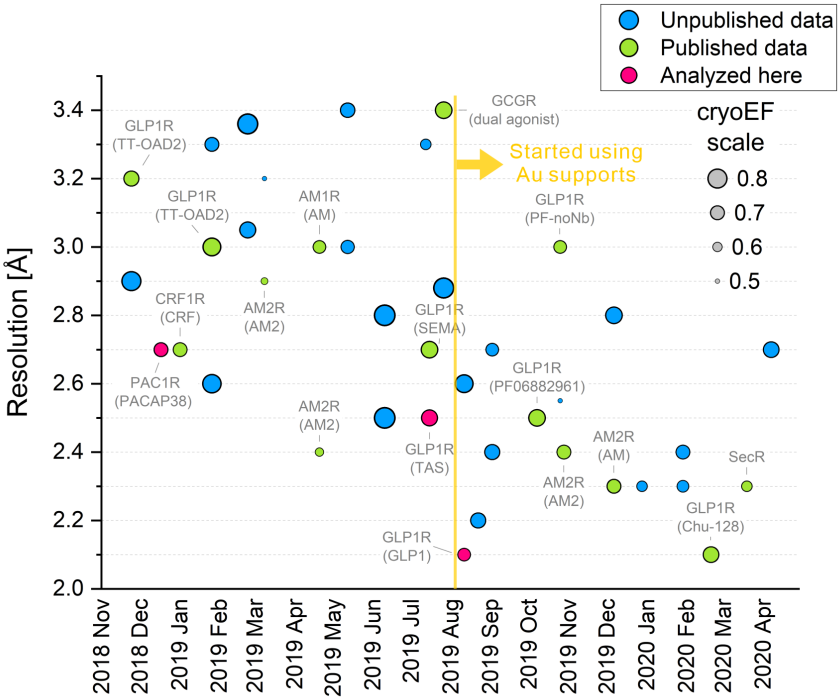

Figure 1. History of our cryo-EM GPCR reconstructions.
Over the year and a half period shown in the plot, there is a general trend towards better resolution with a significant improvement in August 2019, when the sample supports were switched from holey carbon films to gold foil grids.

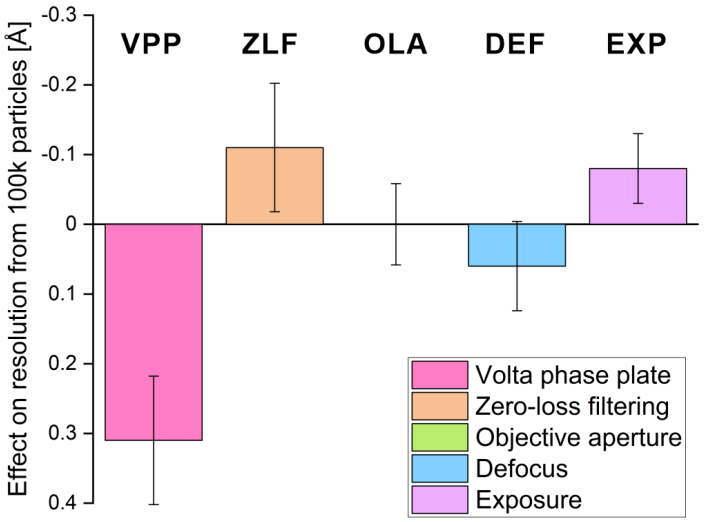

Figure 2. Effect of each experimental parameter on the cryo-EM performance for GPCRs.
VPP: Volta phase plate; ZLF: Zero-loss energy filtering; OLA: Objective lens aperture; DEF: defocus magnitude; EXP: Total electron exposure.