Structural basis of the Borna disease virus (BDV) RNA polymerase

Project Objective:

The primary goal of this proposal is to decipher the structural basis of the Borna disease virus (BDV) synthesis machine, known as the BDV polymerase (L:P) complex.

Preliminary Results:

The BDV polymerase (L:P) catalyzes three enzymatic reactions using a single catalytic subunit (L): RNA polymerization, 5' cap addition, and 5' cap methylation activities[1]. We have successfully expressed and purified full-length BDV L and P proteins using sf21 insect cells. We also prepared the BDV P using *E. coli* cells. We included SDS-PAGE gel and size exclusion chromatography (SEC) data of the prepared proteins. In summary, we demonstrated that we could prepare and purify recombinant full-length BDV L and P proteins (**Fig. 1**).

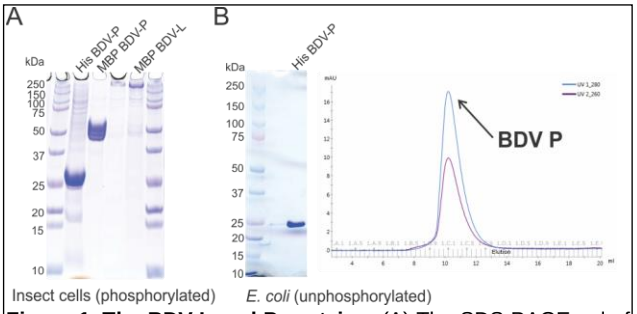

Figure 1. The BDV L and P proteins. (A) The SDS-PAGE gel of the BDV L and P proteins as phosphorylated forms expressed in insect cells. (B) The SDS-PAGE gel and FPLC filtration profile of the BDV P protein as the unphosphorylated form in *E. coli*.

We will use negative stain EM to guide the preparation of homogenous biological samples. We will screen the sample conditions, optimize the data collection strategy, and collect and process data to obtain the preliminary cryo-EM analysis. Briefly, we first screen and optimize the buffer and freezing conditions of the cryo-EM specimen. To prepare cryo-specimens for high-resolution cryo-EM studies, we will test different compositions of the buffers, various freezing conditions (e.g., glow discharging time, sample volume, humidity, blotting time), and different types of grids (e.g., Quantifoil, or Au grids). After data acquisition, we will follow the standard procedure to calculate 2D class averages and 3D reconstructions of the complex with a combination of program suites cryoSPARC, RELION, and cisTEM[2-4]. Once the maximum attainable resolution of the 3D map is obtained, we will use Coot[5] to build and refine the molecular model. We will perform refinement procedures using CCP4 and PHENIX programs[6,7]. In recent exciting preliminary data, we have successfully obtained a 4.6 Å reconstruction of the apo BDV L protein (**Fig. 2**), and we plan to collect and analyze more high-quality data of BDV L protein alone and the polymerase (L:P) to reach the atomic resolution. These studies will provide key structural insights into the mechanisms of BDV RNA synthesis.

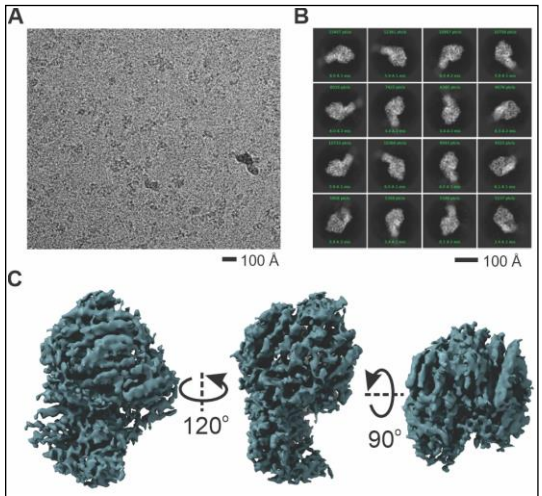

Figure 2. The preliminary cryo-EM analysis of the BDV L protein. **(A)** Raw image of BDV L. **(B)** 2D class averages of BDV L. **(C)** Preliminary 3D reconstruction of the BDV L at 4.6 Å.

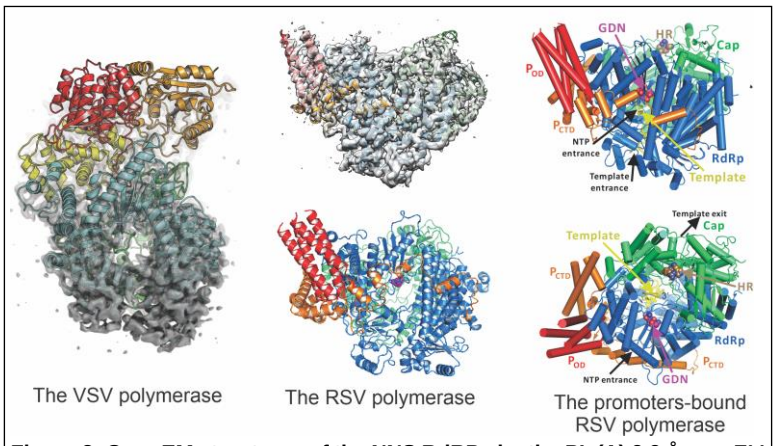

Figure 3: Cryo-EM structures of the NNS RdRPs by the PI. (A) 3.8 Å cryo-EM structure of the VSV RdRP (Liang et al. *Cell*, 2015). **(B)** 3.67 Å cryo-EM structure of the *apo* RSV RdRP (Cao et al. *Nat Comm,* 2020). **(C)** 3.40 Å and 3.41 Å cryo-EM structures of the promoters-bound RSV RdRP (Cao et al. *Nat,* 2023).

In summary, we have demonstrated the feasibility of preparing the cryo specimens of the BDV L proteins. We have successfully determined multiple high-resolution structures of the polymerases from NNS RNA viruses using single-particle cryo-EM[8-10] (**Fig. 3**). We will use a similar strategy to prepare the BDV L:P complex. In light of the exciting preliminary results, we anticipate there are some degrees of flexibility of the BDV L:P. To reach the atomic resolution, we expect to obtain a large dataset(s) of the complex assemblies. Therefore, we wish to request 300KeV Titan Krios time for high-resolution cryo-EM data collection at NCCAT. Once we collect sufficient data, we will obtain the maximum attainable resolution reconstruction of the BDV polymerase using cryo-EM.

References:

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