Structural Basis of the Nipah Virus (NiV) Polymerase

Project Objective:

The primary goal of this proposal is to decipher the structural basis of the NiV synthesis machine, in particular, the NiV polymerase (L:P).

Preliminary Results:

We have successfully determined the cryo-EM structures of the *apo* RSV polymerase (L:P) (Cao *et al.*, Nat Comm 2020) (**Fig. 1**). We will use similar strategies to prepare the NiV L:P complex. So far, we have successfully co-expressed and co-purified full-length NiV L:P using sf21 insect cells. We included the SDS-PAGE gel of the prepared NiV polymerase (L:P) complex (**Fig. 2**). We have also adapted and established an *in vitro* polymerization assay and demonstrated the activities of the NiV polymerase (**Fig. 3**). We had negative stain EM images of the NiV L:P complex. Preliminary data suggested that the NiV L:P complex showed reasonable homogeneity similar to that of the *apo* RSV L:P complex, and we anticipate we will obtain high-resolution data using our purified samples.

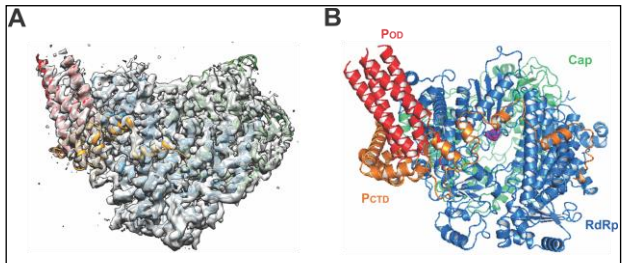

Figure 1: Cryo-EM structure of the RSV polymerase (L:P). (A) 3.67 Å cryo-EM 3D reconstruction. **(B)** Structure of the RSV polymerase (L:P). Cao *et al.*, Nat Comm 2020.

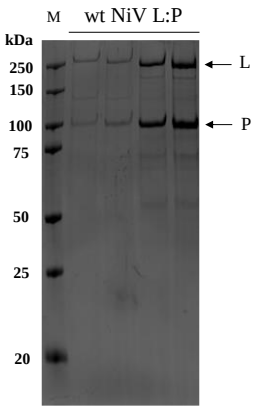

Figure 2: Preparation of the NiV polymerase. SDS-PAGE gel shows the expression of full-length NiV L:P.

For the cryo-screening, we have prepared the cryo-grids for the NiV L:P complex using freezing conditions similar to those of the *apo* RSV L:P complex. The initial cryo-screen revealed reasonable ice thickness and ice gradient in the cryo grids (**Fig. 4A, B**). The preliminary screen showed the particles are readily visible in the grid holes (**Fig. 4C**). In summary, we have demonstrated the feasibility of preparing the cryo specimens of the NiV L:P complex, and we are continuing to optimize the conditions.

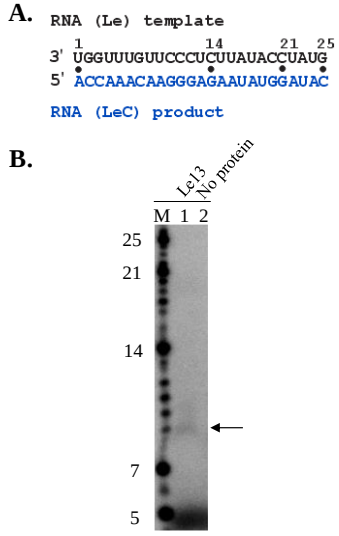

Figure 3: Reconstitution of NiV RNA polymerization assay i*n vitro*. (A) The RNA (Le) template and RNA (LeC) product. The different lengths of templates are indicated. **(B)** *in vitro* polymerization assay with Le13 and result with no protein added as a control.

In light of the exciting preliminary results, we anticipate there are some degrees of flexibility of the NiV L:P complex, and such complex may be a portion of the total particles. To reach the atomic resolution, we expect to obtain large datasets of the NiV polymerase complex. Therefore, we wish to request 300KeV Titan Krios time for high-resolution cryo-EM data collection at NCCAT. Once we succeed in collecting sufficient data, we will optimize the strategy to obtain the maximum attainable resolution reconstruction of the NiV polymerase using cryo-EM and perform model building and validation. These studies will provide key structural and molecular mechanisms of the NiV polymerase.

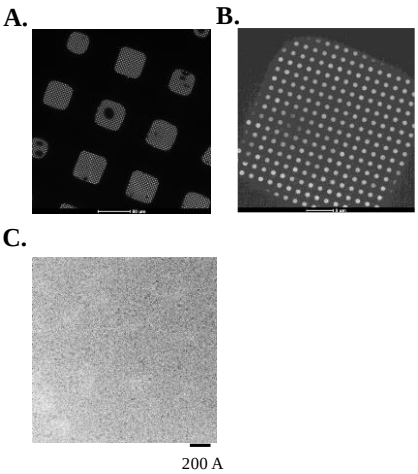

Figure 4: Preliminary cryo-EM analysis of NiV polymerase complex. (A) The low magnification view of the grid. **(B)** The medium magnification view of a representative grid square. **(C)** The raw image of the L:P complex.