Structural Basis of the Respiratory Syncytial Virus Polymerase Complex

Project Objective:

The primary goal of this proposal is to decipher the structural basis of the RSV synthesis machine, in particular, the RSV polymerase (L:P) complex with its RNA template.

Preliminary Results:

Recently, we have successfully determined a 3.67 Å cryo-EM structure of the *apo* RSV polymerase (L:P) using the 200kV Arctica with Gatan BioQuantum at Emory (Cao *et al.*, Nat Comm 2019, accepted) (**Fig. 1**). We will use a similar strategy to prepare the RSV L:P complex. We have successfully co-expressed and co-purified full-length RSV L:P using sf21 insect cells. We included SDS-PAGE gel and size exclusion chromatography data of the prepared RSV polymerase (L:P) complex (**Fig. 2**). We have also adapted and established an *in vitro* polymerization assay and compared the length impact of template sequences. We defined the minimal length for *de novo* RNA synthesis of RSV polymerase (Cao *et al.*, in revision) (**Fig. 3**). We used and will continue to use the *in vitro* polymerization assay to guide the selection of appropriate RNA template(s) for the RSV L:P. We had negative stain EM images of both RSV L:P and L:P:RNA complex. Preliminary data suggested that after the incubation with selected RNA template, the RSV L:P:RNA complex showed reasonable homogeneity and similar to that of *apo* RSV L:P complex.

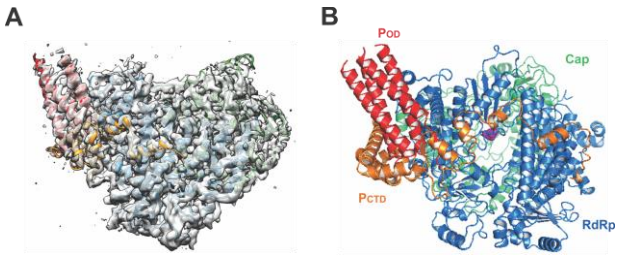

Figure 1: Cryo-EM structure of the RSV polymerase. (A) 3.67 Å cryo-EM 3D reconstruction. **(B)** Structure of the RSV polymerase (L:P). Cao *et al.*, Nat Comm 2019, accepted.

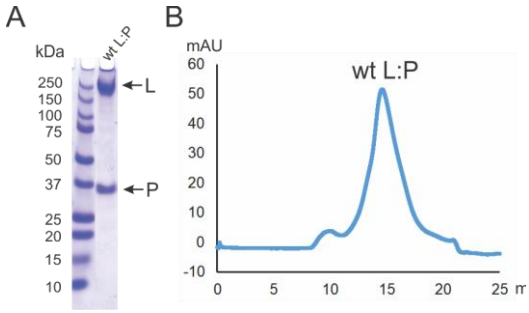

Figure 2: Preparation of the RSV polymerase. (A) SDS-PAGE gel shows the expression of full length RSV L:P. **(B)** The gel filtration shows the homogeneity of the polymerase.

We have also prepared the cryo-grids for the RSV L:P:RNA complex using similar freezing conditions as that 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**). We have acquired a small cryo-EM dataset and performed 2D class averages. The class averages show both similar and different views compared to that of *apo* RSV L:P complex (Cao *et al.*, Nat Comm 2019, accepted) (**Fig. 4D**). In summary, we have demonstrated the feasibility of preparing the cryo specimens of the RSV L:P:RNA complex, and we are continuing to optimize the conditions.

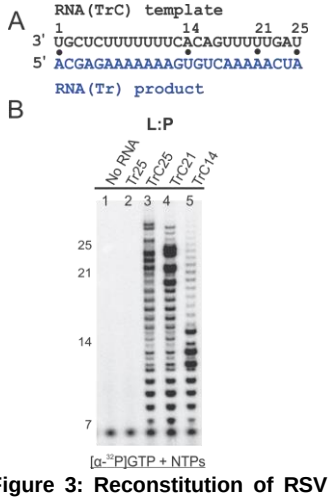

Figure 3: Reconstitution of RSV RNA polymerization assay *in vitro*. (A) The RNA (TrC) template and RNA (Tr) product. The different lengths of templates are indicated. **(B)** *in vitro* polymerization assay with different lengths of templates and product as a control.

In light of the exciting preliminary results, we anticipate there are some degrees of the flexibility of the RSV L:P:RNA complex, and such complex may be a portion of the total particles. To reach the atomic resolution, we expect to obtain 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 succeed in collecting sufficient data, we will optimize the strategy to obtain the maximum attainable resolution reconstruction of the RNA bound RSV polymerase using cryo-EM and perform model building and validation.

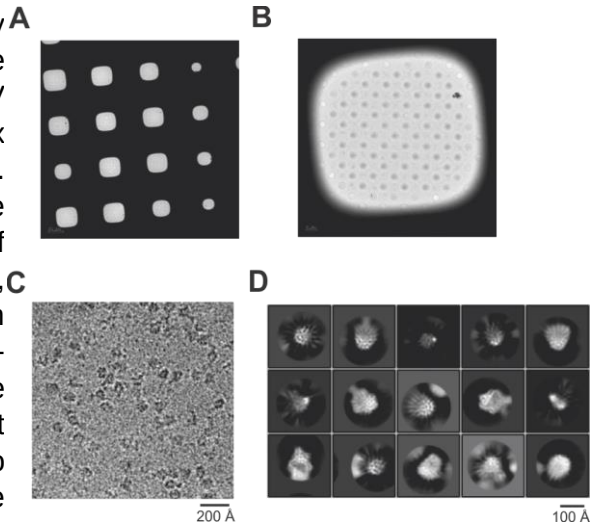

Figure 4: Preliminary cryo-EM analysis of RSV polymerase complex with its RNA (L:P:RNA). (A) The low magnification view of the grid. **(B)** The medium magnification view of a representative grid square. **(C)** The raw image of L:P:RNA complex. **(D)** The class averages show both similar and different views to that of *apo* RSV L:P complex.