張栢恒

PETER CHEUNG

# 03 EMERGING INFECTIOUS DISEASES AND MICROBIOTA



**Principal Investigator**

Peter Cheung

**Team members**

Wing Ying Au, Xinzhou Xu

## Research Progress Summary

### RNA Virus as Public Health Concern

RNA viruses are a major player in zoonotic disease transmission with severe public health consequences (Binder et al. *Science.* 1999; 284; 1311-1313). Zoonotic transmission resulting from the increasing human interaction with wild environments has led to a number of pandemics originated from wildlife reservoirs, as was seen with the emergence of the H1N1 and the highly pathogenic H5N1 Influenza A Virus (IAV), the Severe Acute Respiratory Syndrome Coronavirus (SARS) in 2003 and 2019, Bunyaviruses, Enterovirus 71 (EV71), Dengue, Japanese Encephalitis Virus, Hepatitis C Virus. Modern medicine has struggled to overcome and predict their biological variety and high adaptation rates that allow them to adapt to new host species and antiviral therapeutics. The replication of the viral genetic material by almost all RNA viruses is error prone. This trait is advantageous to the virus, ensuring sustained adaptivity to the host (Boivin et al. *Journal of Biological Chemistry*. 2010; 285: 28411-28417; Domingo et al. *Microbiology and Molecular Biology Reviews*. 2012; 76: 159-216) and the environments, and Peter Cheung has shown it to be critical for host transmission (Luk et al. *Journal of Virology*. 2015; 89: 9939-9951) and pathogenesis (Cheung et al. *Nature Communications*. 2014; 5: 4794). Thus, it is of paramount importance to understand the vital processes that govern viral replication and adaptation to human population.

## Viral Polymerase as Engine for Replication

The RNA-dependent RNA polymerase (RdRP) is the central machinery for viruses' transcription and genome replication processes. RdRP lacks proofreading (except coronavirus), thus relying on viral RdRP for maintaining genome integrity. RdRP synthesizes long nucleotide acid chains via template-directed biosynthesis using nucleotide triphosphates (NTPs) as substrates (Fig. 1A).

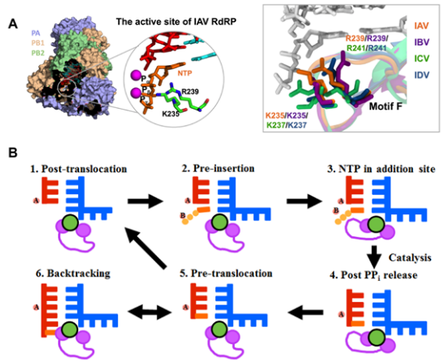
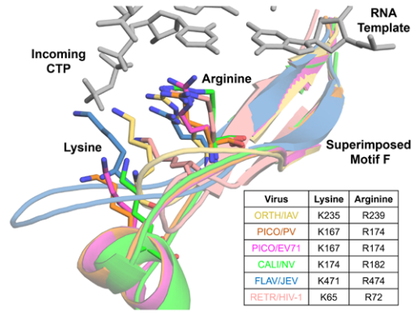



**Fig. 1.** Viral RdRP cycles through different functional states for nucleotide addition. (A) Left: The cryo-EM structure of IAV elongation complex comprised of PA, PB1 and PB2 domains. Right: The active site with NTP bound and conserved motifs. (B) The five functional states and the conformational transitions in the nucleotide addition cycle (NAC).

The catalytic process of RdRP is mainly composed of two stages: initiation and elongation. The initiation stage is unstable, and requires the precise control at the synthesis initiation site, at which point the incorporation of several nucleotides allow conversion to a stable elongation stage. The elongation stage consists of thousands of cycles of nucleotide additions (NAC). For each NAC, RdRP undergoes multiple functional states with conformational changes (Fig. 1B). NAC consists of four steps: NTP binding, closing of active site, phosphoryl transfer, and translocation.

RdRP catalytic core contains the pre-A (also known as F) and A-E motifs. Structure-based alignment with RNA- and DNA-dependent RNA polymerases, including viruses from Picornaviridae, Flaviviridae, Caliciviridae, and Retroviridae families shows a well-conserved configuration in Motif F (Fig. 2), which has been speculated and showed by us (Xu et al. *Nucleic Acids Research.* 2021; 49: 8796-8810) and others to play essential roles in controlling the rate and fidelity of viral transcription (Yin and Steitz. *Cell*. 2004; 116: 393-404).




| Virus | Lysine | Arginine |
|---|---|---|
| ORTH/IAV | K235 | R239 |
| PICO/PV | K167 | R174 |
| PICO/EV71 | K167 | R174 |
| CALI/NV | K174 | R182 |
| FLAV/JEV | K471 | R474 |
| RETR/HIV-1 | K65 | R72 |

**Fig. 2. Structural alignment of RdRp active sites** of *Picornaviridae/Poliovirus* (PICO/PV PDB: 3OLA), Enterovirus 71 (EV71 PDB: 5F8I), *Caliciviridae*/ Norwalk Virus (CALI/ NV PDB: 3BSO), *Flaviviridae*/ JEV (FLAV/ JEV PDB: 4MTP), and *Retroviridae*/ HIV-1 (RETR/HIV-1 PDB: 4MTP)z aligned to IAV RdRP complex (PDB: 6SZV). Critical K and R residues on Motif F are coloured. Grey atoms are incoming CTP pairing with the template.

## Viral Polymerase as Driver for Diversity

Proper NTP substrates readily cause active site closure for NTP incorporation, whereas incorrect substrates would lead to instability (Gong and Peersen. *Proceedings of the National Academy of Sciences USA.* 2010; 107: 22505-22510; Shi et al. *Nucleic Acids Research*. 2020; 48: 1392-1405; Wang et al. *Nature Communications*. 2020; 11: 2605). Thus, polymerase sites, regions, or interactions that coordinate NTP and subsequently affect impact active site closure may affect fidelity. The choice of the correct NTP matched with the template nucleoside is critical for preserving the fidelity of the synthesis. However, low-level mismatch occurrences in replication might enable RNA viruses to deal with selection pressure during adaptation or co-evolution with their hosts (Crotty et al. *Nature Medicine*. 2000; 6: 1375-1379).

## Previous Work Done in Polymerase NAC and Fidelity

Due to its vital role for viral replication, RdRP is an essential target for antiviral therapy. Peter and his team have spent over a decade to study polymerase NAC and reveal the structural basis of how polymerases maintain replicational fidelity by developing and employing state-of-the-art enzymology assays to quantify RdRP replication and cleavage activities of eukaryotes and vial polymerases (Tse et al. *Nature Catalysis*. 2019; 2: 228-235; Wang et al. *Biophysical Chemistry*. 2021; 277: 106652; Xu et al. *Nucleic Acids Research.* 2021; 49: 8796-8810; Zhang et al. *Accounts of Chemical Research*. 2016; 49: 687-694).

At the virus level, their work has revealed how RNA viruses adapt to the host environment to cause disease (Cheung et al. *Nature Communications*. 2014; 5: 4794), provided novel targets for antiviral therapies, and revealed RdRP mutations conferring drug resistance to NTP analogues (Choy et al. *Antiviral Research*. 2020; 178: 104786; Wang et al. *Biophysical Chemistry*. 2021; 277: 106652). At the structural level, their work (Xu et al. *Nucleic Acids Research.* 2021; 49: 8796-8810) using Molecular Dynamics (MD) simulations and enzymology identified critical fidelity control mechanisms, which lies in the catalytic core or active site of RdRP incorporating to the newly synthesised RNA (Xu et al. *Nucleic Acids Research.* 2021; 49: 8796-8810). Peter employed a mechanism-insight driven approach by developing a novel enzymology assay tightly integrated with MD simulation of the RdRP structure to identify a mutation K235R in the catalytic core of the RdRP within Motif F that confer both increased polymerase activity and enhanced fidelity (Fig. 2), the first such RdRP ever discovered.

## Previous Work Done in Elucidating the Inhibition Mechanism of NuA to Viral RdRps

Peter and his team have profound experiences in NuA's inhibition of IAV and SARS-CoV-2 RdRps. For example, they discovered how ribavirin inhibits IAV viral replication by mutagenesis (Cheung et al. *Nature Communications*. 2014; 5: 4794). They discovered the first Ribavirin-resistant IAV virus with enhanced transcriptional fidelity (Cheung et al. *Nature Communications*. 2014; 5: 4794). Using this tightly coupled platform, they elucidated the mechanisms by which NTP analogue (NuA) Favipiravir terminates RNA synthesis by RdRP and induces mutation once the NTP analogue is incorporated into the newly synthesised strand (Wang et al. *Biophysical Chemistry*. 2021; 277:106652). They are amongst the first to demonstrate that the NTP analogue Remdesivir suppresses live SARS-CoV-2 replication (Choy et al. *Antiviral Research*. 2020; 178: 104786). Then, they elucidated structurally how Remdesivir inhibits transcription by delayed chain termination by inhibiting RdRp NSP12 translocation and cleavage by exoribonuclease NSP14 (Zhang et al. *Physical Chemistry Chemical Physics*. 2021; 23: 5852-5863). These findings lay a solid foundation for us to investigate how NuA interferes with the kinetics of IAV RdRp.

## Diagnostics for SARS-CoV-2

Improved diagnostics are needed to manage the ongoing COVID-19 pandemic. Recently, the research team enhanced the color changes and sensitivity of colorimetric SARS-CoV-2 RT-LAMP assays based on triarylmethane dyes. They determined a mechanism for the color changes and obtained sensitivities of 10 RNA copies per microliter (Suarez et al. *Analytical Method.* 2022; 14; 378-382). They performed meta-analysis to comprehensively analyse three standard nucleic acid assays, namely digital PCR (dPCR), quantitative PCR (qPCR), and LAMP. They demonstrated interestingly that all three tests consistently perform better with pharyngeal swabs utilising SARS-CoV-2 open reading frame 1ab primer with RNA extraction. dPCR was demonstrated to be the most sensitive, followed by qPCR and LAMP. However, their accuracy does not vary considerably. Instead, accuracy relies on experimental settings, meaning that additional efforts should be dedicated to optimising the experimental setups for the nucleic acid assays. Hence, the findings of Peter's team might be a reference for improving and creating a standard nucleic acid test technique that is applicable in laboratories worldwide (Au and Cheung, *Lancet Microbe*. 2021; 2: E704-E714).

# Research and Scholarship

## Reviewer of Journal / Conference

| Member's Name | Details | |
|---|---|---|
| | Role | Journal / Conference |
| Peter Cheung | Reviewer | Microbiology Spectrum |
| | | Emerging Infectious Diseases |
| | | Biochemical Engineering Journal |

## Grants and Consultancy

| Name | Project Title | Funding Source | Start Date (dd/mm/yyyy) | End Date (dd/mm/yyyy) | Amount (HK$) |
|---|---|---|---|---|---|
| Peter Cheung | Establishment of a Parallel Platform for the Mechanistic Studies of Function and Inhibition of Influenza Polymerase | Research Grants Council – General Research Fund | 15/07/2018 | 14/07/2021 | 505,298 |
| | Establishment of Highly Accurate Nuclear Run-on RNA Sequencing Platforms to Study the Genome-wide Mechanisms of Transcription and Mutation by Influenza Polymerase | Research Grants Council – General Research Fund | 01/07/2019 | 30/06/2022 | 558,272 |

## Publications

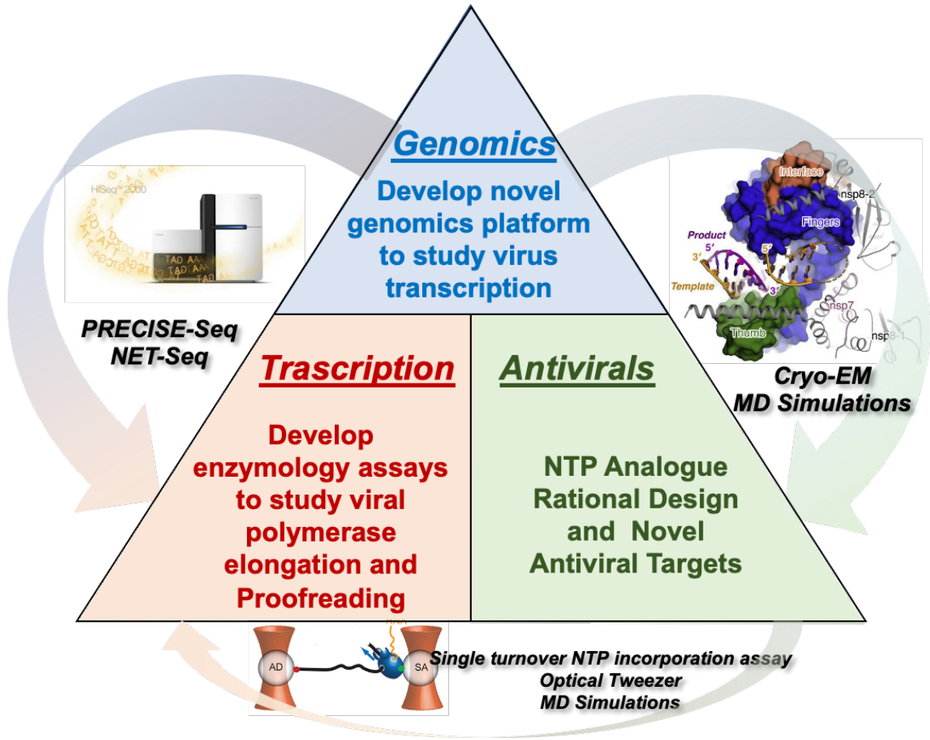
### A. Journal Papers


1. Suarez GD, Suarez DA, Kiu Tang YY, Zhang J-X, Li J, Nagl S, Cheung PP-H. Uncovering mechanisms of RT-LAMP colorimetric SARS-CoV-2 detection to improve assay reliability. *Analytical Methods*. doi:10.1039/d1ay01395e. (Epub ahead of print)

2. Au WY, Cheung PPH. Diagnostic performances of common nucleic acid tests for SARS-CoV-2 in hospitals and clinics: A systematic review and meta-analysis. *The Lancet Microbe*. 2021;2(12):e704-e714. doi:10.1016/s2666-5247(21)00214-7. (Review) (Epub ahead of print)

3. Xu X, Zhang L, Tung Sem Chu J, Wang Y, Wing Hong Chin A, Hang Chong T, Dai Z, Lit Man Poon L, Pak-Hang Cheung P, Huang X. A novel mechanism of enhanced transcription activity and fidelity for influenza A viral RNA-dependent RNA polymerase. *Nucleic Acids Research*. 2021;49(15):8796-8810. doi:10.1093/nar/gkab660.

4. Wang Y, Yuan C, Xu X, Chong TH, Zhang L, Cheung PPH, Huang X. The mechanism of action of T-705 as a unique delayed chain terminator on influenza viral polymerase transcription. *Biophysical Chemistry*. 2021;277(April):106652. doi:10.1016/j.bpc.2021.106652. (Epub ahead of print)


To better understand the structural mechanisms underlying the highly complex processes of SARS-CoV-2 gene expression and replication, genomic data must be linked to polymerase structure. Peter Cheung and his team propose to bridge this genomics-structural divide by utilizing their highly interdisciplinary expertise in genomics, bioinformatics, cryo-EM, computational biology, chemical synthesis, enzymology, and virology.

***Source: Peter Cheung***