Contribution ID: **36** Type: **Poster**

Revealing the architecture and dynamics of SARS-CoV-2 RTC

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded RNA virus that caused an outbreak of coronavirus disease in 2019 (COVID-19). The replication of the viral genome is facilitated by the replication/transcription complex (RTC), in which nonstructural proteins (nsps) are the principal contributors. Therefore, unraveling the functional properties and structural organization of nsps will deepen our understanding of the evolutionary success of the virus and aid in developing preventative and curative drug strategies against SARS-CoV-2.

The primary aim of this research project is to reveal the spatial configuration and topology of non-structural proteins (nsps) and reconstruct a complete RTC while preserving its native conformation. This will be achieved by transforming bacterial and mammalian cell lines, expressing and purifying nsps, and analyzing them using native top-down mass spectrometry (nTDMS). nTDMS enables structural investigation of proteins and protein complexes while preserving their native, folded conformation. This methodology allows for the detection of transient protein-protein interactions, assembly and disassembly of nsps within the RTC. Additionally, nTDMS can identify post-translational modifications (PTMs) of proteins. Comparing PTMs across these expression systems will provide valuable insights into the role of PTMs in SARS-CoV-2 pathogenesis.

We have developed a protocol for performing affinity purification directly in an nMS-compatible buffer, significantly reducing purification time while maintaining optimal protein yield. Using this optimized methodology, we successfully purified multiple nsps, including nsp10, nsp14, nsp15, and nsp16. Our results revealed protein-protein interactions between nsp10, nsp14, and nsp16, as well as the homohexamer formation of nsp15.

As a future direction, we plan to analyze the remaining nsps in both bacterial and mammalian expression systems and examine their PTMs using nTDMS. This approach will provide deeper insights into the dynamic nature of the RTC and the functional roles of nsps in viral replication and transcription.

User consent

yes

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Session Classification: Poster Session 1