Submission Date: 06/13/2025

2024 Academic Year Bio-SPMs Collaborative Research
Research Report Summary

Title of the research project		Characterization of the conformational dynamics of Influenza A virus ribonucleoprotein complexes during genome replication	
PI (Person in charge of the research project)	Name	Borja Ibarra	
	Affiliated Institution and Department/Division/etc.	IMDEA Nanoscience	
	Position	Tenured research professor	
Bio-SPMs that you used (Check the boxes)		☐	Atomic resolution/3D-AFM
		x	High-speed AFM
		☐	SICM
		☐	AFM for Cell Measurement
Collaborative NanoLSI Faculty Members		Dr. Shingo Fukuda	

Replication of Influenza A virus genome is orchestrated by ribonucleoprotein complexes (RNPs), which are composed of viral RNA polymerases (RNApol) and nucleoproteins (NP). While the transcription mechanism of these RNPs has been extensively studied, the **real-time structural dynamics during replication** and the specific roles of host cofactors such as ANP32A remain poorly understood. This study aimed to elucidate these dynamics and address how structural transitions within RNPs influence replication efficiency.

We employed **high-speed atomic force microscopy (HS-AFM)** to directly visualize the **real-time conformational dynamics** of recombinant Influenza A virus ribonucleoprotein complexes (rRNPs) during genome replication. Recombinant rRNPs, assembled from purified viral RNA polymerase and nucleoproteins on short RNA templates, initially formed an **annular architecture** with two RNA polymerases visible. During active replication, a **transient compaction** of the rRNPs was observed, accompanied by a height increase. Post-replication, the rRNPs reverted to their original ring-like conformation, suggesting **cyclical structural remodeling** during replication. To validate these observations, **transmission electron microscopy (TEM)** was used to generate three-dimensional reconstructions of replication intermediates, confirming the presence of transient height increases that mirror the dynamic transitions captured by HS-AFM.

Kinetic analysis revealed that replication proceeds up to 4-times **slower than transcription** under identical nucleotide conditions. This suggests that replication involves additional kinetic barriers or structural constraints not present during transcription. The influence of **RNA secondary structure** on replication efficiency was also investigated by incorporating modified nucleotides. The destabilizing modifications enhanced replication rates, while the stabilizing modifications slowed them, confirming that **nascent RNA folding modulates replication dynamics**.

*This form (Form 3) will be open on the NanoLSI website in the following academic year.

*Note that this form should be prepared in one A4-size paper.

*Submission Deadline: May 9, 2025 (Friday). Submit it as a PDF file.

*Submission Destination: the person in charge of Bio-SPMs collaborative research at WPI-NanoLSI, Kanazawa University

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