**Single-Molecule Analysis of the Influenza Virus Replication Initiation Mechanism**

Nicole C. Robb[1], Aartjan J.W. te Velthuis[2], Ervin Fodor[3], Achillefs N. Kapanidis[1]

[1]Physics, University of Oxford, Oxford, United Kingdom
[2]Pathology, University of Cambridge, Cambridge, United Kingdom
[3]Pathology, University of Oxford, Oxford, United Kingdom

Influenza viruses have a segmented viral RNA (vRNA) genome, which is replicated by the viral RNA-dependent RNA polymerase (RNAP). Replication initiates on the vRNA 3' terminus, producing a complementary RNA (cRNA) intermediate, which serves as a template for the synthesis of new vRNA. RNAP structures show that the 3' vRNA template can adopt more than one configuration; it can be bound in a pre-initiation state on the surface of the RNAP, as well as in an initiation state in the active site of the RNAP. No information is available on 3' cRNA binding, although a crystal structure of the RNAP with a short 5' cRNA terminus shows that the conformation of the first twelve residues of the 5´ cRNA is virtually identical to that of the vRNA 5´ terminus. We have used single-molecule Fo¨ rster resonance energy transfer (smFRET) on surface-immobilised initiation complexes to probe the RNA conformations adopted during RNAP binding and initial replication. We show that in the absence of nucleotides, the RNAP-bound 3' terminus of the vRNA promoter exists in dynamic equilibrium between the pre-initiation and initiation conformations. Analysing the dynamics of immobilised RNAP/RNA complexes in real-time has provided dwell times for the two states. Nucleotide addition stabilises the 3' vRNA in the active site and results in unwinding of the duplexed region of the promoter. Intriguingly, and in contrast to the vRNA promoter, the cRNA promoter is stably bound, with limited dynamics, suggestive of differences in the initiation mechanisms for the two promoters. Our data provide novel insights into the dynamic motions of RNA that occur during initial influenza replication and has implications for our understanding of the replication mechanisms of similar pathogenic viruses.