

**P045** Mechanistic studies of programmed ribosomal frameshifting

**Emily Nikolic<sup>1</sup>, Christina Mayer<sup>2</sup>, John Flanagan<sup>2</sup>, Robert Gilbert<sup>2</sup>, Ian Brierley<sup>1</sup>**

*<sup>1</sup>Department of Pathology, University of Cambridge,*

*<sup>2</sup>Division of Structural Biology, University of Oxford*

Many viral and some cellular mRNAs contain programmed -1 ribosomal frameshifting signals that instruct ribosomes to change reading frame at a defined point. The mRNA signal that specifies frameshifting has two components: a heptanucleotide slippery sequence where frameshifting occurs, and an essential stimulatory RNA structure, typically a pseudoknot but occasionally a stem-loop. The mechanism of frameshifting is not fully understood, but likely involves direct interaction between the stimulatory structure and the ribosome, perturbing the elongation cycle as the slippery sequence is being decoded. Recently, the cryo-EM structure of ribosomes stalled at the frameshift signal of infectious bronchitis virus (IBV) was solved. This revealed several features specific to pseudoknot-stalled complexes, and suggested that the frameshift event occurred during translocation. We have purified ribosomes paused at the pseudoknot of beet western yellows virus (BWYV) and the two-stem helix of HIV-1, stimulatory structures of known three-dimensional architecture. We showed that both these structures are able to pause a significant proportion of translating ribosomes, sufficient for the isolation of frameshift intermediates for structural analysis. Preliminary cryo-EM analysis of these complexes shows the stimulatory structures stalled in the mRNA entry channel, and higher resolution analysis will hopefully allow us to further unravel the mechanism of frameshifting.