

**P030** Biophysical analysis of eukaryotic translation initiation factor-4A (eIF4A)

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eIF4A is an ATP dependant RNA helicase that is thought to unwind secondary structure in the 5'UTR of messenger RNAs, hence facilitating the binding and scanning of the 40S ribosomal subunit during eukaryotic translation initiation<sup>1</sup>.

Here, a novel Atomic Force Microscope (AFM) force spectroscopy assay was developed to detect the presence of a stem-loop on single RNA molecules. The effect of eIF4A on the frequency and strength of this stem-loop within the RNA population was investigated under conditions of varying ATP, with a view to determining the ATP-dependence of helicase-catalysed unwinding.

The AFM assay involves suspending the RNA molecule end-to-end between the AFM tip and stage by attaching the 5' end to a gold surface and then picking up the biotinylated 3' end with a streptavidin-function-alised AFM tip.

Force-extension curves with discontinuity features corresponding to removal of secondary structure were collected and the addition of eIF4A reduced the frequency and unwinding force of these features in a concentration-dependant manner. Furthermore, the addition of eIF4A co-factor eIF4B and increasing ATP concentration caused further decreases in the frequency and magnitude of the discontinuity.

More traditional bulk biochemical data from ATPase and helicase unwinding assays was also obtained as a comparison.