

**P042** Purification, crystallization and functional characterization of Retinoic Acid Inducible Gene-I

**Jung-Hoon James Choo, Martin Moncrieffe, Annett Schoenemeyer, Peter Morley and Nick Gay**

*University of Cambridge, GlaxoSmithKline*

Retinoic acid inducible gene-I (RIG-I) is a cytoplasmic RNA helicase, that binds to viral RNA and initiates antiviral responses by signalling through the interferon (IFN) pathway. Full length recombinant human RIG-I was cloned and expressed in insect Sf9 cells, using the baculovirus system. SDS-Polyacrylamide Gel Electrophoresis (PAGE) and Native PAGE shows that RIG-I forms higher order aggregates **in addition to the monomeric form**. RIG-I was purified using tandem affinity chromatography, anion exchange and size exclusion chromatography. Purified RIG-I is subjected to a range of crystallization conditions.

RIG-I, its signalling component  $\Delta$ RIG-I and the adaptor protein Interferon Beta Promoter Stimulator-1 (IPS-1) were also transiently over expressed in mammalian human embryonic kidney 293 cells, and IFN- $\beta$  and NF $\kappa$ B activation was measured using luciferase based assay. When the Hepatitis C Virus (HCV) protease NS34A, which cleaves IPS-1, was co-expressed, the activation of IFN signalling pathway was abrogated; however the effect could be reversed by the addition of NS34A protease inhibitor. This indicates that IPS-1 is the critical adaptor for the RIG-I signalling pathway and suggests that **NS34A can be successfully targeted for HCV infection**.