Studies on the HIV-1 Nef protein

Matthew Bentham and Mark Harris

HIV-1 Nef is a 205 amino acid N-terminally myristoylated protein that plays a critical role in viral pathogenesis. Myristoylation is an eukaryotic specific co-translational modification that is catalysed by a ribosomal associated enzyme - N-myristoyltransferase (NMT). Two projects are ongoing. Firstly, we are attempting to understand the mechanisms by which Nef interacts with cellular membranes – using a combination of *in vitro* liposome binding assays and sucrose gradient fractionation of lysates from Nef-expressing cells we have determined that both the myristate and basic amino acids near the N-terminus of the protein are required. In further experiments, we have anchored Nef irreversibly to the membrane with a C-terminal farnesyl tag and shown that this abrogates the ability of Nef to down-modulate cell-surface CD4.

Secondly, we are raising RNA aptamers to native, myristoylated Nef. The latter can be co-expressed in *E.coli* with N-myristoyl transferase to generate large amounts of purified myristoylated Nef. This has been used to select pools of randomised RNA aptamers and we are currently in the process of characterising these aptamers. They will be tested for the ability to inhibit Nef functions – both *in vitro* (using ELISA based protein-protein interaction assays to measure effects on the interactions of Nef with cellular SH3 domains or CD4 cytoplasmic tail), and *in vivo*. The latter include FACS analysis of down-modulation of cell surface CD4 by Nef, and effects of Nef on virus replication - these experiments are being carried out in the Category III containment facility.

Publication:

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Collaborators:

Kalle Saksela, University of Tampere, Finland
Matthias Geyer, Max Planck Institute, Dortmund
J Victor Garcia, University of Texas at Dallas

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