Fondation Innovations en Infectiologie

Mutations of the influenza polymerase: understanding how animal influenza viruses become highly infectious human pathogens

Project coordinator : **Darren HART**

Laboratoire de Biologie structurale des interactions entre virus et cellule-hôte

UMR5233 - CNRS - UJF – EMBL

hart@embl.fr

There is worldwide concern at how circulating animal influenza viruses have crossed the species barrier to become pandemic, human infectious strains. Swine influenza strains have already achieved this and we remain concerned that the more pathogenic avian influenza viruses may follow. Numerous studies have highlighted potentially important mutations for inter-species transmission and several are found in the heterotrimeric influenza polymerase that replicates and transcribes the viral genome. The polymerase PB2 subunit contains a number of such positions, notably residue 627 which is glutamic acid in the current swine/avian viruses but lysine in most other human-adapted strains. Polymerase mutations affect the efficiency of viral replication in different host species and account for infectivity in different species, but the molecular mechanisms are unknown – as are most details of polymerase function. To address this, we used robotics to screen a total of 60,000 random *pb2* gene fragments to identify previously unidentified constructs expressing milligrams of protein in *E. coli*. We were thus able to crystallise and determine, by X-ray crystallography, the first influenza polymerase structures.

Recently, we identified an additional expressible C-ter region containing many of the known host determinant sites, including 627. We determined several structures revealing how all positions were located on the domain surface, suggesting interactions with viral proteins or host factors. The 627 position is solvent-exposed in both the human (Lys) and swine/avian (Glu) variants, respectively either reinforcing or disrupting a striking positively charged surface patch.

The aim of this project is to characterise the nature of the interactions between PB2 and viral or host proteins. We will combine biophysical and structural approaches to study our unique recombinant influenza polymerase domains to explain how these mutations generate human-infectious viruses.