UNIVERSITY OF LEEDS

ADDITIONAL INFORMATION

Background to the Programme, Composition of the Research Team and Facilities to Support our Research

Project Title: Exploiting the power of heterologous expression in plants to discover new virus structure

Background

Viral capsids play an essential role in the replication cycle of viruses, protecting the genetic material of the virus during the transmission of infections both within and between host organisms. Capsids are the vehicle that interacts with the cell, with the vectors responsible for transmission, and its usually the capsid proteins against which defense responses are mounted, be it through adaptive or innate immunity. For this reason, many vaccines are based either on inactivated virus particles or on recombinant virus-like particles (VLPs). Furthermore, the ability of viral capsids to elicit such an immune response is exploited to produce antibody-based diagnostic reagents. Most recently, VLPs have been exploited in biotechnology for purposes such as bioimaging and drug delivery.

Given this enormous importance in so many contexts, there has been huge interest in understanding the structure of virus capsids. Such studies have historically depended on isolating the virus of interest, propagating it safely in multi-milligram quantity followed by X-ray crystallography. In the plant world, many pathogens that endanger food security are ‘phloem-limited’ and exceptionally difficult to isolate in the quantities needed.

Recent advances however, give us an unprecedented opportunity to solve new virus structures. The first is the development of a system for heterologous expression of viral coat proteins (CP) in plants, which can generate VLPs which are structurally and immunogenically identical to infectious viruses. The second advance is the tremendous improvements in resolution (and throughput) achievable by single particle cryo-EM. Finally, we now have the ability to rapidly generate protein-based ‘Affimer’ affinity reagents that recognize the VLPs. Using these new technologies together, it will be possible to identify a potential new virus, transiently express its capsid protein, solve its structure, and generate a binding reagent, without ever having to grow a pure culture of the virus.

In essence, this is the aim of this post: to determine the structures of new viruses and VLPs generated by heterologous expression in plants, to generate new binding reagents that could be used to diagnose authentic virus infections, and generate new research tools to manipulate the authentic viruses in the lab.

Research Facilities

Both UoL and JIC provide outstanding facilities to underpin our programme. The Astbury Centre for Structural Molecular Biology in Leeds is an interdisciplinary research hub focused on understanding life in molecular detail. ACSMB has >70 academic members, with expertise in physics, chemistry, medicine and biology. ACSMB hosts state-of-the-art facilities for MS, crystallography, NMR, force spectroscopy and fluorescence, and provides a vibrant environment for structural molecular biology, with experts in biophysics and structure, chemical biology, membrane biology, virology, enzymology and bioninformatics. The proposed project will exploit the state-of-the-art facilities for cryo-EM (and NMR) at the new ABSL, recently established with a £17m strategic investment from UoL, with ~£1.5m from the Wellcome Trust. The EM facility is superbly equipped, and includes two Titan Krios EMs: one with a Falcon-3 direct detector, and the other with an energy-filtered Gatan K2, and Volta phase plate. Specimens will be frozen on either a Leica EM GP or FEI Vitrobot, and optimized using TF20 & T12 EMs. The JIC has all the facilities necessary for the creation of the multiple constructs that will underpin this project and the containment greenhouse facilities required for their plant-based transient expression in N. benthamiana.

Recent Relevant Publications

Recent publications from the Ranson and Lomonossoff groups relevant to the project advertised include:

Marsian, J., Fox, H., Bahar, M.W., Kotecha, A., Fry, E.E., Stuart, D.I., Macadam, A.J., Rowlands, D.J. & Lomonossoff, G.P. (2017). Plant-made polio type-3 stabilised VLPs – a candidate synthetic polio vaccine. *Nature Comms.,* **DOI: 10.1038/s41467-017-00090-w**

Patel, N., White, S.J., Thompson, R.F., Weiß, E.U., Bingham, R., Zlotnick, A., Dykeman, E., Twarock, R., Ranson, N.A. & Stockley, P.G. (2017). The HBV RNA pregenome encodes specific interactions with the viral core protein that can promote nucleocapsid assembly*. Nature Microbiology,* **DOI:10.1038/nmicrobiol.2017.98**

Hesketh, E. L., Meshcheriakova, Y., Thompson, R. F., Lomonossoff, G. P., & Ranson, N. A. (2017). The structures of a naturally empty cowpea mosaic virus particle and its genome-containing counterpart by cryo-electron microscopy. *Scientific Reports*, ***7***(1), 539. **DOI:10.1038/s41598-017-00533-w**

Huynh, N., Hesketh, E.L., Saxena, P., Meshcheriakova, Y., Ku, Y-C., Hoang, L., Johnson, J.E., Ranson, N.A., Lomonossoff, G.P. & Reddy, V.S. (2016) Crystal structure and proteomics analysis of empty virus like particles of Cowpea mosaic virus. *Structure,* **DOI: 10.1016/j.str.2016.02.011.**

Thompson, R.F., Walker, M.L., Siebert, C.A., Muench, S.P. & Ranson, N.A. (2016). Electron microscopy methods in the wake of the 'resolution revolution'. *Methods*, **DOI:10.1016/j.ymeth.2016.02.017.**

Hesketh, E.L., Meshcheriakova, Y., Dent, K.C., Saxena, P., Thompson, R.F., Cockburn, J.J.B, Lomonossoff, G.P. & Ranson, N.A. (2015). Mechanisms of assembly and genome packaging in an RNA virus revealed by high-resolution cryo-EM. *Nature Comms.,* **DOI:10.1038/ncomms10113**

A full list of recent publications from the Ranson group, and information about the Astbury Centre for Structural Molecular Biology can be found at: http://www.astbury.leeds.ac.uk