Searching for the dengue virus Achilles heel

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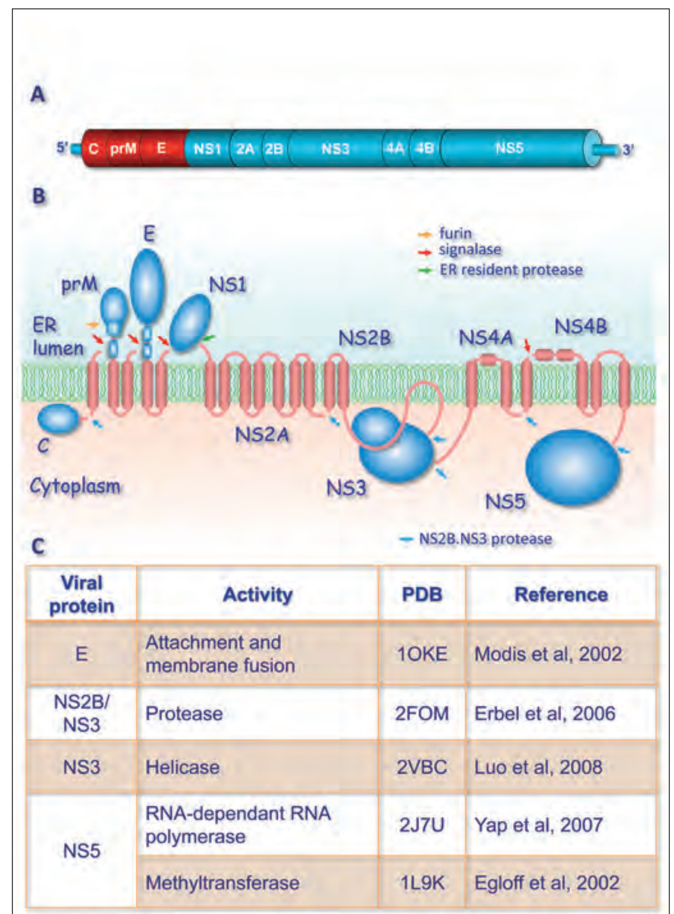
Dengue viruses are a major public health problem throughout the tropical world, with up to 100 million people infected annually. Infection can result in acute febrile illness (dengue fever) and in severe cases is associated with abnormalities in vascular permeability and haemostasis (dengue haemorrhagic fever) that can lead to sudden and fatal hypovolemic shock (dengue shock syndrome). The incidence of dengue has steadily increased over the last two to three decades such that it is now endemic throughout much of the tropics and is the leading cause of infant mortality in some South-East Asian countries. Australia has not escaped this territorial expansion of dengue, with regular epidemic outbreaks now occurring in North Queensland. The epidemic that lasted for most of the summer of 2008–2009 involved the circulation of all four dengue virus serotypes and more than 1,000 confirmed cases. Coupled with the potential impact that climate change may have in increasing the range of its mosquito vector, there is growing concern that dengue may become endemic in Australia. Considerable challenges have accompanied the development of vaccine strategies for dengue and this has reinforced the importance of the complementary development of antiviral therapies. Part of our dengue research efforts has been focused on identifying viral targets for inhibitor design.

The flaviviruses are a large group of mostly insect-borne viruses, many of which pose a significant global human health threat. Most notable amongst these are the dengue viruses (DENV), West Nile virus (WNV), Japanese encephalitis (JEV) and yellow fever virus (YFV). While vaccines have been developed for some, no effective therapeutic drugs are available to treat infection by any of these agents.

Extensive research over the last decade has seen an explosion in our understanding of virus replication and the engagement of viral proteins with specific host cell machinery. As a direct result of these studies, multiple targets for therapeutic intervention have been identified. Not only do these include viral proteins but also components of the host cell pathways that are essential for viral replication. Although the targeting of host cell machinery for inhibition may seem counter-intuitive, the redundancies in many cellular functions and the requirement for only short-term treatment in acute infections make this a viable, albeit challenging strategy. A recent genome-wide RNA interference screen in insect cells has revealed more than 100 host cell



Viral protein	Activity	PDB	Reference
E	Attachment and membrane fusion	1OKE	Modis et al, 2002
NS2B/ NS3	Protease	2FOM	Erbel et al, 2006
NS3	Helicase	2VBC	Luo et al, 2008
NS5	RNA-dependant RNA polymerase	2J7U	Yap et al, 2007
	Methyltransferase	1L9K	Egloff et al, 2002

Figure 1. Dengue virus encoded proteins. A: flavivirus genome strategy. B: flavivirus polyprotein is expressed in association with the endoplasmic reticulum (ER) and co-translationally cleaved by host and viral proteases. C: functional activities and crystal structures of dengue virus proteins [2-6].

factors required for dengue virus production, 42 of which have homologues in human cells [1]. These host cell targets are now the subject of intense investigation.

The flavivirus genome encodes 10 separate proteins (three structural and seven non-structural) that are initially expressed as one long polyprotein that is co-translationally cleaved by a viral protease, NS2B/NS3 and host proteases (Figures 1A and B). The determination of the atomic structures of many of these proteins in recent years has now provided a solid basis for inhibitor design (Figure 1C). Our studies have focused on the viral encoded protease NS2B/NS3 and the virion surface glycoprotein E, which mediates fusion between the viral and host cell membranes. Both are essential for viral replication and represent attractive targets for the development of inhibitors.

Fusion between the viral and cellular membranes is an integral feature of flavivirus entry into cells. The virion glycoprotein E mediates this process as it undergoes low pH-induced conformational changes. These structural transitions in E are obvious targets for inhibitor design. One of the initial structural changes is movement around a putative hinge between domains I and II (Figure 2A) that is thought to expose and present an internal fusion peptide for association with the host cell membrane. We have identified potential small molecule inhibitors by *in silico* screening of compound libraries via computational docking into the hydrophobic pocket within this hinge domain (Figure 2B). When tested in an antiviral assay, a number of the selected compounds were found to inhibit virus replication, with low μM potency. Not surprisingly, the high level of conservation of the E protein structure across the flaviviruses was reflected by the activity of these lead compounds against other members of the group, including DENV, WNV and YFV [7]. These and other small compound leads are currently undergoing further refinement to improve antiviral potency.

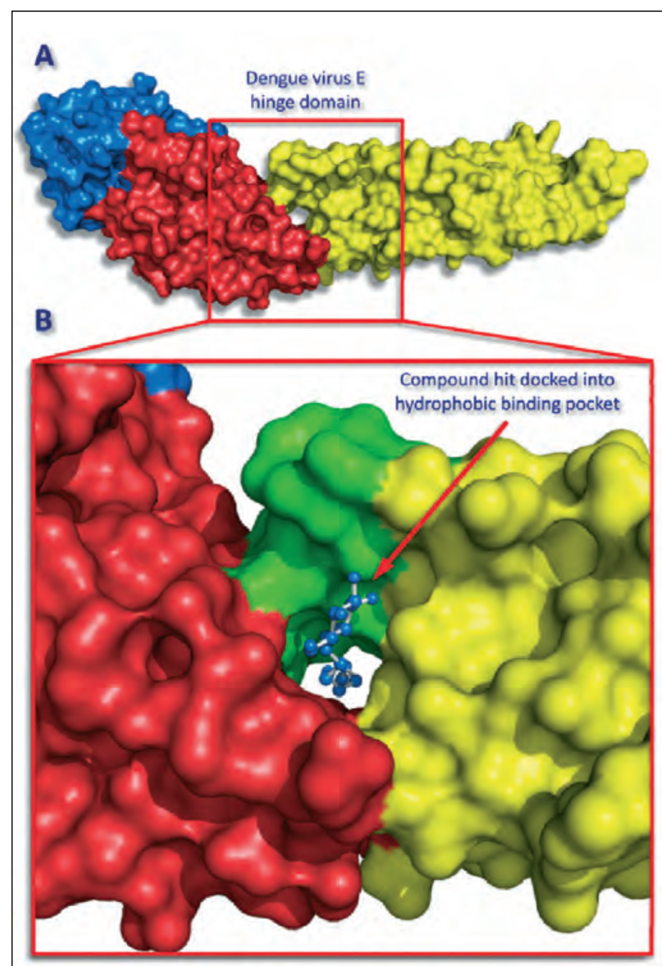


Figure 2. Dengue virus envelope (E) protein. A: Surface representation of an E monomer. Domains I (red), II (yellow) and III (blue). Hydrophobic pore through the hinge domain between domains I and II can be clearly seen. B: a low μM fusion inhibitor docked into the hydrophobic pocket. Domain II loop that occludes the hydrophobic pocket in the compound free form of E is shown in green.

The flavivirus-encoded protease NS2B/NS3 is essential for viral replication and represents another attractive target for the development of small molecule inhibitors. We have already described the design, expression and enzymatic characterisation of catalytically active dengue and WNV recombinant proteases and laid the groundwork for antiviral drug discovery by identifying key substrate, protease and cofactor residues for catalytic activity (see [8]). We have generated a series of lead inhibitors with high potency, have shown their antiviral activity in cell culture and have determined the crystal structure of selected inhibitor/protease complexes [9, 10]. However, the discovery and further development of inhibitors for the flavivirus protease based on substrate interactions has posed significant challenges. While these substrate-based inhibitors have been the primary focus of our group to date, an approach based on NS2B cofactor displacement could prove to be an effective alternative. To better understand the role of the NS2B cofactor in protease activation we conducted a mutagenesis screen through the entire 42-residue central domain of NS2B that is known to interact with NS3 [11]. Two critical sites were identified for which the majority of substitutions were found to significantly decrease proteolytic activity (one of these is shown in Figure 3A). These findings, in combination with crystallographic studies, provided mechanistic insights to the structural and functional role the cofactor may play in the substrate-bound and free protease complexes as well as providing novel sites for targeting of antiviral inhibitors. Following the identification of these two key activation sites, *in silico* screening and docking experiments of small compound libraries were again conducted with selected compounds tested for inhibitor activity. One of these *in silico* hits is shown in Figure 3B docked into the protease crystal structure. A number of leads have now been identified which are currently undergoing optimisation to increase potency.

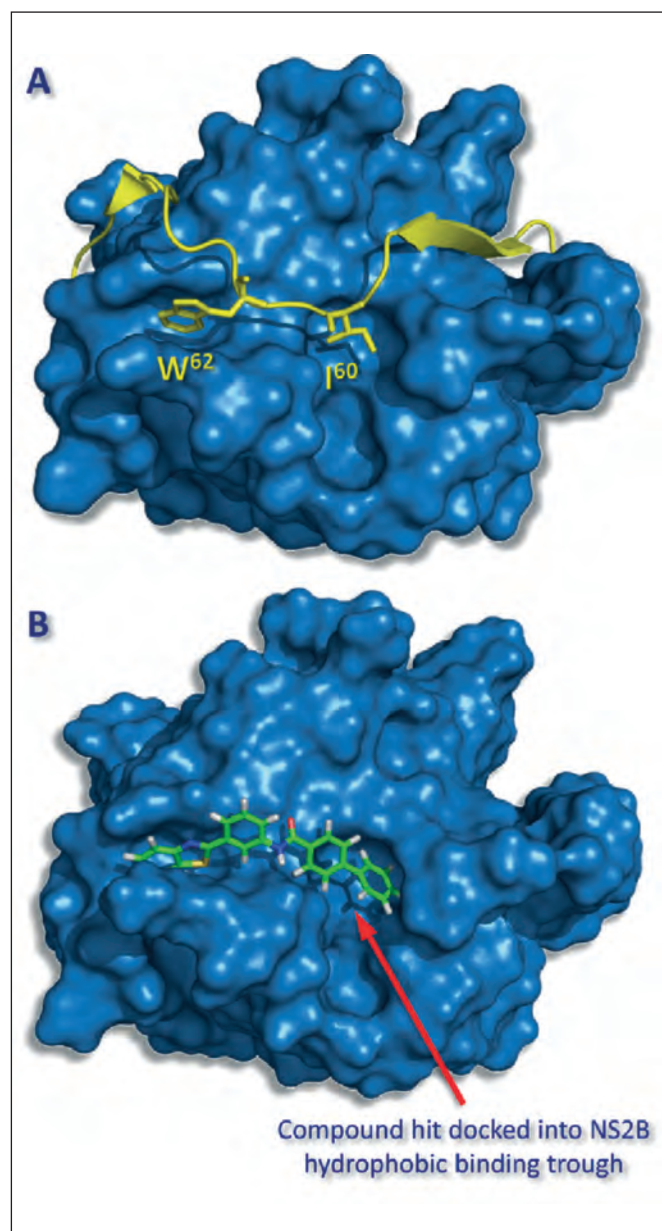


Figure 3. Flavivirus protease NS2B/NS3. A: NS2B cofactor (yellow) showing two highly conserved residues that interact with the NS3 protease (blue) and are essential for protease activity. B: a low μM inhibitor docked into the hydrophobic trough comprising the NS2B interacting interface. Modelling kindly provided by Martin Stoermer.

The challenge we face in developing any therapeutic approach for the flaviviruses is the need for intervention early in acute infection. This is particularly true for dengue infections, where the onset of severe clinical disease occurs at a time when viral load is already falling. Consequently, early diagnosis will play a key role in effective implementation of antiviral therapy. We have developed an effective diagnostic assay for dengue infection based on early detection of the secreted flavivirus protein NS1 in patient plasma [12]. This assay is now in commercial use in dengue epidemic areas around the world and will comprise an important part of the clinical trigger for implementation of antivirals when they finally hit the clinic.

Acknowledgements

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Biography

Paul Young is a Professor of Virology in the School of Chemistry and Molecular Biosciences at the University of Queensland, where he has been a member of academic staff since 1991. His research is focused on the molecular biology of dengue and respiratory syncytial viruses as well as in the development of diagnostic, vaccine and antiviral strategies. He has been a tertiary educator for more than 25 years and was the recipient of the 2008 ASM David White Excellence in Teaching Award.