

# **Structural and functional analysis of the Hepatitis C Virus p7 ion channel**

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A Thesis Submitted to the Board of the Faculty of Biological Sciences,  
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## Structural and functional analysis of the Hepatitis C Virus p7 ion channel

Thomas F.A. Whitfield, Christ Church, D.Phil. Thesis, Trinity Term 2008

### Abstract

The Hepatitis C virus (HCV) encodes the protein p7 that oligomerises to form an ion channel. The 63 amino acid long p7 monomer is an integral membrane protein predominantly found in the endoplasmic reticulum. Although it is currently unknown whether p7 is incorporated into secreted virions, its presence is crucial for the release of infectious virus. The molecular and biophysical mechanism employed by the p7 ion channel is largely unknown.

The biophysical and structural characteristics of p7 were analyzed *in vitro*.

Synthetic HCV p7 peptides of native length and charge oligomerise in artificial lipid bilayers, leading to reproducible single ion channel opening events that feature two main and one sub-conductance states. HCV p7 channels can assemble into both hexamers and heptamers, as shown by transmission electron microscopy and analytical ultracentrifugation.

The influence of the lipid environment on p7 structure, function, and topology was investigated. We combined three sets of data and suggest that p7 adopts an  $\alpha$ -helical hairpin structure in phosphatidylethanolamine (PE)-rich bilayers, and an L-shaped topology in phosphatidylcholine (PC)-rich bilayers.

Furthermore we studied the effect of various ion channel inhibitors on the function and structure of p7 by a variety of different techniques including an improved *in vitro* electrophysiology assay, synchrotron radiation circular dichroism (SRCD) spectroscopy and analytical ultracentrifugation.

In contrast to amantadine, long alkylchain iminosugar derivatives rapidly eliminate HCV from cell culture and inhibit ion channel activity *in vitro* at micromolar concentrations. Long alkyl chain iminosugar derivatives inhibit ion channel activity by preventing p7 monomers from oligomerising rather than by blocking the pore of assembled channels.

This novel mechanism of ion channel inhibition is predicted to target all HCV genotypes with the same efficiency and to reduce the probability of viral escape mutants occurring.

The drug-peptide interaction of iminosugars with p7 was studied by performing a mutational analysis. The results suggest an interaction between these inhibitors with the aromatic amino acid Y42, at the beginning of transmembrane helix 2.

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