

**THESIS TITLE:**

Role of Hepatitis A Virus non-structural proteins 2B and 3C in virus infectivity and development of putative antivirals against HAV

**STUDENT'S NAME:**

KAMALIKA BANERJEE

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**ABSTRACT:**

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## ABSTRACT

Hepatitis A Virus (HAV) is a mild pathogen which causes acute jaundice and is typically self-limiting. HAV infects hepatocytes or the liver cells and spreads through the faeco-oral route. The disease is rarely fatal, however, it could lead to acute liver failure in elderly people. Over the years, improved sanitation conditions and decreased protective immunity in developed countries along with expensive vaccines, prevalence of vaccine-resistant strains and lack of specific treatment have led to increase in the number of HAV outbreaks world-wide.

HAV belongs to the *Picornaviridae* family, which has positive-sense single-stranded monopartite RNA genome enclosed within a non-enveloped capsid. Most of the pathobiology regarding this family has been obtained from poliovirus, a prototype picornavirus. However, HAV has shown distinctive features in different aspects of its virus life-cycle, leading HAV to be taxonomically assigned in a separate genus, *Hepatovirus*. The single open-reading frame (ORF) of HAV encodes a large polyprotein that eventually cleaves into viral precursors, structural and non-structural proteins. Structural proteins formed, upon translation and cleavage, are VP1, VP2, VP3 and VP4 whereas non-structural proteins formed are 2B, 2C, 3A, 3B, 3C and 3D.

Viral pathogenesis involves antagonistic interactions between viral and host proteins. One of the non-structural proteins, HAV 3C is the only protease of the virus. It is crucial for viral polyprotein processing and subsequent production of structural and non-structural proteins. 3C protease and its precursors are responsible for the cleavage of several host cellular proteins that eventually help in maintaining the viral life cycle and subsequently result in a persistent infection. 3C also tends to cleave several host proteins of the innate immune system, thus abrogating the first line of defense against the virus. Hence, it is a viable antiviral target.

Our first objective was to identify lead compounds that could be tested as effective drugs against HAV in the future. Structure-based drug designing was carried out and nine potentially efficacious compounds were screened. Experimental validation of these small-molecule inhibitors eventually led us to identify three of them as the most viable lead compounds having the lowest inhibition constants and reduced cytotoxicity. These compounds not only showed significant therapeutic potential against 3C protease of HAV, but also against Rhinovirus 3C. Extended computational analysis showed these could possibly be used as lead compounds for further testing against this

family of proteases. This work thus highlights the feasibility of designing and developing generic drugs against the 3C proteases of the picornavirus family.

Our second objective was to understand a virus-host cell interaction of HAV 3C protease with RelA/p65, a significant component of the NF- $\kappa$ B signaling pathway which is a part of the mammalian innate immune system. Using a combination of biochemical and computational approaches, we found that HAV 3C protease cleaves the C-terminal region of p65, thus disrupting host's innate immune response.

Our third objective examines the role of 2B, another non-structural protein of HAV, in viral pathogenicity. The amphipathic  $\alpha$ -helix of 2B is known to be a viroporin in poliovirus, coxsackie virus, rhinovirus and encephalomyocarditis virus, and is responsible for causing membrane alterations and cell permeability. In HAV, a single point mutation in 2B (A216V) has led to 10 to 20 fold replication in infected cells. There have been conflicting reports regarding the localization of picornaviral 2B proteins, hence, we carried out extensive immunofluorescence studies on mammalian cells to see the localization of HAV 2B in subcellular organelles. We found that HAV 2B localized mostly to ER, very slightly to the Golgi complex, while almost no localization was observed in plasma membrane and mitochondria. However, we observed that HAV 2B, unlike analogous proteins of other picornaviruses, localizes significantly to the nuclear envelope. We also did not observe any significant differences in subcellular localization of 2B wild-type and 2B A216V mutant. Our molecular dynamics simulation studies also showed that 2B A216V mutant is slightly more stable than 2B wild-type, which could contribute towards the 2B A216V containing virus being more replication-competent.