Biochemical and Structural Aspects of Endosomal Escape by Porcine Circo Virus 2 (PCV2)

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Porcine circovirus 2 (PCV2) is a non-enveloped immunosuppressive virus that infects pigs and is the etiological agent of several diseases and syndrome that are collectively referred to as porcine circovirus-associated disease (PCVAD) [1]. PCV2 is promiscuous in that it infects and destroys nearly every tissue and organ within its natural host. It infects and induces immunosuppression in multiple species of the animal kingdom, and is capable of infecting and replicating in human cells in culture, properties that give PCV2 the propensity of becoming zoonotic and a deterrent to xenotransplantation [2]. Xenotransplantation from swine to human is becoming increasingly attractive due to the shortage of organs from human donors. Understanding the detailed mechanisms by which PCV2 interacts with its cellular hosts is of significant importance for the development of therapeutics to intervene with PCV2 infection. Moreover, PCV2 is the smallest known pathogenic virus and thus provides the opportunity to understand the minimal components needed by an autonomously replicating virus [3].

The present study focuses to understand the detailed mechanisms by which PCV2 capsid protein interacts with the endosomal membrane to enter cytoplasm of host cell. The mechanism of viral escape and release of genomic material into host cell following internalization or endocytosis of viral particle, is still not completely understood in non-enveloped virus [4].

We have assessed the ability of PCV2 capsid protein to disrupt membranes using biochemical assays and Cryo-Electron Microscopy. For this, liposomes composed of phosphatidylcholine have been used as model membranes loaded with high concentration of self-quenching fluorescent dye. Liposome disruption assays were carried out using microplate reader wherein, liposomes and PCV2 capsid protein/ Virus like particles (VLPs) were incubated together and change in fluorescence was measured. Our results exhibit PCV2 capsid protein induced concentration and pH dependent release of dye from these model membranes (Fig. 1). The dye release is more for PCV2 capsid proteins assembled into Virus like particles (VLPs) than individual monomeric capsid PCV2. The results emphasize the importance of assembly formation by capsid protein for the endosomal escape. Additionally, we are analyzing the probable role of membrane destabilization by 1-40 N-terminal residues of PCV2 capsid that is rich in positively charged residues like arginine and lysine. For this we are using different constructs of PCV2 capsid like PCV2 mutant without 1-40 N-terminal residues and synthetically produced peptide with N-term 1-40 residues.

We are using Cryo-Electron Microscopy and Cryo-Electron Tomography for determining the density maps of VLP-liposome complex to structurally understand the membrane interacting partner in the capsid protein and mode of action responsible for fusion or lysis of liposomes. Lacey grids with ultra hin carbon on the top were used to prepare the cryo-grids blotted with Liposome-VLP complex and vitrified using vitrobot. Data was collected at SEMC New York Structure Biology Center, NY. The tilt series were collected bi-directionally from -60° to 60° starting from 0° degrees with a tilt increment of 3° at

64,000 mag and pixel size of 1.36Å. Appion-protomo tool for doing fiducial less alignment was done for collected tilt series gave tomograms that clearly exhibit VLPs bound at the liposomal surface (Fig. 2) [5]. We are further doing sub-tomogram averaging of these VLP-liposome complexes to obtain sub-nanometer density map for clearer structural features.

References:
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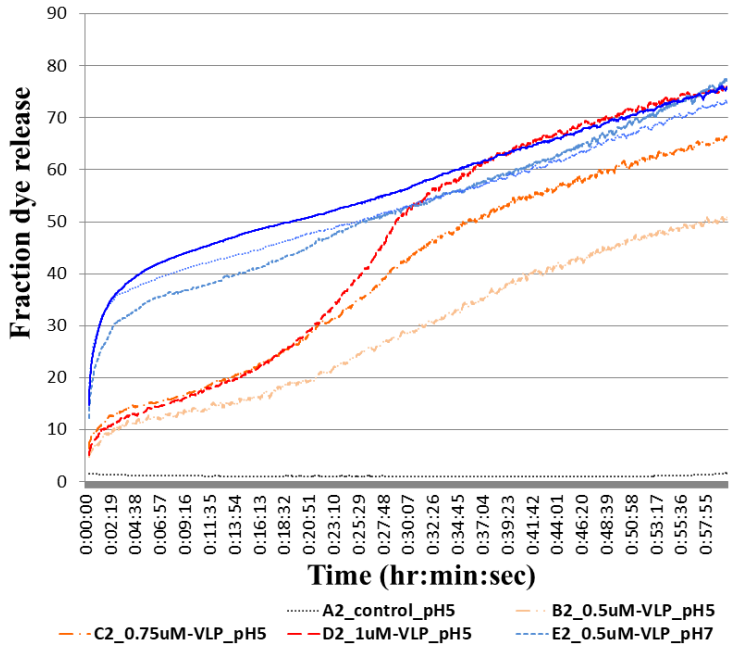

Figure 1. Fraction dye release from the sulphorhomaine B loaded liposomes at pH 5 & pH 7 at different concentrations of PCV2-VLPs measured by flurescence dequenching as a function of time.

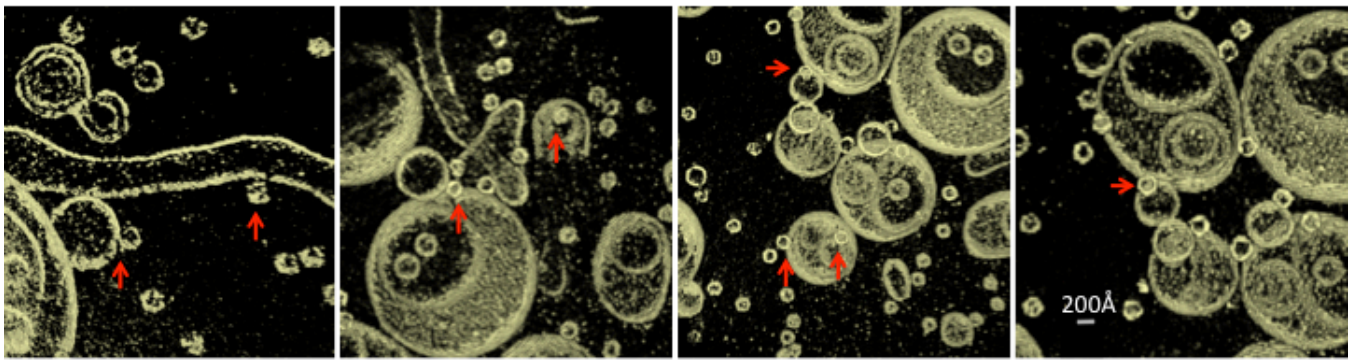

Figure 2. Set of 4 tomograms showing the VLPs bound to liposomes marked by red arrows.