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A Gateway into Understanding the Unique Vertex of Samba Virus

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Giant viruses, including the *Mimiviruses*, *Pandoraviruses*, and *Pithoviruses*, near a micron in diameter and are so large they can be visualized with classical light microscopy. Samba Virus was initially isolated from a tributary of the Amazon River in Brazil, and is a putative member of the *Mimiviridae* family [1]. Samba Virus is the first, giant virus isolated from Brazil to date, and infects *Acanthamoeba castellani*, a causative agent of amoebic keratitis and encephalitis [2]. Most non-enveloped icosahedral viruses have a symmetry mismatch that facilitates genome exit, and for giant viruses there are two known varieties—corks and 5-fold gates [3]. Mimivirus and Samba Viruses are thought to release their genomes into infected hosts by physically opening its capsid at a unique vertex, which houses a 5-fold gateway structure called the "stargate." Evidence for a stargate structure was first identified in Mimivirus, and later also seen in Samba Virus [4, 5]. Both Mimivirus and Samba Virus stargates are sealed prior to infection by a macromolecular "starfish" complex [5, 6]. Here we report evidence for Samba Virus particles including identifying the stargate and starfish structures, as well as factors that contribute to Samba Virus stargate opening, as triggered *in vitro*.

We utilized cryo-electron microscopy, including two-dimensional single particle imaging, cryo-electron tomography, and "bubblegram" imaging, to visualize the Samba Virus unique vertex, including the stargate and starfish complex [5]. Virus particles were imaged in a JEOL JEM-2200FS TEM operating at 200 keV, using low-dose conditions controlled by SerialEM with the use of an in-column Omega Energy Filter, operating at a slit width of 35 eV. Micrographs were recorded using a Direct Electron DE-20 camera (Direct Electron, LP, San Diego, CA, USA), cooled to −40 °C. Movie correction was performed on whole frames using the Direct Electron software package, v2.7.1. Tilt series were acquired using SerialEM at 15 frames per second for 45 frames per tilt angle, along a tilt range of ±55° with images acquired every 2°. Tilt series were acquired at 8000× nominal magnification (6.87 Å/pixel). Tilt series alignment was performed using IMOD and standard tomographic reconstruction practices, using SIRT. Additionally, we performed fluorescence microscopy of native particles, differentially stained to visualize the dsDNA genome, membranes, and capsid layers [5] using 1 μg/mL 4',6-diamino-phenylindole (DAPI, DNA) and 0.1 μg/mL fluorescein isothiocyanate (FITC, protein). Virus particles were imaged using a Zeiss Axio Observer A1 microscope (100×, 1.45 NA) outfitted with an Axiocam ICc5 camera. DAPI fluorescence was imaged with Zeiss filter set 49 and FITC fluorescence was imaged with Zeiss filter set 38 HE. Micrographs were then processed using Zeiss Zen software.

Various *in vitro* chemical treatments were then used to stimulate particles, and remove the starfish seal complex, priming the viral particles for infection. Seal-less particles demonstrated significantly increased infectivity in cell culture, suggesting a role of the starfish in shielding the Samba Virus unique vertex prior to the moment of infection. We present a working model for the structure and composition of Samba Virus, including a view of their unique vertices [7].

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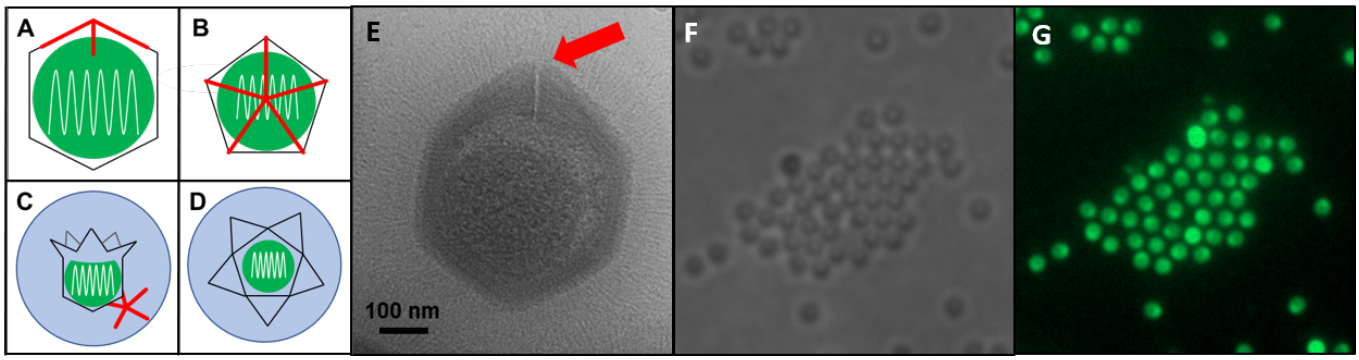

Figure 1. Working model of the Samba Virus infection processes. Side view (A) and top view (B) of the virion depicting the external seal complex (red), nucleocapsid (green), and dsDNA genome (white). Side view (C) and top view (D) of the virion in a phagosome/lysosome after the seal complex has been removed. (E) Cryo-electron micrograph of a seal-less particle. Red arrow highlights the capsid opening. Bright field (F) and DAPI fluorescence (G) micrographs of Samba Virus particles.

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