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Microscopic Evidence for a Stargate Structure in the Giant Virus, Samba Virus.

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With global particle numbers estimated around 10^{31}, viruses play a critical role in life on Earth. Viruses, as a whole, exhibit a large amount of diversity, which can be exemplified by the disparity in the size of viral particles. The Goliaths of the viral world, giant viruses, including the Mimiviruses, Pandoraviruses, and Pithoviruses, near a micron in diameter and dwarf smaller, more common viruses such as Rhinovirus (~30 nm). Samba Virus, a putative member of *Mimiviridae*, was isolated from a tributary of the Amazon River in Brazil [1]. With a genome size of 1.2 Mbp and a total particle diameter (capsid + fibers) of approximately 780 nm, Samba Virus is the first, and largest, giant virus isolated from Brazil to date. Samba Virus infects *Acanthamoeba castellani*, a causative agent of amoebic keratitis and encephalitis [2], and, much like Mimivirus [3], is thought to enter the host cell via phagocytosis. Mimivirus releases its genome by physically opening its capsid at a unique vertex, called the "stargate" [4], which is sealed prior to infection by a macromolecular "starfish" complex [5]. Here we report evidence for Samba Virus stargate and starfish structures, visualized through the use of electron microscopy.

Cryo-electron micrographs of Samba Virus virions reveal particles with regular morphologies (Figure 1A) which may be surrounded by a layer of external fibers (Figure 1B). Some Samba Virus particles exhibit capsids that have opened at a unique vertex, suggesting the presence of a Samba Virus stargate (Figure 2A). Samba Virus particles with an open stargate vertex no longer contain the DNA genome and represent approximately 5% of the preparation visualized through cryo-electron microscopy. Cryo-electron micrographs also revealed free-floating starfish-like structures (Figure 2B), which appear to have been released from Samba Virus particles, perhaps facilitating the opening of the capsid at the stargate vertex. Scanning electron microscopy, used to corroborate the evidence for a stargate provided by cryo-EM, also displayed Samba Virus particles with a capsid opening at a single, unique vertex (Figure 2C).

Further evidence for the Samba Virus stargate vertex was obtained through the use of "bubblegrams" [6]. Briefly, "bubblegrams" require imaging a vitrified sample until the particles begin to exhibit radiation damage caused by exposure to the electron beam. Differences in radiation sensitivity amongst proteins can be used to distinguish proteins within a sample and to orient particles with unique features which are not readily visualized through electron microscopy [6]. When subjected to large quantities of radiation (~100 electrons/$Å^2$), Samba Virus particles exhibit a 5-fold symmetric radiation damage pattern (Figure 2D). A radiation damage pattern with 5-fold symmetry suggests the presence of a protein complex separate from the capsid protein, reminiscent of macromolecular starfish complex. In summation, Samba Virus shares features with Mimivirus, namely the presence of the stargate and starfish structures. The presence of these structures in a second giant virus may speak to the universality of the stargate and starfish structures amongst giant viruses.

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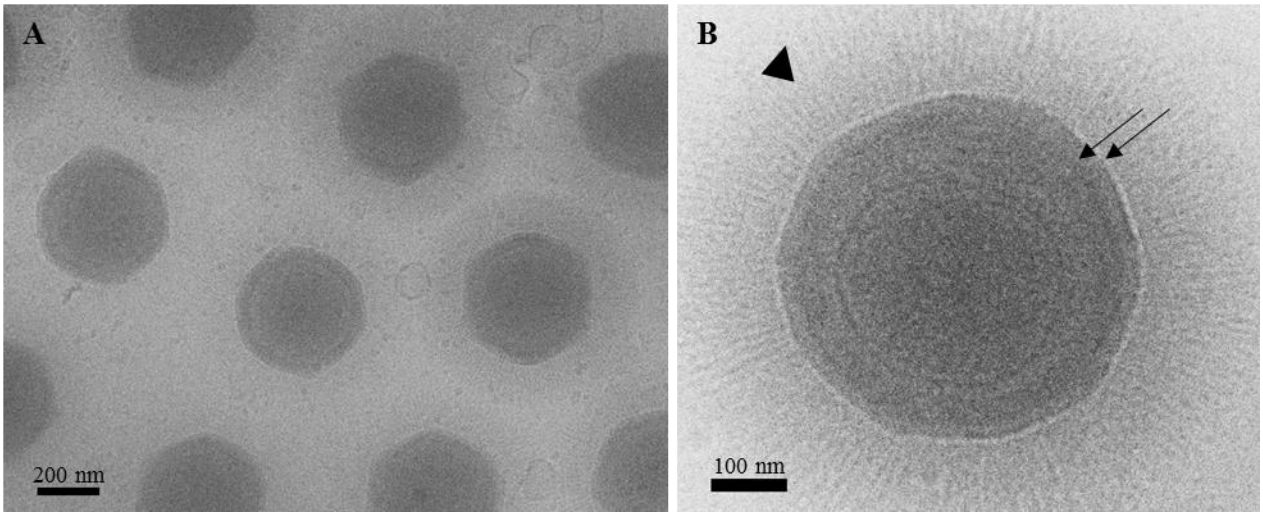

Figure 1. A) Micrograph of vitrified Samba Virus particles imaged on a DE-20 direct electron detector at 10,000X magnification (5.23 Å/pixel) in a JEOL 2200-FS microscope operated at 200 keV with the use of an Omega Energy Filter. B) Enlarged view of a Samba Virus particle exhibiting a multi-layered capsid (arrows) surrounded by a layer of external fibers (arrowhead).

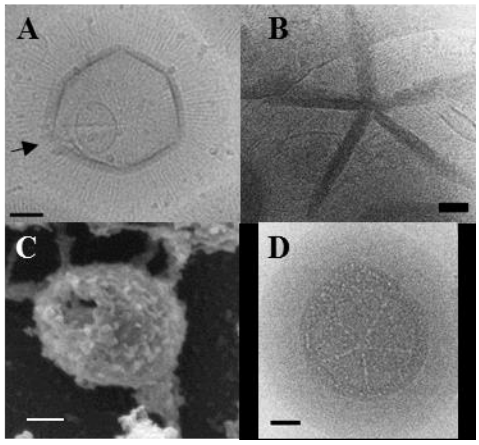

Figure 2. Microscopic evidence for the Samba Virus stargate structure. All scale bars represent 100 nm. A) Cryo-electron micrograph of a Samba Virus particle, devoid of DNA and exhibiting an opening in the capsid at the stargate vertex (arrow). B) Cryo-electron micrograph of a free-floating Samba Virus starfish seal complex. C) Scanning electron micrograph of Samba Virus particle depicting an open vertex. Samba Virus particles were imaged at 110,000X magnification in a JEOL 7500F microscope following critical point drying and iridium coating. D) Cryo-electron micrograph of a Samba Virus bubblegram depicting a 5-fold symmetric radiation damage pattern.

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