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Recent insights into the structural characterisation of Herpes Simplex Virus
fusion protein, gB
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19 Herpesviruses infect many vertebrate and at least one invertebrate hosts. They 20 include over 100 viruses, of which eight cause human infections, with Herpes Simplex Virus (HSV) being one of them. HSV is a model system for the Herpesvirus 21 family and has two serotypes, HSV-1 and HSV-2, that globally infect approximately 22 90% of the population. HSV inflicts lifelong infections by establishing latency in the 23 24 host and undergoes periodic reactivations that can spread the virus. These infections 25 normally manifest as mucocutaneous infections including keratitis, gingivostomatitis 26 and genital warts. Furthermore, infection with HSV-2 increases the risk of acquiring and transmitting HIV [1]. Specific antivirals limit the impact of HSV but none cure 27 28 infection. Coupled with the lack of a preventative vaccine, this virus will continue to afflict the population, making it a global health burden of high priority. 29 30 31 HSV has a linear DNA genome of approximately 152Kb packaged tightly in an 32 icosahedral capsid, which is 15nm thick and 125nm in diameter. The capsid itself is 33 encased in a matrix of 20 proteins (the tegument), that lies beneath a host derived lipid envelope decorated with 10-12 glycoproteins [2]. Therefore, the size and 34 35 complexity of HSV make structural studies extremely challenging. 36 37 As with all enveloped viruses, HSV infection begins with entry, a process that 38 requires fusion of cellular and viral membranes. While the molecular details are still 39 not known, all events are thought to follow the fusion-through-hemifusion pathway [3]. The basic principle posits that the fusion of two lipid membranes is 40 thermodynamically favorable and that the high kinetic barrier is overcome when free 41 42 energy is released as the fusion protein undergoes a series of conformational changes. These changes bring the membranes close together, inducing membrane 43 44 curvature, hemifusion (where only the outer leaflets are fused), and finally full fusion 45 [4]. 46 47 HSV membrane fusion is mediated by four glycoproteins: the primary receptor binding protein gD, a covalently linked heterodimer gH/gL, and the fusion protein, 48 49 gB. HSV fusion begins with the interaction of gD with a cellular receptor. This interaction induces a conformational change in gD, prompting gH/gL to activate gB. 50 Successive rearrangements of gB, from its initial metastable pre-fusion conformation 51 52 to the more energetically favoured post-fusion conformation, lead to membrane curvature and disruption of cellular membranes, resulting in viral capsid release into 53 54 the host cell [5]. 55 Several structures of gD exist, including unliganded gD and in complex with its 56 receptors (reviewed in [6]) and for a partially activated form of gH/gL [7]. However, 57 58 only the post-fusion structure of gB has been solved [8]. This is because all purified 59 forms of gB adopt the post-fusion conformation, and attempts to change this have been unfruitful [9]. This leaves an important gap in the knowledge of the HSV 60 lifecycle. 61

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HSV-1 gB is comprised of 904 residues and is a trimer in the post-fusion 63 conformation. Side views depict it as a three-lobed structure. The truncated post-64 fusion structure identifies five domains that place the two fusion loops in domain I. 65 Both domains I and V are at the "base" of the protein, in close proximity to the viral 66 67 membrane. Domain II, the central lob, is postulated to mediate interactions with 68 gH/gL and is connected to the trimeric coiled-coil, domain III. Domain IV, the "crown", resides at the top, tethered to domain II by domain III [10]. The N-terminus (residues 69 70 31-102, putatively domain VI), is not resolved in the crystal structure due to its flexibility. Amino acids 730-904, which are missing in the purified proteins used for 71 72 crystallographic studies, include the cytoplasmic tail, the transmembrane domain and the membrane-proximal region, all of which are involved in virus fusion and 73 74 infectivity.

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76 Viral fusion proteins are categorized into three distinct groups: I, II and III. As a class 77 III fusion protein, gB is composed of α -helices and β -sheets, and contains two fusion loops per protomer. Class III fusion proteins are found in Herpesviruses, Vesicular 78 79 stomatitis virus (VSV) and Baculovirus. The VSV fusion protein, G, is the best characterized class III fusion protein and its post-fusion form shares features similar 80 81 to gB [11, 12]. Based on the structures of pre- and post-fusion G, Gallagher et al. created an in silico model for pre-fusion gB [13, 14]. To generate it, they proposed 82 83 that gB's pre-fusion domain arrangements are similar to G in its pre-fusion 84 conformation, and accordingly gB's fusion loops would point toward the viral membrane. Therefore, by analogy to G, during the transition from its pre- to post-85 fusion conformation, the fusion loops would first relocate to the top of gB, to interact 86 87 with the target membrane. Further conformational changes would position the fusion 88 loops of gB close to the transmembrane domains, leading to the merging of the cell 89 and virus membranes. This model is supported by an in-depth structural study using 90 fluorescent proteins (FP) to map gB's domains, which suggested that regions allowing insertion of the FPs are exposed [14]. 91 92

93 A second model of pre-fusion gB was recently proposed by Zeev-Ben-Mordehai et al. [15]. This was generated using cryo-electron microscopy (cryo-EM) to image 94 microvesicles expressing full-length gB. Cryo-EM allows imaging of specimens at 95 96 atomic or molecular resolution in close-to-native conditions. gB expressed in 97 microvesicles adopted two different conformations: an elongated post-fusion form, and a compact form, putatively pre-fusion gB. They then calculated a 3D average of 98 the compact form, fitting two post-fusion domains of gB (domains I and II) into the 99 average. Based on VSV G, and like Gallagher et al., they assumed that the domains 100 101 of gB are similar in the pre- and post-fusion conformations. The resulting model suggests that gB's fusion loops (within domain I) point away from the viral 102 membrane. Therefore, to produce fusion, gB would extend so that the fusion loops 103 could reach the target membrane, and then conformational changes, similar to the 104 ones proposed by Gallagher et al., would merge the cell and virus membranes. 105 106

107 Recently, we augmented the microvesicle strategy [15] to produce gB in its prefusion form [16]. Using cryo-EM, we imaged vesicles expressing full-length gB bound 108 to monovalent antibody fragments that do not possess an Fc region (Fabs) and to 109 whole antibodies, along with gB containing genetically encoded FP insertions. Since 110 111 the Fabs, antibodies and FPs were visible by cryo-EM, we used them as landmarks 112 to map the position of gB domains in its pre-fusion conformation. According to our experimental data, we proposed that, initially, gB has the fusion loops pointing 113 114 toward the viral membrane [16], thereby agreeing with the model proposed by Gallagher et al. Additionally, some samples trapped intermediate conformations of 115 gB, providing insights about how the pre- to post-fusion transitions could take place. 116 117 Based on these intermediate conformations, we suggested that the fusion loops of gB, which initially point toward the viral membrane, are relocated to the top of the 118 molecule as a second step in the fusion process, while gB maintains a compact 119 120 conformation. This intermediate conformation would therefore be similar to the one proposed by Zeev-Ben-Mordehai et al., reconciling the two models for two 121 conformations of qB. More data will be needed to unequivocally unravel the pre-122 123 fusion structure of gB and its transition to the post fusion form, thereby elucidating 124 the mechanism of fusion. 125 In conclusion, while the pre-fusion structure of gB still poses a challenge to structural 126

biologists, the advances in structural determination techniques and the ability to 127

- 128 produce gB in conformations other than post-fusion, are bringing us closer to the
- 129 answer. This structure will help with rational drug design and vaccine development to
- 130 tackle HSV infection.
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138 References

- 139 1. WHO. Herpes Simplex Virus. 2017 [cited 2017 17/01]; Available from: http://www.who.int/mediacentre/factsheets/fs400/en/. 140
- Grunewald, K., et al., Three-dimensional structure of herpes simplex virus from crvo-141 2. electron tomography. Science, 2003. 302(5649): p. 1396-8. 142
- 143 3. Chernomordik, L.V. and M.M. Kozlov, Mechanics of membrane fusion. Nat Struct Mol 144 Biol, 2008. 15(7): p. 675-83.
- Harrison, S.C., Viral membrane fusion. Nat Struct Mol Biol, 2008. 15(7): p. 690-8. 145 4.
- Eisenberg, R.J., et al., Herpes virus fusion and entry: a story with many characters. 146 5. 147 Viruses, 2012. 4(5): p. 800-32.
- Krummenacher, C., et al., Entry of herpesviruses into cells: the enigma variations. 148 6. 149 Adv Exp Med Biol, 2013. 790: p. 178-95.
- 7. Chowdary, T.K., et al., Crystal structure of the conserved herpesvirus fusion 150 151 regulator complex gH-gL. Nat Struct Mol Biol, 2010. 17(7): p. 882-8.

- Stampfer, S.D., et al., Structural basis of local, pH-dependent conformational
 changes in glycoprotein B from herpes simplex virus type 1. J Virol, 2010. 84(24): p.
 12924-33.
- Vitu, E., et al., Extensive mutagenesis of the HSV-1 gB ectodomain reveals
 remarkable stability of its postfusion form. J Mol Biol, 2013. 425(11): p. 2056-71.
- Cooper, R.S. and E.E. Heldwein, Herpesvirus gB: A Finely Tuned Fusion Machine.
 Viruses, 2015. 7(12): p. 6552-69.
- 159 11. Roche, S., et al., Structures of vesicular stomatitis virus glycoprotein: membrane
 160 fusion revisited. Cell Mol Life Sci, 2008. 65(11): p. 1716-28.
- 161 12. Roche, S., et al., Structure of the prefusion form of the vesicular stomatitis virus glycoprotein G. Science, 2007. **315**(5813): p. 843-8.
- 163 13. Atanasiu, D., et al., Dual split protein-based fusion assay reveals that mutations to
 164 herpes simplex virus (HSV) glycoprotein gB alter the kinetics of cell-cell fusion
 165 induced by HSV entry glycoproteins. J Virol, 2013. 87(21): p. 11332-45.
- 166 14. Gallagher, J.R., et al., Functional fluorescent protein insertions in herpes simplex
 167 virus gB report on gB conformation before and after execution of membrane fusion.
 168 PLoS Pathog, 2014. **10**(9): p. e1004373.
- 169
 15. Zeev-Ben-Mordehai, T., et al., Two distinct trimeric conformations of natively
 170 membrane-anchored full-length herpes simplex virus 1 glycoprotein B. Proc Natl
 171 Acad Sci U S A, 2016. **113**(15): p. 4176-81.
- 172 16. Fontana, J., et al., The Fusion Loops of the Initial Prefusion Conformation of Herpes
 173 Simplex Virus 1 Fusion Protein Point Toward the Membrane. MBio, 2017. 8(4).

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