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# Conformational Dynamics Related to Membrane Fusion Observed in Single Ebola GP Molecules †

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**Abstract:** The Ebola virus (EBOV) envelope glycoprotein (GP) is a membrane fusion machine required for virus entry into cells. Following the endocytosis of EBOV, the GP1 domain is cleaved by cellular cathepsins in acidic endosomes, exposing a binding site for the Niemann-Pick C1 (NPC1) receptor. The NPC1 binding to the cleaved GP1 is required for entry, but how this interaction translates to the GP2 domain-mediated fusion of viral and endosomal membranes is not known. Here, using a virus-liposome hemifusion assay and single-molecule Förster resonance energy transfer (smFRET)-imaging, we found that acidic pH, $Ca^{2+}$, and NPC1 binding act synergistically to induce conformational changes in GP2 that drive lipid mixing. Acidic pH and $Ca^{2+}$ shift the GP2 conformational equilibrium in favor of an intermediate state primed for NPC1 binding. GP1 cleavage and NPC1 binding enable GP2 to transition from a reversible intermediate to an irreversible conformation, suggestive of the post-fusion 6-helix bundle. Thus, the GP senses the cellular environment to protect against triggering prior to the arrival of EBOV in a permissive cellular compartment.

**Keywords:** virus entry; membrane fusion; Ebola virus; single-molecule FRET; conformational dynamics