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475 - Combining Hydrogen-deuterium Exchange Mass Spectrometry (hdx-ms) And Mass Photometry (mp) to Investigate Ebola Virus Entry Mechanisms

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Ebola virus (EBOV) entry into human cells is mediated by its trimeric fusion glycoprotein GP through internalization into the lysosomal compartment, where GP undergoes pH-dependent proteolytic cleavage by cathepsin B and L prior to NPC1 receptor binding. During entry, GP undergoes stepwise conformational changes that are difficult to study by the most common structural techniques, therefore these molecular mechanisms have so far remained unknown. We combined HDX-MS and MP to uncover the molecular bases of GP-mediated entry of the two major infectious species, Zaire and Sudan EBOV. HDX-MS unveils conformational rearrangements of GP and its structural transition in the endosomes when cleaved by cathepsin B and L. Specifically, the receptor binding site progressively switches from a more occluded to a more open conformation, but in a species-specific manner. MP and HDX-MS together provide insights in the GP cleavage products and their relative enzymatic rates as a function of pH, revealing Zaire GP being cleaved faster than Sudan GP. Furthermore, by HDX-MS we could observe that the binding strength of GP to its receptor varies with Sudan>Zaire subspecies. Overall, by employing biophysical measurements, we refine the Ebola virus entry model and highlight how the virus optimized specific steps of the entry process in the various species/variants that correlate to outbreak severity.