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Assessment of crowding by viral spike protein Neuraminidase as a potential driver for membrane budding

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Influenza A is a common viral infection spreading among humans and still remains a major societal challenge resulting in annual, global outbreaks with numerous casualties. A critical step in virus dissemination is the budding from the plasma membrane of infected cells. Despite extensive efforts, the mechanism underlying this viral budding event remains largely unsolved, however, the viral proteins NA, HA, M1 and M2 are known to be implicated in the facilitation of viral budding. In recent years, stochastic lateral pressure amongst membrane proteins, known as protein crowding, has evolved as a putative and effective driver of membrane bending and tubulation. The bulky ectodomains of the spike proteins NA and HA renders crowding a promising mechanism involved in the progression of viral budding. To gather evidence for this mechanism, optical tweezers were used to probe membrane bending in living cells expressing viral proteins. Specifically, membrane tethers were pulled from HEK cells transiently expressing NA, employing optical tweezers to allow for sensitive assessment of the membrane budding potential. Parallel imaging with confocal fluorescence microscopy allowed for correlation of the protein density with the force needed to extract membrane tethers. The results show a decrease in the tether equilibrium force as membrane coverage of NA increases, indicating an effect of crowding. Unexpectedly however, it was found that the average force (17.47 pN) measured for NA membrane tethers is significantly larger than for control cells (10.95 pN). This difference between transfected and wildtype cells is postulated to arise from a variability in membrane mechanics between the two groups, possibly due to side effects of the transfection protocol or due to an increase in the membrane bending rigidity (κ) upon expression of the NA transmembrane domain in the membrane. Conclusively, the presented assay is appropriate for the study of membrane crowding, and offers a novel method for quantitatively assessing the budding effect from viral envelope proteins.

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