

SELF-ASSEMBLY OF INFLUENZA HEMAGGLUTININ: STUDIES OF ECTODOMAIN AGGREGATION BY *IN SITU* ATOMIC FORCE MICROSCOPY

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We have used *in situ* tapping mode atomic force microscopy (AFM) to study the structural morphology of two fragments of the influenza hemagglutinin protein bound to supported bilayers. The two proteins that we studied are the bromelain-cleaved hemagglutinin (BHA), corresponding to the full ectodomain of the hemagglutinin protein and FHA2, the 127 amino acid N-terminal fragment of the HA2 subunit of the hemagglutinin protein. While BHA is water soluble at neutral pH and is known to bind to membranes via specific interactions with a viral receptor, FHA2 can only be solubilized in water with an appropriate detergent. Furthermore, FHA2 is known to readily bind to membranes at neutral pH in the absence of a receptor. Our *in situ* AFM studies demonstrated that, upon binding to supported bilayers at neutral pH, both these proteins self-assemble into oligomeric structures. *In situ* acidification resulted in further association of the FHA2 without a large perturbation of the bilayer. In contrast, BHA remained largely unaffected by acidification, except in areas of exposed mica where it aggregated on the mica. Remarkably, these results are consistent with previous observations that FHA2 promotes membrane fusion while BHA only induces liposome leakage at low pH.