

POSTER 50**MODULATION OF HIV-1 PROTEASE AUTOPROCESSING BY CHARGE PROPERTIES OF SURFACE RESIDUE 69 UNDER DIFFERENT CONTEXTS**

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The HIV protease is initially translated as part of the Gag-Pol precursor in the infected cell and the cleavages releasing the protease from the precursor are essential for the production of mature dimeric protease that is indispensable for viral infectivity. In order to define viral determinants that are important for the protease maturation, we engineered a bacterial expression plasmid encoding for a chimeric protein consisting of GST fused to a mini protease precursor, the N-terminal transframe region (p6^{pol}) and a pseudo wild type protease, followed by FLAG peptide. The resulting fusion precursor was competent for autoprocess in *E. coli* releasing at least two soluble cleavage products: the N-terminal GST-containing moiety and the C-terminal FLAG-tagged mature protease. With this model system, we demonstrate that protease maturation was abolished when H69, a surface residue on mature protease, was replaced with a negatively-charged glutamate; while mutations to other neutral or basic amino acids had no significant impact on protease autoprocessing. Biochemical analyses revealed that H69E mutation significantly delayed protease precursor maturation in an *in vitro* autoprocessing assay; while properly refolded mature H69E mutant showed slightly increased dimer dissociation constant, and reduced catalytic activity by about two fold. We conclude that charge properties of residue 69 modulate protease maturation probably by influencing productive folding of the precursor.

Mutation H69E also inhibited protease maturation (> 10-fold) in a pNL4-3 derived provirus, in which the pNL protease sequence was replaced by the pseudo wild type (WT^{pse}) protease carrying H69E mutation. Interestingly, H69E mutation alone in the context of pNL backbone reduced protease maturation by less than one-fold, suggesting H69E effect is environment dependent. Compared to the pNL 4-3 derived protease sequence, the WT^{pse} protease carries 6 mutations (Q7K, L33I, N37S, L63I, C67A, and C95A). Mutagenesis analyses mapped C67 and C95 as the primary determinants that dampened the inhibitory effect of H69E. However, the rescue effect of C67/C95 was completely abolished when H69 was replaced by aspartic acid even in the pNL backbone. Taken together, we suggest that residue 69 along with other amino acids such as C67 and C95 play a regulatory role in protease maturation.