

## FUNCTIONAL ANALYSIS OF A NOVEL 3-FOLD CTD-CTD INTERFACE IN THE HIV-1 CAPSID

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Mature HIV-1 particles contain a conical capsid that encloses the viral RNA genome and performs essential functions in the viral life cycle. Previous structural analyses of two- and three-dimensional arrays provided a molecular model of the capsid protein (CA) hexamer and revealed three interfaces in the lattice. To search for novel intermolecular interactions involved in HIV-1 capsid function, we performed a cryoEM study of a tubular assembly of CA. By docking atomic 3D structures of individual domain of CA into the cryoEM density map, a novel CTD-CTD interface at the local three-fold axis was identified. To validate the structural model, pairs of Cys mutations (P207C/ T216C) were engineered at positions predicted to lie at the 3-fold interface. Oxidation of mutant virions resulted in accumulation of a novel disulfide-linked protein corresponding to a trimer of CA. The trimer was also produced upon oxidation of recombinant CA P207C/ T216C protein following assembly *in vitro*. Examination of the trimer interface revealed several amino acids (K203 and Q219) previously shown to be critical for stabilizing the viral capsid. Therefore, we tested the effects of additional CTD mutations in this interface. Mutations E213A or E213Q at the trimer interface resulted in decreased viral infectivity and elevated capsid stability compared to wild type. E213 mutant particles exhibited mature viral capsids resembling those in wild type virions under electron microscopy. This study provides genetic evidence for the existence of a novel 3-fold CTD-CTD interface in the HIV-1 capsid that plays a critical role in viral uncoating.