

STRUCTURE OF FULL-LENGTH HEXAMERIC HIV-1 CA: A MOLECULAR JIGSAW PUZZLE SOLVED BY HYBRID METHODS

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HIV assembly and replication proceed through formation of morphologically distinct immature and mature viral capsids that are organized by the Gag polyprotein (immature) and by the fully processed CA protein (mature). The Gag polyprotein is composed of three folded polypeptides (MA, CA, and NC) and three smaller peptides (SP1, SP2, and p6) that function together to coordinate membrane binding and Gag-Gag lattice interactions in immature virions. Following budding, HIV maturation is initiated by proteolytic processing of the spherically-arranged Gag polyprotein. The resulting dramatic morphological changes result in formation of the distinctive conical capsid and culminate in particles that are infectious. The mature capsid can be modeled as fullerene structures composed of exactly 12 CA pentamers and about 1,500 copies of CA arranged in a quasi-hexagonal lattice. The lack of strict symmetry in the capsid has precluded traditional high-resolution X-ray and NMR structure analysis, and we have used model systems with two-dimensional (2D) and three-dimensional (3D) symmetry to gain insight into the mature capsid lattice. Electron cryomicroscopy (cryoEM) and image analysis of 2D crystals of CA yielded a 3D density map with an in-plane resolution of 9 Å (1). Rods of density corresponding to α -helices served as fiducials for docking high-resolution structures for the individual N-terminal domains (NTDs) and C-terminal domains (CTDs) of CA. The resulting cryoEM-based model guided the design of intermolecular disulfide bonds to generate hexamers of full-length CA (2). The mutants were screened to identify those that retained wild-type assembly properties and could be purified as stable hexamers. Extensive 3D crystallization trials culminated in atomic resolution structures of full-length CA hexamers from two different crystal forms, the highest at 1.9 Å resolution. To verify that the mutagenesis strategy did not perturb the structures, we also determined a lower-resolution crystal structure of wild-type CA fused to the stable hexameric protein, CcmK4. Preservation of the CA conformation between the wild-type fusion protein and the crosslinked constructs gave us confidence that the high-resolution structures were biologically relevant. The CA hexamer is composed of a relatively rigid inner ring of NTD subunits, surrounded by a mobile belt of CTD subunits. Interfaces that stabilize the NTD ring are highly hydrated, and this may be a key feature that allows the use of the same interfaces to form the quasi-equivalent pentamer. Pair-wise interactions between the CTDs link adjacent hexamers, and mobility of the CTD belt is likely to be an underlying mechanism for generating the variably curved lattice in authentic capsids. HIV-1 CA is a potential therapeutic target, and high-resolution structures are now essential for any drug discovery efforts. Indeed, the crystal structures reveal the details on how specific compounds sterically interfere with capsid assembly. (Supported by NIH R01 GM066087 and NIH P50 GM082545)

1. Barbie K. Ganser-Pornillos, Anchi Cheng, and Mark Yeager, Structure of Full-length HIV-1 CA: A Model for the Mature Capsid Lattice. *Cell* 131: 70-79 (2007).
2. Owen Pornillos, Barbie K. Ganser-Pornillos, Brian N. Kelly, Yuanzi Hua, Frank G. Whitby, David Stout, Wesley I. Sundquist, Christopher P. Hill, and Mark Yeager, X-ray Structures of the Hexameric Building Block of the HIV Capsid. *Cell* 137:1282-1292 (2009).