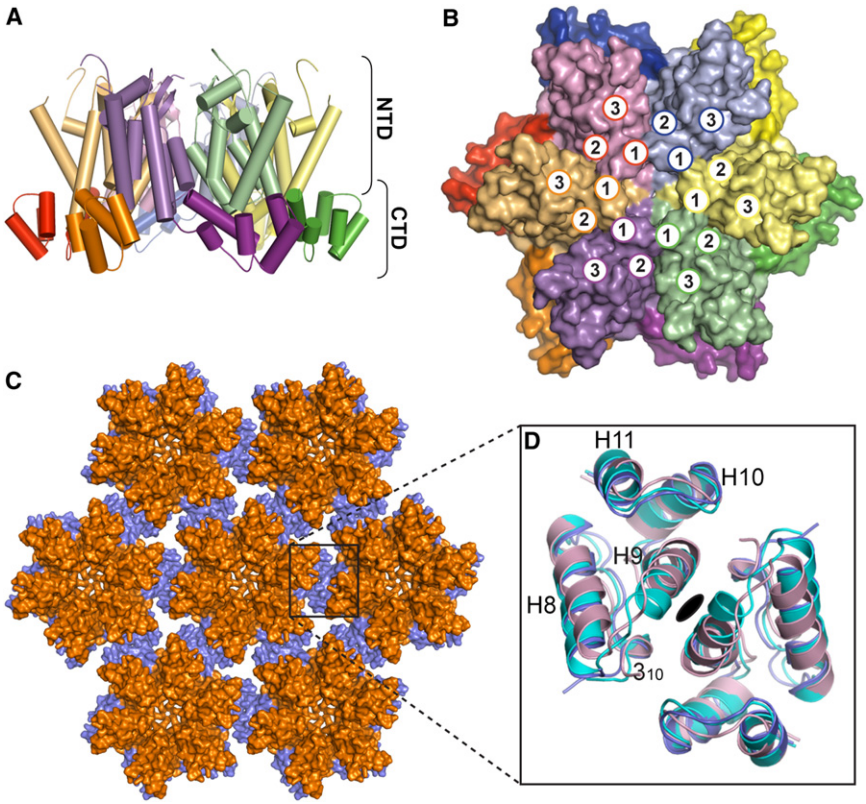

Figure 2. Structures of the HIV-1 CA Hexamer

(A) Side view of a crosslinked hexamer. Each protomer is in a different color, with the NTDs in muted shades and the CTDs in brighter shades. The NTD and CTD layers are indicated.

(B) Top view of a crosslinked hexamer, colored as in (A). The positions of the first three helices of each protomer are indicated by numbered circles. These form a helical barrel at the core of the hexamer.

(C) Top view of one sheet in the CcmK4-templated CA crystals, which recapitulates the hexameric lattice of authentic capsids at its planar limit. The NTDs are colored orange, and the CTDs are blue. This view emphasizes that interactions between neighboring hexamers are mediated only by the CTD.

(D) Top view of the CTD-CTD interface that connects neighboring hexamers, as seen in the CcmK4-templated (cyan) and crosslinked hexagonal crystals (blue), and superimposed with the isolated full-affinity CTD dimer (pink) (Worthylake et al., 1999). The black oval represents the twofold symmetry axis. We speculate that the slight differences in domain orientations across the dyad arise from the W184A and M185A mutations in the crystallized constructs. The average deviations for all Cα positions are cyan/pink = 2.4 Å, blue/pink = 2.8 Å, and cyan/blue = 0.9 Å.

other, and resemble the X-ray structure of the isolated full-affinity CTD dimer (pdb code 1a43) (Worthylake et al., 1999), although the agreement in the relative orientations of the CTDs about the dyad is not exact (Figure 2D). This dimer interface is distinctly different from those seen in a shorter CTD construct (2a8o) (Gamble et al., 1997), or in the presence of an assembly inhibitor (2buo) (Ternois et al., 2005), or upon mutation-induced domain swapping (2ont) (Ivanov et al., 2007) (data not shown).

Previous studies have suggested conformational plasticity in the tertiary fold of the CTD (Alcaraz et al., 2007; Bartonova et al., 2008; Berthet-Colominas et al., 1999; Bhattacharya et al., 2008; Ivanov et al., 2007; Ternois et al., 2005; Wong et al., 2008), and this flexibility is also evident in our structures of crosslinked CA. In one crystal form, the N-terminal two-thirds of helix 9 and the preceding loop are poorly ordered, and in the second crystal form this region adopts two distinct conformations. In one conformation, the loop trajectory places it close to the neighboring NTD, allowing polar side chains to participate in the hydrogen bond network at the NTD-CTD interface (Figure S5A). This CA CTD configuration has not been observed previously. In the second conformation, the loop resembles the position seen in the 1a43 monomer and does not contact the NTD (Figure S5B). We identified three additional areas of structural variability (data not shown): the major homology region (MHR) hairpin in two subunits appeared to have an alternative conformation with a noncanonical hydrogen-bonding network, which we did not attempt to model because of ill-defined density; the native C198-C218 disulfide bond appeared to exist in both the reduced and oxidized forms, with varying occupancies for each protomer, and helix 10 was characterized by variable and ill-defined densities across the different subunits, indicative of positional disorder and mobility. It is likely that the range of conformations seen in the crystals reflects both the natural plasticity of the CTD and amplified flexibility arising from the W184A and M185A mutations within helix 9.

Hexamer-Stabilizing Interactions between NTDs

The first three helices of CA contain the NTD-NTD-interacting residues, which form a loose 18 helix barrel at the center of the hexamer (Figure 2B). This interface contains a small hydrophobic core of aliphatic side chains, which include M39 and A42 (indicated in Figure 3B). These residues were previously shown by mutagenesis to be critical for both CA assembly in vitro and viral infectivity in vivo (Ganser-Pornillos et al., 2004; von Schwedler et al., 1998, 2003). Despite these limited hydrophobic interactions, the bulk of the interface is created by hydrophilic contacts. In particular, numerous ordered water molecules bridge polar side chain and backbone atoms throughout the entire interface, forming a pervasive hydrogen-bonding network (Figure 3C). Bridging waters were also observed in the hexameric X-ray structure of the isolated NTD of MLV CA (Mortuza et al., 2004), suggesting that heavily solvated interfaces may be a general property of retroviral CA hexamer interfaces. To our knowledge, similar water-rich interfaces have not been seen previously in nonretroviral capsid assemblies.

A very recent cryoEM study showed that the CA hexamer and pentamer of Rous sarcoma virus are quasi-equivalent, with pentamers formed by simply removing a protomer from the hexamer and closing the ring (Cardone et al., 2009). By analogy, helices