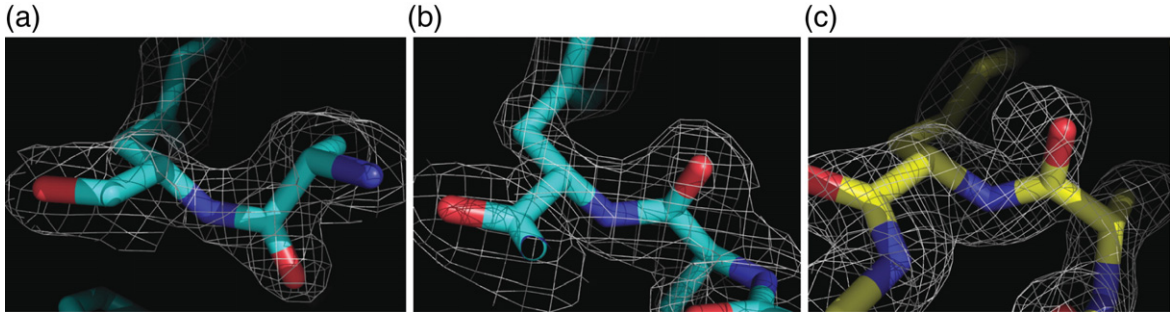

Figure 5. Buried Phe32 conformation seen in the absence of CAP-1 is associated with backbone strain. (a) Example of a *cis* Ala31–Phe32 peptide. (b) Example of a *trans* Ala31–Phe32 peptide. (c) The Ala31–Phe32 in the structure crystallized in the presence of CAP-1 (*trans*). Density is shown for simulated annealing omit maps.[61]

hexamer. The six C termini, which lead to the CA^C dimerization domains, are located at the bottom of the hexamer model (Figure 6(a)).

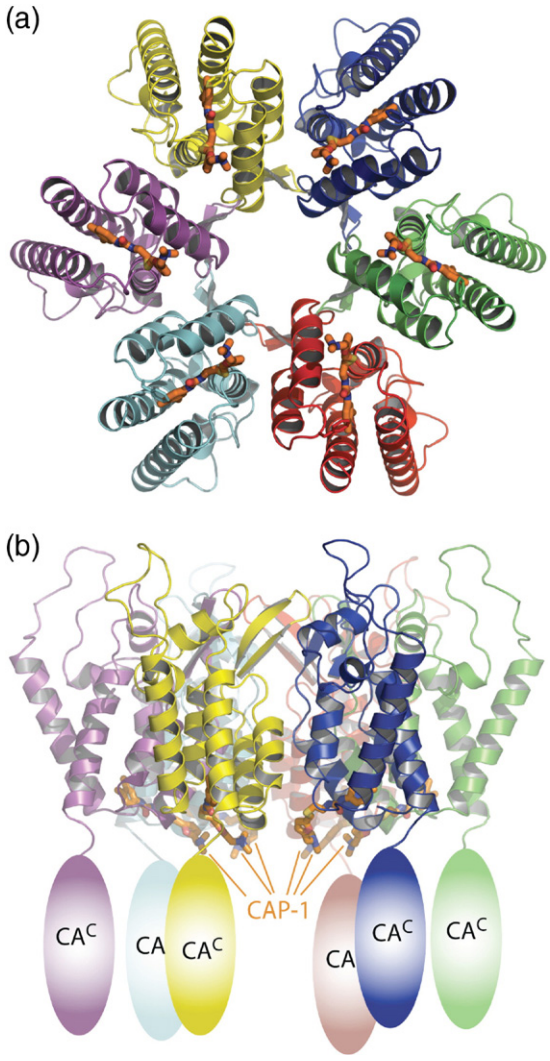

Figure 6. (a) Model of the HIV-1 CA^N hexamer of the mature capsid lattice modeled on the MLV CA^N structure.[17] CAP-1 is shown in stick representation. (b) Orthogonal view. The approximate location of CA^C is indicated.

The CAP-1 binding site and Phe32 are located on the bottom (inner) surface of the CA^N hexamer model. Binding of CAP-1 would not obviously impact CA^N hexamer formation because the nearest approach of a modelled CAP-1 atom to the adjacent CA^N subunit is ~5 Å, and occurs between a flexible CA^N side-chain and the exposed and presumably mobile CAP-1 furyl ring. In addition, CAP-1 does not appear to induce significant conformational changes away from its binding site. Similarly, it is difficult to envision how CAP-1 binding to CA^N would alter CA^C dimerization. It therefore seems most likely that CAP-1 binding inhibits formation of the third intermolecular interface, between C^N and CA^C domains of adjacent molecules within the hexamer. The importance of CA^N:CA^C interactions was initially suggested by genetic analyses of the RSV CA protein[24] and by hydrogen exchange experiments,[21,22,40] which indicated that several of the conserved residues adjacent to HIV-1 CA Phe32 participate in an intermolecular CA^N-CA^C interface upon capsid assembly.[21] Furthermore, the flexible side-chain of CA^N Lys70 is susceptible to chemical cross-linking with Lys182 on the CA^C domain of a second CA molecule during mild alkylation of assembled tubes,[21] and mutation of Lys70 inhibits CA tube assembly *in vitro*.[41]

Very recently, Ganser-Pornillos & Yeager (unpublished results) have visualized this third CA^N-CA^C interface at moderate (9 Å) resolution in cryo-EM reconstructions of 2D crystals of hexagonal CA arrays. Their studies show that CA^C domains pack in a groove between the N-terminal end of CA^N helix 4 and the C-terminal end of CA^N helix 7, i.e. in the region distorted by binding of CAP-1. Although side-chain detail is not available at the current resolution, it seems likely that the conformational changes that we observe upon CAP-1 binding would inhibit this packing interaction. One possibility is that exposure of the Phe32 side-chain, displacement of the CA^N helix 7 Tyr145 side-chain and repacking of the His62 side-chain against the N-terminal end of CA^N helix 4 might alter the surface of CA^N in a manner that inhibits its interactions with CA^C. An alternative possibility is that, during capsid assembly, CA^C binding to CA^N normally triggers a conformational change similar