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Structure of the T=3 Hepatitis B Virus Capsid at 3.6Å Resolution

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Hepatitis B virus is a major cause of liver disease, with some 400 million chronically infected people, worldwide. The virion consists of a lipoprotein envelope surrounding an icosahedral capsid containing the genome. The HBV capsid, also called the core antigen (HBcAg), is unusual in that it exhibits two sizes. The majority have a triangulation number T=4 and consist of 120 dimers. The remainder are smaller, with T=3 and 90 dimers. The T=4 capsid was the first particle to be reconstructed to subnanometer resolution by cryo-EM (1, 2). Its building-blocks are dimers paired through the formation of a 4-helix bundle from two α-helical hairpins, Structure determination was completed a year later with a crystal structure at 3.4 Å resolution (3). The present study aims to determine the structure of the T=3 capsid to comparably high resolution by single-particle cryo-EM.

Capsids were prepared by expressing the 149-residue "assembly domain" in *E. coli* and purified as described (4). To separate the T=3 capsids, this material was concentrated by ultrafitration and applied to a 5-30% sucrose gradient formed by tilted-tube rotation and centrifuged in a Beckman SW28 rotor. The T=3 capsid band was recovered from below by bent-tube collection, concentrated by ultrafiltration, and re-chromatographed by gel filtration. The sample was vitrified and imaged on a FEI F30 Polara transmission electron microscope operating at 200 keV, with defocus settings between 0.8 and 1.7 μm and a magnification of 69,000x. A total of ~ 28,000 particles were picked from 294 scanned micrographs and reconstructed as described (5). 16,000 particles were included in the final reconstruction which has a resolution of ~ 3.6 Å according to the "gold standard" version of the Fourier shell correlation coefficient.

Grayscale sections of the T=3 capsid are shown in Figure 1 (panels 1 and 2). The α-helices that make up the capsid framework are clearly defined. The main feature of the outer surface is the protruding 25Å-long spikes made up of paired α-helical hairpins. The long helices have evident helical features in terms of grooves that follow the helical geometry and protruding densities for some side-chains. The density of the connecting loops of the hairpins – the so-called “immunodominant loops” - is significantly fainter (white arrow in Figure 1), indicative of local mobility. The transverse section (panel 3) resolves the four helices clearly. T=3 capsid has three quasi-equivalent variants of the assembly domain (A, B, C in Fig. 2 - left panel) which differ markedly from each other and from the four quasi-equivalent variants on the T=4 capsid (A, B, C, D) in their affinities for monoclonal antibodies (6). On this basis, they are anticipated to exhibit significant structural nuances among the variants. The molecular structure was determined by using "flexible fitting" to adapt dimers from the T=4 crystal structure to best fit the T=3 cryo-EM map. The most pronounced difference detected at this stage of the analysis is lateral displacement of the α4-helix of the C-subunit from its position in the T=4 capsid (arrows in Figure 2). This indicates flexing in the 4-helix bundle centered on the dimer interface as the protein adapts to the greater curvature of the T=3 capsid. In contrast, the respective A-subunits which are clustered around the 5-fold axes are essentially indistinguishable.

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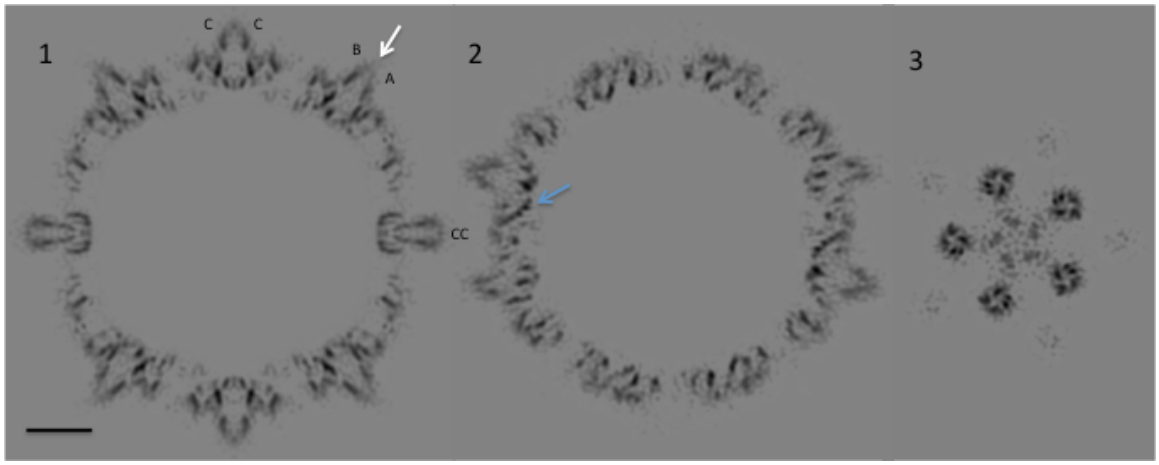

Figure 1. Grayscale sections of the T=3 HBV capsid viewed along a 2-fold axis. A C-C dimer and an A-B dimer are labeled. The sections labeled 1, and 2 are respectively slices 240 (central), and 265. The section labeled 3 is slice 391 of 5-fold-view, showing five 4-helix bundles. The densities marked with blue arrows have evident helical features. The white arrow marks the fainter density of an "immunodominant loop". Scale bar = 50Å.

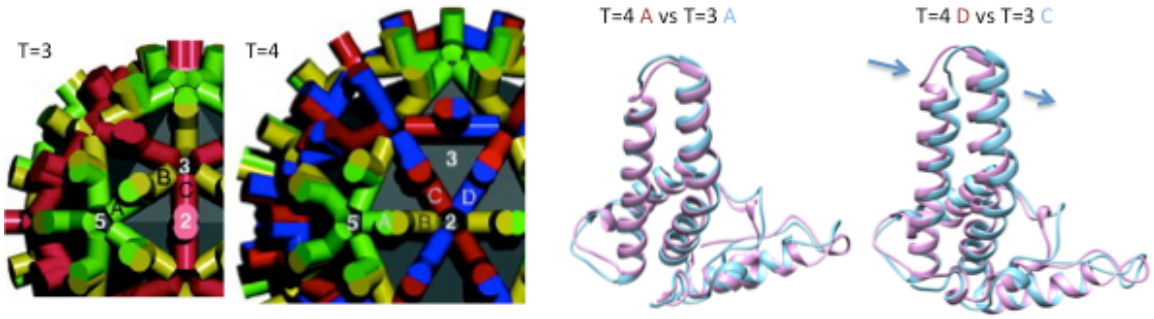

Figure 2. Left: models of dimer packing in T=3 and T=4 capsids showing the positions of the various subunits (A, B, C, etc), adapted from (6). Right: Comparison of the A-subunits of the T=4 capsid (PDB 1QGT) and the T=3 capsid (present reconstruction) and between the D-subunit of the T=4 capsid and the C-subunit of the T=3 capsid. The relative displacement of the helical hairpin is marked with blue arrows.

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