

## An N-terminal extension to the hepatitis B virus core protein forms a poorly ordered trimeric spike in assembled virus-like particles

### ABSTRACT

Virus-like particles composed of the core antigen of hepatitis B virus (HBcAg) have been shown to be an effective platform for the display of foreign epitopes in vaccine development. Heterologous sequences have been successfully inserted at both amino and carboxy termini as well as internally at the major immunodominant epitope. We used cryogenic electron microscopy (CryoEM) and three-dimensional image reconstruction to investigate the structure of VLPs assembled from an N-terminal extended HBcAg that contained a polyhistidine tag. The insert was seen to form a trimeric spike on the capsid surface that was poorly resolved, most likely owing to it being flexible. We hypothesise that the capacity of N-terminal inserts to form trimers may have application in the development of multivalent vaccines to trimeric antigens. Our analysis also highlights the value of tools for local resolution assessment in studies of partially disordered macromolecular assemblies by cryoEM.

**Keyword:** Virus-like particle; Vaccine; Hepatitis B virus; Cryo-electron microscopy; Three-dimensional reconstruction; Local resolution