Dissecting HBc protein domains essential for Hepatitis B Virus Capsid Assembly with the help of quantitative fluorescence microscopy.

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Hepatitis B Virus is a 42 nm diameter hepatotropic DNA virus with an outer envelope sheltering a 30 nm diameter icosahedral capsid. This capsid corresponds to the oligomerization of the core protein (HBc) and is filled by a partially double stranded DNA. A number of studies support that in addition to its structural functions, HBc also has pleiotropic function in HBV cycle and is a critical actor in virus replication, thus making it a target in the search for anti-viral molecules.

HBc contains 183 residues and is composed of two major domains. The N terminal domain (NTD, residues 1-140) is essential for the self-assembly of the particle. The C terminus (CTD) corresponds to unstructured basic and phosphorylated peptide interacting with the Pol DNA polymerase. Between NTD and CTD, a small spacer peptide (141-149) has been proposed to regulate capsid assembly.

To date, studies of the HBV capsid assembly and the functions of the HBc protein domains have relied mainly on electron microscopy and in vitro experiments using proteins purified from E. coli or insect cells.

Here, we visualized and monitored HBV capsid assembly in live cultured cells. To do so, we used Huh7 cells transiently expressing HBc together with HBc derivatives harbouring a fluorescent reporter at position 79-81. We were then able to follow the behavior of these proteins in vivo under the microscope by using FRET by FLIM and Fluorescence Fluctuation Spectroscopy. Classical autocorrelation analysis always exhibited a single species model even in the situation of potential mixed populations of monomers and oligomers. We thus developed an analysis pipeline of the Photon Counting Histogram through fitting the experimental data with a two-species model, allowing us to distinguish mixed populations. We could therefore characterize the capacity of several truncated versions of the HBc protein to form oligomers and dissect the requirement of the HBc subdomains in capsid assembly in live conditions.

Most notably, we were able to estimate the concentration of HBc protein at which capsid assembly occurs and show it is independent from cellular compartment. The loss of the C-terminal domain is detrimental for the assembly and not rescued by increasing the protein concentration.

Overall, our results shines new light on the spatio-temporal behaviour of the HBc protein and provides useful tools for more in-depth studies of HBc oligomerization and regulation, and of HBV capsid assembly.