The use of biological model systems behaving in a native fashion is crucial to gain meaningful insights into biological processes. The HIV-1 Gag protein assembles at the plasma membrane into virus-like particles (VLPs) when expressed in mammalian cells. VLP assembly and budding have been studied using fluorescently labelled Gag (GagFP), but the impact of the fluorescent label on Gag assembly has been controversial. As shown by electron microscopy, expressing GagFP alone leads to aberrant VLP morphology [1]. Intriguingly, fluorescence fluctuation spectroscopy measurements show no change in size of budded VLPs formed from GagFP [2]. However, the impact on the morphology of VLPs has not been quantified. This is due to one main limitation: the size of VLPs, which are too small to resolve using diffraction-limited fluorescence imaging.

We address this limitation by studying the morphology of VLPs at the plasma membrane as a function of the fluorescent label used, with superresolution imaging. Photoactivated localization microscopy (PALM) of fixed mammalian cells expressing Gag labelled with mEos or the larger tandem dimeric variant tdEos is performed to parameterize Gag cluster shapes at the plasma membrane. We determine amongst other quantities their radius, labelling density, aspect ratio, centre of mass and radial distribution. We do not observe a significant effect on the morphology of forming VLPs, but we do measure an increase in VLP size with increasing label size. This quantitative analysis of Gag cluster morphology aims to address the existing controversies and establish a suitable model system to further investigate Gag assembly using live-cell PALM.