UNDER THE LENS

Coming together during viral assembly

Christof Hepp and Nicole C. Robb

This month's Under the Lens discusses how super-resolution microscopy has been used to answer fundamental questions about the assembly mechanisms of enveloped viruses. What — if anything — helps the major components of a virus particle find each other?

Enveloped viruses are surrounded by a lipid bilayer, which are formed as nascent virions bud through cellular membranes. Virus assembly at the plasma membrane is an essential part of the viral life cycle, and its regulation is a potential target for antiviral therapies. It is generally accepted that the accumulation of viral matrix proteins at the plasma membrane is sufficient to drive the formation of virus-like particles (VLPs), while viral glycoproteins are incorporated into the particles to mediate host cell entry. Viral glycoproteins are thought to be directed to assembly sites through interactions with viral matrix proteins; however, precise information on the distribution, organisation and dynamics of the viral components is needed to determine whether this association is random, or the result of specific regulatory interactions. Two recent studies have sought to address these questions by imaging Nipah virus (NiV) and HIV-1 assembly at nanometre-scale resolution.

Single molecule localization microscopy methods take advantage of the sequential activation and ultra-precise localization of photoswitchable fluorophores to reconstruct a super-resolved image. Liu et al.[1] utilised three-dimensional stochastic optical reconstruction microscopy (STORM) imaging combined with sample drift correction to image host cells transfected with plasmids that express the NiV matrix protein (M), attachment glycoprotein (G) and fusion protein (F). The researchers found that clusters of F and G glycoproteins did not significantly co-localise, and were randomly distributed on the plasma membrane regardless of the presence or absence of M. Moreover, imaging of purified VLPs showed that the concentration of F and G on the virion surface was dependent on cell surface expression levels, suggesting that the incorporation levels of these proteins is unregulated. These findings suggest a model for the assembly of NiV particles in which the incorporation of glycoproteins into nascent virions is not driven by M, but rather an undirected, stochastic process.

In a separate study, Buttler et al.[2] used inferometric photoactivation localization microscopy (iPALMto obtain super-resolved images of cell-associated HIV-1 particles. During HIV-1 infection, the matrix domain of the Gag polyprotein oligomerizes into a lattice to drive the formation VLPs at assembly sites, and viral envelope (Env) glycoproteins assemble within this lattice[3]. Steric trapping of the long cytoplasmic tail domain of Env (Env-CT) between Gag proteins is thought to retain Env at assembly sites, and because only a small number (7–14 molecules) of Env is present on individual released particles, this incorporation is likely to be tightly regulated. The authors showed that Env predominantly clusters at the neck region of VLPs, suggesting that Env is incorporated into the Gag lattice late during assembly. Three dimensional super-resolution microscopy of infected cells showed that Env was predominantly retained intracellularly, possibly to prolong incorporation into VLPs and limit its density on individual virus particles. Furthermore, single particle tracking revealed that the Env-CT domain decreased Env mobility within the plasma membrane, supporting a model in which steric trapping supports the incorporation of Env into nascent particles. Whereas Liu et al.'s study provides evidence for stochastic incorporation of glycoproteins into NiV virions, Buttler et al.'s results point to a tightly regulated assembly pathway that limits cell surface exposure and the incorporation of Env into HIV-1 virions.

In summary, the comparison of these two studies highlights important differences in enveloped virus assembly mechanisms, and illustrates the strength of super-resolution microscopy techniques in taking advantage of precise spatial information to discover subtle regulation mechanisms that could not be observed using conventional light microscopy methods.

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1. Liu, Q. et al. A stochastic assembly model for Nipah virus revealed by super-resolution microscopy. *Nat. Commun.* **9**, 3050 (2018).
2. Buttler, C. A. et al. Single molecule fate of HIV-1 envelope reveals late-stage viral lattice incorporation. *Nat. Commun.* **9**, 1861 (2018).
3. Freed, E.O. HIV-1 assembly, release and maturation. *Nat. Rev. Microbiol.* **13**, 484 (2015).

Competing interests

The authors declare no competing interests.