Combining FRET imaging with single-virus particle tracking to elucidate the uncoating process of Dengue virus

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Abstract:

Single-particle tracking is a real-time imaging technique that can monitor successfully individual virus entry and trafficking behavior in live cell [1]. However, the scale of virus uncoating involved virus-host membrane fusion is within dozens nanometer. It is smaller than optical resolution limitation and failed to detect by single-particle tracking. In order to resolve this limitation, we designed the Förster resonance energy transfer (FRET) pair fluorophore labeling virus particles using DiI as a FRET donor and DiD as a FRET acceptor and tracked this virus particle in living cells. We hypothesized that during viral uncoating, the distance between DiI and DiD in virus envelope was elevated, resulting in the decrease of FRET efficiency. According to this hypothesis, uncoating events of DENV cells can be observed by FRET acceptor bleaching imaging. In addition, the treatment of various drugs including Bafilomycin A1, a vacuolar-type H$^+$-ATPase inhibitor, and 3-MA, a suppressor of autophagic initiation caused the change of DENV uncoating process, suggesting that DENV uncoating events are occur in autophagosomes. In summary, by combining FRET and single-virus particle tracking approaches, we demonstrate that autophagy may participate in DENV trafficking and uncoating during viral infection.

Reference: