# Fluorescence lifetime imaging reveals single-cell resolution of metabolic control of HIV-1 entry and its link to membrane lipid order and tension.

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How viruses modulate the cell's bioenergetic state during entry remains to be investigated. Furthermore, it is unknown whether distinct metabolic states of host cells affect the success of viral entry. We developed an assay utilising FRET-based biosensors (i.e. of ATP:ADP ratio[1] and lactate[2]) to evaluate the effect of viral entry on their fluorescence lifetimes in TZM-bl and primary $CD4^+$ T-cells. Lifetimes of single cells were recorded before and after addition of HIV-1 pseudoviruses (i.e. HIV-$1_{JRFL}$). These pseudoviruses encapsulated Vpr-BlaM to perform fusion assays. Lifetime measurements from cells expressing either the ATP:ADP ratio or the lactate biosensor were used to determine relative metabolite concentrations during entry. These same cells were screened with β-lactamase and β-galactosidase assays to correlate lifetime changes with fusion-positivity and/or infection-positivity. Notably, cells exposed to HIV-$1_{JR\text{-}FL}$ decisively increased their [ATP]:[ADP] ratio and lactate concentrations. These global metabolic changes correlated with virion fusion. Cells with a lower basal [ATP]:[ADP] ratio prior to virus addition were less likely to demonstrate changes in metabolic state during entry, indicating a relationship between the metabolic state of the cell and viral entry. To confirm this, we show that cells treated with 2-deoxy-d-glucose (2-DG) had substantially less fusion, and viruses were arrested at hemifusion during treatment. Additionally, cells treated with 2-DG possessed less plasma membrane cholesterol. However, the addition of cholesterol to the plasma membrane partially rescued this block to fusion. Lifetime imaging of Flipper-TR[3] and MSS[4] plasma membrane biosensors revealed a link between the glycolytic activity of the cell, membrane order and tension, indicating glycolytically-inhibited cells possessed a decreased membrane order and increased membrane tension, both negative regulators of endocytic entry[5]. We therefore tracked viral-induced changes in global host cell metabolism during fusion and productive infection in real-time at the single-cell level. Our results indicate that HIV-$1_{JR\text{-}FL}$ fuse with glycolytically-active cells and this activity is linked to plasma membrane cholesterol abundance and tension. Moreover, we show that HIV-$1_{JR\text{-}FL}$ actively alters the host metabolic profile during viral entry.

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