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Replication Independent Virus Entry Assay Based on Enzyme Complementation

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Studies of viral entry often rely on the detection of post-entry parameters such as viral replication or the expression of a reporter, rather than on measuring entry per se. The lack of assays to actually detect the entry event hampers the analysis of this important process.

Here we describe a method that allows us to monitor entry of the membrane enveloped mouse hepatitis coronavirus (MHV) without relying on replication dependent signal amplification. The entry assay makes use of the minimal complementation of β-galactosidase. It comprises a deficient enzyme fragment, which is expressed by the target cell in the cytosol. A small complementing peptide is fused to the intravirion C-terminal end of the spike membrane fusion protein. Upon fusion of the virus with the cell membrane, enzymatic activity is reconstituted which can be measured in live mammalian cells by flow cytometry.

We were able to produce a stable recombinant MHV virus that is minimally attenuated compared to the wild type. We proved that the method used is independent of viral replication and that viral fusion can be detected already at low MOI.

This entry assay may be adapted to other viruses and could provide a viable alternative to replication dependent entry assays.

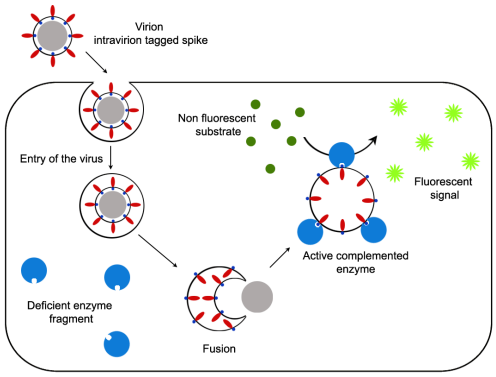

Figure1: Scheme of the entry assay

Keywords: Virus Entry, Murine Hepatitis Virus, Enzyme Complementation, Entry Monitoring