Real-time monitoring of viral replication by fluorescence resonance energy transfer (FRET)-based molecular probes

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The ability to detect infectious viruses is of critical importance for medical diagnostic and environmental/agricultural protection. Current methods to assess the presence of infectious viruses are based on detecting the production of cytopathic effects from mammalian cell culture and can take up to weeks before positive identification. Viral proteases play a key role in the production of infectious virus particles by proteolytic cleavage of viral polyproteins and are the prime targets for therapeutic agents. Our lab has recently developed several fluorescence resonance energy transfer (FRET)-based molecular probes to detect the presence of viral proteases as an indication of viral infection. The specific approach shown here was to generate a CdSe-ZnS core-shell quantum dot (QD)-modified, protease-specific (Hepatitis A virus 3C protease) protein module that can be used as a FRET substrate for probing protease activity, and fluorescent protein as the FRET acceptor for the QD donor. For intracellular delivery, a cell-penetrating Tat peptide was flanked to the FRET probes to enable real-time monitoring of viral infection in living cells. Fluorescence microscopy was used to directly visualize infected cells. The specific nature of these probes enables their utility for rapid diagnostic of viral infection.