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Abstract

Genetically Encoded Fluorescent Probes for Imaging of Intracellular Localization and Activity of SARS-CoV-2 Proteins †

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Abstract: Since December 2019, the problem caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has grown into a global threat. The search for new treatment strategies is strongly associated with both fundamental research into the mechanisms of the virus life cycle and the development of new screening platforms for antiviral drug candidates. In this project, we labeled SARS-CoV-2 membrane proteins M, E, and S and studied their localization in mammalian cell compartments using fluorescent microscopy. We tested the N- and C-oriented sensor designs and different fluorescent proteins. Additionally, we successfully visualized the early stages of M protein transport in real time. We found that the M protein localizes in cell lysosomes, which supports the recent hypothesis that β-coronaviruses use lysosomal organelles for egress instead of the traditional Golgi-mediated secretory pathway. Further, we plan to study the interactions between M, E, and S using the FRET method. In addition, we developed several types of FRET-based and translocational sensors to track SARS-CoV-2 PLpro protease and measure its activity in living cells. The preliminary experiments showed the expected increase in donor fluorescence after proteolysis of the PLpro site between the FRET-pair. The results of the current project will provide unique information on the spatial-temporal dynamics and interaction between SARS-CoV-2 membrane proteins during the viral lifecycle. The developed system for the real-time visualization of PLpro activity can potentially serve as a basis for safe cell antiviral drug screening platforms. The proposed strategy for studying viral proteins combines two important properties. Firstly, research has been conducted on human living cells, which is closely approximated to native conditions in contrast to in vitro experiments. Secondly, the experimental system lacks interaction with a functional virus which makes it completely safe for the researcher.

Keywords: SARS-CoV-2; coronavirus; COVID-19; genetically encoded probes; live cell imaging; FRET; fluorescence microscopy

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