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 to a global threat. The search for new treatment strategies is strongly associated with both fundamental research on mechanisms of virus life cycle and development of new screening platforms for anti-viral drug candidates.

In this project we labelled SARS-CoV-2 membrane proteins M, E, S to track protein intracellular localization and study protein-protein interactions in mammalian cells using fluorescent microscopy.

In addition, we developed several types of FRET-based and translocation sensors to track SARS-CoV-2 PLpro protease and measure its activity in living cells.

The proposed strategy for studying viral proteins combines two important advantages. Firstly, research is 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.

Conclusions

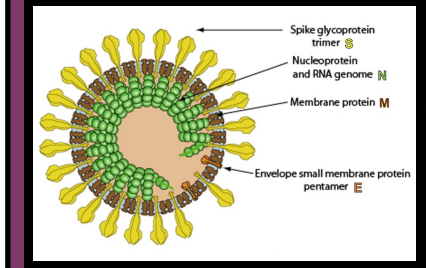
1. E protein accumulates in cell lysosomes and endosomes. S protein is distributed in the form of a network (obviously, endoplasmic reticulum).

2. M protein localizes in two compartments – Golgi and lysosomes. Untagged M shows a tendency for oligomerization.

3. Two types of sensors (soluble FRET-sensor and membrane-associated translocation sensors) for real-time detection of PLpro activity in live cells are developed. Testing in mammalian cells demonstrated a robust readout.

Results

SARS-CoV-2 structural proteins



SIB Swiss Institute of Bioinformatics

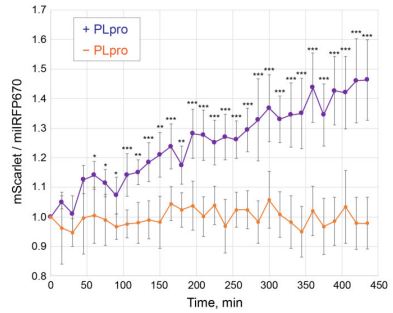
Labeling of SARS-CoV-2 E and S proteins

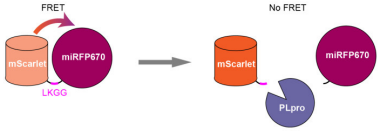
N-terminal mNeonGreen fluorescent protein + hTPA signal sequence
Confocal microscopy, HeLa cells

E protein accumulates in lysosomes and endosomes

S protein in the ER

Red FRET sensor for SARS-CoV-2 PLpro activity

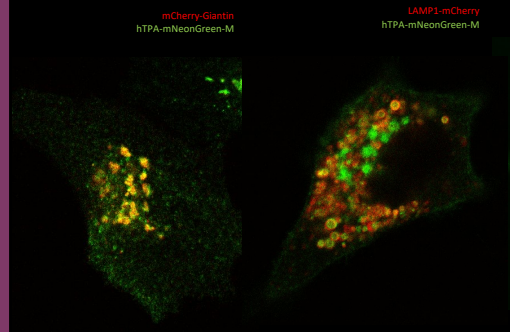




1.3-1.6-fold increase in the mScarlet/iRFP670 signal ratio several hours after induction of PLpro expression

Labeling of SARS-CoV-2 M protein

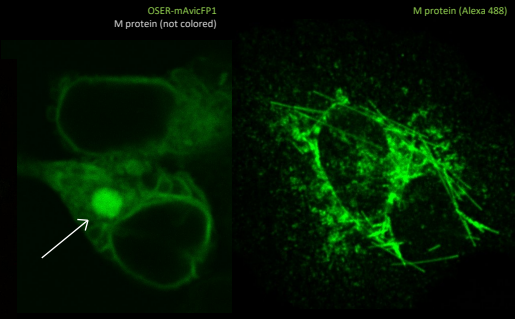
N-terminal mNeonGreen fluorescent protein + hTPA signal sequence
Confocal microscopy, HeLa cells



M protein in cell Golgi

M protein in cell lysosomes

Untagged M protein forms oligomers in mammalian cells

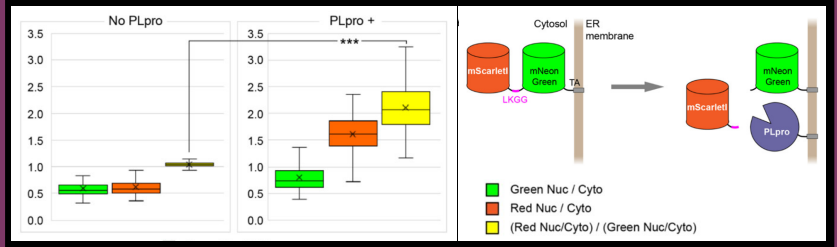


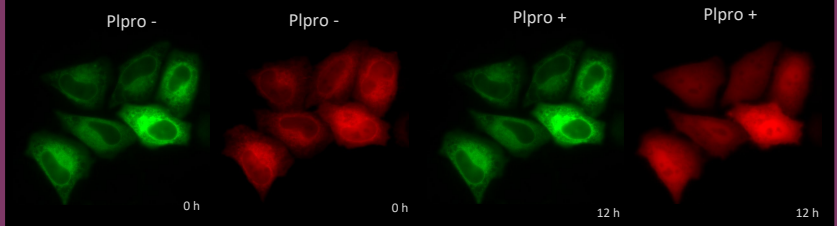
Whorl structures in HEK293T cells

Fibril formation in HeLa cells; immunostaining

M protein predominantly localizes in cell Golgi but in the later stages in some cells it forms clusters of vesicles that colocalize with lysosomal markers. Ability to form OSER whorl structures and fibril formation demonstrates a tendency for oligomerization. It may serve as the driving force for the membrane fusion and virion formation.

Translocation sensor for SARS-CoV-2 PLpro activity





After PLpro activation green signal (mNeonGreen) stays connected to the ER, while red signal (mScarletI) changes its intracellular localization: the ER network disappears and the red signal becomes distributed in the cytoplasm. HeLa cells.

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