Live click labelling with Tri*PPP*ros

For any questions regarding this protocol, feel free to send an email to info@deep-dv.org

Purpose

The Tri*PPP*ro system facilitates live imaging of cellular and viral genomes through the use of a pronucleotide linked to a dienophile and a fluorogenic linked to a diene. These components undergo a catalysator-free inverse electron demand Diels Alder reaction with rapid reaction kinetics without the need for fixation and permeabilization of the cells, allowing the performance of live cell experiments (Sterrenberg, Stalling et al., Angewandte Chemie 2023, https://doi.org/10.1002/ange.202308271).

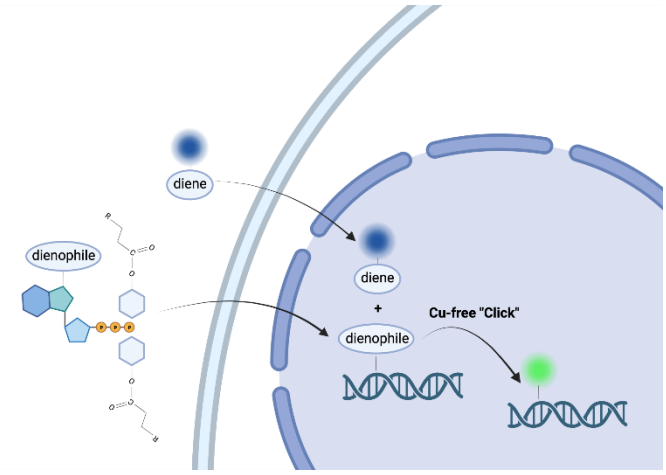

Figure 1: Graphical Scheme of labeling genomes with the Tri*PPP*ro system.

Notes

- To obtain the TriPPPros and their corresponding dyes, please reach out to Iven Knaack (Research Group of Prof. Dr. Chris Meier, Institute of Organic Chemistry, University of Hamburg).
- Cells should have a confluency of 70-80% before proceeding with the click labeling.
- Samples can be fixed in 4% PFA after completing the click reaction.

Reagents

Reagent/Material	Description	Reference/Vendor	Cat.no.
µ-slide 8 well high glass bottom	Glass coverslip bottom microscopy 8-well chamber slide	ibidi	80807
DMEM	Supplement with 10% FBS	Sigma-Aldrich	D6429
FBS Superior		Merck	F7524
VP SFM (1x)	Virus production serum-free medium, supplement with 20 ml GlutaMAX™ Supplement	ThermoFisher	11681020
GlutaMAX™ Supplement		ThermoFisher	35050061
Hoechst 33342	Stains DNA	ThermoFisher	H3569
Tri*PPP*ro 2TCOa-dCTP	$1*10^{-2}$ mol/l	UHH, AK Meier	
Click-Fluorophore	$1.73*10^{-3}$ mol/l, HD656, excitation maximum: 656 nm	UHH, AK Meier	IK341

Live click labelling with Tri*PPP*ros Protocol

Day 0

Seed cells (e.g. Vero cells, 2,5*10^4 cells / well) in 10% FBS DMEM in ibidi 8-well plate

Day 1

-x h Infect cells if applicable

0 h Wash cells 3x in serum-free medium (VP SFM)
Add 5 µM Tri*PPP*ro in serum-free medium (VP SFM)
-> 1000 µl VP SFM + 0.5 µl Tri*PPP*ro

2 h Wash cells 3x in in serum-free medium (VP SFM)
Add 5 µM IK341 dye and Hoechst (1:1000, if necessary) in serum-free medium (VP SFM)
-> 1000 µl VP SFM + 2.9 µl IK341

2.30 h Live cell imaging with a confocal microscope, e.g. Spinning Disk microscope
Use a 647 nm laser for imaging the Tri*PPP*ro-labelled genomes

Results

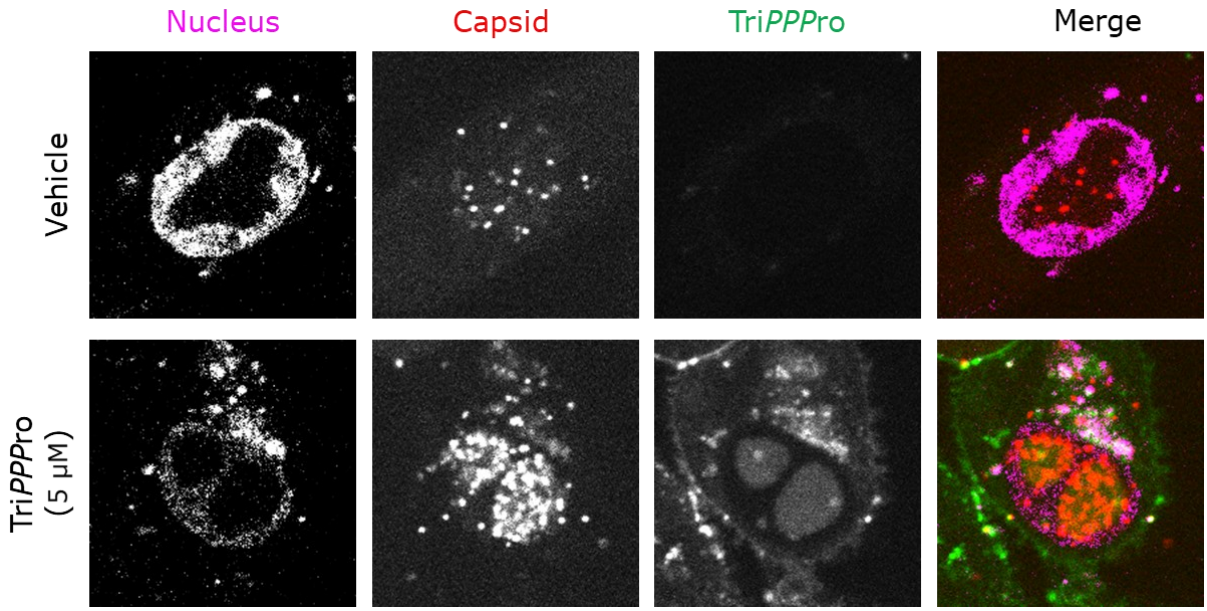

Figure 2: Live click labeling of nascent viral genomes in living cells. Vero cells were infected with HSV-1 VP26_mCherry at an MOI of 10. 1 hpi medium was changed and 4 hpi cells were pulsed with 5 µM Tri*PPP*ro-dCTP for 2 hours. Scalebar 10 µm.