Supporting Information

for

An *in situ* labelling chemistry of respiratory syncytial virus by employing the biotinylated host-cell membrane protein for tracking the early stage of virus entry

Lin Ling Zheng,[a] Xiao Xi Yang,[b] Yue Liu,[a] Xiao Yan Wan,[b] Wen Bi Wu,[b] Ting Ting Wang,[b] Qiang Wang,[b] Shu Jun Zhen,[a] Cheng Zhi Huang*[a, b]

[a] Key Laboratory of Luminescent and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China

[b] College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, China. E-mail: chengzhi@swu.edu.cn

1. Experimental details

1.1 Cell culture and virus propagation

HEp-2 cells were cultured in RPMI 1640 (Hyclone) medium supplemented with 10% fetal bovine serum (FBS, Hyclone), 100 U/mL penicillin G, and 100 μg/mL streptomycin sulfate. Human RSV strain Long (Guangzhou Biotest bioengineering Co., LTD, China) was propagated in monolayer cultures of HEp-2 cells in the presence of 2% FBS at 37 ^{o}C for 2-3 days. When the cytopathic effect of RSV became visible, the cell culture was subjected to 2 rounds of freeze-thaw cycles to release viruses and the cell debris was removed by centrifugation at 3000 rpm at 4 ^{o}C for 10 min.

1.2 Preparation of biotin-labelled RSV

For the direct labelling, the harvested viruses were directly incubated with EZ-Link sulfo-NHS-LC-Biotin (Biotin-LC-NHS, Thermo Scientific) for 2 h to allow biotinylation of viruses. The excessive Biotin-LC-NHS was removed by a NAP-5 desalting column (GE healthcare). While for in situ labelling method, viruses were labelled during propagation. Viruses were first introduced to monolayer HEp-2 cells at 37 ^{o}C for 2 h to allow viruses binding. Then, the infected cells were incubated with 1 mg/mL Biotin-LC-NHS for 30 min at room temperature to make cell surface present biotin molecules and washed three times with PBS plus 100 mM glycine to quench and remove excess biotinylation reagent and byproducts. Cells were cultured in RPMI 1640 medium (2% FBS) for virus propagation. After 48 h post-infection, the cell cultures were subjected to 2 rounds of freeze-thaw cycles to release viruses and the cell debris was removed by centrifugation at 3000 rpm at 4 ^{o}C for 10 min.

1.3 Virus titer assays

The titer of RSV was determined by 50% tissue culture infective dose (TCID50). Before infection, HEp-2 cells were cultured in 96-well plates in RPMI 1640 medium supplemented with 2% FBS for 24 h to reach 70-80% confluence. After that, virus samples were serially diluted with RPMI 1640 and then introduced to the cell monolayers. After incubation for 2 h at 37^{o}C, the infected cells were cultured in RPMI 1640 supplemented with 2% FBS in 5% CO_2 incubator for 6-8 days. Finally, the TCID50 was calculated by Formula of Reed-Muench.

1.4 Immunofluorescence assay

HEp-2 cells were cultured in the 35 mm glass-bottom dishes (NEST. Corp.) over 24 h. Then the cells were incubated with viruses or biotinylated viruses at a multiplicity of infection (MOI) of 7 for 30 min at 4 ^{o}C, and washed with ice-cold PBS for 3 times. After that, cells were incubated with 1 nM SA-QDs (Jiayuan Co., China) for 10 min at 4 ^{o}C and fixed with 4% paraformaldehyde for 20 min. After being blocked with 2% BSA for 1 h, cells were incubated with the mouse monoclonal antibody against RSV envelope protein G (Abcam) for 1.5 h at 37 ^{o}C. Finally, cells were incubated with DyLight 488-conjugated goat anti-mouse IgG (Thermo Scientific) for 1 h at 37 ^{o}C and washed three times with PBS. Fluorescent images were acquired using an Olympus IX-81 inverted microscope equipped with an Olympus IX2-DSU confocal scanning system and a Rolera-MGi EMCCD. Colocalization analysis were performed with Image-Pro Plus software and confirmed by

eye. SA-QDs (605 nm) was excited at 340-390 nm and detected with BA595-615 nm. DyLight 488 was excited at 470-490 nm and detected with a barrier filter BA510-550 nm.

1.5 One-step growth curve

HEp-2 cells were incubated with control RSV and biotin-labelled RSV at an MOI of 7 for 2 h. Medium supernatant was taken at 12, 24, 36, 48, 60, 72 and 96 h after infection, and stored at -80 ^{o}C. The infectivity of supernatants collected at different infection times was determined by TCID50 to achieve one-step growth curve.

1.6 Preparation and imaging of the dual-labelled RSV

HEp-2 cells were cultured in RPMI 1640 medium supplemented with 2% FBS for 24 h and then inoculated with RSV for 2 h at 37 ^{o}C. Subsequently, the infected cells were biotinylated with 1 mg/mL Biotin-LC-NHS for 30 min at room temperature and washed three times with PBS plus 100 mM glycine. Then, cells were maintained in RPIM 1640 supplemented with SYTO RNA stain at a final concentration of 1.0 μM. At 48 h post-infection, the infected cells were subjected to 2 rounds of freeze-thaw cycles to release viruses and the cell debris was removed by centrifugation at 3000 rpm at 4 ^{o}C for 10 min. The harvest dual-labelled virus was then introduced to HEp-2 cells cultured in 35 mm glass-bottom dishes at 50% confluence at 4 ^{o}C for 30 min. The cells were washed three times and incubated with 1 nM SA-QDs for 10 min at 4 ^{o}C. Then, the cells were washed and imaged by Olympus IX-81 microscope. Hoechst 33258 was excited at 360-370 nm and detected with BA420-460 nm. SA-QDs (605 nm) was excited at 340-390 nm and detected with BA595-615 nm.

1.7 Real-time fluorescence imaging of labelled virus

HEp-2 cells were plated in 35 mm glass-bottom dishes over 24 h to reach 50% confluence. The cells were incubated with biotin-labelled virus or dual-labelled virus for 30 min at 4 ^{o}C to allow virus binding, and then incubated with 1 nM SA-QDs for 10 min at 4^{o}C to allow QDs binding to virus. The cells were washed three times with PBS between two successive steps. Finally, the samples were transferred to a stage-top incubator (Tokai Hit Co., Ltd.) combined with the Olympus IX-81 inverted microscope and shifted from 4^{o}C to 37 ^{o}C. Live cell time lapse image acquisition were taken by in vivo 3.2/3D Analyzer Suite 6.2 software (MedicaCybernetics, Inc.). SYTO RNA stain was excited at 470-490 nm and detected with a barrier filter BA510-550 nm.

2. Additional Figures

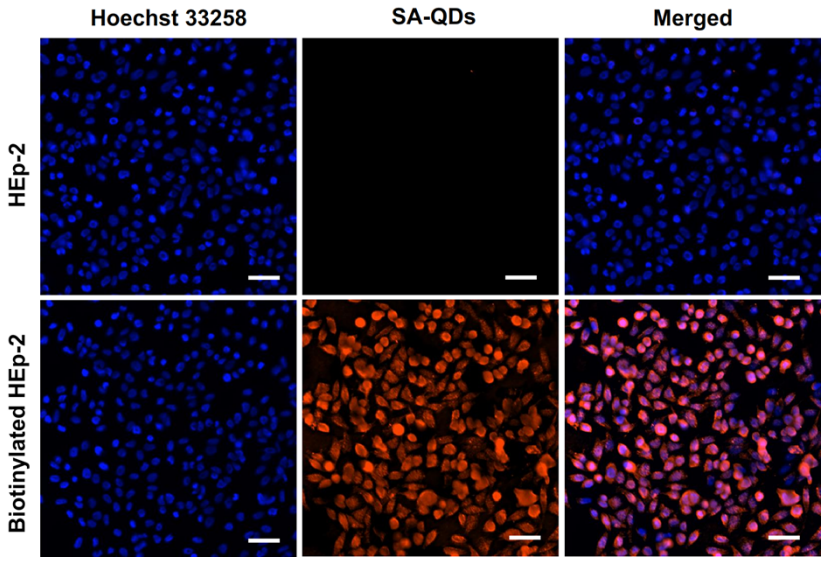

Fig. S1 Characterization of the efficient biotinylation of HEp-2 cells. Fluorescence images of non-biotinylated and biotinylated HEp-2 cells after incubation with SA-QDs (red) at 4 °C for 10 min. The nucleuses of the cells were stained with Hoechst 33258 (blue). Scale bar, 50 μm.

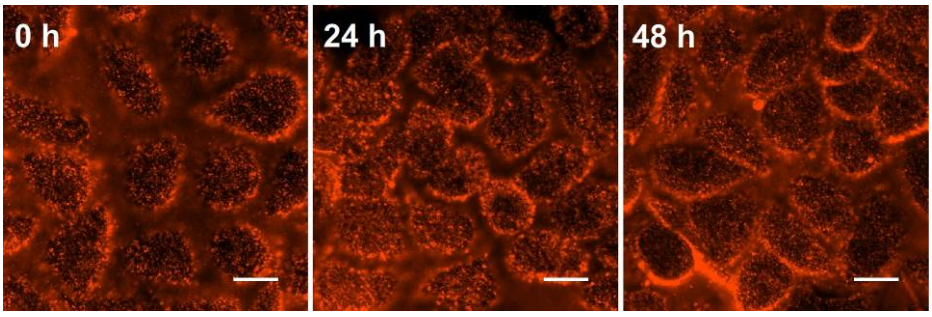

Fig. S2 Fluorescence imaging of biotinylated cells cultured for 0 h, 24 h or 48 h, respectively. HEp-2 cells were biotinylated, then the cells were incubated with SA-QDs either immediately or after varied times of cultivation at 37°C. Scale bar, 10 μm.

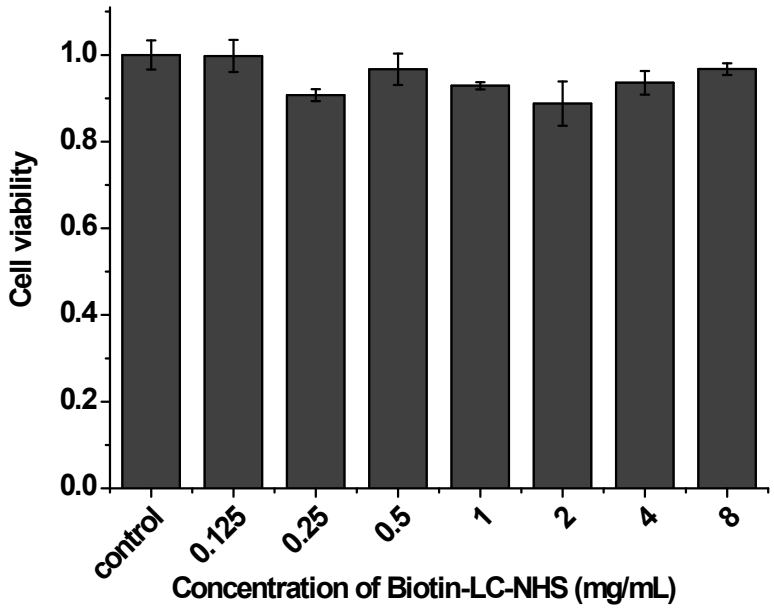

Fig. S3 CCK-8 detection of the viability of HEp-2 cells. The HEp-2 cells were biotinylated with Biotin-LC-NHS at different concentrations for 30 min, then cultured for 48 h. The viability of HEp-2 cells was detected by Cell Counting Kit-8.

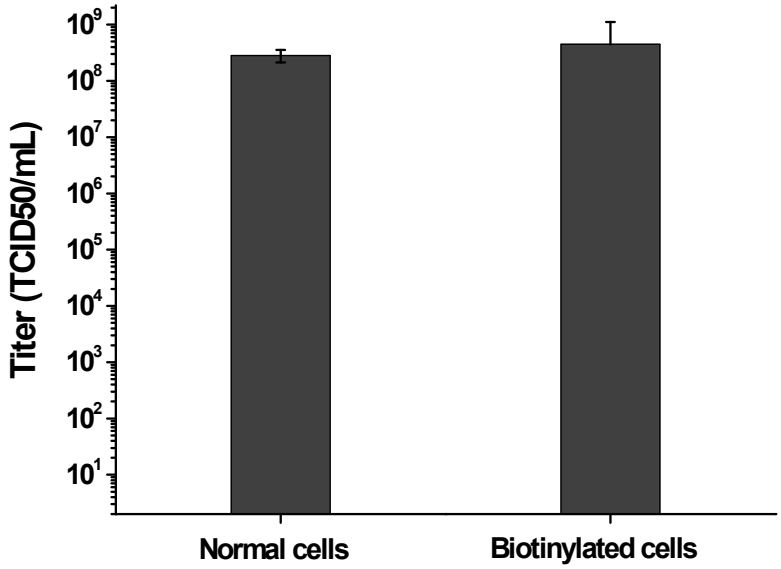

Fig. S4 Titer of virus obtained from normal cells and biotinylated cells. The viral infectivity of harvested viruses was evaluated by TCID50.

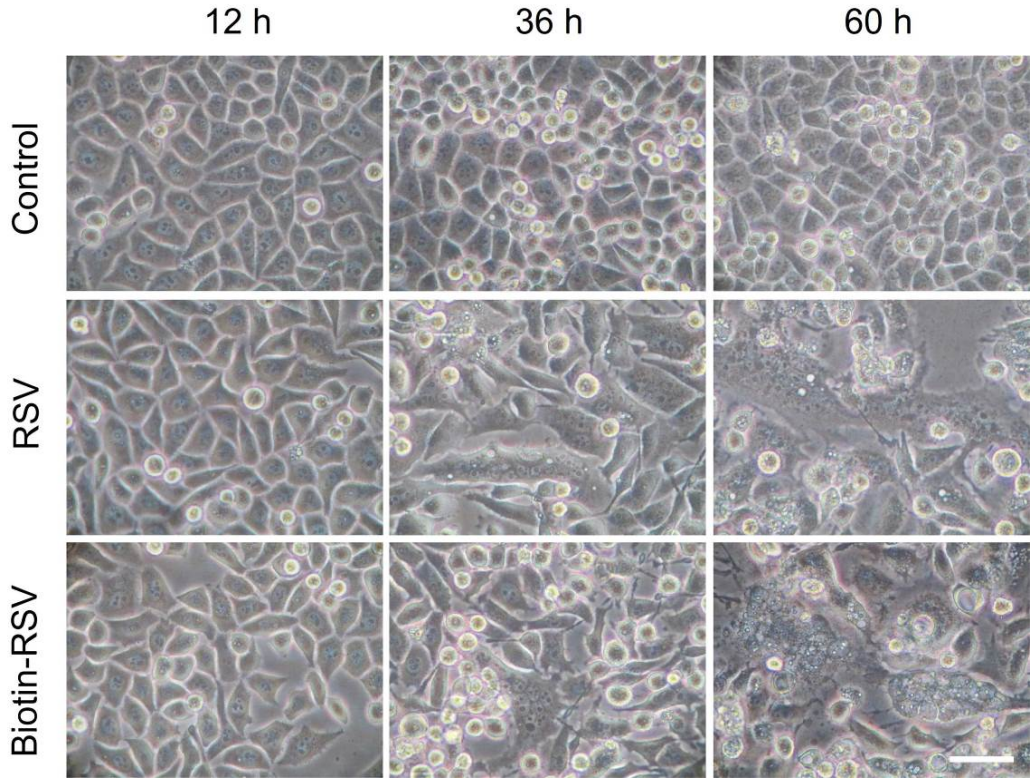

Fig. S5 Microscopic imaging of HEp-2 cells infected by native RSV and biotin-labelled RSV for various times. Scale bar, 40 μm.

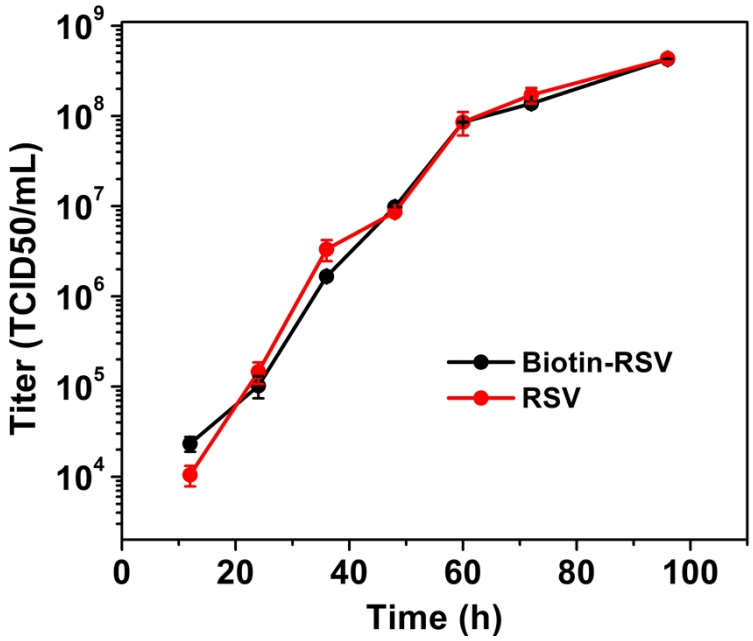

Fig. S6 One-step growth curve of RSV (red) and biotin-RSV (black). The titer of RSV was determined by TCID50 and calculated by Formula of Reed-Muench.

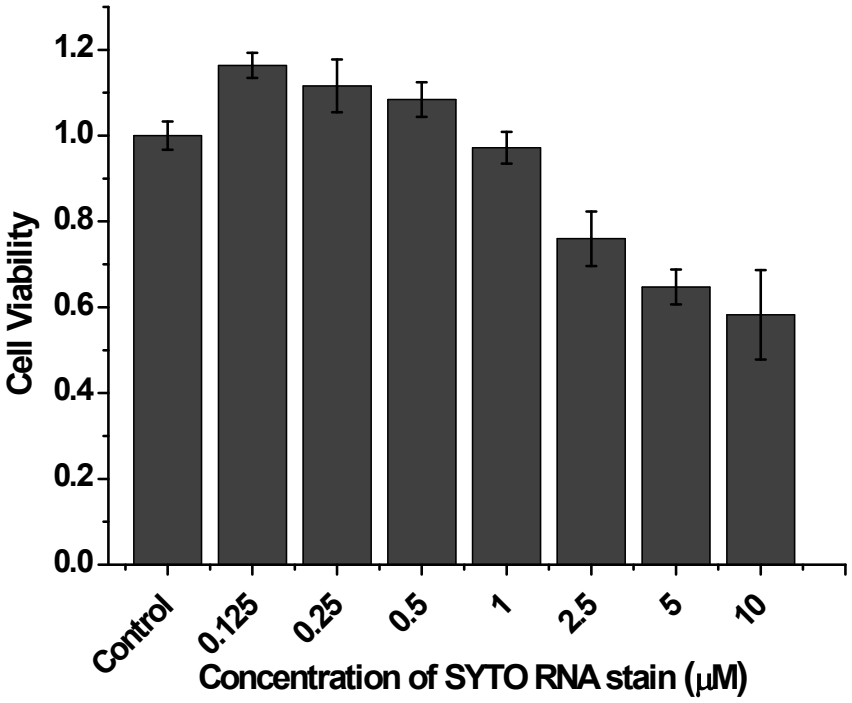

Fig. S7 CCK-8 detection of the viability of HEp-2 cells. The HEp-2 cells were incubated with SYTO RNA stain at different concentrations for 48 h, the viability of HEp-2 cells were detected by Cell Counting Kit-8.

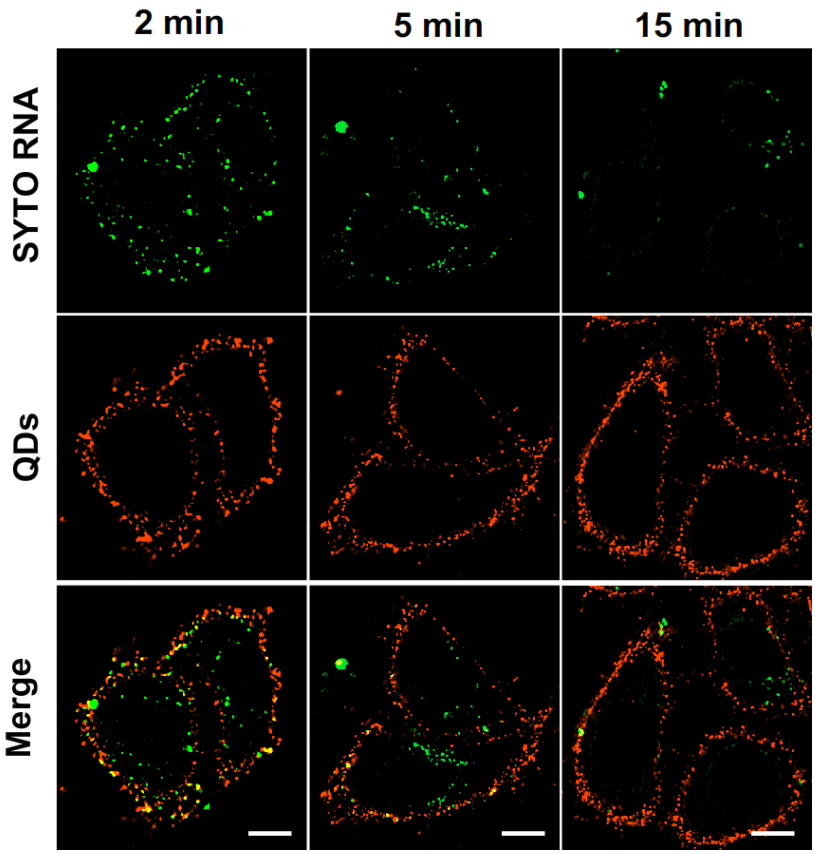

Fig. S8 Fluorescence imaging of HEp-2 cells at 2-15 min post-infection with dual-labelled RSV. Each sample was only imaged at one time point. Scale bar, 10 μm. To eliminate the potential effect of photo-damage on the labelled virus and ensure that the disappearance of fluorescence of SYTO RNA stain was not due to the photo-bleaching, we also adopted another imaging procedure that acquired the image of different samples at each time point, such that cells would not be exposed to the excitation light prior to imaging and only imaged once (Fig. S8). The result was similar to time-lapse imaging. The viruses began to lose their RNA immediately after temperature rise and almost all of the viruses lost the RNA within 30 min. These results revealed that the genome release of RSV in cells was a rapid and efficient event and it occurs closed to the plasma membrane of the host cells.