Supporting Information

Surface Labeling of Enveloped Viruses Assisted by Host Cells

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MATERIALS AND METHODS

The purification of PrV. PrV virions and biotin labled virions were purified in the same way. Briefly, when the cytopathic effect (CPE) was completed, the culture medium was harvested. After three alternations of freezing and thawing, PrV virions were centrifuged for 10 min at 17,700 g to remove cell debris. Virions were further purified through 60%, 30% and 10% sucrose (in PBS) gradients centrifugation for 2 h at 200,000 g (4 ^{o}C) with a Beckman Optima L-100 ultracentrifuge. The substances at the interface between the 30% and 60% sucrose layers were collected, diluted in PBS, and ultracentrifuged for 2 h at 200,000 g to remove sucrose. After resuspension of the pellet in PBS, the virion stocks were frozen and stored at -70 ^{o}C.

Cell labeling and imaging. Biotinylated Vero cells and normal cells were cultured in 96-well culture plates, respectively. After one day's culture, the medium was removed and the cells were washed with PBS. Then, the cells in each well were incubated with 1 μg streptavidin-Cy3 conjugates (Cy3-SA, Invitrogen) in 100 μL PBS. The nonspecific absorption was blocked with 2% BSA (w/v) in PBS before the addition of Cy3-SA. After incubation for 30 min, the rest Cy3-SA was removed and rinsed with PBS. The cells were observed using a Nikon TE2000-U inverted fluorescence microscope. The fluorescence signals were observed with TXR filter (EX: 510-560 nm, DM: 575 nm, BA 590 nm) and GFP filter (EX: 460-500 nm, DM 505 nm, BA: 510-560 nm), respectively. The bright field images were captured under DIC mode. Images were collected and analyzed using QCapture software.

Flow cytometry analysis of cellular biotinylation kinetics. In biotinylated kinetics analysis, after culturing in the Biotin-Cap-PE contained medium for 0 h, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h,

and 36 h, the Vero cells were labeled by Cy3-SA and analyzed with flow cytometry (Beckman counter). All samples were counted over 10,000 cells and all data was processed using WinMDI and Origin 8.1.

MTT assay for the Biotin-Cap-PE cytotoxicity. Biotin-Cap-PE of different concentrations (0.04 mg mL^{-1} to 0.078 μg mL^{-1}) was added to Vero cells. After an additional 24 hours, the cells were washed with PBS and a solution of 1 mg mL^{-1} MTT (3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) in DMEM without phenol red. After culturing four hours at 37 °C, the medium was removed and the purple precipitate was dissolved in 50% (v/v) DMSO/isopropanol. The intensity of the purple color was measured as the absorbance at 570 nm minus background at 660 nm. Viability was determined by comparison with control cultures of normal Vero cells without the exposure to Biotin-Cap-PE.

Confocal imaging of the biotinylated cells. Both Vero cells and biotinylated cells were cultured in 35 mm cell culture plates with normal DMEM medium and DMEM medium added Biotin-Cap-PE, respectively. After one day of culture, 1 μL H33342 (5 mol L^{-1}, Beyotime) was added to the plate to stain the DNA of the cells. After incubation for 30 min, the medium was removed and washed with PBS. The cells were incubated with 1 μg Cy3-SA in 200 μL PBS at 4 ^{o}C for 30 min after the blocking with 2% (w/v) BSA. After removing the rest Cy3-SA, the cells were observed under Andor Revolution XD Microscopy System using a 100× oil immersion lens. The excitation light sources of the system were diode-pumped solid-state (DPSS) lasers. In all the cellular experiments, the cells were maintained at 37 ^{o}C using an environmental control system (Solent Scientific). The H33342 channel was excited by laser 404 nm with filter

447 nm (415 nm-480 nm) and the Cy3-SA was excited by laser 561 nm with filter 605 nm (595 nm-615 nm). The bright field images were captured under DIC mode. All the images were recorded and processed with Andor iQ (Andor Technology PLC).

For the acquirement of the movie on the Biotin-Cap-PE stained by Cy3-SA, cells were maintained at 37 ^{o}C using an environmental control system. The exciting light and filter were the same as that for biotinylated cell imaging mentioned above. Videos were acquired using a 100× oil immersion lens with 20 ms exposure for H33342, 50 ms exposure for Cy3 (binning mold 2×2).

Extraction of virions inside infected biotinylated Vero cells. After infected by PrV for 20 h, the infected biotinylated Vero cells were collected by the centrifuge (10 min at 1,000 rpm, 4 ^{o}C) and rinsed for three times with 1× PBS. Following resuspension of the pellet in PBS, the infected cells were freezed (-70 ^{o}C), remelted (0 ^{o}C) and repeated over three times to release the mature virions inside cells. Then the cell debris was removed by low-speed centrifugation for 10 min at 17,700 g and 4 ^{o}C. The virions were further purified by density gradient centrifugation as mentioned in the article.

Preparation of SA-PEG-magnetic beads. About 443.42 μg sufo-NHS-biotin (1μmol, Pierce) and 3.400 mg NH2-PEG-COOH (1μmol, Laysan Bio) were dissolved in dried DMF and incubated overnight in room temperature. Then 19.1 mg 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (100 μmol, EDC, sigma) and 230.2 mg N-Hydroxysuccinimide (2μmol, NHS, Acros) were added directly to the reaction solution and incubated for 4 h. Finally, the newly produced biotin-PEG-NHS was immidiately added into the Amino-Adembeads (1 mg mL^{-1} in PBS, Ademtech) to prepare

biotin-PEG-magnetic beads. The reaction completed after incubation for 4h under shaking,. Then the biotin-PEG-magnetic beads were magneticly collected, washed for three times with ultrapure water and redispersed in PBS. The SA-PEG-magnetic beads were produced by adding excess streptavidin (7.5 μg) into the biotin-PEG-magnetic beads (1 mg).

To measure the biotin binding ability of SA-PEG-magnetic beads, a standard curve of the fluorescence of biotin-4-Fluorescein *vs.* its concentration was firstly plotted by measuring the fluorescence of different concentrations from 0.03 mg L^{-1} to 1 mg L^{-1}. Then, 10 μL stock solution of SA-PEG-magnetic beads, 90 μL biotin-4-Fluorescein (1 μg μL^{-1}) and 100 μL 1× PBS containing 2% (w/v) BSA were added to a 200 μL centrifuge tube. After mixed and incubated for 30 min, the SA-PEG-magnetic beads were magneticly separated and the amount of biotin-4-Fluorescein in the tube before and after the capture by SA-PEG-magnetic beads was measured. According the standard curve, the reduction of the fluorescence was $1.60 \times 10^7 \pm 3.73 \times 10^5$ CPS, therefore the Biotin binding ability was 5.03 ± 0.12 pmol μL^{-1}. The fluorescence was detected and collected with NanoLog – Spectrofluorometer (Horiba Jobin Yvon Inc). The data disposal process was done with Origin Pro 8.1.

PCR and Electrophoresis Identification of PrV. Before amplification, virus samples were boiled for several minutes so that DNA could be released. The PCR was carried out in a mixture of 5 μL dNTPs (2.5 mM each), 5μL 10× PCR buffer, 2.5 units of taq DNA polymerse (Takara), 5 pmol forward primer (5'-GCCAGCCGTACACGCAG-3', Shanghai Sangon), 5 pmol reverse primer (5'-CCGTAGCAGAGCTCCCG-3', Shanghai Sangon), 5 μL virus sample and sterile water. The amplification with a PCR thermocycler (Biometra UNO II Thermoblock) is consisted of one cycle at 94 ^oC for 5 min followed by 30 cycles at 94 ^oC for 1 min, 60 ^oC for

1 min and 72 ^{o}C for 1 min. The PCR was ended with a final extension step of 10 min at 72 ^{o}C. All the PCR products were analyzed by electrophoresis through 1.5% (w/v) agarose gels in 0.5 × TAE (0.02M Tris-acetate, 0.001M EDTA, pH8.0).

VIDEO LEGEND

Video S1. The bustling movement of Biotin-Cap-PE in cellular membrane traffic.

The bustling movement of red fluorescence (Biotin-Cap-PE labeled by Cy3-SA) suggested that Biotin-Cap-PE could spontaneously join in the whole cellular membrane traffic systems and transported to viral assemble site. This is the basement of the proposed strategy.

FIGURE CAPTIONS

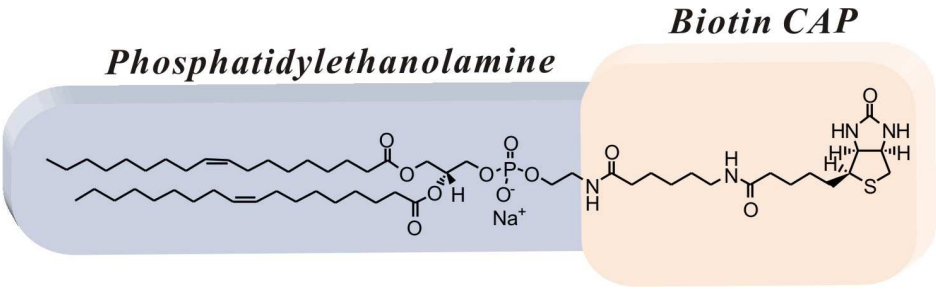
Figure S-1. The chemical structure of Biotin-Cap-PE. The pink part was the biotin cap and a 6-carbon spacer between the biotin and PE. The light blue part was PE.

Figure S-2. Preparation of SA-PEG-magnetic beads and its agglutination with biotinylated PrV virions. (a) The preparation of SA-PEG-magnetic beads. (b) SA-PEG-magnetic beads agglutinated and formed large complexes after incubating with biotinylated PrV virions.

Figure S-3. PCR detection of biotinylated PrV in the magnetic supernatants and precipitates after capture. Sample 1 was PCR products of biotinylated PrV; sample 2 and 3 were the PCR products for the magnetic supernatant and precipitate of biotinylated PrV respectively; sample 4 and 5 were the PCR products for sample 3 and 4 respectively.

Figure S-4. The cytopathic effects in continuous culture for biotinylated PrV virus. The PrV was cultured over 5 generations in biotinylated Vero cells. For each generation, the infected biotinylated Vero cells were observed after 24 h of infection. All the infected Vero cells turn round and expressed GFP in cytoplasm.

Figure S-5. Colocalization assay for biotinylated poxvirus and baculovirus. The DNA was stained by SYTO 82 (green) and the Biotin-Cap-PE was labeled by Alexa-Fluor 660-SA (red). The upper row was for biotinylated poxvirus and the lower row was for biotinylated baculovirus. Scale bar, 10 μm.



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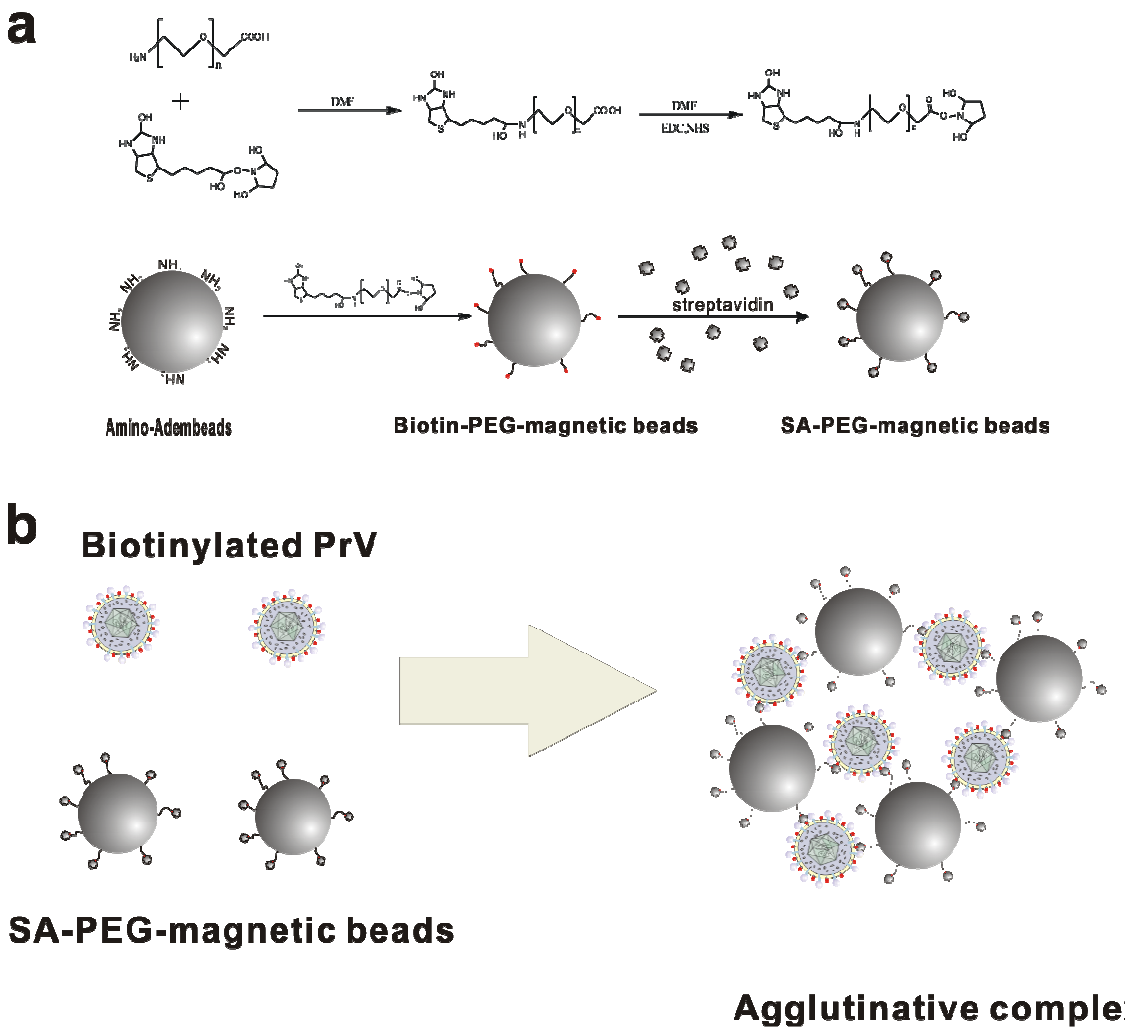

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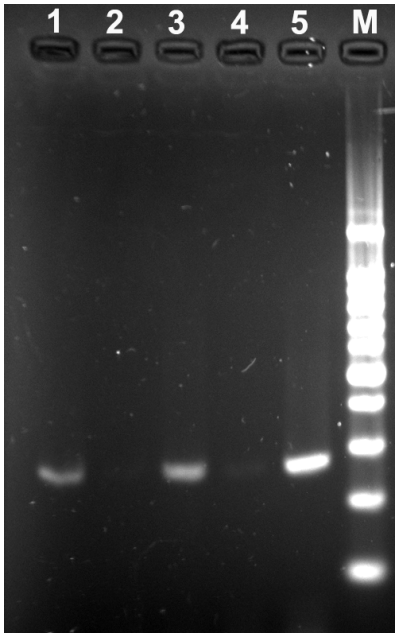

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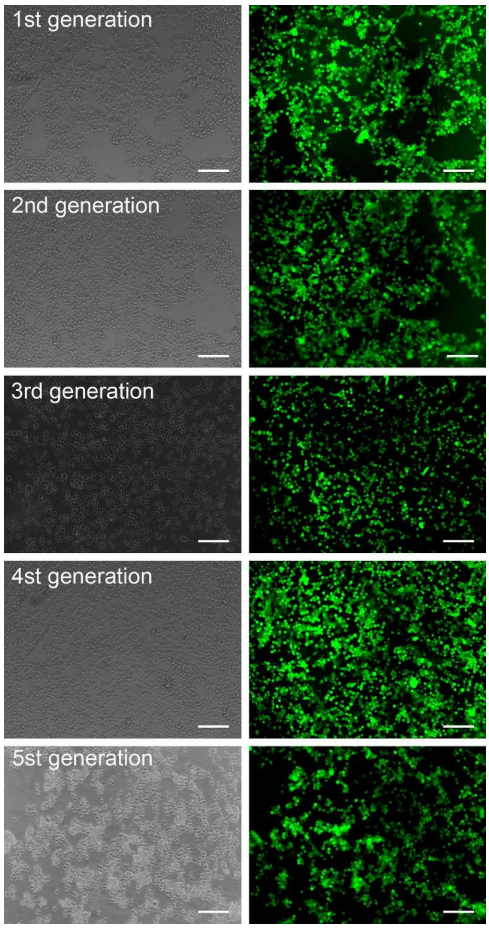
To verify whether the supernatant contained PrV virions, the PCR products of magnetic supernatants were used as template to perform a second round PCR. As the figure S-4 show, after the first round PCR, the expected PCR products (294 bp) can be only detected in the precipitate. Then the PCR products of magnetic supernatants were used as template to perform a second round PCR. After two round PCR, a weak band could be observed in the lane 4, which verified the magnetic supernatant contained trace PrV virions. Therefore, the absence of the expected PCR fragments for the magnetic supernatant in electrophoresis was because the PCR in this complex experiment condition is not enough sensitive to detect trace non-bound PrV in the magnetic supernatant, which also met the high biotinylation efficiency.



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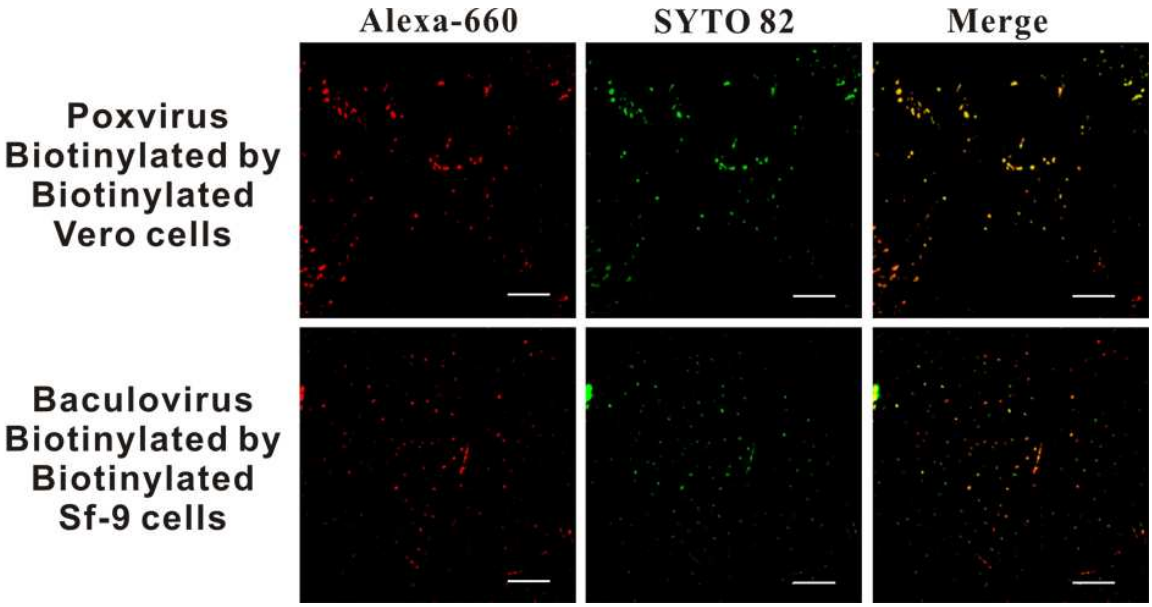

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