Supporting information for

The protein corona does not influence receptor-mediated targeting of virus-like particles

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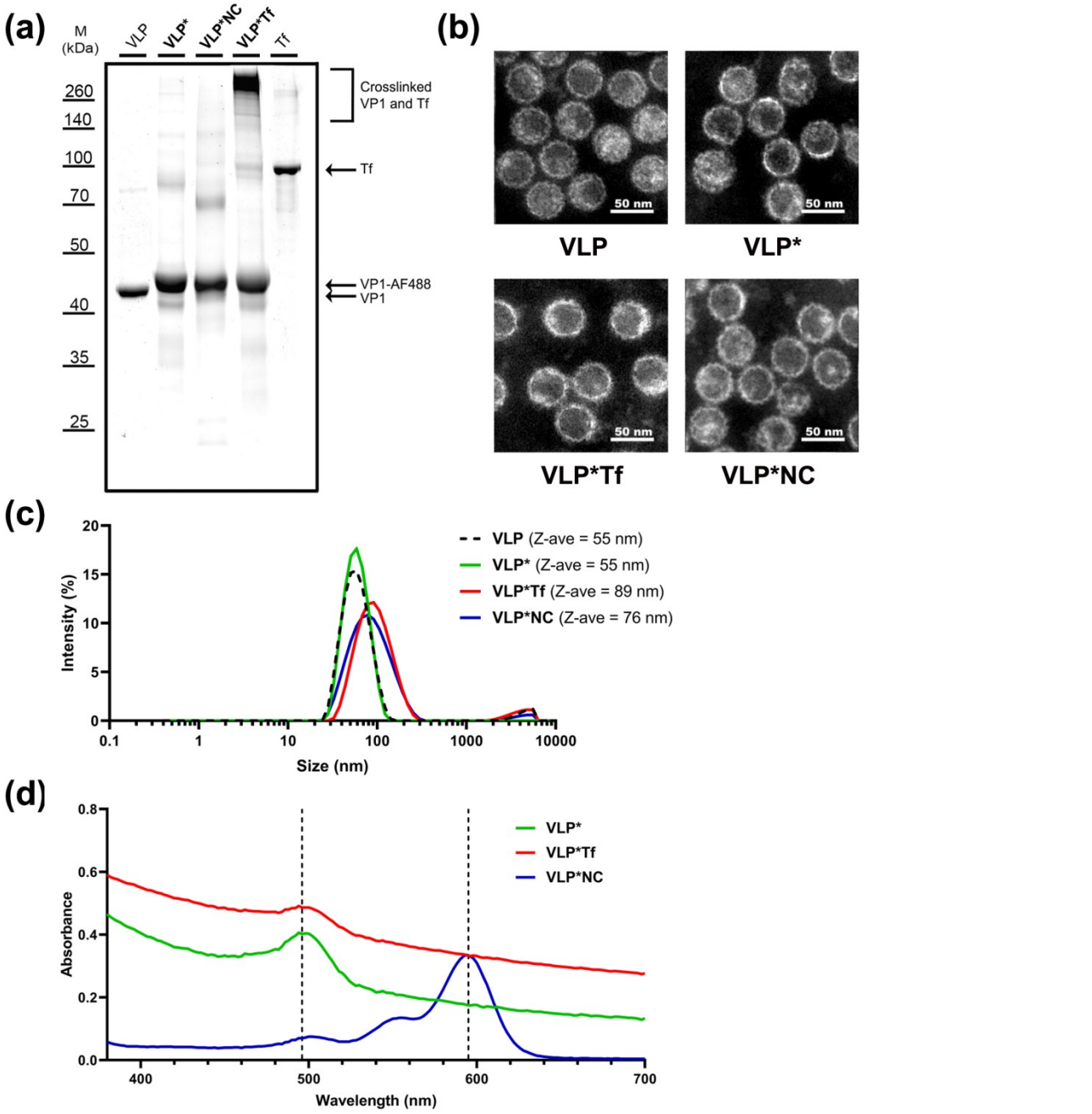

Figure S1. Characterization of VLP*Tf: (a) VLP, VLP*, VLP*NC, VLP*Tf and Tf protein were separated on 4-15% SDS-PAGE and stained with Coomassie Brilliant Blue. The mobility of the major capsid protein VP1 (~45 kDa) was slightly reduced in VLP* (see arrow VP1-AF488) samples. VP1 molecules crosslinked with Tf in the VLP*Tf sample appeared as high molecular weight complexes above the 140 kDa zone. The positions of native and modified VP1 and Tf are indicated with arrows on the right, and molecular weights are indicated on the left. (b) Representative TEM images of VLP, VLP*, VLP*Tf and VLP*NC visualized by negative staining show that all VLP variants are intact. Scale bar = 50 nm. (c) Hydrodynamic diameters of VLP, VLP*, VLP*Tf and VLP*NC measured by DLS diluted with buffer B. Increase in hydrodynamic radius of VLP*Tf is slightly higher than previously observed[1], possibly due to the higher coverage of Tf molecules on the surface of VLP*; polydispersity indexes were: 0.244, 0.086, 0.253 and 0.217, respectively. (d) Extinction UV-vis spectra of VLP*, VLP*NC and VLP*Tf. The absorption maxima of Alexa Fluor 488 (496 nm) and Texas Red (595 nm) are indicated with vertical dotted lines.

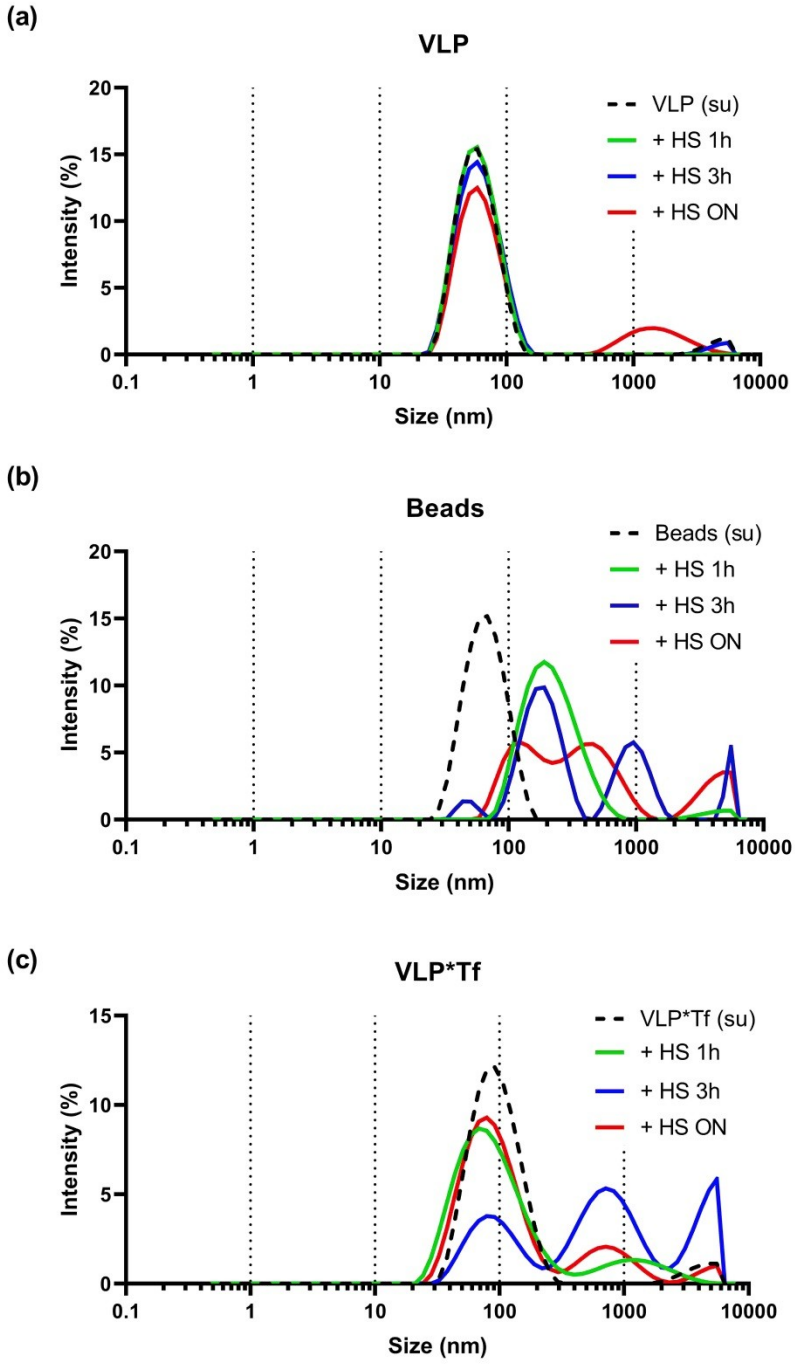

Figure S2. DLS of particles treated with human serum. Hydrodynamic diameters of (a) MPyV VLPs, (b) beads and (c) VLP*Tf before (black dashed line, su – serum untreated) and after 1 h (green), 3 h (blue) and overnight (red) incubation with HS followed by ultracentrifugation through sucrose cushion. The multimodal size distribution observed in samples indicates aggregation. DLS samples were prepared in buffer B, with the exception of serum untreated beads that were measured in water.

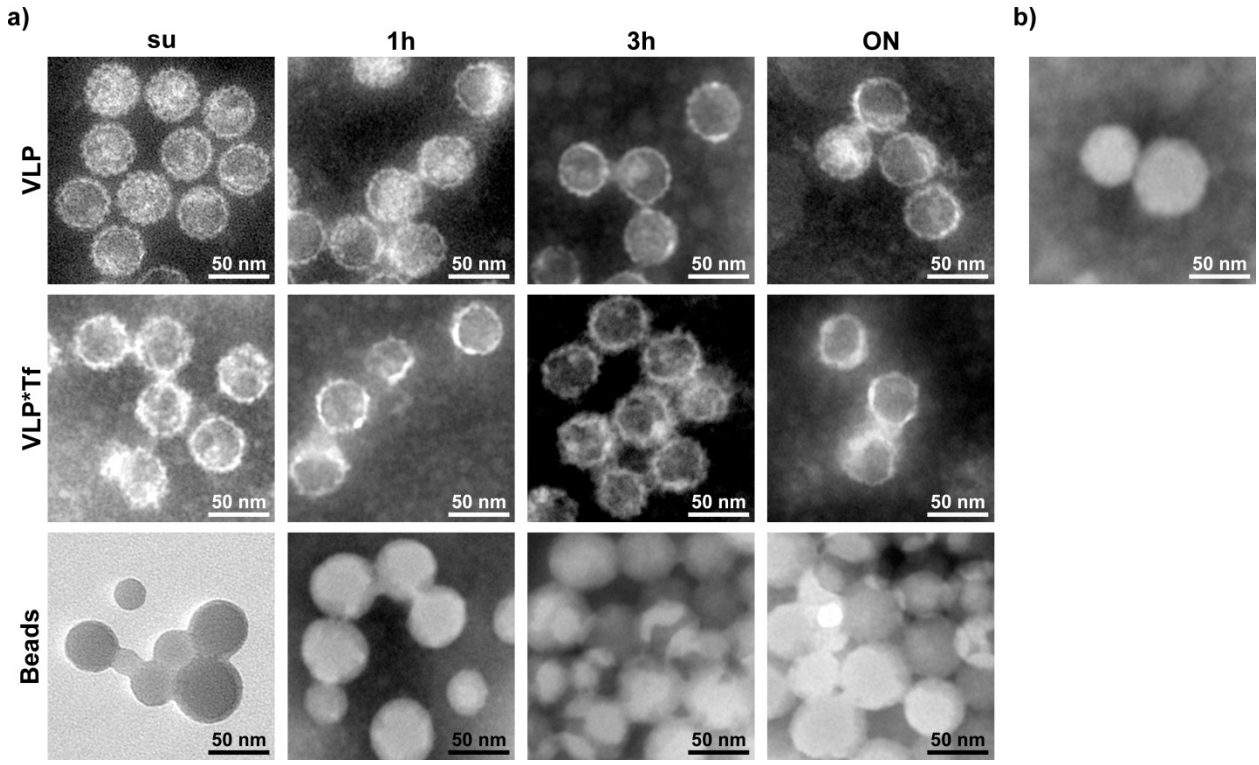

Figure S3. Electron microscopy of particles treated with human serum. (a) Representative TEM images of VLPs, VLP*Tf and beads visualized by negative staining. Images of samples without serum addition, after incubation in 55% human serum for 1 h, 3 h or overnight (ON) with subsequent purification step by 1-h ultracentrifugation through 20% sucrose cushion are shown. Scale bar = 50 nm (b) Control sample of 55% human serum was centrifuged through 20% sucrose cushion for 3 h and was visualized by negative staining. TEM images showed complexes of serum proteins formed during the process.

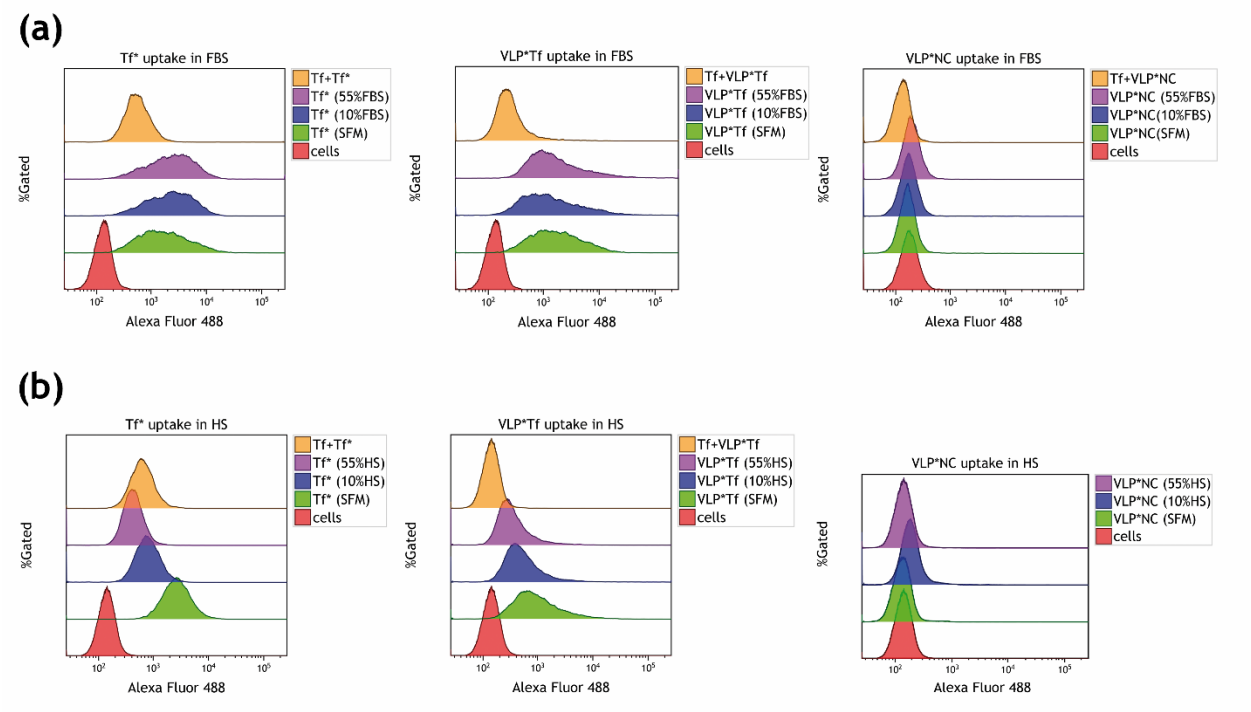

Figure S4. Flow cytometry study of VLPs uptake by U2OS cells – histograms. An overlay of representative histograms from flow cytometry measurements presented in Fig. 3. Uptake of Tf*, VLP*Tf and VLP*NC into U2OS cells was analyzed in (a) FBS and (b) HS. Histogram overlays display autofluorescence of non-treated cell (cells, red), fluorescence of cells treated with Tf*, VLP*Tf, and VLP*NC in serum free media (green), in 10% sera (blue), in 55% sera (violet), or in 10% sera after pre-incubation with non-fluorescent human Tf (orange). Data were analyzed with Kaluza Analysis Software (v1.5a).

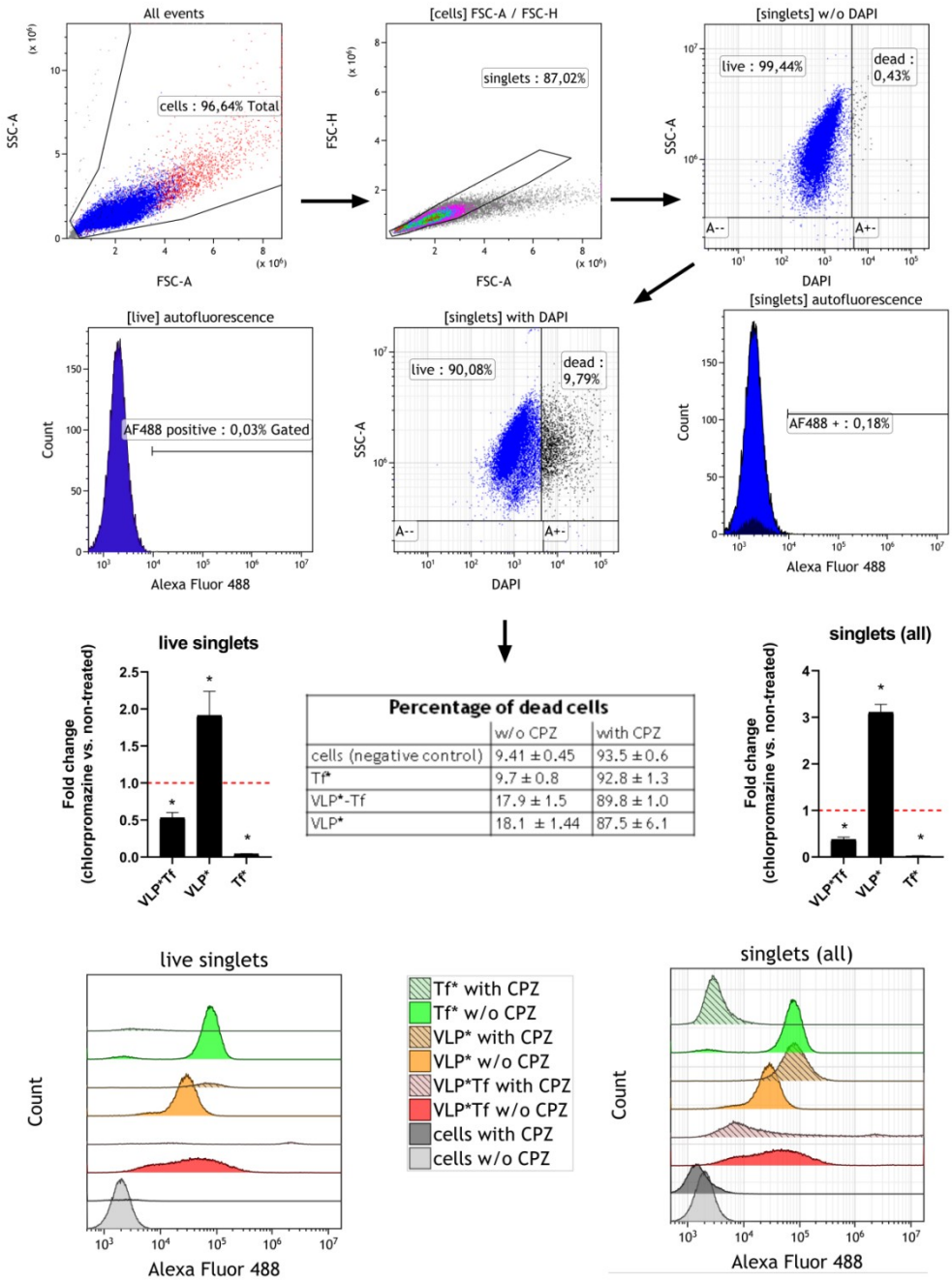

Figure 5. Quantitative analysis of VLP*Tf, VLP* and Tf* binding in the presence of chlorpromazine (CPZ) – gating strategy for flow cytometry analysis and the results. HeLa cells were exposed to VLP*, VLP*Tf and Tf* in complete media with 10% FBS in the absence or presence of CPZ (30 µg/mL) for 30 minutes and processed for flow cytometry as specified in methods. Cells were first gated for singlets (FSC-H vs. FSC-A). Singlets were further analyzed for their uptake of the DAPI to determine live versus dead cells. The results for all samples are shown in the table. Singlets (data on the right) or subpopulation of live singlets (data on the left) were analyzed for median of Alexa Fluor 488 fluorescence intensity (MFI). The gating strategy is shown for non-treated cells and the autofluorescence of those cells was subtracted from all sample values. The graphs represent the fold change of MFI in CPZ treated samples over the MFI measured in the respective sample without the inhibitor treatment. The values of cells exposed to VLP*Tf , VLP* or Tf* without CPZ were set as 1. Asterisks indicate statistical significance (*$p < 0.05$) in pairwise comparisons of each sample with and without CPZ by one-tailed Mann-Whitney test ($n = 3$). Error bars represent standard deviation. An overlay of histograms from data merged from triplicate flow cytometry measurements is presented in the bottom part of the figure. Data were analyzed with Kaluza Analysis Software (v1.5a).

SUPPLEMENTARY METHODS.

Preparation of chemically modified VLPs. The procedure for chemical modification of MPyV VLPs has been described previously (for details, see[1,2]). Briefly, unmodified VP1 VLPs were dialyzed against 0.1 M HEPES, pH 7.9, and treated with NHS-Alexa Fluor 488 (final concentration of 34 nM, 0.2 equivalents per surface lysine) at room temperature overnight with gentle shaking (250 rpm, TS-100C, Thermo-Shaker, Biosan). Excess dye was removed by dialysis against 0.1 M HEPES, pH 7.9 (4 °C, overnight with two buffer changes). Part of the prepared VLP mixture was used for subsequent conjugation of molecules, and the rest was purified and concentrated by centrifugation through two successive 20% sucrose cushions (35,000 rpm, 3 h, SW41 rotor, Beckman) and dissolved in buffer B (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.01 mM $CaCl_2$), yielding the VLP* conjugate.

Labeled VLPs in 0.1 M HEPES, pH 7.9, were further modified with the heterobifunctional linker propargyl-NHS ester (35-fold excess per surface lysine, in 10% DMSO) by incubation at room temperature overnight with gentle shaking (250 rpm, TS-100C, Thermo-Shaker, Biosan). Excess reagents were removed by dialysis against 0.1 M HEPES, pH 7.4 (4 °C, overnight, first two buffer changes contained 10% DMSO), and concentrated by centrifugation through two successive 20% sucrose cushions, providing VLP-alkyne conjugate.

Click reactions contained VLP-alkyne (51.5 nM), in 0.1 M HEPES buffer, pH 7.4, containing 10 mM copper sulfate, 100 mM aminoguanidine, 50 mM THPTA (synthesized according to a previously published procedure[3]), freshly prepared 100 mM sodium ascorbate, and either Tf-azide (12.6 μM) or Texas Red-azide (37.1 μM). Copper sulfate and THPTA were mixed in a separate tube in a 1:5 concentration ratio prior to addition to the reaction mixture. The reaction mixture was well-sealed, mixed, and allowed to stand undisturbed at room temperature for 3 h. Excess transferrin or Texas Red was removed from the resulting VLP conjugates by dialysis (cellulose ester membrane, 300 kDa) against 0.1 M HEPES, pH 7.4 (4 °C, overnight), and buffer B (4 °C, overnight). The VLP-conjugates (VLP*Tf and VLP*NC) were purified and concentrated by centrifugation through two successive 20% sucrose cushions.

Preparation of transferrin conjugates. Transferrin-azide was prepared according to a previously published procedure.[1] Briefly, human holo-transferrin (Tf, 30 mg, 390 nmol) was dissolved in 0.1 M acetate buffer, pH 5.5, to a concentration of 2 mg/mL (15 mL). $NaIO_4$ was slowly added to cooled Tf solution (final concentration of 1 mM). The mixture was incubated on ice in the dark for 30 min and washed four times with 0.1 M HEPES, pH 7.4, using an ultrafiltration cell. The resulting Tf-aldehyde was resuspended in 10 mL HEPES and incubated with 3-aminooxypropyl-1-azide (16.2 mg, 140 μmol dissolved in 500 μL DMSO) for 5 h at room temperature with gentle mixing. Excess 3-aminooxypropyl-1-azide was removed by two cycles of ultrafiltration in 0.1 M HEPES, pH 8, and the reaction mixture was washed four times with water before lyophilization. The solution was freeze-dried to obtain Tf-azide. The theoretical maximum of azide groups present in one transferrin molecule is four. Transferrin conjugated to Alexa Fluor 488 (Tf*), used as a positive control in our confocal and flow cytometry studies, was from a previously prepared stock.[1]

UV-Vis spectroscopy. Spectra were recorded using an Epoch microplate spectrophotometer (BioTek Instruments). The particle concentration was adjusted to a minimum of 150 μg/mL before measurement.

Competitive ELISA. Transferrin was loaded into the wells (5 μg/well in 100 μL) of an ELISA plate (Nunc™ MaxiSorp™ flat-bottom 96-well plates) and left to coat overnight. The wells were washed four times with wash buffer (PBS containing 0.1% Tween-20) and blocked by 5% milk in the volume of 200 μL/well, for 1 h at RT. Dilution series of serum samples or transferrin was prepared in the wells and then anti-transferrin antibody was added (final dilution 1:12,500) in the final volume of 100 μL and incubated

for 1 hour. Then the wells were washed four times and secondary antibody conjugated with horseradish peroxidase was added (GαM-HRP, diluted 1:1,000) for 30 min at RT. Wells were again washed four times with wash buffer and ABTS substrate was added (50 μL/well) and the absorbance was measured by ELISA reader. Values of IC_{50} were determined with GraphPad Prism 8.0.1. software as follows: IC_{50} of pure transferrin 3.377 μM, IC_{50} of HS (H6914, Sigma-Aldrich) equals to 133 μM of transferrin and IC_{50} of HS-0 equals to 3.323 μM of transferrin.

Flow cytometry. HeLa cells were seeded in a 12-well plate (130,000 cells per well). Cells were incubated overnight in DMEM medium with 10% FBS at 37 ˚C, 5% CO2. The following day, the medium was aspirated, and the cells were incubated in 400 μL of serum-free medium for 30 min at 37 °C, 5% CO2. Then, medium was replaced with complete medium (10% FBS) with or without chlorpromazine (30 μg/mL). After incubation for 1 h at 37 °C, 5% CO2, cells were treated for 30 min with Tf*(246.4 nM), VLP*Tf or VLP* (106 VLPs per cell, 1.1 nM) at 37 °C, 5% CO2, then washed twice with PBS. Next, the cells were washed with Versen and harvested by trypsinization. Trypsin activity was subsequently blocked by soybean trypsin inhibitor (1 mg/mL). The cells were washed once with PBS and resuspended in 300 μL PBS with 0,5% BSA After addition of DAPI (1 μM) samples were analyzed with a CytoFLEX S Flow Cytometer (Beckman Coulter) with collection of 10,000 events per sample. The data were analyzed using Kaluza 1.5a software (Beckman Coulter).

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(3) Hong, V., Presolski, S. I., Ma, C., and Finn, M. G. (2009) Analysis and Optimization of Copper-Catalyzed Azide-Alkyne Cycloaddition for Bioconjugation. *Angewandte Chemie International Edition 48*, 9879–9883.