# Supporting Information



# Multivalent Display and Receptor-Mediated Endocytosis of Transferrin on Virus-Like Particles

Deboshri Banerjee,[a] Allen P. Liu,[b] Neil R. Voss,[b] Sandra L. Schmid,*[b] and M. G. Finn*[a]

cbic_201000125_sm_miscellaneous_information.pdf

**General.**

*Instrumentation.* Size-exclusion fast protein liquid chromatography (FPLC) analyses were performed on an AKTA Explorer (Amersham Pharmacia Biotech) equipped with a Superose-6 column. $^1$H NMR spectrum for **2** was recorded on a Varian AM200 spectrometer in $CDCl_3$. Samples for transmission electron micrsocopy (TEM) were prepared by depositing 20 μL sample aliquots on 200-mesh carboncoated copper grids, followed by one wash with 20 μL distilled water and staining with 20 μL of 2% uranyl acetate. The TEM grids were glow discharged prior to sample deposition to prevent aggregation. Images were obtained from a Philips CM100 electron microscope. Dynamic light scattering (DLS) measurements were obtained on a DynaPro Plate Reader (Wyatt) with Dynamics software. Flow cytometry was performed on BD FACS Vantage with Diva option using the 568 nm laser line from a Kryton/ Argon laser, 630/20 emission filter, and a 100 μm nozzle. Data was analyzed in FlowJo software. Total internal reflection fluorescence (TIRF) microscopy was performed using a home-built through-the-objective TIRF system on Nikon TE2000, using procedures reported earlier.[1]

*Reagents.* Human holo-transferrin (98%) was purchased from Sigma. The fluorescent labeling dye Alexa Fluor® 568 (Molecular Probes) was purchased from Invitrogen. NHS ester of the azide linker and compound **4** were synthesized following previously reported methods.[2] Q® coat protein was expressed and purified according to the method described previously.[3] All other reagents were commercially available and used as received, unless otherwise mentioned.

**Formation of Transferrin alkyne**

Successful introduction of alkyne functionality to transferrin was verified by subsequent reaction with an azide-functionalized fluorescein derivative using a recently reported optimized CuAAC bioconjutation conditions that employs copper sulfate, ascorbic acid and a triazolyl ligand (Figure S1).[2a] SDS-PAGE analysis of both Tfn alkyne and Tfn fluorescein was carried out using bovine serum albumin (BSA) as a standard molecular weight marker. The band corresponding to Tfn-fluorescein was visible under UV illumination, thus confirming the presence of alkyne moieties in transferrin that can react with the azide groups in fluorescein via CuAAC click reaction.

---

1. A.P. Liu, D. Loerke, S.L. Schmid, G. Danuser, *Biophys. J.* **2009**, *97*, 1038-1047.
2. (a) V. Hong, S.I. Presolski, C. Ma, M.G. Finn, *Angew. Chem., Int. Ed.* **2009**, *48*, 9879-9883; (b) S. Sen Gupta, J. Kuzelka, P.
Singh, W.G. Lewis, M. Manchester, M.G. Finn, *Bioconjugate Chem.* **2005**, *16*, 1572-1579.
3. V. Hong, A.K. Udit, R.A. Evans, M.G. Finn, *ChemBioChem* **2008**, *9*, 1481-1486.

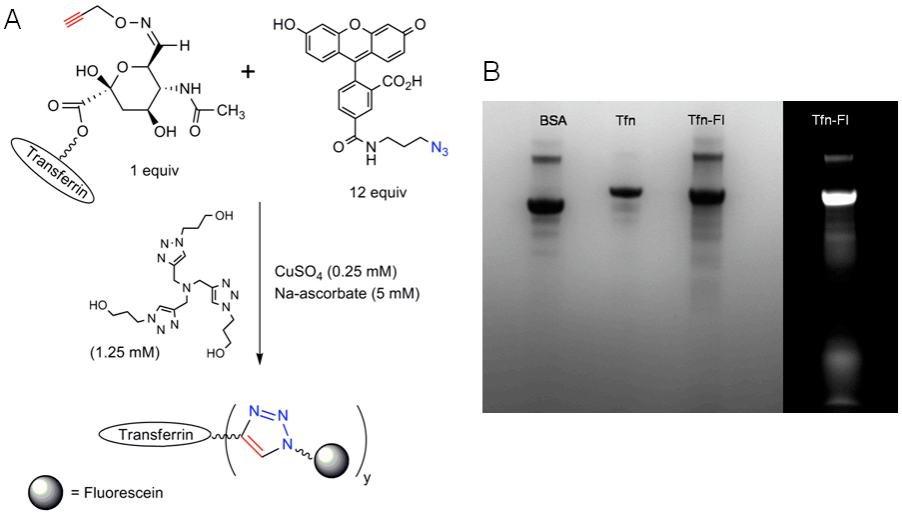


**Figure S1.** (A) CuAAC bioconjugation of transferrin-alkyne (1 equiv) with an azide-functionalized fluorescein derivative (12 equiv). (B) SDS-PAGE of BSA (standard), transferrin and transferrin-fluorescein conjugate are shown here. On the left (light background) is the gel visualized after SimplyBlue staining. On the right (dark background) is the gel illuminated by UV light prior to staining.

## Preparation of Qβ(mCherry)-Tfn conjugate

Qβ-mCherry particles were kindly supplied by Mr. Steven Brown in our laboratory, prepared by a two-plasmid method reported earlier.[4] These red-fluorescent VLPs were derivatized with azide groups by reaction with an NHS-ester of the azide linker shown in Figure S2. Qβ(mCherry)-azide was then reacted with Tfn-alkyne using a recently reported optimized CuAAC bioconjutation conditions that employs copper sulfate, ascorbic acid and a triazolyl ligand (Figure S2).[2a] The final transferrin-conjugated product was purified by size exclusion FPLC, as shown in Figure S2. The protein-virus conjugate was found to elute much faster than its wild-type counterpart, indicating an increase in particle size upon conjugation with the protein.

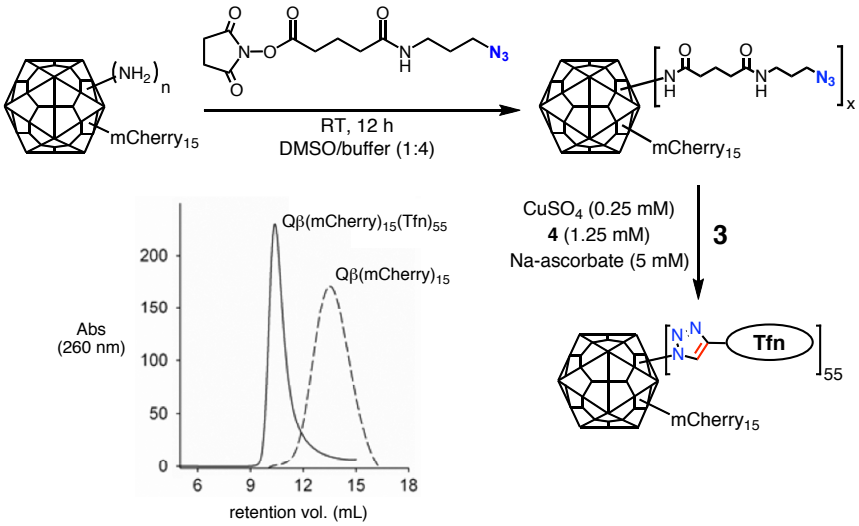


**Figure S2.** Schematic representation for the preparation of Qβ(mCherry)-Tfn conjugate from Qβ(mCherry)-azide and transferrin-alkyne by CuAAC "click" reaction. FPLC traces of the starting and final conjugate particles are shown on the bottom left.

4. S.D. Brown, J.D. Fiedler, M.G. Finn, *Biochemistry* **2009**, *48*, 11155-11157.

**Preparation of Qβ-Tfn conjugates 6a-c with varying numbers of transferrins per particle**

As shown in Figure 5, the fluorescent dye (Alexa Fluor® 568) labeled Qβ-azide was reacted with pre-mixed solutions of Tfn-alkyne and propargyl alcohol to generate three different Qβ-Tfn conjugates (**6a-6c**). In each reaction, the total VLP concentration was maintained at a constant of 1 mg/mL ($7 \times 10^{-5}$ M), while three different concentrations of transferrin-alkyne ($2 \times 10^{-6}$ M, $1.2 \times 10^{-5}$ M, and $7 \times 10^{-5}$ M) were used. Until steric limitations were reached at the highest loading, the CuAAC reactivity of the two alkynes were approximately the same, in spite of their great difference in size. Thus, 2 μM transferrin + 68 μM propargyl alcohol (reaction **a**) gave rise to 5±1 attached Tfn proteins out of approximately 720 available azide groups on the particle. Similarly a mixture of 12 μM transferrin + 58 μM propargyl alcohol produced particles bearing 25±3 attached Tfn per particle out of 720 sites.

The conjugates were purified by size-exclusion FPLC and the protein concentrations were estimated using Bradford protein assay. SDS-PAGE analysis of the three different conjugates at similar concentrations showed bands of gradually increasing intensity with increasing protein load. Densitometry analysis revealed the presence of approx. 5, 25 and 40 transferrin residues per particle. Dynamic light scattering (DLS) measurements indicated the hydrodynamic radii of the conjugates to be 16.2, 17.1 and 18.1 nm, respectively, as compared to that of 14.2 nm for the wild-type particles.

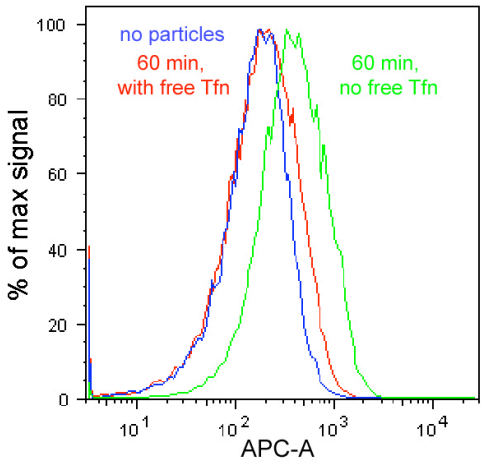


**Figure S3**. FACS analysis of binding and internalization of Qβ-Tfn particle **6c**, showing strong activity after 60 minutes incubation with BSC-1 cells (green *vs.* blue traces), and the complete inhibition of internalization in the presence of excess (1 mg/mL) free transferrin (red *vs.* blue traces).

**Electron Microscopy**

Samples were preserved in a thin layer of vitreous ice on 2.0x0.5 μm C-Flat holey carbon films (Protochips, Inc.) Grids were cleaned immediately prior to use in a Solarus plasma cleaner (8 seconds, 25% $O_2$, 75% Ar). Samples were prepared by applying a drop (~3 μL) of the undiluted sample suspension to the plasma cleaned grid, blotting away with filter paper and immediately proceeding with vitrification in liquid ethane, using an FEI Vitrobot (4C, 95% RH). Data were acquired using a Tecnai F20 Twin transmission electron microscope operating at 120 kV, using a dose of ~20 $e^-/Å^2$ and a nominal underfocus ranging from 1.5 to 3.0 μm. All images were recorded with a Tietz F415 4k x 4k pixel CCD camera (15 μm pixel) using the Leginon data collection software.[5] 348 images were automatically collected at a nominal magnification of 80,000X at a pixel size of 0.105 nm at the

5. C. Suloway, J. Pulokas, D. Fellmann, A. Cheng, F. Guerra, J. Quispe, S. Stagg, C. S. Potter, B. Carragher, *J. Struct. Biol.* **2005**, *151*, 41-60.

specimen level. Experimental data were processed using the Appion software package,[6] which interfaces with the Leginon database infrastructure. 3,554 particles were manually selected within Appion. The contrast transfer function (CTF) was estimated and corrected including astigmatism using ACE2, a variation of the software previously described.[7] Particles were extracted from the CTF corrected images with a box size of 512 pixels and binned by 2 to a box size of 256 pixels. Particles were iteratively centered using the EMAN cenalignint program[8] that discarded 1,315 inconsistent particles, leaving 2,239 particles for refinement. A 3D reconstruction was then carried out using EMAN for 8 rounds of projection matching, 4 rounds at angular increments of 3 and then 4 rounds at 2 degrees. Resolution was assessed by calculating the even-odd Fourier Shell Correlation (FSC) at a cutoff of 0.5, which provided a value of 17.4 Å resolution.

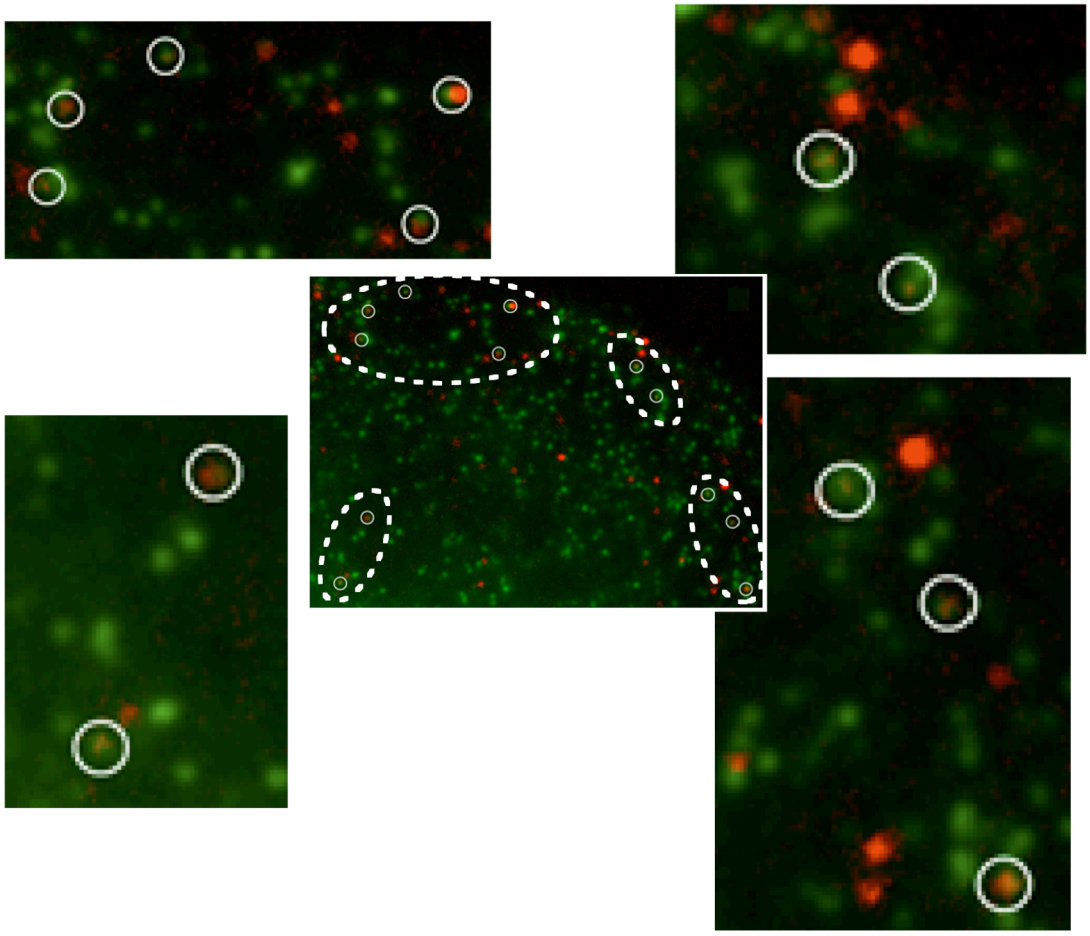

**Figure S4**. Expanded views of epi-fluorescence microscopy images of VLP particles **5b** (red) incubated for 5 min at 37°C with EGFP-Clc (green) BSC1 cells (analogous to Figure 4D).

6. (a) G. C. Lander, S. M. Stagg, N. R. Voss, A. Cheng, D. Fellmann, J. Pulokas, C. Yoshioka, C. Irving, A. Mulder, P.-W. Lau, D. Lyumkis, C. S. Potter, B. Carragher, *J. Struct. Biol.* **2009**, *166*, 95-102. (b) N. R. Voss, D. Lyumkis, A. Cheng, P.-W. Lau, A. Mulder, G. C. Lander, E. J. Brignole, D. Fellmann, C. Irving, E. L. Jacovetty, A. Leung, J. Pulokas, J. D. Quispe, H. Winkler, C. Yoshioka, B. Carragher, C. S. Potter, *J. Struct. Biol.* **2010**, in press (PMID: 20018246).
7. S. P. Mallick, B. Carragher, C. S. Potter, D. J. Kriegman, *Ultramicroscopy* **2005**, *104*, 8-29.
8. S. J. Ludtke, P. R. Baldwin, W. Chiu, *J. Struct. Biol.* **1999**, *128*, 82-97.