# Oriented Insertion of phi29 *N*-Hexahistidine-tagged gp10 Connector Protein Assemblies into $C_{20}$BAS Bolalipid Membrane Vesicles

Seok-Hee Hyun, Hee-kwon Kim, Jong-Mok Kim and David H. Thompson*

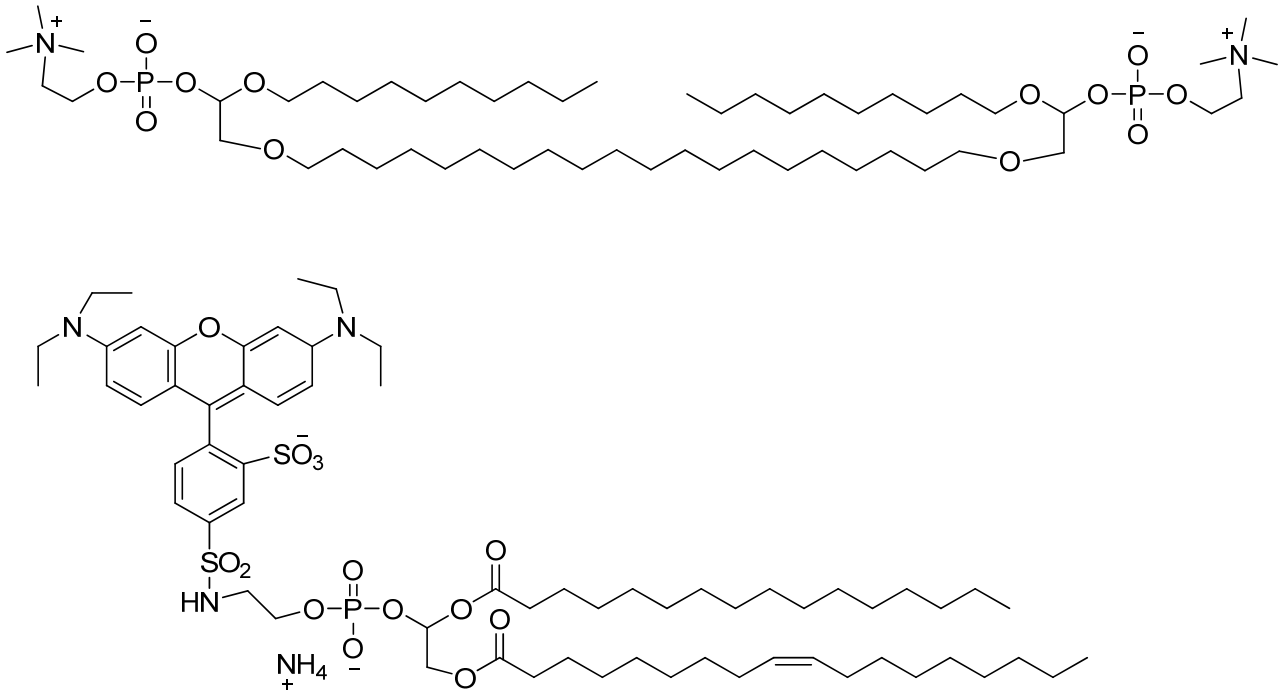
Department of Chemistry, Purdue University, West Lafayette, IN 47907

*Supporting Information*

1. Experimental Methods
2. Synthesis of $(t\text{-BOC})_3$NTA-poly(ethylene oxide[14]), Compound 5 (NTA-PEG)
3. Glass surface modification and transplantation protocol
4. TEM
5. AFM

**Experimental Methods**

The *N*-his$_6$-gp10 connector protein isolated from phi29 DNA packaging motor was a gift from the lab of Professor P. Guo. 2,2′-Di-*O*-decyl-3,3′-di-*O*-(1″,20″-eicosanyl)-bis-(*rac*-glycero)-1,1′-diphosphocholine ($C_{20}$BAS) was synthesized as previously described.[1,2] 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanol amine-*N*-(rhodamine B) (Rh-POPE) was purchased from Avanti Polar Lipids. All solvents were distilled under $N_2$ from an appropriate drying agent; all reactions were conducted under a dry $N_2$ atmosphere.



***Scheme S1.*** Host membrane lipid $C_{20}$BAS (top) and the fluorescent label used for monitoring the vesicle experiments, Rh-POPE (bottom).

Stock solutions of cholesterol, POPC, Rh-POPE and $C_{20}$BAS in $CHCl_3$ were prepared and mixed to give the appropriate ratio of lipids desired (i.e., 69:30:1 $C_{20}$BAS:Chol:Rh-POPE or 89:10:1 $C_{20}$BAS:POPC:Rh-POPE). The solvent was removed under a gentle Ar stream to produce a thin lipid film; residual $CHCl_3$ was removed under a 100 μm vacuum overnight. Then, the film was hydrated by adding 20 mM phosphate buffered saline

(PBS, 0.15 M NaCl, pH=7.4) before probe sonication at 60W for 2 min to produce small unilamellar vesicles (SUV). The final $C_{20}$BAS concentration was 2-3 mM and the average vesicle diameter ranged between 80 and 150 nm as measured by dynamic light scattering (DLS).

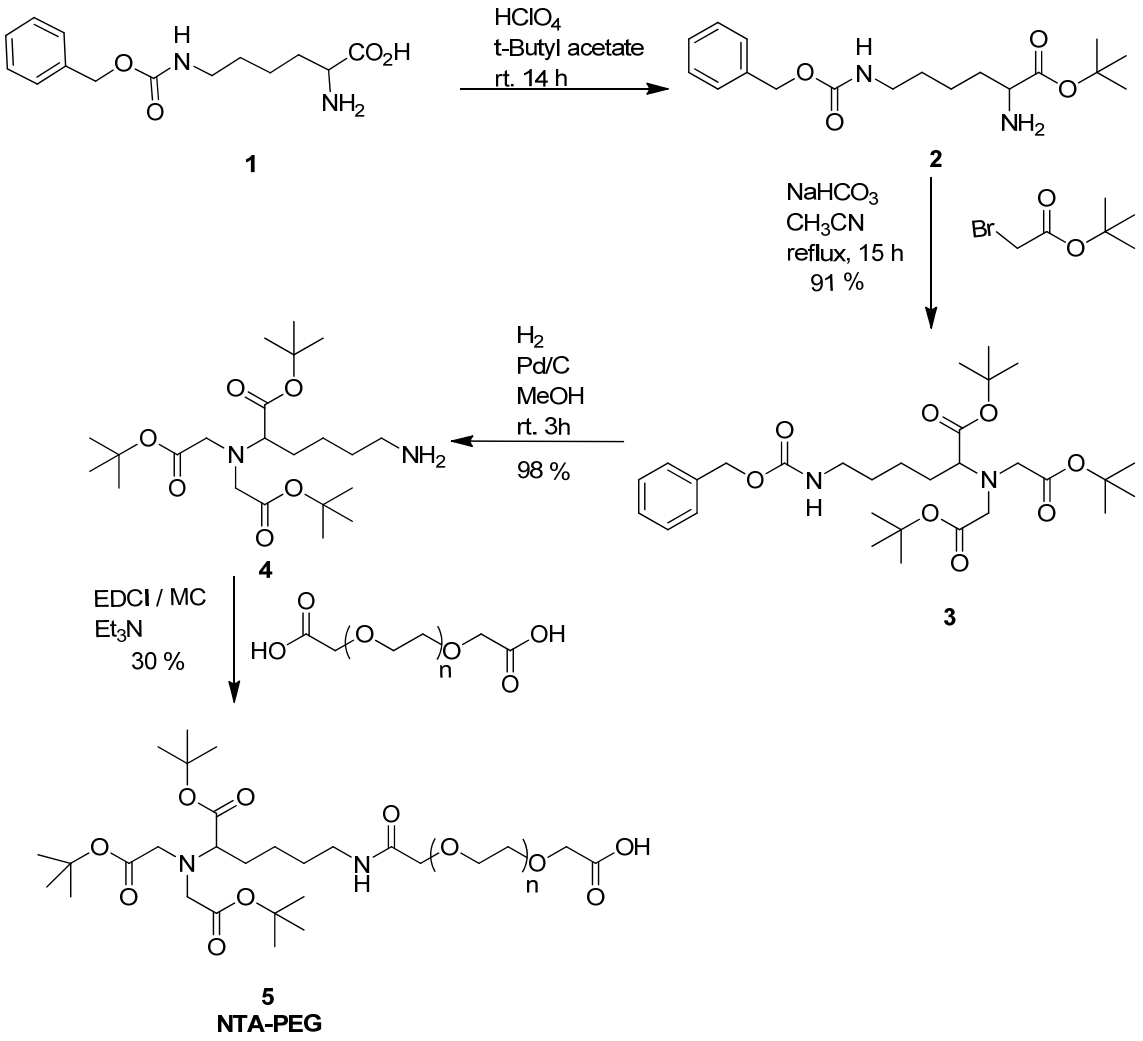
Adhesive rubber sheets (0.2 mm thick with a 0.5 cm diameter hole, 3-4 holes/slide) were mounted onto the $Ni^{2+}$:NTA-PEG-grafted glass surfaces. *N*-$his_6$-gp10 connector protein solution (200 μL, ~100 ng/mL) was loaded inside the hole and sealed with a second glass slide before incubation at 4 °C for 18 h. The excess protein was removed by careful rinsing five times with PBS. The preformed vesicle solution (200 μL) was added to the connector protein-captured surface and incubated for 5 h at 23 °C before removal of the excess vesicles via careful rinsing with PBS. The resulting surfaces were imaged by confocal laser scanning microscopy (CLSM, Bio-Rad MC 1024, Bio-Rad Laboratories, Hercules, CA) before and after vesicle addition. The connector-vesicle surfaces were then treated with imidazole buffer solution (2M, pH 7.4) for 1-2 min before re-imaging via CLSM to assess the vesicle distribution within the samples.

**Synthesis of (*t*-BOC)$_3$NTA-poly(ethylene oxide[14]), Compound 5 (NTA-PEG).**

*Synthesis of t-butoxy-$N^{\varepsilon}$-(benzyloxycarbonyl)-$N^{\alpha}$,$N^{\alpha}$-bis(t-butoxycarboethoxy)lysinate (3).* To a stirred solution of $N^{\varepsilon}$-benzyloxycarbonyl-L-lysine (**1**, 6.5 g, 23.18 mmol) in *t*-butyl acetate (80 mL) was added $HClO_4$ (3.01 mL). The mixture was stirred at 23 °C for 14 h and then extracted with $H_2O$ (150 mL) and 0.5N HCl solution (150 mL). The combined aqueous solutions then were adjusted to pH 9 with 10% $K_2CO_3$ solution and extracted with $CH_2Cl_2$ (3 × 100 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated to give **2** as an oil.

Compound **2** was dissolved in $CH_3CN$ (150 mL) before addition of $NaHCO_3$ (4.29 g, 51.07 mmol) and *t*-butyl bromoacetate (13.58 g, 69.64 mmol). The mixture was heated at reflux for 15 h before cooling to 23 °C, concentrating the mixture under reduced pressure and extracting the residue with ethyl acetate (2 × 150 mL). The organic layer was washed with saturated NaCl solution (2×100 mL), dried over anhydrous $MgSO_4$, evaporated and purified by silica gel flash chromatography using 4:1 hexane:EtOAc as eluent to give **3** (11.62 g, 91%) as a colorless oil. $^1$H NMR ($CDCl_3$, 300 MHz) δ 7.30-7.35 (m, 5H), 5.08 (s, 2H), 3.44 (dd, *J* = 17.4 & 11.1 Hz, 4H), 3.30 (t, *J* = 7.35 Hz, 1H), 3.20 (m, 2H), 1.62 (m, 2H), 1.53 (m, 4H), 1.45 (s, 9H), 1.43 (s, 18H); $^{13}$C NMR

($CDCl_3$, 75 MHz) δ 172.2, 171.2, 157.0, 137.34, 128.9, 128.6, 128.4, 81.64, 81.2, 66.9, 65.6, 54.4, 41.3, 30.6, 29.8, 28.7, 28.6, 23.5; MS (positive ESI): found 565.06 $(M+H)^+$, *m/z* calc'd for $C_{30}H_{48}N_2O_8$ = 564.71.




***Scheme S2.*** Synthesis scheme for the preparation of (*t*-BOC)$_3$NTA-PEG600-COOH.

*Synthesis of t-butoxy $N^{\alpha}$,$N^{\alpha}$-bis(t-butoxycarboethoxy)lysinate (4).* Palladium on carbon (0.5 g of 10 wt% Pd/C) was added to a solution of **3** (5 g, 9.09 mmol) in MeOH (100 mL). After stirring the mixture at 23 °C for 3 h under 1 atm $H_2$, the solution was filtered and evaporated under reduced pressure to give **4** (3.83 g, 98 %). $^1$H NMR ($CDCl_3$, 300 MHz) δ 3.45 (dd, *J* = 17.7 & 12.3 Hz, 4H), 3.30 (t, *J* = 7.35 Hz, 1H), 2.69 (t, J=6.15 Hz, 2H), 1.79 (m, 2H), 1.64 (m, 4H), 1.45 (s, 9H), 1.44 (s, 18H); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 171.9, 170.2, 81.5, 80.1, 64.0, 53.2, 41.4, 32.0, 30.1, 27.7, 27.6, 22.7; MS (positive ESI): found 431.05 $(M+H)^+$, *m/z* calc'd for $C_{22}H_{42}N_2O_6$ = 430.57.

*Synthesis of t-butoxy $N^{\alpha}$,$N^{\alpha}$-bis(t-butoxycarboethoxy)lysine-$N^{\varepsilon}$-carboxamide-PEG600-COOH (5).* PEG600 diacid (5 g, 8.33 mmol) and EDCI (2.4 g, 12.49 mmol) were dissolved in 150 mL dichloromethane. Compound **4** (4.66 g, 6 mmol) and triethylamine (12.5 mmol) were added and the reaction stirred for 1 d at 23 °C. The reaction mixture then was washed with deionized water (50 mL) and the solvent removed by rotary evaporation. The residue was purified by silica gel column chromatography using 10:10:0.5 $CH_2Cl_2$:MeCN:MeOH as eluent. Bis-NTA-PEG was isolated as the first fraction, while the desired product, Compound **5**, was isolated as the second component. $R_f$ = 0.6; yield = 1.85 g, 30%. $^1$H NMR ($CDCl_3$, 300 MHz) δ 6.95 (t, 1H), 4.2 (s, 2H), 4.0 (s, 2H), 3.1-3.9 (m, ca. 48H), 3.4-3.57 (dd, 4H), 3.2-3.6 (m, 3H), 1.65 (m, 2H), 1.55 (m, 2H), 1.43 (s, 27H).

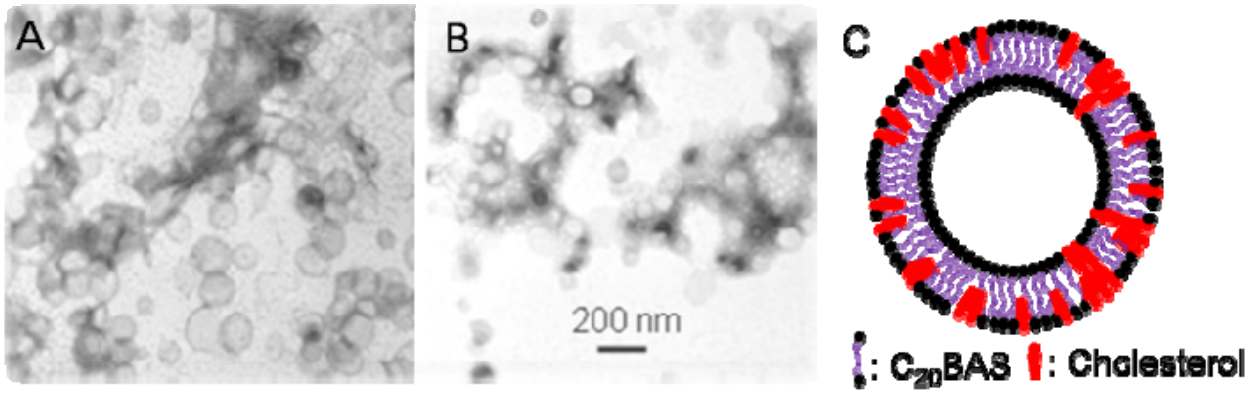
**Glass surface modification and transplantation protocol**

Clean glass surfaces were treated with piranha solution (7:3 $H_2SO_4$:$H_2O_2$) for 20 min (**Caution:** piranha solutions are extremely corrosive and potentially explosive) and rinsed extensively with deionized water. The surfaces then were immersed into an aqueous 1% 3-aminopropyltriethoxysilane (APTES) solution in water for 3 hr, before washing by immersion in a 1:1 $H_2O$:2-propanol sonication bath (3 x 5 min with a complete solvent change with each wash), and drying under Ar. The amine-modified glass was immersed in a $CH_2Cl_2$ solution of NTA-PEG, EDCI and $Et_3N$ (1 mM each) and the solution stirred under Ar at 23 °C overnight. The modified glass surfaces then were rinsed carefully with $CH_2Cl_2$ before immersion into 10% $Ac_2O$ in $CH_2Cl_2$ for 18 h to cap the unreacted surface amines. Next, the surfaces were rinsed with $CH_2Cl_2$, immersed in 30% TFA in $CH_2Cl_2$ solution for 6 h to cleave the NTA protecting groups, and then washed via sonication bath using $CH_2Cl_2$ and deionized water (3 x 5 min each). The surfaces were then activated for his-tag protein binding by immersion in 20 mM $NiSO_4$ solution for 1 h, before washing carefully with deionized water and drying under an Ar stream. These surfaces were then exposed to $his_6$-gp10 connector in 10 mM phosphate-buffered saline (PBS) solution for 12 to 20 h before rinsing with the same PBS buffer solution. These $his_6$-gp10 connector-modified surfaces were then exposed to 9:1 $C_{20}$BAS:POPC vesicles (labeled with 1% Rh-POPE; 10 mM lipid vesicles were prepared by probe sonication at 50 W for 2 min) for 5 h at room temperature before rinsing with PBS buffer solution to remove unbound vesicles. Displacement of the connector from the surface was achieved by adding a small aliquot of 2 M

imidazole stock solution to the surface-immobilized vesicle solution (net 10-fold dilution of imidazole) and incubating for 10 min before rinsing with PBS and analyzing by microscopy.

**TEM**

A drop of the imidazole-treated connector-vesicle sample was placed onto a carbon-coated Cu grid and the excess solution removed by filter paper. Next, a drop of negative staining solution (1% sodium phosphotungstate) was placed on the grid, the excess solution removed by filter paper, and the sample dried overnight in a dessicator. The grids were then transferred to a Philips EM 10 microscope and imaged at 80 kV. All samples had a final $C_{20}$BAS bolalipid concentration of 2-3 mM.



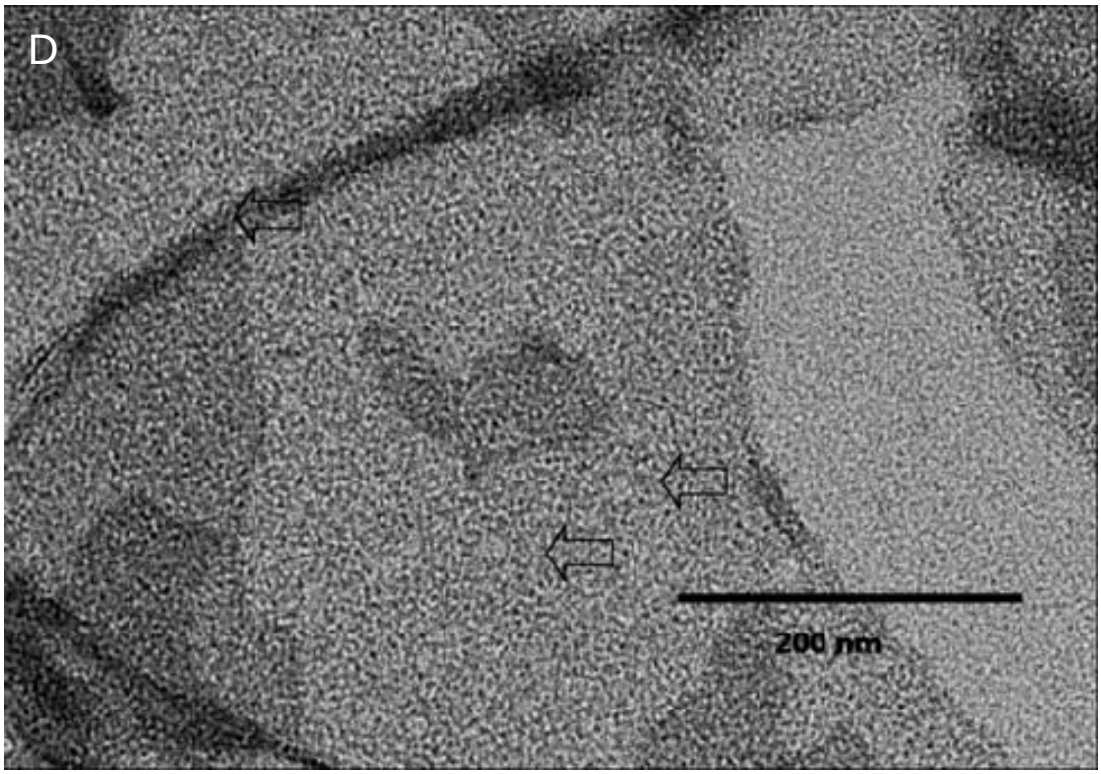



***Figure S1.*** TEM images of vesicles stained by 1% $UO_2OAc_2$. A: 30 mol% Chol in $C_{20}$BAS; B: 10 mol% POPC in $C_{20}$BAS; C: Model showing the presumed enrichment of monopolar lipid (Chol in this drawing) on the outer vesicle surface to accommodate curvature in the planar membranes preferred by centrosymmetric $C_{20}$BAS. D: High-magnification TEM image of *N*-$his_6$-gp10 connector arrays (open arrows) embedded within 9:1 $C_{20}$BAS:POPC membranes.

**AFM**

The surface-bound connectors on $Ni^{2+}$:NTA-PEG-modified glass were imaged via tapping mode AFM (WiTec Instruments, Ulm, Germany). The AFM tips (NSC15/AlBS, MikroMasch, USA) used had a typical uncoated probe tip radius of 10 nm or less, the cantilevers had a spring constant of 40 N/m, and the images were recorded with a scan rate of 1 Hz over a selected area of 1 $\mu$m × 1 $\mu$m.

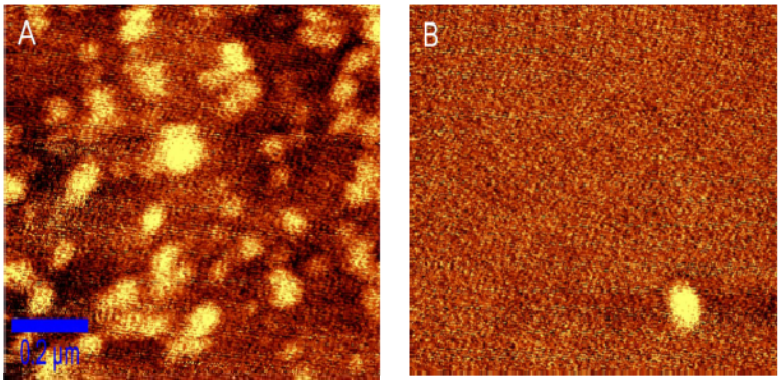



***Figure S2.*** Tapping mode AFM images of glass surfaces modified with *N*-$his_6$:$Ni^{2+}$:NTA-PEG gp10 after exposure to 9:1 $C_{20}$BAS:POPC vesicles (A) and after exposure of surface A to 2 M imidazole (B). Both images were collected at the same magnification. Scale bar = 200 nm.

**References**

(1) Svenson, S.; Thompson, D. H. *J. Org. Chem.* **1998**, *63*, 7180-7182.
(2) Di Meglio C.; Rananavare, S. B.; Svenson, S.; Thompson, D. H. *Langmuir* **2000**, *16*, 128-133.