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

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Abstract: Ni^{2+}:NTA−PEG600-grafted glass surfaces are capable of immobilizing *N*-his_6 gp10 connector protein assemblies from the phi29 DNA packaging motor and mediating their transplantation into bolalipid vesicles whose membrane thickness is compatible with the hydrophobic domain of the gp10 assemblies.

In an attempt to resolve the safety issues of viral vectors and the inefficiency issues of nonviral vectors, we are attempting to combine their advantages into an efficacious hybrid system through the use of vectorially oriented, membrane-embedded, three-component DNA packaging motors within a bioresponsive host vesicle. The ATP-actuated DNA packaging motor from phi29 virus was chosen to test this concept since one component, the 440 kD gp10 dodecamer, can be readily expressed and purified in histidine-tagged form, has a structure known to 2.1 Å resolution,[1] and has been investigated for potential use in numerous applications.[2,3] Although oligomeric protein assemblies have been incorporated into membranes for controlled transport applications,[4] we are not aware of any previous efforts to transplant part of a functional viral motor into vesicle membranes with orientation control. The alternating hydrophilic−hydrophobic−hydrophilic trilayer structure of the gp10 connector array stem region (Figure 1) promotes aggregation in aqueous solutions via intermolecular association of the 2 nm band of hydrophobic residues.[3] Therefore, incorporation of the phi29 connector array into vesicle membranes requires strategies that will accommodate both the hydrophobic domain in the dodecamer array and the orientation necessary assembly of the other two motor components, pRNA and gp16 ATPase, to enable DNA packaging into the vesicle host. This work describes the development of a strategy for transplanting the gp10 dodecamer arrays into vesicle membranes with orientation control using nitrilotriacetic acid−polyethylene glycol (NTA−PEG)-modified surfaces. The well-known property of PEG toward reducing nonspecific adsorption of proteins and vesicles onto solid substrates,[5] as well as the deployment of a distal NTA unit for specific capture[6] and orientation of *N*-his_6-gp10, are key features of this approach.

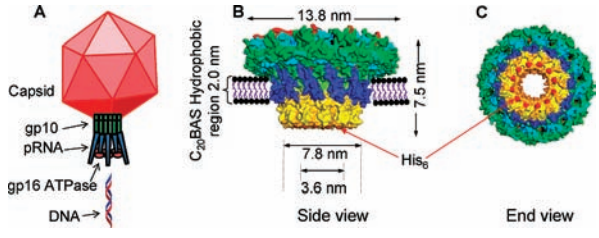

Figure 1. (A) Model of the phi29 packaging motor showing the three motor components and their relationship with the capsid. (B) Conceptual diagram of the phi29 *N*-his_6-gp10 connector protein assembly in a C_{20}BAS bolalipid membrane. (C) Exposed his_6 sites at the narrow N-terminal end of the gp10 assembly that are available for Ni^{2+}:NTA capture.

The method used for transplanting the gp10 array into C_{20}BAS bolalipid vesicles is shown in Figure 2. The key to orientational control is fixation of the gp10 N-terminus to the glass surface via a short water-soluble polymer tether.[7] A bias toward single insertions of *N*-his_6-gp10 connectors into C_{20}BAS vesicles is achieved by producing low surface coverages of NTA−PEG, so surface-mediated reconstitution occurs with only a single immobilized gp10 connector array.

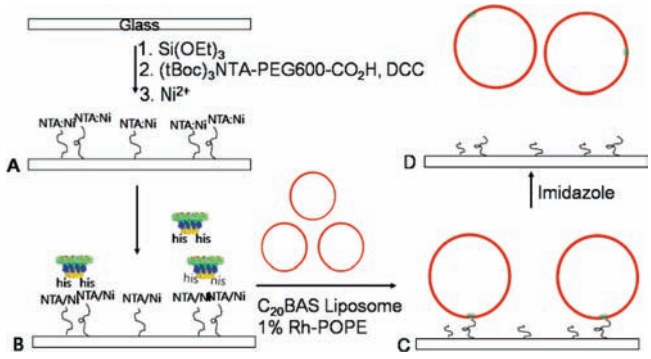

Figure 2. Transplantation strategy for incorporating oriented gp10 phi29 DNA motors into C_{20}BAS bolalipid vesicles. (A) Surface modification of glass with Ni^{2+}:NTA−PEG600. (B) Immobilization and orientation of *N*-his_6-gp10 connector protein via Ni^{2+}:NTA−PEG600 chelation. (C) Transplantation of oriented *N*-his_6-gp10 connector protein assembly into C_{20}BAS vesicles via surface-mediated reconstitution. (D) Release of immobilized C_{20}BAS vesicles via Ni^{2+} stripping with imidazole. Final assembly of a functional phi29 motor would involve docking pRNA and gp16 ATPase with the vesicles obtained from D.

C_{20}BAS bolalipid was chosen as the host membrane for the phi29 DNA motor since it has a hydrophobic thickness similar to that of the gp10 connector array stem domain (Figure 1) and is known to accommodate other hydrophobic peptides and integral membrane proteins.[8] Since C_{20}BAS prefers to adopt planar membranes in the absence of additional lipid components that can help stabilize a membrane structure with appreciable curvature,[9] the inclusion of either cholesterol (Chol) or 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was attempted in order to enable the formation of stable C_{20}BAS bolalipid-rich vesicles. Transmission electron microscopy (TEM) experiments (Figure S1 in the Supporting Information) confirmed that 7:3 C_{20}BAS/cholesterol and 9:1 C_{20}BAS/POPC mixtures form stable vesicles upon probe sonication, in good agreement with dynamic light scattering data showing that 80−160 nm particles are formed by this procedure. These findings are also consistent with previous reports of homogeneous dispersions formed by binary C_{20}BAS/POPC mixtures at low POPC molar ratios.[10] We infer from these results that the monopolar lipids preferentially occupy the voids that would otherwise be present on the outer surface of the membranes formed by C_{20}BAS in a transmembrane conformation,[11] thus stabilizing the curvature in the bolalipid vesicle membrane.

Confocal laser scanning microscopy (CLSM) images collected at NTA−PEG-grafted glass surfaces revealed little or no nonspecific binding of the Ni^{2+}:NTA−PEG surface (Figure 3A), but extensive punctate surface staining was apparent on samples that had been pretreated with *N*-his_6-gp10 connector arrays (Figure 3B). Adjustment of the confocal plane to image the bulk phase revealed very little rhodamine (Rh) fluorescence in solution due to the absence of unbound vesicles. Addition of 2 M imidazole solution to these surfaces and incubation for 10 min prior to CLSM imaging revealed the opposite behavior, i.e., little or no surface fluorescence (Figure 3C) and extensive punctate Rh fluorescence in the bulk solution (Figure 3D). TEM images of the imidazole (Im)-desorbed vesicles showed the presence of membrane-associated features whose size and shape are consistent with gp10 connector arrays (Figure 4 and Figure S1). We infer from these observations that oriented insertion of *N*-his_6-gp10 connector proteins occurred when they were exposed to small unilamellar C_{20}BAS bolalipid vesicles. This conclusion is supported by the following observations: (1) absence of vesicle-associated fluorescence in samples that were not exposed to connector protein (Figure 3A); (2) displacement of vesicle-associated Rh fluorescence upon Im stripping of Ni^{2+} from the his_6:Ni^{2+}:NTA−PEG interaction (Figure 3D); and (3) highly unfavorable interactions between the water-soluble PEG tether and the hydrophobic core of the C_{20}BAS membrane that would be expected if the *N*-his_6 terminus were oriented within the inner lumen of the vesicle. Similar results were obtained when 7:3 C_{20}BAS/Chol vesicles were used in the surface-mediated reconstitution process. Taken together, these findings suggest that the sequence depicted in Figure 2 is a viable strategy for vectorial transplantation of *N*-his_6-gp10 connector arrays into host vesicle membranes of appropriate hydrophobic thickness. The reversible adsorption of 9:1 C_{20}BAS/POPC vesicles from Ni^{2+}:NTA−PEG-grafted surfaces after transplantation of *N*-his_6 gp10 connector protein was also confirmed by atomic force microscopy (AFM). AFM images collected after rinsing with Im solution revealed a surface with few remaining vesicles (Figure S2). This result supports the conclusions drawn from the CLSM experiments.

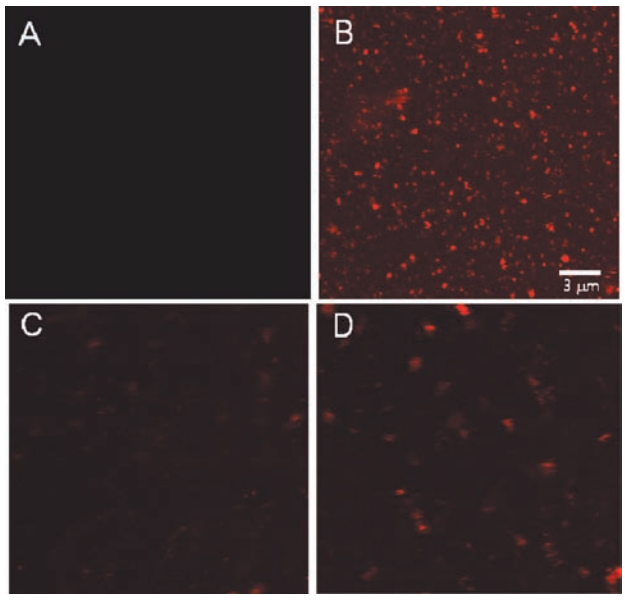

Figure 3. CLSM images of Ni^{2+}:NTA−PEG-modified glass surfaces after exposure to 1% Rh−POPE-labeled 9:1 C_{20}BAS/POPC vesicles. (A) Surface image taken after protein-free surfaces were exposed to bolalipid vesicles (control). (B) Surface image taken after sequential exposure of the surface to *N*-his_6 gp10 connector and bolalipid vesicles, respectively. (C) Surface image of sample B taken after Im addition. (D) Solution image of sample B after Im addition.

Since we seek the production of vesicles with a single gp10 array implanted in the membrane, low NTA−PEG600 surface coverages are required for enabling adequate spacing between individual surface-captured proteins in order to minimize vesicle−vesicle encounters at the interface during connector array insertion into the membrane. Increases in either the 3-aminopropyltriethoxysilane concentration or the treatment time produced surfaces with very large vesicles (>10 μm diameter) that were difficult to displace from the surface, even at very high Im concentrations (CLSM data not shown). We infer from these findings that high surface coverages of gp10 connector protein lead to vesicle−vesicle fusion during the surface-mediated reconstitution step. Since these very large vesicles are presumed to possess numerous his_6:Ni^{2+}:NTA surface interactions, they would be expected to be difficult to competitively displace from the surface with Im.

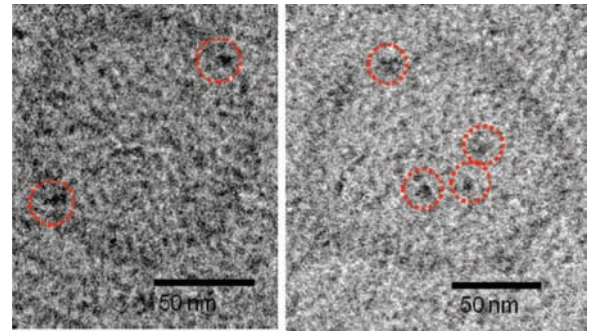

Figure 4. CryoTEM images of *N*-his_6-gp10 connector array-containing 9:1 C_{20}BAS/POPC vesicles produced as shown in Figure 2. The membrane-embedded gp10 arrays are highlighted with dotted circles.

In conclusion, Ni^{2+}:NTA−PEG600-grafted glass surfaces are capable of immobilizing *N*-his_6-gp10 from the phi29 DNA packaging motor. C_{20}BAS bolalipid vesicles stabilized by incorporation of 10 mol % POPC or 30 mol % cholesterol can produce reconstituted vesicles with transplanted connector arrays embedded in the membrane upon exposure of the *N*-his_6 gp10-primed surfaces to sonicated C_{20}BAS dispersions. These oriented gp10 vesicles were detached by stripping the surface with excess Im, resulting in a construct that is primed for subsequent pRNA and gp16 ATPase binding to enable ATP-driven DNA packaging. Development of this simple DNA packaging motor implantation strategy is an important first step toward the practical realization of nonviral vectors whose DNA cargo can be controllably encapsulated within a carrier system having properties that can be readily optimized for in vivo performance.

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Supporting Information Available: Experimental methods and TEM and AFM images. This material is available free of charge via the Internet at http://pubs.acs.org.

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