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Artificial Transport Vesicles

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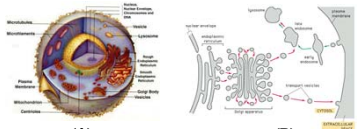
Abstract

Lipid tubules and vesicles are important structures associated with the transport of biomaterials in cells. Their formation is based on the pulling activity of membrane-bound kinesin processing on microtubule (MT) networks. We are inspired to build nanoscale transport systems modeled after biological processes using giant unilamellar vesicles (GUVs) coupled with the kinesin-MT complex. To generate transport vesicles we are exploring the use of membrane domains to achieve vesicle scission.

Background

Cell structure

Golgi bodies are integral components of the cell, responsible for organizing and packaging material for transport within the cell (Fig. 1).



(A) (B)

Fig. 1. (A) Cut away schematic structure of eukaryotic cell. **(B)** Vesicle trafficking between the Golgi body, endoplasmic reticulum (ER), and the plasma membrane [1, 2].

Transport vesicles from the Golgi

In vitro studies with isolated Golgi showed transport vesicles formed through the pulling activity of membrane-bound kinesin on microtubules (Fig. 2).

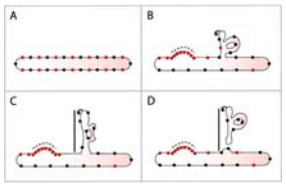


Fig. 2. Transport vesicle formation in Golgi transport. **(A)** Golgi membrane before tube formation. **(B)** Components are sorted into two domains one of which bends, forming the precursor transport tube that attaches to kinesin. **(C)** The kinesin attaches to MT, and pulls the Golgi membrane, forming a tube. **(D)** Transport vesicle forms via scission from the Golgi [3].

Kinesin-pulled Nanotubes

Others have shown that biotinylated kinesin bound to GUV membrane via streptavidin-coated beads are capable of generating lipid nanotubes (Fig. 3).

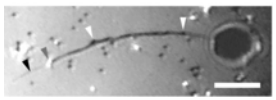
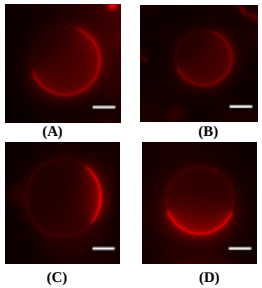


Fig. 3. Tube (white arrows) extending from a GUV via kinesin bead interaction with MT (black arrow). Scale bar 5 μm [4].

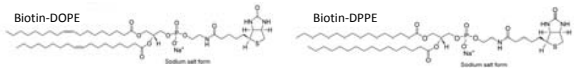
Results

In an effort to generate functionalized lipid domains in giant vesicles for kinesin-MT experiments we studied several biphasic membrane architectures. Using two commercially available biotinylated lipids our hope was to selectively partition biotin to either liquid ordered (L_o) or liquid disordered (L_d) regions, depending on lipid structure (Fig. 4).



(A) (B) (C) (D)

Fig. 4. The L_d phase (bright red region of membrane) was enriched in DPhPC and membrane dye TRTIC-DHPE (0.03%), while the L_o phase (dark) was enriched in DPPC and cholesterol. Biotinylated lipids biotin-DPPE and biotin-DOPE were expected to partition to the L_o and L_d phase, respectively. Scale bar: 5 μm. Compositions are as follows:
(A) Sample 1, 0.38 DPhPC / 0.04 biotin-DPPE / 0.24 cholesterol / 0.34 DPPC.
(B) Sample 2, 0.38 DPhPC / 0.11 biotin-DPPE / 0.24 cholesterol / 0.27 DPPC.
(C) Sample 3, 0.18 DPhPC / 0.02 biotin-DOPE / 0.40 cholesterol / 0.40 DPPC
(D) Sample 4, 0.14 DPhPC / 0.06 biotin-DOPE / 0.40 cholesterol / 0.40 DPPC



Determining the L_o to L_d area ratio

ImageJ software was used to measure the surface are of the L_o domain to the total vesicle area (Fig. 5).

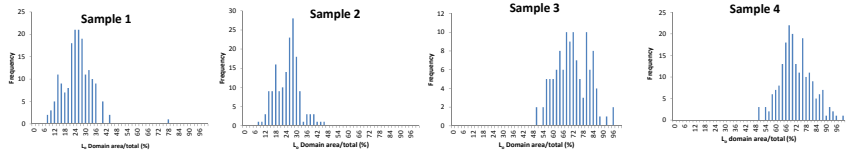
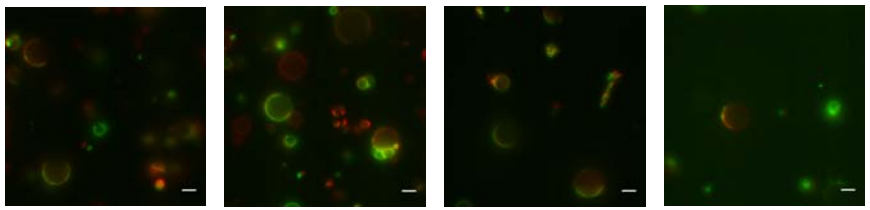


Fig. 5. Distribution of surface area ratios. Samples 1 (n=174) and 2 had similar distribution profiles, peaking between 20 and 30%, consistent with the compositions. Sample 2 (n=163) contained more biotin-DPPE. The distribution of samples 3 (n=115) and 4 peaked between 65 and 75%, also consistent with the compositions. Sample 4 (n=201) contained more biotin-DOPE.

Avidin-FITC binding to lipid domains

FITC-labeled avidin was used to assess specificity of binding to lipid domains. Avidin-FITC bound preferentially to the L_d domain for samples 1-3, despite where the biotinylated lipids were predicted to partition (Fig. 6). Sample 4 vesicles showed no domain selectivity for protein binding.



(A) (B) (C) (D)

Fig. 6. Two-color (false) fluorescence images showing the membrane (red) and membrane-bound avidin-FITC (green). The ratio of L_o to total vesicle area did not change after addition of protein. **(A)** Sample 1. Protein and membrane fluorescence were colocalized in the L_d domain. Protein was expected to bind to the L_o (dark) domain. **(B)** Sample 2. Protein and membrane fluorescence were colocalized. Some vesicles did not exhibit protein fluorescence. Protein was expected to bind to the L_o (dark) domain. **(C)** Sample 3. Protein and membrane fluorescence were colocalized. **(D)** Sample 4. Protein and membrane fluorescence were colocalized. Some structures only had protein fluorescence. Scale bar 5 μm.

Conclusions

Vesicle domain ratios were found to be consistent with membrane composition and did not vary following protein affinity. However, protein binding specificity to lipid domains was not achieved. Interestingly, avidin-FITC preferentially bound to L_d domains regardless of the type of biotinylated lipid. This suggests that either biotin-DPPE does not selectively partition to L_o domains or the protein favors L_d phase membranes.

Future Directions

Improved control over biotin localization

Based on previous work we have designed a new lipid, which should partition selectively to the L_o domain (Fig. 7).

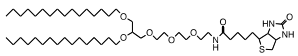
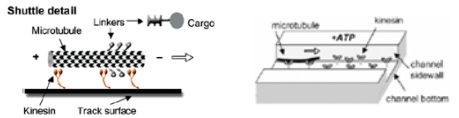


Fig. 7. Novel glycero-ether linked lipid, DP-biotin, is proposed to preferentially partition to the L_o domain of GUVs.

Inverted motility assay with domain-structured GUVs for transport units

Further development of system for cargo transport to specific destinations. Kinesin is strategically placed in structured surfaces to guide MTs bound with cargo pulled from GUVs (Fig. 8).



(A) (B)

Fig. 8. Transport system. **(A)** Cargo is bound to MTs and is transported by kinesin-MT interaction. **(B)** Kinesin is strategically placed in channels to guide loaded MTs to destination [5, 6].

References

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