Anchoring giant plasma membrane vesicles to a surface for novel biosensing

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Purpose

Giant plasma membrane vesicles (GPMVs) are unilamellar bodies that can be easily isolated from mammalian cells. SNAP-tag is a protein tag which binds covalently with benzylguanine (BG) derivatives, and is also capable of being fused to a protein of interest. Currently, specifically anchoring cellular material remains a challenge for bioengineering. The purpose of our project was to create a benzylguanine-covered surface to immobilize GPMVs expressing SNAP-tag fused to A_{2A}, a G-protein coupled receptor. The ability to anchor vesicles from mammalian cells could be instrumental for the development of functional biosensors. Using this novel anchoring system, a wide variety of compounds could be quickly detected via binding to an exogenously expressed protein of interest within the same membrane environment.

Experimental Design

Harvesting $pSNAP_f$-A_{2A}-expressing GPMVs Wild-type human embryonic kidney (HEK) cells were cultured in 10% FBS-containing DMEM media at 37°C. Cells were transfected with purified DNA for A_{2A} or $pSNAP_f$-A_{2A} using Lipofectamine 2000 in Opti-MEM. After 24-48 hours, cells were vesiculated through addition of 25 mM paraformaldehyde (PFA) and 2 mM dithiothreitol (DTT).

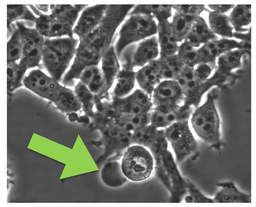
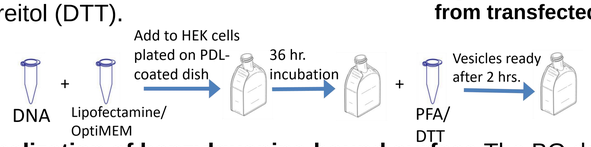
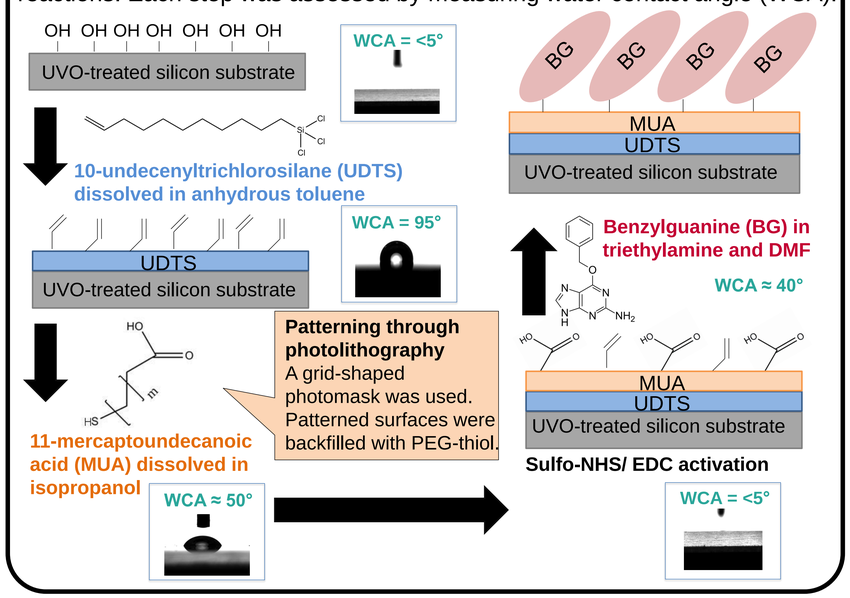


Figure 1. Isolation of vesicles from transfected HEK cells.



Functionalization of benzylguanine-bound surface The BG derivative-bound surface was assembled through a series of thiol-ene chemistry click reactions. Each step was assessed by measuring water contact angle (WCA).



SNAP-tag fused A_{2A} protein successfully expressed in HEK cells

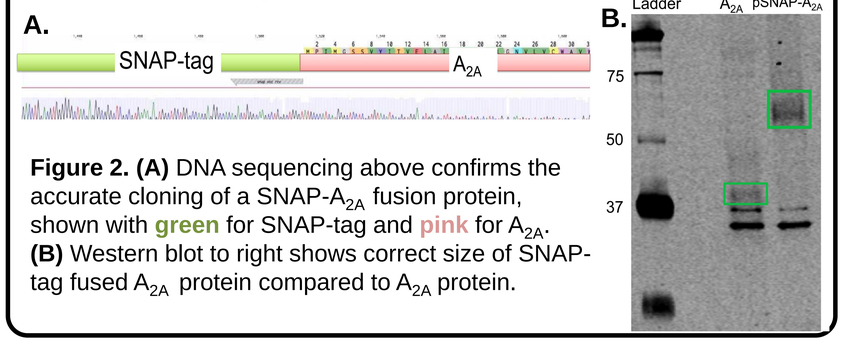


Figure 2. (A) DNA sequencing above confirms the accurate cloning of a SNAP-A_{2A} fusion protein, shown with **green** for SNAP-tag and **pink** for A_{2A}. **(B)** Western blot to right shows correct size of SNAP-tag fused A_{2A} protein compared to A_{2A} protein.

SNAP-tag expressing GPMVs immobilized by BG-functionalized surface

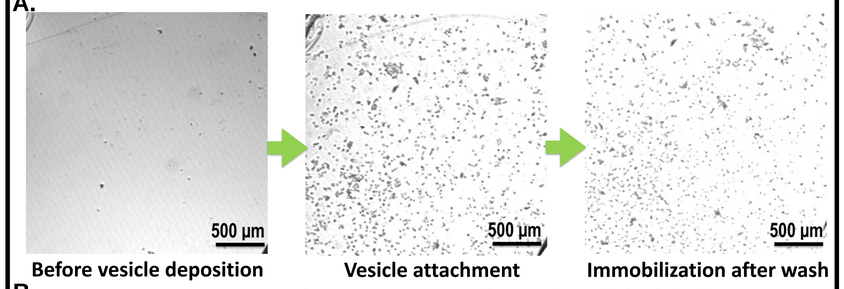


Figure 3. Recording of vesicle binding on BG-bound and PEG-thiol bound surfaces demonstrates specific GPMV binding to BG-surface. (A) Above figures demonstrate SNAP-tag expressing GPMVs binding to benzylguanine through comparison of vesicle attachment after wash to surface before vesicle deposition. **(B)** Figure to left illustrates GPMV-resistance to binding on negative control surface functionalized with PEG-thiol.

Examining GPMV-bound surface storage conditions

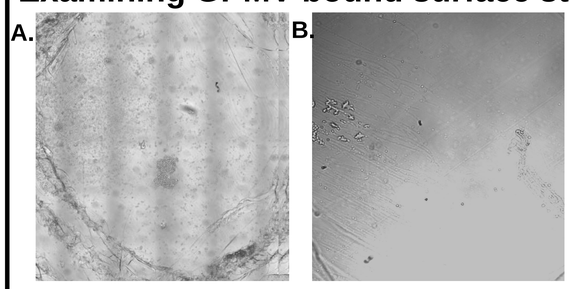
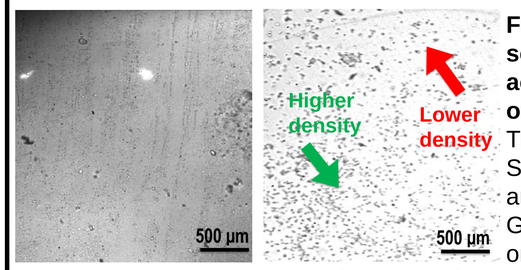


Figure 4. Comparison of GPMV-bound surface condition after horizontal and vertical storage. (A) Horizontally-stored surfaces retained vesicle immobilization. **(B)** Vertically-stored surfaces lost GPMV-binding.

GPMVs formed attachment gradient



No visible variation in A_{2A}-expressing GPMV binding on BG-functionalized surface

Areas of higher and lower densities of SNAP-tag expressing GPMV binding on BG-functionalized surface

Figure 5. GPMVs may be selectively bound according to a gradient of BG-fuctionalization. The interaction between SNAP-tag and BG led to areas of greater and lesser GPMV-binding, as GPMVs only expressing A_{2A} failed to form a gradient. The BG-gradient exhibits a forceful attraction of GPMVs expressing SNAP-tag.

Discussion

- Successful construction of a BG-functionalized surface through thiol-ene chemistry that can bind SNAP-tag transfected GPMVs.
- Portability and durability of GPMV-bound surface evidenced by continued immobilization by BG and appearance of GPMV viability after horizontal storage.
- Surfaces widely available: surface modification is replicable, and GPMVs are readily available through only a two hour vesiculation procedure.
- GPMVs were strongly attracted to BG-functionalized surface and consequently formed an attachment gradient, through the mechanism behind this force should be further characterized.
- Photomasks were incapable of reproducing exact patterns for GPMV-binding; further attempts to precisely determine vesicle attachment are needed.
- General applicability to biosensing should be tested through fluorescence resonance energy transfer (FRET)-activated receptor binding to future proteins of interest.

Conclusion

We successfully created a novel portable BG-functionalized surface with the ability to immobilize GPMVs expressing a fusion protein, solidifying the basic technique for creation of a biosensor. Future work will involve characterizing biological receptor binding of various molecules to the SNAP-tag to activate FRET and release consequent visible color change to detect human disease.

Acknowledgements

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