A2: Investigation of dynamic nano-structures in biomimetic membranes

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Presentations

- 8 oral IRTG
- 8 posters IRTG
- 4 oral other
- 3 posters other

Publications

[1] A.Weinberger et al., Biophysical Journal 105, 154ff (2013).

[2] A.Weinberger et al., submitted to PNAS

[3] N.Mathaiyan, A.Weinberger et al., in preparation

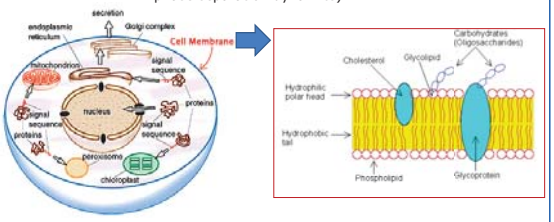
Other activities

- Participation in Science Day (fête de la Science)
- Strasbourg Workshop on Membrane Biophysics (SWOMB event)
- 42nd IFF Spring School, February 2011, Jülich, Germany
- Boulder Summer School "condensed matter and material physics", July-August 2012, Boulder, Colorado, USA
  Poster Prize
- GDR 3070: Physique de la cellule au tissu. Journées plénières, October 2012, Autrans (Grenoble), France
- Visit to Chilkoti's lab, Duke University, Durham, North Carolina, USA

Motivation

Glycolipids

- Are cell membrane components
- Both chemical groups - lipids (responsible for shapes and behavior of cell membrane) and carbohydrates are covalently bonded
- serve as epitopes (membrane receptors) for cell to cell communication
- effects biophysical properties of the membrane (fluidity, phase separation dynamics).



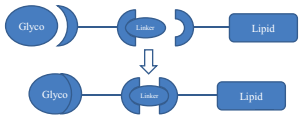
Challenge: Obtain stable, selectively permeable and functional biomimetic membranes for sensor and separation purposes

→The assembly of glycolipids into defined supramolecular architectures at molecular scale, requires the synthesis of new multifunctional building blocks, composed of several modules.

Glycolipids synthesis

Flexible system of lipids and carbohydrates building block allow the combinatorial arrangement of the building blocks and the adjustment of their distances.

→ Bioorthogonal click-chemistry leads to generate a glycolipids library.



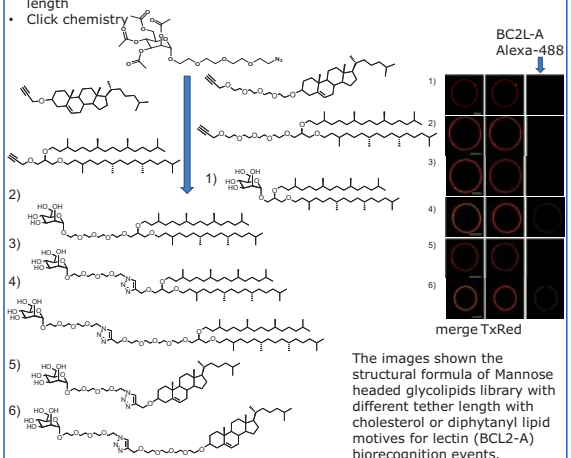
Click chemistry

- high yield and purity
- simple reaction conditions
- tolerance of oxygen and water
- simple product isolation

Glycoepitopes modified with different linkers/tethers or lipid motives allows the precise tuning of their interaction with the membrane. It also address spatiotemporal questions in signalling events.

O-Linked Glycolipids Library

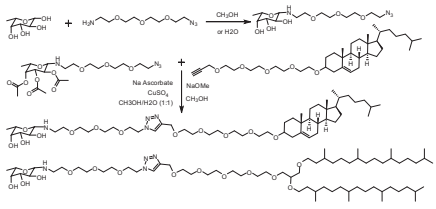
- Protected carbohydrates (mannose and fucose)
- Carbohydrate building block with clickable functional group azide
- Alkyne functionalized lipids (phytanol and cholesterol) with different tether length
- Click chemistry



The images shown the structural formula of Mannose headed glycolipids library with different tether length with cholesterol or diphytanyl lipid motives for lectin (BCL2-A) biorecognition events.

N-Linked Glycolipids Library

- Unprotected and unactivated carbohydrates
- Heterobisfunctional linker of tetraethylene glycol
- Alkyne functionalized lipids with tether length
- Click chemistry



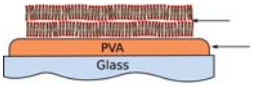
Glycolipids characterization

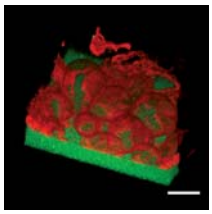
Giant unilamellar vesicles (GUVs) are good biomimetic membrane model. Motivated by the difficulties that we encountered to make GUVs containing charged lipids, we developed a new method for designing giant vesicles based on gel-assisted swelling.

- efficient and rapid growth of vesicles without any special equipment needed.
- GUVs composed of various lipids could be grown on a PVA-substrate and successfully collected for further studies.

PVA-assisted formation of GUVs for peculiar lipids

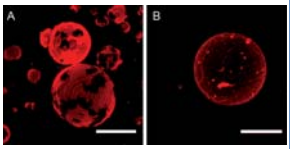
Figures: Using a PVA support below the lipid bilayer stacks facilitate lipid hydration and GUV growth.





Obtained GUV are free of polymer.
→ Well adapted method for the encapsulation of biomolecules
Any external energy input is required:
→ Minimization of the risk of degradation of involved substances.

Figure: Comparison between electroformation (A) and gel assisted growth (B) of GUV containing charged lipids.



The above images clearly show the advantage of this new method in order to obtain "undamaged" GUVs.

Glycolipids ability to form GUVs and binding efficiency

The PVA gel assisted growth method was particularly adapted to obtain GUVs from the new synthesized glycolipids. We tested all lipids synthesized in order to distinguish which one are able to form GUVs and their protein binding ability. GUVs with various amounts of glycolipids were formed and their binding potential to fluorescently labeled Concanavalin A was studied.

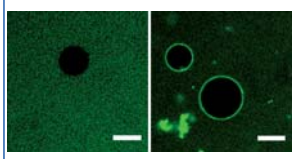
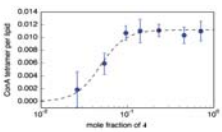


Figure: Left: Pure DPhPC- GUVs do not show any corona. Indeed ConA only binds specifically to mannose groups. Right: FITC- labeled ConA can bind to pure GLDPh1 GUVs. Scale bars 20 µm.

From fluorescence analysis we show that when the glycolipid is immersed in the bilayer, the length of the linker located between the tails and the lipid head is crucial for binding proteins such as Concanavalin A.

Langmuir monolayer compression isotherms were recorded from the synthesized glycolipids at different temperatures and with various amount of glycolipids within the monolayer. Concanavalin A was present in bulk solution. The area per lipid was measured at a pressure where monolayer transfers to substrate are usually performed. From the data one can extract the number of concanavalin A tetramer per lipid.

Figure: Binding of Concanavalin A to one of the newly synthesized glycolipids which was able to form stable bilayers, when varying the amount of the glycolipids within the monolayer. Results indicate that above 0,1 mole fraction of glycolipids, Concanavalin A are close packed onto the monolayer.



Conclusion & outlook

- Synthesized new glycolipids with different lipid anchors and tether length, from both unprotected and protected carbohydrates, building a lipid library.
- Developed a new, costless, and effective method to design GUVs from film precursor of peculiar lipids.
- The in-situ fluorescence microcopy experiments show that the Lectin bio-recognition clearly depends on the tether length.
- Biophysical studies on N-linked glycolipid molecules and the synthesis of complex carbohydrates are under progress.
- Translocation across the GUVs membrane would be made possible if the lipid composition (including glycolipids) allows structuration and local deformation of the model membrane.
- The IRTG project nucleated a new research thematic with numerous international collaborations (ANR kBT with ENSCBP Bordeaux France, A. Chilkoti's lab, North Carolina, USA).
- Refer renewal proposal projects A1, A2 and A3.