On the biomolecular interaction with bilayer and non-lamellar biomembranes

Tommy Nylander[a], Marie-Louise Ainalem[b], Pauline Vandoolaeghe[a,c], Marité Cárdenas[d], Richard A. Campbell[e], Hanna Wacklin[e], Robert K. Thomas[f], Fredrik Tiberg[g] and Adrian R. Rennie[h]

[a]*Physical Chemistry, Lund University, Lund, Sweden*

[b]*ESS Scandinavia AB, Lund, Sweden.*

[c]*Present affiliation: Syngenta, Basel, Switzerland.*

[d]*Nanoscience center, Copenhagen University, Denmark*

[e]*Institut Laue-Langevin, Grenoble, France*

[f]*Physical & Theoretical Chemistry, University of Oxford,, UK*

[g]*Camurus AB, Lund, Sweden*

[h]*Department of Physics, Uppsala University, Sweden*

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Interfacial behavior of drug delivery vehicles are important for

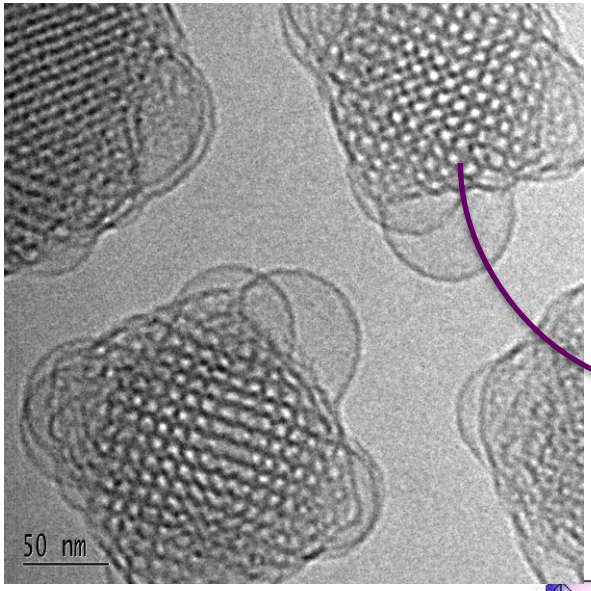
- the uptake of the drugs
- the biocompatibility of the delivery system
- stability upon storage.

Lipid based self-assembled structures

Has a large potential as drug delivery systems:

- Large capability to solubilise drugs
- Can be dispersed into nano-sized liquid crystalline particles
- Large variability of structures and morphologies - not only vesicles

What happens when such a liquid crystalline nano particle meets a biological (model) membrane?



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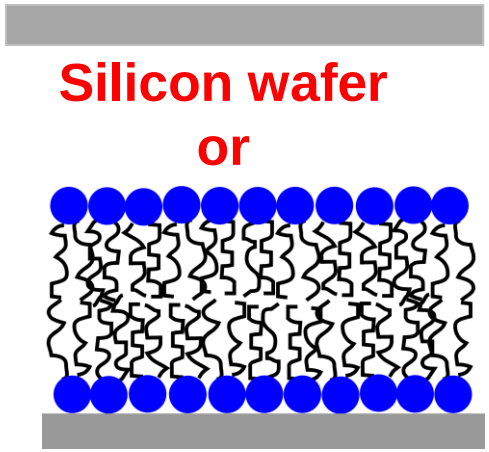
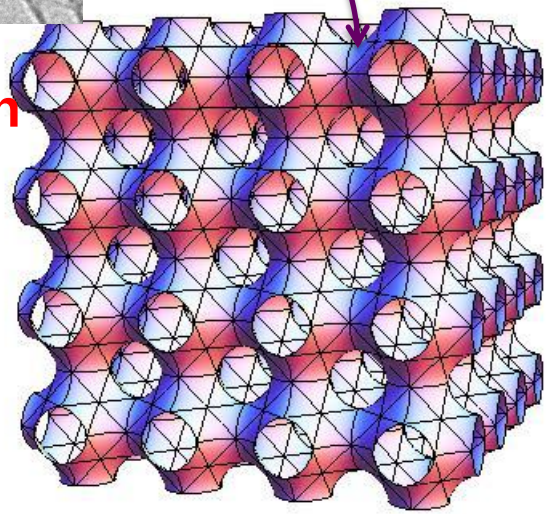


Silicon wafer

or

Dioleoylphosphatidylcholine (DOPC) supported bilayer

Glycerol monoolein (GMO) based dispersions (Cubosome®)



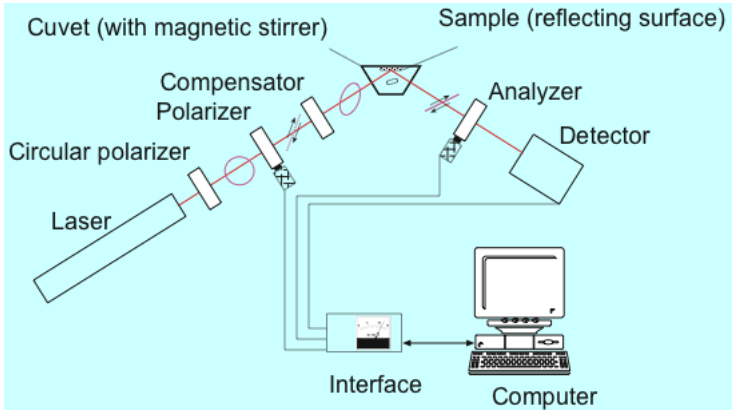
Bicontinuous cubic phase: Curved lipid bilayer

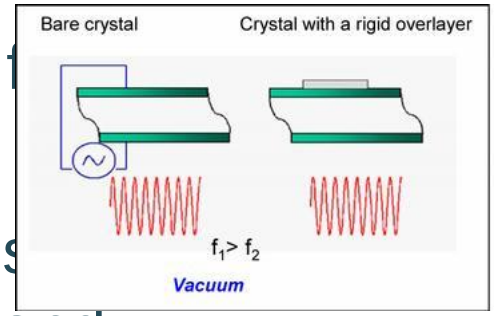
Main techniques used

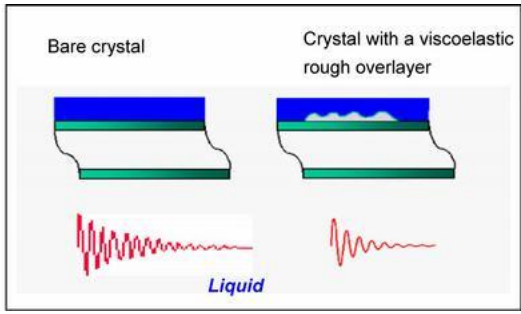
- Ellipsometry gives “optical” thickness and “dry” mass versus time
- QCM-D gives “wet” mass with coupled water and

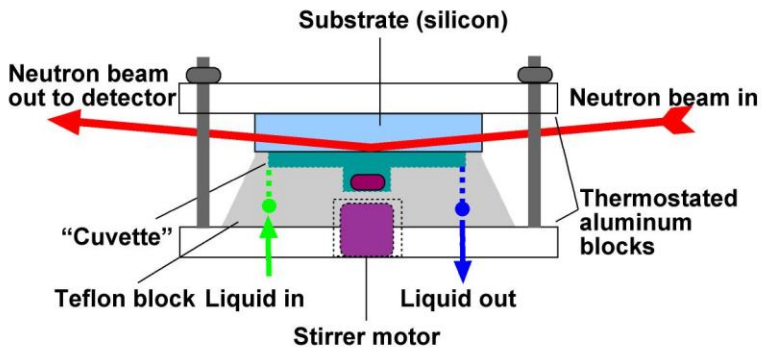
  dissipation measures the viscoelastic properties of the f
- Neutron Reflectometry gives dens

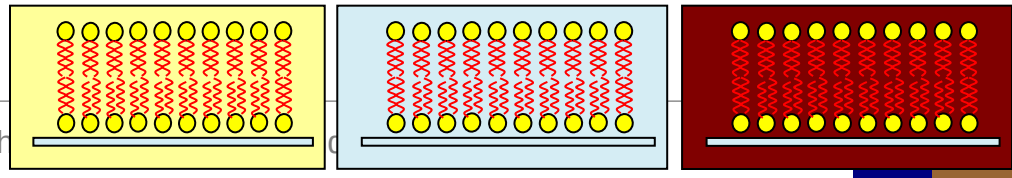
  profile of the interfacial layer and selective deuteration + contrast matching gives composition



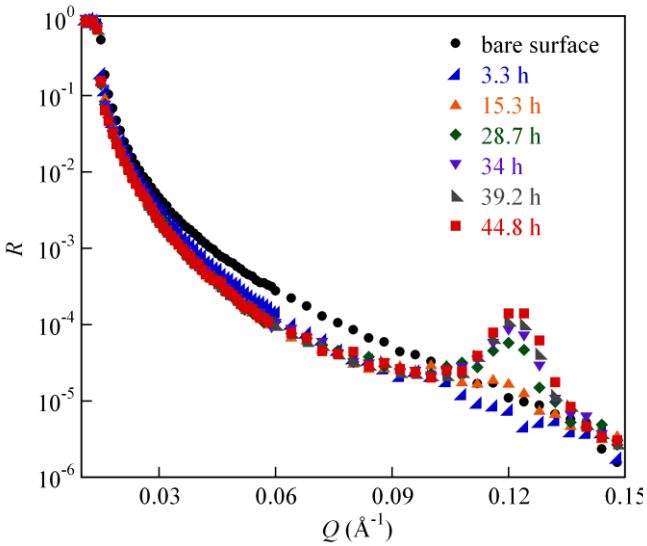


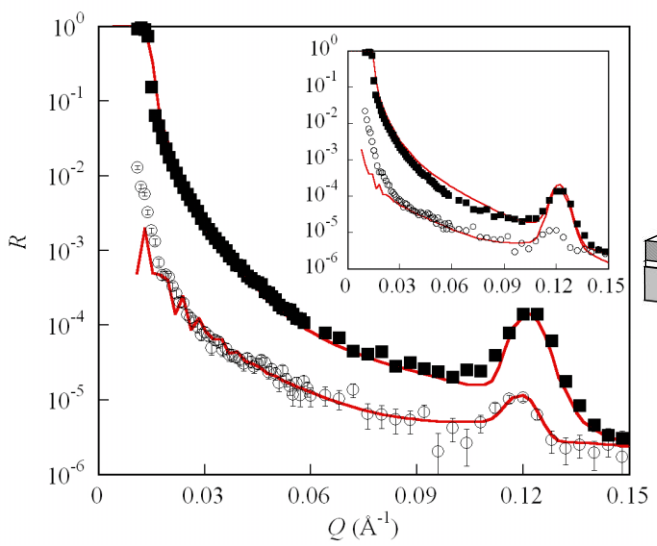


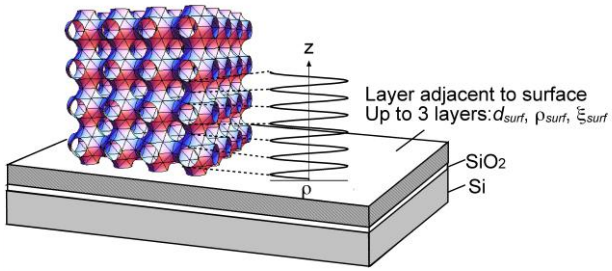




CPNP layer structure on silica from neutron reflectivity

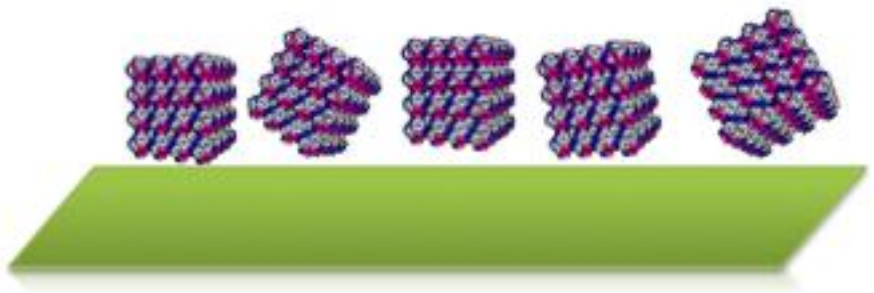






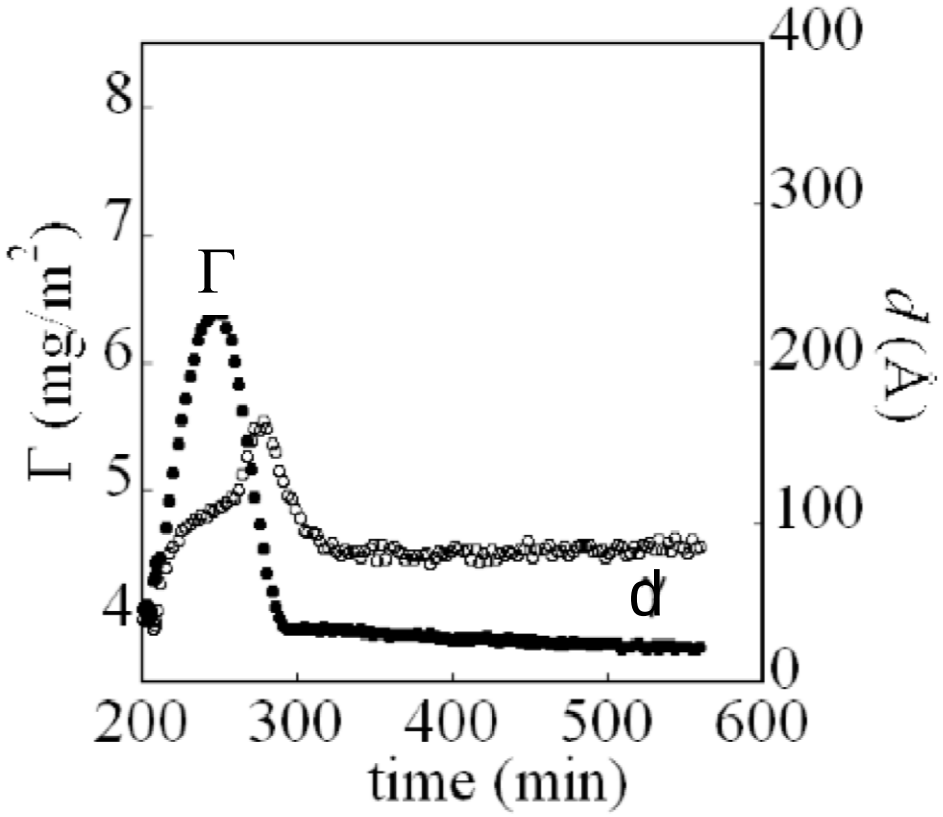
- Single layer adjacent to the surface + repeating structure
- SLD following a sinusoidal function versus *z*

3D nanoscopic lipid based surface layer by means of depositing different types of lipid based liquid crystalline nanoparticles (LCNP)



- Surface concentration of particles on a negatively charged silica surface can be increased by
- decreasing the pH below the iep of the surface
- change the surface charge by
  - adsorbing a cationic polymer (chitosan)
  - chemically modify the surface by means of silanization, using 3-aminopropyltrethoxysilane.

ADSORPTION OF CPNP ON DOPC BILAYER-ellipsometry

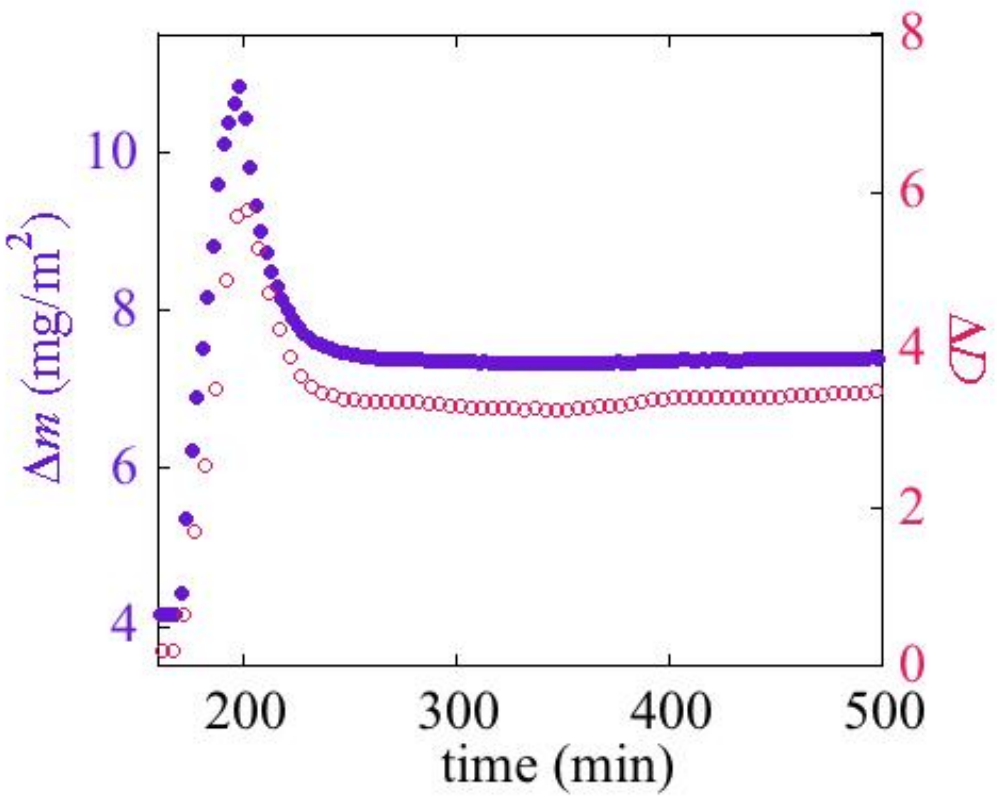


- Initially observed a strong adsorption of the particles on the DOPC bilayer.
- Subsequent decrease in the adsorbed amount

⇒Adsorption/desorption of particles?

P. Vandoolaeghe, R. A. Campbell, A. R. Rennie, R. K. Thomas, F. Tiberg, F. Höök, G. Fragneto, T. Nylander Adsorption of cubic liquid crystalline nanoparticles on model membranes. Soft Matter, 2008 4, 2267–2277.

ADSORPTION OF CPNP ON DOPC BILAYER-QCM-D



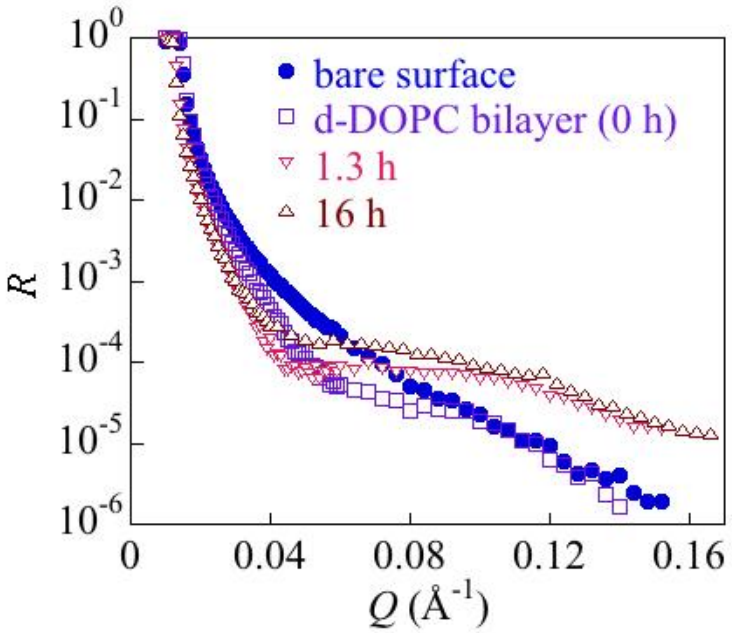
- Maxima after one hour in the wet mass and change in dissipation (as for adsorbed amount from ellipsometry)
- Change in dissipation is large and significantly more than from molecular adsorption

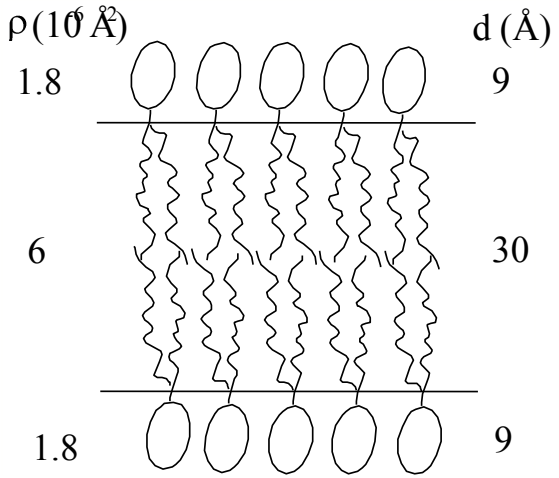
⇒Adsorption/desorption of particles!

WHY?

ADSORPTION OF CPNP ON d-DOPC BILAYER-neutron reflectivity (low CPNP conc)

Reflectivity in D_2O of d-DOPC (deuterated chains) bilayer after adding CPNP





Initial Bilayer

Addition 0.05 mg/mL CPNP at pH 4

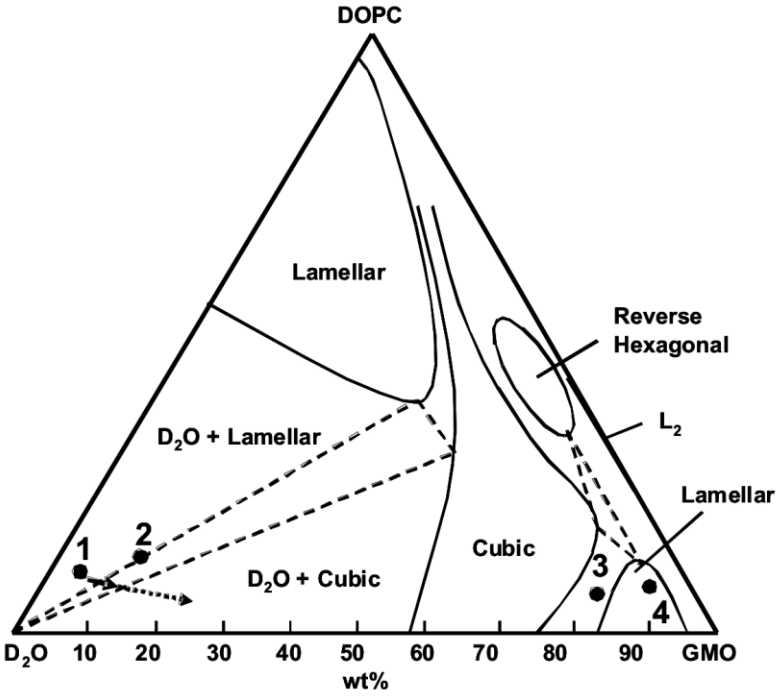
Integrity of bilayer seems to be kept but composition changes 20% solvent, 72% GMO, 8% DOPC

=>Lipid exchange occur

ADSORPTION OF CPNP ON d-DOPC BILAYER

Phase-diagram of D_2O-DOPC-GMO

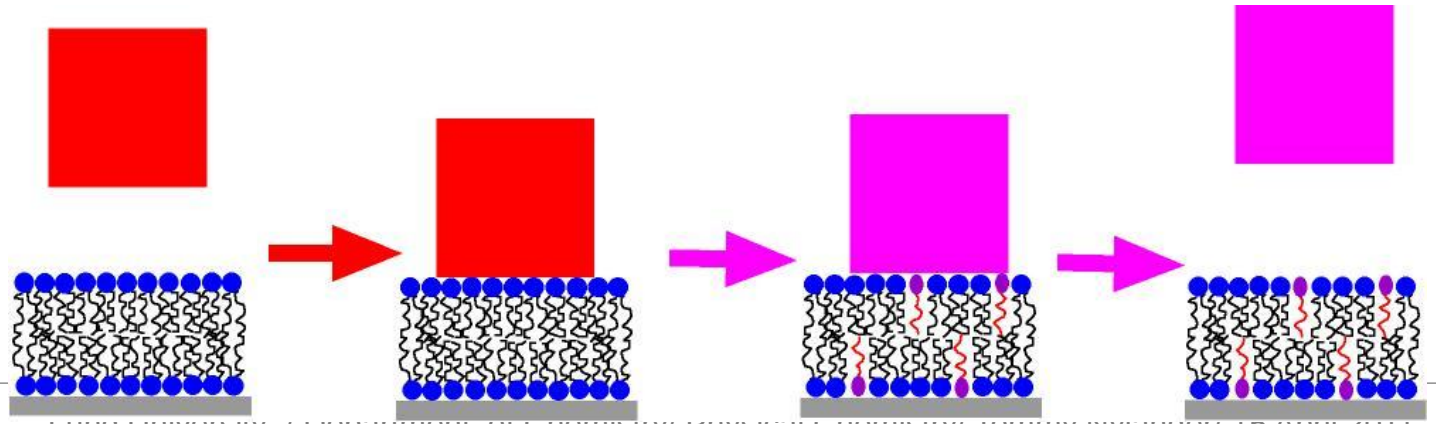
(from Gutman et al)



Lipid exchange occur when CPNP attach to bilayer

This make interaction between CPNP less favorable

=> Detachment of some particles



Other studies

- Change in structure verified by:
  - SAXD studies of the interaction between DOPC vesicles and various types of CPNP
- Exchange mechanism at the interface trigger release of CPNP form lipid bilayer:
  - Release slower if the bilayer is made of DPPC.
  - Exchange depends on bilayer coverage.

CAN THIS CONCEPT BE EXPLOITED FOR TARGET RELEASE TO MEMBRANES?

Gene delivery and DNA compaction and cell membrane interaction

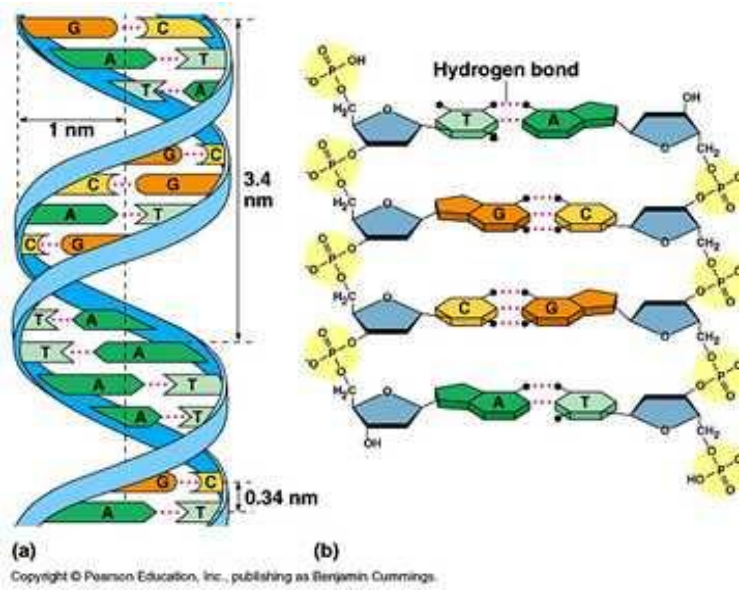
DNA (Deoxy ribo nucleic acid)

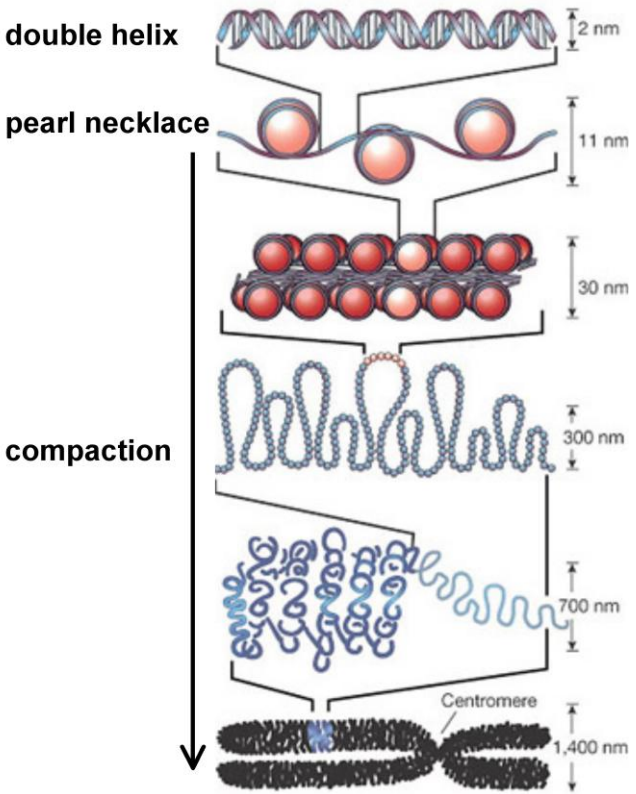
Highly Negatively charged polyelectrolyte

Long L= 1.478 μm

and

Stiff (R_G= 155 nm. l_p= 50 nm)





Material used

Luciferase T7 Control DNA (4331)

Linearized using Pdm1

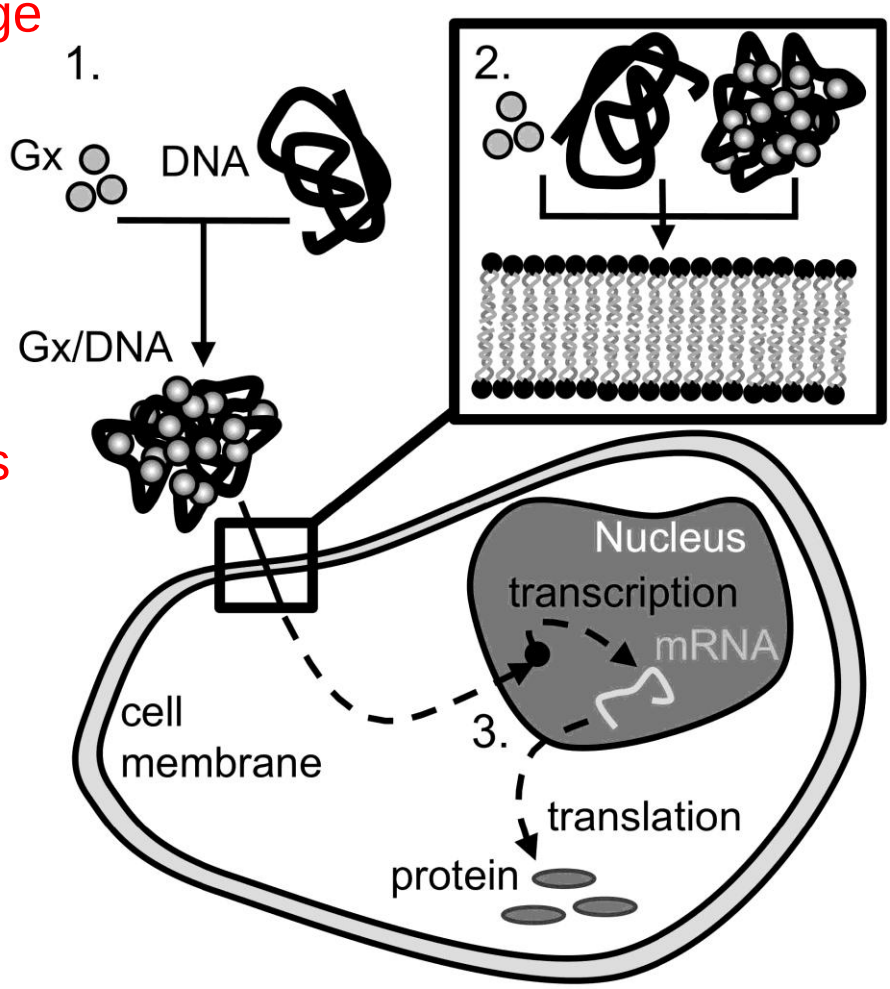
Gene therapy requires that the large hydrophilic highly (negatively) charged DNA crosses the lipid membrane

Is this possible?

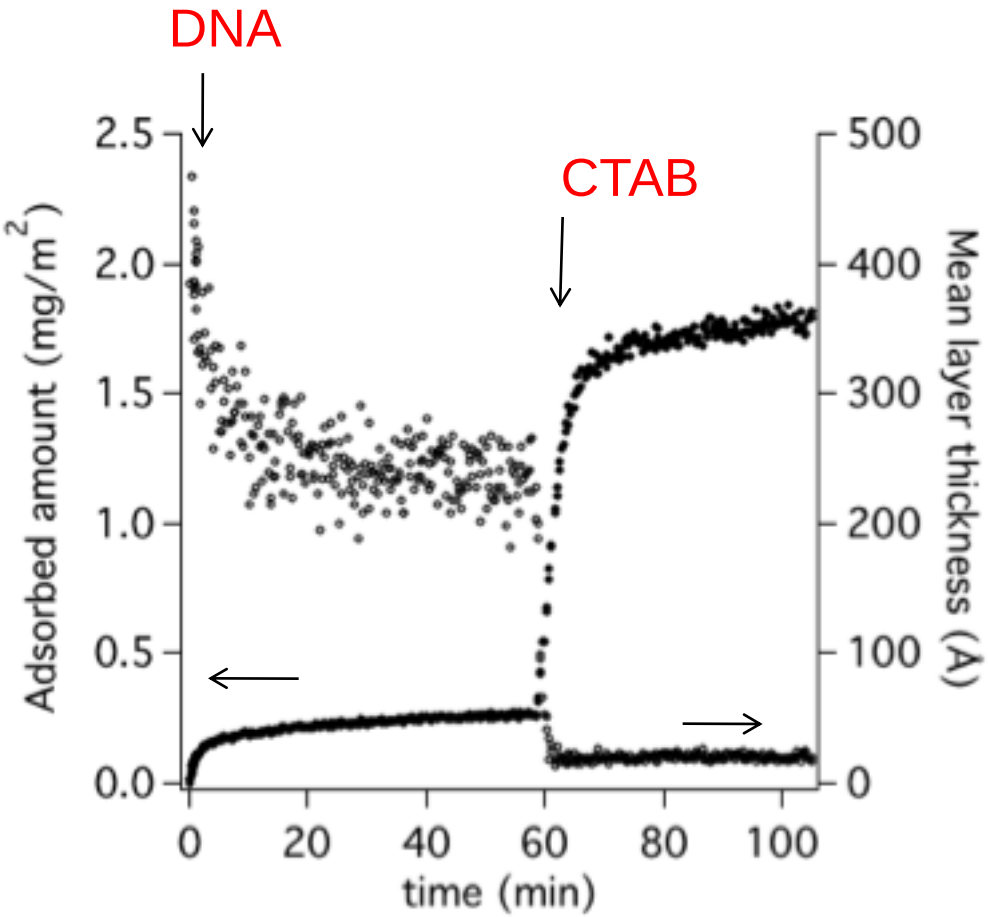
- Compacting DNA with oppositely charge dendrimers (and surfactants)
  - Reducing size
  - Reducing charge

We have studied

- Compacting DNA with oppositely charge dendrimers and surfactants
- Transport of dendrimer across supported lipid bilayer
- Interaction of DNA/dendrimer complex with the bilayer

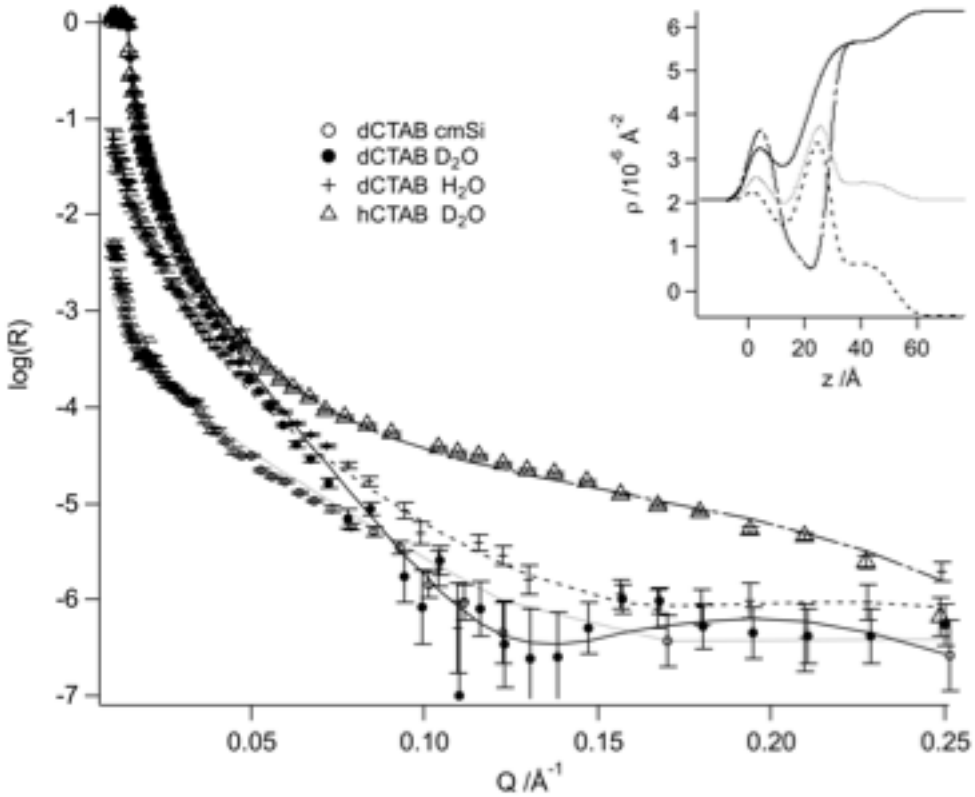


DNA can be compacted by cationic surfactants at interfaces (ellipsometry data)



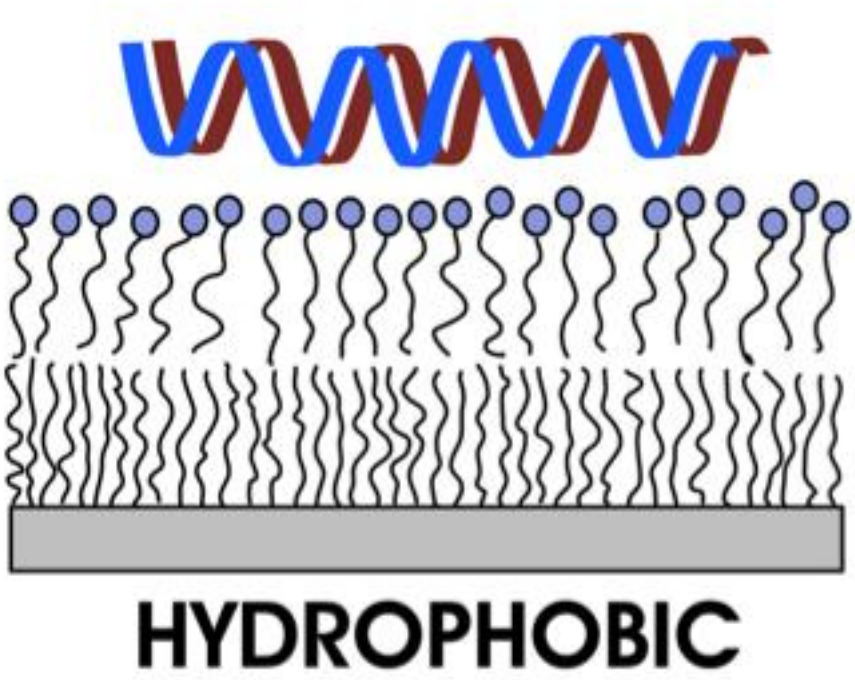
- **DNA and CTAB on hydrophobized silica surface**
  - adsorption of DNA on hydrophobic surface
  - further adsorption and compaction with CTAB

Neutron reflectivity profiles for mixed DNA-CTAB layers on hydrophobized Si crystals (four contrasts)



	Thickness / Å	Solvent penetration %v/v	Roughness / Å
CTAB monolayer	9 ± 1	10 ± 5	3 ± 1
DNA layer	22 ± 2	70 ± 5	3 ± 1

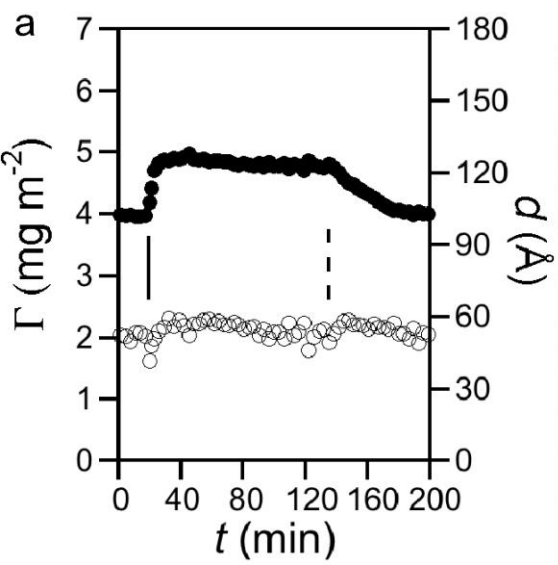
DNA compacted by cationic surfactants (CTAB and DTAB) at hydrophobized silica

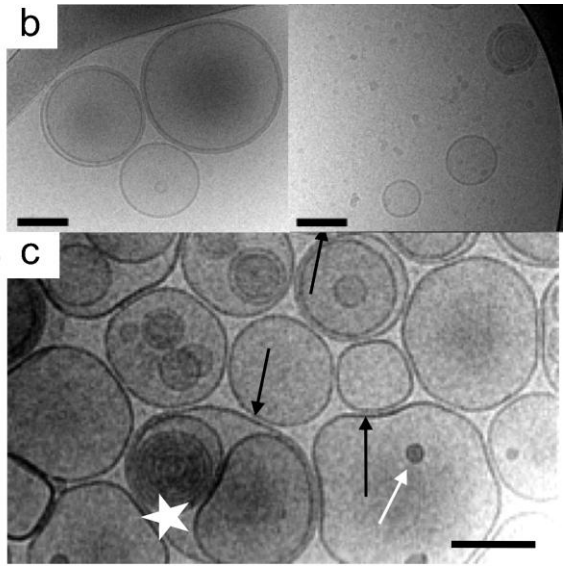


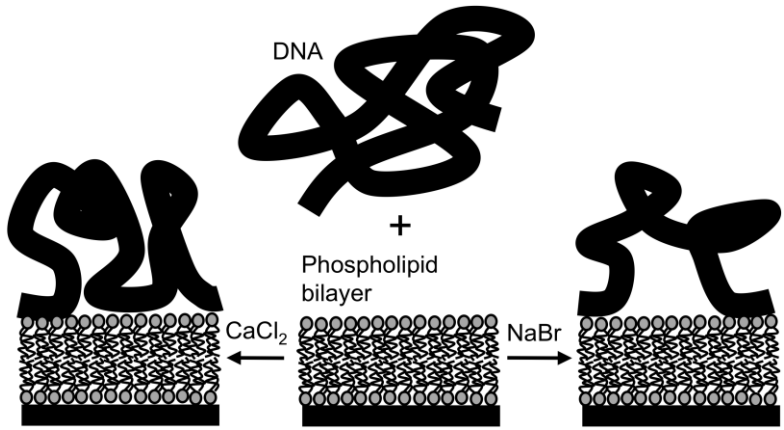
- CTAB monolayer on hydrophobic surface with DNA on top

Marité Cárdenas Gómez, Hanna Wacklin, Richard A. Campbell and Tommy Nylander: *Structure of DNA-cationic surfactant complexes at hydrophobically modified and hydrophilic silica surfaces revealed by neutron reflectometry* In preparation (2011).

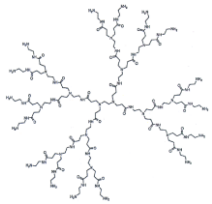
DNA interactions with model biomembranes supported bilayers – zwitterionic DOPC

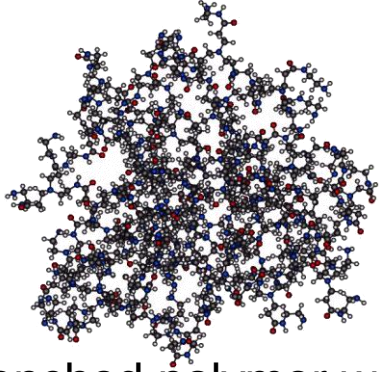






- In situ null ellipsometry and cryo-TEM display association between DNA and zwitterionic phospholipid bilayers in aqueous 10 mM NaBr solutions. Effect enhanced with Ca^{2+}!

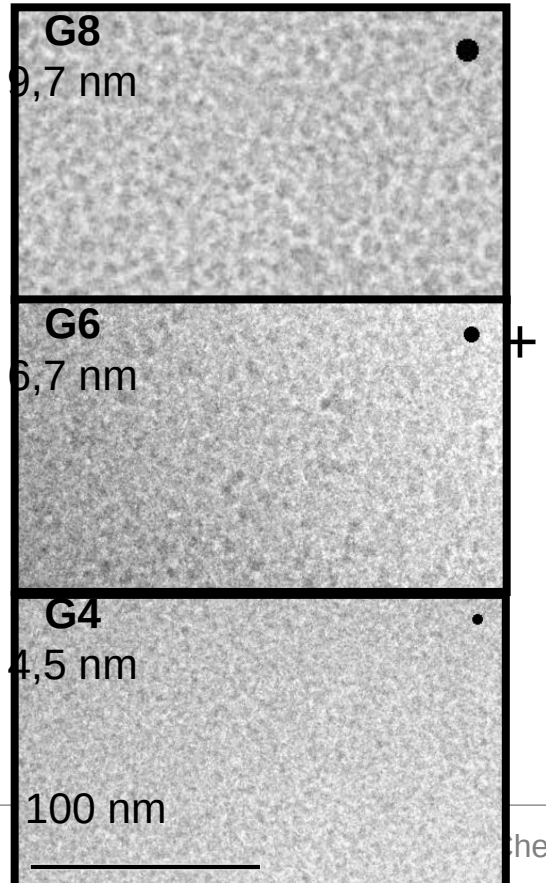




DNA condensation using cationic PAMAM dendrimers (G1-G8) - mimics histones

Cationic radially branched polymer w amido amine groups (ethylenediamine core).
G4 dendrimers: soft, flexible with dense core.

Cryo-TEM images:



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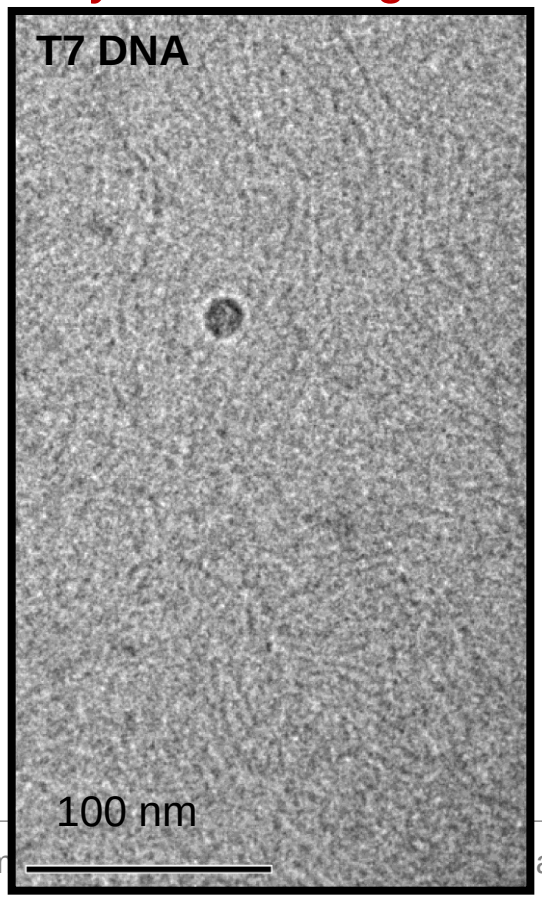

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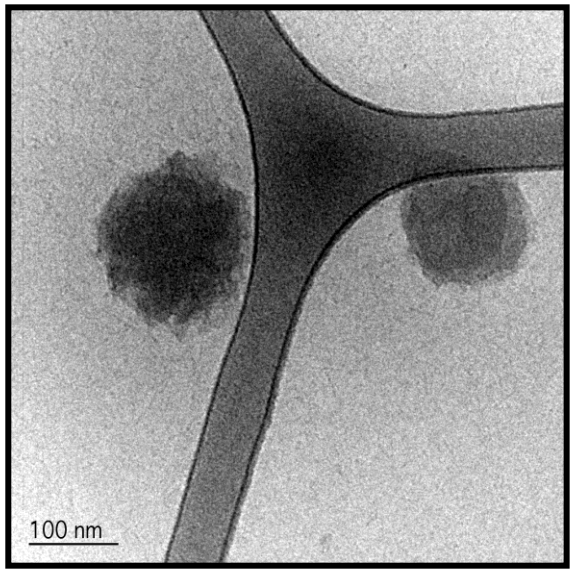
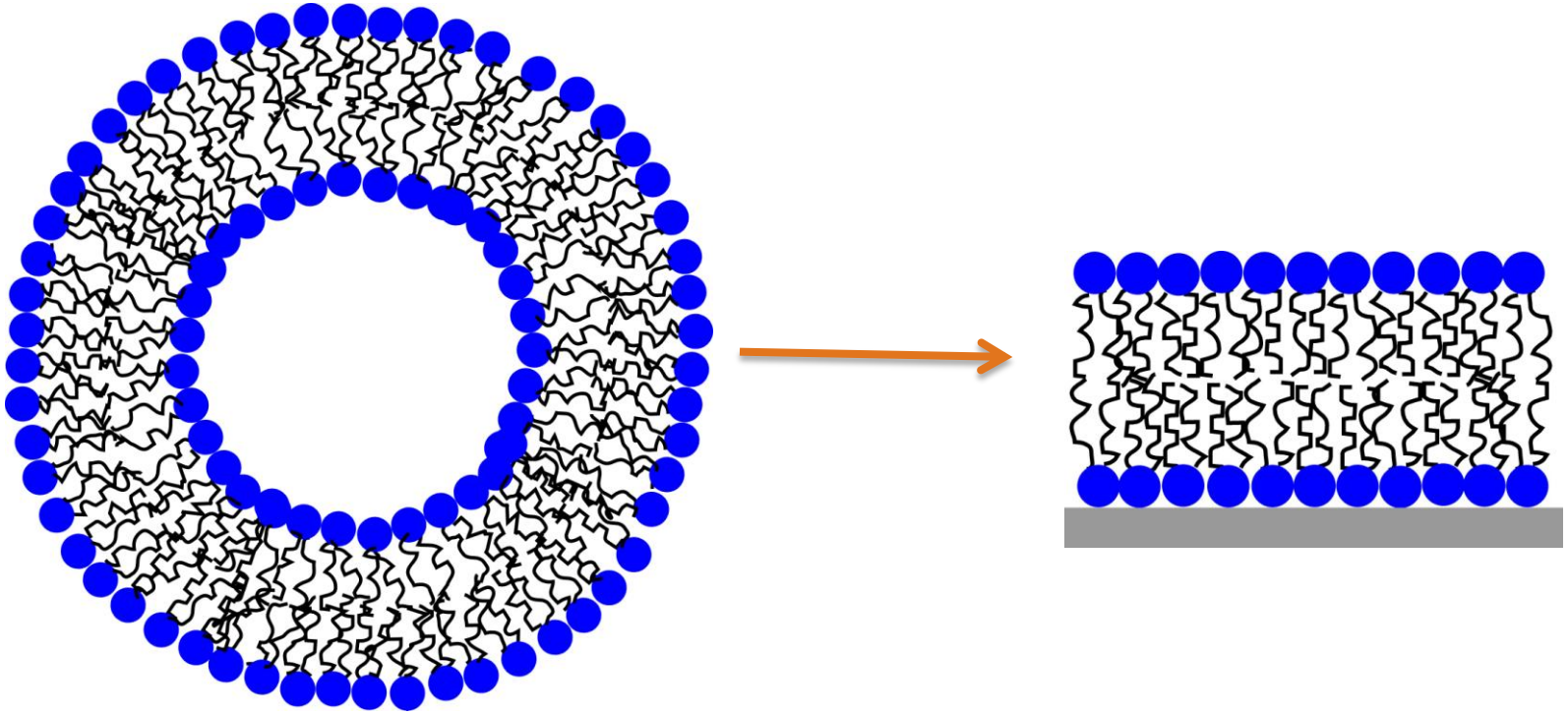


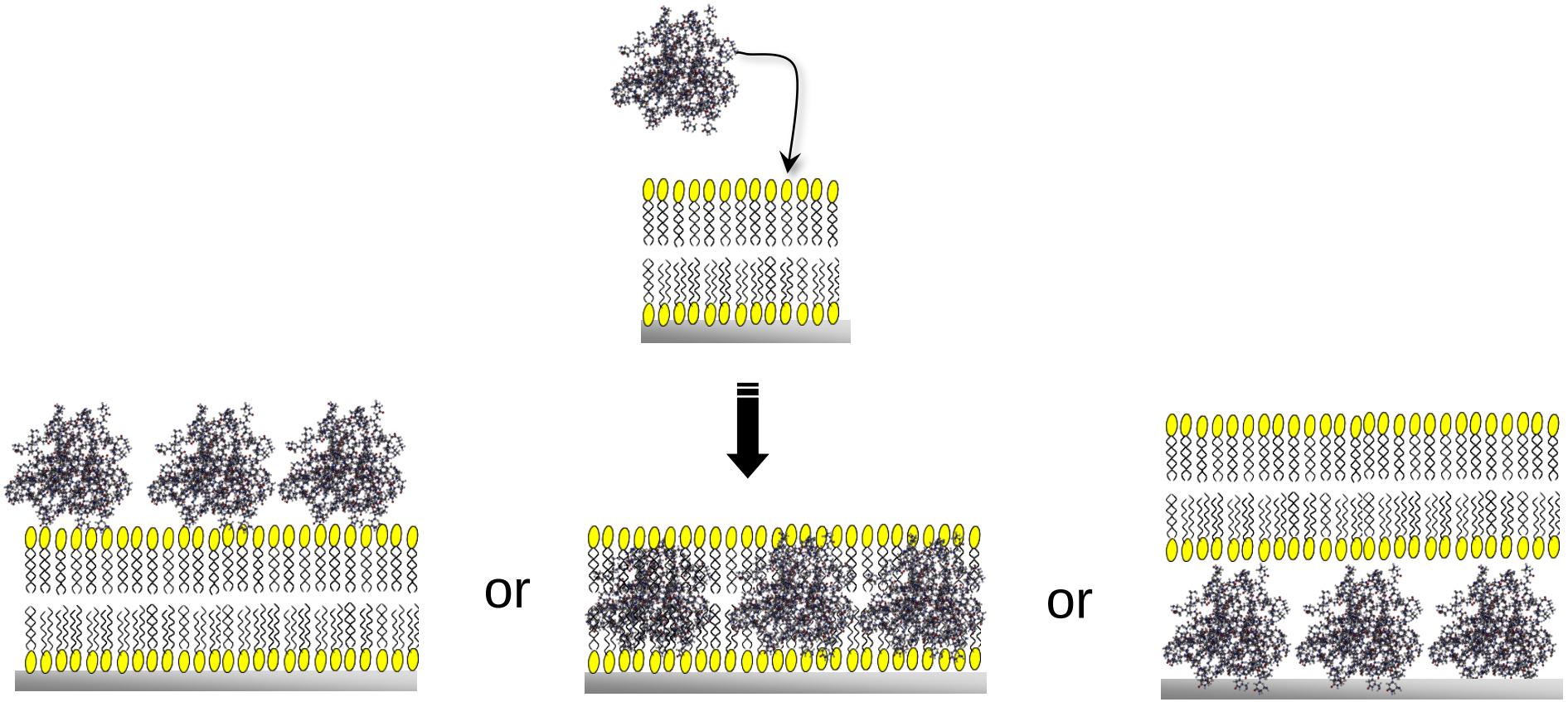
Ainalem et. al. Soft matter, **2009, 5, 2310 - 2320**.

MODEL MEMBRANE:

Bilayer formed from dPOPC by vesicle fusion on a (negatively charged) silicon surface and gave ≈ 95% coverage



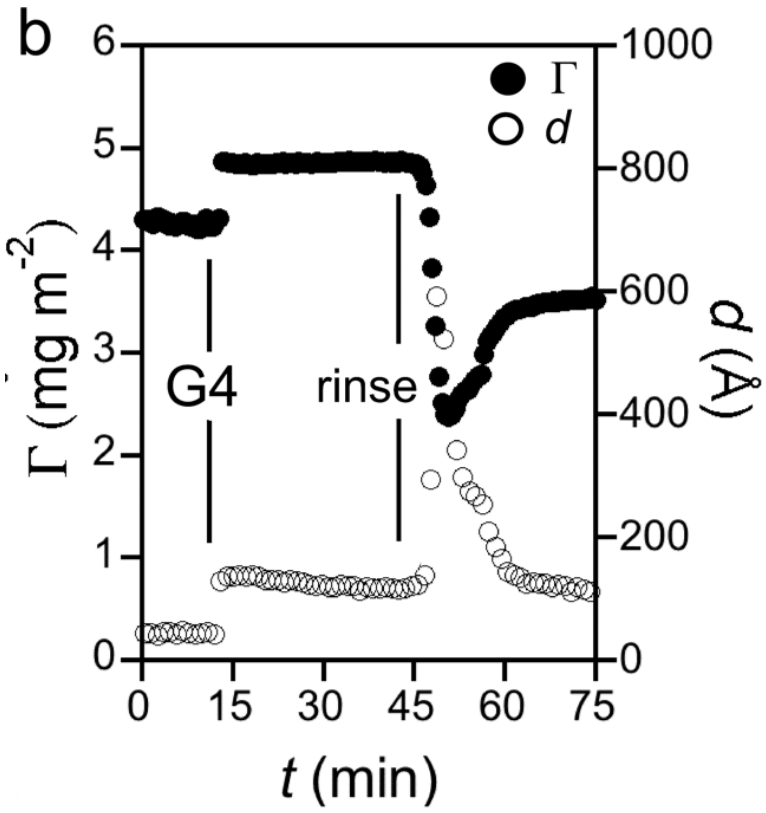
What happens when dendrimers interact with a model cell membrane?



Ainalem, M.-L.; Campbell, R.; Khalid, S.; Gillams, R.; Rennie, A.; Nylander, T. On the Ability of PAMAM Dendrimers and Dendrimer/DNA Aggregates to Penetrate POPC Model Biomembranes. J. Phys. Chem. B. 2010, 114, 7229-7244.

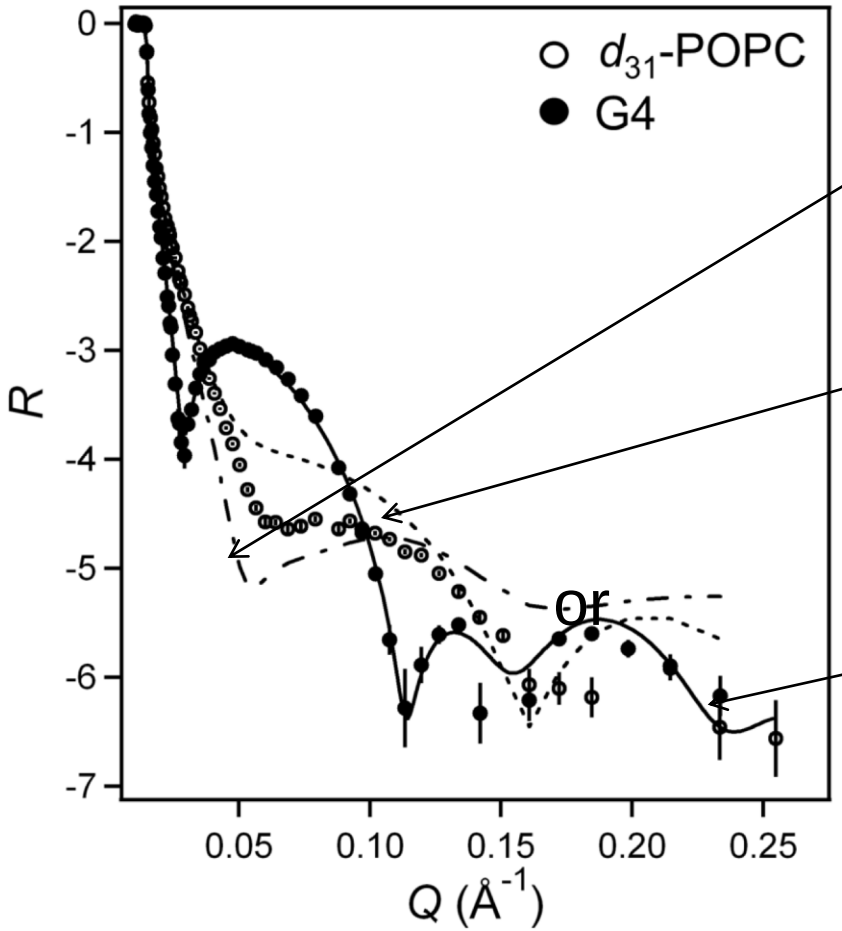
Ellipsometry give change in adsorbed amount but not location

- Increase in thickness and adsorbed amount when adding dendrimers (PAMAM-G4) to lipid (POPC) bilayer



What happens when dendrimers interact with a model cell membrane?

Neutron reflectometry in D_2O



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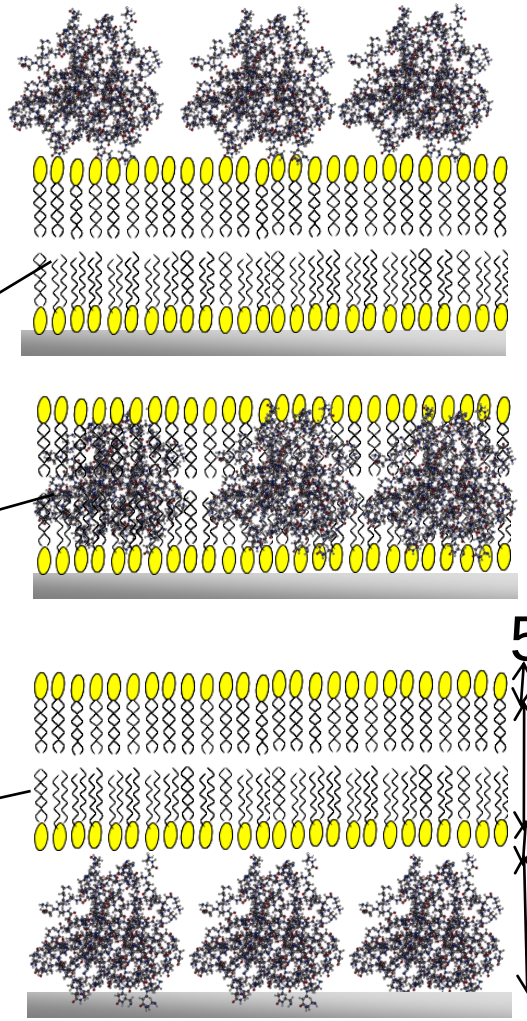


5.5 Å, 19% D_2O

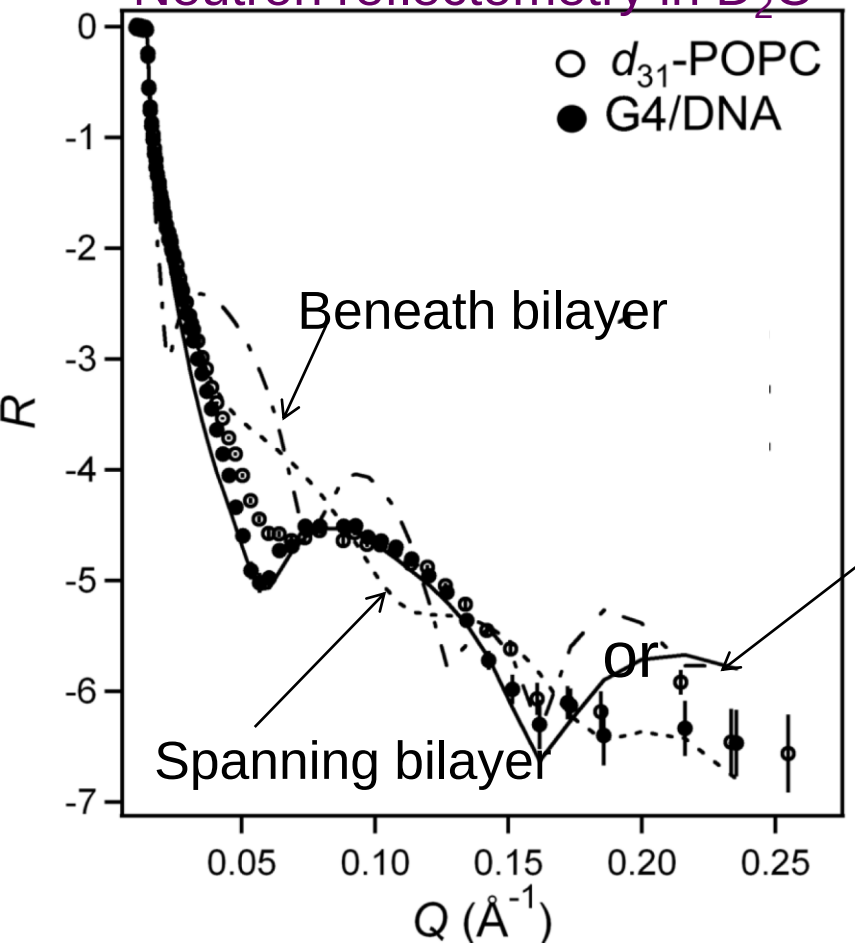
28 Å, 8.4% D_2O

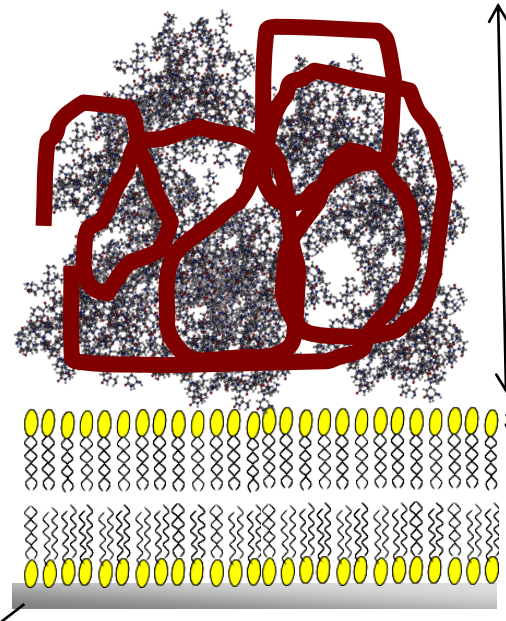
44 Å, 85% D_2O

Only possible fit with dendrimer under bilayer

What happens when DNA/dendrimer complex interact with a model cell membrane?

Neutron reflectometry in D_2O





430 Å, 77% D_2O

5.5 Å, 19% D_2O

28 Å, 8.4% D_2O

- No penetration of the bilayer by G4/DNA complex
- G4/DNA complex sits on top of bilayer

Conclusions

Dendrimer interaction with model membranes

- G2 and G4 penetrates bilayers, but G6 partly destroys bilayer. Data supporting transfecting ability.

Dendrimer/DNA complex interaction with model membranes

- Complex of G4 and G6 do not penetrate bilayers and G6 do not.
- Increasing ionic strength can release (G2) dendrimers form complex which penetrate bilayer

Does the efficiency of dendrimers as transfection agent rest on their ability to penetrate the membrane?

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