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Reflectometry in Soft Matter – Some Examples

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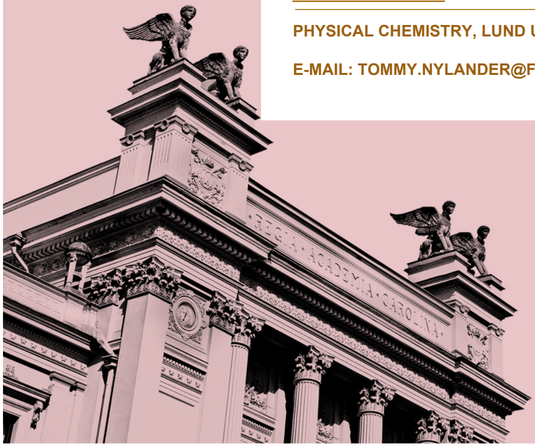

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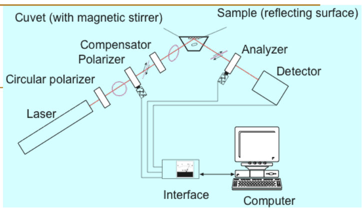
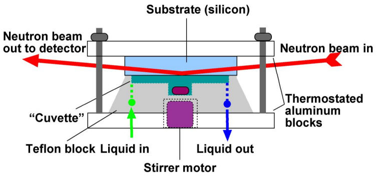
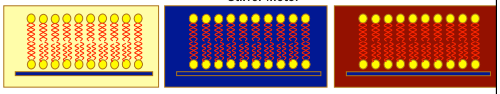
Comparison of surface techniques used

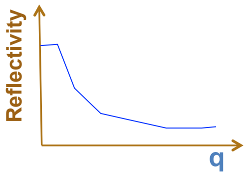
- Ellipsometry gives “optical” thickness and “dry” mass versus time

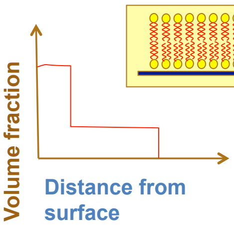


- Neutron Reflectometry gives density profile of the interfacial layer and selective deuteration + contrast matching gives composition



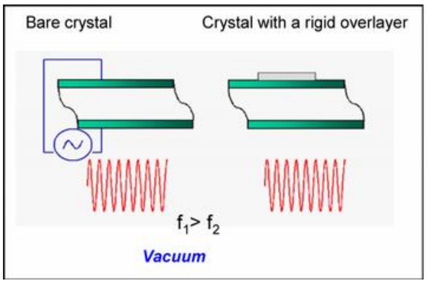


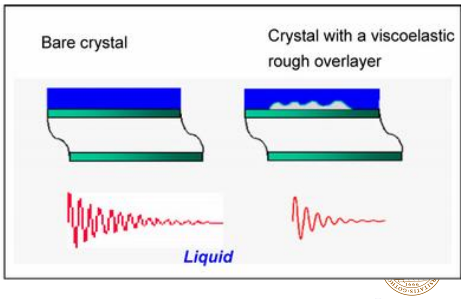




Comparison of surface techniques used II

- QCM-D gives “wet” mass with coupled water and dissipation measures the viscoelastic properties of the film



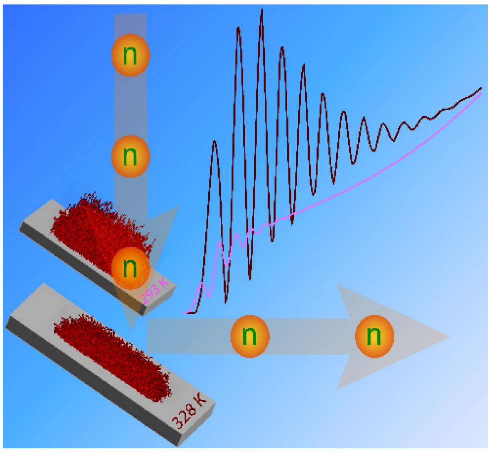


The Goal of Neutron Reflectivity Measurements Is to Infer a Density Profile Perpendicular to a Flat Interface

- In general the results are not unique, but independent knowledge of the system often makes them very reliable
- Frequently, layer models are used to fit the data
- Advantages of neutrons include:
    - Contrast variation (using H and D, for example)
    - Low absorption – probe buried interfaces, solid/liquid interfaces etc
    - Non-destructive
    - Sensitive to magnetism
    - Thickness length scale 10 – 5000 Å

Example scattering from polymer brushes

- Evaluation using box model and Lattice mean-field theory

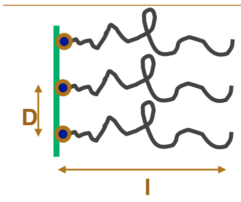


Poly(N-isopropylacrylamide) (pNIPAAM)

- Hydrophilic (water soluble) at low temperature
- Hydrophobic (water insoluble) at high temperature
- Sharp transition at ≈30 ^{o}C

- pNIPAAM brushes have potential use in:
  - Protein affinity separation
  - Sensing applications
  - Mirco- and nanofluid devices

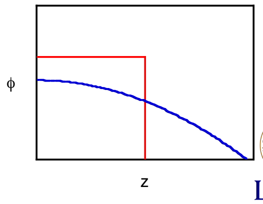
Polymer brushes



D: distance between neighboring chain
l: brush height
$D << 2R_G$ for a brush
• brush properties are only mildly dependent on surface adsorption and solvency

Brush height depends on two competing factors:
1) stretching entropy which strives to decrease the height
2) excluded volume interactions, which favor an increased height

Two simple analytic theories for non-adsorbing surface and good solvent:
1) block segment density profile (Alexander 1977)
2) parabolic segment density profile (Milner, Witten, and Cates (MWC)1988; Zhulina, Priamitsyn, and Borisov (ZPB) 1988)



Modeling of the polymer brushes

- establish model parameters to describing the interactions in an aqueous polymer solution from fitting to experimentally determined phase behavior
- predict polymer brush density profiles using molecular-based lattice mean-field theory, developed by Scheutjens and Fleer, and Gunnar Karlström

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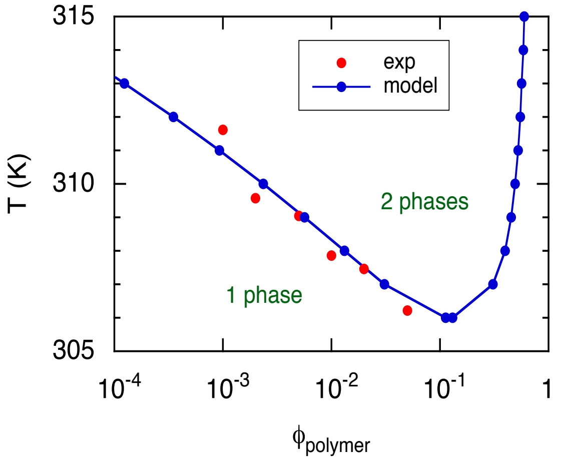
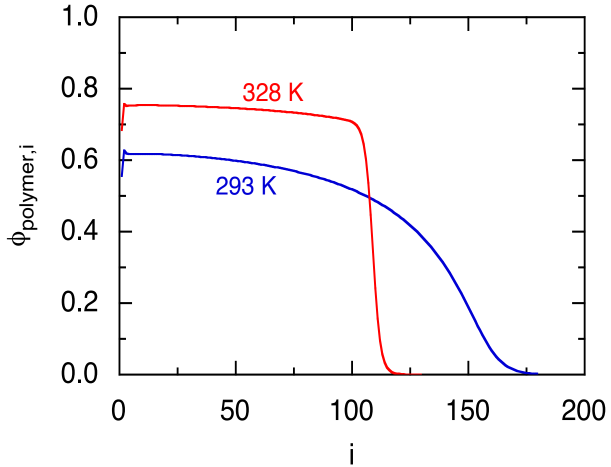
Fit of parameters characterizing the polymer in bulk solution used for modelling polymer brush



Fit of parameters describing interaction and internal degrees of freedom

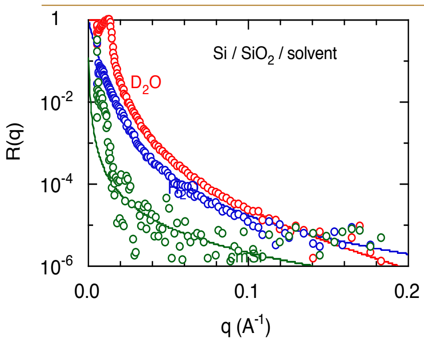
Predicted polymer volume fraction profiles from modelling



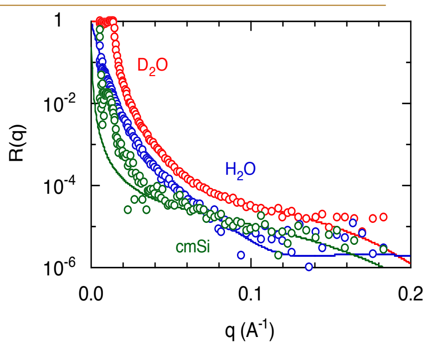
A large structural change at increasing temperature is predicted

First: Surface characterization

Si / SiO$_2$ / solvent



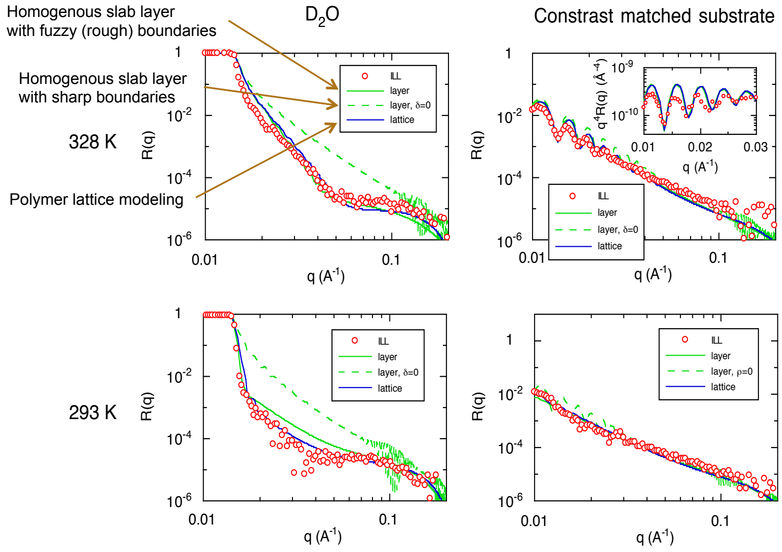
Si / SiO$_2$ / init layer / solvent



layer	ϕ_i	l_i (Å)	δ_i (Å)
Si	1	semi-infinite	0
SiO$_2$	1	**9 ± 2**	0

layer	ϕ_i	l_i (Å)	δ_i (Å)
Si	1	semi-infinite	0
SiO$_2$	1	9	0
Initiator	**0.75 ± 0.05**	**30 ± 3**	0

Grafted polymer brushes on the surfaces: NR data and models

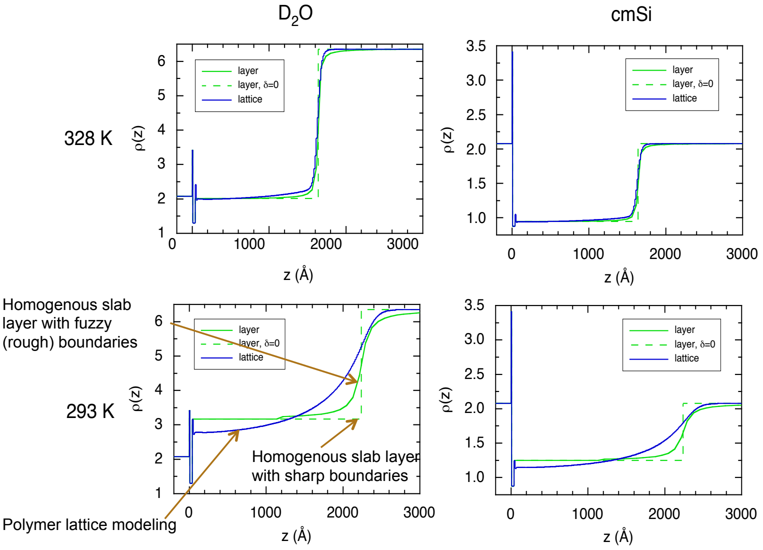


Homogenous slab layer with fuzzy (rough) boundaries

Homogenous slab layer with sharp boundaries

Polymer lattice modeling

Scattering length density profiles obtained by fitting different models to the reflectivity data



Homogenous slab layer with fuzzy (rough) boundaries

Homogenous slab layer with sharp boundaries

Polymer lattice modeling

Parameters

Common to both models

1 fitted parameter

layer	*Volume Fraction, ϕ_i*	*Thickness l_i (Å)*	*Roughness δ_i (Å)*
Si	1	semi-infinite	0
SiO_2	1	9	0
initiator	**0.85 ± 0.05**	30	0

Layer model

5 fitted parameters

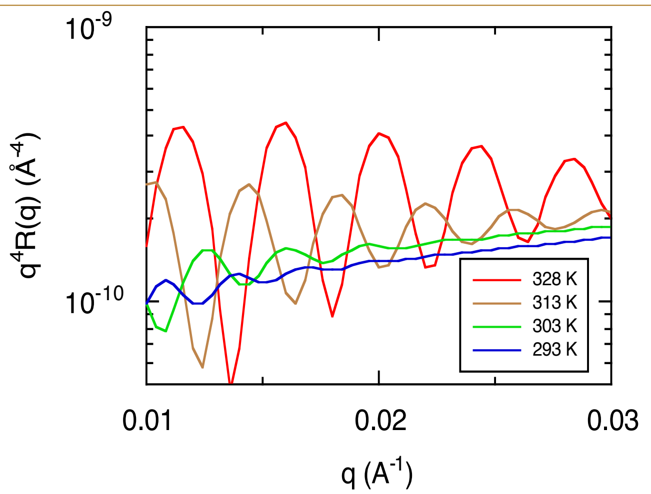
layer	*Volume Fraction, ϕ_i*	*Thickness l_i (Å)*	*Roughness δ_i (Å)*
polymer (328 K)	**0.75 ± 0.05**	**1600 ± 50**	**50 ± 5**
polymer (293 K)	**0.55 ± 0.05**	**2200 ± 50**	**250**

Lattice model

2 fitted parameters

surface interaction	$kT\chi_{\text{surface,water}} = kT\chi_{\text{surface,polymer(polar)}} = 3$ kJ mol^{-1} $kT\chi_{\text{surface,polymer(nonpolar)}} = 0$ kJ mol^{-1}
number of segments	$r_{\text{polymer}} = 1000$
grafting density	σ = **0.08 ± 0.005**
length of lattice site	d = **14.7 ± 0.3** Å

Predicted reflectivity profiles in cmSi



Some comments

- Layer profile
  - Fitting parameters
    - » Scattering length density
    - » Thickness
    - » Roughness
  - Nonzero roughness in the reflectivity model is important
- Lattice mean-field theory
  - Fitting parameters (global)
    - » Length scale
    - » Grafting density (here experimentally unknown)
  - Extensions
    - » Polyelectrolytes (e.g., polyacrylamide), Block copolymers, Adsorbed polymers

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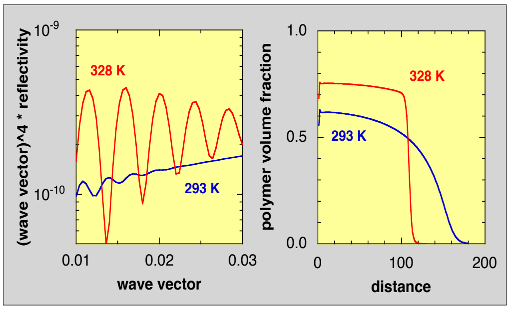
NR of polymer responsive polymer brushes from polymer theory and fitting to experimental neutron reflectivity data on NIPAAM-containing polymers



Calculated neutron reflectivity data for polymers grafted onto a solid surface at different temperatures

Deduced polymer brush volume fraction profiles, illustrating the collapse of the polymer brush at the elevated temperature.

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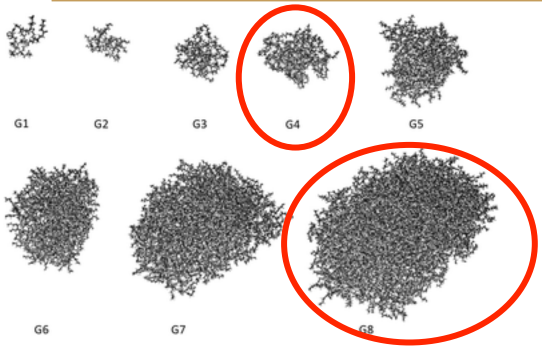
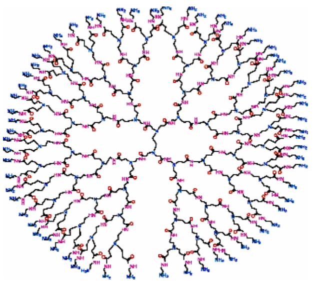
NEUTRON REFLECTOMETRY CAN BE USED TO DETERMINE LAYER COMPOSTION

Interactions of anionic surfactant SDS and cationic PAMAM dendrimers at silica-liquid interface





G4 PAMAM Dendrimer

Poly(amidoamine), PAMAM dendrimer
“Hydrophobic” core and cationic shell

Sodium dodecylsulphate
Anionic surfactant

Aim of the study

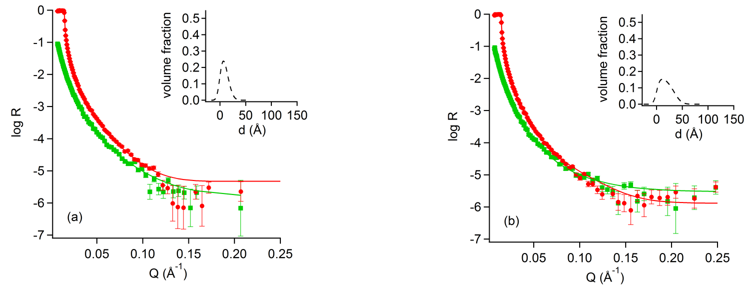
- Provide fundamental understanding of the interactions at surfaces of well-defined hyperbranched polymers and oppositely charged surfactants
- Particular emphasis on non-equilibrium aspects
- Findings relevant for delivery of surface active materials used in consumer products and pharmaceuticals and building of nanostructured materials.

Interactions of PAMAM dendrimers with SDS at the solid-liquid interface

Generation	Theoretical Molecular Weight g mol^{-1}	Hydrodynamic Radius Å	Surface Cation Groups	Density g cm^{-3}	Molecular Volume Å^3
4	14215	24.5	64	1.224	19290
8	233383	66.3	1024	1.231	314900

Neutron reflectivity profiles for PAMAM G4 and G8 dendrimers onto silica

D_2O (●) and H_2O (■)

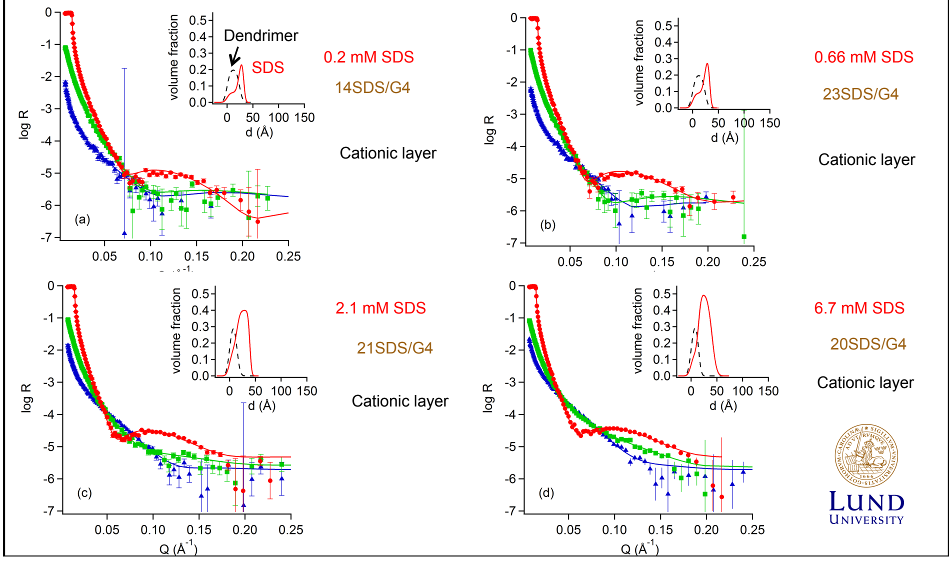


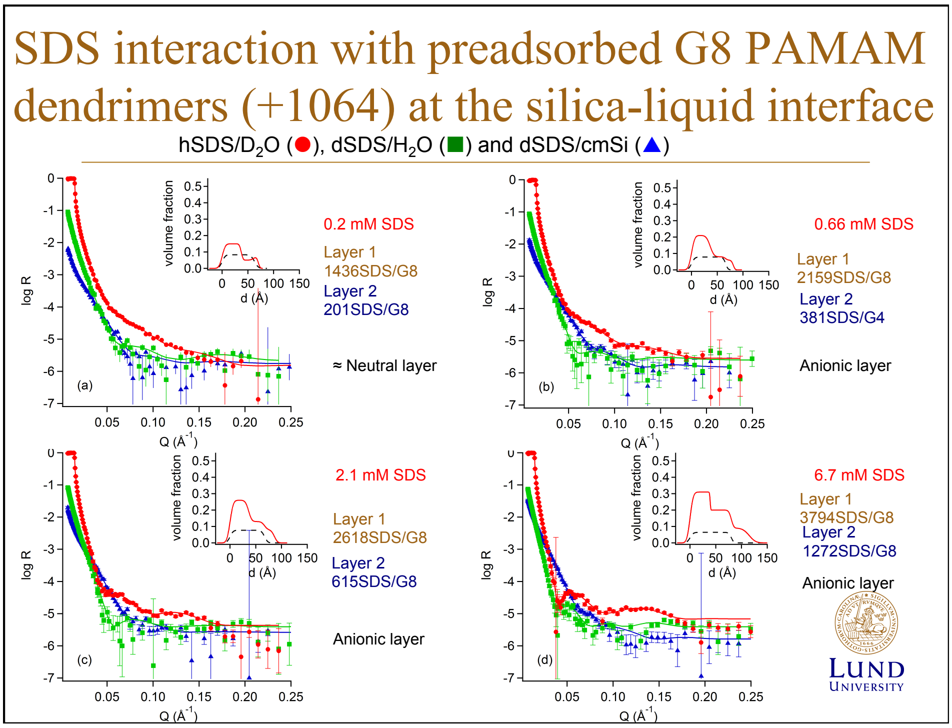
PAMAM G4 dendrimers
1.5 ± 0.1 mg/m^2
13.5±0.1 Å
65% solvent in Layer

PAMAM G8 dendrimers
3.4 ± 0.6 mg/m^2
33±6 Å
84% solvent in Layer

SDS interaction with preadsorbed G4 PAMAM dendrimers (+64) at the silica-liquid interface

hSDS/D_2O (●), dSDS/H_2O (■) and dSDS/cmSi (▲)





SDS interaction with preadsorbed G8 PAMAM dendrimers (+1064) at the silica-liquid interface

hSDS/D$_2$O (●), dSDS/H$_2$O (🟩) and dSDS/cmSi (▲)

0.2 mM SDS

Layer 1
1436SDS/G8
Layer 2
201SDS/G8

≈ Neutral layer

0.66 mM SDS

Layer 1
2159SDS/G8
Layer 2
381SDS/G4

Anionic layer

2.1 mM SDS

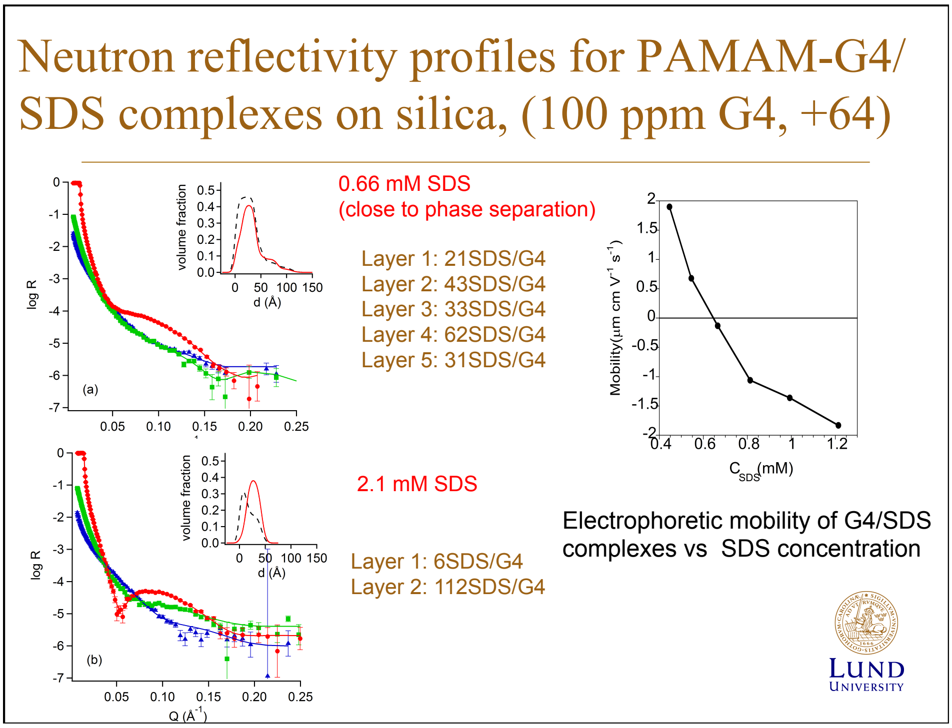
Layer 1
2618SDS/G8
Layer 2
615SDS/G8

Anionic layer

6.7 mM SDS

Layer 1
3794SDS/G8
Layer 2
1272SDS/G8

Anionic layer



Neutron reflectivity profiles for PAMAM-G4/ SDS complexes on silica, (100 ppm G4, +64)

0.66 mM SDS
(close to phase separation)

Layer 1: 21SDS/G4
Layer 2: 43SDS/G4
Layer 3: 33SDS/G4
Layer 4: 62SDS/G4
Layer 5: 31SDS/G4

2.1 mM SDS

Layer 1: 6SDS/G4
Layer 2: 112SDS/G4

Electrophoretic mobility of G4/SDS complexes vs SDS concentration

Mode of surfactant binding controls PAMAM dendrimer/SDS layer structure

- Low SDS concentrations:
  - monomeric surfactant binding due to electrostatic interactions and hydrophobic interactions => expansion of the polymer layer.
- High SDS concentration
  - cooperative surfactant binding => formation of surfactant aggregates or a bilayer-like structure on the polymer layer

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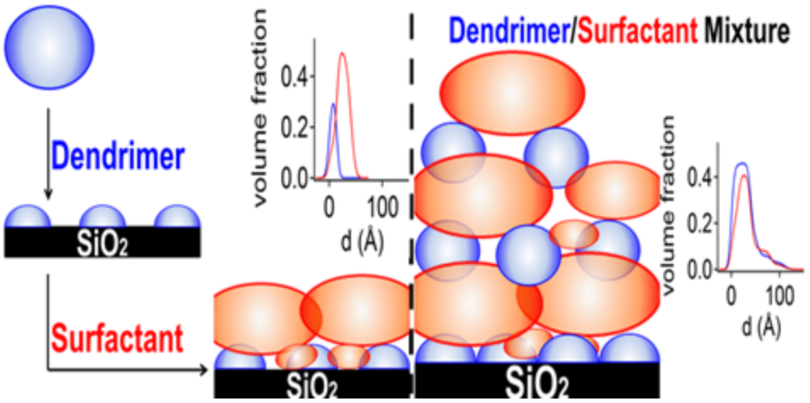
PAMAM dendrimer/SDS at the solid-liquid interface:



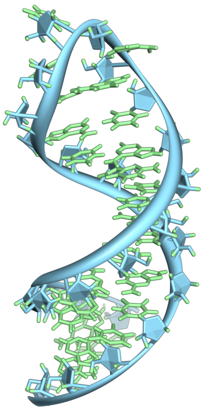
The pathway of adsorption from dendrimer/SDS mixtures determines the layer structure and composition.

NEUTRON REFLECTOMETRY TO QUANTIFY INTERACTIONS AT LIPID MONOLAYERS

Knowledge of the interaction between nucleic acids and phospholipids is important for

- drug delivery
- therapeutics
- synthetic biology
- fundamental understanding of biological mechanisms.

WHY STUDY SHORT RNA OLIGOMERS (miRNA)?



- Important for regulatory properties in the cell, such as cell death, cell proliferation and for the initiation of several types of cancer.
- There are significant amount of phospholipids in the cell nucleus and e.g. RNA in the nucleus is co-localize with these lipids.
- Important for drug delivery, therapeutics, synthetic biology and fundamental understanding of biological mechanisms.

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OBJECTIVE: REVEAL MECHANISM AND NATURE OF INTERACTION BETWEEN NUCLEIC ACIDS AND BIOMEMBRANES

- We have compared interaction of single stranded RNA (10 bases) and double stranded DNA (2000 base pairs)
  - with monolayers (Π= 8 and 28 mN/m) of
    - »zwitterionic DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine)
    - »cationic DODAB (dioctadecyl-dimethyl-ammoniumbromide)
- using surface film balance, fluroscence microscopy and neutron reflectometry.

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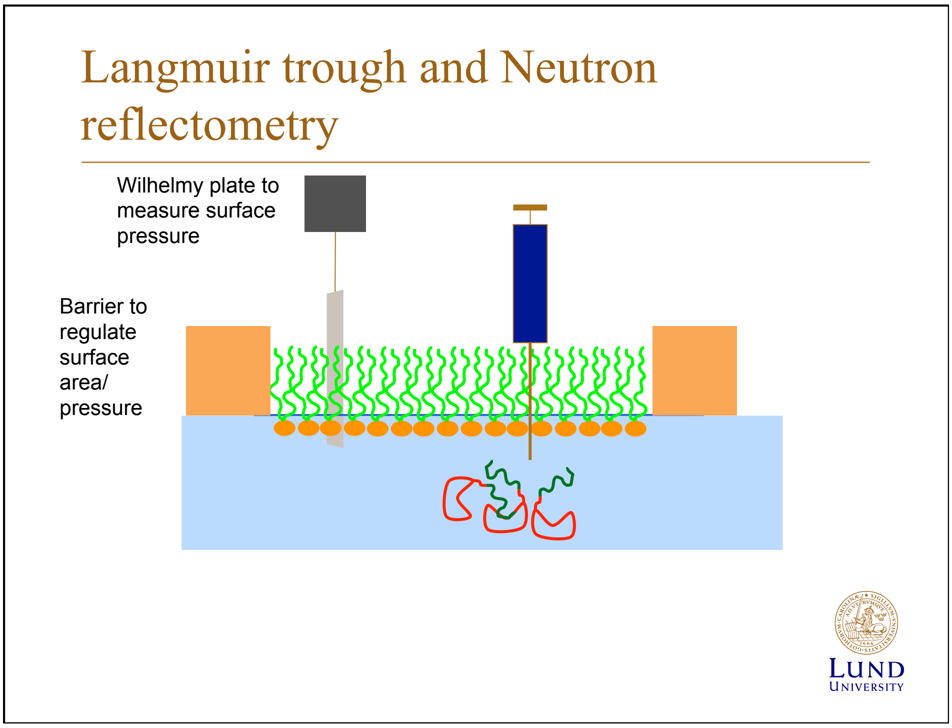
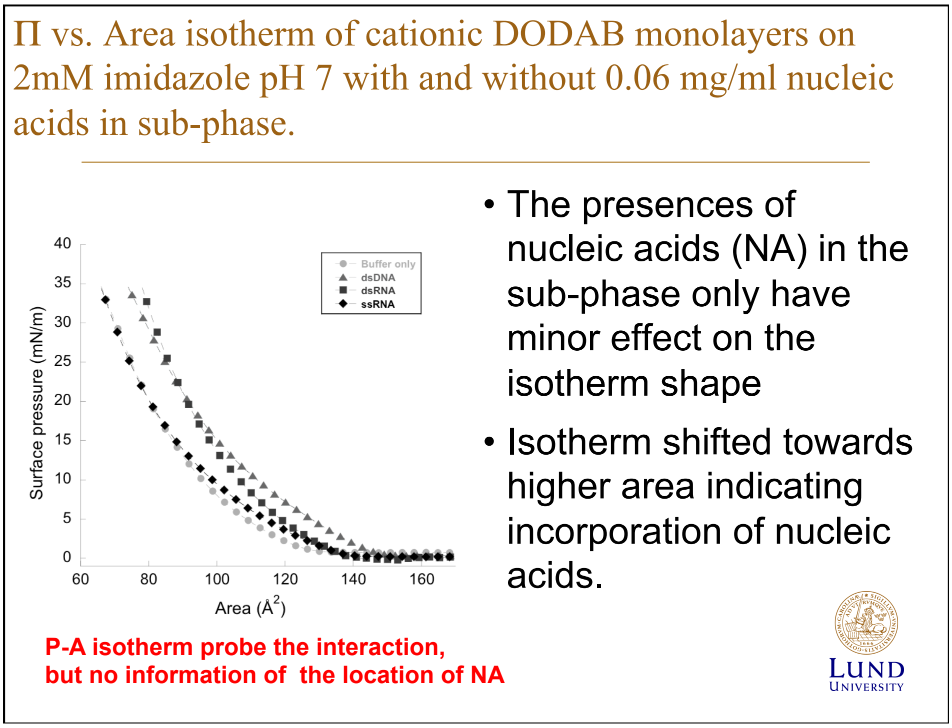


Langmuir trough and Neutron reflectometry

Wilhelmy plate to measure surface pressure

Barrier to regulate surface area/ pressure



Π vs. Area isotherm of cationic DODAB monolayers on 2mM imidazole pH 7 with and without 0.06 mg/ml nucleic acids in sub-phase.

- The presences of nucleic acids (NA) in the sub-phase only have minor effect on the isotherm shape
- Isotherm shifted towards higher area indicating incorporation of nucleic acids.

P-A isotherm probe the interaction, but no information of the location of NA

Neutron reflectometry can provide information on the location of nuclei acids on a lipid monolayer

Michanek, A.; Yanez, M.; Hughes, A.; Wacklin, H.; Nylander, T.; Sparr, E. "RNA and DNA association to zwitterionic and charged monolayers at the air-liquid interface" Langmuir, 2012, 28, 9621–9633.

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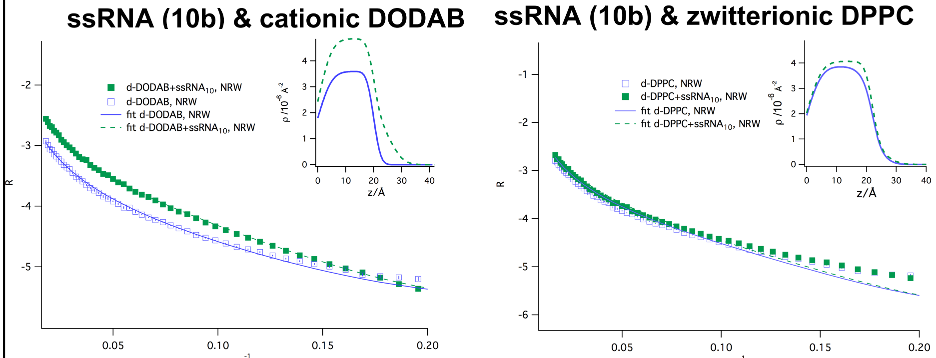
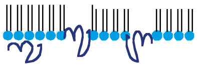
Reflectivity profiles for DODAB & DPPC monolayer on 2mM imidazole, pH 7, with and without 0.06 mg/ml ssRNA

Low surface pressure, Π=8.8 mN/m


ssRNA (10b) & cationic DODAB

ssRNA (10b) & zwitterionic DPPC



ssRNA (10 bases) penetrate DODAB and DPPC at low Π
(Note fits based on several contrasts)

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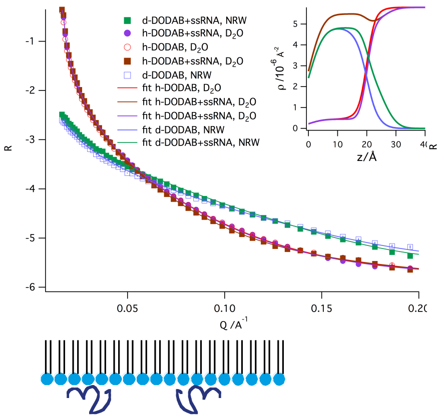
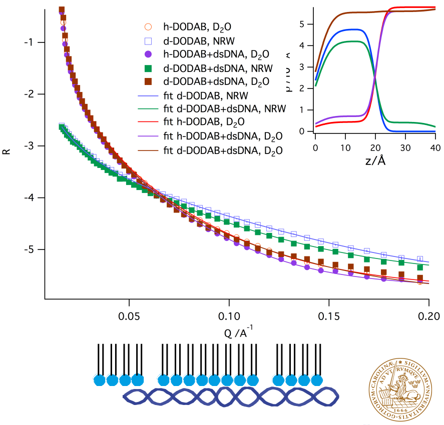
Reflectivity profiles for DODAB monolayer on 2mM imidazole, pH 7, with and without 0.06 mg/ml ssRNA or ds DNA

High surface pressure, Π=28 mN/m

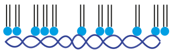
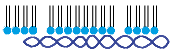
ssRNA (10b)



dsDNA (2000bp)



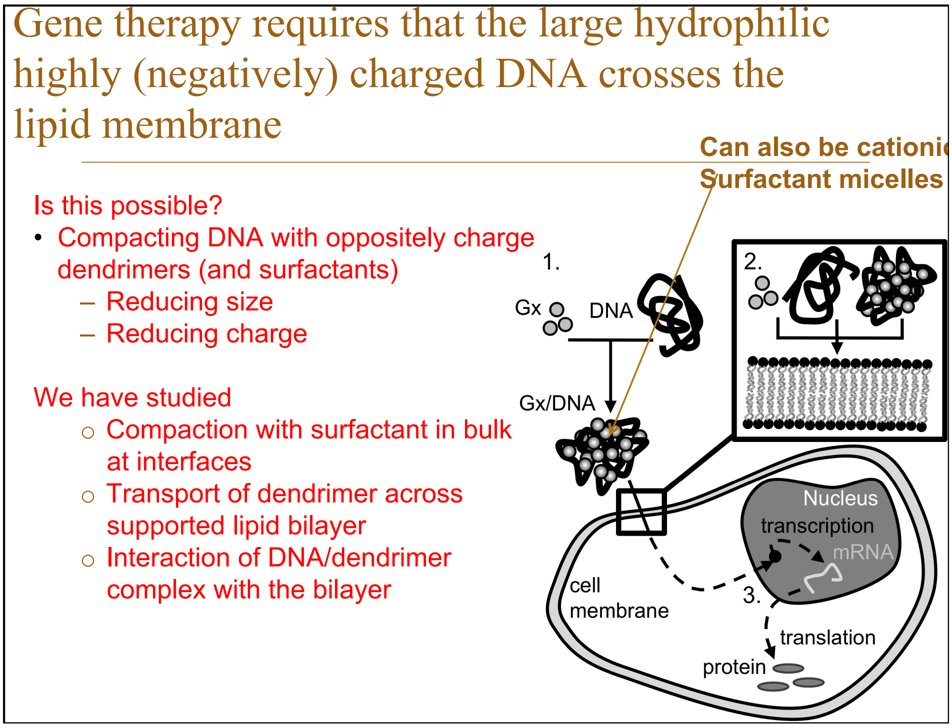
Cationic/ Zwitterionic monolayer

	ssRNA	dsDNA
Low Π	 Penetration/headgroup *Hydrophobic/ Electrostatic & polar*	 Headgroup *Mainly electrostatic & polar*
High Π	 Headgroup *Electrostatic & polar* *weak with zwitterionic*	 Headgroup *Electrostatic & polar* *No adsorption on zwitterionic*

- Hydrophobic interaction plays role in the interaction between nucleic acid and model lipid membranes =>

The state of lipid affect interaction of nucleic acid with membrane

GENE DELIVERY - NEUTRON REFLECTOMETRY CAN PROVE MEMBRANE PENETRATION

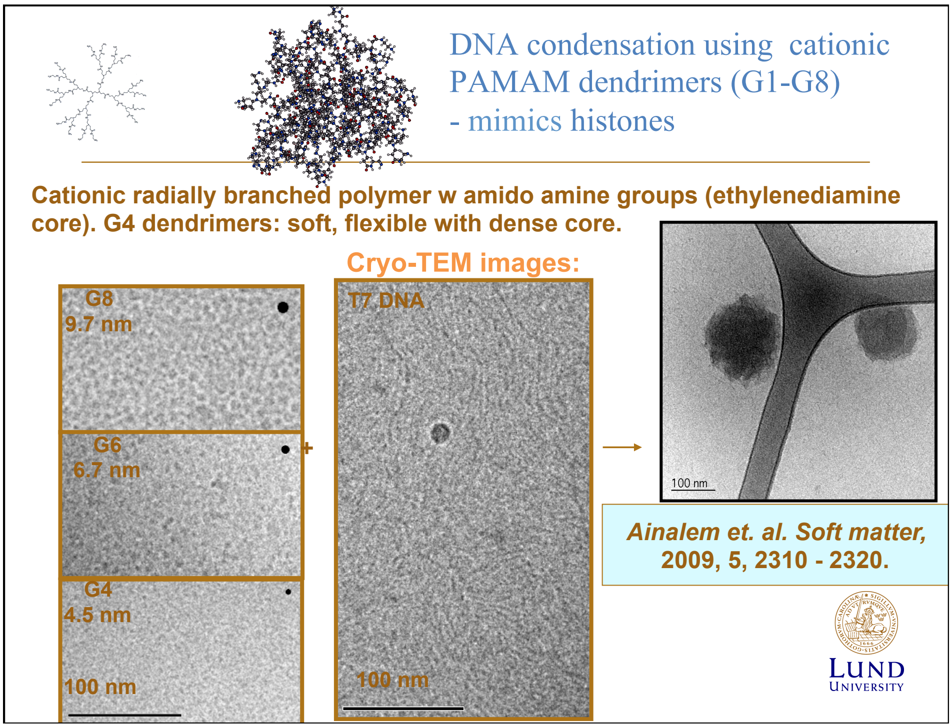


Gene therapy requires that the large hydrophilic highly (negatively) charged DNA crosses the lipid membrane

Can also be cationi
Surfactant micelles

Is this possible?
- Compacting DNA with oppositely charge dendrimers (and surfactants)
  - Reducing size
  - Reducing charge

We have studied
  - Compaction with surfactant in bulk at interfaces
  - Transport of dendrimer across supported lipid bilayer
  - Interaction of DNA/dendrimer complex with the bilayer



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:

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

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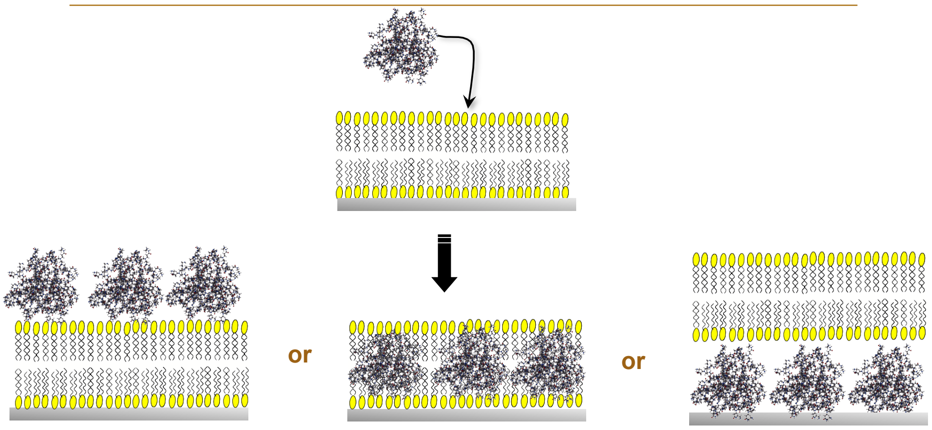
What happens when dendrimers interact with a model cell membrane?



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.

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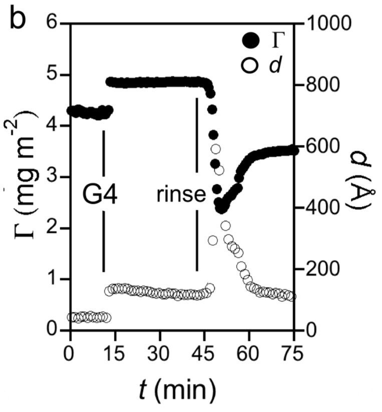
Ellipsometry give change in adsorbed amount but not location

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



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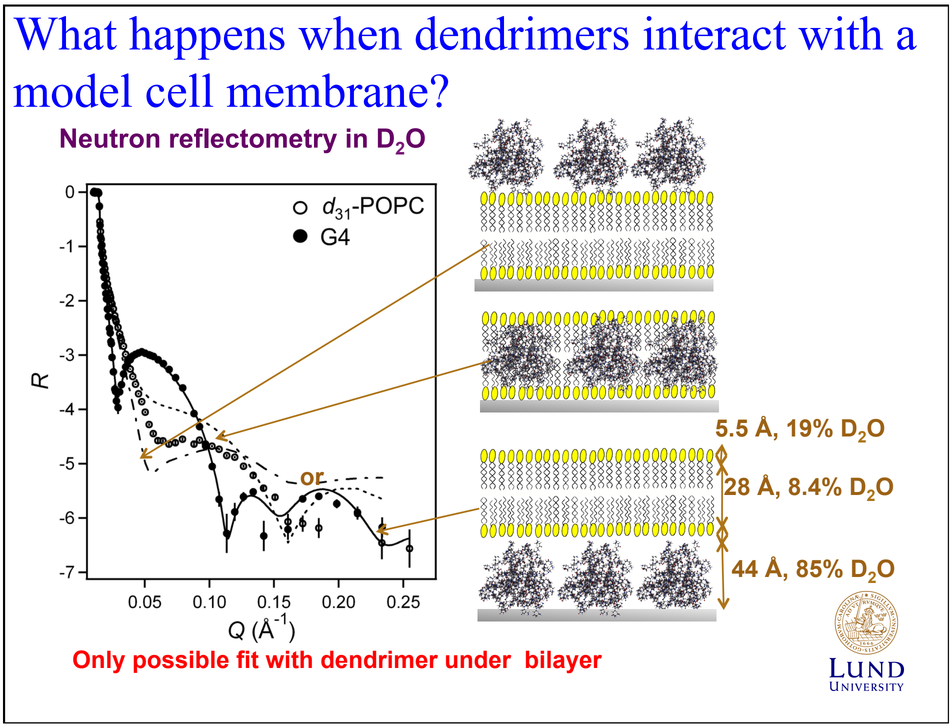

What happens when dendrimers interact with a model cell membrane?

Neutron reflectometry in D_2O

Only possible fit with dendrimer under bilayer

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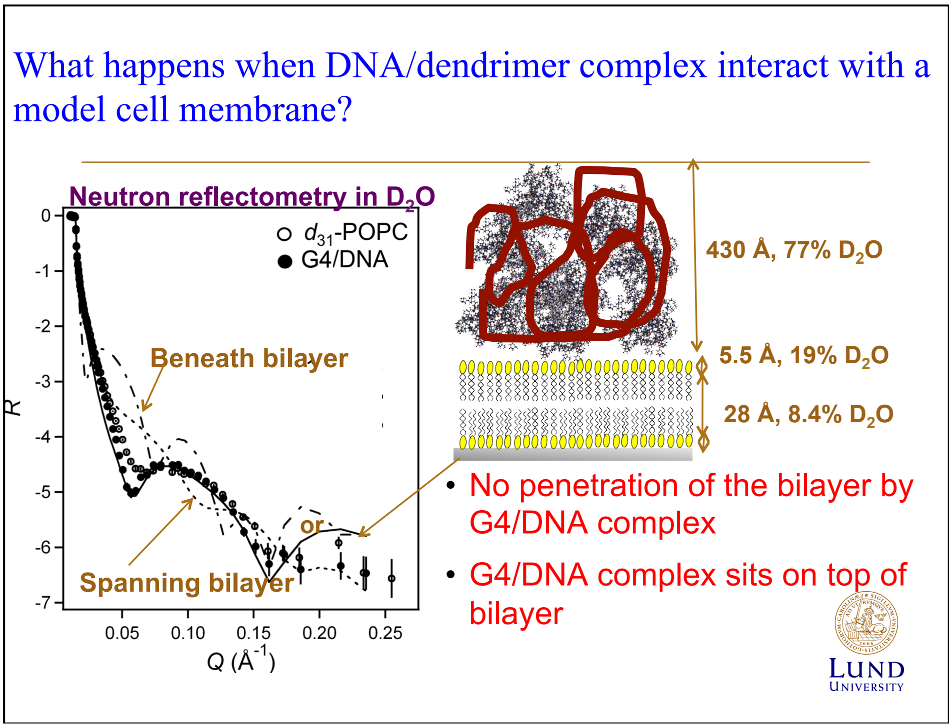

What happens when DNA/dendrimer complex interact with a model cell membrane?

Neutron reflectometry in D_2O

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

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Conclusions

Dendrimer interaction with model membranes

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

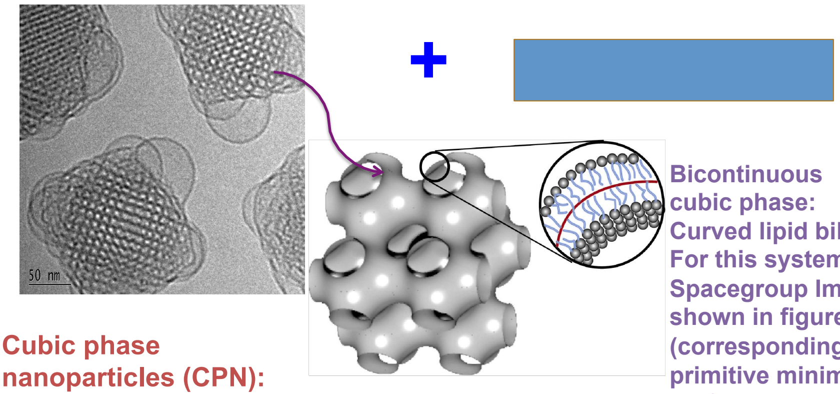
Dendrimer/DNA complex interaction with model membranes

- Complex of G4 and G6 do not penetrate bilayers and G6 do not.

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

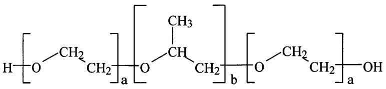
ADSORPTION OF NANO-STRUCTURED PARTICLES - NEUTRON REFLECTOMETRY BRAGG PEAK CAN PROVE SURFACE STRUCTURE

What happens when such a non lamellar liquid crystalline nano-particle meets a flat interface ?



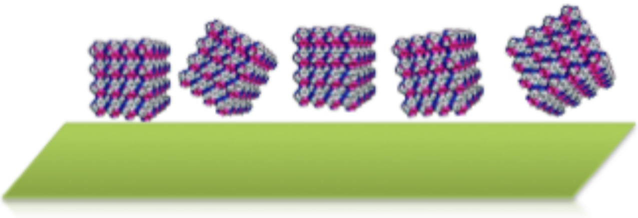
Cubic phase nanoparticles (CPN): Glycerol monoolein (GMO) based dispersions (Cubosome®) stabilised by Pluronics F127

Bicontinuous cubic phase: Curved lipid bilayer For this system Spacegroup Im3m shown in figure (corresponding to a primitive minimal surface) is observed

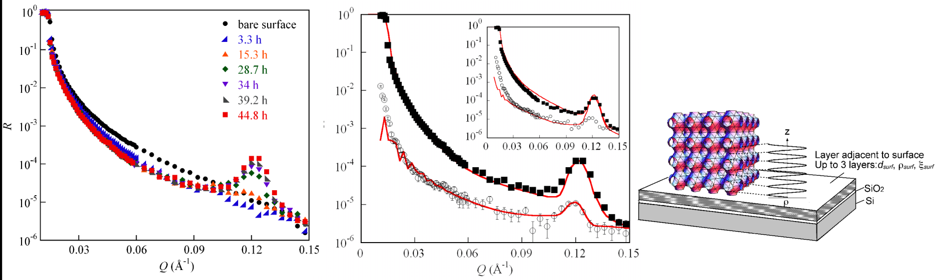


$H{-}\left[O{-}CH_2{-}CH_2\right]_a{-}\left[O{-}CH(CH_3){-}CH_2\right]_b{-}\left[O{-}CH_2{-}CH_2\right]_a{-}OH$

Adsorption on silica surface-simplest model of a biological hydrophilic surface



CPNP layer structure on silica from neutron reflectivity



- Single layer adjacent to the surface + repeating structure
- SLD following a sinusoidal function versus *z* with period (52Å) consistent with unit cell dimensions 135-140 Å with the minimum expected periodicity for the structure of around 46 Å for perfectly aligned crystals.
- The number of repeating units $\mathbf{N}_{\mathrm{rep}}$ = 30 for the cubic structure correspond to total layer thicknesses of 1500 Å, which is close to the size of the particles.

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