Experimental report

09/02/2026

Proposal: 9-12-696 **Council:** 4/2023

Title: Assessing how hydrophilic quantum dots alter the organization of charged lipid bilayers

Research area: Soft condensed matter

This proposal is a resubmission of 9-12-670

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Samples: SiO2
DOTAP
DOPC
DMPG
CdTe

Instrument	Requested days	Allocated days	From	To
FIGARO	0	0		
D17	3	3	23/09/2023	26/09/2023
D22	1	0		

Abstract:

Quantum dots (QDs) are semiconductor nanoparticles with unique optical and electronic properties, with increasing interest as potential nano-theranostic platforms for imaging and sensing. The design and use of QDs requires the understanding of cell-nanoparticle interactions at the molecular level, which depends on surface charge of both membranes and the nanomaterials, and plays a important role in early-stage nanoparticle uptake driven by long-range electrostatic interactions.
Conflicting results on how surface charge affects the efficiency of nanoparticles membrane disruption and cellular uptake have been reported due to the complexity of the cell constituents and environment used in nanoparticle-cell interactions experiments. Motivated by this controversy, we propose the use of neutron reflectometry and small angle neutron scattering to study the interactions between charged artificial lipid bilayers and QDs. Combining well-controlled lipid bilayer model systems with robust time-resolved structural characterization techniques we aim at dissecting the role of charge on nanoparticle-lipid bilayer interactions and assessing the probability of insertion and bilayer disruption.

Experimental Report for 9-12-696

Instrument: D17
Title: Assessing how hydrophilic quantum dots alter the organization of charged lipid bilayers

1. Scientific Objective

The aim of experiment was to determine, with nanometre resolution, how **hydrophilic, negatively charged CdTe quantum dots (QDs)** interact with **supported lipid bilayers (SLBs)** of different surface charge, and to localize the QDs relative to the membrane structure. Neutron reflectometry (NR) was employed to (i) quantify QD adsorption, (ii) resolve their vertical location with respect to the bilayer, and (iii) assess charge-dependent structural perturbations of the membrane, such as hydration and defect formation.

2. Samples

Planar supported lipid bilayers were prepared on polished silicon (111) substrates by vesicle fusion. Silicon substrates were cleaned by solvent sonication followed by plasma treatment and mounted in solid–liquid flow cells allowing controlled liquid exchange and temperature regulation. Three lipid compositions were investigated:

- **DOPC** (zwitterionic)
- **DOTAP** (positively charged)
- **DOPC:DOTAP** (1:1 molar ratio)

All SLBs were formed in TRIS buffer (10 mM Tris, 150 mM KCl, pH 8). Hydrophilic CdTe QDs (core diameter 2.6 nm, –COOH termination) were dispersed in the same buffer at 0.2 mg/mL. At pH 8 the QDs are negatively charged.

3. Neutron Reflectometry Measurements

NR experiments were performed in **time-of-flight mode on D17**. The incident wavelength band ranged from 2 Å to 10 Å. Two incident angles were used: 0.8° and 3.0°.

Each sample was measured under **three isotopic contrast conditions** such as 100% D_2O buffer, 100% H_2O buffer and silicon-matched buffer (68:32 H_2O:D_2O, v/v).

Reflectivity was collected for: (i) the bare silicon substrates, (ii) the SLBs before QD exposure, and (iii) the bilayer after incubation with QDs.

After QD injection, reflectivity was monitored over approximately **4 hours**. No further evolution was observed after the first hour, indicating that adsorption and structural rearrangements occurred rapidly and reached a steady state. Samples were finally rinsed with buffer to remove unbound particles before final measurements in all contrasts.

4. Data Reduction and Analysis

Raw detector images were converted to specular reflectivity curves using COSMOS. Data analysis and model refinement were performed with the Aurore software package.

The supported lipid bilayers were described using a molecularly constrained slab model separating headgroup and tail regions, with lipid molecular volumes fixed from independent densitometry measurements. Simultaneous fitting of all contrast conditions yielded scattering length density (SLD) profiles and corresponding volume fraction profiles ϕ(z) along the surface-normal direction.

For samples exposed to QDs, the pre-existing bilayer structure was used as a starting point, allowing detection of structural changes within the bilayer (hydration, defects) and/or the formation of additional layers attributable to adsorbed QDs. The amount of adsorbed material was quantified using the **equivalent thickness** derived from the volume fraction profiles.

5. NR Results

Prior to QD exposure, all three lipid bilayers formed homogeneous, well-defined SLBs with structural parameters consistent with literature values. DOPC bilayers were the thickest, DOTAP the thinnest, and the mixed DOPC:DOTAP bilayers showed intermediate values, indicating homogeneous lipid mixing. Hydration of both headgroup and tail regions was limited, demonstrating well-packed membranes.

NR revealed a strong dependence of QD interaction on membrane surface charge.

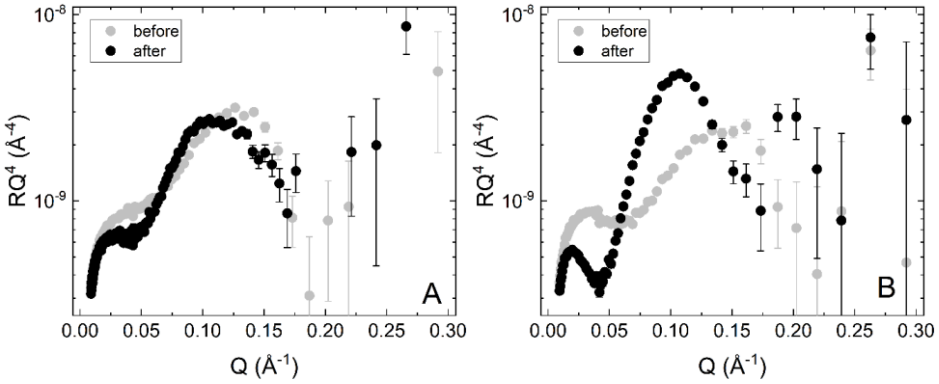

Figure 1. A: *NR data collected in H-buffer before (grey circles) and after (black circles) incubation of CdTe QDs with the DOPC:DOTAP bilayer.* ***B:*** *NR data collected in H-buffer before (grey circles) and after (black circles) incubation of CdTe QDs with the DOTAP bilayer.*

- **DOPC (zwitterionic):**
  Reflectivity curves before and after QD incubation were indistinguishable within experimental uncertainty. No additional layer and no detectable changes in the bilayer SLD profiles were observed, indicating negligible QD adsorption.

- **DOPC:DOTAP:**
  Small but reproducible changes in reflectivity were detected after QD exposure. Analysis

showed the formation of a **diffuse layer on top of the bilayer**, facing the solution, with an SLD compatible with that expected for hydrated CdTe QDs.
This layer was highly hydrated (water volume fraction ≈ 0.86) corresponding to a modest amount of adsorbed QDs. In addition, NR detected a slight increase in hydration within the hydrophobic region of the outer leaflet, consistent with the formation of small defects or partial lipid removal induced by QD adsorption.

- **DOTAP (fully cationic):**
  The largest changes in reflectivity were observed for pure DOTAP bilayers. NR analysis again required an additional top layer with an SLD characteristic of QDs, but with a substantially larger QD volume fraction. The equivalent thickness of the QD-rich layer increased to **~9 Å**, indicating more than twice the adsorbed amount compared to the mixed bilayer. Moreover, pronounced structural perturbations of the bilayer were detected: increased hydration in both outer and inner tail regions and a reduced volume fraction of the outer headgroup layer, indicative of significant membrane disruption and defect formation.

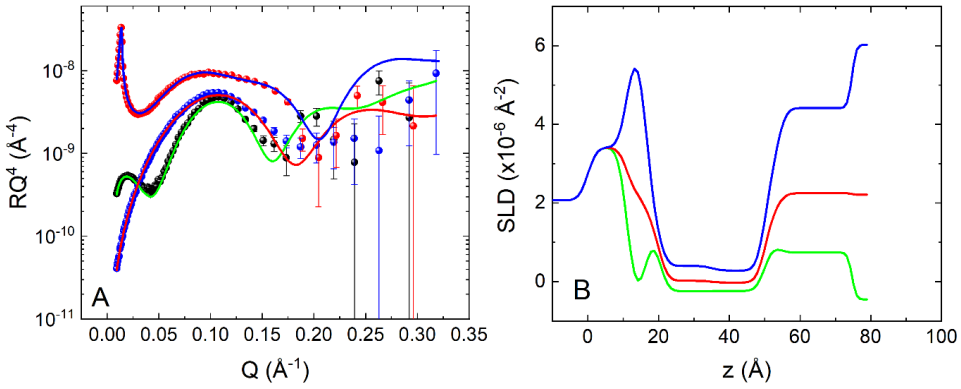

***Figure 2**. **A**: NR data for the DOTAP bilayer measured after rinsing following incubation with CdTe QDs for 4 hours. Data were collected in D-buffer (red symbols), H-buffer (black symbols), and SiM-buffer (blue symbols). They are shown along with the corresponding model curves (solid lines). **B**: SLD profiles corresponding to the model curves shown in panel A. The same color code applies (D-buffer in blue, SiM-buffer in red, and H-buffer in green).*

6. Conclusions

Neutron reflectometry unambiguously demonstrated that the interaction between hydrophilic CdTe QDs and supported lipid bilayers is **governed by electrostatics**.

NR provided quantitative, depth-resolved information showing that QDs adsorb predominantly **on top of the distal leaflet** rather than inserting as intact particles into the bilayer. Increasing membrane charge leads to a higher surface coverage of QDs and to progressively stronger structural perturbations, including enhanced hydration of the hydrophobic core and defect formation.

These results have been published in `Villanueva et al. Journal of Colloid and Interface Science 677 (2025) 620 – 631`