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Formation of nanopores in suspended lipid bilayers using quantum dots

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Abstract. In this work, nanopores are formed in lipid (DOPC:DOPE) membranes suspended across 150 micron apertures by oligomeric aggregation of 12 nm diameter CdSe quantum dots. The bilayer and quantum dot nanopores are simultaneously characterized by low noise electrical current monitoring and epifluorescence microscopy. Suspended lipid bilayers form high resistance gigaseals (>10 GOhm) that serve as barriers to the migration of charged ions and particles. Oligomeric aggregation of quantum dots is observed on the surface of the suspended lipid bilayer in the presence of charge stabilized quantum dot suspensions. The aggregate forms a nanometer scale pore (~2 nm in diameter) in the bilayer resulting in non-quantal ion current bursts. Migration of net neutral Rhodamine B dye (1.6 nm molecular diameter) across the bilayer is measured only in the presence of the aggregates. Potential applications for the non-lithographic fabrication of bilayer nanopores include biochemical detection, DNA sequencing, or cellular drug delivery.

1. Introduction

Nanopores and nanoporous membranes have been explored for DNA studies [1], fuel cell membranes [2], and Coulter counting [3]. Nanopore fabrication methods such as wet etched electron beam lithography nanopores [1], epoxy encased carbon nanotube nanopores [3], U-238 wet etched nanopores [4], and transmembrane protein nanopores [5] can be both time intensive and costly. Other techniques such as dry etched cylindrical nanopores [6, 7] are an improvement because they allow batch processing and form cylindrical pores. However, a technique for forming nanopores without lithography or the challenges associated with biological ion channels is desirable. Here we describe a method for forming nanopores using oligomeric aggregation of quantum dots (QDs) into a suspended lipid bilayer as shown in Figure 1. Lipid bilayers suspended across micro or nanometer scale apertures are often used as biological cell membrane analogs to study the behavior of ion channels [8] and more recently as biochemical sensors in hybrid bio-silicon devices [5, 9].

A lipid molecule consists of a hydrophobic tail and a hydrophilic head. When lipids are ‘painted’ in an aqueous solution across an aperture they spontaneously self-assemble so that the hydrophobic and hydrophilic ends align forming a bilayer [10]. In this work, 12 nm diameter QDs with a CdSe core are added to the aqueous solution surrounding the painted lipid bilayers. The core fluoresces at a peak emission of 525 nm under a broad excitation spectrum. A ZnS shell covers the core to improve the optical properties and a carboxyl coating helps stabilize the QDs in aqueous solution and decrease...
the effects of oxidation. When the QDs are inserted into the bulk solution they aggregate on the bilayer to form nanopores. We monitor the lipid bilayer using epifluorescence microscopy and patch-clamp type measurements of the leakage current across the membrane. Non-quantal, ionic current bursts are observed as well as aggregated QD on the bilayer surface. Similar current bursts have been recorded with streptavidin functionalized and polymer coated QDs, where it was also proposed that QDs can form nanopores [11]. Additionally, it has been observed that polycationic polymeric nanoparticles interact with and disrupt fluid phase domains of lipid membranes [12, 13]. Here, we confirm that QDs form nanopores by imaging the QD aggregation on the bilayer surface and molecular transport of fluorescent dye through the nanopores simultaneously with measurements of current bursts across the bilayer.

2. Experimental Setup and Techniques

The formation of oligomeric QD nanopores is detected using simultaneous optical and electrical measurements as shown in Figure 2. A low noise amplifier (Axopatch 200 B, Axon Instruments, Inc., Union City, CA) with Ag/AgCl electrodes is used to record the electrical currents through the aperture. A standard epifluorescence microscope (TE2000U, Nikon USA, Melville, NY) with 40x, NA 0.6 objective and high quantum efficiency, cooled CCD camera (Cascade IIB, Photometrics, Tucson, AZ), is used to image the QD aggregation and fluorescent dye transport. A Nipkow spinning disk confocal system (CARV II, BD Biosciences, Franklin Lakes, NJ) allows for optical sectioning and reduces the amount of light due to out of focus fluorescent particles reaching the CCD sensor.

Aqueous solution in both the upper and lower baths is 1 M KCl with 20 mM HEPES buffer at pH 7.4. The solution is added such that there is no differential hydrostatic pressure across the bilayer. Two types of lipids are used (Avanti Polar Lipids, Inc., Alabaster, AL), a 4:1 mixture of 1,2-dioleoyl-sn-glycero-3 phosphoethanolamine (DOPE) to 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and a 4:1 mixture of DOPC to 1-acyl-2-acyl-sn-glycero-3-phosphate (DOPA) dissolved in decane. DOPE-DOPC is net neutral and DOPC-DOPA is net negative at pH 7.4. Lipids are painted across a 150 μm delrin aperture (Warner Instruments, Inc., Hamden, CT) using standard Montal-Mueller techniques [10]. After measuring bilayer resistance and capacitance, we add 16 or 160 nM of QDs to the upper bath. Following an insertion wait time of between 1 and 5 minutes, current bursts begin coincident with increased fluorescence on the bilayer due to aggregation of QDs. A bias voltage of 50-200 mV is applied to measure the current across the bilayer, and a 500 mV pulse is used to rupture the bilayer at the end of the experiment.
3. Results and Discussion

Upon the addition of nM concentrations of QDs to the upper bath of the experimental setup shown in Figure 2, current bursts begin concurrently with increases in fluorescence on the bilayer. Figure 3 shows a fluorescence image of the bilayer along with current traces before (a) and after (b) QDs are added. Before the QD are added (Figure 3a) the fluorescence micrographs show uniform low intensity and the current is steady at ~10 pA at 100 mV bias demonstrating the ~10 GΩ seal of the bilayer as measured by applying a ramp voltage. Two minutes after the QD are added, we observe aggregation of QDs on the bilayer surface and unsteady current bursts due to flux of ions across the membrane. The aggregates shown in Figure 3 move with thermal motion demonstrating a fluid bilayer.

![Figure 3](image_url)

**Figure 3.** a) Inverted fluorescence micrograph of lipid bilayer showing no QD aggregates and low steady current. b) Aggregated QD on bilayer recorded simultaneously with current bursts.

![Figure 4](image_url)

**Figure 4.** Current trace and accompanying conductance histogram for applied bias voltage of 100 mV. Peak shown at 2.3 nS.

Figure 4 shows a one second current trace and a resultant conductance histogram. The conductance histogram shows a peak at $G = 2.3$ nS, which corresponds with a 230 pA current at 100 mV bias voltage. We can use this measurement along with a simplified model for nanopore conductance to estimate the size of our nanopores. Although the nanopore will have a complex distribution of ions due to the overlapping electric double layers [14], for simplicity here we model the conductance as,

$$ G = \sigma \frac{\pi d^2}{4D} $$

where $d$ is the expected diameter of the nanopore, $D$ is the diameter of our QDs, and $\sigma$ is the conductivity of the bulk solution. We measure the conductivity of our bulk solution to be $\sigma = 98$ mS/cm and our QDs are 12 nm in diameter. Using the conductance peak of 2.30 nS, we arrive at an expected diameter of $d \approx 1.90$ nm.

We verify nanopore formation by measuring the transport of molecular dye through the nanopores. Rhodamine B (Acros, Geel, Belgium) is used because it is a zwitterionic, net neutral species with an emission peak at 625 nm, sufficiently red shifted from the QDs. A net neutral dye is chosen because the overlapping electric double layers in nanometer pores can result in charge based exclusion for hydrated ions which are significantly smaller than the pore itself [14]. To conduct this experiment, a bilayer is painted and Rhodamine B is added to the upper bath. We measure constant fluorescence in the lower bath as well as a steady current across the bilayer for a period of 30 minutes demonstrating that the dye does not cross the bilayer. QDs are then added to the upper bath corresponding at $t = 0$ of Figure 5. Figure 5 shows the area averaged fluorescence intensity of the Rhodamine B as a function of
The fluorescence in the lower bath increases at the onset of current bursts across the bilayer. At \( t \sim 55 \text{ min} \) the bilayer is deliberately ruptured using a 500 mV pulse which results in saturation of both the optical and electrical detectors. Rhodamine B has an approximate diameter of 1.6 nm (evaluated from molecular structure) [15] which compares well with our expected nanopore diameter of 1.90 nm indicating that the increase in fluorescence in the lower bath is likely due to diffusion of Rhodamine through the QD nanopores.

We are currently investigating possible mechanisms for this phenomenon. One theory suggests that due to the similarity in dipole moment of Alamethicin and QDs, the QDs insert into the bilayer under an applied electric field in a similar manner to the antibiotic Alamethicin [11]. We also expect the electrostatic interactions of the QD and lipids to be important [16]. We have tested this by preparing lipids with the same charge as the QDs (net negative) using a 4:1 DOPC:DOPA mixture. We obtained similar current traces and detected a fluorescence increase on the bilayer that is similar to our previous experiments with net neutral lipids, indicating that the repulsive electrostatic interactions between the bilayer and the QDs do not control the interaction at the tested conditions.

![Figure 5](image_url)

**Figure 5.** a) Fluorescence intensity in the lower bath as a function of time. b) Current bursts due to QD aggregation.

In summary, we present strong evidence that QDs form oligomeric nanopores on suspended lipid bilayers. We simultaneously use optical and electrical measurements showing that QDs aggregate on the bilayer surface and ions conduct through the bilayer upon aggregation of the QDs. We also show that Rhodamine B dye can translocate the bilayer during the QD aggregation. The estimated nanopore diameter is \( \sim 2 \text{ nm} \), making these nanopores potentially useful for biochemical detection, DNA sequencing, and cellular drug delivery. Additional experiments are underway to identify other nanoparticles that may be used to create nanopores and the physicochemical conditions required for nanopore formation.

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