



Electrodeless QCM-D for lipid bilayer applications

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ABSTRACT

An electrodeless quartz crystal microbalance with dissipation monitoring (QCM-D) setup is used to monitor the formation of supported lipid bilayers (SLBs) on bare quartz crystal sensor surfaces. The kinetic behavior of the formation of a 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) SLB on SiO₂ surfaces is discussed and compared for three cases: (i) a standard SiO₂ film deposited onto the gold electrode of a quartz crystal, (ii) an electrodeless quartz crystal with a sputter-coated SiO₂ film, and (iii) an uncoated electrodeless quartz crystal sensor surface. We demonstrate, supported by imaging the SLB on an uncoated electrodeless surface using atomic force microscopy (AFM), that a defect-free, completely covering bilayer is formed in all three cases. Differences in the kinetics of the SLB formation on the different sensor surfaces are attributed to differences in surface roughness. The latter assumption is supported by imaging the different surfaces using AFM. We show furthermore that electrodeless quartz crystal sensors can be used not only for the formation of neutral SLBs but also for positively and negatively charged SLBs. Based on our results we propose electrodeless QCM-D to be a valuable technique for lipid bilayer and related applications providing several advantages compared to electrode-coated surfaces like optical transparency, longer lifetime, and reduced costs.

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1. Introduction

In the past decade, the quartz crystal microbalance with dissipation monitoring technique (QCM-D) has become a widely used method to study various surface processes, especially in biophysics and biochemistry. One of the most frequent, and maybe also most successful model system to which the technique has been applied, is formation of supported lipid bilayers (SLBs) from lipid vesicles (liposomes) of different lipid composition (Keller and Kasemo, 1998; Merz et al., 2008; Richter et al., 2006), their functionalization with various functional biomolecules (Svedhem et al., 2003), and furthermore studies of the interaction of SLBs with biomolecules (Glasmästar et al., 2002; Janshoff et al., 1996; Richter et al., 2005), lipid vesicles (Kunze et al., 2009; Wikström et al., 2008) and cells (Andersson et al., 2003; Svedhem et al., 2003).

SLBs are a versatile model system for mimicking of the much more complex real cell membrane. The advantages of SLBs include reproducible preparation protocols, the possibility to systematically and controllably vary their composition, and the opportunity to apply a variety of surface sensitive probes to measure non-specific and specific interactions at the SLB interface or (ion) transport across the membrane.

Different approaches have been used to prepare SLBs on QCM sensor surfaces. For instance, Au-coated QCM sensors can be modified by chemisorption of a hydrophobic or charged alkanethiolate self-assembled monolayer (SAM), allowing for the subsequent formation of a supported lipid membrane (Faiss et al., 2004; Keller and Kasemo, 1998; Steinem et al., 1996). A more convenient method for SLB preparation is via the vesicle adsorption-rupture-fusion approach on SiO₂-coated QCM or QCM-D sensors, as first reported by Keller and Kasemo (1998), which was followed by intensive studies in several groups, including as well other coatings like SiN₃ or TiO₂ (Keller et al., 2000; Merz et al., 2008; Reimhult et al., 2003; Richter et al., 2006; Rossetti et al., 2005; Seantier et al., 2004).

In all the studies mentioned above, the surface of the quartz crystal sensor has to be modified, first by deposition of a conductive material acting as an electrode, usually gold, and secondly by deposition or chemisorption of another material promoting SLB formation (e.g. SiO₂ or thiolate-SAMs). A question we asked ourselves is if all these preparation steps, i.e. deposition of an electrode plus a layer promoting the SLB formation, are really necessary, since the bare quartz (=SiO₂) crystal surface in principle should work as well, without any additional modifications. This would however, require that the excitation of the oscillation of the QCM-D sensor could be achieved without a top electrode directly on the sensor. Such excitation has been demonstrated by several groups using electrodeless quartz crystals in aqueous solutions (Hu et al., 2003; Nomura et al., 1991; Ogi et al., 2007; Shen et al., 2005). The use of a quartz crystal

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sensor without a top electrode, or without any electrode, or any other material, on either side of the sensor, offers several advantages, including (i) the possibility to combine QCM with optical transmission techniques, as recently demonstrated (Larsson et al., 2009), (ii) longer lifetime of the sensor, since it can be cleaned with aggressive solvents without risk of destroying the electrode, (iii) better reproducibility of experiments because of the better characterization and robustness of the surface, and (iv) simplified sensor design and reduced cost due to fewer processing steps, while not compromising the sensor performance.

The first study of electrodeless QCM by Nomura et al. (1991) reported on the behavior of an electrodeless quartz crystal in aqueous solution, with focus on the dependency of the resonance frequency on temperature and conductivity of the electrolyte. The adsorption of barium sulphate was measured, showing that the frequency change is proportional to the concentration of barium sulphate and thus indicating that the electrodeless QCM set up could be used as a mass sensor in the same way as the normal QCM. This study was followed by theoretical and experimental work done by Shen et al. (2005) focusing on the sensitivity of the electrodeless QCM to the permittivity, conductivity, viscosity and density of the electrolyte, also accompanied by a study of gelatin adsorption. In recent studies, electrodeless QCM has been used to investigate the adsorption of fibrinogen (Hu et al., 2003) and the affinity of the IgG-protein A interaction (Ogi et al., 2007), thereby introducing the electrodeless QCM as a biosensor.

To our knowledge, electrodeless QCM or QCM-D has not been applied to the study of supported lipid membranes before. The introduction of electrodeless QCM-D for such studies is of great interest for the broad research field of lipid membranes and associated areas. In the present study we show that SLBs can be formed directly on a bare quartz crystal used for QCM sensing, and that the kinetics of this process can be followed in the same detail as for conventional QCM sensors with deposited conductive electrodes and additional SiO₂ layers deposited onto the quartz. This is demonstrated for SLB formation using three different lipid compositions. For comparative and optimization reasons, SLB formation was measured on three different surfaces: (i) a commonly used, commercially available QCM-D sensor with a multilayer coating consisting of Cr, Au and SiO₂, (ii) an electrodeless quartz crystal sputter coated with 20 nm SiO₂, and (iii) an uncoated electrodeless quartz crystal surface. Since (i) and (ii) has the same outermost coating, comparison between SLB formation on these two surfaces should reveal difference related to electrodeless versus non-electroded measurements. Similar comparison between (ii) and (iii) should then allow conclusions about possible differences in SLB formation kinetics and quality on the deposited versus single crystalline quartz surfaces. Since the experimental arrangement has four measurement cells operating in parallel these comparisons are simplified and eliminate, e.g. differences in preparations of the vesicle solutions.

2. Experimental

2.1. Materials

Unless otherwise stated, chemicals were obtained from commercial sources and used without further purification. Water was deionized (resistivity > 18 MΩ/cm) and purified using a MilliQ unit (MilliQ plus, Millipore, France).

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-L-serine] (POPS), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-ethylphosphocholine (POEPC) were obtained from Avanti Polar Lipids.

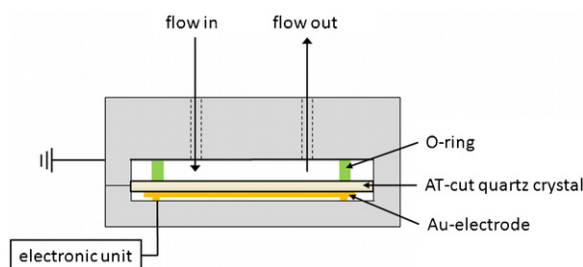


Fig. 1. Schematic drawing of the QCM-D setup.

2.2. Preparation of lipid vesicles

Lipids were dissolved in chloroform to prepare films of the desired composition. The solvent was removed, first under a gentle stream of nitrogen to form a thin lipid film on the wall of a round-bottom flask, and secondly under vacuum for 3 h. The lipid films were hydrated in buffer. After vortexing, the solutions were extruded 11 times through a 30 nm polycarbonate membrane. The resulting vesicle solutions were stored under nitrogen in a fridge.

2.3. QCM sensor surfaces

AT-cut quartz crystals with a fundamental frequency of 5 MHz were purchased from Q-Sense AB (Göteborg, Sweden). All crystals had an electrode (200 nm of Au on a 2 nm thick adhesion layer of Cr) deposited on one side (bottom) of the crystal. Different modifications of the other side (top) of these crystals were considered: (i) stepwise deposition of Cr (2 nm), Au (200 nm), and SiO₂ (100 nm) (Q-Sense AB), (ii) sputtering of 20 nm SiO₂ directly onto the unmodified crystal, (iii) the unmodified, electrodeless single crystal quartz cleaned twice according to the following protocol: immersion of the crystal for 60 s in a piranha solution (70 vol% H₂SO₄ (concentrated) and 30 vol% H₂O₂ (30%)), thorough rinsing with water, and ultrasonication in water (3 × 15 min with rinsing in between). Note that cleaning the crystals in piranha solution was an essential step, as elucidated further in [supplemental information](#). Prior to the experiment, all crystals were cleaned in 10 mM SDS (overnight), rinsed thoroughly with water, dried under nitrogen, and treated in a UV-ozone chamber (3 × 15 min) with rinsing and drying in between.

2.4. Electrodeless QCM-D

The QCM-D measurements were performed in flow mode using a Q-Sense E4 instrument (Q-Sense AB). No modifications of the instrumental set-up were necessary in order to run experiments using electrodeless QCM-D sensors. Fig. 1 depicts a schematic drawing of the QCM-D setup used in the present study.

The QCM-D sensor is mounted in a grounded flow module in such a way that the electrode on the lower side of the sensor is connected to the electronic unit of the instrument while the upper (electrodeless) side of the sensor is exposed to the fluid (volume above the sensor ~40 μl). Using the standard flow module, the distance between the top of the quartz sensor surface and the top of the module is on average 0.62 mm. This allows the top of the grounded module to act as counter electrode to excite the oscillation of the quartz crystal. In this way, the crystal is excited over the liquid gap. Thus the liquid is part of the electronic circuit driving the oscillation of the quartz crystal and therefore the QCM-D signal is more sensitive to changes in liquid conductivity, permittivity, density and viscosity than in regular QCM-D with electrode-coated quartz crystals (Nomura et al., 1991; Zhihong et al., 1991). The conductivity of TRIS (10 mM TRIS, 100 mM NaCl, pH 8) and TRIS-Ca (10 mM TRIS, 100 mM NaCl, 10 mM CaCl₂, pH 8) buffer used in this study is

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