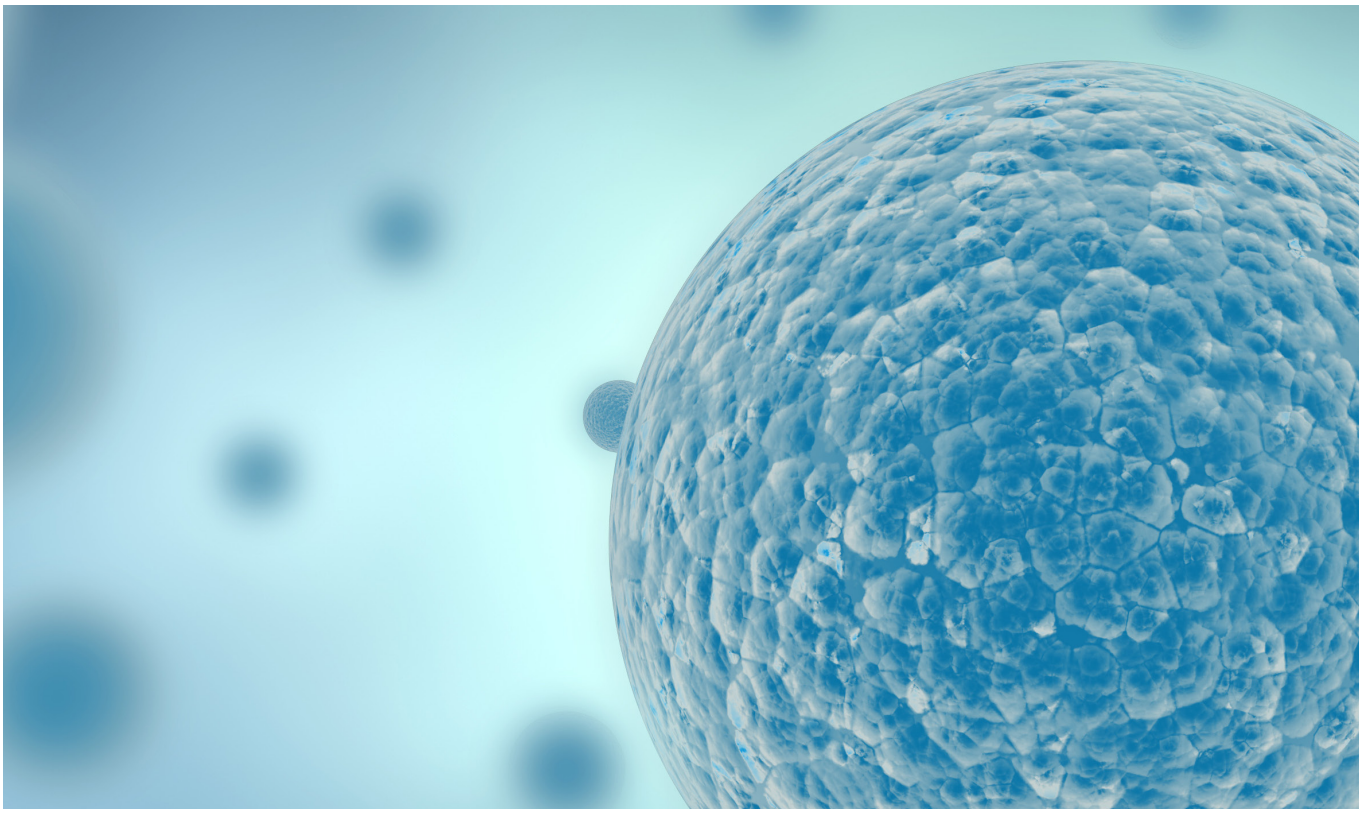
Overview

Characterization of lipid-based systems with QSense QCM-D



Analysis of the dynamics, structure and layer quality

Lipid-based systems are widely used in various fields of research, for example, in the design and development of biosensor platforms, biomaterial coatings and drug delivery applications. In this overview, we present examples of how these lipid-based systems can be characterized using QSense QCM-D technology.

The overview covers analysis of:

1. intact lipid vesicles
2. lipid monolayers
3. lipid bilayers
4. molecular interactions with lipid-based structures

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Explore the world of lipid-based systems

Working with lipid-based structures on solids supports, both the layer formation dynamics and the properties of the formed structure can be of interest. For example, questions to answer could be:

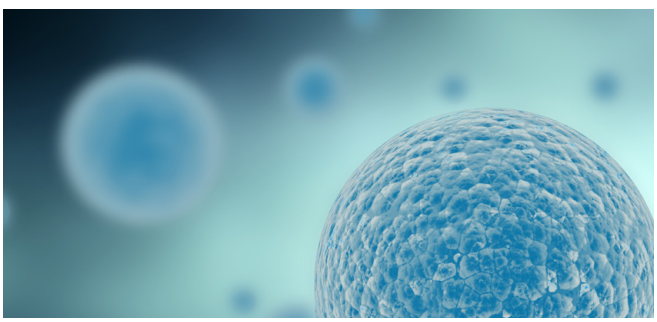
- Are these polymer-grafted liposomes forming a supported membrane?
- What is the quality of the formed bilayer?
- Are there intact vesicles present in the supported membrane?
- Will these proteins bind to the functionalized membrane?
- Is the supported membrane stable over time?

An unsurpassed technology in the characterization of lipid-based systems

QCM-D has been a standard technology in lipid-based research for two decades[1]. Offering real-time information on mass, thickness and viscoelastic properties of surface adhering layers, the technology enables monitoring of interaction dynamics between lipids and the solid support. It also enables characterization of the formed lipid-based structures.

Unveil lipid system structures and configurational changes

The key to be able to measure structural changes, such as the vesicle rupture and fusion process, lies in the ability of QCM-D to measure what is often referred to as "hydrated mass". Whereas the "dry mass", measured by for example optical techniques, refers to the mass of the molecules of interest, the hydrated mass includes both the molecules and the associated solvent. Monitoring the hydrated mass enables detection of conformational changes, such as layer swelling and collapse. In these structural rearrangements, the number of molecules at the surface is the same, but the amount of coupled solvent differs. In the characterization of lipid-based structures, this feature is very helpful as it enables the differentiation between for example lipids arranged as a bilayer and lipids arranged as a vesicle, where the former structure will have large amounts of associated solvent, and the latter will have little.



"The QCM-D information makes it easy to segregate between vesicular layers, lipid monolayers and lipid bilayers. It is also straightforward to monitor the layer formation dynamics, as well as to characterize the quality of the supported membranes."

Characterize lipid-based systems with QSense QCM-D:

- Analyze lipid - solid surface interaction dynamics, e. g. adsorption rate and adsorbed amount
- Detect structural rearrangements, e.g. vesicle rupture and fusion
- Reveal the structure of the lipid system, e.g. intact vesicles, monolayers and bilayers
- Evaluate lipid membrane quality
- Quantify the layer thickness
- Quantify the mechanical properties of the layer

QSense QCM-D is a surface sensitive real-time technology for label-free analysis of surface interactions and reactions. Monitoring changes in resonance frequency, f, and dissipation, D, of a quartz crystal, surface interaction events can be characterized and quantified.

How to interpret the data

Frequency: In general, the frequency, f, provides information about mass changes at the surface. A decrease in f indicates a mass uptake and vice versa.

Dissipation changes: The dissipation, D, provides information about the layer softness. As a rule of thumb, the higher the D, the softer and/or thicker the layer.

1. Characterization of intact lipid vesicles

QCM-D technology enables real-time monitoring of vesicle interaction with solid substrates[1]. The information reveals if, and how, the vesicles adsorb to the surface, e.g. at what rate they adsorb, what structural arrangement they take at the surface and if they stay intact over time or if they rupture. These processes can be particularly interesting to evaluate in situations where, for example, a complex lipid mixture is used or if the vesicles are functionalized with e.g. proteins. Other situations where this information is relevant are, for example, if a specific surface material of the solid support, or the ambient conditions, are evaluated. It is also possible to characterize the vesicular layer to reveal whether they form a vesicle monolayer at the surface or if they aggregate.

Lipid vesicle adsorption is easily identified already in the raw data, where both f and D show large responses, (**Figure 1 A)**. The large shifts indicate that there is a large mass uptake and that the formed layer is very soft, as is the case of a layer of solvent-filled vesicles. The raw data can be quantified to extract mass, thickness and viscoelastic properties of the vesicular layer.

Use QCM-D to, for example: **(Figure 1 B)**:

- Verify the vesicle adsorption process
- Explore the vesicle adsorptions dynamics
- Analyze vesicle stability and/or rupture
- Characterize the vesicle layer thickness, mass and viscoelastic properties
- Optimize conditions (lipid composition, ambient conditions, material of the solid support) to reach the desired result

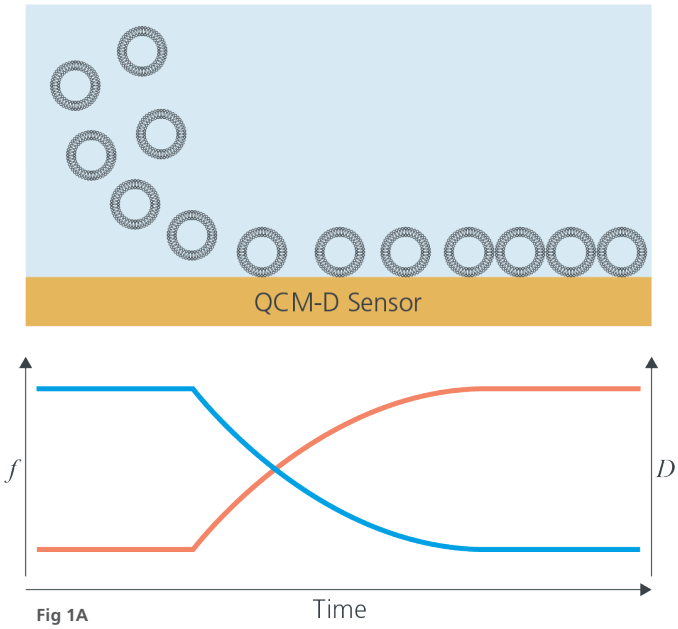


Fig 1A

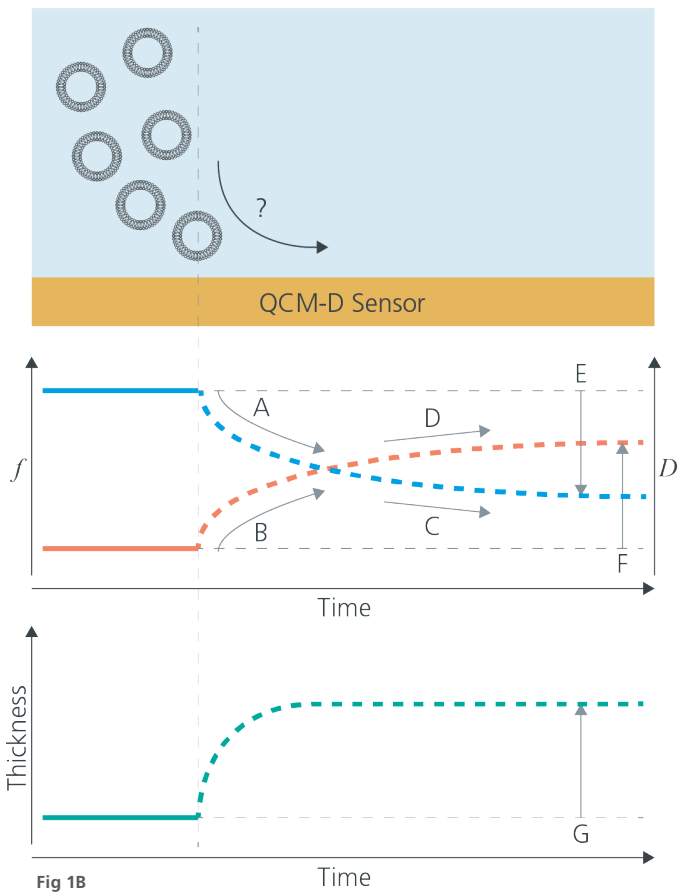


Fig 1B

Figure 1 A-B Schematic illustrations of vesicle adsorption (not to scale).

Fig. A: Adsorption of intact vesicles to a solid support, monitored by QCM-D. When the vesicles adsorb, the frequency curve (blue), decreases, which indicates that the mass increases. The dissipation curve (red), increases, which indicates that a thick and soft layer is forming.

Fig. B: Using QCM-D, questions related to the vesicle adsorption process and the layer properties can be answered, e.g. are the vesicles adsorbing (A, B)? What is the adsorption rate and what is the process (A- D)? Will the vesicles stay intact over time (E, F)? And what is the thickness of the vesicle layer (G)?

2. Characterization of lipid monolayers

Lipid monolayers are typically formed on hydrophobic surface swhere the lipid molecules arrange themselves with the hydrophobic tails facing the surface and the hydrophilic heads facing the solvent. Such layers are very thin and have little associated solvent, which means that the sensed mass is very close to the molecular mass.

QCM-D technology enables real-time monitoring of lipid monolayer formation[1]. The formation process is easily identified by the characteristic change in f corresponding to a mass uptake of a monolayer of lipids, **(Figure 2 A)**. The low change in D indicates that a very thin and rigid layer is formed.

Use QCM-D for example to **(Figure 2 B)**:

- Verify the monolayer formation process
- Explore the lipid monolayer formation dynamics
- Assess the layer quality
- Characterize the layer thickness
- Optimize the conditions to reach the desired result (lipid composition, ambient conditions, material/functionalization of the solid support)

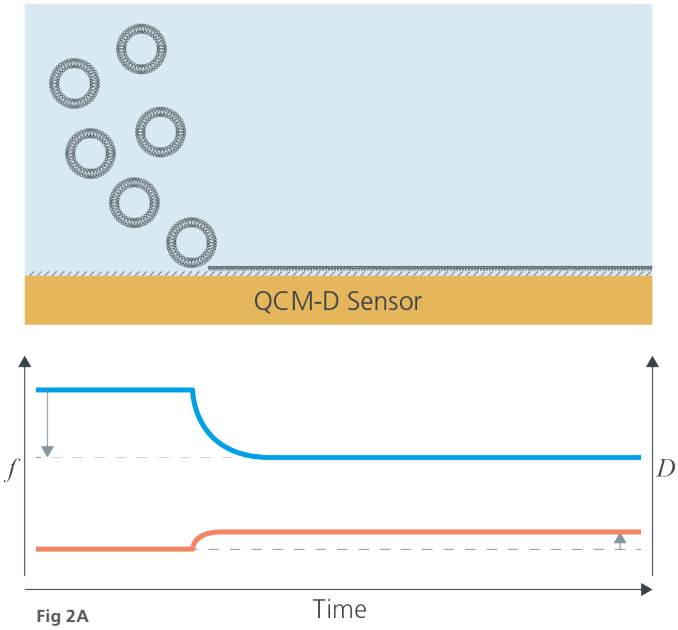


Fig 2A

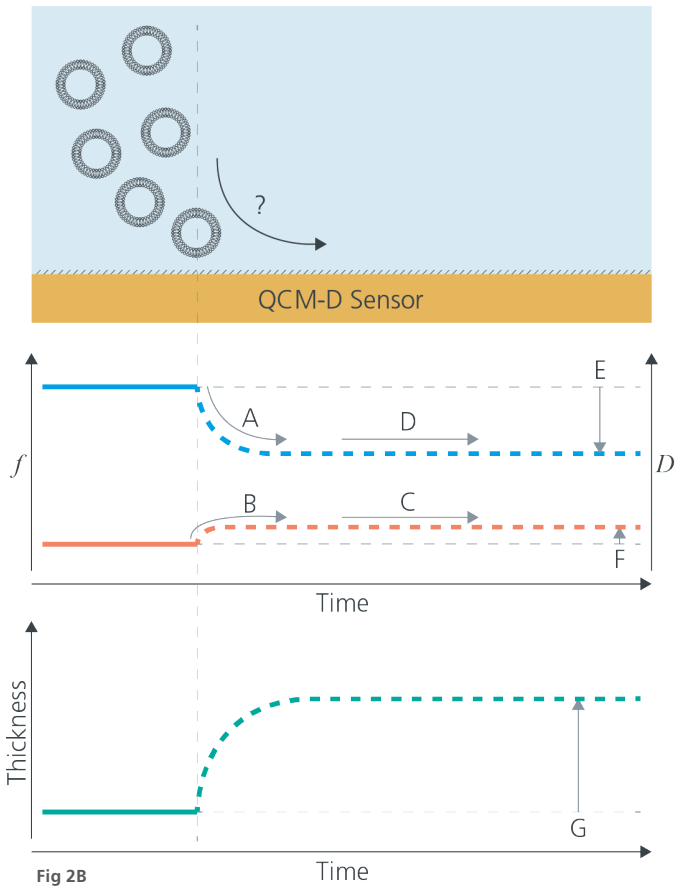


Fig 2B

Figure 2 A-B Schematic illustration of lipid monolayer formation (not to scale)

Fig. A: Formation of a lipid monolayer monitored by QCM-D. Typically, the frequency curve (blue) stabilizes at a well-defined value, indicating a mass uptake corresponding to a monolayer of lipids. The dissipation curve (red), stabilizes at a low value, close to zero, indicating that a very thin and rigid layer has formed.

Fig. B: Using QCM-D, questions related to the monolayer formation process can be answered and the layer can be characterized. For example, is a monolayer forming (A-B)? What is the formation dynamics (A-D)? And what is the quality of the monolayer – are there any intact vesicles present (E-G)?

3. Characterization of supported lipid bilayers

Supported lipid bilayers can be formed in different ways. One approach is via vesicle fusion[1-4]. Thanks to the sensing of hydrated mass, the structural rearrangement from vesicles to a supported lipid bilayer results in unique fingerprints which are straightforward to detect with QCM-D. For example, using zwitterionic vesicles on silica, there is a distinctive two-step behavior revealing the adsorption of intact vesicles at the surface, which then, at a critical surface coverage, rupture and fuse to form a bilayer, **(Figure 3 A)**. The final shifts of f and D are characteristic, f showing twice the mass uptake of a lipid monolayer and D being very low, indicating that a thin and rigid layer is formed.

Not only can it be relevant to verify the bilayer formation and evaluate the quality of the formed bilayer in cases where it is used as a platform for subsequent layer build-up, but it can also be relevant to explore the bilayer formation process if for example vesicles of more complex lipid mixtures are used, or if the ambient conditions are changed.

Use QCM-D for example to **(Figure 3 B)**:

- Verify the bilayer formation process
- Explore the lipid bilayer formation dynamics
- Asses the bilayer quality
- Characterize the bilayer thickness
- Optimize the conditions (lipid composition, ambient conditions, material of the solid support) to reach the desired result

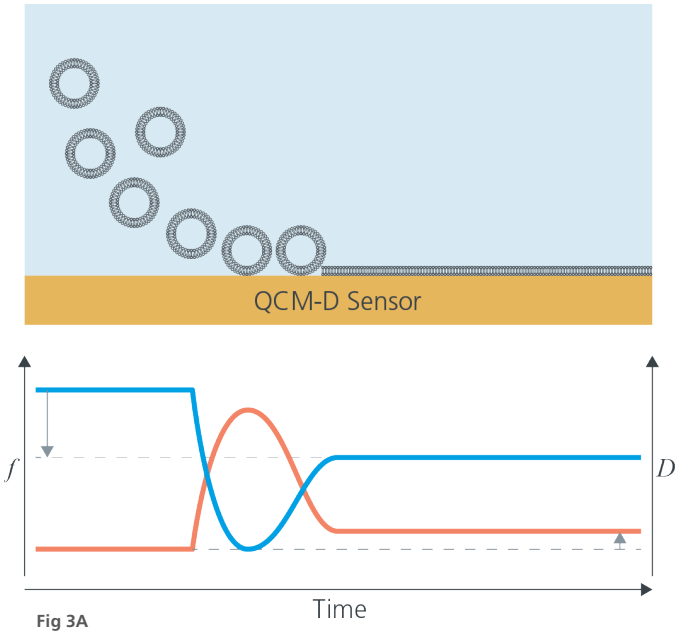


Fig 3A

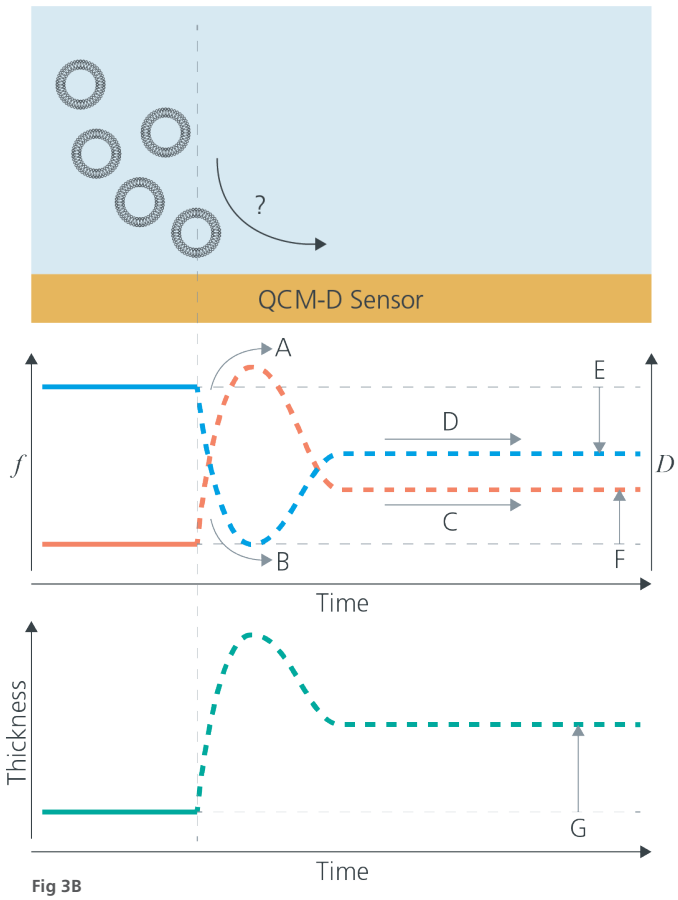


Fig 3B

Figure 3 A-B Schematic illustration of bilayer formation (not to scale).

Fig. A: Formation of a supported lipid bilayer monitored by QCM-D. The f and D signals show the unique fingerprint of vesicle rupture and fusion. First, there is an initial large uptake indicating vesicle adsorption. Next, both the f and D curves turn, indicating that mass is lost and the structure is becoming less soft. Finally, the curves equilibrate at characteristic f and D values, where the frequency is twice that of a lipid monolayer and the dissipation is low, indicating a thin and rigid layer.

Fig. B: Using QCM-D, questions related to the bilayer formation process can be answered and the layer can be characterized. For example, are the vesicles rupturing (A, B)? What is the lipid bilayer formation dynamics (A-D)? What is the quality (E, F) and thickness of the supported lipid bilayer (G)?

4. Characterization of molecular interactions with lipid-based structures

Lipid membranes provide a good environment for biomolecules and are interesting as for example biosensor platforms and cell membrane mimics[5,6]. In this context, the formation of the functionalized supported membrane is only the first step in the experimental protocol. The main interest is the subsequent interaction of the molecules or particles with the bilayer.

QCM-D technology enables real-time monitoring of molecular interaction and binding to functionalized lipid layers, as well as disruption of the membrane. Having verified the formation of a high-quality supported lipid membrane, as described above, it is straightforward to follow subsequent molecular binding or interaction of for example proteins, polymers, nanoparticles or tagged vesicles, via time-resolved changes in f and D, **(Figure 4 A)**.

Use QCM-D for example to **(Figure 4 B):**

- Verify the formation of the supported lipid membrane platform
- Assess the quality of the lipid membrane platform
- Explore or verify the molecular interaction with the lipid membrane (binding rate, bound amount, stability, membrane disruption)
- Characterize the thickness and viscoelastic properties of the bound molecular layer
- Optimize the conditions to reach the desired result

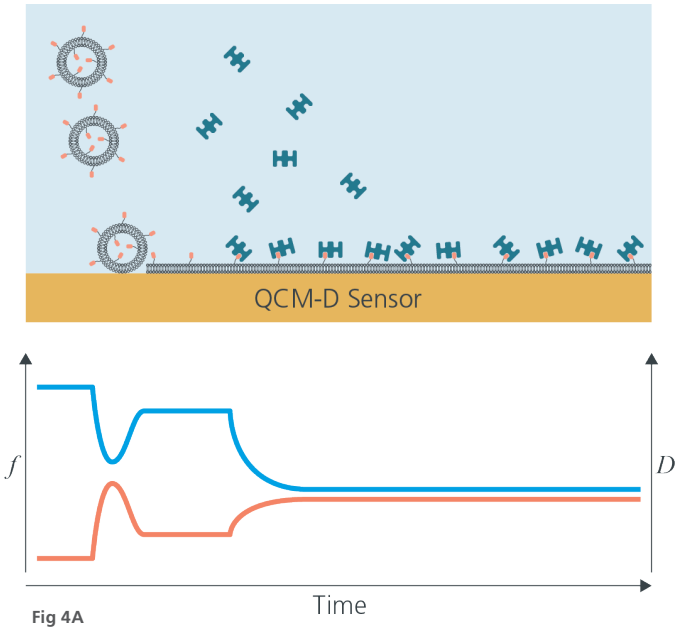


Fig 4A

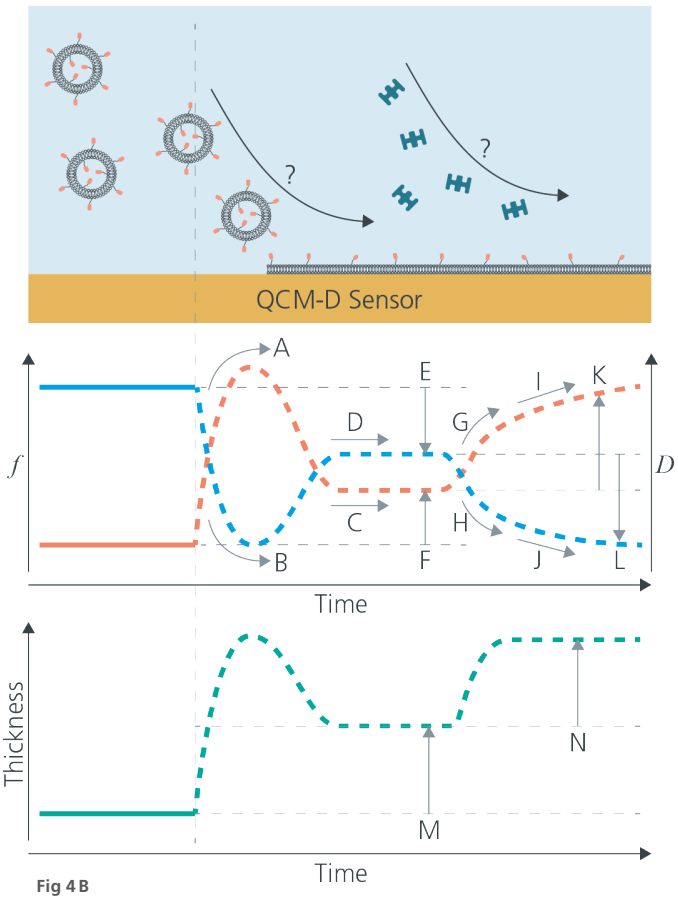


Fig 4 B

Figure 4 A-B Schematic illustration of the formation of a functionalized supported lipid bilayer and subsequent molecular binding (not to scale).

Fig. A: Formation of a functionalized lipid bilayer and subsequent molecular binding monitored by QCM-D. The first part of the f and D data shows the characteristic fingerprint of bilayer formation. Next, molecules that will bind to the functionalized bilayer are introduced. The binding is revealed by the decrease in f, indicating mass uptake, and an increase in D, indicating that a soft/thick layer is formed.

Fig. B: Using QCM-D, questions related to the bilayer formation process and the subsequent binding can be answered. For example, is a high-quality bilayer forming (A-F, M)? Are the molecules binding to the functionalized bilayer (G-H)? At what rate are they binding (I-J)? What is the surface uptake (K-L)? And what is the thickness of the bound molecular layer (N)?

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