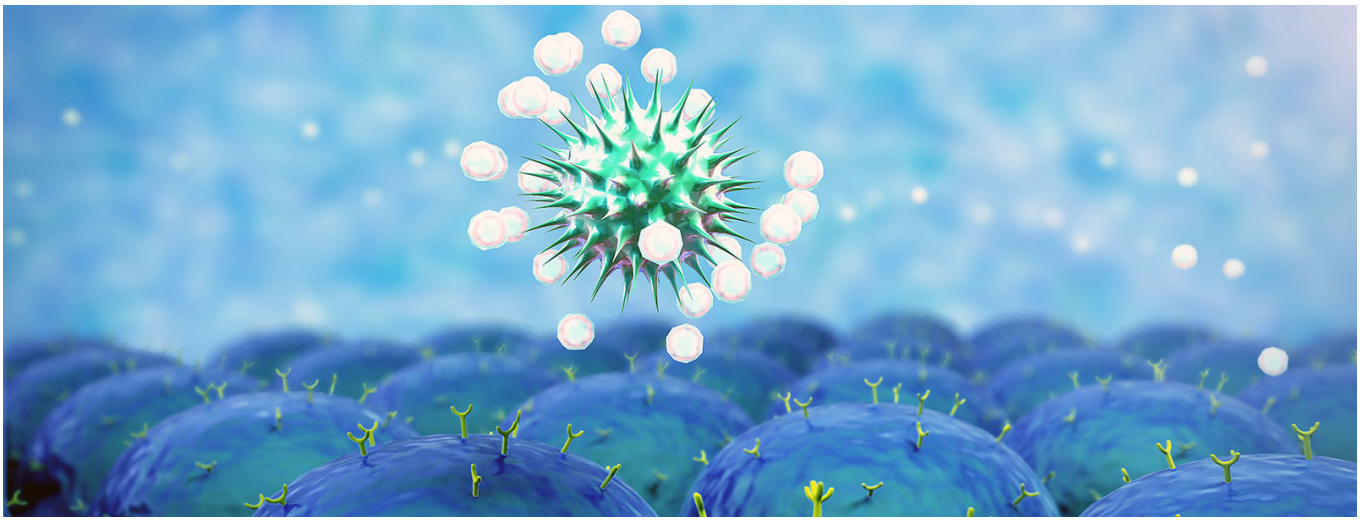
Overview

Characterization of biomolecular interactions

Analysis of adsorption, binding, and enzymatic action with QSense QCM-D



Biomolecular interaction analysis is in focus in a wide range of disciplines. In areas ranging from biochemistry and biotechnology to medicine and nanotoxicology, biomolecular interactions are explored both to gain increased knowledge and understanding of biological systems and functions, and to design products such as pharmaceuticals, sensors and materials.

In this overview, we present how biomolecular interactions can be analyzed using QSense® QCM-D technology and what information QCM-D measurements offer.

The overview covers analysis of:

1. Adsorption/desorption
2. Binding
3. Enzymatic activity
4. Structure, structural change and fibril formation

Explore, characterize and optimize your biomolecular-based systems

Irrespective of whether the objective is to investigate and characterize a biomolecular-based system, such as a protein interaction with a lipid layer, or to characterize the biocompatibility of a biomaterial, or to optimize a drug carrier for a targeted nanomedicine, both the molecular interaction dynamics and the structure of the formed layer are of interest. Working with such systems, questions to answer could for example be:

- Is the surface of this material inert?
- Will the addition of an excipient reduce the protein adsorption to the surface?
- Are there any active/functional binding sites available?
- Will the ligand induce a structural change of the receptor?
- How efficient is the enzymatic activity at this temperature?

Analyze biomolecular interactions and reactions with QSense QCM-D

- Characterize biomolecule adsorption to various surface materials
- Analyze e.g. protein, antibody, DNA interactions
- Characterize enzymatic action
- Study conformational changes of biomolecules when changing solvent conditions or upon binding of compounds
- Optimize solvent conditions to minimize adsorption
- Explore molecular behavior and causes of disease, such as protein misfolding disorders

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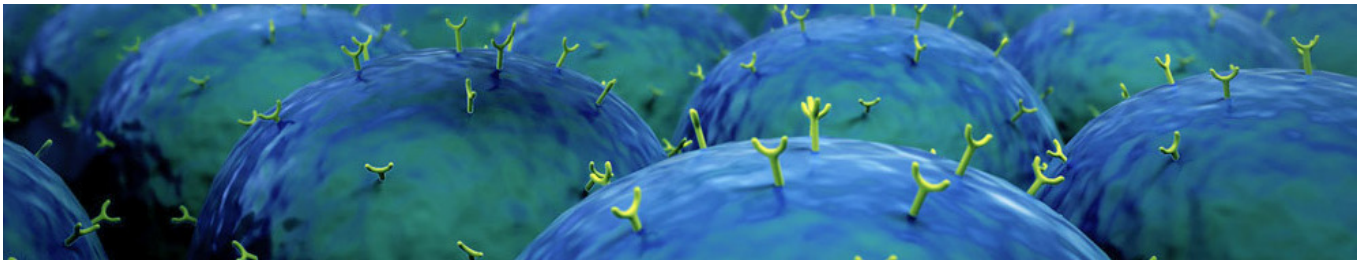


Get the full picture of your surface-molecule interaction

The questions above can be answered using QSense QCM-D. QCM-D is a surface sensitive technology which has been used to analyze biomolecular interactions for two decades [1, 2]. Via time-resolved information on mass, thickness and viscoelastic properties of surface adhering layers, the method can detect and monitor molecular interactions and events in real-time. Interactions and events that can be analyzed are for example adsorption, molecular binding, and structural changes such as crosslinking. Running QCM-D analysis at relevant conditions, varying, for example, the surface material, the temperature, pH, or salt concentration, the behavior of the biomolecular system can be both characterized for deeper understanding and optimized for a target application.

Analyze binding, interaction, and structural changes

QCM-D measures the so called "hydrated mass", which makes it an excellent complement to the optical time-resolved technologies, which are often used to study biomolecular interactions, and which senses "non-hydrated mass". Whereas the non-hydrated mass refers to the mass of the biomolecules, the hydrated mass includes both the molecules and the surrounding solvent. Monitoring the hydrated mass enables not only detection of surface interaction events such as adsorption, desorption and binding, but it also enables the detection of molecular arrangement at the surface and changes thereof. In the analysis and characterization of biomolecular interactions, this feature is very helpful as it enables the differentiation between for example molecules taking a coiled conformation at the surface or an elongated conformation, stretching out from the surface and extending into the bulk. It also enables the detection of structural changes, such as crosslinking, swelling and collapse of the molecules in the layer. In these structural rearrangements, the number of molecules at the surface is the same, but the amount of solvent surrounding the molecules differs. This makes the rearrangement readily detectable with QCM-D.

Below we show examples of what typical data could look like in the different cases. We also discuss what information you could extract from the respective measurement and what questions you could typically answer.

Your question	information provided by QSense QCM-D
Will the interaction take place?	Yes/No
How fast?	Rate of change
How much?	Amounts of mass, thickness and mechanical (viscoelastic) properties (structure)
What process am I looking at?	Changes over time in mass, thickness and mechanicla properties
What is the molecular arrangement?	Thickness and mechanical properties, rigid or soft layer

Table 1. Overview of information related to biomoleculralr intyeractions and reactions that QSense QCM-D can extract

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 charactreized and quantified.

What you can do: Analyze molecular interactions with surfaces and molecules, time-resolved and label-free.

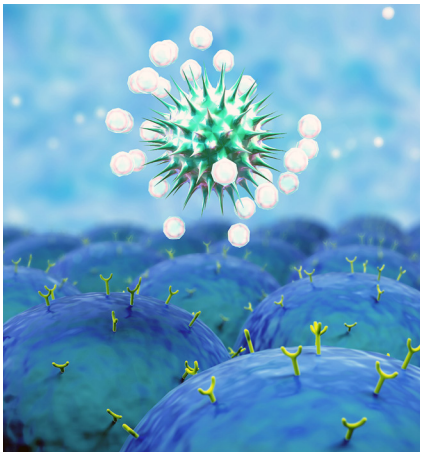
Events that you can analyze:

- Adsorption/desorption
- Binding
- Enzymatic activity
- Layer structure and changes thereof.

How to interpret the data

Frequency changes: 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 lauer softness. As a rule of thumb, the higher the *D*, the softer and/or thicker the layer



1. Adsorption and desorption

As QCM-D monitors mass changes as a function of time, it is an excellent tool to monitor and quantify adsorption to the sensor surface, which shows as a mass increase in the QCM-D data, Fig. 1A. Mass loss, i.e. desorption of material from the surface, is equally straightforward to characterize, Fig 1B. Via the time-resolved QCM-D measurement, it is possible to follow adsorption and desorption processes, how fast they are, and how much material that adsorbs to, or desorbs from, the surface as a function of the molecular, surface and solvent conditions. QCM-D also senses the mechanical properties of the surface-adhering layer, which makes it possible to analyze the arrangement of the adsorbed molecules. For example, is the molecular layer densely or loosely packed? Are the molecules taking a collapsed conformation or do they stretch out from the surface? And is the packing arrangement the same throughout the entire adsorption phase or does it depend for example on surface coverage or any other parameter such as surface material, temperature, salt concentration, pH etc? For more details on the analysis of the layer structure, see section 4 below.

Biomolecular adsorption analysis is relevant for example when assessing protein repellency of a surface, in the characterization of biomolecular interaction with a specific material such as a biomaterial, or in the assessment of drug interaction with for example filter, bags, containers and syringe materials in a pharmacological context.

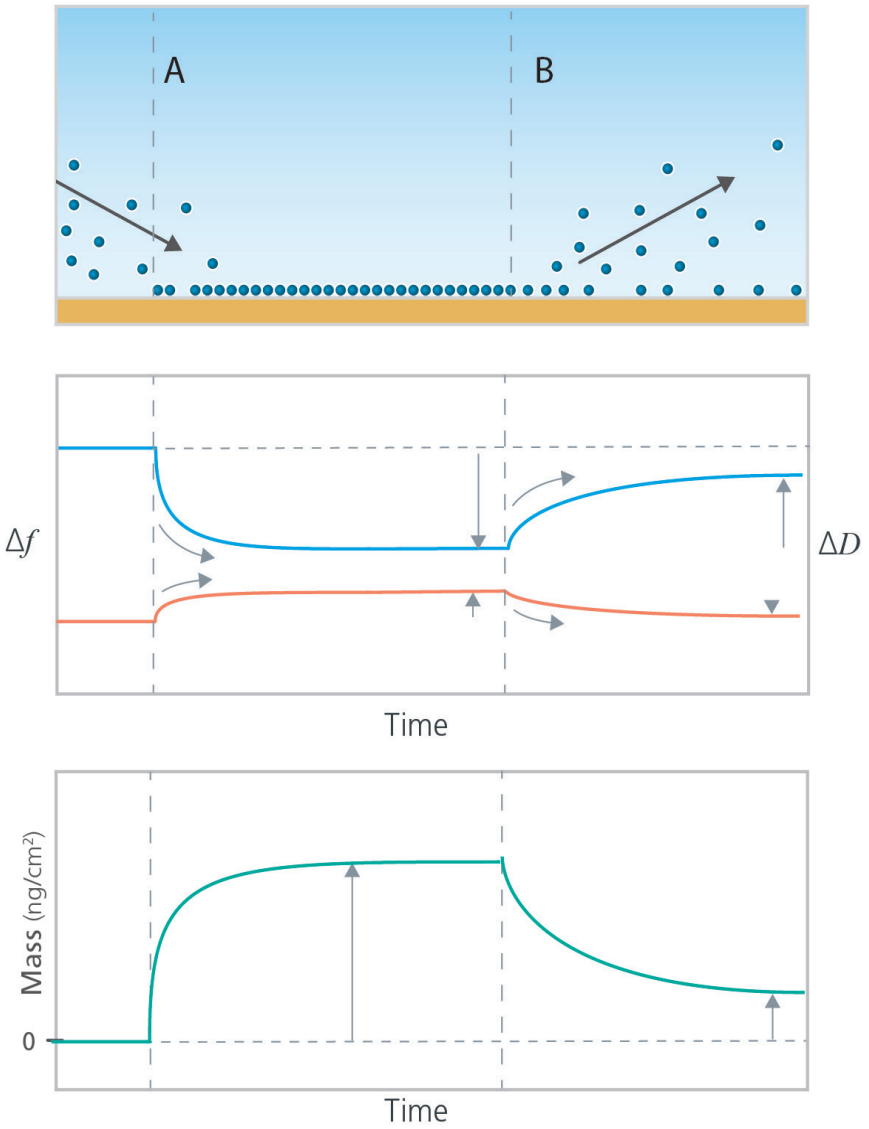


Figure 1. Schematic illustration (top panel) of (A) adsorption and (B) desorption of biomolecules to a surface, characterized by QSense QCM-D (middle panel). The Δ*f* and Δ*D* data reflect mass change and layer softness respectively. As indicated by the grey arrows in the schematic graphs, the time-resolved data makes it possible to follow the adsorption and desorption processes, how fast they are, how much material that is added to and lost from the surface. The amount adsorbed to, and desorbed from, the surface can also be analyzed via quantification of the time-resolved layer mass (bottom panel) or layer thickness (not shown).

Use QCM-D for example to:

- Assess whether a protein adsorbs to a surface material of interest at specific solvent conditions
- Explore the adsorption dynamics
- Quantify the adsorption rate and total adsorbed amount
- Characterize the molecular arrangement at the surface
- Identify conditions that prevent adsorption
- Optimize the surface or solvent conditions to minimize or maximize adsorption.

Vary meaurement conditions such as

- Surface material
- Sample concentration
- Temperature
- Solvent
- pH
- Ionic strength

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2. Characterization of binding

The mass measurements are also suitable to analyze binding. Typically, this would be an analysis of binding to a prefunctionalized surface where the target molecules are immobilized either ex-situ or in situ, Fig 2A. Using QCM-D, the binding process can be monitored in a time- resolved manner, Fig 2B, and the binding rates and bound amounts can be quantified. It is also possible to extract information on the structure of the layer, and if the binding induces a structural change.

Binding analysis would typically be relevant when evaluating the functionality of a protein, in the design of a biosensor, or in the development of drugs or nanomedicines. It is also useful when there is a need to improve the understanding of interaction mechanisms of interest, such as an antibody-antigen interaction.

Use QCM-D for example to:

- Assess whether a biomolecule binds to target molecules immobilized at the surface and at specific solvent conditions
- Explore the binding dynamics
- Quantify the binding rate and bound amount
- Characterize the molecular arrangement at the surface
- Optimize the surface or solvent conditions to minimize or maximize adsorption.
- Identify conditions that prevent adsorption

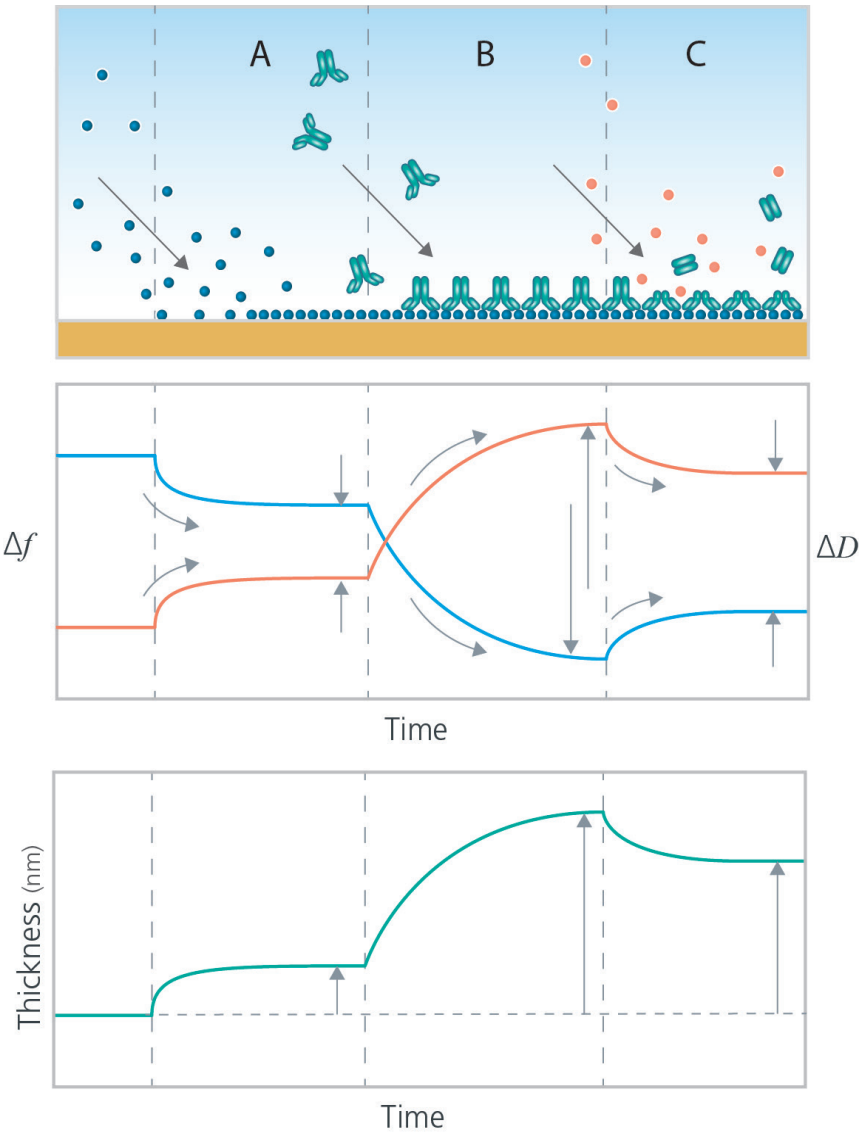

Figure 2. Schematic illustration (top panel) of (A) molecular adsorption, (B) binding and (C) enzymatic action, characterized by QSense QCM-D (middle panel). The Δ*f* and Δ*D* data reflect time-resolved mass uptake and layer softness respectively. As indicated by the grey arrows in the schematic graphs, the time-resolved data makes it possible to follow the adsorption, binding and enzymatic action processes, how fast they are, and how much material that is added to or lost from the surface in the respective process. The absolute amount adsorbed, bound and enzymatically removed can be analyzed via quantification of the time-resolved layer thickness (bottom panel) or layer mass (not shown).

3. Assessment of enzymatic activity

The processes so far discussed have involved mass increase, i.e. molecules adding mass to the surface either via adsorption or binding. Mass loss is equally straightforward to measure. Desorption has already been discussed in section 1. Another process which would show up as a mass loss is enzymatic action. Exposing a prefunctionalized sensor surface to a targeted enzyme, molecule fragments would be cleaved and leave the surface, which would be detected as a loss of mass, Fig 2C. This is useful for example to detect and characterize protease activity. It is also useful in contexts such as hydrolysis of cellulose and soil removal by enzymatic action.

Use QCM-D for example to:

- Assess the enzymatic activity
- Explore the dynamics of the enzymatic activity
- Quantify the removal rate and amount
- Optimize the conditions, such as temperature, to minimize or maximize the enzymatic activity

QCM-D is especially useful for characterization of highly hydrated structures, and for some systems strong QCM-D responses are obtained when optical signals are very weak [3]

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4. Sense structural arrangement and structural changes

As QCM-D senses hydrated mass, i.e. mass including surrounding solvent, it is an excellent tool to analyze the structure and structural changes of the molecular layers. For example, it is possible to detect different structural phases during adsorption and binding, Fig 3A-B, where the molecular conformation could depend on e.g. the surface coverage. It is also possible to analyze changes induced by altered ambient conditions such as temperature and, pH and salt concentration, Fig 3C. Particularly, the ΔD vs Δf plot, where the time parameter has been eliminated, will clearly reveal if there are different phases in e.g. an adsorption process.

Layer conformation can help to get an increased fundamental understanding of biomolecular systems and interaction processes at certain conditions, such as structural change of proteins upon binding of ligand. It can also be useful in the design and evaluation of materials, and in the optimization of interaction or reaction processes, e.g. biomolecular crosslinking.

Another structural process that can be analyzed with QCM-D is fibril formation. Typically, monomers adsorb to the sensor surface whereafter they assemble into larger structures, forming fibrils. Monitoring the time-resolved evolution of mass and viscoelastic properties, the fibrillation can be characterized. This could be useful for example in the understanding and prevention of protein folding disorders where polypeptides aggregate into fibrils, and to study the effect of amyloid growth.

Use QCM-D for example to:

- Characterize the molecular arrangement at the surface during adsorption or binding
- Characterize structural changes as a function of ambient, and solvent conditions
- Verify molecular arrangement
- Optimize the conditions to obtain the desired molecular arrangement
- Characterize the temporal development of fibril formation
- Optimize conditions to prevent fibril formation

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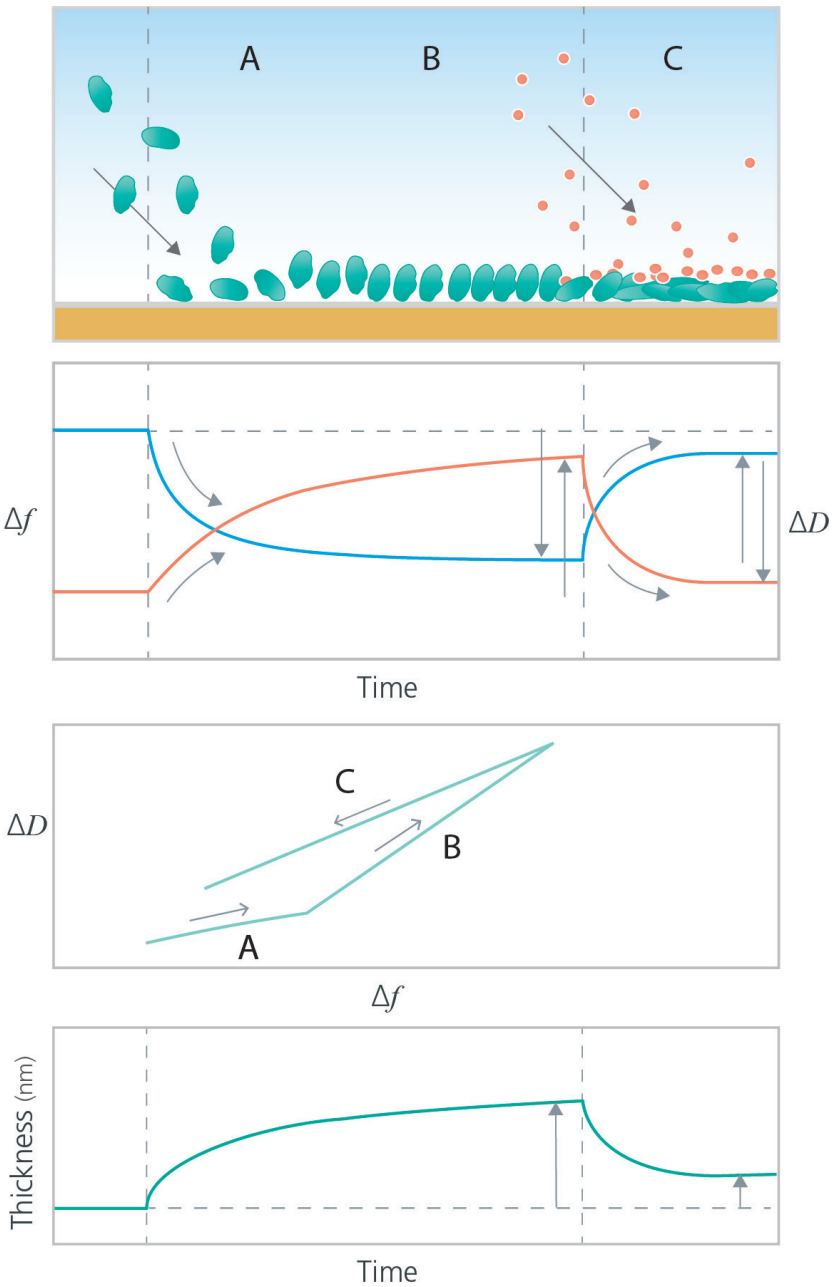

Figure 3. Schematic illustration (top panel) of (A-B) molecular adsorption and (C) structural rearrangement of the molecular layer induced by changes in the ambient solvent conditions, characterized by QSense QCM-D (panel second from top). The Δf and ΔD parameters reflect mass uptake and layer softness respectively. As indicated by the grey arrows in the schematic graphs, the time-resolved data makes it possible to analyze molecular arrangement at the surface and to follow structural changes, how fast they are, and how much material that is added to or lost from the surface in the respective process. The ΔD vs Δf graph (second panel from bottom) shows "layer softness per mass" and reveals structural changes between the phases A-C. Absolute properties of the layer, such as the layer thickness (bottom panel) or layer mass (data not shown) can also be quantified.

References

1. Höök F., et al., Faraday Discuss, 107 (1997), 229-246
2. Höök F., et al., Proc. Natl. Acad. Sci., 95 (1998), 12271-12276
3. Frost R., et al., J. Coll. Int. Sci, 362, (2011), 575-583

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