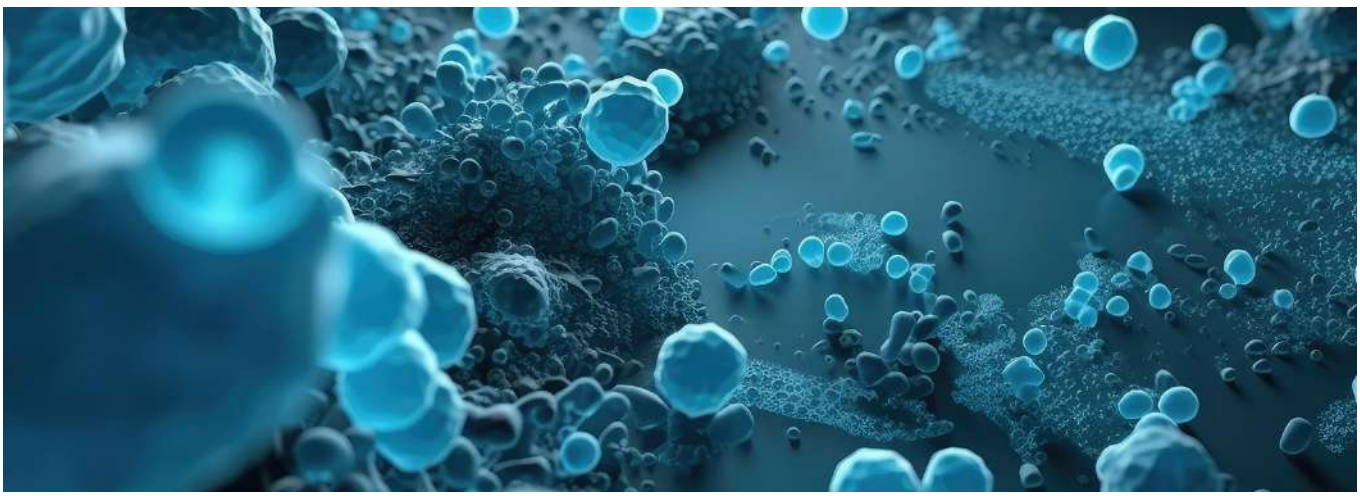
Overview

QSense QCM-D Analysis in Biointerface Science

Measure molecular interactions at surfaces and interfaces in Biotechnology and Biophysics



The interaction of biological macromolecules such as proteins and lipids with material surfaces is critical to biotechnology and biophysics applications. Key examples include protein adsorption that can regulate immune system processes, cell fouling that can cause infections on medical device coatings, and enzymatic and detergent-mediated processes that involve removing adsorbed biomacromolecules from interfaces. Understanding these interactions is important to build fundamental knowledge about biological systems and to realize production goals and biotechnology application opportunities in industrial sectors such as healthcare, cosmetics, environmental science, diagnostics, and pharmaceutics.

There are various measurement tools to characterize biomacromolecular adsorption on material surfaces, which can help to answer questions such as: (1) how much adsorption occurs; (2) how fast adsorption occurs; and (3) how biomacromolecular conformational changes in the adsorbed state. Such information can be useful for designing material surfaces that either promote or inhibit biomacromolecular adsorption and/or conformational changes depending on the application context while it is important to note that not all measurement tools provide the same capabilities.

Indeed, while there are many different types of surface-sensitive measurement tools to study biomacromolecular interaction processes, there are only a few options that do not require labeling of the biomacromolecule and that have rapid data acquisition for real-time kinetic tracking. Furthermore, among label-free measurement techniques in this subset, most options only work with very few types of material surfaces and/or provide limited information about the adsorbed biomacromolecules. In this regard, the quartz crystal microbalance-dissipation (QCM-D) technique stands out as the most complete and versatile, label-free measure-

QSense QCM-D is a surface sensitive time-resolved technology for label free analysis of molecular interactons at surfaces and interfaces. Monitoring changes in resonance frequency, *f*, and dissipation, *D*, of a quartz crystal sensor, processes at the solid-liquid interface can be characterized and quantified.

How to interpret the data

- **Δ*f* provides** information about mass changes at the surface. A decrease indicates mass uptake and vice versa
- **Δ*D* provides** information about the layer softness. As a rule of thumb, the higher the *D*, the softer and/or thicker the layer

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ment tool for characterizing adsorbed biomacromolecules on material surfaces because it is compatible with a wide range of material surfaces and is sensitive to not only the total mass of adsorbed biomacromolecules but also to their conformational properties.[1]

The QCM-D technique is based on an oscillating, piezoelectric quartz crystal sensor chip and one side of the sensor chip is coated with a material of interest such as silica, titania, or gold. Biomacromolecular adsorption onto the material-coated side of the sensor chip can be monitored as a function of time by measuring the resonance frequency (Δf) and energy dissipation (ΔD) properties of the sensor chip. In general, a negative Δf shift corresponds to mass adsorption because added mass slows down the oscillation whereas a positive Δf shift can indicate mass loss from the sensor surface. On the other hand, the ΔD signal is sensitive to the viscoelastic properties of the adsorbed biomacromolecules. A small ΔD shift often indicates that the adsorbed biomacromolecules are rigidly attached to the sensor surface, whereas a large ΔD shift is usually related to a soft adsorbate consisting of adsorbed biomacromolecules and attached solvent molecules. The latter feature is another competitive advantage of the QCM-D technique because it can detect adsorbed biomacromolecular mass and hydrodynamically coupled solvent mass, whereas other measurement options are limited to only detecting adsorbed biomacromolecular mass.

A wealth of information is obtained during QCM-D measurements because the Δf and ΔD signals are simultaneously collected at multiple overtones corresponding to the fundamental resonance frequency of the quartz crystal sensor chip and several odd overtones thereof. For example, if the fundamental resonance frequency is 5 MHz, then data can be collected at 5 MHz, 15 MHz, 25 MHz, 35 MHz, 55 MHz, 65 MHz, and so forth. Data collection at multiple frequencies is a critical advantage of the QCM-D technique because the surface sensitivity of the Δf and ΔD signals depends on the overtone number. For data collected at 5 MHz, the QCM-D measurement responses are sensitive to adsorption events occurring up to ~240 nm away from the sensor surface in aqueous environments. By contrast, for data collected at 65 MHz, the measurement responses are sensitive to adsorption events only within ~70 nm from the sensor surface, which indicates greater surface sensitivity. Importantly, various models have been developed to analyze the QCM-D data from all overtones together in order to extract information about the adsorbed biomacromolecule layer such as thickness, viscosity, and shear modulus.

As such, QCM-D measurements present many options for collecting and analyzing data related to biotechnology and biophysics applications while every application scenario is unique and can benefit from a tailored measurement strategy. The goal of this application package is to introduce some of the latest and most cutting-edge uses of QCM-D technology to study biotechnology and biophysics topics, demonstrating the breadth and depth of scientific insights that are possible to obtain with the QCM-D technique. Five case studies are presented to cover different application scenarios and each one provides a broad overview of the study motivation and measurement concepts while offering detailed remarks about experimental operation, data analysis, and future possibilities that will be helpful for novice and advanced users alike.

QSense Sensors - core of the QCM-D technology

QSense sensors are crafted to cover the various surfaces and materials required in research. The QCM-D technology is highly sensitive, underscoring the importance of using sensors made with precise techniques that meet top standards and quality for biophysics and biotechnology. Our sensors offer a wide range of materials, from metals and glass to polymers. Examples include:

- Silicon dioxide
- Titanium
- Polystyrene
- Borosilicate glass
- PTFE (Amorphous polymer)

Our team of surface experts is proficient in customizing sensors and has extensive experience in coating them with a diverse range of materials. These materials include bio-grade polymers like PVC, PC, PES, PMMA, PP, PE, COP, COC as well as various functional surfaces such as different steel variations and bio-grade alloys.

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Case study 1: Adsorption phenomena

Protein corona formation for lipid nanoparticle development[2]

Motivation:

There is high interest in developing lipid nanoparticles for medical and biotechnology applications such as vaccine delivery, and natural versions of lipid nanoparticles like lipoproteins are important for human health. In the body, the performance of lipid nanoparticles, including where they go and how well they work, depends on the types of serum proteins that bind to the nanoparticle surface and form a corona. However, using conventional biological assays, it is difficult to determine which serum proteins bind to the lipid nanoparticle surface and QCM-D technology has emerged as a promising solution to directly measure serum protein binding to lipid nanoparticle surfaces as illustrated in this case study.[2]

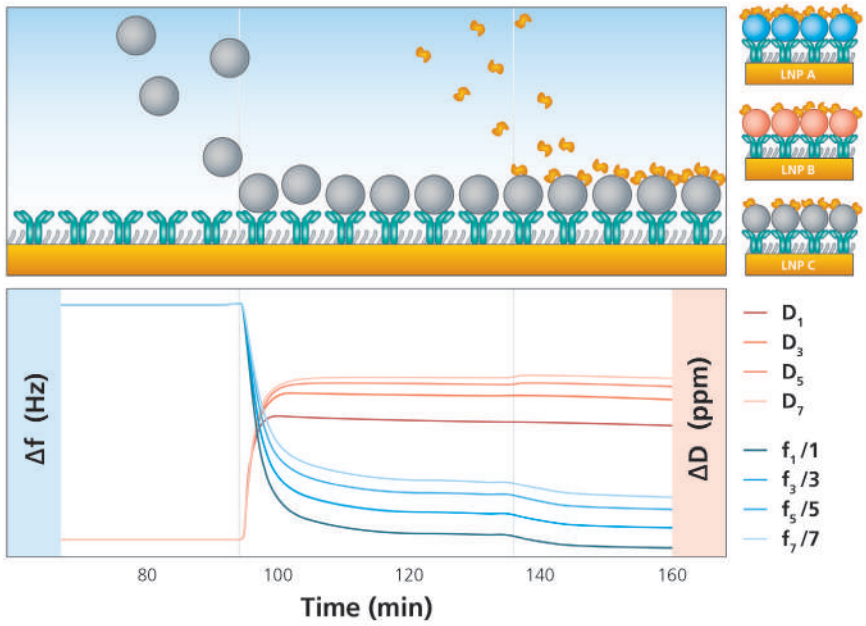

Figure 1. Binding kinetics of human apolipoprotein E (ApoE) to different types of lipid nanoparticles. The QCM-D resonance frequency (Δ*f*) and energy dissipation (Δ*D*) signals were tracked as a function of time to measure lipid nanoparticle attachment to the sensor surface, followed by ApoE addition to the immobilized nanoparticles.

Key Measurement Concepts:

Using biotin-streptavidin chemistry, gold-coated QCM-D sensor chips were functionalized with antibodies specific for human apolipoprotein E (ApoE) – which is a biologically important serum protein – or for polyethylene glycol (PEG) that is widely used in lipid nanoparticle formulation development. The key measurement objective was to measure the selective binding of ApoE- or PEG-coated lipid nanoparticles to the antibody-functionalized sensor chips and, in some cases, the subsequent adsorption of other serum proteins to immobilized nanoparticles.

Scientific Findings:

The anti-ApoE antibody-functionalized sensor chip could selectively bind to ApoE-coated lipoproteins and larger QCM-D signals were detected for the binding of ApoE-coated lipoprotein nanoparticles compared to smaller, free ApoE proteins. It was also possible to detect the selective binding of larger vs. smaller lipoprotein nanoparticle types due to the QCM-D technique's high surface sensitivity. The anti-PEG antibody-functionalized sensor chip could selectively bind to different types of PEG-coated lipid nanoparticles and ApoE protein binding to the immobilized nanoparticles could be studied subsequently. Importantly, the QCM-D measurements showed that the PEG-coated lipid nanoparticle type with the highest biological performance had the greatest amount of ApoE protein binding whereas the PEG-coated lipid nanoparticle type with the lowest biological performance had the smallest amount of ApoE protein binding (Figure 1).

Application Impact:

The QCM-D approach provides a fast, sensitive, and cost-efficient method to screen lipid nanoparticle formulations and to quantify the type and amount of serum protein binding to different lipid nanoparticles, which is correlated with corona formation and biological performance. Such insights related to protein corona formation are difficult to obtain with conventional biological assays and the unique merits of the QCM-D measurement approach can help to predict which nanoparticles might work the best in the human body. QCM-D technology is readily integrable into existing research and development pipelines and can be broadly applied to various classes of biomedical nanoparticles.

Related Applications:

In addition to monitoring nonspecific and specific protein binding to lipid nanoparticles, the QCM-D technique is useful for characterizing the nanomechanical and interfacial properties of lipid nanoparticles, including how they adsorb to and interact with membrane interfaces and other types of surfaces. Various types of lipid-enveloped biological nanoparticles like virus particles and exosomes can also be studied using the QCM-D technique in order to study adsorption properties and receptor-ligand interactions, which can aid molecular diagnostic and pharmaceutical drug development applications.

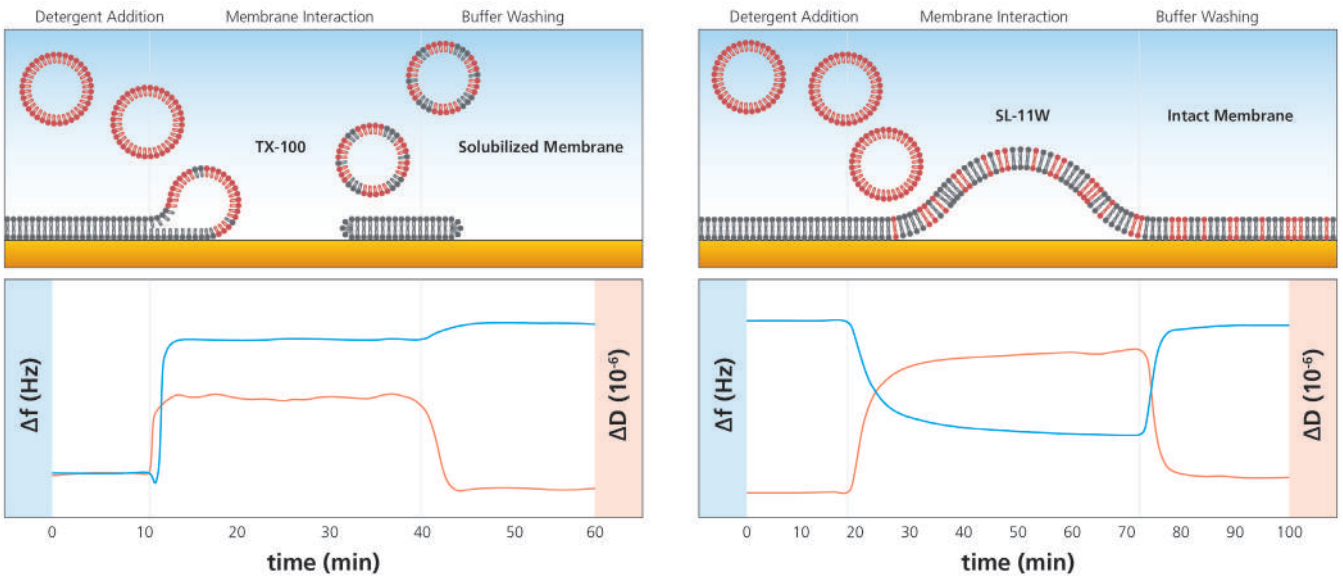

Figure 2. Membrane disruption efficiency of different antimicrobial detergents. A supported lipid bilayer (SLB) platform was fabricated on the sensor surface before Triton X-100 (TX-100) detergent or a replacement candidate (SL-11W) was added. TX-100 quickly caused membrane disruption and complete SLB solubilization whereas SL-11W caused large, temporary membrane morphological changes but not permanent membrane disruption.

Case Study 2: Biomacromolecular Interation

Membrane Disruption Analysis for Antimicrobial Detergent Testing[3]

Motivation:

Detergents play an important role in inhibiting membrane-enveloped pathogens such as bacteria and viruses in industrial bioprocessing and in blood transfusion applications. Until recently, the membrane-disrupting Triton X-100 (TX-100) detergent was the most popular option for pathogen inhibition, however, its use is being phased out due to environmental concerns. There is intense interest in finding antimicrobial detergents to replace TX-100 but it is difficult to develop effective replacements due to the limitations of biological assays. Indeed, it is critical to not only determine whether a new detergent inhibits pathogens as well as TX-100 but to also evaluate how the detergent disrupts lipid membranes—a mechanistic question that QCM-D technology is uniquely capable of answering quickly in a cost-effective manner.

Key Measurement Concepts:

To mimic pathogenic membranes, a supported lipid bilayer (SLB) was first formed on silica-coated QCM-D sensor chips by using the bicelle method.[3] Afterwards, different concentrations of TX-100 detergent or a proposed replacement detergent (SL-11W) were injected into the measurement chamber, and real-time changes in the mass and viscoelastic properties of the SLB platform were monitored. The maximum changes in the SLB properties during the detergent interaction process were quantified along with the final SLB properties after a buffer washing step. This measurement approach enabled determination of the minimum concentration at which each detergent caused membrane disruption in order to define their respective potencies as well as their corresponding speed and type of disruption such as membrane solubilization or binding.

Scientific Findings:

It was identified that TX-100 causes rapid and irreversible membrane solubilization of the SLB platform at detergent concentrations down to 250 µM. By contrast, SL-11W only caused extensive membrane disruption at 2000 µM and higher detergent concentrations, and the effects occurred gradually and were fully reversible. The concentration-dependent QCM-D results indicated that the detergents were only active above their respective critical micelle concentration values, establishing that TX-100 is approximately 8-times more potent than SL-11W. Importantly, the QCM-D measurement outputs demonstrated that the two detergents have distinct mechanisms of membrane disruption and function differently (Figure 2). More specifically, TX-100 caused irreversible membrane solubilization whereas SL-11W caused transient, reversible membrane budding.

Application Impact:

The QCM-D approach provided direct experimental evidence that the two detergents exhibited different potencies and mechanisms of action to disrupt lipid membranes. Such insights can be important for validating detergent performance in order to find functionally equivalent detergents with similar inhibitory efficacies and biophysical mechanisms. While antimicrobial detergents are typically screened in terms of biological performance, the used biological assays are slow, costly, require high technical expertise and safety standards, and provide only limited mechanistic information. QCM-D technology addresses all of these shortcomings by providing a fast and affordable tool to unravel mechanistic information in conventional lab settings.

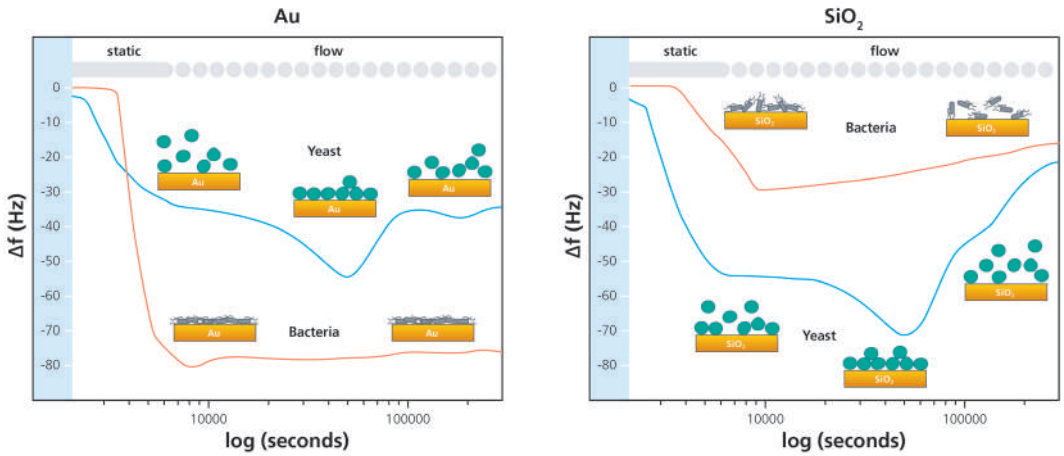

Figure 3. Measuring attachment of prokaryotic and eukaryotic cells to inorganic surfaces. *E. coli* bacterial cells and *S. cerevisiae* yeast cells served as model prokaryotes and eukaryotes, respectively, and were added to gold and silicon dioxide surfaces. The corresponding attachment and detachment kinetics were monitored under static and flow conditions.

Related Applications:

The QCM-D technique is highly sensitive to characterize the interfacial activity of detergents and surfactants at solid-liquid interfaces. In addition to lipid membrane platforms, the solubilizing effects of detergents to remove other types of particulates such as soil and oil from sensor surfaces can also be detected for cleaning applications. The real-time tracking capabilities are also able to unravel more complex biomacromolecular interaction processes such as membrane-peptide interactions related to anticancer and antiviral drug development.

Case Study 3: Biofouling

Eukaryotic vs. Prokaryotic Cell Adhesion and Detachment[4]

Motivation:

Understanding how cells adhere to and detach from different material interfaces is critical to fundamental biology and various biomaterial applications. Both cell-cell and cell-material interactions are important, especially when designing materials for medical implant and biosensing applications. In some cases, it is favorable to engineer material interfaces to resist cell adhesion while, in other cases, it is advantageous to promote the selective adhesion of certain cell types. QCM-D technology is highly useful for studying cell adhesion and detachment because the technique works with a wide range of organic and inorganic material surface types, is label-free, operates under static and flow conditions, and provides high kinetic resolution. In addition, QCM-D data analysis can aid viscoelastic characterization of adhered cell states.

Key Measurement Concepts:

The adsorption kinetics of model eukaryotic cells (*S. cerevisiae* yeast) and model prokaryotic cells (*E. coli* bacteria) onto gold and silicon dioxide surfaces, which have different surface free energies, was studied by the QCM-D technique. The experiments were conducted at different cell concentrations in phosphate-buffered saline with physiologically relevant salt concentrations, and cell adhesion was initially studied under flow conditions before flow was stopped to further probe cell attachment stability in static conditions over longer periods of time—all in the same measurement run. Time-independent plots of the resonance frequency vs. energy dissipation shifts were also prepared to analyze cytoskeletal changes in the adhered cell cytoskeletal structures. Similar experiments were also run with human embryonic kidney (HEK) cells as a more biologically complex eukaryote model.

Scientific Findings:

The results showed that *S. cerevisiae* yeast cells adsorbed to a greater extent on silicon dioxide surfaces than on gold surfaces (Figure 3). Interestingly, in both cases, cell detachment readily occurred during long-term incubation and the extent of detachment was in fact greater in the silicon dioxide case. Similar result trends were also obtained with HEK cells as a second type of tested eukaryotic cell. By contrast, *E. coli* bacterial cells adsorbed much more strongly to both surface types. The overall extent of bacterial cell attachment to gold surfaces was greater than on silicon dioxide surfaces and there was only minor cell detachment even after long-term incubation. Different stages of the cell attachment and detachment processes could also be identified by analysis of the frequency-dissipation plots.

Application Impact:

While various experimental techniques such as microscopy-based options can analyze cell attachment on certain solid surfaces, the QCM-D approach has several key advantages related to quantifying surface adsorption. The QCM-D technique works with various types of optically transparent and opaque surface coatings and does not require cell labeling. This latter point is particularly advantageous when considering that this one study example was able to study yeast, bacterial, and human cells using the same protocol. Another advantage of the QCM-D technique is the ability to monitor cell attachment/detachment in a well-controlled environment for long time periods while being able to controllably adjust the flow conditions.

Related Applications:

The biofouling of material surfaces is an important part of biological recognition processes and can influence the biocompatibility level. This measurement approach is also important for developing antifouling coatings that resist nonspecific cell adhesion, in which case the QCM-D technique can sensitively detect the degree of fouling in a quantitative manner, even if very small. In addition to studying cell-material interactions related to inorganic surfaces, cell interactions with different material surfaces such as lipid membrane and protein coatings can also be monitored and are relevant to tissue engineering and regenerative medicine applications.

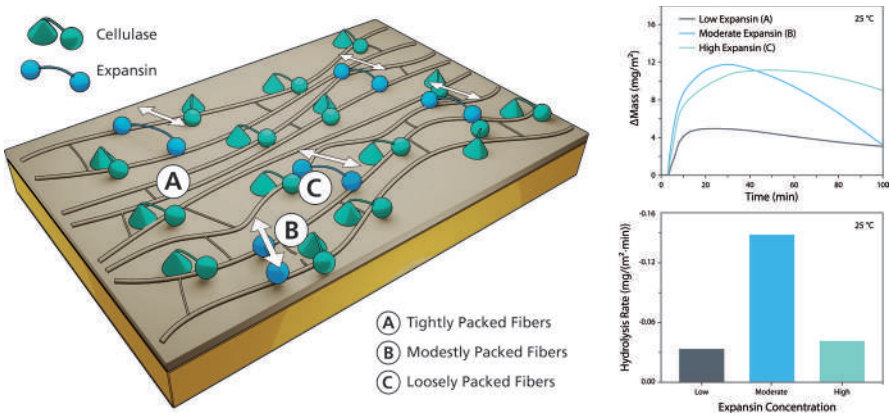

Figure 4. Effect of expansin protein on lignocellulose hydrolysis by cellulase enzyme. Expansin proteins bind to cellulose in order to loosen structure and aid enzyme binding as well. Measured levels of cellulase enzyme-mediated mass change rates, adsorption capacities, and hydrolysis rates in the presence of different expansin protein concentrations.

Case Study 4: Enzyme Activity

Optimizing Enzymatic Hydrolysis of Cellulose[5]

Motivation:

The enzymatic conversion of lignocellulose – the most abundant carbohydrate on the planet – into sugar monomers is an important need for biorefinery and clean energy applications. The binding of cellulase enzymes to cellulose is an important step in the enzymatic process, however, it can be difficult for cellulose to bind effectively, which in turn hinders enzymatic efficiency. There is interest in developing methods to improve efficiency and one promising strategy involves adding nonenzymatic expansin proteins, which can loosen the cellulose structure to aid cellulase binding. The effect of expansin on improving cellulase efficiency has been debated in the scientific literature and QCM-D technology has provided unique capabilities to study the real-time adsorption of cellulase to cellulose-coated surfaces in the presence of expansins along with resulting enzyme activity details.

Key Measurement Concepts:

The real-time enzymatic hydrolysis of thin cellulose films prepared on cationic polymer-modified gold surfaces was studied by the QCM-D technique. The measurement protocol involved first incubating the cellulose-coated surfaces in aqueous buffer to allow the cellulose films to swell until reaching equilibrium condition as indicated by stable QCM-D signals. Then, expansin and one of two cellulase types were injected into the measurement chamber containing the swollen cellulose film, and different expansin concentrations were tested. It was possible to track cellulase and expansin adsorption onto the cellulose film, followed by material desorption due to hydrolytic processes. Various quantitative parameters were evaluated, including initial adsorption rate, maximum adsorption capacity, and hydrolysis rate. The Voigt model was applied to QCM-D data collected at multiple overtones to estimate changes in film thickness and viscosity, and kinetic modeling was also performed.

Scientific Findings:

The QCM-D data demonstrated that protein adsorption occurred first, followed by net mass desorption from the sensor surface. When one cellulase type and expansin were added together, it was discovered that an optimal 1:1 ratio of the two proteins had the highest adsorption rate constant and hydrolytic rate whereas a higher expansin ratio demonstrated competitive binding that impeded enzymatic efficiency (Figure 4). A similar trend in the adsorption rate constant and hydrolytic rate values was observed with the second cellulase type, reinforcing that the presence of expansin can improve the rate of cellulase activity by up to five-fold compared to without expansin but also that excess expansin can lead to sub-optimal rate enhancement.

Application Impact:

The QCM-D technique provided a label-free approach to quantify multiple steps of complex enzymatic processes in order to optimize processing conditions. In this case study, the main focus was on directly verifying the enhancing effect of expansins on cellulase enzymes and also unraveling which mechanistic factors drive the synergism. Importantly, it was possible to discover that expansins work optimally at relatively low concentrations but higher concentrations can impede cellulase binding due to competitive adsorption. These mechanistic details could be discerned readily based on the experimental design and additional parameters like solution pH, temperature, and flow conditions are easy to study with the QCM-D approach.

Related Applications:

There are a wide range of enzymatic processes involving different classes of biomacromolecules that can be studied using the QCM-D technique. Additional examples include proteolytic degradation of protein films and phospholipase hydrolysis of lipid films as well as replicases for genome replication. Such studies can be

useful to elucidate fundamental molecular mechanisms while increasing attention has been placed on using the QCM-D approach to optimize processing conditions to maximize enzymatic performance. In some cases, it is also possible to test potential inhibitors of certain enzymes in order to determine potency and mechanisms of action, which can be relevant to medical and biotechnology applications.

Case Study 5: Interfacial Rheology

Monoclonal Antibody Aggregation on Steel Surfaces[6]

Motivation:

Therapeutic proteins such as monoclonal antibodies are an emerging class of medicine and quality control of the manufacturing and storage processes is critical to developing safe, well-characterized products. When antibodies are produced, they come into contact with various material surfaces such as steel and glass and these protein-material interactions can induce protein aggregation in some cases. The aggregates can be smaller than filtration pore sizes and thus remain in the final product so it is important to characterize potential aggregate formation on different material surfaces and to characterize the material properties of adsorbed antibodies at the solid-liquid interface. In this case study, QCM-D technology is shown to detect antibody fouling on steel surfaces and demonstrates the ability to characterize complex (two-layer) adsorbate formation using physics-based modeling approaches.

Key Measurement Concepts:

The adsorption kinetics of a monoclonal antibody onto stainless steel-coated surfaces was monitored as a function of antibody concentration by the QCM-D technique. The protocol involved a buffer baseline signal, injection of antibody solution at a relatively low flow rate, and then a buffer rinse step at a relatively high flow rate. The data were collected at multiple overtones in order to analyze the adsorbate properties with an appropriate model. During antibody adsorption, the measurement responses were complex and a two-layer Voigt model was applied to interpret the thickness, shear modulus, and interfacial viscosity of each layer. On the other hand, after buffer rinsing, it was possible to apply the simpler Sauerbrey model that assumes a single, rigid adlayer and can directly convert the resonance frequency shift into the adsorbed mass.

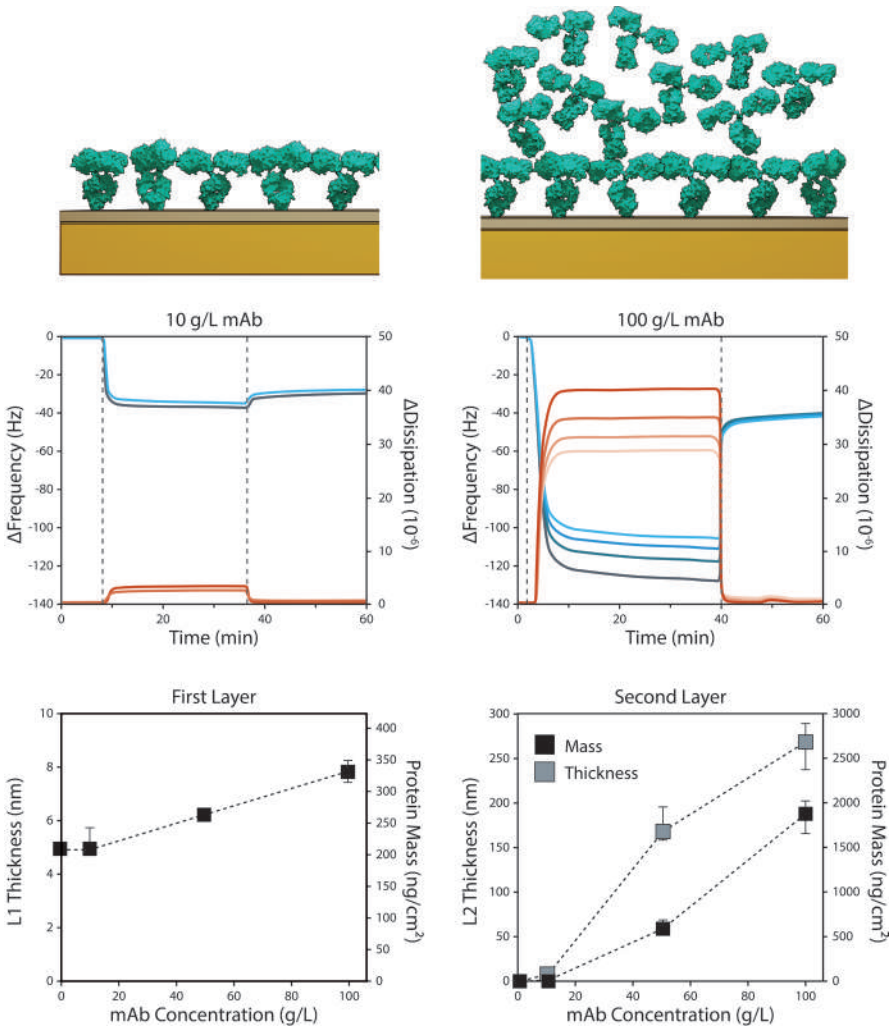

Figure 5. Monoclonal antibody adsorption and aggregation on steel surfaces. Representative QCM-D resonance frequency (Δf) and energy dissipation (ΔD) signals as a function of time at multiple overtones for low and high concentration antibody adsorption. Corresponding layer thicknesses obtained from different QCM-D modeling approaches.

Scientific Findings:

The results showed that antibody adsorption onto steel surfaces occurred in a concentration-dependent manner (Figure 5). At low antibody concentration, a rigid antibody layer of ~5 nm thickness adhered at the solid-liquid interface. At higher concentrations, there was a two-stage adsorption process consisting of a dense, rigid layer of ~7 nm thickness on the steel surface and a much thicker but less dense upper layer of up to ~250 nm thickness. The two-stage adsorption process was directly detected from the time-resolved QCM-D measurement signals while the complex film properties were obtained from subsequent modeling. After buffer rinsing, only rigidly attached antibody molecules remained on the steel surface so it was possible to use the Sauerbrey model for quantitative analysis and there was more remaining adsorbate in high antibody concentration cases than in low concentration cases.

Application Impact:

The QCM-D technique is highly sensitive to the viscoelastic properties of adsorbed biomacromolecules, which enables various modeling options to analyze one- and two-layer films. If the film is laterally homogenous, it is possible to extract quantitative information about film properties such as thickness, shear modulus, and viscosity. This case study shows the potential of rationally applying different modeling approaches in distinct application scenarios while such efforts can also be integrated with changing experimental parameters such as antibody type, solution conditions, and flow conditions. Interestingly, the QCM-D technique was able to detect the presence of the liquid-like upper layer at high antibody concentrations, whereas it was not possible to detect this second layer with neutron reflectivity due to weak contrast.

Related Applications:

Various industrial processes involve biomacromolecules such as proteins contacting material surfaces and the QCM-D technique is adept at characterizing the mass and viscoelastic properties of resulting adsorbates due to protein-material and protein-protein interactions. The capability to probe interfacial rheology can also be useful for studying thin polymer films and polyelectrolyte complexes, including stimuli-responsive systems that can exhibit softer or more rigid properties depending on the environmental conditions.

Interested to learn more?

If you would like to learn more about QSense QCM-D and how it can help you in your work, please reach out and we will tell you more. We would love to hear from you.

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