

TRACKING CHANGES AT THE SURFACE:

Q-Sense E4

rapid characterization of bio-interfaces



●● **Measure the mass**

of molecular layers forming on the surface with nanogram sensitivity. For example, 1% or less of a protein monolayer can be detected.

●● **Structural changes**

to be measured simultaneously so as to distinguish between two similar binding events or observe a phase transition in bound layers.

●● **Real time analysis**

allowing real-time recording and evaluation of kinetics.

●● **Label free**

No need for labelling of molecules, the instrument measures the molecules themselves.

●● **Flexible choice of surface**

including metals, polymers and chemically modified surfaces. Any surface that can be applied as a thin film can be used.

●● **Flow measurements**

Chamber specifically designed for flow measurements in a temperature-controlled environment.

●● **4-Sensor chamber**

allows higher throughput and makes reproducibility easier.

●● **Electrochemistry chamber**

Study electrochemical reactions simultaneously by using an optional electrochemistry chamber.

As the second generation of Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) from Q-Sense, the E4 offers the opportunity to study molecular interactions and molecular adsorption to many different types of surfaces. Applications include proteins, lipids, polyelectrolytes, polymers and cells/bacteria interacting with surfaces or with previously bound molecular layers.

The instrument determines the mass of very thin surface bound layers and simultaneously gives information about their structural (viscoelastic) properties. It is based on the patented QCM-D technique, an extremely sensitive and fast technique providing multi-frequency and dissipation data that are needed to fully understand the state of molecular layers bound to the sensor surface.

Today QCM-D plays a key role in the investigation of biomaterials, development of surfaces for biosensing and biochips and in fundamental research to understand processes taking place on surfaces or in thin films. It is often used as a complement to optical and imaging techniques such as Surface Plasmon Resonance (SPR) and Atomic Force Microscopy (AFM).

Q-Sense E4 is a complete turnkey instrument and includes everything needed to quickly get started and produce high quality data. The instrument has four flow modules, each holding one sensor. The flow modules can be used in any serial or parallel configuration to suit different measurements needs. It also includes software that allows the system to extract the thickness, viscosity and elasticity of adsorbed layers as well as fitting to kinetic models.

● ● ● ● THE E4 MEASUREMENT PROCEDURE



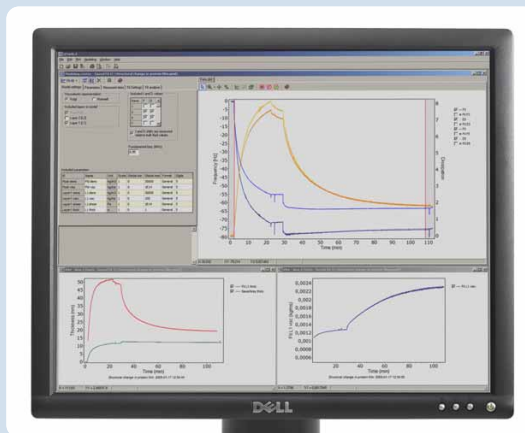
1. Mount quartz crystal sensors in the temperature controlled chamber. Four sensors are fixed in removable flow modules with inlet and outlet. The quartz crystal sensors may be pre-coated with, for example, metals, polymers or SAMs.



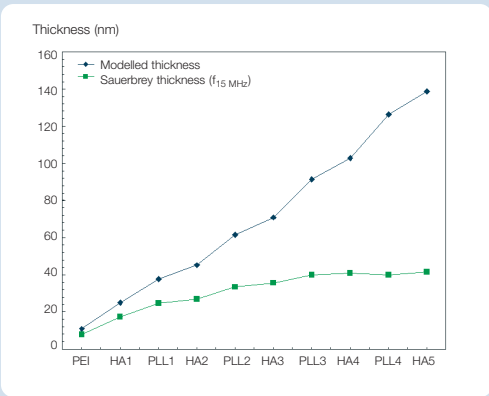
2. Introduce sample and conduct in-situ experiments. The chosen experimental procedure is run; for example, buffer followed by sample A and sample B and back to buffer.



3. Follow results in real time on the computer screen. Frequency changes reflect mass changes taking place on the sensor surface, dissipation changes reflect changes in the adlayer's viscoelastic properties.



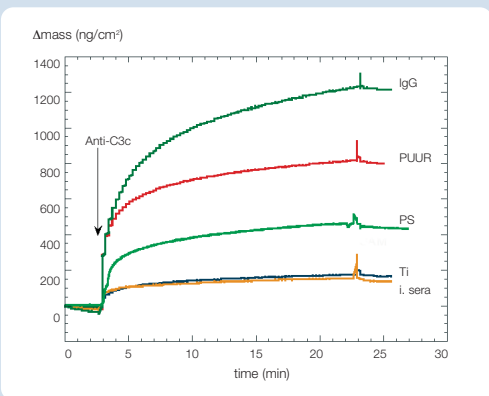
4. Analyse and present results in the software QTools. Extract mass, thickness, viscoelastic properties, kinetic constants, adsorption phases and so on.



Thickness of soft polymer multilayers

The alternate deposition of positively and negatively charged polymers onto solid substrates leads to the formation of so-called polyelectrolyte multilayers. In this example a polysaccharide, hyaluronic acid (HA), and a polyaminoacid, poly-L-lysine (PLL) were used in aqueous solution. The formed multilayer is soft, i.e. water rich, and the outer part of adsorbed layers does not follow the oscillation of the sensor surface. Therefore, the Sauerbrey relation, $\Delta m = -k \Delta f$, strongly underestimates the mass of the multilayer film. However by using multiple frequency and dissipation data and a viscoelastic representation of the film, implemented in software from Q-Sense, it becomes possible to extract mass and thickness of the multilayer.

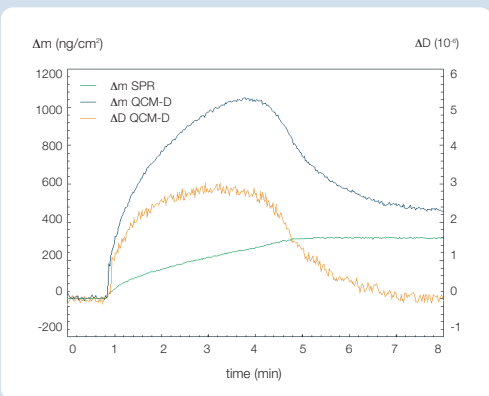
Ref: Picart et al. *Proc. Natl. Acad. Sci. U. S. A.* 99 (2002) p. 12531-12535



Mass of protein molecular layers adsorbed on different surfaces

Here, QCM-D is used as a screening method of biomaterial surfaces. A variety of surface preparations were incubated with human sera that contain the complement factor C3. The amount of bound anti-C3c in the subsequent step as shown in the figure was used as a measure of surface induced complement activation. In this case, the Sauerbrey relation could be used since the dissipation values were low (not shown). Complement activation was found on the pre-adsorbed IgG surface (positive control) and also on polystyrene (PS) and PUUR. The titanium (Ti) surface exhibited a low degree of anti-C3c binding similar to the negative control (heat-inactivated sera).

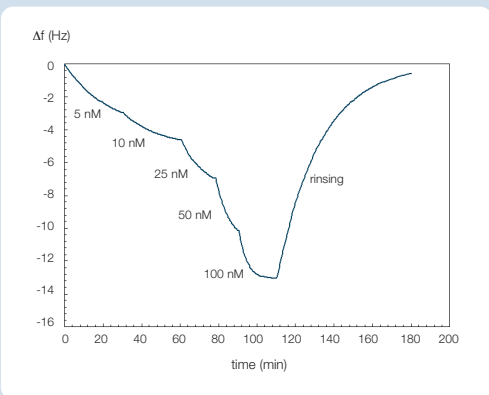
Ref: Sellborn et al. *Colloids and Surfaces B: Biointerfaces* 27 (2003) p. 295-301



Structure and water content of lipid molecular layers

Supported lipid membranes can be formed by the adhesion and spreading of lipid vesicles from aqueous solution on silica surfaces. The QCM-D signal of this process exhibits a peak in the dissipation before the signal returns to almost zero. This response is indicative for a phase transition in the adsorbed layer. Vesicles initially adsorb intact. They rupture and form a lipid membrane only after a critical surface coverage of vesicles is reached. The difference in the mass measured by QCM-D and by surface plasmon resonance (SPR) is due to water trapped inside and between adsorbed vesicles. Using QCM-D and SPR in parallel thus can give a detailed understanding of the complete self-organization process.

Ref: Reimhult et al. *Langmuir* 19 (2003) p. 1681-1691



Kinetics of molecular interactions

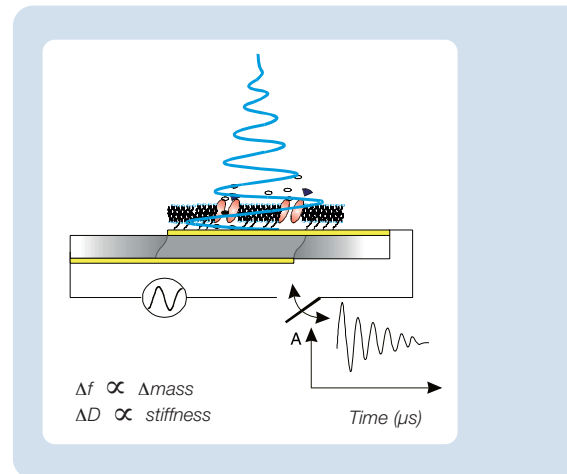
It is possible to estimate kinetic constants by monitoring adsorption upon sequential increase of the bulk concentration followed by monitoring of the desorption at zero bulk sample concentration. In this example linkage of reversibly bound cholesterol-DNA to a lipid bilayer was monitored and kinetic constants were determined to $K_D = 16.7 \pm 4$ nM and $k_{off} = 5.8 \times 10^{-4} \text{ s}^{-1}$ ($k_{off}/k_{on} = K_D$). It is assumed that the amount of coupled water per molecule does not vary with coverage.

Ref: Indriati Pfeiffer et al. *JACS* 126 (2004) p. 10224-10225

The heart of the system is a quartz crystal sensor that consists of a thin disc of crystalline quartz sandwiched between two electrodes. An AC voltage applied over the electrodes induces a small shear oscillation in the sensor at resonance. The oscillation will decay exponentially if the driving AC voltage is turned off. In the QCM-D technique, this decay is recorded and two parameters, the resonance frequency (f) and the dissipation (D) are extracted.

The resonance frequency of the sensor crystal depends on the total oscillating mass. For thin, rigid films the Sauerbrey relation, $\Delta m = -k \Delta f$ may be used to calculate the mass of adsorbed layers following the oscillation of the sensor.

A deposited film that is soft and viscous will dissipate energy (via frictional losses in the film) and the sensor oscillation is damped. The dissipation gives information about the structure of the thin film attached to the sensor. A compact globular protein adsorbed to the surface gives rise to only a low dissipation (rigid film) while an elongated protein with a lot of coupled water results in higher dissipation (soft film).



By using multiple frequency and dissipation data it becomes possible to calculate mass, thickness, viscosity and elasticity, using a viscoelastic model, outside the Sauerbrey regime. These calculations are done in software Q-Tools, included in the E4 system.

● ● ● ● SPECIFICATIONS

Specifications subject to change without notice

Sensors and sample handling system				
Number of sensors	4, also possible to measure using only 1, 2 or 3 sensors			
Volume above each sensor	~ 40 μ l using Q-Sense Flow Module for 5 MHz crystals			
Minimum sample volume	~ 200 μ l			
Working temperature	18 to 45 °C, controlled via the software, stability \pm 0.02 K			
Flow rates	0-1 ml/min			
Cleaning	All parts exposed to liquid can be easily removed and cleaned in e.g. ultrasound bath			
Sensor crystals*	5 MHz, 14 mm diameter, polished, AT-cut, gold electrodes			
Frequency and dissipation characteristics				
Frequency range	1-70 MHz (allows 7 frequencies, up to the 13th overtone, 65 MHz for a 5 MHz crystal)			
Maximum time resolution, 1 sensor, 1 frequency	~ 100 data points per second			
Maximum mass sensitivity in water**	~ 0.5 ng/cm ² (5 pg/mm ²)			
Normal mass sensitivity in water***	~ 1.8 ng/cm ² (18 pg/mm ²)			
Maximum dissipation sensitivity in water**	~ 0.04 x 10 ⁻⁶			
Normal dissipation sensitivity in water***	~ 0.1 x 10 ⁻⁶			
Software				
PC requirements	USB 2.0, Windows 2000, XP			
Input data, analysis software	Multiple frequency and dissipation data			
Output data, analysis software	Modelled values of viscosity, elasticity, thickness and kinetics			
Import/Export	Excel, BMP, JPG, WMF etc			
Dimensions				
	Height (cm)	Width (cm)	Depth (cm)	Weight (kg)
Electronics unit	18	36	21	9
Measurement chamber	12	23	34	8

* Several other sensor materials are available, for example, SiO₂, Titanium, Stainless steel, Polystyrene to mention a few.

** Data from 1 sensor in single frequency mode. 1 data point is collected every 5 seconds. The Sauerbrey relation is assumed to be valid.

*** Data from all 4 sensors in multiple frequency mode (3 harmonics) are collected within 1 second. The Sauerbrey relation is assumed to be valid.

Contact details to US office and to a worldwide network of authorised distributors are found on the Q-Sense web site:

www.q-sense.com