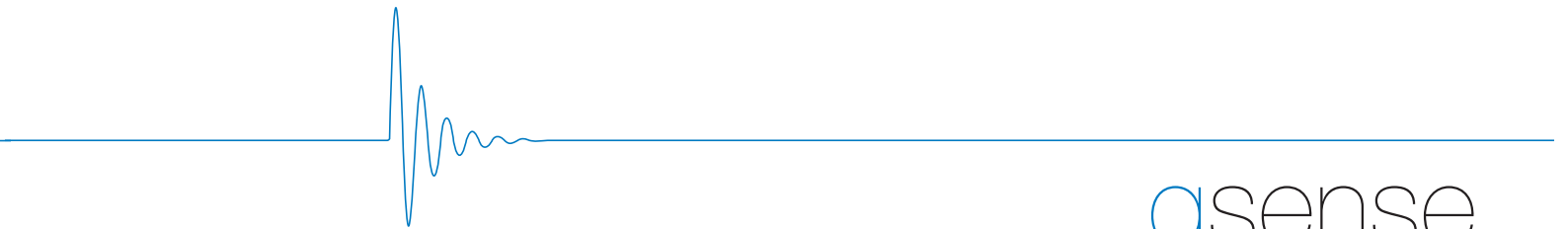
Cleaning & Immobilization Protocols

Sensor Surface	*Recommended Cleaning Protocol*
QSX 301 Gold	A-I
QSX 303 Silicon Dioxide	B
QSX 304 Stainless Steel	C-I
QSX 305 Polystyrene	D
QSX 309 Aluminum Oxide	E-I (or B)
QSX 310 Titanium	C-I (or B)
QSX 311 Tantalum	C-I
QSX 314 Platinum	A-II
QSX 316 Iridium	C-II
QSX 318 Silicon Dioxide 300nm	B
QSX 319 Iron	C-II
QSX 322 Silver	C-II
QSX 324 Tantalum Nitride	C-I
QSX 325 Cerium Oxide	E-I
QSX 326 Iron Oxide	E-II
QSX 327 Hydroxyapatite	E-III
QSX 328 Silicon Nitride	B
QSX 330 Zirconium Oxide	B

For surface materials not listed here we advise testing mild cleaning protocols first.

Instrument	*Recommended Cleaning Protocol*
After each measurement	Daily
Between experiments	Thorough

Surface Preparation	*Recommended Prep. Protocol*
Spin-coating of polymers	Spin
NTA/Ni2+-doped supported lipid bilayer	NTA/Ni
Biotinylated albumin	b-BSA
Biotinylated supported lipid bilayer	b-SLB



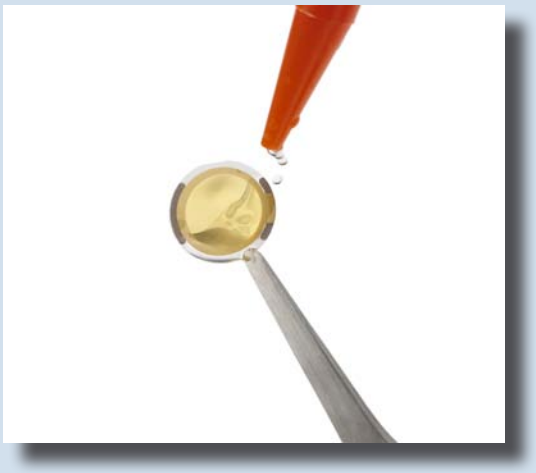
The cleaning protocols described in this document are a collection of standard protocols recommended by Q-Sense for our sensor surfaces and instrument flow path interior.

The suggested protocols are not harmful to the sensor coatings themselves, *however note that there is no guarantee that materials adsorbed onto the coatings are removed - this depends on the coupling chemistry of the adsorbed materials.*

When working with Q-Sense sensors, it is recommended to use the sensor holder in Teflon (Order number QCLH 301) to avoid direct contact with the sensor surfaces as much as possible, and thereby to prolong their life.

When holding a sensor using tweezers, grip it on the edge to avoid scratches to the surface.

By rinsing towards the tweezers, you minimize the risk of transfering contaminants from the tweezers onto the sensor.



UVO

Applicable to:	All sensor surfaces, unless oxidation is un-desired, except for QSX 322 (Ag).
Function:	Removes low molecular weight hydrocarbons through oxidation.

The UV/Ozone treatment is performed in a UV/ozone chamber, in which light is generated at the wavelengths 185 nm and 254 nm. The surface is treated both with the UV wavelength from the lamp and the generation of ozone by breakage of the O-O bond by the 185 nm wavelength. The treatment works in two ways: organic contaminants on the sensor surface are volatilized and readily removed and the underlying surface is slightly oxidized. Note that the treatment does not work for removal of thick organic films.

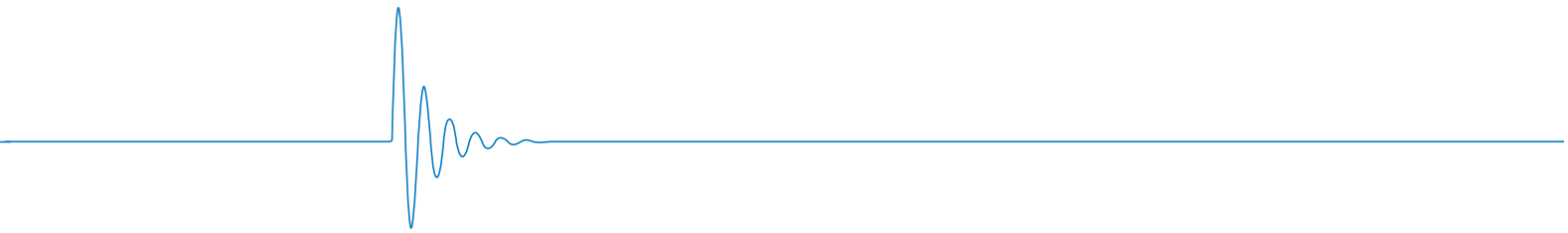
Protocol UVO

1. Place the sensor surfaces in a UV/ozone chamber, approximately 5 mm from the lamp.
2. Turn on the UV lamp for 5-10 minutes. The time is dependent on the power of the lamp. A minimum of 12 mW/cm^2 at 1 inch from a 185/254 nm lamp is recommended.

References

J.R. Vig, *Journal of Vac. Sci. Technol.* A 3 (1985) p1027

Krozer et al, *Journal of Vac. Sci. Technol.* A 15 (1997) p1704



Chemical treatment: Ammonium Peroxide Mix (TL1)

A-I
A-II

Applicable to:	A-I: QSX 301, tweezers A-II: QSX 314
Function:	Efficient removal of organic and biological material through oxidation. The procedure should be carried out under a fume hood, with proper protection (eye wear and gloves).

Protocol A-I

1. UV/ozone treat for 10 minutes (see UVO treatment).
2. Heat a 5:1:1 mixture of milliQ water, ammonia (25%) and hydrogen peroxide (30%) to 75°C, approx. 10 ml is sufficient.
3. Place the sensor in the heated solution for 5 minutes.
4. Rinse with milliQ water. *It is important that the surfaces are kept wet after ammonium-peroxide immersion until they are rinsed well with water.*
5. Dry with nitrogen gas.
6. UV/ozone treat for 10 minutes (see UVO treatment).

Protocol A-II

1. Heat a 5:1:1 mixture of milliQ water, ammonia (25%) and hydrogen peroxide (30%) to 75°C, approx. 10 ml is sufficient.
2. Place the sensor in the heated solution for 5 minutes.
3. Rinse with milliQ water. *It is important that the surfaces are kept wet after ammonium-peroxide immersion until they are rinsed well with water.*
4. Dry with nitrogen gas.
5. Rinse with 99% ethanol.
6. Dry with nitrogen gas.

Reference (A-I)

W. Kern et al, *RCA Review* 31 (1970) p187

Surfactant Treatment: Sodium Dodecyl Sulfate

B

Applicable to:	QSX 303, QSX 328, QSX 330
Function:	Removes organic and biological material through lowering of interfacial tension. Works well for removal of proteins and lipids.

Protocol B

1. UV/ozone treat for 10 minutes (see UVO treatment).
2. Prepare a solution of 2% Sodium Dodecyl Sulfate (SDS) in milliQ water.
3. Immerse the sensor surfaces in the solution for 30 min in room temperature.
4. Rinse with milliQ water. *It is important that the surfaces are kept wet after SDS immersion until they are rinsed well with water.*
5. Dry with nitrogen gas.
6. UV/ozone treat for 10 minutes (see UVO treatment).

References

K. Harewood et al, *Anal. Biochem.* 55 (1973) p573

J. Penfold et al, *Langmuir* 18 (2002) p5755

Surfactant Treatment: Hellmanex

C-I
C-II

Applicable to:	C-I: QSX 304, QSX 310, QSX 311, QSX 324 C-II: QSX 316, QSX 319, QSX 322
Function:	Removes organic and biological material through lowering of interfacial tension. Hellmanex gives alkaline solutions and may etch glassware and quartz. Works well for removal of proteins and lipids; is somewhat more aggressive than SDS treatment.

Protocol C-I:

1. Immerse the sensor surfaces in 1% Hellmanex II (see www.hellma-worldwide.com) for 30 minutes at room temperature. QSX 304 and QSX 311 can be kept in the solution for 12 hours.
2. Rinse with milliQ water.
3. Dry with nitrogen gas.
4. Sonicate in 99% ethanol for 10 minutes.
5. Rinse with milliQ water.
6. Dry with nitrogen gas.
7. UV/ozone treat for 10 minutes (see UVO treatment).

Protocol C-II:

1. Immerse the sensor surfaces in 1% Hellmanex II (see www.hellma-worldwide.com) at room temperature for 30 minutes (QSX 322); 3 hours (QSX 319); or 12 hours (QSX 316).
2. Rinse with milliQ water.
3. Dry with nitrogen gas.
4. Sonicate in 99% ethanol for 10 minutes.
5. Rinse with milliQ water.
6. Dry with nitrogen gas.

Reference

A.-C. Olofsson et al, *Appl. Environ. Microbiol.* 71 (2005) p2705 (QSX 304 sensors only)

D

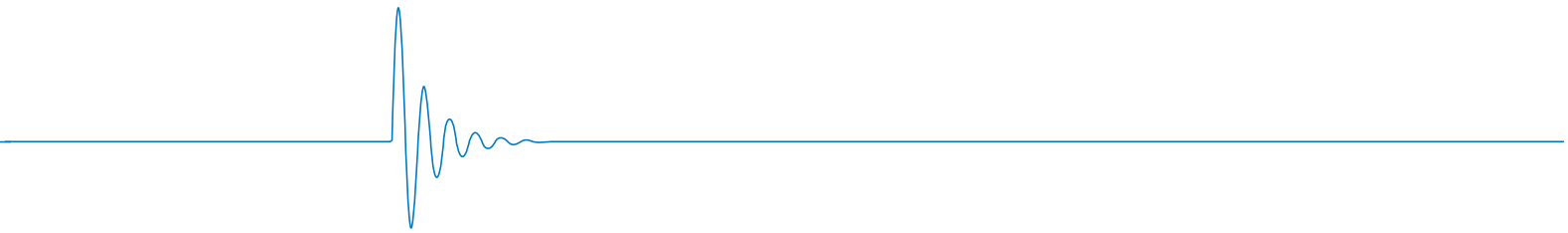
Applicable to:	QSX 305
Function:	Removes organic and biological material through lowering of interfacial tension. Works well for removal of proteins and lipids.

Protocol D

1. Prepare a solution of 1% Deconex 11 (see www.borer.ch) in milliQ water.
2. Immerse the sensor surfaces in the solution for 30 min at 30°C temperature.
3. Rinse with milliQ water.
4. Keep in milliQ water for at least 2 hours.
5. Rinse with 99% ethanol.
6. Dry with nitrogen gas.

Reference

A. Naderi et al, *Langmuir* 22 (2006) p7639



Solvent Treatment: Alcohol

E-I
E-II
E-III

Applicable to:	E-I: QSX 309, QSX 325 E-II: QSX 326 E-III: QSX 327
Function:	Removes organic and biological material through dissolution.

Protocol E-I:

1. Sonicate the sensor surfaces in 99% ethanol for 15 minutes.
2. Rinse with milliQ water.
3. Dry with nitrogen gas.
4. UV/ozone treat for 10 minutes (see UVO treatment).

Protocol E-II:

1. Sonicate the sensor surfaces in 99% ethanol for 15 minutes.
2. Rinse with milliQ water.
3. Dry with nitrogen gas.

Protocol E-III:

1. UV/ozone treat for 10-20 minutes (see UVO treatment).
2. Immerse the sensor surfaces in 99% ethanol for 30 minutes.
3. Rinse with milliQ water.
4. Dry with nitrogen gas.
5. UV/ozone treat for 10-20 minutes (see UVO treatment).

References

K. D. Kwon et al, *Environ. Sci. Technol.* 40 (2006) p27739 (QSX 309)
Q-Sense and Promimic (QSX 327)

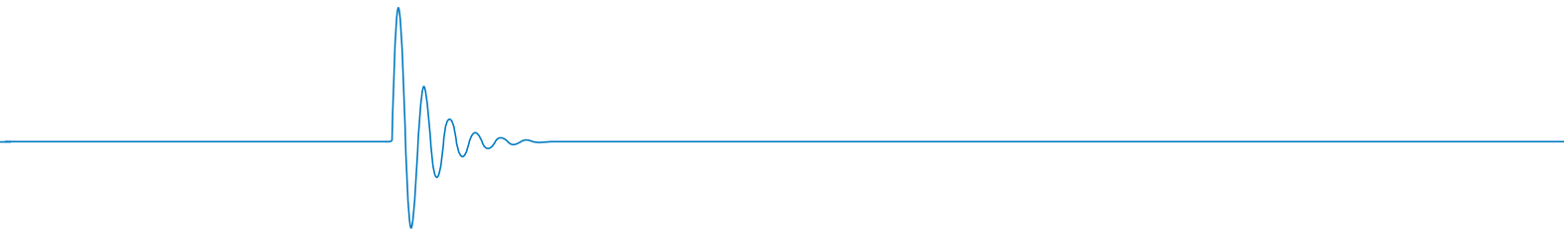
Daily

Applicable to:	E4, E1, D300
Function:	Removes organic and biological material through lowering of interfacial tension.

A common reason for drifting measurements and unexplained behaviors is contamination of the flow system itself. After each measurement it is important to clean the system and to finish with a pure water rinse.

Protocol Daily

1. Mount a sensor surface into the chamber / flow module(s).
2. Pump approximately 20 ml of 2% Hellmanex II through the system (heating to 30°C could improve the cleaning effect).
3. Pump 100 ml of milliQ water through the system.
4. Empty the chamber / flow module from liquid and dry visible parts with nitrogen gas.



Thorough

Applicable to: E4, E1

Function: Removes organic and biologic material through lowering of interfacial tension.

Protocol Thorough:

1. Open the module and unscrew the 6 screws holding the flow part together, using a PH-1 screwdriver.
2. Pry or twist the two metal pieces apart, and remove sealing gasket and o-ring.

3. Immerse all flow parts in Sodium Dodecyl Sulfate (SDS), dissolved to 2% in milliQ water. ***Note*** *that the contact block with the two electrode pins should never be immersed in any cleaning solution!*
4. Sonicate while heating to 40°C.
5. Rinse with milliQ water.
6. Dry with nitrogen gas.
7. Optionally, replace gasket and o-ring.
8. Reassemble. Take care not to overtighten the 6 M2 screws.

Spin

Applicable to: Any sensor surface

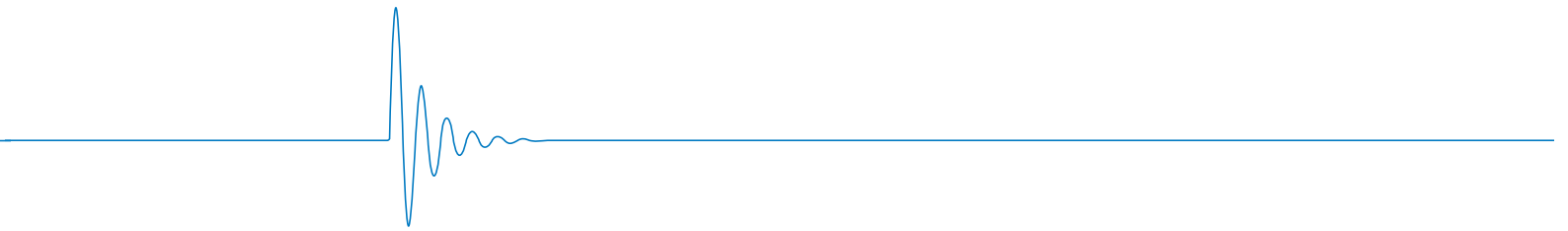
Function: Forms a thin even polymer coating.

By varying the concentration and spinning velocity, different thicknesses can be achieved. Since the solvent used, typically are hazardous chemicals, spin-coating should always be performed under a ventilated hood, with proper protection (eye wear and gloves).

Protocol

1. UV/ozone treat the sensor crystals for 10 minutes (see UVO treatment).
2. Prepare a polymer solution according to the table below.
3. Place the sensor crystal on the spin coater.
4. Apply 2-3 droplets of the polymer solution.
5. Spin the crystal at 2000 rpm for 20 seconds.
6. Check that no polymer residues have contaminated the backside of the sensor. If any, clean this excess polymer off with a cotton stick soaked in solvent.
7. Evaporate all solvents by placing the crystals in an oven at ~80ºC for 30 minutes.

Polymer	Solvent	Conc. w/w
PS	Toluene	0.5%
PMMA	Dichloromethane	0.5%
PC	Dichloromethane	0.5%



Surface Functionalization: NTA/Ni^{2+} Complex

NTA/Ni

Applicable to:	QSX 303
Function:	Forms a supported phospholipid bilayer, doped with NTA/Ni2+ complexes.

Materials

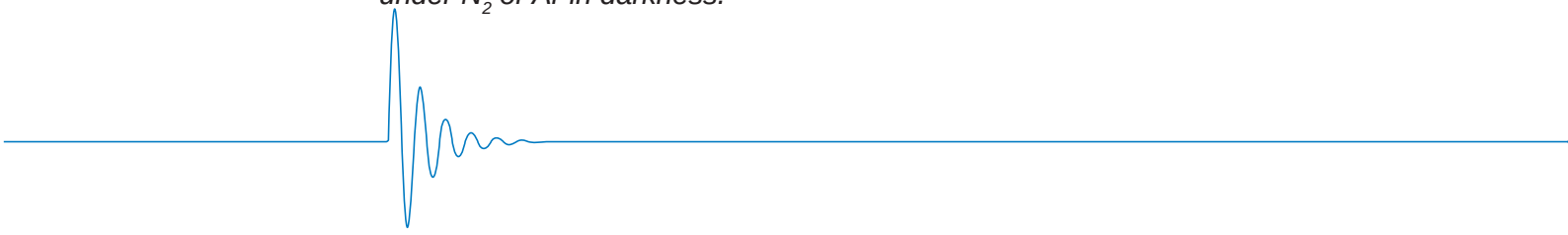
- SiO_2-coated Q-Sense sensor crystals [QSX 303].
- Palmitoyl-Oleoyl Phosphatidylcholine (POPC) [Avanti Polar Lipids, USA, p/n 850457, MW 760 g/mol]
- Nitriloacetic acid (NTA) synthesized with lipid structures (DOGS) [Avanti Polar Lipids, USA, p/n 790528, MW 1015.40 g/mol]
- $NiCl_2$
- Sodium Dodecyl Sulphate, 0.4%
- Tris buffer, 10 mM, with NaCl, 100 mM/300 mM, pH 8.0

1. Cleaning of the sensor surface

Immerse the SiO_2-coated sensor crystal(s) in SDS, 0.4% for 2 hours, and rinse thoroughly in MilliQ water afterwards. Dry with N_2 gas. Treat with UV/ozone for 15 minutes to remove any further hydrocarbon contaminations. Rinse again with MilliQ water and dry with N_2 gas. *Note: it is important that the surfaces are kept wet after SDS immersion until they are well rinsed with water.*

2. Preparation of functionalized vesicles

Dissolve 95mol% of the synthetic lipid POPC and 5mol% DOGS-NTA in chloroform in a beaker. Remove the chloroform solvent by drying under N_2 gas stream for 1 hour. Redissolve the lipids in a buffer of 100 mM Tris, 100 mM NaCl, pH 8.0, to a final concentration of 8.5 mg/ml. Sonicate the lipids in a bath sonicator until the solution turns clear. In order to reduce the vesicle size distribution to around 25 nm Ø, centrifuge the sonicated liquid at 65,000 rpm for 4.5 h, remove the top 100 µl fraction and collect the rest of the supernatant. *Sonication adds the energy necessary to overcome the barrier for the vesicle formation. When this happens, the solution changes from milky white to almost clear. Vesicle solution should be stored in 4°C under N_2 or Ar in darkness.*



3. Application of lipid bilayers

De-gas the (Tris 100 mM, NaCl 300 mM, pH 8.0)-buffer (e.g. by sonication) to avoid air bubble formation when it is flowed through the QCM-D chamber/module. Inject 40 µl of the NTA-doped vesicle solution in 2 ml of the buffer. *The vesicles will now split up and form supported bilayers. An initial dip in frequency and mirrored peak in dissipation will confirm the adsorption of intact vesicles before decomposing into a bilayer. A successful formation should cause a frequency shift of around -26 Hz (normalized) and a final D-shift close to zero.*

4. Activation of NTA-groups

Dissolve $NiCl_2$ in the de-gassed (Tris 100 mM, NaCl 300 mM, pH 8.0) to a saturated solution (e.g. 2.5 mM) and rinse over the sensor crystal for 5 minutes. *The excessive amount of $NiCl_2$ will activate the NTA-groups to form the NTA-Ni^{2+} complex. The NTA chelate agent couples to Ni^{2+} at 4 positions, and leaves 2 positions for interactions with other molecules.*

The obtained NTA/Ni-doped lipid bilayer is now available for protein interactions via the unoccupancies on the Ni^{2+} ion. It has e.g. been shown that the amino acid Histidine has a certain affinity to the NTA/Ni complex, especially when appearing as an oligo-histidine with 6 residues in a row. Further methods for anchoring of proteins via His-tags can be found elsewhere.

Since non-functionalized supported lipid bilayers are found to be resistant to protein adsorption, the surface is an excellent platform for controlled immobilization and coupling events.

Removal of coupled proteins/ Ni^{2+} ions

After completed measurement, the sensor crystal can be rinsed with either EDTA or Imidazole for a couple of minutes for re-access to the NTA-surface. Bound proteins and Ni^{2+} ions are then removed.

References:

- S. A. Lauer, “Development and Characterization of Ni-NTA-Bearing Microspheres”, *Cytometry* vol 48 (2002) p136-145 – *Includes molecular structures.*
- *C.* Larsson et al, “Gravimetric antigen detection utilizing antibody-modified lipid bilayers”, *Analytical Biochemistry* vol 345 (2005) p72-80
- K. Glasmästar et al., ”Protein Adsorption on Supported Phospolipid Bilayers”, *J. of Colloid and Interface Science* vol 246 (2002) p40-47 – *discusses the inertness of lipid bilayers.*

Surface Functionalization: Biotinylated Albumin

b-BSA

Applicable to:	QSX 301
Function:	Forms a linker surface for Streptavidin/biotin coupling chemistry.

Materials

- Au-coated Q-Sense sensor crystals [QSX 301]
- Biotin-labeled Bovine Serum Albumin, 8-16 mol biotin per mol albumin [Sigma-Aldrich, p/n A8549]
- Streptavidin, [Sigma-Aldrich, p/n S4762, MW 60,000 kDa] or Neutravidin [Pierce Biotechnology, p/n 31000]
- Tris buffer, 10 mM, NaCl, 100 mM, pH 8.0

1. Cleaning of the sensor surface

Heat a 5:1:1 mixture of Milli-Q water, ammonia (25%), and hydrogen peroxide (30%) ("APM" mixture) to 75°C. Place the Au-coated sensor crystal(s) in the heated solution for 5 minutes. Rinse thoroughly in MilliQ water afterwards and dry with N_2 gas. Treat with UV/ozone for 15 minutes to remove any further hydrocarbon contaminations. Rinse again with MilliQ water and dry with N_2 gas. *Note: it is important that the surfaces are kept wet after APM immersion until they are well rinsed with water.*

2. Application of Biotinylated Albumin

Dissolve biotinylated Albumin to10 µg/ml in de-gassed MilliQ water. Rinse the solution over the sensor, mounted in the chamber/module. *A successful adsorption should result in a frequency shift of around -15 Hz (normalized) and a D-shift close to zero.*

3. Application of Streptavidin

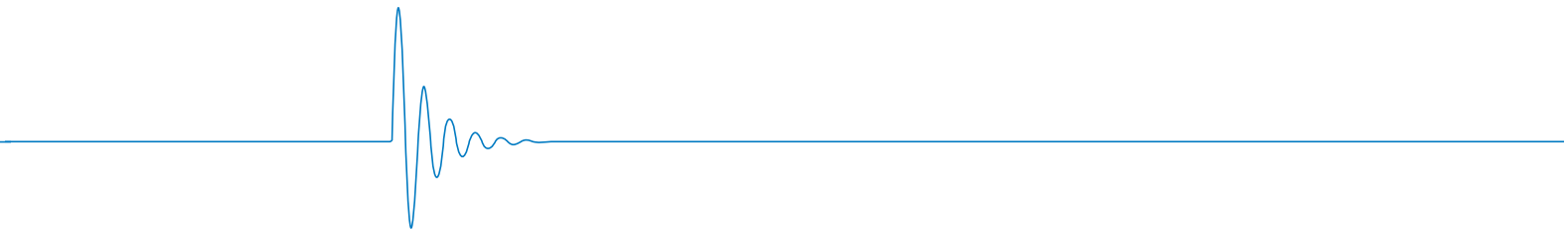
Dissolve Streptavidin OR Neutravidin, 10 µg/ml in de-gassed buffer (Tris 10 mM, NaCl 100 mM). *A successful binding should also here cause a frequency shift of around -15 Hz (normalized) and a D-shift <1 E-6.*

The obtained Streptavidin/Neutravidin layer is now available for protein interactions via further biotin coupling.

It should be noted that the inertness of biotinylated Albumin might not be as reliable as e.g. of phospholipid bilayers. As to what Q-Sense knows, unspecific binding of molecules directly to free spots on the solid surface may occur in the next measurement step.

Reference

S. Svedhem et al, “Patterns of DNA-labeled and scFv-Antibody-Carrying Lipid Vesicles Directed by Material-Specific Immobilization of DNA and Supported Lipid Bilayer Formation on an Au/SiO2 Template”, *ChemBioChem* (2003), No 4



Surface Functionalization: Biotinylated Lipid Bilayer

b-SLB

Applicable to:	QSX 303
Function:	Forms a linker surface for Streptavidin/biotin coupling chemistry.

Materials

- SiO_2-coated Q-Sense sensor crystals [QSX 303]
- Biotin-labeled Phospholipids (Biotin Cap DPPE) [Avanti Polar Lipids, USA, p/n 870277]
- Palmitoyl-Oleoyl Phosphatidylcholine (POPC) [Avanti Polar Lipids, USA, p/n 850457, MW 760 g/mol]
- Streptavidin, [Sigma-Aldrich, p/n S4762, MW 60,000 kDa]
- Tris buffer, 10 mM, NaCl, 100 mM, pH 8.0

1. Cleaning of the sensor surface

Immerse the SiO_2-coated sensor crystal(s) in SDS, 0.4% for 2 hours, and rinse thoroughly in MilliQ water afterwards. Dry with N_2 gas. Treat with UV/ozone for 15 minutes to remove any further hydrocarbon contaminations. Rinse again with MilliQ water and dry with N_2 gas. *Note: it is important that the surfaces are kept wet after SDS immersion until they are well rinsed with water.*

2. Preparation of functionalized vesicles

Dissolve 95mol% of the synthetic lipid POPC and 5mol% Biotin Cap DPPE in chloroform in a beaker. Remove the chloroform solvent by drying under N_2 gas stream for 1 hour. Redissolve the lipids in a buffer of 10 mM Tris, 100 mM NaCl, pH 8.0, to a final concentration of ~10 mg/ml. Sonicate the lipids in a bath sonicator until the solution turns clear. Centrifuge the sonicated liquid at 65,000 rpm for 4.5 h, remove the top 100 µl fraction and collect the rest of the supernatant, which now content small unilamellar vesicles. *Sonication adds the energy necessary to overcome the barrier for the vesicle formation. Vesicle solution should be stored in 4°C under N_2 or Ar in darkness.*

3. Preparation of Streptavidin

Dissolve Streptavidin in de-gassed buffer (Tris 10 mM, NaCl 100 mM, pH 8.0) to approx. 10 µg/ml.

4. Application of lipid bilayers

De-gas the (Tris 10 mM, NaCl 100 mM, pH 8.0)-buffer (e.g. by sonication) to avoid air bubble formation when it is flowed through the QCM-D chamber/module. Dilute the vesicle solution in buffer by a factor of 100 and inject it. *The vesicles will now split up and form supported bilayers. A successful formation should cause a frequency shift of around -26 Hz (normalized) and a final D-shift close to zero. An initial dip in frequency and mirrored peak in dissipation will confirm the adsorption of intact vesicles before decomposing into a bilayer.*

5. Application of Streptavidin

When the lipid bilayer adsorption has stabilized, expose the surface to Streptavidin. *A successful binding should cause a frequency shift of around -25 Hz (normalized) and a D shift of ~0.5 E-6.*

The obtained Streptavidin layer is now available for protein interactions, via further biotin coupling. Since non-functionalized supported lipid bilayers are found to be resistant to protein adsorption, the surface is an excellent platform for controlled immobilization and coupling events.

References

- F. Höök et al., ”Characterization of PNA and DNA Immobilization and Subsequent Hybridization with DNA Using Acoustic-Shear-Wave Attenuation Measurements”, *Langmuir* (2001) 17, p8305-8312
- J. Salafsky et al., “Architecture and Function of Membrane Proteins in Planar Supported Bilayers: A Study with Photosynthetic Reaction Centers”, *Biochemistry* (1996) 35, p14773-14781
- K. Glasmästar et al., ”Protein Adsorption on Supported Phospolipid Bilayers”, *J. of Colloid and Interface Science* vol 246 (2002) p40-47 – *discusses the inertness of lipid bilayers.*
- C. Larsson, Lic. Thesis at Chalmers University of Technology, Applied Physics, Sweden 2003, “Functionalized Lipid Assemblies for Biosensing Applications”