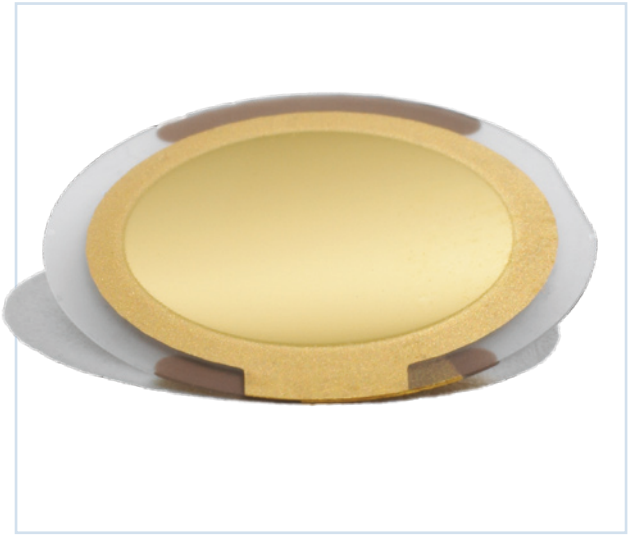
Q-Sense Biotin Functionalized Sensor

Label-free measurement of biomolecular interactions can be conveniently performed by immobilizing one of the interacting species onto the surface of a sensor. Q-Sense Biotin Functionalized Sensor, QSX 339, enables immobilization via the commonly used high affinity interaction between Biotin and Streptavidin [1-4]. The affinity between Biotin and Streptavidin is the highest of any known biological ligand pair, K_a=2.3x10^{13} M^{-1}.



Q-Sense delivers QSX 339 as single sensors dark-packed to minimize light exposure that would otherwise degrade the Biotin functionality. QSX 339 can be used with your streptavidin or avidin analogue of choice (please note that these proteins are not provided by Q-Sense).

Sensor Specifications

Surface chemistry:	Short stranded poly ethylene glycol (PEG) thiols creating mixed self assembled monolayers exposing biotin groups on Q-Sense gold coated sensors (QSX 301).
Binding:	-22 ± 3 Hz (a monolayer) of Streptavidin reproducibly and proven robustness for incorporation of different biotinylated proteins.
Specificity:	No non-specific binding detected when incubated in fetal bovine serum with protein concentration ~ 40 mg/ml for 30 min.
Usage:	The sensors should be mounted into the instrument directly from the box without prior cleaning since this can affect the adsorbed thiol monolayer. It is sufficient to rinse with water/buffer in situ before measuring.
Storage:	Stable for > 8 weeks (90 % retained Streptavidin binding activity, see Figure 2) when stored sealed in the dark at low temperatures (2-8 oC). Note that thiols have a tendency to oxidise when exposed to light and excess air.

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Streptavidin has four binding sites for Biotin and thus acts as a linker between the biotinylated sensor surface and a biotinylated analyte. In Figure 1 an example is shown where biotinylated protein-A has been immobilized that specifically binds to immunoglobulin (Ig) antibodies. This enables studies of an antibody-antigen interaction, as seen in Figure 1.

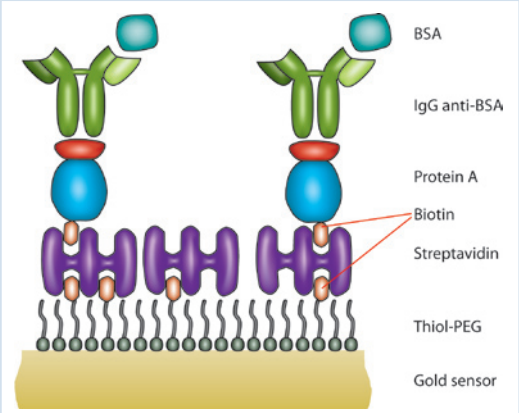

Figure 1: Example of suitable layer build-up for antigen-antibody interaction studies.

Effectivness of Biotin activity after storage

	0 w	4 w	8 w
Streptavidin, f	**-22.6** ± 0.28	**-20.55** ± 0.21	**-20.85** ± 0.21
Streptavidin, D	**-0.12** ± 0.06	**-0.08** ± 0.03	**0.09** ± 0.07
Biotin-BSA, f	**-17.1** ± 0.14	**-16.1** ± 0.42	**-17.0** ± 0.35
Biotin-BSA, D	**0.66** ± 0.03	**0.64** ± 0.03	**0.69** ± 0.03

Table 1: Frequency (f) and dissipation (D) shifts obtained at binding of Streptavidin and subsequently biotin-BSA as a measure of the Biotin activity. QSX 339 proves to be stable for 8 weeks of storage in dark and at low temperatures(fridge).

To prove that the Q-Sense Biotin Functionalized Sensor was still active after 8 weeks of storage, immobilization of Streptavidin and the subsequent binding of biotinylated Bovine Serum Albumin (biotin-BSA) were performed. The level of binding of these proteins, which corresponds to the level of binding activity of QSX 339, was measured with QCM-D. The values shown in Table 1 are Dissipation and frequency shifts obtained at these immobilizations. These sensors were stored dark and at low temperatures (fridge) and retained up to 90 % of the binding activity after 4 and 8 weeks. Also, the associated dissipation shifts have the same characteristics as at starting point.

References

1. Edvardsson, M., et al., *QCM-D and Reflectometry Instrument: Applications to Supported Lipid Structures and Their Biomolecular Interactions*. Analytical Chemistry, 2009. 81(1): p. 349-361.

2. Glasmastar, K., et al., *Protein adsorption on supported phospholipid bilayers.* Journal of Colloid and Interface Science, 2002. 246(1): p. 40-47.

3. Hook, F., et al., *Characterization of PNA and DNA immobilization and subsequent hybridization with DNA using acoustic-shear-wave attenuation measurements*. Langmuir, 2001. 17(26): p. 8305-8312.

4. Larsson, C., M. Rodahl, and F. Hook, *Characterization of DNA immobilization and subsequent hybridization on a 2D arrangement of streptavidin on a biotin-modified lipid bilayer supported on SiO2*. Analytical Chemistry, 2003. 75(19): p. 5080-5087.