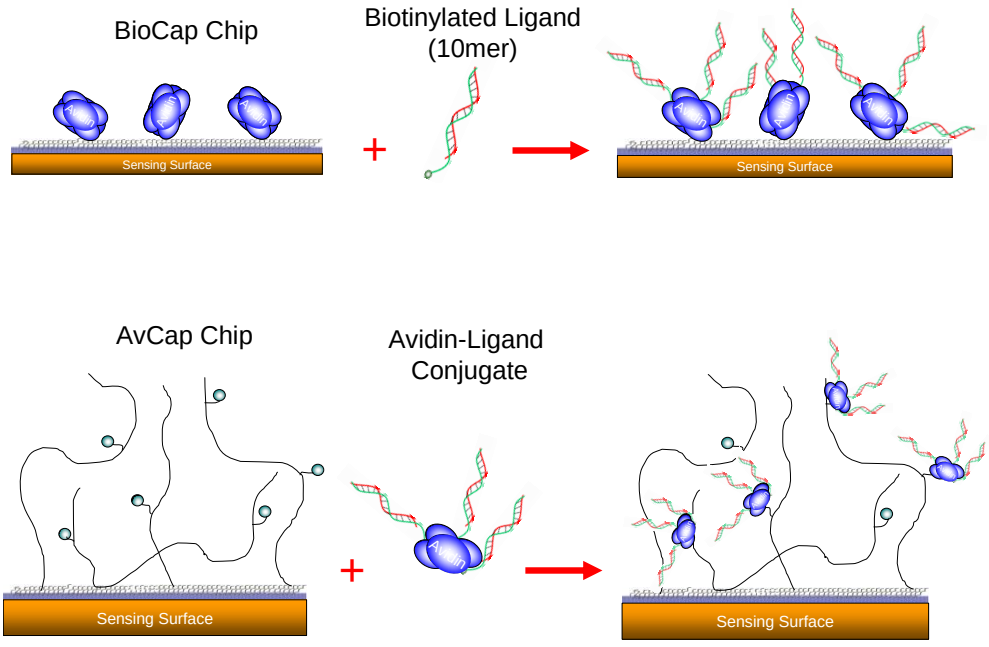
IMMOBILIZATION BY AVIDIN-BIOTIN (BioCap and AvCap BIOSENSORS)

TECHNICAL NOTES

The immobilization of biomolecules by avidin-biotin based methods is common in SPR studies due to its excellent robustness and simplicity. Both the BioCap chip and AvCap chip exploit this interaction for reliable immobilization of ligand. The illustration below depicts the immobilization scheme for both of these biosensors.



Benefits Of Avidin-Biotin Methods

- Highly efficient capture in a wide range of pH
- Requires very low quantities of ligand (nanomolar concentrations)
- A wide variety of biotinylated reagents are available commercially
- Easy-to-use biotinylation kits are also available
- Single step immobilization
- Surfaces possess far lower electrostatic charge compared to COOH series sensors

BioCap Biosensors

The BioCap sensor is prepared from a planar carboxylated surface (i.e. COOH2 biosensor) with pre-immobilized Neutravidin. Biotinylated ligand is captured onto this surface with extremely high affinity (~10^{-12} M). We have chosen Neutravidin as it exhibits very low non-specific binding. This affinity capture method is non-reversible as conditions necessary to reverse the interaction cause denaturation of the Neutravidin. Alternatively, Streptavidin can be manually immobilized onto a COOH2 biosensor.

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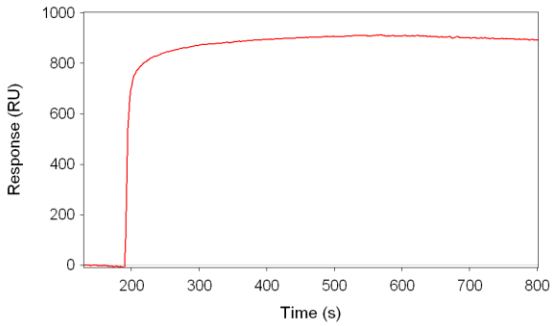


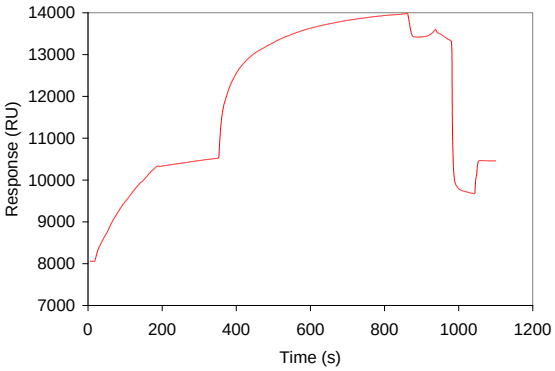
A typical immobilization onto a BioCap sensor is shown below. Biotinylated Protein A was prepared in running buffer and injected over the BioCap chip surface for 6 minutes. The baseline was stable upon completion of the injection (i.e. t = 650sec) confirming negligible dissociation of the tightly bound protein A.

For optimum immobilization it is best if each ligand molecule possesses a single biotin that is tethered via a short PEG chain[1]. We recommend using biotin-$(PEO)_3$-NHS, or similar derivative when biotinylated ligands.

AvCap Biosensors

The AvCap surface is comprised of pre-immobilized biotin on a planar carboxylated sensor (ie. COOH2). It is supplied with the biotinylated surface ready for the user to immobilize any ligand of interest conjugated with avidin. These conjugates may be prepared by any suitable means as long as at least one free biotin binding site remains per conjugate molecule. The simplest approach is to prepare the conjugates by pre-incubating Neutravidin with a biotinylated ligand. The mole ratio of the mixture should be adjusted such that conjugates are formed in good yield but free biotin binding sites remain for linking to the AvCap chip.

It is also possible to prepare conjugates by chemical cross-linking allowing the biotin binding sites to be used exclusively for affinity capture to the hydrogel. For example, incubating a thiolated ligand with Neutravidin-maleimide will yield conjugates without requiring purification. These conjugates can then be injected over the AvCap surface. If the ligand lacks a free thiol group then one may be introduced by reaction with 2-iminothiolane which converts an amine to a thiol. A mouse IgG-Neutravidin conjugate was prepared as described above and injected over the AvCap chip and the resulting response curve is shown below.



The injection was terminated when approximately 2500 RU of conjugate was bound. A polyclonal anti-mouse antibody was subsequently injected giving a binding response of almost 3000 RU thereby confirming successful immobilization of mouse IgG. Furthermore, the bound anti-mouse IgG could be completely removed by an injection of dilute acid.

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[1] Papalia, G., Myszka, D.G. Anal. Biochem. **403** (2010) p. 30-35