AVIDIN-BIOTIN BASED IMMOBILIZATION (BioCap and AvCap CHIPS)

TECHNICAL NOTES

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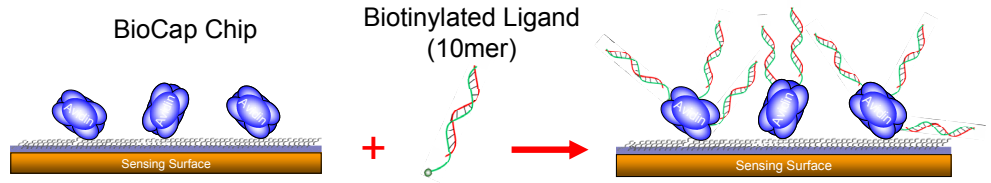
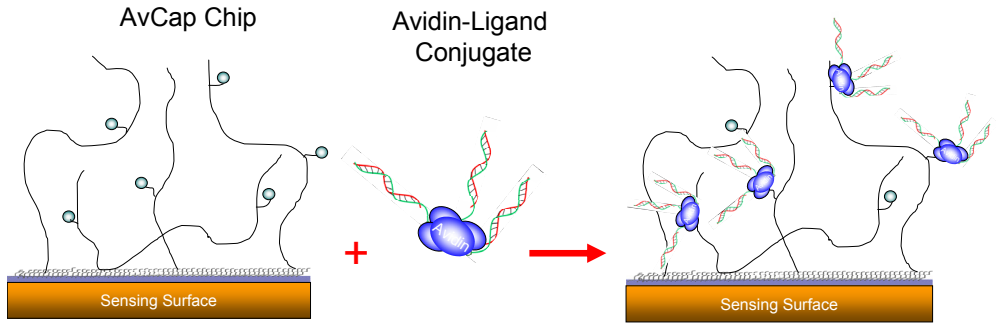
The immobilization of biomolecules by avidin-biotin based methods remains popular today due to its excellent robustness and simplicity. Both the BioCap chip and AvCap chip exploit this technology for reliable immobilization of ligand. The illustration shown below depicts the immobilization scheme for both of these approaches. Key benefits include:

- Does not depend on the isoelectric point of the protein
- Requires very low quantities of ligand
- A wide variety of biotinylated reagents are available commercially
- Easy-to-use biotinylation kits are also available
- The immobilization requires a single injection
- Can accurately control the concentration of bound conjugate immobilized by terminating the injection when the desired R_{max} is reached
- Surfaces possess far lower electrostatic charge compared to the COOH chips
- A single biotinylation reaction usually yields enough product to allow an almost unlimited number of immobilizations

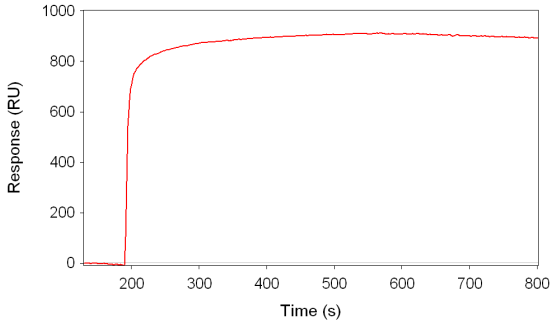




BioCap CHIP PROPERTIES

The BioCap Chip is prepared from a planar carboxylated surface (i.e. COOH1 Chip) by immobilizing neutravidin onto the surface by amine coupling. Biotinylated ligand will be 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. If streptavidin is known to be perform better for your interaction system then simply immobilize streptavidin onto our COOH1 chip.

A typical immobilization onto a BioCap chip 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) indicating negligible dissociation of the tightly bound protein A.

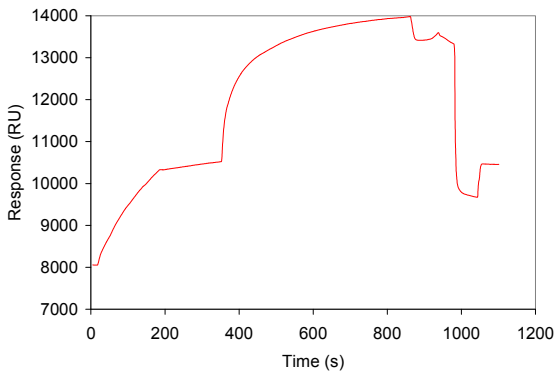


For optimum immobilization it is best if each ligand molecule possesses a single biotin that is tethered via a short PEG chain. When biotinylating ligand we recommend using biotin-$(PEO)_3$-NHS, or similar derivative. A hydrophilic spacer arm is optimum.

AvCap CHIP

This surface is prepared by covalent immobilization of biotin into a three dimensional hydrogel matrix bound to the sensing surface. It is supplied with the biotinylated hydrogel pre-attached allowing the user to immobilize any ligand-avidin conjugate of interest. 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 neutraavidin 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. It may be useful to consider partial blocking of the neutravidin with free biotin to prevent tetravalant cross-linking within the hydrogel. We recommend using commercially available Neutravidin-maleiimide from Pierce Chem. Comp. Inc. to form such conjugates. Incubating a thiolated ligand with neutravidin-maleiimide will yield conjugates without requiring purification. These conjugates are then injected over the AvCap surface. If the ligand lacks a free thiol group then one may be introduced by conversion of an amine to a thiol by reaction with 2-iminothiolane. This reaction is highly quantitative and does not require a molar excess of 2-iminothiolane. Therefore purification by dialysis is not required. In fact this process of thiolation, conjugate synthesis and conjugate immobilization can be completed in less that 1h and requires only a few fluid transfer steps for completion. 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 2500RU of conjugate was bound. A polyclonal anti-mouse antibody was subsequently injected giving a binding response of almost 3000RU thereby confirming successful immobilization of mouse IgG. Furthermore, the bound anti-mouse IgG could be completely removed by an acid pulse. If the ligand and/or analyte possess a high molecular weight then 1:1 conjugates are recommended in order to avoid steric hindrance.

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