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IMMOBILIZATION BY AMINE COUPLING (COOH1 & COOH2 CHIPS)

TECHNICAL NOTES

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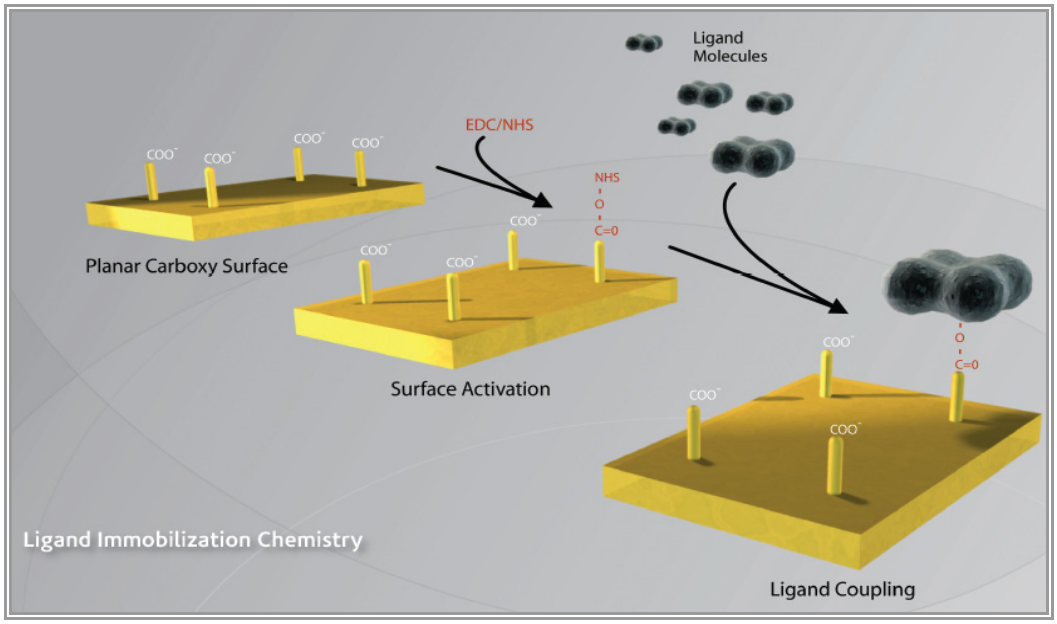
Immobilization of ligand via amine coupling is applicable to a wide variety of biomolecules since amine groups are common. The ligand is immobilized randomly yet we have found that this method usually yields high quality results. Therefore, directed immobilization via a unique site, while desirable, is not usually a requirement.



The most common approach is the use of an aqueous mixture of N-hydroxysuccinimide (NHS) and 1-Ethyla-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) to activate carboxyl groups to yield amine reactive esters[1]. This procedure has several advantages including:

- Highly versatile as the vast majority of biomolecules may be immobilized without derivatization, or without requiring tags
- Produces a highly stable covalent bond that prevents ligand from leaching from the surface.
- Is effective over a wide pH range
- Does not require exposure of the biomolecule to harsh conditions
- Immobilization conditions are easily controlled to prevent excessive cross-linking with the surface
- Reagents may be prepared and stored frozen for a few months

OPTIONAL PRE-CONCENTRATION

Pre-concentration allows in-situ immobilization of biomolecules where low concentrations of biomolecules are available. The method exploits electrostatics to bring about a pre-concentration of a positively charged biomolecule onto an electronegative surface. This process includes:

- Activating the surface carboxyl groups to NHS esters while retaining sufficient un-activated carboxyl groups to generate a strong electrostatic potential
- Adjusting the pH of the biomoleule solution below its isoelectric point
- The salt concentration of the buffer is kept low in order to prevent dampening of the electrostatic fields
- Pre-concentrated biomolecules form covalent bonds by reacting with surface NHS esters

[1] Staros, Wright and Swingle (1986), Anal. Biochem., 156(1):, 220-2

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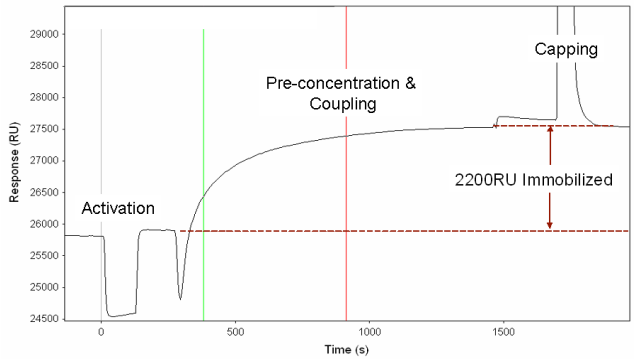
TYPICAL IMMOBILIZATION SEQUENCE

The immobilization of neutravidin is shown in the response curve below. The surface is activated with a short pulse of EDC/NHS. Neutravidin is then pre-concentrated onto the surface and covalently coupled.



Finally, an ethanolamine solution is injected to remove any weakly bound material and also serves to cap residual NHS esters. In this case a mass equivalent to 2200RU of neutravidin was immobilized onto a planar carboxylated surface. The entire sequence was complete is just over 30 min.

LIGAND HETEROGENEITY

Analyte heterogeneity is a term used to describe a mixed population of immobilized ligand molecules. It has been found that biomolecules immobilized in a three dimensional hydrogel will often suffer from steric hindrance causing some loss in binding activity[2]. In the worst case extensive cross-linking between the hydrogel and the ligand can completely obscure the ligand causing complete loss of analyte binding. In other cases a mixed population of ligand is present on the surface where some are freely accessible to analyte, others completely obscured and with any number of intermediate states. High quality kinetic analysis demands that these effects are minimized otherwise the complex binding response curves that will result from such a surface will not obey any interaction model and kinetic constants cannot be determined.

Fortunately it is usually possible to prevent ligand heterogeneity from interfering with kinetic analysis. We have determined that a two dimensional surface provides an environment where excessive cross-linking to the surface is prevented. Ligand heterogeneity will depend on the number of amine groups, the size of the ligand and the location of these amines relative to the analyte binding site. If only a few amines are present and are close to the analyte binding site then we may expect a significant degree of analyte heterogeneity. Despite these concerns amine coupling remains the most popular method of immobilization in biosensing and has been successful in countless applications. In cases where complex data sets are recorded we suggest considering a more directed immobilization strategy. In fact repeating a kinetic analysis over a range of surfaces, or immobilization methods, is the best means of identifying these artifacts.

OPTIONAL MODIFICATIONS

If you possess a ligand with a polysaccharide component it may be possible to oxidize it to produce a reactive aldehyde. Simply immobilize carbohydrazide by amine coupling and then pre-concentrate and couple the aldehyde containing ligand. The resulting hydrazone bond is relatively stable but can be reduced for improved stability. This method often preserves analyte binding activity as the polysaccharide usually does not participate in binding the analyte and can be considered to act as a spacer. Immobilization of ligand via a thiol-maleiimide reaction is also a good option for acidic ligands. Reversible disulfide bonds can also be employed where stable, but reversible covalent, bonds are required. If a thiol is not present on the ligand then one may be introduced by reaction with 2-iminothiolane without subsequent purification.

[2] Löfås, Johnsson, Tegendal and Rönnberg (1993), Colloids and Surfaces B Biointerfaces, 1, 83-89.

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