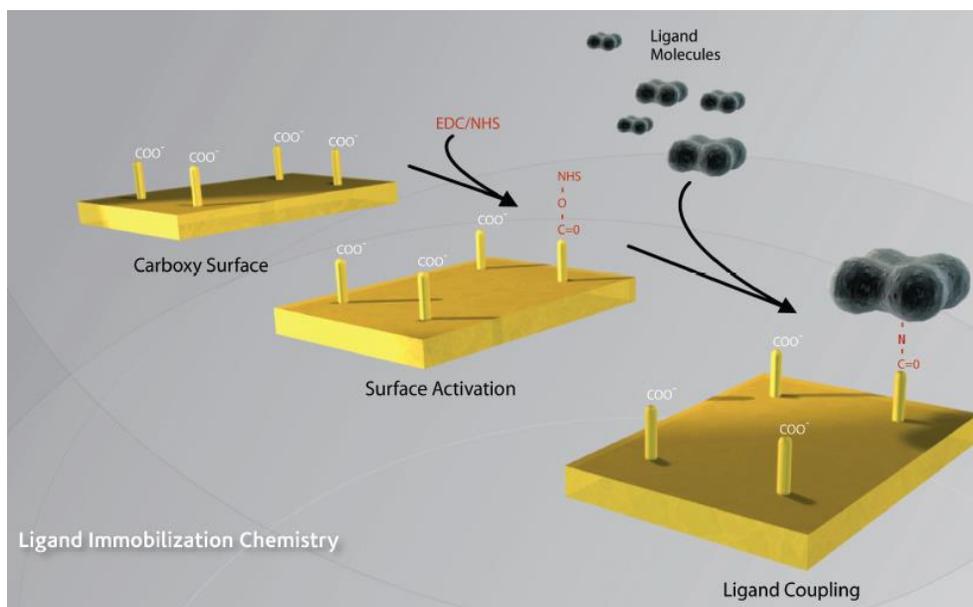


IMMOBILIZATION BY AMINE COUPLING (COOH1, COOH2, COOHV, COOH5 BIOSENSORS)

Immobilization of ligands via amine coupling is applicable to a wide variety of biomolecules since amine groups are common. Proteins often possess multiple Lysine residues which can be used for forming an amide bond with a carboxylated sensor surface. The SensiQ COOH series sensors enable this mode of amine coupling of proteins and other biomolecule targets.



The most common approach is the use of an aqueous mixture of N-Hydroxysuccinimide (NHS) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) to activate carboxyl groups to yield amine reactive esters¹. 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

Pre-concentration Effect

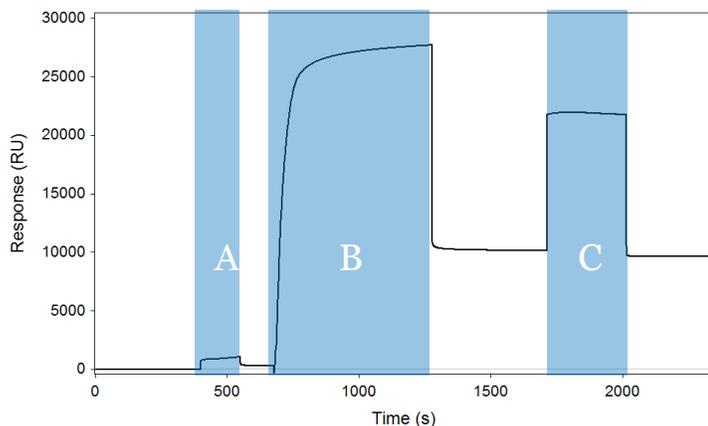
Pre-concentration allows in-situ immobilization of biomolecules where low concentrations of biomolecules are available. The method exploits electrostatics to bring about a pre-concentration of a positively charged biomolecule onto an electronegative surface. This process includes:

- Adjusting the pH of the biomolecule solution below its isoelectric point
- Reducing the concentration of salts in the buffer to prevent electrostatic field dampening
- Biomolecules attract to the sensor giving a significant increase in surface concentration
- Pre-concentrated biomolecules form covalent bonds by reacting with surface NHS esters

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

Immobilization Procedure

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



Finally, an Ethanolamine solution is injected to remove any weakly bound material and also serves to cap residual NHS esters (Inject C). In this case a mass equivalent to 10,000 RU of Neutravidin was immobilized onto a COOH₅ surface. The entire sequence was complete in just over 30 min.

Ligand Heterogeneity

Ligand 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 activity². 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

The COOH series sensors can be used to immobilize other affinity ligands for directed capture of specific molecules from crude samples. For example, protein G can be immobilized using amine coupling to a COOH sensor thereby producing a reusable capture surface for many Immunoglobulins. The COOH series sensor is a highly versatile surface with many potential means for ligand immobilization.

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