AMINATED SURFACE

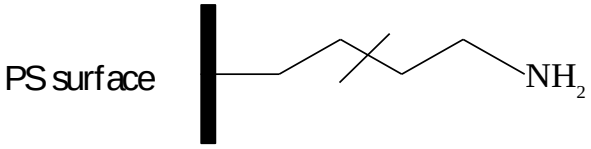
TECHNICAL NOTE N. 6

Introduction to the preparation and use of aminated surfaces for immunological assays

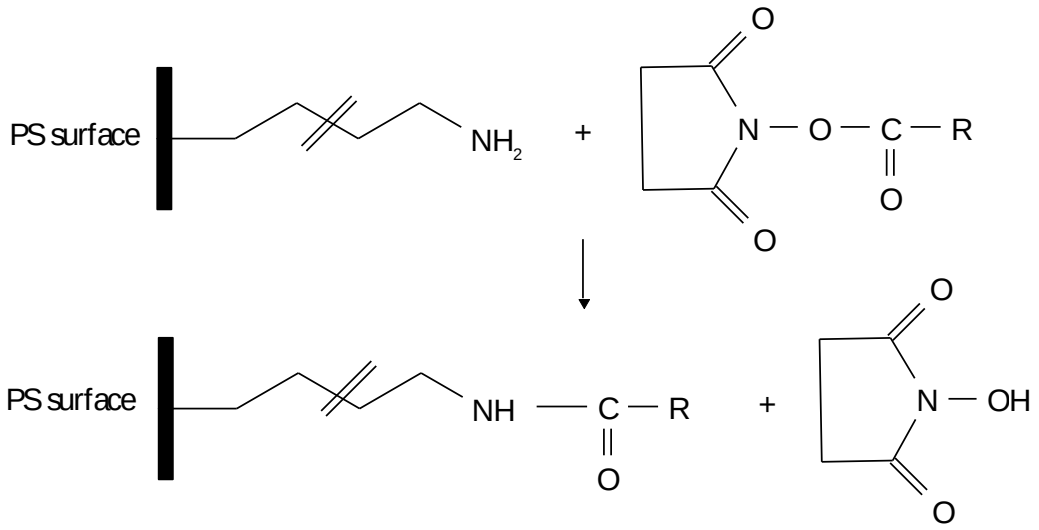
Direction for use

Several recipes are routinely used for the coupling of biological molecules to amino groups. Specific directions for use require the knowledge of the intended application. As general guideline, the interaction between the amino group on the surface and the functional group of the molecule to be bound is based on covalent binding mediated by homo and heterofunctional crosslinkers.
In particular, Ethyldiethylaminopropylcarbodiimide (EDC), with or without the addition of N-hydroxysuccinimide, is a powerful coupling agent of the carboxylic group of the molecule with the amino group of the surface.
If the biomolecule to be bound contains ε amino groups of lysine, the simplest method is coupling via Glutaraldehyde, with the formation of a stable amine linkage by reduction with Sodium Cyanoborohydride.
Other crosslinkers for this purpose are Dimethylpimelidate and Dissucinimidyl suberate.
Biomolecules containing thiolic groups, as Fab-SH or peptides with cystein at terminal end, can exploit the large number of maleimido groups containing crosslinkers as Succinimidyl 4-(N-maleimidomethyl)cycloexane-1-carboxylate (SMCC) for reacting with the amino group.

Schematic chemical and physical configuration of Biomat NH_2 surface



example of reaction scheme:
an arbitrary NHS estherified compound (R) covalently combines with Biomat NH_2 surface through NHS splitting off

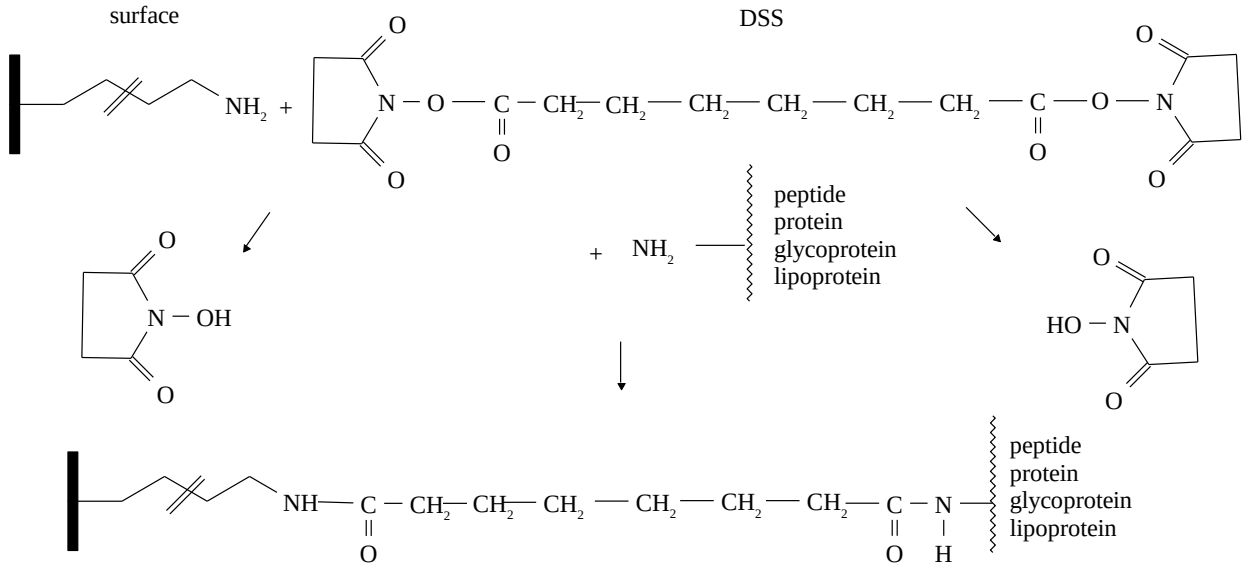


Hereunder are some examples of coupling agents to be used for covalent coating of the Biomat NH_2 surface with reactive groups

A. Disuccinimidyl suberate (DSS).

This symmetric (homobifunctional) linker is capable of linking compounds containing secondary or primary amino groups, and can thus be used for covalent immobilisation of peptides, proteins, glycoproteins, lipoproteins

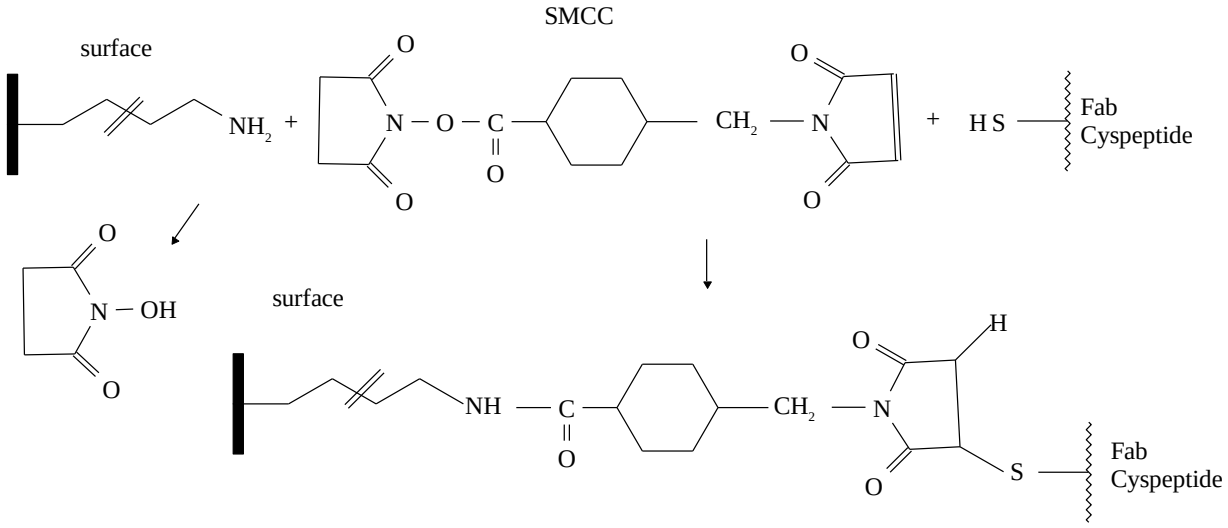
Reaction A



B. Sulfosuccinimidyl maleimidomethyl cyclohexane carboxylate (SMCC).

This heterobifunctional linker is capable of linking compounds with SH-containing compounds. It can be used especially for covalent immobilisation of Fab-SH-antibody fragments or terminally cysteinized antigenic peptides, thereby exposing the active ends of these compounds to the liquid phase.

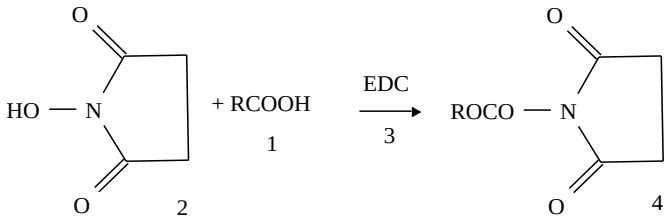
Reaction B

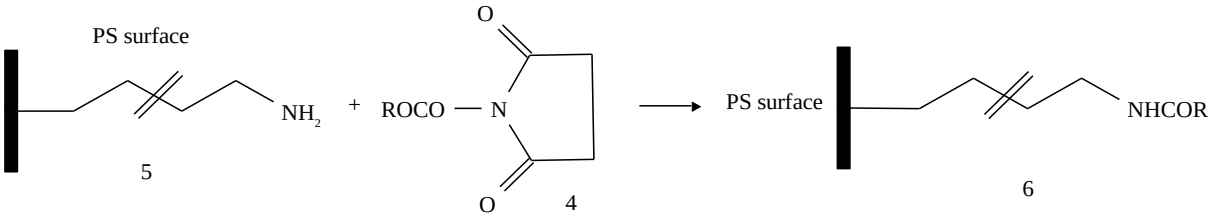


C. N-Hydroxysulfosuccinimide (Sulfo-NHS) or N-Hydroxysuccinimide (NHS) combined with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide Hydrochloride(EDC).

The EDC linker combined with sulfo-NHS is capable of linking small peptides (M.W. around 1000) via their carboxyl group to the NH-activated strips surfaces.

Reaction C





1. Peptide
2. Sulfo NHS (or NHS)
3. EDC
4. Intermediate active compound resulting from the reaction
5. Biomat NH_2 surface
6. Peptide covalently immobilised on Biomat NH_2 surface