AMINATED SURFACE

TECHNICAL NOTE N. 12

General directions for the use of surfaces with amino and carboxylic groups

Biomat has developed modified polystyrene surfaces introducing chemical groups such as NH_2 and COOH.

These groups are able to covalently bind compounds to the plastic surface. The optical properties of polystyrene remain unchanged, allowing to use the modified surfaces as powerful tools for diagnostic assays.

These surfaces offer the possibility to

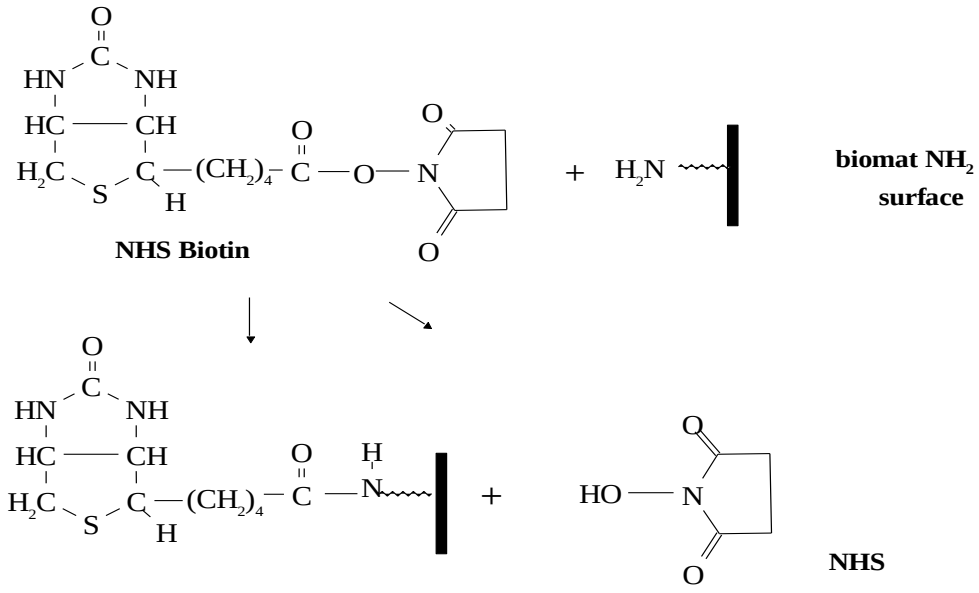
- covalently immobilize small molecules which only bind weakly or not at all by physical adsorption
- orientate the immobilization of molecules in a defined way on the solid phase.

Hereunder are some examples of application that can be used as guidelines to enable users to develop their own bio-specific assays.

1. Coupling of NHS-activated compounds

A very simple and easy application of Biomat NH_2 surfaces is coupling of molecules that have been activated by esterification with N-hydroxysuccinimide derivatives (NHS). In our experiment an-Hydroxysuccinimide active ester of biotin links immediately via its carbonilic group to the surface amino groups as shown in figure 1.

Figure 1



Preparation of reagents and buffers

Materials

Solid phase:	Biomat plates	MT02F2-AM1 (primary amino groups) MG01F-HB (high binding capacity)
ε-Caproylamido-biotin-N-hydroxysuccinimide ester (NHS- biotin)	BIO-SPA	Cat No. B002-61
Dimetilformamide (DMFO)	Fluka	Cat No. 40250
Tween® 20	Merck	Cat No. 822184
Streptavidin	BIO-SPA	Cat. No. S002-60
Streptavidin-peroxidase conjugate	BIO-SPA	Cat. No. SB01-61
BSA	Intergen	Cat. No. 3100
TMB peroxidase substrate	Kirkegard & Perry	Cat. No. 50-76-05

NHS-Biotin stock solution

NHS-biotin	6mg
DMFO	2 ml

NHS-Biotin solution 150µg/ml

NHS Biotin stock solution	500µl
PBS 0.1M pH 7.2+0.15% Tween® 20 →10ml	

NHS-Biotin solution 100µg/ml

NHS Biotin stock solution	333µl
PBS 0.1M pH 7.2+0.15% Tween® 20 →10ml	

NHS-Biotin solution 50µg/ml

NHS Biotin stock solution	167µl
PBS 0.1M pH 7.2+0.15% Tween® 20 →10ml	

NHS-Biotin solution 10µg/ml

NHS Biotin stock solution	33µl
PBS 0.1M pH 7.2+0.15% Tween® 20 →10ml	

Streptavidin-mix

Streptavidin	50µg
Streptavidin-peroxidase	1µg
PBS-BSA 1%	10ml

Experiment

1. Add 100µl NHS-biotin solutions 150-100-50-10 µg/ml and 0.1M PBS + Tween® 20 0.15% pH 7.2 as 0 µg/ml to the wells (with primary amines and HB). Seal the wells with adhesive tape to prevent evaporation
2. Incubate overnight at room temperature
3. Empty the wells and wash with 0.1M PBS + Tween® 20 0.05%, pH 7.2 four times
4. Add 100µl of streptavidin mix to each well and incubate 30 minutes at room temperature
5. Empty the wells and wash with 0.1M PBS + Tween® 20 0.05%, pH 7.2 four times
6. Add 100 µl /well of TMB substrate solution and incubate 10 minutes at room temperature
7. Stop the substrate reaction by adding 100 µl of sulphuric acid 1 N and read the optical density values at 450 nm

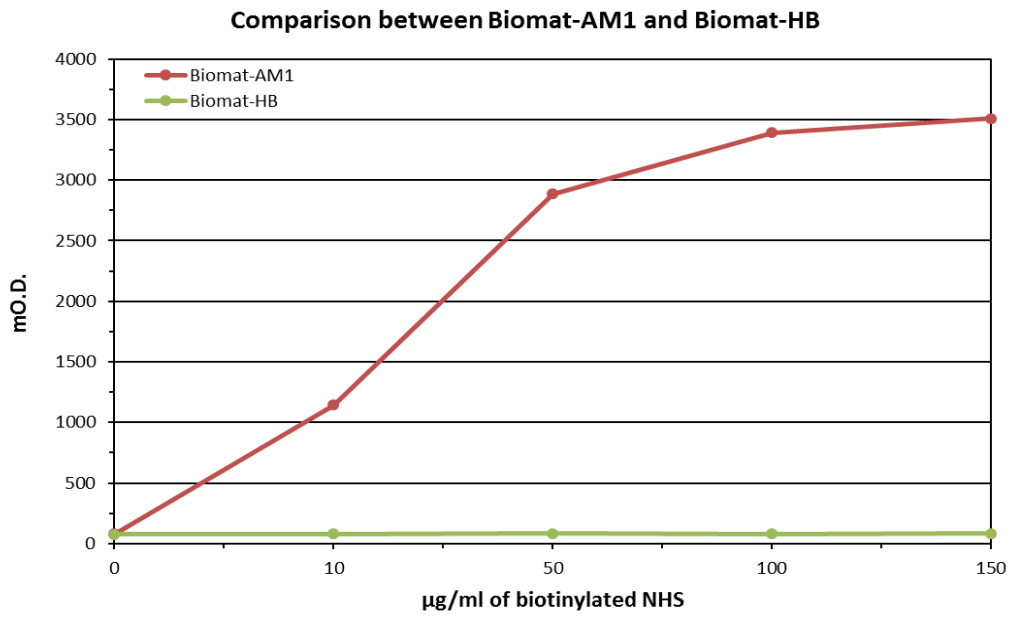
Results

The results (see fig. 2) show a clear correlation between the concentration of NHS-biotin added to the wells and the amount of biotin bound to the Biomat NH_2 surface.

On the other side no biotin is bound onto the plate without primary amino groups grafted to its surface, showing that passive adsorption of neither biotin nor enzyme conjugate occurs.

We therefore conclude that NHS-Biotin has indeed been covalently bound to the amino groups present on the Biomat NH_2 surface.

Figure 2

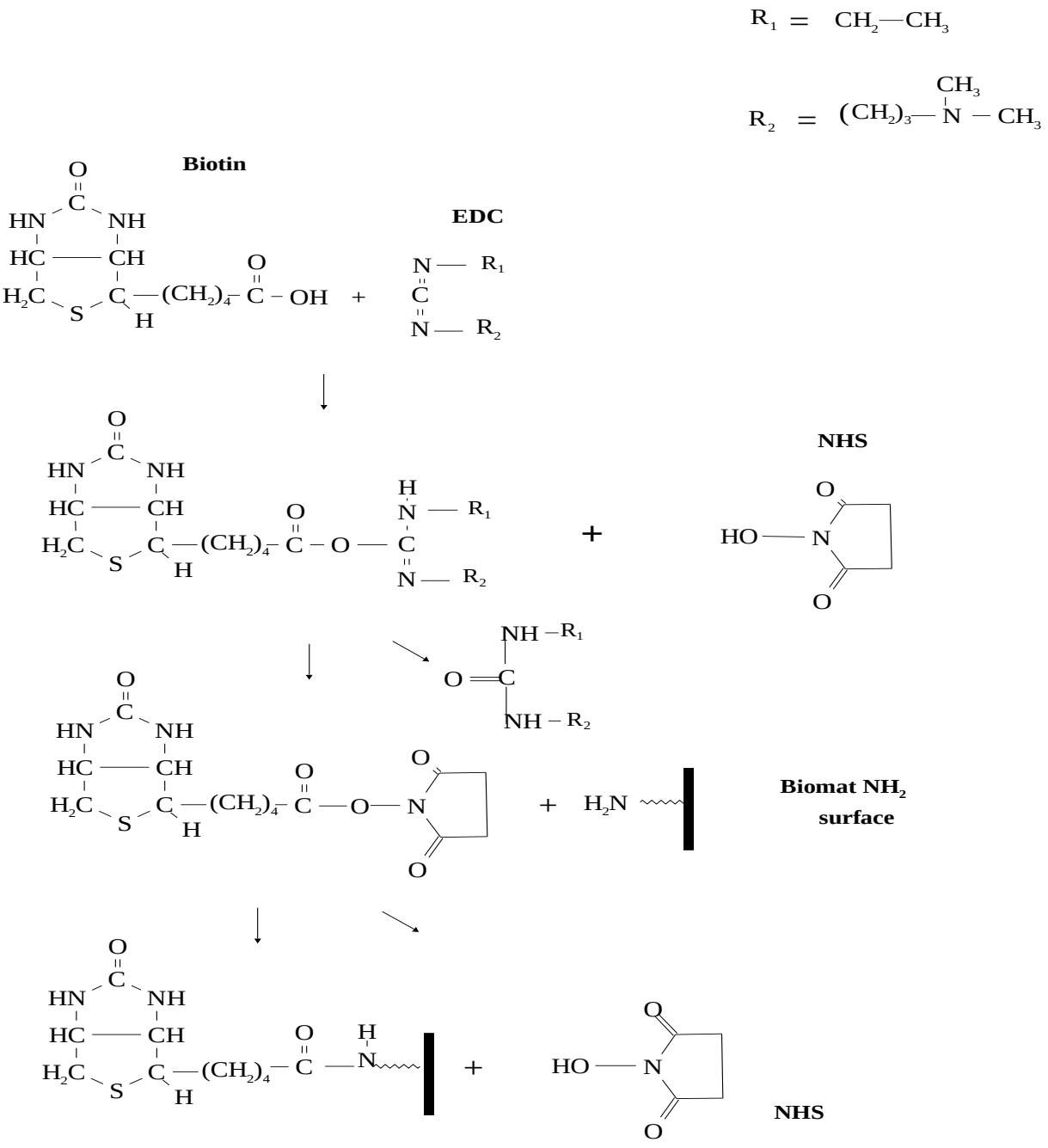


2. Coupling hapten or peptide, having a carboxylic group, to Biomat NH_2 surface

The carboxylic group presents in a molecule with a low molecular weight, such as a hapten or a peptide, binds to Biomat NH_2 through formation of amide bonds between the carboxylic group presents in the molecule and the surface amino group by the combined action of carbodiimide and N-hydroxysuccinimide.

The figure 3 shows the reaction scheme for coupling of the hapten, biotin, through its available carboxylic group.

Figure 3



Biotin coupled to Biomat NH_2 surface

Preparation of reagents and buffers

Materials

Solid phase:	Biomat plates	MG02F-AM1(primary amino groups) MG01F-HB (high binding capacity)
d-Biotin	Sigma	Cat. No. B 4501
1-Ethyl-3-(3 dimethylaminopropyl)-carbodiimide (EDC)	Sigma	Cat. No. E 1769
Sulfo-N-hydroxysuccinimide (sulfo-NHS)	Fluka	Cat. No. 56485
Dimethylsulfoxide (DMSO)	Merck	Cat. No. 2931
Tween® 20	Merck	Cat. No. 822184
Streptavidin	BIO-SPA	Cat. No. S002-60
Streptavidin-peroxidase conjugate	BIO-SPA	Cat. No. SB01-61
BSA	Intergen	Cat. No. 3100
TMB peroxidase substrate	Kirkegard & Perry	Cat. No. 50-76-05

Biotin stock solution

d-Biotin	7.8 mg
DMSO	0.5 ml
Distilled water	0.5 ml

EDC solution

EDC	5.8 mg
Distilled water	to 10 ml

Biotin/NHS solution

Biotin stock solution	500µl
Sulfo-NHS	3.45 mg
Distilled water+0,30% Tween® 20	to 10 ml

Streptavidin-mix

Streptavidin	50 µg
Streptavidin-peroxidase	1µg
PBS-BSA 1%	10ml

Experiment

1. Add 50 µl of distilled water to each well, apart from wells in column 2. Then add 100 µl of Biotin-NHS solution to all wells in column 2
2. Dilute by transferring 50 µl from the wells in column 2 to column 3, mix, transfer 50 µl from column 3 to column 4, mix and proceed in this way up to column 12
3. To start reaction: add 50 µl of EDC solution to each column. In blank experiment (column 1) add 50 µl of distilled water instead of EDC
4. Incubate at room temperature for 2 hours
5. Empty the wells and wash with 0.1M PBS+0.05% Tween® 20 pH 7.2 four times
6. Add 100µl of streptavidin mix to each well and incubate for 30 minutes at room temperature
7. Empty the wells and wash with 0.1M PBS+0.05% Tween® 20 pH 7.2 four times
8. Add 100 µl of TMB substrate solution to each well and incubate for 10 minutes at room temperature
9. Stop the substrate reaction by adding 100 µl of sulphuric acid 1 N and read the optical density values at 450 nm

Results

The results of this experiment (figure 4) clearly show that the molecule (biotin) is bound in a detectable way to the Biomat NH_2 (cod. AM1) whereas no detection could be obtained on Biomat HB.

The results indicate that a covalent coupling has taken place between the carboxylic group in the biotin and the primary amino group grafted on the Biomat NH_2.The results (data not displayed) point out that without adding carbodiimide the covalent binding of biotin does not occur.

Figure 4