

## TECHNOTE 201

# Conjugation of Biomolecules to the Surface of Maleimide Functionalized Particles

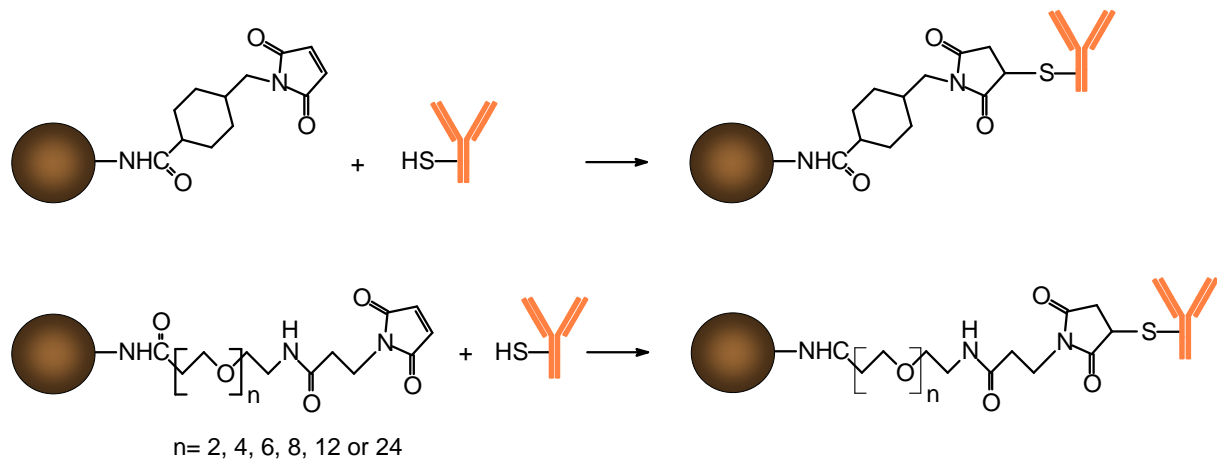
### micromod Partikeltechnologie GmbH

Friedrich-Barnewitz-Str. 4  
D-18119 Rostock  
Germany

Telephone: + 49 381 / 54 34 56 10  
Fax: + 49 381 / 54 34 56 20  
E-mail address: [info@micromod.de](mailto:info@micromod.de)

### Introduction

An efficient method for the conjugation of antibodies, proteins, peptides or other biomolecules to particle surfaces is based on the reaction of maleimide functionalized particles with thiolated biomolecules under mild conditions [1]-[5]. This method leads to an optimal orientation of the immobilized biomolecules and preserves their biological functionality to a high degree [6]. The maleimide groups on the particle surface can be introduced via a rigid spacer or via flexible PEG chains of different lengths (see Technote 202):



### Protocol

The protocol is given for the coating of a fixed amount of 25 mg of particles with a special protein or antibody. It can be varied in the scale according to your individual requirements.

#### Material:

- suspension of maleimide functionalized particles containing 25 mg of particles (For maleimide functionalization of aminated particles see Technote 202.),
- PBS-EDTA buffer (0.01 M PBS buffer, pH = 7.4, 1 mM EDTA ),
- 0.5 mg protein or antibody,
- 14 mM 2-iminothiolan solution,
- 20 mM D-cysteine hydrochloride solution in 0.01 M PBS buffer,
- 0.01 M PBS buffer (pH=7.4),
- PD Miditrap® G-25 column (GE Healthcare).

## Procedure:

1. Thiolation of the antibody or protein (see Pierce/Thermo Fisher Scientific Technote 26101)
  - suspend 0.5 mg protein or antibody to a final volume of 984  $\mu$ l PBS-EDTA buffer,
  - add 16  $\mu$ l of freshly prepared 2-iminothiolane solution in water,
  - shake the protein solution for one hour at room temperature,
  - equilibrate a PD Mditrap<sup>®</sup> G-25 column with PBS-EDTA buffer,
  - add the protein/ antibody solution (1 ml) to the column,
  - let the sample enter the packed bed completely,
  - discard the flow-through,
  - place a reaction tube under the column,
  - elute with 1.5 ml PBS-EDTA buffer and collect the eluate.
2. Protein conjugation to the particles
  - mix the maleimide functionalized particles (25 mg), that are suspended in PBS-EDTA buffer, with the thiolated protein or antibody,
  - incubate the suspension with continuous mixing for 3 hours at room temperature,
  - add 200  $\mu$ l of 20 mM cysteine solution in 0.01 M PBS buffer,
  - incubate the suspension with continuous mixing for one hour at room temperature,
  - wash the particles three times by centrifugation (Technote 100), magnetic separation (Technote 101), size exclusion chromatography (Technote 102) or dialysis (Technote 103) with 0.01 M PBS buffer (pH=7.4),
  - resuspend the particles in 1 ml PBS-buffer (pH = 7.4),
  - stabilize the suspension by addition of 20  $\mu$ l 1% sodium azide solution if necessary.

## Note:

This protocol is intended to provide general guidelines for the binding of biomolecules or related compounds. Further optimization may be required in order to achieve optimal functionality and stability from case to case.

## References

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