TECHNOTE 202

Maleimide Functionalization of Aminated Particles

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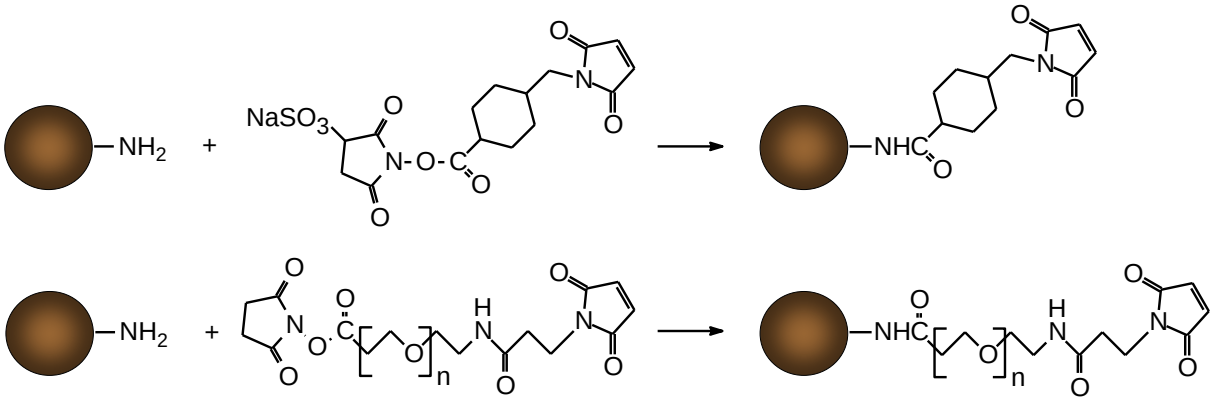
Introduction

Maleimide functionalized particles are very efficiently for the binding of thiolated biomolecules, like antibodies, proteins, peptides or other biomolecules. Maleimide groups can be easily introduced on the surface of aminated particles with spacers of different flexibility and length to achieve an optimal orientation of the conjugated biomolecules, that preserves their biological activity [1]:



Protocol

The protocol is given for the maleimide functionalization of a fixed amount of 25 mg of aminated particles. It can be varied in the scale according to your individual requirements.

Material:
- suspension of amino functionalized particles containing 25 mg of particles,
- 10 x PBS-EDTA buffer (0.1 M PBS buffer, pH = 7.4, 0.01 M EDTA),
- PBS-EDTA buffer (0.01 M PBS buffer, pH = 7.4, 1 mM EDTA),
- NHS/ maleimide crosslinker, e.g. sulfo-SMCC (sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate) or SM(PEG)n crosslinkers (NHS-PEG$_n$-Maleimide (n=2, 4, 6, 8, 12 or 24), Pierce/Thermo Fisher Scientific).

Procedure:

- add 10 x PBS-EDTA buffer to the suspension of 25 mg of amino functionalized particles to get a final buffer concentration of 0.01 M PBS buffer (pH = 7.4, 1 mM EDTA),

- dissolve 12.5 µmol of the NHS/ maleimide crosslinker of your choice, e.g. sulfo-SMCC (5.5 mg), in 100 µl DMSO and add the solution to the particle suspension,
- incubate the suspension with continuous mixing for one hour at room temperature,
- wash the particles twice with 0.01 M PBS buffer (pH=7.4) by centrifugation (Technote 100), magnetic separation (Technote 101) or size exclusion chromatography (Technote 102),
- resuspend the particles in 1 ml PBS-EDTA buffer.

Note:
This protocol is intended to provide general guidelines for the maleimide functionalization of different types of particles. Further optimization may be required in order to achieve optimal functionality and stability from case to case.

Reference

1. Grüttner C., Müller K., Teller J., Westphal F., Foreman A. R., Ivkov R., Synthesis and antibody conjugation of magnetic nanoparticles with improved specific power absorption rates for alternating magnetic field cancer therapy. *J. Magn. Magn. Mat.* **2007,** 311:181-186.