

Nanoparticle-based immunodetection of the tumor marker CD30

S. Dembski¹, T. Klockenbring², C. Gellermann¹, F. Kampmeier², G. Breuer², H. Hansen³, S. Barth²

¹Fraunhofer ISC, Neunerplatz 2, 97082 Wuerzburg/Germany

²Fraunhofer IME, Forckenbeck Str. 6, 52074 Aachen/Germany

³University Hospital Cologne, Department of Internal Medicine I, Kerpener Str. 62, 50924 Cologne/Germany

Introduction

Luminescent nanoparticles are promising tools for a wide range of biological and medical applications. Here we present our recent activities in the fabrication and biofunctionalization of luminescent silica nanoparticles (NP) and in the development of novel nanoparticle-based CD30 immunodetection assays. CD30 is a 120 kDa trans-

membrane molecule that is overexpressed on activated lymphocytes, hematologic malignancies and inflammatory disorders, such as hepatitis and HIV. The extracellular domain is constitutively shedded from the membrane. Elevated serum levels of the soluble form sCD30 are regarded as tumor markers for Hodgkin disease and other related disorders.

Synthesis of Luminescent Nanoparticles (NP)



Solutions of dye labelled silica nanoparticles

TEM micrograph of dye labelled SiO₂-NP

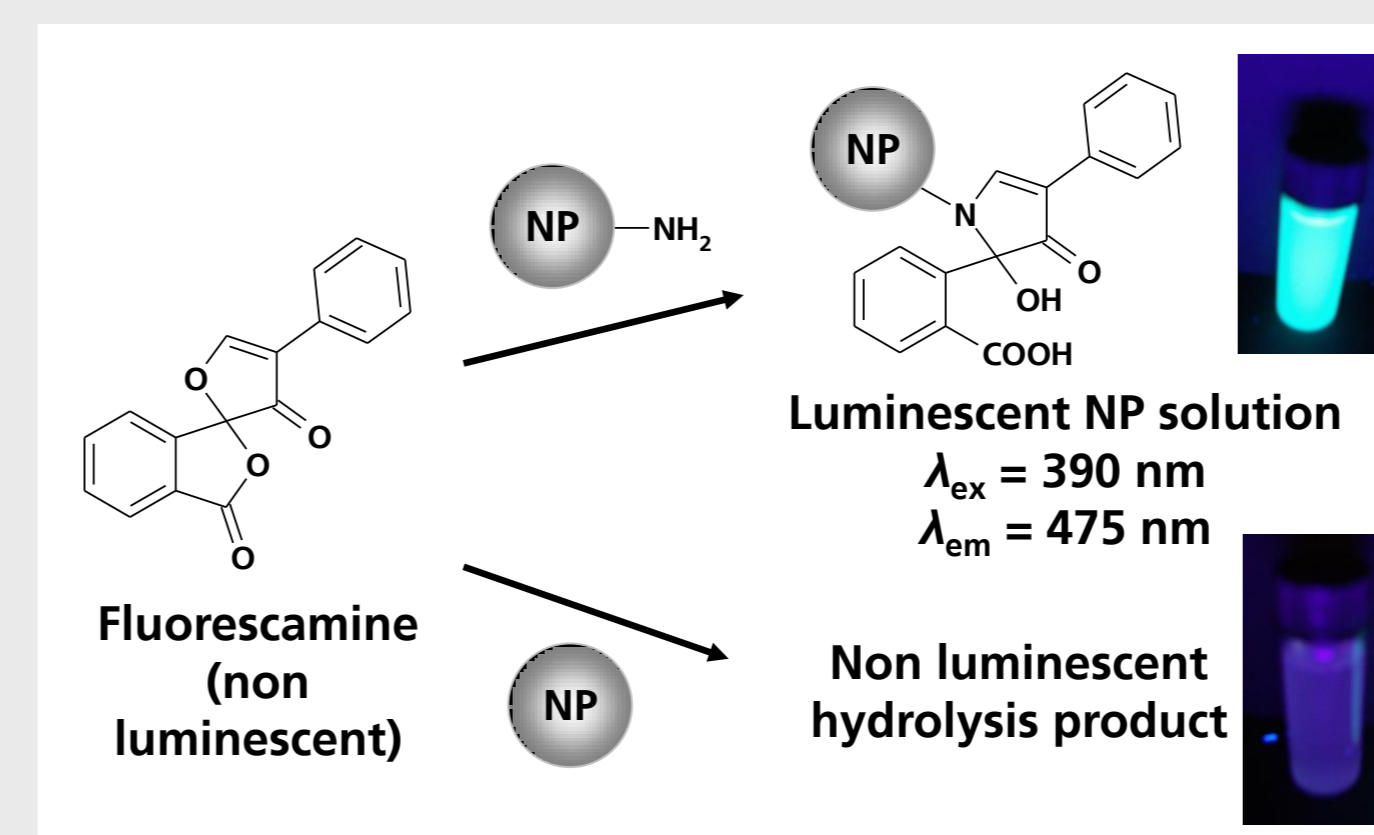
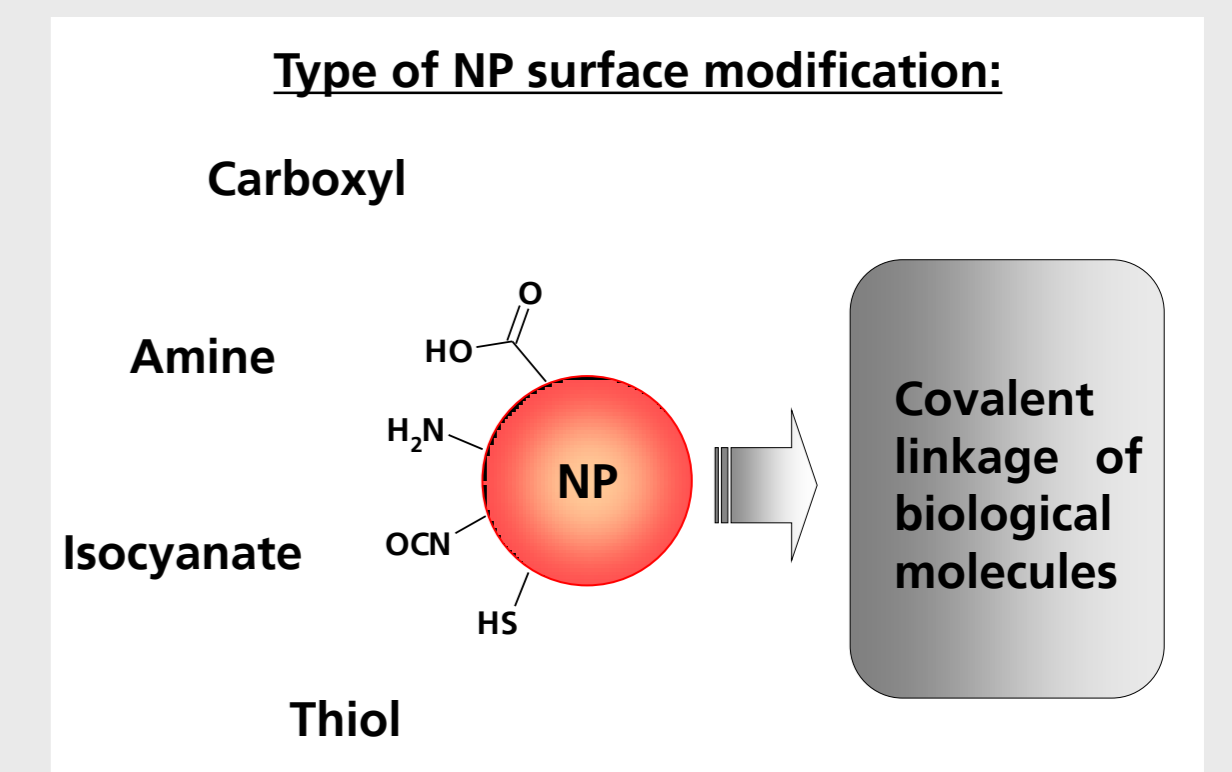
Synthesis of monodisperse, luminescent dye labelled silica NP by sol-gel technology (d = 60 – 160 nm)

Incorporation of various organic dyes into the SiO₂-matrix by covalent attachment:

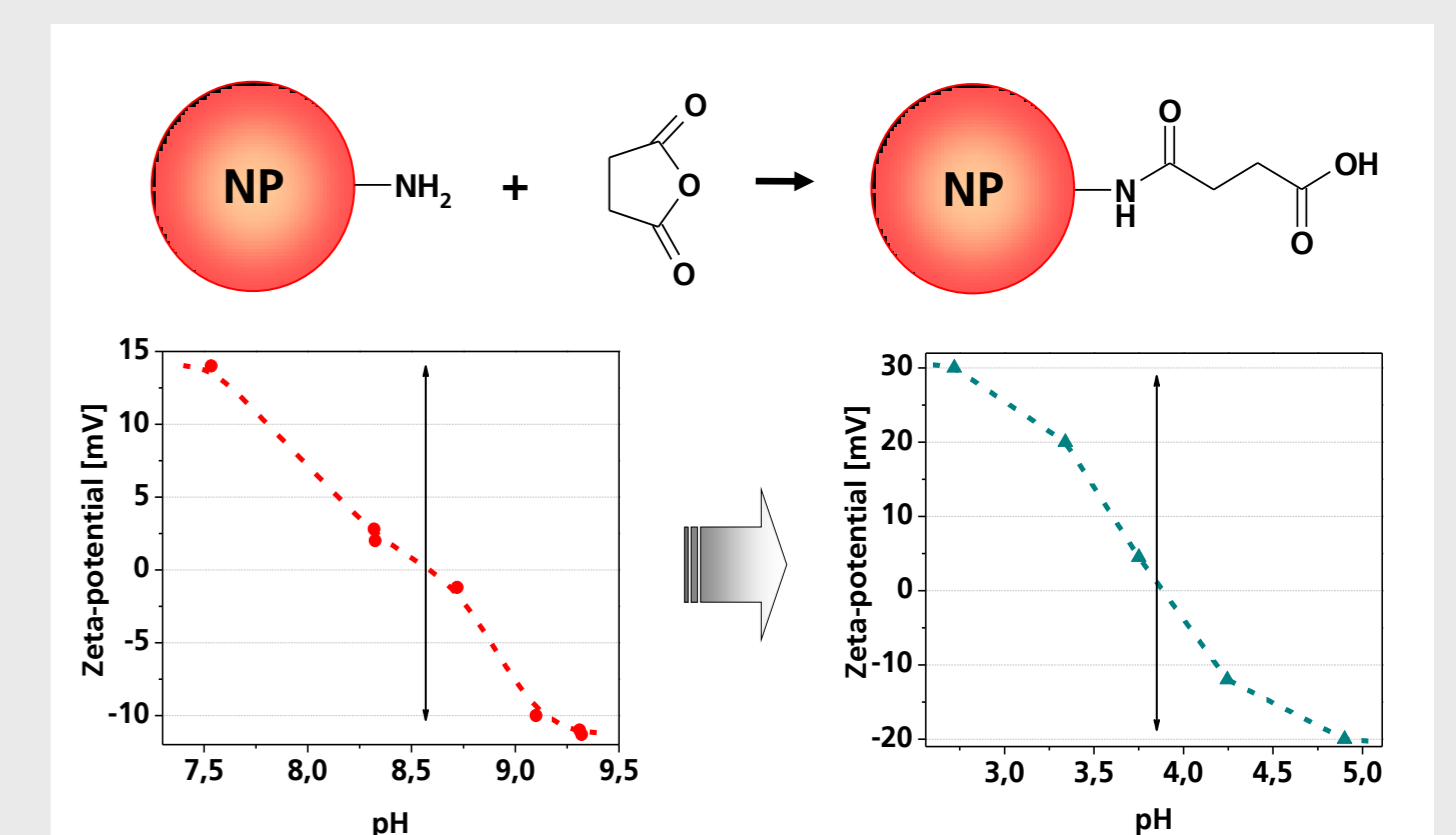
- increases the resistance to photobleaching
- prevents dye leakage

Surface Modification of Nanoparticles

- Subsequent introduction of reactive functionalities to the surface of NP
- Systematic adjustment of spacer length and type of chemical functionality, depending on the application
- Qualitative and quantitative analysis of NP surface coverage with chemical functionalities

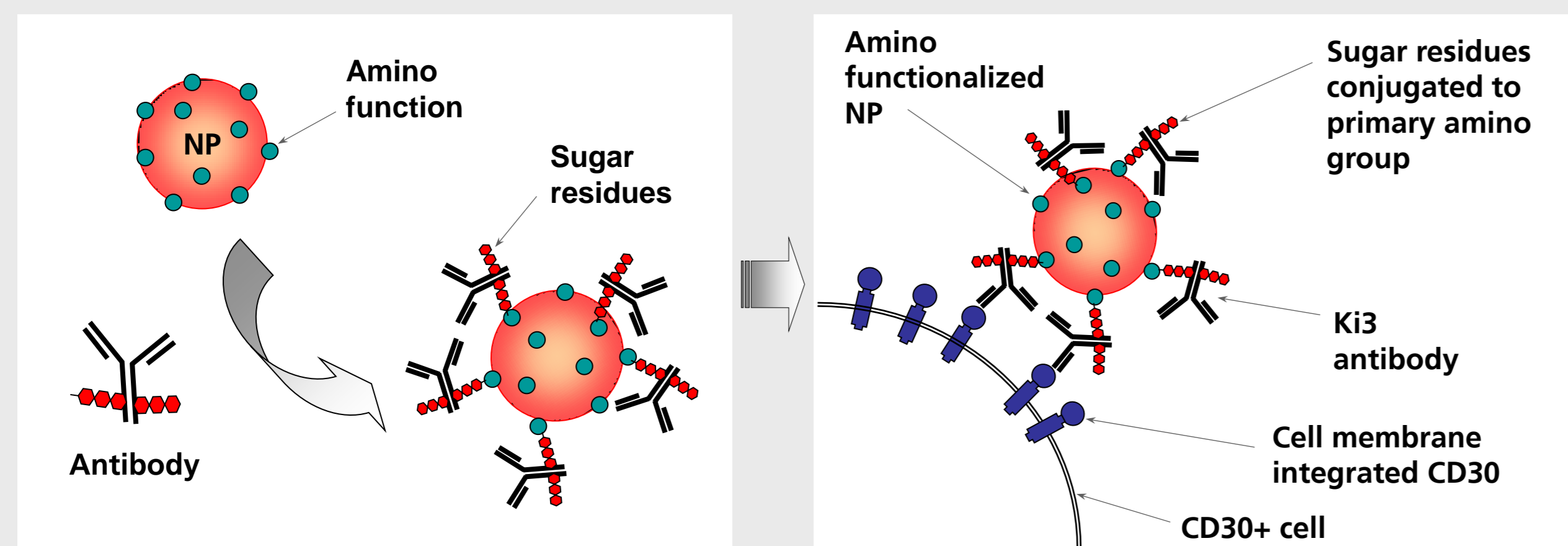


Analysis of NP surface coverage with amino functionalities by the reaction with Fluorescamine

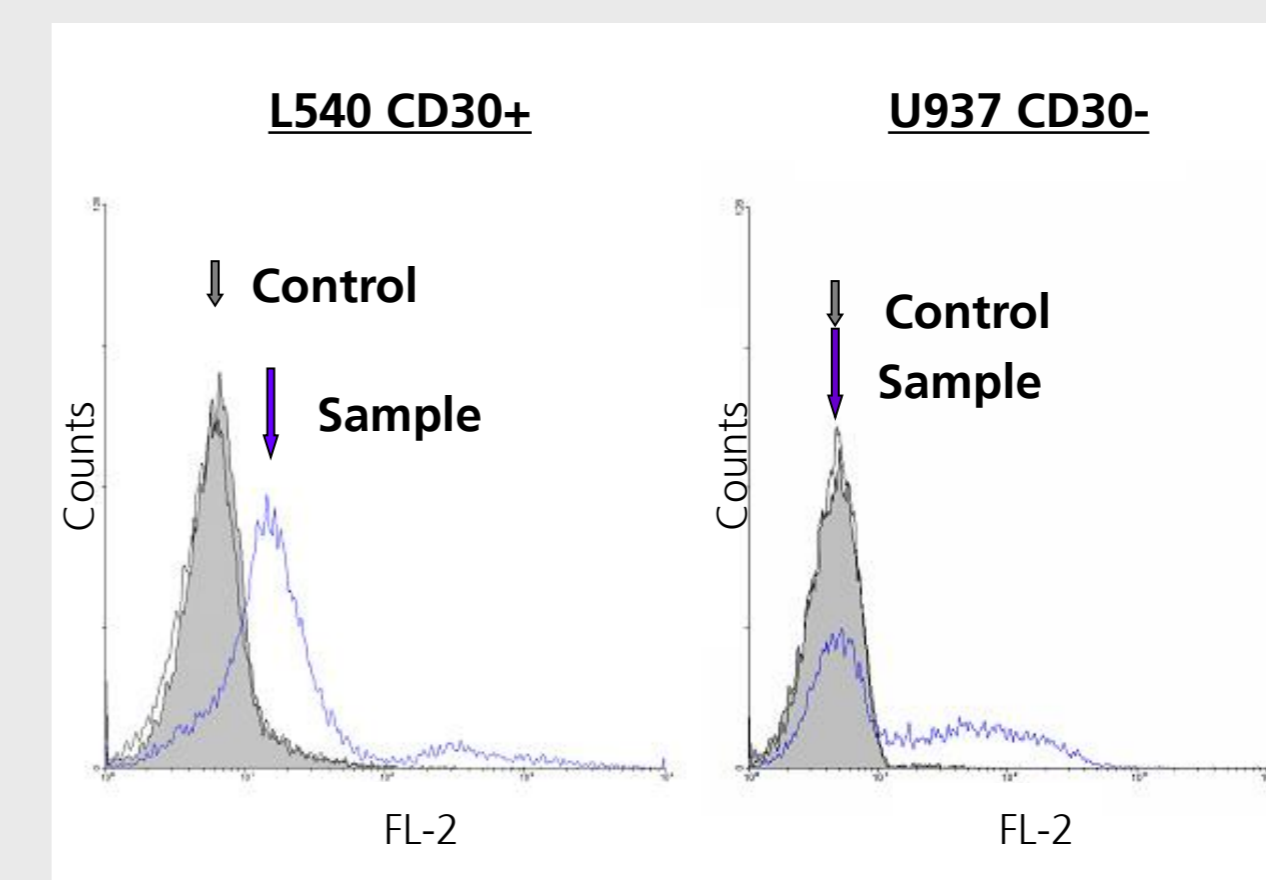


ζ -potential as a function of the pH: change of the isoelectrical point (IP) after transformation of amino functionalized silica NP (d = 60 nm) to carboxylated NP by addition of succinic anhydride

Biofunctionalization and Nanoparticle-based immunodetection of CD30 and sCD30



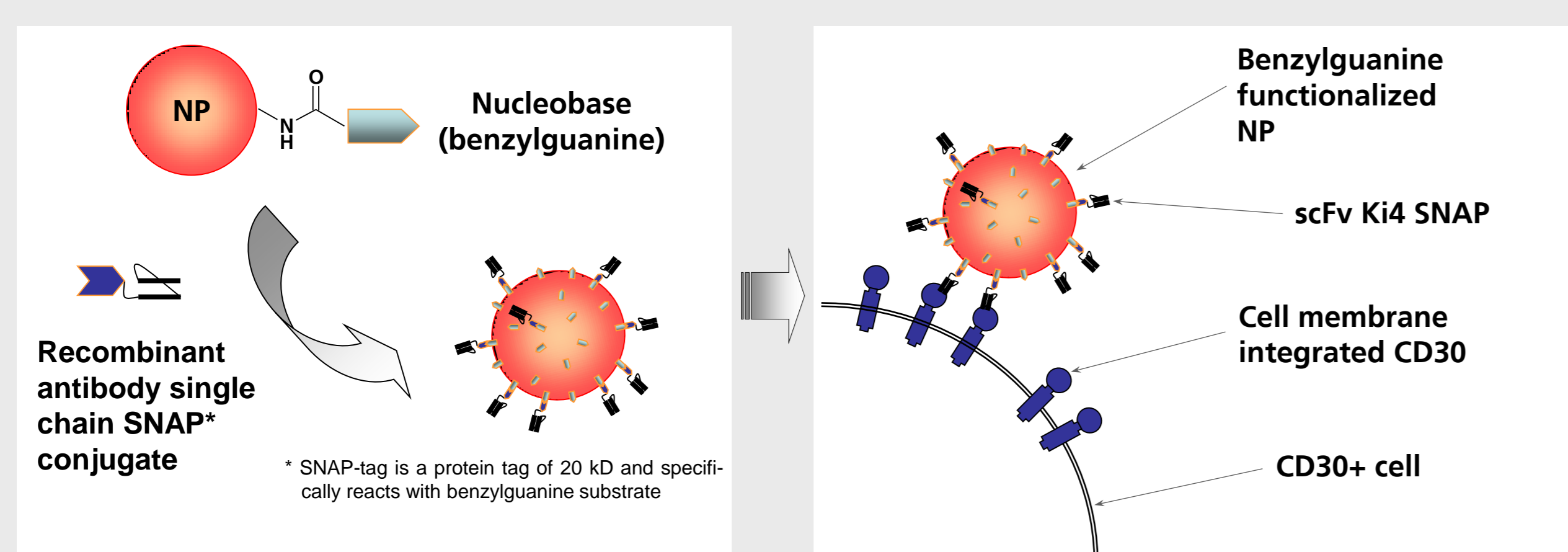
Oriented conjugation complex generated by full length antibodies via the aldehyd group of sugar residues and the amino functionalities on NP surface



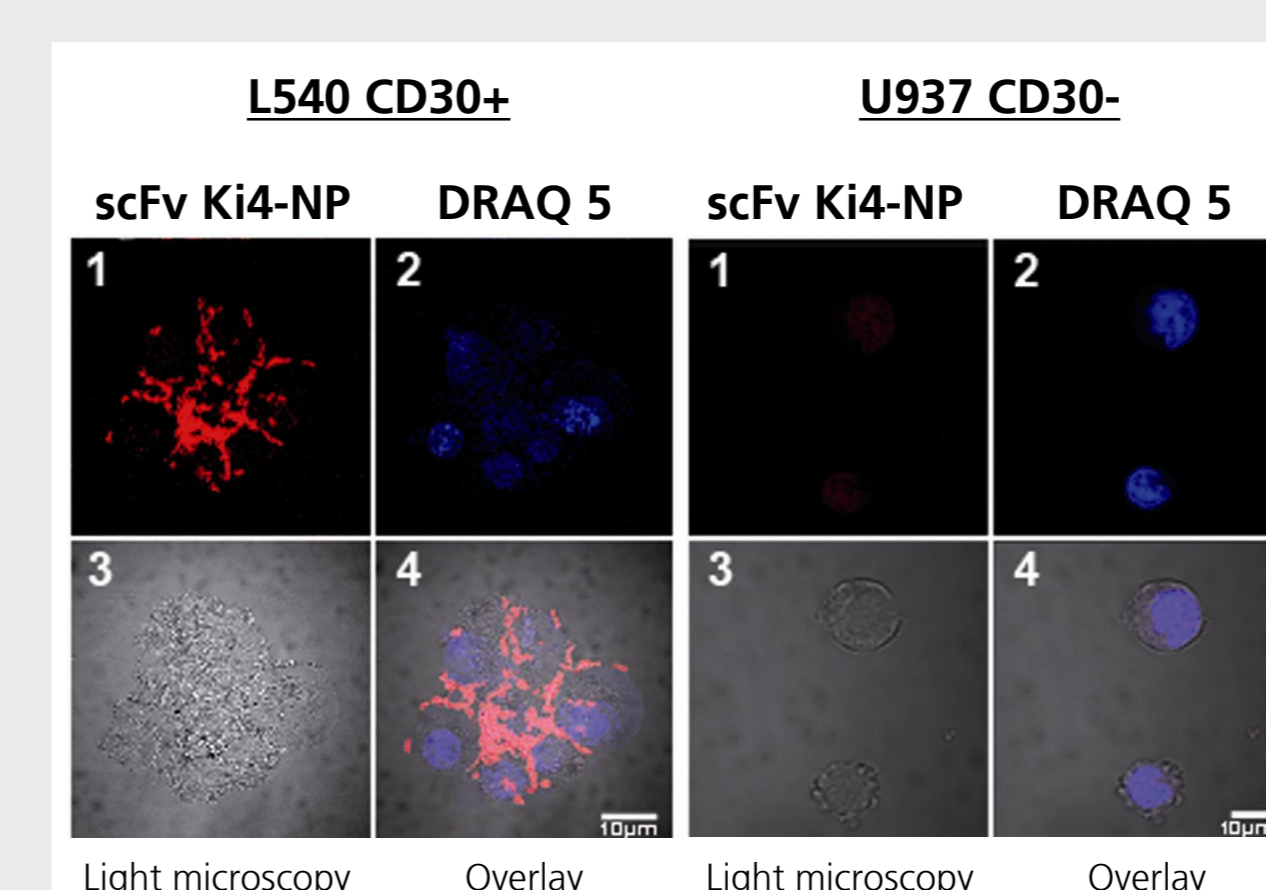
Application: flow cytometry

Detection: membrane-bound CD30

Flow cytometry binding analysis of CD30+ and CD30- cell lines, excitation 488 nm, emission measured by FL-2



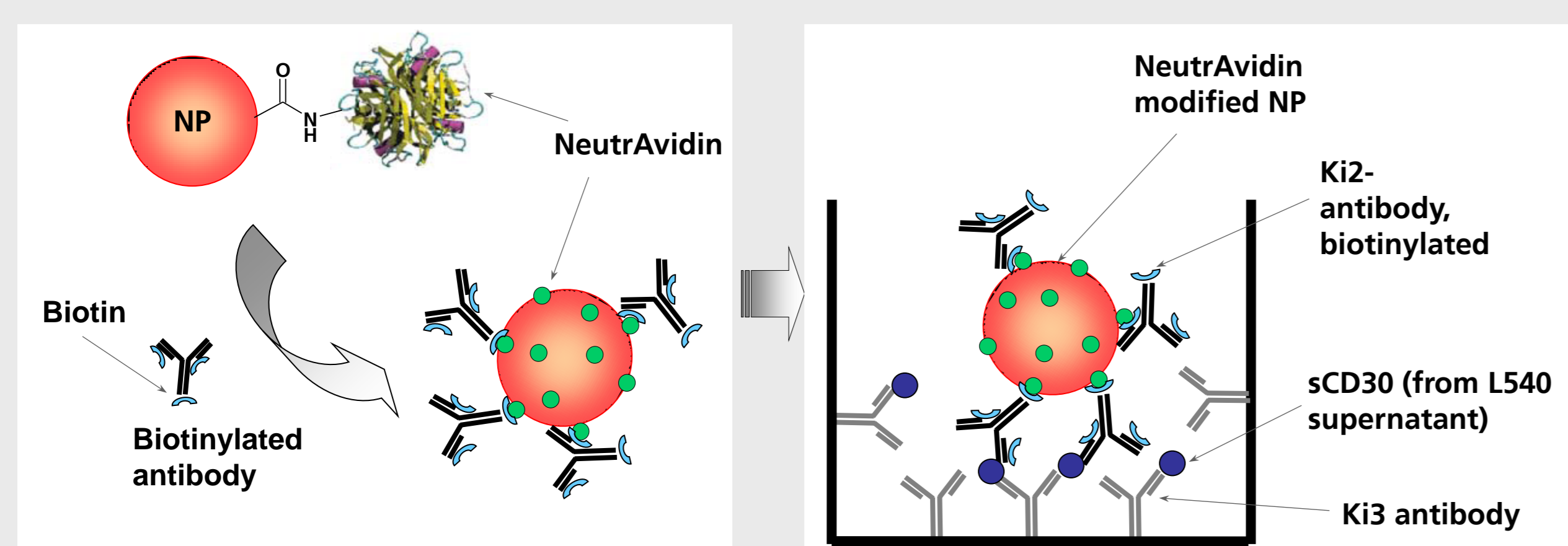
Oriented conjugation of recombinant antibody single chain Ki4 SNAP tag fusions via benzylguanine modified NP



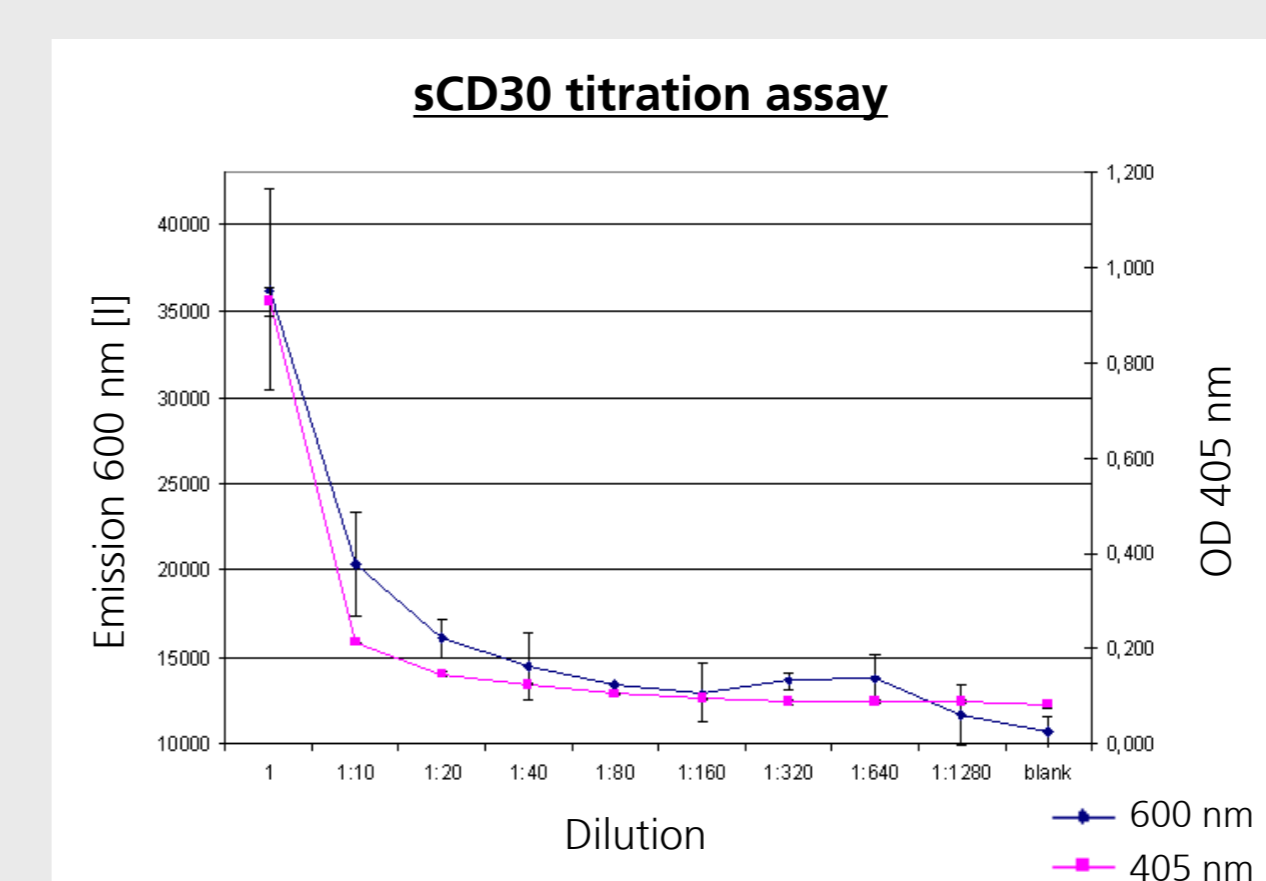
Application: confocal microscopy

Detection: membrane-bound CD30

Binding analysis by confocal microscopy: CD30+ and CD30- cell lines were stained for nuclei (blue) and CD30 (red)



Coupling of full length antibody-biotin conjugates to NeutrAvidin coated NP



Application: solid phase assay

Detection: soluble CD30

Solid phase assay: serial dilutions of sCD30 containing cell culture supernatant were analysed by a sandwich ELISA (red line) and an analogous antibody NP set-up (blue line)

Conclusion

- Organic dye labelled silica NP have been proved to meet all requirements for different immuno assay applications
- Oriented and non-oriented functional coupling of antibodies and recombinant formats were realized by appropriate chemical surface modifications of the NP

Outlook

- Qualitative surface analysis of coverage density with carboxyl functionalities
- Analysis of cell physiological parameters, e.g. cytotoxicity and internationalization

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