The goal of this project is to site-specifically functionalize nanobodies with a 'click' functionality, to allow an oriented and covalent coupling to a complementary functionalized surface. A methodology is proposed in which an unnatural amino acid is introduced in the protein structure, with applications towards biosensor chips and affinity-based chromatography.

Innovation in the field of functionalized protein-based biomaterials strongly demands for improved immobilization techniques. A stable and oriented surface coupling is required, since the efficiency of surface biofunctionalization depends on the physico-chemical properties of the biomolecules after their immobilization. Conventional techniques like physical adsorption, random covalent coupling and binding through affinity tags generally result in low performance biolayers. An improved binding affinity, sensitivity and reproducibility can be achieved by coupling Nanobodies in an oriented and covalent coupling.

**Characteristics of VHH Nanobodies**

Nanobodies are single-domain antibody fragments, derived from camilidae Heavy Chain Antibodies. Advantages include high stability, small size (15 kDa) and strong antigen binding capacity.

In this research a Nanobody against the LOX-1 cardiovascular disease biomarker will be used.

**LOX-1 Amber construct**

The TAG stop codon is introduced at the C-terminus of LOX-1. Codons around TAG: optimized for nonsense suppression.

Expression of 'clickable' Nanobodies

**Cytoplasmic**

- JX33 cells
- RF1 knockout
- Improved amber suppression

**Periplasmic**

- WK6 cells
- Disulfide bridge formation in periplasm

LOX-1

Expression 4h at 37°C

Purification: His$_6$ Ni-NTA SDS-PAGE

Clear band visible at 15kDa

Alkyn introduced into LOX-1

**Expanding the genetic repertoire of E. coli**


Cotransformation pEvol-pLys-FA with the Nanobody vectors.

Incorporation unnatural lysine with alkyne functionality.

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rekabka.hansen@uhasselt.be

Tel: +3211268300

Institute for Materials Science Universiteit Hasselt | Campus Diepenbeek Agoralaan Bld. D | B-3590 Diepenbeek