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SITE-SPECIFIC FUNCTIONALIZATION OF NANOBODIES USING NONSENSE SUPPRESSION FOR UNIFORMLY LOADED BIOSENSOR SURFACES

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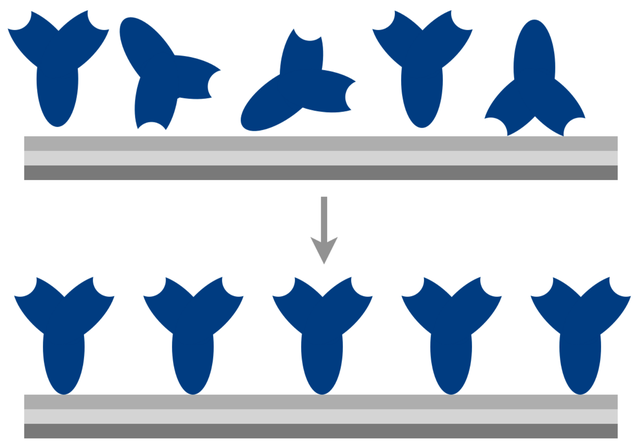
The goal of this project is to site-specifically functionalize nanobodies with a 'click' functionality, to allow an oriented and covalent coupling to a complementary functionalized surface. A methodology is proposed in which an unnatural amino acid is introduced in the protein structure, with applications 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[1]. An improved binding affinity, sensitivity and reproducibility can be achieved by coupling Nanobodies in an oriented and covalent coupling.

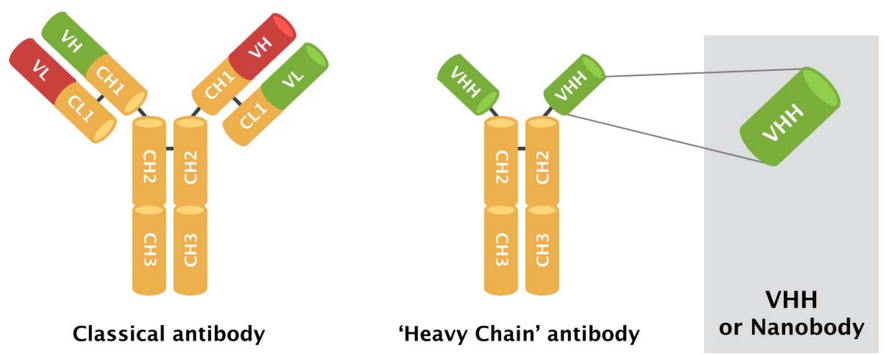


Characteristics of VHH Nanobodies

Nanobodies are single-domain antibody fragments, derived from camilidae Heavy Chain Antibodies[2].

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[3]

His_6-tag only attached when suppression of **TAG**

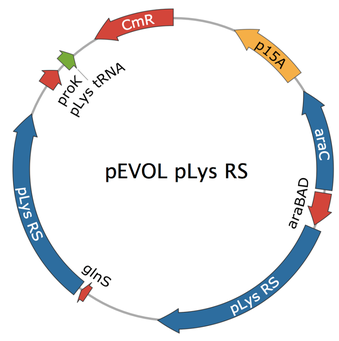
Ni-NTA purification

Expanding the genetic repertoire of *E. coli*

Mutation of pLys-RS gene (Y306A, Y384F): larger tRNA synthetase binding pocket[4].

Cotransformation pEvol-pLys-FA with the Nanobody vectors.

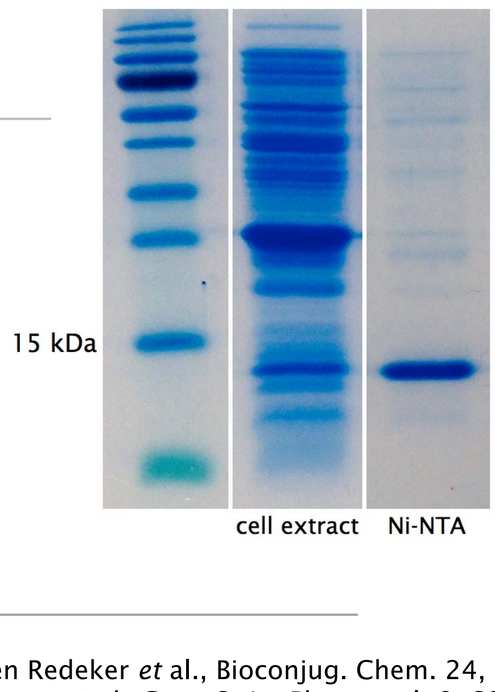
Incorporation unnatural lysine with alkyne functionality.



Expression of 'clickable' Nanobodies

Cytoplasmic

- JX33 cells
- RF1 knockout
- Improved amber suppression



LOX-1
Expression 4h at 37°C
Purification: His_6 Ni-NTA
SDS-PAGE

Clear band visible at 15kDa

Alkyne introduced into LOX-1

Periplasmic

- WK6 cells
- Disulfide bridge formation in periplasm

LOX-1 expressed without alkyne

- further optimalisation needed

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