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Oriented Llama antibodies as versatile detection element

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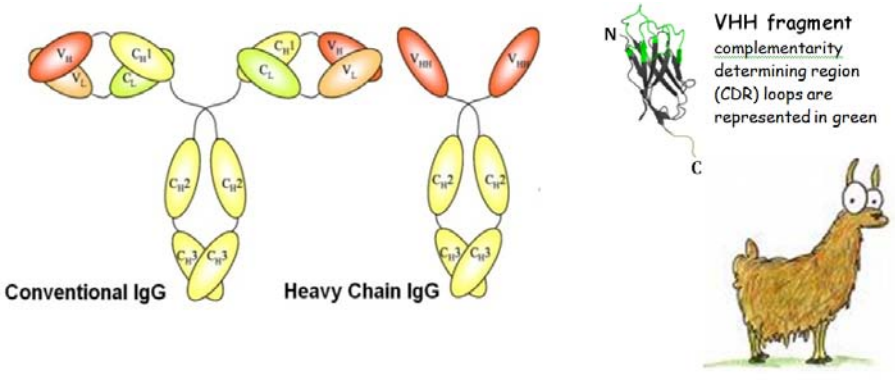
A robust detection method that enables a sensitive and reproducible analysis of targets is the driving force towards biosensors. The on-going miniaturization in this field yields sensors with nanometer-sized dimensions, which limits the size and quantity of deposited detector elements. Llama antibodies are the smallest antibodies available today, and can serve as a detector element for biosensors.

Conventional antibodies are large, complex molecules, but llamas possess smaller heavy chain antibodies. Heavy chain fragments (VHH's) display elevated conformational stability and are small in size. Therefore we aim to couple llama antibody domains (VHHs) oriented onto surfaces.



VHH fragments have been selected and are easy to engineer. In vivo biotinylation using the BirA enzyme1 was performed to functionalize the C-terminus of VHH's. Biotinylated VHH's (BT-VHH's) can be immobilized in an oriented way onto streptavidin coated surfaces. In Surface Plasmon Resonance (SPR) analysis orientation of VHH's result in a 2-9 time higher response compared to randomly immobilized BT-VHHs. Experiments also revealed that the biotin streptavidin bond is not stable under performed regeneration conditions. Several cycles of binding and regeneration lead to a strong loss in signal intensity.

To generate a stable biosensor, covalent coupling is important. To thisend VHH's have been functionalized with the methionine analog azidohomoalanine[2] to yield azide(N_3) bearing VHHs. An azide can selectively reacted with an alkyne by Strain promoted Azide-Alkyne 1,3-dipolar Cycloaddition (SPAAC) orCu(I)-catalyzed Azide-Alkyne 1,3-dipolar Cycloaddition (CuAAC)[3], known as click reactions. To investigate accessibility of azide groups, VHH's were reacted with alkyne-PEG_{5000} . A shift in size confirmed that alkynyl-PEG_{5000} was coupled to azide functionalized VHHs.

Selectivity was improved by mutating VHH's, to obtain one single azide at the C-terminal end. Next, these functionalized VHHs will be oriented onto alkyne functionalized surfaces via the azide group. Orientation lowers the antigen detection limit and covalent bound VHHs represent a stable detection element.

1. Saerens et al. 2005 AnalChem 77(23):7547-7555
2. Kiick et al. 2002 Proc Nat AcadSci USA 99(1):19-24
3. Rostovtsev et al. 2002 AngewChem Inter Ed 41(14):2596-2599

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