[P2.003]

Site-specific functionalization of nanobodies using nonsense suppression for uniformly loaded biosensor surfaces

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Innovation in the field of functionalized protein-based biomaterials, with potential application towards biosensor chips and affinity-based chromatography, strongly demand 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 biolayers with decreased activity, stability and reproducibility. In an attempt to overcome these problems, a methodology is proposed in which an unnatural amino acid is introduced in the protein structure. The aim of is this research is to site-specifically functionalize nanobodies in order to immobilize them on surfaces in a highly oriented and covalent manner. The focus is placed on nanobodies, which are single-domain antibody fragments with the right characteristics for biosensing applications. Nanobodies targeting Vascular Cell Adhesion Molecule-1 (NbVCAM1) and Lectin-like oxidized LDL receptor-1 (NbLOX-1) will be modified using the *in vivo* nonsense suppression technique in *E. coli*. Nonsense suppression was chosen because it involves the introduction of a stop codon into the protein sequence at any strategically selected location. This in contrast to other site-selective techniques that only allow for protein modification at one of the termini. We will combine the *M. mazei* pyrrolysine $tRNA_{CUA}$ with a mutated anticodon that recognizes the amber stopcodon (UAG) and the *M. mazei* pyrrolysine synthetase with an unnatural lysine containing a ‘clickable’ alkyne moiety. The well-known copper-catalysed Huisgen azide-alkyne 1,3-cycloaddition can be carried out with high efficiencies under physiological conditions and will therefore be used to covalently couple the modified nanobodies to different azidified surfaces. The activity of the nanobodies after surface coupling will be analysed using ELISA, ellipsometry and SPR. In this way a general method towards the development of homogeneously coated and highly sensitive bioactive surfaces is created.

Keywords: Nonsense suppression, Nanobody, 'Click' chemistry, Bioactive surfaces