In Vivo Synthesis of "Click" Functionalized Nanobodies for Advanced Biosensing platforms

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Goal

The goal of this project is the development of a generic *in vivo* method for the site-specific modification of proteins with a "click" functionality in order to allow an orientated, covalent coupling on a complementary functionalised surface for the design of innovative, bioactive materials.

Introduction

The immobilisation of proteins on solid surfaces is an important step towards numerous applications in research fields like e.g. proteomics, biomedical implants, drug delivery and biosensing systems. The strategies, available nowadays, allow the formation of either an orientated or a covalent bond, but not both. An interesting method to obtain a site-specific *and* orientated coupling of proteins is by means of "click" chemistry, of which the azide/alkyne cycloaddition is a well-known example. The "click" functional groups will be introduced into the proteins by *in vivo* methods, i.e. by using 'nonsense suppression' [1]. For this, a genetically encoded, mutant, orthogonal *E. coli* tyrosyl-tRNA synthetase (EcTyrRS)/$tRNA_{CUA}$ pair will be created and added to the genetic repertoire of *S. cerevisiae* for the incorporation of "click" functionalised phenylalanine. To obtain the correct mutant EcTyrRS, a library will be developed and screened. The benefit of this strategy is that it allows to produce proteins that contain a genetically encoded orthogonal functional group (i.e. alkyne or azide) on a single, strategically chosen position.

Which protein model system?

Nanobodies

The serum of Camelids contains, besides classical antibodies, also a large fraction of so called 'heavy chain' antibodies (HCAbs) *(figure 1)*. These HCAbs lack the light chain and the antigen-binding domain is reduced to one variable domain, the VHH [2]. The cloned and isolated VHH domain, or nanobody, is a very stable, relatively small protein, encoded by a single gene and has an activity comparable to classical antibodies. These characteristics makes them perfect to be used as a modelsystem. In this research the nanobody BCII10 will be used [3], of which the 3D structure is well-known and is representative for the class of nanobodies.

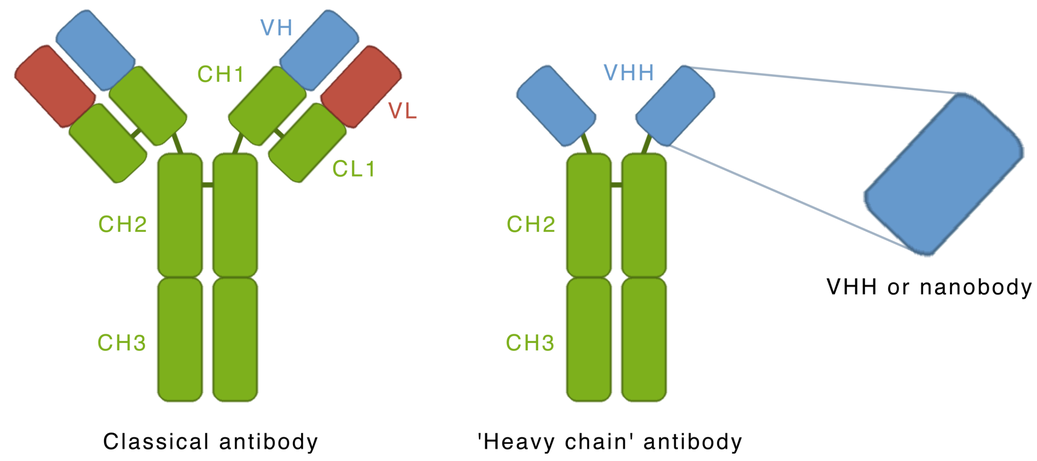

Figure 1: Comparison of the Classical antibody, the 'Heavy chain' antibody (HCAb) and the VHH or nanobody

Methodology

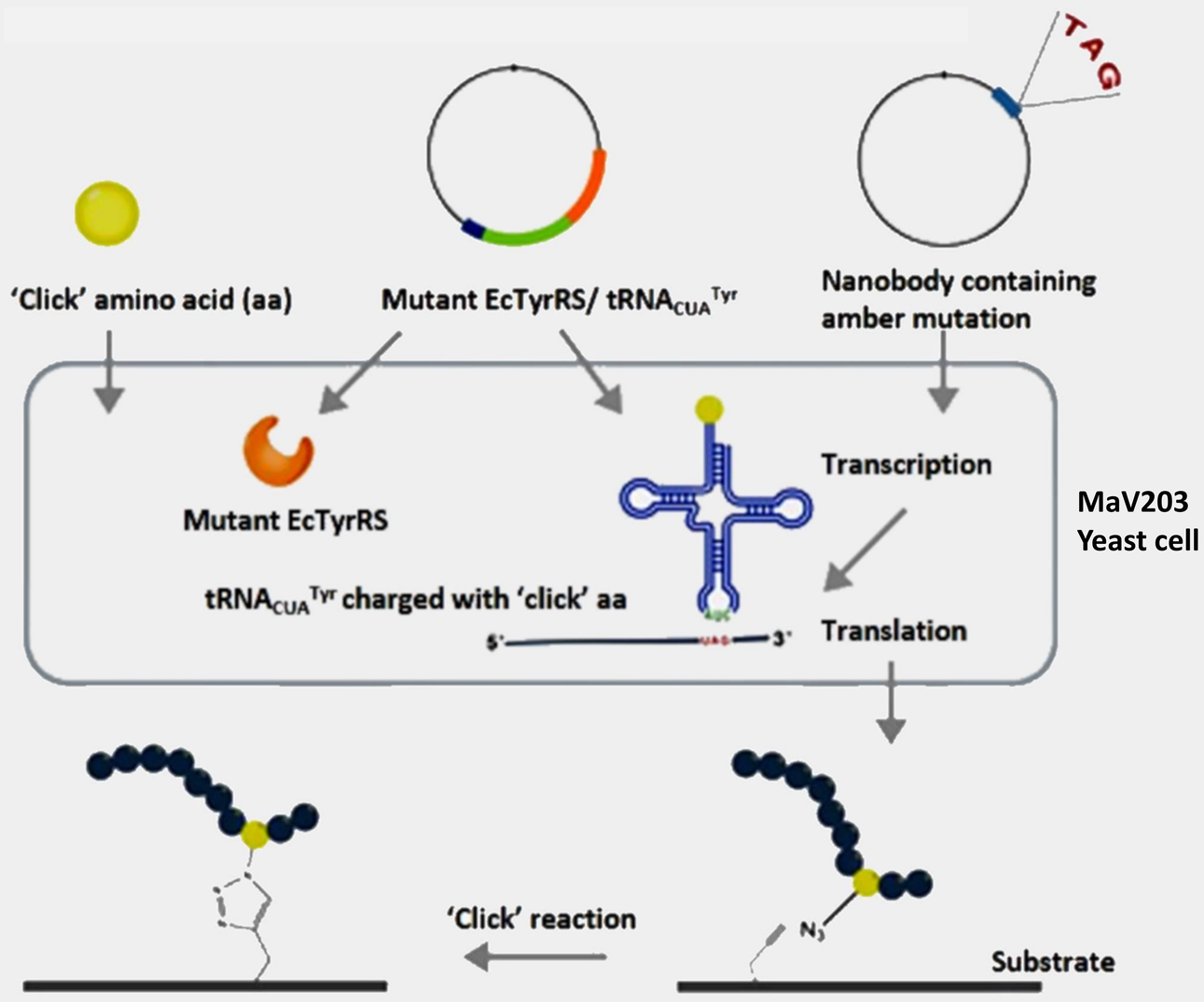

Figure 2: Method used for producing site specifically modified nanobodies in *S. cerevisiae* for the oriented and covalent coupling to surfaces.

Verifying nonsense suppression using GFP

MaV203 cells were transformed with the vectors:

- **pEcTyrRS/tRNA$_{CUA}^{Tyr}$** , containing the mutant EcTyrRS and the tRNA$_{CUA}^{Tyr}$
- **pTEF/GFP_TAG**, containing the GFP gene with an amber codon at position 48.

In case amber suppression is successful, full length GFP is cytoplasmatically expressed and the cells will show fluorescence. The MaV203 cells were grown in either the presence or absence of 1 mM *p*-azidophenylalanine, in order to verify if amber suppression occurred and whether it only happened with the incorporation of the modified amino acid. MaV203 cells transformed with the pTEF/GFP vector, containing wildtype GFP, were used as a positive control.

Figure 3 shows the cell pellets of the different conditions. It is evident that amber suppression is only successful in the presence of *p*-azidophenylalanine, suggesting that the EcTyrRS/tRNA$_{CUA}^{Tyr}$ pair selectively incorporates the modified amino acid as a response to the amber codon.

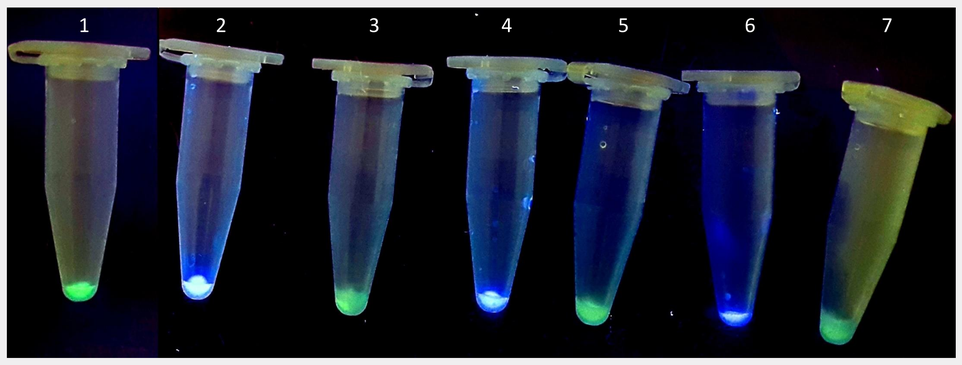

Figure 3: pellet of MaV203 cells, exposed to UV light, containing 1. pTEF/GFP (positive controle); 2, 4, 6: pEcTyrRS/tRNA$_{CUA}^{Tyr}$ + pTEF/GFP grown without *p*-azidophenylalanine; 3, 5, 7: pEcTyrRS/tRNA$_{CUA}^{Tyr}$ + pTEF/GFP grown with 1mM *p*-azidophenylalanine.

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References:

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