Sortase A-mediated protein ligation for site-specific 'click' functionalization and oriented coupling of nanobodies to biosensor material surfaces or contrast probes.

Duy Tien Ta[1], Erik Steen Redeker[1], Wanda Guedens[1] and Peter Adriaensens[1,2]

1) Biomolecule Design Group, Institute for Materials Research (IMO), Hasselt University, B-3590 Diepenbeek, Belgium
2) Applied and Analytical Chemistry, Institute for Materials Research (IMO), Hasselt University, B-3590 Diepenbeek, Belgium

Campus Diepenbeek · Agoralaan – Gebouw D, 3590 Diepenbeek, Belgium

Introduction

Nanobodies (Fig. 1) – minimized camelidae variable domains of single-domain antibodies – have numerous advantages over conventional antibodies: small size (± 15 kDa), ease of genetic manipulation and expression in *E. coli*, high stability and strong antigen binding capacity compared to the full-length antibody [1].

Staphylococcus aureus sortase A catalyzes the transpeptidation process in which the glycine in the protein LPETG motif is removed, and a peptide bond is subsequently formed between the carboxyl group of threonine and the amine group of an oligoglycine containing target [2].

In this study, we use sortase A as an *in vitro* strategy for site-specific protein modification and demonstrate this for the nanobody targeting Vascular Cell Adhesion Molecule 1 (NbVCAM1) as a model protein.

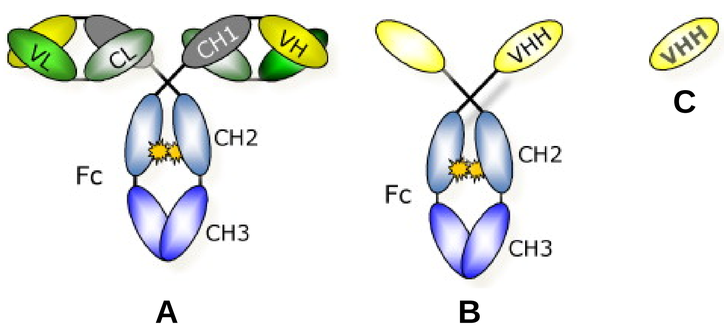

Figure 1. Structural diagram of (A) canonical antibody; (B) camelidae single-domain heavy chain antibody; and (C) nanobody (VL: variable domain of light chain, VH: variable domain of heavy chain; CL: constant domain of light chain; CH: constant domain of heavy chain; VHH: variable domain of heavy chain of heavy chain antibody) [3].

Methodology

Expression of NbVCAM1-LPETG(His)$_6$ and Sortase A

The NbVCAM1 gene with the LPETG signal and the *S. aureus* sortase A gene are separately expressed in *E. coli*. The genes are engineered with a His_6-tag to enhance subsequent purification.

In vitro modification and immobilization of NbVCAM1-LPETG(His)$_6$

The recombinant nanobody is subject to a sortase A-mediated ligation to contrast labeling probes [4], such as biotin, or further ligated to other oligoglycine containing targets including 'clickable' biomolecules or material surfaces (Fig. 2). The activity of the modified or immobilized nanobodies will be tested by measuring their antigen-binding capacity using ELISA, ellipsometry and SPR.

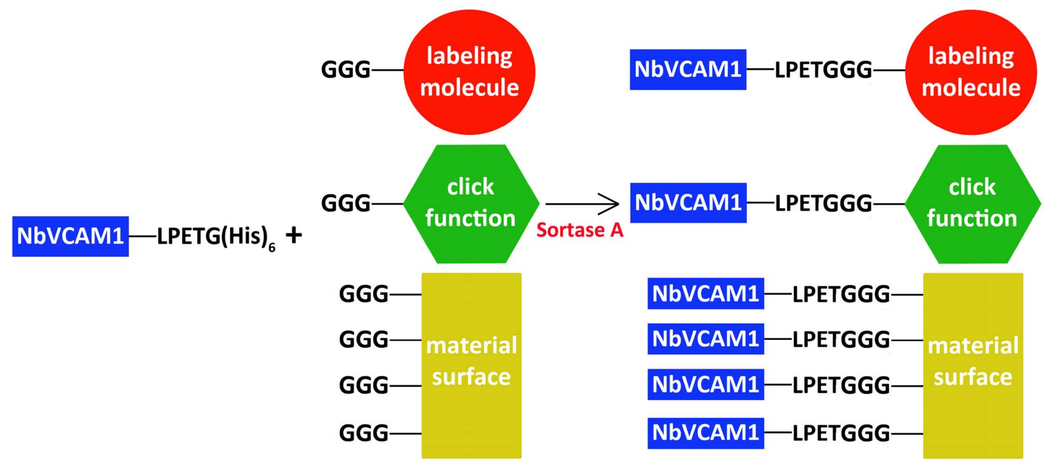

Figure 2. Sortase A-mediated ligation of NbCAM1 with various oligoglycine targets.

Sortase A-mediated biotinylation of NbVCAM1-LPETG(His)$_6$

The NbVCAM1 containing the LPETG motif is successfully and site-specifically labeled with a biotin molecule in a sortase-A catalyzed transpeptidation reaction with triglycine-biotin (Fig. 3A). A product yield of almost 100% was achieved after 10 hours incubation at 37°C when using 32 μM enzyme (Fig. 3B).

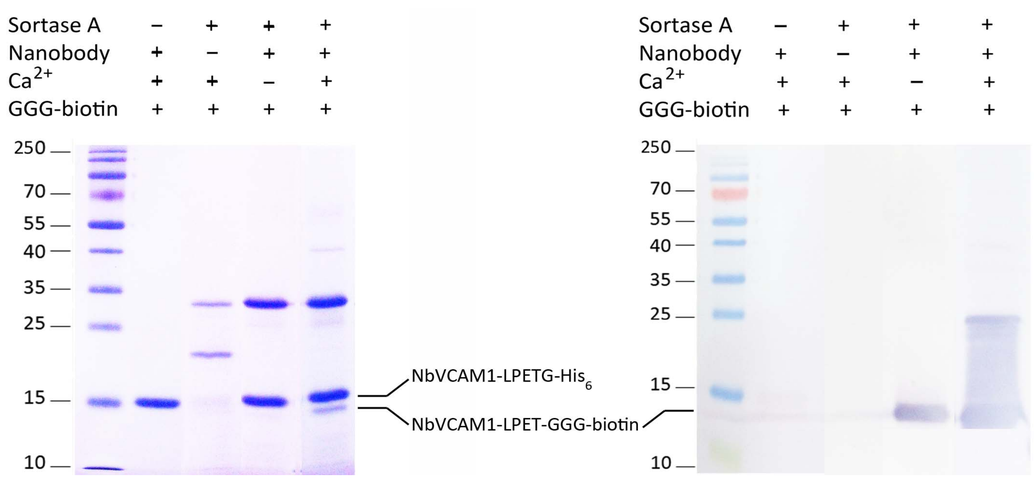

Figure 3. Sortase A-mediated biotinylation of the VCAM1-targeting nanobody. **(A)** A reaction mixture of 20 μM NbVCAM1-LPETG-His_6 is incubated with 4 μM sortase A, 100 μM $CaCl_2$ and 40 μM triglycine-biotin for 24 hours at 37°C. The mixture is then resolved on SDS-PAGE gel (left panel), and then blotted on a PVDF membrane and visualized with streptavidin-alkaline phosphatase (right panel). **(B)** Time course kinetics study of the *in vitro* biotinylation of NbVCAM1-LPETG-His_6 under sortase A catalysis. The reaction was performed with different concentrations of sortase A. The product was detected by Western blot and the yield was quantified by densitometric analysis using ImageJ software (http://rsb.info.nih.gov/ij/).

Conclusion

The enzymatic biotinylation of NbVCAM1 using sortase A is performed at mild conditions with promisingly high specificity. This study paves the way to a site-specific modification of the nanobody with a 'click' function, resulting in homogeneously modified proteins for future 'click' chemistry-mediated coupling onto material surfaces. This in order to obtain a surface covered with highly oriented nanobodies via stable covalent bonds.

Acknowledgement

This work is funded by the FWO project G.0581.12N. The authors gratefully thank Prof. Dr. Muyldermans (Vrije Universiteit Brussel, Belgium) for kindly supplying the vector pHEN6(c):*pelB*-NbVCAM1-His_6 to be used in this research. The authors further appreciate the support from the BioMiMedics consortium established in the framework of Interreg IVa in the Euregio Meuse-Rhine region.

References

[1] L. K. Swee *et al*. *PNAS* **110** (2013), 4: 1429 – 1433.
[2] M. Dumouli *et al*. *Protein Sci* **11** (2002), 3: 500 – 515.
[3] S. Muyldermans *et al*. *Vet Immunol Immunop* **128** (2009) 178 – 183.
[4] T. Proft. *Biotechnol Lett.* **32** (2010) 1 – 10.
[5] http://rsb.info.nih.gov/ij/

duytien.ta@uhasselt.be