Sortase A-mediated functionalization of nanobodies toward surface coupling

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Introduction

Staphylococcus aureus sortase A catalyzes the transpeptidation process in which the glycine in the protein LPXTG 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. The LPETG signal sequence was reported to give the best transpeptidation efficiency [1].

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

Nanobodies (Fig. 1) – minimized variable domains of single-domain antibodies found in camelidae – have numerous advantages over conventional antibodies: small size of ± 15 kDa, ease of genetic manipulation and expression in *E. coli*, high stability and strong antigen capacity compared to the full-length antibodies [2].

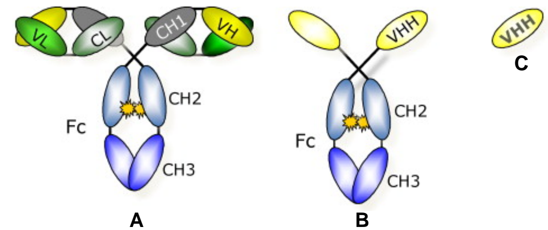

Figure 1. Structural diagram of (A) canonical antibody; (B) camelidae single-domain heavy chain antibody; and (C) nanobody (VL: variable domain of light chain, VH: variable domain of heavy chain; CL: constant domain of light chain; CH: constant domain of heavy chain; VHH: variable domain of heavy chain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

Methodology

Expression of NbVCAM1 and Sortase A

The NbVCAM1 with the LPETG signal and the *S. aureus* sortase A are separately expressed in *E. coli*. The genes are engineered with a His_6-tag to enhance subsequent purification (Fig. 2A and 2B). Both proteins are expressed at high yield on a multi-milligram scale.

In vitro modification and immobilization of NbVCAM1

The recombinant nanobody will be subject to a sortase A-mediated ligation to different oligoglycine containing targets in order to construct e.g. biofunctionalized surfaces, contrast labeling probes [4] and 'clickable' biomolecules (Fig. 2C). The activity of the modified or immobilized VCAM1 nanobodies will be tested by measuring their antigen-binding capacity using ELISA and SPR.

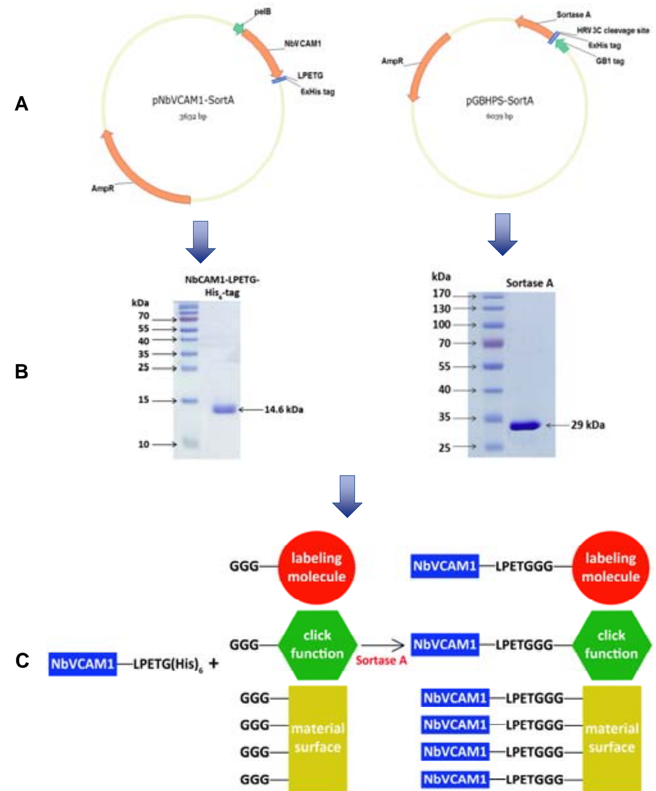

Figure 2. Schematic strategy for functionalization of VCAM1 nanobody: (A) expression constructs for NbVCAM1 and *S. aureus* sortase A, (B) SDS-PAGE analysis of purified NbVCAM1 and sortase A; and (C) sortase A-mediated ligation of NbCAM1 with various oligoglycine targets.

Conclusion

The enzymatic modification will be performed at mild conditions with promisingly high specificity. This will result in a homogeneously modified protein population for future oriented covalent coupling onto material surfaces.

References

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