Sortase A as an enzymatic tool for site-specific modification and oriented surface coupling of nanobodies

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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].

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

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

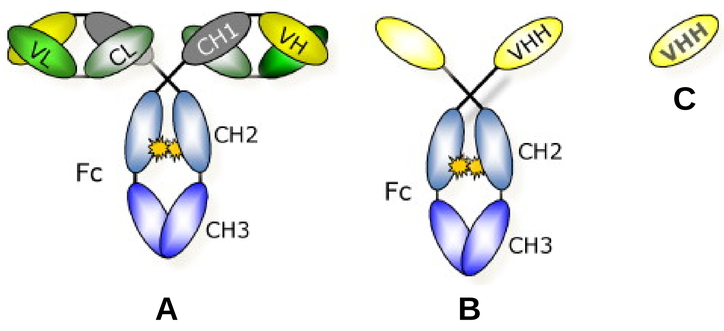

Figure 1. Structural diagram of (A) canonical antibody; (B) camelidae single-domain heavy chain antibody; and (C) nanobody (VL: variable domain of light chain, VH: variable domain of heavy chain; CL: constant domain of light chain; CH: constant domain of heavy chain; VHH: variable domain of heavy chain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

Methodology

Expression of NbVCAM1 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).

In vitro modification and immobilization of NbVCAM1

The recombinant nanobody will be subjected 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-targeting nanobodies will be tested by measuring their antigen-binding capacity using ELISA, ellipsometry and SPR.

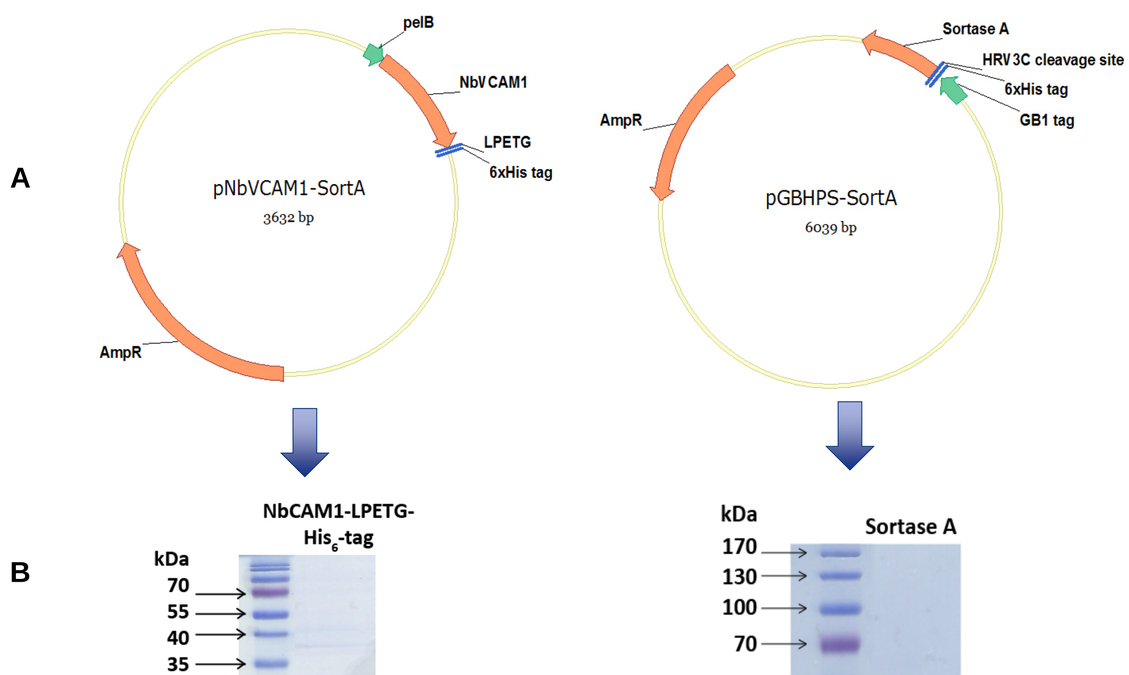

Figure 2. Strategic description for functionalization of VCAM1-targeting nanobody using sortase A: (A) expression constructs for NbVCAM1 and *S. aureus* sortase A, (B) SDS-PAGE analysis of purified NbVCAM1 and sortase A; and (C) sortase A-mediated ligation of NbCAM1 with various oligoglycine targets.

Results

The NbVCAM1 containing the LPETG motif is successfully and specifically labeled with a biotin in a sortase-A catalyzed transpeptidation reaction with triglycine-biotin (Figure 3).

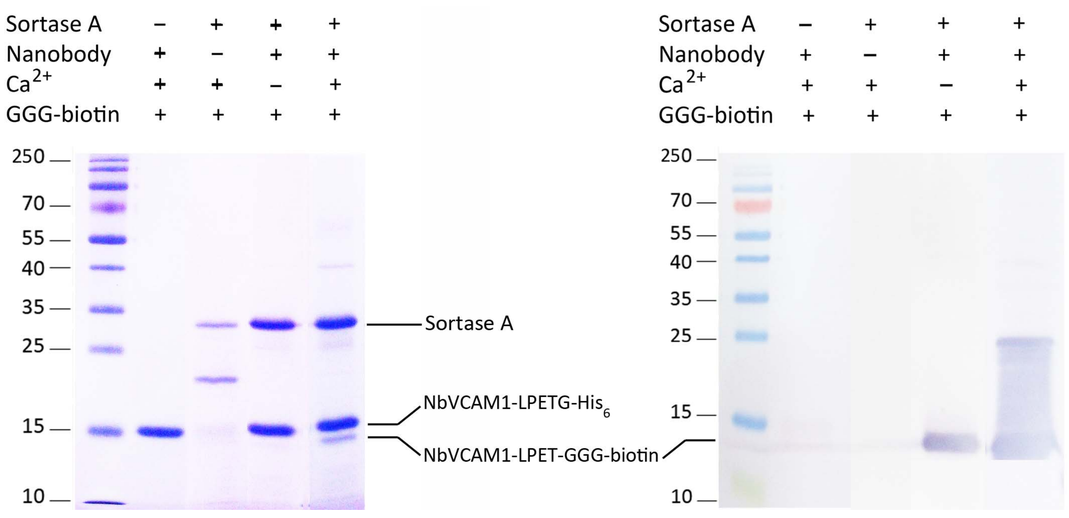

Figure 3. Sortase A-mediated biotynylation of the VCAM1-targeting nanobody. 20 μM NbVCAM1-LPETG-His_6 is incubated with 4 μM sortase A, 100 μM $CaCl_2$ and 40 μM triglycine-biotin for 24 hours at 37°C. The mixture is then resolved on SDS-PAGE gel (left panel), and then blotted on a PVDF membrane and visualized with streptavidine-alkaline phosphatase (right panel).

Conclusion

The enzymatic biotinylation is performed at mild conditions with promisingly high specificity. Future modification of the nanobody with a 'click' function will result in site-specifically modified nanobodies enabling 'click' chemistry-mediated covalent and oriented coupling to material surfaces, and so paving the way to homogeneously bio-functionalized materials.

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References

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