A RATIONALLY DESIGNED AND HIGHLY VERSATILE EPITOPE TAG FOR NANOBODY-BASED PURIFICATION, DETECTION AND MANIPULATION OF PROTEINS

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ABSTRACT
Specialized epitope tags are widely used for detecting, manipulating or purifying proteins, but often their versatility is limited. Here, we introduce the ALFA-tag, a novel, rationally designed epitope tag that serves an exceptionally broad spectrum of applications in life sciences while outperforming established tags like the HA, FLAG or myc tags. The ALFA-tag forms a small and stable α-helix that is functional irrespective of its position on the target protein in prokaryotic and eukaryotic hosts. We developed a nanobody (NbALFA) binding ALFA-tagged proteins from native or fixed specimen with extremely high affinity. It is ideally suited for super-resolution microscopy, immunoprecipitations and Western blotting, and also allows *in-vivo* detection of proteins. By solving the crystal structure of the complex we were able to design a nanobody mutant (NbALFA^{PE}) that permits efficient one-step purifications of native ALFA-tagged proteins, complexes and even entire living cells using peptide elution under physiological conditions.

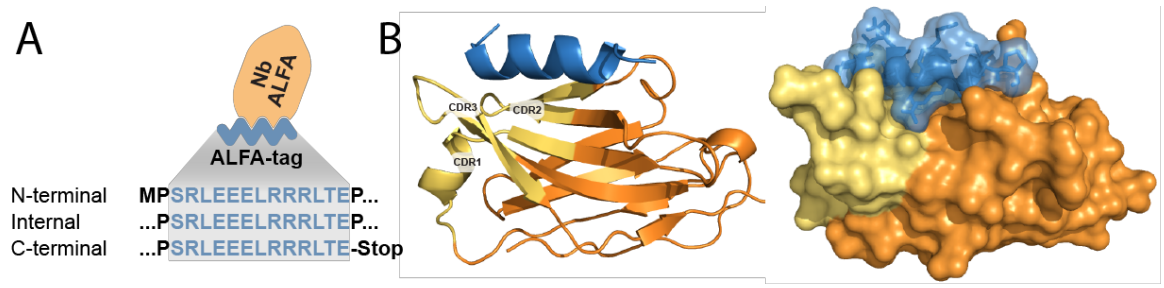

Figure 1: ALFA-tag sequence at various positions in the protein of interest (A). The crystal structure of the ALFA-tag bound to the anti-ALFA single-domain antibody (B).