

INTRODUCING SPAAC AND SPIEDAC COUPLING CHEMISTRY INTO THE VCAM-1 TARGETING NANOBODY FOR ADVANCED MEDICAL BIOMATERIALS

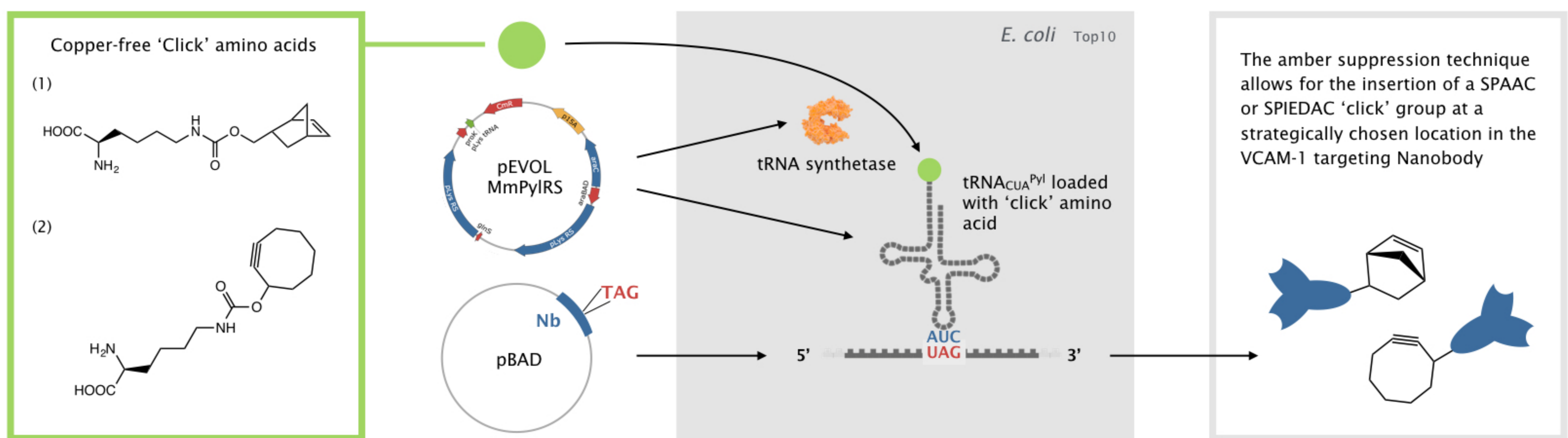
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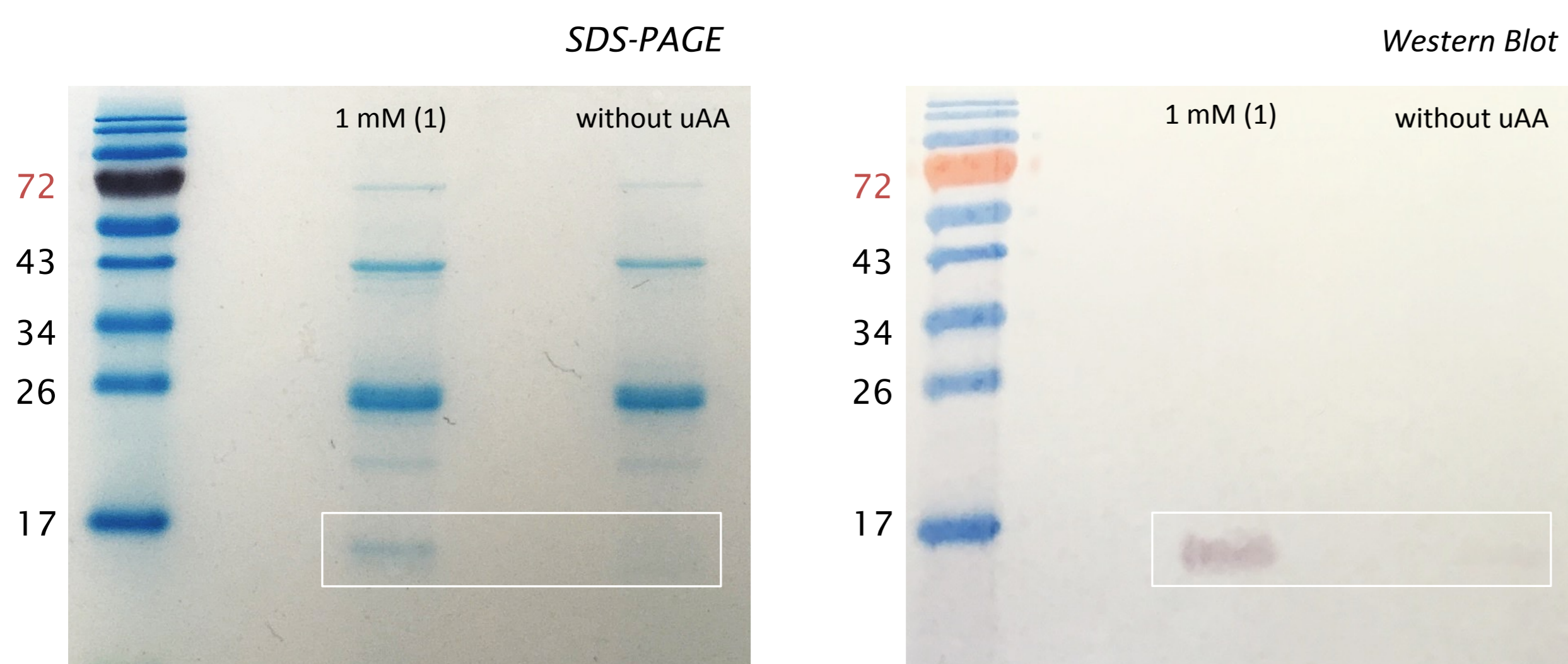
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The goal of this project is to site-specifically functionalize nanobodies with a copper-free ‘click’ functionality, to allow an oriented and covalent coupling to a complementary functionalized surface. A methodology is proposed in which an unnatural amino acid is introduced in the protein structure, with applications towards biosensor chips and affinity-based chromatography.

Expanding the genetic repertoire of *E. Coli* with copper-free ‘click’ chemistry



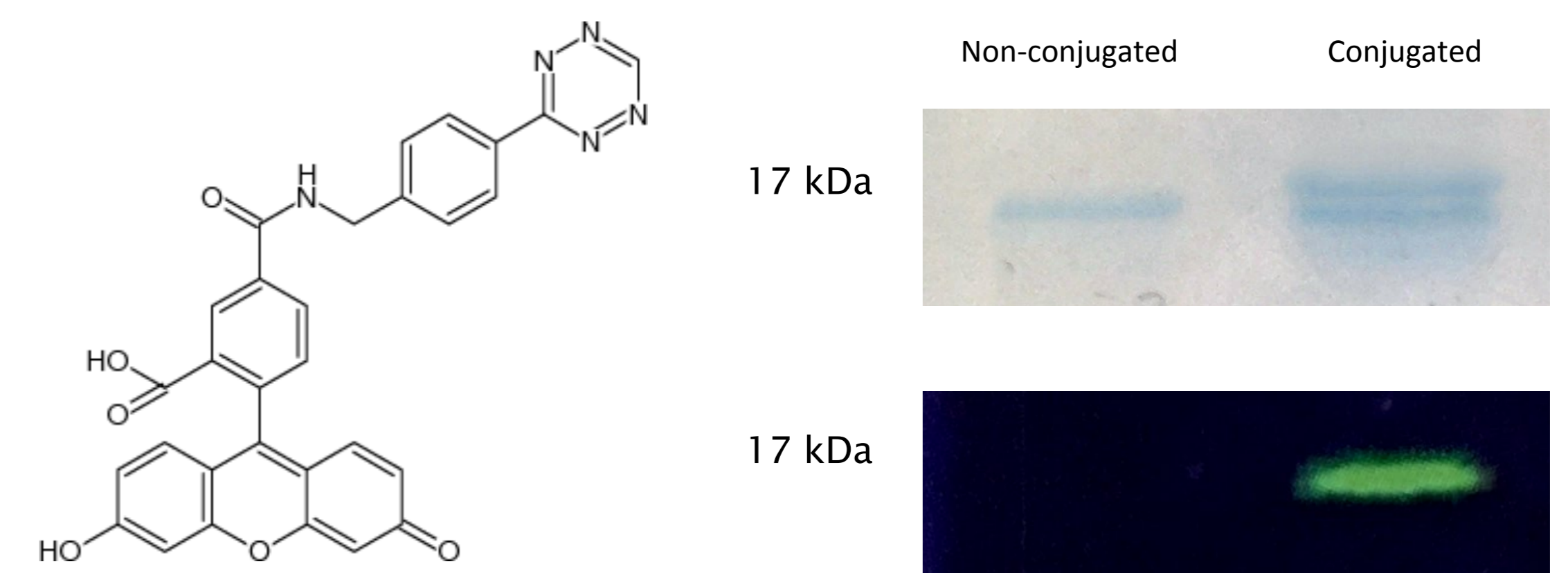
Norbornene functionalized *anti*-VCAM1 Nanobodies



The *anti*-VCAM1 gene was ligated into the pBAD vector. The TAG codon was inserted between the Nb gene and a C-terminal His-tag.

Expression conditions in Top10F['] were similar to above. 1 mM final volume of (1) was added. The cell lysate was analysed with SDS and Western Blot targeting the C-terminal His-tag.

The *E. coli* cell lysate was incubated with a fluorescent dye containing a ‘click’ functional group (tetrazine-5-FAM). The Nanobodies with a norbornene functionality conjugated with the dye, resulting in two bands on the SDS-PAGE. The conjugated Nanobodies were visualised under fluorescent light.



Optimisation amber codon location

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VCAM_WT  CCATGGCCAGGTGCAGCTGCAGGAGTCTGGGGAGGCTCGGTCCAGACTGGAGGGTCTCTGAGACTCTCCTGCGCAGCCTCTGGATACACCAATAGTAT  100
VCAM_TAG  CCATGGCCAGGTGCAGCTGCAGGAGTCTGGGGAGGCTCGGTCCAGACTGGAGGGTCTCTGAGACTCTCCTGCGCAGCCTCTGGATACACCAATAGTAT  100
VCAM_TAG_Pott  CCATGGCCAGGTGCAGCTGCAGGAGTCTGGGGAGGCTCGGTCCAGACTGGAGGGTCTCTGAGACTCTCCTGCGCAGCCTCTGGATACACCAATAGTAT  100

VCAM_WT  CATGTACATGGCCTGGTTCGCGCAGGCTCCAGGGAAGAACGCGGAGGGGGTGCAGCTATAAGATTTCCCGATGATAGTGCCTATTATGCGCGCTCCGTG  200
VCAM_TAG  CATGTACATGGCCTGGTTCGCGCAGGCTCCAGGGAAGAACGCGGAGGGGGTGCAGCTATAAGATTTCCCGATGATAGTGCCTATTATGCGCGCTCCGTG  200
VCAM_TAG_Pott  CATGTACATGGCCTGGTTCGCGCAGGCTCCAGGGAAGAACGCGGAGGGGGTGCAGCTATAAGATTTCCCGATGATAGTGCCTATTATGCGCGCTCCGTG  200

VCAM_WT  AAGGGCGGATTCACCATTTCCCAAGCAACGCCAAGAACACGGGTGTATCTGCAATGAACAACCTGAATCCTGAGGACACTGCCATGTACTACTGTGCAG  300
VCAM_TAG  AAGGGCGGATTCACCATTTCCCAAGCAACGCCAAGAACACGGGTGTATCTGCAATGAACAACCTGAATCCTGAGGACACTGCCATGTACTACTGTGCAG  300
VCAM_TAG_Pott  AAGGGCGGATTCACCATTTCCCAAGCAACGCCAAGAACACGGGTGTATCTGCAATGAACAACCTGAATCCTGAGGACACTGCCATGTACTACTGTGCAG  300

VCAM_WT  CGCGGTCTGCGCGGTACAGTTTTGCTGGAACGACCCCAAGTAACTATAACTACTGGGGCAGGGGACCCAGGTCAACCGTCTCCTCA-----  386
VCAM_TAG  CGCGGTCTGCGCGGTACAGTTTTGCTGGAACGACCCCAAGTAACTATAACTACTGGGGCAGGGGACCCAGGTCAACCGTCTCCTCATAG-----  389
VCAM_TAG_Pott  CGCGGTCTGCGCGGTACAGTTTTGCTGGAACGACCCCAAGTAACTATAACTACTGGGGCAGGGGACCCAGGTCAACCGTCTCCTCACAGAAAGTAGAAGAA  400

VCAM_WT  - CACCACCATCACCATCACTAACTCGAG  413
VCAM_TAG  - CACCACCATCACCATCACTAACTCGAG  416
VCAM_TAG_Pott  TCACCACCATCACCATCACTAACTCGAG  428
    
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The location of the amber stop codon has an influence on the efficacy of the amber suppression. Therefore, different constructs were made to optimize the yield of modified nanobodies.

The mutations were made with megaprimer PCR. The constructs will be tested in expression experiments.

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