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

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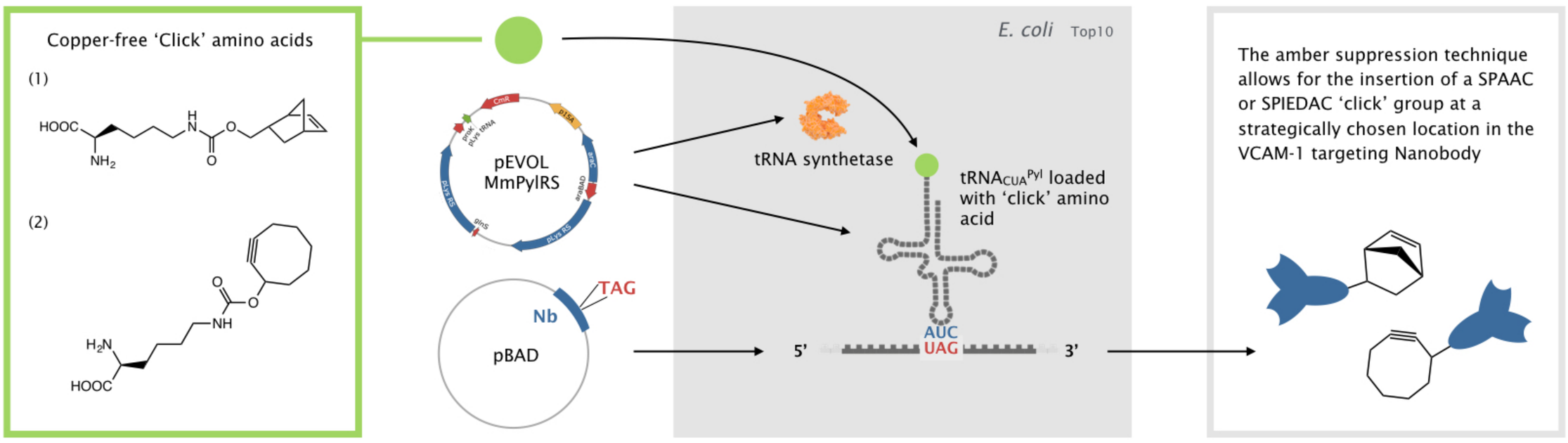
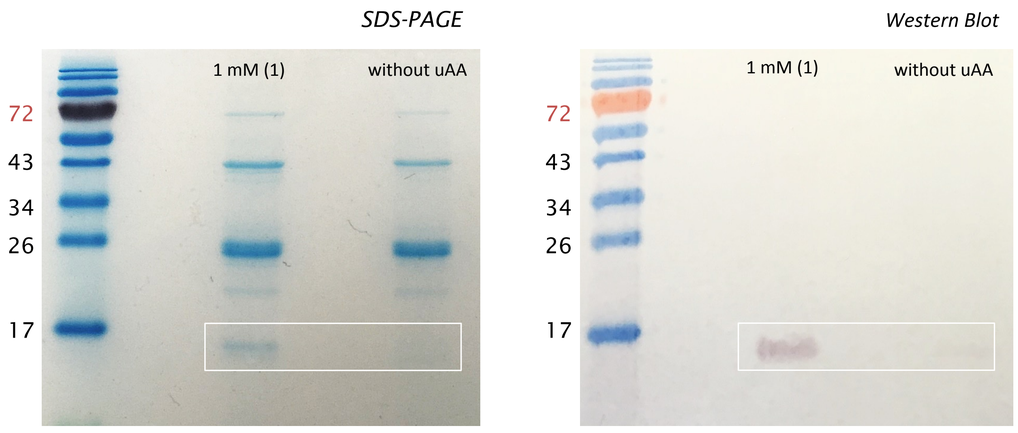
The goal of this project is to site-specifically functionalize nanobodies with a copper-free 'click' functionality, to allow an oriented and covalent coupling to a complementary functionalized surface. A methodology is proposed in which an unnatural amino acid is introduced in the protein structure,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

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



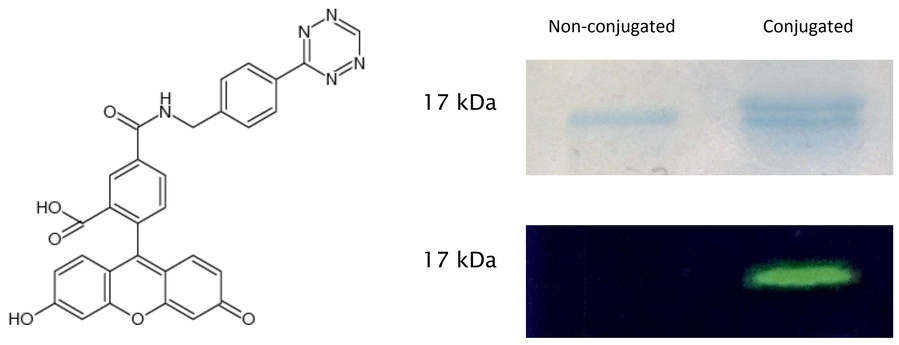
Norbornene functionalized *anti*-VCAM1 Nanobodies



The *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.

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.



Optimisation amber codon location

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                                 20                  40                  60                  80                 100
                                  |                   |                   |                   |                   |
      VCAM_WT  CCATGGCCCAGGTGCAGCTGCAGGAGTCTGGGGGAGGCTCGGTCCAGACTGGAGGGTCTCTGAGACTCTCCTGCGCAGCCTCTGGATACACCAATAGTAT 100
      VCAM_TAG CCATGGCCCAGGTGCAGCTGCAGGAGTCTGGGGGAGGCTCGGTCCAGACTGGAGGGTCTCTGAGACTCTCCTGCGCAGCCTCTGGATACACCAATAGTAT 100
VCAM_TAG_Pott CCATGGCCCAGGTGCAGCTGCAGGAGTCTGGGGGAGGCTCGGTCCAGACTGGAGGGTCTCTGAGACTCTCCTGCGCAGCCTCTGGATACACCAATAGTAT 100
                                120                 140                 160                 180                 200
                                  |                   |                   |                   |                   |
      VCAM_WT  CATGTACATGGCCTGGTTCCGCCAGGCTCCAGGGAAGAAGCGCGAGGGGGTCGCAGCTATAAGATTTCCCGATGATAGTGCCTATTATGCCGGCTCCGTG 200
      VCAM_TAG CATGTACATGGCCTGGTTCCGCCAGGCTCCAGGGAAGAAGCGCGAGGGGGTCGCAGCTATAAGATTTCCCGATGATAGTGCCTATTATGCCGGCTCCGTG 200
VCAM_TAG_Pott CATGTACATGGCCTGGTTCCGCCAGGCTCCAGGGAAGAAGCGCGAGGGGGTCGCAGCTATAAGATTTCCCGATGATAGTGCCTATTATGCCGGCTCCGTG 200
                                220                 240                 260                 280                 300
                                  |                   |                   |                   |                   |
      VCAM_WT  AAGGGCCGATTCACCATTTCCCACGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAACCTGAATCCTGAGGACACTGCCATGTACTACTGTGCAG 300
      VCAM_TAG AAGGGCCGATTCACCATTTCCCACGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAACCTGAATCCTGAGGACACTGCCATGTACTACTGTGCAG 300
VCAM_TAG_Pott AAGGGCCGATTCACCATTTCCCACGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAACCTGAATCCTGAGGACACTGCCATGTACTACTGTGCAG 300
                                320                 340                 360                 380                 400
                                  |                   |                   |                   |                   |
      VCAM_WT  CGCGGTCGTCGCCGTACAGTTTTGCCTGGAACGACCCCAGTAACTATAACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA---------------- 386
      VCAM_TAG CGCGGTCGTCGCCGTACAGTTTTGCCTGGAACGACCCCAGTAACTATAACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCATAG----------- 389
VCAM_TAG_Pott CGCGGTCGTCGCCGTACAGTTTTGCCTGGAACGACCCCAGTAACTATAACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCACAGAAGTAGAAGAA 400
                                420
                                  |
      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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