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Highly Sensitive Fluorescent Nonnatural Amino Acids That Can Be Incorporated into Specific Positions of Streptavidin

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HIGHLY SENSITIVE FLUORESCENT NONNATURAL AMINO ACIDS THAT CAN BE INCORPORATED INTO SPECIFIC POSITIONS OF STREPTAVIDIN

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A small and highly fluorescent nonnatural amino acid that carries an anthraniloyl group was synthesized and incorporated into specific positions of streptavidin. The positions of the fluorescent amino acid were directed by CGGG/CCCG four-base codon/anticodon pair. The tRNA containing the four-base anticodon and charged with the fluorescent amino acid, was prepared as shown in Figure 1. In brief, the key intermediate (pdCpA-linked amino acid) **3** was synthesized and coupled with the tRNA_{CCCG}(-CA) with T4 RNA ligase. Then, the aminoacylated tRNA **4** was mixed with in vitro biosynthesizing system of *E. coli* S30 lysate together with the mutant streptavidin mRNAs containing the four-base

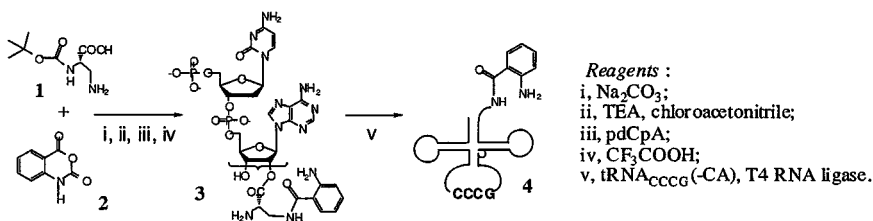


FIGURE 1

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codon. Some of the nonnatural mutant proteins were obtained in excellent yield and retained strong biotin binding activity. The fluorescence wavelength and intensity of the fluorophore at the 120th position of the streptavidin changed sensitively by the biotin binding. The results indicate that the nonnatural amino acid can be applied as highly sensitive microenvironmental probe for site-specific labeling of proteins.