Abstract

Antibodies are frequently used in many applications and they are often modified with a reporter group such as a fluorophore, biotin, a hapten or an enzyme. This type of modification of a protein might lead to unwanted alteration of the stability and activity. Hence, controlled covalent modification is beneficial. In a previous study, a strategy to site-specifically and covalently label antibodies by exploiting a selective IgG binding domain, the Z domain, for antibody labeling was presented. A photoreactive reagent with high affinity to the Fc was produced. The protein domain was decorated with a biotin on a lysine at position 58. By using this synthetic domain, covalent and specific conjugation was proven possible. In order to further optimize the system, the possibility to obtain higher signals has been investigated. To evaluate the accessibility of the biotin when directly linked to a lysine in the protein backbone, the incorporation of a PEG-linker between the Z domain and biotin were tested. Moreover, the introduction of a second biotin into the IgG-binding domain was made. The data achieved show that extending the space between the protein domain and the biotin group does not increase the accessibility of the biotin. However, by decorating the Z domain with two biotins instead of one, the signal intensity was increased with 100 %.