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Halotag System-Novel Protein Technology

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Introduction

Generally, HaloTag includes the HaloTag protein and differential functional groups are present alongwith selected HaloTag ligands. Protein of interest (POI) gets fused to HaloTag protein easily. The HaloTag ligands bind to the HaloTag fusions with covalent bonds and are specific in nature.

In this example there is studied the expression of HaloTag protein and ligand of wild and mutant type dehalogenase on protein expression. The intermediates were formed and their catalysis was studies by adding bases which effects the reaction in wild but there was no effect on mutant type intermediate. A comparison studies shows that the reactions remain the same [1].

Halotag which is an artificially developed HaloalkaneDehalogenase can form ester bonds with a chloro-alkane functionalized surface was used with thiol chemistry to achieve this goal. For the covalent attachment of proteins to surface, the procedure of using a Halotag is becoming popular. As an example we can look at the successful use of fusion proteins and terminal Halotag to label live cells and purify proteins with increased purity and high yield. Another example is the use of Halotag in single molecule force spectroscopy to immobilize poly Filamin to beads through DNA spacers and poly 127 polyprotein to mica surfaces [2].

Although covalent anchoring extends the lifetime, has high detachment forces, increased tethering time and increased range of forces to which a single molecule can be exposed, due to the little knowledge about Halotag its potential use in single molecule experiments is limited. The denaturation of Halotag under certain conditions poses both advantages and disadvantages. One advantage of Halotag as compared to other covalent attachment techniques is its mechanical fingerprint caused due to its extension up to its chloroalkane anchoring point and unfolding. The number of trapped amino acids within the protein fold and the direction of the pulling force must also be taken into consideration when looking at the response of Halotag to the denaturing forces. The interference of Halotag on the studied process might pose the biggest disadvantage for this process [3]. Investigation into the effects of the refolding and unfolding of two model proteins and the methods employed to attach proteins using chemistry and HaloTag-chloroalkane anchoring is reported here [4]. Analytical tools were developed to verify the specific HaloTag attachment to chloroalkane-functionalized surfaces. Unfolding mechanisms of Halotag caused by force-clamp AFM and force extension are discussed here. By uncoupling its refolding and unfolding from these proteins of interest, the mechanical properties of Halotag are used to show the usage of HaloTag to investigate mechanical properties of other proteins. Through measure folding of Halotag fused polyproteins and Halotag anchoring a guide for successfully employing covalent attachment of polyproteins is provided.

Protein-protein interaction

Protein-Protein interaction occurs during the cellular signalling while protein-DNA interaction requires complex techniques. The HaloTag technology has limited the research in order to save time allowing scientists to visualize the interaction among intracellular and extracellular transmembrane proteins by using micro patterning techniques. Halotag technique adaptation helped them to examine the epidermal growth factor receptor present in living cells to measure the dissociation contents of several protein complexes. Halotag has been used for the investigation of interactions between protein-DNA by using highly effective methods for the analysis of human genes [5].

Protein-DNA interactions

Designing of well-defined structures is favored by the incomparable specificity of various sequences of DNA oligonucleotides and base pairing. Oligonucleotides are practical for use in multiple applications due to their mechanical stability and ready availability. Effective drug delivery is enabled by the therapeutic application of conjugates composed of proteins or peptides and single stranded DNA. DNA-protein and protein-protein interactions are also an application of this. The fabrication of protein arrays also use DNA-protein conjugates which provide universal and reversal binding surface, enhanced binding capacity and increased reversibility.

Bio analytical immune assays also use DNA-protein conjugates to help in signal generation. The ultrasensitive immune assays have been enabled by immuno-RCA (rolling circle amplification) and immuno-PCR (polymerase chain reaction) by measuring the amplification product formed by conjugated oligonucleotide. Analyte recognition uses two separate oligonucleotide-binder conjugates which are used in proximity ligation assays. Template is formed by the ligation of two sequences for nucleic acid amplification after the analyte-bound antibodies bring the conjugated oligonucleotide sequences. Using direct luminescence-based detection reporter-labeled oligonucleotide-binder conjugates have been used. The production of the DNA-protein conjugates and their high performance in assays is enabled by conjugation chemistry. A widely used and well established method is the covalent conjugation using cross-linkers to primary amine or sulfhydral groups of the protein. Because of the availability of several sites in a protein the conjugation is uncontrollable. The amount of oligonucleotide per antibody may vary from batch to batch and the random oligonucleotide conjugation to a protein may interfere with analyte recognition. The modification of recombinant binders to enable site-specific coupling and stoichiometric conjugation overcomes this problem.

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