Split Peroxidases for Detection of Protein-Protein Interactions

Technology #16082

Applications

This technology is a novel split peroxidase-based system for the detection of protein-protein interactions and has applications in the fields of cell and molecular biology, drug discovery, and diagnostics.

Problem Addressed

Split protein reporter systems have potential applications for research of cell signaling events, detection of interactions between different cell types, sensing of environment pollutants, quantification of biomarkers, and diagnosis of disease states, including allergies and infectious diseases. Most existing split protein reporter systems are limited in that they enable detection by only a single modality (i.e. fluorescent or colorimetric or chemiluminescent). Furthermore, many split protein reporters are limited in their sensitivity of detection, with each detection event generating only a weak luminescent signal.

Technology

Split peroxidase reporters, such as split horseradish peroxidase (HRP) and split enhanced ascorbate peroxidase 2 (APEX2), overcome these limitations because once the full-length peroxidase is reconstituted, it is a versatile catalyst that generates signal for a wide array of detection modalities, including fluorescence, colorimetric readouts, and chemiluminescence. Furthermore, the peroxidases can generate contrast through a reaction with diaminobenzidine (DAB), generating a polymer that can be visualized with nanometer resolution using electron microscopy, or they can be utilized for spatially restricted proteomic mapping through proximity tagging with the probe biotin phenol.

This technology is based on the generation of a split-peroxidase system, where the peroxidase enzyme is split into two components that, alone, are catalytically inactive. Proteins of interest can be tagged with these peroxidase components. When distinct proteins of interest interact, the two complementary components of the peroxidase enzyme are brought into proximity. These components associate to form the complete, active enzyme that can catalyze a wide array of reactions, enabling diverse detection modalities. Split peroxidases can be expressed in mammalian, yeast, and bacterial cells.

Advantages

- Readout from protein-protein interactions is highly sensitivity owing to catalytic amplification by the reconstituted peroxidase enzyme
- Flexible system - split peroxidase tags can be fused to any protein of interest, and a variety of reagents and detection tools can be used to detect peroxidase activity
- Enables high-resolution detection of intercellular protein-protein interactions

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Intellectual Property

IP Type: Granted US Patent

IP Title: Split peroxidases and methods of use

IP Number: US 9,969,987

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IP Title: Split peroxidases and methods of use

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Categories For This Invention:

Life Sciences
Research Tools
Expression Systems
Protein & Protein Chemistry
Reagent
Transgenic

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Publications:

A Split Horseradish Peroxidase for the Detection of Intercellular Protein-Protein Interactions and Sensitive Visualization
Nature Biotechnology
May 30, 2016. 34(7), 774-780.
Evolution of Split APEX2 Peroxidase
ACS Chem. Biol.
March 8. 2019. 14, 619–635.

External Links:

Ting Lab
http://www.tinglab.org/
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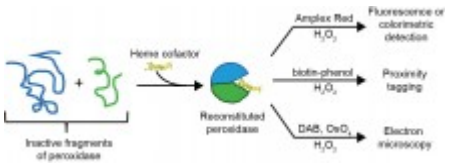
Image Gallery:



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