Biological and Biophysical Technologies to Explore Proton Pump Inhibitors

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DESCRIPTION

The functions of many proteins depend on their interactions with other proteins or their own copies, and these PPIs (Proton Pump Inhibitors) are crucial for life processes. Thus, understanding the structure, dynamics and function of PPIs is important for elucidating the molecular mechanisms of various biological processes and diseases. However, PPIs are often challenging to study experimentally, as they can be transient, weak, dynamic, or involve large and complex assemblies. Therefore, there is a need for developing and applying novel biological and biophysical technologies to explore PPIs in a reliable and comprehensive manner. Biological technologies are methods that use living systems or their components to study PPIs.

Biological technologies

Yeast Two-Hybrid (Y2H) system is a widely used technique that exploits the modular nature of transcription factors in yeast. The interaction between two proteins of interest (X and Y) is detected by fusing them to the DNA-Binding Domain (BD) and the Activation Domain (AD) of a transcription factor, respectively. If X and Y interact, they bring together the BD and AD, which activate the expression of a reporter gene. The strength of the interaction can be quantified by measuring the reporter gene activity.

Co-Immunoprecipitation (Co-IP) is a technique that uses antibodies to isolate a protein of interest (X) and its interacting partners from a cell lysate or a tissue extract. The antibody that recognizes X is immobilized on a solid support, such as beads or columns, and incubated with the sample. The bound proteins are then washed and eluted from the support, and analyzed by methods such as (Sodium dodecyl-sulfate polyacrylamide gel electrophoresis Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) or mass spectrometry.

Fluorescence Resonance Energy Transfer (FRET) is a technique that uses the transfer of energy between two fluorescent molecules (donor and acceptor) to measure the distance and orientation between them. The donor and acceptor are attached

to two proteins of interest (X and Y), either genetically or chemically. If X and Y are close enough (<10 nm), the donor can transfer energy to the acceptor upon excitation, resulting in a decrease in donor fluorescence and an increase in acceptor fluorescence. The efficiency of FRET depends on the distance and orientation between the donor and acceptor, which can be used to infer the interaction between X and Y.

Biophysical technologies

Nuclear Magnetic Resonance (NMR) spectroscopy is a technique that uses the magnetic properties of atomic nuclei to obtain information about the structure, dynamics and interactions of molecules. NMR can be used to study PPIs by measuring changes in chemical shifts, relaxation rates, cross-relaxation rates or dipolar couplings of nuclei upon binding. NMR can also provide structural information about PPIs by using techniques such as chemical shift mapping, transferred NOE or residual dipolar coupling.

Surface Plasmon Resonance (SPR) spectroscopy is a technique that uses the phenomenon of surface plasmons, which are collective oscillations of electrons at a metal-dielectric interface. SPR can be used to study PPIs by immobilizing one protein of interest (X) on a metal surface, such as gold or silver, and flowing another protein of interest (Y) over it. The binding of Y to X changes the refractive index at the interface, which alters the angle of incidence at which surface plasmons are excited. This angle can be measured by monitoring the intensity of reflected light at different angles. SPR can provide information about the kinetics, affinity and thermodynamics of PPIs.

X-ray crystallography is a technique that uses the diffraction of Xrays by crystals to determine the three-dimensional structure of molecules. X-ray crystallography can be used to study PPIs by crystallizing complexes of two or more proteins of interest (X and Y), and collecting diffraction data from them. The diffraction data can be processed by using mathematical methods such as Fourier transform or maximum likelihood to obtain an electron density map, which can be interpreted by fitting atomic models. These are some examples of biological and biophysical technologies that can be used to explore PPIs. Each technology

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has its own advantages and limitations, depending on factors such as sensitivity, specificity, throughput, resolution and

applicability. Therefore, it is often necessary to combine multiple technologies to obtain a comprehensive picture.