OPEN ACCESS Freely available online

Enzyme Engineering

ISSN: 2329-6674

Commentary

Analyzing Protein-Protein Interaction by using Crosslinking Methods

James George*

Department of Biological Sciences, Purdue University, West Lafayette, IN, 47907, USA

DESCRIPTION

Most protein-protein interactions occur briefly and are ephemeral under physiological conditions, making it challenging to study them. By covalently attaching protein-protein complexes together as they interact, crosslinking reagents, also known as crosslinkers, offer the analytical solution to capture even fleeting, weak interactions for subsequent isolation and characterisation.

In vivo or *in vitro* crosslinking can be carried out. The particular requirements of the project will determine whether *in vitro* or *in vivo* crosslinking should be used. During *in vivo* crosslinking, proteins are crosslinked in their natural state; whereas, during *in vitro* crosslinking, proteins may be denatured.

In vivo crosslinking

Proteins are crosslinked during *in vivo* crosslinking, which eliminates the possibility of false positive interactions or a loss of protein complex stability. When reacting with proteins found inside the cell membrane, hydrophobic and lipid-soluble crosslinking reagents are frequently utilised; whereas, hydrophilic, water-soluble crosslinking reagents are used to crosslink proteins found on the cell membrane, such as plasma-anchored receptors. Although proteins are still in their native state, the intricacy of proteins in cells makes it difficult to optimise *in vivo* crosslinking, and it is still possible for nonspecific crosslinking to happen. Applying crosslinking reagents with shorter spacer arms will solve the nonspecific crosslinking issue.

In vitro crosslinking

The four types of lipids that make up LNPs (Lipid Nanoparticle) are an ionizable cationic lipid (whose positive charge binds to negatively charged mRNA), a PEGylated lipid (for stability), a phospholipid (for structure), and cholesterol. LNPs are included in mRNA vaccines for SARS-CoV-2 (the virus that causes COVID-19). Neutral ionizable amino lipids were produced as a result of the positively charged lipid's rapid immune system clearance.

Crosslinking methods

Chemical crosslinking: By creating chemical connections between specific amino acid functional groups of two or more biomolecules that occur in close proximity as a result of their interaction, crosslinking chemicals covalently link together interacting proteins, domains, or peptides. Depending on the particular needs of the projects, there are a variety of commercial chemical crosslinkers available, including those that are homobifunctional or heterobifunctional, long or short in arm length, cleavable or not, water-soluble or insoluble. While heterobifunctional crosslinkers target various functional groups on various proteins for higher variety or specificity, homobifunctional molecules target the same group on the protein.

It is also possible to create crosslinker molecules with cleavable components like esters or disulfide bonds, which can be used to reverse or break the connection by adding hydroxylamine or reducing agents, respectively. Crosslinkers can either be hydrophobic to allow passage through the cell membrane or into hydrophobic protein domains or hydrophilic to only allow crosslinking in watery compartments.

Photoreactive crosslinking: More advanced crosslinker designs were developed that incorporate photoreactive groups, which react at specific times and only in response to exposure to UV light. This is because the addition of crosslinkers to cell suspensions or cell lysates may result in the formation of numerous unspecific crosslinks in addition to the target protein-protein interaction. Through a two-step procedure, heteroobifunctional crosslinkers having a chemical crosslinking group at one end and a photoreactive group at the other end can be used to specifically react to the target proteins.

Correspondence to: Dr. James George, Department of Biological Sciences, Purdue University, West Lafayette, IN, 47907, USA, E-mail: georgejames@upm.edu

Received: 01-Nov-2022, Manuscript No. EEG-22-20766; **Editor assigned:** 03-Nov-2022, Pre QC No. EEG-22-20766 (PQ); **Reviewed:** 18-Nov-2022, QC No. EEG-22-20766; **Revised:** 25-Nov-2022, Manuscript No. EEG-22-20766 (R); **Published:** 02-Dec-2022, DOI: 10.35248/2329-6674.22.11.199

Citation: George J (2022) Analyzing Protein-Protein Interaction by using Crosslinking Methods. Enz Eng. 11:199