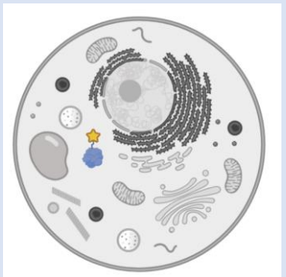
The Arginine-Phenylglyoxal Peptide Tag (APP-tag) – A Ubiquitous Strategy for Protein Bioconjugation In Vivo

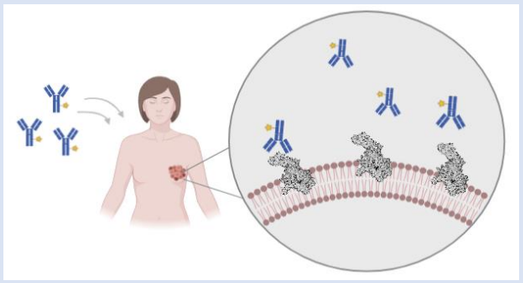
Olivia Steiner, Dr. Rebecca Scheck
Tufts University Department of Chemistry

BACKGROUND

Protein bioconjugation is a chemical technique that is used to attach molecules to proteins. It has important implications in both proteomics and drug development. Protein bioconjugation techniques can be implemented to visualize the localization and abundance of specific proteins in live cells. Understanding protein function aids researchers in gaining an in-depth understanding of biochemical pathways and the knowledge they gain can be used to manipulate these pathways. Furthermore, the emerging class of drugs coined antibody-drug conjugates (ADCs) are dependent on novel protein bioconjugation strategies to link drug molecules to antibodies which can deliver drug payloads to specific locations in the body.



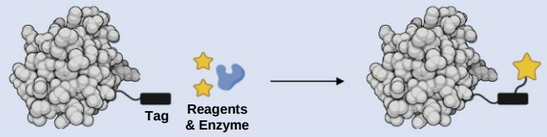
Visualizing proteins *in vivo*



ADCs for targeted drug delivery

SITE-SPECIFIC PROTEIN BIOCONJUGATION STRATEGIES

Chemical Modification of Canonical Amino Acids – Chemical reagents specifically target one amino acid for chemical modification**.**



Enzymatic Modification of Canonical Amino Acids – Enzymes recognize a specific tag attached to a POI and chemically modify the tag.

Chemical Modification of Noncanonical Amino Acids – Unnatural amino acids (UAAs) with unique chemical handles are engineered into proteins and chemically modified.

REFERENCES

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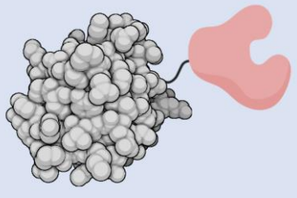
(5) Created with BioRender.com

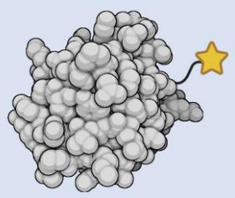
PROTEIN BIOCONJUGATION STRATEGIES

Fusion Proteins



Proteins of interest (POI) can be fused to an additional protein for study in vivo. One of the most common examples of a fusion protein is the Green Fluorescent Protein which can be incorporated into a POI and visualized under UV-light[1,2]. Enzymes can also serve as fusion proteins. The SNAP-tag is the O^6-alkylguanine-DNA alkyl transferase (AGT) enzyme fused to a protein of interest. The natural substrate of AGT can be derivatized with a variety of chemical handles to incorporate numerous functionalities, or even fluorescent molecules, into POIs.[3] A few other examples of fusion protein tags include the HaloTag, CLIP-tag, and FLAG tag.

Site-Specific Bioconjugation



Site-specific protein bioconjugation is the attachment of a molecule to a precise location within a protein by utilizing the reactivity of a specific amino acid. For example, lysine and cysteine are two of the most popular amino acids targeted for protein bioconjugation because of their nucleophilic properties and high abundance in proteins. Targeting lysine for protein bioconjugation has been utilized to successfully create one of the first approved ADCs trastuzumab which is a targeted treatment for breast cancer[4]. However, targeting a single amino acid is a challenge because proteins are made up of twenty amino acids with disparate functionalities.

ARGININE-PHENYLGLYOXAL PEPTIDE TAG (APP-Tag)

The goal of the arginine-phenylglyoxal peptide tag is to mitigate some of the challenges associated with current protein bioconjugation strategies. One common limitation of protein bioconjugation strategies is that they are not universal; they must be adapted to fit the specific protein of interest being studied. For example, there are many protein bioconjugation strategies that target the amino acid lysine, however the efficacy of the technique is dependent on where, and how many, lysine residues are accessible within the protein being studied. The arginine-phenylglyoxal peptide tag would be a short peptide tag that could be incorporated into any protein of interest. Furthermore, several of the protein bioconjugation strategies available are not applicable in vivo because the chemistry they employ is not compatible with living systems. However, the arginine-phenylglyoxal peptide tag would be functional in live cells because it utilizes chemistry that emulates glycation, which is a naturally occurring cellular process. Arginine can become post-translationally modified by the small molecule methylglyoxal, and for my project, I am using the molecule 4-azidophenyl glyoxal to modify arginine. Since the two molecules are structurally similar, 4-azidophenyl glyoxal should also be compatible for use in live cells. Below is a reaction scheme for the arginine-phenylglyoxal peptide tag.