

BIOID VS. BIOID2

TURBOID VS. MINITURBO

Comparing Protein Labeling Technologies

Protein-protein interactions (PPIs) are essential for cellular processes such as signaling, regulation and structural integrity. Mapping these interactions provides insights into cellular function and aids in identifying potential therapeutic targets.

Proximity labeling technologies enable the tagging of proteins in living cells and capturing interactions in real-time within their natural environment. This allows researchers to study complex and dynamic cellular networks more effectively.

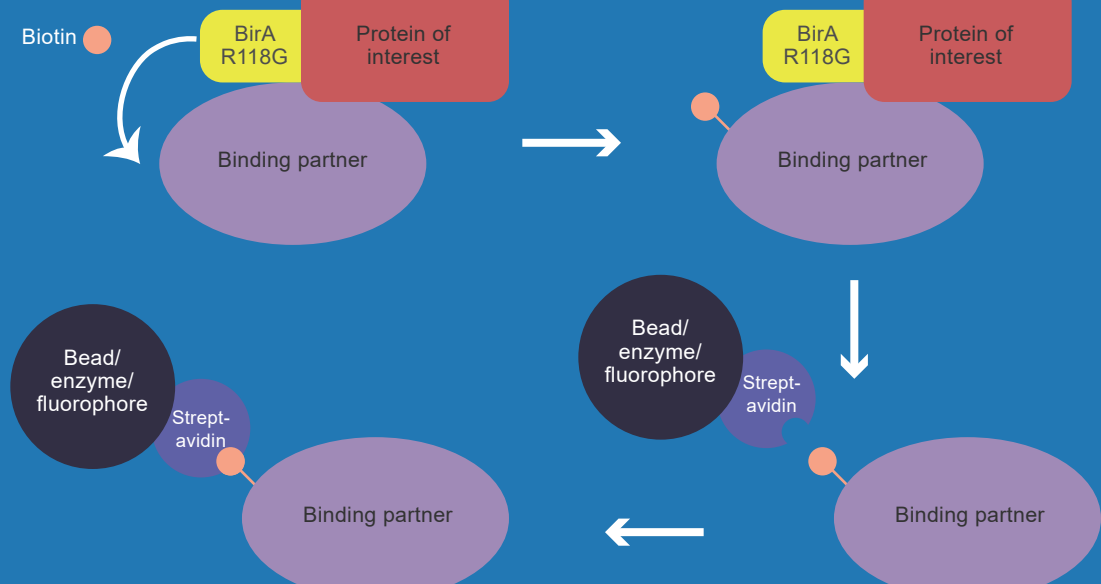
Traditional methods, such as co-immunoprecipitation, often fail to capture transient or weak interactions and can disturb the natural cellular environment. In contrast, proximity labeling techniques like BioID and TurboID preserves the cellular context and offering a more precise representation of protein interactions.

BioID

BioID (Proximity-dependent Biotin Identification) is a method for proximity labeling that identifies protein-protein interactions within living cells. Developed by Roux et al. in 2012, BioID uses a mutated E. coli biotin ligase enzyme (BirA) to biotinylate proteins that are near a protein of interest, allowing researchers to isolate and identify interacting partners.

Working Mechanism:

In BioID, the BirA* enzyme is fused to the protein of interest. When expressed in cells, BirA* catalyzes the covalent attachment of biotin to lysine residues on proteins that are spatially close to the fusion protein. The biotinylated proteins can then be isolated using streptavidin-coated beads, which have a high affinity for biotin. These isolated proteins are subsequently identified via mass spectrometry, allowing researchers to map protein interaction networks. The process typically requires 18-24 hours for sufficient biotinylation to occur, making it suitable for studying relatively stable or long-term interactions.



Advantages

- **Living Cell Labeling:** BioID can be employed to label proteins in living cells, preserving the native cellular environment.
- **No Cross-Linking Required:** The absence of cross-linking agents reduces potential artifacts.
- **High-Throughput Screening:** Suitable for large-scale studies and high-throughput screening.

Limitations

- **Extended Labeling Time:** BioID typically requires 18-24 hours for effective labeling, which may not be suitable for dynamic studies.
- **Potential Fake Positive Results:** BioID may cause non-specific biotinylation, which could lead to background noise and affect data accuracy.

BioID2

BioID2 is an enhanced version of BioID, designed to overcome some of the limitations of the original method. It employs a smaller, engineered biotin ligase from Aquifex aeolicus, which minimizes interference with the target protein's function or localization, providing higher specificity and versatility for studying protein-protein interactions.

BioID2 employs a smaller BirA* enzyme to achieve more precise labeling. This reduction in enzyme size minimizes interference with cellular functions and improves overall labeling efficiency.

Advantages

- **Reduced Protein Interference:** The smaller enzyme reduces potential disturbances in cellular processes.
- **Improved Efficiency:** Enhanced expression and labeling efficiency in living cells.

Limitations

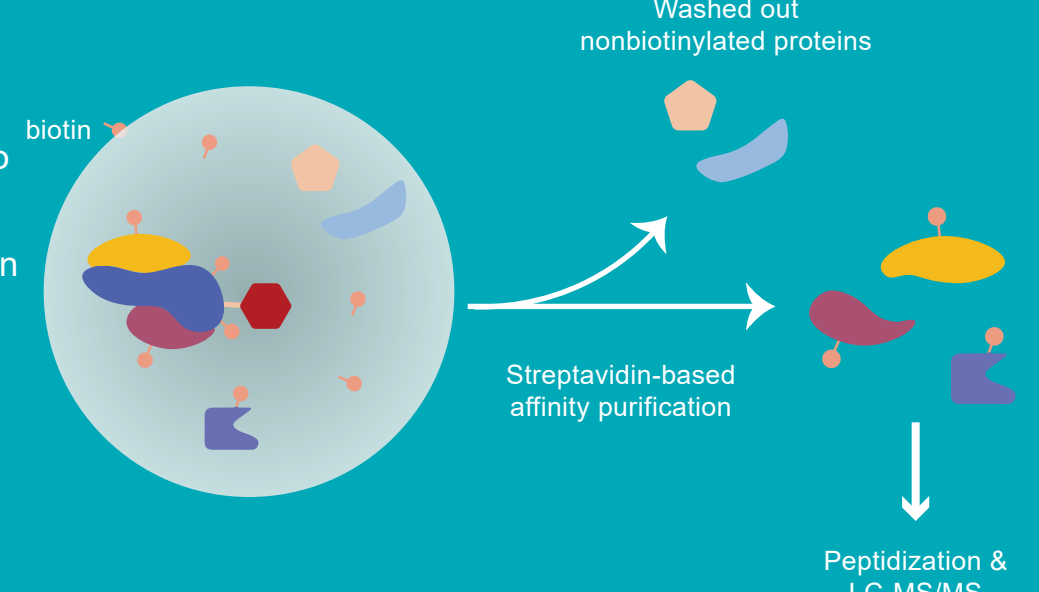
- **Slow Response Speed:** Despite improvements, BioID2 still faces the challenge of slower labeling times compared to newer technologies.

TurboID

TurboID presents a major advancement over BioID and BioID2, designed to improve the speed and efficiency of biotin labeling. TurboID was developed by Branon et al. in 2018 as a more active variant of the biotin ligase, enabling rapid labeling of proteins in vivo. This technology is particularly beneficial for studying dynamic processes and transient interactions.

Working Mechanism:

TurboID operates on the same basic principle as BioID, where a biotin ligase is fused to the protein of interest to label nearby proteins. However, TurboID's engineered biotin ligase is much more efficient, allowing biotinylation to occur in as soon as 10 minutes to 1 hour. This rapid labeling capability makes TurboID ideal for capturing transient protein interactions and for applications requiring high temporal resolution. However, the heightened enzymatic activity of TurboID can lead to increased background noise due to the potential for non-specific labeling.



Advantages

- **Fast Labeling:** TurboID provides rapid protein labeling, ideal for studies requiring swift data acquisition.
- **Enhanced Spatiotemporal Accuracy:** The improved speed allows for better analysis of dynamic cellular processes.

Limitations

- **Higher Non-Specific Labeling Risk:** The rapid tagging may lead to higher instances of non-specific biotinylation.
- **Background Signal Issues:** Extensive biotinylation can sometimes result in elevated background signals.

miniTurbo

miniTurbo is a variant of TurboID, designed to retain the speed of TurboID while reducing the size of the biotin ligase. The smaller size of miniTurbo makes it ideal for labeling proteins in confined spaces within cells, such as within specific organelles or densely packed protein complexes.

Working Mechanism:

miniTurbo functions similarly to TurboID but with a smaller biotin ligase that reduces potential interference with the protein's function or localization. The miniTurbo ligase is fused to the protein of interest, and upon activation, it biotinylates nearby proteins within minutes (10 minutes to 1 hour). This makes miniTurbo highly effective for studying protein interactions in specific subcellular regions or in experiments where the size of the labeling enzyme could be a limiting factor.

Advantages

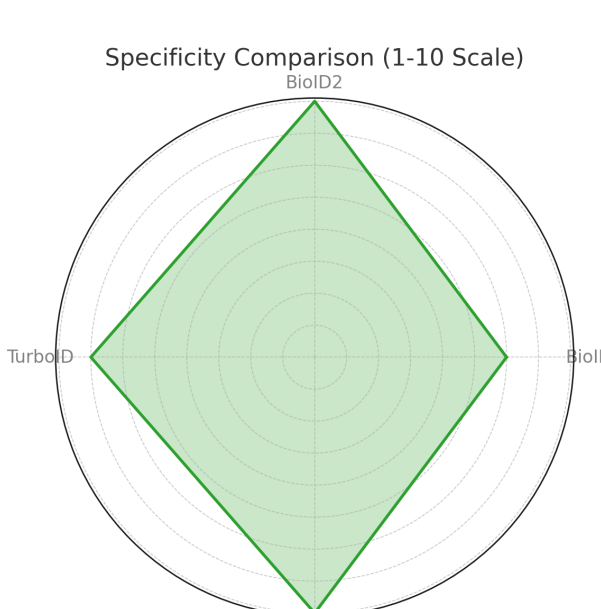
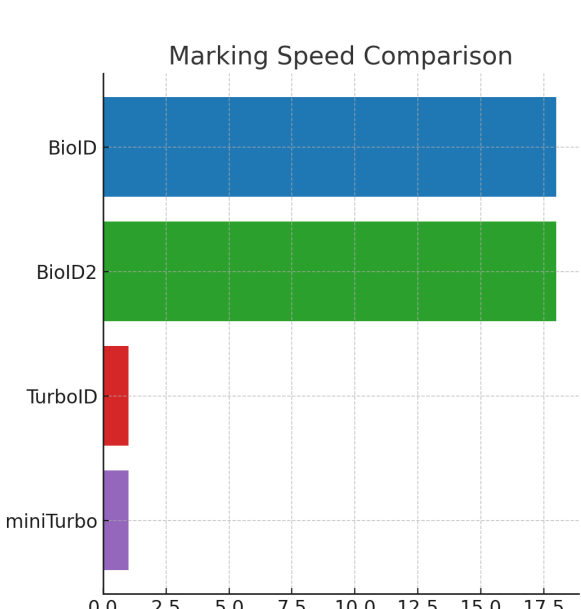
- **Subcellular Labeling:** Suitable for studying proteins in specific cellular compartments.
- **Fast Labeling Speed:** Comparable to TurboID, facilitating quick data acquisition.
- **Lower Non-Specific Labeling Risk:** The smaller enzyme minimizes the risk of non-specific interactions.

Limitations

- **Background Signal Issues:** There may still be challenges with background signals, although less pronounced than with TurboID.

How to Choose?

Feature	BioID	BioID2	TurboID	miniTurbo
Reaction Time	18-24 h	18-24 h	10 min - 1 h	10 min - 1 h
Specificity	Moderate	High	Moderate	High
Labeling Efficiency	Slow	Slow	Fast	Fast
Size of Biotin Ligase	Large	Small	Large	Small
Best for	Stable protein-protein interactions	Confined spaces, smaller complexes	Dynamic and transient protein interactions	Spatially restricted regions (organelles)
Strengths	Reliable for long-term interaction studies	Reduced steric hindrance in crowded spaces	Fast labeling, ideal for transient interactions	Small size, fast labeling in confined spaces



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