Docket #: S22-487

LIGHT CONTROLLED PROXIMITY LABELING WITH LOV-TURBO

Technology Reference
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Stanford ref. no. S22-487

Researchers at Stanford University have developed a new approach for proximity labeling via optogenetic control.

Allosteric regulation is common in naturally occurring enzymes in living tissues. However, despite its ability to improve spatial and temporal control, allostery has yet to be used extensively in artificially engineered enzymes.
One artificially engineered enzyme, TurboID, achieves broad proximity-dependent biotinylation. While Turbo is useful for mapping interactomes and organelle proteomes both in vivo and in vitro, one important drawback is that Turbo's substrate, biotin, is endogenously expressed in most tissues of living organisms. This endogenous expression severely limits its temporal specificity. Furthermore, poor genetic integration and targeting can additionally contribute to the limited spatial specificity of Turbo and other proximity labeling enzymes. This limitation can be highly dependent on cell type, cellular compartment, and proteins of interest. Further methods are needed to improve the spatial specificity of proximity labeling via Turbo.

Stage of Development
Research -

in vivo

Stage of Research
The inventors have developed a light-regulated version of Turbo, dubbed LOV-Turbo. This enzyme was engineered through a combination of structure-guided design, screening and directed evolution to express a light-dependent domain. LOV-Turbo

has virtually undetectable activity in darkness but activates within seconds of weak blue light illumination. Additionally, LOV-Turbo has an extremely wide dynamic range even in the presence of high amounts of biotin. The inventors have proven in a peer-reviewed study that this enzyme can efficiently and accurately perform proximity labeling in yeast, bacteria, and the mouse brain. This is especially impressive considering that neurons have a relatively high burden of biotin and are therefore more prone to confounding background noise in the original TurboID system. The applications of LOV-Turbo are vast, exciting, and represent a step forward in the field of enzyme engineering.

Applications

- Enhanced spatial and temporal specificity when compared to other proximity-labeling enzymes.
- Ability to perform pulse-chase experiments to explore protein shuttling between different cellular compartments.
- LOV-Turbo can be activated by the luciferase NanoLuc via BRET to selectively biotinylate protein subcomplexes.

Advantages

- Comparable activity to the original TurboID enzyme
- Increases temporal and spatial specificity when compared to other proximity labeling enzymes.
- Provides additional avenues for the interrogation of cellular and molecular biology.

Publications

- Lee, SY., Cheah, J.S., Zhao, B. et al. "Engineered allostery in light-regulated LOV-Turbo enables precise spatiotemporal control of proximity labeling in living cells." Nat Methods 20, 908–917 (2023).

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