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IMPROVED VARIANTS OF TEV PROTEASE FOR BIOTECHNOLOGICAL APPLICATIONS

Researchers at Stanford and the Chan Zuckerberg Biohub have developed methods for producing protease variants with increase catalytic efficiency.

Proteases are ubiquitous in biology, and their peptide bond cleavage activities have been harnessed for a wide range of biotechnological applications. TEV (Tobacco Etch Virus) protease is widely used and appealing due to its high sequence specificity for the TEV cleavage site (TEVcs) and orthogonality to mammalian systems. However, a major limitation of TEV protease is its slow catalysis.

Stage of Research
The inventors have developed methods for producing proteases with increased catalytic efficiency or catalytic rates using a directed evolution platform. Their platform employs a pair of photoinducible fusion proteins and subsequent reporter tools to assess light-activated and proximity-dependent protease activity. The inventors demonstrate that improved TEV variants can be used as sequence-specific transcription factor release tools in response to calcium and light in FLARE (Fast Light- and Activity-Regulated Expression), or GPCR activation and light in SPARK (Specific Protein Associated tool giving transcriptional Readout with rapid Kinetics).

Applications

- Improved protease tools for use in existing biotechnology applications
- Improved TEV variants for increased temporal resolution in sequence-specific release applications, such as FLARE or SPARK

Advantages

- Directed evolution and iterative selection is useful for producing proteases having increased substrate cleavage rates with each round of selection.
- Methods provide the advantage of enabling kinetic selection for fast protease catalysts
- Variants maintain high sequence specificity while improving catalytic turnover

Publications

- Sanchez MI and Ting AY. Directed evolution improves the catalytic efficiency of TEV protease. Nat Methods. 2020 Feb;17(2):167-174. Doi: 10.1038/s41592-019-0665-7.

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