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HUH fusion enzymes for multiplex protein labeling

HUH fusion tags are novel, robust endonucleases that allow proteins and DNA to be linked covalently in one simple step

IP Status: US Patent Issued # 10,717,773

Applications

- Covalently linking proteins and nucleic acids
- Multiplex “one pot” protein labeling
- Molecular machine assembly
- DNA based drug delivery
- Synthesis of DNA scaffolds for various biotechnology applications

Key Benefits & Differentiators

- **Less disruptive to protein function:** because HUH are small proteins composed of only approximately 100 amino acids
- **Diverse range of targets:** HUH proteins are able to recognize specific DNA sequences of standard nucleotides (instead of modified bases) and over 20 HUH proteins are known
- **Increased stability:** HUH proteins use tyrosine as the catalytic residue which is less prone to deactivation by oxidation

Technology Overview

Self-labeling enzymes such as the SNAP-tag, CLIP-tag, and HALO-tag are popular systems for specific protein-DNA bioconjugation. While these fusion tags are useful and efficient, they require the incorporation of expensive modified nucleotides bases and additional purification steps, which is costly and time-consuming. In addition, current protein tagging solutions use catalytic amino acids vulnerable to deactivation and are more likely to interfere with protein function due to the large size of the fusion enzymes used. Ultimately, current technologies offer a limited number of orthogonal or non-cross reactive self-labeling enzymes, which pose a bottleneck for development of multiplexed applications reliant on protein-DNA linkages, such as protein barcoding.

To address this gap researchers at the University of Minnesota have developed a suite of HUH enzymes (HUH-tags) for covalent linkage of proteins and nucleic acids. HUH are small proteins (~100 amino acids) found in viruses and mobile DNA elements which recognize and cleave single stranded DNA in a sequence-specific manner. These very small proteins can be engineered to bind many DNA sequences with minimal interference and utilize, more stable chemistry than pre-existing protein tags. Multiple HUH tags have been tested and work very robustly, and these tags can be used to fuse multiple types of proteins to nucleic acids (nuclear, cytoplasmic and cell-surface proteins). The chemistry works both in-vitro and in mammalian cells and multiple proteins can be labeled simultaneously in “one pot” reactions. The large number of available HUH enzymes expand the possibilities for orthogonal protein labeling and enable related applications in nucleic acid origami, molecular machine assembly, DNA-based

Technology ID
20150143

Category
Life Sciences/Biologics
Life Sciences/Biomarkers
Life Sciences/Biochemicals & Small Molecules
Life Sciences/Research Tools

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drug delivery, and gene editing applications.

Phase of Development

TRL: 3-4

The versatility of HUH-tags have been demonstrated in multiple applications: For enhancement of HDR, to increase efficiency of Cas9-mediated gene editing To create Antibody-AAV capsid composites for altered AAV cell tropism The construction of doubly DNA-tethered proteins for single-molecule studies In cellular imaging in live and fixed cells.

Desired Partnerships

This technology is now available for:

- License
- Sponsored research
- Co-development

Please contact our office to share your business' needs and learn more.

Researchers

- **Wendy Gordon, PhD** Associate Professor, Department of Biochemistry, Molecular Biology, and Biophysics

References

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