Bright and Photostable Cyanine Dye Tags for Research Applications

Protein tags with high affinity and selectivity for off-the-shelf commercially-available fluorescent dyes that enhance their spectral properties.

Technology

The NYU innovators have developed genetically encoded, high affinity protein tags specific for two widely used sulfo-cyanine dyes, Cy3 and Cy5, to enhance their spectral performance in common research applications. As described in a bioRxiv preprint (*Sasazawa et al. 2024*), directed evolution was performed on a yeast cell surface display library of single-chain variable fragments (scFvs), isolating two unique proteins that bind and stabilize sulfonated derivatives of cyanine (Cy3 or Cy5) with high affinity and selectivity. These photostability enhancing proteins against cyanine (PEPCy) dyes have enhanced fluorescence lifetimes, molecular brightness and photostability compared to their cognate cyanine dyes. The PEPCy3-Cy3 complex showed a 6-fold enhancement in molecular brightness, while the PEPCy5-Cy5 complex exhibited a modest 7% enhancement. Each PEPCy tag displayed high affinity (nanomolar and picomolar range) and highly specificity for specific sulfo-cyanine dyes with no cross-reactivity. The utility of the PEPCy tags was successfully demonstrated in proof-of-concept microscopy experiments using two different classes of cell surface proteins in mammalian cells (the β2 adrenergic receptor and sodium channel Nav1.7) and bacteria (Intimin). In summary, these next-generation PEPCy tags allow for superior visualization of protein targets of interest and can be leveraged in basic research applications and drug development efforts.

Background

The demand for durable fluorescent markers to visualize protein targets of interest is growing, spurring advancements in the development of photostable dyes. In particular, efforts have been made to develop highly photostable rhodamine and cyanine dyes that incorporate modifications to enhance their brightness, photostability, spectral properties, and cell permeability. Despite their advantages, the use of cyanine dyes for visualization applications has been limited due to a lack of robust methods to conjugate these dyes to proteins of interest. Current methods use HaloTag (HT) or SNAPTag to covalently target haloalkane and benzylguanine conjugated derivatives of cyanine dyes; however, these conjugates are expensive and not readily accessible to the research community. Therefore, there is an unmet need to develop new, non-covalent, genetically-encoded tags with high affinity and selectivity against off the shelf commercially-available dyes that enhance their spectral properties.

Development Status

The researchers are actively making new variants of PEPCy optimized for different organelle specificity, as well as for binding to other cyanine derivatives. Further optimization for gel-based assays is ongoing.

Applications

Technology ID

SAU02-01

Category

Life Sciences/Biochemicals & Small Molecules

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Life Sciences/Research tools/Reagents

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Learn more

- **Use as a research tool in visualization experiments for a protein or peptide target of interest:** Single-molecule microscopy, live-cell imaging, in-gel protein/peptide detection.
- **Biochemical assays:** Can be used for Western blots and pull-down using Cy3 or Cy5 conjugated beads.

Advantages

- **Enhanced photostability:** PEPCy tags decrease dye photobleaching to permit superior visualization.
- **High compatibility:** PEPCy tags can be developed with high affinity and selectivity against off-the-shelf commercial cyanine dyes.
- **Improved fluorescence properties:** PEPCy tags improve molecular brightness and fluorescence lifetimes of cyanine dyes.
- **Ease of use:** Minimal incubation time, wash-free labeling, and non-cross reactivity with other dyes.
- ***In vivo* applications:** Fast in-gel protein detection by fluorescence.

Intellectual Property

NYU has filed a U.S. provisional patent application covering the sequence of the protein tags and their methods of use in microscopy and biochemical applications for visualizing and detecting proteins or peptides of interest.

References

1. Moeka Sasazawa, Afroze Chimthanawala, Rui Zeng, Danah Kim, Katherine Buchan, Ming Zhang, Saumya Saurabh , https://www.biorxiv.org/content/10.1101/2024.07.03.601615v1