University of Colorado
TECHNOLOGY TRANSFER OFFICE

Research Tool: High Throughput Screening for Next-Generation Fluorescent Proteins

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Issued patent, available for licensing.

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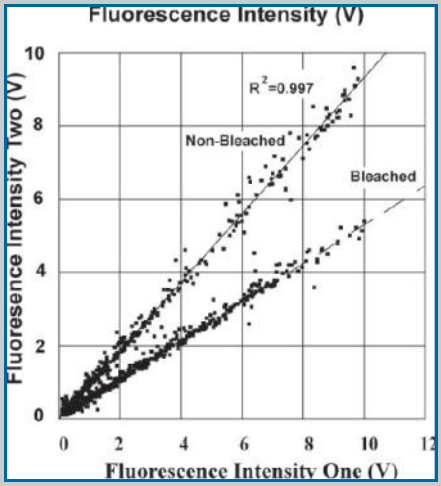
Background

Genetically encodable fluorescent proteins (FPs) have enabled exploration of cellular dynamics with unprecedented resolution. While FPs have proven invaluable tools for cell biology, there is a continual push to improve their photophysical properties. The discovery that mutations near the chromophore alter the photophysical properties of FPs rapidly led to a range of mutants with hues that now span from the near-UV to the IR. Despite their widespread use, all FPs, especially red FPs (RFPs), suffer from photon-limiting complex photophysical phenomena, such as decreased brightness, accelerated irreversible photobleaching, and a propensity to enter long-lived dark-states. In fluorescent proteins, photobleaching occurs by both reversible and irreversible pathways, both of which may limit photon output. Irreversible photobleaching tends to limit the imaging duration and signal output in conventional fluorescence microscopy and prevent their widespread use in single-molecule or low-copy fluorescence imaging. In contrast, reversible photobleaching recovers a fraction of the initial fluorescence intensity upon elimination of the excitation source, thereby complicating data analysis and introducing deviations in otherwise quantitative assays. Therefore, new techniques are needed to investigate correlations between sequence and irreversible and reversible photobleaching properties in order to develop FPs with greater photostability.

Technology

A University of Colorado research group led by Ralph Jimenez has combined microfluidics and spectroscopy to develop a versatile microfluidic platform capable of measuring and quantifying the magnitudes of reversible and irreversible photobleaching on individual mammalian cells at high-throughput under a range of excitation intensities. By employing a series of spatially separated excitation beams, the irreversible component of photobleaching was isolated. A mixture of red fluorescent proteins was assayed, and mCherry was identified as the variant with the lowest degree of irreversible photobleaching.



Applications

This technology will be useful for investigating correlations between sequence diversity and photophysical diversity in FPs and complements existing methods for fluorescent protein analysis, thereby facilitating the development of next-generation fluorescent proteins for single-molecule research.

Key Documents

Optically Integrated Microfluidic Cytometers For High Throughput Screening Of Photophysical Properties of Cells or Particles. U.S. 8618510, issued Dec. 31, 2013.

Microfluidic cell sorter for use in developing red fluorescent proteins with improved photostability. Lab Chip, 2013,13, 2320-2327. *PDF available upon request.*