【Grant-in-Aid for Scientific Research (S)】

Broad Section E

Title of Project： **Directed Evolution of a Palette of Optogenetic and Chemi-Optogenetic Indicators for Multiplexed Imaging of Cellular Metabolism**

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Research Project Number：19H05633　Researcher Number：40831318
Keyword：Protein Engineering, Fluorescence, Microscopy, Cell Biology, Metabolites, Neuroscience, Cancer

【Purpose and Background of the Research】

A healthy cell is like a well-organized city which runs smoothly due to a steady supply of input energy (e.g., food, petrol, and electricity) and reliable lines of communication (e.g., phones, internet, and newspapers). If one of these energy inputs or lines of communication is interrupted, the organization of the city is grossly affected and its productive contributions to the country are disrupted. Similarly, growing evidence indicates that many important human diseases have causes, or consequences, that relate to changes in the way cells (the cities) of the body (the country) acquire or use biological energy. One example is that cancer cells consume sugars very differently than healthy tissues. Another example is that many neurodegenerative diseases involve detrimental changes in brain energy metabolism. The aim of this research is to develop tools for multiparameter visualization of the full metabolism of a cell. These tools will provide insight into numerous diseases by enabling us to visualize how individual cells are generating and using biological energy.

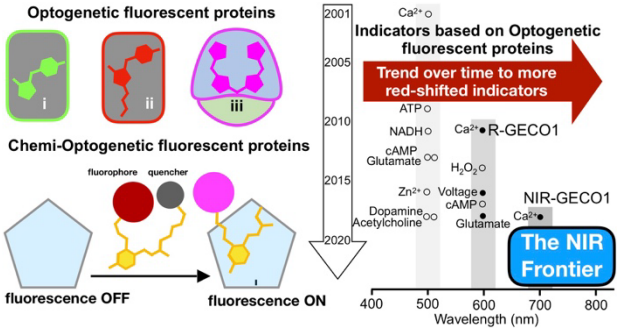

Figure 1. Optogenetic (i. green FP; ii. red FP; and iii. near-infrared FP) and chemi-optogenetic fluorophores.

【Research Methods】

The most powerful tools for visualizing dynamic processes in cells are optogenetic fluorescent proteins (FPs) that absorb one color of light and emit (fluoresce) a different color of light. We have extensive expertise at converting optogenetic FPs into indicators for the visualization of neuronal signalling. In this work we propose to build upon this foundation and develop metabolite indicators in a variety of colors, including near-infrared (NIR). NIR is very safe and very low energy, and therefore enables the imaging of indicators deeper into tissue than is possible using visible wavelengths of light. To create NIR indicators we will use both optogenetic FPs as well as chemi-optogenetic FPs based on proteins that bind to designed NIR fluorophore ligands.

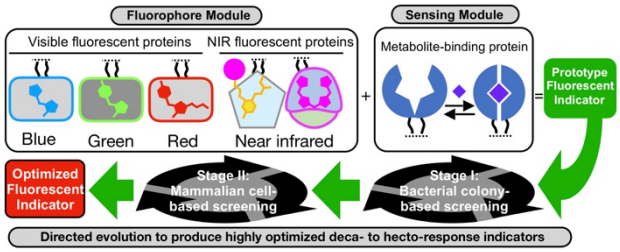

Figure 2. An innovative work flow for the design and optimization of modular, multicolor indicators.

【Expected Research Achievements and Scientific Significance】

By the conclusion of this project, we will have developed an improved toolbox of indicators that can will enable future insights into the mechanisms of debilitating neural and metabolic disorders. We will distribute these tools freely and broadly to accelerate research in as many countries and health areas as possible.

【Publications Relevant to the Project】

- Y. Qian *et al.*, "A genetically encoded near-infrared fluorescent calcium ion indicator", *Nat. Methods*, **2019**, 16, 171–174.
- Y. Shen *et al.*, "Genetically encoded fluorescent indicators for imaging intracellular potassium ions", *Commun. Biol.*, **2019**, 2, 18.
- J. Wu *et al.*, "Genetically Encoded Glutamate Indicators with Altered Color and Topology", *ACS Chem. Biol.*, **2018**, 13, 1832–1837.

【Term of Project】

FY2019-2023

【Budget Allocation】

155,000 Thousand Yen

【Homepage Address and Other Contact Information】

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