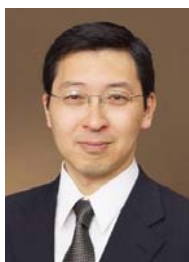


## 【Grant-in-Aid for Scientific Research (S)】

### Integrated Disciplines (Complex Systems)



#### Title of Project : Development of Fluorescent Probes with Molecular Evolution Engineering

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Research Project Number : 15H05723 Researcher Number : 80237198

Research Area : Complex Systems

Keyword : Fluorescent probe

#### 【Purpose and Background of the Research】

Since Green fluorescent protein (GFP) has been cloned, the fluorescent proteins and the imaging techniques are getting important for biological and medical research. We have been developing genetically encoded calcium indicators (GECIs) such as G-CaMPs and R-CaMPs (Figure 1). Nowadays, these GECIs are widely used in many model animals for reporting cellular activities. On the other hand, peptide aptamers are peptides that bind to molecules with high specificity. In recent years, because of the molecular recognition capability of peptide aptamers of biological material, biotechnological and medical application of peptide aptamers has been studied. In this research, we are going to combine our fluorescent reporter technology and the peptide aptamers, so that new fluorescent probes that have high-affinity binding to a variety of substrates can be rapidly generated.

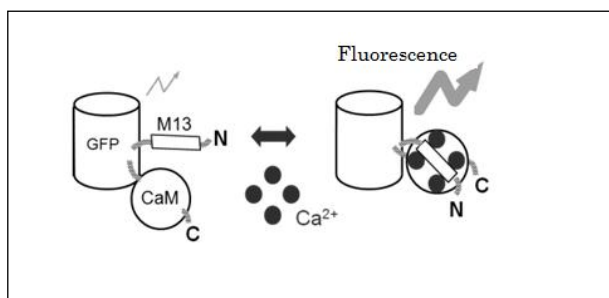


Figure 1 G-CaMP

#### 【Research Methods】

This research has three parts, (1) development of fluorescent probe with functional peptide aptamers, (2) development of fluorescent probes with random peptide aptamers, (3) development of new screening techniques.

The fluorescent reporter moiety of G-CaMP will be fused with the peptide aptamers that are known to be functional as binding proteins or the random peptide aptamers, that will be selected with new screening technique afterward.

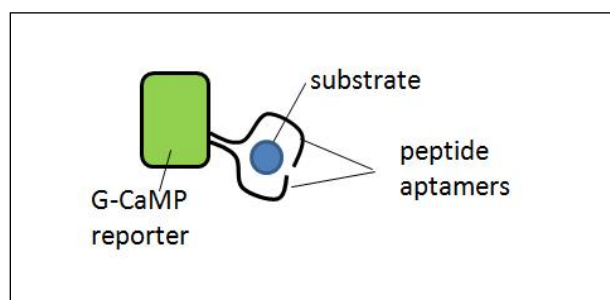


Figure 2 New probe

#### 【Expected Research Achievements and Scientific Significance】

Imaging techniques are getting more and more important in biology, medicine, and pharmacology. In this research, we are going to establish new techniques for developing fluorescent probes. With the new methodology, high-performance fluorescent probes will be rapidly generated. These fluorescent probes will be able to be used in biology, basic research in medicine, and drug discovery, so that this research makes contribution to development of human society.

#### 【Publications Relevant to the Project】

Nakai J, Ohkura M, Imoto K: A high signal-to-noise  $\text{Ca}^{2+}$  probe composed of a single green fluorescent protein. *Nat Biotechnol* 19, 137-141, 2001.

Inoue M, Takeuchi A et al: Rational design of a novel high-affinity, ultrafast, red calcium indicator R-CaMP2. *Nat Methods* 12, 64-70, 2015.

【Term of Project】 FY2015-2019

【Budget Allocation】 154,500 Thousand Yen

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