Development of a chemi-genetic fluorescent indicator for imaging of intracellular sodium ion concentration

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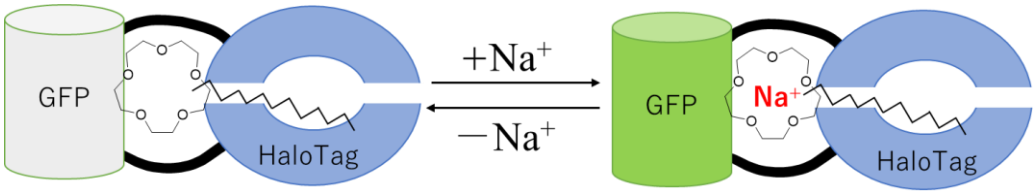
Sodium ion concentration gradients inside and outside of cells play important roles in living organisms. Therefore the development of highly sensitive and non-invasive fluorescent Na^+ indicators is essential for understanding those biological phenomena. The most widely used intracellular sodium indicator now is SBFI, which was developed in the 1980s, and has the limitations of short excitation wavelength, low brightness, and cytotoxicity.[1] Recently, hybrids of polypeptides and synthetic molecules, called chemi-genetic molecules, are attracting attention as a new scaffold of fluorescent indicators.[2] However, there is no report of chemi-genetic indicators for Na^+.

In this study, we aim to develop a new fluorescent chemi-genetic indicator by combining green fluorescent protein (GFP) with a synthetic sodium chelator via HaloTag (a self-labeling protein reactive towards chloroalkanes).[3] By using GFP as a fluorescent moiety, we expect that the problems of excitation wavelength, quantum yield, and toxicity can be overcome. The structure of the indicator is as follows: a circular permutated GFP is inserted into the HaloTag protein, and a chloroalkane attached to a sodium-chelator (crown ether) is used to label the protein. When the sodium ion binds to the chelator, we expect it changes the chromophore environment of GFP and causes a fluorescence change (see Figure). We first synthesized a ligand by combining an amino crown ether and a chloroalkane terminated with a carboxylic acid. We then introduced random mutations into the HaloTag-GFP protein and measured their fluorescence response to Na^+ after incubation with the ligand. By repeating this cycle several times, we obtained an optimized protein that shows 30% fluorescence change to 100 mM [Na^+].

In future work, we will further improve the sensor performance by synthesizing a new organic molecule with higher affinity to Na^+ and also optimizing the protein by directed evolution, to develop an indicator suitable for intracellular Na^+ concentration (about 15 mM).



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