

## Whole Cell Sensors for the Detection of Molecules Dissolved in Seawater

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### Summary

With the remarkable growth of the chemical industry, river and soil pollution by various toxic components is progressing throughout the world, and the flow of various harmful molecules into seawater is becoming serious. In Japan, seawater is used as a source of minerals and salt. It is necessary to monitor the presence/absence of heavy metals and toxic substances (e.g., arsenic) in the seawater used for quickly and accurately. In this study, we aimed to create a whole cell sensor that specifically targets various metal ions. Since metal ions cannot pass through cell membranes, we attempted to develop a technology for constructing artificial proteins that can freely transmit the information. We focused on the non-allosteric phenomenon called ligand-induced folding in our sensor configuration.

First, we displayed enzymes and metal-binding proteins on the outer domains of membrane proteins, and by destabilizing the junctions with random mutations, we succeeded in redesigning membrane proteins into the conditionally functional ones to the ligands, cofactors, and/or substrates of the proteins and/or enzymes displayed outside the cell.

We then tethered reporter proteins, such as transcription factors, to the membrane proteins so that they are in the cytosolic spaces. The functional output of the reporter proteins was found to be dependent on the molecular recognition of the protein displayed on the other side of the membrane. By replacing the input component displayed to the periplasmic space, we could have created different artificial signal transduction devices where information flows from extracellular space to intracellular protein functions, by way of transmembrane proteins. With this remarkable plug and play-ability and unnecessary for designing allosteric mechanisms, the method described herein could rapidly provide the arrays of sensory tools to synthetic biologists as well as for those who need to monitor the chemical analysis of seawater.