Cadmium is a heavy metal very harmful to many species of organisms. The pollution of cadmium brings a serious problem for human. For example, ‘itai-itai’ disease in Japan was caused by cadmium poisoning. Nagahama, where our institute is located, is near to Biwa Lake and one of famous rice fields in Japan. If the rice field contains some level of cadmium, harvested rice grain should contain cadmium. For our safe, the cadmium in rice field must be removed. For this purpose, we have planned to collect the cadmium efficiently with the recombinant E. coli (Catching E. coli). Catching E. coli traps cadmium on the outer membrane. This E. coli produces outer membrane protein (Ag43) fused with the heavy metal binding protein (metallothionein).

In doing so, cadmium will be caught and released without killing E. coli itself. But E. coli was going away from cadmium that was proven by our experiment. We had an idea to use chemotaxis factor to go toward cadmium.

So we created another kind of recombinant E. coli (Gathering E. coli) that produces aspartic acid, which is a positive chemotaxis factor for E. coli. Therewith, both E. coli were gathered to the cadmium spot. We characterized the Gathering E. coli whether or not this E. coli synthesizes aspartic acid and releases in the media in the presence of cadmium ion.

### Results

#### Swarming Assay

E. coli, swimming away from cadmium E. coli (w)

E. coli, swimming toward aspartic acid

L: cadmium ion (100 mM, 4 μL)
R: H₂O (4 μL)
Incubation: 110 hours
Temperature: 30 °C

E. coli, swimming away from cadmium E. coli (w)

This graph shows the negative chemotaxis profile of E. coli by cadmium ion. Assuming that cadmium is spotted at the center of “swarming plate” and E. coli shows negative chemotaxis for cadmium, E. coli cells were split away from the spotted point.

E. coli, swimming toward aspartic acid

This graph shows the positive chemotaxis profile of E. coli by aspartic acid. Assuming that aspartic acid was spotted at the center of “swarming plate” and E. coli shows positive chemotaxis for aspartic acid, E. coli cells were gathering to the spotted point.

#### TLC Assay

Analysis of aspartic acid synthesized by E. coli (JM109/BBa_K1342001) by TLC

- No addition of cadmium culture (diluted at 1:50 by medium (lane1) and 1:100 (lane2))
- Addition of cadmium ion (250 μM) no diluted by medium (lane3)
- Diluted at 1:50 (lane4), 1:100 (lane5) and 1:200 (lane6).

Estimation of aspartic acid content in spot Each intensity of spots indicating the content of aspartic acid, was estimated by Image J.

### Systems

Gathering E. coli, in which the construct was illustrated as above, can have the cadmium-trap protein on the outer membrane. The protein consists of a fusion of metallothionine (MT), SmtA, and adventitia protein (Ag43), one of outer membrane proteins, and a signal peptide (SP) of Ag43. The genetic origin of SmtA is Synechococcus PCC. 7002.

### Policy & Practice

We participated in “The 86th Annual Meeting of The Genetics Society of Japan at Nagahama Institute of Bio-Science and Technology in September 17th-20th, 2014”. We proposed the workshop of synthetic biology and invited the general public, iGEM Teams (UT-Tokyo, Kyoto, Osaka, Hokkaido, U. TMU-Tokyo, Tokyo Tech and Gifu), researchers and instructors of the synthetic biology there, because we wanted to know and learn more about synthetic biology and their societies.