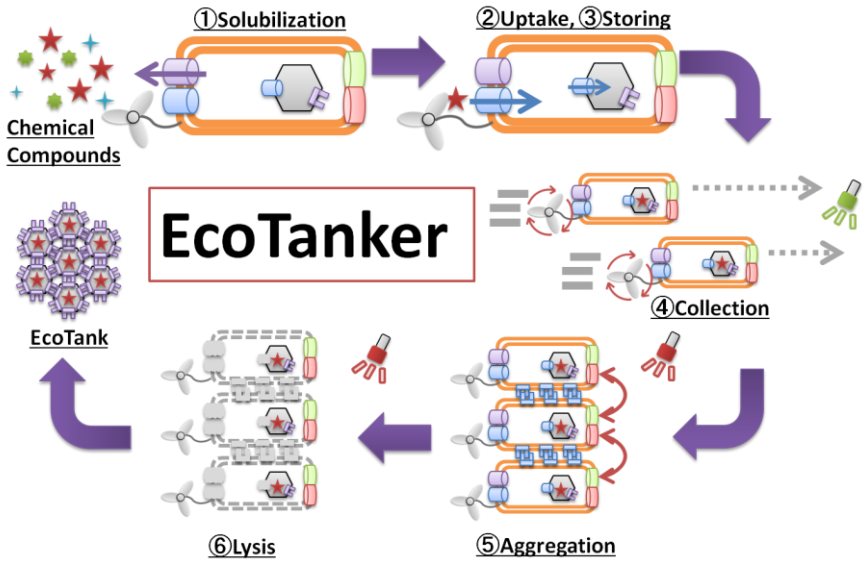
EcoTanker

We propose an entirely new micromachine that will enable us to extract target compounds selectively (and potentially even convert them into value-added products) and deliver them to a desired location. The entire process will be automated and controlled with light signals. As this *E. coli* micro machine behaves like a tanker, we named it EcoTanker.

The EcoTanker will serve two goals, to extract valuable chemicals and to clean up the environment of toxic chemicals. The EcoTanker will be designed to extract and deliver specific chemicals from locations exploited by the oil industry. The EcoTanker may be particularly useful in the recently devastated Gulf coast, as well as where more moderate environmental damage has occurred.



The EcoTanker will consist of 7 devices (explained in Project section)

① Solubilization Device
② Uptake Device
③ Storage or Reactor Tank
④ Phototaxis Device
⑤ Photocontrol Device
⑥ Self-aggregation Device
⑦ Autolysis Device

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Project

① Solubilization Device

Extraction of chemicals from oil contaminated environment may be rendered difficult by their low solubility. The EcoTanker includes a device to solubilize the chemicals with biosurfactants. We have focused on rhamnolipid which is produced by *Pseudomonas aeruginosa* PAO1 and can solubilize PAH(Polyaromatic hydrocarbon) such as naphthalene or phenanthrene. Rhamnolipid expression in *E.coli* BL21(DE3) using pET28-RhlA and pBluescript-RhlB has been reported (Kun et al.(2008), and is expected to also be possible using BioBrick plasmids.

[$P_{const.low}$-RBS-*RhlB*-$P_{const.high}$-RBS-*RhlA*-Term]

② Uptake Device

After solubilization of PAH, the EcoTanker will selectively uptake the desired components. Several microbes have been found in tarsands and oilsands, such as *Pseudomonas sp*.(Obho et al.(2006)). Bacteria that can assimilate specifically certain types of asphaltenes, resins, other aromatic compounds, and saturates have also been reported (Seo et al.(2009)). We will design uptake devices that mimic the natural uptake mechanism, such as channels (e.g. OmpW(Hong et al.(2006)) and transporters (e.g. PcaK (Nichols and Harwood (1997)) that have been found to function in *E.coli*.

③ Storage or Reactor Tank

Following the uptake of bitumen extracts, the EcoTanker will store these compounds inside a compartment within the EcoTanker. This EcoTank will be constructed from a bacterial micro compartment (BMC), which is a protein shell-like structure. Several BMCs have been reported, and each BMC has the ability accumulate a specific compound as well as catalyze a specific chemical reaction (e.g. 1,2-propanediol utilization(Parsons et al.(2010)). In addition to storing specific chemical compounds,

BMCs may also be modified to serve as a bioreactor to change chemical extracts into value-added products. The EcoTank will be modified with special peptides that will cause them to self-aggregate upon cell lysis to simplify the harvesting of the EcoTank.

[$P_{const.high}$-RBS-*PduB-J-K-N-U*-RBS-*Peptide-PduA*-RBS-*PduC-Peptide*-Term]

④ **Phototaxis Device**

After uptake and storage of specific bitumen compounds, the EcoTankers can be guided by light signals to a desired location for easy harvesting. We plan to modify the EcoTanker with the phototaxis machinery from Halophilic archea, which alter their swimming behavior in response to changes in light intensity and color using visual pigment-like sensory rhodopsin (SRs). A fusion chimeric protein (*NpSRII-NpHtrII-Tar*) was constructed and succeeded in mediating retinal-dependent phototaxis response in *E. coli* (Jung et al.(2001)). EcoTanker will recruit this fusion chimera to acquire phototaxis ability.

⑤ ⑥ **Photocontrol Device & Self-aggregation Device**

For easy harvesting, the EcoTankers will be signaled to self-aggregate using a photocontrol device (green-light actuator) to induce the expression of an aggregation device (antigen 43). We designed both devices in our previous iGEM competition (Tokyo-Nokogen- 2009).

[$P_{const.high}$-RBS-*ho1*-RBS-*pcyA*-RBS-*cph8*-Term]

[$P_{Red\ Light}$-RBS-*Antigen 43*-Term]

⑥ **Autolysis Device**

A previously designed auto-lysis device (Holin & Endolysin, Tokyo-Nokogen-2009) will be used for the liberation of the EcoTanks, which will in turn self-aggregate. The EcoTanks can then be harvested and processed to extract the desired chemical component.

[P_{Quorum} -RBS-*T4 endolysin*-RBS-*lamda holin*-Term]

Members

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Our team 'Tokyo-NoKoGen' belongs to the Department of Biotechnology and Life Science at Tokyo University of Agriculture and Technology. Our first challenge with the competition was in iGEM-2009, where we designed the *Escherichia coli* Auto Protein Synthesizer (ESCAPES) for the easy preparation of target proteins. ESCAPES eliminates the need for the tiresome steps of centrifugation and sonication, thus making the prtotein preparation more convenient, economical, and ecological. ESCAPES comprises four devices: 1. Green-light actuator, 2. self-aggregation, 3. auto-lysis, and 4. signal counter. These devices will be incorporated into our iGEM-2010 project EcoTanker.

We continue to participate in iGEM, as we view it as a valuable experience for students to develop a project idea, and to plan and carry out a project within a team. iGEM also helps us develop our communication skills by interacting with other team members as well as with the international scientific and nonscientific community. We also see iGEM as a good opportunity to promote our school and our sponsor(s)

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