Phosphate pollution in waterways and water treatment plants is a major problem. Removal of phosphate from wastewater is required to treat phosphate-containing discharge to reduce eutrophication, algal blooms and "dead zones" in lakes, rivers and coastal marine ecosystems. IGEM HKU 2013 aims at removing or reducing the levels of inorganic phosphate from a system or environment by employing engineered bacteria E. capsii, capable of accumulating phosphate in the form of polyphosphate.

**PolyP Synthesis – PPK1**

Polyphosphate kinase 1 (PPK1) is the most extensively studied polyP-synthesizing enzyme and has been detected in a wide range of prokaryotes. It catalyzes the transfer of the terminal phosphate of ATP to an active-site histidine residue, the initial step in the processive synthesis of a long PolyP chain.

**Bacterial Microcompartment**

**Bacterial Microcompartments (MCP)** are formed by proteins, enclosing enzymes and cofactors for carbon fixation or various forms of fermentative metabolism.

To isolate polyP synthesis from the surrounding polyP degrading enzymes, we expressed the Eut MCP (BBa_K311004) from iGEM Minnesota 2010 and localized PPK1 into the MCP by adding a signal sequence to the enzyme, thereby enabling efficient polyP synthesis.

**Results:**

(1) **PPK1 expression**

We successfully cloned ppp1 gene from K. oralis and T. forsythia and engineered E. coli to overexpress PPK1 under the regulation of T7 promoter. (BBa_K1217002 & K1217003)

We also fused the signal sequence to the PPK1 so that it can be localized into the Eut MCP. (BBa_K1217004 & BBa_K1217005)

(2) **Eut MCP assembly and Surface Tagging**

We add a 6-His tag to the N terminus of Euts monomer to enable surface tagging of the whole MCP. We also created 2 versions of Eut MCP: MCP endocoded by EutS hexamers only and native MCP encoded by 5 structural genes (EutsMNLK). (BBa_K1217015, K1217016, K1217017)

(3) **Phosphate removal and PolyP synthesis**

We localized PPK1 into two versions of MCP and test for their efficiency in phosphate uptake from the medium and PolyP synthesis. We found that they have higher efficiency compared with the control.

**Enzymes against PolyP Synthesis**

Metabolic reactions are dynamic, maintaining a homeostasis inside the bacterial cells. PolyP synthesis by PPK1 may be counteracted by numerous "enemies" inside the bacteria, e.g. exopolyphosphatase (PPX) which catalyses the processive hydrolytic cleavage of Pi from the end of the polyP chain.

**MCP Surface Tagging**

We try to investigate the potential of MCP as a delivery vehicle. To enable specific targeting, we try to displace designed sequence on MCP’s surface. We found Euts monomer is suitable for tagging at its N terminus. We hypothesize fusing a designed targeting peptide to the Euts N termini will be presented on the exterior surface of the MCP, at the same time, pose little steric effect on the MCP assembly.

**References:**