CUPID strives to use engineered obligate mutualism to expand the reach of synthetic biology to multicellular, multispecies machines. As proof of concept, we propose uniting the model organisms *E. coli* and *S. cerevisiae*. The system for codependence relies on the inducible expression of an enzyme (IGPD) required for histidine biosynthesis in each organism. In yeast, expression of the gene encoding IGPD is induced by mating pheromone. In the *E. coli*, it is induced by lactate. Yeast mating pheromone is provided by the *E. coli* as a surface bound polypeptide, while yeast consitutively expresses lactate dehydrogenase to provide lactate to the *E. coli*. We propose that selective pressure on medium lacking histidine could lead to formation of a stable exosymbiont—a first step toward the development of stable multicellular, multispecies microbial machines.

**Mechanism of Codependence:**

MFα activates mating pheromone response turning on expression of yeast IGPD gene.

Lactate secreted by the yeast turns on expression of *E. coli* IGPD gene.

---

**Yeast Construct:**

- **FUS1** The FUS1 promoter is activated by phosphorylated STE12 in the pheromone response pathway. Genetic circuits can be made mating-sensitive by using it as a promoter.
- **IGPD** This essential gene has been knocked out in the strain, meaning that the only way the yeast can survive is if the mating factor response pathway has been activated.
- **LDH** Lactate dehydrogenase catalyzes the reaction between lactate and pyruvate. This allows yeast to produce and export lactate into its environment.

---

**E. coli Construct:**

- **INPNC** A modified ice nucleation protein allows for the expression of mating factor α on the surface.
- **MF alpha** Mating factor α is a pheromone secreted by α type yeast which activates the mating pheromone response in mating factor a haploids.
- **Lac** A lactate sensitive promoter will activate gene expression in *E. coli* in response to the presence of lactate.
- **IGPD** The homologous version of this gene in *E. coli*, HisB has been knocked out in the strains being used. This means that the only way the *E. coli* can survive is if it is close to the yeast and absorbing the lactate that the yeast is producing.

---

**Characteristics of Interest**

- Aggregate Size
- Synchronization
- Binding Affinity

---

**Future Research:**

- Grow Together Under Selective Pressure
- Transform Yeast and *E. coli*

---

**Potential Outcomes**

- Dynamic Stability
- Exosymbiont
- Unstable Population