**ABSTRACT:** Some eukaryotic cells, such as white blood cells, have the amazing ability to sense specific external chemical signals and move toward those signals. This behavior, known as chemotaxis, is a fundamental biological process crucial to such diverse functions as development, wound healing and immune response. In our project, we used a synthetic biology approach to manipulate signaling pathways that mediate chemotaxis in two model organisms: HL-60 (neutrophil-like) cells and the slime mold, Dictyostelium discoideum.

In doing so, we have demonstrated that we can regulate both the navigation and speed of our cells, as well as harness their ability to carry a payload. Through our manipulations, we hope to better understand how these systems work, and eventually to build or reprogram cells that can perform useful tasks. Imagine, for example, therapeutic nanorobots that could home to a directed site in the body and execute complex, user-defined functions (e.g., kill tumors, deliver drugs). Such cellular robots could be revolutionary biotechnological tools.

**Why cellular robots as therapeutics?**

For treating localized diseases, cellular “robots” (smart, motile vehicles, inspired by our own immune cells) would have several key advantages over traditional (small molecule-based) medicine:  
1. Move directly and specifically to a target.  
2. Make decisions/perform computation.  
3. Execute user-defined functions, deliver cargo.

Can we build a prototype cell-bot whose movement we can control?  

**CHALLENGES:** Engineer NAVIGATION, SPEED, and PAYLOAD of cells.

**CHASSIS:** HL-60 (neutrophil-like cells) and Dictyostelium discoideum.

**NAVIGATION Movement to new targets**

**APPROACH:** Express new receptors (GPCRs) in HL-60 cells.  

**RESULT:** Chimeric receptor functions in chemotaxis; may be possible to convert other Gq-coupled receptors into chemotaxis sensors, thus broadening our range of potential targets.

**NAVIGATION Tuning sensitivity**

**APPROACH:** Control number of receptors at the plasma membrane by attaching recycling modules to GPCR.

**RESULT:** Attaching modules that regulate endocytosis recycling can increase or decrease sensitivity of GPCR (model: endocytosis controls number of receptors at plasma membrane).

**SPEED Accelerators and brakes**

(con’t) Building Accelerators and Brakes.

Screened over 100 combinations of our localization and catalytic domains for their effect on motility.

One example: a strong BRAKE

**E Delivering a PAYLOAD**

Once a cellular robot has detected and traveled to a site of disease in the human body, it should be able to perform a useful task, like delivering imaging reagents or therapeutic drugs.

**APPROACH:** Conjugate cells to polystyrene beads using Concanavalin A (ConA).

**RESULT:** By rewiring cell polarity we created 7 brakes and 1 accelerator.

**RESULT:** Have successfully tethered fluorescent bead “cargoes” to HL-60 cells. Cells can transport cargo.

**VISION Example Future Application**

**TARGET:** Cell-bots to find Carcinoid tumors  
- Found in gut and lungs; often malignant  
- Small and very hard to find  
- Secret high levels of serotonin (neuroactive hormone detected by Gq-coupled GPCR)

**APPROACH:** Use engineered cells to deliver imaging reagents or drugs to diseased areas.

**CONCLUSIONS**

We were able to:

1. Engineer cells to NAVIGATE to new GPCR signals  
2. Tune INPUT SENSITIVITY by linking different recycling modules to receptors  
3. Control SPEED by modifying polarization feedback circuits  
4. Make cells carry a PAYLOAD of beads

We have made progress towards a cellular robot platform for diverse therapeutic functions.