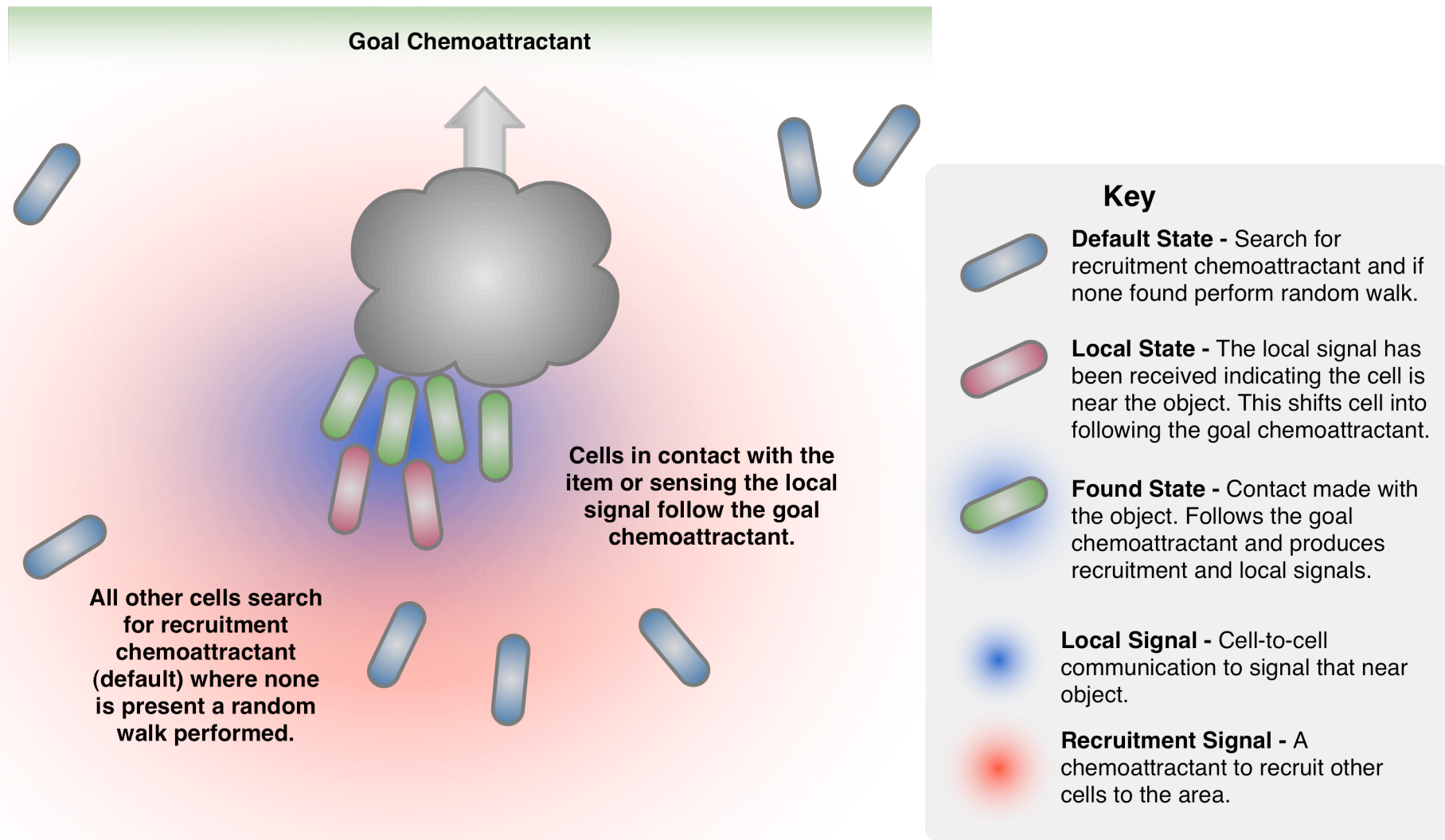


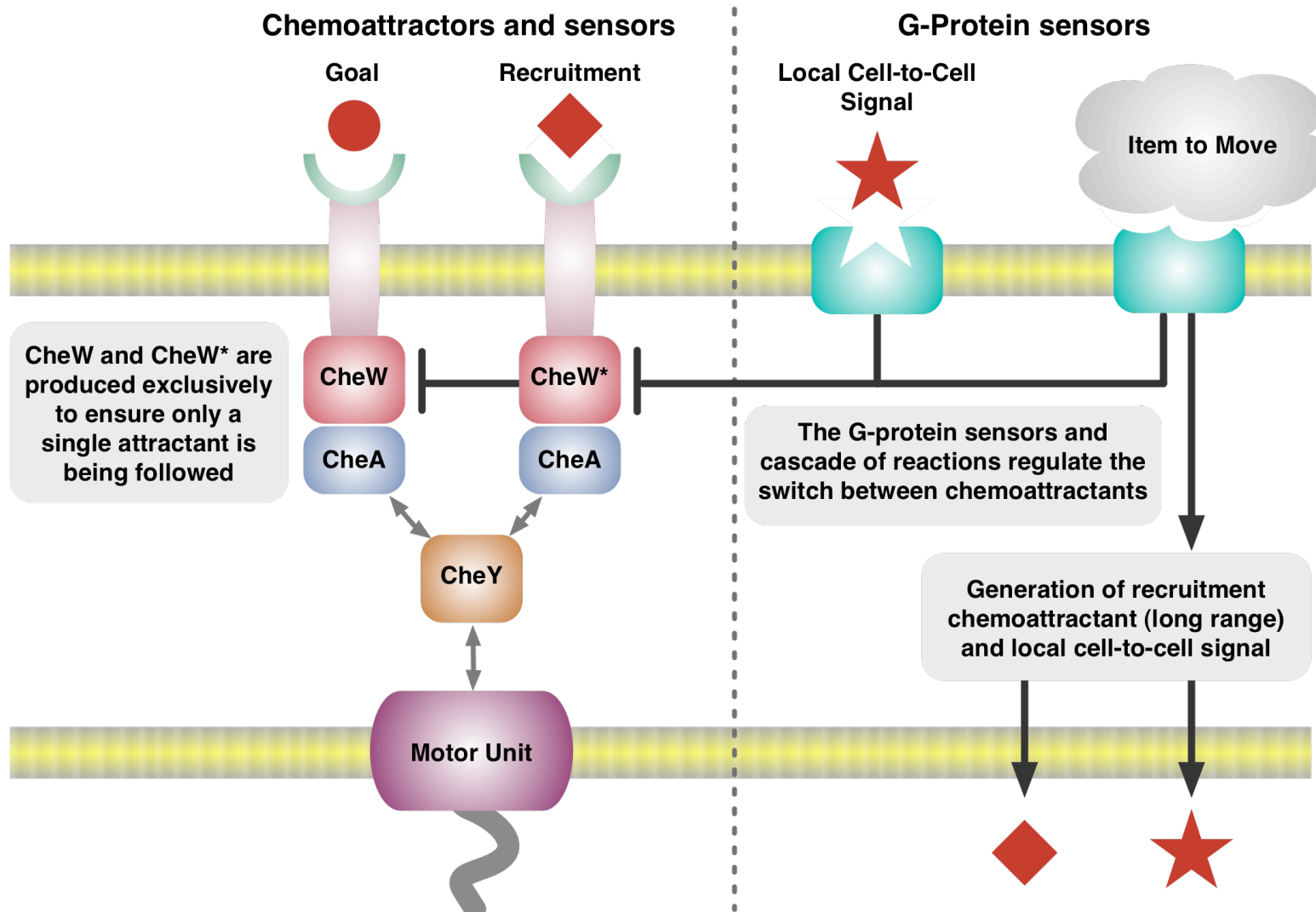
Previous iGEM Chemotaxis Projects

- **Remote Controlled Bacteria** – University California San Francisco 2006
 - Switchable chemoattractant (*E.coli* contains five different receptors)
 - Alter the signaling pathways
 - Orthogonal interactions designed and CheW and CheW mutant created
 - By switching the expression of CheW/CheW mutant different receptors enable
- **Pattern Formation Using Mobility** – Cambridge 2006
 - Free swimming bacteria
 - Bi-directional communication with quorum sensing
 - Green and Red populations (GFP, RFP)
 - Battle occurs when sufficient numbers, losers defect
- **Seek and Destroy *E.coli*** – Rice 2006
 - Link quorum sensing with chemotaxis
 - Move towards *B.subtilis* pheromone
 - Signal goal reached with GFP or gram-positive lethal proteins
- **X-Y Chemotaxis** – Bangalore 2006
 - 2D Control of movement using 3 different chemoattractors
 - Problems with chemoreceptors interfering with each other

Co-operative Movement Overview



Possible Implementation



Chemotaxis

- Switchable chemoattractant
 - Use previous work by University California San Francisco (2006) *“Remote controlled bacteria”*
 - Existing parts for orthogonal CheW/CheW* and full sensors:
 - **BBa_J56004**: Tar, aspartate receptor mutated to bind with BBa_J56001
 - **BBa_J56007**: PheTar, phenylalanine receptor mutated to respond to phenylalanine
 - **BBa_J56001**: CheW_mut, mutated to bind with orthogonal partner BBa_J56004
 - **BBa_J56018**: CheW, wild-type CheW
 - Need further information on how successful the project was as parts library does not contain any user reviews
- Control of expression
 - Expression of CheW/CheW* needs to be linked to control mechanism used by other sensors (G-proteins)
- Chassis selection
 - UCSF used chassis from Parkinson Lab with all other receptors knocked out to ensure no interference

Sensors

- G-protein coupled receptors (GPCR)
 - Loads! (800-1000 genes) – Normally in Eukaryotes
 - 5 Families
 - Glutamate
 - Rhodopsin
 - Adhesion
 - Secretin
 - Frizzled
 - Majority of ligands are proteins e.g. hormones, Wnts.
 - Rhodopsin is activated on absorption of a photon of light.
 - Need 2 local signaling molecules (photons, pheromones?)
 - Particle
 - Won't degrade easily.
 - Fluoresces? (maybe use Rhodopsin)
 - Size (modelling to check physics)
 - Local signal
 - Won't diffuse readily
 - Secretin GPCR detecting protein ligands
 - Eukaryotic protein production in prokaryotes?
- Histidine Kinase Receptor (need transduction of signal)
- FeoB – G protein dependant Fe II uptake

Control

- Choosing sensor
 - Transcription of CheW/CheW*
 - Unsure of regulation of the expression of CheW (in normal case)
 - Unsure how we can link GPCR to transcriptional change
 - Altering activity of CheA
 - Need to check that CheAs are unique as only want to interact with single type
 - Normally CheA sequestered via P1. On GPCR activation P1a conformation is altered, releasing CheA.
 - Altering methylation of chemotatic receptor → CheB
 - Controls sensitivity of receptor
 - Selective CheB required
 - Need to inhibit/promote one type on GPCR activation