We're from Melbourne
Components required:

1. Buoyant Bacteria
2. Red Photosensor
3. Blue Photosensor
4. AND gate
5. Adhesion
Stoke’s Law, \( f = 6\pi \mu r \)  
Radius of bacteria = 0.5µm

viscosity of media = 1x10\(^{-3}\) Pa.s

Then, Stokes-Einstein Relationship

\[
D = \frac{k_B T}{f} \langle \vec{R}(t)^2 \rangle
\]
Boltzmann’s constant

Frictional force

Temperature (K)

Mean squared Displacement

Diffusivity

From Brownian motion: 3D random walk

\[
t = \frac{\langle \vec{R}(t)^2 \rangle}{6D}
\]
Time

Diffusivity

Therefore, for 1 bacteria to move 1mm takes 4.5 days

… and for a clump 10 bacteria across to move 1mm takes 45 days
If there is enough adhesion, bacteria should effectively not move within the time scale of our experiments.
Floatation
• From *Bacillus megaterium*

• Protein polymer creates a coat that embodies air, making bacteria float.
• Floatation function encoded by a gene cluster 6kb in length.
• Gene cluster can function as a polycistron.
<table>
<thead>
<tr>
<th>Protein</th>
<th>AA Length</th>
<th>Proposed Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>gvpB</td>
<td>88</td>
<td>Main structural protein</td>
</tr>
<tr>
<td>gvpF</td>
<td>255</td>
<td>Nucleation</td>
</tr>
<tr>
<td>gvpG</td>
<td>88</td>
<td>Present in immunoblots of vesicles</td>
</tr>
<tr>
<td>gypJ</td>
<td>100</td>
<td>Change in coil size with development</td>
</tr>
<tr>
<td>gvpK</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>gypL</td>
<td>269</td>
<td>Coiled coil nucleation</td>
</tr>
<tr>
<td>gvpN</td>
<td>308</td>
<td>Vesicle number regulation</td>
</tr>
<tr>
<td>gvpQ</td>
<td>157</td>
<td>Negative regulation of vesicle production</td>
</tr>
<tr>
<td>gvpR</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>gvpS</td>
<td>95</td>
<td>Similar to gvpB and gvpJ</td>
</tr>
<tr>
<td>gvpT</td>
<td>292</td>
<td></td>
</tr>
<tr>
<td>gvpU</td>
<td>138</td>
<td></td>
</tr>
</tbody>
</table>
GvP Biobrick: Plan

- Remove restriction sites
- Make biobricks out of all genes
- Add prefix and suffix
Results

- Four unintended (silent) mutations in 6kb
- 11x40bp repeat in GvPL, but doesn’t seem to affect buoyancy
- BBa_I750016
Results

Day 0

Day 3 (72 hours)

PLASMID pNL29

INDUCED WITH IPTG

After mutagenesis plasmid 41C

INDUCED WITH IPTG
Red Light
We attempted to use the Cph8 Red light receptor from the registry. (BBa_M30109)

Requires PcyA and ho1
Blue Light
Blue Light Receptor

Absorbance for Cph1  \( \text{A}_{\text{Max}} = 660\text{nm} \)

Absorbance for SopII  \( \text{A}_{\text{Max}} = 500\text{nm} \)


Blue Light Photosensor

As was done with Cph8, we made a Photoreceptor-kinase chimera
SopII–HtrII

Transmembrane protein complex, inducing flagellar motion in response to light (phototaxis)
Fusion with Tsr Kinase from *E. coli* works – a two-component signal transduction is initiated in *E. coli*.

• Exists as a part, but not in distribution
• Ordered from GENEART, and optimised sequences. They have have been submitted as ComPalt, ComAalt
Selection of fusion sites
• Fusions created by PCR stitching
• Have created the chimeras, but still characterizing.
AND gate
Integration: The AND gate

Red Light Receptor

Blue Light Receptor

OmpR

P-OmpR

cI repressor

ComA

P-ComA

R00 82  B00 34  C00 51  B0010  B0012  R00 51  B00 34  B0010  B0012  B0010  B0012

OUT PUT
• Currently, our construct is designed to express a reporter gene (GFP)

• Will show that genes under the control of srfA are up-regulated only at the intersection of the two light beams.
Adhesion
Possible Adhesion Systems

- Surface expression of Ig-like molecules
- Intimin
- Fos-Jun adhesion molecules (McGill University)
Medical Applications

• Possible applications in building scaffolds in tissue engineering
• Cadherins
Progress…

Floatation Device is now a Biobrick

Still working on putting together the light responsive system and coupling it to the adhesion molecules.
• Joe (for his super-competent cells)
• Lei Xing
• Heung-Chin Cheng & Paul Gooley, and their respective labs
• University of Melbourne
  – Dept of Biomedical Engineering
  – Dept of Biochemistry and Molecular Biology
  – Bio21 Institute
James Spudich
Maura Cannon
Alan Grossman