Circuits, Sensors & Cell-Free Systems

1A What would your synthetic cell do? What is the input and what is the output.

The input to my synthetic cell would be EM fields, and the output would be color changes (e.g mKate, and GFP on three different promotors). [1] [2]. In this way, EM fields could change colored "pixels." Given some genetic memory and pattern boundary circuitry over many cells, a simple message or picture could be displayed.

1B Could this function be realized by cell free Tx/Tl alone, without encapsulation?

To some extent yes, but for measurement, reproducibility, and localization of effect, there needs to be encapsulation.

1C Could this function be realized by genetically modified natural cell?

Very likely so – most of my references refer to E coli specifically - but not as easily, and there would likely be silenced to a large degree.

1D Describe the desired outcome of your synthetic cell operation.

In the presence of low frequency ~60Hz, 8-80uT EMF, the hsp70 promotor will increase expression of mKate or GFP, with the remaining fluorescent protein being produced by the matA,safA, or chbB promotors (to be experimentally determined with the specific synthetic cell – all show potential for greatly increased 100x or higher expression under UHF EMFs) at .1 – 10 THz (magnitude to be determined, not to exceed ~2-3mW/cm^2).

2. Design all components that would need to be part of your synthetic cell.

2A What would be the membrane made of?

Phospholipids + cholesterol.

2B What would you encapsulate inside? Enzymes, small molecules.

Cell free Tx/Tl system (including all required bases, enzymes, etc), DNA with genes for protein expression.

2C Which organism your tx/tl system will come from? is bacterial OK, or do you need mammalian system for some reason? (hint: for example, if you want to use small molecule modulated promotors, like Tet-ON, you need mammalian).

Bacterial; all promotors could function to some degree in E coli.

2D How will your synthetic cell communicate with the environment? (hints: are substrates permeable? or do you need to express membrane channel?)

EMF fields will act on promotors – the relationship of how this works should be investigated further in controlled environments anyway.

3. Experimental details

3A List all lipids and genes (bonus: find the specific genes; for example, instead of just saying “small molecule membrane channel” pick actual gene)

Lipids: POPC, cholesterol

Enzymes: bacterial cell free tx/tl

Genes: hsp70 and the matA, safA, or chbB promotor, mKate and GFP

3B How will you measure the function of your system?

Measure GFP, mKate output of the cells, via real time expression or flow cytometry. Alternatively, use enzymatic reporter and measure bulk output.

[1] DANILS. SERDYUKOV et al. Study on the effects of terahertz radiation on gene networks of Escherichia coli by means of fluorescent biosensors. Vol. 11, No. 9 /1 September 2020 /Biomedical Optics Express 5258.

[2] Rodríguez-De la Fuente, Abraham O et al. “Effect of 60 Hz electromagnetic fields on the activity of hsp70 promoter: an in vivo study.” *Cell biology international reports* vol. 19,1 e00014. 26 Mar. 2012, doi:10.1042/CBR20110010

Experimental Setup:

We are using Biobits to design a transcription/translation process that produces a fluorescent RNA called Broccoli.

Basic Setup:

Reagents	Intermediate stock concentration	Working stock concentration
Nuclease free water		
Transcription buffer	10x	1x
NTP mix	20mM	4mM
Template	50uM	0.14uM
Pyrrophosphatase	10x	1x
Homemade T7	125x	1x
RNAse inhibitor	50	1
DFHBI	100mM	0.1mM

Basic reaction:

Tx-TI Master Mix Formulation (Component)	Example TxTI 15ul Reaction (uL)	35 rxn's of MM (uL)
Mg-glu 1 M	0.18	6.30
K-glu 3 M	0.65	22.75
DTT 0.1 M	0.15	5.25
Energy Mix	1.5	52.50
Amino Acids	1.5	52.50
RNAse Inhinitor A (murine) 40k U/mL	0.3	10.50
T7 homemade (1.5 uM Final) e.g. @ 35 uM Stock	0.643	22.50
Water	3.58	125.30
Cell Extract	5.00	175.00
MM/reaction	13.50	N/A
Template	1.50	N/A

Negative Control: Water. Expect no color.

Positive Control A: Follow steps for DNA A - We should see the reaction turn red after the entire process has been conducted.

Positive Control B: Follow steps for DNA B - We should see the reaction turn red after the entire process has been conducted.

Test 1: Add low concentration Purmomycin (.1X) – Translation may occasionally be stopped early, resulting in slightly more green in red.

Test 2: Add high concentration Purmomycin (10X) – Translation may be stopped entirely, resulting in much more green.

Test 3: Add low concentration Kanamycin (.1X) – binds to ribosome, obstructs mRNA reading – translation slightly impeded, so solution is slightly more green.

Test 4: Add high concentration Kanamycin (10X) – binds to ribosome, obstructs mRNA reading – translation slightly impeded, so solution is much more green.

Test 5: Add low concentration Rifamycin (.1X). Prohibit translation (less RNA polymerase), resulting in more green.