FZL
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Yeast-Based Microplastics Sensing and Remediation Modeling

To detect and combat plastic pollution, especially from widespread fragments that are small enough to be ingested, I proposed a yeast-based solution. In the device I designed, there are genetically engineered yeast cells, which are able to sense, classify and quantify microplastics (MPs) in water with G protein-coupled receptors (GPCR), a dCas9-based toggle system and a focus on one MP, polyethylene terephthalate (PET). An enzyme, PETase, has been found to break PET down to mainly mono(2-hydroxyethyl) terephthalic acid (MHET) as well as some amounts of terephthalic acid (TPA) and ethylene glycol (EG).

Assumptions

1) The loss of the plasmids or the copy-variation is negligible.
2) The mutational rate of the genome integration is negligible.
3) The differences of genome integration at different loci are negligible.
4) The system is homogeneous without the consideration of diffusion and all yeast cells are identical.
5) The cell-growth, division and death are negligible.
6) The proteins will function properly in the water samples.
7) The yeast cells have tolerance to MPs as well as its degraded products at all stages.
8) The exogenous enzymes are optimized to the same level of performances in the yeast cells.
9) Each individual yeast cell has abundant RNA polymerases and ribosomes. All mRNAs are full piece, meaning not none-functional mRNAs. All ribosomal translation rates are the same.
10) All GPCRs and signals are orthogonal, meaning each signal and receptor pair with decent specificity.
11) The initial condition is set to be all GPCRs already expressed with exactly one receptor of each kind for each cell at t = 0 s.
12) PET_{aseout} or other kinds of enzymes for the initial MPs breaking down act homogeneously on different length of MPs polymer mixture. For instance, k_PETase is the same for $PET_{n=100}$ and $PET_{n=99}$.

Models

The circuit toggle switch model with the dCas9 mutual inhibition:

$$\frac{dCas9-|mKate}{dt} = \frac{k\_Syn_{Cas9-|mKate}}{1+\ Cas9-|GFP^{coorp1}} - k\_deg_{Cas9-|mKate} \cdot\ Cas9-|mKate$$

$$\frac{dCas9-|GFP}{dt} = \frac{k\_Syn_{Cas9-|GFP}}{1+\ Cas9-|mKate^{coorp2}} - k\_deg_{Cas9-|GFP} \cdot\ Cas9-|GFP$$

The GPCR signaling pathway model:

$PET_n + H_2O + PETase_{out} \xrightarrow{k\_PETase} PET_{n-1} + MHET + PETase_{out}$

$MHET + H_2O + MHETase_{out} \xrightarrow{k\_MHETase} TPA + EG + MHETase_{out}$

$TPA \xrightarrow{k\_TPA\_meta} \emptyset$

$MHET + GPCR_{MHET} \xrightarrow{k\_on\_MHET} MHET \cdot GPCR_{MHET}$; $MHET \cdot GPCR_{MHET} \xrightarrow{k\_off\_MHET} MHET + GPCR_{MHET}$

$MHET \cdot GPCR_{MHET} \xrightarrow{k\_MHET\_tln} mRNA_{PETase\text{-}MHETase\text{-}GFP\text{-}Cas9\text{-}|mKate}$

$mRNA_{PETase\text{-}MHETase\text{-}GFP\text{-}Cas9\text{-}|mKate} \xrightarrow{k\_txn} PETase_{in} + MHETase_{in} + GFP + Cas9\text{-}|mKate$

$PETase_{in} \xrightarrow{k\_port\_PETase} PETase_{out}$; $PETase_{out} \xrightarrow{k\_deg\_PETaseout} \emptyset$

$MHETase_{in} \xrightarrow{k\_port\_MHETase} MHETase_{out}$; $MHETase_{out} \xrightarrow{k\_deg\_MHETaseout} \emptyset$

$GFP \xrightarrow{k\_deg\_GFP} \emptyset$; $mKate \xrightarrow{k\_deg\_mKate} \emptyset$

Calculations for Parameter Tweaking (the basis for "Educated Guess")

$k\_Syn_{Cas9-|mKate} = k\_txn \cdot mRNA_{PETase\text{-}MHETase\text{-}GFP\text{-}Cas9\text{-}|mKate}$

$= k\_txn \cdot k\_MHET\_tln \cdot (MHET \cdot GPCR_{MHET})$

As k_on_MHET to k_off_MHET ratio is $2\times10^8\ M^{-1}$, it should be valid to be considered as fast dynamic compared to the transcription step. With this assumption moving forward, the amount of $MHET \cdot GPCR_{MHET}$ can be approximated as a constant if it were only in the binding dynamics, where:

$\frac{d\ \text{MHET} \cdot \text{GPCR}_{\text{MHET}}\ for\ binding}{dt}$ = 0, meaning:

k_on_MHET · MHET · $GPCR_{MHET}$ - k_off_MHET · (MHET · $GPCR_{MHET}$ for binding) = 0, thus: MHET · $GPCR_{MHET}$ for binding = $\frac{\text{k\_on\_MHET}}{\text{k\_off\_MHET}}$ · MHET · $GPCR_{MHET}$

Similarly, another assumption such that MHET is at steady state could be made. MHET concentration could be approximated by $\frac{d\ \text{MHET}\ for\ meta}{dt}$ = 0, meaning:

k_PETase · PET_n- k_MHETase · MHET = 0 and thus MHET = $\frac{\text{k\_PETase}}{\text{k\_MHETase}}$ · PET_n. Therefore,

$k\_Syn_{Cas9\ -|\ mKate}$= k_txn · k_MHET_tln · $\frac{\text{k\_on\_MHET}}{\text{k\_off\_MHET}}$ · $\frac{\text{k\_PETase}}{\text{k\_MHETase}}$ · PET_n · $GPCR_{MHET}$ = 50 nM/s

$k\_Syn_{Cas\ 9\ -|\ GFP}$ = k_txn · k_tln = 50 nM/s

Based on literature that k_txn is 10 times slower than k_tln, k_txn = $\sqrt{50/10}$ = 2.24 with arbitrary units and k_tln = 22.4 with arbitrary units.

k_MHET_tln · $\frac{10^{-2}\ nM^{-1} \cdot min^{-1}}{0.05\ min^{-1}}$ · $\frac{\text{k\_PETase}}{4.25 \cdot 10^{-3}\ nM^{-1}}$ · PET_n · $GPCR_{MHET}$ = k_tln = 22.4

Modify $GPCR_{MHET}$ to be the combination of associated rate constants divided by PET_n.

The parameters actually used in the model, N/A = not available:

Parameter	Meaning	Values Used	Values from Literature
k_{PETase}	Rate of PETase digesting PET_{100}	10^{-4} $nM^{-1} \cdot s^{-1}$	N/A
$k_{MHETase}$	Rate of MHETase digesting MHET	4.25 $\mu M^{-1} \cdot s^{-1}$	$kcat/k_M$ = (31 ± 0.8 s^{-1}) / (7.3 ± 0.6 μM) = 4.25 $\mu M^{-1} \cdot s^{-1}$
k_TPA_meta	Rate of TPA metabolism	10^{-3}	N/A
k_{on_MHET}	Rate of MHET binds to $GPCR_{MHET}$	10^{7} $M^{-1} \cdot min^{-1}$	Tunable; Estimated from the literature
k_{off_MHET}	Rate of MHET dissociates from MHET · $GPCR_{MHET}$	0.05 min^{-1}	
k_{MHET_tln}	Rate of MHET triggered translation, including MHET · $GPCR_{MHET}$ signaling cascade	2.24 $nM^{-1} \cdot s^{-1}$	0.1 nM/h
k_{tln}	Rate of transcription from DNA	2.24 $nM^{-1} \cdot s^{-1}$	0.1 nM/h
k_{txn}	Rate of translation from mRNA	0.224 $nM^{-1} \cdot s^{-1}$	0.01 nM/h
k_{port_PETase}	Rate of PETase transported from intracellular to the water sample	3.4/60 $nM^{-1} \cdot s^{-1}$	3.4 min^{-1}
$k_{port\_MHETase}$	Rate of MHETase transported from intracellular to the water sample	3.4/60 $nM^{-1} \cdot s^{-1}$	3.4 min^{-1}
$k_{GFP\ deg}$	Rate of GFP degradation	10^{-3} $nM^{-1} \cdot s^{-1}$	ln2/~5hrs, estimated from the literature but tunable with degradation tags
$k_{mKate\ deg}$	Rate of mKate degradation	10^{-2} $nM^{-1} \cdot s^{-1}$	
k_{PETase_outdeg}	Rate of PETase_out degradation	10^{-3} $nM^{-1} \cdot s^{-1}$	
$k_{MHETase\_outdeg}$	Rate of MHETase_out degradation	10^{-3} $nM^{-1} \cdot s^{-1}$	
$k_{Cas9byBonA\_deg}$	Rate of dCas9 -| GFP degradation	10^{-1} $nM^{-1} \cdot s^{-1}$	
$k_{Cas9byAonB\_deg}$	Rate of dCas9 -| mKate degradation	10^{-3} $nM^{-1} \cdot s^{-1}$	
corpCas9byBonA	Cooperativity of dCas9 -| GFP onto GFP DNA	1.6	1.6 from the current literature but could be engineered to be even higher
corpCas9byAonB	Cooperativity of dCas9 -| mKate onto mKate DNA	1.6	

The variables and their meanings:

Variables	Meaning
PETn	None-degraded PET concentration
PETn-1	PETase degraded PET concentration
MHET	MHET concentration
TPA	TPA concentration
EG	EG, ethylene glycol, $C_2H_6O_2$, concentration

$PETase_{out}$	PETase concentration in water sample
$PETase_{in}$	PETase intracellular concentration
$MHETase_{out}$	MHETase concentration in water sample
$MHETase_{in}$	MHETase intracellular concentration
mKate	mKate concentration
GFP	GFP concentration
dCas9 -| mKate	mKate repressing dCas9 concentration
dCas9 -| GFP	GFP repressing dCas9 concentration
$GPCR_{MHET}$	Free GPCR specific to MHET
$MHET \cdot GPCR_{MHET}$	MHET bounded GPCR, initiating signals
$mRNA_{PETase\_MHETase\_GFP\_dCas9 -\| mKate}$	The concentration for the mRNA coded for PETase – MHETase – GFP – dCas9 -| mKate

Files

BioSystem.m, Compositor.m, Const.m, MATLAB_sandbox.m, part_composition_setup.m, Part.m, Pulse.m, and Rate.m are part of the biomolecular simulation setup.

NoGPCR.m plots the simulations for the system with three different dCas9 cooperativities without the GPCR setup to determine the best cooperativity for the downstream case with GPCR. The plots are adjusted to the axes best represent the data.

WithGPCR.m plot the individual simulation result for the full system with different starting concentrations of PETn (none-degraded PET).

DiffGFPmKatePETnEG.m plots the overlay of GFP concentration, mKate concentration, PETn consumption and EG production. At the same time, the function obtains the minimum and maximum concentrations of GFP and mKate and the corresponding time achieving those.

ToggleComp.m compares the GFP and mKate concentrations with and without mKate calibration or called toggle system.

References

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