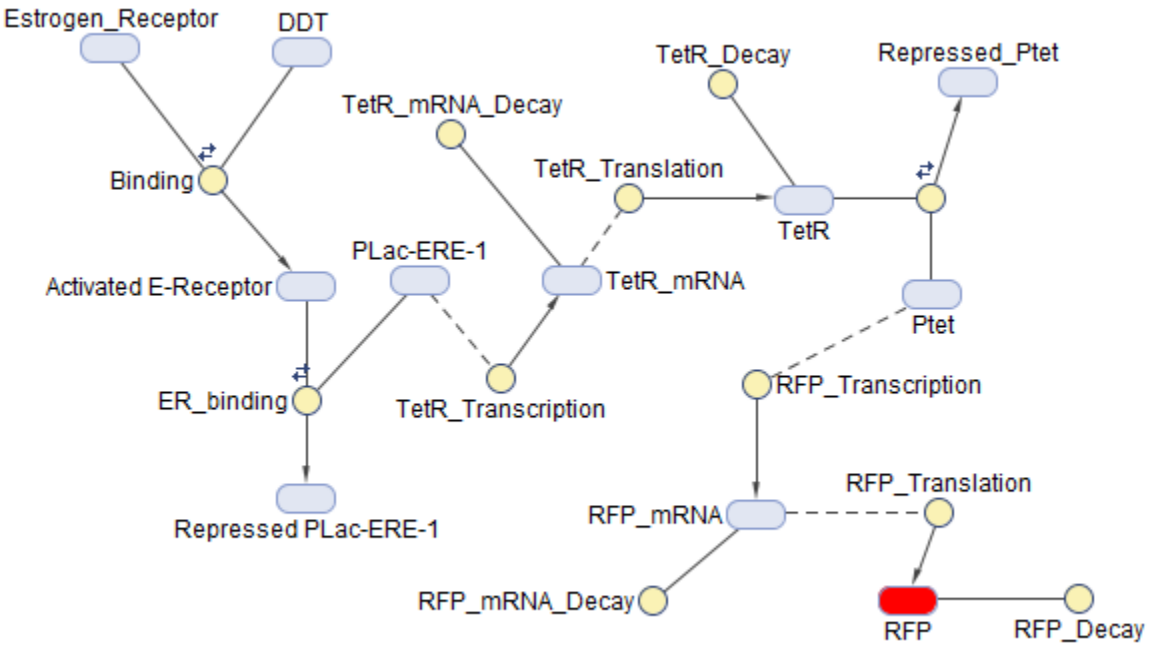
Math Modelling: First Steps

To help guide the specific choices for our design, and find out how well it might work in determining DDT concentrations, we developed math models using Matlab's SimBiology tool. The three designs highlighted on our engineering success page were modelled here.

Model of the initial design



In this model, the expression of RFP is controlled by DDT levels by way of a TetR inverter. We initially sought to find the appropriate parameters for the BioBricks used in our initial design (I13521 and K123002). We found some of the following sources relevant for our model from previous iGEM teams and literature on Estrogen receptor, such as (in no particular order):

TU Delft, 2013 [[http://2013.igem.org/Timer_Plus_Sumo]]

mRNA decay: 0.231 / minute

TetR protein decay: 0.1386 / minute

Imperial College London, 2016 [[http://2016.igem.org/Team:Imperial_College/Model]]

mRNA decay: 0.139 / minute

UNESP Brazil, 2018 [[http://2018.igem.org/Team:Unesp_Brazil/Model]]

mRNA decay: 4.1x10^-3 / second, or 0.25 / minute

Northwestern University Team, 2018 [[http://2018.igem.org/Team:Northwestern/Model]]

mRNA decay: 3.8x10^-3 / second, or 0.23 / minute

Carnegie Mellon, 2014 [[http://2014.igem.org/Team:Carnegie_Mellon/SensorModel]]

Rate of DDT/Estrogen Binding: 1.3x10^-3 / nM*second

Rate of DDT/Estrogen Dissociation: 1.2x10^-3 / second

Diffusion rate of DDT through cell membrane: 1.7x10^-2 / second

RFP transcription rate (mRNA production): 8.8x10^-1 / nM* second

RFP mRNA decay: 4.3x10^-3 / second, or 0.258 / minute

RFP translation rate: 9.0x10^-3 / second, or 0.54 / minute

RFP protein decay: 8.30x10^-4 / second, or 0.498 / minute

HZAU-China, 2019 [[https://2019.igem.org/Team:HZAU-China/Model]]

TetR transcription rate: 4x10^-3 / second, or 0.24 per minute

TetR mRNA decay: 9x10^-3 / second, or 0.54 per minute

TetR translation rate: 2.36x10^3 / second

TetR protein decay: 6.31x10^-1 / second

TetR Promoter Repression Rate (Promoter Binding): 1x10^-1 / second

Literature Search, EPA Consulation

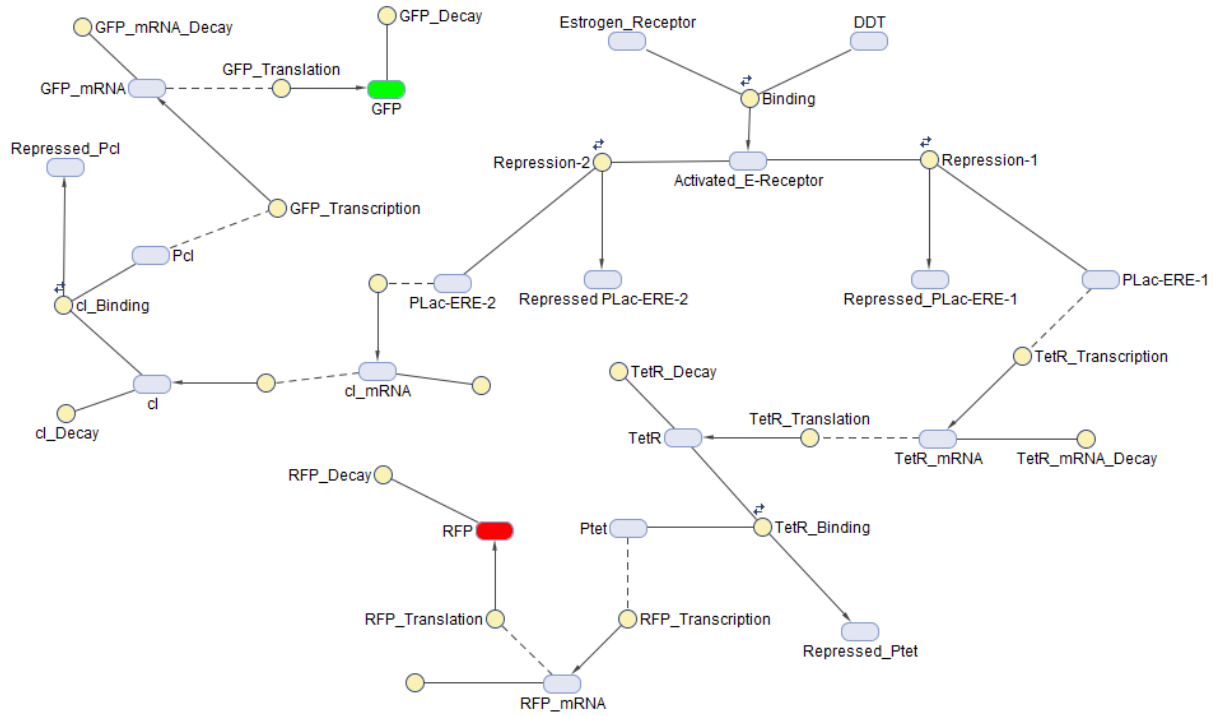
Detection target: 1ppm of DDT

Activated Estrogen Receptor, DNA Binding Kd: 1.0x10^-1 nM

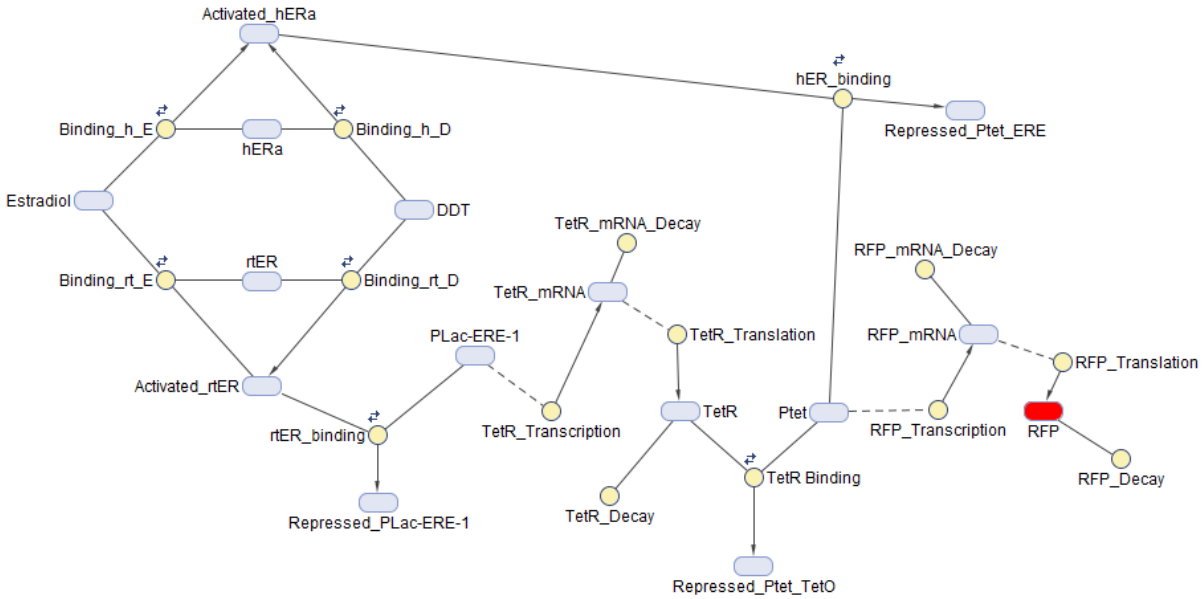
From the above, we tried different combinations as well as averages (mRNA decay of 0.23 / minute). Our preliminary attempts to incorporate these values gave incoherent results. Therefore, we are planning on fitting or optimizing relative numbers for our parameters such as TetR transcription or decay, similar to the approach exemplified by this model by Imperial (2009) [[https://openwetware.org/wiki/IGEM:IMPERIAL/2009/Encapsulation/Modelling/Timer1]] This way, we can determine how much stronger TetR expression needs to be compared to RFP expression to get the appropriate dynamic. These insights will lead to a more informed design choice - perhaps modulating ribosome binding sites, protein stabilities, or choice of inverter.

Likewise, results from our wet-lab experiments will help us sort out which of the parameters above should be included in the model, and which are not appropriate.

Expanded Models



In this model, DDT levels control two different inverters, which in turn control GFP or RFP outputs. The parameters we will need to optimize here will be the transcription of the inverters - the pLac inverter should be much more sensitive to the levels of DDT. Optimizing these relative values might tell use how many tandem repeats of the ERE to include on the GFP module - the more EREs, the greater the chance that DDT binding the receptor will result in repression of the cI inverter component, which in turns will lead to GFP expression.



Here, we are modelling another design that will lead to a biosensor that can discriminate between DDT and estrogen (or other xenoestrogens). In this case, we would need to optimize the circuit based on the differential binding affinities of estrogen receptors from different species (such as human and rainbow trout) to estradiol or DDT.