Synthetic Biology

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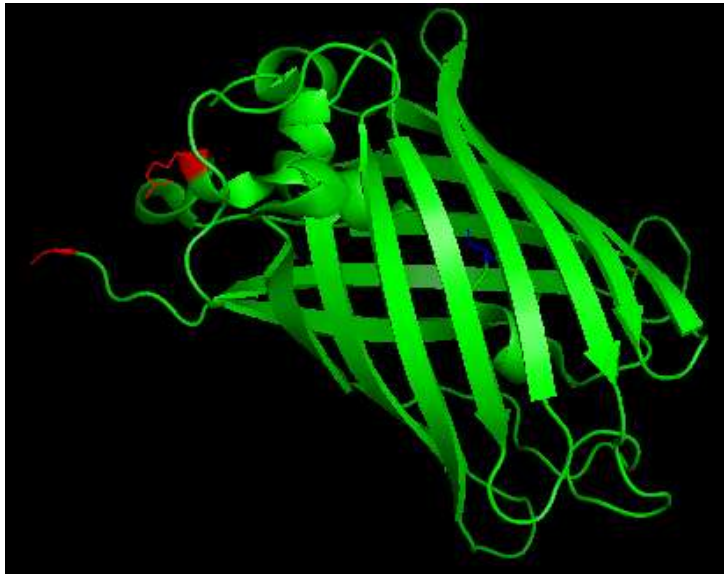
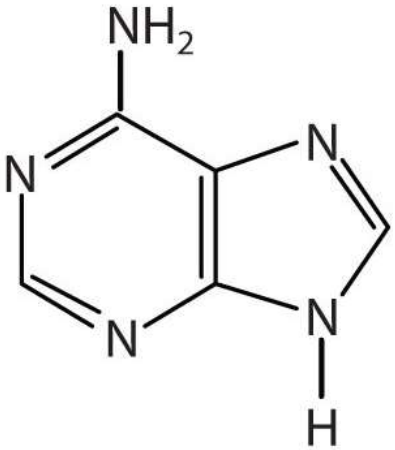
iGEM 2017

What is Biochemistry?

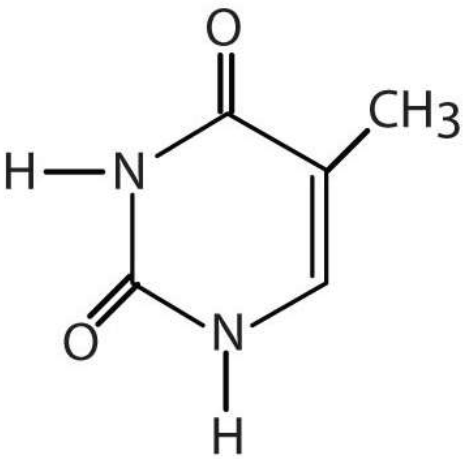
- Some things you learn in A level Chemistry and Biology:
  - Organic chemistry
  - Metabolism
  - Transcription
  - Translation
  - Cell structure
  - Biological molecules
- Some things you don't learn…
  - Mechanisms of enzyme action
  - Physics behind biological processes
  - Cell signalling
  - Protein structures



DNA to genes to proteins

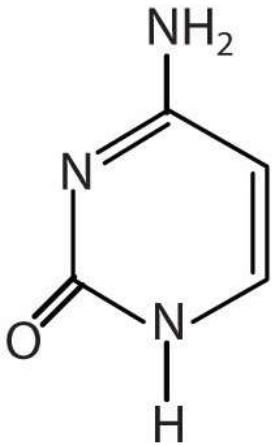


A



T

C



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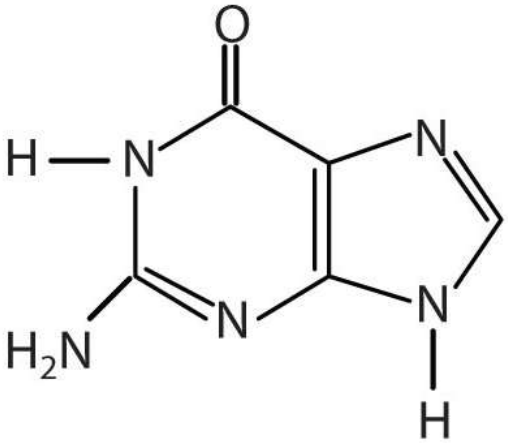


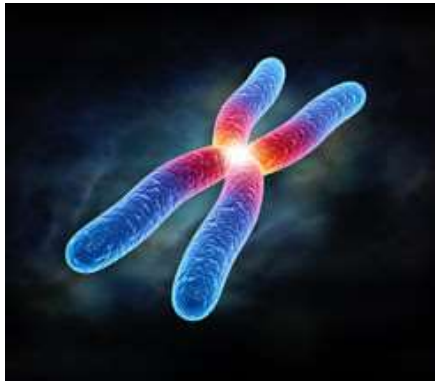
DNA to RNA…

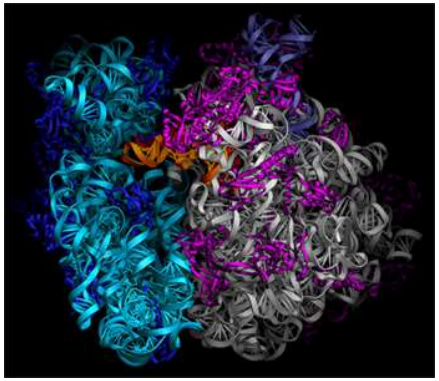
DNA

- Uses T instead of U
- More stable
- Only exists as chromosomes
- Keeps information safe in the nucleolus

RNA

- Uses U instead of T
- Less stable
- Can exist as small strands
- Carries genetic information throughout the nucleus and cell





Promoters, Repressors, and Polymerase

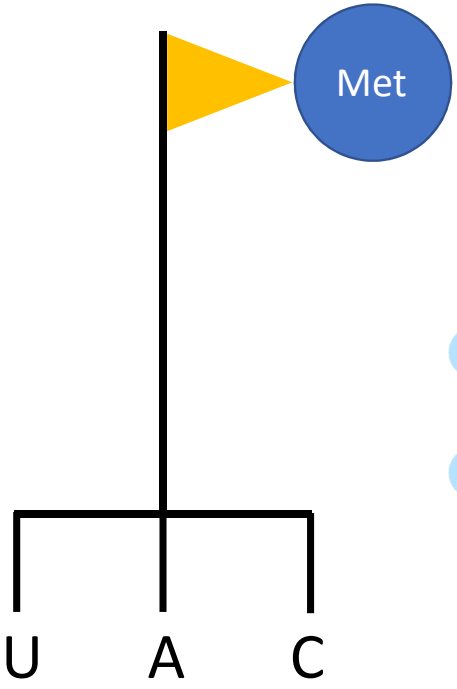
- DNA needs to be converted into RNA
- This requires a start site where the polymerase (which copies the DNA into RNA) can bind
- There can be DNA sequences around the start site which either enhance the binding of the polymerase or decrease it
- These sequences can also recruit proteins which can interact with polymerase and help it find where to go, or block the polymerase completely so it can't get to the DNA
- Promoters enhance polymerase binding to DNA, repressors decrease polymerase binding to DNA

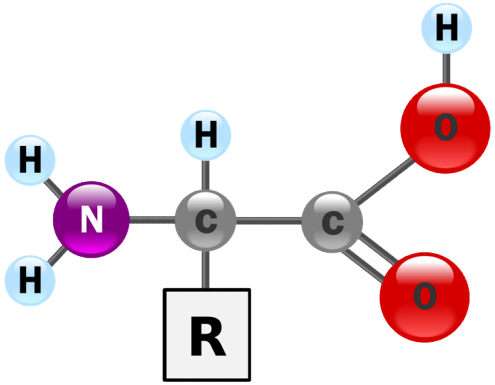
Codons and tRNA

ATG = Methionine (START)

AAA or AAG or AAC or AAT = Alanine

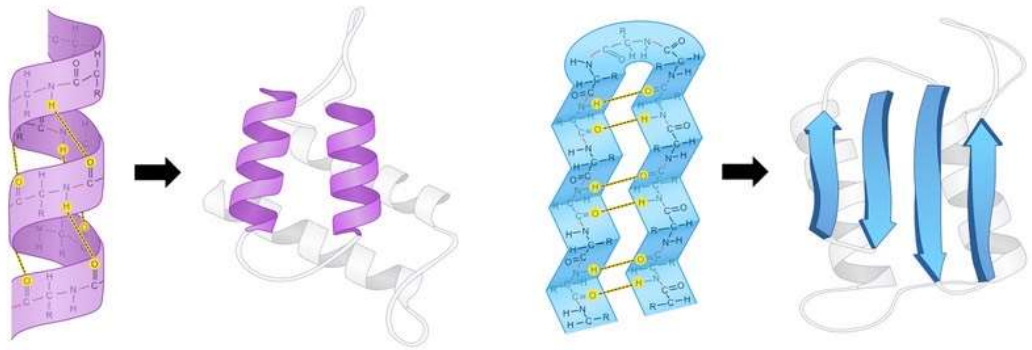
TAA or TAG or TGA = STOP





Building the protein

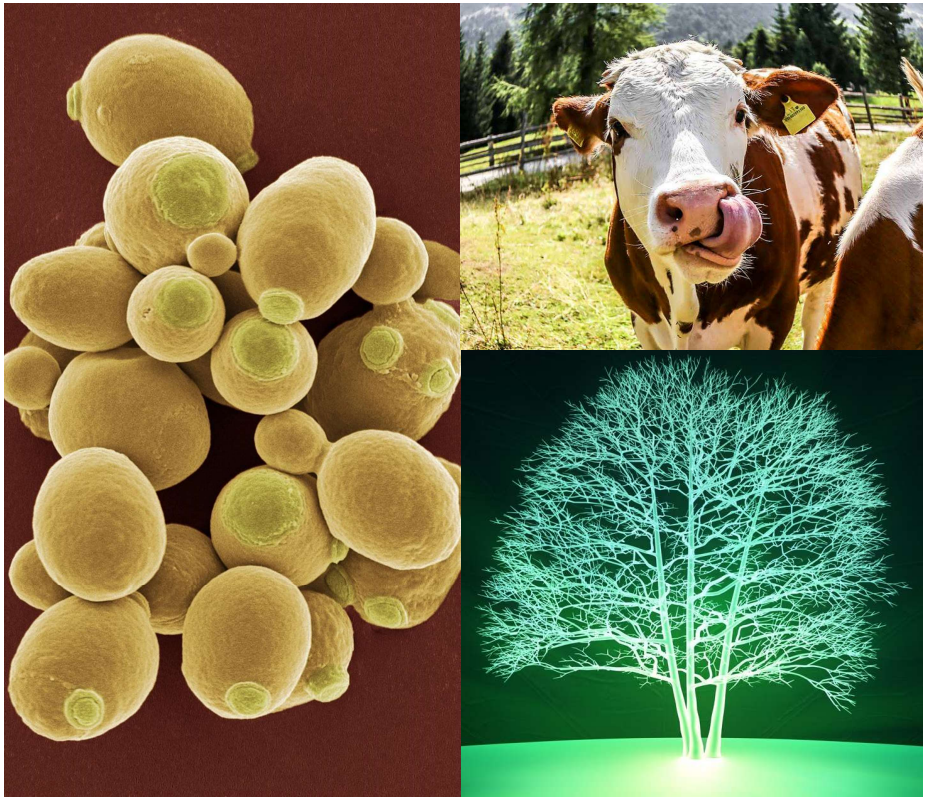
- Ribosome makes peptide bonds between amino acids
- Primary structure – amino acid sequence
- Secondary structure – alpha helices and beta strands/sheets
- Tertiary structure – folds of the helices and sheets eg Ig fold
- Quaternary structure – more than one protein coming together, may include non-protein elements like metals



What is Synthetic Biology?

- Designing a system to fit your needs, by taking ‘parts’ from a variety of sources – bacteria, plants, or even humans
- A ‘part’ can be a protein (including enzymes) or a DNA sequence encoding something that isn’t a protein (like a promoter)
- The end result is usually a biological circuit, that converts a specific input into a specifically defined output
- In an ideal world, you would design something well and it would work right away… but biology doesn’t like doing this!
- Multi-disciplinary – incorporates biochemists and mathematicians and engineers and chemists and more…

What can we use synthetic biology for?

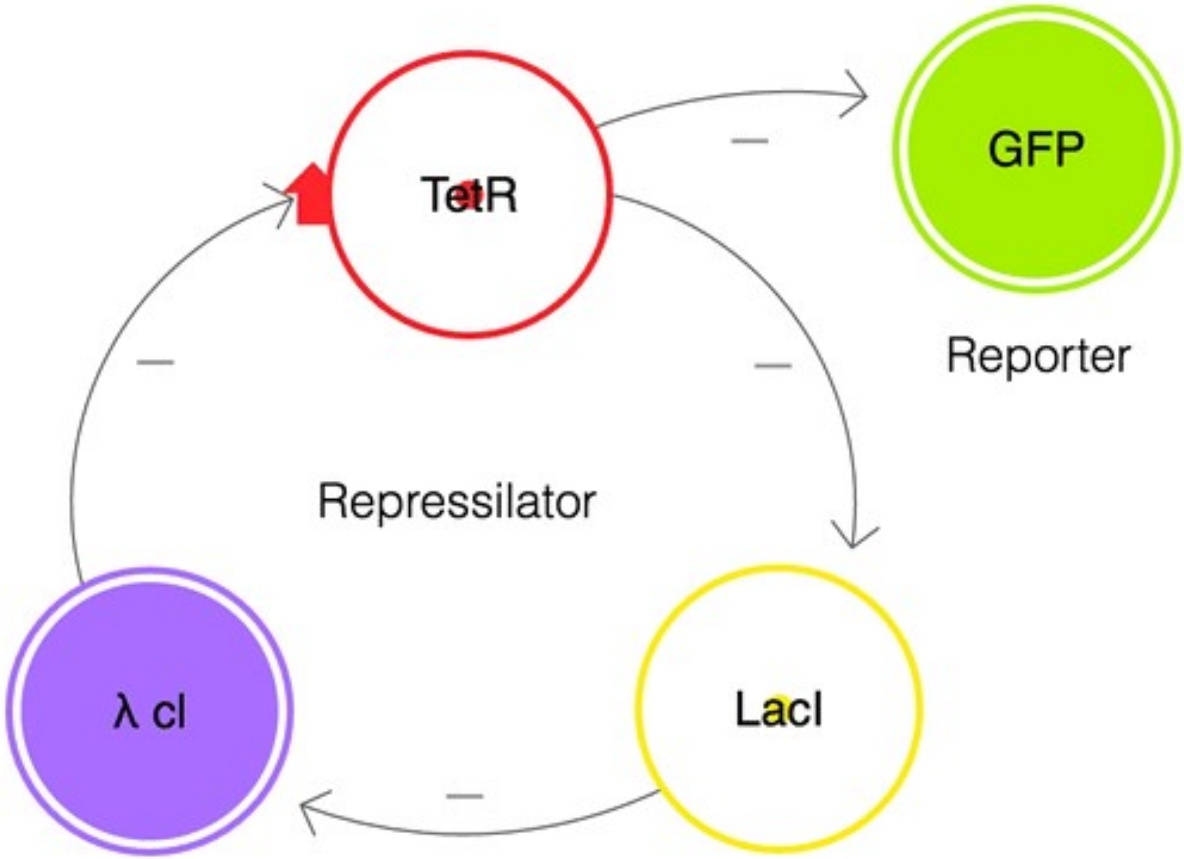


- Insulin production
- Opiate production
- Toxin biosensors for water sources
- Luminescent plants for street lights
- DNA-based logic gates
- Introducing new amino acids

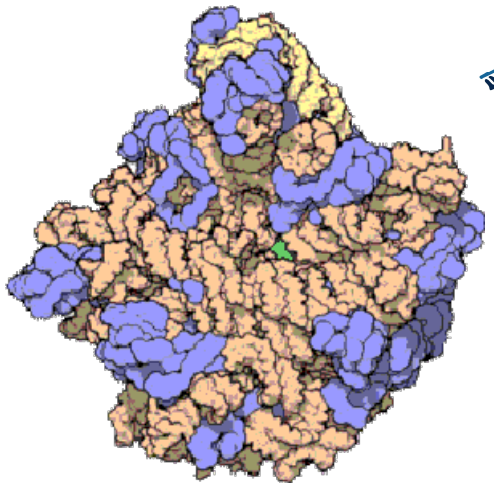
More applications (fun)

- Bacterial ‘photography’ (TinkerCell software)
- *E. coli* perfumes and flavourings (Ginkgo Bioworks)
- Repressilator
- Fluorescent protein art (#fluorescentfriday)

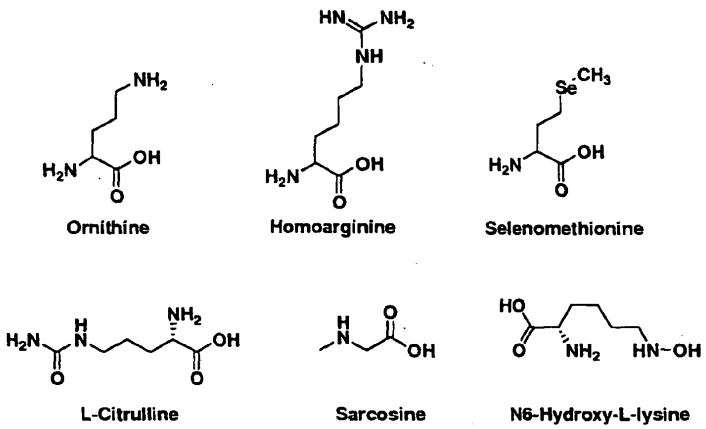
Repressilator



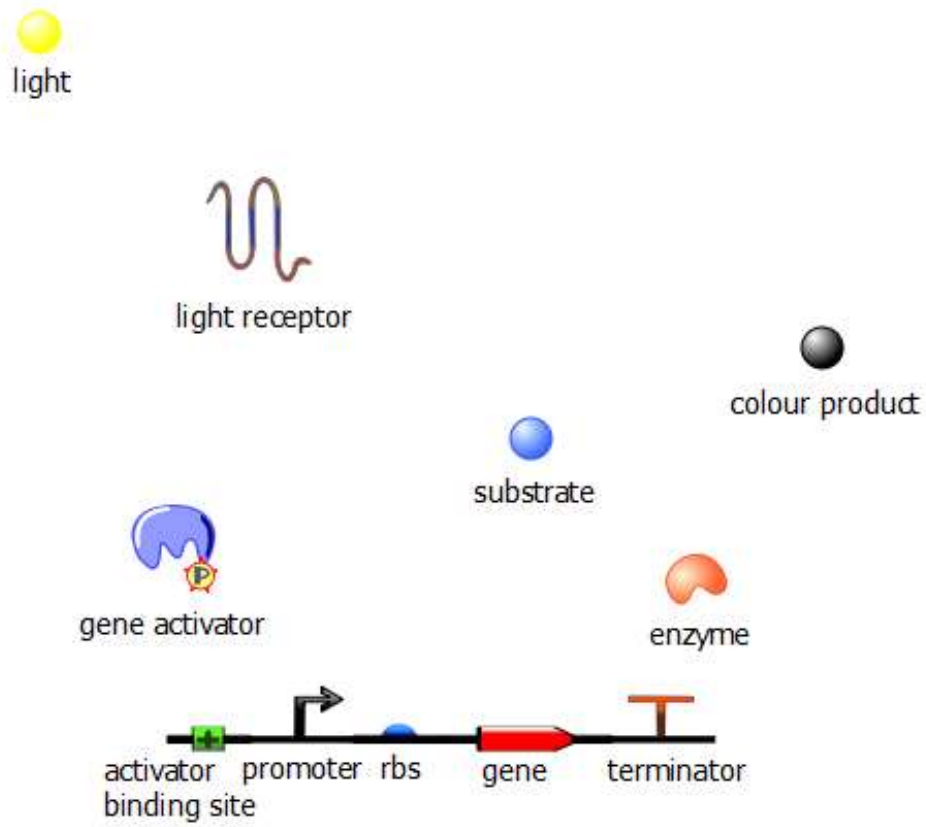
Orthogonal ribosomes



- Zoe's favourite potential use!
- '3D printing' using ribosomes
- Read mRNAs not normally recognised in cells
- It hasn't got very far… yet
- Now the development of o-ribosomes is well-documented, main limitation is mutating other components using biased evolution (tRNA, enzymes)



Bacterial Photography – Tinkercell



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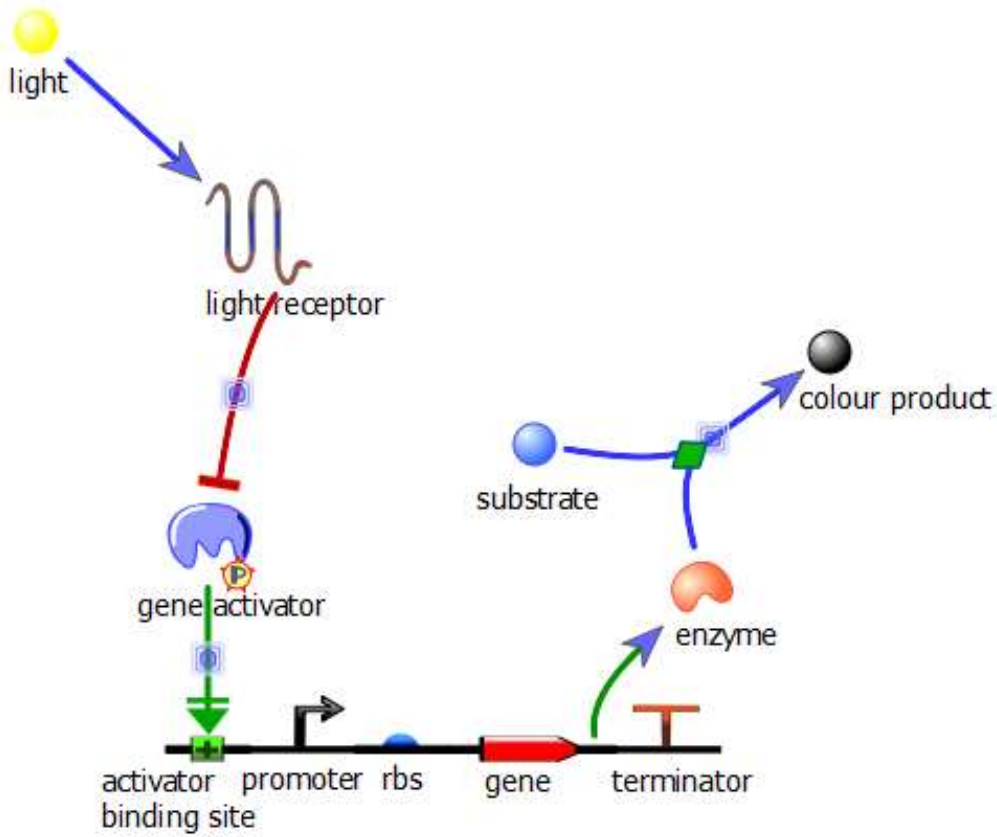

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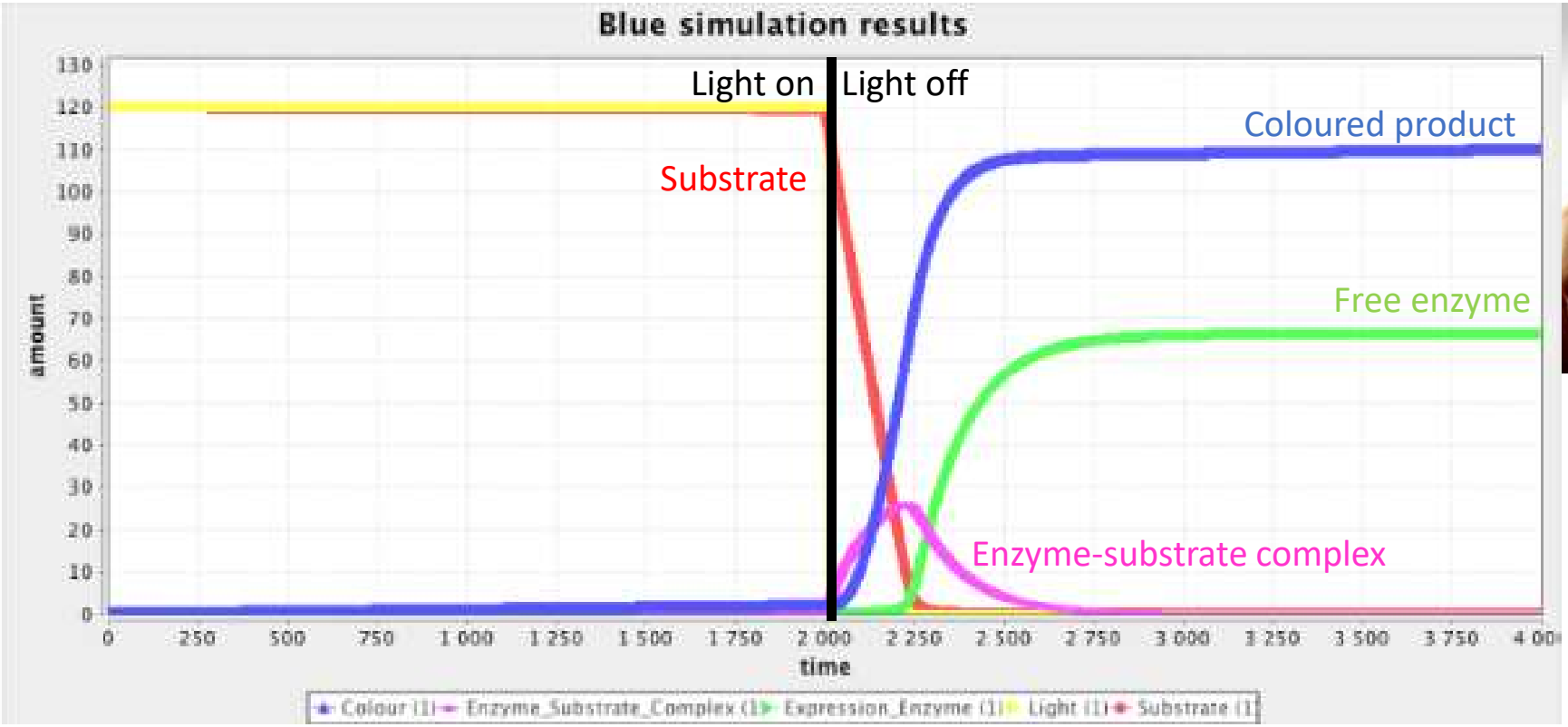
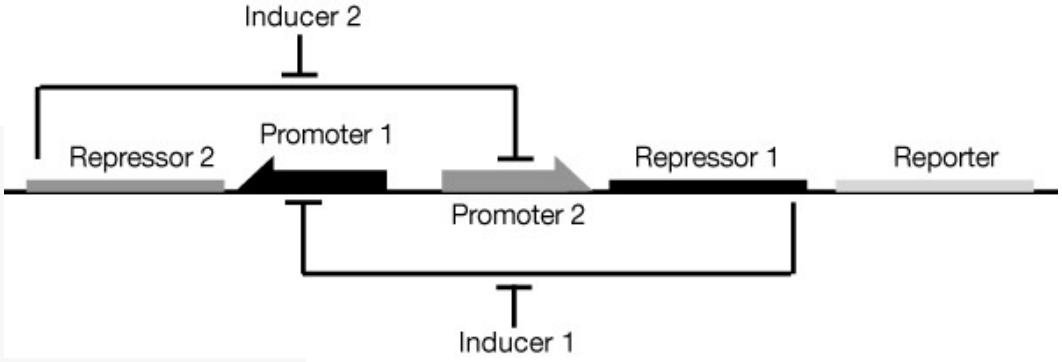
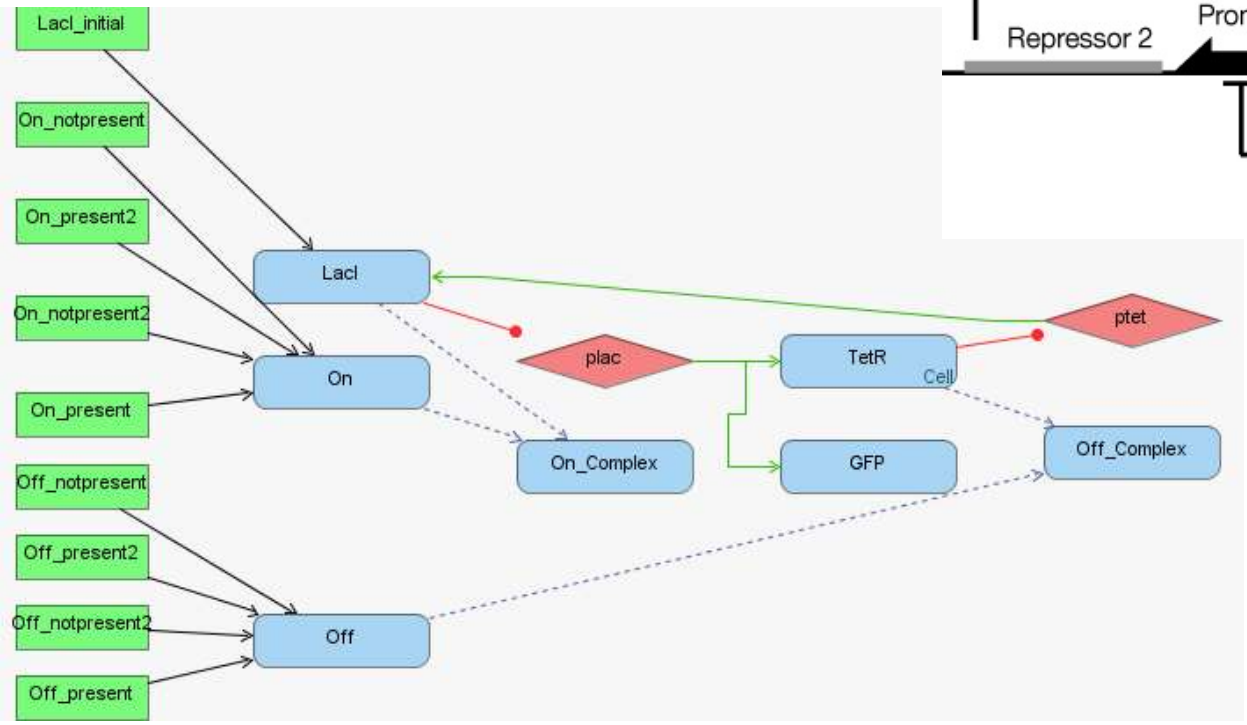


Genetic Toggle Switch





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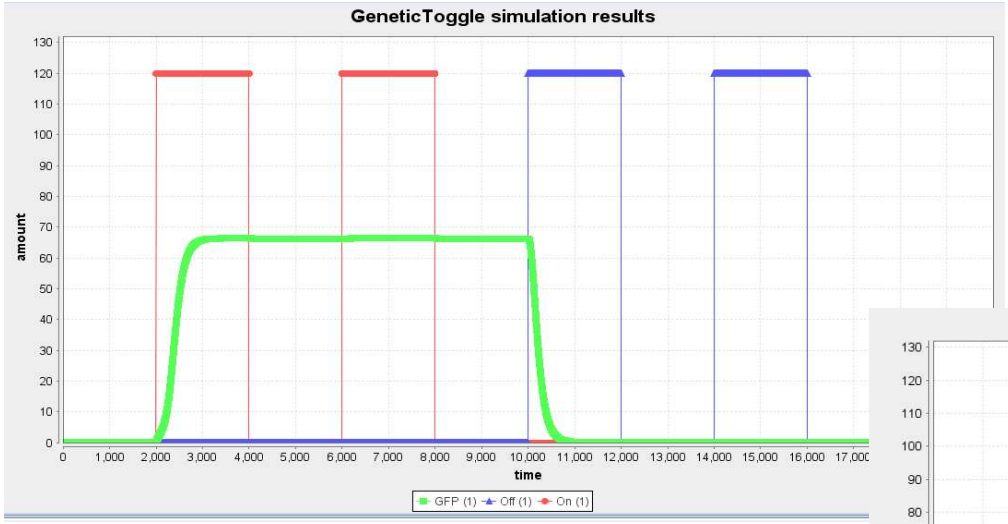
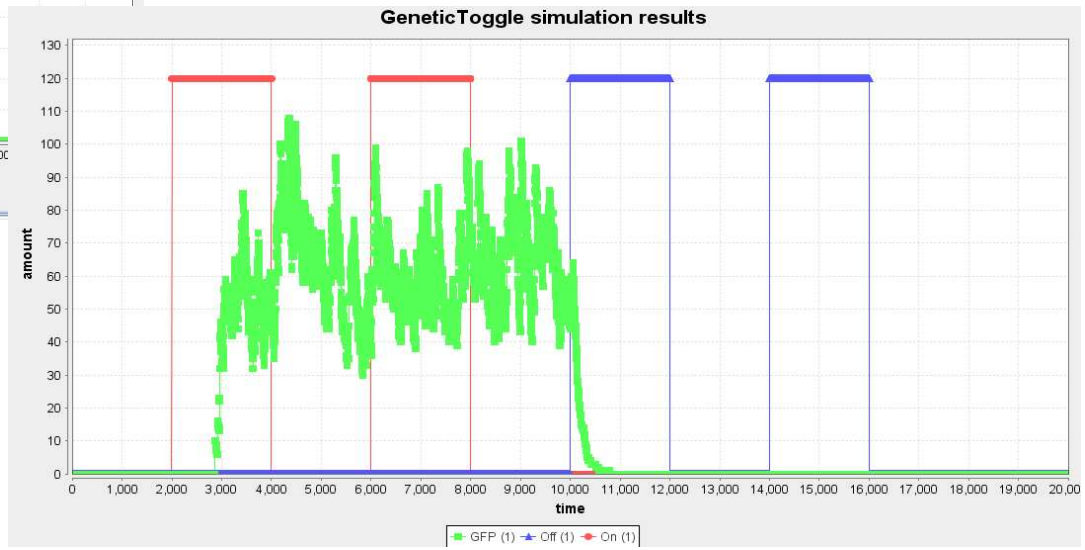
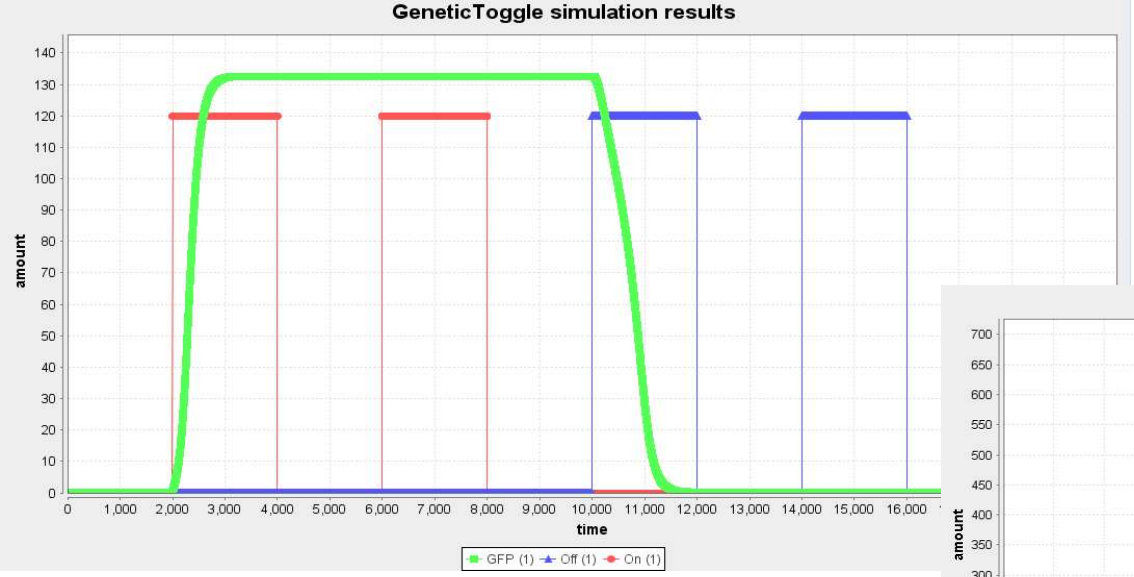


- Why do these two graphs look different when they are modelling the same thing?

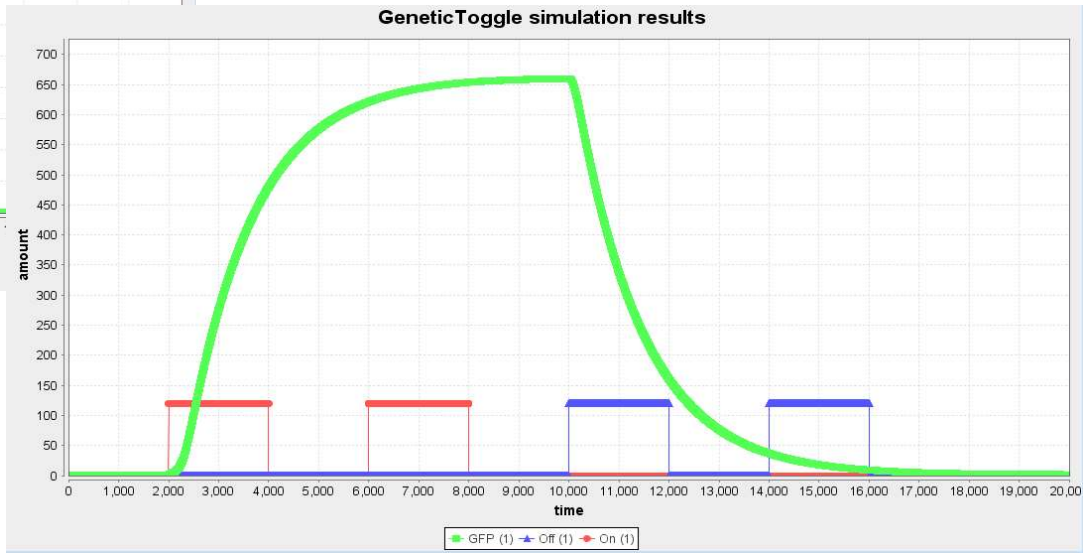


- Which is better? Why?

Genetic Toggle Switch



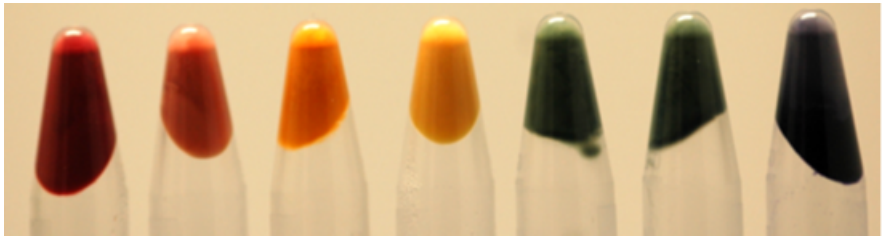
- These graphs both show the system modified to have a higher output. Which is better?



- What does the steepness of the curve show and why does it change?

The International Genetically Engineered Machine Competition

- For the past ten years, iGEM has been open to students from around the world to 'compete' to produce the best synthetic biology project
- Three competitions: high school, undergraduates, postgraduates
- A variety of streams: diagnostics, foundational advance, therapeutics, environment, manufacturing and more
- Past projects:
  - Enzymes to produce coloured products
  - Co-culturing bacteria
  - New drugs for UTIs



History of iGEM

- Started at MIT
- Aims to develop young scientists to think in a multi-disciplinary fashion – not just limiting themselves to a pure strand
- Many past iGEM competitors have gone on to start companies, work as post-docs etc
- Oxford have had a team since 2014 and have won a gold medal every year (so no pressure)
- The Grand Prize is what every team wants to win – a shiny Lego brick to represent the ‘biobricks’ every team works with

Past Oxford iGEM projects

- DCMation (2014.igem.org/Team:Oxford)
  Using bacteria to clean up environmental pollution

- SolUTIon (2015.igem.org/Team:Oxford)
  Using bacteria to fight bacterial UTIs

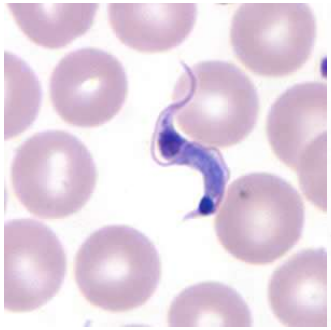
- Cure (2016.igem.org/Team:Oxford)
  Making a probiotic for a specific disease

Oxford iGEM 2017 – See cruzi

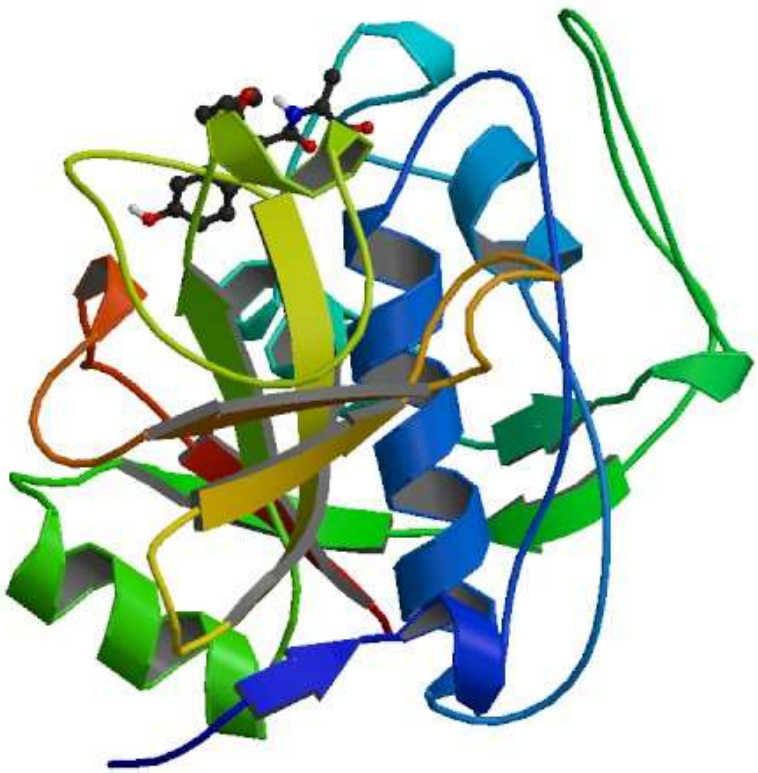
- Cell-free biosensor for a tropical parasite
- Two proposed systems
  - DNA-based
  - Protein-based
- Aim is to detect a protease (enzyme) in the blood which is produced by the parasite
- Output is a peptide called hirudin (from leeches) that stops blood clotting

Chagas Disease – *Trypanosoma cruzi*

- Caused by a parasite called *T. cruzi*
- Parasite is spread by a ‘kissing bug’
- Lives in the blood
- Causes damage to heart and intestines
- Shortens lifespan – generally 30 years after infection, person dies
- Has two phases, acute and chronic
- Huge issue in Latin America

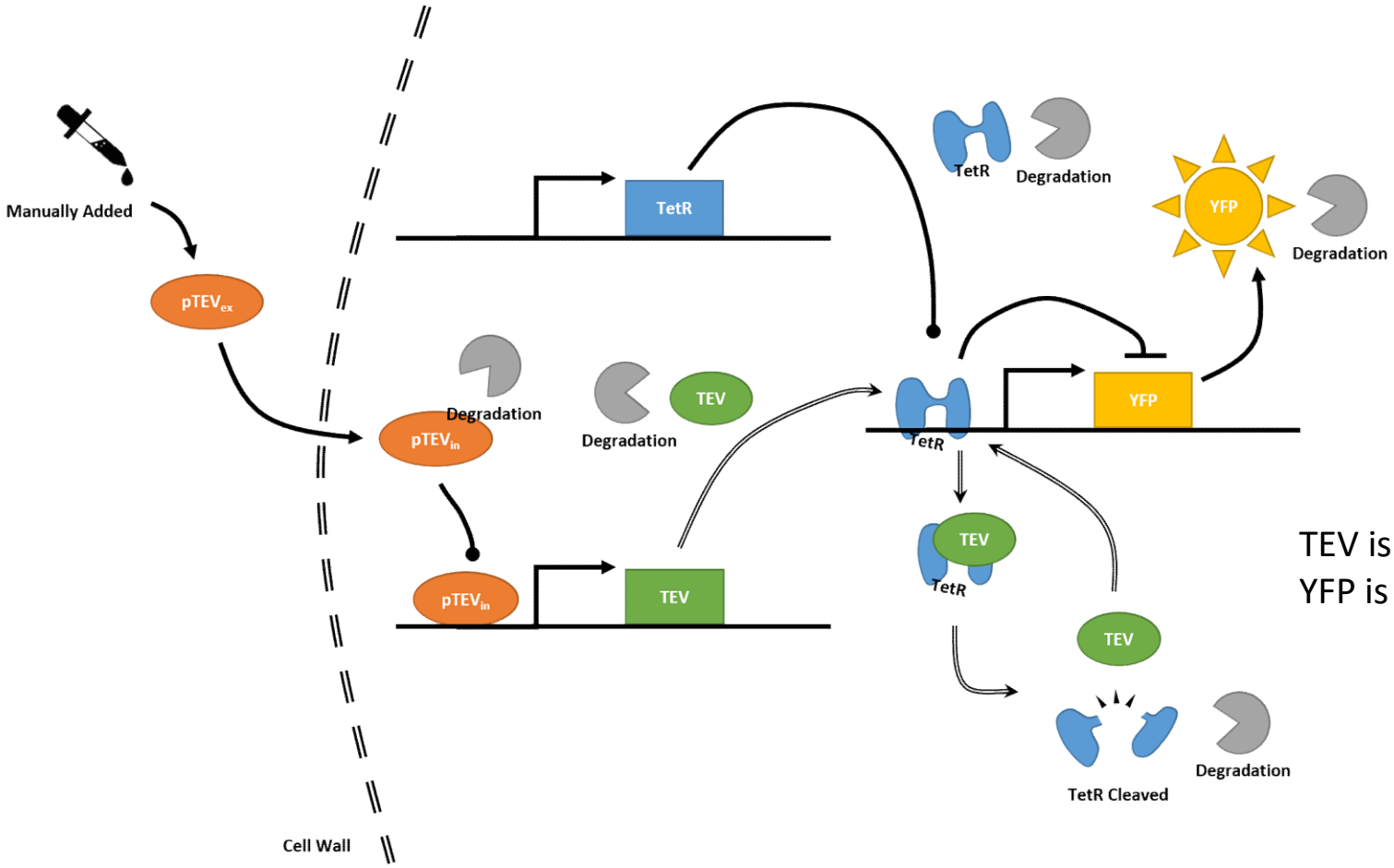


Cruzipain (and other proteases)



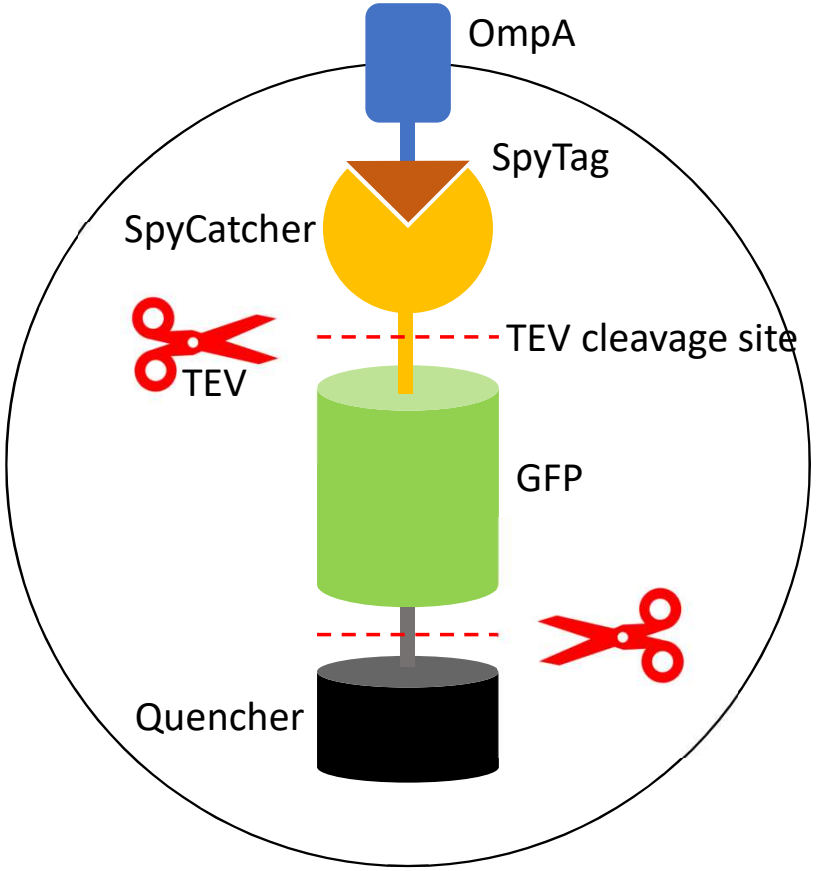
- Proteases are enzymes that cleave peptide bonds
- ‘Specific’ proteases cut up proteins by recognising sequences e.g. TEV protease recognises ENLYFQG (amino acid sequence)
- Cruzipain is a specific protease only released by *T. cruzi*
- We are using it to cut linkers between different protein components to release output proteins
- These linkers will not be cut if the protease is not present

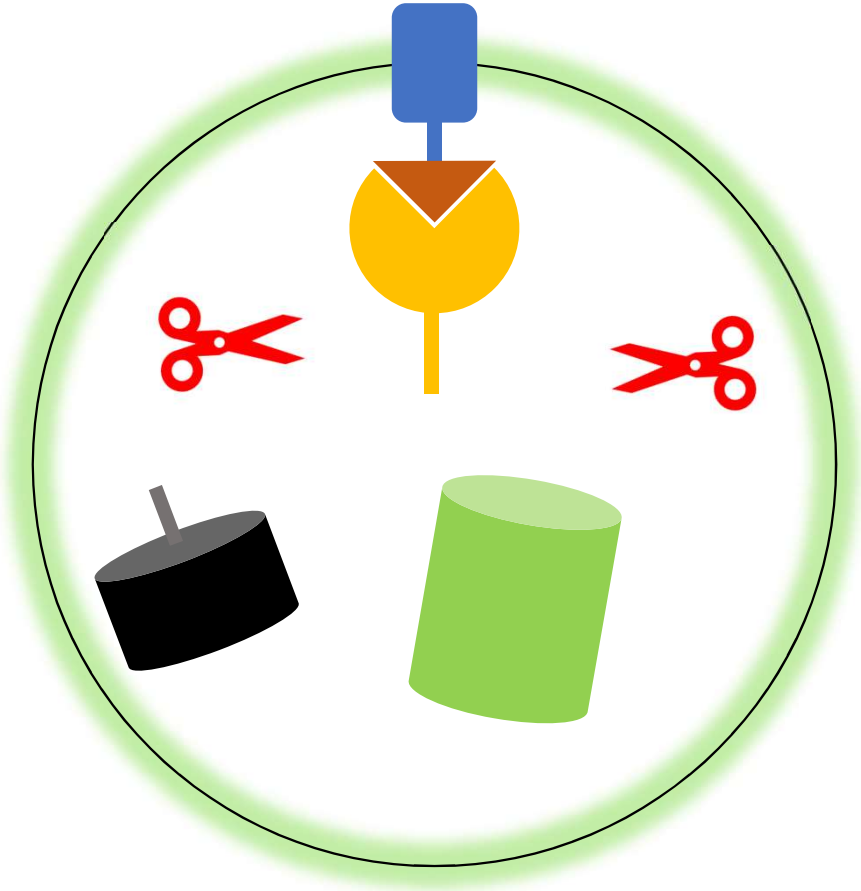
Overview of our model – DNA-based



TEV is modelling cruzipain
YFP is modelling hirudin

Overview of our model – Protein-based



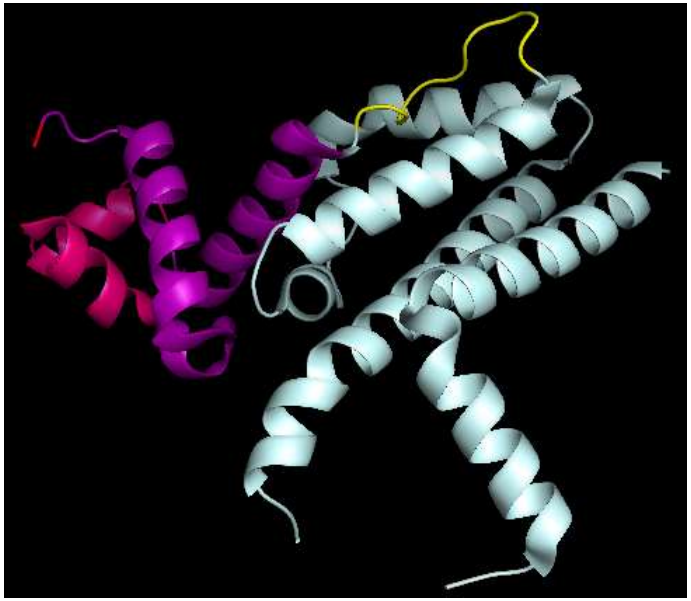


Overview of the biosensor

- Cell-free parts or vesicles will be freeze-dried onto paper
- Small piece of paper will be added to a blood sample
- Leave the sample for ~2 hours including a vesicle breaking step
- See if the blood has clotted or not

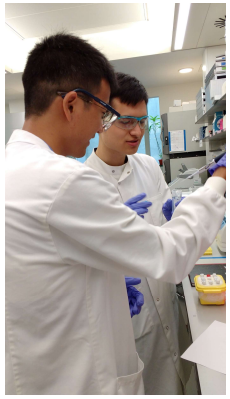
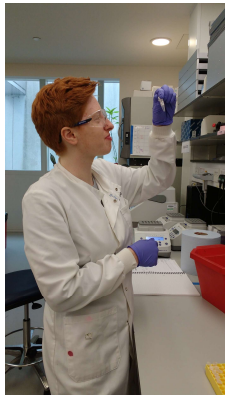
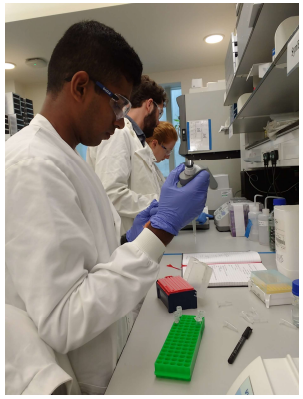
  Clotting = no Chagas detected

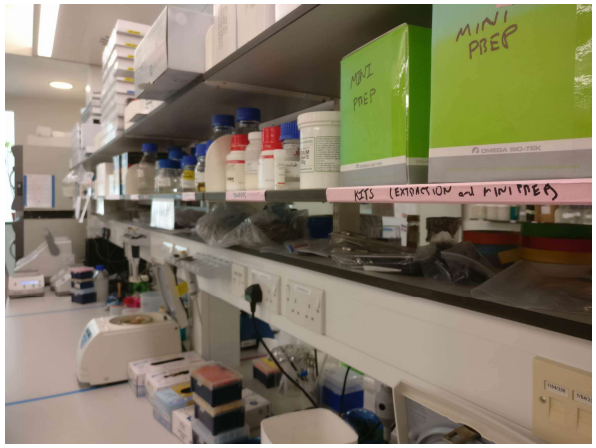
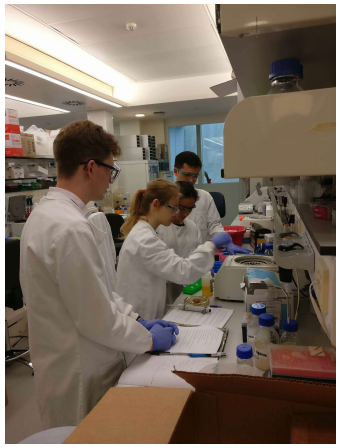
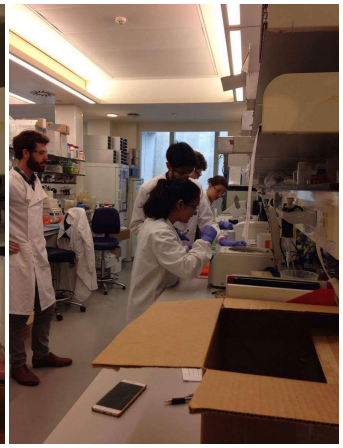
  No clotting = Chagas detected



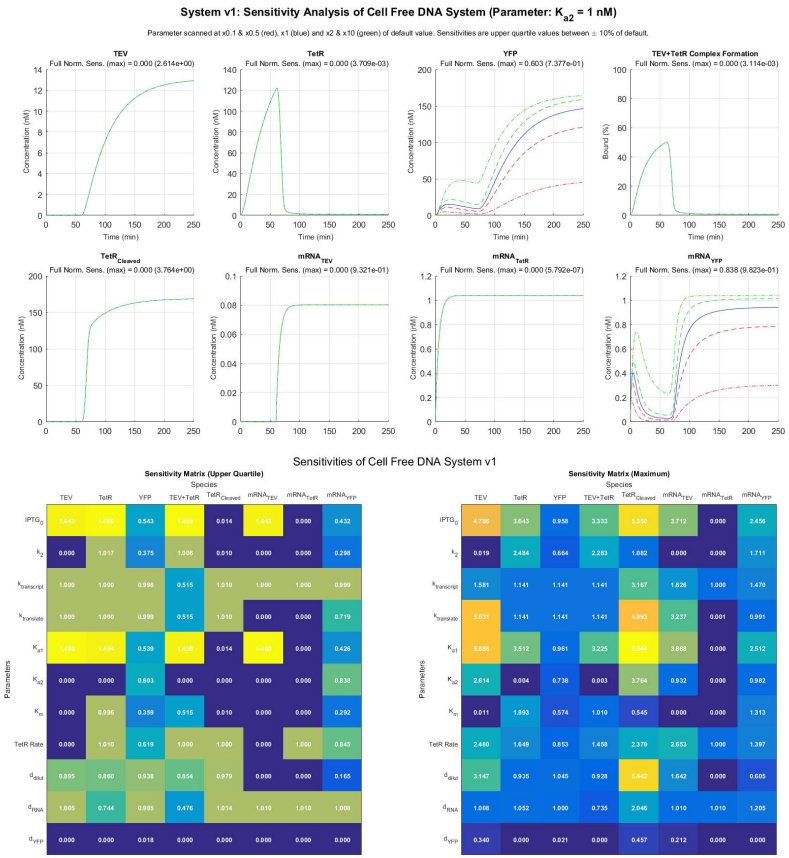
Lab work



iGEM - 2017
Black n' Red

MINI PREP
KITS (EXTRACTION and MINI PREP)




Modelling


System v1: Sensitivity Analysis of Cell Free DNA System (Parameter: K_{a2} = 1 nM)
Parameter scanned at x0.1 & x0.5 (red), x1 (blue) and x2 & x10 (green) of default value. Sensitivities are upper quartile values between ± 10% of default.
TEV
Full Norm. Sens. (max) = 0.000 (2.614e+00)
TetR
Full Norm. Sens. (max) = 0.000 (3.709e-03)
YFP
Full Norm. Sens. (max) = 0.603 (7.377e-01)
TEV+TetR Complex Formation
Full Norm. Sens. (max) = 0.000 (3.114e-03)
$TetR_{Cleaved}$
Full Norm. Sens. (max) = 0.000 (3.764e+00)
$mRNA_{TEV}$
Full Norm. Sens. (max) = 0.000 (9.321e-01)
$mRNA_{TetR}$
Full Norm. Sens. (max) = 0.000 (5.792e-07)
$mRNA_{YFP}$
Full Norm. Sens. (max) = 0.838 (9.823e-01)
Concentration (nM)
Bound (%)
Time (min)

Sensitivities of Cell Free DNA System v1
Sensitivity Matrix (Upper Quartile)
Sensitivity Matrix (Maximum)
Species
TEV
TetR
YFP
TEV+TetR
$TetR_{Cleaved}$
$mRNA_{TEV}$
$mRNA_{TetR}$
$mRNA_{YFP}$
Parameters
$IPTG_0$
k_2
$k_{transcript}$
$k_{translate}$
K_{a1}
K_{a2}
K_m
TetR Rate
d_{dilut}
d_{RNA}
d_{YFP}

Find out more

Ethics of Synthetic Biology

- Who should be allowed to do synthetic biology?
- What should we be allowed to engineer?
- Where should systems be applied?
- Should the public know when something is made using synbio?
- Some potential uses:

  Bacteria making biofuels

  Bacteria making flavourings for foods

  Yeast producing drugs

  Hybrid plants to produce high yield in poor conditions

What does the future hold?

- Greater focus on using synthetic biology in diagnostics
- Move towards more cell-free systems
- Much more automation of lab procedures
- Increased speed of production
- More accurate modelling
- Integration with systems such as Raspberry Pi

Q&A

- Questions about the biology?
- Questions about life at Oxford?
- Questions about iGEM?
- Questions about synthetic biology?