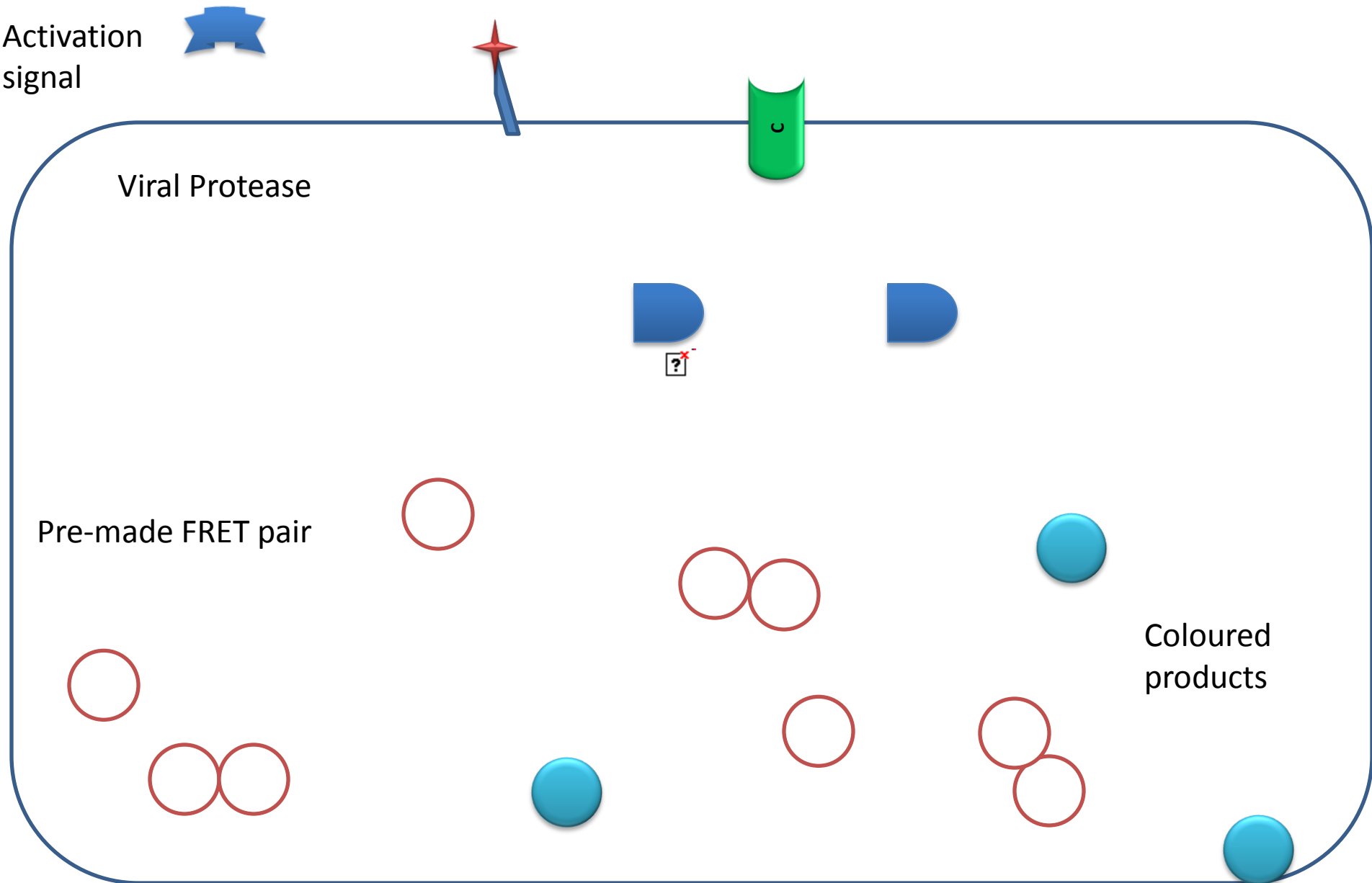
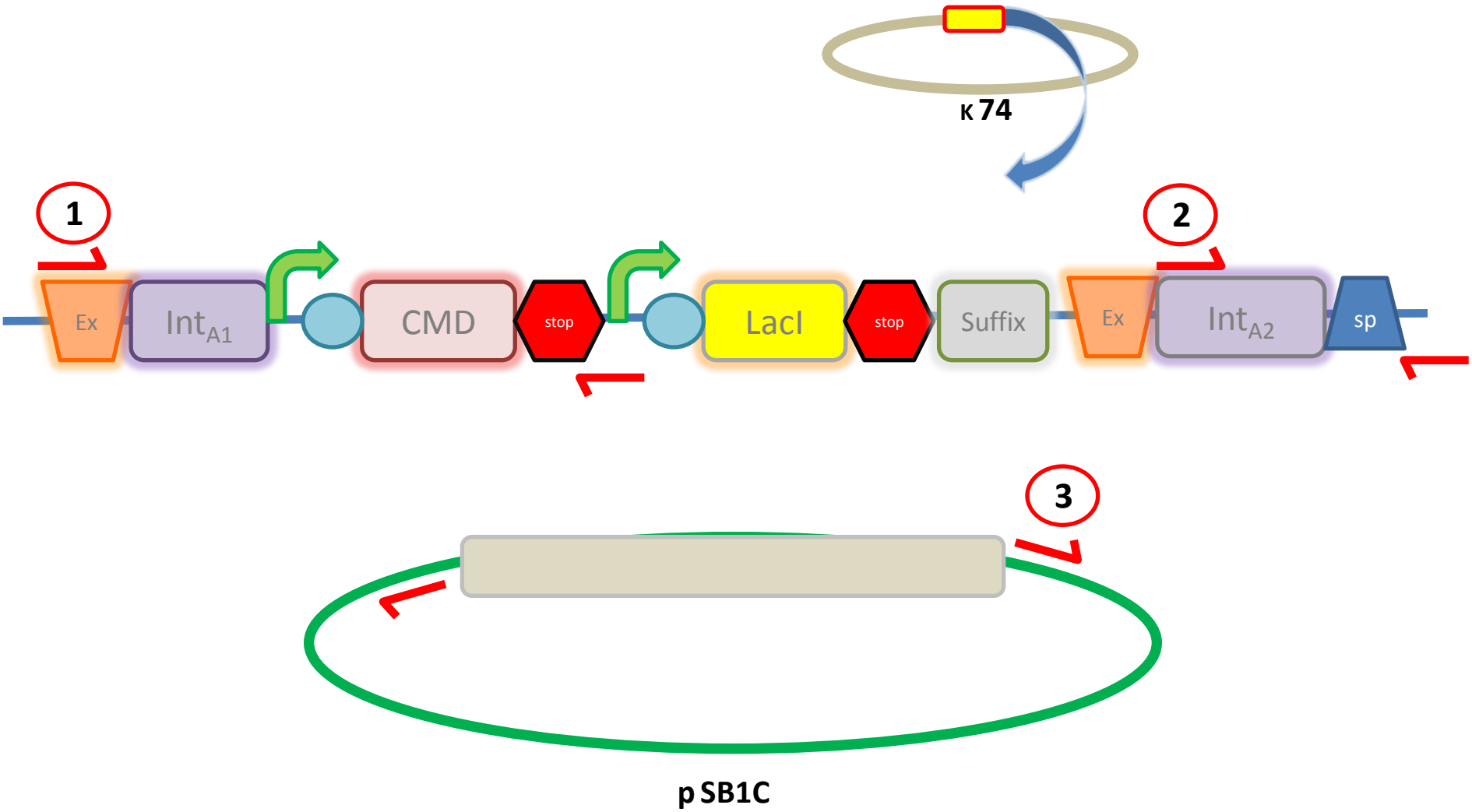
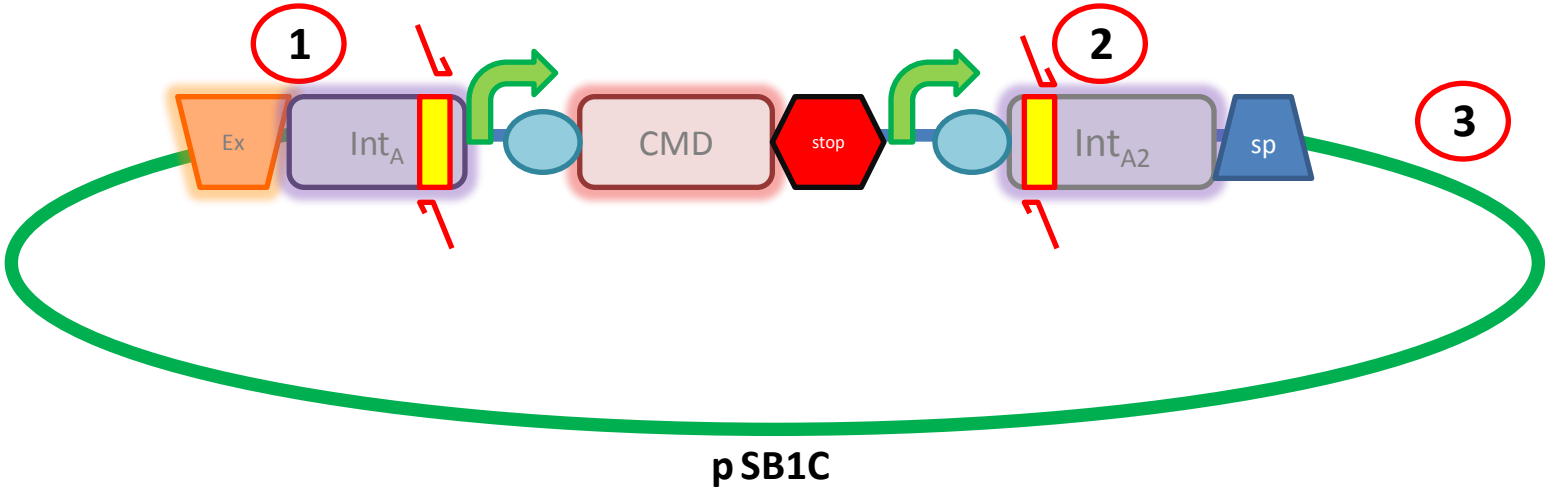
Baccelerator

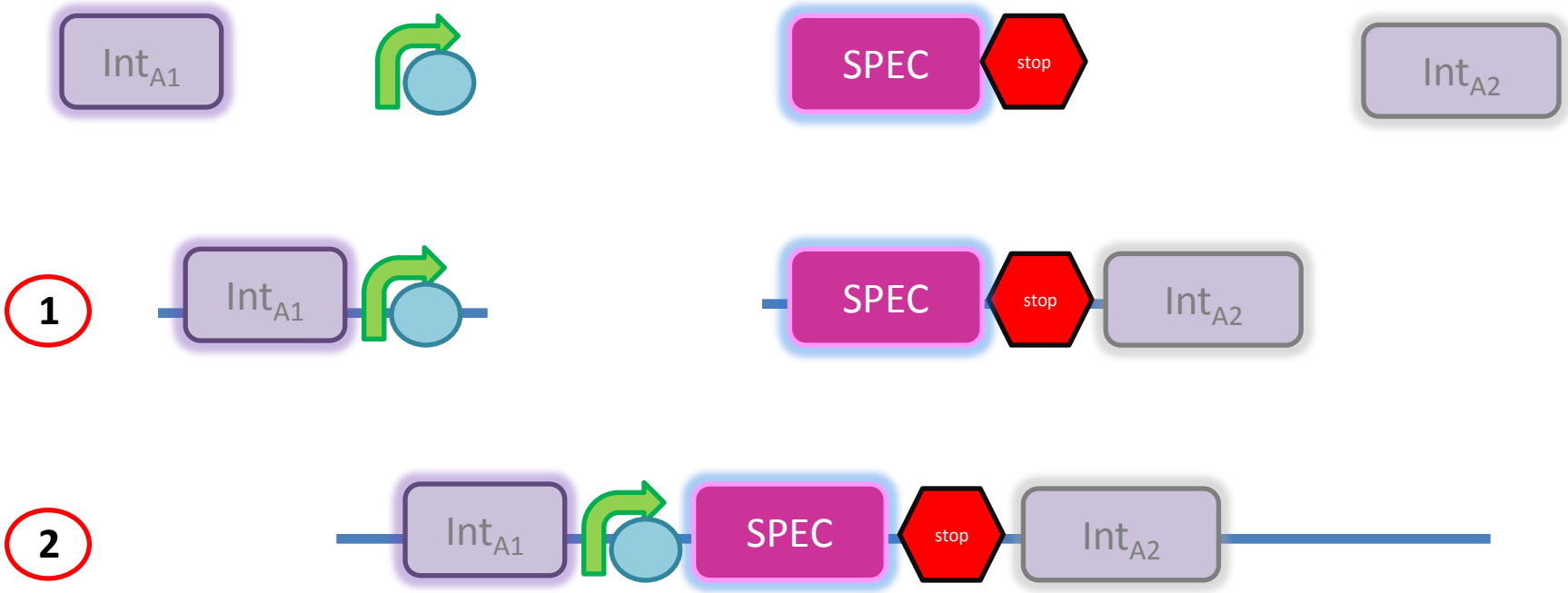
- Overview
- Assembly strategy
- Testing strategy
- Issues
- Modelling

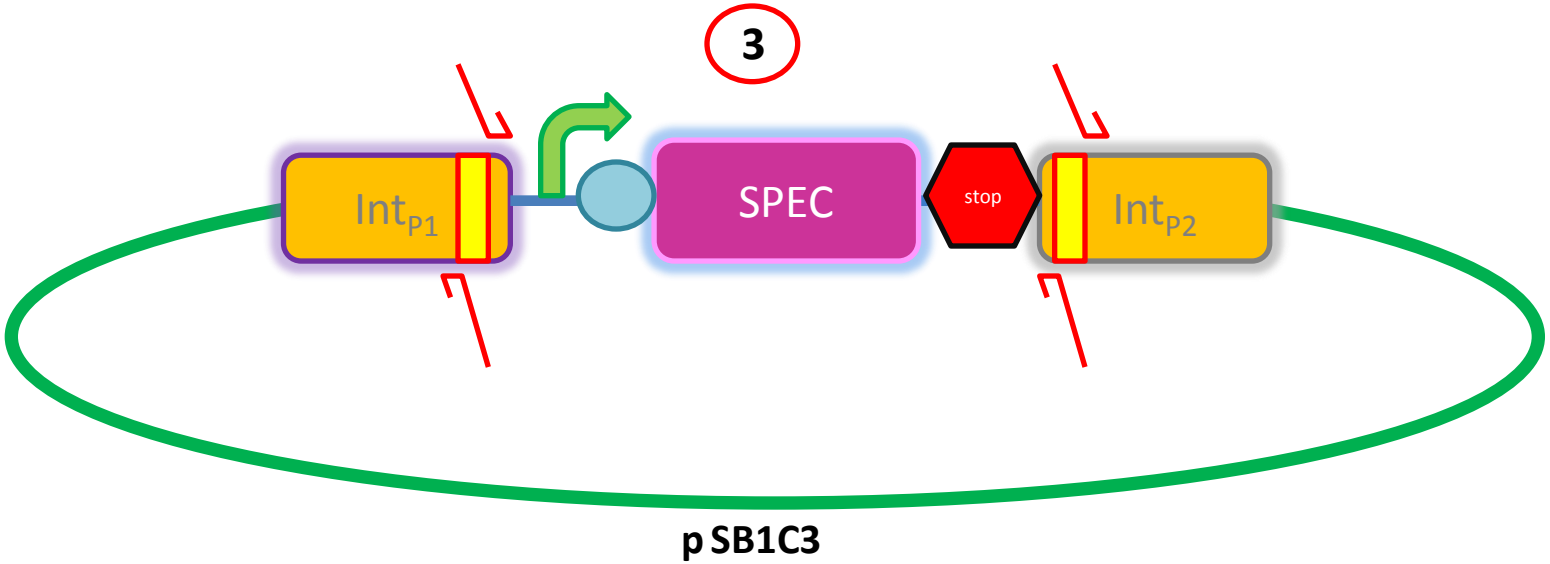
Current Mechanism



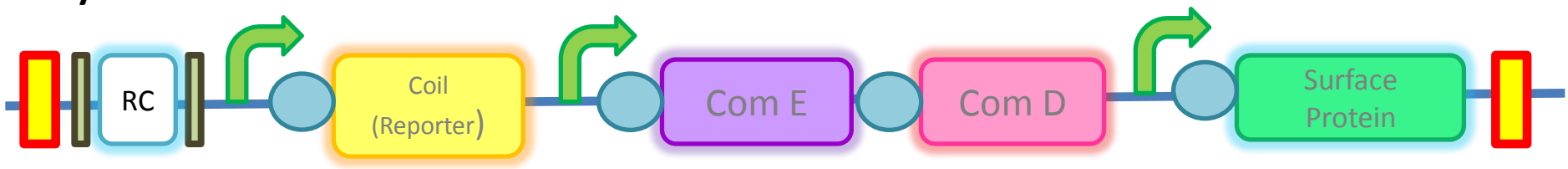

κ 74
1
2
Ex
Int A1
CMD
stop
LacI
stop
Suffix
Ex
Int A2
sp
3
p SB1C


1
2
3
Ex
Int$_A$
CMD
stop
Int$_{A2}$
sp
p SB1C


Int$_{A1}$
SPEC
stop
Int$_{A2}$
1
Int$_{A1}$
SPEC
stop
Int$_{A2}$
2
Int$_{A1}$
SPEC
stop
Int$_{A2}$


3
Int$_{P1}$
SPEC
stop
Int$_{P2}$
p SB1C3

Pyrd


RC
Coil
(Reporter)
Com E
Com D
Surface
Protein

AmyE


RC
LacI
stop
TEV
Lac I
binding
Coil
(Reporter)

Surface Display

Cell Wall Proteins

- CwlC
  - 765bp
  - 2 cell wall binding repeats
- LytC
  - 1488bp
  - 3 cell wall binding repeats

Previous Research

- Everything except the CWBs removed
- Both successfully attached to a lipase
- Retained function on the cell surface
- When both combined, 36% of cell wall protein

- *B. sub* native so can perform PCR
- Linker/peptide can be attached to primer

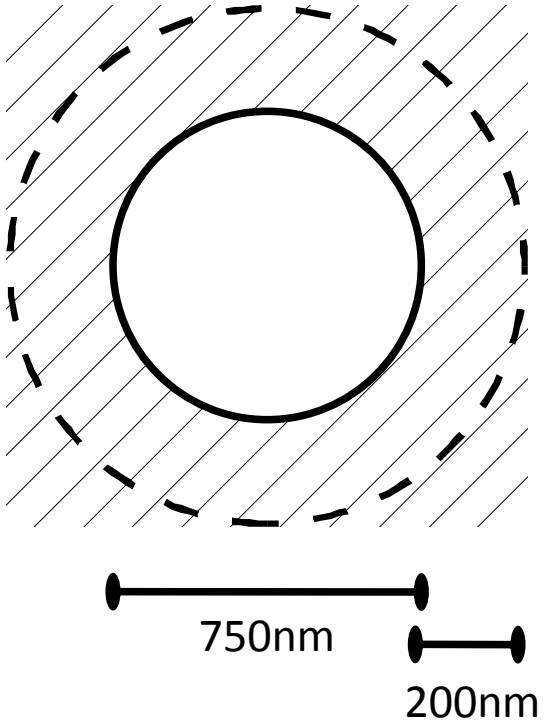
Options

Cell Wall Binding Domain	Protein catalytic domain	Linker	Number of AIP repeats	AIP	His-Tag
CWBD (CwlB)	LytC	Linker	1	AIP	-
CWBD	LytC	Linker	1	AIP	His-Tag
CWBD	LytC	Linker	12	AIP	-
CWBP	LytC	Linker	12	AIP	His-Tag
CWBD	-	Linker	1	AIP	-
CWBD	-	Linker	1	AIP	His-Tag
CWBD	-	Linker	12	AIP	-
CWBD	-	Linker	12	AIP	His-Tag
CWBD (CwlC)	CwlC	Linker	1	AIP	-
CWBD	CwlC	Linker	1	AIP	His-Tag
CWBD	CwlC	Linker	12	AIP	-
CWBP	CwlC	Linker	12	AIP	His-Tag
CWBD	-	Linker	1	AIP	-
CWBD	-	Linker	1	AIP	His-Tag
CWBD	-	Linker	12	AIP	-
CWBD	-	Linker	12	AIP	His-Tag
CWBD single CwlC	-	Linker	1	AIP	-
CWBD	-	Linker	1	AIP	His-Tag
CWBD	-	Linker	12	AIP	-
CWBD	-	Linker	12	AIP	His-Tag

Testing

- Increased salt concentration disrupts CWB
  - Can test for production and rough localisation
- Attach His-tag and linker
  - Can test for precise localisation and cleavage

Modelling

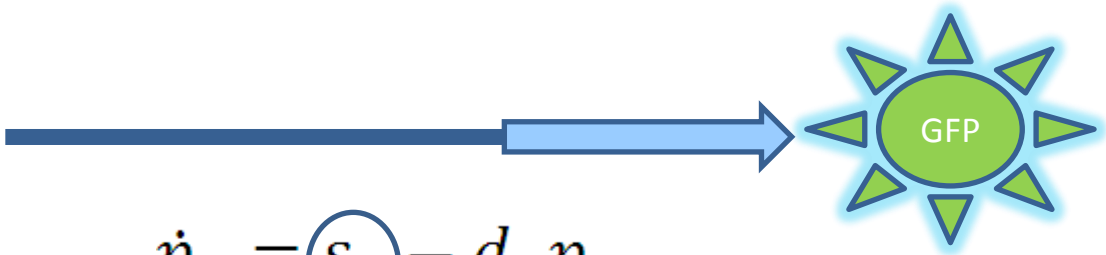


- 10 ng/ml is the required conc. of the peptide to activate ComD
- 2.24 kDa is the mass of the peptide
- If there is total cleavage in a small area, resultant conc. is 1000x required

Additional

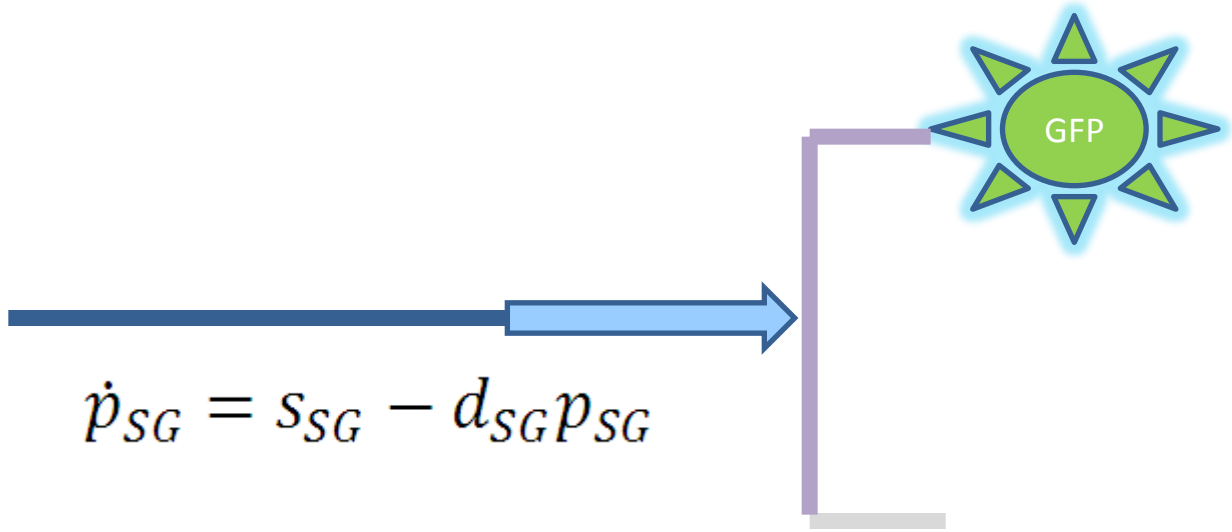
- Two BioBricks
  - Schistosoma protease specific linker/signal, which could be formed into a series of parts responding to different parasites
  - Cell wall binding segment, which when attached would allow you to display anything on the surface of *B. sub*

Modelling

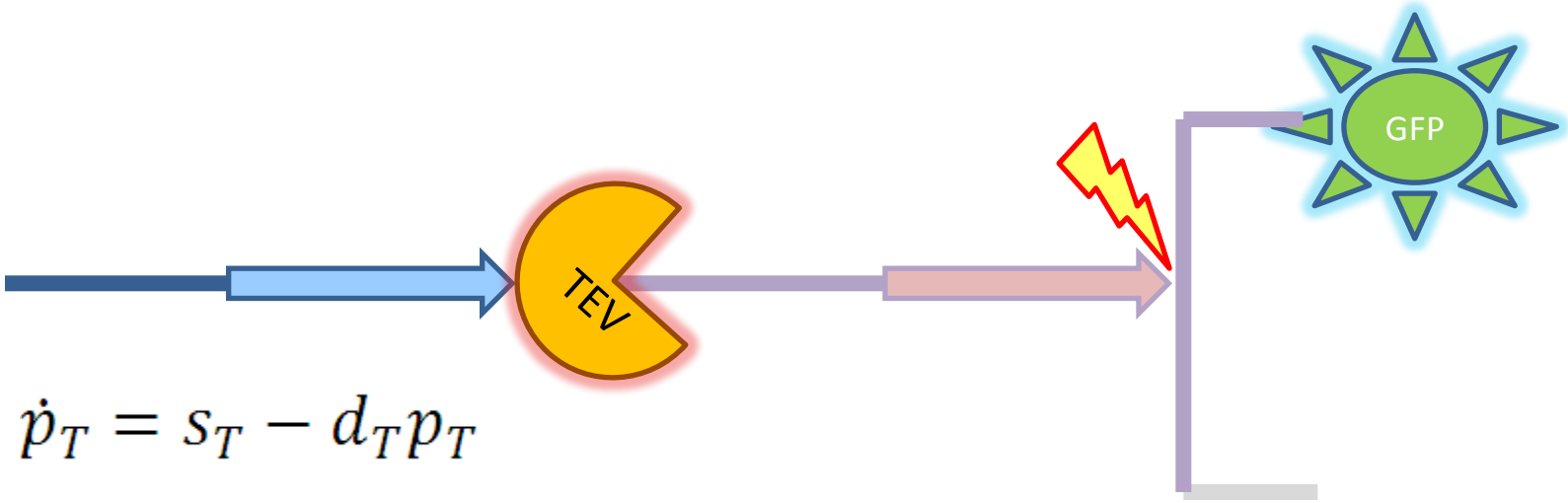


$$\dot{p}_G = s_G - d_G p_G$$

$$\frac{k_T k_{TO}}{d_{TO}}$$

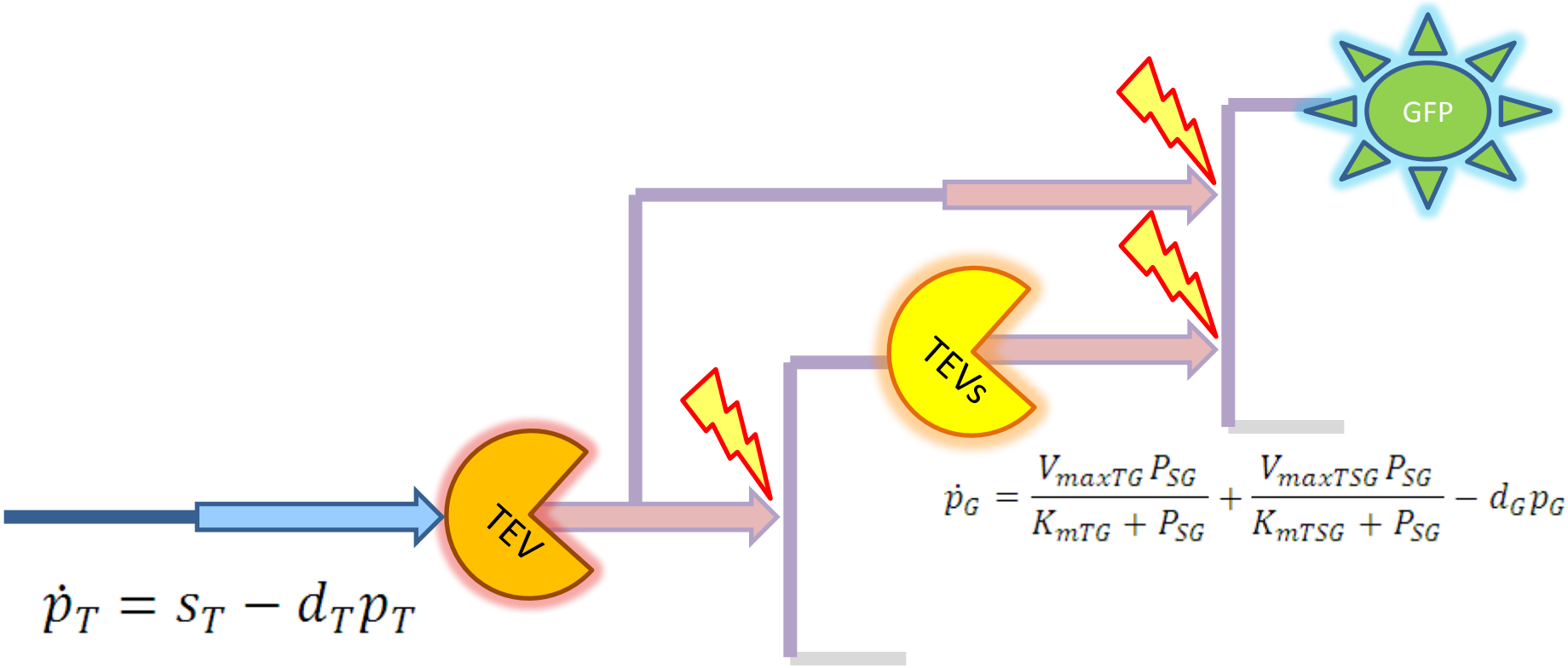


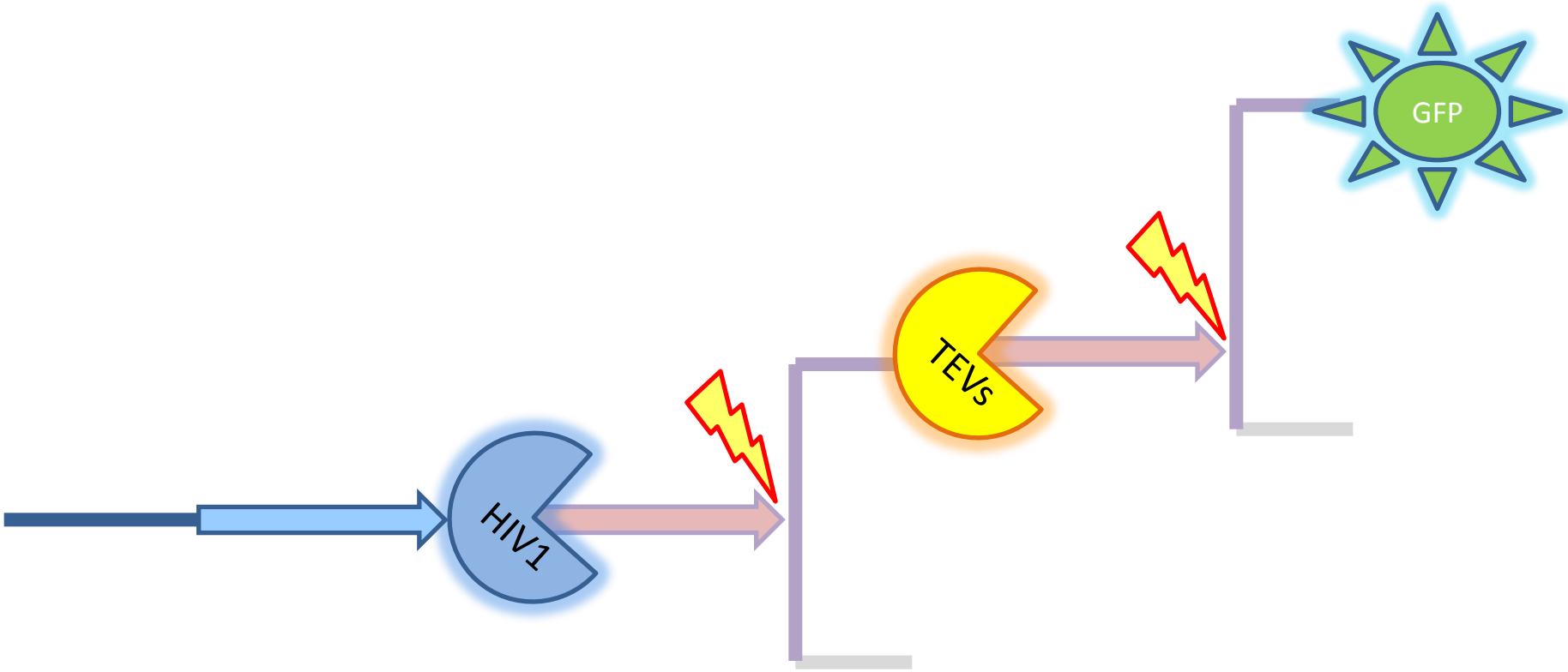
$$\dot{p}_{SG} = s_{SG} - d_{SG}p_{SG}$$

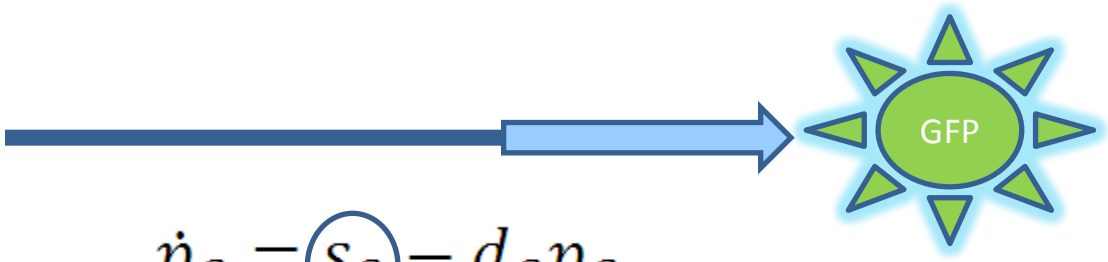


$$\dot{p}_T = s_T - d_T p_T$$

$$\dot{p}_G = \frac{V_{maxT}P_{SG}}{K_{mT} + P_{SG}} - d_G p_G$$

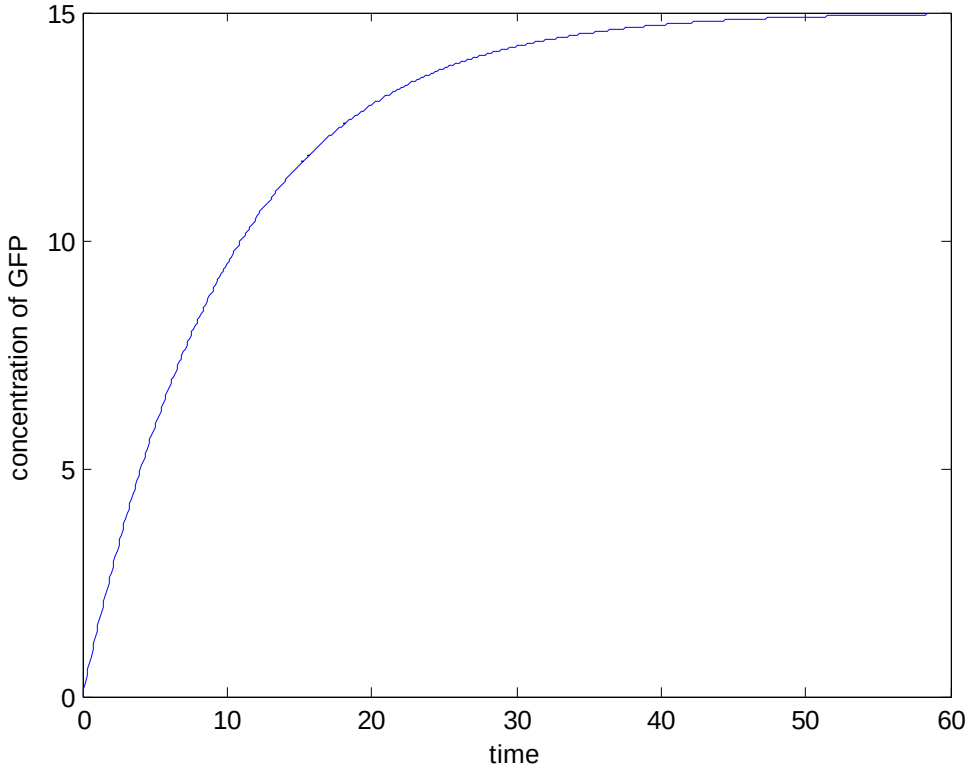

GFP
TEVs
TEV
$\dot{p}_G = \frac{V_{maxTG} P_{SG}}{K_{mTG} + P_{SG}} + \frac{V_{maxTSG} P_{SG}}{K_{mTSG} + P_{SG}} - d_G p_G$
$\dot{p}_T = s_T - d_T p_T$
$\dot{p}_{TS} = \frac{V_{maxT} P_{ST}}{K_{mTG} + P_{ST}} - d_{TS} p_{TS}$

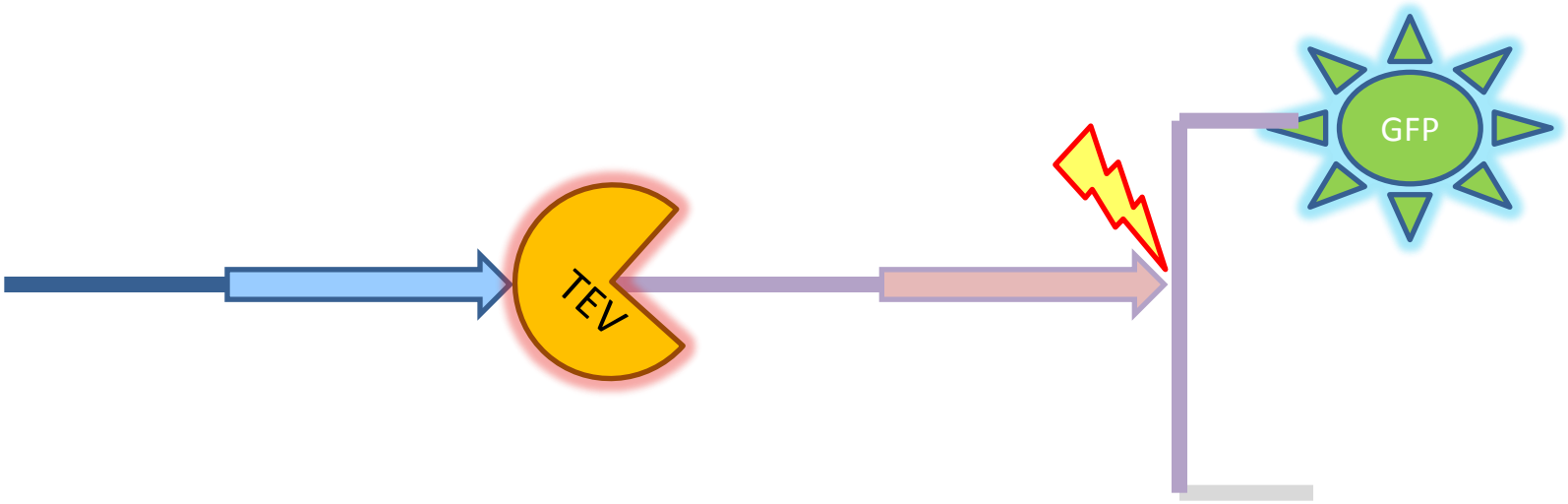

HIV1
TEVs
GFP

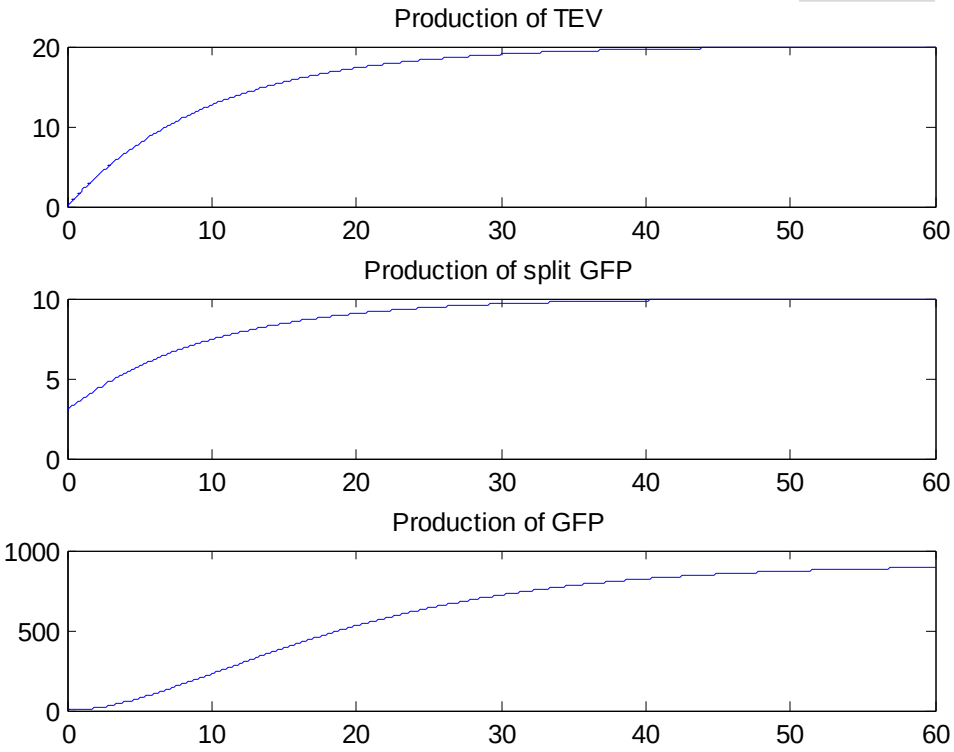


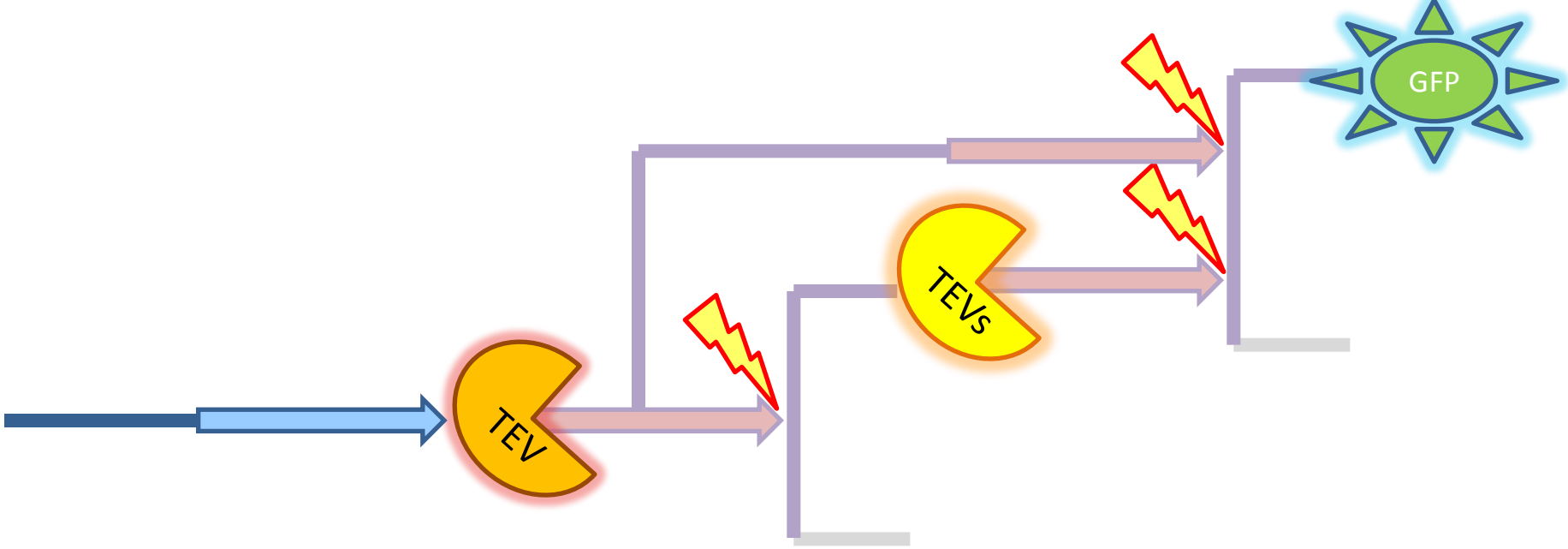
$$\dot{p}_G = s_G - d_G p_G$$

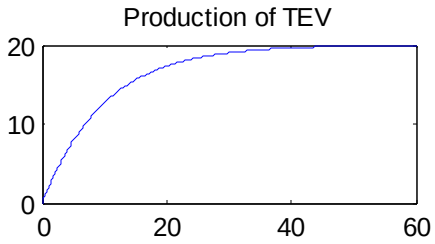
$$\frac{k_T k_{TO}}{d_{TO}}$$

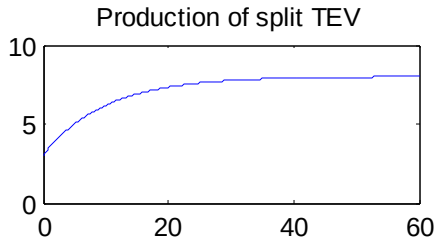


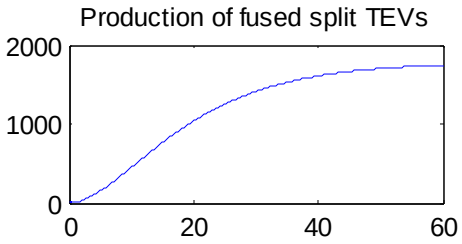

TEV
GFP

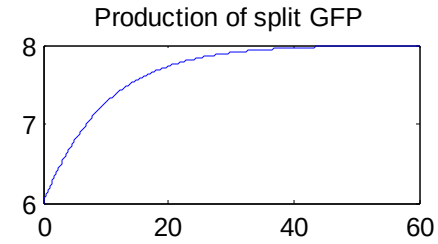

Production of TEV
20
10
0
0
10
20
30
40
50
60
Production of split GFP
10
5
0
0
10
20
30
40
50
60
Production of GFP
1000
500
0
0
10
20
30
40
50
60

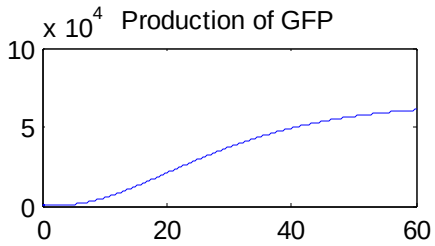

GFP
TEVs
TEV

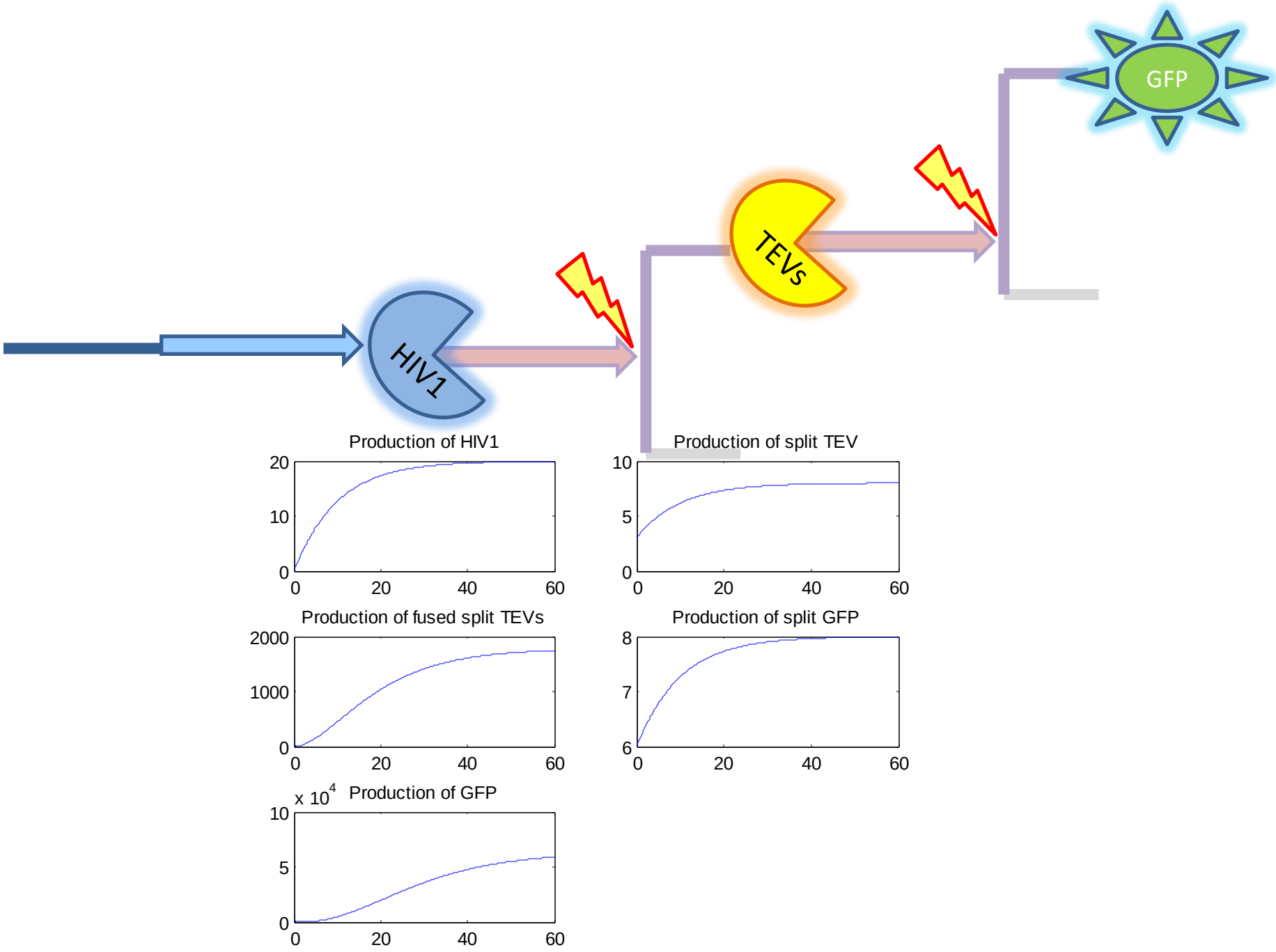

Production of TEV
20
10
0
0
20
40
60


Production of split TEV
10
5
0
0
20
40
60

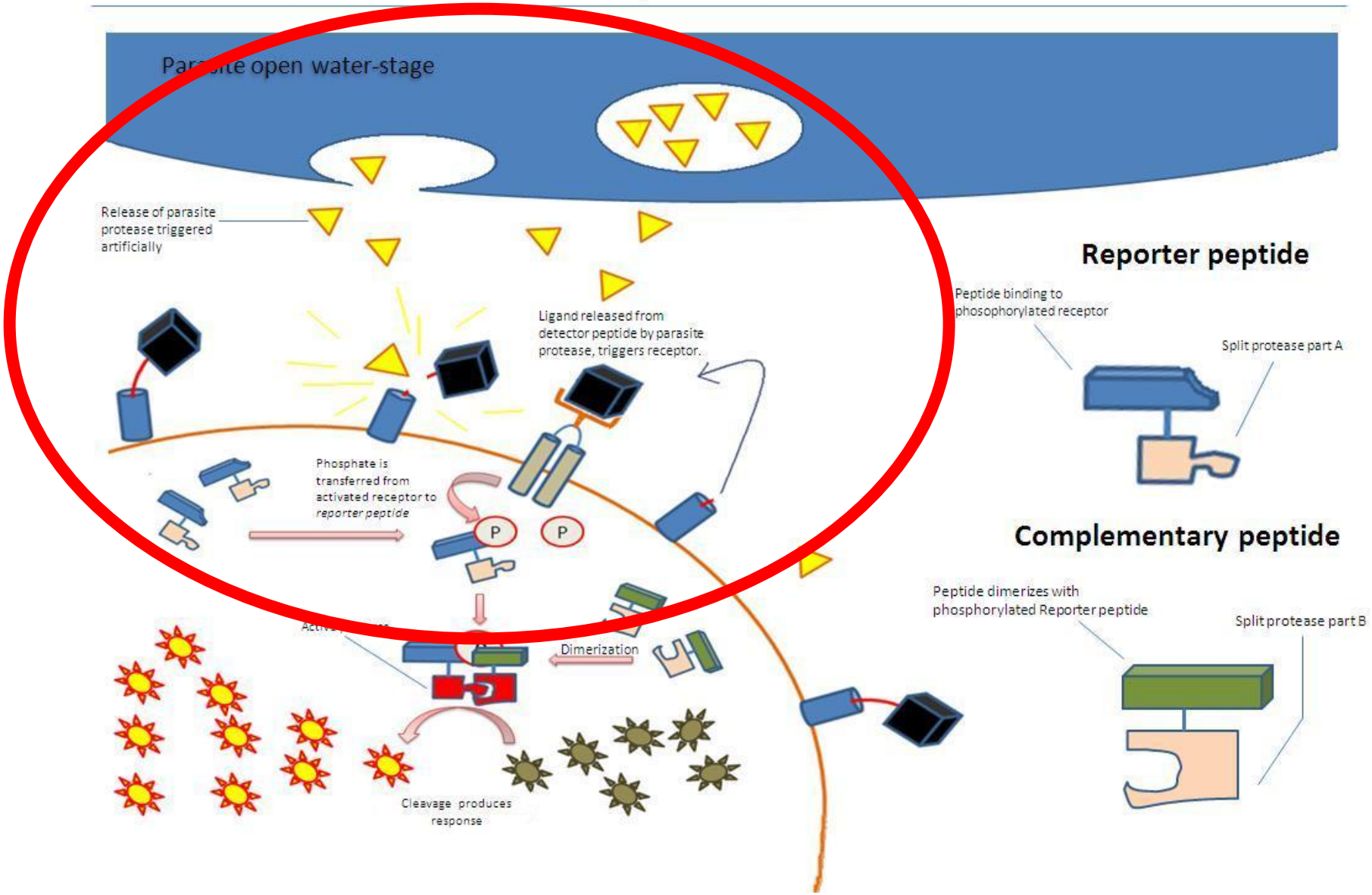

Production of fused split TEVs
2000
1000
0
0
20
40
60


Production of split GFP
8
7
6
0
20
40
60

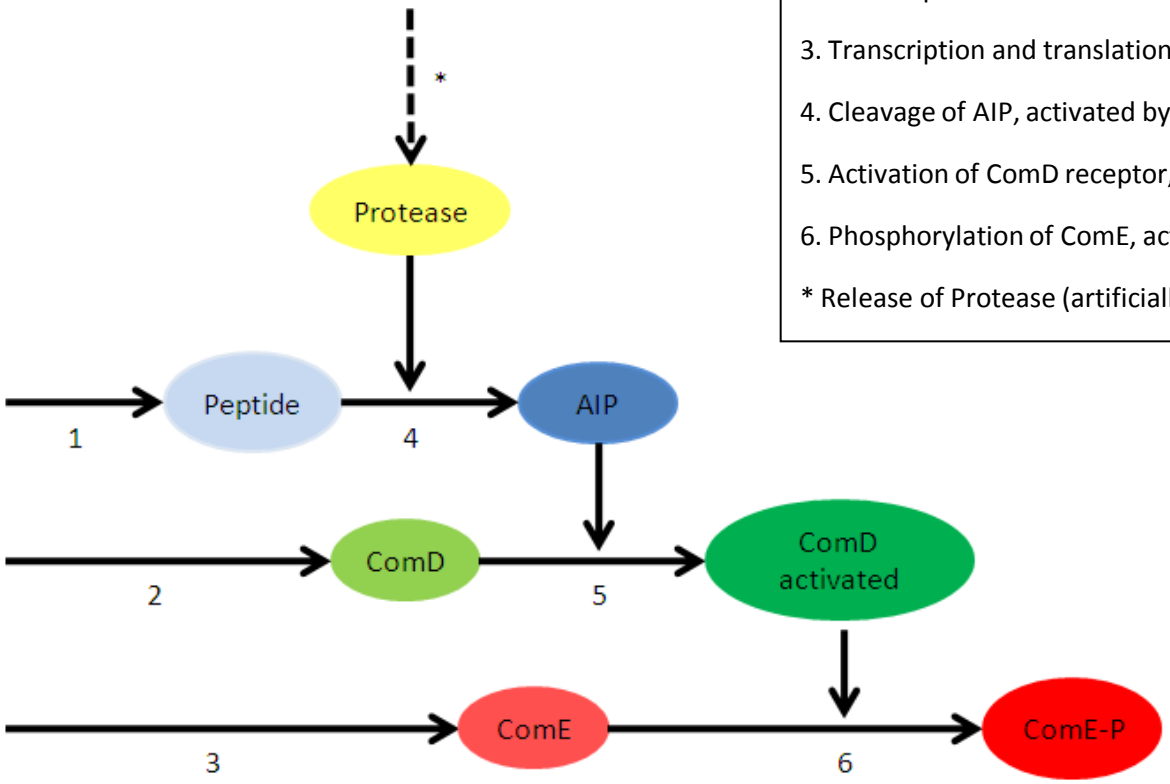

x 10^4
Production of GFP
10
5
0
0
20
40
60


GFP
TEVs
HIV1
Production of HIV1
20
10
0
0
20
40
60
Production of split TEV
10
5
0
0
20
40
60
Production of fused split TEVs
2000
1000
0
0
20
40
60
Production of split GFP
8
7
6
0
20
40
60
x 10^4
Production of GFP
10
5
0
0
20
40
60

Fast Response Module



The System



1. Transcription and translation of Protease detector Peptide
2. Transcription and translation of ComD
3. Transcription and translation of ComE
4. Cleavage of AIP, activated by Protease
5. Activation of ComD receptor, reaction activated by AIP
6. Phosphorylation of ComE, activated by ComD receptor

* Release of Protease (artificially triggered)

Equations

- 1. Transcription and translation of Protease detector Peptide

$$\dot{p}_{peptide} = s_{1,peptide} - d_{1,peptide} p_{peptide}$$

- 2. Transcription and translation of ComD

$$\dot{p}_{ComD} = s_{2,ComD} - d_{2,ComD} p_{ComD}$$

- 3. Transcription and translation of ComE

$$\dot{p}_{ComE} = s_{2,ComE} - d_{2,ComE} p_{ComE}$$

Equations

- 4. Cleavage of AIP, activated by Protease

$$\dot{p}_{AIP} = k_{1,AIP}\frac{[Protease]}{K_{AIP}+[Protease]} - d_{1,AIP}p_{AIP}$$

- 5. Activation of ComD receptor, reaction activated by AIP

$$\dot{p}_{ComD-A} = k_{1,ComD-A}\frac{[AIP][ComD]}{K_{comD-A}+[ComD]} - d_{1,ComD-A}p_{ComD-A}$$

- 6. Phosphorylation of ComE, activated by ComD receptor

$$\dot{p}_{ComE-P} = k_{1,ComE-P}\frac{[ComD-A]}{K_{ComE-P}+[ComD-A]} - d_{1,ComE-P}p_{ComE-P}$$