GTPassay

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GTP enzymatic assay

Based on docking GTP affinity a preliminary enzymatic assay was performed.
Sugerencia de lia... buscar niveles intracelulares de GTP (Bionumbers DB)
Intracellular GTP concentration in glucose-fed, exponentially growing E. coli Bacteria Escherichia coli 4.9 mM 104697
Aumentar al orden de mM
Bennett BD, Kimball EH...

Poner como control Gtp en buffer sin enzima
EDTA, 0.1mM DTT probar quitar estos componentes

Preliminar dGTP assay conditions:

$1\mu M$ dGTP (Invitrogen)
TrpF Buffer (50mM Tris-HCL buffer pH8.0, 5% glycerol, 0.5 mM EDTA, 0.1mM DTT)
50nM PriA vs 50 nM PriA D11A

Method: Fluorimetry (black box) Excitation wavelength: 255 nm Emission wavelength: 334 nm

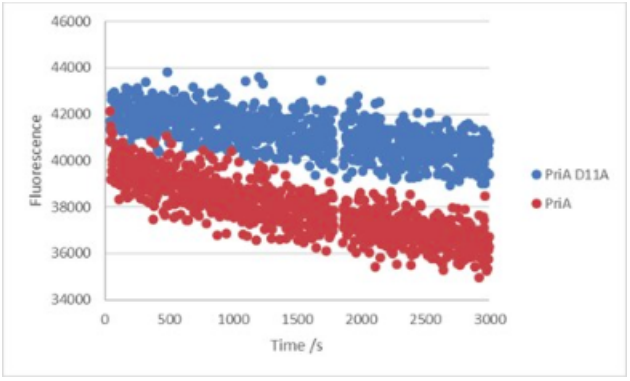


Figure 1: Scoe and non functional Scoe PriA acting on dGTP

Preliminar assays to test enzyme activity (Ernesto)

To test preliminary results we perform the following activity test assays.

We will use $160\mu l$ as final reaction volume.
$<150\mu l$ Buffer
$5\mu l$ substrate at $50\mu M$ (Stock at $1.6mM$)
$5\mu l$ enzyme at $2.5\mu M$ (Stock at $80\mu M$)

Now I will calculate substrate stock concentration needed to obtain a final concentration of $50\mu M$ on a final volume of $160\mu l$ adding $5\mu l$ of substrate.
$C_1 = \frac{50\mu M \times 160\mu l}{5\mu l} = 1600\mu M = 1.6mM$
So we need to prepare an stock of $1.6mM$ of substrate concentration. Commercial stock is at $100mM$, so on a first dilution 1:10 named C_0, ($90H_20$ μl and 10 mul GTP) we obtain our lab stock at $10mM$.

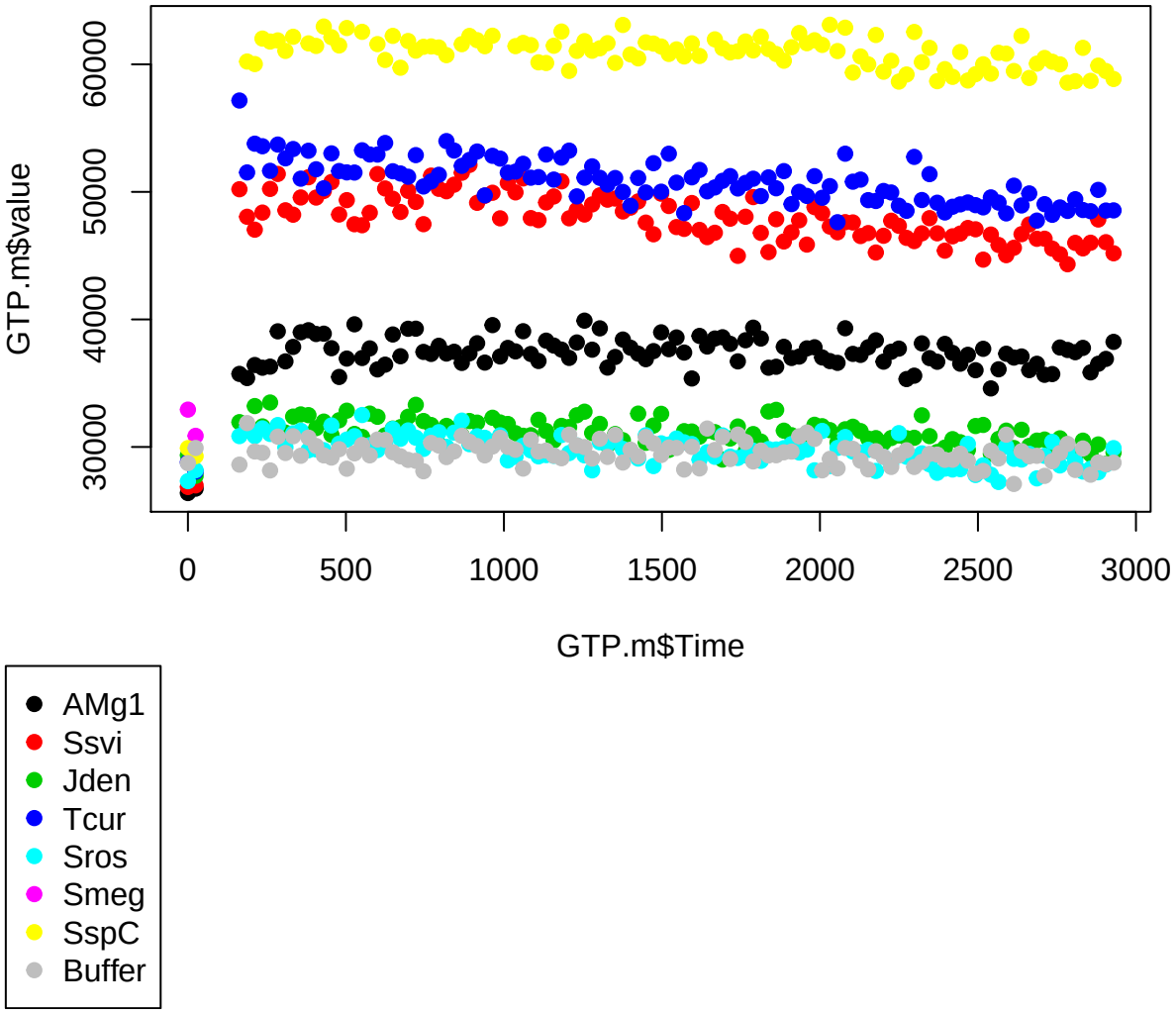
To obtain $1.6mM$ again the formula $C_1V_1 = C_2V_2$ was used. $100\mu l$ were prepared thinking on 10 reactions of $5\mu l$ each one.
$V_1 = \frac{1.6mM \times 100\mu l}{10mM} = 16\mu l$
so $16\mu l$ substrate was diluted on 84 μl of H_2O.

Next I will explain calculus of enzyme stock concentration needed in order to obtain a final concentration of $2.5\mu l$ on a final volume of $160\mu l$ adding $5\mu l$ of enzyme.
$C_1 = \frac{2.5\mu M \times 160\mu l}{5\mu l} = 80\mu M$
So we need to prepare an stock of $80\mu M$ of enzyme concentration. An example was calculated using again the formula $C_1V_1 = C_2V_2$.

Example
We will prepare $20\mu l$ of stock because we need $15\mu l$ for three reactions. A little excess must ALWAYS be prepared for pipeting needs.
Streptomyces sp Mg1 enzyme concentration obtained was $461\mu M$, so to obtain $20\mu l$ we must add: $V_1 = \frac{80 \times 20}{461}\mu l = 3.4\mu l$
$3.4\mu l$ of enzyme to $16.6\mu l$ TrpF reaction buffer.
Generalizing:
$C_1 = x\mu M$, $V_1 = y$, $C_2 = 80\mu M$, $V_2 = 20\mu l$

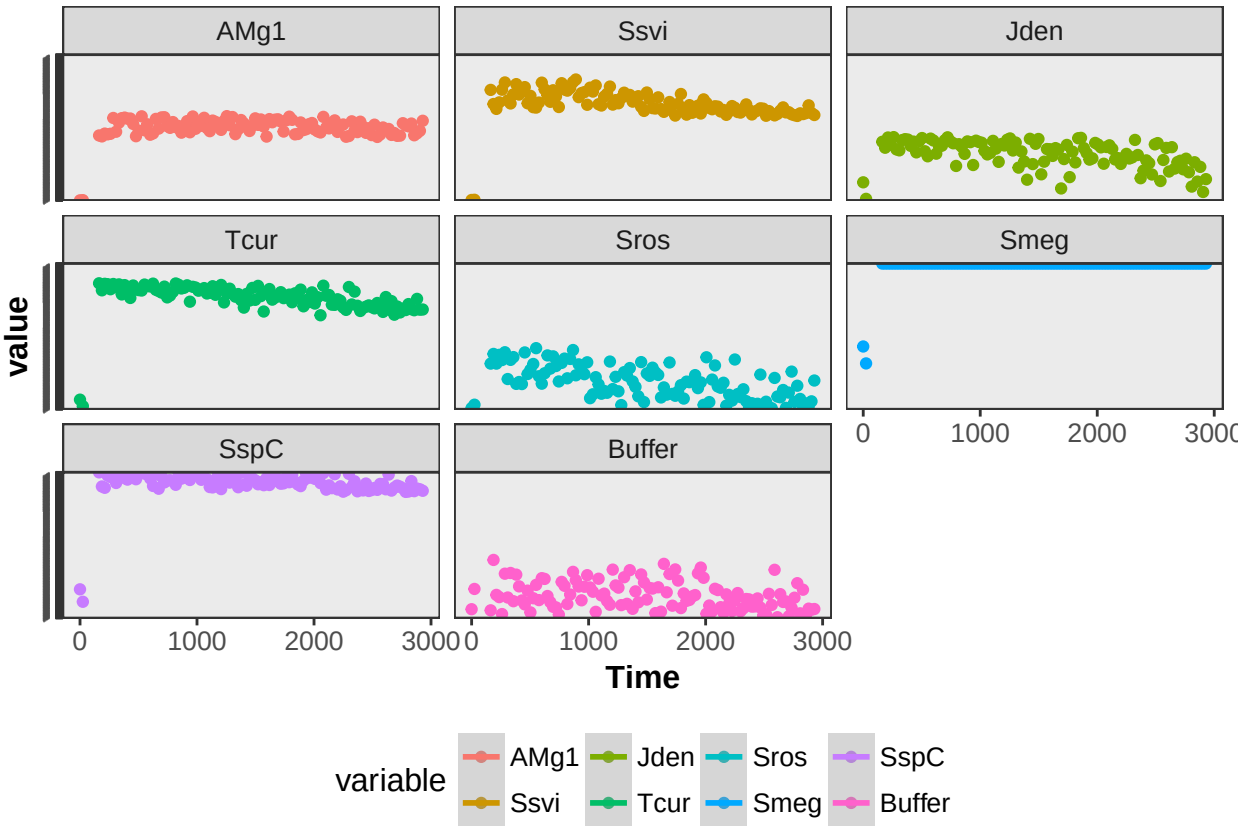
Enzyme	from Organism	Concentration	Enzyme μl	Buffer μl
PriB	SMg1	$461\mu M$	3.4	16.6
PriB	Ssvi	$100\mu M$	16	4
PriB	JDen	$215\mu M$	7.4	12.6
PriA	Tcur	$335\mu M$	4.7	15.3
PriA	Sros	$450\mu M$	3.5	16.5
PriA	Smeg	$370\mu M$	4.3	15.7
PriB	Sspc	$1346\mu M$	1.1	18.9

Results



the two enzymes with major activity were:
Thermomonospora curvata thermophilic Actinobacteria from Thermomonosporaceae genus, it can be found in compost and participate in the active degradation of cellulose [@chertkov_complete_2011]. Jonesia trpF shows no activity

Jonesia denitrificans is classified as a pathogenic organism for animals, reported genome was originally isolated from cooked ox blood [@pukall_complete_2009].


AMg1
Ssvi
Jden
Tcur
Sros
Smeg
SspC
Buffer
value
0
1000
2000
3000
Time
variable
AMg1
Ssvi
Jden
Tcur
Sros
Smeg
SspC
Buffer

Karina second attempt to obtain enzyme kinetics.

Now we will try substrate at different concentrations.

GTP is commercially available at stocks of $100mM$ We want 8 different concentrations between 0 and 50mM on a final volume of $160\mu l$. We chose 0,0.5,1,1.5,5,10,20,50 as the eight points.

Dilutions were made to reach this concentrations. $C_{stock} = 100mM$ dil0 Stock original $C_0 = 10mM = 10000\mu M$ dil1 1:10 primera dilucsio la llame dil0 Stock de nelly $C_1 = 1000\mu M$ dil2 1:10 $C_2 = 100\mu M$ dil3 1:10 $C_3 = 10\mu M$ dil4 1:10 $C_4 = 1\mu M$ dil5 1:10

$[S] = [dGTP] = 10000\mu M$
$[E] = 50nM$

Concentration (μM)	Buffer (μl)	Enzyme (μl)	dGTP (μl)
0	156.1	3.9	0 dil4
0.5	148.1	3.9	8 dil4
1	140.1	3.9	16 dil4
1.5	132.1	3.9	24 dil4
5	76.1	3.9	80 dil4
10	140.1	3.9	16 dil3
20	124.1	3.9	32 dil3
50	76.1	3.9	80 dil3

Calculate your own GTP data

```
#PriA_stock=48.5 ##uM D11A
PriA_stock=40 ##uM Scoe
type="Scoe"
hole_vol=170 ##ul
PriA_final=1 ##uM final concentration on hole
#PriA_final=.050 ##uM = 50 nM final concentration on hole
PriA_vol=hole_vol*PriA_final/PriA_stock
PlateColum=2
##
GTP_stock=100000000 ##100,000,000nM-> 100,000uM-> 100mM
```

```
GTPdata <- read.table(header=TRUE, text='
  GTP_Dilution
  3
  3
  3
  4
  4
  4
  4
  5
')
```

```
PriA<-rep(PriA_vol,8)  ##

##Use following line when mM concentrations are needed
#GTP_mM<-rev(c(0,0,.5,1,2.5,5,10,15))  # en 150  eliminamos la enzima columna
#GTPdata["GTP_uM"]<-GTP_mM*1000

##Use following line when uM concentrations are needed, please comment when no needed
GTP_uM<-rev(c(0, .5,1,2,5, 10,25,50))
GTPdata["GTP_uM"]<-GTP_uM

#GTP_uM<-rev(c(0,0,.0005,.0001,2.5,5,10,15))  # en 150  eliminamos la enzima columna

#GTPdata["GTP_uM"]<-GTP_uM

#Calculating volumes accordi
GTPdata["Vol"] <-1000*hole_vol*GTPdata["GTP_uM"]/(GTP_stock*10**(-1*GTPdata["GTP_Dilution"])) # That cr

GTPdata["PriA_ul"]<-round(PriA_vol,digits=2)

# As an example, the new column receives
GTPdata$Buffer <- round(160-GTPdata$Vol-GTPdata$PriA_ul,digits=2)

kable(GTPdata)
```

GTP_Dilution	GTP_uM	Vol	PriA_ul	Buffer
3	50.0	85.0	4.25	70.75
3	25.0	42.5	4.25	113.25
3	10.0	17.0	4.25	138.75
4	5.0	85.0	4.25	70.75
4	2.0	34.0	4.25	121.75
4	1.0	17.0	4.25	138.75
4	0.5	8.5	4.25	147.25
5	0.0	0.0	4.25	155.75

GTP Stock 100 mM PriA final 1 μM