A Gemcitabine Intracellular Model, Parameter Estimation and ML workflow
Sample Work

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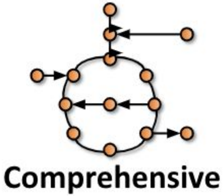
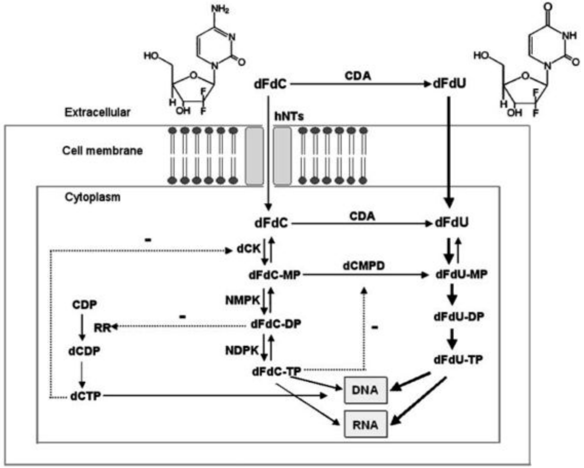
GEMCITABINE PATHWAY: MODELING RATIONALE

- How Well understood is Gemcitabine pathway?
- Is it possible to build a generalized model that can explain different cell line's behavior?



MODELING RATIONALE

- Gemcitabine strong In vitro and in vivo antitumor activity.
- Inhibition of DNA Synthesis, specifically it inhibits ribonucleotide reductase and it inhenders DNA synthesis and thus cell dies.
- Typically is given weekly for 3 weeks



Enzymes:

dCK(Deoxycytidine kinase**)**
RR(Ribonucleotide Reductase)
dCMPD(Deoxycytidylate Deaminase)
5'-NT(5'-Nucleotidase)
CDA(Cytidine Deaminase)
NDPK(ucleoside Diphosphate Kinase)
NMPK(Nucleoside Monophosphate Kinase)

Metabolites:

- **dCTP(**Deoxycytidine triphosphate)
- **CDP**(Cytidine Diphosphate)
- **dCTP(**Deoxycytidine diphosphate)

DATA 1 *Heinemann V, 1(1992)*

CCRF-CEM Human T- Lymphoblast cell line dFdCTP and its metabolites.

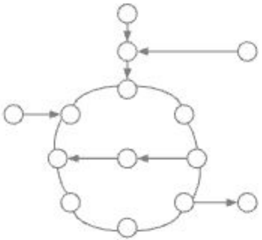
- ❑ **dFdC** was incubated in vitro for 2 hrs. in RPMI at (uM) = [0.1 0.3   1   10].
- ❑ Cells washed in drug free medium after 2hrs.
- ❑ Intracellular and Extracellular Concentrations are measured after 2hrs.

Physical Properties of the experiment:

Mean Cell Volume = 9.43 x 10 ^-11 L/cell

Number of Cells Incubated = 2 x 10 ^6 Cells

Volume medium = Unknown(Extracellular)

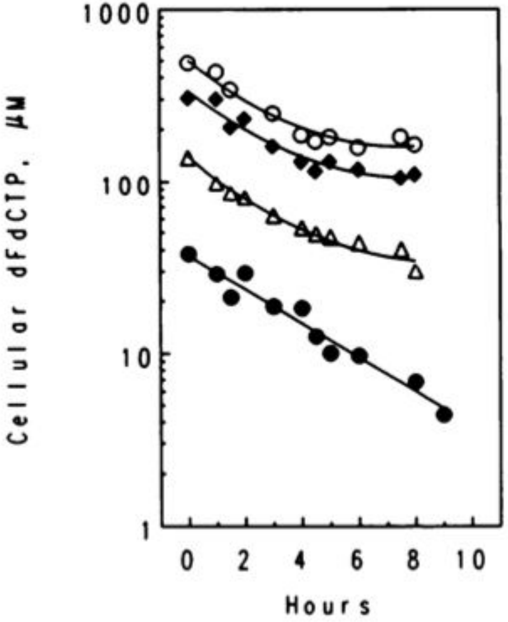


Sparse data

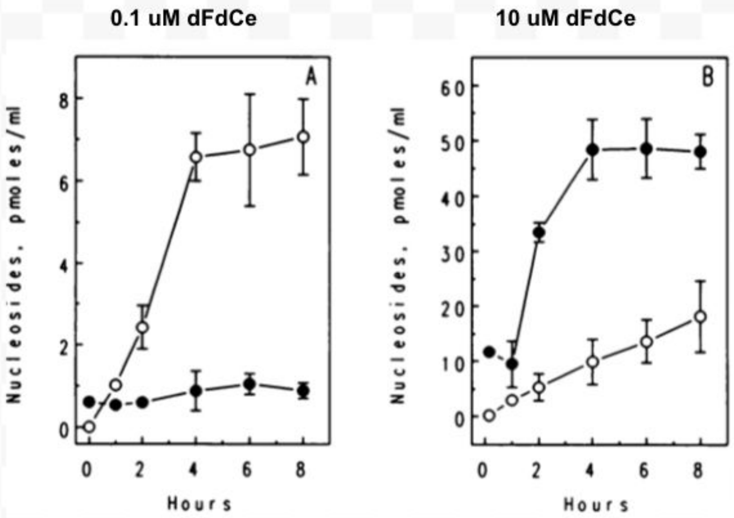
DATA 1 continued

Heinemann V, 1(1992)

Intracellular uM dFdCTP after 2 hrs



Extracellular dFdU(White)and dFdC(Black) after 2 hrs



dFdCe incubation

[0.1, 0.3, 1.0 or 10 uM] after 2hrs.

DATA 2: VELTKMAN Study (Veltkamp 2008)

HepG2, A549, and MDCK cells were incubated with dFdC or dFdU

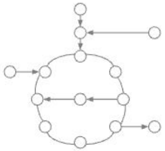
HepG2 cells :

- ❑ 5 nmol/L dFdC + THU,
- ❑ dFdC 0.5 uM + THU
- ❑ dFdU 500 uM and
- ❑ 0.5 uM dFdU for 24 hr.

Physical Properties of Experiment:

Tetrahydrouridine (THU) is a CDA competitive inhibitor Ki ~ 110 nM
HepG2 (12 x 10^6 cells) 1 mg protein activity -> uM = pmol/mg *4.90
HepG2 Volume~ 17 fL
Parameter "a" (Vmedium/Vcells) in the in vitro study experiment -> **a =49000 (Vm/Vcells)**

Note: "a" for Heinemann study is not known because Vmedium is not known

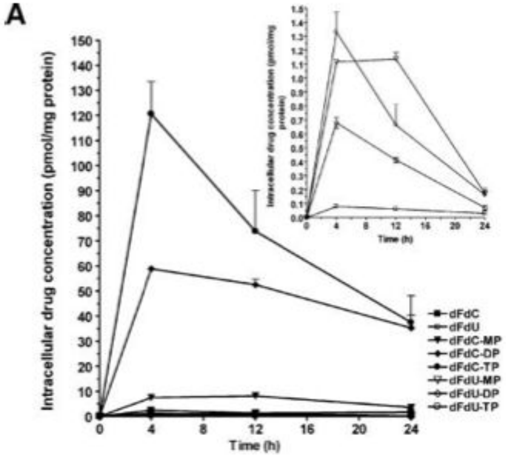


Sparse data

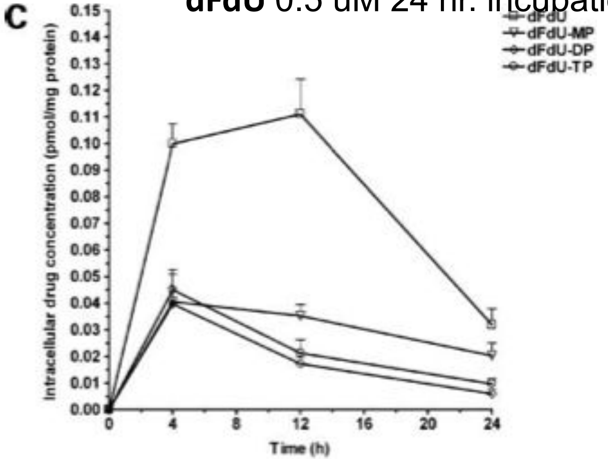
DATA 2: VELTKMAN STUDY Veltkamp 2008)

Metabolite Concentrations (pmol/mg protein). Decoupling the system.

dFdC 0.5 uM 24 hr incubation + THU



dFdU 0.5 uM 24 hr. incubation.



DATA 2: VELTKMAN STUDY

	dFdC	dFdU	dFdC-MP	dFdC-DP	dFdC-TP	dFdU-MP	dFdU-DP	dFdU-TP
HepG2								
5 nmol/L dFdC + THU								
C_{max} (pmol/mg protein)	0.2 ± 0.01	0.03 ± 0.01	0.5 ± 0.05	1.4 ± 0.2	2.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.04	0.3 ± 0.03
AUC (h pmol/mg protein)	6.3 ± 0.5	1.0 ± 0.1	14 ± 0.7	44 ± 5.4	45 ± 5.4	6.7 ± 1.4	4.0 ± 0.7	5.0 ± 0.4
0.5 μmol/L dFdC + THU								
C_{max} (pmol/mg protein)	2.5 ± 0.4	0.1 ± 0.01	8.2 ± 0.3	59 ± 1.0	121 ± 13	1.1 ± 0.04	0.7 ± 0.04	1.3 ± 0.1
AUC (h pmol/mg protein)	38 ± 0.2	1.2 ± 0.2	148 ± 2.3	1,090 ± 64	1,687 ± 253	19 ± 0.7	8.6 ± 0.4	16 ± 2.2
$t_{1/2}$ (h)	n.d.	13 ± 1.9	10 ± 2.1	33 ± 18	12 ± 0.7	6.0 ± 0.5	6.0 ± 0.6	7.0 ± 0.9
DNA (pmol/μmol)	n.a.	n.a.	n.a.	n.a.	1,600 ± 300	n.a.	n.a.	20 ± 1.0
RNA (pmol/μmol)	n.a.	n.a.	n.a.	n.a.	400 ± 30	n.a.	n.a.	10 ± 2.0
0.5 μmol/L dFdU								
C_{max} (pmol/mg protein)	n.d.	0.1 ± 0.01	n.d.	n.d.	n.d.	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
AUC_{0-24} (h pmol/mg protein)	n.d.	1.9 ± 0.2	n.d.	n.d.	n.d.	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
$t_{1/2}$ (h)	n.d.	10 ± 0.9	n.d.	n.d.	n.d.	21 ± 7.0	8.0 ± 0.8	9.0 ± 2.0
DNA (pmol/μmol)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	10 ± 2.0
RNA (pmol/μmol)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	6.0 ± 1.0
500 μmol/L dFdU								
C_{max} (pmol/mg protein)	n.d.	74 ± 9.0	n.d.	n.d.	n.d.	45 ± 3.0	21 ± 2.6	15 ± 3.4
AUC_{0-24} (h pmol/mg protein)	n.d.	2,090 ± 167	n.d.	n.d.	n.d.	1,098 ± 58	557 ± 45	309 ± 63
A549								
0.5 μmol/L dFdC + THU								
C_{max} (pmol/mg protein)	0.8 ± 0.1	0.3 ± 0.1	4.6 ± 1.0	26 ± 4.6	64 ± 13	0.1 ± 0.03	0.2 ± 0.06	0.3 ± 0.03

CELL ENTRY

1 Cell Entry

$$uM = \frac{pmol}{mg} * \frac{(CellsIncubated)\,(1mg)}{12 * 10^6} * \left(\frac{1umol}{10^6 pmol}\right) * \left(\frac{1cell}{17 * 10^{-15}}\right)$$

$$uM \approx \frac{pmol}{mg} * 4.90$$

$$a = \frac{Vmedium}{VCell} \approx 49000$$

$$\frac{dFdC_e}{dt} = -\frac{1}{a} * \frac{VmaxdFdC_e \frac{dFdC_e}{KmdFdC_e}}{\left(1 + \frac{dFdCe}{KmdFdC_e} + \frac{dFdC}{KmdFdC} + \frac{dFdUe}{KmdFdU_e} + \frac{dFdU}{KmdFdU}\right)}$$

$$\frac{dFdC}{dt} = \frac{a}{1} * \frac{VmaxdFdC * \frac{dFdC}{KmdFdC}}{\left(1 + \frac{dFdCe}{KmdFdC_e} + \frac{dFdC}{KmdFdC} + \frac{dFdUe}{KmdFdU_e} + \frac{dFdU}{KmdFdU}\right)} + (Pcflux)$$

$$\frac{dFdU_e}{dt} = -\frac{1}{a} * \frac{VmaxdFdU_e * \frac{dFdU_e}{KmdFdU_e}}{\left(1 + \frac{dFdCe}{KmdFdC_e} + \frac{dFdC}{KmdFdC} + \frac{dFdUe}{KmdFdU_e} + \frac{dFdU}{KmdFdU}\right)}$$

$$\frac{dFdU}{dt} = \frac{a}{1} * \frac{VmaxdFdU * \frac{dFdU}{KmdFdU}}{\left(1 + \frac{dFdCe}{KmdFdC_e} + \frac{dFdC}{KmdFdC} + \frac{dFdUe}{KmdFdU_e} + \frac{dFdU}{KmdFdU}\right)} + (Puflux)$$

Vmaxcda	360 uM/hr
Kmcda	95.7 uM
VdCK	8.94 uM/hr
KmdCK	4.2 uM
Km dFdCMP to dFdCDP	581 uM
Ki dFdCTP on dCMPD	460 uM
Ki dCTP on dCK	2 to 5 uM
Ki dFdCDP on RR	0.3 to 4 uM

dFdCTP elimination

2 DNA Elimination

$$\frac{dFdCTP}{dt} = (FluxdFdCTP) - \frac{\frac{VmaxdFdCTP*dFdCTP}{KmdFdCTP}}{(1 + \frac{dFdCTP}{KmdFdCTP} + \frac{dFdUTP}{KmdFdUTP} + \frac{dCTP}{KmdCTP})}$$

$$\frac{dFdUTP}{dt} = (FluxdFdUTP) - \frac{\frac{VmaxdFdUTP*dFdUTP}{KmdFdUTP}}{(1 + \frac{dFdCTP}{KmdFdCTP} + \frac{dFdUTP}{KmdFdUTP} + \frac{dCTP}{KmdCTP})}$$

CELL ENTRY

```
#state dfdce 1positive 1 negative
dx[4] =( (1/a)*((vmhout*x[5]/kmhout)/(1.0 + x[4]/kmhin + x[5]/kmhout+ x[10]/kmuhout + x[9]/kmuhin  ) ) -   #dfdce
       ( (vmhin*x[4]/kmhin) /(1.0 + x[4]/kmhin + x[5]/kmhout+ x[10]/kmuhout + x[9]/kmuhin   )) ) ;  #dfdce -> dfc

#state dfdc 2positive 3 negative
dx[5] = (  a*( (vmhin*x[4]/kmhin) /(1.0 + x[4]/kmhin + x[5]/kmhout+ x[10]/kmuhout + x[9]/kmuhin) ) + #dfdce -> dfc
        ((vmaxdfdcmp2dfdc*x[6])/(kmdfdcmp2dfdc + x[6]))  - #dfdcmp -> dfdc
       ((vmhout*x[5]/kmhout)/(1.0 + x[4]/kmhin + x[5]/kmhout+ x[10]/kmuhout + x[9]/kmuhin)) - #dfdc->dfdce
        ((vmaxdfdc2dfdcmp*x[5])/(kmdfdc2dfdcmp*(1.0 + x[3]/kinhdctp) + x[5])) -  #dfdc -> dfdcmp
       ( (vmaxdfdc2dfdu*x[5])/(kmdfdc2dfdu*(1.0 + x[10]/kinhdfdu) + x[5] ) ) #dfdc-> dfdu
         ); #dfdc-> dfdce

#state dfdcmp 2positive 3 negative
dx[6]  = ( ((vmaxdfdc2dfcmp*x[5])/(kmdfdc2dfdcmp*(1.0 + x[3]/kinhdctp) + x[5])) + #dfdc -> dfdcmp
         ( (vmaxdfdcdp2dfdcmp*x[7] ) /( kmdfdcdp2dfdcmp + x[7]   ) ) - #dfdcdp -> dfdcmp
        ((vmaxdfdcmp2dfdcdp*x[6])/(kmdfdcmp2dfdcdp + x[6])) -  #dfdcmp -> dfdcdp
        ((vmaxdfdcmp2dfdump*x[6])/(kmdfdcmp2dfdump*(kinhdfdctp/(kinhdfdctp + x[8])))) - #dfdcmp ->dfdump
        ((vmaxdfdcmp2dfdc*x[6])/(kmdfdcmp2dfdc + x[6]) )   ) #dfdcmp ->dfdc

#state dfdcdp  2positive 2 negative
```

2 DNA Elimination

$$\frac{dFdCTP}{dt} = (FluxdFdCTP) - \frac{\frac{VmaxdFdCTP*dFdCTP}{KmdFdCTP}}{(1 + \frac{dFdCTP}{KmdFdCTP} + \frac{dFdUTP}{KmdFdUTP} + \frac{dCTP}{KmdCTP})}$$

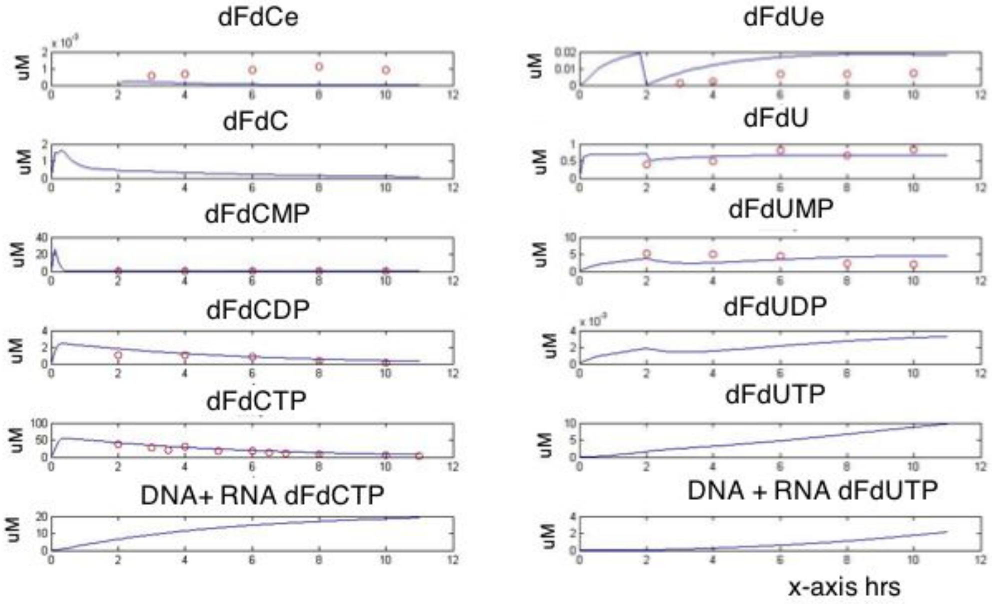
$$\frac{dFdUTP}{dt} = (FluxdFdUTP) - \frac{\frac{VmaxdFdUTP*dFdUTP}{KmdFdUTP}}{(1 + \frac{dFdCTP}{KmdFdCTP} + \frac{dFdUTP}{KmdFdUTP} + \frac{dCTP}{KmdCTP})}$$

Julia Representation

dFdCTP, dCTP, dFdCTP ELIMINATION

```
#state dfdcdp  2positive 2 negative
dx[7] =(((vmaxdfdcmp2dfdcdp*x[6])/(kmdfdcmp2dfdcdp + x[6])) +  #dfdcmp -> dfdcdp
          ((vmaxdfdctp2dfdcdp*x[8])/(kmdfdctp2dfdcdp + x[8])) -             #dfdctp -> dfdcdp
        ((vmaxdfdcdp2dfdctp*x[7])/(kmdfdcdp2dfdctp + x[7])) -   #dfdcdp -> dfdctp
          ((vmaxdfdcdp2dfdcmp*x[7])/(kmdfdcdp2dfdcmp + x[7] ) ) ); #dfdcdp -> dfdcmp  )
```

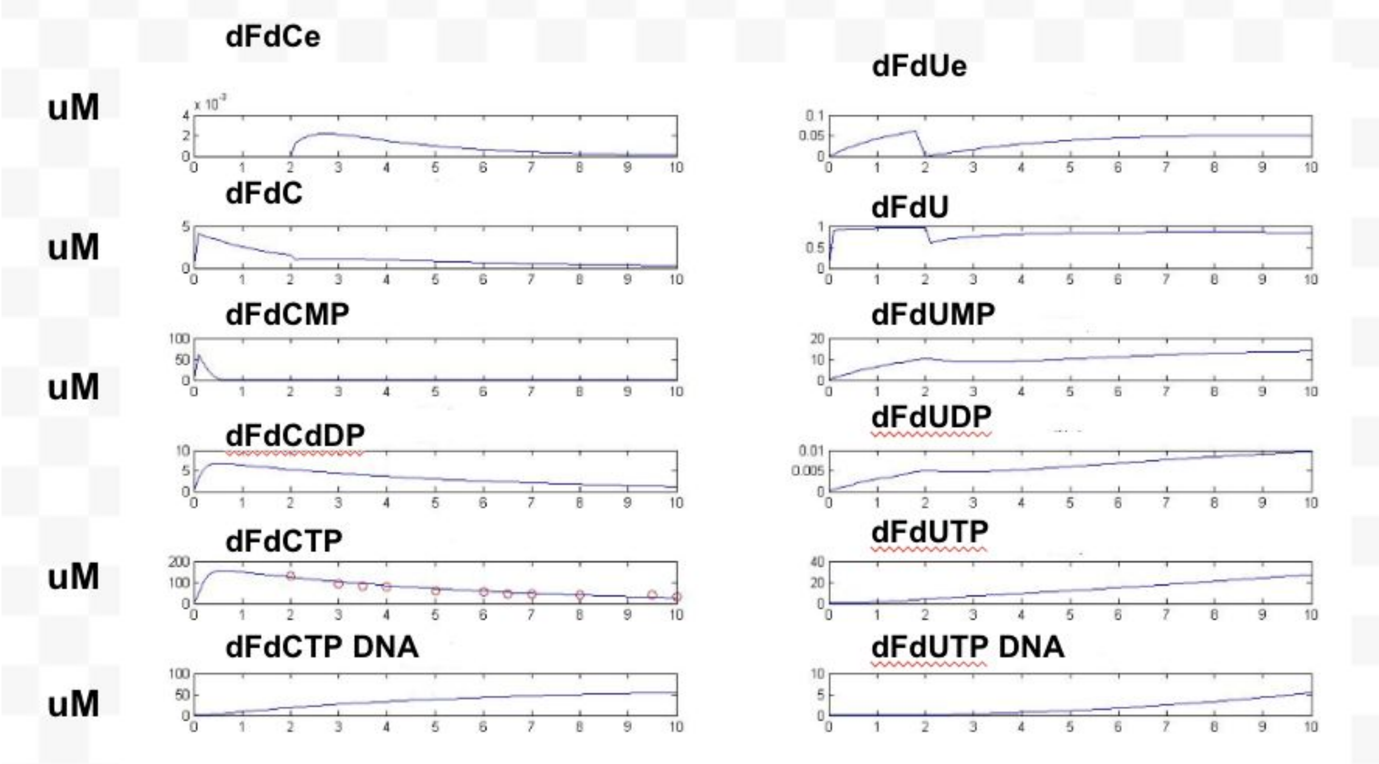
DATA 1: 0.1 uM dFdC incubation



x-axis = hours

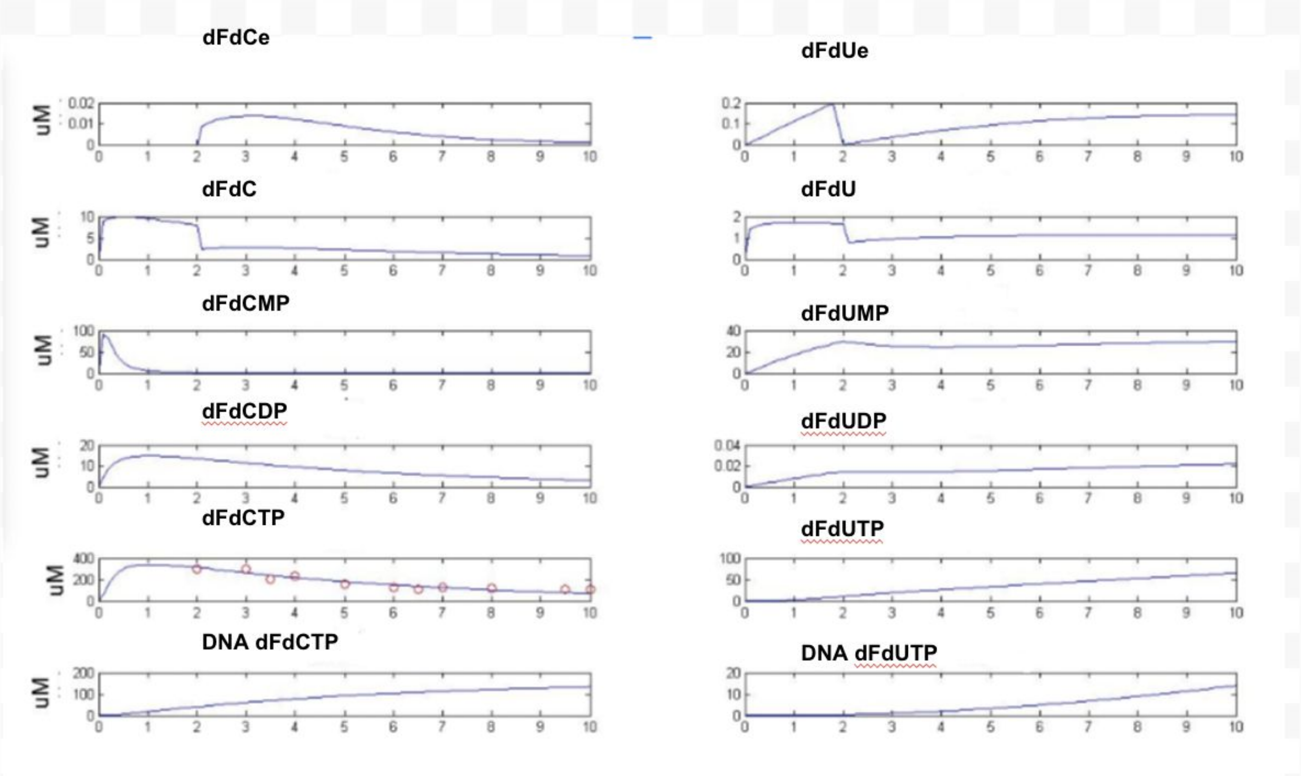
dFdCe decrement explains Maximum Concentration reached before 2 hrs(incubation time)

DATA 1: 0.3 uM dFdC incubation



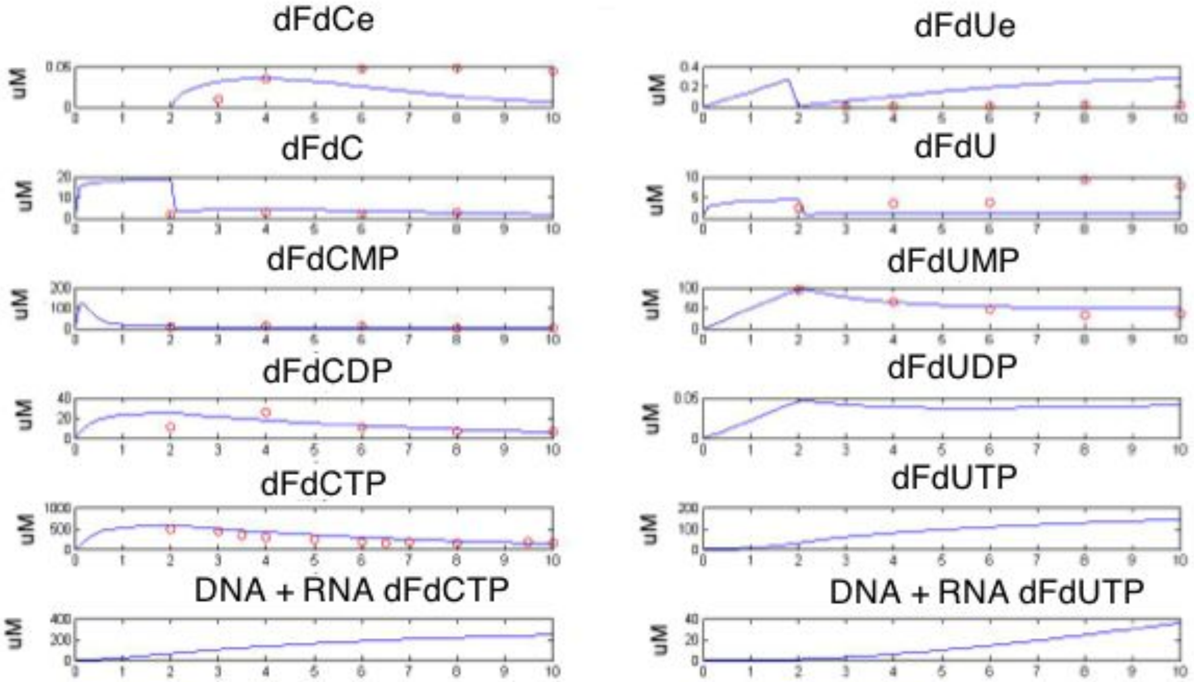
x-axis = hours

DATA 1: 1 uM dFdC incubation



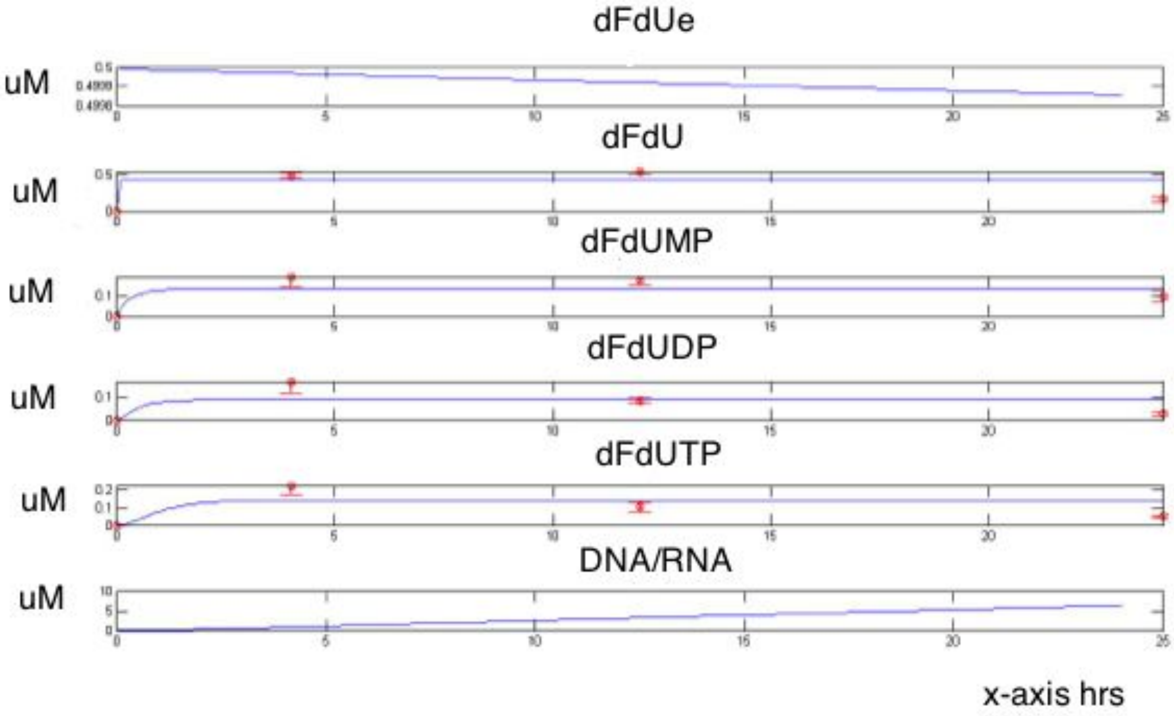
x-axis = hours

DATA 1: 10 uM dFdC incubation

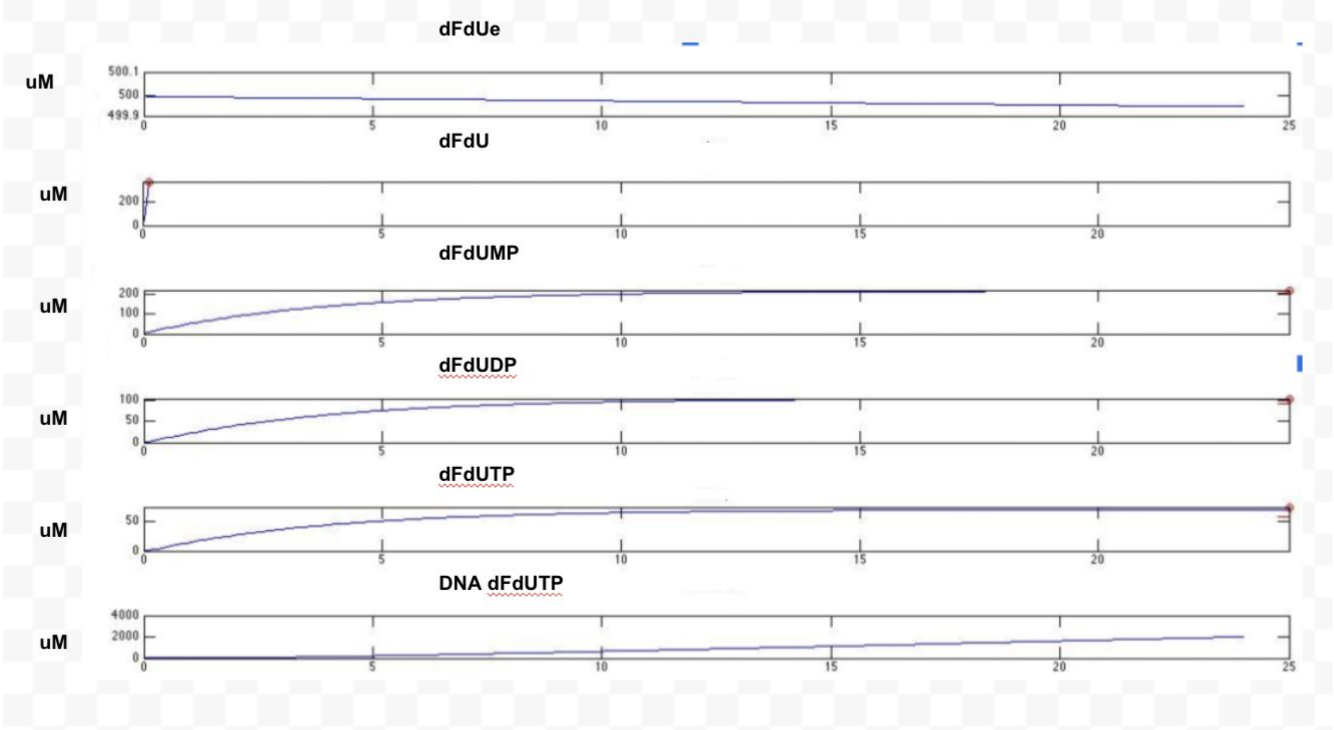


x-axis = hours

DATA 2: dFdU 0.5 uM

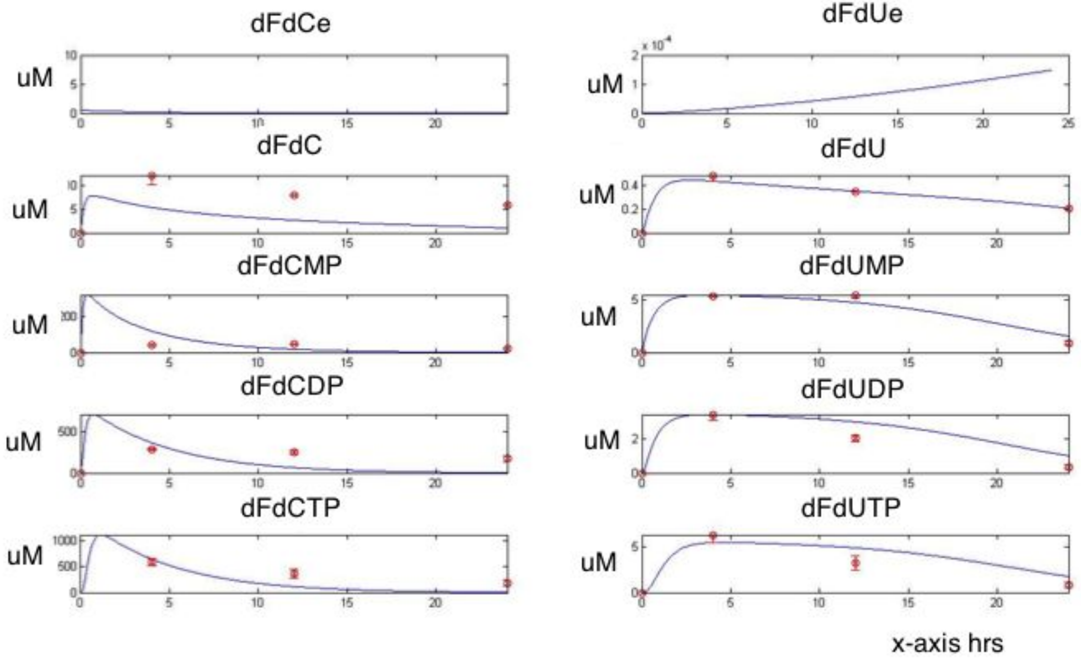


DATA 2: 500 uM dFdU



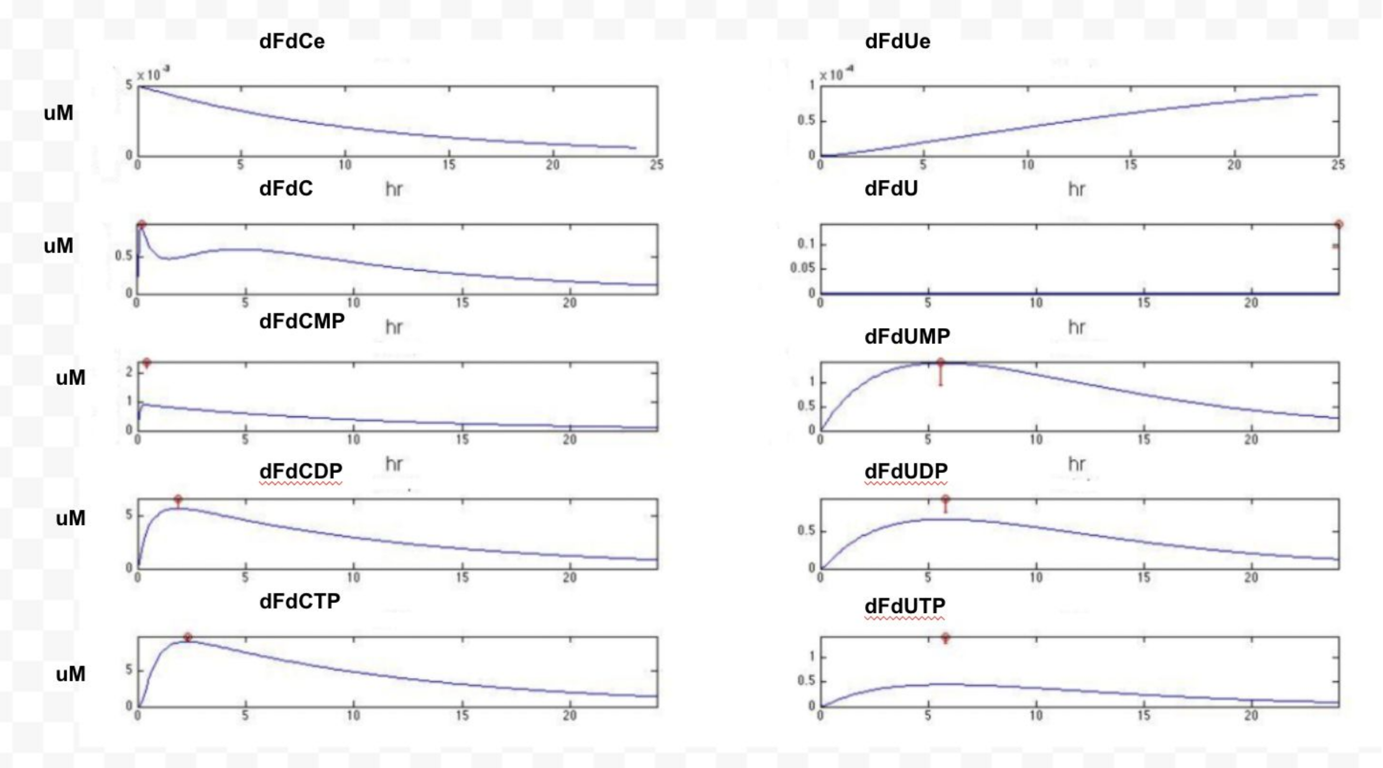
x-axis = hours

DATA 2: dFdCe 0.5 uM



x-axis = hours

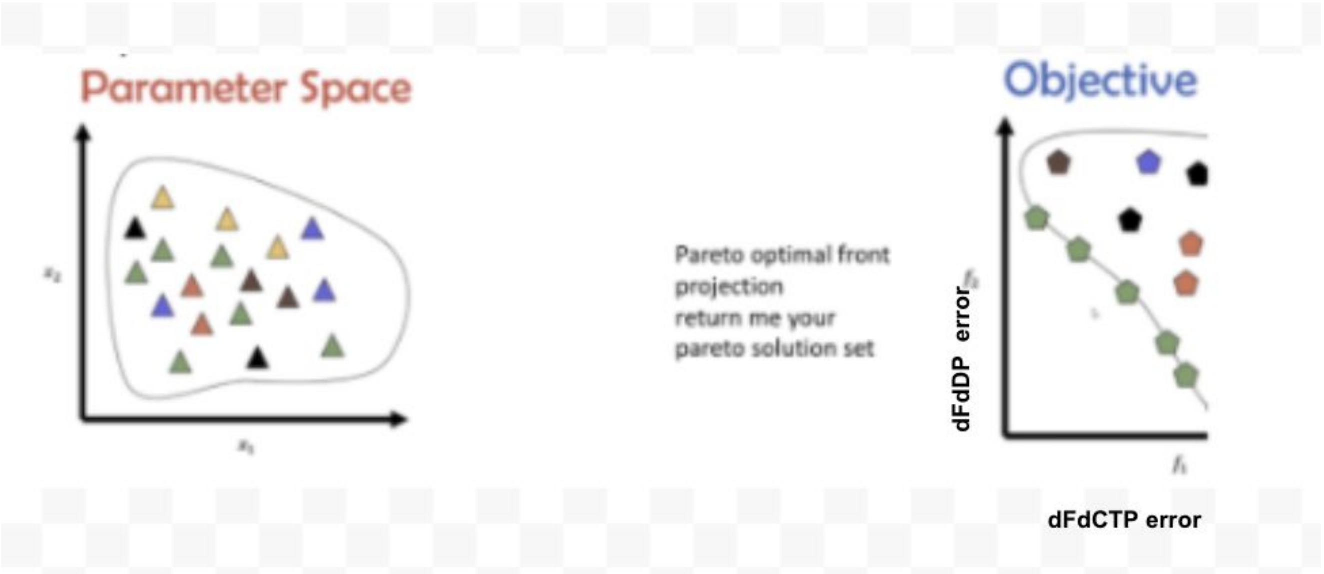
DATA 2: 5 nM dFdC



x-axis = hours

Good Solutions

	Y65I	Y73I	B
	Float64	Float64	String
1	49000.0	49000.0	a
2	0.005	0.0001	kcdp2dcdp
3	0.01	0.0100767	kinhbydfdcdp
4	0.1	1.97201	kdcdp2dctp
5	0.0001	10.0	kdctpelim
6	505.857	0.793568	vmhin
7	290.76	852.684	vmhout
8	686.142	709.147	kmhin
9	2.0	476.271	kmhout
10	964.366	1047.57	vmuhin
11	1000.0	259.096	vmuhout
12	329.446	387.285	kmuhin
13	900.0	917.727	kmuhout
14	806.652	638.749	vmaxdfdcmp2dfdcv
15	564.806	855.233	kmdfdcmp2dfdc
16	928.309	1000.0	vmaxdfdc2dfdcmp
17	5.0	5.0	kmdfdc2dfdcmp
18	500.0	5.0	kinhdctp
19	0.01224	400.0	vmaxdfdc2dfdu
20	95.7	95.7	kmdfdc2dfdu
21	343.436	573.129	kinhdfdu
22	561.647	1000.0	vmaxdfdc2dfcmp
23	0.235061	0.119926	vmaxdfdcdp2dfdcmp
24	400.0	400.0	kmdfdcdp2dfdcmp
25	998.497	1000.0	vmaxdfdcmp2dfdcdp
26	100.18	580.365	kmdfdcmp2dfdcdp



SPEEDING UP ODE

Speeding up processes

$$\mathbf{J} = \begin{bmatrix} \dfrac{\partial \mathbf{f}}{\partial x_1} & \cdots & \dfrac{\partial \mathbf{f}}{\partial x_n} \end{bmatrix} = \begin{bmatrix} \nabla^{\mathrm{T}} f_1 \\ \vdots \\ \nabla^{\mathrm{T}} f_m \end{bmatrix} = \begin{bmatrix} \dfrac{\partial f_1}{\partial x_1} & \cdots & \dfrac{\partial f_1}{\partial x_n} \\ \vdots & \ddots & \vdots \\ \dfrac{\partial f_m}{\partial x_1} & \cdots & \dfrac{\partial f_m}{\partial x_n} \end{bmatrix}$$

Subject/Item	MATLAB	SciPy	deSolve	DifferentialEquations.jl	Sundials
Language	MATLAB	Python	R	Julia	C++ and Fortran
Selection of Methods for ODEs	Fair	Poor	Fair	Excellent	Good
Efficiency*	Poor	Poor****	Poor***	Excellent	Excellent
Tweakability	Fair	Poor	Good	Excellent	Excellent
Event Handling	Good	Good	Fair	Excellent	Good**
Symbolic Calculation of Jacobians and Autodifferentiation	None	None	None	Excellent	None
Complex Numbers	Excellent	Good	Fair	Good	None
Arbitrary Precision Numbers	None	None	None	Excellent	None
Control Over Linear/Nonlinear Solvers	None	Poor	None	Excellent	Excellent
Built-in Parallelism	None	None	None	Excellent	Excellent

```
F.jacobian([y1, y2])
```

$$\begin{array}{cc} & \text{dFdCTP} \qquad\qquad\qquad\qquad\qquad\qquad \text{DNA} \\ \begin{array}{l} \text{dFdCTP/dt} \\[2em] \text{dFdCTP DNA/dt} \end{array} & \begin{bmatrix} \dfrac{vmaxdfdctp2dfdcdp y_1}{(kmdfdctp2dfdcdp+y_1)^2} - \dfrac{vmaxdfdctp2dfdcdp}{kmdfdctp2dfdcdp+y_1} - \dfrac{vmaxdfdctpelim}{kmdfdctpelim\left(\frac{dCTP}{kmdCTP}+1.0+\frac{y_1}{kmdfdctpelim}\right)} + \dfrac{vmaxdfdctpelim y_1}{kmdfdctpelim^2\left(\frac{dCTP}{kmdCTP}+1.0+\frac{y_1}{kmdfdctpelim}\right)^2} & 0 \\ \dfrac{vmaxdfdctpelim}{kmdfdctpelim\left(\frac{dCTP}{kmdCTP}+1.0+\frac{y_1}{kmdfdctpelim}\right)} - \dfrac{vmaxdfdctpelim y_1}{kmdfdctpelim^2\left(\frac{dCTP}{kmdCTP}+1.0+\frac{y_1}{kmdfdctpelim}\right)^2} & 0 \end{bmatrix} \end{array}$$

y1=dFdCTP

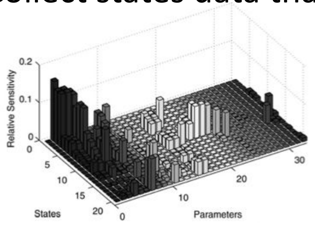
- ❑ SIMULATION FAILS DUE TO PARAMETER CHOSEN
- ❑ ODE OPTION /studying cases.

SymPy in Python outputs the Jacobian; Similarly Modeling ToolKit in Julia can as well

PROPOSED FRAMEWORK

1. **Mechanistic models vs less Complex Models**
2. **Parameter Sampling**
3. **Local Sensitivity Analysis vs. Global Sensitivity analysis (i.e Variance Methods)**

Multiple inputs can affect a state different than other
Collect states data that is sensitive



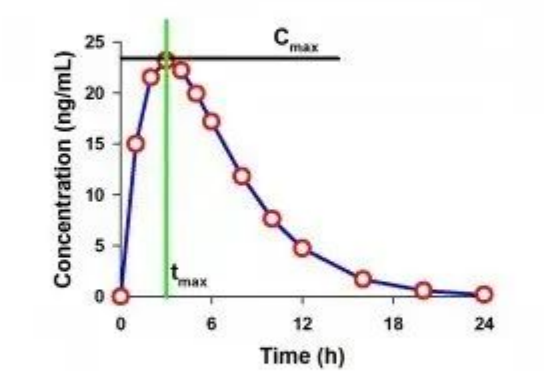
Model defined in terms of ODEs $\boxed{\frac{dy}{dt} = f(y,p)}$ $y \in \mathbb{R}^n$ state vector, $p \in \mathbb{R}^m$ parameter vector

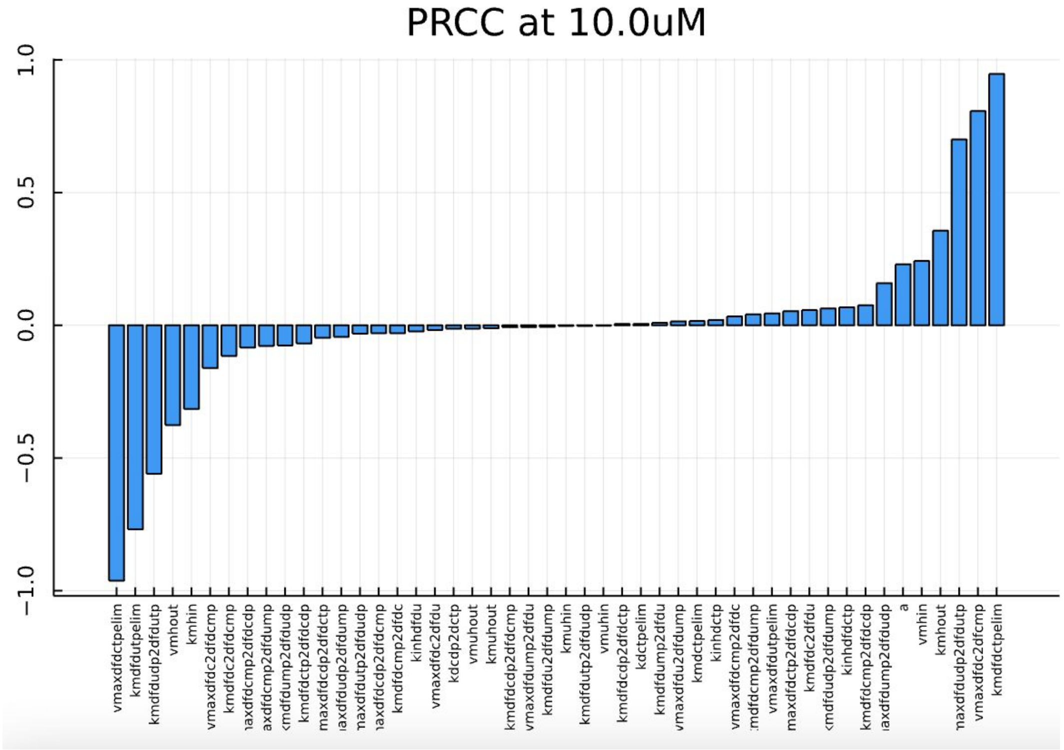
dose-response relationship (**drr**): change in **output** as a function of a **parameter**

$y_i(p_k)$ → keep remaining parameters at a fixed value $p_{j \neq k}$ (local drr)

$y_i(p_k)$ → average over remaining parameters → conditional expectation $E_{p_{j \neq k}}(y_i | p_k)$

PRCC at 10 uM for dFdCTP % reduction from Cmax

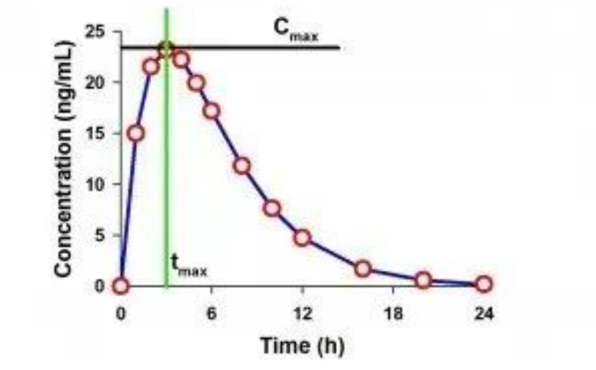


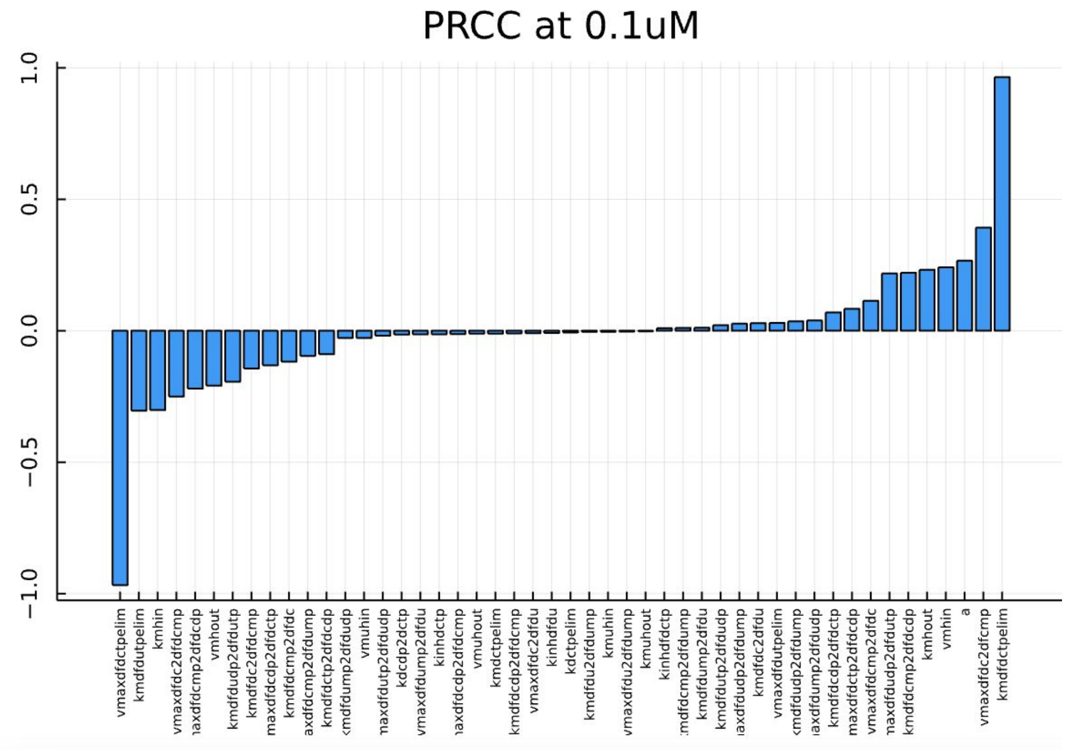


Cmax⟶ C | t= 24 h
% Decrease

N = 2000 Samples

PRCC at 0.1 uM for dFdCTP % reduction from Cmax

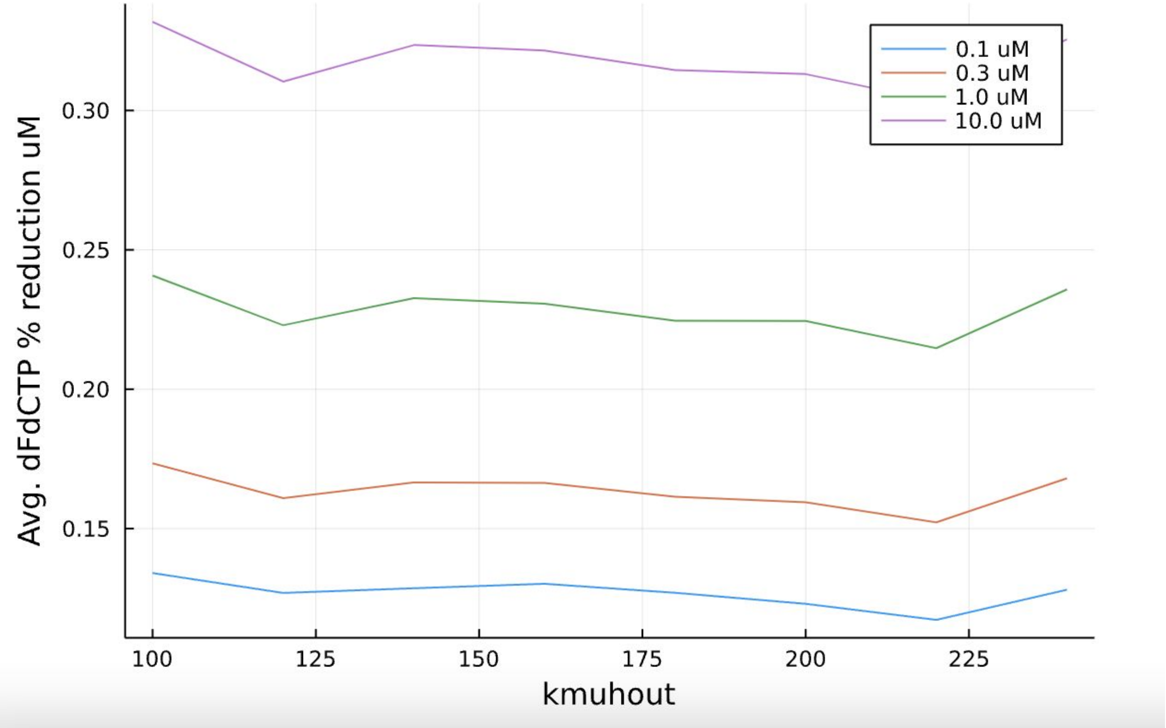




Cmax⟶ C | t= 24 h
% Decrease

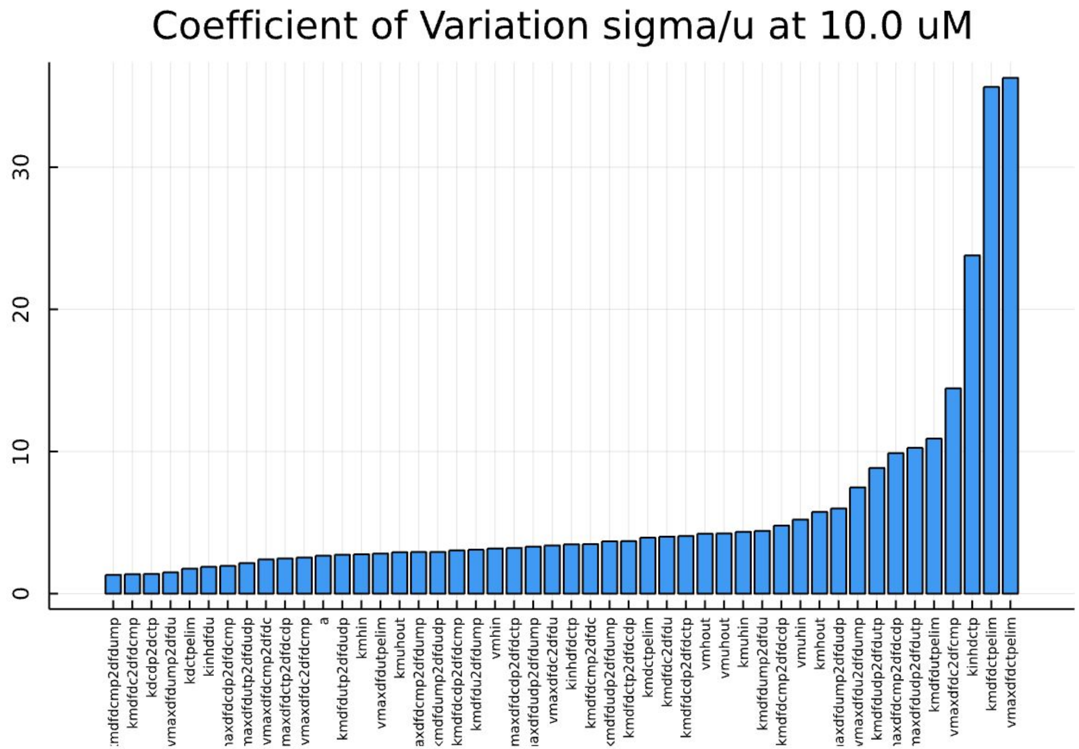
N = 2000 Samples

DOSE RESPONSE % dFdCTP Reduction



N = 2000 Samples

Coefficient of Variation of Dose Response sorted at 10 uM



N = 2000 Samples