IKKKa $dy(1) = K_a \, B(KN - y(1)) \cdot ka20/(ka20 + y(9)) - Ki \, y(1)$

IKKn $dy(2) = k4(KNN - y(2) - y(3) - y(4)) - K1 \, y(1)^2 y(2)$

IKKa $dy(3) = K1 \, y(1)^2 y(2) - K3 \, y(3)(K2 + y(9))/K_2$

IKKi $dy(4) = K3 \, y(3)(K2 + y(9))/K_2 - K4 \, y(4)$

IKBp $dy(5) = a2 \, y(3) \, y(11) - t_p \, y(5)$

NFkB|IKBp $dy(6) = a3 \, y(3) \, y(14) - t_p \, y(6)$

NFkB $dy(7) = c6a \, y(14) - a1 \, y(7) \, y(11) + t_p \, y(6) - i1 \, y(7)$

NFkBn $dy(8) = -a1 \cdot K_v \, y(12) \, y(8) + i1 \, y(7)$

A20 $dy(9) = c_4 \, y(10) - c_5 \, y(9)$

A20t $dy(10) = c_1 \, G_{A20} - c_3 \, y(10)$

IkB $dy(11) = -a2 \, y(3) \, y(11) - a1 \, y(11) \, y(7) + c_4 \, y(13) - c5a \, y(11) - i1a \, y(11) + e1a \, y(12)$

IkBn $dy(12) = -a1 \cdot kv \cdot y(12) \cdot y(8) + i1a \cdot y(11) - e1a \, y(12)$

IKBt $dy(13) = c1 \, G_{IkB} - c3 \, y(13)$

NFkB|IkB $dy(14) = a1 \, y(11) \cdot y(7) - c6a \, y(14) - a_3 \, y(3) \, y(14) + e2a \, y(15)$

NFkB|IkBn $dy(15) = a1 \, kv \, y(12) \, y(8) - e2a \, y(15)$

TNFext $dy(16) =$

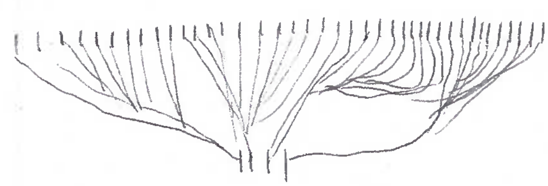
$c_{15r}A - c_{3r} \, y(17) + c1r \, GR$

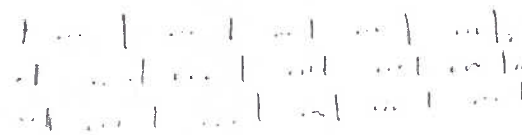
Code Table with variables

READ ME.txt

1000 bottles

all but 1 need to drink each bottle





Translation Protocol of each cell line of cytokines.

Find concentration. For STAT 3-10... Find types of receptors.

Windows Printer Test Page

You have correctly installed your Microsoft PWG Raster Class Driver on DESKTOP-3A4R1TE.

PRINTER PROPERTIES

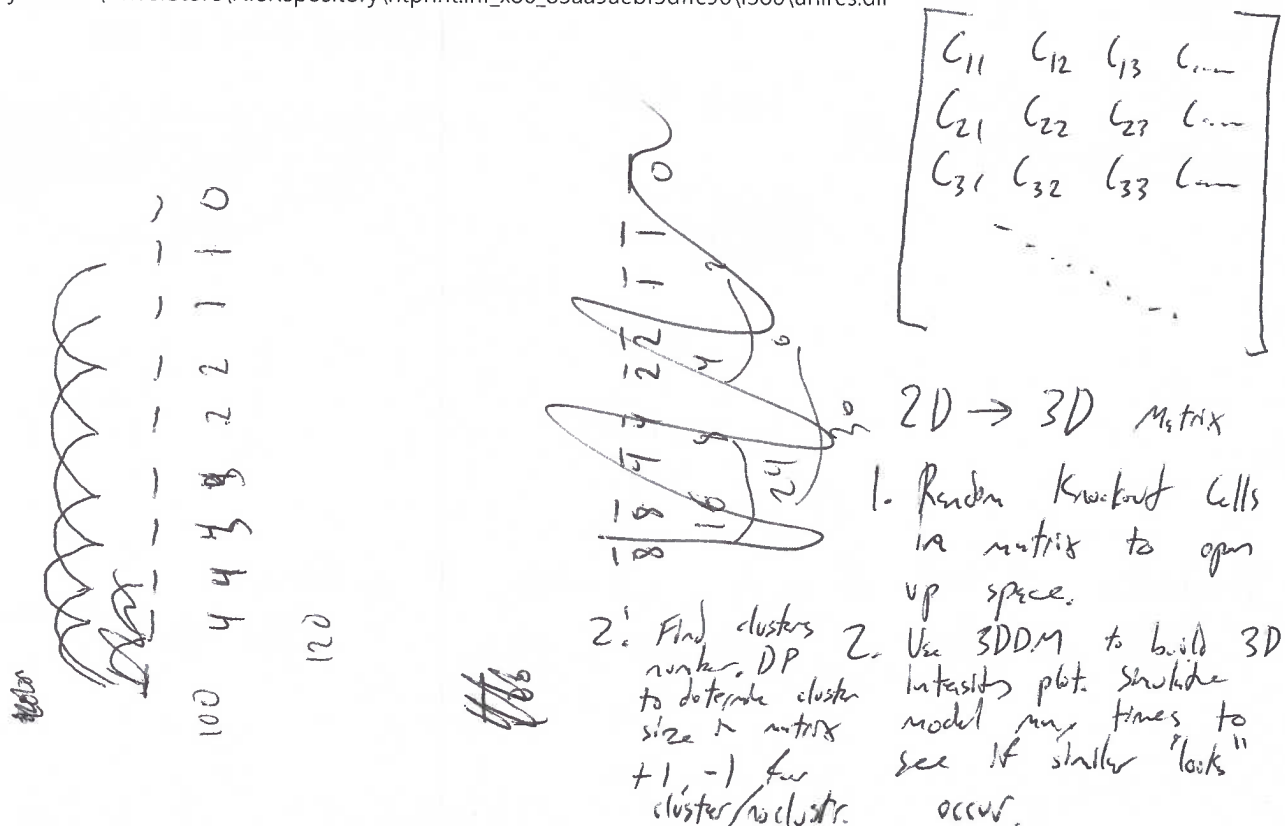
Submitted Time:	12:59:25 PM
Date:	5/30/2019
User Name:	UOFI\sarkar
Computer Name:	DESKTOP-3A4R1TE
Printer Name:	Brother DCP-L2550DW series Printer
Printer Model:	Microsoft PWG Raster Class Driver
Color Support:	No
Port Name(s):	WSD-cd119b1f-c64f-4057-a54d-6e83d6a4b9b2
Data Format:	RAW
Printer Share Name:	Brother DCP-L2550DW series Printer
Print Processor:	winprint
OS Environment:	Windows NT x86

PRINT DRIVER PROPERTIES

Driver Name:	Microsoft PWG Raster Class Driver
Driver Type:	Type 4 - User Mode
Driver Version:	10.0.17763.1

ADDITIONAL PRINT DRIVER FILES

C:\WINDOWS\System32\DriverStore\FileRepository\prnms007.inf_x86_d694ba8720fe35d3\I386\MSPWGR-manifest.ini
C:\WINDOWS\System32\DriverStore\FileRepository\prnms007.inf_x86_d694ba8720fe35d3\I386\MSPWGR-pipelineconfig.xml
C:\WINDOWS\System32\DriverStore\FileRepository\ntprint4.inf_x86_34808b1c386b5dc1\I386\PWGRRenderFilter.dll
C:\WINDOWS\System32\DriverStore\FileRepository\ntprint.inf_x86_83aa9aebf5dffc96\I386\StdNames.gpd
C:\WINDOWS\System32\DriverStore\FileRepository\ntprint.inf_x86_83aa9aebf5dffc96\I386\unires.dll



```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%
%    Main Program for
%
%    STOCHASTIC SIMULATIONS OF NF-kB PATHWAY
%    S. Tay et al. 2010 Nature
%
%    Calls: Model, Parameters, AllCellPlotting and AvarageCellPlotting
%
%    After running MainFile you can run
%    AllCellPlotting and AvarageCellPlotting
%
%    Saves all data in 'last' - can be used to make plots latter on
%
%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

clear;                  %reset all
clc;                    %clear comand window
starttime=clock;        %current time
rand('twister', sum(1000*clock));


%#######################################
%########## Simulation setup ##########
%#######################################

TNF=10;                             % TNF dose

ANa=2; AN=2; ANR=2;                 % ANa=2 - # IKBa alleles, AN=2 - # A20 alleles, ANR=2 - # Reporter gene alleles

%Set AN=0 to study A20 knockout%

N=5;                                % number of cell to be simulated

%#########################################################################
%####### Simulation time points                                    #######
%#### Various time protocols can be studied within this frame  #######
%#########################################################################

t000=10*3600;           %10h randomization of initial conditions
t00=10*3600;            %10h equilibrium waiting time

t0=50*60;               % 1 step, time when TNF is being introduced into the system (in seconds)

```

```
46 tw1=5*60;              % 2 step, length of TNF stimulation
47 te1=100*60;            % 3 step length of first break   (White breaks: 3600s,
6000s, 12000s, our break 170*60s)
48
49 tw2=5*60;              % 4 step length of second TNF stimulation
50 te2=100*60;            % 5 step length of second break
51
52 tw3=5*60;              % 6 step length of third TNF stimulation
53 te3=100*60;            % 7 step length of third break
54
55 % ###########################################################
56
57
58 tt=1000;              % forword time for ODEs solving
59
60 YYY=0;                          %matrix of average, all variables y0(i)(t)
61 NFKB=0;                         %total nuclear NF-kB
62 GGa=0; GG=0;  GGT=0; GGR=0;     %status of Ikba,  A20, TNF and reporter genes
63 Bb=0;                           %number of active receptors
64 MM=0;
65 NFF=0;
66
67 for i=1:N              %beginning the mean loop
68
69     %###################################
70     %####### Initial conditions #######
71     %###################################
72
73     i                    %cell nummber
74
75
76     [NF0,NF1,NF2,M0,M1,M2,k4,ka20,AB,kv,q1,q2,c1,c3,c4,c5,k1,k2,k3,a1,a2,a3,c1a,
c5a,c6a,i1,i1a,e1a,e2a,dt,tp,KN,KNN,ka,ki,kb,kf,Tdeg,q1r,q2r,q2rr,c1r,c1rr,c3r]
=Parameters;
77
78     %%% Randomizations of total TNF receptors and NF-kB levels %%%%%%
79
80
81      NF=round(NF0*exp(NF2+randn*NF1))                    %NF-kB level
82      while NF > 10*NF0
83      NF=round(NF0*exp(NF2+randn*NF1))
84      end
85
86     %NF=NF0;     %uncomment to remove extrinsic noise
87
88    % Lognormal distribution with Median=NF0, Mean=NF0*Exp(NF1^2/2), %
89    % Variance = NF0^2 * (Exp (NF1^2 -1) * Exp(NF1^2)
90
91
```

Time ODE Increment

Not used.

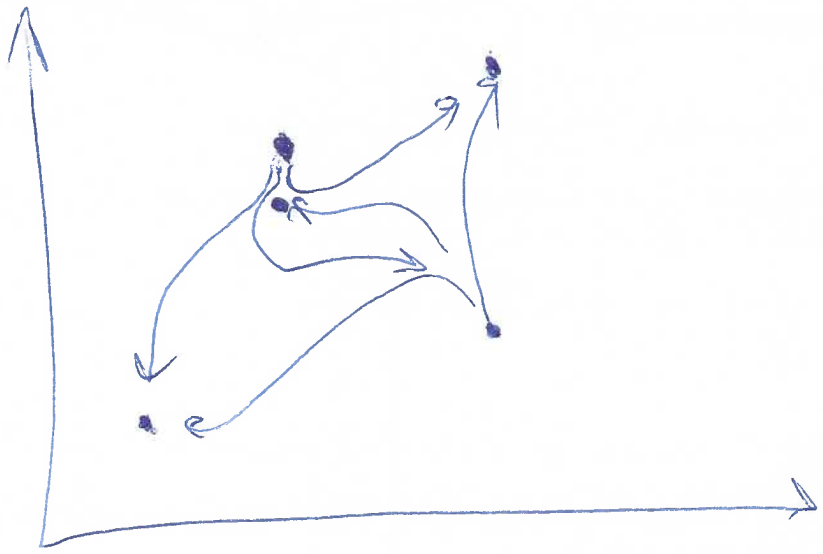
← TNFR1 receptors

← NF-kB level

Loop through each cell

Keep within order of magnitude.

```
92      M=round(M0*exp(M2+randn*M1))                % number of TNFR1 receptors
93      while M > 10*M0
94      M=round(M0*exp(M2+randn*M1))
95      end
96
97      %M=M0;          %uncomment to remove extrinsic noise
98
99      % Lognormal distribution with Median=M0, Mean=M0*Exp(M1^2/2), %
100     % Variance = M0^2 * (Exp (M1^2 -1) * Exp(M1^2)
101
102     %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
103
104
105
106     y0=zeros(1,19);      %initial conditions set to zero and next:
107
108     y0(14)=NF;           %NF-kB is given in cytoplasmic complex(IkBa|NFkB) (total
NF-kB kept constant), standard = 10^5
109     y0(2)=2*10^5;        %initial IKKn, total IKK kept constant
110     y0(11)=0.14*y0(14); %free cytoplasmic IkBa protein
111     y0(12)=0.06*y0(14); %free nuclear IkBa protein
112     y0(13)=10;           %IkBa mRNA
113     y0(10)=10;           %10 A20 mRNA
114     y0(9)=10000;         %10000 A20 protein
115
116
117     y0(10)=AB*y0(10);
118     y0(9)=AB*y0(9);
119
120     Ga=0;                % initial status of IkBa promoter
121     G=0;                 % initial status of A20 promoter
122     GT=0;                % initial status of TNF promoter
123     GR=0;                % initial status of reporter gene promoter
124     B=0;                 % initial number of active receptors
125     yy0=y0;              % initial conditions y0(i)
126
127     %#####################################################################
128     %####### -1 step - randomization of initial condition  ########
129     %#####################################################################
130
131     realtime=0;                          %simulated time
132     phase=round(rand*t000/dt)*dt;        %random initial time (dt -simulation time
step -10s)
133     tspan=[0:dt:tt];                     %time for which the solution is derived to
find the switching time, tt=1h
134
135     while (realtime<phase)
136         [T0,Y0]=ode23tb(@Model,tspan,yy0,[],Ga,G,GR,B);
137         Yact=Y0(:,8);                    %amount of NF-kBn
```



```
138         Yin=Y0(:,12);                   %amount of IkBan
139         TR=Y0(:,16);                    %TNF level
140         Gax=Ga;Gx=G;GRx=GR;Bx=B;
141         [mk,Ga,G,GR,B]=StatusChange(AN,ANa,ANR,TR,Gax,Gx,GRx,Bx,Yact,Yin,M) ;   %↙
function determining the change of gene status, calls statuschange
142
143         tc=T0(mk);                      %time when the status changes
144         yy0=Y0(mk,:);                   %transfer of initial conditions to the next↙
iteration
145         realtime=realtime+tc;
146     end;
147
148     if (realtime>phase)
149         nn=(realtime-phase)/dt;
150         yy0=Y0(mk-nn,:);
151         Ga=Gax;G=Gx;GR=GRx;B=Bx;
152     end;                                %status before the last change it occured↙
outside of the time interval
153
154     clear Yact Yin Y0 T0 nn mk phase tc;
155
156     %#####################################################
157     %###### 0 step - waiting for "equilibrium"    #######
158     %#####################################################
159
160     realtime=0;
161
162   * while (realtime<t00)
163         [T0,Y0]=ode23tb(@Model,tspan,yy0,[],Ga,G,GR,B);
164         Yact=Y0(:,8);                   %amount of NF-kBn
165         Yin=Y0(:,12);                   %amount of IkBan
166         TR=Y0(:,16);                    %TNF level
167         Gax=Ga;Gx=G;GRx=GR;Bx=B;
168         [mk,Ga,G,GR,B]=StatusChange(AN,ANa,ANR,TR,Gax,Gx,GRx,Bx,Yact,Yin,M);  %↙
function determining the change of gene status, calls statuschange
169
170         tc=T0(mk);                      %time when the status changes
171         yy0=Y0(mk,:);                   %transfer of initial conditions to the next↙
iteration
172         realtime=realtime+tc;
173     end;
174
175     if (realtime>t00)
176         nn=(realtime-t00)/dt;
177         yy0=Y0(mk-nn,:);
178         Ga=Gax;G=Gx;GR=GRx;B=Bx;
179     end;                                %status before the last change it occured↙
ouside of the time interval
180
```

```
181     clear Yact Yin Y0 T0 nn mk tc;
182
183     %#########################################
184     %###### 1 step- still no TNF #######
185     %#########################################
186
187     realtime=0;
188     ga=[Ga];g=[G];gR=[GR];                %saves activity of IkBa A20 reporter genes
189     bb=[B];                               %saves number of active receptors
190     Y=yy0;                                %variables where single cell run is stored
191     T=zeros(1,1);
192
193     while (realtime<t0)
194         [T0,Y0]=ode23tb(@Model,tspan,yy0,[],Ga,G,GR,B);
195         Yact=Y0(:,8);                     %amount of NF-kBn
196         Yin=Y0(:,12);                     %amount of IkBan
197         TR=Y0(:,16);                      %TNF level
198         Gax=Ga;Gx=G;GRx=GR;Bx=B;
199         [mk,Ga,G,GR,B]=StatusChange(AN,ANa,ANR,TR,Gax,Gx,GRx,Bx,Yact,Yin,M); %function determining the change of gene status, call statuschange
200         tc=T0(mk);                        %time when the status changes
201         yy0=Y0(mk,:);                     %transfer of initial conditions to the next iteration
202         Y=[Y;Y0(2:mk,:)];                 %rows from 2 to mk, all columns
203         T=[T;T0(2:mk)+realtime];
204         ga=[ga;Gax*ones(mk-1,1)];
205         g=[g;Gx*ones(mk-1,1)];
206         gR=[gR;GRx*ones(mk-1,1)];
207         bb=[bb;Bx*ones(mk-1,1)];
208         realtime=realtime+tc;
209     end;
210
211
212         nn=(realtime-t0)/dt;
213         x=size(Y);
214         Y=Y(1:(x(1)-nn),:);
215         T=T(1:(x(1)-nn));
216
217         Y0(mk-nn,16)=TNF;                 %setting TNF ON for the next step
218
219         yy0=Y0(mk-nn,:);
220         x1=length(ga);
221         ga=ga(1:x1-nn);g=g(1:x1-nn);gR=gR(1:x1-nn);bb=bb(1:x1-nn);
222         Ga=Gax;G=Gx;GR=GRx;B=Bx;
223
224
225     %#########################################
```

Initialization

Because Y is cumulative to be the union. Therefore, only start at 2.

Plot summary in loop: Accumulation pattern.

[...] Length is used. Cumulative Length

Cumulative

IC

Consider TNF in last position as IC.

Could be size(yy)

x1(1)

Rewrite

Cumulative

Should be same as X.

Same states not changed during 1-st step.

```
226     %######  2 step TNF on 1 time        #######
227     %#####################################
228
229
230     realtime=t0;
231
232     while (realtime<t0+tw1)
233        [T0,Y0]=ode23tb(@Model,tspan,yy0,[],Ga,G,GR,B);
234         Yact=Y0(:,8);                  %amount of NF-kBn
235         Yin=Y0(:,12);                  %amount of IkBan
236         TR=Y0(:,16);                   %TNF level
237         Gax=Ga;Gx=G;GTx=GT;GRx=GR;Bx=B;
238        [mk,Ga,G,GR,B]=StatusChange(AN,ANa,ANR,TR,Gax,Gx,GRx,Bx,Yact,Yin,M);  %↙
function determining the change of gene status, call statuschange
239         tc=T0(mk);                     %time when the status changes
240         yy0=Y0(mk,:);                  %transfer of initial conditions to the next↙
iteration
241         Y=[Y;Y0(2:mk,:)];              %rows from 2 do mk, all columns
242         T=[T;T0(2:mk)+realtime];
243         ga=[ga;Gax*ones(mk-1,1)];
244         g=[g;Gx*ones(mk-1,1)];
245         gR=[gR;GRx*ones(mk-1,1)];
246         bb=[bb;Bx*ones(mk-1,1)];
247         realtime=realtime+tc;
248     end;
249
250
251         nn=(realtime-t0-tw1)/dt;
252         x=size(Y);
253         Y=Y(1:(x(1)-nn),:);
254         T=T(1:(x(1)-nn));
255
256         Y0(mk-nn,16)=0;          %setting TNF OFF for the next step
257
258         yy0=Y0(mk-nn,:);
259         x1=length(ga);
260         ga=ga(1:x1-nn);g=g(1:x1-nn);gR=gR(1:x1-nn);bb=bb(1:x1-nn);
261         Ga=Gax;G=Gx;GT=GTx;GR=GRx;B=Bx;
262
263
264     %#########################################
265     %###### 3 step TNF washed out 1 time ###########
266     %#########################################
267
268
269     realtime=t0+tw1;
270
271      while (realtime<t0+tw1+te1)
272         [T0,Y0]=ode23tb(@Model,tspan,yy0,[],Ga,G,GR,B);
```

```
273         Yact=Y0(:,8);                   %amount of NF-kBn
274         Yin=Y0(:,12);                   %amount of IkBan
275         TR=Y0(:,16);                    %TNF level
276         Gax=Ga;Gx=G;GRx=GR;Bx=B;
277         [mk,Ga,G,GR,B]=StatusChange(AN,ANa,ANR,TR,Gax,Gx,GRx,Bx,Yact,Yin,M);  %↙
function determining the change of gene status, call statuschange
278         tc=T0(mk);                      %time when the status changes
279         yy0=Y0(mk,:);                   %transfer of initial conditions to the next↙
iteration
280         Y=[Y;Y0(2:mk,:)];               %rows from 2 do mk, all columns
281         T=[T;T0(2:mk)+realtime];
282         ga=[ga;Gax*ones(mk-1,1)];
283         g=[g;Gx*ones(mk-1,1)];
284         gR=[gR;GRx*ones(mk-1,1)];
285         bb=[bb;Bx*ones(mk-1,1)];
286         realtime=realtime+tc;
287     end;
288
289
290         nn=(realtime-t0-tw1-te1)/dt;
291         x=size(Y);
292         Y=Y(1:(x(1)-nn),:);
293         T=T(1:(x(1)-nn));
294
295         Y0(mk-nn,16)=TNF;           %setting TNF ON for the next step
296
297         yy0=Y0(mk-nn,:);
298         x1=length(ga);
299         ga=ga(1:x1-nn);g=g(1:x1-nn);gR=gR(1:x1-nn);bb=bb(1:x1-nn);
300         Ga=Gax;G=Gx;GR=GRx;B=Bx;
301
302
303
304     %##########################################
305     %###### 4 step TNF on for 2 time ##############
306     %##########################################
307
308
309     realtime=t0+tw1+te1;
310
311     while (realtime<t0+tw1+te1+tw2)
312         [T0,Y0]=ode23tb(@Model,tspan,yy0,[],Ga,G,GR,B);
313         Yact=Y0(:,8);                   %amount of NF-kBn
314         Yin=Y0(:,12);                   %amount of IkBan
315         TR=Y0(:,16);                    %TNF level
316         Gax=Ga;Gx=G;GRx=GR;Bx=B;
317        [mk,Ga,G,GR,B]=StatusChange(AN,ANa,ANR,TR,Gax,Gx,GRx,Bx,Yact,Yin,M);  %↙
function determining the change of gene status, call statuschange
318         tc=T0(mk);                      %time when the status changes
```

```
319         yy0=Y0(mk,:);                %transfer of initial conditions to the next iteration
320         Y=[Y;Y0(2:mk,:)];            %rows from 2 do mk, all columns
321         T=[T;T0(2:mk)+realtime];
322         ga=[ga;Gax*ones(mk-1,1)];
323         g=[g;Gx*ones(mk-1,1)];
324         gR=[gR;GRx*ones(mk-1,1)];
325         bb=[bb;Bx*ones(mk-1,1)];
326         realtime=realtime+tc;
327     end;
328
329
330         nn=(realtime-t0-tw1-te1-tw2)/dt;
331         x=size(Y);
332         Y=Y(1:(x(1)-nn),:);
333         T=T(1:(x(1)-nn));
334
335         Y0(mk-nn,16)=0;          %setting TNF OFF for the next step
336
337         yy0=Y0(mk-nn,:);
338         x1=length(ga);
339         ga=ga(1:x1-nn);g=g(1:x1-nn);gR=gR(1:x1-nn);bb=bb(1:x1-nn);
340         Ga=Gax;G=Gx;GR=GRx;B=Bx;
341
342     %##########################################
343     %###### 5 step TNF washed out 2 time ###########
344     %##########################################
345
346
347     realtime=t0+tw1+te1+tw2;
348
349       while (realtime<t0+tw1+te1+tw2+te2)
350         [T0,Y0]=ode23tb(@Model,tspan,yy0,[],Ga,G,GR,B);
351         Yact=Y0(:,8);                %amount of NF-kBn
352         Yin=Y0(:,12);                %amount of IkBan
353         TR=Y0(:,16);                 %TNF level
354         Gax=Ga;Gx=G;GRx=GR;Bx=B;
355         [mk,Ga,G,GR,B]=StatusChange(AN,ANa,ANR,TR,Gax,Gx,GRx,Bx,Yact,Yin,M);  %function determining the change of gene status, call statuschange
356         tc=T0(mk);                   %time when the status changes
357         yy0=Y0(mk,:);                %transfer of initial conditions to the next iteration
358         Y=[Y;Y0(2:mk,:)];            %rows from 2 do mk, all columns
359         T=[T;T0(2:mk)+realtime];
360         ga=[ga;Gax*ones(mk-1,1)];
361         g=[g;Gx*ones(mk-1,1)];
362         gR=[gR;GRx*ones(mk-1,1)];
363         bb=[bb;Bx*ones(mk-1,1)];
364         realtime=realtime+tc;
```

```
365     end;
366
367
368         nn=(realtime-t0-tw1-te1-tw2-te2)/dt;
369         x=size(Y);
370         Y=Y(1:(x(1)-nn),:);
371         T=T(1:(x(1)-nn));
372
373         Y0(mk-nn,16)=TNF;          %setting TNF ON for the next step
374
375         yy0=Y0(mk-nn,:);
376         x1=length(ga);
377         ga=ga(1:x1-nn);g=g(1:x1-nn);gR=gR(1:x1-nn);bb=bb(1:x1-nn);
378         Ga=Gax;G=Gx;GR=GRx;B=Bx;
379
380
381     %########################################
382     %###### 6 step TNF on for the 3 time ###########
383     %########################################
384
385
386     realtime=t0+tw1+te1+tw2+te2;
387
388      while (realtime<t0+tw1+te1+tw2+te2+tw3)
389          [T0,Y0]=ode23tb(@Model,tspan,yy0,[],Ga,G,GR,B);
390         Yact=Y0(:,8);                %amount of NF-kBn
391         Yin=Y0(:,12);                %amount of IkBan
392         TR=Y0(:,16);                 %TNF level
393         Gax=Ga;Gx=G;GRx=GR;Bx=B;
394        [mk,Ga,G,GR,B]=StatusChange(AN,ANa,ANR,TR,Gax,Gx,GRx,Bx,Yact,Yin,M);  %↙
function determining the change of gene status, call statuschange
395         tc=T0(mk);                   %time when the status changes
396         yy0=Y0(mk,:);                %transfer of initial conditions to the next↙
iteration
397         Y=[Y;Y0(2:mk,:)];            %rows from 2 do mk, all columns
398         T=[T;T0(2:mk)+realtime];
399         ga=[ga;Gax*ones(mk-1,1)];
400         g=[g;Gx*ones(mk-1,1)];
401         gR=[gR;GRx*ones(mk-1,1)];
402         bb=[bb;Bx*ones(mk-1,1)];
403         realtime=realtime+tc;
404     end;
405
406
407         nn=(realtime-t0-tw1-te1-tw2-te2-tw3)/dt;
408         x=size(Y);
409         Y=Y(1:(x(1)-nn),:);
410         T=T(1:(x(1)-nn));
411
```

```
        Y0(mk-nn,16)=0;         %setting TNF OFF for the next step

        yy0=Y0(mk-nn,:);
        x1=length(ga);
        ga=ga(1:x1-nn);g=g(1:x1-nn);bb=bb(1:x1-nn);
        Ga=Gax;G=Gx;GR=GRx;B=Bx;

    %#########################################
    %####### 7 step TNF washed out 3 time ###########
    %#########################################


    realtime=t0+tw1+te1+tw2+te2+tw3;

      while (realtime<t0+tw1+te1+tw2+te2+tw3+te3)
          [T0,Y0]=ode23tb(@Model,tspan,yy0,[],Ga,G,GR,B);
        Yact=Y0(:,8);                %amount of NF-kBn
        Yin=Y0(:,12);                %amount of IkBan
        TR=Y0(:,16);                 %TNF level
        Gax=Ga;Gx=G;GRx=GR;Bx=B;
       [mk,Ga,G,GR,B]=StatusChange(AN,ANa,ANR,TR,Gax,Gx,GRx,Bx,Yact,Yin,M); % function determining the change of gene status, call statuschange
        tc=T0(mk);                   %time when the status changes
        yy0=Y0(mk,:);                %transfer of initial conditions to the next iteration
        Y=[Y;Y0(2:mk,:)];            %rows from 2 do mk, all columns
        T=[T;T0(2:mk)+realtime];
        ga=[ga;Gax*ones(mk-1,1)];
        g=[g;Gx*ones(mk-1,1)];
        gR=[gR;GRx*ones(mk-1,1)];
        bb=[bb;Bx*ones(mk-1,1)];
        realtime=realtime+tc;
    end;


        nn=(realtime-t0-tw1-te1-tw2-te2-tw3-te3)/dt;
        x=size(Y);
        Y=Y(1:(x(1)-nn),:);
        T=T(1:(x(1)-nn));

        Y0(mk-nn,16)=TNF;         %setting TNF ON for the next step

        yy0=Y0(mk-nn,:);
        x1=length(ga);
        ga=ga(1:x1-nn);g=g(1:x1-nn);gR=gR(1:x1-nn);bb=bb(1:x1-nn);
        Ga=Gax;G=Gx;GR=GRx;B=Bx;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

```

```
459
460      YYY=YYY+Y;
461      GGa=GGa+ga;
462      GG=GG+g;
463      GGR=GGR+gR;
464      Bb=Bb+bb;
465
466  MM(i)=M;
467  NFF(i)=NF;
468
469  %##############################
470  %####### DATA FOR PLOTS #######
471  %##############################
472
473
474      XXX(i,:,:)=Y(:,:);
475      XB(i,:)=bb(:);
476      XG(i,:)=g(:);
477      XGa(i,:)=ga(:);
478      XGR(i,:)=gR(:);   % data for all cells
479
480  end;                  % end of the Main LOOP
481
482  clear tspan tspan1 g ga T0 Y0 Yact Yin Y y0 yy0 tindex;
483
484
485
486  YYY=YYY/N;                          %average over population
487  GGa=GGa/N;
488  GG=GG/N;
489  GGR=GGR/N;
490  Bb=Bb/N;
491  T=(T-t0)/60;
492
493
494  % % Data Processing : Counts Cells Responding to First and Second Pulse
495  % % designated for two pulses only
496  %
497  % RecetorsAverage=sum(MM)/N
498  % NFaverage=sum(NFF)/N
499  %
500  % NFKB=XXX(:,:,8)+XXX(:,:,15);
501  % both=0;
502  % onlyfirst=0;
503  % onlysecond=0;
504  %
505  % both2=0;
506  % onlyfirst2=0;
507  % onlysecond2=0;
```

Set to new variable

Accumulate Pattern

Per Cell

Per Cell

Cell Loop

Time in Minutes

```
508 %
509 % for i=1:N
510 %     s=0;
511 %     f=0;
512 %     s2=0;
513 %     f2=0;
514 %     aa=NFKB(i,:);
515 %     c1=size(aa);
516 %     c=round(c1(2)/2);
517 %     a1=aa(i:c);
518 %     a2=aa(c:2*c-1);
519 %
520 % if max(a1)>NF0/10
521 %     f=1;
522 % end
523 % if max(a1)>NF0/5
524 %     f2=1;
525 % end
526 % if max(a2)>NF0/10
527 %     s=1;
528 % end
529 % if max(a2)>NF0/5
530 %     s2=1;
531 % end
532 %     both=both+f*s;
533 %     onlyfirst=onlyfirst+f-f*s;
534 %     onlysecond=onlysecond+s-f*s;
535 %
536 %     both2=both2+f2*s2;
537 %     onlyfirst2=onlyfirst2+f2-f2*s2;
538 %     onlysecond2=onlysecond2+s2-f2*s2;
539 % end
540 %
541 % Threshold=0.1
542 %
543 % both
544 % onlyfirst
545 % onlysecond
546 % any=both+onlyfirst+onlysecond
547 %
548 % Threshold=0.2  % Used for this study
549 %
550 % both2
551 % onlyfirst2
552 % onlysecond2
553 % any2=both2+onlyfirst2+onlysecond2
554
555 %%% End of data processing %%%
556
```

```
557
558 simulation_time=etime(clock,starttime) %simulation time seconds
559 save last
560 AllCellPlotting
561
```

```
%#########################################################################
%####### Changes the values of the discrete variables             #######
%### First time to the next reaction is determined, then the reaction ####
%#########################################################################

function [mk,Ga,G,GR,B]=StatusChange(AN,ANa,ANR,TRx,Gax,Gx,GRx,Bx,Yact,Yin,M)

Ga=Gax;G=Gx;GR=GRx;B=Bx;
%calls Parameters
[NF0,NF1,NF2,M0,M1,M2,k4,ka20,AB,kv,q1,q2,c1,c3,c4,c5,k1,k2,k3,a1,a2,a3,c1a,c5a,
c6a,i1,i1a,e1a,e2a,dt,tp,KN,KNN,ka,ki,kb,kf,Tdeg,q1r,q2r,q2rr,c1r,c1rr,c3r]
=Parameters;

%   Yact= amount of NFkBn -Y(:,8)
%   Yin = amount of IKBa  -Y(:,12)
%   mk index (time) of gene status change

ro=(ANa-Gax)*q1*Yact+Gax*(q2*Yin)+(AN-Gx)*q1*Yact+Gx*(q2*Yin)+(ANR-GRx)
*q1r*Yact+GRx*(q2r*Yin+q2rr)+(M-Bx)*(kb*TRx)+Bx*kf; %total propensity function

roint=dt*cumtrapz(ro);          % propensity function integrated
fd=1-exp(-roint);               % Distribution of the switching time

r=rand;
if (fd(length(fd))<r)

    mk=length(fd);
end;

if (fd(length(fd))>=r)

a=abs(fd-r);
mk=find([a-min(a)]==0);         % mk= index (time) of next reaction
clear a fd r roint;

%#########################################################
%####### Determining which reaction takes place #######
%#########################################################

p1a=(ANa-Gax)*q1*Yact(mk);   % risk of NF-kB association to IKBa site at time mk
p2a=Gax*(q2*Yin(mk));        % risk of  NF-kB dissociation from IkBa at time mk

p1=(AN-Gx)*q1*Yact(mk);      % risk of NF-kB association to A20 site
p2=Gx*(q2*Yin(mk));          % risk of  NF-kB dissociation from A20 site

p1r=(ANR-GRx)*q1r*Yact(mk); % risk of NF-kB association to reporter gene
p2r=GRx*(q2r*Yin(mk)+q2rr); % risk of  NF-kB dissociation from reporter gene

p3=(M-Bx)*kb*TRx(mk);   % risk of TNFR1-TNF binding
```

```
47 p4=Bx*kf;                % rist of TNFR1 inactivation
48
49 ss=(p1a+p2a+p1+p2+p1r+p2r+p3+p4);
50 p1a=p1a/ss;p2a=p2a/ss;
51 p1=p1/ss;p2=p2/ss;
52 p1r=p1r/ss;p2r=p2r/ss;
53 p3=p3/ss;p4=p4/ss;
54
55 rnumber=rand;
56 if (rnumber<p1a)                                    Ga=Ga+1;end;    %IKBa
activates
57 if (rnumber>=p1a)&(rnumber<p1a+p2a)                 Ga=Ga-1;end;    %IKBa
inactivates
58
59 if (rnumber>=p1a+p2a)&(rnumber<p1a+p2a+p1)                  G=G+1;end;      %A20
activates
60 if (rnumber>=p1a+p2a+p1)&(rnumber<p1a+p2a+p1+p2)           G=G-1;end;      %A20
inactivates
61
62 if (rnumber>=p1a+p2a+p1+p2)&(rnumber<p1a+p2a+p1+p2+p1r)            GR=GR+1;
end;     %reporter gene activates
63 if (rnumber>=p1a+p2a+p1+p2+p1r)&(rnumber<p1a+p2a+p1+p2+p1r+p2r)    GR=GR-1;
end;     %reporter gene inactivates
64
65 if (rnumber>=p1a+p2a+p1+p2+p1r+p2r)&(rnumber<p1a+p2a+p1+p2+p1r+p2r+p3)
B=B+1;end;      %receptor activation
66 if (rnumber>=p1a+p2a+p1+p2+p1r+p2r+p3)&(rnumber<p1a+p2a+p1+p2+p1r+p2r+p3+p4)
B=B-1;end       %receptor deactivation
67
68 end;
```

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%                                                          %
%        Function includes system of ODEs describing       %
%        NF-kB regulatory pathway.                         %
%        Substrates are coded as follows:                  %
%                                                          %
%        y(1)   IKKKa active                               %
%        y(2)   IKKn   neutral                             %
%        y(3)   IKKa   active                              %
%        y(4)   IKKi   inactive                            %
%        y(5)   phospho-IkBa cytoplasmic                   %
%        y(6)   phospho-IkBa|NFkB cytoplasmic              %
%        y(7)   NFkB  cytoplasmic                          %
%        y(8)   NFkBn  nuclear                             %
%        y(9)   A20                                        %
%        y(10)  A20t                                       %
%        y(11)  IkBa                                       %
%        y(12)  IkBan                                      %
%        y(13)  IkBat                                      %
%        y(14)  (IkBa|NFkB) cytoplasmic                    %
%        y(15)  (IkBan|NFkBn) nuclear                      %
%        y(16)  extracellular TNF                          %
%                                                          %
%        y(17)  mRNA reporter                              %
%                                                          %
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

function dy=Model(t,y,Ga,G,GR,B)

[NF0,NF1,NF2,M0,M1,M2,k4,ka20,AB,kv,q1,q2,c1,c3,c4,c5,k1,k2,k3,a1,a2,a3,c1a,c5a,c6a,i1,i1a,e1a,e2a,dt,tp,KN,KNN,ka,ki,kb,kf,Tdeg,q1r,q2r,q2rr,c1r,c1rr,c3r]=Parameters;

%###########################################################

dy=zeros(19,1);

dy(1)=ka*B*(KN-y(1))* ka20/(ka20+y(9))-ki*y(1);                 % active IKKK kinase
dy(2)=-y(1)^2*k1*y(2)+k4*(KNN-y(2)-y(3)-y(4));                  % neutral IKK
dy(3)=y(1)^2*k1*y(2)-k3*y(3)*(k2+y(9))/k2;                      % free active IKK
dy(4)=k3*y(3)*(k2+y(9))/k2-k4*y(4);                             % inactive IKK
dy(5)=a2*y(3)*y(11)-tp*y(5);                                    % Phospo-IkBa cytoplasmic
dy(6)=a3*y(3)*y(14)-tp*y(6);                                    % cytoplasmic (phospho-IkBa|NF-kB)
```

```
42  dy(7)=c6a*y(14)-a1*y(7)*y(11)+tp*y(6)-i1*y(7);                                  %↙
free cytoplasmic NFkB
43  dy(8)=i1*y(7)-a1*kv*y(12)*y(8);                                                 %↙
free nuclear NFkB
44  dy(9)=c4*y(10)-c5*y(9);                                                         %↙
cytoplasmic A20
45  dy(10)=c1*G-c3*y(10);                                                           %↙
A20 transcript
46  dy(11)=-a2*y(3)*y(11)-a1*y(11)*y(7)+c4*y(13)-c5a*y(11)-i1a*y(11)+e1a*y(12);     %↙
free cytoplasmic IkBa
47  dy(12)=-a1*kv*y(12)*y(8)+i1a*y(11)-e1a*y(12);                                   %↙
free nuclear IkBan
48  dy(13)=c1a*Ga-c3*y(13);                                                         %↙
IkBa transcript
49  dy(14)=a1*y(11)*y(7)-c6a*y(14)-a3*y(3)*y(14)+e2a*y(15);                         %↙
cytoplasmic (IkBa|NFkB) complex
50  dy(15)=a1*kv*y(12)*y(8)-e2a*y(15);                                              %↙
nuclear (IkBa|NFkB) complex
51  dy(16)=-Tdeg*y(16);                                                             %↙
extracellular TNF
52  dy(17)=c1rr+c1r*GR-c3r*y(17);                                                   %↙
Reporter transcript
53
54
55
```

```
% %#########################################################################
% %#####    PARAMETERS corresponding to S. Tay el al. 2010,  Nature     ##
% %#####   Some frequently changed parameters are defined in MainFile   ##
% %#########################################################################


function [NF0,NF1,NF2,M0,M1,M2,k4,ka20,AB,kv,q1,q2,c1,c3,c4,c5,k1,k2,k3,a1,a2,a3,c1a,c5a,c6a,i1,i1a,e1a,e2a,dt,tp,KN,KNN,ka,ki,kb,kf,Tdeg,q1r,q2r,q2rr,c1r,c1rr,c3r]=Parameters

% ########################################################
dt=10;              %simulation step s
% ########################################################

kv=5;          %kv=5,

%####### Randomization of Receptors and NF-kB levels ############

M0=5000;  % 2000 mean number of TNFR1 receptors assumed for 3T3 cells (our experiment)
          % Assumed M0=10000 for MEFS, M0=5000 for SK-N-AS, 500 for HeLa to acount
          % for different cell sensitivities
M1=sqrt(2);
M2=-1;

 % Lognormal distribution with Median=M0*Exp(M2), Mean=M0*Exp(M2+M1^2/2)=M0
 % Variance = M0^2 * (Exp (M1^2 -1) * Exp(2*M2+M1^2)


NF0=10^5; % mean NF-kB
NF1=1/sqrt(2);
NF2=-1/4;

% Lognormal distribution with Median=NF0*Exp(NF2), Mean=NF0*Exp(NF2+NF1^2/2)=NF0
% Variance = NF0^2 * (Exp (NF1^2 -1) * Exp(2*NF2+NF1^2)


%#########################################################
%##### Parametrization for the genes and receptor part ######
%#########################################################

%####### Receptors activation #######

kb=1.2*10^-5;        %default 1.2*10^-5 = receptor activation rate
kf=1.2*10^-3;        %default 1.2*10^-3 = receptor inactivation rate

%####### A20 IkBa Promoters binding #######

```

```
46 q1=4*10^-7;      %default 4*10^-7 - NF-kB atatching at A20 and IkBa site
47 q2=10^-6;        %default 10^-6 - IkBa inducible detaching from A20 and IkBa site
48
49
50 %#########################################################
51 %####### Parametrization for the deterministic part #######
52 %#########################################################
53
54
55 Tdeg=7.7*10^-4; % TNF loss
56                 % 2*10^-4  for 10ng (t1/2=60min)
57                 % 7*10^-4  for 1ng,
58                 % 7.7*10^-4 for 0.1ng
59                 % 8.3*10^-4 for 0.01ng
60
61                 % use 2*10^-4 for experiments in other than microfluidics
62
63 %####### Transduction pathway #######
64
65 KN=10^5;          %default 10^5 - total number of IKKK kinase molecules,
Assumption
66 KNN=2*10^5;       %default 2*10^5 - total number of IKK kinase molecules,
Assumption
67 ka=2*10^-5;       %default 2*10^-5 - IKKK kinase activation rate (at most 1/s),
Assumption
68 ki=0.01;         %default 0.01 - IKKK kinase inactivation rate, Assumption
69
70 %####### A20 and IKK #######
71
72 AB=1;             %A20 on (or off)
73
74 c0=0.1;          %default 0.1 - inducible A20 and IkBa mRNA synthesis, Assumption
75 c1=AB*c0;        %inducible A20 mRNA synthesis
76 c3=0.00075;      %default 0.00075 - A20 and IkBa mRNA degradation rate
77 c4=0.5;          %default 0.5 - A20 and IkBa translation rate, FIT
78 c5=0.0005;       %default 0.0005 - A20 degradation rate, FIT
79 ka20=10^5;       %default 10^5 - A20 TNFR1 block, FIT
80 k2=10000;        %default 10000 - IKKa inactivation caused by A20, FIT
81 k1=6*10^-10;     %default 6*10^-10; IKKn activation caused by active IKKK,
Assumption
82 k3=0.002;        %default 0.002 - IKKa inactivation, FIT
83 k4=0.001;        %default 0.001 - IKKii transformation, FIT
84
85 %####### IkB alpha #######
86
87 AA=1;            %IkBa on (or off)
88 c1a=AA*c0;       %inducible IkBa mRNA synthesis
89 a1=5*10^-7;      %default 5*10^-7 - IkBa*NFkB association, Assumption
90 a2=10^-7;        %default 10^-7 - IkBa phosphoryation due to action of IKKa, FIT
```

```
 91 a3=5*10^-7;        %default 5*10^-7 - (IkBa|NFkb) phosphorylation due to action of IKKa,  FIT
 92 tp=0.01;           %default 0.01 - degradation of phospho-IkBa and phospho-IkBa complexed to NF-kB, FIT
 93 c5a=0.0001;        %default 0.0001 - IkBa degradation rate
 94 c6a=0.00002;       %default 0.00002 - spontaneous (IkBa|NFkB) degradation of IkBa complexed to NF-kB
 95
 96
 97 %######## Reporter gene  ##############
 98
 99 q1r=1*10^-7;        %default 1*10^-7 NF-kB ataching at reporter gene site
100 q2r=1*10^-7;        %default 1*10^-7 inducible NF-kB detaching from reporter gene site
101 q2rr=1*10^-3;       %default 1*10^-3 spontaneous NF-kB detaching from reporter gene site
102
103
104 c1r=0.05;           %default 0.05 Reporter gene mRNA inducible synthesis
105 c1rr=0.001;         %default 0.001 Reporter gene mRNA constitutive synthesis
106 c3r=0.001;          %various considered,  Reporter mRNA degradation rate
107
108
109 %####### Transport #######
110
111 i1=0.01;           %default 0.01 - NFkB nuclear import, FIT
112 e2a=0.05;          %default 0.05 - (IkBa|NFkB) nuclear export, FIT
113 i1a=0.002;         %default 0.002 - IkBa nuclear import, FIT
114 e1a=0.005;         %default 0.005 - IkBa nuclear export, FIT
115
```