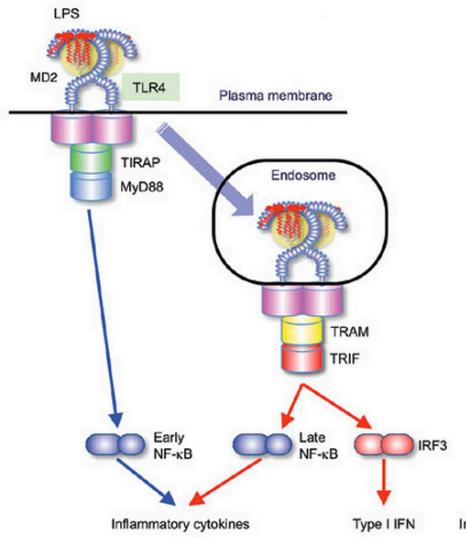
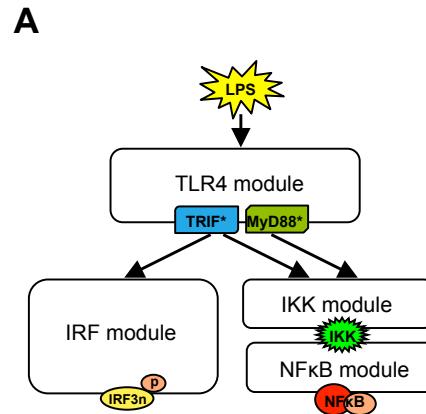


TLR4 is special

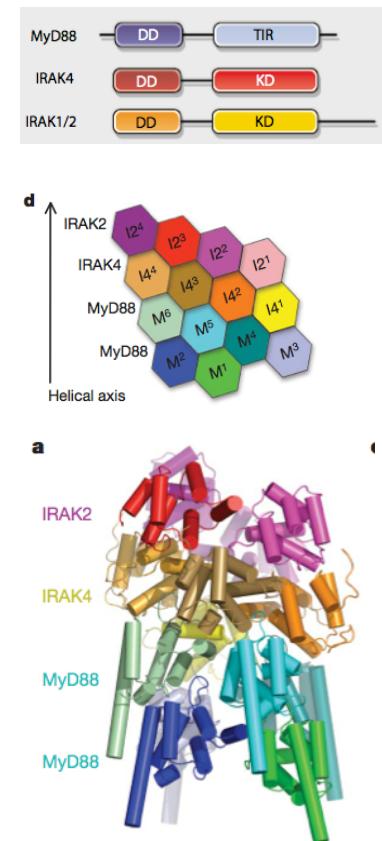
Spatial



Topological



Structural



Dynamical features in the sub-pathways (quantitatively)

Molecular details in shaping the dynamics

Current conclusions and/or hypothesis:

Dynamical features in the sub-pathways (prediction):

- MyD88 sub-pathway determine the response speed.
- TRIF sub-pathway determine the response duration(linearly increase to log dose).
- Will strengthen by single cell data (first peak, duration, integral)

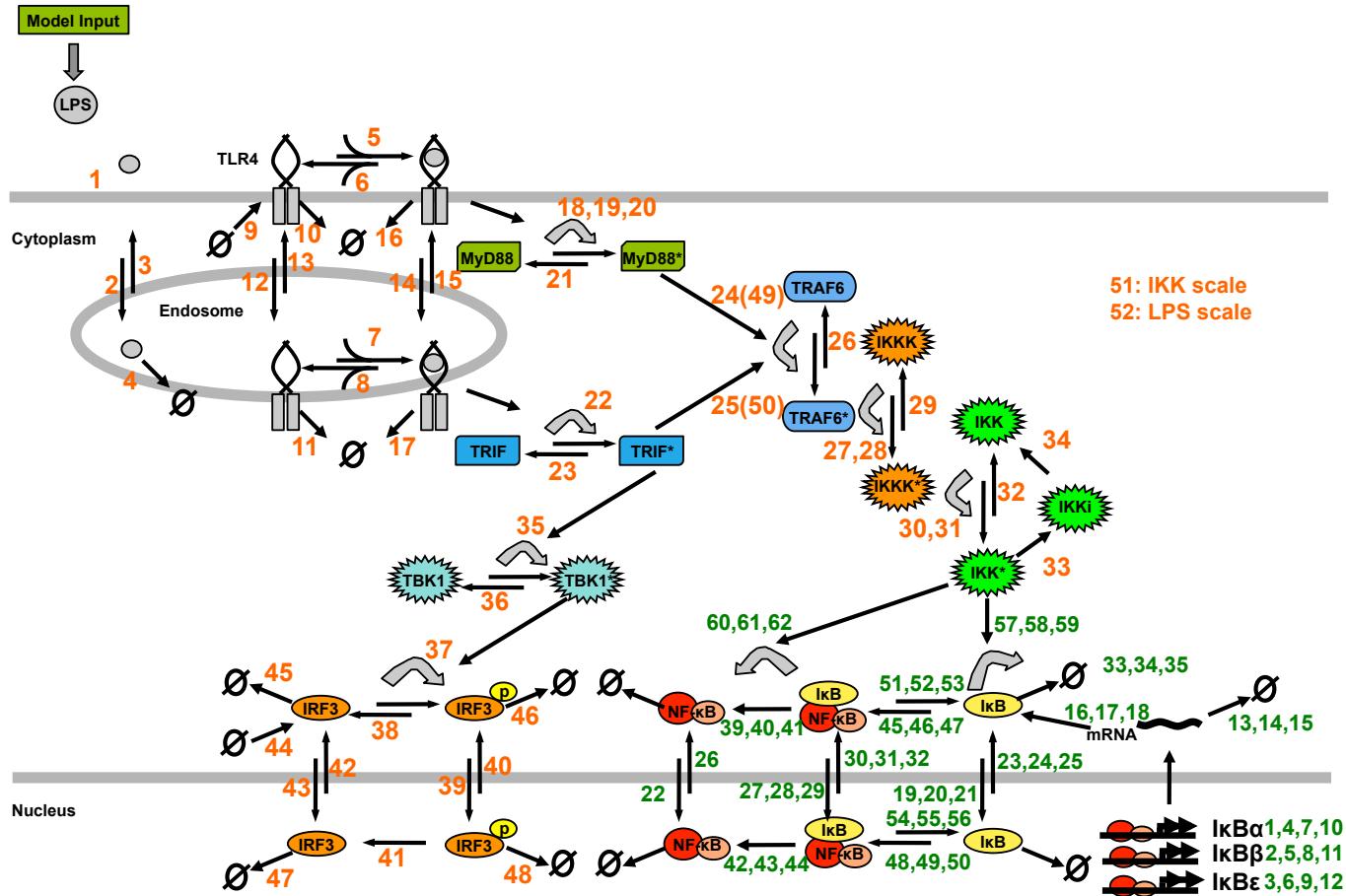
Molecular details in shaping the dynamics (MyD88):

- Myddosome formation produce threshold in signaling activation
- ~~The ligand induced shuttling is responsible for the duration specificity~~
- Threshold provides switch-like first peak behavior

Stochasticity happens at the level of sub-pathway activation can explain single cell data

- Stochastic activation in Trif alone can generate ~~two clusters~~ heterogeneous post-first-peak behavior (very stochastic, due to endocytosis??)
- But the stochasticity in MyD88 will bring in viability in response speed (very robust)

The TLR4 model diagram



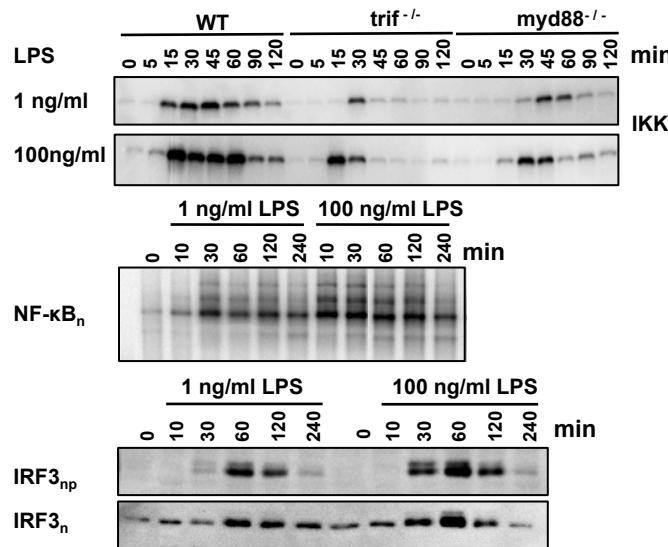
3 compartments

41 species

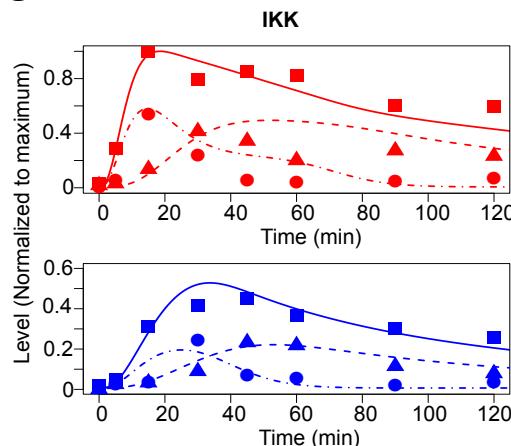
100 Reactions

Model validation and experimental data

B

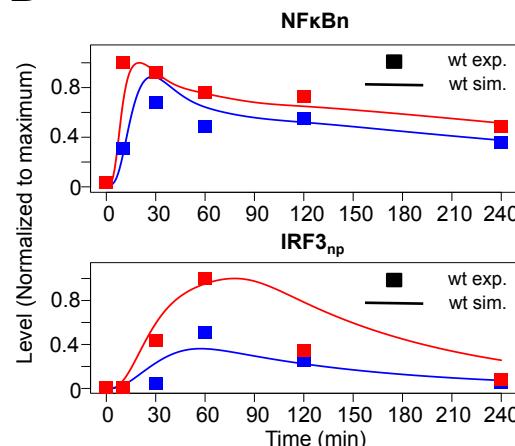


C



Red: 100 ng/ml LPS; Blue: 1 ng/ml LPS
 ■ wt exp. ■ wt sim.
 ▲ mko exp. — mko sim.
 ● tko exp. - - - tko sim.

D

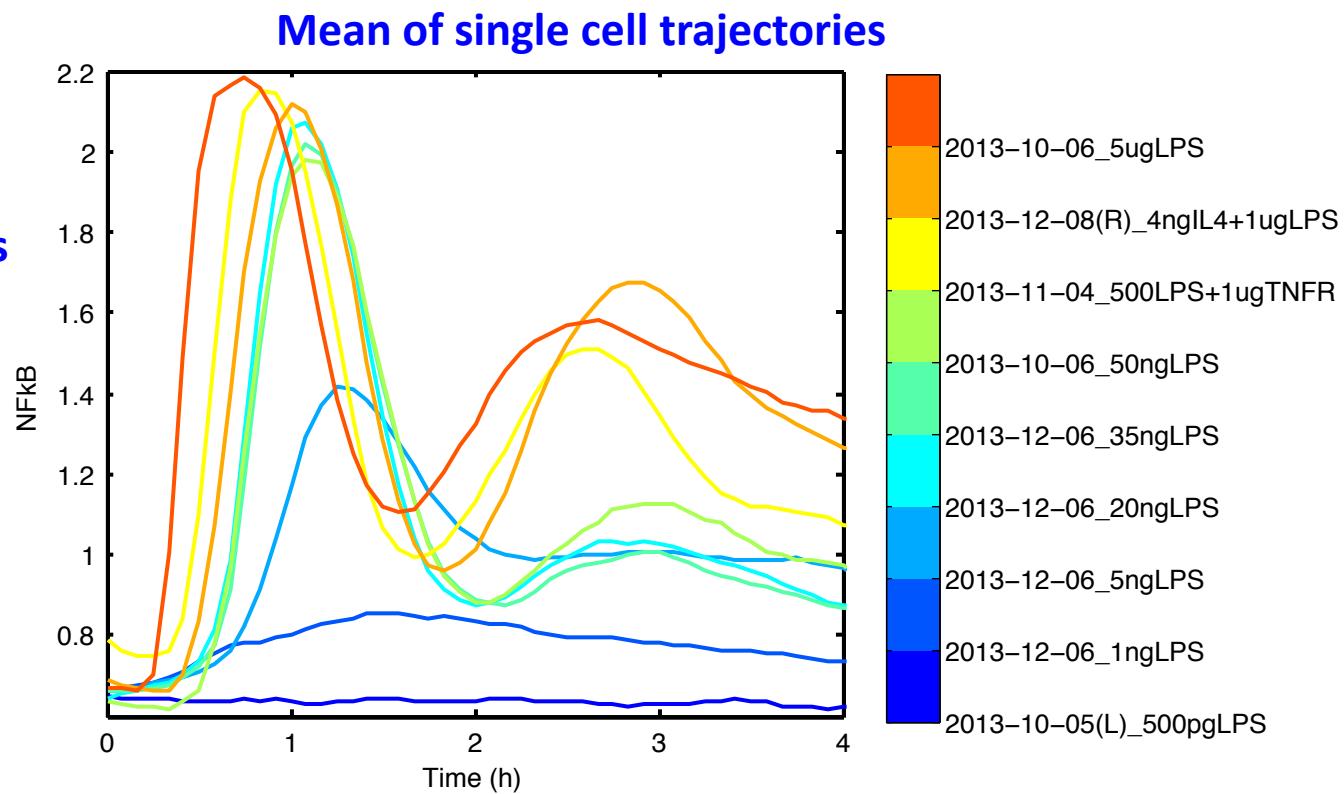
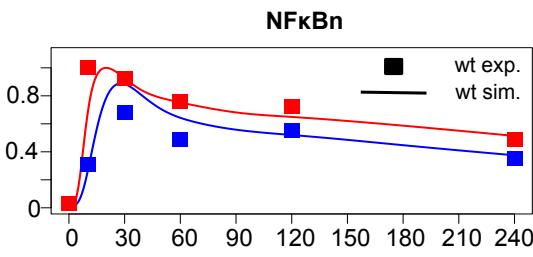


Red: 100 ng/ml LPS; Blue: 1 ng/ml LPS

Challenge: Fit the mean behavior of single cell data for first 4 hours

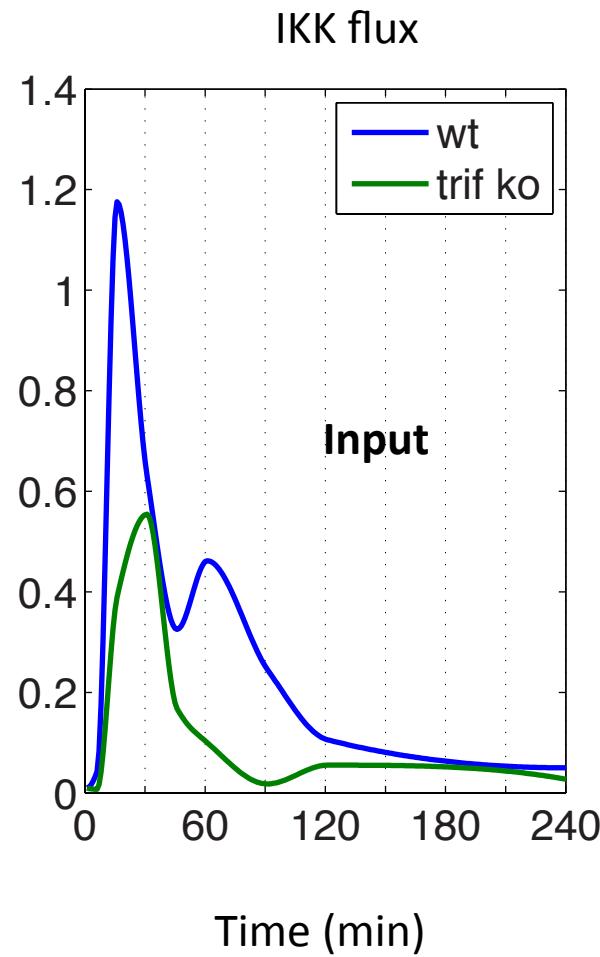
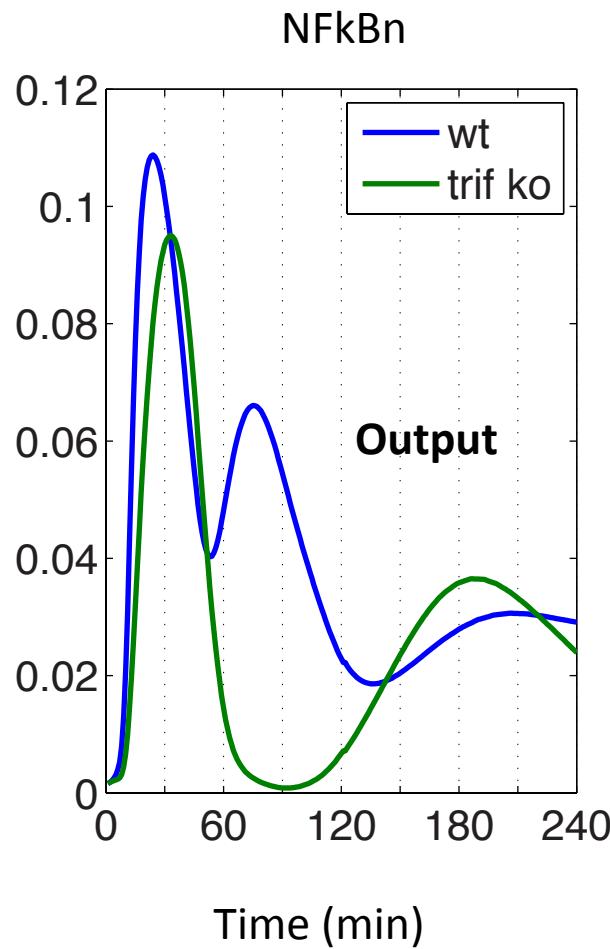
- Change as little as possible (**find key parameters**)
- NFkB module
- Negative feedback is the key

EMSA data and simulations

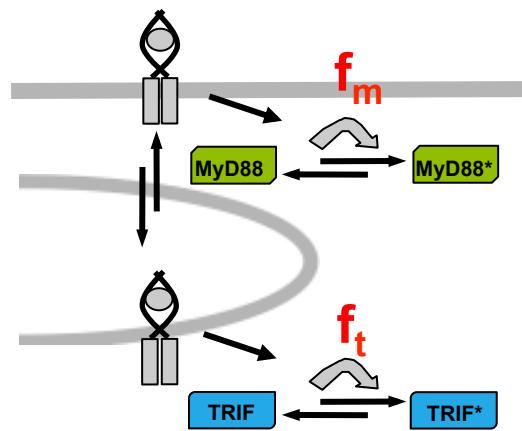


➤ Two peaks

The NFkB module is trending to be oscillation.



To capture the variability in single cell data

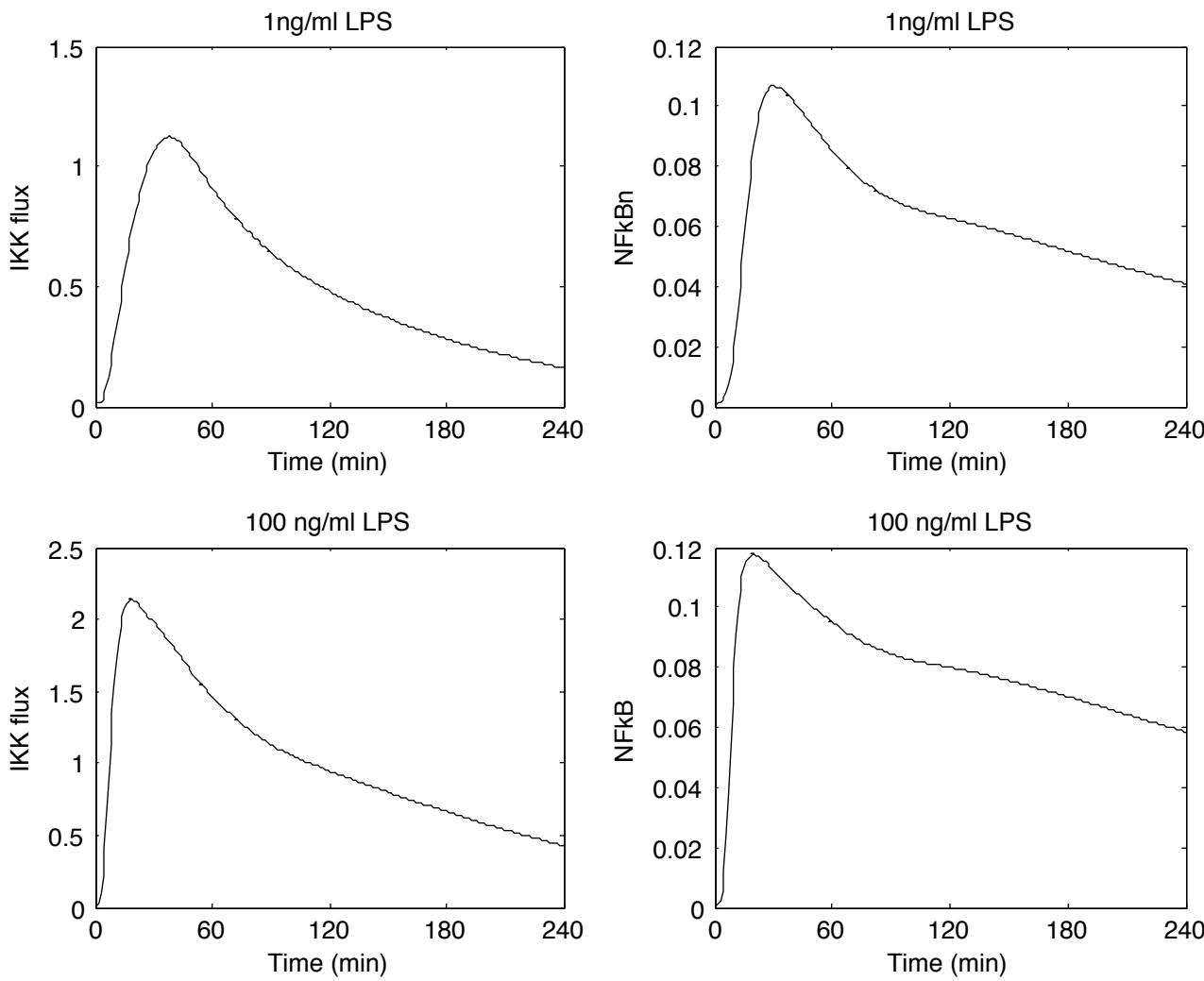


+

Another key parameter

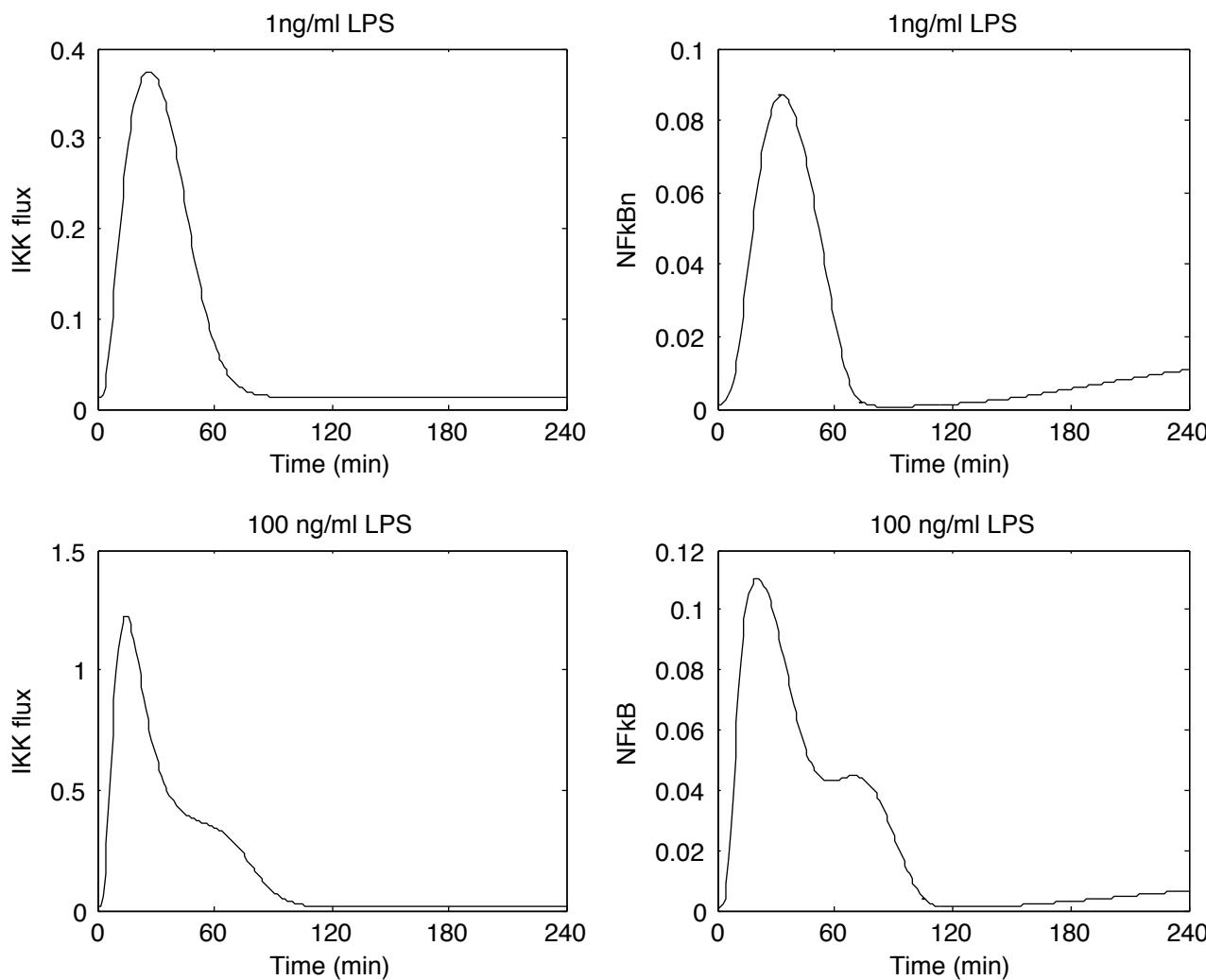
Randomize those parameters
(log-normal distribution or other)

Diminish the oscillation: I increased the IKK flux by a modifier



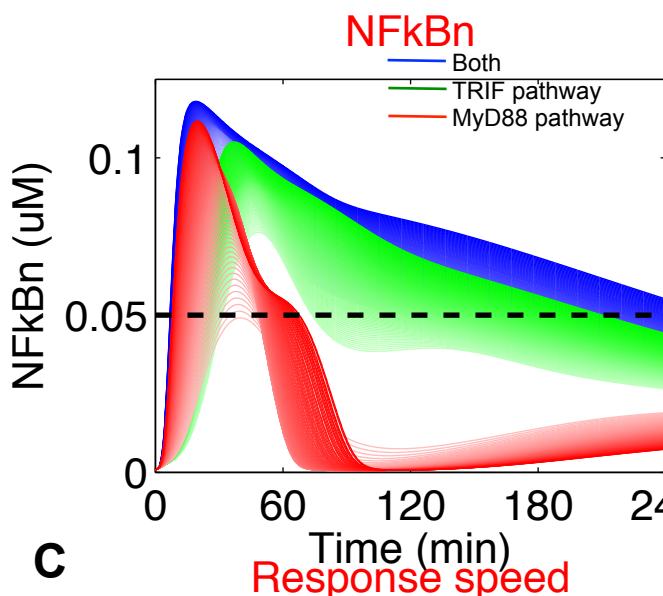
Diminish the oscillation: I increased the IKK flux by a modifier

trif ko

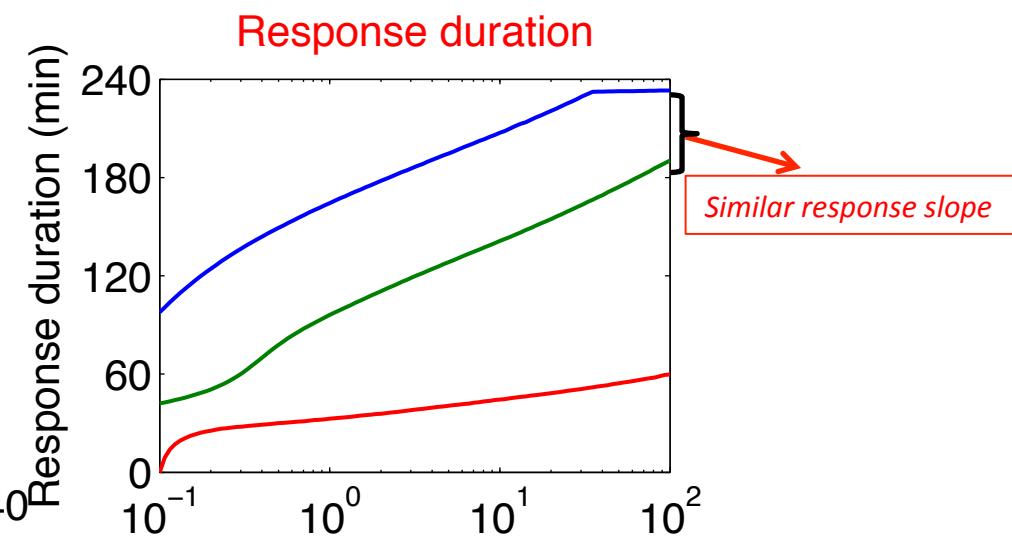


Dynamics features in sub-pathways: duration, responsive speed

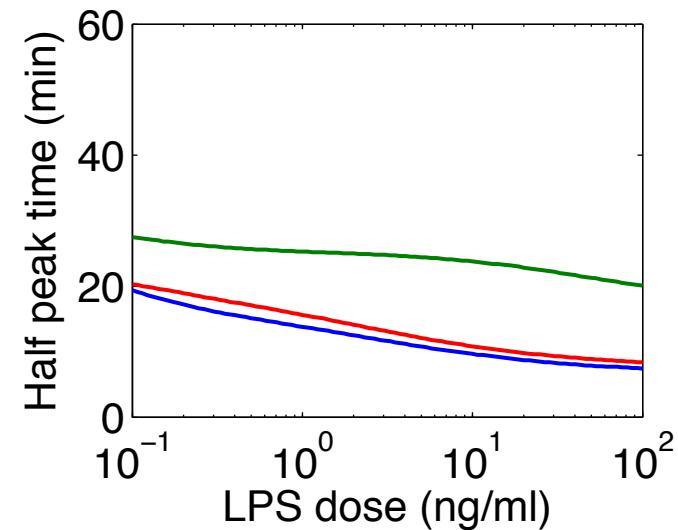
A



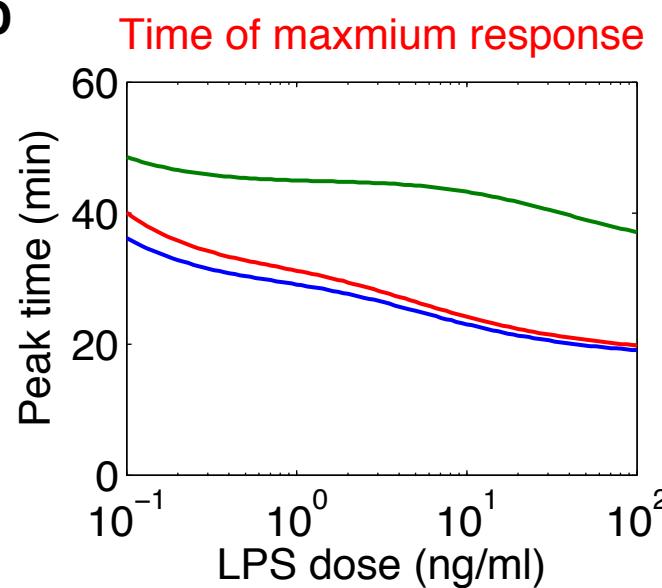
B



C



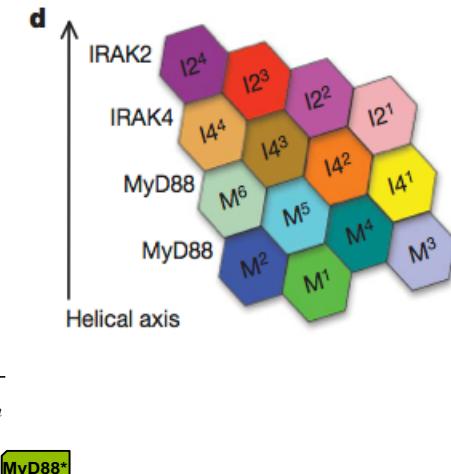
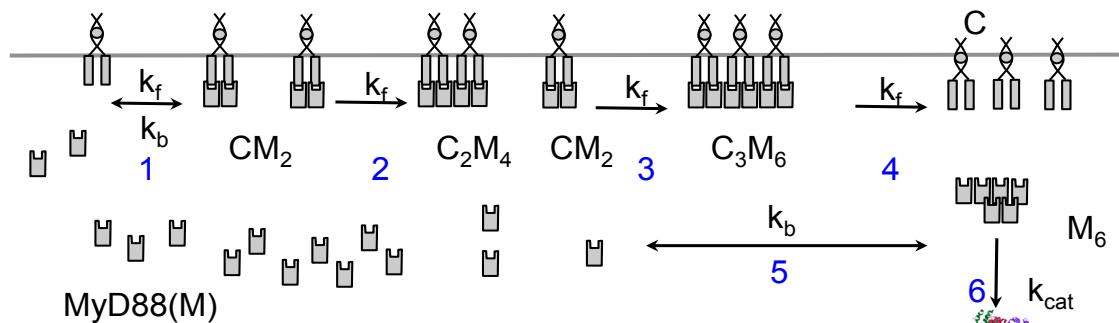
D



Myddosome formation produce kinetic threshold in signaling activation (1)

Input

Ligand-Receptor complex (C)



Output

MyDDosome model

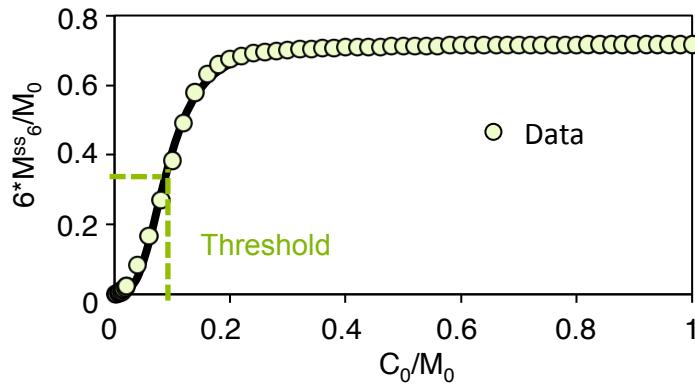
```

dM/dt      <- -2*k1*C*M + 2*k_1*CM2 + 6*kd*M6
dC/dt      <- k_1*CM2-k1*C*M + 3*k4*C3M6
dCM2/dt    <- k1*C*M - k_1*CM2 - 2*k2*CM2^2 - k3*CM2*C2M4
dC2M4/dt   <- k2*CM2^2 - k3*CM2*C2M4
dC3M6/dt   <- k3*C2M4*CM2 - k4*C3M6
dM6/dt     <- k4*C3M6 - k5*M6
  
```

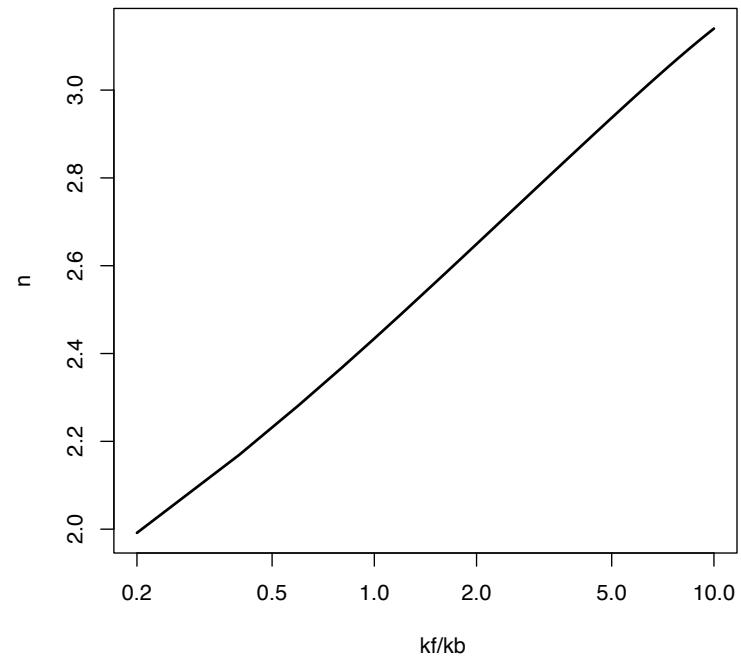
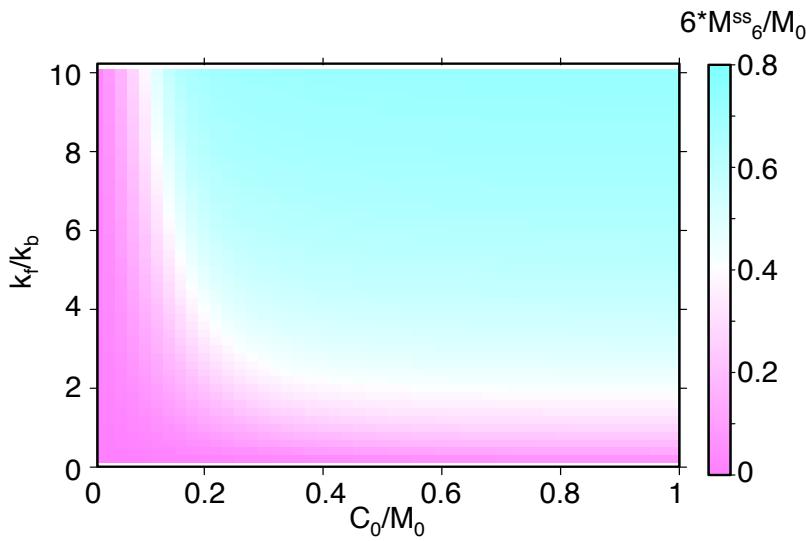
$k1=kf$, $k_1=kb$, $k2=kf$, $k3=kf$, $k4=kf$, $k5=kb$
 $kf=1$, $kb=0.1$
 $M0=10$, $C0=from\ 0\ to\ 10$

Myddosome formation produce kinetic threshold in signaling activation (2)

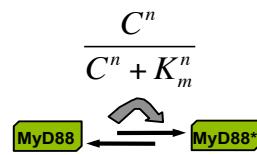
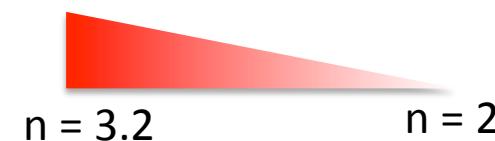
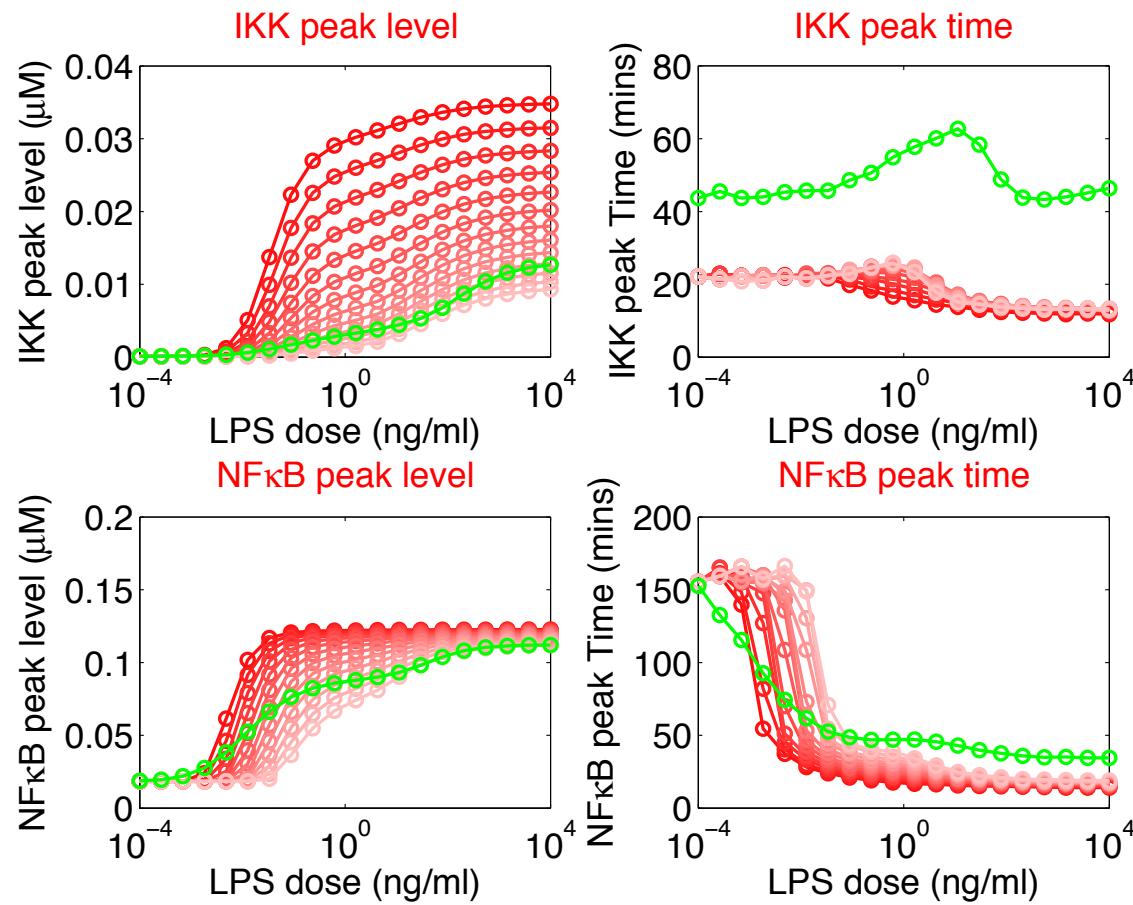
A



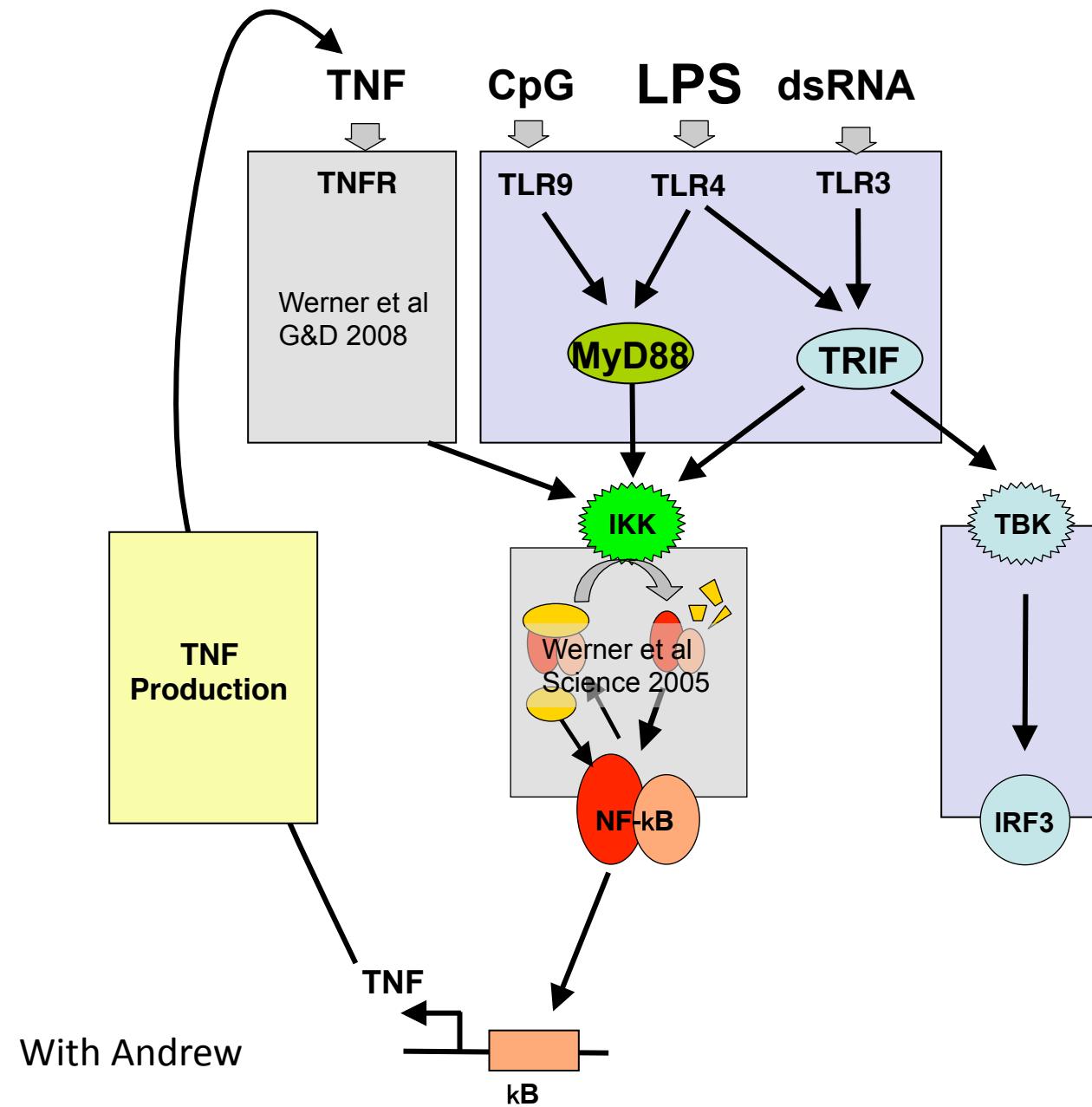
B



The kinetic threshold in MyD88 activation produce the switch-like behavior in the first peak

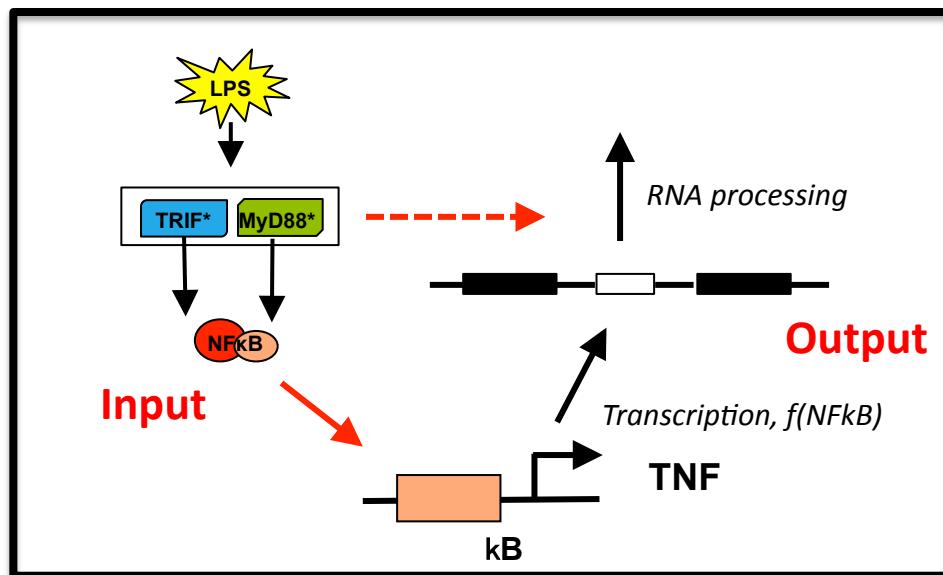


What's next?



Part 1: TNF production module

Module1 : Transcription + RNA processing



Equation

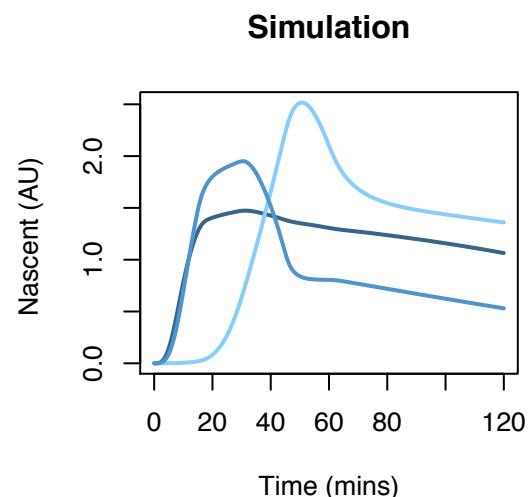
$$\frac{d[Nascent]}{dt} = V_{tr} \frac{[NF\kappa B_n]^{n_H}}{[NF\kappa B_n]^{n_H} + K_{dtr}^{n_H}} - k_{pr}[Nascent]$$

Initial condition

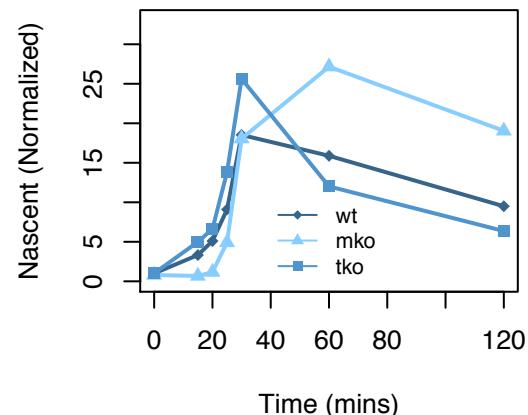
$$[Nascent]_{t=0} = \frac{V_{tr}}{k_{pr}} \frac{[NF\kappa B_n]_{t=0}^{n_H}}{[NF\kappa B_n]_{t=0}^{n_H} + K_{dtr}^{n_H}}$$

Parameters ($n_p = 5$)

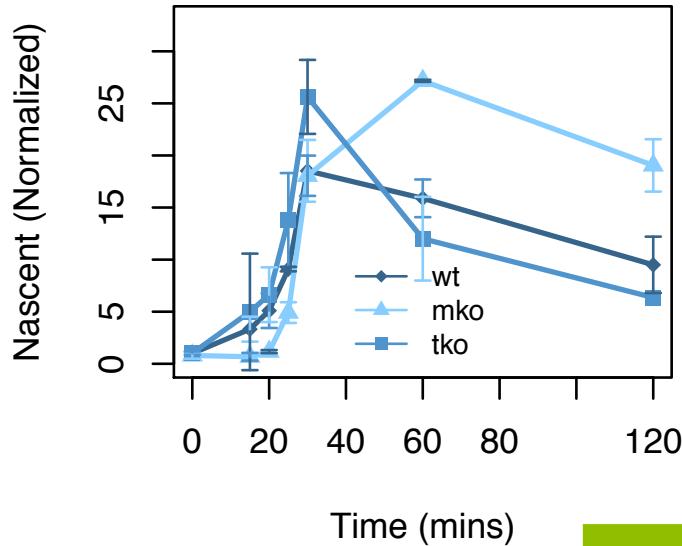
$$V_{tr} = 1(\text{fixed}), K_{dTr} = 0.65, n_H = 2, k_{pr}^{wt} = 0.4 \text{ min}^{-1}, k_{pr}^{mko} = \frac{k_{pr}^{wt}}{4.2}, k_{pr}^{tko} = \frac{k_{pr}^{wt}}{1.5}$$



Experiment



Experiment



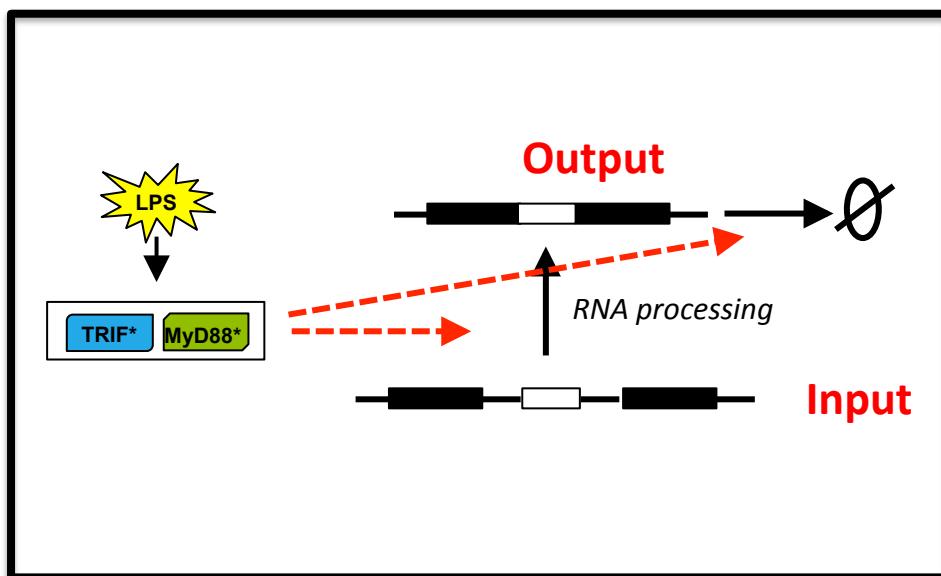
Score function (modified chi square)

$$\chi_m^2 = \frac{1}{n_F - n_p - 1} \sum_{i=1}^{n_F} \frac{(F_i^s - F_i^e)^2}{\sigma_i^2}$$

Table of features ($n_F=9$)

Index(i)	Feature	Value(F_e^i)	Error(σ_i)
1	Peak time (wt)	30 min	5 min
2	Peak time (mko)	60 min	10 min
3	Peak time (tko)	30 min	5 min
4	Peak_wt/Peak_mko	0.68	0.11
5	Peak_wt/peak_tko	0.75	0.22
6	Wt(60)/tko(60)	1.55	0.67
7	Wt(60)/mko(60)	0.59	0.07
8	Wt(120)/mko(120)	0.52	0.21
9	Wt(120)/tko(120)	1.49	0.43

Module 2: RNA processing + stabilization



Equation

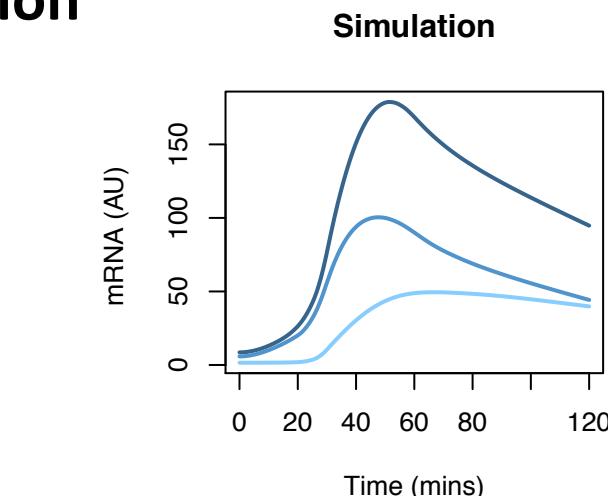
$$\frac{d[mRNA]}{dt} = k_{pr}[Nascent] - k_{deg\ mRNA}[mRNA]$$

Initial condition

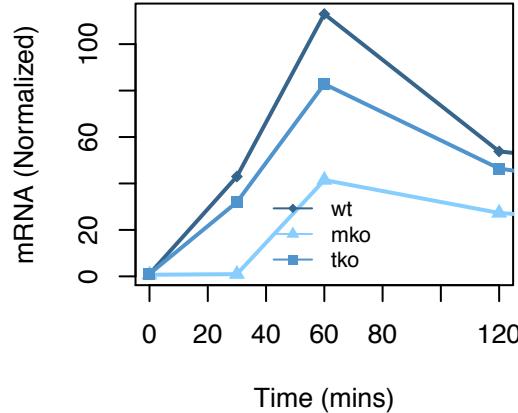
$$[mRNA]_{t=0} = \frac{k_{pr}}{k_{deg\ mRNA}} [nascent]_{t=0}$$

Parameters ($n_p = 5$)

$$k_{pr}^{wt} = 0.6 \text{ min}^{-1}, k_{pr}^{mko} = \frac{k_{pr}^{wt}}{4.5}, k_{pr}^{tko} = \frac{k_{pr}^{wt}}{1.5}, k_{deg\ mRNA} = 0.07 \text{ min}^{-1}, k_{deg\ mRNA}^{wt/mko}$$

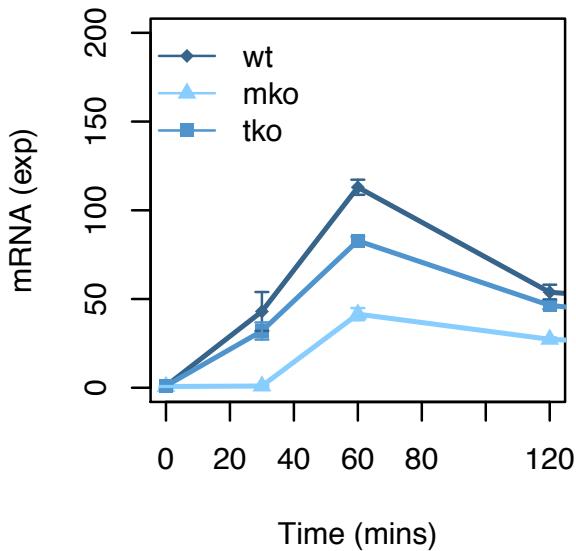


Experiment



$$k_{deg\ mRNA} = \begin{cases} -\frac{0.05}{30}t + 0.07 \text{ min}^{-1} & (0 \leq t < 30 \text{ min}) \\ \frac{0.05}{30}(t - 30) + 0.02 \text{ min}^{-1} & (30 \text{ min} \leq t < 60 \text{ min}) \\ 0.07 \text{ min}^{-1} & (60 \text{ min} \leq t) \end{cases}$$

Experimental data



Score function (modified chi square)

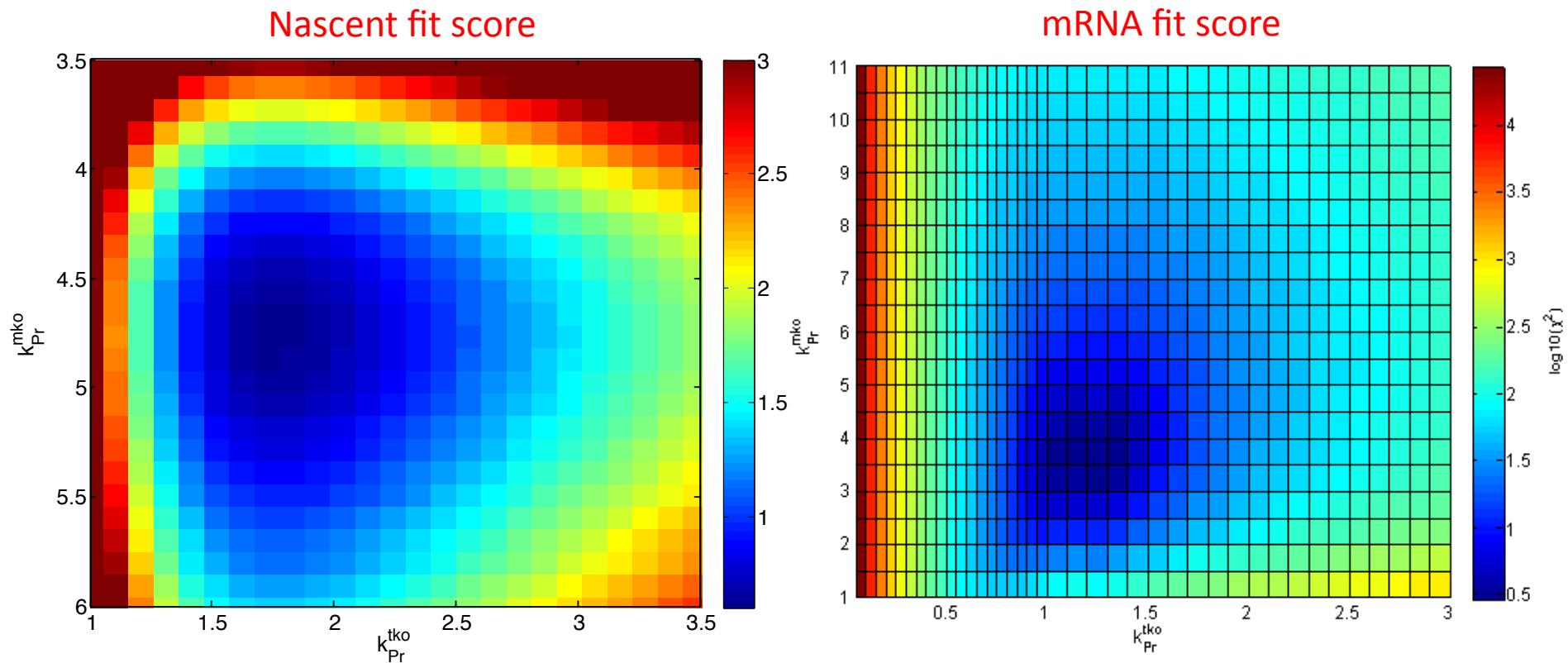
$$\chi_m^2 = \frac{1}{n_F - n_p - 1} \sum_{i=1}^{n_F} \frac{(F_i^s - F_i^e)^2}{\sigma_i^2}$$

Table of features ($n_F=7$)

Index(i)	Feature	Value(F_i^s)	Error(σ_i)
1	Peak time (wt)	60 min	10 min
2	Peak time (mko)	60 min	10 min
3	Peak time (tko)	60 min	10 min
4	Peak_tko/Peak_wt	0.73	0.05
5	Peak_mko/peak_tko	0.50	0.06
6	wt_120/mko_120	1.98	0.23
7	Wt_120/tko_120	1.17	0.14

How well the model capture the features.

Score heat map for processing rate in different genotypes



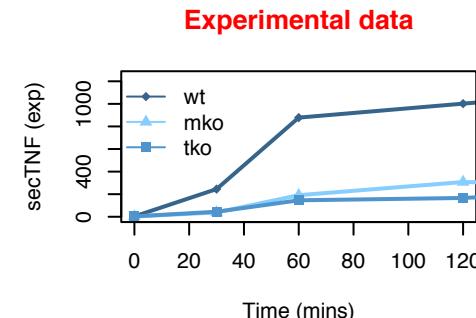
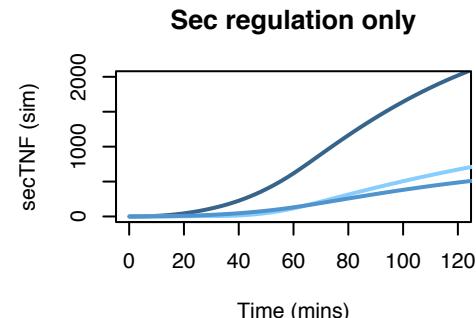
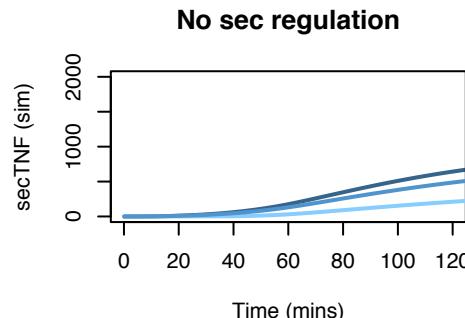
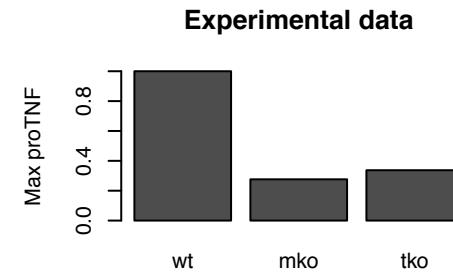
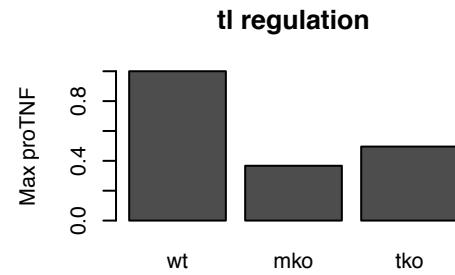
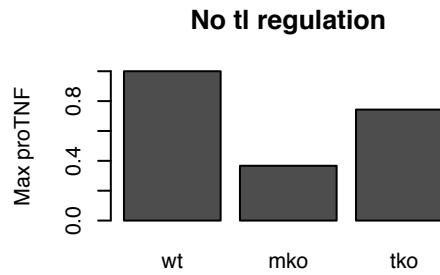
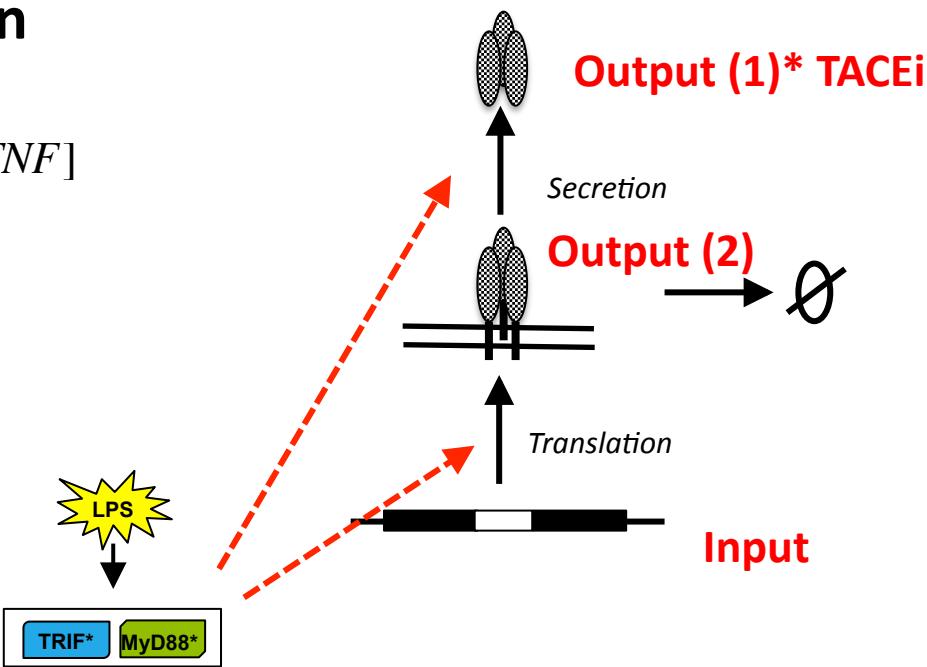
Module 3: Translation + Secretion

$$\frac{d[proTNF]}{dt} = k_{tl}[mRNA] - k_{degP}[proTNF] - k_{sec}[proTNF]$$

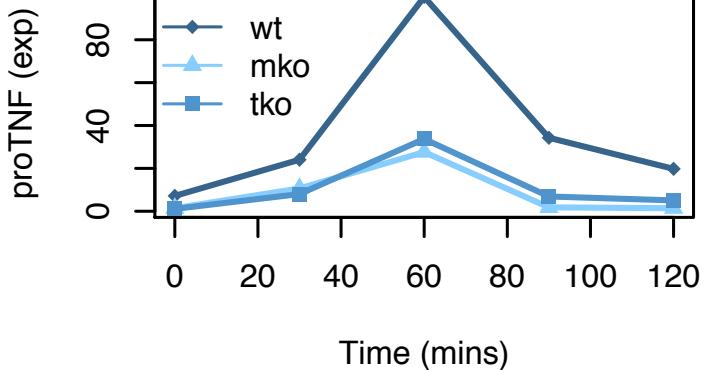
$$[secTNF](t) = \int_0^t k_{sec}[proTNF] dt$$

$$k_{tl}^{wt/mko} = 0.05 \text{ min}^{-1}, k_{tl}^{tko} = \frac{k_{tl}^{wt}}{1.5}, k_{degP} = 0.07 \text{ min}^{-1},$$

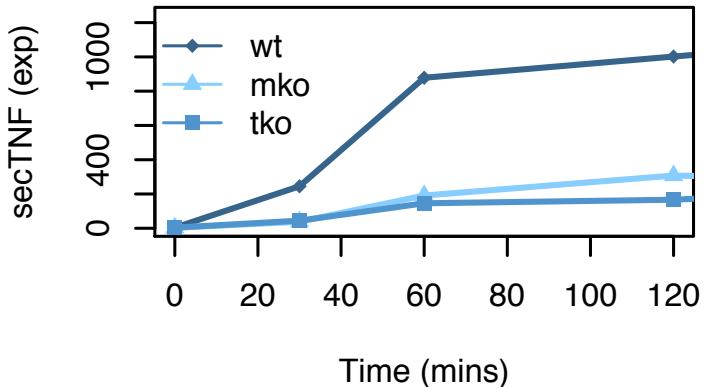
$$k_{sec}^{wt/mko} = 0.07 \text{ min}^{-1}, k_{sec}^{tko} = \frac{k_{sec}^{wt}}{5}$$



Experimental data



Experimental data

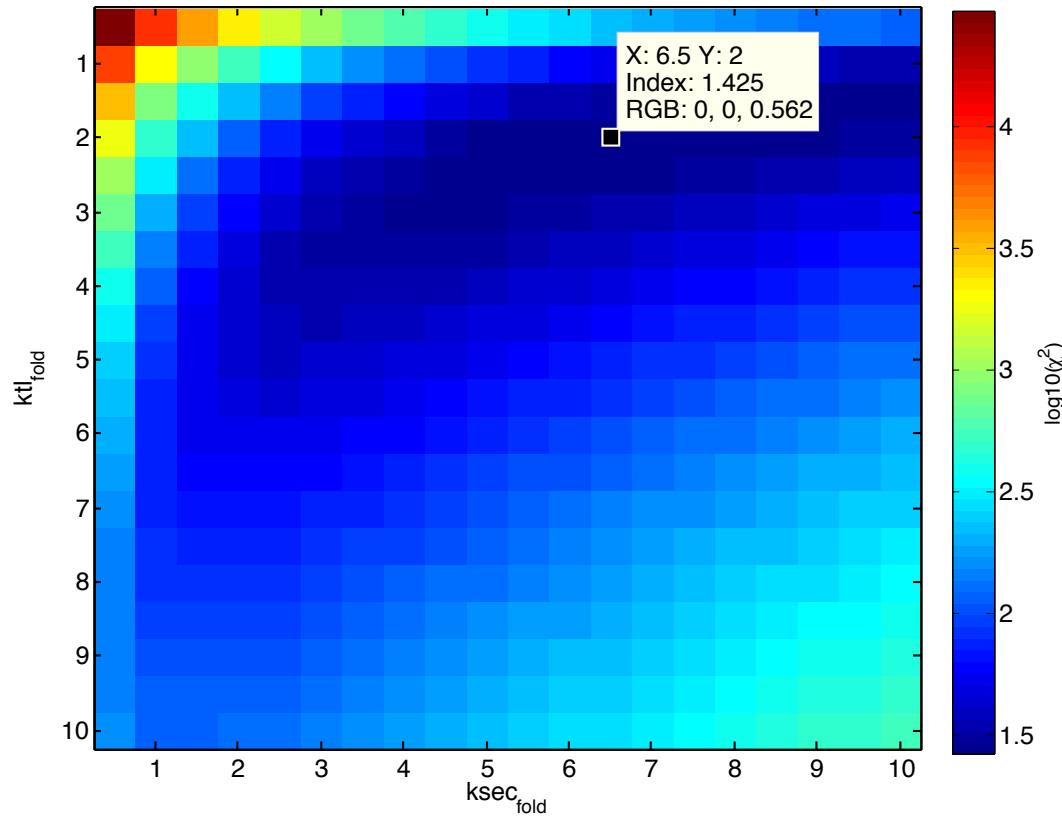


Index(i)	Feature	Value(F^s_i)	Error(σ_i)
1	Peak time (wt)	60 min	10 min
2	Peak time (mko)	60 min	10 min
3	Peak time (tko)	60 min	10 min
4	Peak_tko/Peak_wt	0.34	0.06
5	Peak_mko/peak_tko	0.82	0.14
6	wt_120/Peak_wt	0.20	0.04
7	Wt_120/tko_120	3.9	0.65
8	wt_120/mko_120	14	2.3
9	Tko_30/mko_30	0.75	0.12

Index(i)	Feature	Value(F^s_i)	Error(σ_i)
1	tko_60/wt_60	0.17	0.03
2	Mko_60/tko_60	1.4	0.54
3	Tko_120/wt_120	0.17	0.03
4	Mko_120/tko_120	1.9	0.76
5	wt_120/wt_60	1.2	0.16

How well the model capture the features.

Score heat map for different parameters.



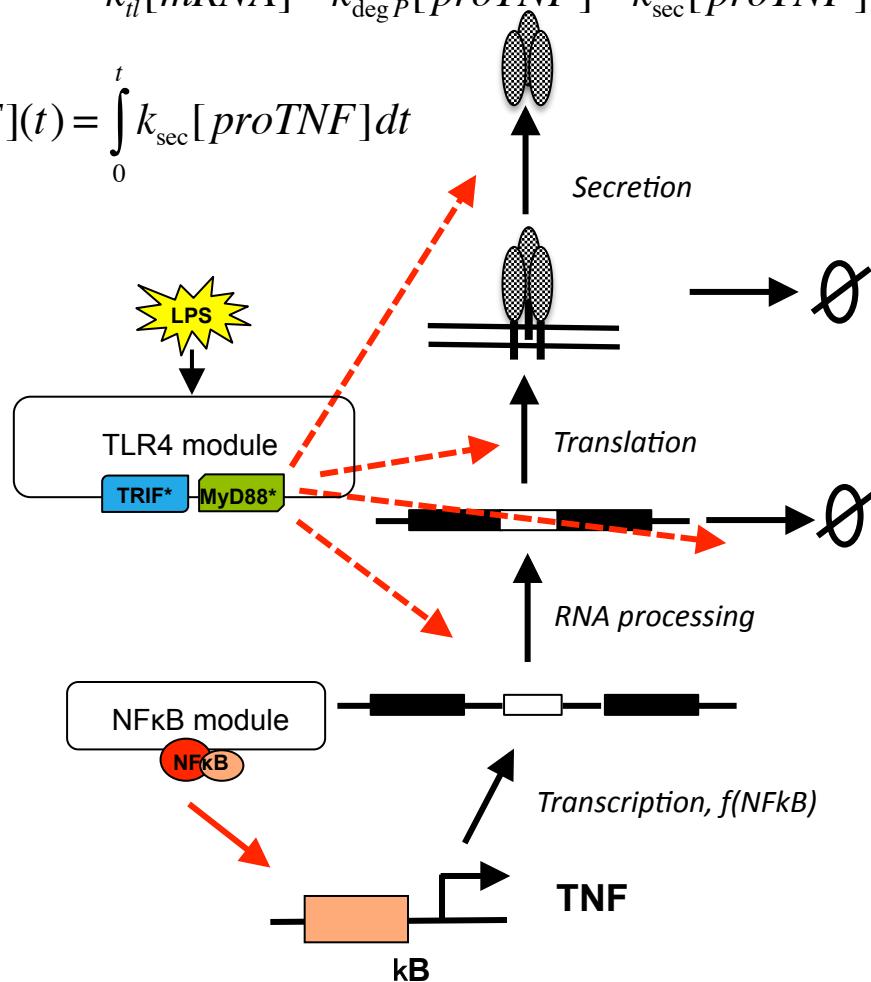
Module 1 to 3, together (NFκB as input, 4 outputs)

$$\frac{d[Nascent]}{dt} = V_{tr} \frac{[NF\kappa B_n]^{n_H}}{[NF\kappa B_n]^{n_H} + K_{dtr}^{n_H}} - k_{pr}[Nascent]$$

$$\frac{d[mRNA]}{dt} = k_{pr}[Nascent] - k_{deg\ mRNA}[mRNA]$$

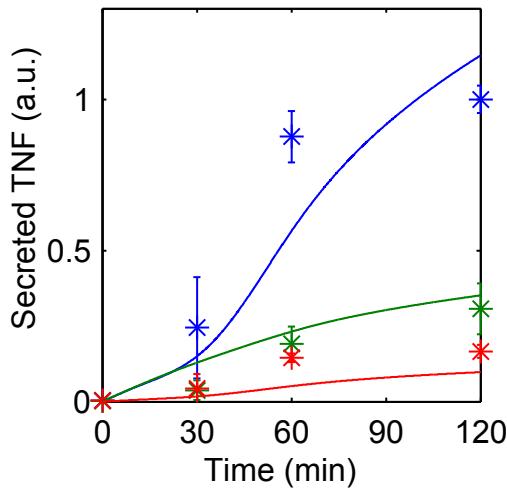
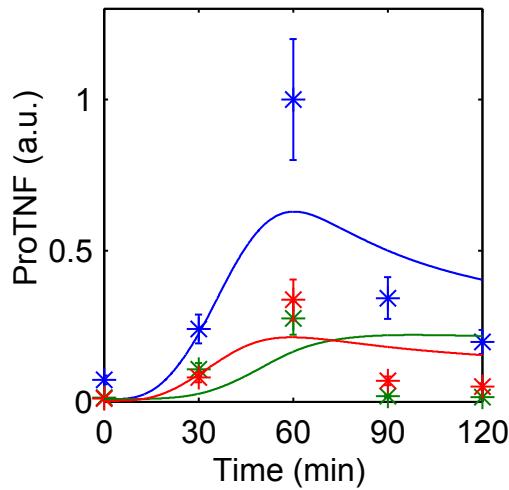
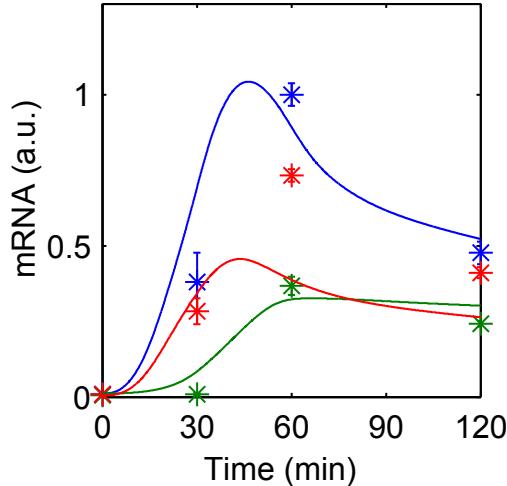
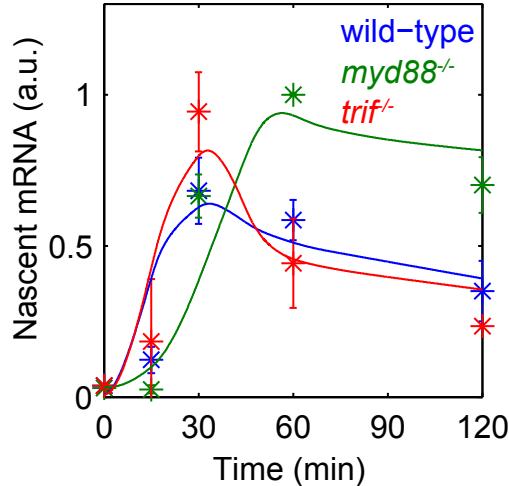
$$\frac{d[proTNF]}{dt} = k_{tl}[mRNA] - k_{deg\ P}[proTNF] - k_{sec}[proTNF]$$

$$[sec\ TNF](t) = \int_0^t k_{sec}[proTNF] dt$$



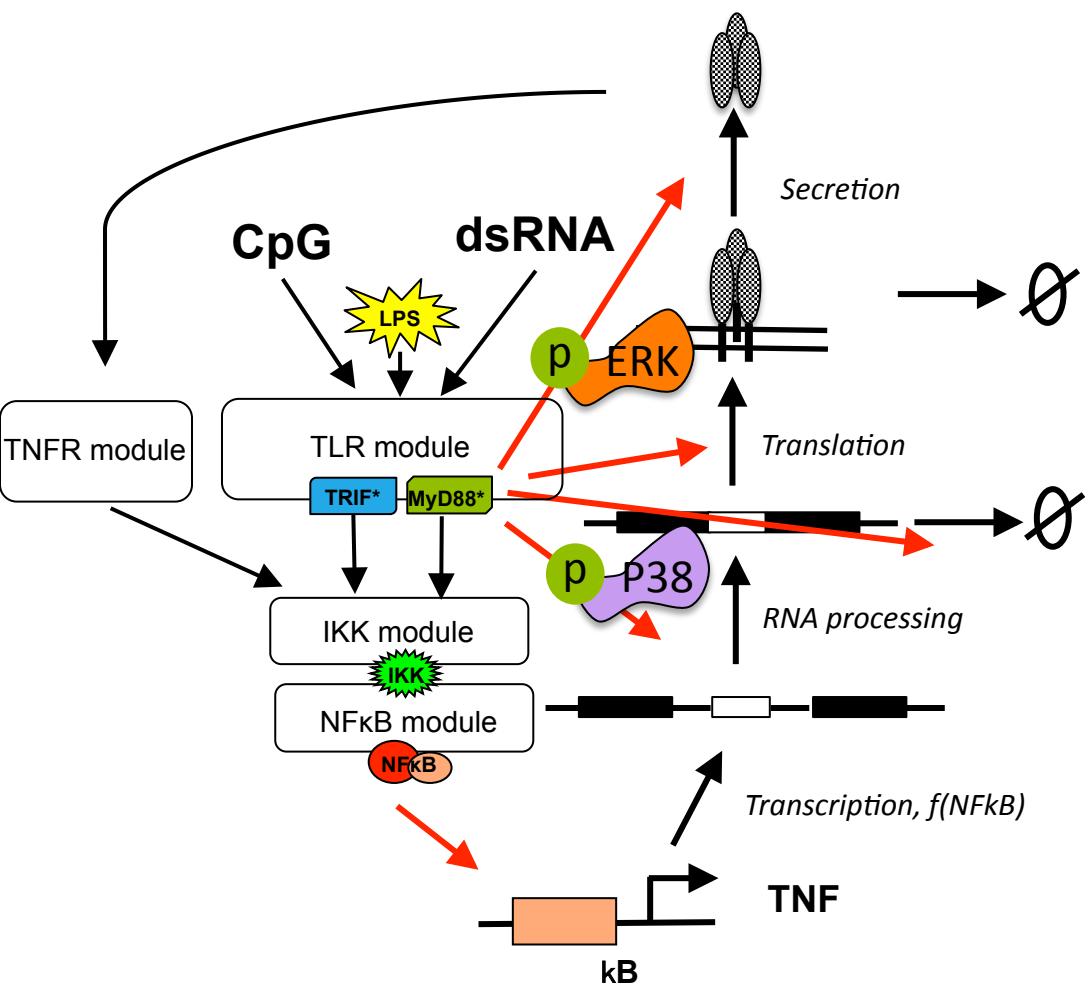
ID	Name	Values
1	Kd_tr	0.65
2	Kd_tr_fold	2
3	V_tr	1
4	k_pr	0.4
5	k_sec	0.07
6	k_tl	0.05
7	kdeg_m	0.02
8	kdeg_p	0.07
9	n	2
10	pr_fold_mko	4.2
11	pr_fold_tko	1.5
12	sec_fold	5
13	tl_fold	1.5

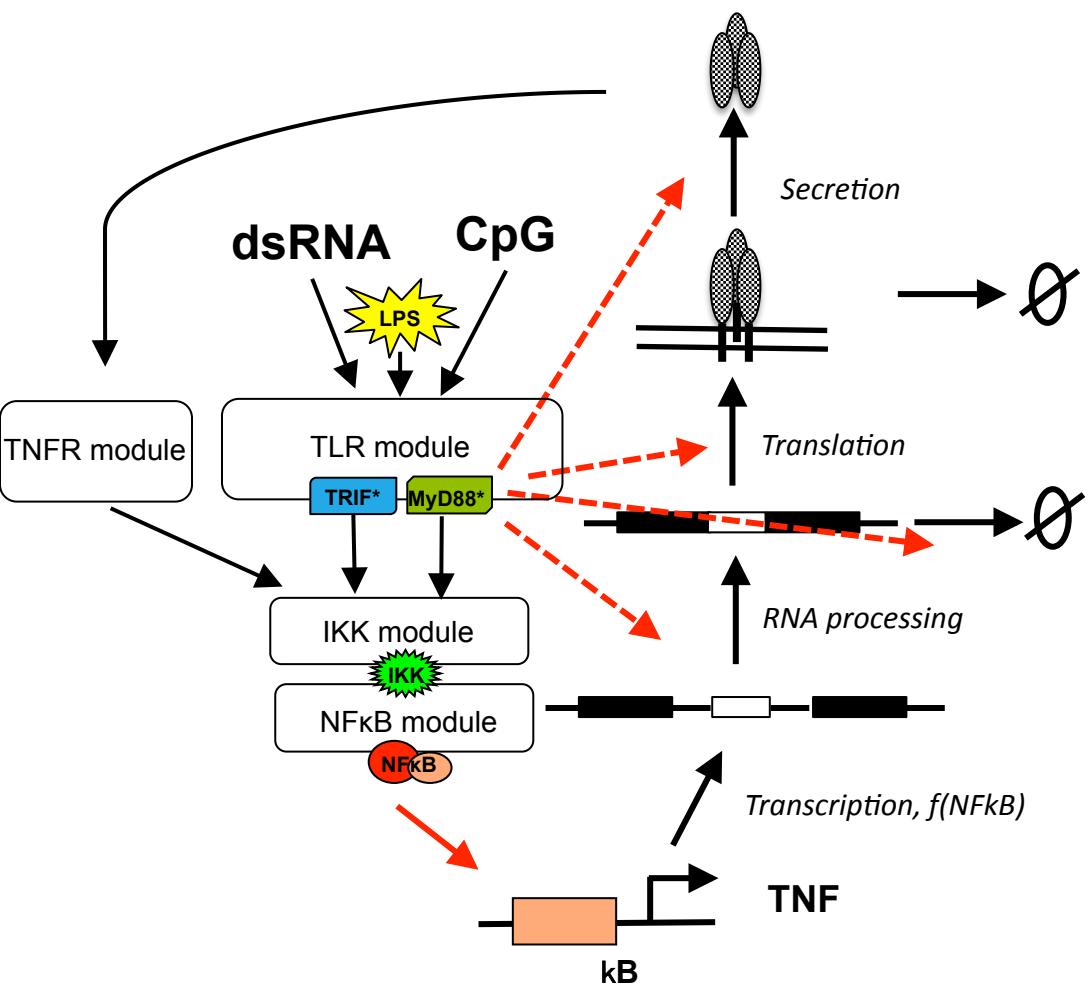
Module 1 to 3, together (NF κ B as input, 4 outputs)

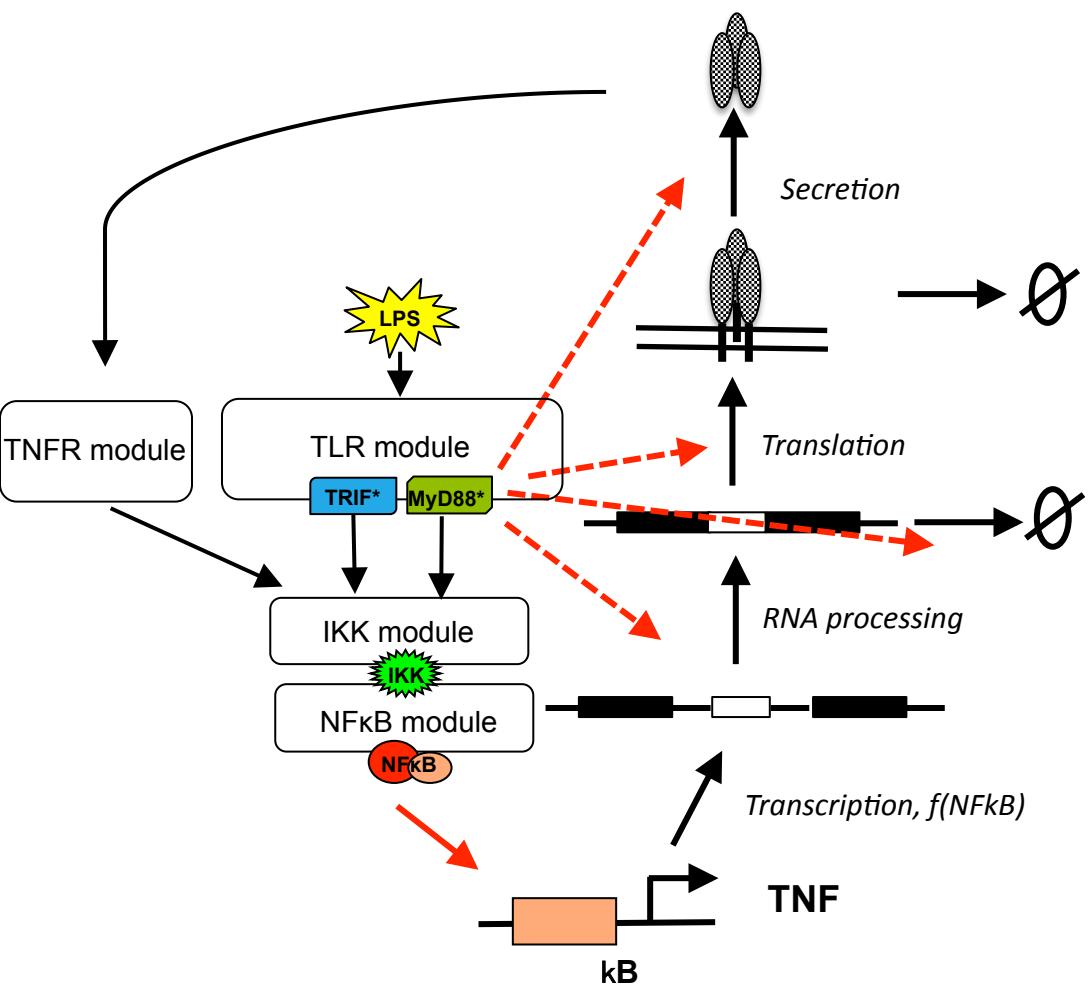


ID	Name	Values
1	Kd_tr	0.65
2	Kd_tr_fold	2
3	V_tr	1
4	k_pr	0.4
5	k_sec	0.07
6	k_tl	0.05
7	kdeg_m	0.02
8	kdeg_p	0.07
9	n	2
10	pr_fold_mko	4.2
11	pr_fold_tko	1.5
12	sec_fold	5
13	tl_fold	1.5

Part 2: Linking all modules together







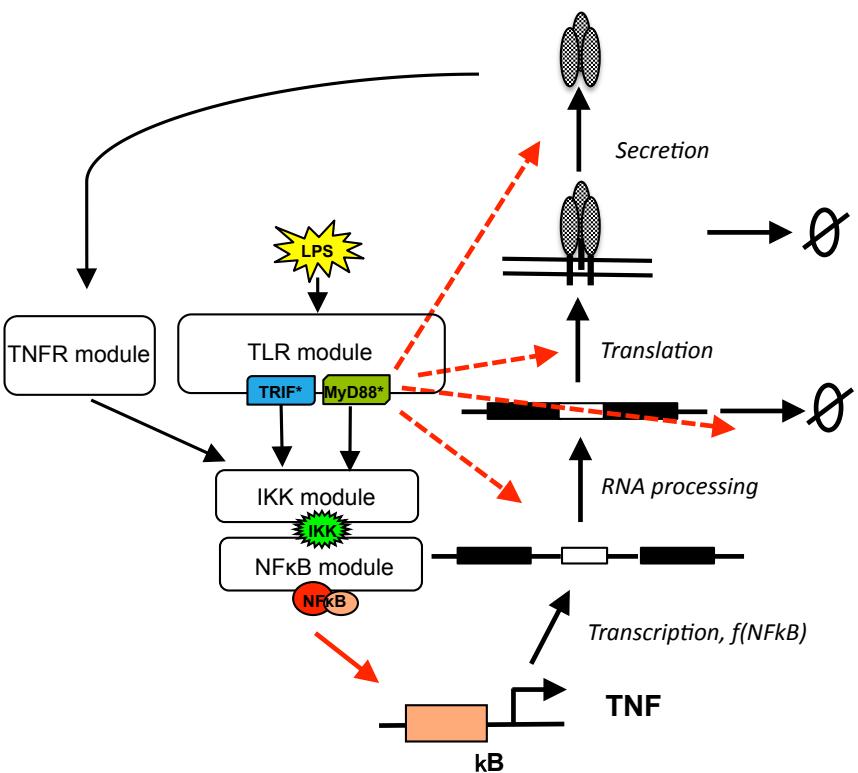
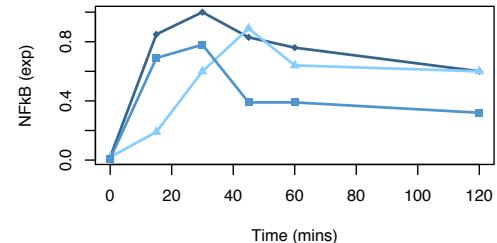
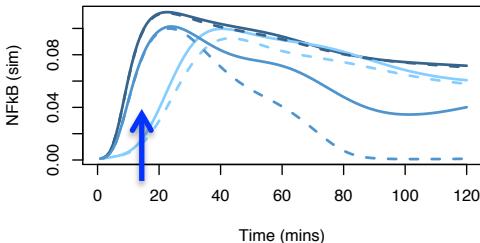
Linking with TLR4 model (LPS as input)

TNF feedback contribute to the 60mins -120mins activity in myd88 pathway but not trif

Dashed lines: without TNF feedback
 Solid lines: with TNF feedback

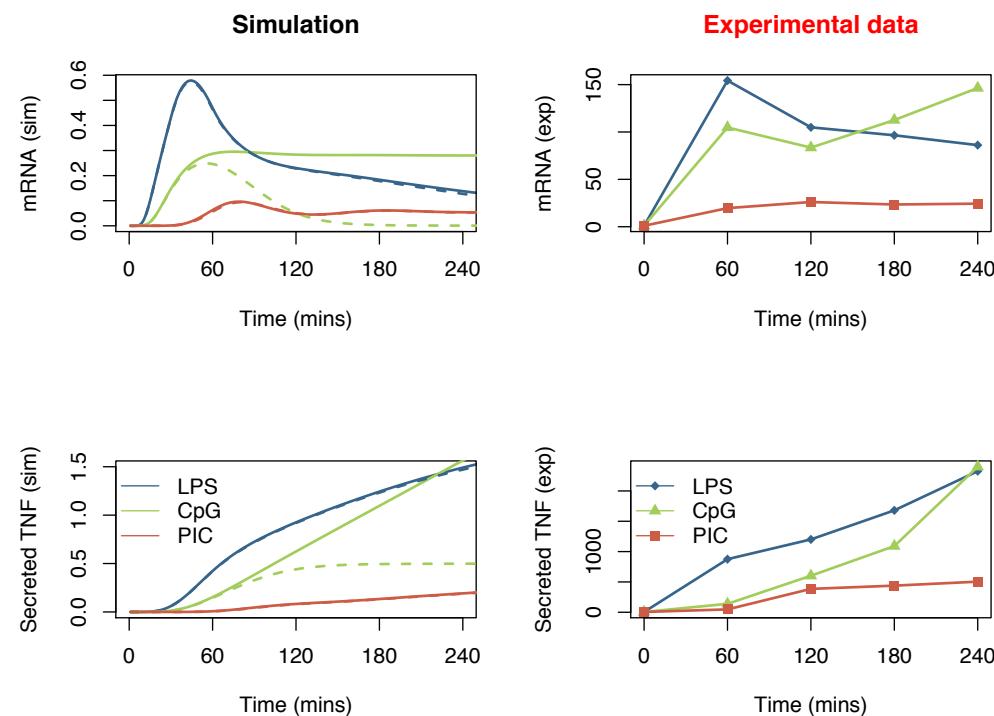
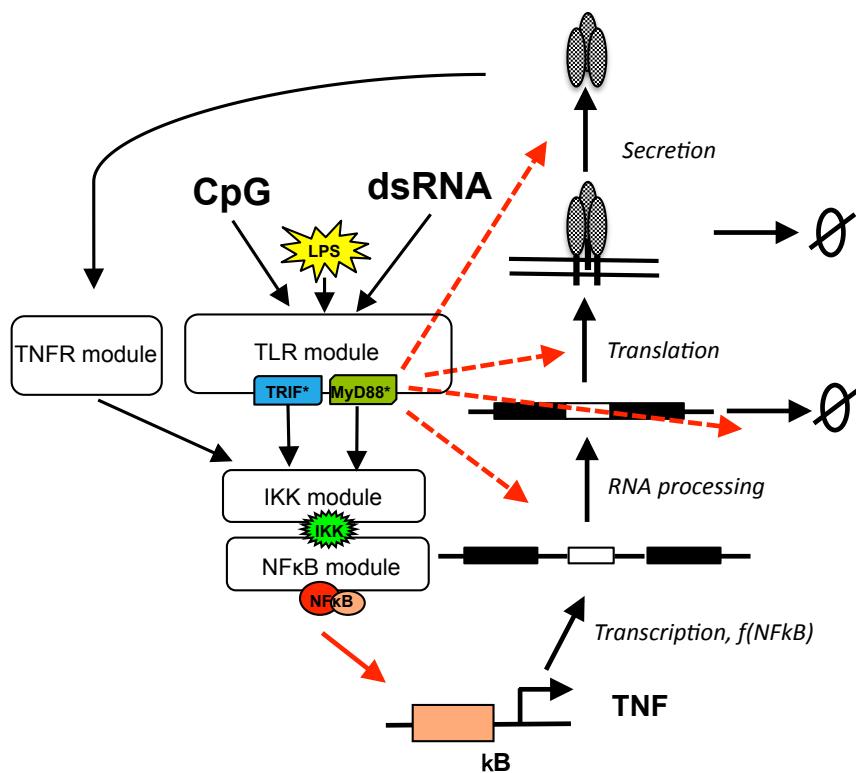
Experimental data

Simulation



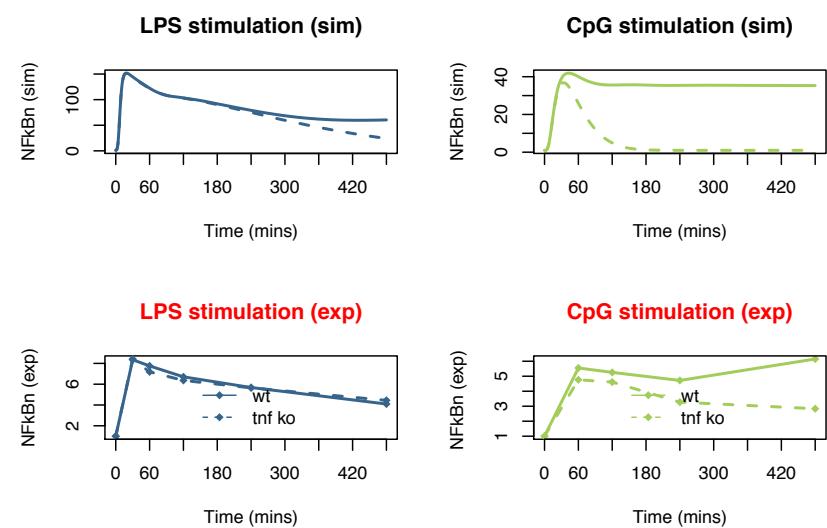
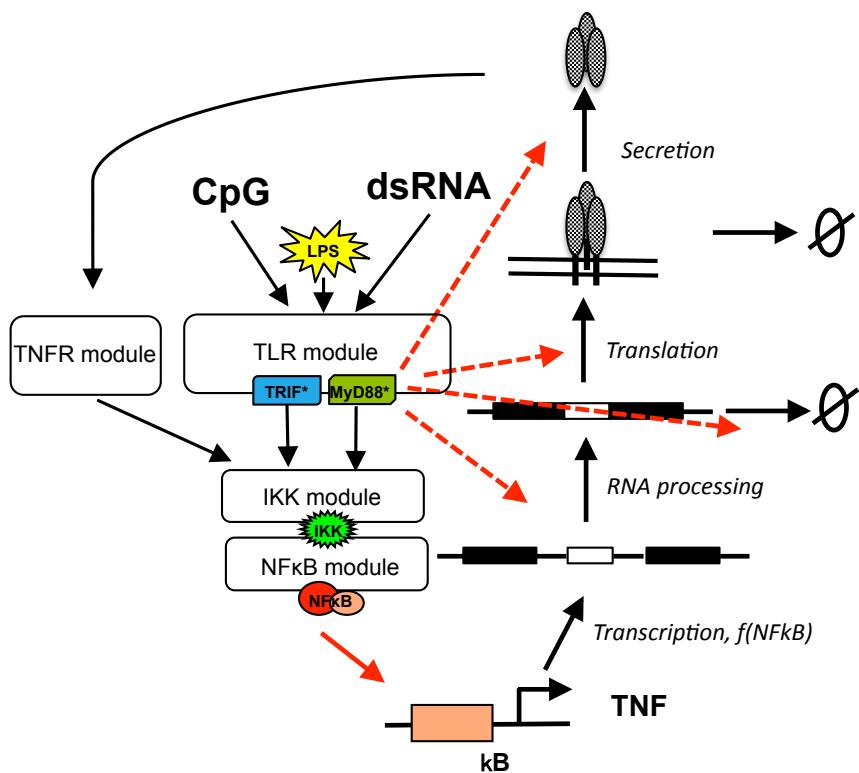
TNF feedback contribute to myd88 pathway but not trif

Validate by CpG and PIC stimulation (1)



TNF feedback contribute to myd88 pathway but not trif

Validate by CpG and PIC stimulation (2)



Summary for this project.

1. Cytokine production is tightly regulated at different molecular level.

- We constructed the mathematical model in each module.
- We also tested different regulation mechanisms by the model.
- We linked all the modules together.
- We linked all the modules with TLR4 model (but seems not match *trif* *ko* late phase for the NFkB activity, why?).

2. TNF feedback is critical in determining stimulus specificity in the TLR signaling.

- Linking TLR4 model + TNF production model + TNF receptor model can capture all the data, suggesting the TNF feedback is more important in MyD88 mediated signaling.
- Testing this idea, by studying CpG and PIC, which are mediated by MyD88 and TRIF respectively.
 - We see big difference in CpG responses but not PIC (readouts: TNF mRNA and Elisa). Adding CpG and PIC module into the big model confirms the difference.
 - The big model also suggested upstream NFkB should also see the same result
 - We tested in NFkB activity
- Lastly, functional study (image and RNAseq)