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***Computational Modeling***

**A. Description of the overall modeling construction.**

A Flow Chart is used to show the overall modeling construction process.

**Constructing the model for the TNF production module.** The fit is then optimization of the score function. Because our model here is pretty simple and not so many paramters, we can scan the interested parameter region to get the best fit. Given the model is simple, we use a local optimization function in MATLAB to experimental measurements. First, we try to use the same parameters for all genotypes. Then try to get the knockout parameters. We start with TNF production model. The model construction procedure for this module is kinda straight forward. We first construct the ODEs for the component step by step, following the centra dogama. We used the measured activity as input and output data to calaribe the models. Then we linked all the parts together, and using NFkB as a input and verify all the outputs at the same time. In order to get best result, a little bit adjustment is performed. We first assume different knockouts are sharing the same parameters with wild-type. It turns out at least some of the parameters are genotype dependent, suggesting tightly regulations by the adapters at different steps cytokine secretions. Then we begin to explore such regulations step by steps. For this model, Eqn. Step1 is to identify genotype-specific parameters, which can be considered as predictions. Step2 is to test predictions by experiments or finding references to support this. Step 3 is find the parameters values and verlidate the model.

**Then we link the TNF production model with the TLR model and existed TNFR model to prediction responses to LPS, CpG, and PIC.** At this stage, we compared results with and without includeing TNFR model, i.e. *tnfr-/-* condition to test and predict whether autocrine functions in a stimulus-specific manner.

**B. TNF production model**

The model equations are as follows:







In order to achieve a good fit, we used root-mean-square deviation (RMSD) as a score function to measure the discrepancy between our simulation and the experimental data. RMSD is defined as:



, in which n is the number of data points. Dmi is the simulation, dei is the the experimental data.

**Predicting mature mRNA dynamics.** We started the parameter exploration from Eqn (1.2), because we have measured mRNA half-lives leaving only one parameter kpr. The input is nascent mRNA and interpreted using the same time steps as numerical solving the equations. The mRNA degradation rates kdegmRNA are measured by the using atemyson B. Actually no free parameters in this step. But with the constraints with the previous nascent RNA we can determine the parameters.

The mRNA degradation rate is measured at 30 mins post stimulation. As suggested by the data, the mRNA half-life seems controlled by the p38 activity. The p38 activity seems to be reach at peak at 30 mins and return to basal at the 60 mins. So we assumed a linear dependence of the p38 with the degradation rate for the wild-type and myd88-/- and constant at the trif-/-  (Eqn. ).



**a) Same processing rate cannot capture the lowest mRNA profile in MyD88-/-.** The predicted mRNA in MyD88-/- will be higher than wild-type and trif-/- after 60mins. This is due to the nascent mRNA is highest in myd88-/-. The fitting is not dependent of processing rate, because kpr functions as a scaling factor only. We scaled to the maximum in wild-type, which eliminated the effects of kpr. And wild-type and trif-/- fits relatively well. This means, in myd88-/-, the processing is less efficient. We introduce a parameter fold\_pr\_mko to capture the fold of reduction in kpr mko.

**b) fold\_pr\_mko>2 is suggested by the data.** Data cannot determine one exact value for fold\_pr\_mko, but only a range larger than 2, because the shape of RMSD.

In summary:

* Processing rate is not determinable by mrna data alone
* Reduction of processing in MyD88-/-. Fold of reduction >= 2.5
* Fold\_pr\_tko should be less than 4.

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**Supplemental Figure2. Predicting mRNA dynamics based on nascent suggesting a decrease of processing in myd88-/-.**

1. **Same processing rate cannot capture the lowest mRNA profile in MyD88-/-.**

**Predicting nascent mRNA dynamics.** Then we move back to the eqn.(1.1). The input is the EMSA data for the NFkB activities. The output is the nascent RNA concentration. It was suggested that the transcription factor binds to the kB site and have a non-linearity activation. So we use a hill equation to describe the transcription regulation. kpr<g> is the processing rate constant in one of the genotype: wild-type, trif-/- and MyD88-/-. The mRNA processing is modeled as first order master action kinetics. The maximum transcription rate Vtr is set as 1 for simplicity. The EC50 Kdtr has the same unit of the NFkB, which is relative fold of the basal condition of the wild-type. Vtr =1, kdtr = 0.5, k\_pr = 0.4.

**a) fold\_pr\_tko >2 predict much higher nascent mRNA in myd88-/-, hints a loss of transcription efficiency in myd88-/-.** We model this effect by introduce a fold\_increase in the nfkb dissociation affinity Kdtr (fold\_kdtr\_mko). After introducing this factor, we can fit both nascent and mRNA for trif-/- and wild-type data.

**b) The requirement of trif in processing.**

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fit_tko_same_pr.pdf

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**Supplemental Figure 3. After introducing fold increase in the promoter dissociation, we are able to capture both nascent and mRNA data**

1. Same processing rate cannot capture the peak of nascent mRNA production in trif -/-. Left: RMSD heat-map. Right: best fit result.
2. By introducing a fold reduction in processing rate for the trif-/-, we can fit both nascent and mRNA data for trif-/-.

**Predicting proTNF and secreted TNF.**

Here heatmap confirms the no-dependence of transcriptional rate (Vtr).

In summary, we indentified four parameters: Kmtr, fold\_pr\_mko, fold\_pr\_tko, fold\_kmtr\_mko to be fit for all the nascent and mRNA data. We ran a optimization algorithm, based on the contraints we have learned so far, we can get best fit results as shown below:

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***Fig. best fit for nascent and mRNA together.***

|  |  |  |  |
| --- | --- | --- | --- |
| Index(i) | Feature | Value(Fei) | Error(σi) |
| 1 | Vtr | 30 min | 5 min |
| 2 | Kdtr | 60 min | 10 min |
| 3 | kpr | 30 min | 5 min |
| 4 | kdegm | 0.68 | 0.11 |
| 5 | n | 0.75 | 0.22 |
| 6 | kdegp | 1.55 | 0.67 |
| 7 | ksec | 0.59 | 0.07 |
| 8 | Fold\_pr\_mko | 0.52 | 0.21 |
| 9 | Fold\_pr\_tko | 1.49 | 0.43 |
| 10 | Fold\_kdtr\_mko |  |  |
| 11 | Fold\_tl\_tko |  |  |

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Figs. The wild-type nascent mRNA data constrains the kpr around 0.1/min, Km is larger than 1.

* Tko cannot get a higher peak in nascent than wild-type if they have the same processing rate.

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**2) TNF mature mRNA**



**figs. Simulating mRNA suggested reduction of processing rate in myd88-/-.**

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**Fig S2. The simulated mRNA profile when have same processing rate in knockouts. Lines are 1.5 fold; dashed lines are 3 fold; dots are 4.5 fold less process rate in knockouts.**

* Different prcessing rates in knockouts.

We tested the simulated mRNA profile under the hypothesis that MyD88 and TRIF contribute equally to the mRNA processing. The result shows that mRNA unconsistent with the experimental data (Fig S2). The most unconsistence is the trif-/-, in which condition, the mRNA is less stable than the wild-type. That’s why mRNA is much lower than the *wild-type* and myD88-/-. This turns out in order to get a higher mRNA level in trif-/- than myd88-/-, they can not have the same

**Fig S3. Fitting for mRNA profiles. (A) The score heat-map of processing fold reduction in myd88-/- and trif-/-. (B) Best fit at fold\_mko= 4.5, fold\_tko = 1.1.**

process rates. Or imply a higher process rates in trif-/- than myd88-/-. In other words, MyD88 seems contribute more than TRIF in TNF mRNA processing. The heatmap of fitting score confirmed this conclusion (Fig S3).



We can tested the different stabilization regime presenting the different stabilization regulation. We assume the mRNA is all stable or unstable. We set the corresponding the TNF mRNA degradation rate as 0.02 min-1 or 0.07 min-1.

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**FigS4. The unsuccessful fitting assume no stabilization**

The unmatch happens at the wild-type and trif-/-. It is becaucse the 4.5 less processing rate in the myd88-/-, the level of mRNA is low at myd88-/-. The mRNA is higher than the wild-type at the peak level, because higher nascent in trif-/- and the same processing rate. If the stabilization is lost at trif-/-, then the levl of mRNA in trif-/- will match the experimental data.

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**Figs mRNA and nascent together.**

So till now, the model constructing suggesting 1) MyD88 contributes to the processing more than TRIF 2) TRIF contribute to the stabilization not MyD88.

Now we came back to the nascent mRNA module. So the analysis revealed a low processing rate at myd88 knockout. However the nfkb level is close to the wild type. Then we can anticipate that the level of nascent mRNA in myd88 knockout should be far more than the wild-type (fig s). How can we get a reduced level of nascent in myd88-/-. One possible clue comes from the chromation remodeling process is myd88 dependent (Xie et al., 2013). We modeling this by introduce a fold reduction parameter in the Kd of the transcription hill equation in MyD88-/-.

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**FigS5. Unbalanced nascent mRNA profiles predicted by the model and fixed by MyD88 contributes to transcription.**

So after adjusting for processing, transcription, the nascent and mature mRNA are fine fited by the model.

**3) proTNF and secreted TNF**



The proTNF is produced by translation from mRNA and also degrades or secret if it isn’t degraded. The secrected TNF is measured by ELISA. And assume no degradation of the secreted TNF. So the secreted TNF is the integral of the screted TNF as at the beginning assume no TNF in the media. Because the proTNF measurement is under the condition of using TASE inhibitor, the proTNF simulation by set ksec = 0.

The wild-type data for the proTNF constrains the kdegp >=0.4.

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***figs. Same ktl and kdegp cannot capture the relationships in proTNF.***

The main disagreement is the predicted higher level of proTNF in trif-/-. This suggested TRIF plays a role in translation.

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***figs. After introduce fold\_tl\_tko, we inproved the fitting for trif-/-.***

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*Figs. Same rate constant predict higher secretion in trif-/-.*

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***figs with fold\_tl\_tko, the proTNF and secTNF in trif-/- can fit the data***

We connected all the models for the steps by the NFkB as input. To validate the simulations.

So till now, the model constructing suggesting 1) TRIF contributes to the protein translation

**The TLR-TNFR model**

The TLR model and TNFR model share the same component of IKK cycle and NFkB module, different in the receptor modules. TNFR requires a trimerization and the model detailed describes how trimerization works. The TLR model need a spatial translocation to bifurcate the signal into two adaptor- MyD88 and TRIF. Both MyD88 and TRIF activates TRAF6, at which node it merges with TNFR signaling.

**The TLR4 model**

LPS =>LPSpm, LPSpm => LPSen; LPSen=>LPS;LPSen=>

TLR4pm + LPSpm <--> TLR4LPSpm

TLR4en + LPSen <-->TLR4LPSen

**Adding PIC**

TLR3 <-->

TLR3 + PIC <--> TLR3:PIC

TRIF -> TRIF\*; TLR3:PIC

TRIF\* -> TRIF

**Adding CpG:**

TLR9 <-->

TLR9 + CpG <--> TLR9:CpG

MyD88 -> MyD88\*; TLR9:CpG

MyD88\* -> MyD88

**Linking the TNF production model with TLR-TNFR model with and with TNF feedback**

Because the outputs of the TLR model is the NFkB, it can directly connect with TNF production model in previous section. Also TNF is able to activate the cell in auto-/para-crine manner, we also includes the TNFR module from (Werner et al. 2008) .