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Effects of two negative feedback loops on NF-κB signaling: noise-induced oscillation

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Outline

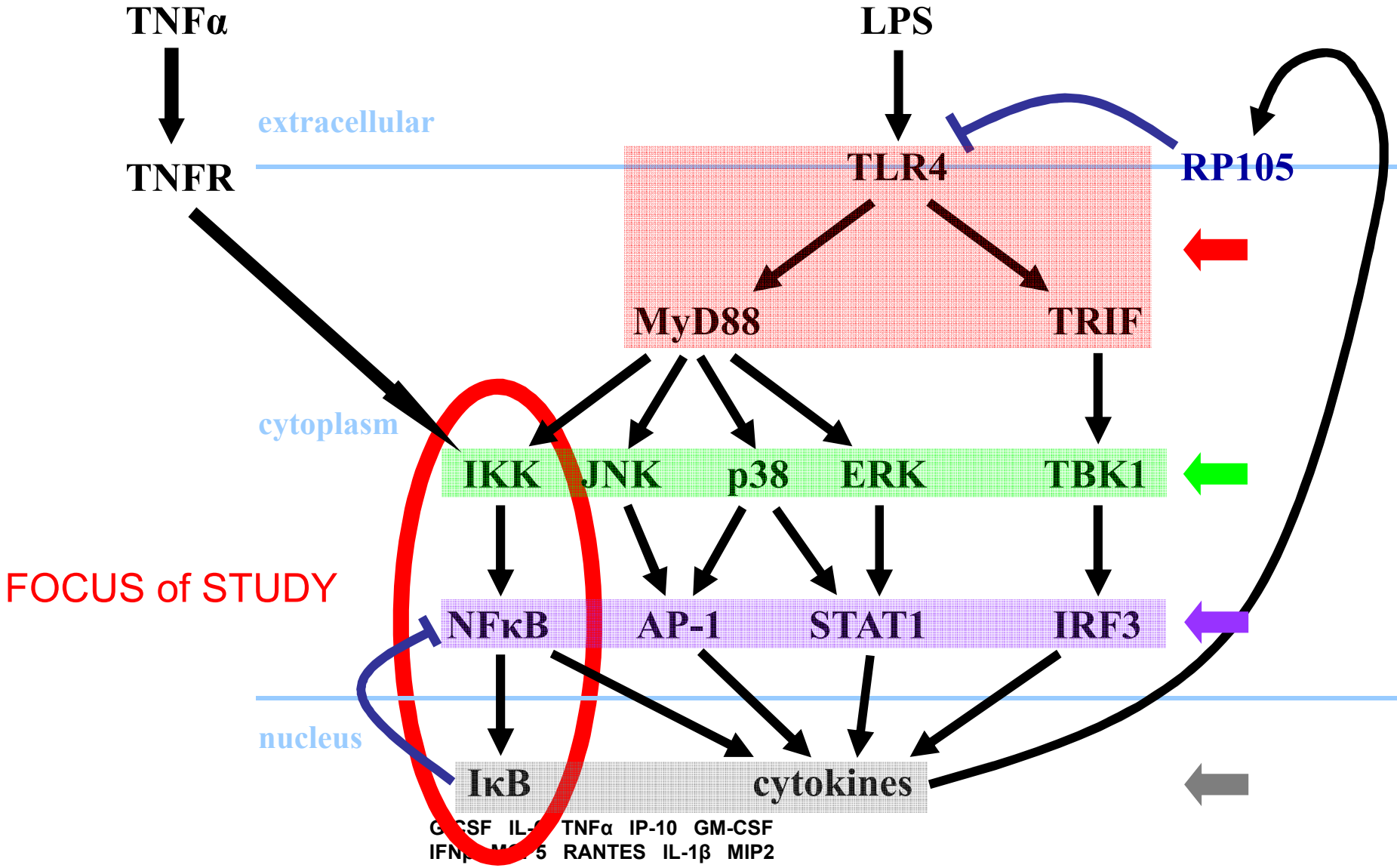
1. Computational model of NF-κB signaling network

2. Sensitivity analysis

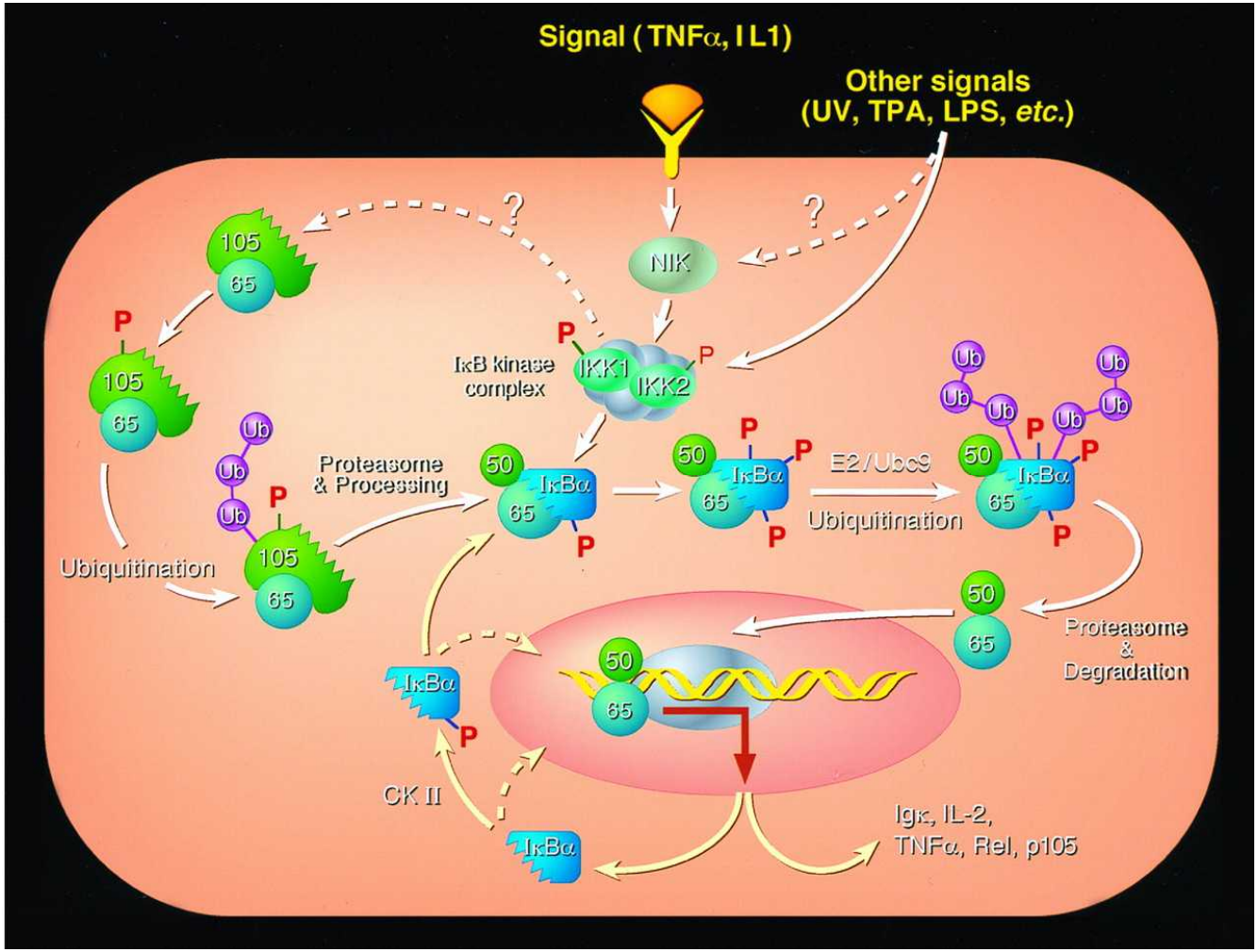
3. Predictions from deterministic model:
   a) Statistical ensemble of NF-κB dynamic patterns
   b) LPS dose-dependent NF-κB translocation time
   c) LPS dose-NF-κB response curve

4. Role of noise on oscillatory behavior of NF-κB shuttling: noise-induced oscillation of NF-κB shuttling

TNFR & TLR4 signaling lead to NF-κB response



Overview of NF-κB signal transduction network

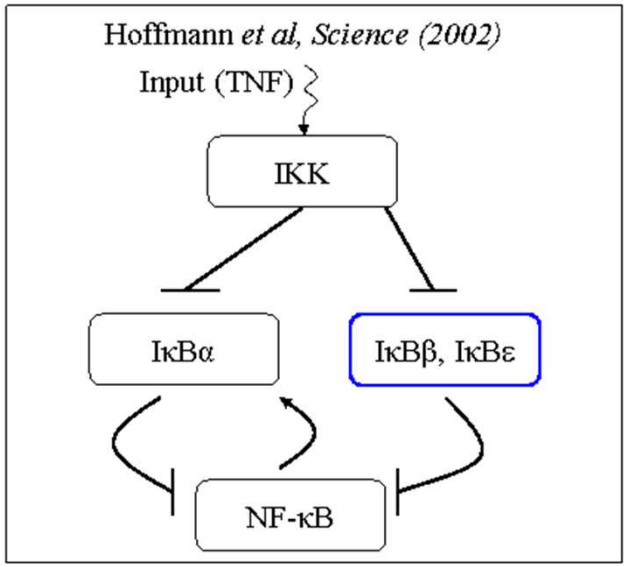


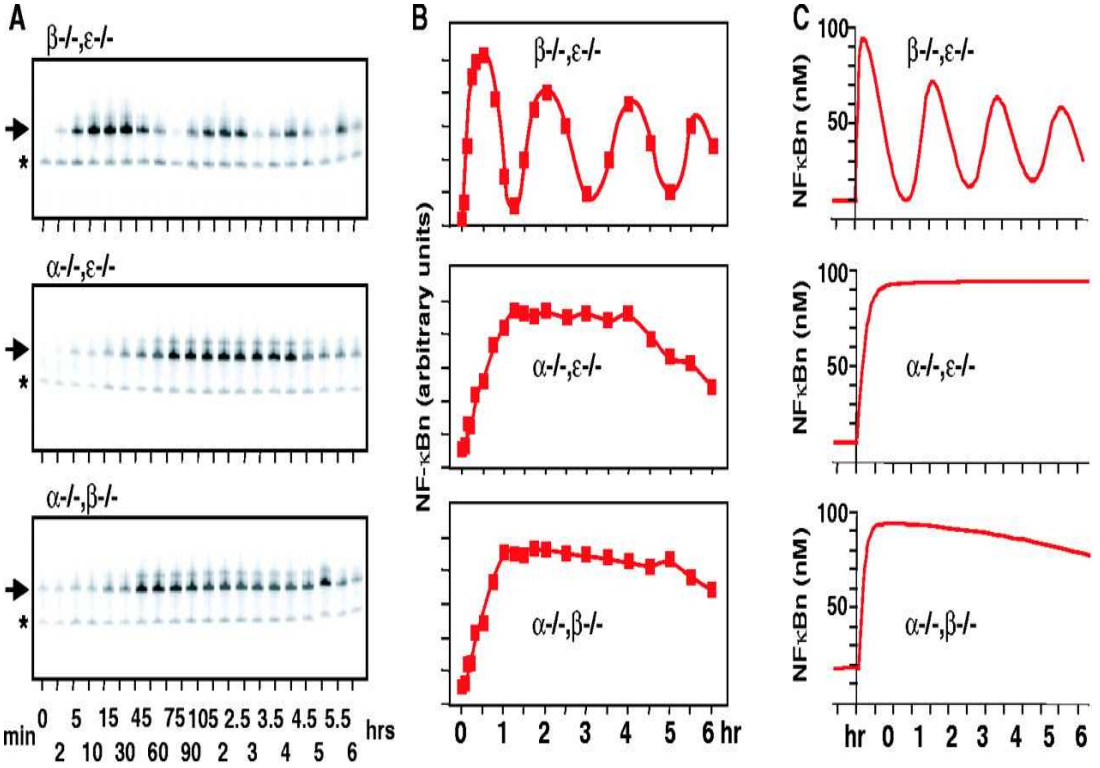
Key players:

- IKK
- p50/p65 (NF-κB)
- IκBα
- IκBβ
- IκBε
- A20

I. M. Verma *et al* PNAS 94, 11759 (1997)

Dynamic patterns of NF-κB: IκBα-driven oscillation of NF-κB



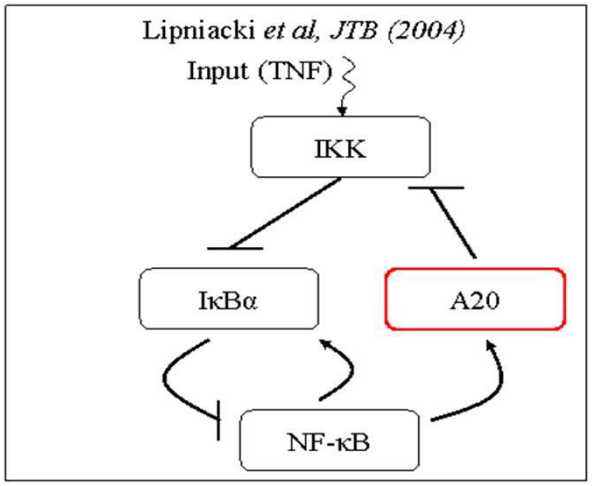


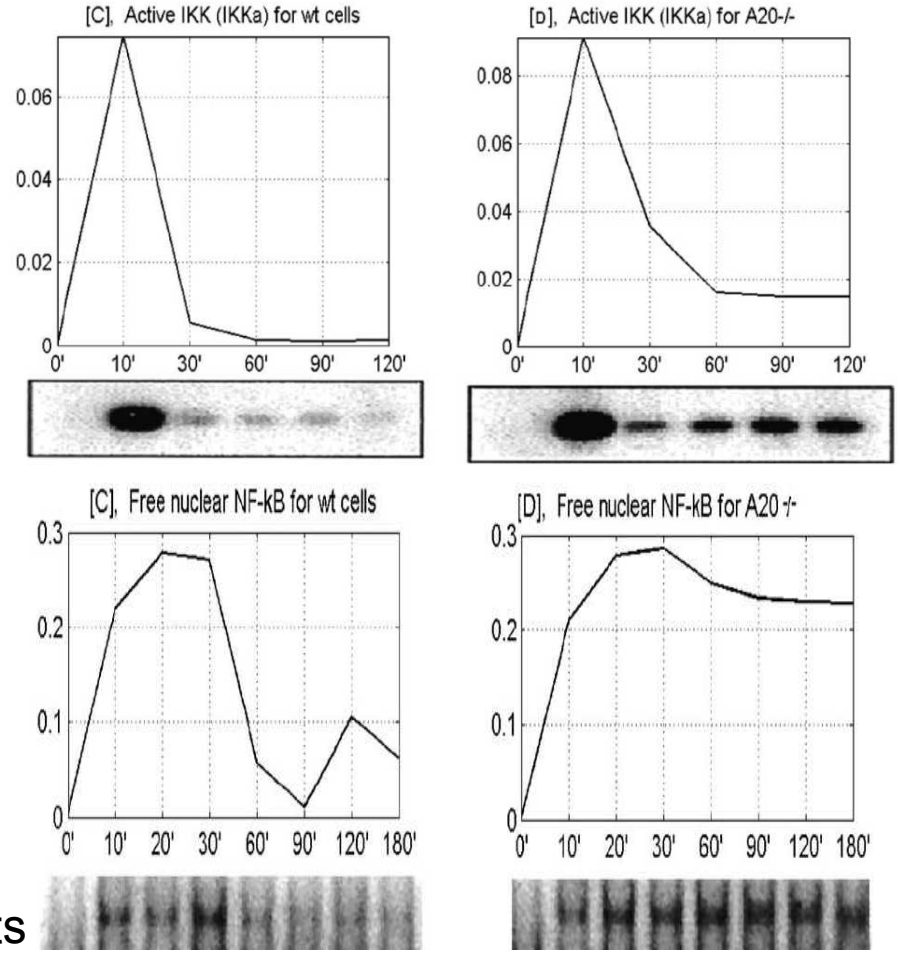
Hoffmann *et al.*
Science, 298:1241 (2002)

Mouse fibroblasts

In IκBε & IκBβ knock out mice, NF-κBn is more oscillatory!

Additional negative regulator of NF-κB: A20





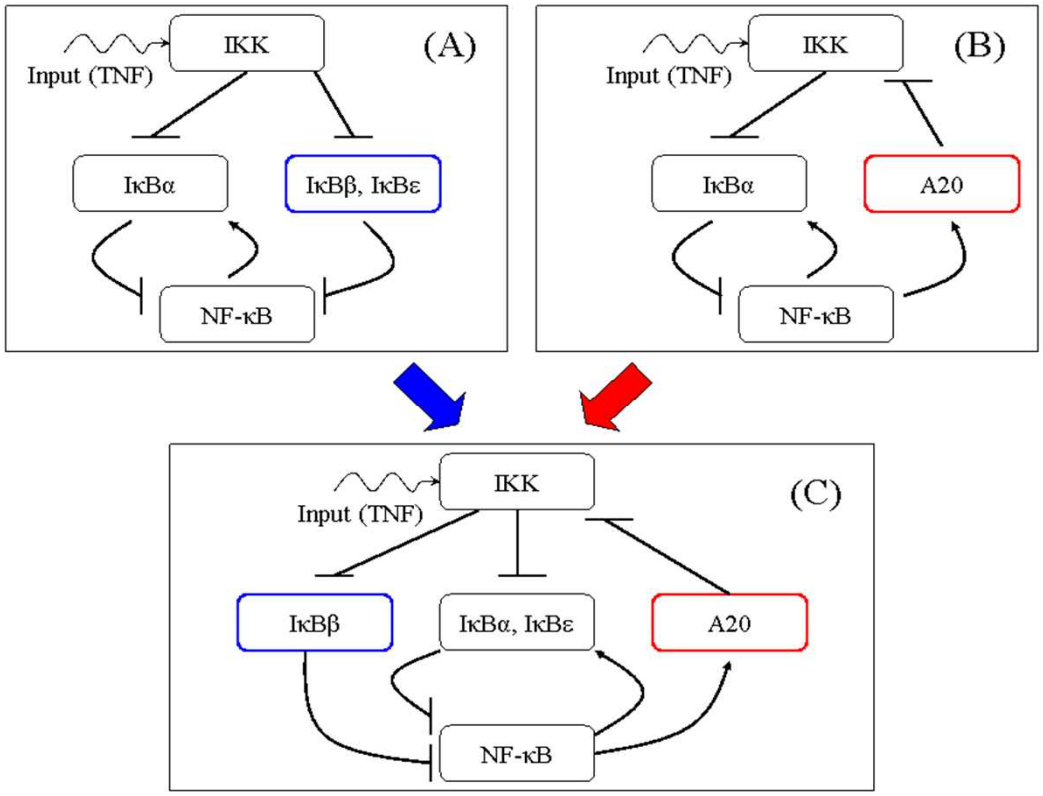
Lee *et al.* Science 289:2350 (2000)
Lipniacki *et al.* JTB 228:195 (2004)

Mouse fibroblasts

In A20 knock out mice, NF-κBn level remains up high!

Our up-to-date hybrid NF-κB Signaling network

Hoffmann *et al.*
Science, 298:1241
(2002)

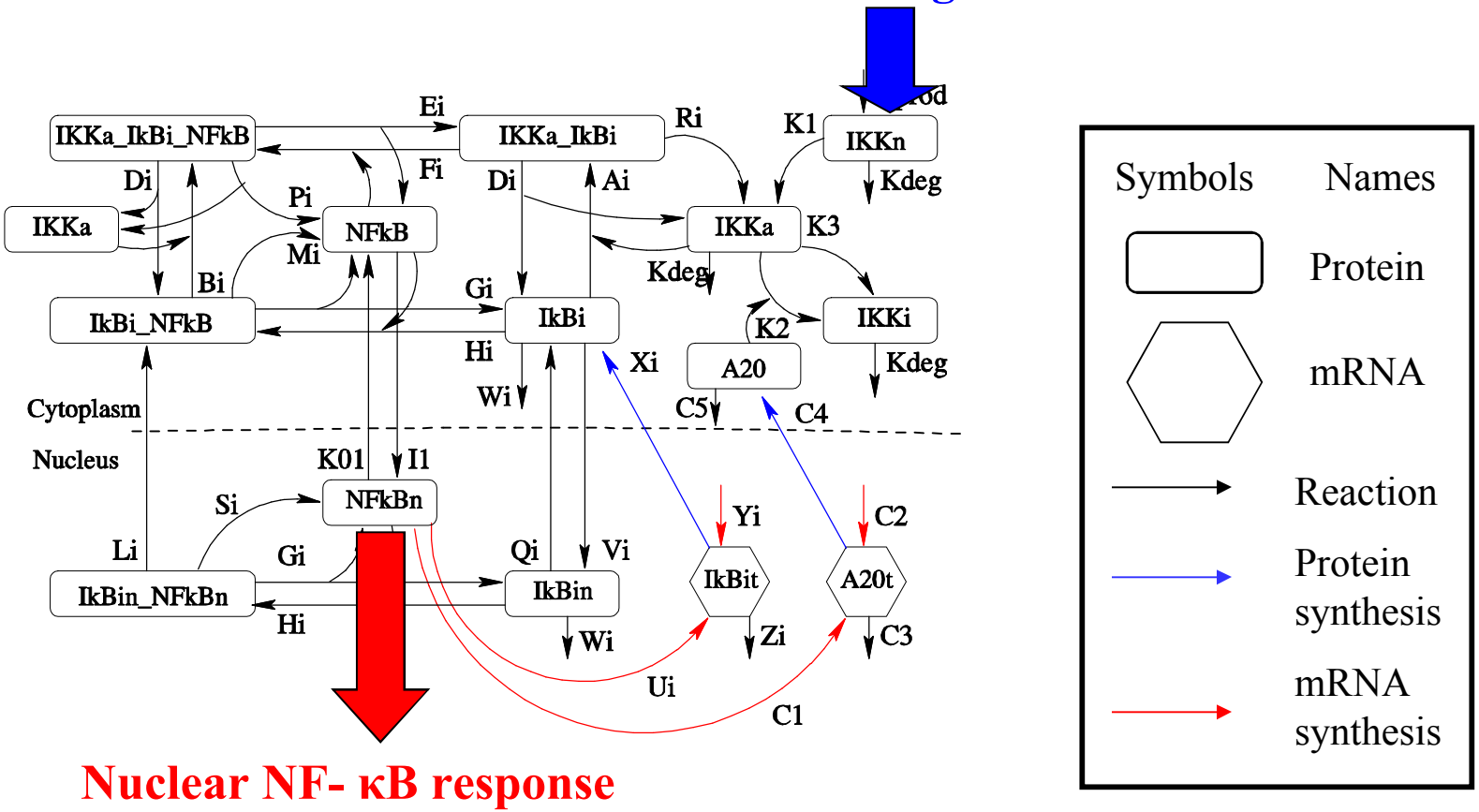


Lipniacki *et al.*
JTB 228:195
(2004)

Ingredients: IKK, NF-κB, IκBα, IκBβ, IκBε, A20, and their compounds

Hybrid model of NF-κB signaling network

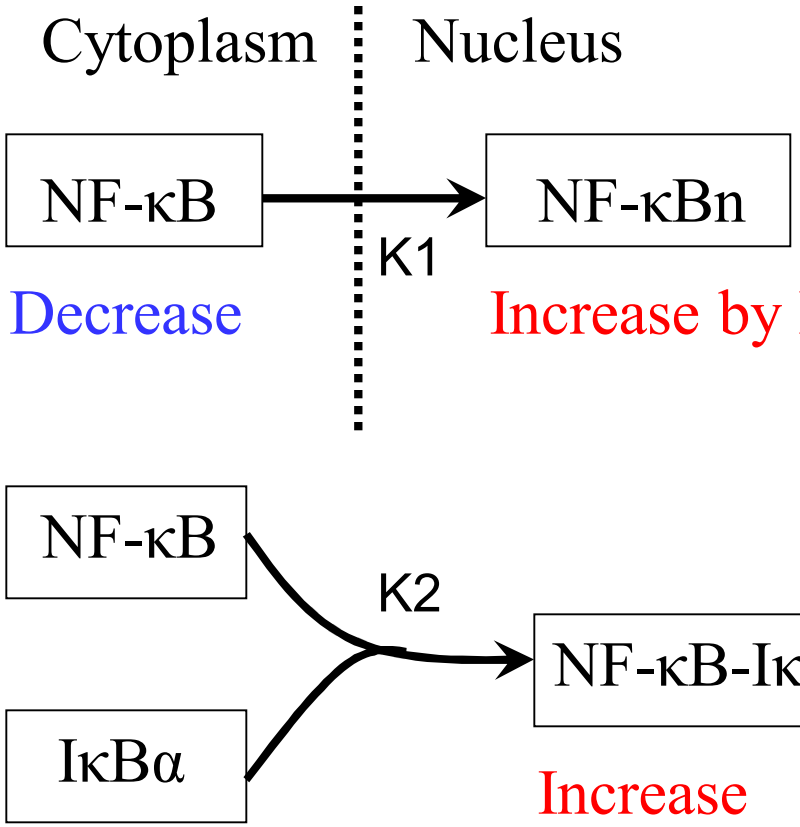
TLR signal ← Persistent LPS



Nuclear NF- κB response

- Signal is transduced from TLR to IKK, and then regulated in IKK-IκB- NF-κB module.
- A system of ordinary differential equations with 28 species & 70 reactions_

Translation from hybrid network to a system of ODE

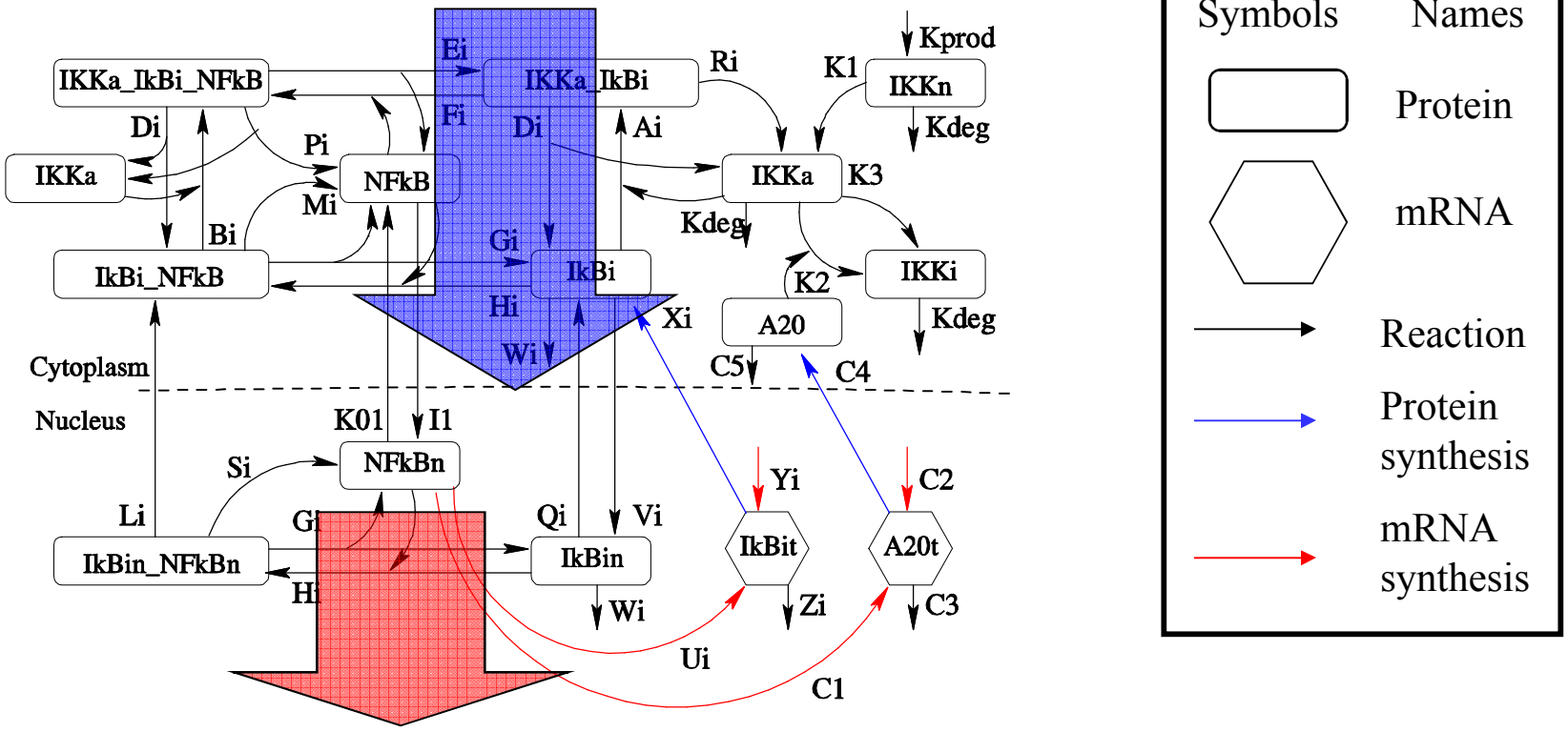


$$d[\text{NF-}\kappa\text{B}]/dt = -K1[\text{NF-}\kappa\text{B}]$$
$$d[\text{NF-}\kappa\text{Bn}]/dt = K1Kv[\text{NF-}\kappa\text{B}]$$

$$d[\text{NF-}\kappa\text{B}]/dt = -K2[\text{NF-}\kappa\text{B}][\text{I}\kappa\text{B}\alpha]$$
$$d[\text{I}\kappa\text{B}\alpha]/dt = -K2[\text{NF-}\kappa\text{B}][\text{I}\kappa\text{B}\alpha]$$
$$d[\text{NF-}\kappa\text{B-I}\kappa\text{B}\alpha]/dt = K2[\text{NF-}\kappa\text{B}][\text{I}\kappa\text{B}\alpha]$$

Sensitivity analysis of hybrid NF-κB signaling network model

Input: Perturbation of kinetic rate variables



Output: Change in dynamic features of nuclear NF- κB response

Sampling of 71 input variables of the hybrid network model

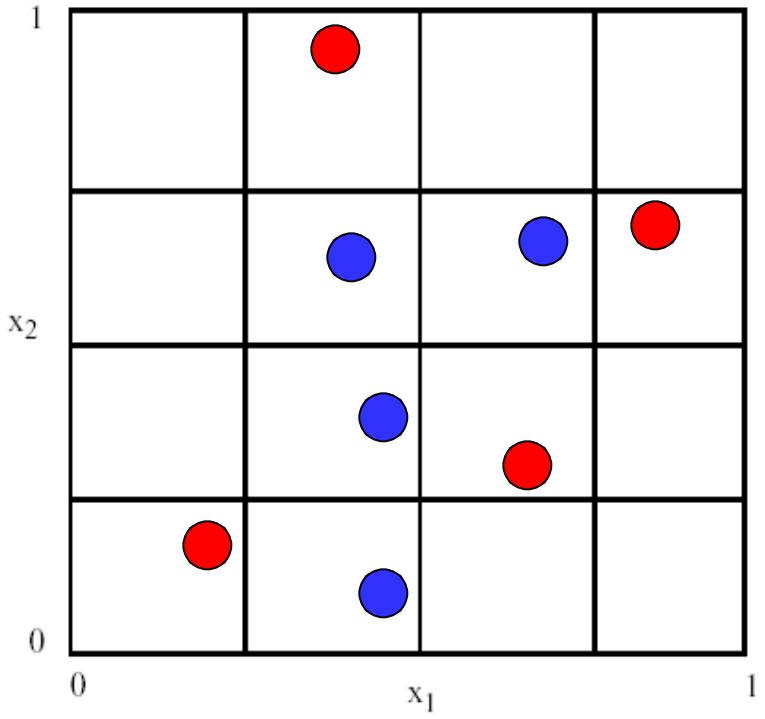
- 70 kinetic rate variables + 1 initial condition
- Sample 71 input variables by Latin Hypercube Sampling according to assumed joint distribution
  - Uniform or lognormal distribution
  - Interval size for uniform distribution:

    Kinetic rate variable x in (xo (1-f), xo (1+f))

    xo is a nominal value and f =70%
- Typical sample size: 1,000 to 10,000 ODE simulations

Latin Hypercube Sampling according to uniform distribution

Example:



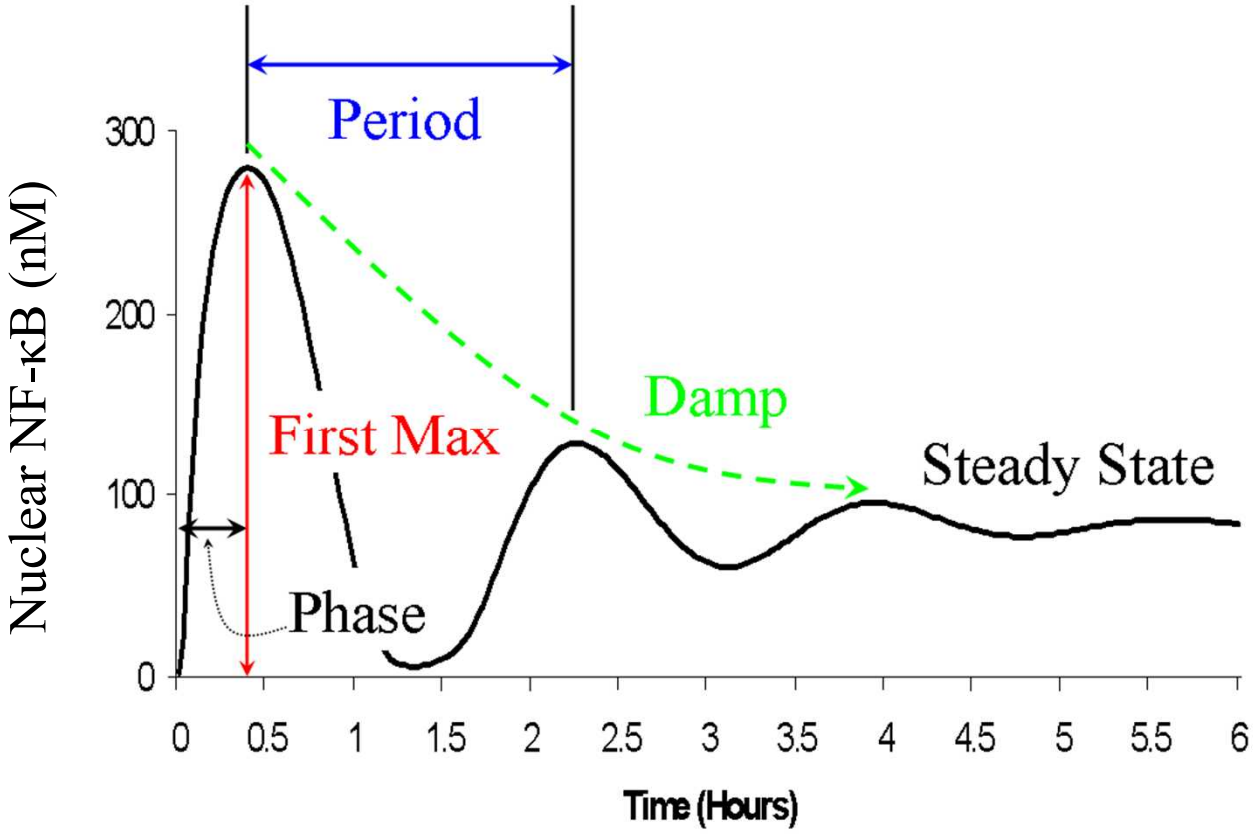
Random

Latin Hypercube Sampling

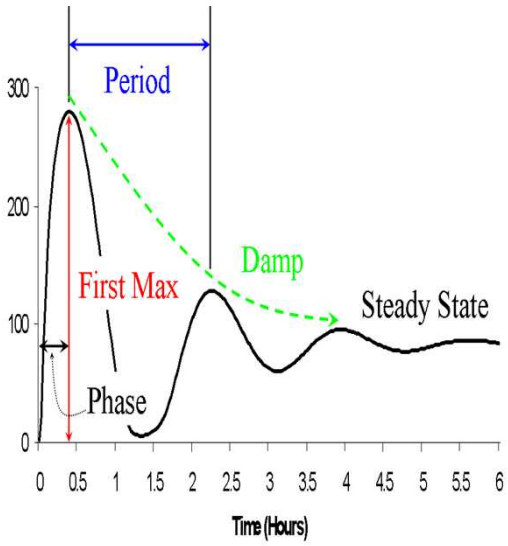
Question:

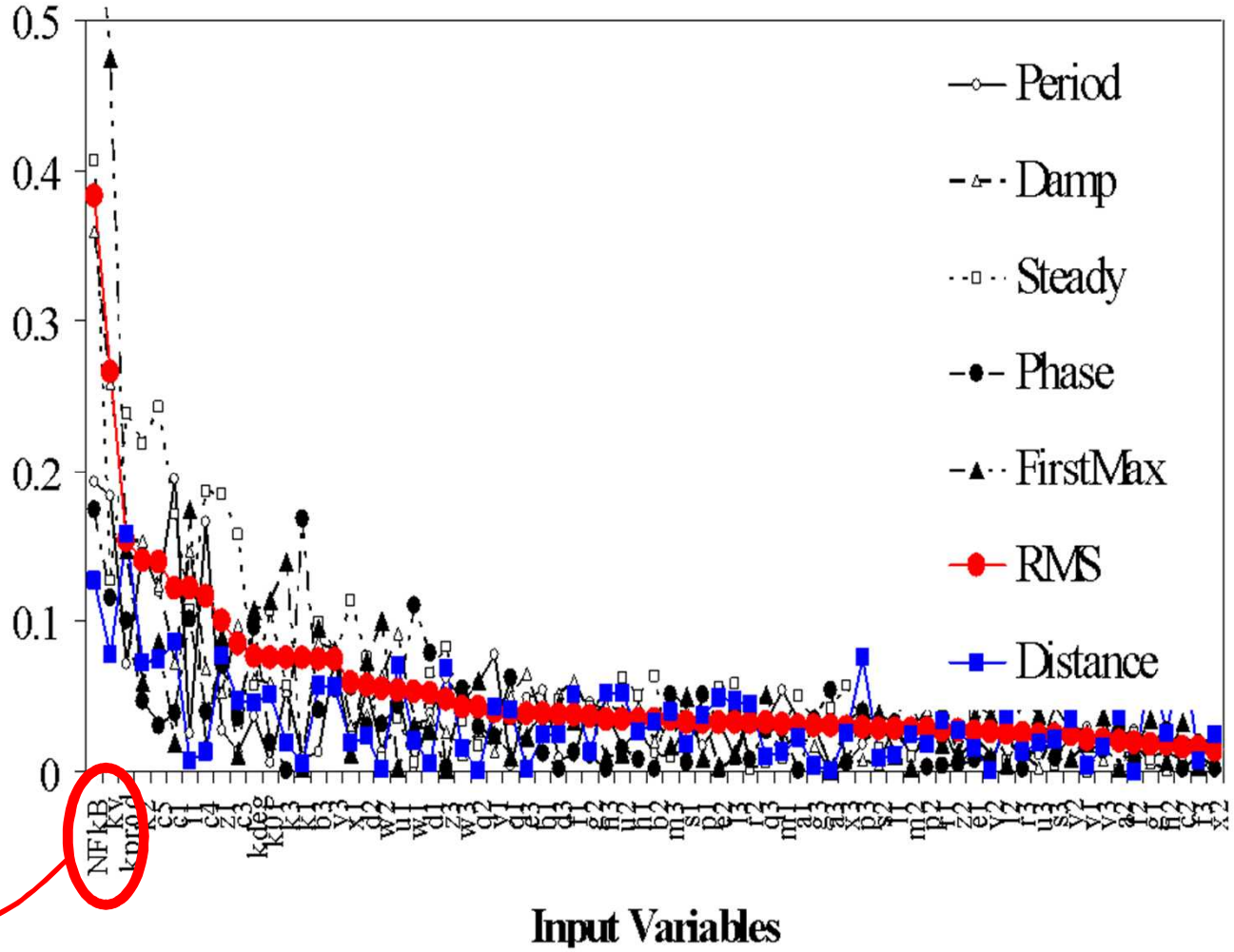
What are the most influential kinetic rates on NF-κB response?

Quantification of nuclear NF-κB response with five dynamic features



Correlation between kinetic rate variables and dynamic features



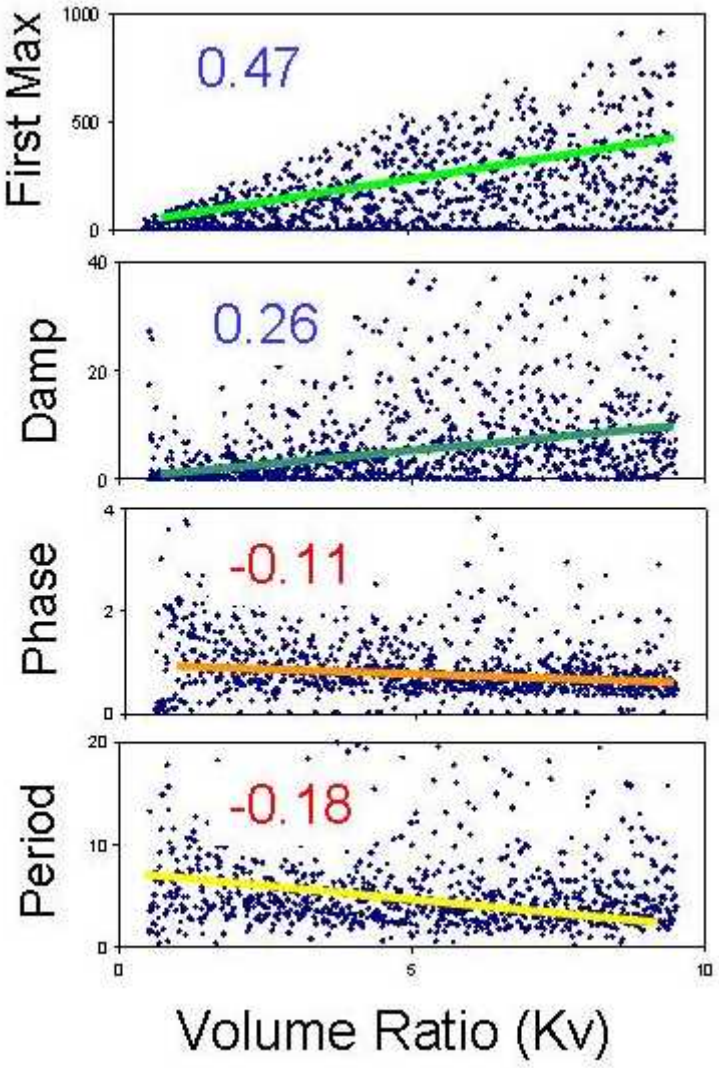


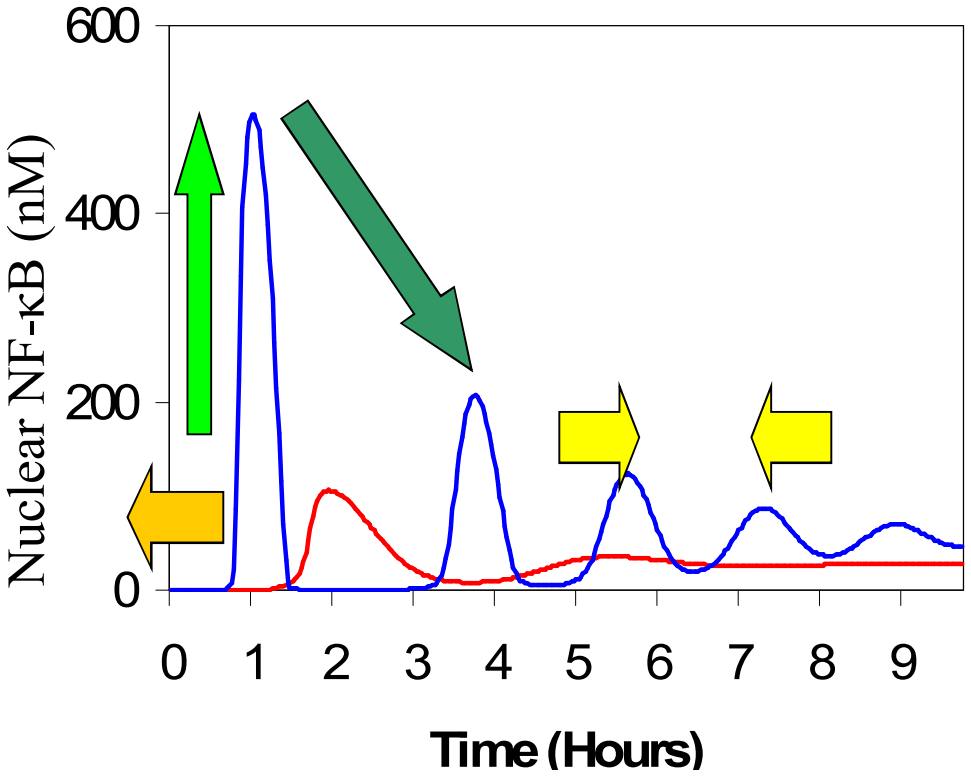
1. NF-κB
2. Kv

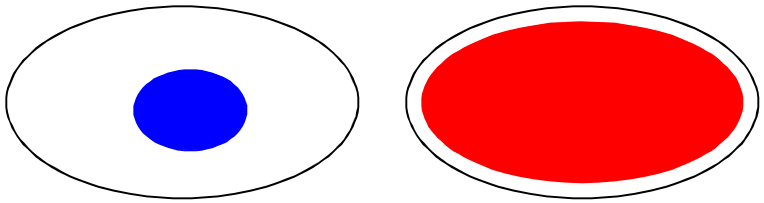
Question:

What is the typical NF-κB response to variation of the most influential input variables?

Dependence of NF-κB dynamic features on volume ratio of cytoplasm to nucleus

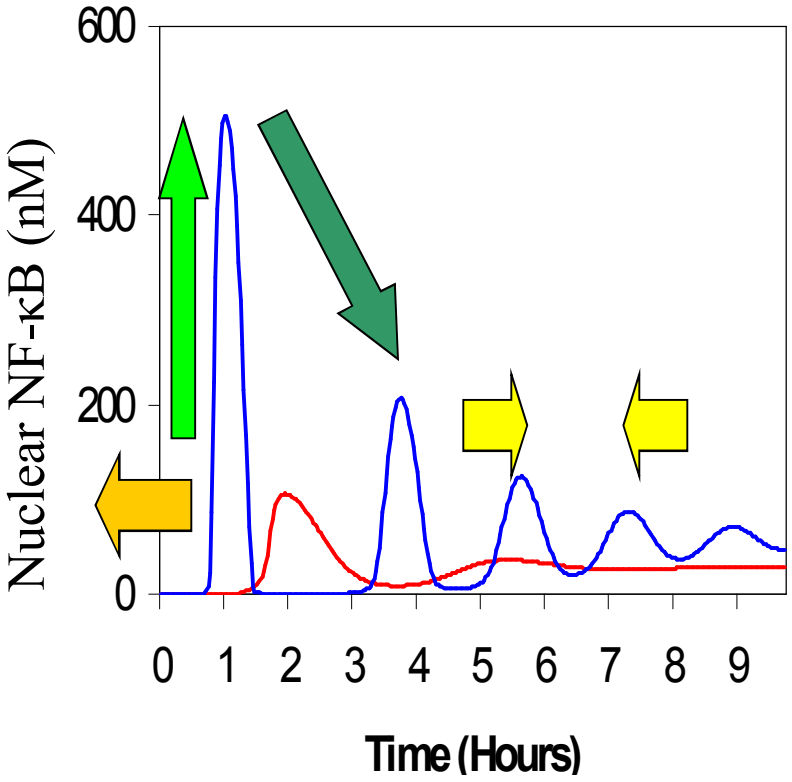


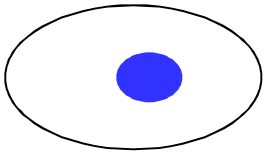




High Kv=10 Low Kv=2

Dependence of NF-κB response on volume ratio of cytoplasm to nucleus

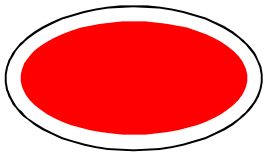




High volume ratio:

Strong and fast NF-κB response

with more temporal modulation

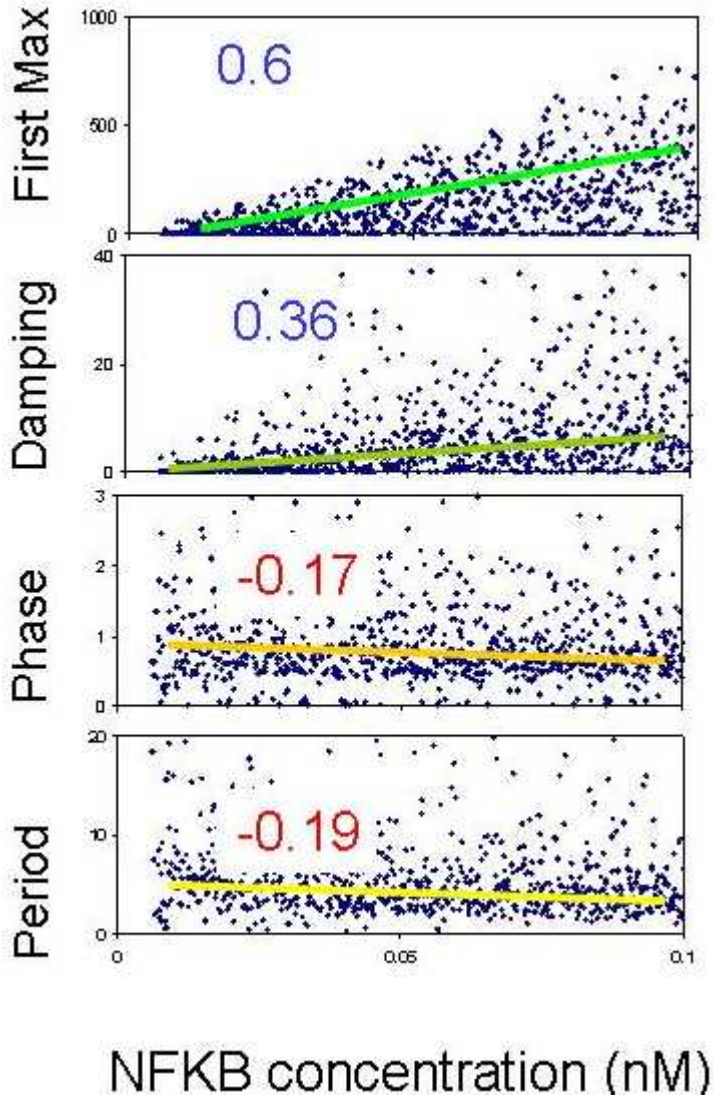


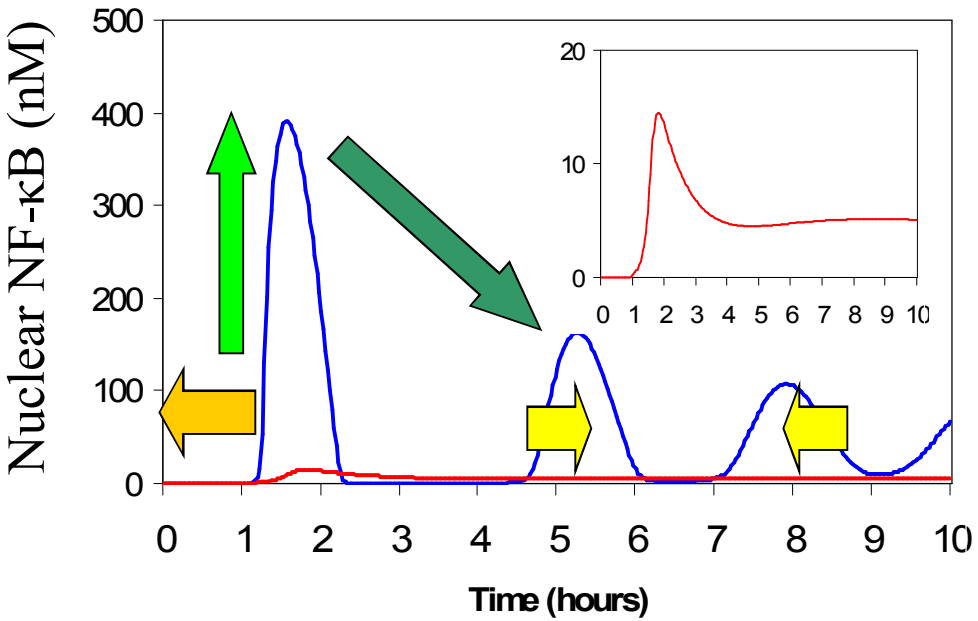
Low volume ratio:
Weak and slow NF-κB response
with less temporal modulation

Why?

Higher volume ratio (smaller nucleus size)
→ higher nuclear NF-κB concentration
→ higher production of IκBα & A20
→ Stronger negative feedback

Dependence of NF-κB dynamic features on total NF-κB concentration





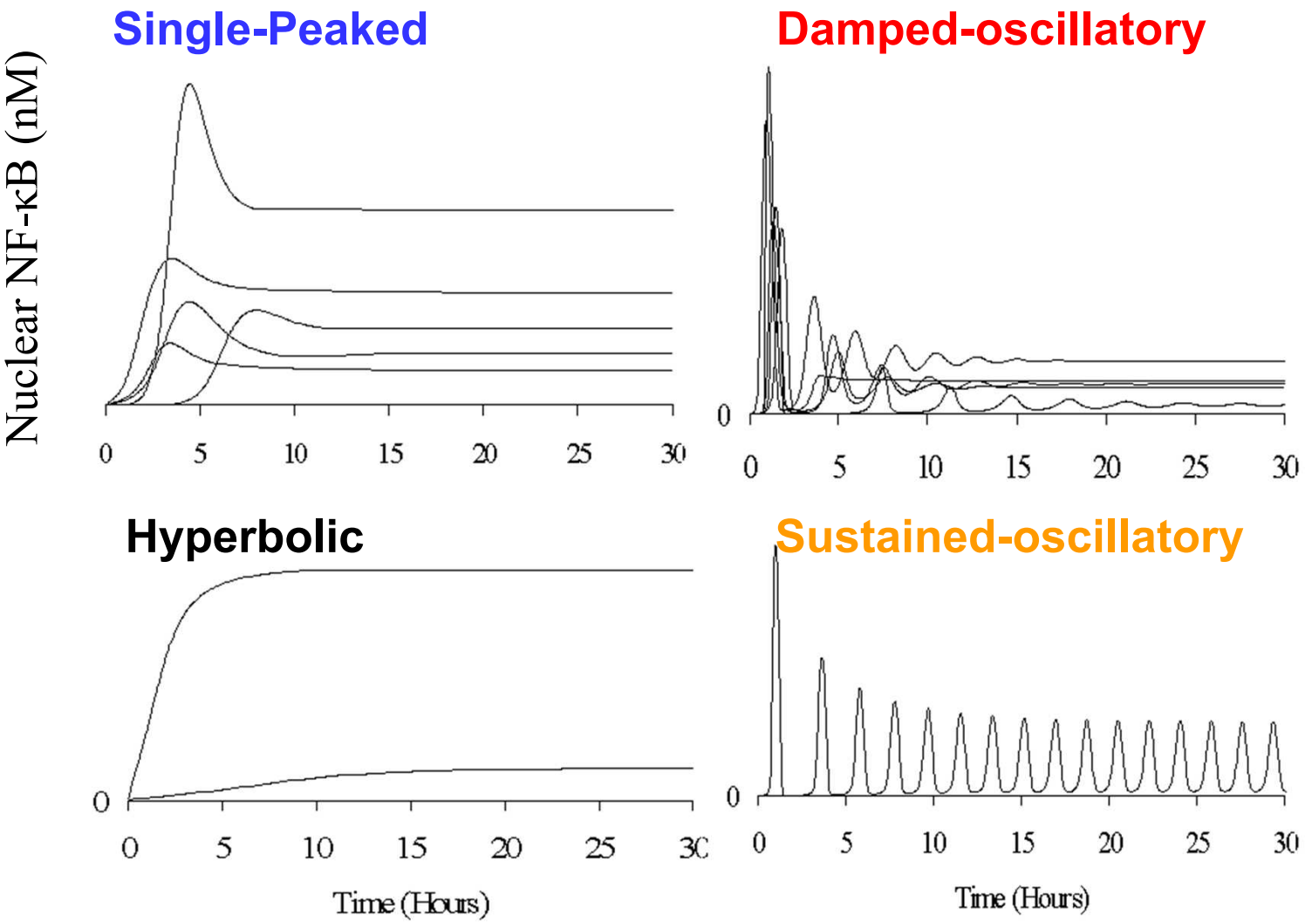
High NF-κB concentration:
Strong and fast NF-κB response with more temporal modulation

Low NF-κB concentration:
Weak and slow NF-κB response with less temporal modulation

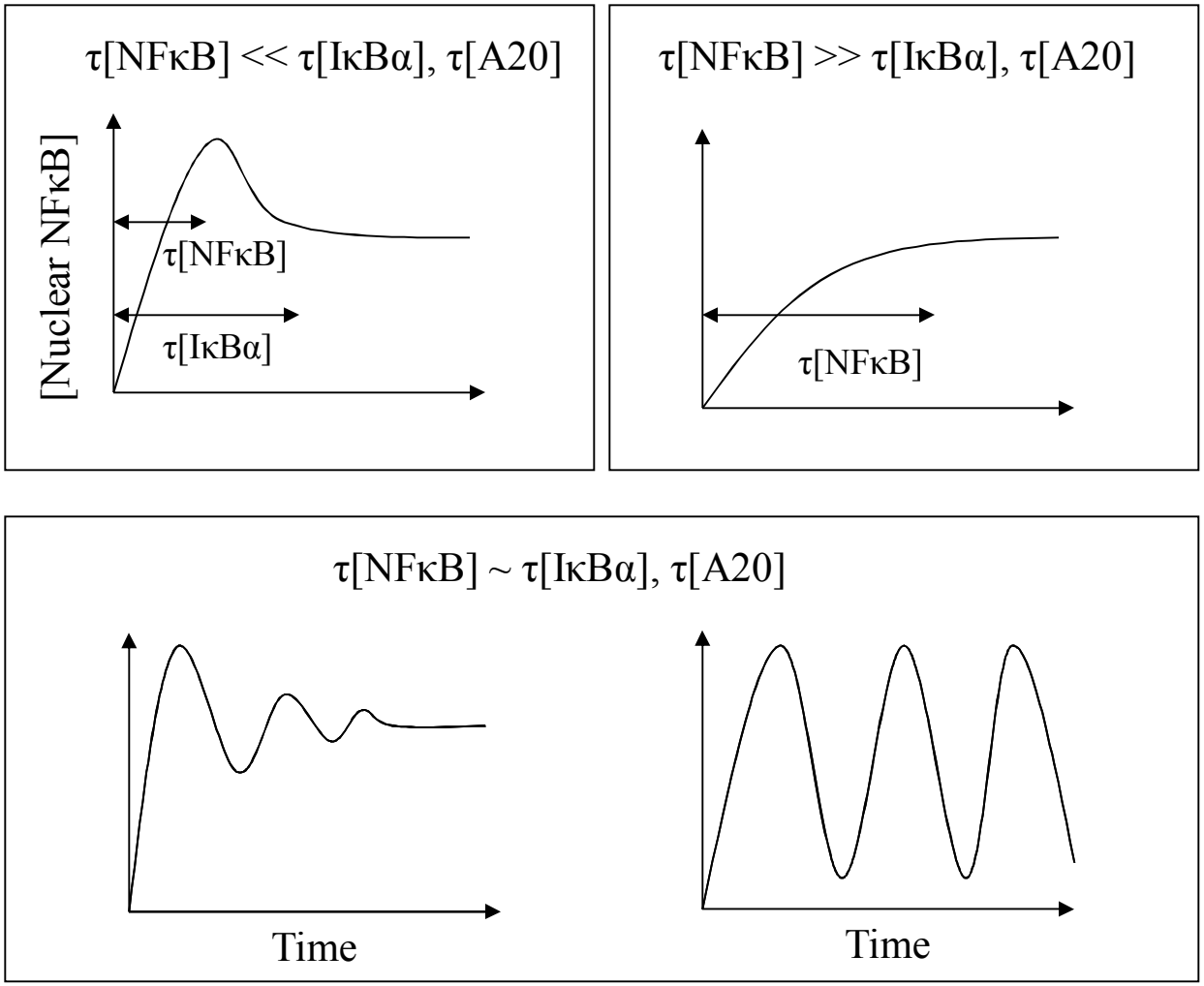
Question:

What is statistical ensemble of NF-κB response?

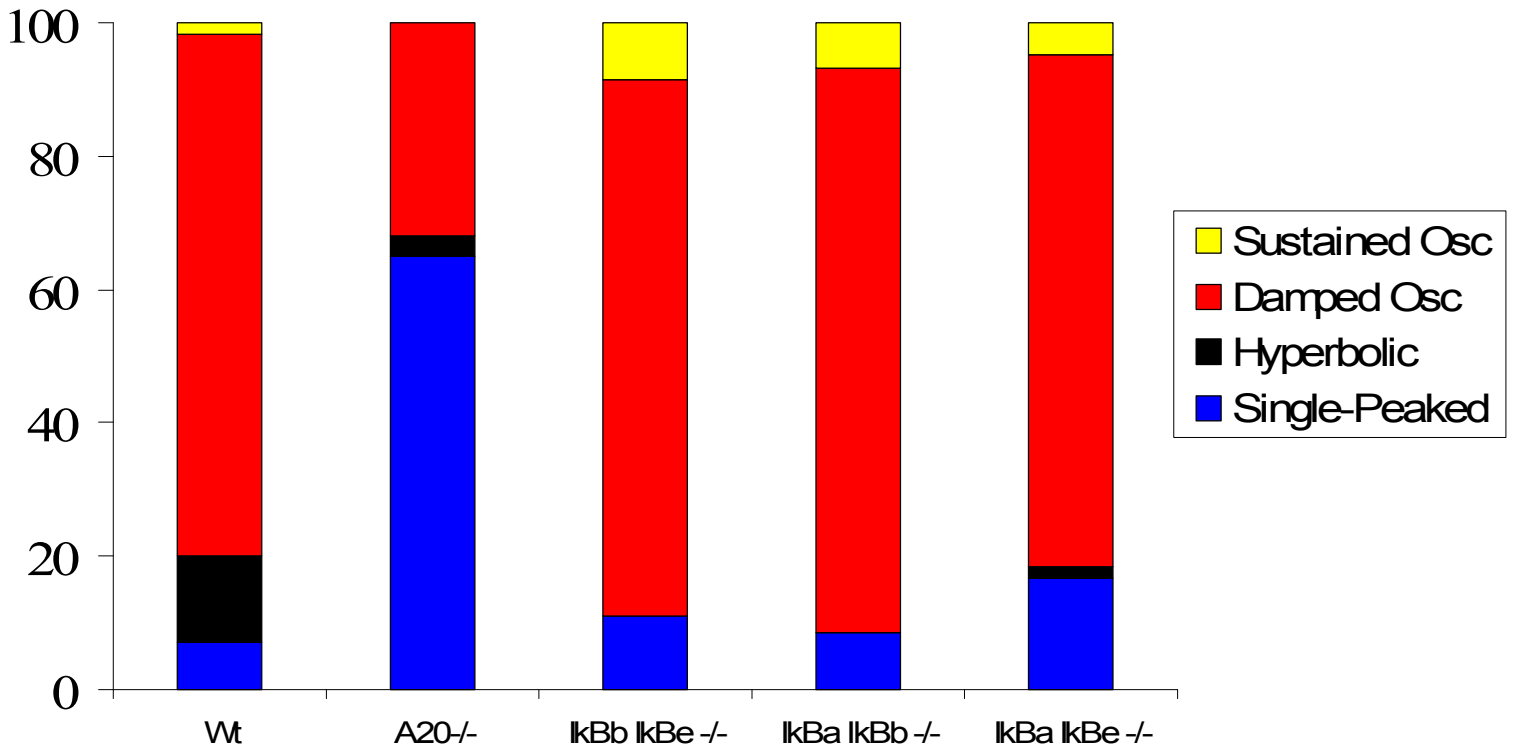
Four basic dynamic patterns of NF-κB response



Four basic dynamic patterns depends on protein response time τ



Distributions of dynamic patterns of NF-κB response



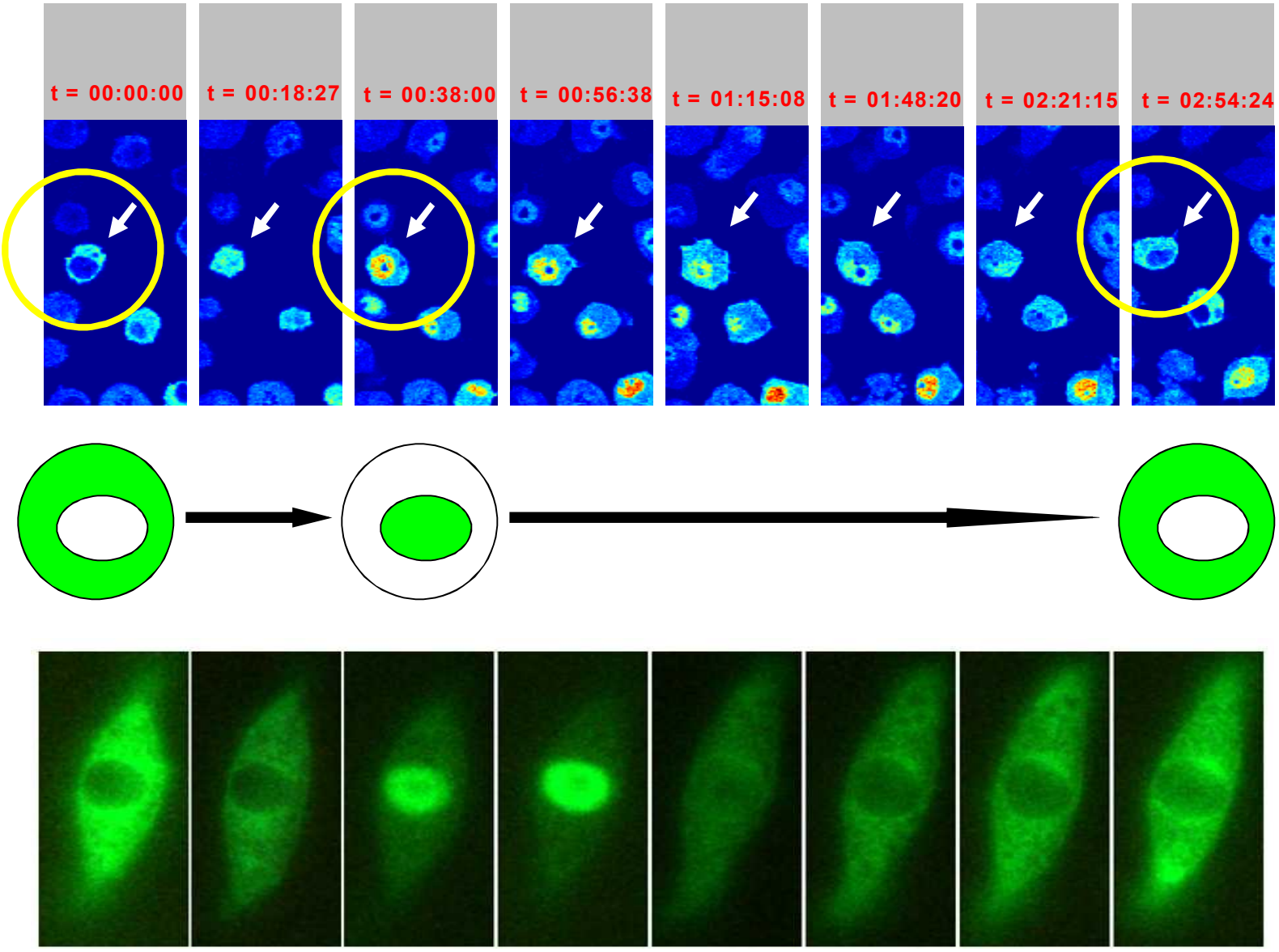
- Signal strength=large ; number of samples=1000; interval size= 80%
- Most probable dynamic patterns are most observable & even robust against fluctuations of reaction rates.

Question:

What are the conditions for experimentally observable oscillation of NF-kB in vivo?

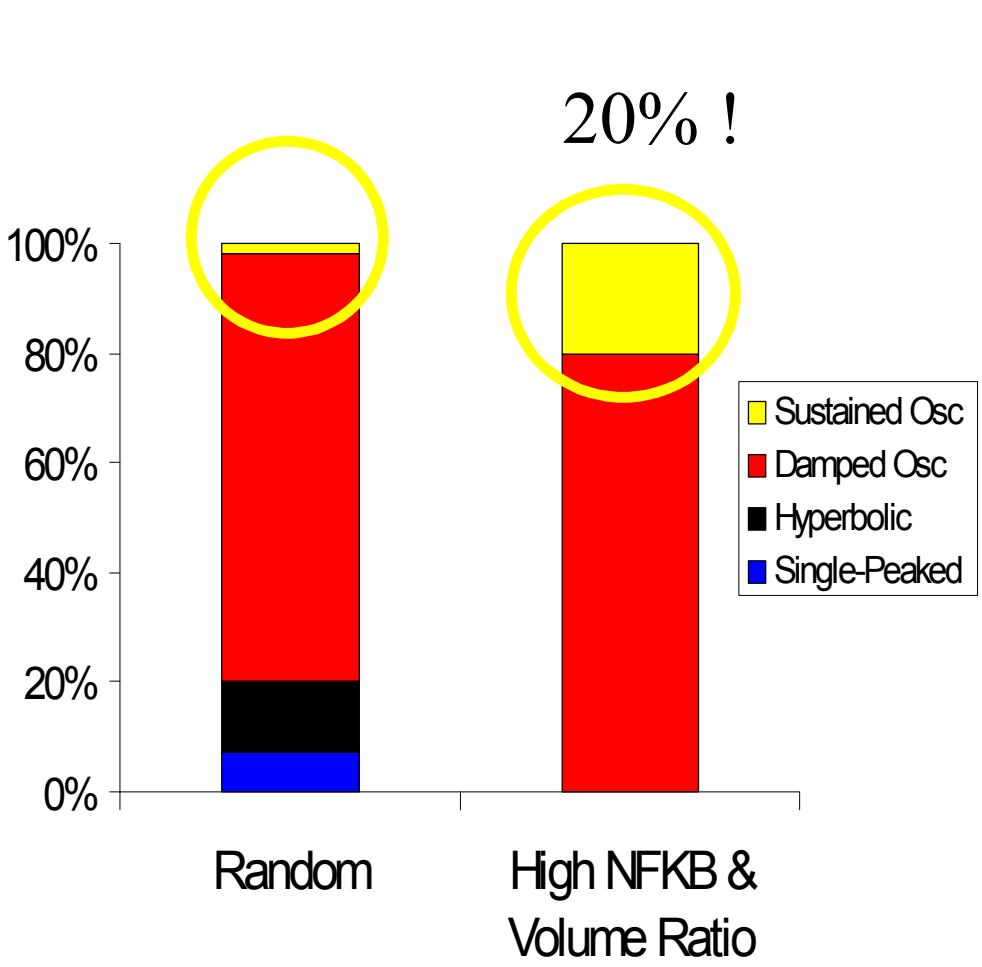
(With Full Hybrid Model)

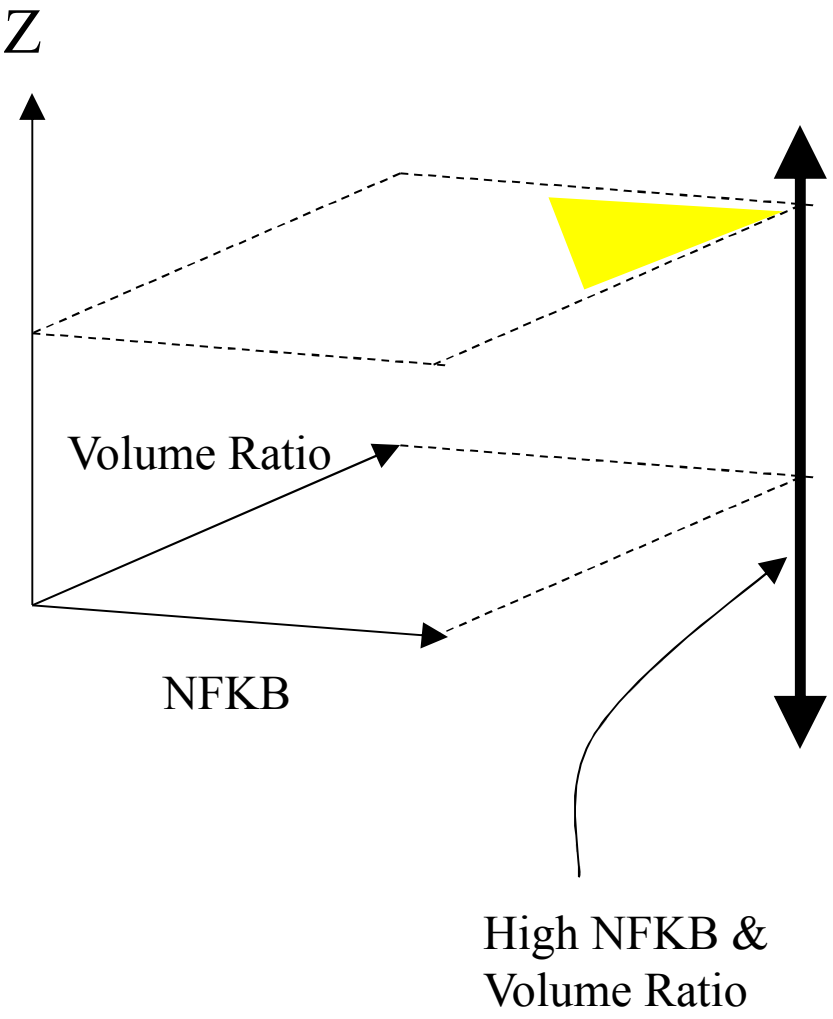
RAW cells challenged with persistent LPS stimulus



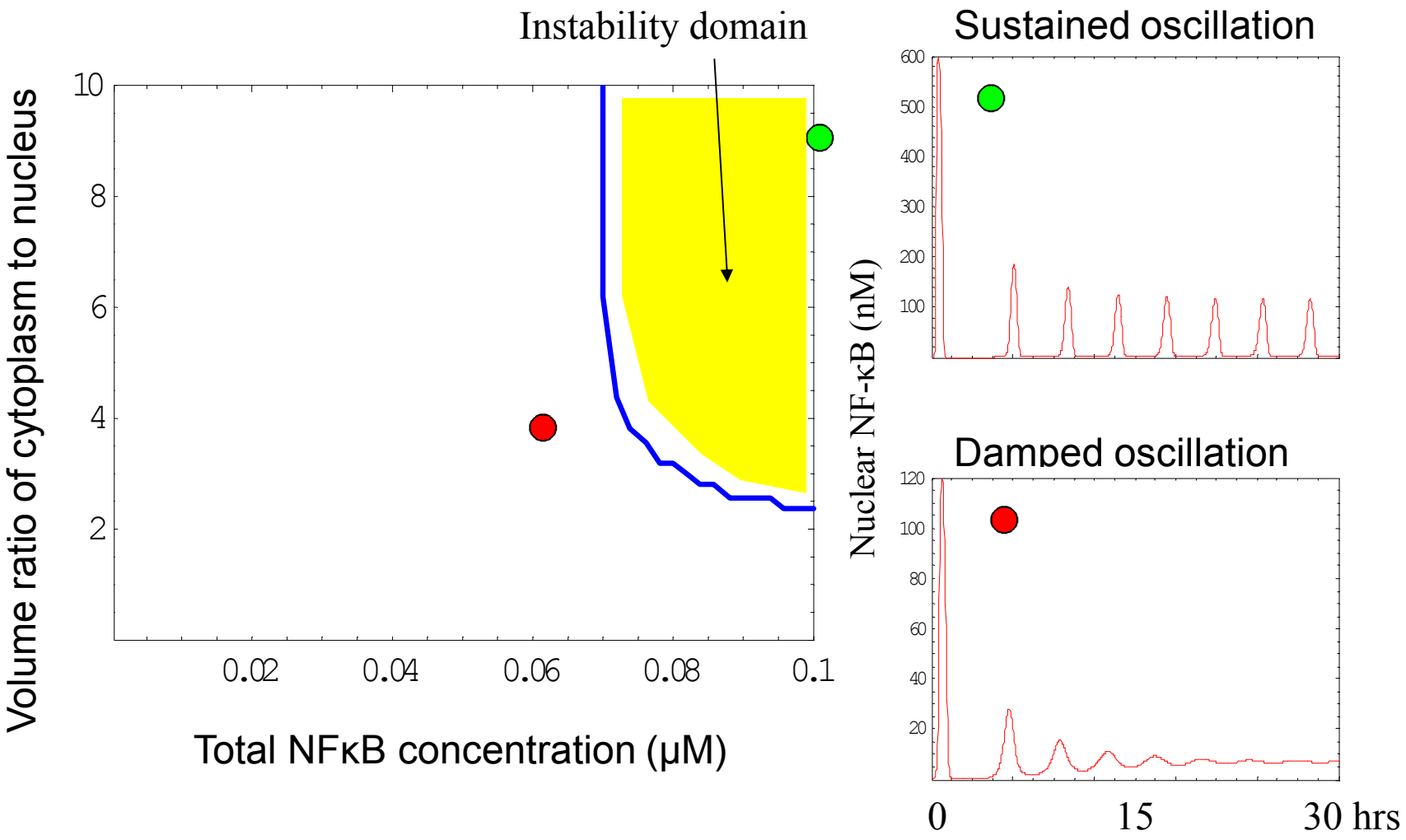
Unpublished, Jens Poschet, Bryan Carson, Sandia National Labs.

Distribution of NF-κB dynamic patterns for high total NF-κB concentration & volume ratio

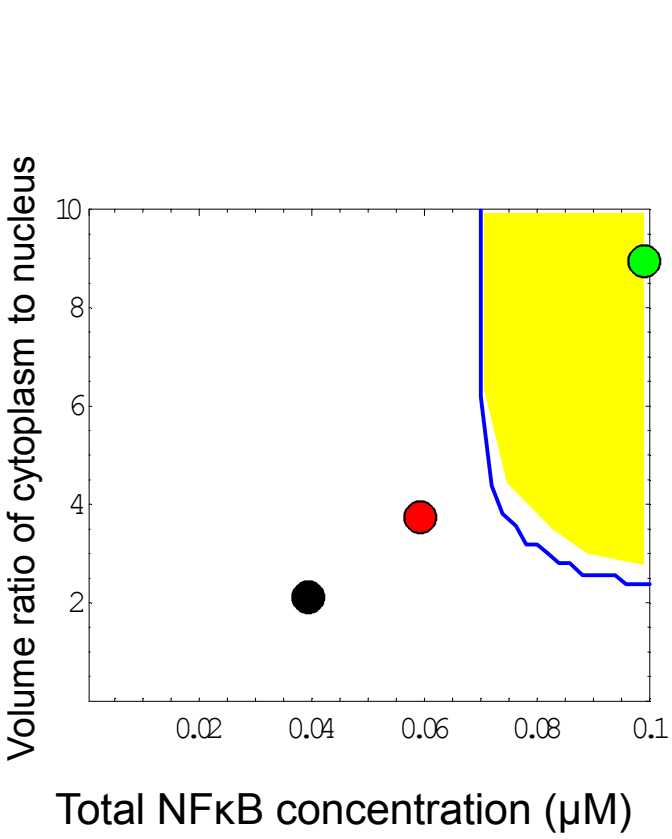


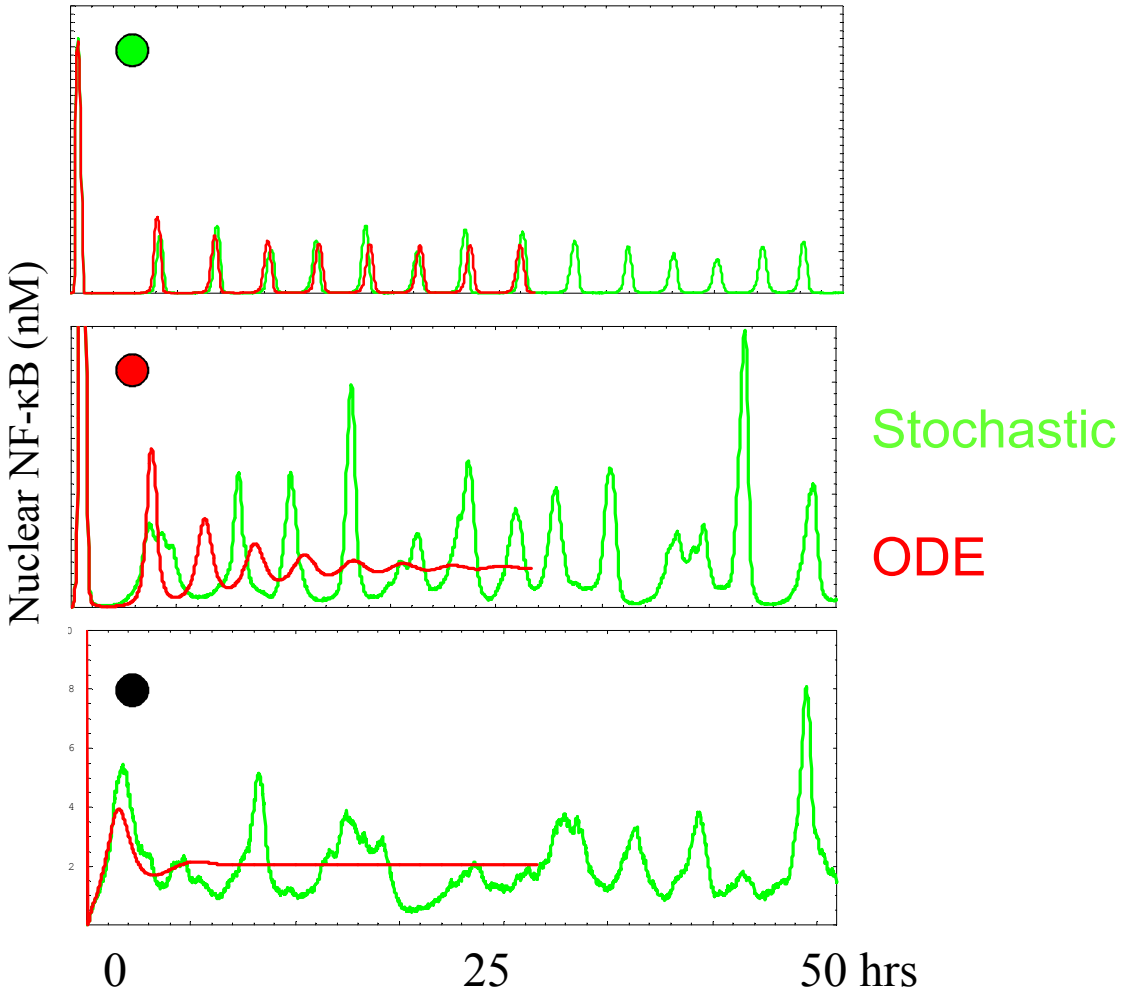


Bifurcation Diagram: with Deterministic Full Hybrid Model



Can noise induce NF-κB oscillation?



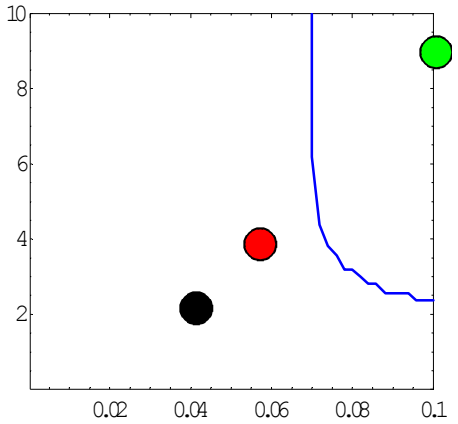


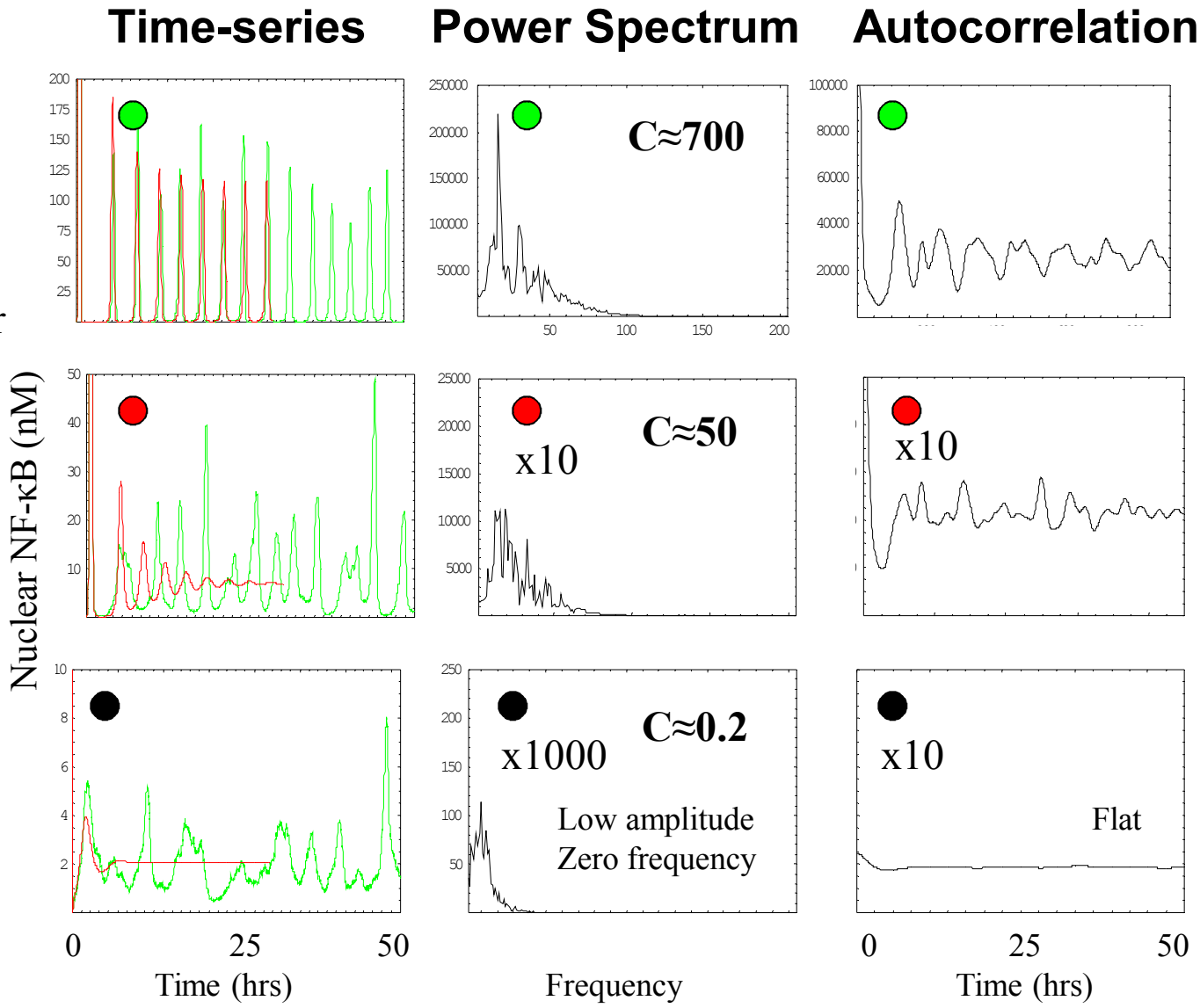
For Gillespies simulations, cytoplasm volume of RAW cells, 1643 μm^3, is used.

Criteria for amplified noise-induced oscillation

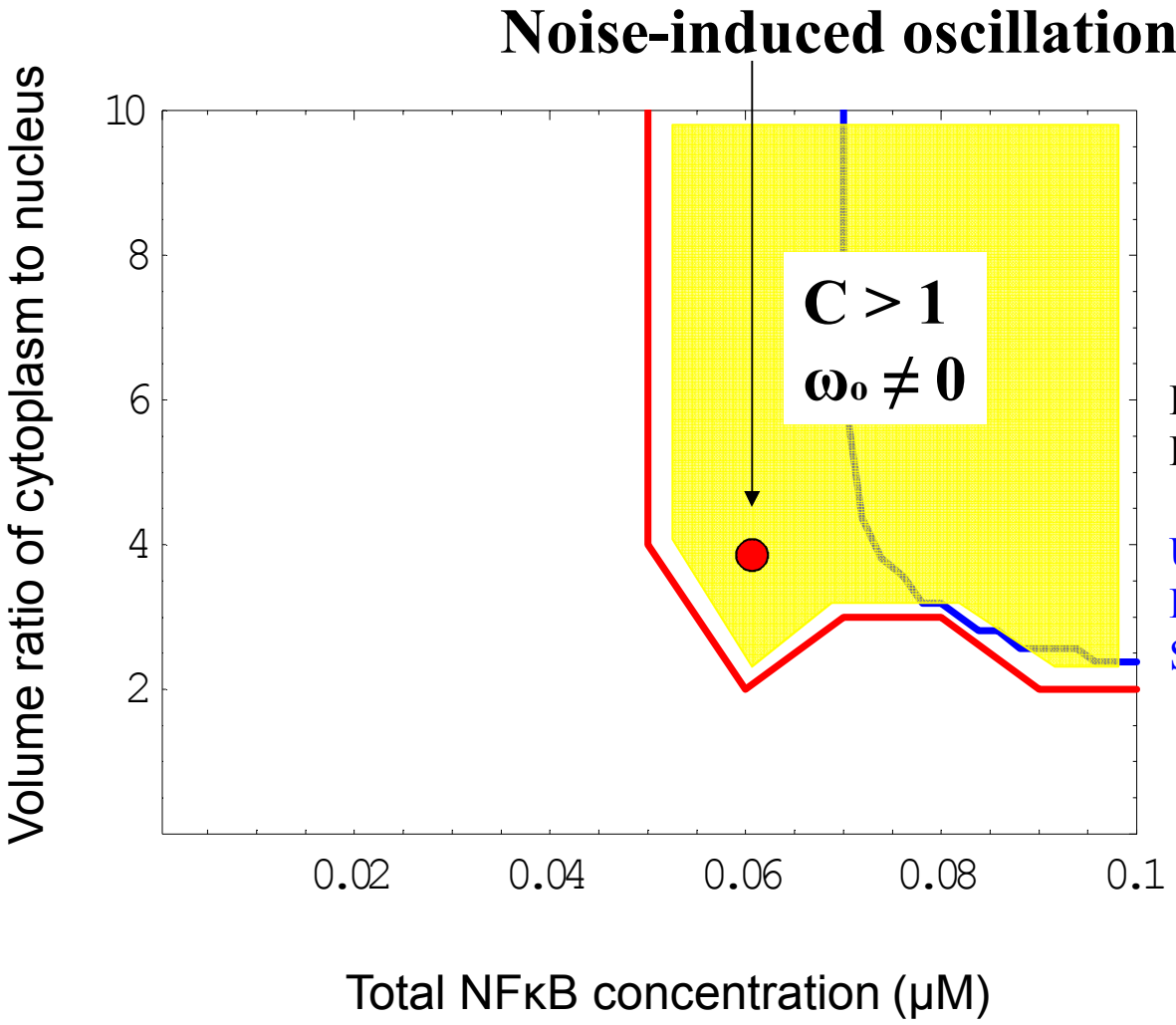
C=R/√N

R: peak amplitude
N: total NFKB number





Noise expands oscillatory domain: With Stochastic Full Hybrid Model



HeLa cells: volume ratio = 2.8
RAW cells: volume ratio = 2.5

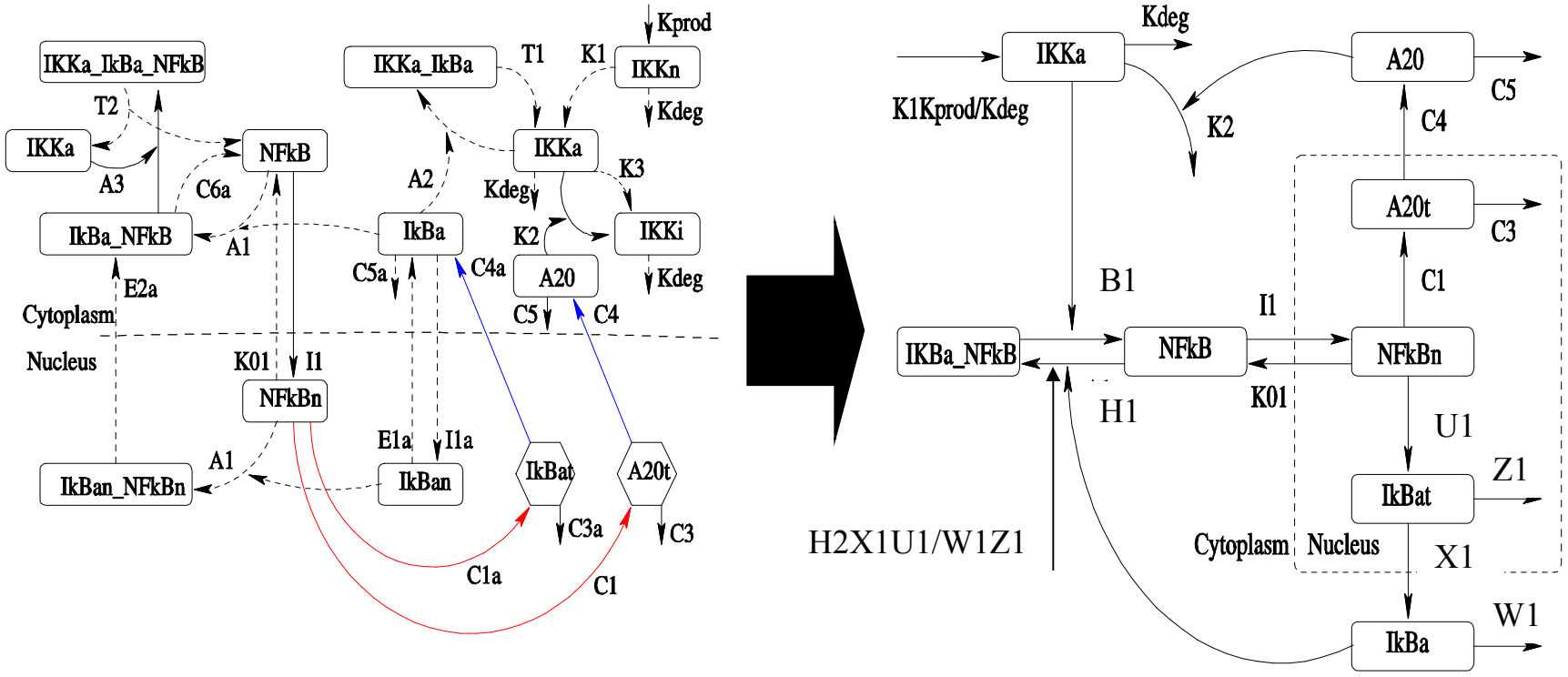
Unpublished,
Howland Jones, Bryan Carson,
Sandia National Labs.

Question 6:

What are the conditions for experimentally observable oscillation of NF-kB in vivo?

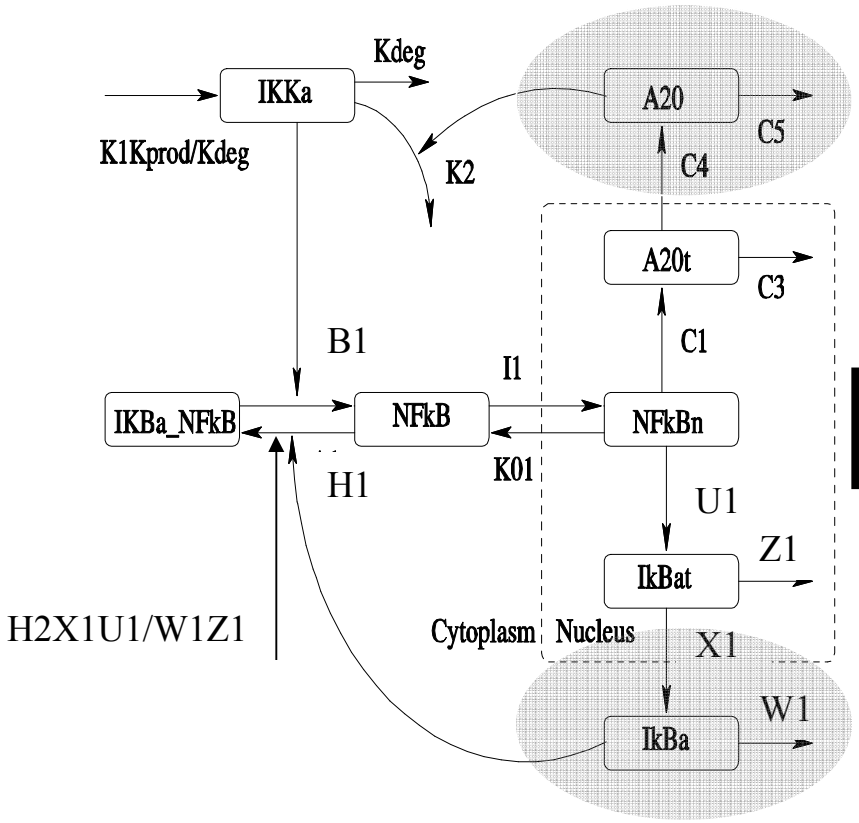
(With Minimal Model)

Model reduction: Renormalization of kinetic rate variables

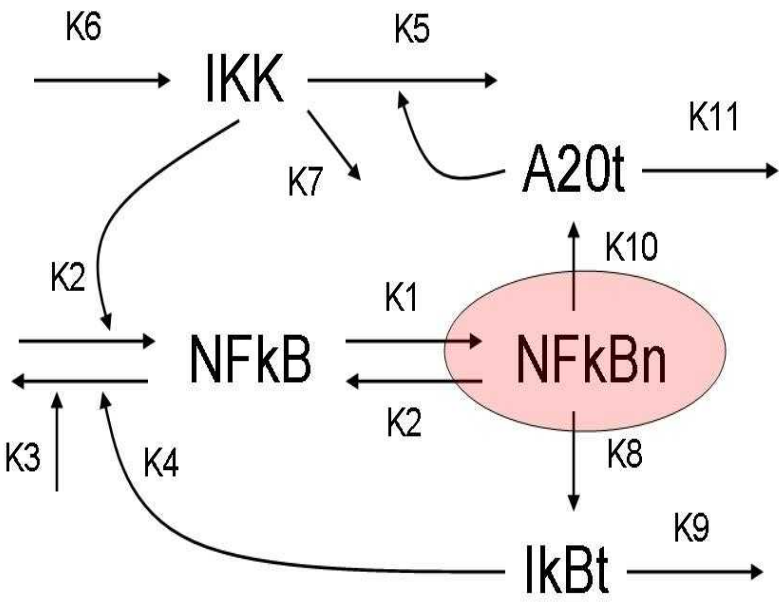


Full Hybrid Model **Intermediate Model**

Model reduction: Renormalization of kinetic rate variables

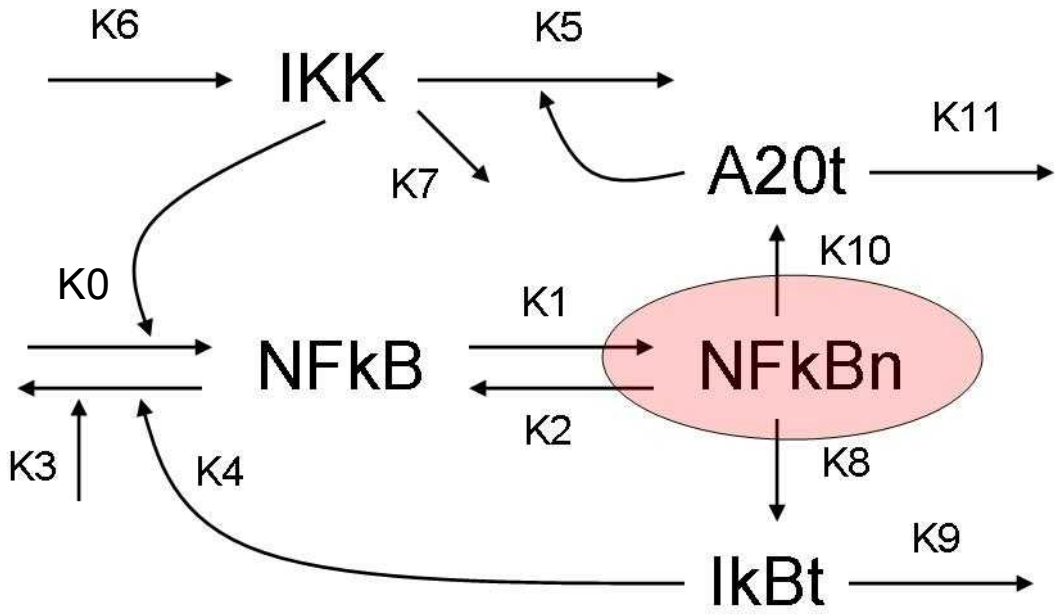


Intermediate Model



Minimal Model

Stochastic two compartmental model: Explicitly include total NF-κB and two compartments



System-size expansion of Master equation

Van Kampen Ansatz:

$X = x\,Vc + \xi x\,Vc^{(1/2)}$: species in cytoplasm

$Y = y\,Vn + \xi y\,Vn^{(1/2)}$: species in nucleus

Macroscopic concentration

Gaussian correction

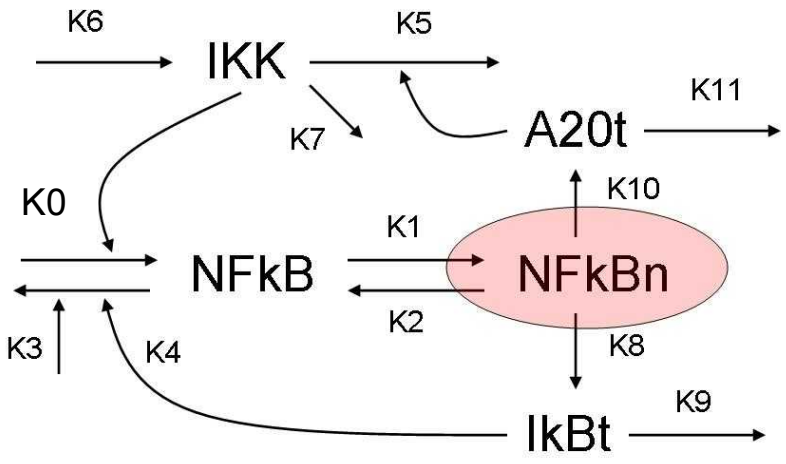
$d\,P(X,\ldots)/dt = d\,\pi\,(\xi x\,,\ldots)/dt$

$= \text{Terms}\,(V^{(1/2)}) + \text{Terms}\,(V^{(0)}) + O(V^{(-1/2)})$

Macroscopic Equations

Langevin Equations

Emergence of macroscopic equations: Terms (V^(1/2))



d [NFkB] / dt = - K1[NFkB] + K2 [NFkBn] / kv

- K3 [NFkB] – K4 [NFkB] [IKBt]

+ Ko [IKK] (NFkB_TOT – [NFkB] – [NFkBn] / kv)

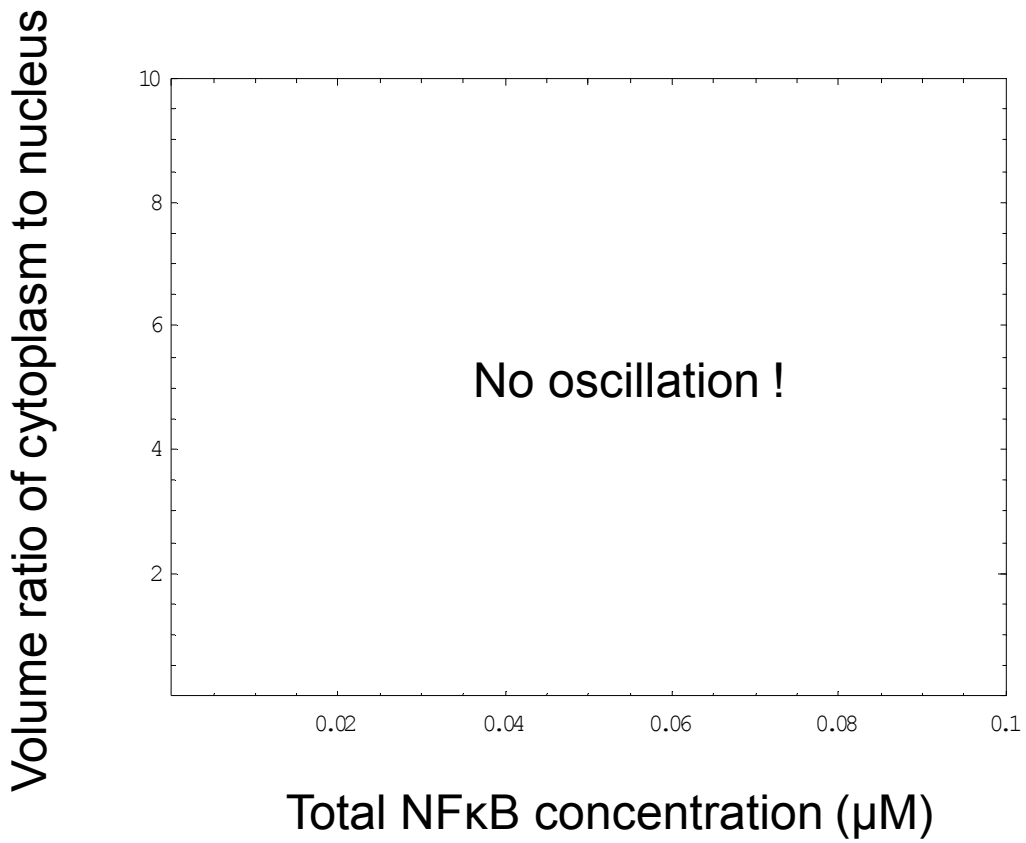
d [NFkBn] / dt = K1 kv [NFkB] – K2 [NFkBn]

d [IkBt] / dt = K8 [NFkBn] / kv –K9 [IkBt]

d [A20t] / dt = K10 [NFkBn]/kv – K11 [A20t]

d [IKK] / dt = K6 – K5 [IKK] [A20t] – K7 [IKK]

Bifurcation diagram: with Deterministic Minimal model



A single positive fixed point is stable for the kinetic rate variables under our consideration.

Linear Fokker Planck equation: Terms(V^(0))

$d\,\pi(\xi_\alpha,\dots)/dt = -\sum \partial (A_\alpha\,\pi) / \partial\,\xi_\alpha + (1/2) \sum B_{\alpha\beta}\,\partial\text{^}(2)\,\pi / \partial\,\xi_\alpha\,\partial\,\xi_\beta$

A_α is a linear function of ξ: $A_\alpha = \sum M_{\alpha\beta}\,\xi_\beta$ where "**M**" is a matrix without ξ.

"**B**" is noise covariance matrix, responsible for amplification of noise.

Conversion to Langevin equations for simplicity of analysis:

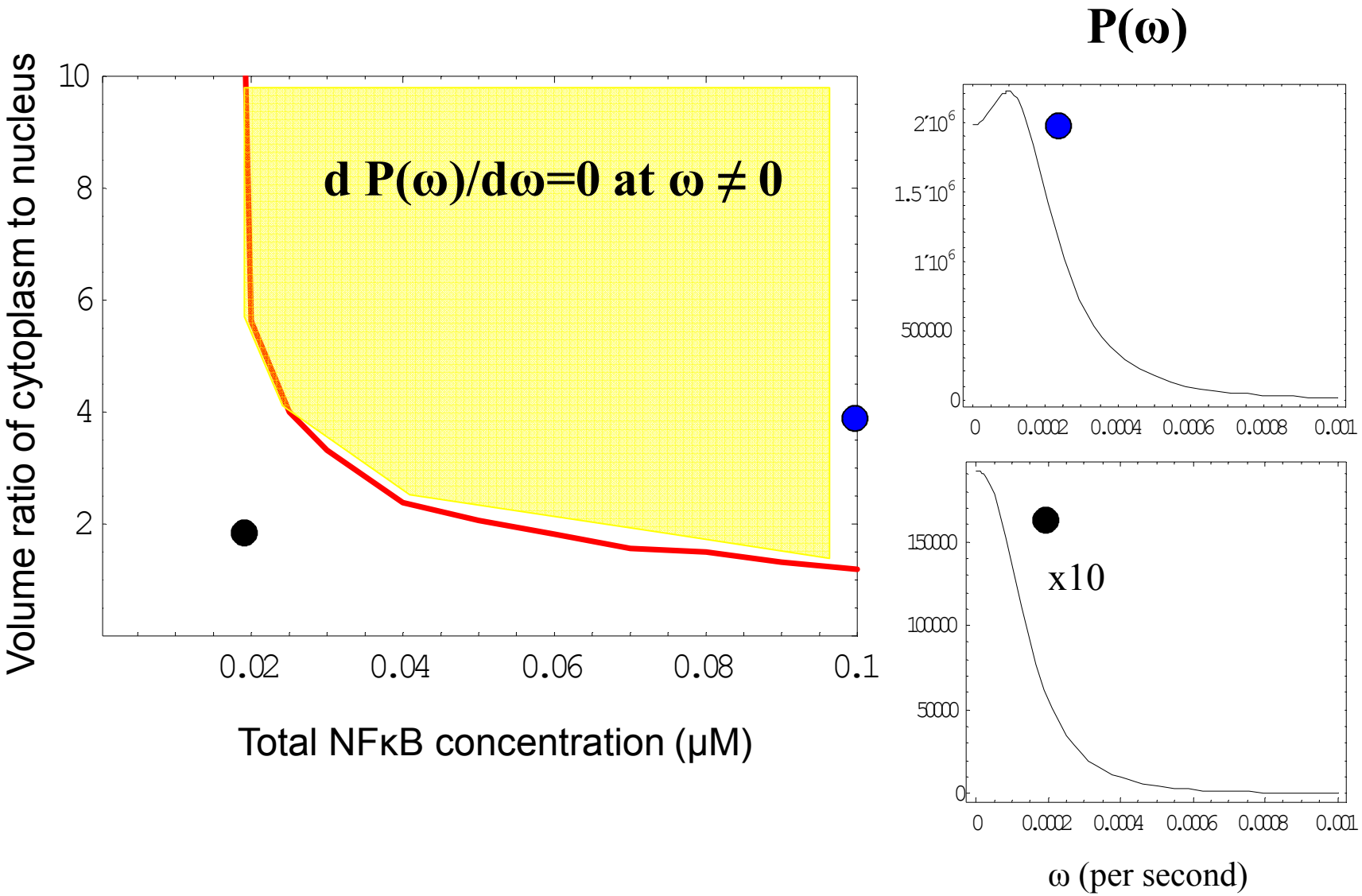
$d\,\boldsymbol{\xi}\,(t)/\,dt = \mathbf{M}\,\boldsymbol{\xi}\,(t) + \boldsymbol{\eta}\,(t)$ where $\langle\eta_\alpha\,(t)\,\eta_\beta\,(t')\rangle = B_{\alpha\beta}\,\delta\,(t-t')$

Power Spectrum :

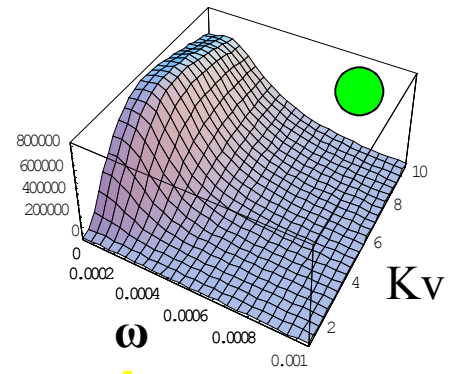
$P_\alpha\,(\omega) = \langle\,\xi_\alpha\,(\omega)\,\xi_\alpha^*(\omega)\rangle = \sum\sum\,(-\,i\omega\boldsymbol{E} - \boldsymbol{M})\,\text{^}(-1)_{\alpha\beta}\,B_{\beta\gamma}\,(-i\omega\boldsymbol{E} - \boldsymbol{M}\,)^*_{\gamma\alpha}$

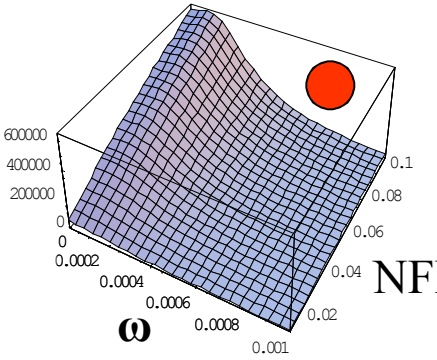
We calculate power spectrum from ***M*** and ***B*** matrices.

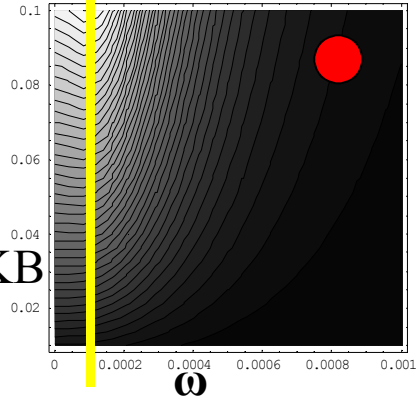
Noise-induced oscillatory domain: with Stochastic Minimal Model

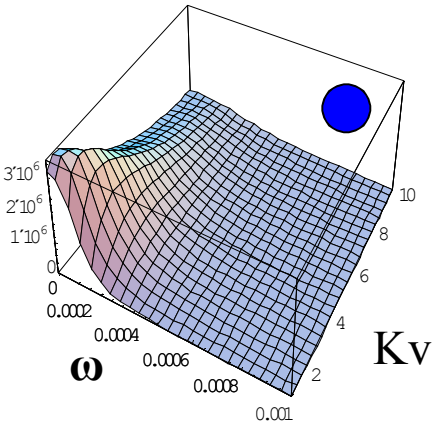


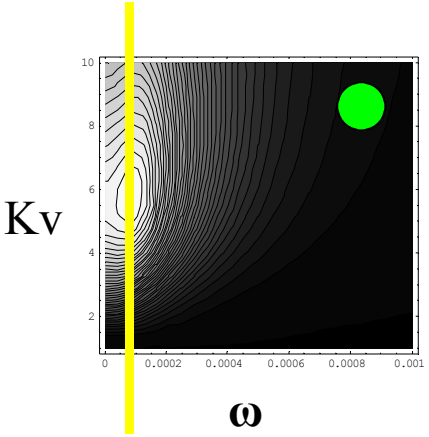
Period of noise-induced oscillation is independent of total NF-κB concentration and volume ratio





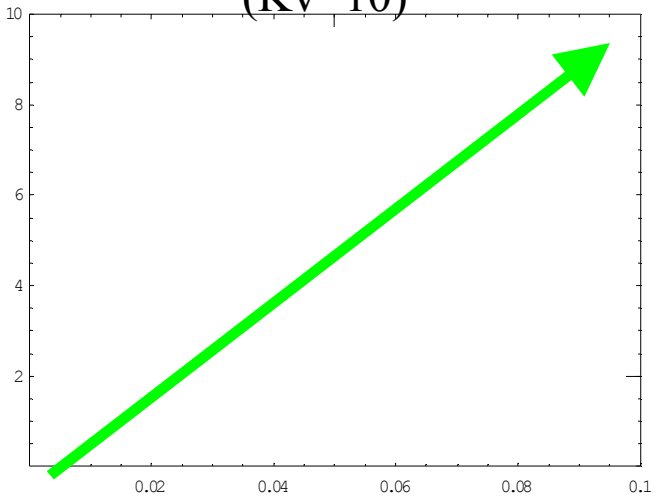


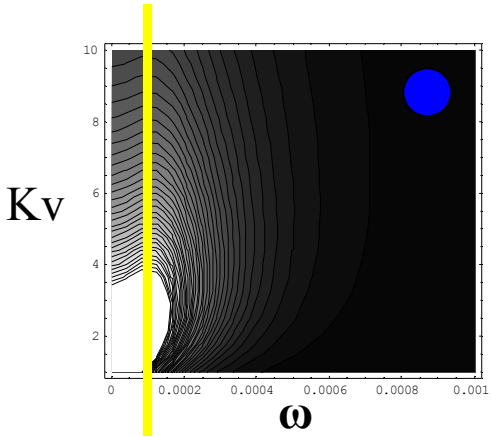




Volume ratio of cytoplasm to nucleus

(Kv=10)





Total NFκB concentration (μM)

(NFKB=0.1)

Conclusion

1. Sensitivity analysis reveals that the NF-κB dynamics critically depends on total NF-κB concentration and volume ratio of cytoplasm to nucleus.

2. Deterministic full hybrid model generates the dynamic instability when both total NF-κB concentration and volume ratio are large.

3. Noise expands the instability domain of NF-κB, i.e., emergence of noise-induced oscillation of NF-κB at its natural frequency.

4. Stochastic minimal model qualitatively reproduces the noise-induced oscillation of NF-κB whereas its deterministic counterpart has only stable fixed point.

Collaborators

Sandia National Laboratories:

Dr. Jean-Loup Faulon

Dr. Steve Plimpton

Dr. Laura Swiler

Extra Slides

Sensitivity Analysis: List of important reactions and species

Computational Model

Important Kinetic rate variables:

- Protein concentration of [NF-κB]
- Volume ratio of cytoplasm to nucleus
- mRNA synthesis of [IkBα] & [A20]
- Protein synthesis of [IkBα] & [A20]
- Degradation/production of IKK
- A20-induced inactivation of IKK
- IKK-NFκB-IκBα complex formation
- Transport of NFkB & IkBα

Experiments

Important Biochemical Species:

- Protein [NFkB]
- Volume ratio of cytoplasm to nucleus
- mRNA [IkBα]
- mRNA [A20]
- Protein [IkBα]
- Protein [A20]
- Protein [free IKKβ]
- Protein [IKK_IkBα_NFkB]

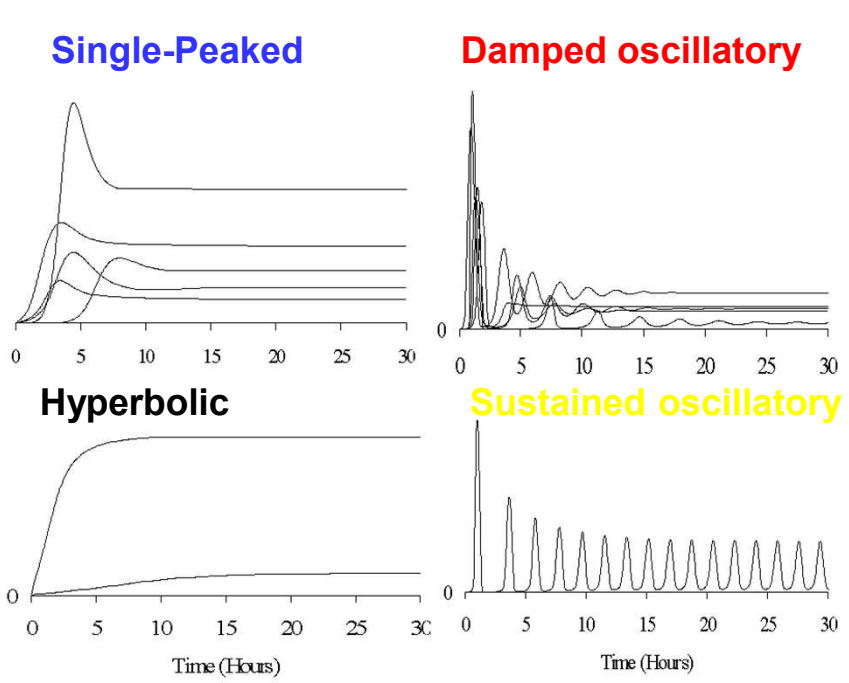
Some are currently being measured by Bio-Core & Platform Core.

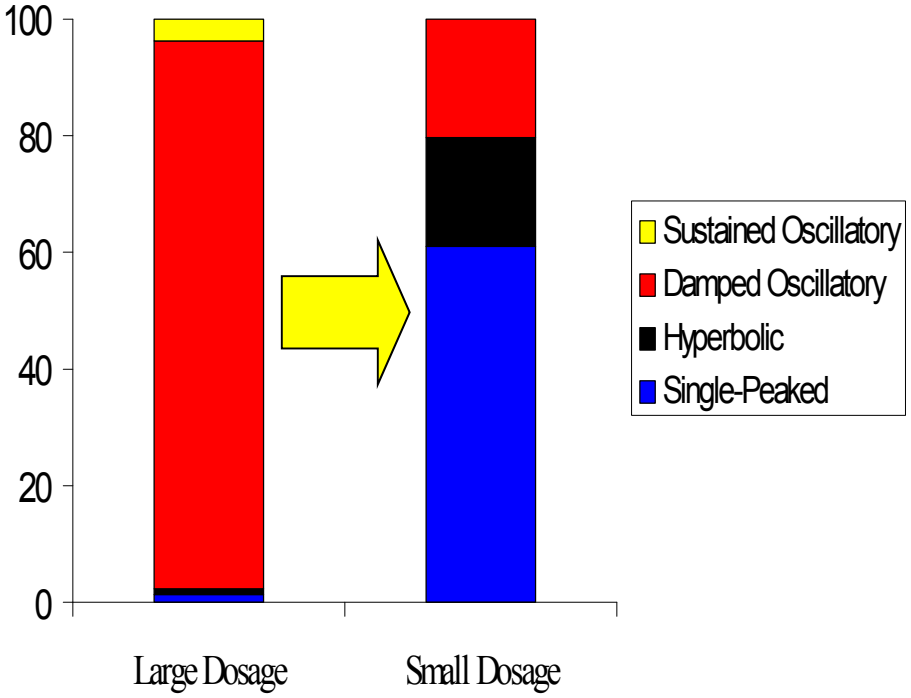
NF-κB Response for Macrophages

Question:

Does LPS dosage level change the distribution of NF-κB dynamic patterns?

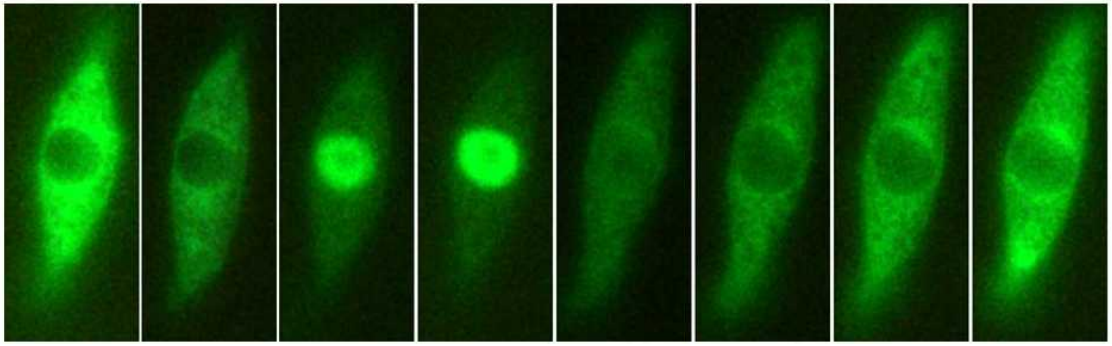
LPS dosage amount changes distribution of dynamic patterns of NF-κB response

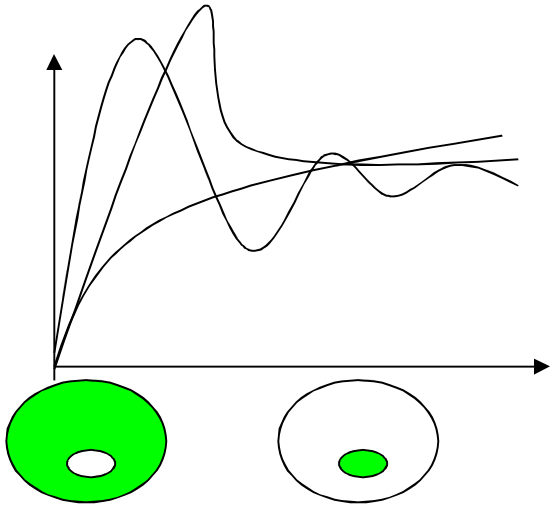




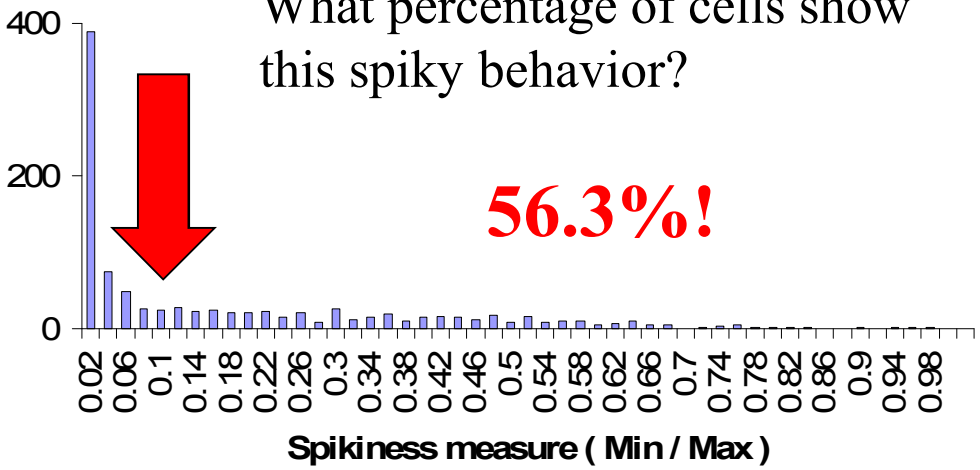
- For large dosage, damped oscillatory pattern is exclusively most probable.
- For small dosage, single-peaked and hyperbolic patterns are most probable.

Translocation of RelA-GFP in macrophages challenged with LPS



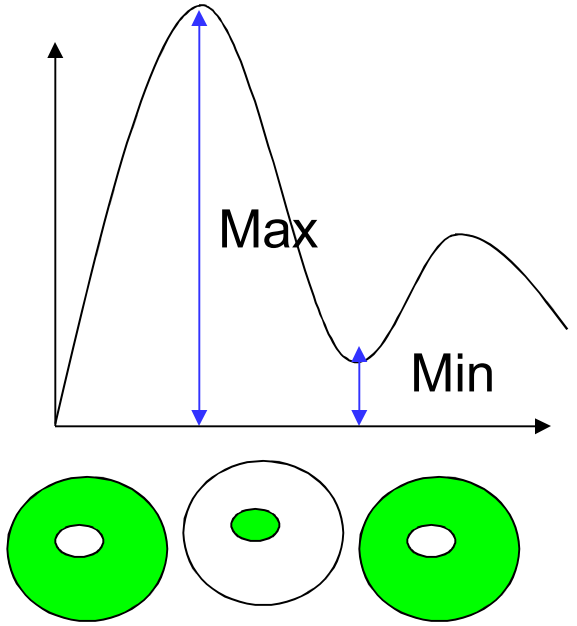


RelA-GFP in and out of nucleus only in 10% of cells



What percentage of cells show this spiky behavior?

56.3%!



NF-κB Response for Macrophages

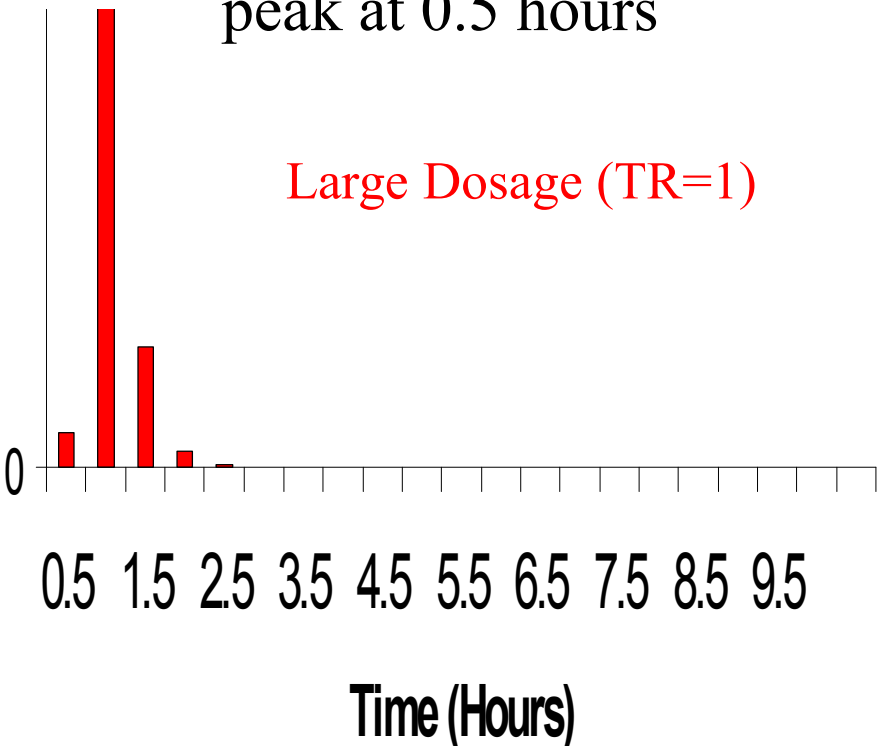
Question:

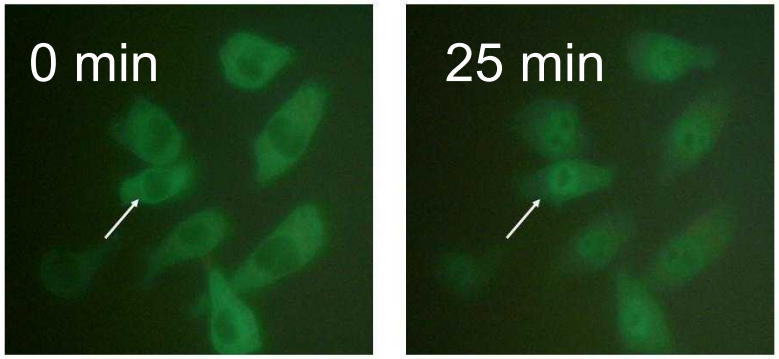
Does different LPS dosage level affect NF-κB translocation time?

Average NF-κB translocation time is equal to a phase of nuclear NF-κB profile

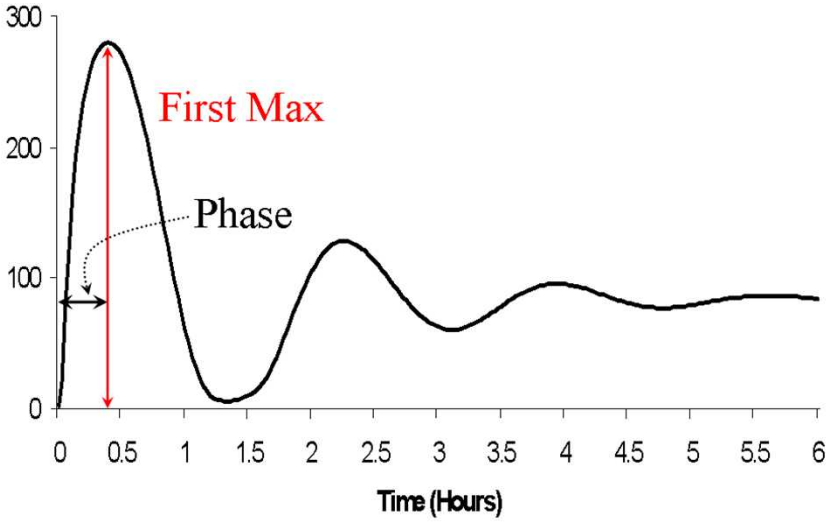
Distribution of phase: peak at 0.5 hours

Large Dosage (TR=1)



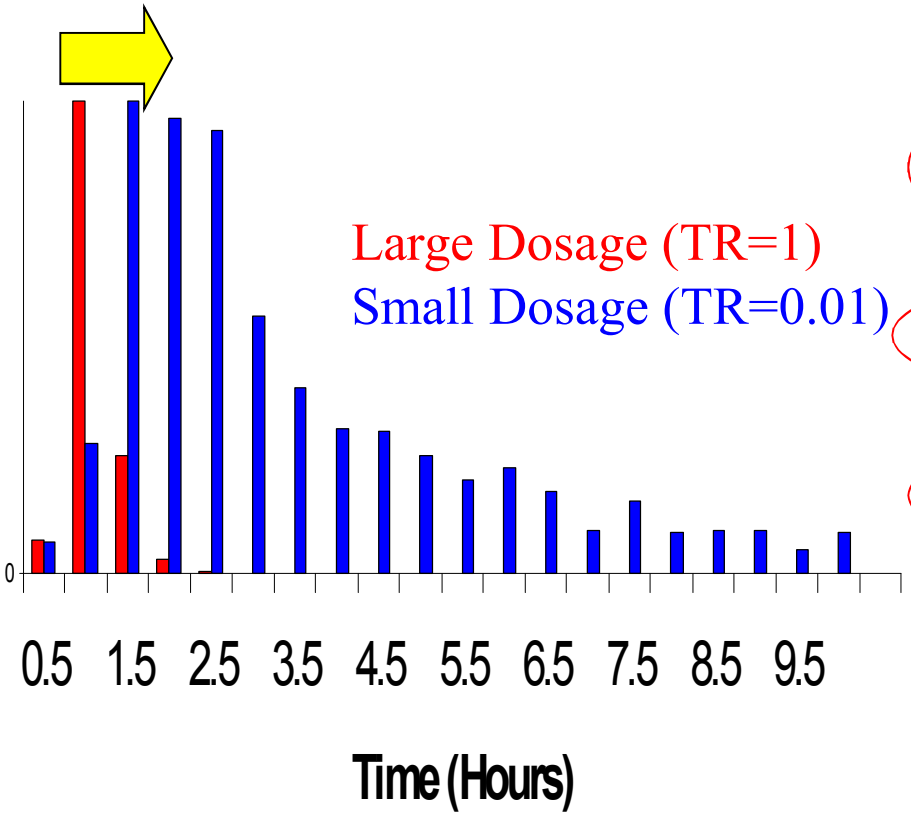


Macrophage stimulated with 1 μM Y. pesitis LPS



Different LPS dosage amount induces a shift of the distribution of NF-κB translocation time

Shift from 0.5 hours to 1 hour



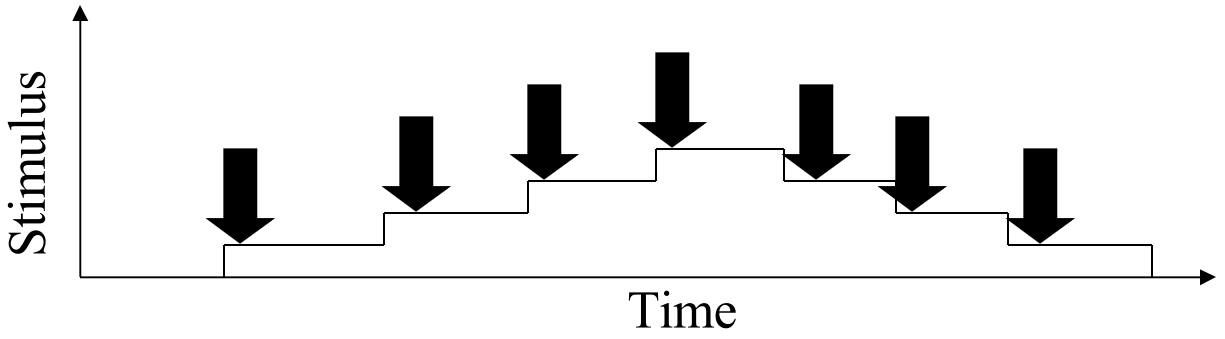
LPS	15min	25min	45min	60min	90min
1μM E. coli	X				
1nM E.coli		X			
1μM 21°C YP	X				
1nM 21°C YP		X			
1μM 37°C YP		X			
1nM 37°C YP			X		

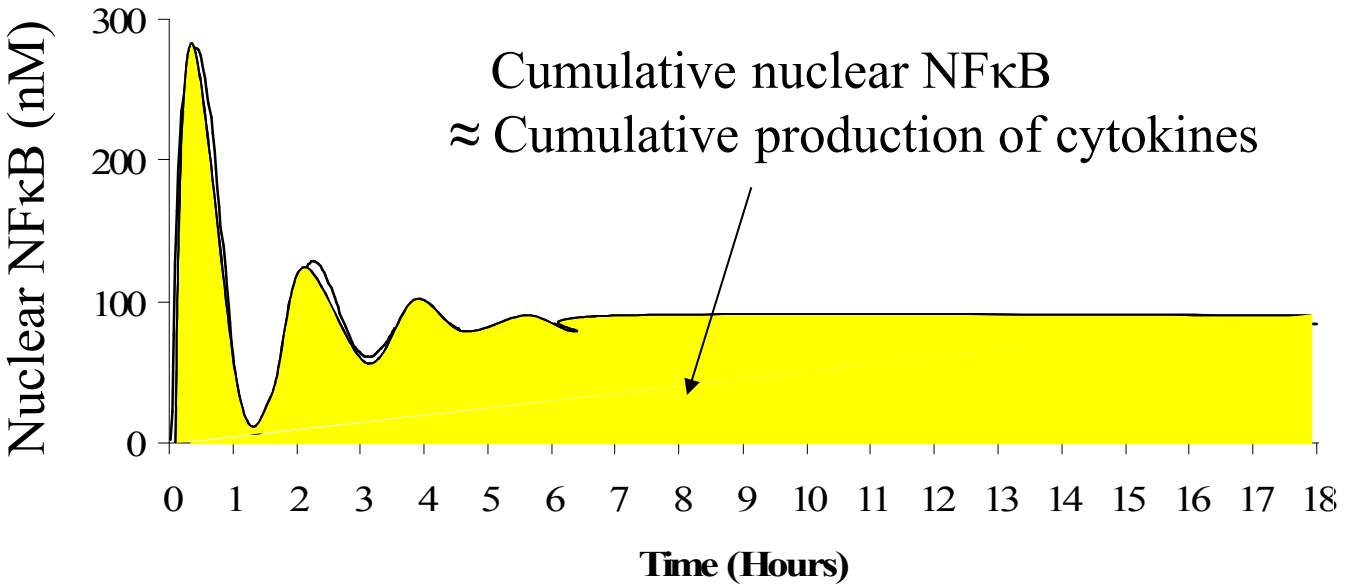
NF-κB Response for Macrophages

Question:

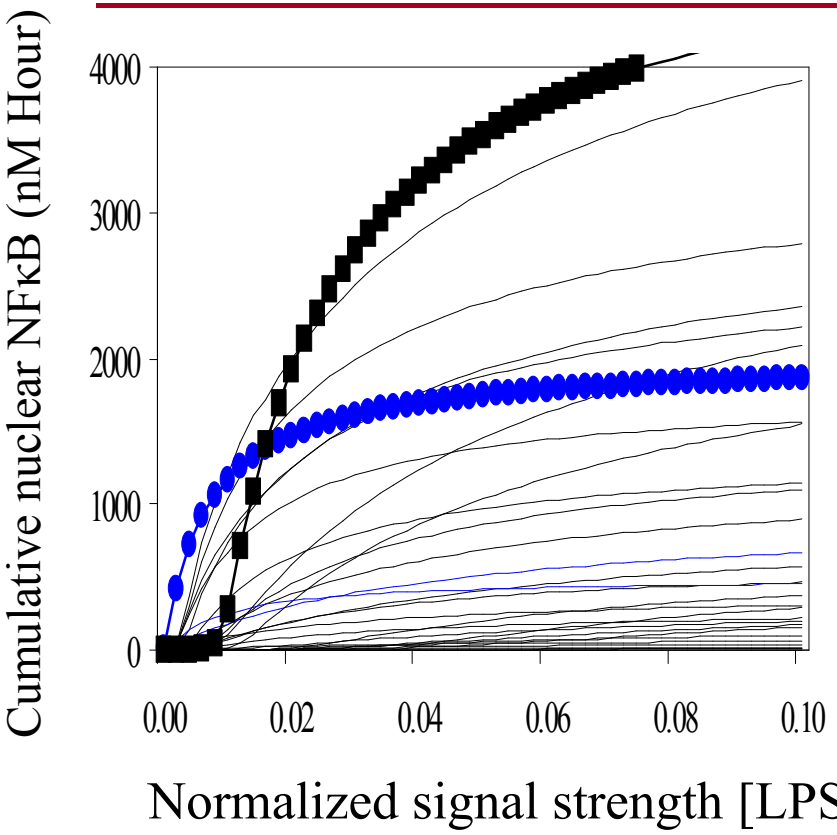
What is the shape of LPS dose-response curve?

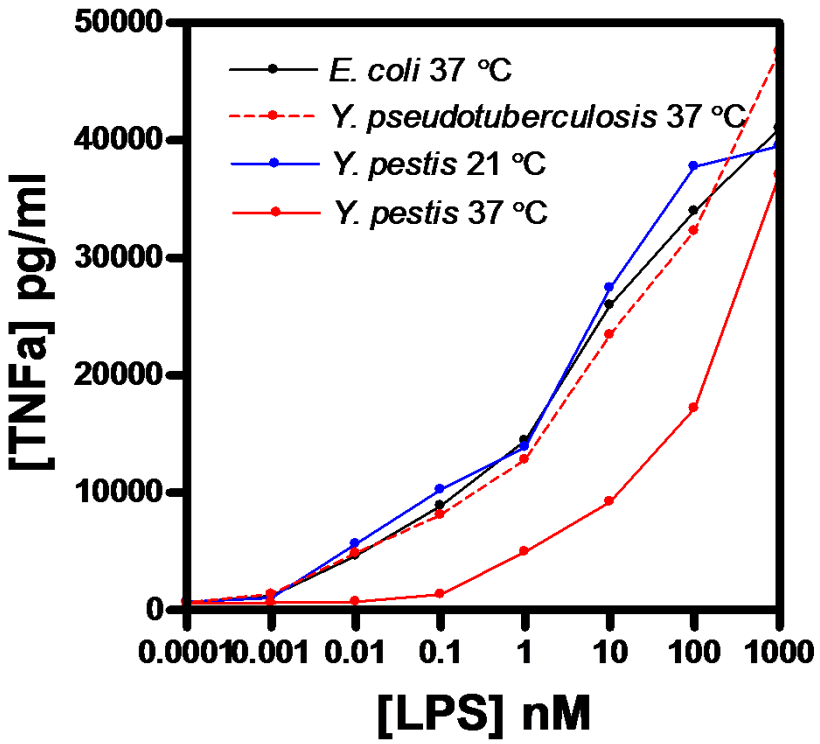
NFκB response to different LPS dosage levels



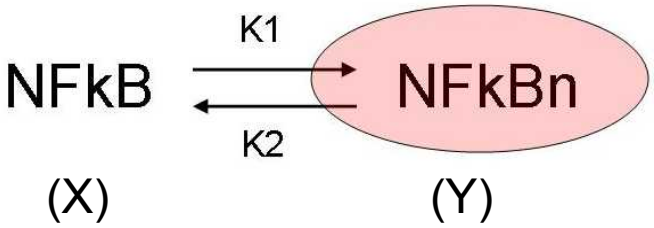


Dose-response curve with sigmoidal shape



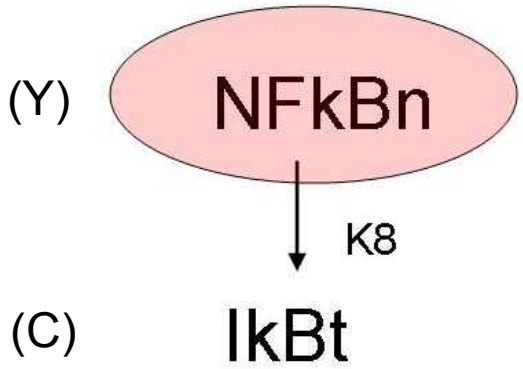


- Sigmoidal 92%; Hyperbolic 8%; Hysteresis 0%
- Sigmoidal shape == switching behavior of immune response
- No hysteresis == a single steady state and no memory



Transition probability per unit time:
K1 Vc (NFkB/Vc)
K2 Vn (NFkBn/Vn)

M-equation:
dP(X,Y)/dt = K1 (Ex^(+1)Ey^(-1)-1) X P(X,Y)
+ K2 (Ex^(-1)Ey^(+1)-1) Y P(X,Y)



Transition probability per unit time:
K8 Vn (NFkBn/Vn)

M-equation:
dP(Y,C)/dt = K8 (Ec^(-1)-1) Y P(X,Y)