SAND2007-3942C

Effects of two negative feedback loops on NF-κB signaling

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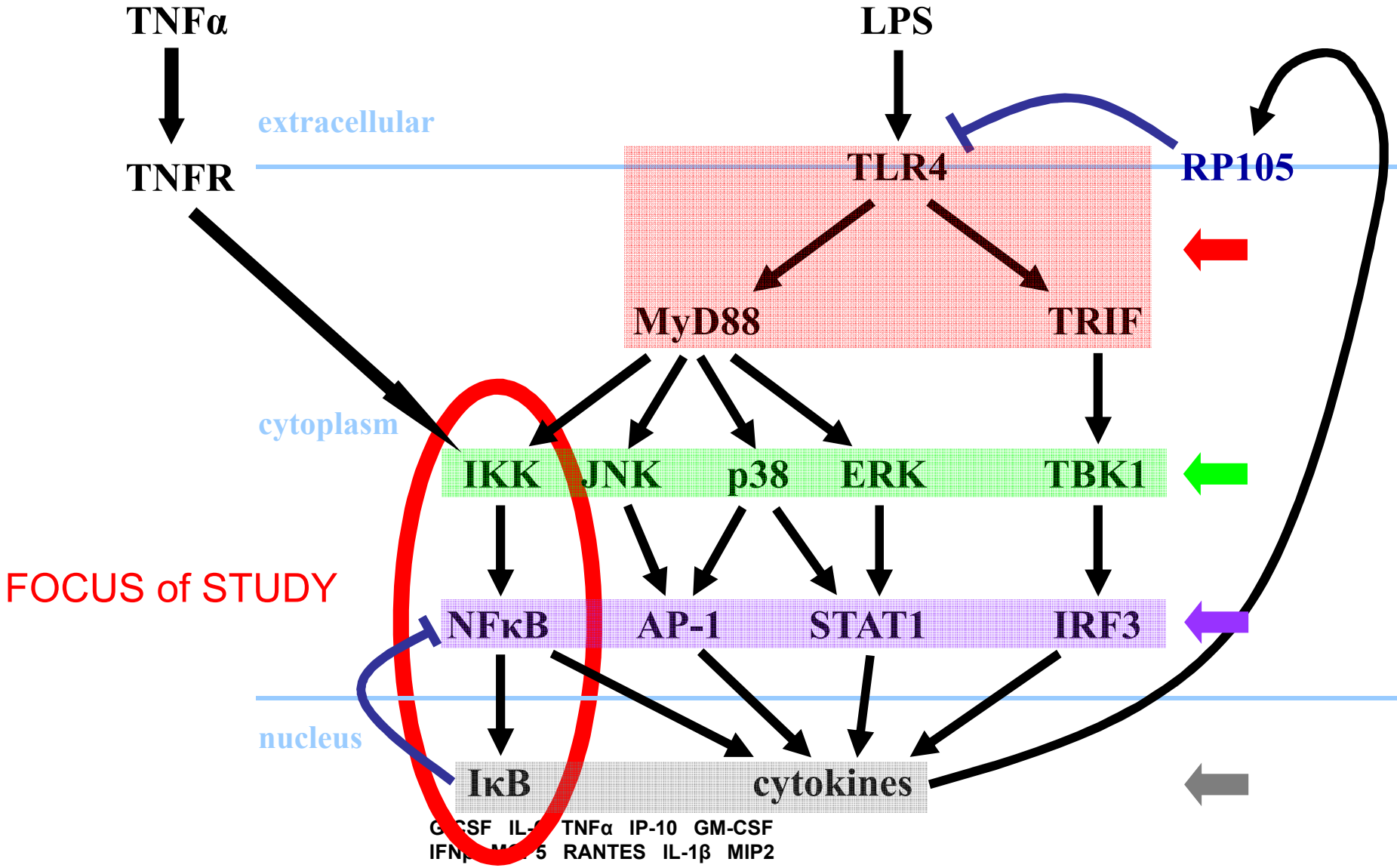
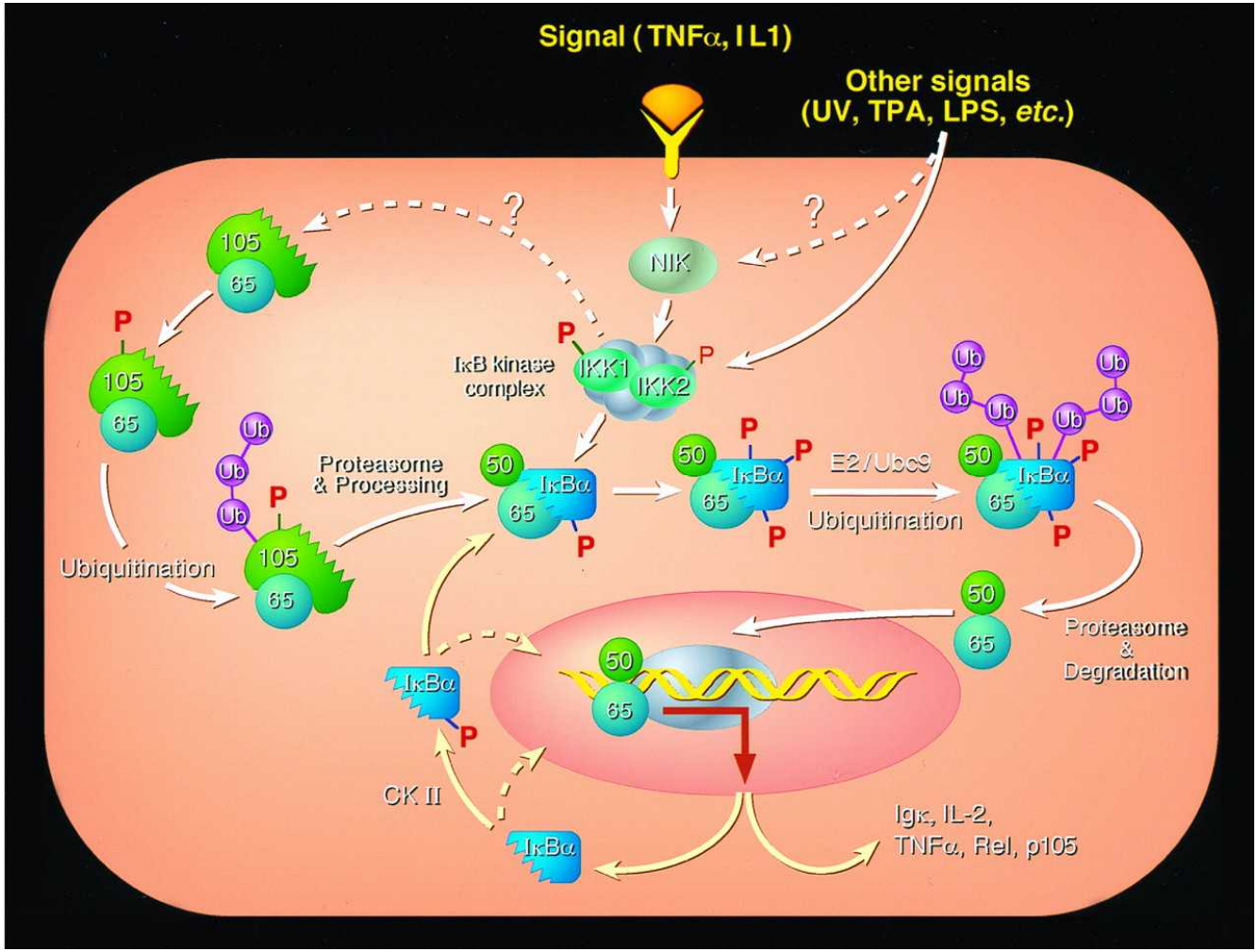
TNFR & TLR4 signaling lead to NF-κB response



Overview of NF-κB signal transduction network

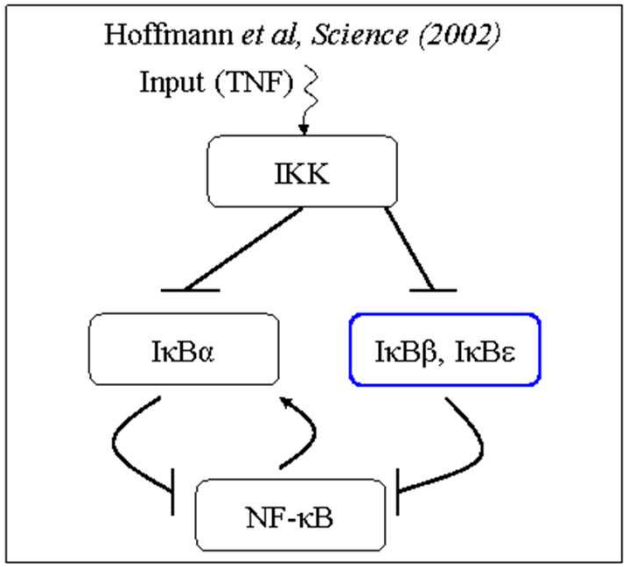


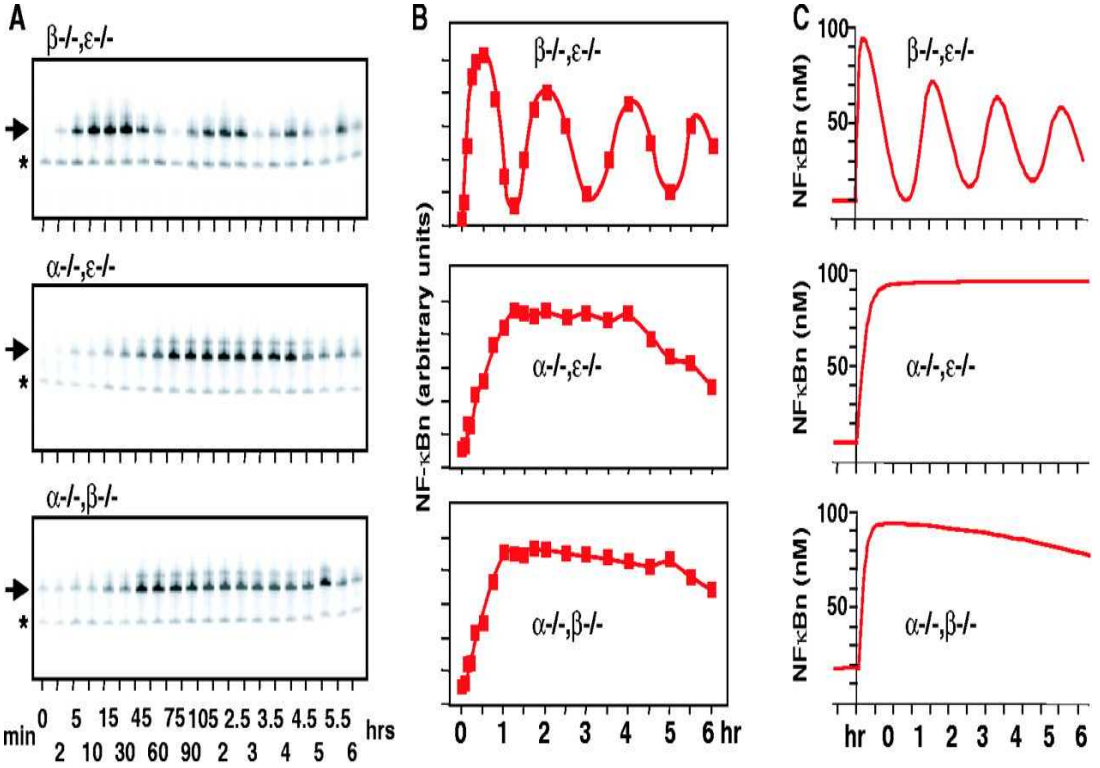
Key players:

- 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



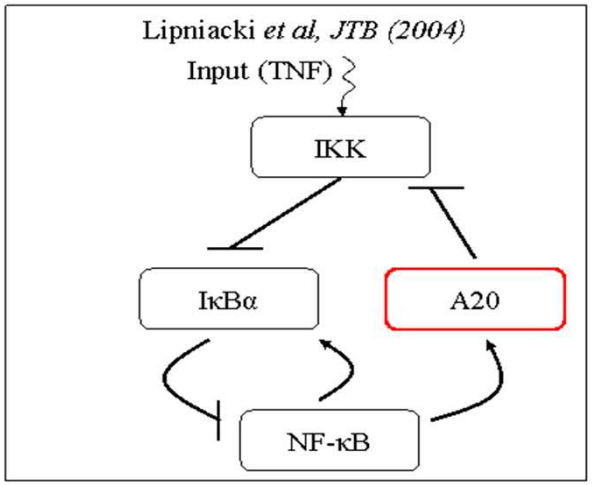


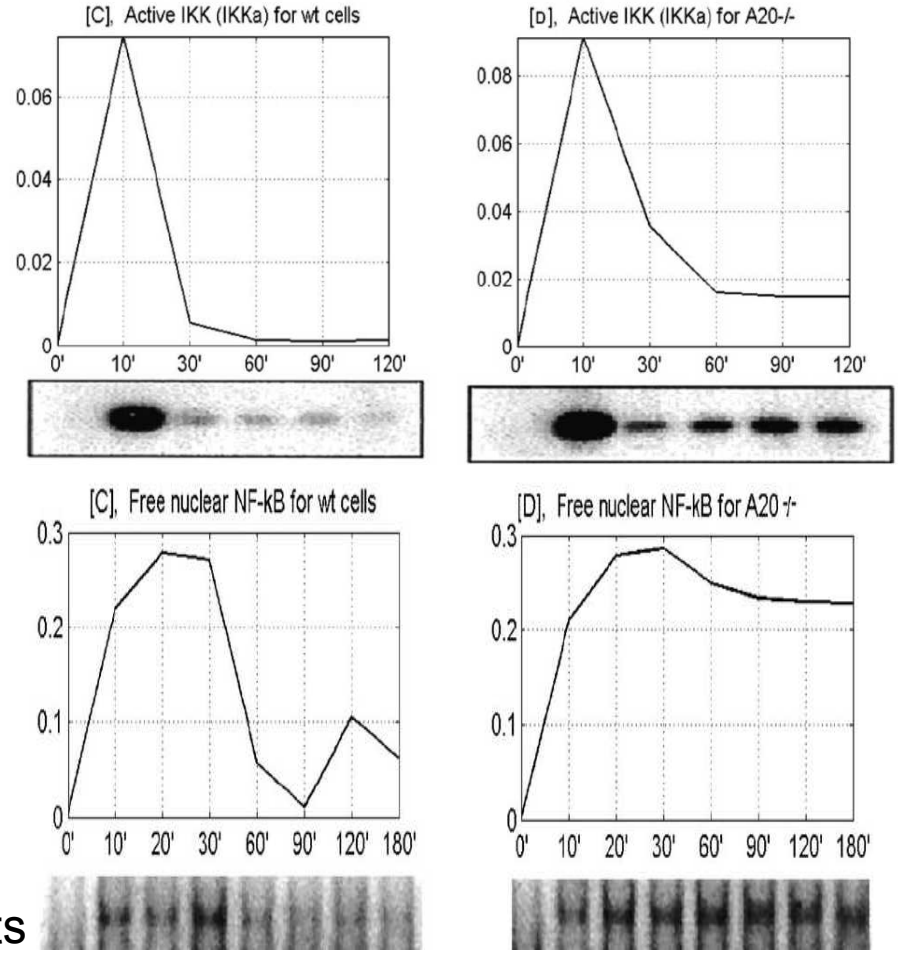
Hoffmann *et al.*
Science, 298:1241 (2002)

Mouse fibroblasts

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

Additional negative regulator of NF-κB: A20





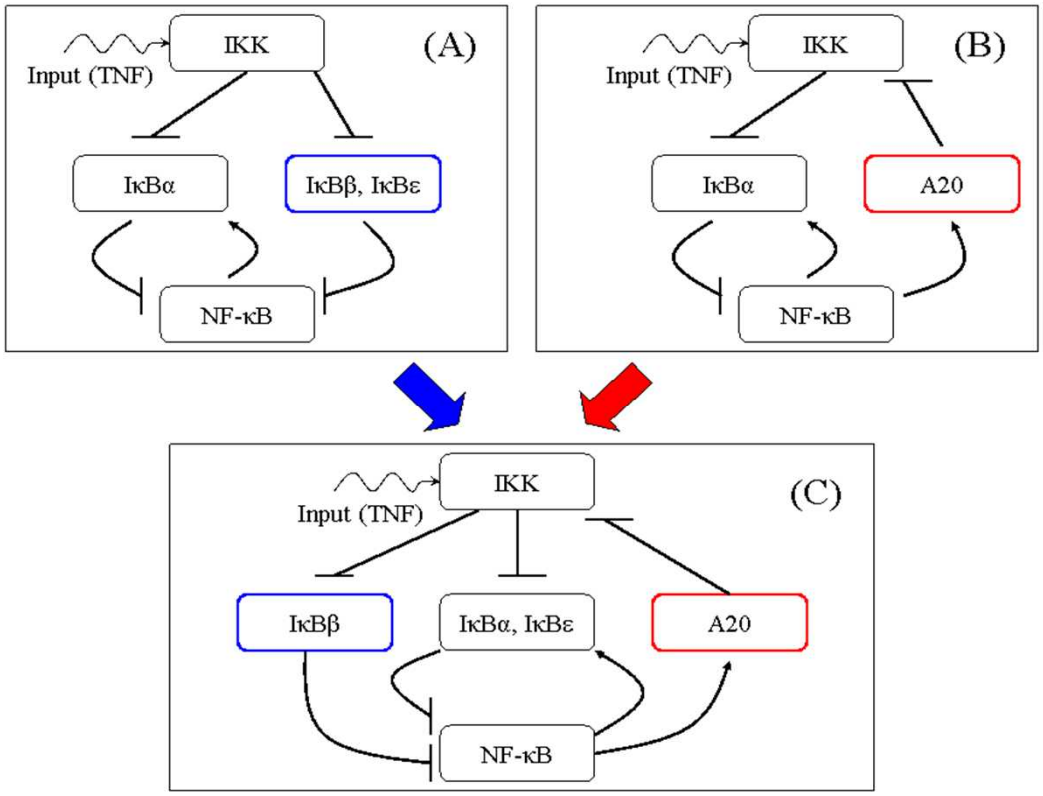
Lee *et al.* Science 289:2350 (2000)
Lipniacki *et al.* JTB 228:195 (2004)

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)

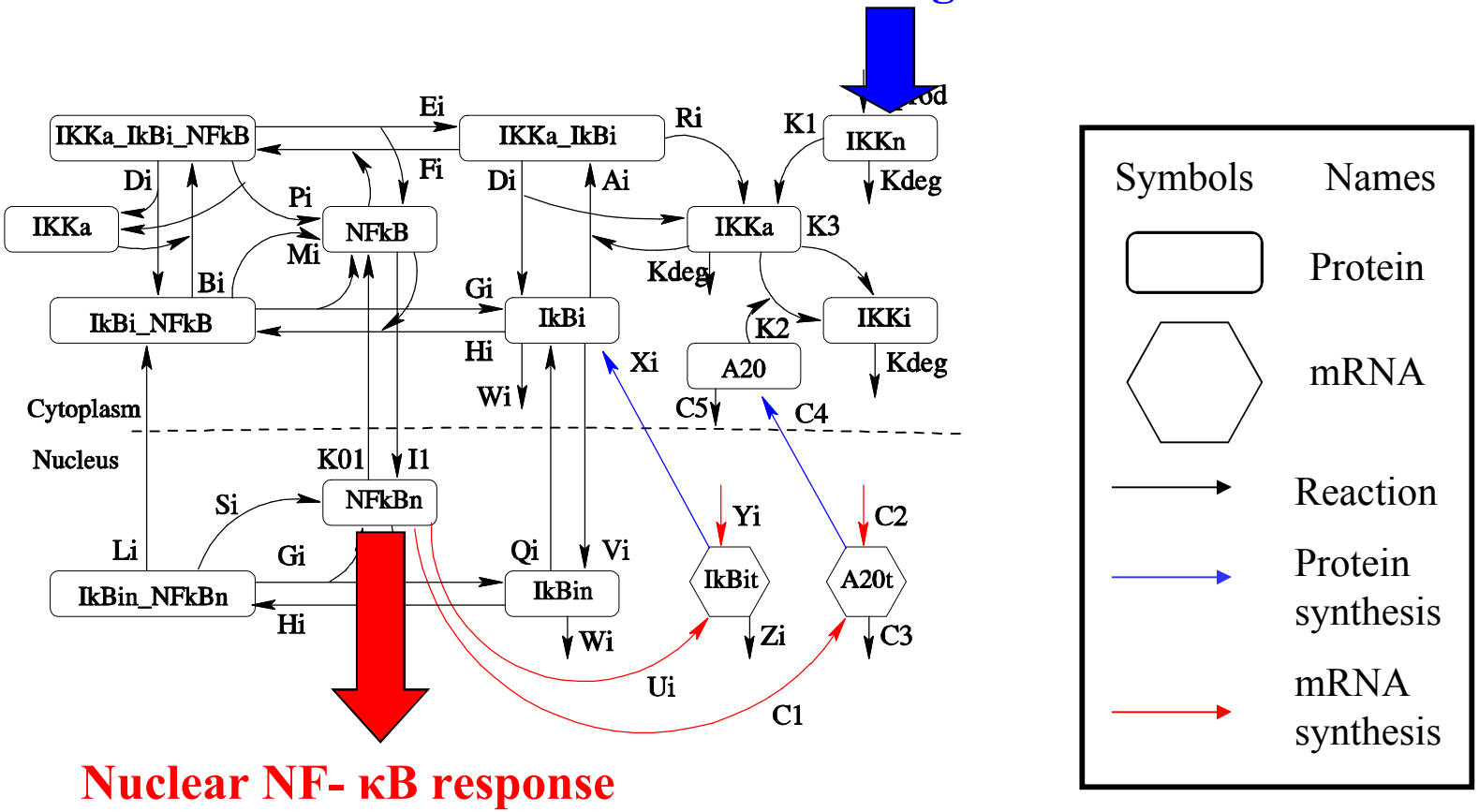


Lipniacki *et al.*
JTB 228:195
(2004)

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

Hybrid model of NF-κB signaling network

TLR signal ← Persistent LPS

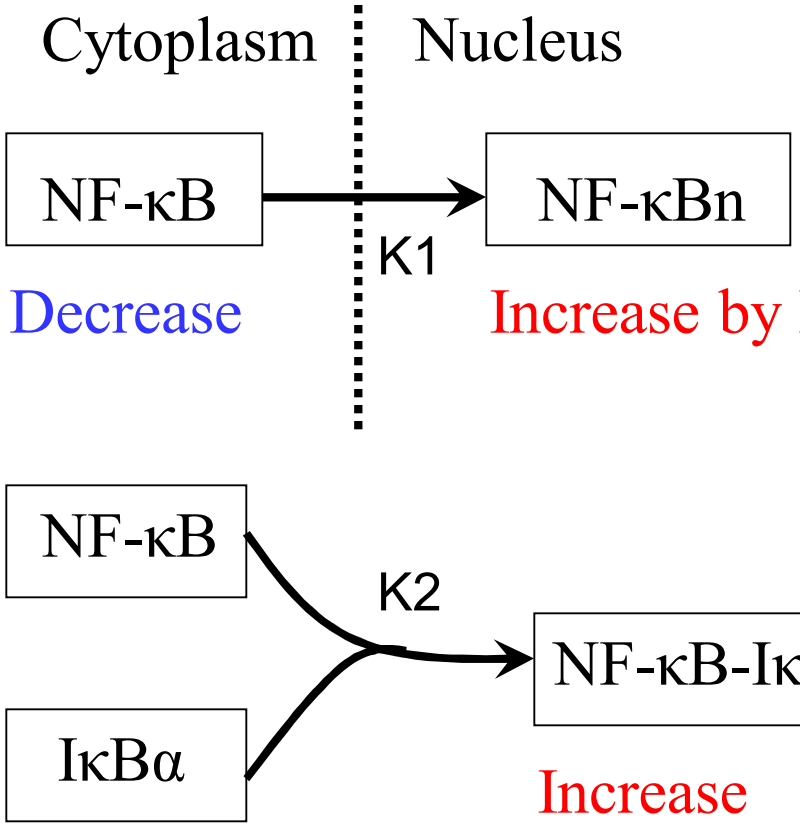


Nuclear NF- κB response

Symbols	Names
	Protein
	mRNA
→	Reaction
→	Protein synthesis
→	mRNA synthesis

- 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

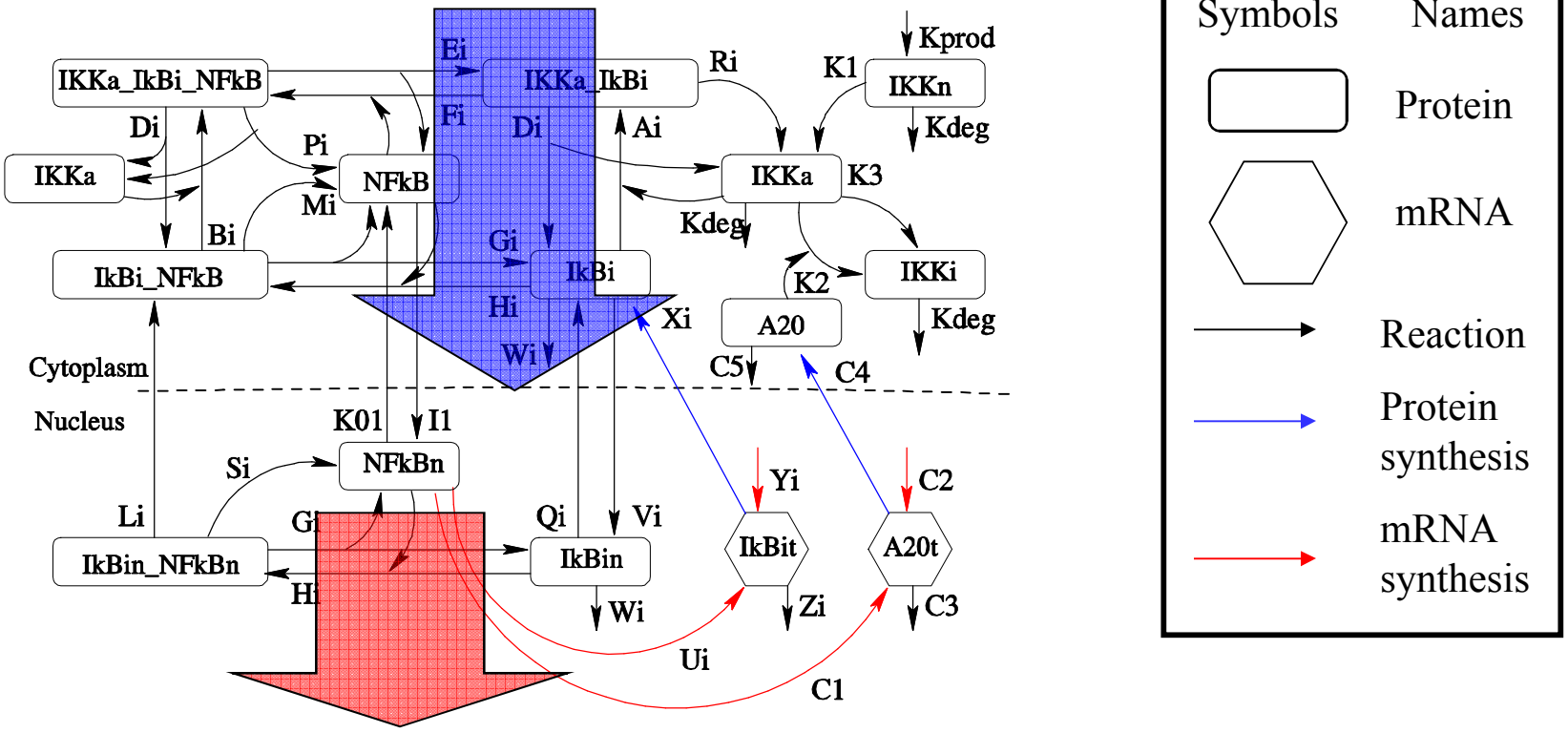


$$d[\text{NF-}\kappa\text{B}]/dt = -K1[\text{NF-}\kappa\text{B}]$$
$$d[\text{NF-}\kappa\text{Bn}]/dt = K1Kv[\text{NF-}\kappa\text{B}]$$

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

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

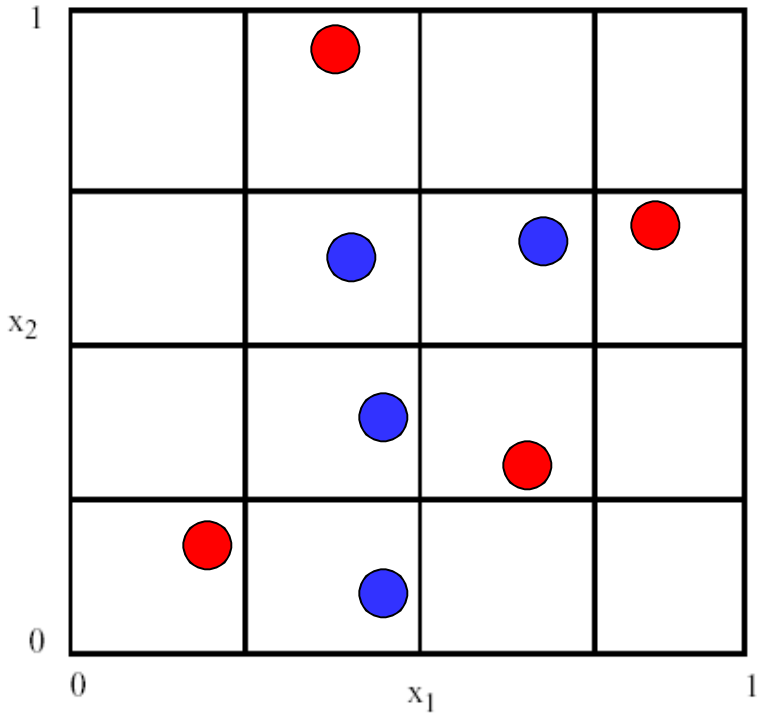
- 70 kinetic rate variables + 1 initial condition
- Sample 71 input variables by Latin Hypercube Sampling according to assumed joint distribution
  - Uniform or lognormal distribution
  - Interval size for uniform distribution:

    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:



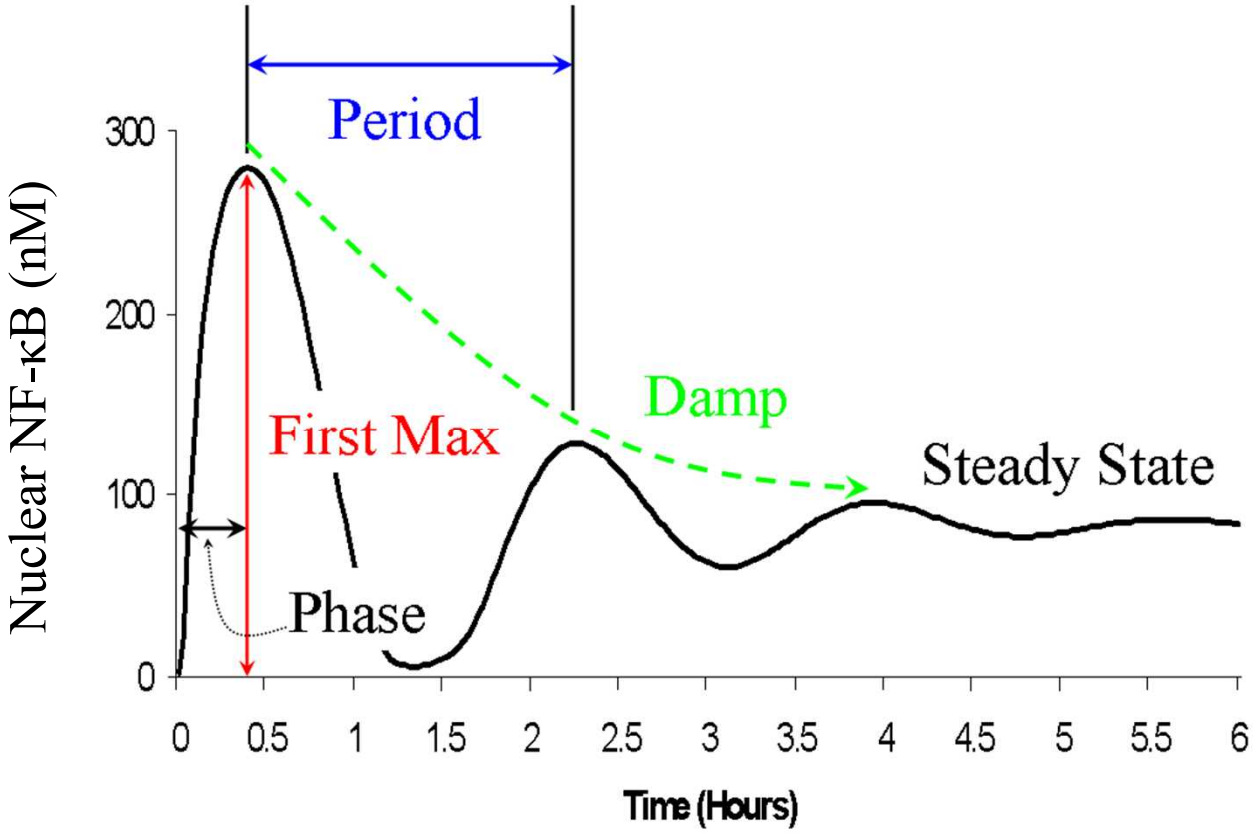
Random

Latin Hypercube Sampling

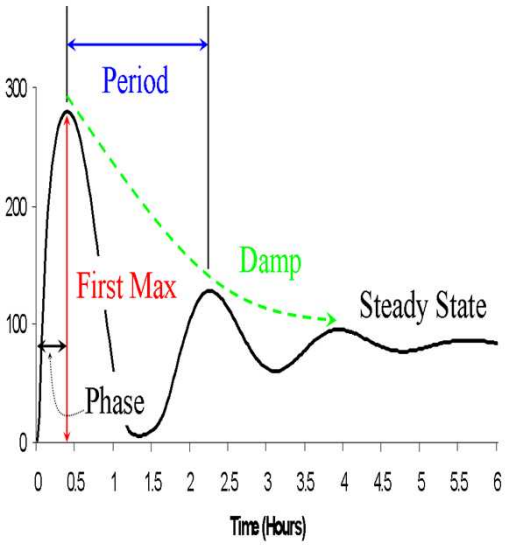
Question 1:

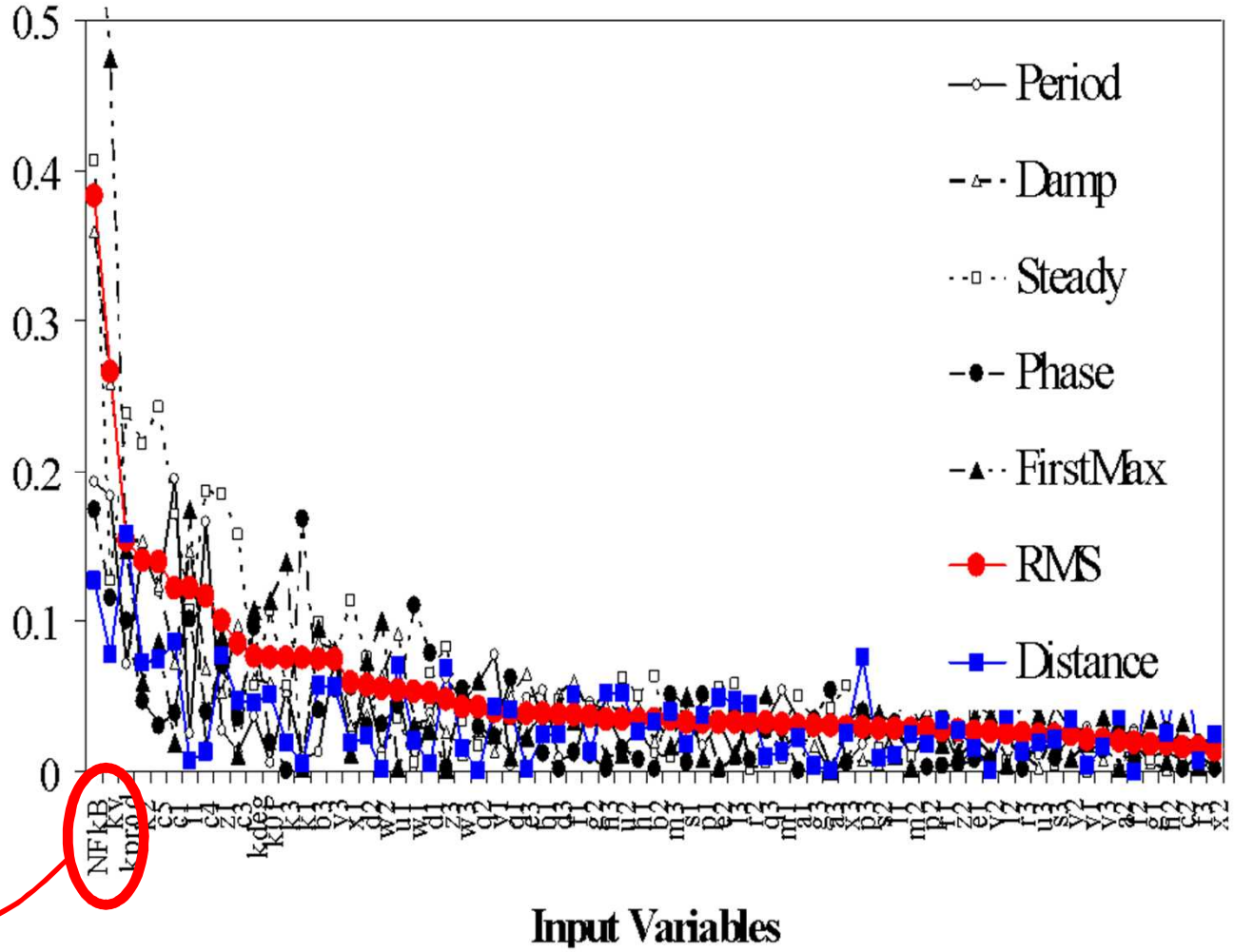
What are the most influential kinetic rates on NF-κB response?

Quantification of nuclear NF-κB response with five dynamic features



Correlation between kinetic rate variables and dynamic features



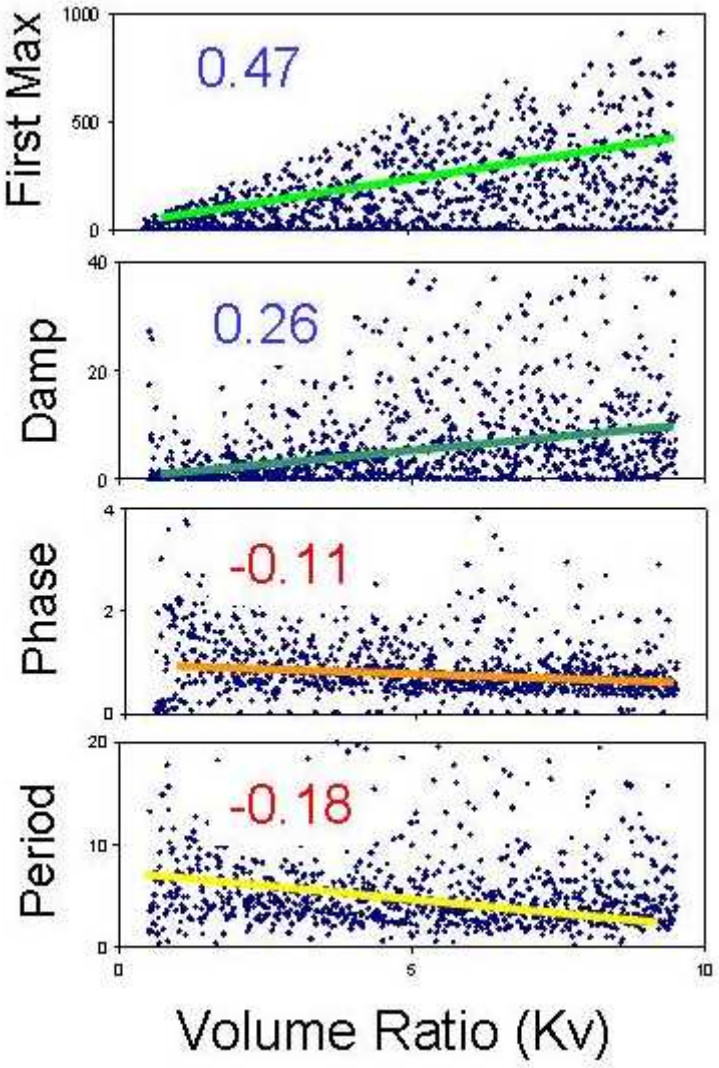


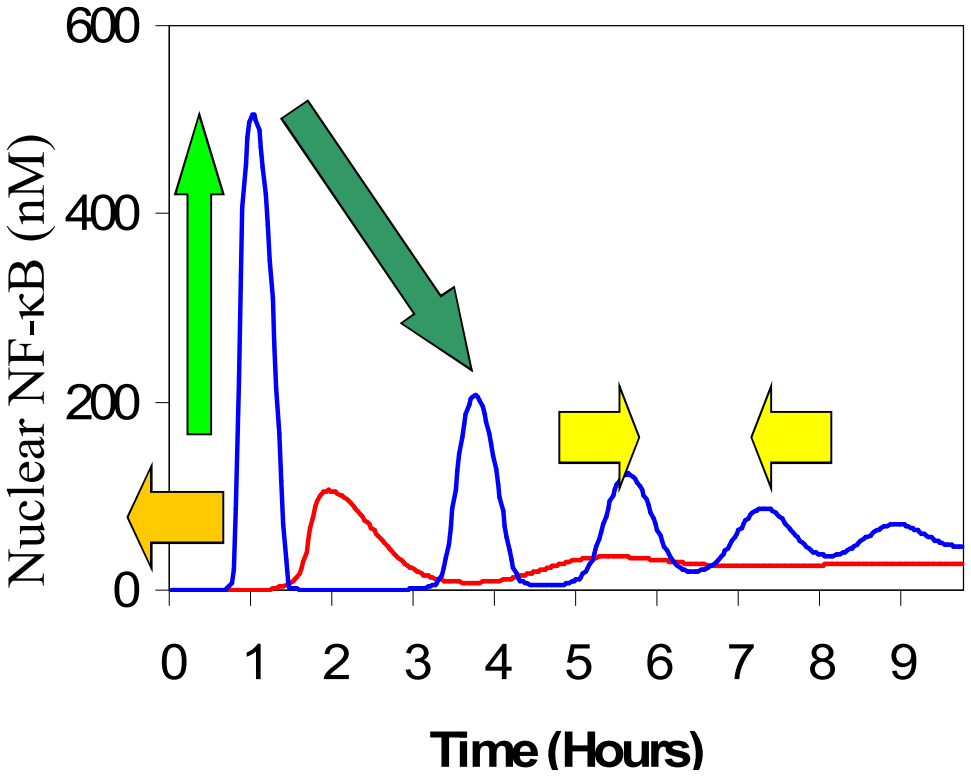
1. NF-κB
2. Kv

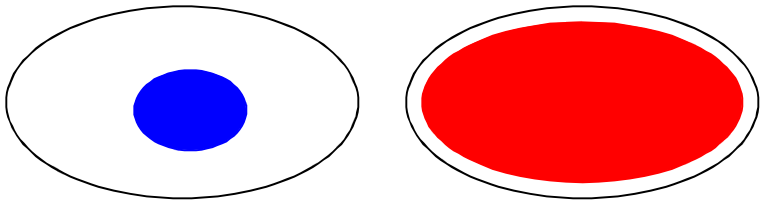
Question 2:

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

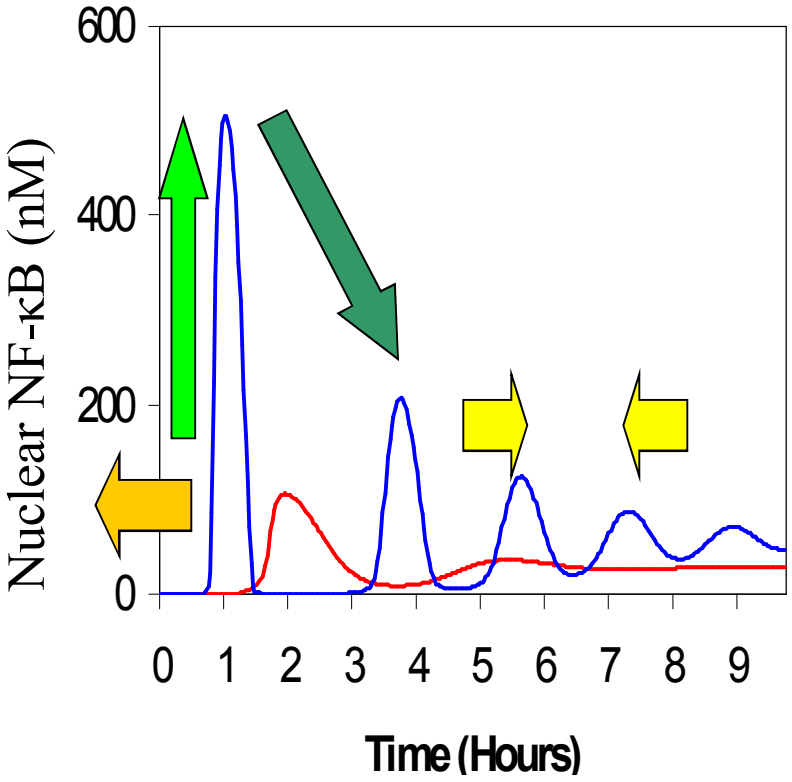


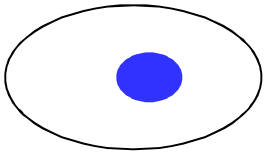




High Kv=10 Low Kv=2

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

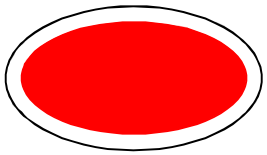




High volume ratio:

Strong and fast NF-κB response

with more temporal modulation

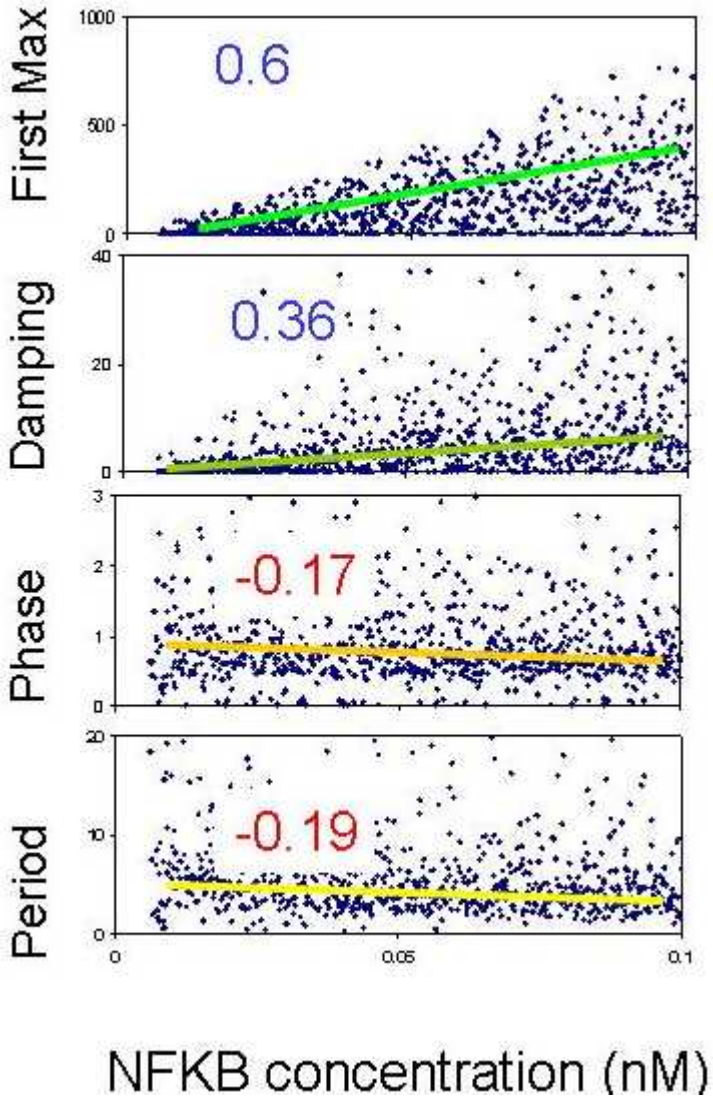


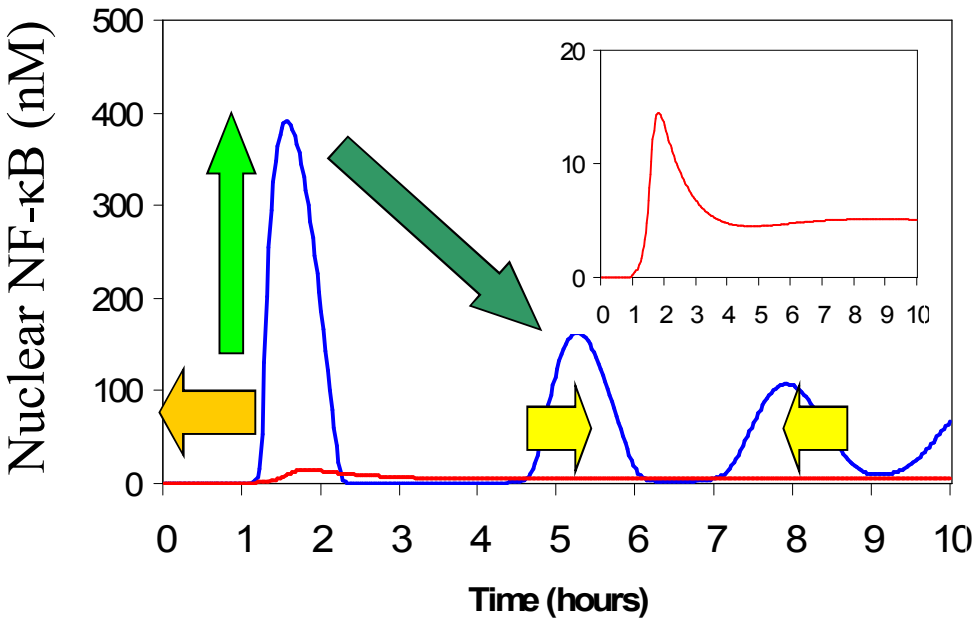
Low volume ratio:
Weak and slow NF-κB response
with less temporal modulation

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





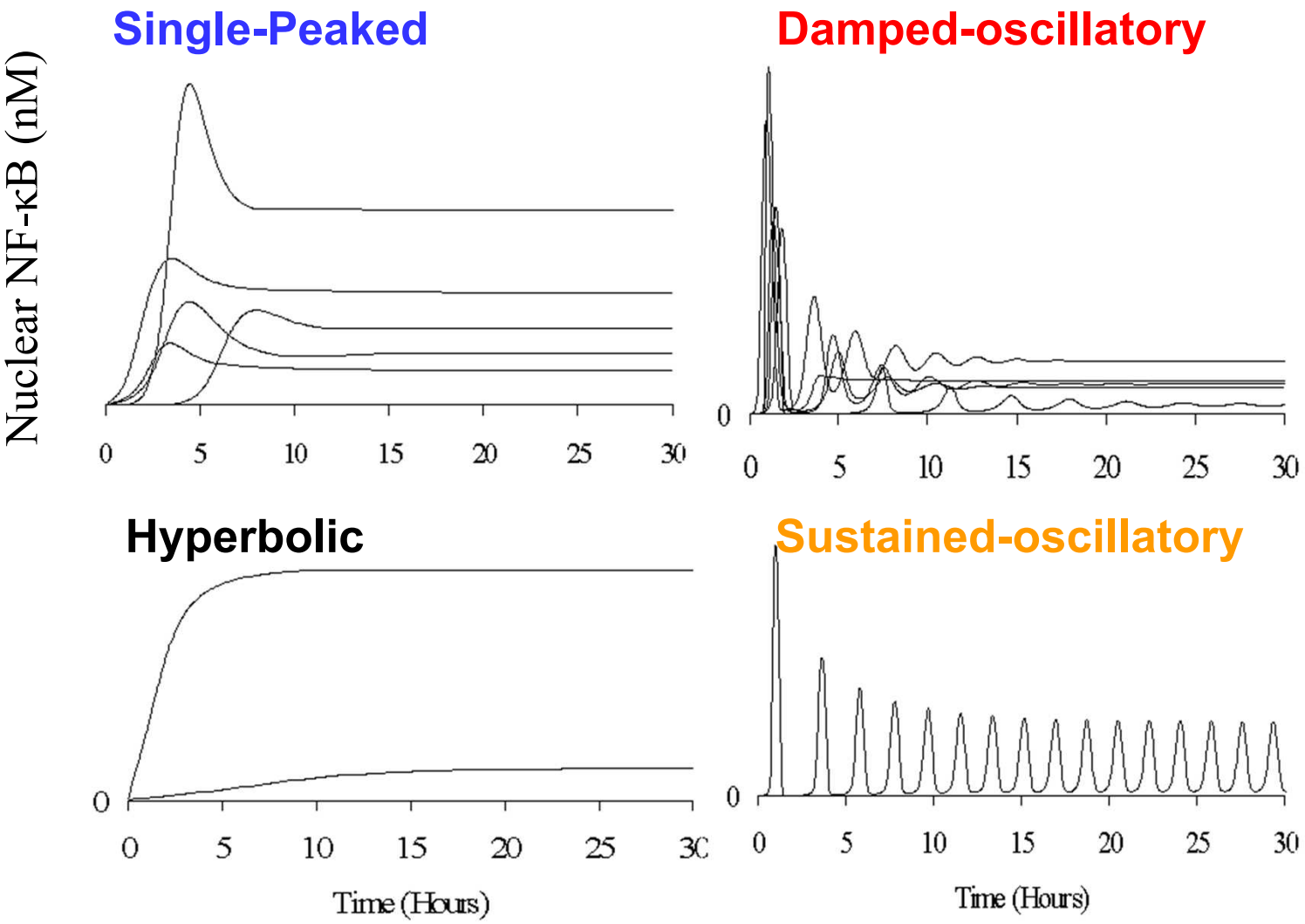
High NF-κB concentration:
Strong and fast NF-κB response with more temporal modulation

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

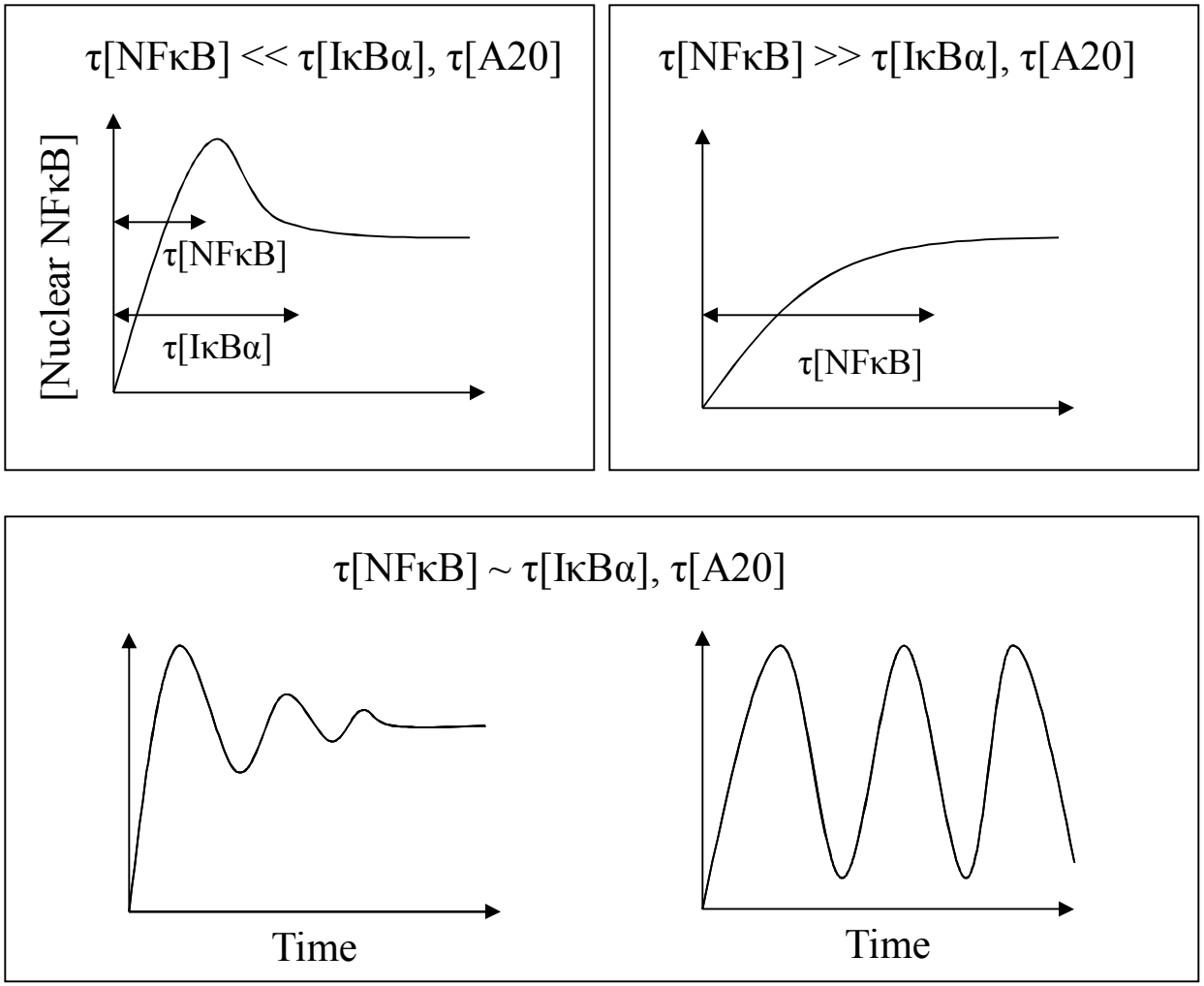
Question 3:

What is statistical ensemble of NF-κB response?

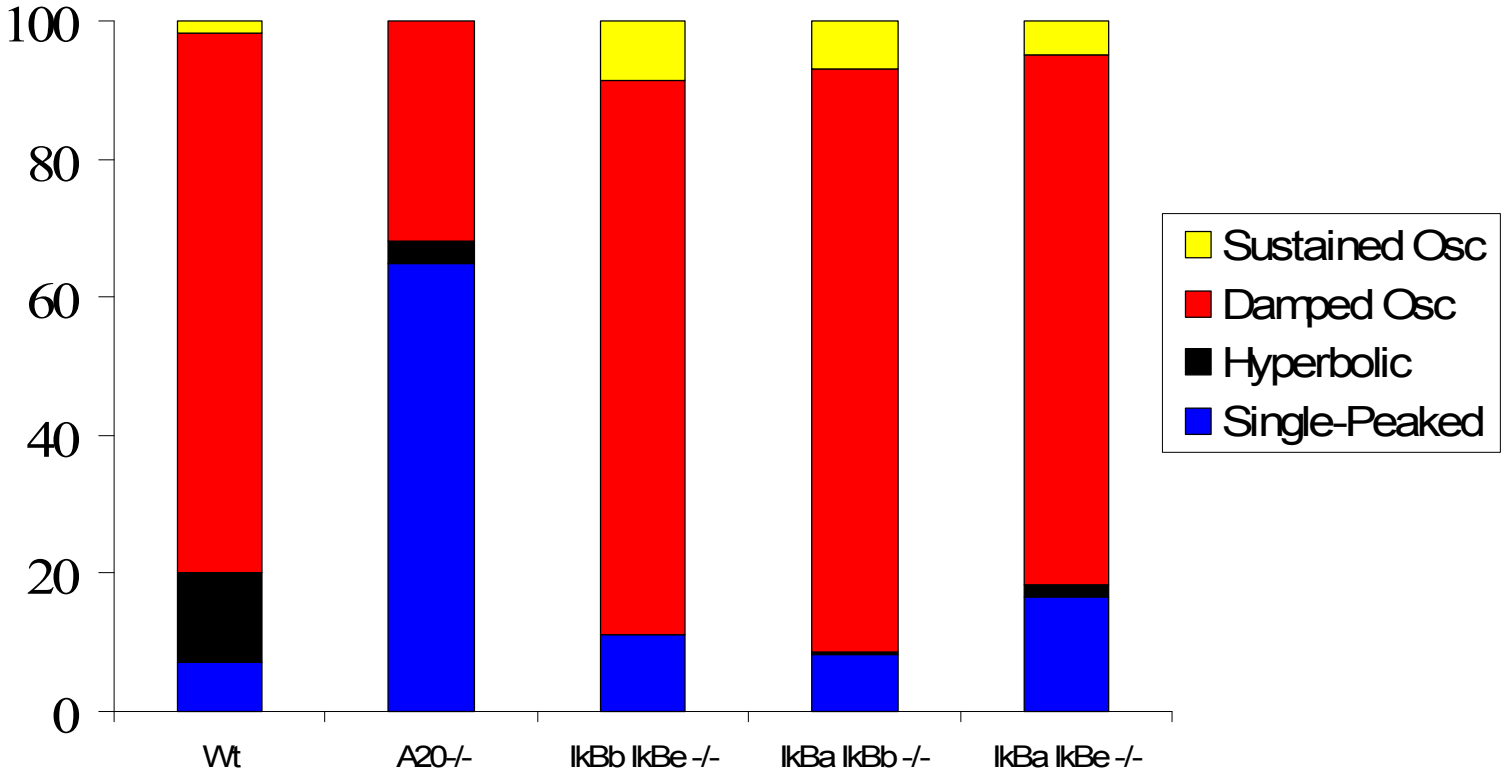
Four basic dynamic patterns of NF-κB response



Four basic dynamic patterns depends on protein response time τ



Distributions of dynamic patterns of NF-κB response



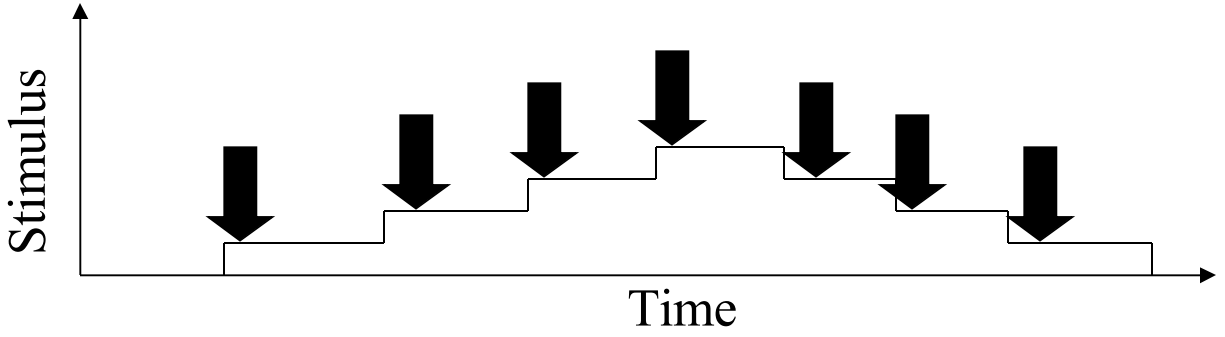
- Signal strength=large ; number of samples=1000; interval size= 80%
- Most probable dynamic patterns are most observable & even robust against fluctuations of reaction rates.

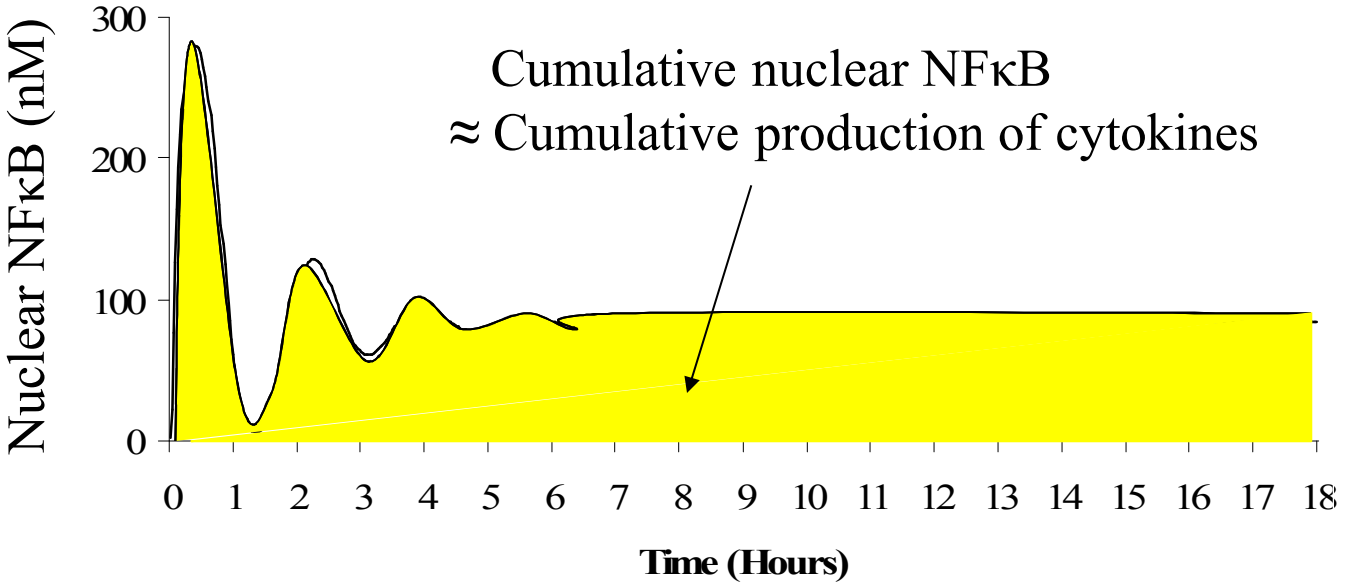
NF-κB Response for Macrophages

Question 4:

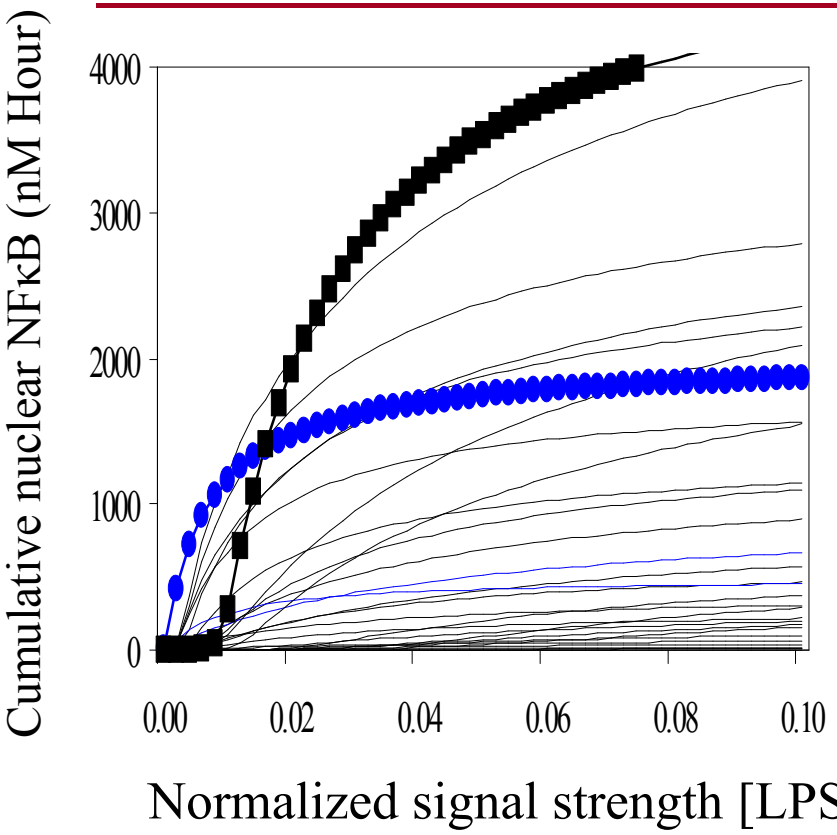
What is the shape of LPS dose-response curve?

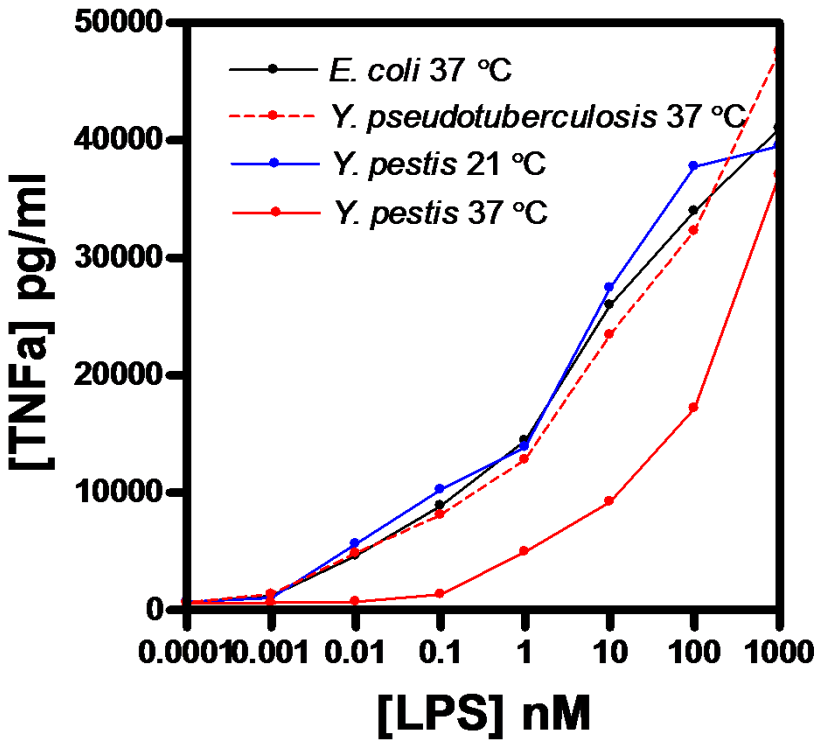
NFκB response to different LPS dosage levels





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

NF-κB Response for Macrophages

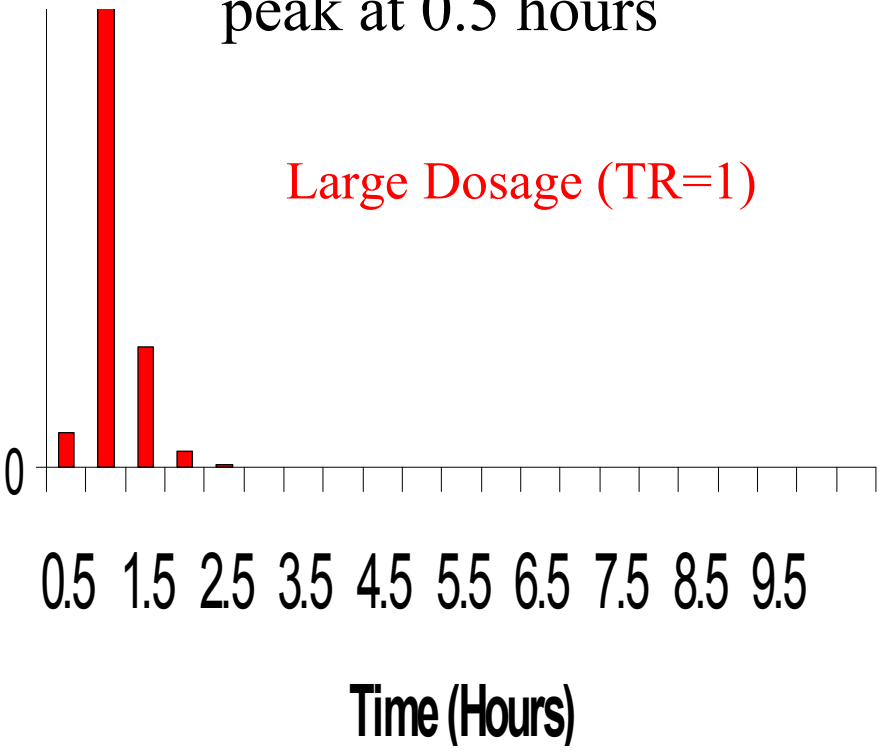
Question 5:

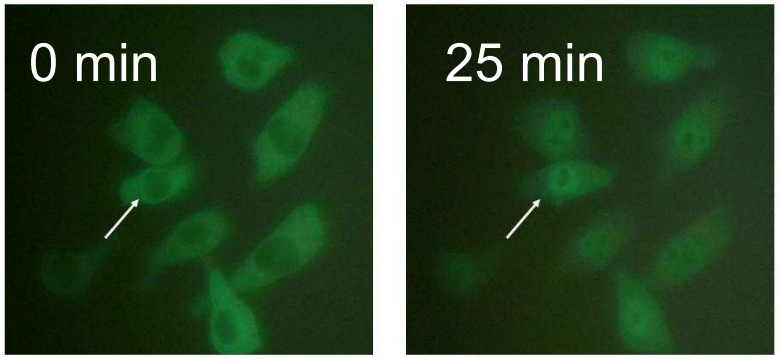
Does different LPS dosage level affect NF-κB translocation time?

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

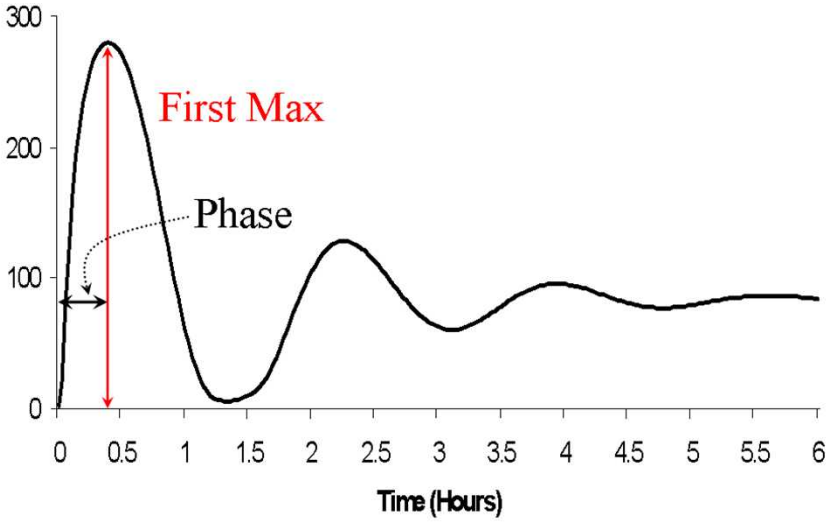
Distribution of phase: peak at 0.5 hours

Large Dosage (TR=1)

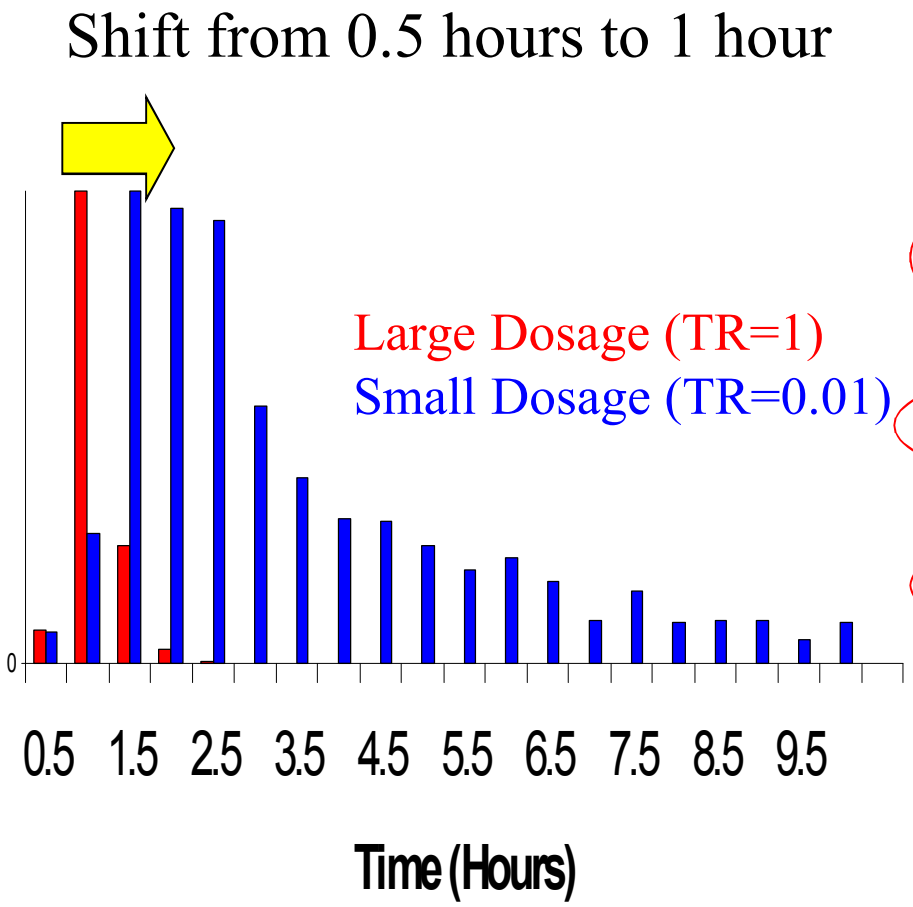




Macrophage stimulated with 1 μM Y. pesitis LPS



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



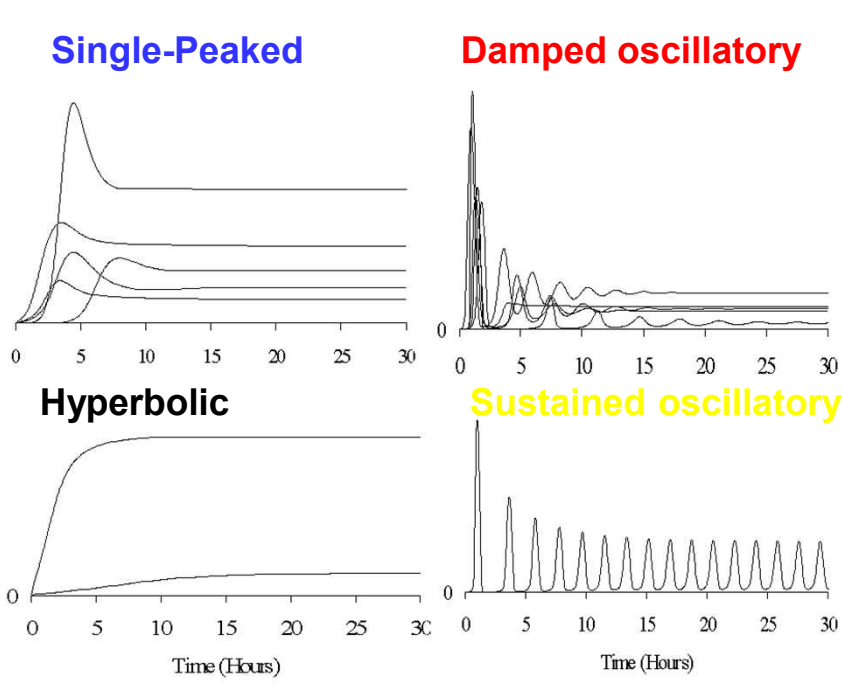
LPS	15min	25min	45min	60min	90min
1μM E. coli	X				
1nM E.coli		X			
1μM 21°C YP	X				
1nM 21°C YP		X			
1μM 37°C YP		X			
1nM 37°C YP			X		

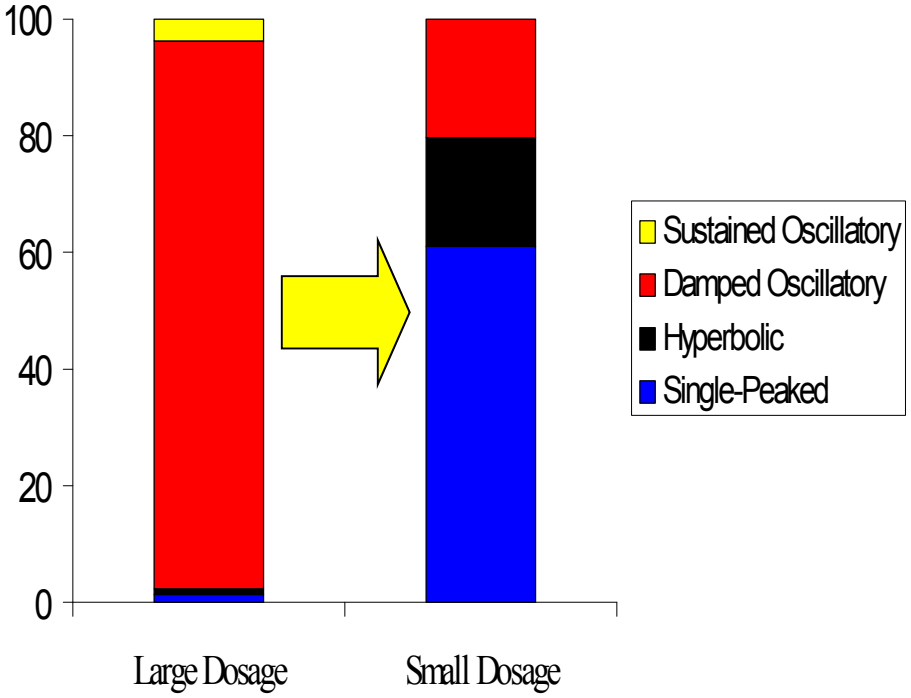
NF-κB Response for Macrophages

Question 6:

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

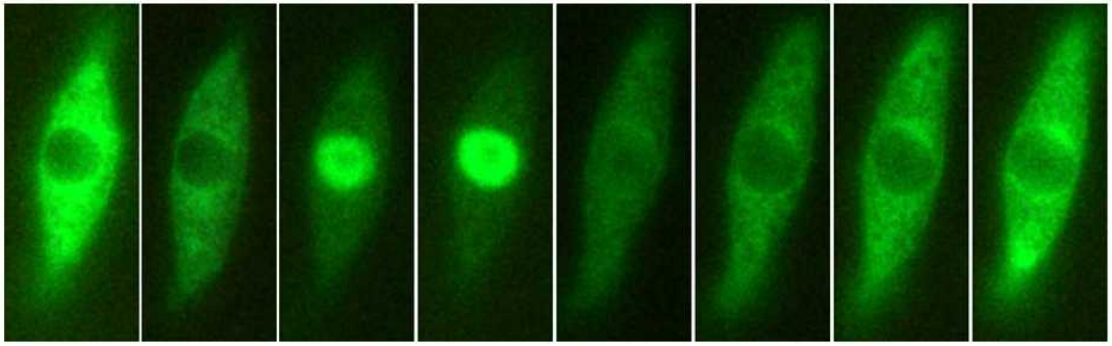
LPS dosage amount changes distribution of dynamic patterns of NF-κB response

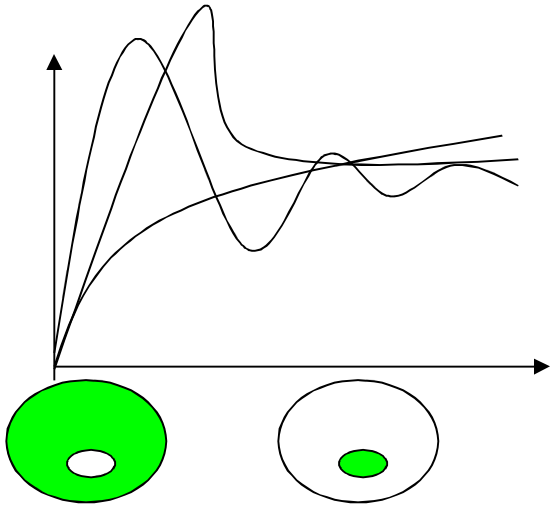




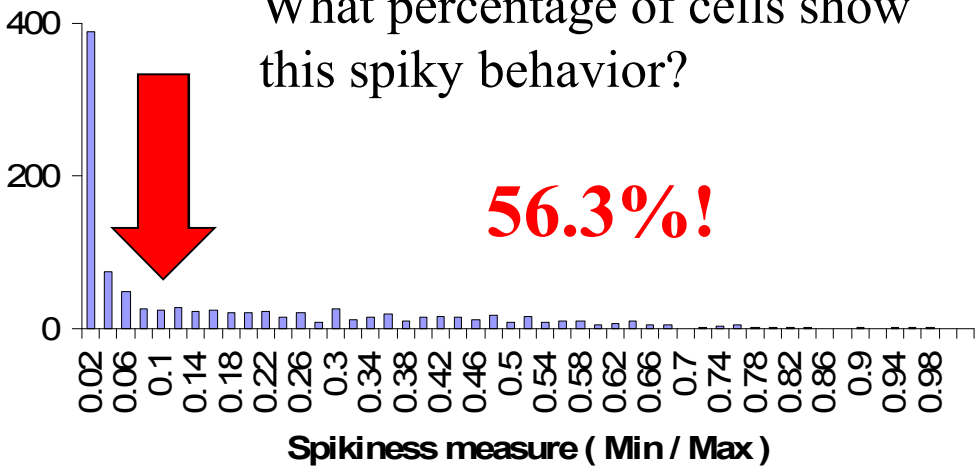
- For large dosage, damped oscillatory pattern is exclusively most probable.
- For small dosage, single-peaked and hyperbolic patterns are most probable.

Translocation of RelA-GFP in macrophages challenged with LPS



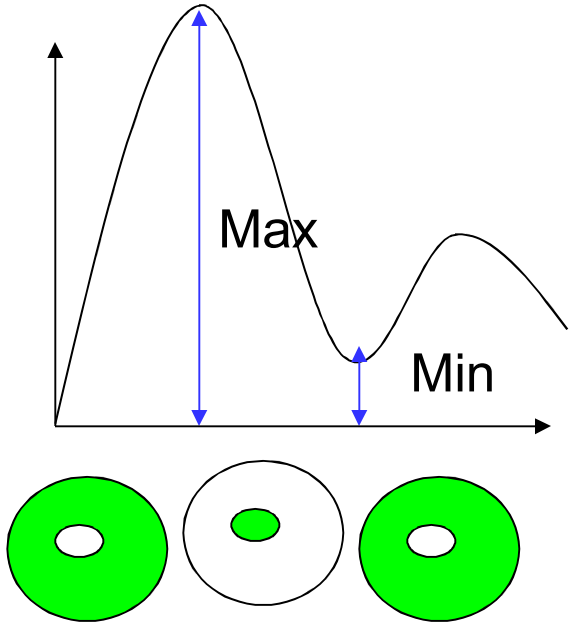


RelA-GFP in and out of nucleus only in 10% of cells



What percentage of cells show this spiky behavior?

56.3%!

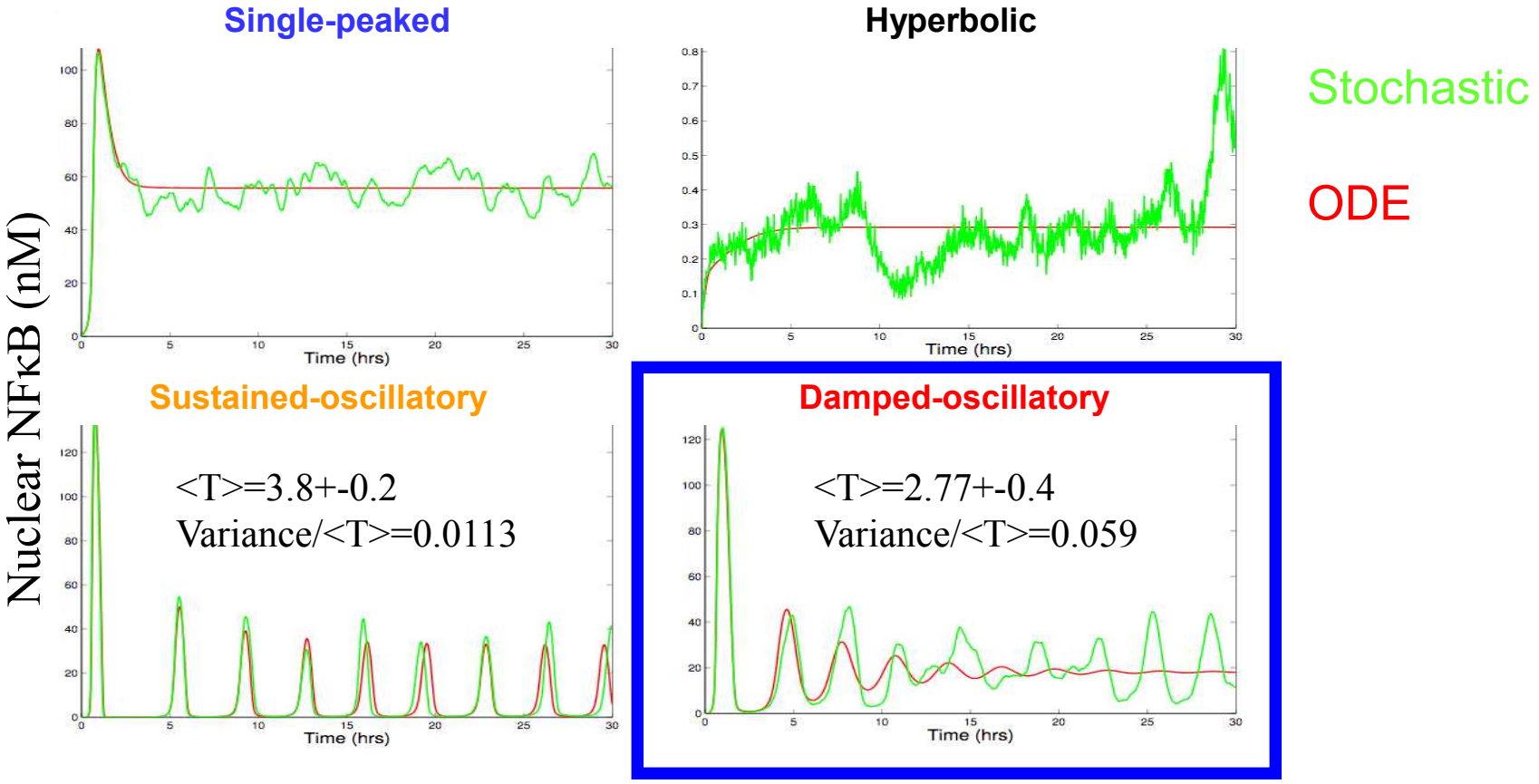


NF-κB Response for Macrophages

Question 7:

Could stochastic noise change dynamic patterns of NF-κB response?

Noise doesn't change most of dynamic patterns of NF-κB response, BUT…



- Noise can induce oscillation out of damped-oscillation

Conclusion

- Kinetic rate variables are ranked in order of their importance to the NF-κB response: volume ratio & total NF-κB.
- We predict a dose-response curve with sigmodial shape for macrophages.
- We predict the statistical distribution of the four dynamic patterns of nuclear NF-κB for macrophages.
- We predict that different LPS dosage amount induces a shift of the distribution of NF-κB translocation time.
- We predict that noise can induce NF-κB oscillations.