Stochastic gene expression. NF-kappaB model

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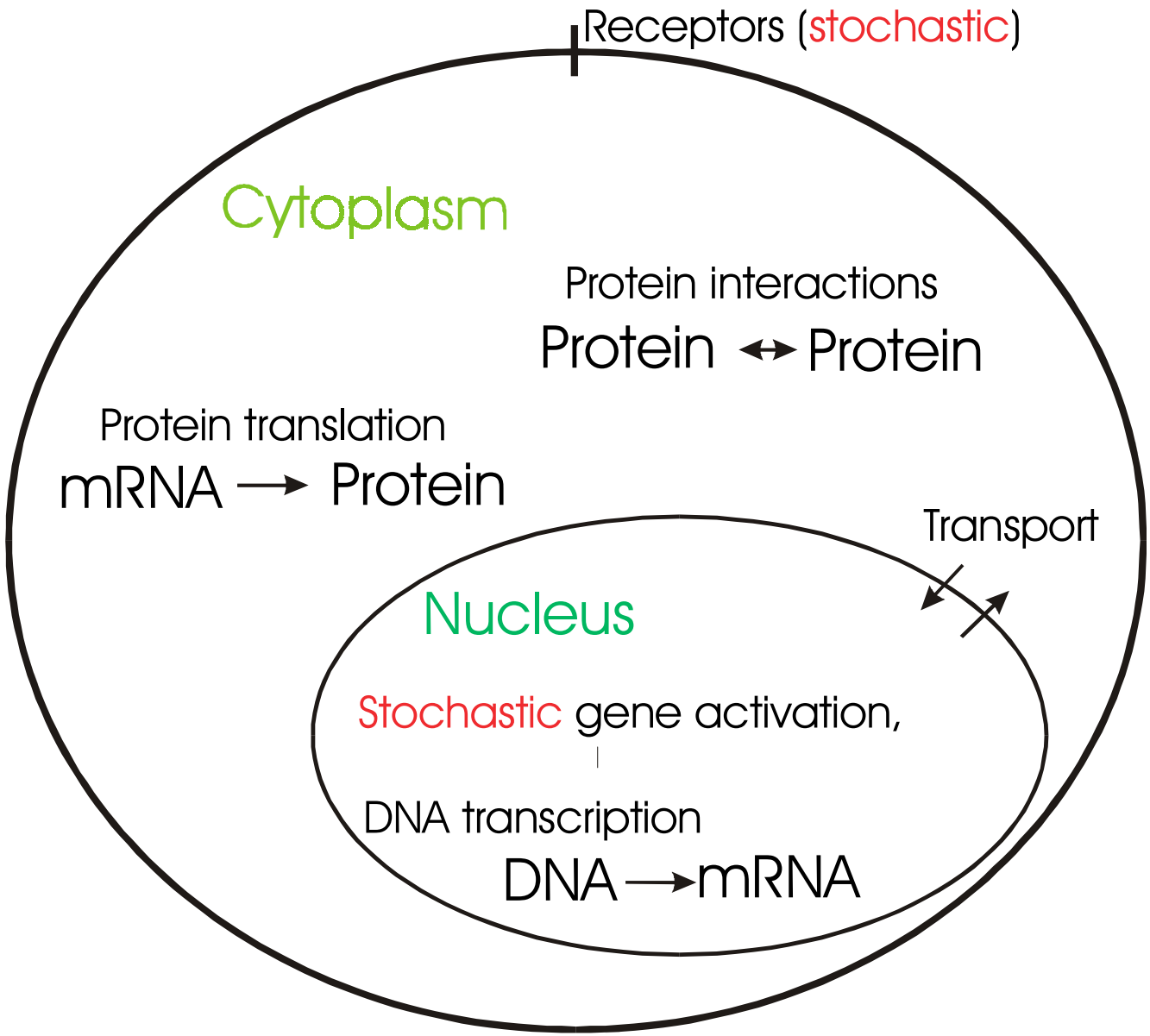
EUKARYOTIC CELL

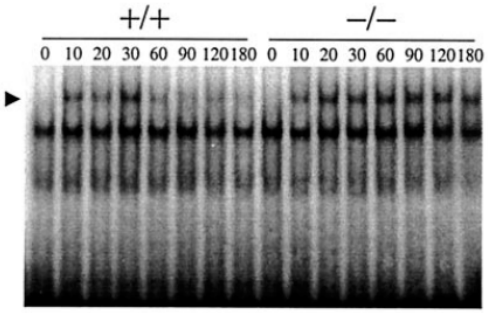


Experiments

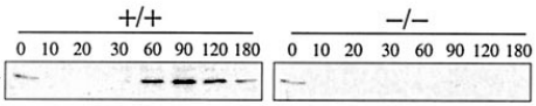
Population

Single cell

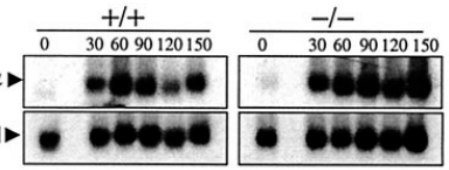
EMSA



WB



NB



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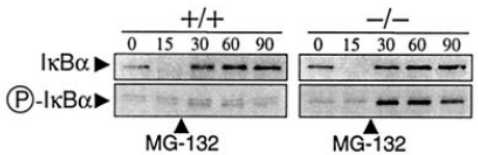
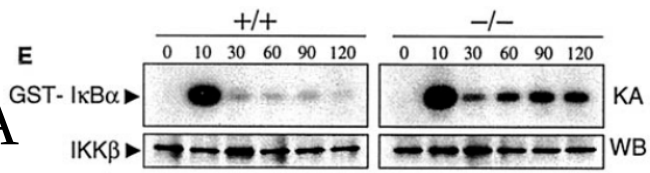
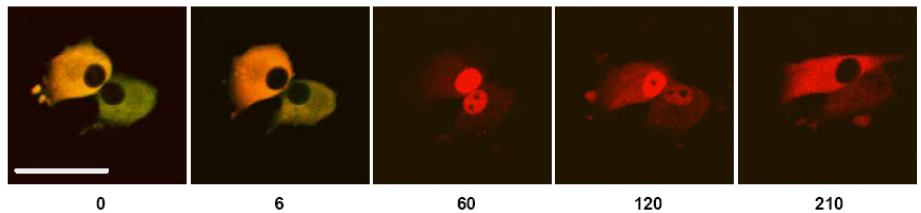


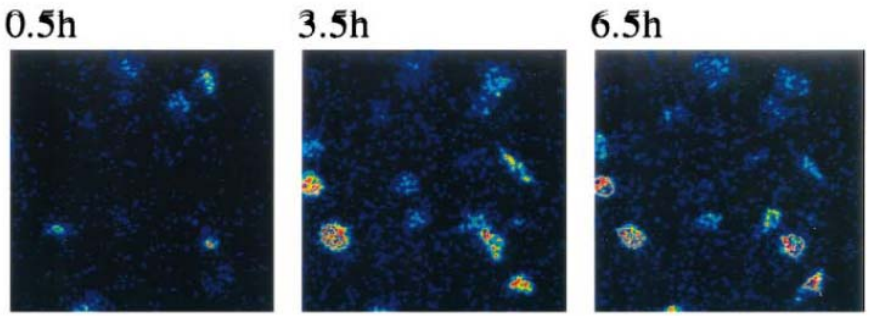
KA



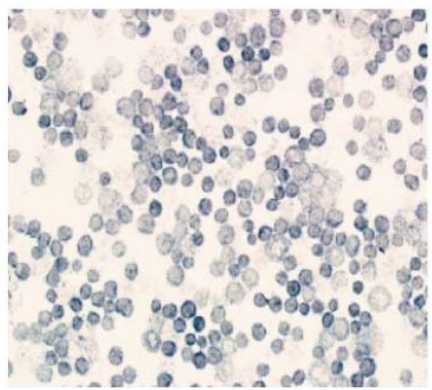
Fluorescent proteins



Luciferase

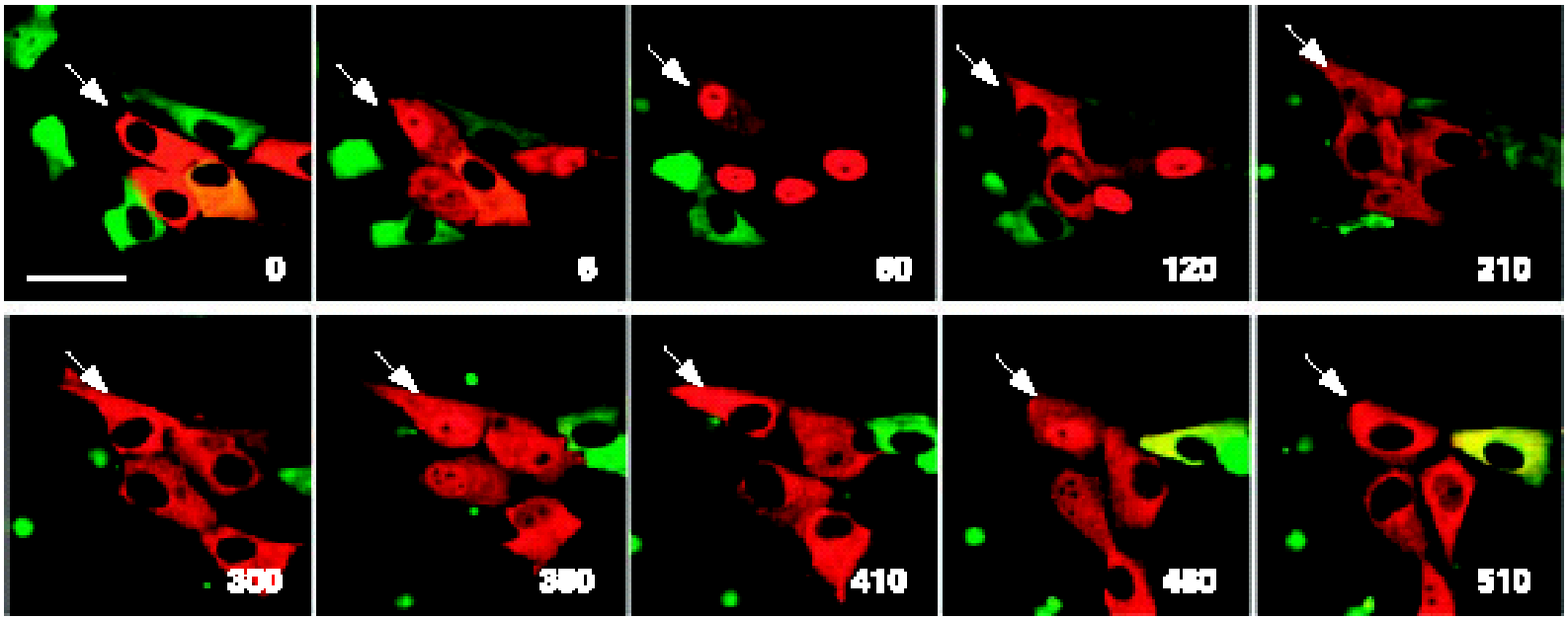


In situ hybridization



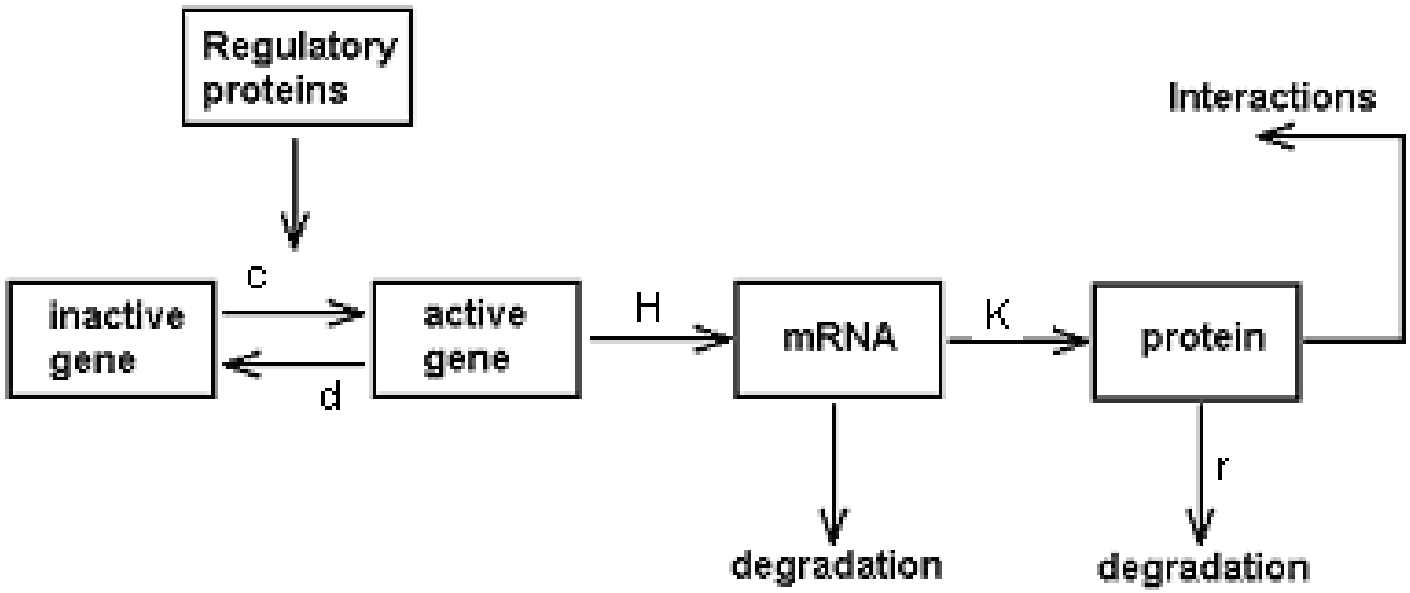
Nelson et al, Science 2004 (M.R.H. White group)

TNF stimulation



SK-N-AS (human S-type neuroblastoma cells) expressing
RelA-DsRed (RelA fused at C-terminus to red fluorescent protein)
and
IkBa-EGFP (IkBa fused to the green fluorescent protein)

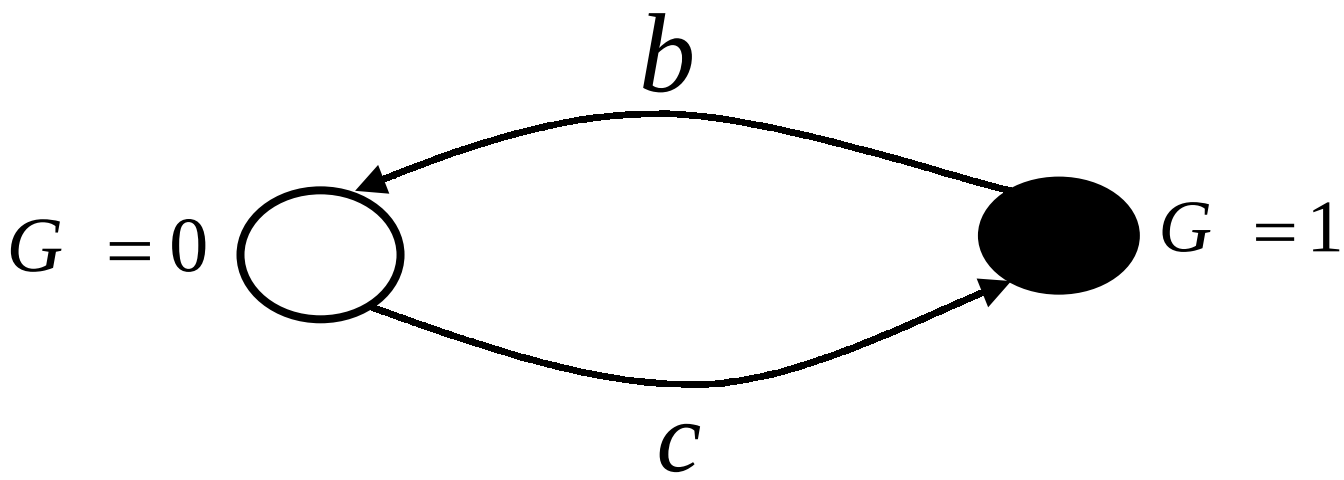
Simplified schematic of gene expression



- The number of molecules involved:

$$1 \leq DNA \leq mRNA \leq protein \leq 10^6$$

Stochastic gene activation



G is a Markov process.

Piece-wise deterministic Markov process

Continuous approximation
for mRNA and protein levels

$x := mRNA$

$y := protein$

$$\frac{dx(t)}{dt} = HG - x(t)$$

$$\frac{dy(t)}{dt} = Kx(t) - ry(t)$$

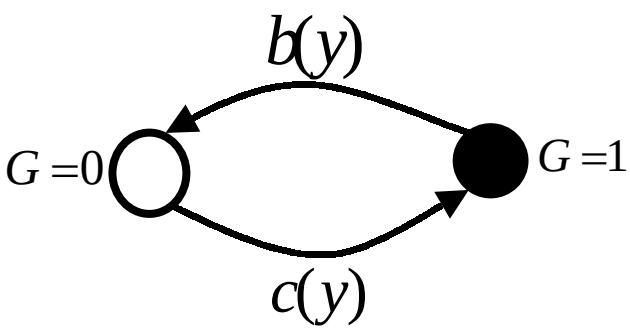
$$(G = 0) \xrightarrow{c(y)} (G = 1),$$

$$(G = 1) \xrightarrow{b(y)} (G = 0)$$

Probability density functions

$$f(x,y,t)\Delta x\Delta y = \Pr[(x(t)\in(x,x+\Delta x), y(t)\in(y,y+\Delta y), G(t)=0]$$
$$g(x,y,t)\Delta x\Delta y = \Pr[(x(t)\in(x,x+\Delta x), y(t)\in(y,y+\Delta y), G(t)=1]$$

$$\frac{\partial f}{\partial t} + div[\, f(\frac{dx}{dt}|_{G=0}, \frac{dy}{dt})] = -c(y)\, f + b(y) g$$
$$\frac{\partial g}{\partial t} + div[g(\frac{dx}{dt}|_{G=1}, \frac{dy}{dt})] = c(y)\, f - b(y) g$$



$$\frac{dx(t)}{dt} = HG - x(t)$$
$$\frac{dy(t)}{dt} = Kx(t) - ry(t)$$

Partial differential equations for probability density functions

f(x,y,t) and g(x,y,t)

$$\frac{\partial f}{\partial t} - \frac{\partial}{\partial x}(xf) + \frac{\partial}{\partial y}((Kx - ry)f) = -c(y)f + b(y)g$$

$$\frac{\partial g}{\partial t} + \frac{\partial}{\partial x}((H - x)g) + \frac{\partial}{\partial y}((Kx - ry)g) = c(y)f - b(y)g$$

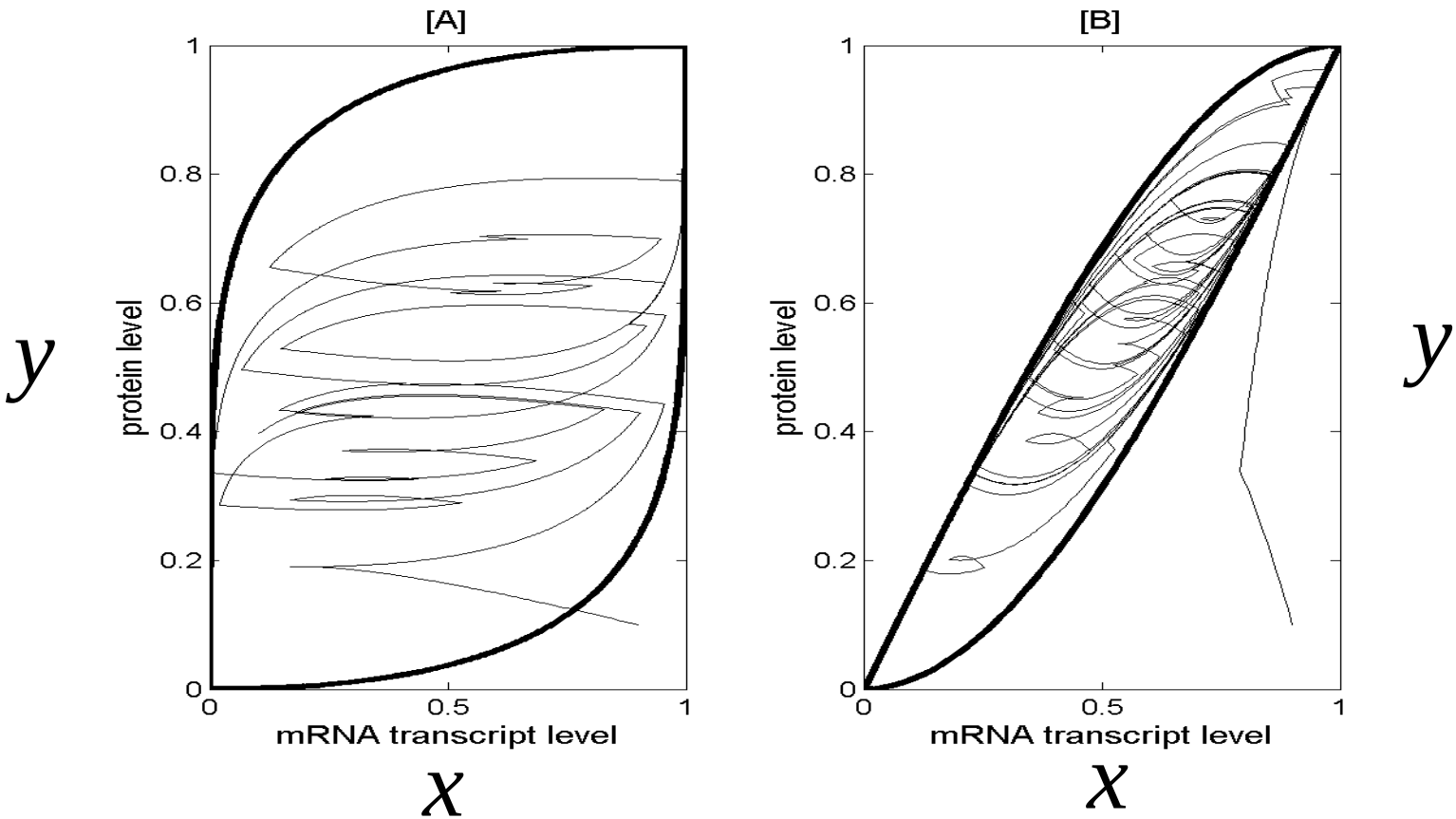
Asymptotic stability

The stationary distributions

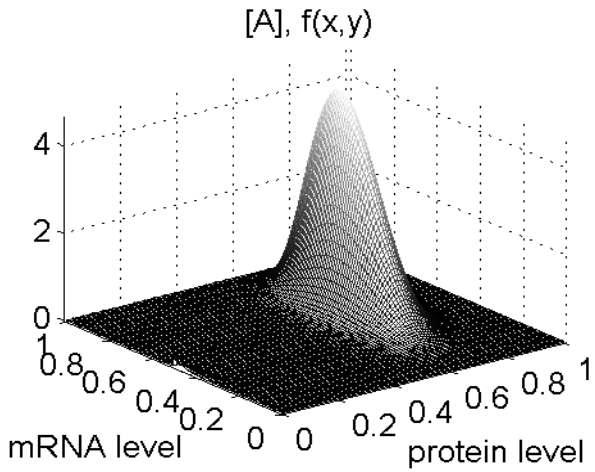
$$\frac{dx(t)}{dt} = HG - x(t)$$

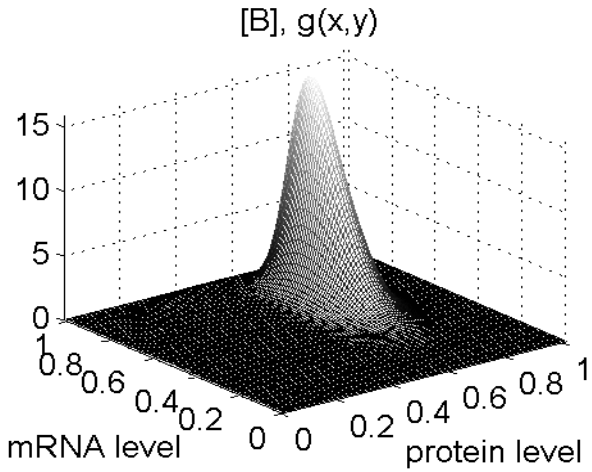
$$\frac{dy(t)}{dt} = Kx(t) - ry(t)$$

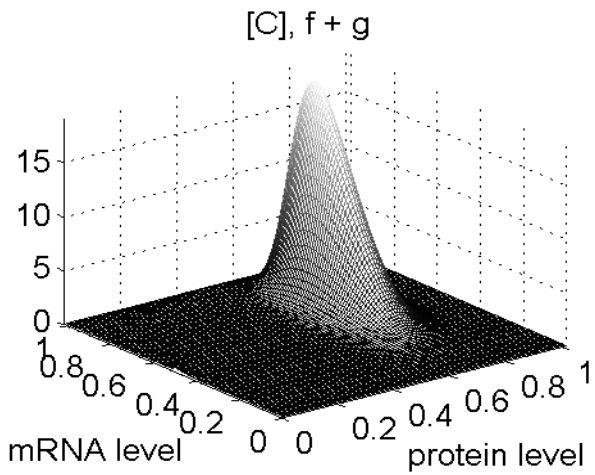
$$\mathrm{supp}\,[f(x,y)] = \mathrm{supp}\,[g(x,y)] = D(r)$$



Stationary distributions – numerical solution

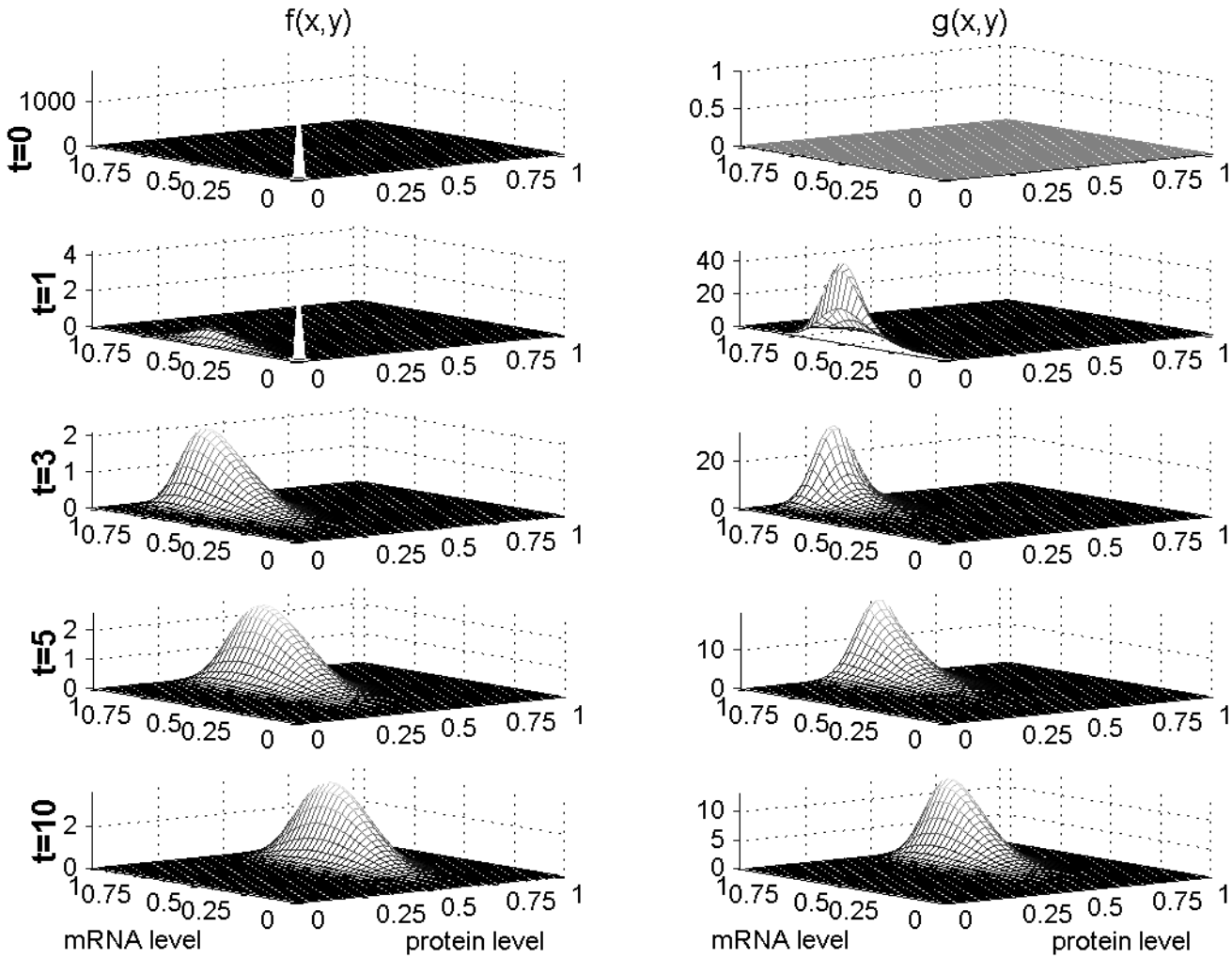






Negative feedback: $c = 6, \quad b = 3y, \quad r = 0.2$

Time evolution



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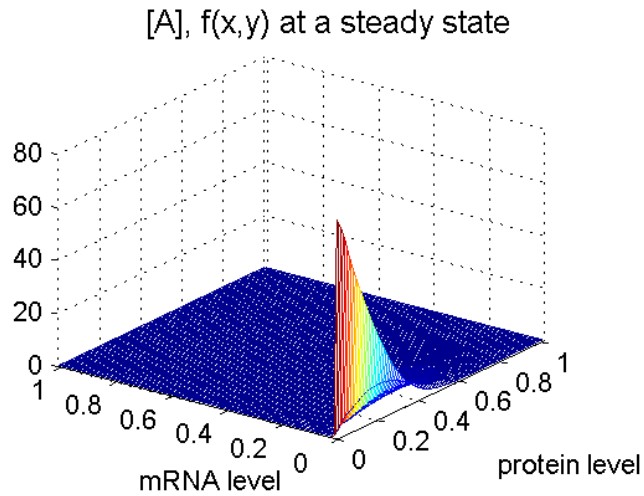
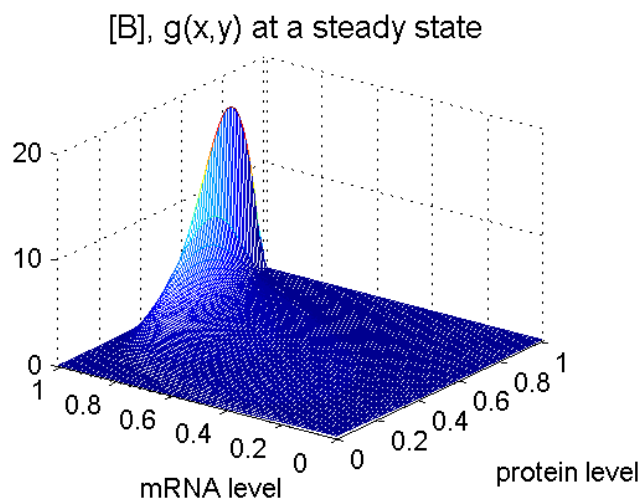
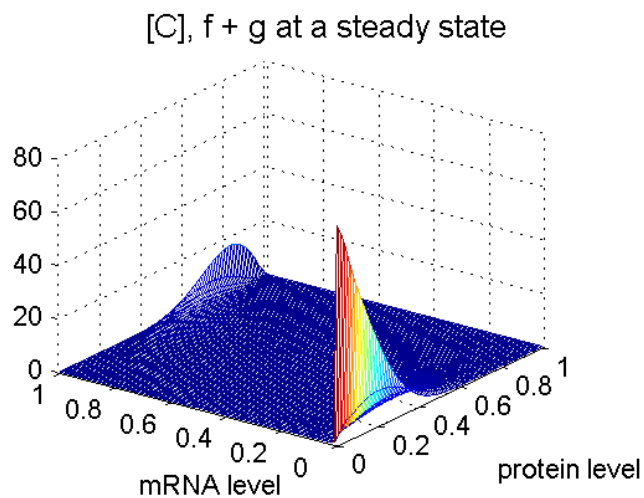
Stationary distributions – numerical solution



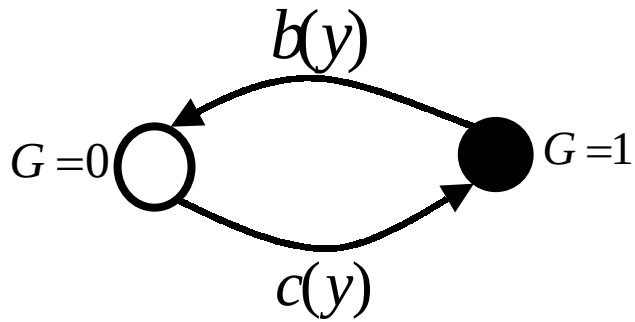




Positive feedback: $c = 0.2 + 0.5y, \quad b = 0.5, \quad r = 0.2$

Protein (y) synthesized directly from the gene

$$\frac{dy}{dt} = G - y$$



$$\frac{df(y,t)}{dt} + \frac{d}{dy}(-y \cdot f) = -c(y)f + b(y)g$$

$$\frac{dg(y,t)}{dt} + \frac{d}{dy}((1-y) \cdot g) = c(y)f - b(y)g$$

Stationary solution:

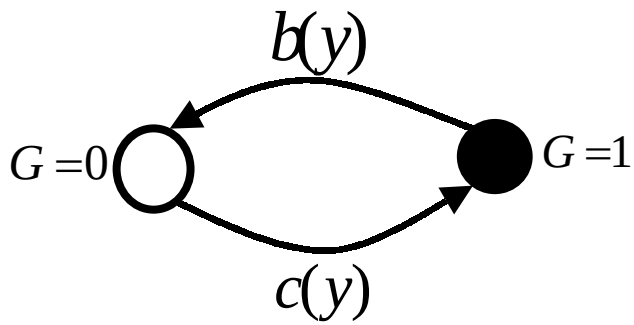
$$f(y) = A\exp\left[\int_0^y \frac{b(z)}{1-z} + \frac{c(z)-1}{z} dz\right]$$

$$g(y) = \frac{Ayf(y)}{(1-y)}$$

Deterministic approximation

$$\frac{dy(t)}{dt} = E(G) - y(t),$$

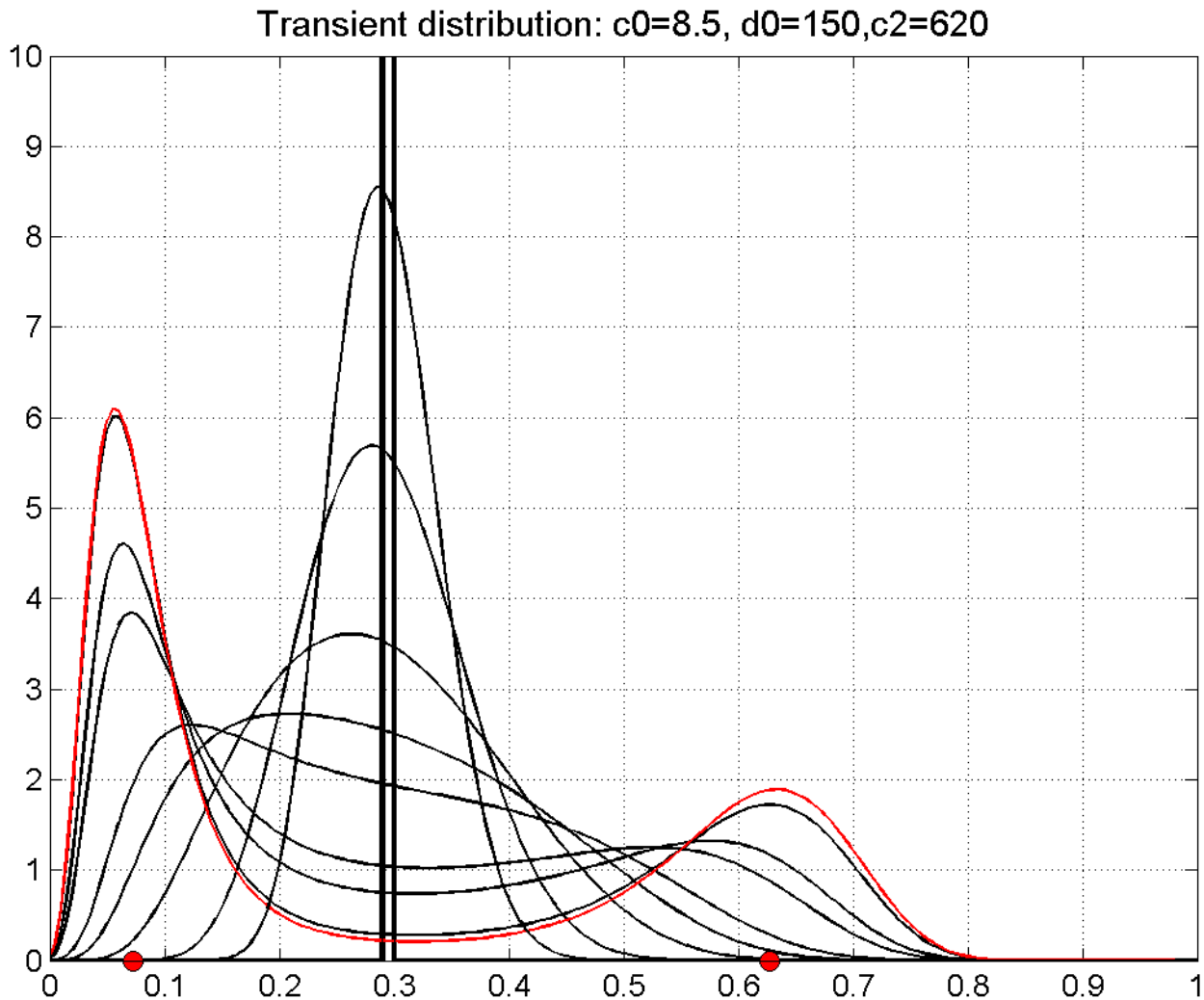
$$E[G] = \frac{c(y)}{c(y) + d(y)},$$



For $c(y) = c_0 + c_1 y + c_2 y^2, \quad b(y) = b_0 + b_1 y + b_2 y^2$

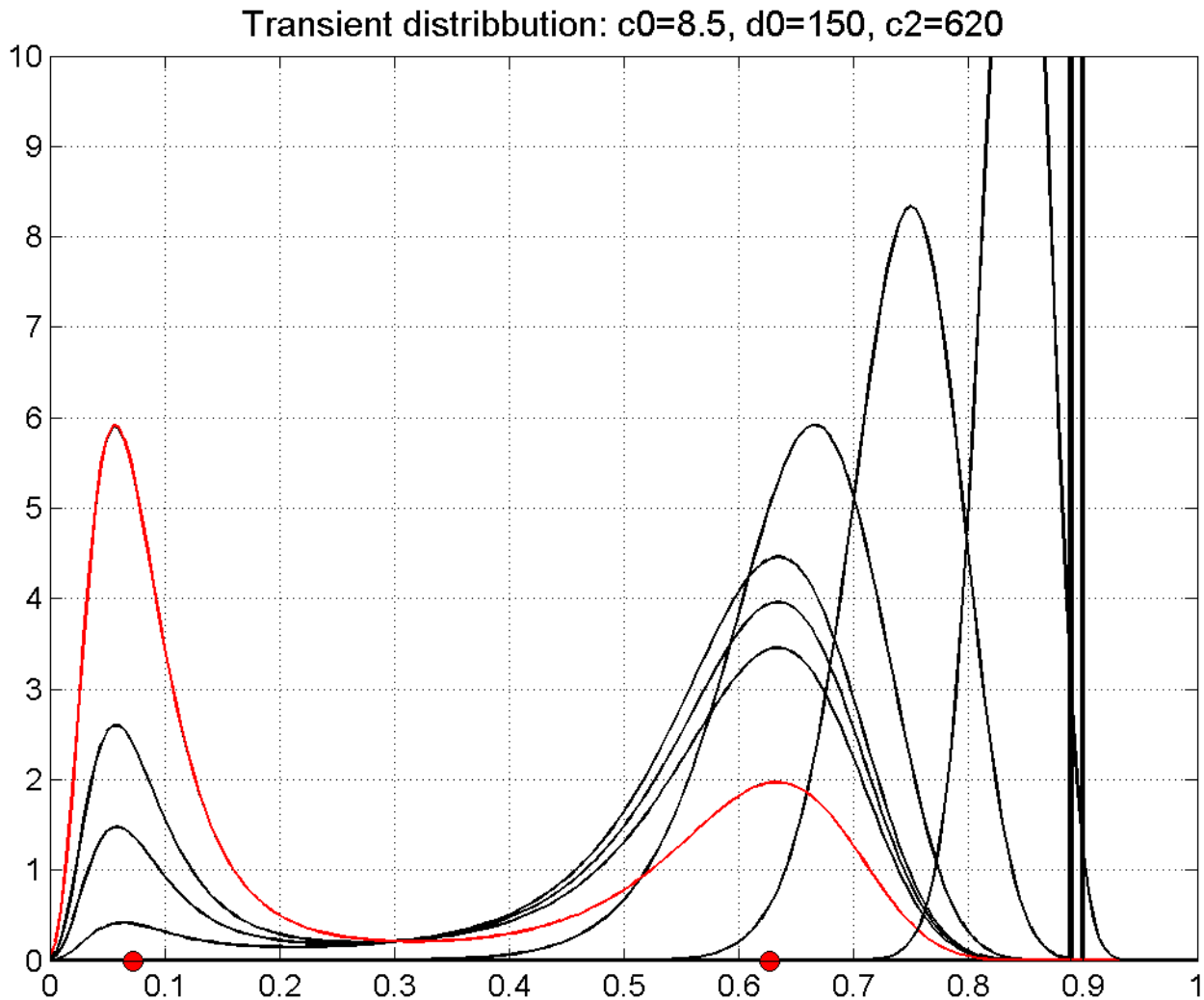
the system has one or two stable steady states depending on the parameters.

Transient probability density functions



Stable deterministic solutions are at 0.07 and 0.63

Transient probability density functions

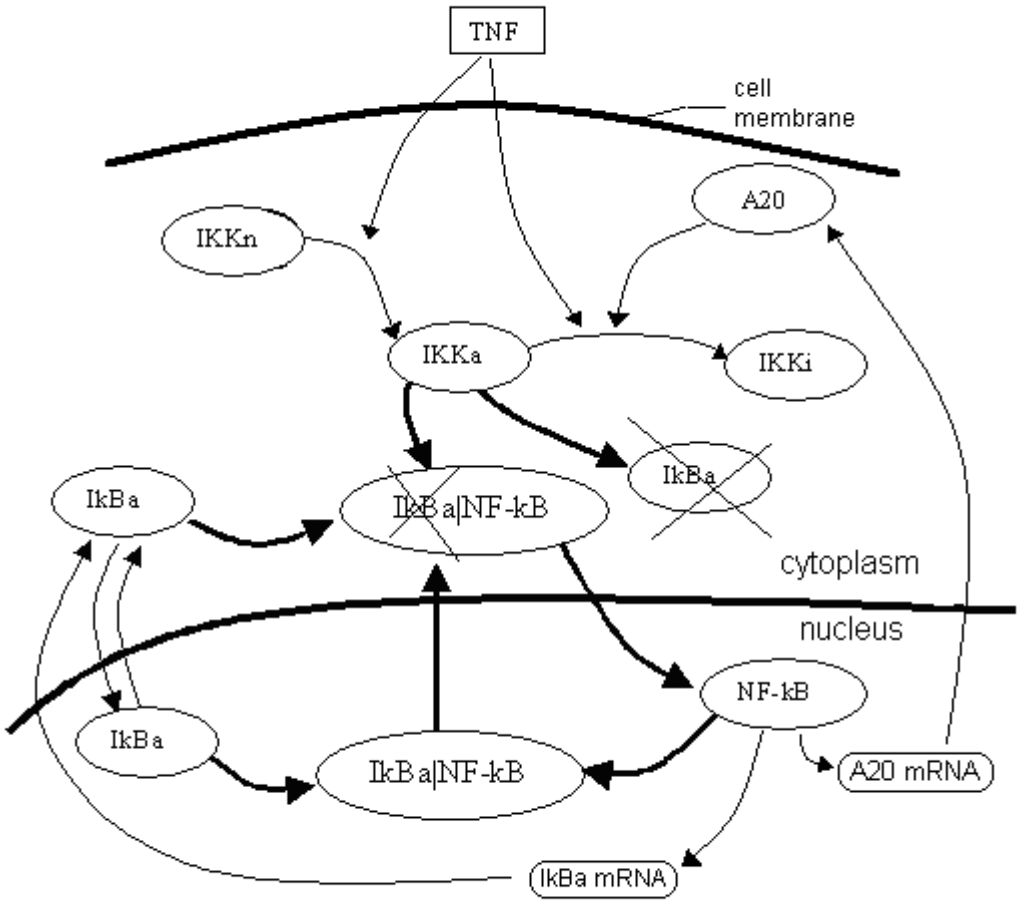


Stable deterministic solutions are at 0.07 and 0.63

Two feedback model of NF-κB dynamics

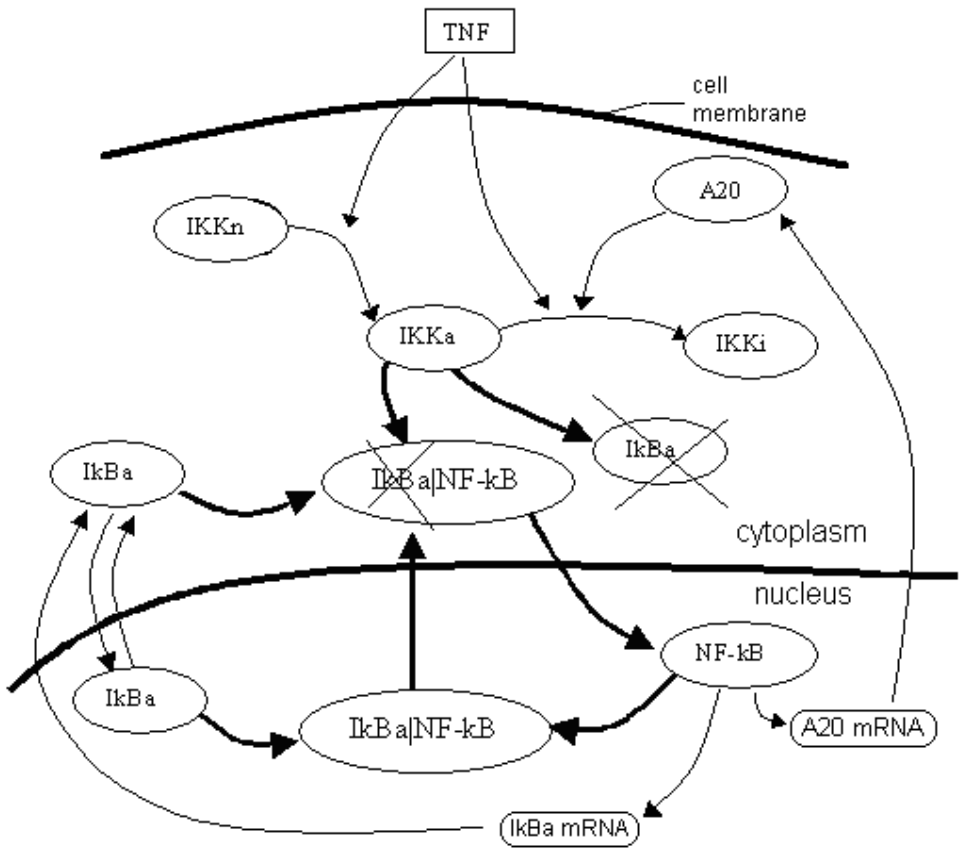
- Key players:
  - NF-κB (transcription factor)
  - IκBα (inhibits NF-κB)
  - IKK (destroys IκBα)
  - TNF (activates IKK)
  - A20 (inactivates IKK)

- Feedbacks
  - NF-κB promotes transcription of IκBα
  - NF-κB promotes transcription of A20



The model: processes considered

- IKK activation, IKKn->IKKa
- IKK inactivation, IKKa->IKKi
- Synthesis of protein complexes
- Catalytic degradation of IκBα
- mRNA transcription
- mRNA translation
- Transport between compartments



Modeling:

14 ODEs + Stochastic switches for gene activities.

ODEs

$$\frac{\mathrm{d}}{\mathrm{dt}} IKKn(t) = k_{prod} - k_{\deg} \cdot IKKn(t) - T_R \cdot k_1 \cdot IKKn(t) \quad (1)$$

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{dt}} IKKa(t) = {} & T_R \cdot k_1 \cdot IKKn(t) - k_2 \cdot IKKa(t) - T_R \cdot k_3 \cdot IKKa(t) \cdot A20(t) - k_{\deg} \cdot IKKa(t) \\ & - a_2 \cdot IKKa(t) \cdot I\kappa B\alpha(t) + t_1 \cdot (IKKa \mid I\kappa B\alpha)(t) \\ & - a_3 \cdot IKKa(t) \cdot (I\kappa B\alpha \mid NF\kappa B)(t) + t_2 \cdot (IKKa \mid I\kappa B\alpha \mid NF\kappa B)(t)\end{aligned} \quad (2)$$

$$\frac{\mathrm{d}}{\mathrm{dt}} IKKi(t) = k_2 \cdot IKKa(t) - k_{\deg} \cdot IKKi(t) + T_R \cdot k_3 \cdot IKKa(t) \cdot A20(t) \quad (3)$$

$$\frac{\mathrm{d}}{\mathrm{dt}} (IKKa \mid I\kappa B\alpha)(t) = a_2 \cdot IKKa(t) \cdot I\kappa B\alpha(t) - t_1 \cdot (IKKa \mid I\kappa B\alpha)(t) \quad (4)$$

$$\frac{\mathrm{d}}{\mathrm{dt}} (IKKa \mid I\kappa B\alpha \mid NF\kappa B)(t) = a_3 \cdot IKKa(t) \cdot (I\kappa B\alpha \mid NF\kappa B)(t) - t_2 \cdot (IKKa \mid I\kappa B\alpha \mid NF\kappa B)(t) \quad (5)$$

$$\frac{\mathrm{d}}{\mathrm{dt}} NF\kappa B(t) = c_{6a} \cdot (I\kappa B\alpha \mid NF\kappa B)(t) - a_1 \cdot I\kappa B\alpha(t) \cdot NF\kappa B(t)$$
$$+ t_2 \cdot (IKKa \mid I\kappa B\alpha \mid NF\kappa B)(t) - i_1 \cdot NF\kappa B(t) \qquad (6)$$

$$\frac{\mathrm{d}}{\mathrm{dt}} NF\kappa B_n(t) = i_1 \cdot NF\kappa B(t) - a_1 \cdot k_v \cdot I\kappa B\alpha_n(t) \cdot NF\kappa B_n(t) \qquad (7)$$

$$\frac{\mathrm{d}}{\mathrm{dt}} A20(t) = c_4 \cdot A20_t(t) - c_5 \cdot A20(t) \qquad (8)$$

$$\frac{\mathrm{d}}{\mathrm{dt}} A20_t(t) = c_1 \cdot G(t) - c_3 \cdot A20_t(t) \qquad (9)$$

$$\frac{\mathrm{d}}{\mathrm{dt}} I\kappa B\alpha(t) = -a_2 \cdot IKKa(t) \cdot I\kappa B\alpha(t) - a_1 \cdot I\kappa B\alpha(t) \cdot NF\kappa B(t)$$
$$+ c_{4a} \cdot I\kappa B\alpha_t(t) - c_{5a} \cdot I\kappa B\alpha(t) - i_{1a} \cdot I\kappa B\alpha(t)$$
$$+ e_{1a} \cdot I\kappa B\alpha_n(t) \qquad (10)$$

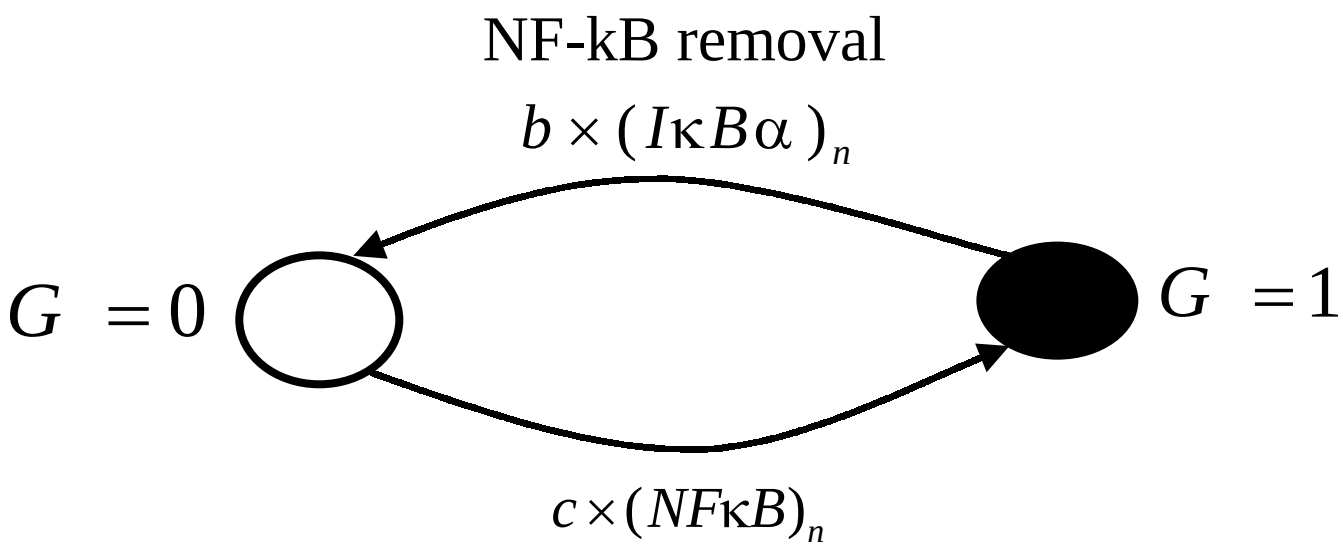
$$\frac{\mathrm{d}}{\mathrm{dt}} I\kappa B\alpha_n(t) = -a_1 \cdot k_v \cdot I\kappa B\alpha_n(t) \cdot NF\kappa B_n(t) + i_{1a} \cdot k_v \cdot I\kappa B\alpha(t) - e_{1a} \cdot I\kappa B\alpha_n(t) \qquad (11)$$

$$\frac{\mathrm{d}}{\mathrm{dt}} I\kappa B\alpha_t(t) = c_{1a} \cdot G_a(t) - c_{3a} \cdot I\kappa B\alpha_t(t) \qquad (12)$$

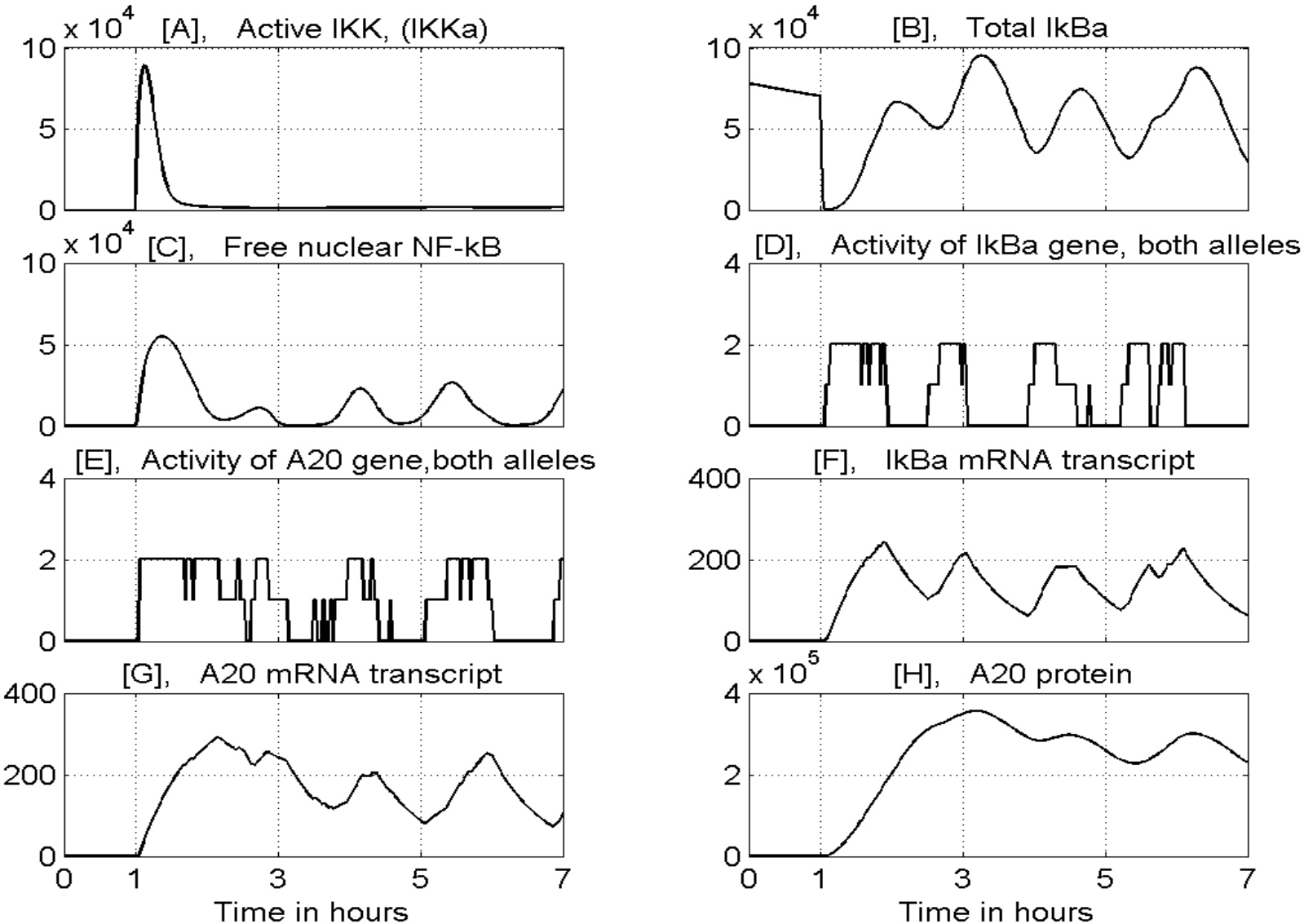
$$\frac{\mathrm{d}}{\mathrm{dt}} \left(I\kappa B\alpha \mid NF\kappa B\right)(t) = a_1 \cdot I\kappa B\alpha(t) \cdot NF\kappa B(t) - c_{6a} \cdot \left(I\kappa B\alpha \mid NF\kappa B\right)(t)$$
$$- a_3 \cdot IKKa(t) \cdot \left(I\kappa B\alpha \mid NF\kappa B\right)(t) + e_{2a} \cdot I\kappa B\alpha_n(t) \cdot NF\kappa B_n(t) \qquad (13)$$

$$\frac{\mathrm{d}}{\mathrm{dt}} \left(I\kappa B\alpha_n \mid NF\kappa B_n\right)(t) = a_1 \cdot k_v \cdot I\kappa B\alpha_n(t) \cdot NF\kappa B_n(t) - e_{2a} \cdot I\kappa B\alpha_n(t) \cdot NF\kappa B_n(t) \qquad (14)$$

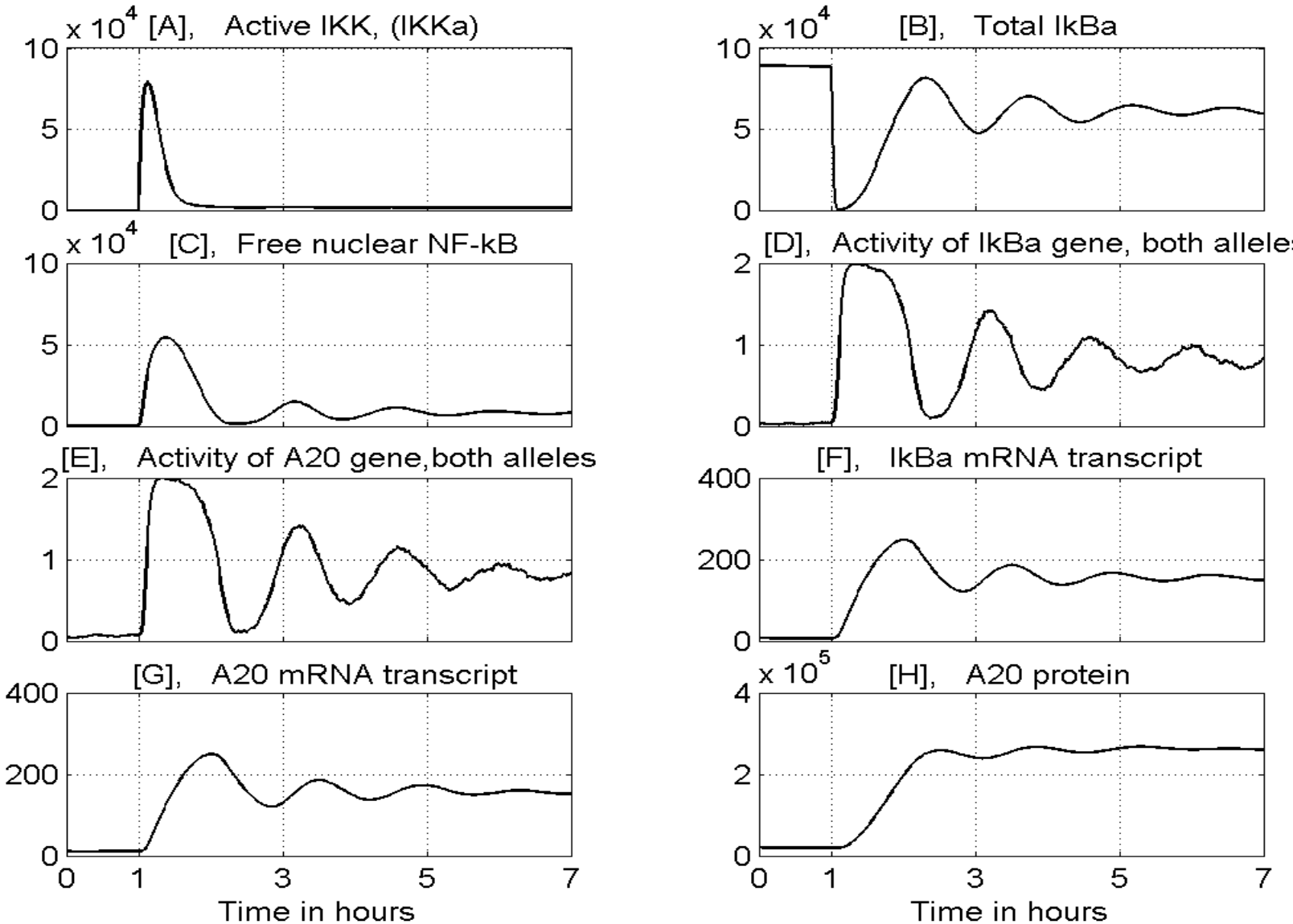
Stochastic switch



Single cell, TNF stimulated

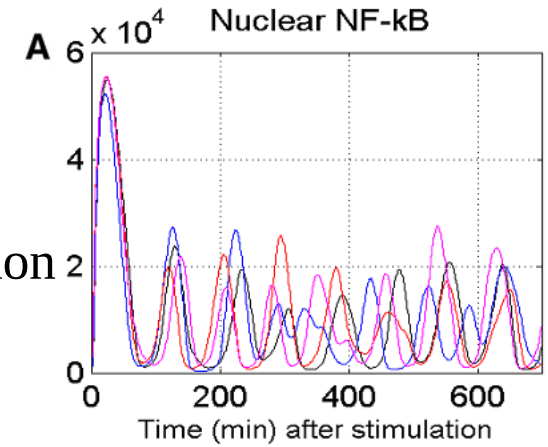


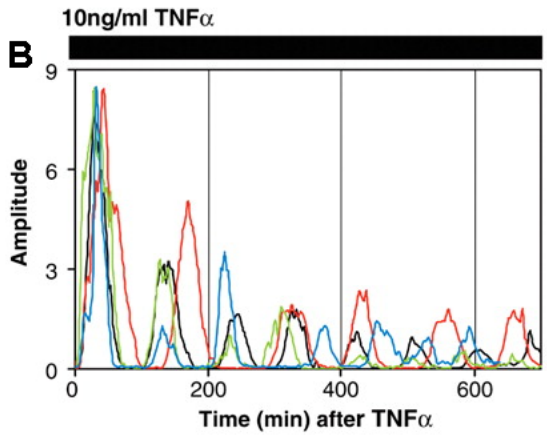
Outcome averaged over 500 cells



Comparing model predictions with single cell experiment,
Nelson et al, Science 2004 (M.R.H. White group)

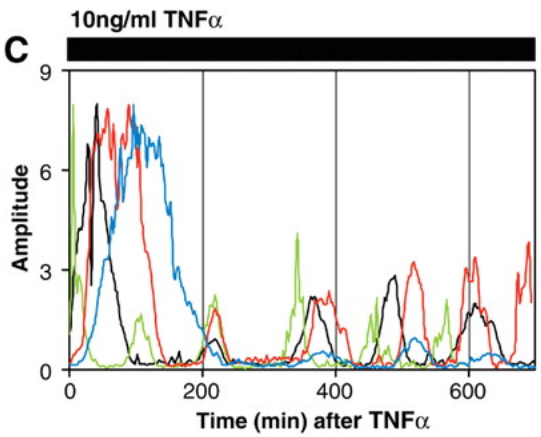
Model,
no
overexpression

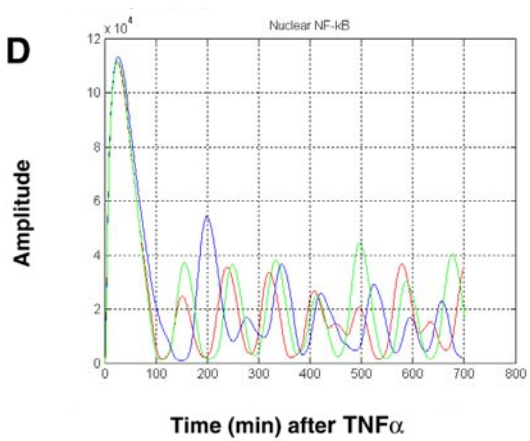




SK-N-AS cells
expressing
RelA-DsRed
3-5 fold
overexpression

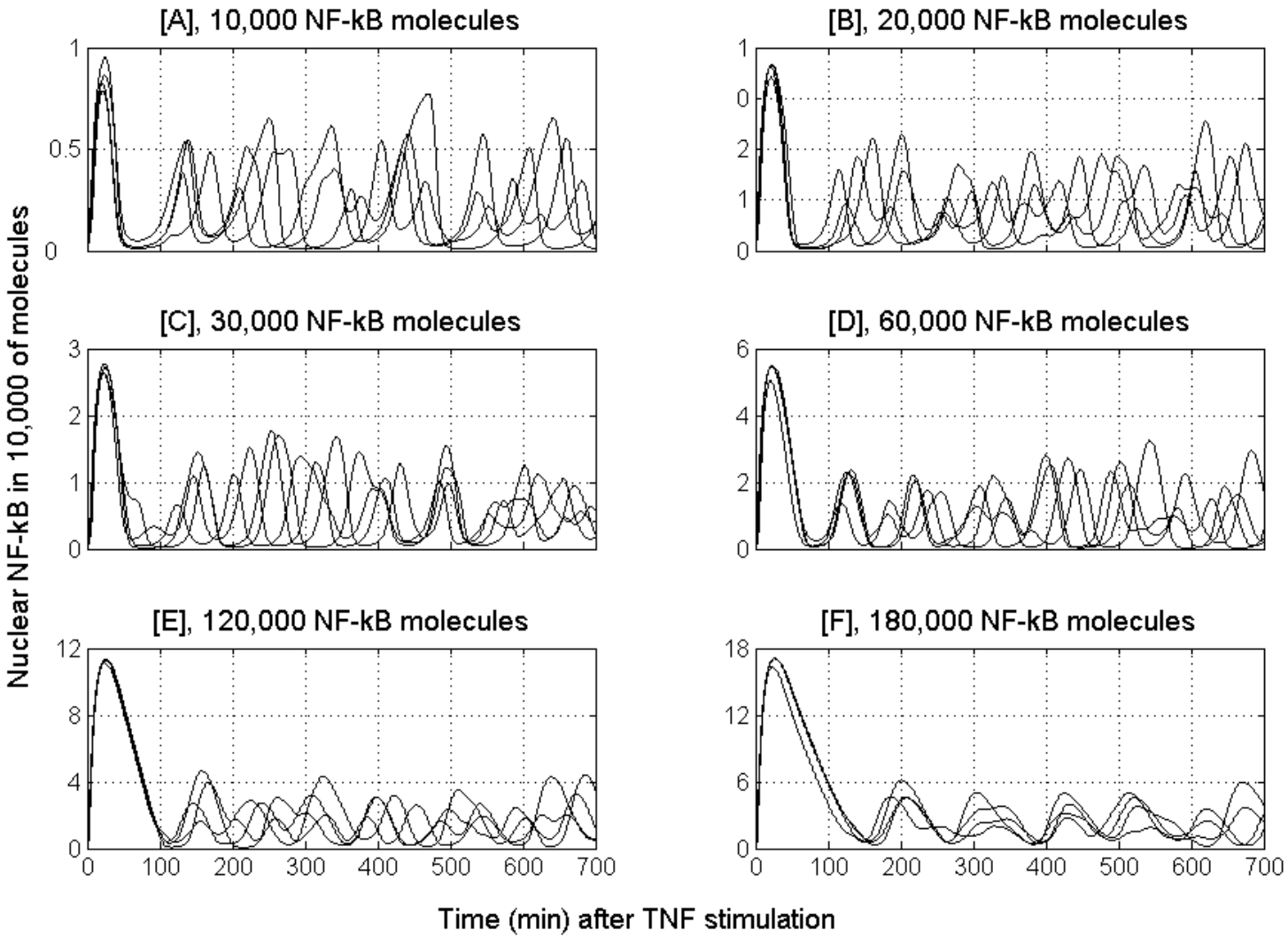
SK-N-AS
cells
coexpressing
RelA-DsRed
and
IkBa-EGFP



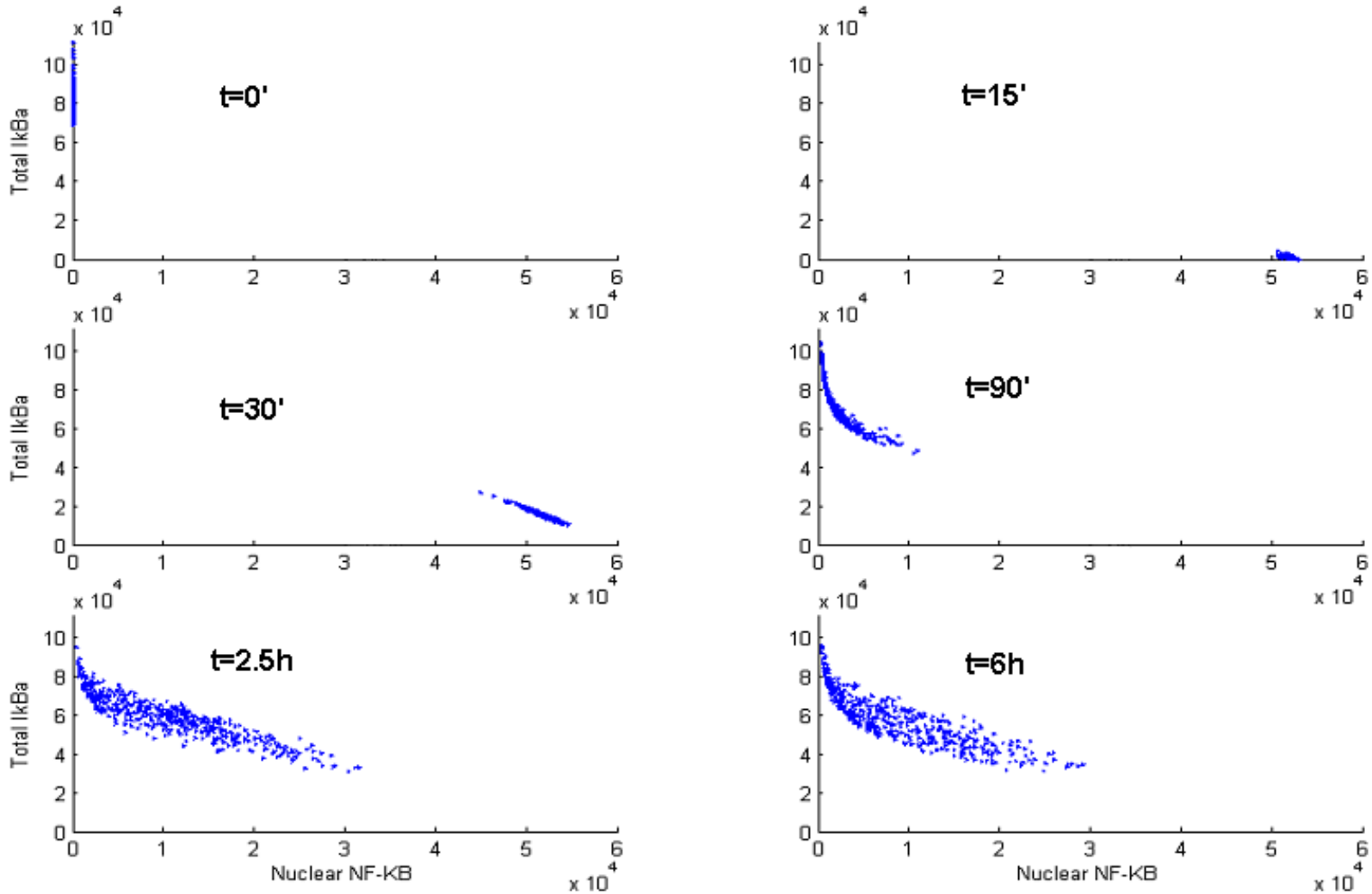


Model,
two-fold
overexpression

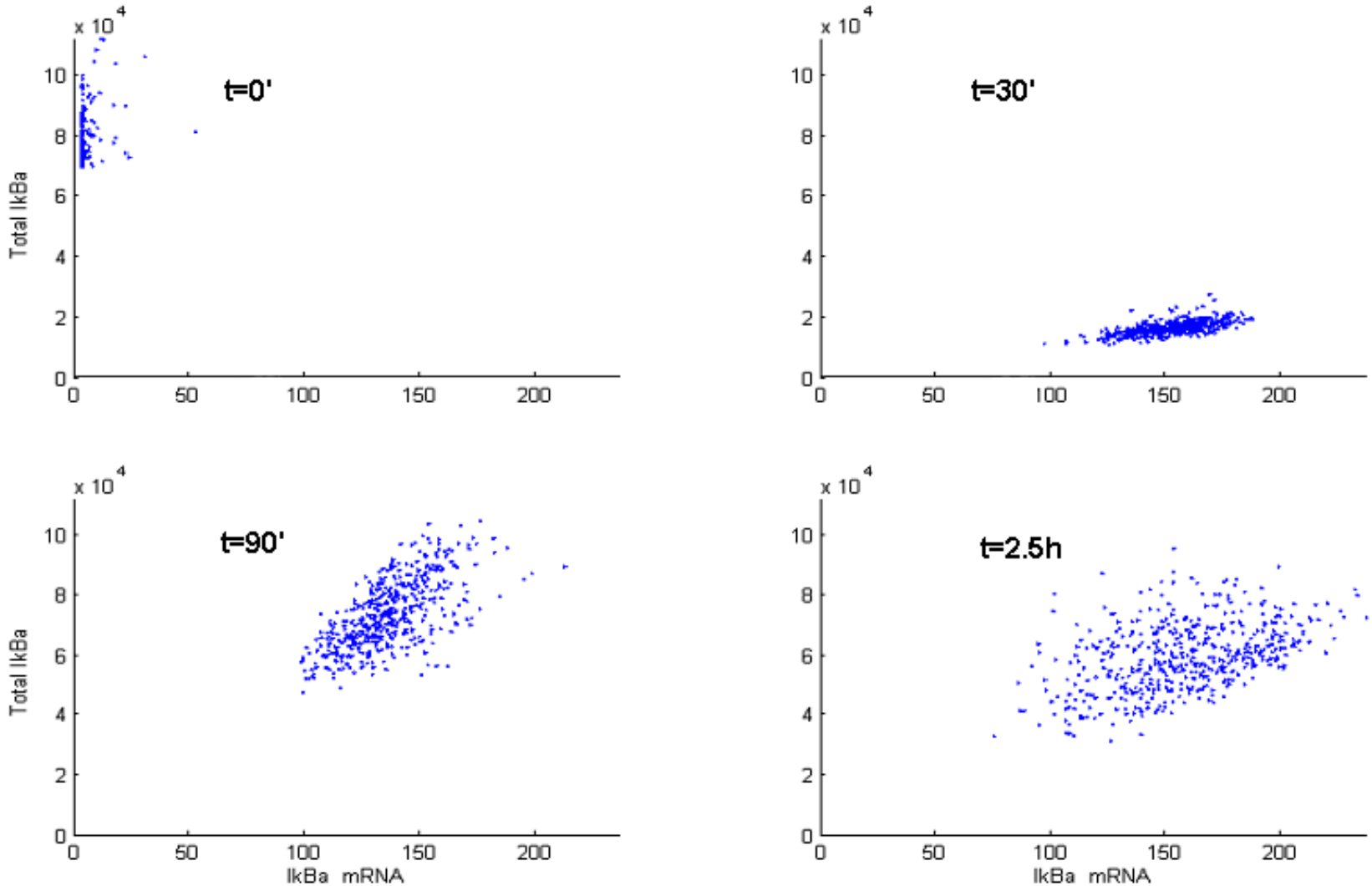
Low period and relative amplitude sensitivity to the level of NF-kB, here changed 18-fold.



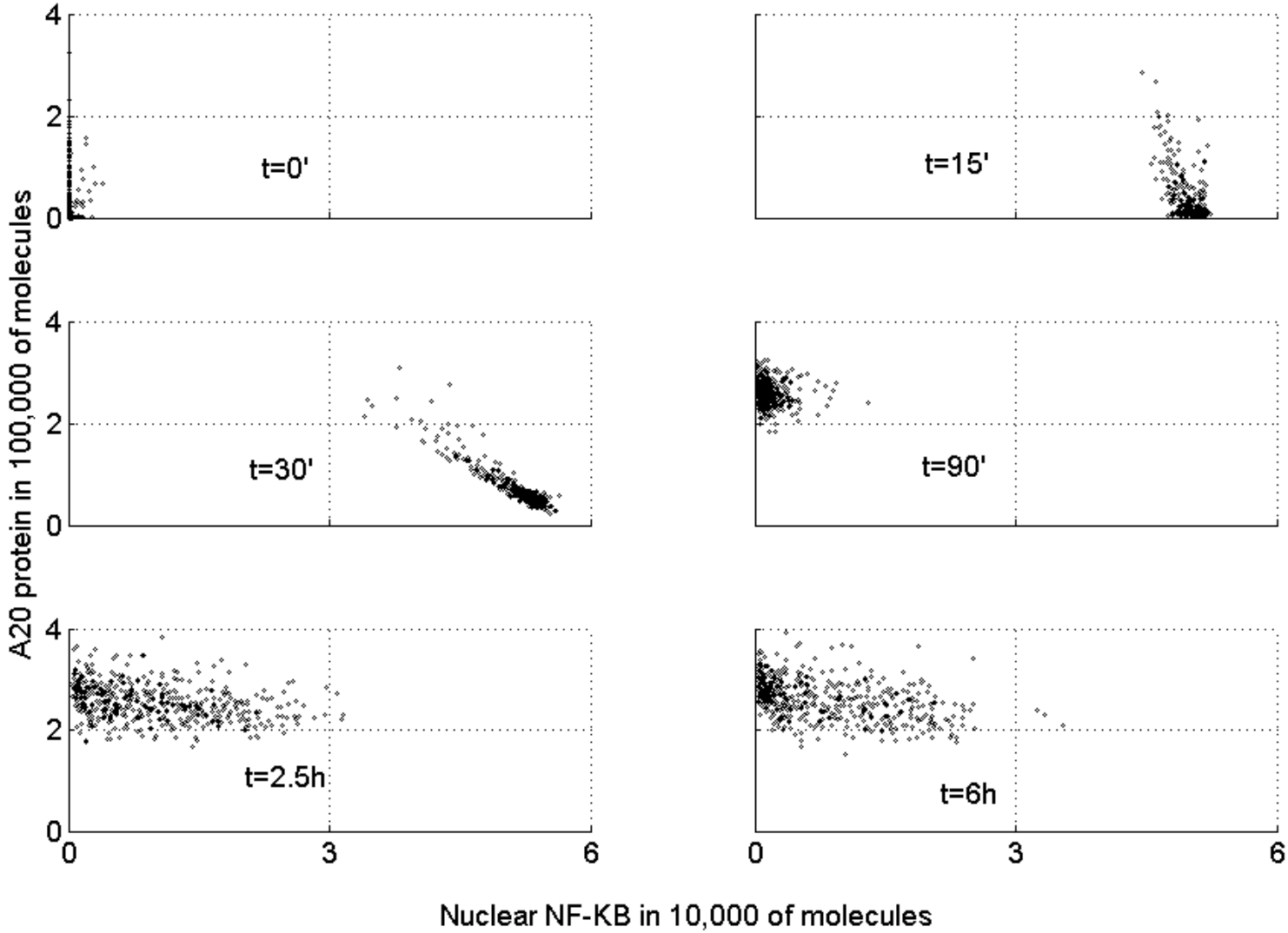
Scatter plots of total IκBα vs. NF-κB$_n$



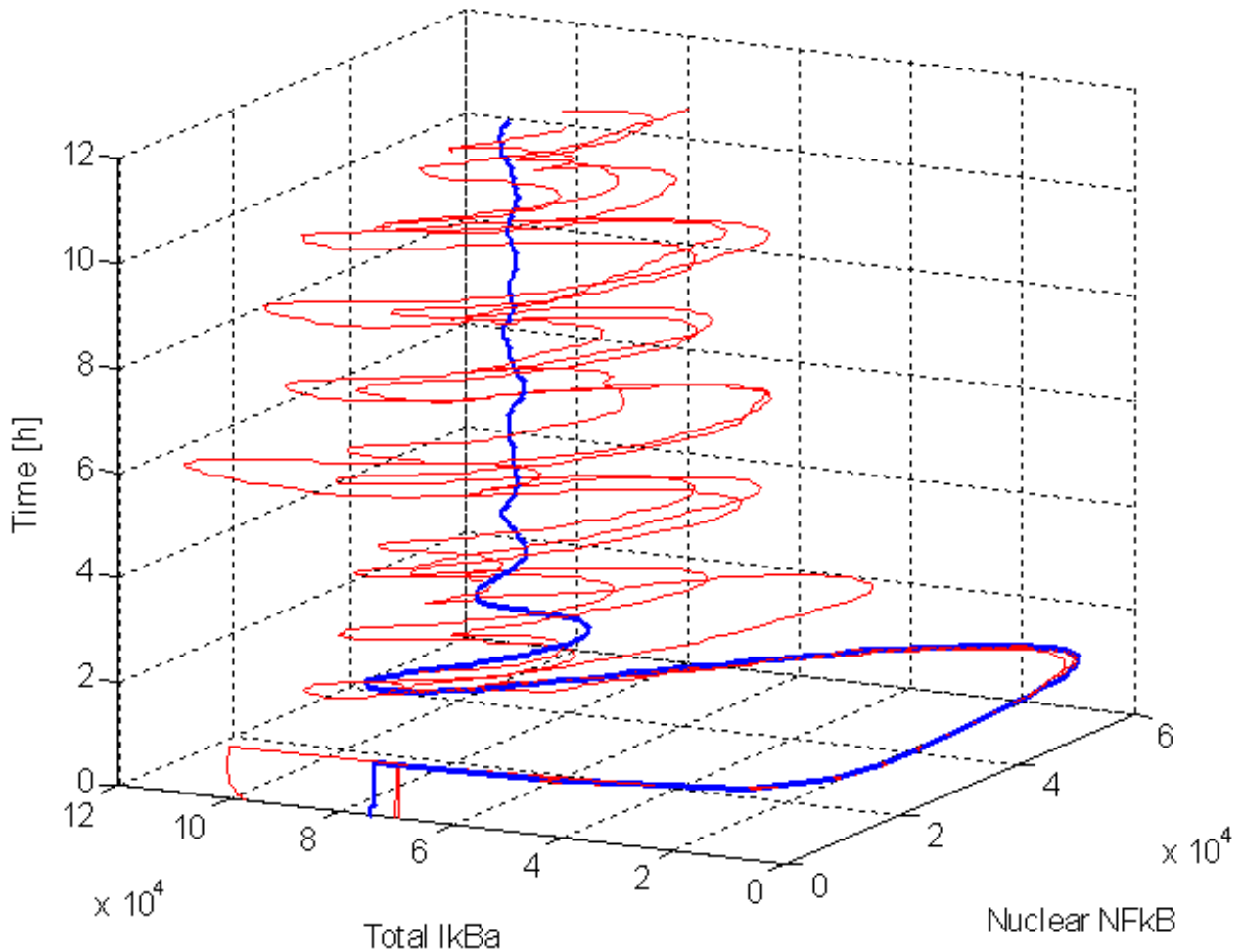
Scatter plots of total IκBα vs. IκBα mRNA



Scatter plots of total A20 vs. NF-κB$_n$



Trajectories projected on (IκBα,NF-κB$_n$,time) hiperplane, red: 3 single cells, blue: cell population



Any single cell trajectory differs from the “averaged” trajectory

Conclusions

- **The two-feedback-model traces satisfactorily data from wild type and A20-deficient cells**:

-mRNAs of A20 and IκBα,
-NF-κB nuclear binding (pulse-like and persistent stimulation),
-IκBα cytoplasmic protein,
-IKK and IKK kinase activity.

- **The single cell kinetics differs from the kinetics averaged kinetics**.

- The persistent NF-kB oscillations are due to switching NF-kB dependent inhibitors (A20 and IκBα) *ON* and *OF*.

- The single cell oscillations are key to persistent NF-kB activity. NF-kB is activated in cytoplasm, but acts in nucleus.

Conclusions cont.

Stochasticity as a way of defense:

First 1.5h: same for all cells (inflamatory genes), then different (late genes activation)

Single cell modeling:

Fast reactions channels (large number of molecules): determinstic reaction-rate equations

Slow reaction channels (small number of molecules): stochastic modeling

References

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T. Lipniacki, P. Paszek, A.R. Brasier, B.A. Luxon, M. Kimmel, Stochasticity in early immune response. *Biophysical Journal* **90** (2006) 725-742.

P. Paszek, T. Lipniacki, A. R. Brasier, B. Tian, D. E. Nowak, M. Kimmel "Stochastic effects of multiple regulators on expression profiles in Eukaryotes", *J. Theor. Biol* **233** (2005) 423-433.

B. Hat, P. Paszek, M. Kimmel, K. Piechor, T. Lipniacki, How the number of alleles influences gene expression, *J. Stat. Phys*. submitted

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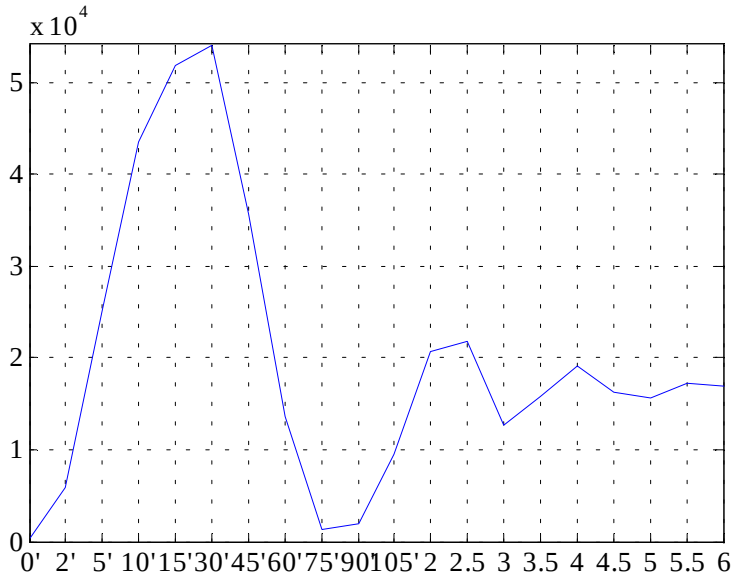
A. Bobrowski, T. Lipniacki, K. Pichor, R. Rudnicki Asymptotic behavior of distributions of mRNA and protein levels in a model of stochastic gene expression *J. Math. Anal. App*. submitted

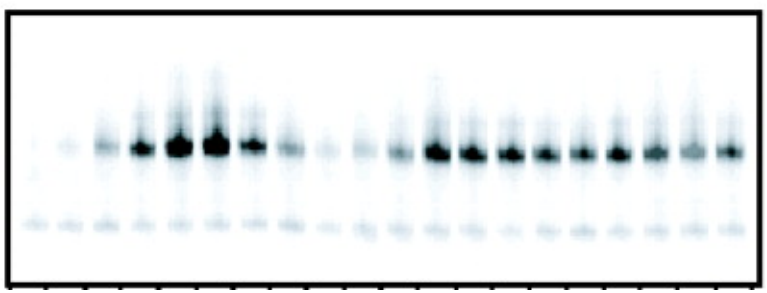
Supported by NHLBI and KBN Grants.

Hoffmann, Levchenko, Scott, Baltimore, 2002, Science, 298, p.1241

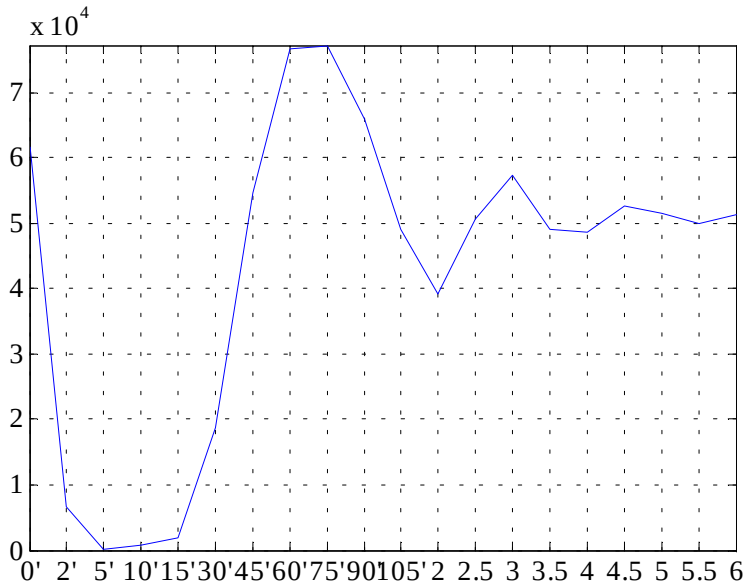
- Constant TNF (mouse fibroblasts)

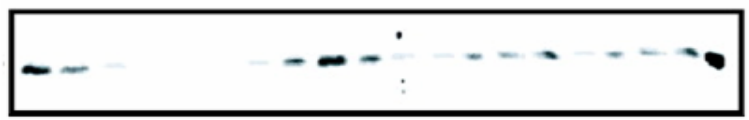
Nuclear NF-κB





Cytoplasmic IκBα

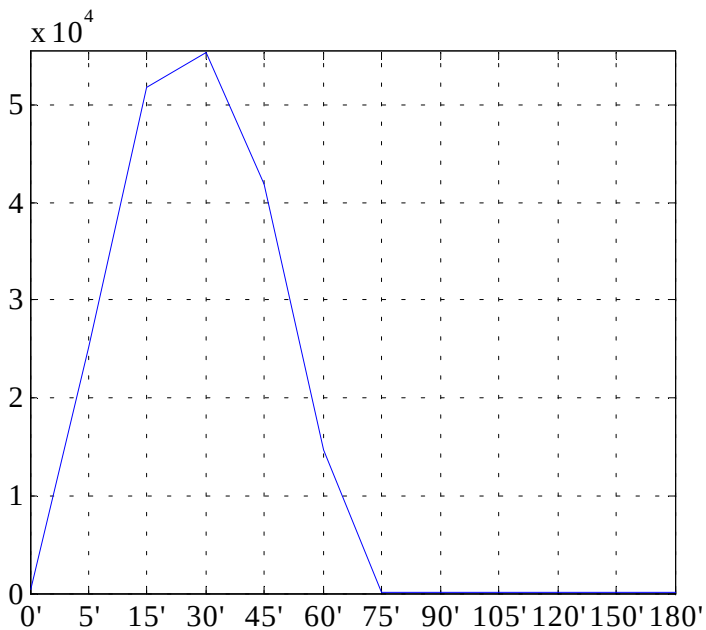




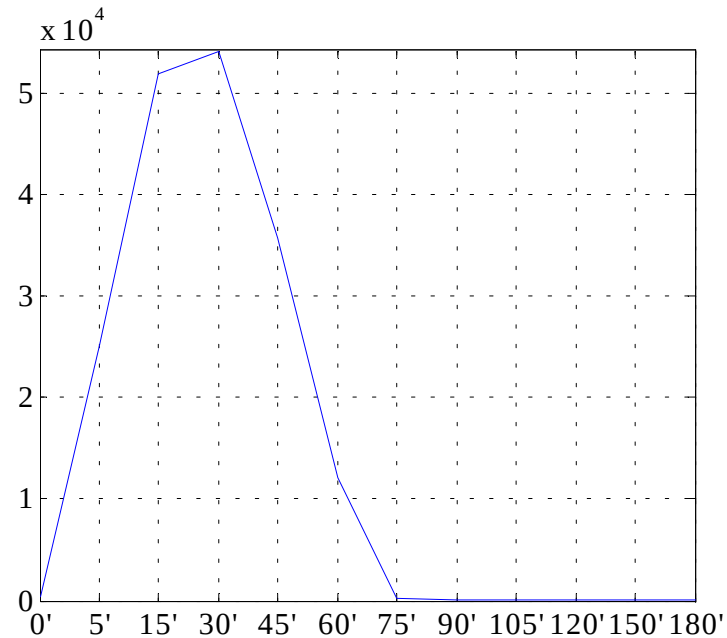
Hoffmann et al., 2002, Science, 298, p.1241

- TNF pulse (mouse fibroblasts)

Nuclear NF-κB (15 min. pulse)



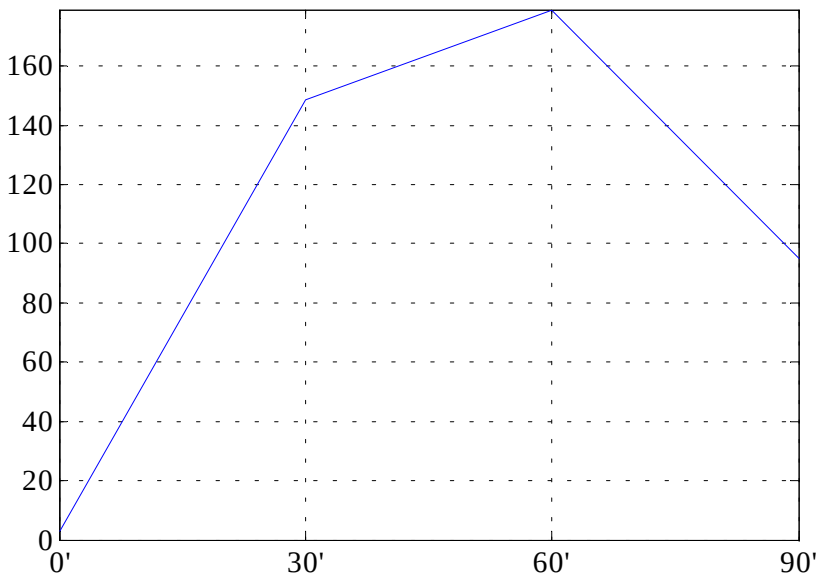
Nuclear NF-κB (1 hr. pulse)

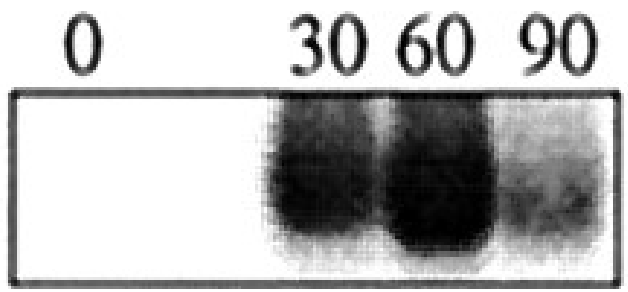


Lee, Boone, Chai, Libby, Chien, Lodolce, Ma, 2000, Science, 289, p. 2350

- Constant TNF (mouse fibroblasts)

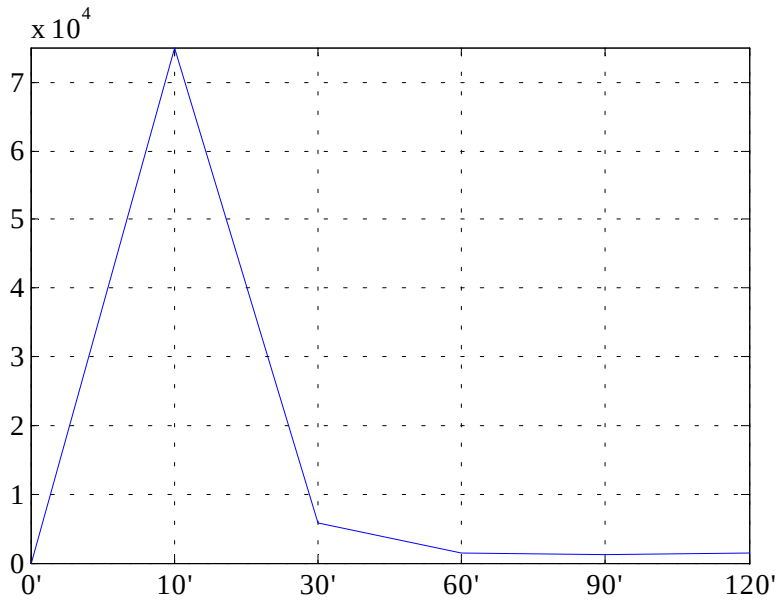
A20 message level



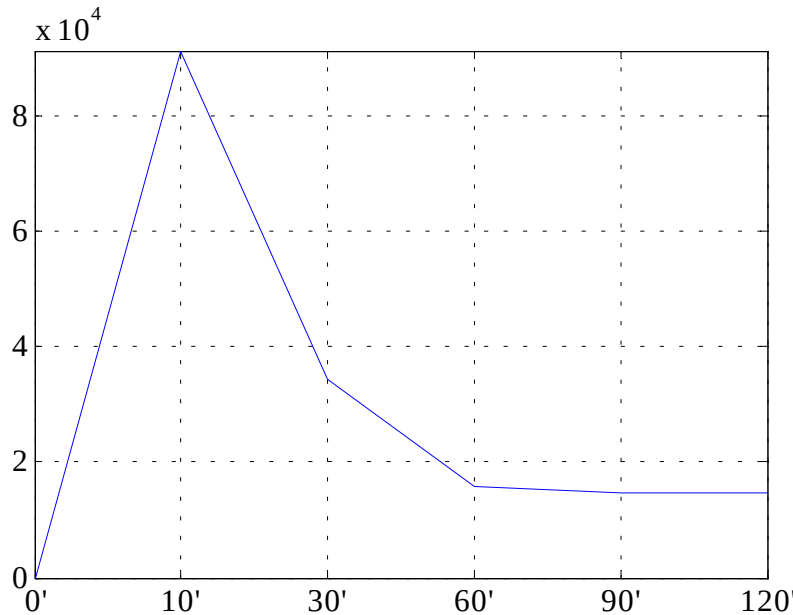


IKK kinase activity, Lee et al., 2000

Wild Type cells

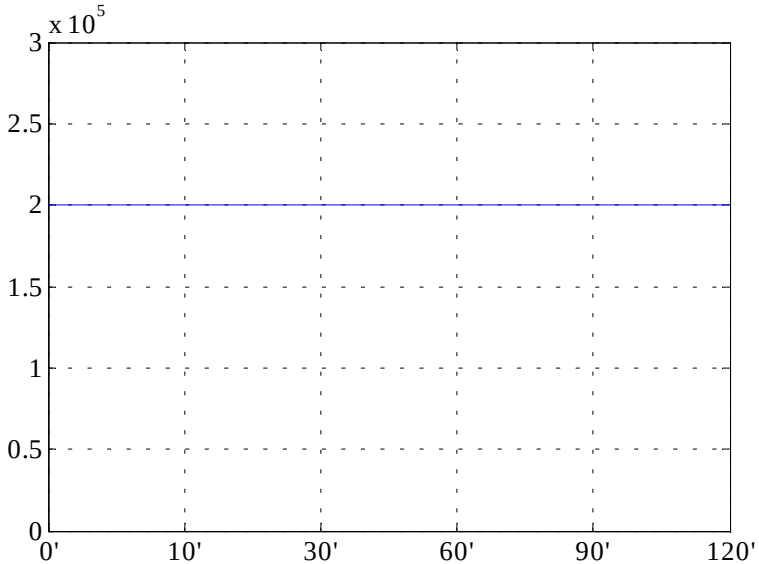


A20-/- cells

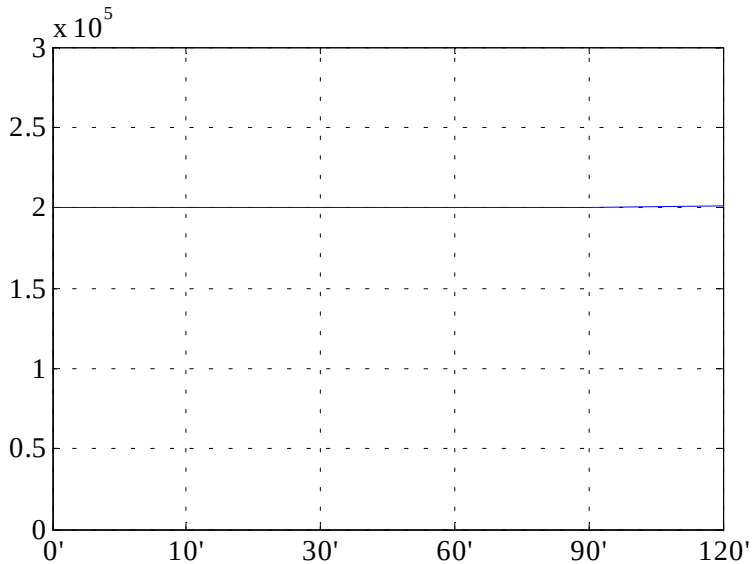


IKKβ protein, Lee et al., 2000

Wild type cells

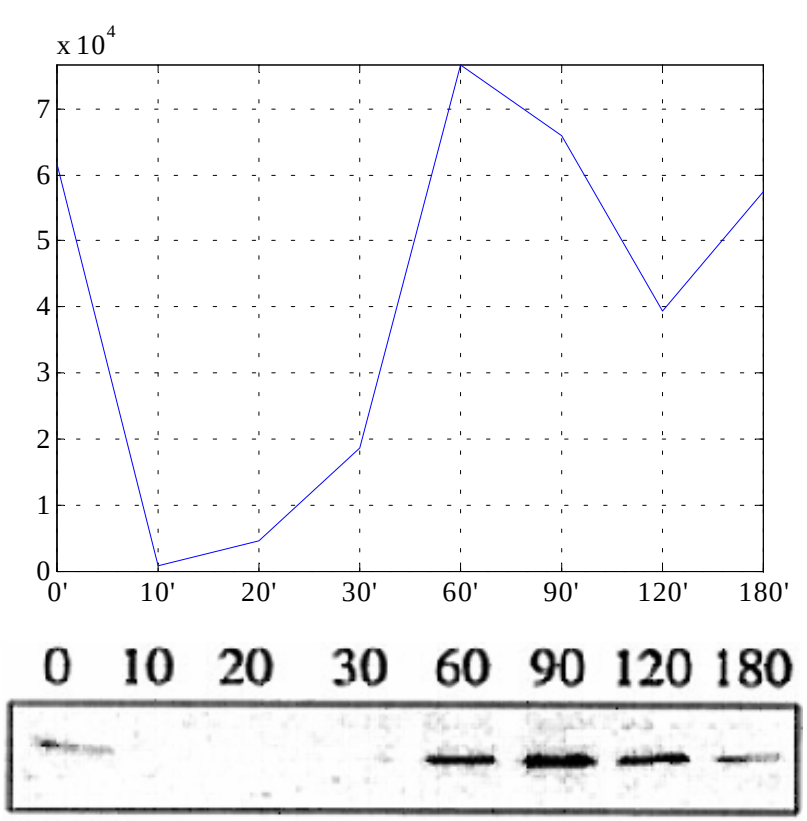


A20 -/- cells



Cytoplasmic IκBα, Lee et al., 2000

Wild type cells



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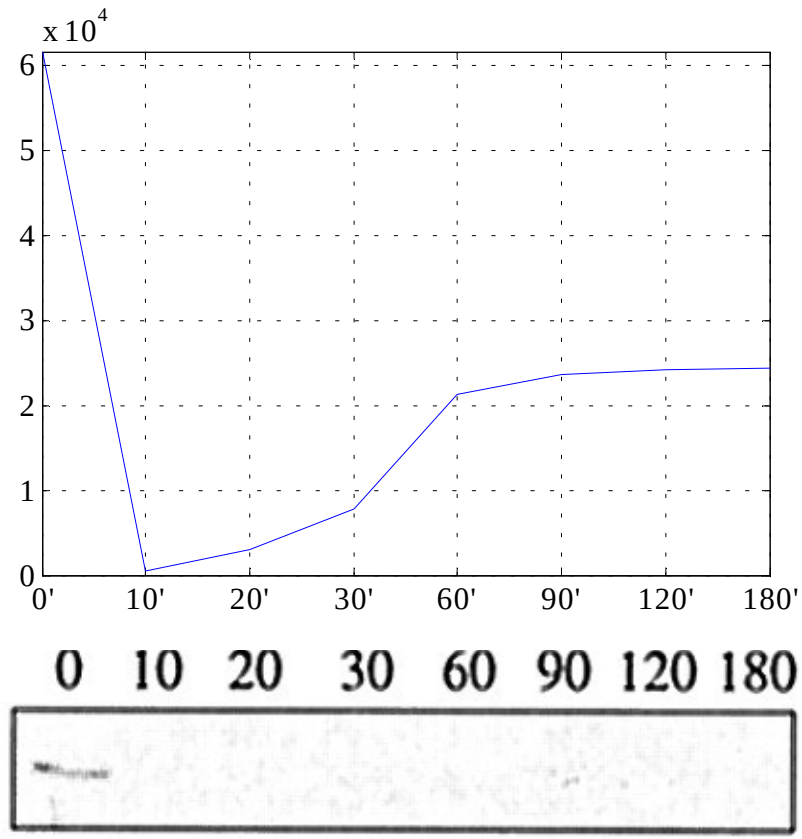
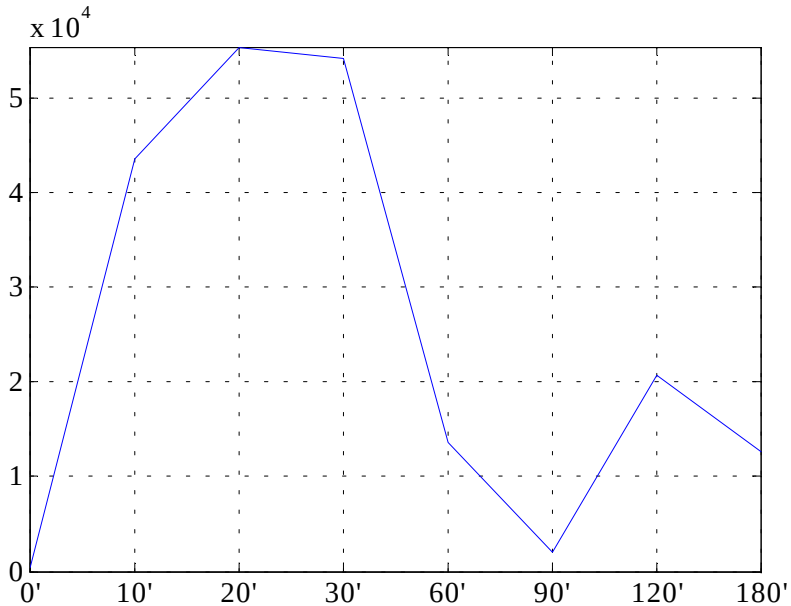


Nuclear NF-κB, Lee et al., 2000

Wild type cells



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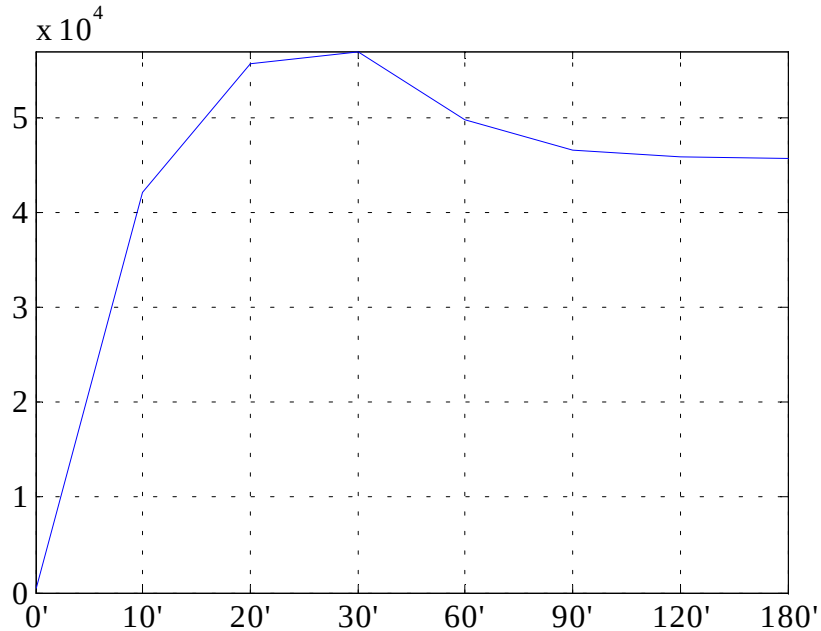
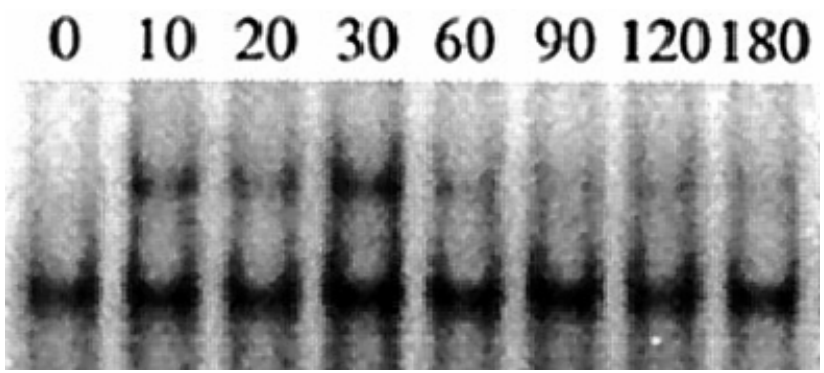
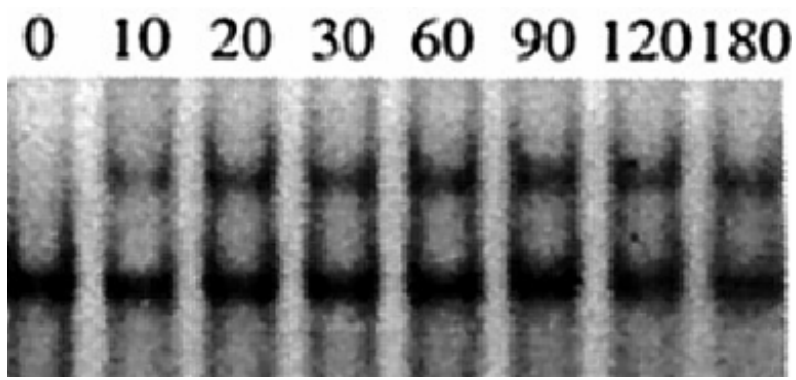
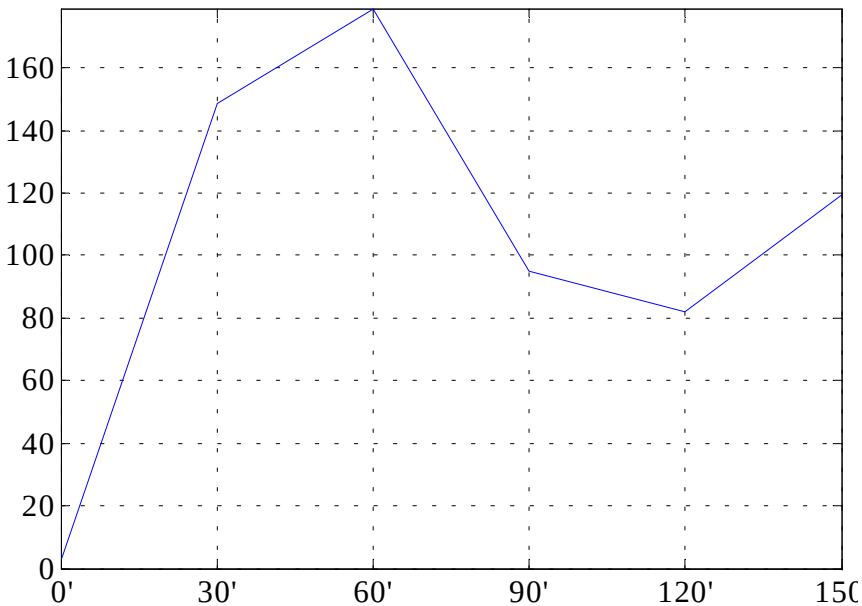


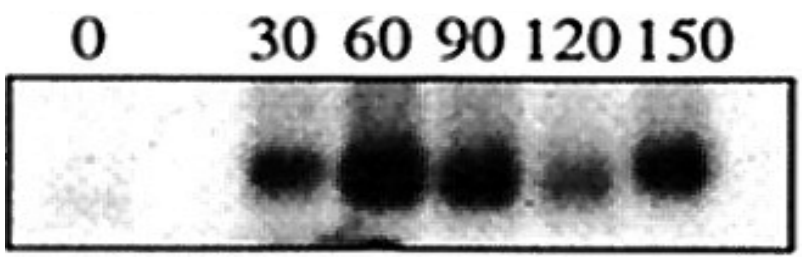




mRNA IκBα, Lee et al., 2000,

Wild type cells





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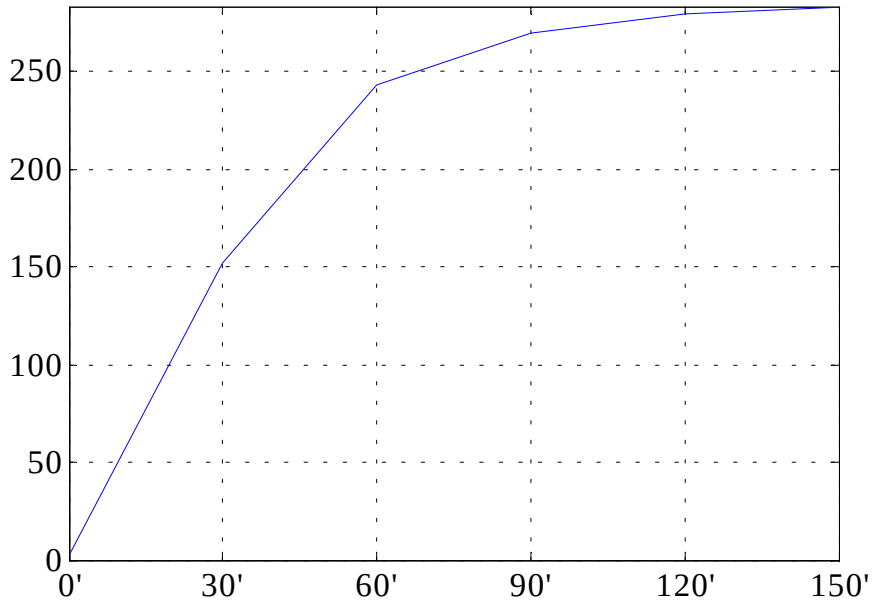

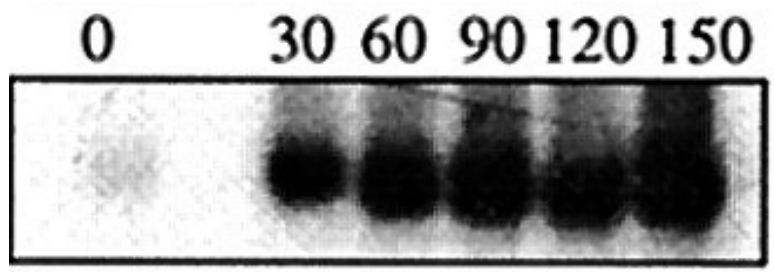

Note: Low level of cytoplasmic IκBα, high mRNA level