SUPPLEMENTARY INFORMATION

Table of Content

Text for the chapter "Flux through the NPC is robust precisely because all reactions go through the same pore"

Here we present the model for metabolic control analysis of NPC transport. The nuclear import rate of each particular signaling protein x_i (with y_i represented its imported form) through the NPC is described by a reversible kinetic equation incorporating competitive inhibition by other cargo from both sides of the nuclear membrane:

$$v_i = \frac{V_{MAX} \cdot \frac{x_i}{K_{x_i}} \cdot \left(1 - \frac{y_i}{x_i}\right)}{1 + \frac{x_i}{K_{x_i}} + \sum_{\substack{k=1 \\ k \neq i}}^{n} \frac{x_k}{K_{x_k}} + \sum_{k=1}^{n} \frac{y_k}{K_{y_k}}}.$$

Here v_i is the transport rate of species x_i (and hence also of y_i); V_{MAX} and the K_{xk} are constant rate characteristics of transport of species k.

For large n, several simplifications apply, i.e. when many other cargo proteins compete for transport and their individual concentrations become small relative to the total cargo concentration. First, the nuclear import rate v_i becomes linear with respect to x_I and the corresponding elasticity coefficient, i.e. the log-log dependence of rate on concentration (Burns et al, 1985) approaches 1, whenever the gradient of substance i across the nuclear membrane is sizeable, i.e. $x_i >> y_i$:

$$v_i \approx_{n>>1} \frac{V_{MAX}}{1 + \sum_{\substack{k=1 \\ k \neq i}}^{n} \frac{x_k}{K_{x_k}} + \sum_{k=1}^{n} \frac{y_k}{K_{y_k}}} \frac{x_i}{K_{x_i}} \cdot \left(1 - \frac{y_i}{x_i}\right)$$

$$\varepsilon_{x_i}^{v_i} \equiv \left(\frac{\partial \ln v_i}{\partial \ln x_i}\right)_{x_j} \approx 1 - \frac{y_i}{x_i - y_i} \approx_{if\ y_i << x_i} 1$$

Second, the rate becomes independent of the concentration of any specific other cargo molecule including the already imported forms, causing all cross elasticity coefficients to become zero:

$$v_i = \frac{V_{MAX} \cdot \frac{x_i}{K_{x_i}} \cdot \left(1 - \frac{y_i}{x_i}\right)}{1 + \frac{x_j}{K_{x_j}} + \sum_{\substack{k=1 \\ k \neq j}}^{n} \frac{x_k}{K_{x_k}} + \frac{y_j}{K_{y_j}} + \sum_{\substack{k=1 \\ k \neq j}}^{n} \frac{y_k}{K_{y_k}}} \approx_{n>>1} \frac{V_{MAX} \cdot \frac{x_i}{K_{x_i}} \cdot \left(1 - \frac{y_i}{x_i}\right)}{1 + \sum_{\substack{k=1 \\ k \neq j}}^{n} \frac{x_k}{K_{x_k}} + \sum_{\substack{k=1 \\ k \neq j}}^{n} \frac{y_k}{K_{y_k}}} \approx$$

$$\approx_{if\ y_i << x_i} \frac{V_{MAX} \cdot \frac{x_i}{K_{x_i}}}{1 + \sum_{\substack{k=1 \\ k \neq j}}^{n} \frac{x_k}{K_{x_k}} + \sum_{\substack{k=1 \\ k \neq j}}^{n} \frac{y_k}{K_{y_k}}},$$

$$\varepsilon_{y_j}^{v_i} \equiv \left(\frac{\partial \ln v_i}{\partial \ln y_j}\right)_{x_j} \approx 0, \forall j \neq i; \varepsilon_{y_i}^{v_i} \approx -\frac{y_i}{x_i - y_i} \approx_{if\ y_i << x_i} 0; \varepsilon_{x_j}^{v_i} 0, \forall j \neq i$$

This shows if there are many active pathways (the condition that *n* be large), all regulatory influences between the pathways have elasticity coefficients that are very close to zero. Consequently, all pathways become independent of each other. Therefore, all the flux control of any path resides completely with its component enzymes:

$$C^{J_{\text{flux pathway j}}}_{\text{enzymes pathway k}} = 0; for\ j \neq k$$

$$C^{J_{\text{flux pathway j}}}_{\text{enzymes pathway j}} > 0$$

$$\sum_{i=1}^{\substack{\text{all enzymes in}\\ \text{pathway j}}} C_i^{J_j} = 1$$

Partly because the NPC import is irreversible but mostly because of the saturation effect itself, the reactions after import that sense the concentration of the imported form, become so-called 'slave enzymes', which only control the concentration of their own imported form in the nucleus but not its flux into the nucleus (Bakker et al, 2000) (Fig. 4B; control by 'output' is small). A possibly important exception to this arises when NR accumulates in the nucleus after import, thereby reducing the amount of NR in the cytosol. The NPC itself has positive control over all fluxes (approximately 0.5 in Fig. 4B). Cytosolic activities of a particular pathway have little, negative control on the flux through any another pathway (cross control by input is small and negative in Fig. 4C). Because they exert negative concentration control on x and y, nuclear processes control other pathway fluxes positively if at all (Fig. 4C, gray line). Because no steady-state flux exchange occurs between pathways, all flux control coefficients within a pathway sum to 1 (dashed line in Fig. 4B). It turns out that even though all pathways run through the same pore, perturbation of any one pathway does not much influence the flux through the other pathways. It only influences the flux through the perturbed pathway itself. Thus, the flux of each pathway is robust precisely because all reactions go through the same pore.

Figures

Fig. 1S

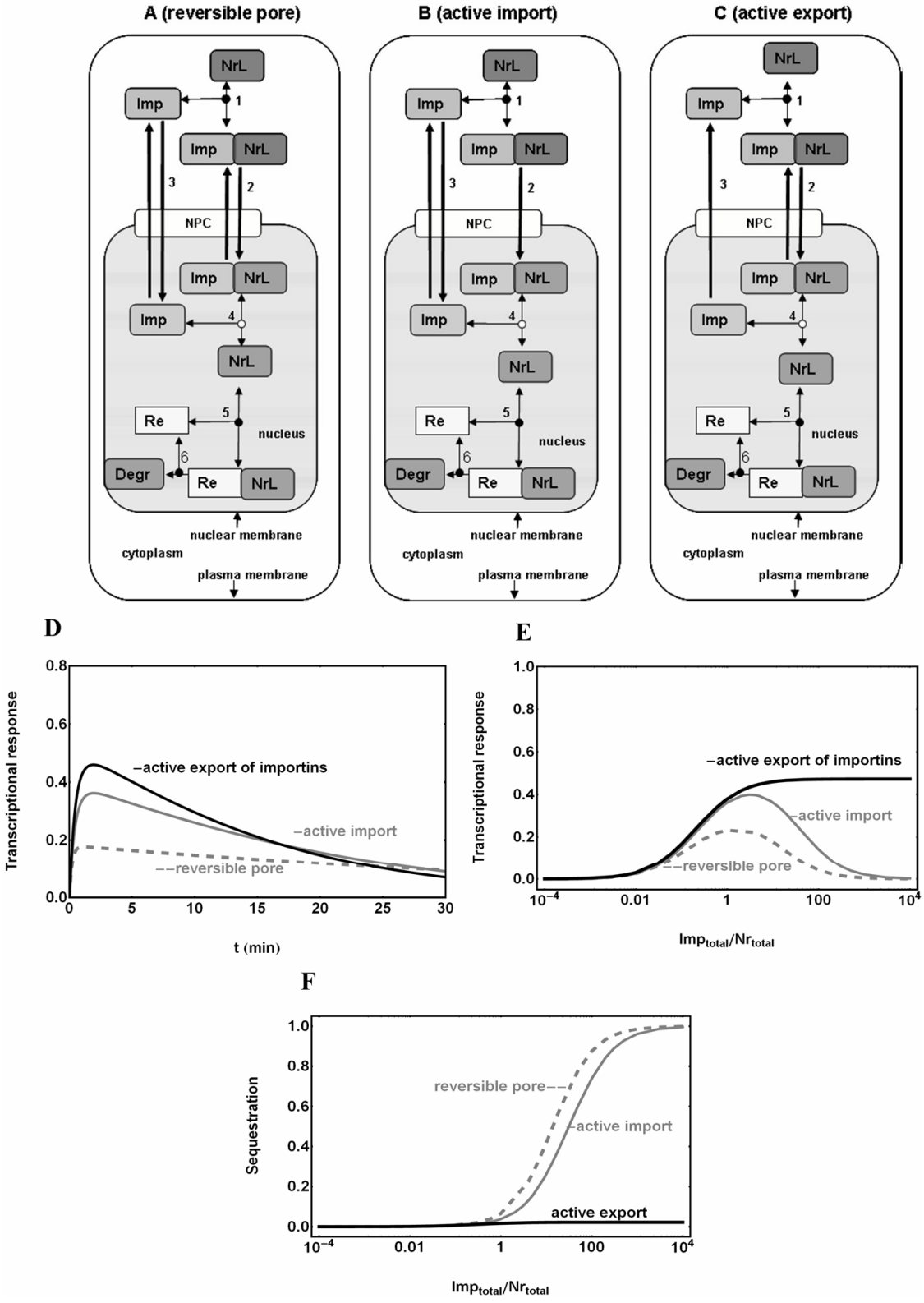


Fig. 1S. Active export of importins rather than active import of the importin-NR complex is advantageous in enhancing the transcriptional response also when there is degradation of NR.

Three alternative network designs are depicted: (**A)** Passive facilitated diffusion (reversible pore) of NR across the nuclear membrane; (**B)** Active nuclear import (irreversible pump) of importin-NR complex; (**C)** Active export (irreversible pump) of importins from the nucleus. (**D)** Time course of the transcriptional response (ratio $ReNrL/Re_{total}$) at total importin concentration of 0.1×10^{-12} *nmoles*. The transcriptional response represents the fraction of REs complexed to the NRL. There is a peak of transcriptional response (maximal transcriptional response). **(E)** Maximal transcriptional response (ratio $ReNrL/Re_{total}$) as function of the total concentration of importins (for designs A-C; note the logarithmic importin concentration axis). The transcriptional response is inhibited by high concentrations of importin in models A and B. **(F)** Sequestration (defined as the ratio $NrL_{total}Imp_{total}/NrL_{total}$) as function of the total concentration of importins (for designs A-C; note the logarithmic importin concentration axis). A high importin concentration leads to a high fraction of the NR fraction being bound to importins, but only in models A and B. In model C active export of importins keeps the NR-importin complex concentration in the nucleus low. Cytoplasmic and nuclear compartment volume, resp. 1.55×10^{-12} L and 0.45×10^{-12} L (Riddick and Macara, 2007), and the total concentrations of NRL and RE were set to realistic values (1.31×10^{-12} nmoles of NRLs and 1.67×10^{-12} nmoles of Res, respectively, per cell). The rate constants for complex formation of NRs with importins and of NRs with REs were chosen as diffusion-limited ($k_{association}$ = 60 $nM^{-1}min^{-1}$; resp. K_d = 5 nM and K_d = 1 nM); nuclear import and nuclear export is described by mass action kinetics with Kappa = 5.4×10^{-12} [l/min]. Rate equations and additional parameters are given in Table 9 of Supplementary Information. Abbreviations: **NrL** – liganded NR (for example, GR); **Imp-** importins, **NrLImp-** liganded NR bound with importins; **Re -** RE for NR on DNA; **ReNrL-** RE on DNA bound with activated NR; **NPC** – nuclear pore complex; **Degr** – degraded NR.

The model is available in JWS Online and can be simulated in a web browser: http://jjj.biochem.sun.ac.za; http:// jjj.bio.vu.nl; http://jjj.mib.ac.uk (Snoep and Olivier, 2002; Olivier and Snoep, 2004). The model can be found via "author search", 'kolodkin7'. The model can be also accessed directly via: http://jjj.bio.vu.nl/webMathematica/Examples/run.jsp?modelName=kolodkin7 or at any of the other servers listed above. Please note that values of parameters for nuclear import/ export rates are set for reversible transport. Simulations of receptor (importin) pump require parameter values from Supplementary Table 9.

Fig. 2S

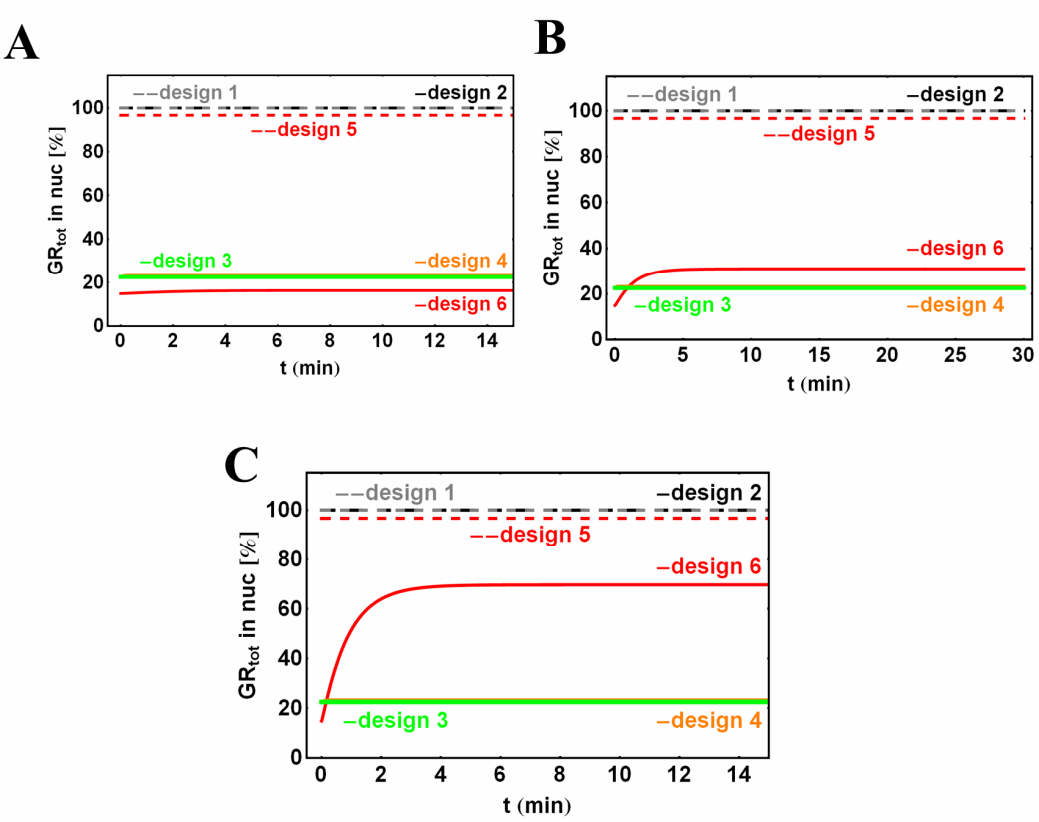


Fig. 2S. Time courses of the concentration of the total NR in the nucleus for the six network designs. Nuclear transport of liganded and unliganded NR in design 6 is fitted to GR case (model for Figure 2 of Supplementary Information). **(A)** Upon 0.005 nM of DEX addition. **(B)** Upon 0.1 nM of DEX addition. **(C)** Upon 1 nM of DEX addition. Rate equations and additional parameters are given in Table 10 of Supplementary Information.

Models can be found via the "author search", 'kolodkin'. Models can be also accessed directly via: http://jjj.bio.vu.nl/webMathematica/Examples/run.jsp?modelName=kolodkinX, with X ranging from 1 to 6 respectively for design 1 to design 6 (at each of the servers listed above). Note: Figure 2D cannot be reproduced with online simulations, which allow determining the net flux of ligand (as a sum of import and export fluxes) but not the time course of import flux alone. Please contact the authors for more details. Figure 2G can be reproduced by populating design 6 model with parameters from Supplementary Table 10.

Fig. 3S

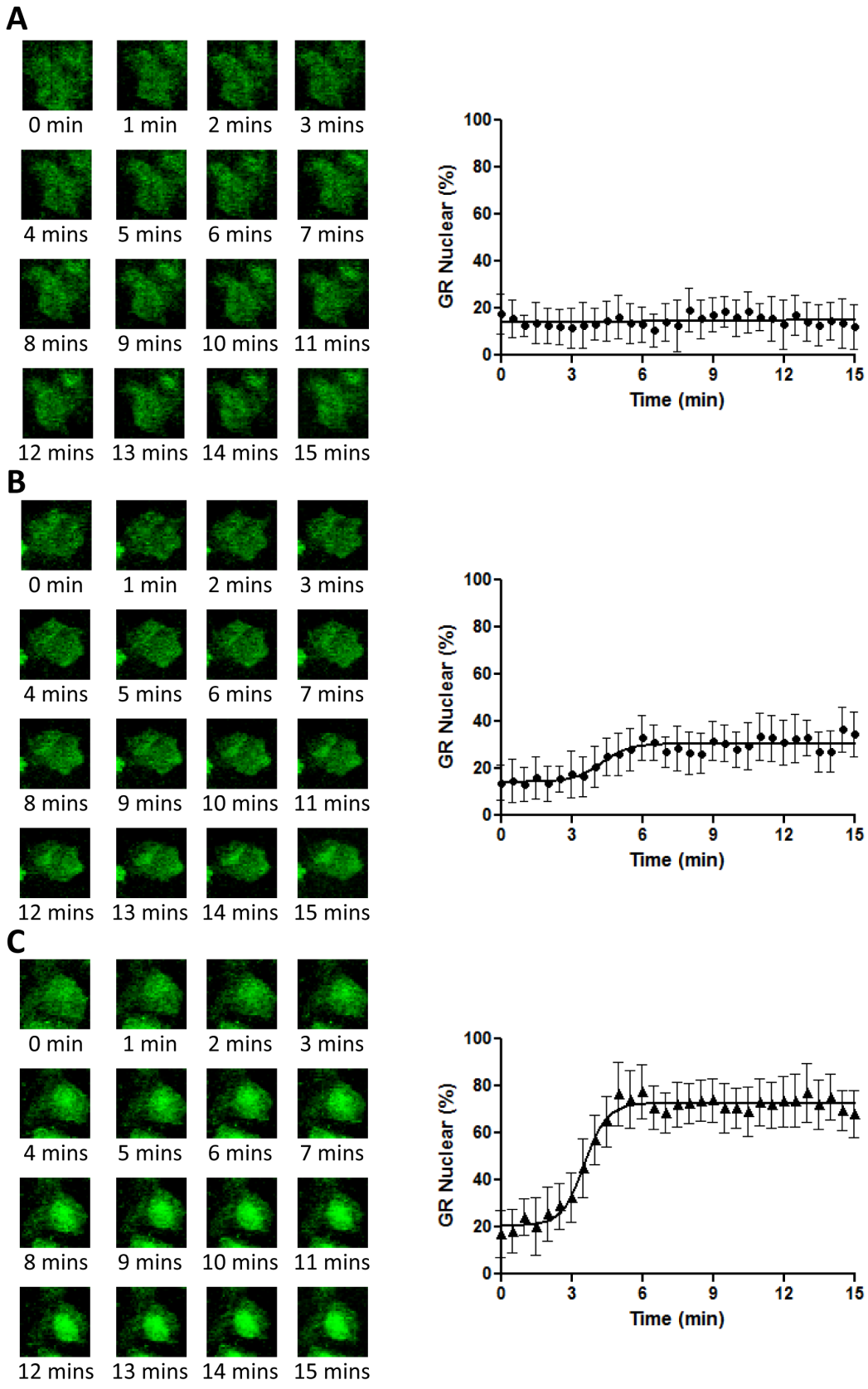

A
0 min
1 min
2 mins
3 mins
4 mins
5 mins
6 mins
7 mins
8 mins
9 mins
10 mins
11 mins
12 mins
13 mins
14 mins
15 mins
GR Nuclear (%)
Time (min)
B
C

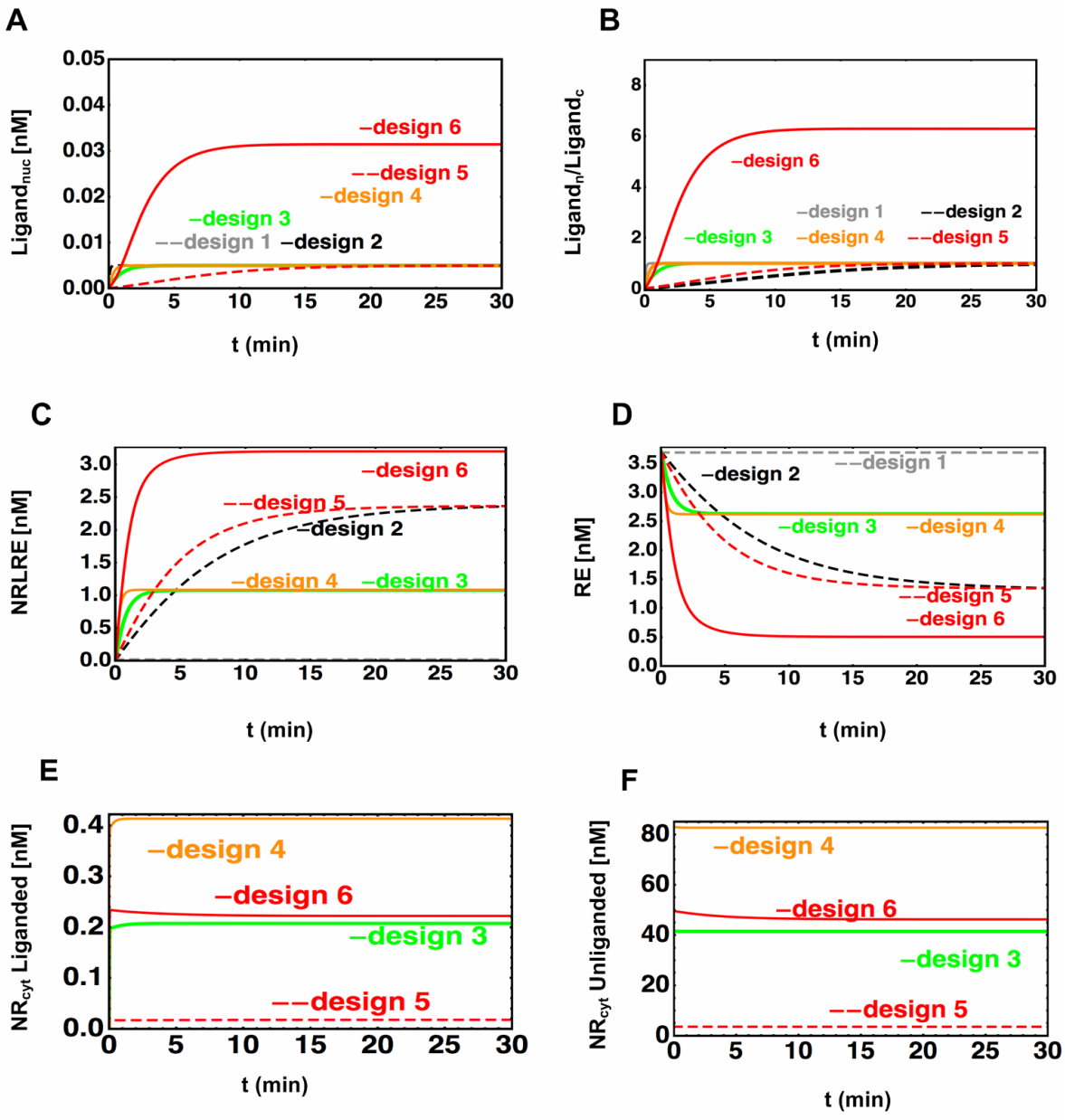
Fig. 3S. Nuclear translocation of GR-GFP following exposure to varying concentrations of dexamethasone. Huh7 were transiently transfected with an over-expression plasmid for GR-GFP and then exposed to **(A)** 0.005 nM **(B)** 0.1 nM or. **(C)** 1 nM of DEX. Nuclear:cytoplasmic localisation of GR is presented graphically in a representative cell, and as the average nuclear localisation over time for at least ten cells (error bars = SEM). Data is representative of three independent experiments.

Supplementary Methods

Huh7 cells, a human hepatoma cell line (a kind gift from Dr Steve Hood, GlaxoSmithKline), were seeded into 8-chambered microscope slides (BD Biosciences, Erembodegem, Belgium) at a concentration of 12,500 cells/well and incubated at 37ºC for 24 hrs in a humidified container for attachment. FuGENE 6-mediated DNA transfections, using 83ng DNA/chamber were performed as described previously [El-Sankary et al, 2001], using serum-free medium for the six-hour transfection period; this was then replaced with fresh, complete medium, containing charcoal-stripped serum, for the remaining culture period. Following 48 hours incubation, cells were visualized using a Zeiss Axiovert LSM510 microscope. Following addition of dexamethasone at the indicated concentration, cell fields were imaged in every 30s for 15 mins, using a 2.6µs pixel time. Total and nuclear fluorescence from at least ten cells for each treatment condition were analysed using ImageJ v1.43.

SIMULATION RESULTS FOR ALL SPECIES IN ALL MODELS

Fig. 4S



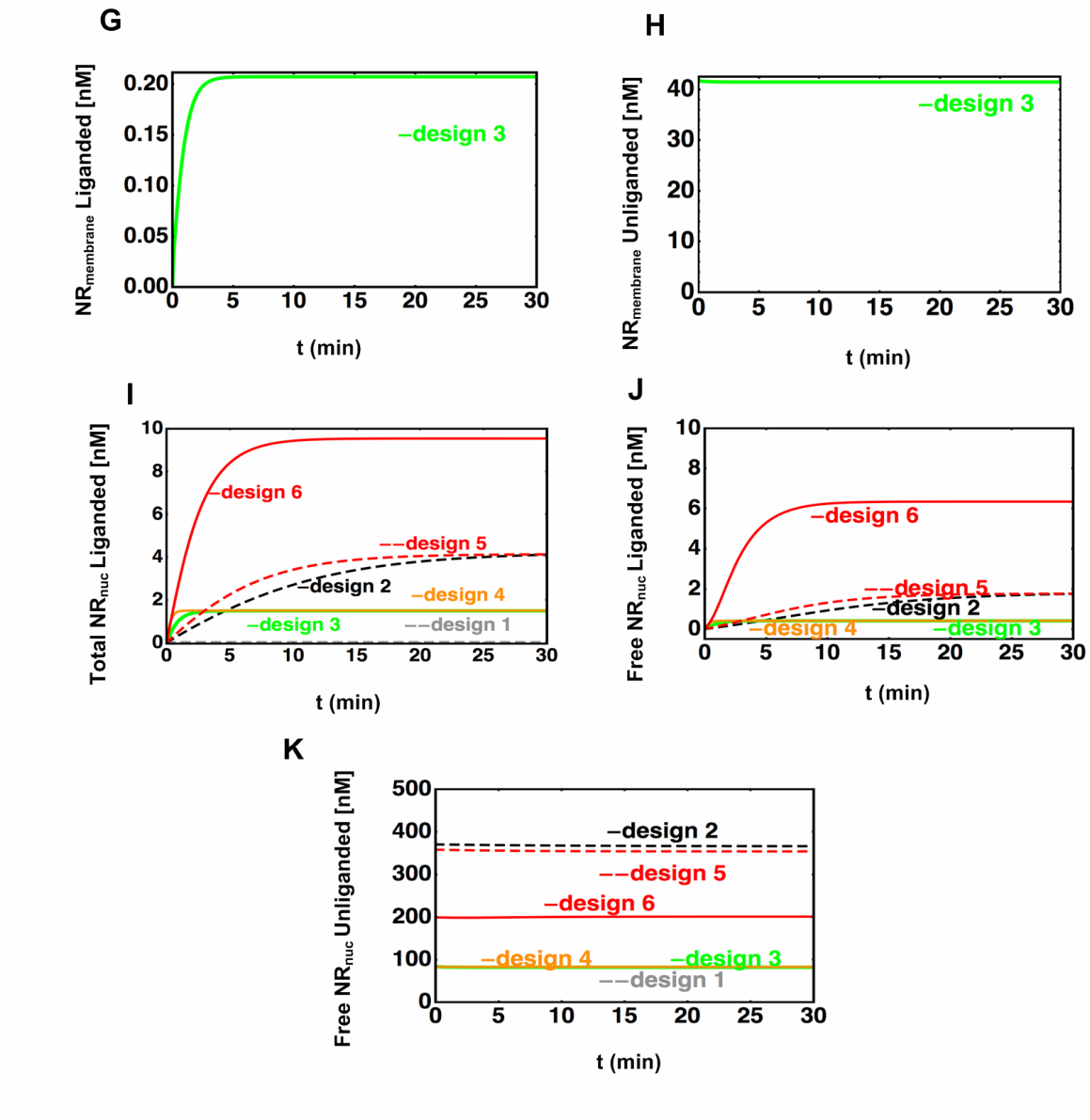


Fig. 4S. Simulation results for all species in all models of Figure 2

Fig. 5S

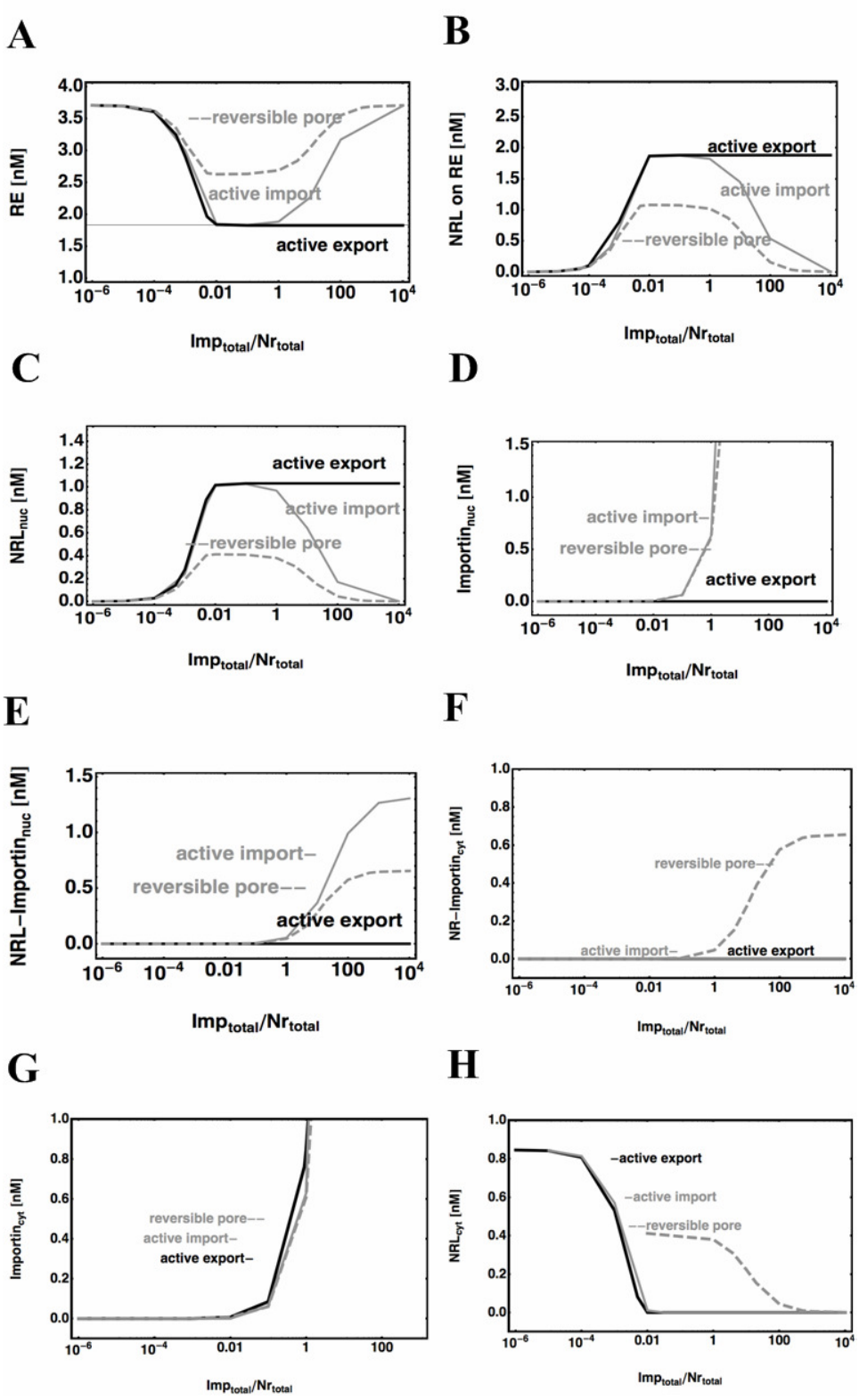


Fig. 5S. Simulation results for all species in all models of Figure 3

Fig. 6S

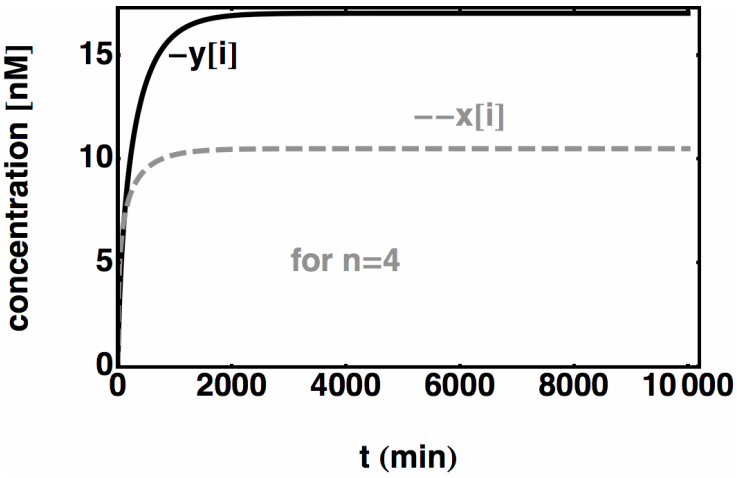


Fig. 6S. Simulation results for all species in all models of Figure 4 (x and y for n=4)

Tables (rate equations and parameters of models)

Table 1

Design 1: just the role of NR

Reactions		Parameters
v_1	Binding of nuclear ligand to NR tightly bound to the nuclear RE on the DNA: $\left(k_{1f} \cdot \mathrm{Re}\, Nr_n(t) \cdot L_n(t) - k_{1b} \cdot \mathrm{Re}\, NrL_n(t)\right) V_{nuc}$ [nmoles/min]	k_{1f}= 60 $nM^{-1}min^{-1}$; k_{1b}=60 min^{-1}
v_2	Ligand diffusion between cytosol and nucleus: $Kappa_2 \cdot \left(L_c - L_n(t)\right)$ [nmoles/min]	$Kappa_2$=*$\kappa_{Ligand\ diffusion}$=$32\times10^{-12}$ L/min; L_c = 0.005 nM
Balance equations		
$dL_n(t)/dt$	$(v_2-v_1)/V_{nuc}$ [nM/min]	
$dReNr_n(t)/dt$	$(-v_1)/V_{nuc}$ [nM/min]	
$dReNrL_n(t)/dt$	$(+v_1)/V_{nuc}$ [nM/min]	
Conserved Moieties		
$DNA_{total} \cdot V_{nuc}$	$\mathrm{Re}\, Nr_n(t) \cdot V_{nuc} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$DNA_{total} \cdot V_{nuc}$ *=1000*$10^9/N_A$ = 1.67×10^{-12} nmoles (10^3 molecules/cell)*
Initial conditions		
$\mathrm{Re}\, Nr_n(0)$	DNA_{total} [nM]	
$\mathrm{Re}\, NrL_n(0)$	0 [nM]	
$L_n(0)$	0 [nM]. The addition of ligand was modeled as the increase of its fixed concentration in the outer cellular membrane (L_c) from 0 to 0.005 nM (and maintained constant at the latter level), where the concentrations are quantified as the aqueous concentrations in the cytosol immediately adjacent to the plasma membrane; we shall assume a rapid equilibration of the ligand between this aqueous phase and the plasma-membrane phase. The concentration of ligand in the nucleus (L_n(t)) was treated as aqueous only, i.e. in terms of the total number of molecules divided by the volume of the nucleus.	
Volumes: V_{nuc}=0.45×10^{-12} l; V_{cyt}=1.55×10^{-12} l; V_{cell}=2×10^{-12} l; N_A=Avogadro's number=6.02×10^{23}		

We describe a spherical cell of radius 7.85 µm, with a spherical nucleus of radius 4.75 µm. Consequently, $Area_{nuc}$ (area of nuclear membrane) =280 μm^2=2.8·$10^{-8}dm^2$, Dist (distance between cytoplasmic and nuclear membrane)=3.1 µm=3.1·10^{-5}dm ; V_{nuc}=450 μm^3, $V_{cytoplasm}$=1575 μm^3, and V_{cell}=2025 μm^3.

$D_{Protein}$ =6·$10^{-9}dm^2$/min (diffusion coefficient for protein (Kholodenko et al, 2000a).
D_{Ligand}=36·$10^{-9}dm^2$/min (calculated from the Stokes-Einstein equation by comparing with $D_{Protein}$).

*$\kappa_{Ligand\ diffusion}$= $D_{Ligand} \times \frac{Area_{nuc}}{Dist}$ [litre/min= dm^3/min]=32.5pL/min=32.5·10^{-12} L/min.

Table 2

Design 2: NR functioning as NR only

Reactions		Parameters
v_1	Nuclear RE binding NRL: $\left(k_{1f} \cdot \mathrm{Re}_n(t) \cdot NrL_n(t) - k_{1b} \cdot \mathrm{Re}\, NrL_n(t)\right) V_{nuc}$ [nmoles/min]	k_{1f}= 60 $nM^{-1}min^{-1}$; k_{1b}=60 min^{-1}
v_2	NR binding nuclear ligand: $\left(k_{2f} \cdot Nr_n(t) \cdot L_n(t) - k_{2b} \cdot NrL_n(t)\right) V_{nuc}$ [nmoles/min]	k_{2f}= 60 $nM^{-1}min^{-1}$; k_{2b}=60 min^{-1}
v_3	Ligand diffusion between cytosol and nucleus: $Kappa_3 \cdot \left(L_c - L_n(t)\right)$ [nmoles/min]	$Kappa_3$=*$\kappa_{Ligand\ diffusion}$=$32\times10^{-12}$ l/min; L_c = 0.005 nM
Balance equations		
$dL_n(t)/dt$	$(v_3-v_2)/V_{nuc}$ [nM/min]	

dRe(t)/dt	$-v_1/V_{nuc}$ [nM/min]	
$dReNrL_n(t)/dt$	$+v_1/V_{nuc}$ [nM/min]	
dNr_n/dt	$-v_2/V_{nuc}$ [nM/min]	
dNrLn(t)	$(+v_2-v_1)/V_{nuc}$ [nM/min]	
Conserved Moieties		
$DNA_{total} \cdot V_{nuc}$	$\mathrm{Re}\, Nr_n(t) \cdot V_{nuc} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$DNA_{total} \cdot V_{nuc} = 1000*10^9/N_A = 1.67\times10^{-12}$ *nmoles (10^3 molecules/cell)*
$NR_{total} \cdot V_{nuc}$	$Nr_n(t) \cdot V_{nuc} + NrL_n(t) \cdot V_{nuc} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$NR_{total} \cdot V_{nuc} = 10^5*10^9/N_A = 167\times10^{-12}$ *nmoles (10^5 molecules/cell)*
Initial conditions		
$\mathrm{Re}\, Nr_n(0)$	DNA_{total} [nM]	
$\mathrm{Re}\, NrL_n(0)$	0 [nM]	
$Nr_n(0)$	NR_{total} [nM]	
$NrL_n(0)$	0 [nM]	
$L_n(0)$	0 [nM]. The addition of ligand was modeled as the increase of its fixed concentration in the outer cellular membrane (L_c) from 0 to 0.005 nM (and maintained constant at the latter level), where the concentrations are quantified as the aqueous concentrations in the cytosol immediately adjacent to the plasma membrane; we shall assume a rapid equilibration of the ligand between this aqueous phase and the plasma-membrane phase. The concentration of ligand in the nucleus ($L_n(t)$) was treated as aqueous only, i.e. in terms of the total number of molecules divided by the volume of the nucleus.	
Volumes: $V_{nuc}=0.45\times10^{-12}$ l; $V_{cyt}=1.55\times10^{-12}$ l; $V_{cell}=2\times10^{-12}$ L; N_A=Avogadro's number $=6.02\times10^{23}$		

We describe a spherical cell of radius 7.85 µm, with a spherical nucleus of radius 4.75 µm. Consequently, $Area_{nuc}$ (area of nuclear membrane) =280 $\mu m^2=2.8\cdot10^{-8} dm^2$, Dist (distance between cytoplasmic and nuclear membrane)=3.1 µm=$3.1\cdot10^{-5}$dm ; V_{nuc}=450 μm^3, $V_{cytoplasm}$=1575 μm^3, and V_{cell}=2025 μm^3.

$D_{Protein}$ =$6\cdot10^{-9} dm^2$/min (diffusion coefficient for protein (Kholodenko et al, 2000a).
D_{Ligand}=$36\cdot10^{-9} dm^2$/min (calculated from the Stokes-Einstein equation by comparing with $D_{Protein}$).

$^*\kappa_{\text{Ligand diffusion}} = D_{Ligand} \times \frac{Area_{nuc}}{Dist}$ [litre/min= dm^3/min]=32.5pL/min=$32.5\cdot10^{-12}$ L/min.

Table 3

Design 3: NR functioning both as NR in the nucleus and as cytosolic shuttling protein

	Reactions	Parameters
$\mathbf{v_1}$	Nuclear RE binding NRL: $\left(k_{1f} \cdot \mathrm{Re}_n(t) \cdot NrL_n(t) - k_{1b} \cdot \mathrm{Re}\, NrL_n(t)\right) V_{nuc}$ [nmoles/min]	k_{1f}= 60 $nM^{-1}min^{-1}$; k_{1b}=60 min^{-1}
$\mathbf{v_2}$	NR binding nuclear ligand in the nucleus: $\left(k_{2f} \cdot Nr_n(t) \cdot L_n(t) - k_{2b} \cdot NrL_n(t)\right) V_{nuc}$ [nmoles/min]	k_{2f}= 60 $nM^{-1}min^{-1}$; k_{2b}=60 min^{-1}
$\mathbf{v_3}$	Mass Action: NR near the nuclear membrane (but in the cytoplasm) binding ligand from the nucleus: $\left(k_{3f} \cdot Nr_m(t) \cdot L_n(t) - k_{3b} \cdot NrL_m(t)\right) \cdot V_{cyt}$ [nmoles/min]	k_{3f}= 60 $nM^{-1}min^{-1}$; k_{3b}=60 min^{-1}
$\mathbf{v_4}$	Mass Action: NR that sits near plasma membrane binds ligand from the plasma membrane. $\left(k_{4f} \cdot Nr_c(t) \cdot L_c - k_{4b} \cdot NrL_c(t)\right) \cdot V_{cyt}$ [nmoles/min]	k_{4f}= 60 $nM^{-1}min^{-1}$; k_{4b}=60 min^{-1}
$\mathbf{v_5}$	Passive diffusion of Nr between cytoplasma close to plasma membrane and cytoplasma close to nuclear membranes $Kappa_5 \cdot \left(Nr_c(t) - Nr_m(t)\right)$ [nmoles/min] Please note we modeled the diffusion as a single movement from	$Kappa_5$=$^{**}\kappa_{\text{Protein diffusion}}$=$5.4\times10^{-12}$ L/min

	close to the plasma membrane where the nuclear receptor was present at concentration Nr_c to a position close to the nuclear membrane where the nuclear receptor had a concentration Nr_m.	
v_6	Passive diffusion of NrL between plasma and nuclear membranes: $Kappa_6 \cdot (NrL_c(t) - NrL_m(t))$ [nmoles/min] Please note we modeled the diffusion as a single movement from close to the plasma membrane where the nuclear receptor was present at concentration NrL_c to a position close to the nuclear membrane where the nuclear receptor had a concentration NrL_m.	$Kappa_6 = **\kappa_{Protein\ diffusion} = 5.4 \times 10^{-12}$ l/min
v_7	Ligand diffusion between cytosol and nucleus: $Kappa_7 \cdot (L_c - L_n(t))$ [nmoles/min]	$Kappa_7 = *\kappa_{Ligand\ diffusion} = 32 \times 10^{-12}$ l/min; L_c = 0.005 nM
Balance equations		
$dL_n(t)/dt$	$(v_7 - v_3 - v_2)/V_{nuc}$ [nM/min]	
$dRe(t)/dt$	$-v_1/V_{nuc}$ [nM/min]	
$dReNrL_n(t)/dt$	$+v_1/V_{nuc}$ [nM/min]	
dNr_n/dt	$-v_2/V_{nuc}$ [nM/min]	
$dNrL_n(t)$	$(+v_2 - v_1)/V_{nuc}$ [nM/min]	
$dNrL_m(t)$	$(+v_3 + v_6)/V_{cyt}$ [nM/min]	
dNrLc(t)	$(+v_4 - v_6)/V_{cyt}$ [nM/min]	
dNr_c/dt	$(-v_5 - v_4)/V_{cyt}$ [nM/min]	
dNr_m/dt	$(+v_5 - v_3)/V_{cyt}$ [nM/min]	
Conserved Moieties		
$DNA_{total} \cdot V_{nuc}$	$\mathrm{Re}\, Nr_n(t) \cdot V_{nuc} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$DNA_{total} \cdot V_{nuc} = 1000 * 10^9 / N_A = 1.67 \times 10^{-12}$ *nmoles (10^3 molecules/cell)*
$NRC_{CytTotal} \cdot V_{cyt}$	$Nr_m(t) \cdot V_{cyt} + Nr_c(t) \cdot V_{cyt} + NrL_m(t) \cdot V_{cyt} + NrL_c(t) \cdot V_{cyt}$ *[nmoles/cell]*	$NR_{total} \cdot (V_{nuc} + V_{cyt}) = 10^5 * 10^9 / N_A = 167 \times 10^{-12}$ *nmoles (10^5 molecules/cell)*
$NR_{NucTotal} \cdot V_{nuc}$	$Nr_n(t) \cdot V_{nuc} + NrL_n(t) \cdot V_{nuc} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$NR_{total} \cdot (V_{nuc} + V_{cyt}) = 10^5 * 10^9 / N_A = 167 \times 10^{-12}$ *nmoles (10^5 molecules/cell)*
Initial conditions		
$\mathrm{Re}\, Nr_n(0)$	DNA_{total} [nM]	
$\mathrm{Re}\, NrL_n(0)$	0 [nM]	
$Nr_n(0)$	NR_{total} [nM]	
$NrL_n(0)$	0 [nM]	
$Nr_m(0)$	NR_{total} [nM]	
$NrL_m(0)$	0 [nM]	
$Nr_c(0)$	NR_{total} [nM]	
$NrL_c(0)$	0 [nM]	
$L_n(0)$	0 [nM]. The addition of ligand was modeled as the increase of its fixed concentration in the outer cellular membrane (L_c) from 0 to 0.005 nM (and maintained constant at the latter level), where the concentrations are quantified as the aqueous concentrations in the cytosol immediately adjacent to the plasma membrane; we shall assume a rapid equilibration of the ligand between this aqueous phase and the plasma-membrane phase. The concentration of ligand in the nucleus ($L_n(t)$) was treated as aqueous only, i.e. in terms of the total number of molecules divided by the volume of the nucleus.	
Volumes: $V_{nuc} = 0.45 \times 10^{-12}$ L; $V_{cyt} = 1.55 \times 10^{-12}$ L; $V_{cell} = 2 \times 10^{-12}$ L; N_A=Avogadro's number = 6.02×10^{23}		

We describe a spherical cell of radius 7.85 μm, with a spherical nucleus of radius 4.75 μm. Consequently, $Area_{nuc}$ (area of nuclear membrane) =280 $\mu m^2 = 2.8 \cdot 10^{-8} dm^2$, Dist (distance between cytoplasmic and nuclear membrane)=3.1 μm=$3.1 \cdot 10^{-5}$dm ; V_{nuc}=450 μm^3, $V_{cytoplasm}$=1575 μm^3, and V_{cell}=2025 μm^3.

$D_{Protein}$ =6·10^{-9}dm^2/min (diffusion coefficient for protein (Kholodenko et al, 2000a).
D_{Ligand}=36·10^{-9}dm^2/min (calculated from the Stokes-Einstein equation by comparing with $D_{Protein}$).

$^{*}\kappa_{Ligand\ diffusion} = D_{Ligand} \times \frac{Area_{nuc}}{Dist}$ [litre/min= dm^3/min]=32.5pL/min=32.5·10^{-12} L/min.

$^{**}\kappa_{Protein\ diffusion} = D_{Protein} \times \frac{Area_{nuc}}{Dist}$ [litre/min= dm^3/min]=5.4 pl/min=5.4·10^{-12} L/min.

Table 4

Design 4: NR functioning both as NR and as shuttle from plasma membrane all the way to the DNA

Reactions		**Parameters**
$\mathbf{v_1}$	Nuclear RE binding NRL: $\left(k_{1f} \cdot \mathrm{Re}_n(t) \cdot NrL_n(t) - k_{1b} \cdot \mathrm{Re}\, NrL_n(t)\right) \cdot V_{nuc}$ [nmoles/min]	k_{1f} = 60 nM^{-1}min^{-1}; k_{1b} = 60 min^{-1}
$\mathbf{v_2}$	NR binding nuclear ligand in the nucleus: $\left(k_{2f} \cdot Nr_n(t) \cdot L_n(t) - k_{2b} \cdot NrL_n(t)\right) \cdot V_{nuc}$ [nmoles/min]	k_{2f} = 60 nM^{-1}min^{-1}; k_{2b} = 60 min^{-1}
$\mathbf{v_3}$	Mass Action: NR that sits near plasma membrane binds ligand from the plasma membrane. $\left(k_{3f} \cdot Nr_c(t) \cdot L_n(t) - k_{3b} \cdot NrL_c(t)\right) \cdot V_{cyt}$ [nmoles/min]	k_{3f} = 60 nM^{-1}min^{-1}; k_{3b}= 60 min^{-1}
$\mathbf{v_4}$	Passive transport of NR across nuclear membrane: $Kappa_4 \cdot \left(Nr_c(t) - Nr_n(t)\right)$ [nmoles/min]	Kappa$_4$ = $^{**}\kappa_{Protein\ diffusion}$ = 5.4×10^{-12} L/min
$\mathbf{v_5}$	Passive transport of NRL across nuclear membrane: $Kappa_5 \cdot \left(NrL_c(t) - NrL_n(t)\right)$ [nmoles/min]	Kappa$_5$= $^{**}\kappa_{Protein\ diffusion}$= 5.4×10^{-12} L/min
$\mathbf{V_6}$	Ligand diffusion between cytosol and nucleus: $Kappa_6 \cdot \left(L_c - L_n(t)\right)$ [nmoles/min]	Kappa$_6$= $^{*}\kappa_{Ligand\ diffusion}$= 32×10^{-12} L/min; L_c = 0.005 nM
Balance equations		
$dL_n(t)/dt$	$(v_6-v_2)/V_{nuc}$ [nM/min]	
$dRe(t)/dt$	$-v_1/V_{nuc}$ [nM/min]	
$dReNrL_n(t)/dt$	$+v_1/V_{nuc}$ [nM/min]	
dNr_n/dt	$(+v_4-v_2)/V_{nuc}$ [nM/min]	
$dNrL_n(t)$	$(+v_2+v_5-v_1)/V_{nuc}$ [nM/min]	
dNr_c/dt	$(-v_3-v_4)/V_{cyt}$ [nM/min]	
$dNrL_c/dt$	$(+v_3-v_5)/V_{cyt}$ [nM/min]	
Conserved Moieties		
$DNA_{total} \cdot V_{nuc}$	$\mathrm{Re}\, Nr_n(t) \cdot V_{nuc} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$DNA_{total} \cdot V_{nuc}$ = = *1000*10^9/N_A = = 1.67×10^{-12} nmoles (10^3 molecules/cell)*
$NR_{total} \cdot (V_{nuc} + V_{cyt})$	$Nr_n(t) \cdot V_{nuc} + Nr_c(t) \cdot V_{cyt} + NrL_n(t) \cdot V_{nuc} + NrL_c(t) \cdot V_{cyt} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$NR_{total} \cdot (V_{nuc} + V_{cyt})$ =10^5*10^9/N_A = 167×10^{-12} nmoles (10^5 molecules/cell)*
Initial conditions		
$\mathrm{Re}\, Nr_n(0)$	DNA_{total} [nM]	
$\mathrm{Re}\, NrL_n(0)$	0 [nM]	
$Nr_n(0)$	$NR_{total} \cdot (V_{nuc} + V_{cyt}) \times \frac{k_{4f}}{k_{4f} \cdot V_{nuc} + k_{4b} \cdot V_{cyt}}$ [nM]	
$NrL_n(0)$	0 [nM]	

$Nr_c(0)$	$NR_{total}\cdot(V_{nuc}+V_{cyt})\times\frac{k_{4b}}{k_{4f}\cdot V_{nuc}+k_{4b}\cdot V_{cyt}}$ [nM]
$NrL_c(0)$	0 [nM].
$L_n(0)$	0 [nM]. The addition of ligand was modeled as the increase of its fixed concentration in the outer cellular membrane (L_c) from 0 to 0.005 nM (and maintained constant at the latter level), where the concentrations are quantified as the aqueous concentrations in the cytosol immediately adjacent to the plasma membrane; we shall assume a rapid equilibration of the ligand between this aqueous phase and the plasma-membrane phase. The concentration of ligand in the nucleus ($L_n(t)$) was treated as aqueous only, i.e. in terms of the total number of molecules divided by the volume of the nucleus.
Volumes: $V_{nuc}=0.45\times10^{-12}$ l; $V_{cyt}=1.55\times10^{-12}$ l; $V_{cell}=2\times10^{-12}$ l; N_A=Avogadro's number$=6.02\times10^{23}$	

We describe a spherical cell of radius 7.85 µm, with a spherical nucleus of radius 4.75 µm. Consequently, $Area_{nuc}$ (area of nuclear membrane) =280 $\mu m^2=2.8\cdot10^{-8}dm^2$, Dist (distance between cytoplasmic and nuclear membrane)=3.1 µm=$3.1\cdot10^{-5}$dm ; V_{nuc}=450 μm^3, $V_{cytoplasm}$=1575 μm^3, and V_{cell}=2025 μm^3.

$D_{Protein}=6\cdot10^{-9}dm^2$/min (diffusion coefficient for protein (Kholodenko et al, 2000a).
$D_{Ligand}=36\cdot10^{-9}dm^2$/min (calculated from the Stokes-Einstein equation by comparing with $D_{Protein}$).

$^{*}\kappa_{\text{Ligand diffusion}}=D_{Ligand}\times\frac{Area_{nuc}}{Dist}$ [litre/min= dm^3/min]=32.5pL/min=$32.5\cdot10^{-12}$ L/min.

$^{**}\kappa_{\text{Protein diffusion}}=D_{Protein}\times\frac{Area_{nuc}}{Dist}$ [litre/min= dm^3/min]=5.4 pl/min=$5.4\cdot10^{-12}$ L/min.

Table 5

Design 5: NR functioning as NR and imported actively from the cytoplasm

Reactions		Parameters
$\mathbf{v_1}$	Nuclear RE binding NRL: $\left(k_{1f}\cdot \mathrm{Re}_n(t)\cdot NrL_n(t)-k_{1b}\cdot \mathrm{Re}\, NrL_n(t)\right)\cdot V_{nuc}$ [nmoles/min]	k_{1f}= 60 $nM^{-1}min^{-1}$; k_{1b}=60 min^{-1}
$\mathbf{v_2}$	NR binding nuclear ligand: $\left(k_{2f}\cdot Nr_n(t)\cdot L_n(t)-k_{2b}\cdot NrL_n(t)\right)\cdot V_{nuc}$ [nmoles/min]	k_{2f}= 60 $nM^{-1}min^{-1}$; k_{2b}=60 min^{-1}
$\mathbf{v_3}$	Mass Action: NR that sits near plasma membrane binds ligand from the plasma membrane. $\left(k_{3f}\cdot Nr_c(t)\cdot L_n(t)-k_{3b}\cdot NrL_c(t)\right)\cdot V_{cyt}$ [nmoles/min]	k_{3f}= 60 $nM^{-1}min^{-1}$; k_{3b}=60 min^{-1}
$\mathbf{v_4}$	Active transports of NR across nuclear membrane: $Kappa_{4importin}\cdot Nr_c(t)-Kappa_{4exportin}\cdot Nr_n(t)$ [nmoles/min]	$Kappa_{4importin}=5.4\times10^{-12}$ L/min; $Kappa_{4exportin}=0.054\times10^{-12}$ L/min
$\mathbf{v_5}$	Active transports of NRL across nuclear membrane: $Kappa_{5importin}\cdot NrL_c(t)-Kappa_{5exportin}\cdot NrL_n(t)$ [nmoles/min]	$Kapp_{5importinf}=5.4\times10^{-12}$ L/min; $Kappa_{5exportin}=0.054\times10^{-12}$ L/min
$\mathbf{V_6}$	Ligand diffusion between cytosol and nucleus: $Kappa_6\cdot\left(L_c-L_n(t)\right)$ [nmoles/min]	$Kappa_6=^{*}\kappa_{\text{Ligand diffusion}}=32\times10^{-12}$ L/min; L_c = 0.005 nM
Balance equations		
$dL_n(t)/dt$	$(v_6-v_2)/V_{nuc}$ [nM/min]	
$dRe(t)/dt$	$-v_1/V_{nuc}$ [nM/min]	
$dReNrL_n(t)/dt$	$+v_1/V_{nuc}$ [nM/min]	
dNr_n/dt	$(+v_4-v_2)/V_{nuc}$ [nM/min]	
$dNrL_n(t)$	$(+v_2+v_5-v_1)/V_{nuc}$ [nM/min]	
dNr_c/dt	$(-v_3-v_4)/V_{cyt}$ [nM/min]	
$dNrL_c/dt$	$(+v_3-v_5)/V_{cyt}$ [nM/min]	
Conserved Moieties		

$DNA_{total} \cdot V_{nuc}$	$\mathrm{Re}\, Nr_n(t) \cdot V_{nuc} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$DNA_{total} \cdot V_{nuc}$ *=1000*10^9/N_A = 1.67×10^{-12} nmoles (10^3 molecules/cell)*
$NR_{total} \cdot (V_{nuc} + V_{cyt})$	$Nr_n(t) \cdot V_{nuc} + Nr_c(t) \cdot V_{cyt} + NrL_n(t) \cdot V_{nuc}$ $+ NrL_c(t) \cdot V_{cyt} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$NR_{total} \cdot (V_{nuc} + V_{cyt})$ *=10^5*10^9/N_A = 167×10^{-12} nmoles (10^5 molecules/cell)*
Initial conditions		
$\mathrm{Re}\, Nr_n(0)$	DNA_{total} [nM]	
$\mathrm{Re}\, NrL_n(0)$	0 [nM]	
$Nr_n(0)$	$NR_{total} \cdot (V_{nuc} + V_{cyt}) \times \frac{k_{4f}}{k_{4f} \cdot V_{nuc} + k_{4b} \cdot V_{cyt}}$ [nM]	
$NrL_n(0)$	0 [nM]	
$Nr_c(0)$	$NR_{total} \cdot (V_{nuc} + V_{cyt}) \times \frac{k_{4b}}{k_{4f} \cdot V_{nuc} + k_{4b} \cdot V_{cyt}}$ [nM]	
$NrL_c(0)$	0 [nM]	
$L_n(0)$	0 [nM]. The addition of ligand was modeled as the increase of its fixed concentration in the outer cellular membrane (L_c) from 0 to 0.005 nM (and maintained constant at the latter level), where the concentrations are quantified as the aqueous concentrations in the cytosol immediately adjacent to the plasma membrane; we shall assume a rapid equilibration of the ligand between this aqueous phase and the plasma-membrane phase. The concentration of ligand in the nucleus ($L_n(t)$) was treated as aqueous only, i.e. in terms of the total number of molecules divided by the volume of the nucleus.	
Volumes: V_{nuc}=0.45×10^{-12} l; V_{cyt}=1.55×10^{-12} l; V_{cell}=2×10^{-12} L; N_A=Avogadro's number=6.02×10^{23}		

We describe a spherical cell of radius 7.85 μm, with a spherical nucleus of radius 4.75 μm. Consequently, $Area_{nuc}$ (area of nuclear membrane) =280 μm^2=2.8·10^{-8}dm^2, Dist (distance between cytoplasmic and nuclear membrane)=3.1 μm=3.1·10^{-5}dm ; V_{nuc}=450 μm^3, $V_{cytoplasm}$=1575 μm^3, and V_{cell}=2025 μm^3.

$D_{Protein}$ =6·10^{-9}dm^2/min (diffusion coefficient for protein (Kholodenko et al, 2000a).
D_{Ligand}=36·10^{-9}dm^2/min (calculated from the Stokes-Einstein equation by comparing with $D_{Protein}$).

$^*\kappa_{\text{Ligand diffusion}} = D_{Ligand} \times \frac{Area_{nuc}}{Dist}$ [litre/min= dm^3/min]=32.5pL/min=32.5·10^{-12} L/min.

Table 6

Design 6: NR functioning as NR and preferential active import of NRL into the nucleus

	Reactions	Parameters
$\mathbf{v_1}$	Nuclear RE binding NRL: $\left(k_{1f} \cdot \mathrm{Re}_n(t) \cdot NrL_n(t) - k_{1b} \cdot \mathrm{Re}\, NrL_n(t)\right) \cdot V_{nuc}$ [nmoles/min]	k_{1f}= 60 nM^{-1}min^{-1}; k_{1b}=60 min^{-1}
$\mathbf{v_2}$	NR binding nuclear ligand: $\left(k_{2f} \cdot Nr_n(t) \cdot L_n(t) - k_{2b} \cdot NrL_n(t)\right) \cdot V_{nuc}$ [nmoles/min]	k_{2f}= 60 nM^{-1}min^{-1}; k_{2b}=60 min^{-1}
$\mathbf{v_3}$	Mass Action: NR that sits near plasma membrane binds ligand from the plasma membrane. $\left(k_{3f} \cdot Nr_c(t) \cdot L_n(t) - k_{3b} \cdot NrL_c(t)\right) \cdot V_{cyt}$ [nmoles/min]	k_{3f}= 60 nM^{-1}min^{-1}; k_{3b}=60 min^{-1}
$\mathbf{v_4}$	Active transports of NR across nuclear membrane: $Kappa_{4importin} \cdot Nr_c(t) - Kappa_{4\exp ortin} \cdot Nr_n(t)$ [nmoles/min]	$Kappa_{4f}$=0.216×10^{-12} L/min; $Kappa_{4b}$=0.054×10^{-12} L/min
$\mathbf{v_5}$	Active transports of NRL across nuclear membrane:	$Kapp_{5umportin}$ =5.4×10^{-12} L/min;

	$Kappa_{5importin} \cdot NrL_c(t) - Kappa_{5exportin} \cdot NrL_n(t)$ [nmoles/min]	$Kappa_{5exporin}=0.054\times10^{-12}$ L/min
V_6	Ligand diffusion between cytosol and nucleus: $Kappa_6 \cdot (L_c - L_n(t))$ [nmoles/min]	$Kappa_6=*\kappa_{Ligand\ diffusion}=32\times10^{-12}$ L/min; L_c = 0.005 nM
Balance equations		
$dL_n(t)/dt$	$(v_6-v_2)/V_{nuc}$ [nM/min]	
$dRe(t)/dt$	$-v_1/V_{nuc}$ [nM/min]	
$dReNrL_n(t)/dt$	$+v_1/V_{nuc}$ [nM/min]	
dNr_n/dt	$(+v_4-v_2)/V_{nuc}$ [nM/min]	
$dNrL_n(t)$	$(+v_2+v_5-v_1)/V_{nuc}$ [nM/min]	
dNr_c/dt	$(-v_3-v_4)/V_{cyt}$ [nM/min]	
$dNrL_c/dt$	$(+v_3-v_5)/V_{cyt}$ [nM/min]	
Conserved Moieties		
$DNA_{total} \cdot V_{nuc}$	$\mathrm{Re}\, Nr_n(t) \cdot V_{nuc} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$DNA_{total} \cdot V_{nuc} = 1000*10^9/N_A = 1.67\times10^{-12}$ *nmoles (10^3 molecules/cell)*
$NR_{total} \cdot (V_{nuc} + V_{cyt})$	$Nr_n(t) \cdot V_{nuc} + Nr_c(t) \cdot V_{cyt} + NrL_n(t) \cdot V_{nuc} + NrL_c(t) \cdot V_{cyt} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$NR_{total} \cdot (V_{nuc} + V_{cyt}) = 10^5*10^9/N_A = 167\times10^{-12}$ *nmoles (10^5 molecules/cell)*
Initial conditions		
$\mathrm{Re}\, Nr_n(0)$	DNA_{total} [nM]	
$\mathrm{Re}\, NrL_n(0)$	0 [nM]	
$Nr_n(0)$	$NR_{total} \cdot (V_{nuc} + V_{cyt}) \times \frac{k_{4f}}{k_{4f} \cdot V_{nuc} + k_{4b} \cdot V_{cyt}}$ [nM]	
$NrL_n(0)$	0 [nM]	
$Nr_c(0)$	$NR_{total} \cdot (V_{nuc} + V_{cyt}) \times \frac{k_{4b}}{k_{4f} \cdot V_{nuc} + k_{4b} \cdot V_{cyt}}$ [nM]	
$NrL_c(0)$	0 [nM]	
$L_n(0)$	0 [nM]. The addition of ligand was modeled as the increase of its fixed concentration in the outer cellular membrane (L_c) from 0 to 0.005 nM (and maintained constant at the latter level), where the concentrations are quantified as the aqueous concentrations in the cytosol immediately adjacent to the plasma membrane; we shall assume a rapid equilibration of the ligand between this aqueous phase and the plasma-membrane phase. The concentration of ligand in the nucleus ($L_n(t)$) was treated as aqueous only, i.e. in terms of the total number of molecules divided by the volume of the nucleus.	
Volumes: $V_{nuc}=0.45\times10^{-12}$ l; $V_{cyt}=1.55\times10^{-12}$ l; $V_{cell}=2\times10^{-12}$ L; N_A=Avogadro's number$=6.02\times10^{23}$		
*****Different regimes of predominant localization and shuttling rate of Nr were used for Fig. 2G:** nuclear, high shuttling (red solid line): $Kappa_{4f}=5.4\times10^{-10}$ l/min; $Kappa_{4b}=5.4\times10^{-12}$ l/min; $Kapp_{5f}=2.7\times10^{-8}$ l/min; $Kappa_{5b}=5.4\times10^{-12}$ l/min nuclear, low shuttling (red dashed line): $Kappa_{4f}=5.4\times10^{-14}$ l/min; $Kappa_{4b}=5.4\times10^{-16}$ l/min; $Kapp_{5f}=2.7\times10^{-12}$ l/min; $Kappa_{5b}=5.4\times10^{-16}$ l/min mixed, high shuttling (black solid line): $Kappa_{4f}=5.4\times10^{-12}$ l/min; $Kappa_{4b}=5.4\times10^{-12}$ l/min; $Kapp_{5f}=2.7\times10^{-10}$ l/min; $Kappa_{5b}=5.4\times10^{-12}$ l/min mixed, low shuttling (black dashed line): $Kappa_{4f}=5.4\times10^{-16}$ l/min; $Kappa_{4b}=5.4\times10^{-16}$ l/min; $Kapp_{5f}=2.7\times10^{-14}$ l/min; $Kappa_{5b}=5.4\times10^{-16}$ l/min cytoplasmic, high shuttling (blue solid line): $Kappa_{4f}=5.4\times10^{-12}$ l/min; $Kappa_{4b}=5.4\times10^{-10}$ l/min; $Kapp_{5f}=2.7\times10^{-10}$ l/min; $Kappa_{5b}=5.4\times10^{-10}$ l/min cytoplasmic, low shuttling (blue dashed line):		

$Kappa_{4f}=5.4\times10^{-16}$ l/min; $Kappa_{4b}=5.4\times10^{-14}$ l/min; $Kapp_{5f}=2.7\times10^{-14}$ l/min; $Kappa_{5b}=5.4\times10^{-14}$ l/min

We describe a spherical cell of radius 7.85 µm, with a spherical nucleus of radius 4.75 µm. Consequently, $Area_{nuc}$ (area of nuclear membrane) =280 $\mu m^2=2.8\cdot10^{-8}dm^2$, Dist (distance between cytoplasmic and nuclear membrane)=3.1 µm=$3.1\cdot10^{-5}$dm ; V_{nuc}=450 μm^3, $V_{cytoplasm}$=1575 μm^3, and V_{cell}=2025 μm^3.

$D_{Protein}$ =$6\cdot10^{-9}dm^2$/min (diffusion coefficient for protein (Kholodenko et al, 2000a).
D_{Ligand}=$36\cdot10^{-9}dm^2$/min (calculated from the Stokes-Einstein equation by comparing with $D_{Protein}$).

$^*\kappa_{Ligand\ diffusion}= D_{Ligand} \times \frac{Area_{nuc}}{Dist}$ [litre/min= dm^3/min]=32.5pL/min=$32.5\cdot10^{-12}$ L/min.

Table 7

Model for Fig. 3

Reactions		**Parameters**
$\mathbf{v_1}$	NRL binding importins in the cytoplasm: $(k_{1f}\cdot NrL_c(t)\cdot IMP_c(t)-k_{1b}\cdot NrLIMP_c(t))\cdot V_{cyt}$ [nmoles/min]	k_{1f}= 60 $nM^{-1}min^{-1}$; k_{1b}=300 min^{-1}
$\mathbf{v_2}$	Nuclear transport of importins complexed with NR: $Kappa_{2f}\cdot NrLIMP_c - Kappa_{2b}\cdot NrLIMP_n$ [nmoles/min]	reversible pore (black dashed line): $Kappa_{2f}=5.4\times10^{-12}$ l/min; $Kappa_{2b}=5.4\times10^{-12}$ l/min active import of NrLImp complex (grey solid line): $Kappa_{2f}=5.4\times10^{-12}$ l/min; $Kappa_{2b}$=0 L/min active export of IMP (black solid line): $Kappa_{2f}=5.4\times10^{-12}$ l/min; $Kappa_{2b}=5.4\times10^{-12}$ l/min
$\mathbf{v_3}$	Nuclear transport of importins: $Kappa_{3f}\cdot IMP_c(t)-Kappa_{3b}\cdot IMP_n(t)$ [nmoles/min]	reversible pore (black dashed line): $Kappa_{3f}=5.4\times10^{-12}$ l/min; $Kappa_{3b}=5.4\times10^{-12}$ l/min active import of NrLImp complex (grey solid line): $Kappa_{3f}=5.4\times10^{-12}$ l/min; $Kappa_{3b}=5.4\times10^{-12}$ l/min active export of IMP (black solid line): $Kappa_{3f}$=0 L/min; $Kappa_{3b}=5.4\times10^{-12}$ l/min
$\mathbf{v_4}$	NRL binding importins in the nucleus: $(k_{4f}\cdot NrL_n(t)\cdot IMP_n-k_{4b}\cdot NrLIMP_n(t))\cdot V_{nuc}$ [nmoles/min]	k_{4f}=60 $nM^{-1}min^{-1}$; k_{4f}=300 min^{-1}
$\mathbf{v_5}$	Nuclear RE binding NRL: $(k_{5f}\cdot NrL_n(t)\cdot \mathrm{Re}_n(t)-k_{5b}\cdot \mathrm{Re}\,NrL_n(t))\cdot V_{nuc}$ [nmoles/min]	k_{5f}=60 $nM^{-1}min^{-1}$; k_{5f}=60 min^{-1}
Balance equations		
$dNrL_c(t)/dt$	$-v_1/V_{cyt}$ [nM/min]	
$dImp_c(t)/dt$	$(-v_1-v_3)/V_{cyt}$ [nM/min]	
$dImpNrL_c(t)/dt$	$(+v_1-v_2)/V_{cyt}$ [nM/min]	
$dNrL_n(t)/dt$	$(+v_4-v_5)/V_{nuc}$ [nM/min]	
$dImp_n(t)/dt$	$(+v_3-v_4)/V_{nuc}$ [nM/min]	
$dImpNrL_n(t)/dt$	$(+v_2+v_4)/V_{nuc}$ [nM/min]	

dRe_n/dt	$-v_5/V_{nuc}$ [nM/min]	
$dReNrL_n/dt$	$+v_5/V_{nuc}$ [nM/min]	
Conserved Moieties		
$DNA_{total} \cdot V_{nuc}$	$\mathrm{Re}\, NrL_n(t) \cdot V_{nuc} + \mathrm{Re}_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$DNA_{total} \cdot V_{nuc} = 1000*10^9/N_A =$ *1.67×10^{-12} nmoles (10^3 molecules/cell)*
$NR_{total} \cdot (V_{nuc} + V_{cyt})$	$NrL_n(t) \cdot V_{nuc} + NrL_c(t) \cdot V_{cyt} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ $+ NrLIMP_c(t) \cdot V_{cyt} + NrLIMP_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$NR_{total} \cdot (V_{nuc} + V_{cyt}) =$ *1.31×10^{-12} nmoles*
$IMP_{total} \cdot (V_{nuc} + V_{cyt})$	$NrLIMP_n(t) \cdot V_{nuc} + NrLIMP_c(t) \cdot V_{cyt}$ $+ IMP_c(t) \cdot V_{cyt} + IMP_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	variable
	Initial conditions	
DNA (0)	DNA_{total} [nM]	
$NrL_c(0)$	$\frac{NR_{total} \cdot (V_{nuc} + V_{cyt})}{V_{cyt}}$ [nM]	
$Imp_c(0)$	$IMP_{total} \cdot (V_{nuc} + V_{cyt}) \times \frac{k_{3b}}{k_{3f} \cdot V_{nuc} + k_{3b} \cdot V_{cyt}}$ [nM]	
$ImpNrL_c(0)$	0 [nM]	
$NrL_n(0)$	0 [nM]	
$Imp_n(0)$	$IMP_{total} \cdot (V_{nuc} + V_{cyt}) \times \frac{k_{3f}}{k_{3f} \cdot V_{nuc} + k_{3b} \cdot V_{cyt}}$ [nM]	
$ImpNrL_n(0)$	0 [nM]	
Volumes: $V_{nuc}=0.45\times10^{-12}$ l; $V_{cyt}=1.55\times10^{-12}$ l; $V_{cell}=2\times10^{-12}$ l; N_A=Avogadro's number=6.02×10^{23}		
Various regimes of Nr nucleo-cytoplasmic transport (reactions 2 and 3): (Fig. 3 A) reversible pore (black dashed line): $Kappa_{2f}=5.4\times10^{-12}$ l/min; $Kappa_{2b}=5.4\times10^{-12}$ l/min; $Kappa_{3f}=5.4\times10^{-12}$ l/min; $Kappa_{3b}=5.4\times10^{-12}$ l/min (Fig. 3 B) active import of NrLImp complex (grey solid line): $Kappa_{2f}=5.4\times10^{-12}$ l/min; $Kappa_{2b}=0$ l/min; $Kappa_{3f}=5.4\times10^{-12}$ l/min; $Kappa_{3b}=5.4\times10^{-12}$ l/min (Fig. 3 C) active export of IMP (black solid line): $Kappa_{2f}=5.4\times10^{-12}$ l/min; $Kappa_{2b}=5.4\times10^{-12}$ l/min; $Kappa_{3f}=0$ l/min; $Kappa_{3b}=5.4\times10^{-12}$ l/min		

Table 8

Model for Fig. 4

Reactions		Parameters
$\mathbf{v_{1i}}$	Product inhibition (The S/Ks term in the denominator is neglected) $\frac{V_{MAX_1}' \cdot \frac{s}{K_s'}}{1 + \frac{x_i(t)}{K_{1x}} + \frac{s}{K_s'}}$ [nM/min]which was approximated by:	$V_{MAX_1} = 500$ nM min^{-1}; $K_s = 50$ $K_{1x} = 0.1$ nM; $s = 1$ nM;

	$\frac{V_{MAX_1} \cdot \frac{s}{K_s}}{1+\frac{x_i(t)}{K_{1x}}}$	
$\mathbf{v_{2i}}$	$\frac{V_{MAX_2} \cdot \frac{x_i(t)}{K_{2x}}}{1+\sum_{k=1}^{n}\frac{x_i(t)}{K_{2x}}+\sum_{k=1}^{n}\frac{y_i(t)}{K_{2y}}}$ [nM/min]	$V_{MAX_2}=1$ nM min^{-1}; $K_{2x}=1$ nM; $K_{2y}=1$ nM
$\mathbf{v_{3i}}$	$\frac{V_{MAX_3} \cdot \frac{y_i(t)}{K_{3y}}}{1+\frac{y_i(t)}{K_{3y}}}$ [nM/min]	$V_{MAX_3}=0.1$ nM min^{-1}; $K_{3x}=1$ nM; $K_{3y}=1$ nM
Balance equations		
ds(t)/dt	0 (considered an infinite reservoir)	
$dx_i(t)/dt$	$(+v_{1i}-v_{2i})$ [nM/min]	
$dy_i(t)/dt$	$(+v_{2i}-v_{3i})$ [nM/min]	
Initial conditions		
s(0)	1 [nM]	
$x_i(0)/dt$	0 [nM]	
$y_i(0)/dt$	0[nM]	

Table 9

Model for Figure 1 of Supplementary Information

Reactions		Parameters
$\mathbf{v_1}$	NRL binding importins in the cytoplasm: $(k_{1f} \cdot NrL_c(t) \cdot IMP_c(t) - k_{1b} \cdot NrLIMP_c(t)) \cdot V_{cyt}$ [nmoles/min]	k_{1f} = 60 $nM^{-1}min^{-1}$; k_{1b} = 300 min^{-1}
$\mathbf{v_2}$	Nuclear transport of importins complexed with NR: $Kappa_{2f} \cdot NrLIMP_c - Kappa_{2b} \cdot NrLIMP_n$ [nmoles/min]	reversible pore (black dashed line): $Kappa_{2f} = 5.4 \times 10^{-12}$ l/min; $Kappa_{2b} = 5.4 \times 10^{-12}$ l/min active import of NrLImp complex (grey solid line): $Kappa_{2f} = 5.4 \times 10^{-12}$ l/min; $Kappa_{2b}$ = 0 L/min active export of IMP (black solid line): $Kappa_{2f} = 5.4 \times 10^{-12}$ l/min; $Kappa_{2b} = 5.4 \times 10^{-12}$ l/min
$\mathbf{v_3}$	Nuclear transport of importins: $Kappa_{3f} \cdot IMP_c(t) - Kappa_{3b} \cdot IMP_n(t)$ [nmoles/min]	reversible pore (black dashed line): $Kappa_{3f} = 5.4 \times 10^{-12}$ l/min; $Kappa_{3b} = 5.4 \times 10^{-12}$ l/min active import of NrLImp complex (grey solid line): $Kappa_{3f} = 5.4 \times 10^{-12}$ l/min; $Kappa_{3b} = 5.4 \times 10^{-12}$ l/min active export of IMP (black solid line): $Kappa_{3f}$ = 0 l/min; $Kappa_{3b} = 5.4 \times 10^{-12}$ l/min
$\mathbf{v_4}$	NRL binding importins in the nucleus: $(k_{4f} \cdot NrL_n(t) \cdot IMP_n - k_{4b} \cdot NrLIMP_n(t)) \cdot V_{nuc}$ [nmoles/min]	k_{4f}= 60 $nM^{-1}min^{-1}$; k_{4f} = 300 min^{-1}
$\mathbf{v_5}$	Nuclear RE binding NRL: $(k_{5f} \cdot NrL_n(t) \cdot \mathrm{Re}_n(t) - k_{5b} \cdot \mathrm{Re}\,NrL_n(t)) \cdot V_{nuc}$ [nmoles/min]	k_{5f} = 60 $nM^{-1}min^{-1}$; k_{5f} = 60 min^{-1}
$\mathbf{v_6}$	Degradation of NR after being bound with RE (RE): $(k_{6f} \cdot \mathrm{Re}\,NrL_n(t)) \cdot V_{nuc}$ [nmoles/min]	K_{6f} = 0.1 min^{-1}
Balance equations		
$dNrL_c(t)/dt$	$-v_1/V_{cyt}$ [nM/min]	
$dImp_c(t)/dt$	$(-v_1-v_3)/V_{cyt}$ [nM/min]	
$dImpNrL_c(t)/dt$	$(+v_1-v_2)/V_{cyt}$ [nM/min]	
$dNrL_n(t)/dt$	$(+v_4-v_5)/V_{nuc}$ [nM/min]	
$dImp_n(t)/dt$	$(+v_3-v_4)/V_{nuc}$ [nM/min]	
$dImpNrL_n(t)/dt$	$(+v_2+v_4)/V_{nuc}$ [nM/min]	
dRe_n/dt	$(-v_5+v_6)/V_{nuc}$ [nM/min]	
$dReNrL_n/dt$	$(+v_5-v_6)/V_{nuc}$ [nM/min]	
$dDegr_n/dt$	$+v_6/V_{nuc}$ [nM/min]	
Conserved Moieties		
$DNA_{total} \cdot V_{nuc}$	$\mathrm{Re}\,NrL_n(t) \cdot V_{nuc} + \mathrm{Re}_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$DNA_{total} \cdot V_{nuc}$ *=1000*10^9/N_A = 1.67×10^-12 nmoles (10^3 molecules/cell)*
$NR_{total} \cdot (V_{nuc} + V_{cyt})$	$NrL_n(t) \cdot V_{nuc} + NrL_c(t) \cdot V_{cyt} + \mathrm{Re}\,NrL_n(t) \cdot V_{nuc} + NrLIMP_c(t) \cdot V_{cyt} + NrLIMP_n(t) \cdot V_{nuc} + Degr_n$	$NR_{total} \cdot (V_{nuc} + V_{cyt})$ = *1.31×10^-12 nmoles*

	[nmoles/cell]	
$IMP_{total} \cdot (V_{nuc} + V_{cyt})$	$NrLIMP_n(t) \cdot V_{nuc} + NrLIMP_c(t) \cdot V_{cyt} + IMP_c(t) \cdot V_{cyt} + IMP_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	Variable; *For Fig. 1S D:* $IMP_{total} \cdot (V_{nuc} + V_{cyt}) = 0.1 \times 10^{-12}$ *nmoles*
	Initial conditions	
DNA (0)	DNA_{total} [nM]	
$NrL_c(0)$	$\frac{NR_{total} \cdot (V_{nuc} + V_{cyt})}{V_{cyt}}$ [nM]	
$Imp_c(0)$	$IMP_{total} \cdot (V_{nuc} + V_{cyt}) \times \frac{k_{3b}}{k_{3f} \cdot V_{nuc} + k_{3b} \cdot V_{cyt}}$ [nM]	
$ImpNrL_c(0)$	0 [nM]	
$NrL_n(0)$	0 [nM]	
$Imp_n(0)$	$IMP_{total} \cdot (V_{nuc} + V_{cyt}) \times \frac{k_{3f}}{k_{3f} \cdot V_{nuc} + k_{3b} \cdot V_{cyt}}$ [nM]	
$ImpNrL_n(0)$	0 [nM]	
$Degr_n(0)$	0 [nM]	
Volumes: $V_{nuc}=0.45\times10^{-12}$ l; $V_{cyt}=1.55\times10^{-12}$ l; $V_{cell}=2\times10^{-12}$ l; N_A=Avogadro's number$=6.02\times10^{23}$		
Various regimes of Nr nucleo-cytoplasmic transport (reactions 2 and 3): (Fig. 3A) reversible pore (black dashed line): $Kappa_{2f}=5.4\times10^{-12}$ l/min; $Kappa_{2b}=5.4\times10^{-12}$ l/min; $Kappa_{3f}=5.4\times10^{-12}$ l/min; $Kappa_{3b}=5.4\times10^{-12}$ l/min (Fig. 3B) active import of NrLImp complex (grey solid line): $Kappa_{2f}=5.4\times10^{-12}$ l/min; $Kappa_{2b}=0$ l/min; $Kappa_{3f}=5.4\times10^{-12}$ l/min; $Kappa_{3b}=5.4\times10^{-12}$ l/min (Fig. 3C) active export of IMP (black solid line): $Kappa_{2f}=5.4\times10^{-12}$ l/min; $Kappa_{2b}=5.4\times10^{-12}$ l/min; $Kappa_{3f}=0$ L/min; $Kappa_{3b}=5.4\times10^{-12}$ l/min		

Table 10

Model for Figure 2S of Supplementary Information (GR specific model)

Reactions		Parameters
$\mathbf{v_1}$	Nuclear RE binding NRL: $\left(k_{1f} \cdot \mathrm{Re}_n(t) \cdot NrL_n(t) - k_{1b} \cdot \mathrm{Re}\, NrL_n(t)\right) \cdot V_{nuc}$ [nmoles/min]	k_{1f}= 60 $nM^{-1}min^{-1}$ (diffusion limited); k_{1b}=60 min^{-1} (in oreder kd=1 nM (Drouin et al, 1992))
$\mathbf{v_2}$	NR binding nuclear ligand: $\left(k_{2f} \cdot Nr_n(t) \cdot L_n(t) - k_{2b} \cdot NrL_n(t)\right) \cdot V_{nuc}$ [nmoles/min]	k_{2f}= 60 $nM^{-1}min^{-1}$(diffusion limited); k_{2b}=60 min^{-1}(Marissal-Arvy et al, 1999))
$\mathbf{v_3}$	Mass Action: NR that sits near plasma membrane binds ligand from the plasma membrane. $\left(k_{3f} \cdot Nr_c(t) \cdot L_n(t) - k_{3b} \cdot NrL_c(t)\right) \cdot V_{cyt}$ [nmoles/min]	k_{3f}= 60 $nM^{-1}min^{-1}$(diffusion limited); k_{3b}=60 min^{-1}(Marissal-Arvy et al, 1999))
$\mathbf{v_4}$	Active transports of NR across nuclear membrane: $Kappa_{4importin} \cdot Nr_c(t) - Kappa_{4\exp ortin} \cdot Nr_n(t)$ [nmoles/min]	$Kappa_{4f}$=0.6×10^{-12} L/min (in the range of nuclear import of Smad protein (Schmierer et al, 2008);; $Kappa_{4b}$=1×10^{-12} L/min (fitted)
$\mathbf{v_5}$	Active transports of NRL across nuclear membrane: $Kappa_{5importin} \cdot NrL_c(t) - Kappa_{5\exp ortin} \cdot NrL_n(t)$ [nmoles/min]	$Kapp_{5f}$=5×10^{-12} L/min (fitted); $Kappa_{5b}$=0.04×10^{-12} L/min (fitted)
$\mathbf{V_6}$	Ligand diffusion between cytosol and nucleus: $Kappa_6 \cdot (L_c - L_n(t))$ [nmoles/min]	$Kappa_6$=*$\kappa_{\text{Ligand diffusion}}$=32×$10^{-12}$ L/min; L_c = 0.005 nM
Balance equations		
$dL_n(t)/dt$	$(v_6-v_2)/V_{nuc}$ [nM/min]	
$dRe(t)/dt$	$-v_1/V_{nuc}$ [nM/min]	
$dReNrL_n(t)/dt$	$+v_1/V_{nuc}$ [nM/min]	
dNr_n/dt	$(+v_4-v_2)/V_{nuc}$ [nM/min]	
$dNrL_n(t)$	$(+v_2+v_5-v_1)/V_{nuc}$ [nM/min]	
dNr_c/dt	$(-v_3-v_4)/V_{cyt}$ [nM/min]	
$dNrL_c/dt$	$(+v_3-v_5)/V_{cyt}$ [nM/min]	
Conserved Moieties		
$DNA_{total} \cdot V_{nuc}$	$\mathrm{Re}\, Nr_n(t) \cdot V_{nuc} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$DNA_{total} \cdot V_{nuc}$ = *1000*10^9/N_A = 1.67*×10^{-12} *nmoles (10^3 molecules/cell)* (de Kloet et al, 2000)
$NR_{total} \cdot (V_{nuc} + V_{cyt})$	$Nr_n(t) \cdot V_{nuc} + Nr_c(t) \cdot V_{cyt} + NrL_n(t) \cdot V_{nuc} + NrL_c(t) \cdot V_{cyt} + \mathrm{Re}\, NrL_n(t) \cdot V_{nuc}$ *[nmoles/cell]*	$NR_{total} \cdot (V_{nuc} + V_{cyt})$ = *10^5*10^9/N_A = 167*×10^{-12} *nmoles (10^5 molecules/cell)* (Nordeen et al, 1989; van Steensel et al, 1995)
Initial conditions		
$\mathrm{Re}\, Nr_n(0)$	DNA_{total} [nM]	
$\mathrm{Re}\, NrL_n(0)$	0 [nM]	
$Nr_n(0)$	$NR_{total} \cdot (V_{nuc} + V_{cyt}) \times \frac{k_{4f}}{k_{4f} \cdot V_{nuc} + k_{4b} \cdot V_{cyt}}$ [nM]	
$NrL_n(0)$	0 [nM]	
$Nr_c(0)$	$NR_{total} \cdot (V_{nuc} + V_{cyt}) \times \frac{k_{4b}}{k_{4f} \cdot V_{nuc} + k_{4b} \cdot V_{cyt}}$ [nM]	
$NrL_c(0)$	0 [nM]	
$L_n(0)$	0 [nM]. The addition of ligand was modeled as the increase of its fixed concentration in the outer cellular membrane (L_c) from 0 to 0.005 nM (and maintained constant at the latter level), where the concentrations are quantified as the aqueous concentrations in the cytosol immediately adjacent to the plasma membrane; we shall assume a rapid equilibration of the ligand between this aqueous phase and the plasma-membrane	

	phase. The concentration of ligand in the nucleus ($L_n(t)$) was treated as aqueous only, i.e. in terms of the total number of molecules divided by the volume of the nucleus.
Volumes: $V_{nuc}=0.45\times10^{-12}$ l; $V_{cyt}=1.55\times10^{-12}$ l; $V_{cell}=2\times10^{-12}$ L; N_A=Avogadro's number$=6.02\times10^{23}$ (Riddick and Macara, 2007)	

We describe a spherical cell of radius 7.85 µm, with a spherical nucleus of radius 4.75 µm. Consequently, $Area_{nuc}$ (area of nuclear membrane) =280 μm^2=$2.8\cdot10^{-8} dm^2$, Dist (distance between cytoplasmic and nuclear membrane)=3.1 µm=$3.1\cdot10^{-5}$dm ; V_{nuc}=450 μm^3, $V_{cytoplasm}$=1575 μm^3, and V_{cell}=2025 μm^3.

$D_{Protein}$ =$6\cdot10^{-9} dm^2$/min (diffusion coefficient for protein (Kholodenko et al, 2000a).
D_{Ligand}=$36\cdot10^{-9} dm^2$/min (calculated from the Stokes-Einstein equation by comparing with $D_{Protein}$).

$^*\kappa_{Ligand\ diffusion}= D_{Ligand} \times \frac{Area_{nuc}}{Dist}$ [litre/min= dm^3/min]=32.5pL/min=$32.5\cdot10^{-12}$ L/min.

SENSITIVITY ANALYSIS OF MAIN CONCLUSIONS

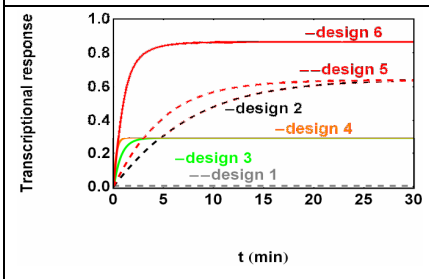
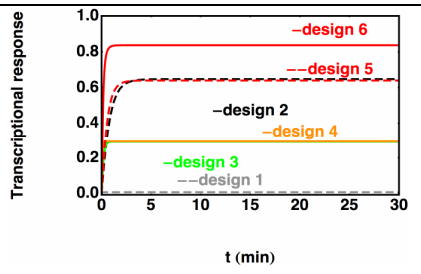
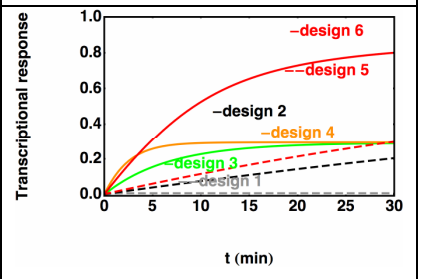
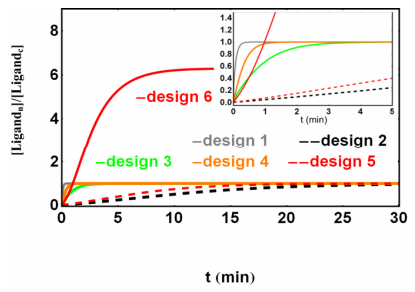
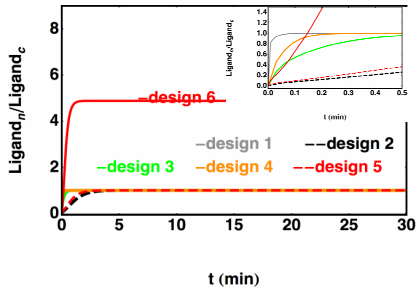
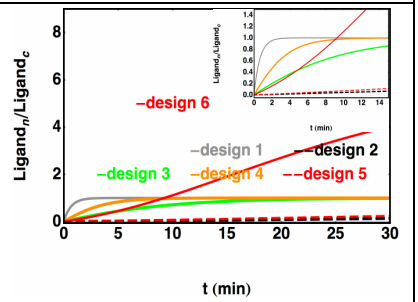
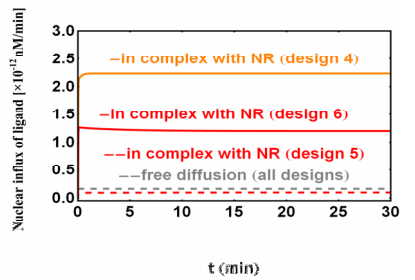
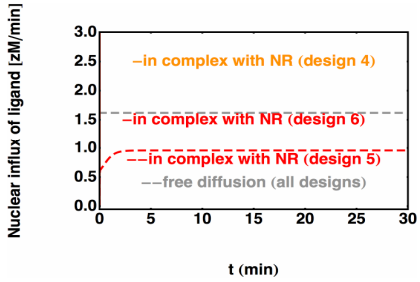
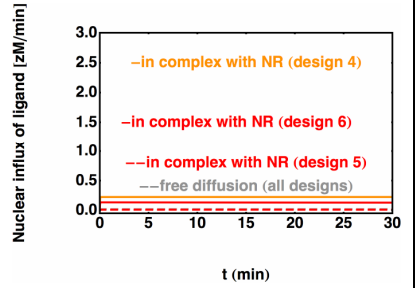
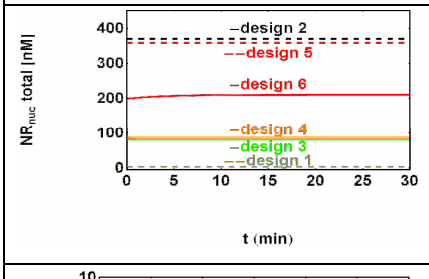
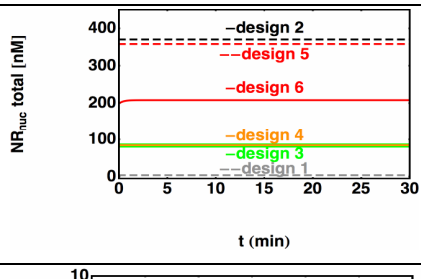
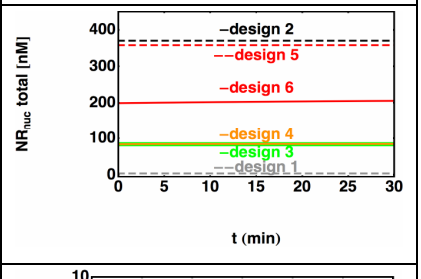
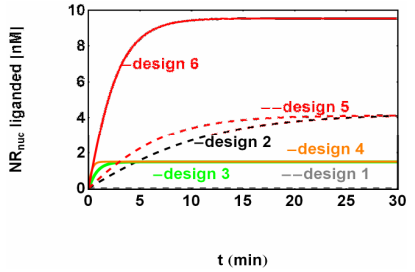
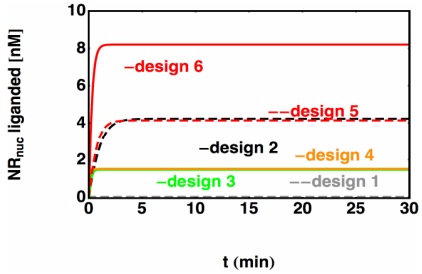
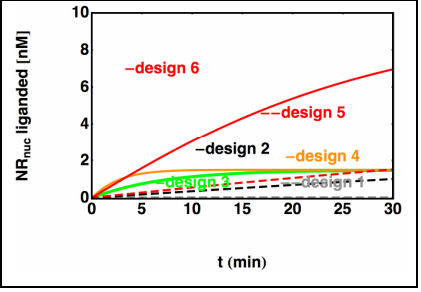
We have varied parameter values and checked whether the conclusions persisted. Our conclusions were mostly robust for up to five-fold changes in parameter values, but the precise details are given below.

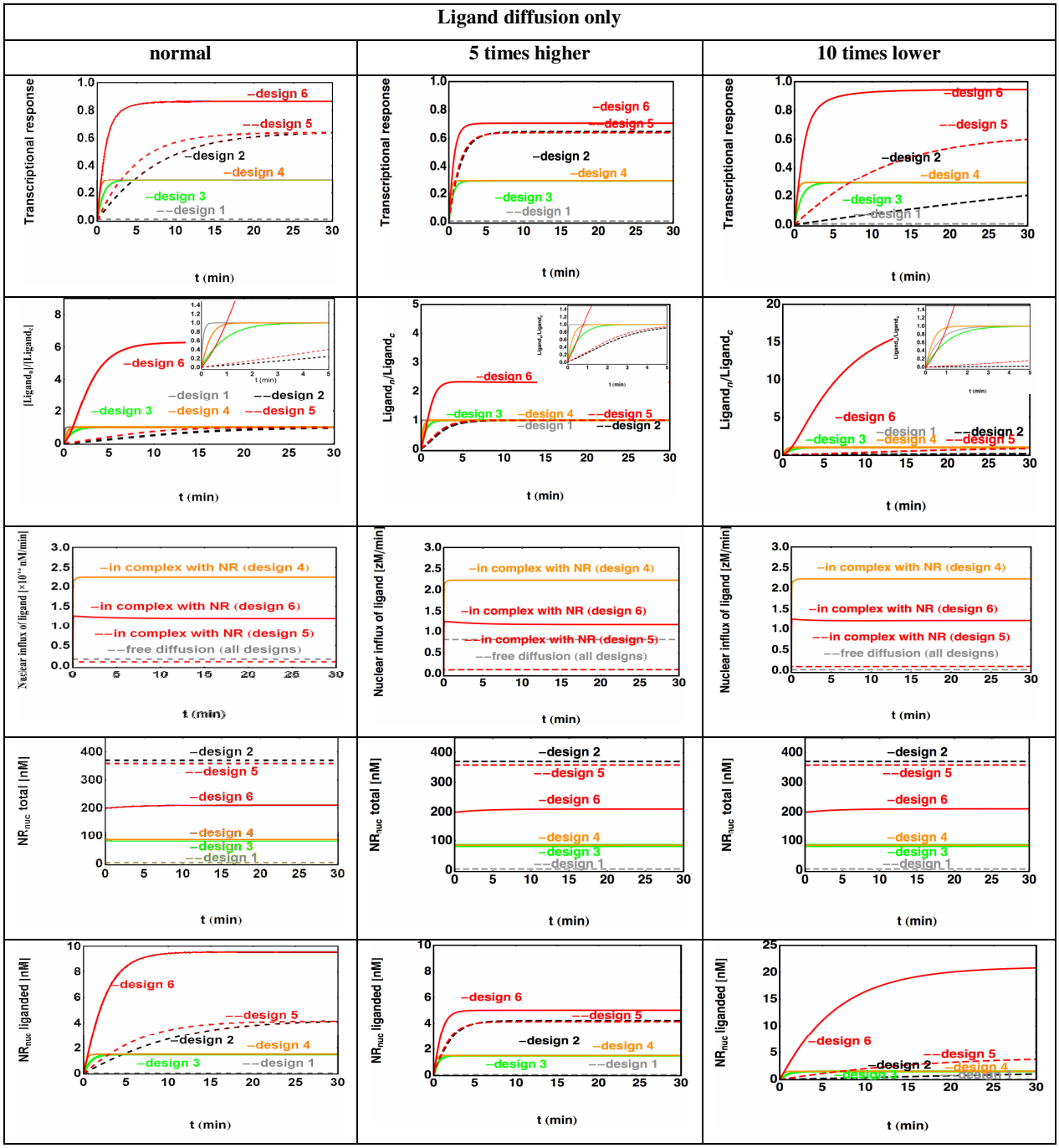
Figure 2: Design 6 is the most advantageous. This conclusion was not affected by at least 5 fold perturbation of any single parameter in the model. The only exception was related to the rate of nuclear import of liganded receptor. If active nuclear import of liganded receptor in design 6 is decreased more then 3 fold, then the advantages of the design 6 comparing with the design 2 almost disappear. This fits well in the context of the main messages of our manuscript. Indeed, an advantageous feature of the design 6 is exactly the active import of ligand into the nucleus achieved by preferential nuclear import of the ligand bound receptor.

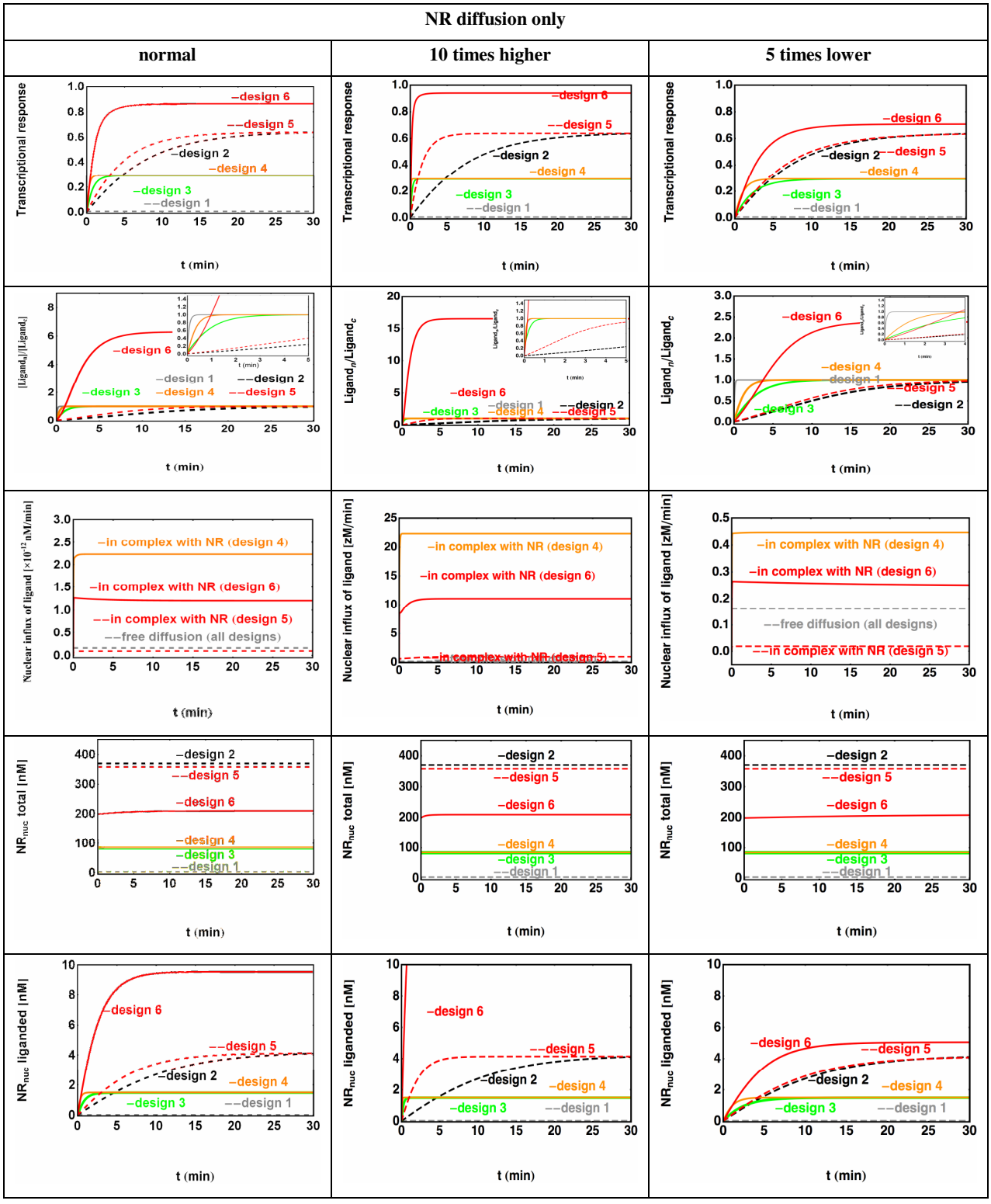
Figure 3: Active export of importins prevents sequestration of the receptor in the nucleus by importins. This conclusion was not affected by l0 fold perturbation of any single parameter in the model.

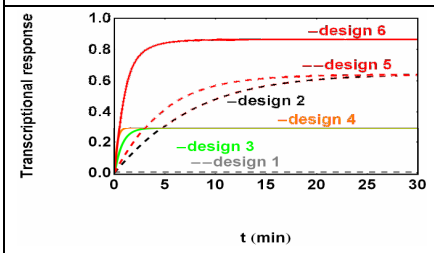
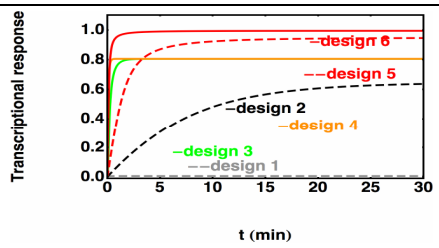
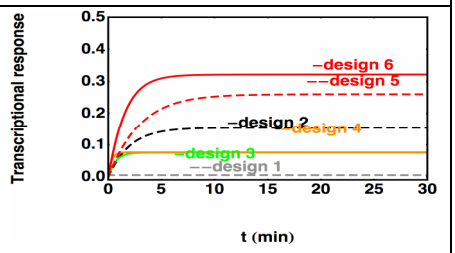
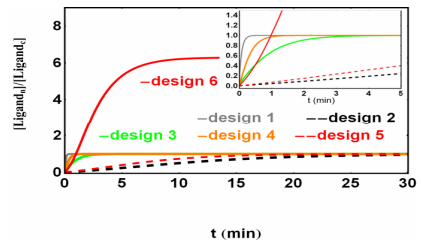
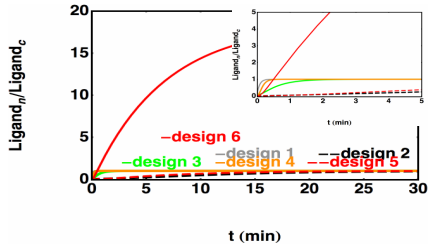
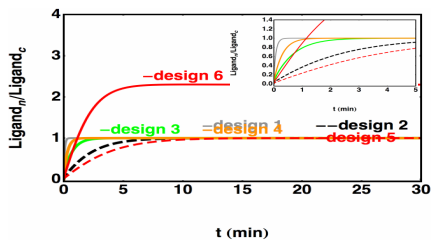
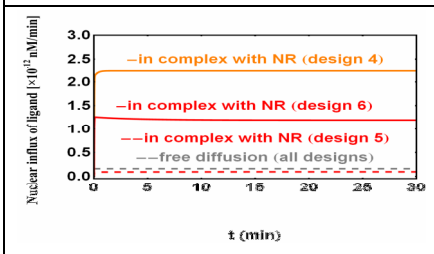
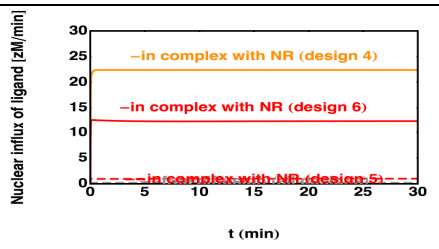
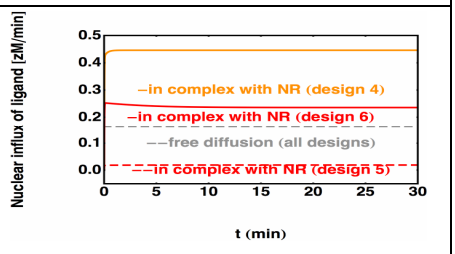
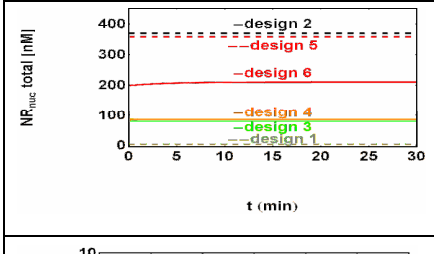
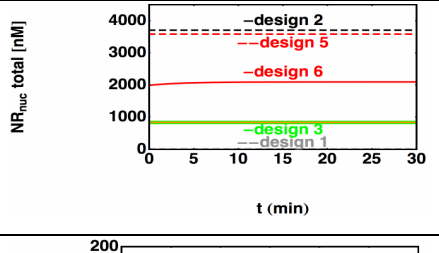
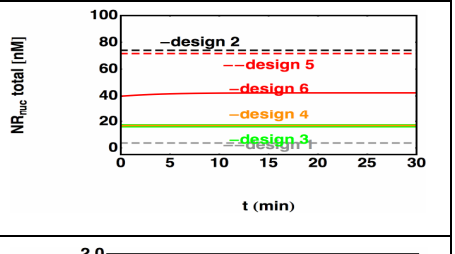
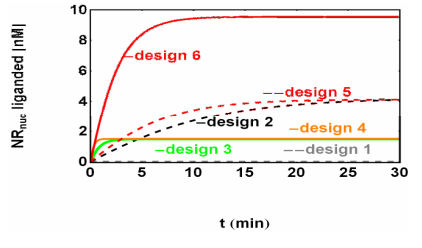
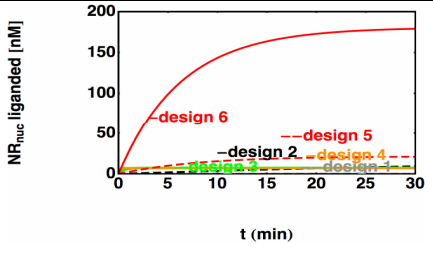
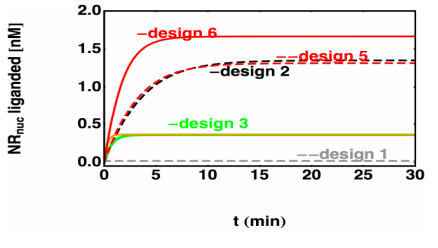
Figure 4: flux through the NPC may be robust even if all pathways run through the same pore. This conclusion was not affected by l0 fold perturbation of any single parameter in the model.

Table 11 SENSITIVITY ANALYSIS OF MAIN CONCLUSIONS FOR FIG. 2

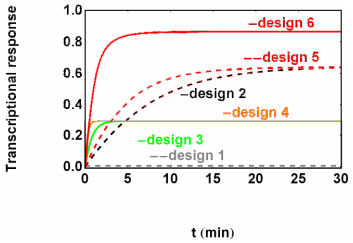
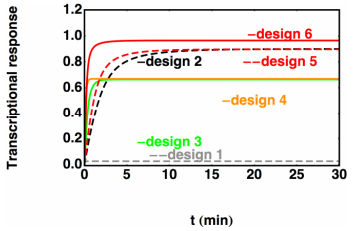
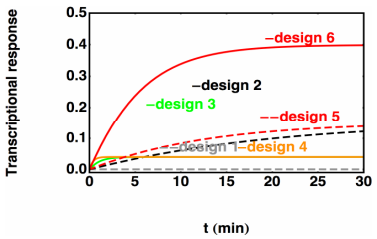
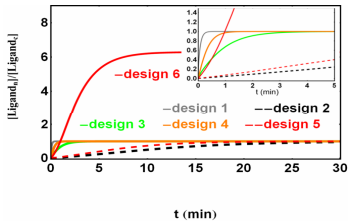
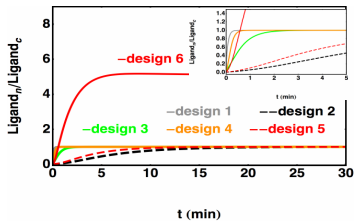
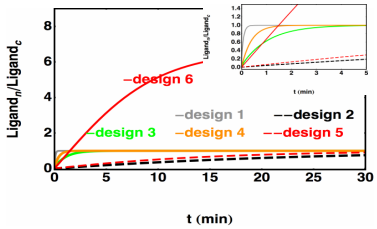
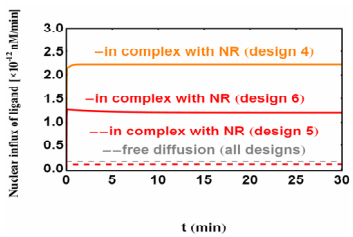
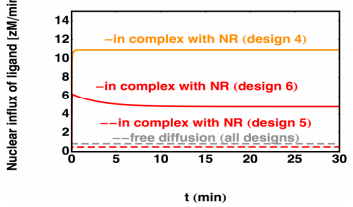
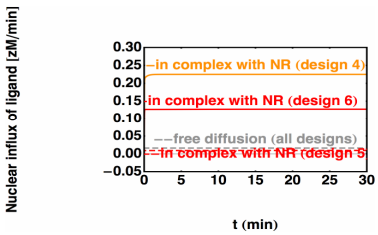
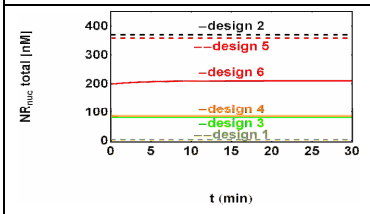
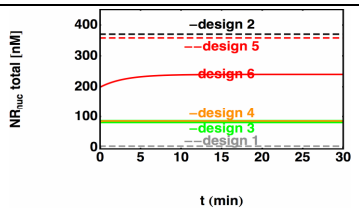
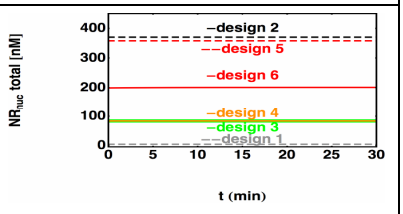
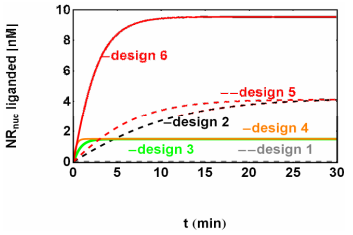
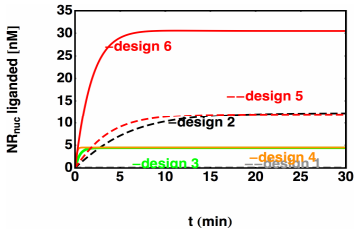
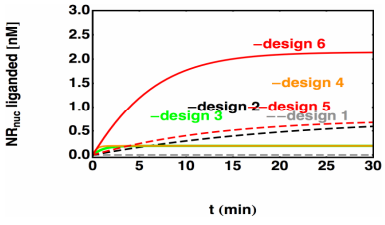
NR and Ligand diffusion (Ligand diffusion/NR diffusion ratio = 6 as calculated from Stokes-Einstein equation)		
normal	**10 times higher**	**10 times lower**
		
		
		
		
		

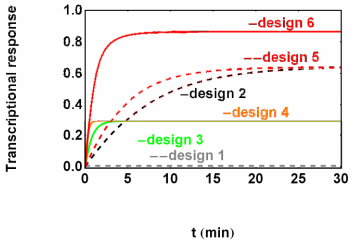
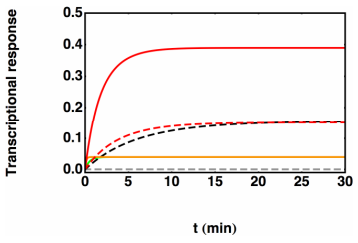
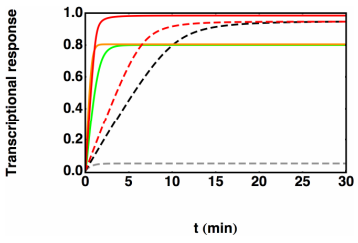
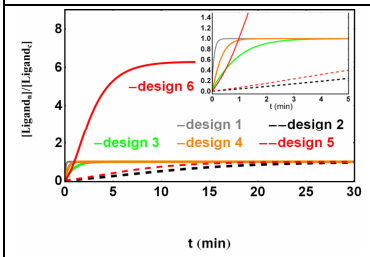
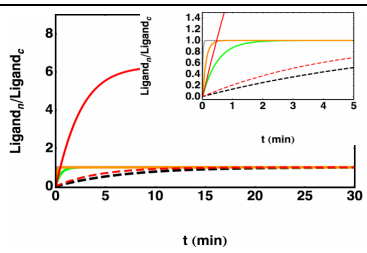
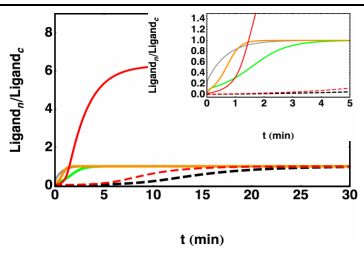
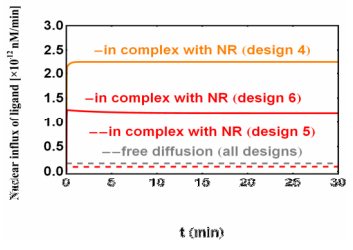
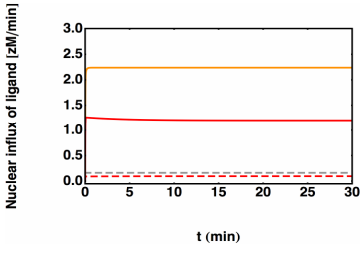
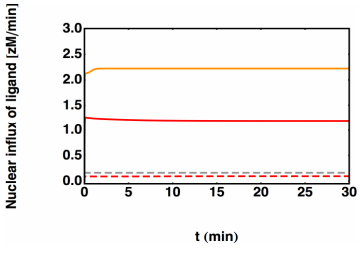
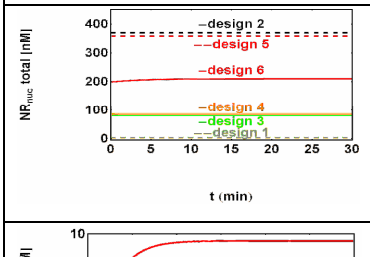
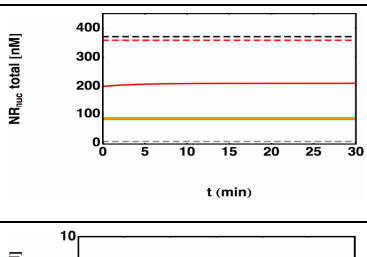
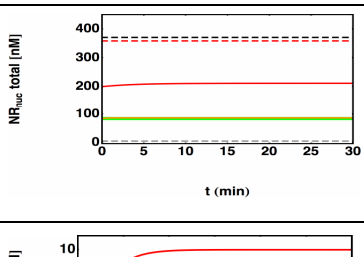
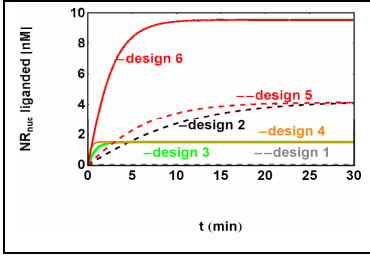
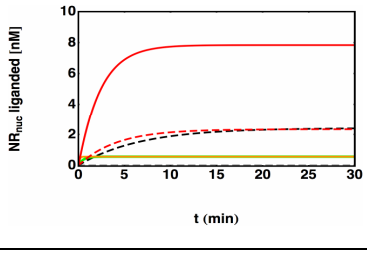
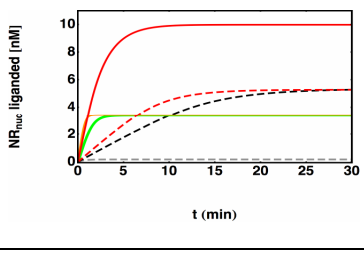

Ligand diffusion only
normal
5 times higher
10 times lower
Transcriptional response
t (min)
design 6
design 5
design 2
design 4
design 3
design 1
[Ligand_n]/[Ligand_c]
Ligand_n/Ligand_c
in complex with NR (design 4)
in complex with NR (design 6)
in complex with NR (design 5)
free diffusion (all designs)
Nuclear influx of ligand [zM/min]
NR_nuc total [nM]
NR_nuc liganded [nM]

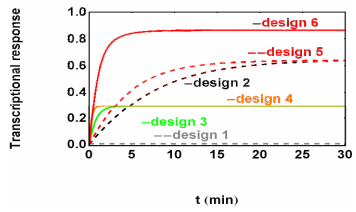
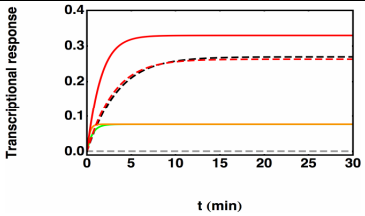
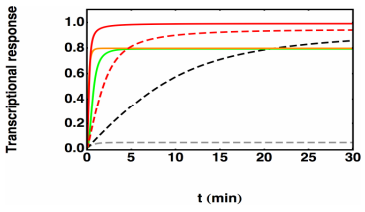
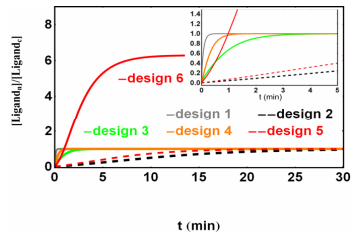
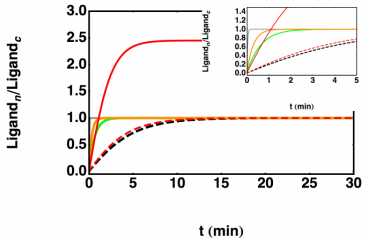
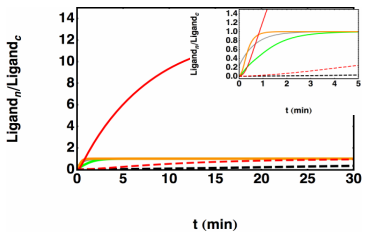
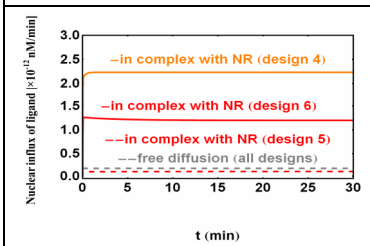
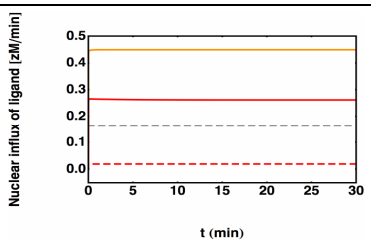
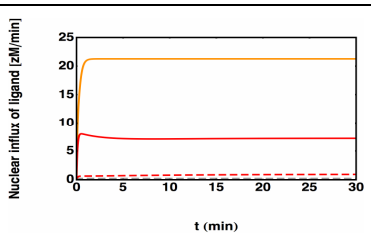
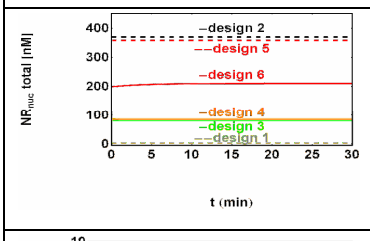
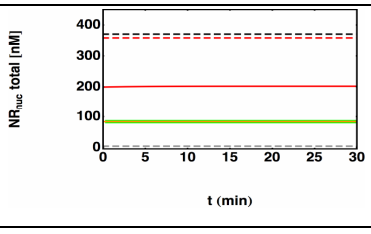
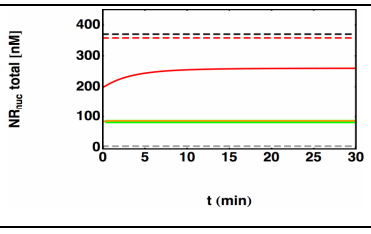
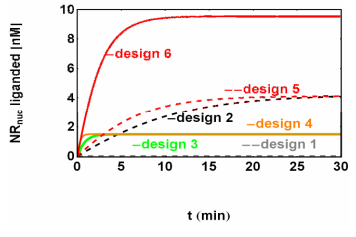
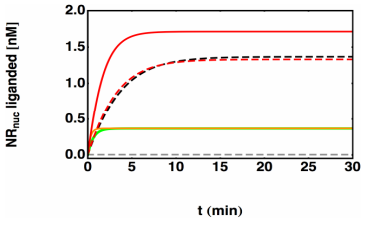
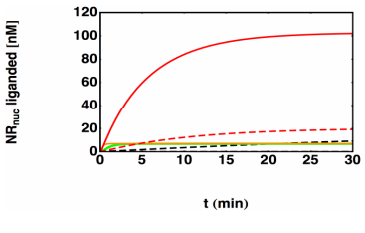

NR diffusion only
normal
10 times higher
5 times lower
Transcriptional response
t (min)
design 6
design 5
design 2
design 4
design 3
design 1
[Ligand_n]/[Ligand_c]
Ligand_n/Ligand_c
Nuclear influx of ligand [×10^-12 nM/min]
Nuclear influx of ligand [zM/min]
in complex with NR (design 4)
in complex with NR (design 6)
in complex with NR (design 5)
free diffusion (all designs)
NR_nuc total [nM]
NR_nuc liganded [nM]

NR concentration (also means the change in the ratio NR/DNA and NR/Ligand) for designs 2-6		
normal	**10 times higher**	**5 times lower**
		
		
		
		
		

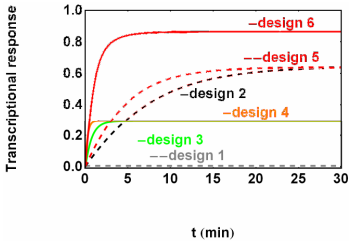
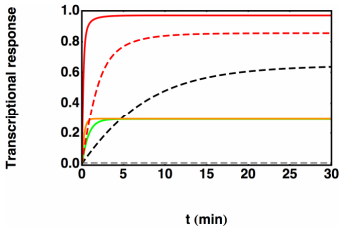
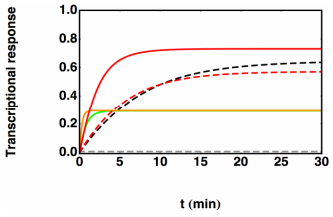
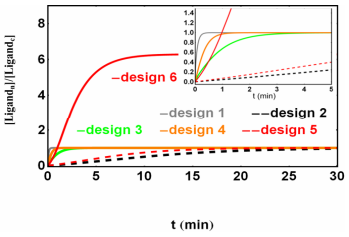
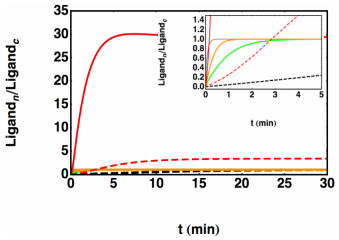
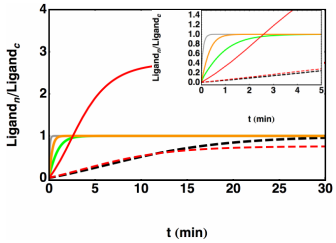
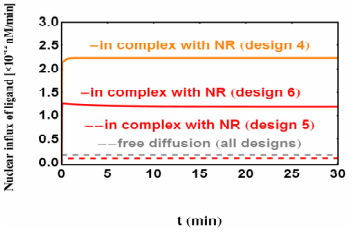
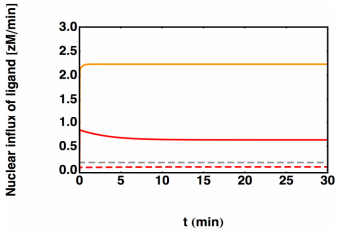
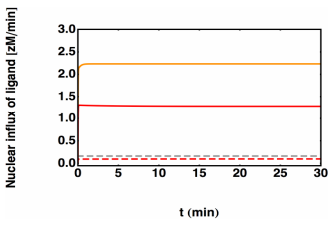
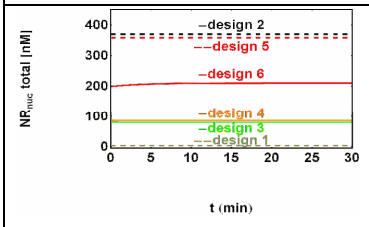
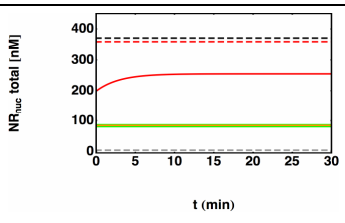
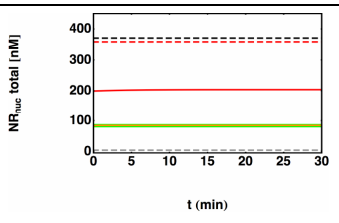
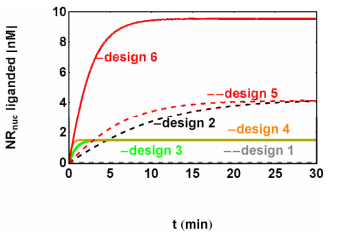
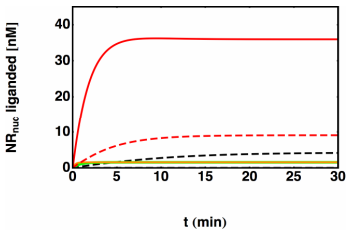
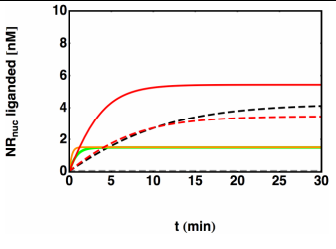
Ligand concentration (also means the change in the ratio Ligand/NR; Ligand/DNA) for designs 1-6

normal	5 times higher	10 times lower
		
		
		
		
		

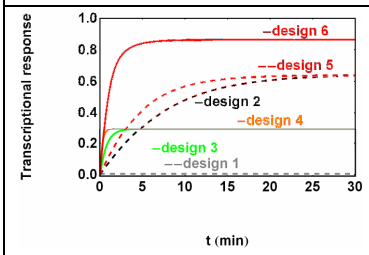
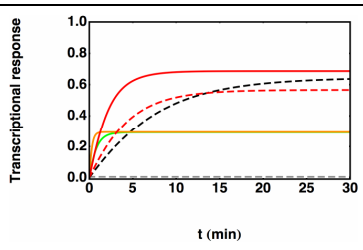
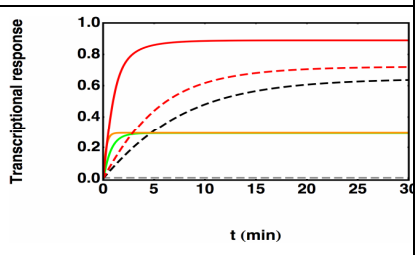
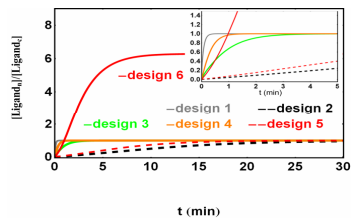
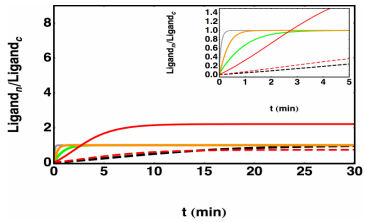
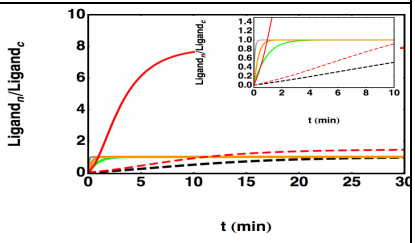
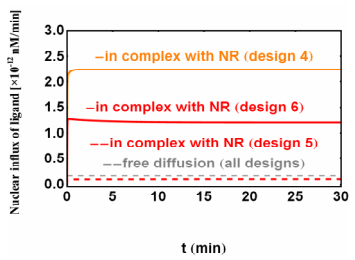
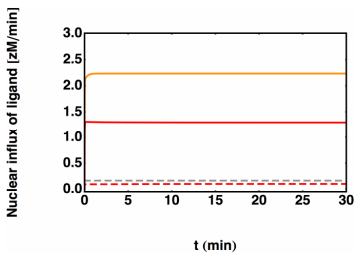
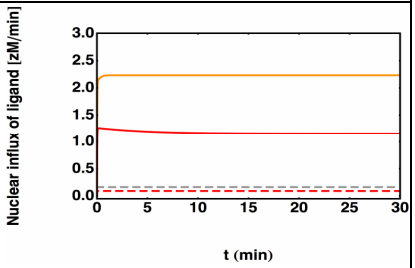
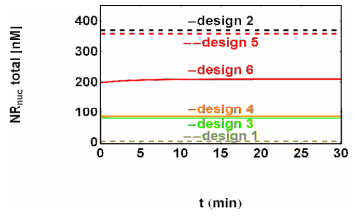
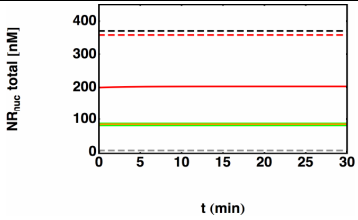
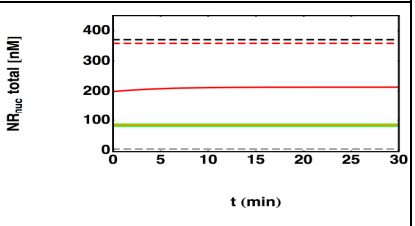
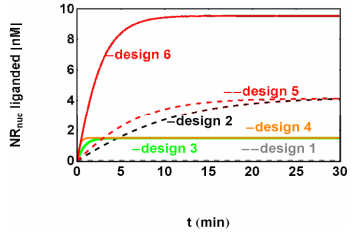
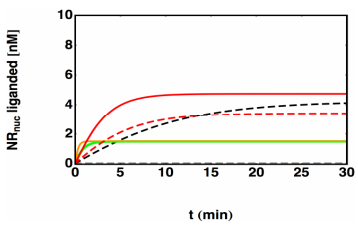
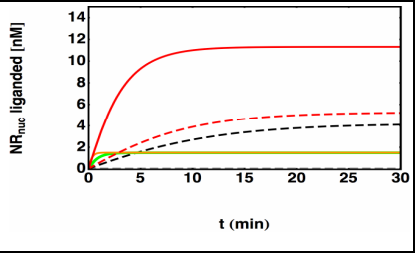
K_d Receptor-DNA binding, designs 2-6 (k_{1b})		
normal	**10 times higher**	**10 times lower**
		
		
		
		
		

K_d Receptor-Ligand binding design 1 (k_{1b}); design 2 (k_{2b}); design 3 (k_{2b}; k_{3b}; k_{4b}); designs 4-6 (k_{2b}; k_{3b})		
normal	**5 times higher**	**10 times lower**
		
		
		
		
		

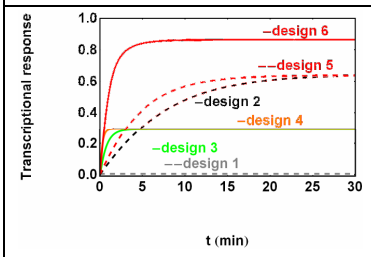
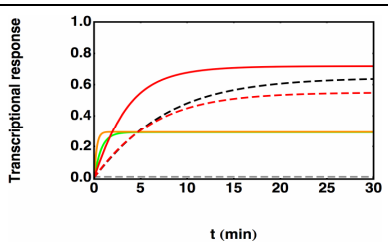
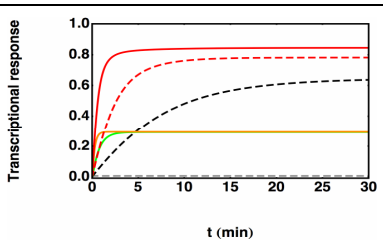
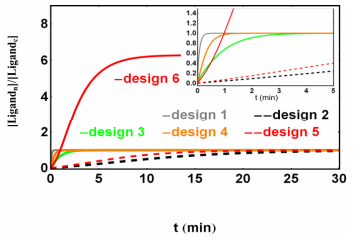
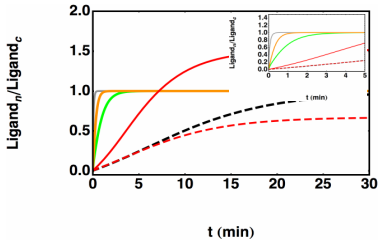
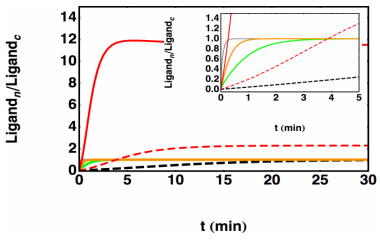
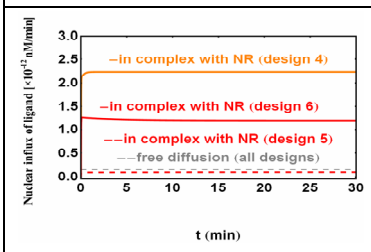
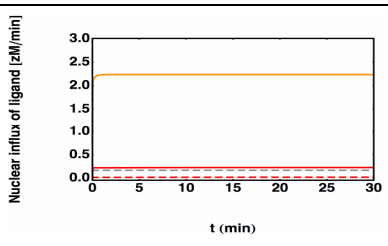
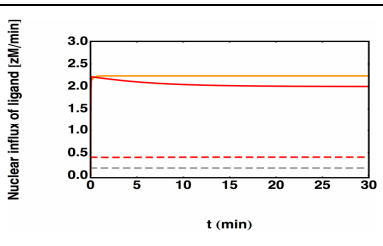
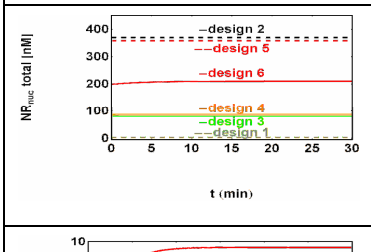
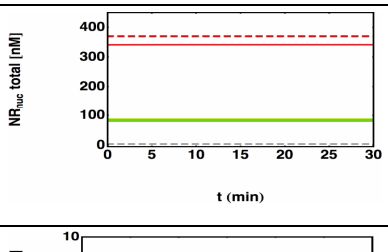
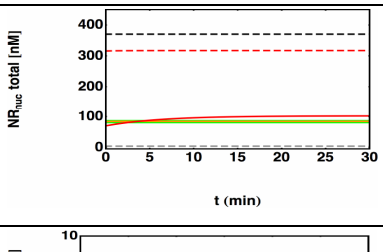
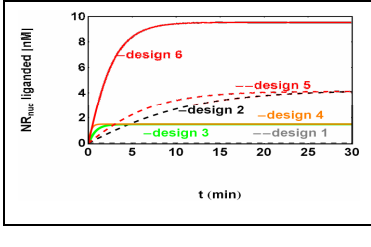
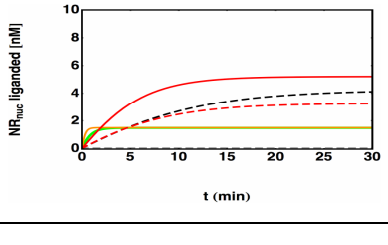
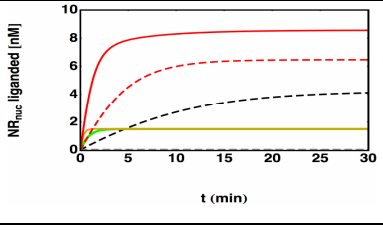
Liganded NR nuclear import, designs 5-6 (k_{5f})

normal	10 times higher	3 times lower
		
		
		
		
		

Liganded NR nuclear export, designs 5-6 (k_{5b})

normal	10 times higher	10 times lower
		
		
		
		
		

Unliganded NR nuclear import, designs 5-6 (k_{5f})

normal	10 times higher	5 times lower
		
		
		
		
		

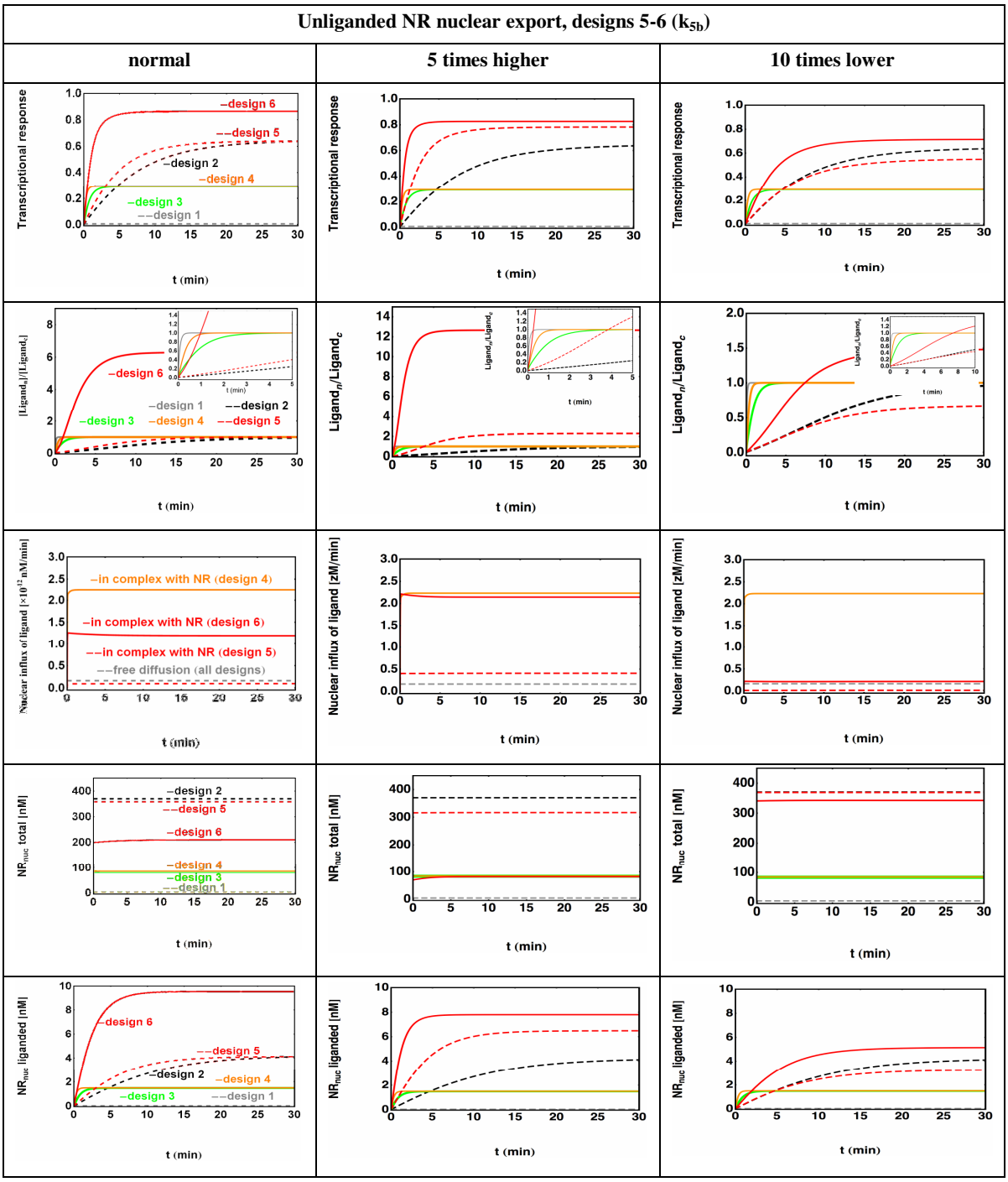
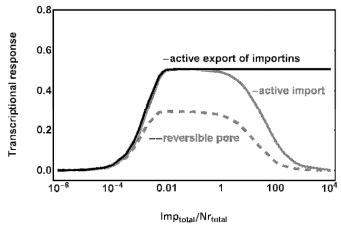
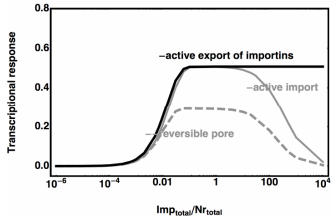
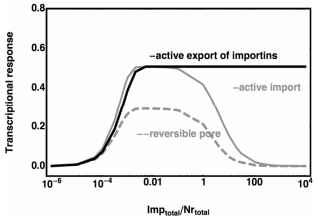
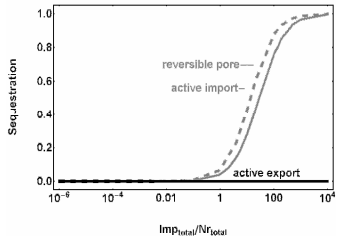
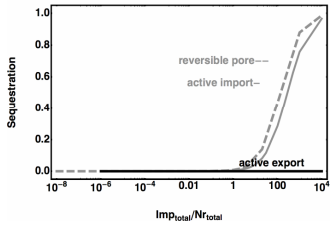
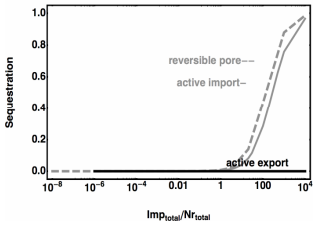
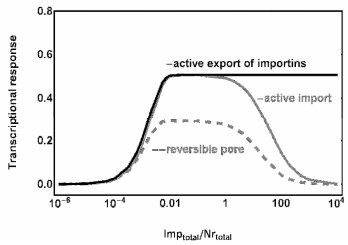
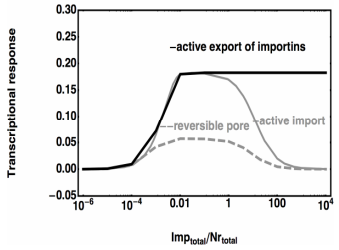
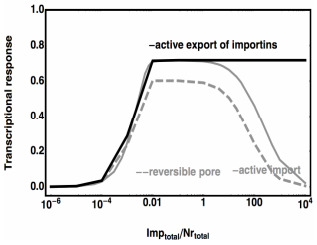
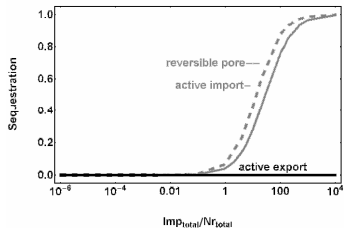
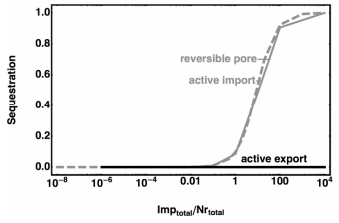
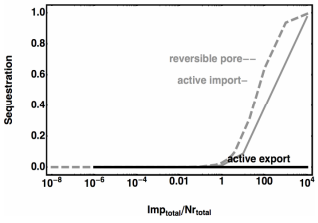
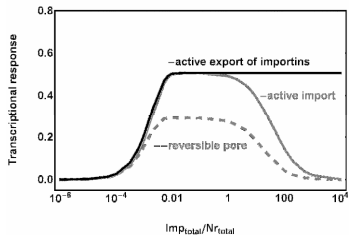
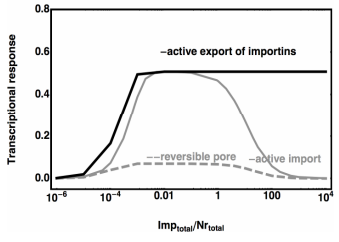
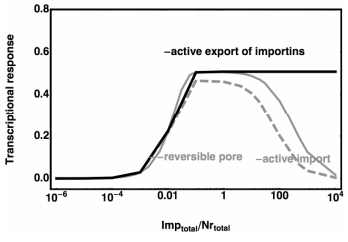


Table 12 SENSITIVITY ANALYSIS OF MAIN CONCLUSIONS FOR FIG. 3

K_d Importin - Liganded NR (k_{1b} and k_{4b})		
normal	**10 times higher**	**10 times lower**
		
		
K_d RE - Liganded NR (k_{5b})		
normal	**10 times higher**	**10 times lower**
		
		
Transport **Reversible pore (k_{2f}, k_{2b}, k_{3f}, k_{3b},)** **Active import (k_{2f}, k_{3f}, k_{3b},) (k_{2b}=0)** **Active export of importins (k_{2f}, k_{2b}, k_{3f}) (k_{3b}=0)**		
normal	**10 times higher**	**10 times lower**
		

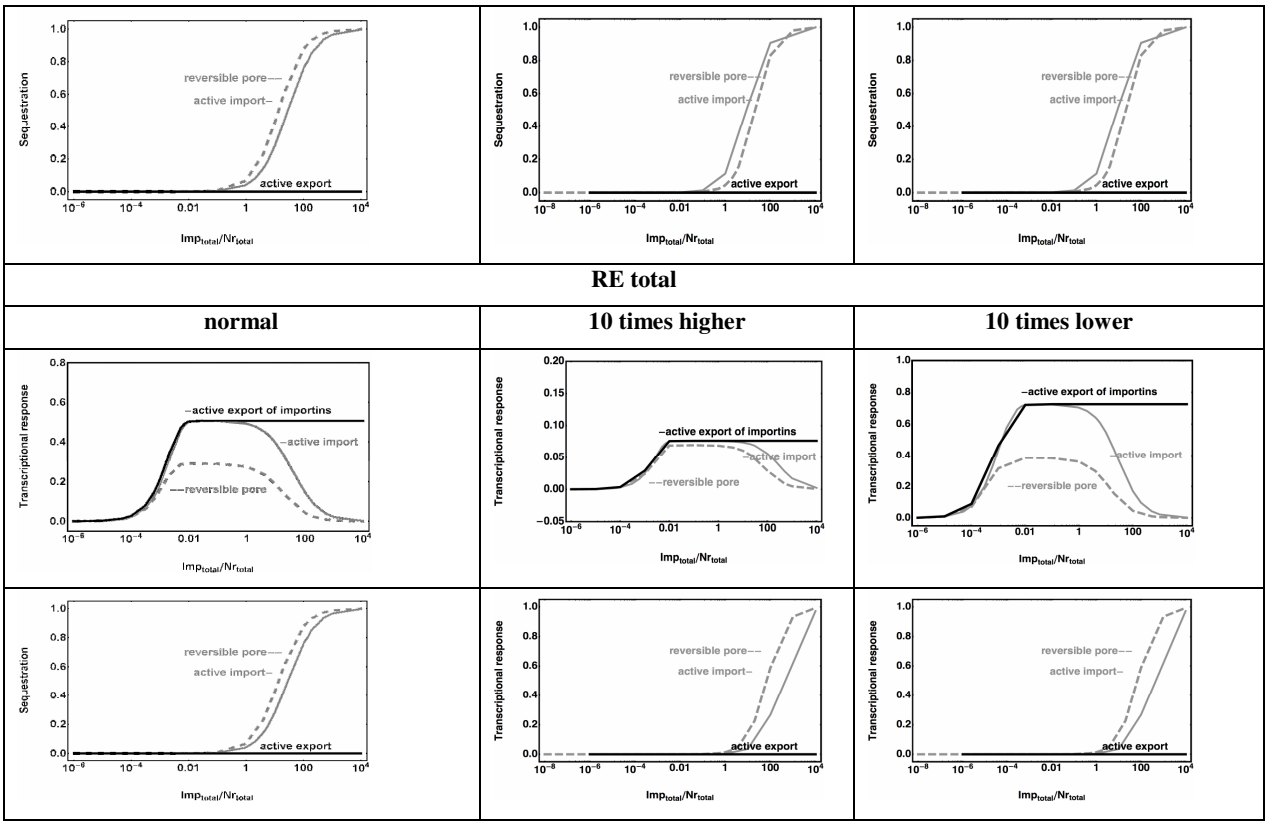
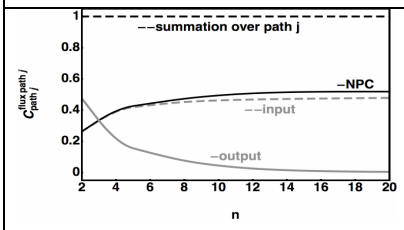
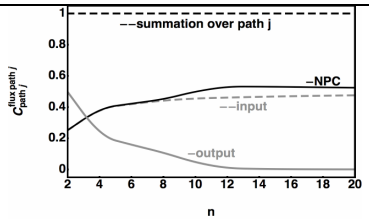
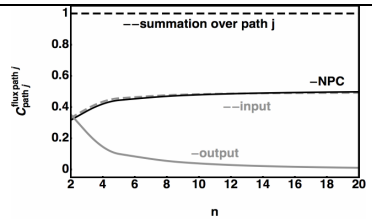
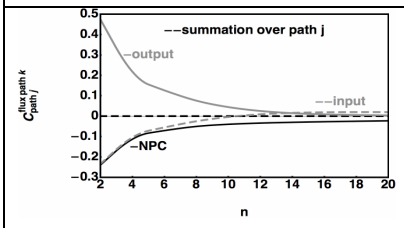
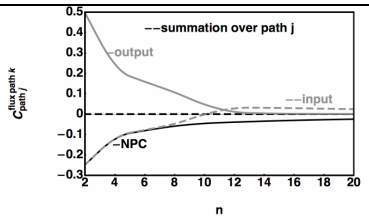
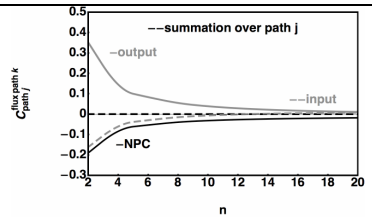
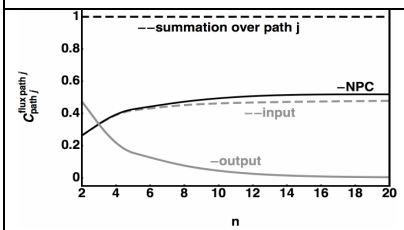
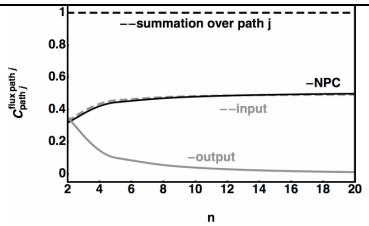
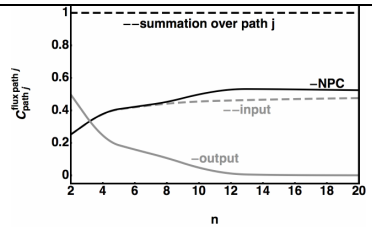
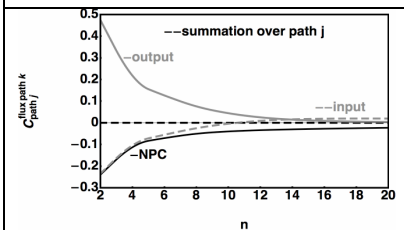
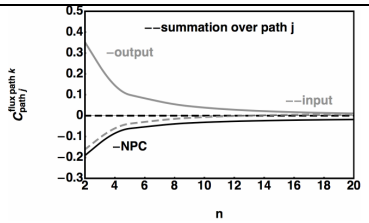
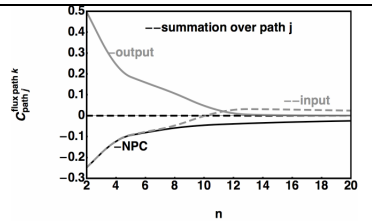
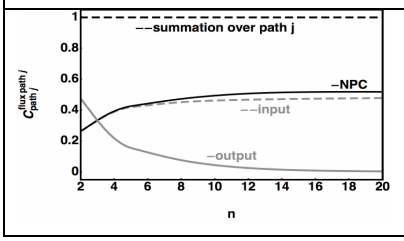
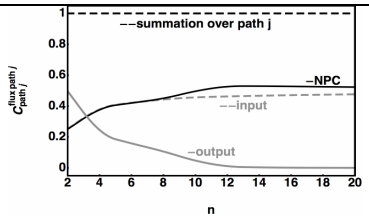
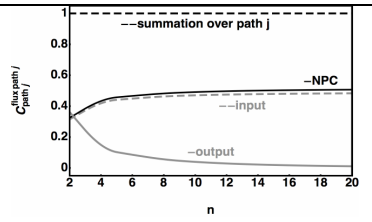
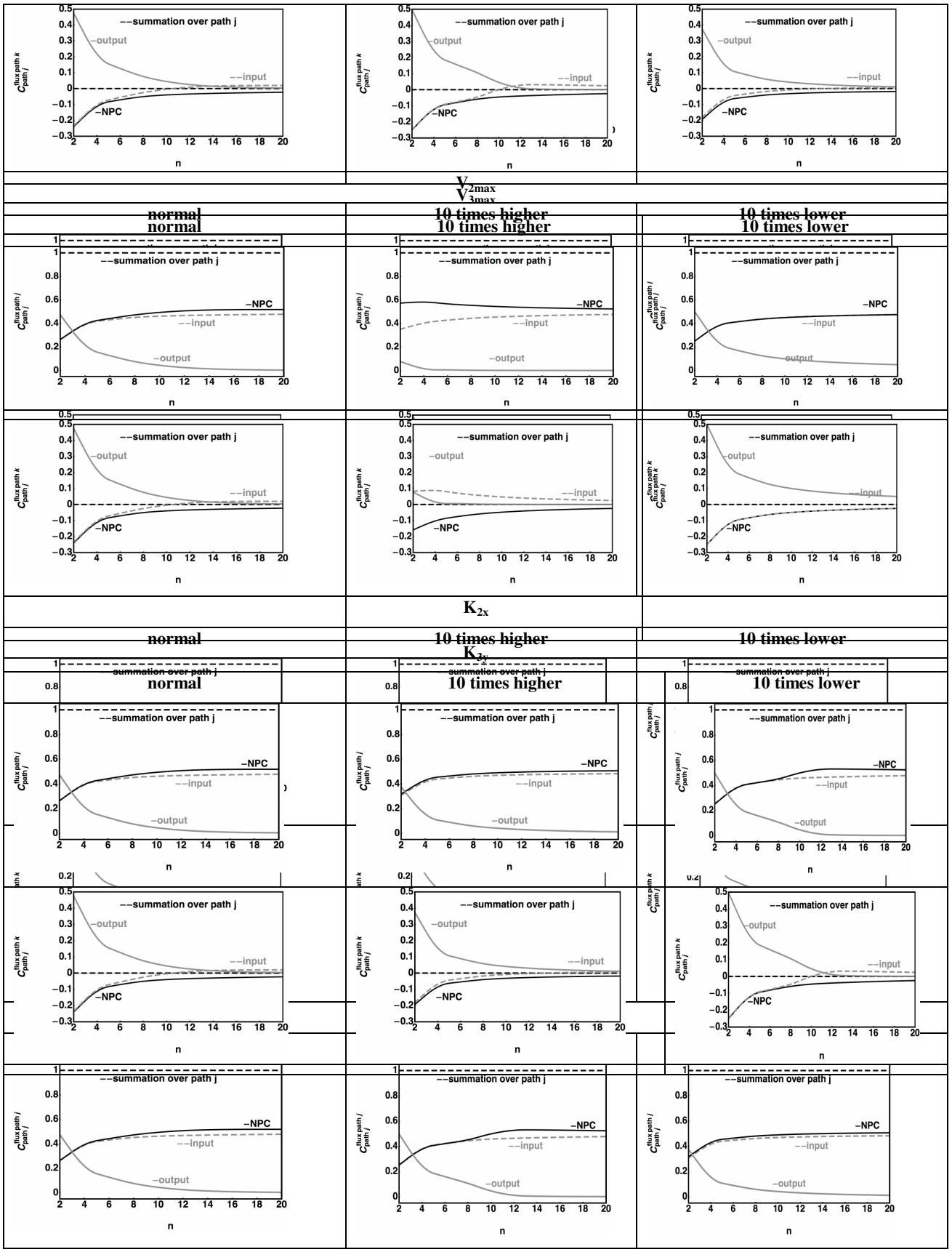

Sequestration
reversible pore
active import
active export
Imp_total/Nr_total
RE total
normal
10 times higher
10 times lower
Transcriptional response
active export of importins
active import
reversible pore

Table 13 SENSITIVITY ANALYSIS OF MAIN CONCLUSIONS FOR FIG. 4

V_{1max}		
normal	**10 times higher**	**10 times lower**
		
		
K_{1s}		
normal	**10 times higher**	**10 times lower**
		
		
K_{1x}		
normal	**10 times higher**	**10 times lower**
		


summation over path j
output
input
NPC
n
V_{2max}
V_{3max}
normal
10 times higher
10 times lower
K_{2x}
K_{3y}

Supplementary References

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