DRAFT: NCT

RA

2022-01-25

1 Introduction

TODO(1): intro

We are particularly interested in the nuclear-to-cytoplasmic contrast (henceforth, N:C contrast) of certain molecular species at steady state: $\mathsf{Ran}\cdot\mathsf{GTP}$, $\mathsf{Imp\beta}$, CAS, and NLS.

Abbreviations. FG-nups = FG-nucleoporins; NCT = nucleocytoplasmic transport; NPC = nuclear pore complex; NE = nuclear envelope; IBB = importin beta binding; ODE = ordinary differential equations; SPR = surface plasmon resonance;

2 NCT models

2.1 GSR'03 model of NCT

Ran gradient. The Ran gradient, i.e. the nuclear accumulation of $\mathsf{Ran}\cdot\mathsf{GTP}$, is the base layer of nucleocytoplasmic transport. We implement it as the "minimal Ran gradient system" from [GSR03]. The equations are recapitulated in §5.1 and the constants are collected in Table 2. Following [GSR03], the "dynamic capacity" Ex is an optional maximal steady-state (positive) flux of nuclear $\mathsf{Ran}\cdot\mathsf{GTP}$ to cytoplasmic $\mathsf{Ran}\cdot\mathsf{GDP}$, which we determine using the additional equation (24). The fluxes are in units of concentration/time (µM/s). The ones across the nuclear boundary have positive sign when exiting the nucleus and are normalized to the nuclear volume. Thus, the *amount* exiting the nucleus per unit of time is $\mathsf{flux}\times V_{\mathrm{nuc}}$.

Simulating the ODE across the scenarios of [GSR03] we obtain results that are sufficiently close to the original, see Table 3. Importantly, an order of 1000-fold nuclear enrichment of $\mathsf{Ran}\cdot\mathsf{GTP}$ is sustained in steady-state. Moreover, the dynamic capacity clocks in at around 0.6 µM/s in most cases, meaning the Ran gradient is established within seconds. Therefore, we will replace the whole Ran gradient layer by a virtual pump

$$\text{cytoplasmic } \mathsf{Ran}\cdot\mathsf{GDP} \longrightarrow \text{nuclear } \mathsf{Ran}\cdot\mathsf{GTP} \quad \text{with kinetic rate } 0.1\,\mathrm{s}^{-1}. \tag{1}$$

This rate is chosen conservatively (a concentration of 1 µM of cytoplasmic $\mathsf{Ran \cdot GDP}$ generates a flux of 0.1 µM/s) but will be sufficient for our purposes.

Code #1.

Coupling to Impβ-mediated transport. A coupling of the Ran gradient to importin–cargo transport was proposed in [GSR03, Fig. 6A]. We now formulate a version of it. The following equations comprise the handling of cargo by $\mathsf{Imp}\beta$ in the cytoplasm,

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Imp\beta \cdot\ Ran \cdot GTP}]_{\mathrm{cyt}} = -\mathsf{R}_{\mathsf{cyt}} + \mathsf{F}_{\mathsf{Imp\beta \cdot Ran \cdot GTP}} \frac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} - \mathsf{GAP}_{\mathsf{Imp\beta}} + \mathsf{Knockoff}_{\mathsf{cyt}} \tag{2a}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Imp\beta}]_{\mathrm{cyt}} = +\mathsf{R}_{\mathsf{cyt}} + \mathsf{C}_{\mathsf{cyt}} + \mathsf{F}_{\mathsf{Imp\beta}} \frac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} + \mathsf{GAP}_{\mathsf{Imp\beta}} \tag{2b}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Imp\beta \cdot\ Cargo}]_{\mathrm{cyt}} = -\mathsf{C}_{\mathsf{cyt}} + \mathsf{F}_{\mathsf{Imp\beta \cdot Cargo}} \frac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} - \mathsf{Knockoff}_{\mathsf{cyt}} \tag{2c}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Cargo}]_{\mathrm{cyt}} = +\mathsf{C}_{\mathsf{cyt}} + \mathsf{F}_{\mathsf{Cargo}} \frac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} + \mathsf{Knockoff}_{\mathsf{cyt}} \tag{2d}$$

with the fluxes

$$\mathsf{R}_{\mathsf{cyt}} := -k^{\mathrm{R}}_{\mathrm{on}}[\mathsf{Imp\beta}][\mathsf{Ran \cdot GTP}]_{\mathrm{cyt}} + k^{\mathrm{R}}_{\mathrm{off}}[\mathsf{Imp\beta \cdot\ Ran \cdot GTP}]_{\mathrm{cyt}} \tag{3a}$$

$$\mathsf{C}_{\mathsf{cyt}} := -k^{\mathrm{C}}_{\mathrm{on}}[\mathsf{Imp\beta}][\mathsf{Cargo}]_{\mathrm{cyt}} + k^{\mathrm{C}}_{\mathrm{off}}[\mathsf{Imp\beta \cdot\ Cargo}]_{\mathrm{cyt}}. \tag{3b}$$

The forward flux of the reaction

$$\mathsf{Imp\beta \cdot\ Cargo} + \mathsf{Ran \cdot GTP} \rightleftharpoons \mathsf{Imp\beta \cdot\ Ran \cdot GTP} + \mathsf{Cargo} \tag{4}$$

is called Knockoff. It is modeled as a one-way reaction with forward rate k_{knockoff}. The GSR equations are modified accordingly:

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Ran \cdot GDP}]_{\mathrm{cyt}} = (18\mathrm{a}) + \mathsf{GAP}_{\mathsf{Imp\beta}} \tag{18a′}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Ran \cdot GTP}]_{\mathrm{cyt}} = (18\mathrm{b}) + \mathsf{R}_{\mathsf{cyt}} - \mathsf{Knockoff}_{\mathsf{cyt}} \tag{18b′}$$

Analogous nuclear equations (without GAP) are implemented but are omitted here. Analogously to (22a)/(22b) we have the additional nuclear-to-cytoplasmic diffusion fluxes

$$\mathsf{F}_{\mathsf{Imp\beta \cdot Ran \cdot GTP}}, \quad \mathsf{F}_{\mathsf{Imp\beta}}, \quad \mathsf{F}_{\mathsf{Imp\beta \cdot Cargo}}, \quad \mathsf{F}_{\mathsf{Cargo}} \tag{5}$$

with the permeability constants given in Table 1.

SPR experiments of [Cat+01] indicated that the IBB domain of importin-α binds importin-β and undergoes a conformational change,

$$\mathsf{A} + \mathsf{B} \rightleftharpoons \mathsf{AB} \rightleftharpoons \mathsf{A^*B}. \tag{6}$$

We therefore assume the analogous reaction

$$\mathsf{Cargo} + \mathsf{Imp\beta} \underset{\mathsf{k}_{d1}}{\overset{\mathsf{k}_{a1}}{\rightleftharpoons}} \mathsf{Cargo \cdot Imp\beta} \underset{\mathsf{k}_{d2}}{\overset{\mathsf{k}_{a2}}{\rightleftharpoons}} \mathsf{Cargo^* \cdot Imp\beta}. \tag{7}$$

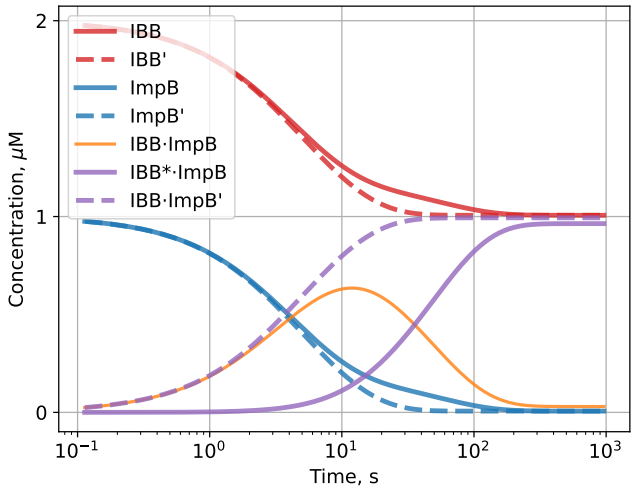

Figure 1: Stand-alone simulation of (6) starting with 2 μM IBB and 1 μM Impβ with the constants (8). The dashed counterpart is the effective system of the form $\mathsf{A}+\mathsf{B} \rightleftharpoons \mathsf{AB}$, cf. §2.1.

(3a)	$k^{\mathrm{R}}_{\mathrm{on}} = 0.096\,\mu\mathrm{M}^{-1}\,\mathrm{s}^{-1}$, $k^{\mathrm{R}}_{\mathrm{off}} = 4.8\times10^{-6}\,\mathrm{s}^{-1}$	[GSR03, Supp. Table A], [RM05, Table II]
(3b)	$k^{\mathrm{C}}_{\mathrm{on}} = 0.11\,\mu\mathrm{M}^{-1}\,\mathrm{s}^{-1}$, $k^{\mathrm{C}}_{\mathrm{off}} = 7.2\times10^{-4}\,\mathrm{s}^{-1}$	[Cat+01, Table I], [RM05, Table II]
(4)	$k_{\mathrm{knockoff}} = 2\times10^{-2}\,\mu\mathrm{M}^{-1}\mathrm{s}^{-1}$	[RM05, Table II]
(5)	$D_{\mathsf{Impβ}\cdot\mathsf{Ran}\cdot\mathsf{GTP}} = 0.07\,\mathrm{s}^{-1}$, $D_{\mathsf{Impβ}} = 0.4\,\mathrm{s}^{-1}$ $D_{\mathsf{Impβ}\cdot\mathsf{Cargo}} = 0.25\,\mathrm{s}^{-1}$, $D_{\mathsf{Cargo}} = 5\times10^{-4}\,\mathrm{s}^{-1}$	[RM05, Table III]

Table 1: Constants for the Impβ-mediated transport from §2.1.

Examples of the kinetic constants are available in [Cat+01, Table I], e.g.,

$$k_{a1} = 0.11\,\mu\mathrm{M}^{-1}\,\mathrm{s}^{-1}, \quad k_{d1} = 0.024\,\mathrm{s}^{-1}, \qquad k_{a2} = 0.024\,\mathrm{s}^{-1}, \quad k_{d2} = 7.4\times10^{-4}\,\mathrm{s}^{-1}, \tag{8}$$

for an IBB domain binding to Impβ. The intermediate state in (6) is transient on a moderately relevant time-scale, see Fig. 1 (code #2). Therefore, in the present model we lump the complexed states together and take $k^{\mathrm{C}}_{\mathrm{on}} := k_{a1}$ and $k^{\mathrm{C}}_{\mathrm{off}} := k_{d1}\frac{k_{d2}}{k_{a2}+k_{d2}}$ as the effective kinetic constants for (3b), cf. Table 1.

With the constants from Table 1, the steady-state of the model (reached after some 10^4 s) is reported in Fig. 2. Nuclear accumulation of free cargo is 37-fold. Sensitivity analysis shows that, in relative terms, the final nuclear concentration of free cargo depends most strongly on k_{knockoff}. Doubling k_{knockoff} almost doubles the nuclear concentration. Code #3.

This model predicts a slight accumulation of Imgβ in the nucleus, with Impβ · Ran · GTP contributing most of the excess. We improve on it in §2.3.

2.2 GTP hydrolysis and role of RanBP1

According to [LM97, Fig. 4A], Impβ blocks hydrolysis of Ran · GTP by RanGAP but RanBP1 rescues it for most part. Similarly, [BG97] showed that RanBP1 transiently detaches Ran

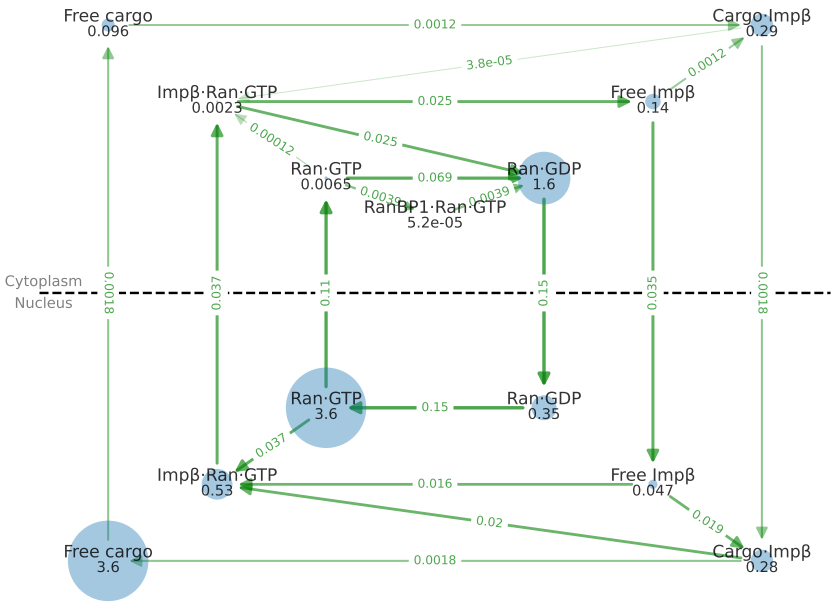

Figure 2: Steady-state of the transport system from §2.1 with conditions of Table 1. The free cargo shows 37-fold accumulation in the nucleus; total nuclear to total cytoplasmic cargo is 10-fold. Units are µM for species and $\mu M\,s^{-1}$ for fluxes. Initial conditions: $[\mathsf{Ran}\cdot\mathsf{GDP}]_{cyt} = 5\,\mu M$, $[\mathsf{Imp\beta}]_{cyt} = 1\,\mu M$, $[\mathsf{Cargo}]_{cyt} = 3\,\mu M$, all else zero.

from the complex $\mathsf{Kap}\cdot\mathsf{Ran}\cdot\mathsf{GTP}$ (where Kap can be importin β, transportin or CAS), whereupon hydrolysis by RanGAP disassembles the complex; and that efficient disassembly of $\mathsf{Imp\beta}\cdot\mathsf{Ran}\cdot\mathsf{GTP}$ required $\mathsf{RanBP1}$ *and* $\mathsf{Imp\alpha}$ [BG97, §3.2, cf. Fig. 4], [FBR97]. Importantly, Kaps and RanBP1 bind Ran at distinct sites [BG97, p.253].

Further, [See+03, Fig. 13] characterizes the kinetics of the formation of the complex between $\mathsf{Ran}\cdot\mathsf{GTP}$, $\mathsf{RanBP1}$ and RanGAP and the hydrolysis. In particular, the release rate of the γ-phosphate, on the order or $10\,s^{-1}$ in [See+03, Fig. 13], is barely influenced by $\mathsf{RanBP1}$, which instead stimulates the association of Ran with RanGAP. A computational model of hydrolysis with these parameters qualitatively reproduces the experimental data from [LM97, Fig. 4A]. We omit the details that can be found in Code #4.

For simplicity, we will take the constant

$$\mathsf{GTP}\text{ hydrolysis rate of }0.1\,s^{-1} \tag{9}$$

for all cytoplasmic-side species containing $\mathsf{Ran}\cdot\,\mathsf{GTP}$, and no GTP hydrolysis elsewhere. This should be compared with the effective Ran gradient rate (1).

2.3 NPC as compartment

Introduction. It has been observed TODO(2): ref that Impβ accumulates inside the NPCs as they bind to the FG-nups, suggesting a regulatory role, and possibly shuttling the cargo across the pore repeatedly. To account for this we propose a model with cytoplasm, nucleus and the NPCs as three compartments. The following dual observation is essential (cf. [Hof20]):

1. cytoplasmic and nuclear species initially react with NPC components in proportion to the number of NPCs, and
2. the observed fluorescence signal from Kaps accumulating inside the NPCs correlates with the total volume or the capacity of the NPC channel (rather than their number).

The model includes the following main components:

- Generic NLS cargo. TODO(3): on NLS
- Impα TODO(4): on ImpA
- Impβ TODO(5): on ImpB
- CAS TODO(6): on CAS
- The NPCs are described as vacant NPC channel space, the cytoplasm-facing opening NPC(c) and the nucleus-facing opening NPC(n). To transition the nuclear pore, a species has to bind to the opening, transition into the channel, bind to the other opening, and unbind on the other side. This allows us to model the capacity of the NPC and the dwelling time (but we make no distinction between different transiting species). There is no directionality, i.e. a species currently residing in the channel is equally likely to bind to either opening next.
- Ran · GTP in the nucleus and Ran · GDP in the cytoplasm. The consumption of Ran · GTP that have transitioned to the cytoplasmic side by hydrolysis is compensated by an effective pump as in (1). The hydrolysis itself is described by one effective kinetic rate as in (9).

An overview of the model is shown in Fig. 3. The computational results for this model are summarized in Fig. 4. The code is found in Code #5.

TODO(7): Use a particular checkpoint throughout

Main reactions. Here we comment on the main reactions of the model. For the complete set we refer to Fig. 3 as well as TODO(8): Link to checkpoint on github.io.

The cytoplasmic NLS cargo associates with free Impα · Impβ before binding to the cytoplasmic side of the NPC, or with those already attached there. On the nuclear side, the "knockoff"

reaction releases the cargo (the suffix (n) is omitted):

$$\mathsf{Ran}\cdot\mathsf{GTP} + \mathsf{NLS}\cdot\mathsf{Imp}\alpha\cdot\mathsf{Imp}\beta\cdot\mathsf{NPC} \longrightarrow \mathsf{Ran}\cdot\mathsf{GTP}\cdot\mathsf{Imp}\beta\cdot\mathsf{NPC} + \mathsf{Imp}\alpha + \mathsf{NLS}. \tag{10}$$

TODO(9): More reactions

Baseline parameters. Here we comment on the choice of selected model parameters, such as concentrations and kinetic rates. The complete set of parameters is recorded in [TODO(10): Link to checkpoint base model].

We estimate the total NPC channel capacity as 2000 NPCs per nuclear envelope times 300 Kaps per NPC TODO(11): refs for those numbers. Note that

$$1\,\mathrm{pL} \times 1\,\mu\mathrm{M} \approx 300 \times 2000\,\mathrm{units}, \tag{11}$$

which implies a concentration of $1\,\mu\mathrm{M}$ in a computational volume of $1\,\mathrm{pL}$. The computation itself is performed in amounts (rather than concentration), so to convert apparent concentration of Kaps at the NE we estimate TODO(12): automate insertion of this volume

$$\text{the volume of apparent NE fluorescence as } 0.01\,\mathrm{pL}. \tag{12}$$

TODO(13): complete section

Variants. We have defined a handful of scenarios to illustrate how parameter choice affects the steady-states. The results are summarized in Fig. 4. All parameter changes are reported in [TODO(14): Link to the checkpoint parameter table].

- Increase $\mathsf{Imp}\beta$. TODO(15): Why
- Increase also the number of NPC. TODO(16): Why
- Starting from the baseline scenario, we substantially decrease the initial concentration of $\mathsf{Imp}\alpha$. In this scenario we obtain a clear N:C contrast for total CAS in the steadstate, in line with experimental evidence from [**?**] TODO(17): CAS contrast ref.
- Turn off $\mathsf{Ran}\cdot\mathsf{GTP}$ regeneration, starting from the baseline scenario. We obtain a breakdown of N:C contrast for all species, as expected.

3 Conclusions

TODO(18): conclude

4 Old stuff dump – ignore

The crux is now that the nuclear envelope, having small volume, has a high concentration of NPCs. At the nuclear envelope we posit the reactions

$$\mathsf{Imp\beta}_i + \mathsf{NPC}_{\mathsf{vacant}} \rightleftharpoons \mathsf{Imp\beta \cdot NPC} \tag{13a}$$

$$\mathsf{Cargo \cdot Imp\beta}_i + \mathsf{NPC}_{\mathsf{vacant}} \rightleftharpoons \mathsf{Cargo \cdot Imp\beta \cdot NPC} \tag{13b}$$

TODO(19): ref with $k_{\text{on}} = 10^{-3}\,\mu\text{M}^{-1}\,\text{s}^{-1}$ and $k_{\text{off}} = 10^{-4}\,\text{s}^{-1}$, as well as

$$\mathsf{Cargo}_i + \mathsf{Imp\beta \cdot NPC} \rightleftharpoons \mathsf{Cargo \cdot Imp\beta \cdot NPC} \tag{14}$$

with $k^{\text{C}}_{\text{on}} = 0.11\,\mu\text{M}^{-1}\,\text{s}^{-1}$ and $k^{\text{C}}_{\text{off}} = 7.2 \times 10^{-4}\,\text{s}^{-1}$, where i can be "cytoplasmic" or "nuclear". This envelope compartment is in diffusive exchange with cytoplasm ($i = \text{cyt}$) and nucleus ($i = \text{nuc}$) with the permeability constant $D = 1\,\text{s}^{-1}$. In both, we also allow

$$\mathsf{Cargo} + \mathsf{Imp\beta} \rightleftharpoons \mathsf{Cargo \cdot Imp\beta} \tag{15}$$

with the same $k^{\text{C}}_{\text{on}}/k^{\text{C}}_{\text{off}}$. For simplicity, we assume $[\mathsf{RanGTP}] = 3\,\mu\text{M}$ and $[\mathsf{RanGDP}] = 2\,\mu\text{M}$ are maintained at fixed concentrations and are only relevant at the envelope, where we have

$$\text{cargo knockoff:}\quad \mathsf{RanGTP}_{\mathsf{nuc}} + \mathsf{Cargo \cdot Imp\beta \cdot NPC} \longrightarrow \mathsf{Cargo} + \mathsf{RanGTP \cdot Imp\beta \cdot NPC} \tag{16}$$

$$\text{GTP hydrolysis:}\quad \mathsf{RanGTP \cdot Imp\beta \cdot NPC} \longrightarrow \mathsf{RanGDP}_{\mathsf{cyt}} + \mathsf{P} + \mathsf{Imp\beta \cdot NPC}. \tag{17}$$

For hydrolysis we assume the reaction rate $v_{\text{GAP}}/(1+K_{\text{GAP}}/[\mathsf{RanGTP \cdot Imp\beta \cdot NPC}])$, similarly to (23a). We take $v_{\text{GAP}} = 0.07\,\mu\text{M}\,\text{s}^{-1}$ and $K_{\text{GAP}} = 0.1\,\mu\text{M}$. This reaction rate about $100\times$ smaller than in (23a) but in view of TODO(20): ref, this seems more realistic and has little effect on the course of the simulation.

Starting from $[\mathsf{Cargo}]_{\text{cyt}} = 1\,\mu\text{M}$ and $[\mathsf{Imp\beta}]_{\text{cyt}} = [\mathsf{Imp\beta}]_{\text{nuc}} = 0.5\,\mu\text{M}$, this model predicts a 6-fold accumulation of total cargo in the nucleus in steady-state. Meanwhile, the concentration of $\mathsf{Imp\beta}$ at the envelope is approximately $10^3\,\mu\text{M}$.

Code #6.

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5 Appendix

5.1 Minimal Ran gradient system

Here we recapitulate the minimal Ran gradient system from [GSR03, Fig. 2], cf. §2.1. The following account for the cytoplasmic species. Here, $[\dots]$ abbreviates the (cytoplasmic) concentration of the complex $\mathsf{RanBP1}\cdot\mathsf{Ran}\cdot\mathsf{GTP}$. Ex is an additional potentially useful flux of nuclear $\mathsf{Ran}\cdot\mathsf{GTP}$ to cytoplasmic $\mathsf{Ran}\cdot\mathsf{GDP}$, set by default to zero.

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Ran}\cdot\mathsf{GDP}]_{\mathrm{cyt}} = \mathsf{F}_{\mathsf{Ran}\cdot\mathsf{GDP}}\frac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} + \mathsf{GAP} + \mathsf{GAP}_{\mathsf{RanBP1}} + \mathsf{Ex}\frac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} \tag{18a}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{cyt}} = \mathsf{F}_{\mathsf{Ran}\cdot\mathsf{GTP}}\frac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} - \mathsf{GAP} - k_{\mathrm{on}}^{\mathrm{rbp}}[\mathsf{RanBP1}][\mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{cyt}} + k_{\mathrm{off}}^{\mathrm{rbp}}[\dots] \tag{18b}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{RanBP1}\cdot\mathsf{Ran}\cdot\mathsf{GTP}] = -\mathsf{GAP}_{\mathsf{RanBP1}} + k_{\mathrm{on}}^{\mathrm{rbp}}[\mathsf{RanBP1}][\mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{cyt}} - k_{\mathrm{off}}^{\mathrm{rbp}}[\dots] \tag{18c}$$

The following account for the nuclear species. As in [GSR03], E denotes free $\mathsf{RCC1}$.

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Ran}\cdot\mathsf{GDP}]_{\mathrm{nuc}} = -\mathsf{F}_{\mathsf{Ran}\cdot\mathsf{GDP}} + r_8[\mathsf{IntC}] - r_1[\mathsf{E}][\mathsf{Ran}\cdot\mathsf{GDP}]_{\mathrm{nuc}} \tag{19a}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{nuc}} = -\mathsf{F}_{\mathsf{Ran}\cdot\mathsf{GTP}} + r_4[\mathsf{IntA}] - r_5[\mathsf{E}][\mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{nuc}} - \mathsf{Ex} \tag{19b}$$

The nucleotide-exchange reaction $\mathsf{Ran}\cdot\mathsf{GDP} + \mathsf{GTP} \rightleftharpoons \mathsf{Ran}\cdot\mathsf{GTP} + \mathsf{GDP}$ is catalyzed by $\mathsf{RCC1}$. It is modeled as in [Kle+95, Fig. 6] / [GSR03, Fig. 1] with three intermediates. Note that it depends on the availability of GDP and GTP.

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{IntA}] = -(r_4 + r_6)[\mathsf{IntA}] + r_5[\mathsf{E}][\mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{nuc}} + r_3[\mathsf{GTP}][\mathsf{IntB}] \tag{20a}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{IntB}] = r_6[\mathsf{IntA}] + r_2[\mathsf{IntC}] - (r_3[\mathsf{GTP}] + r_7[\mathsf{GDP}])[\mathsf{IntB}] \tag{20b}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathsf{IntC}] = -(r_2 + r_8)[\mathsf{IntC}] + r_1[\mathsf{E}][\mathsf{Ran}\cdot\mathsf{GDP}]_{\mathrm{nuc}} + r_7[\mathsf{GDP}][\mathsf{IntB}] \tag{20c}$$

Constraints on the total concentration:

$$\text{Free RCC1}: \qquad [\mathsf{E}] = \mathsf{RCC1}_{\mathsf{total}} - ([\mathsf{IntA}] + [\mathsf{IntB}] + [\mathsf{IntC}]) \tag{21a}$$

$$\text{Free RanBP1}: \qquad [\mathsf{RanBP1}] = \mathsf{RanBP1}_{\mathsf{total}} - [\mathsf{RanBP1}\cdot\mathsf{Ran}\cdot\mathsf{GTP}] \tag{21b}$$

Gradient-driven fluxes from the nucleus to the cytoplasm:

$$\mathsf{F}_{\mathsf{Ran.GTP}} = D_{\mathsf{Ran}\cdot\mathsf{GTP}}\left([\mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{nuc}} - [\mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{cyt}}\right) \tag{22a}$$

$$\mathsf{F}_{\mathsf{Ran.GDP}} = D_{\mathsf{Ran}\cdot\mathsf{GDP}}\left([\mathsf{Ran}\cdot\mathsf{GDP}]_{\mathrm{nuc}} - [\mathsf{Ran}\cdot\mathsf{GDP}]_{\mathrm{cyt}}\right) \tag{22b}$$

RanGAP hydrolyzes the γ-phosphate of $\mathsf{Ran}\cdot\mathsf{GTP}$. This is more efficient when $\mathsf{Ran}\cdot\mathsf{GTP}$ is bound to $\mathsf{RanBP1}$ [Bis+95], reducing the IC50 seven-fold [GSR03, Table I, p. 1091].

$$\mathsf{GAP} = k_{\mathsf{GAP}}[\mathsf{RanGAP}]/(1 + K_{\mathsf{GAP}}/[\mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{cyt}}) \tag{23a}$$

$$\mathsf{GAP}_{\mathsf{RanBP1}} = k'_{\mathsf{GAP}}[\mathsf{RanGAP}]/(1 + K'_{\mathsf{GAP}}/[\mathsf{RanBP1}\cdot\mathsf{Ran}\cdot\mathsf{GTP}]) \tag{23b}$$

To determine the dynamic capacity Ex at steady-state we introduce the additional equation:

$$\frac{\mathrm{d}}{\mathrm{d}t}\mathsf{Ex} = k_{\mathsf{Ex}}\,[\mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{nuc}}, \quad k_{\mathsf{Ex}} := 10\,\mathrm{s}^{-2}, \quad \text{initial} \quad \mathsf{Ex} := 0\,\mu\mathrm{M}\,\mathrm{s}^{-1}. \tag{24}$$

(18a)	$V_{\text{nuc}} = 1.2\,\text{pl}, \quad V_{\text{cyt}} = 1.8\,\text{pl}$	[GSR03, Table II]
(18a)	initial condition $[\mathsf{Ran}\cdot\mathsf{GDP}]_{\text{cyt}} = 5\,\mu\text{M}$	[GSR03, Table II]
(18b)–(18c)	$k_{\text{on}}^{\text{rbp}} = 0.3\,\mu\text{M}^{-1}\,\text{s}^{-1}, \quad k_{\text{off}}^{\text{rbp}} = 4\times10^{-4}\,\text{s}^{-1}$	[GSR03, Supp. Table A]
(19a)–(20c)	$r_1 = 74\,\mu\text{M}^{-1}\,\text{s}^{-1}, \quad r_8 = 55\,\text{s}^{-1}$ $r_7 = 11\,\mu\text{M}^{-1}\,\text{s}^{-1}, \quad r_2 = 21\,\text{s}^{-1}$ $r_3 = 0.6\,\mu\text{M}^{-1}\,\text{s}^{-1}, \quad r_6 = 19\,\text{s}^{-1}$ $r_5 = 100\,\mu\text{M}^{-1}\,\text{s}^{-1}, \quad r_4 = 55\,\text{s}^{-1}$	[GSR03, Supp. Table A] [Kle+95, Fig. 6]
(20a)–(20c)	$[\mathsf{GTP}] = 500\,\mu\text{M}, \quad [\mathsf{GDP}] = 1.6\,\mu\text{M}$	[GSR03, Table II]
(21a)	$\mathsf{RCC1}_{\mathsf{total}} = 0.7\,\mu\text{M}$	[GSR03, Supp. Table B]
(21b)	$\mathsf{RanBP1}_{\mathsf{total}} = 2\,\mu\text{M}$	[GSR03, Fig. 4]
(22a) (22b)	$D_{\mathsf{Ran}\cdot\mathsf{GTP}} = 0.03\,\text{s}^{-1}$ $D_{\mathsf{Ran}\cdot\mathsf{GDP}} = 0.12\,\text{s}^{-1}$	[GSR03, Table II]
(23a)	$k_{\mathsf{GAP}} = 10.6\,\text{s}^{-1}, \quad K_{\mathsf{GAP}} = 0.7\,\mu\text{M}$	[GSR03, Supp. Table A]
(23b)	$k'_{\mathsf{GAP}} = 10.8\,\text{s}^{-1}, \quad K'_{\mathsf{GAP}} = 0.1\,\mu\text{M}$	[GSR03, Table I]
(23a)–(23b)	cytoplasmic $[\mathsf{RanGAP}] = 0.7\,\mu\text{M}$	[GSR03, Table II / ST B]

Table 2: Constants for the "standard simulation condition" of §2.1 at 25 °C. Except for (18a), all species are initialized to zero at $t = 0$.

Condition	Affected parameters	Nuclear RanGTP, µM	Cytoplasmic RanGTP, nM	Dynamic capacity, µM/s
"Standard"	See Table 2	4.26 (4.3)	7.75 (7.7)	0.59 (0.60)
Omission of RanBP1	$\mathsf{RanBP1}_{\mathsf{total}} := 0$	4.27 (4.3)	8.13 (8.1)	0.59 (0.60)
200% RCC1	$\mathsf{RCC1}_{\mathsf{total}}$	3.95 (4.0)	7.17 (7.1)	0.59 (0.60)
50% RCC1	$\mathsf{RCC1}_{\mathsf{total}}$	4.31 (4.3)	7.82 (7.7)	0.58 (0.60)
10% RCC1	$\mathsf{RCC1}_{\mathsf{total}}$	3.59 (3.6)	6.50 (6.4)	0.46 (0.48)
1% RCC1	$\mathsf{RCC1}_{\mathsf{total}}$	1.40 (1.4)	2.52 (2.5)	0.075 (0.08)
GTP:GDP = 500:0	$[\mathsf{GDP}] := 0\,\mu\text{M}$	4.80 (4.8)	8.72 (8.6)	0.59 (0.60)
GTP:GDP = 500:50	$[\mathsf{GDP}] := \frac{1}{10}[\mathsf{GTP}]$	0.98 (0.8)	1.76 (1.5)	0.57 (0.58)
GTP:GDP = 500:500	$[\mathsf{GDP}] := [\mathsf{GTP}]$	0.12 (0.12)	0.22 (0.21)	0.34 (0.34)
Saturating NTF2	$D_{\mathsf{Ran}\cdot\mathsf{GDP}} := 0.48\,\text{s}^{-1}$	5.12 (5.1)	9.32 (9.2)	2.18 (2.2)
No NTF2	$D_{\mathsf{Ran}\cdot\mathsf{GDP}} := D_{\mathsf{Ran}\cdot\mathsf{GTP}}$	2.55 (2.5)	4.60 (4.5)	0.15 (0.16)
200% RanGAP	$[\mathsf{RanGAP}]$	4.27 (4.3)	3.95 (3.9)	0.59 (0.60)
50% RanGAP	$[\mathsf{RanGAP}]$	4.26 (4.3)	14.9 (14)	0.59 (0.60)
50% permeability	$D_{\mathsf{Ran}\cdot\mathsf{GTP}}$	4.91 (4.9)	4.44 (4.4)	0.59 (–)
200% permeability	$D_{\mathsf{Ran}\cdot\mathsf{GTP}}$	3.41 (3.4)	12.4 (12.3)	0.59 (–)
400% permeability	$D_{\mathsf{Ran}\cdot\mathsf{GTP}}$	2.46 (2.5)	18.0 (17.8)	0.59 (–)

Table 3: Steady-state concentrations for the simulation scenarios from [GSR03, Table II/III], with their results shown in brackets. Value for $D_{\mathsf{Ran}\cdot\mathsf{GDP}}$ is from [GSR03, Fig. 3].

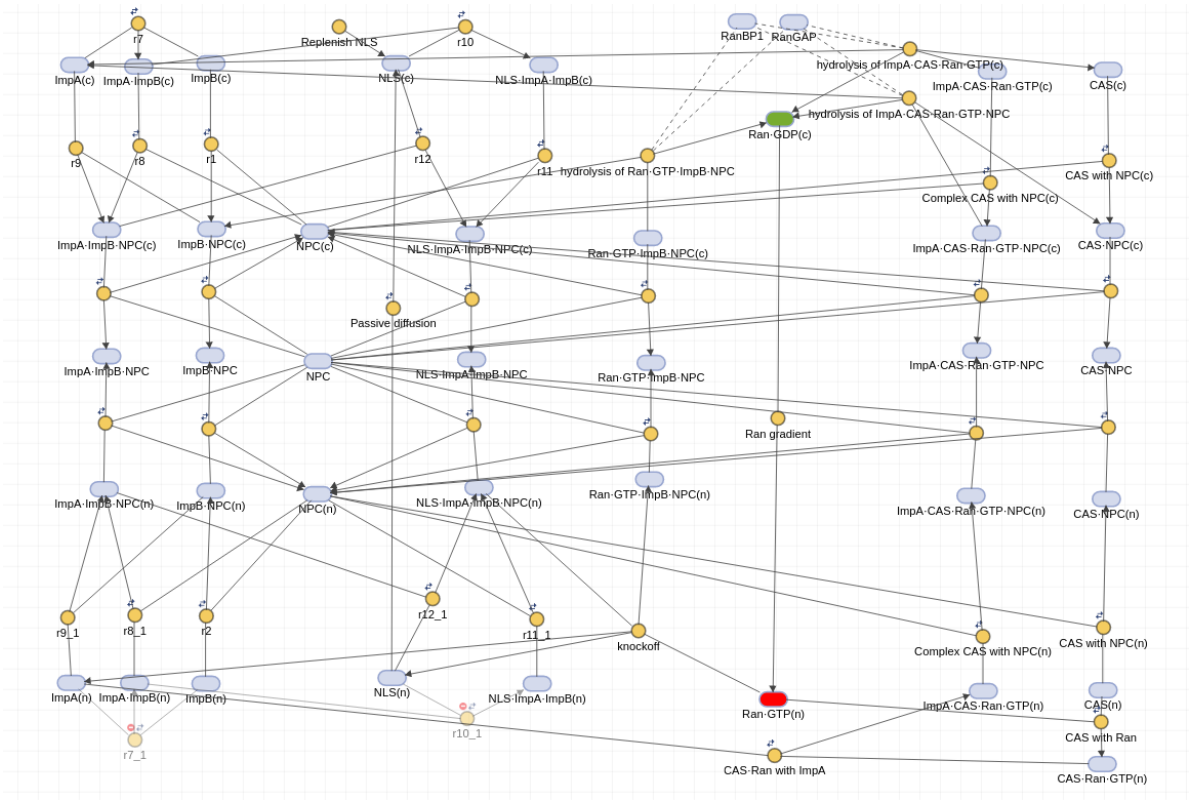
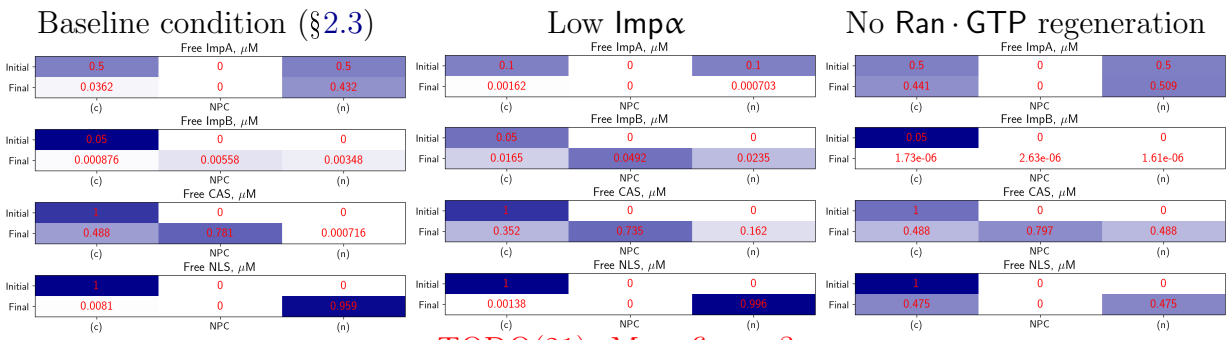


Figure 3: SimBiology diagram for the model "NPC as compartment" from §2.3.



TODO(21): More figures?
TODO(22): Consider displaying on a landscape page

Figure 4: Initial states and steady-states for the model "NPC as compartment" from §2.3. For more full-size figures, see TODO(24): Link to the full set

5.2 NPC as compartment

5.3 List of codes

	page	https://github.com/numpde/nct1/tree/ ...
#1	p.2	main/code/20210225-GSR/v1
#2	p.3	main/code/20210407-Rearrangement
#3	p.3	main/code/20210225-GSR/v2
#4	p.4	main/code/20210403-StickyPore/c_rangap-sequence
#5	p.5	main/code/20211018-Appli
#6	p.7	main/code/20210403-StickyPore

6 TODO

TODOs:

1. p.1. intro
2. p.5. ref
3. p.5. on NLS
4. p.5. on ImpA
5. p.5. on ImpB
6. p.5. on CAS
7. p.5. Use a particular checkpoint throughout
8. p.5. Link to checkpoint on github.io
9. p.6. More reactions
10. p.6. Link to checkpoint base model
11. p.6. refs for those numbers
12. p.6. automate insertion of this volume
13. p.6. complete section
14. p.6. Link to the checkpoint parameter table
15. p.6. Why
16. p.6. Why
17. p.6. CAS contrast ref
18. p.6. conclude

19. p.7. ref
20. p.7. ref
21. p.12. More figures?
22. p.12. Consider displaying on a landscape page
23. p.**??**. Link to the full set
24. p.12. Link to the full set