DRAFT: NCT

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2021-04-02

1 NCT models

Abbreviations: ODE = ordinary differential equations.

1.1 GSR'03 model of NCT

Ran gradient. First we implement the "minimal Ran gradient system" from [GSR03]. The equations are shown in Table 1 and the constants are collected in Table 2. The "dynamic capacity" Ex is an optional maximal steady-state (positive) flux of nuclear $\mathsf{Ran \cdot GTP}$ to cytoplasmic $\mathsf{Ran \cdot GDP}$, which we determine using the additional equation

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

The fluxes are in units of concentration/time ($\mathrm{\mu M\,s^{-1}}$). The ones across the nuclear boundary have positive sign when exiting the nucleus and are normalized to the nuclear volume. 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, a 1000-fold nuclear enrichment of $\mathsf{Ran \cdot GTP}$ is sustained in steady-state.

Code: d56d16f/code/20210225-GSR/v1

Coupling to transport. A coupling of the Ran gradient to importin–cargo transport was proposed in [GSR03, Fig. 6A]. We formulate a version of it in Table 4.

With the constants from Table 5, the steady-state of the model (reached after some $10 \times 10^4\,\mathrm{s}$) is reported in Fig. 1. Nuclear accumulation of free cargo is over 20-fold. Sensitivity analysis shows that, in relative terms, the final nuclear concentration of free cargo depends most strongly on k_{knockoff} (and the volume of the nucleus). In particular, doubling k_{knockoff} almost doubles the concentration.

Code: 2a2199d/code/20210225-GSR/v2

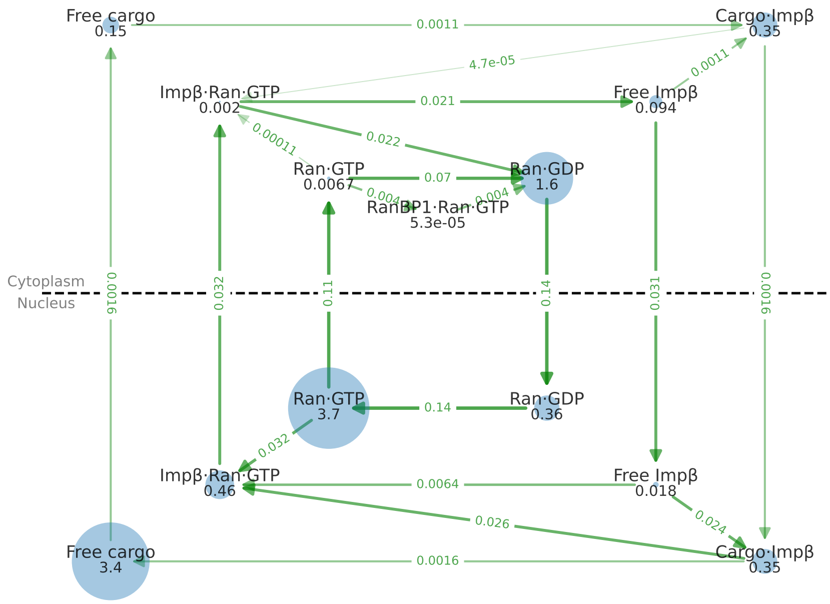

Figure 1: Steady-state of the transport system from §1.1 / Table 4 with conditions of Table 5. The free cargo shows a 20-fold accumulation in the nucleus. Units are µM for species and $\mu\mathrm{M\,s^{-1}}$ for fluxes. Initial conditions: $[\mathsf{Ran}\cdot\mathsf{GDP}]_{\mathrm{cyt}} = 5\,\mu\mathrm{M}$, $[\mathsf{Imp}\beta]_{\mathrm{cyt}} = 1\,\mu\mathrm{M}$, $[\mathsf{Cargo}]_{\mathrm{cyt}} = 3\,\mu\mathrm{M}$, all else zero.

TODO(1): ? [Cat+01] and [RM05] discuss the reaction $\mathsf{Imp}\beta\cdot\mathsf{Cargo} \rightleftharpoons \mathsf{Imp}\beta^{*}\cdot\mathsf{Cargo}$

The following account for the cytoplasmic species. Here, $[\ldots]$ abbreviates the (cytoplasmic) concentration of the complex $\mathsf{RanBP1} \cdot \mathsf{Ran} \cdot \mathsf{GTP}$.

$$\begin{aligned}
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Ran} \cdot \mathsf{GDP}]_{\mathrm{cyt}} &= \mathsf{F}_{\mathsf{Ran} \cdot \mathsf{GDP}} \tfrac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} + \mathsf{GAP} + \mathsf{GAP}_{\mathsf{RanBP1}} + \mathsf{Ex} \tfrac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} && \text{(2a)} \\
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Ran} \cdot \mathsf{GTP}]_{\mathrm{cyt}} &= \mathsf{F}_{\mathsf{Ran} \cdot \mathsf{GTP}} \tfrac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} - \mathsf{GAP} - k_{\mathrm{on}}^{\mathrm{rbp}}[\mathsf{RanBP1}][\mathsf{Ran} \cdot \mathsf{GTP}]_{\mathrm{cyt}} + k_{\mathrm{off}}^{\mathrm{rbp}}[\ldots] && \text{(2b)} \\
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{RanBP1} \cdot \mathsf{Ran} \cdot \mathsf{GTP}] &= -\mathsf{GAP}_{\mathsf{RanBP1}} \qquad + k_{\mathrm{on}}^{\mathrm{rbp}}[\mathsf{RanBP1}][\mathsf{Ran} \cdot \mathsf{GTP}]_{\mathrm{cyt}} - k_{\mathrm{off}}^{\mathrm{rbp}}[\ldots] && \text{(2c)}
\end{aligned}$$

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

$$\begin{aligned}
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Ran} \cdot \mathsf{GDP}]_{\mathrm{nuc}} &= -\mathsf{F}_{\mathsf{Ran} \cdot \mathsf{GDP}} + r_8[\mathsf{IntC}] - r_1[\mathsf{E}][\mathsf{Ran} \cdot \mathsf{GDP}]_{\mathrm{nuc}} && \text{(3a)} \\
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Ran} \cdot \mathsf{GTP}]_{\mathrm{nuc}} &= -\mathsf{F}_{\mathsf{Ran} \cdot \mathsf{GTP}} + r_4[\mathsf{IntA}] - r_5[\mathsf{E}][\mathsf{Ran} \cdot \mathsf{GTP}]_{\mathrm{nuc}} - \mathsf{Ex} && \text{(3b)}
\end{aligned}$$

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.

$$\begin{aligned}
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{IntA}] &= -(r_4 + r_6)[\mathsf{IntA}] + r_5[\mathsf{E}][\mathsf{Ran} \cdot \mathsf{GTP}]_{\mathrm{nuc}} + r_3[\mathsf{GTP}][\mathsf{IntB}] && \text{(4a)} \\
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{IntB}] &= r_6[\mathsf{IntA}] + r_2[\mathsf{IntC}] - (r_3[\mathsf{GTP}] + r_7[\mathsf{GDP}])[\mathsf{IntB}] && \text{(4b)} \\
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{IntC}] &= -(r_2 + r_8)[\mathsf{IntC}] + r_1[\mathsf{E}][\mathsf{Ran} \cdot \mathsf{GDP}]_{\mathrm{nuc}} + r_7[\mathsf{GDP}][\mathsf{IntB}] && \text{(4c)}
\end{aligned}$$

Constraints on the total concentration:

$$\begin{aligned}
\text{Free } \mathsf{RCC1}: &\qquad [\mathsf{E}] = \mathsf{RCC1}_{\mathsf{total}} - ([\mathsf{IntA}] + [\mathsf{IntB}] + [\mathsf{IntC}]) && \text{(5a)} \\
\text{Free } \mathsf{RanBP1}: &\qquad [\mathsf{RanBP1}] = \mathsf{RanBP1}_{\mathsf{total}} - [\mathsf{RanBP1} \cdot \mathsf{Ran} \cdot \mathsf{GTP}] && \text{(5b)}
\end{aligned}$$

Gradient-driven fluxes from the nucleus to the cytoplasm:

$$\begin{aligned}
\mathsf{F}_{\mathsf{Ran.GTP}} &= D_{\mathsf{Ran} \cdot \mathsf{GTP}} \left([\mathsf{Ran} \cdot \mathsf{GTP}]_{\mathrm{nuc}} - [\mathsf{Ran} \cdot \mathsf{GTP}]_{\mathrm{cyt}}\right) && \text{(6a)} \\
\mathsf{F}_{\mathsf{Ran.GDP}} &= D_{\mathsf{Ran} \cdot \mathsf{GDP}} \left([\mathsf{Ran} \cdot \mathsf{GDP}]_{\mathrm{nuc}} - [\mathsf{Ran} \cdot \mathsf{GDP}]_{\mathrm{cyt}}\right) && \text{(6b)}
\end{aligned}$$

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].

$$\begin{aligned}
\mathsf{GAP} &= k_{\mathsf{GAP}}[\mathsf{RanGAP}]/(1 + K_{\mathsf{GAP}}/[\mathsf{Ran} \cdot \mathsf{GTP}]_{\mathrm{cyt}}) && \text{(7a)} \\
\mathsf{GAP}_{\mathsf{RanBP1}} &= k'_{\mathsf{GAP}}[\mathsf{RanGAP}]/(1 + K'_{\mathsf{GAP}}/[\mathsf{RanBP1} \cdot \mathsf{Ran} \cdot \mathsf{GTP}]) && \text{(7b)}
\end{aligned}$$

Table 1: The minimal Ran gradient system from [GSR03, Fig. 2]. 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.

(2a)	$V_{\text{nuc}} = 1.2\,\text{pl}, \quad V_{\text{cyt}} = 1.8\,\text{pl}$	[GSR03, Table II]
(2a)	initial condition $[\mathsf{Ran}\cdot\mathsf{GDP}]_{\text{cyt}} = 5\,\mu\text{M}$	[GSR03, Table II]
(2b)–(2c)	$k_{\text{on}}^{\text{rbp}} = 0.3\,\mu\text{M}^{-1}\,\text{s}^{-1}, \quad k_{\text{off}}^{\text{rbp}} = 4\times10^{-4}\,\text{s}^{-1}$	[GSR03, Supp. Table A]
(3a)–(4c)	$r_1 = 74\,\mu\text{M}^{-1}\,\text{s}^{-1}, \quad r_8 = 55\,\text{s}^{-1}$ $r_7 = 11\,\mu\text{M}^{-1}\,\text{s}^{-1}, \quad r_2 = 21\,\text{s}^{-1}$ $r_3 = 0.6\,\mu\text{M}^{-1}\,\text{s}^{-1}, \quad r_6 = 19\,\text{s}^{-1}$ $r_5 = 100\,\mu\text{M}^{-1}\,\text{s}^{-1}, \quad r_4 = 55\,\text{s}^{-1}$	[GSR03, Supp. Table A] [Kle+95, Fig. 6]
(4a)–(4c)	$[\mathsf{GTP}] = 500\,\mu\text{M}, \quad [\mathsf{GDP}] = 1.6\,\mu\text{M}$	[GSR03, Table II]
(5a) (5b)	$\mathsf{RCC1}_{\mathsf{total}} = 0.7\,\mu\text{M}$ $\mathsf{RanBP1}_{\mathsf{total}} = 2\,\mu\text{M}$	[GSR03, Supp. Table B] [GSR03, Fig. 4]
(6a) (6b)	$D_{\mathsf{Ran}\cdot\mathsf{GTP}} = 0.03\,\text{s}^{-1}$ $D_{\mathsf{Ran}\cdot\mathsf{GDP}} = 0.12\,\text{s}^{-1}$	[GSR03, Table II]
(7a) (7b)	$k_{\mathsf{GAP}} = 10.6\,\text{s}^{-1}, \quad K_{\mathsf{GAP}} = 0.7\,\mu\text{M}$ $k'_{\mathsf{GAP}} = 10.8\,\text{s}^{-1}, \quad K'_{\mathsf{GAP}} = 0.1\,\mu\text{M}$	[GSR03, Supp. Table A] [GSR03, Table I]
(7a)–(7b)	cytoplasmic $[\mathsf{RanGAP}] = 0.7\,\mu\text{M}$	[GSR03, Table II / ST B]

Table 2: Constants for the "standard simulation condition" of §1.1 at 25 °C. Except for (2a), 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].

The following equations comprise the handling of cargo by $\mathsf{Imp}\beta$ in the cytoplasm.

$$
\begin{aligned}
\mathsf{R}_{\mathsf{cyt}} &:= -k_{\mathrm{on}}^{\mathrm{R}}[\mathsf{Imp}\beta][\mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{cyt}} + k_{\mathrm{off}}^{\mathrm{R}}[\mathsf{Imp}\beta\cdot\ \mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{cyt}} && (8a)\\
\mathsf{C}_{\mathsf{cyt}} &:= -k_{\mathrm{on}}^{\mathrm{C}}[\mathsf{Imp}\beta][\mathsf{Cargo}]_{\mathrm{cyt}} + k_{\mathrm{off}}^{\mathrm{C}}[\mathsf{Imp}\beta\cdot\ \mathsf{Cargo}]_{\mathrm{cyt}} && (8b)\\
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Imp}\beta\cdot\ \mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{cyt}} &= -\mathsf{R}_{\mathsf{cyt}} + \mathsf{F}_{\mathsf{Imp}\beta\cdot\mathsf{Ran}\cdot\mathsf{GTP}}\tfrac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} - \mathsf{GAP}_{\mathsf{Imp}\beta} + \mathsf{Knockoff}_{\mathsf{cyt}} && (8c)\\
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Imp}\beta]_{\mathrm{cyt}} &= +\mathsf{R}_{\mathsf{cyt}} + \mathsf{C}_{\mathsf{cyt}} + \mathsf{F}_{\mathsf{Imp}\beta}\tfrac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} + \mathsf{GAP}_{\mathsf{Imp}\beta} && (8d)\\
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Imp}\beta\cdot\ \mathsf{Cargo}]_{\mathrm{cyt}} &= -\mathsf{C}_{\mathsf{cyt}} + \mathsf{F}_{\mathsf{Imp}\beta\cdot\mathsf{Cargo}}\tfrac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} - \mathsf{Knockoff}_{\mathsf{cyt}} && (8e)\\
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Cargo}]_{\mathrm{cyt}} &= +\mathsf{C}_{\mathsf{cyt}} + \mathsf{F}_{\mathsf{Cargo}}\tfrac{V_{\mathrm{nuc}}}{V_{\mathrm{cyt}}} + \mathsf{Knockoff}_{\mathsf{cyt}} && (8f)
\end{aligned}
$$

The flux of the reaction

$$\mathsf{Imp}\beta\cdot\ \mathsf{Cargo} + \mathsf{Ran}\cdot\mathsf{GTP} \rightleftharpoons \mathsf{Imp}\beta\cdot\ \mathsf{Ran}\cdot\mathsf{GTP} + \mathsf{Cargo} \qquad (9)$$

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

$$
\begin{aligned}
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Ran}\cdot\mathsf{GDP}]_{\mathrm{cyt}} &= (2a) + \mathsf{GAP}_{\mathsf{Imp}\beta} && (2a')\\
\tfrac{\mathrm{d}}{\mathrm{d}t}[\mathsf{Ran}\cdot\mathsf{GTP}]_{\mathrm{cyt}} &= (2b) + \mathsf{R}_{\mathsf{cyt}} - \mathsf{Knockoff}_{\mathsf{cyt}} && (2b')
\end{aligned}
$$

Analogous nuclear equations (without GAP) are implemented but are omitted here. Analogously to (6a)/(6b) we have the additional nuclear-to-cytoplasmic diffusion fluxes

$$\mathsf{F}_{\mathsf{Imp}\beta\cdot\mathsf{Ran}\cdot\mathsf{GTP}},\quad \mathsf{F}_{\mathsf{Imp}\beta},\quad \mathsf{F}_{\mathsf{Imp}\beta\cdot\mathsf{Cargo}},\quad \mathsf{F}_{\mathsf{Cargo}}. \qquad (10)$$

Table 4: Equations for the coupling of the minimal Ran gradient system from §1.1 to importin-mediated cargo transport.

(8a)	$k_{\mathrm{on}}^{\mathrm{R}} = 0.096\,\mathrm{\mu M^{-1}\,s^{-1}}$, $k_{\mathrm{off}}^{\mathrm{R}} = 4.8\times10^{-6}\,\mathrm{s^{-1}}$	[GSR03, Supp. Table A], [RM05, Table II]
(8b)	$k_{\mathrm{on}}^{\mathrm{C}} = 0.49\,\mathrm{\mu M^{-1}\,s^{-1}}$, $k_{\mathrm{off}}^{\mathrm{C}} = 0.017\,\mathrm{s^{-1}}$	[Cat+01, below Fig. 3], [RM05, Table II]
(9)	$k_{\mathrm{knockoff}} = 2\times10^{-2}\,\mathrm{\mu M^{-1}s^{-1}}$	[RM05, Table II]
(10)	$D_{\mathsf{Imp}\beta\,\cdot\,\mathsf{Ran}\,\cdot\,\mathsf{GTP}} = 0.07\,\mathrm{s^{-1}}$, $D_{\mathsf{Imp}\beta} = 0.4\,\mathrm{s^{-1}}$ $D_{\mathsf{Imp}\beta\,\cdot\,\mathsf{Cargo}} = 0.25\,\mathrm{s^{-1}}$, $D_{\mathsf{Cargo}} = 5\times10^{-4}\,\mathrm{s^{-1}}$	[RM05, Table III]

Table 5: Constants for the $\mathsf{Imp}\beta$-mediated transport from §1.1 / Table 4.

References

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TODOs:

1. p.1. ? [Cat+01] and [RM05] discuss the reaction $\mathsf{Imp}\beta \cdot \mathsf{Cargo} \rightleftharpoons \mathsf{Imp}\beta^* \cdot \mathsf{Cargo}$

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