Nuclear FRAP Model

Model definition

I propose the following parameterization for the Nuclear FRAP model.

tPB_i: time of PB i, with $1 \le i \le$ number of FRAPs
Δt: time between consecutive data points

n[t]: number of nuclear molecules at time t
c[t]: number of cytoplasmic molecules at time t
effVn: nuclear effective volume, that is where the molecules can diffuse.
effVc: cytoplasmic effective volume.
effVfrac = effVc / effVn: cytoplasmic to nuclear effective volume ratio
kI: Import rate (has volume units)
kE: Export rate (has volume units)
PBn: fraction of nuclear fluorescence photobleached in each FRAP
PBc: fraction of cytoplasmic fluorescence photobleached in each FRAP
deltaPBn1: extra PBn of first FRAP as compared to following FRAPs
deltaPBc1: extra PBc of first FRAP as compared to following FRAPs
PBfrac = PBc/PBn: ratio of cytoplasmic to nuclear photobleaching
n0: number of nuclear fluorescent proteins at the start of the experiment
c0: number of cytoplasmic fluorescent proteins at the start of the experiment
tot0 = n0 + c0: total number of fluorescent proteins at the start of the experiment
n_i: number of nuclear fluorescent molecules just after the PB i
c_i: number of cytoplasmic fluorescent molecules just after the PB i
i: PB index. i=0 if ($t < tPB_1$), i=1 if ($t \ge tPB_1$ & $t < PB_2$), ...
j: time course index, identifies all data point acquired in the same “movie” or file.

geomVn: nuclear geometric volume, relevant for microscopy
geomVc: cytoplasmic geometric volume, relevant for microscopy
geomVfrac = geomVc / geomVn: cytoplasmic to nuclear geometric volume ratio
γ: proportionality constant between molecule concentration in geomV and fluorescence signal, assuming perfect focus.
focusN[t]: function describing the nuclear focus during the experiment
focusC[t]: function describing the cytoplasmic focus during the experiment
auto0n: non photoblechable nuclear autofluorescence amount (in units of fluorescence signal)
auto0c: non photoblechable cytoplasmic autofluorescence amount (in units of fluorescence signal)
auto1n: photoblechable nuclear autofluorescence at the start of the experiment amount (in units of fluorescence signal)
auto1c: photoblechable cytoplasmic autofluorescence at the start of the experiment amount (in units of fluorescence signal)
autoPBn: fraction of nuclear photoblechable autofluorescence photobleached in each FRAP
autoPBc: fraction of cytoplasmic photoblechable autofluorescence photobleached in each FRAP

the “recovery” model after each FRAP is given by the ODEs

$$\left\{n'[t] == -kE\,\frac{n[t]}{effVn} + kI\,\frac{c[t]}{effVc},\ c'[t] == kE\,\frac{n[t]}{effVn} - kI\,\frac{c[t]}{effVc},\ n[tPB_i] == n_i,\ c[tPB_i] == c_i\right\}$$

that have analytic solution

$$n[t] = \frac{(c_i + n_i)\,\text{effVn}\,kI + (n_i\,\text{effVc}\,kE - c_i\,\text{effVn}\,kI)\,e^{\left(-\frac{kE}{\text{effVn}} - \frac{kI}{\text{effVc}}\right)t}}{\text{effVc}\,kE + \text{effVn}\,kI}$$

$$c[t] = c_i + n_i - n[t]$$

Assuming that at the start of the experiment the system is in steady state we have

$$n0 = \frac{\text{tot0}\,\text{effVn}\,kI}{\text{effVc}\,kE + \text{effVn}\,kI}$$

$$c0 = \frac{\text{tot0}\,\text{effVc}\,kE}{\text{effVc}\,kE + \text{effVn}\,kI}$$

The initial conditions after each frap are given by

$$n[\text{tPBi}] = \begin{cases} n[\text{tPBi} - \Delta t]\,(1 - \text{PBn} - \text{deltaPBn1}) & i == 1 \\ n[\text{tPBi} - \Delta t]\,(1 - \text{PBn}) & i > 1 \end{cases}$$

$$c[\text{tPBi}] = \begin{cases} c[\text{tPBi} - \Delta t]\,(1 - \text{PBc} - \text{deltaPBc1}) & i == 1 \\ c[\text{tPBi} - \Delta t]\,(1 - \text{PBc}) & i > 1 \end{cases}$$

Note that we allow for the first PB to produce a larger drop in fluorescence than the subsequent ones, as this is what we observe experimentally. The model has to be solved in chunks of time. (we could get an analytical expression, but it would be quite messy).

$$\text{if } t < \text{tPB}_1$$

$$n[t] = \frac{\text{tot0}\,\text{effVn}\,kI}{\text{effVc}\,kE + \text{effVn}\,kI}$$

$$c[t] = \frac{\text{tot0}\,\text{effVc}\,kE}{\text{effVc}\,kE + \text{effVn}\,kI}$$

$$\text{if } t >= \text{tPB}_i \;\&\; t < \text{tPB}_{i+1}\ \Big\{$$

$$n_i = \begin{cases} n[\text{tPBi} - \Delta t]\,(1 - \text{PBn} - \text{deltaPBn1}) & i == 1 \\ n[\text{tPBi} - \Delta t]\,(1 - \text{PBn}) & i > 1 \end{cases};$$

$$c_i = \begin{cases} c[\text{tPBi} - \Delta t]\,(1 - \text{PBc} - \text{deltaPBc1}) & i == 1 \\ c[\text{tPBi} - \Delta t]\,(1 - \text{PBc}) & i > 1 \end{cases};$$

$$n[t] = \left((c_i + n_i)\,\text{effVn}\,kI + (n_i\,\text{effVc}\,kE - c_i\,\text{effVn}\,kI)\,e^{\left(-\frac{kE}{\text{effVn}} - \frac{kI}{\text{effVc}}\right)t}\right) \Big/ (\text{effVc}\,kE + \text{effVn}\,kI);$$

$$c[t] = c_i + n_i - n[t];\Big\}$$

A second part of the model relates to the “microscopy effects”. The observed fluorescence is proportional to the concentration of molecules in the “geometrical volume”, not the “effective volume”. (I’m assuming the fluorescence signal has been corrected for imaging PB). Taking into account the autofluorescence and the focus, the expression for the fluorescence level as a function of n[t] and c[t] is given by

$$Fn[t] = \text{focusN}[t]\left(\gamma\frac{n[t]}{\text{geomVn}} + \text{auto1n}\,(1 - \text{autoPBn})^i + \text{auto0n}\right)$$

$$Fc[t] = \text{focusC}[t]\left(\gamma\frac{c[t]}{\text{geomVc}} + \text{auto1c}\,(1 - \text{autoPBc})^i + \text{auto0c}\right)$$

To avoid identifiably issues, I adimensionalized the equations to get rid of some parameters. First I define the adimentional rate parameters by dividing by the cytoplasmic effective volume

$$\varkappa I = \frac{kI}{\text{effVc}} \Rightarrow kI = \varkappa I\,\text{effVc}$$

$$\kappa E = \frac{kE}{effVc} \Rightarrow kE = \kappa E\, effVc$$

Using this in the equation for n[t]

$$n[t] = \frac{(c_i + n_i)\,\kappa I + (n_i\,\kappa E\; effVfrac - c_i\,\kappa I)\, e^{(-\kappa E\, effVfrac - \kappa I)\, t}}{\kappa E\, effVfrac + \kappa I};$$

where

$$effVfrac = \frac{effVc}{effVn}$$

for the focus we have that $0 \le focusN \le 1$ and $0 \le focusC \le 1$, but because we can't know when we are in perfect focus, I define the relative focus by dividing by the nuclear focus at time zero

$$\phi n[t] = \frac{focusN[t]}{focusN[0]}$$

$$\phi c[t] = \frac{focusC[t]}{focusN[0]}$$

$$Fn[t] = \phi n[t]\, focusN[0] \left(\gamma \frac{n[t]}{geomVn} + auto1n\, (1 - autoPBn)^i + auto0n\right)$$

$$Fc[t] = \phi c[t]\, focusN[0] \left(\gamma \frac{c[t]}{geomVc} + auto1c\, (1 - autoPBc)^i + auto0c\right)$$

Second, I adimentionalize the fluorescence variables and parameters, or really I just express the amounts in terms of nuclear fluorescence in the nuclear focus of time zero.

$$\eta[t] = \gamma\, focusN[0] \frac{n[t]}{geomVn} \Rightarrow n[t] = \eta[t] \frac{geomVn}{\gamma\, focusN[0]}$$

$$\xi[t] = \gamma\, focusN[0] \frac{c[t]}{geomVn} \Rightarrow c[t] = \xi[t] \frac{geomVn}{\gamma\, focusN[0]}$$

$$T0 = \gamma\, focusN[0] \frac{tot0}{geomVn}$$

$$\alpha 1n = focusN[0]\, auto1n$$
$$\alpha 1c = focusN[0]\, auto1c$$
$$\alpha 0n = focusN[0]\, auto0n$$
$$\alpha 0c = focusN[0]\, auto0c$$

plugging this into the fluorescence equations we get

$$Fn[t] = \phi n[t] \left(\eta[t] + \alpha 1n\, (1 - autoPBn)^i + \alpha 0n\right)$$

$$Fc[t] = \phi c[t] \left(\frac{\xi[t]}{geomVfrac} + \alpha 1c\, (1 - autoPBc)^i + \alpha 0c\right)$$

where

$$geomVfrac = \frac{geomVc}{geomVn}$$

I'll assume that the focus for the nucleus and the cytoplasm is constant during a movie. Otherwise, there are identifiable problems between the focus drift parameters and the kinetic parameters of the model.

$$\phi n[t] = \phi n_j$$
$$\phi c[t] = \phi c_j$$

where t ϵ movie$_j$. I will use second order polynomials evaluated at the time of the PB to model the fluctuations in the focus. Noting that ϕn[0]=1 we have

$$\phi n_j = 1 + an1\left(tPB_j - tPB_1\right) + an2\left(tPB_j - tPB_1\right)^2$$

$$\phi c_j = ac0 + ac1\left(tPB_j - tPB_1\right) + ac2\left(tPB_j - tPB_1\right)^2$$

So in summary, noting that the equations for n and c are linear and therefore don't depend on the units they are expressed in, we have

$$\text{if } t < tPB_1$$
$$\eta[t] = \frac{T0\,\kappa I}{\kappa E\,effVfrac + \kappa I}$$
$$\xi[t] = \frac{T0\,\kappa E\,effVfrac}{\kappa E\,effVfrac + \kappa I}$$
$$\text{if } t >= tPB_i \;\&\; t < tPB_{i+1}\left\{\right.$$
$$\eta_i = \begin{cases} \eta[tPB_i - \Delta t]\,(1 - PBn - deltaPBn1) & i == 1 \\ \eta[tPB_i - \Delta t]\,(1 - PBn) & i > 1 \end{cases};$$
$$\xi_i = \begin{cases} \xi[tPB_i - \Delta t]\,(1 - PBc - deltaPBc1) & i == 1 \\ \xi[tPB_i - \Delta t]\,(1 - PBc) & i > 1 \end{cases};$$
$$\eta[t] = \frac{(\xi_i + \eta_i)\,\kappa I + (\eta_i\,\kappa E\,effVfrac - \xi_i\,\kappa I)\,e^{(-\kappa E\,effVfrac - \kappa I)\,t}}{\kappa E\,effVfrac + \kappa I};$$
$$\xi[t] = \xi_i + \eta_i - \eta[t];\left.\right\}$$

$$Fn[t] = \phi n_j\left(\eta[t] + \alpha 1n\,(1 - autoPNn)^i + \alpha 0n\right)$$

$$Fc[t] = \phi c_j\left(\frac{\xi[t]}{geomVfrac} + \alpha 1c\,(1 - autoPBc)^i + \alpha 0c\right)$$

$$\phi n_j = 1 + an1\left(tPB_j - tPB_1\right) + an2\left(tPB_j - tPB_1\right)^2$$

$$\phi c_j = ac0 + ac1\left(tPB_j - tPB_1\right) + ac2\left(tPB_j - tPB_1\right)^2$$

So the free parameters I have to use in the fit are

1) κEeffVfrac = combined kE × effVfrac to avoid identifiable issues
2) κI
3) T0
4) PBn
5) PBc (or PBfrac)
6) deltaPBn1
7) deltaPBc1
8) geomVfrac
9) α1n
10) autoPBn
11) α0n
12) an1
13) an2
14) α1c
15) autoPBc

16) α0c
17) ac0
18) ac1
19) ac2

To be compatible with previous notation, I go back to latin letters, BUT KEEPING THE INTERPRETATION OF THE GREEK LETTERS!

$$\mathbf{n} \equiv \eta$$
$$\mathbf{c} \equiv \xi$$
$$\mathbf{tot0} \equiv \mathbf{T0}$$
$$\mathbf{kI} \equiv \kappa\mathbf{I}$$
$$\mathbf{kE} \equiv \kappa\mathbf{E}$$
$$\mathbf{Vfrac} \equiv \mathbf{effVfrac}$$
$$\mathbf{kEVfrac} \equiv \kappa\mathbf{EeffVfrac}$$
$$\mathbf{auto1n} \equiv \alpha\mathbf{1n}$$
$$\mathbf{auto0n} \equiv \alpha\mathbf{0n}$$
$$\mathbf{auto1c} \equiv \alpha\mathbf{1c}$$
$$\mathbf{auto0c} \equiv \alpha\mathbf{0c}$$

so we get the following equations (assuming deltaPBc1 = deltaPBn1 PBfrac)

$$\text{if } \mathbf{t} < \mathbf{tPB_1}$$
$$\mathbf{n[t]} = \frac{\mathbf{tot0\,kI}}{\mathbf{kEVfrac + kI}}$$
$$\mathbf{c[t]} = \frac{\mathbf{tot0\,kEVfrac}}{\mathbf{kEVfrac + kI}}$$
$$\text{if } \mathbf{t} >= \mathbf{tPB_i} \;\&\; \mathbf{t} < \mathbf{tPB_{i+1}} \left\{ \right.$$
$$\mathbf{n_i} = \begin{cases} \mathbf{n[tPB_i - \Delta t]\,(1 - PBn - deltaPBn1)} & \mathbf{i == 1} \\ \mathbf{n[tPB_i - \Delta t]\,(1 - PBn)} & \mathbf{i > 1} \end{cases};$$
$$\mathbf{c_i} = \begin{cases} \mathbf{c[tPB_i - \Delta t]\,(1 - (PBn + deltaPBn1)\,PBfrac)} & \mathbf{i == 1} \\ \mathbf{c[tPB_i - \Delta t]\,(1 - PBn\,PBfrac)} & \mathbf{i > 1} \end{cases};$$
$$\mathbf{n[t]} = \frac{\mathbf{(c_i + n_i)\,kI + (n_i\,kEVfrac - c_i\,kI)}\, e^{\mathbf{(-kEVfrac - kI)\,t}}}{\mathbf{kEVfrac + kI}};$$
$$\mathbf{c[t]} = \mathbf{c_i + n_i - n[t]}; \left. \right\}$$
$$\mathbf{Fn[t]} = \mathbf{focusN_j}\left(\mathbf{n[t] + auto1n\,(1 - autoPNn)^i + auto0n}\right)$$
$$\mathbf{Fc[t]} = \mathbf{focusC_j}\left(\frac{\mathbf{c[t]}}{\mathbf{geomVfrac}} + \mathbf{auto1c\,(1 - autoPBc)^i + auto0c}\right)$$
$$\mathbf{focusN_j} = \mathbf{1 + an1}\left(\mathbf{tPB_j - tPB_1}\right) + \mathbf{an2}\left(\mathbf{tPB_j - tPB_1}\right)^2$$
$$\mathbf{focusC_j} = \mathbf{ac0 + ac1}\left(\mathbf{tPB_j - tPB_1}\right) + \mathbf{ac2}\left(\mathbf{tPB_j - tPB_1}\right)^2$$

With free parameters for the fit

1) kEVfrac
2) kI
3) tot0
4) PBn
5) PBfrac
6) deltaPBn1
7) geomVfrac

8) auto1n
9) autoPBn
10) auto0n
11) an1
12) an2
13) auto1c
14) autoPBc
15) auto0c
16) ac0
17) ac1
18) ac2

For example, if we assume

PBfrac = 0, no citoplasmic PB
deltaPBn1 = 0, no extra PB in first FRAP
autoPBn = 1, all photobleachable nuclear autofluorescence PB in first FRAP
auto0n = 0, no non-photobleachable nuclear autofluorescence
auto1c = 0, no photobleachable cytoplasmic autofluorescence
autoPBc = 0, no cytoplasmic PB
ac0 = 1, initial cytoplasmic focus equivalent to initial nuclear focus
ac1 = 0, no cytoplasmic focus drift
ac2 = 0, no cytoplasmic focus drift

resulting in 9 free parameters for the fit
1) kEVfrac
2) kI
3) tot0
4) PBn
5) geomVfrac
6) auto1n
7) an1
8) an2
9) auto0c

Model Fit

The parameters kEVfrac, kI and geomVfrac are scanned in log scale. Therefore I define the parameters

log10_kEVfrac = log10 (kEVfrac)
log10_kI = log10 (kI)
log2_geomVfrac = log2 (geomVfrac)

which are scanned lineally.

geomVfrac is not well defined by the fluorescence data alone. I has identifiability problems with tot0. Chanching the value of geomVfrac influences the value of kI and kEVfrac, as it changes the nuclear fraction. Therefore, we decided to obtain an independent estimate of geomVfrac, based on the cell's images. This can be done for each cell, or use a distribution for the population. We implemented a "ridge regression", where the cost function penalizes departures from the estimated geomVfrac. To do this, we define two new parameters.

log2_geomVfrac_mean = best estimation for log2_geomVfrac
log2_geomVfrac_sd = standard deviation for the estimation of log2_geomVfrac

Note that the distribution of geomVfrac is assumed to be lognormal. Experimental data supports this assumption. The cost function is defined basically as in Cedersund and Roll 2008

$$\mathbf{V(p)} = \sum_{\mathbf{i}}\sum_{\mathbf{j}} \frac{\left(\mathbf{y_i[t_j]} - \mathbf{\hat{y}_i^M[t_j]}\right)^2}{\boldsymbol{\sigma}_{\mathbf{i}}^{\mathbf{2}}\mathbf{[t_j]}} + \sum_{\mathbf{k}} \boldsymbol{\alpha}_{\mathbf{k}}\, \mathbf{h_{pen}}\left[\mathbf{p_k} - \mathbf{p_k^g}\right]$$

where $h_{\text{pen}}[x] = x^2$ and $\alpha_k = \frac{1}{\sigma_k^2}$

In this case there is only one variable over which to penalize (k=1), namely log2_geomVfrac. So we have

$$p_1^g = \texttt{log2\_geomVfrac\_mean}$$

$$\alpha_1 = \frac{1}{\sigma_1^2} = \frac{1}{(\texttt{log2\_geomVfrac\_sd})^2}$$

the cost function is therefore

$$\mathbf{V(p)} = \sum_{\mathbf{i}}\sum_{\mathbf{j}} \frac{\left(\mathbf{y_i[t_j]} - \mathbf{\hat{y}_i^M[t_j]}\right)^2}{\boldsymbol{\sigma}_{\mathbf{i}}^{\mathbf{2}}\mathbf{[t_j]}} + \frac{(\mathbf{log2\_geomVfrac} - \mathbf{log2\_geomvfrac\_mean})^2}{(\mathbf{log2\_geomVfrac\_sd})^2}$$

this statistic is assumed to follow a chi square distribution.