FRAP model of nucleocytoplasmic translocation applied to nuclear export

As stated in the main text, the passive diffusion fluxes are expressed by:

$$J_p^{C \to N} = P_{NES} C_{NES}^C \qquad (S1)$$

$$J_p^{N \to C} = P_{NES} \chi_{NES}^N C_{NES}^N \qquad (S2)$$

where $J_p^{C \to N}$ and $J_p^{N \to C}$ are the passive fluxes, P_{NES} is the *permeability coefficient* of the NE for free NES-GFP, C_{NES}^C and C_{NES}^N are the average concentrations of the entire NES-GFP pool in cytoplasm and nucleus, and χ_{NES}^N is the molar fraction of NES-GFP pool not engaged in a complex with the exportins in nucleus, respectively.
The active export flux is instead given by:

$$J_a^{N \to C} = v_{NES}^{N \to C} \left(1 - \chi_{NES}^N \right) C_{NES}^N \qquad (S3)$$

$v_{NES}^{N \to C}$ represents the first order rate constant of active export. At steady state:

$$J_p^{C \to N} + J_p^{N \to C} + J_c^{N \to C} = 0 \qquad (S4)$$

If we define:

$$K_{eq} = \frac{C_{NES}^N}{C_{NES}^C} \qquad (S5)$$

we obtain from S1-S3:

$$K_{eq} = \frac{P_{NES}}{v_{NES}^{N \to C} \left(1 - \chi_{NES}^N \right) + P_{NES} \chi_{NES}^N} \qquad (S6)$$

We now define the *excess active flux* of the exported cargo $\Phi^{N \to C}$ as:

$$\Phi^{N \to C} = P_{NES} \left(\frac{1}{K_{eq}} - 1 \right) C_{NES}^N \qquad (S7)$$

$\Phi^{N \to C}$ is an experimentally measurable parameter and it corresponds to the global N→C flux (passive+active) minus the theoretical passive N→C flux of NES-GFP in absence of exportin. As stated in the main text and reported in (1) $\Phi^{N \to C}$ can be computed from the monexponential fitting parameters of recovered FRAP curves and the nuclear radius. By combining S6 and S7, we get the fundamental equation of our model:

$$\Phi^{N \to C} = \left(v_{NES}^{N \to C} - P_{NES} \right) \left(1 - \chi_{NES}^N \right) C_{NES}^N \qquad (S8)$$

For very large C_{NES}^N, the product $\left(1 - \chi_{NES}^N \right) C_{NES}^N$ converges to C_{Ex}^N, i.e. all the exportin receptor is saturated. We always found out $v_{NES}^{N \to C} \gg P_{NES}$ (see below), and eq. S8 becomes in this limit:

$$\Phi^{N \to C} \approx v_{NES}^{N \to C} C_{Ex}^N = V_m^{N \to C} \quad \text{for } C_{NES}^N \to \infty \qquad (S9)$$

$V_m^{N \to C}$ is the *truly intrinsic dynamic parameter of nuclear export,* because it quantifies the translocation efficiency, in terms of exported molecule per second, of the exportin-NES complex through the NPC.

When the exportins are not fully saturated by NES ($\chi_{NES}^N > 0$), $\Phi^{N \to C}$ is a function of C_{NES}^N. Indeed, χ_{NES}^N is connected to C_{NES}^N through the mass action law together with the mass conservation balance. The simplest case occurs when a 1:1 complex is formed; we have:

$$1 - \chi_{NES}^{N} = \frac{\beta - \left(\beta^2 - 4C_{NES}^{N} C_{Ex}^{N}\right)^{0.5}}{2C_{NES}^{N}} \qquad (S10)$$

where $\beta = \left(C_{NES}^{N} + C_{Ex}^{N} + K_{D}^{*}\right)$, C_{Ex}^{N} is the global nuclear concentration of exportin and K_{D}^{*} is the intranuclear dissociation constant of the NES-GFP·Ex complex (the asterisk denotes that the dissociation constant is in principle different from the actual binding constant measured *in vitro* owing to the intranuclear environment: see main text for further discussion on this point).

If a reasonable estimate of C_{Ex}^{N} is assumed, fitting of experimental $\Phi^{N \to C}$ *vs.* C_{NES}^{N} curve to eq. S8 and S10 yields both $V_{m}^{N \to C}$ and K_{D}^{*}. Accordingly, the thermodynamic as well as the kinetic features of the active export can be fully identified. It is worth noting that we always assumed $C_{Ex}^{N}=[CRM1]_{N}=0.3$ μM after Riddick et al. (2). Note that $C_{Ex}^{N}=0.3$ μM leads to $v_{NES}^{N \to C}=620\pm145$ μm^3/s, a much higher value than P_{NES} ($\bar{P}_{NES}=9.5\pm5$ μm^3/s), thus justifying the assumption underlying eq. S9.

1. Cardarelli, F., Bizzarri, R., Serresi, M., Albertazzi, L., and Beltram, F. (2009) *J Biol Chem* **284**, 36638-36646
2. Riddick, G., and Macara, I. G. (2007) *Mol Syst Biol* **3**, 118