Appendix D

Conformational switch model

The so called conformation switch model was firstly proposed by Okamura *et al* [17], based on their experimental study of NFAT1 phosphorylation states:

1. NFAT1 can assume two different structures, an active conformation and an inactive conformation.
2. The phosphorylation state of certain residues affects the global conformation of the portein. Therefore the probabilities of assuming the active and inactive conformations depend on the phosporylation state.
3. The active conformation is favored in the dephosporylated state, while the inactive conformation is favored in the phosphorylated state
4. The active conformation is imported into the nucleus, the inactive conformation is exported from the nucleus.

Later, Salazar *et al* further developed this concept into a fully mathematical form [18].

By describing the conformational switch model as a protein network and using the rapid equilibrium approximation [18], we can use the following equation to describe the kinetics of the total nuclear Crz1 fraction which is denoted by Crz(t) [12]:

$$\frac{dCrz(t)}{dt} = d \cdot \phi \cdot (1 - Crz(t)) - f \cdot \psi \cdot Crz(t) \tag{D.1}$$

where d denotes the import rate constant, f denotes the export rate constant, ϕ denotes the ratio of the fraction of the cytosolic active conformation over the total cytosolic fraction and ψ denotes the ratio of the fraction of nuclear inactive conformation over the total nuclear fraction. Concerning the number of cytosolic active, a_n, and inactive conformations, i_n, with n phosphorylated residues, ϕ can be calculated as follows [12]:

$$\begin{aligned}\phi &= \frac{\sum_{n=0}^{N} a_n}{\sum_{n=0}^{N}(a_n + i_n)} \\ &= \frac{1}{1 + \sum_{n=0}^{N} i_n / \sum_{n=0}^{N} a_n} \\ &= 1/(1 + L_0 \cdot \frac{(\lambda k/c)^{N+1} - 1}{\lambda k/c - 1} \cdot \frac{k/c - 1}{(k/c)^{N+1} - 1}) \end{aligned} \tag{D.2}$$

where k and c denote kinase and calcineurin activity in the cytosol respectively. Capital letters K and C are the corresponding activity in the nucleus. N is the number of relevant regulatory phosporylation sites, experimental data shows that $N = 13$ in the case of NFAT1. L_0 denotes the basic equilibrium constant and λ is the increment factor. The small case letters a_n and i_n $(n = 0, 1, 2...N)$ denote cytosolic active and inactive conformations with n phosphorylated residues respectively [12].

Under the assumption of $k/c = K/C$ we can calculate $\psi = 1 - \phi$. Therefore ϕ can be regarded as a function of k/c. If we assume that the kinase level is a constant and further express the concentration of activated calcineurin, CaN(t), in dimensionless units relative to this constant, then:

$$\phi(k/c) = \phi(1/(c/k)) = \phi(1/CaN(t)) \tag{D.3}$$

Now we can rewrite the kinetics equation **??** of the total nuclear fraction Crz1 as follows:

$$\frac{dCrz(t)}{dt} = d \cdot \phi(1/CaN(t)) \cdot (1 - Crz(t)) - f \cdot (1 - \phi(1/CaN(t))) \cdot Crz(t) \tag{D.4}$$

d denotes the import rate constant, f denotes the export rate constant and ϕ denotes the ratio of the fraction of the cytosolic active conformation over the total cytosolic fraction:

$$\phi(y) = 1/(1 + L_0 \cdot \frac{(\lambda y)^{N+1} - 1}{\lambda y - 1} \cdot \frac{y - 1}{(y)^{N+1} - 1}) \tag{D.5}$$