Appendix A

A.1 Combinatorics for subunits

Measurements on the IP_3 receptor have revealed that a minimum number of subunits h_m needs to be activated for Ca^{2+} conductance (Bezprozvanny et al. 1991). A single IP_3R possesses a non zero open probability only if at least h_m subunits are in the state 10. Consequently the number of open channels and thus the Ca^{2+} concentration depends on the arrangement of activable subunits n_{10} on the receptors. Activation in the cell occurs of course for a subunit already associated with a certain receptor. Whereas the mean was used earlier we derive the distribution of open channels resulting from such a random scattering of activable subunits. and its properties. To this aim we consider N receptors with h subunits each. Let $n_i, i = 1, \ldots, h$ denote the number of receptors with i activable subunits, then the number of possible configurations for a given set $\{n_i\} := \{n_1, \ldots, n_h\}$ that satisfies

$$n_0 + \ldots + n_h = N\,, \quad n_1 + 2n_2 + \ldots + hn_h = n_{10} \tag{A.1}$$

is

$$M\left(\{n_i\}\right) := \frac{N!}{n_0! \cdots n_h!} \binom{h}{0}^{n_0} \binom{h}{1}^{n_1} \cdots \binom{h}{h}^{n_h}. \tag{A.2}$$

The fraction represents the number of permutations for the set $\{n_i\}$, whereas the binomial coefficients take into account the number of ways how to distribute i activable subunits on a single receptor. Evaluating the total number of configurations yields

$$\Gamma = \sum_{\{n_i\}}^{\star} M(\{n_i\}) = \binom{hN}{n_{10}}\,, \tag{A.3}$$

which complies with the combinatorics of choosing n_{10} from hN possible subunits. The asterisk indicates the summation with the restrictions of equation (A.1).

Knowing the normalization Γ the probability distribution of n_j for a fixed value of $j \in \{0, h\}$ is given by

$$\begin{aligned} p(n_j) =& \frac{1}{\Gamma} \sum_{\substack{\{n_i\} \\ i \neq j}}^{\star} \frac{N!}{n_0! \cdots n_k!} \binom{h}{0}^{n_0} \binom{h}{1}^{n_1} \cdots \binom{h}{h}^{n_h} \\ =& \frac{1}{\Gamma} \binom{N}{n_j} \binom{h}{j}^{n_j} \sum_{\substack{\{n_i\} \\ i \neq j}}^{\star} (N - n_j)! \prod_{\substack{l=0 \\ l \neq j}}^{h} \frac{1}{n_l!} \binom{h}{l}^{n_l} . \end{aligned} \tag{A.4}$$

Equation (A.4) is most conveniently computed as

$$p(n_j) = \frac{1}{\Gamma} \binom{N}{n_j} \binom{h}{j}^{n_j} \frac{1}{n_{10}!} \frac{d^{n_{10}}}{dz^{n_{10}}} f(z) \Big|_{z=0} , \tag{A.5}$$

where we used the generating function

$$\begin{aligned} f(z) =& \sum_{\substack{\{n_i\} \\ i \neq j}}' \tilde{N}! \prod_{\substack{l=0 \\ l \neq j}}^{h} \frac{1}{n_l!} \left[\binom{h}{l} z^l \right]^{n_l} \\ =& \sum_{i=0}^{\tilde{N}} \sum_{l=0}^{hi} \binom{\tilde{N}}{i} \binom{hi}{l} \left[- \binom{h}{j} \right]^{\tilde{N}-i} z^{l+j(\tilde{N}-i)} . \end{aligned} \tag{A.6}$$

Here the prime denotes the restriction

$$n_0 + \ldots + n_{j-1} + n_{j+1} + \cdots + n_h = N - n_j =: \tilde{N} . \tag{A.7}$$

In the case $j = 0$ the derivatives in equation (A.5) can be performed explicitly, so that

$$p(n_0) = \frac{1}{\Gamma} \binom{N}{n_0} \sum_{j=0}^{\tilde{N}} \binom{\tilde{N}}{j} \binom{jh}{n_{10}} (-1)^{\tilde{N}-j} . \tag{A.8}$$

Due to particle hole symmetry we obtain the distribution function for n_h by setting $n_{10} = Nh - n_{10}$ and substituting n_0 with n_h in eq.(A.8):

$$p(n_h) = \frac{1}{\Gamma} \binom{N}{n_h} \sum_{j=0}^{\tilde{N}} \binom{\tilde{N}}{j} \binom{jh}{Nh - n} (-1)^{\tilde{N}-j} . \tag{A.9}$$

To gain further insight into the probability distributions we calculate the first two moments. For the average we start with

$$\langle n_j \rangle = \frac{1}{\Gamma} \sum_{\{n_i\}}^{\star} n_j M , \tag{A.10}$$

because a closed expression for the probability distribution is only available for the two cases presented above. Defining the corresponding generating function

$$f(z) := \sum_{\{n_i\}}^{\dagger} n_j \rho z^l \,, \quad l = n_1 + \ldots + h n_h \,, \tag{A.11}$$

where the dagger indicates the restriction $n_0 + \ldots + n_h = N$ we find

$$\langle n_j \rangle = \frac{1}{n_{10}!} \frac{d^{n_{10}}}{dz^{n_{10}}} f(z) \Big|_{z=0} = \frac{N}{\Gamma} \binom{h}{j} \binom{h(N-1)}{n_{10}-j} . \tag{A.12}$$

In the limit $N \to \infty, n_{10} \to \infty$ we recover the result from (Bär et al. 2000). Analogously evaluation of the second moments results in

$$\langle n_l n_k \rangle = \frac{N(N-1)}{\Gamma} \binom{h}{l} \binom{h}{k} \binom{h(N-2)}{n_{10}-l-k} + \delta_{k,l} \frac{N}{\Gamma} \binom{h}{l} \binom{h(N-1)}{n_{10}-l} . \tag{A.13}$$

Applying these general expressions to IP$_3$Rs requires values for h, h_m and N. The tetrameric structure of the receptor ensues $h = 4$. However, previous results by different groups are based on $h = 3$. We therefore compute the statistics for both cases. Experiments on a single channel have shown four conductance levels, each a multiple of 20pS, with a predominance of opening to the third level (Bezprozvanny et al. 1991, Watras et al. 1991). Thus we set $h_m = 3$. The number of receptors in a cluster has not been measured yet, but an estimate by Swillens and Dupont yields $N = 25$ (Swillens et al. 1999).

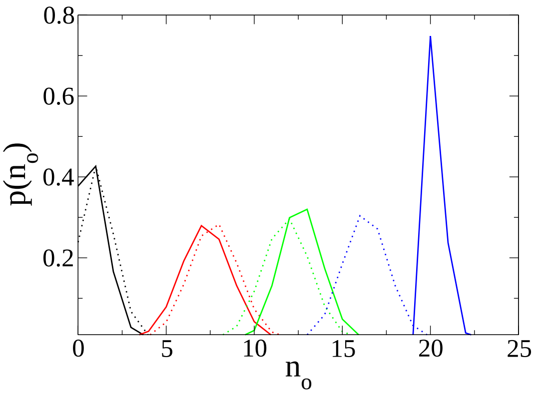

Figure A.1: Probability distribution $p(n_o)$ for $n_o = n_3, h = 3$ (full lines) and $n_o = n_3 + n_4, h = 4$ (dotted lines) for $N = 25$ and different n_{10}. Values of n_{10} read 25 (black), 50 (red), 60 (green) and 70 (blue).

The probability distributions $p(n_3 + n_4)$ with $h = 4$ and $p(n_3)$ with $h = 3$ are depicted in figure A.1. They both agree very well. This is also supported by their mean and variance as shown in figure A.2. In the left panel we also include the postition of the maxima of the distributions indicated by dots. They closely follow the average. Due to the narrowness of the distributions demonstrated by the small variance as well as the accordance between the mean and the maximum we calculate the number of open channels n_c from the average for a given value of n_{10}:

$$n_c^{(3)} = Nr^3 \frac{n_{10}}{3N} \frac{n_{10}-1}{3N-1} \frac{n_{10}-2}{3N-2}, \tag{A.14}$$

$$n_c^{(3,4)} = Nr^3 \frac{n_{10}}{4N} \frac{n_{10}-1}{4N-1} \frac{n_{10}-2}{4N-2} \left[\frac{n_{10}-3}{4N-3}(4-3r) + \left(1 - \frac{n_{10}}{4N}\right) \right] \tag{A.15}$$

Here $r := I/(I+d_1)$ denotes the fraction of subunits in the activable state 10 that are activated (Falcke et al. 2000b). The subscripts (3) and $(3,4)$ indicate that we used $p(n_3), h = 3$ and $p(n_3 + n_4), h = 4$ for averaging, respectively. Note that in the limit $N \to \infty, n_{10} \to \infty$ equations (A.14),(A.15) reduce to the well known expressions of the deterministic description.

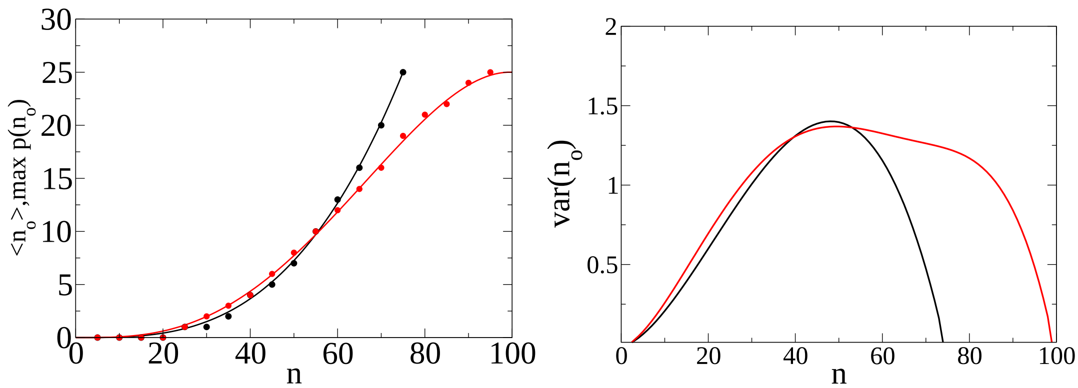

Figure A.2: Mean (left) and variance (right) of n_o for $n_o = n_3, h = 3$ (black) and $n_o = n_3 + n_4, h = 4$ (red). The left panel shows the position of $\max p(n_o)$ as dots.

Studies on puffs indicate that on average 5 channels open (Sun et al. 1998). In this regime there is no significant difference between $p(n_3 + n_4)$ and $p(n_3)$. Therefore we consider a channel to be open when 3 out of 3 subunits are in the activated state. Hence we apply equation (A.14) which can be further simplified by approximating all the denominators by $3N$ because of $3N \gg 1$.

A.2 Proof of equation (4.39)

This section deals with the proof of equation (4.39). It is based on the identity

$$\sum_{k=0}^{j} \binom{j}{k} \frac{(-1)^k}{2k+1} = \frac{2^{2j}\,(j!)^2}{(2j+1)!}\,. \tag{A.16}$$

We transform the left hand side of equation (A.16) according to

$$\sum_{k=0}^{j} \binom{j}{k} (-1)^k \int_0^1 t^{2k} dt = \int_0^1 \sum_{k=0}^{j} \binom{j}{k} (-t^2)^k dt = \int_0^1 \left(1-t^2\right)^j dt\,. \tag{A.17}$$

It can be simplified with Euler's Beta function $B(z,w)$. From its definition

$$B(z,w) := \int_0^1 t^{z-1}(1-t)^{w-1} dt \tag{A.18}$$

follows

$$\int_a^b (t-a)^{z-1}(b-t)^{w-1} dt = (b-a)^{z+w-1} B(z,w)\,. \tag{A.19}$$

Hence we express the integral in equation (A.17) through

$$\int_0^1 \left(1-t^2\right)^j dt = \frac{1}{2}\int_{-1}^1 (t+1)^j (1-t)^j dt = 2^{2j} B(j+1, j+1)\,. \tag{A.20}$$

According to (Abramowitz and Stegun 1974) the Beta function is related to the Gamma function $\Gamma(z)$ via $B(z,w) = \Gamma(z)\Gamma(w)/\Gamma(z+w)$, so that we find

$$\sum_{l=0}^{j} \binom{j}{l} \frac{(-1)^l}{2l+1} = 2^{2j} \frac{\Gamma(j+1)^2}{\Gamma(2j+2)} = \frac{2^{2j}\,(j!)^2}{(2j+1)!} \tag{A.21}$$

due to $n! = \Gamma(n+1)$. Expanding the right hand side yields

$$\frac{2^{2j}\,(j!)^2}{(2j+1)!} = \frac{2\cdot 1}{2}\cdot\frac{2}{3}\cdot\frac{2\cdot 2}{4}\cdot\frac{2}{5}\cdot\frac{2\cdot 3}{6}\cdots\frac{2\cdot j}{2j}\cdot\frac{2}{2j+1} j! = \frac{j!}{\left(\frac{3}{2}\right)_j}\,. \tag{A.22}$$

This proofs equation (4.39) when we use $j! = (1)_j$.

A.3 Numerical methods

The geometry of the IP_3R cluster imposes considerations on the discretization. As stated above the radius of the active area measures only tens of nanometers but the outer boundary is 5-100 μm away. A constant grid size that sufficiently resolves the dynamics in the cluster would lead to an enormous calculational effort. To reduce computation time we use a grid with non uniform spacing. The mesh size is small enough for $r \leq a_0$ and saturates at a larger value in the bulk. It entails that the usual discretization of the radial Laplacian

$$\nabla_r^2 = \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial}{\partial r}\right) = \frac{\partial^2}{\partial r^2} + \frac{2}{r}\frac{\partial}{\partial r}\,. \tag{A.23}$$

cannot be applied. Let $\{r_i\}$ denote the set of grid points, $dr_i := r_i - r_{i-1}$ the spacing and u_i an approximation to the concentration profile. Then a second order scheme for equation (A.23) reads

$$L(u_i) = \frac{1}{r_i^2}\left\{\left[r_i + \frac{dr_{i+1}}{2}\right]^2 \frac{u_{i+1} - u_i}{dr_{i+1}} - \left[r_i - \frac{dr_i}{2}\right]^2 \frac{u_i - u_{i-1}}{dr_i}\right\}\frac{2}{dr_{i+1} + dr_i}\,. \tag{A.24}$$

Moreover we adopt a first order scheme for the time integration and 50% of the stability criterion (Press et al. 2002).