Appendix: Quantitative modeling of the molecular steps underlying shut-off of rhodopsin's activity in rod phototransduction

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A Boundary condition at photoisomerization position

For free aqueous diffusion of cGMP, Fick's Second Law is

$$\frac{\partial cG}{\partial t} = D\frac{\partial^2 cG}{\partial x^2}\,. \tag{A.1}$$

Within the rod outer segment, though, longitudinal diffusion is 'baffled' by the stack of disks, and as a result only a fraction $f_{\rm A}$ of the cross-sectional area A is available for diffusion, and only a fraction $f_{\rm V}$ of the envelope volume is cytoplasmic [1]. Furthermore, as invoked by Lamb & Pugh [2], the cytoplasm is envisaged to have a buffering capacity BP for cGMP. As a result, the modified diffusion equation is

$$\frac{\partial cG}{\partial t} = D_{\rm cG}\frac{\partial^2 cG}{\partial x^2} \tag{A.2}$$

where the effective longitudinal diffusion coefficient $D_{\rm cG}$ is

$$D_{\rm cG} = \frac{f_{\rm A}}{f_{\rm V}\,BP}\,D\,. \tag{A.3}$$

From Fick's First Law, the longitudinal flux of cGMP along the outer segment (which can occur only via the available area $f_{\rm A}A$) is given by

$$\text{Longitudinal flux} = -D\,f_{\rm A}\,A\,N_{\rm Av}\,\frac{\partial cG}{\partial x} \tag{A.4}$$

where the true diffusion coefficient D, rather than the effective diffusion coefficient $D_{\rm cG}$, must be used, and where Avogadro's number $N_{\rm Av}$ converts the units to molecules $\rm s^{-1}$. At a disk that has received a single photoisomerization, the rate of hydrolysis of cGMP is

$$\text{Hydrolysis rate} = E^*(t)\,\frac{k_{\rm cat}}{K_{\rm m}}\,cG(x,t) = E^*(t)\,\frac{\frac{1}{2}k_{\rm CAT}}{K_{\rm m}}\,cG(x,t) \tag{A.5}$$

where $k_{\rm cat} = \frac{1}{2}k_{\rm CAT}$ is the maximal rate of hydrolysis by a single $\rm G^*{-}E^*$, and $k_{\rm CAT}$ is the maximal rate of hydrolysis by the fully-activated PDE dimer.

In the symmetrical case, with a single photoisomerization occurring at the middle of the outer segment ($x = x_0$), the magnitude of the unidirectional flux on either side of this point is half the total hydrolytic rate, so that from Eqns (A.4) and (A.5)

$$D\,f_{\rm A}\,A\,N_{\rm Av}\,\left.\frac{\partial cG}{\partial x}\right|_{x_0^+} = \frac{1}{2}\,E^*(t)\,\frac{\frac{1}{2}k_{\rm CAT}}{K_{\rm m}}\,cG(x_0,t)\,. \tag{A.6}$$

From Eqn (A.3) we have

$$D\,f_{\rm A} = D_{\rm cG}\,f_{\rm V}\,BP\,D \tag{A.7}$$

and substitution into Eqn (A.6) gives

$$D_{\rm cG}\,f_{\rm V}\,A\,BP\,N_{\rm Av}\,\left.\frac{\partial cG}{\partial x}\right|_{x_0^+} = \frac{1}{2}\,E^*(t)\,\frac{\frac{1}{2}k_{\rm CAT}}{K_{\rm m}}\,cG(x_0,t) \tag{A.8}$$

or

$$\left.\frac{\partial cG}{\partial x}\right|_{x_0^+} = \frac{E^*(t)}{2\,D_{\rm cG}}\left[\frac{\frac{\frac{1}{2}k_{\rm CAT}}{K_{\rm m}}}{f_{\rm V}\,A\,BP\,N_{\rm Av}}\right]cG(x_0,t)\,. \tag{A.9}$$

Formulation of Lamb & Pugh (1992)

For the term in [ ] above, we note that Lamb & Pugh [2] defined $\beta_{\rm sub}$ in their Eqn (4.4) as

$$\beta_{\rm sub} = \frac{\frac{\frac{1}{2}k_{\rm CAT}}{K_{\rm m}}}{V_{\rm cyto}\,N_{\rm Av}\,BP} \tag{A.10}$$

where the cytoplasmic volume $V_{\rm cyto}$ is given by

$$V_{\rm cyto} = f_{\rm V}\,A\,L\,. \tag{A.11}$$

Hence

$$\beta_{\rm sub}\,L = \frac{\frac{\frac{1}{2}k_{\rm CAT}}{K_{\rm m}}}{f_{\rm V}\,A\,N_{\rm Av}\,BP} \tag{A.12}$$

where the right hand side of the equation above is the term in [ ] in Eqn (A.9). Hence the latter equation may be rewritten as

$$\left.\frac{\partial cG}{\partial x}\right|_{x_0^+} = \frac{E^*(t)\,\beta_{\rm sub}\,L}{2\,D_{\rm cG}}\,cG(x_0,t) \tag{A.13}$$

which is presented as Eqn (4.11) in the Theory section, and which is exactly equivalent to Eqn (B 1) of Lamb & Pugh [2].

Formulation of Gross, Pugh & Burns (2012)

In a variant of the symbols, Gross et al [3] defined $\beta_{\rm idv}$ (see p. 1781) as

$$\beta_{\rm idv} = \frac{\frac{k_{\rm cat}}{K_{\rm m}}}{V_{\rm id}\, N_{\rm Av}} = \frac{\frac{\frac{1}{2}k_{\rm CAT}}{K_{\rm m}}}{V_{\rm id}\, N_{\rm Av}} \tag{A.14}$$

where the interdiscal cytoplasmic volume $V_{\rm id}$ is given by

$$V_{\rm id} = f_{\rm V}\, A\, \delta \tag{A.15}$$

with $\delta = L/N_{\rm disks}$ being the mean interdisk spacing. Hence

$$\beta_{\rm idv}\, \delta = \frac{\frac{\frac{1}{2}k_{\rm CAT}}{K_{\rm m}}}{f_{\rm V}\, A\, N_{\rm Av}} \tag{A.16}$$

or

$$\frac{\beta_{\rm idv}\, \delta}{BP} = \frac{\frac{\frac{1}{2}k_{\rm CAT}}{K_{\rm m}}}{f_{\rm V}\, A\, BP\, N_{\rm Av}} \tag{A.17}$$

where the right hand side is again the term in [ ] in Eqn (A.9). Accordingly, Eqn (A.9) may be rewritten in the terminology of Gross et al [3] as

$$\left.\frac{\partial cG}{\partial x}\right|_{x_0^+} = \frac{E^*(t)\, \beta_{\rm idv}\, \delta}{2\, D_{\rm cG}\, BP}\, cG(x_0, t)\,. \tag{A.18}$$

We noticed that this equation differs from Eqn (6) of [3], which instead gives the denominator as $4\, D_{\rm cG}$. As the buffering power BP is not mentioned anywhere in Gross et al [3], it is clear that they assumed $BP = 1$. Accordingly, in their terminology, the required boundary condition is

$$\left.\frac{\partial cG}{\partial x}\right|_{x_0^+} = \frac{E^*(t)\, \beta_{\rm idv}\, \delta}{2\, D_{\rm cG}}\, cG(x_0, t)\,. \tag{A.19}$$

Eqn (6) of Gross et al [3] differs from this equation by a factor of 2, and in our view this occurred because they incorrectly invoked an additional volume fraction of $\frac{1}{2}$. As a result, their subsequent analysis of 'rogue' responses actually calculated $2\beta_{\rm idv}$, instead of $\beta_{\rm idv}$.

Finally, we note that

$$\beta_{\rm idv}\, \delta = \beta_{\rm sub}\, L \tag{A.20}$$

so that

$$\beta_{\rm idv} = N_{\rm disks}\, \beta_{\rm sub} \tag{A.21}$$

where $N_{\rm disks} = L/\delta$ is the number of disks in the outer segment.

Appendix References

1. Lamb TD, McNaughton PA, Yau K-W. Spatial spread of activation and background desensitization in rod outer segments. J Physiol 1981; 319:463-496.

2. Lamb TD, Pugh EN Jr. A quantitative account of the activation steps involved in phototransduction in amphibian photoreceptors. J Physiol 1992; 449:719-757.

3. Gross OP, Pugh EN Jr, Burns ME. Spatiotemporal cGMP dynamics in living mouse rods. Biophys J 2012; 102:1775-1784.