

Supplemental material 1

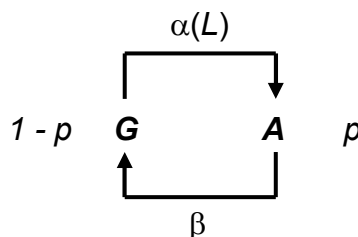


Fig. S1 The simple photocycle model consists of two states, the ground state (G) where the channel gate is closing and the activated state (A) where the gate is open (Fig. S1). The macroscopic rate constant from G to A , $\alpha(L)$, is a function of light power density (L), the photon density. The macroscopic rate constant from A to G , β , is not dependent on light. If the molecule is in the state A with a probability p , the current amplitude I is related to p by the following relationship,

$$I = i N p, \quad (1)$$

where, N is the number of molecules in a cell and i is the current produced by a single molecule. When $L = 0$, all the molecules are in G ($p = 0$). Upon turning on the light with a given intensity L , the probability increases by Δp in a period of Δt in a relation as follows.

$$\Delta p = \alpha(L) (1 - p) \Delta t - \beta p \Delta t. \quad (2)$$

That is,

$$\Delta p = \alpha(L) \Delta t - [\alpha(L) + \beta] p \Delta t, \quad (3)$$

$$dp/dt = \alpha(L) - [\alpha(L) + \beta] p. \quad (4)$$

Therefore, during the turning-on transition, p increases with a time constant of $[\alpha(L) + \beta]^{-1}$. This gives the ON rate constant of photocurrent activation as a function of L ,

$$\text{ON rate constant} = \alpha(L) + \beta. \quad (5)$$

When light is turned off, $\alpha(L) = 0$ in equation (4). That is,

$$dp/dt = -\beta p. \quad (6)$$

Since p decreases with a time constant of β^{-1} during the turning-off transition, the OFF rate constant of photocurrent is equal to β .

$$\text{OFF rate constant} = \beta. \quad (7)$$

On the other hand at the steady-state ($dp/dt = 0$), the steady state current, I_{ss} is equal to,

$$I_{ss} = i N p_{ss} = i N \alpha(L) / [\alpha(L) + \beta]. \quad (8)$$

If G to A is a single photon reaction,

$$\alpha(L) = \varepsilon \phi L, \quad (9)$$

where the constant ε is a molar absorption coefficient and the constant ϕ is a quantum yield. Therefore, the ON rate constant is predicted to be a first order relation of L ,

$$\text{ON rate constant} = \varepsilon \phi L + \beta. \quad (10)$$

If the relationship (9) is also applied to (8), we can predict that

$$I_{ss} = i N \varepsilon \phi L / (\varepsilon \phi L + \beta). \quad (11)$$

Using Lineweaver-Burk plot, the following relationship is predicted between $(I_{ss})^{-1}$ and L^{-1} as,

$$(I_{ss})^{-1} = (i N)^{-1} \{1 + (\beta \varepsilon^{-1} \phi^{-1}) L^{-1}\}, \quad (12)$$

which gives the linear line with a x-intersection at $(-\beta^{-1} \varepsilon \phi)$ and a y-intersection at $(i N)^{-1}$. Therefore, both relationships (10) and (12) enables one to estimate $(\varepsilon \phi)$, the value representing the intrinsic light sensitivity of the molecule.

Supplementary material 2

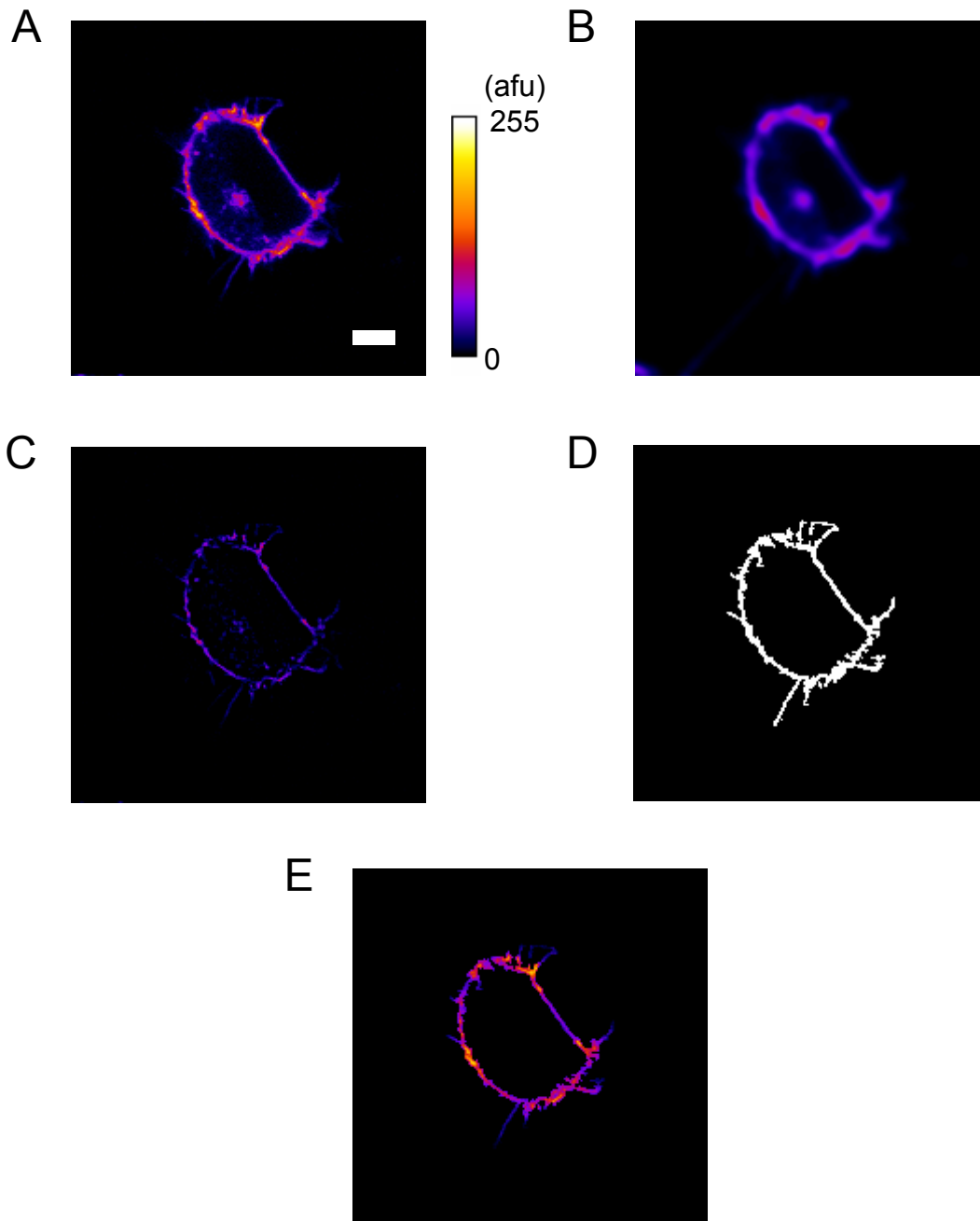


Fig. S2 Algorithm to identify a cell's contour.

(A) A confocal images of the representative HEK293 cell expressing Venus-conjugated ChR2(1-315). Scale bar, 5 μ m. The fluorescence intensity is scaled by the arbitrary fluorescent unit (afu) and expressed in pseudocolor ratings. (B) The 2-pixel Gaussian-filtered image. (C) The difference image between A and B. This difference is expected to be large in a region where the spacial change of fluorescence is large. (D) The cell's contour is thus dichotomously extracted using ImageJ's wand (tracing) tool. This image gives the number of pixels in the plasma membrane region. (E) Multiplied image of A and D. The sum fluorescence is calculated using values given to all pixels in this image.