

S1 Text. Estimation of endosomal Ca²⁺ content and release rate

For the estimation of the Ca²⁺ amount that can be released from an endosome upon illumination the following parameters are assumed:

| | | |
|----------|--|--|
| C_i | Cytosolic Ca ²⁺ concentration | 100 nM |
| C_e | Endosomal Ca ²⁺ concentration [2] | 40 μM |
| σ | Single channel conductance of CatCh [1] | 140 fS |
| n | Estimated number of proteins [3] | 500 / μm ² |
| A | Endosome surface | 3.1416 μm ² |
| r | Endosome radius | 0.5 μm |
| p | open probability | 0.5 |
| E | Membrane potential Endosome [4] | 20 mV |
| z | valence of Ca ²⁺ | 2 |
| e | elementary charge | 1.60218 * 10 ⁻¹⁹ C |
| N_A | Avogadro constant | 6.02214*10 ²³ mol ⁻¹ |

The total amount of Ca²⁺ ions present in one endosome can be estimated assuming a spherical shape:

$$n_{Ca^{2+}} = \frac{4}{3}\pi r^3 * c_e * N_A = \frac{4}{3}\pi(5 * 10^{-7})^3 m^3 * 0.04 \frac{mol}{m^3} * 6.02214 * 10^{23} mol^{-1} = 12613$$

The rate at which ions leave the endosome through tCXCR4/CatCh into the cytosol right upon illumination of the cell can be estimated by the following formula:

$$J_{Ca^{2+}} = E * n * A * \sigma * p * (ze)^{-1} = 0.02 V * 500 * 3.1416 * 140 * 10^{-15} C(sV)^{-1} * 0.5 * (2 * 1.60218 * 10^{-19} C)^{-1} = 6.86 * 10^6 s^{-1}$$

Thus, the amount of calcium released from the endosomes is mainly limited by the number of ions present rather than by the permeability of CatCh.

1. Kleinlogel S, Feldbauer K, Dempski RE, Fotis H, Wood PG, Bamann C, et al. Ultra light-sensitive and fast neuronal activation with the Ca²⁺-permeable channelrhodopsin CatCh. *Nat Neurosci.* 2011;14: 513–518. doi:10.1038/nn.2776
2. Lloyd-Evans E, Waller-Evans H, Peterneva K, Platt FM. Endolysosomal calcium regulation and disease. *Biochem Soc Trans.* 2010;38: 1458–1464. doi:10.1042/BST0381458
3. Zimmermann D, Zhou A, Kiesel M, Feldbauer K, Terpitz U, Haase W, et al. Effects on capacitance by overexpression of membrane proteins. *Biochem Biophys Res Commun.* 2008;369: 1022–1026. doi:10.1016/j.bbrc.2008.02.153
4. Koivusalo M, Steinberg BE, Mason D, Grinstein S. In situ Measurement of the Electrical Potential Across the Lysosomal Membrane Using FRET. *Traffic.* Blackwell Publishing Ltd; 2011;12: 972–982. doi:10.1111/j.1600-0854.2011.01215.x