

References

1. Catterall, W. A., Perez-reyes, E., Snutch, T. P. & Striessnig, J. International Union of Pharmacology. XLVIII. Nomenclature and Structure-Function Relationships of Voltage-Gated Calcium Channels. **57**, 411–425 (2005).
2. Catterall, W. A. Structure and Regulation of Voltage-Gated Ca²⁺ Channels. *Annu. Rev. Cell Dev. Biol.* **16**:521–55 (2000).
3. Bean, B. P. The action potential in mammalian central neurons. **8**, 18–20 (2007).
4. Catterall, W. A. NIH Public Access. **67**, 915–928 (2011).
5. Armstrong, D. L., Erxleben, C. & White, J. A. Patch clamp methods for studying calcium channels. *Methods Cell Biol.* **99**, 183–197 (2010).
6. Bronner, F. Extracellular and Intracellular Regulation of Calcium Homeostasis. *Sci. World J.* **1**, 919–925 (2001).
7. Dolphin, A. C. A short history of voltage-gated calcium channels. *Br. J. Pharmacol.* **147 Suppl**, S56–S62 (2006).
8. Fatt, B. Y. P. & Katz, B. The Electrical Properties of Crustacean Muscle Fibres. *The. Current* (1952).
9. Hagiwara, S., Ozawa, S. & Sand, O. Voltage clamp analysis of two inward current mechanisms in the egg cell membrane of a starfish. *J. Gen. Physiol.* **65**, 617–644 (1975).
10. Catterall, W. A. Interactions of Presynaptic Ca²⁺ Channels and Snare Proteins in Neurotransmitter Release. 144–159 (1999)
11. Dolphin, A. C. Biochimica et Biophysica Acta The $\alpha_2\delta$ subunits of voltage-gated calcium channels ☆. *BBA - Biomembr.* **1828**, 1541–1549 (2013).
12. Campbell, K. P., Leung, A. T. & Sharp, A. H. The biochemistry and molecular biology of the dihydropyridine-sensitive calcium channel. **2236**, (1988).
13. Sharp, A. H. & Campbell, K. P. Characterization of the 1, 4-Dihydropyridine Receptor Using Subunit-specific Polyclonal Antibodies. **264**, 2816–2825 (1989).
14. Moss, F. J. *et al.* The novel product of a five-exon stargazin-related gene abolishes Cav2.2 calcium channel expression. **21**, (2002).

15. Ellis SB, Williams ME, Ways NR, Brenner R, Sharp AH, Leung AT, Campbell KP, McKenna E, Koch WJ, Hui A, et al. Sequence and expression of mRNAs encoding the alpha 1 and alpha 2 subunits of a DHP-sensitive calcium channel. *Science*. Sep 23;241(4873):1661-4. (1988)
16. Day NC, Shaw PJ, McCormack AL, Craig PJ, Smith W, Beattie R, Williams TL, Ellis SB, Ince PG, Harpold MM, Lodge D, Volsen SG. Distribution of alpha 1A, alpha 1B and alpha 1E voltage-dependent calcium channel subunits in the human hippocampus and parahippocampal gyrus. *Neuroscience*. Apr;71(4):1013-24 (1996)
17. Breustedt, J., Vogt, K. E., Miller, R. J., Nicoll, R. A. & Schmitz, D. α 1E - Containing Ca^{2+} channels are involved in synaptic plasticity. **100**, (2003).
18. Dietrich, D. *et al.* Functional Specialization of Presynaptic Cav2.3 Ca^{2+} Channels. **39**, 483–496 (2003).
19. Stotz, S. C., Jarvis, S. E. & Zamponi, G. W. Functional roles of cytoplasmic loops and pore lining transmembrane helices in the voltage-dependent inactivation of HVA calcium channels. 263–273 (2003). doi:10.1113/jphysiol.2003.047068
20. Fox, a P., Nowycky, M. C. & Tsien, R. W. Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. *J. Physiol.* **394**, 149–172 (1987).
21. Snutch, T. P., Peloquin, J., Mathews, E. & Mcrory, J. E. Molecular Properties of Voltage-Gated Calcium Channels. (2005).
22. L  v  que, C. *et al.* Purification of the N-type Calcium Channel Associated with Syntaxin and Synaptotagmin: A complex implicated in synaptic vesicle exocytosis. *J. Biol. Chem.* **269**, 6306–6312 (1994).
23. Buraei, Z. & Yang, J. The Beta Subunit of Voltage-Gated Calcium Channels. *Physiol. Rev.* **90**, 1461–1506 (2010).
24. Ellinor, P. T., Yang, J., Sather, W. A., Zhang, J. & Tsien, R. W. Ca^{2+} Channel Selectivity at a Single Locus for High-Affinity Ca^{2+} Interactions. **15**, 1121–1132 (1995).
25. Lansman, J. B., Hess, P. & Tsien, R. W. Blockade of Current through Single Calcium Channels by Cd^{2+} , Mg^{2+} , and Ca^{2+} Voltage and Concentration Dependence of Calcium Entry into the Pore. **88**, (1986).

26. Bourinet, E. *et al.* The $\alpha 1E$ Calcium Channel Exhibits Permeation Properties Similar to Low-Voltage-Activated Calcium Channels. **16**, 4983–4993 (1996).
27. Sheng, Z., Ret, J., Takahashi, M. & Catterall, W. A. Identification of a Syntaxin-Binding Calcium Channels. Site on N-Type. **13**, 1303–1313 (1994).
28. Asadi, S., Javan, M., Ahmadiani, A. & Sanati, M. H. Alternative splicing in the synaptic protein interaction site of rat Cav2.2 ($\alpha 1B$) calcium channels: Changes induced by chronic inflammatory pain. *J. Mol. Neurosci.* **39**, 40–48 (2009).
29. Cassidy, J. S., Ferron, L., Kadurin, I., Pratt, W. S. & Dolphin, A. C. Functional exofacially tagged N-type calcium channels elucidate the interaction with auxiliary $\alpha 2\delta$ -1 subunits. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 8979–84 (2014).
30. De Waard, M., Pragnell, M. & Campbell, K. P. Ca^{2+} channel regulation by a conserved beta subunit domain. *Neuron* **13**, 495–503 (1994).
31. Pragnell, M. *et al.* Calcium channel beta-subunit binds to a conserved motif in the I-II cytoplasmic linker of the alpha 1-subunit. *Nature* **368**, 67–70 (1994).
32. Scott, V. E. S. *et al.* Beta subunit heterogeneity in N-type Ca^{2+} channels. *J Biol Chem* **271**, 3207–3212 (1996).
33. Dolphin, A. C. Calcium channel auxiliary $\alpha 2\delta$ and β subunits: trafficking and one step beyond. *Nat. Rev. Neurosci.* **13**, 664–664 (2012).
34. Maximov, A. & Bezprozvanny, I. Synaptic targeting of N-type calcium channels in hippocampal neurons. *J. Neurosci.* **22**, 6939–6952 (2002).
35. Size, T., Hell, J. W., Westenbroek, R. E., Elliott, E. M. & Catterall, W. A. Differential Phosphorylation, Localization, and Function of Distinct $\alpha 1$ Subunits of Neuronal Calcium Channels. **22625**, 282–293 (1994)
36. Ruiz, R. *et al.* Active zones and the readily releasable pool of synaptic vesicles at the neuromuscular junction of the mouse. *J. Neurosci.* **31**, 2000–2008 (2011).
37. Verhage, M. & Toonen, R. F. Regulated exocytosis: merging ideas on fusing membranes. *Curr. Opin. Cell Biol.* **19**, 402–408 (2007).
38. Neher, E. Introduction: Regulated exocytosis. *Cell Calcium* **52**, 196–198 (2012).
39. Becherer, U. & Rettig, J. Vesicle pools, docking, priming, and release. *Cell Tissue Res.* **326**, 393–407 (2006).
40. Heuser, J. E. & Jahn, R. Neurotransmitter release -four years of SNARE complexes. 310–315

41. Ahn, R. E. J. Assembly and disassembly of a ternary complex of synaptobrevin, syntaxin, and SNAP-25 in the membrane of synaptic vesicles. **94**, 6197–6201 (1997).
42. Fasshauer, D. et al. Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs. **95**, 15781–15786 (1998).
43. Sutton, R. B., Fasshauer, D., Jahn, R. & Brunger, A. T. Crystal structure of a SNARE complex involved in synaptic ° resolution exocytosis at 2. 4 Å. *Nature* **395**, 347–353 (1998).
44. Chen, Y. A., Scheller, R. H. & Medical, H. H. SNARE-MEDIATED MEMBRANE FUSION. **2**, 98–106 (2001).
45. Rettig, J. *et al.* Isoform-specific interaction of the alpha1A subunits of brain Ca²⁺ channels with the presynaptic proteins syntaxin and SNAP-25. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 7363–7368 (1996).
46. Harkins, A. B., Cahill, A. L., Powers, J. F., Tischler, A. S. & Fox, A. P. Deletion of the synaptic protein interaction site of the N-type (CaV2.2) calcium channel inhibits secretion in mouse pheochromocytoma cells. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 15219–15224 (2004).
47. Cohen, R. & Atlas, D. R-TYPE VOLTAGE-GATED Ca²⁺ CHANNEL INTERACTS WITH SYNAPTIC PROTEINS AND RECRUITS SYNAPTOTAGMIN TO THE PLASMA MEMBRANE OF XENOPUS OOCYTES. **128**, 831–841 (2004).
48. Watanabe, H. *et al.* Involvement of Ca²⁺ channel synprint site in synaptic vesicle endocytosis. *J. Neurosci.* **30**, 655–660 (2010).
49. Yokoyama, C. T., Sheng, Z. H. & Catterall, W. a. Phosphorylation of the synaptic protein interaction site on N-type calcium channels inhibits interactions with SNARE proteins. *J. Neurosci.* **17**, 6929–6938 (1997).
50. Bennett, M. K., Calakos, N. & Scheller, R. H. Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. *Science* **257**, 255–259 (1992).
51. Misura, K. M. S., Scheller, R. H. & Weis, W. I. Three-dimensional structure of the neuronal-Sec1 ± syntaxin 1a complex. **2**, 355–362 (2000).
52. Bezprozvanny, I., Zhong, P., Scheller, R. H. & Tsien, R. W. Molecular determinants of the functional interaction between syntaxin and N-type Ca²⁺ channel gating. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 13943–13948 (2000).

53. Jarvis, S. E., Barr, W., Feng, Z. P., Hamid, J. & Zamponi, G. W. Molecular determinants of syntaxin 1 modulation of N-type calcium channels. *J. Biol. Chem.* **277**, 44399–44407 (2002).
54. Trus, M. D. The transmembrane domain of syntaxin 1A negatively regulates voltage-sensitive Ca (2⁺) channels. doi:10.1016/S0306-4522(01)00083-5 (2001)
55. Davies, J. N., Jarvis, S. E. & Zamponi, G. W. Bipartite syntaxin 1A interactions mediate CaV2.2 calcium channel regulation. *Biochem. Biophys. Res. Commun.* **411**, 562–568 (2011).
56. Dulubova, I. *et al.* A conformational switch in syntaxin during exocytosis: role of munc18. **18**, 4372–4382 (1999).
57. Brunger, A. T. VESICLE FUSION IN NEURONS. (2001).
58. Ullrich, A. *et al.* Dynamical Organization of Syntaxin-1A at the Presynaptic Active Zone. 1–22 (2015). doi: 10.1371/journal.pcbi.1004407
59. Tucker, W. C. & Chapman, E. R. Role of synaptotagmin in Ca²⁺ -triggered exocytosis. **13**, 1–13 (2002).
60. Fernandez-Chacon, R. *et al.* Synaptotagmin I function as a calcium regulator of release probability. (2001). doi:10.1038/35065004
61. Sheng, Z. H., Yokoyama, C. T. & Catterall, W. a. Interaction of the synprint site of N-type Ca²⁺ channels with the C2B domain of synaptotagmin I. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 5405–5410 (1997).
62. Sheng ZH, Rettig J, Cook T, Catterall WA. Calcium-dependent interaction of N-type calcium channels with the synaptic core complex. *Nature*. Feb 1;379(6564):451-4. (1996)
63. Leveque, C. *et al.* Interaction of Cysteine String Proteins with the α 1A Subunit of the P / Q-type Calcium Channel *. **273**, 13488–13492 (1998).
64. Seagar, M. *et al.* Interactions between proteins implicated in exocytosis and voltage-gated calcium channels. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **354**, 289–297 (1999).
65. Magga, J. M. *et al.* Cysteine String Protein Regulates G Protein Modulation of N-Type Calcium Channels. **28**, 195–204 (2000).
66. Chan, A. W. *et al.* Calcium Channel Interacting Protein ND SC RIB. **6950**, (2007).

67. Coppola, T., Magnin-lu, S., Gattesco, S., Schiavo, G. & Regazzi, R. Direct Interaction of the Rab3 Effector RIM with Ca²⁺ Channels, SNAP-25, and Synaptotagmin. **276**, 32756–32762 (2001).
68. Jarvis, S. E., Zamponi, G. W., Jarvis, S. E. & Zamponi, G. W. Interactions between presynaptic Ca²⁺ channels, cytoplasmic messengers and proteins of the synaptic vesicle release complex. **22**, 519–525 (2001).
69. Herlitze, S., Garcia, D. E., Mackie K, Hille B, Scheuer T, Catterall WA. Modulation of Ca²⁺ channels by G-protein beta gamma subunits. *Nature* Mar 21;380(6571):258-62 (1996)
70. Yoon, E., Gerachshenko, T., Spiegelberg, B. D., Alford, S. & Hamm, H. E. G Interferes with Ca²⁺ -Dependent Binding of Synaptotagmin to the Soluble N - Ethylmaleimide-Sensitive Factor Attachment Protein Receptor (SNARE) Complex. 1210–1219 (2007). doi:10.1124/mol.107.039446.
71. Hole, W. & Eric, R. Are the presynaptic membrane particles the calcium channels. **78**, 7210–7213 (1981).
72. Sieber, J. J., Willig, K. I., Heintzmann, R., Hell, S. W. & Lang, T. The SNARE motif is essential for the formation of syntaxin clusters in the plasma membrane. *Biophys. J.* **90**, 2843–2851 (2006).
73. Sieber JJ, Willig KI, Kutzner C, Gerding-Reimers C, Harke B, Donnert G, Rammner B, Eggeling C, Hell SW, Grubmüller H, Lang T. Anatomy and dynamics of a supramolecular membrane protein cluster. *Science*. Aug 24;317(5841):1072-6. (2007)
74. Wolter, S. Super-resolution Imaging Reveals the Internal Architecture of Nano-sized Syntaxin Clusters Super-Resolution Imaging Reveals the Internal Architecture of. (2012). doi:10.1074/jbc.M112.353250
75. Yang, L. *et al.* Secretory Vesicles Are Preferentially Targeted to Areas of Low Molecular SNARE Density. *PLoS One* **7**, (2012).
76. Squid, C. I. N. & Synapse, G. PRESYNAPTIC CALCIUM CURRENTS IN SQUID GIANT SYNAPSE. **33**, (1981).
77. Zucker, R. S. Exocytosis: A Molecular and Physiological Perspective. **17**, 1049–1055 (1996).
78. Abdrakhmanov, M. M., Petrov, A. M., Grigoryev, P. N. & Zefirov, A. L. Depolarization-Induced Calcium- Independent Synaptic Vesicle Exo- and Endocytosis at Frog Motor Nerve Terminals. **5**, 77–82 (2013).

79. Chad, J. E. & Eckert, R. Calcium domains associated with individual channels can account for anomalous voltage relations of CA-dependent responses. *Biophys. J.* **45**, 993–999 (1984).
80. Zucker, R. S. & Fogelson, a L. Relationship between transmitter release and presynaptic calcium influx when calcium enters through discrete channels. *Proc. Natl. Acad. Sci. U. S. A.* **83**, 3032–3036 (1986).
81. John H. Byrne Ruth Heidelberger M. Neal Waxham From Molecules to Networks, 3rd Edition, An Introduction to Cellular and Molecular Neuroscience, Academic Press (2014)
82. Bingham, J. P., Mitsunaga, E. & Bergeron, Z. L. Drugs from slugs-Past, present and future perspectives of ω -conotoxin research. *Chem. Biol. Interact.* **183**, 1–18 (2010).
83. Terlau, H. & Olivera, B. M. Conus venoms: a rich source of novel ion channel-targeted peptides. *Physiol. Rev.* **84**, 41–68 (2004).
84. Lewis, R. J., Dutertre, S., Vetter, I. & Christie, M. J. Conus venom peptide pharmacology. *Pharmacol. Rev.* **64**, 259–98 (2012).
85. Feng, Z. P. *et al.* Residue Gly1326 of the N-type Calcium Channel α 1B Subunit Controls Reversibility of ω -Conotoxin GVIA and MVIIA Block. *J. Biol. Chem.* **276**, 15728–15735 (2001).
86. Yarotskyy, V. & Elmslie, K. S. omega-conotoxin GVIA alters gating charge movement of N-type (CaV2.2) calcium channels. *J. Neurophysiol.* **101**, 332–340 (2009).
87. Berecki, G. *et al.* Analgesic (omega)-conotoxins CVIE and CVIF selectively and voltage-dependently block recombinant and native N-type calcium channels. *Mol. Pharmacol.* **77**, 139–148 (2010).
88. Sousa, S. R., Vetter, I. & Lewis, R. J. Venom peptides as a rich source of Cav2.2 channel blockers. *Toxins (Basel).* **5**, 286–314 (2013).
89. Witcher, D. R., De Waard, M. & Campbell, K. P. Characterization of the purified N-type Ca²⁺ channel and the cation sensitivity of omega-conotoxin GVIA binding. *Neuropharmacology* **32**, 1127–1139 (1993).
90. Wagner, J. a, Snowman, a M., Biswas, a, Olivera, B. M. & Snyder, S. H. Omega-conotoxin GVIA binding to a high-affinity receptor in brain: characterization, calcium sensitivity, and solubilisation. *J. Neurosci.* **8**, 3354–3359 (1988).

91. Yarotsky, V. & Elmslie, K. S. Interference between two modulators of N-type (CaV2.2) calcium channel gating demonstrates that ω -conotoxin GVIA disrupts open state gating. *Biochim. Biophys. Acta - Biomembr.* **1798**, 1821–1828 (2010).
92. Lakowicz, J. R. Principles of Fluorescence Spectroscopy, Plenum Press (1983)
93. Road, S. The discovery of aequorin and green fluorescent protein. **217**, 3–15 (2005).
94. Tsien, R. Y. the Green Fluorescent. *Annu. Rev. Biochem.* **67**, 509–544 (1998).
95. Patterson, G. H. et al. A Photoactivatable GFP for Selective Photolabeling of Proteins and Cells. **297**, 1873–1878 (2002).
96. Ando, R., Hama, H., Yamamoto-Hino, M., Mizuno, H. & Miyawaki, A. An optical marker based on the UV-induced green- to-red photoconversion of a fluorescent protein. **2–7** (2002).
97. Ando, R., Mizuno, H. & Miyawaki, A. Regulated Fast Nucleocytoplasmic Shuttling Observed by Reversible Protein Highlighting. **306**, (2004).
98. Sluder, G., Wolf, D. Methods in Cell Biology Volume 114, Digital Microscopy 4th Edition, Elsevier (2013)
99. Cole, R., Brown, C. M., Cole, R. W., Jinadasa, T. & Brown, C. M. Measuring and interpreting point spread functions to determine confocal microscope resolution and ensure quality control Measuring and interpreting point spread functions to determine confocal microscope resolution and ensure quality control. *Nat. Protoc.* **6**, 1929–1941 (2011).
100. Heintzmann, R. & Ficz, G. Breaking the resolution limit in light microscopy. *Methods Cell Biol.* **114**, 525–544 (2013).
101. Pawley, J., Handbook of Biological Confocal Microscopy, Springer (1995)
102. Jose M. Moran-Mirabal (2013). Advanced-Microscopy Techniques for the Characterization of Cellulose Structure and Cellulose-Cellulose Interactions, Cellulose - Fundamental Aspects, Dr. Theo G.M. van De Ven (Ed.), InTech, DOI: 10.5772/56584
103. Klar, T. A, Engel, E. & Hell, S. W. Breaking Abbe's diffraction resolution limit in fluorescence microscopy with stimulated emission depletion beams of various shapes. *Phys. Rev. E. Stat. Nonlin. Soft Matter Phys.* **64**, 66613 (2001).
104. Klar, T. A, Jakobs, S., Dyba, M., Egner, A & Hell, S. W. Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 8206–8210 (2000).

105. Tønnesen, J. & Nägerl, U. V. Superresolution imaging for neuroscience. *Exp. Neurol.* **242**, 33–40 (2013).
106. Neupane, B. *et al.* Continuous-Wave Stimulated Emission Depletion Microscope for Imaging Actin Cytoskeleton in Fixed and Live Cells. 24178–24190 (2015). doi:10.3390/s150924178
107. Moffitt, J. R., Osseforth, C. & Michaelis, J. Time-gating improves the spatial resolution of STED microscopy. *Opt. Express* **19**, 4242–4254 (2011).
108. Vicidomini, G. *et al.* Sharper low-power STED nanoscopy by time gating. *Nat. Methods* **8**, 571–573 (2011).
109. Beier, H. T. & Ibey, B. L. Experimental Comparison of the High-Speed Imaging Performance of an EM-CCD and sCMOS Camera in a Dynamic Live-Cell Imaging Test Case. **9**, (2014).
110. Axelrod, D. Total internal reflection fluorescence microscopy in cell biology [Review]. *Traffic* **2**, 764–774 (2001).
111. Axelrod, D. Cell-substrate contacts illuminated by total internal reflection fluorescence. *J. Cell Biol.* **89**, 141–145 (1981).
112. Steyer, J. a, Horstmann, H. & Almers, W. Transport, docking and exocytosis of single secretory granules in live chromaffin cells. *Nature* **388**, 474–478 (1997).
113. Oheim, M., Loerke, D., Stühmer, W. & Chow, R. H. The last few milliseconds in the life of a secretory granule. 83–98 (1998).
114. Owen, D. M. *et al.* PALM imaging and cluster analysis of protein heterogeneity at the cell surface. *J. Biophotonics* **3**, 446–454 (2010).
115. Rust, M. J., Bates, M. & Zhuang, X. imaging by stochastic optical reconstruction microscopy (STORM). **3**, 793–795 (2006).
116. Heilemann, M. *et al.* Subdiffraction-Resolution Fluorescence Imaging with Conventional Fluorescent Probes **. 6172–6176 (2008). doi:10.1002/anie.200802376
117. Linde, S. Van De, Sauer, M. & Heilemann, M. Subdiffraction-resolution fluorescence imaging of proteins in the mitochondrial inner membrane with photoswitchable fluorophores. *J. Struct. Biol.* **164**, 250–254 (2008).
118. Heilemann, M., Linde, S. Van De, Mukherjee, A. & Sauer, M. Super-Resolution Imaging with Small Organic Fluorophores. 6903–6908 (2009). doi:10.1002/anie.200902073

119. Dempsey, G. T., Vaughan, J. C., Chen, K. H., Bates, M., Zhuang, X., *Methods, N. HHS Public Access.* **8**, 1027–1036 (2012).
120. Vaughan, J. C. *et al.* Phosphine-Quenching of Cyanine Dyes as a Versatile Tool for Fluorescence Microscopy Phosphine-Quenching of Cyanine Dyes as a Versatile Tool for Fluorescence Microscopy. (2013). doi:10.1021/ja3105279
121. Linde, S. Van De *Et al.* Direct stochastic optical reconstruction microscopy with standard fluorescent probes. **6**, 34–38 (2011).
122. Endesfelder, U. *et al.* Chemically induced photoswitching of fluorescent probes- A general concept for super-resolution microscopy. *Molecules* **16**, 3106–3118 (2011).
123. Bates, W. M., Huang, B., Dempsey, G. T., Zhuang, X., Multicolor Super-resolution Imaging with Photo-switchable Fluorescent Probes, *Science* 2007 September 21; 317(5845): 1749–1753. doi:10.1126/science.1146598.
124. Jones, S. A., Shim, S., He, J. & Zhuang, X. Fast, three-dimensional super-resolution imaging of live cells. **8**, (2011).
125. Bost, A., Pasche, M., Schirra, C. & Becherer, U. Super-resolution microscopy in studying neuroendocrine cell function. *Front. Neurosci.* **7**, 222 (2013).
126. Deschout, H. *et al.* Precisely and accurately localizing single emitters in fluorescence microscopy. **11**, 253–266 (2014).
127. Cheezum, M. K., Walker, W. F. & Guilford, W. H. Quantitative Comparison of Algorithms for Tracking Single Fluorescent Particles. **81**, 2378–2388 (2001).
128. Thompson, R. E., Larson, D. R. & Webb, W. W. Precise Nanometre Localization Analysis for Individual Fluorescent Probes. **82**, 2775–2783 (2002).
129. Betzig, E. *et al.* Imaging Intracellular Fluorescent Proteins at Nanometre Resolution. **x**, 1642–1646 (2006).
130. Manley, S. *et al.* High-density mapping of single-molecule trajectories with photoactivated localization microscopy. *Nat. Methods* **5**, 155–157 (2008).
131. Shroff, H., Galbraith, C. G., Galbraith, J. A. & Betzig, E. Live-cell photoactivated localization microscopy of nanoscale adhesion dynamics. **5**, 417–423 (2008).
132. Kenworthy, A. K. *et al.* cell surface. **165**, 735–746 (2004).
133. Hess Girirajan T, Mason M, S. Ultra-High Resoluion Imaging by Fluorescence Photoactivation Localization Microscopy. *Biophys. J.* **91**, 4258–4272 (2006).

134. Deschout, H., Shivanandan, A., Annibale, P., Scarselli, M. & Radenovic, A. Progress in quantitative single-molecule localization microscopy. *Histochem. Cell Biol.* **142**, 5–17 (2014).
135. Duncan, R. R., Bergmann, A., Cousin, M. A., Apps, D. K. & Shipston, M. J. Multi-dimensional time-correlated single photon counting (TCSPC) fluorescence lifetime imaging microscopy (FLIM) to detect FRET in cells. *J. Microsc.* **215**, 1–12 (2004).
136. Poland, S. P. *et al.* A high speed multifocal multiphoton fluorescence lifetime imaging microscope for live-cell FRET imaging. *Biomed. Opt. Express* **6**, 277 (2015).
137. Becker, W. Fluorescence lifetime imaging – techniques and applications. **247**, 119–136 (2012).
138. Becker, W., Bergmann, M. A., König, K., Benndorf, K., Biskup, C. Fluorescence Lifetime Imaging by Time-Correlated Single-Photon Counting. **66**, 58–66 (2004).
139. Duncan, R. R. Europe PMC Funders Group Fluorescence lifetime imaging microscopy (FLIM) to quantify protein-protein interactions inside cells. **34**, 679–682 (2007).
140. Lleres, D., Swift, S. & Lamond, A. I. Detecting Protein-Protein Interactions In Vivo with FRET using Multiphoton Fluorescence Lifetime Imaging Microscopy (FLIM). *Curr. Protoc. Cytom.* (2007). doi:10.1002/0471142956.cy1210s42
141. Ishikawa-ankerhold, H. C., Ankerhold, R. & Drummen, G. P. C. Advanced Fluorescence Microscopy Techniques—FRAP, FLIP, FLAP, FRET and FLIM. 4047–4132 (2012). doi:10.3390/molecules17044047
142. Stryer, L. & Haugland, R. P. Energy transfer: a spectroscopic ruler. *Proc. Natl. Acad. Sci. U. S. A.* **58**, 719–726 (1967).
143. Albertazzi, L., Arosio, D., Marchetti, L., Ricci, F. & Beltram, F. Quantitative FRET Analysis with the E0 GFP-mCherry Fluorescent Protein Pair. 287–297 (2009). doi:10.1111/j.1751-1097.2008.00435.x
144. Catterall, W. A. Voltage-Gated Calcium Channels. *Cold Spring Harb. Perspect. Biol.* **3**, 1–24 (2011).
145. Fernandez, I. *et al.* Three-dimensional structure of an evolutionarily conserved N-terminal domain of syntaxin 1A. *Cell* **94**, 841–849 (1998).

146. Wu, J. et al. Structure of the voltage-gated calcium channel Ca. *Structural Biology* (2015)
147. Soong, T. W. *et al.* Structure and functional expression of a member of the low voltage-activated calcium channel family. *Science* **260**, 1133–6 (1993).
148. Loechner, K. J. et al. Calcium Currents in a Pituitary Cell Line (AtT-20): Differential Roles in Stimulus-Secretion Coupling. *Endocrinology* vol.137, no. 4 (1996)
149. Greene, L. A., Tischlert, A. S. & Kuffler, S. W. Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor (sympathetic neurons/cell culture/catecholamines/differentiation/neurites). *Cell Biol.* **73**, 2424–2428 (1976).
150. Janigro, D., Maccaferri, G. & Meldolesi, J. Calcium channels in undifferentiated PC 12 rat pheochromocytoma cells. *Current* **255**, 398–400 (1989).
151. Lang, T. Imaging SNAREs at work in ‘unroofed’ cells--approaches that may be of general interest for functional studies on membrane proteins. *Biochem. Soc. Trans.* **31**, 861–864 (2003).
152. <http://csmedia2.corning.com/LifeSciences/media/pdf/SPC-354240.pdf>
153. <https://svi.nl/SamplingRate>
154. <https://svi.nl/NyquistRate>
155. <http://www.abberior.com/shop/Labels-by-Application/Confocal-Epi-SIM/Abberior-CAGE-552::13.html>
156. Manders, E. M. M. et al. Measurement of co-localization of objects in dual-color confocal images. *Journal of Microscopy* 169, 375–382 (1993).
157. Ovesný, M., Křížek, P., Borkovec, J., Svindrych, Z. & Hagen, G. M. ThunderSTORM: a comprehensive ImageJ plug-in for PALM and STORM data analysis and super-resolution imaging. *Bioinformatics* **30**, 2389–2390 (2014).
158. Lagache, T., Lang, G., Sauvonnnet, N. & Olivo-Marin, J.-C. Analysis of the Spatial Organization of Molecules with Robust Statistics. *PLoS One* **8**, e80914 (2013).
159. Rubin-Delanchy, P. *et al.* Bayesian cluster identification in single-molecule localization microscopy data. *Nat Meth* **12**, 1072–1076 (2015).
160. Visser, A. J. W. G., Westphal, A. H., Skakun, V. V & Borst, J. W. GFP as potential cellular viscosimeter. *Methods Appl. Fluoresc.* **4**, 35002 (2016).

161. Young, M. E., Carroad, P. A. & Bell, R. L. Estimation of Diffusion Coefficients of Proteins. *Biotechnol. Bioeng.* **22**, 947–955 (1980).
162. Cruickshank Miller, C. The Stokes-Einstein Law for Diffusion in Solution. *Math. Phys. Character* **106**, 724–749 (1924).
163. Einstein, A. On the Motion of Small Particles Suspended in a Stationary Liquid, as Required by the Molecular Kinetic Theory of Heat. *Ann. Phys.* **322**, 549–560 (1905).
164. Barg, S., Knowles, M. K., Chen, X., Midorikawa, M. & Almers, W. Syntaxin clusters assemble reversibly at sites of secretory granules in live cells. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 20804–9 (2010).
165. Knowles, M. K. *et al.* Single secretory granules of live cells recruit syntaxin-1 and synaptosomal associated protein 25 (SNAP-25) in large copy numbers. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 20810–20815 (2010).
166. Honigsmann, A. *et al.* Phosphatidylinositol 4,5-bisphosphate clusters act as molecular beacons for vesicle recruitment. *HHS Public Access.* **20**, 679–686 (2013).
167. Chenouard, N. *et al.* Objective comparison of particle tracking methods. *Nat. Methods* **11**, 281–289 (2014).
168. Wilson, R. S. *et al.* Automated single particle detection and tracking for large microscopy datasets. *R. Soc. Open Sci.* **3**, 160225 (2016).
169. Dun, A. R. *et al.* Navigation through the Plasma Membrane Molecular Landscape Shapes Random Organelle Movement. *Curr. Biol.* **27**, 408–414 (2017).
170. Schlangen, I. *et al.* Marker-Less Stage Drift Correction in Super-Resolution Microscopy Using the Single-Cluster PHD Filter. *IEEE J. Sel. Top. Signal Process.* **10**, 193–202 (2016).
171. Smal, I., Loog, M., Niessen, W. & Meijering, E. IEEE TRANSACTIONS ON MEDICAL IMAGING Quantitative Comparison of Spot Detection Methods in Fluorescence Microscopy. **29**, 282–301 (2009).
172. Mahler, R. P. (2003). Multitarget Bayes filtering via first-order multitarget moments. *IEEE Transactions on Aerospace and Electronic systems*, 39(4), 1152-1178.
173. Meijering, E., Dzyubachyk, O. & Smal, I. *Methods for cell and particle tracking. Methods in Enzymology* **504**, (Elsevier Inc., 2012).

174. Wang, S.-Q., Song, L.-S., Lakatta, E. G. & Cheng, H. Ca²⁺ signalling between single L-type Ca²⁺ channels and ryanodine receptors in heart cells. *Nature* **410**, 592–596 (2001).
175. Miyawaki, A. et al. Fluorescent indicators for Ca²⁺ based on green fluorescent proteins and calmodulin. *Nature* **388**, 882–887 (1997).
176. Zhao Y. *et al.* An Expanded Palette of Genetically Encoded Ca²⁺ Indicators. Pub Med Central CANADA. *Clin. Neurophysiol.* **123**, 106–116 (2011).
177. Serulle, Y., Sugimori, M. & Llinás, R. R. Imaging synaptosomal calcium concentration microdomains and vesicle fusion by using total internal reflection fluorescent microscopy. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 1697–702 (2007).
178. Dawidowski, D. & Cafiso, D. S. Allosteric control of syntaxin 1a by munc18-1: Characterization of the open and closed conformations of syntaxin. *Biophys. J.* **104**, 1585–1594 (2013).
179. Schermelleh, L., Heintzmann, R. & Leonhardt, H. A guide to super-resolution fluorescence microscopy. **190**, 165–175 (2010).
180. Kornreich, B. G. The patch clamp technique: Principles and technical considerations. *J. Vet. Cardiol.* **9**, 25–37 (2007).
181. Demuro, A. & Parker, I. Optical single-channel recording: Imaging Ca²⁺ flux through individual N-type voltage-gated channels expressed in *Xenopus* oocytes. *Cell Calcium* **34**, 499–509 (2003).
182. Demuro, A. & Parker, I. Imaging the activity and localization of single voltage-gated Ca (2+) channels by total internal reflection fluorescence microscopy. *Biophys. J.* **86**, 3250–3259 (2004).
183. Liu, Ch., Hermann, T. E. Communication Characterization Calcium Ionophore *. 5892–5894 (1978).
184. Morgan, a J. & Jacob, R. Ionomycin enhances Ca²⁺ influx by stimulating store-regulated cation entry and not by a direct action at the plasma membrane. *Biochem. J.* **300 (Pt 3)**, 665–672 (1994).
185. Simon, S. M. & Llinás, R. R. Compartmentalization of the sub membrane calcium activity during calcium influx and its significance in transmitter release. *Biophys. J.* **48**, 485–498 (1985).
186. Tohse, N., Meszaros, J., Sperelakis, N., MÇsz†ros, J. & Sperelakis, N. Developmental changes in long-opening behaviour of L-type Ca²⁺ channels in embryonic chick heart cells. *Circ Res* **71**, 376–384 (1992).

187. (<http://imagej.net/T-functions>)
188. Dulhunty, A. F. & Gage, P. W. Effects of cobalt, magnesium, and cadmium on contraction of rat soleus muscle. **56**, 1–14 (1989).
189. Marom, M., Hagalili, Y., Sebag, A., Tzvier, L. & Atlas, D. Conformational changes induced in voltage-gated calcium channel Cav1.2 by BayK 8644 or FPL64176 modify the kinetics of secretion independently of Ca²⁺ influx. *J. Biol. Chem.* **285**, 6996–7005 (2010).
190. Lebrun, P. & Atwater, I. Effects of the calcium channel agonist, BAY K 8644, on electrical activity in mouse pancreatic B-cells. *Biophys. J.* **48**, 919–930 (1985).
191. Shen, J.-B., Jiang, B. & Pappano, A. J. Comparison of L-Type Calcium Channel Blockade by Nifedipine and/or Cadmium in Guinea Pig Ventricular Myocytes. *J. Pharmacol. Exp. Ther.* **294**, 562–570 (2000).
192. McCleskey, E. W., Fox, A. P., Feldman, D. & Tsien, R. W. Different types of calcium channels. *J. Exp. Biol.* **124**, 177–90 (1986).
193. Shuai, J. & Parker, I. Optical single-channel recording by imaging Ca²⁺ flux through individual ion channels: Theoretical considerations and limits to resolution. *Cell Calcium* **37**, 283–299 (2005).
194. Paredes, R. M., Etzler, J. C., Watts, L. T. & Lechleiter, J. D. NIH Public Access. *Imaging* **46**, 143–151 (2009).
195. Pérez Koldenkova, V. & Nagai, T. Genetically encoded Ca²⁺ indicators: Properties and evaluation. *Biochim. Biophys. Acta - Mol. Cell Res.* **1833**, 1787–1797 (2013).
196. Gather, M. C. & Yun, S. H. Lasing from Escherichia coli bacteria genetically programmed to express green fluorescent protein. *Opt. Lett.* **36**, 3299–3301 (2011).
197. Maiman, Stimulated Optical Radiation in Ruby, *Nature* (1960).
198. Aitken, C. E., Marshall, R. A. & Puglisi, J. D. An oxygen scavenging system for improvement of dye stability in single-molecule fluorescence experiments. *Biophys. J.* **94**, 1826–1835 (2008).
199. Ellis-Davies, G. C. R., Kaplan, J. H. & Robert, J. Laser Photolysis. **70**, (1996).
200. <http://kirschner.med.harvard.edu/files/bionumbers/Sizes%20of%20various%20cells.pdf>

201. Houel, J. *et al.* Autocorrelation analysis for the unbiased determination of power-law exponents in single-quantum-dot blinking. *ACS Nano* **9**, 886–893 (2015).
202. Kouri, T. & Mehta, D. Experimental Algorithms. *Exp. Algorithms* **6630**, 157–168 (2011).
203. Miki, T. *et al.* Numbers of presynaptic Ca²⁺ channel clusters match those of functionally defined vesicular docking sites in single central synapses. *Proc. Natl. Acad. Sci.* 201704470 (2017). doi:10.1073/pnas.1704470114
204. Berjukow, S. *et al.* Endogenous calcium channels in human embryonic kidney (HEK293) cells. *Br. J. Pharmacol.* **118**, 748–54 (1996).
205. Di Virgilio, F., Milani, D., Leon, A., Meldolesi, J. & Pozzan, T. Voltage-dependent activation and inactivation of calcium channels in PC12 cells. Correlation with neurotransmitter release. *J Biol Chem* **262**, 9189–9195 (1987).
206. Szabo, Z., Obermair, G. J., Cooper, C. B., Zamponi, G. W. & Flucher, B. E. Role of the synprint site in presynaptic targeting of the calcium channel CaV2.2 in hippocampal neurons. *Eur. J. Neurosci.* **24**, 709–718 (2006).
207. Spafford, J. D. & Zamponi, G. W. Functional interactions between presynaptic calcium channels and the neurotransmitter release machinery. *Curr. Opin. Neurobiol.* **13**, 308–314 (2003).
208. Shivanandan, A., Deschout, H., Scarselli, M. & Radenovic, A. Challenges in quantitative single molecule localization microscopy. *FEBS Lett.* **588**, 3595–3602 (2014).
209. Neher, E. Vesicle pools and Ca²⁺ microdomains: New tools for understanding their roles in neurotransmitter release. *Neuron* **20**, 389–399 (1998).
210. Roberts, W. M., Jacobs, R. A. & Hudspeth, A. J. Colocalization of ion channels involved in frequency selectivity and synaptic transmission at presynaptic active zones of hair cells. *J. Neurosci.* **10**, 3664–84 (1990).
211. Rickman, C. & Duncan, R. R. Munc18/syntaxin interaction kinetics control secretory vesicle dynamics. *J. Biol. Chem.* **285**, 3965–3972 (2010).
212. Mackenzie, M. D., Cialowicz, K. I., Hamilton, C., Martin, K. J., Saleeb, R. S., Duncan, R. R., Kar, A. K. Femtosecond Pumped Biological Laser for Use In Fluorescence Studies. (in submission)
213. Wu, M. M., Llobet, A. & Lagnado, L. Loose coupling between calcium channels and sites of exocytosis in chromaffin cells. *J. Physiol.* **587**, 5377–5391 (2009).

214. Garciaa-Saez, A. J. & Schwille, P. Surface analysis of membrane dynamics. *Biochim. Biophys. Acta - Biomembr.* **1798**, 766–776 (2010).
215. Lorén, N. *et al.* Fluorescence recovery after photobleaching in material and life sciences: putting theory into practice. *Q. Rev. Biophys.* **in press**, 323–387 (2015).
216. Malengo, G. *et al.* Fluorescence Correlation Spectroscopy and Photon Counting Histogram on membrane proteins: Functional dynamics of the GPI-anchored Urokinase Plasminogen Activator Receptor. **13**, 1–24 (2009).
217. Kreytzerger, A. J. B. *et al.* Reconstitution of calcium-mediated exocytosis of dense-core vesicles. *Sci. Adv.* **3**, e1603208 (2017).
218. Tytgat, H. L. P. *et al.* Endogenous biotin-binding proteins: An overlooked factor causing false positives in streptavidin-based protein detection. *Microb. Biotechnol.* **8**, 164–168 (2015).
219. Ellis-Davies, G. C. & Kaplan, J. H. Nitrophenyl-EGTA, a photolabile chelator that selectively binds Ca²⁺ with high affinity and releases it rapidly upon photolysis. *Proc. Natl. Acad. Sci. U. S. A.* **91**, 187–191 (1994).
220. Jurkat-Rott, K. & Lehmann-Horn, F. The impact of splice isoforms on voltage-gated calcium channel $\alpha 1$ subunits. *J. Physiol.* **554**, 609–619 (2004).
221. Bleakman, D. *et al.* Characteristics of a human N-type calcium channel expressed in HEK293 cells. *Neuropharmacology* **34**, 753–765 (1995).
222. Shaw, G. *et al.* Preferential transformation of human neuronal cells by human adenoviruses and the origin of HEK 293 cells. *FASEB J.* **16**, 1805–1807 (2002).
223. Chen, Y. A. *et al.* Calcium Regulation of Exocytosis in PC12 Cells. *J. Biol. Chem.* **276**, 26680–26687 (2001).
224. Westerink, R. & Ewing, A. The PC12 cell as model for neurosecretion. *Acta Physiol.* **192**, 273–285 (2008).
225. Niemeyer, B. A. *et al.* Ion channels in health and disease. 83rd Boehringer Ingelheim Fonds International Titisee Conference. *EMBO Rep.* **2**, 568–573 (2001).
226. Perret, D. & Luo, Z. Targeting voltage-gated calcium channels for neuropathic pain management. *Neurotherapeutics* **6**, 679–692 (2009).
227. Chapman, E. R., Desai, R. C., Davis, A. F. & Tornehl, C. K. Delineation of the oligomerization, AP-2 binding, and synprint binding region of the C2B domain of synaptotagmin. *J. Biol. Chem.* **273**, 32966–32972 (1998).

228. Ramachandran, S. *et al.* Structure and Permeability of Ion-channels by Integrated AFM and Waveguide TIRF Microscopy. *Sci. Rep.* **4**, 4424 (2014).
229. Chan, A. W., Khanna, R., Li, Q. & Stanley, E. F. Munc18: A presynaptic transmitter release site N type (CaV2.2) calcium channel interacting protein. *Channels* **1**, 11–20 (2007).
230. Mochida, S., Sheng, Z.-H., Baker, C., Kobayashi, H. & Catterall, W. a. Inhibition of Neutotransmission by Peptides Containing the Synaptic Protein Interaction Site of N-type Ca²⁺ Channels. *Neuron* **17**, 781–788 (1996).