Abstract

Calcium ions in the human body are responsible for cell homeostasis. In addition, many cell functions, such as synaptic transmission, hormone and neuropeptide release are triggered by calcium. Ca^{2+} concentration must be therefore carefully regulated by voltage-gated calcium channels. Exocytosis is the process of the fusion of the secretory vesicle with the plasma membrane. For effective and fast exocytosis, vesicles must be docked near calcium channels. The probability of the release of synaptic vesicles is hypothesized to increase with the number of proximal calcium channels. N-type (Cav2.2) calcium channels cooperate directly with SNARE proteins and synaptotagmin through the specific synprint site, which is located on the main pore forming subunit. In recent years the amount of research on calcium channels has markedly increased, but there are still limitations in the methods used to study Ca^{2+}. This results in a loss of information with regard to the true location of a point source that is emitting light and therefore the proteins we want to localize. The development of super-resolution techniques allows imaging of these proteins closer to the molecular scale, enabling us to better understand these cellular processes.

The results presented in this thesis aim to understand the distribution and behaviour of N-type calcium channels across the cell membrane, using advanced microscopy techniques to image below the diffraction limit. Our findings revealed that the synprint site has an influence on Cav2.2 calcium channel cluster patterning and behaviour. The results demonstrate no direct interaction between Cav2.2 calcium channels and syntaxin-1A. The experiments with a genetically encoded calcium indicator fused to a SNARE protein together with TIRF microscopy present a promising method to examine the calcium "activity" across the plasma membrane.

Findings presented in this thesis introduce a new angle of looking at the interaction of syntaxin-1A with the synprint motif of Cav2.2 calcium channels. Taken together the results can create a novel model of the distribution and behaviour of Cav2.2 calcium channels in the secretory cells and it is recommended that these methods are employed more widely in the future to investigate ion channel distribution and function in cells.

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I would like to dedicate this thesis to my parents, two amazing mathematicians and to my two little sisters, for their endless love and support in all my decisions. Thank you for believing in me.

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Preliminaries

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Abbreviations

AID	Subunit interaction domain at the α_1 cytoplasmic linker of the I-II loop
AOBS	Acousto-optical beam-splitter
APD	Avalanche photodiode
AtT-20	Mouse pituitary tumour cell line (AtT-20/D16-16)
BME	Mercaptoethanol
BSA	Bovine Serum Albumin
Cav2.2	N-type voltage-gated calcium channels, full length variant
Cav2.2(18a)	N-type voltage-gated calcium channels, without 18a exon which encodes 21 aa in the synaptic protein interaction site
CLSM	Confocal laser scanning microscope
CSP	Vesicle-associated cysteine string proteins
CW	Continuous-wave
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dSTORM	Direct Stochastic optical reconstruction microscopy
DTT	Dithiothreitol
EGFP	Enhanced GFP
EGTA	Ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid
EM	Electron Multiplying
EMCCD	Electron Multiplying Charge-Coupled Device
ESI-MS	Electrospray ionisation mass spectrometry
FBS	Foetal Bovine Serum
FCS	Fluorescence correlation spectroscopy
FLIM	Fluorescence lifetime imaging microscopy
Fluo-3	Chemical calcium indicator
FRAP	Fluorescence recovery after photobleaching
FRET	Förster resonance energy transfer
FWHM	Full width at half maximum
Gcamp	Genetically encoded calcium indicator
GFP	Green fluorescent protein
Gly	Glycine
gSTED	Time-gated Stimulated emission depletion microscopy

H3	Carboxy-terminal SNARE domain of syntaxin-1A
Habc	N-terminal regulatory domain of syntaxin-1A
HEK293	Human embryonic kidney cells
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPC-1	Anti-Syntaxin antibody [STX01 (HPC-1)]
HPLC	High-performance liquid chromatography method
HVA	High-voltage activated
LVA	Low-voltage activated
M1/M2	Manders coefficient for the pixels above the channel 1/2
MCP	Micro Channel Plate
MEA	Mercaptoethilamine
NA	Numerical Aperture
NGF	Nerve growth factor
PACherry	Photoactivatable mCherry
PALM	Photoactivated localisation microscopy
PC12	Adrenal pheochromocytoma cell line
PFA	Paraformaldehyde
PHD	Probability Hypothesis Density filter
PI	Propidium Iodide
PMT	Photomultiplier tube detector
PSF	Point spread function
Q-SNARE	tSNARE, plasma (target) membrane SNARE
R-GECO	Genetically encoded calcium
R-SNARE	vSNARE vesicle membrane SNARE
Rim-1	Rab3-interacting molecule
ROI	Region of interest
RT	Room temperature
sCMOS	Scientific Complementary Metal Oxide Semiconductor
SMD	Single molecule detection
SNAP-25	Synaptosomal-associated protein of 25 kDa
SNARE	Soluble NSF (N-ethylmaleimide-sensitive fusion protein) attachment protein receptor
SNR	Signal-to-noise ratio
SPAD	Single Photon Avalanche Diode detector

sptPALM	Single-particle tracking PALM
STED	Stimulated emission depletion microscopy
STORM	Stochastic optical reconstruction microscopy
TCEP	Tris (2-carboxyethyl) phosphine
TCSPC	Time-Correlated Single Photon Counting
TIRF	Total internal reflection fluorescence microscopy
TTX	Tetrodotoxin
UV	Ultraviolet
VAMP-2	Synaptobrevin-2 (vesicle-associated membrane protein)
WLL	White light laser

Publications

Navigation through the Plasma Membrane Molecular Landscape Shapes Random Organelle Movement, A.R.Dun, G.Lord, R.S.Wilson, D.M.Kavanagh, **K.Cialowicz**, S.Sugita, S.Park, L.Yang, A.M.Smyth, A.Papadopulos, C.Rickman, R.R.Duncan, Current Biology 27(3), January 2017, DOI: 10.1016/j.cub.2016.12.002

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Vitamin C for stabilising biological lasers. (Conference Presentation), A.A. Kar, M.D.Mackenzie, **K.Cialowicz**, R.Saleeb, R.R.Duncan, Conference Paper, April 2016, DOI: 10.1117/12.2211720, Conference: SPIE BiOS