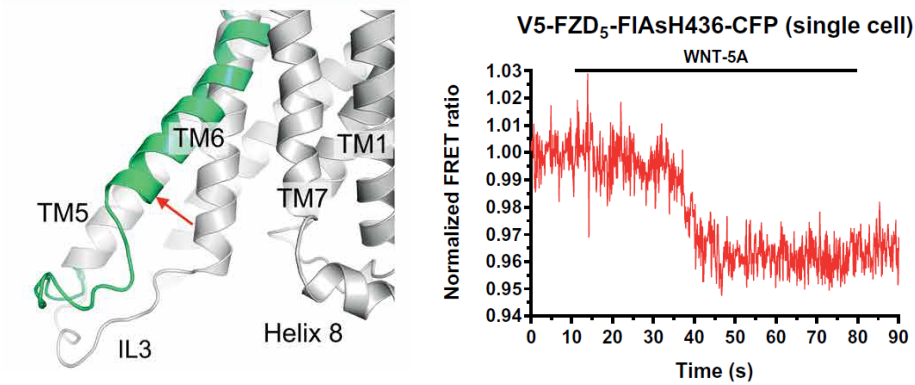
# BioPen® Applications

## G-PROTEIN COUPLED RECEPTOR ACTIVATION IN SINGLE CELLS




Conformational change of tranfmembrane helix 6 of $FDZ_5$ from inactive to active conformation (left). Single cell expressing $FDZ_5$ undergoes activation when exposed to WNT-5A using the BioPen dequenching the FRET donor (right).

G-protein coupled receptors (GPCR) are a class of transmembrane receptor proteins that are involved in a wide range of physiological processes. Central to the classification of the GPCRs is the ability to activate G-proteins following binding of an agonist to receptor. Frizzleds (FZDs) are a ubiquitously expressed type of transmembrane receptor that share many features with GPCRs, but whose inclusion into that category is disputed due to the lack of evidence for functional G-protein activation. Hoffmann and co-workers use the BioPen to study single cells that express the receptor $FZD_5$ and show that the protein exhibits the functional hallmarks of a GPCR. Both the activation of $FZD_5$ and of a G-protein was investigated using fluorescence resonance energy transfer (FRET). BioPen was used to stimulate individual live-cells with the $FZD_5$ agonist WNT-5A. Because BioPen allows rapid exchange of the microenvironment around the cell, the authors were able to record the time-resolved activation of $FZD_5$ in single cells, which occurs 10-20 s after addition of WNT-5A and which appears as a dequenching of the FRET donor. A similar assay was used for G-protein activation, where dissociation of the G-protein subunits was used as a marker. Here, a dequenching of the FRET donor following ligand addition was again observed. Together, this together shows that WNT-5A not only activates $FZD_5$, but that receptor activation by the agonist also leads to subsequent activation of the G-protein. This completed the link between ligand, receptor and G-protein activation and confirms that $FZD_5$ can be classified as a GPCR.

*Wright S. C. et al., $FZD_5$ is a $G\alpha_q$-coupled receptor that exhibits the functional hallmarks of prototypical GPCRs. Science Signaling (2018)*

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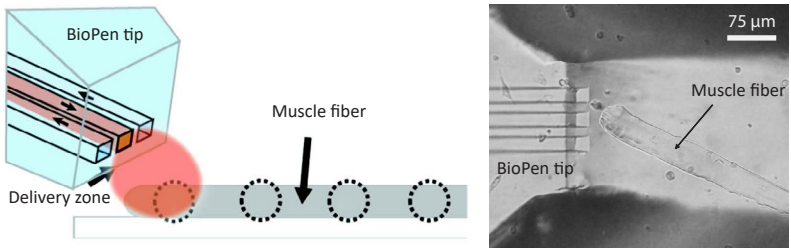
## SINGLE MUSCLE FIBER PHYSIOLOGY



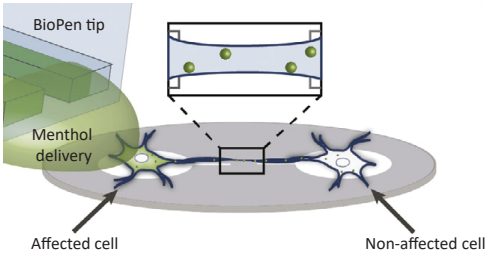

Exposure of FCCP by Using the BioPen to the end or side of a muscle fiber induces localized depolarisation.

Using the BioPen, compounds were delivered locally to the end or side of single adult mouse skeletal muscle fibres to test whether changes in mitochondrial membrane potential were transmitted to more distant located mitochondria. Mitochondrial membrane potential was monitored with tetramethylrhodamine ethyl ester (TMRE). Cytosolic free [$Ca^{2+}$] was monitored with fluo-3. A pulse of carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) applied to a small area of the muscle fibre (30 µm in diameter) which produced a rapid decrease in the mitochondrial TMRE signal (indicative of depolarization) to 38% of its initial value. After washout of FCCP, the TMRE signal partially recovered. At distances greater than 50 µm away from the site of FCCP application, the mitochondrial TMRE signal was unchanged. Similar results were observed when two sites along the fibre were pulsed sequentially with FCCP. After a pulse of FCCP, cytosolic [$Ca^{2+}$] was unchanged and fibres contracted in response to electrical stimulation. These results indicate that extensive networks of interconnected mitochondria do not exist in skeletal muscle. Furthermore, the limited and reversible effects of targeted FCCP application with the BioPen highlight its advantages over bulk application of compounds to isolated cells.

*Bruton J. et al., Usage of a localised microflow device to show that mitochondrial networks are not extensive in skeletal muscle fbres. PloS One (2014)*

## CELL-TO-CELL MOLECULAR TRANSPORT

The BioPen was utilized to target individual cells within interconnected networks, to investigate the cell-cell connections which spanned fabricated Teflon structures. Intercellular transport between HEK 293 cells, expressing the ion channel TRPM8, was measured by monitoring the changes in calcium concentration within individual cells. Menthol, in the presence of a $Ca^{2+}$ spiked buffer solution, was delivered to one cell in a network to activate the TRPM8 ion channels. Utilizing the pre-fluorescent dye Calcium green-1, allows $Ca^{2+}$ ion transport though the connections to be visualized and monitored. Measurement of the molecular transport, enabled the characteristics of cell protrusions crossing small microgaps to be established.



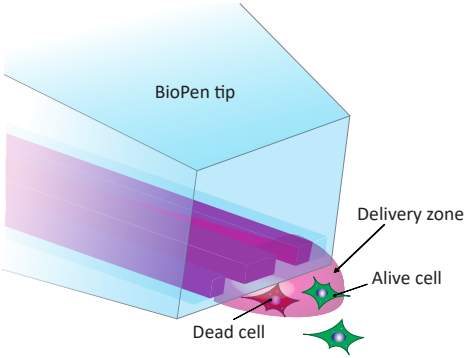

Using the BioPen to deliver menthol to only one of the connected cells to activate TRPM8 ion channels. The activation allows visualization of calcium transport throught tubular interconnections.

*Zhang H. et al., Cellular communication via directed protrusion growth: critical length-scales and membrane morphology. Nano Commu. Net. (2015)*

# BioPen® Applications

## SINGLE-CELL VIABILITY

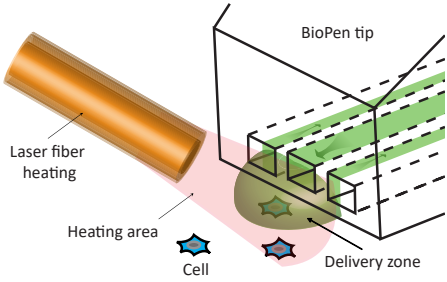
A novel approach for determining the viability of individual cells in an adherent cell culture was developed by using the BioPen, in combination with a multi-component fluorescent response assay. Through directed exposure of the target cells to a pore-forming agent, the membrane permeability was controlled, where the extent of poration depended heavily on the cell type and life-cycle. The cell viability was able to be assessed 60s after the poration, by simultaneous exposure to fluorescein diacetate and propidium iodide solution. Viable and non-viable cells were distinguished by the fluorescence emission of the two dyes within 10s of the application. Hundreds of cells from four different cell lines, NG108-15, HEK 293, PC12, and CHO, were investigated within two days. These results demonstrate that the BioPen system is a facile, rapid, and reliable means to determine the viability in single-cell experiments.




Determination of individual cells viability by exposure to multi-component fluorescent agents using the BioPen.

*Xu S. et al., A rapid microfluidic technique for integrated viability determination of adherent single cells. Anal. Bioanal. Chem. (2015)*

## SINGLE-CELL ENZYMOLOGY



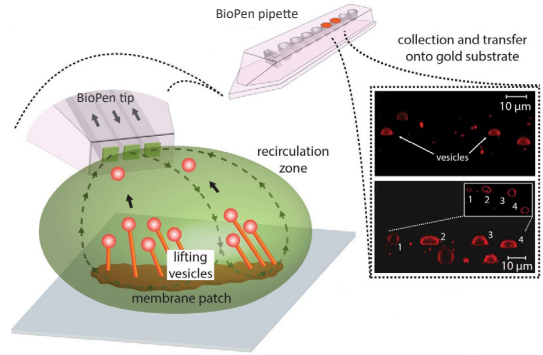

Using the BioPen in combination with a laser heating system to probe single-cell enzymology.

A novel approach for the study of single-cell intracellular enzyme activity at various temperatures was accomplished by using the BioPen in combination with a localized laser heating probe. A pore-forming agent $\alpha$-hemolysin and an enzyme substrate fluorescein diphosphate (FDP) were delivered to individual cells by using the BioPen, to subsequently control the cell membrane permeability and target delivery of the FDP substrate. While adjusting the local temperature using a laser heating system, the activity of the enzyme alkaline phosphatase was monitored by following the production of the fluorescent product from the FDP substrate. The quantitative estimates for the intracellular alkaline phosphatase activity at 5 different temperatures in different cell lines were generated by constructing temperature-response curves of enzymatic activity at the single-cell level. Enzymatic activity was determined rapidly after cell permation, generating five-point temperature-response curves within just 200 s.

*Xu S. et al., A heating-superfusion platform technology for the investigation of protein function in single cells. Anal. Chem. (2015)*

# BioPen® Applications

## COLLECTING VESICLES

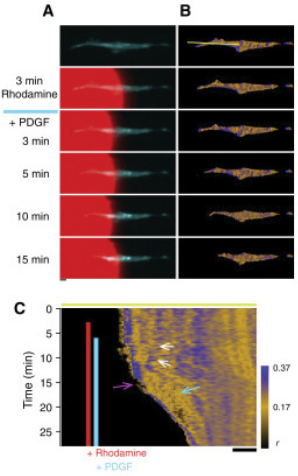



Using the BioPen to separate and retrieve vesicles.

The published findings report the spontaneous transformation of lipids land on a surface to protocell formation. By using the BioPen, surface adhered lipid vesicles were exposed to hydrodynamic flow in the range of 10-100 nl/s. The effective hydrodynamic flow in the exposure zone causes loosely surface-adhered objects to separate, and they can be collected in BioPen pipette wells. By means of this setup, the surface-adhered vesicles were separated from the membrane patch, collected and subsequently transferred onto gold substrates.

*Köksal E. S. et al., Nanotube-Mediated Path to Protocell Formation. ACS Nano (2019)*

## INDUCING THE RETRACTION OF PROTRUSION IN SINGLE-CELLS




Using the BioPen to induce the retraction of a cell protrusion.

In this study, Regulatory Light Chain (RLC) phosphorylation was investigated during retraction of cell protrusion using a Förster Resonance Energy Transfer (FRET) approach. To induce the retraction, the cell protrusion was stimulated with local delivery of a Platelet-Derived Growth Factor (PDGF) using the BioPen PRIME system. PDGF stimulation resulted in the retraction of a cell protrusion initiating 5 min into the treatment (Figures A and B). The morphology of the cell outside of the treatment area did not undergo substantial alterations. Before retraction, the tip of the protrusion contained stable high-anisotropy phosphorylated regions (Figure B). Retraction proceeded through a multi-phasic process similar to those during random migration. Initially, cytoskeletal movements were observed 10–20 µm deep into the protrusion (Figure C, white arrows).

*M. L. et al., A Genetically Encoded Biosensor Strategy for Quantifying Non-muscle Myosin II Phosphorylation Dynamics in Living Cells and Organisms. Cell Reports (2018)*