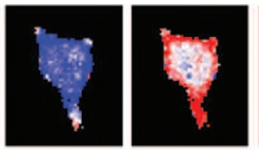
BIOSENSORS

Voltage, absolutely

Biophys. J. **106**, 639–648 (2014)



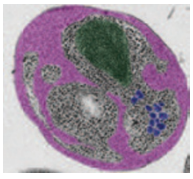
ELSEVIER

Fluorescence-based biosensors exist for monitoring numerous cellular processes, including visualization of single neuronal action potentials *in vivo*. Fluorescence-based biosensors of membrane voltage (V_m) exist but use intensity-based measurements, which cannot report an absolute numerical value. Also, these biosensors suffer from the inability to measure slow shifts in resting voltage, as, for instance, that which occurs during apoptosis. To explore potential alternatives for voltage sensing, Hou *et al.* sought a biosensor based on the light-powered proton pump Arachaerhodopsin 3 (Arch) in which voltage controls the equilibrium between a fluorescent state, protonated at the retinal Schiff base, and a nonfluorescent deprotonated state. The counterion to the Schiff base, Asp95, has a key role in modulating protonation. Screening a library of Arch mutants at Asp95 found that the D95H variant showed the best voltage-sensitive fluorescence. Reasoning that a change in illumination wavelength at constant voltage would change the photostationary distribution of the Arch^{D95H} conformational ensemble in time, the authors monitored the trajectory of the relaxation from a fluorescence distribution induced by blue wavelength light ('pump') to a new distribution at orange wavelength ('probe') and concluded that, indeed, the relaxation between photostationary conformations could report on V_m. Once the pump-probe parameters were optimized, the authors used principal component analysis to parameterize voltage-dependent changes in fluorescence transients and concluded that their Arch^{D95H} biosensor had an accuracy of ~10 mV. Therefore, the time-based biosensor could prove useful in cases where V_m shifts are minimal or slow. *MB*

METABOLISM

Contain yourself

Appl. Environ. Micro. doi:10.1128/aem.03887-13
J. Biol. Chem. doi:10.1074/jbc.m113.531236



KENT MCDONALD, ONUR ERBILGIN AND CHERYL KERFELD

Bacterial microcompartments (BMCs) serve to isolate certain enzymatic pathways from the rest of the cell. BMCs performing five different functions have been characterized, including the archetypical carbon fixation by RuBisCO in the carboxysome, yet bioinformatics analysis suggests additional functions await discovery. Two new studies now investigate these computational clues. Wheatley *et al.* focused on an unusual gene within some carboxysome operons homologous to pterin-utilizing enzymes. Bioinformatics, structural and functional analysis confirmed that the protein, newly named acRAF, was not enzymatically active. Instead, the authors suspected it might be functionally equivalent to RbcX, a protein found in carboxysome operons that do not contain acRAF, which chaperones RuBisCO assembly by stabilizing dimers en route to functional hexadecamers. Indeed, RuBisCO assembly in the presence of the newly named acRAF and the chaperone GroELS greatly increased compared to assembly in the presence of GroELS alone. Erbilgin *et al.*, in contrast, investigate the function of an uncharacterized microcompartment conserved in *Planctomycetes* and *Verrucomicrobia*, bacteria known to be associated with algae rich in sulfated polysaccharides. Directed gene knockouts demonstrated this 'PV BMC' (shown in blue in the false-colored image) was important for the metabolism of several carbon sources, particularly L-fucose, L-rhamnose and the physiologically relevant polysaccharide fucoidan. The authors also confirmed the presence of BMC-like structures in cells grown on fucose or rhamnose but not glucose. These studies expand our understanding of these intriguing organelles. *CG*

CELL BIOLOGY

Too hot to handle

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Many bacteria are able to adjust the fluidity of their lipid membranes in response to changes in external temperature. For example, when *Bacillus subtilis* experience low temperatures, the histidine kinase DesK phosphorylates DesR, which then activates the transcription of a fatty acid desaturase; this desaturase catalyzes the formation of double bonds in the membrane lipids, thereby restoring membrane fluidity. At higher temperatures, DesK acts as a phosphatase, removing the phosphate group from phospho-DesR to halt the transcription of the desaturase. Inda *et al.* now report that a highly conserved stretch of positively charged amino acids, found between the thermosensor-containing transmembrane (TM) domain and the cytoplasmic catalytic domain of DesK, adopts distinct conformations in response to temperature-dependent changes in the thickness of the lipid membrane. When the temperature decreases, the conformation of this 'linker region' changes from a disordered, membrane-associated peptide to a helical structure that can enter the lipid bilayer to form a continuous α-helix with the TM domain. Disruption of this continuous α-helix (i.e., at higher temperatures) leads to conformational changes in the cytoplasmic catalytic domain of DesK, switching it from a kinase to a phosphatase. Similar mechanisms may be used to help regulate the activity of transient receptor potential channels, mechanosensitive channels and other membrane proteins. *JMF*

STEM CELLS

Four steps to insulin

Cell Stem Cell **14,** 228–236 (2014)

Fibroblasts can undergo reprogramming into different cell types through the introduction of genetic factors or small molecules, but creating functional β cells, which would find utility in treating Type I diabetes (T1D), has remained challenging. Li *et al.* found that they could generate mature pancreatic-like cells from fibroblasts through four distinct stages using transcription factors and growth factors. However, the efficiency of producing insulin-expressing cells was low. To increase this efficiency, the authors strove to identify small molecules from a known drug collection that could enhance production of these cell types at each stage. The addition of known epigenetic modifiers Bix-01294 and pVc to fibroblasts at an early stage increased the number of definitive endoderm progenitors, whereas the combination of four small molecules—retinoic acid, pVc, an inhibitor of TGF-β signaling (A83-01) and an inhibitor of Hedgehog signaling (LDE225)—could substitute for the use of growth factors in generating pancreatic precursors. Finally, the addition of SB203580 (an inhibitor of p38-MAPK signaling) and pVc at later stages increased the production of pancreatic-like cells. To verify that these pancreatic-like cells were functional, the authors transplanted these cells near the kidney capsule in a T1D mouse model and could detect rescue of blood glucose levels. Thus, the generation of functional β cells from fibroblasts could be a useful approach to treat both T1D and T2D. *GM*