Brownian Dynamics. We modeled the carboxylate-rich selectivity filter of calcium channels with 8 independent half-charged oxygens confined in the central region, and computed ions' trajectories self-consistently evaluating the electrostatic forces acting on the ions at every timestep. Such forces were evaluated solving Poisson's equation with a Boundary Element Method to deal with dielectric boundaries, called Induced Charge Computation method (ICC). A transmembrane potential was included as a spatially constant component of the electric field, a good approximation to a fully consistent treatment, see Crozier et al. (Biophys. J. 81:3077) and Hollerbach and Eisenberg (Langmuir, 18:3626). Boundary conditions for ionic concentrations in the intra- and extra-cellular domain were imposed by a Grand Canonical-Monte Carlo algorithm. We simulated different concentrations of CaCl₂ added to NaCl solution only on one side of the membrane. Ion permeation was investigated under physiological conditions, using different sub-millimolar calcium concentrations and different transmembrane potentials. Channel selectivity and conductance were determined by electrostatic forces, ionic repulsion due to charge crowding, and gradients of concentration and potential.

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Real-Time Modulation of Zebrafish Cone Phototransduction by Whole-Cell Delivery of zGCAP3 and of its Monoclonal Antibody
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Regulation of excitation and adaptation in photoreceptors of the vertebrate retina strongly depends on the cytoplasmic Ca²⁺ concentration and its interplay with Ca²⁺ sensor proteins like recoverin, calmodulin and the activating proteins (GCAPs) of guanylate cyclase (GC) (Scholten and Koch, 2011). Of the four GCAP isoforms exclusively transcribed in cones (zGCAP3, 4, 5 and 7), we investigated the physiological function of zGCAP3 in green-sensitive cones of zebrafish, by recording the effect on the photoreponse waveform by cystosol injection of exogenous zGCAP3 (to simulate “real time” protein overexpression), and its monoclonal antibody (to simulate protein knock-down). To identify a suitable antibody we screened several hybridoma fluids with respect to specificity and affinity towards zGCAP3, using immunoblotting and surface plasmon resonance (SPR) spectroscopy. The global fitting of an overlay of SPR sensograms obtained with increasing antibody concentrations gave a Ca²⁺-independent Kd of 12 nM for the interaction of zGCAP3 with the antibody. Exogenous proteins were incorporated with a precise timing in the zebrafish cone cytosol by an internal perfusion system coupled to a pressure-polished patch pipette (Benedusi et al. 2011). Typical whole-cell recordings lasting even more than 20 min did not show any significant change in light sensitivity, dark current amplitude, response kinetics and light adaptation, proving also that the enzymatic cascade was not perturbed by the recording protocol. Injection of anti-zGCAP3 caused the complete shutdown of the dark current, indicating that zGCAP3 plays a major role in regulating GC. Injection of purified zGCAP3 did not alter the photoreponse, indicating that the target GC was already saturated with endogenous zGCAP3. Benedusi M, Aquila M, Milani A and Rispoli G (2011). Eur Biophys J 40: 1215-23.

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Effects of Redox Environment on Calcium Alternans in Isolated Rabbit Cardiomyocytes
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Cardiac alternans is a multifactorial phenomenon linked to cardiac arrhythmias. At the cellular level cardiac alternans is defined by beat-to-beat alternations in contraction amplitude (mechanical alternans), action potential duration (electrical or action potential duration alternans) and Ca transient amplitude (Ca alternans) at constant stimulation frequency. The aim of this project was to characterize the effect of changes in the cellular redox environment on Ca alternans in cardiac myocytes. Single myocytes (from New Zealand White rabbits) were isolated enzymatically by retrograde Langendorff perfusion. Ca alternans were induced by incrementally increasing the pacing frequency (electrical stimulation) until stable Ca alternans occurred. The frequency at which stable Ca alternans were observed varied from cell to cell and ranged from 1 to 2.5 Hz at room temperature. Global cytosolic Ca transients were measured with Indo-1. In some experiments, cytosolic Ca alternans and intra-SR Ca alternans were simultaneously measured with the fluorescent Ca indicators Rhod-2 and Fluo-5N, respectively. Confocal microscopy was used to measure Ca sparks with Fluo-4.

Reducing agents dithiotreitol and reduced glutathione partially abolished Ca and mechanical alternans by restoring diastolic Ca and Ca transient amplitudes. A decreased sarcoplasmic reticulum (SR) Ca release flux but not Ca content, together with a decreased Ca spark frequency, suggest that reducing agents normalized alternans through effects on the SR Ca release channel (ryanodine receptor type-2). Addition of a membrane permeant superoxide dismutase mimetic, Tempol, had little effect on Ca alternans, suggesting the possible role of dithiotreitol directly acting on the ryanodine receptor. These data highlight that the redox state of the cell may be important in the generation of Ca and mechanical alternans during oxidative stress.