

**Background:** Nesiritide (B-type natriuretic peptide) improves hemodynamic function and heart failure status for patients with decompensated congestive heart failure. However, studies associated the use of nesiritide with an increased risk of sudden death, to date, little is known of the underlying mechanisms. In this study, the effect of BNP on action potential duration and underlying electrophysiologic mechanisms were investigated in rat hearts.

**Method:** Wistar rats were anesthetized with sodium pentobarbital (40mg/kg) and injected with BNP-32 (12µg/kg) from abdominal vena cava. EKG was recorded using electrodes inserted into the subcutaneous layer of the paws. Action potentials from 8 rat left ventricles were recorded by a high-resolution optical mapping with voltage-sensitive dye RH237 in Langendoff perfusion system. Transient outward potassium current of rat ventricular myocytes was recorded by the whole cell configuration of patch clamp technique.

**Results:** BNP-32, at a clinically relevant concentration, prolonged corrected QT interval ( $QT_c = QT / \sqrt{RR}$ ) obviously in adult rats, whereas heart rate was comparable during and after BNP-32 treatment. BNP-32 (0.1µM) increased action potential duration at 50% ( $APD_{50}$ ) repolarization ( $45.62 \pm 4.45$ ms,  $P < 0.01$ ) and this effect persisted after 15 min of washout ( $57.71 \pm 12.62$ ms,  $P < 0.05$ ) compared to baseline (BL:  $41.22 \pm 2.88$ ms). In control group of 6 rat hearts,  $APD_{50}$  had no obvious changes over the same time period without BNP perfusion. The peak transient outward potassium current at +60mV was significantly reduced ( $7.11 \pm 4.97$  to  $2.85 \pm 3.30$  pA/pF,  $n=6$ ;  $P < 0.05$ ) by 0.01µM BNP-32, and then partially recovered to  $5.85 \pm 4.12$  pA/pF ( $n=6$ ,  $P < 0.05$ ) after washout of BNP-32.

**Conclusion:** BNP prolongs action potential duration and reduces transient outward potassium current in rat hearts, which might contribute to BNP-induced increase of death risk in decompensated heart failure patients.

## 2126-Plat

### The Mitochondrial Bioenergetic Phenotype for Protection from Ischemia in Sur2-Mutant Mice

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ATP-sensitive potassium channels ( $K_{ATP}$ ) in mitochondria are postulated to play a key role to protect mitochondria and myocytes from cardiac ischemic insult. The sulfonylurea receptor-2 (SUR2) is a subunit of  $K_{ATP}$  in sarcolemma, although its role in mitochondrial physiology is unclear. Mice where the SUR2 gene was disrupted (SUR2 mutant) have been shown to be constitutively protected from ischemic injury.

We characterized the bioenergetic phenotype of mitochondria in SUR2 mutant mice to gain insight into mechanisms of protection from ischemia.

Membrane potential ( $\Delta\Psi_m$ ),  $Ca^{2+}$  uptake, and reactive oxygen species (ROS) generation were studied in the isolated mitochondria by fluorescence based assays and  $K^+$ -influx was studied by volume measurements. Mitochondrial respiration was studied in normoxia and after hypoxia-reoxygenation. Myocyte protection against metabolic inhibition was also investigated.  $\Delta\Psi_m$  was depolarized ( $53.37 \pm 1.5$  vs  $48.4 \pm 1.8$  %), tolerance to  $Ca^{2+}$  loading was increased ( $163 \pm 26$  vs  $116 \pm 23$  µM), and ROS generation was increased ( $9.3 \pm 0.4$  vs  $7.4 \pm 0.6$  FU/sec) in the SUR2 mutant mitochondria compared to wild type (Wt). SUR2 mutant mitochondria had greater swelling ( $30.2 \pm 3.1$ %) compared with Wt ( $14.5 \pm 0.6$ %) indicating greater  $K^+$  influx. SUR2 mutant mitochondria recovered better from post hypoxia-reoxygenation than Wt as measured by the respiration control index (RCI). Finally, the SUR2 mutant myocytes viability was better protected against metabolic inhibition.

We concluded that SUR2 plays a key role in mitochondrial mechanisms of protection from ischemia by altering a potassium conductance consistent with a mitochondrial  $K_{ATP}$  and causing a protected mitochondrial phenotype.

## 2127-Plat

### Multiple Mechanisms of hERG Liability: $K^+$ Current Inhibition, Disruption of Protein Trafficking, and Apoptosis Induced by Amoxapine

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The antidepressant amoxapine has been linked to QT prolongation, acute heart failure, and sudden death. Drug binding to cardiac hERG (Kv11.1) potassium channels causes prolonged repolarization and induces apoptosis. This study was designed to investigate amoxapine effects on hERG currents, hERG protein trafficking, and hERG-associated apoptosis in order to elucidate molecular mechanisms underlying cardiac side effects of the drug. hERG channels were expressed in *Xenopus laevis* oocytes and HEK 293 cells, and potassium currents

were recorded using patch clamp and two-electrode voltage clamp electrophysiology. Protein trafficking was evaluated in HEK 293 cells by Western blot analysis, and cell viability was assessed by immunocytochemistry and colorimetric MTT assay. Amoxapine caused acute hERG blockade in oocytes ( $IC_{50} = 21.6$  µM) and in HEK 293 cells ( $IC_{50} = 5.1$  µM). Mutation of residues Y652 and F656 attenuated hERG blockade, suggesting drug binding to a receptor inside the channel pore. Channels were mainly blocked in open and inactivated states, and voltage-dependence was observed with reduced inhibition at positive potentials. Amoxapine block was reverse frequency-dependent and resulted in accelerated and leftward-shifted inactivation. Furthermore, amoxapine caused chronic reduction of hERG trafficking into the cell surface membrane ( $IC_{50} = 15.3$  µM). Finally, the antidepressant drug triggered apoptosis in cells expressing hERG channels. Triple mechanisms of hERG liability associated with a single compound, are revealed. Amoxapine causes direct hERG current inhibition and disruption of hERG protein trafficking. Furthermore, the drug induces apoptosis of cells expressing hERG potassium channels.

## 2128-Plat

### Cardiac Glycoside Chronotropic and Arrhythmogenic Effects in Sinus Atrial Nodal Pacemaker Cells (SANC) Occur Along a Continuum of Electrochemical Gradients of $Na^+$ ( $E_{Na}$ ) and $Ca^{2+}$ ( $E_{Ca}$ )

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Cardiac glycosides reduce  $E_{Na}$  and  $E_{Ca}$  (due to an increase of  $Na^+$  via Na-K pump inhibition, and of  $Ca^{2+}$  due to a secondary reduction in Ca efflux for Na influx via NaCa exchange). Here we show that exposure of single rabbit SANC to the cardiac glycoside, digoxigenin (10-20µM) results in a continuum of time-dependent effects. Within 30s to 1 min, the rate of rhythmic spontaneous action potentials (AP) increases by 20% ( $n=3$ ) and this is associated with an earlier occurrence (reduced period) of local sub-membrane  $Ca^{2+}$  releases (LCR's) during diastolic depolarization, detected by confocal  $Ca^{2+}$  imaging. Approximately 1-3 minutes following AP rate acceleration, LCR period lengthens by 40%, accompanied by a similar reduction in the rhythmic AP rate. The changes in LCR period during the biphasic changes in rhythmic AP firing rate increase are highly correlated with the changes in AP cycle length ( $R^2=0.98$ ). A progressive increase in the steady level of diastolic  $Ca^{2+}$  beneath the surface membrane then ensues usually within 4 to 6 additional minutes, LCR's became undetectable, and dysrhythmic and chaotic AP firing occurs. Numerical model simulations (Maltsev-Lakatta model, AJP 2009) in which  $Na_i$  was increased progressively 5-15mM during glycoside exposure reproduced the experimental results. That rate and rhythm regulation of SANC AP firing during cardiac glycoside exposure occurs along a continuum of  $E_{Na}/E_{Ca}$  is in agreement with repeated observations over the last decade, showing that the SANC spontaneous AP firing rate is critically dependent on the timing of acute changes in sub-membrane  $E_{Ca}$  during DD caused by LCR occurrence (LCR period) that accelerates DD by activation of an inward Na/Ca exchange current.

## 2129-Plat

### Calcium Currents in Chronically Dysfunctional Pig Myocardium

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Chronic reduction in coronary artery blood flow due to stenosis results in reduced myocardial contractile function, termed "hibernating" myocardium. This change involves electrical remodeling and a propensity for sudden cardiac death in the absence of infarction. We used a pig model of chronic left anterior descending artery stenosis to study calcium currents associated with the hibernating myocardium. We developed a cell isolation technique from punch biopsies of the left ventricle. This yields calcium-tolerant isolated myocytes with brick shaped morphology and clear striation patterns. Myocyte length from hibernating myocardium averaged  $145 \pm 7.7$  (n=15) vs.  $124 \pm 4.8$  µm (n=42) for control, suggesting cellular hypertrophy. The cell shortening of hibernating cells was also reduced compared to the remote region from  $6.4 \pm 1\%$  (n=21) to  $4.45 \pm 1\%$  (n=11) suggesting that the reduced contractility seen in the hibernating region was preserved in the isolated myocytes. Furthermore, cells isolated from hibernating myocardium had significantly higher numbers of premature contractions 4/15 for hibernating vs. 0/19 for control suggesting a cellular propensity for arrhythmias. The L-type calcium channel current ( $I_{Ca,L}$ ) in myocytes from hibernating myocardium ( $1.35 \pm 0.08$  pA/pF, n=22) was reduced compared to normal myocardium ( $1.96 \pm 0.07$  pA/pF, n=33;  $P < 0.01$ ). The voltage dependence of steady state activation and inactivation were nearly

identical. Isoproterenol (100 nM) shifted the activation curves nearly identically for both groups, but the ability of isoproterenol to enhance the current was decreased from a  $4.1 \pm 0.48$  fold increase to a  $2.64 \pm 0.37$  ( $p < 0.05$ ). Finally, the recovery rate for calcium current was reduced by hibernation, reflecting an approximate 20 mV shift. These changes in L-type calcium current and isoproterenol response may explain the reduced contractility of hibernating myocytes and the increased the likelihood of sudden cardiac arrhythmias.

## Platform AD: Fluorescence Spectroscopy

### 2130-Plat

#### Proton Transfer and Hydrogen-Bond Interactions Determine the Fluorescence Quantum Yield of Bacteriophytochrome, a Novel Deep-Tissue Fluorescent Probe

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Phytochromes are red-light photoreceptors that regulate a variety of responses and cellular processes. The phytochrome light activation mechanism involves isomerization around the C15=C16 double bond of an open-chain tetrapyrrole chromophore, resulting in a flip of its D-ring. In an important new development, bacteriophytochrome (Bph) has been engineered for use as a fluorescent marker in mammalian tissues (Shu et al. Science 2009). Bph fluoresces at ~720 nm, a wavelength less prone to scattering that can penetrate more deeply into tissue than light emitted by GFP-derived fluorescent proteins. The Bph chromophore biliverdin is a naturally occurring cofactor in mammalian tissue that covalently binds to a conserved cysteine in Bph, and hence BPhs can readily be genetically encoded. BPh photochemistry is thus of considerable significance for biomedical technology. Here we report that an unusual Bph, P3 from *Rps. palustris*, is highly fluorescent. We identify the factors that determine the fluorescence and isomerization quantum yields of P3 through the application of ultrafast spectroscopy to wild-type and mutants of P3 and a classical Bph, P2. The excited-state lifetime of biliverdin in P3 was significantly longer at 330 - 500 ps than in P2, and accompanied by a significantly reduced isomerization quantum yield. H/D exchange reduces the rate of decay from the biliverdin excite state by a factor of 1.4 and increases the isomerization quantum yield. Comparison of the properties of the P2 and P3 variants in relation to X-ray structures shows that the quantum yields of fluorescence and isomerization are determined by excited-state deprotonation of biliverdin at the pyrrole rings, in competition with hydrogen-bond rupture between the biliverdin D-ring and the apoprotein. This work provides a basis for structure-based conversion of BPh into an efficient near-IR fluorescent marker.

### 2131-Plat

#### Fine Tuning the Optical Properties of Green to Red Photoconvertible Fluorescent Proteins

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Dendra2 is an engineered, monomeric GFP-like protein that belongs to a subclass of fluorescent proteins undergoing irreversible photoconversion from a green- to a red-emitting state upon exposure to purple-blue light. We have measured the X-ray structure of the green species of Dendra2 and performed a comprehensive characterization of the optical absorption and fluorescence properties of the protein in both its green and red forms. The structure, which is very similar to those reported for the closely related proteins EosFP and Kaede, revealed a local structural change next to the chromophore, involving mainly Arg66 and a water molecule. We propose that this structural change explains the blue shift of the absorption and emission bands, as well as the markedly higher pKs of the hydroxyphenyl moiety of the chromophore, which were determined as 7.1 and 7.5 for the green and red species, respectively. The 20-fold enhancement of the neutral species in Dendra2 at physiological pH accounts for the observed higher photoconversion yield of this protein in comparison to EosFP.

### 2132-Plat

#### Color Hues in Fluorescent Proteins with the Same Chromophore are due to Internal Quadratic Stark Effect

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Intrinsically fluorescent proteins (FPs) exhibit broad variations of absorption and emission colors and are available for different imaging applications. The physical cause of the absorption wavelength change in series of mutants with the same chromophore structure, but different surrounding, is however not understood. Here we study the FP series with acylimine-containing red chromophore, in which the absorption maximum varies from 540 nm to 590 nm, and a series of green FPs with phenolate chromophore, where the absorption peak shifts from 450 to 500 nm. We use two-photon absorption spectroscopy to show that the different colors in each series can be explained by quadratic Stark effect due to variations of the strong local electric field within the beta barrel. The model allows us to experimentally access the chromophore parameters, such as vacuum transition frequency ( $\nu_0$ ) and vacuum changes of permanent dipole moment ( $\Delta\mu_0$ ) and polarizability ( $\Delta\alpha_0$ ) upon excitation. Using this purely experimental and all-optical approach, we estimate, for the first time to our knowledge, the amplitudes of the internal electric field (namely its projection on  $\Delta\mu_0$ ) in a protein. These values amount 10 to 100 MV/cm in the mFruits series. Although these fields appear to be very large, they fall well in the range previously estimated theoretically for different other proteins, and are still 1 - 2 orders of magnitude smaller than the fields required to ionize the chromophore. Our model brings simplicity to a bewildering diversity of fluorescent protein properties, and it suggests a new way to sense electrical fields in biological systems. On the other hand, it opens up the way to create two-photon brighter FP probes by tuning internal electric field with smart mutagenesis around the chromophore.

### 2133-Plat

#### TIRF-Based FRET with One Base-Pair Resolution

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Single-molecule FRET (smFRET) is commonly used as a "nanoscale ruler" for the measurement of biomolecular distances and distance changes. However, the limits of FRET resolution for measurements on surface-immobilized molecules have not been rigorously explored. Using total-internal reflection fluorescence (TIRF) microscopy on a set of DNA standards and advanced image analysis software, we have quantified and extended the limits of FRET resolution associated with the use of electron-multiplying CCD (EMCCD) cameras. For such measurements, we derived a novel theoretical description of the major sources of noise (photon shot noise, background, CCD noise and pixelation effects); we find excellent agreement between our experimental results and predictions from theory and Monte Carlo simulations. For FRET measurements on a truly single-molecule basis (as opposed to measurements on an ensemble of single molecules), analysis of the experimental noise allows us to predict a resolution of 4% FRET within the linear FRET range (20-80%), sufficient to directly observe a distance difference equivalent to one DNA base-pair separation (3.4 Å). For FRET distributions obtained from an ensemble of single molecules (which exhibits broadening due to presence of static heterogeneity), we demonstrate the ability to distinguish between distances differing by as little as 2 base pairs (~7 Å). Current work focuses on real-time observation of single-base-pair translocation steps of Escherichia coli RNA polymerase within single early transcription-elongation complexes; such observations are crucial for understanding the mechanisms of DNA and RNA polymerases. Our work paves the way for ultra-high resolution studies of processes involving conformational changes and protein translocation on nucleic acids.

### 2134-Plat

#### Single-Molecule STED with Photostable Fluorophores

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With recent advantage in the development of far-field super-resolution microscopy, further development depends crucially on improved fluorescent probes. We combined STED microscopy with fluorophore stabilization through a reducing and oxidizing system (ROXS) and demonstrate significant improvement of photostability. We show that this improvement can be exploited either for repetitive measurements necessary for 3D or dynamic STED imaging or for resolution enhancement through the application of higher STED beam intensities. Accordingly, a lateral resolution below 30 nm is demonstrated for single organic fluorophores immobilized in aqueous buffer.

### 2135-Plat

#### Fluorescence Correlation Spectroscopy Elucidates the Pathway of RNA Interference

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