for intermediates along the reaction pathway of DHFR to elucidate the conformational changes and the variation of the electrostatic microenvironments during catalysis.

2254-Symp
Using Quantum Mechanics in Biological Structure Refinement
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The starting point for structure-based drug design (SBDD) efforts is a high quality structural model obtained using X-ray crystallography or NMR spectroscopic techniques. In most instances classical tools are used as structural surrogates in X-ray and NMR refinement protocols in order to improve the parameter to observation ratio realized from these experimental techniques. While classical approaches are useful structural surrogates, they do suffer from a number of issues that affect their performance including: electrostatic modeling, parameter defects and missing parameters. The way in which these issues can be mitigated is to use more robust structural theories like quantum mechanical (QM) methods, which have had a tremendous impact on our understanding of "small" chemical and biological systems. In this talk we will focus on the application of ab initio QM methods to refine protein/ligand complexes for use in SBDD applications using NMR and X-ray methods. We will discuss the computational details and describe several uses of QM in structure refinement efforts using NMR and X-ray datasets. The strengths and weaknesses of a QM approach in structure refinement will be discussed as well as future prospects of this strategy.

2255-Symp
Classical and Mixed Quantum Mechanical/Molecular Mechanical (Qm/Mm) Simulations of G Protein Coupled Receptors
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We use a combination of classical and quantum mechanical (QM/MM) simulation methods to study the structural, dynamical and optical properties of the prototypical G protein coupled receptor (GPCR) rhodopsin along the photocycle and assess the possible role of aggregation and lipid interaction for early signal transduction [1-3].
Our simulations predict structures of the early photointermediates batho and lumi that are in good agreement with the available experimental data. In addition we are able to predict a structure of the Blue shifted intermediate BSI, for which no experimental high-resolution structure is available yet. Applying techniques from machine learning, we are also able to identify the main factors responsible for the distinct color shifts between the early intermediates.
Comparison of our results with those obtained for the other GPCRs, β1 and β2 adrenergic receptors [4-6] helps to characterize some of the common features as well as variations among different members of Class A GPCRs.
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Platform: Excitation-Contraction Coupling

2256-Plat
Biochemical, Cellular and Electrophysiological Characterization of HMCL-7304 a Human Skeletal Muscle-Derived Cell Line
Ori Rokach[1], Nina D. Ullrich[2], Martin Rausch[3], Haiyan Zhou[4], Francesco Muntoni[4], Francesco Zorzato[1,5], **Susan Treves**[1,5].
[1]Basel University Hospital, Basel, Switzerland, [2]University of Bern, Bern, Switzerland, [3]Novartis Biomedical Institute, Basel, Switzerland, [4]Dubowitz Neuromuscular Center, London, United Kingdom, [5]University of Ferrara, Ferrara, Italy.
In the past years a number of techniques have been developed to isolated single muscle fibers from small rodents allowing detailed investigations of the excitation contraction (EC) coupling mechanism at the ultrastructural, biochemical and cellular levels in normal and pathological conditions. Because of their high degree of differentiation and specialization however, it is difficult to maintain differentiated muscle fibers in culture for more than a few days and it is nearly impossible to obtain mature fibers starting from precursor satellite cells. However, starting from newborn mice one can obtain cultures of contracting and striated myotubes that can be used for a number of manipulations. As to human muscle cells, primary cultures can be obtained in vitro by culturing satellite cells from biopsies and differentiating them into myotubes, but there is a clear necessity to develop cell lines from control and diseased individuals which will develop into myotubes. In the present investigation we characterized for the first time the excitation contraction coupling machinery of HMCL-7304 an immortalized human skeletal muscle cell line. Intracellular Ca^{2+} measurements showed a normal response to pharmacological activation of the ryanodine receptor whereas super resolution structured illumination microscopy (3D-SIM) revealed a low level of structural organization of ryanodine receptors and dihydropyridine receptors. Interestingly, the expression levels of several transcripts of proteins involved in calcium homeostasis and differentiation indicate that the cell line has a phenotype closer to that of slow twitch than fast twitch muscles. These results point to the potential application of such human muscle-derived cell lines to the study of neuromuscular disorders; in addition they may serve as a platform for the development of therapeutic strategies aimed at correcting defects in calcium homeostasis due to mutations in genes involved in calcium regulation.

2257-Plat
Structural and Binding Studies of the Cav1.1 β1A Subunit
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Excitation-contraction (EC) coupling in skeletal muscle requires a physical coupling between the voltage-gated calcium channel (Cav1.1) in the surface membrane and the skeletal ryanodine receptor (RyR1) Ca^{2+} release channel in the membrane of the sarcoplasmic reticulum Ca^{2+} store. Although the exact molecular mechanism of EC coupling is unresolved, both the α1s and β1a subunits of Cav1.1 are essential for this process. The β1a subunit has a modular structure consisting of SH3/guanylate kinase (GK) domains separated by a variable hook region. The GK domain binds with high affinity to the I-II loop of the α1 subunit, but the functional significance of the SH3 domain remains undefined.
Until now the structure of the Cav1.1 β1a subunit has not been experimentally determined, but other Cav β-isoform structures have suggested that the SH3 binding site is occluded, preventing binding to polyproline-rich partners. This prediction is at odds with our findings that show the Cav1.1 β1a subunit and the α1s subunit II-III loop interact (Kd = ~3 μM). We demonstrate that this interaction takes place through the SH3 domain of the β1a subunit and a proline-rich region of the α1s II-III loop, which has previously been shown to be critical for skeletal-type EC-coupling (1). Through mutational studies we demonstrate that isoform-specific differences in the SH3 RT loop enable the interaction of the β1a SH3 domain with proline-rich binding motifs.
Our determination of the crystal structure of Cav1.1 β1a provides the first opportunity to examine differences between this isoform and other published structures. In light of this novel structure and binding data, we discuss the specific role of the β1a subunit in EC coupling and its relationship with the Cav1.1 α1 subunit and RyR1.
1. Kugler, G. et al (2004). J Biol Chem 279(6): 4721-4728.

2258-Plat
Dissecting Roles of Cav1.2 (α1C) Intracellular Loops in Cardiac Excitation-Contraction Coupling
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Cardiac excitation-contraction (EC) coupling relies on Ca^{2+}-induced Ca^{2+} release (CICR) enabled by an intimate relationship between L-type (CaV1.2) channels and ryanodine receptors (RyRs) at dyadic junctions. The determinants underlying CaV1.2/RyR functional proximity responsible for effective CICR are unknown, but likely entail protein interactions involving one or more intracellular loops of CaV1.2 pore-forming α1C subunit. We hypothesized that over-expressing α1C intracellular loops that play a critical role in CaV1.2/RyR communication would disrupt CICR in cardiomyocytes. We used adenoviruses to overexpress CFP-tagged α1C intracellular loops and termini (NT, I-II, II-III, III-IV, CT) in cardiomyocytes and determined their impact on field-stimulation-evoked rhod-2-reported Ca^{2+} transients. Over-expressed NT, II-III, and III-IV loops had minimal effect on CICR. By contrast, over-expressed I-II and CT produced dramatic disruption of EC coupling characterized by two distinct signatures: a sharp augmentation in CICR failure, and an