This implies that *C. elegans* can develop into a system to study the molecular and genetic basis of KCNE-mediated muscle contractility and disease states.

1750-Plat KCNE2 Gene Structure and Direct Genomic Regulation by Estrogen

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K^+ channels are protein complexes made of pore-forming α-subunits and regulatory β-subunits, whose expression can be regulated by sex hormones. In particular, KCNE2 β-subunit forms complexes with a variety of K^+ channel α-subunits suggesting a broad spectrum of physiological roles, though its regulation by estrogen is unknown. To investigate whether *mouse* KCNE2 gene is regulated by estrogen, we experimentally identified KCNE2 transcription start sites and delineated its gene structure. We found a new exon (0) and mRNA with a retained intron. Analysis of 5′-flanking sequence to exon 0 revealed a promoter region with multiple vicinal start sites, a TATA sequence for transcriptional regulation and the presence of a quasi-perfect estrogen responsive element (ERE). Estrogen treatment stimulated KCNE2 promoter activity in a dose-dependent manner and the estrogen antagonist ICI 182,780 blocked estrogen stimulation. A direct genomic mechanism was demonstrated by: i) the lack of estrogen-responsiveness using a DNA-binding domain mutant estrogen receptor and by mutating the KCNE2 ERE, and ii) binding of estrogen receptor to the KCNE2-ERE. In vivo, KCNE2 transcripts were also upregulated in ovariectomized mice by estrogen treatment and the upregulation was prevented by ICI 182780. We conclude that estrogen upregulates KCNE2 transcription via a direct genomic regulatory mechanism finely tuning the cardiac excitability.

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1751-Plat The Role Of MiRP1 (*kcne2*) In Murine Ventricular Repolarization

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The MiRP1 ancillary subunit, encoded by the *KCNE2* gene, functionally co-assembles *in vitro* with a range of cardiac-expressed voltage-gated potassium (Kv) channel α subunits including hERG, KCNQ1 and Kv4.2. *KCNE2* mutations associate with inherited and acquired forms of human long QT syndrome, yet its relevance to cardiac physiology in any species is still questioned. Here, we disrupted the murine *kcne2* gene to begin to address this controversy. Neither echocardiography nor myocyte electron microscopy revealed abnormalities in *kcne2* (−/ −) mice. In transthoracic pacing studies, in contrast to *kcne2* (+/+) mice, *kcne2* (−/ −) mice were resistant to lengthening of the ventricular effective refractory period by 4-aminopyridine (4-AP). In whole-cell voltage-clamp studies of left ventricular myocytes, *kcne2* disruption reduced the density of two currents: $I_{to,f}$ (~25%) and $I_{K,slow}$ (~50%) (n = 14–23 cells per group; $p < 0.05$). In left ventricular apex and/or septal myocytes, *kcne2* disruption halved the 4-AP (50 μM) sensitive component of $I_{K,slow}$ current (generated by Kv1.5), and diminished by ~20 % the HpTx (300 nM) sensitive current ($I_{to,f,}$ - generated by Kv4 subunits). Currents sensitive to 25 mM TEA were unchanged. Western blots revealed MiRP1 expression in *kcne2* (+/+) but not (−/ −) murine ventricles. Quantitative Western blots from left ventricular membrane fractions revealed a ~50% reduction ($p < 0.05$) in Kv1.5 protein in *kcne2* (−/ −) mice compared to wild-type, with no significant changes in Kv1.4, Kv2.1, Kv4.2 or Kv4.3 protein expression (n = 3 x 10 hearts per group). Further, MiRP1 co-immunoprecipitated with Kv4.2 but not Kv4.3 or Kv1.4 in *kcne2* (+/+) murine ventricular membrane fractions. We conclude that MiRP1 contributes to murine ventricular repolarization by direct modulation of Kv4.2 ($I_{to,f}$). Also, in *kcne2* (−/ −) ventricles, Kv1.5 protein membrane expression is significantly reduced, diminishing the 4-AP sensitive component of $I_{K,slow}$.

Platform AI: Cryoelectron Microscopy & Reconstruction

1752-Plat Development Of Phase Plates Of Electron Microscopes For Their Biological Application

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To visualize transparent materials, transmission electron microscopy(TEM) traditionally has employed a phase contrast method, defocus phase contrast, that is commonly used in high resolution studies of inorganic materials(1). Unfortunately, a fundamental flaw in the application of this method to biological specimens is the difficulty of reconciling contrast and resolution. One approach for surmounting this problem would be to employ a phase contrast method that utilizes phase plates, similar to the method used to visualize transparent objects by light microscopy. Theoretically, electron microscopy is compatible with three different types of phase plates(2), the thin film, electrostatic, and magnetic. However, designing functional phase plates has been arduous process that has

suffered from unavoidable technical obstacles such as the thin-film phase plate charging and difficulties associated with micro-fabrication of electrostatic and magnetic phase plates. Recently, Nagayama and colleagues obtained higher contrast images for unstained biological specimens in their vitrified state with thin-film phase plates(3). I report the state of the art in functional phase plates that focuses on the thin-film type. First, the methodology of phase contrast TEM is introduced. Second, recent studies imaging vitrified specimens such as proteins(4), proteolipids(5), cyanobacteria(6) and neurons with two kinds of thin-film phase plates are reviewed. Third, I discuss electron loss, a disadvantage of thin-film phase plates. Last, I review advanced phase plate technologies to overcome the electron loss with elaborate designs of electrostatic and magnetic phase plates.

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1753-Plat Molecular Dynamics Flexible Fitting (MDFF) - A Novel Method for Cryo-EM Maps Refinement

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Recent advances in experimental techniques for structural studies of biomacromolecules resulted in development of the cryo-EM method that allows obtaining low-resolution structural information about a biomolecule at various stages of its biological activity. In this work we present a new theoretical approach to refinement of this low-resolution structure in order to obtain high-resolution all-atom conformation of a molecule in the state captured in cryo-EM experiments. In our method we deform high-resolution, all-atom PDB structure so as it fits into a cryo-EM contour of the same molecule captured in the cryo-EM experiment in different conformation. Our methodology is based on the molecular dynamics technique, in which we modified expression for the potential energy so as it incorporates additional effective potential. This additional potential V^{MDFF} is calculated as $k(1\text{-}C.C.)$, where k is a constant allowing to modify magnitude of deforming forces and $C.C.$ is the correlation coefficient that describes overlap between the experimental cryo-EM map and synthetic map generated from the all-atom structure. Presence of the additional effective potential in the force field expression results in additional forces acting on all the atoms of the system. These forces deform the all-atom structure in such a way that the correlation coefficient is maximized, which is equivalent to maximizing the overlap between the cryo-EM and synthetic maps. Our results show that using MDFF technique we are able to obtain all-atom structures with 1.6Å resolution from the cryo-EM maps of 8Å resolution. Additionally, since the deformation is done with MD technique, we are able to get conformational evolution of molecules. In case of proteins important patterns in their secondary structure are preserved, which can be used as a criterion for evaluation of the MDFF fit quality.

1754-Plat Methods For Aligning And For Averaging 3D Volumes With Missing Data

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The visibility and resolution of a tomographic reconstruction containing multiple copies of discrete particles can be enhanced by averaging subtomograms after they are corrected aligned. However, the "missing wedge" in electron tomography can easily lead to erroneous alignment. We have explored a Fourier space cross-correlation method with a proper weighting scheme to align and average different sets of volumetric data, each of which has different missing data due to the limited specimen tilts. This approach depends neither on a preexisting template, nor an exact knowledge of the geometry, orientation, or amount of the missing data. This paper introduces a procedure where the missing data might be gradually "filled in" by consecutively aligning and averaging volumes with different orientations of their missing data. We have validated these techniques by a set of simulated data with various symmetries and extent of missing data. We have also successfully applied these procedures to experimental cryo-electron tomographic data (Chang et al., 2007; Schmid et al., 2006).

1755-Plat Sculptor: A Program for Visualization and Segmentation of Electron Tomographic Reconstructions

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Macromolecular machines carry out many cellular functions, understanding fundamental processes therefore involves studying the structural and functional properties of these large sub-cellular systems. Recent advances in electron tomography make it now possible to capture not only isolated particles but also pleiomorphic structures, parts of whole cells or organelles. The low-resolution volumetric reconstructions are often composed out of numerous substructures, which have to be identified and separated for further analysis.

The segmentation of large 3D volumetric maps into regions defining individual components is a well studied problem in related disciplines like medical image-processing. Although various algorithms and semi-interactive solutions were proposed, a direct application to tomographic maps is typically not feasible due to the low signal-to-noise levels and the large size of the volumetric reconstructions.

We propose a novel semi-interactive solution based on our visualization and analysis software "Sculptor". Sculptor facilitates

an interactive exploration of the volumetric data by iso-surface and GPU-based direct volume rendering.

After visual identification of a substructure, the user can select the object and thereby trigger a local pattern matching analysis. Reduced models of the substructures are docked into the region identified in the previous step, whereby the position and orientation are subsequently refined optimizing a Hessian-based scoring function. Longer filamentous structures are described utilizing a deformable model which can be parameterized to follow closely the bending parameters observed in experimental studies.

The docked models are then utilized for generating a mask, defining the local segmentation of the component. The proposed semi-interactive approach avoids the long run-times and the sensitivity to noise of fully-automatic segmentation methods, and is at the same time - due to the automatic local search - less subjective and ambiguous than fully interactive tools.

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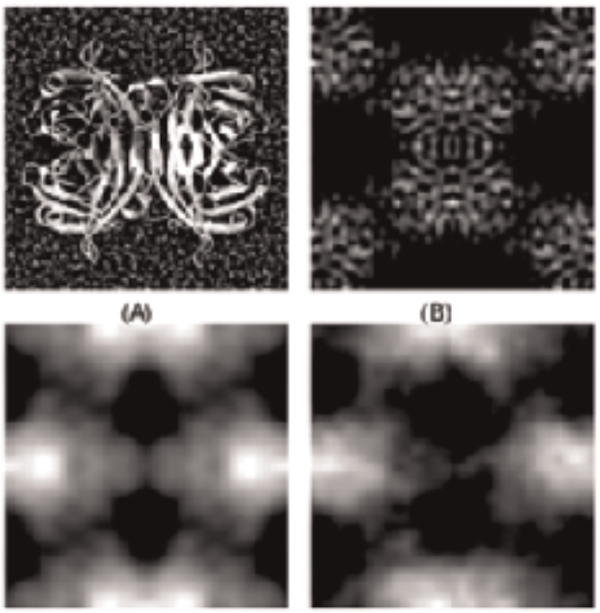

Figure (A) Molecular representation of the atomic model of the hydrated streptavidin tetramer. (B) Projection map of a unit cell (82.3Å*82.3Å) (2 tetramers) based on (A). (C) Convoluted with the CTF (computed for 200 keV; defocus=2.56 µm; B- factor 100 Å^2) which reversed the contrast. (D) The averaged projection map from cryo-EM data.

1756-Plat Absolute Calibration Of Cryo-EM Image Formation Using 2d Streptavidin Crystals

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In electron cryomicroscopy(cryo-EM) the phase-contrast image intensity is a reflection of the electron wave phase shift χ due to electrostatic potentials of the specimen. In the weak-phase approximation, variations in the intensity *I* are given by I=I0*[1 +2*m*χ*sin(ξ)], where I0 is the incident beam intensity and sin (ξ) is the spatial-frequency-dependent contrast transfer function (CTF). The scaling factor *m* is theoretically equal to 1, but in practice it takes a smaller value. Our goal is to estimate *m* for a given imaging situation, making use of a quantitative model of electron scattering by the streptavidin crystal. Up to now, atomic models for comparison with cryo-EM images have been constructed in vacuum based on published PDB coordinates from X-ray diffraction; this gives far too much contrast between the protein and the surround. Here, molecular dynamics(MD) simulations of streptavidin in a water box were used to compute a projection map of a hydrated streptavidin tetramer. This map was fitted to the cryo-EM image of a streptavidin crystal, thereby determining *m*. A self-consistency check was performed by using this factor to predict a liposome image from the MD-computed electron scattering by a lipid bilayer.

Figure (A) Molecule representation of the atomic model of the hydrated streptavidin tetramer. (B) Projection map of a unit cell (82.3Å*82.3Å) (2 tetrmers) based on(A). (C) Convoluted with the CTF (computed for 200 keV; defocus = 2.56 µm; B- factor 100Å^2) which reversed the contrast.(D). The averaged projection map from cryo-EM data.

1757-Plat Assembly And Supramolecular Organization Of The Septin Hetero-oligomers And Filaments Of The Yeast, Saccharomyces Cerevisiae

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Septin proteins assemble into a cytoskeletal polymer, which structure has not yet been characterized. Septin cytoskeleton is essential for cytokinesis, the last step in cell division. In budding yeast filamentous rings of septins have been visualized at the bud neck and constrict it in order to separate the mother and daughter cells. We are using yeast septins that self-assemble in vitro and are amenable to structural and functional analysis. Mitotic *Saccharomyces cerevisiae* cells express five septins: Cdc3, Cdc10, Cdc11, Cdc12, and Shs1/Sep7. All, but Shs1, are essential for cell division. Using electron microscopy of negatively-stained samples, we have observed that at low salt concentrations (50 mM KCl), the Cdc3-Cdc10-Cdc11-Cdc12 septin complex forms long paired filaments and bundles. At higher salt concentrations (300 mM KCl) short, linear rods can be observed. We have examined the ultrastructure of this “core” septin complex by electron microscopy. Single particle

averaging and image analysis confirms the octameric nature of the hetero-oligomer and reveals an unexpected linear arrangement (rod) of the subunits. The position and identity of each subunit in the rod is determined using immuno-EM with antibodies directed against each septin and MBP or GFP proteins fusionned with septin subunits.. Shs1, the non-essential yeast septins is now being added so that our model can be made more complete and even closer to the *in vivo* situation. Alternatively we are also studying the structure and behavior of partial complexes and complexes containing mutant proteins, this in the aim of judging the relevance of different septins and septin domains to the architecture of the assembly unit and its propensity to self-assemble. The kinetic of assembly of the filaments is also studied by scattering methods in various ionic and nucleotide conditions.

1758-Plat Desmosome Structure in Intact Cells by Electron Cryotomography

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Demosomes are intercellular junctions that mediate cell-cell interactions and provide mechanical strength to cellular tissues. They are composed of transmembrane glycoproteins (cadherins) that directly bind one another to establish the intercellular bond, and a heterogeneous protein plaque in the cytoplasm that couples the cadherins to the intermediate filament network. Electron tomography provides a method for generating 3D structures of these complex molecular assemblies, from which we are trying to deduce the molecular interactions that characterize the desmosome. A previous electron tomographic study of freeze-substituted, plastic sections offered the first 3D insight into the organization of the extracellular cadherin domains. These results suggested flexibility in the binding interface between individual cadherins, which led to the formation of pleimorphic groups within the extracellular space. This result contrasted with projection images of frozen-hydrated vitreous sections, which revealed straight, rod-like densities with an apparent 5 nm periodicity. To better define their native structure, we have developed a new technique for preparing desmosomes in the frozen-hydrated state that avoids the technical challenges of cryoultramicrotomy. In particular, we have flash-frozen intact keratinocytes which were cultured on electron microscopic grids. This approach involves minimal manipulation of the samples and provides images of the entire desmosome rather than thin sections. Although cell bodies are too thick for electron microscopy, desmosomes initially form between thin filopodia that extend between the cell borders. Thus, we have been able to use a 300 kV electron microscope equipped with an in-column energy filter to acquire suitable images. Projection images of these desmosomes reveal features comparable to those previously observed in frozen-hydrated sections. We are currently recording tomographic reconstructions to elucidate the 3D organization of these densities and ultimately the molecular interactions of the constituent proteins.

1759-Plat Study of Magainin-induced Pores in Phospholipid Vesicles

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Magainin is a peptide antibiotic that interacts directly with the lipid membrane in the target cell. The prevalent mode of action for Magainin and related peptides is formation of large nonselective pores in the membrane leading to cell lysis. A number of methods, like Circular Dichroism, solid state NMR, Raman Scattering, Neutron Scattering, etc., have been used to infer this mode of action. However, none of those methods has directly observed the structure of an individual pore, and some puzzling inconsistencies exist in the current models. Here we present a new method for studying peptide/lipid interactions, which employs Cryo-EM to image Magainin-induced pores in phospholipid vesicles. Since the size of the putative pores is quite small for direct visualization under the dose limitations that exist for Cryo-EM, we are also analyzing the Cryo-EM data in a scattering context. That is, performing 'virtual' electron scattering experiments through analysis of the power spectra of selected vesicles. Unlike X-ray and Neutron Scattering experiments, this technique permits selective masking of the regions of the specimen for which the power spectrum is computed. This permits, for example, isolation of the in-plane power spectrum from the transverse power spectrum. While we eventually intend to directly image individual pores, as a first step, this permits observation of the effects of Magainin in bulk specimen, and relates Cryo-EM results to previous X-ray and Neutron Scattering experiments.

Platform AJ: Mitochondrial Channels & Calcium Signaling

1760-Plat Cytoskeleton Regulates Mitochondria Respiration Through A Tubulin-VDAC Direct Interaction

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Mitochondria have long been known to localize within the tubulin-microtubule network in heart and many other cells (Appaix et al., 2003). It is also well-known that in permeabilized cardiac cells the apparent Km for exogenous ADP in the control of mitochondrial respiration is significantly higher than in isolated mitochondria. It has been suggested that the low permeability of the mitochondria outer membrane (MOM) for ATP and ADP in cells is due to interaction of mitochondria with some cytoplasmic proteins (Saks et al., 2003). Here, for the first time, we demonstrate that tubulin is the factor which controls MOM permeability by regulating VDAC, the major channel of MOM. By direct measurements we show that nanomolar concentrations of mammalian tubulin induce highly voltage-sensitive reversible closure of VDAC channels reconstituted into planar phospholipid membranes. Analysis of VDAC single channel fluctuations in the presence of tubulin shows that channel closure occurs at very low potentials (as low as 10 mV)