PNAS Plus Significance Statements

Design and formulation of functional pluripotent stem cell-derived cardiac microtissues

Nimalan Thavandiran, Nicole Dubois, Alexander Mikryukov, Stéphane Massé, Bogdan Beca, Craig A. Simmons, Vikram S. Deshpande, J. Patrick McGarry, Christopher S. Chen, Kumaraswamy Nanthakumar, Gordon M. Keller, Milica Radisic, and Peter W. Zandstra

Robust and predictive in vitro models of human cardiac tissue function could have transformative impact on our ability to test new drugs and understand cardiac disease. Despite significant effort, the generation of high-fidelity adult-like human cardiac tissue analogs remains challenging. In this paper, we systematically explore the design criteria for pluripotent stem cell-derived engineered cardiac tissue. Parameters such as biomechanical stress during tissue remodeling, input-cell composition, electrical stimulation, and tissue geometry are evaluated. Our results (pp. E4698–E4707) suggest that a specified combination of a 3D matrix-based microenvironment, uniaxial mechanical stress, and mixtures of cardiomyocytes and fibroblasts improves the performance and maturation state of in vitro engineered cardiac tissue.

Free-energy landscape of protein oligomerization from atomistic simulations

Alessandro Barducci, Massimiliano Bonomi, Meher K. Prakash, and Michele Parrinello

Oligomeric proteins, comprising two or more associating polypeptide chains, represent a large fraction of cellular proteins. In particular, many proteins self-associate into homooligomers to gain functional advantages. Our understanding of the oligomerization at the molecular level is currently limited because intermediates that occur in the process are short-lived in most occasions, precluding a direct experimental characterization. Using molecular dynamics simulations, enhanced by a sampling method developed in our group, we obtained an atomistic description of the assembly of an evolutionary-optimized trimeric protein. Our results (pp. E4708–E4713) are in excellent agreement with available experimental data and extend the current view of oligomerization by showing the importance of the apparently contradictory requirements of monomer preorganization and conformational flexibility.

Circular dichroism and site-directed spin labeling reveal structural and dynamical features of high-pressure states of myoglobin

Michael T. Lerch, Joseph Horwitz, John McCoy, and Wayne L. Hubbell

High hydrostatic pressure facilitates the characterization of functionally relevant, but sparsely populated, excited conformational states of proteins by reversibly increasing their equilibrium population. Here (pp. E4714–E4722), high-pressure instrumentation for circular dichroism and developments in high-pressure site-directed spin-labeling EPR are reported, and a combination of EPR and circular dichroism is used to map pressure-populated structural changes in various states of myoglobin. The data reveal that the high-pressure molten globule (MG) of apomyoglobin at neutral pH retains native-like helical content despite a fluctuating tertiary fold, an MG state of holomyoglobin populated at low pH and high-pressure retains ligand-binding capacity, and a transient folding intermediate of apomyoglobin is populated under similar conditions at equilibrium.

Rho GTPases orient directional sensing in chemotaxis

Yu Wang, Hiroshi Senoo, Hiromi Sesaki, and Miho Iijima

During chemotaxis, cells recognize an extracellular chemical gradient and produce amplified intracellular responses independently of the actin cytoskeleton. This process is called directional sensing and observed as the activation of Ras GTPase and the production of phosphatidylinositol (3,4,5)-triphosphate (PIP3) toward higher concentrations of chemoattractants. How directional sensing is controlled is largely unknown. In our current study (pp. E4723–E4732), we demonstrate that a Rho GTPase (RacE) and a Rho guanine nucleotide exchange factor (GxcT) are required for the orientation of directional sensing independently of feedback from the actin cytoskeleton and cell polarity in *Dictyostelium*, and reveal a previously unknown role for Rho GTPases in intracellular signaling upstream of Ras activation and PIP3 production.

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Exposure of MC4R to agonist in the endoplasmic reticulum stabilizes an active conformation of the receptor that does not desensitize

Susana Granell, Brent M. Molden, and Giulia Baldini

Melanocortin-4 receptor (MC4R) is a cell-surface hormone receptor in the brain that is central to the control of appetite. Upstream pathways sensing energy balance in the organism lead to the secretion of α-melanocyte–stimulating hormone (α-MSH), which stimulates MC4R activity and leads to decreased appetite. Previously, it was found that MC4R becomes resistant to treatment with extracellular agonists by a mechanism that may involve desensitization of the receptor, arguing against their usefulness in treating obesity. In this study, we show that exposing MC4R to α-MSH in the endoplasmic reticulum leads to constant signaling of MC4R without desensitization. Our results (pp. E4733–E4742) indicate that the cellular localization where agonist binding initially takes place affects the conformation and the desensitization properties of MC4R, suggesting a target for therapy.

Nonmuscle myosin II powered transport of newly formed collagen fibrils at the plasma membrane

Nicholas S. Kalson, Tobias Starborg, Yinhui Lu, Aleksandr Mironov, Sally M. Humphries, David F. Holmes, and Karl E. Kadler

Collagen is the most abundant protein in vertebrates and is the building block of strong tissues such as tendons, skin, and bones. The fibrils can be millimeters long and occur in the extracellular matrix as a scaffold for tissue growth. Important questions remain unanswered about how cells assemble and transport the fibrils. We show here (pp. E4743–E4752) that collagen fibril assembly can occur at the plasma membrane in structures called fibripositors. We show that fibripositors are a nonmuscle myosin II (NMII)-dependent mechanical interface between the actinomyosin machinery and the extracellular matrix; thus, we propose a new function for NMII. A unique mechanism of fibril transport is presented as a basis for studies of tissue morphogenesis and conditions including wound healing and fibrosis.

Saposins modulate human invariant Natural Killer T cells self-reactivity and facilitate lipid exchange with CD1d molecules during antigen presentation

Mariolina Salio, Hemza Ghadbane, Omer Dushek, Dawn Shepherd, Jeremy Cypen, Uzi Gileadi, Michael C. Aichinger, Giorgio Napolitani, Xiaoyang Qi, P. Anton van der Merwe, Justyna Wojno, Natacha Veerapen, Liam R. Cox, Gurdyal S. Besra, Weiming Yuan, Peter Cresswell, and Vincenzo Cerundolo

Understanding how to optimize lipid-loading onto CD1d molecules is important to better harness invariant natural killer T (iNKT) cells' central role at the interface between innate and adaptive immunity. We report (pp. E4753–E4761) that the lipid transfer proteins saposins play an essential role in modulating human iNKT cell autoreactivity to antigen-presenting cells activated by inflammatory stimuli. Lipid-loading occurs in an endo-lysosomal compartment, where saposins work as "lipid editors," capable of fine-tuning loading and unloading of CD1d molecules and increasing the off-rate of CD1d-bound lipids.

Metastatic castration-resistant prostate cancer reveals intrapatient similarity and interpatient heterogeneity of therapeutic kinase targets

Justin M. Drake, Nicholas A. Graham, John K. Lee, Tanya Stoyanova, Claire M. Faltermeier, Sudha Sud, Björn Titz, Jiaoti Huang, Kenneth J. Pienta, Thomas G. Graeber, and Owen N. Witte

Metastatic castration-resistant prostate cancer (CRPC) remains incurable due to the lack of effective therapies. The need to identify new actionable targets in CRPC is crucial as we begin to examine the resistance mechanisms related to androgen withdrawal. Here (pp. E4762–E4769), we report an unbiased quantitative phosphoproteomic approach to identify druggable kinases in metastatic CRPC. These kinase activation patterns revealed intrapatient similarity and interpatient heterogeneity across a large panel of targets. Interestingly, these kinase activities are not a result of mutation but rather pathway activation within the tumors themselves. The observation that similar kinase activities are present in most if not all anatomically disparate metastatic lesions from the same patient suggests that CRPC patients may benefit from individualized, targeted combination therapies.

Coxiella burnetii effector protein subverts clathrin-mediated vesicular trafficking for pathogen vacuole biogenesis

Charles L. Larson, Paul A. Beare, Dale Howe, and Robert A. Heinzen

The vesicular trafficking pathways required for generation of the phagolysosome-like vacuole occupied by *Coxiella burnetii* are poorly defined, and no pathogen effectors of vesicular trafficking are known. Here, we reveal an important role for clathrin-mediated vesicular trafficking in *Coxiella* vacuole formation and identify a type 4B secretion system effector protein [*Coxiella* vacuolar protein A (CvpA)] that engages this pathway. *C. burnetii* CvpA traffics through the endocytic recycling compartment, and endocytic sorting motifs within CvpA bind the clathrin adaptor complex AP2. Mutation of *cvpA*, or depletion of AP2 or clathrin, significantly restricts *Coxiella* replication. Thus, our results (pp. E4770–E4779) reveal a effector–clathrin interaction that benefits pathogen replication.

Nuclear import of APOBEC3F-labeled HIV-1 preintegration complexes

Ryan C. Burdick, Wei-Shau Hu, and Vinay K. Pathak

We observed (pp. E4780–E4789) that fluorescent protein-tagged host restriction factors APOBEC3F (A3F) and APOBEC3G (A3G) remain associated with HIV-1 preintegration complexes (PICs) in the nuclei of infected cells. Using A3F labeling as a tool to visualize PICs, we determined that (*i*) reverse transcription is not required for nuclear import of PICs, indicating that a viral core uncoating event associated with reverse transcription, and the central DNA flap that forms during reverse transcription, are not required for nuclear import; (*ii*) viral core stability mutations dramatically reduce association of PICs with the nuclear envelope as well as diminish their nuclear import; and (*iii*) most nuclear PICs remain close to the nuclear envelope and are not distributed throughout the nuclei.

Genetic regulation of vesiculogenesis and immunomodulation in *Mycobacterium tuberculosis*

Poonam Rath, Chengdong Huang, Tao Wang, Tianzhi Wang, Huilin Li, Rafael Prados-Rosales, Olivier Elemento, Arturo Casadevall, and Carl F. Nathan

Bacteria stimulate host cells in part via secreted products, some of which are packaged in membrane vesicles (MV). MV released by the major human pathogen *Mycobacterium tuberculosis* (Mtb) carry lipoprotein LpqH, a major agonist for host Toll-like receptor 2 (TLR2). This study (pp. E4790–E4797) identifies a gene, *rv0431*, which appears to regulate mycobacterial MV formation, and therefore we suggest it be named "vesiculogenesis and immune response regulator" (*virR*). This gene encodes a protein that includes a unique fold, as determined by NMR spectroscopy, and a disordered domain suggestive of participation in a higher-order complex. By restraining the release of most of the material released by Mtb that activates host cells through TLR2, VirR reduces Mtb's immunostimulatory potential and increases its virulence.

Estimating functional connectivity in an electrically coupled interneuron network

Pepe Alcami and Alain Marty

Certain classes of central neurons, notably interneurons, are linked together by electrical synapses made by gap junctions, which play an important role in network function. Modeling electrically coupled interneuron networks has been limited by a lack of knowledge of the number of cells directly connected to a reference neuron and of the extent of charge redistribution in the network. We show (pp. E4798–E4807) that capacitive currents can be used to derive such information. We illustrate this method, using the network formed by cerebellar molecular layer interneurons. We establish a quantitative model of the network, and we estimate the influence of gap junctions on network excitability. The method may be generally applicable to sparsely electrically coupled networks, particularly if they are planar.

Reconstruction of protein networks from an atlas of maize seed proteotypes

Justin W. Walley, Zhouxin Shen, Ryan Sartor, Kevin J. Wu, Joshua Osborn, Laurie G. Smith, and Steven P. Briggs

Here we report deep, quantitative, and replicated proteome analysis of a developing multicellular organism. We quantified protein abundance and levels of protein phosphorylation during development of the maize seed. The depth and quantitative nature of the data enabled a network-based approach to identify kinase-substrate relationships as well as the reconstruction of biochemical and signaling networks that underpin seed development and seed storage product production. We found (pp. E4808–E4817) that many of the most abundant proteins are not associated with detectable levels of their mRNAs and vice versa. These data significantly add to our understanding of seed development and facilitate knowledge-based crop improvement.