Immune cell screening of a nanoparticle library improves atherosclerosis therapy

The immune system plays an essential role in the pathophysiology of major diseases such as atherosclerosis, diabetes, and cancer, which has inspired the development of numerous small molecules to modulate immune cells, intending to create immunotherapies for these diseases. Tissue- and cell-specific delivery of these small molecules is the key to transform these compounds to safe, potent immunotherapies. In this study, we present an in vivo nanoparticle screen approach that involves designing and evaluating a library of nanoparticles with distinct immune cell targeting specificity. This study carries out a systematic in vivo immune cell screening to create effective nanoparticle-based immunotherapy for modulating the pathological immune cells in atherosclerosis. (See pp. E6731–E6740.)

Thermodynamic origin of surface melting on ice crystals
Ken-ichiro Murata, Harutoshi Asakawa, Ken Nagashima, Yoshinori Furukawa, and Gen Szaki

Phase transitions of ice are a major source of a diverse set of natural phenomena on Earth. In particular, quasi-liquid layers (QLLs) resulting from surface melting are recognized to be key players involved in various natural phenomena spanning from making snowballs to electrification of thunderclouds. With the aid of in situ observations with our advanced optical microscopy combined with two-beam interferometry, we elucidate a thermodynamic origin of the formation of QLLs and their unique wetting behavior (pseudo-partial wetting and wetting transitions) on ice surfaces. We show that QLLs are a metastable transient state formed through vapor growth and sublimation of ice that are absent at equilibrium. (See pp. E6741–E6748.)

Design and characterization of a nanopore-coupled polymerase for single-molecule DNA sequencing by synthesis on an electrode array
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DNA sequencing has been dramatically expanding its scope in basic life science research and clinical medicine. Recently, a set of polymer-tagged nucleotides were shown to be viable substrates for replication and electronically detectable in a nanopore. Here, we describe the design and characterization of a DNA polymerase–nanopore protein construct on an integrated chip. This system incorporates all four tagged nucleotides and distinguishes single-tagged-nucleotide addition in real time. Coupling protein catalysis and nanopore-based detection to an electrode array could provide the foundation of a highly scalable, single-molecule, electronic DNA-sequencing platform. (See pp. E6749–E6756.)

Inositol polyphosphates intersect with signaling and metabolic networks via two distinct mechanisms
Mingxuan Wu, Lucy S. Chong, David H. Perlman, Adam C. Resnick, and Dorothea Fiedler

Inositol polyphosphates and pyrophosphates are ubiquitous eukaryotic messengers and are involved in numerous cellular processes, including insulin signaling and cell migration. Nevertheless, annotation of the specific signaling events underlying these processes has been stymied by the lack of suitable tools. By applying chemically synthesized affinity reagents, we comprehensively annotated inositol polyphosphate binding proteins in the model organism Saccharomyces cerevisiae, revealing a role for these molecules in nucleotide metabolism, ribosome biogenesis, and phosphorylation-based signaling. Furthermore, the reagents enabled the magnesium-dependent isolation of targets of protein pyrophosphorylation, a posttranslational modification mediated...
by the inositol pyrophosphates. The protein targets are diverse in function and highlight the complex regulation of cellular signaling and metabolic networks by inositol pyrophosphates. (See pp. E6757–E6765.)

Structural basis of recognition of farnesylated and methylated KRAS4b by PDE6
Sriarathyaranyan Dharmaiah, Lakshman Bindu, Timothy H. Tran, William K. Gillette, Peter H. Frank, Rodolfo Ghirlando, Dwight V. Nisley, Dominic Esposito, Frank McCormick, Andrew G. Stephen, and Dhirendra K. Simanshu

Despite the significant progress made in the last few years toward targeting phosphodiesterase-δ (PDEδ) for KRAS (Kirsten rat sarcoma isoform)-driven cancers, there is no structural information available on posttranslationally modified KRAS4b in complex with PDEδ. The KRAS4b–PDEδ structure reported here provides the structural details of the protein–protein interaction interface and the atomic details of the hypervariable region of KRAS4b. Structural comparison of the two crystal forms allowed identification of a 5-aa-long sequence motif in KRAS4b that could allow PDEδ to bind to both farnesylated and geranylgeranylated KRAS4b. Structural insights obtained from this study could be used to guide the development of improved and more specific inhibitors of the KRAS4b–PDEδ complex. (See pp. E6766–E6775.)

Unidirectional allosteroy in the regulatory subunit Rlx facilitates efficient deactivation of protein kinase A
Cong Guo and Huan-Xiang Zhou

Activation and deactivation of protein kinase A (PKA) are triggered by cAMP binding to and unbinding from two tandem domains of the regulatory subunit. Evidence indicates that both binding and unbinding of two cAMPs are ordered. Whereas sequential binding to the inactive holoenzyme complex can be attributed to masking of one binding site by the catalytic subunit, sequential unbinding from the regulatory subunit appears related to unidirectional allosteric communication between the two domains, although the mechanism for unidirectionality has been a mystery. Here, we present a solution through molecular dynamics simulations. One of the two cAMPs acts as a bridge between the domains and thereby gates interdomain communication. Directionality of allostery can facilitate PKA deactivation and may have broad functional importance. (See pp. E6776–E6785)

Molecular lock regulates binding of glycine to a primitive NMDA receptor
Alvin Yu, Robert Alberstein, Alecia Thomas, Austin Zimmet, Richard Grey, Mark L. Mayer, and Albert Y. Lau

Glycine-activated ionotropic glutamate receptors (iGluRs) encoded in ctenophore genomes are evolutionary precursors to NMDA receptors, which play important roles in synaptic plasticity. Ctenophore iGluRs feature a distinct interdomain salt bridge in the ligand-binding domain, a molecular lock, that thus far has not been found in iGluRs of other organisms. We use a combination of crystallographic, biochemical, electrophysiological, and computational approaches to elucidate the role of this molecular lock in the ctenophore iGluR. We find that perturbations to the lock can tune receptor kinetics and thermodynamics over very broad ranges. We also find that the strategic location of the lock may be the basis for the ligand-binding domain’s extraordinarily high affinity for glycine. (See pp. E6786–E6795.)

Avilamycin and evernimicin induce structural changes in rProteins Ul16 and CTC that enhance the inhibition of A-site tRNA binding
Miri Kupkin, Itai Wekselman, Donna Matzov, Zohar Eyal, Yael Diskin Posner, Haim Rozenberg, Ella Zimmerman, Anat Bashan, and Ada Yonath

Resistance to antibiotics poses a serious threat in contemporary medicine. Avilamycin and evernimicin, polysaccharide antibiotics belonging to the orthosomycin family, possess inhibitory activity against multidrug-resistant pathogenic strains of Enterococci, Staphylococci, and other Streptococci gram-positive bacteria by paralyzing ribosomes function in protein biosynthesis. The crystal structures of the large ribosomal subunit from the eubacteria Deinococcus radiodurans in complex with avilamycin and evernimicin revealed their binding sites at the entrance to the A-site tRNA accommodating corridor, thus illuminating the mechanisms of their translation inhibition. Analysis of the binding interactions of these antibiotics depicted the features enabling their species discrimination (namely, selectivity) and elucidated the various mechanisms by which pathogens use single mutations to acquire resistance to those drugs. (See pp. E6796–E6805.)

Activation of SAT1 engages polyamine metabolism with p53-mediated ferroptotic responses
Yang Ou, Shang-Jui Wang, Dawei Li, Bo Chu, and Wei Gu

Although it is commonly accepted that p53-mediated cell-cycle arrest, apoptosis, and senescence all serve as major mechanisms of tumor suppression, accumulating evidence indicates that other activities of p53, such as ferroptosis, are also critical for tumor suppression. However, the molecular mechanisms by which p53-mediated ferroptosis operates are not completely understood. In this study, we discovered that p53 can execute ferroptotic cell-death responses by directly activating its target gene SAT1, coded for the spermidine/spermine N’-acetyltransferase 1. These data indicate a regulatory role of p53 in polyamine metabolism and reveal that p53-mediated activation of SAT1 contributes significantly to ferroptotic responses. Thus, p53 may engage multiple metabolic pathways with ferroptotic cell-death responses for tumor suppression. (See pp. E6806–E6812.)

Matrix mechanics controls FHL2 movement to the nucleus to activate p21 expression
Naotaka Nakazawa, Anesh R. Sathe, G. V. Shivashankar, and Michael P. Sheetz

Substrate rigidity has important roles for physiological processes, such as stem cell differentiation and cell growth. Although substrate rigidity clearly modulates gene expression, the mechanism of rigidity control of gene expression remains unknown. Our work reveals that four-and-a-half LIM domains (domain discovered in the proteins, Lin11, Isl-1, and Mec-3) 2 moves from adhesion sites to the nucleus on soft substrates through focal adhesion kinase activity and up-regulates p21 gene expression. Thus, we show a
molecular pathway for inhibiting cell growth on soft substrates. (See pp. E6813–E6822.)

Oxidative stress in oocytes during midprophase induces premature loss of cohesion and chromosome segregation errors
Adrienne T. Perkins, Thomas M. Das, Lauren C. Panzera, and Sharon E. Bickel

Chromosome segregation errors during female meiosis, the leading cause of birth defects and miscarriages, increase dramatically as women age. Because oxidative damage increases with age and oocytes age during a woman’s lifetime, one factor that may contribute to increased segregation errors in older women is damage of the protein linkages (cohesion) required for accurate segregation. In support of this hypothesis, we find that inducing oxidative stress in Drosophila oocytes during meiotic prophase causes a significant increase in segregation errors due to premature loss of cohesion. These data demonstrate that oxidative stress during the stage at which human oocytes remain arrested for decades can cause meiotic segregation errors and offer insight into why cohesion deteriorates with age. (See pp. E6823–E6830.)

Neural tube morphogenesis in synthetic 3D microenvironments
Adrian Ranga, Mehmet Girgin, Andrea Meinhardt, Dominic Eberle, Massimiliano Caiazzo, Elly M. Tanaka, and Matthias P. Lutolf

In vitro organoids have become widely used model systems in basic research and for therapeutic applications due to their ability to recapitulate key elements of in vivo form and function. However, their full potential remains unfulfilled as a result of the poorly defined matrices in which they are grown. Here, we use modular synthetic 3D matrices to show that early neural morphogenesis can be precisely controlled by the extracellular microenvironment. Our approach is broadly applicable to gain a broader understanding of the multifactorial 3D cell–matrix interactions that coordinate multicellular growth and differentiation, and opens up avenues to discover and dissect the unique microenvironments that control morphogenesis in various organoid systems. (See pp. E6831–E6839.)

Spemann organizer gene Goosecoid promotes delamination of neuroblasts from the otic vesicle
Husniye Kantarcı, Andrea Gerberding, and Bruce B. Riley

Neurons that innervate the inner ear originate as neuroblasts in the otic vesicle, the epithelial precursor of the inner ear. Neuroblasts subsequently delaminate from the otic epithelium to complete differentiation near the hindbrain. Despite growing understanding of otic neurogenesis, the mechanism by which neuroblasts delaminate from the otic vesicle is unknown. Here we show that delamination is triggered by Goosecoid (Gsc), a homeobox gene famously discovered as the first known regulator of the “Spemann” embryonic organizer. Gsc is expressed in the otic vesicle in a region overlapping with neuroblasts, inducing localized epithelial-to-mesenchymal transition (EMT). Hence, regulation of cellular dynamics appears to be a general function of Gsc during otic neurogenesis as well as in the embryonic organizer. (See pp. E6840–E6848.)

Structural basis for nonneutralizing antibody competition at antigenic site II of the respiratory syncytial virus fusion protein

Respiratory syncytial virus is a highly contagious human pathogen, infecting the majority of infants before age 2 y, and is the leading cause of viral bronchiolitis and viral pneumonia in infants and children. An approved prophylactic humanized mouse monoclonal antibody, palivizumab, is currently the standard-of-care treatment for immunocompromised individuals, and competition with palivizumab has been proposed as the basis for measuring effective immune responses for vaccine trials. Using a combination of X-ray crystallography, hydrogen-deuterium exchange, and saturation alanine mutagenesis scanning, we show the structural basis for neutralization by a human antibody at the palivizumab antigenic site. Furthermore, we describe nonneutralizing antibodies that directly compete with palivizumab and another human antibody 14N4. Taken together, the data presented provide unique concepts in structure-based vaccine design. (See pp. E6849–E6858.)

Dynamic translation regulation in Caulobacter cell cycle control
Jared M. Schrader, Gene-Wei Li, W. Seth Childers, Adam M. Perez, Jonathan S. Weissman, Lucy Shapiro, and Harley H. McAdams

The Caulobacter cell cycle is controlled by a genetic circuit that dynamically regulates transcription of nearly 20% of the genome; however, the role of translational control of cell cycle progression is unexplored. To understand the contribution of translational regulation, we measured both mRNA and translation levels at multiple stages of the cell cycle. We found that cell cycle-dependent translational regulation is important for hundreds of genes and also that the positioning of regulatory proteins to a specific cell pole is coordinated by the timing of their synthesis. The cell cycle-regulatory pathway that controls translation rates is linked to the regulatory circuit that controls transcription rates of cell cycle-regulated genes. (See pp. E6859–E6867.)

Human cortical–hippocampal dialogue in wake and slow-wave sleep
Anish Mitra, Abraham Z. Snyder, Carl D. Hacker, Mrinal Pahwa, Enzo Tagliazucchi, Helmut Laufs, Eric C. Leuthardt, and Marcus E. Raichle

Reciprocal cortical–hippocampal signaling is widely believed to underlie consolidation of declarative memories. By investigating human fMRI and electrocorticography during both wake and slow-wave sleep (SWS), we find, first, that δ-band activity and infraslow activity propagate in opposite directions between the hippocampus and cortex. Second, both δ activity and infraslow activity reverse propagation directions between the hippocampus and the cortex across wake and SWS. These results highlight reciprocal communication between frequencies, and constitute direct evidence for the reversal of the human cortical–hippocampal dialogue across wake and SWS. (See pp. E6868–E6876.)
Glutamate-induced RNA localization and translation in neurons

Local translation in dendrites of neurons has been shown to be important for neuronal function and synaptic biology. We imaged changes in the localization of β-actin mRNA and protein in dendritic spines. Our results showed that activating specific synapses can drive changes in the localization of endogenous mRNA and the translation of reporter RNA in dendrites of hippocampal neurons. Enhancing our understanding of the spatial and temporal kinetics of mRNA localization in dendrites informs local protein synthesis in neurons. These results provide direct evidence of protein synthesis away from the soma and allow us to determine how the kinetics of mRNA localization and translation could influence synaptic physiology and plasticity. (See pp. E6877–E6886.)

Structural and functional significance of water permeation through cotransporters
Thomas Zeuthen, Edurne Gorraitz, Ka Her, Ernest M. Wright, and Donald D. F. Loo

Transport of water and polar solutes across membranes play an important physiological role, and it is widely appreciated that many membrane transport proteins are permeable to water. Molecular dynamics studies of sodium-coupled glucose cotransporters (SGLTs) indicate that water flows through the sugar transport pathway. We test this hypothesis by mutating residues lining the sugar transport pathway through SGLT1, and measuring the changes in the permeability of water and urea before and after their chemical modification. Mutation of outer and inner gate residues and residues involved in sugar binding confirms that water and urea permeate the glucose transport pathway, suggesting that water is involved in sugar transport. SGLT1 is physiologically important in determining osmotic flow across the small intestine. (See pp. E6887–E6894.)

Twenty-four-nucleotide siRNAs produce heritable trans-chromosomal methylation in F1 Arabidopsis hybrids
Ian K. Greaves, Steven R. Eichten, Michael Groszmann, Aihua Wang, Hua Ying, W. James Peacock, and Elizabeth S. Dennis

We show that the changes in DNA methylation that occur in F1 hybrids of Arabidopsis are mostly dependent on the presence of 24-nt siRNAs at the locus. The methylation change at a locus results in the two alleles becoming similar to each other in methylation pattern. The methylation changes occur through the processes of trans-chromosomal methylation and trans-chromosomal demethylation. These altered methylation states can be inherited in the F2 generation and can be associated with changes in levels of gene activity, which may contribute to the phenotypic heterogeneity in the F2. (See pp. E6895–E6902.)