PNAS Plus Significance Statements

Catalytic enzymes are active matter

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Catalysis and mobility of reactants in fluid are normally thought to be decoupled. Violating this classical paradigm, this paper presents the catalyst laws of motion. Comparing experimental data to the theory presented here, we conclude that part of the free energy released by chemical reaction is channeled into driving catalysts to execute wormlike trajectories by piconewton forces performing work of a few k_BT against fluid viscosity, where the rotational diffusion rate dictates the trajectory persistence length. This active motion agitates the fluid medium and produces antichemotaxis, the migration of catalyst down the gradient of the reactant concentration. Alternative explanations of enhanced catalyst mobility are examined critically. (See pp. E10812–E10821.)

Structural consequences of hereditary spastic paraplegia disease-related mutations in kinesin

Mandira Dutta, Michael R. Diehl, José N. Onuchic, and Biman Jana

Motor proteins are important biological machines responsible for cellular transport. Malfunctioning of them causes several neurodegenerative diseases. We searched for a molecular-level answer for malfunctioning kinesin, which causes hereditary spastic paraplegia (HSP) disease. Using explicit solvent simulation, the thermodynamic integration (TI) method, and bioinformatics analysis, we explored how four HSP mutants of kinesin perturb microtubule (MT) binding and motor dimerization. Taking these observations into account, we developed a coarse-grained structure-based model to reveal the effect of these mutations on kinesin's order–disorder transition, which leads to the processivity and directionality of kinesin. Our study potentially uncovers a molecular-level picture of the role of some HSP mutants and its broad aspect in kinesin mechanochemistry. (See pp. E10822–E10829.)

Microfluidics in structured multimaterial fibers

Rodger Yuan, Jaemyon Lee, Hao-Wei Su, Etgar Levy, Tural Khudiyev, Joel Voldman, and Yoel Fink

The development of microfluidics, or the study of fluid behavior and manipulation at the microscale, has largely been catalyzed by microfabrication techniques based on a planar chip format. While immensely powerful, planar fabrication methods have inherent restrictions that prevent the realization of relatively simple channel structures, such as nonrectangular cross-sectional geometries and arbitrary cross-sectional materials placement. This study introduces a microfluidics fabrication method based on a fiber format that enables the construction of microchannels with highly tunable cross-sectional geometries and a broad range of materials. This approach allows for degrees of freedom in the design and function of microfluidic systems, thereby extending the reach of realizable microfluidic devices. (See pp. E10830–E10838.)

Bifunctional amyloid-reactive peptide promotes binding of antibody 11-1F4 to diverse amyloid types and enhances therapeutic efficacy

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Amyloidosis is characterized by the deposition of pathologic protein fibrils in organs and tissues and is associated with ~25 disorders, including Alzheimer's disease and a systemic disease resulting from the aggregation of immunoglobulin light chains. Present treatments focus on reducing production of amyloid precursor proteins, but recent studies using amyloid-reactive antibodies have shown them capable of removing amyloid and improving organ function. To expand the utility of one such clinical antibody, designated 11-1F4, we have developed a synthetic, bifunctional peptide that binds many types of amyloid and mediates the recruitment of antibody to diverse amyloid types. This peptide approach may be used to enhance the efficacy and utility of existing antibodies, leading to broad-spectrum therapeutics and improved patient care. (See pp. E10839–E10848.)

microRNA-378 promotes autophagy and inhibits apoptosis in skeletal muscle

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Muscle wasting and weakness can be observed under either physiological or pathological conditions, which are partly due to an imbalance between autophagy ("self-eating") and apoptosis ("self-killing"). How microRNAs coordinate autophagy and apoptosis in the metabolic regulation of cell death remains largely unknown. This work identifies miR-378 as a critical component of metabolic checkpoints, which integrates

metabolic information into an adaptive response to reduce the propensity of myocytes to undergo apoptosis by enhancing autophagy and suppressing apoptosis via directly targeting phosphoinositide-dependent protein kinase 1 and Caspase 9, respectively. Our study highlights a crucial role of miR-378 in maintaining normal muscle homeostasis by orchestrating autophagy and apoptosis processes and provides a potential therapeutic target to treat myopathies. (See pp. E10849–E10858.)

Discs large 1 controls daughter-cell polarity after cytokinesis in vertebrate morphogenesis

Yuwei Li, Jason A. Junge, Cosimo Arnesano, Garrett G. Gross, Jeffrey H. Miner, Rex Moats, Richard W. Roberts, Don B. Arnold, and Scott E. Fraser

An integrative approach is presented for studying cell biology in vivo, assessing protein dynamics and cell behavior, and offering in situ analyses of cytokinesis, daughter-cell polarity, and stereotyped tissue morphogenesis. Tagging endogenous Discs Large 1 (Dlg1) in cartilage using intrabody technology permits in situ 3D time-lapse imaging and reveals Dlg1 enrichment at the midbody during cytokinesis. Functional significance is tested by disrupting Dlg1 multimerization and its midbody localization by using an ablating intrabody, DLGE3. Building on prior work on Dlg1 in epithelia, our work reveals that Dlg1 propagates cell polarity in proliferative mesenchymal tissues and suggests that multiple mechanisms act in concert at distinct phases of the cell cycle to transmit and maintain cell polarity. (See pp. E10859–E10868.)

p53 mutants cooperate with HIF-1 in transcriptional regulation of extracellular matrix components to promote tumor progression

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Expression in cancer cells of novel proteins generated by mutations in the *TP53* gene is an important prognostic factor; however, how p53 mutants promote cancer progression is largely unknown. Here, we describe a molecular mechanism of gain-of-function by mutant p53 in hypoxic non-small cell lung cancer (NSCLC) cells. We identified the existence of a hypoxia-inducible factor-1 (HIF-1)/mutant p53 complex, exerting transcriptional control of a specific subset of protumorigenic genes, codifying for extracellular matrix (ECM) components. Employing in vivo cancer models and analyzing clinical material, we demonstrate that these ECM components substantially contribute to the synergistic protumorigenic activity of p53 mutants and HIF-1. Our data indicate that HIF-1/mutant p53 cross-talk is an innovative potential therapeutic target to treat advanced NSCLC. (See pp. E10869–E10878.)

Role of sexual imprinting in assortative mating and premating isolation in Darwin's finches

Peter R. Grant and B. Rosemary Grant

It is important to preserve the potential for future speciation because global biodiversity is being rapidly degraded. A key question is how incipient species become reproductively isolated from each other. Here we provide evidence that two species of Darwin's finches choose mates on the basis of learning morphological features of their parents and possibly from inherited preferences. The evidence for imprinting is stronger in sons than in daughters and for imprinting by both sons and daughters is stronger on fathers, which sing, than on mothers, which do not. Imprinting establishes a barrier to interbreeding between morphologically different species. The barrier is leaky, species occasionally hybridize, and the hybrids can give rise to a new species based on learned mate preferences. (See pp. E10879–E10887.)

Caspase-8 induces cleavage of gasdermin D to elicit pyroptosis during *Yersinia* infection

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Here we demonstrate that *Yersinia* YopJ-induced murine macrophage death involves caspase-8–induced cleavage of both gasdermin D (GSDMD) and gasdermin E (GSDME). The ensuing cell death is rapid, morphologically is similar to pyroptosis, and induces IL-1 release. Recently, both GSDMD and GSDME were reported to be critical effectors of caspase-1/11–driven pyroptosis and caspase-3–dependent secondary necrosis, which prompted the redefinition of pyroptosis as cell death-mediated by gasdermin activation. Our work extends these studies and shows that activation of caspase-8 in the context of TAK1 inhibition results in cleavage of both GSDMD and GSDME, leading to pyroptotic-like cell death. Further study will be needed to determine whether caspase-8 cleaves GSDMD directly or via intermediate substrates. (See pp. E10888–E10897.)

Switchable control over in vivo CAR T expansion, B cell depletion, and induction of memory

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Chimeric antigen receptor (CAR) T cell therapy represents a powerful strategy in immuno-oncology. Nevertheless, associated life-threatening toxicities and chronic B cell aplasia have underscored the need to control engineered T cells in the patient. To address these challenges, we have previously developed a switchable CAR (sCAR) T cell platform that allows dose-titratable control over CAR T cell activity by using antibody-based switches. Here, we demonstrate in a syngeneic murine model that the switchable platform can impart antitumor efficacy while dissociating long-term persistence from chronic B cell aplasia. Further, the functional reversibility of the switchable platform can be leveraged to incorporate "rest" phases through cyclical dosing of the switch to enable the induction of a robust central memory population for in vivo, on-demand expansion of sCAR T cells. (See pp. E10898–E10906.)

Cell engineering with microfluidic squeezing preserves functionality of primary immune cells in vivo

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Ex vivo manipulation of primary cells is critical to the success of this emerging generation of cell-based therapies, such as chimeric antigen receptor T cells for the treatment of cancer and CRISPR for the correction of developmental diseases. However, the limitations of existing delivery approaches may dramatically restrict the impact of genetic engineering to study and treat disease. In this paper, we

compared electroporation to a microfluidic membrane deformation technique termed "squeezing" and found that squeezed cells had dramatically fewer side effects than electroporation and gene expression profiles similar to those of unmanipulated cells. The significant differences in outcomes from the two techniques underscores the importance of understanding the impact of intracellular delivery methods on cell function for research and clinical applications. (See pp. E10907–E10914.)

IL-15 enhanced antibody-dependent cellular cytotoxicity mediated by NK cells and macrophages

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Previously we demonstrated that IL-15 by continuous infusion at 2 μg/kg/d for 10 days induced a 38-fold increase in circulating natural killer (NK) cells and a 358-fold increase in $CD56^{bright}$ NK cells. In the present study we demonstrated that IL-15 enhanced antibody-dependent cellular cytotoxicity (ADCC) of tumor-directed monoclonal antibodies in two systems. Both NK cells and macrophages were required for optimal therapeutic responses. These studies support clinical trials of IL-15 combined with tumor-directed monoclonal antibodies. In translation of this study, a phase I trial of IL-15 combined with alemtuzumab has been opened for patients with adult T cell leukemia (ATL) NCT02689453. (See pp. E10915–E10924.)

Preferred end coordinates and somatic variants as signatures of circulating tumor DNA associated with hepatocellular carcinoma

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Cell-free DNA fragmentation is a nonrandom process. We showed that cell-free DNA fragments with ends at certain genomic coordinates had higher likelihoods of being derived from hepatocellular carcinoma. Other coordinates were associated with cell-free DNA molecules originating from the liver. Quantitative assessment of cell-free DNA molecules bearing these respective groups of end signatures correlated with the amounts of tumor-derived or liver-derived DNA in plasma. There were millions of tumor-associated plasma DNA end coordinates across the genome. Due to their high prevalence, they were more readily detectable than somatic mutations as a cancer signature in plasma. Hence, detection of tumor-associated plasma DNA ends may offer a cost-effective means of capturing evidence for the presence of cancer through liquid biopsy assessment. (See pp. E10925–E10933.)

Structural basis for anthrax toxin receptor 1 recognition by Seneca Valley Virus

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Anthrax toxin receptor 1 (ANTXR1), also known as Tumor Endothelial Marker 8, is overexpressed on the surface of tumor cells in over 60% of human cancers. A serious drawback for developing specific ligands for targeted therapy against ANTXR1 is the cross-reactivity with ANTXR2. Recently, ANTXR1 was identified as the high-affinity cellular receptor for Seneca Valley Virus (SVV). SVV has shown promising results as an oncolytic agent in clinical trials, and this discovery offers a powerful biomarker for selecting patient response to treatment. The identification of specific interaction sites between SVV and ANTXR1 lays the foundation to construct potent virus mutants with specific cancer tropism that can escape host antibody response and to expand the development of both antiangiogenic and anticancer antibody therapy. (See pp. E10934–E10940.)

Cell-autonomous requirement of TDP-43, an ALS/FTD signature protein, for oligodendrocyte survival and myelination

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TDP-43 is the defining pathological hallmark protein for two overlapping adult-onset neurodegenerative diseases, amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). TDP-43 proteinopathies are also found in other major neurodegenerative diseases, such as Alzheimer's disease, further highlighting the pivotal role of TDP-43 in the nervous system. Curiously, TDP-43 aggregates are also found in oligodendrocytes, which provide myelination and metabolic support for the neurons. Here, we show that TDP-43 is indispensable, in a cell-autonomous manner, for the proper functioning of mature oligodendrocytes, in particular, myelination and cell survival. Specifically, TDP-43 depletion leads to RIPK1-mediated necroptosis of mature oligodendrocytes and down-regulation of myelin proteins that are essential for myelination but exerts no apparent toxicity on motor neurons. (See pp. E10941–E10950.)

Anterograde and retrograde signaling by an *Aplysia* neurotrophin forms a transsynaptic functional unit

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Intermediate- and long-term synaptic plasticity generally require coordinated pre- and postsynaptic mechanisms. In our companion paper, we found that presynaptic autocrine signaling by an *Aplysia* neurotrophin (ApNT) forms part of a positive feedback loop during the consolidation of learning-related synaptic plasticity. Here we report that ApNT also acts through both anterograde and retrograde signaling, so that the pre- and postsynaptic compartments act as one functional unit. (See pp. E10951–E10960.)

Gene-guided discovery and engineering of branched cyclic peptides in plants

Roland D. Kersten and Jing-Ke Weng

In the past decade, the number of publicly available plant genomes and transcriptomes has steadily increased. Inspired by this genetic resource, we developed a genome-mining approach for the rapid discovery of plant ribosomal peptides from genome-sequenced plants. Herein, we introduce the hypotensive lyciumins as a class of branched cyclic ribosomal peptides in plants and show that they are widely distributed in crop and forage plants. Our results suggest that lyciumin biosynthesis is coupled to plant-specific BURP domains in their precursor peptides and that lyciumin peptide libraries can be generated *in planta*. This discovery sets the stage for gene-guided discovery of peptide chemistry in the plant kingdom and therapeutic and agrochemical applications of lyciumins. (See pp. E10961–E10969.)

Functional and evolutionary genomic inferences in *Populus* through genome and population sequencing of American and European aspen

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We performed de novo, full-genome sequence analysis of two *Populus* species, North American quaking and Eurasian trembling aspen, that contain striking levels of genetic variation. Our results showed that positive and negative selection broadly affects patterns of genomic variation, but to varying degrees across coding and noncoding regions. The strength of selection and rates of sequence divergence were strongly related to differences in gene expression and coexpression network connectivity. These results highlight the importance of both positive and negative selection in shaping genome-wide levels of genetic variation in an obligately outcrossing, perennial plant. The resources we present establish aspens as a powerful study system enabling future studies for understanding the genomic determinants of adaptive evolution. (See pp. E10970–E10978.)

NRG1 functions downstream of EDS1 to regulate TIR-NLR-mediated plant immunity in *Nicotiana benthamiana*

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Plants employ nucleotide-binding leucine-rich repeat (NLR) immune receptors to recognize pathogen effectors and to activate effector-triggered immunity (ETI). The Toll/IL-1 receptor-NLR (TNL) protein (Roq1) recognizes the effectors XopQ and HopQ1 in an Enhanced Disease Susceptibility 1 (EDS1)-dependent way in *Nicotiana benthamiana*. Interestingly, we found that the coiled-coil NLR protein N requirement gene 1 (NRG1) is required for activation of ETI by the TNLs Roq1 and Recognition of *Peronospora parasitica* 1. NRG1 interacts with EDS1 and acts downstream of Roq1 and EDS1 to mediate XopQ/HopQ1-triggered ETI. In addition, Roq1, EDS1, and NRG1 mediate XopQ-triggered transcriptional changes in *N. benthamiana* and regulate resistance to *Xanthomonas* and *Pseudomonas* species that carry the effectors XopQ or HopQ1. This study suggests that NRG1 may be a conserved key component in TNL-mediated signaling pathways. (See pp. E10979–E10987.)

The in silico human surfaceome

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Despite the fundamental importance of the surfaceome as a signaling gateway to the cellular microenvironment, it remains difficult to determine which proteoforms reside in the plasma membrane and how they interact to enable context-dependent signaling functions. We applied a machine-learning approach utilizing domain-specific features to develop the accurate surfaceome predictor SURFY and used it to define the human in silico surfaceome of 2,886 proteins. The in silico surfaceome is a public resource which can be used to filter multiomics data to uncover cellular phenotypes and surfaceome markers. By our domain-specific feature machine-learning approach, we show indirectly that the environment (extracellular, cytoplasm, or vesicle) is reflected in the biochemical properties of protein domains reaching into that environment. (See pp. E10988–E10997.)