PNAS Plus Significance Statements

Ab initio dynamics and photoionization mass spectrometry reveal ion–molecule pathways from ionized acetylene clusters to benzene cation

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The formation of benzene and its cation constitutes a likely gateway to polycyclic aromatic hydrocarbons, which are the bridge to larger carbonaceous material such as soot in combustion processes and interstellar dust. Our paper reports computational and experimental results that address the long-standing puzzle of how ion–molecule reactions involving small unsaturated organics, such as acetylene, which is widespread in the interstellar medium, can lead to benzene cation. We present insights into the facile way in which $C_6H_6^+$ products, including benzene cation, can be accessed after ionization of cold isolated neutral clusters, and show that there is a catalytic role for what are nominally spectator acetylene molecules. (See pp. E4125–E4133.)

Body sway reflects leadership in joint music performance

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People perform tasks in coordination with others in daily life, but the mechanisms are not well understood. Using Granger causality models to examine string quartet dynamics, we demonstrated that musicians assigned as leaders affect other performers more than musicians assigned as followers. These effects were present during performance, when musicians could only hear each other, but were magnified when they could also see each other, indicating that both auditory and visual cues affect nonverbal social interactions. Furthermore, the overall degree of coupling between musicians was positively correlated with ratings of performance success. Thus, we have developed a method for measuring nonverbal interaction in complex situations and have shown that interaction dynamics are affected by social relations and perceptual cues. (See pp. E4134–E4141.)

Polyketide mimetics yield structural and mechanistic insights into product template domain function in nonreducing polyketide synthases

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Product template (PT) domains from fungal nonreducing polyketide synthases (NR-PKSs) are responsible for controlling the aldol cyclizations of poly-β-ketone intermediates during polyketide biosynthesis. Our ability to understand the high regioselective control that PT exerts is hindered by the inaccessibility of unstable poly-β-ketones for in vitro studies. We describe here the crystallographic application of "atom replacement" mimetics in which isoxazole rings linked by thioethers mimic the alternating sites of carbonyls in the poly-β-ketone intermediates. The probe contains a heptaketide mimetic tethered to a modified 4′-phosphopantetheine, which provides important empirical evidence for the PT-catalyzed cyclization mechanism. These findings afford a view of a polyketide "atom-replaced" mimetic in a NR-PKS active site that could prove general for other PKS domains. (See pp. E4142–E4148.)

Fold-change detection and scale invariance of cell–cell signaling in social amoeba

Keita Kamino, Yohei Kondo, Akihiko Nakajima, Mai Honda-Kitahara, Kunihiko Kaneko, and Satoshi Sawai

Recent works have hinted at an ability of cells to respond in the exact same manner to a fold change in the input stimulus. The property is thought to allow cells to function properly regardless of changes in the absolute concentrations of signaling molecules. Despite its general importance, however, evidence has remained scarce. The present work demonstrated that, in the social amoeba *Dictyostelium*, a response to cell–cell communication molecules is fold-change dependent and that this property is tightly linked to the condition that allows them to oscillate collectively, and thus to organize into a multicellular form. Such properties may be of importance for robustness of other developmental systems where oscillatory signaling plays a pivotal role in defining multicellular organization. (See pp. E4149–E4157.)

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String method solution of the gating pathways for a pentameric ligand-gated ion channel

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High-resolution structures of pentameric ligand-gated ion channels have created an opportunity to discover the mechanisms of rapid synaptic transduction in the brain. This study describes the mechanisms of allosteric channel gating using string method simulations, applied to a complete atomistic ion channel, combined with a transition analysis approach to extract free-energy surfaces from swarms of trajectories. We reproduce pH-modulated activity of the channel, identify the molecular interactions associated with interdomain communication, and quantify the energetics of the gating process. These results provide general mechanistic understanding of the function of pentameric ligand-gated channels, with potential applications in the design of improved anesthetics, neuromodulatory drugs, antiparasitics, and pesticides. (See pp. E4158–E4167.)

Atypical interactions of integrin $\alpha_V\beta_8$ with pro-TGF-β1

Jianchuan Wang, Xianchi Dong, Bo Zhao, Jing Li, Chafen Lu, and Timothy A. Springer

Integrins are cell-surface molecules that link extracellular ligands to the cytoskeleton. Force exerted by the cytoskeleton that is resisted by the ligand is thought to be important in activating the integrin by stabilizing an extended-open conformational state with high affinity for ligand. However, integrin $\alpha_V\beta_8$ does not interact with the cytoskeleton in the same way. Here, we show that, although the closely related integrins $\alpha_V\beta_8$ and $\alpha_V\beta_6$ bind the same ligand, pro-TGF-β1, their conformational responses to ligand binding and regulation by metal ions are quite different. These differences correlate with their distinct linkage to the cytoskeleton. (See pp. E4168–E4174.)

Dynamic microtubules regulate cellular contractility during T-cell activation

King Lam Hui and Arpita Upadhyaya

T-cell activation is an essential event in the adaptive immune response to fight against infections. T cells were recently shown to be mechanosensitive, and to exert forces actively on the opposing antigen-presenting cell during activation. However, the molecular basis of force generation by T cells is poorly understood. Here, we report a molecular mechanism that involves the T-cell microtubule (MT) and actomyosin cytoskeletons, which interact through Rho GTPase activity. Our study highlights the role played by the MT cytoskeleton in regulating actomyosin-generated forces, which play a key role in T-cell activation. (See pp. E4175–E4183.)

Ectopic protein interactions within BRD4–chromatin complexes drive oncogenic megadomain formation in NUT midline carcinoma

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Chromatin factors generally act within large, multisubunit complexes; thus, identifying both their normal and aberrant interactors in cancer should provide important information regarding potential targets for therapeutic intervention. Here, we apply this principle to analysis of BRD4–NUT, a fusion oncoprotein that drives an aggressive subtype of squamous cell cancer. We identify ZNF532 as a prominent BRD4–NUT–interacting protein in an established NUT midline carcinoma patient cell line, and independently discover ZNF532 fused directly to NUT in a newly analyzed patient. Like BRD4–NUT, ZNF532–NUT forms unusually large (100-kb to 1-Mb) domains of hyperactive chromatin, including at the *MYC* locus, and drives self-reinforcing regulatory loops that are likely to be a powerful strategy for the growth advantage of cancer cells. (See pp. E4184–E4192.)

Hsp104 disaggregase at normal levels cures many [*PSI*$^+$] prion variants in a process promoted by Sti1p, Hsp90, and Sis1p

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Prions (infectious proteins) pose a substantial risk to yeast, as they do for humans. Overproduction of the disaggregase, Hsp104, has long been known capable of curing [*PSI*$^+$], a prion based on amyloid formation by the Sup35 protein. We find that most [*PSI*$^+$] variants arising spontaneously in the absence of this Hsp104 overproduction curing activity are cured when that activity is restored at normal levels. This activity is thus an antiprion system, largely protecting the cells from prion formation by this protein. (See pp. E4193–E4202.)

Selective targeting of point-mutated KRAS through artificial microRNAs

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Recently, small interfering RNAs have been used to specifically target point-mutated KRAS, yet without sufficiently discriminating its wild-type counterpart. Here, we describe an innovative approach based on the development of artificial microRNAs able to efficiently target mutated KRAS, leaving their normal counterpart unaffected and preventing major side effects. (See pp. E4203–E4212.)

Aggregation of thrombin-derived C-terminal fragments as a previously undisclosed host defense mechanism

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The work summarized in this paper is based on the simple but unexpected observation that addition of lipopolysaccharide (LPS) or bacteria to human wound fluids leads to precipitation of protein aggregates, a phenomenon not observed in plasma. Using a broad mix of technologies ranging from biophysical, biochemical, and microbiological methods to fluorescence and electron microscopy, and from in silico modeling to studies on wound materials, we demonstrate here a previously undisclosed role of C-terminal thrombin fragments of about 11 kDa, involving LPS- and bacteria-induced aggregation and scavenging, facilitating clearance and microbial killing. Our findings provide a link between the major coagulation factor thrombin, innate immunity, and amyloid formation. (See pp. E4213–E4222.)

Structural basis for cancer immunotherapy by the first-in-class checkpoint inhibitor ipilimumab

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Biologics represent a major class of therapeutics for the treatment of malignancies, autoimmune diseases, and infectious diseases. Ipilimumab is the first-in-class immunotherapeutic for blockade of

CTLA-4 and significantly benefits overall survival of patients with metastatic melanoma. The X-ray crystal structure of the ipilimumab:CTLA-4 complex defines the atomic interactions responsible for affinity and selectivity and demonstrates that the therapeutic action of ipilimumab is due to direct steric competition with the B7 ligands for binding to CTLA-4. (See pp. E4223–E4232.)

Erythritol is a pentose-phosphate pathway metabolite and associated with adiposity gain in young adults

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The prevention of weight gain in adulthood is a public health challenge, particularly given the difficulty of losing weight. Data on freshmen were collected at the beginning and end of the academic year, and baseline blood samples were studied to find markers of incident weight gain. A metabolite, erythritol, was elevated at the beginning of the year in freshmen who went on to gain weight, fat, and abdominal fat compared with freshmen with stable weight. Erythritol is a sugar substitute low-calorie sweetener, and prior studies claimed no endogenous synthesis. We report a previously unrecognized metabolism of glucose to erythritol, and given the association between erythritol and weight gain, research is needed to understand whether and how this pathway contributes to weight gain risk. (See pp. E4233–E4240.)

Hypoxia treatment reverses neurodegenerative disease in a mouse model of Leigh syndrome

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Inherited or acquired defects in mitochondria lead to devastating disorders for which we have no effective general therapies. We recently reported that breathing normobaric 11% O_2 prevents neurodegeneration in a mouse model of a pediatric mitochondrial disease, Leigh syndrome. Here we provide updated survival curves of mice treated with varying doses of oxygen and explore eventual causes of death. We explore alternative hypoxia regimens and report that neither intermittent nor moderate hypoxia regimens suffice to prevent neurological disease. Finally, we show that hypoxia can not only prevent, but also reverses the brain lesions in mice with advanced neuropathology. Our preclinical studies will help guide future clinical studies aimed at harnessing hypoxia as a safe and practical therapy. (See pp. E4241–E4250.)

Coronavirus nonstructural protein 15 mediates evasion of dsRNA sensors and limits apoptosis in macrophages

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Macrophages are immune cells equipped with multiple double-stranded RNA (dsRNA) sensors designed to detect viral infection and amplify innate antiviral immunity. However, many coronaviruses can infect and propagate in macrophages without activating dsRNA sensors. Here we present a function of murine coronavirus nonstructural protein 15 in preventing detection of viral dsRNA by host sensors. We show that coronaviruses expressing a mutant form of nonstructural protein 15 allow for activation of dsRNA sensors, resulting in an early induction of interferon, rapid apoptosis of macrophages, and a protective immune response in mice. Identifying the strategies used by viruses to evade detection provides us with new approaches for generating vaccines that elicit robust innate immune responses and protective immunity. (See pp. E4251–E4260.)

mTORC1 promotes proliferation of immature Schwann cells and myelin growth of differentiated Schwann cells

Bogdan Beirowski, Keit Men Wong, Elisabetta Babetto, and Jeffrey Milbrandt

The myelination of axons is essential for neuronal wiring and normal nervous system functions. In the peripheral nervous system, Schwann cells (SCs) form myelin sheaths around axons during nerve development. Such myelination is compromised in a number of diseases. Hence, identification and understanding of the key pathways regulating SC development and myelinogenesis are essential for therapeutic progress. Here we uncover two separate roles of the cellular signaling node mTORC1 (mechanistic target of rapamycin complex 1) for regulating the development of SCs and subsequently the growth of myelin sheaths. Moreover, we demonstrate that defective SCs possess a remarkable plasticity to remyelinate axons via mTORC1. Thus, manipulating mTORC1 activity in diseased SCs could be therapeutically beneficial. (See pp. E4261–E4270.)

Maturation arrest in early postnatal sensory receptors by deletion of the miR-183/96/182 cluster in mouse

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MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression posttranscriptionally. The evolutionarily conserved miR-183/96/182 cluster, consisting of three related miRNAs, is highly expressed in maturing sensory receptor cells. However, its role in the functional maturation of sensory receptors has not been adequately addressed due to the lack of appropriate in vivo models. We show that deletion of miR-183/96/182 in mice leads to severe deficits in vision, hearing, balance, and smell. These deficits arise from defects in the timing and completion of terminal differentiation in sensory receptor cells associated with dysregulation of networks of genes involved in key processes, such as chromatin remolding and ciliogenesis. Thus, the miR-183/96/182 cluster has an essential role for the maturation of sensory receptors. (See pp. E4271–E4280.)

Common general anesthetic propofol impairs kinesin processivity

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Kinesins are major transporters of cargos toward the cell periphery. They are highly expressed in the CNS, and their dysfunction leads to a wide range of human pathologies, including neurodevelopmental and neurodegenerative diseases, ciliopathies, epilepsy, and birth defects. We have discovered that the widely used general anesthetic propofol shortens the distance that kinesins travel, but their velocity remains unchanged. These results suggest that propofol is not binding at the ATP site or allosteric sites that affect ATP turnover, leading to the conclusion that the allosteric sites form on microtubule association. We postulate that general anesthetics bind specifically to transport kinesins and/or the kinesin–β-tubulin interface, and diminish their ability to transport critical cargos, thereby contributing to the pleiotropic state of anesthesia. (See pp. E4281–E4287.)

Exclusion of alternative exon 33 of $Ca_V1.2$ calcium channels in heart is proarrhythmogenic

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To directly address in vivo significance of the altered $Ca_V1.2$ channel property arising from alternative splicing, we generated $Ca_V1.2$ exon 33-specific knockout (exon $33^{-/-}$) mice. Here, we showed that the exclusion of alternative exon 33 altered $Ca_V1.2$ biophysical property, leading to greater I_{Ca} density. This increase in current density induced prolongation of ventricular cardiomyocyte action potential duration, and the cardiomyocytes exhibited increased early afterdepolarizations and autonomous action potentials—hallmarks of arrhythmias. In vivo, exon $33^{-/-}$ mice had increased occurrences of premature ventricular contractions, tachycardia, and lengthened QT interval. As such, exclusion of exon 33 of the $Ca_V1.2$ channel is proarrhythmogenic. Although failing human hearts had greater inclusion of exon 33, it is unclear whether the inclusion is compensatory, neutral, or damaging. (See pp. E4288–E4295.)

Chromosome-level genome assembly and transcriptome of the green alga *Chromochloris zofingiensis* illuminates astaxanthin production

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The growing human population generates increasing demand for food and energy. Microalgae are a promising source of sustainable bioproducts whose production may not exacerbate worsening environmental problems. The green alga *Chromochloris zofingiensis* has potential as a biofuel feedstock and source of high-value nutraceutical molecules, including the carotenoid astaxanthin. We present a high-quality, chromosome-level assembly of the genome by using a hybrid sequencing approach with independent validation by optical mapping. Our analyses of the genome and transcriptome, in addition to experiments characterizing astaxanthin production, advance understanding of the green lineage and carotenoid production, and enhance prospects for improving commercial production of *C. zofingiensis*. (See pp. E4296–E4305.)

The wisdom of crowds for visual search

Mordechai Z. Juni and Miguel P. Eckstein

Simple majority voting is a widespread, effective mechanism to exploit the wisdom of crowds. We explored scenarios where, from decision to decision, a varying minority of group members often has increased information relative to the majority of the group. We show how this happens for visual search with large image data and how the resulting pooling benefits are greater than previously thought based on simpler perceptual tasks. Furthermore, we show how simple majority voting obtains inferior benefits for such scenarios relative to averaging people's confidences. These findings could apply to life-critical medical and geospatial imaging decisions that require searching large data volumes and, more generally, to any decision-making task for which the minority of group members with high expertise varies across decisions. (See pp. E4306–E4315.)