PNAS Plus Significance Statements

Inhibited proton transfer enhances Au-catalyzed CO_2-to-fuels selectivity

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Renewable electricity can be stored in the energy-dense bonds of carbon-based fuels via the electroreduction of CO_2. CO_2 reduction in aqueous electrolytes suffers efficiency losses because of the simultaneous reduction of water to H_2. Rational design of selective CO_2-to-fuels catalysts requires direct knowledge of the electrode surface structure during turnover and how electrons and protons couple to direct product selectivity. Using model Au catalysts, we uncover the complex heterogeneity in CO surface binding equilibria and the differential proton coupling requirements for CO vs. H_2 production. We assemble the spectroscopic and kinetic data to construct a mechanistic model that predicts that impeding proton transfer to the surface is an effective strategy for improving CO_2-to-fuels catalyst selectivity. (See pp. E4585–E4593.)

Stochastic ice stream dynamics

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Ice streams form the backbone of the flow field of ice sheets. They are known to exhibit a complex spatio-temporal dynamics, which is largely not well understood. Understanding the controls on such dynamics is crucial to sea level change projections as well as to the interpretation of paleorecords. Our contribution unravels the impact of climate variability on the temporal dynamics of ice streams. In particular, we show that minimal, and at the same time realistic, random fluctuations due to climate variability are capable of producing unprecedented behaviors in ice stream dynamics. These results may open a new perspective on the past and future behavior of ice sheets. (See pp. E4594–E4600.)

Pamidronate functionalized nanoconjugates for targeted therapy of focal skeletal malignant osteolysis

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Malignant osteolysis associated with inoperable primary bone tumors and multifocal skeletal metastases remains a challenging clinical problem in cancer patients. The outgrowth of residual cancer cells within the bone microenvironment despite aggressive multimodality therapies necessitates the discovery and validation of novel strategies for treating osteotropic solid tumors. We report on pamidronate functionalized polylactide nanoparticles for the targeted treatment of focal malignant osteolysis by delivering doxorubicin specifically to the bone tumor microenvironment. Improved efficacy was demonstrated in a preclinical orthotopic mouse model of osteosarcoma. Most importantly, through the inclusion of dogs with naturally developing osteosarcoma, biocompatibility, biodistribution, and anticancer activities at clinically relevant dosages were demonstrated in a large mammalian modeling system. (See pp. E4601–E4609.)

Adaptation of the symbiotic *Mesorhizobium*–chickpea relationship to phosphate deficiency relies on reprogramming of whole-plant metabolism

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Inorganic phosphate (Pi) is an essential constituent for nodule performance. Consequently, symbiotic efficiency in dinitrogen (N_2)-fixing legumes, where nodules act as a sink for Pi, is affected by Pi availability. Low Pi availability results in major inhibition of symbiotic performance; thus, understanding the adaptive responses of nodule metabolism to Pi deficiency is crucial to improve symbiotic efficiency under Pi-limited conditions. This study reports the key mechanisms responsible for decreasing nodule activity under Pi deficiency and examines whether nodule responses to low Pi availability are mediated by changes in the metabolism of other organs of nodulated plants. These findings can be used to develop chickpea cultivars and perhaps, other leguminous crops with effective symbiotic efficiency under Pi-limited conditions through genetic engineering. (See pp. E4610–E4619.)

Cell size and fat content of dietary-restricted *Caenorhabditis elegans* are regulated by ATX-2, an mTOR repressor

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Dietary restriction is a metabolic intervention that extends the lifespan and reduces animal size and fat content. We have used *Caenorhabditis elegans* to demonstrate that the homolog of human *ATXN2*, *atx-2*, is a major regulator of the animal response to dietary restriction. Down-regulation of *atx-2* in

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dietary-restricted animals leads to increased animal size and fat levels, as well as accelerated development. Surprisingly, it does not affect the extended lifespan of dietary-restricted animals. These findings are relevant to mammals because Ataxin-2 knockout mice exhibit adult-onset obesity, owing to an unknown mechanism. *atx-2* negatively regulates the mechanistic target of rapamycin pathway via its interaction with a GDP dissociation inhibitor β. Forced activation of this pathway may have therapeutic potential for obesity. (See pp. E4620–E4629.)

Structures of mammalian ER α-glucosidase II capture the binding modes of broad-spectrum iminosugar antivirals

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Most pathogenic enveloped viruses crucially depend on the quality control (QC) machinery in the endoplasmic reticulum (ER) of the host cell. ERQC inhibitors therefore have the double potential benefit of targeting a wide variety of viruses ("broad-spectrum antivirals") without the risk of losing efficacy due to escape mutations in the viral genome. Our recent work has proven that inhibition of the central enzyme of ERQC, α-glucosidase II (α-GluII), is sufficient for antiviral activity against dengue fever in vitro and in vivo. Here, we show how antiviral inhibitors bind to portions of α-GluII that are unique to this enzyme, and we open the way to the development of potent and selective antivirals against existing and emerging infectious disease. (See pp. E4630–E4638.)

Numerous proteins with unique characteristics are degraded by the 26S proteasome following monoubiquitination

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A substrate-conjugated polyubiquitin chain is accepted as the "canonical" proteasomal degradation signal. Using a cellular (human and yeast) proteomic screen in the exclusive presence of nonpolymerizable ubiquitin, we show that a large group of proteins is degraded by the proteasome following monoubiquitination. The screen also unraveled polyubiquitin-dependent substrates, as they are stabilized in the presence of this ubiquitin mutant. Notably, monoubiquitination- and polyubiquitination-dependent substrates display distinct important characteristics. Monoubiquitinated proteins are of lower molecular mass and of lesser structural disorder. The two groups can be assigned to defined cellular pathways. Furthermore, some of the characteristics are confined to either human or yeast cells, suggesting that the mechanism of action/recognition of the ubiquitin system in the two organisms are different somehow. (See pp. E4639–E4647.)

Hydrogen isotopes in individual amino acids reflect differentiated pools of hydrogen from food and water in *Escherichia coli*

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Hydrogen isotope (δ^2H) values of bulk tissues have become valuable tracers for studying migration and movement patterns in animals, but the biochemical mechanism for how hydrogen is incorporated into heterotrophic organisms is not well understood. We grew *Escherichia coli* as a model organism on two different substrates, and then measured δ^2H values of individual amino acids (AAs) in cellular material. The δ^2H values of AAs were highly variable within a simple microbial culture. Using isotopic fractionation models, we show that AA δ^2H provide tracers of an organism's environmental (e.g., drinking) water, as well as its food, information of prime interest to ecologists. The work is also of significance to microbial physiologists studying metabolic pathways in microbes from extreme environments. (See pp. E4648–E4653.)

Impact of a homing intein on recombination frequency and organismal fitness

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Parasitic interactions can result in changes to the host's behavior in a way that promotes the distribution or life cycle of the parasite. Inteins are molecular parasites found in all three domains of life. Here we look at the influence of an intein in the DNA polymerase on a population of halophilic archaea in simulations, in experiments, and in the wild. This intein has a fitness cost that is higher than expected for a self-splicing genetic element. In these populations, where mating is independent of host replication, the intein increases the recombination rate between cells with and without inteins. This modification may contribute to the long-term persistence of these genetic parasites, despite the fitness burden they impart on their host. (See pp. E4654–E4661.)

Peripheral tolerance can be modified by altering KLF2-regulated Treg migration

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Tregs are necessary to prevent autoimmunity; however, these same cells suppress tumor-specific immune responses and contribute to malignancy. Before Treg-based therapies are devised to treat these diseases, it is important to understand how Tregs function under physiological conditions. We now report that Tregs carry out their immune-suppressive functions in secondary lymphoid organs (e.g. spleen, lymph nodes) and factors that impair or enhance Treg homing to these sites diminish or increase self-tolerance, respectively. Importantly, Treg migration patterns are regulated by Kruppel-like factor 2 (KLF2), and increasing expression of this transcription factor within the Treg compartment promotes self-tolerance. The present study demonstrates that Treg trafficking to lymphoid tissues underpins peripheral tolerance, which can be modified by targeting KLF2 with therapeutic drugs. (See pp. E4662–E4670.)

AIM2 inflammasome is activated by pharmacological disruption of nuclear envelope integrity

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Inflammasomes are cellular sensors of harmful situations such as the presence of microbes or alterations in cellular homeostasis. Upon activation, inflammasomes trigger the proteolytic maturation and release of inflammatory cytokines to initiate immune and repair responses. The assembly of inflammasome relies on a diverse repertoire of sensor proteins that can detect specific stimuli. For example, the AIM2 inflammasome is activated by the

presence of microbial DNA within the cytosol. In this paper, alterations of the nuclear envelope integrity are found to cause the exposure of nuclear DNA in the cytosol to promote the activation of the AIM2 inflammasome. Nuclear envelope stress can therefore directly engage innate immune sensors to elicit inflammation. (See pp. E4671–E4680.)

Hhip haploinsufficiency sensitizes mice to age-related emphysema

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Genome-wide association studies (GWAS) have been very successful in discovering genetic loci associated with complex traits. However, only few studies applied murine models to investigate how GWAS genes contribute to human lung diseases. Motivated by GWAS linking Hedgehog interacting protein (*HHIP*) to emphysema and impairments in lung function, this study demonstrated that *Hhip*$^{+/-}$ mice developed spontaneous emphysema and lung function impairment over time. Moreover, emphysema, associated with increased oxidative stress in *Hhip*$^{+/-}$ lungs, was prevented by treating the mice with the antioxidant, *N*-acetyl cysteine (NAC). This post-GWAS functional study connects aging-related diseases, molecular mechanisms, and potential therapy in a genetic haploinsufficient murine model, which may lead to improvements in understanding pathophysiologic concepts of alveolar loss related to aging. (See pp. E4681–E4687.)

Deubiquitinase Usp8 regulates α-synuclein clearance and modifies its toxicity in Lewy body disease

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A cardinal feature of Parkinson's disease pathology is the aggregation of α-synuclein in ubiquitin-positive inclusions termed Lewy bodies, yet the composition of ubiquitin conjugates in these inclusions and their role in α-synuclein pathobiology remain unclear. Here we demonstrate that α-synuclein inclusions contain K63-linked ubiquitin chains, which are strikingly reduced in dopaminergic neurons. In these neurons, the deubiquitinase Usp8 is present in Lewy bodies, and its content is related inversely to the extent of K63-linked ubiquitination. Our mechanistic studies in vitro and in flies indicate that Usp8 interacts with and deconjugates K63-linked ubiquitin chains on α-synuclein, prolonging its half-life and increasing its toxicity. Thus, Usp8 appears to be a critical factor determining α-synuclein levels that could be targeted for therapies. (See pp. E4688–E4697.)

N-terminal domain of complexin independently activates calcium-triggered fusion

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Synaptic neurotransmitter release is an essential process for communication between neurons. Neurotransmitter release occurs upon an action potential, resulting in a local Ca^{2+}-concentration increase in the presynaptic terminal that triggers synaptic vesicle membrane fusion with the plasma membrane. Synaptic vesicle fusion is orchestrated by neuronal soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), synaptotagmin, complexin, and other factors. Here, we found that the complexin N-terminal domain binds to membranes and that it can be substituted with the fusion peptide of influenza virus hemagglutinin, resulting in similar activation of Ca^{2+}-triggered fusion as wild-type complexin in a reconstituted vesicle fusion system. We conclude that similar fusion elements and principles are used in different contexts of biological membrane fusion. (See pp. E4698–E4707.)

The neural chaperone proSAAS blocks α-synuclein fibrillation and neurotoxicity

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Aberrant protein homeostasis (proteostasis) is increasingly recognized as a major causal factor in the development of protein aggregates, including aggregation of α-synuclein in Parkinson's disease. Cellular proteins known as chaperones control proteostatic processes and are strongly associated with many different neurodegenerative diseases. We show here that an abundant brain- and endocrine-specific secretory chaperone known as proSAAS (named after four residues in the amino terminal region) exhibits potent antiaggregant effects on α-synuclein aggregation, and we identify a region of the protein that is necessary for this antiaggregant effect. We further show that proSAAS expression is able to stop α-synuclein from killing dopaminergic cells in a nigral cell model of Parkinson's disease. This work demonstrates an important role for the proSAAS protein in blocking aggregation in neurodegeneration. (See pp. E4708–E4715.)

Hair cells use active zones with different voltage dependence of Ca^{2+} influx to decompose sounds into complementary neural codes

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We hear sounds varying in intensity over six orders of magnitude using spiral ganglion neurons (SGNs), each of which changes its firing rates over only a fraction of this range. Somehow, the SGNs with different dynamic ranges collectively encode the full range of sound levels represented in the receptor potential of the inner hair cell (IHC) in the mammalian cochlea. Our study, combining subcellular imaging, mouse genetics, and auditory systems physiology, offers a unifying synaptic hypothesis for wide dynamic range sound encoding in the spiral ganglion. We propose that IHCs, from one receptor potential but via presynaptic active zones that vary in the voltage dependence of Ca^{2+} influx, generate complementary codes on sound pressure level in functionally distinct SGNs. (See pp. E4716–E4725.)

Depression-like behavior in rat: Involvement of galanin receptor subtype 1 in the ventral periaqueductal gray

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The pathophysiology of depression remains unclear, but accumulated evidence implicates disturbances in monoaminergic transmission in the brain. Several studies suggest that members of the diverse family of neuropeptides may also be involved. In the rat, the neuropeptide galanin is coexpressed with noradrenaline and serotonin, and modulates the signaling of these neurotransmitters. Here, we explored a possible role of galanin and its receptors in a rat model of depression based on chronic mild stress using quantitative

real-time PCR combined with viral-mediated delivery of galanin receptor 1 (Galr1) siRNA. Our results indicate involvement of the GALR1 receptor subtype in the ventral periaqueductal gray in depression-like behavior, possibly representing a novel target for antidepressant therapy. (See pp. E4726–E4735.)

PSD-95 stabilizes NMDA receptors by inducing the degradation of $STEP_{61}$

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NMDA receptors (NMDARs) are principal regulators of synaptic signaling in the brain. Modulation of NMDARs' function and trafficking is important for the regulation of synaptic transmission and several forms of synaptic plasticity. Postsynaptic density protein 95 (PSD-95) acts as a scaffolding protein and stabilizes the surface and synaptic expression of NMDARs, whereas striatal-enriched protein tyrosine phosphatase (STEP), a brain-specific protein tyrosine phosphatase, dephosphorylates and destabilizes NMDARs via endocytosis. We now demonstrate that PSD-95 binds to $STEP_{61}$ and promotes its degradation via the proteasome, thereby stabilizing surface expression of NMDARs. We have revealed a dynamic role for PSD-95 in sculpting protein content at excitatory synapses that is distinct from its canonical role as a scaffolding protein. (See pp. E4736–E4744.)

Demographic noise can reverse the direction of deterministic selection

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Demographic stochasticity—the population-level randomness that emerges when the timing of birth, death, and interaction events is unpredictable—can profoundly alter the dynamics of a system. We find that phenotypes that pay a cost to their birth rate to modify the environment by increasing the global carrying capacity can be stochastically selected for, where they would otherwise be deterministically disfavored. Our results hold for a general class of mathematical models but we use a model of public good production for illustration. In this case, demographic stochasticity is exploited by populations of cooperators to turn selection in their favor; it therefore operates as a mechanism that supports the evolution of public good production. (See pp. E4745–E4754.)