PNAS Plus Significance Statements

On the role of water density fluctuations in the inhibition of a proton channel

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Hv1, a voltage-gated proton channel, is an emerging pharmacological target implicated in many pathological conditions, including cancer and ischemic brain damage. We used the recently published experimental structure of Hv1 to generate structural models of relevant conformational states. Thermodynamic analyses of pore waters shed light on the molecular underpinnings of Hv1 druggability. We exploit this information to suggest possible optimizations of known inhibitors and identify a potential binding site located at the exit of the proton path. The resulting molecular picture paves the way for the discovery of novel Hv1 inhibitors and outlines a general approach for identifying druggable binding sites in ion channels. (See pp. E8359–E8368.)

Kinetic isotope effects reveal early transition state of protein lysine methyltransferase SET8

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We developed an MS-based method to determine kinetic isotope effects and binding isotope effects on protein lysine methyltransferase SET8-catalyzed monomethylation. These parameters, coupled with steady-state kinetics and molecular modeling, outlined the reaction path of SET8-catalyzed methylation. Upon the formation of the *S*-adenosyl-L-methionine–SET8–histone 4 lysine 20 intermediate complex followed by lysine deprotonation, the reaction goes through an early, asymmetrical transition state (TS) with the small engagement of the C-N bond and the partial dissociation of the C-S bond. This TS structure is distinct from the known TS structures of other protein lysine methyltransferases (PKMTs) and thus presents the feasibility to design selective TS analog inhibitors against PKMTs. The developed techniques can also be generally applicable to examining other protein methylation and posttranslational modifications. (See pp. E8369–E8378.)

Molecular profiling of single circulating tumor cells from lung cancer patients

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There exists an urgent need for minimally invasive molecular analysis tools for cancer assessment and management, particularly in advanced-stage lung cancer, when tissue procurement is challenging and gene mutation profiling is crucial to identify molecularly targeted agents for treatment. High-throughput compartmentalization and multigene profiling of individual circulating tumor cells (CTCs) from whole-blood samples using modular gene panels may facilitate highly sensitive, yet minimally invasive characterization of lung cancer for therapy prediction and monitoring. We envision this nanoplatform as a compelling research tool to investigate the dynamics of cancer disease processes, as well as a viable clinical platform for minimally invasive yet comprehensive cancer assessment. (See pp. E8379–E8386.)

Self-organization of actin networks by a monomeric myosin

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Actomyosin networks are central to a broad range of cellular motile processes, including cell polarization and collective cell migration during morphogenesis and development. Myosin-IXa is critically involved in these processes. Using fluorescence spectroscopy, total internal reflection fluorescence, and electron microscopy, we demonstrate that myosin-IXa assembles actin filaments into highly ordered lattices. The actin filaments of parallel polarity are connected by myosin-IXa in distinct conformations and at a repeat distance of 36 nm across the network. The myosin-IXa–induced actin lattices introduce orientated actin tracks and a network of regularly spaced platforms for localized Rho-GTPase–activating protein activity in cell polarization and collective cell migration. (See pp. E8387–E8395.)

Assembly of long error-prone reads using de Bruijn graphs

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When the long reads generated using single-molecule sequencing (SMS) technology were made available, most researchers were skeptical about the ability of existing algorithms to generate high-quality assemblies from long errorprone reads. Nevertheless, recent algorithmic breakthroughs resulted in many successful SMS sequencing projects. However, as the recent assemblies of important plant pathogens illustrate, the problem of assembling long error-prone reads is far from being resolved even in the case of

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relatively short bacterial genomes. We propose an algorithmic approach for assembling long error-prone reads and describe the ABruijn assembler, which results in accurate genome reconstructions. (See pp. E8396–E8405.)

No growth stimulation of Canada's boreal forest under half-century of combined warming and CO_2 fertilization

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Limited knowledge about the mechanistic drivers of forest growth and responses to environmental changes creates uncertainties about the future role of circumpolar boreal forests in the global carbon cycle. Here, we use newly acquired tree-ring data from Canada's National Forest Inventory to determine the growth response of the boreal forest to environmental changes. We find no consistent boreal-wide growth response over the past 60 y across Canada. However, some southwestern and southeastern forests experienced a growth enhancement, and some regions such as the northwestern and maritime areas experienced a growth depression. Growth–climate relationships bring evidence of an intensification of the impacts of hydroclimatic variability on growth late in the 20th century, in parallel with the rapid rise of summer temperature. (See pp. E8406–E8414.)

Neutrophils induce proangiogenic T cells with a regulatory phenotype in pregnancy

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Neutrophils are typically known as short-lived cells that act as the first line of defense in response to pathogens. However, emerging data indicate that neutrophils have wider implications in the immune system and have a direct influence on the ensuing immune response. Establishment of successful pregnancy requires immune tolerance at the maternal–fetal interface. Aberrations in normal placental development can lead to complications, including preeclampsia. In this study, we examined a role for maternal neutrophils in maintaining normal pregnancy through their interactions with T cells, resulting in a population of T cells that are both regulatory and proangiogenic and are required for normal placental development. Such interactions are absent in patients with preeclampsia, suggesting a potential therapeutic target for pregnancy-related pathologies. (See pp. E8415–E8424.)

Brief treatment with a highly selective immunoproteasome inhibitor promotes long-term cardiac allograft acceptance in mice

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The potential of proteasome inhibitors to prevent transplant rejection and to treat other immune disorders is hindered by mechanism-based toxicity from inhibition of constitutive proteasomes. Here, we demonstrate that briefly, reversibly, and selectively inhibiting the immunoproteasome prolonged the survival of transplanted hearts in mice and allowed long-term survival when combined with single-dose CTLA4-Ig. Immunoproteasome inhibition noncytotoxically reduced T-cell proliferation and the numbers of effector T cells in the allograft and draining nodes while increasing T-cell expression of exhaustion markers. The immunoproteasome thus appears to play a role in suppressing induction of T-cell exhaustion. Selective inhibition of the immunoproteasome may be a potential treatment option for the management of transplant rejection. (See pp. E8425–E8432.)

Mutant p53 promotes tumor progression and metastasis by the endoplasmic reticulum UDPase ENTPD5

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p53 mutations are the most frequent genetic alteration in cancer and are often indicative of poor patient survival prognosis. The most prevalent missense mutations lead to a "gain of function" (GOF) that actively drives tumor progression, metastasis, and therapy resistance. Our study links the mutant p53 (mutp53) GOF to enhanced N-glycoprotein folding via ectonucleoside triphosphate diphosphohydrolase 5 (ENTPD5) in the calnexin/calreticulin cycle of the endoplasmic reticulum. Mutp53 thus increases expression of prometastatic cell surface proteins, such as receptors and integrins, not only quantitatively but also qualitatively, with respect to N-glycosylation state. Our study reveals N-glycoprotein quality control in the endoplasmic reticulum as an indispensable mechanism underlying the progression of tumors with GOF mutp53 that could provide new possibilities for treating prognostically challenging p53-mutated cancers. (See pp. E8433–E8442.)

Hierarchical CRMP2 posttranslational modifications control NaV1.7 function

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The voltage-gated sodium channel NaV1.7 is important for electrogenesis in sensory neurons. Insertion within the membrane is required for function of NaV1.7. However, the mechanisms determining how NaV1.7 is trafficked to neuronal cell membranes are poorly understood. Here, we elucidate a signaling program involving a complex and intriguing posttranslational modification regime of collapsin response mediator protein 2 (CRMP2), an NaV1.7-binding protein. NaV1.7 surface localization and currents are controlled by CRMP2 modifications. Activity of NaV1.7 is thought to modulate neuronal excitability that codes for several sensory modalities, including chronic pain, as inferred from human pain disorders caused by mutations in NaV1.7 channels. Understanding the role of cross-talk between CRMP2 modifications in modulation of NaV1.7 activity opens routes to exploit this system for pain. (See pp. E8443–E8452.)

Nogo receptor blockade overcomes remyelination failure after white matter stroke and stimulates functional recovery in aged mice

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White matter stroke is a common clinical problem that leads to widespread cognitive and sensorimotor deficits. The incidence of white matter stroke is sharply age-associated; imaging studies indicate that, over age 80, virtually all of us will have white matter strokes. Little is known of the repair processes after white matter stroke. Here, we report the tissue repair processes after white

matter stroke and identify Nogo receptor 1 (NgR1) ligands as inhibitors. We show that white matter repair and functional recovery are indeed possible in aged brains when an engineered NgR1 decoy receptor is systemically administered after white matter stroke, even at chronic periods in this disease. (See pp. E8453–E8462.)

Resting-state hemodynamics are spatiotemporally coupled to synchronized and symmetric neural activity in excitatory neurons

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Resting-state functional connectivity mapping exploits correlations in the functional magnetic resonance imaging (fMRI) blood oxygen level-dependent (BOLD) signal across the brain. However, results are difficult to interpret without an understanding of the neural correlates of these hemodynamic fluctuations. This work uses mice in which neural activity and brain hemodynamics can be mapped simultaneously. We show that resting-state hemodynamics can be predicted from spontaneous neural activity and correspond to a series of driven increases in local blood volume, coupled with spontaneous, bilaterally symmetric fluctuations in excitatory neural activity. This result provides reassurance that resting-state functional connectivity has neural origins. The network-like spontaneous neural activity visualized here represents an underexplored feature of neural activity in the awake brain. (See pp. E8463–E8471.)

Alterations in the neuropeptide galanin system in major depressive disorder involve levels of transcripts, methylation, and peptide

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Depression is a seriously disabling disorder, twice as common in women as in men. Lack of efficacy of existing pharmacotherapies in subsets of patients has led to an intensive search for new targets for antidepressant development, including receptors for neuropeptides such as galanin (GAL). In this study, we explore GAL and its three receptors, $GAL_{1–3}$, comparing postmortem brain regions from depressed suicide patients and controls. Using quantitative PCR and bisulfite pyrosequencing, we report significant changes in the transcript and DNA methylation levels of *GAL* and galanin receptor 3 (*GALR3*) in the locus coeruleus and dorsal raphe nucleus, two regions important for mood regulation. Our findings suggest GAL_3 involvement in depressive disorder, making it a possible drug target for this disease. (See pp. E8472–E8481.)

Palmitoylation regulates glutamate receptor distributions in postsynaptic densities through control of PSD95 conformation and orientation

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Synaptic AMPA-type and NMDA-type glutamate receptors (AMPARs and NMDARs) have different dynamic characteristics critical for synaptic plasticity. We find that the posttranslational modification, palmitoylation, changes the conformation of postsynaptic density protein 95 (PSD95), a major synaptic scaffold, promoting interactions with AMPARs and NMDARs. In synapses, we measured the conformation and orientation of palmitoylated PSD95 relative to the scaffold, synapse-associated protein 97 (SAP97), and found that changing PSD95 palmitoylation altered PSD95 and AMPAR levels, but not NMDAR levels. We conclude that palmitoylation regulates PSD95 conformation and retention of AMPAR and NMDARs at synapses. Differences in PSD95 palmitoylation appear to occur when AMPARs and NMDARs are in separate synaptic domains, likely contributing to differences in AMPAR and NMDAR dynamics in synapses. (See pp. E8482–E8491.)

Reducing future fears by suppressing the brain mechanisms underlying episodic simulation

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Humans possess the remarkable ability to recombine details of divergent memories into imaginings of future events. Such imaginings are useful, for example, because they foster planning and motivate farsighted decisions. Importantly, recurrently imagining feared situations can also undermine our well-being and may even contribute to the development of anxiety. Here, we demonstrate that fearful imaginings about the future can be inhibited by neural mechanisms that help to suppress the past. Importantly, suppression reduces later apprehensiveness about the feared events, a benefit that was diminished in individuals with greater trait anxiety. This pattern suggests that the observed inhibition mechanism serves to control people's future fears and its disruption may foster psychological disorders characterized by intrusive prospective thoughts. (See pp. E8492–E8501.)