# PNAS Plus Significance Statements

## Negative cooperativity in the nitrogenase Fe protein electron delivery cycle

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Nitrogenase catalyzes $N_2$ reduction to ammonia, the largest N input into the biogeochemical nitrogen cycle. This difficult reaction involves delivery of electrons from the Fe protein component to the catalytic MoFe protein component in a process that involves hydrolysis of two ATP per electron delivered. MoFe contains two catalytic halves, each of which binds an Fe protein. The prevailing picture has been that the two halves function independently. Here, it is demonstrated that electron transfer (ET) in the two halves exhibits negative cooperativity: Fe→MoFe ET in one-half partially suppresses ET in the other. These findings thus show that conformational coupling in nitrogenase not only gates ET within each half, as shown previously, but introduces negative cooperativity between the two halves. (See pp. E5783–E5791.)

## Diabetic wound regeneration using peptide-modified hydrogels to target re-epithelialization

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Current treatments for diabetic chronic wounds fail to achieve effective therapeutic outcomes. The majority of these treatments focus on angiogenesis, but diabetes often involves endothelial dysfunction. A hallmark of regenerative wound healing is rapid, effective re-epithelialization. In this study, we present QHREDGS (glutamine-histidine-arginine-glutamic acid-aspartic acid-glycine-serine), a prosurvival peptide derived from angiopoietin-1, as a therapeutic candidate that targets re-epithelialization. Immobilized QHREDGS peptide promoted cell survival against hydrogen peroxide stress and collective cell migration of both normal and diabetic human keratinocytes in vitro. The clinical relevance was demonstrated further in type 2 diabetic mice: A single treatment with a low QHREDGS dose immobilized in chitosan–collagen was effective in promoting wound healing, and a single high-dose peptide treatment outperformed a clinically approved porous collagen dressing. (See pp. E5792–E5801.)

## Decoding how a soil bacterium extracts building blocks and metabolic energy from ligninolysis provides road map for lignin valorization

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Lignin is the only renewable and abundant polymer on the earth with aromatic units as its building blocks; however, it remains as an untapped resource. The current approach to making the biofuel industry cost competitive with the petroleum industry is to derive more value from the lignin. In this study we combined the unique approaches of both chemical engineering and biology to gain a deeper understanding of the metabolism of a soil bacterium, *Sphingobium* sp. SYK-6, that enables it to survive on lignin-derived monomers and oligomers. Understanding the central metabolism of SYK-6 will enable researchers to redesign the metabolic pathways of *Sphingobium* sp. SYK-6 more effectively to provide a renewable route for the production of products currently sourced from petrochemicals. (See pp. E5802–E5811.)

## Calmodulin in complex with the first IQ motif of myosin-5a functions as an intact calcium sensor

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Myosin-5a is a molecular motor that functions as a cargo transporter in cells. The motor function of myosin-5a is regulated by calcium via the calmodulin bound to the first isoleucine-glutamine (IQ) motif (IQ1) of myosin-5a. Here, we solve the crystal structure of a truncated myosin-5a containing the motor domain and the IQ1 complexed with calcium-bound calmodulin. Comparison of the structures of the IQ1 complexed with calmodulin with or without bound calcium reveals the calcium-induced conformational changes of calmodulin. We demonstrated that calmodulin continuously associates with the IQ1 during that calcium transition and that the IQ1 binding substantially changes the thermodynamic and kinetics of calcium transition in calmodulin. These findings provide insight into the mechanism by which calcium regulates myosin-5a. (See pp. E5812–E5820.)


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## Structural definition of the lysine swing in *Arabidopsis thaliana* PDX1: Intermediate channeling facilitating vitamin $B_6$ biosynthesis

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Multifunctional enzymes have been shown to recruit distinct domains for their reactions, remodel active sites, or connect different sites by substrate channeling to facilitate the multitude of transformations taking place. Pyridoxine synthase (PDX1) of the vitamin $B_6$ biosynthesis machinery is a remarkable enzyme that alone has a polymorphic catalytic ability designated to two active sites, the coordination of which is unclear. Here structural snapshots allow us to describe a lysine swinging arm mechanism that facilitates serviced substrate transfer and demonstrates how an enzyme can couple distinct chemistry between active sites, dispensing with the need for extra domains, substrate tunneling, or transfer of coenzyme bound intermediates. The work provides an elegant example of simplicity at work in nature's sea of complexity. (See pp. E5821–E5829.)

## Structural basis for norovirus neutralization by an HBGA blocking human IgA antibody

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Attachment to cellular glycans is a critical process in cell entry for several viruses. Antibodies that block this essential step can serve as neutralizing antibodies. Among human noroviruses (NoVs), serum antibodies that block histo-blood group antigen (HBGA) binding serve as correlates of protection. Escape from neutralization with evolving human NoVs (HuNoVs) through antigenic variation and differential HBGA binding is suggested to form a basis for the emergence of new strains. Currently, we are aware of no structural insights into antibody-mediated HBGA blockade or neutralization, or how emerging strains escape such neutralization. Our study reveals how a human IgA monoclonal antibody binds and blocks HBGA binding and indicates how other strains escape host immunity, laying the structural framework for understanding the immune correlates of protection against HuNoVs. (See pp. E5830–E5837.)

## Dynamics of *Escherichia coli*'s passive response to a sudden decrease in external osmolarity

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Mechanosensation is central to life. Bacteria, like the majority of walled cells, live and grow under significant osmotic pressure. By relying on mechanosensitive regulation, bacteria can adapt to dramatic changes in osmotic pressure. Studying such mechanical sensing and control is critical for understanding bacterial survival in a complex host and natural environment. Here, we investigate the fundamental design principles of *Escherichia coli*'s passive mechanosensitive response to osmotic downshocks by implementing single-cell high-resolution imaging. We explain the observed cell volume changes by modeling flux of water and solutes across the cell membrane. A better characterization of bacterial mechanosensitive response can help us map their reaction to environmental threats. (See pp. E5838–E5846.)

## Strain analysis of protein structures and low dimensionality of mechanical allosteric couplings

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Regulation of biochemical activity is essential for proper cell growth and metabolism. Many proteins' activities are regulated by interactions with other molecules binding some distance away from the proteins' active sites. In such allosteric proteins, active sites should thus be mechanically coupled to spatially removed regulatory regions. We studied crystal and NMR structures of proteins in various regulatory and ligand-binding states. We calculated and analyzed distributions of strains throughout several proteins. Strains reveal allosteric and active sites and suggest that quasi-two-dimensional strained surfaces mediate mechanical couplings between them. Strain analysis of widely available structural data can illuminate protein function and guide future experimental investigation. (See pp. E5847–E5855.)

## Nanoparticles size-dependently initiate self-limiting NETosis-driven inflammation

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The current widespread exposure of humans to natural as well as man-made nanomaterials due to the deployment of nanoparticles (NPs) as food additives, as vaccine- or drug-delivery vehicles, and in diagnostic procedures encourages the evaluation of their interaction with the innate immune system. Understanding how organisms cope with hydrophobic and chemically inert particulate matter, which is excluded from metabolic processing, is of major importance for interpreting the responses associated with the use of NPs in the biosphere. The containment of NPs within neutrophil-derived aggregates locally orchestrates the resolution of inflammation. Overriding this mechanism bears the risk of inducing chronic inflammation and causing tissue damage. (See pp. E5856–E5865.)

## Mechanism of inhibition of the tumor suppressor Patched by Sonic Hedgehog

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The Hedgehog-signaling pathway plays key roles in animal development and physiology. Insufficient Hedgehog signaling causes birth defects, whereas uncontrolled signaling is implicated in cancer. Signaling is triggered by the secreted protein, Sonic Hedgehog, which inhibits the membrane protein Patched1, leading to pathway activation. Despite its fundamental importance, we do not understand how Sonic Hedgehog inhibits Patched1. Here, we uncover a critical interaction between the fatty-acid–modified N-terminal portion of Sonic Hedgehog and Patched1, and we demonstrate that it is necessary and sufficient for Patched1 inhibition during Hedgehog signaling. This interaction explains impairment of a Sonic Hedgehog mutant causing a congenital brain malformation (holoprosencephaly) and oncogenic activity of Patched1 mutants responsible for a human cancer syndrome. (See pp. E5866–E5875.)

## Molecular organization of cytokinesis nodes and contractile rings by super-resolution fluorescence microscopy of live fission yeast

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Cell division occurs by the assembly and constriction of a ring of actin and myosin; however, the organization of proteins within cytokinetic apparatus is still unknown. Without better information about the organization of contractile rings, we cannot understand how they assemble or constrict. In fission yeast, cytokinetic proteins are first recruited around the equator as cortical spots, called nodes, that coalesce into a ring. Here we used high-speed quantitative fluorescence photoactivation localization microscopy to obtain a molecular model of this basic cytokinetic unit. Nodes are discrete structures with distinct distributions of six different proteins. Nodes persist in contractile rings and move around the circumference as the ring constricts. (See pp. E5876–E5885.)

## Autonomous translational pausing is required for *XBP1u* mRNA recruitment to the ER via the SRP pathway

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Unconventional splicing of unspliced X-box–binding protein 1 (*XBP1u*) mRNA on endoplasmic reticulum (ER) is an important process by which signals are transferred from the ER to the nucleus to maintain ER homeostasis. Newly synthesized XBP1u protein drags its own mRNA as a ribosome nascent chain complex to the ER; however, its precise location and molecular mechanism of ER recruitment remain unknown. We show that translational pausing of XBP1u is necessary for the recognition of the internal signal sequence of XBP1u by canonical secretory machinery. Interestingly, most XBP1u targeted to the ER was not imported into the ER lumen but was associated with the ER membrane, suggesting a noncanonical mechanism by which mRNA substrates are targeted to the ER for unconventional splicing. (See pp. E5886–E5895.)

## Single-cell dynamics and variability of MAPK activity in a yeast differentiation pathway

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The yeast mating pathway has long served as a prototypical signal transduction system, but key questions regarding intracellular signaling dynamics remain, including how signaling takes place and is used over time during the differentiation response. Utilizing a single-cell FRET reporter approach, this study indicates that signaling dynamics is governed by complex feedback interactions and that distinct MAPK activity patterns promote early and late phases of mating differently. Whereas submaximal activity is maintained through cell-cycle arrest and mating partner engagement, activity intensification and rapid loss coincide with cell polarization and fusion of prezygote cells. These findings provide new insights into the signaling mechanisms underlying how yeast cells sense and then commit to a nearby mating partner. (See pp. E5896–E5905.)

## WASH drives early recycling from macropinosomes and phagosomes to maintain surface phagocytic receptors

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Macropinocytosis is a way for cells to engulf large volumes of their extracellular fluid. This process allows immune cells to sense their environment and detect antigens, but can also supply nutrients to both cancer cells and some unicellular organisms. However, little is known about the fate of the membrane components internalized via macropinosomes. This study explains how cells avoid the bulk digestion of their surface proteins. We identify several core components of this pathway and show that they are required for early recycling from macropinosomes. We demonstrate that this pathway is crucial for cells undergoing continuous macropinocytosis to maintain surface protein levels and is therefore physiologically important for such cells to sustain normal functions. (See pp. E5906–E5915.)

## Three-dimensional localization of T-cell receptors in relation to microvilli using a combination of superresolution microscopies

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T lymphocytes play a central role in cell-mediated immunity. Their surfaces are covered by narrow and short protrusions called microvilli. It is not known whether there is a role for microvilli in the immune response. To shed light on this question, we probed the location of T-cell receptors (TCRs), the molecules that initiate the immune response of T cells, with respect to the 3D structure of microvilli. Superresolution optical microscopy showed that TCRs are highly concentrated on microvilli. Previous studies stressed the role of small clusters of TCRs in the immune process; our study provides a natural explanation as to how these clusters form. (See pp. E5916–E5924.)

## Integrating biogeochemistry with multiomic sequence information in a model oxygen minimum zone

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Modern molecular sequencing is beginning to provide great insight into microbial community structure and function at ecosystem scales. However, the quantitative integration of multiomic sequence information (DNA, mRNA, and protein) and geochemical processes has so far been elusive. Here, we develop a biogeochemical model that integrates geochemistry and multiomic sequence information to explain key metabolic processes in the oxygen-starved waters of Saanich Inlet, a model ecosystem for studying microbial community responses to oxygen minimum zone expansion. Our model largely explains DNA, mRNA, and protein distributions and sheds light on the metabolic networks coupling carbon, sulfur, and nitrogen transformations across a redox gradient. Our approach is extensible to other biogeochemical models incorporating feedbacks of global change on ecosystem functions. (See pp. E5925–E5933.)

## High-fat diet modifies the PPAR-γ pathway leading to disruption of microbial and physiological ecosystem in murine small intestine

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Our study aimed at exploring the intersection of high-fat diet, mucosal immune defenses, and microbiota. It remains unclear how diet imbalance toward excessive fat intake leads to secondary pathological effects on host physiology through the

microbiota. We show that a short period of consumption of high-fat diet alters the small-intestinal defenses and that the biochemistry of the ileum is drastically modified, leading to physiological changes close to that observed in cystic fibrosis. We identified peroxisome proliferator-activated receptor-γ as major regulator of mucosal defenses upon exposure to fat excess. As a result, our work provides a fundamental understanding of the underlying cause of severe chronic disorders associated with Western diet. (See pp. E5934–E5943.)

## Optimal activation of Fc-mediated effector functions by influenza virus hemagglutinin antibodies requires two points of contact

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The mechanism of how antiviral antibodies induce Fc–FcγR effector functions remains to be fully elucidated. Although the ability to activate effector functions is attributed to antibody isotype, this does not fully address why identical isotypes have different capabilities to stimulate effector function. We show that antibodies that target the influenza virus hemagglutinin (HA) require a second intermolecular interaction to optimally activate effector cells. We demonstrate that the receptor-binding domain of the HA is required to bind to sialic acid expressed on the surface of effector cells to optimize effector cell activation. This finding provides a basic understanding of how an optimal antibody-dependent cell-mediated response against influenza virus is achieved and may allow for better vaccine design. (See pp. E5944–E5951.)

## Cell cycle progression in *Caulobacter* requires a nucleoid-associated protein with high AT sequence recognition

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In all organisms, morphological and functional diversity is the product of cell type-specific genetic programs. Asymmetric cell division in *Caulobacter* yields daughter cells that differ functionally due to the differential read-out of their genomes. Here, we report the discovery of GapR, a conserved DNA-binding protein required for cell cycle progression. We show that GapR only associates with DNA sequences of high adenine and thymine (AT) content, colocating with cell cycle master regulators that control genes mediating swarmer cell development. GapR protein distributes asymmetrically, accumulating on the compacted chromosome of the daughter swarmer cell compartment prior to division. We argue that *Caulobacter* has co-opted a protein that associates with AT-rich DNA to provide spatial control during an asymmetric cell division. (See pp. E5952–E5961.)

## Molecular mechanism of $Zn^{2+}$ inhibition of a voltage-gated proton channel

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$Zn^{2+}$ inhibition of voltage-gated proton (Hv1) channels has important physiological roles, such as quiescence of sperm in the male reproductive system. Here, we show that $Zn^{2+}$ binds to different states of Hv1, and we propose a possible mechanism for $Zn^{2+}$ inhibition of Hv1. Several residues are found to be involved in $Zn^{2+}$ binding, and we provide detailed information about how these residues contribute to the functional effect of $Zn^{2+}$ binding. This study provides valuable information for future drug development for Hv1 channels. (See pp. E5962–E5971.)

## Network architecture of the cerebral nuclei (basal ganglia) association and commissural connectome

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The cerebral nuclei together with the cerebral cortex form the cerebral hemispheres that are critically important for the control of voluntary behavior and motivation. Network analysis of microscopic connectional data collected since the 1970s in a small, intensely studied mammal provides a new way to understand overall design features of circuitry coordinating activity in the various parts of the cerebral nuclei on both sides of the brain. Basically, intracerebral nuclei circuitry is organized into four modules on each side of the brain, with connections within and between modules on one side being quite dense and connections between the cerebral nuclei on either side being quite sparse. The results provide insight into cerebral nuclei structure and function. (See pp. E5972–E5981.)

## Brassinosteroids participate in the control of basal and acquired freezing tolerance of plants

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Cold stress is an influential environmental factor that affects plant distribution and can strongly limit crop productivity. Plants have evolved sophisticated signaling cascades that enable them to withstand chilling or even freezing temperatures. These cascades alter the biochemical composition of cells for protection from damage caused by low-temperature stress. In addition, cold stress has a profound impact on plant morphologies, causing growth repression and reduced yields. In this work we reveal that the brassinosteroids, a class of steroid hormones that is known for its role in growth control, also confers freezing tolerance in plants and describe regulatory circuits that contribute to this activity. Implications for the breeding of cold-resistant plants are discussed. (See pp. E5982–E5991.)