PNAS Plus Significance Statements

Carrier dynamics and the role of surface defects: Designing a photocatalyst for gas-phase CO_2 reduction

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In this work, we investigate the role of defects on the electronic and photocatalytic properties of $In_2O_{3-x}(OH)_y$ nanoparticles that have been shown to effectively reduce CO_2 to CO via the reverse water–gas shift reaction under light. To understand how such defects affect photogenerated electrons and holes in these materials, we studied the relaxation dynamics of these nanoparticles with varying concentration of defects. This analysis showed that higher defect concentrations result in longer excited-state lifetimes, which are attributed to improved charge separation and correlate well with the observed trends in the photocatalytic activity. (See pp. E8011–E8020.)

Near-atomic structural model for bacterial DNA replication initiation complex and its functional insights

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Chromosomal DNA replication is initiated by unwinding duplex DNA via formation of dynamic higher-order protein–DNA complexes. Whereas biochemical analyzes have been performed for a long time, structural dynamics in those processes still remain elusive due to their complex nature. Here, using hybrid approaches of computational modeling and biochemical assays, we explore dynamic structures of the entire machinery and significance of spatial arrangement of every component in the replication initiation complex of *Escherichia coli*. We show the structural model of the crucial core part of the complex at near-atomic resolution. The complex is composed of three subcomplexes, and spatial arrangements of those as well as their components are important for efficient replication initiation. (See pp. E8021–E8030.)

Stability, folding dynamics, and long-range conformational transition of the synaptic t-SNARE complex

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Intracellular membrane fusion is mediated by coupled folding and assembly of three or four soluble *N*-ethylmaleimide–sensitive factor attachment protein receptor (SNARE) proteins into a four-helix bundle. A rate-limiting step is the formation of a partial complex containing three helixes called the target (t)-SNARE complex on the target plasma membrane. The t-SNARE complex then serves as a template to guide stepwise zippering of the fourth helix, a process that is further regulated by other proteins. The synaptic t-SNARE complex readily misfolds. Consequently, its conformation, stability, and dynamics have not been well understood. Using optical tweezers and theoretical modeling, we elucidated the folding intermediates and kinetics of the t-SNARE complex and discovered a long-range conformational switch of t-SNAREs during SNARE zippering, which is essential for regulated SNARE assembly during synaptic vesicle fusion. (See pp. E8031–E8040.)

Mechanism of the intrinsic arginine finger in heterotrimeric G proteins

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The α-subunit of heterotrimeric G proteins is a molecular switch that mediates a great number of physiological processes such as vision, smelling, and blood pressure regulation. A GTPase-activating protein (GAP) [e.g. regulator of G protein signaling 4 (RGS4) in the case of $G\alpha_{i1}$] regulates the off-switch by catalyzing GTP hydrolysis. Here, we present the molecular reactions of GAP catalysis at atomic resolution using a combination of FTIR spectroscopy and biomolecular simulations. In contrast to X-ray structures, not GTP analogs but GTP itself is used. This approach is crucial to reveal now a previously undescribed GAP mechanism for Gα. A key player of the hydrolysis reaction, called the arginine finger, is pushed from a monodentate γ-GTP coordination toward a bidentate α-γ-GTP coordination by RGS4, and thereby catalyzes GTP-hydrolysis. (See pp. E8041–E8050.)

Elucidating the druggable interface of protein–protein interactions using fragment docking and coevolutionary analysis

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Protein–protein interfaces have become an emerging class of molecular targets for the design of therapeutic drugs. However, major challenges exist for the correct identification of binding sites on the protein surface as well as drug-like modulators of protein–protein interaction. An integrated approach using molecular fragment docking and coevolutionary analysis is presented to face these challenges. This approach

can accurately predict and characterize the binding sites for protein–protein interactions as well as provide clusters of bound, fragment-sized molecules on the druggable regions of the predicted binding site. These bound, molecular fragments can be chemically combined to create candidate drugs. (See pp. E8051–E8058.)

MyTH4-FERM myosins have an ancient and conserved role in filopod formation

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Filopodia are actin-based structures used by cells to sense chemical stimuli and promote adhesion to the extracellular environment during the development of multicellular organisms. Filopod formation in evolutionarily distant organisms requires MyTH4-FERM (myosin tail homology 4-band 4.1, ezrin, radixin, moesin; MF) myosins that consist of a motor domain paired with a tail domain that binds cytoskeletal and membrane proteins. Mutational analysis identified the minimal requirements for MF myosin function in filopod formation and revealed that the key features are conserved between amoebozoan and metazoan MF myosins. These findings have implications for understanding the fundamental principles of how filopodia form and how MF myosins function in phylogenetically distant organisms. (See pp. E8059–E8068.)

Model for the architecture of caveolae based on a flexible, net-like assembly of Cavin1 and Caveolin discs

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The surface of mammalian cells contains abundant plasma membrane invaginations termed "caveolae." Caveolae are important for various cellular functions, e.g. signaling, membrane regulation, and vesicular trafficking. Assembly and stability of caveolae depends on a protein coat formed by complexes of cavin and caveolin proteins whose structure has remained elusive. To understand the architecture of caveolae, we structurally analyzed cavin and caveolin complexes and visualized caveolae in cells by electron cryomicroscopy. A regular polyhedron emerged as the most likely architectural principle of caveolae. Polyhedra provide the underlying principle in many biological structures in which spherical curvature is imposed by proteins. We suggest a model for the caveolar coat architecture based on regularly arranged, net-like cavin assemblies and disc-shaped caveolin oligomers. (See pp. E8069–E8078.)

Planar cell polarity signaling in the uterus directs appropriate positioning of the crypt for embryo implantation

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Blastocyst implantation is a complex process that coordinates reciprocal embryo–uterine interactions. The uterus is demarcated by mesometrial (A)–antimesometrial (AM) domains; implantation occurs at regularly spaced intervals along the longitudinal axis of the AM pole, within specialized implantation chambers (crypts). The organized crypt formation in the rodent uterus was first observed in 1901, but the mechanism driving this phenomenon remained unknown. We found that planar cell polarity (PCP) signaling coordinates luminal epithelial evaginations toward the AM domain to form crypts for embryo homing and implantation in mice. Disruption of PCP signaling by inactivation of Vang-like protein 2, but not Vang-like protein 1, in the uterus disturbs the regular formation of epithelial evaginations and crypts, disrupting implantation and decidualization and severely compromising pregnancy outcomes. (See pp. E8079–E8088.)

Evaluating early-warning indicators of critical transitions in natural aquatic ecosystems

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Early-warning indicators (EWIs), statistical metrics of system resilience, have been hypothesized to provide advance warning of sudden shifts in ecosystems, or so-called "regime shifts." Here we tested this hypothesis for four commonly used EWIs. We used empirical time series from five freshwater ecosystems with documented sudden, persistent transitions hypothesized to represent critical transitions. EWIs were detected in several of these long-term records, and in some cases several years before the transition; however, these EWIs varied in reliability, and agreement between indicators was low. Moreover, their applicability was strongly limited by the requirement for ecosystem-specific knowledge of transition-generating mechanisms and their drivers to choose relevant state variables for analysis. (See pp. E8089–E8095.)

Selective sweep suggests transcriptional regulation may underlie *Plasmodium vivax* resilience to malaria control measures in Cambodia

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In Cambodia, where *Plasmodium vivax* and *Plasmodium falciparum* are coendemic and intense multimodal malaria-control interventions have reduced malaria incidence, *P. vivax* malaria has proven relatively resistant to such measures. We performed comparative genomic analyses of 150 *P. vivax* and *P. falciparum* isolates to determine whether different evolutionary strategies might underlie this species-specific resilience. Demographic modeling and tests of selection show that, in contrast to *P. falciparum*, *P. vivax* has experienced uninterrupted growth and positive selection at multiple loci encoding transcriptional regulators. In particular, a strong selective sweep involving an AP2 transcription factor suggests that *P. vivax* may use nuanced transcriptional approaches to population maintenance. Better understanding of *P. vivax* transcriptional regulation may lead to improved tools to achieve elimination. (See pp. E8096–E8105.)

DNA methylome of the 20-gigabase Norway spruce genome

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There are two main groups of land plants, flowering plants (also referred to as angiosperms) and gymnosperms. Compared with angiosperms, gymnosperms have larger genomes, often approximately 20 Gb, and have a higher abundance of transposons and other repetitive elements that are silenced by DNA methylation. Here, we present a whole genome single-base resolution

DNA methylation analysis of the important conifer Norway spruce (*Picea abies*), providing an important resource for the epigenetic study of this species. We show that the Norway spruce genome is heavily methylated because of high transposon content. In addition, we also show that somatic embryogenesis cultures used in the industry show altered DNA methylation patterning. (See pp. E8106–E8113.)

Global analysis of genomic instability caused by DNA replication stress in *Saccharomyces cerevisiae*

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One important source of genomic instability associated with tumor cells is DNA replication stress. In the current study, replication stress was induced in yeast by a 10-fold reduction in the level of the replicative DNA polymerase δ. By DNA microarray analysis and high-throughput DNA sequencing, we showed that this stress resulted in very high rates of both large (aneuploidy, mitotic recombination, deletions and duplications, and translocations) and small (point mutations and small insertion/deletions) genetic alterations. Some of these changes resulted in a selective growth advantage of the cells, demonstrating the role of elevated genetic instability in the rapid evolution of cells in challenging growth conditions. (See pp. E8114–E8121.)

Permissive roles of cytokines interleukin-7 and Flt3 ligand in mouse B-cell lineage commitment

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The generation of different blood lineages is regulated by hematopoietic cytokines, either in an instructive or in a permissive manner. The cytokines Interleukin-7 and fms-like tyrosine kinase-3 (Flt3) ligand are required for B-cell development but their precise mode of action remains controversial. Our study has addressed the role of these cytokines in B-cell commitment by analyzing the progenitor stage where B-cell commitment occurs in mice overexpressing one of the two cytokines in the absence of the other. Our results demonstrate a permissive role for both cytokines in B-cell commitment. Interleukin-7 promotes survival of progenitors instead of up-regulation of B-cell commitment factors early B-cell factor 1 (Ebf1) and paired box 5 (Pax5), as previously hypothesized, whereas Flt3 ligand facilitates progenitor expansion by inducing their proliferation. (See pp. E8122–E8130.)

Egr2 and Egr3 in regulatory T cells cooperatively control systemic autoimmunity through Ltbp3-mediated TGF-β3 production

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Transcription factors early growth response gene 2 (Egr2) and Egr3 have long been regarded as negative regulators of T-cell activation. *Egr2* is also known as a susceptibility gene for systemic lupus erythematosus characterized by dysregulated humoral immune responses to autoantigens. Previously, we reported that Egr2-expressing $CD4^+CD25^-LAG3^+$ regulatory T cells regulate lupus pathogenesis via production of TGF-β3. However, the role of Egr2 and Egr3 in the regulation of humoral immunity is unclear. Here we report that Egr2 and Egr3 regulate germinal center reactions by promoting TGF-β3 production from regulatory T cells. Egr2 and Egr3 induce the expression of latent TGF-β binding protein 3 (Ltbp3), which is required for TGF-β3 secretion. These findings suggest that Egr2 and Egr3 in T cells may be potential novel therapeutic targets for autoantibody-mediated autoimmune diseases. (See pp. E8131–E8140.)

Identifying species of symbiont bacteria from the human gut that, alone, can induce intestinal Th17 cells in mice

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Th17 cells accumulate in the gut, where they mediate barrier defenses and repair but can also provoke inflammatory disease. In mice, segmented filamentous bacteria (SFB) is sufficient to induce Th17 cells in the gut, but functionally analogous microbes in humans have not been defined. Here, we identified *Bifidobacterium adolescentis* as one of several human symbiont bacterial species that could, alone, induce Th17 cells in the small intestine of mice. *B. adolescentis* and SFB exhibited overlapping but also distinct activities, suggesting multiple routes to intestinal Th17 induction. Like SFB, *B. adolescentis* exacerbated autoimmune arthritis, arguing for its pathological relevance. Our results help to inform the search for therapeutic targets in diseases associated with Th17 responses and mucosal dysfunction. (See pp. E8141–E8150.)

TNFα-stimulated gene-6 (TSG6) activates macrophage phenotype transition to prevent inflammatory lung injury

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We found that TNFα-stimulated gene-6 (TSG6), a secreted 30-kDa immunomodulatory protein, resolved LPS-induced inflammatory lung injury by shifting macrophages from a proinflammatory to an anti-inflammatory phenotype. Macrophages from mice genetically deficient in TSG6 failed to transition, demonstrating the essential role of TSG6 in mediating macrophage plasticity. The finding that TSG6 induced the marked transition in anti-inflammatory macrophages lays the foundation for its therapeutic application. (See pp. E8151–E8158.)

Second generation noninvasive fetal genome analysis reveals de novo mutations, single-base parental inheritance, and preferred DNA ends

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We explored the limit of noninvasive prenatal testing by performing genome-wide sequencing of maternal plasma DNA at 195× and 270× haploid genome coverages. Combined with the use of a series of bioinformatics filters, fetal de novo mutations could be detected with a positive predictive value that was two orders of magnitude higher than previously reported. A de novo *BRAF* mutation was noninvasively detected in a case with cardiofaciocutaneous syndrome. The maternal inheritance of the fetus could be ascertained on a genome-wide level without the use of maternal haplotypes, hence greatly increasing the resolution of such analysis. Finally, we showed that certain genomic locations were overrepresented at the ends of plasma DNA fragments with fetal or maternal selectivity. (See pp. E8159–E8168.)

Stretchable multichannel antennas in soft wireless optoelectronic implants for optogenetics

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Soft, multichannel antennas enable wireless, battery-free operation of fully implantable optoelectronic systems designed for use in studies of brain function. These systems support independent, remote control of multiple light-emitting diodes that inject into targeted regions of the deep brain, where they separately stimulate activity in genetically and spatially discrete neural circuits, via the use of the techniques of optogenetics. These capabilities represent significant advancements over alternative technology approaches for this important branch of neuroscience research. In vivo studies using optimized systems demonstrate wireless control of two different brain regions and distinct activation of subpopulations of neurons using separately activated light sources associated with these subdermal devices. (See pp. E8169–E8177.)

Distinct cortical and striatal actions of a β-arrestin–biased dopamine D2 receptor ligand reveal unique antipsychotic-like properties

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Schizophrenia is a debilitating psychiatric disorder characterized by positive, negative, and cognitive symptoms. Current antipsychotic drugs, including D2 receptor (D2R) partial agonist aripiprazole, antagonize excess striatal dopamine (DA) neurotransmission and reverse positive symptoms but are not efficacious at reversing cortical-related cognitive symptoms. Here, we show using pharmacological, behavioral, and electrophysiological approaches that a β-arrestin2 (βarr2)-biased D2R ligand has opposite antagonist and agonist actions in the striatum and cortex, respectively. This phenomenon is regulated by differential expression levels of signal transducer proteins G protein-coupled receptor kinase 2 and βarr2. Thus, D2R-βarr2–biased ligands have the potential to simultaneously target excess striatal and deficient cortical DA neurotransmission and provide more broadly effective therapies for schizophrenia. (See pp. E8178–E8186.)

Tau prions from Alzheimer's disease and chronic traumatic encephalopathy patients propagate in cultured cells

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The progressive nature of neurodegenerative diseases is due to the spread of prions, misfolded infectious proteins, in the brain. In tauopathies, the protein tau misfolds, causing several diseases, including Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE). Here we created a panel of mammalian cell lines expressing a fragment of tau fused to yellow fluorescent protein. Each cell line selectively detects tau prions that are misfolded into self-propagating conformations; such cells permit identification of minute differences among tauopathies. For example, tau prions in AD and CTE are distinct from prions in other tauopathies such as Pick's disease and progressive supranuclear palsy. These insights are likely to contribute to the development of future therapeutics. (See pp. E8187–E8196.)

C-terminal domain (CTD) phosphatase links Rho GTPase signaling to Pol II CTD phosphorylation in *Arabidopsis* and yeast

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Rho GTPase and polymerase II (Pol II), two key molecules involved in cellular signaling and transcription in eukaryotic organisms, have been separately studied for more than 2 decades without evidence showing their functional linkage. We provide genetic and biochemical evidence linking these two molecules in an intracellular signaling pathway. Rho GTPases in *Arabidopsis* and yeast can modulate the phosphorylation status of the Pol II C-terminal domain (CTD) by inhibiting the CTD phosphatases. Our finding renders strong support for a direct or "shortcut" model in transcriptional control. Compared with the classical transcriptional activator/repressor-mediated indirect model, this shortcut model of targeting the core of Pol II likely provides an efficient transcriptional control to rapidly bring about the broad changes in gene expression. (See pp. E8197–E8206.)