# PNAS Plus Significance Statements

## How reduced excitonic coupling enhances light harvesting in the main photosynthetic antennae of diatoms

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Photosynthetic energy transfer must remain robust within the disordered protein environment. A high degree of robustness is generally obtained using molecular exciton states, which are excited states delocalized over a few pigments. These states provide several advantages, including a reduced probability of energy trapping in unfavorable sites, which would diminish the energy transfer efficiency. This study combines single-molecule spectroscopy and quantum-mechanical simulations to explore the different strategy of the main light-harvesting complexes of diatoms to enhance robustness. In the absence of strong exciton interactions, the pigment energies are more susceptible to protein structural changes, but the complexes seem to use these fluctuations to switch frequently into low-energy states with improved light-harvesting properties. (See pp. E11063–E11071.)

## Ultrafast 25-fs relaxation in highly excited states of methyl azide mediated by strong nonadiabatic coupling

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Highly excited states of neutral molecules behave qualitatively differently than the lower excited states that are commonly studied in photochemistry. Such states are involved in ionospheric and astrochemical phenomena, as well as in detonation processes. However, highly excited states are poorly understood due to experimental and theoretical challenges in probing their complex dynamics. Here, we apply vacuum-UV femtosecond laser sources and an imaging photoelectron–photoion coincidence spectrometer to directly probe the surprisingly fast 25-fs reaction pathway of the energetic molecule methyl azide. Combined with advanced calculations, we conclude that the electronic relaxation is driven by strong nonadiabatic coupling and that population transfer occurs along a seam well above the minimum energy conical intersection. (See pp. E11072–E11081.)

## Designing flexible 2D transition metal carbides with strain-controllable lithium storage

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The discovery of MXenes opens an opportunity on flexible energy storage. We explored systematically several factors including metal species, layer thicknesses, functional group, strain, and Li concentration on the mechanical and electrochemical properties of 2D transition metal carbides (TMCs). Taking the electrode polarization into account, we found several critical factors that govern the ionic mobility on the surface of 2D TMCs. Under multiaxial loadings, the electrical conductivity, high ionic mobility, low equilibrium voltage with good stability, excellent flexibility, and high theoretical capacity offered bare 2D TMCs the potential to be ideal flexible anode materials, whereas the surface functionalization degraded the transport mobility and increased the equilibrium voltage. General rules are proposed to identify the optimal candidate based on a combined analysis of these critical parameters. (See pp. E11082–E11091.)

## Global biogeochemical cycle of vanadium

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Human emissions of vanadium to atmosphere exceed natural sources by a factor of 1.7 and are destined to rise dramatically as we switch to the use of heavy oils, tar sands, and bitumen as combustion sources. Breathing vanadium-rich aerosols has unknown but potentially adverse health impacts. The human impacts on the global vanadium cycle parallel impacts on the global cycles for Pb and Hg. (See pp. E11092–E11100.)

## Population is the main driver of war group size and conflict casualties

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Recent views on violence emphasize the decline in proportions of war groups and casualties to populations over time and conclude that past small-scale societies were more violent than contemporary states. In this paper, we argue that these trends are better explained through scaling relationships between population and war group size and between war


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group size and conflict casualties. We test these relationships and develop measures of conflict investment and lethality that are applicable to societies across space and time. When scaling is accounted for, we find no difference in conflict investment or lethality between small-scale and state societies. Given the lack of population data for past societies, we caution against using archaeological cases of episodic conflicts to measure past violence. (See pp. E11101–E11110.)

## Molecular and functional resemblance of differentiated cells derived from isogenic human iPSCs and SCNT-derived ESCs

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Patient-specific pluripotent stem cells (PSCs) can be derived by two nuclear reprogramming methods: somatic cell nuclear transfer (SCNT) using unfertilized eggs and transcription factorbased reprogramming (i.e., induced pluripotent stem cells, iPSCs). The direct comparison of differentiated cells generated by SCNT and iPSC has yet to be assessed. In this study, we employ cutting-edge technologies to evaluate the similarities and differences between isogenic human iPSCs and SCNT-ESC derivatives. We provide proof-of-concept that differentiated cells derived from human iPSCs are comparable to nuclear transfer-derived ESC counterparts with regard to transcriptional, epigenetic, physiological, and pharmacological features, given that they are genetically identical. We conclude that human iPSCs are capable of replacing SCNT for generating differentiated cells for drug testing and disease modeling. (See pp. E11111–E11120.)

## Comparative transcriptomics as a guide to natural product discovery and biosynthetic gene cluster functionality

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Genomics has revealed that even well-studied bacteria maintain many more biosynthetic gene clusters (BGCs) predicted to encode specialized metabolites than expected based on product discovery. These orphan BGCs are often assumed to be transcriptionally silent. Here,we show that a majority of the 46 BGCs observed in four strains of the marine actinomycete *Salinispora* are transcribed at levels that should facilitate product detection. In five cases, similar BGCs were differentially expressed among strains, suggesting that simple presence or absence analyses are not good predictors of metabolic output. Highly expressed BGCs were bioinformatically linked to their products, including a series of salinipostins not previously reported from *Salinispora pacifica*. Subsequent genetic experiments established a formal link between salinipostins and their cognate BGC. (See pp. E11121–E11130.)

## Discovery of the leinamycin family of natural products by mining actinobacterial genomes

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Leinamycin (LNM) is a promising anticancer drug lead, yet no analog has been isolated since its discovery nearly 30 y ago. By mining bacterial genomes, we discovered 49 potential producers of LNM-type natural products, the structural diversity of which was predicted based on bioinformatics and confirmed by in vitro characterization of selected enzymes and structural elucidation of the guangnanmycins and weishanmycins. These findings demonstrate the power of the discovery-based approach to combinatorial biosynthesis for natural product discovery and structural diversity. New members of the LNM family of natural products should greatly facilitate drug discovery and development. The LNM-type biosynthetic machineries provide outstanding opportunities to dissect and mimic Nature's strategies for combinatorial biosynthesis and natural product structural diversity. (See pp. E11131–E11140.)

## Rapid, direct activity assays for Smoothened reveal Hedgehog pathway regulation by membrane cholesterol and extracellular sodium

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The Hedgehog pathway is critical in development and disease, but how cells respond to the secreted Hedgehog signal remains mysterious. A key step involves the regulation of the seven-transmembrane oncoprotein Smoothened by the 12-pass transporter-like Hedgehog receptor Patched1. We investigate the model that Patched1 is an ion-driven transporter of an endogenous lipidic Smoothened ligand. Whereas Patched–Smoothened regulation has traditionally been studied through indirect, downstream pathway readouts, we developed rapid, direct functional assays to dissect this step in simplified cell-based and in vitro systems. Cholesterol, a major membrane lipid, constitutively activates purified Smoothened by engaging its membrane-spanning region. Patched1 activity depends on extracellular $Na^+$, suggesting that transmembrane $Na^+$ gradients, universal among metazoans, might power Patched1 transporterlike activity in Smoothened regulation. (See pp. E11141–E11150.)

## Molecular mechanism for the subversion of the retromer coat by the *Legionella* effector RidL

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Deciphering microbial virulence mechanisms is of fundamental importance for the treatment of infectious diseases. *Legionella* pneumophila, the causative agent of Legionnaires' pneumonia, hijacks a variety of host cell factors during intracellular growth. Herein, we uncovered the molecular mechanism by which the *L. pneumophila* effector RidL targets the host VPS29, a scaffolding protein of endosome-associated sorting machineries. Using X-ray crystallography, we determined the structure of RidL, both alone and in complex with retromer. We found that RidL uses a hairpin loop similar to that present in cellular ligands to interact with retromer. This sophisticated molecular mimicry allows RidL to outcompete cellular ligands for retromer binding, explaining how *L. pneumophila* utilizes the endosomal sorting machinery to facilitate targeting of effector proteins. (See pp. E11151–E11160.)

## Cytoplasmic $Cl^-$ couples membrane remodeling to epithelial morphogenesis

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Calcium-activated chloride channels (CaCCs) serve important physiological functions, including modulation of signal processing

of neurons in the central and peripheral nervous system. In this study, we uncover a previously underappreciated role for TMEM16A, an evolutionarily conserved CaCC, in regulating cytoplasmic chloride homeostasis and membrane remodeling events in nonexcitable epithelial tissues. TMEM16A-mediated intracellular chloride homeostasis can modulate the partitioning of membrane phosphoinositides and endocytic transport, providing a new mechanism that controls membrane dynamics, a key property of eukaryotic cell membranes important for numerous cellular processes in development and diseases. (See pp. E11161–E11169.)

## Daple coordinates organ-wide and cell-intrinsic polarity to pattern inner-ear hair bundles

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Each hair cell of our auditory and vestibular systems transduces stimuli into electrical signals through its mechanosensitive hair bundle. Because the bundle is responsive along only a single axis, its orientation is crucial. Two systems determine hair-bundle polarity: planar cell polarity proteins, which establish axes along which hair cells are oriented, and the proteins Gαi and LGN. Investigating how these two systems are coordinated so that each hair bundle is appropriately aligned, we identified Daple. In mutants lacking Daple, hair bundles are misoriented and misshapen, a phenotype suggestive of both organ-wide and cell-intrinsic defects. Our study indicates how Daple interacts with proteins of the two systems and proposes a model for its role in determining hair-bundle polarity. (See pp. E11170–E11179.)

## Regulatory networks specifying cortical interneurons from human embryonic stem cells reveal roles for CHD2 in interneuron development

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In the human cerebral cortex, activities of excitatory neurons are balanced by local inhibition provided by cortical interneurons (cINs). Although disrupted cIN development contributes to neurodevelopmental disorders, molecular networks controlling this process were largely unknown. Here, we refined protocols for differentiating human embryonic stem cells into functional cINs. We defined gene-expression programs underlying cIN development and direct targets of the NKX2-1 transcription factor in this process, identifying potential regulators. These included *CHD2*, a gene mutated to cause human epilepsies. Accordingly, CHD2 deficiency impaired cIN development and altered later cIN function, while CHD2 and NKX2-1 could coregulate cIN gene expression by cobinding shared genomic regulatory regions. This work defines key features of both normal and disrupted cIN development. (See pp. E11180–E11189.)

## Troy/TNFRSF19 marks epithelial progenitor cells during mouse kidney development that continue to contribute to turnover in adult kidney

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Chronic kidney disease is a worldwide public health problem on the rise for which no curative treatments are available. Progressive kidney disease can be viewed as an imbalance between renal cell damage and repair. A better understanding of progenitor cells involved in kidney development and replacement of damaged cells in adult homeostasis may identify new therapeutic targets. Here, we describe *Troy* as a marker gene for epithelial progenitor cells. Lineage tracing shows that $Troy^+$ cells contribute to kidney development. $Troy^+$ cells have a high organoid-forming capacity, which is a stem cell characteristic. Tracing of $Troy^+$ cells in adult kidney shows that the cells contribute to kidney homeostasis, predominantly of the collecting duct, and regeneration. (See pp. E11190–E11198.)

## Zooplankton can actively adjust their motility to turbulent flow

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Zooplankton possess narrow swimming capabilities, yet are capable of active locomotion amid turbulence. By decoupling the relative velocity of swimming zooplankton from that of the underlying flow, we provide evidence for an active adaptation that allows these small organisms to modulate their swimming effort in response to background flow. This behavioral response results in reduced diffusion at substantial turbulence intensity. Adjusting motility provides fitness advantage because it enables zooplankton to retain the benefits of self-locomotion despite the constraints enforced by turbulence transport. Vigorous swimming and reduced diffusion oppose turbulence advection, can directly affect the dispersal of zooplankton populations, and may help these organisms to actively control their distribution in dynamic environments. (See pp. E11199–E11207.)

## Ser7 of RNAPII-CTD facilitates heterochromatin formation by linking ncRNA to RNAi

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Some noncoding RNAs (ncRNAs) transcribed by RNA polymerase II (RNAPII) affect gene expression by altering chromatin structures. Since transcriptional regulation by ncRNA is critically important in developmental process and diseases, clarification of the principles ensuring the locus-specific chromatin regulation is of great interest. Here, we found that in *Schizosaccharomyces pombe* Ser7 of the C-terminal domain (CTD) of RNAPII is involved in locus-specific siRNA amplification within heterochromatin and facilitates heterochromatin formation. Ser7 and a chromodomain protein Chp1, which binds to H3K9 methylation and RNAs, cooperatively promote chromatin retention of the nascent heterochromatic RNAs (hRNAs) across heterochromatin. Our findings present a principle of epigenetic regulation by ncRNAs in which the RNAPII CTD links hRNA transcription to RNAi for heterochromatin formation. (See pp. E11208–E11217.)

## Effects of mutation and selection on plasticity of a promoter activity in *Saccharomyces cerevisiae*

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From seasonal variation in the color of butterfly wings to trees bending toward the light, organisms often change in response to their environment. These changes, known as phenotypic plasticity, can result from differences in how genes are expressed among environments. Mutations causing environment-specific changes in gene expression provide raw material for phenotypic plasticity, but their frequency, effect size, and direction of effects among environments are not well understood. This study shows that mutations

in the promoter of a yeast metabolic gene often display environment-dependent effects on gene expression and that these environment-dependent effects have been shaped by selection in natural populations. (See pp. E11218–E11227.)

## ATG-dependent phagocytosis in dendritic cells drives myelin-specific CD4$^+$ T cell pathogenicity during CNS inflammation

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How autoreactive CD4$^+$ T cells recognize their target antigen and induce sustained inflammation in organ-specific autoimmune diseases is incompletely understood. In an experimental model of multiple sclerosis, we show that accumulation of myelin-specific CD4$^+$ T cells within the CNS and subsequent clinical disease development requires autophagy protein (ATG)-dependent phagocytosis in dendritic cells (DCs). Absence of ATG-dependent phagocytosis in DCs abrogates myelin presentation to CD4$^+$ T cells following phagocytosis of oligodendroglial cells, and its pharmacological inhibition delays the onset and reduces the clinical severity of experimental autoimmune encephalomyelitis. Thus, DCs use ATG-dependent phagocytosis for enhanced presentation of myelin antigen during autoimmune CNS inflammation, thereby linking oligodendrocyte injury with antigen processing and autoimmune T cell pathogenicity. (See pp. E11228–E11237.)

## Sclerostin influences body composition by regulating catabolic and anabolic metabolism in adipocytes

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Sclerostin exerts profound control over skeletal metabolism by regulating the osteoanabolic Wnt/β-catenin signaling pathway. In this study, we demonstrate that in addition to a dramatic increase in bone mass, Sost$^{-/-}$ mice as well as those treated with a sclerostin-neutralizing antibody exhibit a reduction in white adipose tissue mass and are protected from high fat diet feeding. This effect is associated with an increase in fatty acid oxidation and reduced de novo fatty acid synthesis in adipocytes due to increased Wnt/β-catenin signaling. (See pp. E11238–E11247.)

## Clinical, genetic, and structural basis of apparent mineralocorticoid excess due to 11β-hydroxysteroid dehydrogenase type 2 deficiency

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Apparent mineralocorticoid excess, a rare autosomal recessive disorder characterized by low renin hypertension, may display a severe or mild phenotype in patients. The variability in clinical presentation stems from different extents of impairment of the 11β-hydroxysteroid dehydrogenase type 2 (HSD11B2) enzyme arising from distinct mutations in the encoding gene. The computational model of the HSD11B2 protein that we constructed here will be useful in predicting disease severity for newly reported missense mutations in this gene. (See pp. E11248–E11256.)

## Human genetic variation alters CRISPR-Cas9 on- and off-targeting specificity at therapeutically implicated loci

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CRISPR-Cas9 holds enormous potential for therapeutic genome editing. Effective therapy requires treatment to be efficient and safe with minimal toxicity. The sequence-based targeting for CRISPR systems necessitates consideration of the unique genomes for each patient targeted for therapy. We show using 7,444 whole-genome sequences that SNPs and indels can reduce on-target CRISPR activity and increase off-target potential when targeting therapeutically implicated loci; however, these occurrences are relatively rare. We further identify that differential allele frequencies among populations may result in population-specific alterations in CRISPR targeting specificity. Our findings suggest that human genetic variation should be considered in the design and evaluation of CRISPR-based therapy to minimize risk of treatment failure and/or adverse outcomes. (See pp. E11257–E11266.)

## Impact of insecticide resistance in *Anopheles arabiensis* on malaria incidence and prevalence in Sudan and the costs of mitigation

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Emerging insecticide resistance in malaria vectors could presage a catastrophic rebound in malaria morbidity and mortality. In areas of moderate levels of resistance to pyrethroids, long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) with a carbamate insecticide were significantly more effective than IRS with pyrethroid insecticide. The impact on the effectiveness of LLINs could not be quantified. The incremental cost of using a carbamate insecticide to which vectors are susceptible was US $0.65 per person protected per year, which is considered acceptable by international standards. While the WHO recommends that different interventions, where possible, should use different insecticide classes, these data alone should not be used as the basis for a policy change in vector control interventions. (See pp. E11267–E11275.)

## Identification of cancer genes that are independent of dominant proliferation and lineage programs

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Large, multidimensional "landscaping" projects have provided datasets that can be mined to identify potential targets for subgroups of tumors. Here, we analyzed genomic and transcriptomic data from human breast tumors to identify genes whose expression is enriched in tumors harboring specific genetic alterations. However, this analysis revealed that two other factors, proliferation rate and tumor lineage, are more dominant factors in shaping tumor transcriptional programs than genetic alterations. This discovery shifted our attention to identifying genes that are independent of the dominant proliferation and lineage programs. A small subset of these genes represents candidate targets for combination cancer therapies because they are druggable, maintained after treatment with chemotherapy, essential for cell line survival, and elevated in drug-resistant stem-like cancer cells. (See pp. E11276–E11284.)

## Mechanism by which arylamine *N*-acetyltransferase 1 ablation causes insulin resistance in mice

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Insulin resistance in liver and skeletal muscle are major factors in the pathogenesis of type 2 diabetes; however, the molecular mechanism or mechanisms responsible for this phenomenon have not been established. Recently, an association of a single-nucleotide polymorphism in the human *N*-acetyltransferase 2 (Nat2) gene with insulin resistance in humans was found. Here, we show that the murine ortholog Nat1 knockout (KO) mice manifested whole-body insulin resistance associated with marked increases in liver and muscle lipid content. Nat1 KO mice also displayed reduced whole-body energy expenditure and reduced mitochondrial activity. Taken together, these studies demonstrate that Nat1 deletion promotes reduced mitochondrial activity and is associated with ectopic lipid-induced liver and muscle insulin resistance. (See pp. E11285–E11292.)

## Brain urea increase is an early Huntington's disease pathogenic event observed in a prodromal transgenic sheep model and HD cases

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We present evidence for the presymptomatic dysregulation of urea metabolism in Huntington's disease (HD). We identified increased levels of a urea transporter transcript and other osmotic regulators in the striatum of our prodromal sheep model of HD and a concomitant increase in striatal and cerebellar urea. Elevated urea was also detected in brain tissue from postmortem HD cases, including cases with low-level cell loss, implying that increased brain urea in HD is not just a product of end-stage cachexia. Disruption of urea metabolism is known to cause neurologic impairment and could initiate neurodegeneration and the symptoms of HD. Our findings suggest that lowering brain levels of urea and/or ammonia would be a worthwhile therapeutic target in HD. (See pp. E11293–E11302.)

## A transient dopamine signal encodes subjective value and causally influences demand in an economic context

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A central tenet of economics is that as the price of a commodity increases, its demand goes down because individuals choose to buy less. Mounting evidence supports a role for the neuromodulator dopamine in representing subjective value. We investigated the role of dopamine in valuation by presenting rats with a reward across a range of prices. We showed that dopamine concentration decreased with price and increasing release using optogenetic manipulations-altered price sensitivity. Increasing release prior to reward delivery made animals more sensitive to price, whereas increasing release at reward delivery made animals less sensitive to price. These data extend the notion that dopamine release events encode subjective value and further demonstrate that increasing dopamine release causally modifies price sensitivity. (See pp. E11303–E11312.)

## FKBP12 contributes to α-synuclein toxicity by regulating the calcineurin-dependent phosphoproteome

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Calcineurin is an essential $Ca^{2+}$-dependent phosphatase in all eukaryotes. Whether calcineurin can be endogenously regulated by factors other than $Ca^{2+}$ and calmodulin is not known. Using a model of Parkinson's Disease (PD) as a surrogate for high pathophysiological calcineurin activity and employing a shotgun proteomic approach, we show that the isomerase FKBP12 physiological regulates calcineurin activity by facilitating dephosphorylation of proteins involved in vesicle recycling. Using a rodent model of PD, partial inhibition of the functional interaction between FKBP12 and calcineurin blocks the phosphatase activity toward critical vesicle recycling proteins at nigral presynaptic terminals conferring strong neuroprotection. Our work reassigns to FKBP12 a novel mechanism that supports toxicity in a PD model by modulating calcineurin's phosphatase activity with therapeutic implications. (See pp. E11313–E11322.)

## Noncanonical thyroid hormone signaling mediates cardiometabolic effects in vivo

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This study changes our understanding of how thyroid hormone acts. Thyroid hormone receptors are considered typical nuclear receptors that bind to DNA and, after binding, alter the expression of their target genes and regulate physiological responses. Nevertheless, we show that thyroid hormone still mediates important physiological effects in mice expressing mutant receptors that cannot bind DNA. These are predominantly linked to energy metabolism and include glucose and triglyceride concentrations, body temperature, locomotor activity, and heart rate. This study provides in vivo evidence that thyroid hormone receptors mediate physiologically relevant effects that are independent of DNA binding and direct activation of gene expression. (See pp. E11323–E11332.)