PNAS Plus Significance Statements

History of art paintings through the lens of entropy and complexity

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The critical inquiry of paintings is essentially comparative. This limits the number of artworks that can be investigated by an art expert in reasonable time. The recent availability of large digitized art collections enables a shift in the scale of such analysis through the use of computational methods. Our research shows that simple physics-inspired metrics that are estimated from local spatial ordering patterns in paintings encode crucial information about the artwork. We present numerical scales that map well to canonical concepts in art history and reveal a historical and measurable evolutionary trend in visual arts. They also allow us to distinguish different artistic styles and artworks based on the degree of local order in the paintings. (See pp. E8585–E8594.)

Entropic forces drive clustering and spatial localization of influenza A M2 during viral budding

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For influenza virus to release from the infected host cell, controlled viral budding must finalize with membrane scission of the viral envelope. Curiously, influenza carries its own protein, M2, which can sever the membrane of the constricted budding neck. Here we elucidate the physical mechanism of clustering and spatial localization of the M2 scission proteins through a combined computational and experimental approach. Our results provide fundamental insights into how M2 clustering and localization interplay with membrane curvature, membrane lateral stresses, and lipid bilayer phase behavior during viral budding to contribute to virion release. (See pp. E8595–E8603.)

Dynamic process connectivity explains ecohydrologic responses to rainfall pulses and drought

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In the face of changing climate, weather variability, and land cover, it is important to understand how ecosystem components vary jointly to determine how the system responds as a whole. While previous works have identified thresholds along climate gradients regarding precipitation, soils, and vegetation, we relate these thresholds to shifts in connectivity between variables, captured through joint variability, to better understand whole-system attributes of resilience, sensitivity, and vulnerability. We use data from flux tower transects along elevation gradients to address the relationship between joint connectivity and energy, water, and carbon flux responses to changes in moisture availability. This analysis reveals differences in joint variability between locations that can help explain responses to disturbances such as rain events or drought. (See pp. E8604–E8613.)

Structural and mechanistic analysis of the arsenate respiratory reductase provides insight into environmental arsenic transformations

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Microbial arsenate respiration enhances the mobility of arsenic and contributes to the poisoning of tens of millions of people worldwide. Our ability to quantitatively predict how microbial activities shape arsenic geochemistry depends on a detailed understanding of how the enzymes that catalyze arsenate reduction work under environmentally relevant conditions. The structural and kinetic findings of the Arr enzyme complex reported here both help rationalize its extracytoplasmic localization and allow us to predict that the rate of arsenate release from minerals likely constrains its activity in sedimentary environments. Moreover, this work illustrates that engineering environmental bacteria to overexpress their native proteins can be straightforward, a strategy that may advance the study of enzymes that are challenging to express in traditional hosts. (See pp. E8614–E8623.)

β-Subunit of the voltage-gated Ca^{2+} channel Cav1.2 drives signaling to the nucleus via H-Ras

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The L-type voltage-gated calcium channel Cav1.2 mediates depolarization-triggered signaling cascades that regulate neuronal-specific transcription factors such as CREB and immediate-early genes. We demonstrate that the interaction of the intracellular β-subunit of the channel with H-Ras is indispensable for depolarization-triggered gene activation. The binding of the recombinant β-subunit to H-Ras and H-Ras pulldown assays confirms the ability of H-Ras to

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physically interact with the β-subunit. We show that gene transcription also requires the binding of Ca^{2+} to the channel pore and is calcium-influx independent. These results delineate Cav1.2–H-Ras interaction by extracellular signaling as a mode of rapid induction of gene transcription. They expand the repertoire of Cav1.2 metabotropic signaling triggered by depolarization-induced conformational changes, which require channel-pore occupancy and are calcium-influx independent. (See pp. E8624–E8633.)

De novo formation of an aggregation pheromone precursor by an isoprenyl diphosphate synthase-related terpene synthase in the harlequin bug

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Many insects release volatile terpenes for chemical communication. However, the biosynthetic origin and evolution of these infochemicals are mostly unknown. We show that the harlequin bug, *Murgantia histrionica*, a stink bug pest (Hemiptera) of crucifer crops, produces a terpene aggregation pheromone by an enzyme that is unrelated to microbial and plant terpene synthases. *M. histrionica* terpene synthase activity is highly sex- and tissue-specific and makes a sesquiterpene alcohol, so far unknown in animals, as pheromone precursor. The enzyme evolved from ancestral isoprenyl diphosphate synthases and provides new evidence for de novo biosynthesis of terpenes in hemipteran insects. Knowledge of pheromone biosynthesis in stink bugs may lead to the development of new controls of these pests. (See pp. E8634–E8641.)

Conformational changes in Arp2/3 complex induced by ATP, WASp-VCA, and actin filaments

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Arp2/3 complex consists of actin-related proteins 2 and 3 with five other subunits. It forms actin filament branches under the regulation of ATP, actin monomers, actin filaments, and a nucleation-promoting factor (NPF). Here we used fluorescence spectroscopy and EM to characterize conformational changes in Arp2/3 complex along the pathway of actin filament branch formation. ATP binding to Arp2/3 complex causes small local changes in Arp2 and Arp3. NPF binding causes a larger conformational change that moves Arp2 closer to Arp3 and favors binding to the side of an actin filament, which allows further rearrangement of the subunits in the complex and growth of the new branch. (See pp. E8642–E8651.)

QTY code enables design of detergent-free chemokine receptors that retain ligand-binding activities

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The QTY (glutamine, threonine, and tyrosine) code-designed detergent-free chemokine receptors may be useful in many applications. The QTY variants may be useful not only as reagents in deorphanization studies but also for designing biologics to treat cancer and autoimmune or infectious diseases. The QTY code allows membrane proteins to be systematically designed through simple, specific amino acid substitutions. The QTY code is robust and straightforward: It is the simplest tool to carry out membrane protein design without sophisticated computer algorithms. Thus it can be used broadly. The QTY code has implications for designing additional G protein-coupled receptors and other membrane proteins or, potentially, for rendering water-insoluble and aggregated proteins soluble. (See pp. E8652–E8659.)

Long noncoding RNA *NEAT1* (nuclear paraspeckle assembly transcript 1) is critical for phenotypic switching of vascular smooth muscle cells

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Many occlusive vascular diseases in humans are largely dependent upon vascular smooth muscle cell (VSMC) phenotypic switching from a contractile to a proliferative phenotype, contributing to the formation of intimal lesions that eventually block the blood flow. Previous studies showed that the long noncoding RNA (lncRNA) *NEAT1* is critical for tumorigenesis. In this report, we showed that *NEAT1* expression was not only induced in VSMCs during phenotypic switching but functionally was critical for the smooth muscle phenotypic change. Our study demonstrates an unexpected role of the lncRNA *NEAT1* in VSMCs and suggests that *NEAT1* is a novel therapeutic target for treating occlusive vascular diseases in humans. (See pp. E8660–E8667.)

BRAF/MAPK and GSK3 signaling converges to control MITF nuclear export

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Signaling pathways ultimately exert their influence on cell behavior by regulating the activity of transcription factors that drive gene expression programs associated with specific cell phenotypes. How transcription factors integrate the outputs from multiple independent signaling events to coordinate cell behavior is a key issue. Here, we identify a regulated nuclear export signal in the lineage survival oncogene and cell fate-determining factor MITF. The regulated export signal integrates the outputs from the MAPK signaling pathway with those regulating GSK3 that play key roles in development and disease. The regulation of MITF nuclear export provides a means by which these key signaling pathways tune MITF activity that, in turn, controls cell identity in development and disease. (See pp. E8668–E8677.)

Physical foundations of biological complexity

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Living organisms are characterized by a degree of hierarchical complexity that appears to be inaccessible to even the most complex inanimate objects. Routes and patterns of the evolution of complexity are poorly understood. We propose a general conceptual framework for emergence of complexity through competing interactions and frustrated states similar to those that yield patterns in striped glasses and cause self-organized criticality. We show that biological evolution is replete with

competing interactions and frustration that, in particular, drive major transitions in evolution. The key distinction between biological and nonbiological systems seems to be the existence of long-term digital memory and phenotype-to-genotype feedback in living matter. (See pp. E8678–E8687.)

Global analysis of mutations driving microevolution of a heterozygous diploid fungal pathogen

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Evolution acts on mutations that naturally arise within the genome and are shaped both by intrinsic genomic features and by the cellular environment. We catalog the mutations arising in a heterozygous diploid yeast during passaging in vitro and in the mammalian host. We establish genome-wide mutation rates and reveal that "microscale" changes (base substitutions and short-track recombination events) are the primary drivers of microevolution, although chromosomal-level changes also occur in specific host environments. Our results define mutation hotspots, including those adjoining recombination tracts, and indicate that many mutations are purged from the population due to purifying selection. Together, these data provide a high-resolution picture of how the heterozygous diploid genome of a fungal pathogen undergoes evolution over short time scales. (See pp. E8688–E8697.)

Adjuvant effect of the novel TLR1/TLR2 agonist Diprovocim synergizes with anti–PD-L1 to eliminate melanoma in mice

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Adjuvants enhance adaptive immune responses, sometimes through unknown mechanisms, and can be used to augment both humoral and cellular responses to cancer antigens. We report the immunological effects of the synthetic chemical adjuvant Diprovocim, which targets the innate immune receptor TLR1/TLR2 in mice and humans. Diprovocim displayed strong adjuvant activity in mice, particularly abetting cellular immune responses. Immunization against a genetically engineered tumor-specific antigen, ovalbumin, when adjuvanted with Diprovocim, inhibited growth of B16 melanoma and prolonged survival in the presence of immune checkpoint blockade by anti–PD-L1; 100% of mice responded to treatment. Our data suggest Diprovocim boosts the success of anti–PD-L1 treatment by increasing the number and activation of tumor-specific CTLs capable of responding to this checkpoint inhibitor. (See pp. E8698–E8706.)

Structure of a patient-derived antibody in complex with allergen reveals simultaneous conventional and superantigen-like recognition

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We have discovered that a human antibody can simultaneously bind two molecules of antigen, in this case a grass pollen allergen, one in a conventional manner and the other unconventionally. The two allergen molecules also bridge two identical antibodies. These observations challenge the dogma that one antibody recognizes only a single antigen/allergen epitope. The mechanism of antibody cross-linking seen here may explain the potency of certain allergens in triggering an allergic reaction, extending our understanding of the nature of allergenicity and informing the design of hypoallergenic molecules for allergen immunotherapy. This dual reactivity and potential for cross-linking surface immunoglobulin on B cells suggests mechanisms by which human autoimmune and other diseases might be initiated. (See pp. E8707–E8716.)

Mechanisms of enhanced drug delivery in brain metastases with focused ultrasound-induced blood–tumor barrier disruption

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Improved penetration along with accurate prediction and mechanistic understanding of anticancer agent delivery across the blood–brain/blood–tumor barrier (BBB/BTB) are essential for the rational development of effective therapeutic strategies in intracranial malignancies. In this study, we provide insights in drug pharmacokinetics in brain metastases after focused ultrasound-induced BBB/BTB disruption by integrating quantitative microscopy with mathematical modeling. We demonstrate that focused ultrasound-induced BBB/BTB disruption contributes to enhanced interstitial convective transport in solid tumors, in addition to alleviating vascular barriers, and provide evidence of improved penetration of nontargeted and antibody-targeted chemotherapies. Together, our work provides a unified framework for prospective, quantitative, and mechanistic investigation of the penetration of anticancer drugs across the BBB/BTB in brain tumors. (See pp. E8717–E8726.)

Precursor proadrenomedullin influences cardiomyocyte survival and local inflammation related to myocardial infarction

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Myocardial infarction (MI) is one of the leading causes of death worldwide and is characterized by apoptosis and inflammation. While increased adrenomedullin (ADM) levels after MI are associated with disease severity, ADM infusion leads to anti-apoptotic effects, suggesting a self-protective mechanism. ADM is cleaved from a full-length precursor protein (ProADM), a putatively inactive prohormone. Our data show that ProADM is biologically active by reducing apoptosis to a similar extent as ADM. In contrast to ADM, ProADM has proinflammatory effects on cardiac fibroblasts but antiinflammatory effects on activated leukocytes. We assume that ProADM induces local inflammation but attenuates exaggerated inflammation. Our data suggest that both proteins are beneficial during MI by regulating inflammation and reducing apoptosis of cardiomyocytes. (See pp. E8727–E8736.)

Circular DNA tumor viruses make circular RNAs

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Circular RNAs (circRNAs) play critical physiologic functions, but it is not known whether human DNA viruses express circRNAs.

We surveyed Epstein–Barr virus (EBV) and Kaposi's sarcoma herpesvirus (KSHV) tumors and cell lines, and found specific circRNAs expressed from both viruses. EBV circular BamHI A rightward transcripts (circBARTs) were expressed in all EBV tumor latency forms, including all EBV-infected posttransplant lymphoproliferative disease tumors tested, whereas EBV circBHLF1 and circLMP2 were more variably expressed. KSHV expressed circvIRF4 constitutively in primary effusion lymphoma cell lines, while the polyadenylated nuclear locus promiscuously generated variable, inducible, and bidirectional circRNAs. Tumor virus circRNAs can be long-lived, unique tumor biomarkers that may also open new research opportunities into understanding how these viruses cause cancer. (See pp. E8737–E8745.)

Palmitoylation enables MAPK-dependent proteostasis of axon survival factors

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Neurons extend long structures called axons that are highly susceptible to damage and undergo degeneration in many neurological disorders. One strategy for treating these diseases is by elevating the abundance of axon survival factors that suppress axon death. Herein, we describe a protein homeostasis network that regulates axon degeneration by tuning the local levels of axon survival factors. In particular, we find that small-molecule inhibitors targeting a MAPK stress pathway protect axons from pathological degeneration by elevating the local abundance of axon survival factors NMNAT2 and SCG10. Furthermore, we discover that intracellular location imparts sensitivity to distinct protein homeostasis networks. Inactivating multiple nodes in this protein homeostasis network confers maximal therapeutic potential in diseases of axon degeneration. (See pp. E8746–E8754.)

Distinct roles of prefrontal and parietal areas in the encoding of attentional priority

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During search, information about the similarity of stimuli to the searched-for target is thought to be encoded in spatial priority maps, which signal the behavioral relevance of stimuli across the visual field. The frontal eye field (FEF) and the lateral intraparietal area (LIP) have both been reported to hold priority maps, but it is unknown whether the two areas have identical or distinct roles in encoding attentional priority. Here, we show that whereas LIP responses reflect the similarity of stimuli to the target, FEF responses integrate perceptual relevance with oculomotor decisions. Moreover, although feature-based attention effects are stronger within FEF, they emerge at similar latencies in subpopulations of the two areas, implying parallel processing of priority information. (See pp. E8755–E8764.)

Early postnatal behavioral, cellular, and molecular changes in models of Huntington disease are reversible by HDAC inhibition

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In Huntington disease (HD) gene carriers the disease-causing mutant *Huntingtin* (m*HTT*) is already present during early developmental stages, but, surprisingly, HD patients develop clinical symptoms only many years later. While a developmental role of *Huntingtin* has been described, so far new therapeutic approaches targeting those early neurodevelopmental processes are lacking. Here, we show that behavioral, cellular, and molecular changes associated with m*HTT* in the postnatal period of genetic animal models of HD can be reverted using low-dose treatment with a histone deacetylation inhibitor. Our findings support a neurodevelopmental basis for HD and provide proof of concept that pre-HD symptoms, including aberrant neuronal differentiation, are reversible by early therapeutic intervention in vivo. (See pp. E8765–E8774.)

Human iPSC-derived trigeminal neurons lack constitutive TLR3-dependent immunity that protects cortical neurons from HSV-1 infection

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We previously demonstrated that induced pluripotent stem cell (iPSC)-derived cortical neurons from HSV-1 encephalitis patients with Toll-like receptor 3 (TLR3) pathway deficiencies are highly susceptible to HSV-1, due to impairment of cell autonomous TLR3-IFN immunity. In this study we present a protocol for efficient derivation/purification of trigeminal ganglion (TG) neurons from human iPSCs. The resulting TG neurons are of sensory identity and exhibit robust biological function. We also show that TG neurons and cortical neurons play distinct roles in host defense against HSV-1 in the central nervous system: unlike cortical neurons, TG neurons are vulnerable to HSV-1 because they require preemptive induction of TLR3-, IFN-α/β–mediated immunity. This is an important step to further our understanding of the HSV-1 encephalitis disease mechanism. (See pp. E8775–E8782.)

Transcriptional switch for programmed cell death in pith parenchyma of sorghum stems

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Sugar and ethanol productivity from the sugar juice of grass stems depends on their water content. Pith parenchyma cells function as a water storage tissue in plant stems, and the death of these cells reduces stem water content. In this study, we identified a gene, long referred to as *D*, in a promising energy grass, *Sorghum bicolor*, that is responsible for reducing stem water content. *D* and its *Arabidopsis* ortholog encode master transcriptional switches that induce programmed death of stem pith parenchyma cells by activating autolytic enzymes. Identifying *D* as the gene involved in programmed death of plant pith parenchyma cells will provide an approach to breeding crops for sugar and ethanol production. (See pp. E8783–E8792.)

Mechanistic insights into plant SUVH family H3K9 methyltransferases and their binding to context-biased non-CG DNA methylation

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Plant SUVH family H3K9 methyltransferases play a key role in connecting the two epigenetic silencing marks, DNA methylation and H3K9me2. However, the regulation of SUVH protein activities and their precise role in the regulation of DNA methylation remains unclear. In this research, we performed a comprehensive investigation into the structure, biochemistry, and in vivo targeting characteristics of SUVH histone methyltransferases. For binding methylated DNA, we reveal that the SUVH family proteins possess a unique thumb loop-dependent base-flipping mechanism. For methyltransferase function, we reveal that SUVH6 is regulated by a dynamic autoinhibitory domain. Finally, our in vitro DNA-binding assays combined with ChIP-seq data uncover mechanisms to help explain context-biased non-CG DNA methylation in plants. (See pp. E8793–E8802.)

Feedback-mediated signal conversion promotes viral fitness

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How biological systems robustly sustain differentiated states is under active study. Here, we find that human cytomegalovirus, a herpesvirus that is a major cause of birth defects, encodes a transcriptional positive-feedback loop that temporally extends transient activation signals carried in the incoming viral particle to sustain the viral lytic expression cycle. Attenuation of this feedback loop severely impacts virus fitness, suggesting a new antiviral target that may extend to other herpesviruses. (See pp. E8803–E8810.)