PNAS Plus Significance Statements

Wafer-recyclable, environment-friendly transfer printing for large-scale thin-film nanoelectronics

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The ability to endow the surface of existing objects with desired electronic features enables many emerging applications such as Internet of Things (IoT). Despite significant progress in the optimization of thin-film materials and construction schemes, the realization of high-performance thin-film electronics on arbitrary place through a wafer-scale batch process is limited. This paper presents a wafer-recyclable, environment-friendly transfer printing process that enables the physical delivery of large-scale thin-film integrated circuits with various combinations of active nanomaterials from their fabrication wafer to nearly any kind of places. Detailed experimental and theoretical studies reveal the essential attributes of this approach. Demonstrations in electronics and sensors realized on the surface of commercial appliances to meet the user-specific needs illustrate the utility. (See pp. E7236–E7244.)

Theoretical search for heterogeneously architected 2D structures

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The bottom-up assembly of deterministic structures by lattice cell structures for surpassing properties of individual components or their sums by orders of magnitude is of critical importance in materials by design. Here, we present a theoretical strategy in the search and design of heterogeneously architected 2D structures (HASs) by assembling arbitrarily shaped basic lattice structures and demonstrate that an extremely broad range of mechanical properties can be achieved. This strategy allows designing HASs through interface properties of close relevance to assembly patterns and bonding connections between basic lattice structures. Studies using extensive numerical experiments validate the robust, reliable, and lucrative strategy of searching and designing HASs and offer quantitative guidance in the discovery of emerging 2D superstructures. (See pp. E7245–E7254.)

Valuation of knowledge and ignorance in mesolimbic reward circuitry

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Humans desire to know what the future holds. Yet, at times they decide to remain ignorant (e.g., reject medical screenings). These decisions have important societal implications in domains ranging from health to finance. We show how the opportunity to gain information is valued and explain why knowledge is not always preferred. Specifically, the mesolimbic reward circuitry selectively treats the opportunity to gain knowledge about favorable, but not unfavorable, outcomes as a reward to be approached. This coding predicts biased information seeking: Participants choose knowledge about future desirable outcomes more than about undesirable ones, vice versa for ignorance, and are willing to pay for both. This work demonstrates a role for valence in how the human brain values knowledge. (See pp. E7255–E7264.)

Pupil mimicry promotes trust through the theory-of-mind network

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Trusting others is central for cooperative endeavors to succeed. To decide whether to trust or not, people generally make eye contact. As pupils of interaction partners align, mimicking pupil size helps them to make well-informed trust decisions. How the brain integrates information from the partner and from their own bodily feedback to make such decisions was unknown because previous research investigated these processes separately. Herein, we take a multimethod approach and demonstrate that pupil mimicry is regulated by the theory-of-mind network, and informs decisions of trust by activating the precuneus. This evolutionary ancient neurophysiological mechanism that is active in human adults, infants, and chimpanzees promotes affiliation, bonding, and trust through mimicry. (See pp. E7265–E7274.)

Genetic analysis of social-class mobility in five longitudinal studies

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Genome-wide association study (GWAS) discoveries about educational attainment have raised questions about the meaning of the genetics of success. These discoveries could offer clues about biological mechanisms or, because children inherit genetics and social class from parents, education-linked genetics could be spurious correlates of socially transmitted advantages. To distinguish between these hypotheses, we studied social mobility in five cohorts from three countries. We found that people with more education-linked genetics were more successful compared with parents and siblings. We also found mothers' education-linked genetics predicted their children's attainment over and above the children's own genetics, indicating an environmentally mediated genetic effect. Findings reject pure social-transmission explanations of education GWAS discoveries. Instead, genetics influences attainment directly through social mobility and indirectly through family environments. (See pp. E7275–E7284.)

Delineating the role of cooperativity in the design of potent PROTACs for BTK

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Proteolysis targeting chimera (PROTAC)-based protein degradation is an emerging field that holds significant promise for targeting the "undruggable" proteome: the vast majority of the proteins that do not exhibit enzymatic activity and are thereby not amenable to classical inhibition. Despite significant progress, a thorough mechanistic characterization of biochemical determinants that underpin efficient PROTAC activity is lacking. Here we address one such question: Is positive cooperativity necessary for potent protein degradation? Through a collection of independent techniques, we show that within a Bruton's tyrosine kinase/cereblon PROTAC system, potent knockdown correlates with alleviation of steric clashes in the absence of thermodynamic cooperativity. This result broadens the scope of PROTAC applications and affects fundamental design criteria across the field. (See pp. E7285–E7292.)

Evolutionary repurposing of a sulfatase: A new Michaelis complex leads to efficient transition state charge offset

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The versatility of promiscuous enzymes plays a key role in the evolution of catalysts. This work addresses the molecular mechanism of repurposing a promiscuous enzyme by laboratory evolution and reveals that mutations distinct from the catalytic machinery reshaped the active site. Evolution fine-tuned binding of a previously disfavored Michaelis complex (E·S), repositioning the promiscuous substrate to enable better charge offset during leaving group departure in the transition state. The functional transition relies on maintaining the reactivity of existing catalytic groups in a permissive active-site architecture, able to accommodate multiple substrate binding modes, without requiring changes in conformational dynamics. Such a parsimonious route to higher efficiency illustrates a molecular scenario in which catalytic promiscuity facilitates short adaptive pathways of evolution. (See pp. E7293–E7302.)

Deep mutational analysis reveals functional trade-offs in the sequences of EGFR autophosphorylation sites

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Phosphorylation of tyrosine residues in the cytoplasmic tail of the epidermal growth factor receptor (EGFR) by its kinase domain propagates a rich variety of information downstream of growth factor binding. The amino acid sequences surrounding each phosphorylation site encode the extent of phosphorylation as well as the extent of binding by multiple effector proteins. By profiling the kinase activity of EGFR alongside the binding specificities of an SH2 domain and a PTB domain for thousands of defined phosphorylation site sequences, we discovered that the sequences surrounding the phosphorylation sites in EGFR are not optimal and that discrimination against phosphorylation by cytoplasmic tyrosine kinases such as c-Src and c-Abl is likely to have shaped the evolution of these sequences. (See pp. E7303–E7312.)

Monovalent ions modulate the flux through multiple folding pathways of an RNA pseudoknot

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The assembly mechanism of RNA, vital to describing its functions, depends on both the sequence and the metal ion concentration. How the latter influences the folding trajectories remains an important unsolved problem. Here, we examine the folding pathways of an RNA pseudoknot (PK) with key functional roles in transcription and translation, using a combination of experiments and simulations. We demonstrate that the PK, consisting of two hairpins with differing stabilities, folds by parallel pathways. Surprisingly, the flux between them is modulated by monovalent salt concentration. Our work shows that the order of assembly of PKs is determined by the relative stability of the hairpins, implying that the folding landscape can be controlled by sequence and ion concentration. (See pp. E7313–E7322.)

Cholesterol promotes Cytolysin A activity by stabilizing the intermediates during pore formation

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Pore-forming toxins (PFTs) are the largest class of bacterial exotoxins mediating virulence. Soluble toxin monomers oligomerize upon binding to cellular membrane and convert to stable membrane-integrated pores, causing cell death. This conversion

to an active form occurs in absence of extrinsic factors and is governed solely by molecular determinants in the protein and target membrane. Here we demonstrate the existence of cholesterol-binding motifs in ClyA, which stabilize structural intermediates in the assembly pathway in presence of cholesterol. Our finding elucidates the basis for selective targeting of the toxin to eukaryotic membranes. Molecular engineering of these signatures could advance application of PFTs in cytolytic therapy. (See pp. E7323–E7330.)

Kv2 potassium channels form endoplasmic reticulum/plasma membrane junctions via interaction with VAPA and VAPB

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In addition to functioning as a delayed-rectifier K^+ channel, Kv2.1 interacts with the cortical endoplasmic reticulum (ER) in hippocampal neurons to form somatic ER/plasma membrane (ER/PM) junctions. Neuronal activity and insult induce Kv2.1 release from the cortical ER and subsequent ER withdrawal from the PM. Neuronal ER/PM contacts represent >10% of the cell surface and play roles in membrane trafficking, the regulation of burst firing, Ca^{2+} homeostasis, and control of PM lipid. We report here that Kv2 channel–VAMP-associated protein (VAP) interaction tethers the cortical ER to the PM via a noncanonical FFAT motif contained within the channel C terminus. Since VAPs have a wide-ranging interactome, Kv2-induced ER remodeling and VAP concentration at ER/PM contacts likely play a central role in neuronal physiology. (See pp. E7331–E7340.)

Direction of flagellum beat propagation is controlled by proximal/distal outer dynein arm asymmetry

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The motile flagellum/cilium is found across eukaryotic life, and it performs critical functions in many organisms including humans. A fundamental requirement for a motile flagellum is that it must undergo the appropriate waveform for its specific function. Much is known about the generation of asymmetry in flagellum movement; however, it is unknown how a motile flagellum specifies where waves should start and whether waves should go from base to tip, or from tip to base. We show here in two flagellum model organisms (the human parasites *Trypanosoma brucei* and *Leishmania mexicana*) that differences in the outer dynein arms between the distal and proximal regions of the flagellum determine wave propagation direction and are generated and maintained by the flagellum growth machinery. (See pp. E7341–E7350.)

Enhanced expression of MycN/CIP2A drives neural crest toward a neural stem cell-like fate: Implications for priming of neuroblastoma

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Neuroblastoma is a neural crest-derived pediatric cancer that develops in the embryonic peripheral nervous system (PNS). Studies of PNS progenitors have failed to uncover how tumors initiate or fully recapitulate the most aggressive forms of the disease. Previous transcriptome analysis reveals similarity between some neuroblastoma samples and neural stem cells. Here, we show that ectopic expression of MycN in the neural crest domain of the developing neural tube biases neural crest stem cells toward a more CNS neural stem cell-like fate and thus results in improperly specified neural crest cells. This may play a role as a priming event for tumor initiation, thus providing useful insights into understanding the mechanism behind neuroblastoma formation. (See pp. E7351–E7360.)

Role of carbon allocation efficiency in the temperature dependence of autotroph growth rates

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To predict how plant growth rate will respond to temperature requires understanding how temperature drives the underlying metabolic rates. Although past studies have considered the temperature dependences of photosynthesis and respiration rates underlying growth, they have largely overlooked the temperature dependence of carbon allocation efficiency. By combining a mathematical model that links exponential growth rate of a population of photosynthetic cells to photosynthesis, respiration, and carbon allocation; to an experiment on a freshwater alga; and to a database covering a wide range of taxa, we show that allocation efficiency is crucial for predicting how growth rates will respond to temperature change across aquatic and terrestrial autotrophs, at both short and long (evolutionary) timescales. (See pp. E7361–E7368.)

Stress-testing the relationship between T cell receptor/peptide-MHC affinity and cross-reactivity using peptide velcro

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T cells recognize their targets through the T cell receptor (TCR). The affinity of a typical receptor for an agonist peptide-major histocompatibility complex (pMHC) molecule is extremely weak, and TCRs are known to be cross-reactive for related peptides. However, there are known TCR/pMHC interactions that occur at weaker affinities, such as in thymic selection and recognition of self-antigens, yet little is known about the identity of these peptides. We show that TCR/pMHC interactions of extremely low affinities remain highly specific, which informs of the nature of extremely weak affinity ligands. We also show that a peptide "velcro" can induce peptide-dependent T cell activation, providing a method for increasing the potency of a target, which is useful in immunotherapy. (See pp. E7369–E7378.)

Stem cell-derived clade F AAVs mediate high-efficiency homologous recombination-based genome editing

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Precise genomic correction of pathogenic mutations is an attractive therapeutic strategy. Here, we show that a unique class of stem cell-derived nonpathogenic virus, hematopoietic stem

cell-derived adeno-associated virus vector (AAVHSC), mediates precise genome editing at unprecedented efficiencies in primary human cells, including postmitotic cells, and in vivo. Unlike the majority of current editing platforms, genome editing by AAVHSC is uniquely based on homologous recombination (HR), requiring no exogenous nucleases. AAVHSC-mediated editing is seamless, with no evidence of on-target insertion/deletion mutations common to nuclease-based platforms. Efficient in vivo editing was achieved by i.v. injection of AAVHSC editing vectors. The combination of efficient and precise HR-based genome editing coupled with the superior in vivo transduction properties of AAV facilitates progress toward in vivo therapeutic gene editing. (See pp. E7379–E7388.)

Insights into bacterial lipoprotein trafficking from a structure of LolA bound to the LolC periplasmic domain

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The outer membrane of Gram-negative bacteria presents a selectively permeable barrier to the environment and is the first line of defense against antibiotics and other antimicrobial agents. Maintenance of the outer membrane relies on lipoproteins delivered by the LolABCDE system, making the Lol proteins attractive targets for the development of new antimicrobial compounds. During trafficking, lipoproteins are extracted from the cytoplasmic membrane by the LolCDE complex, transported across the periplasm by LolA, and integrated into the outer membrane by LolB. Here, we describe structural features underpinning the interaction between LolA and LolCDE. The structure of LolA bound to the periplasmic domain of LolC provides an arresting molecular snapshot of a key intermediate in the bacterial lipoprotein trafficking pathway. (See pp. E7389–E7397.)

Mouse maternal protein restriction during preimplantation alone permanently alters brain neuron proportion and adult short-term memory

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Maternal protein malnutrition during pregnancy and lactation compromises brain development, with lasting consequences for motor and cognitive function. However, the importance of nutrition on early brain development is unknown. We have previously shown that maternal low-protein diet confined to the preimplantation period (Emb-LPD) in mice, with normal nutrition thereafter, is sufficient to induce cardiometabolic and locomotor behavioral abnormalities in adult offspring. Here, we report that Emb-LPD and sustained LPD reduce neural stem cells (NSCs) in the fetal brain. Moreover, Emb-LPD causes remaining NSCs to upregulate neuronal differentiation in compensation beyond control levels and increase cortex thickness and neuron ratio, leading to adult memory deficits. These data demonstrate that poor maternal nutrition from conception adversely affects brain development and adult memory. (See pp. E7398–E7407.)

Aspirin binds to PPARα to stimulate hippocampal plasticity and protect memory

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Aspirin, one of the most widely used medications worldwide, binds to PPARα ligand-binding domain at the Tyr314 residue to up-regulate hippocampal plasticity via transcription of CREB. Accordingly, low-dose aspirin also improved hippocampal function in an animal model of Alzheimer's disease via PPARα. These results delineate a new receptor of aspirin through which it may protect memory and learning. (See pp. E7408–E7417.)

Time-resolved neural reinstatement and pattern separation during memory decisions in human hippocampus

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One of the biggest computational challenges the memory system faces is to disambiguate highly similar experiences while at the same time preserving and reinstating prior memories. Remarkably, hippocampal processes have been implicated in both of these functions. However, how this is accomplished is unknown. Leveraging the spatiotemporal resolution of electrocorticography, we found evidence for memory reinstatement in both the hippocampus and occipitotemporal cortex. Interestingly, when a current experience was very similar but not identical to a prior one, occipitotemporal cortical activity still showed reinstatement of the prior memory, but hippocampal activity differentiated or disambiguated these two similar experiences. (See pp. E7418–E7427.)

Phosphodiesterase 2 inhibition preferentially promotes NO/guanylyl cyclase/cGMP signaling to reverse the development of heart failure

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The morbidity and mortality associated with heart failure (HF) are unacceptably high. Cyclic guanosine-3′,5′-monophosphate (cGMP) plays a key role in preserving cardiac structure and function, and therapeutically targeting cGMP in HF has shown promise in experimental models and patients. Phosphodiesterases (PDEs) metabolize and curtail the actions of cGMP (and cAMP), and increased PDE activity is thought to contribute to HF pathogenesis. Herein, we show that inhibition of one specific isoform, PDE2, enhances the salutary effects of cGMP in the context of HF, and that this beneficial action facilitates a distinct pathway, driven by nitric oxide, that is impaired in this disorder. These observations validate PDE2 inhibitors as a demonstrable means of boosting cardiac cGMP and advancing HF therapy. (See pp. E7428–E7437.)

Protease-activated receptor-2 in endosomes signals persistent pain of irritable bowel syndrome

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Activated G protein-coupled receptors (GPCRs) internalize and can continue to signal from endosomes. The contribution of endosomal signaling to human disease is unknown. Proteases that are generated in the colon of patients with irritable bowel syndrome (IBS) can cleave protease-activated receptor-2 (PAR_2) on nociceptors to cause pain. We evaluated whether PAR_2 generates signals in endosomes of nociceptors that mediate persistent hyperexcitability and pain. Biopsies of colonic mucosa from IBS patients released proteases that induced PAR_2 endocytosis, endosomal signaling, and persistent hyperexcitability of nociceptors. When conjugated to the transmembrane lipid cholestanol, PAR_2 antagonists accumulated in endosomes and suppressed persistent hyperexcitability. The results reveal the therapeutic potential of endosomally targeted PAR_2 antagonists for IBS pain, and expand the contribution of endosomal GPCR signaling to encompass processes that are relevant to disease. (See pp. E7438–E7447.)

Environmental limits of Rift Valley fever revealed using ecoepidemiological mechanistic models

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Vector-borne diseases represent complex infection transmission systems; previous epidemiological models have been unable to formally capture the relationship between the ecological limits of vector species and the dynamics of pathogen transmission. By making this advance for the key disease, Rift Valley fever, we are able to show how seasonally varying availability of water bodies and ambient temperatures dictate when the mosquito vector populations will persist and importantly, those sets of conditions resulting in stable oscillations of disease transmission. Importantly, under the latter scenario, short-term health control measures will likely fail, as the system quickly returns to the original configuration after the intervention stops. Our model, therefore, offers an important tool to better understand vector-borne diseases and design effective eradication programs. (See pp. E7448–E7456.)