PNAS Plus Significance Statements

Random subsets of structured deterministic frames have MANOVA spectra

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A frame (overcomplete set of vectors) represents an analog coding scheme. Deterministic frame constructions offer useful codes for communication and signal processing tasks. When the coded signal only uses a random subset of the frame vectors (for example, in compressed sensing), the coding quality is determined by the typical covariances within subsets of frame vectors. We provide a method to calculate functions of these typical covariances, which predict specific performance measures of the corresponding coding scheme. Our method uses a universality property: for many well-known deterministic and random frames, typical covariances within subsets of frame vectors do not depend on the frame and are described by the MANOVA (multivariate ANOVA) ensemble, a classical object in statistics and random matrix theory. (See pp. E5024–E5033.)

Broken flow symmetry explains the dynamics of small particles in deterministic lateral displacement arrays

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Deterministic lateral displacement (DLD) is a technique for size fractionation of particles in continuous flow that has shown great potential for biological and clinical applications. Several theoretical models have been proposed to explain the trajectories of different-sized particles in relation to the geometry of the pillar array, but experimental evidence has demonstrated that a rich class of intermediate migration behavior exists, which is not predicted by models. In this work, we present a unified theoretical framework to infer the trajectory of particles in the whole array on the basis of trajectories in the unit cell. This framework explains many of the unexpected particle trajectories reported in literature and can be used to design arrays for the fractionation of particles, even at the smallest scales reaching the molecular realm. We also performed experiments that verified our predictions, even at the nanoscales. Using our model as a set of design rules, we developed a condenser structure that achieves full particle separation with a single fluidic input. (See pp. E5034–E5041.)

Cupriphication of gold to sensitize d^{10}–d^{10} metal–metal bonds and near-unity phosphorescence quantum yields

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Closed-shell metal–metal bonds are experimentally and theoretically substantiated as bona fide polar-covalent/ligand-unassisted/ground-state Cu(I)–Au(I) d^{10}–d^{10} bonds. Counterintuitively, replacing some of the gold content with copper leads to stronger metal–metal bonding (despite the lesser relativistic effects in Cu vs. Au) and higher phosphorescence quantum efficiency [despite much lower spin-orbit coupling constants (ξ_{SOC}) for 3d vs. 5d orbitals]. The former results are attributed to greater orbital mixing between (n+1)s/p orbitals of one metal with filled nd^{10} orbitals of the other metal in the heterometallic vs. homometallic system, whereas the near-unity photoluminescence quantum yields and higher extinction coefficients in mixed-metal (Au/Cu) heterometallic vs. homometallic systems are attributed to symmetry reduction. The latter results are promising for enabling societally useful technologies (e.g., Ir-/rare-earth-free LEDs). (See pp. E5042–E5051.)

Peripheral modifications of [Ψ[CH_2NH]Tpg^4]vancomycin with added synergistic mechanisms of action provide durable and potent antibiotics

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In a quest for antibiotics that may display durable clinical lifetimes, analogs of the glycopeptide antibiotics, including vancomycin, have been designed that not only directly overcome the molecular basis of existing vancomycin resistance but also contain two added peripheral modifications that endow them with two additional independent mechanisms of actions not found in the parent antibiotics. It is shown that such peripherally and binding pocket-modified vancomycin analogs display little propensity for acquired resistance by vancomycin-resistant Enterococci and that both their antimicrobial potency and durability against such challenges follow trends (three > two > one mechanisms of action) that are now predictable. (See pp. E5052–E5061.)

Interface-induced multiferroism by design in complex oxide superlattices

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Developments in synthesis and characterizing artificially structured materials have greatly advanced the possibility to explore new states of matter in material science. Recent discoveries show that new quantum states can be achieved at heterointerfaces with various electric and mechanical boundary conditions. It remains an open question of how to design ultrathin layers with properties inaccessible in bulk phases that are amenable to technological applications. In this work, we grow heterostructures with extremely high-quality interfaces shown by state-of-the-art atomically resolved electron microscopy and spectroscopy. This combination allows us to identify an interface-induced structure that stabilizes ferromagnetism. Coupled with theory, we provide a conceptually useful recipe to design low-dimensional materials with unique functionalities, in line with the loop "make, measure, model." (See pp. E5062–E5069.)

Network dynamics of social influence in the wisdom of crowds

Joshua Becker, Devon Brackbill, and Damon Centola

Since the discovery of the wisdom of crowds over 100 years ago theories of collective intelligence have held that group accuracy requires either statistical independence or informational diversity among individual beliefs. Empirical evidence suggests that allowing people to observe the beliefs of others leads to increased similarity of individual estimates, reducing independence and diversity without a corresponding increase in group accuracy. As a result, social influence is expected to undermine the wisdom of crowds. We present theoretical predictions and experimental findings demonstrating that, in decentralized networks, social influence generates learning dynamics that reliably improve the wisdom of crowds. We identify general conditions under which influence, not independence, produces the most accurate group judgments. (See pp. E5070–E5076.)

Immunoengineering nerve repair

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Annually, more than 250,000 Americans suffer from a peripheral nerve injury, which results in a loss of function and a compromised quality of life. The current clinical gold standard to bridge long, nonhealing nerve gaps, the autograft, has several drawbacks. Therefore, there is a clear and urgent unmet clinical need for an alternative approach that can match or exceed autograft performance. Here we investigated the regenerative effect of fractalkine, a chemokine that preferentially recruits reparative monocytes in the synthetic nerve conduit. Our method of bridging gaps enhanced axonal regeneration and muscle reinnervation and showed results comparable to those observed in autografts. (See pp. E5077–E5084.)

Computationally optimized deimmunization libraries yield highly mutated enzymes with low immunogenicity and enhanced activity

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Nature produces a variety of proteins that could be tapped for therapeutic applications. This paper focuses on the bacterial enzyme β-lactamase, a component of antibody-directed enzyme prodrug therapies designed to activate cytotoxic prodrugs selectively at sites of malignancy. Unfortunately, like many other non-human proteins, β-lactamase evokes an antidrug immune response that limits its clinical potential. This paper demonstrates that a multiobjective library-design method enables incorporation of mutations throughout the protein, modifying portions that trigger immune recognition while simultaneously preserving stability and catalytic activity. The libraries were inherently enriched in beneficial variants, and they produced numerous candidates that were both highly functional and immunologically stealthy. The method is general purpose and could enable functional deimmunization of other biotherapeutic agents. (See pp. E5085–E5093.)

Mouse model of hematogenous implant-related *Staphylococcus aureus* biofilm infection reveals therapeutic targets

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Hematogenous implant-related infections are an important clinical problem because bacteria spread from the bloodstream to a previously well-functioning implant and result in infectious complications and failure of a medical device or prosthesis. To study these infections, we developed a preclinical animal model of a *Staphylococcus aureus* hematogenous implant infection with the capability to monitor noninvasively and longitudinally the dissemination of the bacteria from the blood to a surgically placed orthopedic implant. Using this model, α-toxin and clumping factor A were identified as key factors that contributed to the pathogenesis of these infections by promoting biofilm formation. Finally, neutralizing antibodies against these factors provided a targeted, non-antibiotic alternative approach to help prevent these difficult-to-treat and costly infections. (See pp. E5094–E5102.)

Trigger loop of RNA polymerase is a positional, not acid–base, catalyst for both transcription and proofreading

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Synthesis of new RNA and removal of incorrect nucleotides during proofreading by RNA polymerase involve the transfer of two protons. Here, we show that a polymerase component, the trigger loop, does not directly mediate proton transfer during these reactions, as previously proposed. Instead, the trigger loop plays a central role in transcription as a positional catalyst, by orienting the reactants and promoting the polymerase backtracking necessary for proofreading. This positional-catalyst model of trigger-loop function explains its diverse effects on polymerase catalysis and reconciles contradictory reports in the literature. By establishing that the trigger loop is not an acid–base catalyst, our results also guide the search for alternative proton donors and acceptors for reactions in the active site of polymerase. (See pp. E5103–E5112.)

Cas1 and the Csy complex are opposing regulators of Cas2/3 nuclease activity

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Prokaryotes have adaptive immune systems that rely on CRISPRs (clustered regularly interspaced short palindromic repeats) and

diverse CRISPR-associated (*cas*) genes. Cas1 and Cas2 are conserved components of CRISPR systems that are essential for integrating fragments of foreign DNA into CRISPR loci. In type I-F immune systems, the Cas2 adaptation protein is fused to the Cas3 interference protein. Here we show that the Cas2/3 fusion protein from *Pseudomonas aeruginosa* stably associates with the Cas1 adaptation protein, forming a 375-kDa propeller-shaped Cas1–2/3 complex. We show that Cas1, in addition to being an essential adaptation protein, also functions as a repressor of Cas2/3 nuclease activity and that foreign DNA binding by the CRISPR RNA-guided surveillance complex activates the Cas2/3 nuclease. (See pp. E5113–E5121.)

Spacer capture and integration by a type I-F Cas1–Cas2-3 CRISPR adaptation complex

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CRISPR-Cas systems provide prokaryotic adaptive immunity against invading genetic elements. For immunity, fragments of invader DNA are integrated into CRISPR arrays by Cas1 and Cas2 proteins. Type I-F systems contain a unique fusion of Cas2 to Cas3, the enzyme responsible for destruction of invading DNA. Structural, biophysical, and biochemical analyses of Cas1 and Cas2-3 from *Pectobacterium atrosepticum* demonstrated that they form a 400-kDa complex with a $Cas1_4$:$Cas2\text{-}3_2$ stoichiometry. Cas1–Cas2-3 binds, processes, and catalyzes the integration of DNA into CRISPR arrays independent of Cas3 activity. The arrangement of Cas3 in the complex, together with its redundant role in processing and integration, supports a scenario where Cas3 couples invader destruction with immunization—a process recently demonstrated in vivo. (See pp. E5122–E5128.)

Selective oxidation of aliphatic C–H bonds in alkylphenols by a chemomimetic biocatalytic system

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Selective oxidation of aliphatic C–H bonds in alkylphenols is important for both structural derivatization and biological degradation of these fundamental chemicals. However, significant problems are persistently associated with the chemical methods for this oxofunctionalization. In this study, we developed a unique chemomimetic biocatalytic system that is capable of selectively oxidizing *p*- and *m*-alkylated phenols in a controllable manner, overcoming the challenges faced by similar chemical oxidation. The structural and bioinformatics analyses of the central P450 biocatalyst CreJ suggest that its substrate flexibility and reaction selectivity could be further leveraged. This novel alkylphenol biooxidation system may hold great potential for application in pharmaceutical, biomanufacturing, and environmental industries once upscaled systems can be further developed in the future. (See pp. E5129–E5137.)

Efficient, ultra-high-affinity chromatography in a one-step purification of complex proteins

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Protein purification is a primary step and the basis for numerous biochemical and biomedical studies. It is particularly crucial for high-resolution structural analysis and industrial protein production, where it has to meet the high-yield, high-purity, and high-activity (HHH) requirement. However, the HHH purification of many proteins or protein complexes remains a difficult, target-dependent, and multistep process. The ultra-high-affinity (CL7/Im7) purification system described in this work allows for one-step HHH purification of a wide range of traditionally challenging biological molecules, including eukaryotic, membrane, toxic, and DNA/RNA-binding proteins and complexes. It might emerge as an efficient, universal tool for high-throughput isolation of many significant biological systems to advance modern biological studies as well as manufacturing of therapeutic proteins. (See pp. E5138–E5147.)

Retroviral host range extension is coupled with Env-activating mutations resulting in receptor-independent entry

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The emergence of novel infectious diseases is often associated with cross-species virus transmission. Virus host range is owed, in large part, to genetic variation of its envelope gene. The illustrative example presented here is based on a structural study of the Rous sarcoma virus (RSV) avian retrovirus passaged twice through rodents. RSV heterotransmission was enabled by spontaneous envelope gene mutations, which allow virus entry even into human and other receptor-free cells. Through three independent biochemical assays, we established that these mutations caused conformational change similar to the one that follows virus–receptor interaction. The newly identified envelope mutations responsible for the broad retrovirus host range contribute to our understanding of retroviruses' pathogenicity and their ability to cross the transclass barriers. (See pp. E5148–E5157.)

Deubiquitinating enzyme VCIP135 dictates the duration of botulinum neurotoxin type A intoxication

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Botulinum neurotoxins (BoNTs) are the most potent biological toxins. These proteases function by cleaving SNARE proteins, leading to paralysis and death from respiratory failure. BoNT serotype A (BoNT/A) has the most prolonged symptoms due to an extraordinarily stable catalytic light chain (LCA). BoNT/A intoxication can occur through ingestion (either sporadically or from a common food source), as a consequence of a clinical mishap, or potentially through bioterrorism, which would require mass mechanical ventilation. We report that LCA persistence is due to a particular deubiquitinating enzyme that binds a specific region of LCA and prevents its ubiquitin-dependent proteasomal degradation. These findings represent an essential step toward developing targeted molecular approaches to reducing morbidity and mortality from this toxin. (See pp. E5158–E5166.)

Optogenetic control of mitochondrial metabolism and Ca^{2+} signaling by mitochondria-targeted opsins

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Mitochondrial functions depend on the steep H^+ electrochemical gradient ($\Delta\mu H^+$) across their inner membrane. The available tools for

controlling this gradient are essentially limited to inhibitors of the respiratory chain or of the H^+ ATPase or to uncouplers, poisons plagued by important side effects and that lack both cell and spatial specificity. We show here that, by transfecting cells with the cDNA encoding channelrhodopsins specifically targeted to the inner mitochondrial membrane, we can obtain an accurate and spatially confined, light-dependent control of mitochondrial membrane potential and, as a consequence, of a series of mitochondrial activities ranging from electron transport to ATP synthesis and Ca^{2+} signaling. (See pp. E5167–E5176.)

Biomechanical coupling facilitates spinal neural tube closure in mouse embryos

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Neurulation has been intensively studied in lower vertebrates, but marked species differences call into question the relevance of these models for human neural tube (NT) closure. Here, using mouse embryos, we demonstrate that mammalian neural fold apposition results from constriction of the open posterior NT, which is biomechanically coupled to the zippering point by an F-actin network. Using the *Zic2* mutant model, we show that genetic predisposition to spina bifida, which likely underlies most human cases, directly affects the biomechanics of closure. We also identify a NT closure point at the caudal end of the embryo. Many spina bifida cases correspond to this anatomic portion of the NT, suggesting that this closure point may be important in humans as well. (See pp. E5177–E5186.)

How temporal patterns in rainfall determine the geomorphology and carbon fluxes of tropical peatlands

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A dataset from one of the last protected tropical peat swamps in Southeast Asia reveals how fluctuations in rainfall on yearly and shorter timescales affect the growth and subsidence of tropical peatlands over thousands of years. The pattern of rainfall and the permeability of the peat together determine a particular curvature of the peat surface that defines the amount of naturally sequestered carbon stored in the peatland over time. This principle can be used to calculate the long-term carbon dioxide emissions driven by changes in climate and tropical peatland drainage. The results suggest that greater seasonality projected by climate models could lead to carbon dioxide emissions, instead of sequestration, from otherwise undisturbed peat swamps. (See pp. E5187–E5196.)

Skin-specific regulation of SREBP processing and lipid biosynthesis by glycerol kinase 5

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We discovered a previously unrecognized regulator of cholesterol biosynthesis, glycerol kinase 5 (GK5), which functions exclusively in the skin independently of cholesterol regulation in other tissues. GK5 negatively regulates the processing and nuclear localization of sterol regulatory element binding proteins, transcription factors that control expression of virtually all cholesterol synthesis enzymes. Excessive amounts of cholesterol, triglycerides, and ceramides were found in the skin of GK5-deficient mice. These mice displayed alopecia (hair loss) caused by impaired hair growth and maintenance, for which proper amounts of cholesterol and other skin lipids are necessary. (See pp. E5197–E5206.)

Genome-wide CRISPR screen identifies HNRNPL as a prostate cancer dependency regulating RNA splicing

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Alternative RNA splicing and the spliceosome machinery have been implicated in cancer progression. A genome-wide CRISPR screen identified the RNA processing factor heterogeneous nuclear ribonucleoprotein L (HNRNPL) as required for prostate cancer growth by regulating alternative RNA splicing and circular RNA formation. HNRNPL and its RNA clients are overexpressed during prostate cancer progression, supporting their potential role as therapeutic targets. (See pp. E5207–E5215.)

Systems-guided forward genetic screen reveals a critical role of the replication stress response protein ETAA1 in T cell clonal expansion

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T cells are required for control of many intracellular infections, and a critical component of T cell immunity is the proliferative expansion of effector T cells upon stimulation. Using a forward-based genetic screen, we identify the mouse *Etaa1* gene as critically important for T cell proliferative expansion after vaccination and during infection. Consistent with recent findings that ETAA1 prevents DNA damage during proliferation, our data demonstrate elevated DNA damage within *Etaa1*-deficient effector T cells, which likely leads to cell death. This phenotype is restricted to effector T cell proliferation, with T cell development and other immune parameters remaining normal. Thus, ETAA1 may represent a novel drug target to selectively suppress pathological T cell responses in transplantation or autoimmunity. (See pp. E5216–E5225.)

Maintenance of antiangiogenic and antitumor effects by orally active low-dose capecitabine for long-term cancer therapy

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Withdrawal of antiangiogenic drugs leads to tumor revascularization, even rebound effects of tumor angiogenesis, and potential metastasis. Long-term maintenance therapy with antiangiogenic drugs is crucial for achieving maximal clinical benefits for cancer patients. There are no antiangiogenic drugs that are clinically available for maintenance therapy. We show that orally active capecitabine at an extremely low and nontoxic dose displays a potent antiangiogenic effect that can be used for maintenance therapy. Importantly, the low-dose capecitabine in a sequentially therapeutic setting following anti-VEGF drugs produces remarkable anticancer effects. If successfully proven in clinical settings, our therapeutic regimen would be beneficial for millions of cancer patients. Our findings will shift the paradigm of current antiangiogenic therapy for treatment of human cancer patients. (See pp. E5226–E5235.)

Electron-shuttling antibiotics structure bacterial communities by modulating cellular levels of c-di-GMP

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In cells residing within communities, limitation for electron acceptors (e.g., oxygen) leads to redox imbalance and hampers metabolism. *Pseudomonas aeruginosa* bacteria in biofilms produce antibiotics called phenazines that facilitate redox balancing. When phenazines and oxygen are not sufficiently available, *P. aeruginosa* biofilms increase production of ECM, which increases surface area-to-volume ratio and access to oxygen. Here, we describe RmcA, a protein that regulates matrix production in response to phenazine exposure. RmcA contains four sensing domains and two domains that modulate levels of a small-molecule inducer of matrix secretion. These results provide molecular insight into mechanisms underpinning the adaptability of *P. aeruginosa* biofilms, which likely contributes to their persistence in diverse hosts and clinical settings. (See pp. E5236–E5245.)

Numbers of presynaptic Ca^{2+} channel clusters match those of functionally defined vesicular docking sites in single central synapses

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Release–docking sites have been considered from a morphological point of view as the sites where synaptic vesicles dock and fuse with the active-zone membrane. Functionally, docking sites limit the maximum number of vesicles released per action potential, determining synaptic strength. Until now it has not been possible to establish a clear link between morphological and functional aspects of docking sites in mammalian brain synapses. Recent data suggest that calcium channels form several clusters in the active zone. Here, we show that the number of the clusters matches that of functional docking sites and both parameters change in parallel with age and synaptic size. Based on these results we propose a one-to-one correspondence between docking sites and calcium channel clusters. (See pp. E5246–E5255.)

Regulation of spinogenesis in mature Purkinje cells via mGluR/PKC-mediated phosphorylation of CaMKIIβ

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The cerebellar cortex, and its sole output, Purkinje cells, is essential for motor coordination and learning. Dendritic spines of Purkinje cells are the primary sites of cerebellar synaptic plasticity. Therefore, maintenance of spine structure of mature Purkinje cells is a critical aspect of cerebellar functions, but the underlying mechanisms remain unclear. Here we described an activity-dependent regulatory mechanism of spines in Purkinje cells. We found that F-actin cross-linking activity of Ca^{2+}/calmodulin-dependent protein kinase II β isoform is controlled by protein kinase C-mediated phosphorylation, which is triggered by group I metabotropic glutamate receptor signaling. Defective function of the phosphorylation leads to excess spine development in mature Purkinje cells. Our findings revealed a mechanism for proper maintenance of cerebellar Purkinje cell spines. (See pp. E5256–E5265.)

Specific targeting of TGF-β family ligands demonstrates distinct roles in the regulation of muscle mass in health and disease

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Myostatin, via activation of the Smad2/3 pathway, has long been recognized as the body's major negative regulator of skeletal muscle mass. In this study, however, we demonstrate that other TGF-β proteins, particularly activin A and activin B, act in concert with myostatin to repress muscle growth. Preventing activin and myostatin signaling in the tibialis anterior muscles of mice resulted in massive hypertrophy (>150%), which was dependent upon both the complete inhibition of the Smad2/3 pathway and activation of the parallel bone morphogenetic protein (BMP)/Smad1/5 axis. Using this approach in models of muscular dystrophy and cancer cachexia increased muscle mass or prevented muscle wasting, respectively, highlighting the potential therapeutic advantages of complete inhibition of Smad2/3 ligand activity in skeletal muscle. (See pp. E5266–E5275.)