PNAS Plus Significance Statements

State-space multitaper time-frequency analysis

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Rapid growth in sensor and recording technologies is spurring rapid growth in time series data. Nonstationary and oscillatory structure in time series is commonly analyzed using timevarying spectral methods. These widely used techniques lack a statistical inference framework applicable to the entire time series. We develop a state-space multitaper (SS-MT) framework for time-varying spectral analysis of nonstationary time series. We efficiently implement the SS-MT spectrogram estimation algorithm in the frequency domain as parallel 1D complex Kalman filters. In analyses of human EEGs recorded under general anesthesia, the SS-MT paradigm provides enhanced denoising (>10 dB) and spectral resolution relative to standard multitaper methods, a flexible time-domain decomposition of the time series, and a broadly applicable, empirical Bayes' framework for statistical inference. (See pp. E5–E14.)

Initial elevation bias in subjective reports

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People's reports of their own thoughts, feelings, and behaviors are essential assessment tools in biomedical and social science. When subjective states have been studied over time, however, researchers have often observed an unpredicted and puzzling decrease with repeated assessments. Our results across multiple outcomes in four field experiments suggest that this pattern is due to an initial elevation bias. This effect is larger for reports of internal states than for behaviors and for negative mental states and physical symptoms than for positive states. This initial elevation bias needs to be considered in all types of research using subjective reports. (See pp. E15–E23.)

Functions of maize genes encoding pyruvate phosphate dikinase in developing endosperm

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Mutations affecting the transcription factor encoded by the gene *o2* are important in maize agriculture because they result in improved grain nutritional quality. The mutations also cause detrimental effects by reducing kernel hardness and diminishing agronomic quality and food applications. The undesirable characteristics are not fully understood because the *o2* product regulates multiple targets that could contribute to the phenotype. This study investigated one target that had not been previously mutated, pyruvate phosphate dikinase (PPDK), and showed that PPDK deficiency in isolation causes the negative phenotype associated with reduced kernel hardness. Thus, maize improvement may be better accomplished by targeting individual metabolic pathways determining protein and amino acid balance rather than pleiotropic regulators such as the *o2* transcription factor. (See pp. E24–E33.)

Artificial antibody created by conformational reconstruction of the complementary-determining region on gold nanoparticles

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Mimicking protein-like specific interactions and functions has been a long-pursued goal in nanotechnology. The key challenge is to precisely organize nonfunctional surface groups on nanoparticles into specific 3D conformations to function in a concerted and orchestrated manner. Here, we develop a method to graft the complementary-determining regions of natural antibodies onto nanoparticles and reconstruct their "active" conformation to create nanoparticle-based artificial antibodies that recognize the corresponding antigens. Our work demonstrates that it is possible to create functions on nanoparticles by conformational engineering, namely tuning flexible surface groups into specific conformations. Our straightforward strategy could be used further to create other artificial antibodies for various applications and provides a new tool to understand the structure and folding of natural proteins. (See pp. E34–E43.)

Structure of the chlorovirus PBCV-1 major capsid glycoprotein determined by combining crystallographic and carbohydrate molecular modeling approaches

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The three-dimensional structure of Vp54, the major capsid protein of the chlorovirus *Paramecium bursaria* chlorella virus (PBCV-1), was determined by combining two powerful techniques, X-ray diffraction and carbohydrate molecular modeling. This strategy resolved the limitations posed by each technique alone and increases our understanding of how glycans fold,

their flexibility, and the type of interactions they exert within the protein component. (See pp. E44–E52.)

Structure of sexual networks determines the operation of sexual selection

Grant C. McDonald and Tommaso Pizzari

Sexual selection is a powerful evolutionary force, but debate persists over its strength and quantification. We argue that current approaches ignore the structure of the sexual network. We capture this network structure with a metric we call "mating assortment" that precisely and exhaustively captures the indirect as well as direct relationship between a male's promiscuity and that of his sexual partners. We show that mating assortment is highly variable in nature and use simulations to reveal that such variation plays a key—but so far unappreciated—role in determining the strength of sexual selection on males. Our results provide a clear and quantitative method for studying sexual selection in the many mating systems in which both polygyny and polyandry co-occur. (See pp. E53–E61.)

In vivo inhibition of tryptophan catabolism reorganizes the tuberculoma and augments immune-mediated control of *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis induces the expression of the indoleamine 2,3-dioxygenase (IDO) enzyme, which catabolizes tryptophan. Tryptophan metabolites potently suppress host immunity. The present study demonstrates that blockade of IDO activity reduces both clinical manifestations of tuberculosis (TB) as well as microbial and pathological correlates of the human TB syndrome in macaques. In granulomas, T cells localize in the periphery, and are unable to access the core, where bacilli persist. Inhibiting IDO activity altered granuloma organization such that more T cells translocated to the lesion core and exhibited highly proliferative signatures. Our results identify a highly efficient immunosuppressive mechanism at play in the granuloma environment that aids in *M. tuberculosis* persistence. The ability to modulate this pathway with safe and approved compounds could, however, facilitate chemotherapy-adjunctive host-directed therapy approaches for the control of TB. (See pp. E62–E71.)

Affimer proteins inhibit immune complex binding to FcγRIIIa with high specificity through competitive and allosteric modes of action

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Autoimmune disease pathogenesis is driven by inflammation, induced partly by IgG autoantibody-containing immune complexes binding to Fc gamma receptors (FcγRs). These receptors are valid therapeutic targets in the treatment of autoimmunity. FcγRIIIa is one of a family of highly homologous receptors for IgG antibodies; previous attempts at therapeutic blockade have resulted in off-target effects involving cells that express the almost identical protein FcγRIIIb. Here we report the identification of functionally specific protein-based inhibitors (Affimer proteins) of FcγRIIIa and the structural/functional basis of their selectivity. As molecular research tools FcγRIIIa-specific Affimer proteins provide the ability to block IgG interaction with a single receptor. Our findings suggest that highly selective protein-based blocking agents that may have therapeutic applications can be readily produced. (See pp. E72–E81.)

Synergistic anti-HCV broadly neutralizing human monoclonal antibodies with independent mechanisms

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More than 71 million people are infected with HCV, and eradication of this pandemic will likely require a vaccine. Induction of broadly neutralizing monoclonal antibodies (bNAbs) is a goal of vaccine development, but no single bNAb neutralizes all strains of HCV. Here, we measured neutralizing activity of 35 combinations of neutralizing monoclonal antibodies (NAbs), showing that some NAbs form combinations with greater neutralizing breadth than any individual bNAb. One combination was also exceptionally potent because it blocks virus binding to three different HCV receptors. These data suggest that full-length envelope protein might have an advantage as a vaccine antigen relative to truncated protein or single-epitope scaffolds, since it might induce combinations of NAbs that are synergistic, with complementary neutralizing breadth. (See pp. E82–E91.)

Metagenomics-guided analysis of microbial chemolithoautotrophic phosphite oxidation yields evidence of a seventh natural CO_2 fixation pathway

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Phosphite (HPO_3^{2-}) is the most energetically favorable biological electron donor known, but only one organism capable of growing by phosphite oxidation has been previously identified. Here, we describe a phosphite-oxidizing bacterium that can grow with CO_2 as its sole electron acceptor, and we propose a metabolic model in which inorganic carbon is assimilated via the reductive glycine pathway. Although the reductive glycine pathway has previously been identified as a "synthetic" carbon fixation pathway, this study provides evidence that it may actually function as a natural autotrophic pathway. Our results suggest that phosphite may serve as a driver of microbial growth and carbon fixation in energy-limited environments, particularly in aphotic environments lacking alternative terminal electron acceptors. (See pp. E92–E101.)

Behavioral state modulates the ON visual motion pathway of *Drosophila*

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Animal visual systems are typically thought of by analogy to cameras—sensory systems providing continuous information streams that are processed through fixed algorithms. However, studies in flies and mice have shown that visual neurons are dynamically and adaptively retuned by the behavioral state of the animal. In *Drosophila*, prominent higher-order neurons in the visual system respond more strongly to fast-moving stimuli once the animal starts walking or flying. In this study, we systematically investigated the neurobiological mechanism governing the behavioral-state modulation of directionally selective neurons in *Drosophila*. We show that behavioral activity modifies the physiological properties of critical neurons in this visual motion circuit and that neuromodulation by central feedback neurons recapitulates these effects. (See pp. E102–E111.)