PNAS Plus Significance Statements

Linguistic positivity in historical texts reflects dynamic environmental and psychological factors

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For nearly 50 y social scientists have observed that across cultures and languages people use more positive words than negative words, a phenomenon referred to as "linguistic positivity bias" (LPB). Although scientists have proposed multiple explanations for this phenomenon—explanations that hinge on mechanisms ranging from cognitive biases to environmental factors—no consensus on the origins of LPB has been reached. In this research, we derive and test, via natural language processing and data aggregation, divergent predictions from dominant explanations of LPB by examining it across time. We find that LPB varies across time and therefore cannot be explained simply as the product of cognitive biases and, further, that these variations correspond to fluctuations in objective circumstances and subjective mood. (See pp. E7871–E7879.)

Discovery of cofactor-specific, bactericidal *Mycobacterium tuberculosis* InhA inhibitors using DNA-encoded library technology

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The increasing prevalence of multidrug-resistant strains of tuberculosis has created an urgent need for novel therapies to treat tuberculosis infections. Here we have demonstrated the successful utilization of the DNA-encoded X-Chem technology for the discovery inhibitors of *Mycobacterium tuberculosis* enoyl–acyl-carrier protein (ACP) reductase, InhA, a validated target for the treatment of tuberculosis. The identified inhibitors are cofactor specific and have activity in multiple cellular assays. Crystal structures of representative compounds from five chemical series revealed that the compounds bind adjacent to the NADH cofactor and adopt a variety of conformations, including two previously unreported binding modes. The compounds identified may serve as useful leads in the development of new antibacterial drugs with efficacy against multidrug-resistant tuberculosis. (See pp. E7880–E7889.)

Substrate recognition and catalysis by GH47 α-mannosidases involved in Asn-linked glycan maturation in the mammalian secretory pathway

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Asn-linked glycosylation of newly synthesized polypeptides occurs in the endoplasmic reticulum of eukaryotic cells. Glycan structures are trimmed and remodeled as they transit the secretory pathway, and processing intermediates play various roles as ligands for folding chaperones and signals for quality control and intracellular transport. Key steps for the generation of these trimmed intermediates are catalyzed by glycoside hydrolase family 47 (GH47) α-mannosidases that selectively cleave α1,2-linked mannose residues. Despite the sequence and structural similarities among the GH47 enzymes, the molecular basis for residue-specific cleavage remains obscure. The present studies reveal enzyme–substrate complex structures for two related GH47 α-mannosidases and provide insights into how these enzymes recognize the same substrates differently and catalyze the complementary glycan trimming reactions necessary for glycan maturation. (See pp. E7890–E7899.)

Molecular evidence of keratin and melanosomes in feathers of the Early Cretaceous bird *Eoconfuciusornis*

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We report fossil evidence of feather structural protein (beta-keratin) from a 130-My-old basal bird (*Eoconfuciusornis*) from the famous Early Cretaceous Jehol Biota, which has produced many feathered dinosaurs, early birds, and mammals. Multiple independent molecular analyses of both microbodies and associated matrix recovered from the fossil feathers confirm that these microbodies are indeed melanosomes. We use transmission electron microscopy and immunogold to show localized binding of antibodies raised against feather protein to matrix filaments within these ancient feathers. Our work sheds new light on molecular constituents of tissues preserved in fossils. (See pp. E7900–E7907.)

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Paired quantitative and qualitative assessment of the replication-competent HIV-1 reservoir and comparison with integrated proviral DNA

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A reservoir of latently infected cells poses the greatest challenge to HIV-1 eradication. Efforts to develop strategies to eliminate the reservoir have been hampered, in part, by the lack of a precise understanding of the cellular and molecular nature of this reservoir. We describe a new method to analyze the replication-competent latent reservoir quantitatively and qualitatively. We find that over 50% of the replication-competent viruses in the reservoir form part of groups with identical *env* sequences. However, a negative correlation exists between integrated proviral clones and replication-competent viruses, such that the larger the proviral clone, the lower is its probability of representing a replication-competent virus. (See pp. E7908–E7916.)

MIF allele-dependent regulation of the MIF coreceptor CD44 and role in rheumatoid arthritis

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High-expression alleles of the cytokine macrophage migration inhibitory factor (MIF) are associated with severe joint destruction in autoimmune arthritis, but the mechanism for this effect is unknown. High-genotypic *MIF*-expressing joint fibroblasts produce high levels of MIF under inflammatory stimulation to up-regulate the surface expression of the MIF signaling coreceptor CD44 and promote its alternative splicing into invasive, tumor-associated isoforms, which contribute to the invasive and tissue-destructive character of the rheumatoid joint synovium. These findings support a precision medicine approach to the treatment of rheumatoid arthritis by pharmacologically targeting the MIF pathway in high-genotypic *MIF*-expressing patients. (See pp. E7917–E7926.)

An oligotrophic deep-subsurface community dependent on syntrophy is dominated by sulfur-driven autotrophic denitrifiers

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Microorganisms are known to live in the deep subsurface, kilometers below the photic zone, but the community-wide metabolic networks and trophic structures (the organization of their energy and nutritional hierarchy) remain poorly understood. We show that an active subsurface lithoautotrophic microbial ecosystem (SLiME) under oligotrophic condition exists. Taxonomically and metabolically diverse microorganisms are supported, with sulfur-driven autotrophic denitrifiers predominating in the community. Denitrification is a highly active process in the deep subsurface that evaded recognition in the past. This study highlights the critical role of metabolic cooperation, via syntrophy between subsurface microbial groups, for the survival of the whole community under the oligotrophic conditions that dominate in the subsurface. (See pp. E7927–E7936.)

Proteomics and comparative genomics of *Nitrososphaera viennensis* reveal the core genome and adaptations of archaeal ammonia oxidizers

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Ammonia-oxidizing archaea (AOA), key players in global biogeochemical cycles, represent a heterogeneous group with a broad environmental distribution. Understanding their activity and physiology is of great importance due to the impact of the overuse of agricultural fertilizers on the N cycle and the production of the greenhouse gas N_2O during nitrification. Despite their prominent ecological role, little is known about the fundamental metabolic processes of AOA. Here, we show that AOA of marine and terrestrial environments share unique and well-conserved pathways of carbon and nitrogen metabolism, and we raise hypotheses about missing steps in these pathways. Our approach also highlights the extensive environmental adaptations of the soil clade, including the capacity for cell surface modifications, carbohydrate conversions, detoxification, and biofilm formation. (See pp. E7937–E7946.)

Reconstitution of a *Mycobacterium tuberculosis* proteostasis network highlights essential cofactor interactions with chaperone DnaK

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The proteostasis pathway may be a source of new drug targets in *Mycobacterium tuberculosis* (Mtb). The conserved protein chaperone DnaK is essential in *Mycobacterium smegmatis* and predicted to be essential in Mtb. DnaK is regulated by cofactors, J proteins and nucleotide exchange factor GrpE. In contrast to most bacterial pathogens, Mtb has two J proteins, DnaJ1 and DnaJ2. Here, we characterize in vitro activities of Mtb DnaK, DnaJ1, DnaJ2, and GrpE, the disaggregase ClpB, and the small heat shock protein Hsp20, in reactivation of a protein aggregate. We found that DnaJ1 and DnaJ2 are individually dispensable, but collectively essential and mutations in a conserved motif of each result in cellular loss of function. These findings will help in identifying and characterizing inhibitors of Mtb's proteostasis network. (See pp. E7947–E7956.)

Context-dependent memory traces in the crab's mushroom bodies: Functional support for a common origin of high-order memory centers

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Mushroom bodies (MBs) are higher brain structures of several invertebrate groups, vastly studied in insects as a key structure involved in memory processes. Moreover, MBs and the vertebrate pallium have been proposed to share an ancestral common origin. In crustaceans, the hemiellipsoid bodies (HBs) are proposed to be homologues of the insect MBs. However, functional evidence for the involvement of HBs in memory processes is lacking. Here, in the crab *Neohelice*, we show memory traces in the HBs that, as for MBs, reflect the context attribute of memory. These results extend the homology based on anatomy and gene expression to the functional level. Consequently, present data support the hypothesis of a common origin for the arthropods' high-order memory centers. (See pp. E7957–E7965.)

Emergence of an abstract categorical code enabling the discrimination of temporally structured tactile stimuli

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What are the neural codes that allow the discrimination of two vibrotactile stimulus patterns of equal mean frequency? We recorded single-neuron activity in primary somatosensory (S1) and dorsal premotor (DPC) cortex while trained monkeys performed a challenging pattern discrimination task. We found a faithful representation of the stimuli in S1 and a heavily transformed, more abstract, and highly varied set of responses in DPC. Most notably, in addition to memory-related activity and responses encoding the monkeys' choices, the DPC data included a large set of categorical neurons that code specific combinations of past and present stimuli and, at the same time, are strongly predictive of the monkeys' behavior. (See pp. E7966–E7975.)

Loss of β-adrenergic–stimulated phosphorylation of $Ca_V1.2$ channels on Ser1700 leads to heart failure

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Calcium entry initiates contraction in cardiac myocytes, and altered expression of voltage-gated calcium channel 1.2 ($Ca_V1.2$) causes heart failure in mice. Here we show that reducing β-adrenergic regulation of $Ca_V1.2$ by mutation of a PKA site in the C-terminal domain causes age-related heart failure. Dual mutation of a nearby casein-kinase II phosphorylation site accelerated heart failure. The PKA level was increased; PKA-mediated phosphorylation of ryanodine receptor type-2, phospholamban, and troponin-I was increased; the calcium pool in the sarcoplasmic reticulum was increased; and the activity of the calcium-dependent phosphoprotein phosphatase calcineurin was persistently elevated. These changes in mice with a mutation at the PKA site Ser1700 (SA mice) suggest that compensatory mechanisms may initially enhance contractility but eventually cause increased sensitivity to cardiovascular stress and heart failure. (See pp. E7976–E7985.)

Mechanism of gating by calcium in connexin hemichannels

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Connexin channels are ubiquitous, providing pathways for movement of molecules between cells (junctional channels) and for release of molecular effectors into the extracellular environment (plasma membrane hemichannels). To maintain an adequate permeability barrier, hemichannels are tightly regulated by normal extracellular Ca^{2+} to be closed under most conditions. Connexin mutations that disrupt hemichannel regulation by Ca^{2+} cause human pathologies due to aberrantly open hemichannels. Here we elucidate molecular mechanisms of gating by Ca^{2+} in hemichannels: Ca^{2+} binding causes a reorganization of specific interactions within the connexin protein that lead to a closed channel. Further, we show that the actual "gate" is deeper into the pore from where Ca^{2+} binds. The interactions involved are conserved across connexins, pointing to a general mechanism. (See pp. E7986–E7995.)

Root nodule symbiosis in *Lotus japonicus* drives the establishment of distinctive rhizosphere, root, and nodule bacterial communities

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Legumes are known as pioneer plants colonizing marginal soils, and as enhancers of the nutritional status in cultivated soils. This beneficial activity has been explained by their capacity to engage in symbiotic relationship with nitrogen-fixing rhizobia. We performed a community profiling analysis of *Lotus japonicus* wild type and mutants to investigate the role of the nodulation pathway on the structure of the root-associated bacterial microbiota. We found that several bacterial orders were almost entirely depleted from the mutant roots, and that an intact symbiosis is needed for the establishment of taxonomically diverse and distinctive bacterial communities in the root and rhizosphere. Our findings imply that a symbiosis-linked bacterial community, rather than dinitrogen-fixing rhizobia alone, contributes to legume growth and ecological performance. (See pp. E7996–E8005.)