PNAS Plus Significance Statements

Evaluating optimal therapy robustness by virtual expansion of a sample population, with a case study in cancer immunotherapy

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A successful cancer therapy induces a strong antitumor response while causing minimal side effects. The heterogeneous nature of cancer observed across different regions of the primary tumor, across metastatic sites, across time, and across patients makes designing such a successful therapy challenging. Both standard of care and finely tailored treatment protocols run the risk of not exhibiting a robust antitumor response in the face of these uncertainties. Here we introduce a platform for exploring this robustness question using treatment response data from a sample population. Our method integrates these experimental data with statistical and mathematical techniques, allowing us to quantify therapeutic robustness. Using this approach, we identified a robust therapeutic protocol that combines oncolytic viruses with an immunotherapeutic vaccine. (See pp. E6277–E6286.)

Local destabilization, rigid body, and fuzzy docking facilitate the phosphorylation of the transcription factor Ets-1 by the mitogen-activated protein kinase ERK2

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This study provides experimental support for the mechanism of proximity-mediated catalysis by a mitogen-activated protein (MAP) kinase. It describes how the transcription factor Ets-1 uses the cumulative effects of two suboptimal docking interactions, rather than a single canonical docking interaction, to enable a unique bipartite mechanism of recognition of the MAP kinase ERK2. This mode of interaction between Ets-1 and ERK2 facilitates the formation of a highly productive complex that not only induces the proximity of Ets-1 phospho-acceptor (T38) to the ERK2 active site, but does so in optimal fashion, thereby promoting efficient phospho-transfer. (See pp. E6287–E6296.)

Identification of a vesicular ATP release inhibitor for the treatment of neuropathic and inflammatory pain

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Although the incidence of chronic pain is estimated at 20–25% worldwide, optimal drug treatment regimens with few side effects have yet to be developed. In this study, we demonstrated that clodronate is a potent and selective inhibitor of vesicular ATP release that attenuates neuropathic and inflammatory pain unrelated to bone abnormalities. Clodronate was more effective and associated with comparatively fewer side effects than existing drugs. Thus, the nonopioid agent clodronate may represent a unique treatment strategy for chronic pain via inhibition of vesicular ATP release, suggesting that clodronate may be effective in the treatment of several diseases involving purinergic chemical transmission, including inflammatory diseases, diabetes, and neurological disorders. Our study identifies a transporter-targeted analgesic drug. (See pp. E6297–E6305.)

Effect of directional pulling on mechanical protein degradation by ATP-dependent proteolytic machines

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Directional degradation is an important feature of energy-dependent intracellular proteolysis in all cells. Proteolytic machines can unravel protein substrates from either terminus depending on the site of initial recognition. Here, we use single-molecule optical trapping to probe how the bacterial AAA+ proteases ClpXP and ClpAP mechanically degrade a model substrate, titin^{I27}, from the N terminus. N-terminal unfolding of this substrate is often accomplished with one or a few power strokes and is much faster than C-terminal unfolding, although translocation is only mildly affected by the direction. Our results highlight the paramount role of local stability in protein degradation and provide clues as to how the placement of degradation signals on a substrate may evolve to minimize the energetic cost of degradation. (See pp. E6306–E6313.)

Symmetry-related proton transfer pathways in respiratory complex I

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Complex I is a redox-driven proton pump, central to aerobic energy conversion in most living organisms. To elucidate the mechanism of its pumping machinery, we need a detailed molecular picture of how access across the membrane is established and regulated. In this work we find that proton pathways in complex I form at symmetry-related locations near broken transmembrane helices. The channel opening allows influx of water molecules, catalyzing rapid Grotthuss-type proton transfer reactions. The

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hydration of these channels is sensitive to the protonation state of conserved buried lysine residues, which are in turn coupled to conformational changes in conserved ion pairs within each subunit. Our results provide mechanistic insight into the function of the long-range proton-pumping machinery in complex I. (See pp. E6314–E6321.)

Sequential eviction of crowded nucleoprotein complexes by the exonuclease RecBCD molecular motor

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Chromosomes are crowded places, and any nucleic acid motor proteins that act on DNA must function within these crowded environments. How crowded environments affect motor protein behaviors remains largely unexplored. Here, we use single-molecule fluorescence microscopy to visualize the ATP-dependent motor protein RecBCD as it travels along crowded DNA molecules bearing long tandem arrays of DNA binding proteins. Our findings show that RecBCD can push through highly crowded protein arrays while evicting the proteins from DNA. This behavior on crowded DNA is distinct from a previously described mechanism by which RecBCD disrupts single isolated nucleoprotein complexes. These findings may provide insights into how other types of motor proteins travel along crowded nucleic acids. (See pp. E6322–E6331.)

Intrinsically disordered chromatin protein NUPR1 binds to the C-terminal region of Polycomb RING1B

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We describe the interaction between an intrinsically disordered protein (IDP), NUPR1, and the well-folded C-terminal region of RING1B protein of the Polycomb complex, involving residues Ala33 and Thr68 of the IDP. These observations are significant because they raise the possibility that NUPR1 aids the function of the members of the Polycomb complex, playing an active role in carcinogenesis. (See pp. E6332–E6341.)

Decoupling of size and shape fluctuations in heteropolymeric sequences reconciles discrepancies in SAXS vs. FRET measurements

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Conformational properties of unfolded and intrinsically disordered proteins (IDPs) under native conditions are important for understanding the details of protein folding and the functions of IDPs. The average dimensions of these systems are quantified using the mean radius of gyration and mean end-to-end distance, measured by small-angle X-ray scattering (SAXS) and single-molecule Förster resonance energy transfer (smFRET), respectively, although systematic discrepancies emerge from these measurements. Through holistic sets of studies, we find that the disagreements arise from chemical heterogeneity that is inherent to heteropolymeric systems. This engenders a decoupling between different measures of overall sizes and shapes, thus leading to discrepant inferences based on SAXS vs. smFRET. Our findings point the way forward to obtaining comprehensive descriptions of ensembles of heterogeneous systems. (See pp. E6342–E6351.)

Conserved gene regulatory module specifies lateral neural borders across bilaterians

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The lateral neural plate border (NPB) gives rise to the neural crest, one of the precursors of the peripheral nervous system (PNS) and generally considered an evolutionary innovation of the vertebrate lineage. Recently, it has been reported that a rudimentary neural crest exists in protovertebrate *Ciona*, but whether this is true in other invertebrates and there is conserved molecular machinery specifying the NPB lineage are unknown. We present evidence that orthologs of the NPB specification module specify lateral neural progenitor cells in several invertebrates, including worm, fly, and tunicate. We propose that an ancient lateral neuroblast gene regulatory module was coopted by chordates during the evolution of PNS progenitors. (See pp. E6352–E6360.)

Insights into the red algae and eukaryotic evolution from the genome of *Porphyra umbilicalis* (Bangiophyceae, Rhodophyta)

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Fossil evidence shows that red algae (Rhodophyta) are one of the most ancient multicellular lineages. Their ecological, evolutionary, and commercial importance notwithstanding, few red algal nuclear genomes have been sequenced. Our analyses of the *Porphyra umbilicalis* genome provide insights into how this macrophyte thrives in the stressful intertidal zone and into the basis for its nutritional value as human food. Many of the novel traits (e.g., cytoskeletal organization, calcium signaling pathways) we find encoded in the *Porphyra* genome are extended to other red algal genomes, and our unexpected findings offer a potential explanation for why the red algae are constrained to small stature relative to other multicellular lineages. (See pp. E6361–E6370.)

Autophagy-related protein Vps34 controls the homeostasis and function of antigen cross-presenting CD8α^+ dendritic cells

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Dendritic cells (DCs) of the immune system are critical for displaying foreign antigens to T lymphocytes, a process called "antigen presentation." This process may involve Vacuolar protein sorting 34 (Vps34), a protein implicated in diverse cellular processes, including endocytosis, an extracellular product uptake system, and autophagy, an intracellular degradation system. Here we have generated and analyzed mice in which the *Vps34* gene is specifically knocked out in DCs. These animals displayed defects in the survival and function of a subset of DCs specialized in presenting antigens from dead cells to T cells. Thus our findings have revealed a critical contribution of Vps34 in DC functions that

may have important implications for targeting this pathway for therapeutic purposes. (See pp. E6371–E6380.)

Visualizing context-dependent calcium signaling in encephalitogenic T cells in vivo by two-photon microscopy

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Before invading the central nervous system, encephalitogenic T cells cross a series of microenvironments where they interact with local cells. T-cell activation was visualized by specific calcium signals using a combination of a genetic calcium reporter, Twitch1, and in vivo two-photon microscopy. In peripheral immune organs, short-lived calcium signaling indicated antigen-independent interactions. By contrast, in the CNS, saturated long-lived calcium signaling was induced by endogenous autoantigens presented by a subset of local antigen-presenting cells. Because T-cell trafficking is controlled at serial checkpoints, our findings may help to identify therapeutic targets for preventing CNS inflammation. (See pp. E6381–E6389.)

Inhibition of complement C5 protects against organ failure and reduces mortality in a baboon model of *Escherichia coli* sepsis

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Complement activation occurs when bacteria invade the circulating blood, leading not only to removal of the pathogen but also to inflammation, organ damage, and poor prognosis for septic patients. We used a baboon model of *Escherichia coli* bacteremia to determine the effects of a C5 inhibitor on bacteriolysis, bacteria clearance, and sepsis progression. We observed that complement-mediated bacteriolysis has a detrimental effect by inducing release of LPS and fulminant inflammation. Inhibition of C5 cleavage and subsequent formation of the lytic terminal complex C5b-9 diminished LPS release, blocked sepsis-induced inflammation, decreased the associated consumptive coagulopathy, and protected organ function. Overall, treatment with C5 inhibitor significantly improved the survival of septic baboons, suggesting a potentially important strategy to treat bacteremic sepsis. (See pp. E6390–E6399.)

A distinct subpopulation of CD25$^-$ T-follicular regulatory cells localizes in the germinal centers

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T-follicular regulatory (Tfr) cells, a subset of Foxp3-expressing regulatory T (Treg) cells, have a critical role in the control of antibody responses. Whereas Treg cells express CD25 and are dependent on IL-2, Tfr cells also express the transcription factor BCL6 that is inhibited by IL-2 in T-follicular helper (Tfh) cells. In this report, we find that mature Tfr cells in the germinal centers or circulating in human blood down-regulate CD25 and gain a transcriptional signature mixed between Tfh cells and Treg cells while retaining their regulatory function. These cells represent an IL-2–independent branch of effector Treg cells losing CD25 expression but gaining increased expression of Tfh-related markers, such as BCL6 and CXCR5, in both mice and humans. (See pp. E6400–E6409.)

Intracellular zinc activates KCNQ channels by reducing their dependence on phosphatidylinositol 4,5-bisphosphate

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M-type (Kv7, KCNQ) potassium channels are powerful regulators of neuronal and muscular excitability and are validated drug targets for the treatment of excitability disorders. The plasma membrane phosphoinositide phosphatidylinositol 4,5-bisphosphate (PIP_2) is required to maintain M channel activity. We report that intracellular free zinc directly and reversibly augments the activity of recombinant and native M channels by reducing or virtually abolishing the channel requirement for PIP_2, thereby permitting a PIP_2-gated ion channel to operate independently of this important signaling molecule. Given the growing recognition of zinc as an intracellular second messenger, this phenomenon might represent a hitherto unknown pathway of M channel modulation and provide a fresh strategy for design of M channel-targeting drugs. (See pp. E6410–E6419.)

Inhibition of the integrated stress response reverses cognitive deficits after traumatic brain injury

Austin Chou, Karen Krukowski, Timothy Jopson, Ping Jun Zhu, Mauro Costa-Mattioli, Peter Walter, and Susanna Rosi

Traumatic brain injury (TBI) is a leading cause of long-term neurological disability and affects an ever-growing population. Currently, there are no effective treatments for patients suffering from chronic TBI-induced cognitive impairments. Here, we found that suppression of the integrated stress response (ISR) with a drug-like small-molecule inhibitor, ISRIB, rescued cognition in two TBI mouse models, even when administered weeks after injury. Consistent with the behavioral results, ISRIB restored long-term potentiation deficits observed in TBI mice. Our data suggest that targeting ISR activation could serve as a promising approach for the treatment of chronic cognitive dysfunction after TBI. (See pp. E6420–E6426.)

Prostaglandin dehydrogenase is a target for successful induction of cervical ripening

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Prostaglandin E_2 (PGE_2), a cervical ripening agent, mediates unique EP2 receptor signaling pathways in human cervical stromal cells targeting its own synthesis by increasing cyclooxygenase-2 (COX-2) and PGE synthase (PTGES) expression and decreasing its metabolism by loss of its degradative enzyme 15-hydroxy prostaglandin dehydrogenase (15-PGDH). Here, we show that down-regulation of 15-PGDH is crucial for PGE_2-induced cervical ripening and preterm birth. This report details unique mechanisms of PGE_2 action in the cervix and serves as a catalyst for (*i*) use of PGDH inhibitors to initiate, or amplify, PGE_2-mediated cervical ripening and (*ii*) EP2 receptor antagonists, histone deacetylase 4 (HDAC4) inhibitors, or 15-PGDH activators to prevent preterm cervical ripening and preterm birth. (See pp. E6427–E6436.)

Functional selectivity for face processing in the temporal voice area of early deaf individuals

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Here, we show that deaf individuals activate a specific and discrete subregion of the temporal cortex, typically selective to voices in hearing people, for visual face processing. This reorganized "voice"

region participates in face identity processing and responds selectively to faces early in time, suggesting that this area becomes an integral part of the face network in early deaf individuals. Observing that face processing selectively colonizes a region of the hearing brain that is functionally related to identity processing evidences the intrinsic constraints imposed to cross-modal plasticity. Our work therefore supports the view that, even if brain components modify their sensory tuning in case of deprivation, they maintain a relation to the computational structure of the problems they solve. (See pp. E6437–E6446.)

Diverse continuum of CD4$^+$ T-cell states is determined by hierarchical additive integration of cytokine signals

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Understanding the logic by which cells respond to complex signal combinations is challenging. We used CD4$^+$ T cells as a model system to study signal integration by systematically mapping their differentiation in response to a large number of cytokine combinations. We find that, in response to varied cytokine mixtures, cells coexpress lineage-specifying proteins at diverse levels, such that the cell population spans a continuum of intermediate states between canonical cell phenotypes. Mathematical modeling explains these results using hierarchical summation of cytokine inputs and correctly predicts population response to new input conditions. These findings suggest that complex cellular responses can be effectively described using relatively simple hierarchical summation rules, providing a framework for prediction of cellular responses to signal combinations. (See pp. E6447–E6456.)

Cellular trade-offs and optimal resource allocation during cyanobacterial diurnal growth

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Cyanobacteria are important players in Earth's biogeochemical cycles and a promising resource for the synthesis of renewable raw materials. Of particular interest are the cellular organization that enables fast growth and the corresponding intracellular limits on growth rates. Here, we develop a constraintbased computational model of phototrophic growth to investigate the optimal allocation of cellular resources in a diurnal light environment. The model-derived optimal metabolite partitioning during diurnal growth is in qualitative agreement with recent experimental data. Our results suggest that phototrophic metabolism at fast growth rates is highly optimized and strongly dependent on the timing characteristics of enzyme synthesis. Furthermore, we demonstrate that the experimentally observed pattern of glycogen accumulation is in agreement with predictions based on optimal resource allocation. (See pp. E6457–E6465.)

Dynamic responses of the adrenal steroidogenic regulatory network

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Our ability to respond to stress depends on a remarkably dynamic process of hormone secretion. The rapid release of glucocorticoid hormones from the adrenal glands is critical to mount such an efficient response to stress, particularly during inflammation. Although many key factors involved in this process have been studied, the way in which these factors interact dynamically with one another to regulate glucocorticoid secretion has not been investigated. Here, we develop a mathematical model of the regulatory network controlling glucocorticoid synthesis and, by combining this with in vivo experiments, show how this network governs changes in adrenal responsiveness under basal unstressed physiological conditions and under exposure to inflammatory stress. (See pp. E6466–E6474.)