PNAS Plus Significance Statements

Mapping the stochastic response of nanostructures

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A challenge in nanotechnology is that nanoscale structures exhibit a rate-dependent and apparently random (stochastic) load-deflection behavior when repeated experimental measurements are compared. The variability in mechanical response is due to the extreme nonconvexity of the nanostructure's energy versus structure function. The many minima of this function correspond to equilibrium states, accessible during loading, that create many possible pathways. Traditional atomistic simulation techniques cannot systematically address this complexity. Here (pp. E1678–E1686), we explore a new method based on constructing an "equilibrium map" (akin to a phase diagram) that efficiently and systematically explores a nanostructure's stochastic, rate-dependent response to specified loading conditions. We demonstrate the method's capabilities and its surprisingly complex results for the case of a nanoslab of nickel under uniaxial compression.

Architecture and assembly of the archaeal Cdc48·20S proteasome

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From microbes to humans, proteolytic machines called proteasomes cleave proteins that are damaged or unnecessary into peptide fragments. Proteasomes minimally consist of the barrel-like 20S peptidase and an AAA+ ring, which harnesses chemical energy to unfold and translocate proteins into the 20S chamber for degradation. Here (pp. E1687–E1694), we determine the architecture of a recently discovered proteasome, Cdc48·20S, by electron microscopy. A continuous axial channel allows translocation through the double AAA+ rings of Cdc48 into the 20S chamber. A model in which dynamic "wobbling" of the AAA+ unfoldase relative to 20S is necessary for function is ruled out for Cdc48·20S by electron-microscopy results showing coaxial alignment of Cdc48 and 20S and by the proteolytic activity of cross-linked complexes.

Conserved electron donor complex Dre2–Tah18 is required for ribonucleotide reductase metallocofactor assembly and DNA synthesis

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Ribonucleotide reductases (RNR) play a critical role in supplying cellular deoxynucleotide pools. Nucleotide reduction by class Ia RNR requires a diferric-tyrosyl radical cofactor, which is a target of anticancer agents. How this essential cofactor is assembled in vivo is not well understood. We show here (pp. E1695–E1704) that a conserved protein complex composed of the Fe-S–requiring Dre2 and the diflavin-requiring Tah18, previously shown to donate electrons for Fe-S cluster assembly for proteins found in the cytosol and nucleus, also is required for RNR cofactor assembly. Deficiency in this complex leads to activation of both the DNA-damage checkpoint and the iron regulon, linking iron homeostasis to maintenance of genome stability. These findings may provide new insights into development of RNR-targeted therapeutics.

Measurement of histidine pK_a values and tautomer populations in invisible protein states

Alexandar L. Hansen and Lewis E. Kay

Electrostatic interactions in proteins play significant roles in conferring stability and in dictating function. Histidine residues are particularly important because their side chains can serve as both acids and bases over the physiological pH range and as both hydrogen bond donors and acceptors. Solution NMR spectroscopy is a powerful method for studying these residues in highly populated ground-state conformers. Here (pp. E1705–E1712) we develop a strategy for extending such studies to sparsely populated, short-lived protein states that can also play a significant role in defining protein function. An application to an invisible folding intermediate of the Im7 protein is provided, where site-specific pK_a values of histidine residues have been determined that explain the strong pH-dependent stability differences between native and intermediate states.

Initial steps of inactivation at the K^+ channel selectivity filter

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C-type inactivation represents a key process that governs cellular K^+ channel activity. Although C-type inactivation seems to be inextricably linked with dissociation of K^+ from the channel's pore, the structural connection between K^+ dissociation and initiation of C-type inactivation has been unclear. Here (pp. E1713–E1722), we combine electrophysiology and molecular simulation of MthK, a prototypical K^+ channel of known structure, to determine relations between K^+ dissociation and entry into the inactivated state. We find that Ca^{2+} can bind to a site in the pore favored by outward movement of K^+. K^+ subsequently dissociates, favoring a conformational change to the inactivated state. This study, thus, establishes a direct link between K^+ dissociation and initiation of C-type inactivation.

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Generation of multiciliated cells in functional airway epithelia from human induced pluripotent stem cells

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Pulmonary disease is the third highest cause for morbidity and mortality worldwide. Studies of human lung disease in vivo or in vitro are currently limited. Using induced pluripotent stem cells (pp. E1723–E1730), we developed a step-wise differentiation protocol ending in an air–liquid interface to generate a pseudostratified polarized layer of endodermal-derived epithelial cells (forkhead box protein $A2^+$ and NK2 homeobox 1^+). This layer includes Clara cells with Clara cell 10 kD-positive vesicles, mucin 5A/C-positive goblet cells, multiciliated cells, and isolated cells that have forskolin-induced chloride currents sensitive to cystic fibrosis transmembrane regulator inhibitor 172. The development of this model will enable the future study of many lung diseases (especially those where defective cilia are involved, such as primary ciliary dyskinesia) that have been difficult to study in human models from a developmental perspective.

Cellular heterogeneity profiling by hyaluronan probes reveals an invasive but slow-growing breast tumor subset

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Tumor heterogeneity is a poorly understood phenomenon. We lack biomarkers for identifying aggressive primary tumor subsets that give rise to metastases and impact early cancer detection and treatment. Many solid tumors are known to accumulate hyaluronan (HA), a glycosaminoglycan, which is also produced by the tumor cells themselves. We report (pp. E1731–E1739) a quantitative approach for uncovering breast cancer heterogeneity using fluorescent HA to detect differential binding patterns to CD44 and RHAMM/HMMR receptors. This approach permits identification of tumor-cell subsets that bind high levels of HA, and may be applicable to other ligands/receptors and disease models. Despite representing the invasive/metastatic subset of parental tumors, unexpectedly, the high HA-binding subset was slow-growing, and is thus likely to be a source of dormancy and relapse.

Brf1 posttranscriptionally regulates pluripotency and differentiation responses downstream of Erk MAP kinase

Frederick E. Tan and Michael B. Elowitz

Intercellular signaling pathways strongly regulate gene expression in uncommitted precursor stem cells, but the mechanisms through which these signaling pathways regulate gene targets often remain unclear. We address this question in mouse embryonic stem cells (mESCs) and highlight the importance of AU-rich element mRNA-binding proteins as regulatory intermediates of intercellular signaling. We show (pp. E1740–E1748) that the FGF/Erk MAP kinase signaling pathway strongly influences the expression of Brf1, a member of the Zfp36 protein family that is known to bind and destabilize its mRNA targets. Brf1 physically binds many pluripotency and differentiation-associated mRNAs. Moderate changes in its expression compromise self-renewal capacity and bias fate commitment, thus providing a posttranscriptional link between intercellular signaling activity and gene expression in mESCs.

Humoral response to a viral glycan correlates with survival on PROSTVAC-VF

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Because individual cancer patients differ considerably in their clinical benefits from immunotherapies, early indicators of response could help physicians personalize treatments. Unfortunately, conventional clinical response criteria can be misleading for cancer vaccines. Herein (pp. E1749–E1758), we show that early humoral responses to xenogenic Forssman disaccharide displayed on PROSTVAC-VF's viral vectors correlate with long-term survival of vaccinated prostate cancer patients. The survival correlation for anti-Forssman responses was observed consistently when PROSTVAC-VF was used either as monotherapy or combined with the radiopharmaceutical Quadramet. Monitoring postvaccination anti-Forssman humoral responses could offer a simple indicator of response many months before conventional clinical response criteria become reliable. Finally, this study suggests that modifying glycans may improve poxvirus-based vaccines even when not specifically designed to target glycans.

Diverse mutational pathways converge on saturable chloroquine transport via the malaria parasite's chloroquine resistance transporter

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This study provides detailed insights into the workings of a protein that is a key determinant of drug resistance in the malaria parasite. We found (pp. E1759–E1767) that two main lineages of mutational routes lead to chloroquine transport via the chloroquine resistance transporter (PfCRT) and that a low level of chloroquine transport is conferred by as few as two mutations. However, the attainment of full transport activity is a rigid process that requires the mutations be added in a specific order to avoid decreases in chloroquine transport. Our finding that diverse forms of mutant PfCRT are all limited in their capacity to transport chloroquine indicates that resistance should be overcome by reoptimizing the chloroquine dosage.

Evolutionary pathway to increased virulence and epidemic group A *Streptococcus* disease derived from 3,615 genome sequences

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Epidemics of microbial infections are a considerable threat to human and animal health. Analysis of 3,615 genome sequences (pp. E1768–E1776), coupled with virulence studies in animals, permitted us to delineate the nature and timing of molecular events that contributed to an ongoing global human epidemic of infections caused by group A *Streptococcus*, the "flesh-eating" pathogen. We clarified decades-long uncertainty about the timing and sequence of genomic alterations that underpinned the global epidemic. Analyses of this type are crucial for developing better strategies to predict and monitor strain emergence and epidemics, formulate effective protective public health maneuvers, and develop or modify vaccines.

Transformation of the cerebellum into more ventral brainstem fates causes cerebellar agenesis in the absence of *Ptf1a* function

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The contribution of cell fate misspecification to human brain disorders is poorly understood. The cerebellum, a major center of motor and sensory coordination, is frequently malformed in humans. During development it arises from dorsal hindbrain, but a long-standing question has been how the cerebellum is established along the dorsal–ventral axis of the neural tube. Here (pp. E1777–E1786) we identified the gene encoding pancreatic transcription factor PTF1A, which is inactivated in patients with cerebellar agenesis, as the first gene regulating the ventral limit of the cerebellum. We describe transformation of cerebellar neurons into more ventral extracerebellar fates as a novel mechanism of cerebellar agenesis. Our data provide some of the strongest evidence reported to date for a critical role of cell fate misspecification in a human brain developmental phenotype.

Homeostasis of functional maps in active dendrites emerges in the absence of individual channelostasis

Rahul Kumar Rathour and Rishikesh Narayanan

Voltage-gated ion channels and their subcellular localization profiles mediate several continuous maps of neuronal physiological properties and confer astounding computational capabilities on single neurons. How do neurons with complex morphologies maintain these functional maps despite constant turnover of and plasticity in the ion channels that mediate them? We addressed this question through a computational framework spanning channels and measurements from the cell body and dendrites of hippocampal neurons. Our results (pp. E1787–E1796) demonstrate that individual channel properties or their densities need not be maintained at constant levels in achieving overall homeostasis of several coexistent functional maps. We suggest collective channelostasis, where several channels regulate their properties and expression profiles in an uncorrelated manner, as an alternative for accomplishing homeostasis of functional maps.

Thalamocortical rhythms during a vibrotactile detection task

Saskia Haegens, Yuriria Vázquez, Antonio Zainos, Manuel Alvarez, Ole Jensen, and Ranulfo Romo

When a near-threshold sensory stimulus is presented, a sensory percept may or may not be produced. The exact process by which neural activity elicits subjective perception is a long-standing open question. Here, we ask what role brain oscillations in early sensory regions play in this cognitive process, as oscillations are thought to reflect population dynamics, indicative of the state of a brain network. We found (pp. E1797–E1805) task-related modulations in oscillatory activity in both the somatosensory thalamus and primary sensory cortex, which were correlated with the perceptual decision process and subsequent behavioral response. We conclude that these early sensory regions, in addition to their primary sensory functions, may be actively involved in perceptual decision making.

Control of vacuolar dynamics and regulation of stomatal aperture by tonoplast potassium uptake

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Rapid fluxes of K^+ and other osmolytes in guard cells control the opening and closing of stomata and thereby gas exchange and transpiration of plants. Despite the well-established role of the plasma membrane of guard cells in stomatal function, osmolyte uptake into the cytosol represents only a transient step to the vacuole, as more than 90% of the solutes accumulate in these organelles. We show (pp. E1806–E1814) that the tonoplast-localized K^+/H^+ exchangers mediate the vacuolar accumulation of K^+ in guard cells, and that activity of these transporters controls not only stomatal opening but also stomatal closure. We also establish vacuolar K^+/H^+ exchange as a critical component involved in vacuolar remodeling and the regulation of vacuolar pH during stomatal movements.