PNAS Plus Significance Statements

Energetic cost of building a virus

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Viruses rely entirely on their host as an energy source. Despite numerous experimental studies that demonstrate the capability of viruses to rewire and undermine their host's metabolism, we still largely lack a quantitative understanding of an infection's energetics. However, the energetics of a viral infection is at the center of broader evolutionary and physical questions in virology. By enumerating the energetic costs of different viral processes, we open the door to quantitative predictions about viral evolution. For example, we predict that, for the majority of viruses, translation will serve as the dominant cost of building a virus, and that selection, rather than drift, will govern the fate of new genetic elements within viral genomes. (See pp. E4324–E4333.)

Spread of Zika virus in the Americas

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Mathematical and computational modeling approaches can be essential in providing quantitative scenarios of disease spreading, as well as projecting the impact in the population. Here we analyze the spatial and temporal dynamics of the Zika virus epidemic in the Americas with a microsimulation approach informed by high-definition demographic, mobility, and epidemic data. The model provides probability distributions for the time and place of introduction of Zika in Brazil, the estimate of the attack rate, timing of the epidemic in the affected countries, and the projected number of newborns from women infected by Zika. These results are potentially relevant in the preparation and analysis of contingency plans aimed at Zika virus control. (See pp. E4334–E4343.)

Controllable load sharing for soft adhesive interfaces on three-dimensional surfaces

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In transfer printing, robotics, and precision manufacturing, adhesion-controlled grasping of complex 3D surfaces is very challenging because the adhesive must be soft enough to enable intimate contact under light pressure but stiff enough to support high load and fracture strength. We address this dilemma by replacing the adhesive with a pressurized microfiber array that enables independent control of 3D conformability and bond strength. This architecture exhibits enhanced and robust adhesion on various sizes of 3D and deformable surfaces. In contrast to other microfiber adhesives, it has the area scalability of the natural gecko footpad. These features suggest that the proposed soft-gripping system can outperform conventional adhesive systems for a broad range of surface shapes and length scales. (See pp. E4344–E4353.)

Large-moment antiferromagnetic order in overdoped high-T_c superconductor $^{154}SmFeAsO_{1-x}D_x$

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Using neutron powder diffraction measurements on the bulk highest-T_c superconductor $^{154}SmFeAsO_{1-x}D_x$, we discovered a new antiferromagnetic (AFM) phase in the electron-overdoped regime, $x \geq 0.56$ (AFM2). The magnetic moment on Fe in AFM2 reaches 2.73 μ_B/Fe, which is the largest in all the nondoped iron-based antiferromagnets reported so far. Our theoretical calculations reveal that the AFM2 phase in $SmFeAsO_{1-x}H_x$ originates in the kinetic frustration of the Fe-3d_{xy} orbital, in which the Fe-3d_{xy} nearest-neighbor hopping parameter becomes zero. The unique phase diagram, i.e., the highest-T_c superconducting phase adjacent to the strongly electron-correlated phase in heavily electron-doped regime (not nondoped regime), yields important clues to the unconventional origins of superconductivity. (See pp. E4354–E4359.)

EHD2 restrains dynamics of caveolae by an ATP-dependent, membrane-bound, open conformation

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The EHD2 protein controls the association of membrane pits termed caveolae to cell surfaces. Caveolae are implicated in muscle, pulmonary, and lipid disorders. We establish functionally, and structurally, how EHD2 cycles between an active, membrane-bound state and an inactive state in solution. We present an approach to resolve the structure of proteins in their membrane-bound state, which is difficult to obtain otherwise. A dramatic conformational change of EHD2 upon membrane binding is demonstrated. ATP binding is required for partial membrane insertion and subsequent oligomerization. In solution, internal regulatory regions inhibit the conformational change. This stringently regulated

mechanistic cycle might be prototypical for a large family of proteins involved in membrane fission and may open avenues to control the process in vivo. (See pp. E4360–E4369.)

Control of Hsp90 chaperone and its clients by N-terminal acetylation and the N-end rule pathway

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We found that the yeast Hsp90 chaperone system is greatly impaired in *naa10Δ* cells, which cannot N-terminally acetylate a majority of normally N-terminally acetylated proteins, including Hsp90 and its cochaperones. Hsp90 clients, including Chk1, Kar4, Tup1, Gpd1, Ste11, and even the Hsp90 chaperone (Hsc82) itself, became short-lived substrates of the Arg/N-end rule pathway in *naa10Δ* cells. Ubr1 targets the Chk1 kinase through its internal degron. Interactions of Hsp90 with Chk1 could be detected in wild-type but not in *naa10Δ* cells. These results revealed a major role of N-terminal acetylation in the Hsp90-mediated protein homeostasis and showed that a number of Hsp90 clients are previously unknown substrates of the Arg/N-end rule pathway. (See pp. E4370–E4379.)

Ubiquitin- and ATP-dependent unfoldase activity of P97/VCP•NPLOC4•UFD1L is enhanced by a mutation that causes multisystem proteinopathy

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The ATPase p97 plays an important cellular role by extracting proteins modified with ubiquitin (Ub) from membranes, chromatin, or protein complexes. However, the unstable and complicated nature of p97 substrates has hindered a detailed study of mechanism. To overcome these issues, we developed Ub-GFP as a fluorescent reporter of p97 activity. When Ub-GFP is conjugated with ubiquitin chains, p97 and its cofactor NPLOC4-UFD1L unfold it in an ATP-dependent manner, explicitly demonstrating that p97 is an unfoldase. We also show that a p97 mutation associated with multisystem proteinopathy has enhanced unfoldase activity, which suggests a novel approach to disease therapy. Our method opens the door for future studies of p97 mechanism that were until now not feasible. (See pp. E4380–E4388.)

Kinematics of the lever arm swing in myosin VI

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Myosin VI (MVI), a molecular motor whose malfunction is linked to deafness, moves on the actin filament fueled by ATP. The chemomechanical transduction culminates in a power stroke, in which the motor domain undergoes a conformational transition exaggerated by the lever arm. We performed simulations of the MVI power stroke, showing that the lever arm undergoes a nearly free rotational diffusion that is only weakly biased by the rest of the motor. Our model yields a molecular picture of the MVI power stroke that is in quantitative agreement with experiments showing evidence for the pliancy of the lever arm. Our findings provide insights into the broad step-size distribution of MVI. (See pp. E4389–E4398.)

Translation and folding of single proteins in real time

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How proteins fold natively with efficient fidelity while being synthesized remains largely unexplored. Understanding protein synthesis on a single-molecule level is of particular interest to the life sciences and relevant for various diseases. Although protein synthesis and folding are well-studied subjects, cotranslational folding has been proven difficult to observe. Using optical tweezers, we measured the mechanics of synthesis and simultaneous folding in real time. We found that cotranslational folding occurs at predictable locations, exerting forces on the nascent polypeptide. Furthermore, we show that transient pauses and gradual slowing of translation occur in particular locations along the protein sequence, facilitating native secondary-structure formation. Thus, the rate of synthesis is inherently coupled to cotranslational folding, assuring reliable and fast native folding. (See pp. E4399–E4407.)

Direct observation of structure and dynamics during phase separation of an elastomeric protein

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An increasing number of proteins have been shown to undergo liquid–liquid phase separation in response to changes in their environment, resulting in formation of a dense protein-rich phase (coacervate), and plays an important role in several systems regulating the growth and development of cells and tissues. Determining the effects of phase separation on protein structure and dynamics is critical for understanding how it modulates protein function. However, structural studies have been limited by the intrinsic disorder and decreased mobility of coacervated proteins. We report direct observation of protein structure and dynamics during the phase transition of an elastomeric protein. Despite large changes in dynamics, coacervation has little effect on protein structure, such that intrinsic disorder is retained. (See pp. E4408–E4415.)

Control of metastatic niche formation by targeting APBA3/Mint3 in inflammatory monocytes

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Metastasis complicates cancer treatment. This process is intricately orchestrated by both cancer and normal cells. Thus, new anti-metastasis drugs targeting normal cells that interact with cancer cells constitutes a promising approach, as long as body homeostasis is unaffected. Here, we focused on a unique feature of metastasis-promoting monocytes/macrophages: their dependence on aerobic glycolysis for energy production. APBA3 promotes aerobic glycolysis by activating HIF-1 in macrophages. APBA3 depletion decreases ATP production by approximately 60% in macrophages without affecting other cells. We show that depletion of APBA3 in monocytes suppresses their recruitment to the metastatic niche and E-selectin induction in endothelial cells, resulting in metastasis inhibition. Thus, targeting APBA3 may be useful for preventing metastasis, with few side effects. (See pp. E4416–E4424.)

Spatially restricted dental regeneration drives pufferfish beak development

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Teleost fishes have evolved a wonderful array of diverse dentitions. The highly derived order Tetraodontiformes exhibits the most unique dental forms among teleosts. The novel beak-like dentition of the pufferfish develops through a drastic shift in dental morphology during ontogeny. A simple first-generation tooth set is followed by the repetitive development of multiple elongated jaw-length tooth bands, which fuse together over time to form the characteristic beak. A restriction of the tooth-regenerative process in all but four tooth sites, coupled with the maintenance of lifelong stem cells for perpetual tooth development,

is essential for the formation of this unique dentition. In pufferfish, regeneration plays a vital role in producing this novel dental form from highly conserved developmental underpinnings. (See pp. E4425–E4434.)

Long-read sequencing uncovers the adaptive topography of a carnivorous plant genome

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Carnivorous plants capture and digest animal prey for nutrition. In addition to being carnivorous, the humped bladderwort plant, *Utricularia gibba*, has the smallest reliably assembled flowering plant genome. We generated an updated genome assembly based on single-molecule sequencing to address questions regarding the bladderwort's genome adaptive landscape. Among encoded genes, we segregated those that could be confidently distinguished as having derived from small-scale versus whole-genome duplication processes and showed that conspicuous expansions of gene families useful for prey trapping and processing derived mainly from localized duplication events. Such small-scale, tandem duplicates are therefore revealed as essential elements in the bladderwort's carnivorous adaptation. (See pp. E4435–E4441.)

Alterations in cellular metabolism triggered by *URA7* or *GLN3* inactivation cause imbalanced dNTP pools and increased mutagenesis

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The duplication of the genetic information (DNA) requires high accuracy to prevent potentially deleterious genetic alterations (mutations). The fidelity of this reaction depends on DNA polymerase selectivity and proofreading functions, postreplicative mismatch repair (MMR), and the abundance of dNTPs, the building blocks of DNA. Here, in a genome-wide screen in budding yeast, we uncovered a group of genes required for high-fidelity DNA replication. When these genes are absent, cells are prone to incorporate incorrect nucleotides, and consequently they heavily rely on DNA polymerase functions and MMR to prevent severe hypermutability. These findings suggest that similar genetic interactions could play a role in human cancer, where inactivation of these genes might act as "minidrivers" that facilitate tumor evolution. (See pp. E4442–E4451.)

Loss of the homologous recombination gene *rad51* leads to Fanconi anemia-like symptoms in zebrafish

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The homologous recombination protein RAD51 has been extensively studied in prokaryotes and lower eukaryotes. However, there is a significant lack of knowledge of the role of this protein and its regulation in an in vivo context in vertebrates. Here we report the first viable vertebrate mutant model of *rad51* in zebrafish. These mutant fish enabled us to confirm the recently discovered role of *RAD51* in Fanconi anemia pathogenesis. We report that p53-linked embryonic stem cell defects directly lead to hematological impairments later in life. Comutation of *rad51* with *p53* rescues the observed hematological defects, but predisposes the fish to early tumor development. The application of this model opens new possibilities to advance Fanconi anemia drug discovery. (See pp. E4452–E4461.)

Probing the lithium-response pathway in hiPSCs implicates the phosphoregulatory set-point for a cytoskeletal modulator in bipolar pathogenesis

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One-third of bipolar disorder (BPD) patients are lithium-responsive (LiR) for unknown reasons. Were lithium's target to be identified, then BPD's pathogenesis might be unraveled. We identified and mapped the "lithium-response pathway," which governs the phosphorylation of *CRMP2*, a cytoskeleton regulator, particularly for dendritic spines: hence, a neural network modulator. Although "toggling" between inactive (phosphorylated) and active (nonphosphorylated) CRMP2 is physiologic, the "set-point" in LiR BPD is abnormal. Lithium (and other pathway-modulators) normalize that set-point. Hence, BPD is a disorder not of a gene but of the posttranslational regulation of a developmentally critical molecule. Such knowledge should enable better mechanistically based treatments and bioassays. Instructively, lithium was our "molecular can-opener" for "prying" intracellularly to reveal otherwise inscrutable pathophysiology in this complex polygenic disorder. (See pp. E4462–E4471.)

Involvement of a gut–retina axis in protection against dietary glycemia-induced age-related macular degeneration

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Food is medicine, and diet impacts the risk for and progression of age-related macular degeneration AMD, but we have few clues as to why. We found that wild-type mice fed a high-glycemic-index diet similar in composition to the Western diet developed a disease state that resembles dry AMD. To gain insight into the mechanism, we used LC-MS– and NMR-based metabolomics to discover diet-, metabolic-, and AMD-associated phenotypes. These studies revealed changes in the gut microbiota that altered the production of metabolites that protected against AMD, including serotonin. Changing the diet to a low-glycemic-index diet, even late in life, arrested the development of AMD, offering dietary interventions for AMD. (See pp. E4472–E4481.)

Embryonic transcription factor SOX9 drives breast cancer endocrine resistance

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Resistance to endocrine treatment remains a significant clinical obstacle. *ESR1* mutations were found to be the mechanism of

endocrine resistance in a substantial number of patients with metastatic ER-positive breast. However, these mutations are primarily linked to aromatase inhibitor resistance and are not strongly associated with tamoxifen resistance. Herein, we show that tamoxifen treatment promotes a RUNX2–ER complex, which mediates an altered ER cistrome that facilitates the up-regulation of SOX9. We show that up-regulation of SOX9, an embryonic transcription factor with key roles in metastases, is a driver of endocrine resistance in the setting of tamoxifen treatment. Our data provide putative targets for the development of new strategies to treat tamoxifen-resistant breast cancer. (See pp. E4482–E4491.)

Thiophene antibacterials that allosterically stabilize DNA-cleavage complexes with DNA gyrase

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The spread of multidrug-resistant bacteria constitutes a significant unmet medical need. Fluoroquinolone antibiotics have been compromised by resistance mutations in their targets: DNA gyrase and topoisomerase IV. Using biochemical and genetic techniques, we have identified and characterized a class of antibacterials which transforms DNA gyrase into toxic DNA-cleavage complexes, similar to fluoroquinolones, but with a distinct mechanism of action. X-ray crystallography shows that the inhibitors access a previously unexploited pocket in gyrase, leading to their activity against fluoroquinolone-resistant bacteria and providing a strategy to target bacterial topoisomerases. (See pp. E4492–E4500.)

Development of visual category selectivity in ventral visual cortex does not require visual experience

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The brain's ability to recognize visual categories is guided by category-selective ventral-temporal cortex (VTC). Whether visual experience is required for the functional organization of VTC into distinct functional subregions remains unknown, hampering our understanding of the mechanisms that drive category recognition. Here, we demonstrate that VTC in individuals who were blind since birth shows robust discriminatory responses to natural sounds representing different categories (faces, scenes, body parts, and objects). These activity patterns in the blind also could predict successfully which category was visually perceived by controls. The functional cortical layout in blind individuals showed remarkable similarity to the well-documented layout observed in sighted controls, suggesting that visual functional brain organization does not rely on visual input. (See pp. E4501–E4510.)

Critical roles of DNA demethylation in the activation of ripening-induced genes and inhibition of ripening-repressed genes in tomato fruit

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DNA methylation is generally considered an epigenetic mark for transcriptional gene silencing. In this work, we generated loss-of-function mutant alleles of *SlDML2*. We characterized the mutant fruits that failed to ripen and discovered that *SlDML2* is required for the demethylation and activation of genes important for fruit ripening, including genes involved in fruit pigment and flavor synthesis, ethylene synthesis and signaling, and cell wall hydrolysis. Unexpectedly, we found that SlDML2-mediated DNA demethylation is also necessary for fruit ripening-induced repression of hundreds of genes involved in photosynthesis and cell wall synthesis and organization. Our study has therefore revealed a broad and critical role of DNA methylation as an activation mark for the expression of many genes in a eukaryotic organism. (See pp. E4511–E4519.)