PNAS Plus Significance Statements

Interferometric mapping of material properties using thermal perturbation

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Rapid, accurate, and nondestructive mapping of material properties is of great interest in many fields, with applications ranging from detection of defects or other subsurface features in semiconductors to estimating temperature rise in various tissue layers during laser therapy. We demonstrate the speed and precision of two interferometric techniques, quantitative phase imaging and phase-resolved optical coherence tomography, in recording optical phase changes induced by energy deposition in various materials. Such phase perturbations can be used to infer sample properties, ranging from absorption and temperature maps to distribution of electric field or resistivity. We derive the theoretical sensitivity limits of such techniques and demonstrate their applicability to the mapping of absorption coefficients, temperature, and electric fields in synthetic and biological samples. (See pp. E2499–E2508.)

Exploring functional pairing between surface glycoconjugates and human galectins using programmable glycodendrimersomes

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Cells are decorated with charged and uncharged carbohydrate ligands known as glycans, which are responsible for several key functions, including their interactions with proteins known as lectins. Here, a platform consisting of synthetic nanoscale vesicles, known as glycodendrimersomes, which can be programmed with cell surface-like structural and topological complexity, is employed to dissect design aspects of glycan presentation, with specificity for lectin-mediated bridging. Aggregation assays reveal the extent of cross-linking of these biomimetic nanoscale vesicles—presenting both anionic and neutral ligands in a bioactive manner—with disease-related human and other galectins, thus offering the possibility of unraveling the nature of these fundamental interactions. (See pp. E2509–E2518.)

Multiscale approach reveals that *Cloudina* aggregates are detritus and not in situ reef constructions

Akshay Mehra and Adam Maloof

Little is known about how the Ediacaran index fossil *Cloudina* lived and what impact it had on its surroundings. This uncertainty is due to the fact that *Cloudina* often is preserved with the same mineralogy as the rocks in which it is found; the lack of density contrast means that traditional imaging techniques cannot be used to reconstruct and measure in situ *Cloudina* populations. Recently, researchers have suggested that *Cloudina* was a framework reef builder that actively adapted to changing environmental conditions. In this paper, we use a serial grinding and imaging technique to produce 3D models of *Cloudina* aggregates. Along with detailed field observations, we demonstrate that *Cloudina* populations are detritus and not in situ growth. (See pp. E2519–E2527.)

Brain-to-brain coupling during handholding is associated with pain reduction

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The mechanisms that underlie social touch analgesia are largely unknown. Here, we apply a hyperscanning approach with real-life interaction of dyads to examine the association between brain-to-brain coupling and pain relief. Our findings indicate that handholding during pain increases the brain-to-brain coupling network that correlates with the magnitude of the analgesia and the observer's empathic accuracy. These findings make a unique contribution to our understanding of physiological mechanisms of touch-related analgesia. (See pp. E2528–E2537.)

Structural basis of transcriptional stalling and bypass of abasic DNA lesion by RNA polymerase II

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Abasic DNA lesions are one of the most abundant types of DNA lesions and are frequent byproducts of normal cellular metabolism, and they represent intermediates in the base excision repair pathway. These DNA lesions can lead to DNA mutations and transcription errors and block replication and transcription. The molecular basis for RNA polymerase II (Pol II) stalling and bypass of abasic lesion remains elusive due to a lack of atomic structural information. Here we reported the structural snapshots of Pol II

stalling and bypass of abasic DNA lesion in a step-wise manner and elucidated how bypass of the lesion leads to the errors in RNA transcripts. These results provide important mechanistic insight into understanding the biological consequences of these abundant mutagenic DNA lesions. (See pp. E2538–E2545.)

Effects of maturation on the conformational free-energy landscape of SOD1

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Copper, zinc superoxide dismutase 1 (SOD1) is an enzyme involved in free radical scavenging in the cell. Despite the high stability of the mature protein, mutations, covalent modifications, and increased populations of immature forms result in instability and misfolding of SOD1 that is neurotoxic. Here, we study how the structure and dynamics of SOD1 change as a function of maturation. We find that as SOD1 progresses to its final active conformation, the free-energy landscapes for each successive state along the maturation pathway appear to become smoother with less exploration of higher energy conformations. Our results suggest that maturation may occur via a series of steps in which the transiently populated structures of preceding states successively become the dominant conformations. (See pp. E2546–E2555.)

Bone degradation machinery of osteoclasts: An HIV-1 target that contributes to bone loss

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Bone deficits are frequent complications observed in HIV-1–infected patients. Our study demonstrates that HIV-1 infects osteoclasts, the cells specialized in bone degradation, using different models including HIV-1–infected humanized mice. We decipher the cellular mechanisms by which HIV-1 contributes to enhanced bone degradation in human osteoclasts, showing that the virus modifies the structure and function of the sealing zone, the bone resorption machinery of osteoclasts. We identify the viral protein Nef as the key factor responsible for such effects. As a proof-of-concept, we correlate bone deficit in transgenic Nef-expressing mice with enhanced osteoclast activity. Therefore, our findings provide formal evidence that osteoclasts constitute HIV-1 host target cells, contributing to bone deficits in vivo. (See pp. E2556–E2565.)

A comprehensive genomic history of extinct and living elephants

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Elephantids were once among the most widespread megafaunal families. However, only three species of this family exist today. To reconstruct their evolutionary history, we generated 14 genomes from living and extinct elephantids and from the American mastodon. While previous studies examined only simple bifurcating relationships, we found that gene flow between elephantid species was common in the past. Straight-tusked elephants descend from a mixture of three ancestral populations related to the ancestor of African elephants, woolly mammoths, and present-day forest elephants. We detected interbreeding between North American woolly and Columbian mammoths but found no evidence of recent gene flow between forest and savanna elephants, demonstrating that both gene flow and isolation have been central in the evolution of elephantids. (See pp. E2566–E2574.)

Expanded cellular clones carrying replication-competent HIV-1 persist, wax, and wane

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The HIV-1 latent reservoir cannot be eradicated by antiretroviral therapy (ART). The reservoir is a major barrier to cure. To characterize the mechanisms that contribute to persistence of the latent reservoir, we examined clonally expanded cell populations carrying replication-competent HIV-1 and followed them longitudinally. Expanded clones harboring replication-competent HIV-1 were identified in all study participants, but these clones emerge and wane on a time scale of years. A similar pattern was identified in viruses sampled from residual viremia. The findings suggest that the latent reservoir is likely to be maintained through expansion driven by antigens and cytokines, and that the expansion is balanced with a constant cell loss. (See pp. E2575–E2584.)

T_{reg} cells limit IFN-γ production to control macrophage accrual and phenotype during skeletal muscle regeneration

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Skeletal muscle relies on its regenerative capacity to recover after acute injury. Immune-system cells, notably macrophages and regulatory T cells, play critical roles during muscle regeneration. This study addressed the impact of regulatory T cells on macrophages during muscle repair. In a mouse model of acute injury, regulatory T cells controlled the composition and phenotype of muscle macrophages during muscle repair by limiting production of the inflammatory cytokine, interferon-γ, produced by natural killer and effector T cells. Thus, we uncovered an interferon-γ–centered regulatory loop that can be further explored as a gateway to improved muscle therapies. (See pp. E2585–E2593.)

Coamplification of *miR-4728* protects *HER2*-amplified breast cancers from targeted therapy

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In *HER2*-amplified breast cancers, HER2 inhibitors have been very successful as adjuvant therapy but not as monotherapy. Here, we demonstrate that coamplification of a *HER2* intronic miRNA causes intrinsic resistance to HER2 inhibitors by indirectly down-regulating the pro-apoptotic NOXA. Importantly, coinhibition with MCL-1 inhibitors overcomes this resistance. (See pp. E2594–E2603.)

Host biotin is required for liver stage development in malaria parasites

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Malaria parasites require certain host nutrients for growth and survival. In this project, we examined the role of the human vitamin biotin in all stages of the malaria life cycle. We cultured blood- and liver-stage malaria parasites in the absence of biotin and found that, whereas blood-stage replication was unaffected, liver-stage parasites deprived of biotin were no longer capable of establishing a blood-stage infection. Interestingly, biotin depletion resulted in more severe developmental defects than the genetic disruption of parasite biotin metabolism. This finding suggests that host biotin metabolism also contributes to parasite development. Because neither the parasite nor the human host can synthesize biotin, parasite infectivity may be affected by the nutritional status of the host. (See pp. E2604–E2613.)

Role of a single noncoding nucleotide in the evolution of an epidemic African clade of *Salmonella*

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Invasive nontyphoidal *Salmonella* disease is a major and previously neglected tropical disease responsible for an estimated ~390,000 deaths per year in Africa, largely caused by a variant of *Salmonella* Typhimurium called ST313. Despite the availability of >100,000 *Salmonella* genomes, it has proven challenging to associate individual SNPs with pathogenic traits of this dangerous bacterium. Here, we used a transcriptomic strategy to identify a single-nucleotide change in a promoter region responsible for crucial phenotypic differences of African *S.* Typhimurium. Our findings show that a noncoding nucleotide of the bacterial genome can have a profound effect upon the pathogenesis of infectious disease. (See pp. E2614–E2623.)

Karyopherin α-3 is a key protein in the pathogenesis of spinocerebellar ataxia type 3 controlling the nuclear localization of ataxin-3

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Ataxin-3 is the affected protein in the neurodegenerative disorder spinocerebellar ataxia type 3 (SCA3). Nuclear ataxin-3 has been linked to disease progression and formation of aggregates. Our present findings implicate karyopherin alpha 3 (KPNA3) in the in vitro transport of ataxin-3 and in the SCA3-related phenotypes in *Drosophila* and mouse models. We have demonstrated that altering transport proteins has an effect on both pathogenic mechanisms (e.g., the intracellular localization and the formation of aggregates) and key features of ataxin-3 toxicity such as anxiety, total activity, and gait abnormalities. A better appreciation of this cellular mechanism can enhance our understanding of polyglutamine diseases and the role of nuclear/cytoplasmic compartments in toxicity and clearance of ataxin-3. (See pp. E2624–E2633.)

Identification of a highly neurotoxic α-synuclein species inducing mitochondrial damage and mitophagy in Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disease linked to the misfolding and aggregation of a protein called "α-synuclein." α-Synuclein aggregates found in the brains of PD patients are called "Lewy bodies" and "Lewy neurites." We discovered the existence of a type of α-synuclein aggregate, smaller than previously described and conformationally distinct, that we called "pα-syn*." Pα-syn* was present in neuronal cultures and mice brains injected with recombinant α-synuclein fibrils as well as in the brains of PD patients. We showed that pα-syn* is made of trimmed α-synuclein resulting from a failed cellular attempt to degrade fibrillar α-synuclein aggregates. We found that pα-syn* is a major neurotoxic species inducing mitochondrial damage, fission, and mitophagy, therefore constituting a central player in PD pathogenesis. (See pp. E2634–E2643.)

Target selectivity of septal cholinergic neurons in the medial and lateral entorhinal cortex

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Acetylcholine is a key modulator of hippocampal and entorhinal cortex (EC) function. The majority of cholinergic projections targeting these structures originate in the basal forebrain complex, specifically the medial septum. Many studies focused on the behavioral effects involving these projections, but there still is a paucity regarding their connectivity in the target area. Here we provide this missing link. By combining optogenetics with whole-cell recordings in superficial EC layers, we identified the synaptic target cells of septal cholinergic neurons. This level of analysis is an important step toward a better understanding of the modulatory action of acetylcholine in EC in vivo. (See pp. E2644–E2652.)

Monitoring ligand-dependent assembly of receptor ternary complexes in live cells by BRETFect

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Many biological processes, including signal transduction pathways and gene expression regulation, require the assembly of multisubunit protein complexes in a temporally coordinated fashion. However, approaches to studying the dynamics and properties of multimeric complex assembly currently remain limited. We have developed a method to monitor assembly of trimeric complexes in live cells, using a specific combination of bioluminescence- and fluorescence-based energy transfer. We illustrate the potential of this technology to reveal ligand-activated assembly of ternary protein complexes involving nuclear receptors or G protein-coupled receptors (GPCRs) and their effectors or regulators. We show that this methodology uniquely enables the functional characterization of nuclear receptor heterodimers and the demonstration of corecruitment of effector proteins to a GPCR. (See pp. E2653–E2662.)