PNAS Plus Significance Statements

Quantitative nanoscale imaging of orientational order in biological filaments by polarized superresolution microscopy

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Central biological processes in cells and tissues are intrinsically governed by the structural order of biomolecular assemblies. It is thus a key factor to decipher how these assemblies organize in complex molecular organizations, from the nanometric to the macroscopic scale. Polarized microscopy can access such information; however, signals are spatially averaged over the optical diffraction limit and are contaminated by the fluorophores' orientational flexibility of their linker to the biomolecules. By bringing polarized fluorescence down to superresolution microscopy using single-molecule localization, we show that structural imaging can be scaled down to nanometric scales and is able to discriminate fluorophores' flexibility from biomolecules' orientational order. We demonstrate nanoscale structural imaging in fundamental biological filament organizations. (See pp. E820–E828).

Integrated in vivo and in vitro nascent chain profiling reveals widespread translational pausing

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The synthesis of a protein takes tens of seconds to a few minutes, in which amino acids are polymerized linearly. Nonuniform progression of this elongation process is thought to be important for the subsequent fates of newly synthesized proteins. However, there have been few attempts to profile elongation intermediates, polypeptidyl–tRNAs, directly. Here we attempted to detect systematically the accumulation of tRNA-linked nascent chain intermediates during the translation of *Escherichia coli* proteins in vivo and in vitro. The results revealed the widespread occurrence of translational pausing in a manner correlated with the subcellular localization and solubility properties of proteins. Our in vivo/in vitro integrated nascent chain profiling provides groundwork information for our understanding of genetic message translation into functional proteins. (See pp. E829–E838).

Fluoroquinolone interactions with *Mycobacterium tuberculosis* gyrase: Enhancing drug activity against wild-type and resistant gyrase

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Moxifloxacin and other fluoroquinolone antibacterial agents are important antituberculosis therapeutic agents. Fluoroquinolones kill *Mycobacterium tuberculosis*, the causative agent of tuberculosis, by increasing levels of DNA breaks generated by gyrase, an essential type II topoisomerase that regulates DNA topology. As fluoroquinolone use in antituberculosis regimens is becoming more pronounced, understanding the basis of drug–gyrase interactions and resistance is becoming more important. By using a mechanism-based chemical biology approach, our work identified critical drug features that mediate fluoroquinolone interactions with *M. tuberculosis* gyrase and determined the biochemical basis for fluoroquinolone resistance caused by the most common clinical mutations in gyrase. These findings allowed us to identify a moxifloxacin derivative that displays enhanced activity against WT gyrase and maintains high activity against clinically relevant resistant enzymes. (See pp. E839–E846).

Complexation and coacervation of like-charged polyelectrolytes inspired by mussels

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Conventional coacervates can form on mixing oppositely charged polyelectrolytes in aqueous solutions, due to electrostatic attraction between the oppositely charged polymers. This report describes the first instance (to the best of our knowledge) of complexation and coacervation of two positively charged polyelectrolytes by overcoming longer-range electrostatic repulsion. The molecular force measurements and theoretical simulations demonstrate that the complexation of the like-charged coacervate is most likely driven by strong cation–π interactions inspired by marine mussel adhesives. This like-charged coacervation mechanism provides new insights into biological self-assembly processes and a new paradigm for engineering strong, reversible interactions between polymers underwater, which has various potential

applications like encapsulation and dispersion of particles and cells. (See pp. E847–E853).

Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters

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Conventional models of cancer progression propose that single cells leave the primary tumor, enter the circulation, and seed clonal metastases. However, metastases can contain multiple clones, raising the question: How do polyclonal metastases form? We demonstrate that cancer cells seed distant organs as cohesive clusters, composed of two molecularly distinct subpopulations, whose proportions vary systematically during metastasis. We establish that collective dissemination is a frequent mechanism for metastasis and identify a molecular program in the most invasive, keratin 14^+ ($K14^+$) cancer cells, regulating cell–cell adhesion, cell–matrix adhesion, and immune evasion. We demonstrate that this metastatic phenotype is dependent upon K14 expression. Understanding the molecular basis of collective dissemination may therefore enable novel prognostics and therapies to improve patient outcomes. (See pp. E854–E863).

Functional malignant cell heterogeneity in pancreatic neuroendocrine tumors revealed by targeting of PDGF-DD

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Emerging evidence suggest that the cellular composition of tumors is highly heterogeneous. Subclonal species of malignant cells may account for variability in therapeutic responses and for relapse following treatments. However, little is known about the molecular drivers of specific subsets of cancer cells. Herein, we identify expression of platelet-derived growth factor receptor beta (PDGFRβ) as a previously unrecognized feature of a minor malignant cell population in pancreatic neuroendocrine tumors. By the use of mice genetically deficient for *Pdgfd*, we reveal a crucial and nonredundant function for signaling by platelet-derived growth factor (PDGF)-DD in promoting functional tumor heterogeneity by providing growth-stimulatory cues. Taken together, the use of drugs targeting PDGFRβ signaling, such as the approved targeted therapy sunitinib, may affect the functional intratumoral cross talk in pancreatic neuroendocrine tumors. (See pp. E864–E873).

Toll-like receptor-5 agonist, entolimod, suppresses metastasis and induces immunity by stimulating an NK-dendritic-$CD8^+$ T-cell axis

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Innate immune modulators can generate a potent antitumor T-cell response and are thus a desirable approach to immunotherapy. However, their use is limited by the risk of induction of acute inflammation. In this regard, bacterial flagellin is unique among other innate immune modulators because of a significantly safer cytokine profile induced upon activation by its target, Toll-like receptor 5 (TLR5). We show here that systemic administration of entolimod, a pharmacologically optimized flagellin derivative, induces a cascade of cell–cell signaling resulting in mobilization to the liver of various components of innate and adaptive immunity, followed by suppression of liver metastases and development of long-term antitumor immune memory. Thus, TLR5 agonists can be considered as an organ-specific immunotherapy for the treatment and prevention of metastases. (See pp. E874–E883).

TLR4/MD-2 activation by a synthetic agonist with no similarity to LPS

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The Toll-like receptor 4 (TLR4)/myeloid differentiation factor 2 (MD-2) complex recognizes lipopolysaccharide (LPS) on Gram-negative bacteria to induce an innate immune response. Neoseptins, chemically synthesized peptidomimetics that bind and activate the mouse TLR4 (mTLR4)/MD-2 complex independent of LPS, were discovered through unbiased screening and reverse genetic studies, and improved by chemical modification. NMR and X-ray crystallography of the TLR4/MD-2/Neoseptin-3 complex determined the mechanism by which Neoseptin-3 activates mTLR4/MD-2 and triggers myeloid differentiation primary response gene 88- and Toll-interleukin 1 receptor domain-containing adaptor inducing IFN-beta-dependent signaling. Neoseptin-3 binds as a dimer within the hydrophobic pocket of MD-2, contacting residues distinct from those contacted by LPS or lipid A, yet triggering a conformational change very similar to that elicited by LPS or lipid A. Natural peptides might conceivably produce similar effects. (See pp. E884–E893).

miR-H28 and miR-H29 expressed late in productive infection are exported and restrict HSV-1 replication and spread in recipient cells

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Herpes simplex virus-1 (HSV-1) is transmitted by contact between the infected tissues of a transmitter and those of an uninfected recipient. Effective spread requires that the lesions be small and not repulsive in appearance. For successful transmission HSV must control both its replication and its spread from infected to uninfected cells. Here we report that two HSV microRNAs (miRNAs) made late in productive infection are exported in exosomes. Ectopic expression in transfected cells before infection reduces the synthesis of viral gene products and the spread from infected to uninfected cells. The miRNAs accumulate in neurons after latent virus is induced to reactivate. The miRNAs conform to the expectations of gene products that control viral replication and spread. (See pp. E894–E901).

Distinct signaling of *Drosophila* chemoreceptors in olfactory sensory neurons

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Drosophila is a popular model system for the study of olfaction. However, the physiological properties of its olfactory sensory neurons, both intrinsic and responsive, remain unclear. We have succeeded, for the first time, in patch-clamp recording from targeted *Drosophila* OSNs, revealing the distinct signaling of odorant receptors (Ors) and ionotropic receptors (Irs). We found that Ir-driven receptor currents did not adapt, whereas Or responses strongly adapted. Surprisingly, although Or adaptation increased odor sensitivity at high odor backgrounds, it reduced odor sensitivity at low backgrounds. Adaptation permeates all senses, and the finding of dynamic gain control by adaptation in

Drosophila Or-expressing OSNs sheds light on the understanding of adaptation in other sensory systems. (See pp. E902–E911).

Induction of de novo α-synuclein fibrillization in a neuronal model for Parkinson's disease

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Although it has been established for over 100 years that Lewy bodies (LBs) represent the major pathological hallmark of Parkinson's disease (PD), we still do not know why these fibrillar intraneuronal inclusions of α-synuclein (α-Syn) protein form, or how they contribute to disease progression. One of the major causes underlying this gap in knowledge is the lack of animal models that reproduce the formation of fibrillar LB-like inclusions. In this study, we show that the absence of human α-Syn (hα-Syn) fibrillization into LBs in mice can be attributed to interactions between hα-Syn and its endogenously expressed mouse α-Syn homologue. Moreover, we provide well-characterized primary neuronal and in vivo models that recapitulate the main molecular feature of PD, bona fide α-Syn fibrillization. (See pp. E912–E921).

Differential vesicular sorting of AMPA and $GABA_A$ receptors

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In neurons most fast excitatory neurotransmission is mediated by AMPA receptors, which cluster at excitatory synapses primarily on dendritic spines. Fast synaptic inhibition is largely mediated by $GABA_A$ receptors, which cluster at inhibitory synapses mainly on the soma and dendritic shafts. It is unclear how these receptors are segregated and delivered to specific locations on the plasma membranes. Here we directly studied the constitutive exocytosis of AMPA and $GABA_A$ receptors and demonstrate that they are initially sorted into different vesicles in the Golgi apparatus and inserted into distinct domains of the plasma membrane. Their exocytosis is dependent on distinct Rab GTPases and SNARE complexes. Our results reveal fundamental mechanisms underlying the sorting of excitatory and inhibitory neurotransmitter receptors in neurons. (See pp. E922–E931).

G protein-gated I_{KACh} channels as therapeutic targets for treatment of sick sinus syndrome and heart block

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The "sick sinus" syndrome (SSS) is characterized by abnormal formation and/or propagation of the cardiac impulse. SSS is responsible for about half of the total implantations of electronic pacemakers, which constitute the only currently available therapy for this disorder. We show that genetic ablation or pharmacological inhibition of the muscarinic-gated K^+ channel (I_{KACh}) prevents SSS and abolishes atrioventricular block in model mice without affecting the relative degree of heart rate regulation. We propose that "compensatory" genetic or pharmacological targeting of I_{KACh} channels may constitute a new paradigm for restoring defects in the balance between inward and outward currents in pacemaker cells. Our study may thus open a new therapeutic perspective to manage dysfunction of formation and conduction of the cardiac impulse. (See pp. E932–E941).