PNAS Plus Significance Statements

How adaptive immunity constrains the composition and fate of large bacterial populations

Madeleine Bonsma-Fisher, Dominique Soutiére, and Sidhartha Goyal

Complex communities of microorganisms are important ecological forces and phages are integral components of microbial populations. Among the many bacterial defense mechanisms against phages, CRISPR-Cas is unique in its ability to learn from past infections by storing pieces of phage DNA (called spacers) in its own genome to neutralize future infections. Our work shows that the rank abundance distribution of spacers across the whole bacterial population, which is readily accessed using genomic sequencing, may provide a phenomenological observable that reflects important structural aspects of bacterial populations. This study lays out a path toward a phenomenological framework for understanding microbial dynamics and may provide insights into complex and diverse natural populations where microscopic modeling is plagued by overparameterization and overfitting. (See pp. E7462–E7468.)

Selection of an ASIC1a-blocking combinatorial antibody that protects cells from ischemic death

Min Qiang, Xue Dong, Zhao Zha, Xiao-Kun Zuo, Xing-Lei Song, Lixia Zhao, Chao Yuan, Chen Huang, Pingdong Tao, Qin Hu, Wei-Guang Li, Wanhui Hu, Jie Li, Yan Nie, Damiano Buratto, Francesco Zonta, Peixiang Ma, Zheng Yu, Lili Liu, Yi Zhang, Bei Yang, Jia Xie, Tian-Le Xu, Zhihu Qu, Guang Yang, and Richard A. Lerner

Unfortunately, the need for ideal medical treatment of acute ischemic stroke is still largely unmet. One of the contributing factors to the deleterious neuronal death is the opening of acid-sensing ion channels (ASICs) at reduced pH, which in turn, activates other calcium-permeable channels that initiate the catastrophic cascade. Here, we report the discovery of an antibody that blocks the ASIC1a with high specificity and potency. Infusion of this antibody reduces the damaged area from brain ischemia in the murine stroke model. We hypothesize that using antibodies to target ASIC1a is a valid approach for future stroke therapy. The antibody that we report here has the potential to be further developed as drug candidate. (See pp. E7469–E7477.)

Switching of the folding-energy landscape governs the allosteric activation of protein kinase A

Jeneffer P. England, Yuxin Hao, Lihui Bai, Virginia Glick, H. Courtney Hodges, Susan S. Taylor, and Rodrigo A. Maillard

Mutations, deletions, or gene fusions in protein kinases have been associated with the development of many diseases in humans and have led to the emergence of the protein kinase family as an important therapeutic drug target. In the cell, kinase activity is often turned on and off allosterically by intramolecular regulatory domains, flexible linkers, or other interacting proteins. Here, we use single-molecule optical tweezers to investigate the mechanism of allosteric regulation of the cAMP-dependent PKA. This approach allowed us to determine the thermodynamic driving forces that enable PKA to transduce cAMP-binding signals to turn on its kinase activity. (See pp. E7478–E7485.)

Mavacamten stabilizes an autoinhibited state of two-headed cardiac myosin

John A. Rohde, Osha Roopnarine, David D. Thomas, and Joseph M. Muretta

Small-molecule allosteric effectors designed to target and modulate striated and smooth myosin isoforms for the treatment of disease show promise in preclinical and clinical trials. Beta-cardiac myosin is an especially important target, as heart disease remains a primary cause of death in the United States. One prevalent type of heart disease is hypertrophic cardiomyopathy (HCM), which is hypothesized to result from dysregulated force generation by cardiac myosin. Mavacamten is a potent cardiac myosin ATPase activity inhibitor that improves cardiac output in HCM animal models. Our results show that mavacamten selectively stabilizes a two-headed–dependent, autoinhibited state of cardiac myosin in solution. The kinetics and energetics of this state are consistent with the autoinhibited superrelaxed state previously observed only in intact sarcomeres. (See pp. E7486–E7494.)

Many-body effect determines the selectivity for Ca^{2+} and Mg^{2+} in proteins

Zhifeng Jing, Chengwen Liu, Rui Qi, and Pengyu Ren

Metal ions have important biological functions and are associated with diseases including cancer and neurodegenerative disorders. The fundamental question of

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metal ion selectivity in proteins has received continued interest over the past decades. Compared with Na^+/K^+, the selectivity for Mg^{2+}/Ca^{2+} is less well understood. Although Mg^{2+} is a better charge acceptor, calcium-binding proteins with highly charged binding pockets can selectively bind Ca^{2+} against a much higher concentration of Mg^{2+}. Here we show that this selectivity is dictated by the many-body polarization effect, which is a cost arising from the dense packing of multiple residues around the metal ion. By combining geometric constraint and the many-body effect, it is possible to fine-tune the selectivity for metal ions of different sizes. (See pp. E7495–E7501.)

Electrostatic lock in the transport cycle of the multidrug resistance transporter EmrE

Josh V. Vermaas, Susan B. Rempe, and Emad Tajkhorshid

EmrE is a small membrane transporter found in *Escherichia coli* that exports drug-like molecules from the cell, contributing to antibiotic resistance. In EmrE, as well as in the wider small-multidrug resistance transporter family, a specific anionic amino acid (E14) has been implicated in governing the conformational changes that export drugs. However, due to sparse structural information, the exact interactions remain unidentified. Through interactive molecular dynamics to incorporate existing cryo-electron microscopy data, we create a fully refined atomic model of EmrE. We then embed this model in a lipid bilayer and evaluate the interactions within EmrE under different loading states. We find that E14 makes specific hydrogen bonds to neighboring residues, coupling observed experimental phenomena to interactions at the atomic scale. (See pp. E7502–E7511.)

Tethered multifluorophore motion reveals equilibrium transition kinetics of single DNA double helices

Matthias Schickinger, Martin Zacharias, and Hendrik Dietz

Understanding cellular functions and dysfunctions often begins with quantifying the interactions between the binding partners involved in the processes. Learning about the kinetics of the interactions is of particular importance to understand the dynamics of cellular processes. We created a tethered multifluorophore motion assay using DNA origami that enables over 1-hour-long recordings of the statistical binding and unbinding of single pairs of biomolecules directly in equilibrium. The experimental concept is simple and the data interpretation is very direct, which makes the system easy to use for a wide variety of researchers. Due to the modularity and addressability of the DNA origami-based assay, our system may be readily adapted to study various other molecular interactions. (See pp. E7512–E7521.)

Tumor promoter TPA activates Wnt/β-catenin signaling in a casein kinase 1-dependent manner

Zijie Su, Jiaxing Song, Zhongyuan Wang, Liang Zhou, Yuqing Xia, Shubin Yu, Qi Sun, Shan-Shan Liu, Liang Zhao, Shiyue Li, Lei Wei, Dennis A. Carson, and Desheng Lu

The phorbol ester 12-*O*-tetra-decanoylphorbol-13-acetate (TPA) is a well-known tumor promoter in two-stage mouse skin carcinogenesis, but the exact mechanism by which TPA promotes tumorigenesis remains elusive. This study discovered that TPA could stabilize CK1ε, enhance its kinase activity, and induce phosphorylation of LRP6, resulting in the formation of CK1ε–LRP6–axin1 complex, which may bypass the requirement of Wnt–Fzd–Dvl complex. TPA also increased the interaction between β-catenin and TCF4E in a CK1ε/δ-dependent way, and finally led to activation of the Wnt/β-catenin pathway. Our findings reveal a pathway by which TPA activates the Wnt/β-catenin signaling cascade. This pathway may represent a common mechanism for the tumor-promoting activity of some carcinogenic agents. (See pp. E7522–E7531.)

Waves cue distinct behaviors and differentiate transport of congeneric snail larvae from sheltered versus wavy habitats

Heidi L. Fuchs, Gregory P. Gerbi, Elias J. Hunter, and Adam J. Christman

Many marine populations grow and spread via larvae that disperse in ocean currents. Larvae can alter their physical transport by swimming vertically or sinking in response to environmental signals. However, it remains unknown whether any signals could enable larvae to navigate over large scales. We studied larval responses to water motions in closely related snails, one from turbulent coastal inlets and one from the wavy continental shelf. These two species reacted similarly to turbulence but differently to waves, causing their transport patterns to diverge in wavy, offshore regions. Contrasting responses to waves could enable these similar species to maintain separate spatial distributions. Wave-induced behaviors provide evidence that larvae may detect waves as both motions and sounds useful in navigation. (See pp. E7532–E7540.)

Warming reverses top-down effects of predators on belowground ecosystem function in Arctic tundra

Amanda M. Koltz, Aimée T. Classen, and Justin P. Wright

Organisms' responses to climate change can result in altered species interactions, with cascading effects on communities and ecosystems. Understanding these processes is especially relevant in the rapidly warming Arctic, where faster decomposition of stored soil carbon is expected to result in positive carbon feedbacks to the atmosphere. We provide evidence that warmer temperatures alter the cascading effects of wolf spiders, an abundant and widespread predator, on ecosystem functioning. Specifically, we find that warming tends to reverse the effect of high spider densities on fungal-feeding Collembola and ultimately leads to slower decomposition rates. Our work demonstrates that climate change can alter the nature of predator effects on decomposition, resulting in unexpected changes in ecosystem function with potentially important global implications. (See pp. E7541–E7549.)

Inferring the shape of global epistasis

Jakub Otwinowski, David M. McCandlish, and Joshua B. Plotkin

How does an organism's genetic sequence govern its measurable characteristics? New technologies provide libraries of randomized sequences to study this relationship in unprecedented detail for proteins and other molecules. Deriving insight from these data is difficult, though, because the space of possible sequences is enormous, so even the largest experiments sample a tiny minority of sequences. Moreover, the effects of mutations may combine in unexpected ways. We present a statistical framework to analyze such mutagenesis data. The key assumption is that mutations contribute in a simple way to some unobserved trait, which is related to the observed trait by a nonlinear mapping. Analyzing three proteins, we show that this model is easily interpretable and yet fits the data remarkably well. (See pp. E7550–E7558.)

Identifying a large number of high-yield genes in rice by pedigree analysis, whole-genome sequencing, and CRISPR-Cas9 gene knockout

Ju Huang, Jing Li, Jun Zhou, Long Wang, Sihai Yang, Laurence D. Hurst, Wen-Hsiung Li, and Dacheng Tian

Finding the genes that control a complex trait is difficult because each gene may have only minor phenotypic effects. Quantitative trait loci mapping and genome-wide association study techniques have been developed for this purpose but are laborious and time-consuming. Here we developed a method combining pedigree analysis, whole-genome sequencing, and CRISPR-Cas9 technology. By sequencing the parents and descendants of IR8, the Green Revolution "miracle rice," we identified many genes that had been retained in the pedigree by selection for high yield. Knockout and knockdown studies showed that a large proportion of the identified genes are essential or have phenotypic effects related to production. Our approach provides a powerful means for identifying genes involved in a complex trait. (See pp. E7559–E7567.)

Unbiased classification of mosquito blood cells by single-cell genomics and high-content imaging

Maiara S. Severo, Jonathan J. M. Landry, Randall L. Lindquist, Christian Goosmann, Volker Brinkmann, Paul Collier, Anja E. Hauser, Vladimir Benes, Johan Henriksson, Sarah A. Teichmann, and Elena A. Levashina

Mosquito blood cells are central players of immunity against the vector-borne pathogens that devastate the lives of millions of people worldwide. However, their molecular identity and classification remain controversial. By applying single-cell RNA sequencing and high-content imaging flow cytometry, we defined the molecular fingerprint of a subset of mosquito blood cells and characterized two transcriptionally distinct blood cell populations that resemble previously described cell types. Surprisingly, cell population analyses at a single-cell level uncovered an active molecular transfer between the two cell types that may contribute to cellular diversity and plasticity seen across biological systems. (See pp. E7568–E7577.)

Analysis of $CD8^+$ T cell response during the 2013–2016 Ebola epidemic in West Africa

Saori Sakabe, Brian M. Sullivan, Jessica N. Hartnett, Refugio Robles-Sikisaka, Karthik Gangavarapu, Beatrice Cubitt, Brian C. Ware, Dylan Kotliar, Luis M. Branco, Augustine Goba, Mambu Momoh, John Demby Sandi, Lansana Kanneh, Donald S. Grant, Robert F. Garry, Kristian G. Andersen, Juan Carlos de la Torre, Pardis C. Sabeti, John S. Schieffelin, and Michael B. A. Oldstone

Zaire ebolavirus (EBOV) is a viral pathogen of significant global health concern best exemplified by more than 28,000 human infections during the recent West African epidemic. Examining immunity in EBOV disease survivors has been historically difficult due to the occurrence of only small outbreaks in remote regions of central Africa. Consequently, little data exist describing EBOV-specific T cell responses during human infection. We examined virus-specific $CD8^+$ T cell immunity in 32 Sierra Leonean survivors of the 2013–2016 epidemic. $CD8^+$ T cells against the nucleoprotein dominated the EBOV-specific responses in this group, while a minority of individuals harbored memory $CD8^+$ T cells against the EBOV-GP. Our data have implications in designing EBOV vaccines that can elicit cell-mediated immunity in a large group of individuals. (See pp. E7578–E7586.)

Liquid crystalline bacterial outer membranes are critical for antibiotic susceptibility

Nicolò Paracini, Luke A. Clifton, Maximilian W. A. Skoda, and Jeremy H. Lakey

Gram-negative bacteria possess an outer membrane (OM) which reduces antibiotic effectiveness. The OM has a dense outer layer of lipopolysaccharide (LPS), a glycolipid which is directly targeted by the last-resort antibiotic polymyxin B (PmB). However, we lack accurate molecular data on the PmB–OM interaction to inform future drug development. Here we study the PmB–OM interaction in vitro and show that in vivo results are reproduced only when the LPS is in a liquid crystalline phase, which occurs at body temperature. These findings not only explain the temperature dependence of PmB function but also support the notion that bacteria actively control the viscosity of their outer membranes as growth temperatures vary. (See pp. E7587–E7594.)

Selective visual representation of letters and words in the left ventral occipito-temporal cortex with intracerebral recordings

Aliette Lochy, Corentin Jacques, Louis Maillard, Sophie Colnat-Coulbois, Bruno Rossion, and Jacques Jonas

The left ventral occipito-temporal cortex (VOTC) is a critical part of the reading circuitry. We made measurements with intracerebral electrodes in 37 participants to understand whether this region contains functionally separated brain loci for processing letters and words. Letter-selective responses are found in much of VOTC. Responses to word forms are absent in posterior VOTC but are present and intermingled with letter-specific responses in left anterior VOTC. The results are inconsistent with a hierarchical model in which posterior regions uniquely perform letter identification functions and increasingly anterior regions perform increasingly complex linguistic functions. (See pp. E7595–E7604.)

Top-down, contextual entrainment of neuronal oscillations in the auditory thalamocortical circuit

Annamaria Barczak, Monica Noelle O'Connell, Tammy McGinnis, Deborah Ross, Todd Mowery, Arnaud Falchier, and Peter Lakatos

Our results indicate that nonhuman primates detect complex repeating acoustic sequences in a continuous auditory stream, which is an important precursor for human speech learning and perception. We demonstrate that oscillatory entrainment, known to support the attentive perception of rhythmic stimulus sequences, can occur for rhythms defined solely by stimulus context rather than physical boundaries. As opposed to acoustically driven entrainment by rhythmic tone sequences demonstrated previously, this form of entrainment relies on the brain's ability to group auditory inputs based on their statistical regularities. The internally initiated, context-driven modulation of excitability in the medial pulvinar prior to A1 supports the notion of top-down entrainment. (See pp. E7605–E7614.)

"Shepherd's crook" neurons drive and synchronize the enhancing and suppressive mechanisms of the midbrain stimulus selection network

Florencia Garrido-Charad, Tomas Vega-Zuniga, Cristián Gutiérrez-Ibáñez, Pedro Fernandez, Luciana López-Jury, Cristian González-Cabrera, Harvey J. Karten, Harald Luksch, and Gonzalo J. Marín

In a crowded environment, animals must direct their behavior to the stimulus with the highest priority at each moment. Stimulus selection seems to develop from competitive interactions between concurrent neural inputs. In the avian midbrain, a neural network that reciprocally connects the optic tectum to the isthmi forms a simple neural mechanism for stimulus competition, where

neurons mediating enhancement and suppression of tectal inputs are located in separate isthmic nuclei. Here, we show that collaterals of the same tectal neurons simultaneously drive these nuclei, synchronizing both antagonistic processes. These results contribute to our understanding of how distinctive neurons integrate inputs from different sources to drive in parallel several concurrent neural processes underlying a complex behavior. (See pp. E7615–E7623.)

Synaptotagmin oligomerization is essential for calcium control of regulated exocytosis

Oscar D. Bello, Ouardane Jouannot, Arunima Chaudhuri, Ekaterina Stroeva, Jeff Coleman, Kirill E. Volynski, James E. Rothman, and Shyam S. Krishnakumar

Synaptotagmin (Syt) is the primary calcium ion (Ca^{2+}) sensor for regulated exocytosis. It couples Ca^{2+} binding to soluble *N*-ethylmaleimide–sensitive factor attachment protein receptor-catalyzed fusion, but how this happens is unclear. Here, using a targeted mutation combined with a single-vesicle fusion optical assay, we show that the recently discovered structural feature of Syt to self-oligomerize is essential for Ca^{2+} coupling of vesicular fusion. This suggests an elegant yet simple model in which these Syt oligomers formed at the interface of the docked vesicle physically prevent fusion until the influx of Ca^{2+}. (See pp. E7624–E7631.)

A population of gut epithelial enterochromaffin cells is mechanosensitive and requires Piezo2 to convert force into serotonin release

Constanza Alcaino, Kaitlyn R. Knutson, Anthony J. Treichel, Gulcan Yildiz, Peter R. Strege, David R. Linden, Joyce H. Li, Andrew B. Leiter, Joseph H. Szurszewski, Gianrico Farrugia, and Arthur Beyder

Mechanical forces are important for normal gastrointestinal tract function. The enterochromaffin cells in the gastrointestinal epithelium have been proposed, but not previously shown, to be specialized sensors that convert forces into serotonin release, and serotonin released from these cells is important for normal gastrointestinal secretion and motility. The findings in this study show that some enterochromaffin cells are indeed mechanosensitive, and that they use mechanosensitive Piezo2 channels to generate an ionic current that is critical for the intracellular Ca^{2+} increase, serotonin release, and epithelial fluid secretion. (See pp. E7632–E7641.)

Mice harboring the human *SLC30A8* R138X loss-of-function mutation have increased insulin secretory capacity

Sandra Kleiner, Daniel Gomez, Bezawit Megra, Erqian Na, Ramandeep Bhavsar, Katie Cavino, Yurong Xin, Jose Rojas, Giselle Dominguez-Gutierrez, Brian Zambrowicz, Gaelle Carrat, Pauline Chabosseau, Ming Hu, Andrew J. Murphy, George D. Yancopoulos, Guy A. Rutter, and Jesper Gromada

The zinc transporter SLC30A8 is primarily expressed in islets of the endocrine pancreas. Human *SLC30A8* loss-of-function mutations protect against type 2 diabetes. However, *Slc30a8* knockout mice do not show this protection. We have generated a mouse model mimicking a common protective human *SLC30A8* loss-of-function allele. This mouse model shows a beneficial effect of loss of SLC30A8 function on β-cell biology. In particular, mice carrying the protective R138X allele have an increased capacity to secrete insulin in high-glucose conditions. Understanding the signaling mechanisms regulating insulin secretion in the R138X mice could provide novel insights into β-cell biology, and may lead to the identification of therapeutic targets for the treatment of diabetes. (See pp. E7642–E7649.)