PNAS Plus Significance Statements

Understanding shape entropy through local dense packing

Greg van Anders, Daphne Klotsa, N. Khalid Ahmed, Michael Engel, and Sharon C. Glotzer

Many natural systems are structured by the ordering of repeated, distinct shapes. Understanding how this happens is difficult because shape affects structure in two ways. One is how the shape of a cell or nanoparticle, for example, affects its surface, chemical, or other intrinsic properties. The other is an emergent, entropic effect that arises from the geometry of the shape itself, which we term "shape entropy," and is not well understood. In this paper (pp. E4812–E4821), we determine how shape entropy affects structure. We quantify the mechanism and determine when shape entropy competes with intrinsic shape effects. Our results show that in a wide class of systems, shape affects bulk structure because crowded particles optimize their local packing.

The mechanism of Torsin ATPase activation

Rebecca S. H. Brown, Chenguang Zhao, Anna R. Chase, Jimin Wang, and Christian Schlieker

Torsin activity critically depends on accessory cofactors that activate their ATPase activity by a poorly understood mechanism. This study (pp. E4822–E4831) establishes the mechanistic framework for Torsin activation, which relies on a complementation of the fragmentary Torsin active site. Given these unusual properties and the fact that Torsin activation is dysregulated in the congenital movement disorder primary dystonia, our results suggest that pharmacological manipulation of Torsin activation may present a novel therapeutic opportunity.

Developing functional musculoskeletal tissues through hypoxia and lysyl oxidase-induced collagen cross-linking

Eleftherios A. Makris, Donald J. Responte, Nikolaos K. Paschos, Jerry C. Hu, and Kyriacos A. Athanasiou

The inadequate mechanical properties of engineered tissues have prevented related therapies from clinical translation. Collagen cross-links correlate with the mechanical integrity of tissues; however, addressing the weakness of neotissues through enhancing collagen cross-links has not received the attention it deserves. The present study (pp. E4832–E4841) demonstrates, both in vitro and in vivo, that improvements in the mechanical properties of native and engineered tissues can be attained using endogenous (hypoxia-mediated) and exogenous application of lysyl oxidase, which is the enzyme responsible for collagen cross-linking. By promoting an ∼16-fold increase in collagen cross-linking and, concomitantly, an approximately fivefold enhancement in the neotissue's mechanical properties, this work creates new prospects for regenerative medicine. The methods developed here work across a spectrum of collagen-rich tissues and are clinically applicable.

Hedgehog-regulated atypical PKC promotes phosphorylation and activation of Smoothened and Cubitus interruptus in *Drosophila*

Kai Jiang, Yajuan Liu, Junkai Fan, Garretson Epperly, Tianyan Gao, Jin Jiang, and Jianhang Jia

Hedgehog (Hh) signaling by Smoothened (Smo) is mediated by phosphorylation and cell surface/cilium accumulation, but how the localization of Smo is controlled remains poorly understood. We show that the atypical PKC (aPKC)–partition defective 6 (Par6) complex promotes Hh signaling by phosphorylating Smo and regulating Smo basolateral accumulation in addition to phosphorylating the transcription factor cubitus interruptus (Ci). Our results (pp. E4842–E4850) demonstrate direct involvement of aPKC in Hh signaling beyond its role in cell polarity and suggest that basolateral accumulation of Smo is critical for its activity. Abnormal activation of Smo results in several types of cancers, and Smo can easily acquire drug resistance through mutations. A better understanding of the mechanisms of Smo regulation is critical to developing more effective therapeutic treatments for cancers caused by Smo dysregulation.

Mating-type switching by chromosomal inversion in methylotrophic yeasts suggests an origin for the three-locus *Saccharomyces cerevisiae* system

Sara J. Hanson, Kevin P. Byrne, and Kenneth H. Wolfe

Saccharomyces cerevisiae undergoes a programmed DNA rearrangement to switch between mating types a and alpha. The origins of this complex and multifaceted process, which requires three copies of the mating-type (*MAT*) locus (with two silenced), have remained unknown. In this study (pp. E4851–E4858) we present a mechanism for mating-type switching in methylotrophic yeasts that shares a common origin with the well-characterized system in *S. cerevisiae* but has simpler components. This system requires only two copies of the *MAT* locus, with one copy transcriptionally repressed by proximity to centromeric or telomeric chromatin. Switching between the mating types occurs by recombination between inverted-repeat sequences flanking the *MAT* loci. This system suggests an ancestral mechanism for mating-type switching in yeasts.

Phylotranscriptomic analysis of the origin and early diversification of land plants

Norman J. Wickett, Siavash Mirarab, Nam Nguyen, Tandy Warnow, Eric Carpenter, Naim Matasci, Saravanaraj Ayyampalayam, Michael S. Barker, J. Gordon Burleigh, Matthew A. Gitzendanner, Brad R. Ruhfel, Eric Wafula, Joshua P. Der, Sean W. Graham, Sarah Mathews, Michael Melkonian, Douglas E. Soltis, Pamela S. Soltis, Nicholas W. Miles, Carl J. Rothfels, Lisa Pokorny, A. Jonathan Shaw, Lisa DeGironimo, Dennis W. Stevenson, Barbara Surek, Juan Carlos Villarreal, Béatrice Roure, Hervé Philippe, Claude W. dePamphilis, Tao Chen, Michael K. Deyholos, Regina S. Baucom, Toni M. Kutchan, Megan M. Augustin, Jun Wang, Yong Zhang, Zhijian Tian, Zhixiang Yan, Xiaolei Wu, Xiao Sun, Gane Ka-Shu Wong, and James Leebens-Mack

Early branching events in the diversification of land plants and closely related algal lineages remain fundamental and unresolved questions in plant evolutionary biology. Accurate reconstructions of these relationships are critical for testing hypotheses of character evolution: for example, the origins of the embryo, vascular tissue, seeds, and flowers. We investigated (pp. E4859–E4868) relationships among streptophyte algae and land plants using the largest set of nuclear genes that has been applied to this problem to date. Hypothesized relationships were rigorously tested through a series of analyses to assess systematic errors in phylogenetic inference caused by sampling artifacts and model misspecification. Results support some generally accepted phylogenetic hypotheses, while rejecting others. This work provides a new framework for studies of land plant evolution.

Development of covalent inhibitors that can overcome resistance to first-generation FGFR kinase inhibitors

Li Tan, Jun Wang, Junko Tanizaki, Zhifeng Huang, Amir R. Aref, Maria Rusan, Su-Jie Zhu, Yiyun Zhang, Dalia Ercan, Rachel G. Liao, Marzia Capelletti, Wenjun Zhou, Wooyoung Hur, NamDoo Kim, Taebo Sim, Suzanne Gaudet, David A. Barbie, Jing-Ruey Joanna Yeh, Cai-Hong Yun, Peter S. Hammerman, Moosa Mohammadi, Pasi A. Jänne, and Nathanael S. Gray

Inhibitors of the FGF receptors (FGFRs) are currently under clinical investigation for the treatment of various cancers. All currently approved kinase inhibitors eventually are rendered useless by the emergence of drug-resistant tumors. We used (pp. E4869–E4877) structure-based drug design to develop the first, to our knowledge, selective, next-generation covalent FGFR inhibitors that can overcome the most common form of kinase inhibitor resistance, the mutation of the so-called "gatekeeper" residue located in the ATP-binding pocket. We also describe a novel kinase inhibitor design strategy that uses a single electrophile to target covalently cysteines that are located in different positions within the ATP-binding pocket. These results have important implications for the design of covalent FGFR inhibitors that can overcome clinical resistance.

Aag-initiated base excision repair promotes ischemia reperfusion injury in liver, brain, and kidney

Mohammad R. Ebrahimkhani, Ali Daneshmand, Aprotim Mazumder, Mariacarmela Allocca, Jennifer A. Calvo, Nona Abolhassani, Iny Jhun, Sureshkumar Muthupalani, Cenk Ayata, and Leona D. Samson

Ischemia reperfusion (I/R)-induced tissue injury and inflammation encompasses a wide range of human disease, including stroke, hepatic and renal failure, and myocardial infarction. Generation of highly reactive oxygen and nitrogen species during I/R results in DNA damage that is subject to numerous DNA repair processes. Base excision repair (BER) initiated by various DNA glycosylases is critical for the repair of reactive oxygen and nitrogen species (RONS)-induced DNA damage. Our data (pp. E4878–E4886) describe a new paradigm wherein the Aag BER DNA glycosylase enzyme promotes, rather than prevents, tissue injury and inflammation in liver, brain, and kidney following I/R. This finding reveals a detrimental facet of DNA repair during inflammation and presents a novel target for controlling I/R-induced injury.

CDK5 activator protein p25 preferentially binds and activates GSK3β

Hei-Man Chow, Dong Guo, Jie-Chao Zhou, Guan-Yun Zhang, Hui-Fang Li, Karl Herrup, and Jie Zhang

CDK5 and GSK3β are recognized as interrelated kinases; they share a strong structural resemblance, and both are known tau kinases that contribute to the etiology of Alzheimer's disease. We report here (pp. E4887–E4895) that p25 but not p35, the normal cyclin-like activator of CDK5, unexpectedly binds to GSK3β in the AXIN-binding region. The binding of p25 increases GSK3β activity and alters its substrate specificity. Results, both in vivo and in vitro, suggest that many of the effects of p25 previously assumed to be due to hyperactivation of CDK5 must now be reexamined for the potential role of altered GSK3β activity. This result carries important implications for how we approach disease-modifying strategies for the treatment of Alzheimer's and other neurodegenerative diseases.

Activity-dependent PI(3,5)P_2 synthesis controls AMPA receptor trafficking during synaptic depression

Amber J. McCartney, Sergey N. Zolov, Emily J. Kauffman, Yanling Zhang, Bethany S. Strunk, Lois S. Weisman, and Michael A. Sutton

Defects in biosynthesis of the signaling lipid phosphatidylinositol 3,5-bisphosphate [PI(3,5)P_2] are associated with profound neurodegeneration and early mortality in both humans and mice. However, surprisingly little is known about the functions of this lipid in cells, including neurons, where its loss has the most dramatic impact. Prompted by the striking localization of mammalian homolog of yeast vacuole segregation mutant (Vac14), part of the PI(3,5)P_2 synthesis complex, to excitatory synapses, we developed new tools to measure and manipulate PI(3,5)P_2 synthesis in hippocampal neurons. We find (pp. E4896–E4905) that dynamic changes in PI(3,5)P_2 synthesis impose bidirectional changes on synaptic strength by regulating AMPA-type glutamate receptor trafficking and that activity-dependent regulation of this lipid is crucial for enduring forms of synaptic depression, findings that implicate PI(3,5)P_2-dependent signaling as a critical synaptic regulatory pathway.