# PNAS Plus Significance Statements

## Unraveling the chromophoric disorder of poly(3-hexylthiophene)

Alexander Thiessen, Jan Vogelsang, Takuji Adachi, Florian Steiner, David Vanden Bout, and John M. Lupton

Ideal photovoltaic cells would be black, absorbing all of the Sun's radiation, whereas Nature's machinery for solar energy harvesting—photosynthesis—looks green. Organic semiconductor devices, based on molecular building blocks, lie conceptionally between the extremes of inorganic and photosynthetic light harvesting. How can organic solar cells appear almost black if they are based on molecular units? Using single-molecule spectroscopy, we identify (pp. E3550–E3556) the fundamental electronic building blocks of organic solar cells and reveal that discrete molecule-like transitions scatter over the entire visible spectrum. The fundamental molecular unit is narrowband, but disorder induces a continuum reminiscent of that characterizing highly ordered inorganic crystals.

## Origin and provenance of spherules and magnetic grains at the Younger Dryas boundary

Yingzhe Wu, Mukul Sharma, Malcolm A. LeCompte, Mark N. Demitroff, and Joshua D. Landis

This study ties the spherules recovered in Pennsylvania and New Jersey to an impact in Quebec about 12,900 y ago at the onset of Younger Dryas. Our discovery resulted from an exhaustive search that examined the question of whether there is any evidence of extraterrestrial platinum group metals present in the bulk sediments, magnetic grains, and spherules recovered from the Younger Dryas boundary (YDB). We find (pp. E3557–E3566) that the spherules are likely quenched silicate melts produced following the impact at the YDB. The source of spherule osmium, however, is likely terrestrial and not meteorite derived.

## Inherited human sex reversal due to impaired nucleocytoplasmic trafficking of SRY defines a male transcriptional threshold

Yen-Shan Chen, Joseph D. Racca, Nelson B. Phillips, and Michael A. Weiss

Mutations in human *SRY* (sex determining region on Y chromosome) associated with somatic sex reversal provide a model for the perturbation of a genetic switch in organogenesis. Inherited alleles, associated with either testicular or ovarian differentiation, provide unique probes of threshold biochemical properties, defining mechanistic borders between functional and nonfunctional transcription factors. This study (pp. E3567–E3576) exploited two such alleles to demonstrate that bidirectional nucleocytoplasmic trafficking (import–export shuttling) enables robust operation of this switch via phosphorylation at a site external to the DNA-binding motif of the transcription factor. In accordance with studies of intersexual mice, our results suggest that human SRY functions at the edge of ambiguity.

## Nitric oxide synthase domain interfaces regulate electron transfer and calmodulin activation

Brian C. Smith, Eric S. Underbakke, Daniel W. Kulp, William R. Schief, and Michael A. Marletta

The biological role of nitric oxide (NO) in mammalian physiology is now well established as a signaling molecule in the cardiovascular and nervous systems and as a chemical component in the host response to infection. NO is synthesized by the enzyme NO synthase (NOS). The structure of the entire NOS enzyme has not been solved, but the structure of isolated domains has been reported. In this study, we use a mass spectrometry approach (hydrogen–deuterium exchange) to find interaction surfaces of the native protein. These results (pp. E3577–E3586) were then used to generate NOS models, which revealed interaction surfaces that mediate NOS activity. These interacting surfaces provide insight into the conformational changes and residues necessary for regulating NOS activity.

## Synthesis and dissolution of hemicatenanes by type IA DNA topoisomerases

Shun-Hsiao Lee, Grace Ee-Lu Siaw, Smaranda Willcox, Jack D. Griffith, and Tao-Shih Hsieh

A hemicatenane conjoins two DNA duplexes through a single-strand interlock. It has been proposed that hemicatenanes are important intermediates for replication, repair, and recombination. However, the biochemical analysis of hemicatenanes is hampered by the relative inaccessibility of such structures. We report here (pp. E3587–E3594) that a DNA topoisomerase III (Top3) from a hyperthermophilic archaeum can carry out synthesis and dissolution of hemicatenanes. We also show that a complex of human Top3α, Bloom helicase (Blm), and RecQ-mediated genome instability protein 1 and 2 has a biochemical function of reversing this hemicatenation. Our results demonstrate that type IA topoisomerases can regulate the formation of hemicatenane structures.

## Mechanism for activation of mutated epidermal growth factor receptors in lung cancer

Monica Red Brewer, Cai-Hong Yun, Darson Lai, Mark A. Lemmon, Michael J. Eck, and William Pao

This is a unique report of receptor tyrosine kinase (RTK) "superacceptor" activity in which mutated EGFRs associated with lung cancer preferentially adopt the "acceptor" or "receiver" position in the presence of WT epidermal growth factor receptor (EGFR) or ErbB-2. The mechanism of superacceptor activity is defined by biochemical reconstitution data in combination with the first crystal structure of the L834R/T766M (L858R/T790M in alternate numbering) mutant EGFR kinase asymmetric dimer in an active conformation. The data imply (pp. E3595–E3604) that mutant/wild-type interactions play a key role in tumorigenesis as well as sensitivity of cells to various EGFR tyrosine kinase inhibitors, which could be therapeutically important. Notably, none of the previous studies involving mutated EGFR have studied the contribution of WT EGFRs in heterogeneous cell populations, although in nearly all instances wild-type EGFR alleles are preserved within EGFR mutant tumor cells.

## Nonspecific bridging-induced attraction drives clustering of DNA-binding proteins and genome organization

Chris A. Brackley, Stephen Taylor, Argyris Papantonis, Peter R. Cook, and Davide Marenduzzo

We use molecular dynamics to simulate reversible binding of proteins to DNA and uncover an unexpected force driving DNA compaction and protein aggregation. In the absence of any explicit interactions between proteins, or between templates, we find proteins aggregate spontaneously to locally organize the genome. The simulations reproduce the structures seen experimentally when small bivalent proteins assemble into rows (like bacterial H-NS protein), larger proteins with eight binding sites into irregular strings (like octameric nucleosomal cores in chromatin fibers), and still-larger complexes representing RNA polymerase II and a transcription factor (NFκB) into clusters surrounded by loops (like transcription factories). We suggest (pp. E3605–E3611) clustering is driven by an entropic bridging-induced attraction that minimizes bending and looping penalties in the template.

## Protease homolog BepA (YfgC) promotes assembly and degradation of β-barrel membrane proteins in *Escherichia coli*

Shin-ichiro Narita, Chigusa Masui, Takehiro Suzuki, Naoshi Dohmae, and Yoshinori Akiyama

Outer membrane proteins (OMPs) are involved in important cellular activities in Gram-negative bacteria. Although the *bepA* (formerly *yfgC*) gene encoding a putative metalloprotease has been implicated in quality control of OMPs, its specific function remains unclear. This study (pp. E3612–E3621) reveals that BepA promotes assembly of LptD, an OMP involved in the transport of lipopolysaccharides, which undergoes intramolecular disulfide rearrangement during its biogenesis. BepA also promotes degradation of incorrectly folded LptD. BamA, another OMP involved in OMP assembly, is also degraded in a BepA-dependent manner in the absence of periplasmic chaperone SurA. BepA thus controls the quality of OMPs by promoting either the biogenesis or elimination of OMPs, depending on their folding state.

## Tissue- and cell-type–specific manifestations of heteroplasmic mtDNA 3243A>G mutation in human induced pluripotent stem cell-derived disease model

Riikka H. Hämäläinen, Tuula Manninen, Hanna Koivumäki, Mikhail Kislin, Timo Otonkoski, and Anu Suomalainen

Mitochondrial DNA (mtDNA) mutations are a common cause of human inherited diseases and manifest with exceptional clinical heterogeneity and tissue specificity, the molecular basis of which are largely unknown. We produced induced pluripotent stem cells (iPSCs) from patients carrying the most common mtDNA mutation, m.3243A>G. During reprogramming, the cells underwent an mtDNA bottleneck, mimicking that in epiblast specification. We differentiated iPSCs to heteroplasmic human cells and tissues with isogenic nuclear background and show that the disease manifestation depends on cellular context. We show (pp. E3622–E3630) that upon neuronal differentiation, complex I is actively degraded by an autophagy-mediated mechanism. Our data indicate that cellular context actively modifies mtDNA segregation and manifestations, and that complex I is actively down-regulated in neurons with m.3243A>G mutation.

## Priming of jasmonate-mediated antiherbivore defense responses in rice by silicon

Mao Ye, Yuanyuan Song, Jun Long, Ruilong Wang, Scott R. Baerson, Zhiqiang Pan, Keyan Zhu-Salzman, Jiefen Xie, Kunzheng Cai, Shiming Luo, and Rensen Zeng

Silicon (Si) is the second most abundant element in soil, and it can increase plant resistance against many abiotic and biotic stresses. The jasmonate (JA) signaling pathway plays a crucial role in mediating antiherbivore defense responses in plants. Our work (pp. E3631–E3639) shows that Si primes JA-mediated antiherbivore defense responses and increases rice resistance to the leaffolder caterpillar and that Si accumulation in rice leaves is mediated by the JA pathway, suggesting a strong interaction between Si and JA in rice defense against insect herbivores. This interaction may represent a widespread mechanism by which Si enhances plant resistance against biotic stresses in Si-accumulating plants.

## Low-copy *piggyBac* transposon mutagenesis in mice identifies genes driving melanoma

Thomas K. Ni, Sean F. Landrette, Robert D. Bjornson, Marcus W. Bosenberg, and Tian Xu

Passenger mutation rates are highly elevated in many human cancers, posing a significant hurdle for the identification of cancer-driving genes. In this study, we screened for melanomas in mice using a unique, low-copy transposon mutagenesis system that can induce tumors with few somatic mutations. We show (pp. E3640–E3649) that our experimental system accurately recapitulated the genetic basis of human melanomas with only five somatic mutations, thus circumventing hundreds of unrelated passengers. Using cross-species comparative analyses and functional studies in human cells, we identified three previously undescribed genes involved in melanoma and several dozen candidate genes. Our study demonstrates that the low-copy transposon mutagenesis approach can facilitate the identification of cancer-driving genes that are masked by high passenger mutation rates.

## Live cell imaging shows reversible assembly of the TatA component of the twin-arginine protein transport system

Felicity Alcock, Matthew A. B. Baker, Nicholas P. Greene, Tracy Palmer, Mark I. Wallace, and Ben C. Berks

The twin-arginine translocation (Tat) pathway transports folded proteins across a membrane without significant ion leakage. The mechanism by which Tat is able to carry out this challenging feat is unclear. We used direct imaging of fluorescent protein-tagged Tat components in bacterial cells (pp. E3650–E3659) to show that the TatA element of the Tat system undergoes substrate- and proton motive force-dependent oligomerization. Thus the Tat transporter element is assembled on demand, avoiding the need to seal the transporter between translocation events.

## Dissociable effects of surprise and model update in parietal and anterior cingulate cortex

Jill X. O'Reilly, Urs Schüffelgen, Steven F. Cuell, Timothy E. J. Behrens, Rogier B. Mars, and Matthew F. S. Rushworth

This study investigates the brain mechanisms by which people disregard their previous beliefs about their environment and start forming new beliefs. Surprising events are often a signal that one's previous beliefs are no longer valid. Using brain imaging, we identified separate brain systems involved in dealing with the immediate consequences of surprise (i.e., reprogramming actions) and in updating one's beliefs about the environment to predict future events accurately. We present (pp. E3660–E3669) a mathematical and neuroanatomical model of how brains adjust to change in their environment that may inform our understanding of neurological disorders in which this adjustment process fails.

## Independent circuits in the basal ganglia for the evaluation and selection of actions

Marcus Stephenson-Jones, Andreas A. Kardamakis, Brita Robertson, and Sten Grillner

The activity in the dopaminergic reward system of the brain is concerned with the evaluation of actions and is controlled from the lateral habenulae, in all vertebrates investigated, from lamprey to primates. This study (pp. E3670–E3679) considers the mechanisms by which the lateral habenulae is controlled. We show here that a particular group of nerve cells conveys excitatory drive to the lateral habenulae, which, in turn, receive excitatory input from pallium (cortex in mammals) and inhibitory control from a specific compartment (striosomes) in the basal ganglia. This control system is critical for value-based decisions, important in all groups of vertebrates.