PNAS Plus Significance Statements

Multiscaled exploration of coupled folding and binding of an intrinsically disordered molecular recognition element in measles virus nucleoprotein

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Quantitative understanding of how coupled binding and folding occurs for intrinsically disordered molecular recognition element (MoRE) critical for function is still challenging. By developing an integrated approach, we provided physical quantification of folding and binding energy landscapes of a MoRE at atomistic level. Our predictions (pp. E3743–E3752) are in remarkable agreements with the experiments, and lead to the recognition mechanism via conformational selection, followed by induced folding. We provided an explanation for the underlying connections among "downhill folding," "molten globule," and "intrinsic disorder." We proposed a "kinetic divide-and-conquer" mechanism to understand the high specificity without high affinity in intrinsic disordered protein binding.

Template-constrained macrocyclic peptides prepared from native, unprotected precursors

Kenneth V. Lawson, Tristan E. Rose, and Patrick G. Harran

Cyclic peptides and peptidomimetics are valuable tools in biomedical research. This paper (pp. E3753–E3760) describes chemistry to convert linear, unmodified peptides directly into stable, templated macrocycles. The ring-closing reaction is an allylic substitution catalyzed by palladium(0). It requires no tailored amino acid residues or protecting groups. It proceeds rapidly at room temperature and largely independent of product-ring size and composition. The catalysis shows broad scope and predictable chemoselectivity while engaging functional groups native to peptides. These methods could be applied broadly and have special utility for those attempting to perturb biological systems with unique small molecules.

Organellar oligopeptidase (OOP) provides a complementary pathway for targeting peptide degradation in mitochondria and chloroplasts

Beata Kmiec, Pedro F. Teixeira, Ronnie P.-A. Berntsson, Monika W. Murcha, Rui M. M. Branca, Jordan D. Radomiljac, Jakob Regberg, Linda M. Svensson, Amin Bakali, Ülo Langel, Janne Lehtiö, James Whelan, Pål Stenmark, and Elzbieta Glaser

Import of proteins to mitochondria and chloroplasts is essential for organelle biogenesis and organism survival. Proteins to be imported contain an N-terminal peptide targeting the protein to the correct organelle. The targeting peptides are cleaved off after the completed import. Because the free targeting peptides are potentially toxic to organellar activities, they must be removed. Here (pp. E3761–E3769) we report the identification and characterization of a unique mitochondrial and chloroplastic oligopeptidase, organellar oligopeptidase, that provides a complementary pathway for the degradation of targeting peptides and also participates in general organellar quality control mechanisms degrading the peptides produced from complete protein degradation.

Structural features of Argonaute–GW182 protein interactions

Janina Pfaff, Janosch Hennig, Franz Herzog, Ruedi Aebersold, Michael Sattler, Dierk Niessing, and Gunter Meister

MicroRNAs (miRNAs) are short RNA molecules that negatively regulate the expression of protein-coding genes in many eukaryotes. In order to do so, miRNAs interact with a member of the Argonaute (Ago) protein family and guide it to partially complementary sequences on mRNAs. Ago proteins interact with a member of the GW182 protein family, which, in turn, recruits additional factors and coordinates all downstream steps. In our study (pp. E3770–E3779), we have characterized Ago–GW182 protein interactions using biochemical and biophysical methods. We define the interaction surfaces on GW182 and Ago proteins and provide a model for the binding mechanism and specificity.

Regulated structural transitions unleash the chaperone activity of αB-crystallin

Jirka Peschek, Nathalie Braun, Julia Rohrberg, Katrin Christiane Back, Thomas Kriehuber, Andreas Kastenmüller, Sevil Weinkauf, and Johannes Buchner

The small heat shock protein αB-crystallin functions as an archetypical and ubiquitous molecular chaperone. It is an integral part of the cellular proteostasis system and associated with human diseases such as Alzheimer's disease, myopathy, cataract, and multiple sclerosis. The molecular architecture of αB-crystallin follows an intriguing construction plan characterized by a dynamic oligomer equilibrium. Here (pp. E3780–E3789), we exploited phosphorylation mimetics as a tool to switch the protein to an activated functional state by a shift in the conformational ensemble. Using cryo-EM and image processing, we defined the structures of the activated αB-crystallin ensemble. Biochemical analysis revealed that, on activation, the N-terminal regions gain flexibility and solvent accessibility. This allows enhancing the activity of αB-crystallin and promoting its cooperation with the Hsp70 system.

Structural basis of regulation and oligomerization of human cystathionine β-synthase, the central enzyme of transsulfuration

June Ereño-Orbea, Tomas Majtan, Iker Oyenarte, Jan P. Kraus, and Luis Alfonso Martínez-Cruz

Cystathionine β-synthase (CBS), the pivotal enzyme of the transsulfuration pathway, regulates the flux through the pathway to yield compounds such as cysteine, glutathione, taurine, and H_2S that control the cellular redox status and signaling. Our crystal structures of the full-length wild-type and D444N mutant human CBS enzymes show a unique arrangement of the regulatory CBS motifs, thus making it possible to infer how the enzyme is stimulated by its allosteric activator S-adenosyl-L-methionine and how native tetramers are formed. The structure (pp. E3790–E3799) will allow modeling of numerous mutations causing inherited homocystinuria and the design of compounds modulating CBS activity.

Enhanced group II intron retrohoming in magnesium-deficient *Escherichia coli* via selection of mutations in the ribozyme core

David M. Truong, David J. Sidote, Rick Russell, and Alan M. Lambowitz

Mobile group II introns are bacterial retrotransposons. They consist of an autocatalytic intron RNA ("ribozyme") and an intron-encoded reverse transcriptase and were likely ancestors of spliceosomal introns and retroelements in eukaryotes. Although active in bacteria, group II introns function inefficiently in eukaryotes, where lower Mg^{2+} concentrations decrease their ribozyme activity and constitute a natural barrier to group II intron proliferation within nuclear genomes. By using an *Escherichia coli* Mg^{2+}-transport mutant, we selected mutations near the intron RNA's active site that enhance group II intron function at low Mg^{2+} concentrations. Our results (pp. E3800–E3809) have implications for ribozyme mechanisms, evolution, and biotechnology.

SUMOylation is essential for sex-specific assembly and function of the *Caenorhabditis elegans* dosage compensation complex on X chromosomes

Rebecca R. Pferdehirt and Barbara J. Meyer

Dosage compensation equalizes X-chromosome transcription between nematode males (1X) and hermaphrodites (2X) via a dosage compensation complex (DCC) that binds hermaphrodite X chromosomes to repress transcription by half. We show (pp. E3810–E3819) that several DCC subunits are modified by the small ubiquitin-like modifier SUMO in response to the signal that triggers DCC assembly onto X. DCC assembly and function require SUMOylation. DCC subunit DUMPY-28 also acts in condensin complexes essential for chromosome segregation, but its SUMOylation is DCC-specific. We propose that specific signals trigger DCC protein SUMOylation, stimulating robust complex formation. SUMOylation facilitates distinct activities of proteins that function in multiple complexes.

Actin-related protein2/3 complex regulates tight junctions and terminal differentiation to promote epidermal barrier formation

Kang Zhou, Andrew Muroyama, Julie Underwood, Rebecca Leylek, Samriddha Ray, Scott H. Soderling, and Terry Lechler

This study (pp. E3820–E3829) used a genetic approach to probe the function of the F-actin nucleating Arp2/3 complex in skin development. Although the Arp2/3 complex was expected to be involved in morphogenesis and cell adhesion, loss of its activity did not result in architectural problems in the epidermis. However, Arp2/3 was essential for epidermal function. We revealed two unexpected functions for the Arp2/3 complex in the epidermis that are critical for tissue physiology: tight junction assembly/function and the regulation of YAP activity.

Endothelin-2 signaling in the neural retina promotes the endothelial tip cell state and inhibits angiogenesis

Amir Rattner, Huimin Yu, John Williams, Philip M. Smallwood, and Jeremy Nathans

Two distinct and interconvertible types of endothelial cells are present during blood vessel growth: tip cells at the growing front of the vascular network and stalk cells behind the front. In the present study (pp. E3830–E3839), overexpression of Endothelin-2, a peptide previously implicated in the control of blood pressure, is shown to promote the tip cell fate and arrest vascular growth within the mouse retina. Genetic experiments show that this effect requires Endothelin receptor A expression in the neural retina, implying the existence of a retina-derived regulator of vascular growth and development that is under Endothelin control.

Critical role of segment-specific packaging signals in genetic reassortment of influenza A viruses

Boris Essere, Matthieu Yver, Cyrille Gavazzi, Olivier Terrier, Catherine Isel, Emilie Fournier, Fabienne Giroux, Julien Textoris, Thomas Julien, Clio Socratous, Manuel Rosa-Calatrava, Bruno Lina, Roland Marquet, and Vincent Moules

Genetic reassortment is one of the main mechanisms by which pandemic viruses emerge during influenza A coinfection, but little is known about the molecular mechanisms affecting this process. Here, we studied genetic reassortment between a human and an avian influenza A strain, focusing on the generation of reassortant viruses containing the avian HA gene, which have pandemic potential. We found (pp. E3840–E3848) that this genetic process was strongly biased, and we show that packaging signals are crucial for genetic reassortment and that suboptimal compatibility between the segment-specific packaging signals of the two parental viruses limits the emergence of reassortant viruses.

Active output state of the *Synechococcus* Kai circadian oscillator

Mark L. Paddock, Joseph S. Boyd, Dawn M. Adin, and Susan S. Golden

For circadian clocks to modulate a daily cycle of metabolic and behavioral processes, temporal information must be transmitted to output pathways. In cyanobacteria, the circadian oscillator is composed of three Kai proteins that mediate cyclic phosphorylation of KaiC. We determined (pp. E3849–E3857) that a specific phosphostate of KaiC promotes genome-wide circadian output responses. A model that includes temporal feedback control of this KaiC phosphostate can accurately simulate key features of observed output responses of the two major contrasting promoter types. This study provides a unique perspective on the complex regulatory output responses of circadian oscillators.

Simultaneous modeling of visual saliency and value computation improves predictions of economic choice

R. Blythe Towal, Milica Mormann, and Christof Koch

Many everyday decisions require viewing displays with several alternatives and then rapidly choosing one, e.g., choosing a snack from a vending machine. Each item has objective visual properties, such as saliency, and subjective properties, such as value. Objective and subjective properties are usually studied independently. We have implemented a single integrated paradigm that links perceptual with economic decision making by having subjects search through visual displays to choose a single food item that they want to eat. We demonstrate (pp. E3858–E3867) that two linked accumulator models, one modeling perceptual and the other modeling economic decisions, account for subjects' viewing patterns and choices.

Sensorimotor structure of *Drosophila* larva phototaxis

Elizabeth A. Kane, Marc Gershow, Bruno Afonso, Ivan Larderet, Mason Klein, Ashley R. Carter, Benjamin L. de Bivort, Simon G. Sprecher, and Aravinthan D. T. Samuel

Small animals such as *Drosophila* provide an opportunity to understand the neural circuitry for complex behaviors from sensory input to motor output without gaps. Here, we define the algorithms for *Drosophila* larva phototaxis (i.e., the maps between sensory input and motor output) by quantifying the movements of individual animals responding to a battery of illumination conditions. Surprisingly, the distinct rules that define different components of the overall photosensory response begin to segregate at the first synapses after the photoreceptor cells. These results (pp. E3868–E3877) lay the foundation for mapping the circuits for phototaxis in the compact nervous system of the larva by first elucidating the algorithms that define behavior and then mapping these algorithms to specific circuit pathways.

Dendritic growth gated by a steroid hormone receptor underlies increases in activity in the developing *Drosophila* locomotor system

Maarten F. Zwart, Owen Randlett, Jan Felix Evers, and Matthias Landgraf

Why do bigger animals have bigger brains, and how do they get them? We find in *Drosophila* larvae that motoneuron dendrites, the branched structures receiving information from other neurons, grow as the animal gets bigger, and that this is regulated on a cell-by-cell basis by a specific isoform of a steroid hormone receptor, whose functions were unknown. As these dendrites enlarge, they form more connections with presynaptic partners, leading to greater levels of neuronal activity. We propose (pp. E3878–E3887) that these nerve cells increase their activity to compensate for the demands of a bigger body.

ABI3 controls embryo degreening through Mendel's *I* locus

Frédéric Delmas, Subramanian Sankaranarayanan, Srijani Deb, Ellen Widdup, Céline Bournonville, Norbert Bollier, Julian G. B. Northey, Peter McCourt, and Marcus A. Samuel

Occurrence of mature green seeds in oil-seed crops such as canola and soybean causes severe losses in revenue. Retention of chlorophyll in seeds can be an undesirable trait as it affects seed maturation, seed oil, and meal quality. We show that the abscisic acid (ABA, plant hormone) dependent transcription factor ABSCISIC ACID INSENSITIVE 3 (ABI3), confers seed degreening by regulating Mendel's stay-green genes. This study (pp. E3888–E3894) unveils a new role for ABI3 in removal of seed chlorophyll in addition to its functions in embryo maturation and conferring desiccation tolerance. This pathway could be manipulated to tackle the cold-induced green seed problem in oil-seed crops.