# PNAS Plus Significance Statements

## Extending density functional embedding theory for covalently bonded systems

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Quantum mechanics (QM) simulations increasingly help researchers understand phenomena in chemistry, geoscience, material science, and biology. Unfortunately, the exact solution of the relevant QM equations is not available; consequently approximate yet accurate QM methods must be developed. In many situations, e.g., heterogeneous catalysis and enzymatic reactions, although the entire system is too large to treat with high-level QM, the relevant phenomena happen in a confined space. Quantum embedding theories can study exactly such cases by using highly accurate but expensive QM methods to simulate only the region of interest, while using fast but less accurate approximations to treat the surroundings. In this article, we present a unique, accurate quantum embedding theory applicable to covalently bonded systems. (See pp. E10861–E10870.)

## Effect of removing Kupffer cells on nanoparticle tumor delivery

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Nanomaterials are developed for treating and diagnosing cancer, but only 0.7% (median) are delivered to a solid tumor. To address this delivery problem, we are examining each biological barrier to determine its impact on tumor delivery. Because the liver sequesters up to 70% of nanomaterials, in this study, we asked, if liver Kupffer cells were removed, what is the impact on tumor delivery? While we demonstrate that the tumor delivery increased up to 150 times, we achieved 2% for nanomaterials of different size, material, and tumor type. This suggests the need to focus on tumor pathophysiology to increase delivery efficiency, since this approach led to a greater availability of nanoparticles in the blood, but 98% did not accumulate in solid tumors. (See pp. E10871–E10880.)

## Development and validation of a high-throughput transcriptomic biomarker to address 21st century genetic toxicology needs

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Standard in vitro assays to assess genotoxicity frequently generate positive results that are subsequently found to be irrelevant for in vivo carcinogenesis and human cancer risk assessment. Currently used follow-up methods, such as animal testing, are expensive and time-consuming, and the development of approaches enabling more accurate mechanism-based risk assessment is essential. We developed an in vitro transcriptomic biomarker-based approach that provides a robust biomarker reflecting stress-signaling responses. The biomarker correctly identifies the vast majority of irrelevant genotoxicity results from in vitro chromosome damage assays. TGx-DDI, a multigene biomarker for DNA damage-inducing agents, is the first biomarker that not only shows convincing interlaboratory and intralaboratory reproducibility, but also performs accurately in a system suitable for high-throughput screening. (See pp. E10881–E10889.)

## Structural insights into how GTP-dependent conformational changes in a metallochaperone UreG facilitate urease maturation

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Our work provides insights into how cells solve the problem of delivering nickel, a toxic metal, to the active site of a metalloenzyme such as urease. Urease, a nickel-containing enzyme, is a virulence factor for *Helicobacter pylori*, which infects half of the human population and causes peptic ulcers. Supported by structural and biochemical evidence, we present a paradigm on how a metallochaperone UreG couples GTP hydrolysis/binding to allosterically control the binding/release of nickel ions and to switch protein-binding partners along the metal-delivery pathway so that the nickel ions are passing from one metallochaperone to another, without releasing the "free" toxic metal to the cytoplasm. (See pp. E10890–E10898.)

## Aminoglycoside interactions and impacts on the eukaryotic ribosome

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Aminoglycosides are well known as antibiotics that target the bacterial ribosome. However, they also impact the eukaryotic translation mechanism to promote read-through of premature termination codons (PTCs) in mRNA. Aminoglycosides are therefore considered as potential therapies for PTC-associated human diseases. Here, we performed a comprehensive

study of the mechanism of action of aminoglycosides in eukaryotes by applying a combination of structural and functional approaches. Our findings reveal complex interactions of aminoglycosides with eukaryotic 80S ribosome caused by their multiple binding sites, which lead to inhibition of intersubunit movement within the human ribosome that impact nearly every aspect of protein synthesis. (See pp. E10899–E10908.)

## pH-sensitive vibrational probe reveals a cytoplasmic protonated cluster in bacteriorhodopsin

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The vectorial transport of protons across membranes by pumps is central to cellular bioenergetics. A persistent problem in their study is the technical unfeasibility to simultaneously resolve the dynamics of all the relevant proton transfer steps by the same method, that is, those within the protein as well as those involving protonation changes of the aqueous medium, currently relying on complementary methods to map both. Here, we solved this limitation and monitored both internal and external protonation changes during the proton-pump mechanism of bacteriorhodopsin by time-resolved infrared spectroscopy. Our findings reveal inconsistencies with the proton uptake mechanism accepted for the last 25 years, highlighting the need for simultaneous and comprehensive monitoring of protonation changes to resolve the molecular mechanism of ion pumps. (See pp. E10909–E10918.)

## Molecular chaperones maximize the native state yield on biological times by driving substrates out of equilibrium

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Molecular chaperones have evolved to assist the folding of proteins and RNA, thus avoiding the deleterious consequences of misfolding. Thus, it is expected that increasing chaperone concentrations should enhance the yield of native states. While this has been observed in GroEL-mediated protein folding, experiments on *Tetrahymena ribozyme* folding assisted by CYT-19 surprisingly show the opposite trend. Here, we reconcile these divergent experimental observations by developing a unified theory of chaperone-assisted protein and RNA folding. We show that these ATP-fueled machines drive their substrates out of equilibrium, maximizing the nonequilibrium native yield in a given time rather than the absolute yield or folding rate. The theory predicts that in vivo the number of chaperones is regulated to optimize their functions. (See pp. E10919–E10927.)

## STRIP1, a core component of STRIPAK complexes, is essential for normal mesoderm migration in the mouse embryo

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Striatin-interacting phosphatases and kinases (STRIPAK) complexes can regulate the cytoskeleton and cell migration in cell lines, but their roles in vivo in mammals are not known. Here, we show that mouse embryos that lack striatin-interacting protein 1 (STRIP1), a core component of STRIPAK complexes, arrest at midgestation with striking morphological defects. *Strip1* mutants lack a trunk, and both paraxial and axial mesoderm fail to elongate along the anterior–posterior body axis. Mesodermal cells from *Strip1* mutants have defects in actin organization, focal adhesions, and cell migration that can account for the failure of normal mesoderm migration. The findings demonstrate that STRIPAK is a critical regulator of mammalian cell migration and is likely to have important roles in tumor progression as well as development. (See pp. E10928–E10936.)

## Mapping local and global variability in plant trait distributions

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Currently, Earth system models (ESMs) represent variation in plant life through the presence of a small set of plant functional types (PFTs), each of which accounts for hundreds or thousands of species across thousands of vegetated grid cells on land. By expanding plant traits from a single mean value per PFT to a full distribution per PFT that varies among grid cells, the trait variation present in nature is restored and may be propagated to estimates of ecosystem processes. Indeed, critical ecosystem processes tend to depend on the full trait distribution, which therefore needs to be represented accurately. These maps reintroduce substantial local variation and will allow for a more accurate representation of the land surface in ESMs. (See pp. E10937–E10946.)

## *Kras* mutant genetically engineered mouse models of human cancers are genomically heterogeneous

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*RAS* mutant cancers represent a large unmet clinical need. *Kras* mutant genetically engineered mouse models (GEMMs) of cancer recapitulate disease characteristics and are relied upon preclinically to validate targets and test therapies. Our integrative analysis of GEMM tumors revealed significantly evolved genetic heterogeneity, a common feature of human tumors that undermines therapeutic responses. Moreover, interspecies comparative analyses showed the extent of gene-level fidelity between altered oncogenes and tumor suppressors. The genomic diversity represents an unrecognized opportunity to identify therapeutically susceptible genomic subsets preclinically. Moreover, this more-thorough understanding of the unappreciated complexity in these model systems ultimately allows for better interpretation and translatability of preclinical GEMM data for the benefit of cancer patients. (See pp. E10947–E10955.)

## CD1b-restricted GEM T cell responses are modulated by *Mycobacterium tuberculosis* mycolic acid meromycolate chains

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Tuberculosis is a major global pandemic responsible for more deaths than any other infectious disease, yet no effective vaccine exists. Here, we demonstrate CD1b expression within human

tuberculous granulomas, supporting a role for CD1b lipid antigen presentation in host immunity to infection. CD1b presents mycolates, the dominant *Mycobacterium tuberculosis* (Mtb) cell wall lipid class and key virulence factors, to αβ T cells. We reveal that mycolate tail moieties, distal to the head group, are antigenic determinants for the conserved human germline-encoded mycolyl lipid-reactive (GEM) T cell receptors (TCRs). Computational simulations suggest a putative mechanism whereby lipid-ligand dynamics within CD1b regulate GEM-TCR activity. This work provides insights for the development of major histocompatibility complex (MHC)-independent Mtb lipid vaccines, including those that target GEM T cells. (See pp. E10956–E10964.)

## Point-of-care device to diagnose and monitor neonatal jaundice in low-resource settings

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Neonatal jaundice, a condition caused by the accumulation of bilirubin in the bloodstream, affects approximately half of all newborns. In high-resource settings, babies with elevated serum bilirubin levels are identified through routine hospital laboratory testing. When identified, jaundice is easily treated using blue-light phototherapy. Low-cost, rugged phototherapy lights have been developed and shown to be effective in low-resource settings. However, jaundice regularly goes undetected in these settings due to a lack of diagnostic tools to measure bilirubin levels. Left untreated, jaundice can lead to permanent neurological damage and mortality, the vast majority of which currently occurs in low-resource settings. In this paper, we present a low-cost method to measure total bilirubin at the point of care in low-resource settings. (See pp. E10965–E10971.)

## DNA replication timing alterations identify common markers between distinct progeroid diseases

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We show that the temporal order of replication (replication timing, RT), normally an extremely stable cell type-specific chromosomal property, is altered in cells from two different premature aging (progeroid) diseases. By converting patient cells to stem cells and redifferentiating them as a model of disease progression, we identified the *TP63* gene as one of the earliest RT alterations and altered RT was associated with abnormal *TP63* gene expression. *TP63* mutations have been linked to other diseases that share clinical features of progeroid syndromes. These findings introduce an approach for disease marker discovery, identify molecular abnormalities distinguishing progeroid diseases from natural aging, and point to *TP63* as a molecular link to the pathophysiological manifestations of progeroid diseases. (See pp. E10972–E10980.)

## Epigenetic therapy activates type I interferon signaling in murine ovarian cancer to reduce immunosuppression and tumor burden

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Therapies that activate the host immune system have shown tremendous promise for a variety of solid tumors. However, in most cancer types, fewer than half of patients respond to these immunotherapies. We propose epigenetic therapy as a mechanism to sensitize tumors to immune checkpoint therapy. We have shown that inhibiting DNA methylation triggers a viral defense pathway in tumors. Here we show that epigenetic therapy in a mouse model of ovarian cancer increases the numbers of activated immune cells, and that this is dependent on the interferon antiviral response. The combination of epigenetic therapy and immune checkpoint blockade leads to the greatest reduction in tumor burden and increase in survival, and may hold the greatest promise for patients. (See pp. E10981–E10990.)

## An endogenous retroviral envelope syncytin and its cognate receptor identified in the viviparous placental *Mabuya* lizard

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Retroviral envelope gene capture and exaptation for a placental function has been demonstrated in mammals. Remarkably, placental structures have also emerged on rare occasions in nonmammalian vertebrates, resulting in related modes of reproduction. The *Mabuya* lizard, which emerged 25 Mya, possesses a placenta closely related to that of mammals. Here, we identified a specific retroviral envelope gene capture that shows all the characteristic features of a bona fide mammalian syncytin, being conserved in *Mabuya* evolution, expressed in the placenta, and fusogenic. Together with the present identification of its cognate receptor, these results show that syncytin capture is not restricted to mammals and is likely to be a major driving force for placenta emergence. (See pp. E10991–E11000.)

## Enigmatic origin of the poxvirus membrane from the endoplasmic reticulum shown by 3D imaging of vaccinia virus assembly mutants

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Poxviruses cause human and epizootic diseases and are employed as vaccine vectors. The present investigation provides insights into a key step in poxvirus replication, the assembly of infectious virus particles. Enveloped viruses acquire membranes from cellular organelles; nevertheless, the source of the poxvirus membrane has been an enigma. Poxvirus assembly occurs in cytoplasmic factories, and the first recognizable structures are membrane crescents without discernible connections to cellular membranes. The key to demonstrating connections was isolation of vaccinia virus mutants that are defective in assembly. Electron tomographic analyses of cells infected with the mutants unambiguously demonstrated continuity between viral membranes and the endoplasmic reticulum and suggested that viral proteins induce or stabilize membrane scissions during a normal infection. (See pp. E11001–E11009.)

## Lipid bilayer mediates ion-channel cooperativity in a model of hair-cell mechanotransduction

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Hearing relies on molecular machinery that consists of springs stretched by mechanical stimuli and mechanosensitive ion channels responding to the generated tension. Reproducing the experimental data theoretically without requiring unrealistically large

conformational changes of the channels has been a longstanding hurdle. Here, we propose and develop a model with two mobile channels per spring, coupled by elastic forces within the membrane. The relative motion of the channels following their cooperative opening and closing produces the required change in spring extension. This study lies at the interface between the fields of membrane mechanics and mechanotransduction in the inner ear. It describes a physiological function for the bilayer-mediated cooperativity between mechanosensitive ion channels in a vertebrate sensory system. (See pp. E11010–E11019.)

### Disease onset in X-linked dystonia-parkinsonism correlates with expansion of a hexameric repeat within an SVA retrotransposon in *TAF1*

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The genetic basis of X-Linked dystonia-parkinsonism (XDP) has been difficult to unravel, in part because all patients inherit the same haplotype of seven sequence variants, none of which has ever been identified in control individuals. This study revealed that one of the haplotype markers, a retrotransposon insertion within an intron of *TAF1*, has a variable number of hexameric repeats among affected individuals with an increase in repeat number strongly correlated with earlier age at disease onset. These data support a contributing role for this sequence in disease pathogenesis while further suggesting that XDP may be part of a growing list of neurodegenerative disorders associated with unstable repeat expansions. (See pp. E11020–E11028.)

### Evidence for sortilin modulating regional accumulation of human tau prions in transgenic mice

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Human neurodegenerative diseases such as Alzheimer's disease develop in a highly stereotyped fashion, suggesting intrinsic differences among brain regions determine whether they are affected or spared. Here, we employed a widely used line of transgenic (Tg) mice expressing mutant (P301S) human tau and exhibiting robust tauopathy. By examining brain regions affected by and spared of tau prions, we found that localization of human tau prion formation in Tg mice arises from regional inhibition of tau prion replication. Using a cell-based bioassay, we identified an inhibitor of tau prion propagation in the forebrain. These discoveries may lead to the identification of key mediators of brain vulnerability involved in human tauopathies, a promising strategy for the development of targeted therapeutics for these diseases. (See pp. E11029–E11036.)

### Divergence of regulatory networks governed by the orthologous transcription factors FLC and PEP1 in Brassicaceae species

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Developmental programs of higher plants show plasticity to environmental signals. In the Brassicaceae, the transcription factor (TF) FLOWERING LOCUS C (FLC) represses reproduction until plants are exposed to winter cold. Here we define the target genes of FLC in two species in different lineages of the Brassicaceae and compare the target sequences across the family. Fewer than 20% of target genes were conserved between the species examined, and genes involved in flowering were overrepresented among these. By contrast, many of the nonconserved target genes were involved in stress responses. We propose that, for TFs like FLC, which control environmental responses of plants, core sets of targets are conserved between species, but the majority change rapidly during evolution. (See pp. E11037–E11046.)

### Encoding model of temporal processing in human visual cortex

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How is temporal information processed in human visual cortex? To address this question, we used fMRI and a two temporal channel-encoding model. This approach not only explains cortical responses for time-varying stimuli ranging from milliseconds to seconds but finds differential temporal processing across human visual cortex. While motion-sensitive regions are dominated by transient responses, ventral regions that process the content of the visual input surprisingly show both sustained and transient responses, with the latter exceeding the former. This transient processing may foster rapid extraction of the gist of the scene. Importantly, our encoding approach marks a transformative advancement in the temporal resolution of fMRI, as it enables linking fMRI responses to the timescale of neural computations in cortex. (See pp. E11047–E11056.)