

Jonathan Bogan, M.D., 2000 Smith Award Recipient
Yale School of Medicine

An insulin-stimulated proteolytic mechanism links energy expenditure with glucose uptake

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Mechanisms to coordinately regulate oxidative metabolism and glucose transport into cells are not well described. In muscle and fat, insulin mobilizes GLUT4 glucose transporters to the cell surface in part by stimulating the site-specific endoproteolytic cleavage of TUG proteins. Here, we show that the TUG C-terminal cleavage product enters the nucleus, binds the transcriptional regulators PGC-1 α and PPAR γ , and increases oxidative metabolism, thermogenic protein expression, and energy expenditure. The PPAR γ 2 Pro12Gly polymorphism, which confers reduced diabetes risk, enhances TUG binding. The TUG cleavage product stabilizes PGC-1 α , and makes both proteins susceptible to an Ate1 arginyltransferase -dependent degradation mechanism. We conclude that TUG cleavage coordinates energy expenditure with glucose uptake, and that alterations in this pathway may contribute to metabolic disease.

Laurie A. Boyer, Ph.D., 2008 Pew Scholar, 2009 Smith Award Recipient
Massachusetts Institute of Technology

Regulation of Transcriptional Dynamics in Controlling Developmental Decisions

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Precise control of transcription is crucial to ensure proper development in all metazoans and faulty regulation of this process underpins many diseases. In most cases, however, we lack fundamental knowledge of how upstream signals cross-talk with the transcriptional machinery to specify cell fate. We investigate how multi-scale regulatory inputs are translated into quantitative transcriptional outputs during mammalian development. A deep learning of this regulatory code has the potential to predict how perturbations in these networks lead to disease. In particular, we focus on the interplay between non-coding RNA, DNA binding factors, and chromatin remodelers with an emphasis on understanding how these regulators coordinate to promote lineage commitment. Our recent work has also begun to uncover how metabolic signaling drives transcriptional programs and ultimately terminal differentiation during mammalian heart development. For example, cardiac muscle cells (cardiomyocytes; CMs) undergo a major metabolic shift during embryogenesis toward fatty acid beta oxidation after birth. This change in CM metabolism coincides with dramatic changes in transcriptional and epigenetic programs that coincide with maturation of mitochondria and sarcomere structure as the cells become post-mitotic and lose their ability for repair in response to injury. By combining single-cell genetic, genomic, and imaging approaches, our preliminary work shows that maintenance of spatiotemporal regulation of redox signaling is critical for specifying the transcriptional programs that drive CM maturation during postnatal heart development. More broadly, we expect that this investigation will uncover novel mechanisms for understanding how metabolism is coupled to regulation of gene expression states in diverse developmental contexts.

David K. Breslow, Ph.D., 2018 Smith Award Recipient
Yale University

Deconstructing the Cellular Antenna: Primary Cilium Disassembly and Control of Cell Growth

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The primary cilium is a protrusion from the cell surface that serves as an organizing center for diverse signaling pathways. Cilia are required for embryonic development, and inherited ciliary defects cause pediatric disorders known as ciliopathies. Cilia are found on most cells in the human body, but cilia are not constantly present; rather, they undergo regulated disassembly prior to mitosis and re-assemble after cell division. This regulated disassembly is widely observed across evolution and appears to be a key event in the cell cycle, as cilia are never observed on mitotic cells. Recent evidence further indicates that cilium disassembly controls cell cycle progression, acting like a checkpoint that blocks proliferation until disassembly is completed. Deregulation of cilium disassembly may thus contribute to mitotic errors, uncontrolled cell growth and cancer. With support from a Smith Award, we are investigating several key questions, including: what signals trigger cilium disassembly, what factors enable disassembly of the cilium's complex structural elements, how does an intact cilium delay cell cycle progression, and how do errors in these processes contribute to disease? To address these questions, we are applying new tools including high-resolution imaging of cilium disassembly, genome-wide screening to identify genes that control cilium disassembly, and biochemical reconstitution of cilium disassembly. Preliminary findings indicate that centriolar proteins and tumor suppressors regulate cilium disassembly. Together, these studies will provide a foundational characterization of cilium disassembly and of the critical connections between this process, the cell cycle, and disease.

Abhishek Chatterjee, Ph.D., 2013 Smith Award Recipient
Boston College

An optimized platform for universal genetic code expansion
Katherine T. Grasso, James S. Italia, Megan Yeo, Abhishek Chatterjee
Boston College

Site-specific incorporation of noncanonical amino acids (ncAAs) has emerged as a powerful technology to probe and manipulate protein function *in vivo*. This technology uses engineered aminoacyl-tRNA synthetase (aaRS)/tRNA pairs to incorporate ncAAs in response to nonsense codons. A key limitation is the requirement of using alternate aaRS/tRNA pairs for ncAA incorporation in prokaryotes and eukaryotes, which are engineered using different directed evolution platforms. This limits the scope of the technology for application in eukaryotes. Recently, we developed universal genetic code expansion platforms that can be used for ncAA incorporation in both *E. coli* and mammalian cells. Our strategy relies on the creation of “altered translational machinery” (ATM) *E. coli* strains where one of the endogenous aaRS/tRNA pairs is functionally replaced with a eukaryotic or archaeal counterpart. The “liberated” aaRS/tRNA pair can then be used for ncAA incorporation in ATM *E. coli* and eukaryotes. This strategy was employed to create an ATM-tyrosyl (ATMY) strain but the reintroduction of the “liberated” *E. coli* tyrosyl-tRNA as a UAG suppressor in ATMY *E. coli* led to cross-reactivity with the endogenous glutaminyl-tRNA synthetase, restricting the activity range of aaRS’ that could be selected. We used directed evolution of the *E. coli* tyrosyl-tRNA_{UAG} to eliminate cross-reactivity, enabling selection of ncAA-selective aaRS mutants with a broad range of activities. We further developed a directed evolution scheme to improve the activity of first-generation engineered aaRS mutants. The utility of this selection system was demonstrated by developing highly efficient incorporation systems for a variety of ncAAs in mammalian cells.

Stephanie Dougan, PhD, 2015 Smith Award Recipient, 2016 Pew-Stewart Scholar
Dana-Farber Cancer Institute

Destruction of MHC class I negative tumors through T cell-dependent reprogramming of mononuclear phagocytes

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Loss of MHC class I and IFNg sensing are major causes of primary and acquired resistance to checkpoint blockade immunotherapy. The cellular (c)-IAPs regulate classical and alternative NF-κB signaling. Induction of non-canonical NF-κB signaling with IAP antagonists mimics costimulatory signaling, augmenting anti-tumor immunity. We now show that induction of non-canonical NF-κB signaling induces T-cell dependent immune responses even in b2m-/ tumors, demonstrating that direct CD8 T cell recognition of tumor cell expressed MHC class I is not required. Instead, T cell-produced cytokines reprogram both mouse and human macrophages to be tumoricidal. In wild type mice, but not mice incapable of antigen-specific T cell responses, IAP antagonism reduces tumor burden by increasing phagocytosis of tumor cells. Efficacy is augmented by combination with CD47 blockade. Activation of non-canonical NF-κB stimulates antigen-specific responses toward tumors that are resistant to checkpoint blockade due to loss of MHC class I or IFNg sensing.

Patrick Emery, Ph.D., 2002 Smith Award Recipient

Sleep Modulation By Glial Transporters In *Drosophila*

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In this study, we examined the role of astrocytes in sleep using *Drosophila melanogaster*. A set of astrocyte-enriched genes was screened for sleep defects using RNAi or genome editing. From this screen, we identified two mutants in genes encoding transporters with opposing effects on sleep: While a hypomorphic mutation in the gene encoding the GABA transporter (GAT), caused extended sleep, null mutants for CG7888, led to a reduction in sleep. Tissue specific rescue experiments showed that both genes act in glia and more specifically in astrocytes to affect sleep behavior.

In *gat* mutants glial clearance of the neurotransmitter GABA is expected to be impaired, which would lead to the accumulation of GABA at the synaptic cleft. As a result, mutant flies exhibited more consolidated and longer sleep bouts. Interestingly, the excessive sleeping phenotype was suppressed, when combined with a mutation disrupting *wake*, a gene that regulates the level of GABA_A receptor (RDL) and facilitates its localization to the plasma membrane in PDF neurons. This suggests that *gat* functions upstream of *wake*. In contrast, a null mutant of CG7888 exhibited reduced sleep. CG7888 is highly homologous to carriers in the mammalian SLC36 family, whose members include proton-assisted amino acid transporters (PATs). In stably transfected HEK cells, CG7888 is detected both at the plasma membrane and in rab11-positive endosomes. Radiotracer uptake assays revealed that, similar to PAT2, proline, glutamate, and glutamine can serve as CG7888 substrates. We are exploring the possibility that this transporter contributes to glutamate-glutamine recycling in glia.

Jianmin Gao, Ph.D., 2007 Smith Award Recipient
Boston College

Protein Surface Recognition via Chemically Enhanced Phage Display

Wenjian Wang, Samantha Cambray and Jianmin Gao
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Proteins are appealing targets for drug development and disease biomarkers discovery. However, it is challenging to target undruggable proteins which typically lack a targetable binding pocket. Phage display, a powerful technique for peptide library screening, allows facile identification of peptide ligands for many targets with high affinity and specificity. A novel phage library that incorporate dynamic covalent binding motif, 2-acetylphenylboronic acid (2-APBA), has been developed by our lab. In this report, the potential of APBA-dimer library to discover peptide probes for specific proteins is demonstrated. Ligands with low micromolar K_d and specificity were identified after three rounds of panning. Instead of binding to the active site, these peptides are believed to anchor on the surface of the protein.

Elena O. Gracheva, PhD, 2018 Smith Odyssey Award Recipient
Yale School of Medicine

Osmolyte depletion and thirst suppression allow hibernators to survive for months without water

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Hibernation is a fascinating physiological phenomenon during which animals must rely solely on the management of internal resources for long-term survival of up to nine months. Winter hibernation in small rodents is characterized by torpor and arousal; animals cycle between weeks-long torpor bouts of severe hypothermia and hypometabolism and short interbout arousals, during which major physiological parameters return to normal for up to 12-18h. We use the thirteen-lined ground squirrel as a study system to characterize and identify adaptive mechanisms that regulate fluid balance throughout the year, including during hibernation when water is unavailable. The neural, hormonal, and molecular adaptations identified here provide novel insights into hibernation physiology and mammalian fluid homeostasis in general. These findings contribute to a more comprehensive understanding of neural and hormonal mechanisms that allow life to persist under extreme internal and external conditions beyond those currently tolerated by non-hibernators like humans and mice. Physiological and mechanistic insights gained from hibernators have numerous and far-reaching medical applications, such as increased organ survival time for transplant surgeries, faster recovery from ischemia and reperfusion injuries, and better methods for therapeutic hypothermia.

Paul Greer, Ph.D., 2018 Smith Award Recipient and 2019 Searle Scholar
University of Massachusetts Medical School

Diverse Chemosensory Roles of MS4A Chemosensors

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Our lab is interested in the fundamental question of how animals sense and interpret interoceptive and exteroceptive chemical signals to generate appropriate organismal responses. To address this problem, we are leveraging our recent discovery of a novel family of chemosensor, encoded by the *Ms4a* family of genes, which participates in both interoception and exteroception. We find that a subset of *Ms4a* family members is expressed within the primary sensory neurons of the mammalian olfactory system, where they are responsible for detecting external, ethologically relevant odorant cues. By contrast, a second subset of *Ms4a* family members is expressed within microglia, the resident immune cells of the central nervous system, where they play a critical role in regulating inflammatory responses within the brain. Here, we report our latest findings characterizing how MS4A chemoreceptors translate chemical stimuli into organismal action in both of these contexts.

Aaron N. Hata, M.D., Ph.D., 2017 Smith Award Recipient
Massachusetts General Hospital Cancer Center

Impact of Therapy-Induced APOBEC3A on Tumor Heterogeneity and Evolution of Acquired Drug Resistance To Lung Cancer Targeted Therapies

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Massachusetts General Hospital Cancer

Molecularly targeted therapies have transformed the treatment of lung cancers that harbor genetic alterations in the EGFR and ALK genes. Unfortunately, responses are temporary and acquired drug resistance inevitably develops leading to disease progression. We have observed that in some patients, the evolution of acquired drug resistance coincides with the accumulation of new mutations caused by APOBEC, a family of enzymes known to play a role in viral defense as well as cancer development. We hypothesize that induction of mutational processes such as APOBEC-mediated cytidine deamination by EGFR and ALK targeted therapies increases intratumoral genetic heterogeneity and facilitates the evolution of acquired drug resistance. To investigate this, we modeled the evolution of drug resistant clones in EGFR and ALK cell lines and observed increased expression and activity of APOBEC3A in response to targeted therapy. Drug tolerant persister cells (DTPs) that survived during therapy maintained APOBEC3A expression and accumulated double strand DNA breaks (DSBs) which could lead to genomic instability. In fully resistant clones that emerged from DTPs, we observed APOBEC signature and elevated copy number changes and genomic rearrangements. Surprisingly, overexpression of APOBEC3A enhanced the survival of DTPs, and conversely, deletion of APOBEC3A suppressed the emergence of DTPs. Together, these results suggest that lung cancer targeted therapies induce expression of APOBEC3A leading to genomic instability, which in turn may accelerate the evolution of acquired drug resistance.

Ya-Chieh Hsu, Ph.D., 2014 Smith Award Recipient, 2017 Pew Scholar, and
2018 Smith Odyssey Award Recipient
Harvard University

Psychological Stress Drives Melanocyte Stem Cell Exhaustion Through Activation Of The Sympathetic Nervous System

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Psychological stress negatively affects tissue homeostasis and regeneration, but whether and how stress perception leads to profound changes in tissue biology remains poorly understood. Here, we investigate this question in the skin. Psychological stress has been anecdotally associated with hair graying, but a scientific evidence linking the two is lacking. By adapting approaches to induce stress in mice, including physical pain and restraining, we showed that psychological stress leads to gray hair formation through rapid depletion of melanocyte stem cells (MeSCs). Combining denervation, endocrine surgeries, cell ablation, and cell-type specific gene deletions, we showed that stress-induced hair graying is independent of stress hormones or the immune system, but relies on the activation of the sympathetic nervous system. Sympathetic nerve terminals innervate the MeSC niche. Under stress, sympathetic nerve activation leads to burst release of neurotransmitter norepinephrine, which targets MeSCs directly. Norepinephrine drives MeSCs proliferation, leading to their rapid exhaustion. Inhibition of MeSC proliferation or MeSC-specific deletion of norepinephrine receptors rescue stress-induced hair graying. Our study shows that psychological stress-induced neural activity can alter somatic stem cells directly, and identifies strategies that might be exploited for therapeutic purposes in the future.

Shantanu Jadhav, Ph.D., 2019 Smith Odyssey Award Recipient
Brandeis University

Memory Replay in the Hippocampal-Prefrontal Network for Spatial Learning and Decision Making

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Brandeis University

Memories allow us to learn and recall our experiences to guide daily behavior, and memory deficits remain an intractable consequence of several neuropsychiatric disorders. Neural synchronization between two key brain regions, the hippocampus and prefrontal cortex, is thought to be important for storage and recall of memories, but the physiological mechanisms that mediate this coordination remain unclear. One process that could play a key role is "memory replay", prominently seen in spatial memory tasks, during which hippocampal neuronal populations reactivate stored patterns representing sequences of behavioral experiences in either forward or reverse order. We and others have recently discovered coordination between hippocampal-prefrontal networks during memory replay. To address how this coordination supports memory-guided behavior, we continuously monitored neural activity using longitudinal recordings of hippocampal-prefrontal ensembles in rats throughout learning of a spatial-choice task. We found that reverse and forward hippocampal sequences at goal locations replay all available past and future choices, respectively, underlying an internal cognitive search that can support learning and planning of behavioral paths towards goals. Coordinated hippocampal-prefrontal replay can further read-out these sequences to distinguish correct past and future choices, supporting the ability to recall specific past experiences to guide future decisions. Additionally, reverse replay of past paths and forward replay of future paths exhibited opposing learning gradients. Our findings reveal a role of coordinated hippocampal-prefrontal replay in retrospective evaluation for learning, and prospective planning for memory recall and decision making.

Ankur Jain, Ph.D., 2018 Smith Award Recipient
Whitehead Institute for Biomedical Research

Identifying Cellular Suppressors of RNA Aggregation

Joshua Park, Michael Das, Ankur Jain
Whitehead Institute for Biomedical Research

Aggregation of proteins is a well-studied phenomenon that has implications ranging from basic biology to human disease. Our lab has discovered that like proteins, RNA in a cell is also susceptible to aggregation. Mutations associated with several rare neurological diseases such as Huntington disease, myotonic dystrophy, and amyotrophic lateral sclerosis, result in RNA that form aggregates. Here, our goal is to identify genes and small molecules that may inhibit RNA aggregation. We have developed a high-throughput microscopy-based assay where we can quantitatively measure the efficacy of an applied perturbation in reducing the RNA aggregates. In pilot studies, we screened ~6000 FDA approved small molecule drugs. We found that inhibitors of cyclin-dependent kinases (CDK) and heat shock protein 90 (HSP90) diminished RNA aggregation, and in some cases, sequestered the RNA in the nucleus. In parallel studies, we are conducting genetic screens to identify endogenous proteins that abate RNA aggregation under physiological conditions. These experiments will help us identify the cellular defense mechanisms against RNA aggregation and may reveal novel modifiers of neurological disease.

Aleksandar D. Kostic, Ph.D., 2017 Smith Award Recipient
Joslin Diabetes Center and Harvard Medical School

Building a Genetic Understanding of the Microbiome in Health and Diabetes

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The Smith Family grant to the Kostic lab has been instrumental in spurring a lively research program, which I highlight here. Our research centers around building genetic tools and resources for the human microbiome and applying them to understand diabetes. We have conducted a metagenomic meta-analysis of the human microbiome and discovered the existence of the “singleton” gene, enabling us to estimate the size of the microbiome gene universe (235M in the gut, 87M in the oral cavity). We have paired this expansive gene catalog with machine learning approaches to make a novel predictor of human phenotypes that rivals human genomics. By applying this workflow to archaeological specimens, we have reconstructed hundreds of draft genomes of the ancient human gut microbiome. On the experimental side, we have built genetic tools in a “model microbiome” composed of eight organisms that together rescue many phenotypic deficits of germ-free animals. By building these tools, we have enabled the discovery of novel secreted factors by which the microbiome elicits immune tolerance from the host. By studying the microbiomes of individuals with long-term type 1 diabetes (T1D), we have identified genetic features that correlate with residual insulin production and established that a related microbiome-derived protein can induce beta cell expansion *ex vivo*. We have established that vaccination against a prominent bacterial cell wall constituent, PNAG, can prevent onset of T1D in a mouse model. Finally, we demonstrate that high-dose probiotics decrease post-meal blood glucose spikes by sequestering sugar, a potential therapeutic remedy to a major problem in diabetes.

Philip J. Kranzusch, Ph.D., 2016 Smith Award Recipient
Harvard Medical School, Dana-Farber Cancer Institute

Poxvirus immune nucleases are descended from ancient insect virus cGAS-STING antagonists

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Cytosolic DNA triggers innate immune responses by activating cyclic GMP–AMP synthase (cGAS) to produce the cyclic dinucleotide second messenger 2'3'-cGAMP. We recently discovered the enzyme poxvirus immune nuclease (poxin), a vaccinia virus effector that cleaves 2'3'-cGAMP and restricts immune activation during infection. Surprisingly, distant poxin homologs exist outside mammalian pathogens, encoded in the genomes of diverse insect viruses and host insects in the order Lepidoptera (moths and butterflies). Here we use an iterative structural biology and bioinformatic approach to define poxins as an ancient family of enzymes dedicated to inhibition of cGAS-STING signaling. The poxin family includes seven clades that arose through horizontal transfer events between viral pathogens and through domestication within host insect genomes. Viral and host poxins share structural homology with proteases from (+)ssRNA viruses, and the most divergent poxin homologs map to unclassified insect RNA viruses in the family *Flaviviridae*. Recombinant *Flaviviridae* poxins retain selective 2'3'-cGAMP degradation supporting a possible RNA viral origin of this diverse enzyme family. Structural and biochemical analysis of divergent poxin homologs defines clade-specific remodeling of active site residues, altered rates of 2'3'-cGAMP degradation, and enzyme adaptation to distinct immunoregulatory roles in host and viral genomes. Together, our data reveal mammalian poxviruses captured an effector from an insect host-pathogen arms race, highlighting how pathogens tap into rich sources of environmental genetic diversity to gain the upper-hand against their hosts.

Joshua Kritzer, Ph.D., 2009 Smith Award Recipient
Tufts University

Using the Chloroalkane Cell Penetration Assay (CAPA) to Study the Cytosolic Localization of RNA Therapeutics

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Tufts University

A critical obstacle for RNA therapeutics is the lack of understanding of the characteristics that affect cell uptake, cytosolic localization, and intracellular trafficking. RNA with specific chemical modifications are able to access the cytosol without an external delivery method, but the mechanisms by which these molecules penetrate the cell membrane are unknown. Current methods for measuring the cellular uptake of RNA are imprecise and largely qualitative, which severely limits our understanding of, and control over, this therapeutic strategy. The drawbacks of existing methods highlight the importance of a quantitative and high throughput assay to uncover information about the cytosolic access of therapeutically-relevant molecules. To solve this problem, the Kritzer lab has developed the chloroalkane penetration assay (CAPA), which can quantitatively compare the cytosolic penetration of exogenously added molecules. We have previously used CAPA to examine the relative cytosolic access of peptides, and here we apply CAPA to RNA for the first time. CAPA provides cell penetration profiles for therapeutically relevant RNA molecules, and we are using it to screen for structural changes that improve RNA penetration. We present CAPA as a means to study the mechanism of uptake, degree of endosomal escape, and subcellular localization of therapeutic RNA molecules. Enhancing cell penetration would improve the therapeutic effect of biologically active molecules. Better control over the cellular fate of RNAs would be transformative in the design of new therapies for currently untreatable genetic diseases.

Galit Lahav, Ph.D., 2005 Smith Award Recipient
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Combined p53 dynamics and post-translational modifications determine subsequent protein expression and cell fate

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The transcription factor p53 is activated by a diverse repertoire of cellular stresses and subsequently induces the expression of response pathway genes governing diverse cell fates (e.g. cell cycle arrest, apoptosis). Temporal changes in p53 levels (*dynamics*) following activation, as well as post-translational modifications (*PTMs*) on p53, are two known distinct mechanisms by which differential target gene expressions are selected. The collective effects of disparate p53 dynamics and PTMs then regulate the expression of target genes and proteins, which in turn alter cell fates. Understanding how PTMs cooperate with dynamical changes in p53 protein levels to regulate gene expression will help us understand how p53 coordinates cell fate decisions. Here we show that PTMs on p53 are not static in response to DNA damage, but instead also change dynamically. Furthermore, we have begun to resolve the spectrum of PTMs on individual p53 proteins to elucidate how combinatorial PTMs affect p53 function. The results described here have important implications for the understanding how p53 coordinates cell fate decisions in response to various cellular signaling.

Anthony Letai, M.D., Ph.D., 2004 Smith Award Recipient
Dana-Farber Cancer Institute

Dynamic BH3 profiling as pharmacodynamic biomarker for the activity of BH3 mimetics

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Pharmacodynamic (PD) biomarkers provide information about the pharmacologic effects of a drug on its target, i.e. to assess whether a given agent is engaging its target in the expected manner. There is currently no convenient PD marker for BH3 mimetics. BH3 profiling (BP) is a functional assay developed to measure cells susceptibility to apoptosis and cell's dependence on certain Bcl-2 anti-apoptotic proteins for survival. Dynamic BH3 profiling (DBP) measures the changes of BP signals after drug perturbations. In this study, we studied whether DBP with lymphoid cells can serve as a PD biomarker for the activity of BH3 mimetics.

Using DBP and cell lines with defined dependency, we first showed that BH3 mimetics BCL201 (S55746) and S63845 are highly selective for Bcl-2 or Mcl-1, respectively. Next, we treated primary human lymphoid cells with BCL201 and then conducted DBP. We asked if pretreatment with BCL201 altered mitochondrial sensitivity to different BH3 peptides. After treatment with 1 μ M BCL201, an amount consistent with achievable levels in vivo, T cell sensitivity to the Mcl-1 selective MS1 peptide, but not Bad peptide or Bfl-1 selective FS1 peptide, increased significantly, from 20% to 51%. These results suggest that BCL201 treatment increases T cell dependency on Mcl-1 and that DBP of T cells with MS1 peptide may serve as a PD marker for BCL201 activity. Similar results were also observed in B cells.

Next, we conducted DBP on healthy T cells treated with S63845. T cell mitochondrial sensitivity to MS1 peptide or Bcl-xL selective HRK peptide did not change. However, the treatment increased mitochondrial sensitivity to the Bad and FS1 peptides significantly, boosting the signal from 10.2% to 57.2% and 46.8% respectively, suggesting DBP of T cells with Bad or FS1 peptides can be robust PD markers for S63845 activity. This results also indicate that S63845 treatment augmented T cell dependency on Bcl-2 and Bfl-1 for survival.

Currently, we are investigating whether DBP can be used as a PD biomarker for in vivo studies. Our preliminary data suggest that DBP works well across different species (rat, mouse, and human). More importantly, DBP can detect changes in mitochondrial apoptotic signaling of ex vivo rat cells after in vivo treatment with BH3 mimetics.

Conclusions: Exposure to BH3 mimetics causes changes in mitochondrial apoptotic signaling of T/B cells which are readily detectable by DBP. DBP with lymphoid cells may provide a robust PD biomarker for clinical trials testing BH3 mimetics, either as monotherapy or in combination with other agents.

Gene-Wei Li, Ph.D., 2016 Searle Scholar, 2017 Smith Award Recipient, 2017 Pew Scholar
Massachusetts Institute of Technology

Uncoupled transcription-translation defines regulatory genomes of Gram-positive bacteria

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Kinetically coupled transcription and translation set up a cornerstone of gene regulation in *Escherichia coli*. However, whether coupling of these two fundamental processes is a general principle across the bacterial world remains unknown. Here we show that RNA polymerases (RNAPs) consistently outpace the pioneering ribosomes in the Gram-positive model bacterium *Bacillus subtilis*. Uncoupled transcription-translation profoundly impacts the placement and regulatory roles of both Rho-dependent and intrinsic transcription terminators, thereby defining the landscape of gene regulation in *B. subtilis*. Bioinformatic analysis further revealed widespread signatures of discoordinate ribosome-RNAP movements among Gram-positive bacteria. These results indicate that transcription-translation coupling is not a hallmark of bacterial gene expression and help explain the prevalence of ribosome-mediated transcriptional attenuation in *E. coli* and RNA-mediated attenuation in *B. subtilis*.

Maofu Liao, Ph.D., 2016 Smith Award Recipient, 2019 Smith Odyssey Award Recipient
Harvard Medical School

Structure and mechanism of the cation–chloride cotransporter NKCC1

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Cation-chloride cotransporters (CCCs, also known as the SLC12 family) mediate electroneutral transport of chloride, potassium, and/or sodium across the membrane. They play critical roles in regulating cell volume, controlling ion absorption and secretion across epithelia, and maintaining intracellular chloride homeostasis. Within this family of transporters NKCC mediates cotransport of Na⁺, K⁺, and Cl⁻ in a 1:1:2 stoichiometry. In this study we utilize single-particle cryo-EM to determine the structure and conformational dynamics of NKCC1. Several ion-binding sites are identified directly in the cryo-EM map and are fully supported by molecular dynamics simulation. Multibody refinement was used to visualize the principal components of inter-domain motion, providing critical insights into the ion transport mechanism.

Sibongile Mafu, Ph.D., 2018 Smith Award Recipient
University of Massachusetts – Amherst

Discovery of Plant- Derived Antifungals

Ghislain Fotso Wabo, Ayousha Shahi, Sibongile Mafu
University of Massachusetts – Amherst

Opportunistic fungal infections pose an increasing threat to public health. Effective treatment therapies are hindered by a combination of challenges including an increasing population of immunocompromised individuals and a limited range of drug classes that effectively control plant pathogens. Plant fungal pathogens also pose an increasing threat to global food resources through loss of agricultural yield and postharvest contamination. However, plants have developed intricate mechanisms to defend themselves against pathogen attack often through specialized metabolites that mediate interactions between the plant and its environment. As plants are continually challenged by ever evolving plant pathogens and evolved successful means to mitigate most fungal infection attempts, they offer a great potential in searching for plant derived antifungals.

The University of Massachusetts Amherst houses a collection of plant cell cultures from diverse taxonomic and geographical origin, presenting a unique resource for the rediscovery of plant natural products. We have developed pathogen specific elicitation conditions of the cell lines combined with high throughput profiling methods to mine the plant cell culture library. Elicited extracts are evaluated for their chemical composition through bioassay guided fractionations towards identification of lead compounds. We have complemented identification of lead compounds through evaluating known phytoalexins (defense compounds microbes) from agricultural crops as well ethnobotanical plants. This is foundational work towards our future goal of future integration of transcriptomics and proteomic approaches to investigate the underlying mechanisms of lead compounds.

Amity Manning, Ph.D., 2017 Smith Award Recipient
Worcester Polytechnic Institute

Exploiting Epigenetic Sensitivities of Cells Lacking the pRB Tumor Suppressor

Margaret M. Pruitt, L. Gregory Gutierrez-Zamalloa, Amity L. Manning
Worcester Polytechnic Institute

Maintenance of genome stability is sensitive to the regulation of chromatin structure such that alterations in chromatin compaction contribute to the generation of DNA damage and compromise DNA damage repair. Such changes in the regulation of chromatin can also corrupt centromere structure and function and lead to defects in mitotic chromosome segregation. Prior work from our lab and others has shown cells lacking the Retinoblastoma tumor suppressor pRB exhibit altered chromatin structure. This series of studies has implicated chromatin changes in subsequent replication defects, DNA damage, and mitotic errors. To better define the role of pRB in the regulation of chromatin structure and to exploit the resulting genome instability as a druggable target in pRB-deficient cancer cells, we have performed an imaging-based screen to identify epigenetic regulators of chromatin structure that preferentially impact pRB-deficient cells. Results of our imaging based screen have identified a sensitivity of pRB deficient cells to PARP inhibition. Cells lacking pRB and in which PARP has been inhibited experience replication stress, with regions of the genome still undergoing replication shortly prior to mitosis. These very late replicating regions become susceptible to DNA damage upon mitotic onset and correspond with both an increase in mitotic defects and a failure to reenter the cell cycle. In this way pRB deficient cells are exquisitely sensitive to PARP inhibition, suggesting that PARPs may be clinically relevant targets in pRB-deficient cancers.

Babak Momeni, Ph.D., 2017 Smith Award Recipient
Boston College

Using transposon insertion sequencing to identify molecular mechanisms of microbial interaction

Samantha Dyckman and Babak Momeni
Boston College

Microbial communities are comprised of different species populations exerting facilitatory and inhibitory interactions on each other. These interactions can shape the community by encouraging (e.g. by supplying metabolites) or hindering (e.g. releasing inhibitory byproducts) the growth of species. While it is possible to identify whether one species facilitates or inhibits the growth of another species, identifying the molecular mechanism of such interactions remains challenging. To uncover microbial interaction mechanisms, we propose to use transposon insertion sequencing (Tn-Seq) to identify genes that are important in interaction between two species. To evaluate this methodology, we construct fluorescently labeled *Escherichia coli* strains with engineered contact-dependent inhibition (CDI). With direct contact, the CDI cell injects a toxin into target cells to inhibit their growth, while its own kin are protected from harm by producing an immunity protein that neutralizes the CDI toxin. The machinery for this interaction has been previously studied in detail. We first assess the interactions between a CDI strain and a target strain by monitoring their fluorescence in cocultures. We will then create a mutant library and use a recently developed method, random bar code transposon-site sequencing (RB-TnSeq), to quantify the fitness of different mutants of the target strain when growing with the CDI strain. We anticipate that the results will confirm the known molecular mechanisms of CDI action and potentially reveal other genes involved in this interaction as a proof of principle.

Nick Rhind, Ph.D., 2002 Smith Award Recipient
University of Massachusetts Medical School

The Mechanism of Size Control in Fission Yeast

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The regulation of size is a fundamental, yet currently mysterious, cellular function. Efforts to identify a signaling system that measures cell size and conveys that information to the cell-cycle machinery have been unsuccessful. Instead, we propose that size control is an intrinsic function of the basic cell cycle machinery.

Using both population-level and single cell assays we have shown that two key positive regulators of the G2/M transition—Cdc13, the B-type cyclin that forms CDK with the Cdc2 kinase, and Cdc25, the phosphatase that activates CDK—accumulate in a size dependent manner. That is, unlike most proteins which maintain a constant concentration as cells grow, Cdc13 and Cdc25 increase in concentration as cells get bigger. Since both Cdc13 and Cdc25 are dose-dependent activators of mitosis, their size-dependent accumulation provides a mechanism by which cells can regulate their size.

We have shown that Cdc25 is regulated transcriptionally. We have gone on to show that a 100-bp promoter is sufficient to drive size-specific expression of Cdc25. This promoter contains motifs conserved within the promoters of the three other fission yeast species. Cdc13, on the other hand, is regulated translationally or post translationally. Its mRNA maintains a constant, size-independent concentration, but produces a size-dependent concentration of protein. We have localized the determinants of size-dependent expression to the 5' UTR or the ORF. We will continue to narrow down their location, be they in the transcript or protein.

Silvi Rouskin, Ph.D., 2016 Smith Award Recipient
Whitehead Institute, Massachusetts Institute of Technology

Co-transcriptional RNA secondary structure and its role in alternative splicing

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Silvi Rouskin
Whitehead Institute

Alternative splicing occurs in nearly all human genes and is a major contributor to our proteomic diversity and gene expression regulation. These splicing decisions have important consequences in human health and diseases. Co-transcriptional RNA secondary structures at splice junctions have long been proposed to play a role in regulating pre-mRNA splicing: by 1) affecting spliceosomal accessibility to the pre-mRNA, 2) interacting with splicing factors and 3) promoting long-range RNA interactions in a protein-independent fashion. A well-characterized example is the human microtubule-associated protein Tau (MAPT), where a predicted stem-loop structure at the 5' splice site of exon 10 affects the exon inclusion rate. Mutations that destabilize the 5' splice site structure promote exon inclusion and are strongly linked to several neurodegenerative diseases. However, it is unclear whether examples like this are a general rule or represent a singular case of structure affecting splicing. Currently, limited approaches exist to probe co-transcriptional RNA secondary structures *in vivo*. We have developed a method based on chemical probing coupled with nascent- RNA labelling and next-generation sequencing to identify both gene-specific and genome-wide co-transcriptional RNA structures. Our preliminary data revealed major differences between nascent and *in vitro* secondary structure of tau pre-mRNA, supporting the need for *in vivo* probing to understand splicing regulation. In addition, genome-wide analysis of our approach confirmed enrichment of nascent RNA sequences, enabling us to globally assess the role of pre-mRNA structure in splicing.

Sichen (Susan) Shao, Ph.D., 2017 Smith Award Recipient
Harvard Medical School

Mechanistic dissection of tail-anchored membrane protein triage

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Harvard Medical School

Newly-synthesized proteins must be sorted to the correct cellular destinations or be promptly degraded if they become mislocalized. How cells accurately triage nascent proteins between biosynthesis and degradation is a fundamental question in biomedical science. We use tail-anchored (TA) membrane proteins as a model to study protein triage mechanisms. TA proteins contain a single C-terminal transmembrane domain that routes them to different target membranes to perform essential cellular functions such as vesicular trafficking and forming protein channels. We have established biochemical systems that reconstitute TA protein sorting through a multi-chaperone complex and the selective insertion of TA proteins into two cellular organelles, the endoplasmic reticulum (ER) and mitochondria. These reagents and experimental systems enable precise mechanistic dissection and structural analyses using high-resolution electron cryo-microscopy (cryo-EM). Covalently trapping transient interactions of mitochondrial TA proteins using site-specific photocrosslinking has identified new candidate factors that may mediate specific TA protein sorting to different organelles. Ongoing work aims to determine how these factors and structural insights contribute to TA protein targeting and quality control to ensure high-fidelity protein localization.

Matthew Shoulders, Ph.D., 2013 Smith Award Recipient
Massachusetts Institute of Technology

The Biophysical Cost of Influenza Immune Escape Can be Addressed by Host Chaperones

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Protein homeostasis (proteostasis) networks, along with fundamental biophysics, govern accessible evolutionary pathways for proteins. Acquisition of adaptive amino acid mutations during evolution is often kinetically or thermodynamically destabilizing, and chaperones from the proteostasis machinery can rescue these destabilizing mutations for endogenous client proteins. Not surprisingly, rapidly evolving viral proteins also require folding assistance, perhaps more than native clients. The extent of viral protein's ability to tolerate mutations defines the success of their escape from antiviral drugs and immunity. We investigated viral protein mutational tolerance in different host cell proteostasis environments by pairing the high throughput deep mutational scanning method with stress-independent perturbation of the host's proteostasis components. The study revealed that chaperone depletion compromises the fitness of an adaptive influenza variant, and an additional temperature increase mimicking fever renders the biophysically unstable immune escape variants disfavored, such as the Pro283 nucleoprotein variant that is structurally unstable yet allows influenza A to escape from human myxovirus resistance protein A (*PLoS Biology* 2018). Hence, influenza can potentially hijack host chaperones to address viral protein mutations that cause folding defects but facilitates host cell immune escape. When folding assistance is limited the magnitude of biophysical defect outweighs the positive selection pressure from the host's antiviral factors, making the virus highly vulnerable to immune suppression. Therefore, host chaperones play a central role in facilitating influenza adaptation at the host-pathogen interface, a finding with far-reaching implications for viral host-switching and resistance development.

Sarah Slavoff, Ph.D., 2019 Smith Odyssey Award Recipient
Yale University

Dynamic profiling of 5'-to-3' RNA decay reveals mechanistic integration of multiple regulators

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Yale University

5'-to-3' RNA decay is a mechanism of cytoplasmic RNA degradation and quality control that proceeds via RNA decapping by Dcp2 followed by 5'-to-3' exonucleolytic digestion by Xrn1. This process has long been known to be tightly spatiotemporally regulated in human cells by multiple coactivator proteins, including Dcp1A and EDC4, as well as by phase separation into cytoplasmic RNA-protein granules known as P-bodies. However, key questions remain unanswered. Are additional regulatory factors yet to be discovered? Is this pathway highly substrate-specific, or does it regulate the entire cellular transcriptome? Here we describe the application of a high-sensitivity temporally resolved approach for global RNA stability quantitation, Timelapse-seq, to identify (1) the function of a novel small protein regulator of mammalian 5'-to-3' RNA decay, NBDY, and (2) the molecular determinant(s) defining substrates of the human RNA decapping protein Dcp2.

Mansi Srivastava, Ph.D., 2016 Smith Family Foundation and Searle Scholars Program
Harvard University

Using a New Model System to Study the Dynamics and Regulation of Pluripotent Stem Cells

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Andrew Gehrke, Katy Loubet-Senear, Amber Rock, Hafsa Sadiq, Mansi Srivastava
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Wound repair and regeneration are fundamental features of animal biology. In humans and other vertebrates, virtually all wounds can be healed yet only some will result in subsequent regeneration of tissues lost to injury (e.g., liver in humans, tail fin in fish). In contrast, many other animal species can regenerate extensively and have the capacity to replace all tissues of the adult animal (e.g., planarians, cnidarians). Underlying these differential regenerative capacities are different stem cell systems; highly regenerative species often harbor pluripotent adult stem cells that can differentiate into virtually any cell type, whereas vertebrates have lineage-restricted adult stem cells. We seek to identify the molecular mechanisms that control specification, maintenance, and differentiation of pluripotent stem cells *in vivo*. We focus our studies on the acel worm *Hofstenia miamia*, a highly regenerative member of the sister lineage to all other bilaterians, that has a large population of pluripotent stem cells called neoblasts. *Hofstenia* offers many advantages as a new model system for mechanistic studies of regeneration, including accessible and manipulable embryos. We applied single-cell RNA-sequencing (scRNASeq) to obtain a detailed atlas of cell types in *Hofstenia*, which has revealed complex maintenance and differentiation dynamics of the stem cell population as the worms age and regenerate. Further, scRNASeq data for developing embryos revealed candidate genes that may be required for the specification of neoblasts. We developed methods for transgenesis in *Hofstenia*, which will enable us to test the hypotheses generated by scRNASeq. In particular, we will use transgenic lineage tracing to follow the dynamics and differentiation potentials of single stem cells as they allow amputated fragments to regenerate an entire organism.

Beth Stevens, Ph.D., 2008 Smith Award Recipient
Boston Children's Hospital

Prefrontal Cortical Development Over Adolescence in Mouse

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The prefrontal cortex (PFC) is implicated in the pathogenesis of cognitive dysfunction in psychiatric and neurodegenerative disease. While PFC development is known to occur during adolescence, precisely how brain circuit matures remains unclear. To understand how disease risk factors (both genetic and environmental) shape PFC trajectory, we must first establish parameters that define healthy development. Here, we employ anatomic and electrophysiological techniques to map changes in PFC of the mouse. First, we performed whole-cell slice electrophysiology and recorded miniature excitatory postsynaptic currents (mEPSCs) across weeks of postnatal development. We found that the frequency and amplitude of mEPSCs undergo an extended period of maturation that took months to reach their stable state in adulthood. Next, we used immunohistochemical approaches to track the density and localization of specific inputs across cortical layers. Most notably, our preliminary evidence suggests a reorganization of inhibitory puncta onto the superficial layers of the PFC. Lastly, we combined whole-cell recordings with electrical stimulation of presynaptic inputs to track how excitatory and inhibitory synapses alter their strength and anatomical targets across this period. Consistent with our anatomical results, there is an increase in the strength of inhibition that may underlie many circuit and behavioral changes during this period. In future experiments, we will combine cell-type specific markers to enhance our understanding of how these inputs change onto recognized cell-types in the PFC. Together, these results provide a timeline of how inputs to the PFC mature across the full adolescent window and provide the basis for which perturbations to genes and environment can be compared.

Shobha Vasudevan, Ph.D., 2010 Smith Award Recipient
Massachusetts General Hospital

A specialized post-transcriptional program in chemoresistant, quiescent cancer cells

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Quiescent (G0) cells are a clinically relevant fraction in cancers, which include dormant cancer stem cells, and resist clinical therapy. G0 cells reveal extensive changes in gene expression at the protein and translation levels. We previously identified that the translation mechanism is altered in G0. G0 cancer cells show similar proteome and translatome to cells isolated post-chemotherapy, implicating specific, post-transcriptionally- and translationally-regulated genes in chemoresistance. These data suggest that specialized post-transcriptional mechanisms in G0 cancer cells regulate a distinct translatome to mediate chemoresistance.

To understand the role of post-transcriptional regulation in chemoresistance, we conducted global RNA, translational and proteome profiling in chemoresistant G0 cancer cells. We find that chemotherapy or G0 induction leads to DNA-damage-responsive ATM and stress signaling, which alter post-transcriptional and translational mechanisms. ATM and stress-activated p38 MAPK modify a distinct post-transcriptional regulatory mechanism to increase AU-rich-element-bearing pro-inflammatory gene mRNAs and decrease canonical translation. Both rate limiting steps of canonical translation—mRNA cap recognition and tRNA recruitment—are altered by stress signaling and low mTOR activity that activates the cap complex inhibitor, eIF4EBP to impair canonical translation. Instead, specialized translation mechanisms, RNA and ribosome modifiers are increased. This permits specific expression of immune and survival genes. Significantly, inhibiting these regulators—prior to chemotherapy—decreases chemoresistance in leukemic cells and patient samples, without affecting normal cells. This correlates with stress signals in early G0, which elicits subsequent chemoresistance. These studies reveal a transient, pro-inflammatory subpopulation in cancer, enabled by DNA-damage- and stress-regulated post-transcriptional and translational mechanisms, which mediates chemoresistance.

Jessica Whited, Ph.D., 2013 Smith Award Recipient, 2019 Smith Odyssey Award Recipient
Harvard University

Eya2 promotes cell cycle progression by regulating DNA damage response during vertebrate limb regeneration

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DNA replication and cell division are integral parts of tissue regrowth in highly regenerative animals like salamanders. These processes are also the cause of senescence or tumorigenesis in human cultured stem cells and the main target of drug treatments in cancer patients. How salamanders accomplish progenitor cell proliferation while faithfully maintaining genomic integrity and regenerative potential remains elusive. Understanding the underlying mechanisms may inform strategies for more efficient utilization of stem cells in regenerative medicine as well as novel approaches for controlling cancer. Here we found an innate DNA damage response mechanism that is activated upon injury, and we studied its role during regeneration by ablating the function of one of its components, Eyes absent 2. Using eya2 knockout axolotls, we found that DNA damage signaling through the H2AX histone variant was de-regulated, especially within the proliferating limb progenitors during limb regeneration. In these animals, cell cycle progression at the G1/S and G2/M checkpoints was impaired and regeneration rate was reduced. However, their limbs ultimately regenerated and their cells maintained their genomic integrity, indicating a developmentally-acquired compensatory mechanism for coping with the loss of Eya2. Our RNA-seq analysis revealed that this ability is likely imparted by the upregulation of DNA damage response pathways which in turn slow cell cycle dynamics in the eya2 knockout animals during regeneration. This developmentally-wired resiliency could, however, be overridden by acute pharmacological inhibition of the Eya2 phosphatase activity in wild-type animals, leading to a breakdown in genomic integrity accompanied by increased levels of apoptosis and cell cycle arrest. Together, our data indicate that highly-regenerative animals employ a robust DNA damage response pathway which involves regulation of H2AX phosphorylation via Eya2 to facilitate proper cell cycle progression upon injury.

Wesley P. Wong, Ph.D., 2019 Smith Odyssey Award Recipient
Boston Children's Hospital

Single-Molecule Mechanical Fingerprinting of Biomolecules with DNA Nanoswitch Caliper

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Single-molecule methods for measuring distance have provided an unprecedented view into the structure and dynamics of biomolecules. However, long-standing challenges in determining the spatial arrangement of multiple residues within single biomolecules, and the general need for purified homogeneous samples, have limited their applications to proteomics and structural biology. By combining structural DNA nanotechnology with single-molecule force spectroscopy (SMFS), we have developed a mechanically reconfigurable DNA Nanoswitch Caliper (DNC), able to measure multiple coordinates in single biomolecules within heterogeneous samples. Using dual-trap optical tweezers, we have demonstrated distance measurements with sub-angstrom level of precision for DNA constructs, single-molecule fingerprinting of synthetic and natural peptides, and discrimination of different post-translational modifications. We anticipate that DNC could enable a novel route for single-cell proteomics and 3D structural fingerprinting of biomolecules and nanoscale structures.