Bioengineering and Biomedicine

From bedside to clinical trials and company creation, how one patient's journey in improving the outcomes of their disease has just begun

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Cystic Fibrosis is a genetic disease that affects multiple organ systems. The severity of the disease is due to the genetic mutations a patient may carry. In my case, a rare mutation, diagnosis came later in life with the advent of widespread genetic sequencing. This disease has affected my reproductive health, ability for physical activity and general happiness. I have benefitted from advances in reproductive health as well as the recent approval of Vertex's Trikafta which was not indicated for my genetic mutation which has changed my life. As a successful investor, I am now privileged to help create biotechnology companies that I hope will directly impact my health outcomes for other patients. I am participating in a clinical trial for the latest therapeutic in development for CF. I will describe how I have benefited from scientific advancements, actively participate in therapeutic trials and aim to be involved in creating the next class of diagnostics and medicines.

Cytidine deaminases APOBEC3C and APOBEC3D promote DNA replication stress resistance in pancreatic cancer

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Pancreatic cancer is among the most refractory cancers and a leading cause of cancer death. A first line of treatment for advanced or metastatic pancreatic cancer is the DNA replication stress-inducing agent gemcitabine (2',2'-difluorodeoxycytidine), often used in combination with the microtubule stabilizing agent paclitaxel. Although gemcitabine has been the standard of care for pancreatic cancer for greater than two decades, most tumours exhibit intrinsic or acquired resistance to gemcitabine regimens. It is largely unknown how pancreatic cancer cells respond to the DNA replication stress produced by gemcitabine and why different pancreatic cancer cells display differential responses to the same treatment.

To address these knowledge gaps, we performed genome-wide CRISPR-Cas9 screens on pancreatic cancer cells with different genetic backgrounds and gemcitabine sensitivities. Our screens recalled known vulnerabilities to gemcitabine, in addition to novel shared and genotype-specific vulnerabilities. Of particular interest, we found that knockout of two cytidine deaminases, *APOBEC3C* and *APOBEC3D*, hypersensitized pancreatic cancer cells to gemcitabine. *A3C* and *A3D* encode members of the APOBEC3 family of cytidine deaminases involved in the restriction of viruses and endogenous retroelements by DNA and mRNA editing. We show that *A3C* and *A3D* expression is strongly induced by gemcitabine treatment and that A3C and A3D promote gemcitabine resistance in pancreatic cancer cells by deamination of deoxycytidines in ssDNA to promote replication fork re-start.

Our work provides the first comprehensive genetic network of targets that contribute to the sensitivity and resistance to gemcitabine and defines a novel role for A3C and A3D in maintaining genome stability, advancing our molecular understanding of the DNA replication stress response in pancreatic cancer.

The Emerging Roles of Live-Cell Assays and Artificial Intelligence in Cancer Drug Development and Precision Therapy

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During my talk I'll present our recent unpublished data showing the integration of Cyclica's artificial intelligence-driven computational tools with two high-throughput, live-cell drug discovery technologies recently developed in my lab - Mammalian Membrane Two-Hybrid Drug Screening (MaMTH-DS) and Split Intein Mediated Protein Ligation (SIMPL). By progressively screening a cohort of currently 'undruggable' protein targets such as EGFR-triple mutant and KRAS, we obtained valuable prospective validation of our integrated platform and rapidly discovered actionable compounds, establishing a robust pipeline against challenging disease targets associated with untreatable cancers.

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Sigma-1 receptor – a role in neuronal signaling and neurodegeneration

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The sigma 1 receptor (S1R) is a 223 amino acid-long transmembrane endoplasmic reticulum (ER) protein. Agonists of S1R demonstrated neuroprotective effects in variety of preclinical models and there are several on-going clinical trials of S1R agonists in neurodegenerative disorders. However, signaling functions of S1R are poorly understood. In our recent studies we tested the hypothesis that biological activity of S1R in cells can be explained by its ability to interact with cholesterol. By performing experiments in reduced reconstitution systems, we demonstrate direct effects of cholesterol on S1R clustering. We identify a novel cholesterol-binding motif in the transmembrane region of human S1R. Mutations of this motif impair association of recombinant S1R with cholesterol beads, affect S1R clustering in vitro and disrupt S1R subcellular localization. Further, we found that S1R agonists cause disruption of S1R clusters. Based on these results we propose that S1R-cholesterol interactions enable the formation of cholesterol-enriched microdomains in the ER membrane. We further propose that S1R agonists enable the disassembly of these cholesterol-enriched microdomains and the release of accumulated proteins such as ion channels, signaling receptors, and trophic factors from the ER. We also propose that these cholesterol-enriched microdomains form the basis for formation of membrane contact sites between ER and other subcellular organells such as mitochondria and plasma membrane

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Mitochondrial Contributions To Cancer: Causes, Consequences, & Coincidence

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Mitochondria created an evolutionary advantage for eukaryote and metazoan organization, and their impact on cell biology extends from anabolic and catabolic metabolism to determining the final moments of cell survival by engaging apoptosis. Throughout the last decade, interest in studying how mitochondria influence cancer cell biology led our laboratory to identify mechanisms linking oncogenic signaling (i.e., $BRAF^{V600E}$ / $NRAS^{G12V}$) to multiple mitochondria-centric processes within malignant cells including altered mitochondrial dynamics, oxidative phosphorylation, and chemosensitivity. More recently, we focused on exploring how oncogenes intersect upon mitochondrial biology prior to transformation – which will likely provide molecular details into pre-malignant cell biology and early stages of disease. We commonly position our studies in the context of melanoma as we have extensive experience with primary human melanocytes, integrated cohorts of patient RNA-seq datasets and tissues, and multiple in vitro and in vivo models of early and late disease At present, we are investigating the implications of chronic mitochondrial division in oncogene-induced senescence, the mitochondrial unfolded protein response, and the immunobiology of melanoma in situ. Our discussion will provide new molecular insights into how mitochondrial biology impacts on the cell biology of melanoma, and informs the immune landscape of primary tumors.

Characterization and improvement of a nicotine degrading flavoenzyme

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Nicotine dependence is currently responsible for one in seven deaths worldwide, but methods of escaping this global addiction have limited efficacy. The flavoenzyme nicotine oxidoreductase (NicA2) has shown promise as an injectable treatment for nicotine addiction in rat studies.[1] It works by removing nicotine from the bloodstream before it reaches the brain. Unfortunately, this enzyme re-oxidizes very poorly with O_2, severely limiting nicotine turnover.[2] *In vitro*, NicA2 has a k_{cat} of only 0.006 s^{-1}.[3] This necessitates the injection of clinically unrealistic amounts of enzyme in order to have the desired therapeutic effect. Using a genetic selection, we isolated variant forms of NicA2 that increase the O_2-dependent activity of NicA2 more than 100-fold. The mutations in these variants map to five hotspot locations that surround the active site. We evaluate the pharmacokinetic properties of increased activity variants in a rat model. Characterization of the resulting increased activity variants has allowed us to gain insight about how oxygen utilization can emerge in evolution, while also providing more clinically viable forms of NicA2.

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IzoView: The Advantages of Contrast Enhanced Cone-Beam Breast Computed Tomography for 3-Dimensional Imaging of the Breast for Diagnostic Imaging

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Mammography and ultrasound remain the principal tools for diagnostic breast imaging. Compression of the 3D structure of the breast is necessary for 2D mammography imaging. Over the past decade, mammography systems have in general been augmented with tomosynthesis, which uses a small number of mammographic projections covering an angular range of 15-50° (depending on vendor), and these data are reconstructed to produce pseudo-tomographic images (~2.5D). Mammography as well as tomosynthesis suffer from a significant loss of sensitivity in women with high breast density, because overlapping tissues can obscure the detection of a tumor, if present. The aggressive compression required for these modalities can also lead to breast implant rupture. Izotropic is building a dedicated cone-beam breast computed tomography (bCT) system, based on four successive prototypes from the laboratory of Dr. J.M. Boone [1–3]. This system will be the first x-ray device that is completely self-shielded for breast imaging. The bCT system acquires ~500 images in 10 seconds, which are reconstructed into a high-resolution volume data set – a true 3D depiction of the breast. Breast CT overcomes the challenges of visualizing overlapping structures, eliminates the need for breast compression, and with the injection of contrast agent can demonstrate both physiologic function as well as breast anatomy. Informed by previous studies at UC Davis, we will demonstrate in the Izotopic system that the ability to precisely visualize breast tissue in 3 dimensions with contrast enhancement is superior to the current standard-of-care breast diagnostic examination. This approach will largely mimic the sensitivity of breast MRI at a fraction of the cost, and with much better spatial resolution. This new platform will enable robotic guided biopsies, allow better visualization of implants, and provide tumour classification based on morphology and physiologic enhancement patterns. Additionally, it will permit local staging and provide information on treatment response in the neoadjuvant setting.

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Reversible protein aggregation regulates cell growth in response to environmental stress conditions

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Protein aggregation has historically been viewed as an irreversible, deleterious process responsible for several pathologies, including neurodegenerative diseases. However, recent evidence in yeast suggests that aggregation of an increasing number of proteins is a reversible, highly-regulated physiological mechanism used by cells to adapt to several stress conditions. Indeed, our recent work identified reversible aggregation of Cdc19, the major isoform of yeast pyruvate kinase, as a critical factor contributing to the adaptation of cells to carbon starvation. Available suggest that its reversible aggregation protects Cdc19 from unscheduled degradation to allow efficient re-initiation of growth after stress release. However, the molecular structure and cellular mechanisms regulating assembly and disassembly of reversible aggregates remain poorly understood. Moreover, it remains to be determined whether reversible aggregation is conserved in mammalian cells, and whether reversible aggregates serve as precursors for toxic, irreversible amyloids. We have used a combination of genetic, biochemical and mass spectrometry-based approaches to tackle these questions, and recent progress on these efforts will be presented.

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Conformational models of APP processing by gamma secretase based on analysis of pathogenic mutations

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Proteolytic processing of amyloid precursor protein (APP) by β and γ secretases leads to generation of Aβ40 (non-amyloidogenic) and Aβ42 (amyloidogenic) peptides. Presenilin-1 (PS1) or presenilin-2 (PS2) play a role of catalytic subunit of γ-secretase. Multiple familial AD (FAD) mutations in APP, PS1, or PS2 result in increased Aβ42:Aβ40 ratio and accumulation of toxic Aβ42 oligomers and plaques in patient brains. In this study we performed molecular modeling of APP complex with γ-secretase and analyzed potential effects of FAD mutations in APP and PS1. Based on structural analysis of known γ-secretase structures we proposed that APP can form a complex with γ-secretase in 2 potential conformations – M1 and M2. In conformation M1 transmembrane domain of APP forms a contact with perimembrane domain that follows the transmembrane domain 6 (TM6) in PS1 structure. In conformation M2 transmembrane domain of APP forms a contact with transmembrane domain 7 (TM7) in PS1 structure. By analyzing effects of PS1-FAD mutations on local protein disorder index, we discovered that these mutations increase conformational flexibility of M2 and reduce conformational flexibility of M1. Based on these results we proposed that M2 conformation, but not M1 conformation, of γ secretase complex with APP leads to amyloidogenic (Aβ42-generating) processing of APP. Our results also suggest that specific inhibitors of Aβ42 production could be potentially developed by selectively targeting M2 conformation of γ secretase complex with APP.

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Integrative analysis of lung cancer identifies distinct proteotypes associated with altered metabolism and patient outcomes

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Non-small cell lung cancer (NSCLC) is the leading cause of cancer deaths worldwide. Only a fraction of NSCLC harbor actionable driver mutations and there is an urgent need for patient-derived model systems that will enable the development of new targeted therapies. NSCLC and other cancers display profound proteome remodeling compared to normal tissue that is not predicted by DNA or RNA analyses. Here, we generate 137 NSCLC patient-derived xenografts (PDXs) that recapitulate the histology and molecular features of primary NSCLC. Proteome analysis of the PDX models reveals 3 adenocarcinoma and 2 squamous cell carcinoma proteotypes that are associated with different patient outcomes, protein-phosphotyrosine profiles, signatures of activated pathways and candidate targets, and in adenocarcinoma, stromal immune features [1]. Altered iron regulation is identified as a key determinant of NSCLC aggressiveness associated with patient outcome [2]. These findings portend proteome-based NSCLC classification and treatment and support the PDX resource as a viable model for the development of new targeted therapies.

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Machine Learning for Understanding Molecular Complexity in Precision Medicine

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Abstract:

We are faced with a flood of molecular and clinical data. We are measuring interactions between various bio-molecules in and around a cell that form large, complex systems. Patient omics datasets are also increasingly becoming available. These systems-level network data provide heterogeneous, but complementary information about cells, tissues and diseases. The challenge is how to mine them collectively to answer fundamental biological and medical questions. This is nontrivial, because of computational intractability of many underlying problems on networks (also called *graphs*), necessitating the development of approximate algorithms (heuristic methods) for finding approximate solutions.

We develop artificial intelligence (AI) methods for extracting new biomedical knowledge from the wiring patterns of systems-level, heterogeneous biomedical networks. Our *graphlet*-based and other methods uncover the patterns in molecular networks and in the multi-scale organization of these networks indicative of biological function, translating the information hidden in the network topology into domain-specific knowledge. We also introduce a versatile data fusion (integration) machine learning (ML) framework to address key challenges in precision medicine from the wiring patterns of biomedical network data: better stratification of patients, prediction of driver genes in cancer, and re-purposing of approved drugs to particular patients and patient groups, including Covid-19 patients. Our new methods stem from novel network science algorithms coupled with graph-regularized non-negative matrix tri-factorization (NMTF), a machine learning technique for dimensionality reduction, inference and co-clustering of heterogeneous datasets. We utilize our new framework to develop methodologies for performing other related tasks, including disease re-classification from modern, heterogeneous molecular omic data, inferring new Gene Ontology relationships, aligning multiple molecular networks, and uncovering new cancer mechanisms.

Systems Biologics: Large-Scale Engineering of Modulators of Protein Networks

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In recent years, genomics technologies have revolutionized basic research and are also having a significant impact on understanding, predicting and diagnosing disease. Over the same period, the biologics revolution, led by therapeutic antibodies, has greatly expanded our ability to target proteins that drive cancer and other diseases. To date, however, the academic genomics revolution and the industrial biologics revolution have not been combined, so that the vast amounts of data generated by genomics technology have not been effectively translated to drug development, which remains a slow, case-by-case process. We have developed an approach that we call "systems biologics", which combines large-scale systems biology with the development of new antibody drugs. The efficient pipeline extends from basic research through translational science, and it constitutes a new model for research and drug development. Through this model, cutting-edge systems biology basic research can be seamlessly translated into systems biologics: novel, multi-functional drugs and diagnostics that take advantage of the complexities of human biology revealed by genomics data.

Systems Biologics in Network Biology

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The interplay of environment and genome on the traits of an organism are reflected in how variations in either act on the biochemical networks that underlie all cellular processes. Current evidence suggests that predicting how environmental or genome variation affect specific cellular processes is most accurately determined by their effects on biochemical networks of the cell. It is impossible to measure, let alone predict, how entire molecular networks function, but we can choose useful surrogates of the network to act as reporters, such as protein interaction networks (PINs). We have developed general strategies to measure spatiotemporal dynamics of PINs in living cells, using Protein-fragment Complementation Assays (PCA) to measure PINs at whole proteome scales (Tarassov et al., 2008) and for smaller subsets of interactions, response to environmental perturbations and to map novel biochemical pathways and predict genes associated with human diseases (Macdonald et al., 2006; Messier et al., 2013; Stynen et al., 2018). I will present a simple and global strategy to map out gene functions and target pathways of drugs, toxins, or protein biologics based on ‘‘homomer dynamics’’ protein-fragment complementation assays (hdPCA), a method that captures the integrated fate of a gene following a genetic or environmental perturbation, from transcription to protein to post-translational modification (Stynen et al., 2018). I will then present recent developments of methods to measure and manipulate proteins, their abundances, and both abundance and protein-protein interaction stoichiometries, at a proteome-wide level and in any cell lines. These are allowing us to decipher mechanisms of action of biologics, and drugs and gene variations, on biochemical processes. They are also revealing details about the thermodynamics of PINs, providing insights into passive and active origins of energy and information propagation in living cells.

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Pulse labeling reveals the tail end of protein folding in the cell

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Over two million proteins are synthesized every minute in a rapidly dividing human cell. Some of these proteins will spontaneously reach their native state even while being translated and within a few milliseconds, whereas other proteins will only do so with the help of chaperone proteins and on a longer time span. Failure to correctly fold can result in an inactive protein and is associated with numerous neurodegenerative diseases, in which protein misfolding and aggregation is observed. To broaden our understanding on how protein folding is regimented at the cellular level, we probed which newly translated proteins are more thermo-sensitive and aggregate upon heat stress. These newly synthesized thermo-sensitive proteins correspond to a subset of abundant, short, and highly structured proteins. Notably, these proteins display a tendency to form β-sheet secondary structures and are enriched for chaperone binding motifs, suggesting a higher demand from chaperone-assisted folding. Importantly, these proteins are only thermos-sensitive following synthesis and not once "matured". One possibility is that there is a tradeoff, where more time is needed for a subset of proteins to fold and mature into a conformation that is then more stable upon stress.

Using Deep Learning to Reveal Polypharmacology for Small Molecule Discovery

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Artificial Intelligence (AI) holds promise in ushering in a new era of drug discovery. MatchMaker™ is a deep learning approach trained on drug target interaction (DTI) data mapped to protein structures to predict binding complementarity of small molecules to proteins. MatchMaker routinely screens chemical spaces on the order of 10^9 molecules across the structurally characterized proteome – supplemented by AlphaFold structures – to provide a unique glimpse into the polypharmacology of evaluated molecules. Applying MatchMaker to known active small molecules reveals their putative interactions with proteins that may be pivotal for its biological activity. Revealing potential protein binders is particularly useful in cell-based or phenotypic-based experiments where the mechanism of action is unknown. Exemplifying this application is our work with EMI1[1], a molecule initially discovered using MaMTH-DS (mammalian membrane two-hybrid drug screening) that was found to block activation of EGFR triple mutant independent of kinase activity. Using EMI1's predicted polypharmacology as a guide, additional small molecules were generated to phenocopy the activity of EMI1. A small molecule hit was found that served as the basis for a series of compounds that, like EMI1, were able to block EGFR triple mutant activation in cell-based assays.

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A TLR-to-STING-to-UPR pathway modulated by Parkinson's disease-related proteins regulates the transition between innate to adaptive immunity

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While the contribution of inflammation in the pathological process leading to Parkinson's disease (PD) is firmly established, a growing body of evidence also supports a role for the adaptive arm of the immune system in the disease. We have shown that the PD-proteins PINK1 and Parkin regulate the presentation of mitochondrial antigens on MHC I molecules during inflammation, a process referred to as MitAP (**Mit**ochondrial **A**ntigen **P**resentation). We proposed that the over-activation of the MitAP pathway, in the absence of PINK1 for example, is part of an autoimmune response enabling the recognition and attack of dopaminergic neurons by cytotoxic T cells. The emerging concept that PD-proteins regulate, in part, both inflammation and antigen presentation led us to investigate the role these proteins may be playing in the transition from innate to adaptive immunity during stress. Our data indicate that this transition is triggered by multiple stress sensors along a TLR4-to-STING-to-UPR pathway. Remarkably, PD-proteins, including LRRK2, actively regulate this process, highlighting their role in the control of a balanced immune response during stress. Understanding the role played by the immune system in PD will lead to the development of novel immune-based therapeutic approaches.

Pathophysiology and Novel Therapies for Uremic Cardiomyopathy

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Chronic kidney disease (CKD) has reached epidemic proportions globally. The presence of CKD greatly elevates the risk of death and by far the most common cause of death is cardiac disease consisting clinically of heart failure and dysrhythmias and pathologically of cardiac hypertrophy and fibrosis. The cardiomyopathy in CKD can be affected by the traditional Framingham cardiovascular risk factors. However, the collective metabolic derangements in CKD, termed uremia, beget uremic cardiomyopathy which is a multifactorial disorder of the myocardium. The underlying metabolic causes of uremic cardiomyopathy is diverse and we have focused on three pathobiologic intermediates- Klotho deficiency, excess fibroblast growth factor (FGF)-23, and phosphotoxicity. Klotho deficiency results from the inability of the failing kidney to synthesize and supply the body with Klotho. The reason for the massive increase in FGF23 production from bone cells in CKD is unclear. Phosphotoxicity results from the inadequate renal phosphate excretion in CKD. We provide associative and interventional rodent data the each of the above three condition can individually and synergistically cause uremic cardiomyopathy. At this juncture, the preclinical data needs to be translated into human studies. Although Klotho therapy, FGF23 blockade, and phosphate control do not represent the totality of interruption of the pathobiology of uremic cardiomyopathy, but constitute three major pillars of reducing mortality in CKD.

Modeling the role of the gut in Parkinson's Disease

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Parkinson's disease is a neurodegenerative disorder associated with the progressive loss of dopaminergic neurons in specific regions of the brain. While the mechanisms leading to neuron cell death in Parkinson's disease are still poorly understood, host genetics and environmental factors are viewed as playing a key role. The gut-brain axis has long been suspected to play a role in this complex disease, with a growing body of evidence now supporting the notion that intestinal inflammation is an important driver of Parkinson's Disease development. We have recently developed a new mouse model to examine the link between these pathologies. Although mutations in the gene *PINK1* in humans are associated with a high risk of developing Parkinson's Disease, *Pink1* knockout mice are generally healthy and display no Parkinson's-like symptoms. We previously showed that intestinal infection with the Gram-negative intestinal pathogen *Citrobacter rodentium* in *Pink1* knockout mice leads to the emergence of motor symptoms that can be reversed by treatment with L-DOPA. Here we show, using single cell RNA sequencing, that loss of *Pink1* has profound impacts on the early response to intestinal infection. These findings provide a window to better understand how dysregulation of inflammatory events in the gut can trigger the development of Parkinson's Disease in genetically susceptible individuals.