In Vitro Cell.Dev.Biol.—Animal
DOI 10.1007/s11626-013-9612-6

Animal Symposia and Workshops

A-1

The 'Meta' Era of Microbiology: Cheaper Sequencing and Bigger Data. D. A. ANTONOPOULOS. Institute for Genomics and System Biology, Argonne National Laboratory, Argonne, IL 60439. Email: dion@anl.gov

The study of natural microbial communities is undergoing a transformation owing to the constant decline in DNA sequencing costs. Microbial ecology, like many other biological disciplines, has likewise been transformed because of the scale of sequence data captured on a per sample basis. These improvements in sequencing technology have made metagenomic analysis of microbial communities feasible on a scale commensurate with the genetic content present. Initially the key challenge had been gene annotation since the sheer amount of diversity present in natural microbial communities created an extremely large search space – in other words, database searching is not limited to a single reference organism. Improvements in data organization and computational strategies have tempered what would otherwise have been an unsustainable approach to data analysis. However, the main challenge now is linking different data types (metadata) in order to make meaningful inferences about the functions that different communities encode and exhibit.

A-2

Dietary Manipulation of the Gut Microbiota and Its Implications for Tailored Therapies. SUZANNE DEVKOTA. Joslin Diabetes Center/Harvard Medical School, 1 Joslin Pl., Boston, MA 02215. Email: Suzanne.Devkota@joslin.harvard.edu

The interplay of dietary macronutrients with the intestinal lumen alters the microbial environment, and thus host-microbe interactions, in ways that are not always in a favorable, mutualistic fashion. Specifically, with regards to inflammation, experimental and clinical observations have implicated a detrimental impact of environmental/microbial factors on the etiopathogenesis of autoimmune and inflammatory diseases in individuals with a background of genetic susceptibility. Thus, now more than ever, we are realizing that specific intestinal microbes can metabolize and react to a wide array of dietary compositions that, in turn, markedly alter microbial populations. Recent evidence has shown certain dietary fats that are prevalent in Western diets are capable of precipitating colonic inflammation, for example, through their actions on the enteric microbiota. On a background of genetic susceptibility, these microbial changes can impact host immune homeostasis and increase risk for disease. This serves as a new paradigm for studying host-microbe interactions with potential implications for future medical care.

A-3

Diversity, Networking and Metabolic Potential of the Digestive Tract of the Healthy Individuals. JACQUES IZARD. Department of Microbiology, The Forsyth Institute/Harvard School of Dental Medicine Cambridge, MA. Email: jizard@forsyth.org

The human body co-exists and co-evolved with myriads of microbes associated with both health and disease. They represent a powerful component of our ability to digest, fend of pathogens and balance our immune system. The focus has been for many years on diseases and pathogens, avoiding the question what microbiota is needed in health. In conjunction with the Human Microbiome Project, we examined the bacterial populations of the oral cavity and of the lower digestive tract. Investigating the microbiome diversity, we found four distinct microbial community types among the ten different body habitats examined. Each group contained several bacteria known to be abundant in the healthy oral cavity and gut, and organisms thought to be mostly environmental, but established inhabitants of the human body. In contrast, bacteria known to include pathogenic members were well represented in this disease-free population. The metabolic potential of those bacteria

interacting with mucosal surface had a core functional component as well as variations in abundance at particular body habitats, particularly for uptake of specific sugars. As those bacteria are present in complex populations, we examined both co-occurrence and co-exclusion of organism within and between body sites. We established a benchmark of the microbiome normal structure and variation.

A-4

Mechanisms of Cellular Trafficking That Tailor Toll-like Receptor 2 (TLR2) Responses. TANJA PETNICKI-OCWIEJA. Tufts Medical Center, Infectious Diseases, 800 Washington Street, Boston, MA 02111. Email: tpetnickiocwieja@tuftsmedicalcenter.org

Host cell responses are tailored to the infecting microbe. Although a relatively simple concept, a continuing area of research has been the elucidation of how a handful of immune receptors generate such a wide range of inflammatory signatures. Cell biology has been shown to be crucial in customizing these responses. Depending on cellular location, signaling results in marked differences in cytokine profile. Our studies with *Borrelia burgdorferi* have allowed us to contribute to a shifting paradigm in our understanding of the role of cellular compartmentalization in Toll-like receptor (TLR2) signaling. In our current work, we explore the relevance of specific motifs within cell surface molecules and the role of intracellular trafficking molecules to define internalization and sorting into intracellular compartments. We show that the β1 integrin affects *B. burgdorferi* dependent TLR2 signaling and internalization of the pathogen through the NPXY motifs in their cytoplasmic domains. Mutations in the tyrosine residue in two of these motifs significantly decrease signaling of *B. burgdorferi* and TLR2 ligands. In addition, we show that this decrease is due to a defect in internalization of *B. burgdorferi* and TLR2-ligands attached to beads. Upon internalization of *B. burgdorferi* or TLR2 ligand-beads, adaptor protein (AP) complexes in the endosomal vesicular pathway play an important role in inflammatory responses induced by these ligands, as cells from knockout mice show a significant decrease in either TLR2 ligand-beads or *B. burgdorferi*- induced signaling. The mode of internalization is crucial to proper trafficking since free TLR2 ligand is not affected in the same way as *B. burgdorferi* or TLR2 ligand-beads and the endosomal adaptor complexes seem to be crucial in mediating proper internalization. Our study is the first to identify a role for the integrin NPXY motif in the internalization of *B. burgdorferi* and describe vesicular trafficking of *B. burgdorferi* and TLR2.

A-5

Solving Redundancy in a Bacterial Pathogen Using iMAD, a Novel Strategy for Dissecting Complex Interactions. TAMARA O'CONNOR. Sackler School of Biomedical Sciences, Dept of Molecular Biology and Microbiology, Tufts University, 136 Harrison Avenue, Boston, MA 02111. Email: tamara.oconnor@tufts.edu

The outcome of many parasitic relationships is decided by an elaborate game of molecular chess. Understanding the interaction between a pathogen and its host requires the analysis of the molecular events operating in both organisms and the causal relationships acting at the interface between them. Many pathogens however, employ multiple strategies to manipulate a single host cell process, confounding the identification of critical virulence determinants. Insertional mutagenesis and depletion (iMAD) is a genetic screening strategy that integrates bacterial mutagenesis and host RNA interference to systematically identify genetic interactions between two organisms. This technique has been used to resolve redundancy amongst the arsenal of proteins secreted by the intracellular bacterial pathogen Legionella pneumophila that are responsible for disease. Hierarchial clustering based on common behavioral patterns of individual bacterial mutants has allowed us to define collections of bacterial proteins that target common host pathways, identify sets of proteins that have redundant functions and predict specific defects in host cell biology resulting from bacterial mutant dysfunction. While iMAD has been used to dissect a process plagued by redundancy, its utility extends to any interface between two organisms involving numerous interactions

A-7

Laying the Groundwork for the Development of Validated In Vitro Test Methods for Regulatory Toxicology. JOHN W. HARBELL. Email: John.Harbell@sbcglobal.net

Traditionally toxicology has relied on the use of surrogate species to predict the impact of chemicals on the species of interest, man. Generally, the surrogate species (i.e., rodents and lagomorphs) were chosen for convenient manipulation in the laboratory rather than a demonstrated physiological match to the human. Most regulatory toxicology follows this pattern of test system selection. It is the response in the surrogate species alone, rather than a predicted response in humans, that drives regulatory decisions. Over the past several decades, there has been a strong interest in replacing the surrogate animal tests with physiologically relevant in vitro assays. This drive came from concerns about reproducibility (within and

across laboratories) (Weil & Scala 1971, Tox App Sci 19:276–360) and predictive capacity relative to the human experience (Freeberg et al., 1986, Fund & Appl Tox 7(4):626–634). In vitro programs were developed to address topical endpoints (eye and skin irritation), systemic toxicity, hepatic toxicity and metabolism, pyrogen determination, and muscle/tissue irritation as well as large scale drug screening programs. In almost 30 years of work, a number of key principles have been developed that have moved the field forward. 1) It is key to understand the physiological changes responsible for and predictive of the toxicity manifested in vivo. The in vitro assay needed to measure one or more of these primary changes. 2) The prediction models which translate the measured in vitro endpoint into a prediction of the in vivo change moved from binary (yes/no) predictions (i.e., mutagen/nonmutagen), to models designed to address a continuum of responses. 3) Early more informal evaluation programs for assessing the predictive capacity of assays (Balls e al, 1995, Tox In Vitro 9(6):871–929) have given way to formal validation programs with detailed protocols, in-laboratory training, and coded test materials (Spielmann et al., 1998, Tox In Vitro 12:305–327). 4) The test materials are chosen to represent the range of modes of toxic action, physical forms and other variables that might be experienced in testing for regulatory purposes. From the resulting data, the "validated" predictive capacity is developed. 5) Finally, positive and negative controls are required to show the consistent behavior of the test system and assay execution over time. This presentation will focus on successes (and some setbacks) of past approaches, guiding principles developed from these programs and how those principles pertain to the use of current in vitro methods and to the development of future in vitro methods.

A-8

Current In Vitro Test Methods for Classification and Labeling of Chemicals and Products. H. A. RAABE. Institute for In Vitro Sciences, Inc., 30 W. Watkins Mill Road, Suite 100, Gaithersburg, MD 20878. Email: hraabe@iivs.org

With the impetus of legislative and societal mandates to reduce or replace the use of animals for chemical and product safety testing, several non-animal test methods have been validated and approved for chemical hazard assessment and for classification and labeling purposes. Indeed, over the past 25 years, in vitro gene damage and mutation assays have routinely been used for qualitatively identifying potential chemical hazard, particularly since these assays provide a high positive predictive capacity in a timely and cost-effective manner. The relevance of the dose and exposure modeling of these relatively simple cell-based models, for risk assessment, has often been brought into question, particularly when improbable doses drive the positive responses. Several cell-based approaches have also been adopted for estimating systemic or organ-specific toxicity of chemicals, (e.g., liver disease, cardiotoxicity, dermal and retinal phototoxic reactions, etc.). However, the absence of pharmacodynamics modeling in these simple cell-based models, these current techniques have found limited application in the regulatory arena. More often than not, they are used to identify hazards which subsequently drive further animal-based studies. More recently, considerable efforts have been applied to developing and refining the exposure modeling for in vitro assays. The goal is to more readily produce the continuum of in vivo responses. The greatest successes have been realized in the development and validation of test methods using ex vivo and 3-D reconstructed human tissues for eye and skin corrosion, irritation, and permeation. Here, the dose and exposure scenarios allow the application of chemicals, formulations, or products to the test system in the same manner as what might be experienced in vivo, and where the effects are generally limited to local events. Based upon the successful validation or post hoc performance evaluation of many of these test methods, individual international regulatory agencies have endorsed or accepted test results from selected assays, while the Organization for Economic Co-operation and Development (OECD) has adopted several globally-recognized Test Guidelines (TG) for regulatory classification and labeling purposes. The adoption of these 3-D target tissue models has been particularly successful since these models effectively address tissue barrier function and diffusion kinetics. Furthermore, they are composed of target-specific cells at relevant states of stratification and differentiation, utilize simple but relevant cell-based endpoints, and allow for graded responses commensurate with the inherent toxicity and permeation characteristics of the active chemicals. These techniques and equipment can be readily acquired by interested laboratories. Select non-animal test methods accepted for regulatory classification and labeling of chemicals and products will be presented, with a focus upon their intended applications, inherent limitations, use in integrated testing strategies, and their potential for use in non-regulatory arenas.

A-9

Tox21 Overview and Update. W. CASEY. National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, PO Box 12233, Research Triangle Park, NC 27709. Email: warren.casey@nih.gov

"Tox21" is collaboration between the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP), the National Center for Advancing Translational Sciences (NCATS), National institutes of

Health Chemical Genomics Center (NCGC), the Environmental Protection Agency (EPA), and the Food and Drug Administration. The goal of Tox21 is to move toxicology from a predominantly observational science to a predictive science which uses a broad range of target-specific, mechanism-based in silico and in vitro methods to aid with hazard identification and risk assessment. Phase I of the Tox21 program, in which 1400 to 2800 compounds were screened across more than 70 high-throughput assays, is now complete. Phase II screening is currently underway, with the aim to test a 10 K library of chemicals in approximately 30 assays per year. This talk will provide a brief overview of the Tox21program and provide updated information on data generated to date, as well as plans for future testing.

A-10

RalA: A Double Edged Sword in Skin Squamous Cell Carcinoma. ADAM G. SOWALSKY[1], Addy Alt-Holland[2], Yulia Shamis[3], Jonathan Garlick[4], and Larry Feig[5]. [1]Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; [2]Department of Endodontics, Tufts University School of Dental Medicine, Boston, MA; [3]Mount Sinai School of Medicine, New York, NY; [4]Department of Oral and Maxillofacial Pathology, Tufts University School of Dental Medicine, Boston, MA; and [5]Department of Biochemistry, Tufts University School of Medicine, Boston, MA. Email: asowalsk@bidmc.harvard.edu

Squamous cell carcinomas represent 90 % of head and neck cancers and ~25 % of skin cancers. It is well-documented that progression of these cancers to a malignant state requires the epithelial cells to alter their adhesiveness to acquire motile and invasive properties. However, many studies have suggested that invasiveness is also dependent upon the tumor microenvironment, specifically the fibroblasts that populate the stroma underlying the developing tumor. In order to study the contribution of the tumor microenvironment, consisting of intercellular and stromal interactions, to the transition from a precancerous to cancerous state, we utilized an in vitro bioengineered tissue model of Ras-mediated squamous cell carcinoma. In this system, human Ras-transformed, skin-derived, E-cadherin-suppressed keratinocytes are used to populate the engineered epithelium while human dermal fibroblasts populate the underlying collagen matrix. These keratinocytes form an epidermis that mimics the earliest stage of malignant disease, as they begin to invade across the basement membrane into the dermal layer. We have recently shown that in Ras-expressing keratinocytes, RalA plays a tumor-supporting role, such that downregulation of RalA is necessary and sufficient for this transition to malignancy. However, in the underlying, supporting fibroblasts, RalA displays an opposite, tumor promoting function. Specifically, we find that RalA knockdown in these fibroblasts blocks the invasive phenotype of adjacent tumorigenic keratinocytes. In the fibroblasts, this inhibition of tumor cell invasion is due, at least in part, to the decreased secretion of the pro-invasive cytokine hepatocyte growth factor (HGF) that leads to upregulation of E-cadherin in the neighboring epithelial tumor cells. While HGF signaling is necessary for invasion, complementation with recombinant HGF is not sufficient to restore an invasive phenotype. This suggests that RalA plays a critical role in mediating the invasive potential of epithelial cancer cells through the secretion of multiple cytokines by the fibroblasts. Thus, targeting the RalA pathway in the stromal cells may be a novel therapeutic strategy with the dual advantage of blocking the secretion of several pro-invasive cytokines in normal, rather than potentially genetically-unstable, cancer cells.

A-11

The Role of Dab2 in Directing the Pathogenesis of Human Skin Squamous Cell Carcinoma. J. D. BALEJA[1], S. Pore[2], E. Bingham[2], A. G. Maione[1], J. Garlick[2], and A. Alt-Holland[2]. [1]School of Medicine and [2]School of Dental Medicine, Tufts University, Boston, MA. Email: jim.baleja@tufts.edu

Loss of the tumor suppressor E-cadherin is a hallmark of the advanced stages of squamous cell carcinoma (SCC), but the mechanisms that regulate the transition of a premalignant lesion to SCC are still elusive. The adaptor protein Disabled-2 (Dab2) may regulate this transition by controlling the trafficking of surface proteins, such as β1 integrins, that are involved in cell adhesion, migration and growth factor signaling. Here we studied the role of Dab2 in mediating molecular interactions, in skin SCC tumor cell behavior in three-dimensional (3D) human engineered tissues in vitro, and in tumor progression in vivo. Dab2 expression in 3D engineered tissues harboring either human E-cadherin competent (II-4) or E-cadherin suppressed (II-4-Ecad-) skin SCC cells, and in SCC tumors developed from these tissues in mice, was determined by IF and IHC analyses. The ability of Dab2 to interact with Eps15-homology domain containing proteins (Eps15 and Intersectin) was studied using GST-pulldown assays.The expression levels of β1 integrins, Dab2 and other endocytic proteins were determined in cell lysates by WB analysis, and Dab2 depletion was achieved by cell transfection with si-Dab2-RNA. Reduced protein expression of Dab2, Eps15, Intersectin, EEA1, and Rab5, and elevated expression of β1 integrins were found in II-4-Ecad- cells, when compared to II-4 cells. Dab2 interacted directly with both Eps15 and Intersectin. Loss of Dab2 expression in II-4-Ecad- cells was preserved in the epithelial layer of 3D engineered tissues harboring these cells and in the development of aggressive SCC tumors in

vivo. Si-RNA-mediated Dab2 depletion resulted in reduction of Dab2, Eps15 and Rab5 in II-4-Ecad- cells, while it caused an increase of Eps15 in II-4 cells. Moreover, Dab2 loss reverted the phenotype of well-organized, II-4 colonies and led to cell spreading characteristic of II-4-Ecad- cultures. We conclude that the tumor-promoting effect of E-cadherin suppression in skin SCC is mediated, at least in part, by decreased Dab2 expression and altered endocytosis.

A-12

Organ-cultured Human Skin to Study Squamous Epithelial Cell Invasion. J. VARANI. Department of Pathology, University of Michigan Medical School, Med. Sci. 1, 1301 Catherine St., Ann Arbor, MI 48109–5602. Email: varani@umich.edu

Biopsies of normal human skin can be maintained in organ culture under serum-free, growth factor-free conditions with preservation of normal histological structure and biochemical function for several days. When organ-cultured skin is exposed to exogenous growth factors that act through the epidermal growth factor (EGF) receptor, epithelial cells in the base of the epidermis undergo a proliferative response. Concomitantly, the basement membrane separating the epithelium from the stroma is eroded and epithelial cells penetrate the stroma. That is, invasion occurs. Stromal invasion is accompanied by altered expression of cell-cell and cell-matrix adhesion receptors and by an up-regulation of matrix metalloproteinases (MMPs) including MMP-1 (collagenase-1; interstitial collagenase) without a measurable change in MMP-8 (collagenase-2; neutrophil collagenase) or MMP-13 (collagenase-3). MMP suppression with tissue inhibitor of metalloproteinases-1 prevents invasion. Suppression of the mitogen-activated protein kinase (MAPK) cascade inhibits proliferation and suppresses MMP-1 production. Stromal invasion is blocked. These data suggest that organ-cultured human skin may provide a useful tool for elucidating mechanistic events that bring about stromal invasion by epithelial cells.

A-13

The Biodiversity of the Caribbean Is a Rich Source of Health and Wealth That Can Be Sustainably Developed to Benefit Both Donor and Receiver Nations. S. A. MITCHELL. The Biotechnology Centre, University of the West Indies, Mona Campus, Kingston 7, Jamaica, WEST INDIES. Email: sylvia.mitchell@uwimona.edu.jm; sylviamitchell.biotech@gmail.com

The Caribbean encompasses over 25 main islands and several main-land countries which have a Caribbean shore-line; it is among the top 8 of the world's 25 'hotspots' across the globe. The Caribbean contains 2.3 % and 2.9 % of the world's flora and fauna, respectively, on only 0.15 % of the Earth's surface and these are in danger of extinction due to the multiplying effects of population, economic and climate change factors. It is estimated that the plant biodiversity in this 'hotspot' of 230,000 sq km is an estimated 13,000 species with 6,500 endemics, and 205 genera with one family endemic to the region. Although the full total of how many of these species are medicinal may never be completely tallied, good estimates are emerging, a few examples are Jamaica (366), Martinique (251), Eastern Cuba (170), and Barbados (115). A percentage of these plants have been screened for bioactivty of various sorts and tested for drug-herb interactions by in vitro tests using CYP liver enzymes. A team of academics called TRAMILERS are testing recipes made traditionally in the region for efficacy and safety; they also maintain a Caribbean pharmacopeia and a regularly updated herbarium database online. TRAMIL's database includes 9,017 traditionally used folk medicinal recipes made from plants in 126 families, 517 genus and 800 species (568 of these are native). The species/recipes used in the most countries in the region include guava (*Psidium guajava*; diarrhoa), lemon grass (*Cymbopogon citratus*; gripe, fever), cerasee (*Mormordica charantia*; fever), bitter orange (*Citrus aurantifolia*; gripe, fever, diarrhoea), semi-contract (*Chenopodium ambrosiodies*, intestinal worms), garlic (*Allium sativum*) and aloe (*Aloe vera*). In Cuba, traditional and 'modern' medicne are fully integrated but they are less well integrated elsewhere in the Caribbean but still enjoy a high percentage of use. Development of in vitro protocols to multiply key plants and phytochemicals via micropropagation and somatic embryogenesis have also been developed for several plants such as for guinea hen weed, turmeric, ginger, aloe, sarsaparilla, chainy root and *Piper amalago*. The implications of this rich biodiversity source for future development to benefit both the Caribbean and its partners will be presented.

A-14

Chemoprevention by Resveratrol: Targets and Mechanisms. J. M. WU and T. C. Hsieh. Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, NY. Email: Joseph_Wu@nymc.edu

Cancer is the leading cause of death among men and women. Evidence implicates cancer as a multi-stage and several decade long disease process, amenable to intervention using dietary and pharmacological agents. Bioactive food component, such as those found in grapes may provide these chemopreventive benefits. The long-term goal of our

laboratory is to evaluate the efficacy of grape-derived polyphenol resveratrol in prevention of prostate cancer and to glean insights by which resveratrol exerts its anti-prostate cancer activities and mechanisms. We have used a panel of cultured human prostate cancer cells as a model to test our central working hypothesis that treatment by resveratrol will suppress cancer cell proliferation, restore the induction of apoptosis, and modulate the expression of prostate specific genes, notably, the androgen receptor and prostate specific expression. Moreover, we also advance the thesis that the anti-prostate cancer activities are mediated by molecular targets that subserve as sensors and mediators of activities of resveratrol. Using a combination of affinity chromatography and mass spectrometry, we have identified several cellular targets of resveratrol, including the protein quinone reductase NQO2. Evidence supporting the role of NQO2 as a sensor and mediator of effects of resveratrol in prevention of prostate cancer will be summarized and presented.

A-15

Ethnomedicinal Opportunities for Human Health from Jamaican-grown Tropical Herbs: Anti-cancer Guinea Hen Weed (*Petiveria alliacea*), Bitter Herbs, Root Tonics, Nurtaceuticals, Spices and Cold Bushes. S. A. MITCHELL. The Biotechnology Centre, University of the West Indies, Mona Campus, Kingston 7, Jamaica, WEST INDIES. Email: sylvia.mitchell@uwimona.edu.jm; sylviamitchell.biotech@gmail.com

Ethnomedicinal recipes gathered over the last decade in Jamaica, and associated research by the University of the West Indies, has identified several medicinal plants with the potential for human health. Of 334 medicinal plants identified between 1948 and 2001, 193 had been tested for various levels of bioactivity. Crude extracts of 80 of these plants and at least 29 extracted phytochemicals were bioactive. The top herbs found to be bioactive include John Charles (*Hyptis verticillata*), neem (*Azadirachta indica*), shame-mi-lady (*Mimosa pudica*), breadfruit (*Artocarpus altilis*), kidney bush (*Bontia daphoides*), ackee (*Blighia sapida*) and spirit weed (*Eryngium foetidum*) with the family Piperaceae being particularly bioactive. Guinea hen weed (*Petiveria alliacea*) has since been found to contain several phytochemicals including a potent anti-cancer chemical: dibenzyl trisulphide (DTS). Of more than 1,400 plant extracts, guinea hen weed was among 34 plants which were active against cancer. DTS was the most active and was able to differentiate between normal and cancer cells, killing only the cancerous cells. This plants has also been found to stimulate the immune system, fight infection (broad spectrum antimicrobial activity), relieve pain (has COX-1 inhibitory properties) and lower blood sugar levels. Bitter herbs such as neem, aloe (*Aloe vera*), cerasee (*Momordica charantia*) and rice bitters (*Andrographis paniculata*) are being used for blood sugar and obesity control. Root tonic plants such as sarsaparilla (*Smilax regelii*), chainy root (*Smilax balbisiana*), ramoon (*Trophis racemosa*) and medina (*Alysicarpus vaginalis*) as well as noni (*Morinda citrifolia*) and aloe are used as nutraceuticals for building strength and vitality. Locally grown spices such as ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), nutmeg (*Myristica fragrans*), cinnamon (*Cinnamomum zeylanicum*) and pimento (*Pimento racemosa*) have had increasing research and clinical trials applied to them which have identified a myriad of health uses. Cold bushes (cold, fever, asthma) have the largest number of plants being used, these include Jack-in-the-bush (*Eupatorium odoratum*), lime (*Citrus aurantifolia*), eucalyptus, fever grass (*Cymbopogon citratus* & *C. flexuosus*) and John Charles. Some of these plants have a lot of research accomplished already while others are not well-known, the least known are the endemics for which preliminary research has indicated have a high relative bioactivity. Recent research on these plants will be highlighted. The difficulties, challenges and opportunities for further development of these plants for human health will be discussed.

A-16

Constrained Peptides as Probes for In Vitro Biology. JOSHUA KRITZER. Tufts University School of Arts and Sciences, Department of Chemistry, Medford, MA. Email: Joshua.Kritzer@tufts.edu

Short peptides can be potent and selective tools for in vitro biology, but poor affinity, proteolytic susceptibility, and poor cell penetration can limit the usefulness of peptide probes. Structural rigidification is a proven method for improving these properties. We have demonstrated that constrained peptide macrocycles can be further stabilized by introducing side-chain-to-side-chain staples. This process produces peptide bicycles with higher affinity, selectivity, and resistance to degradation. We have applied this strategy to G1, an 11-residue peptide macrocycle that binds the Src homology 2 (SH2) domain of growth-factor-bound protein 2 (Grb2). Several peptide bicycles were synthesized entirely on-resin with high yields. Two rounds of iterative design produced peptide bicycle BC1, which is 60-fold more potent than G1 and 200-fold more selective with respect to binding a related SH2 domain. Also, BC1 is completely intact after 24 hours in buffered human serum, conditions under which G1 is completely degraded, and is cell-penetrant. The structure, function, cell penetration, and

applications of peptide macrocycles and bicycles bearing phosphotyrosine mimics will be discussed, as well as late-breaking applications of this strategy to other systems.

A-17

Biosensors for Biomarkers: Measuring Kinase Activity Using Chemical Biology. L. L. PARKER. Purdue University Center for Cancer Research, Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, West Lafayette, IN 47907. Email: llparker@purdue.edu

Our research program is broadly directed at assay development for post-translational modifications (PTMs), particularly to measure intracellular phosphorylation by tyrosine kinases. Protein tyrosine kinases play key roles in disease and are particularly important in cancer: mutations in several protein tyrosine kinase genes have been identified as drivers of many tumor types and drugs targeted at inhibiting these enzymes represent ~20 % (>$9 billion) of the current oncology market. We use a "decoy" substrate biosensor approach in which an artificial, optimized substrate peptide is designed to report the activity of a specific enzyme or enzyme family in living cells. Delivery is achieved using cell-penetrating peptides and enzymatic modification is measured using a range of readout strategies—some that require extraction of the cell contents and some that leave the cell intact. Targeting the function of the enzyme in its intracellular environment preserves protein-protein interaction-, localization- and scaffolding-dependent aspects of its activation, and decoy substrates provide a snapshot of enzymatic activity that circumvents the need to understand the functional consequences of every endogenous substrate site. We are also developing multiplex-compatible readouts so we can use a suite of biosensors for different enzymes to profile whole pathways. Through proof-of-concept development and initial applications to basic and translational questions we have established the potential of this approach and laid the groundwork of a substrate development workflow to expand our repertoire of biosensors for kinases and other enzymes in current and future efforts.

A-18

Solutions for Microfluidics: Novel Interconnects, Precision Fluid Delivery, and Alternatives to the Classical Incubator. T. N. CORSO[1], C. K. Van Pelt[1], A. Rose[1], J. P. Morgan[2], A. D. Stroock[2], and S. Y. Rabbany[3]. [1]CorSolutions, 95 Brown Road, Box 1007, Ithaca, NY 14850; [2]School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY 14853; and [3]School of Engineering and Applied Science, Hofstra University, Hempstead, NY 11549. Email: tcorso@mycorsolutions.com

For "cells-on-a-chip" approaches to become adopted as an industry standard, the support hardware surrounding these microfluidic devices must be developed and standardized. Current areas of difficulty include making connections to the microdevices, as well as providing controlled and precise fluid delivery. We report here a novel interconnect technology for coupling microfluidic, "cells-on-a-chip" devices with pumps and detectors of the macro-world. The interconnects offer reliability, compatibility with all substrate materials, ease-of-use with little training, flexibility for use with chips having varied architectures, chemical compatibility, allowance of maximum field of view for optical assessment, remain leak-free over a wide-range of flow rates and backpressures, low cost, and have potential for automation. Additionally we will show a means for providing accurate flow control to the cultures on-chip. With the critical role that flow rate plays on the cell seeding process used to establish a microfluidic culture and on shear stress in maintaining cultures, it is advantageous to have a highly accurate means of fluidic control. It will be shown that when Human Umbilical Vein Endothelial Cells (HUVECs) are seeded onto optically transparent flow cells and subjected to shear stress using our pump, the cells align, in the direction of flow, in response to the controlled laminar flow of media as delivered by the pump. Finally the integration of pumps and connectors into a platform capable of providing a unique means of environmental control, as an alternative to a classical incubator, will be discussed.