Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-231-2825.

1. **Grantee Institution:** University of Pittsburgh- of the Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 1/1/2011-12/31/2014

3. **Contact Person (First Name, M. I., Last Name, Degrees):** Margaret C. McDonald, PhD

4. **Grant Contact Person's Telephone Number:** 412-383-7474

5. **Grant SAP Number:** 4100054875

6. **Project Number and Title of Research Project:** 01—Cellular Systems in Biology in Cancer Drug Discovery

7. **Start and End Date of Research Project:** 1/1/2011-12/31/2013

8. **Name of Principal Investigator for the Research Project:** D. Lansing Taylor, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 4,018,983

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Taylor	PI	2.6% Yr1; 2.8% Yr2; 2.7% Yr3	37,076
Bahar	Co-PI	5% Yr1,Yr2, Yr3	36,689
Wipf	Co-PI	5% Yr2; 11% Yr3	38,429
Gough	Researcher	63% Yr1; 64% Yr2; 73% Yr3	344,749
Vernetti	Researcher	100% Yr1; 94% Yr2; 89% Yr3	497,793
Boltz	Researcher	17% Yr1; 100% Yr2; 100% Yr3	391,548
Lezon	Researcher	33% Yr3	25,629
Shrivastava	Researcher	17% Yr1; 28% Yr2; 82% Yr3	104,116
McDermott	Researcher	19% Yr1; 23% Yr2; 23% Yr3	47,057
Iyer	Researcher	75% Yr1; 100% Yr2; 100% Yr3	199,377
Cheng	Researcher	23% Yr3	16,950
Chen	Research Associate	66% Yr3	61,471
Gur	Postdoc	14% Yr1; 10% Yr2	12,420
Paris	Postdoc	40% Yr1	17,488
Zumot	Postdoc	8% Yr2; 33% Yr3	25,895
Zhao	Postdoc	50% Yr1; 50% Yr2; 33% Yr3	69,939
Grabowski	Administrator	41% Yr1; 38% Yr2; 41% Yr3	120,715
Reese	Technician	92% Yr1; 71% Yr2; 40% Yr3	115,906
Shun	Statistician	7% Yr2; 15% Yr3	23,751
Wagner	Technician	33% Yr2; 23% Yr3	19,975
Zaidins	Technician	28% Yr3	11,269

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Multidrop Combi Reagent Dispenser	The Multidrop is a relatively inexpensive but versatile system for microplate preparation. In particular, it is a reliable system for plating a consistent number of cells per well. Since cell density can affect cell response to	22,040

	stimulus, repeatability of plating is a critical factor in assay repeatability.	
LED Light Engine Module for Arrayscan-VTI HCS Reader	The LED light engine upgrade on the Arrayscan VTI replaced an unstable arc lamp light source. The upgrade was essential to minimize system-induced variability in the data.	24,120

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds awarded:
InCell 6000 High Content Instrument for Cellular Systems Biology	☑NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:__)	September 2011	$816,270	$502,020

A 3D Biomimetic Liver Sinusoid Construct for Predicting Physiology and Toxicity	☑NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:__)	January 2012	$4,526,347	$2,328,281
Quantitative Cellular Systems Pharmacology of Protein-Protein Interaction	☑NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:___)	January 2012	$7,024,310	Not Funded
Discovery and Optimization of Inhibitors for STAT3 Activation for the Treatment of Squamous Cell Carcinoma of the Head and Neck (TO6) Milestone 4	☐NIH ☑ Other federal (specify:_SAIC / Leidos____ ☐ Nonfederal source (specify: _)	January 2012	$795,030	$795,030
Chemical Inducers of ATF2-Mediated Apoptotic Activity in Melanoma	☐NIH ☑ Other federal (specify:_SAIC / Leidos_____) ☐ Nonfederal source (specify:___)	September 2012	$567,655	Not Funded
Modeling Cellular Heterogeneity for Development of Improved Therapies	☑NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:___)	October 2012	$370,710	Not Funded
Modeling the Biochemical Basis of Cell Differentiation	☐NIH ☐ Other federal (specify:_____) ☑ Nonfederal source (specify: Paul G. Allen)	October 2012	$883,538	Not Funded
Identification of Small-Molecules That Selectively Kill HPV-Associated Tumor Cells	☐NIH ☐ Other federal (specify:_____) ☑ Nonfederal source (specify: ____UPMC____)	December 2012	$78,492	$78,492
Discovery and Optimization of Inhibitors for STAT3 Activation for the Treatment of Squamous	☐NIH ☑ Other federal (specify:_SAIC / Leidos_____) ☐ Nonfederal	March 2013	$788,974	$788,974

Cell Carcinoma of the Head and Neck (TO6) Milestone 5	source (specify:___)			
Breast Cancer TMA	☐NIH ☐ Other federal (specify:_____) ☑ Nonfederal source (specify: _GE Global Research)	August 2013	$35,000	$35,000
Comprehensive Program for Breast Cancer Metastasis Treatment and Therapeutic Development	☐NIH ☑ Other federal (specify:__DOD__) ☐ Nonfederal source (specify:___)	September 2013	$2,029,212	Not Funded
Center for Quantitative Systems Pharmacology	☑NIH ☐ Other federal (specify:____) ☐ Nonfederal source (specify:___)	November 2013	$15,591,085	Not Funded
Resource for Organotypic Models for Predictive Toxicology	☐NIH ☑ Other federal (specify:_EPA____) ☐ Nonfederal source (specify: __)	January 2014	$1,047,480	Pending
A 3D Biomimetic Liver Sinusoid Construct for Predicting Physiology and Toxicity	☑NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:___)	May 2014	$ 4,748,348	$4,252,709
Integrating Patient-Derived 3D Spatiotemporal Experimental and Computational Models To Deconvolute Cancer Heterogeneity and Progression	☑NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:___)	May 2014	$862,223	Pending
Informatics Tools for Tumor Heterogeneity in Multiplexed Fluorescence Images	☑NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:___)	June 2014	$608,499	Pending

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X__ No_____

If yes, please describe your plans:

As shown in the table above, we continue to aggressively pursue grants to maintain funding for these programs. We have already had some success, but we will continue to submit grant applications and establish collaborations at the University of Pittsburgh, with the pharmaceutical industry, and with additional academic institutions (see Item 16 below).

12. Future of Research Project. What are the future plans for this research project?

We are continuing the program in cancer-therapeutics development, with a shift in focus to metastatic breast cancer. We are actively seeking research grant funding for ongoing support of the breast cancer research program. We are also expanding our research on computational modeling, especially with respect to heterogeneity analysis, to address polypharmacology in drug discovery, development, and diagnostics.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male	1		3	
Female			1	1
Unknown				
Total	**1**		**4**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	1		4	1
Unknown				
Total	**1**		**4**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White	1			
Black				
Asian			1	1
Other			3	
Unknown				
Total	**1**		**4**	**1**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This project was instrumental in bringing together scientists from the Departments of Computational and Systems Biology and Chemistry, as well as the University of Pittsburgh Cancer Institute. These scientists, together with the University of Pittsburgh Drug Discovery Institute (UPDDI), are continuing to apply multidisciplinary approaches to cancer research, diagnostics, and therapies. This project was also a key component of the "IN Cell 6000 High-Content Instrument for Cellular Systems Biology" grant. The (GE) IN Cell 6000 and methods of analysis developed for this project were instrumental in securing the funding for the "3D Biomimetic Liver Sinusoid Construct for Predicting Physiology and Toxicity" project, which is being used as a model of a metastatic cancer niche as well as a toxicity model. Faculty and postdoctoral/graduate students funded under this program continue to pursue projects and submit grant applications focused on research and modeling of cancer systems. In summary, this project was leveraged to establish a multidisciplinary program within UPDDI that is focused on a quantitative systems pharmacology approach to cancer. Our program continues to expand in scope—most recently including biomedical engineers from Carnegie Mellon University who have expertise in tissue engineering and printing.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (. e.g., entire university, entire hospital system)?

Yes_____X____ No_____

If yes, please describe the collaborations:

John Wikswo, PhD, biological physicist, Vanderbilt University: Collaboration on the design of organ-model systems for safety testing, including model scaling, microfluidic devices, and micro-detection systems

Johnathan Himmelfarb, MD, nephrologist, University of Washington: Safety testing, especially with respect to kidney toxicity

Mark Donowitz, MD, gastroenterologist, Johns Hopkins University: Safety assessment, especially with respect to the role of the gut in compound absorption and metabolism

Mary Estes, PhD, molecular biologist, Baylor University: Safety testing, especially with respect to the role of the gut in absorption and metabolism

Evrogen: Development and potential marketing of cellular biosensors

Nortis Inc.: Design and production of microfluidic chips for safety assessment

16(B) Did the research project result in commercial development of any research products?

Yes____X_____ No_____

If yes, please describe commercial development activities that resulted from the research project:

We are testing the performance of the liver-safety models in microfluidic devices in development at Nortis Inc. We believe this Nortis collaboration is likely to result in a design that is optimized for liver-safety assessment.

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period

that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

The Major Goal as stated in the proposal:
The project focuses on the development of a research program to discover and develop small-molecule anticancer therapeutics. At the University of Pittsburgh, novel chemistries are combined with cellular systems biology and computational pharmacology approaches to drug discovery. The overriding goal of this project is to further enhance the broad scientific capabilities at the University of Pittsburgh in these research areas and to deploy them for the development of novel anticancer drug candidates and, ultimately, commercial drugs.

Progress toward project goal:
To achieve this goal, project funds were used to bring together a team of experienced medicinal chemists, drug discovery biologists, and computational biologists to establish a research program in cellular systems biology (CSB). Funds also were used to establish collaborations with cancer biologists to apply a systems-based approach to the development of new cancer therapies. Our approach to CSB was to develop sophisticated multiparameter high-content screening (HCS) assays and computational models that provide insight into

complex cellular systems; such assays hold particular relevance for diseases that result from the abnormal functioning of those systems.

Phenotypic pathway assays (like the signal transducer and activator of transcription-3 [STAT3] assay) provide a robust readout without the need to select a particular target. In addition, these assays can be multiplexed with other pathway assays for more specific readouts. In this project, we developed a multiplexed STAT3, STAT1 (signal transducer and activator of trascription-1) assay to characterize compound activity with respect to these two pathways—and, ultimately, to identify compounds that can restore the balance between these two pathways with opposite action in tumorigenesis. Further examples of multiparameter assays are the highly multiplexed panels developed for assessing cardio- and hepato-toxicities.

For the first two years, we focused on assay and computational-model development in the context of specific drug development projects and biological drivers (details below and in the annual reports). Work during the third year focused on the application of these tools, establishing targeted research programs in breast and head and neck cancers in the University of Pittsburgh Drug Discovery Institute (UPDDI), and securing additional funding for these collaborations. In addition, we continued efforts to develop CSB tools, including more sophisticated models of safety assessment and 3D cancer models.

An important measure of the project's success (and validation of its importance) is indicated by the additional funding leveraged through the developments and collaborations established during the project term. Additional research grant funding enabled by this program totaled over $30 million (listed above), of which nearly $5 million was awarded during the term of this project; an additional $4.4 million in grants was based on program-related work but was awarded following the December 2013 end of this project.

In particular, the grant titled "A 3D Biomimetic Liver Sinusoid Construct for Predicting Physiology and Toxicity," which is aimed at constructing a microfluidic liver organoid with HCS readouts of mechanisms of action, was a direct extension of the early safety-assessment panel developed in this project. Though this organ model currently serves as a platform for early safety assessment, preliminary experiments show its promise as a metastatic cancer niche model.

Important cancer research collaborations have been established as a direct result of this program. A collaboration on head and neck cancer with Jennifer Grandis, MD, (now at the University of California, San Francisco) is focused on the selection and optimization of hits from a pathway screen for inhibitors of an important cancer target, STAT3. Other significant collaborations include breast cancer research with Adrian Lee, PhD, at the University of Pittsburgh Cancer Institute, and work with GE Healthcare on the development of novel cancer diagnostics using highly multiplexed, single-cell data from the company's MultiOmyx platform.

We described our approach to quantitative systems pharmacology (QSP) in a high-profile book, *The Molecular Basis of Cancer* (submitted 2013). Our book chapter provides a

complete description and strategy for QSP implementation (Gough, Lezon et al. 2014). We also published an analysis of cellular heterogeneity in the STAT3 signaling pathway and the development of heterogeneity indices that can be incorporated into large-scale biology projects, including screening for drug discovery and development of improved diagnostics (work completed in 2013) (Gough, Chen et al. 2014).

The number of papers published during this grant period was limited, in part due to the time required for preparation, review, and publication; however, in addition to the publications identified here, others are listed in section 20B.

Overall, the CURE grant funding was successful in launching a major UPDDI program that will fundamentally influence the development of cancer diagnostics and therapies at the University of Pittsburgh, GE, and beyond.

Objectives (as stated in the proposal):
The objective of this project is to advance anticancer drug discovery by developing three classes of targets: (1) improved modulators of microtubule dynamics, (2) transcription factors (e.g., STAT3), and (3) modulators of the interaction between two critical proteins (e.g., Myc-Max) in an important cellular signaling pathway (Myc is also a transcription factor).

Functional, cell-based models using human cell lines, primary cells, and, ultimately, validated stem cell-derived cells will be developed and investigated using medium-throughput and high-content light microscope imaging methods, as well as mechanistic probe molecules, to define the systemic response to both commercially available and novel drug candidates. Computational methods will be developed and applied to assess the protein-protein and protein-inhibitor interactions at the molecular structural level and to relate the cellular responses to known patterns of gene and protein responses, cellular pathways, and protein-protein interaction networks. Critical attention will be given to polypharmacology effects for early safety assessment and compound prioritization. Promising compounds will be further developed by lead optimization and pharmacokinetics studies, including animal models.

Progress toward Objectives:
(1) As noted in our 2013 annual report, we discontinued work on novel microtubule (MT) stabilizers because of the challenges associated with improving the chemical synthesis that was originally developed by Dennis Curran, PhD. The relatively complex synthesis made the development of new analogs of the MT stabilizers difficult; and the success of the STAT3 inhibitor project, as discussed below, provided a much better opportunity.
(2) The STAT3 transcription factor inhibition project provided a significant opportunity for CSB development; therefore, it was a major focus of effort in this project for the chemistry, bioassay, and computational biology teams. While a National Cancer Institute (NCI) contract supported the standard screening and chemistry, the contract was limited to delivering on the milestones.

    Project effort was focused on the development and application of CSB tools, including: designing a method for evaluating the reproducibility of cellular imaging systems;

development and application of heterogeneity indices for identifying and monitoring heterogeneity in cellular activity; development of a computational model of the STAT3 pathway; and establishment of improved criteria for rank ordering compounds in structure-activity relationships (SAR) that take into account both potency (IC50) and heterogeneity in the response. CSB methods developed for the STAT3 pathway screen are sufficiently general to provide broad applicability to future UPDDI phenotypic screening projects. More importantly, these methods will be an essential component for all cancer research projects.

While significant progress was made toward the objectives of this project, additional effort will be required to complete CSB tools development to assess protein-protein and protein-inhibitor interactions at the molecular-structural level and to relate cellular responses to known patterns of gene and protein responses, cellular pathways, and protein-protein interaction networks. These methods, which are being developed in collaboration with scientists in the Department of Computational and Systems Biology, were ongoing when this project term ended. Development will continue with funding from future grants.

(3) As stated in the 2013 annual report, an alternate source of funding was identified for the Myc-Max project. Work is progressing under a grant from Johnson and Johnson.

Specific Aims (as stated in the proposal):
Aim 1: To advance the use of functional CSB profiling as secondary screens to assist in the selection of lead series for recently developed dictyostatin analogues as MT stabilizers. The goal is to develop a novel drug candidate with the same potency as paclitaxel for MT stabilization and induction of cell-cycle arrest, but with less drug resistance and fewer off-target effects. Lead series selection will be assisted based on CSB profiling of at least six of the NCI-60 cell lines, with a panel of at least 10 cellular pathway and functional cellular parameters, including MT stabilization, with high-content screening (HCS) measurements. This CSB approach will identify both potency for the target and the extent of significant off-target effects before going into animal models and preclinical testing.

Aim 2: To advance the use of CSB profiling as secondary screens to assist in the selection of lead series from hits identified in primary screens measuring the activation of STAT3 by using an HCS assay that measures the translocation of phosphorylated STAT3-Y705 from the cytoplasm into the nucleus. Lead series selection will be assisted based on CSB profiling of at least six of the NCI-60 cell lines, with a panel of at least 10 cellular pathway and functional cellular parameters, including STAT3 and STAT1 activation by translocation to the nucleus and other HCS measurements. This CSB approach will identify both potency for the target and the extent of significant off-target effects before going into animal models and preclinical testing.

Aim 3: To advance the use of CSB profiling as secondary screens to assist in the selection of lead series from hits identified in primary screens measuring the interaction between Myc-Max *in vitro*. Lead series selection will be assisted based on CSB profiling of at least six of the NCI-60 cell lines, with a panel of at least 10 cellular pathway and functional cellular

parameters. A biosensor of Myc-Max interaction will be developed and included as part of the CSB profile.

Aim 4: To implement a cell-based and predictive early safety-assessment platform using rodent primary liver cells and the human cell line HepG2 in a CSB profiling protocol to assist in prioritization and/or de-risking lead series compounds identified in efficacy profiling outlined in Aims 1-3.

Aim 5: To develop and apply computational pharmacology tools at the molecular and systems levels to assess the druggability of target proteins, assist in compound selection and prioritization using both high-throughput virtual screening and molecular simulations with ligand- or target-based approaches, and reduce the complex efficacy profiling and early safety-assessment profiling data sets to actionable subsets.

Summary of Progress and Results toward the Specific Aims:
Aims 1-3 each had two components. The first was to advance the use of functional CSB profiling as secondary screens to assist in the selection of lead series, and the second was to apply functional CSB profiling to important biological systems. Three important cancer biology targets were proposed: (Aim 1) cell-cycle arrest, (Aim 2) inhibition of STAT3 activation, and (Aim 3) disruption of the Myc-Max interaction.

<u>Aims 1-3, Component 1—Advance the use of CSB profiling:</u>
Understanding how seemingly identical cells respond differently to drug treatment is a significant challenge to CSB application in drug discovery. In cancer, the prevalence of intratumor genetic and phenotypic heterogeneity, results from clonal evolution, epigenetic plasticity, and variation in tumor microenvironments suggest that a single drug targeting a single driver is not likely to control disease progression adequately. Tumor microenvironment complexity, which extends to stromal and immune cells, may contribute significantly to the development of treatment resistance. Efforts to recapitulate the *in vivo* tumor microenvironment in physiologically relevant models will require analytical approaches that address the heterogeneity in the models. However, cellular heterogeneity is not limited to cancer cells but is also exhibited in “normal” clonal cell lines, and the impact of heterogeneity extends from basic biology to drug discovery and diagnostics. The foundation of CSB has to be data that reliably capture biological variability for incorporation in the analysis and models.

To address this challenge, we developed a systematic approach to generate data for CSB modeling, starting with tools to characterize the reproducibility of HCS systems, including image-analysis algorithms. We continued with the development of three general indices that can be used to monitor, characterize, and compare the biological heterogeneity of cellular systems. Assessing protein-protein and protein-inhibitor interactions at the molecular structural level (as well as modeling the cellular responses to known patterns of gene and protein responses, cellular pathways, and protein-protein interaction networks) requires data that accurately represent the heterogeneity of the cellular system of interest. Tools developed in this project form a basis for, and are essential to, CSB profiling. These tools provide a means to distinguish between biological and system variability.

To understand HCS system reproducibility, we took advantage of quality control standards and methods for cell analysis that are well established for flow cytometry systems (FCS). We have designed a protocol for routine HCS systems characterization by using parallel HCS- and FCS-based studies on standard fluorescent beads and cells. This protocol can be used to optimize data reproducibility, provide a baseline measure of performance, and for routine monitoring of system performance. A protocol for illumination correction using a flatfield standard fluorophore solution was developed, tested, and routinely applied to optimize imaging reproducibility. Table 1 shows results of a comparison between flow- and imaging-intensity measures on standard beads and cells. Imaging intensity CVs (coefficient of variation) were somewhat higher, most likely due to the variation in the distribution of bead or cell intensity over the pixels. These measures were still small, however, relative to the heterogeneity observed in STAT3 activation. Future analysis will include a deconvolution step to improve the reproducibility of imaging intensity measures.

Table 1. Reproducibility of Intensity Measures

Method	Sample	CV(%)	Ratio	S(%)	N
BD Spec	2 µm Beads	2.3	-	-	-
Flow	2 µm Beads	2.8	2.0	-	8,681
Imaging	2 µm Beads	5.2	2.0	-	13,156
Flow	Cal33 Cells	6.2	1.8	31	9,522
Imaging	Cal33 Cells	8.0	1.9	28	10,814

To address heterogeneity in cellular phenotypes, we developed a set of indices that are used to identify, quantify, and characterize heterogeneity in a way that can be easily integrated into all screening and cellular profiling. We also developed an optimal data representation, the “Histo-Box Plot,” to analyze the full range of heterogeneity in the data, when it is identified. Design and use of our heterogeneity indices is discussed in detail in Gough, Chen et al. (2014). We used activation of STAT3 as a model system for developing and testing indices and show how these heterogeneity indices can be used in high throughput biology and drug discovery to quantify, compare, and flag studies in which: (1) there is a high degree of variability in the cellular responses, (2) results suggest there is more than one subpopulation, or (3) there are more than the expected number of outliers.

Using this approach, we found that heterogeneity in STAT3 activation varies between cell lines and between modes of activation. Interleukin-6 (IL-6) induces a bimodal activation of STAT3 in Cal33 head and neck squamous cell carcinoma (HNSCC) cells, while Oncostatin M (OSM) induces a unimodal activation of STAT3. In MCF10A breast fibrocystic epithelial cells, both IL-6 and OSM induce unimodal activation of STAT3. In contrast, MDA-MB-468 breast cancer cells exhibit unimodal response to OSM but essentially no response to IL-6.

In summary, after developing an approach to characterizing and optimizing the performance of the imaging systems, we were able to identify and characterize biological heterogeneity in the STAT3 activation pathway. We demonstrated that the heterogeneity is not a cell cycle

effect and not a result of variation in IL-6 receptor level (by Western blot, data not shown). Determining the biological source of the heterogeneity will be best approached through model-driven experimentation. We are currently refining models to drive hypothesis generation and experimentation.

Aim 4: Early safety assessment panel

We have developed six "fit-for-purpose" models to screen for cardiotoxicity, hepatotoxicity, metabolic liver clearance, and drug metabolites that are used as an early safety-assessment panel in UPDDI. We have established assays using primary human hepatocytes, rodent hepatocytes, and HepG2 cells in the microplate format. In addition, we developed a zebrafish assay for cardiotoxicity, a microsomal metabolic drug stability assay, and a human hepatocyte assay for drug clearance and metabolism.

The novel zebrafish embryo cardiotoxicity model is a moderate throughput screen, in the industry standard microplate format, with the capability to use low-power, white-light video microscopy and computational image processing to identify potential pro-arrhythmia drugs. The detailed protocols and test results from the development of these assays is documented in previous annual reports. We have applied this panel of early safety assays in the following collaboration projects:

We screened 38 compounds with magneto optical test (MOT) assays for cardiotoxicity (zebrafish heartbeat assay) and hepatotoxicity (rat hepatocyte and HepG2 assays) in collaboration with Dr. Thomas Smithgall's laboratory (Nef inhibitor program). We identified oxidative stress as a possible cardio- and hepato-toxic mechanism of action for the lead compound of the SAR series. Additional analysis of the SAR identified two "hot spots" on the core compound that induced toxicity when an electron withdrawing group was added in these locations. This finding prompted us to redesign the compound with electron donating groups and other possible chemical modification to remove or diminish the toxic hot spots (Iyer, Zhao et al. 2014).

We screened 28 compounds for Dr. Lee McDermott's glutaminase inhibitor project with the HepG2 assay, human hepatocyte assay, and aqueous solubility. In addition, five compounds were selected to screen through the metabolic stability mass spec assay. Results from these studies gave Dr. McDermott information to use in a grant application to fund further chemical synthesis and *in vitro* efficacy efforts.

We screened 19 histone deacetylase (HDAC) compounds for the Dr. Neil Hukriede (University of Pittsburgh) and Dr. Mark deCastecker's (Vanderbilt) acute kidney injury project. Early drug-safety assays were conducted to screen for effects of HDAC inhibitors on the heart rate and the production of reactive oxygen species (ROS) in zebrafish. Compounds were also screened for toxicity in the rat hepatocyte and HepG2 assays, as well as metabolic stability in the mouse microsomal screen.

Five of these compounds of interest were tested further for metabolic stability and metabolite identification using human microsomes. Data from this effort were used to develop a grant proposal to conduct preclinical (rodent) *in vivo* pharmacology, toxicity, and pharmacokinetic

(PK) studies on the lead candidate compound for possible investigational new drug (IND) submission.

In summary, details of the early safety-assessment panel, developed with funding from this project, can be found in our annual reports. The panel has become a UPDDI standard and has helped to drive SAR in several projects to develop compounds with fewer toxic liabilities, which should increase their probability of making it to the clinic and, ultimately, into the marketplace.

Aim 5: Computational Pharmacology tools.

We have taken several approaches to modeling data generated in this project. To extract information on subpopulation responses, we have implemented ModFit, a program developed employing a Gaussian Mixture Model (GMM) approach to segmenting multiparameter phenotypic distributions. This program is discussed in our 2012 report, which includes information on two applications of statistical network models to the characterization of mechanistic variability. First, higher-order statistics reveal finer details of cellular populations and facilitate segmentation of these cells into subpopulations with differential drug sensitivity, based on variation in functional response. This capability is especially important in cancer therapies because cells that do not respond to a treatment will ultimately lead to a loss of treatment efficacy. Second, higher-order statistics applied to multidimensional cellular data enable the characterization of local network topology variations in subpopulations. Understanding these observations will suggest complementary pathways that can be targeted to produce more effective combination therapies.

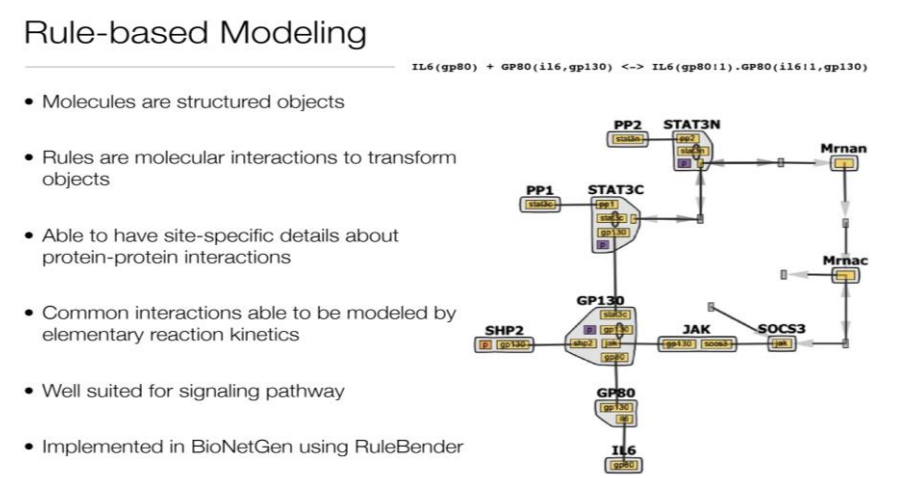

Figure 1. An Initial Rule-based Model of STAT3 activation by IL6.

We have also collaborated on the development of a liver-focused panel of 150 compounds that have been assembled as a reference safety database. This database will be used to develop predictive models of human toxic liabilities using the panel of assays developed in Aim 4. In particular, this database will be used to accumulate information on pathways, compounds, and target proteins, which will be combined with physiological data collected from the models.

HCS data generated in Aims 1-3 are currently being used to refine a model of STAT3 pathway activation. Using a published model (Singh, Jayaraman et al. 2006) as a starting point, a rule-based model of STAT3 activation by IL-6 was constructed using BioNetGen (Faeder, Blinov et al. 2009), a modeling tool developed at the University of Pittsburgh. An example of fit quality is shown in Figure 2. Note that this initial model only fits average cellular activity; it does not reproduce heterogeneity. Strategies for incorporating heterogeneity into pathway models are currently being evaluated. Alternative modeling approaches based on statistical-network models were discussed in previous reports.

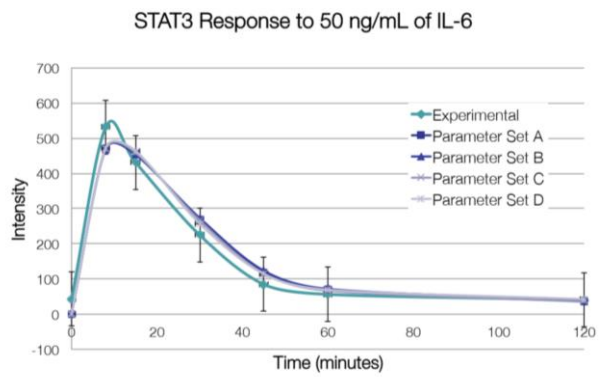

Figure 2. Response of the initial Rule-based model of STAT3 activation by IL6, compared to experimental data.

Improved models that account for cellular heterogeneity will be important to understanding the role of pathway interactions in cellular responses in more complex microenvironments, *in vivo* and *in vitro*, and in physiologically relevant disease and organ models, as well as patient samples.

Aim 5 of this project is the least developed because (1) modeling was delayed until by the optimized data from the STAT3 assays became available and (2) some development and testing are required to determine the most effective approach to building heterogeneity into the models. Additional funding will be sought to continue this avenue of inquiry.

Impact of Project Accomplishments

Recent studies suggest that knowledge of tumor composition and the response of component subpopulations to single drugs, in conjunction with computational and experimental modeling, can identify drug combinations that minimize the outgrowth of resistant subpopulations in tumors, while enhancing tumor-free survival in mice (Pritchard, Bruno et al. 2013, Zhao, Pritchard et al. 2014). Importantly, the experimentally validated computational simulations demonstrated that the predicted optimal drug combination depended on whether the entire tumor population, or only a particular subpopulation, was examined. These results emphasize the need to identify and incorporate intratumor heterogeneity and the expected evolutionary trajectories into rational drug combination design. Models are in development but will require additional funding to complete.

The development of comprehensive, unbiased, target-based mutagenesis and genome-wide gain- and loss-of-function technologies that can anticipate clinically relevant resistance represents another approach to modeling and therapeutically addressing tumor heterogeneity (Garraway and Janne 2012, Wood, Konieczkowski et al. 2012). It is likely that the future of personalized cancer medicine will involve a comprehensive bioinformatics analysis of a

biopsy that can reveal distinct cell populations (Beckman, Schemmann et al. 2012, Fedele, Tothill et al. 2014) and usage of an established drug-genotype database that will allow clinicians to computationally determine optimal, patient-specific, combination therapies. We hypothesize that heterogeneity analysis will be essential to the implementation of a QSP-driven approach. Such an approach would include the highly coordinated, parallel optimization of complementary lead structures, with each structure having a clinically relevant resistance profile that is addressed by its counterpart. This QSP-driven approach, we believe, will lead to polypharmacologic therapies that effectively provide sustainable remissions and higher cure rates.

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Beckman, R. A., G. S. Schemmann and C. H. Yeang (2012). "Impact of genetic dynamics and single-cell heterogeneity on development of nonstandard personalized medicine strategies for cancer." Proceedings of the National Academy of Sciences of the United States of America **109**(36): 14586-14591.

Faeder, J. R., M. L. Blinov and W. S. Hlavacek (2009). Rule-based modeling of biochemical systems with BioNetGen. Methods in Molecular Biology: Systems Biology. I. V. Maly. Totawa, NJ, Humana Press.

Fedele, C., R. W. Tothill and G. A. McArthur (2014). "Navigating the challenge of tumor heterogeneity in cancer therapy." Cancer Discov **4**(2): 146-148.

Garraway, L. A. and P. A. Janne (2012). "Circumventing cancer drug resistance in the era of personalized medicine." Cancer Discov **2**(3): 214-226.

Gough, A., T. Lezon, J. R. Faeder, C. Chennubhotla, R. F. Murphy, R. Critchley-Thorne and D. L. Taylor (2014). High-Content Analysis with Cellular and Tissue Systems Biology: a Bridge between Cancer Cell Biology and Tissue-Based Diagnostics. The Molecular Basis of Cancer.

J. Mendelsohn, P. M. Howley, M. A. Israel, J. W. Gray and C. Thompson, Elsevier.
Gough, A. H., N. Chen, T. Y. Shun, T. R. Lezon, R. C. Boltz, C. E. Reese, J. Wagner, L. A. Vernetti, J. R. Grandis, A. V. Lee, A. M. Stern, M. E. Schurdak and D. L. Taylor (2014). "Identifying and Quantifying Heterogeneity in High Content Analysis: Application of Heterogeneity Indices to Drug Discovery." PLoS One **9**(7): e102678.

Iyer, P. C., J. Zhao, L. A. Emert-Sedlak, K. K. Moore, T. E. Smithgall and B. W. Day (2014). "Synthesis and structure-activity analysis of diphenylpyrazolodiazene inhibitors of the HIV-1 Nef virulence factor." Bioorg Med Chem Lett **24**(7): 1702-1706.

Pritchard, J. R., P. M. Bruno, L. A. Gilbert, K. L. Capron, D. A. Lauffenburger and M. T. Hemann (2013). "Defining principles of combination drug mechanisms of action." Proc Natl Acad Sci U S A **110**(2): E170-179.

Singh, A., A. Jayaraman and J. Hahn (2006). "Modeling regulatory mechanisms in IL-6 signal transduction in hepatocytes." Biotechnol Bioeng **95**(5): 850-862.

Wood, K. C., D. J. Konieczkowski, C. M. Johannessen, J. S. Boehm, P. Tamayo, O. B. Botvinnik, J. P. Mesirov, W. C. Hahn, D. E. Root, L. A. Garraway and D. M. Sabatini (2012). "MicroSCALE screening reveals genetic modifiers of therapeutic response in melanoma." Science signaling **5**(224): rs4.

Zhao, B., J. R. Pritchard, D. A. Lauffenburger and M. T. Hemann (2014). "Addressing Genetic Tumor Heterogeneity through Computationally Predictive Combination Therapy." Cancer Discovery **4**(2): 166-174.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No. "

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No. ")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible

subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5. 0. 5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Identifying and Quantifying Heterogeneity in High Content Analysis: Application of Heterogeneity Indices to Drug Discovery	Gough, A. H., N. Chen, T. Y. Shun, T. R. Lezon, R. C. Boltz, C. E. Reese, J. Wagner, L. A. Vernetti, J. R. Grandis, A. V. Lee, A. M. Stern, M. E. Schurdak and D. L. Taylor	PLOS ONE	March 2014	☐Submitted ☐Accepted ☑Published
2. Synthesis and Structure-Activity Analysis of Diphenylpyrazolodia zene Inhibitors of the	Iyer, P. C., J. Zhao, L. A. Emert-Sedlak, K. K. Moore, T. E. Smithgall and B. W. Day	BioOrganic and Medicinal Chemistry Letters	January 2014	☐Submitted ☐Accepted ☑Published

HIV-1 Nef Virulence Factor				

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

Additional papers that are the result of work funded by this grant currently in preparation include: (1) an analysis of heterogeneity in the STAT3 SAR studies (led by Gough), (2) development of a function-based approach to characterization of heterogeneity (led by Lezon), and (3) a paper on the imaging-based zebrafish cardiotoxicity assay as a component of the early safety assessment panel of assays (led by Vernetti).

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable. " Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The impact of this research is not immediate but will be important in future studies. The analysis of cell-population distributions (rather than a simple mean population response as is the current practice) will be essential in many drug discovery and development projects, as well as in diagnostics, drug and chemical safety, and basic research. Recent studies suggest that knowledge of tumor composition and the response of component subpopulations to single drugs, in conjunction with computational and experimental modeling, can identify drug combinations that minimize the outgrowth of resistant subpopulations in tumors while enhancing tumor-free survival in mice. Importantly, experimentally validated computational simulations have demonstrated that the optimal drug combination predicted depended on whether the entire tumor population, or only a particular subpopulation, was examined. These results emphasize the need to incorporate intratumor heterogeneity into rational drug combination design. We hypothesize that heterogeneity analysis will be essential to the implementation of an approach to therapy that includes the highly coordinated, parallel optimization of complementary lead structures, where each structure has a clinically relevant resistance profile that is addressed by its counterpart, leading to polypharmacologic therapies that provide sustainable remissions and higher cure rates.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were

no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable. " Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No____X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No. ")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person.
DO NOT EXCEED FOUR PAGES.

NAME D. Lansing Taylor	POSITION TITLE Director, University of Pittsburgh Drug Discovery Institute Allegheny Foundation Professor of Computational

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Maryland	BS	1968	Zoology
State University of New York at Albany	PhD	1973	Cell Biology
Woods Hole Marine Biological Laboratory	Postdoctoral Fellow	1973-74	Biophysics

A. Personal Statement

I began my academic career as an Assistant Professor at Harvard University and remained at Harvard until 1982, developing and using novel fluorescence-based reagents and imaging technologies to investigate fundamental cellular processes. I then moved to Carnegie Mellon University as a Professor of Biological Sciences and as Director of the Center for Fluorescence Research in the Biomedical Sciences. In 1991, I became the Director of the National Science Foundation-funded Center for Light Microscope Imaging and Biotechnology, and in 1995, I was named Vice Dean of CMU's Division of Molecular Sciences. I continued to develop reagent and imaging technologies, while applying the technologies to understand fundamental processes in cells and tissues. Alan Waggoner and I co-founded Biological Detection Systems (BDS) to commercialize the multi-color cyanine dyes and research imaging platforms and it was acquired by Amersham-now GE Life Sciences. A great limitation of imaging technology in the 1990's was the very slow and interactive nature of image data management and analysis. I left CMU to found Cellomics, Inc., the company that developed High Content Screening (also called automated microscopy). Fully automated microscopy was the foundation for a shift from focusing primarily on generating images to generating large-scale, quantitative data on cells. I was CEO of this company from 1996 through 2003 when it became part of Thermo Fisher. I then founded a third company, Cellumen, which developed a predictive safety assessment platform using primary hepatocytes, multiplexed panels of reagents, reference safety databases and computational biology. I was CEO of Cellumen from 2004 until 2010 when it became part of Cyprotex. I also co-founded Cernostics, Inc., a fluorescence-based, tissue systems pathology company. I hold >25 U.S. patents, including six focused on cell-based imaging. I returned to academia at the end of 2010 to continue my academic interests which now link large-scale cell, tissue and human, biomimetic, tissue-engineered model profiling with computational and systems biology to optimize drug discovery and diagnostics. I am also developing computational tools to identify and quantify heterogeneity. As the Director of the Drug Institute, I am focusing our efforts in Quantitative Systems Pharmacology in order to change the paradigm in drug discovery, development and diagnostics.

B. Positions and Honors <u>Present</u>

<u>Professional Positions</u>

2010 – *present* Allegheny Foundation Professor of Computational and Systems Biology

2010 – *present* Director, University of Pittsburgh Drug Discovery Institute, Pittsburgh, PA

1999 – *present* Adjunct Professor, Carnegie Mellon University, Pittsburgh, PA

Honors

2011	International Society for Advancement in Cytometry: Mack J. Fulwyler Award	Outstanding Innovation by invention or a Career of Innovative Science
2007	Society of Biomolecular Sciences	Achievement Award for Dev High Content Screening
2007	American Institute for Medical and Biomedical Engineering	Elected Fellow
2004	Pittsburgh Life Sciences Greenhouse	Pioneer Award (Creation of Companies)
2003	National Science Foundation	Pioneer Award -Recognition for Vision and leadership for Science & Technology Centers
2002	Carnegie Science Center Awards	Entrepreneur of the Year
2001	Ernst & Young, PA	Entrepreneur of the Year
1996	Computer World-Smithsonian Award	Achievement in Biomedical Imaging Science
1995	Carl Zeiss Award	Cell Biology Research with Microscopy
1990 – 2000	NIH	Merit Award

C. Recent Peer-reviewed Publications (from >140 peer-reviewed articles, 18 reviews and 7 book Chapters-Does not include > 25 patents)

1. Gough A, Chen N, Shun TY, Lezon T, Boltz RC. Reese, Wagner J, Grandis J, Lee A, Stern A, Schurdak M, **Taylor DL.** Identifying and Quantifying Heterogeneity in High Content Analysis: Application of Heterogeneity Indices to Drug Discovery. PLoS ONE. 2014;9(7) e102678.
2. Taylor DL. A new vision for drug discovery and development. Eur Pham Rev. 2012;17(6):20-24.
3. Bale SS, Vernetti L, Jindal R, Senutovitch N, Bhushan A, Gough A, McCarty W J, Bakan A, Hegde M, Shun TY, Golberg I, DeBiasio R, Usta O B, Yarmush M L ,**Taylor DL**. 2014. In Vitro Platforms for Evaluating Liver Toxicity. J. Expt Biol and Med. (In Press).
4. Bhushan A, Bale SS, McCarty WJ , Hegde M, Jindal R, Golberg I, Usta OB, Yarmush ML, Senutovitch N, Vernetti L, Gough A, Bakan A , Shun TY, DeBiasio R, **Taylor DL**. Towards a 3D Microfluidic Liver Platform for Predicting Drug Efficacy and Toxicity in Humans. 2013. J Stem Cell Res & Therapy 4 (Suppl): S16
5. Gough AG, Lezon T, Faeder JR, Chennubhotla C, Murphy RF, Critchley-Thorne R, **Taylor DL**. High content analysis and cellular and tissue systems biology: a bridge between cancer cell biology and tissue- based diagnostics. In/The Molecular Basis of Cancer, 4th Ed. (Mendelsohn J, Howley PM, Israel MA, Gray JW, Thompson CB) Elsevier, NY. 2014.

BIOGRAPHICAL SKETCH

NAME Ivet Bahar	Distinguished Professor and John K Vries Chair
eRA COMMONS USER NAME	**Dept of Computational & Systems**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
Bogazici University, Istanbul, Turkey	BS	1980	Chemical Engineering
Bogazici University, Istanbul, Turkey	MS	1983	Chemical Engineering
Istanbul Technical University, Turkey	PhD	1986	Chemistry

A. Personal Statement

As the Founding Chair of the Department of Computational and Systems Biology at the University of Pittsburgh (Pitt), School of Medicine, and the Pitt Founding Director of the Joint PhD program in Computational Biology between Pitt and Carnegie Mellon University, I am dedicated to initiating and establishing collaborative and interdisciplinary research and training initiatives. My lab introduced and developed models and methods for exploring complex biological systems dynamics at multiple scales, which assist in the translation of basic science findings to therapeutic applications. In particular, we pioneered the introduction and extensions of elastic network models for exploring the machinery of biomolecular systems, in addition to developing methods for efficient examination of protein-substrate and protein-inhibitor interactions.

B. Positions and Honors

Present Position

6/2013 -- Distinguished Professor of Computational & Systems Biology, U of Pittsburgh (Pitt)
2/2010 -- Professor and John K. Vries Chair, Dept of Computational & Systems Biology, Pitt
01/2011-- Associate Director, University of Pittsburgh Drug Discovery Institute

Past Positions

2010 -2013 Co-Director, Clinical & Translational Sci Institute, Molecular & Systems Modeling Center
2004 -2010 Founding Chair, Department of Computational Biology, School SOM, Pitt
2005-2010 John K. Vries Chair, Department of Computational Biology, SOM, Pitt
2005-2009 Founding Director, PhD Program in Comput Biology, Joint Pitt & Carnegie Mellon U
2001–2004 Founding Director, Center for Computational Biology & Bioinformatics, SOM, Pitt
2001 -2004 Professor, Department of Molecular Genetics & Biochemistry, SOM, Pitt
1993-2001 Professor, Chemical Engineering Department, Bogazici University
1992-2000*(summers)* Fogarty Fellow/Visiting Scientist, Exp & Comp Biol Lab, Div of Basic Sci, NCI, NIH
1992-2000 Director, Polymer Research Center, Bogazici University
1986-1987, 87-93 Assistant Prof, Associate Professor, Chemical Engineering Dept, Bogazici University
1989-1998 Visiting Scientist, Ecole Supérieure de Physique et Chimie (ESPCI), Lab de Physico Structurale et Macromol (PCSM), Paris, France; *several short-term visits between*.

Honors and Memberships

2014 Chancellor's Distinguished Research Award (Pitt)
2010 - 2012 Executive Board Member (Elected), Biophysical Society
2008 - 2012 Council Member (Elected), Biophysical Society

Recent Professional Activities

2013 Review Committee Member, Intern Human Frontier Sci Program, Strasbourg, France
2013 *Review Panel Member*, NIH Director's New Innovator Award Phase I Mail Review

Other Scientific Activities

6/2014 *Elected Chair*, Gordon Research Conference on Biopolymers.
8/2013 *Featured Speaker*, Ion Channel Meeting, Oleron, France
5/2013 *Keynote Speaker*, Symposium 'Comput Biology: Then and Today" Weizmann Inst, Israel

C. Selected peer-reviewed publications *(from 220+ papers, 15 chapters & 1 edited book - H index: 58)*

- Liu, B, Bhatt DZ, Oltvai ZN, Greenberger JS**, Bahar, I** Significance of p53 dynamics in regulating apoptosis in response to ionizing radiation, and polypharmacological strategies. *Scientific Reports* **4**, 6245 (2014).
- Chu CT, Ji J, Dagda RK, Jiang JF, Tyurina YY, Kapralov AA, Tyurin VA, Yanamala N, Shrivastava IH, Mohammadyani D, Wang KZQ, Zhu J, Klein-Seetharaman J, Balasubramanian K, Amoscato AA, Borisenko G, Huang Z, Gusdon AM, Cheikhi A, Steer EK, Wang R, Baty C, Watkins S, **Bahar I**, Bayir H, Kagan VE (2013) Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells *Nature Cell Biology***15**: 1197-1205 PMID: 24036476
- Jiang J, Bakan A, Kapralov AA, Ishara Silva K, Huang Z, Amoscato AA, Peterson J, Krishna Garapati V, Saxena S, Bayir H, Atkinson J, **Bahar I**, Kagan VE (2014) Designing inhibitors of cytochrome c/cardiolipin peroxidase complexes: mitochondria-targeted imidazole-substituted fatty acids *Free Radic Biol Med***71C**: 221-230 PMID: 24631490
- Gur M, Madura JD, **Bahar I** (2013) Global Transitions of Proteins Explored by a Multiscale Hybrid Methodology: Application to Adenylate Kinase *Biophys J***105**: 1643-1652 PMID: 24094405
- Cheng MH**, Bahar I** (2013) Coupled Global and Local Changes Direct Substrate Translocation by Neurotransmitter-Sodium Symporter Ortholog LeuT *Biophys J* **105**: 630-639. PMID: 23931311
- Zomot E**, Bahar I** (2013) Intracellular Gating in an Inward-facing State of Aspartate Transporter GltPh Is Regulated by the Movements of the Helical Hairpin HP2 *J Biol Chem* **288**: 8231-8237. PMID: 23386619
- Liu Y, **Bahar I** (2012) Sequence Evolution Correlates with Structural Dynamics *Mol Biol Evol* **29**: 2253-63.PMID: 22427707
- Zomot E, **Bahar I** (2012) A conformational switch in a partially unwound helix selectively determines the pathway for substrate release from antiporter CaiT *J Biol Chem* **287**: 31823-32. PMID: 22843728
- Lezon TR, **Bahar I.** (2012) Constraints imposed by the membrane selectively guide the alternating access dynamics of the glutamate transporter GltPh. *Biophys J,* **102**: 1331-40. PMID: 22455916
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- Jiang J, Shrivastava IH, Watts SD, **Bahar I,** Amara SG. (2011) Large collective motions regulate the functional properties of glutamate transporter trimers. *Proc Natl Acad Sci USA*, **108**: 15141-6. PMID: 21876140
- Sukumaran M, Rossmann M, Shrivastava I, Dutta A, **Bahar I**, Gregor IH. (2011) Dynamics and allosteric potential of the AMPA receptor N-terminal domain. *EMBO J,* **30**: 972-82. PMID: 21317871
- **Bahar I**. (2010) On the functional significance of soft modes predicted by coarse-grained models for membrane proteins. *J Gen Physiol* **135**:563-73 PMID: 20513758
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- Marcos E, Crehuet R, **Bahar I**. (2010) On the conservation of the slow conformational dynamics within the amino acid kinase family: NAGK the paradigm. *PLoS Comput Biol* **6**: e1000738 PMID: 20386738

D. Research Support <u>Ongoing Research Support</u>

1P41GM103712 (PI: Bahar) 9/24/2012-7/31/2017
BTRR on High Performance Computing for Multiscale Modeling of Biological Systems

NIH U19AI68021 (Greenberger) 9/1/2010-8/30/2015
Mitochondrial Targeting Against Radiation Damage
Role: PI of the Computational Systems Pharmacology Core

NIH-NIGMS 1R01GM099738 (PI: Bahar) 3/26/2012-12/31/2015
Continued Development of Protein Dynamics Software ProDy

NIH 1P30DA035778 (PI: Bahar, Xie, Xing) 8/1/2014-7/31/2019

NIDA Center of Excellence OF Computational Drug Abuse Research (CDAR)

NIH-NHGRI U54HG007934 (PI: Cooper, Bahar, Berg) 9/15/14-9/14/19
Center for Causal Modeling and Discovery of Biomedical Knowledge from Big Data

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Wipf, Peter	Distinguished University Professor
eRA COMMONS USER NAME (credential, e.g., agency login)	
pxwipf	

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Univ. of Zürich, Dept. of Chem., Zürich,	Dipl. Chem.	1980-84	Chemistry & Biochemistry
Univ. of Zürich, Dept. of Chem., Zürich,	Ph. D.	1984-87	Organic Chemistry
Univ. of Virginia, Dept. of Chem., Charlottesville,	Postdoc	1988-90	Synthetic Chemistry

D. Personal Statement.

I have led an independent research group since 1990 and published more than 500 papers and patents in several research areas relevant to the proposed specific aims. My group has pursued medicinal chemistry projects since 1995, and one of our compounds, PX-866, is currently in Phase II clinical trials for the treatment of cancer. I was one of the original founders of the University of Pittsburgh Drug Discovery Institute, and still serve as an Associate Director of the UPDDI. Since 2002, I am the Director of the Center for Chemical Methodologies and Library Development (UPCMLD), and since 2009, I am the co-Leader of the University of Pittsburgh Chemical Diversity Center (UPCDC), a Participant in NCI's Chemical Biology Consortium. Since 2012, I am also a co-leader of the Molecular Cancer Therapeutics Program at the University of Pittsburgh Cancer Institute. My contributions to the scientific literature and my ongoing discovery & development projects clearly demonstrate my commitment to interdisciplinary biomedical research, as well as my ability to interact with a diverse set of colleagues in biology, chemistry, pharmacology, and medicine. My group remains committed to harnessing the power of small molecules in the search for innovative cancer treatments.

E. Positions and Honors.

1990-1995 Assistant Professor, Department of Chemistry, University of Pittsburgh, Pittsburgh, PA
1995-1997 Associate Professor, Department of Chemistry, University of Pittsburgh, Pittsburgh, PA
1997-2004 Professor, Department of Chemistry, University of Pittsburgh, Pittsburgh, PA
2001- Professor, Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA
2002- Adjunct Professor, Department of Chemistry, Duke University, Durham, NC
2002- Director, Center for Chemical Methodologies & Library Development, University of Pittsburgh
2004- Distinguished University Professor, University of Pittsburgh, Pittsburgh, PA
2012- Co-Leader, UPCI Molecular Therapeutics and Drug Discovery Program, Pittsburgh, PA.
2014- Professor, Department of Bioengineering, Swanson School of Engineering, Pittsburgh, PA.

Selected Other Experience and Professional Memberships

2009- ACS Medicinal Chemistry Letters, Associate Editor
2007- Organic Reactions, Inc., Board of Directors
2005- Chemical Biology & Drug Design, Editorial Board
2002-2010 Organic Syntheses, Inc., Board of Editors
2002-2006 Organic & Biomolecular Chemistry, Associate Editor for North America
1998-2003 NIH Medicinal Chemistry Study Section, Member, and Chair

Selected Honors

2015-2018 Chair-Elect, Chair, and Retiring Chair of the Section on Pharmaceutical Sciences of the American Association for the Advancement of Science

2014 Humboldt Research Award of the Alexander von Humboldt Foundation

2013 Harry and Carol Mosher Award of the Santa Clara Valley Section of the ACS
2013 Edward W. Morley Award
2012 ACS Pittsburgh Award
2010 Fellow of the American Chemical Society (ACS)
2009 ACS Guenther Award in the Chemistry of Natural Products
2008 Chancellor's Distinguished Research Award
2004 Fellow of the Royal Society of Chemistry (FRSC)
2003 International Society for Heterocyclic Chemistry (ISHC) Katritzky Award
2002 Fellow of the American Association for the Advancement of Science (AAAS)
1998 Arthur C. Cope Scholar Award
1995 Camille Dreyfus Teacher-Scholar Award
1995 Merck Young Investigator Award
1995 Zeneca Award for Excellence in Chemistry
1994-1999 NSF Presidential Faculty Fellow
1994-1996 Alfred P. Sloan Research Fellow
1993-1995 Eli Lilly Grantee

C.Recent Peer-Reviewed Publications (from a total of 460)

"**Synthesis of Heterocyclic Triads by Pd-Catalyzed Cross-Couplings and Evaluation of their Cell- Specific Toxicity Profile**." Salamoun, J.; Anderson, S.; Burnett, J. C.; Gussio, R.; Wipf, P. *Org. Lett.* **2014**, *16*(7), 2034-2037. PMCID: PMC3983320.

"**New Pyrazolopyrimidine Inhibitors of Protein Kinase D as Potent Anticancer Agents for Prostate Cancer Cells**." Tandon, M.; Johnson, J.; Li, Z.; Xu, S.; Wipf, P.; Wang, Q. J. *PLoS One* **2013**, *8*, e75601. PMCID: PMC3781056.

"**Protein Kinase D as a Potential Chemotherapeutic Target for Colorectal Cancer**." Wei, N.; Chu, E.; Wipf, P.; Schmitz, J. C. *Mol. Cancer. Ther.* **2014**, *13*, 1130-1141. PMCID: PMC4019967.

"**The Hsp70 Modulator MAL3-101 Inhibits Merkel Cell Carcinoma**." Adam, C.; Baeurle, A.; Brodskly, J. L.; Wipf, P.; Schrama, D.; Becker, J. C.; Houben, R. *PLoS ONE* **2014** *9*(4), e92041. PMCID: PMC3973671.

"**Growth Arrest by the Antitumor Steroidal Lactone Withaferin A in Human Breast Cancer Cells is Associated With Down-Regulation and Covalent Binding at Cysteine 303 of □-Tubulin**." Antony, M. L.; Lee, J.; Hahm, E.-R.; Kim, S.-H.; Marcus, A. I.; Kumari, V.; Ji, X.; Yang, Z.; Vowell, C. L.; Wipf, P.; Uechi, G. T.; Yates, N. A.; Romero, G.; Sarkar, S. N.; Singh, S. V. *J. Biol. Chem.* **2014**, *289*, 1852-1865. PMCID: PMC3894360.

"**Targethunter: An in Silico Target Identification Tool for Predicting Therapeutic Potential of Small Organic Molecules Based on Chemogenomic Database**." Wang, L.; Ma, C.; Wipf, P.; Liu, H.; Su, W.; Xie, X.-Q. *AAPS J.* **2013**, *15*, 395-406. PMCID: PMC3675739.

"Stochastic Voyages into Uncharted Chemical Space Produce a Representative Library of All Possible Drug-like Compounds." Virshup, A. M.; Contreras-García, J.; Wipf, P.; Yang, W.; Beratan, D. N. *J. Am. Chem. Soc.* **2013**, *135*, 7296-7303. PMCID: PMC3670418.

"**A Targeted Library Screen Reveals a New Inhibitor Scaffold for Protein Kinase D**." Tandon, M.; Wang, L.; Xu, Q.; Xie, X.; Wipf, P.; Wang, Q. J. *PLoS One* **2012**, *7*(9), e44653. PMCID: PMC3445516.

"**Targeting of XJB-5-131 to Mitochondria Suppresses Oxidative DNA Damage and Motor Decline in a Mouse Model of Huntington's Disease**." Xun, Z.; Rivera-Sanchez, S.; Ayala-Penã, S.; Lim, J.; Budworth, H.; Skoda, E. M.; Robbins, P. D.; Niedernhofer, L. J.; Wipf, P.; McMurray, C. T. *Cell Rep.* **2012**, *2*(5), 1137-1142. PMCID: PMC3513647.

"**Global Lipidomics Identifies Cardiolipin Oxidation as a Mitochondrial Target for Redox Therapy of Acute Brain Injury**." Ji, J.; Kline, A. E.; Amoscato, A.; Arias, A. S.; Sparvero, L. J.; Tyurin, V. A.; Tyrina, Y. Y.; Fink, B.; Cheng, J. P.; Alexander, H.; Clark, R. S. B.; Kochanek, P. M.; Wipf, P.; Kagan, V. E.; Bayir, H. *Nat. Neurosci.* **2012**, *15*(10), 1407-1413. PMCID: PMC3697869.

"**Synthesis and Structure-Activity Relationships of Benzothienothiazepinone Inhibitors of Protein Kinase D**." Bravo-Altamirano, K.; George, K. M.; Frantz, M.-C.; Lavalle, C. R.; Tandon, M.; Sharlow, E. R.; Lazo, J. S.; Wang, Q.; Wipf, P. J. *ACS Med. Chem. Lett.* **2011**, *2*, 154-159. PMCID: PMC3100199.

"**Discovery of Diverse Small Molecule Chemotypes with Cell-Based PKD1 Inhibitory Activity**." Sharlow, E. R.; Mustata Wilson, G.; Close, D.; Leimgruber, S.; Tandon, M.; Reed, R. B.; Shun, T. Y.; Wang, Q. J.; Wipf, P.; Lazo, J. S. *PLoS One* **2011**, *6*, e25134. PMCID: PMC3187749.

"**Design, Synthesis, and Biological Evaluation of PKD Inhibitors**." George, K. M.; Frantz, M.-C.; Bravo- Altamirano, K.; Lavalle, C. R.; Tandon, M.; Leimgruber, S.; Sharlow, E. R.; Lazo, J. S.; Wang, Q. J.; Wipf, P. *Pharmaceutics* **2011***, 3,* 186-228*.* PMCID: PMC3261798.