Response Form for the Final Performance Review Report—
Mellon Pitt Corporation 2008F*

1. Name of Grantee: Mellon Pitt Corporation

2. Year of Grant: 2008 Formula Grant

A. For the overall grant, briefly describe your grant oversight process. How will you ensure that future health research grants and projects are completed and required reports (Annual Reports, Final Progress Reports, Audit Reports, etc.) are submitted to the Department in accordance with Grant Agreements? If any of the research projects contained in the grant received an "unfavorable" rating, please describe how you will ensure the Principal Investigator is more closely monitored (or not funded) when conducting future formula funded health research.

Grant oversight and reporting for Mellon Pitt Corporation (MPC) is shared by the University of Pittsburgh's Office of the Senior Vice Chancellor for the Health Sciences (OOSVC) and Carnegie Mellon University's Quality of Life Technology Center. Both offices distribute a training manual to investigators that clearly states PA DOH's reporting requirements. We also underscore the importance of complying with the requirement that all publications resulting from these projects acknowledge PA DOH and that publications that acknowledge the Commonwealth be directly related to the specific project described in the strategic plan.

For each research project contained in the grant, please provide a response to items B-D as listed on the following page(s). When submitting your response please include the responses for all projects in one document. The report cannot be submitted as a ZIP file, because the Department's exchange server will remove it from the email. If the report exceeds 2MB, please contact the Health Research Program for transmittal procedures: 717-783-2548.

* Please note that for grants ending on or after July 1, 2007, grantees' Final Performance Review Reports, Response Forms, and Final Progress Reports ***will be made publicly available on the CURE Program's Web site.***

Project Number: 0864101
Project Title: Computational and Experimental Approaches for Predicting miRNAs and their Targets
Investigator: Hinman, Veronica F.

B. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in Section B using the following format. As you prepare your response please be aware that the Final Performance Review Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

<u>Reviewer 1:</u>

1. The investigators have made substantial progress on Aim 1; however, more development and details concerning the boosting algorithm for prediction of miRNA targets in Aim 2 is needed.

<u>Reviewer 2:</u>

1. Conduct the critical evaluation of the most widely used methods with regards to sensitivity and specificity as described in the specific aims. Provide more evidence of how the supervised learning approach offers significant advantage.

2. Apply target prediction to novel miRNAs discovered in sea urchins and validate them to prove the methods are effective.

3. Establish more links towards how the impact of these algorithms is important to human health.

<u>Reviewer 3:</u>

Major weaknesses:
1. The stated objectives are too ambitious, especially Aim 2, miRNA target prediction.
2. No publication or software were generated; therefore, it is difficult to evaluate the progress.

Recommendations:
1) Publish the results as soon as possible.
2) Conduct luciferase reporter assay to validate the interesting microRNAs.
3) Knock-in microRNAs to sea urchin embryo and do expression profiling to get a list of putative targets, then combine with seed-match to further filter out confident miRNA targets.

Response:
<u>Reviewer 1</u>:

Our collaborator, Takis Benos, PhD, has continued to work toward this aim after the grant period. Dr. Benos is in the final stages of manuscript preparation to publish data regarding the algorithm for prediction of miRNA targets.

Reviewer 2:

1. Please see response to Reviewer 1.
2. Our group has performed this evaluation, which is ongoing. Preliminary data were recently published in:

Kadri S, Hinman VF, Benos PV. RNA deep sequencing reveals differential microRNA expression during development of sea urchin and sea star. PLoS ONE. 2011;6(12):e29217. Epub 2011 Dec 28.

Unfortunately, this publication does not acknowledge CURE funding.

We have also made great progress in establishing a working antibody for argonaute, which we use for high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITs-CLIP) analyses. This project was a major part of the work undertaken by graduate student, as we realized that having an initial data set was an important part of the computational analysis. We are currently optimizing conditions for HITs-CLIP and will soon send samples out for sequencing. Once we have obtained these data, we will work with Dr. Benos on the target prediction alogrithms.

3. Knowledge of miRNA targets is very poor in any system, especially in human development. Echinoderms are an invertebrate model system for understanding vertebrate development. We have identified miRNAs in sea urchins that are found in humans, but not in other model systems, such as *Drosophila* (see *PLoS ONE* publication above). These echinoderms are thus an excellent system in which to study human miRNAs. When we have fully developed the HITs-CLIP method in sea urchins, we will be in a powerful position to understand the role of these miRNAs in human development.

Reviewer 3:

The *PLoS ONE* paper listed above and a second publication (below) describe software generated from this project:

Kadri S, Hinman V, Benos PV. HHMMiR: Efficient de novo prediction of microRNAs using hierarchical hidden Markov models. BMC Bioinformatics. 2009 Jan 30;10 Suppl 1:S35.

In this publication, we describe HHMMiR, a novel approach for *de novo* miRNA hairpin prediction in the absence of evolutionary conservation. Our method implements a Hierarchical Hidden Markov Model (HHMM) that uses region-based structural and sequence information of miRNA precursors.

Recommendations:

This work is ongoing. The expression profiling has been done and is described in the *PLoS ONE* paper listed above. The target prediction is progressing as our HITs-CLIP experiments continue to improve.

C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.

Response: This project received a favorable rating.

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: None.

Project Number: 0864102
Project Title: High-Throughput Estimation and Modeling of Protein Filament Distributions from Microscope Images
Investigator: Rohde, Gustavo K.

B. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in Section B using the following format. As you prepare your response please be aware that the Final Performance Review Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

<u>Reviewer 1</u>: None

<u>Reviewer 2</u>:
1. The initial proposal was vague on the methods to be employed and how they would be validated. Secondly, while this work is interesting and may yield insights into principles of cellular organization, a clear case was not made for existing needs for such a method in medical diagnostic or assay applications. For instance, if I was a cancer researcher and had access to a perfect version of this software that could reproduce or report the distributions of features of microtubule network in a population of cells, how would I use it to diagnose a disease state or treat it more safely/effectively?

<u>Reviewer 3</u>:
1. A major weakness was the lack of progress on Aim 2.

2. Modeling of dynamic networks of tubulin and actin has been developed by other groups. It was not apparent from the project description that this literature was familiar to the investigators.

3. Few details on methodologies to be utilized in Aim 2 were given.

Response:

<u>Reviewer 2</u>:
We regret that the reviewer found our research plan to be vague. We described a new method for modeling microtubule distributions in cells, as well as approaches for estimating the model parameters that best fit a given image. During the course of the project, we were able to extract these findings from both real and simulated (for validation) images. The applications could be many. Those who wish to screen for drugs that stabilize microtubules so as to slow cell division, tumor growth, and metastasis could find the method useful in providing more quantitative information (rather than simply visualizing images). Furthermore, our work provides a valuable tool for biologists who wish to study the distribution of microtubules in different cell lines but may not have a quantitative method to study a large set of cells imaged under microscopes.

Reviewer 3:

1. It is true that during the short duration of this funding period, our work focused mainly on Aim 1. Since that time, however, the task of modeling the distribution of mitrotubule networks in live HeLa cells has been achieved. The modeling procedure has also been expanded to include time dynamics. Sample results are available at (http://cellorganizer.org/) and manuscripts are currently in preparation.

2. Our research plan focused on modeling the spatial distribution of microtubules in fixed and live cells. The reviewer mentioned "modeling of dynamic networks." While we are familiar with some such efforts, they do not typically include any information about the sub-cellular organization of such networks; rather, they usually focus on dynamics, as the reviewer mentioned. We believe ours is the first approach toward modeling the spatial distribution of such filaments in cells.

3. Please see response to Reviewer 2.

C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.

Response: This project received an outstanding rating.

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: None.

Project Number: 0864103
Project Title: Computational Modeling of miRNA Involvement in Pulmonary Gene Networks
Investigator: Benos, Panagiotis

B. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in Section B using the following format. As you prepare your response please be aware that the Final Performance Review Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

<u>Reviewer 1:</u>

1. A description of the performance of the new algorithm relative to existing methods should be provided.

2. A description of how the algorithm has or hasn't resulted in new hypotheses about the regulation of IPF-related gene expression should be provided.

<u>Reviewer 2:</u>

1. There is the relative lack of progress and evidence that the objectives have been met. Clear documentation needs to be provided to allow for assessment of progress.

2. Another weakness is the descriptive nature of the study and lack of more substantive relevance to the modeling of IPF in the second specific aim. Assumptions are made vis-a-vis molecular mechanisms based on ChIP data, but the presented evidence provided no insight as to actual functional significance of the binding interaction data. Moreover, there is no provision to confirm functional significance. Actual experimental data need to be provided to allow confirmation of the validity of the algorithm and its predictive capacity.

3. There is insufficient appreciation of known molecular mechanisms of what the cell data is supposed to model - EMT in IPF. A more direct participation by the collaborator (Dr. Kaminski) is essential to correct this deficiency.

<u>Reviewer 3:</u>

1. A549 cells are lung carcinoma cells and have already undergone epithelial mesenchymal transition. The findings made using these cells, particularly studies related to epithelial mesenchymal transition and miRNA-transcription factor interaction with TGF-beta treatment, may be problematic because that may not occur in non-transformed lung epithelial cells. Therefore, using primary lung epithelial cells is highly appreciated.

2. miRNAs predominantly contribute to posttranscriptional events; therefore, proposed experiments that focus on the transcription factor may yield only partial information.

3. Preliminary results are not properly described; therefore, it is very difficult to appreciate the broader application of the proposed study.

4. Fibroblasts obtained from the fibrotic lung tissues significantly change their phenotypes including the potential to proliferate with passages. It is not clear what passage cells will be tested for the study.

5. Proposed experiments lack alternative approaches if the proposed experiments in Aim 1 failed to make significant inroads. In that case, it is not clear how the PI will continue with the proposed experiments in Aim 2.

6. The PI is highly talented and has broad knowledge in computational aspects of the study; however, he appears to have limited experience with lung fibrosis.

Response:

Reviewer 1:

1. A comparative performance of new and existing methods would be difficult, as there is no gold standard dataset to be used for the evaluation. However, a project has been recently initiated in collaboration with the lab of David Kreil, PhD, Boku University, Austria, to use The Cancer Genome Atlas (TCGA) data and perturbated datsets to evaluate all existing algorithms, including mirConnX.

2. The ideas used to develop mirConnX are the ones on which we based our analysis for the identification of feed-forward loops related to epithelial to mesenchymal transition (EMT). The results of this analysis are now published in Pandit, Corcoran, *et al.*, *Am J Respir Crit Care Med*, (2010), 182:220-229. PMID: 20395557.

Reviewer 2:

1. In addition to the publication listed above, the work described in our final report was published in this paper:

G.T. Huang, C. Athanassiou, P.V. Benos. mirConnX: condition-specific mRNA-microRNA network integrator. *Nucl Acids Res*, (2011), 39:W416-423. PMID: 21558324

Regrettably, neither publication acknowledged CURE funding.

2. We do not entirely understand what the reviewer meant in this particular critique. In general, we agree that biochemical validation of computational predictions is always needed. However, this part of our project focused on the development of computational methods and tools and their application to idiopathic pulmonary fibrosis (IPF)-related datasets. There was no validation included in the original research plan, nor funds to support these types of experiments. We note, however, that some validation data are published in our 2010 paper (Pandit, Corcoran, et al).

3. Please see response above and the aforementioned publication.

Reviewer 3:

1. A549 are adenocarcinoma lung cells that, when untreated, maintain their epithelial phenotype. If, however, the cells are treated with TGF-beta, they become mesenchymal. The epithelial or mesenchymal nature of the cells is defined by certain protein markers, as we show in the Pandit, Corcoran, et al., 2010 publication.

2. The chromatin immunoprecipitation (ChIP) assays we refer to are those with antibodies against members of the AGO family. These are the proteins that carry miRNAs to their corresponding targets.

3. Please see the two publications listed above.

4. Although we do not know for certain at which passage the cells were, experiments by our collaborator (Naftali Kaminski, MD) had all of the necessary controls, which are detailed in the Pandit, Corcoran, *et al.* publication.

5. It is true that Aim 2 proceeds in uncharted territory. There are many factors that could influence the progress of IPF, a disease of which we are only now beginning to understand the mechanisms. The computational analysis will go hand-in-hand with the biochemical experimentation that will be conducted with our long-term collaborator, Dr. Kaminski, an international expert in IPF. Our previous record and publications show we can work together with alternative strategies, if our main strategy should fail.

   Examples of previous collaborative publications include the two papers we mention above as well as the following:

   D.L. Corcoran, K.V. Pandit, B. Gordon, A. Bhattacharjee, N. Kaminski, P.V. Benos. Features of mammalian microRNA promoters emerge from polymerase II chromatin immunoprecipitation data. *PLoS One* (2009), 4:e5279. PMID: 19390574

   A.S. Bais, N. Kaminski, P.V. Benos. Finding subtypes of transcription factor motif pairs with distinct regulatory roles. *Nucleic Acids Res*, (2011), 39:e76. PMID: 21486752

   Y. Zhang, D. Handley, T. Kaplan, H. Yu, A.S. Bais, T. Richards, K.V. Pandit, P.V. Benos, N. Friedman, O. Eickelberg, N. Kaminski. High throughput determination of TGFβ1/SMAD3 targets in A549 lung epithelial cells. *PLoS One*, (2011), 6:e20319. PMID: 21625455

6. We thank the reviewer for the kind words. We agree that Dr. Benos has a lack of experience in lung fibrosis research; however, the computational development in this study was conducted in collaboration with Dr. Kaminski, an international expert in lung fibrosis.

C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.

Response: This project received a favorable rating.

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: None.

Project Number: 0864104
Project Title: Modeling the Interactions of Dual Specificity Phosphatases with Inhibitors
Investigator: Bahar, Ivet

B. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in Section B using the following format. As you prepare your response please be aware that the Final Performance Review Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

<u>Reviewer 1:</u>

1. No peer-reviewed manuscripts or grant applications were submitted on the project. Publication of the results is important.

2. Aim 2 was not directly addressed in the work undertaken. Whether the current methodology is insufficient to address the aim of estimating entropic contributions to binding could be addressed, as well as how binding energies can be predicted most accurately for this system.

3. Methods employed should be well validated for their ability to address the investigators' specific questions. Validation is needed for others to be convinced by the results.

<u>Reviewer 2:</u>

1. Validation of normal mode predictions by comparison with experimental/computational measurements of protein dynamics and changes upon complex formation is needed.

2. Correlations are not necessarily causal. The Bahar group excels at data mining and finding correlations. These are not necessarily causal, but they can be used to form hypotheses which must then be tested experimentally. Here, the Bahar group should forge stronger collaborations with experimental spectroscopists to test the hypotheses that could be generated.

3. This project represents a very naive approach to the complexity of binding interactions, particularly the entropy of binding. Displacement of a bond water can be a significant contributor to the free energy of binding of an inhibitor.

4. Considerable experimental data from isothermal titration calorimetry for series of inhibitors binding to therapeutic targets exists in the literature. These would be an excellent source with which to validate the approach.

<u>Reviewer 3:</u>

1. This is an outstanding use of formula grant funds. The science is important, the training for young scientists in this laboratory is superb, and the success attained is unusual for formula grants of this type. I would recommend a continuation of funding for this laboratory.

Response:

Reviewer 1:

1. We recently submitted two manuscripts to peer-reviewed journals. These publications acknowledge CURE funding.

Title: Structural features for allosteric inhibition of DUSP6 identified by molecular dynamics simulations and in vivo SAR studies
Authors: Vasiliy N Korotchenko, Manush Saydmohammed, Vollmer L Laura, Ahmet Bakan, Kyle Sheetz, Karl T Debiec, Kristina A Greene, Christine S Agliori, Greg Gibson, Simon C Watkins, Ivet Bahar, Billy W Day, Andreas Vogt, and Michael Tsang
Journal: Chemistry & Biology
Submitted: March 1, 2012; under revision

Title: Druggability Assessment of Allosteric Proteins by Dynamics Simulations in the Presence of Probe Molecules
Authors: Ahmet Bakan, Neysa Nevins, Ami S. Lakdawala, and Ivet Bahar
Journal: Journal of Chemical Theory and Computation
Submitted: February 8, 2012; accepted with revisions on April 2, 2012

We have made significant progress in development of dual-specificity phosphatase (DUSP) inhibitors and establishment of DUSP druggability and submitted two refereed journal articles. In particular, we have established a structure-activity-relationship for DUSP6 inhibitor (E)-2-benzylidene-3-(cyclohexylamino)-2,3-dihydro-1H-inden-1-one (BCI) by improving our model and design and synthesis of new analogs. The interactions of BCI at the DUSP6 allosteric pocket were refined using the Poisson-Boltzmann Surface Area (PBSA) scoring method implemented in the docking program FRED (OpenEye, Santa Fe, NM). This method incorporates electrostatic interactions and desolvation effects that play important roles in ligand binding to polar sites. The new approach identified key hydrogen-bonding interactions (Fig. 1A) that were not observed in the previous model. The new model was used to design 31 analogs, which were synthesized and tested in zebrafish and cell-based assays. Weakening of the predicted hydrogen bond between BCI and Arg293 (Fig. 1A) by reduction of the carbonyl oxygen (Fig. 1B) abolished the activity of the compound in support of the new model. The new series of compounds also included analogs that lacked whole organism toxicity while retaining the activity comparable to that of BCI. These results reinforce the notion that DUSPs are druggable through allosteric mechanisms. A manuscript is submitted to the journal *Chemistry & Biology*. The manuscript also contains results from molecular dynamics (MD) and druggability simulations of DUSP6. The motions observed in the simulations are in accord with previously presented dynamics of DUSP6, modeled using elastic network models (ENMs). The druggability method, on the other hand, is also presented in a manuscript submitted to the *Journal of Chemical Theory and Computation*.

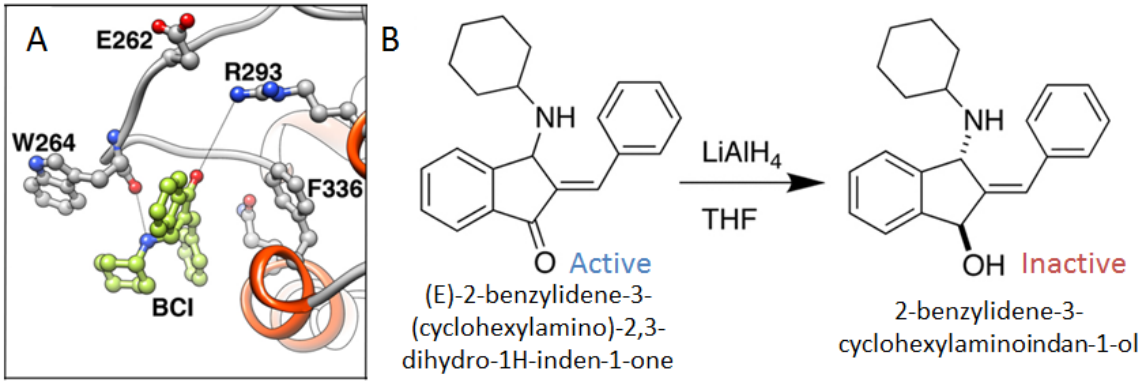

Figure 1. Key DUSP6-inhibitor interactions and their experimental interrogation. A. BCI docking pose that was used to guide analog design studies is shown. The pose was prioritized using PBSA scoring and predicts hydrogen bonding interactions between BCI and Arg293 side-chain and Trp293 backbone carbonyl. **B.** The improved model was tested by making analogs that would weaken key interactions. The reduction of BCI carbonyl oxygen abolished the activity of BCI in support of the new docking mode.

2. We have made progress in aim 2 toward understanding the contributions of different factors to the binding of DUSP inhibitors. We have investigated different methods for scoring inhibitor interactions. By using the PBSA method, we have improved our models and established a structure-activity relationship for a DUSP6 analog series. Our work on delineating entropic and enthalpic contributions to binding free energy will continue and will also include dynamics simulations to complement our proposed strategy. The results will be published as they are available.

3. The published papers present experimental support for interaction models and validation for new methods.

<u>Reviewer 2</u>:

1. We have performed MD simulations and observed that the motions predicted by normal mode analysis are also present in long-scale unbiased MD simulations, validating the approach we have taken. Part of the simulation results are presented in the *Chemistry & Biology* manuscript. We plan to submit an extended discussion and comparative evaluation of these results for dynamics and druggability of DUSPs in another manuscript.

2. The motions that we modeled for DUSPs have been known to be accessible to the DUSP6 and are indirectly supported by our *in vitro* experiments. As our primary focus is discovery of DUSP inhibitors and development and improvement of interaction models, we have not used our efforts to study DUSP dynamics using experimental approaches. However, we collaborate with experimental groups for validation of our predictions for membrane proteins, such as receptors and transformers, for which prediction of such motions have a higher impact.
3. The new method we have developed for druggability calculations incorporates explicit solvent molecules and accounts for entropic and dynamics effects in binding free energy

calculations. The approach has been extensively validated and results have been submitted to *Journal of Chemical Theory and Computation*. We have also started using simulation methods for studying inhibitor binding with the PBSA method, which accounts for the desolvation effect in binding.

Reviewer 3:
We thank the reviewer for his/her encouraging words.

C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.

Response: This project received a favorable rating.

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: We thank the reviewers for their insightful comments. We have addressed the issues they have raised as discussed above and have submitted new papers presenting validated methods and results supported by experiments.