

Pennsylvania Department of Health Final Performance Summary Report Formula Grants

Overview of the Health Research Project Performance Review Process and Criteria

An applicant that receives a health research grant under Tobacco Settlement Act / Act 77 of 2001, Chapter 9, is subject to a performance review by the Department of Health upon completion of the research project. The performance review is based on requirements specified by Act 77 and criteria developed by the Department in consultation with the Health Research Advisory Committee.

As part of the performance review process, each research project contained in a grant is reviewed by at least three experts who are physicians, scientists or researchers. Reviewers are from the same or similar discipline as the research grant/project under review and are not from Pennsylvania. Reviewers use the applicant's proposed research plan (strategic plan), the annual progress report and final progress reports to conduct the review. A grant that receives an unfavorable performance review by the Department may be subject to a reduction in funding or become ineligible for health research funding in the future. The overall grant evaluation rating is based on the ratings for the individual research projects contained in the grant.

This performance review report contains the outcome of the review for the grant as a whole (outstanding, favorable, or unfavorable), strengths and weaknesses of each research project, as well as recommendations for future improvement.

The following criteria were applied to information submitted by research grant recipients:

- **Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?**
 - Did the project meet the stated objectives?
 - Were the research design and methods adequate in light of the project objectives?
 - Consider these questions about data and empirical results: Were the data developed sufficiently to answer the research questions posed? Were the data developed in line with the original research protocol?
 - If changes were made to the research protocol, was an explanation given, and, if so, is it reasonable?
 - Consider (only for clinical research projects) the extent of laboratory and clinical activities initiated and completed and the number of subjects relative to the target goal.
 - Were sufficient data and information provided to indicate or support the fact that the project met its objectives or made acceptable progress?
 - Were the data and information provided applicable to the project objectives listed in the strategic research plan?

- **Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?**
 - What is the significance of this project for improving health?
 - Consider the value of the research completed towards eventual improvement in health outcomes.
 - Consider any changes in risk factors, services provided, incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of impact and effectiveness of the research being conducted.
 - Consider any major discoveries, new drugs and new approaches for prevention, diagnosis and treatment, which are attributable to the completed research project.
 - What are the future plans for this research project?

- **Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?**
 - If leveraging of funds were expected, did these materialize?
 - Are the researchers planning to apply for additional funding in the future to continue or expand the research?

- **Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted/filed?**
 - If any of the above listed were expected, did these materialize?
 - Are the researchers planning to submit articles to peer-reviewed publications, file for any licenses, or patents or begin any commercial development opportunities in the future?
 - Consider the number/quality of each.

- **Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?**
 - Were there improvements made to infrastructure?
 - Were any new investigators added or were any researchers brought into the institution to help carry out this research?
 - Were funds used to pay for research performed by pre- or post-doctoral students?

- **Criterion 6 - Did the project lead to collaboration with research partners outside the institution, or new involvement with the community?**
 - Are the researchers planning to begin any collaborations as a result of the research?
 - For clinical research only: consider the number of hospitals and health care professionals involved and the extent of penetration of the studies throughout the region or the Commonwealth.

Overall Evaluation Rating

An overall evaluation rating is assigned to each research project. The rating reflects the overall progress the project attained in meeting the stated goals and objectives. The rating is based on a scale of 1–3, with 1 being the highest. An average rating is obtained from all the reviews (minimum of 3) of each project and is the basis for the determination of the final overall rating for each project as follows:

1.00 – 1.33 = *Outstanding*

1.34 – 2.66 = *Favorable*

2.67 – 3.00 = *Unfavorable*

The grant level rating is an average rating from all projects as above. The numerical rating appears in parentheses for the grant and each project in the ***Overall Grant Performance Review Rating*** section of the report.

Overall Grant Performance Review Rating

Grant Rating: Favorable (1.75)

Project Rating:

Project	Title	Average Score
0864101	Computational and Experimental Approaches for Predicting miRNAs and their Targets	Favorable (2.00)
0864102	High-Throughput Estimation and Modeling of Protein Filament Distributions from Microscope Images	Outstanding (1.33)
0864103	Computational Modeling of miRNA Involvement in Pulmonary Gene Networks	Favorable (2.00)
0864104	Modeling the Interactions of Dual Specificity Phosphatases with Inhibitors	Favorable (1.67)

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths:

The investigators have made substantial progress on Aim 1 of the grant, which includes 1) using a previously developed algorithm (HHMMiR) to search the sea urchin genome for miRNAs containing stem-loops; 2) validating these predicted miRNAs using transcriptome data; 3) developing a computational extension to HHMMiR (HESD-HMM) to better enable decoding of the miRNA portion of the hairpin loop; 4) using Northern blots to verify presence of mature miRNAs in the sea urchin; and 5) using Illumina sequencing of small RNA libraries in combination with a computational pipeline to verify predicted miRNAs and potentially discover novel miRNAs. Thirteen potentially novel miRNAs were discovered using the miRDeep algorithm as a result.

The progress on Aim 2 of the grant seems to be less substantial, but the preliminary results, based on the proposed boosting algorithm, are exciting, especially concerning the drastic reduction in false positive rates that seem to plague the currently available algorithms.

Weaknesses:

The benefit from the HESD-HMM algorithm is not clear. The listed specificity and sensitivity rates are not an improvement over those mentioned for HHMMiR. The investigators should be more clear on how the performance of the HESD-HMM algorithm was evaluated and more specific on the improvements relative to HHMMiR.

The boosting algorithm for predicting miRNA targets seems to offer substantial improvements over existing algorithms. However, the details concerning the implementation of the algorithm are entirely omitted.

Reviewer 2:

The specific aims of this project were to (1) develop and test algorithms to identify miRNAs from genome sequences and (2) develop and test software tools for predicting miRNA targets.

With regard to Aim 1, the researchers had previously developed an algorithm (HHMMiR) and applied it to (1) whole genome sea urchin, (2) 55k transcriptome regions, and (3) Illumina sequencing data. The only development that they made was to enable HHMMiR to decode the miRNA portion of a predicted hairpin. This development was introduced in their specific aims as "this task should not be difficult;" therefore, I feel as though this objective is not a major advancement to their already existing algorithm.

The authors do not conduct any cross algorithm analysis to see how their algorithm compares for detecting sea urchin microRNAs in comparison to the growing body of existing programs.

The authors should expand in their Final Report how they achieved their sensitivity and specificity estimates. Furthermore, the researchers do not do an adequate job explaining how to account for the fact that only 16 of ~4.5 million single-loop hairpins align with known miRNAs. Can they confirm how many were missed, or how many of these are false positives?

It is also not clear why the authors don't merely conduct homology searches for each of the 55k transcriptome regions to miRBase directly since they do not show any candidates from their program are not confirmed by a homology search to known miRNAs.

For Aim 1, it would have been more appropriate for the researchers to propose "the identification and confirmation of sea urchin miRNAs" as they spent more effort experimentally confirming candidate miRNAs rather than developing and evaluating computational programs for miRNA detection.

For Aim 2, the researchers initially propose to explore TarBase to comprehensively explore existing target prediction tools for the purpose of reducing false positives. In the Annual Review, the authors do not discuss TarBase but instead use immunoprecipitation data from *C. elegans*. There isn't a solid justification for this substitution, or why they didn't use both IP and TarBase to test their methods. The results are also brief as they didn't explore the variability introduced by using different combinations of programs or within their own supervised learning methods. Furthermore, while there was a great deal of validation on sea urchin miRNAs, the researchers started off the target prediction portion of the grant with the statement "the ultimate goal of this project is to make better predictions." Yet they have sparse data and no experimental validation to determine the accuracy or improvements to their ability to predict targets.

Reviewer 3:

The project has two stated aims: developing methods to predict microRNAs in sea urchin, and developing methods to predict miRNA targets. For Aim 1, the applicants improved the previously developed hierarchical HMM method and have adjusted their experimental strategy by using deep sequencing data instead of old tiling array data. The applicant mentioned a manuscript in preparation.

For Aim 2, the applicants described a statistical boosting method that combine predictions from a number of existing prediction algorithms. They tested this in *c. elegans* and showed that such an approach out performs single method; however, the applicant did not test this method in sea urchins.

It appears that the proposed objectives were too ambitious for a project that was only 1.5 years long. Predicting microRNA genes and their targets as challenging, the authors have made progress, especially on Aim 1 since they already had software developed for HHMMiR in the beginning. However, without a manuscript published, it is hard to judge. The same is true for Aim 2. The applicants made progress, but no papers were published or software released.

My conclusion is that the applicants have made sufficient progress, given the level of funding and the length of funding period.

The proposed future research appears optimistic. The data generated and methods developed from this grant have provided a foundation to further expand the investigation of microRNAs in sea urchins.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths:

- 1) The computational pipeline for verifying and discovering new miRNAs can be applied by investigators to other genomes.
- 2) The boosting algorithm for predicting targets of miRNAs offers substantial improvements in false positive rates compared to existing algorithms and will offer substantial benefits to investigators interested in this area.
- 3) The principal investigator (PI) plans to continue and extend her research on miRNAs through an NSF funded grant.

Weaknesses:

None.

Reviewer 2:

MicroRNAs are a growing area of research, and their impact on human health is of growing importance as we learn more about this class of non-coding RNAs. As such, understanding and identifying miRNAs and also accurately predicting miRNA:mRNA interactions will be important. As such, I think this proposal could have a significant contribution.

One of the weaknesses of this project was that they focused on sea urchins, which can serve as a valuable model organism; however, they failed to translate how their approaches and findings can improve health.

Reviewer 3:

Sea urchin is a model organism for chordate development, which has its advantages over other systems such as mice or drosophila. The knowledge of microRNA regulation in sea urchin development will provide insight on the development process of humans.

In the future plan, the applicants proposed to further improve the miRNA prediction and target

prediction algorithms. A more obvious direction is to experimentally validate these microRNA targets using reporter assay or perturbation followed by expression profiling, which would validate individual targets and give a global first-pass of all the genes influenced by particular microRNAs. The applicants did not propose these experiments in the proposal, presumably because these were included in their NSF proposal.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths:

The PI has obtained additional funding from the NSF (\$500K) to continue and expand the research.

Weaknesses:

None.

Reviewer 2:

This project was able to leverage additional funds from the NSF. They do not plan to apply for any additional funds.

Reviewer 3:

The applicant has secured an NSF grant one year after this grant started. The applicant is not applying for additional funds.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths:

The PI listed two publications (one experimental and one computational) as end products from the work supported in part by this grant. Though neither publication was submitted, the total duration of the project was only 1.5 years, and the experimental paper seems to be near completion.

Weaknesses:

The computational paper concerning miRNA target prediction is still in development and has not progressed as far as the experimental paper concerning miRNA predictions.

Reviewer 2:

The authors are planning on submitting a publication, although they haven't yet, and do not indicate the targeted journal.

There are no new drugs, prevention diagnosis, or treatment advances. There are no patents or

licenses.

Reviewer 3:

A major weakness of the project is that no publications were generated, though it was mentioned two were being prepared. No software was released.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths:

- 1) The funds were used to support a graduate student who is enrolled in a joint Ph.D. program in computational biology, involving both the University of Pittsburgh and Carnegie Mellon University.
- 2) The performed research served as preliminary data for a funded NSF grant.

Weaknesses:

None.

Reviewer 2:

A new collaboration was made with Dr. Takis Benos, and they initiated an outreach program.

Reviewer 3:

The project has no equipment funding; therefore, it did not enhance the infrastructure of the institutions.

Collaborations were established with Dr. Benos at the University of Pittsburgh, and one graduate student was trained in the project.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The research involved a joint collaboration between the University of Pittsburgh (Dr. Benos) and Carnegie Mellon University (Dr. Hinman), who served as co-PIs.

Reviewer 2:

None.

Reviewer 3:

The collaborator, Dr. Benos, is from the University of Pittsburgh.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

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.

Reviewer 2:

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 are important to human health.

Reviewer 3:

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.

ADDITIONAL COMMENTS

Reviewer 2:

The research failed to exhaustively compare algorithms to detect miRNAs or make major developments to their existing program. A good deal of effort was spent to identify known and novel microRNAs rather than methods to improve algorithms. Additionally, for spending the time confirming miRNAs, their impact on human health was not made clear.

Aim 2 was extremely sparse with regards to methods, data, and analysis. They used a different data set than initially planned without much explanation. While they stress the importance of predicting miRNA targets, they have compared approaches, explored the variability in combined different methods, learned how to optimize their own approach, or any discoveries that were made were not previously known.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The goal of this project was to develop a software tool that could be used to extract the spatial distribution of the filamentous protein network directly from fluorescence microscopy images. The final outcome met the specific aims of (i) devising methods for quantitative information extraction directly from standard microscopy images in a manner that is amenable to high throughput studies; and (ii) modeling and simulation approaches that will capture the cell-level essential characteristics for protein filament distributions.

At the time of the Final Progress Report, the data were not developed sufficiently to directly answer the research questions. However, the grant is to allow the team to generate pilot studies in order to leverage for future funding. This has been accomplished. The progress made is good.

Reviewer 2:

The project did meet its stated objectives, for the most part. For Aim 1, the investigators have shown the ability to model test data reasonably well. Filament distributions were created, analyzed, and reproduced with moderate to good quality of match to the statistical distributions of features such as number of microtubules, microtubule length distribution, colinearity, etc. Also, a fluorescence image of alpha tubulin in a HeLa cell was analyzed, modeled, and reproduced with reasonably good visual correspondence, though the gathering of tubules into a hub was not reproduced well. There could be an inherent problem in reproducing any single cell image very well when the modeling is aimed at reproducing distributions of feature values across many cells. Equivalently, it is hard to match an individual if the method is designed to match an average over individuals. A paper was published on this work in *Cytometry Part A*.

For Aim 2, the wording was rather confusing, as was true of much of the initial proposal, making it hard to envision what to expect from the work, or what its ultimate value might be to the medical community. However, by demonstrating the ability to model the HeLa microtubule distribution reasonably well, and by publishing their first results, they have definitely gotten a challenging project off the ground. This represents a significant accomplishment during the 18-month time frame of the grant.

Reviewer 3:

Two objectives were mentioned:

1. Development of quantitative methods for characterization of intracellular filament distributions by fluorescence microscopy. Based on the included PDF from the journal *Cytometry Part A*, this objective appears to have been successfully completed using a Bayesian approach.
2. Development of accurate network models to predict cell behavior. As this objective depends on completion of the first objective, it is still incomplete.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The likely beneficial impact of this project that can be directly translated into eventual improvement in health outcomes is minimal. The ability of the team to turn their modeling and simulation approaches is still a long way from being able to accurately predict any cellular event. While the beneficial impact is deemed to be minimal, it is judged to be reasonable in light of the dollars budgeted. More importantly, the bridge fund allowed the team to secure an R01 award from the National Institutes of Health (NIH) to further take one step closer to fruition.

Reviewer 2:

The significance to human health is that the morphology of protein filament networks in human cells, and the remodeling of them, is sensitive to disease states, and in some cases is sensitive to drug therapies. The benefit of developing a model or assay tool that can assess how normal or abnormal the microfilament properties are in a population of cells (one way to describe this research) has not been spelled out in any detail. For instance, if this tool were available now in hospitals and research labs, how would it advance science and medicine? This would be valuable information for the investigators to provide. Certainly, the ability to model this kind of complex morphology is a scientific advancement with potential applications to other image/pattern recognition and cytological questions. In upcoming research, the researchers plan to apply this methodology to specific cell types under different physiological conditions, as well as to organelles.

Reviewer 3:

The tool developed has multiple applications in cell biology. In particular, drugs used in oncology often target tubulin whose functional state as microtubules can be characterized by this technology. The technology should prove useful in high-throughput screens for anticancer agents.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The researchers successfully leveraged an R01 grant from the NIH. Additional funding in the future is also planned.

Reviewer 2:

Funds were leveraged via a new NIH R01 grant for this project. \$800,000 grant was funded by NIH to continue this project which indicates significant support and enthusiasm on the part of NIH for the project and should enable significant work to be accomplished.

Reviewer 3:

A significant NIH grant has been funded based on the preliminary results obtained in this project.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project resulted in a peer-reviewed publication in *Cytometry Part A*. The publication is of acceptable quality. More publications are planned.

Reviewer 2:

A good-quality paper by the investigators was published on the work described, as intended by the milestones they set. A second manuscript on cells treated with the drug Nocodazole was planned for submission in late 2010 (after the Final Progress Report was submitted).

Reviewer 3:

One peer-reviewed publication has been published.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:

There were no additional improvements made to the institutional infrastructure, nor was there any new investigators added to the project. The funds were used to pay for research performed by pre- and post-doctoral students.

Reviewer 2:

A jointly-mentored graduate student, Aabid Shariff, was a key investigator in performing this research, as indicated by his first authorship on the *Cytometry Part A* paper. The new NIH

funding enabled by this seed funding of the project will bring in substantial support \$800,000 to the institution, thus enhancing the quality and capacity for future research.

Reviewer 3:

Whether the project enhanced the quality and capacity for research at the grantee's institution is not evident from report.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project did not lead to collaboration with research partners outside of the institution, and there is no plan to do so in the future.

Reviewer 2:

The project itself is collaborative and via the applications to different cell types is likely to connect with other medically-oriented researchers (though this was not an emphasis of the progress reports and future plans). The computational methods developed in this research will be shared with the community, which will be of benefit to others involved in cell image analysis.

Reviewer 3:

Whether the project led to collaboration with research partners outside of the institution, is not evident from the report.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

None.

Reviewer 2:

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?

Reviewer 3:

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.

Generic Recommendations for the Mellon Pitts Corporation

Reviewer 3:

Results on Aim 1 are impressive and have resulted in NIH funding. Aim 2 remains ill defined.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:

This project sought to evaluate existing computational models and develop new computational approaches to identify miRNA-mRNA regulatory relationships. A web server that instantiates the newly developed algorithm has been developed. A description of the performance of the new algorithm relative to existing methods has not been provided. The investigators highlight that their method predicts a role for miR-34a and miR-10b in downregulating STAT3 and ASXL1 in interstitial pulmonary fibrosis (IPF). However, the saliency of this result, with regard to the predictions made by the method or the potential relevance of this finding to IPF, are not discussed.

Reviewer 2:

This project has two aims directed at development of an algorithm to identify regulatory networks that incorporate miRNAs in addition to standard transcription factors and then apply this to analysis of high throughput data obtained from studies of lung epithelial cells stimulated with TGFbeta as a model of EMT in IPF. The proposed methods are routine and likely to yield the desired algorithm, although the outcome is uncertain with respect to the inclusion of miRNAs where the precise upstream regulatory region is not always clear. The applicant recognized this yet provided no evidence of feasibility with respect to overcoming this potential handicap. This is partly reflected in the relative lack of progress in attaining the stated objectives, and the lack of sufficient data and description to document progress. This is especially of concern in Specific Aim 2 wherein essentially no data are provided to indicate progress.

Reviewer 3:

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression. miRNAs play a major role in multiple basic biological processes such as cell differentiation, development, proliferation, and morphogenesis. Altered miRNAs have been associated with the pathogenesis of pulmonary fibrosis, cardiovascular diseases, cancer, and diabetes to name a few. However, the contribution of miRNA-mediated altered gene expression to various diseases is just beginning to unravel and is poorly understood at the moment. Therefore, the proposed project to develop a computational approach to study miRNA's involvement in complex eukaryotic gene

networks is timely and highly significant. Analysis of high-throughput data collected from lung epithelial cells after stimulation with profibrotic cytokine associated with idiopathic pulmonary fibrosis will certainly provide information regarding the contribution of miRNAs as a proof-of-principle.

Dr. Benos and colleagues proposed to extend existing algorithms that can model interactions between transcription factor genes and miRNA genes using publicly available datasets. In addition, they proposed to develop new algorithms to model gene regulatory networks that involve miRNA genes. Secondly, they will extend their positive findings derived from the first objectives using publicly available datasets to analyze data from lung epithelial cells exposed to TGF-beta to relate to idiopathic pulmonary fibrosis. At the end of the study, Dr. Benos and his research group expected to accomplish 1) a robust method for modeling transcription factor-miRNA interaction networks that contributes to gene expression particularly at the transcriptional level; and 2) high-confidence predictions of miRNA network involvement in pulmonary fibrosis as a proof-of-principle using lung epithelial cell lines responding profibrotic cytokine TGF-beta.

TGF-beta contributes to pulmonary fibrosis via epithelial mesenchymal transition. Thus identification of responsible network components will not only aid the understanding of the progression of pulmonary fibrosis, but will also allow development of preventive and therapeutic strategies by short listing the putative target genes. TGF- β induces SMAD transcription factor and down regulates HMG2A, a target gene of SMAD4 protein. HMG2A is a target of let-7 miRNA. Dr. Benos and his colleagues discovered that TGF-beta inhibits let-7d expression by 80%. These changes are associated with parallel induction of HMG2A. On the contrary, inhibition of let-7d induces HMG2A by 2-2.5 fold between 24-48 h.

These are all very interesting observations and probably contribute to the development of progressive lung diseases like idiopathic pulmonary fibrosis. Having said that, there are many shortcomings with regard to this study design; preliminary data is very poorly described, descriptions provided in the figure legend are incomplete and difficult to understand. For example, Figure 1A, it is not clear what is PCR amplified; in Figure 1B, it is not clear what is target DNA; in Figure 2B, it is not clear whether the cells are also treated with TGF- β after transfection with let-7d inhibitor or just let-7d inhibitor alone.

A549 cell is an adenocarcinoma cell, not a normal lung epithelial cell. There is no justification for the use of a lung carcinoma cell that has already undergone epithelial mesenchymal transition during the process of carcinogenesis. Therefore, it is very difficult to interpret the experimental outcome using A549 cells or the TGF-beta effect on epithelial mesenchymal transition. Another major problem with this study is Aim 2 is completely dependent on the outcome of Aim 1. There are no alternative experimental approaches for Aim 2 if the experiments proposed in Aim 1 failed to yield relevant information.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:

I have noticed that miRNA are emerging as important regulators of gene expression and the possibility of miRNA-based gene-therapy has excited many in the field. Methods to identify the regulatory networks that control complex cellular behaviors in health and disease could greatly improve our ability to develop novel therapeutic interventions that disrupt pathogenic processes. Methods for identifying regulatory networks that include the consequences of miRNA expression alterations are needed. While the project did not have any immediate impact on improving health, it is possible that applying the methods developed here to disease-related datasets could have a positive impact in the future.

Reviewer 2:

The significance of the project is only peripheral to the potential for providing insight into mechanisms important in IPF pathogenesis. The project's argument on this point ignores a body of literature raising important issues on the relevance of EMT to tissue fibrosis. Also, the primary focus of the project is on a theoretical and descriptive level with minimal likely impact on improvement of health.

Reviewer 3:

Idiopathic pulmonary fibrosis is a chronic, progressive, and often fatal pulmonary disease of unknown etiology. Long term survival of patients is very poor with a five year survival rate at a dismal 20%. Anti-inflammatory agents provide only symptomatic relief. Epithelial mesenchymal transition is a significant contributor to the development of idiopathic pulmonary fibrosis. There is no effective treatment available for idiopathic pulmonary fibrosis with lung transplantation being the only available option in more severe cases. The outcome of the proposed study could provide information related to the development and progression of idiopathic pulmonary fibrosis; therefore, the study is highly significant. Having said that, this project is a very high-risk study that may not yield any positive results; however, benefits outweigh risks. Secondly, the PI has been highly productive in his chosen field of research. Besides transcription, mammalian gene expression is controlled at multiple steps after transcription, and miRNAs significantly contribute to post-transcriptional events.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The development of the web server was funded by other unidentified sources. The project leveraged primary data that was generated in collaborator Kaminski's lab through other funding. The investigators state that this method is proven to be useful, and they will apply for additional

funding (together with collaborator Kaminski) to further develop the method and collect and analyze data about IPF-related regulatory networks.

Reviewer 2:

No grant applications have been submitted as a result of this project. However the applicant indicated a plan to do this in the future in order to expand on the utility of any developed algorithm.

Reviewer 3:

The research team has not obtained funding from other sources to expand the proposed research; however, they plan to apply.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Neither of the publications anticipated in the proposal has been submitted, though the investigators are now expecting that the project will lead to three future publications. No patent or commercialization activities are currently envisioned by the investigators.

Reviewer 2:

Manuscripts are planned that would include some of the findings from this project. There is insufficient information provided to adequately judge the potential quality of such manuscripts.

Reviewer 3:

The PI has co-authored a publication related to let-7d in pulmonary fibrosis, which is accepted for publication. The PI is planning to publish a few more.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project funded research by one pre-doctoral student in the investigator's lab.

Reviewer 2:

The major strength here lies in the training of a graduate student in the computational biology program at the grantee's institution. Additionally, some of the activity might have contributed to the research in the collaborators' laboratories.

Reviewer 3:

New hiring was made from the funds provided.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:

No collaborations were planned or are presently being contemplated.

Reviewer 2:

The indicated collaboration was only within the applicant's institution.

Reviewer 3:

Research collaboration has been reported or is planned.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

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.

Reviewer 2:

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.

Reviewer 3:

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 inroad. 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.

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

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project has made significant progress towards understanding the flexibility and druggability of an allosteric binding site on one of the targets, DUSP6, addressing Aim 1. Their approach to Aim 2, addressing the entropic contribution to binding free energy using elastic network models, has substantially changed. The authors instead used molecular dynamics simulations (without providing information on the method used) to address flexibility in the allosteric site, and have not directly addressed the question of entropic contributions to binding energy. More questionable is the calculation of contributions of individual residues to binding free energy (under Aim 2 in the Final Progress Report), with the assumption that adding a series of interaction energies (for which the calculation again was not explained) can result in accurate predictions of a very strong binding affinity.

Most existing methods like those presented in the CSAR session of the fall 2010 American Chemical Society meeting, have average errors of 2 kcal/mol for predicting binding affinity values in protein-ligand complexes. Therefore, accurate prediction of a binding affinity of 0.2nM by adding a series of large, and probably inaccurate terms of magnitude, -2 to -5 kcal/mol, is hard to believe. The methods employed need to be detailed and validated. And while the methods used in the work substantially changed relative to what was proposed, more of an issue is whether the methods now chosen can answer the detailed questions the authors would like to answer. This can only be established by validation, which has not been demonstrated in the progress reports. For instance, the docking tool GOLD (initially proposed for the project), does a poor job of ranking inhibitor candidates using any of its scoring functions, which is freely admitted by those who distribute the software. It is also too slow to screen a large database of compounds. Whether AutoDock is sufficient for the purpose now needs to be addressed. Validation is even more important when so many different modeling techniques/software packages are being combined in a project. The research could benefit more from focusing in and thoroughly addressing one or two questions in terms of best approaches for addressing flexibility and binding affinity prediction for MUSP6, perhaps based on reproducing existing data for a homolog.

An additional point is that a claim made by the PI early in the grant proposal, that other groups had not considered both protein and ligand flexibility for docking and drug discovery, is not true. Many groups have modeled series of protein snapshots using a range of methods, and employed them with full ligand flexibility sampling for docking and screening. Several of these methods are available to the public community and widely used. Their work should be fully acknowledged and cited here.

The milestone for the grant was completing half of Aims 1 and 2 and submitting a paper for publication. In fact, progress was made towards Aim 1, though on a different enzyme from the two that were originally proposed. It is not clear that Aim 2 was actually pursued in a serious way. A publication was provided by the authors relating to metal binding sites, and it is a nice contribution; however, it did not relate to either aim in this proposal. Two manuscript submissions on this project were anticipated at the end of 2010.

Reviewer 2:

The objectives of the project are compelling. The dynamics of macromolecular systems and changes that occur upon complex formation are formidable problems that limit current efforts to predict affinity and specificity. The use of elastic network modeling to address this issue is not novel, nor restricted to the Bahar group (although the citations in the report and Bahar's papers might lead one to a different conclusion). In terms of publications, the group has been productive. The underlying approach, however, suffers from a fundamental limitation—no validation of the normal modes derived by elastic network modeling by experimental, or even MD simulations, is presented. Instead, most of the publications utilize crystal structure data from the PDB without even considering the lack of dynamical information (except for B-factors).

The other collaborations with the Drug Discovery Institute on virtual screening uses commercial software of limited value. One measure of accuracy of the methodology is the percentage of compounds that do not have the predicted activity when tested in the biological system of interest. Normally, using virtual screening, the percentage is 75-80%.

Reviewer 3:

Professor Bahar and his students framed out achievable goals and met them during this project. The overall goal, namely to develop and apply an algorithm to computationally assess peptide surfaces of phosphatases distant from the active site as potential docking surfaces for small molecule inhibitors, was met. A region of MKP-1 was identified as such a surface for drug discovery and design. Overall, this project was an exceptional use of formula funding and demonstrates how valuable such funds can be in extending the reach of a committed laboratory.

Strengths:

A major strength of this project is the importance of the concept as an organizing principle driving new drug discovery. The historical problem with the design of active site ligands against kinase targets has been a cross reaction among the kinase family members, leading to significant off-target toxicities. The same problem applies to design of phosphatase inhibitors. The approach used in this study circumvents the issue because the surfaces away from the active site have a much lower degree of homology between/among family members.

Another major strength of the project has been the commitment to maintain the Molecular mechanics simulations long enough to capture the full range of motion of the domains of the protein, and the skills of the research team in interpreting the flexibility of the loops in MKP-1.

The data brought to bear on the aims of this project were completely germane to the original goals and also to new insights that arose during the progress of these simulations. This project had to adjust to the outcomes of the simulations, and the PI and his group did an admirable job in doing so.

Weaknesses:
None detected.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Discovery of allosteric inhibitors for dual specificity phosphatases will enhance the ability to treat a number of human diseases. The investigators continue to make progress in understanding the allosteric binding site on DUSP6, and the previously discovered allosteric inhibitor, which should facilitate the discovery and design of other compounds that inhibit DUSP6. The software that is being developed by the PI's lab will be shared with other research groups, which is a valuable contribution.

Reviewer 2:

The significance of this project for improving health is minimal in the near term. The focus is on lead discovery and the underlying physical chemistry of molecular interactions. The efficacy of university-based mini-drug companies is limited and often based on a naive understanding of the complexities of drug development and the FDA approval process. Academic groups provide the scientific basis for target selection for a given disease. Once the therapeutic target has been selected and validated, finding chemical leads by virtual screening and/or screening of compound libraries is usually trivial.

Reviewer 3:

Strengths:

The impact of this project is substantial and far-reaching. The computational methods used by the investigators and the degree of success in identifying a surface of the MKP-1 protein that was druggable are completely applicable to virtually any problem in drug discovery.

The value of the MKP-1 protein as a target for cancer therapeutics is still unknown, but the work done under the support of this funding will furnish starting chemical matter that should be sufficiently specific to allow testing of the original hypothesis.

Weaknesses:

No weaknesses were detected. This was a very well done formula grant.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:

An NIH grant proposal will be submitted in the future.

Reviewer 2:

It is not clear how much impact this project had on NIH grants obtained as the Bahar group only seemed to play a support role.

Reviewer 3:

The project did leverage additional funds. An NIH application was submitted as a competing renewal of a U19 grant that was aided by this funding. It was funded. The PI lists another grant submission for May 2010, but this application does not show up at this time as funded by the *Reporter* web site. Professor Bahar does have two other NIH grants currently funded that were undoubtedly aided by the availability of these funds.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:

There were no peer-reviewed publications relating to the project aims.

Reviewer 2:

Multiple peer-reviewed publications are a hallmark of the Bahar group. One publication (Dutta and Bahar, *Structure*, 2010) was included in the progress report.

Reviewer 3:

A manuscript was submitted, accepted and published in *Structure*. It is an excellent contribution in a high-impact journal, and it acknowledged the support of this funding.

It is noted that two other publications appear from this laboratory that were closely related to the theme of this project, one in *Proceedings of the National Academy of Science*, and one in *Nature Chemical Biology*. Both were related to the research completed under this support, and both were excellent. The PI does not claim them as resulting from this funding, but they clearly reflect the atmosphere of his laboratory during this funding period.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The PI's lab continues to make worthwhile contributions to structural modeling methods. The collaboration to test the results of those methods in the context of allosteric inhibitors of DUSP6 is likely to further improve the methodology as well as enhance the panel of inhibitor candidates.

Two graduate students were partially supported by the project funds. One of them worked on the metal-binding site project, and the other on the DUSP6 project.

Reviewer 2:

Collaboration with UPDDI is highly recommended as it provides a mechanism for predictions to be tested.

Reviewer 3:

The PI and his staff constitute a resource for chemoinformatics and computational chemistry at the University of Pittsburgh. This formula grant has been successfully used to advance the capabilities of his group in both of these areas; an expansion of his capabilities that will be very valuable to the university and to the substantial thrust for drug discovery at that institution.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:

New collaborations are not apparent in the progress report, though collaboration with the university's drug discovery institute is testing compounds as DUSP6 inhibitors.

Reviewer 2:

There is one indication of some collaboration with a major pharmaceutical company on one therapeutic application.

Reviewer 3:

The project has led to a useful collaboration with GlaxoSmithKline Pharmaceutical Company. There has been no involvement of the general community at this time.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

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.

Reviewer 2:

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 bound 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.

Reviewer 3:

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.

Generic Recommendations for the Mellon Pitts Corporation

Reviewer 2:

More is not better; adoption of software that has been proven inaccurate in affinity predictions (GOLD, for example) and adding another adjustable parameter related to elastic network modeling is not recommended. Dr. Bahar is effective in generating papers, pictures of plausible binding modes, and support for the UPDDI. From reading this report and his publications, he does not appear to be self-critical regarding the underlying physics and chemistry of molecular

recognition, and it places doubt his ability to provide the paradigm shift that affinity prediction requires.

Reviewer 3:

This type of work has a far-reaching impact that needs to be captured. The combination of this quality computational work, with equally high caliber and intuitive experimental scientists, are the forefront of academic drug development at this time.