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.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 24. Structural Studies of Tight Junction Structure Function and Regulation

7. **Start and End Date of Research Project:** 9/1/2010 - 12/31/2012

8. **Name of Principal Investigator for the Research Project:** John M. Flanagan, 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:

   $ 163,852

   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
Bewley	Co-Investigator	4.3%Y1	5678.60
Bewley	Co-Investigator	.38 %Y2	485.02
Griffin	Research Tech	5% Y1	3652.31
Griffin	Research Tech	8.9%Y2	6657.22
Tash	Post Doc	16.7%Y1	6812.07
Harner	Lab Attendant	7.4%	1667.82

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
Flanagan, John	Professor	10%
Tian, Fang	Assistant Prof	10%

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
SpectraMax Paradigm Multimode Microplate Plate Reader	Provided a platform to conduct assays that were required for this project and others in multiple departments.	$50,490
SP Scientific Slimline Laboratory Freeze Dryer	Allowed sample preparation for structural studies as well as a wide variety of other studies.	$25,395

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 to be awarded:
1 R01 GM094526 Structural Studies of Tight Junction Proteins	✓NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify: _____)	3/2012	$583,998/ first year	$447,016
14-PAF00283 Discovering Novel Atypical PKC Inhibitors as in vivo Chemical Probes	✓NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	11/2013	$1,546,694/ 3y	Awaiting awards statement.

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

Yes_____ No_____X_____

If yes, please describe your plans:

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

We are currently funded to a) continue our structural studies of tight junction function and regulation and b) to optimize a lead compound that targets PKCi, a key regulatory protein in TJ and a validated target for treatment of Diabetic retinopathy.

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				
Female	1	1		
Unknown				
Total	**1**	**1**		

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

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

The addition of critical equipment for high throughput screening and sample preparation has

improved infrastructure for a large number of projects.

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:

We collaborated with Kellogg Eye Institute at the University of Michigan (two collaborative RO1's, one located here at the Penn State University College of Medicine and the other at University of Michigan). We also collaborated with Dr. Alan Fanning at the University of North Carolina Medical School. These collaborations have allowed us to pair high quality structural studies with in vivo and in vitro cell biology to test specific molecular mechanisms of function.

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

Yes_____ No____X_____

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

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.

TJs form the blood-brain and blood-retinal barriers. Loss of barriers increases fluid and solute transport across the brain and retinal vasculature is associated with a number of disease pathologies including brain tumors, stroke, diabetic retinopathy and age-related macular degeneration. In many cases, the increased TJ permeability associated with disease is correlates with changes in Occ phosphorylation and/or membrane localization. We have shown that Occ participates in VEGF regulated TJ remodeling in diabetic retinopathy. However, little is known how this multidomain transmembrane protein functions in this capacity. In two specific aims, we describe experiments to determine the role of the transmembrane (TM) domain and its extracellular loops that appear to be required for TJ formation and regulation. These loops are the binding site for a number of proteins including TGFβ receptor, claudin-1 and JAM-4, and cellular pathogens including Coxsackie virus and HCV. Encouraged by our initial success in the expression of the transmembrane domains of Occ in *E. Coli*, we propose two additional specific aims that seek to understand mechanisms by which both intra- and extracellular signals affect Occ function and alter TJ properties. In these experiments, we will focus on the intracellular domains of Occ which interact with the TJ scaffolding protein ZO-1 and the ubiquitin E3 ligase Itch. The four aims are as follows: *First*, we will optimize the expression, purification and reconstitution conditions for the preparation of a homogeneous sample suitable for structural studies by NMR and X-Ray crystallography. Gel filtration, light scattering, CD and 2D ^{15}N-TROSY will be employed to characterize the preparations. *Second*, we will determine the topology of the Occ through proteolysis, mass spectroscopy in conjunction with H/D exchange and NMR. *Third*, we will use NMR spectroscopy and small angle X-ray scattering to determine the structure of the ZO-1/Occ complex. *Fourth*, we will determine how ZO-1 binding affect the ability of Itch to access and ubiquitinate the C-terminal region of Occludin. These aims represent two distinct but synergistic projects that will advance our knowledge of TJ structure and function.

1. We have identified and begun to characterize key *in vivo* Occ phosphorylation sites that result from VEGF treatment of BREC. These studies required us to develop a novel MS/bioinformatics approach to identify VEGF responsive phosphorylation sites in endogenously expressed Occ.

2. We have shown that VEGF stimulated phosphorylation of Occ S490 regulates TJ properties in BREC cells via an ubiquitin-dependent endosomal recycling pathway. This work is now recognized as a key piece of evidence supporting the hypothesis that Occ is a critical regulator of TJ properties and provides additional support that the ZO-1/Occ interaction is important for regulating TJ properties.

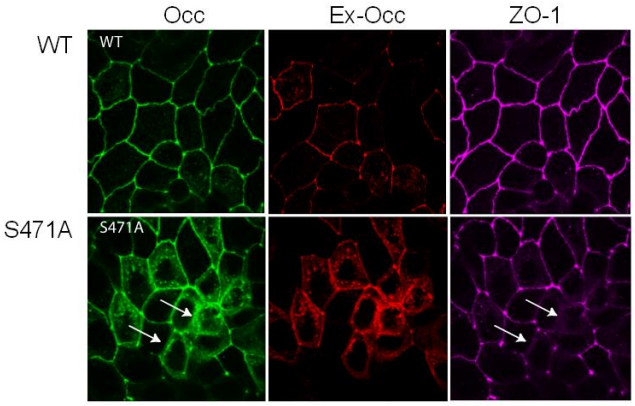


Fig 2. Immuncytochemistry of TJ proteins. Occ (green) and ZO-1 (purple) are organized at cell borders in cells expressing WT occludin (red). Expression of S471A leads to increased cytoplasmic staining and reduced border staining of Occ and ZO-1 (arrows).

3. We have demonstrated a unique role for Occ in mitosis. Specifically, Occ is localized at mitotic centrosomes and phosphorylation at S490 regulates centrosome separation and mitotic entry.

These data are consistent with a requirement for phosphorylation of Y474 in Occ in microtubule reorganization in wound healing.

4. We have demonstrated that S471 in Occ is required for assembly (or to prevent disassembly) of TJs *in vivo* and *in vitro*. Immunocytochemistry of paraformaldehyde fixed cells expressing either WT or S471A Occ reveal stark differences in junction organization. Endogenous Occ and ZO-1 are found at the cell borders as is exogenously expressed WT Occ (Ex-Occ). By contrast, a portion of S471A Occ is found at the cell border but a large fraction is mislocalized with ZO-1 to the cytoplasm (Fig 2, arrows). This is the only known single-site substitution in a TJ protein with this phenotype and *Occ-S471A is a unique tool for dissecting the role of Occ in TJ assembly/disassembly.*

5. We have shown that S471 acts as a central regulator of barrier properties. S471A and S471D mutants were created to prevent or mimic phosphorylation, respectively. A calcium switch assay, which measures the trans-epithelial electrical resistance (TER) was performed in Madine Darby canine kidney (MDCK) cells expressing these mutants or Wt Occ. Using the ECIS system (Fig 3) we observed a modest increase in resistance with overexpression of Occ, as previously observed. However, expression of S471A mutants led to a dramatic loss in the ion barrier. Even 7.5h after calcium repletion the S471A mutant expressing cells still failed to re-establish a tight junction while the WT cells reform the junction by 5h. Expression of S471D yielded resistance nearly identical to the parental line at the 7.5h time point (not shown). Further, the flux of a fluorescent solute tracer (TAMRA 467Da) was increased by 500-1000 fold in the S471A mutant expressing cell lines (not shown). These data suggest a dysregulation of small pore ion flux and increased broken junctions leading to solute flux in the cells overexpressing S471A mutant. To our knowledge there are no other examples of phosphorylation point mutants with such dramatic effect on junction formation.

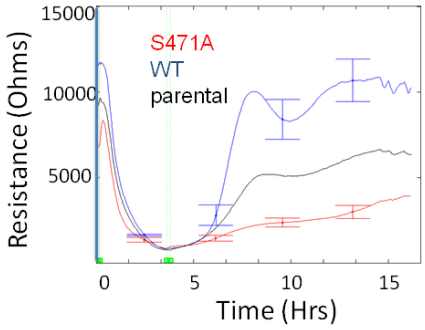


Fig 3. Expression of S471A reduces electrical resistance. Calcium switch assay reveals resistance across the monolayer as measured by ECIS was significantly reduced 7.5h after calcium repletion.

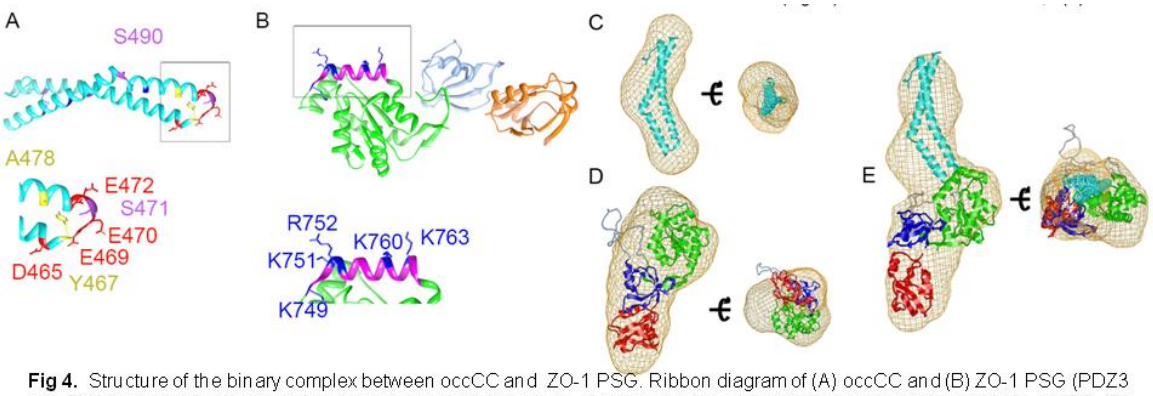


Fig 4. Structure of the binary complex between occCC and ZO-1 PSG. Ribbon diagram of (A) occCC and (B) ZO-1 PSG (PDZ3 red, SH3 blue and GuK green) showing key interfacial residues. Orthogonal views of *ab initio* envelope (beige) of (C) occCC, (D) ZO-1 PSG and (E) binary complex and rigid body fits of the crystal structures. In all cases, the χ^2 for the fits to the SAXS intensities was <1.

6. We have determined the first structure of the binary complex between ZO-1 PSG and occCC domains (Fig 4). ZO-1 PSG/occCCC complexes can be formed at concentrations necessary for structural studies. Data collection required investigator-led improvements to the sample-handling system at the SAXS beamline, now available for all users. The moderate resolution structure clearly identifies the region of interaction in each partner and resolves a long-standing question about the nature of the complex. The structure of the complex also provides a framework to interpret the role of phosphorylation in complex stability. (Published in PNAS).

7. We have shown that occCC competes with Calmodulin for binding to helix V of ZO-1 PSG by SAXS and *in vitro pulldown assays.*

8. We have developed and published a robust RDC-based structure assisted method for NMR resonance assignment in large proteins, including the ZO-1 PSG domain. This method solves the previously outstanding problem of coordinate error that prevented the use of modeled structures, or the structure of distant homologues, in RDC-based resonance assignment.

9. We have generated or obtained a large number of constructs that provide the necessary toolbox for our proposed studies. These include ZO-1 and Occ constructs, and a range of their cellular binding partners (Table 1).

Table 1. Protein Construct Toolbox.

Protein	Residues	Tags	Expression	Mutants	Comments
Occ	1-522	CHis	EC		Full-length
OccTM	48-242	CHis	EC		Marvel
occCtail	210-522	NHis/Gst/Biotin	EC		C-terminal Cytoplasmic
occCC+	382-522	NHis/Gst/Biotin	EC		Linker + coiled coil
occCC	420-522	NHis/Gst/Biotin	EC	Entire acidic loop	Coiled-coil
ZO1Δ889	1-888	NHis	BV		N-terminal half PDZ1-3 SH3GuK U6
ZO1Δ807	1-806	NHis	BV		No U6
7ΔZO1Δ807	8-806	NHis	BV		No N terminus
ZO1Δ641	1-640	NHis	BV		No GuK U6
ZO1Δ503	1-502	NHis	BV		No SH3 GuK U6
ZO1Δ301	1-300	NHis	BV		PDZ1 2
177ΔZO1Δ807	178-806	NHis	BV		PDZ2 PDZ3 SH3 GuK
417ΔZO1Δ888	418-888	NHis	BV		PDZ3 SH3 GuK U6
PSG	418-808	NHis	EC	K749D/R752D/K753E K760E/K763E	PDZ3 SH3 GuK
SG	503-808	NHis	EC		SH3 Guk
PDZ2	183-349	NHis	EC/BV		PDZ2
PDZ3	418-502	NHis	EC		PDZ3
Calmodulin	1-152	No-tag	EC		Binds ZO-1 PSG
PKCζ	1-592	NHis	BV	and N and C-term Δ constructs	Atypical PKC, phosphorylates Occ
TGFβ1R	15–130	N-His	EC		TGFβ1 Receptor
TGFβ2R	7–91	N-His	EC		TGFβ2 Receptor
p85-PI3K	1-724	N-His	BV		P85 subunit of PI3 kinase

10. We propose that additional sites of contact exist between the occCC and longer ZO-1 constructs. Although not observed directly, the structure of the complex suggests a role for the disordered U5 loop in PSG in Occ-binding. Interestingly, the TROSY NMR spectra of the PSG with/without occCC shows a large increase in resonances corresponding to “ordered” structure upon binding occludin (Specific Aim 2) that may be due to U5.

11. We have overexpressed and purified full length Occ and OccTM from an *E. coli* expression system and reconstituted it into nanoliposomal discs. Full-length Occ (Aim 3) opens the possibility of studying complexes that better mimic the *in vivo* situation.

Our collaboration has produced a validated structural/functional working model for the ZO-1 PSG occCC complex that reconciles all relevant biochemical data published by other groups. The structure was not anticipated by the previous modeling studies (Fig 5A) validating our substantial efforts and highlighting the need for further structural studies to further define the details. Analysis of our structure in the context of the current state of knowledge and in the light of our preliminary biochemical and cell biological studies highlights the importance of novel phosphorylation sites in the regulation of TJ by Occ/ZO-1. Our ability to express and characterize a variety of TJ proteins places us in a unique position to advance the structural, biochemical and cell biological understanding of the complex. Ultimately, the planned studies will impact both basic knowledge about TJ's and lead to novel methods to manipulate their properties in disease states.

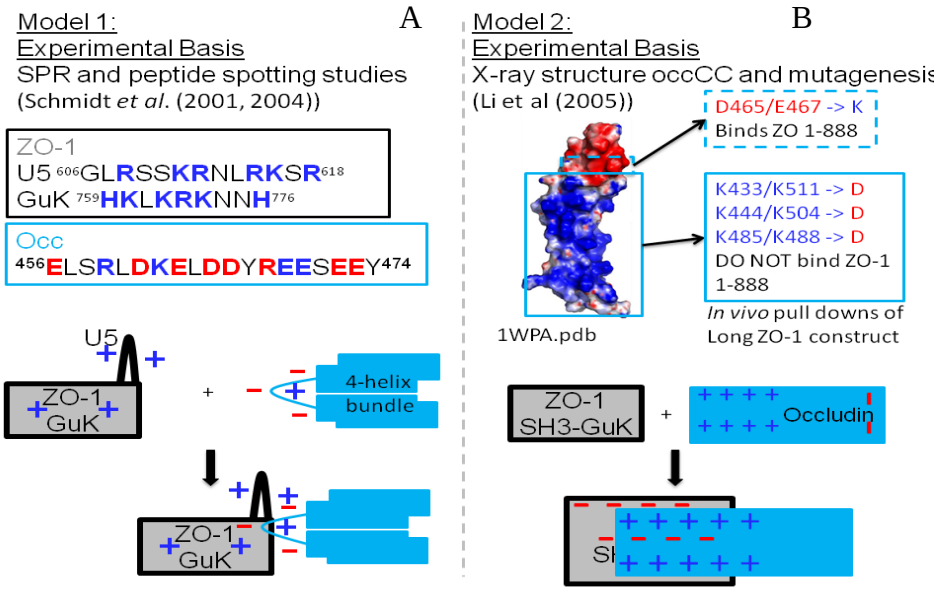


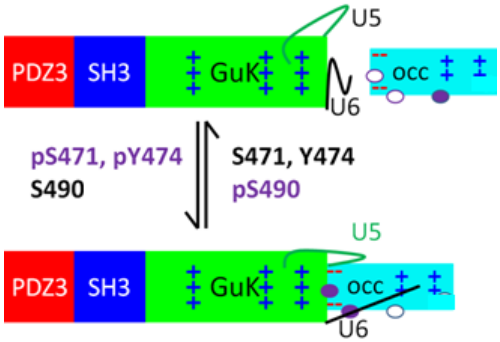
Fig 5. (A) Two models for the Occ ZO-1 binary complex. (B) Model based on validated structure of complex

Overall Rationale

Two different models had been proposed for the Occ ZO-1 complex (Fig 5). The first was proposed before the structures of occCC and ZO-1 PSG were determined. In this model, part of the U5 loop and a short basic region of GuK in ZO-1 bound to a mixed basic/acidic sequence that was predicted to lie in a four helix bundle of the C-terminal sequence of Occ. The tertiary structure was subsequently shown to be incorrect when the crystal structure of occCC was determined.

The second, used charge reversal mutants in occCC to identify residues that block ZO-1 binding (ZO1Δ889) and was conducted in the absense of a ZO-1 PSG structure. This model proposed

that the basic face of occCC interacts with a putative acidic face of the SH3-Guk domains of ZO-1. However, no acidic face was found in the ZO-1 SH3-GuK and ZO-1 PSG structures.



Our low resolution structure of the binary complex resolves the differences in the models and accounts for the biochemical data presented in the prior publications, reconciling the two models into a single consistent testable model that explains why aspects of each were correct. In this model, the acidic head of occCC (identified as a loop in model1) binds to the basic helix V of ZO-1 PSG (identified in model1). The U5 of ZO-1 PSG is a long mixed charge sequence that is disordered in all available structures of ZO-1 PSG (~40 amino acids). We propose that it makes additional interactions with the occCC including the lysine face identified in model2 and the similarly disordered U6 region may contribute (+/-) to binding. Functionally, U5 is required for targeting ZO-1 to TJ and removal of U6 results in ectopically displaced junction strands containing the modified ZO-1, Occ and claudin on the lateral cell membrane. This unified model accounts for the published phosphorylation data. Phosphorylation of S471 and Y474 enhance complex formation and phosphorylation of S490 causes complex disassociation. While this model is consistent and attractive, to fully understand how the different phosphorylation signals favor complex formation or disassembly, we require a more detailed model.

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. The occludin and ZO-1 complex, defined by small angle X-ray scattering and NMR, has implications for modulating tight junction permeability.	Tash BR, B**ewley** MC, Russo M, Keil JM, Griffin KA, Sundstrom JM, Antonetti DA, Tian F, **Flanagan** JM.	PNAS	Jan 2012	☐Submitted ☐Accepted ✓Published

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:

We are currently preparing two manuscripts for submission based upon work partially funded by the Tobacco settlement grant and the recently funded NIH grants.

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.

Based upon our work, we have begun a collaboration to inhibit a key kinase involved in the dysregulation of TJ permeability associated with diabetic retinopathy. In an animal model for this disease, the initial compound prevented the development of neuronal damage associated with pre-retinopathy. Further, we have begun to investigate the role of specific phosphorylation events in endothelial TJ to determine whether these properties can be manipulated for drug uptake into the eye and brain.

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.

We have discovered a novel phosphorylation site in occludin that appears to regulate the formation and stability of TJ's, thereby controlling the permeability.

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.

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PUBLICATIONS

1. Lees JP, Manlandro CM, Picton LK, Tan AZ, Casares S, Flanagan JM, Fleming KG, Hill RB. A designed point mutant in Fis1 disrupts dimerization and mitochondrial fission. J Mol Biol. 2012 Oct 19; 423(2):143-58.
2. Tash BR, Bewley MC, Russo M, Keil JM, Griffin KA, Sundstrom JM, Antonetti DA, Tian F, Flanagan JM. The occludin and ZO-1 complex, defined by small angle X-ray scattering and NMR, has implications for modulating tight junction permeability. Proc Natl Acad Sci U S A. 2012 Jul 3; 109(27):10855-60.
3. Woll MP, De Cotiis DA, Bewley MC, Tacelosky DM, Levenson R, Flanagan JM. Interaction between the D2 dopamine receptor and neuronal calcium sensor-1 analyzed by fluorescence anisotropy. Biochemistry. 2011 Oct 18; 50(41):8780-91.
4. Wang X, Tash B, Flanagan JM, Tian F. RDC derived protein backbone resonance assignment using fragment assembly. J Biomol NMR. 2011 Feb; 49(2):85-98.
5. Fox TE, Bewley MC, Unrath KA, Pedersen MM, Anderson RE, Jung DY, Jefferson LS, Kim JK, Bronson SK, Flanagan JM, Kester M. Circulating sphingolipid biomarkers in models of type 1 diabetes. J Lipid Res. 2011 Mar; 52(3):509-17.
6. Gudleski N, Flanagan JM, Ryan EP, Bewley MC, Parent LJ. Directionality of nucleocytoplasmic transport of the retroviral gag protein depends on sequential binding of karyopherins and viral RNA. Proc Natl Acad Sci U S A. 2010 May 18; 107(20):9358-63.
7. Sudol M, Tran M, Nowak MG, Flanagan JM, Robertson GP, Katzman M. A nonradioactive plate-based assay for stimulators of nonspecific DNA nicking by HIV-1 integrase and other nucleases. Anal Biochem. 2010 Jan 15; 396(2):223-30.
8. Purdy JG, Flanagan JM, Ropson IJ, Craven RC. Retroviral capsid assembly: a role for the CA dimer in initiation. J Mol Biol. 2009 Jun 5; 389(2):438-51
9. Zinnanti WJ, Lazovic J, Griffin K, Skvorak KJ, Paul HS, Homanics GE, Bewley MC, Cheng KC, Lanoue KF, Flanagan JM. Dual mechanism of brain injury and novel treatment strategy in maple syrup urine disease. Brain. 2009 Apr; 132(Pt 4):903-18.
10. Sundstrom JM, Tash BR, Murakami T, Flanagan JM, Bewley MC, Stanley BA, Gonsar KB, Antonetti DA. Identification and analysis of occludin phosphosites: a combined mass spectrometry and bioinformatics approach. J Proteome Res. 2009 Feb; 8(2):808-17.

11. Bewley MC, Graziano V, Griffin K, Flanagan JM. Turned on for degradation: ATPase-independent degradation by ClpP. J Struct Biol. 2009 Feb; 165(2):118-25.
12. De Cotiis DA, Woll MP, Fox TE, Hill RB, Levenson R, Flanagan JM. Optimized expression and purification of myristoylated human neuronal calcium sensor 1 in E. coli. Protein Expr Purif. 2008 Oct; 61(2):103-12.
13. Purdy JG, Flanagan JM, Ropson IJ, Rennoll-Bankert KE, Craven RC. Critical role of conserved hydrophobic residues within the major homology region in mature retroviral capsid assembly. J Virol. 2008 Jun; 82(12):5951-61.
14. Chen KM, Spratt TE, Stanley BA, De Cotiis DA, Bewley MC, Flanagan JM, Desai D, Das A, Fiala ES, Amin S, El-Bayoumy K. Inhibition of nuclear factor-kappaB DNA binding by organoselenocyanates through covalent modification of the p50 subunit. Cancer Res. 2007 Nov 1; 67(21):10475-83.
15. Carkaci-Salli N, Flanagan JM, Martz MK, Salli U, Walther DJ, Bader M, Vrana KE. Functional domains of human tryptophan hydroxylase 2 (hTPH2). J Biol Chem. 2006 Sep 22; 281(38):28105-12.
16. Bewley MC, Graziano V, Jiang J, Matz E, Studier FW, Pegg AE, Coleman CS, Flanagan JM. Structures of wild-type and mutant human spermidine/spermine N1-acetyltransferase, a potential therapeutic drug target. Proc Natl Acad Sci U S A. 2006 Feb 14; 103(7):2063-8. Bewley MC, Graziano V, Griffin K, Flanagan JM. The asymmetry in the mature amino-terminus of ClpP facilitates a local symmetry match in ClpAP and ClpXP complexes. J Struct Biol. 2006 Feb; 153(2):113-28.
17. Howitt J, Bewley MC, Graziano V, Flanagan JM, Freimuth P. Structural basis for variation in adenovirus affinity for the cellular coxsackievirus and adenovirus receptor. J Biol Chem. 2003 Jul 11; 278(28):26208-15.
18. Flanagan JM, Bewley MC. Protein quality control in bacterial cells: integrated networks of chaperones and ATP-dependent proteases. Genet Eng (N Y). 2002; 24:17-47. View in: PubMed
19. Bewley MC, Springer K, Zhang YB, Freimuth P, Flanagan JM. Structural analysis of the mechanism of adenovirus binding to its human cellular receptor, CAR. Science. 1999 Nov 19; 286(5444):1579-83.
20. Huang K, Ghose R, Flanagan JM, Prestegard JH. Backbone dynamics of the N-terminal domain in E. coli DnaJ determined by 15N- and 13CO-relaxation measurements. Biochemistry. 1999 Aug 10; 38(32):10567-77.
21. Huang K, Flanagan JM, Prestegard JH. The influence of C-terminal extension on the structure of the "J-domain" in E. coli DnaJ. Protein Sci. 1999 Jan; 8(1):203-14.
22. Wang J, Hartling JA, Flanagan JM. Crystal structure determination of Escherichia coli ClpP starting from an EM-derived mask. J Struct Biol. 1998 Dec 15; 124(2-3):151-63.
23. Wang J, Hartling JA, Flanagan JM. The structure of ClpP at 2.3 A resolution suggests a model for ATP-dependent proteolysis. Cell. 1997 Nov 14; 91(4):447-56.
24. Shanklin J, DeWitt ND, Flanagan JM. The stroma of higher plant plastids contain ClpP and ClpC, functional homologs of Escherichia coli ClpP and ClpA: an archetypal two-component ATP-dependent protease. Plant Cell. 1995 Oct; 7(10):1713-22.
25. Tolman JR, Flanagan JM, Kennedy MA, Prestegard JH. Nuclear magnetic dipole interactions in field-oriented proteins: information for structure determination in solution. Proc Natl Acad Sci U S A. 1995 Sep 26; 92(20):9279-83.
26. Flanagan JM, Wall JS, Capel MS, Schneider DK, Shanklin J. Scanning transmission electron microscopy and small-angle scattering provide evidence that native Escherichia coli ClpP is a tetradecamer with an axial pore. Biochemistry. 1995 Aug 29; 34(34):10910-7.
27. Hill RB, MacKenzie KR, Flanagan JM, Cronan JE, Prestegard JH. Overexpression, purification, and characterization of Escherichia coli acyl carrier protein and two mutant proteins. Protein Expr Purif. 1995 Aug; 6(4):394-400.
28. Hill RB, Flanagan JM, Prestegard JH. 1H and 15N magnetic resonance assignments, secondary structure, and tertiary fold of Escherichia coli DnaJ(1-78). Biochemistry. 1995 Apr 25; 34(16):5587-96.

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PUBLICATIONS

1. Bewley MC, Tash BR, Tian F, Flanagan JM. . Tissue Barriers. A complex affair: Attraction and repulsion make occludin and ZO-1 function!. 2013; 1(1):e23496.
2. Tash BR, Bewley MC, Russo M, Keil JM, Griffin KA, Sundstrom JM, Antonetti DA, Tian F, Flanagan JM. The occludin and ZO-1 complex, defined by small angle X-ray scattering and NMR, has implications for modulating tight junction permeability. Proc Natl Acad Sci U S A. 2012 Jul 3; 109(27):10855-60.
3. Tooley JE, Khangulov V, Lees JP, Schlessman JL, Bewley MC, Heroux A, Bosch J, Hill RB. The 1.75 Å resolution structure of fission protein Fis1 from Saccharomyces cerevisiae reveals elusive interactions of the autoinhibitory domain. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2011 Nov 1; 67(Pt 11):1310-5.
4. Woll MP, De Cotiis DA, Bewley MC, Tacelosky DM, Levenson R, Flanagan JM. Interaction between the D2 dopamine receptor and neuronal calcium sensor-1 analyzed by fluorescence anisotropy. Biochemistry. 2011 Oct 18; 50(41):8780-91.
5. Fox TE, Bewley MC, Unrath KA, Pedersen MM, Anderson RE, Jung DY, Jefferson LS, Kim JK, Bronson SK, Flanagan JM, Kester M. Circulating sphingolipid biomarkers in models of type 1 diabetes. J Lipid Res. 2011 Mar; 52(3):509-17.
6. Gudleski N, Flanagan JM, Ryan EP, Bewley MC, Parent LJ. Directionality of nucleocytoplasmic transport of the retroviral gag protein depends on sequential binding of karyopherins and viral RNA. Proc Natl Acad Sci U S A. 2010 May 18; 107(20):9358-63.
7. Zinnanti WJ, Lazovic J, Griffin K, Skvorak KJ, Paul HS, Homanics GE, Bewley MC, Cheng KC, Lanoue KF, Flanagan JM. Dual mechanism of brain injury and novel treatment strategy in maple syrup urine disease. Brain. 2009 Apr; 132(Pt 4):903-18.
8. Sundstrom JM, Tash BR, Murakami T, Flanagan JM, Bewley MC, Stanley BA, Gonsar KB, Antonetti DA. Identification and analysis of occludin phosphosites: a combined mass spectrometry and bioinformatics approach. J Proteome Res. 2009 Feb; 8(2):808-17.
9. Bewley MC, Graziano V, Griffin K, Flanagan JM. Turned on for degradation: ATPase-independent degradation by ClpP. J Struct Biol. 2009 Feb; 165(2):118-25.
10. Chen KM, Spratt TE, Stanley BA, De Cotiis DA, Bewley MC, Flanagan JM, Desai D, Das A, Fiala ES, Amin S, El-Bayoumy K. Inhibition of nuclear factor-kappaB DNA binding by organoselenocyanates through covalent modification of the p50 subunit. Cancer Res. 2007 Nov 1; 67(21):10475-83.

11. Bewley MC, Graziano V, Jiang J, Matz E, Studier FW, Pegg AE, Coleman CS, Flanagan JM. Structures of wild-type and mutant human spermidine/spermine N1-acetyltransferase, a potential therapeutic drug target. Proc Natl Acad Sci U S A. 2006 Feb 14; 103(7):2063-8.
12. Ikeguchi Y, Bewley MC, Pegg AE. Aminopropyltransferases: function, structure and genetics. J Biochem. 2006 Jan; 139(1):1-9.
13. Bewley MC, Graziano V, Griffin K, Flanagan JM. The asymmetry in the mature amino-terminus of ClpP facilitates a local symmetry match in ClpAP and ClpXP complexes. J Struct Biol. 2006 Feb; 153(2):113-28.
14. Diep CQ, Peng G, Bewley M, Pilauri V, Ropson I, Hopper JE. Intragenic suppression of Gal3C interaction with Gal80 in the Saccharomyces cerevisiae GAL gene switch. Genetics. 2006 Jan; 172(1):77-87.
15. Pilauri V, Bewley M, Diep C, Hopper J. Gal80 dimerization and the yeast GAL gene switch. Genetics. 2005 Apr; 169(4):1903-14.
16. Bewley MC, Davis CA, Marohnic CC, Taormina D, Barber MJ. The structure of the S127P mutant of cytochrome b5 reductase that causes methemoglobinemia shows the AMP moiety of the flavin occupying the substrate binding site. Biochemistry. 2003 Nov 18; 42(45):13145-51.
17. Marohnic CC, Bewley MC, Barber MJ. Engineering and characterization of a NADPH-utilizing cytochrome b5 reductase. Biochemistry. 2003 Sep 30; 42(38):11170-82. View in: PubMed
18. Howitt J, Bewley MC, Graziano V, Flanagan JM, Freimuth P. Structural basis for variation in adenovirus affinity for the cellular coxsackievirus and adenovirus receptor. J Biol Chem. 2003 Jul 11; 278(28):26208-15.
19. Flanagan JM, Bewley MC. Protein quality control in bacterial cells: integrated networks of chaperones and ATP-dependent proteases. Genet Eng (N Y). 2002; 24:17-47.
20. Bewley MC, Marohnic CC, Barber MJ. The structure and biochemistry of NADH-dependent cytochrome b5 reductase are now consistent. Biochemistry. 2001 Nov 13; 40(45):13574-82.
21. MacGillivray RT, Bewley MC, Smith CA, He QY, Mason AB, Woodworth RC, Baker EN. Mutation of the iron ligand His 249 to Glu in the N-lobe of human transferrin abolishes the dilysine "trigger" but does not significantly affect iron release. Biochemistry. 2000 Feb 15; 39(6):1211-6.
22. Bewley MC, Springer K, Zhang YB, Freimuth P, Flanagan JM. Structural analysis of the mechanism of adenovirus binding to its human cellular receptor, CAR. Science. 1999 Nov 19; 286(5444):1579-83.
23. Bewley MC, Jeffrey PD, Patchett ML, Kanyo ZF, Baker EN. Crystal structures of Bacillus caldovelox arginase in complex with substrate and inhibitors reveal new insights into activation, inhibition and catalysis in the arginase superfamily. Structure. 1999 Apr 15; 7(4):435-48.
24. Bewley MC, Tam BM, Grewal J, He S, Shewry S, Murphy ME, Mason AB, Woodworth RC, Baker EN, MacGillivray RT. X-ray crystallography and mass spectroscopy reveal that the N-lobe of human transferrin expressed in Pichia pastoris is folded correctly but is glycosylated on serine-32. Biochemistry. 1999 Feb 23; 38(8):2535-41.
25. Freimuth P, Springer K, Berard C, Hainfeld J, Bewley M, Flanagan J. Coxsackievirus and adenovirus receptor amino-terminal immunoglobulin V-related domain binds adenovirus type 2 and fiber knob from adenovirus type 12. J Virol. 1999 Feb; 73(2):1392-8.
26. Qin BY, Bewley MC, Creamer LK, Baker EN, Jameson GB. Functional implications of structural differences between variants A and B of bovine beta-lactoglobulin. Protein Sci. 1999 Jan; 8(1):75-83.
27. Jeffrey PD, Bewley MC, MacGillivray RT, Mason AB, Woodworth RC, Baker EN. Ligand-induced conformational change in transferrins: crystal structure of the open form of the N-terminal half-molecule of human transferrin. Biochemistry. 1998 Oct 6; 37(40):13978-86.
28. Qin BY, Bewley MC, Creamer LK, Baker HM, Baker EN, Jameson GB. Structural basis of the Tanford transition of bovine beta-lactoglobulin. Biochemistry. 1998 Oct 6; 37(40):14014-23.

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PUBLICATIONS:

1. Bai H, Pan Y, Tong W, Zhang W, Ren X, Tian F, Peng B, Wang X, Zhang Y, Deng Y, Qin W, Qian X. Graphene based soft nanoreactors for facile "one-step" glycan enrichment and derivatization for MALDI-TOF-MS analysis. Talanta. 2013 Dec 15; 117:1-7.
2. Bao J, Yura RE, Matters GL, Bradley SG, Shi P, Tian F, Bond JS. Meprin A impairs epithelial barrier function, enhances monocyte migration, and cleaves the tight junction protein occludin. Am J Physiol Renal Physiol. 2013 Sep 1; 305(5):F714-26.
3. Tash BR, Bewley MC, Russo M, Keil JM, Griffin KA, Sundstrom JM, Antonetti DA, Tian F, Flanagan JM. The occludin and ZO-1 complex, defined by small angle X-ray scattering and NMR, has implications for modulating tight junction permeability. Proc Natl Acad Sci U S A. 2012 Jul 3; 109(27):10855-60.
4. Pielak GJ, Tian F. Membrane proteins, magic-angle spinning, and in-cell NMR. Proc Natl Acad Sci U S A. 2012 Mar 27; 109(13):4715-6.
5. Fu R, Wang X, Li C, Santiago-Miranda AN, Pielak GJ, Tian F. In situ structural characterization of a recombinant protein in native Escherichia coli membranes with solid-state magic-angle-spinning NMR. J Am Chem Soc. 2011 Aug 17; 133(32):12370-3.
6. Boregowda RK, Lin L, Zhu Q, Tian F, Hu J. Cryptic protein priming sites in two different domains of duck hepatitis B virus reverse transcriptase for initiating DNA synthesis in vitro. J Virol. 2011 Aug; 85(15):7754-65.
7. Wang X, Gill RL, Zhu Q, Tian F. Bacterial expression, purification, and model membrane reconstitution of the transmembrane and cytoplasmic domains of the human APP binding protein LR11/SorLA for NMR studies. Protein Expr Purif. 2011 Jun; 77(2):224-30.
8. Rajeev, K. B., Lin, L., Zhu, Q., Tian, F., Hu, J. M. . Cryptic protein priming sites in duck hepatitis B virus transcriptase for initiating DNA synthesis in vitro. Journal of Virology. 2011; 85:7754-7765.
9. Wang X, Tash B, Flanagan JM, Tian F. RDC derived protein backbone resonance assignment using fragment assembly. J Biomol NMR. 2011 Feb; 49(2):85-98.
10. Demarco ML, Woods RJ, Prestegard JH, Tian F. Presentation of membrane-anchored glycosphingolipids determined from molecular dynamics simulations and NMR paramagnetic relaxation rate enhancement. J Am Chem Soc. 2010 Feb 3; 132(4):1334-8.

11. Tian FF, Zeng C, Ma YF, Guo TH, Chen JM, Chen Y, Cai XF, Li FR, Wang XH, Huang WJ, Wang YZ. Potential roles of Cdk5/p35 and tau protein in hippocampal mossy fiber sprouting in the PTZ kindling model. Clin Lab. 2010; 56(3-4):127-36.
12. Tian FF, Zeng C, Guo TH, Chen Y, Chen JM, Ma YF, Fang J, Cai XF, Li FR, Wang XH, Huang WJ, Fu JJ, Dang J. Mossy fiber sprouting, hippocampal damage and spontaneous recurrent seizures in pentylenetetrazole kindling rat model. Acta Neurol Belg. 2009 Dec; 109(4):298-304.
13. Beel AJ, Mobley CK, Kim HJ, Tian F, Hadziselimovic A, Jap B, Prestegard JH, Sanders CR. Structural studies of the transmembrane C-terminal domain of the amyloid precursor protein (APP): does APP function as a cholesterol sensor? Biochemistry. 2008 Sep 9; 47(36):9428-46.
14. Macnaughtan MA, Tian F, Liu S, Meng L, Park S, Azadi P, Moremen KW, Prestegard JH. 13C-sialic acid labeling of glycans on glycoproteins using ST6Gal-I. J Am Chem Soc. 2008 Sep 10; 130(36):11864-5.
15. Bryson M, Tian F, Prestegard JH, Valafar H. REDCRAFT: a tool for simultaneous characterization of protein backbone structure and motion from RDC data. J Magn Reson. 2008 Apr; 191(2):322-34.
16. Sahu SC, Simplaceanu V, Gong Q, Ho NT, Tian F, Prestegard JH, Ho C. Insights into the solution structure of human deoxyhemoglobin in the absence and presence of an allosteric effector. Biochemistry. 2007 Sep 4; 46(35):9973-80.
17. Wang X, Srisailam S, Yee AA, Lemak A, Arrowsmith C, Prestegard JH, Tian F. Domain-domain motions in proteins from time-modulated pseudocontact shifts. J Biomol NMR. 2007 Sep; 39(1):53-61.
18. Tian F, Zhu CH, Zhang XW, Xie X, Xin XL, Yi YH, Lin LP, Geng MY, Ding J. Philinopside E, a new sulfated saponin from sea cucumber, blocks the interaction between kinase insert domain-containing receptor (KDR) and alphavbeta3 integrin via binding to the extracellular domain of KDR. Mol Pharmacol. 2007 Sep; 72(3):545-52.
19. Thorarensen A, Sarver RW, Tian F, Ho A, Romero DL, Marotti KR. Human serum albumin binding assay based on displacement of a non selective fluorescent inhibitor. Bioorg Med Chem Lett. 2007 Aug 15; 17(16):4646-9.
20. Liu S, Venot A, Meng L, Tian F, Moremen KW, Boons GJ, Prestegard JH. Spin-labeled analogs of CMP-NeuAc as NMR probes of the alpha-2,6-sialyltransferase ST6Gal I. Chem Biol. 2007 Apr; 14(4):409-18.
21. Tong YG, Zhang XW, Geng MY, Yue JM, Xin XL, Tian F, Shen X, Tong LJ, Li MH, Zhang C, Li WH, Lin LP, Ding J. Pseudolarix acid B, a new tubulin-binding agent, inhibits angiogenesis by interacting with a novel binding site on tubulin. Mol Pharmacol. 2006 Apr; 69(4):1226-33.
22. Sarver RW, Gao H, Tian F. Determining molecular binding sites on human serum albumin by displacement of oleic acid. Anal Biochem. 2005 Dec 15; 347(2):297-302.
23. Tian F, Zhang X, Tong Y, Yi Y, Zhang S, Li L, Sun P, Lin L, Ding J. PE, a new sulfated saponin from sea cucumber, exhibits anti-angiogenic and anti-tumor activities in vitro and in vivo. Cancer Biol Ther. 2005 Aug; 4(8):874-82.
24. Tong Y, Zhang X, Tian F, Yi Y, Xu Q, Li L, Tong L, Lin L, Ding J. Philinopside A, a novel marine-derived compound possessing dual anti-angiogenic and anti-tumor effects. Int J Cancer. 2005 May 10; 114(6):843-53.
25. Siriwardena AH, Tian F, Noble S, Prestegard JH. A straightforward NMR-spectroscopy-based method for rapid library screening. Angew Chem Int Ed Engl. 2002 Sep 16; 41(18):3454-7.
26. Amor JC, Seidel RD, Tian F, Kahn RA, Prestegar JH. 1H, 15N and 13C assignments of full length human ADP ribosylation factor 1 (ARF1) using triple resonance connectivities and dipolar couplings. J Biomol NMR. 2002 Jul; 23(3):253-4.
27. Amor, J. C., Seidel III, R. D., Tian, F., Kahn, R. A., Prestegard, J. H. . 1H, 15N and 13C assignments of full length ADP Ribosylation Factor 1 (ARF1) using triple resonance connectivities and dipolar couplings. J Biomol NMR. 2002; 23:253-254.
28. Tian F, Valafar H, Prestegard JH. A dipolar coupling based strategy for simultaneous resonance assignment and structure determination of protein backbones. J Am Chem Soc. 2001 Nov 28; 123(47):11791-6.

29. Prestegard JH, Valafar H, Glushka J, Tian F. Nuclear magnetic resonance in the era of structural genomics. Biochemistry. 2001 Jul 31; 40(30):8677-85.
30. Tian F, Al-Hashimi HM, Craighead JL, Prestegard JH. Conformational analysis of a flexible oligosaccharide using residual dipolar couplings. J Am Chem Soc. 2001 Jan 24; 123(3):485-92.
31. Fowler CA, Tian F, Al-Hashimi HM, Prestegard JH. Rapid determination of protein folds using residual dipolar couplings. J Mol Biol. 2000 Dec 1; 304(3):447-60.
32. Tian F, Fowler CA, Zartler ER, Jenney FA, Adams MW, Prestegard JH. Direct measurement of 1H-1H dipolar couplings in proteins: a complement to traditional NOE measurements. J Biomol NMR. 2000 Sep; 18(1):23-31.
33. Losonczi JA, Tian F, Prestegard JH. Nuclear magnetic resonance studies of the N-terminal fragment of adenosine diphosphate ribosylation factor 1 in micelles and bicelles: influence of N-myristoylation. Biochemistry. 2000 Apr 4; 39(13):3804-16.
34. Tian F, Losonczi JA, Fischer MW, Prestegard JH. Sign determination of dipolar couplings in field-oriented bicelles by variable angle sample spinning (VASS). J Biomol NMR. 1999 Oct; 15(2):145-50.
35. Tian F, Fu R, Cross TA. 13C selective polarization and spin diffusion in a lipid bilayer-bound polypeptide by solid-state NMR. J Magn Reson. 1999 Aug; 139(2):377-81.
36. Tian F, Cross TA. Cation transport: an example of structural based selectivity. J Mol Biol. 1999 Feb 5; 285(5):1993-2003.
37. Tian, F., Bolon, P. J., Prestegard, J. H. Intensity based measurement of homonuclear residual dipolar coupling from CT-COSY. J Am Chem Soc. 1999; 121:7712-7713.
38. Cross TA, Tian F, Cotten M, Wang J, Kovacs F, Fu R. Correlations of structure, dynamics and function in the gramicidin channel by solid-state NMR spectroscopy. Novartis Found Symp. 1999; 225:4-16; discussion 16-22.
39. Tian F, Cross TA. Cation binding induced changes in 15N CSA in a membrane-bound polypeptide. J Magn Reson. 1998 Dec; 135(2):535-40.
40. Tian F, Song Z, Cross TA. Orientational constraints derived from hydrated powder samples by two-dimensional PISEMA. J Magn Reson. 1998 Nov; 135(1):227-31.
41. Tian F, Cross TA. Dipolar Oscillations in Cross-Polarized Peptide Samples in Oriented Lipid Bilayers J Magn Reson. 1997 Mar; 125(1):220-3.
42. Arumugam, S., Pascal, S., North, C. L., Hu, W., Lee, K. C., Ketchem, R. R., Xu, F., Brenneman, M., Kovas, F., Tian, F., Wang, A., Hou, S., Cross, T. A. Conformational trapping in membrane environment: A regulatory mechanism for protein activity? Proc Natl Acad Sci U S A. 1996; 93:5872-5876.
43. Tian, F., Lee, K.-C., Hu, W., Cross, T. A. Monovalent cations transport: Lack of structural deformation upon cation binding. Biochemistry. 1996; 35:11959-11966.
44. Ketchem, R. R., Hu, W., Tian, F., Cross, T. A . Structure and dynamics from solid state NMR spectroscopy. Structure. 1994; 2:699-701.