REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
08-02-2019	Final Report	1-May-2016 - 30-Apr-2017

4. TITLE AND SUBTITLE
Final Report: Mechanical regulation of integrin conformation

5a. CONTRACT NUMBER
W911NF-16-1-0257

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES

Georgia Tech Research Corporation
Georgia Tech Research Corporation
505 Tenth Street NW
Atlanta, GA

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)

U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
69443-LS.4

12. DISTRIBUTION AVAILIBILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Cheng Zhu
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RPPR Final Report
as of 08-May-2019

Agency Code:

Proposal Number: 69443LS **Agreement Number: W911NF-16-1-0257**
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Country:
DUNS Number: 097394084 EIN: 580603146
Report Date: 31-Jul-2017 Date Received: 08-Feb-2019
Final Report for Period Beginning 01-May-2016 and Ending 30-Apr-2017
Title: Mechanical regulation of integrin conformation
Begin Performance Period: 01-May-2016 **End Performance Period:** 30-Apr-2017
Report Term: 0-Other
Submitted By: Ph.D. Cheng Zhu Email: cheng.zhu@bme.gatech.edu
Phone: (404) 894-3269

STEM Degrees: 0 **STEM Participants:** 0

Major Goals: The major goals are to elucidate the mechanical regulation of ligand binding and conformational change of integrin ?v?3 using specific mutations to manipulate its structure.

Accomplishments: The most important results of our studies have been published in two technical papers (1, 2) and a review paper (3), acknowledged the support of this grant.

The first paper used a biomembrane force probe to characterize the bending and unbending conformational changes of single ?V?3 integrins on living cell surfaces in real-time. We measured the probabilities of conformational changes, rates and speeds of conformational transitions, and the dynamic equilibrium between the two conformations, which were regulated by tensile force, dependent on the ligand, and altered by point mutations. These findings provide insights into how ?V?3 acts as a molecular machine and how its physiological function and molecular structure are coupled at the single-molecule level.

The second paper used atomic force microscopy to show the interplay between biochemical regulation and biomechanical regulation of actin–actin interactions. The results support the biological significance of actin catch bonds, as they corroborate reported observations that RhoA and formin switch force-induced actin cytoskeleton alignment. Our study demonstrates how the mechano-regulation of actin dynamics is modulated by biochemical signaling molecules, and suggests that actin catch bonds may be important in cell functions.

1. Chen, Y., H. Lee, H. Tong, M. Schwartz and C. Zhu (2017). "Force regulated conformational change of integrin alphaVbeta3." Matrix Biol 60-61: 70-85.
2. Lee, C. Y., J. Lou, K. K. Wen, M. McKane, S. G. Eskin, P. A. Rubenstein, S. Chien, S. Ono, C. Zhu* and L. V. McIntire* (2016). "Regulation of actin catch-slip bonds with a RhoA-formin module." Sci Rep 6: 35058. (* co-corresponding authors)
3. Chen, Y., L. Ju, M. Rushdi, C. Ge and C. Zhu (2017). "Receptor-mediated cell mechanosensing." Mol Biol Cell 28(23): 3134-3155.

Training Opportunities: he grant money was used to support a postdoctoral scholar, Dr. Hyun-Jung Lee. Dr. Lee completed her training in my lab upon the completion of the project.

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Results Dissemination: The results were published in scientific journals:

1.Chen, Y., H. Lee, H. Tong, M. Schwartz and C. Zhu (2017). "Force regulated conformational change of integrin alphaVbeta3." Matrix Biol 60-61: 70-85.
Lee, C. Y., J. Lou, K. K. Wen, M. McKane, S. G. Eskin, P. A. Rubenstein, S. Chien, S. Ono, 2. C. Zhu* and L. V. McIntire* (2016). "Regulation of actin catch-slip bonds with a RhoA-formin module." Sci Rep 6: 35058. (* co-corresponding authors)
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Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Hyun-Jung Lee
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

ARTICLES:

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Scientific Reports
Publication Identifier Type: DOI Publication Identifier: 10.1038/srep35058
Volume: 6 Issue: 1 First Page #:
Date Submitted: 2/8/19 12:00AM Date Published: 10/1/16 8:00AM
Publication Location:
Article Title: Regulation of actin catch-slip bonds with a RhoA-formin module
Authors: Cho-yin Lee, Jizhong Lou, Kuo-Kuang Wen, Melissa McKane, Suzanne G. Eskin, Peter A. Rubenstein,
Keywords: Biophysical chemistry, Single-molecule biophysics
Abstract: The dynamic turnover of the actin cytoskeleton is regulated cooperatively by force and biochemical signaling. We previously demonstrated that actin depolymerization under force is governed by catchslip bonds mediated by force-induced K113:E195 salt-bridges. Yet, the biochemical regulation as well as the functional significance of actin catch bonds has not been elucidated. Using AFM force-clamp experiments, we show that formin controlled by RhoA switches the actin catch-slip bonds to slip-only bonds. SMD simulations reveal that the force does not induce the K113:E195 interaction when formin binds to actin K118 and E117 residues located at the helical segment extending to K113. Actin catchslip bonds are suppressed by single residue replacements K113E and E195K that interrupt the forceinduced K113:E195 interaction; and this suppression is rescued by a K113E/E195K double mutant (E/K) restoring the interaction in the opposite orientation. These results support the biological significance of
Distribution Statement: 1-Approved for public release; distribution is unlimited.
Acknowledged Federal Support: **Y**

RPPR Final Report
as of 08-May-2019

FINAL REPORT

Project Title: Mechanical Regulation of Integrin Conformation
Contract Number: W911NF-16-1-0257
Dates Covered: From 05/01/2016 to 04/30/2017
Principal Investigator: Cheng Zhu, Ph.D.

Forward:
This project represents a biophysical analysis of the mechanical regulation of ligand binding and conformational change of integrin αvβ3 using specific mutations to manipulate its structure. Integrin-mediated mechanotransduction in cells is hypothesized to relate to ligand binding and conformational change of integrin. Using cells expressing wild-type integrin αvβ3 and several mutants, our results elucidate the relationship between the biophysical determinants of ligand binding, conformational change, and intracellular signaling of integrin αvβ3.

Statement of the problem studied:
1) How does force regulate integrin αVβ3 interaction with ligands?
2) How is integrin conformational dynamics affected by force?
3) How does the force-regulated integrin conformational dynamics interplay with ligand dissociation kinetics?
4) What is the structural basis of these biophysical determinants of ligand binding and conformational change of integrin αVβ3.

Summary of the most important results:
The most important results of our studies have been published in two technical papers (1, 2) and a review paper (3), acknowledged the support of this grant.

The first paper used a biomembrane force probe to characterize the bending and unbending conformational changes of single αVβ3 integrins on living cell surfaces in real-time. We measured the probabilities of conformational changes, rates and speeds of conformational transitions, and the dynamic equilibrium between the two conformations, which were regulated by tensile force, dependent on the ligand, and altered by point mutations. These findings provide insights into how αVβ3 acts as a molecular machine and how its physiological function and molecular structure are coupled at the single-molecule level.

The second paper used atomic force microscopy to show the interplay between biochemical regulation and biomechanical regulation of actin–actin interactions. The results support the biological significance of actin catch bonds, as they corroborate reported observations that RhoA and formin switch force-induced actin cytoskeleton alignment. Our study demonstrates how the mechano-regulation of actin dynamics is modulated by biochemical signaling molecules, and suggests that actin catch bonds may be important in cell functions.

Bibliography:

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