BIOGRAPHICAL SKETCH

NAME Henk L. Granzier	POSITION TITLE Professor of Cellular and Molecular Medicine, Physiology; Norville Endowed Chair
eRA COMMONS USER NAME Granzier	

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
University of Wageningen (The Netherlands)	BS	01/82	Biology
University of Wageningen (The Netherlands)	MS (cum laude)	08/83	Physiology & Biophysics
University of Washington (Seattle, Wa)	PhD	05/88	Bioengineering
University of Texas (Austin, Tx)	postdoc	02/93	Biochemistry

A. Personal Statement

The goal of my research program is to perform research on defining the biological roles of titin in muscle function and to utilize these mechanistic insights for identifying therapeutic targets for muscle disease. I have been in the titin field since my postdoctoral training under Dr. Kuan Wang (Department of Biochemistry, University of Texas at Austin), the discoverer of titin. My postdoctoral research focused on studying titin biomechanics and developing techniques for detection of titin, applying my training in Biochemistry, Bioengineering and Physiology. I have worked on titin as an independent faculty member since 1993 (14 years at Washington State University and the last 10 years at the University of Arizona). In my career so far I have published 205 articles (first author on 38 and last author on 105), many of which were published in high impact journals. During my independent career, I continued to develop and apply novel methodologies (e.g., single molecule force spectroscopy and loaded single cells mechanics) and my laboratory made many discoveries regarding the structure and function of titin in the heart.

Through single molecule and single cell studies we discovered that titin functions as an entropic spring and, importantly, that the spring has distinct sub-segments that provide a unique passive force – sarcomere spring relation to the cardiac myocytes. Additionally we discovered that post-transcriptional processing results in titin isoforms (a fetal cardiac isoform and two classes of adult isoforms) and that through altering the isoform expression ratio passive myocyte stiffness can be tuned. We also developed methods to measure the contribution of titin to tissue stiffness by specifically eliminating titin's contribution to stiffness, leaving the ECM-based stiffness behind. Utilizing this method in comparative studies we showed that at physiological sarcomere lengths titin accounts for the majority of the passive myocardial tension in all investigated mammals (from small rodents to human).

My laboratory has also been at the forefront of discovering multiple post-translational modifications in titin's spring elements, how they tune the stiffness of the myocyte and, importantly how this system is deranged in heart failure patients. We were also the first to make mouse models that delete titin's spring elements and more recently models in which disease causing mutations are introduced. Our work has been at the forefront of the titin field and has influenced research in many laboratories world-wide. Since I became an independent investigator in 1993, I have trained and mentored 12 graduate students (PhD level) and 28 postdoctoral research fellows (16 are in now in academic positions, 2 are Department Heads and 6 are in leading positions in industry) many of whom are productive scientists in the titin field.

In summary, I have a track record of successful and innovative research with consistent funding and productivity with publications in high impact journals.

Examples of several of our recent studies are given below:

a) **Granzier** et al. Deleting titin's IA-junction reveals titin's critical roles in biomechanical sensing and cardiac function. *Proceedings of National Academy of Sciences*, U S A. 2014;111(40):14589-94.

b) Anderson BR, Bogomolovas J, Labeit S, and Henk **Granzier**, H. Single Molecule Force Spectroscopy on Titin Implicates Ig Domain Stability as a Cardiac Disease Mechanism. *J Biol Chem.* 2013;288(8):5303-15.

c) Zile MR, **Granzier** et al. Myocardial stiffness in patients with heart failure and a preserved ejection fraction: contributions of collagen and titin. *Circulation*. 2015;131(14):1247-59.

d) Methawasin M, et al. and **Granzier** 2016. Experimentally Increasing Titin's Compliance Through RBM20 Inhibition Improves Diastolic Function in a Mouse Model of HFpEF. *Circulation*. 2016;134(15):1085-1099. (received an Editorial)

B. Positions and honors

Positions and employment.

1993-2007 1993-1997 Assistant Professor; 1997-2001 Associate Professor; 2002-2007 Full Professor; Department of Physiology, Washington State University (Pullman, WA)
2006-2007 Sabbatical Professor at Max Delbrück Center for Molecular Medicine, Berlin, Germany.
2007-2014 Professor of Physiology, Medical School, University of Arizona
2007-pres. Allan and Alfie Endowed Chair for Heart Disease in Women Research, Sarver heart Center, UA.
2007 pres. Adjunct Professor of Molecular and Cellular Biology, University of Arizona, Tucson.
2014-pres. Professor in Cellular and Molecular Medicine, University of Arizona (UA).
2014-pres. Director of the Small Animal Phenotyping Core Facility, University of Arizona (UA).

Other experiences and professional membership:

2017-pres. Editorial board member of *Circulation: Heart Failure*.
2016 Co organized Symposium on Titin and its Binding Partners (at Loyola University)
2016 Organized symposium at ISHR conference in Buenos Aires, Argentina.
2008-pres. Editorial Board member of *Heart Failure Journal*.
2009-pres. Editorial Board Member of *Animal Physiology*.
2009-pres. Editorial Board Member of *Journal of General Physiology*.
2008-2012 Member of NIH Cardiac Contractility and Heart Failure (CCHF) study section
2008-2010 Chair of AHA Pacific Mountain Affiliate (PMA) Research Committee.
2010-2011 Editor of "Cytoskeleton and the cellular transduction of mechanical strain", Pflügers Archives.
2009-2010 Editor of issue 'Advances in Muscle Physiology', *Journal of Biomedicine and Biotechnology*
2005 Editor of issues 'Muscle Elastic Proteins' in *Journal of Muscle Research and Cell Motility*
2006 Chair of symposium "Spring Molecules," at Congress of Int. Union of Physiological Sciences.
2003 Co-organized International Symposium on Muscle Elastic Proteins. Chiba Japan.
2003 Organized Symposium *Titin Mechanics*. World Congress of Biomechanics, Calgary.
2002 Guest Editor of special issue on elastic proteins for *Journal of Muscle Research and Cell Motility*
2000 Chairman, Biophysical Society, Motility Subgroup
2000 Editor of *Elastic Filaments of the Cell*, 2000. Kluwer Academic/Plenum Publishers, New York
1999 Organized International Conference on Elastic Proteins, Seattle, WA.
2002-pres. Member of 11 PPG review panels (NIA, NHLBI, NIAMS) and 14 Special Emphasis Panels
1995-pres. Member of Biophysical Society of America, American Society for General Physiologists, American Association for the Advancement of Science, International Society of Heart Research, Cardiac Muscle Society, AHA, American Physiological Society Member

Honors

2017 Elected Associate Editor of *Circulation: Heart Failure*.
2014 Editorial Board member of *Journal of General Physiology*.
2011 Fellow of the International Society of Heart Research (FISHR).
2009-2012 Chair of AHA Research Committee (PMA affiliate)
2009 Editorial Board member of *Pflügers Archives - European Journal of Physiology*
2008-2013 Member of NIH Cardiac Contractibility and Heart Failure (CCHF) study section
2007 Allan and Alfie Endowed Chair for Heart Disease in Women Research.
2006 Helmholtz-Humboldt Research Award
2005 Pfizer Award for Research Excellence
2000 Established Investigator of American Heart Association
1995 FIRST award from National Institutes of Health
1994 Biomedical Engineering Research Award from the Whitaker Foundation

1988 Neuromuscular Disease Research Fellowship from Muscular Dystrophy Association of America
1983-1988 Fulbright fellowship from the Netherlands American Commission for Educational Exchange

C. **Contributions to Science**

1. **Titin function in both passive and active muscle.** My initial independent work focused on the physiological role of titin at the single cell level and established for the first time that titin's I-band region functions as a bi-directional spring that develops passive force when cells are stretched above their slack length and restoring force when cells shorten to below slack, see references a) and b). Additionally, we showed that titin's passive force enhances the development of actomyosin-based force at submaximal calcium levels and thereby contributes to the Frank-Starling mechanism of the heart (ref c). For this work we have been an early adapter of multiple novel techniques that include gel electrophoresis and Western blot techniques to study megadalton-sized proteins, single cell mechanics, differential myofilament extraction techniques, muscle X-ray diffraction, and more recently we have created genetically engineered mouse models that have allowed my laboratory to further critically test the physiological roles of titin. An example of such recent work in ref d.
    a) **Granzier, H**., and T. Irving. 1995. Passive tension in cardiac muscle: The contribution of collagen, titin, microtubules and intermediate filaments. *Biophysical Journal,* 68: 1027-1044. This is the first publication from my own laboratory. Citations: 430.
    b) Helmes, M., K. Trombitás, and H.L.M. **Granzier**. 1996. Titin develops restoring force in rat cardiac myocytes. *Circulation Research.* 79(3): 619-626. Citations: 215.
    c) Cazorla O, Wu Y, Irving T, **Granzier** H. 2001. Titin-based modulation of calcium sensitivity of active tension in mouse skinned cardiac myocytes. *Circulation Research*. 2001; 88, 1028-1035. (Article received an Editorial) Citations: 195.
    d) **Henk Granzier**; K Hutchinson; P Tonino; M Methawasin; F Li , R Slater, M Bull, C Saripalli, C Pappas; C Gregorio; J Smith. 2014. Deleting titin's IA-junction reveals titin's critical roles in biomechanical sensing and cardiac function. PNAS, 111(40), 14859-14594. Citations: 31.

2. **Molecular Physiology.** To study the molecular basis by which titin develops passive force, my laboratory was amongst the first to perform single molecule studies on titin. We used both laser-tweezers and atomic force microscopy to manipulate single molecules and characterize their molecular biophysical properties. This work revealed that titin functions as an entropic spring that follows wormlike chain behavior with superimposed unfolding transitions due to reversible domain unfolding events (refs. a-c). We also discovered that titin's I-band region contains distinct spring elements that dominate titin's elasticity at different force levels, providing the sarcomere with a unique passive force-sarcomere length relation (ref. d). This work required development and application of novel techniques and resulted in multiple high-impact and well-received publications.
    a) Kellermayer, M, S Smith, H **Granzier**, and C Bustamante. 1997. Folding-unfolding transitions in single titin molecules characterized with laser tweezers. *Science* 276: 1112-1116. (Article received editorials in *Science* (276:1090-2) and *Nature* (387:233-5).) Citations: 1101
    b) Trombitás, K., M. Greaser, S. Labeit, J.-P. Jin, M. Kellermayer, M. Helmes and H. **Granzier.** 1998. Titin extensibility in situ: entropic elasticity of permanently folded and permanently unfolded molecular segment. *Journal of Cell Biology*. 140: 853-859. Citations: 203.
    c) Kellermayer, M., Smith, S., Bustamante, C., and **Granzier**, H., 2001. Mechanical fatigue in repetitively stretched single molecules of titin. *Biophysical Journal,* 80: 852-863. Citations: 183
    d) Kaori Watanabe, P Nair, M Kellermayer, M Greaser, S Labeit, and Henk **Granzier**. 2002 Molecular mechanics of cardiac titin's PEVK and N2B spring elements. *Journal of Biological Chemistry*;277(13):11549-58. Citations: 116

3. **Turning the molecular spring through differential splicing.** My interest then expanded to include a focus on how titin's passive force can be tuned. We first showed that there is only a single titin gene and that titin's elasticity can be tuned through differential splicing that results in titin isoforms with spring elements that vary in length and composition and consequently have different passive stiffness characteristics (references a -

c). We discovered the adult and fetal cardiac titin isoforms (and named them), and revealed their unique passive force – sarcomere length relations (ref. d). Our publications received multiple Editorials.

a) Cazorla, O., Freiburg, A, Helmes, M. Centner, T., McNabb, M., Trombitás, K. , Labeit, S. and **Granzier**, H., 2000. Differential expression of cardiac titin isoforms and modulation of cellular stiffness. *Circulation Research*, 86, 59-67. Citations: 297

b) Wu, Y., Cazorla, O., Labeit, S. **Granzier** H, 2000 Changes in titin and collagen underlie diastolic stiffness diversity of cardiac muscle. *Journal of Molecular and Cellular Cardiology*, 32, 2151-2161. (Article received an Editorial.) Citations: 178

c) Bang ML, Centner T, Fornoff F, Geach A, Gotthardt M, McNabb M, Witt C, Labeit D, Gregorio C, **Granzier** H, Labeit S. 2001 The complete gene sequence of titin, expression of an unusual ~700 kDa titin isoform and its interaction with obscurin identify a novel Z-line to I-band linking system. *Circulation Research*, 89(11):1065-72. (Selected for cover photo of Journal.) Citations: 430

d) Sunshine M Lahmers, Yiming Wu, D R Call, Siegfried Labeit, and Henk **Granzier**. 2004. Developmental control of titin isoform expression and passive stiffness in fetal and neonatal myocardium. *Circulation Research*;94(4):505-13. (Article received Editorial). Citations: 185

4. **Turning the molecular spring through post-translational modifications.** In subsequent work we focused on posttranslational modifications of titin and discovered that titin's stiffness can also be altered through calcium binding to glutamic acid rich regions in the PEVK spring element, a mechanism that is most prominent in skeletal muscle titin isoforms (ref a). Studies on cardiac titin revealed that titin's stiffness can also be tuned through posttranslational modifications. We discovered that protein kinase A (PKA) phosphorylates the cardiac-specific N2B element, lowering passive stiffness (ref b and c) and that both protein kinase C (PKC) and calmodulin-dependent protein kinase II (CaMKII) phosphorylate the PEVK element, increasing passive stiffness (ref d). This work has had a major impact in the field, currently influencing research directions in laboratories worldwide, work that includes a strong clinical focus.
   a) Labeit,D., Watanabe, K., Witt, C., Wu, Y., Labeit, S., **Granzier** H. 2003 Calcium-dependent molecular spring elements in the giant protein titin. *Proc Natl Acad Sci U S A (PNAS). 11*;100(23):13716-21. Citations: 240
   b) Yamasaki R, Wu Y, McNabb M, Greaser M, Labeit S., and **Granzier** H. 2002 Protein kinase A phosphorylates titin's cardiac-specific N2B domain and reduces passive tension in rat cardiac myocytes. *Circulation Research*, 14; 90(11):1181-8. Citations: 235
   c) Fukuda N, Wu Y, Nair, Preetha, and Henk **Granzier**. 2005. Phosphorylation of titin modulates passive stiffness of cardiac muscle in a titin isoform-dependent manner. *Journal of General Physiology*. 125(3):257-71. (Article received an Editorial) Mar;125(3):249-52). Citations: 133
   d) Carlos Hidalgo, B Hudson, M Greaser, S Labeit and Henk **Granzier**. PKC phosphorylation of titin's PEVK element: a novel and conserved pathway for modulating myocardial stiffness. *Circulation Research,* 2009;105;631-638 (Article received an Editorial) Citations: 159

5. **Clinical relevance of titin.** In collaboration with clinician scientists we have shown that deranged phosphorylation of titin contributes to diastolic dysfunction of the heart. We compared LV myocardial muscle strips obtained from hypertensive patients with and without HFpEF. In the HFpEF group a PKA/PKG site in the N2B element was hypo-phosphorylated whereas one of the PKC sites in the PEVK region was hyper-phosphorylated. Analysis showed that deranged titin phosphorylation occurs during the transition to HFpEF and might be causative for the passive stiffness increase in HFpEF (ref a). We also focused on titin in HFrEF, and discovered isoform switches in DCM (ref b), that truncation mutations in titin cause DCM (ref c) and missense mutations ARVC (ref. d). This work is well cited and continues to be influential in the titin field.
   a) Zile MR, Baicu CF, Ikonomidis JS, Stroud RE, Nietert PJ, Bradshaw AD, Slater R, Palmer BM, Van Buren P, Meyer M, Redfield MM, Bull DA, **Granzier** HL, LeWinter MM. Myocardial stiffness in patients with heart failure and a preserved ejection fraction: Contributions of collagen and titin. Circulation. 2015;131:1247-1259 Citations: 71

b) Nagueh SF, Shah G, Wu Y, King N, Lahmers S, Witt CC, Becker K, Labeit S, and Henk **Granzier**. 2004 Altered titin expression, myocardial stiffness, and left ventricular function in patients with dilated cardiomyopathy. *Circulation*, 110:155-162. (Article received an Editorial) Citations: 278

c) Gerull B, Gramlich M, Atherton J, McNabb M, Sasse-Klaassen S, Seidman JG, Seidman C, **Granzier** H, Labeit S, Frenneaux M, Thierfelder, L. 2002 Titin mutation in familial dilated cardiomyopathy. *Nature Genetics*, 30(2): 201-4. Citations: 415.

d) Matthew Taylor, S Graw, C Barnes, D Slavov, F Brun, B Pinamonti, E Salcedo, W Sauer, S Pixaras, G Sinagra, Henk **Granzier**, Luisa Mestroni. Titin mutations in arrhythmogenic right ventricular cardiomyopathy: a new player in disease pathogenesis. Circulation. 2011;124(8):876-85. Citations: 158

I have published 210 articles that have been cited >16500 and that have an H-factor of 71. For a complete list: https://scholar.google.com/citations?hl=en&user=5zUpVocAAAAJ&view_op=list_works&cstart=0&pagesize=20

Research Support

R01 HL062881 Granzier (PI) 08/01/95-07/31/2021
Function of giant sarcomere matrix proteins in muscle.

R01HL115988 Granzier (PI) 03/01/07-01/31/22
Titin-based adaptations in cardiac function.
The major goals are to study the role of the A-band segment of titin in length sensing and crossbridge cycling kinetics.

R01AR053897 Granzier (PI) 08/01/07-07/31/22
Roles of nebulin in structure and function of skeletal muscle.
The major goals of this project are to study the role of nebulin in muscle contractility.

1R01HL124007 Granzier (MPI with Dr. LeWinter) 09/01/14-06/30/18
Effect of hypertension on cardiac function.
The major goals of this project are to study biopsies from HFpEF patients and evaluate changes in myofibrillar proteins. (MPI with University of Vermont and Washington State University)