BIOGRAPHICAL SKETCH

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NAME: Joseph M Muretta

eRA COMMONS USER NAME (credential, e.g., agency login): jmuretta

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Montana State University	BS	05/2001	Environmental Science
University of Nevada, Reno	PhD	08/2007	Biochemistry
University of Minnesota, Minneapolis	Post-Doctoral	03/2011	Biophysics

A. Personal Statement

My laboratory develops materials for use in DMD dystrophin replacement therapy and for cancer immunotherapy, and drug-discovery. Our efforts are inspired by mechanistic studies of protein structure and function, and are driven by protein biochemistry and biophysics approaches, protein engineering workflows, and small molecule screening. Projects in the lab include developing optimized therapeutic protein sequences for DMD replacement therapy in muscular dystrophy. Other projects focus on engineering allosteric modulators of cell surface receptors, engineering new therapeutic antibodies, antibody-cytokine fusion proteins, and antibody-oligonucleotide conjugates. We use molecular structure determination and computational modeling to direct protein engineering and *in vitro* discovery workflows to identify new protein sequences that can be advanced to the clinic. We subject lead candidates to biochemical and biophysical characterization and collaborate with clinical scientists to perform preclinical testing.

My Lab's work is highly cooperative. In the current project, we are working with **Dr. Murti Salapaka** and **Dr. Ervasti** to develop AI/ML tools for predicting protein mechanics based on protein sequence. This work will provide key insights into the biochemical and biophysical properties of a wide range of proteins including ones we study in our lab such as dystrophins, antibodies, and T-cell receptors.

Enabling technologies in the Muretta Lab directly related to this proposed work include recombinant protein expression in a variety of systems including insect cells and mammalian cells, protein purification and biophysical characterization, and high-precision/throughput approaches in biophysical spectroscopy and protein structure determination (FRET, X-ray crystallography, Cryo-EM, and NRM). Dr. Muretta and Dr. Salapaka have collaborated with Dr. Ervasti for more than 6 years investigating the structural and mechanical determinants of dystrophin function.

Ongoing and recently completed projects that I would like to highlight include:

Sponsored Research Agreement complete
J Muretta MPI
05/01/2019 – 03/31/2024
Sarepta Therapeutics
Engineering Mini-/Micro-Dystrophin for Gene Therapy
Project goals: This project uses approaches in protein engineering and FRET-based detection of protein structural dynamics, X-ray crystallography and electron microscopy, to investigate gene therapy product for the treatment of Duchenne Muscular Dystrophy.

Sponsored Research Agreement
J Muretta MPI
07/01/2023 – 03/31/2026
Sarepta Therapeutics
Structural and molecular determinants of investigational micro-dystrophin stability and efficacy.
Project goals: Structural studies of investigational micro-dystrophins, dystrophin, and utrophin.

Hyundai Hope on Wheels
P Gordon Co-I Peds, J Muretta Co-I BMBB
08/01/2023 – 07/31/2025
Hyundai Hope Foundation award to Children's Hospitals and Clinics of Minnesota.
A novel antibody-drug conjugate against CD179a for the treatment of B-lineage acute leukemia/lymphoma.
Project goals: Develop and optimize antibody-drug conjugates for treatment of B-lineage acute leukemia/lymphoma.

1R33CA272331-01A1
J Muretta MPI
07/01/2023 – 06/30/2026
Engineering Protein Modulators of Notch Activation for T-cell immunotherapy
Project goals: engineer novel protein activators of the Notch family of cell surface receptors to enhance T-cell differentiation from induced pluripotent stem cells

B. Positions, Scientific Appointments, and Honors

Positions & Scientific Appointments

2025-	Assistant Professor, Univ. of Minnesota Dept. of Biochemistry, Molecular Biology and Biophysics.
2024-	Director, Antibody Engineering Shared Resource, Masonic Cancer Center.
2014-	Assistant Professor, Research, Univ. of Minnesota Dept. of Biochemistry, Molecular Biology and Biophysics
2011-13	Research Associate, Univ. of Minnesota Dept. of Biochemistry, Molecular Biology and Biophysics

Other Experience and Professional Memberships

2013-18	American Heart Association
2005-20	Biophysical Society
2003-07	American Diabetes Association

Honors

2017	Biophysical Society Motility Sub-group Co-Chair.
2017-	Biophysical Society Early Careers Committee Member 2017-2018.
2016	Invited Speaker, Muscle and Molecular Motors Gordon Research Conference.
2014	Biophysical Society Motility Sub-group Invited Speaker.
2013	Notable Poster, Motility Subgroup Meeting Biophysical Society Annual Meeting. "The myosin power-stroke gates phosphate release."
2011	Notable Poster, Muscle and Molecular Motors Gordon Research Conference. "Direct detection of the myosin power-stroke using transient time-resolved FRET"

2010 Barnum Postdoctoral Travel Award: “A time-resolved fluorescence spectrometer with sub-millisecond data acquisition time reveals structural transitions in the actomyosin interface.”
2008-11 Ruth L. Kirschstein National Research Service Award (NIH Postdoctoral Fellow): “Spectroscopic probes of smooth-muscle contraction.”
2007 Outstanding Student Poster, FASEB Conference on Glucose Transporter Biology. “Insulin releases Glut4 from static storage compartments into cycling endosomes and increases the rate constant for Glut4 exocytosis”
2005 Outstanding Student Presentation, Granlibakken Research Convocation.
2005 Outstanding Student Presentation, FASEB Conference on Glucose Transporter Biology. “Expression of a synapsin IIb site 1 phosphorylation mutant in 3T3-L1 adipocytes inhibits basal intracellular retention of Glut4”

C. Contributions to Science

1. Protein structural dynamics and kinetics
My lab has investigated the structural and mechanical determinants of dystrophin. Published studies include determination of the atomic structure of dystrophin’s tandem calponin homology actin-binding domain crystalized in a closed structural state. This structure provides atomic details and insights into the mechanism of actin-binding by dystrophin. Our work also includes studies of the mechanical properties of dystrophin and utrophin, in a long-standing collaboration with Dr. James Ervasti.

In other areas of protein structural dynamics, I have developed novel FRET-based approaches for dissecting protein structure-function relationships including sensors for measurement of protein structural dynamics and kinetics. This work spans a wide range of model systems, problems, and disease areas. This approach answers critical questions in the biophysics of protein function and points the way toward novel approaches to therapeutic discovery, and mechanistic insights into disease pathophysiology. Examples of this work include:

Streeter O, Shi K, Vavra J, Aihara H, Ervasti JM, Evans R 3rd, **Muretta JM**. Human dystrophin tandem calponin homology actin-binding domain crystallized in a closed-state conformation. Acta Crystallogr D Struct Biol. 2025 Mar 1;81(Pt 3):122-129. doi: 10.1107/S2059798325001457. Epub 2025 Feb 26. PMID: 40007458; PMCID: PMC11883666.

Hua C, Slick RA, Vavra J, **Muretta JM**, Ervasti JM, Salapaka MV. Two operational modes of atomic force microscopy reveal similar mechanical properties for homologous regions of dystrophin and utrophin. bioRxiv [Preprint]. 2024 May 20:2024.05.18.593686. doi: 10.1101/2024.05.18.593686. PMID: 38826288; PMCID: PMC11142110.

Rohde JA, DD Thomas DD, and JM **Muretta**. Heart failure drug changes the mechano-enzymology of the cardiac myosin powerstroke. Proc Natl Acad Sci U S A. 2017 Feb 21. pii: 201611698. PMC5347578

Rohde JA, O. Roopnarine, DD Thomas, and JM **Muretta**. Mavacamten stabilizes the auto-inhibited state of two-headed cardiac myosin. Proc Natl Acad Sci U S A. 2018 Aug 7;115(32):E7486-E7494. doi: 10.1073/pnas.1720342115. PMC6094135

2. Time-resolved Optical Spectroscopy
I helped develop novel approaches to time-resolved optical spectroscopy that have enabled a powerful new mode of FRET detection, high-throughput nanosecond time-resolved FRET. This approach and the resulting biophysical discoveries that grew from it are answering important structure-function questions in protein biophysics. It has also opened the door to a structure-based platform for high-throughput drug discovery of allosteric modulators and to applications in protein engineering (the focus of my lab’s current work). This expertise is relevant to all Aims of the present proposal.

Muretta, JM, A Kyrychenko, AS Ladokhin, D Kast, GD Gillispie, and DD Thomas. 2010. High -performance time-resolved fluorescence by direct waveform recording. *Rev Sci Instrum*, 81:103101 (1-8). PMC2980540

Petersen, KJ, KC Peterson**,** JM **Muretta**, SE Higgins, GD Gillispie, and DD Thomas. 2014. Fluorescence lifetime plate reader: resolution and precision meet high-throughput. *Rev Sci Instrum* 81:113101-113107. PMC4242087

Muretta, JM, JA Rohde, DO Johnsrud, S Cornea, and DD Thomas. 2015. Direct real-time detection of the structural and biochemical events in the myosin power stroke. Proc Natl Acad Sci U S A. 112:14272-7. PMC4655543

Muretta, JM, AR Thompson, DM Rasmussen, A Majumdar, EB Faber, EF Ruff, DD Thomas, and NM Levinson. 2018. Quantitative conformational profiling of kinase inhibitors reveals origins of selectivity for aurora kinase activation states. *Proc Natl Acad Sci U S A* E11894–E11903. PMC6304972

3. Structure-based, FRET-enabled drug discovery and mechanism of action

Small molecule and protein-based allosteric modulators are becoming increasingly important therapeutics. Discovery efforts have plumbed only a small cross section of the therapeutic potential of small-molecule chemical space and protein sequence space. To discover the small molecule and protein-based allosteric modulators of the future, we need assays that can specifically enrich for therapeutic leads that modulate target structural dynamics in therapeutically meaningful ways. Technologies and approaches we helped develop earlier in my career are helping us tackle this problem. For example, we use high-throughput nanosecond resolved FRET measurements engineered to detect critical protein structural transitions in high-throughput drug discovery efforts performed on up to the multi Million compound library scale or during protein engineering selection workflows. These assays evolve from high-precision measurements repurposed for high-throughput screening workflows. We are using these concepts to direct discovery of allosteric immunotherapeutics.

Muretta JM, Rajasekaran D, Blat Y, Little S, Myers M, Nair C, Burdekin B, Yuen SL, Jimenez N, Guhathakurta P, Wilson A, Thompson AR, Surti N, Connors D, Chase P, Harden D, Barbieri CM, Adam L, Thomas DD. HTS driven by fluorescence lifetime detection of FRET identifies activators and inhibitors of cardiac myosin. SLAS Discov. 2023 Jul;28(5):223-232. PMC10422832.

Ruff, EF, JM **Muretta**, AR Thompson, EW Lake, S Cyphers, SK Albanese, SM Hanson, JM Behr, DD Thomas, JD Chodera, and NM Levinson. 2018. A dynamic mechanism for allosteric activation of Aurora kinase A by activation loop phosphorylation. *ELife* 7:e32766. PMC5849412

Rohde, JA, O Roopnarine, DD Thomas, and JM **Muretta**. 2018. Mavacamten stabilizes an autoinhibited state of two-headed cardiac myosin. *Proc Natl Acad Sci U S A* 115:E7486–E7494. PMC6094135

Lake, EW, JM **Muretta**, AR Thompson, DM Rasmussen, A Majumdar, EB Faber, EF Ruff, DD Thomas, and NM Levinson. 2018. Quantitative conformational profiling of kinase inhibitors reveals origins of selectivity for aurora kinase activation states. Proc Natl Acad Sci U S A, E11894–E11903. PMC6304972

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/pubmed/?term=Muretta+JM