BIOGRAPHICAL SKETCH

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NAME: Margaret M. Stratton

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POSITION TITLE: Assistant Professor of Biochemistry and Molecular Biology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Stonehill College, Easton MA	B.S	05/2005	Biochemistry
SUNY Upstate Medical University, Syracuse NY	Ph.D	05/2010	Biochemistry and Molecular Biology
University of California, Berkeley CA	Postdoc	08/2015	Molecular and Cell Biology

A. Personal Statement

The overarching goal of my lab is to understand memory formation and storage at the protein level. My central hypothesis is that long-lived changes in protein structure and activity provide the molecular framework by which memory is generated and maintained. I am in a unique position to be able to tackle these questions. Many of the components that are crucial to long-term memory formation are known and I am able to exploit the many tools available, and create new tools, to manipulate cells and study mechanistic effects. The missing piece of this puzzle is how these molecular components actually lead to the maintenance of a memory. I have positioned myself to be able to tackle these difficult questions. In 2018 I was awarded an R01 from NIGMS, which has supported my lab in addition to start-up funds from UMass Amherst. This funding facilitated my building a vibrant and productive research program at UMass over the last five years -- I have been successful in recruiting 1 postdoc, 5 PhD students, 2 MS students, and 15 undergraduates. My ambition is to provide mechanistic pictures of complex cellular processes such as memory formation, cardiac pacemaking, and fertilization. My education and training has provided me the molecular background to carry out this work in a methodical and grounded manner. My graduate work with Stewart Loh focused on protein engineering and provided me with a deep understanding of complex protein dynamics and biophysical properties. I capitalized on this understanding during my postdoctoral training with John Kuriyan, where I earned a Jane Coffin Childs fellowship to study structural and biochemical aspects of a complex oligomeric protein (CaMKII), which is known to play a critical role in learning and memory. It was during this time - in a protein crystallography lab - that I became fascinated by the biological roles of CaMKII in memory, cardiac signaling, and fertilization/development. My background in protein chemistry and protein design, along with my cell biology experience, provides me with the skillset necessary to address these questions.

Research Support

R01 GM123157 Stratton (PI)
01/01/18 – 11/30/22 NIH/NIGMS
CaMKII biophysics and its role in LTP

R35 GM145376 Stratton (PI)
09/24/22 – 8/31/27 NIH/NIGMS
Unraveling the molecular events driven by CaMKII in Ca2+-coupled cells

B. Positions, Scientific Appointments, and Honors

Positions

2005-2010	Graduate student in the laboratory of Stewart Loh, Department of Biochemistry and Molecular Biology, SUNY Upstate Medical University
2006-2008	Teaching Assistant, Cell biology, Biochemistry and Neurobiology, SUNY Upstate Medical University
2010-2015	Postdoctoral fellow in the laboratory of John Kuriyan, Department of Molecular and Cell Biology, University of California, Berkeley
2015-present	Assistant Professor, Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst

Honors

2025 Outsanding Research Award (UMass Amherst)
2025 Jennifer Normanly Award for Excellence in Service (UMass Amherst)
2004 Award for travel to the American Chemical Society meeting (Stonehill College)
2004 Research and Development Fellowship, Pfizer Inc.
2005 *Sigma Zeta* Science and Mathematics Honor Society
2005 Bachelor of Science, *cum Laude* (Stonehill College)
2008 Graduate Student Poster Award (Annual meeting of the Protein Society, San Diego, CA)
2008 Graduate Student Best Written Research Proposal Award (SUNY Upstate Medical University)
2010 John Bernard Henry, M.D., Endowed Scholarship Award (SUNY Upstate Medical University)
2011 Jane Coffin Childs Postdoctoral Fellowship

Professional Service and Other Experiences

2024-2026	Conference organizer, FASEB Protein kinases and protein phosphorylation
2006 – present	Member, Protein Society
2017 – present	Membership committee, Protein Society
2019	Session chair, Proteins GRC
2019	Session chair, FASEB Protein kinases and protein phosphorylation
2019	Session chair, Banbury meeting on CaMKII
2020	NIGMS study section for Special Emphasis Panel

C. Contributions to Science

1. *Designing new platforms and utilizing existing platforms to create protein biosensors.* I created a new biosensor platform called alternate frame folding. I converted a small Ca^{2+}-binding protein, calbindin D_{9k}, into a calcium sensor using this novel strategy for generating biosensors. I did this by duplicating the C-terminal portion of the calbindin sequence and ligating this to the N-terminus, creating an additional "frame" of folding. We used an existing template for creating protein kinase biosensors (pioneered by Roger Tsien and Alexandra Newton) to create a new substrate-based sensor for CaMKII activity. This biosensor will significantly enhance our ability to understand endogenous CaMKII and its role in diverse biological pathways.

a. M. M. Stratton*, S. McClendon*, D. Eliezer, and S. N. Loh, (2011) "Structural characterization of two alternate conformations in a Calbindin D_9k-based molecular switch", *Biochemistry*, **50**, 5583-5589.
b. M. M. Stratton, D. M. Mitrea and S. N. Loh, (2008) "A Ca^{2+}-sensing molecular switch based on alternate frame protein folding", *ACS Chem. Biol.* **3**, 723-732.
c. G. Ardestani*, M. West*, T.J. Maresca, R. A. Fissore, M. M. Stratton, (2019) "FRET-based sensor for CaMKII activity (FRESCA): A useful tool for assessing CaMKII activity in response to Ca^{2+} oscillations in live cells", *JBC*, **294**, 11876.

2. *Dissecting the role of endogenous CaMKII in long-term potentiation.* During my postdoctoral work, I reconstituted the frequency-dependent activation of CaMKII using purified proteins. CaMKII, a dodecameric kinase assembly, plays major roles in cardiac and neuronal signaling. Interestingly, it was found that in cells, CaMKII responds to the frequency of Ca^{2+} pulses, and not just the amplitude of the signal. However, the molecular basis of this response was unclear. I developed an *in vitro* assay to reconstitute this frequency-dependent response and discovered that the activation of CaMKII is dependent on the length of the linker connecting the kinase and hub domains in this protein. My work revealed that linker length directly tunes the frequency of Ca^{2+} pulse required for CaMKII activation.

Building on this work, in my own lab we have developed an assay to unambiguously sequence the CaMKII transcripts present in a hippocampal sample. We will continue to use this assay in other tissue samples to begin to sample the heterogeneity present, which has been very difficult to do. We have used these sequences to study how the different sequences of CaMKII relate to activity. We found that the hub domain (the domain responsible for oligomerization) plays a crucial role in regulating activity, which was surprising. This new information provides a potential new route for screening for or designing allosteric modulators.

As we became interested in how the protein domains of CaMKII seems to have a role in in regulating the other domain's activity, hub regulates kinase activity, we became very interested in studying the stability of each domain how it may affect each other's stability. We found that the hub is extremely stable by itself and it is destabilized by the kinase.

a. L. H. Chao, M. M. Stratton, I. H. Lee, J. Levitz, D. Mandell, T. Kortemme, H. Schulman, J. Kuriyan, (2011) "A mechanism for tunable autoinhibition in the structure of a human Ca^{2+}/calmodulin- dependent kinase II holoenzyme", *Cell*, **146**, 732-745.
b. R. Sloutsky*, N. Dziedzic*, M. Dunn, R. Bates, A. P. Torres-Ocampo, B. Page, J. Weeks, M. M. Stratton, (2019) "Heterogeneity in human hippocampal CaMKII transcripts reveals allosteric hub-dependent regulation", *Science Signaling*, **13**, eaaz0240.
c. A.P. Torres-Ocampo, C. Özden, A. Hommer, A. Gardella, E. Lapinksas, A. Samkutty, E. Esposito, S.C. Garman and M.M Stratton, (2020) "Characterizing CaMKIIα holoenzyme stability", *Protein Science*, **29**, 1524-1534.
d. C. Özden, S. MacManus, R. Adafia, A. Samkutty, A.P. Torres-Ocampo, S.C. Garman and M.M. Stratton, (2024) "Ca^{2+}/CaM dependent protein kinase II (CaMKII)α and CaMKIIβ hub domains adopt distinct oligomeric states and stabilities", *Protein Science*, 33, e4960.

3. *Subunit exchange in CaMKII and a novel RAKEC mechanism promotes longevity of the kinase signal.* Subunit exchange and RAKEC may play a role in maintaining long-term memories by extending the activation signal to outlast the rate of protein degradation. It is known that CaMKII plays a major role in long-term potentiation, or long-term memory formation. During my postdoc, I discovered that CaMKII exchanges subunits and discovered that it does so in an activation-dependent manner. These activated subunits and mix with previously unactivated subunits to spread phosphorylation within a complex, revealing a mechanism for stabilizing and spreading the phosphorylation signal that may underlie long-term memory formation. More recently, in collaboration with Yasunori Hayashi's group, we proposed a new mechanism for sustained activation, which we termed RAKEC: reciprocal activation within a kinase-effector complex, where CaMKII maintains an active conformation through interactions with specific substrates.

a. M. M. Stratton*, I. H. Lee*, M. Bhattacharyya, S. M. Christensen, L. H. Chao, H. Schulman, J. T. Groves, J. Kuriyan, (2014) "Activation-triggered subunit exchange between CaMKII holoenzymes facilitates the spread of kinase activity", *eLife*, **3**: e01610.
b. T. Saneyoshi, H. Matsuno, A. Suzuki, H. Murakoshi, N.G. Hedrick, E. Agnello, R. O'Connell, M. M. Stratton, R. Yasuda, Y. Hayashi, (2019) "Reciprocal Activation within a Kinase-Effector Complex Underlying Persistence of Structural LTP" *Neuron*, **102**:1199-1210.
c. Özden, R. Sloutsky, T. Mitsugi, N. Santos, E. Agnello, C. Gaubitz, J. Foster, E. Lapinskas, E.A. Esposito, T. Saneyoshi, B.A. Kelch, S.C. Garman, Y. Hayashi and M.M. Stratton, (2022) "CaMKII binds both substrates and activators at the active site", *Cell Reports*, 40, 111064.

A complete list of published work:
https://www.ncbi.nlm.nih.gov/myncbi/margaret.stratton.1/bibliography/public/

BIOGRAPHICAL SKETCH

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NAME: Ruth Adafia

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

POSITION TITLE: Graduate student

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Ghana, Legon, Ghana	BS	05/2021	Biochemistry, Cell and Molecular Biology
University of Massachusetts, Amherst	Ph.D.	present	Molecular and Cellular Biology

A. Personal Statement

I am a Ph.D. candidate in Molecular and Cellular Biology at the University of Massachusetts Amherst, working with Margaret Stratton. My current research centers on understanding the structural basis for differential Ca^{2+}/calmodulin sensitivity among CaMKII variants using cryo-EM alongside biophysical techniques. This work has broad implications for how isoform-specific structural features of CaMKII decode calcium oscillations to drive cellular function. Over the past two years, I have gained experience with optimizing conditions for cryo-EM sample preparation, and I currently serve as a teaching assistant supporting other users with both plunge-freezing and grid screening on our in-house Tundra microscope at UMass, Amherst. I am also experienced in cryo-EM data analysis using CryoSPARC and CryoDRGN, with a particular interest in probing conformational and compositional heterogeneity in CaMKII variants. I have also contributed to two peer-reviewed publications exploring CaMKII hub domain structure and ligand-induced conformational changes. The collaboration with the NCCAT will provide a valuable opportunity to learn more about the high-energy microscopes and build experience in cryo-EM data collection while advancing my dissertation research. I am excited to work alongside experts in the field, gain exposure to cutting-edge instrumentation, and contribute meaningfully to our understanding of protein structure and function.

B. Positions, Scientific Appointments, and Honors

Positions

2021-2022 Teaching Assistant, Biochemistry, Cell and Molecular Biology, Legon, University of Ghana
2022-2023 Teaching Assistant, Chemistry, University of Massachusetts, Amherst.
2022-Present Graduate student, Molecular and Cellular Biology, University of Massachusetts, Amherst
2025-Present Teaching Assistant, Institute for Applied Life Sciences, University of Massachusetts, Amherst

Professional Services and Other Experiences

2023-2024 Student Vice President, Molecular and Cellular Biology, University of Massachusetts, Amherst
2023-2025 Trainee, Chemistry-Biology Interface (CBI) training program, NIH (T32) funded

C. Contributions to Science

In exploring molecular dynamics of CaMKII, I contributed to work showing characteristic properties and distinct oligomeric states of CaMKIIα and CaMKIIβ hub using mass photometry.

a) Can Özden, Sara MacManus , **Ruth Adafia**, Alfred Samkutty, Ana P Torres-Ocampo, Scott C Garman, Margaret M Stratton. Ca2+/CaM dependent protein kinase II (CaMKII)α and CaMKIIβ hub domains adopt distinct oligomeric states and stabilities. 2024 Apr;33(4): e4960.doi: 10.1002/pro.4960.

CaMKII in previous studies has been shown to be a high affinity binding target of Gamma hydroxybutyrate (GHB) in the brain. To explore effects on CaMKII stoichiometry, the Wellendorph group collaborated with the Stratton lab, where, I helped characterize ligand-induced changes in CaMKII hub domain. This work revealed the GHB anaolog-induced stacking of CaMKIIα hub domain.

b) Dilip Narayanan, Anne Sofie G Larsen, Stine Juul Gauger, **Ruth Adafia**, Rikke Bartschick Hammershøi, Louise Hamborg, Jesper Bruus-Jensen, Nane Griem-Krey, Christine L Gee, Bente Frølund, Margaret M Stratton, John Kuriyan, Jette Sandholm Kastrup, Annette E Langkilde , Petrine Wellendorph , Sara M Ø Solbak. Ligand-induced CaMKIIα hub Trp403 flip, hub domain stacking, and modulation of kinase activity. 2024 Oct;33(10): e5152.doi: 10.1002/pro.5152