

CHEMICAL ENGINEERING

DISTINGUISHED YOUNG SCHOLARS SERIES



MAGGIE FETTIS

July 29, 2019

Postdoctoral Fellow
Georgia Institute of Technology

Advancing Galectin-1 as an Immunotherapeutic

ABSTRACT: Analogous to the proteome or genome, the glycome comprises all carbohydrate structures in an organism. Lectins are carbohydrate binding proteins capable of translating the glycome's sugar code into cell signaling processes that drive homeostatic and disease states, especially in the immunological context. A subset of lectins, galectins are a family of proteins with evolutionarily conserved structures and shared affinities. Broadly, galectins can modulate immune cell behavior, including stimulating or tempering immune responses in homeostasis, autoimmunity, infection, and cancer. Though galectins are potent immunomodulators, translating galectin-based therapies is challenging. This failure in translation is likely due to many factors, including off target effects, repeated dosing strategies leading to immunogenicity, and short serum half-life. Overall, the research program described below combines concepts of synthetic protein modification, a delivery vehicle, and self-assembly to improve the therapeutic potential of galectin-1.

The potential for immunomodulatory clinical use of galectin-1 is challenged by two biochemical features of the protein. The immunomodulatory activity of galectin-1 is driven by a reversible, concentration dependent dimeric structure. Upon dilution, the protein structure disassociates, and loss of immunomodulation is observed. A second property of galectin-1 is that due to 4 surface cysteine residues, it is deactivated in sites of oxidation, such as inflammatory environments. Initially to improve the therapeutic efficacy of galectin-1, we explored site-directed mutagenesis of oxidation sensitive amino acids and stabilization of the dimeric structure through polyethylene glycol crosslinking.[1] Using a mutant of galectin-1 with a single cysteine residue on each monomer, we used polyethylene glycol diacrylate to form a stably dimeric construct with reduced susceptibility to oxidation. With this PEG stabilized protein, we observed more than a 10-fold improvement in lectin activity. This patent-pending research and its presentation have garnered two "Best Oral Presentation" awards and has been presented at numerous national conferences. After promising in vitro

experiments, we moved this therapy in vivo to a model of sterile inflammation with results comparable to galectin-1 not stabilized with PEG. This observation inspired us to look to delivery vehicles and other methods to improve the delivery and decrease the effective dose of galectin-1.

To improve the localization and stability of protein over time, we developed a peptide delivery vehicle derived from the self-assembling, β -sheet fibrilizing peptide, Q11. After peptide assembly into β -sheet nanofibers, these structures can be easily processed using desolvation into micron sized hydrogels, or “microgels”. Previous research has shown that binding galectin-1 to a ligand can improve lectin activity.[2] Using desolvation and Q11 nanofibers appended with a carbohydrate that reversibly binds lectins, we were able to control the release of active protein. Overall, we saw high protein encapsulation efficiency into microgels and successful controlled release when the peptide nanofibers were appended with a carbohydrate.[3] This research has been presented at national conferences and shortly after publication, was highlighted in the “Journal of Materials Chemistry B Hot Papers”.

Finally, we sought to decrease effective dose of galectin-1 through increasing the carbohydrate recognition domain valency via assembly and recombinant fusion with galectin-3.

To this end, we created a chimeric, tetravalent fusion consisting of galectin-1 and galectin-3 with a dimeric assembling domain in the linker region inspired by previous collaborative work.[4,5] Strikingly, this protein more effectively reduced T cell metabolism at very low concentrations than unassembled galectin-1 or its previously described PEG-stabilized variant (Figure 1).[6] This research was provisionally protected by the University of Florida patent office, published, and awarded a “Best Poster Award” at a national conference.

Overall, we envision these engineered galectin variants and delivery vehicles as broadly useful for applications in immunomodulation. Current collaborations are ongoing and are testing the efficacy in vivo of both the PEGylated galectin-1 and the assembled fusion of galectin-1 and galectin-3.

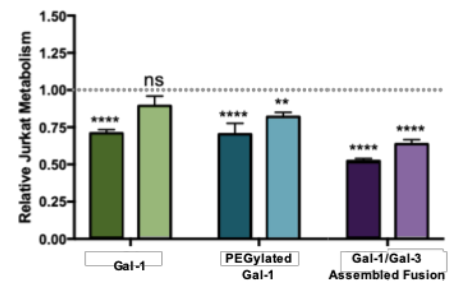


Figure 1. Jurkat T cell metabolism of galectin-1 (Gal-1), PEGylated galectin-1, and Fused and Assembled galectin-1/galectin-3. The darker shades are 5 μ M protein treatment and the lighter shades are 0.5 μ M protein treatment.

BIOGRAPHY: Margaret (Maggie) Fettis earned her PhD from the University of Florida’s Biomedical Engineering department in May 2019 under the mentorship of Dr. Gregory Hudalla. During her graduate studies, Maggie was recognized by several honors including the Attributes of a Gator Engineer Award for Leadership from the Herbert Wertheim College of Engineering and the Emerging Scholar Dissertation Award presented by Association for Academic Women supported by University of Florida President’s Office. Her dissertation research explored carbohydrate decorated biomaterials and proteins that interact with carbohydrates which resulted in numerous publications and patents. She earned her Bachelor of Science in Biomedical Engineering from the University of Rochester in 2008. Between college and graduate school, Maggie researched T cell biology at the Center for Vaccine Biology and Immunology at the University of Rochester Medical Center under the guidance of Dr. Jim Miller. Since July 2019, Maggie has engaged in postdoctoral research with Dr. Susan Thomas at Georgia Institute of Technology.

LECTURE 4:00 - 5:00 (PAA) A118
Happy Hour in Benson Hall Lobby Following

W **CHEMICAL ENGINEERING**
UNIVERSITY of WASHINGTON
Knowledge and solutions for a changing world

1. **Margaret M. Fettis** and Hudalla, Gregory A. (2018) Engineering Reactive Oxygen Species-Resistant Galectin-1 Dimers with Enhanced Lectin Activity. *Bioconjugate Chemistry* 29 (7), 2489-2496.
2. He, J. and Baum, L. (2004) Presentation of galectin-1 by extracellular matrix triggers T cell death. *Journal of Biological Chemistry* 279 (6), 4705-4712.
3. **Margaret M. Fettis**, Yaohua Wei, Antonietta Restuccia, Justin J. Kurian, Shannon M. Wallet, and Gregory A. Hudalla (2016) Microgels with tunable affinity-controlled protein release via desolvation of self-assembled peptide nanofibers. *Journal of Materials Chemistry B* 4 (18), 3054-3064.
4. Shaheen A. Farhadi, Evelyn Bracho-Sanchez, **Margaret M. Fettis**, Dillon T. Seroski, Sabrina L. Freeman, Antonietta Restuccia, Benjamin G. Keselowsky, and Gregory A. Hudalla. (2018) Locally anchoring enzymes to tissues via extracellular glycan recognition. *Nature Communications* 9 (1), 4943.
5. Antonietta Restuccia, **Margaret M. Fettis**, Shaheen Farhadi, Matthew Molinaro, Bryant Kane, Gregory Hudalla. (2018) "Evaluation of Self-assembled Glycopeptide Nanofibers Modified with N,N'-diacetyllactosamine for Selective Galectin-3 Recognition and Inhibition" *ACS Biomaterials Science & Engineering* 4 (10), 3451-3459.
6. **Margaret M. Fettis**, Shaheen A. Farhadi, and Gregory A. Hudalla. (2019) A chimeric, multivalent assembly of galectin-1 and galectin-3 with enhanced extracellular activity. *Biomaterials Science* 7 (5), 1852-1862.