

**Understanding the Chemical Basis of Neuronal Development and  
Communication:**

**I. The Role of Fucose  $\alpha$ (1-2) Galactose Carbohydrates in Neuronal Growth**

**II. Structure-Function Analysis of Chondroitin Sulfate in the Brain**

Thesis by

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In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

2009

(Defended 17 July 2008)

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*...for my family...*

## Acknowledgments

Without the help and support of many people, both scientific and personal, the work in this thesis would not have been possible. I would like to thank my advisor, Linda Hsieh-Wilson, for her advice and guidance. I would also like to thank the members of my committee, Judy Campbell, Mary Kennedy, and Paul Patterson. Without any of them, this thesis would not have come to completion. I would also like to thank Dennis Dougherty, even though he is not technically on my committee, he was like a second mentor and really helped our lab in the beginning, letting us join his group meetings and giving us much advice.

My undergraduate professors deserve special thanks as well since it is because of them that I came to graduate school. Dr. Robert Vellanoweth at Cal State LA was such a great mentor, a young professor with such an amazing mind, brilliant really. He was such an inspiration to me, being of Mexican decent, having gone to Cal State LA himself as an undergrad and then back as a professor leading his own lab and directing his own unique research. It was a wonderful learning experience and he showed me how to design my own experiments and think independently about the projects I worked on. Of course, I am indebted to Dr. Carlos Gutierrez at Cal State LA. I met Dr. Gutierrez as a senior in high school trying to decide which college to go to. He was the first academic professional I ever met, doing “lab research,” which is what I wanted to do, even though at that time I wasn’t even sure what exactly that meant. Dr. Gutierrez is such a wonderful human being, caring, easy going and great, I think it was because of him that I thought I could stick it out in the science field and make it through to get my Ph.D. There are many more wonderful people at Cal State LA that made me getting to Caltech possible,

it's hard to mention everyone. However, I cannot go without special thanks to Vicki Kubo-Anderson. Without Vicki, nothing would be possible. Without Vicki, the biochemistry department would crumble. Without Vicki, we students would be in such a terrible state we wouldn't function either. She was like a mom, a mentor, a counselor, but most importantly, a friend.

The members of the Hsieh-Wilson lab, both past and present, have been a great group to work with. All the original members were unforgettable each in their own way, Raymond Doss, Sarah Tully, Nelly Khidekel, Katherine Poulin-Kerstien, Sherry Tsai, Nathan Lamarre-Vincent and Lori Lee. We helped each other get through some rough and tough times, learning the basics and going from there. Lori Lee was my first partner on the fucose project and, more than that, we became good friends. Sarah Tully did all the synthesis on the chondroitin sulfate project and without her this work couldn't have happened. It was really tough, her getting the synthesis to work and me trying to get those neurons to grow and behave. There were some very long nights and crazy "losing my mind" moments, it was great! Isn't that what science is really about? After the first group of students then came Heather Murrey. What can I say about Heather, other than she is the craziest, most intelligent scientist I think I have ever met. Through the years we have become wonderful friends and I will never forget all the times we've shared, both in and out of lab. Dr. Marian Bryan joined the lab and changed my life. Not only did we share time in the lab, she became my own personal doctor, counselor, therapist and everything in between. Katie Saliba is an amazing chemist and an even better being. She is, through and through, one of the most wonderful, caring people in this world. I would also like to thank everyone else that I have met and worked with. Each and

everyone of you has helped me get to this point: Maria Chiriac, Dr. Stacey Kalovidouris, Dr. Eric Shipp, Dr. Manish Rawat, Tammy Campbell, Dr. Helen Cheng, Dr. Ross Mabon, Monica Luo, Bruce Tai, Rob Moncure, Peter Clark, Claude Rogers, Dr. Mike Chang, Dr. Song-Gil Lee, Dr. Seok-Ho Yu, Dr. Jiang Xia, Jessica Rexach, Jessica Dweck, Long Phan, Gloria Sheng, Joshua Brown, and Joelle Radford. Of course I have to give special mention to some of the newest members in the lab, Young In Oh, Chithra Krishnamurthy, and Arif Wibowo. Although it hasn't been that long, I consider you close friends and I am thankful for all your support.

There are many more people outside of lab who have supported me and helped me survive. First and foremost, I would like to thank my parents, Bonifacia and Guadalupe. It is because of them that I am who I am today and it is for them that I have moved forward and continued my education to this point. My sister Marisol and brother Eric have always provided undying support, ever since my first day in elementary to my last day in graduate school. I know they have thought I was crazy for being in school this long, but they have always been there for me in every way. To all of my nephews and nieces, those here and those to come, this is for you. My dear friend Xiomara Padilla has been wonderful ever since we met. We have grown up together, cried through Quant, made it through graduation and now share our lives over Thai food, Shabu Shabu, chili cheese fries and, of course, Roscoe's. I have to thank Callie Bryan again. She has been the most wonderful friend, helping me get through these last years, pushing me forward, and buying me coffee, or anything else. I also have to thank one of my best friends, Wilbert Preyer. He has supported and believed in me every moment. Thank you all so much from the bottom of my heart, we did it!

## Abstract

Although carbohydrates are known to participate in many important processes including inflammation, cancer metastasis and pathogenic infection, their functional roles are only beginning to be understood on a molecular level. The challenge is that carbohydrates and glycoproteins are inherently difficult to study. Unlike DNA and proteins, carbohydrate structures are not template-encoded, and the modifications are challenging to detect *in vivo* and manipulate for structure-function analyses. As such, new tools are needed to complement the traditional biochemical and genetic approaches in order to advance our understanding of carbohydrates and their physiological roles. Here, we seek to understand the roles of carbohydrates in regulating the structure and function of proteins in the brain. Our major focus will be on two carbohydrate modifications that play important roles in neuronal communication, development and memory storage: fucosylation (Part I) and chondroitin sulfate glycosaminoglycan modifications (Part II).

In Part I, we describe our progress in elucidating the molecular mechanisms by which fucosyl saccharides regulate neuronal communication in the brain. Information flow in the brain is regulated by synapses, which are specialized sites of contact between neurons. Synaptic connections involve numerous molecular recognition events among proteins, carbohydrates, and small molecules. One of the molecules enriched at the synapse is the sugar L-fucose. Previous studies have suggested that fucose $\alpha$ (1-2)galactose (Fuc $\alpha$ (1-2)Gal) saccharides play essential roles in learning and memory. For instance, preventing formation of Fuc $\alpha$ (1-2)Gal linkages has been shown to cause reversible amnesia in animals. Despite these intriguing observations, proteins that

express the Fuc $\alpha$ (1-2)Gal epitope (glycoproteins) or proteins that bind this epitope (lectins) have not been identified. Through the use of several chemical probes, we have established that Fuc $\alpha$ (1-2)Gal associated proteins participate in a novel carbohydrate-mediated pathway for regulating neuronal growth. Additionally, we have found that Fuc $\alpha$ (1-2)Gal glycoproteins are prevalent in the developing brain and that synapsin Ia/Ib are the major Fuc $\alpha$ (1-2)Gal glycoproteins in the adult brain. In our attempts to identify Fuc $\alpha$ (1-2)Gal lectins, we have established that multivalent polymers enhance our ability to capture and characterize such proteins.

In Part II, we describe our efforts toward understanding the role of chondroitin sulfate glycosaminoglycans in neuronal development. Chondroitin sulfate (CS) glycosaminoglycans are linear, sulfated polysaccharides implicated in cell division, neuronal growth, and spinal cord injury. The structural complexity and heterogeneity of CS has hampered efforts to understand its precise biological roles. Although they exist as a heterogeneous mix in nature, it is thought that CS activity is dictated by a sulfation code where distinct sulfation sequences are spatially and temporally regulated and direct the biological activity of CS glycosaminoglycans. We have developed a chemical approach to evaluate the structure-activity relationship of CS as it effects neuronal growth. We generated the first synthetic library of well-defined CS oligosaccharides containing various sulfation sequences and have demonstrated that the CS-E sulfation sequence is a stimulatory motif that promotes the growth of several neuron types. Moreover, we determined that CS-E mediated stimulation of neurite outgrowth was facilitated by activation of midkine/PTP $\zeta$  and BDNF/TrkB signaling pathways.

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### List of Abbreviations

|                       |   |
|-----------------------|---|
| 1D                    | one-dimensional   |
| 2D                    | two-dimensional   |
| 2-dGal                | 2-deoxy-D-galactose   |
| 2-fucosyllactose      | L-fucose $\alpha$ (1-2)galactose $\beta$ (1-4)glucose       |
| 3-dGal                | 3-deoxy-D-galactose   |
| 6-dGal                | 6-deoxy-D-Galactose   |
| AAA                   | <i>Anguilla anguilla</i> agglutinin                         |
| Ab                    | antibody  |
| Ac                    | acetyl, acetate   |
| aq                    | aqueous   |
| BCA                   | bicinchoninic acid  |
| BDNF                  | brain derived neurotrophic factor                           |
| BSA                   | bovine serum albumin  |
| °C                    | degree Celsius  |
| CaCl <sub>2</sub>     | calcium chloride  |
| cAMP                  | cyclic adenosine monophosphate                              |
| CH <sub>3</sub> N     | acetonitrile  |
| CHCl <sub>3</sub>     | chloroform  |
| CMF-HBSS              | Calcium and Magnesium Free Hank's Balanced Salt Solution    |
| CNS                   | central nervous system                                      |
| CO <sub>2</sub>       | carbon dioxide  |
| CRD                   | carbohydrate recognition domain                             |
| CS                    | chondroitin sulfate   |
| ddH <sub>2</sub> O    | double distilled water                                      |
| D-Gal                 | D-galactose   |
| diazirine             | trifluoromethylphenyldiazirine                              |
| DIV                   | days in vitro   |
| DMEM                  | Dulbecco's Minimal Eagle's medium                           |
| DMSO                  | dimethylsulfoxide   |
| DNA                   | deoxyribonucleic acid                                       |
| DP                    | degree of polymerization                                    |
| DRG                   | dorsal root ganglion  |
| DTT                   | dithiothreitol  |
| E18                   | embryonic day 18  |
| ECL                   | enhance chemiluminescence                                   |
| EDTA                  | ethylenediaminetetraacetic acid                             |
| endo N                | endoneuraminidase   |
| EXT1 and 2            | exostosins enzymes 1 and 2                                  |
| FGF                   | fibroblast growth factor                                    |
| FGFR                  | fibroblast growth factor receptor, tyrosine kinase receptor |
| Fuc                   | L-fucose  |
| Fuc $\alpha$ (1-2)Gal | fucose $\alpha$ (1-2) galactose                             |
| FX                    | epimerase-reductase enzyme                                  |

|                    |  |
|--------------------|--|
| g                  | gram, gravitational force                                  |
| GAG                | glycosaminoglycan  |
| Gal                | galactose  |
| GalNAc             | <i>N</i> -acetylgalactosamine                              |
| GDP-fucose         | guanosine diphosphatyl-fucose                              |
| GlcA               | D-glucuronic acid  |
| GlcN               | D-glucosamine  |
| GlcNAc             | <i>N</i> -acetylglucosamine                                |
| Gluc               | glucose  |
| GluR1              | glutamate receptor 1                                       |
| GMD                | GDP-mannose-4,6-dehydratase                                |
| GPC                | gas phase chromatography                                   |
| GTP                | guanosine triphosphate                                     |
| h                  | hour   |
| HIO <sub>4</sub>   | periodate  |
| HRP                | horse-radish peroxidase                                    |
| HS                 | heparan sulfate  |
| IC <sub>50</sub>   | inhibition concentration at 50%                            |
| IdoA               | L-iduronic acid  |
| IgG                | immunoglobulin   |
| IP                 | immunoprecipitated   |
| K <sup>+</sup>     | potassium ion  |
| K <sub>assoc</sub> | association constant                                       |
| KCl                | potassium chloride   |
| kDa                | kilodalton   |
| L                  | liter  |
| LTL                | <i>Lotus tetragonolobus</i> lectin                         |
| LTP                | long-term potentiation                                     |
| M                  | molar  |
| MALDI-TOF          | matrix-assisted laser desorption/ionization time-of-flight |
| MAP2               | microtubule associated protein 2                           |
| MAPK               | mitogen-associated protein kinase                          |
| MEM                | Minimal Eagle's Medium                                     |
| MeOH               | methanol   |
| μg                 | microgram  |
| MgCl <sub>2</sub>  | magnesium chloride   |
| min                | minutes  |
| m                  | milli or meter   |
| μ                  | micro  |
| mol                | mole   |
| MS                 | mass spectrometry  |
| n                  | nano   |
| N                  | normal   |
| Na <sup>+</sup>    | sodium ion   |
| NaCl               | sodium chloride  |
| NaOH               | sodium hydroxide   |

|                                  |   |
|----------------------------------|---|
| NCAM                             | neural cell adhesion molecule                     |
| NDST                             | <i>N</i> -deacetylase- <i>N</i> -sulfotransferase |
| NH <sub>4</sub> HCO <sub>3</sub> | ammonium bicarbonate                              |
| NP-40                            | nonidet P-40 detergent                            |
| NSF                              | <i>N</i> -ethylmaleimide sensitive factor         |
| OEt                              | <i>O</i> -ethyl                                   |
| P2                               | insoluble fraction 2                              |
| PAA                              | polyacrylamide polymer lacking saccharide         |
| PAGE                             | polyacrylamide gel electrophoresis                |
| PAO                              | phenyl arsine oxide                               |
| PAPS                             | 3'phosphoadenosine 5'phosphosulfate               |
| PBS                              | phosphate buffered saline                         |
| PI3-K                            | phosphatidylinositol 3-kinase                     |
| PSA                              | polysialic acid                                   |
| PSD-95                           | post synaptic density protein 95                  |
| PTP $\zeta$                      | protein tyrosine phosphatase zeta                 |
| PVDF                             | polyvinylidene difluoride                         |
| Qeq                              | charge equilibrium                                |
| RNA                              | ribonucleic acid                                  |
| rpm                              | revolutions per minute                            |
| rt                               | room temperature                                  |
| S2                               | soluble fraction 2                                |
| SDS                              | sodium dodecyl sulfate                            |
| SEM                              | standard error of the mean                        |
| Syn KO                           | synapsin knockout                                 |
| TBST                             | tris buffered saline with Tween-20                |
| TNF $\alpha$                     | tumor necrosis factor alpha                       |
| Tris-Cl                          | tris chloride                                     |
| TrkA                             | tyrosine kinase A receptor                        |
| TrkB                             | tyrosine kinase B receptor                        |
| U                                | unit  |
| UEA I                            | <i>Ulex europeaus</i> agglutinin I                |
| UV                               | ultraviolet                                       |
| vol                              | volume  |
| w/v                              | weight per volume                                 |
| WGA                              | wheat germ agglutinin                             |
| WT                               | wild type   |