

# Chemical Probes to Study Fucosylated Glycans

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  
2013  
(Defended 15 April 2013)



## ACKNOWLEDGEMENTS

I would first and foremost like to thank my advisor, Professor Linda Hsieh-Wilson, for her advice and guidance throughout my years at Caltech, without which this work could not have been possible. I would also like to thank the members of my committee, Professors Dennis Dougherty, Shu-ou Shan, and David Tirrell, for their excellent feedback and endless support.

I'd also like to acknowledge my collaborators in the Hsieh-Wilson lab, Dr. Heather Murrey and Jean-Luc Chaubard, who have been critical to the work described. Drs. Sarah Hanson and Sherry Hsu of Dr. Chi-Huey Wong's laboratory at The Scripps Research Institute provided synthetic compounds and helpful guidance necessary for work in described Chapter 1 and 2. Drs. Eric Peters and John Venable at the Genomics Institute of the Novartis Research Institution were instrumental to the work described in Chapter 2, and Rochelle Diamond of the Caltech Flow Cytometry and Cell Sorting Facility was a key collaborator in the work described in Chapter 4. I'd also like to thank all the members of the Hsieh-Wilson lab for help, guidance, and friendship throughout the past several years.

I am indebted to the staff at the Caltech animal facility, especially Jennifer Constanza, Scott Wang, Reyna Sauza, Jeremy Willenborg, Ana Colon, Dr. Karen Lencioni, and Dr. Janet Baer, all of whom have maintained and cared for my mouse colonies as well guided me through new experimental techniques.

Lastly, I'd like to acknowledge my family and friends for their support. I would not be here without the support of my parents and dear friends, and especially that of my fiancé, Benjamin Keith Keitz.

## ABSTRACT

Fucosylated glycans have many critical biological roles, from leukocyte adhesion to host-microbe interactions. However, a molecular level understanding of these sugars has been lacking, in part due to the chemical and structural diversity of glycans that make them challenging to study. In order to gain a deeper understanding of fucosylated glycans, we have explored the use of chemical probes to study these structures. In Chapters 1 and 2, we apply a metabolic labeling technique for the investigation of fucosylated glycans in neurons, where they have been implicated in learning and memory processes. However, the molecular mechanisms by which these sugars influence neuronal processes are not well understood, and only a handful of fucosylated glycoproteins have been identified. In order to facilitate our understanding of these processes, we exploit non-natural fucose analogs to identify the fucose proteome in rat cortical neurons, identifying proteins involved in cell adhesion, neuronal signaling, and synaptic transmission. Moreover, we track fucosylated glycoproteins in hippocampal neurons, and show that fucosylated glycoproteins localize to the Golgi, axons, and dendrites, and are enriched in synapses. In Chapter 4, we report a new chemoenzymatic strategy for the sensitive detection of the  $\text{Fuca}(1-2)\text{Gal}$  epitope, which has been implicated in tumorigenesis as a potential biomarker of cancer progression. We demonstrate that the approach is highly selective for the  $\text{Fuca}(1-2)\text{Gal}$  motif, detects a variety of complex glycans and glycoproteins, and can be used to profile the relative abundance of the motif on live cells, discriminating malignant from normal cells. These approaches represent new potential applications and strategies for the investigation of fucosylated glycans, and expand the technologies available for understanding the roles of this important class of carbohydrates in physiology and disease.

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## LIST OF ABBREVIATIONS

2-dgal	2-deoxy-D-galactose
2-fucosyllactose	L-fucose $\alpha$ (1-2)galactose $\beta$ (1-4)glucose
3-dgal	3-deoxy-D-galactose
4-dgal	4-deoxy-D-galactose
6-dgal	6-deoxy-D-galactose
2-dGlc	2-deoxy-D-glucose
AAA	<i>Anguilla anguilla</i> agglutinin
Ab	antibody
Ac	acetyl, acetate
AgNO <sub>3</sub>	silver nitrate
AlkFuc	Alkynyl-fucose
Aq	aqueous
ATP	adenosine triphosphate
AzFuc	Azido-fucose
Baf. A1	bicinchoninic acid
$\beta$ -Gal	$\beta$ -galactosidase
Bre A	Brefeldin A
BSA	bovine serum albumin
°C	degree Celsius
CaCl <sub>2</sub>	calcium chloride
Cacna2d1	alpha2/delta subunit of the dihydropyridine-sensitive channel
cAMP	cyclic adenosine monophosphate
CAMs	cell adhesion molecules
CDG	congenital disorder of glycosylation
CH <sub>3</sub> CH	acetonitrile
CHCl <sub>3</sub>	chloroform
CHO	Chinese hamster ovary
CMF-HBSS	calcium and magnesium free Hank's Balanced Salt Solution
CNS	central nervous system
CO <sub>2</sub>	carbon dioxide

CRMP-2	collapsin response mediator protein
CV	column volume
ddH <sub>2</sub> O	double distilled water
D-Gal	D-galactose
DIV	days <i>in vitro</i>
DMEM	Dulbecco's Minimal Eagle's medium
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
E18	embryo day 18
EBI-IPI	European Bioinformatics Institute-International Protein Index
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol tetraacetic acid
Endo H	endoglycosidase H
ER	endoplasmic reticulum
FCS	fetal calf serum
Fuc	L-Fucose
Fuc $\alpha$ (1-2)Gal	fucose- $\alpha$ (1,2)-galactose
Fuc $\alpha$ (1-3)Gal	fucose- $\alpha$ (1,3)-galactose
Fuc $\alpha$ (1-4)Gal	fucose- $\alpha$ (1,4)-galactose
Fuc $\alpha$ (1-6)GlcNAc	fucose- $\alpha$ (1,6)- <i>N</i> -acetylglucosamine
FUT1	$\alpha$ (1-2) fucosyltransferase 1
FUT2	$\alpha$ (1-2) fucosyltransferase 2
FUT3	$\alpha$ (1-3,4) fucosyltransferase 3
FUT4	$\alpha$ (1-3,4) fucosyltransferase 4
FUT5	$\alpha$ (1-3) fucosyltransferase 5
FUT6	$\alpha$ (1-3) fucosyltransferase 6
FUT7	$\alpha$ (1-3) fucosyltransferase 7
FUT8	$\alpha$ (1-6) fucosyltransferase 8
FUT9	$\alpha$ (1-6) fucosyltransferase 9
FUT10	putative $\alpha$ (1-3) fucosyltransferase 10
FUT11	putative $\alpha$ (1-3) fucosyltransferase 11
g	gram

Gal	galactose
GalNAc	<i>N</i> -acetylgalactosamine
GDP-fucose	guanosine diphosphatyl-fucose
Glc	glucose
GlcA	D-glucuronic acid
GlcN	D-glucosamine
GlcNAc	<i>N</i> -acetylgalactosamine
GluR1	glutamate receptor 1
GTP	guanosine triphosphate
h	hour
HIO <sub>4</sub>	periodate
hnRNP	heterogeneous ribonucleoprotein
H <sub>2</sub> O	water
HOAc	acetic acid
Hsc/Hsp70	heat shock chaperonin/heat shock protein 70
IACUC	institute of animal care and use committee
IgSF	immunoglobulin superfamily
IgG	immunoglobulin
IP	immunoprecipitated
K <sup>+</sup>	potassium ion
K <sub>m</sub>	Michaelis constant
KCl	potassium chloride
kDa	kilodalton
K <sub>3</sub> Fe(CN) <sub>6</sub>	potassium ferrocyanide
KO	knockout
L	liter
LAC	lectin affinity chromatography
LAD II	leukocyte adhesion deficiency type II
LC/MS <sup>n</sup>	liquid-chromatography mass spectrometry
LTL	<i>Lotus tetragonolobus</i> lectin
LTP	long-term potentiation
M	molar
MALDI-TOF	matrix-assisted laser desorption/ionization time-of-flight

Man	mannose
MAP2	microtubule associated protein
MEM	Minimal Eagle's Medium
MeOH	methanol
μg	microgram
MG132	proteasome inhibitor
MgCl <sub>2</sub>	magnesium chloride
min	minutes
m	milli
μ	micro
mol	mole
MS	mass spectrometry
Munc18	syntaxin-binding protein
MWCO	molecular weight cut-off
n	nano
N	normal
Na <sup>+</sup>	sodium ion
NaCl	sodium chloride
NaOH	hydroxide
Na <sub>2</sub> CO <sub>3</sub>	sodium biocarbonate
NaN <sub>3</sub>	sodium azide
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	sodium thiosulfate
NCAM	neural cell adhesion molecule
NCBI	National Center for Biotechnology Information
NETFD	SDS-neutralization lysis buffer
Neu5Ac	sialic acid
NH <sub>4</sub> HCO <sub>3</sub>	ammonium biocarbonate
NIH	National Institute of Health
NP-40	nonidet P-40 detergent
NPI	neuronal pentraxin I
NSF	<i>N</i> -ethylmaleimide sensitive factor
NTCB	2-nitro-5-thiocyanobenzoic acid
OCAM	olfactory cell adhesion molecule

OEt	<i>O</i> -ethyl
P0	post-natal day 0 mouse or rat pup
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PEPcase	phosphoenolpyruvate carboxylase
PNGase F	<i>N</i> -glycosidase F
POFUT1	<i>O</i> -fucosyltransferase 1
POFUT2	<i>O</i> -fucosyltransferase 2
PSA	polysialic acid, prostate-specific antigen
PSD-95	post synaptic density protein 95
PTM	post-translational modification
PVDF	polyvinylidene difluoride
RNA	ribonucleic acid
rpm	revolutions per minute
rt	room temperature
SDS	sodium dodecyl sulfate
Sec1	noncatalytic $\alpha(1-2)$ fucosyltransferase
SEM	standard error of the mean
SPAAC	strain-promoted azide-alkyne cycloaddition
SynI	synapsin I
Syn KO	synapsin knockout
TBST	tris-buffered saline with Tween-20
TCEP	tris(2-carboxyethyl)phosphine
TEAA	triethylammonium acetate
Tris-Cl	tris chloride
TTX	tetrodotoxin
UEA I	<i>Ulex europeus</i> agglutinin I
UDP	uridyl-diphosphate
UV	ultraviolet
VDAC1	voltage-dependent anion channel 1
vol	volume
w/v	weight per volume
WGA	wheat germ agglutinin

WT  
Xyl

wild type  
xylose

