CHAPTER 3

Fluorescence Detection of Fucosylated Glycans in Neurons

Introduction

Glycans are attractive targets for molecular imaging. As described in Chapter 1, these biopolymers have critical roles in many biological processes, and the cellular "glycome" can report on the physiological state of the cell. The ability to visualize these processes in cellular systems would increase our understanding of biology and accelerate the development of new clinical tools. However, the molecular imaging of glycan structures has been challenging due to their incompatibility with traditional imaging methods. Genetically encoded fluorescent proteins, such as GFP, cannot access specific glycan structures. Though fluorescent antibodies and lectins may have specificity for the glycan of interest, the large size of the protein conjugates can restrict their access into intracellular compartments, or perturb the localization and natural physiology of the glycan of interest. Lectin and antibody-based imaging methods can provide a snapshot of the glycome at a particular point in time, but are difficult to implement in the context of dynamic studies. Additionally, lectins typically have low affinity for the glycan epitope of interest, and require multi-valency for high-avidity binding (1).

Metabolic labeling with non-natural sugar analogs, followed by bioorthogonal detection techniques, fills the need for a chemoselective labeling technique that is compatible with complex cellular systems. Through the selective metabolic incorporation of a bioorthogonal handle, glycans can be tagged yet remain functional with the parameters of normal cell activity. At any point after labeling, these glycans can be selectively and sensitively detected using CuAAc or SPAAC, allowing spatial and temporal resolution of the metabolically tagged glycans. For example, sialic acid-containing glycans have been visualized using metabolic labeling with sialic acid precursors (e.g. ManNAz or alkynyl-ManNAc) (2, 3), or sialic acid derivatives (e.g. SiaNaz, or 9-azido Neu5Ac) (4, 5), in a variety of cellular systems (3, 6), as well as in mice and zebrafish *in vivo* (7, 8). Mucin-type O-linked glycans have been

detected with the metabolic labeling reporter Ac4GalNAz,(9) also in a variety of cellular systems, and mice and zebrafish *in vivo* (8, 10).

Non-natural fucose analogs have also been used in various cell lines to label and visualize fucosylated glycans. Chi-Huey Wong and coworkers utilized the AlkFuc analog to selectively label fucosylated glycoconjugates through the fucose salvage pathway in Hep3B cells and detect them by CuAAC labeling using a fluorescent or biotinylated azide (3). AlkFuc labeled glycoconjugates were visualized throughout the cells, with some colocalization with the Golgi marker WGA lectin. Additionally, the AlkFuc analog has been used to image fucosylated glycans in the *Arabidopsis thaliana* root cell wall (11), visualizing the localization and targeting of fucosylated glycans in growing cell walls and providing insight into the organization and dynamics of the pectin network. Bertozzi and coworkers were able to surmount low levels of metabolic incorporation of the non-natural fucose analogs by utilizing the GDP activated derivative of FucAz (GDP-FucAz) to bypass the biosynthetic machinery and to metabolically label developing zebrafish embryos in the first five days of development (12).

Given the biological relevance of fucosylated glycans in neuronal systems (described in Chapter 1), the fluorescence detection of fucosylated glycoproteins in neurons would have many exciting applications. Fucosylated glycans are reported to be enriched in synapses (13-15), and the cellular machinery responsible for protein glycosylation can be found within dendrites (16), raising the possibility that local protein synthesis and fucosylation may be occurring at synapses in response to neuronal stimulation. Hsieh-Wilson and co-workers have suggested the presence of Fucα(1-2)Gal glycoproteins in hippocampal neurons, using the lectin UEAI (17), although their specific subcellular localization remains to be determined.

In order to gain insight into the subcellular localization and dynamics of fucosylated glycans in neurons, we sought to develop a protocol using non-natural fucose analogs to image fucosylated glycans in neurons. As the ability to resolve the spatiotemporal dynamics of metabolically labeled fucosylated glycans could allow insights into their functional role at synapses, we also sought to develop an assay to

investigate the dynamics of glycoproteins within cells and to potentially measure changes in fucosylated glycoproteins in response to cellular stimuli or perturbations. In the following chapter, we describe efforts to track the subcellular localization of fucosylated glycoproteins in neurons, and to explore their trafficking within the cells.

Results and Discussion

We first determined whether the non-natural fucose analogs could be utilized for selectively imaging fucosylated proteins in neurons. Primary rat hippocampal neurons were cultured on coverslips and treated with 50 to 100 μM AlkFuc, AzFuc or Fuc in DMSO at 1 DIV. We observed that the hippocampal cultures tolerated 100 μM concentrations of AlkFuc and Fuc with only minimal cytotoxicity, whereas AzFuc caused significant cell death. As a result, the studies described only utilize the AlkFuc analog. After washing out excess molecule, neurons were fixed, permeabilized, and blocked with BSA. An additional blocking step with 1 μg/mL streptavidin in BSA was found to be necessary to limit background due to fluorescent streptavidin detection.

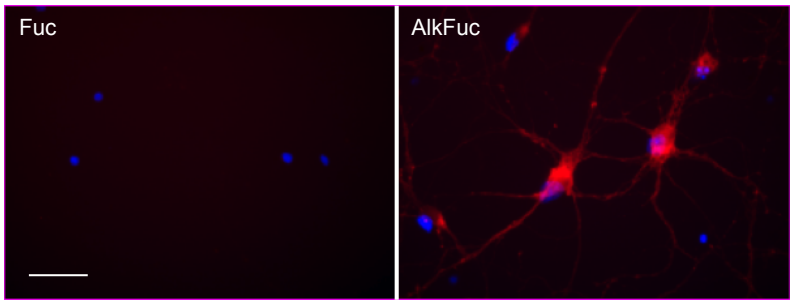


Figure 3.1 Fluorescence detection of AlkFuc labeling. Fuc and AlkFuc labeling (red) in 4 DIV hippocampal neurons after metabolic and CuAAC detection. Nuclei are stained with DAPI (blue). AlkFuc labeling is apparent throughout the soma and processes. Scale bar is 25 μM.

The fixed cells were then subjected to CuAAC labeling with azido-biotin. CuAAC reagents were added to a solution of PBS at 25 mM $CuSO_4$, 1 mM sodium ascorbate, 0.05 mM azido-biotin, and 0.05 mM triazole ligand, and the reaction mixture was added to each coverslip for an overnight incubation at 4 °C.

Subsequent staining with Alexa Fluor 488 conjugated streptavidin revealed extensive fluorescence in the cell body, as well as in the neuronal processes, in cells treated with AlkFuc, but not Fuc (Figure 3.1). In order to determine if AlkFuc labeling was specific to glycoproteins, cultures treated with AlkFuc were fixed and permeabilized utilizing MeOH to disrupt the lipid membrane, prior to detection with CuAAC and Alexa Fluor 488 conjugated streptavidin. Even after lipid disruption, AlkFuc labeling was still apparent throughout the soma and processes, indicating incorporation into glycoproteins (data not shown). Thus, we are capable of specifically labeling fucosylated glycoproteins in the cell body as well as along the neuronal processes of hippocampal cultures.

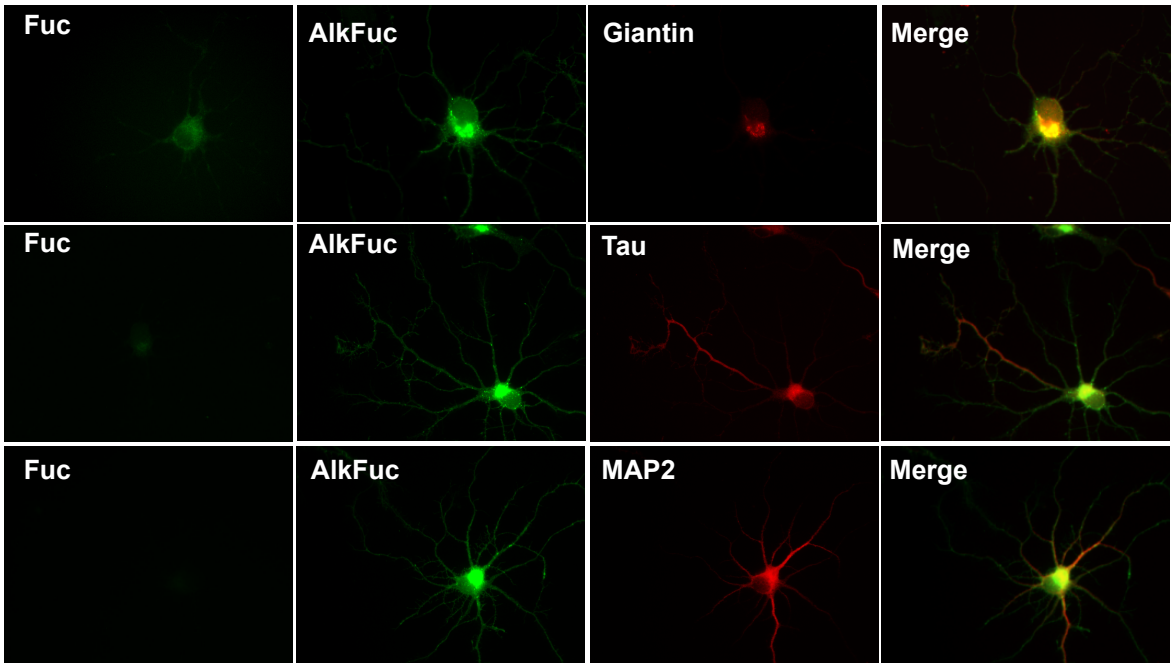


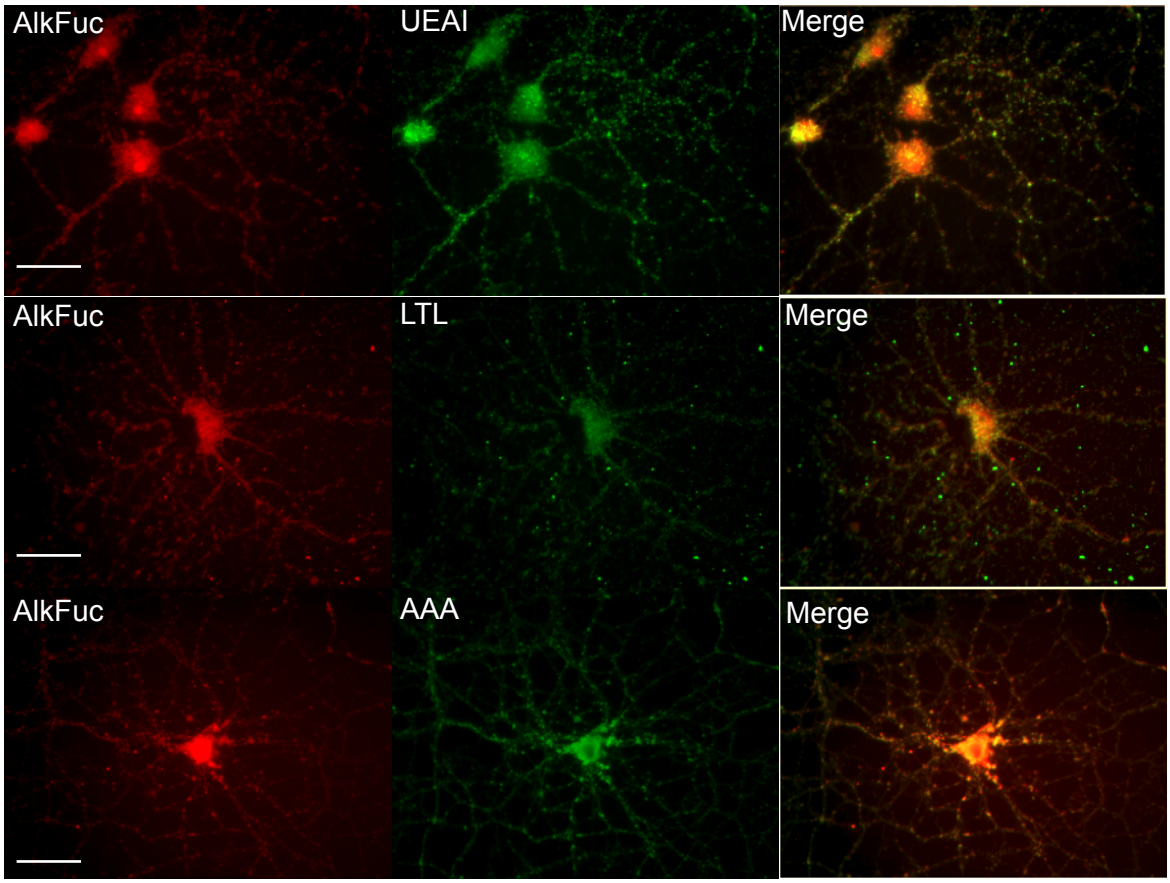
Figure 3.2 Subcellular localization of AlkFuc labeling in 4 DIV neurons. AlkFuc labeling (green) was compared to the Golgi marker Giantin, an axonal marker Tau, and the dendritic marker MAP2 (red). AlkFuc labeling overlayed strongly with the Golgi marker, and also localized to axons and dendrites. Scale bar is 25 μM.

Given that the AlkFuc analog was incorporated into neuronal glycoproteins, we sought to determine the subcellular localization of glycans labeled with AlkFuc. Neurons labeled with AlkFuc were

co-stained with the Golgi apparatus marker Giantin, the dendritic marker MAP2, or the axonal marker tau (Figure 3.2). Strong overlay of Alkfuc labeling with the Golgi marker (Figure 3.2, top) indicated the localization of AlkFuc labeled proteins to the Golgi apparatus, suggesting that fucosylated glycoproteins reside in the Golgi apparatus, consistent with previous results and the notion that fucosylated glycoproteins are synthesized in the GA (3, 18). In addition, we observed labeling of both axons and dendrites in 4 DIV neuronal cultures. Overlay of AlkFuc labeling with both MAP2 and Tau was observed, though AlkFuc labeling was stronger in the MAP2 stained dendrites, indicating that the AlkFuc-labeled glycoproteins were localized to axons and dendrites (Figure 3.2, middle and bottom).

For comparison, 14 DIV hippocampal neurons were also co-stained with the lectins *Ulex europaeus* agglutinin I (UEAI), which binds to Fucα(1,2)Gal glycans (19), *Lotus tetranoglobus* lectin (LTL), which binds to α-linked fucose and the Le^x and Le^y antigens (19), and *Anguilla anguilla* agglutinin (AAA), which binds to Fucα(1-2)Gal structures as well as H antigen types I and II (Figure 3.3) (20). UEAI, LTL, and AAA staining showed overlay with a portion of AlkFuc labeling, consistent with AlkFuc incorporation into fucosylated glycoproteins recognized by fucose specific lectins. However, a subset of AlkFuc labeling, particularly in the processes, was distinct from the staining of each particular lectin, suggesting that the AlkFuc analog labels a broader population of glycans not recognized by the lectins. The majority of fucosylated glycans are reported to exist in complex N-linked structures, which can be modified by fucose in a variety of linkages. The ability to label all fucosylated glycans, irrespective of linkage, would allow a broader investigation into the role of fucosylated glycans.

We also examined whether AlkFuc labeling in 14 DIV hippocampal neurons was enriched in synapses. Hippocampal neurons were labeled for 3 d with AlkFuc or Fuc, subsequently fixed, labeled via CuAAC, and immunostained with various markers. As in younger neurons, we observed the strongest AlkFuc labeling within the Golgi compartment of mature 14 DIV hippocampal neurons. In addition, we examined the colocalization of AlkFuc to synapses with the synaptic markers synapsin I (pre-synaptic



marker), PSD-95 (post-synaptic marker), and spinophilin (post-synaptic marker). Though there was some overlay to synapses indicated by yellow punctae, the majority of AlkFuc labeling was pervasive

Figure 3.3 AlkFuc labeling when compared to fucose-specific lectins. AlkFuc labeling (red) in 14 DIV neurons compared to the lectins UEAI, LTL, and AAA (green).

throughout the processes and did not appear to be highly localized to synapses (Figure 3.4), after 3 d of labeling.

In addition to taking a snapshot of fucosylated glycans in the cell at a particular moment, metabolic labeling provides the opportunity to track the dynamics of glycoproteins inside cells, affording greater insight into changes in subcellular localization in real-time that cannot be obtained using lectins or antibodies. To more closely investigate subcellular dynamics of AlkFuc labeled proteins, we treated hippocampal neurons at 4 DIV with a pulse of 200 μM AlkFuc for 1 h and chased with media lacking

AlkFuc for increasing periods of time before fixing. The resulting fluorescence after CuAAC labeling with azido-biotin and staining with streptavidin conjugated to Alexa Fluor 488 was observed by confocal microscopy. Until 16 h of incorporation, AlkFuc labeling was largely limited to the Golgi, co-localizing with the Golgi marker Giantin (Figure 3.5A), indicating fucosylated glycoproteins were located in the

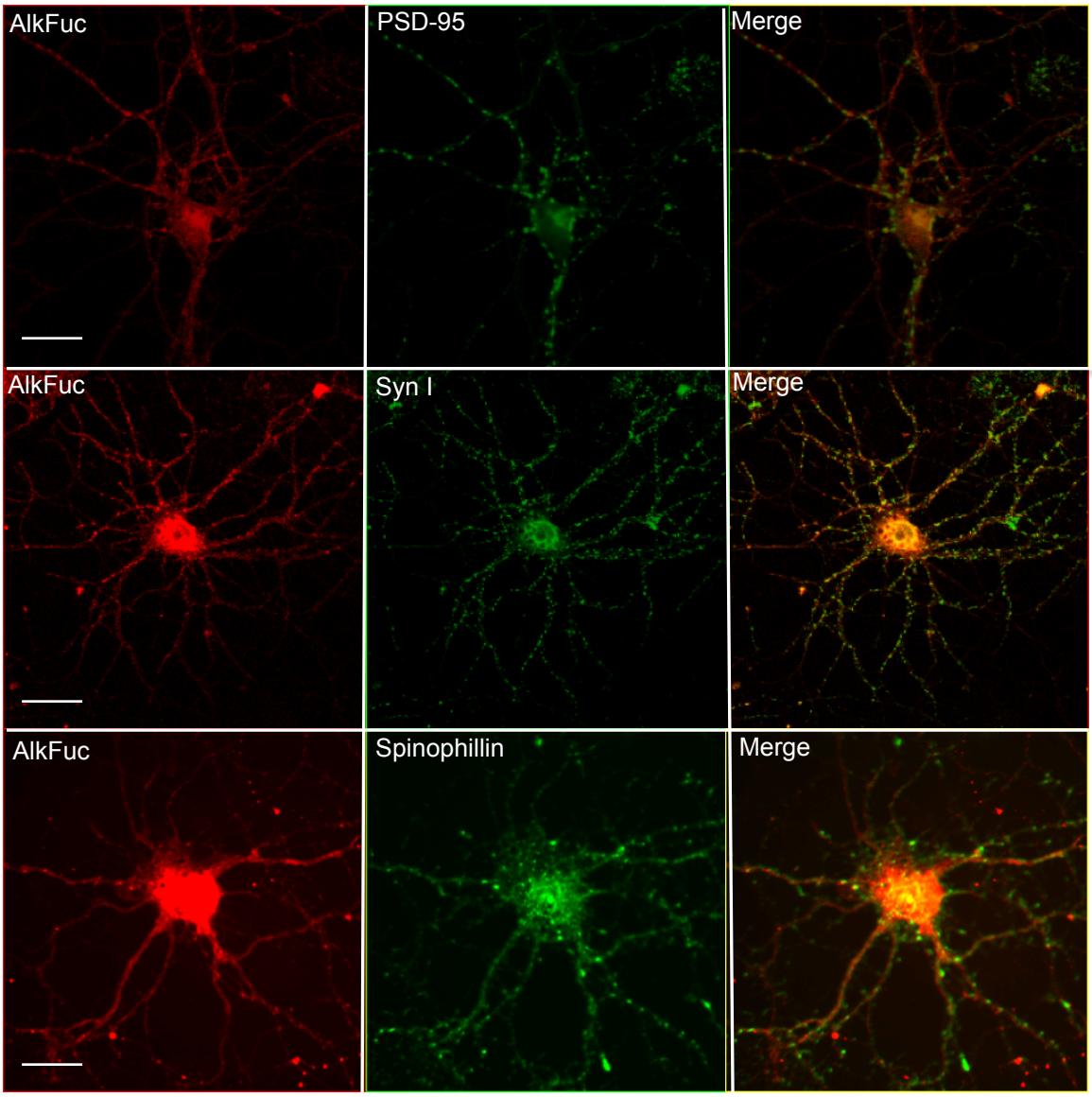


Figure 3.4 AlkFuc labeling in 14D IV neurons compared to the synaptic markers PSD-95, syn I, and spinophillin. AlkFuc overlay with synaptic markers was not apparent after metabolic labeling for 3 d with the AlkFuc analog. Scale bar is 25 μM.

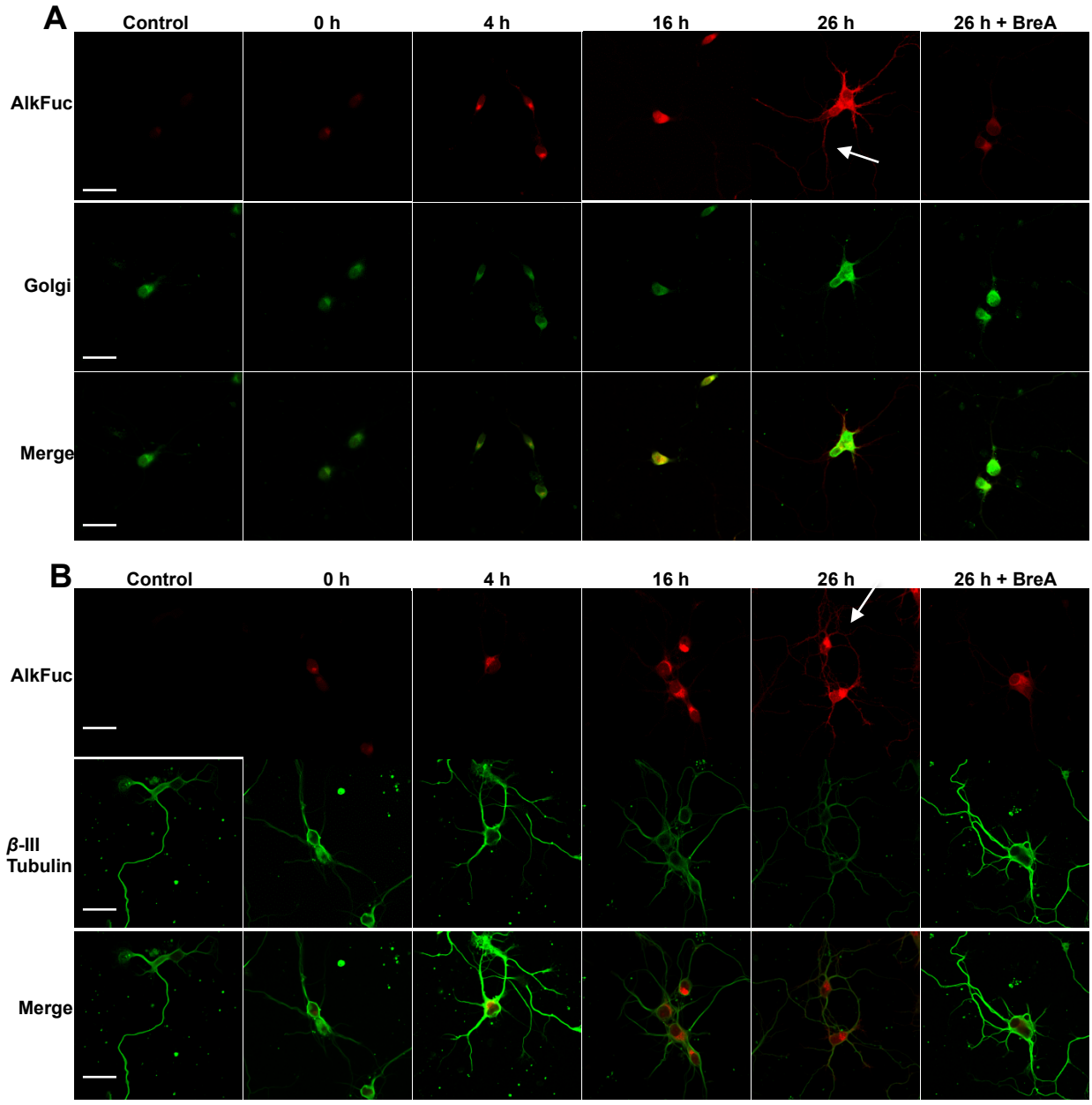


Figure 3.5 Pulse-chase analysis of AlkFuc incorporation in 5 DIV neurons. AlkFuc labeled proteins (red, top row) are detected in the Golgi after a 4 to 16 h chase with untreated media when compared to (A) the Golgi marker, Giantin, or (B) the neuronal marker *β*-III tubulin. Labeled proteins remain largely limited to the Golgi until 16 h after incorporation. Cells treated with a pulse of natural fucose (Fuc) show no labeling. By 26 h after incorporation, AlkFuc labeled glycoproteins are detected in neuronal processes, overlaying with *β*-III tubulin. Treatment with 2 µg/mL Brefeldin A (BreA), a protein secretion inhibitor, leads to diffuse AlkFuc labeling with limited labeling in neuronal processes. Scale bar is 50 µM.

Golgi apparatus (GA). After a 16 to 26 h chase, AlkFuc labeling was evident throughout the processes, overlaying with the cell marker tubulin (Figure 3.5B, arrow), suggesting AlkFuc labeled glycoproteins are delivered to neuronal processes after synthesis in the GA. To validate the conclusion that fucosylated glycoproteins are trafficked from the GA to the processes, we added the secretion inhibitor Brefeldin A (BreA) was added at various time points after the initial pulse of AlkFuc. BreA, a lactone isolated from fungi, interferes with the transport of proteins from the ER to the Golgi by reversibly disassembling the Golgi apparatus (21), and has shown to reversibly inhibit secretory trafficking in neurons (22). In all cases, the addition of BreA disrupted the GA, and AlkFuc labeling was confined to diffuse labeling in the soma, with limited labeling in the processes. Notably, when BreA was added at 16 h after the initial pulse of AlkFuc, the majority of AlkFuc labeling in the processes was abolished (Figure 3.5, right panel), suggesting that trafficking of AlkFuc labeled glycoproteins occurs 16 h after metabolic incorporation.

Intrigued by the possibility that fucosylated glycoproteins are enriched in synapses (23), the pulse-chase study was repeated in 23 DIV hippocampal neurons with formed synapses. After 18 h of incorporation, labeling was pervasive throughout neuronal processes (Figure 3.6A and 3.6B). After a 24 h chase, AlkFuc labeling manifested as distinct punctae, consistent with synaptic localization of labeled fucosylated glycoproteins. When compared to the pre- and post-synaptic markers synapsin I and GluR1, AlkFuc labeling showed significant co-localization with the post-synaptic marker GluR1, suggesting that AlkFuc significantly labeled post-synaptic proteins that are delivered to synapses after 36 hours after synthesis (Figure 3.6A and 3.6B). Alternatively, the signal may be the result of a sufficient accumulation of synaptic proteins by 36 h; the half-life of synaptic proteins, such as GluR1, have been reported to be fairly lengthy, from 1-10 d, depending on the stage of culture and type of neuron (24). Additional examples include NCAM, which has a half-life of 14-20 h in PC12 cells (25), and PSD-95, which has a half-life of 24-36 h (26). The turnover of synaptic glycoproteins has also been reported to be very slow; the half-lives of fucose-labeled glycoproteins of synaptic vesicles and the synaptosomal plasma membrane were noted to be between 26 and 36 days (27). As such, the fluorescence may be due to the

accumulation of long-lived labeled proteins. Curiously, we did not detect any labeling in dendrites in the early time points, which we might have expected given the discovery of dendritic glycosylation machinery (16).

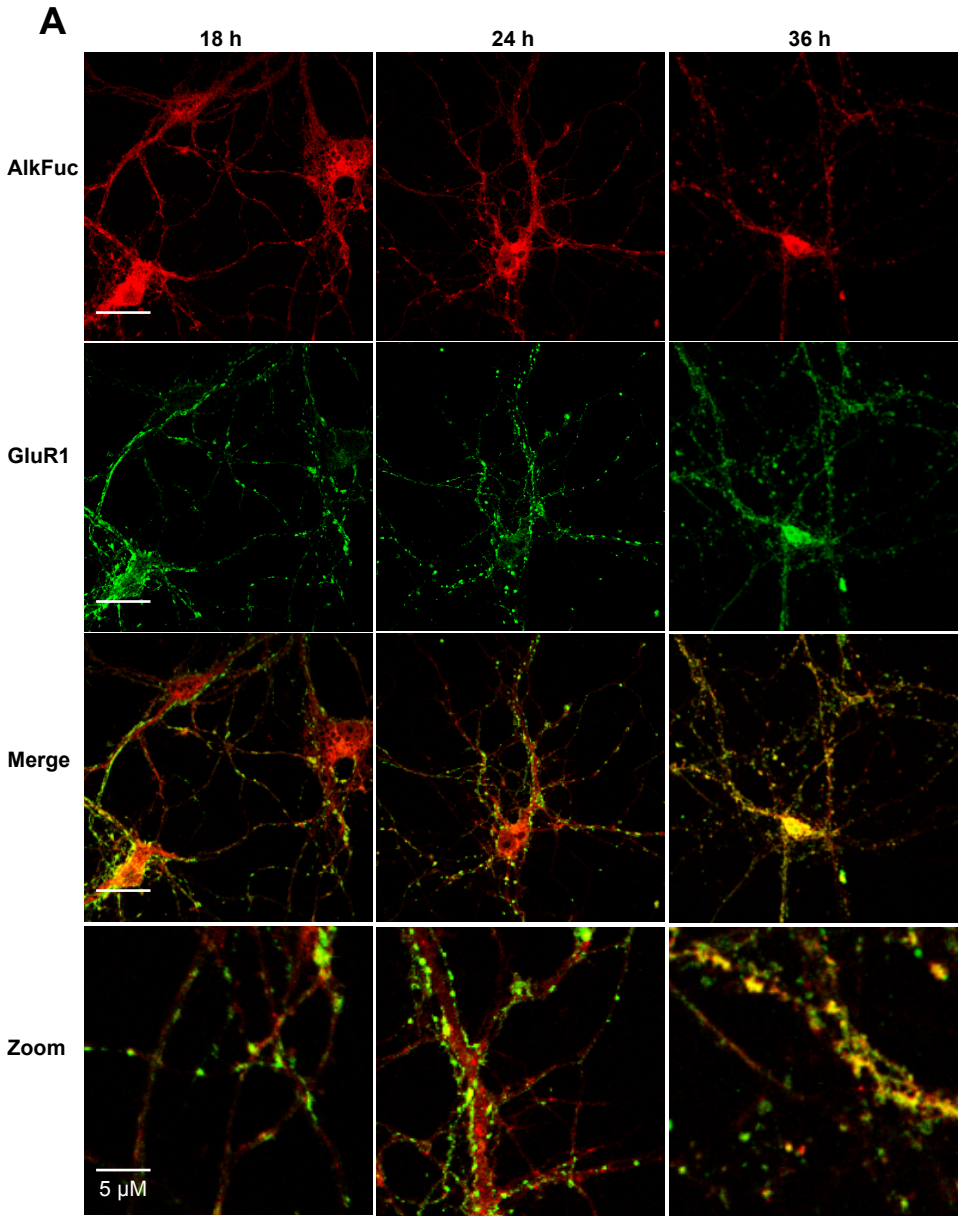
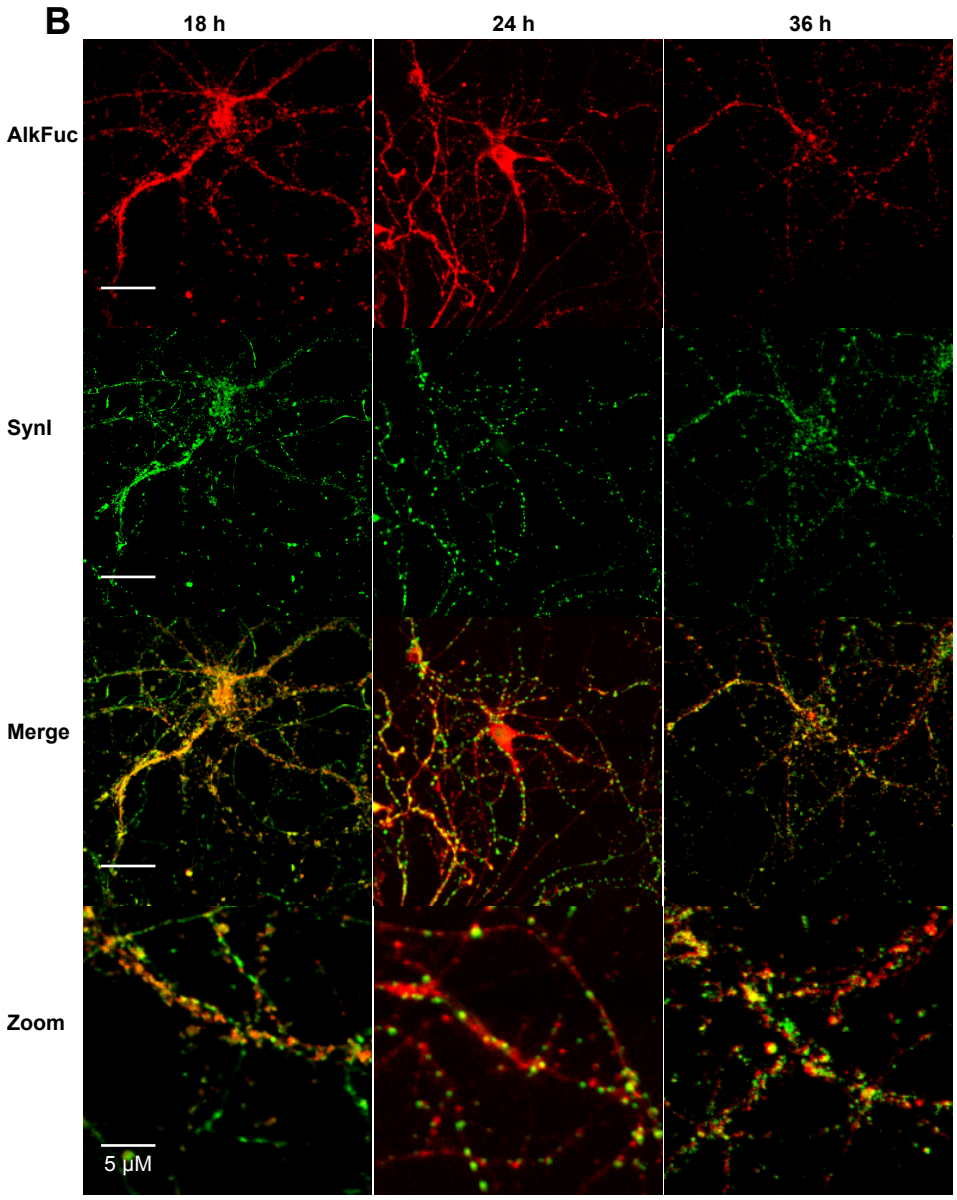


Figure 3.6. Pulse-chase analysis of alknyl-fucose incorporation in 23 DIV neurons. AlkFuc labeled proteins (red, top row) are pervasive through the cell body and processes after an 18 h chase. Between 24 h and 36 h after the chase, AlkFuc labeled glycoproteins manifest as distinct punctae, and co-localize with pre- and post-synaptic markers Syn I (A) and GluR1 (B), suggesting that alknyl-fucose labeled glycoproteins are delivered to the synapses. Scale bar is 25 µM except where indicated.



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In addition to localization and pulse-chase studies, we were interested in investigating fucosylated glycoproteins in response to neuronal stimuli. In order to determine if AlkFuc labeling was perturbed in any way due to neuronal stimulation, hippocampal neurons at 14 DIV were metabolically labeled with AlkFuc and depolarized with KCl. Cultures were labeled with AlkFuc for 2 d prior to a 24 h co-treatment with tetrodotoxin (TTX) to ensure neurons were similarly silenced. AlkFuc labeling in TTX treated neurons was consistent with labeling observed thus far: strong labeling of the Golgi with some labeling apparent in neuronal processes (Figure 3.7). When treated with 50 mM KCl for 6 h, the Golgi was observed to fragment as determined by the Golgi marker Giantin, an interesting phenomenon recently reported to be caused by neuronal hyperexcitation (28), suggesting that neuronal activity serves as a signal to the Golgi. AlkFuc labeling was similarly diffuse and fragmented in the Golgi as a result of KCl depolarization, resulting in punctate labeling throughout the soma and early processes (Figure 3.7, arrow). Further studies are necessary to investigate whether neuronal stimulation leads to increased trafficking of fucosylated glycoproteins and what implications that may have on neuronal growth and morphology.

In summary, we are able to track the subcellular localization of AlkFuc glycoproteins in hippocampal neurons. Additionally, we are able to exploit the AlkFuc analog for pulse-chase investigations into the dynamic localization of AlkFuc labeled glycoproteins, showing that fucosylated glycoproteins are trafficked from the Golgi apparatus and localize to the synapses. The ability to label newly synthesized glycoproteins is a distinct advantage of the metabolic labeling technique over other methods, such as lectin staining or chemoenzymatic detection, and could be utilized to study the dynamics and trafficking of specific glycoproteins.

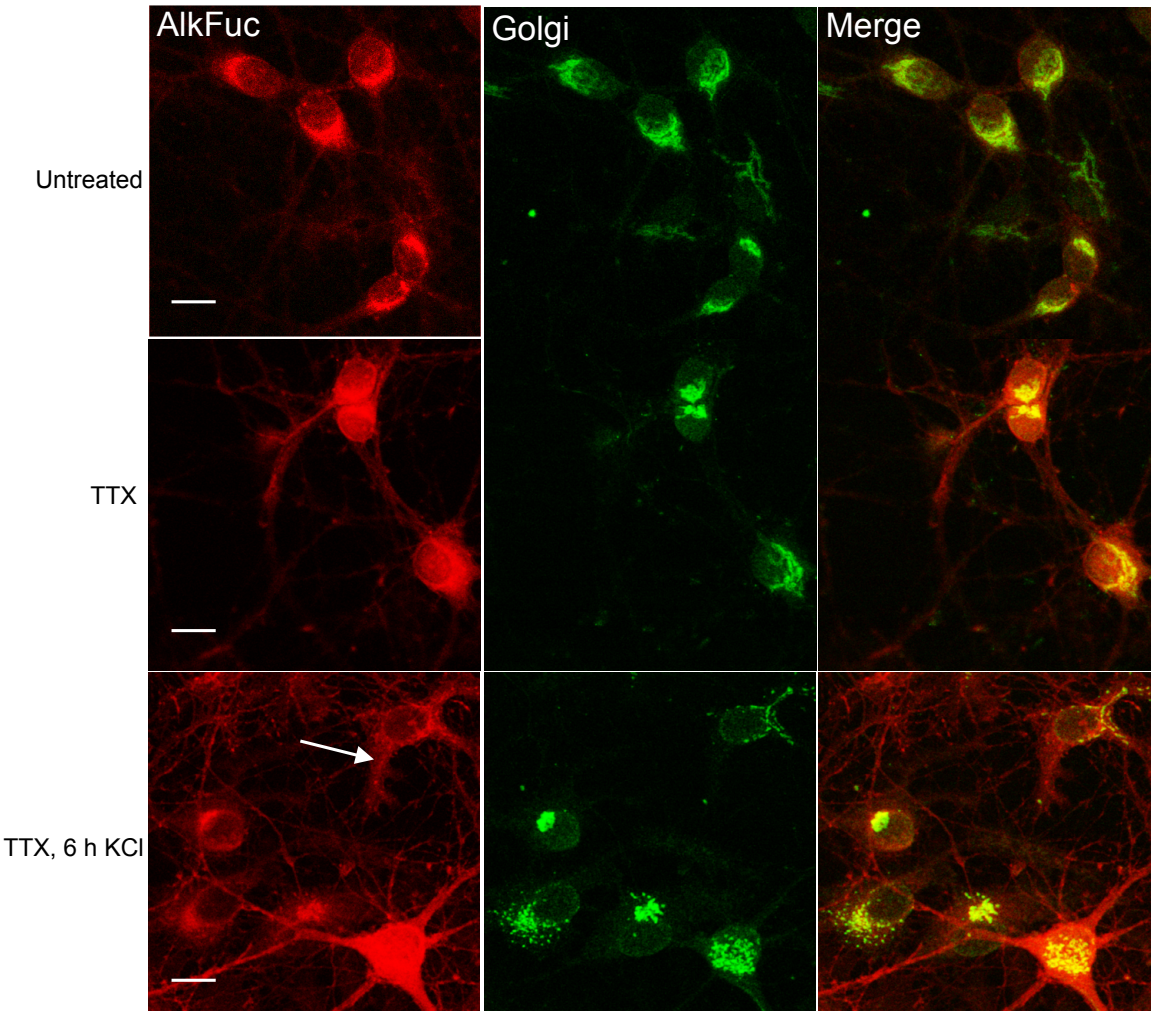

Figure 3.7 AlkFuc labeling after KCl depolarization**.** After KCl depolarization for 6 h, AlkFuc labeling in the Golgi appears diffuse and punctate. Scale bar is 25 µM.

Experimental Procedures

Fluorescent Imaging of Fucosylated Glycans in Hippocampal Neurons. After the indicated number of days in culture, hippocampal neurons on coverslips were treated with 50 µM Fuc or AlkFuc for 3 d. Subsequent to treatment in culture, the media was removed and cells were rinsed once with PBS, fixed and permeabilized in ice cold methanol, and washed twice with PBS. All coverslips were blocked in 3% BSA in PBS for 1 h at rt, followed by 1 µg/mL streptavidin in 3% BSA in PBS for 15 min at rt. Metabolically labeled proteins were tagged by treating the cells with 5 mM triazole ligand, 50 mM sodium ascorbate (Sigma Aldrich), 50 mM $CuSO_4$ (Sigma Aldrich), and 5 mM alkyne-biotin (CHW lab) in PBS (100 µL/coverslip) at 4 °C for 8 h, followed by detection with streptavidin Alexa Fluor 488 (Life

Technologies, 1:1000 in 3% BSA). The primary antibodies anti-synapsin (rabbit, 1:100; Sigma), anti-PSD-95 (mouse, 1:250; Affinity BioReagents), anti-giantin (Santa Cruz, 1:100), anti-NCAM (mouse, 1:100, Sigma) and UEAI conjugated to fluorescein (50μL/mL, Sigma) was added in 3% BSA in PBS, overnight at 4 °C. After the coverslips were washed three times with PBS, fluorophore conjugated secondary antibodies (goat anti-rabbit; 1:500 and goat anti-mouse; 1:500) were added in 3% BSA in PBS for one hour at rt. AlkFuc was detected with streptavidin-Alexa Fluor 488 (1:1000; Life Technologies) or Alexa Fluor 546 (1:1000, Life Technologies) added together with the secondary antibodies. The coverslips were washed three times with PBS and mounted onto slides with Vectashield with DAPI (Vector Labs) and sealed with clear nail polish. Cells were then subjected to fluorescence and confocal microscopy.

Pulse–Chase Analysis of AlkFuc Labeled Proteins. Hippocampal neurons at 23 DIV were treated with 200 μM AlkFuc in supplemented Neurobasal (Invitrogen) for 30 min, after which all the media was replaced with alknyl-fucose free media. Neurons were fixed and permeabilized as described above every two to four hours, and stored in PBS at 4 °C until the termination of the time-course. All coverslips were blocked first in 3% BSA in PBS for 1 h at rt, followed by 0.001 μg/μL streptavidin in 3% BSA in PBS for 15 min at rt. Metabolically labeled proteins were tagged by treating the cells 5 mM triazole ligand, 50 mM sodium ascorbate (Sigma Aldrich), 50 mM CuSO4 (Sigma Aldrich), and 5 mM alkyne-biotin in PBS (100 μL/coverslip)at 4 °C for 8 h, followed by detection with streptavidin Alexa Fluor 488 (Life Technologies, 1:1000 in 3% BSA). Synapsin I, GluR1, and Giantin were detected and the coverslips mounted as described above. Cells were imaged using a 40X or 63X Plan-Achromat objective on a Zeiss Meta510 or 700 inverted microscope.

Detection of AlkFuc labeled Proteins after Neuronal Stimulation. After the indicated number of days in culture, hippocampal neurons on coverslips were treated with 100 μM Fuc or AlkFuc for 3 d. 2

d into the treatment, neuronal cultures were treated with 1 μM tetrodotoxin (TTX; Sigma Aldrich). After 1 d of TTX treatment, neurons were stimulated with 50 mM KCl in PBS (Sigma Aldrich) for 5 min up to 25 h. Subsequent to treatment in culture, the media was removed and cells were rinsed once with PBS, fixed and permeabilized in ice cold methanol, and washed twice with PBS. All coverslips were blocked in 3% BSA in PBS for 1 h at rt, followed by 1 μg/mL streptavidin in 3% BSA in PBS for 15 min at rt. Metabolically labeled proteins were tagged by treating the cells 5 mM triazole ligand, 50 mM sodium ascorbate (Sigma Aldrich), 50 mM $CuSO_4$ (Sigma Aldrich), and 5 mM alkyne-biotin (CHW lab) in PBS (100 μL/coverslip) at 4 °C for 8 h, followed by detection with streptavidin Alexa Fluor 488 (Life Technologies, 1:1000 in 3% BSA). The primary antibodies anti-synapsin (rabbit, 1:100; Sigma), anti-PSD-95 (mouse, 1:250; Affinity BioReagents), anti-giantin (Santa Cruz, 1:100), anti-NCAM (mouse, 1:100, Sigma) and UEAI conjugated to fluorescein (50μL/mL, Sigma) was added in 3% BSA in PBS, overnight at 4 °C. After the coverslips were washed three times with PBS, fluorophore conjugated secondary antibodies (goat anti-rabbit; 1:500 and goat anti-mouse; 1:500) were added in 3% BSA in PBS for one hour at rt. AlkFuc was detected with streptavidin-Alexa Fluor 488 (1:1000; Life Technologies) or Alexa Fluor 546 (1:1000, Life Technologies) added together with the secondary antibodies. The coverslips were washed three times with PBS and mounted onto slides with Vectashield with DAPI (Vector Labs) and sealed with clear nail polish. Cells were then subjected to fluorescence and confocal microscopy.

References

1. Kiessling LL & Pohl NL (1996) Strenght in Numbers: Non-natural polyvalent carbohydrate derivatives. *Chemistry and Biology* 3:71-77.

2. Saxon E & Bertozzi CR (2000) Cell surface engineering by a modified Staudinger reaction. *Science* 287:2007-2010.

3. Hsu TL*, et al.* (2007) Alkynyl sugar analogs for the labeling and visualization of glycoconjugates in cells. *Proc. Natl. Acad. Sci. U.S.A* 104(8):2614-2619.

4. Luchansky SJ, Goon S, & Bertozzi CR (2004) Expanding the diversity of unnatural cell-surface sialic acids. *Chembiochem* 5:371-374.

5. Kosa RE, Brossmer R, & J. GH (1993) Modification of cell surfaces by enzymatic introduction of special sialic acid analogues. *Biochem. Biophys. Res. Commun.* 190:914-920.

6. Luchansky SJ, Argade S, Hayes BK, & Bertozzi CR (2004) Metabolic functionalization of recombinant glycoproteins. *Biochemistry* 43:12358-12366.

7. Prescher JA, Dube DH, & Bertozzi CR (2004) Chemical remodelling of cell surfaces in living animals. *Nature* 430:873-877.

8. Laughlin ST, Baskin JM, Amacher SL, & Bertozzi CR (2008) In Vivo Imaging of Membrane-Associated Glycans in Developing Zebrafish. *Science* 320(5876):664-667.

9. Hang HC, Yu C, Kato DL, & Bertozzi CR (2003) A metabolic labeling approach toward proteomic analysis of mucin-type O-linked glycosylation. *Proc. Natl. Acad. Sci. U.S.A* 100(25):14846-14851.

10. Dube DH, Prescher JA, Quang CN, & Bertozzi CR (2003) Probing mucin-type O-glycosylation in living animals. *Proc. Natl. Acad. Sci. U.S.A.* 103:14846-14851.

11. Anderson CT, Wallace IS, & Somerville CR (2012) Metabolic click-labeling with a fucose analog reveals pectin delivery, architecture, and dynamics in Arabidopsis cell walls. *Proc. Natl. Acad. Sci. U.S.A.* 109(4):1329-1334.

12. Dehnert KW*, et al.* (2011) Metabolic labeling of fucosylated glycans in developing zebrafish. *ACS Chem. Biol.* 6(6):547-552.

13. Murrey HE*, et al.* (2006) Protein fucosylation regulates synapsin Ia/Ib expression and neuronal morphology in primary hippocampal neurons. *Proc. Natl. Acad. Sci. U.S.A* 103(1):21-26.

14. Zanetta JP, Reeber A, Vincendon G, & Gombos G (1977) Synaptosomal Plasma Membrane Glycoproteins. II. Isolation of Fucosyl-Glycoproteins by Affinity Chromatography on the Ulex Europeus Lectin Specific for L-Fucose. *Brain Res.* 138:317-328.

15. Krusius T & Finne J (1977) Structural Features of Tissue Glycoproteins: Fractionation and Methylation Analysis of Glycopeptides Derived from Rat Brain, Kidney, and Liver. *FEBS J.* 78:369-379.

16. Gardiol A, Racca C, & Triller A (1999) Dendritic and postsynaptic protein synthestic machinery. *J. Neurosci.* 19(1):168-179.

17. Kalovidouris SA, Gama CI, Lee LW, & Hsieh-Wilson LC (2005) A role for fucose alpha(1-2) galactose carbohydrates in neuronal growth. *J. Am. Chem. Soc.* 127(5):1340-1341.

18. Becker DJ & Lowe JB (2003) Fucose: biosynthesis and biological function in mammals. *Glycobiology* 13(7):41R-53R.

19. Manimala JC, Roach TA, Li Z, & Gildersleeve JC (2006) High-Throughput Carbohydrate Microarray Analysis of 24 Lectins. *Angew. Chem. Int. Ed. Engl.* 45(22):3607-3610.

20. Baldus SE*, et al.* (1996) Characterization of the binding specificity of *Anguilla anguilla* agglutinin (AAA) in comparison to *Ulex europeaus* agglutinin I (UEA-I). *Glycoconjugate J.* 12(4):585-590.

21. Lippincott-Schwartz J, Yuan LC, Bonifacino JS, & Klausner RD (1989) Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: Evidence for membrane cycling from Golgi to ER. *Cell* 56(5):801-813.

22. Horton AC*, et al.* (2005) Polarized secretory trafficking directs cargo for asymmetric dendrite growth and morphogenesis. *Neuron* 48(5):757-771.

23. Kalovidouris SA, Gama CI, Lee LW, & Hsieh-Wilson LC (2005) A Role for Fucose α(1−2) Galactose Carbohydrates in Neuronal Growth. *J. Am. Chem. Soc.* 127(5):1340-1341.

24. Mammen AL, Hugnair RL, & O'Brien RJ (1997) Redistribution and Stabilization of Cell Surface Receptors during Synapse Formation. *J. Neurosci.* 17(19):7351-7358.

25. Park T-U, Lucka L, Reutter W, & Horstkorte (1997) Turnover Studies of the Neural Cell Adhesion Molecule NCAM: Degradation of NCAM in PC12 Cells Depends on the Presence of NGF. *Biochem. Biophys. Res. Commun.* 234:686-689.

26. Gray NW, Weimer RM, Bureau I, & Svoboda K (Rapid Redistribution of Synaptic PSD-95 in the Neocortex in vivo. *PLoS Biol.*

27. Marko P & Cuenod M (1973) Contribution of the nerve cell body to renewal of axonal and synaptic glycoproteins in the pigeon visual system. *Brain Res.* 62(2):419-423.

28. Thayer DA, Jan YN, & Jan LY (2013) Increased neuronal activity fragments the Golgi complex. *Proc. Natl. Acad. Sci. U.S.A.* 110(4):1482-1487.