N-linked surface glycan biosynthesis, composition, inhibition, and function in cnidarian-dinoflagellate symbiosis

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Abstract

The success of symbioses between cnidarian hosts (e.g. corals and sea anemones) and micro-algal symbionts hinges on the molecular interactions that govern the establishment and maintenance of intracellular mutualisms. As a fundamental component of innate immunity, glycan-lectin interactions impact the onset of marine endosymbioses, but our understanding of the effects of cell surface glycome composition on symbiosis establishment remains limited. In this study, we examined the canonical N-glycan biosynthesis pathway in the genome of the dinoflagellate symbiont *Breviolum minutum* (family Symbiodiniaceae) and found it to be conserved with the exception of the transferase GlcNAc-TII (MGAT2). Using coupled liquid chromatography-mass spectrometry (LC-MS/MS), we characterized the cell surface N-glycan content of *B. minutum*, providing the first insight into the molecular composition of surface glycans in dinoflagellates. We then used the biosynthesis inhibitors kifunensine and swainsonine to alter the glycan composition of *B. minutum*. Successful high-mannose enrichment via kifunensine treatment resulted in a significant decrease in colonization of the model sea anemone *Aiptasia* (*Exaiptasia pallida*) by *B. minutum*. Hybrid glycan enrichment via swainsonine treatment, however, could not be confirmed and did not impact colonization. We conclude that functional Golgi processing of N-glycans is critical for maintaining appropriate cell surface glycan composition and for ensuring colonization success by *B. minutum*.

Introduction

The biodiversity and productivity of coral reef ecosystems relies on the interactions of cnidarian hosts with dinoflagellates in the family Symbiodiniaceae (formerly the genus *Symbiodinium* [1]). These unicellular endosymbionts provide photosynthate to their hosts in return for inorganic carbon and nitrogen and a high light environment which enable the partners to thrive in nutrient-limited waters. In most symbiotic cnidarian species, each generation a host must first engulf potential symbionts, select algae for productive symbiosis in the symbiosome (a modified host vacuole), and allow for proliferation within its tissue. The biological steps that regulate this colonization process are therefore critical to symbiosis establishment and homeostasis. Innate immunity pathways govern the onset of symbiosis when algae first come into contact with their future host cells [2, 3]. Cnidarians rely on expanded innate immune repertoires for defense against pathogens and selection of microbes [4–8]. These immune pathways are often co-opted targets for microorganisms looking to benefit from a host, and therefore offer potential avenues for symbiont coevolution and entry into hosts [9].

The surfaces of most eukaryotic cells are decorated with a complex array of surface sugars that play a major role in cell-cell communication [10]. These cell surface glycans are recognized by carbohydrate-binding proteins, known as lectins, to mediate events such as cell homing, sperm-egg binding, and host-microbe interactions [11–13] (**Figure 1**). Glycans are also a major category of microbe-associated molecular pattern that interact with host pattern recognition receptors that, in turn, regulate innate immunity and symbiosis [2, 14]. Lectins on host cells have the ability to either promote microbial attachment and colonization or to block the interactions required for entry into the host [15–18]. These glycan-lectin interactions form a critical component of host innate immunity that functions in self/non-self discrimination and pathway activation against potential pathogens [19, 20].

While the importance of glycan recognition has been well documented in some host-symbiont associations, the molecular characterization of surface glycans in the family Symbiodiniaceae and their function in symbiosis is just beginning to be uncovered. Other alveolates offer potential insight into glycan-lectin interactions in Symbiodiniaceae mutualisms [21]. For example, the well-studied apicomplexan parasites *Plasmodium falciparum* and *Toxoplasma gondii* have simplified glycomes that lack the longer immunogenic glycans often detected by host immunity [22–24]. The alveolate parasite *Perkinsus marinus*, a member of the

sister phylum to dinoflagellates, has glycans that are recognized during shellfish infection by a host galectin receptor [25]. The prevalent nature of glycan-lectin interactions in parasitic groups that are closely related to dinoflagellates suggests that the glycomes of Symbiodiniaceae may also have evolved to gain access to the host cnidarian environment.

The strongest evidence supporting glycan-lectin recognition during the onset of Symbiodiniaceae mutualisms comes from experimental manipulations of symbiont cells through enzymatic cleavage of surface glycans. Several species of Symbiodiniaceae have been experimentally de-glycosylated, leading to reduced symbiont uptake by larval, juvenile, and adult aposymbiotic animals [26–29]. Though the effects are not entirely consistent from study to study, the data suggest that N-glycans in particular are an important determinant of how rapidly hosts are colonized by symbionts. It remains uncertain whether disruption in the onset of symbiosis is driven primarily by changes in the overall abundance of N-glycans (i.e. quantity) or by changes in the composition of N-glycans (i.e. complexity), particularly the relative proportions of high-mannose, hybrid, and complex N-glycans.

Although recent advances in the field of glycomics have made the direct chemical analysis of carbohydrates feasible, most studies probing the Symbiodiniaceae cell surface have relied on indirect lectin binding to characterize glycan type and abundance, and to mask glycan interactions. For example, a variety of fluorescent FITC-labeled lectin probes were used to examine the cell surface of *Cladocopium* sp. (type C1f) isolated from the coral *Fungia scutaria* [29]. The C1f symbionts were strongly labeled with Concanavalin A (ConA) and Jacalin lectins, which bind α -mannosyl, α -glucosyl and α -galactosyl (Jacalin only) groups. Logan et al. (2010) further examined other genera of Symbiodiniaceae, finding that binding varied by species. *Breviolum minutum* in particular was labeled by a diversity of lectins recognizing mannose, glucose, galactose, N-acetylglucosamine (GlcNAc), and N-acetylgalactosamine (GalNAc) residues. In contrast, Parkinson et al. (2018) used a glycan-binding array of 40 lectins and found only small differences in the glycan profiles between the free-living species *Symbiodinium pilosum* and the mutualistic species *B. minutum*. Both studies provide evidence that Symbiodiniaceae produce surface glycoproteins containing high-mannose as well as hybrid/complex type N-glycans, but it remains unclear which types of N-glycans specifically contribute to recognition for colonization by mutualistic species. Here, we employed liquid

chromatography coupled mass spectrometry (LC-MS/MS) to characterize *B. minutum*'s cell surface N-glycan content on the molecular level.

The N-glycan biosynthesis pathway is well-described in yeast and humans, and a number of natural products have been isolated that serve as inhibitors [30, 31]. If the canonical N-glycan pathway found in animals and plants is conserved in dinoflagellates (as shown in **Figure 1**), it should be possible to use commercially-available inhibitors to alter glycan biosynthesis in Symbiodiniaceae by primarily changing the composition of the glycome instead of altering the overall abundance of N-glycans. Kifunensine is one such molecule, a plant-derived alkaloid, that inhibits the activity of ER Mannosidase I [32]. Because blocking cleavage prevents the synthesis of hybrid and complex glycan structures, this inhibition results in an increase in the proportion of high-mannose type N-glycans (**Figure 1**) [33, 34]. Swainsonine is another small molecule inhibitor that blocks later stage activity of Golgi Mannosidase II [35]. Therefore, swainsonine treatment increases the proportion of hybrid-type N-glycan content and decreases the proportion of complex-type glycans (**Figure 1**) [36, 37]. These two inhibitors have the potential to differentially modify symbiont surface glycan composition allowing one to experimentally test for preferential uptake by cnidarian hosts of algal cells with certain surface glycan profiles.

In this study, we directly determined the structure and abundance of N-glycans from symbiotic *B. minutum*, used small molecule inhibitors to manipulate the glycome of *B. minutum*, and subsequently tested colonization success of these altered algae in model cnidarian hosts.

Materials and methods

Raw data and R code for the following analyses can be found in Supplementary File S1. For complete details regarding colonization and image analysis, protocols can be accessed on protocols.io at [dx.doi.org/10.17504/protocols.io.j8ucrww](https://doi.org/10.17504/protocols.io.j8ucrww).

Identification of N-Glycan Biosynthesis Homologs

Our hypotheses were based on the idea that Symbiodiniaceae share the canonical N-linked glycosylation biosynthesis pathway with other eukaryotes. To strengthen this premise, we first searched for conservation of dolichol-linked precursor oligosaccharide synthesis and ER/Golgi processing pathways in Symbiodiniaceae genomes. We used *Homo sapiens* protein sequences to search for predicted proteins in the Symbiodiniaceae genomes of *Breviolum minutum* [38],

Symbiodinium microadriaticum [39], and *Fugacium kawagutii* [40]. The genomes of *Perkinsus marinus* strain ATCC 50983 [41], *Plasmodium falciparum* strain 3D7 [42], and *Toxoplasma gondii* strain ME49 [43] were also included as closely-related alveolate comparisons. In total, 34 manually annotated protein sequences from UniProtKB were used as queries representing proteins involved in N-glycan precursor synthesis and N-glycan processing in the ER and Golgi [44]. Enzymes that have similar functions but that are involved in degradation were included as well, such as lysosomal α -mannosidase and β -hexosaminidase 1. Predicted proteins for Symbiodiniaceae species were searched on reefgenomics.org, a platform hosting the genomes of several marine organisms including three Symbiodiniaceae genomes [45]. Predicted proteins for the three other alveolate genomes were searched on protists.ensembl.org, a browser for protist genomes [46]. Homologous proteins were considered to be present in a genome if the top protein BLAST hit exceeded the following cutoff: eval $< 1 \times 10^{-5}$, bitscore > 50 , identity score $> 25\%$. T-Coffee was used to align amino acid sequences of putative α -mannosidases from Symbiodiniaceae against α -mannosidase 2 and lysosomal α -mannosidase [47, 48]. Sequences were chosen from *Drosophila melanogaster*, *Homo sapiens*, *Mus musculus*, and *Arabidopsis thaliana* to allow for comparison to previous studies characterizing the active sites of α -mannosidase [37, 49].

Treatment of Algal Cultures

Breviolum minutum (strain Mf1.05b), a homologous or native symbiont found in Aiptasia, was used for all experiments. Algal cultures were maintained in f/2 media in an incubator at 26°C under a 12:12 light:dark cycle and a light intensity of 50 $\mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-2}$. For experimental treatment, algal cultures were reconstituted at $1 \times 10^6 \text{ cells} \cdot \text{mL}^{-1}$ in f/2 media. To cleave N-glycans for dosage and colonization experiments, algal cultures were incubated in a range of concentrations of Peptide:N-glycosidase F for 24 h prior to flow analysis (500, 100, 50, 5, and 0 U PNGase F $\cdot \text{mL}^{-1}$ f/2 media). For biosynthesis inhibition treatment, kifunensine and swainsonine stocks were prepared at concentrations of 50 mM in 100% dimethyl sulfoxide (DMSO) solvent. Cultures were incubated for 1 wk in 1 mM kifunensine or swainsonine in 2% DMSO in f/2 (a 1:50 dilution) or in a 2% DMSO in f/2 vehicle control which was well-tolerated. Pilot experiments tested a range of inhibitor concentrations from 10 μM to 10 mM and tested a range of DMSO concentrations from 2-10% (data not shown.)

164 Identification of N-Glycans via ESI LC-MS/MS

165 *Breviolum minutum* (strain Mfl.05b) was cultured as described above. For enzymatic treatment,
 166 1 L of culture was spun down and treated with Peptide:N-glycosidase F for 72 h (50 U PNGase F
 167 • mL⁻¹ f/2 media). The reactions were stirred at 37 C for 72 h. The glycan sample was dialyzed
 168 using 10k MWCO dialysis tubing in 100 mL ultrapure water; water was changed three times
 169 over the course of 48 h. The glycan sample was lyophilized and 1 mg of lyophilized sample was
 170 reduced and permethylated as previously reported [50–53]. In summary, lyophilized samples
 171 were dissolved in 10 µL of borane-ammonia complex solution (10 mg• mL⁻¹) and incubated in
 172 the water bath at 60°C for 1h. After incubation, 1 mL of methanol was added to the samples and
 173 vacuum dried (LABCONCO Cor. Kansas City, MO). A 1 mL aliquot of methanol was
 174 repeatedly added and dried until borate was completely removed by co-evaporating with
 175 methanol. The reduced sample was redissolved in a 30 µL aliquot of dimethyl sulfoxide
 176 (DMSO), 1.2 µL water, and 20 µL iodomethane. Sodium hydroxide beads (Sigma, St. Louis,
 177 MO; suspended with DMSO) were packed into a spin column, centrifuged at 1800 rpm for 2
 178 min, and then washed with 200 µL of DMSO. Samples were loaded onto the column and
 179 incubated at room temperature for 25 min. Another 20 µL aliquot of iodomethane was added into
 180 the column and incubated for 15 min. After incubation, permethylated glycans were collected by
 181 centrifuging at 1800 rpm for 2 min. Next, 30 µL of acetonitrile (ACN) was added into the
 182 column and centrifuged again to collect the eluent. The ACN eluent was combined with previous
 183 DMSO solution and dried completely.

184 Reduced and permethylated glycans were then subject to nano-C18-LC-MS/MS analysis.
 185 LC-MS analysis was performed on a Dionex 3000 Ultimate nano-LC system (Dionex,
 186 Sunnyvale, CA) interfaced to an LTQ Orbitrap Velos mass spectrometer (Thermo Scientific, San
 187 Jose, CA). A 200 ng aliquot of the lyophilized glycans were injected. Glycans were first retained
 188 on a trap column (Acclaim PepMap 100, 75 µm × 2 mm, 3 µm, Thermo Scientific) and then
 189 separated by a nanoC18 column (Acclaim PepMap 100, 75 µm × 150 mm, 2 µm, Thermo
 190 Scientific). The flow rate was 0.35 µL• min⁻¹. The gradient was attained using mobile phase A
 191 (98% water, 2% ACN, 0.1% formic acid) and mobile phase B (ACN, 0.1% formic acid)
 192 employing the following multi-steps: 20% B for 10 min; 20% - 42% B from 10 to 11 min; 42% -
 193 55% B from 11 to 48 min; 55% - 90% B from 48 to 49 min; 90% B from 49 to 54 min; 90% -
 194 20% B from 54 to 55 min; 20% B from 55 to 60 min.

The resolution of LTQ Orbitrap Velos was set to 100,000. Collision induced dissociation (CID) was applied to achieve tandem mass spectrometry data (MS²) with an injection time of 10 ms, collision energy of 35%, activation Q of 0.25. The data dependent acquisition (DDA) method was used with the top 4 ions detected in the full MS to be picked up for CID fragmentation. The identification and quantitation of glycans were performed by Multiglycan software [54] and then manually checked.

Lectin Labeling and Flow Cytometry

After treatment, 100 μ L algal samples were taken from each treatment, rinsed in 3 mL of filtered artificial seawater (FSW) and centrifuged in 75 mm borosilicate glass tubes (1670 rpm for 5 min). Pellets were resuspended in 50 μ L of 3.3X PBS, prepared from a 10X PBS stock solution (0.02 M NaH₂PO₄, 0.077 M Na₂PO₄, 1.4 M NaCl, pH 7.4). Subsequently, 50 μ L of 2X labeling solution containing lectins conjugated to phycoerythrin (PE) fluorophores was added to each sample at a final concentration of 5 μ g lectin $\cdot$ mL⁻¹ PBS. Lectins were coupled to PE using the Lightning-Link R-PE Antibody Labeling Kit (Novus Biologicals #703-0010); PE was chosen in order to avoid any overlap with algal autofluorescence. Cyanovirin-N lectin (CVN-PE) was used to label high-mannose N-glycans. *Microcystis viridis* lectin (MVL-PE) was used to label core N-glycan residues composed of GlcNAc and mannose [55]. *Phaseolus vulgaris* Phytohaemagglutinin lectin (PHA-L-PE) was used to label complex glycans. Their respective glycan recognition profiles summarized from publicly available glycan array data are shown in **Supplementary Figure 1** (<http://www.functionalglycomics.org>). These lectins were chosen for their micromolar binding affinities and comparatively greater selectivity and cleaner binding profiles. The surface glycan composition of labeled algal symbionts was measured on a CytoFLEX 5L flow cytometer. Algal populations were identified using forward and side scatter, and doublets were removed using FSC-Width in order to avoid overestimating fluorescent intensities of cells. A minimum of 20,000 cells labeled with PE-conjugated lectins were excited at 561 nm and emission was captured with a 585/42 nm bandpass filter. The median fluorescence intensity for each FSC-Width population was averaged for each treatment and compared using ANOVA. The arithmetic mean and geometric mean fluorescence intensity were also averaged by treatment and analyzed.

224 *Colonization Experiments*

225 Aposymbiotic adult *Aiptasia* polyps were obtained after repeated menthol bleaching [56] and
 226 maintenance in the dark. Prior to the beginning of experiments, polyps were individually plated
 227 in 24-well plates and exposed to the same 12:12 light:dark cycle as algal cultures for 1 wk.
 228 Animals were then visually checked under an inverted compound fluorescence microscope for
 229 the presence of any contaminating symbionts, and only those completely lacking symbionts were
 230 used in colonization experiments. Algae from each treatment were rinsed in FSW and
 231 resuspended to 2×10^6 cells $\cdot$ mL⁻¹. Each anemone was inoculated with 1×10^6 cells (0.5 mL of
 232 algal treatment) in a total volume of 1 mL FSW. After 48 h, the wells were rinsed three times
 233 with FSW, and left for 24 h before overnight fixation in 4% paraformaldehyde in 1X PBS. To
 234 prepare for imaging symbiont density, the oral discs with tentacles were dissected from the
 235 columns of each polyp and mounted on slides in a mounting solution (90% glycerol, 10% 1X
 236 PBS) as described previously [57].

237 *Image Analysis*

238 To normalize symbiont density to host surface area, epifluorescence microscopy was used to
 239 capture autofluorescence from the symbionts and the host anemone tissue as detailed previously
 240 [57]. Symbiont chlorophyll autofluorescence was captured under the Cy3 (red) filter, whereas
 241 autofluorescence from host green fluorescence was captured using the GFP (green) filter. Images
 242 of symbiont density after colonization of hosts were captured with a Zeiss AxioObserver A1
 243 microscope and an Axiovert ICm1 camera (Carl Zeiss AG, Jena, Germany). Algal cell numbers
 244 were automatically quantified by setting a predetermined cell radius for the ITCN plugin for Fiji
 245 (ImageJ2). To calculate algal density as a proxy for symbiont colonization, the total algal cell
 246 number was divided by the surface area of host autofluorescence.

247 **Results and Discussion**

248 *N-Glycan Biosynthesis Pathway Conservation in Alveolates*

249 We hypothesized that the pathways underlying N-linked glycoprotein production in
 250 Symbiodiniaceae are similar to those in humans and other well-studied eukaryotes. Our findings
 251 support this hypothesis. We found a large degree of conservation among members of these

groups using BLASTP searches for homologous proteins. We found strong evidence for N-glycan biosynthesis conservation up to MGAT1, an N-acetylglucosaminyltransferase essential for hybrid and complex type glycan processing (**Table 1, Figure 1**). Golgi α -mannosidase 2, the next step in the N-glycan pathway, also appears in a BLASTP search of Symbiodiniaceae genomes, however the sequence hits were more similar to lysosomal α -mannosidase, especially in conserved regions (**Supplementary Figure 2, Supplementary Table 1**). Without functional or subcellular localization data, it is difficult to discern whether this α -mannosidase functions during N-glycan biosynthesis or only during degradation; regardless, it should be inhibited by swainsonine based on conserved residues (**Supplementary Table 1**) [37]. Although all Symbiodiniaceae appear to be missing GlcNAc-TII (MGAT2), the *B. minutum* genome includes transferases GlcNAc-TIII (MGAT 3), GlcNAc-TIV (MGAT 4) and GlcNAc-TV (MGAT5), which are required for bisecting, tri-, and tetra-antennary N-glycans (**Table 1**). The presence of these transferases supports the existence of hybrid glycan biosynthesis in Symbiodiniaceae. With the absence of MGAT2, it remains unclear whether the α -1-6 branch required for complex glycans can be formed [31]. In addition to the majority of enzymes required for N-glycan branching, Symbiodiniaceae genomes contain N-glycan transferases for xylose, fucose, and galactose (**Table 1, Supplementary Table 2**). These moieties are possible targets for the expanded set of cnidarian ficolin-like proteins, fucoselectins, and c-type lectins found in *Aiptasia* [58, 59]. Nevertheless, these Symbiodiniaceae species appear to have the capacity to create high-mannose, hybrid, and potentially complex glycans.

The N-glycan biosynthesis pathway BLASTP protein hits for the Symbiodiniaceae species (*Breviolum minutum*, *Symbiodinium microadriaticum*, and *Fugacium kawagutii*) were most similar to the closely related *Perkinsus marinus*, an endoparasite of oysters (**Table 1**). Among the Symbiodiniaceae, we found the N-glycan biosynthesis pathway in *F. kawagutii* to be the least conserved, which agreed with the initial, less stringent analysis of the N-glycan pathway from the *F. kawagutii* draft genome [40]. This lack of conservation could reflect its phylogenetic position (one of the most derived lineages within the group) and/or ecology: of the three species whose genomes were searched, it is the only one that is exclusively free-living and nonsymbiotic. If hybrid and/or complex N-glycan production is a key to symbiosis establishment and maintenance, there may be less selective pressure to retain these genes in nonsymbiotic species. However, as the Symbiodiniaceae genomes are still in draft form, the absence of

pathways may be a result of differences in sequencing and/or annotation completeness, especially considering the decreased number and shorter length of gene models in the original *F. kawagutii* genome [40, 60]. In examining more evolutionarily distant comparisons, *B. minutum* and *S. microadriaticum* appear to have a greater capacity for N-glycan biosynthesis than many other algal protists, including other dinoflagellates, chlorophytes, diatoms, and haptophytes [40].

The degree of conservation in the N-glycan biosynthesis pathway of the Symbiodiniaceae is striking when compared to the degenerate pathways of the alveolate parasites *Plasmodium falciparum* and *Toxoplasma gondii*. In contrast to the Symbiodiniaceae, *P. falciparum* has severely limited N-glycosylation and O-glycosylation, and is only able to produce the chitobiose core using ALG13 and ALG14 (**Table 1**) [22, 61]. Instead, *P. falciparum* relies on GPI anchors (glycolipids) to stabilize proteins, and uses its own lectins to bind to host cells [62]. The *T. gondii* glycome is predominantly composed of high-mannose (Man₅₋₈(GlcNAc)₂) and shorter paucimannose (Man₃₋₄(GlcNAc)₂) glycans [23]. These degenerate N-glycans are partially a consequence of a loss of ALG glycosyltransferases in both parasites [24] (**Table 1**). The general conservation of precursor synthesis and ER/Golgi processing in the Symbiodiniaceae and *P. marinus* suggests strong differences in N-glycan processing between apicomplexans and Dinozoans (containing dinoflagellates and their sister phylum Perkinsozoa), supporting the hypothesis that among alveolates, only apicomplexans have undergone secondary loss of ALG enzymes [22].

N-Glycan Identification in Breviolum minutum

With the knowledge that N-glycan biosynthesis pathways are present and even conserved in *Breviolum minutum*, we sought to characterize the N-linked glycome. We harvested algal cells, cleaved the N-linked surface glycans, and after per-methylation used LC-MS/MS for glycan analysis [50–53, 63]. Based on molecular weight, retention time, and comparison to carbohydrate standards, the overall cleaved N-linked glycan sample exhibited 52% high-mannose glycans, 12% core-fucosylated glycans, and, to our surprise, 3% sialylated glycans (**Figure 2a**). Overall, 29 glycan structure were identified in the liquid-chromatography coupled mass spectrometry analysis (**Figure 2b**). While the enrichment of high-mannoside glycans has been shown indirectly in *B. minutum* before, this is the first direct, analytical verification of the result [28, 29, 57, 64].

Sialic acid (also called N-acetylneuramic acid) is often considered a hallmark of the human glycome as it can be found mostly on cell surfaces of ‘higher’ invertebrates. In humans, sialic acid modulates a variety of normal and pathological processes. Many pathogens bind to it (e.g. human *Influenza A*, *Vibrio cholerae*, *Plasmodium falciparum*, *Helicobacter pylori*) or express it on their surfaces to interact with human cells (*Neisseria* spp., *Trypanosoma cruzi*) [65]. Sialic acid is rarely found in algae and this is the first report of sialic acid in dinoflagellates [66–68]. Future studies should test whether sialic acid is a crucial component of cnidarian-dinoflagellate symbiosis establishment.

Effects of N-Glycan Removal on Colonization Success in Host Anemones

We expected that glycosidase treatment with PNGase F would decrease the total number of N-linked glycans on the cell surface of *B. minutum* and subsequently reduce colonization success. We measured changes to the algal cell surfaces using fluorescently tagged lectin (CVN-PE) that recognizes glycans highly enriched with $\alpha(1-2)$ -linked dimannosides. After exposure to PNGase F, CVN-PE labeling mean fluorescence intensities decreased, indicating that fewer high-mannoside glycans were present on algal cell surfaces after treatment (**Figure 3a**; ANOVA, Tukey post-hoc test, $p < 0.001$). Aiptasia polyps were then inoculated with glycosidase-treated or untreated cells. After initial colonization, symbiont density was lower in hosts exposed to the cells with reduced N-glycan abundances (**Figure 3b**; t-test, $p < 0.05$).

These results support the importance of N-glycans in host-symbiont interactions during the onset of symbiosis and are consistent with previous studies in Aiptasia and coral larvae. N-glycosidase treatments of algal symbionts blocked colonization of Aiptasia adults by algae, as did treatments with O-glycosidase, trypsin, and α -amylase [28]. Similarly, N-glycosidase and trypsin treatments inhibited symbiont colonization in larvae of the coral *Fungia scutaria* [29]. In contrast, α -amylase had no effect on the percentage of *F. scutaria* larvae colonized or in algal density within individual larvae. In another coral larval study using *Acropora tenuis*, α -amylase and trypsin increased the number of symbiont cells taken up by larvae [26]. Across all enzymatic treatments to date, N-glycosidases have had the most consistent negative effect on colonization rates of Symbiodiniaceae in cnidarian hosts. Enzymatic treatment with trypsin and PNGase F has also been used to decrease affinity of isolated cnidarian lectins to their glycan binding sites on Symbiodiniaceae, supporting the hypothesized function of glycan-lectin interactions in symbiosis

[69, 70]. This consistency across many studies suggests that the overall abundance of N-glycans on the Symbiodiniaceae cell surface impacts the rate of symbiont uptake by cnidarian hosts.

N-Glycan Biosynthesis Manipulation in Breviolum minutum

After confirming the effect of glycan abundance on colonization success, we were interested in understanding how the composition of different types of N-glycans affected the onset of symbiosis. To alter glycan composition, we inhibited the N-glycan biosynthesis pathway at ER and Golgi mannosidase enzymatic steps using the small molecule inhibitors kifunensine and swainsonine (**Figure 4a**). To confirm that the manipulations altered the composition rather than the abundance of N-glycans, we labeled cells with MVL-PE, which binds to structures consisting of Man β (1-4)GlcNAc β (1-4), a common core found in all three N-glycan types (**Figure 4b**). MVL-PE labeling showed a small but statistically significant increase in median fluorescence intensity in the kifunensine treatment compared to untreated cells (**Figure 4c**; ANOVA, Tukey post-hoc test, $p < 0.001$), but showed no difference between arithmetic mean (ANOVA, Tukey post-hoc test, $p = 0.99$) and geometric mean (ANOVA, Tukey post-hoc test, $p = 0.65$) fluorescence intensities. In general there were similar N-glycan abundances between all treatments. This relative stability in glycan abundance allowed us to analyze the remaining lectin binding data as primarily representative of differences in glycan composition.

Kifunensine blocks MAN1, the enzyme responsible for converting high-mannose glycans to hybrid glycans featuring less mannose. To confirm that kifunensine treatment increased the proportion of high-mannose glycans, symbiont cells were labeled with CVN-PE, which binds with α (1-2)-linked dimannosides residing at the branch tips of mannose-rich glycans (**Figure 4a**). Cells treated with kifunensine had the highest median fluorescence intensity of CVN-PE labeling as expected, significantly greater than both swainsonine-treated and untreated cells (**Figure 4d**; ANOVA, $p < 0.05$, $p < 1 \times 10^{-7}$). Swainsonine-treated cells also exhibited significantly greater labeling by CVN for mannosides than untreated cells (**Figure 4d**; ANOVA, $p < 1 \times 10^{-7}$). This was expected, as hybrid glycan structures induced by swainsonine should have more abundant mannose moieties than untreated cells that feature a greater proportion of complex, low-mannose glycans (**Figure 4a**). Analysis of geometric mean fluorescence intensity showed the same significant differences (ANOVA, $p < 1 \times 10^{-7}$) between every comparison except swainsonine and kifunensine treatments (ANOVA, $p = 0.28$), whereas arithmetic mean

fluorescence intensity found no differences between swainsonine and kifunensine treatments (ANOVA, $p = 0.92$) or between swainsonine and untreated cells (ANOVA, $p = 0.10$). Overall, kifunensine had a strong effect on increasing the proportion of high-mannose N-glycans, whereas swainsonine had a weaker effect. The increase in lectin-binding in both treatments supports the presence of active N-glycan biosynthesis inhibition from kifunensine and swainsonine incubation in Symbiodiniaceae cells.

Swainsonine blocks MAN2, the enzyme responsible for converting hybrid glycans to complex glycans featuring even less mannose (**Figure 4a**). To confirm that swainsonine treatment decreased the proportion of complex glycans (and therefore increased the proportion of hybrid glycans), symbiont cells were labeled with PHA-L-PE, which binds Gal β (1-4)GlcNAc linkages from the 2,6 branch found mainly in complex glycans [71]. Symbiont cells exhibited low levels of labeling with PHA-L-PE overall, though increases in median fluorescence intensity were significant compared to unlabeled negative control (**Figure 4e**; ANOVA, $p < 1 \times 10^{-7}$). Opposite to the expectation that swainsonine blocks complex glycan formation, cells treated with swainsonine showed increased, rather than decreased PHA-L-PE labeling compared to untreated cells (**Figure 4e**; ANOVA, $p < 1 \times 10^{-7}$). These significant differences were also present in geometric and arithmetic mean fluorescence intensity comparisons (ANOVA, $p < 1 \times 10^{-7}$). The low levels of staining combined with an absence of complex glycan inhibition suggests differences in later steps of N-glycan biosynthesis among Symbiodiniaceae when compared to the canonical pathway. It is possible that *Breviolum minutum* has alternative pathways to build N-glycans besides the late stage processing in the Golgi apparatus, known in eukaryotes to create N-glycoproteins that can be recognized by PHA-L. Additionally, since the MAN2 found in *B. minutum* is most similar to lysosomal mannosidases (**Supplementary Table 1, Supplementary Figure 2**), swainsonine may have inhibited lysosomal catabolism, contributing to glycan dysregulation [72]. It is also possible that while the treatment failed to decrease complex glycan proportion, it nevertheless may have increased hybrid glycan proportion, as we did not measure hybrid glycans directly. This outcome would be consistent with the elevated mannose-binding we observed directly with CVN-PE in swainsonine-treated cells.

Kifunensine-treated cells were also expected to have a lower proportion of complex glycans, however kifunensine showed no difference in PHA-L-PE labeling compared to untreated cells (**Figure 4e**; ANOVA, $p = 0.29$). This lack of significant change in complex N-

glycan abundance may indicate that the naturally occurring glycans on the Symbiodiniaceae cell surface are not primarily composed of complex N-glycans. Earlier studies on Symbiodiniaceae cell surface glycans found strong PHA-L lectin binding in the family [64]. However, compared to other genera, *B. minutum* exhibited the least amount of binding from PHA-L and ConA, recognizing complex N-glycans and high-mannose glycans, respectively. It is therefore possible that *B. minutum* does not have the ability to produce complex N-glycans through its own N-glycan biosynthesis. However, the presence of additional N-glycan transferases for moieties such as xylose, fucose, and galactose, as well as the detection of these and other ornaments via LC-MS/MS, support the potential for glycan complexity. It cannot be ruled out that *B. minutum* and other Symbiodiniaceae have alternate pathways for producing complex glycans. The O-glycan pathway is currently uncharacterized in Symbiodiniaceae, but it may allow for similar variation in complexity and subsequently play a role in host recognition.

N-Glycan Biosynthesis Inhibition Decreases Symbiont Colonization Success in Host Anemones

Finally, we inoculated aposymbiotic Aiptasia polyps with *Breviolum minutum* cultures treated with the N-glycan biosynthesis inhibitors to determine whether glycan composition has an effect on symbiont colonization rates in naïve hosts. After a fixed exposure period, Aiptasia polyps inoculated with kifunensine-treated cells had a lower symbiont density compared to those inoculated with untreated control cells (**Figure 5**; ANOVA, Dunnett's test, $p < 0.05$). However, animals colonized by swainsonine-treated cells had symbiont densities equivalent to controls (**Figure 5**; ANOVA, Dunnett's test $p = 0.295$).

While lectin masking and enzymatic cleavage experiments have shown that low abundances of high-mannose glycans on symbiont cell surfaces reduce colonization [26–29], our biochemical inhibition data are the first to indicate that elevated proportions of high-mannose glycans can also reduce colonization, suggesting that glycan composition is a key factor modulating symbiosis establishment. Unless the proportion of different glycans is within a specific range, the micro-alga may not be recognized as an appropriate symbiont, providing a potential mechanism for maintaining specificity. An additional possibility is that interactions between lectins and hybrid glycans are selective at a later step after initial recognition of mannose. This would be consistent with the observed difference in colonization success between swainsonine-treated cells (i.e. more high-mannose and hybrid N-glycans) and kifunensine-

treated cells (i.e. more high-mannose N-glycans only). A multi-step glycan recognition pathway could be part of a “winnowing” process of host-symbiont selection similar to that found in other symbioses [73].

In cnidarians, mannose recognition includes a variety of highly conserved mechanisms [74–76]. For example, the lectin-complement pathway is functional in the gastroderm and expression is upregulated in response to bacterial pathogens and algal symbionts [74, 77]. The binding of pattern recognition receptors, such as the coral mannose-binding lectin millectin, to both pathogenic bacteria and members of Symbiodiniaceae suggests a dual role of mannose glycan recognition in cnidarian immunity and symbiosis [17, 78]. Indeed, the activation of host innate immunity during colonization contrasts with the repression of host innate immunity under stable symbiosis conditions [77, 79–81]. Therefore, the response of host cells to symbiont N-glycan abundance and composition may differ depending on symbiotic state and life history stage.

Implications and Future Directions

Our results provide new information about the chemical signaling molecules of *Breviolum minutum*, a mutualistic dinoflagellate symbiont of corals and sea anemones. We verified that the N-linked glycan biosynthesis pathway is mostly present and conserved in the *B. minutum* genome, and we determined the structure and abundance of N-glycans cleaved from the algal surface. While our experiments stress the notion that N-glycans are important for symbiont recognition, we observed a complex algal glycome without clear determinants of symbiosis establishment. The direct approach of manipulating this algal glycome (through cleavage with PNGase F or through biosynthesis inhibition) has had a more consistent effect on colonization than glycan masking approaches [26, 28, 29, 57]. Nevertheless, colonization success was highly variable in the clonal adult hosts that were used in this study, possibly due to small differences in polyp size or states of innate immunity in aposymbiotic adults. Challenging naïve, aposymbiotic larvae may be a more fruitful approach in the Aiptasia system when investigating symbiosis establishment, since larvae allow for greater sample sizes and thus more statistical power to detect subtle differences in colonization [57]. Despite this limitation, the differences observed in glycan characterization and symbiont recolonization support the further use of aposymbiotic Aiptasia adults as a model for understanding processes involved in bleaching recovery. The

potential for variation in N-glycan biosynthesis among Symbiodiniaceae could provide a basis for host-symbiont recognition as well as recolonization dynamics after environmental conditions that result in dysbiosis.

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Figure 1. Canonical N-glycan biosynthesis and N-glycan recognition in animals.

The biosynthesis of N-glycans begins with the transfer of a nucleoside activated N-acetylglucosamine (GlcNAc) to a dolichol membrane anchor (Dol-P-P). A fourteen-sugar precursor structure is created by elongation, in the ER lumen. This structure is then mass-transferred to an Asn residue of a protein undergoing synthesis and aids in protein folding. N-glycans are further processed by glucosidases (MOGS, GANAB) to remove glucose, and then by ER mannosidase I (MAN1) to remove mannose residues. MAN1 enzymes act in both the ER and the Golgi to transform oligomannoside into hybrid glycans by N-acetylglucosaminyltransferase 1 (MGAT1). To create complex glycans, glycoproteins in the Golgi are further cleaved by mannosidase II (MAN2), then a series of transferases add GlcNAc, galactose, and other sugar residues to create complex N-glycans. Small molecules that act as enzymatic inhibitors can be used to block parts of this pathway, for example kifunensine and swainsonine. Pattern recognition receptors (PRRs) from host cells (Mannose-binding lectin, ficolins, etc.) interact with surface glycans on target cells to mediate immunity. In dinoflagellate symbionts, we have limited knowledge regarding the conservation of this general N-glycan biosynthesis pathway found in animals and plants. This N-glycan biosynthesis pathway was adapted from Helenius and Aeibi [82].

Figure 2. Quantitative glycomic analysis in *Breviolum minutum* via LC-MS/MS. **(a)** Proportions of different N-glycan types represented in a sample of enzymatically cleaved *B. minutum* surface glycans. **(b)** Absolute quantitation of N-glycans and their putative structures. Boxed sections indicate zoomed in areas with 20x **(c)** and 10x magnification **(d, e)**. Symbol: blue square – N-Acetylglucosamine (GlcNAc); green circle – mannose (Man); yellow circle – galactose (Gal); purple diamond – N-acetylneuraminic (sialic) acid (NeuAc); hollow diamond – N-Glycolylneuraminic acid (NeuGC); red triangle – fucose (Fuc). Nomenclature: a five digit code is used to represent glycan structures. The 1st number represents the number of HexNAc (GlcNAc); the 2nd number represents the number of Hex (Man + Gal); the 3rd number represents the number of DeoxyHex (Fuc); the 4th number represents the number of sialic acid (NeuAc); the 5th number represents the number of N-Glycolylneuraminic acid (NeuGC). For example, 45120 represents HexNAc₄Hex₅DeoxyHex₁NeuAc₂.

Figure 3. Effect of PNGase F treatment on *Breviolum minutum*.

(a) Cell surface N-glycan removal. After 48 h of PNGase F treatment at different concentrations, glycan abundance on cell surfaces was measured through the binding of fluorescently tagged lectin CVN. Unique letters represent significant differences in mean fluorescence intensity following ANOVA ($p < 0.001$; $n = 2$ replicates per treatment). Untreated cells without fluorescently-labeled lectins were used as a negative control (-), while untreated cells with fluorescently-labeled lectins were used as a positive control (+). Error bars indicate total range.

(b) Colonization rates of untreated and PNGase F-treated algae in adult polyps. Dots represent data from replicate polyps. Asterisks indicate a significant difference between treatments (t -test, $p < 0.05$).

Figure 4. Inhibition of N-glycan biosynthesis in *Breviolum minutum*.

(a) Pharmacological inhibition of the N-glycan biosynthesis pathway with kifunensine (orange) and swainsonine (teal) in the ER and Golgi and the hypothesized effect on cell surface N-glycans in Symbiodiniaceae cells. Parts of the pathway that lack conservation are shown in gray, indicated by a question mark. (b) Glycan recognition sites of the respective lectins within the three types of N-glycans (high-mannose, hybrid, and complex). MVL-PE was used to confirm the core of N-glycans, whereas CVN-PE and PHA-L-PE were used to determine the high-mannose and complex glycan composition, respectively. (c) MVL-PE labeling of core N-glycans. Algae that were incubated for one week with kifunensine, swainsonine, or DMSO (control) were labeled with MVL-PE to quantify N-glycan abundance using median fluorescence intensity (n = 3). No differences in mean fluorescence intensity were found between lectin-labeled treatments. (d) CVN-PE labeling of high-mannose N-glycans. Treated algae were labeled with CVN-PE to quantify relative amount of high-mannose N-glycans using median fluorescence intensity (n = 3-4). Mean fluorescence intensity comparisons resulted in the same significance. (e) PHA-L-PE labeling of complex N-glycans. Treated algae were labeled with PHA-L-PE to quantify relative amount of complex N-glycans using median fluorescence intensity (n = 6-8). Mean fluorescence intensity comparisons resulted in the same significance. Untreated cells without fluorescently-labeled lectins were used as a negative control (-), while untreated cells with fluorescently-labeled lectins were used as a positive control (+). Unique letters represent significant differences in staining percentages (ANOVA, $p < 0.05$).

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Figure 5. Effect of kifunensine and swainsonine symbiont colonization success of host anemones.

Effect of N-glycan biosynthesis inhibition on colonization by *B. minutum* in aposymbiotic adult Aiptasia polyps ($n = 30$). Asterisks indicate a significant difference between treatments (ANOVA, Dunnett's test, $p < 0.05$).

Table 1: N-glycan biosynthesis in alveolates

Description	Protein	B. min.	S. mic.	F. kaw.	P. mar.	T. gon.	P. fal.
Dol-P precursor synthesis							
UDP-N-acetylglucosaminyltransferase subunit	ALG13	+	+	+		+	+
UDP-N-acetylglucosaminyltransferase subunit	ALG14	+	+		+	+	+
Mannosyltransferase-1	ALG1	+	+		+	+	
α -1,3-mannosyltransferase	ALG2	+	+	+	+	+	
α -1,2-mannosyltransferase	ALG11	+	+		+	+	
α -1,3-mannosyltransferase	ALG3	+	+		+		
α -1,2-mannosyltransferase	ALG9	+	+	+	+		
α -1,6-mannosyltransferase	ALG12		+	+	+		
DolP-glucosyltransferase	ALG5	+	+		+	+	
α -1,3-glucosyltransferase	ALG6	+	+	+	+	+	
α -1,3-glucosyltransferase	ALG8	+	+	+	+	+	
α -1,2-glucosyltransferase	ALG10	+	+	+		+	
Oligosaccharyl transferase subunit STT3	STT3	+	+	+	+	+	+
ER Processing							
Mannosyl-oligosaccharide glucosidase	GCS1	+	+		+	+	
Neutral α -glucosidase AB	GANAB	+	+	+	+	+	
ER α -1,2-mannosidase	MAN1B1	+	+	+	+		
Golgi Processing							
α -1,2-mannosidase IA	MAN1A1	+	+	+	+		
α -1,2-mannosidase IB	MAN1A2	+	+	+	+		
(α -1,3) 2- β -GlcNAc transferase I	MGAT1	+	+	+	+		
α -mannosidase 2	MAN2A1						
Lysosomal α -mannosidase	MAN2B1	+	+	+	+		
(α -1,6) β -1,2-GlcNAc transferase II	MGAT2						
(β -1,4) β -1,4-GlcNAc transferase III	MGAT3	+			+		
(α -1,3) β -1,4-GlcNAc transferase IVC	MGAT4C	+	+				
(α -1,6) β -1,6-GlcNAc transferase V	MGAT5	+	+				
Complex							
Xylosyltransferase 1	XYLT1	+	+				
α -1,6-fucosyltransferase 8	FUT8		+	+			
α -1,3-fucosyltransferase 11	FUT11	+	+		+		
α -1,4-fucosyltransferase 13	FUT13	+	+				
β -1,4-galactosyltransferase 1	B4GALT1	+	+	+			
β -1,3-galactosyltransferase 1	B3GALT1	+	+	+	+		
β -galactoside α -2,6-sialyltransferase 1	SIAT1						
β -galactoside α -2,6-sialyltransferase 2	SIAT2						
Paucimannose							
β -hexosaminidase 1	HEXO1	+	+	+	+		

798 **SUPPLEMENTARY MATERIAL**

799 **Supplementary Figure 1. Lectin specificity of MVL, CVN, and PHA-L.**

800 The lectin specificity of (a) MVL, (b) CVN, and (c) PHA-L. is shown as determined from glycan
801 microarray experiments. MVL shows binding affinity for chitobiose core N-glycan motifs [Man-
802 3 to Man-9; α (1-6) and α (1-3)-dimannoside, Man β (1-4) GlcNAc], CVN shows affinity for high-
803 mannose N-glycan motifs [Man-8 and Man-9 only; α (1-2)-dimannoside], and PHA-L shows
804 affinity for complex type N-glycan motifs [Gal- β (1-4)GlcNAc β (1-6)(GlcNAc β (1-2)Man α (1-
805 3)) Man α (1-3)]. Data provided by the Consortium for Functional Glycomics.
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MAN2_DROME 128 GNN-----KMD-PLKYNA-HHKLVFVPHSHNDPGWLCFFBYYQHD-----TKHLLSNAIRHLHDNPEMR
MA2A1_HUMAN 151 GSD-----TME-SNEW-D-TEPLQVFWPHSHNDPGWLKTFNDYRDK-----TQYIFNNVVLKIKEDSSRK
MA2A1_MOUSE 150 GSD-----KYE-ADEW-D-HEPLQVFWPHSHNDPGWLKTFNDYRDK-----TQYIFNNVVLKIKEDSSRK
GMAN2_ARATH 138 GNR-----TYK-DDEW-E-KEKLVFVPHSHNDPGWKLTVBYYQRO-----SRHLDITIVETLSKDSRRK
LMANII_DROME 24 --LRSIQPKASQCQYQSCPT-K-PNMLNVHLLPHTHDDVGWLKTVDOYYYGSETKQKAGVQYILDSVVEALLRDPEKR
MA2B1_HUMAN 38 FLLLAAGARAGGYETCTPT-Q-PNMLNVHLLPHTHDDVGWLKTVDOYYYGKINDQKAGVQYILDSVVEALLRDPEKR
MA2B1_MOUSE 38 FLLLAAPGARAAGYKTCPT-K-PGMLNVHLLPHTHDDVGWLKTVDOYYYGILSDQKASVQYILDSVVSLLLEKPTTR
MANA2_ARATH 22 RMMY-----NT-SHTI-V-PGKLNVMVPHSHDDVGWLKTVDOYYYGSNNSQVACVQNLDSIVPALLADKNRK
Smic4843_SMIC 434 QAAV-----LCS-ALAV-QAEPANVEHVPHTHDDVGWLKTVDOYYTGQNNSORAVVRLILDVVRCLQEQNDRK
symbB.v1.2.0177 35 LLLI-----CSL-LEQI-S-AEQLVHVVCHHDDVGWLKTVDOYYTGQNNSAHAMVHMLDSVVSQSLAANENRT
symbB.v1.2.0177 5 -----HS-----L-----
Skav210588_FKAW 1 -----DPTL-E-AHPLEVENVCHHDDVGWLKTVDOYYAGLNNSQHAMVHMLDSVVRALVAPPSRT
Skav210590_FKAW 1 -----

MAN2_DROME 189 FTTAEI-----SYFARFYHDLGENKKLOKSL-----V-KNCOLEFVTGGWVMPDEANSHRNVLQILEGQTLKQFMNV
MA2A1_HUMAN 211 FINSSEI-----SYLSRWWDIIDIQKKDAVKSL-----ENGQLEFVTGGWVMPDEATPHYFALDQILEGHQLENNIGV
MA2A1_MOUSE 210 FINSSEI-----SYLSRWWDIIDIPKKEAVKSL-----ENGQLEFVTGGWVMPDEATPHYFALDQILEGHQLENNIGV
GMAN2_ARATH 198 FINSSEI-----SYLSRWWDIIDIPKKEAVKSL-----ENGQLEFVTGGWVMPDEATPHYFALDQILEGHQLENNIGV
LMANII_DROME 100 FIYVES-----AFFRWWRKPKPVQEAVRML-----V-ECGRLEFVTGGWVMPDEATPHYFALDQILEGHQLENNIGV
MA2B1_HUMAN 116 FIYVEI-----AFFRWWRKPKPVQEAVRML-----V-ECGRLEFVTGGWVMPDEATPHYFALDQILEGHQLENNIGV
MA2B1_MOUSE 116 FIYVEI-----AFFRWWRKPKPVQEAVRML-----V-ECGRLEFVTGGWVMPDEATPHYFALDQILEGHQLENNIGV
MANA2_ARATH 90 FIYVEQ-----AFFRWWRKPKPVQEAVRML-----V-ECGRLEFVTGGWVMPDEATPHYFALDQILEGHQLENNIGV
Smic4843_SMIC 504 ETVYET-----AFFRWWRKPKPVQEAVRML-----V-ECGRLEFVTGGWVMPDEATPHYFALDQILEGHQLENNIGV
symbB.v1.2.0177 105 ETVYEQ-----AFFRWWRKPKPVQEAVRML-----V-ECGRLEFVTGGWVMPDEATPHYFALDQILEGHQLENNIGV
symbB.v1.2.0177 8 -----
Skav210588_FKAW 62 ETVYEQ-----AFFRWWRKPKPVQEAVRML-----V-ECGRLEFVTGGWVMPDEATPHYFALDQILEGHQLENNIGV
Skav210590_FKAW 1 -----

MAN2_DROME 259 --TPTASWAIIDPFHGSPTMAYLL--KQSGFKNMLIQRTHYSVKKELAQQL--EFVWRQIWDNKG-DTAFTHMMPF--YSY
MA2A1_HUMAN 281 --KPRSGWAIIDPFHGSPTMAYLL--NRAGLSHLLIQRTHYAVKKHFALEKT--EFFWRQIWDNKG-VTDLCHMMPF--YSY
MA2A1_MOUSE 280 --KPRSGWAIIDPFHGSPTMAYLL--NRAGLSHLLIQRTHYAVKKHFALEKT--EFFWRQIWDNKG-VTDLCHMMPF--YSY
GMAN2_ARATH 268 --IPNNSWAIIDPFHGSPTMAYLL--RRMGFENMLIQRTHYELKKDLAQ--KNLEYWRQSWDAME-TTDFVHMMPF--YSY
LMANII_DROME 170 CGRPRVWQIDPFHGSREMASLF-AQMGFDGFFGRIDYQDKDERLMTKNAEMWHGSANLGE-EADLFSGAPY--NNY
MA2B1_HUMAN 186 DGRPRVWQIDPFHGSREMASLF-AQMGFDGFFGRIDYQDKDERLMTKNAEMWHGSANLGE-EADLFSGAPY--NNY
MA2B1_MOUSE 186 DGRPRVWQIDPFHGSREMASLF-AQMGFDGFFGRIDYQDKDERLMTKNAEMWHGSANLGE-EADLFSGAPY--NNY
MANA2_ARATH 160 --TPRIGWQIDPFHGSREMASLF-AQMGFDGFFGRIDYQDKDERLMTKNAEMWHGSANLGE-EADLFSGAPY--NNY
Smic4843_SMIC 574 --APRTGWQIDPFHGSREMASLF-AQMGFDGFFGRIDYQDKDERLMTKNAEMWHGSANLGE-EADLFSGAPY--NNY
symbB.v1.2.0177 175 --APRTGWQIDPFHGSREMASLF-AQMGFDGFFGRIDYQDKDERLMTKNAEMWHGSANLGE-EADLFSGAPY--NNY
symbB.v1.2.0177 8 -----ASIDN--GHPAGGS-----
Skav210588_FKAW 142 --APRTGWQIDPFHGSREMASLF-AQMGFDGFFGRIDYQDKDERLMTKNAEMWHGSANLGE-EADLFSGAPY--NNY
Skav210590_FKAW 1 -----

MAN2_DROME 333 DIPHTCGPDPKVCQDFDKRMGSFGLS-----CPWKVPPTIS--QNVAAASDLL--DQWKKKAELERTNVLIIPIPGDDFRF
MA2A1_HUMAN 355 DIPHTCGPDPKVCQDFDKRIPGGRFG-----CPWGVPPETIHPGNVQSRARML--DQYRKKSLELRTKVLIIPIPGDDFRF
MA2A1_MOUSE 354 DIPHTCGPDPKVCQDFDKRIPGGRFG-----CPWGVPPETIHPGNVQSRARML--DQYRKKSLELRTKVLIIPIPGDDFRF
GMAN2_ARATH 342 DIPHTCGPEPAICQDFDFARMRGFYEL-----CPWGKHPVETTLNVQERALKL--DQYRKKSLELRTNLTIIPIPGDDFRF
LMANII_DROME 245 QAPD-----GFCFDLILCNDAP--II-DGKHSP--NNVKERIDTF--DFAKTQSQY--RTNIIIVTNGGDETY
MA2B1_HUMAN 262 NPPK-----NLCWDVLCVDQPLV-EDPRSP--YNAKELVDYF--NVATAQGRY--RTNHTVMTNGSDRQY
MA2B1_MOUSE 262 NPPK-----NLCWDVLCVDQPLV-EDPRSP--YNAKELVDYF--NVATAQGRY--RTNHTVMTNGSDRQY
MANA2_ARATH 234 EPPP-----GGFYVEITDDSPVVQ--DDPDLF--YNVQERVNAF--AAALDQANITRINHIFTNGTDRY
Smic4843_SMIC 651 GPPA-----GFDWDVTSDDETIQ--DDPALE--YNVKS RVDDF--VAVAMVQANQTRGRHIMMTNGSDRQY
symbB.v1.2.0177 252 GPPD-----GFDWDVTSDDETIQ--DDPALE--YNVKS RVDDF--VAVAMVQANQTRGRHIMMTNGSDRQY
symbB.v1.2.0177 21 -----GFDWDVTSDDETIQ--DDPALE--YNVKS RVDDF--VAVAMVQANQTRGRHIMMTNGSDRQY
Skav210588_FKAW 184 -----GFDWDVTSDDETIQ--DDPALE--YNVKS RVDDF--VAVAMVQANQTRGRHIMMTNGSDRQY
Skav210590_FKAW 1 -----

MAN2_DROME 408 KQTEWQVQVRYERLFEHINSQAH--FNVQAG--GTLQEFDA--HQ-AER-----AG--AEFETLSGDFFTYADR
MA2A1_HUMAN 430 CEYTEWDLQFNYQQLFDYVNSQSK--FKVKIQ--GTLSD--FDA--DK-ADET-----Q-RDKG--SMFVPLSGDFFTYADR
MA2A1_MOUSE 429 SEYTEWDLQFNYQQLFDYVNSQSK--FKVKIQ--GTLSD--FDA--DK-ADET-----Q-RDKG--SMFVPLSGDFFTYADR
GMAN2_ARATH 418 ISIDEAEQFNYQQLFDYVNSQSK--FKVKIQ--GTLSD--FDA--DK-ADET-----Q-RDKG--SMFVPLSGDFFTYADR
LMANII_DROME 306 QAAQVY-----YNNLDKILIRYGNERQANGSN--NLL--STPSCYLKS--HD-A-----GTTVETKSDDFFPYAD
MA2B1_HUMAN 323 ENANMW-----FNNLDKILIRLVNAQQAKGSSVHVLL--STPACYLWE--NK-A-----LT--SVKHDDDFFPYAD
MA2B1_MOUSE 323 ENANMW-----FNNLDKILIRLVNAQQAKGSSVHVLL--STPACYLWE--NK-A-----LT--SVKHDDDFFPYAD
MANA2_ARATH 296 QYAHTW-----YNNLDKILIRLVNAQQAKGSSVHVLL--STPACYLWE--NK-A-----LT--SVKHDDDFFPYAD
Smic4843_SMIC 712 EDAFTW-----YNNLDKILIRLVNAQQAKGSSVHVLL--STPACYLWE--NK-A-----LT--SVKHDDDFFPYAD
symbB.v1.2.0177 313 EDAATW-----YNNLDKILIRLVNAQQAKGSSVHVLL--STPACYLWE--NK-A-----LT--SVKHDDDFFPYAD
symbB.v1.2.0177 21 -----YNNLDKILIRLVNAQQAKGSSVHVLL--STPACYLWE--NK-A-----LT--SVKHDDDFFPYAD
Skav210588_FKAW 184 -----YNNLDKILIRLVNAQQAKGSSVHVLL--STPACYLWE--NK-A-----LT--SVKHDDDFFPYAD
Skav210590_FKAW 1 -----YNNLDKILIRLVNAQQAKGSSVHVLL--STPACYLWE--NK-A-----LT--SVKHDDDFFPYAD

MAN2_DROME 474 SDNHWGYSYTSRPFYKRMORLMHVRRAAEIMSAHWSWDCM-----ARIEEREQARRESLFOHHDHIGTGAKTH
MA2A1_HUMAN 500 DDHWSGYFTSRPFYKRMORMESHRRAAEILYYFALRQHKYKINKFLSSSLYTA TEARRNLGLFOHHDHIGTGAKDW
MA2A1_MOUSE 499 DDHWSGYFTSRPFYKRMORMESHRRAAEILYYFALRQHKYKINKFLSSSPHYTT TEARRNLGLFOHHDHIGTGAKDW
GMAN2_ARATH 496 QDDHWSGYVSRPFYKRMORLEHTRGAEIMSFLLGYCHRIQCEKF-PTSFTYKLTAAARNLALFOHHDHIGTGAKDY
LMANII_DROME 367 PHAWHWGYFTSRPTLKKFERDGNHDLQVCKQLSALAPKKPEEF-----DPHITFMRET LGIMQHHDHIGTGAKER
MA2B1_HUMAN 384 PHQHWIGYFSSRPALKRYERISYNEIQVCNQLALVGLAANVGPY-----GSGDSAPLNEAMAVLQHHDHIGTGAKQH
MA2B1_MOUSE 384 PHMHWIGYFSSRPALKRYERISYNEIQVCNQLALVGLAANVGPY-----GSGDSAPLQEAAMAVLQHHDHIGTGAKQN
MANA2_ARATH 353 INAWHWGYFTSRPALKKRYVRMSAYLAARQLFEFFKGR-SQKG-----PNTDSLADALATAQHHDHIGTGAKQH
Smic4843_SMIC 769 PHQHWIGYFTSRPTLKKYIRDTSAFQVAKQHSVISNQPSQM-----EGNALAEAMGVAAQHHDHIGTGAKQH
symbB.v1.2.0177 371 PHKHWIGYFTSRPALKKYIRDTSAFQVAKQHTTLGA--ASKF-----SGVEKLAEAMGVAAQHHDHIGTGAKQH
symbB.v1.2.0177 21 -----
Skav210588_FKAW 184 -----GAMGVAAQHHDHIGTGAKQH
Skav210590_FKAW 1 -----

MAN2_DROME 545 VVVVDYEQRMQELKACQMVQQSYRRLTKPSI-Y-----SPDIFSFTYTLDDSR----WPGSGVEDSRTTIILGED
MA2A1_HUMAN 580 VVVVDYQRLFHSLMVLEKIGNSAFLILKDKLTY-----DSYSPDTFLEMCLKKSQDSLQPKN-----IIRL-
MA2A1_MOUSE 579 VVVVDYQRLFQSLNSLEKIGDSAFLILKDKKLY-----QSDPSKAFLEMDTKSSQDSLQPKI-----IIQLS
GMAN2_ARATH 575 VVVVDYQRLHHTSLQDLQIFVSKAEVLLGIRHEKE-----KSDQSPSFEEAQMRSKYDARVHK-----PIAAR
LMANII_DROME 437 VALDYARMSVFRACGATTRNAHQTLVQSKDNVKTSAKYVFEFK---TCALLNITS--CPVSE-----
MA2B1_HUMAN 457 VANDYARQLAAGWGPCEVLVSNARLRGFK-----DHFT---FCQQLNISI--CPLSQ-----
MA2B1_MOUSE 457 VVNDYARQLAAGWGPCEVLVSNARLRSHYK-----QNFSSFCRELNISI--CPVSO-----
MANA2_ARATH 421 VANDYARQLAAGWGPCEVLVSNARLRSHYK-----NPTFQ---QCLLLNISI--CPSEVNL-----S
Smic4843_SMIC 838 VTFDYARQLAKRAAALPGVSAAKQLTQAPD-----SSDFV---FCDLRVSV--CAPTQAVG-----K
symbB.v1.2.0177 438 VTFDYARQLSHRSSAGPEVSHALQILMGVRG-----TEDEFT---MCELRVSK--CAATQSVG-----R
symbB.v1.2.0177 21 -----
Skav210588_FKAW 203 VTFDYARQLSHRSSAGPEVSHALQILMGVRG-----
Skav210590_FKAW 1 -----

MAN2_DROME 612 ILPSKHVYVHNTLPHWREQLVDFVSSPFVSVTDLA-NN--PVEAQSPVWSWHHDTLTKT-----IHPQGSTTKRI
MA2A1_HUMAN 644 SAEPRYVYVHNTLPHWREQLVDFVSSPFVSVTDLA-NN--PVEAQSAVWDTA-----NTISETATEI
MA2A1_MOUSE 644 AQEPRYVYVHNTLPHWREQLVDFVSSPFVSVTDLA-NN--PVEAQSAVWNDM-----RTISQAALVEV
GMAN2_ARATH 640 EGNSTYILHNPSQTRREVVTVVNNRAEISVLDSN-WT--CVPSQSPEVQHD-D-----TKLFTGRHRL
LMANII_DROME 498 ANDRFATLHNPLAHTVNEYVRPVPYSNYRIDNK-GV--TESQAVPIPQVLI-DI-----KH-RNSTAKTEI
MA2B1_HUMAN 506 TAARFQIIVNPLGRKVNWVRPVPSEGVFVVKDPN-GR--TVPSDVIIFPSSD-----SQAHPPPEL
MA2B1_MOUSE 506 TSERFQIIVNPLGRKVDQVVRPVPYEGNFIVKDPH-DK--NHSNVMVPSYY-----SETYQDEL
MANA2_ARATH 478 DGKSLIILANPLGKWKVVDVVRPVPVGGDVSVHDSH-GH--EVESQVFPFTDEYV-ALRKYHVEAYLGQS-PTQVPKWL
Smic4843_SMIC 893 TTERSCFTLHNLGHAEELIIEIPWSTEKLEVVSGTRGVQGAVEFQVESLP---SVTNY-----GA-PAGGASKTL
symbB.v1.2.0177 493 NSNRTCFALHNLGLAKERQ-----
symbB.v1.2.0177 21 -----KTL
Skav210588_FKAW 222 -----
Skav210590_FKAW 1 -----

MAN2_DROME 682 FKA-RVFPGLAVYVITISDSKPEHTS-----YASNLLLRKNPTSLPLGQYPEDVKF-GDPREISLRVNGG-PTLAFS-
MA2A1_HUMAN 705 SFRA-HIPELGLKVKIKLESASSNSHL-----ADYVLYKN-KVEDSGIFTIKNMI-NTEE--GITENSFLLRFD-
MA2A1_MOUSE 705 SFRA-HIPELGLKVKIKLESQSSSSHL-----ADYVLYNNDGLAENGIFHVKNMV-DAGD--AITENPFALMFD-
GMAN2_ARATH 702 YKKA-SIPALGLRMYFLANGNVECEKATPSKLKYASEFDPFPCPPP-----Y---SCSKLDND-VTERNEHOTLVEDV
LMANII_DROME 563 VFLATNIPALGYRYIYEKLDSTEGN-T-----RSK-AL-----PKRTSS-VTVGNSHQLGDDT
MA2B1_HUMAN 565 IFSA-SIPALGFSYISYIAQVPRWKFPQ-A-----RAPQPI-----P-RRSWSP--ALTENEHRRATEDP
MA2B1_MOUSE 565 IFPA-SIPALGFSYISYIAKMSDLNHQ-A-----HNLLSR-----PRKHKSHH-VLVLENKYRATEDS
MANA2_ARATH 553 VFSV-TVPELGETIYITSTAKKTGYS--KSYVSN-----ILKGEQS--IINIGHGHKLSST
Smic4843_SMIC 961 VAV-KLEAAGHASYCQTAGVAA--P-----TAKLAN-----IS--QGA--CQALENENKLMFC-
symbB.v1.2.0177 511 -----
symbB.v1.2.0177 25 AEL-KIPALGFGGICQAAGRAL--P-----TPV-V-----ENVTGE--TKVVENDYALFER-
Skav210588_FKAW 222 -----
Skav210590_FKAW 1 -----

MAN2_DROME 753 EQGLKSKQLTQDSPHVVFHFKFLKYGVRS-----HGDRSGAYIFLPNGPASP-VELGQPVVLTGKGLLESSVSVGL
MA2A1_HUMAN 771 QTGLMKQMTKEDGKHHEVNVQFSYGTII-----KRDKSGAYIFLPDGNAPKYVTTTPPFVRTGGRYSEVTCFF
MA2A1_MOUSE 772 RSGLEKMKRKEDSRQHEKVQFLYGTIN-----KRDKSGAYIFLPDGGQGPYSLRPPFVRTGGRYSVTCFL
GMAN2_ARATH 771 KNGSLRKVHRNGSE-TVVGEIIGMY-----SSPESGAYIFLPDGEAQP-VQPDGHVVTSEGLVVEVFSYP
LMANII_DROME 616 -NGELSEITA--DGLTRLVSOEFLIYEGAVGNNAE--FLNRSSGAYIFRPENENKIHA-TDQVEIYKGLVVEVHQKF
MA2B1_HUMAN 619 DTGLLMEIMNMNQQLLPVQRTFVYNASIGDNE--SDQASGAYIFRPNQKFLP-SRWAQSHVVTPLVQEVHQNF
MA2B1_MOUSE 620 GTGLMKLENLEQNLSLPVSQGFVYNASVGDDE--SSQASGAYIFRPNVGKPIP-SRWAQSHVVTALVQEVHQNF
MANA2_ARATH 608 DQCFAINVVGRTSMTEFVKQTFSSYSAINGSNDKEPLIPQNSGAYIFRPNGTFFPIN-EGQVPTITIGGLVVEVHQI
Smic4843_SMIC 1010 -GGLIAN-TDKLSKTSTRAEQSMAYKASVGDA--SSQASGAYIFRPNRSQALPFTGLPFRVFGFLADEVHQV
symbB.v1.2.0177 511 -----
symbB.v1.2.0177 73 -GGLSRVVDKVKNNISTRAEQSMAYGEGSTGNNE--SSQHSGAYIFRPKRQQALPFTGLPFRVFGFLADEVHQSI
Skav210588_FKAW 222 -----
Skav210590_FKAW 1 -----

MAN2_DROME 824 -----PSVHQTIIRGG-----AFERNLVD-----GSLDNTETMFLPHIDSGDIFYTDNGLQFIKRRL-----
MA2A1_HUMAN 843 -----DHVHRVRLYHIQGI-EGQSVEVSNIVD-----RKVYNREIAMISSDKSQNRFYTDNGYQIQPRMTL-----
MA2A1_MOUSE 844 -----EHVHKVRLYNIQGI-EGQSMEVSNIVN-----RNVHNREIMRISSKANNQNRFYTDNGYQIQPRMTL-----
GMAN2_ARATH 837 KTKWEKSPKCKTRLYTGGNTLQDQVVEEIHVELL-GNDFDDRELIVRYKDDDNKKVFYTDNGFQNSRRETY-----
LMANII_DROME 690 -----NDWISQVVRINKDSYA-----EFELVVGPIPDGIGKEVITRFNSDIASDGIERTDSNGREMKRKNHRDWT
MA2B1_HUMAN 694 -----SAWCSQVVRYPQGRHL-----ELEISVGPIPDGTWGEVLSRFDPLETKGRFYTDNNGREIMRRRDYRPTW
MA2B1_MOUSE 695 -----SAWCSQVIRLYKGRHL-----ELEITVVGPIPRDDWGEVLSRFDPLETKKGQFYTDNNGREIMRRDDYRPTW
MANA2_ARATH 687 -----NPWISQITRYKGEHV-----EVEIVGNIPDDGIGKEVITQISSSKSNKTFYTDSSGRDYIKRIRDYRSDW
Smic4843_SMIC 1085 -----GSWISQITRLARGARHV-----EITITVSDIPDDGWGEVLSRISIDHIDGTCYTDNNGREIMRRRDYRPTW
symbB.v1.2.0177 511 -----GSWISQITRLARGARHV-----EITITVSDIPDDGWGEVLSRISIDHIDGTCYTDNNGREIMRRRDYRPTW
symbB.v1.2.0177 148 -----GSWISQITRLARGARHV-----EITITVSDIPDDGWGEVLSRISIDHIDGTCYTDNNGREIMRRRDYRPTW
Skav210588_FKAW 222 -----GSWISQITRLARGARHV-----EITITVSDIPDDGWGEVLSRISIDHIDGTCYTDNNGREIMRRRDYRPTW
Skav210590_FKAW 1 -----GSWISQITRLARGARHV-----EITITVSDIPDDGWGEVLSRISIDHIDGTCYTDNNGREIMRRRDYRPTW

MAN2_DROME 883 ---DKLPIQNNYYPISGMEFEDANT-RLTILTGQPIGG-SSASGEIEMMODRRIASDDERGGQGLDNKP-----
MA2A1_HUMAN 908 ---SKLPIQNNYYPIMAMITQDAKH-RLTILSAQSILGV-SSNSGQTEVIMDRRLQDDNRGEGQGQDNKI-----
MA2A1_MOUSE 909 ---SKLPIQNNYYPIMAMITQDAEH-RLTILSAQSILGV-SSNSGQTEVIMDRRLQDDNRGEGQGQDNKI-----
GMAN2_ARATH 911 ---DKLPIQNNYYPISLAFIQSGNGQRFSSHSRQSIGV-ASKEGWEIEMMDRRLQDDNRGEGQGQDNKI-----
LMANII_DROME 760 SVKINEAMAGNYYPITTKIDEDDTA-RVAILDRAQGG-SSKUGSEIEMMHRRLQDDAFGVGEALNETEY-----
MA2B1_HUMAN 764 KLNQTEPMAGNYYPNTRNITTDGNM-QLTLIDRSQGG-SSRUGSEIEMMHRRLQDDNRGEGQGQDNKI-----
MA2B1_MOUSE 765 TLNQTEPMAGNYYPNTRNITTDGQM-QLTLIDRSQGG-SSRUGSEIEMMHRRLQDDNRGEGQGQDNKI-----
MANA2_ARATH 757 KLDVNQPMAGNYYPNTHGIMQDSKK-EFSVMVDRAFSG-SSVIGQTEIEMMHRRLQDDNRGEGQGQDNKI-----
Smic4843_SMIC 1155 KLKQTEPMAGNYYPITTSIRIRDTAA-QLTLIDVAQGTGCVRGGEIEMMHRRLQDDNRGEGQGQDNKI-----
symbB.v1.2.0177 513 KLREEP-----
symbB.v1.2.0177 218 NFNQTEPMAGNYYPITTSIRIRDEKA-QLTLIDTSQGTGCVRGGEIEMMHRRLQDDNRGEGQGQDNKI-----
Skav210588_FKAW 222 -----
Skav210590_FKAW 1 -----NMHRRLQDDNRGEGQGQDNKI-----

MAN2_DROME 951 -----VLH-----YRLVLEKVNVCVRPSELH-PAGYLTSAAHKASQSLLDPLDKFIFAEN---E---WIGAQCQGG
MA2A1_HUMAN 976 -----TAN-----FRLLLEKRSVANTTEEEK-SVSYPSLISHITSSLMNHFPVIMAN--K---FSSPTLELQCFSP
MA2A1_MOUSE 977 -----TAN-----FRLLLEKRSVNMEEEEKSPVSYPSLISHMTSSFLNHPFLPMVLSGQ---LPSPAFELLSEPL
GMAN2_ARATH 980 -----MTV-----FELLAESNISQDPAANT-NPRNPSLISHLIGAHNLNYPINTFIAKKP---QDISVRVPQYCSAP
LMANII_DROME 831 -----GDGLIARGKHHFFGKSTDR-E---GV-SLKGIERL---TQLEKLLPTWKFFSNMEDYSADEWQTAFNTIFSG
MA2B1_HUMAN 834 -----SCAWRGRHVLDDTAQA-A-----AGHRL---AEQEVLAQ-VVLAPGG-GAAYNLGAPPRTOFSG
MA2B1_MOUSE 835 -----TCDKRGGRHVLSSVSDA-A-----ARHRL---AEQEVLAQ-VVLSLGG-SSPYHSRATPKTOFSG
MANA2_ARATH 830 -----DKCTGLTQGGYYRIDPYGE-A-----KWRRTF---GQEIY-SPLLLAFAQQDDGKPMSS---FGAASFSG
Smic4843_SMIC 1234 KNDQGRHYGPELVTRGVHVFHFGPPAT-A-----ATWRPL---MDRMV-IEVQPFPGGGKAA-----PSVGSFS-
symbB.v1.2.0177 520 -----
symbB.v1.2.0177 297 -QQGQHYGDGLVRCQFHLHFGLTDE-A-----KNWRPL---MDRIY-MEPVPFFTQGA-----V-GKVS-
Skav210588_FKAW 222 -----
Skav210590_FKAW 32 --EPGQHYGPELVRCQFHLHFGSPQT-A-----KSWRPL---MDRLV-MEPAAFFTEGA-----VDMCMFW-

MAN2_DROME 1012 D--HPSAREDDVSVRRLTKS--S-A---KTQRVGYVLHRTNLMQC-----GTPEEHT--QKDDCHLPLN--ARC
MA2A1_HUMAN 1038 I--QSSLECDHLNIRTIQSKVGN-G---HSNEAAILHRKGF-DCRFSSKGTGLFCST-T-QGKLLQKLNKFIYESL
MA2A1_MOUSE 1042 I--QSSLECDHLNIRTIQSKMGK-G---YSDEAAILHRKGF-DCQFSSRGIGLPCST-T-QGKMSLKLKFNKFAVESL
GMAN2_ARATH 1045 I--AKPLECDHINFKVPRPSKYSQOEEEDKPRFALILNRRAWD-SAYCHKGRQVNCTSMANEPVNFSDHFKDLAASKV
LMANII_DROME 896 I--SLVLEKPKHLLTLEPWHENQLIRFEH-----IMENGEDA--SYS-QPVQFNKKNLSAFDEGI
MA2B1_HUMAN 892 I--RRDLPPSHLLTLASWGPVMVLRFEH-----QFVGEDSGRNLS-APVTNIRDLFTFTFTL
MA2B1_MOUSE 893 I--RQELPPQHLLTLARWGPKMILIRFEH-----QFALKEDSDRNLS-SPVTNQNLFQFTFTNYL
MANA2_ARATH 889 I--DPSYSLPDNALLTLQELDDGNGVLRFEH-----LVEVEEDK--ELS-GVASVEKKLEFGKKGKGL
Smic4843_SMIC 1294 I--QGDLEPSSQLTLQWDETHILIRFEH-----QFGIGEDS--ELS-KPVSVDKSLVGATVLSL
symbB.v1.2.0177 520 -----
symbB.v1.2.0177 353 F--LDALPPNELLTLAAWDEKSWLRFEH-----QFGLGEDA--KLS-QPATVLDALFAGAHVTSI
Skav210588_FKAW 222 -----
Skav210590_FKAW 88 F--LKALEENELLTLASWDEKTVLRFEH-----QFGVDEDS--ELS-KPATVLDQTLFAGAKMSL

MAN2_DROME 1073 ERTITLTLQN--LE-----
MA2A1_HUMAN 1110 TPSSLSLMHS--PP-----
MA2A1_MOUSE 1114 VPSSLSLMHS--PP-----
GMAN2_ARATH 1122 KPTSDNLLQE--DM--EILGDDQE-----LP-----
LMANII_DROME 954 RETILDNAWLDES--RELQAPDPEEAFTNYATFSQPAESVHLLSAEKPMGLGVKYADEALPAGQLGAESNRIRRETET
MA2B1_HUMAN 952 QETILVNQLREAA--SRKKTMTNT-----GPT-----
MA2B1_MOUSE 953 QETILANQPLSRA--SRKKTMTNT-----GPT-----
MANA2_ARATH 949 TEMLSLNQERSTMEKKRLVKVEG-----EGS-----
Smic4843_SMIC 1352 EERGIAITRDEVEQRRLPHVEG-----SS-----
symbB.v1.2.0177 524 -----
symbB.v1.2.0177 411 AERGLGTTISSDEVEREINPLDHD-----AVP-----
Skav210588_FKAW 222 -----
Skav210590_FKAW 146 EERGLGTTISSDEVEHREINPLED-----NST-----

1039 **Supplementary Figure 2. Multiple sequence alignment of Mannosidase II sequences.**

1040 A multiple sequence alignment was conducted in T-Coffee using the amino acid sequences of
 1041 Golgi Mannosidase II (MAN2_DROME, MA2A1_HUMAN, MA2A1_MOUSE,
 1042 GMAN2_ARATH) and Lysosomal α -Mannosidase (LMANII_DROME, MA2B1_HUMAN,
 1043 MA2B1_MOUSE, MANA2_ARATH) from *Drosophila melanogaster*, *Homo sapiens*, *Mus*
 1044 *musculus*, and *Arabidopsis thaliana* to the α -Mannosidase found in Symbiodiniaceae species
 1045 *Symbiodinium microadriaticum* (Smic), *Breviolum minutum* (symbB), and *Fugacium kawagutii*
 1046 (Fkaw).

1047

ManII Residue	ManII AA	Conserved Sequence	Importance	Bmin AA	Smic AA	Fkav AA	Lysosomal Mannosidase AA
64	Q	DGGVWKQG	m5 stacking	S	M		K/G/W/M/T/L
90	H	VVPHSHNDPGW	Zn coordination	H	H	H	H
92	D	VVPHSHNDPGW	Zn coordination	D	D	D	D
95	W	VVPHSHNDPGW	m5 stacking	W	W	W	W
204	D	WAIDPFG	nucleophile	D	D	D	D
269	YSY	MPFYSDIPHTCGPE	m5 h-bonding	YRG	YGG	GRR	FSG/FTG/FLG
298,299	P	PP	m5 stacking	DD	DD		DN/DD/ED/DG
340	D	PLGDDFR	m5 stacking	D	D		D
343	R	PLGDDFR	m5 h-bonding	Q	Q		T/Q/H/A
426	R/V	YADR		G	G		G/D/N
431	W	YWS/T		W	W		W
470,471,472	HHD	LFQHHD	Zn coordination, m5 h-bonding	HHD	HHD		HHD
727	Y	GAYLF	m5 h-bonding, stacking	Y	Y		Y

Supplementary Table 1: ManII site conservation in Symbiodiniaceae.

The conserved ManII residue in *Drosophila* is shown on the left, with its corresponding amino acid and conserved sequence. The importance of the residue for binding affinity is shown in the center. Symbiodiniaceae residues are shown for *B. minutum*, *S. microadriaticum*, and *F. kawagutii*. Conserved residues for lysosomal mannosidase amino acids are shown on the right.

N-Glycan Synthesis	Brevivolum minutum				Symbiodinium microadriaticum				Fugacium kawagutii				Perkinsus marinus				Toxoplasma gondii				Plasmodium falciparum			
	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden
ALG13	symB8.v1.2.0 08534.t1 scf fold538.1 size 24240511	86	3.25E-16	43	SmiC27423	110	4.83E-26	49	Skav2 0751 6	97	2.36E-20	47	Pmar_P MAR01 5450	73	0.09	35	TGME4 9_2683 40	167	1.20E-14	40.8	PF3D 7_08 0640	154	4.30E-13	43
ALG14	symB8.v1.2.0 34275.t1 scf fold4398.1 siz e4016812	146	8.76E-42	38	SmiC33514	157	4.79E-46	43					Pmar_P MAR00 0327	105	2.90E-07	52.8	TGME4 9_2070 70	290	2.90E-33	43.4	ALG1 4	156	1.20E-14	44.6
ALG1	symB8.v1.2.0 27383.t1 scf fold2713.1 siz e7494813	137	7.38E-36	38	SmiC38965	272	1.38E-78	41	Skav2 2611 3	47	1.81E-05	30	Pmar_P MAR02 0699	239	9.90E-26	55.8	TGME4 9_2305 90	285	3.30E-32	47				
ALG2	symB8.v1.2.0 08675.t1 scf fold539.1 size 18986417	368	3.85E-115	49	SmiC41638	355	3.39E-116	49	Skav2 0521 0	53	1.30E-06	37	Pmar_P MAR01 3250	190	6.20E-19	55.4	TGME4 9_2277 90	172	2.00E-16	53.2	CLAG 2	75	0.005 6	24
ALG11	symB8.v1.2.0 01761.t1 scf fold695.1 size6 0063219	336	1.02E-104	44	SmiC25097	447	2.90E-140	47	Skav2 0273 1	36	0.07	42	Pmar_P MAR01 8755	191	4.30E-19	41.8	TGME4 9_2469 82	157	2.60E-14	47				
ALG3	symB8.v1.2.0 13786.t1 scf fold975.1 size 15306111	212	5.66E-59	40	SmiC9149	54	3.84E-07	31					Pmar_P MAR02 3648	140	4.00E-12	53.3								
ALG9	symB8.v1.2.0 32558.t2 scf fold3923.1 siz e4828614	220	6.30E-32	51	SmiC34730	311	1.67E-62	38	Skav2 2546 5	57	6.95E-08	41	Pmar_P MAR01 9024	106	4.90E-07	54.3	TGME4 9_3216 60	89	1.20E-04	42.5	GP11 0	78	0.002 7	23.8
ALG12	symB8.v1.2.0 12500.t1 scf fold862.1 size 15711519	39	0.06	31	SmiC23540	258	6.22E-74	42	Skav2 2120 6	235	2.09E-67	35	Pmar_P MAR01 6091	194	1.20E-19	79.5								
ALG5	symB8.v1.2.0 07750.t1 scf fold480.1 size 253386112	231	3.09E-72	45	SmiC39167	243	4.29E-76	46	Skav2 0832 3	39	0.01	29	Pmar_P MAR01 8469	327	3.90E-38	50.8	TGME4 9_2165 40	240	5.80E-26	51.8	DPM 1	83	3.50E-04	30.2
ALG6	symB8.v1.2.0 13301.t1 scf fold937.1 size 15025917	297	2.56E-86	39	SmiC22253	120	1.80E-27	32	Skav2 1015 6	174	1.24E-47	32	Pmar_P MAR02 8409	245	2.80E-27	56.3	TGME4 9_2620 30	118	4.60E-09	45				
ALG8	symB8.v1.2.0 13301.t1 scf fold937.1 size 15025917	129	3.25E-30	31	SmiC30006	284	1.97E-82	43	Skav2 2936 7	62	4.20E-10	55	Pmar_P MAR00 2960	161	3.60E-15	46.9	TGME4 9_2420 30	208	6.20E-22	61.8				
ALG10	symB8.v1.2.0 21974.t1 scf fold1899.1 siz e238565110	208	1.10E-59	33	SmiC4123	158	2.43E-40	31	Skav2 3246 1	210	1.72E-60	32	Pmar_P MAR00 5103	92	1.90E-05	48.6	TGME4 9_2636 90	104	3.30E-07	53.1				
STT	symB8.v1.2.0 13974.t1 scf fold1001.1 siz e14690415	383	1.82E-115	32	SmiC38591	377	1.30E-116	34	Skav2 3448 9	94	1.04E-20	46	Pmar_P MAR02 5427	514	6.60E-65	62.8	TGME4 9_2314 30	520	8.90E-66	63.7	STT3	390	1.60E-47	
GCS1	symB8.v1.2.0 29725.t1 scf fold3288.1 siz e5972811	285	2.35E-55	33	SmiC33937	337	1.18E-41	41	Skav2 1128 2	44	0.001	43	Pmar_P MAR01 7791	151	7.40E-13	59.1	TGME4 9_2420 20	128	1.10E-09	53.2				
GAN48	symB8.v1.2.0 08520.t1 scf fold527.1 size 29307218	563	0	38	SmiC30546	631	0	38	Skav2 0048 4	116	7.40E-28	35	Pmar_P MAR01 6665	992	9.5E-130	54.1	TGME4 9_2530 30	667	1.40E-84	54.5				
MAN1A1	symB8.v1.2.0 25226.t1 scf fold2429.1 siz e7929816	242	1.69E-70	34	SmiC1440	326	6.65E-100	43	Skav2 2259 0	112	5.58E-25	39	Pmar_P MAR02 0391	226	2.30E-23	44.1								

N-Glycan Synthesis	Brevivolum minutum				Symbiodinium microadriaticum				Fugacium kawagutii				Perkinsus marinus				Toxoplasma gondii				Plasmodium falciparum				
	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden	
MAN1A2	sympb8.v1.2.0 25226.t1 scf fold2429.1 siz e7929816	252	1.80E-74	36	Smic1440	306	9.61E-93	35	Skav2 2259 0	112	3.40E-25	40	Pmar_P MAR02 0391	238	3.40E-25	45.1									
MAN1B1	sympb8.v1.2.0 25226.t1 scf fold2429.1 siz e7929816	287	3.80E-87	39	Smic1440	302	1.08E-90	39	Skav2 2259 0	131	3.53E-31	38	Pmar_P MAR02 0391	227	2.40E-23	45	TGME4 9_2644 50	73	0.045	25.5					
MGAT1	sympb8.v1.2.0 37358.t1 scf fold4935.1 siz e2650213	210	9.89E-61	46	Smic24963	230	1.29E-70	30	Skav2 2344 2	86	2.65E-19	54	Pmar_P MAR00 9039	233	5.30E-25	56.3									
MAN2A1	sympb8.v1.2.0 17759.t1 scf fold1355.1 siz e12368811	213	8.96E-58	30	Smic4843	282	5.18E-77	28	Skav2 1058 8	161	1.01E-29	37	Pmar_P MAR02 6493	149	1.80E-12	38									
MAN2B1	sympb8.v1.2.0 17759.t1 scf fold1355.1 siz e12368811	428	5.71E-137	50	Smic4843	762	0.00E+00	44.5	Skav2 1058 8	246	1.55E-56	55	Pmar_P MAR02 6493	524	1.10E-64	58									
MGAT2	sympb8.v1.2.0 14463.t1 scf fold1055.1 siz e14077615	34	1.36E+00	23	Smic19105	40	0.02	26	Skav2 3364 8	33	3.17E+00	25													
MGAT3	sympb8.v1.2.0 02545.t1 scf fold103.1 siz 33105810	51	4.13E-06	27	Smic3340	40	0.02	25	Skav2 1513 5	38	3.00E-02	27	Pmar_P MAR00 7528	106	5.57E-07	34.3	TGME4 9_2077 50	76	0.008 9	39.5					
MGAT4A	sympb8.v1.2.0 33022.t1 scf fold4044.1 siz e4569315	141	8.27E-36	35	Smic12	174	1.29E-44	30																	
MGAT4B	sympb8.v1.2.0 33022.t1 scf fold4044.1 siz e4569315	146	2.86E-37	34	Smic12	153	5.41E-38	29																	
MGAT4C	sympb8.v1.2.0 33022.t1 scf fold4044.1 siz e4569315	174	1.74E-47	36	Smic12	193	2.30E-51	29	Skav2 1180 0	30	9.79E+00	26													
MGAT4D	sympb8.v1.2.0 33022.t1 scf fold4044.1 siz e4569315	100	1.44E-22	30	Smic12	109	2.03E-24	30	Skav2 2580 5	39	1.00E-02	36					PF3D 7_04 1080 0	74	0.006 8	26.7					
MGAT5	sympb8.v1.2.0 27073.t3 scf fold2751.1 siz e7157612	62	4.65E-09	27	Smic31095	57	2.69E-07	26	Skav2 1708 5	31.6	2.12E+00	29													
MGAT5B	sympb8.v1.2.0 27073.t3 scf fold2751.1 siz e7157612	62	7.04E-09	25	Smic31095	65	6.41E-10	25	Skav2 2135 4	36	7.00E-01	27													
XYLT1	sympb8.v1.2.0 20832.t1 scf fold1775.1 siz e10185411	81	1.79E-14	26	Smic32875	89	4.74E-17	26									TGME4 9_3091 60	75	0.03	25.4	PF3D 7_13 4380 0	70	0.091	28.9	
FUT8	sympb8.v1.2.0 27005.t1 scf fold2737.1 siz e7194513	49	2.39E-05	23	Smic28583	52	3.86E-06	25	Skav2 3314 9	56	4.17E-08	25													

N-Glycan Synthesis	Breviolum minutum				Symbiodinium microadriaticum				Fugacium kawagutii				Perkinsus marinus				Toxoplasma gondii				Plasmodium falciparum			
	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden	Gene	Score	Eval	Iden
FUT11	symB8.v1.2.0	91	1.12E-18	32	Smic38835	63	1.56E-09	27	Skav2	32	4.72E+00	50	Pmar_P	110	1.90E-07	44.2								
	20659.t1 scaf fold1748.1 siz e222129 7								1132	0		4081	MAR02											
B4GALT1	symB8.v1.2.0	96	6.25E-22	32	Smic22386	108	2.87E-24	28	Skav2	52	3.61E-07	47	Pmar_P	78	0.0044	43.3	TGME4	83	8.50E-04	42.2	PF3D	68	0.065	28.8
	30619.t1 scaf fold3471.1 siz e56067 7								1032	1		MAR00	MAR00				9_3146	7_06	0990	0				
B4GALT2	symB8.v1.2.0	99	6.16E-23	34	Smic22386	104	3.94E-23	33	Skav2	52	1.65E-07	27	Pmar_P	73	0.021	41.2	TGME4	76	0.005	40.9				
	30619.t1 scaf fold3471.1 siz e56067 7								1032	1		MAR00	MAR00				9_2590	80	6					
B3GALT1	symB8.v1.2.0	66	5.32E-11	27	Smic25670	81	7.36E-16	26	Skav2	65	5.56E-11	26	Pmar_P	96	7.60E-06	28.8	TGME4	72	0.018	38.6				
	31248.t1 scaf fold3605.1 siz e53397 2								2604	8		MAR01	MAR01				9_3215	70						
SIAT1	symB8.v1.2.0	34	1.64E+00	27	Smic2669	33	3.27E+00	33	Skav2	33	1.55E+00	30												
	39159.t1 scaf fold6378.1 siz e18592 2								0734	2														
SIAT2	Smic25787	36	4.30E-01	40	Skav200920	3	5.35E+00	41																
FUT13	symB8.v1.2.0	75	8.77E-14	26	Smic38835	70	7.01E-12	28					Pmar_P	83	9.70E-04	36.6								
	20659.t1 scaf fold1748.1 siz e222129 7												MAR00	3958										
HEX01	symB8.v1.2.0	330	8.82E-103	38	Smic24332	200	1.47E-54	40	Skav2	111	1.26E-25	25	Pmar_P	152	3.00E-13	47.5								
	34859.t1 scaf fold4576.1 siz e37795 4								1581	7			MAR00	9436										

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1069 **Supplementary Table 2: N-glycan biosynthesis datasheet**

1070 N-glycan biosynthesis BLASTp searches were performed in Symbiodiniaceae species and in
1071 related alveolates *Perkinsus marinus*, *Toxoplasma gondii*, and *Plasmodium falciparum*.

1072 Manually annotated N-glycan protein sequences from humans were collected from Uniprot and
1073 queried directly against a target species protein database consisting of gene models. The first
1074 result of each successful search (gene) is shown for each protein query, along with its score, eval,
1075 and % identity.