1 **Detection of glycidic receptors in microalgae using glycodendrons as probes: a new**
2 **tool for studies on cell surface interactions**

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Abstract

Cell recognition, adhesion and internalization are involved in infectious, reproductive and inflammatory processes and are generally mediated by interactions between molecules located in the cell membrane and the extracellular matrix. These processes can decrease proliferation rates and they are well known for bacteria, fungi and animals, but there is a lack of knowledge regarding autotrophic cells. Carbohydrates and proteins (e.g. lectins) are important molecules for cell interactions and information about these molecules is essential to better understand many biological phenomena in uni- or multicellular organisms. Most studies focus on the identification of the carbohydrates present on the cell surface by using labeled lectins. Alternatively, here we present a pioneer research performed by using three different labeled carbohydrates in a multivalent presentation (glycodendrons) to detect the presence of carbohydrate receptors (e.g. lectins) on cell surfaces of twelve algal species. The goal of this study was to detect some specificity in these molecular interactions, but in a reverse way in comparison to that commonly described in the literature. We tested trivalent molecules containing residuals of D-mannose, L-fucose or *N*-acetyl-galactosamine to identify their bindings with the corresponding lectins expressed on cell surfaces. We envisage that our new approach could be an alternative tool for taxonomic and physiological studies on microalgae or even on other groups of organisms. Based on our results, the receptors found in the cell surface of the algal species tend to differ in composition, quantity and distribution. The differences were mainly species-specific, since no patterns were identified at higher taxonomic level. Moreover, like lectins, labeled carbohydrates were proved to be a reliable tool for the study of cell surface composition.

Key words: Cell-interaction, lectins, carbohydrates, algae, fluorescent probes, cell recognition.

1-Introduction

Algae are grown for different purposes and particular culture conditions can optimize their growth or specific products synthesis (Kang et al. 2017). However, under high biomass situations, we can expect increased competition for available light and nutrients. Furthermore, some authors mention that contact between cells, which will increase at higher cell density, is an inhibitory force for algal cell reproduction (Costas et al. 1993). This phenomenon is dependent on cell-cell recognition and has been better understood for animal cells (Costas et al. 1993; McClatchey and Yap 2012). We know almost nothing about cellular interactions in algae and even less about the types of molecules involved in these contacts, but their potential effects should be considered and investigated for better understanding of algal metabolism and for further applications in the process of biomass production of these organisms.

Cell-cell recognition mediates several biological processes by interactions of biomolecules present in the plasmatic membranes (Kresse et al. 2001; Tien et al. 2005). The biomolecular composition of cell surface is unique and typical for each species and, moreover, it can differ among strains or for distinct developmental stages of the same species (Aguilera and Gozález-Gil 2001). Although the importance of cell surface biomolecules was observed for interactions in many taxonomic groups (Perrimon and Bernfiels 2001), we know little about their nature and composition in photosynthesizing organisms (Knox 1992). In the particular case of the microalgae, this knowledge is really scarce.

Among the biomolecules involved in cell interactions, lectins and carbohydrates play a relevant role. Lectins are proteins acting in cell adhesion and recognition processes due to their abilities to interact with carbohydrates in a very specific way (Sharon and Lis 2004). There are several studies showing that this molecular interaction

70 is a key factor governing many cellular phenomena, such as infections, cell growth,

71 symbiosis, predation and reproduction among others (Brandley and Schnaar 1986; Hori

72 et al. 1996; Smith et al. 2003; Wood-Charlson et al. 2006; Khowala et al. 2008; Kikkeri

73 et al. 2009; Espinosa 2010). Based on these interactions, there is an increasing use of

74 labeled lectins as promising and reliable tools for the detection and fast identification of

75 the oligosaccharide components of cell surfaces. This approach has allowed to elucidate

76 the carbohydrates diversity of cell membranes (Muller et al. 1983), the ability of these

77 membranes to interact with molecules or other membranes (Knox 1992), and it can also

78 be useful for taxonomic studies (Cho et al. 2001).

79 Carbohydrates are an abundant component among the molecules found on the

80 cell surface and since they are specific ligands for lectins, they have a major

81 participation in cell interactions (Khowala et al. 2008). Membrane carbohydrates show a

82 high structural variety and the complexity of their multimeric presentation results in a

83 great diversity of molecular information, the glycocode (Kikkeri et al. 2009).

84 Considering these aspects, investigations should not be focused only on labeled lectins

85 as tools to identify cell surface components. These labeled lectins can only clarify a part

86 of the membrane interactions, since they will allow only identify carbohydrates as

87 ligands, with no additional information however about the lectins that are present on the

88 cell surface. Thus, we can envisage that the use of the reverse strategy (using labeled

89 carbohydrates to detect cell surface receptors) could be important in surface cell

90 investigations. For example, fluorescent labeled carbohydrates could be a

91 complementary or an alternative tool to investigate the presence of other key molecules

92 involved in cell-cell interactions (the lectins), an important issue taking into account that

93 lectins isolation and identification is a challenging work (Li et al. 2014). Furthermore,

94 the study on the presence of the two molecules types in membranes could be helpful to

4

evaluate their participation intraspecific or interspecific interactions. We can suppose higher intraspecific importance if both receptors and ligand carbohydrate are present in membrane, while the presence of only one of these molecules could be an evidence of a higher importance for interspecific interactions. To our best knowledge, there is no other study using labeled carbohydrates to evaluate algal lectins, therefore, this new approach could allow the examination of cell surface composition in algae (Tien et al. 2005).

Lee & Lee (1995) and Mammen et al. (1998) suggested that physiologically relevant affinities between lectins and carbohydrates generally are observed only when multivalent bindings occur. Glycodendrimers are monodisperse polymers with molecular structures having a number of branches around an inner core and containing some residual carbohydrate. Glycodendrons are the arms (the structural units) of glycodendrimers. During the last years, the application of these molecules has increased notably in biological studies, in which they are used on different and relevant ways (Roy et al. 2013). Glycodendrimers and glycodendrons are among the most promising molecules for studies on the interactions involving cell surfaces. They are advantageous tools for these studies due to their size (nanometric scale) and mainly because they can present multiple copies of the carbohydrate units in the periphery of these structures, the most exposed portion of the system (Cloninger 2002), which allow them to mimic the membrane oligosaccharides.

Here we synthesized trivalent glycodendrons labeled with a fluorescent tag to evaluate their specificity to interact with the cell surface of 12 different microalgae species. The trivalent molecules were preferred than the monovalent ones, since the interactions can be collectively much stronger and specific than the corresponding monovalent interactions due multivalency effect (Kane 2010). We evaluated the

120 capacity of these systems to differentiate algae species based on the singular lectin

121 composition of their corresponding surfaces.

122

123

124 **2- Material and Methods**

125 *2.1- Synthesis and structure of the glycodendrons*

126　　　　For these experiments, glycodendrimeric molecules were chosen based on our

127 previous works that tested their usefulness as a tool to investigate the surface of

128 dendritic cells. Clear interactions among these molecules and cell surface receptors were

129 demonstrated by these investigations (see Sattin et al. 2010, Ribeiro-Viana et al. 2012,

130 Varga et al. 2014).

131　　　　Here, three different trivalent glycodendrons (Figure 1) were synthesized

132 considering a basic common molecular structure with different carbohydrate units

133 present in the molecule. The glycodendrons are non-charged molecules that were named

134 GD-Man, GD-Fuc and GD-GalNAc and they are formed by a dendritic structure with

135 the more external glycidic portion composed by D-mannose, L-fucose or *N*-

136 acetylgalactosamine, respectively. They are novel molecules synthesized in analytical

137 purity according to a methodology previously described by our group (Ribeiro-Viana

138 2012). Briefly, we have used an alkynyl core and azidoglycosides to be conjugated

139 through a Cu(I) catalyzed azides and alkynes cycloaddition (CuAAC) reaction, a classic

140 *Click Chemistry* reaction furnishing regiospecific 1,4-disubstitued triazoles (Tornøe et

141 al. 2002; Rostovitsev et al. 2002; Kolb et al. 2004). We also synthesized an analogous

142 control dendron, in which we used diethylene glycol instead of a carbohydrate unit. The

143 diethylene glycol provides a soluble control molecule very similar in terms of shape,

144 absence of charge, size and solubility to that of glycodendrons. Considering specificity

6

145 in lectin-carbohydrate interactions, it is expected that the control dendron would not

146 interact with cell surface. To enable us to quantify the interactions between these

147 dendrons and cell surfaces, a fluorophore (BODIPY R6G) was conjugated to the focal

148 position of dendrons (Figure 1) to be detected by fluorescence techniques (Ribeiro-

149 Viana et al. 2012). The BODIPY can be useful in studies on microalgae since it is very

150 stable in aqueous medium (Ni and Wu 2014). Further, when linked to the dendrons, this

151 fluorophore shows excitation and emission bands at 527 and 541 nm respectively,

152 which is a great advantage to perform experiments with microalgae since its emission

153 peak is very different of the chlorophyll emission (650 nm). This fact enables us to

154 produce results with no interference of cell autofluorescence.

155   The fluorescent trivalent glycodendrons and the analogous diethylene glycol

156 were produced by using the classic methods of organic synthesis and they were

157 characterized by Nuclear Magnetic Ressonance (NMR), using a Bruker DRX-300

158 spectrometer, and by mass spectrometry with eletrospray ionization, using a Esquire

159 6000 Bruker Daltonics spectrometer (ESI-MS).

160

161 *2.2- Microalgae cultures*

162   The experiments were performed with 12 species corresponding to 4 taxonomic

163 classes: Bacillariophyceae (*Nitzschia* sp.), Chlorophyceae (*Chlamydomonas* sp.,

164 *Pediastrum duplex*, *Pseudopediastrum boryanum*, *Pediastrum* sp., *Coelastrum*

165 *microporum* and *Coelastrum astroideum*), Trebouxiophyceae (*Chlorella vulgaris* and

166 *Actinastrum hantzschii)* and Zygnematophyceae (*Mougeotia* sp., *Staurastrum iotanum*

167 and *Staurastrum leptocladum*). The species were obtained from the Collection of Algae

168 and Cyanobacteria of the Phycology Laboratory of the Federal University of Minas

169 Gerais, Brazil, where they are cultivated in WC medium (Guilhard & Lorenzen 1972) at

170   $20(\pm1)°C$ and 22 ($\pm0,1$) µmol photons m^{-2} s^{-1}, under a light: dark cycle of 12:12 h. All

171   experiments were performed in the exponential growth phase to assure that cells would

172   not be under any limitation and would present uniform structure and physiology.

173   **2.3- *Exposition of the species to glycodendrons***

174   All glycodendrons and the control trivalent dendron were dissolved in distilled

175   water to a final concentration of 32 µmol L^{-1}. All treatments were performed in

176   triplicate. We collected 3 mL of each culture and distributed 1 mL in each of three

177   microtubes. The material was centrifuged during 5 minutes at 13,792.8 g (13,000 rpm)

178   in a microcentrifuge (Eppendorf 5424). The cells were observed in an optical

179   microscope (Olympus CH30) to verify their integrity. No external or internal alteration

180   was detected. The supernatant was discarded and the pellet in each microtube was

181   resuspended in 25 µL of the aqueous solution containing the dendron to be tested. The

182   microtubes were then kept for 1 hour at 24 °C in a dark chamber. Next, the settled

183   material was collected, put on a slide and covered with a coverslip. The borders of the

184   coverslip were closed with resin. The slides were prepared under low light intensity to

185   avoid alteration or degradation of the fluorophore. The prepared slides were observed

186   on a confocal microscope and photographs were obtained for each treatment.

187

188   **2.4- *Evaluation of the material by confocal microscopy***

189   The cells exposed to glycodendrons were analyzed in a confocal microscope

190   (Zeiss 5 –live) belonging to the Image Acquisition and Processing Center (CAPI-

191   UFMG). For capturing and processing the images, we utilized the software ZEN-2009.

192   The fluorophore in the slides was excited by a laser ray of 532 nm and the fluorescence

193   was captured by a filter receiving waves from 550 to 615 nm. We used the software

194   LSM *IMAGE BROWSER* (Zeiss) for additional preparation of the images. All the results

195 presented here are representative of more than 90% of the individuals observed in the

196 slide (data not presented), since some individuals (less than 10%) did not show the same

197 surface labeling.

198

199 *2.5- Fluorescence quantification*

200 To compare the results obtained for each treatment, we evaluated the

201 fluorescence intensity emitted by the BODIPY in the labeled cells. Fluorescence was

202 quantified after the image was captured in the same confocal microscope and using the

203 software ZEN- 2009. The measurements were made in terms of total emission by cell,

204 for which we delimited the complete area corresponding to the individual in the

205 photographs. We measured the fluorescence intensity for 3 individuals of each species

206 exposed to each glycodendron. For species presenting evident heterogeneous

207 fluorescence in cell surface, the intensity was measured in different regions of the cell to

208 statistically evaluate these differences. For this purpose, measurements were performed

209 in squares, 3 located in the more fluorescent and 3 in the less fluorescent area of the cell

210 surface. Each square had a surface of 2 μm^2. Since comparisons were made for only

211 three observations in each treatment, we assumed that significant differences would

212 only be detected if the variances were small.

213

214 *2.6-Statistical analyses*

215 The total fluorescence by cell (as a total measurement for the entire cell surface)

216 was compared separately for each species to verify if there were different affinities for

217 the three glycodendrons tested for each species. These comparisons were made using a

218 One-Way ANOVA. When significant differences ($P < 0.05$) were detected, we run a

219 Tukey test to identify which specific group's means were different from the others. Data

220 were tested for normality and homocedasticity of residuals, respectively by the Shapiro-

221 Wilk and Brown-Forsythe tests. We used T-test to compare the fluorescence intensity in

222 different parts of the cell surface. The analysis was made separately for each

223 glycodendron only for the four species (*S. leptocladum*, *Pediastrum* sp., *P. boryanum*

224 and *P. duplex*) that presented evident heterogeneous fluorescence intensity in the cell

225 surface. All statistical analyses were performed using the software SigmaStat (version

226 3.5, 2007).

227

228 **3- Results**

229 ***3.1- Detection of glycidic receptors and species-specific molecular bindings***

230   The results obtained using twelve algae species and four different dendrons are

231 summarized in Table 1. GD-GalNAc was the molecule that bound with the largest

232 number of species (10), followed by GD-Man (7). GD-Fuc showed interactions with

233 only 3 species. As expected, the control dendron did not bind with any species (Table

234 1).

235   *Staurastrum leptocladum* showed detectable bindings with all glycodendrons,

236 but the fluorescence was less intense when it was exposed to GD-Fuc (Figure 2, A2, A3

237 and A4). Another species of this genus, *Staurastrum iotanum*, presented bindings with

238 GD-Man and GD-GalNAc, but no interaction with GD-Fuc was observed (Figure 2, B2,

239 B3 and B4). *Coelastrum microporum* (Figure 2, C2, C3 and C4) and *Chlamydomonas*

240 sp. (Figure 2, D2, D3 and D4) presented the same pattern. *Chlorella vulgaris* and

241 *Actinastrum hantzschii* interacted only with GD-GalNAc (Figure 2, E4 and F4,

242 respectively). *Coelastrum astroideum* and *Mougeotia* sp. did not bind with any of the

243 glycodendrons (Table 1, data not shown in photographs).

244   *Pediastrum duplex* and *Pseudopediastrum boryanum* interacted with GD-

245 GalNAc and GD-Man (Figure 2, G3, G4, H3 and H4), while *Pediastrum* sp. showed

246 bindings with all glycodendrons evaluated here (Figure 2, I1, I2 and I3). Finally,

247 *Nitzschia* sp., the unique Bacillariophyceae used in the study, bound with GD-GalNAc

248 and GD-Fuc, but not with GD-Man (Figure 2, J2, J3 and J4).

249   Some species showed an apparent difference in the fluorescence intensities

250 depending on the glycodendron tested and even different parts of the same individual

251 may show distinct intensities. *Staurastrum leptocladum*, for example, was completely

252 covered by the BODIPY fluorescence when exposed to GD-Man, but the bindings with

253 GD-GalNAc and GD-Fuc occurred only in the more central part of the cells, while the

254 branches were not labelled by these dendrons. The fluorescence quantification was

255 performed to compare and analyze in detail the particularity of each interaction.

256

257 *3.2- Fluorescence quantification*

258   The fluorescence emitted by the glycodendrons in the cell surface was quantified

259 and statistically compared (figures 3 and 4). The highest total fluorescence of *S.*

260 *leptocladum* was observed when it was exposed to the D-mannose glycodendron, with

261 values significantly higher than those observed for the control ($F = 6.844$; $P = 0.013$).

262 The interaction with the other glycodendrons resulted in intermediate values of

263 fluorescence, which can be considered as not significant in comparison with those found

264 for the control or the GD-Man. When exposed to GD-GalNAc, *S. iotanum* showed a

265 higher fluorescence intensity ($F = 5.906$; $P = 0.038$) than that observed when it was

266 exposed to GD-Fuc and to the control, but it did not differ from the fluorescence

267 emitted after the exposition to GD-Man. For *C. microporum*, the bindings with GD-

268 GalNAc and GD-Man did not differ from each other and both resulted in significantly

269 higher fluorescence than the observed for GD-Fuc and the control Dendron ($F= 22.2$; P

270 $= 0.002$). *C. vulgaris* showed the highest fluorescence intensity when interacting with

271 GD-GalNAc (F= 160.4; P< 0.001), and showed no difference for the other

272 glycodendrons and the control as well. This pattern was also observed for

273 *Chlamydomonas* sp. (F= 132.6; P < 0.001) and *Actinastrum hantzschii* (F= 92.8; P <

274 0.001). *P. duplex* interacted differently with each glycodendron (F= 86.3; P < 0.001),

275 showing the lowest fluorescence when binding with GD-Fuc, which was not different

276 from the control. *Pediastrum* sp. showed significant higher fluorescence when exposed

277 to GD-GalNAc (F = 7.867; P= 0.009), while fluorescence in the treatments with GD-

278 Man, GD-Fuc and control did not differ from each other. *P. boryanum* showed a

279 significant lower fluorescence (F = 47.281; P < 0.001) for GD-Fuc than that observed

280 for GD-GalNAc and GD-Man, which did not differ from each other. *Nitzschia* sp.

281 showed distinct fluorescence values for each glycodendron (F= 114.2; P < 0.001),

282 showing values not different from the control when was combined with GD-Man and

283 smaller values than those observed for GD-GalNAc and GD–Fuc.

284 The comparisons between the more and less fluorescent part of the cells were

285 performed for *Staurastrum leptocladum*, *Pediastrum* sp*., Pediastrum duplex* and

286 *Pseudopediastrum boryanum* (Figure 4). For *Staurastrum leptocladum*, a significant

287 difference was observed only when this species bound with the D-mannose

288 glycodendron (t = 3.336; P = 0.004). This was also observed for *Pediastrum duplex* (t =

289 5.118 ; P < 0.001). *Pediastrum* sp. showed significant differences between the less and

290 more fluorescent areas for all glycodendrons (t = 4.942, P < 0.001; t = 2.308, P = 0.035

291 and t = 9.157, P < 0.001, for GD-Man, GD-Fuc and GD-GalNAc, respectively). For *P.*

292 *boryanum* we observed a significant difference in cell surface fluorescence (t = 5.235, P

293 < 0.001) only when this species was exposed to GD-GalNAc.

294

295 **4- Discussion**

4.1 - Interactions between the glycodendrons and the algae species

The interactions observed for each glycodendron were species-specific, with no pattern for higher taxonomic groups. Even with some limitation due to the few species studied, our results suggest the existence of great biochemical diversity in algal cell surfaces. The absence of interactions with the control molecule confirms that these interactions take place only through the carbohydrate portion of the dendrons.

Among the twelve species studied here, ten showed interactions with GD-GalNAc and among these species, two (*Chlorella vulgaris* and *Actinastrum hantzschii*) exclusively interacted with this dendron. Although the *N*-acetylgalactosamine is rare and have no clear function in autotrophic cells (Clarke et al. 1988), some interaction of algal surface and this carbohydrate could be expected, since it is found on the cell surface of many other organisms (Khowala et al. 2008; Kushchayev et al. 2012; Kosaka and Heizmann 1989; Kleene and Schachner 2004). It could have a role in algal recognition processes since examples of lectins showing high selectivity for the *N*-acetylgalactosamine monosaccharide were isolated and characterized from algae of different taxonomical groups (Carmichael and Bent 1981; Yamaguchi et al. 1998; Praseptiangga et al. 2012). Thus, the *N*-acetylgalactosamine is rare among algae and common in other organisms and since its receptor seems to be relatively common in algae, the both molecules may be involved in interspecific interactions between algae and heterotrophic organisms.

L-fucose is important for cell-cell interactions in bacteria. *Vibrio cholerae*, for example, infects the cells of human beings and domestic animals by recognizing L-fucose residuals in the host cell surfaces (Ofek and Beachey 1978). However, in our studies with algae, the interactions with GD-Fuc were the less common (3 among the 12 species) and generally less intense. Roberts et al. (2006) used labeled lectins (e.g.

321 *Tetragonolobus purpureus* agglutinin) that specifically bind with L-fucose and showed

322 that algae and heterotrophic protists generally did not possess this carbohydrate at the

323 cell surface. The same pattern for eukaryotic algae and heterotrophic protists could be a

324 consequence from the endosymbiotic origin of algae when a heterotrophic protist

325 ingested an autotrophic cell (Margulis 1970; Zimorski et al. 2014). Since L-fucose and

326 receptors for this carbohydrate are rare in microalgae, we suggest that L-fucose could be

327 less important for recognition or communication among these organisms.

328   The interactions with GD-Man were more frequent (seven among twelve

329 species) than those with GD-Fuc. The bindings between lectins and mannose are not

330 well known for microalgae, but they were reported for other organisms and even for

331 other phototrophs belonging to classes not studied here. For example, the lectin MVN

332 was isolated from the cyanobacterium *Microcystis viridis* and it is highly selective for

333 D-mannose oligosaccharides containing $\alpha(1\rightarrow2)$ linkages (Kehr et al. 2006). The

334 adherence of *Escherichia coli* in mammalian tissues occurs due to the interactions

335 involving D-mannose receptors (Iman et al. 1984). Another example is the recognition

336 of HIV (*Human Immunodeficiency Virus*) by human dendritic cells using the DC-SIGN

337 lectin, a D-mannose receptor (Spear et al. 2003; Geijtenbeek et al. 2000).

338   The absence of mannose receptors in *Nitzschia* sp. cell surface complements the

339 observations by Martin-Cereceda et al. (2007), who did not found D-mannose residuals

340 on the cell surface of other Bacillariophyceae (*Extubocellulus* sp.). From this

341 observation, we suppose D-mannose could be not important for the chemical bindings

342 in diatoms, but information is still scarce to fully support this hypothesis. *Nitzschia* sp.

343 differed from the other algae, since it interacted with GD-Fuc and GD-GalNAc, but not

344 with GD-Man. The origin of diatoms by secondary symbiosis (Margulis 1970; Durnford

345 et al. 1999; Keeling 2010) could result in organisms with a more particular membrane

346 surface composition in relation to the other species, because they belong to

347 phylogenetically more closely related groups derived from primary symbiosis (Gould et

348 al. 2008). Other groups with secondary endosymbiosis should however be tested to

349 confirm this hypothesis. Further, diatoms have a proteic nanopatterned silica cell wall

350 and this is another particularity of *Nitzschia* among the algae used in this study, which

351 could have some effect in the bindings with the dendrons.

352 *Mougeotia* sp. and *C. astroideum* probably have no receptors for the

353 carbohydrates tested. However, the absence of results cannot confirm that the

354 carbohydrate is not important for the recognition of other organisms, but it is evidence

355 that the trivalent presentation is not efficient to promote interactions. It is possible that

356 other multivalent carbohydrate systems with higher valency (more copies of the

357 carbohydrate ligand) would show some level of interaction. In this work using a

358 trivalent presentation of the sugar, we have observed high biochemical diversity, since

359 differences were observed even within the same genus. These observations reinforced

360 the idea that receptor composition in algal cell surface is species-specific, with no

361 pattern related to higher taxonomic group. In a similar study, but using labeled lectins,

362 Muller (1983) did not found a pattern in the interactions with the algal groups studied

363 and also concluded that carbohydrate-lectins bindings could be species-specific in algae.

364 When bindings were observed between a trivalent glycodendron and cells, the

365 results represented more than 90% of the individuals, suggesting that the receptor

366 composition could vary along the life cycle of organisms. This variation can occur

367 according to reproductive cycle (Kim et al. 2007), nutritional (Ramoino 1997; Kremp

368 and Anderson, 2004) or physiological *status* (Tien et al. 2005; Pannof et al. 1988;

369 Aguilera and González-Gil 2001; Waite et al. 1995). However, zero occurrences of

370 labeled cells were observed in cases with no interaction.

371

4.2 - Non-uniform distribution of cell surface receptors

372

373   Since the interactions between *S. leptocladum* and GD-GalNAc or GD-Fuc were

374 weak and only observed in the central part of the cells, it is possible that these

375 carbohydrates could be less relevant in cell recognition processes than GD-Man, which

376 fully covered the cell surface. However, even GD-Man did not show uniform

377 distribution on the cell surface, with higher fluorescence values occurring in the

378 branches. For the colonial species *Pediastrum* sp. the more intense fluorescence

379 corresponds to cell-cell contact areas, independently of the glycodendrons tested,

380 suggesting the existence of a higher number of glycidic receptors in these regions.

381 Therefore, lectins are CAMs (Cell adhesion molecules) (Lasky 1991) and should be

382 related to the cell-cell recognition necessary to colony formation. This role for lectin-

383 carbohydrate bindings was described for other organisms (Espinosa et al. 2010; Kim et

384 al. 2007), such as lichens (Vivas et al. 2010). Here, the highest fluorescence in cell-cell

385 contact regions of the colonial species was observed for GD-Man and GD-GalNAc. D-

386 mannose and *N*-acetylgalactosamine are present on the cell surface of many species

387 (Roberts et al. 2006; Martin-Cereceda et al. 2007). Since the glycodendrons containing

388 these carbohydrates interacted with a higher number of species, we believe that the

389 receptors for these carbohydrates have a wide occurrence in algae. In fact, D-mannose is

390 often reported to be important in cell recognition and interaction for different taxonomic

391 groups (Esquenazi et al. 2003; East and Isacke 2002; Saifuddin et al. 2000). D-mannose

392 units are detected by fluorescent concanavalin A and previous studies reported that this

393 carbohydrate occurs in several species of dinoflagellates, diatoms, prymnesiophytes and

394 other protists (Kremp and Anderson 2004; Wootton et al. 2006; Roberts et al. 2006) .

395

5 - Conclusion

The interactions between the trivalent carbohydrates dendrons and some algal species suggest a different biomolecular composition of the corresponding cell surfaces. We believe that the differences may have some taxonomic value and conclude that the glycodendrons are easily available and useful tools to differentiate species. Furthermore, the knowledge regarding algal cell surface will improve the understanding about microalgae interaction mechanisms with each other and with other organisms, which is a key issue in symbiosis, competition, predation, infection and reproduction of these organisms. All these aspects are all important when thinking about biomass production. GD-GalNAc was more commonly recognized than GD-Man and GD-Fuc, but the specific role of this carbohydrate is not evident for these species. Our study had also generated evidence for the heterogeneity in the glycidic receptors on algal cell surfaces, which is still not clearly understood in literature. In summary, our results indicate that glycodendrons could be useful and trustworthy tools for studies focusing on the differentiation of cell surfaces composition.

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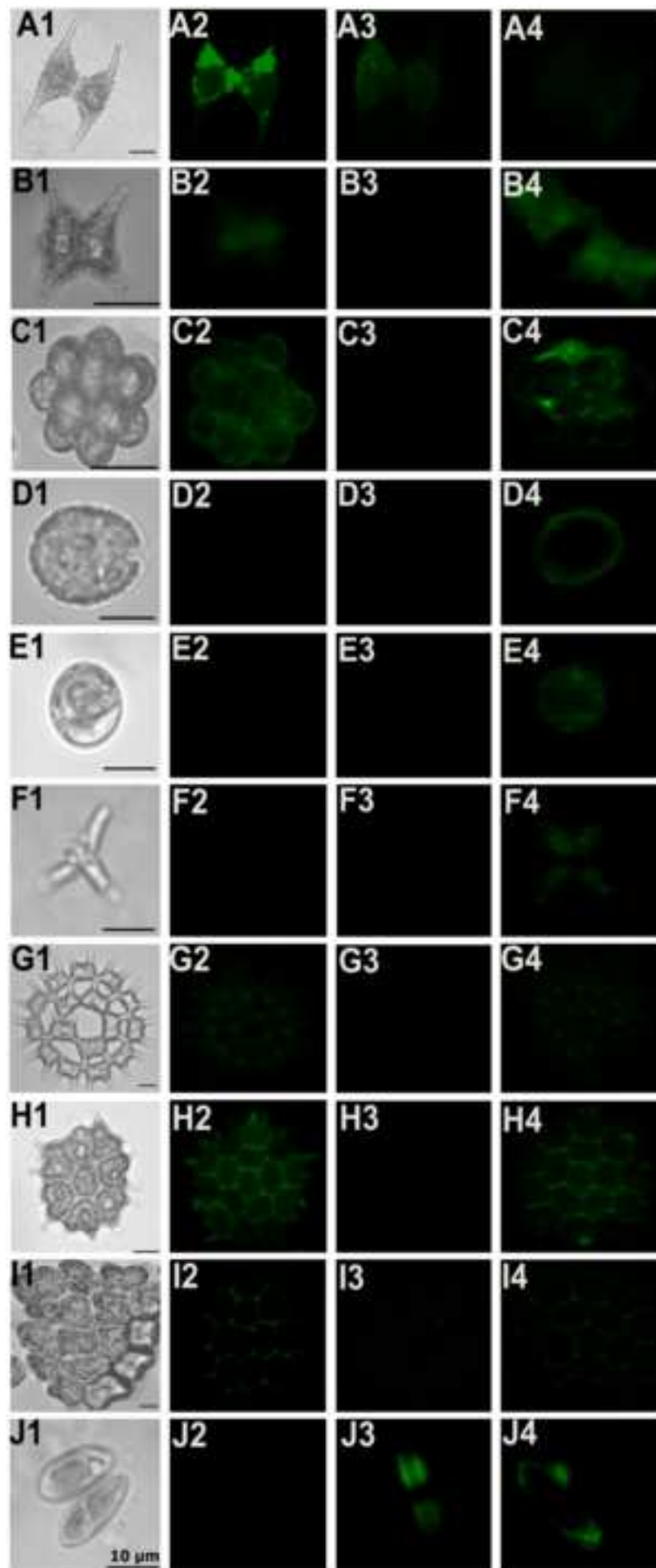
Table 1. Synthesis of the interactions between glycodendrimers and the algal species.

Figure 1. Schematic representation of the chemical structures of the carbohydrates of the glycodendrons. 1) *N*-acetylgalactosamine; 2) L-fucose; 3) D-mannose and 4) diethyleneglycol (control).

Figure 2. Confocal images of the selected algal species and their interactions with the glycodendrons. (A) *Actinastrum hantzschii*; (B) *Coelastrum astroideum*; (C) *Chlamydomonas* sp. ; (D) *Chlorela vulgaris*; (E) *Nitzschia sp*.; (F) *Pseudopediastrum boryanum*; (G) *Pediastrum duplex*.; (H) *Pediastrum* sp.; (I) *Staurastrum iotanum*; (J) *Staurastrum leptocladum*. The first column shows optical microscopy images of the species (A1-J1). The second column shows the interaction fluorescence results with L-fucose (A2- J2). The third with D-mannose (A3-J3) and the fourth with GalNAc (A4-J4).

Figure 3. Total fluorescence values for algal species (A to J denote species. For legend see figure 2). ● controls, ■ D-mannose, ◆ L-fucose, ▲ N-acetylgalactosamine glycodendrons. Bars indicate standard deviation and the letters above the bars indicate significant differences among glycodendrons according to Tukey test. AFU: Arbitrary Fluorescence Units.

Figure 4. Fluorescence observed in different surface regions in the same specimen. (A) *S. leptocladum*, (B) *P. duplex*, (C) *Pediastrum* sp and (D) *P. boryanum*. ◆ D-mannose, ■ L-fucose and ▲ GalNAc glycodendrons. Asterisks indicate significant difference (P<0.05) between regions that reacted more and less with the respective glycodendron. AFU: Arbitrary Fluorescence Units.

line figure
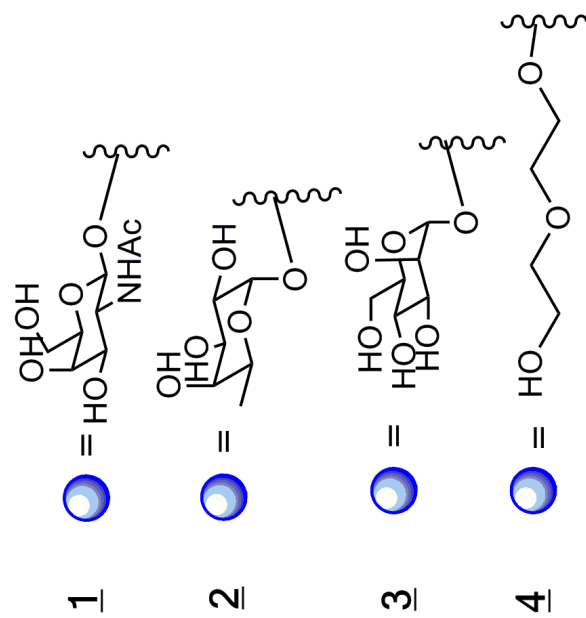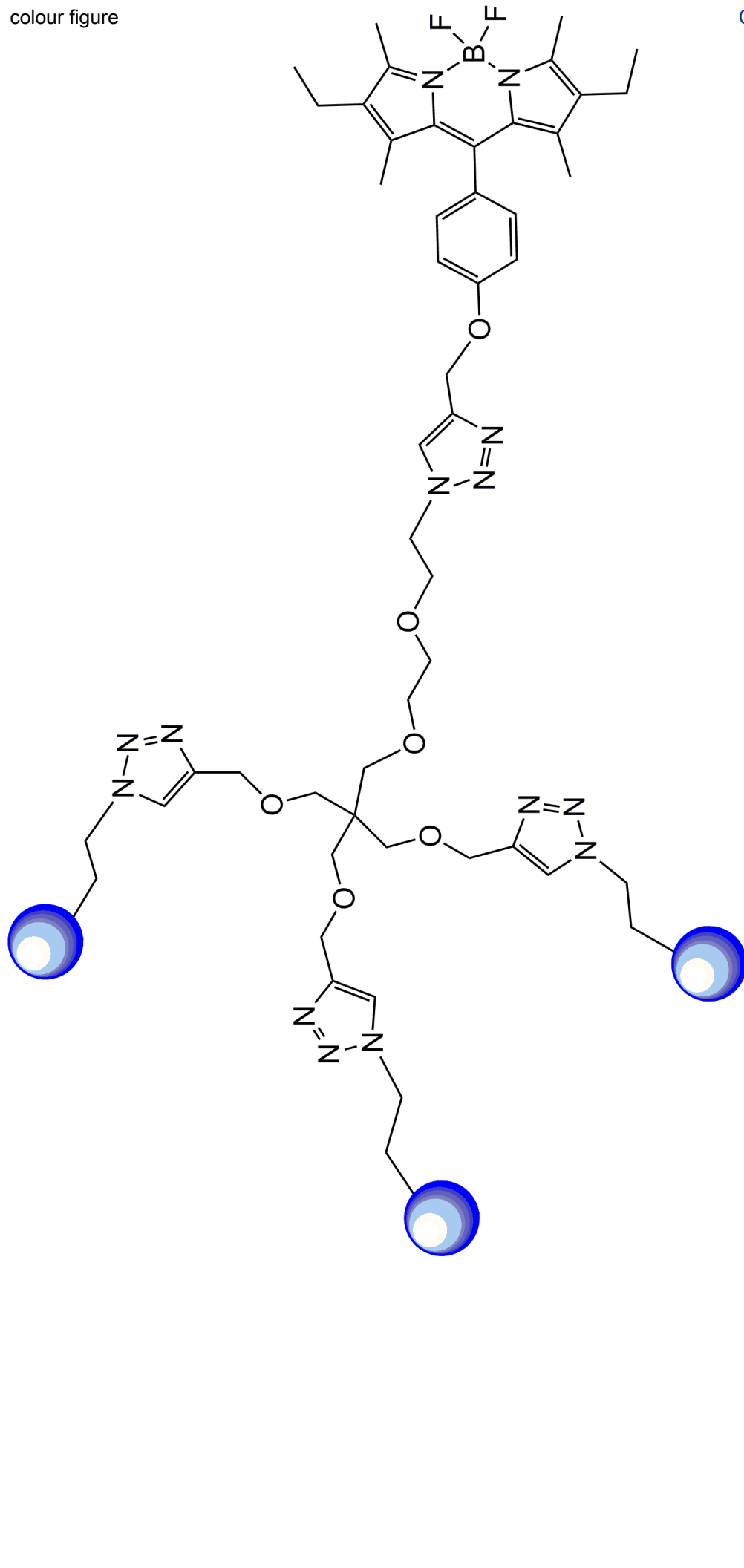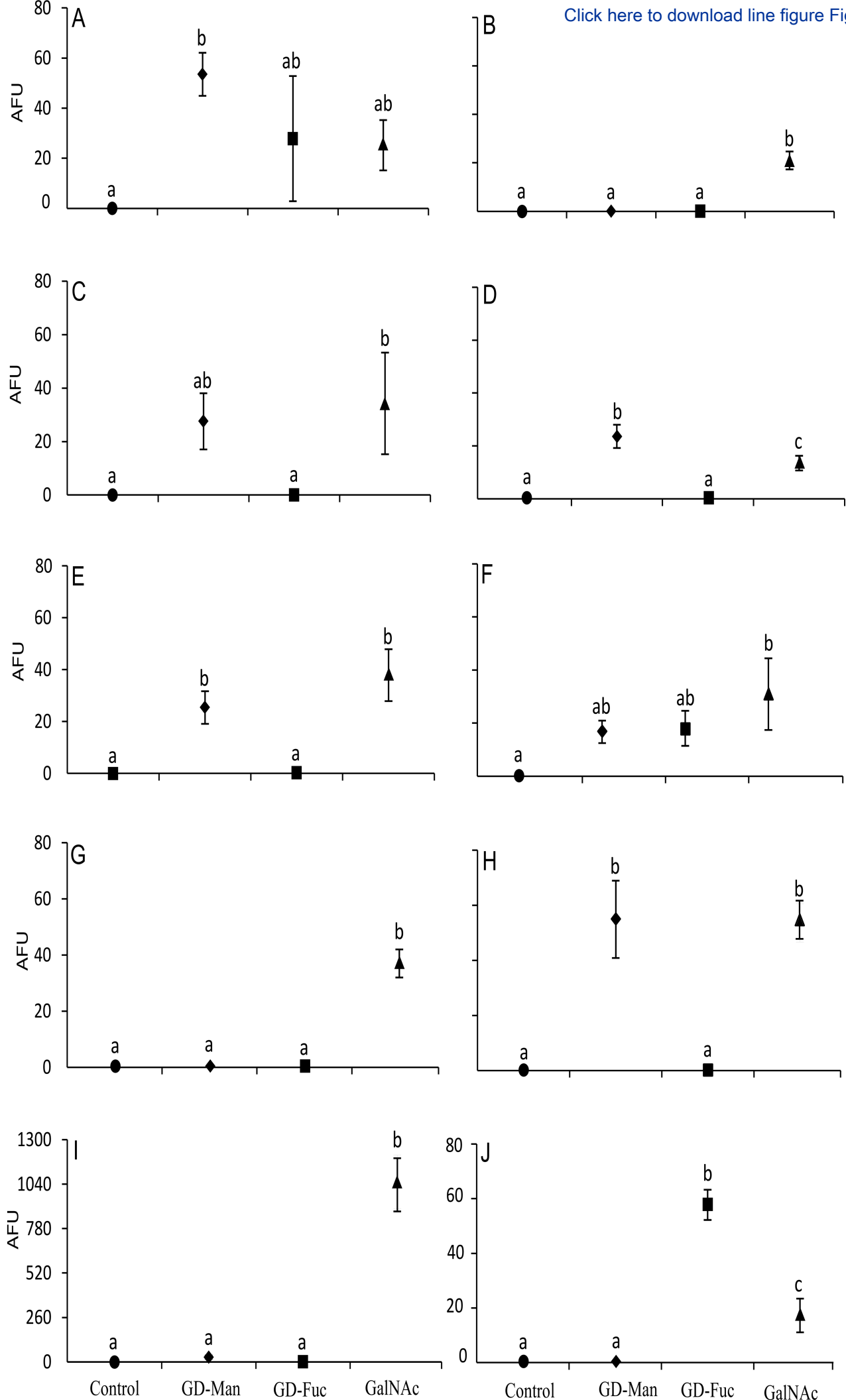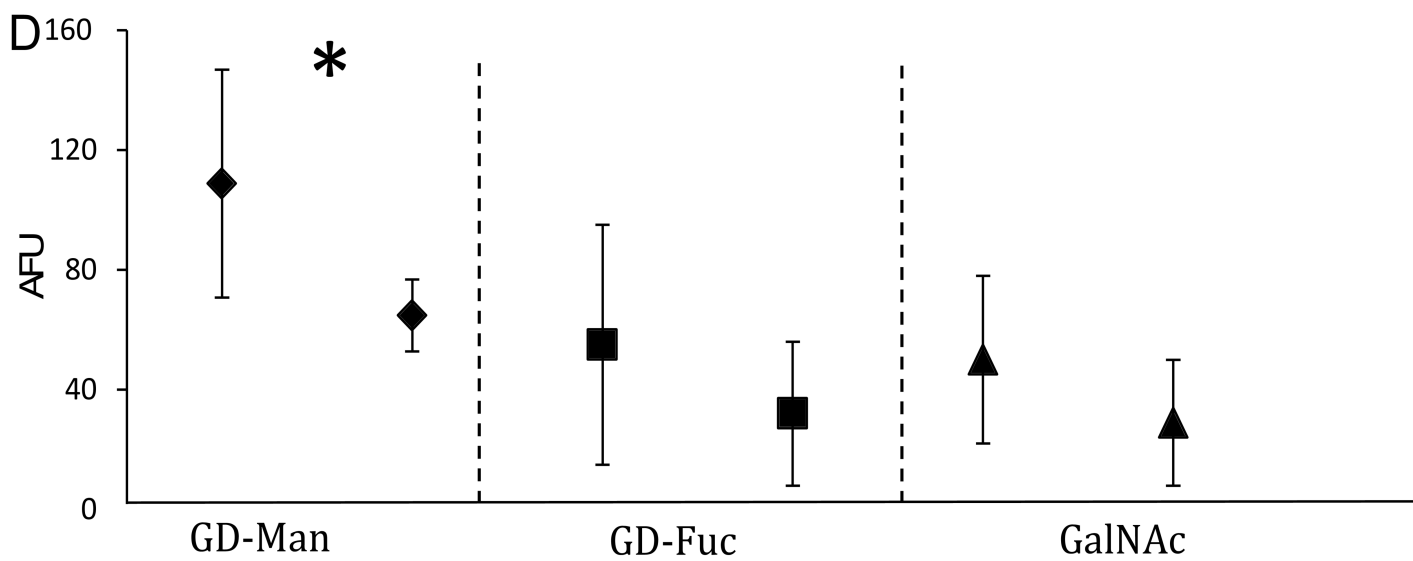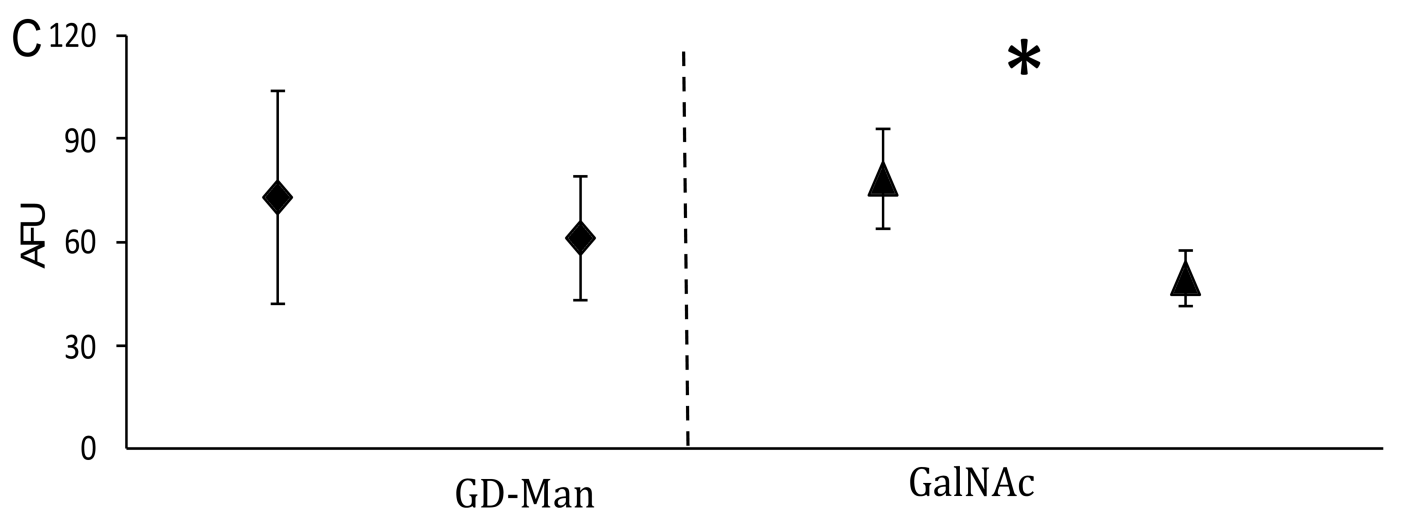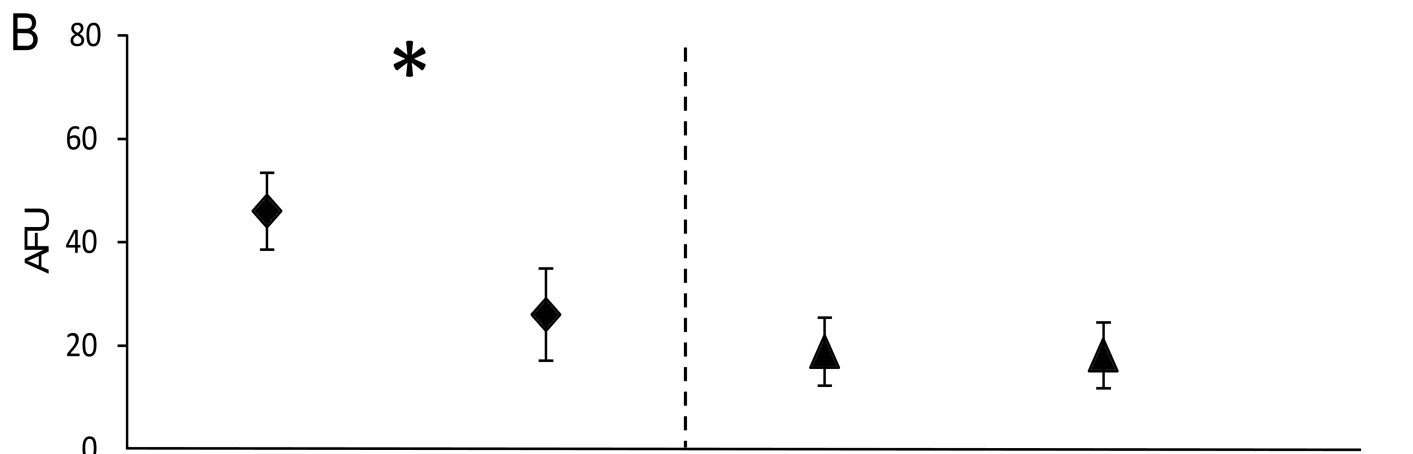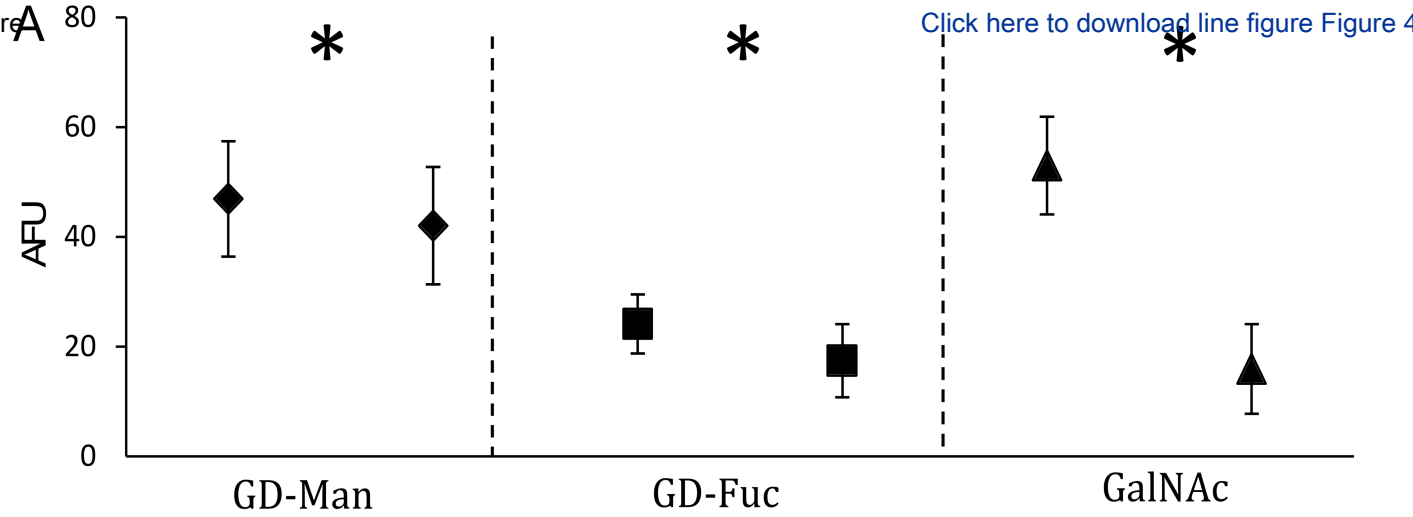
Class	Species	Glycodendrimer			Control
		GD-Man	GalNHAc	GD-Fuc	Diethylene glycol
Trebouxiophyceae	*Actinastrum hantzschii*	-	+	-	-
Trebouxiophyceae	*Chlorella vulgaris*	-	+	-	-
Chlorophyceae	*Chlamydomonas* sp.	+	+	-	-
Chlorophyceae	*Coelastrum microporum*	+	+	-	-
Chlorophyceae	*Pediastrum duplex*	+	+	-	-
Zygnematophyceae	*Staurastrum iotanum*	+	+	-	-
Chlorophyceae	*Pediastrum boryanum*	+	+	-	-
Chlorophyceae	*Pediastrum* sp.	+	+	+	-
Zygnematophyceae	*Staurastrum leptocladum*	+	+	+	-
Zygnematophyceae	*Mougeotia* sp.	-	-	-	-
Chlorophyceae	*Coelastrum astroideum*	-	-	-	-
Bacillariophyceae	*Nitzschia* sp.	-	+	+	-

GalNHAc: N-acetylgalactosamine; Man :D-mannose; Fuc: L-fucose, GD: glycodendron