

Fucanome and Galactanome: Marine Glycomics Contribution

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Abstract

Following the genome, transcriptome and proteome, the glycome has currently launched in biology bringing apparently more challenges than the first projects. The long major conception of carbohydrates as just energetically involved class of biomolecules has fallen apart as innumerable essential biological actions have been raised, impossible to be enlisted all together at once. Such actions involve not only vital roles in cell and physiology but within a different context they also include potent therapeutic actions in coagulation, thrombosis, inflammation, virosis, pathogenesis, tumorigenesis, metastasis and angiogenesis. Glycomics is so extensive project that subdivision is necessary for its progress. Specific segments of research have been created about particular bioactive sugar classes such as sialome for sialic acids, and heparanomics for heparan bioactive domains. Fucanome and galactanome must be also included in the recent glycomics age with respect to the relatively new class of marine fucose- or galactose-composing polysaccharides named sulfated fucans and sulfated galactans. These glycans are very biologically relevant since they show potent pharmacological properties in many of the above-mentioned systems; besides in sea-urchins they are responsible to control a very rare case of carbohydrate-mediated cell signaling event, the acrosome reaction. The structure of these glycans is very restricted to phyla or species of occurrence. Among all marine organisms, only invertebrates and red algae have been known so far to express high molecular weight polymers composed of well-defined structures. These structural features are unique and very rare among any bioactive polysaccharide ever studied. This would make fucanome and galactanome differential glycomics subprojects in terms of structure and phylogeny. This paper not only brings out novel segments and their respective contribution to glycomics but also highlights the great relevance of pharmacological effects of these new glycans in the current glycome era.

Keywords: Galactans; Glycome; Fucan; Pharmacological Action; Marine Polysaccharides

Abbreviations: GAG: Glycosaminoglycan; MSP: Marine sulfated polysaccharides; MW: Molecular weight; SG: Sulfated galactan; SF: Sulfated fucan

How Would Fucanome and Galactanome Fit in the Glycomics' Definition?

Right after the ages of many "omics" projects such as genomics, metagenomics, transcriptomics, proteomics, metabolomics and lipidomics, a novel era has been introduced into biology, now concerning the carbohydrates and their relationships. This new project has been denoted "glycome", and "glycomics" with respect to its study [1-3]. Glycomics seems to bring with it much more challenges and difficulties than the first "omics". For example, an automatic sequencing technique as used for nucleic acids and proteins still does not exist for the case of complex oligosaccharides. Carbohydrates are well-known to be much more structurally complex and flexible than genes, transcripts, lipids and proteins [4], besides the enhanced complexity of the subject due to a higher number of influential factors in glycan biosynthesis [2]. Glycans are products directly dependent on the other "omics" (mainly genome, transcriptome, proteome, and metabolome), synthesized through a non-template driven process well-influenced by exogenous determinants such as secretory machinery, pH, temperature, season, nutrient content, precursor concentrations, metabolites availability and amount, pathological conditions, and others [1,2]. The understanding of the systems of glycan metabolism is beyond the limits of the current technological capacity for analyses and interpretation. Although far away from a totally correct and ultimate definition, the term glycomics roughly stands for profiling the diverse glycan repertoires of certain biological forms (cell, tissue, organ and organism) under specific conditions [2,5]. A more general, simpler, and even comprehensive definition has been the compilation of information about how a collection of glycans (or glycoconjugates) and their respective relationships could be understood in a particular

biological event [1]. This is a broader and more subjective definition, but it certainly depicts a better view of the glycomics' meaning. This last definition might bring a debatable point, however: could the biological action of glycans in organisms or systems, other than those involved in their own metabolism, be assumed as a "particular biological event"? If yes, should pharmacological actions of exogenous non-mammalian glycans in mammalian systems be included in the glycomics project? If so, the marine glycans, sulfated fucans (SF) and sulfated galactans (SG) fit perfectly in this definition due to their large spectrum of therapeutic properties in many biological areas [6-8]. This would make therefore new terminologies like fucanome and galactanome appropriate as subprojects of glycomics. If pharmacological actions of exogenous glycans could not be assumed as part in glycomics, the new terminologies would be validated through the more specific definition of the repertoire of glycan structures in specific biological forms [2,5]. Below we discuss how the chemical and functional properties of these recently discovered marine sulfated polysaccharides (MSP) would be relevant for such incorporation in the glycomics age as well as the significance of their therapeutic applications.

General characteristics of SF and SG molecules

SF and SG molecules are mostly linear, highly negatively charged polysaccharides found exclusively in marine organisms as described

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so far [7]. They have been reported in macroalgae (Rhodophyceae, Phaeophyceae, and Chlorophyceae) [7-11], in echinoderms sea urchins (Echinoidea) [12] and sea cucumbers (Holothuroidea) [13], in marine angiosperms (Angiospermae) [14,15], and in ascidians commonly known as tunicates (Asciacea) [16,17].

In macroalgae, sea cucumber and tunicates, these glycans were reported to be essentially involved in assembling either the cell wall or the body wall [7], where in sea-urchins they are found in the egg jelly coat that surrounds the female gametes, playing thus a crucial role in the initial step of fertilization as primordial inducer of the acrosome reaction [12,18,19]. The acrosome reaction is a very rare case of cell signaling event, being exclusively carbohydrate-triggered and strictly controlled by specific structural features of the MSP [12,18,19]. In marine superior plants, these novel glycans seem to be involved in some osmotic regulations dependent on the salinity of environmental water of the plant's habitat [15].

The occurrence of MSP is very phylogenetically restricted. In brown algae, these MSP are usually found as SF molecules, while in red and green algae and superior plants they are found as SG molecules [7-11]. On the other hand, in invertebrates, both SF and SG molecules can be isolated [12,13,16-19]. The structures of brown algal SF are often very complex. They are branched, heterogeneous, and lack a clear repetitive pattern. One source of heterogeneity is, for example, the random presence of non-fucosyl units along with the fucose-based backbone [7,19]. The SG that occurs in green algae is less complex than the brown algal SF but it is still difficult to arrive at a general view of the structure. For green algal SG molecules only some major components and short patterns can be deciphered, due to the usual absence of a clear regularity [19,20]. Conversely, red algal SG has occasionally shown regular backbones composed of disaccharide units whose sulfation patterns are the major contributors to structural heterogeneity [10,11,19,20]. In marine invertebrates, on the other hand, the MSP frequently appear to be composed of regular repeating oligosaccharide units with a clearly defined sulfation pattern (see structures in Table 1).

Aside from these general structural patterns, the structures of either SG or SF molecules are reported to vary frequently according to the species from which these MSP have been isolated [7]. These results represent a large and specific library of glycans found exclusively in organisms from the sea. The compiled annotation of SF and SG structures with the proper correlation with the respective scientific name of the species of extractions comprises itself an extensive glycomics subproject. The further documentation of biological actions of these relatively new marine glycans either in their own organisms or for pharmacological purposes is complementary and relevant.

Glycomics project sounds actually much bigger than initially realized: fucanome and galactanome in the context of other "glycan-omes"

The structural diversity of carbohydrates is considerably bigger than any other macromolecule. For example, considering only one type of residue, a dimeric form would make just a single type for a peptide, whereas eleven different disaccharides could be made considering only the anomeric configurations (α - and β -) combined with the possible positions for linkages through glycosidic bonds. Considering now a trimeric form, a peptide still makes one single structure whereas the number rises up to 176 for trisaccharides. If, for example, trimers composed of all three different residues are taken into account, the variety in oligosaccharides goes up exponentially. While only six types of heterotriptides are possible, up to 27,648 structures can

be made by three differently linked hexopyranoses [5]. This number would increase immensely if furanosyl form plus variability in D- or L-enantiomers were taken into consideration. If positions of lateral chemical groups like sulfate and phosphate esters, or branches, are included, the number of structural possibilities for an intermediate oligosaccharide, like a deca-saccharide, for example, goes beyond the imagination and current capacity of analyses and interpretation.

Fortunately, the structural variability of naturally occurring polysaccharides and oligosaccharides is greatly restrained by the number of enzymes (mainly transferases) and nucleotide-donors available during the biosyntheses of glycans. Such reduced diversity found normally in nature keeps it still beyond the actual and feasible technological capacity of glycoscientists to sequence an entire intermediate oligomeric chain of certain glycans [26]. The degree of difficulty obviously depends on the length and structural complexity of the carbohydrate chain, but becomes practically unviable when high molecular weight (MW) complex polysaccharides are subjected to structural analysis. This difficulty in sequencing carbohydrates can be exemplified by the great structural diversity in heparin/heparan sulfate molecules, a subclass of the well-known sulfated glycosaminoglycans (GAG) [27]. The differential levels in epimerization of uronic acids (glucuronic versus iduronic acid) plus the variable sulfation patterns (*N*- or *O*-linked, and at differential locations like C2, C3, and C6) enhance enormously the structural heterogeneity in a single polysaccharide chain [28-30]. Such big heterogeneity impairs the full sequencing in a given oligomeric extension and therefore, only size-limited oligosaccharides within bioactive domains like protein-binding sites can be actually structurally mapped [29,30].

Another aggravating factor in the glycomics project is the existence of many numbers of sugar classes, respective subfamilies, and categories of glycoconjugates such as glycoproteins, glycolipids, and proteoglycans. These conjugates have also their individual and complex contribution in glycomics as subprojects like glycoproteomics [31], glycolipidomics [32], and proteoglycanomics [28], respectively. Keeping on the same exemplification of GAG polysaccharides which are studied directly under the glycosaminoglycanome subproject [3] and indirectly studied in proteoglycanome [28] and ultimately in glycome, the polysaccharides chondroitin/dermatan sulfate, keratan sulfate, and hyaluronan participate together with heparin/heparan sulfates as other GAG types enlarging immensely the possibilities of structures, magnifying, therefore, significantly the size of the glycosaminoglycanome subproject only. For example, all GAG compounds are composed of different hexosamines (either *N*-acetylgalactosamine or *N*-acetylglucosamine) and uronic acids (either iduronic or glucuronic acid) or galactose (in the single case of keratan sulfate) and within different sulfation patterns (with the exception of hyaluronan) that can be either *O*- or *N*-linked to the protein. Therefore, the number of possible structures in a chain type is immense.

The expressive number of structures made by such combination of substituents can go absurdly higher when the protein core or mixed GAG types is taken into account in a single proteoglycan type such as syndecan [30], composed of separated chains of heparan sulfates and chondroitin sulfates. The variety of glycan structures becomes completely intractable in glycobiology if we consider all sets of *N*-, or *O*-linked glycoproteins, glycolipids, and peptidoglycans together with the set of proteoglycan structures. Therefore, a subdivision of glycomics is required for the development, and comprehension of this immense "omics" project related to carbohydrates (glycome). The subdivision

Species (Class)	Structure	Ocorrence	Ref.
<i>Ludwigothuria grisea</i> (Holothuroidea)	[\rightarrow 3)- α -L-Fucp-2,4(OSO ₃)-(1 \rightarrow 3)- α -L-Fucp-(1 \rightarrow 3)- α -L-Fucp-2(OSO ₃)-(1 \rightarrow 3)- α -L-Fucp-2(OSO ₃)-(1 \rightarrow) _n	Brazil	[13]
<i>Strongylocentrotus purpuratus</i> II (Echinoidea)	[\rightarrow 3)- α -L-Fucp-2,4di(OSO ₃)-(1 \rightarrow 3)- α -L-Fucp-4(OSO ₃)-(1 \rightarrow 3)- α -L-Fucp-4(OSO ₃)-(1 \rightarrow) _n	USA	[21]
<i>Strongylocentrotus purpuratus</i> I (Echinoidea)	80% [\rightarrow 3)- α -L-Fucp-2,4di(OSO ₃)-(1 \rightarrow) _n and 20% [\rightarrow 3)- α -L-Fucp-2(OSO ₃)-(1 \rightarrow) _n	USA	[21]
<i>Strongylocentrotus franciscanus</i> (Echinoidea)	[3)- α -L-Fucp-2(OSO ₃)-(1 \rightarrow) _n	USA	[22]
<i>Strongylocentrotus droebachiensis</i> (Echinoidea)	[\rightarrow 4)- α -L-Fucp-2(OSO ₃)-(1 \rightarrow) _n	USA, Norway	[23]
<i>Strongylocentrotus pallidus</i> (Echinoidea)	[\rightarrow 3)- α -L-Fucp-2(OSO ₃)-(1 \rightarrow 3)- α -L-Fucp-2(OSO ₃)-(1 \rightarrow 3)- α -L-Fucp-(1 \rightarrow 3)- α -L-Fucp-(1 \rightarrow) _n	USA, Norway	[23]
<i>Lytechinus variegatus</i> (Echinoidea)	[\rightarrow 3)- α -L-Fucp-2(OSO ₃)-(1 \rightarrow 3)- α -L-Fucp-2(OSO ₃)-(1 \rightarrow 3)- α -L-Fucp-4(OSO ₃)-(1 \rightarrow 3)- α -L-Fucp-2,4di(OSO ₃)-(1 \rightarrow) _n	Brazil	[13]
<i>Arbacia lixula</i> (Echinoidea)	[\rightarrow 4)- α -L-Fucp-2(OSO ₃)-(1 \rightarrow 4)- α -L-Fucp-2(OSO ₃)-(1 \rightarrow 4)- α -L-Fucp-(1 \rightarrow 4)- α -L-Fucp-(1 \rightarrow) _n	Brazil	[24]
<i>Echinometra lucunter</i> (Echinoidea)	[\rightarrow 3)- α -L-Galp-2(OSO ₃)-(1 \rightarrow) _n	Brazil	[24]
<i>Glyptosidaris crenularis</i> (Echinoidea)	[\rightarrow 3)- β -D-Galp-2(OSO ₃)-(1 \rightarrow 3)- β -D-Galp-(1 \rightarrow) _n	Japan	[25]
<i>Botryocladia occidentalis</i> (Rodophyta)	[\rightarrow 3)- β -D-Galp-2R ₁ -3R ₂ -(1 \rightarrow 4)- α -L-Galp-2R ₃ -3R ₄ -(1 \rightarrow) _n , where R _# = OSO ₃ or OH, R ₁ and R ₂ = OSO ₃ in ~ 66%, and ~ 33%, respectively.	Brazil	[10]
<i>Gelidium crinale</i> (Rodophyta)	[\rightarrow 3)- β -D-Galp-2R ₁ -4R ₂ -(1 \rightarrow 4)- α -L-Galp-2R ₃ -3R ₄ -(1 \rightarrow) _n , where R _# = OSO ₃ or OH, R ₁ and R ₂ = OSO ₃ in ~ 60%, and ~ 15%, respectively.	Brazil	[11]
<i>Rupia maritima</i> (Angiospermae)	[\rightarrow 3)- β -D-Galp-2(OSO ₃)-(1 \rightarrow 4)- α -D-Galp-(1 \rightarrow 4)- α -D-Galp-(1 \rightarrow 3)- β -D-Galp-4(OSO ₃)-1 \rightarrow) _n	Brazil	[14]
<i>Styela plicata</i> (Ascidiacea)	{ \rightarrow 4)- α -L-Galp-2[\rightarrow 1)- α -L-Galp]-3(OSO ₃)-(1 \rightarrow) _n	Brazil	[17]
<i>Hedmania monus</i> (Ascidiacea)	[\rightarrow 4)- α -L-Galp-3(OSO ₃)-(1 \rightarrow) _n	Brazil	[16]

Table 1: Oligomeric repetitive units of SF and SG polysaccharides from echinoderms sea urchins (Echinoidea), and sea cucumber (Holothuroidea), red algae (Rhodophyta), marine superior plant (Angiospermae), and ascidians, also known as tunicates (Ascidiacea).

presents the best way to make possible in glycobiology the mapping and annotation of such extensive number of structures and functions corresponding to all types of sugars and their respective changes according to physiological, behavioral and environmental influences. In response to the enormity of glycan variety, new terminologies for defining glycomics subprojects have started to appear [5,30]. Among many feasible glycomics sub-categories, the two most popular terms rising recently for particular types of sugars are the sialome [5], and the heparanome [30]. Such projects deal with sialic acids and heparan sulfate bioactive domains respectively, as discussed in the next two paragraphs.

In the case of sialome, it has literally been defined as “a total complement of sialic acid types and linkages, and their mode of presentation on a particular organelle, cell, tissue, organ or organism – as found at a particular time and under specific conditions” [5]. The general conception of the sialome glycomics subproject involves basically the studies of sialylated glycoconjugates, where sialic acids are usually at their outermost edges of glycoproteins or glycolipids. The specific terms that sialome covers are vast and comprise the following: sialic acid core structure, the linkage involved in such residues and in the neighboring sugars, the identity and arrangement of the sialic acids as well as of the neighboring sugars, structural and functional attributes of the sialic acid motifs and of the neighboring sugars, the spatial organization of the sialic acid domains and the neighboring sugars, as well as the global 3D structure of the sialylated glycoconjugates. Another topic and probably the most relevant one is to understand the high levels in cellular surface organization in terms of distribution of sialylated glycoconjugates and their structural and functional differences related to specific conditions like pathological conditions, stress types, environmental effects, and evolution. Sialylated glycoconjugates make the biggest contribution in the sialome, however polymeric forms of sialic acid do exist and they might enhance the complexity of this glycomics subproject. The sialome might be considered a small segment of the glycoproteomics or glycolipidomics subprojects and therefore a very small part of the entire glycomics, but it still represents an enormous project itself in glycoscience.

Heparanomics is another subproject getting more and more

relevance and respect in the glycome era [27-30]. Conventionally, it comprises the study of all biologically active oligosaccharide domain structures in the entire heparanome (either for heparins or for heparan sulfates), and the nature of the molecular interactions involving the bioactive domains of these GAG types with protein ligands that would consecutively trigger fundamental biological actions. Heparanomics primarily aims at the elucidation of internal bioactive oligosaccharide motifs for establishing structure-function relationships. However, this is not a trivial research. The enormous structural heterogeneity of these GAGs as mentioned above represents a huge challenge for structural analysis. This challenge has as positive consequence to push the development of novel strategies and analytical tools in order to try to overcome the barriers in structural characterization of heparin/heparan sulfate active domains [28]. We can even be more audacious in defining heparanomics - it could be defined as the entire sequencing of heparin-like structures in organisms including their parts of occurrence (organs, tissues, cells, and even microcellular domains in the plasmatic membrane surface) in different healthy stages (like embryonic developing periods) or even types and levels of pathological conditions. The sulfation patterns of heparan-like compounds are well-known to change during embryonic development, and in pathological types and progressing levels of illnesses, such as cancer stages [33]. Similar to Sialome, Heparanome sounds itself an enormous and challenging subproject. And it actually comprises just a hierarchical segment of the glycosaminoglycanome and/or proteoglycanome. These latter are themselves subprojects in glycomics, thus showing that heparanome is just an extremely tiny part of the whole glycome project.

Following this same way presented for sialome and heparanome, possible novel “omics” subprojects would be: agaranomics (for agaran), carrageenomics (for carrageenan), dermatanomics (for dermatan sulfates), chondroitinomics (for chondroitin sulfates), among others including also the here proposed fucanomics and galactanomics. If we consider all structural and functional aspects of the above-mentioned glycan categories along their organisms, tissues and biological conditions, we realize that glycomics comprises a much greater project than when it was initially realized or proposed. Subdivision of the glycome is therefore naturally necessary for the progress of such

enormous project. Although subprojects seem less complicate than the total, the big complexity still remains in the subprojects.

The conceptuality of fucanome and galactanome

Fucanome and galactanome would be defined as direct subprojects of glycome, which would comprise the full description and notation of the structures, functions and metabolic relations involved in SF and SG molecules, respectively. The entire structural notation of such MSP related to the names of species and/or phyla comprehend a big library of glycans from the sea. This project of structural notation fits adequately as a subdivision of glycomics. These MSP exhibit structures those are very rare among other polysaccharides. These MSP have been studied only during the last two decades (references in Table 1, for example) and thus they have to be catalogued and broadly exposed to the glycoscientific community worldwide. The additional description of SF and SG functions would be focused both on natural biological actions in the organisms that synthesize them [12,18,19] and on possible medical properties in mammalian systems [12,19,34]. The regulative mechanisms involved in the biosynthesis and expression of MSP structures and consequently their functions should be considered a relevant line of study too since the biosynthetic mechanisms of these MSPs are virtually unknown. However, a consistent body of reports concerning structural and functional changes influenced by either environmental or biological conditions have emerged recently [15, 35-37]. Annual seasons (winter *versus* summer) have been reported to influence the sulfation patterns of sea urchin SF polysaccharides [35]. Salinity levels in the estuary regions in which marine angiosperms naturally inhabit were recently reported to be directly coupled to the density of sulfation of MSP and thus, to the structural properties of the SGs in these superior plants [15]. Seasonal changes are also documented to be influential in structures of algal SF and SG molecules [36]. Metal contamination in polluted areas, for example, has been shown to be influential in MSP expression and abundance in cell wall [37]. The documentation of understandings about these changes is very useful in terms of considering the commercial interest of SF and SG for medical purposes. Understanding these modulating effects consequently gives scientists greater ability to explore and take advantage of these marine glycans either for health care or nutraceuticals production.

The importance of considering therapeutic actions of MSP in glycomics

Besides the natural actions of SF and SG molecules, these glycans have widely been explored due to their medical properties. In fact, they have been targets of study by many international research groups strictly due to their unique and potential therapeutic applications. Glycomics would lose some of its great impact and broad contribution to human society if human healthcare-related properties of glycans were left aside. The importance of such therapeutic use of carbohydrates has already been discussed previously, for example, in the case of GAG molecules in the glycosaminoglycanome subproject [3]. It is worth to remind that only behind the peptide insulin, the carbohydrate heparin is the second most used natural macromolecule in medicine, a GAG type widely explored because of its anticoagulant activity. Heparin naturally occurs in mast cells, and it seems to have a primary key role in inflammation and allergic reactions [38]. However, due to its great affinity to thrombin inhibitors (anti-thrombin and heparin- cofactor II) [39], unfractionated heparin and its low MW derivatives are clinically explored for stopping the clotting processes in patients undergoing extracorporeal circulation in surgery or renal dialysis [40]. The low MW variants of heparin serve also as the primary treatment of deep vein thrombosis. Similar activity has been documented also in SF and

SG, although at lower potencies. [6,7,19,34]. However, as opposed to heparin, which has a demonstrated, high risk of contamination because of its mammalian origin, the MSP has an additional advantage over this GAG type, since they are isolated from marine organisms resulting in lower incidence for contamination of virus and prion [19,34]. Such clinical potencies of MSP should therefore not be left out in fucanome and galactanome as glycome subprojects. Through the broader glycomics definition, the documentation of molecular interactions between carbohydrate structural domains and proteins would also lead to a better comprehension of the biological actions of MSP. Advances in molecular interaction studies involving SF and SG with not only coagulation (co)-factors, but also with selectins, cytokines/chemokines, growth factors, endothelial adhesion-molecules, surface receptors of virus particles and pathogens, are crucial to provide molecular basis for the anticoagulant, antithrombotic, antimetastatic, antitumorogenic, anti-inflammatory, anti-angiogenic, antiviral, and antipathogenic activities of SF and SG molecules [6-8,10,19,34]. The medical applications of other naturally occurring carbohydrates such as agar, carrageenan, chitosan, and chondroitin sulfates should be included in glycomics and overall, would contribute greatly to the growth of the glycome era.

Fucanome and galactanome – differential glycomics subprojects

Table 1 depicts several illustrative examples of SF and SG structures which are composed of well-defined oligosaccharide repeating sequences. The species-specific structures vary in sulfation patterns (but always restricted at 3-O-, 2-O- and/or 4-O-positions), in glycosidic linkages [$\alpha(1\rightarrow3)$, $\alpha(1\rightarrow4)$, and $\beta(1\rightarrow3)$], in variable number of residues along their repetitive oligomeric units (tetrasaccharides, trisaccharides, disaccharides, and monosaccharides), and sometimes presence of short branching segments like the structure of *Styela plicata*. The MW of these polymers, although quite polydisperse, are naturally very high, frequently ranging over 100 kDa. For example, in polymers composed of a repetitive tetrameric unit as observed for the echinoderms *Ludwigothuria grisea* and *Lytechinus variegatus*, the chain extension of such glycans would range approximately more than 100 tetrameric units, if MW > 100 kDa is assumed.

At first glance it is hard to realize the entire structural elucidation of such a long polysaccharide composed of over 400 residues per chain. The full structural sequencing of long polysaccharides with important biological functions would sound apparently impossible to any glycoscientist. However, certain pattern of repetition along the polymeric extensions greatly limits the expected structural complexity (Table 1). This pattern makes the structures more accessible to common analytical practices, manipulation and data interpretation [19,41]. Even though the glycans listed in Table 1 show some simplicity in their structures due to oligomeric repetitive pattern, the diversity of structural forms and sulfation patterns can be still high. Such structural variety, which is constrained by the type of species or phylum, still maintains both identity and the capacity for encoding biological actions through structural features in a species-specific manner. This was previously documented for the species-dependent acrosome reaction in sea urchins [12,18,19]. In this reaction very often only a single SF or SG structure of a given female sea urchin species can induce the acrosome reaction of sperms of the correlated species, avoiding thus the possibility of forming hybridized offspring [12,18]. This specificity is directly controlled by the structural features of MSP (glycosidic linkage type, anomericity, sulfation pattern, monosaccharide type, oligosaccharide extension, and stereochemistry). The acrosome reaction is a quite rare example of cell signaling mechanism triggered

and controlled exclusively by structural features of internal oligomeric motifs in polysaccharides.

Among all high MW sulfated polysaccharides described so far which possess signaling functions in glycobiology like GAGs, the SF and SG molecules are even more unique in terms of regularity of structures [19]. The sulfation pattern of MSP acts as an efficient molecular coding key in sea urchin fertilization. The well-defined structural characteristics of MSP make the structural assignments easier and allow proper correlation of specific functions with structures in the establishment of structure-function relationships of carbohydrates, especially in cases of developing carbohydrate-based therapeutic agents (drug development) [3]. These structural aspects are rare and found essentially in these novel marine glycans (Table 1). This uniqueness would give to both SF and SG molecules a differential status among all signaling polymeric carbohydrates. This places fucanomics and galactanomics as quite distinct subprojects as compared to other "glycan-omics". These hidden glycomics treasures from the sea waters ought to become thus more noticeable among the glycoscientific community. The incorporation of SF and SG molecules as part of glycomics not only gives them an enhanced view in the community to adequately expand the related research, but also enhances credibility to the big glycome project itself. Therefore, the current assumption of Fucanome and Galactanome as new glycomics subprojects is well-justified.

Future Approaches for Fucanome and Galactanome

Because these marine glycans are relatively new in the literature, since only the mid 90's, many tasks have yet to be accomplished in fucanome and galactanome subprojects. Perhaps the first one would be the depositing of structures in an informational and universal public carbohydrate data bank, for which the international community would be able to access openly. The deposition of structures related to the species name and phyla would end up in a valuable library of sugars shared among researchers worldwide.

The second task would be the focused-research on the biosynthetic pathways of SF and SG molecules. As opposed to GAGs in which all steps are well-known at such a level that allows perfect manipulation of the biosynthetic enzymes for different purposes [29,30], the biosynthesis pathways of MSP are virtually unknown. Information about sea urchin (sulfo)transferases and/or nucleotide-sugars as precursors for the SF and SG biosynthesis have to our knowledge not yet been described. The abiotic and biotic influences in their metabolism are apparently an additional unknown. Environmental influences on biosynthesis of MSP represent also a contributing line.

The third task would be attempting to establish some phylogenetic correlation for these MSP. It's clear that only linear polymers occur for echinoderms where echinoidea (sea urchin) structures are limited to a very short set of structural characteristics: only 2-O- and/or 4-Osulfation occurring together with few types of glycosidic bonds (either $\alpha(1\rightarrow3)$, or $\alpha(1\rightarrow4)$, or $\beta(1\rightarrow3)$), as reported [6,7]. This observation raises hypothesis of a possible short number of sulfotransferases in the sea urchin biosynthetic systems, as opposed to a bigger number of biosynthetic enzymes in mammals, as known in GAG biosynthesis [28-30]. In a previous report, we have established a few phylogenetic parameters for SG molecules [6,7]. We noticed that chains of 3-linked β -galactoses are heavily conserved throughout the marine taxonomic groups (among red and green algae, sea angiosperm, clams, sea urchins, and tunicates) with a strong tendency toward 4-sulfation in algae and marine angiosperm, 2-sulfation in invertebrates, and 6-sulfation in various organisms. This indicates that galactosyltransferases seem preserved through the marine species, but sulfotransferases are more

specific. Although 4- and 2- sulfotransferases are hypothetically considered preserved in plants and in invertebrates, respectively, 6-sulfotransferases are broadly dispersed along the marine organisms. This phylogenetic study is still quite preliminary and no correlation with SF structures has been done so far. It is worth to mention that this beginning and speculative phylogenetic view was made just based on structural characteristics of these glycans and thus must be carefully adopted, since sugars are well-known to be products from a non-template driven biosynthetic system. Therefore the observation of structural features of MSP cannot be taken as the only tools to make a phylogenetic correlation since gene expression, transcription levels as amounts of glycan-involved biosynthetic enzymes were not monitored. This phylogenetic task is thus intrinsically dependent on the second task about deciphering the biosynthetic systems concerning the se relatively new marine sulfated glycans.

Another task would be the advanced studies concerning the spatial geometry (3D structural view) of SF and SG molecules as well as their dynamic properties (like general, internal or localized motions). For this, SF- or SG-derived oligosaccharides become ultimately fundamental workpieces, and we have established already an efficient protocol for such production of low-MW derivatives that still retain well-defined molecular structures and biological properties [42,43]. Future experiments using mainly nuclear magnetic resonance spectroscopy (relaxation rates, NOE-based or residual dipolar coupling techniques) should be able to provide information regarding conformation and flexibility of these new glycans. These four proposed tasks are completely new avenues for research constituting a significant contribution not only in fucanome and galactanome but also for the entire glycobiology.

Concluding Remarks

Through this document we made clear the importance in adopting the subdivision of fucanome and galactanome in glycomics. This subdivision would make the MSP-related research line more well-known and promising inside glycobiology. In definition, fucanome and galactanome would comprise subprojects in terms of describing the specific marine glycans composed mostly of sulfated fucosyl and galactosyl units. These glycans have many biological functions either in the naturally occurring marine organisms, such as plastic components of the cell wall in algae, in assembling the body wall in sea cucumbers and ascidians, as osmotic regulators in sea plants, and acting as controller of the sea-urchin acrosome reaction for their species-specific fertilization, or in mammals with alternative pharmacological effects, thus representing valuable examples of carbohydrate-based therapeutic agents in the science of drug development. Their pharmacological actions participate in many systems like coagulation, thrombosis, inflammation, virus and pathogen infections, tumor growth and spreading, vessel formation, among others. The significance of these therapeutic applications in fucanome and galactanome is highly relevant in order to improve glycomics impact as scientific project as well as to give proper recognition of the large spectrum of functions of the marine glycans. The rare structural features of some SF and SG molecules (composition of well-defined and regular repeating oligomeric units) push fucanome and galactanome as unique glycomics subprojects due to the ease of correlating their functions with their structural characteristics. The establishment of this particular structure-function relationship has been harder for other native polysaccharides in glycobiology but more feasible to the marine ones due to the regularity.

Herein, it has become clear also that even though the division

of glycomics into subprojects is really demanding and necessary, the complexity of studying such subdivisions still remains high. High chances are to future include agaranome and carrageenome subprojects inside our proposed galactanome subproject. This would increase the complexity of galactanome itself. The initial thoughts that subdivision of glycomics would significantly reduce the complexity of the system have proved to be not completely true. The glycomics' view turned out to be more a collection of segments than a single scientific project. In analogy to what was previously mentioned: "The Sialome – far more than the sum of its parts" [5], here we reiterate: "The Glycome – bigger than the division of its total". Even though glycoproteomics, peptidoglycanomics, and even proteoglycanomics can be considered intersections between glycomics and proteomics, glycomics seems a much bigger project than firstly realized or created, which certainly justifies the future creation of its own journals suggestively named as Glycomics, Journal of Glycomics, or the Journal of Glycome Research in analogy to the already founded Proteomics, Journal of Proteomics, and Journal of Proteome Research. Others journals regarding glycomics subprojects such as glycosaminoglycanomics and proteoglycanomics would certainly be of interest too. The Journal of Glycobiology, and Journal of Glycomics and Lipidomics, both from OMICS Publishing Group, have already started to significantly contribute toward this development. Hopefully in the near future, other publishing groups start to make their efforts as well.

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Conflict of Interest

The author declares no conflict of interest.

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