Fucanomics & Galactanomics: marine glycans with differential actions in coagulation and thrombosis

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September, 2013
INTRODUCTION: “OMICS” studies and “OME” projects

OMICS - English-language neologism that refers to a field of study in biology.

The related suffix-OME is usually used to address the objects of study of such fields, referring thus to international research projects.

- Genomics (if not the first, one of the first projects to be launched into biology: Human Genome Project at mid-’80s, sheep Dolly from Cloning project at mid-’90s):
  - Pharmacogenomics / - Toxicogenomics / - Psychogenomics /
  - Stem cell genomics / - Epigenomics

- Transcriptomics

- Proteomics:
  - Immunoproteomics / - Nutriproteomics / - Proteogenomics /
  - Structural Genomics / - Pharmacoproteomics

- Metabolomics

- Metabonomics

- Pharmacomicobiomics

- Lipidomics

- Glycomics (one of the latest projects to be launched into biology, 21st century, very challenging)
GLYCOMICS: overview

CHALLENGES: 1) Structural characterization is difficult. Structural diversity and variety are both high, probably higher than any other biomacromolecule. Experimentation and data interpretation are laborious. In addition, previous technology was very limited.

2) Multiple biological functions. For a long time, carbohydrates were considered just a mere class of energetic and structural molecules. Biological roles have been discovered and reported just recently. Innumerous functions have been reported.

Glycomics turned out to be a very extensive project. This is leading to a tendency of subdivision or sectorization to allow the natural flow of the project development. In fact, subprojects, and new terminologies using the suffix-OME are emerging:

| Glycome          | - Glycolipidome                      | - Cerebrosides |
|                 |                                     | - Globosides   |
|                 |                                     | - Gangliosides |
|                 | - Glycoproteome                      | - Sialome (e.g. sialylated gangliosides) |
|                 | - N-linked glycans                   | - Mannomics (e.g. high mannose) |
|                 | - O-Linked glycans                   | - Sialome (e.g. E-selectin Sialyl Lewis*) |
| - Glycosaminoglycanome | - Glycosaminoglycanome | - Heparanome (heparan sulfate) |
|                 | - Galactosaminoglycanome             | - Heparinome (heparin) |
| - Proteoglycanome |                                     | - Dermatanome (dermatan sulfate) |
|                 | (metabolomics related to glycobiology) | - Chondroitinome (chondroitin sulfate) |
| - Glycometabolome | - Peptidoglycanome                   | (bacterial wall heteropolysaccharides) |
| - Galactanome (galactans) | - agaranome (agaran)               | |
| - Fucanome (fucans) | - carrageenome (carrageenan)         | |
|                 | Fungal polysaccharides               | glucuronoxylomannome (e.g. glucuronoxylomannan) |

Table 1: Summarized list of the main glycomics subprojects already in use (bold fonts), about to be used (italic fonts) and possible to be used in the near future (regular fonts).

FUCANOMICS & GALACTANOMICS: overview

➢ **Sulfated fucans (SFs):** marine polysaccharides composed predominantly or essentially of α-L-fucopyranosyl units. Usually highly sulfated. Example: Fucoidan

**Occurrence:**
- cell wall of brown macroalgae
- egg jelly coat of sea urchins
- body wall of sea cucumber

**Function:**
- structural component of the extracellular matrices of the above-mentioned tissues
- triggers the acrosome reaction in sea urchins through a specie-specific way

➢ **Sulfated galactans (SGs):** marine polysaccharides composed essentially of α-L-, α-D-, β-D-galactopyranoses. Usually highly sulfated. Examples: Carrageenans and Agarans

**Occurrence:**
- cell wall of green/red macroalgae
- egg jelly coat of sea urchins
- body wall of ascidians

**Function:**
- structural component of the extracellular matrices of the above-mentioned tissues
- triggers the acrosome reaction in sea urchins through a specie-specific way

Laboratories Worldwide: Brazil (CE, RN, RJ, SP, RS), Argentina, USA, Russia, China, Italy, France and Japan.
FUCANOMICS & GALACTANOMICS: Chemistry vs Phylogeny

Heterogeneous and branched structures of brown algal SFs

A. *F. vesiculosus*  
\[
[2-\alpha-L-Fucp-1]_n \\
\text{proposed by Percival and Ross, 1950.}
\]

B. *F. vesiculosus*  
\[
[3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3]_n \\
\alpha-L-Fucp-1 \quad \text{proposed by Patankar et al. 1993.}
\]

C. *A. nodosum*, *F. evanescens*, *F. vesiculosus*  
\[
[4-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1]_n \\
\text{Current proposition + branches of non sulfated -\alpha-L-Fucp.}
\]

D. *E. kurome*  
\[
[3-\alpha-L-Fucp-1]_n \\
\text{R = H or SO}_3^- \\
\text{R = SO}_3^-
\]

E. *C. filum*  
\[
R^1 = H \text{ or SO}_3^- \text{ or COCH}_3 \\
\text{diR}^2 \\
\text{R}^1 \\
\text{R}^2 \\
[3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3]_n
\]

Pomin V H, and Mourão P A S Glycobiology 2008;18:1016-1027
FUCANOMICS & GALACTANOMICS: Chemistry vs Phylogeny

Regular, homogeneous and well-defined structures of invertebrate SFs

Structures of the repeating units of the α-L-SFs from the cell wall of the sea cucumber (A) and from the egg jelly coats of sea urchins (B-H)

A. L. grisea

\[ \begin{align*}
  & [3-\alpha-L-\text{Fucp}-1 \rightarrow 3-\alpha-L-\text{Fucp}-1 \rightarrow 3-\alpha-L-\text{Fucp}-1 \rightarrow 3-\alpha-L-\text{Fucp}-1]_n \\
  & \quad \text{2} \quad \text{2} \\
  & \quad \text{diSO}_3^- \\
  & \quad \text{SO}_3^- \\
\end{align*} \]

D. A. lixula

\[ \begin{align*}
  & [4-\alpha-L-\text{Fucp}-1 \rightarrow 4-\alpha-L-\text{Fucp}-1 \rightarrow 4-\alpha-L-\text{Fucp}-1 \rightarrow 4-\alpha-L-\text{Fucp}-1]_n \\
  & \quad \text{2} \quad \text{2} \\
  & \quad \text{SO}_3^- \\
  & \quad \text{SO}_3^- \\
\end{align*} \]

B. L. variegatus

\[ \begin{align*}
  & [3-\alpha-L-\text{Fucp}-1 \rightarrow 3-\alpha-L-\text{Fucp}-1 \rightarrow 3-\alpha-L-\text{Fucp}-1 \rightarrow 3-\alpha-L-\text{Fucp}-1]_n \\
  & \quad \text{2} \quad \text{2} \quad \text{2} \quad \text{4} \\
  & \quad \text{diSO}_3^- \quad \text{SO}_3^- \quad \text{SO}_3^- \\
\end{align*} \]

E. S. purpuratus I

\[ \begin{align*}
  & [3-\alpha-L-\text{Fucp}-1]_n \\
  & \quad \text{2} \quad \text{4} \\
  & \quad \text{SO}_3^- \quad \text{~80% SO}_3^- \\
\end{align*} \]

F. S. purpuratus II

\[ \begin{align*}
  & [3-\alpha-L-\text{Fucp}-1 \rightarrow 3-\alpha-L-\text{Fucp}-1 \rightarrow 3-\alpha-L-\text{Fucp}-1]_n \\
  & \quad \text{2} \quad \text{4} \quad \text{4} \\
  & \quad \text{SO}_3^- \quad \text{SO}_3^- \\
  & \quad \text{diSO}_3^- \\
\end{align*} \]

C. S. pallidus

\[ \begin{align*}
  & [3-\alpha-L-\text{Fucp}-1 \rightarrow 3-\alpha-L-\text{Fucp}-1 \rightarrow 3-\alpha-L-\text{Fucp}-1 \rightarrow 3-\alpha-L-\text{Fucp}-1]_n \\
  & \quad \text{4} \quad \text{4} \quad \text{2} \quad \text{2} \\
  & \quad \text{SO}_3^- \quad \text{SO}_3^- \quad \text{SO}_3^- \\
\end{align*} \]

G. S. droebachiensis

\[ \begin{align*}
  & [4-\alpha-L-\text{Fucp}-1]_n \\
  & \quad \text{2} \quad \text{2} \\
  & \quad \text{SO}_3^- \\
\end{align*} \]

H. S. franciscanus

\[ \begin{align*}
  & [3-\alpha-L-\text{Fucp}-1]_n \\
  & \quad \text{2} \\
  & \quad \text{SO}_3^- \\
\end{align*} \]

Pomin V H, and Mourão P A S Glycobiology 2008;18:1016-1027
FUCANOMICS & GALACTANOMICS: Chemistry vs Phylogeny

Algal and invertebrate structures of SGs: heterogeneity vs regularity

Structures of the SGs from (A) red algae, (B) green algae, (C) sea grass (marine angiosperm), and marine invertebrates, such as (D) ascidians (also known as tunicates), and (E) sea urchins.

Pomin V H, and Mourão P A S Glycobiology 2008;18:1016-1027

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FUCANOMICS & GALACTANOMICS: Medical actions

Therapeutic actions in diverse systems: inflammation; hemostasis and vascular biology; angiogenesis; tumor growth, progression and spreading; oxidative stress; infections; and nociception.

MOLECULAR INTERACTIONS IN FUCANOMICS AND GALACTANOMICS: DRUG DEVELOPMENT IN DIFFERENT CLINICAL AREAS

Sulfated fucans & Sulfated galactans

Inflammation

P- and L-selectins

Vascular endothelial growth factor (VEGF)

VEGF receptor

Tumor angiogenesis

Fibroblast Growth Factor (FGF2)

Factors IIa and Xa

Serpins: antithrombin and heparin cofactor II

Coagulation and Thrombosis

Chemokines

FUCANOMICS & GALACTANOMICS: Medical actions

Cellular mechanisms in inflammation

FUCANOMICS & GALACTANOMICS: Medical actions

Anti-inflammatory mechanisms of actions of SFs and SGs

**FUCANOMICS & GALACTANOMICS: Medical actions**

*Anti-angiogenic and antitumoral mechanisms of actions of SFs and SGs*

FUCANOMICS & GALACTANOMICS: Medical actions

Molecular and cell mechanisms in coagulation/thrombosis
FUCANOMICS & GALACTANOMICS: Medical actions

Molecular mechanisms of actions of SFs and SGs in anticoagulation/antithrombosis

FUCANOMICS & GALACTANOMICS: Medical actions

Molecular mechanisms of actions of SFs and SGs in anticoagulation/antithrombosis
FUCANOMICS & GALACTANOMICS: Chemistry

Regular structures enable accurate and advanced structure-function relationships

FUCANOMICS & GALACTANOMICS: Medical Actions

Regular structures enable accurate and advanced structure-function relationships

Table I. Anticoagulant Activities of Marine Invertebrate and Algal Sulfated Fucans and Sulfated Galactans Measured by APTT and by IC₅₀ for Thrombin (IIa) and Factor Xa Inhibition in the Presence of Antithrombin (AT) or Heparin Cofactor II (HCII)

<table>
<thead>
<tr>
<th>Polysaccharide</th>
<th>Source</th>
<th>Structure (Figure)</th>
<th>APTT (IU mg⁻¹)</th>
<th>IC₅₀ (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Linked sulfated α-L-fucans</td>
<td>S. purpuratus (I)</td>
<td>1E-I</td>
<td>76</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>S. purpuratus (II)</td>
<td>1E-II</td>
<td>10</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>S. pallidus</td>
<td>1C</td>
<td>18</td>
<td>&gt;500</td>
</tr>
<tr>
<td></td>
<td>L. variegatus</td>
<td>1B</td>
<td>3</td>
<td>&gt;500</td>
</tr>
<tr>
<td></td>
<td>S. franciscanus</td>
<td>1G</td>
<td>~2</td>
<td>&gt;500</td>
</tr>
<tr>
<td></td>
<td>L. grisea</td>
<td>1A</td>
<td>&lt;1</td>
<td>&gt;500</td>
</tr>
<tr>
<td>4-Linked sulfated α-L-fucans</td>
<td>S. droebachiensis</td>
<td>1F</td>
<td>&lt;1</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>A. lixula</td>
<td>1D</td>
<td>~2</td>
<td>150</td>
</tr>
<tr>
<td>Sulfated α-L-galactans</td>
<td>E. lucunter</td>
<td>2A</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>H. monus</td>
<td>2C</td>
<td>~2</td>
<td>&gt;500</td>
</tr>
<tr>
<td></td>
<td>S. plicata</td>
<td>2B</td>
<td>&lt;1</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Algal sulfated galactans</td>
<td>B. occidentalis</td>
<td>2D</td>
<td>93</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>G. crinale</td>
<td>65</td>
<td>0.02</td>
<td>25</td>
</tr>
</tbody>
</table>

a The activity is expressed as international U mg⁻¹ using a parallel standard curve based on the International Heparin Standard (193 U mg⁻¹).

FUCANOMICS & GALACTANOMICS: STRUCTURE vs FUNCTION

Advanced structure-function relationships: SUGAR TYPE-DEPENDENT ANTICOAGULANT ACTION

(i) *E. lucunter*

![Chemical Structure of E. lucunter](image1)

(h) *S. franciscanus*

![Chemical Structure of S. franciscanus](image2)

## FUCANOMICS & GALACTANOMICS: STRUCTURE vs FUNCTION

### Advanced structure-function relationships: SUGAR TYPE-DEPENDENT ANTICOAGULANT ACTION

Table I. Anticoagulant Activities of Marine Invertebrate and Algal Sulfated Fucans and Sulfated Galactans Measured by APTT and by IC\textsubscript{50} for Thrombin (IIa) and Factor Xa Inhibition in the Presence of Antithrombin (AT) or Heparin Cofactor II (HCII)\textsuperscript{21, 48}

<table>
<thead>
<tr>
<th>Polysaccharide</th>
<th>Source</th>
<th>Structure (Figure)</th>
<th>APTT (IU mg\textsuperscript{-1})</th>
<th>IC\textsubscript{50} (μg ml\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>Ila/AT  Ila/HCI  Xa/AT</td>
</tr>
<tr>
<td>3-Linked sulfated α-L-fucans</td>
<td>S. purpuratus</td>
<td>1E-I</td>
<td>76</td>
<td>0.3 0.3 2</td>
</tr>
<tr>
<td></td>
<td>(I)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. purpuratus</td>
<td>1E-II</td>
<td>10</td>
<td>0.9 2 ND</td>
</tr>
<tr>
<td></td>
<td>(II)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. pallidus</td>
<td>1C</td>
<td>18</td>
<td>&gt;500 &gt;500 &gt;500</td>
</tr>
<tr>
<td></td>
<td>L. variegatus</td>
<td>1B</td>
<td>3</td>
<td>&gt;500 &gt;500 &gt;500</td>
</tr>
<tr>
<td></td>
<td>S. franciscanus</td>
<td>1G</td>
<td>~2</td>
<td>&gt;500 &gt;500 250</td>
</tr>
<tr>
<td></td>
<td>L. grisea</td>
<td>1A</td>
<td>&lt;1</td>
<td>&gt;500 &gt;500 &gt;500</td>
</tr>
<tr>
<td>4-Linked sulfated α-L-fucans</td>
<td>S. droebachiensis</td>
<td>1F</td>
<td>&lt;1</td>
<td>ND ND ND</td>
</tr>
<tr>
<td></td>
<td>A. lixula</td>
<td>1D</td>
<td>~2</td>
<td>150 150 &gt;500</td>
</tr>
<tr>
<td>Sulfated α-L-galactans</td>
<td>E. lucunter</td>
<td>2A</td>
<td>20</td>
<td>3 6 20</td>
</tr>
<tr>
<td></td>
<td>H. monus</td>
<td>2C</td>
<td>~2</td>
<td>&gt;500 &gt;500 &gt;500</td>
</tr>
<tr>
<td></td>
<td>S. plicata</td>
<td>2B</td>
<td>&lt;1</td>
<td>&gt;500 &gt;500 &gt;500</td>
</tr>
<tr>
<td>Algal sulfated galactans\textsuperscript{15, 19}</td>
<td>B. occidentalis</td>
<td>2D</td>
<td>93</td>
<td>0.02 1.1 2.5</td>
</tr>
<tr>
<td></td>
<td>G. crinale</td>
<td>65</td>
<td></td>
<td>0.02 25 1.5</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The activity is expressed as international U mg\textsuperscript{-1} using a parallel standard curve based on the International Heparin Standard (193 U mg\textsuperscript{-1}).

Structures of the complexes between different SP (red, yellow) and AT (blue). (A) ternary complex between AT, thrombin (gold) and a heparin derivative (PDB ID 1TB6); (B) AT bonded to the synthetic pentasaccharide (PDB ID 1E03); (C) final structure from a 5 ns MD of AT complexed to a SF decasaccharide with pyranose rings; (D) final structure from a 5 ns MD of AT complexed to a SG decasaccharide with pyranose rings. For (B)–(D), two orientations of the complexes are presented.

**FUCANOMICS & GALACTANOMICS: STRUCTURE vs FUNCTION**

*Advanced structure-function relationships: SULFATION PATTERN-DEPENDENT ANTICOAGULANT ACTION*

\[
\begin{align*}
(m) \quad & B. \text{ occidentalis} \\
& (R_1 \text{ and } R_2 = \text{SO}_3^\cdot \text{ in } \pm66\% \text{ and } \pm33\%, \text{ respectively}) \\
(n) \quad & G. \text{ crinale} \\
& (R_1 \text{ and } R_2 = \text{SO}_3^\cdot \text{ in } \pm60\% \text{ and } \pm15\%, \text{ respectively})
\end{align*}
\]

**FUCANOMICS & GALACTANOMICS: STRUCTURE vs FUNCTION**

*Advanced structure-function relationships: SULFATION PATTERN-DEPENDENT ANTICOAGULANT ACTION*

Table I. Anticoagulant Activities of Marine Invertebrate and Algal Sulfated Fucans and Sulfated Galactans Measured by APTT and by IC\textsubscript{s0} for Thrombin (IIa) and Factor Xa Inhibition in the Presence of Antithrombin (AT) or Heparin Cofactor II (HCII)*21, 48*

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<td></td>
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<td>1E-II</td>
<td>10</td>
<td>0.9 2 ND</td>
</tr>
<tr>
<td></td>
<td><em>S. pallidus</em></td>
<td>1C</td>
<td>18</td>
<td>&gt;500 &gt;500 &gt;500</td>
</tr>
<tr>
<td></td>
<td><em>L. variegate</em></td>
<td>1B</td>
<td>3</td>
<td>&gt;500 &gt;500 &gt;500</td>
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<tr>
<td></td>
<td><em>A. lixula</em></td>
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<td>150 150 &gt;500</td>
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<tr>
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*The activity is expressed as international U mg\textsuperscript{-1} using a parallel standard curve based on the International Heparin Standard (193 U mg\textsuperscript{-1}).

Advanced structure-function relationships: SULFATION TYPE-DEPENDENT ANTICOAGULANT ACTION

FUCANOMICS & GALACTANOMICS: STRUCTURE vs FUNCTION

Advanced structure-function relationships: SULFATION TYPE-DEPENDENT ANTICOAGULANT ACTION

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<th>APTT (IU mg$^{-1}$)</th>
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<th>IIa/AT</th>
<th>IIa/HCII</th>
<th>Xa/AT</th>
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<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>L. variegatus</td>
<td>1B</td>
<td>3</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>S. franciscanus</td>
<td>1G</td>
<td>~2</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>250</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>L. grisea</td>
<td>1A</td>
<td>&lt;1</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>S. droebachiensis</td>
<td>1F</td>
<td>&lt;1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4-Linked sulfated α-L-fucans</td>
<td>S. droebachiensis</td>
<td>1F</td>
<td>&lt;1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sulfated α-L-galactans</td>
<td>A. lixula</td>
<td>1D</td>
<td>~2</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>&gt;500</td>
</tr>
<tr>
<td>E. lucunter</td>
<td>2A</td>
<td>20</td>
<td>3</td>
<td>6</td>
<td>6</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>H. monus</td>
<td>2C</td>
<td>~2</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>S. plicata</td>
<td>2B</td>
<td>&lt;1</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>Algal sulfated galactans</td>
<td>B. occidentalis</td>
<td>2D</td>
<td>93</td>
<td>0.02</td>
<td>1.1</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>G. crinale</td>
<td>65</td>
<td>0.02</td>
<td>25</td>
<td>25</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
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</table>

a The activity is expressed as international U mg$^{-1}$ using a parallel standard curve based on the International Heparin Standard (193 U mg$^{-1}$).

FUCANOMICS & GALACTANOMICS: 1ST CONCLUSIONS

- SFs and SGs are essentially found in marine organisms such as macroalgae, sea urchins and sea cucumbers, and ascidians.

- Unlike algal SFs and SGs, the invertebrate molecules have well-defined chemical structures which allow accurate and advanced structure-function relationships.

- Although SFs and SGs may exhibit many clinical activities, the mechanisms of actions are known just for inflammation, coagulation/thrombosis and tumoral angiogenesis.

- In inflammation, SGs and SFs can block chemokines, P- and L-selectins. In hemostasis, they block the factors IIa and Xa. In tumoral angiogenesis, they inhibit VEGF and bFGF.

- 2-sulfated 3-linked α-galactan is an anticoagulant polysaccharides, whereas 2-sulfated 3-linked α-fucan is not. Their conformational binding preference to serpins are different.

- 2,4-di-sulfation and maybe 4-sulfation in 3-linked α-SFs have shown a beneficial effect on the anticoagulation, whereas 2-sulfation alone is certainly deleterious. Ongoing NMR relaxation studies will facilitate further explanations.

- Our dataset strongly support the the fact that the clinical actions of SFs and SGs are not a mere consequence of sulfation degrees, but intimately correlated to specific features: anomeric configuration, monosaccharide type, sulfation positions or patterns, glycosylation position, molecular weights, and so forth.
FUCANOMICS & GALACTANOMICS: STRUCTURE DETERMINATION

NMR structure determination of a new red algal SG (Acanthophora muscoides)
FUCANOMICS & GALACTANOMICS: STRUCTURE PROPOSITION

Structures of SGs from Acanthophora muscoides and Botriocladia occidentalis

α-Galp units

β-Galp units

Unit A (≈ 32%)

Unit B (≈ 43%)

Unit C, R = H
Unit c, R = CH₃ (minor)

Unit D (≈ 22%)

Unit E (≈ 30%)

Unit F (≈ 22%)

Unit G (≈ 26%)

 VS

α-Galp units

β-Galp units

R₁ = H or SO³⁻
R₂ = H (≈ 33%) or SO³⁻ (≈ 66%)
R₃ = H (≈ 66%) or SO³⁻ (≈ 33%)
FUCANOMICS & GALACTANOMICS: MW DIFFERENCES

Structures of Acanthophora muscoides and Botriocladia occidentalis

A

Retention Time - min

Refractive Index

B

Origin

UFH - + B. occidentalis - A. muscoides - LMWH
SERPIN-DEPENDENT ANTICOAGULANT ACTIVITIES

- APTT in serpin-containing plasma
  - A

- AT/IIa
  - B

- AT/Xa
  - C

- HCII/IIa
  - D

Residual activity vs. µg mL⁻¹
FUCANOMICS & GALACTANOMICS: ANTITHROMBOSIS

ARTERIAL AND VENOUS MODELS, AND BLEEDING EFFECTS

Diagram showing:
A. Occlusion time (min) vs. Blood loss - µL
B. Thrombus weight (%) vs. Blood loss - µL
C. Comparison of thrombus weight (%) for different treatments (Control, Heparin, B. occidentalis, B. occidentalis, A. muscoides)

Graph legend:
- Square: A m
- Circle: UFH
- Blue square: B occ (LMW)
- Red triangle: B occ
FUCANOMICS & GALACTANOMICS: PROTHROMBOTIC EFFECTS

Activation of factor XII

![Graph showing the activation of factor XII with different concentrations of Heparin, B. occidentalis, and A. muscoides. The graph displays Vmax – mOD min⁻¹ on the y-axis and concentrations of 1 and 50 µg mL⁻¹ on the x-axis.](image-url)
Unlike the SG from *B. occidentalis*, the recently characterized SG from *A. muscoides* is structurally very complex and heterogeneous. Besides sulfation at different positions, anhydro sugars and methyl ethers are also observed;

The MW of *B. occidentalis* was reduced to allow a comparative study in terms of structure-function relationships in anticoagulation and antithrombosis with the natural low-MW of *A. muscoides*;

The native SG from *B. occidentalis* has high anticoagulant activity due to its significant activity on serpins (AT and HCII) and proteases (mostly thrombin). It also shows high serpin-independent anticoagulant activity. The low-MW form lost both activities. SG from *A. muscoides* has just no anticoagulant activity regardless the plasma type.

SG from *A. muscoides* is curiously a potent antithrombotic agent at the arterial model, whereas the one from *B. occidentalis* is not. Both red algal SGs are antithrombotic at the venous model, however, at only small doses. SG from *B. occidentalis* is prothrombotic at higher doses due to its capacity in activate factor XII.

As opposed to heparin which is highly hemorrhagic, neither one of the red algal SGs have shown this side effect.

Through this comparative study using red algal SGs, we have dissociated the anticoagulant (both serpin-dependent and serpin-independent), antithrombotic (both arterial and venous) and hemorrhagic effects, as compared with the standard heparin.
FUCANOMICS & GALACTANOMICS: ACKNOWLEDGEMENTS

- Prof. Paulo A.S Mourão
- Prof. Norma Maria Barros Benevides
- Prof. Ana Cristina Vilela-Silva
- Prof. Ana Paula Valente
- Prof. Fabio Ceneviva Almeida
- Stephan Nicollas de Oliveira
- Gustavo Ramalho dos Santos
- Bianca Fernandes Glauser
- Ismael Lino Nilo de Queiroz
- Bruno Pedrosa Fontes
- Ana Luiza Quinderé
- Eros Falcão
- Gabriel Baptista de Sá