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

Vitor H. Pomin

**Laboratório de Coagulação &
Trombose**

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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):**
  - **- Cognitive Genomics / - Comparative Genomics / - Functional Genomics / - Metagenomics / - Nutritional Genomics /**
  - **- Pharmacogenomics / - Toxicogenomics / - Psychogenomics /**
  - **- Stem cell genomics / - Epigenomics**
- **Transcriptomics**
- **Proteomics:**
  - **- Immunoproteomics / - Nutriproteomics / - Proteogenomics /**
  - **- Structural Genomics / - Pharmacoproteomics**
- **Metabolomics**
- **Metabonomics**
- **Pharmacomicrobiomics**
- **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	- **Sialome** (e.g. sialylated gangliosides)
	- **Glycoproteome**	- *N*-linked glycans	- Mannomics (e.g. high mannose)
		- *O*-Linked glycans	- **Sialome** (e.g. E-selectin Sialyl Lewis^x)
	- **Proteoglycanome**	- **Glycosaminoglycanome**	- **Heparanome** (heparan sulfate)
			- *Heparinome* (heparin)
		- *Galactosaminoglycanome*	- *Dermatanome* (dermatan sulfate)
			- *Chondroitinome* (chondroitin sulfate)
	- **Glycometabolome**	(metabolomics related to glycobiology)	
	- *Peptidoglycanome*	(bacterial wall heteropolysaccharides)	
	- *Galactanome* (galactans)	- agaranome (agaran)	
		- carrageenome (carrageenan)	
	- *Fucanome* (fucans)		
	- Fungal polysaccharides	- glucuronoxylomannome (e.g. glucuronoxylomannan)	

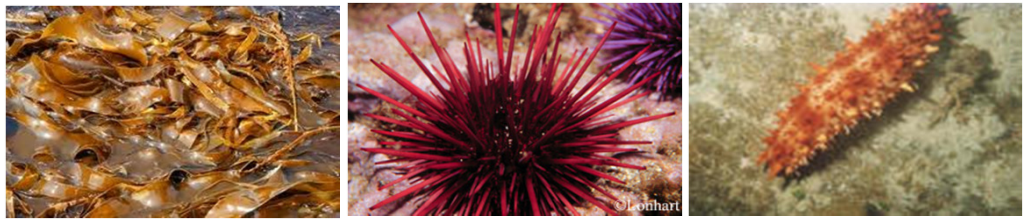
Table 1: Summarized list of the main glycomics subprojects already in use (bold fonts), about to be used (italic fonts) and possible to be used in the near future (regular fonts).

Citation: Pomin VH (2011) Current Glycomics´ Approaches: Subprojects and Journals. J Glycom Lipidom 1:102e. doi:10.4172/2153-0637.1000102e

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**

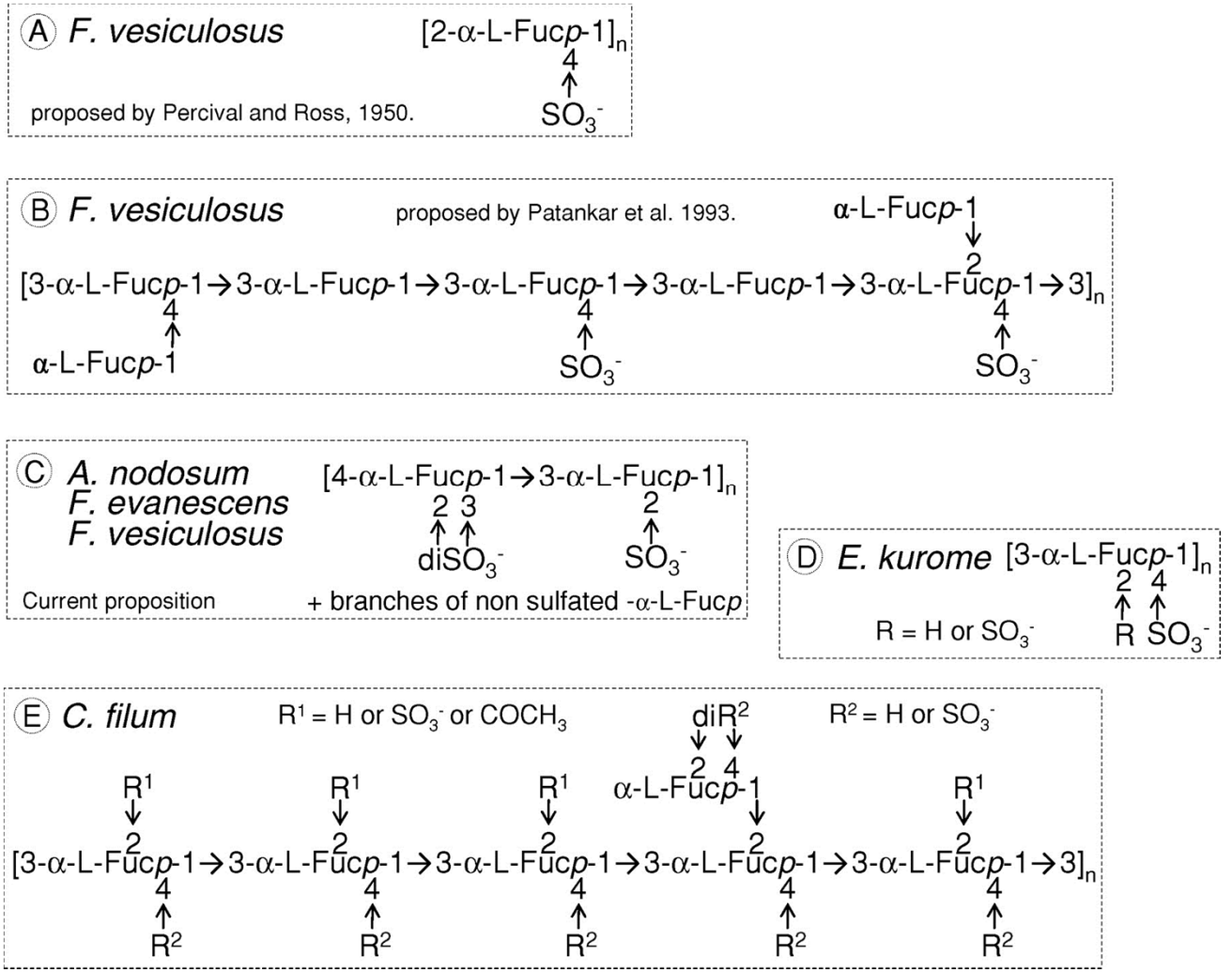
Function:

- **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



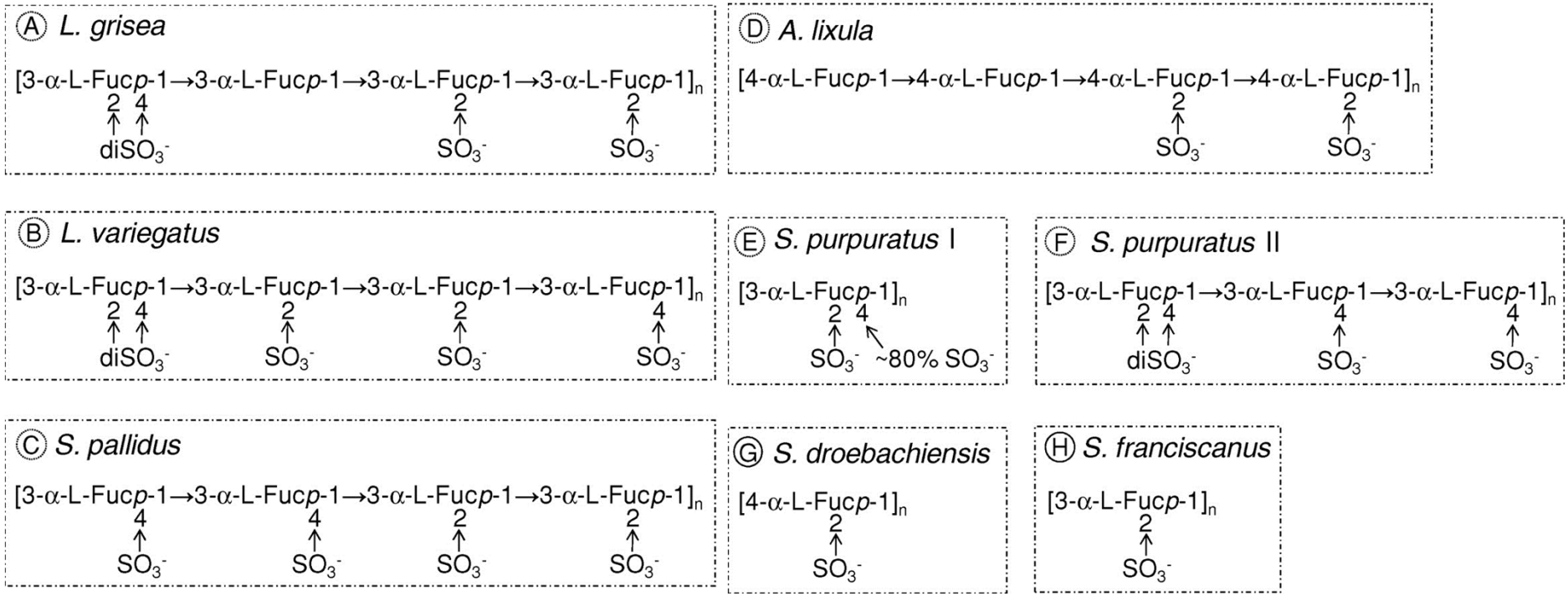
Pomin V H , and Mourão P A S Glycobiology 2008;18:1016-1027

GLYCOBIOLOGY

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)



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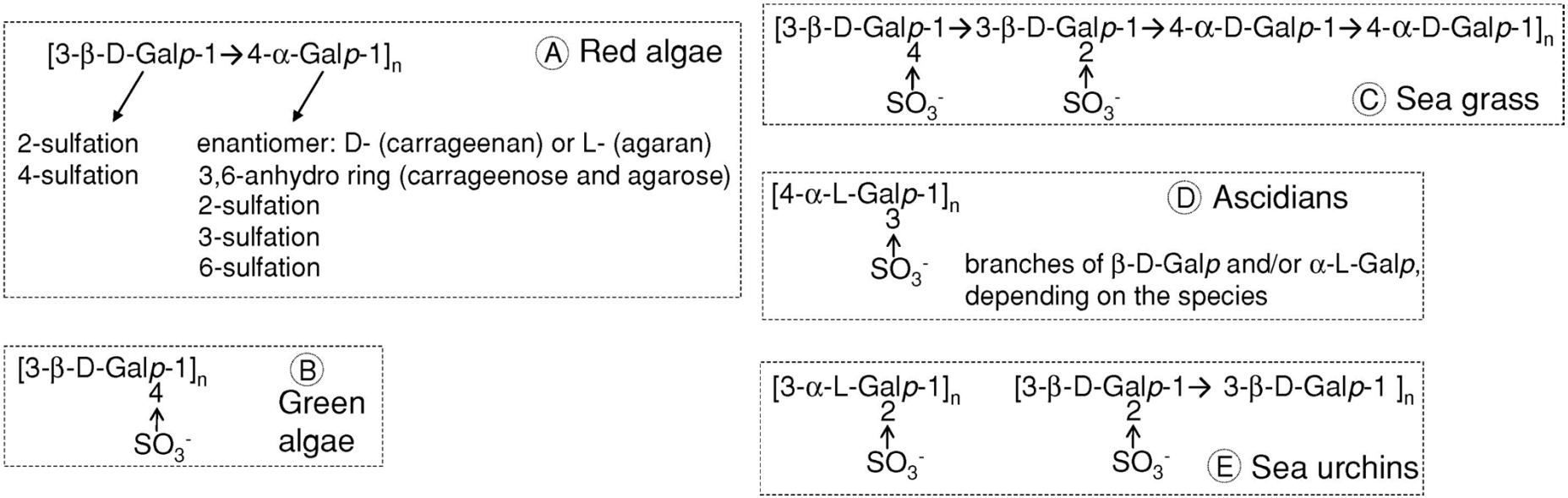
GLYCOBIOLOGY

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.



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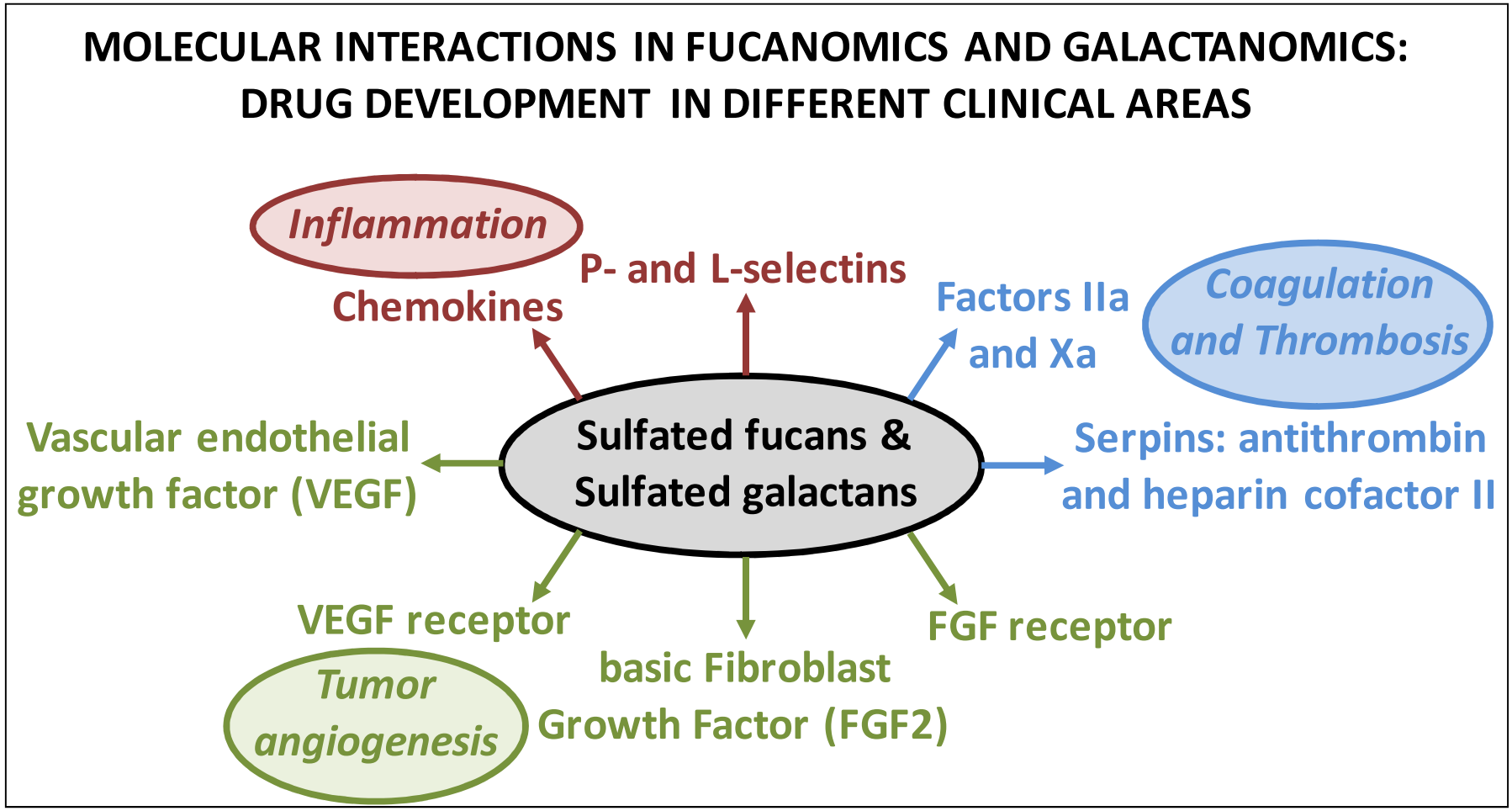
GLYCOBIOLOGY

FUCANOMICS & GALACTANOMICS: Medical actions

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



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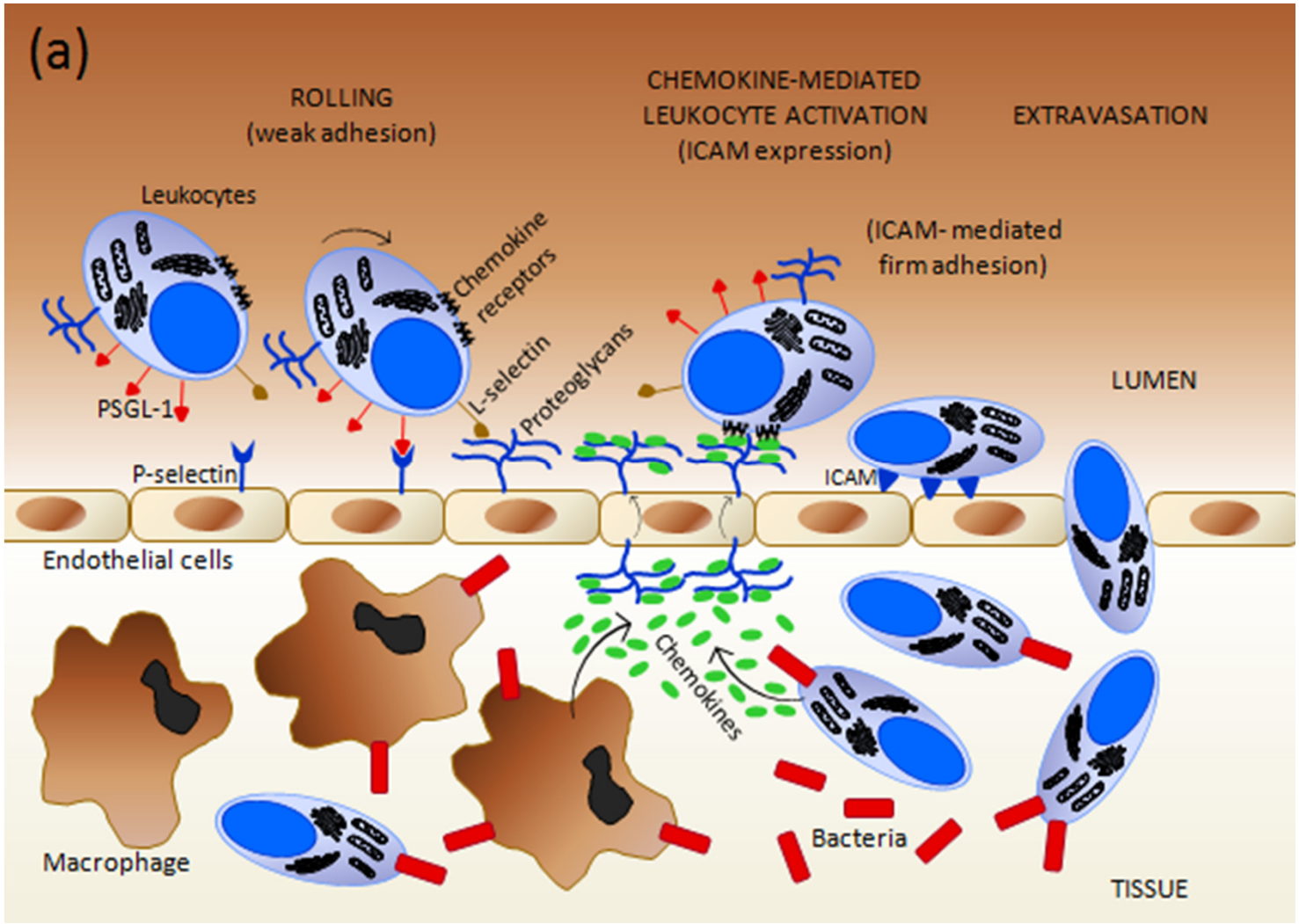
FUCANOMICS & GALACTANOMICS: Medical actions

Cellular mechanisms in inflammation



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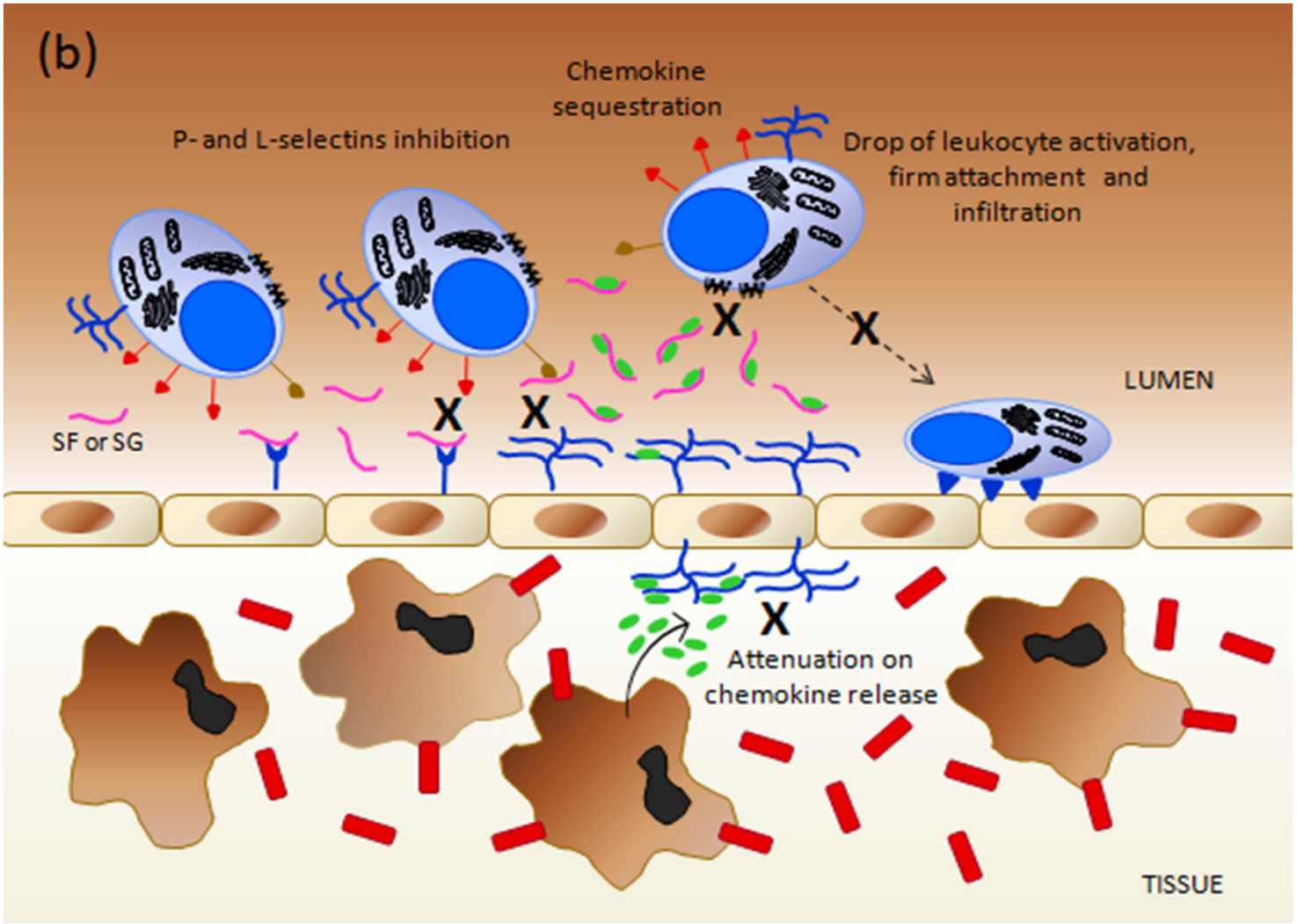
FUCANOMICS & GALACTANOMICS: Medical actions

Anti-inflammatory mechanisms of actions of SFs and SGs



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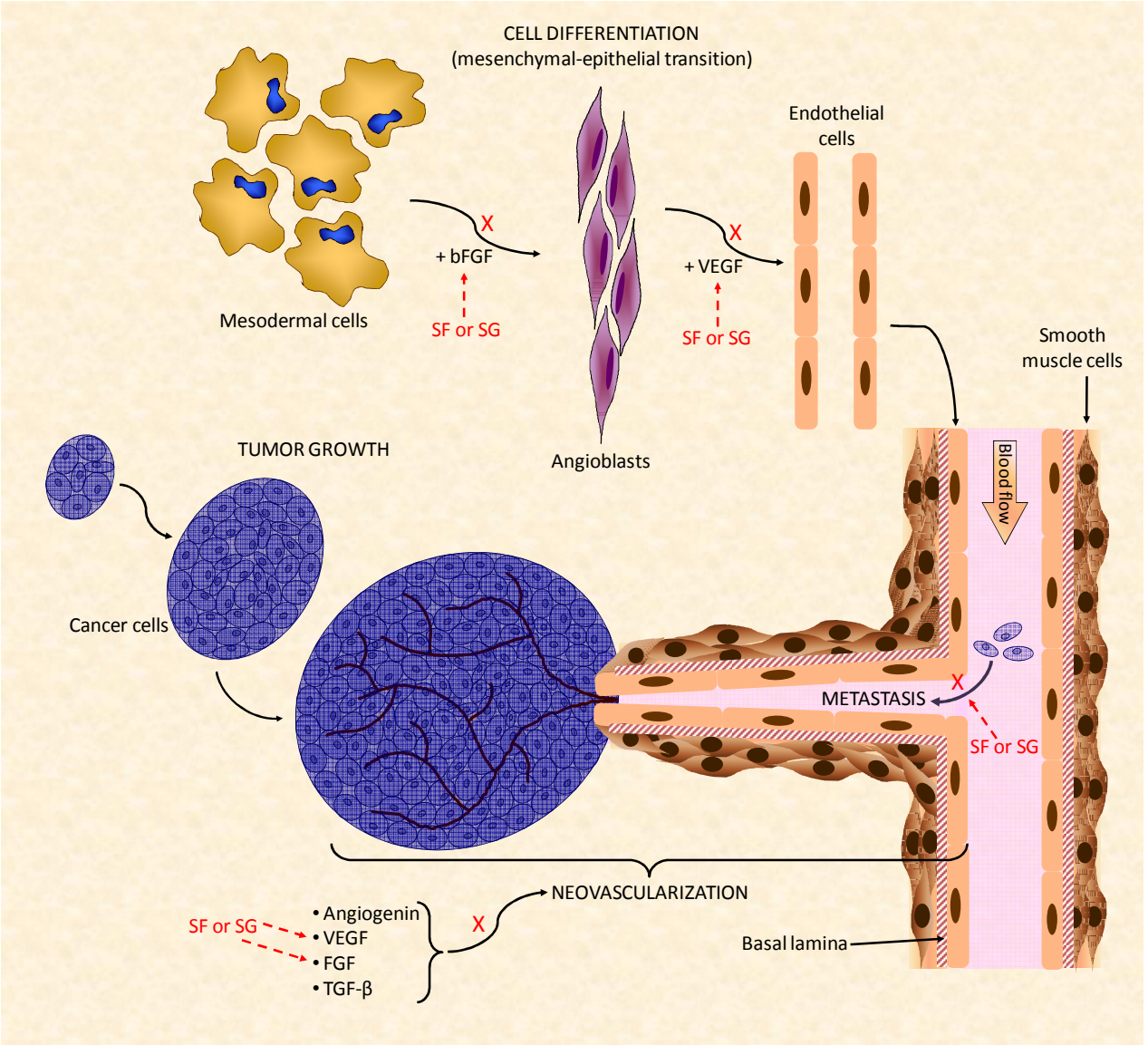
FUCANOMICS & GALACTANOMICS: Medical actions

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



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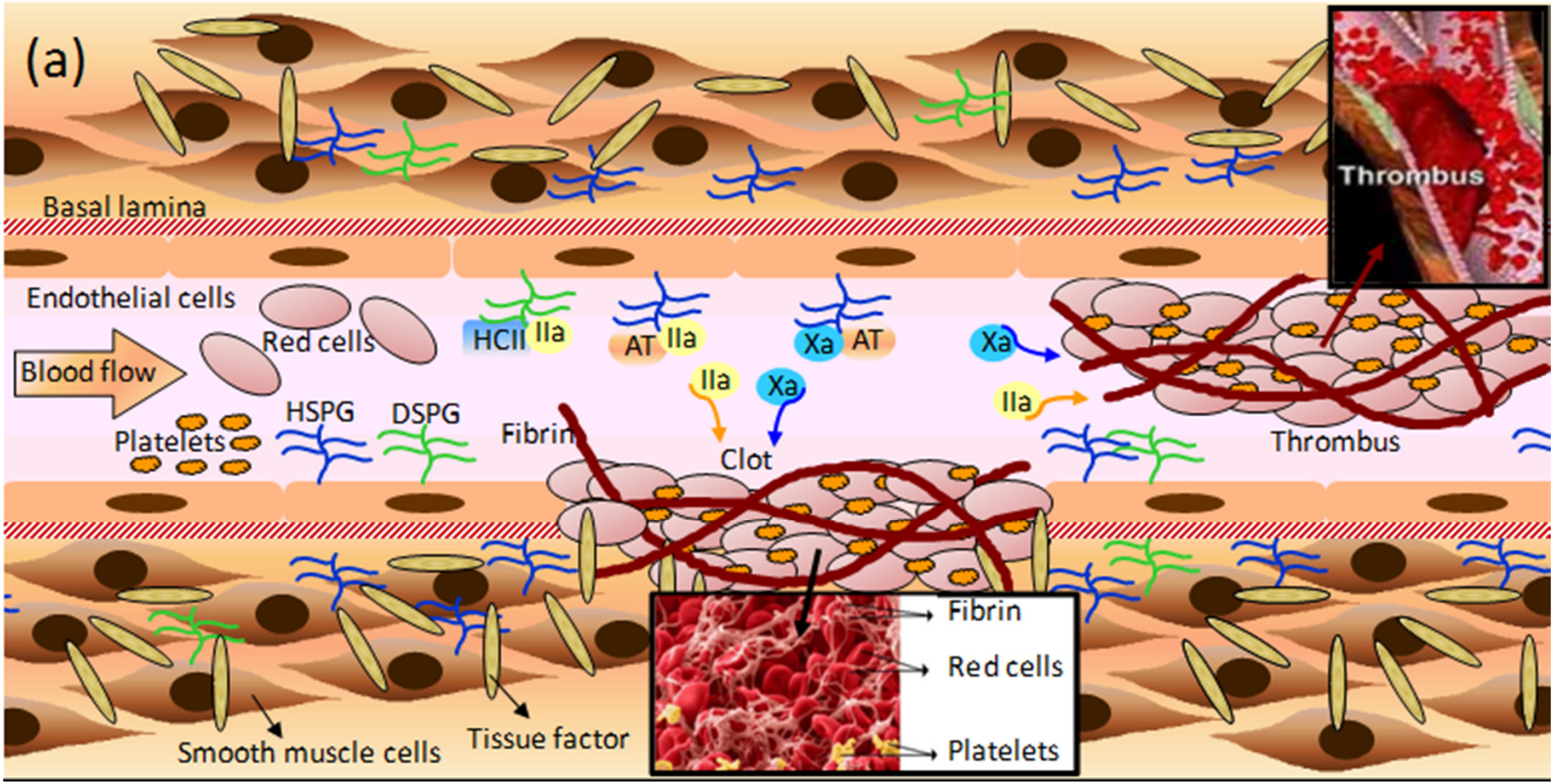
FUCANOMICS & GALACTANOMICS: Medical actions

Molecular and cell mechanisms in coagulation/thrombosis



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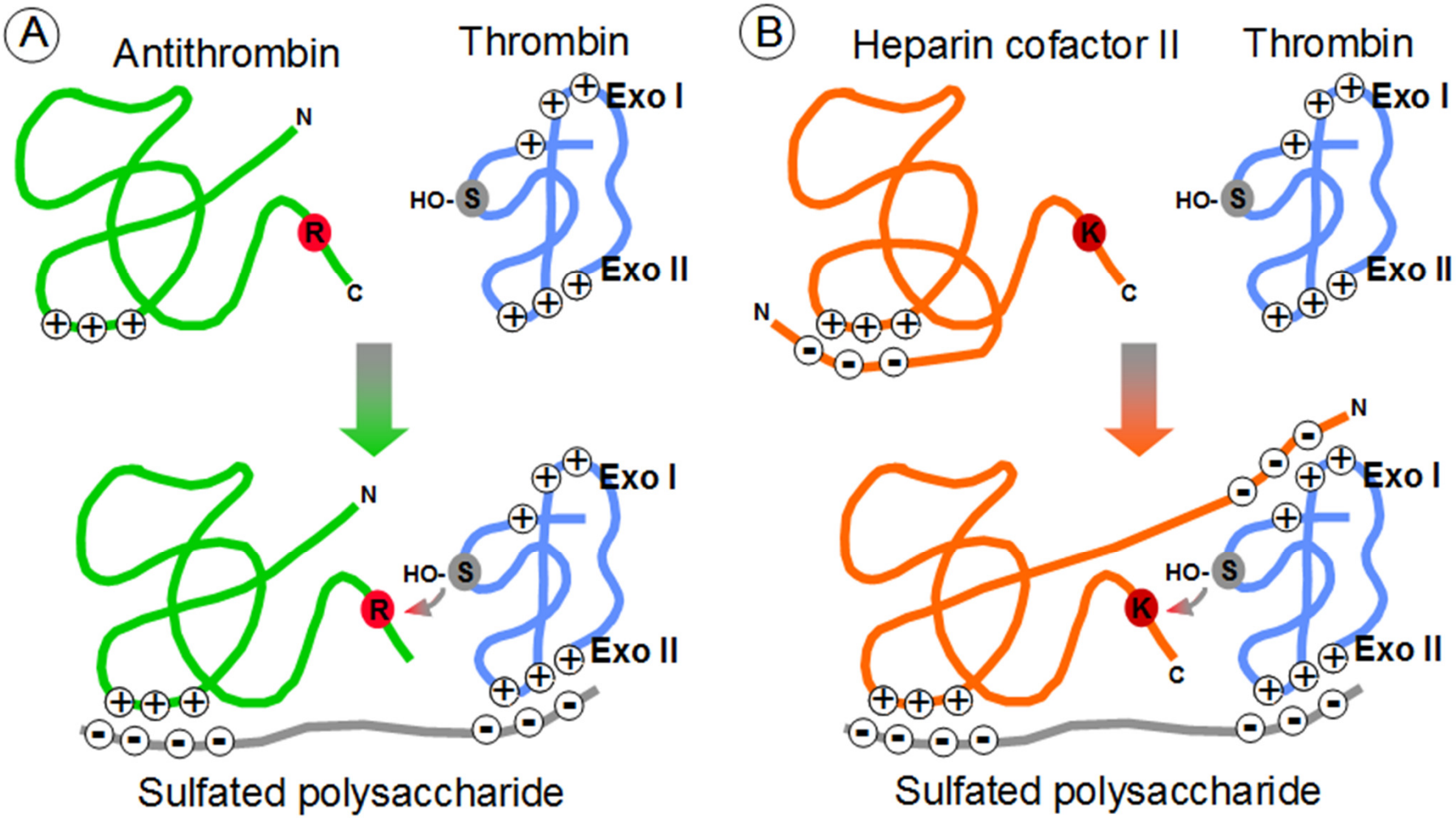
FUCANOMICS & GALACTANOMICS: Medical actions

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



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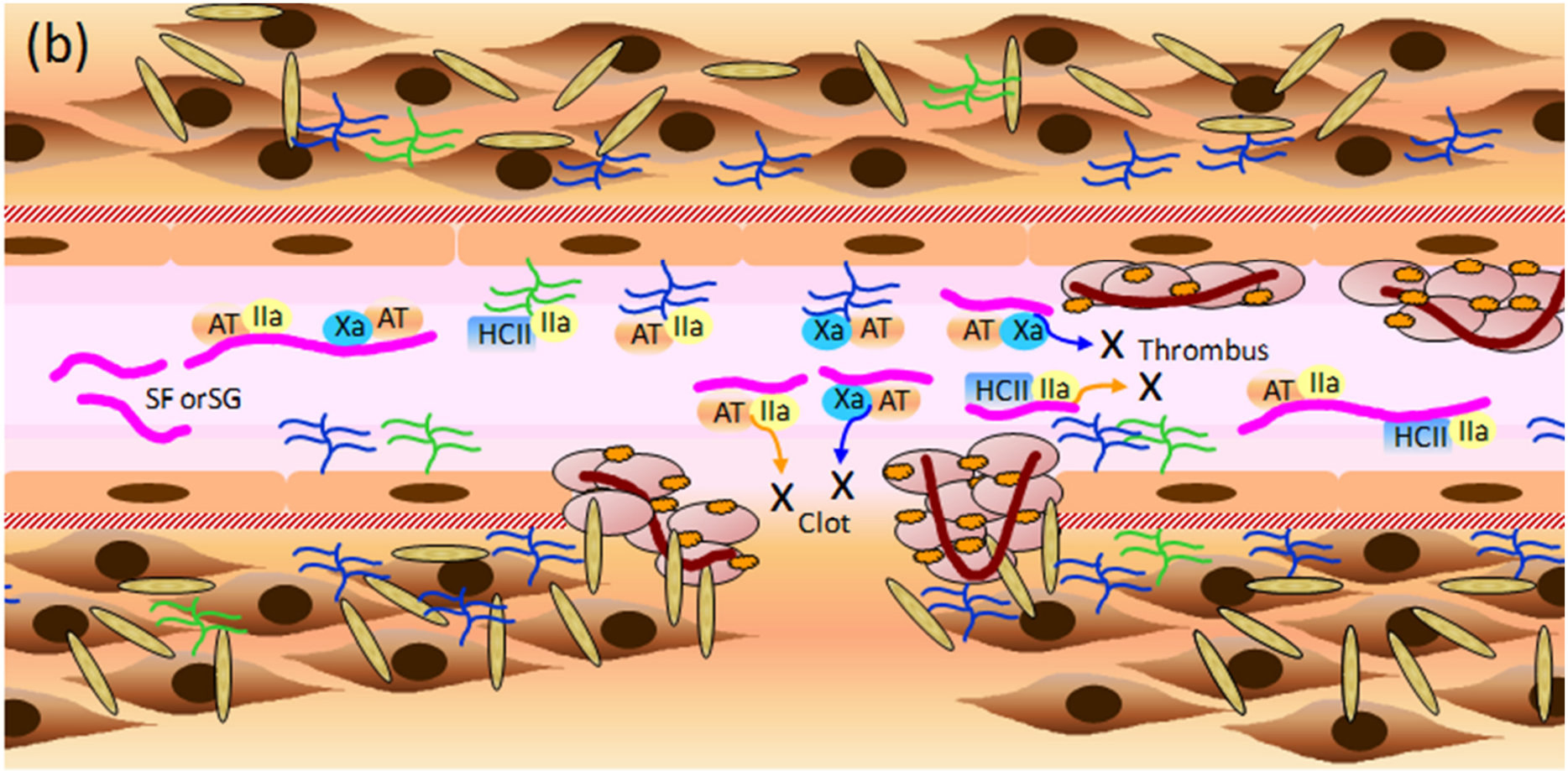

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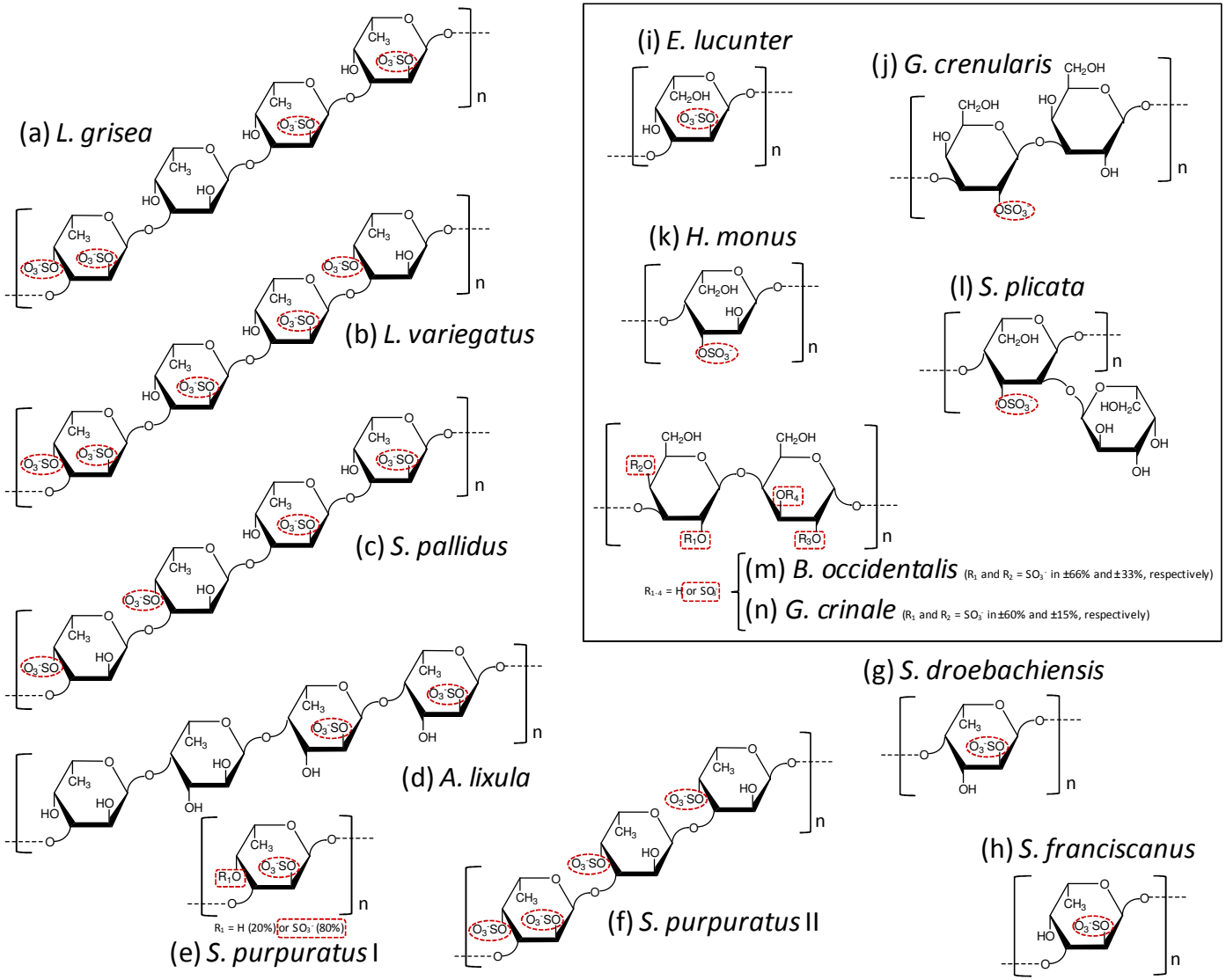
FUCANOMICS & GALACTANOMICS: Chemistry

Regular structures enable accurate and advanced structure-function relationships



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FUCANOMICS & GALACTANOMICS: Medical Actions

Regular structures enable accurate and advanced structure-function relationships

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	H. monus	2C	~2	>500	>500	>500
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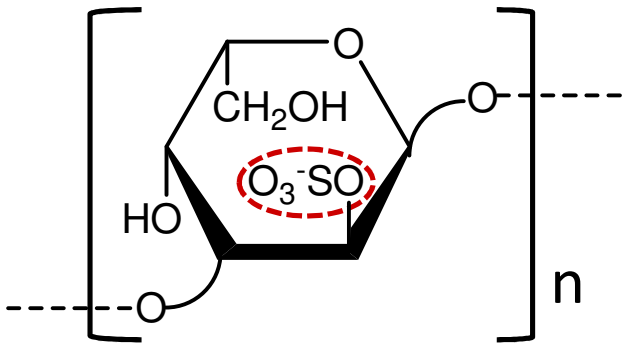
FUCANOMICS & GALACTANOMICS: STRUCTURE vs FUNCTION

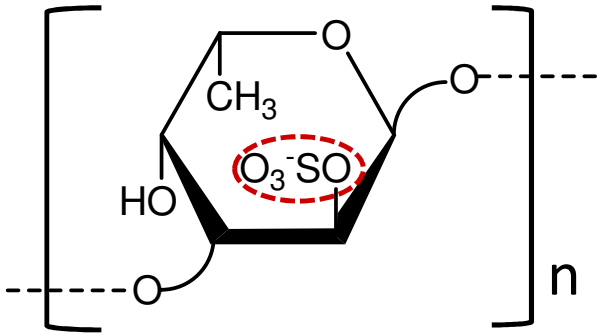
Advanced structure-function relationships: ***SUGAR TYPE-DEPENDENT ANTICOAGULANT ACTION***

(i) *E. lucunter*



VS

(h) *S. franciscanus*



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4-Linked sulfated α-L-fucans	*S. droebachiensis*	1F	<1	ND	ND	ND
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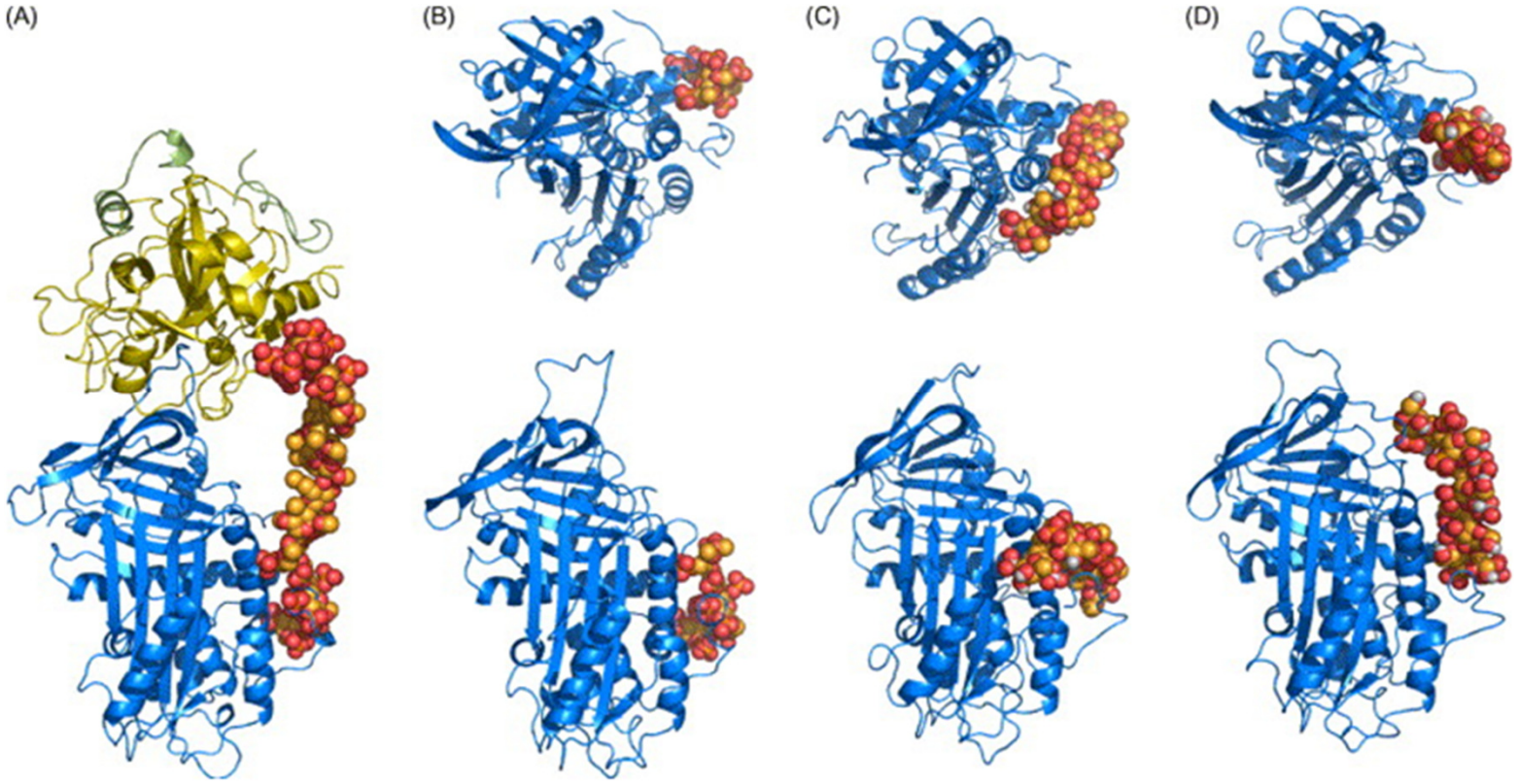
FUCANOMICS & GALACTANOMICS: STRUCTURE vs FUNCTION

Advanced structure-function relationships: ***CONFORMATION-DEPENDENT ANTICOAGULANT ACTION***



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.

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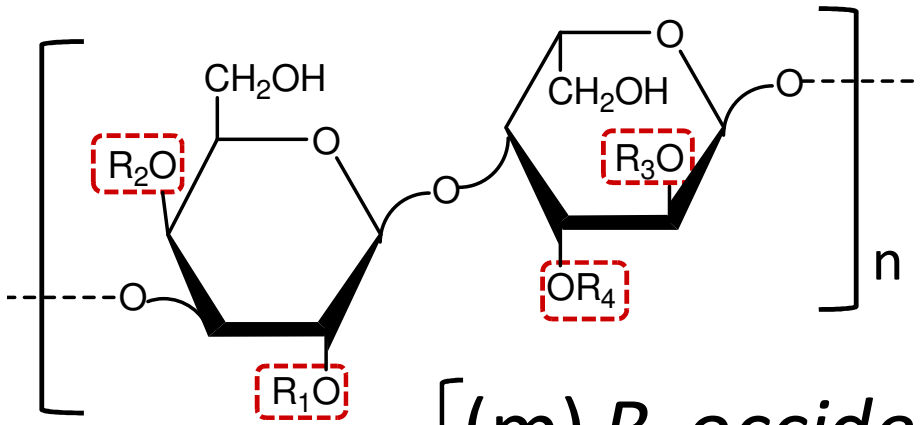
FUCANOMICS & GALACTANOMICS: STRUCTURE vs FUNCTION

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



R_{1-4} = H or SO_3^-

(m) *B. occidentalis* (R_1 and R_2 = SO_3^- in ±66% and ±33%, respectively)

(n) *G. crinale* (R_1 and R_2 = SO_3^- in ±60% and ±15%, respectively)

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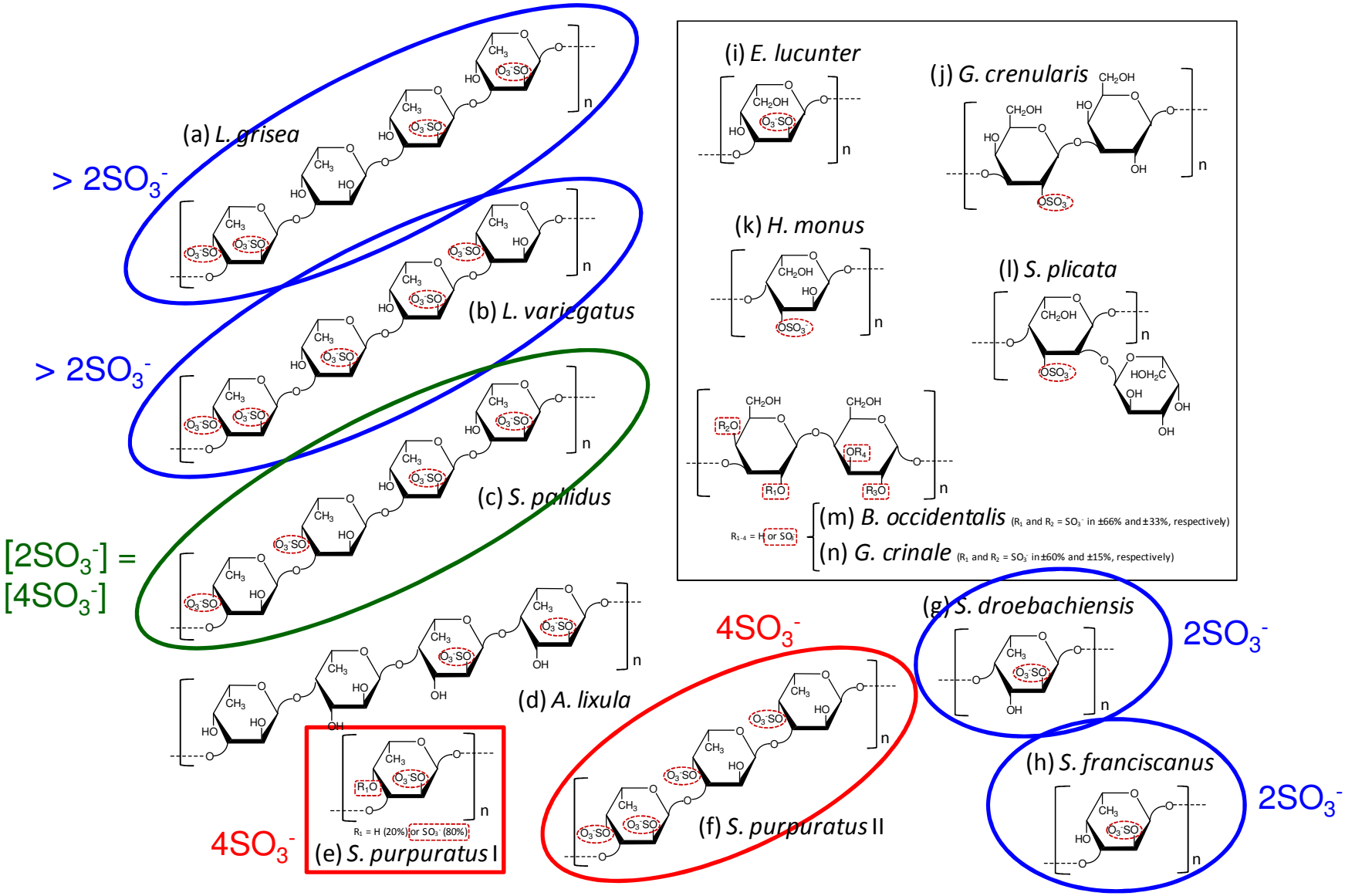
FUCANOMICS & GALACTANOMICS: STRUCTURE vs FUNCTION

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



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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.**

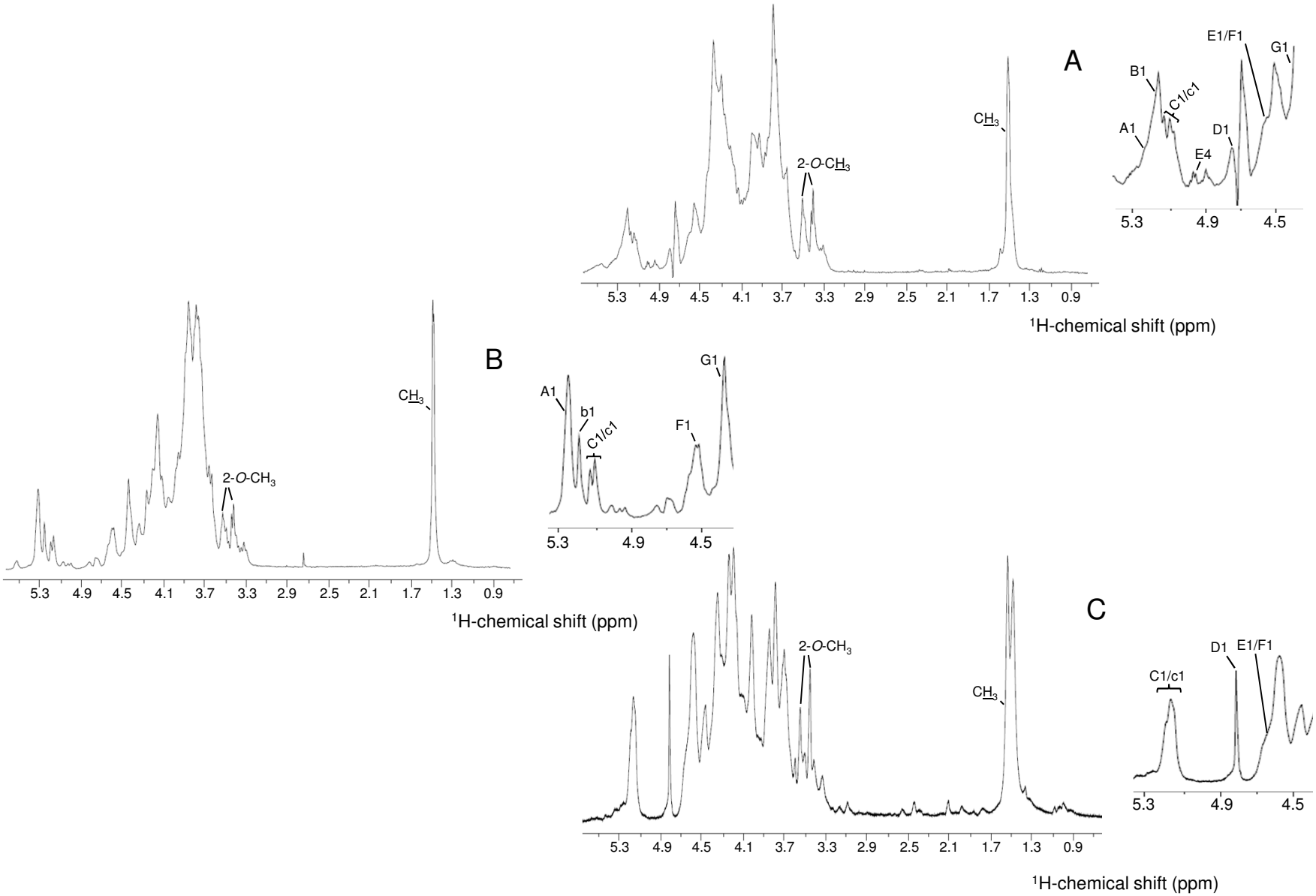
- **2-sulfated 3-linked α-galactan is an anticoagulant polysaccharides, whereas 2-sulfated 3-linked α-fucan is not. Their conformational binding preference to serpins are different.**

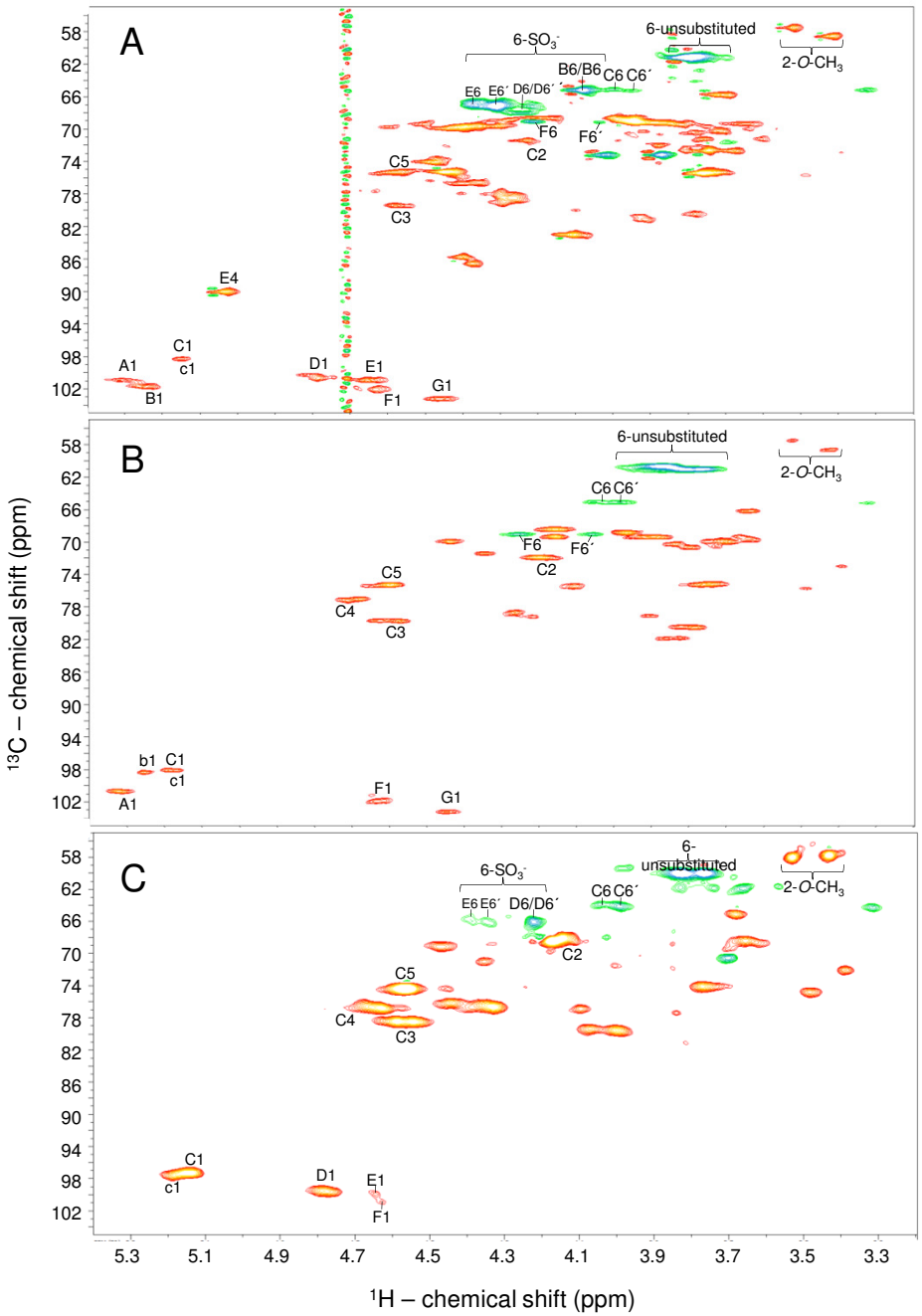
- **2,4-di-sulfation and maybe 4-sulfation in 3-linked α-SFs have shown a benefitial effect on the anticoagulation, whereas 2-sulfation alone is certainly deleterious. Ongoing NMR relaxation studies will facilitate further explanations.**

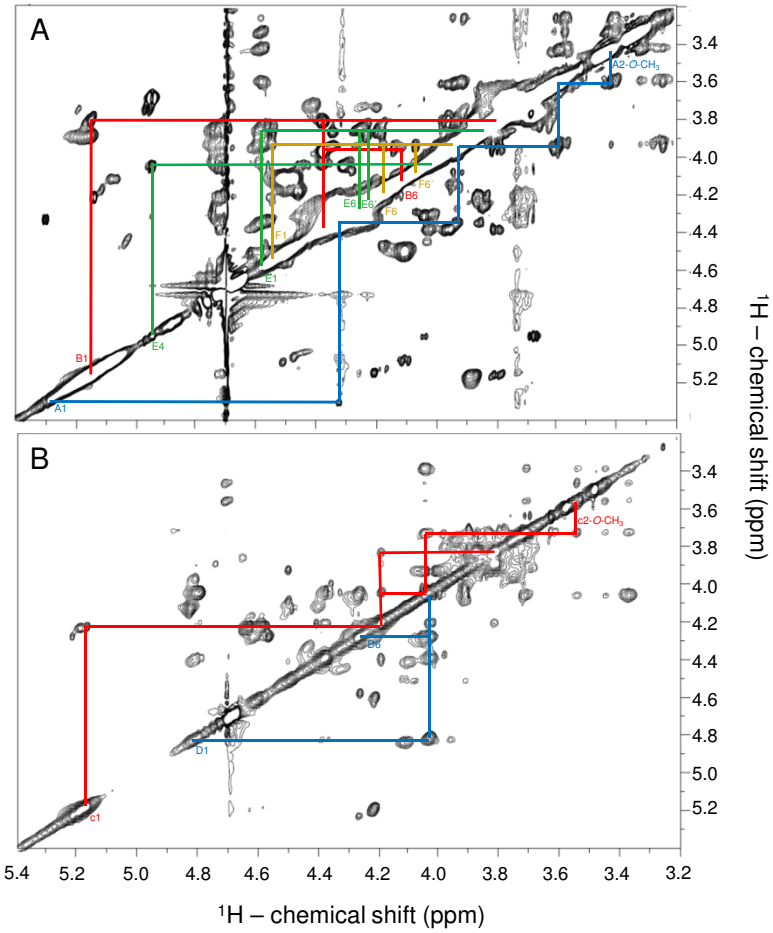
- **Our dataset strongly support the the fact that the clinical actions of SFs and SGs are not a mere consequence of sulfation degrees, but intimately correlated to specific features: anomeric configuration, monosaccharide type, sulfation positions or patterns, glycosylation position, molecular weights,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

FUCANOMICS & GALACTANOMICS: STRUCTURE DETERMINATION

NMR structure determination of a new red algal SG (Acanthophora muscoides)

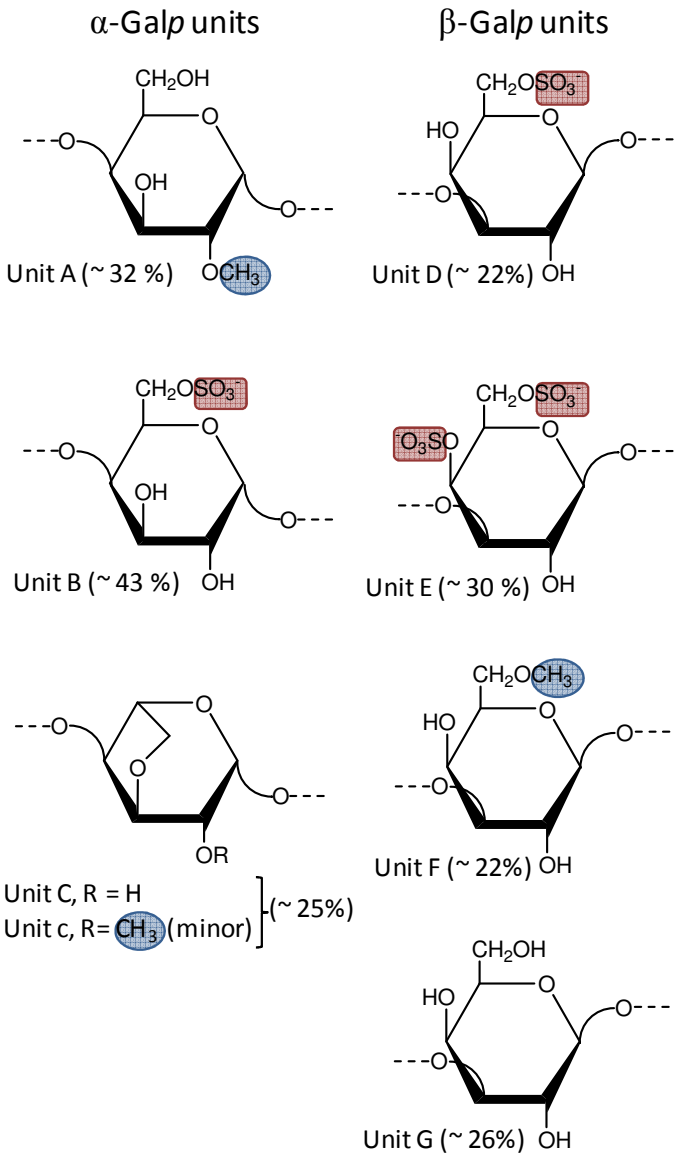



A
B
C
6-SO3-
6-unsubstituted
2-O-CH3
B6/B6
C6 C6´
E6 E6´ D6/D6´
F6
F6´
C2
C5
C3
E4
A1
C1
c1
B1
D1
E1
F1
G1
b1
C4
6-unsubstituted
2-O-CH3
6-SO3-
6-unsubstituted
E6 E6´ D6/D6´
C6 C6´
13C – chemical shift (ppm)
1H – chemical shift (ppm)

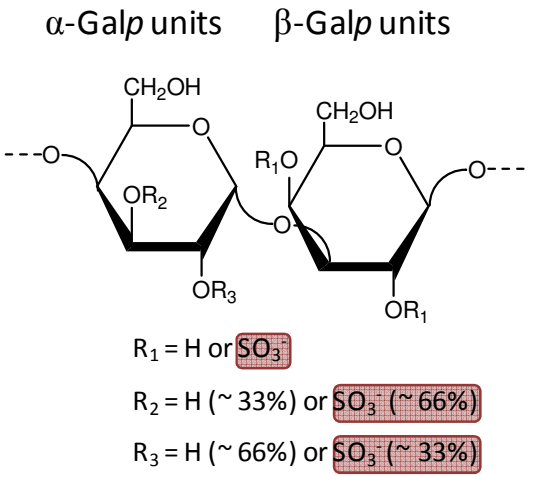

A
B
A2-O-CH3
c2-O-CH3
B1
A1
E4
E1
F1
F6´
B6
E6
E6´
F6
D1
c1
1H – chemical shift (ppm)
1H – chemical shift (ppm)

FUCANOMICS & GALACTANOMICS: STRUCTURE PROPOSITION

Structures of SGs from Acanthophora muscoides and Botriocladia occidentalis

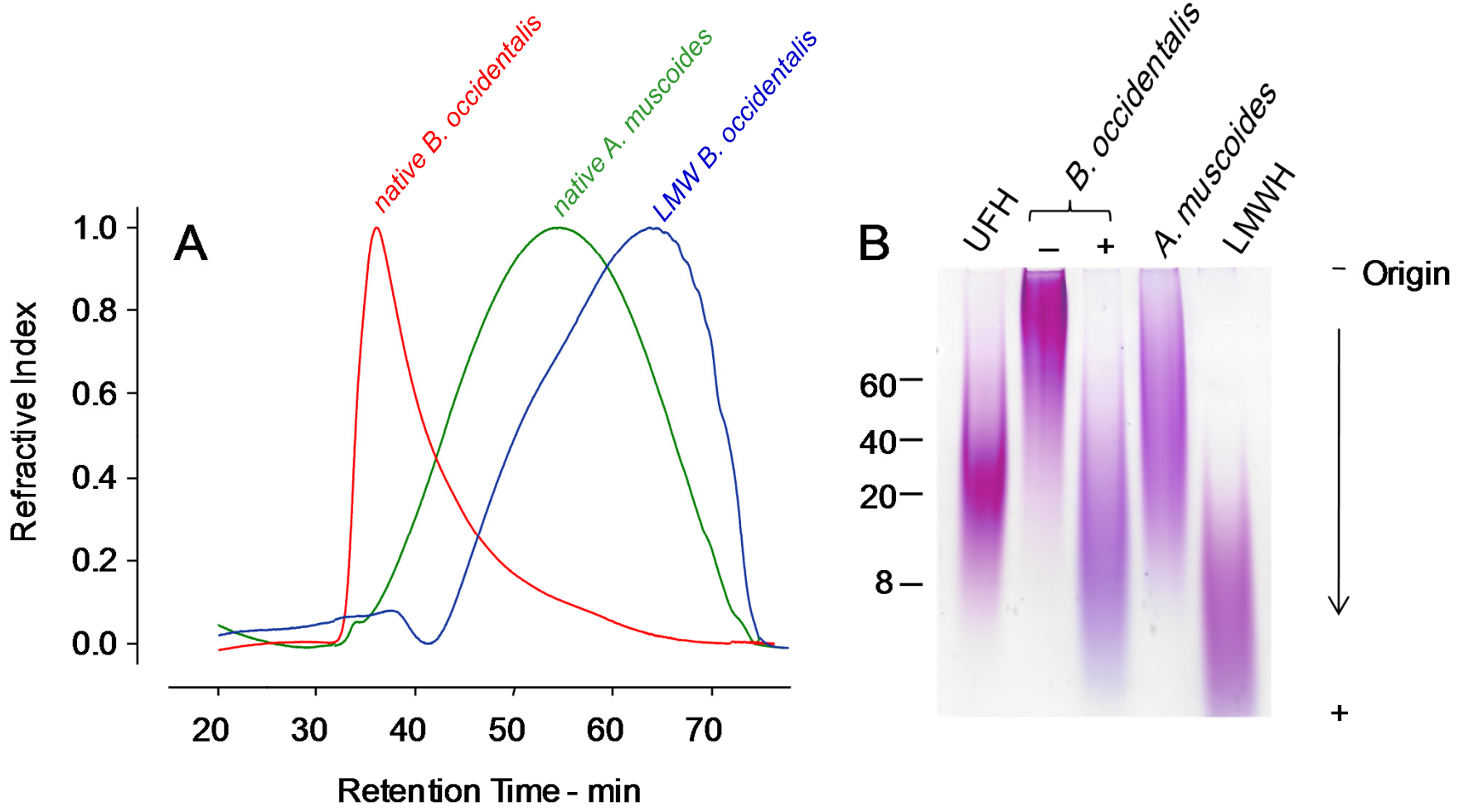


VS



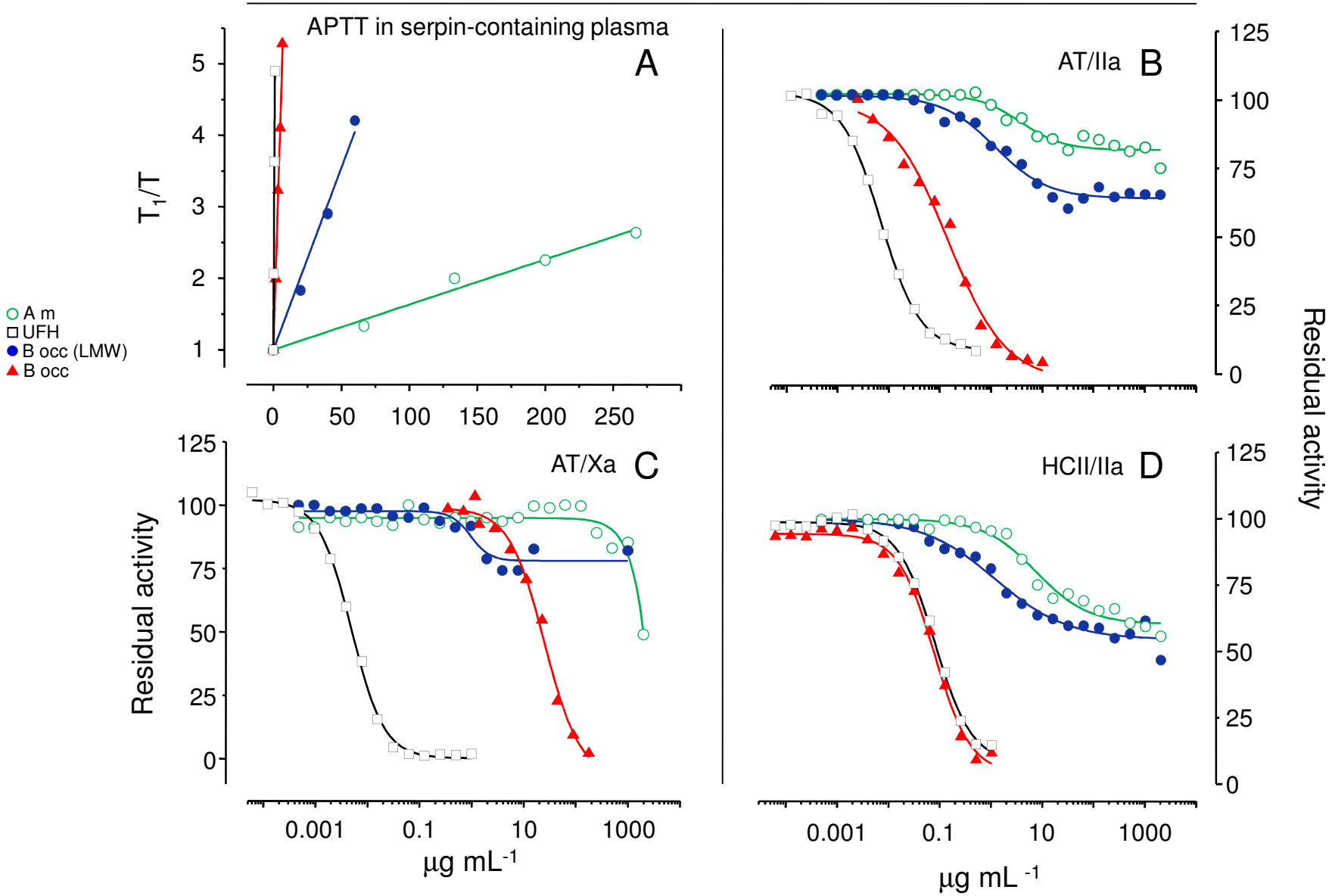
FUCANOMICS & GALACTANOMICS: MW DIFFERENCES

Structures of Acanthophora muscoides and Botriocladia occidentalis



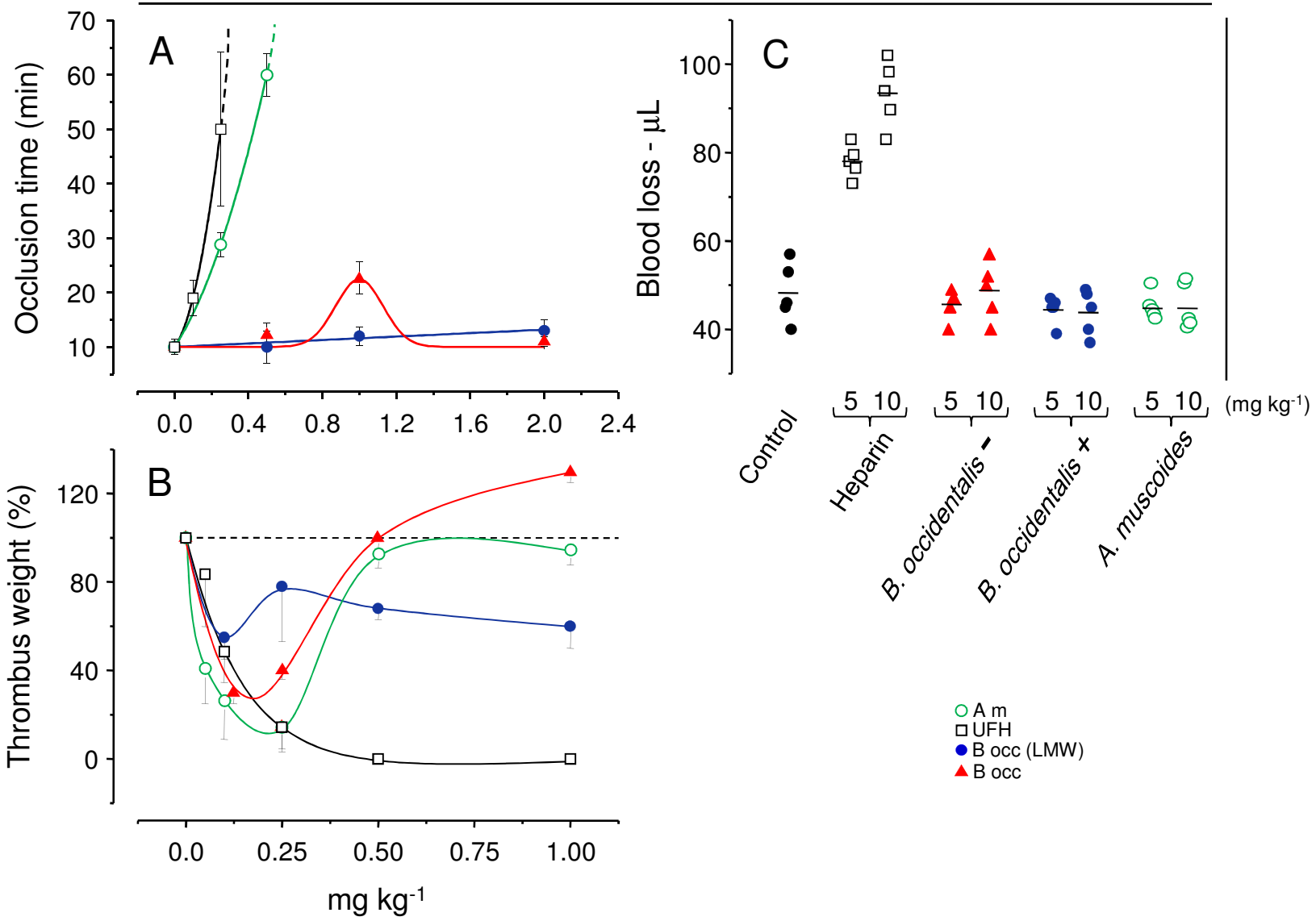
FUCANOMICS & GALACTANOMICS: ANTICOAGULATION

SERPIN-DEPENDENT ANTICOAGULANT ACTIVITIES



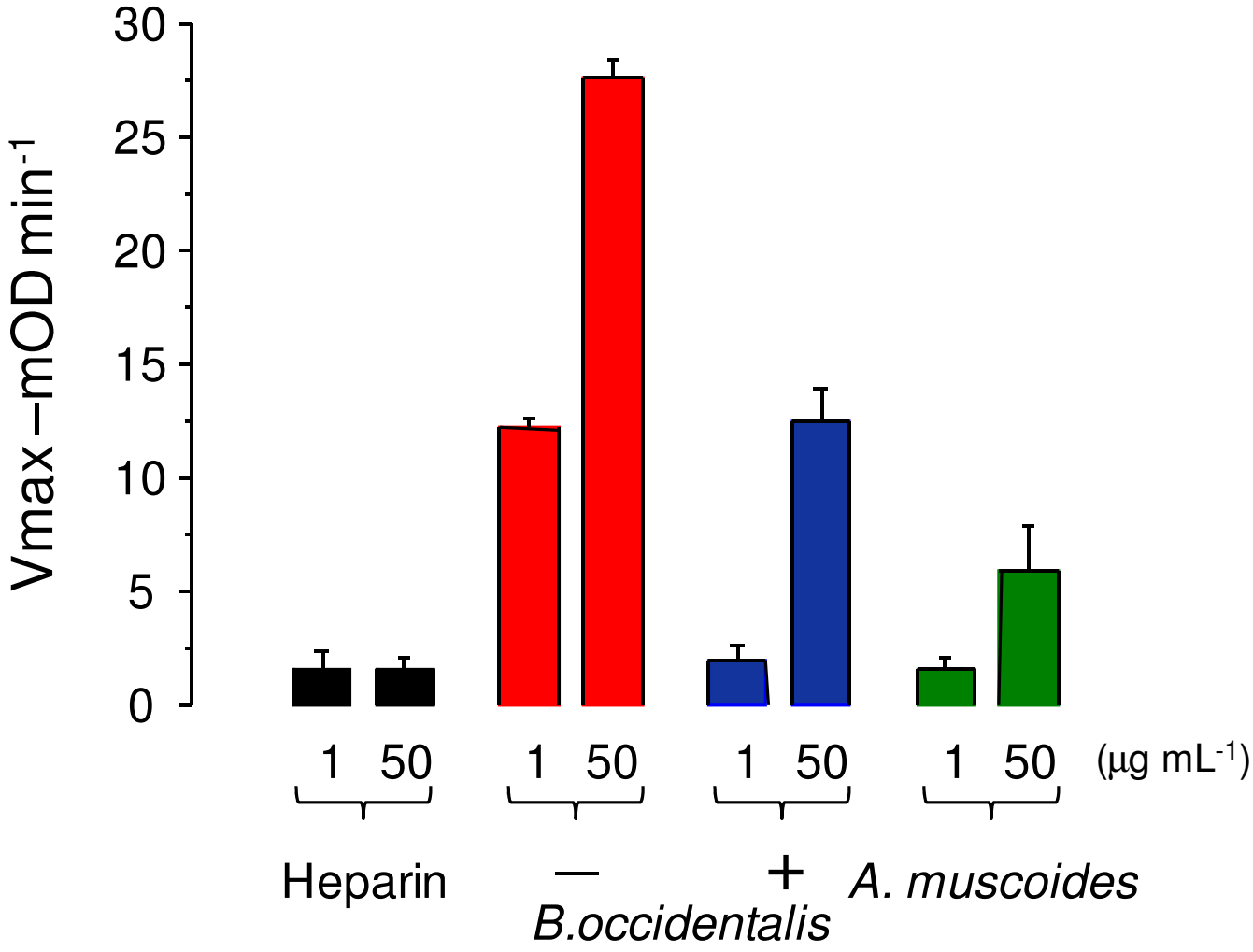
FUCANOMICS & GALACTANOMICS: ANTITHROMBOSIS

ARTERIAL AND VENOUS MODELS, AND BLEEDING EFFECTS



FUCANOMICS & GALACTANOMICS: PROTHROMBOTIC EFFECTS

Activation of factor XII



FUCANOMICS & GALACTANOMICS: 2rd CONCLUSIONS

- **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 hemorragic, 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 hemorragic effects, as compared with the standard heparin.**

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- Gabriel Baptista de Sá

Universidade Federal do Rio de Janeiro

HUCFF Hospital Universitário Clementino Fraga Filho

IBqM Instituto de Bioquímica Médica UFRJ

CNRMN

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico

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