Sulfated polysaccharides ensure a carbohydrate-based mechanism for species recognition during sea urchin fertilization

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Comparison between the sulfated fucans from invertebrates and algae

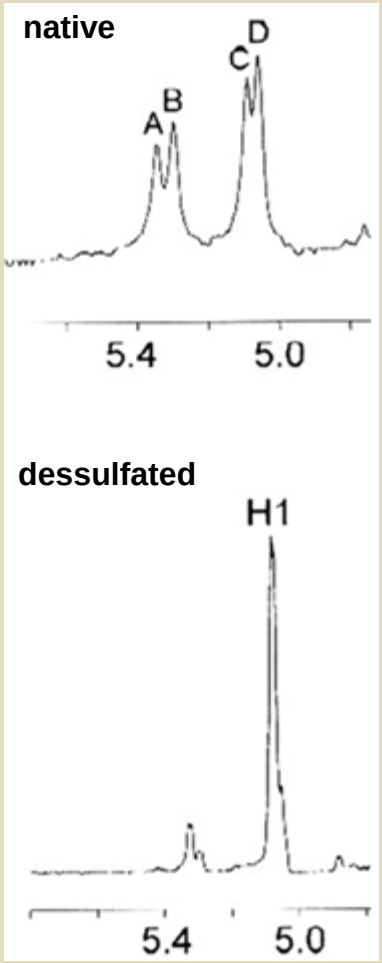
a) Sulfated fucans from marine algae → **Heterogeneous strucuture**

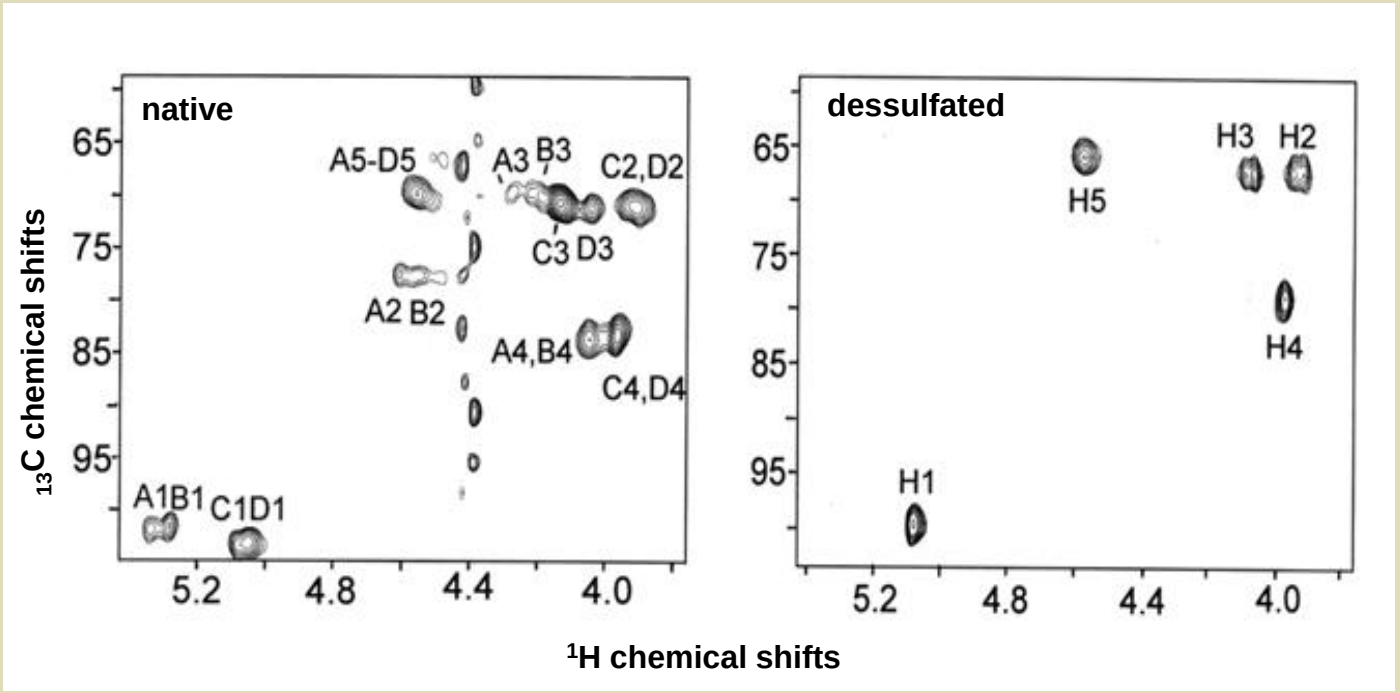
b) Sulfated fucans from marine invertebrates

- → **Homogeneous structure.**
- → **Repetitive units.**
- → **Glycosylation and sulfation sites vary among the different species.**

I) Structures of the sulfated polysaccharides from marine invertebrates

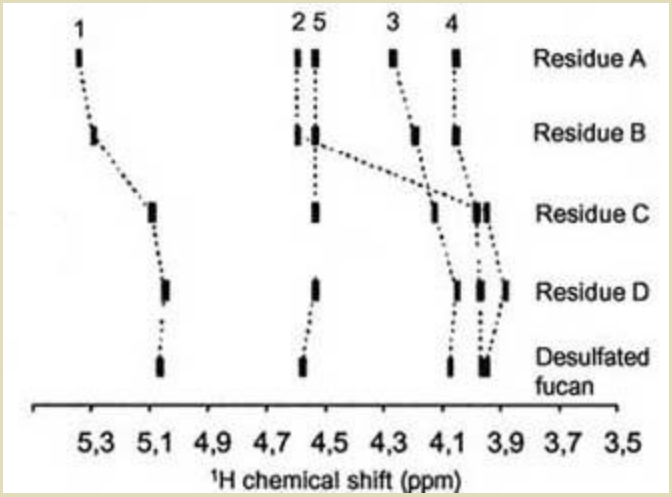
Approach used to determine the structure of a sulfated polysaccharide (an example from the sulfated fucan of *A. lixula)*

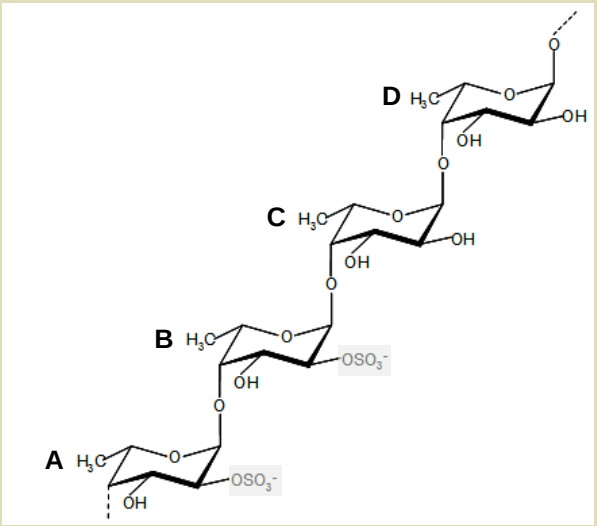


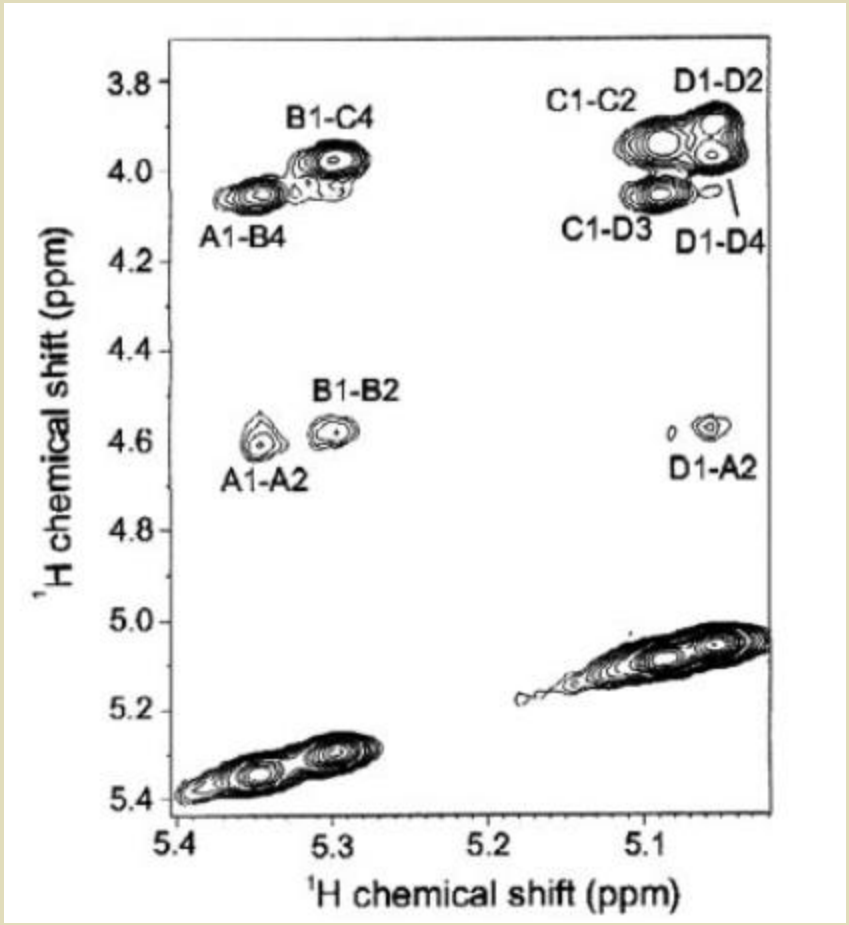


	Methylation Analysis	
	native	desulfated
2,3-Me$_2$Fuc	49%	71%
3-MeFuc	53%	29%

Approach used to determine the structure of a sulfated polysaccharide (an example from the sulfated fucan of *A. lixula)*

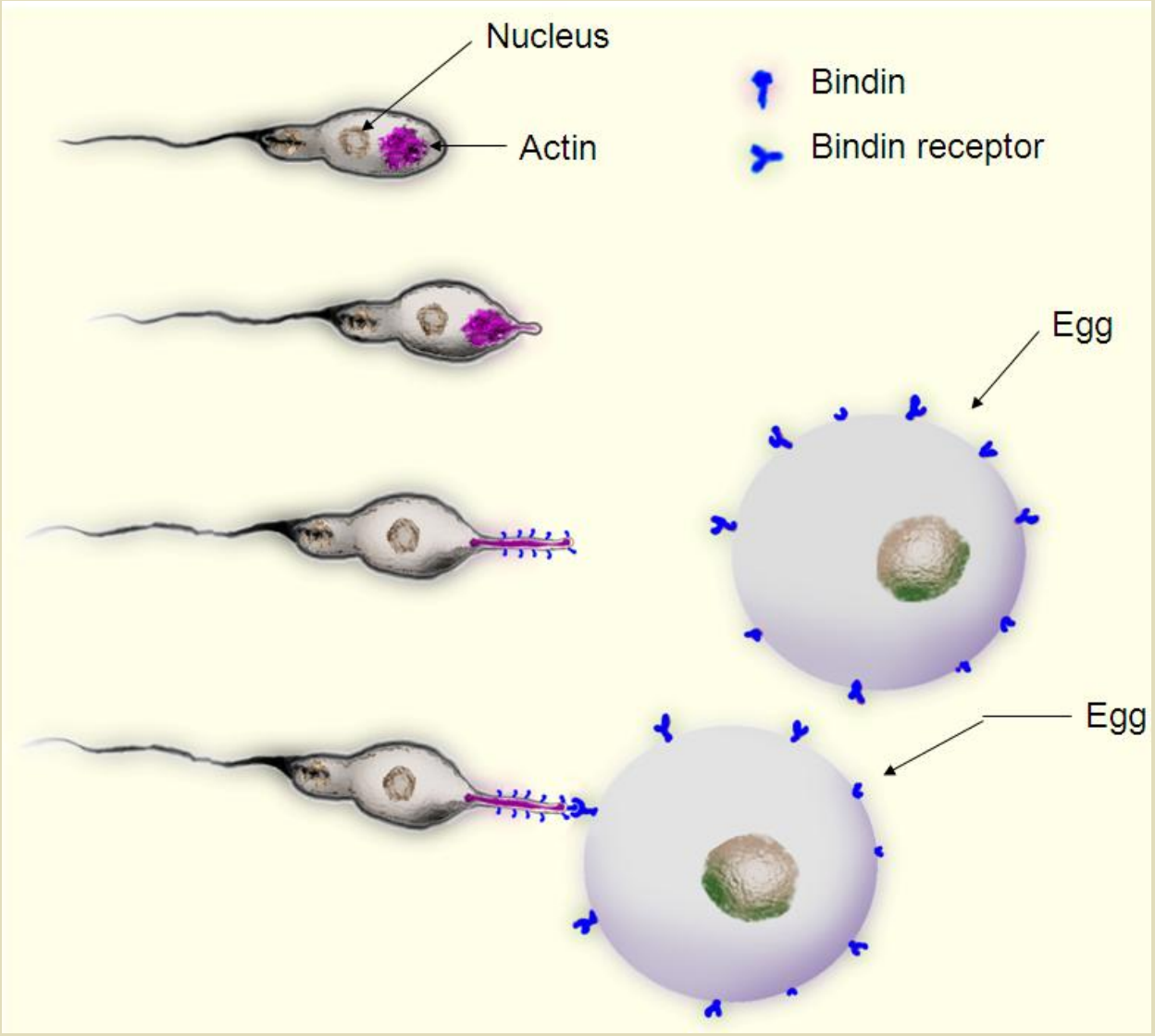




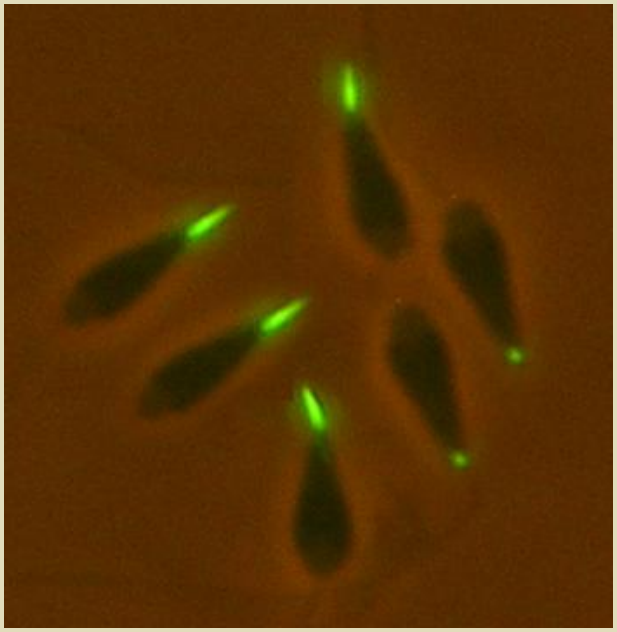


II) What is the biological function of sulfated polysaccharides in sea urchins?

1947: Jean C. Dan described the acrosome reaction

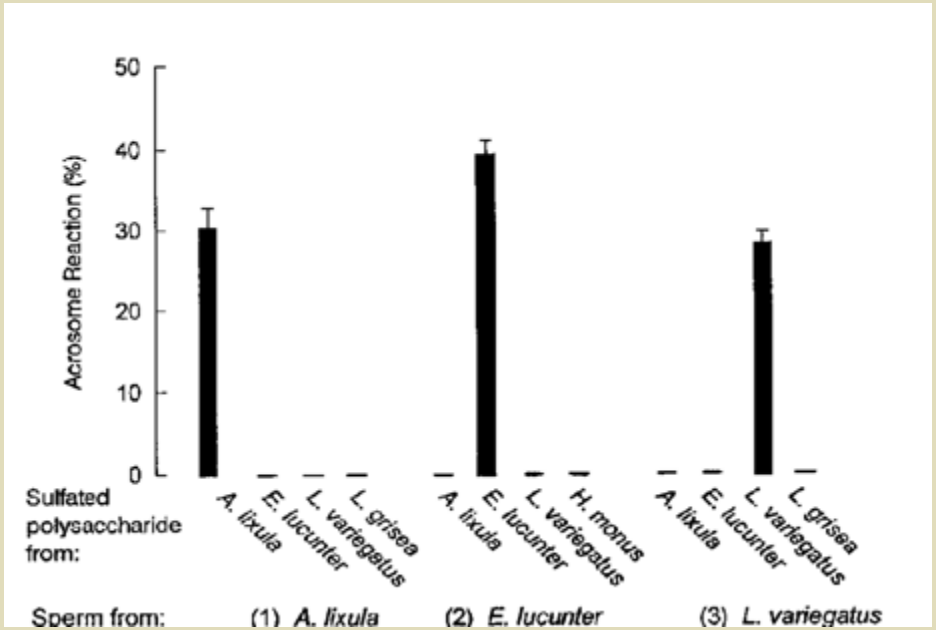


A skepticism that sulfated polysaccharides could induce such a specific reaction

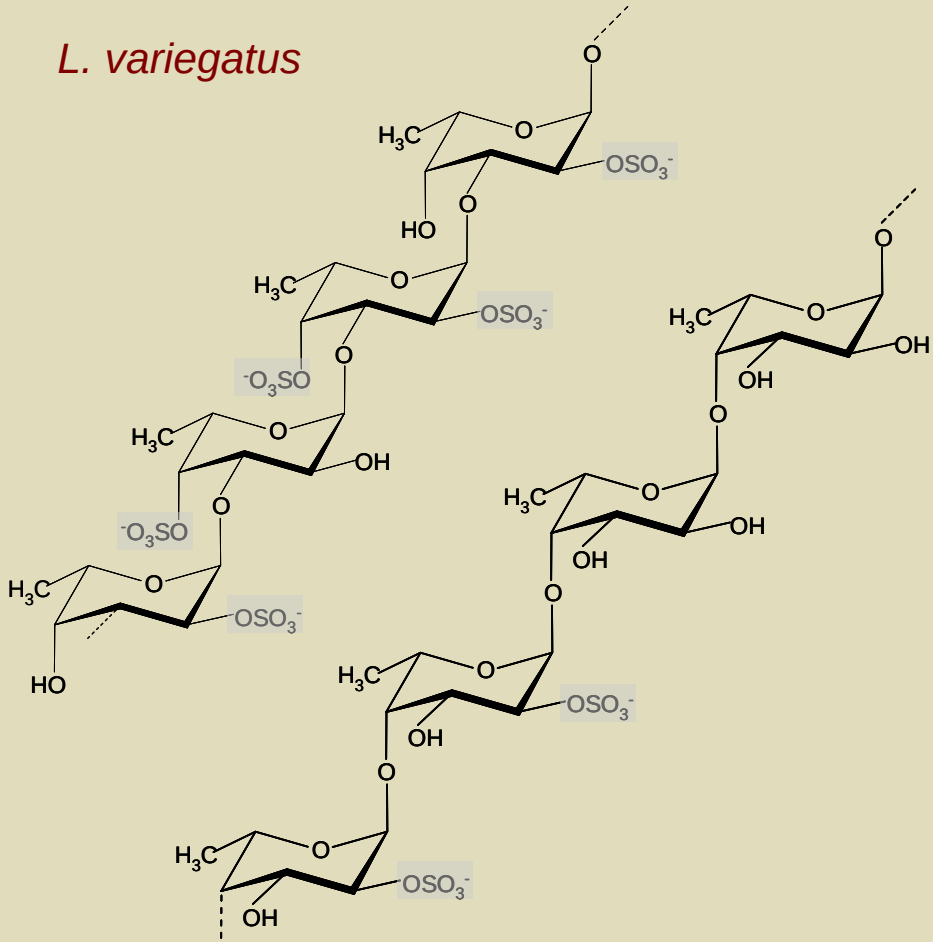


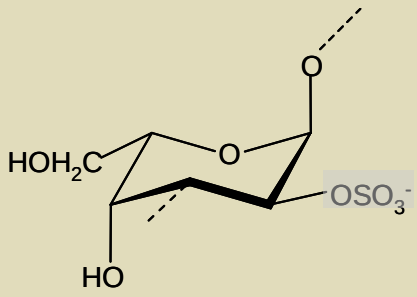
Keller and Vacquier (*Dev. Biol*. 162:304-312, 1994): "...sulfated fucans had no significant acrosome reaction-inducing activity. Instead, acrosome reaction inducing activity was associated only with two glycoproteins."

Sulfated polysaccharides are species-specific inducers of acrosomal reaction in sperm of sea urchins



L. variegatus



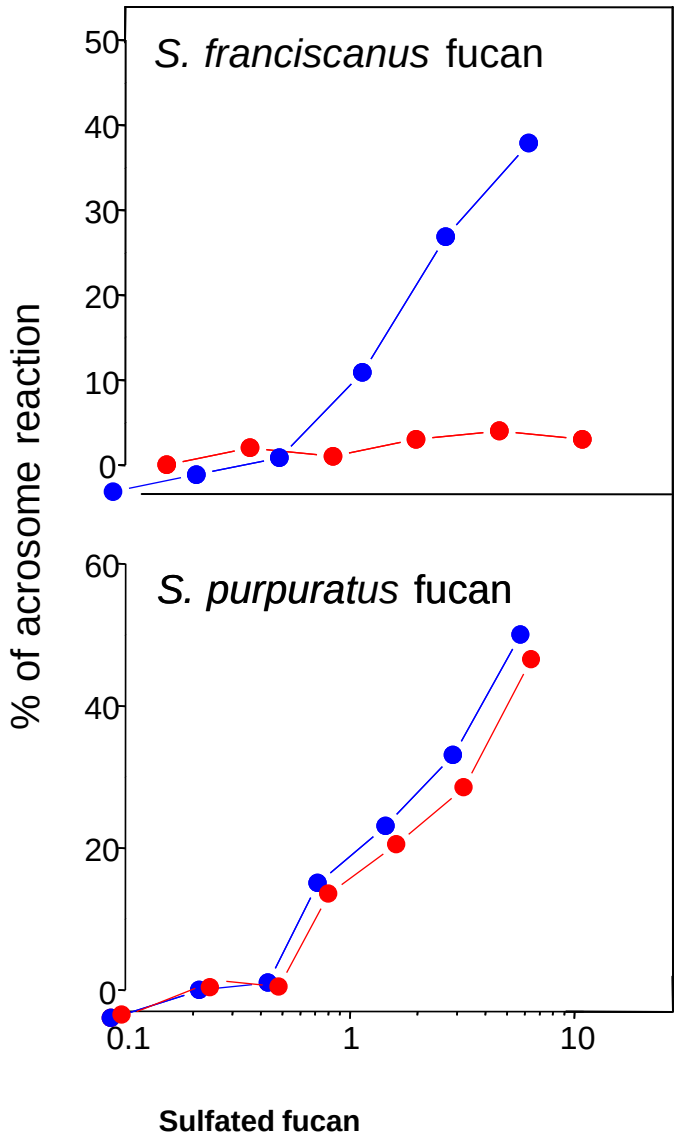


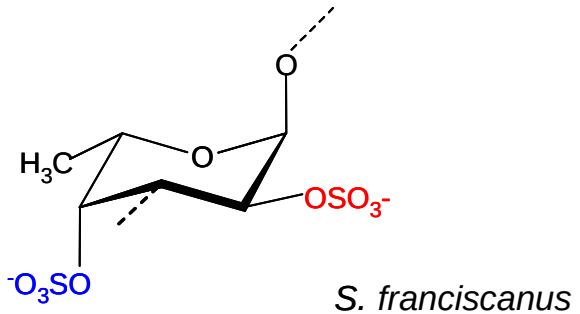
E. lucunter

A. lixula

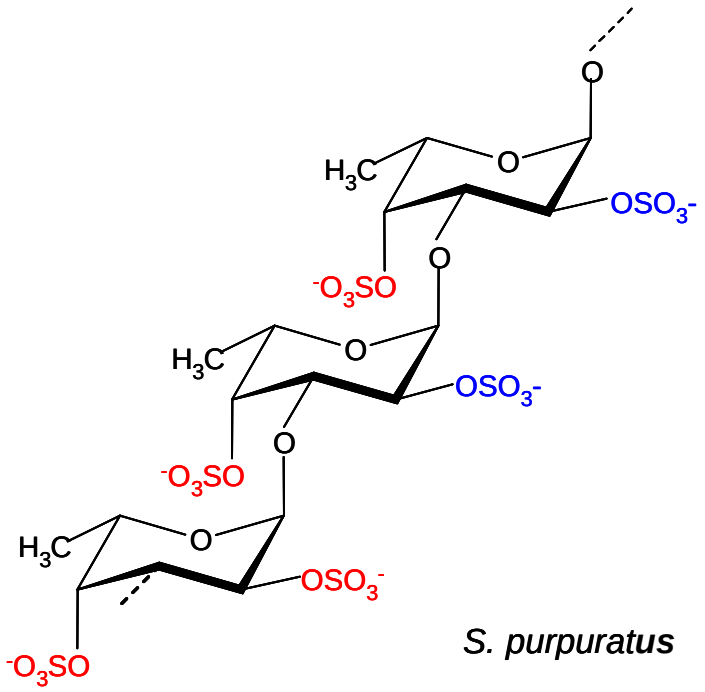
III) Structure *vs.* biological activity of the sea urchin polysaccharides

Effect of sulfation pattern



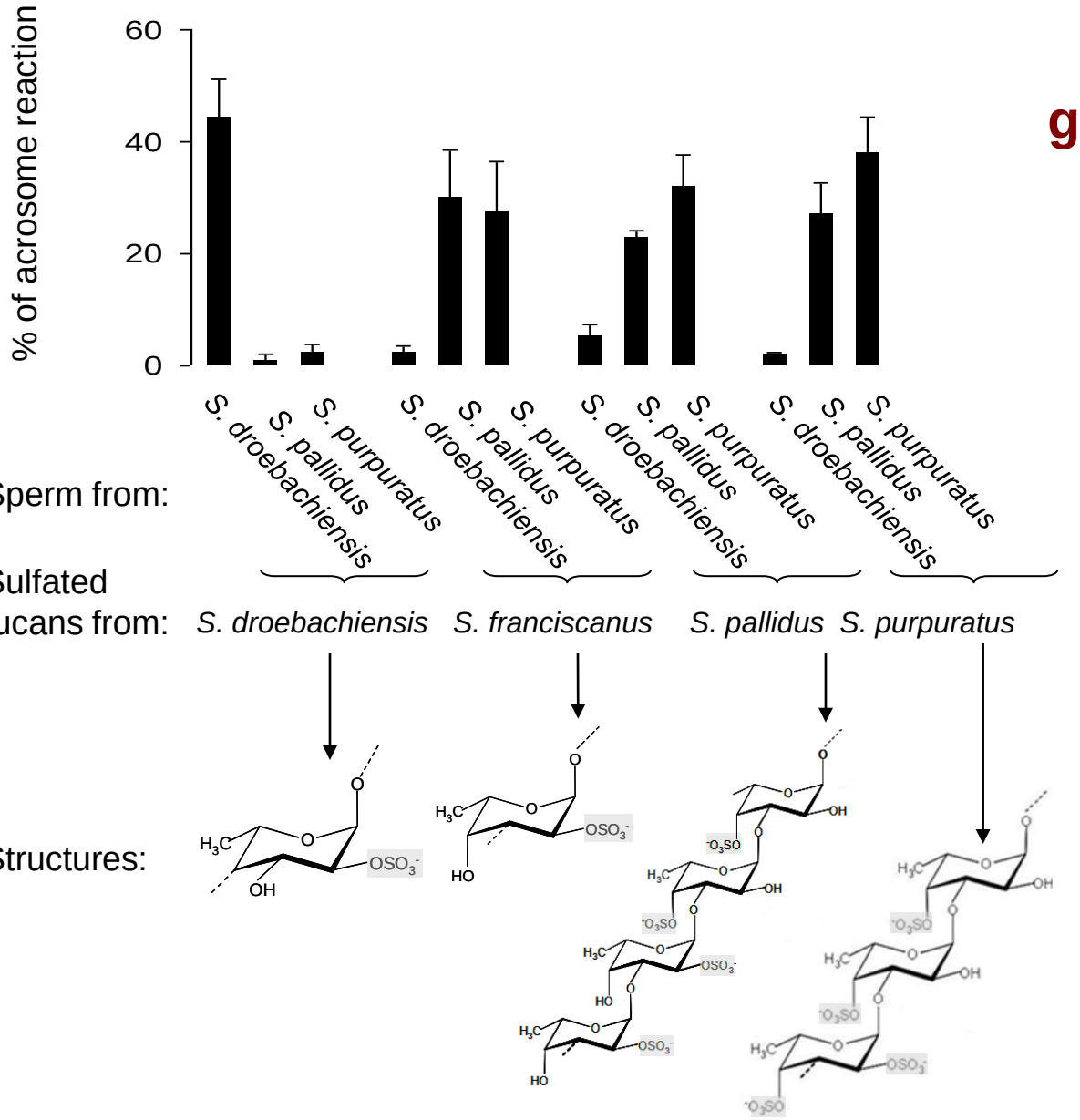


S. franciscanus

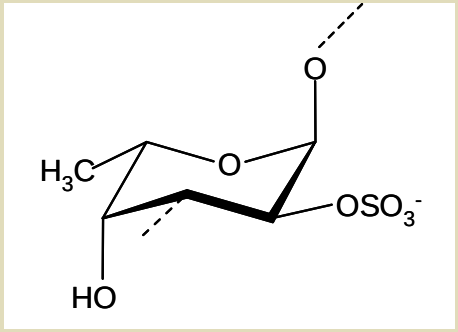


S. purpuratus

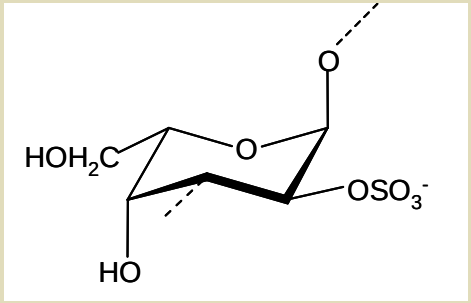
Position of the glycosidic linkage



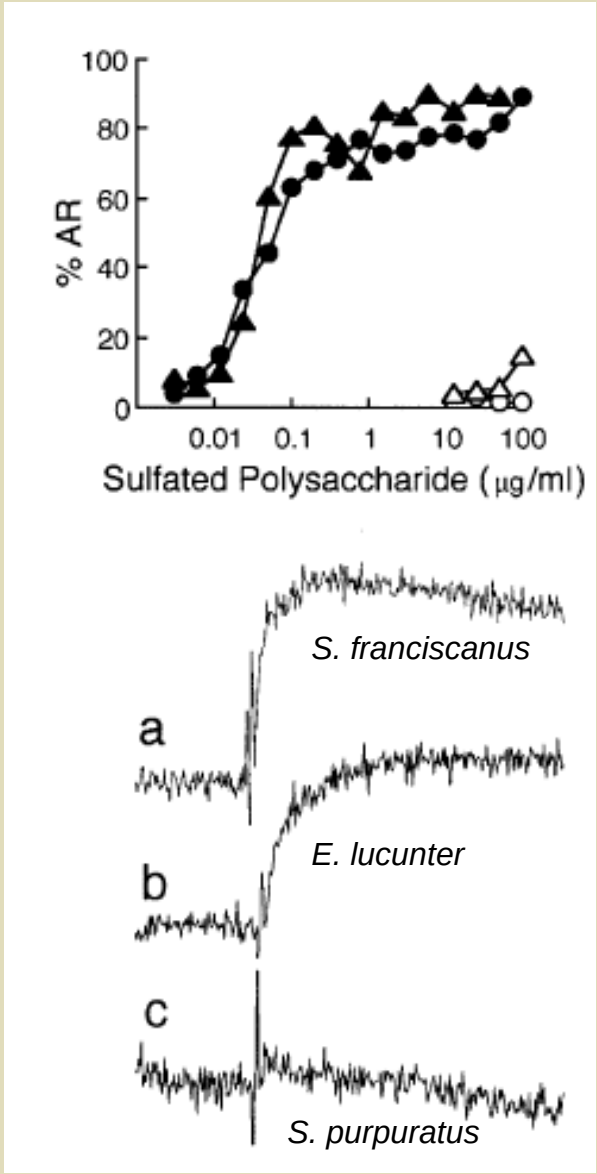
Sulfated α-fucan *vs.* sulfated α-galactan



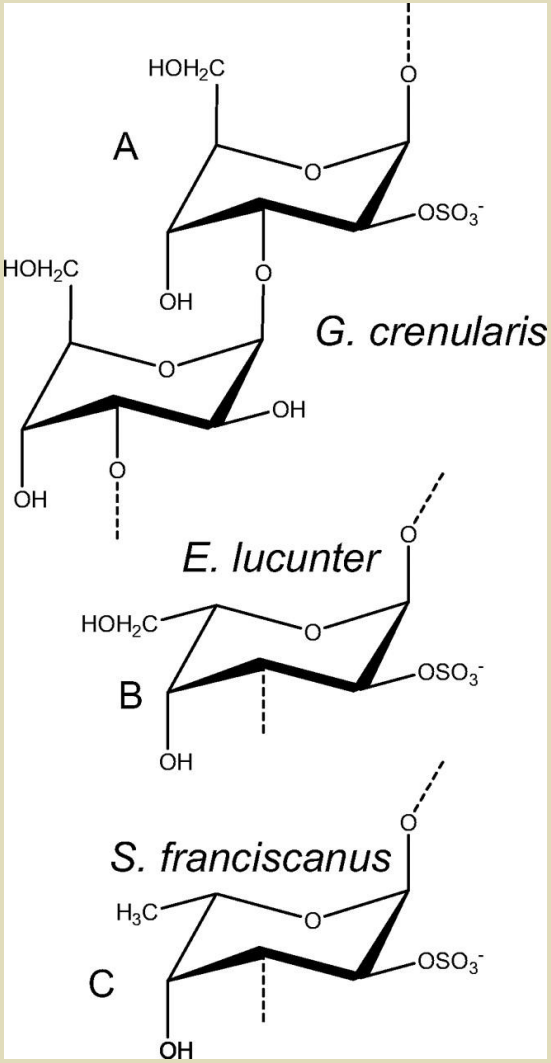
S. franciscanus

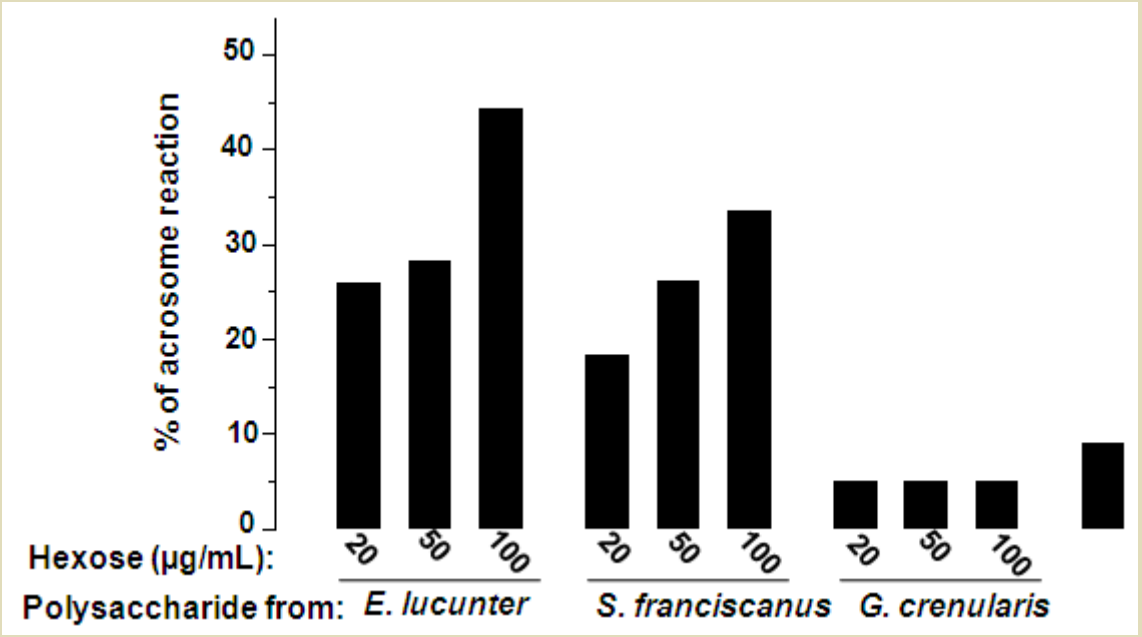


E. lucunter



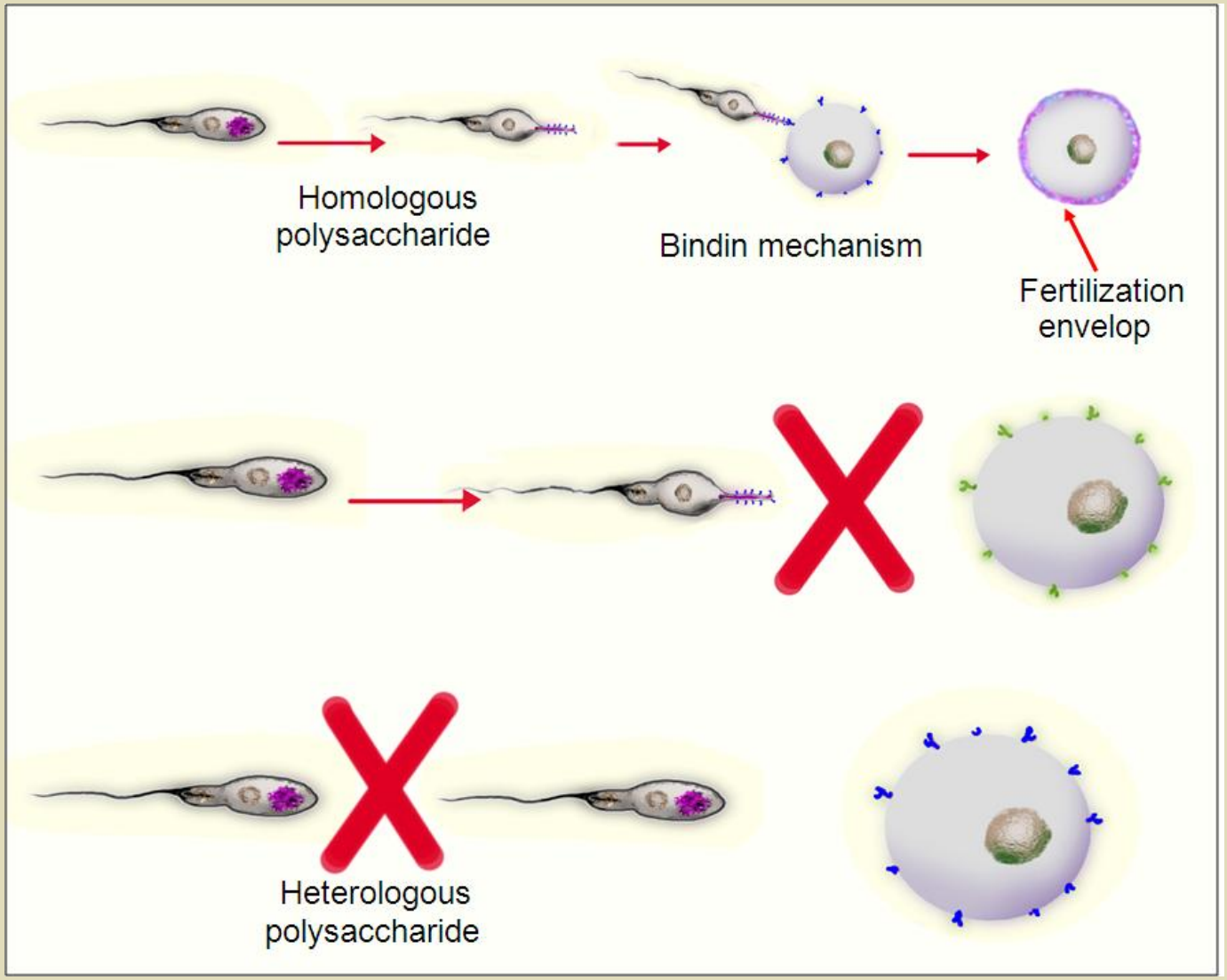
Sulfated α-galactan *vs.* sulfated β-galactan



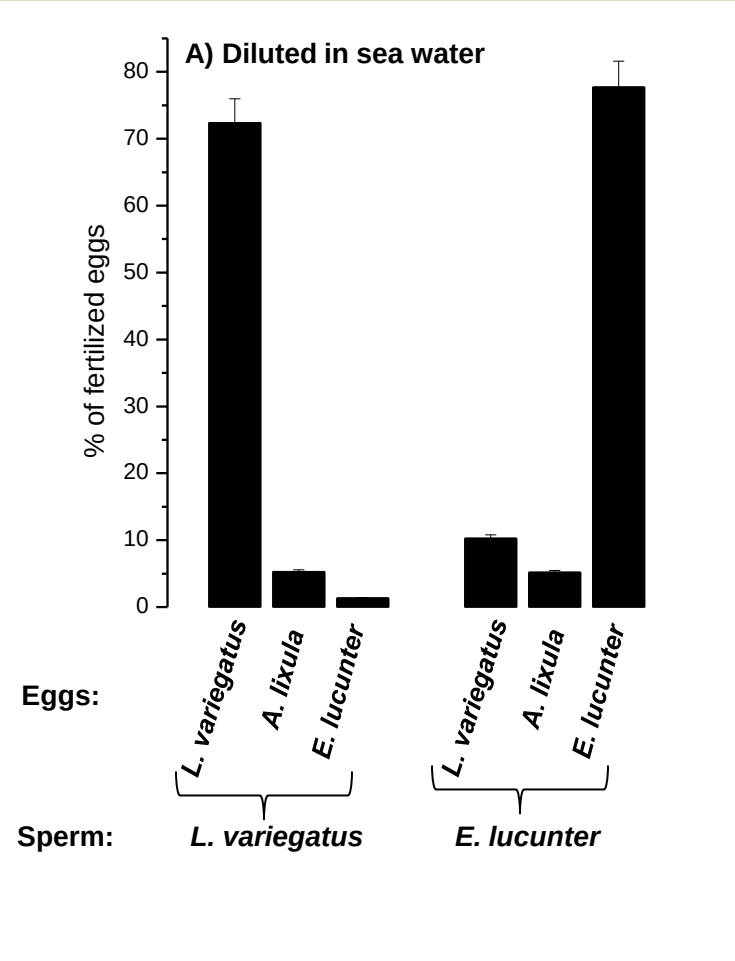


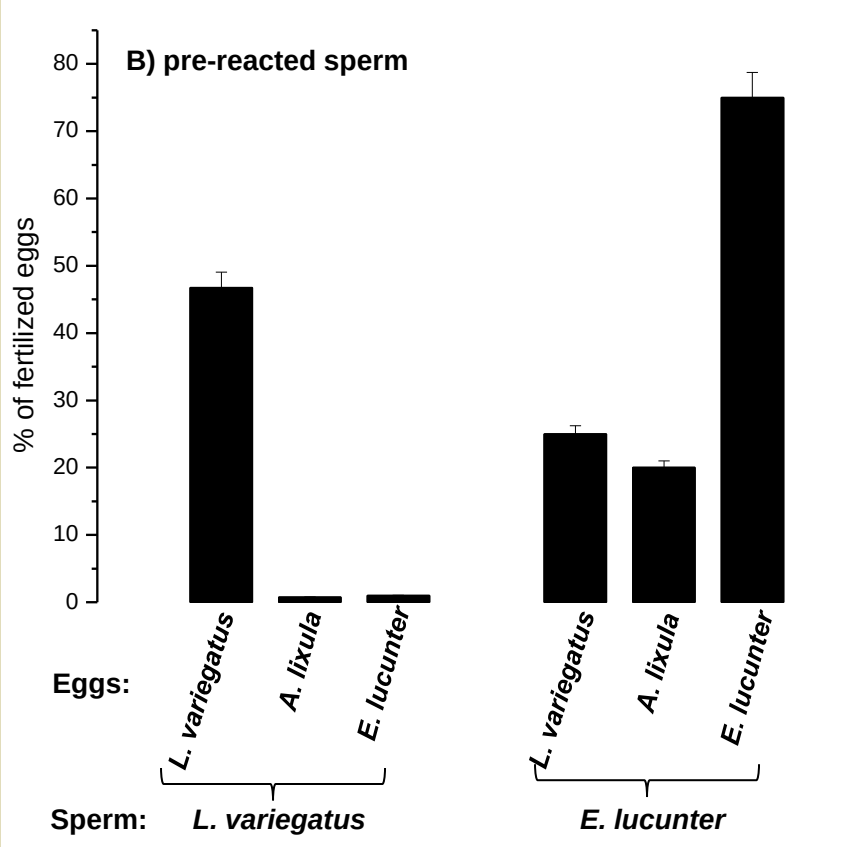
	Fertilization envelope formed			
	Sulfated α-galactan from *E. lucunter*		Sulfated β-galactan from *G. crenularis*	
	0	4 mg/ml	0	4 mg/ml
	% of total eggs			
Experiment 1	98.7	93.0	98.5	3.3
Experiment 2	99.1	96.7	98.0	0.5
Experiment 3	98.2	98.1	98.3	0.5
Mean ± S.D.	98.7 ± 0.5	95.9 ± 2.6	98.3 ± 0.3	1.4 ± 0.9

IV) Two mechanisms of sperm – egg recognition in sea urchins

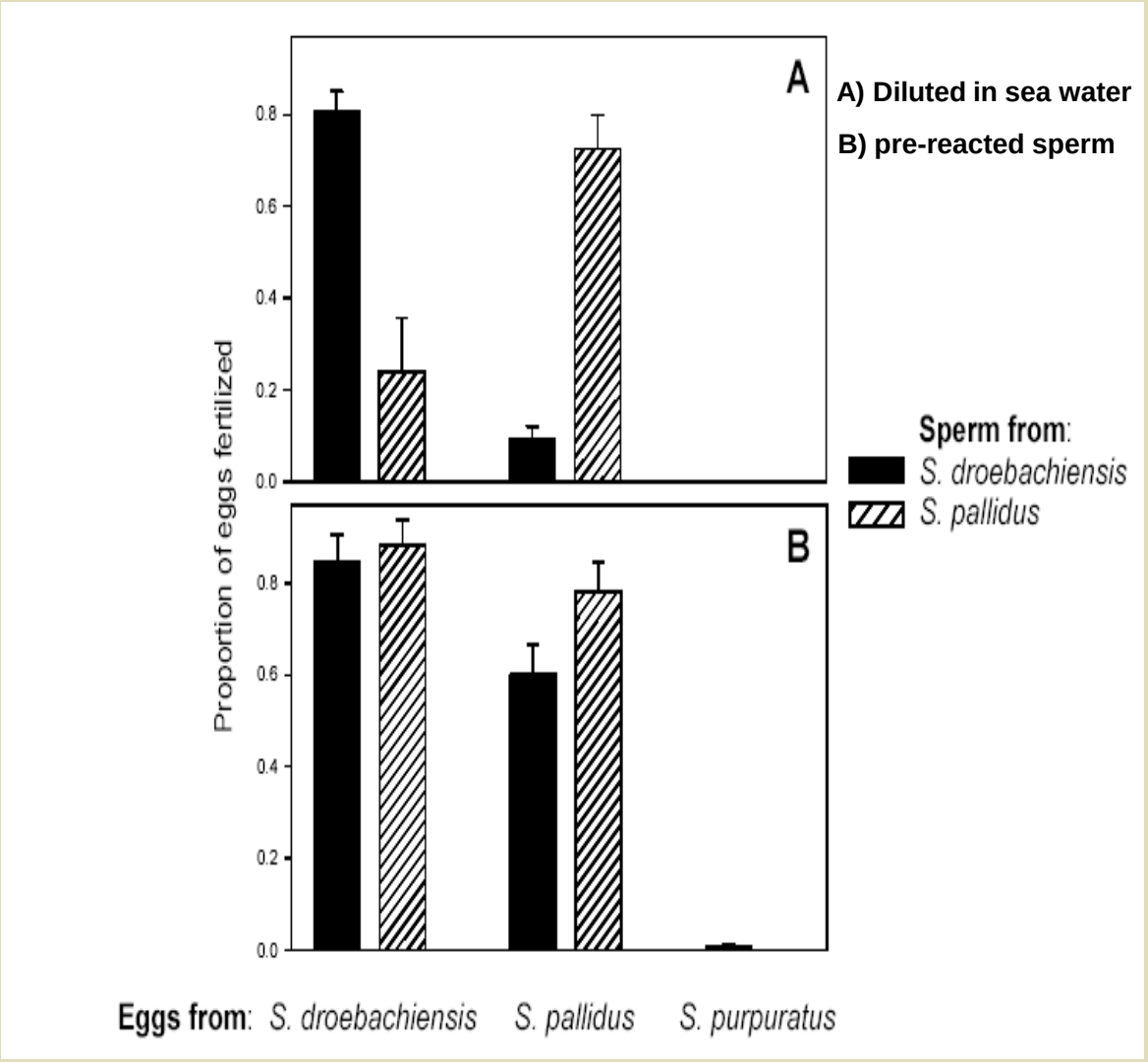


Species-specificity in the fertilization based on the bindin mechanism





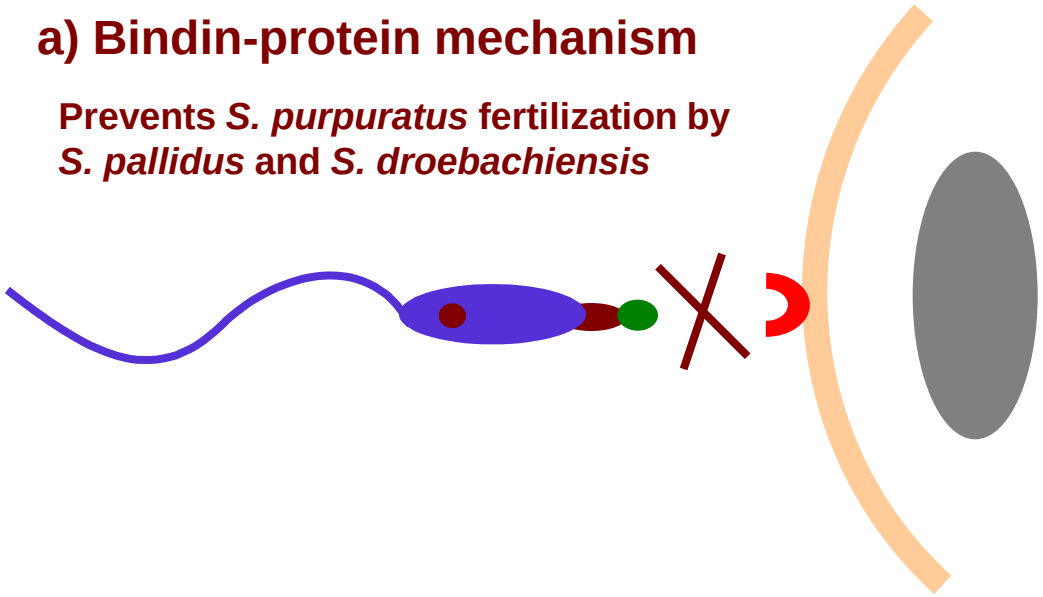
Fertilization and induction of acrosome reaction in sea urchins of the genus *Strongylocentrotus*



Two mechanisms of sperm-egg recognition in sea urchins

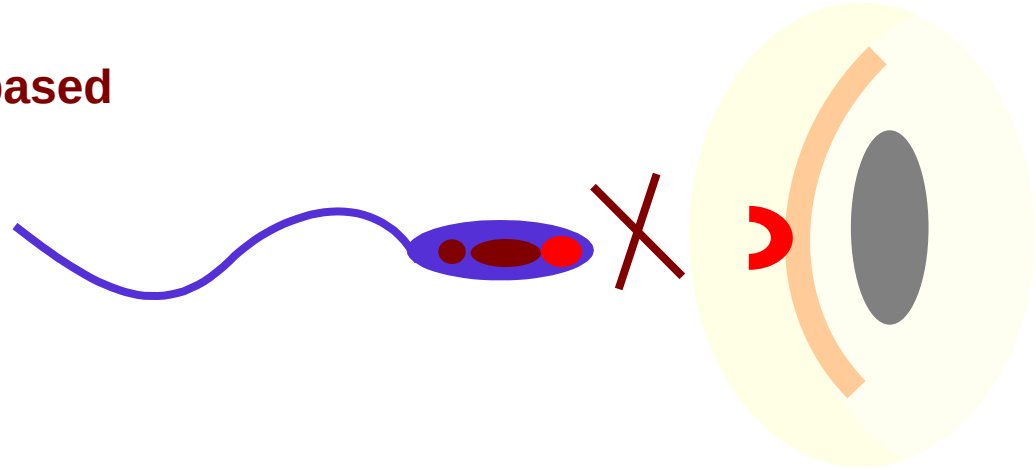
a) Bindin-protein mechanism

Prevents *S. purpuratus* fertilization by *S. pallidus* and *S. droebachiensis*

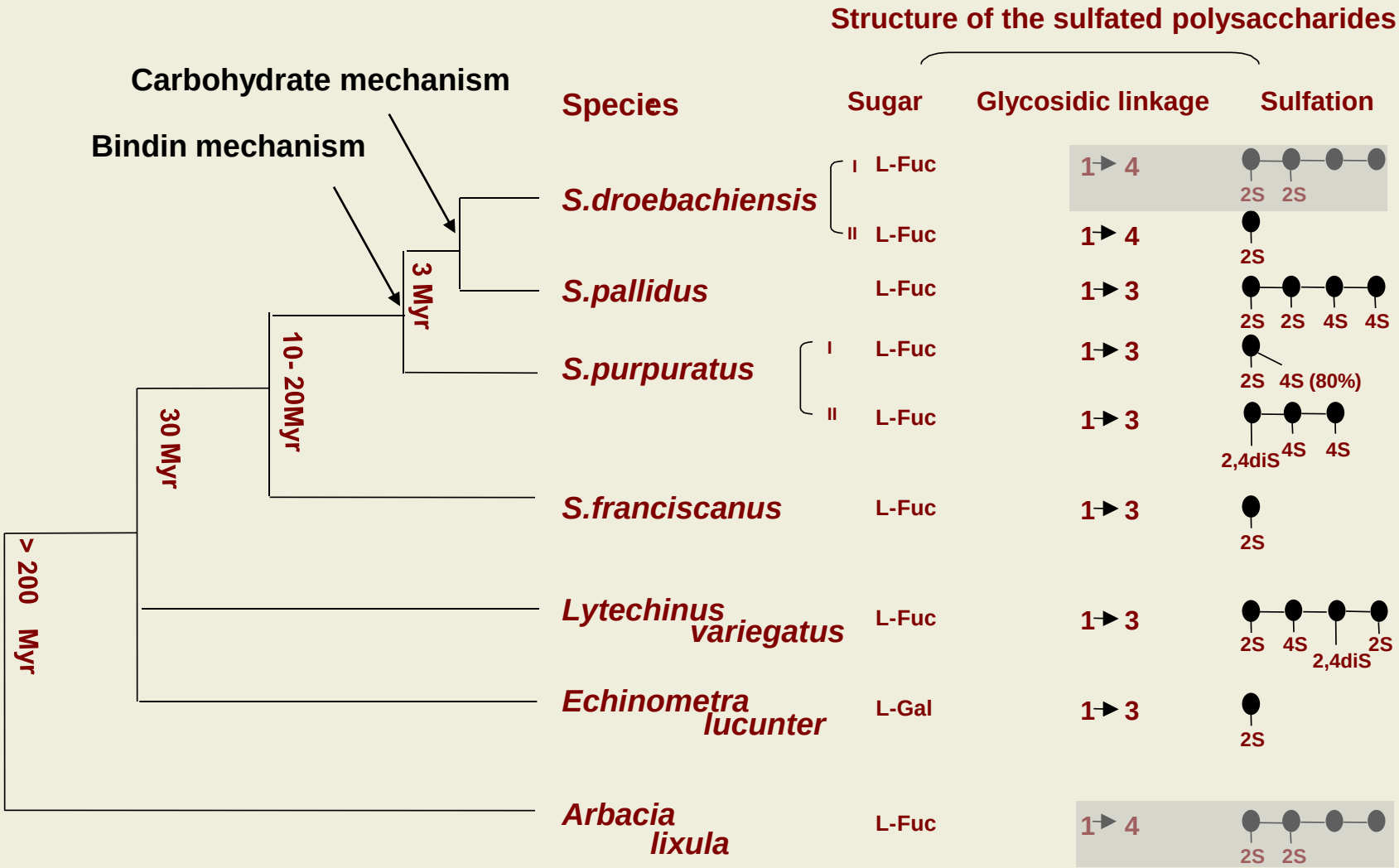


b) Sulfated polysaccharide-based mechanism

Prevents *S. droebachiensis* and *S. pallidus* intercrosses



Sulfated fucans : another avenue for speciation?

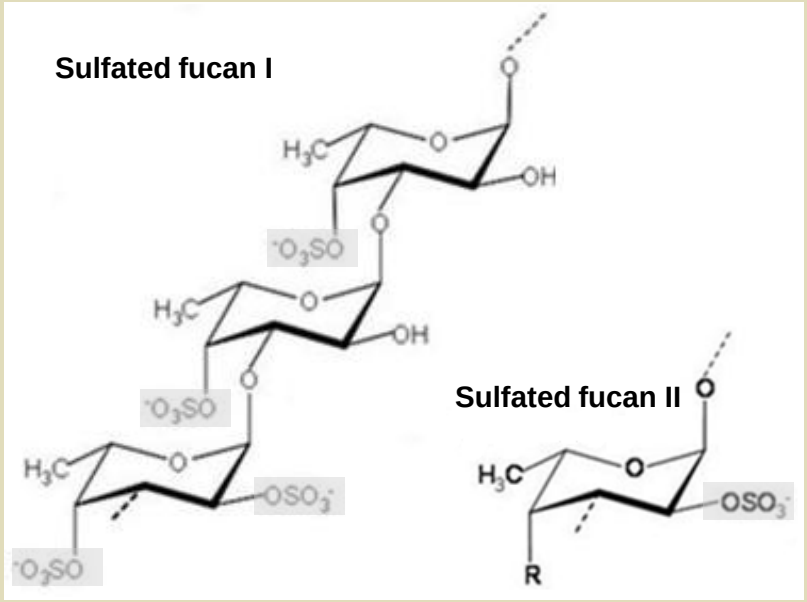


Myr = Million years of evolutionary divergence

V) Isoforms of sulfated fucans

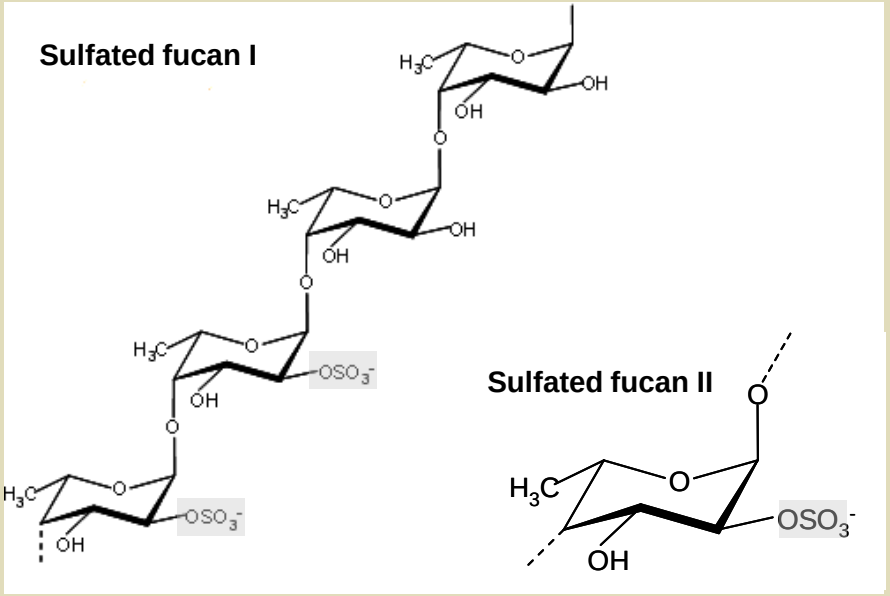
Physiological irrelevant isoforms of sulfated fucans

a) S. purpuratus



71 Individual females, 40 had eggs with sulfated fucans II, 22 had eggs with sulfated fucan I and 9 had eggs with both fucans.

b) S. droebachiensis



Pacific (USA): 13 individual females had sulfated fucan II, 9 had eggs with sulfated fucan I.

Atlantic (Norway): 9 females contains only sulfated fucan II.

The two isoforms of sulfated fucans induce the acrosome reaction with similar potency in homologous sperm

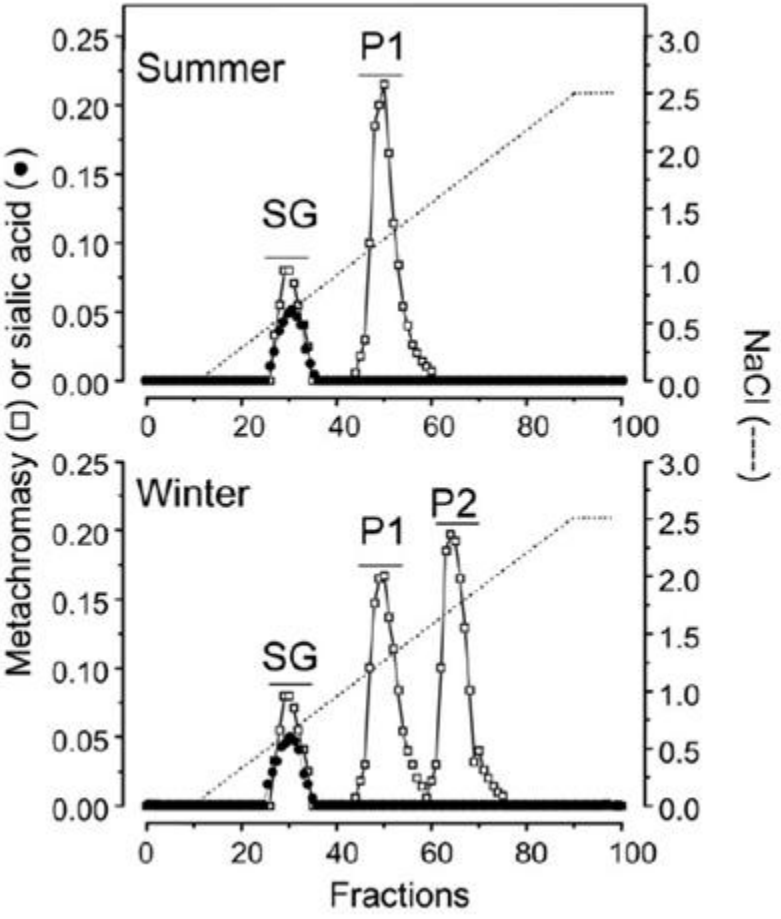
Table 2. Comparison of acrosome reaction–inducing activities of the two isotypes of sulfated fucans (I and II) from *Strongylocentrotus droebachiensis* egg jelly

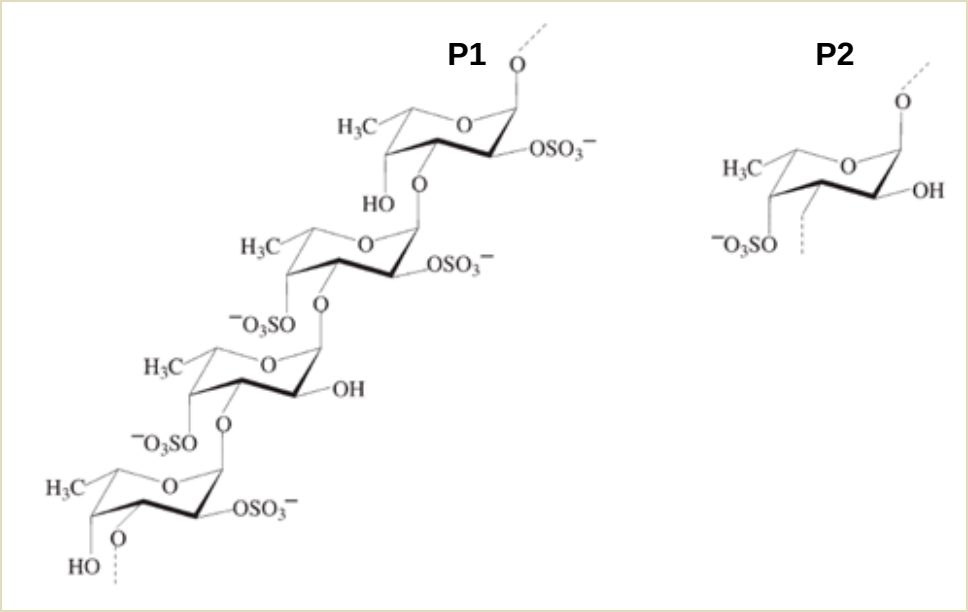
Sperm from	% AR ± SE[1] Sulfated Fucan I[2]	% AR ± SE[1] Sulfated Fucan II[2]	Paired *t*-Test for Comparison of Means	*P*
Pacific				
S. droebachiensis	45.3 ± 5.8	38.3 ± 9.2	$t = 0.712$	0.503
Atlantic				
S. droebachiensis	40.6 ± 6.5	44.2 ± 10.1	$t = -0.577$	0.580
S. pallidus	1.7 ± 0.3	1.0 ± 1.0	$t = 1.177$	0.305
S. purpuratus	0.67 ± 0.3	2.3 ± 1.5	$t = -1.387$	0.300

[1]% AR = percent of sperm having undergone the acrosome reaction (AR) in response to 100 μg hexose/ml of purified sulfated fucan, ± standard error (SE). The sperms' responses to the two isotypes did not differ significantly.
[2]The structures of sulfated fucan I and sulfated fucan II are shown in Fig. 5 and Fig. 4, respectively.

These isotypes of sulfated fucans could represent diferentiation within the species that might be a predecessor of incipient sympatric speciation

Physiological relevant isoforms of sulfated fucans in *Lytechinus variegatus*

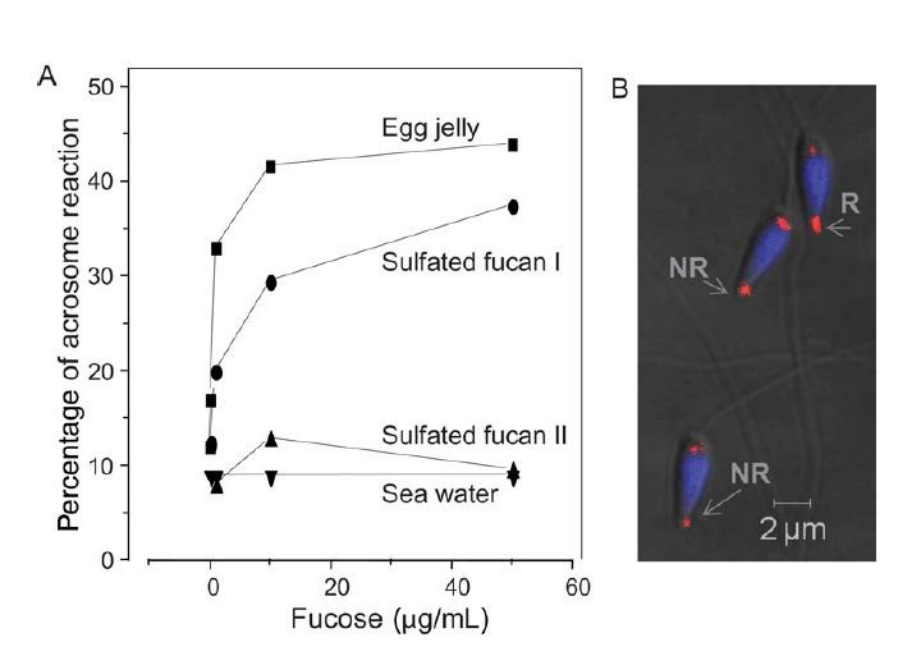




***Summer*: 70 females collected, all shown exclusively sulfated fucan P1.**

***Winter:* 45 females collected, 28 expressed predominantely isotype P1 and 17 secreted isotype P2.**

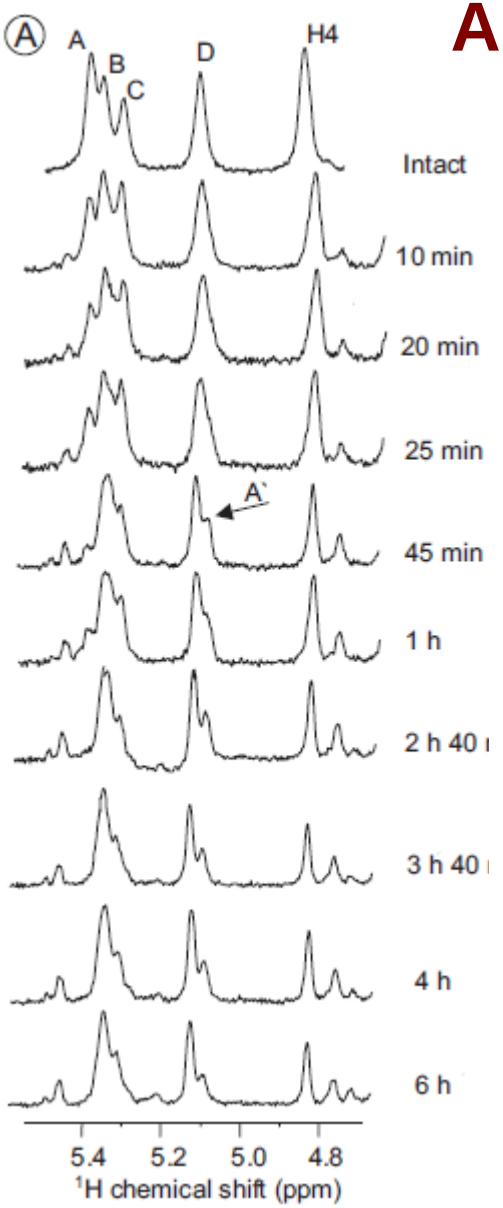
Induction of the acrosome reaction by the two sulfated fucan isoforms

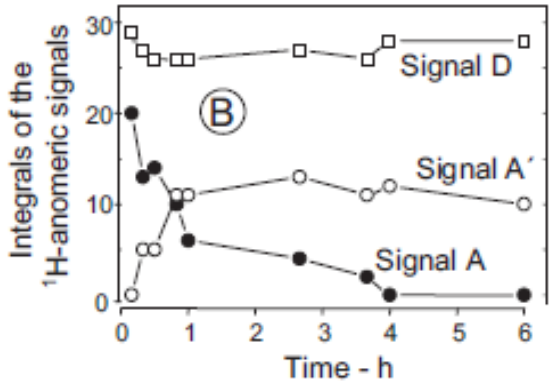


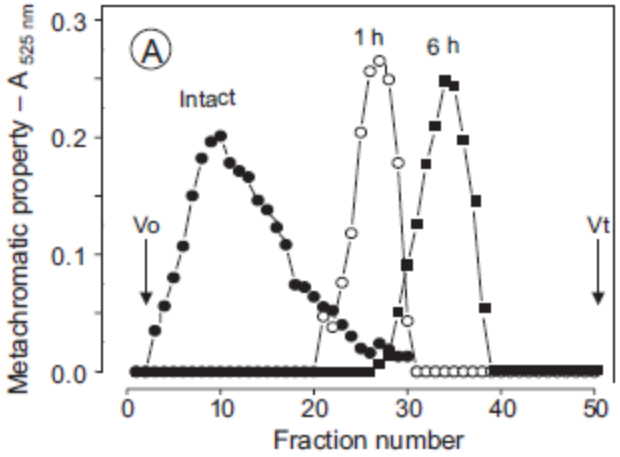
The two isotypes of sulfated fucans in the egg jelly of *L. variegatus*, which differ in their biological activity, maybe involved in the periodicity of the reprodutive cycle of the invertebrate

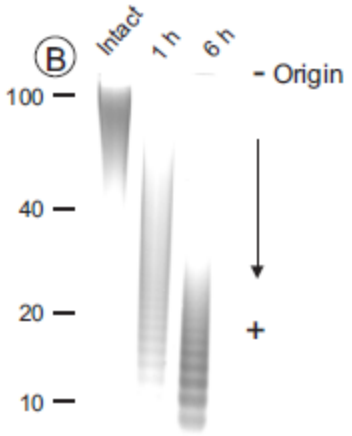
VI) Preparation of oligosaccharides from the sulfated fucans

A selective 2-desulfation reaction

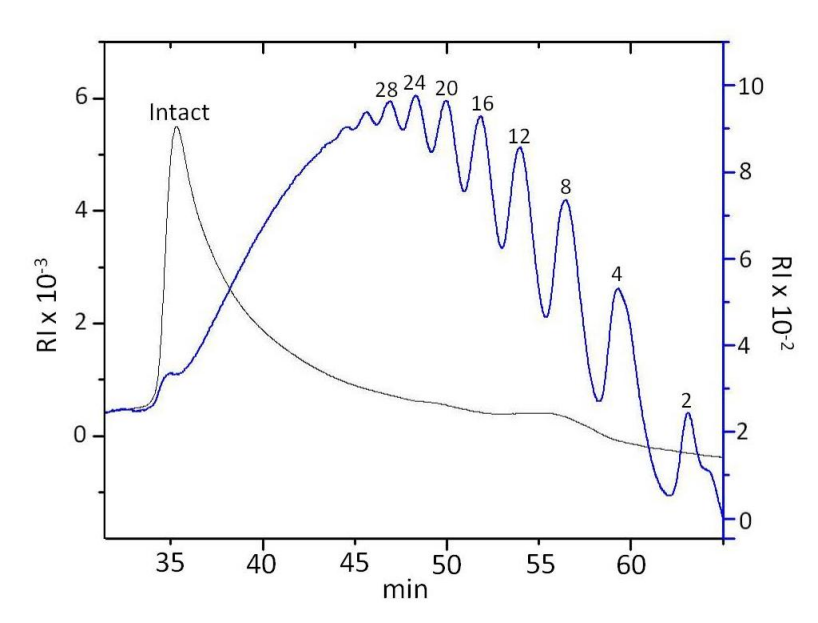


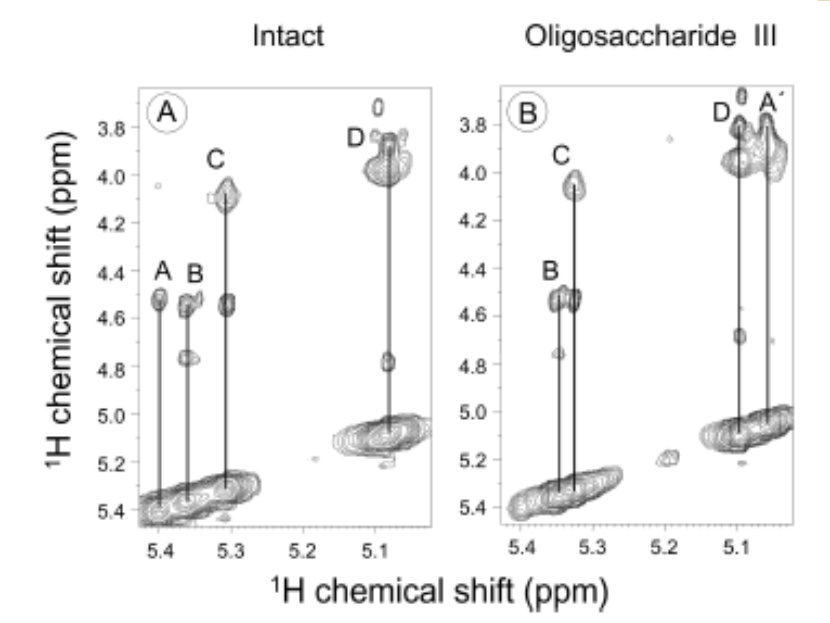






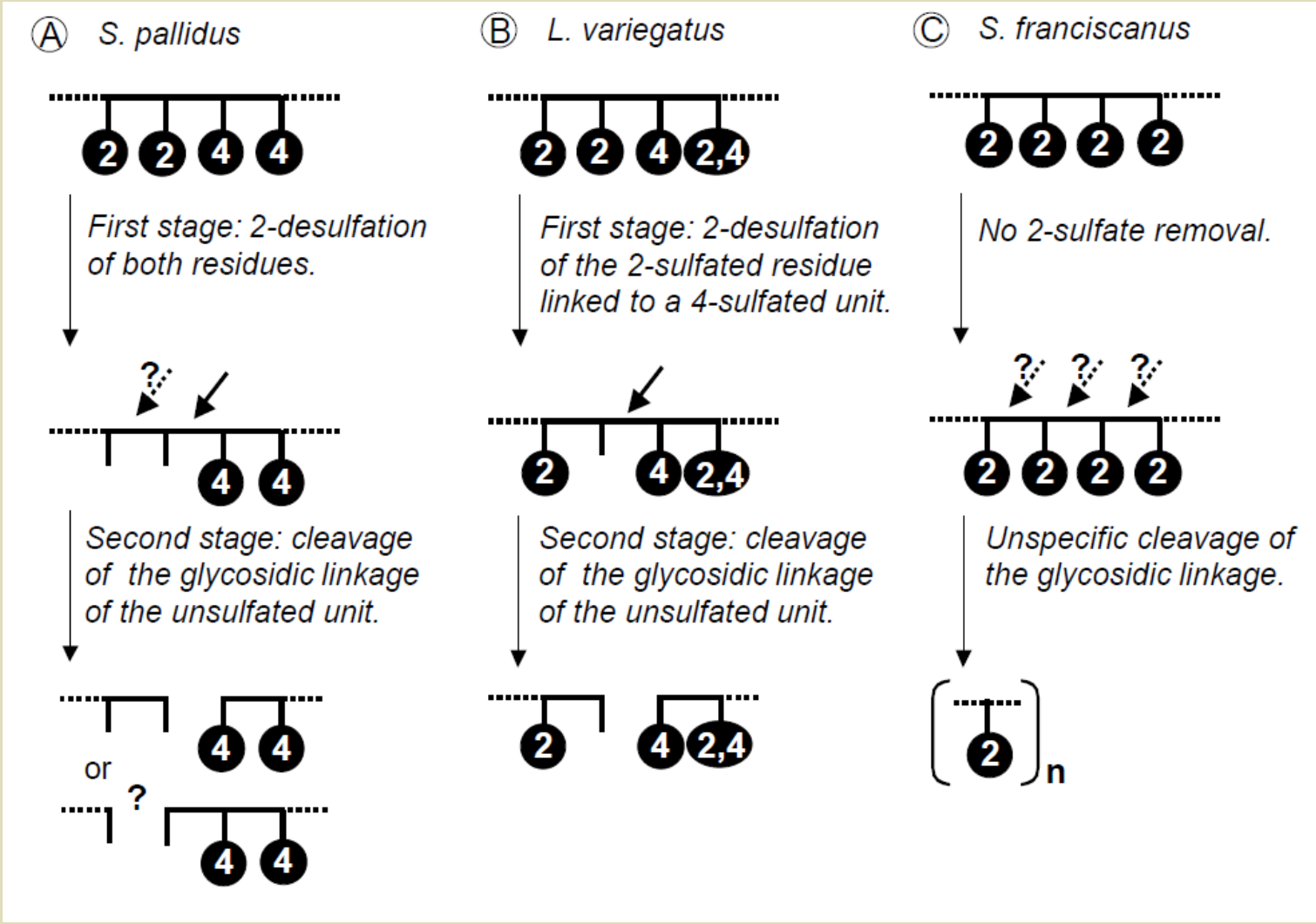
An alternative for preparing tailored sulfated oligosaccharides



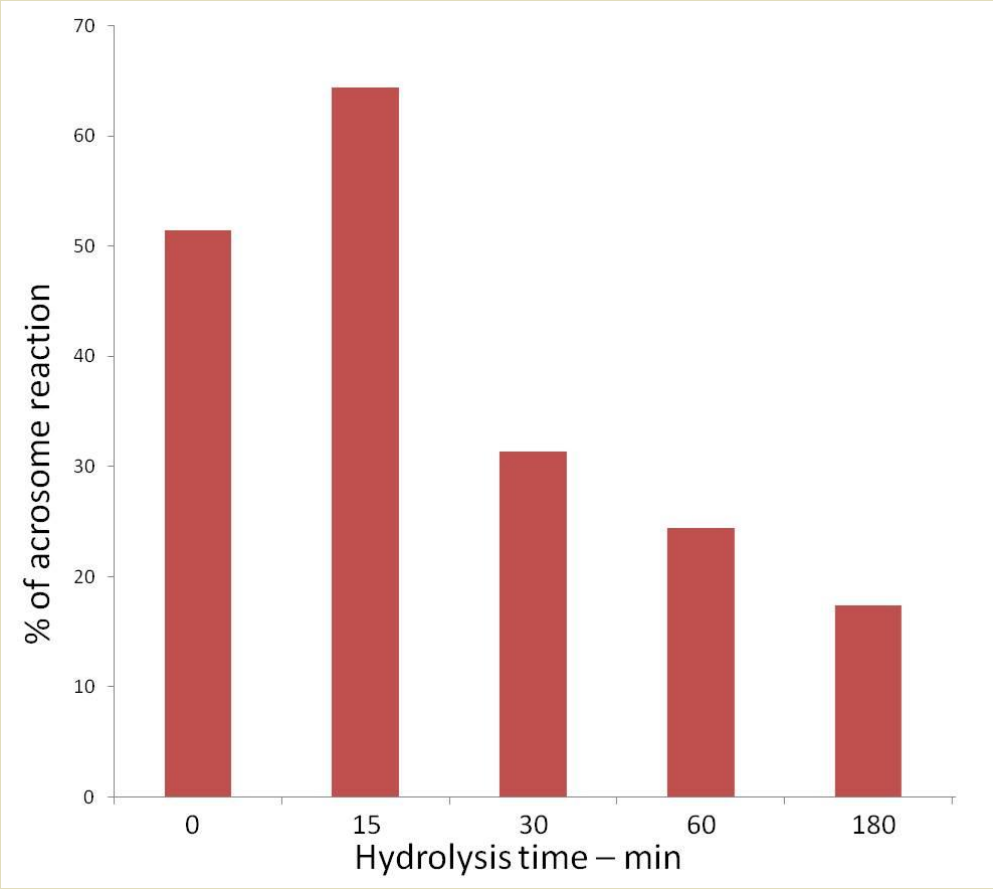


Maldi-MS suggests that oligosaccharides IV has four tetrasaccharides units composed of 4-fucose and 4 sulfated ester- each

Mild acid hydrolysis of sulfated fucans: a selective 2-desulfation reaction and na alternative for preparing tailored sulfated oligosaccharides

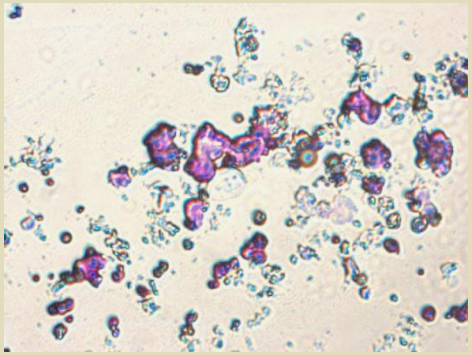


Decrease of molecular size reduces the effect of the sulfated fucan as inducer of acrosome reaction



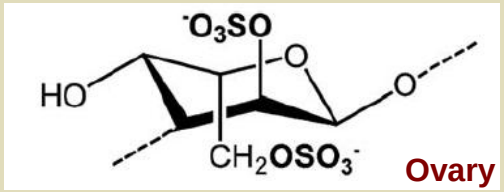
VII) Biosynthesis of the sea urchin polysaccharides

Biosynthesis of the sulfated galactan

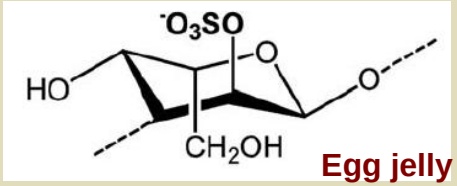


Accessory cells

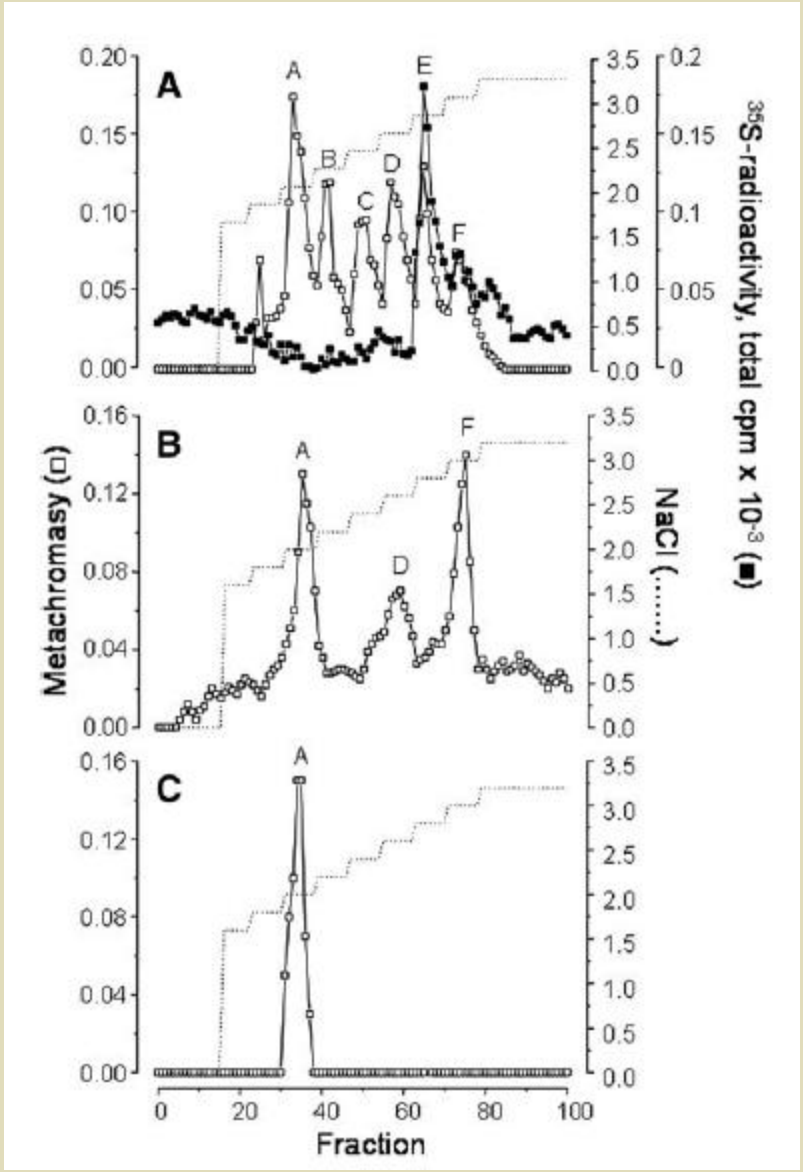
Oocyte



Ovary

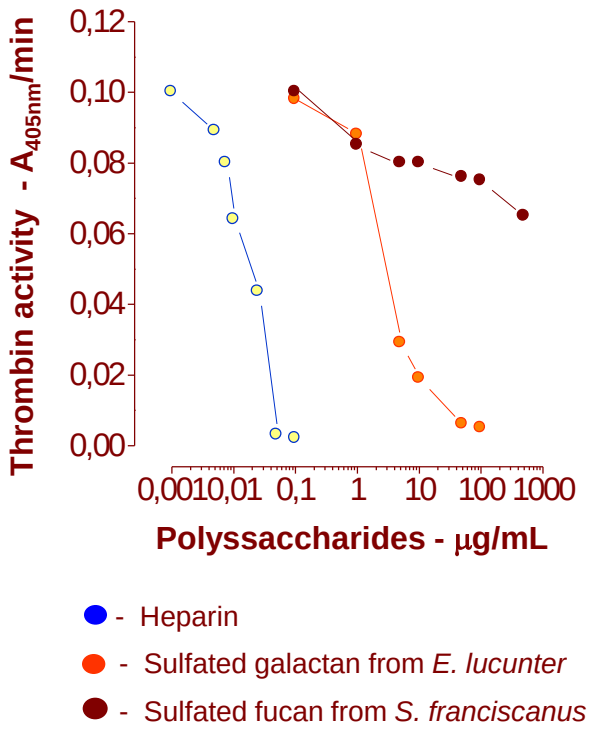


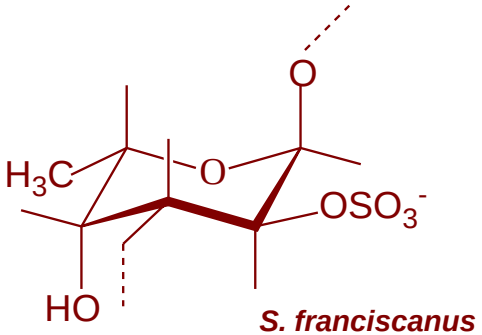
Egg jelly



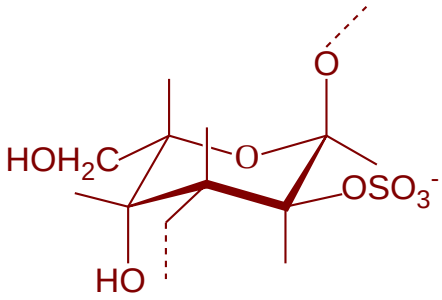
VIII) Medical applications

Interaction of sulfated polysaccharides with serpin (antithrombin)



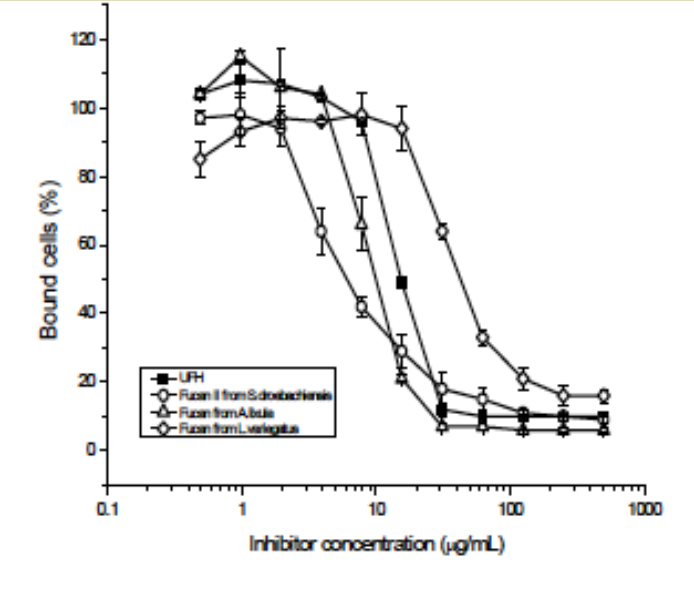


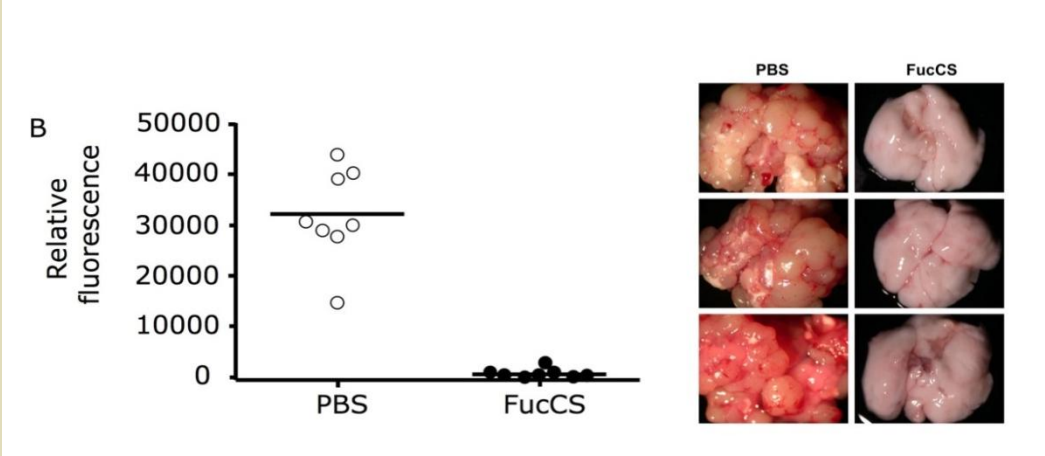
S. franciscanus



E. lucunter

Interaction of sulfated polysaccharides with P-selectin. Effect on tumorigenesis





Conclusions

1. The jelly coat surrounding sea urchin eggs is not a simple accessory structure.

2. It contains sulfated polysaccharides which modulate cell-cell recognition and species specificity leading to exocytose of the acrosome vesicle, the acrosome reaction.

3. The sulfated polysaccharide-mediated mechanism co-exists with that of bindin and its receptor in the egg.

Conclusions

4. The invertebrate polysaccharides can also be assayed as alternative anticoagulant and antitumoral agents and represent a new source of therapeutic agents.

5. The biological actions of sulfated polysaccharides do not simply depend on their negative charge density, but are also influenced by their structural features (sugar type, specific positons of sulfation and glycosilation...).

Challenges

1. Test of oligosaccharides as inducers of the acrosome reaction and as therapeutic agents.

2. Identification of receptor for the sulfated polysaccharides in the sperm membrane.

3. Identification of the metabolic pathways involved in the biosynthesis of the egg jelly polysaccharides.

Acknowledgements

Ana Cristina E.S. Vilela-Silva
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