



THE MOLECULAR BASIS OF THE ACTIVATION OF FIBROBLAST GROWTH FACTORS BY GLYCOSAMINOGLYCANS

FESTKOLLOQUIUM

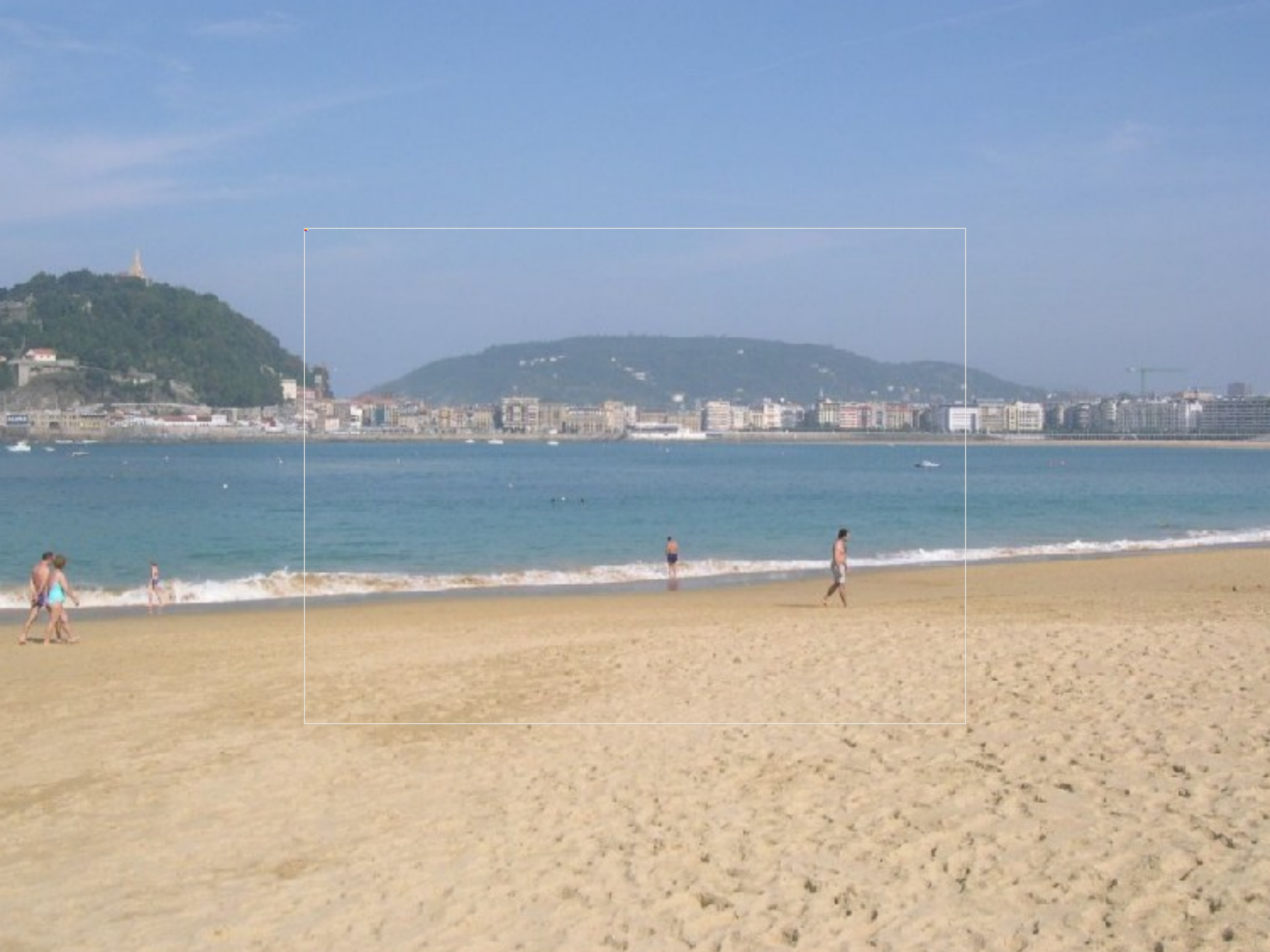
on the occasion of the 85th Birthday of

Prof. Dr. rer. nat. Hans Paulsen, Hamburg 2007















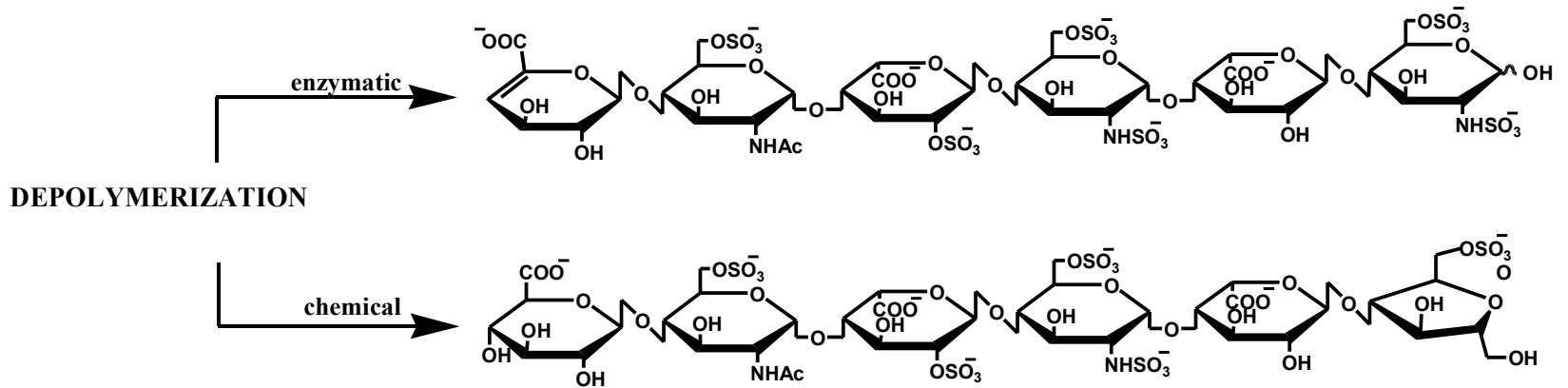
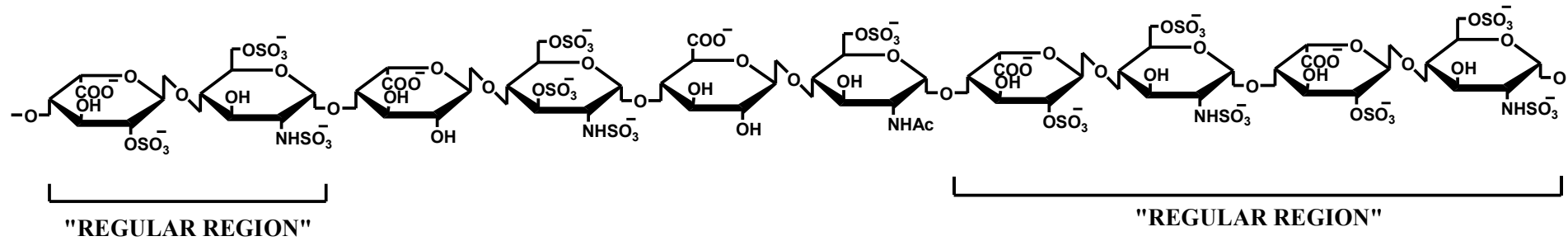
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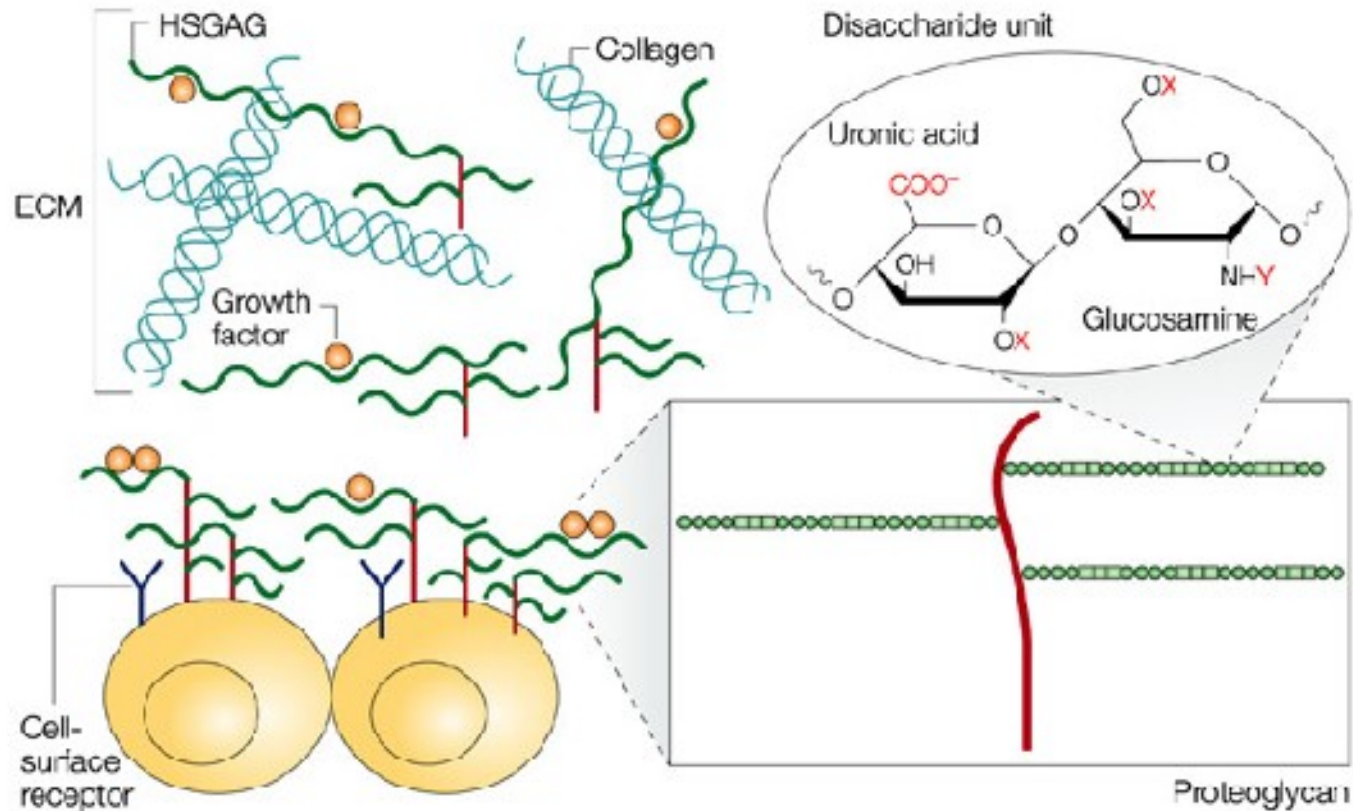
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Structure of heparin



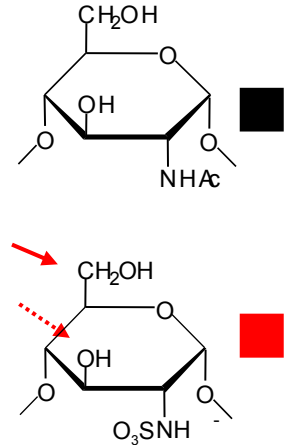
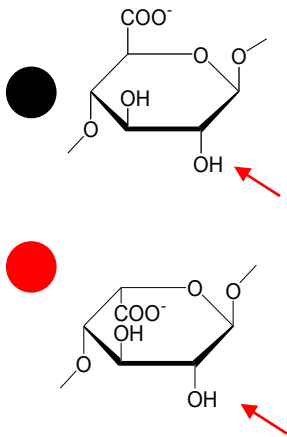
Structure and biology of heparan-sulphate glycosaminoglycans



Nature Reviews | Cancer

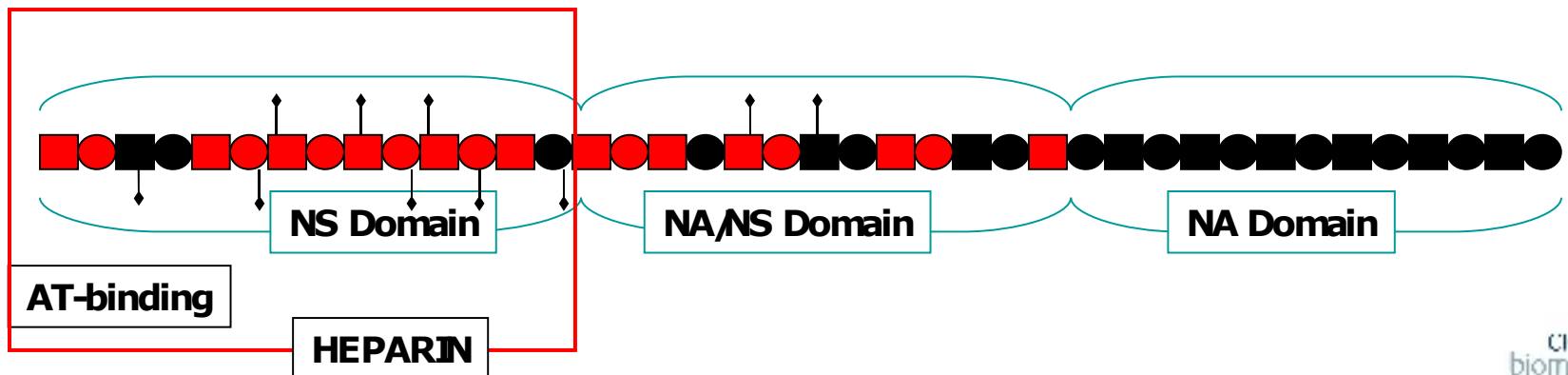
CHEMICAL STRUCTURE OF HEPARIN-HS

Uronic acid (1-4) glucosamine

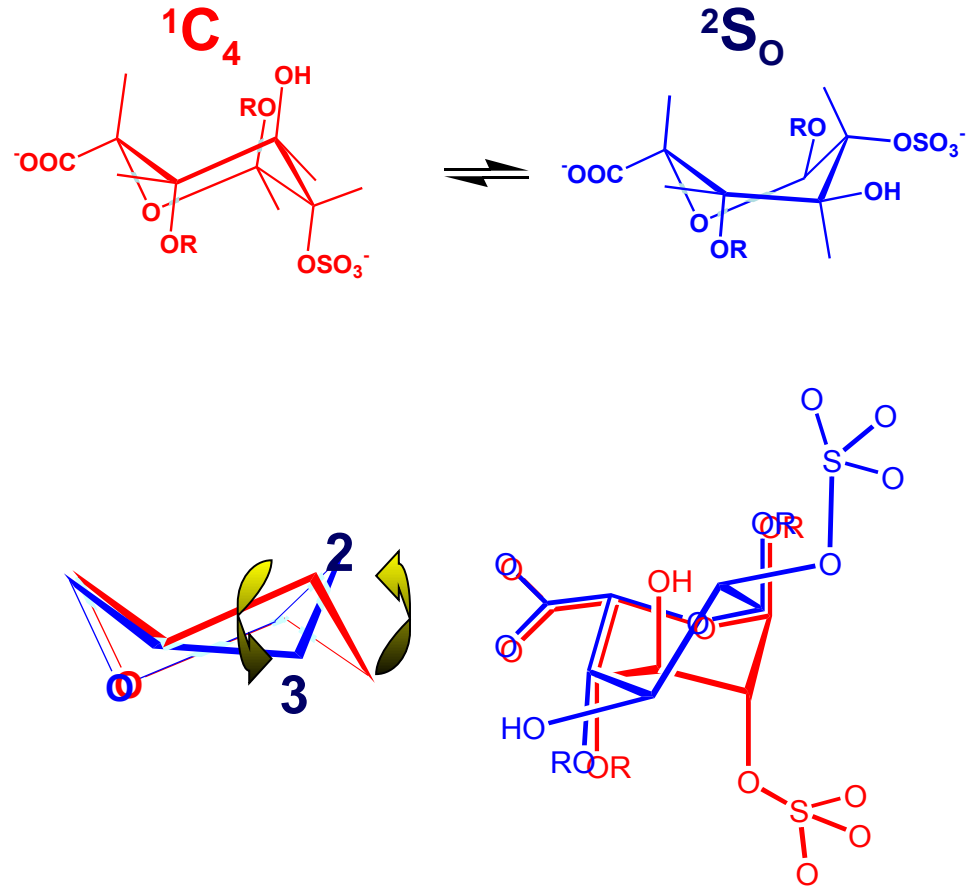
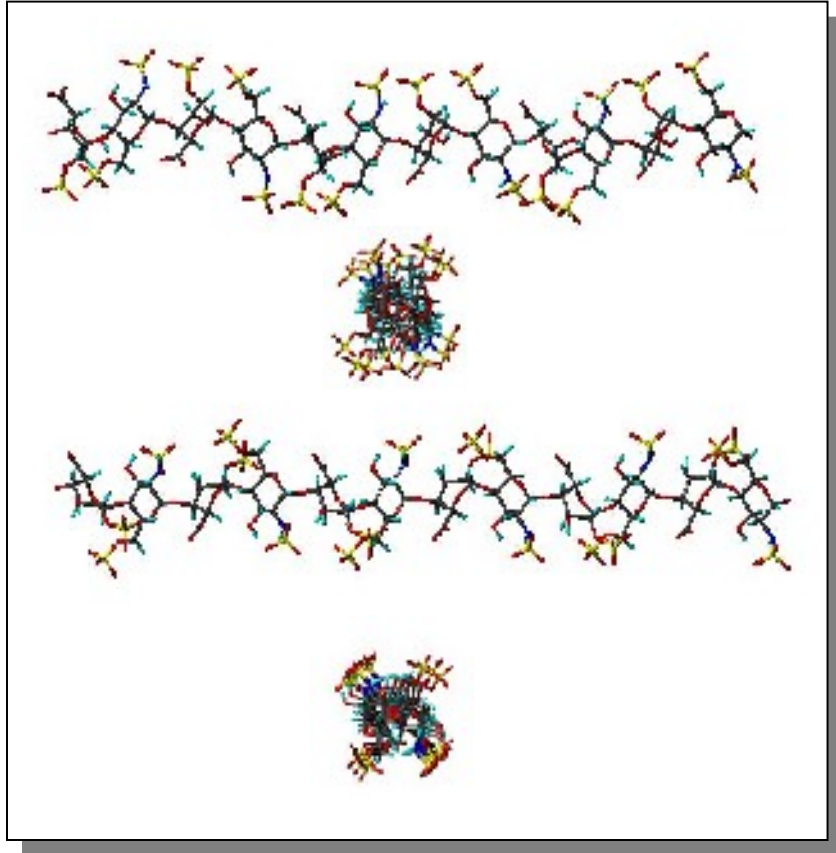


D-glucuronic	2-Sulfate	D-glucosamine	N-Sulfate
L-iduronic	2-Sulfate		6-Sulfate
			N-acetyl
			3-Sulfate
			free amine

Domain	Major sequence	Exclusive sequence
NA	GlcA - GlcNAc	GlcNH ₂
NS	GlcA / IdoA - 6S-GlcNS	2S-IdoA , 3S-GlcN
NA/NS	NA + NS	

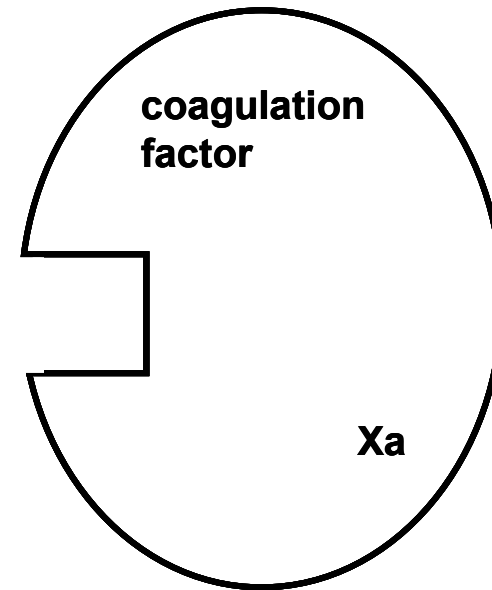
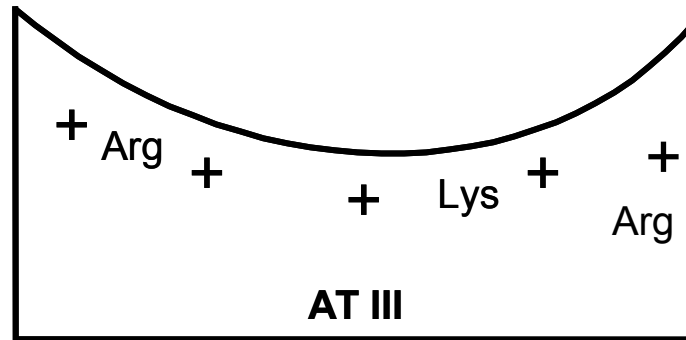
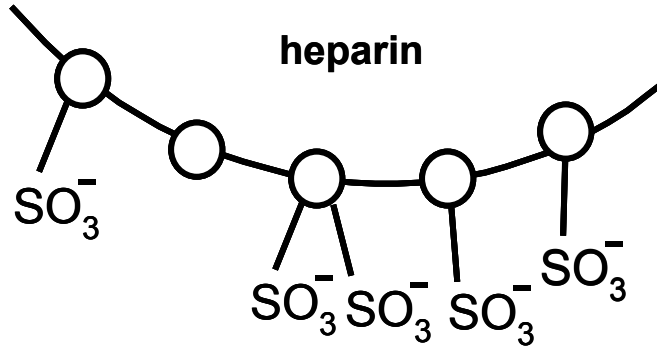


THREE DIMENSIONAL STRUCTURE OF HS-GAGs

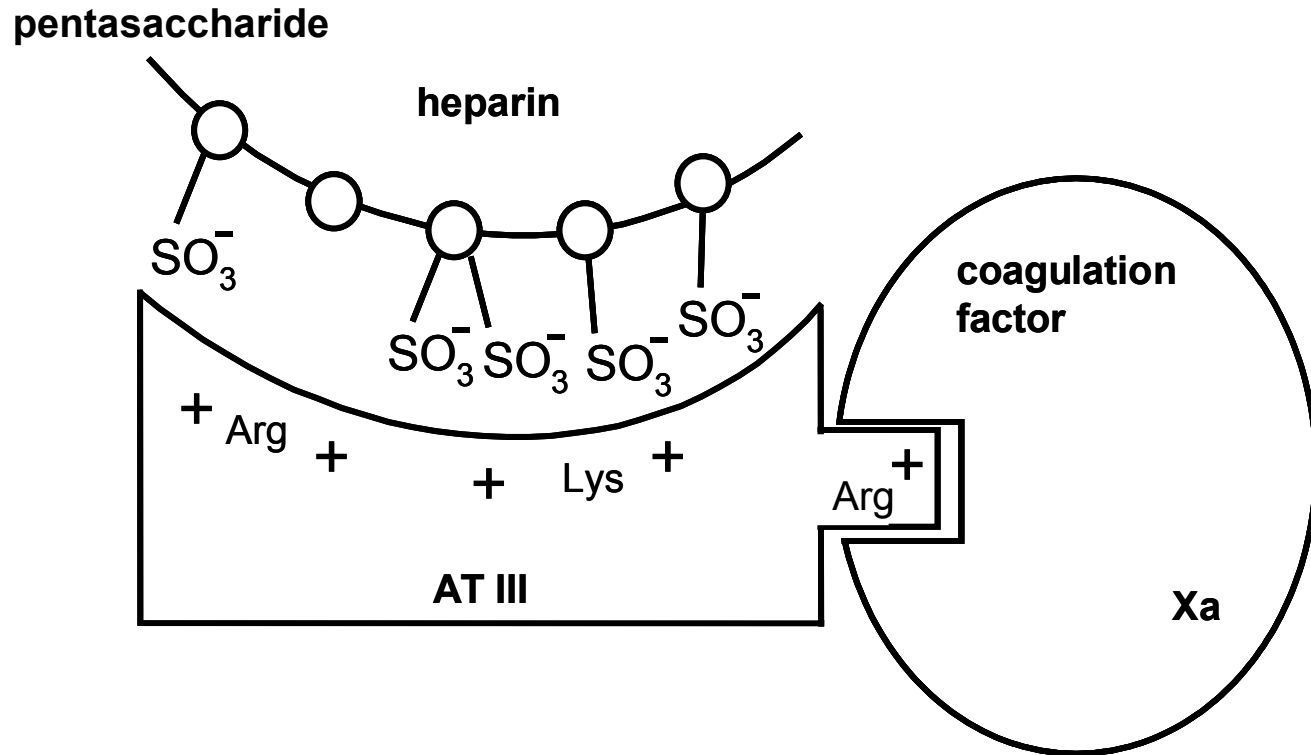


The interaction of heparin with AT III

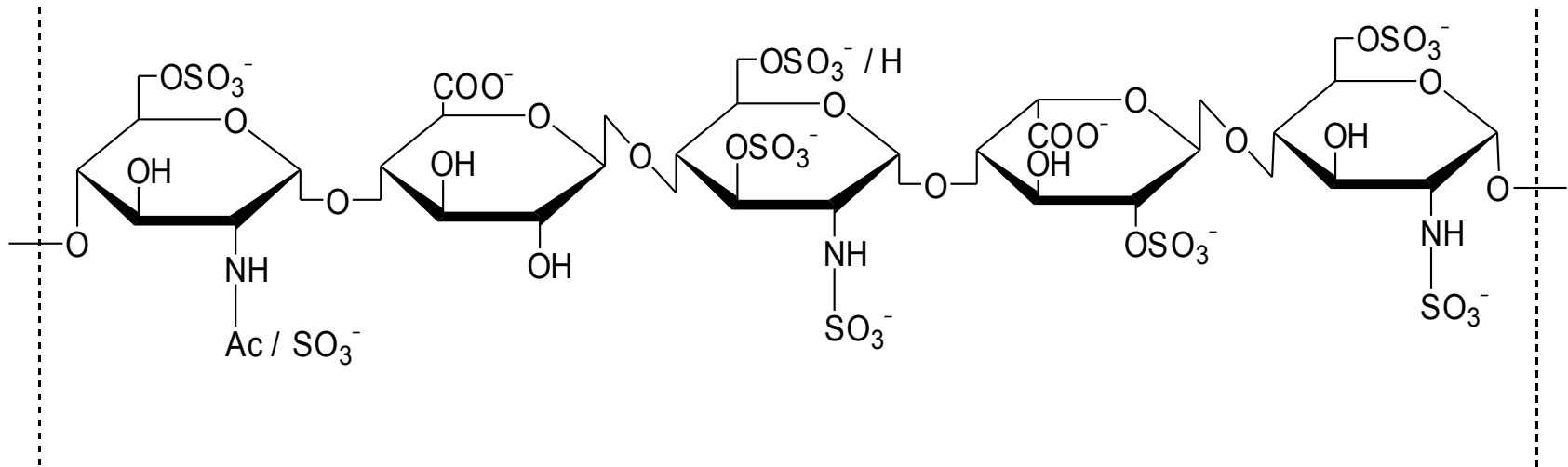
pentasaccharide



The interaction of heparin with AT III



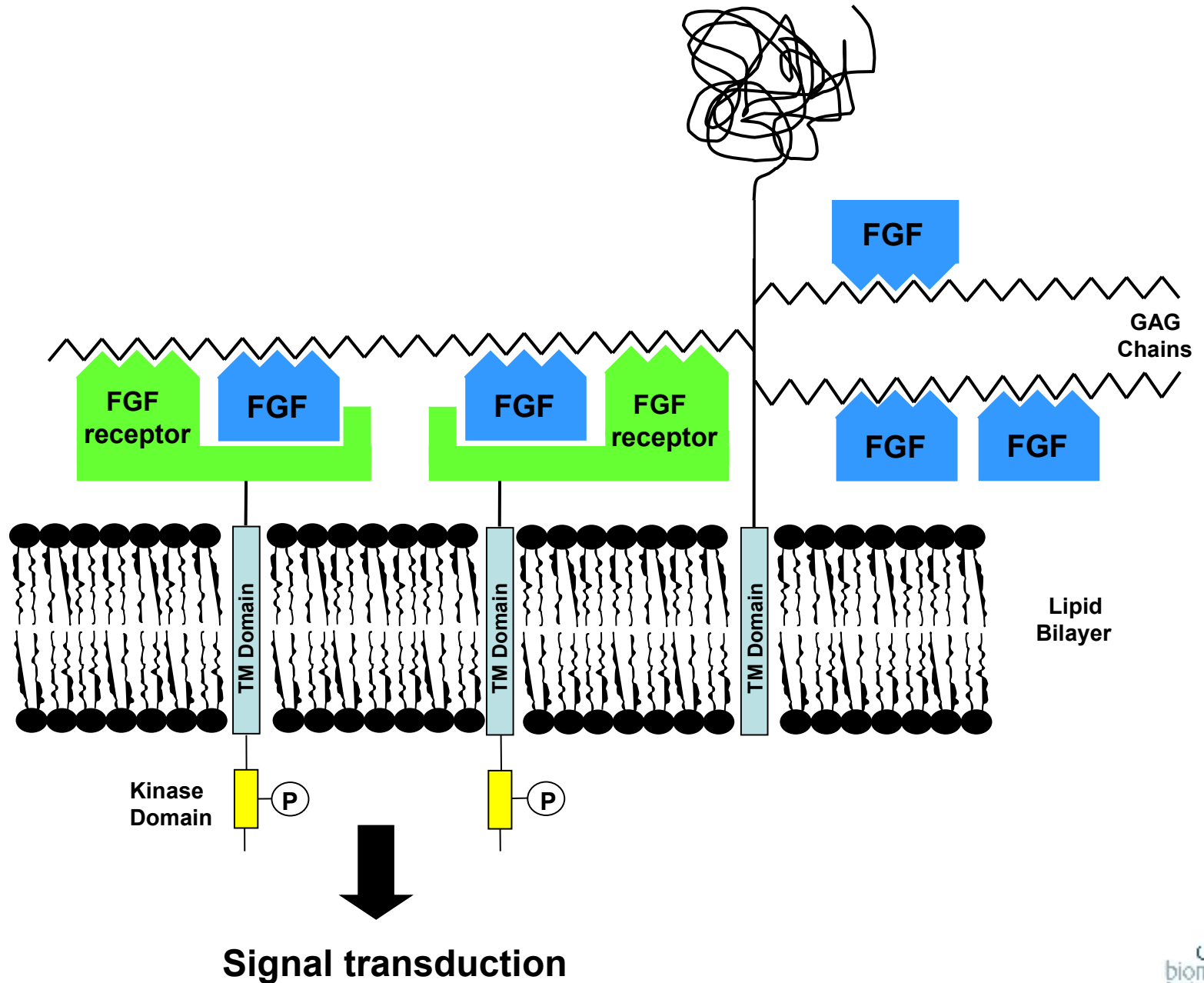
Structure of the AT III binding pentasaccharide



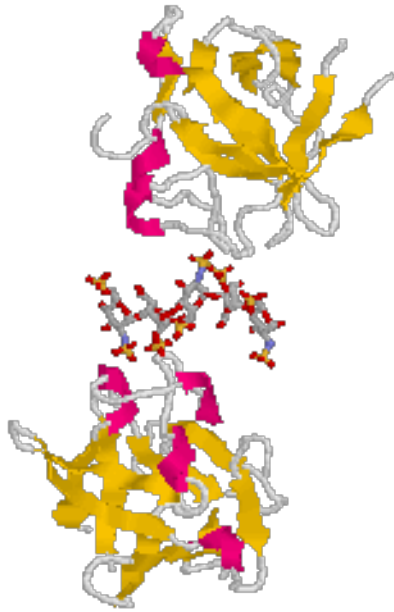
Characteristics of selected heparin-binding proteins

Heparin-binding protein	Physiological/Pathological role	Characteristics of heparin binding			
		K_d	Oligosaccharide size	Sequence features	Function
<i>proteases/esterases</i>					
AT III	coagulation cascade serpin	ca. 20 nM	5-mer	GlcNS6S3S	enhances
SLPI	inhibits elastase and cathepsin G	ca. 6 nM	12-mer to 14-mer	IS	enhances
C1 INH	inhibits C1 esterase	ca. 100 nM	-	HS	enhances
VCP	protects host cell from complement	nM	-		unclear
<i>growth factors</i>					
FGF-1	cell proliferation, differentiation, morphogenesis, and angiogenesis	nM	4-mer to 6-mer	IdoA2S-GlcNS6S	activates signal transduction
FGF-2	(same as FGF-1)	nM	4-mer to 6-mer	IdoA2S-GlcNS	(same as FGF-1)
<i>chemokines</i>					
PF-4	inflammation and wound healing	nM	12-mer	HS/LS/HS	inactivates heparin
IL-8	pro-inflammatory cytokine	ca. 6 μ M	18-mer to 20-mer	HS/LS/HS	promotes
SDF-1 α	pro-inflammatory mediator	ca. 20 nM	12-mer to 14-mer	HS	localizes
<i>lipid-binding proteins</i>					
Annexin II	receptor for TPA and plasminogen, CMV and tenascin C	ca. 30 nM	4-mer to 5-mer	HS	unclear
Annexin V	anticoagulant activity; influenza and hepatitis B viral entry	ca. 20 nM	8-mer	HS	assembles
ApoE	lipid transport; AD risk factor	ca. 100 nM	8-mer	HS	localizes
<i>pathogen proteins</i>					
HIV-1 gp120	viral entry	0.3 μ M	10-mer	HS	inhibits
CypA	viral localization and entry	-	-	-	inhibits
Tat	transactivating factor, primes cells for HIV infection	ca. 70 nM	6-mer	HS	antagonizes
HSV gB and gC	viral entry into cell	-	-	-	inhibits
HSV gD	viral entry and fusion	-	-	GlcNH ₂ 3S	inhibits
Dengue virus envelope protein	viral localization	ca. 15 nM	10-mer	HS	inhibits
Malaria CS protein	sporozoite attachment to hepatocytes	ca. 40 nM	10-mer	HS	inhibits
<i>adhesion proteins</i>					
selectins	adhesion, inflammation, and metastasis	μ M	\geq 4-mer	HS with GlcNH ₂	blocks
vitronectin	cell adhesion and migration	μ M	-	-	removes
fibronectin	adhesion and traction	μ M	8-mer to 14-mer	HS with GlcNS	reorganizes
HB-GAM	neurite outgrowth in development	ca. 10 nM	16-mer to 18-mer	HS	mediates
AP	in amyloid plaque	μ M	4-mer	HS	assembles

Schematic Diagram of a Ternary Complex FGF-GAG-FGFR



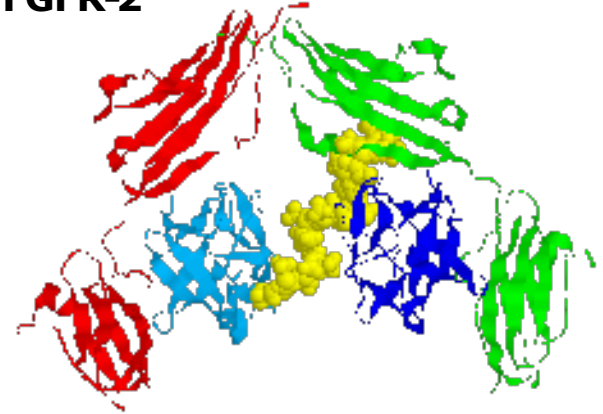
FGF Oligomerization and Ternary Complexes



**FGF2 –
FGFR-1**



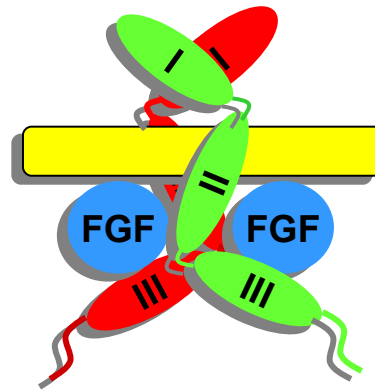
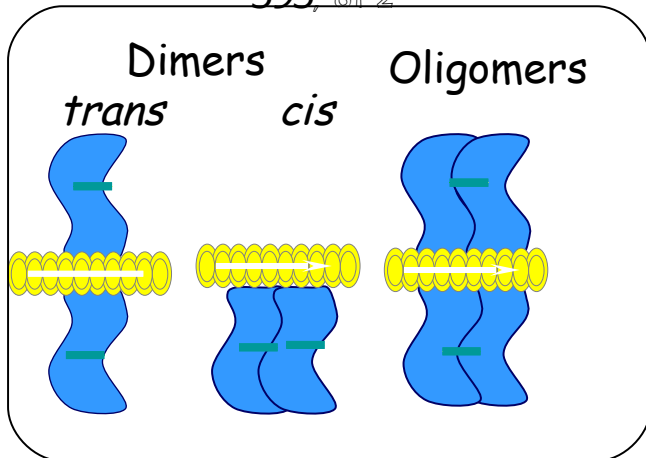
**FGF1 –
FGFR-2**



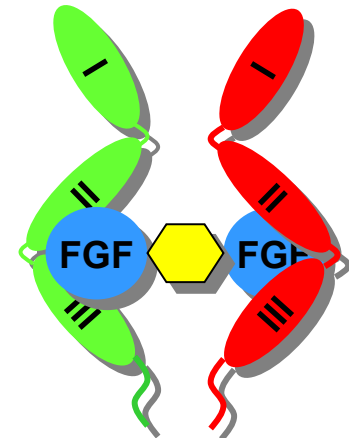
J. SCHLESSINGER, *Mol. Cell*, **2000**,
6, 743

L. PELLEGRINI, *Nature*, **2000**, 407,
1029

A. D. DIGABRIELE, *et al*, *Nature*, **1998**,
393, 812

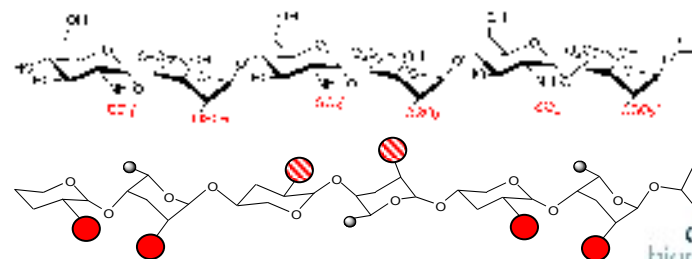
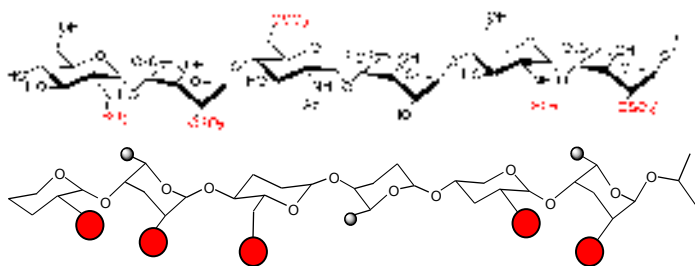
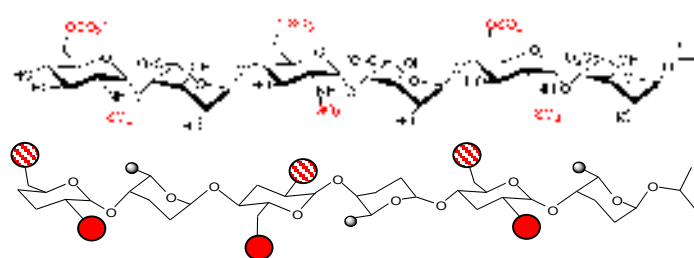
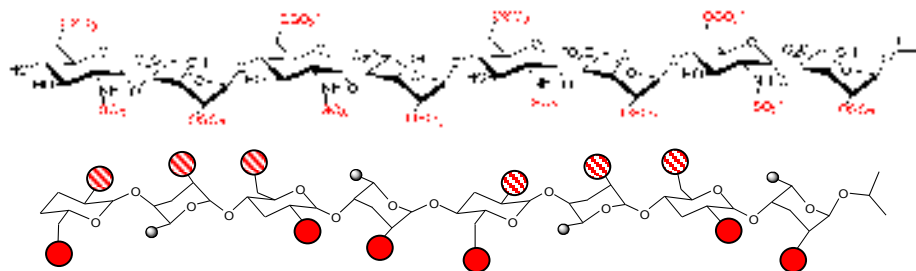
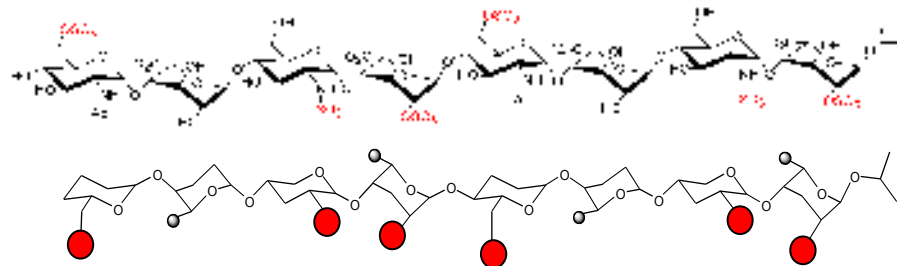
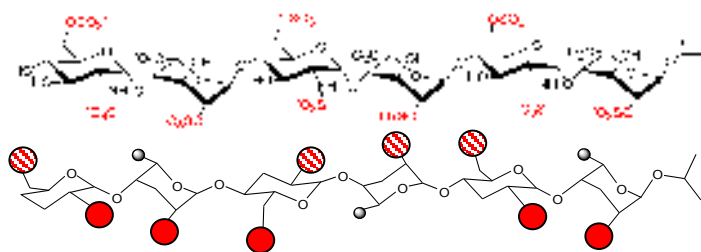


FGF
FGFR
FGFR
HS GAG

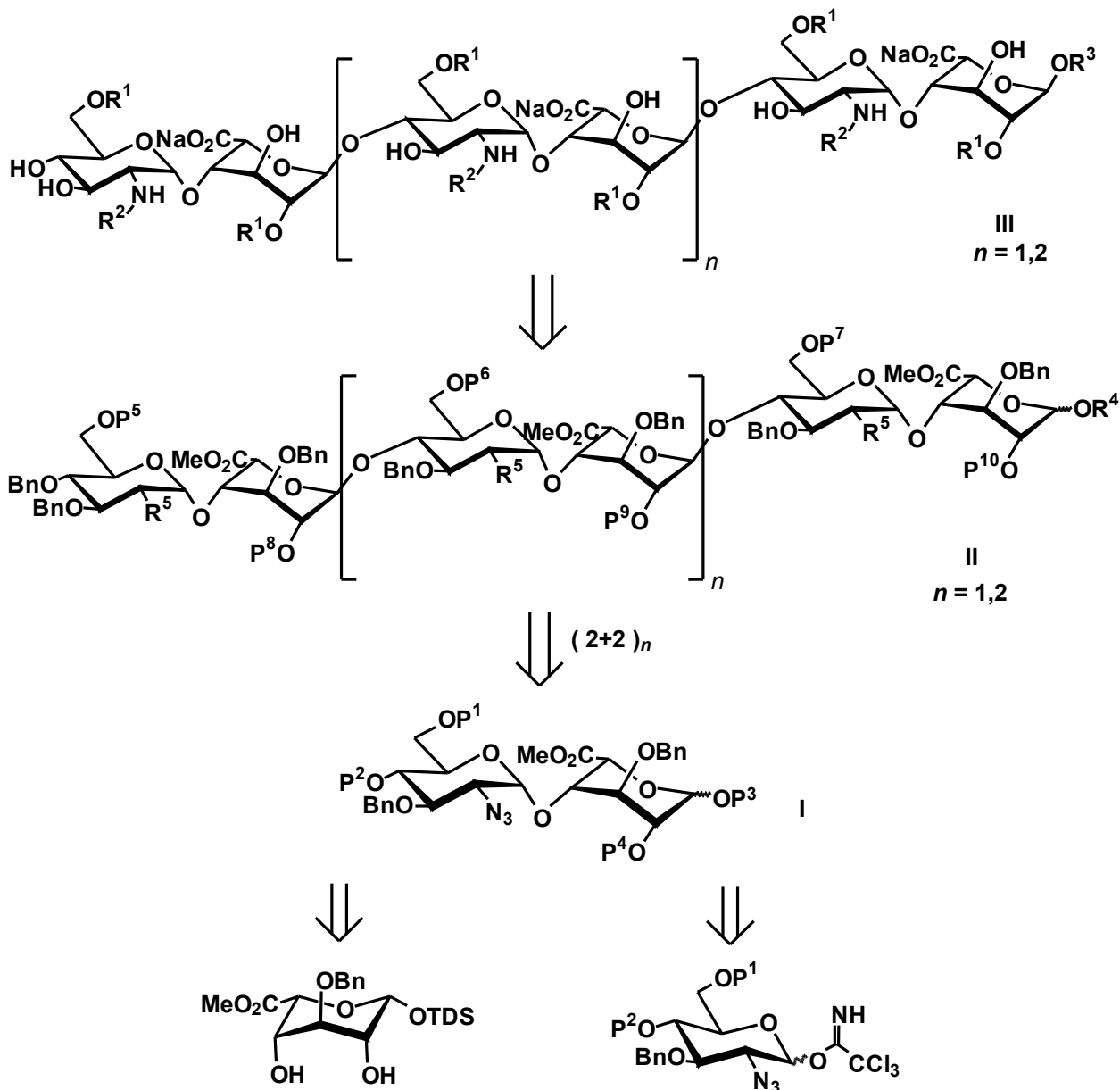


Heparin-like oligosaccharides

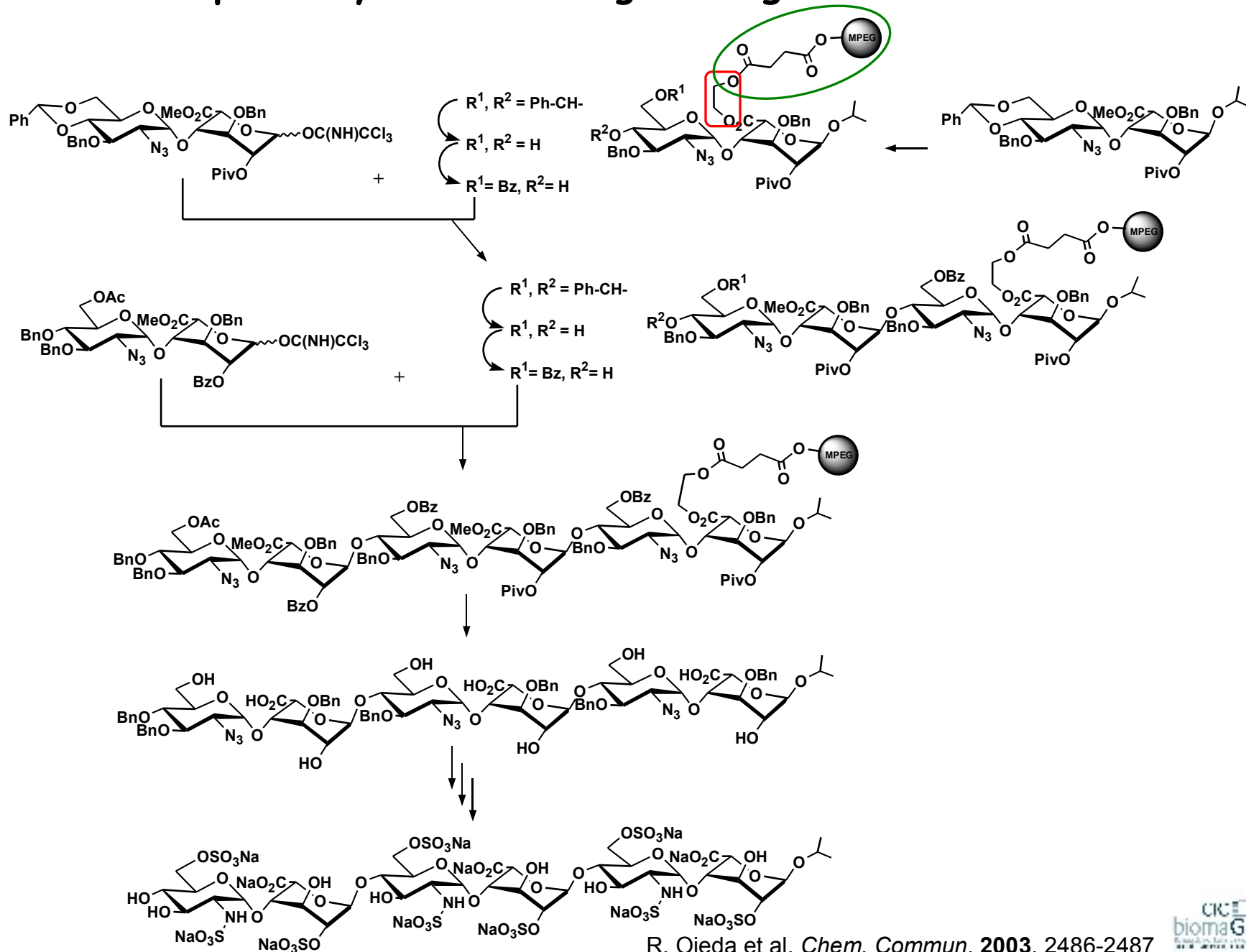
F E D C B A



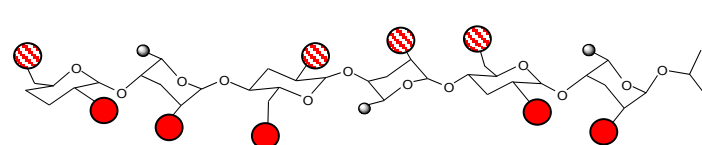
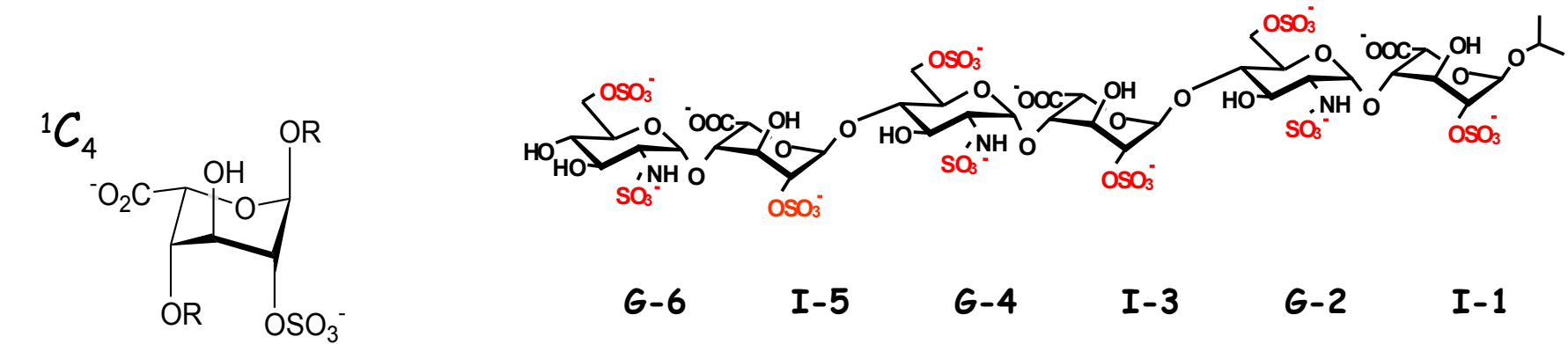
Retrosynthetic Analysis



Solid phase synthesis of regular region hexasaccharide

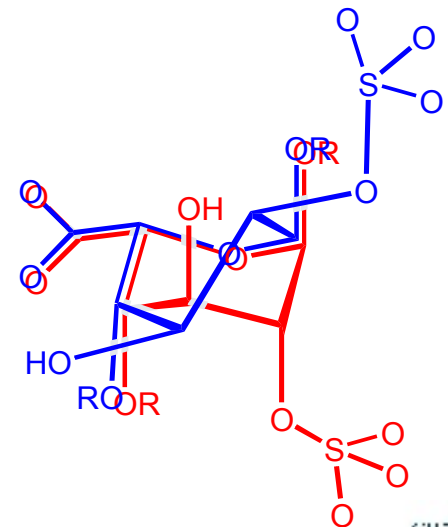


NMR Studies of the regular region hexasaccharide Conformation of the iduronate units

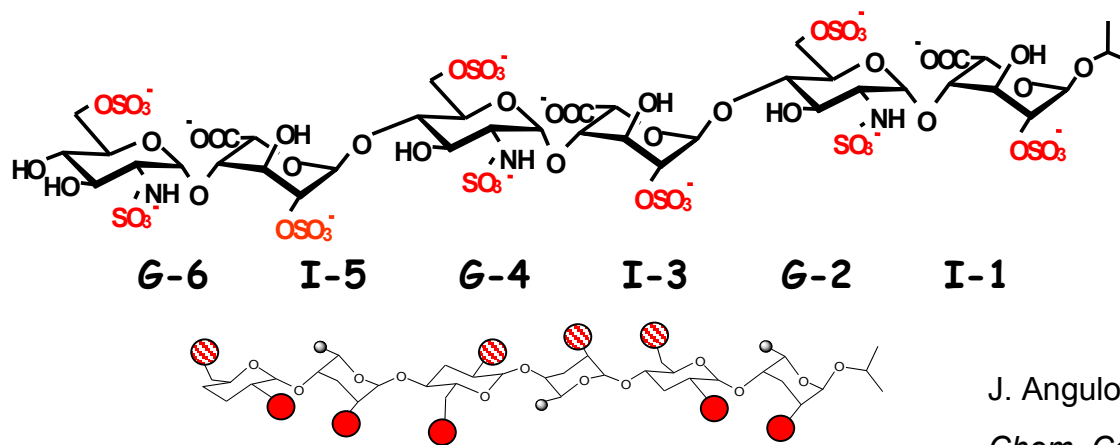


Conformational populations

	${}^1C_4 : {}^2S_0$
IdoA-1	70:30
IdoA-3	69:31
IdoA-5	72:28

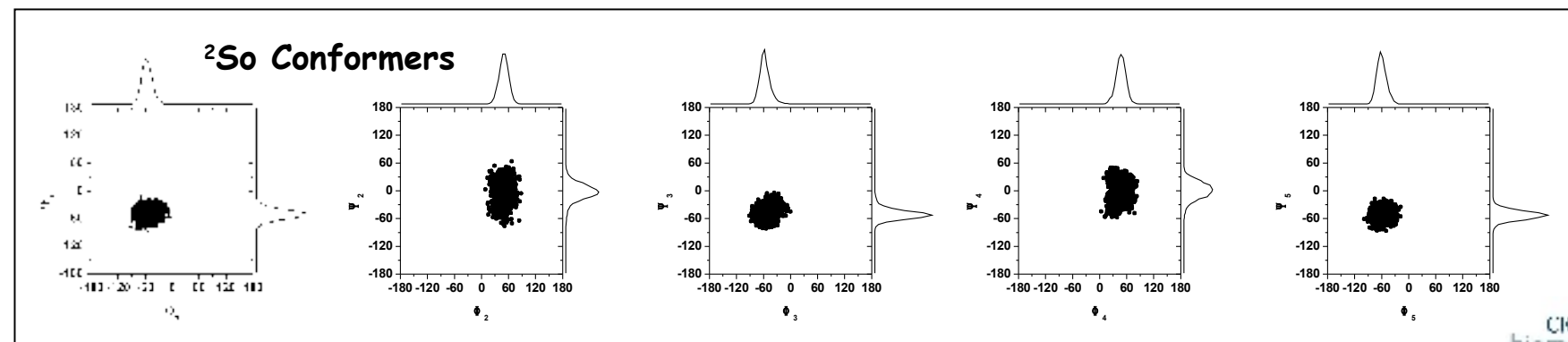
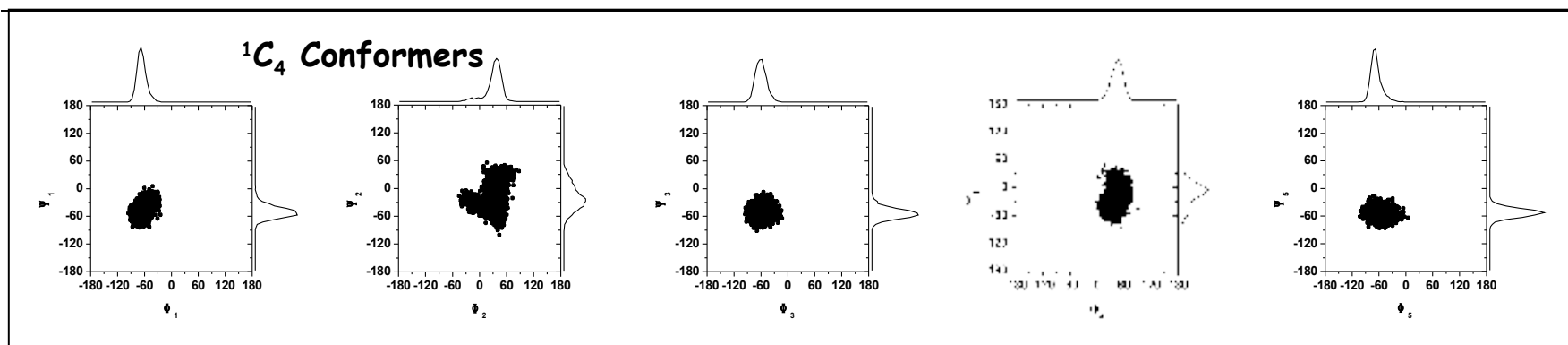


Molecular dynamics (PME) of the regular region hexasaccharide

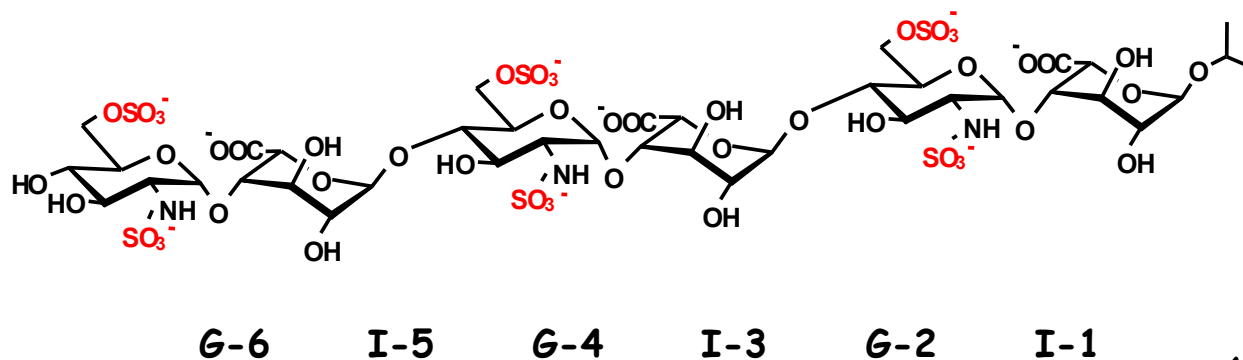


J. Angulo et al.

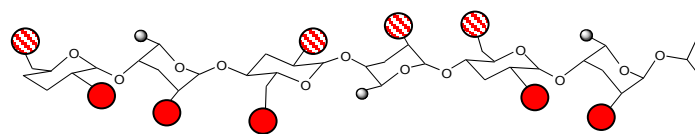
Chem. Commun. **2003**, 1512-1513



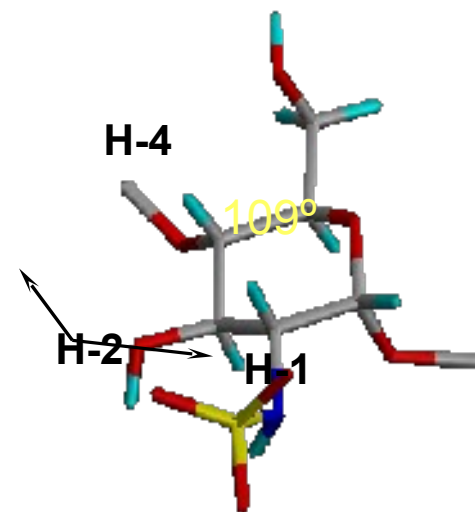
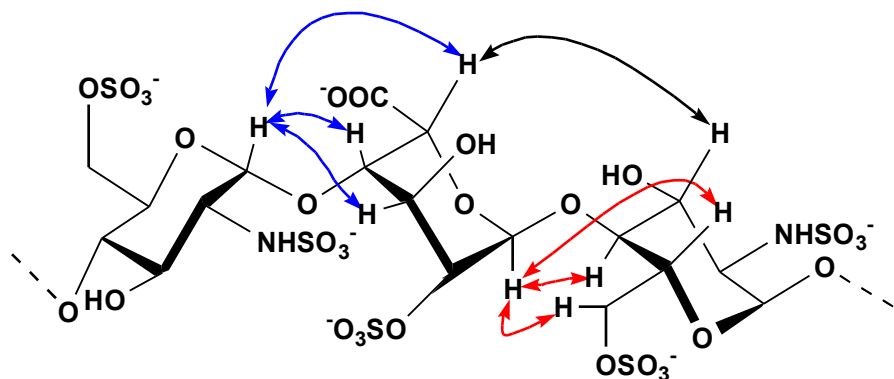
NMR Studies of the Regular Region Hexasaccharide



Anisotropy

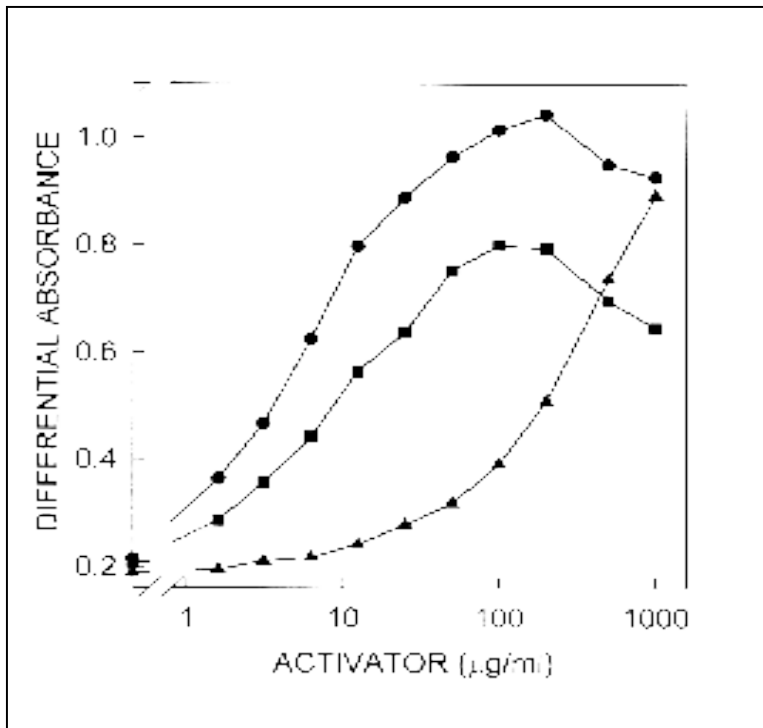
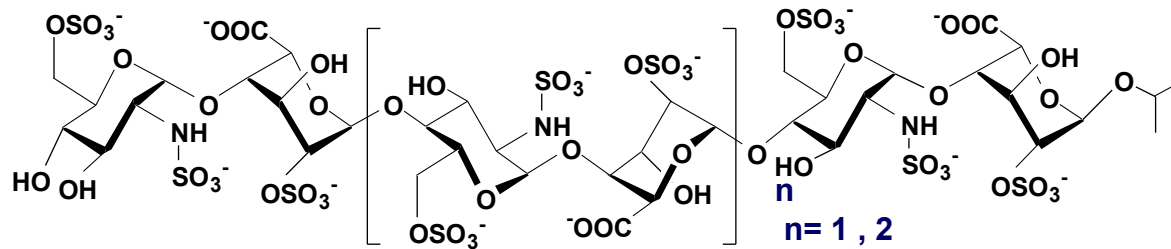


Interglycosidic NOEs



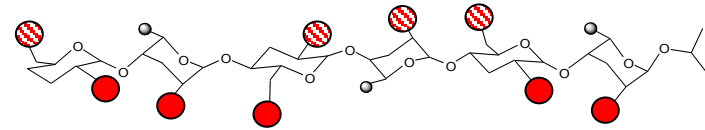
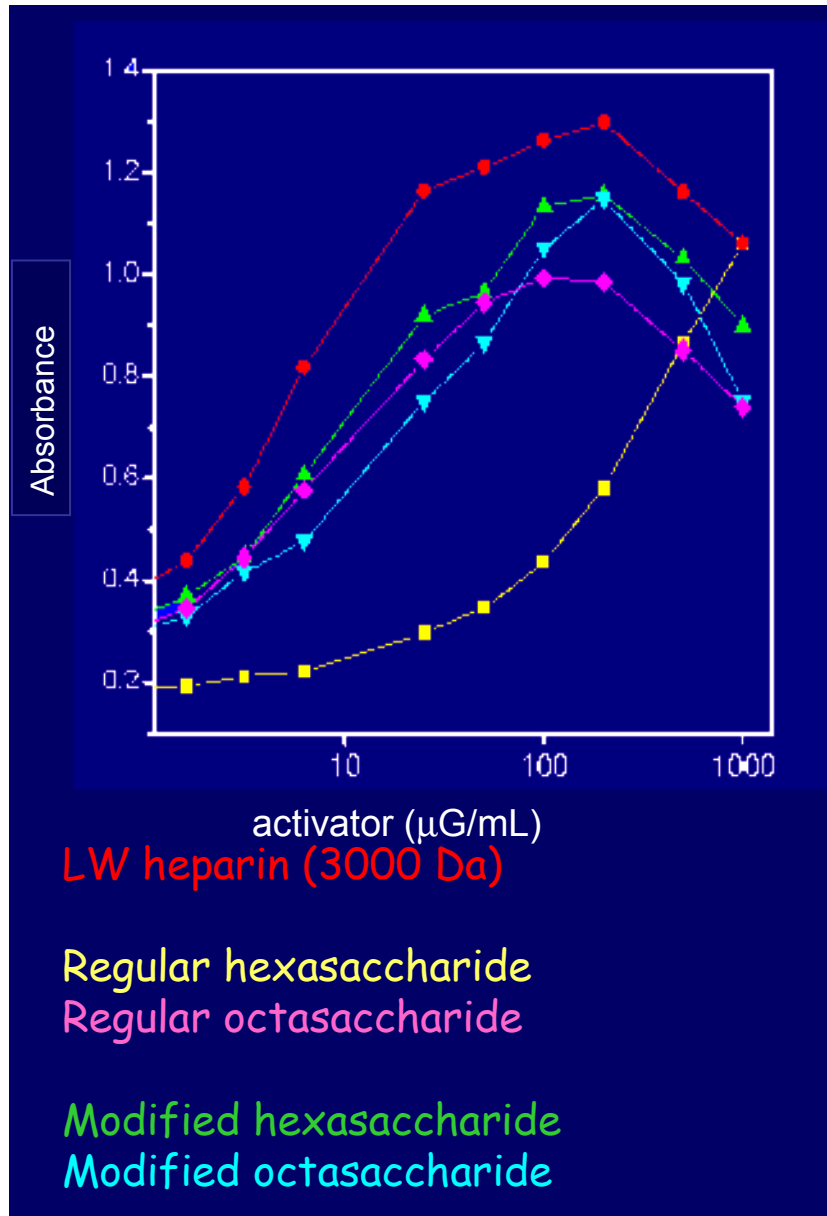
Pair	Distance (Å)	$\tau_{\text{isotropic}}$ (ps)
H1 - H2	2.4	700
H2 - H4	2.5	1300

Mitogenic activity of the regular region oligosaccharides

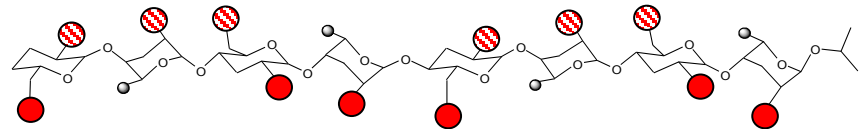


- LM heparin (3000 Da)
- ▲ HEXASACCHARIDE
- OCTASACCHARIDE

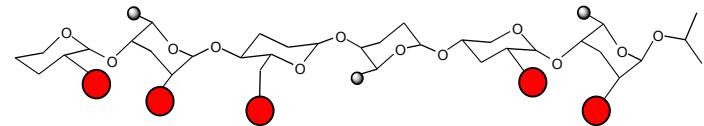
Mitogenic activity



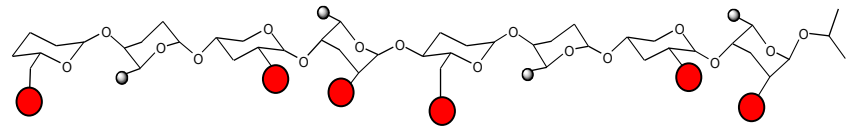
Regular hexasaccharide



Regular octasaccharide

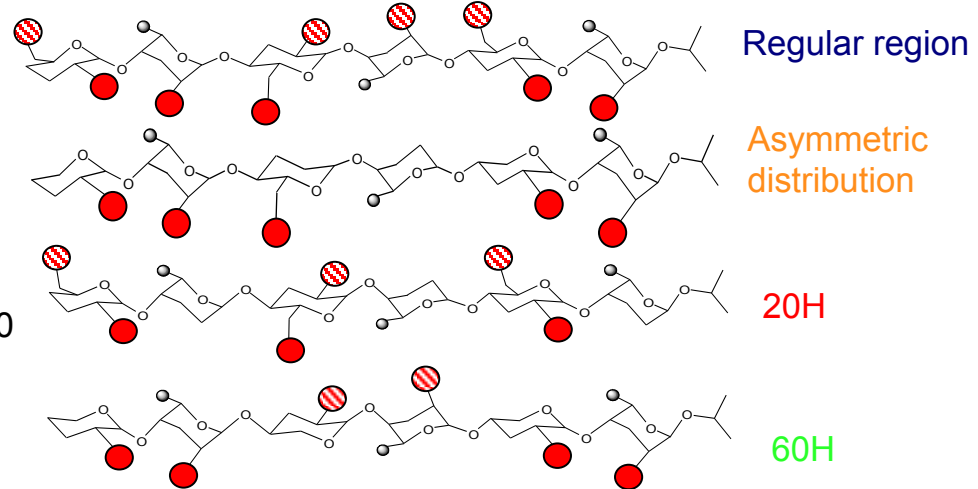
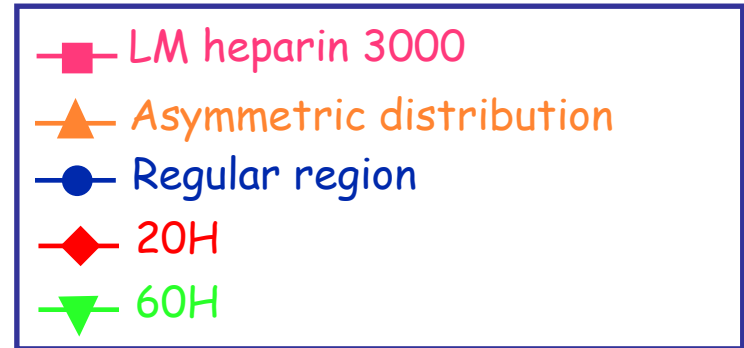
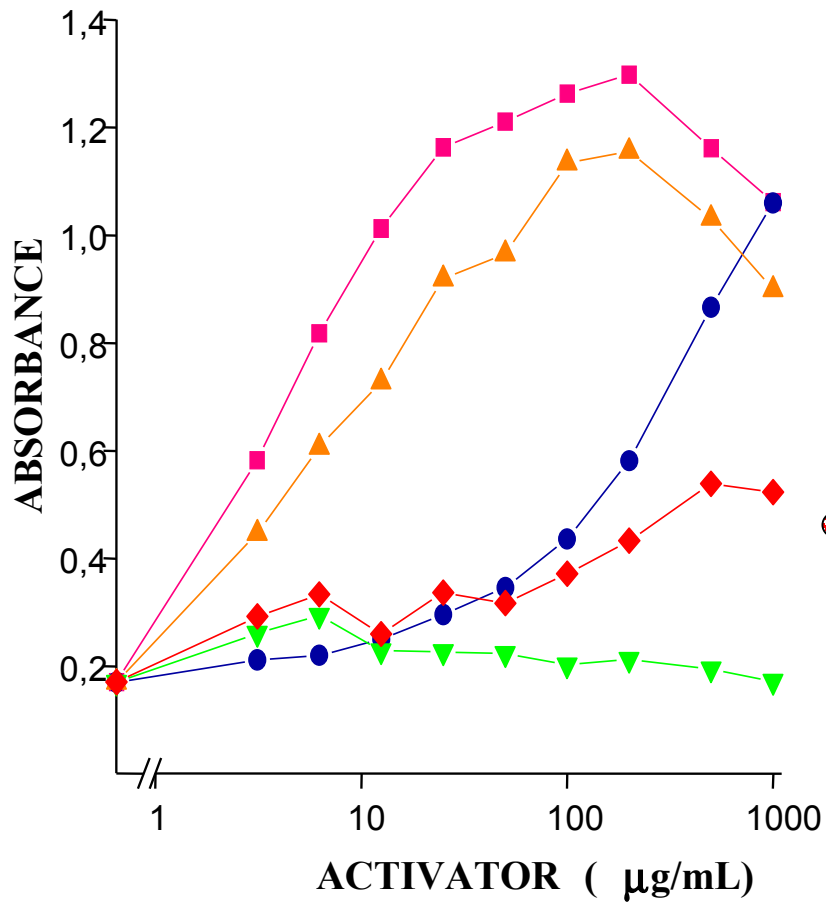


Asymmetric hexasaccharide

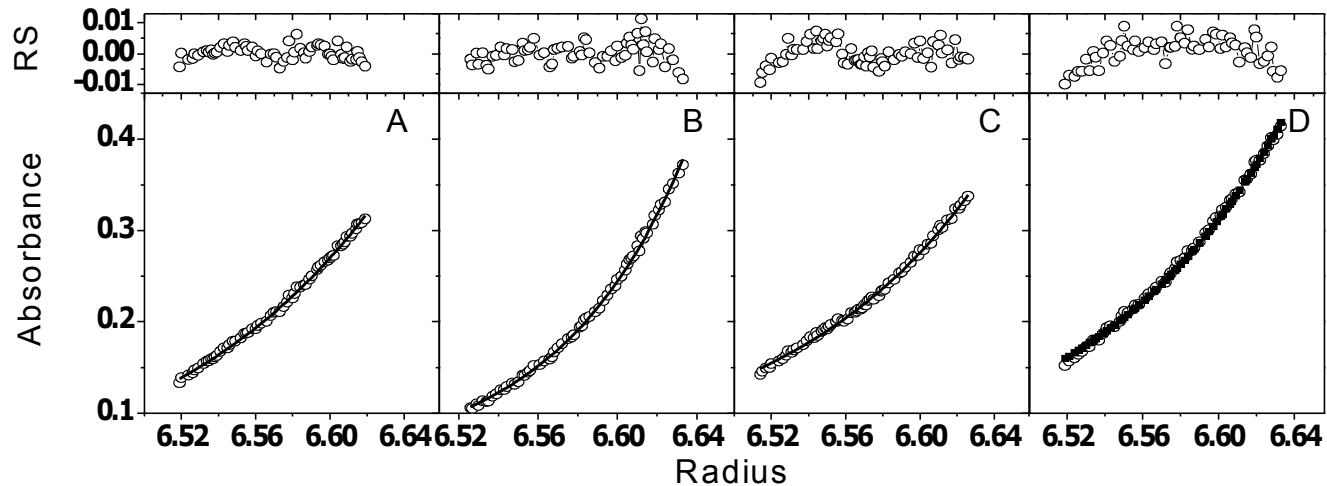


Asymmetric octasaccharide

Mitogenic Activity

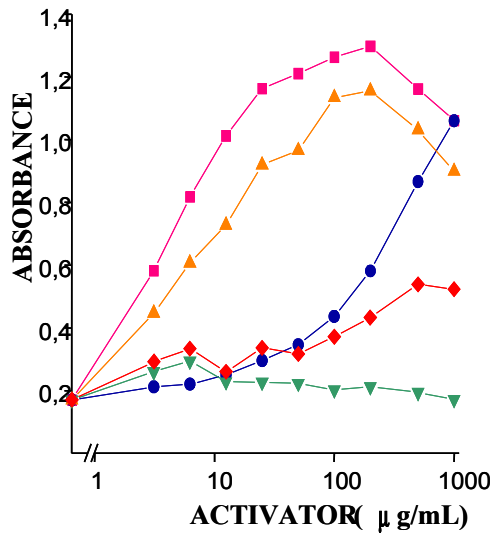


Sedimentation equilibrium curves of FGF-1 in the presence of synthetic oligosaccharides

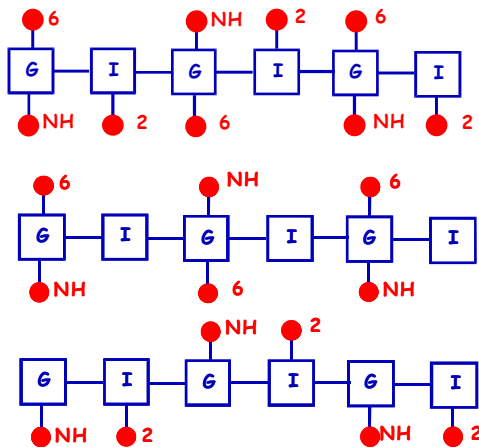
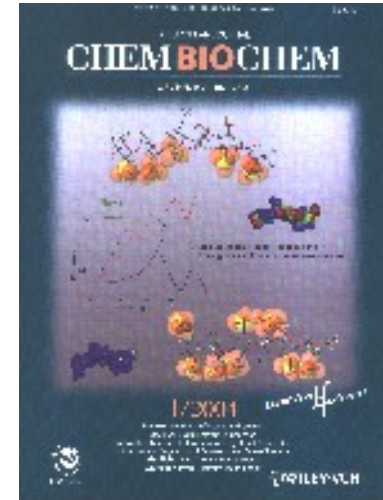
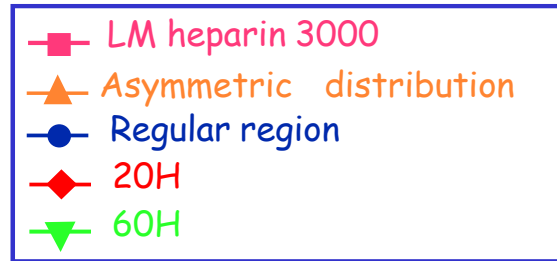


A: FGF-1 (Mw = 16640 ± 885)
B: FGF-1 + LMWH (Mw = 30180 ± 1940)
C: FGF-1 + Hexasaccharide (Mw = 18500 ± 540)
D: FGF-1 + Octasaccharide (Mw = 19130 ± 790)

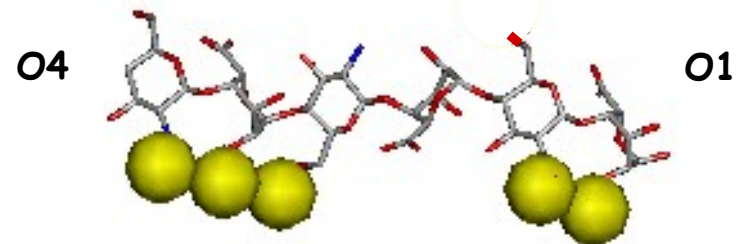
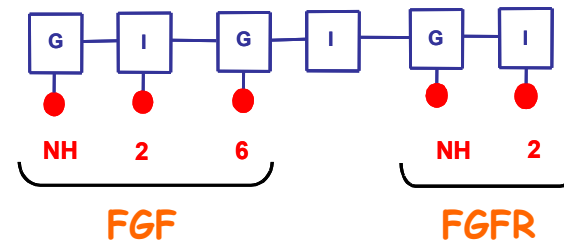
FGF1 activation by synthetic heparin oligosaccharides

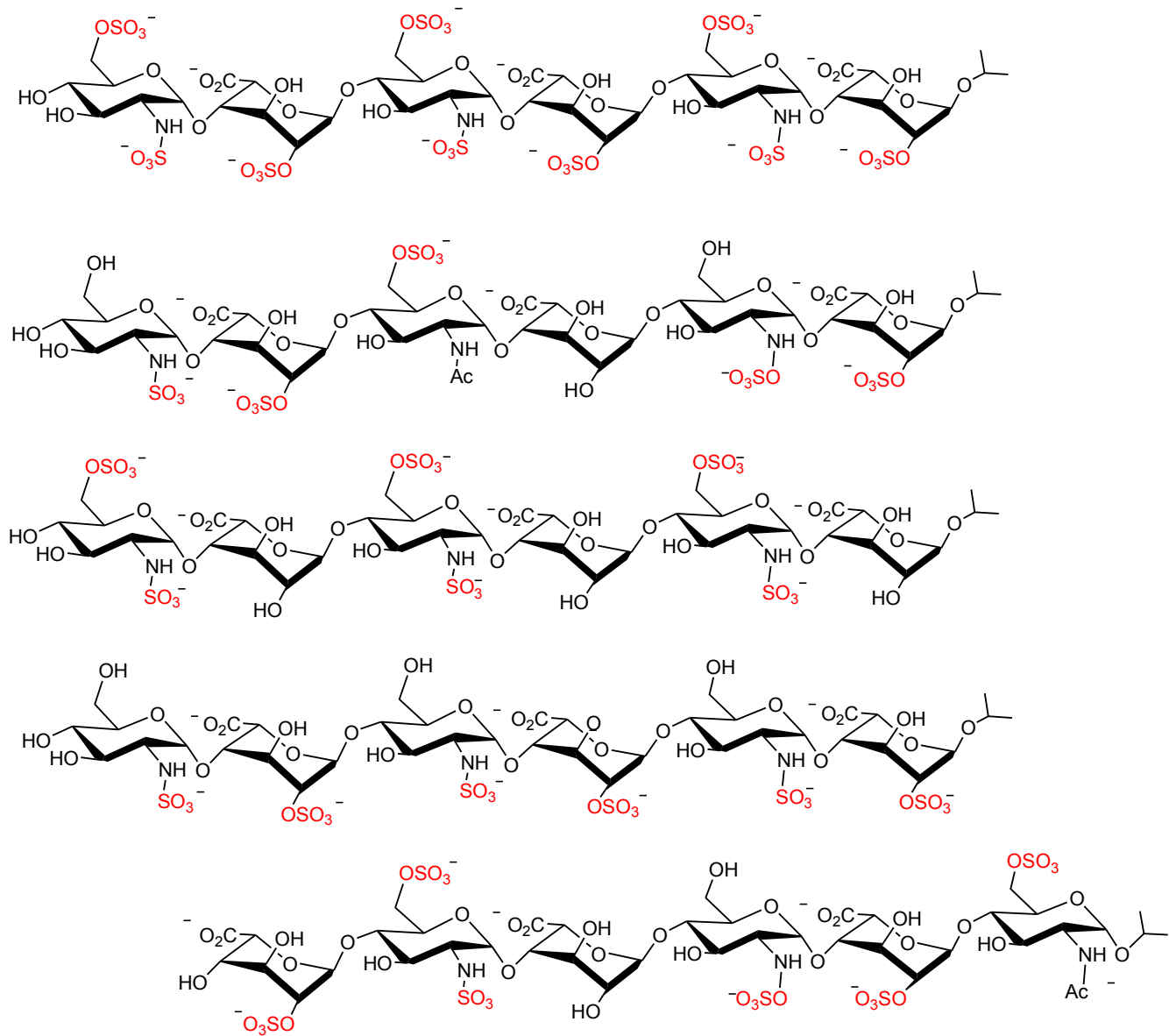


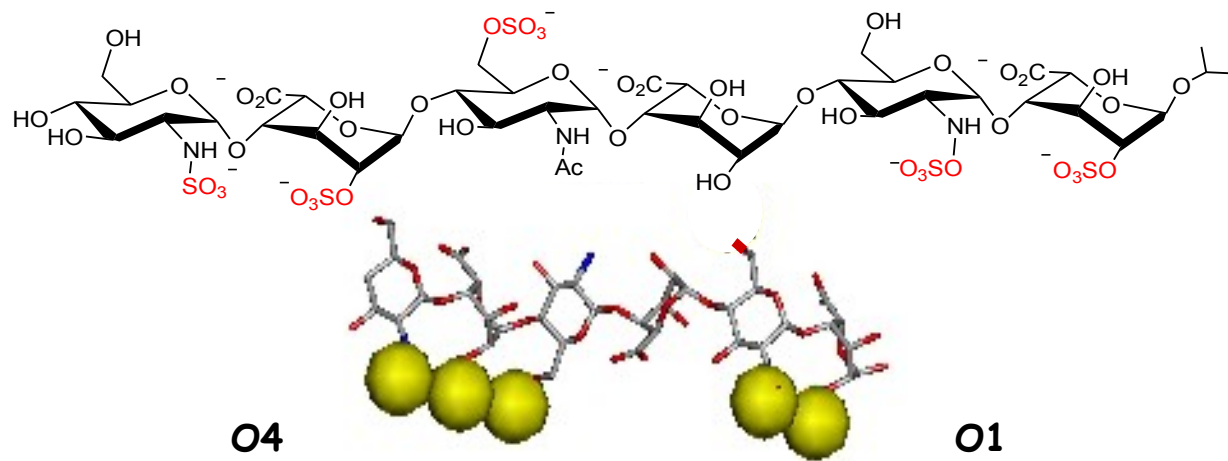
ChemBioChem, 2004, 5, 55-61



Regular region



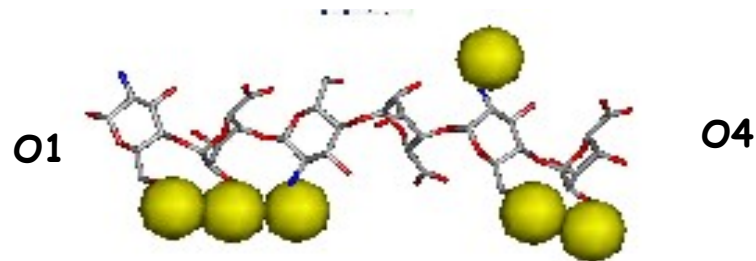




FGF-1 Activator

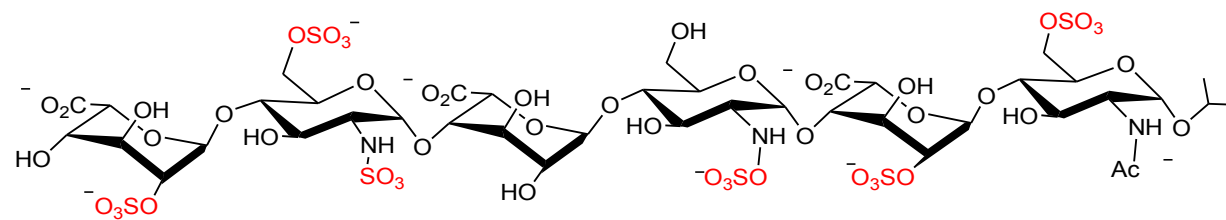
O4

O1

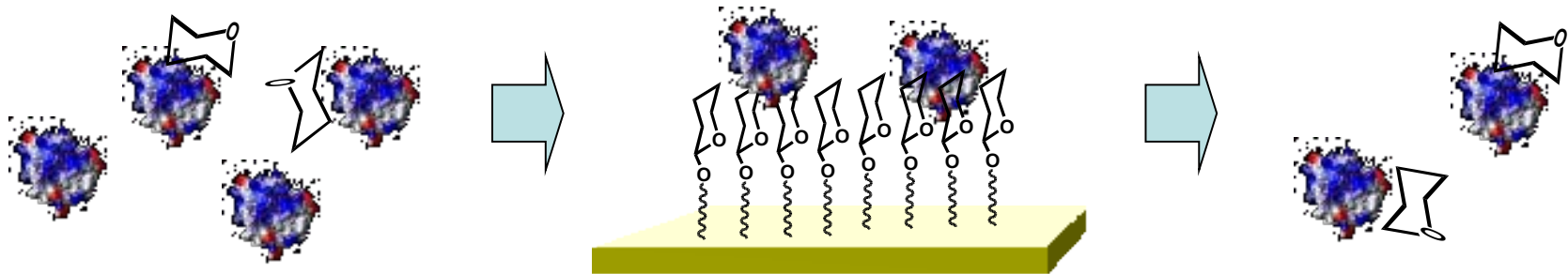


O1

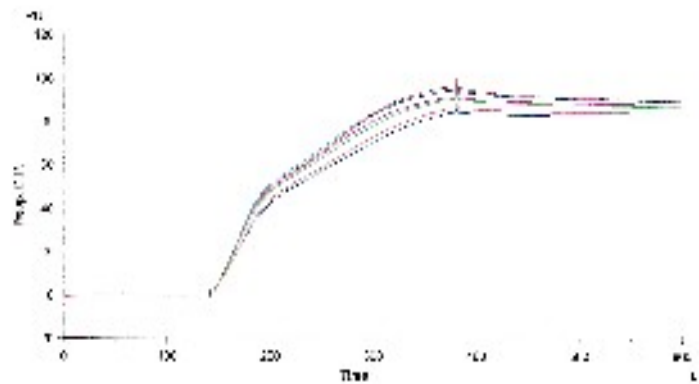
O4



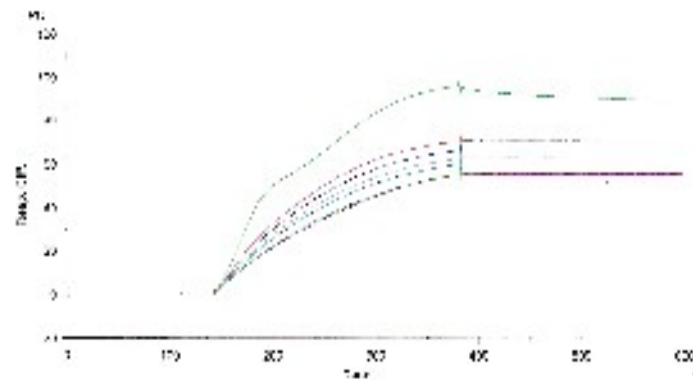
FGF-2 Inhibitor



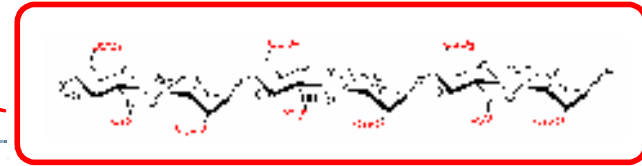
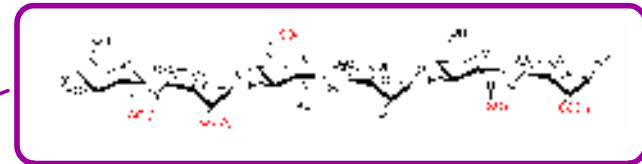
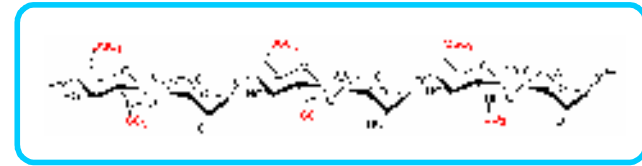
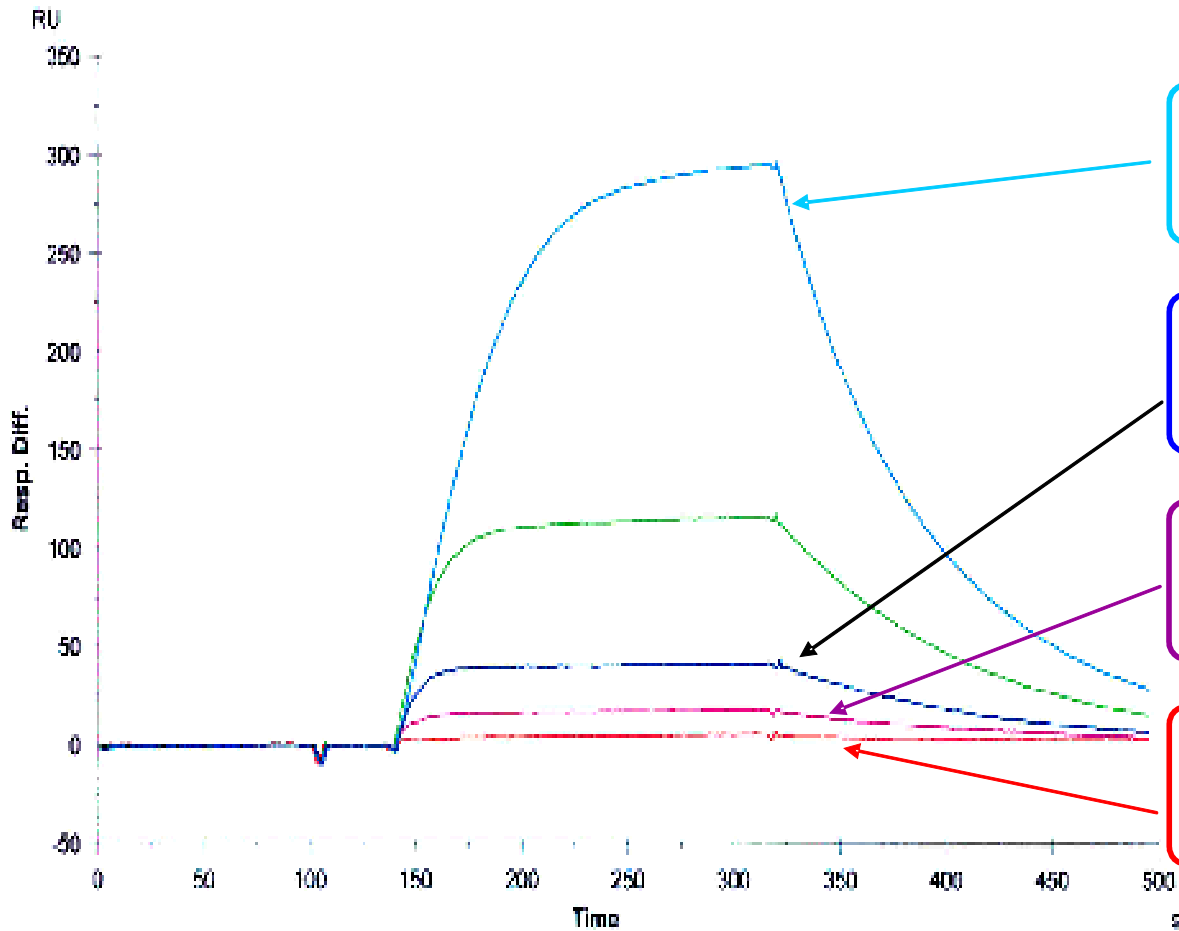
LOW BINDING



HIGH BINDING

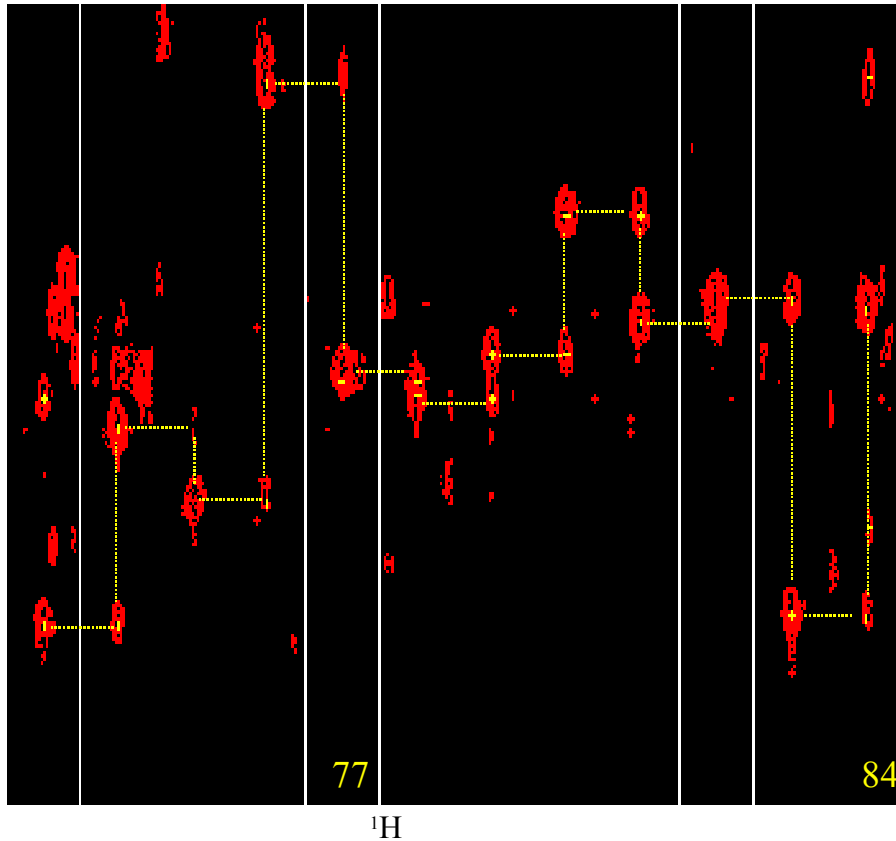
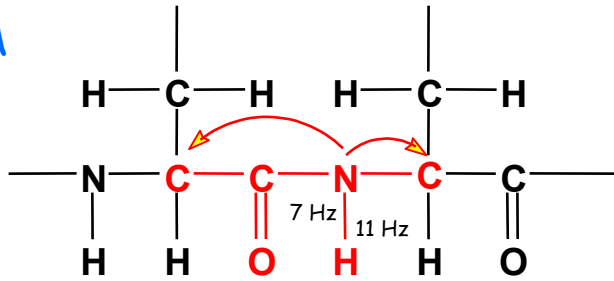


SPR: FGF-1 Binding Constants

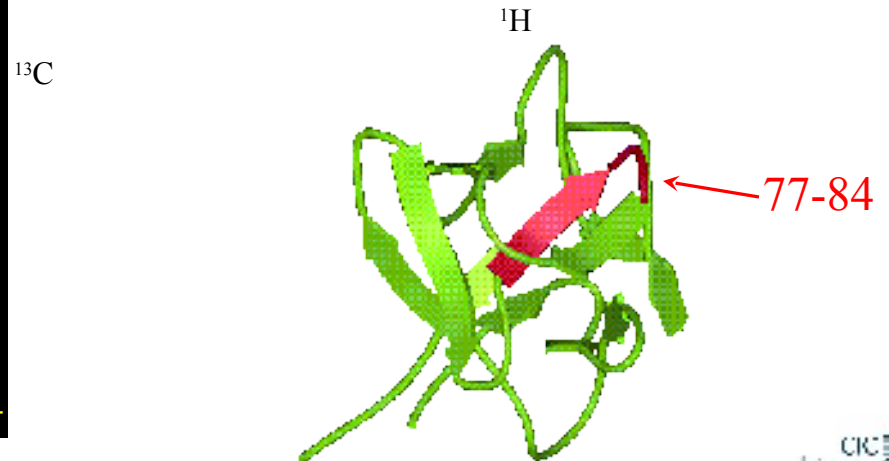
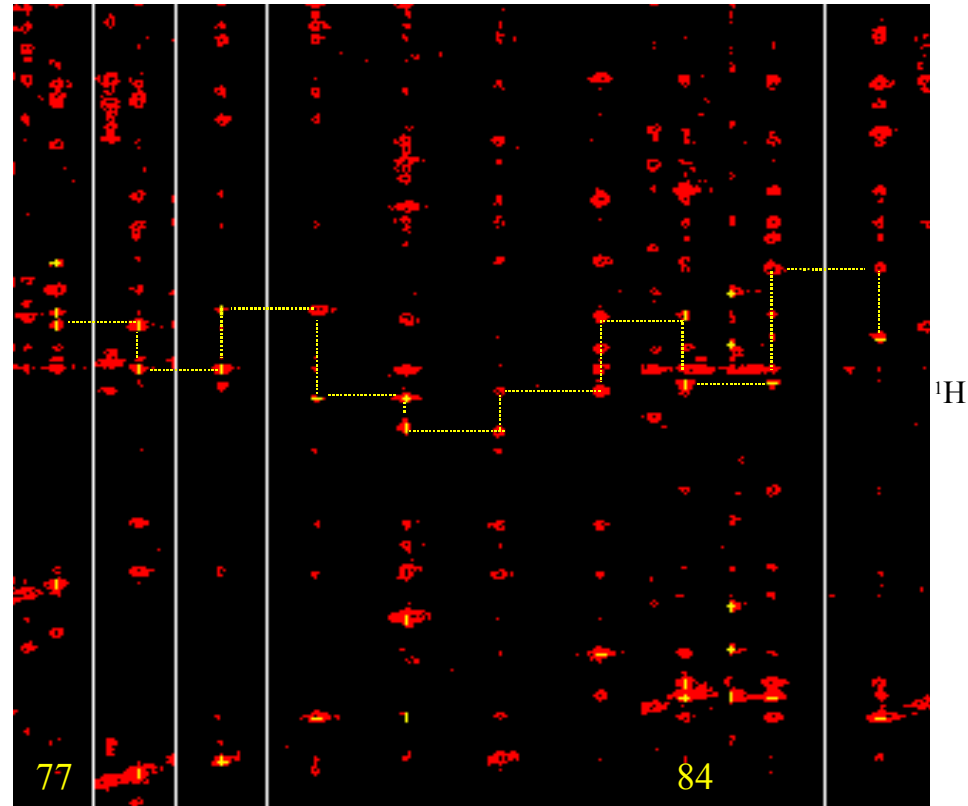


ASSIGNMENT of 3D-experiments

HNCA



HSQC-NOESY ¹⁵N-¹H



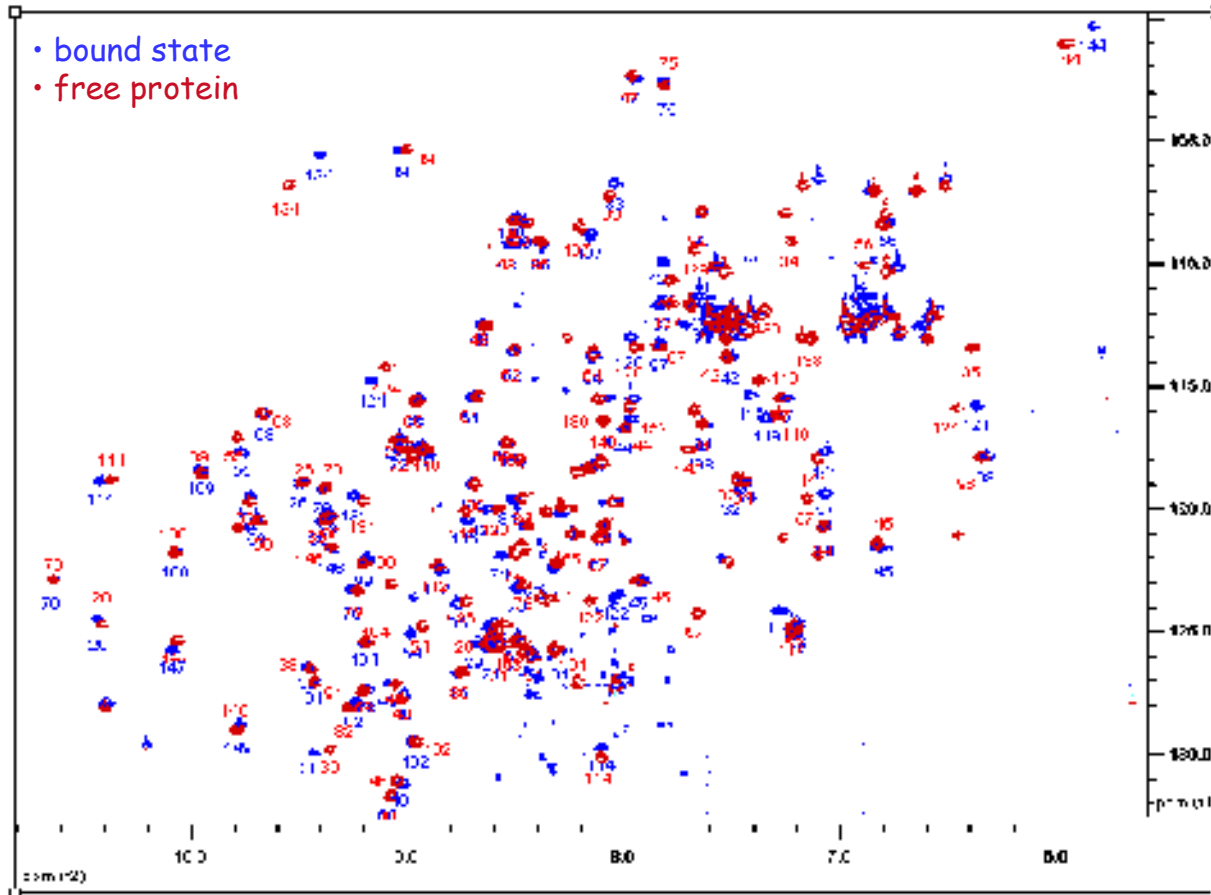
Stereoview of the best fit superpositions of the structure of asymmetric hexasaccharide-bound FGF-1



Superposition of 20 structures
rmsd=0.94 Å
1432 NOEs : 260 sequential
667 medium range
505 long range

BINDING SITE MAPPING

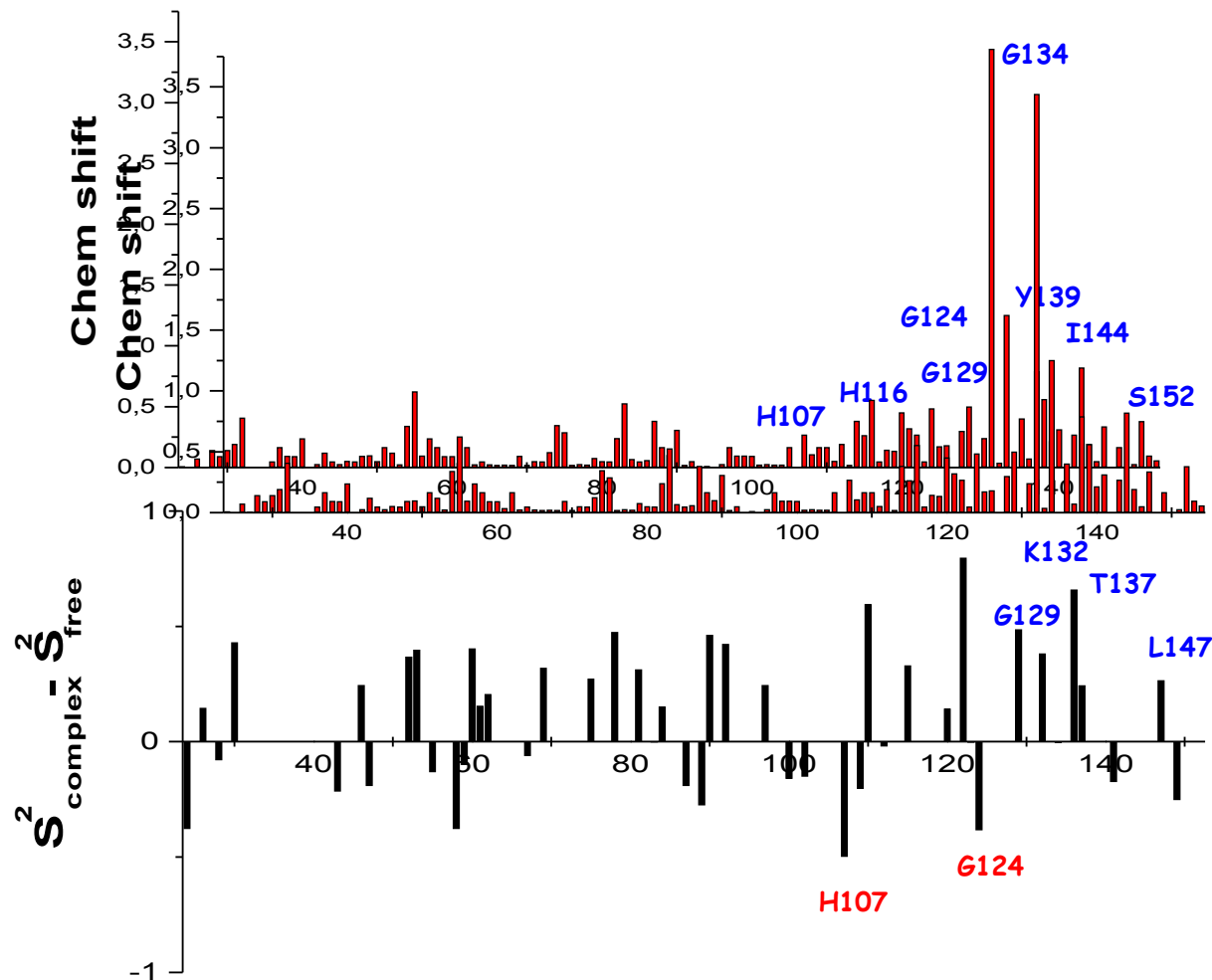
^{15}N - ^1H -HSQC



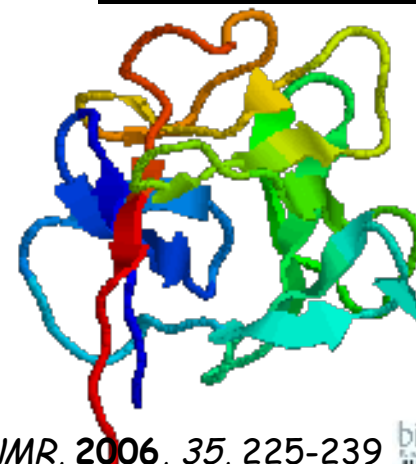
Binding site



The flexibility of the complex



Element	ΔS^2
LOOP 1	0.16
SHEET 4	0.16
TURN 3	0.26
SHEET 5	0.14
SHEET 6	0.13
LOOP 2	0.28
TURN 6	-0.16
SHEET 10	0.18
TURN 7	0.34
SHEET 11	0.20
LOOP 4	0.12
SHEET 12	0.14

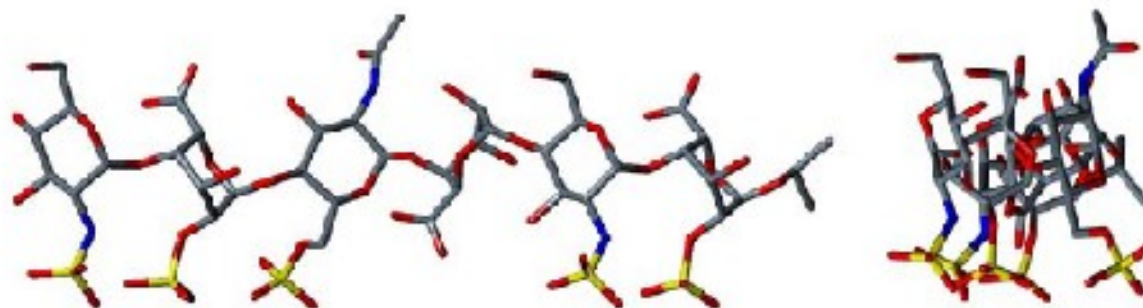
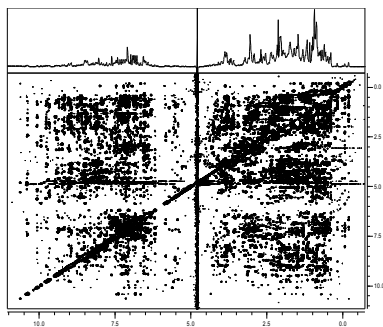
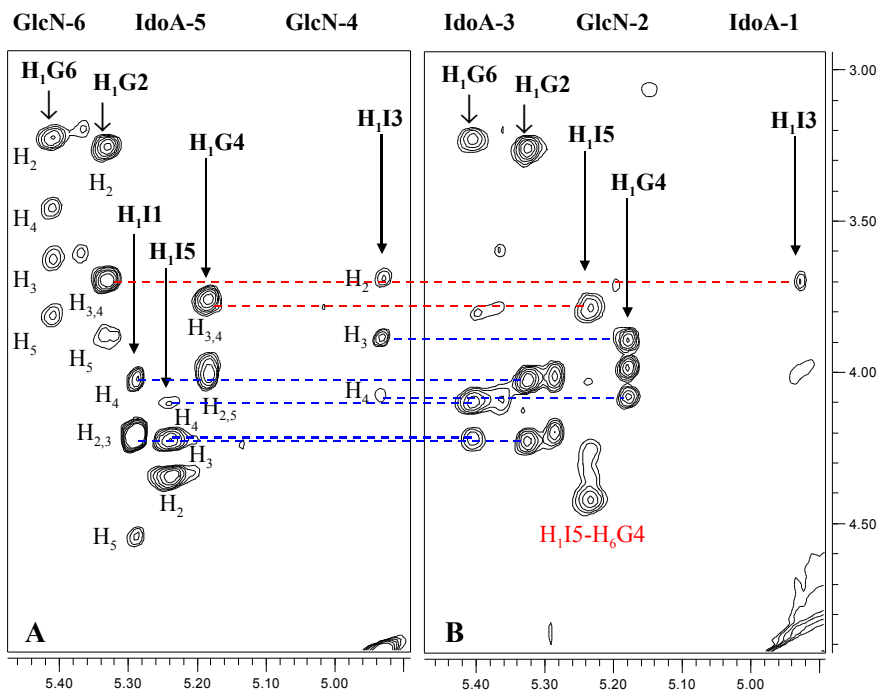
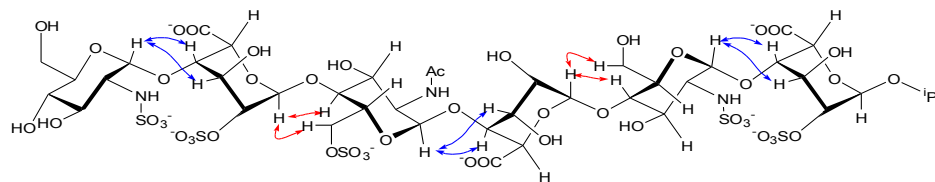
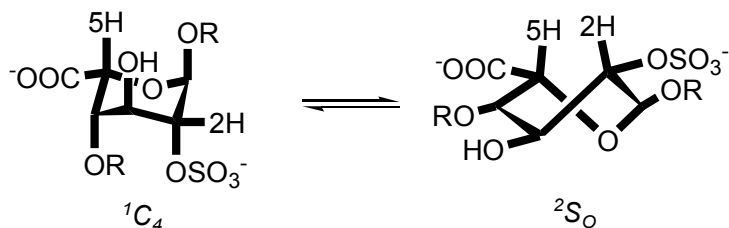


Conformational entropy loss amounts to ca. 56 kJ/mol

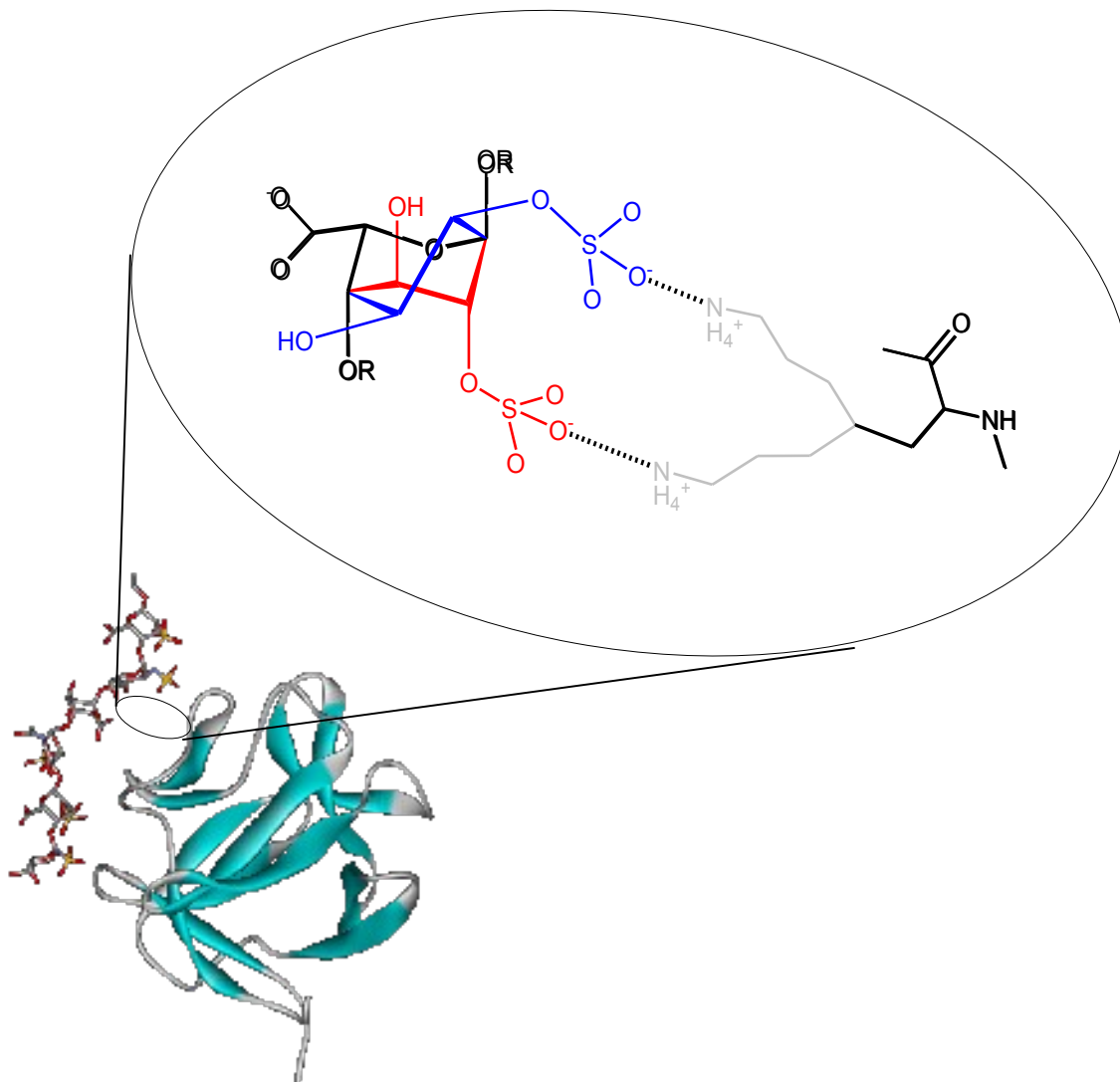
A. Canales, 2004

The structure of the bound hexasaccharide

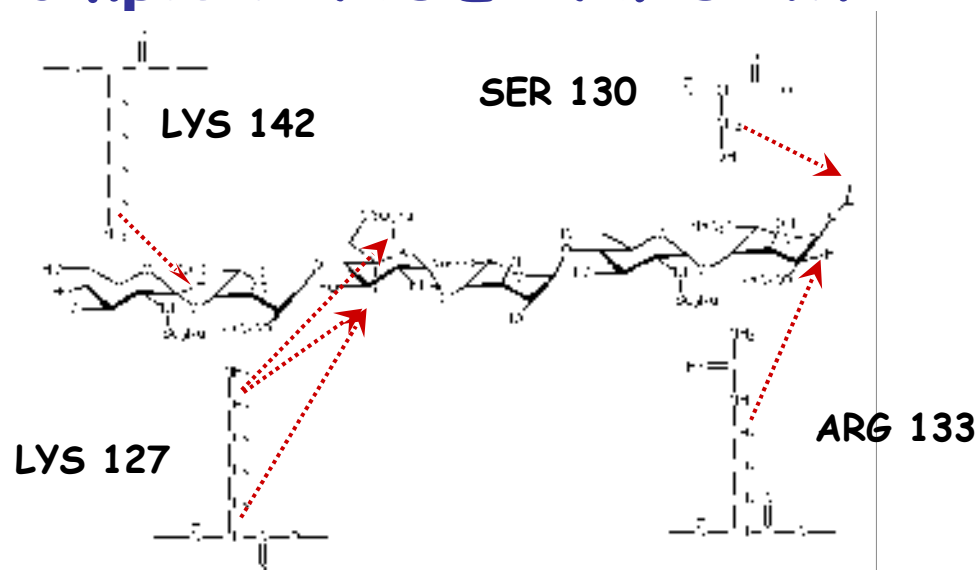
^{13}C -filtered- NOESY
CHAIR-BOAT
EQUILIBRIUM IN THE
BOUND STATE



Dynamics of the hexasaccharide within the Complex



The Complex: NOE INFORMATION



AUTODOCK

First docking run:

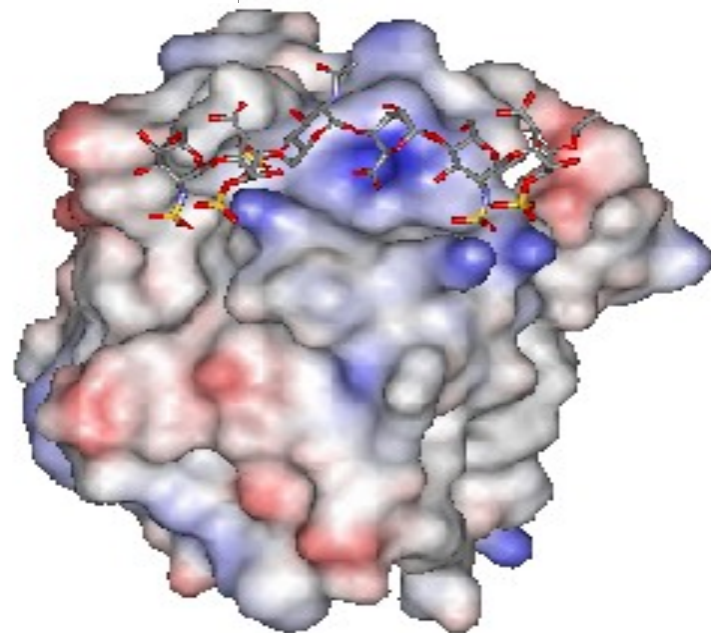
Global search for the binding site

Second step:

local search of the binding site
(rigid ligand)

Third step:

local search of the binding site
(flexible ligand)



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