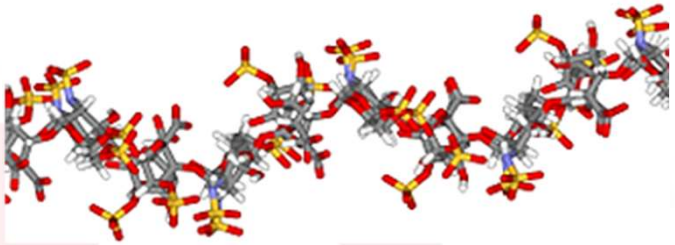
Institut de Biologie Structurale

Structure & Activités des Glycosaminoglycanes (SAGAG)



Preparation, structural characterization and biological assessment of Heparan Sulfate derived oligosaccharides

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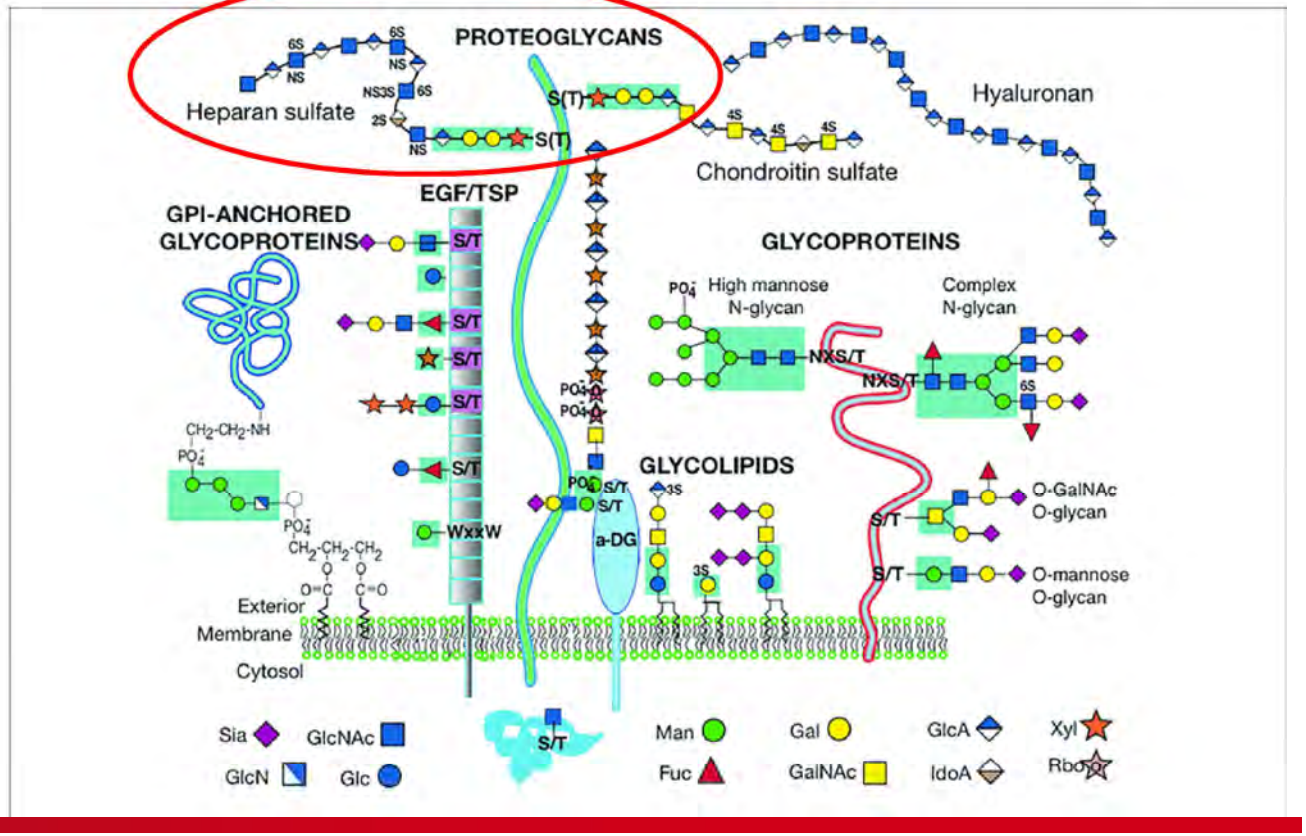
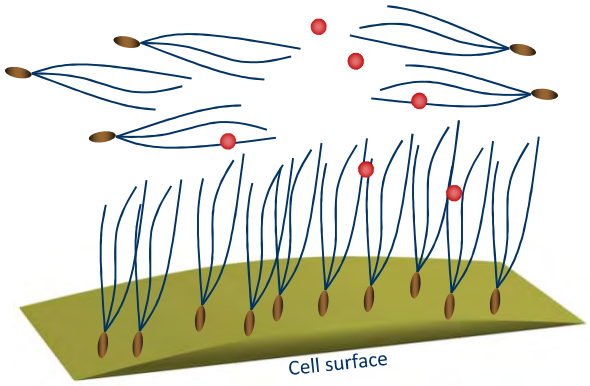
Glyco@Alps
Univ. Grenoble Alpes

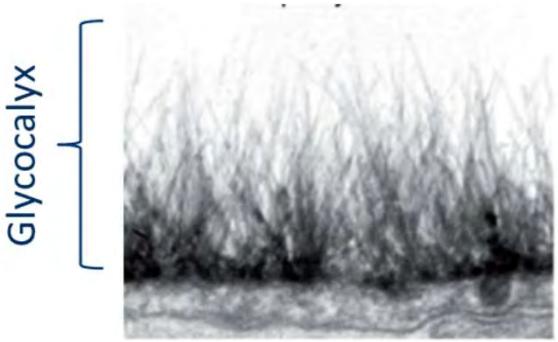
Structural Glycosciences Summer School, 7th of June 2023

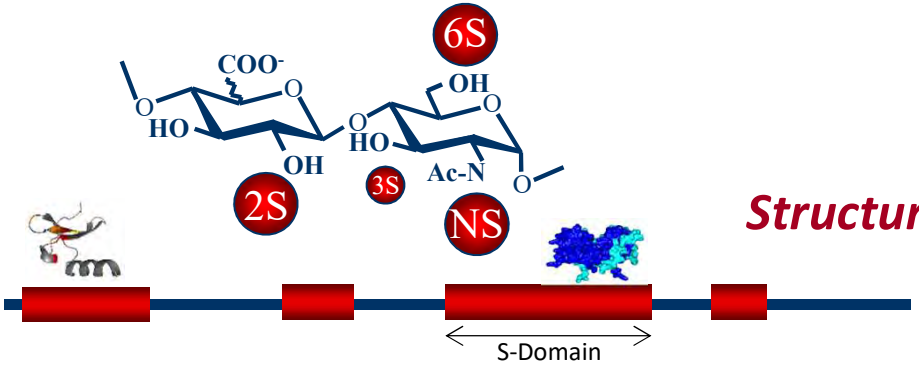
GAGs in the world of glycans



Heparan Sulfate (HS) Proteoglycans







HS

Localization

Structure

Protein core

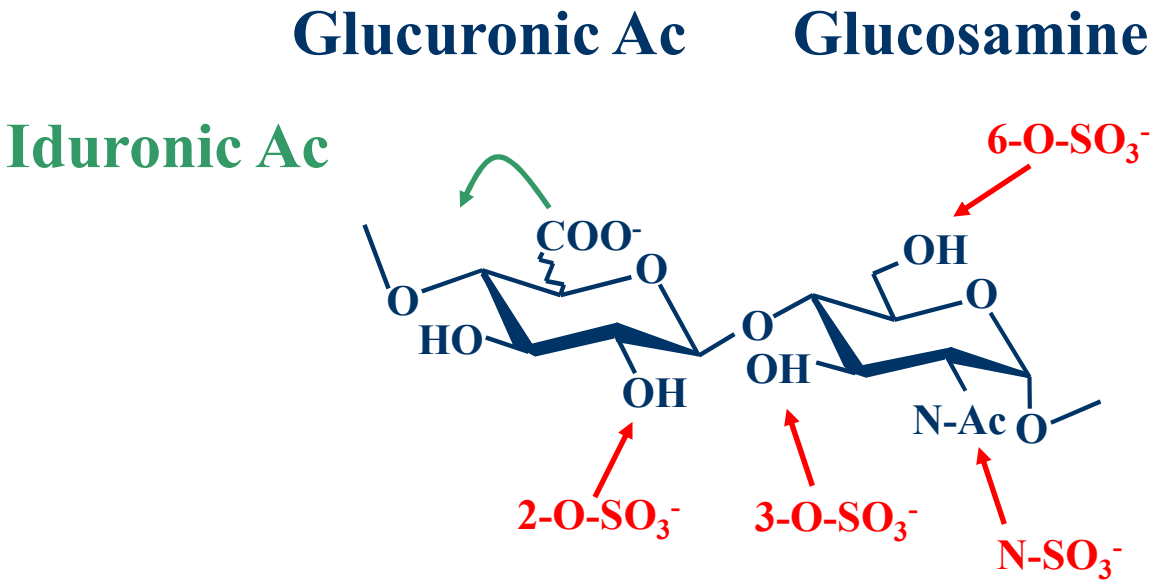
Function

- Growth factors
- Cytokines
- Chemokines
- Adhesion molecules
- Matrix proteins
- Enzymes
- Enzyme inhibitors
- Pathogens
- etc...

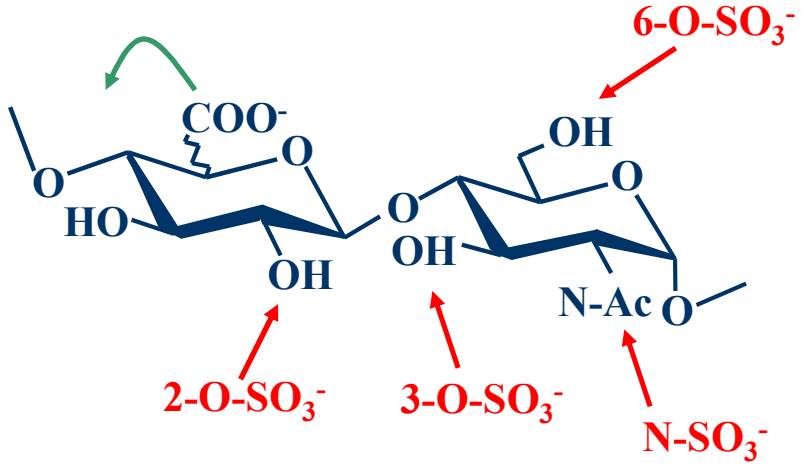
- Proliferation
- Differentiation
- Activation
- Migration
- Tissue cohesion
- Coagulation
- Infection
- etc...

S-Domain => >10^9 possible saccharide sequences

Structure of Heparan sulfate (HS)



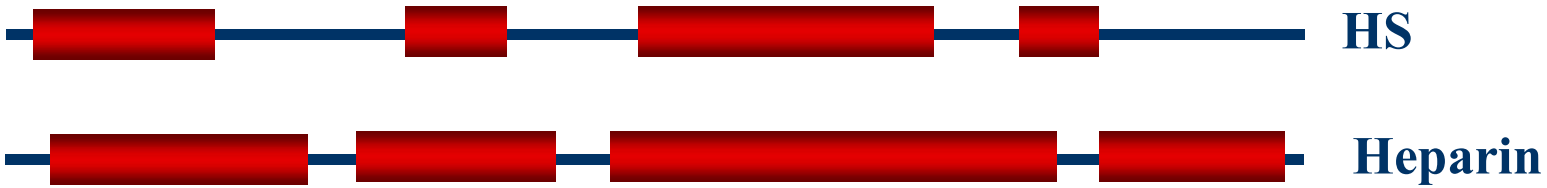
Structure of Heparan sulfate (HS)



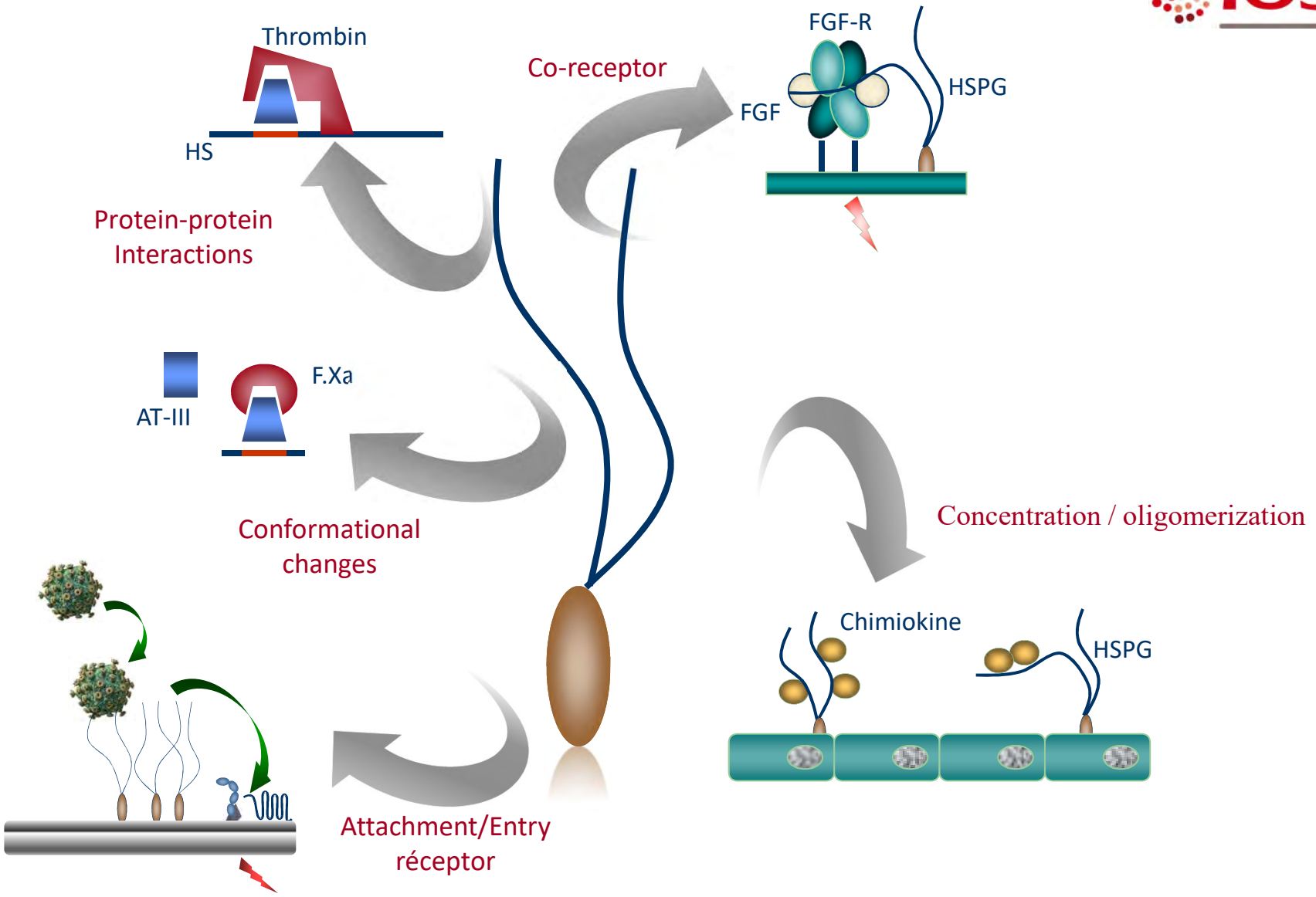
48 possible disaccharides

=> 48^2 = 2304 tetrasaccharides

=> 48^6 = $12x10^9$ dodecasaccharides

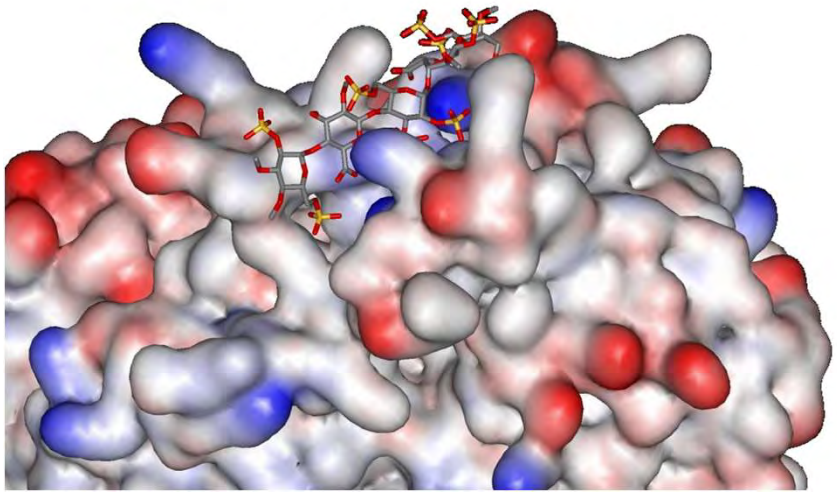


Regulatory activities of HS

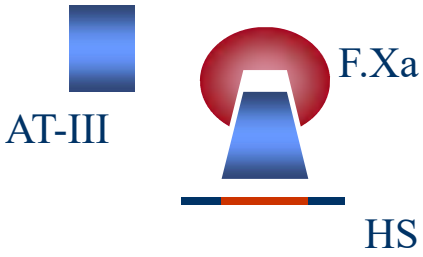


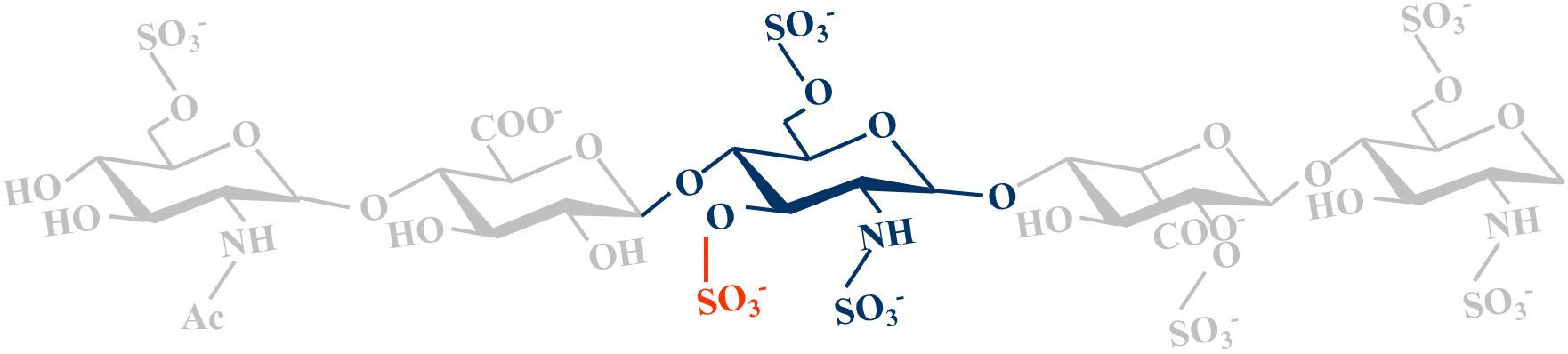
The AT-III/HS binding model

Importance of precise sulfate groups

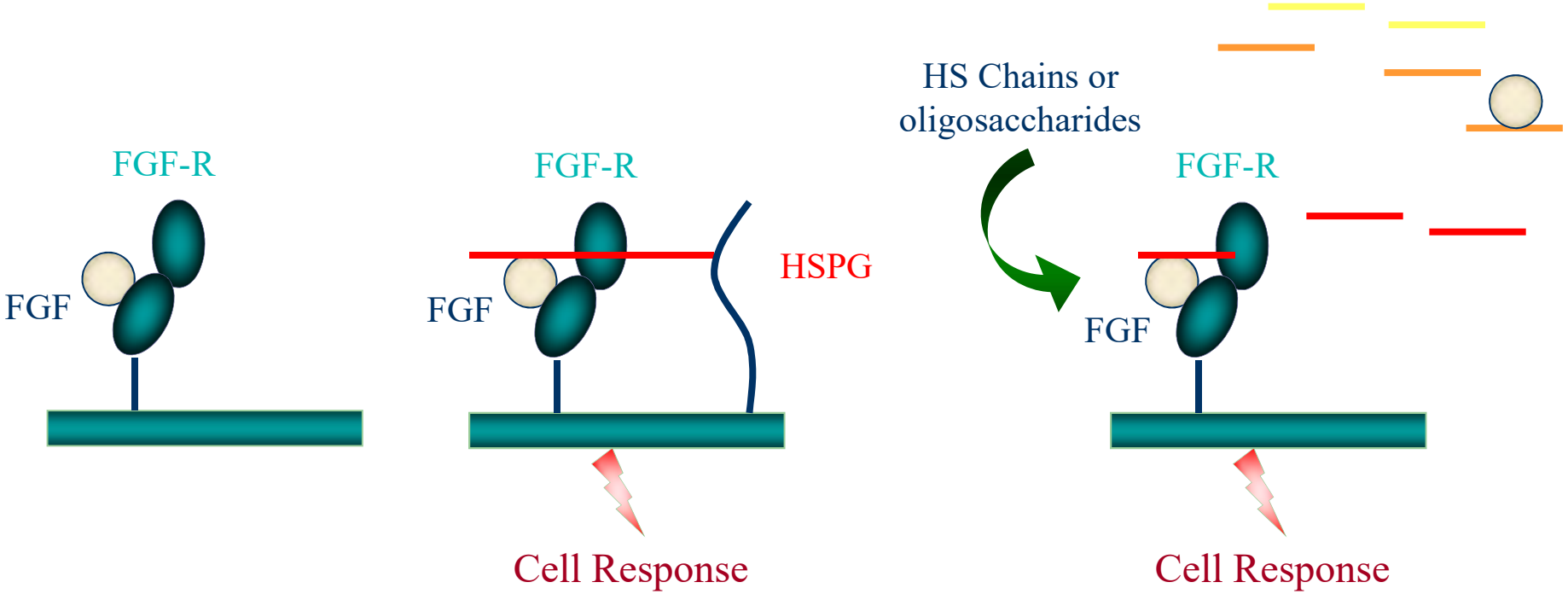


ATIII- pentasaccharide complex
Jin *et al.*, 1997. *PNAS* **94**, 14683-88





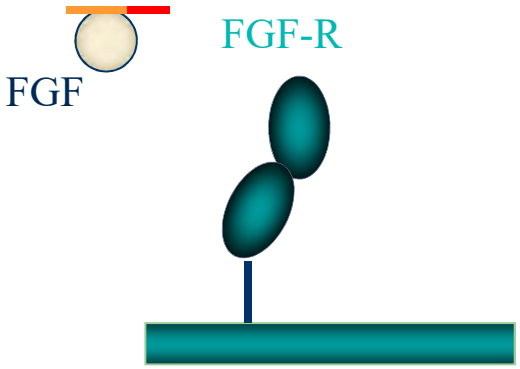
The FGF-2/HS binding model



Interaction FGF-2/HS

Binding to FGF-2

- S Domain
- Minimum size : dp6
- Importance of NS
- Importance of IdoA
- Importance of 2S



Activation of FGF-2

- S Domain
- Minimum size : dp10
- Importance of NS
- Importance of IdoA
- Importance of 2S
- Importance of 6S

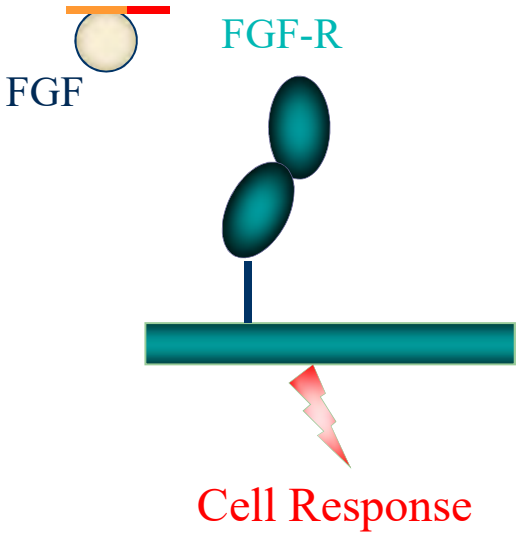
Interaction FGF-2/HS

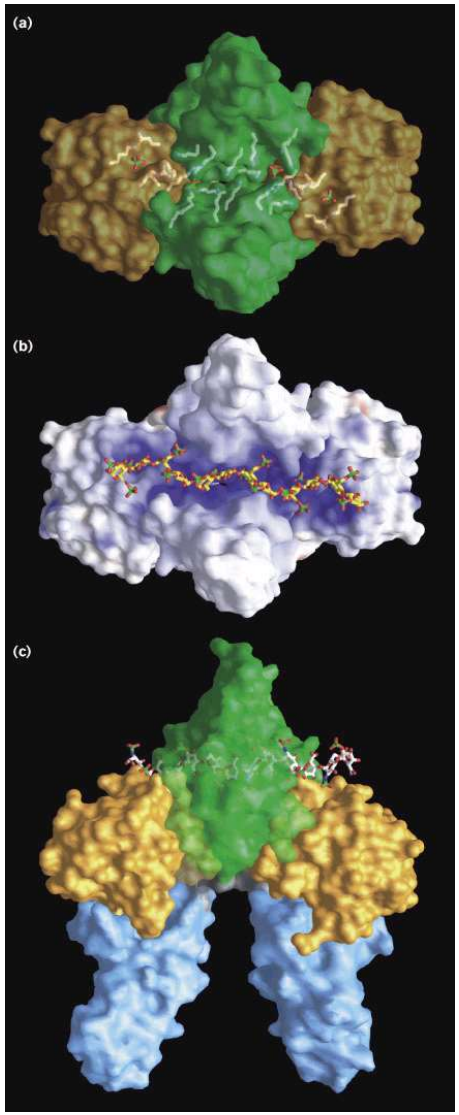
Binding to FGF-2

- S Domain
- Minimum size : dp6
- Importance of NS
- Importance of IdoA
- Importance of 2S

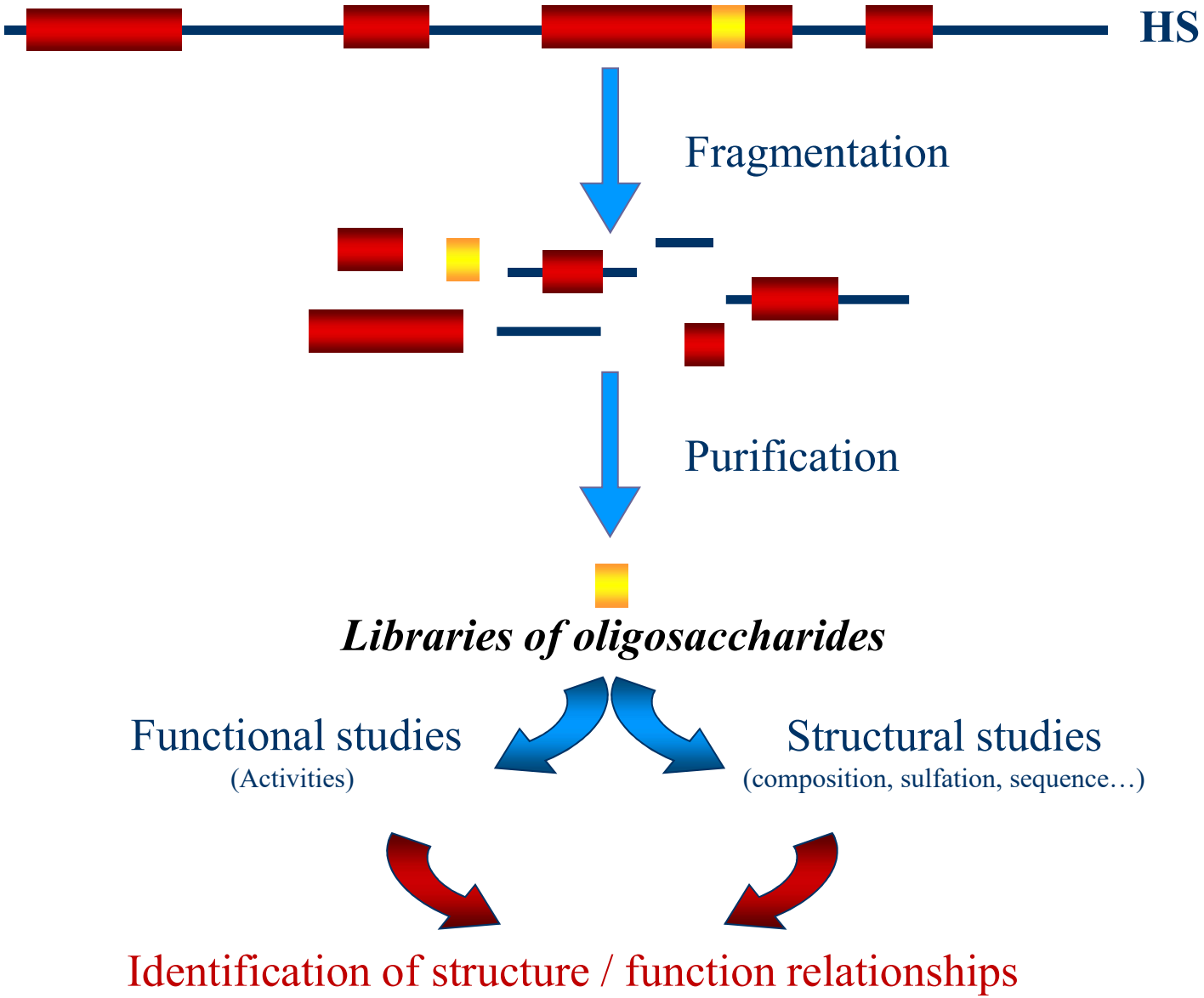
Activation of FGF-2

- S Domain
- Minimum size : dp10
- Importance of NS
- Importance of IdoA
- Importance of 2S
- Importance of 6S

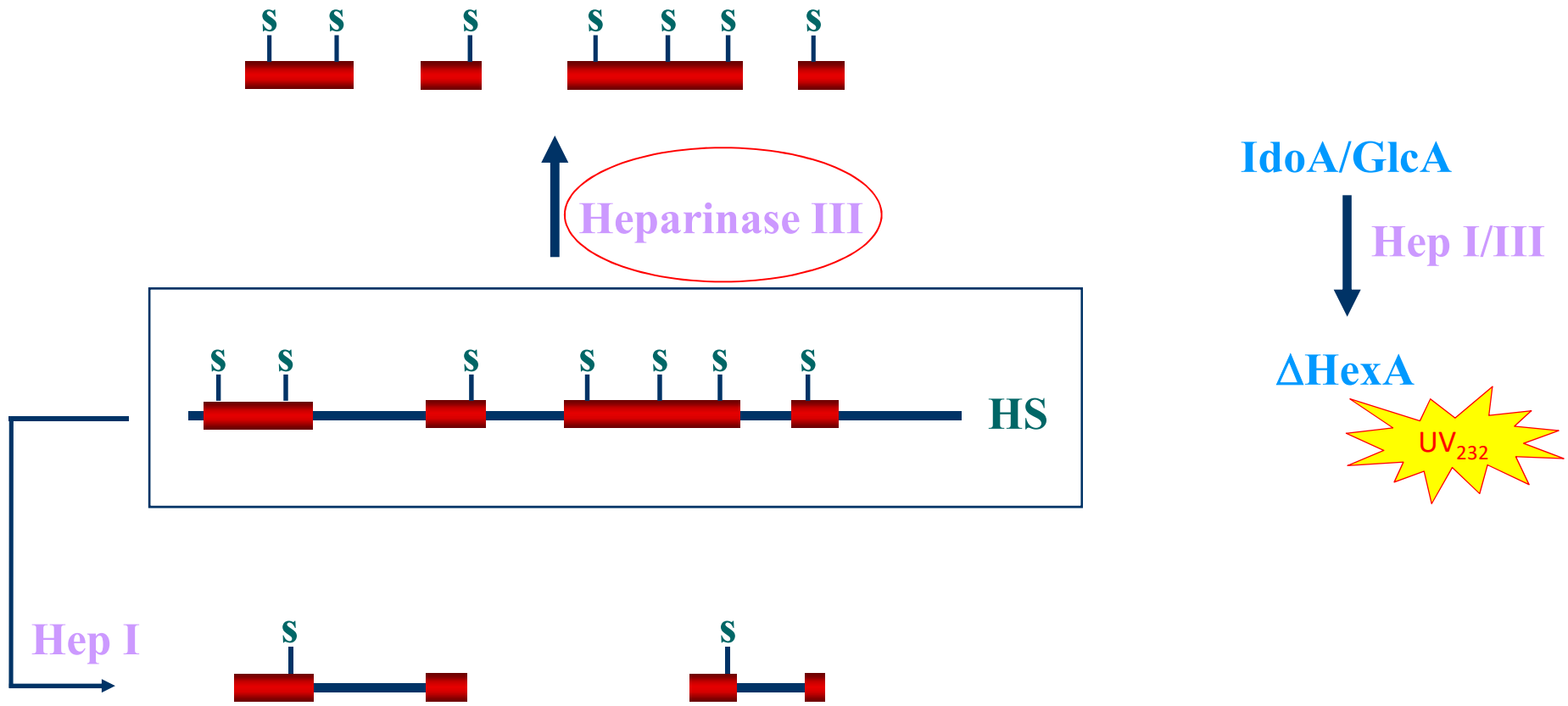




Preparation of HS oligosaccharides

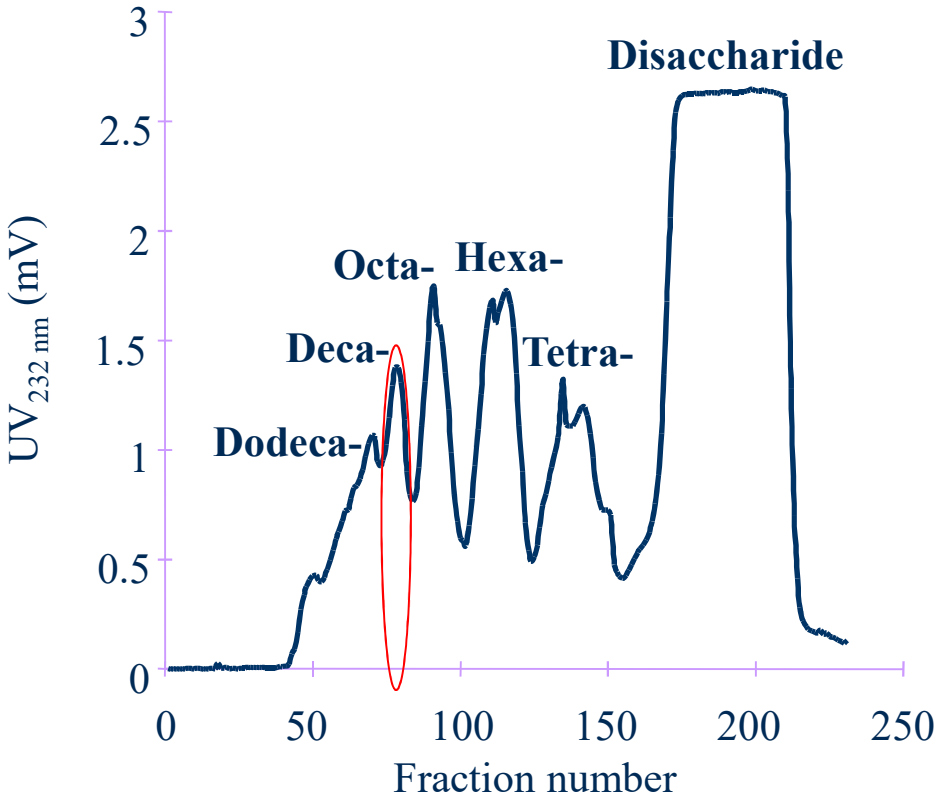


Depolymerization of HS chains



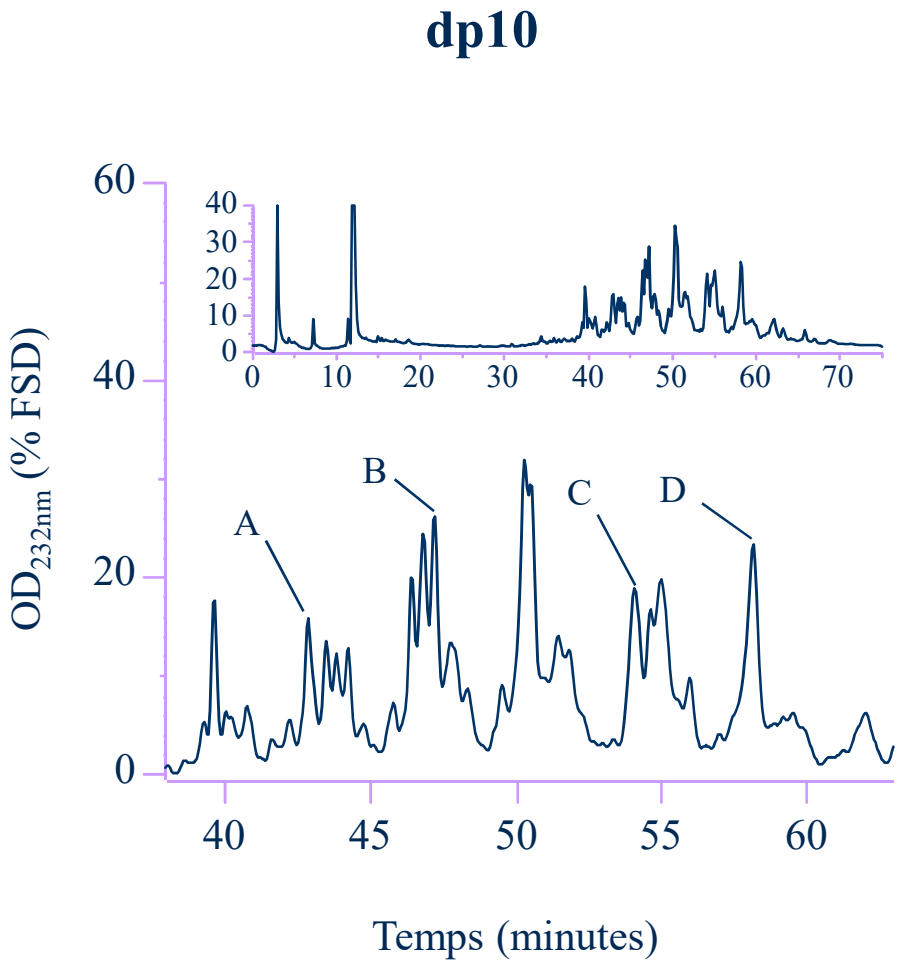
Purification of HS oligosaccharides

1st step: separation according to size

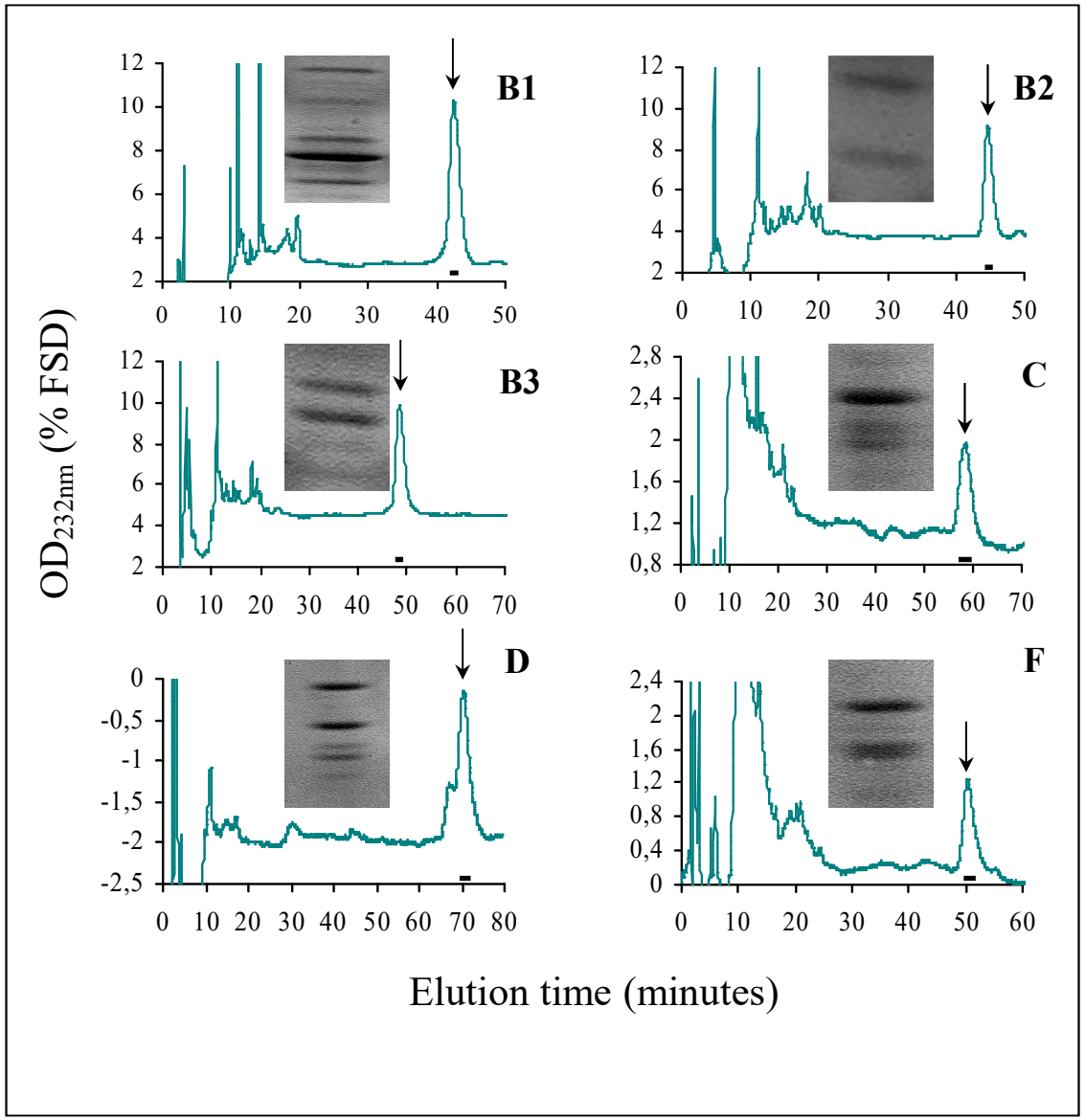


Depolymerization of HS chains

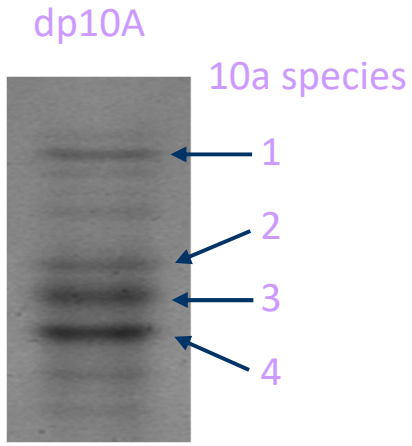
2nd step: separation according to charge



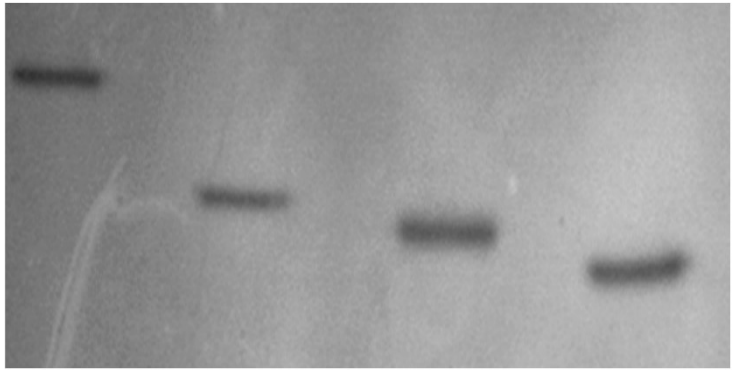
Purity of HS oligosdaccharides



PAGE Based purification of HS oligosdaccharides



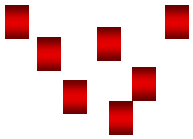
Sous-espèces de dp10A 1 2 3 4



Vivès *et al.*, 2001. *Biochem. J.* **354**, 141-7

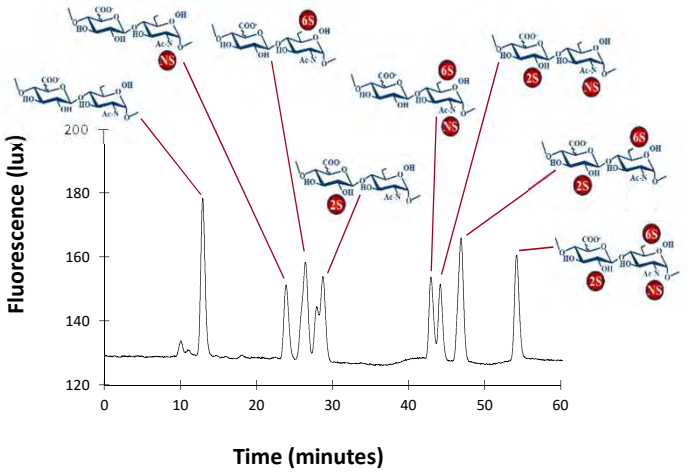
Structural analysis of HS oligosaccharides

Hep III+ Hep I



HS disaccharides

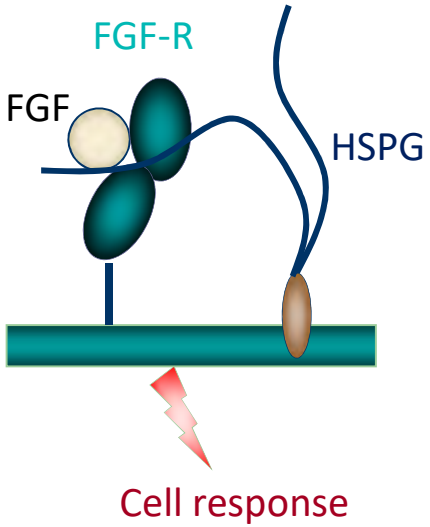
SAX HPLC analysis

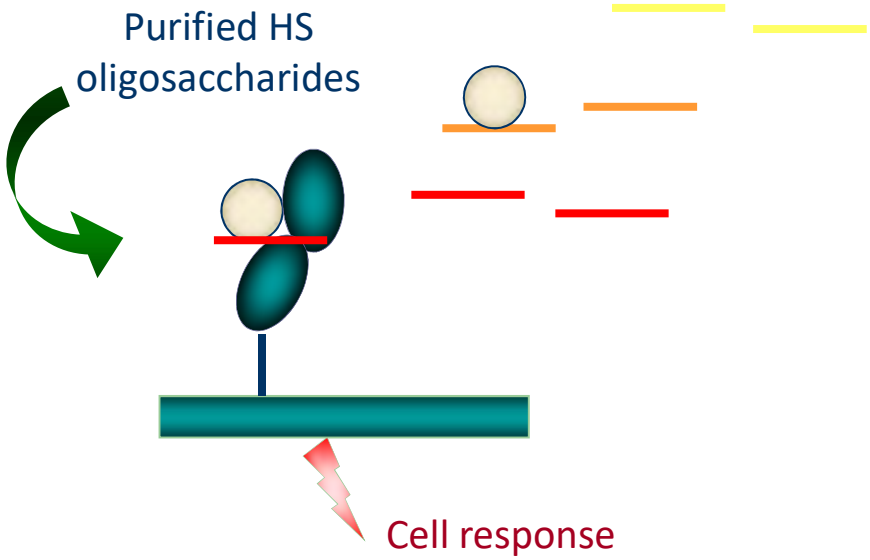


Disaccharide (% of total)	RIdp10A	RIdp10B	RIdp10C	RIdp10D
Δ HexA-GlcNAc	16.3	15.9	10.5	7.7
Δ HexA-GlcNAc,6S	8.3	9.3	11.9	15.4
Δ HexA-GlcNS	21.2	16.8	17.9	12.6
Δ HexA-GlcNS,6S	4.2	7.5	6.6	10.5
Δ HexA,2S-GlcNS	40.7	37.9	29.9	14.9
Δ HexA,2S-GlcNS,6S	7.2	12.4	20.9	36.8
Δ HexA,2S-GlcNAc	2.1	nd	2.3	2.0
6-O-SO_3 /oligosaccharide	0.98	1.47	1.98	3.14
2-O-SO_3 /oligosaccharide	2.51	2.52	2.66	2.69
N-SO_3 /oligosaccharide	3.67	3.74	3.78	3.75

Functional analysis of HS oligosaccharides

❖ Promotion of FGF-2 activity by HS oligosaccharides





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ΔHexA-GlcNS,6S	4.2	7.5	6.6	10.5
ΔHexA,2S-GlcNS	40.7	37.9	29.9	14.9
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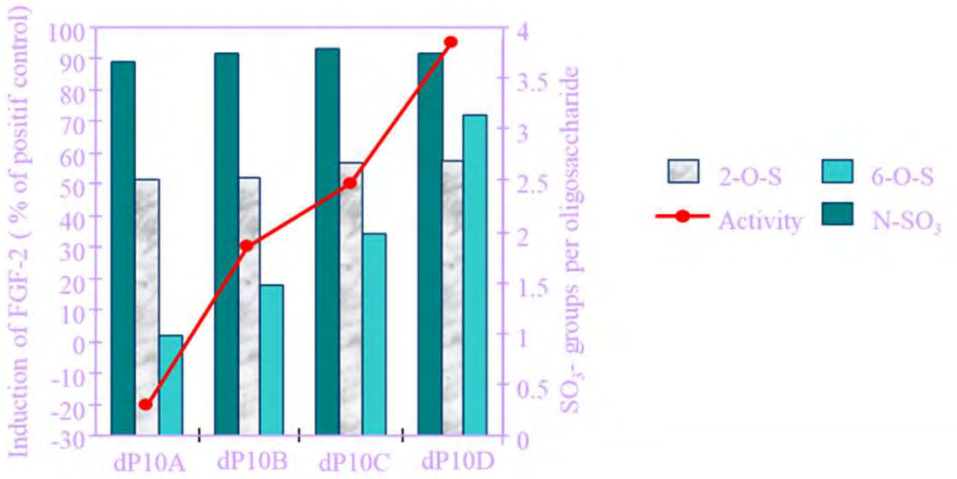
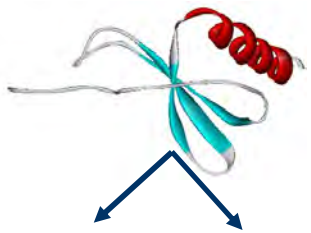


Chemokines…



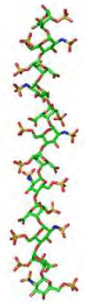
GPCR

Integrin activation

Cell signaling

Cytoskeleton reorganization

GAG



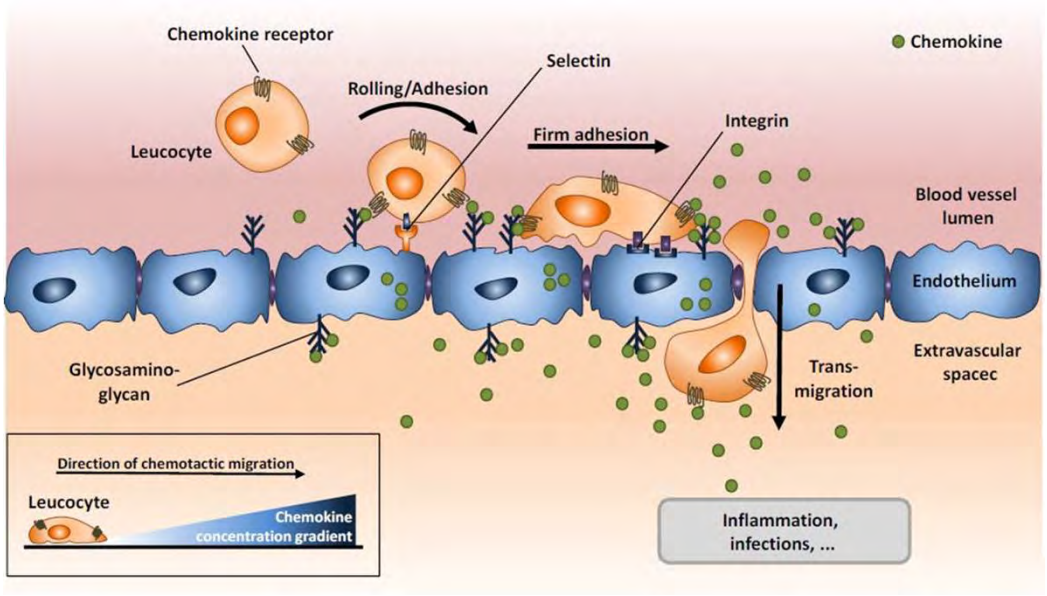
Tissue localization

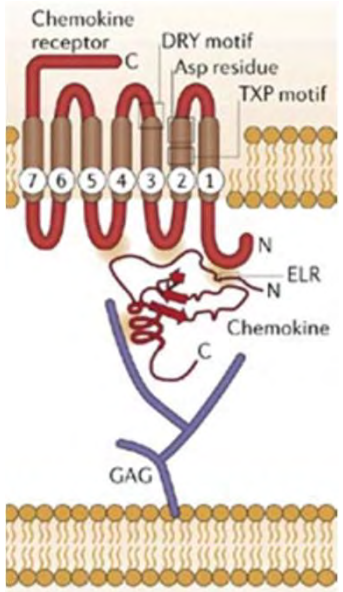
Structural changes

Protection/activation

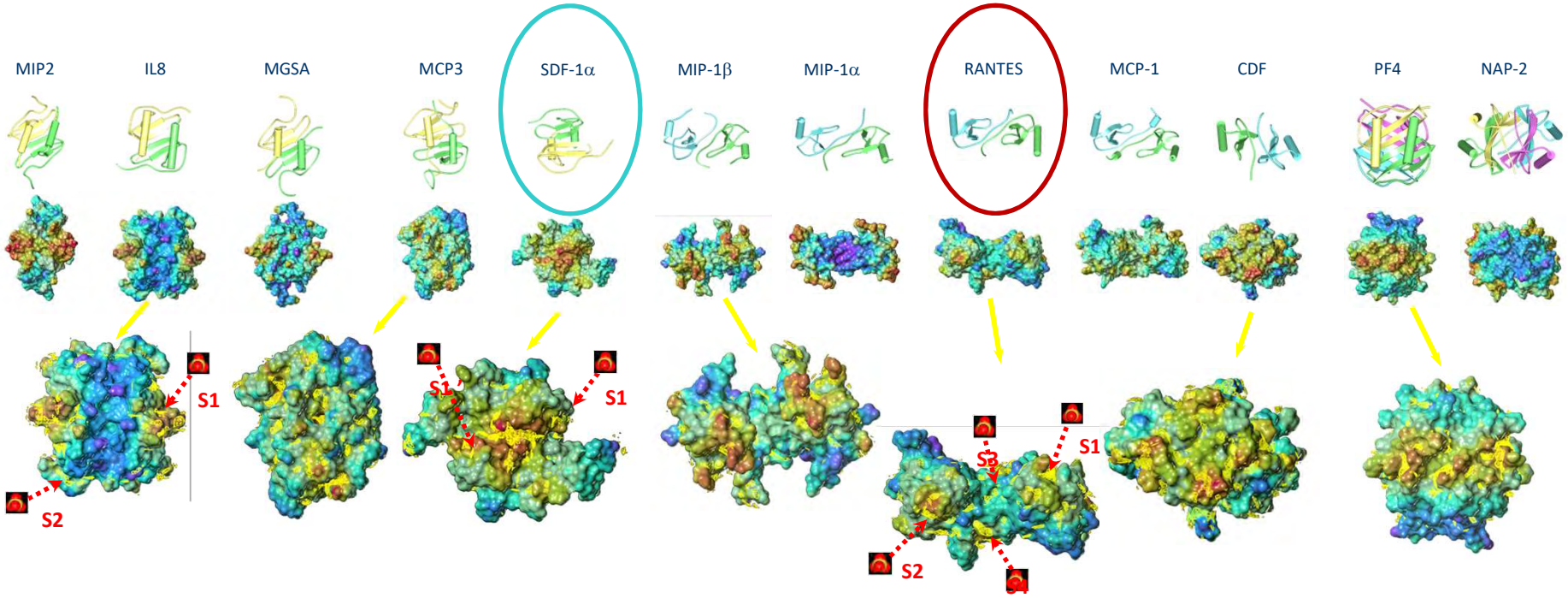
Oligomerization

Receptor presentation



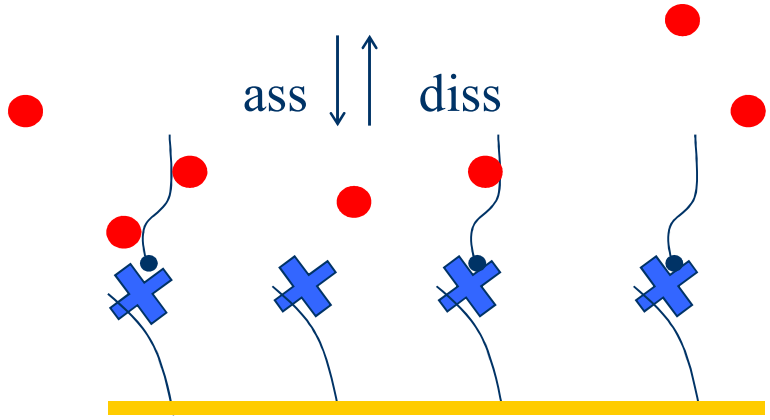


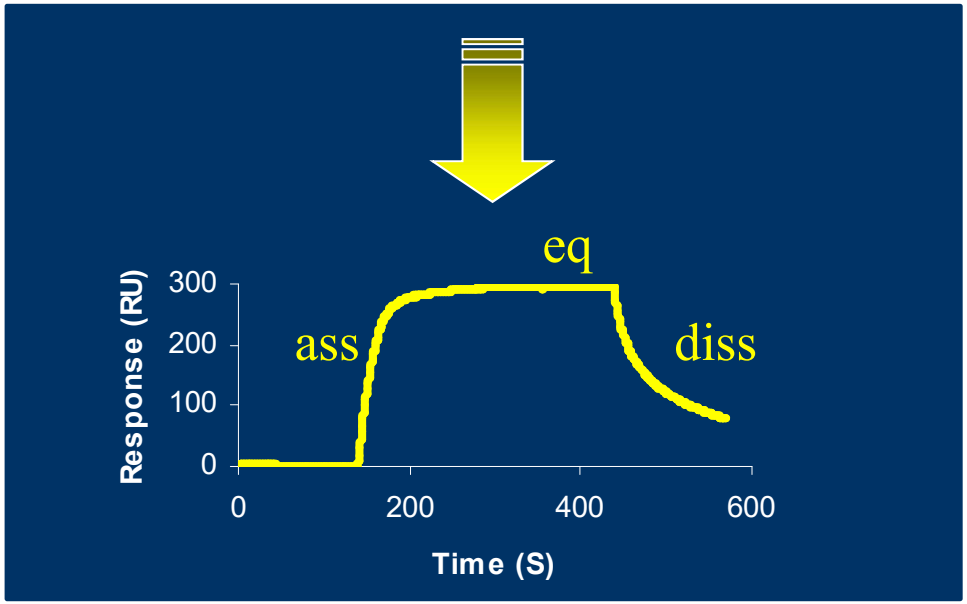
Chemokine/HS interactions



Analysis of HS/protein interactions

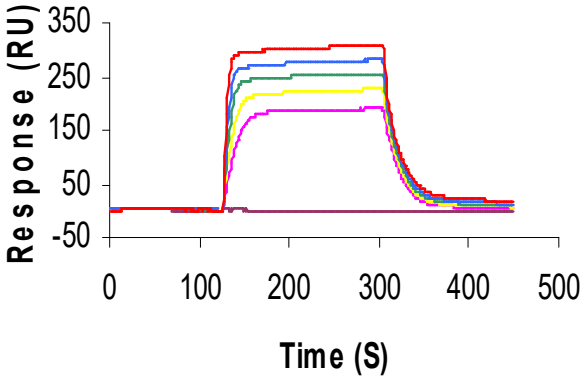
SPR (surface plasmon resonance)

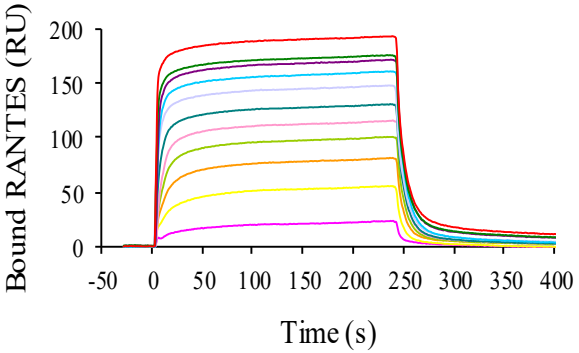




Chemokine /HS interactions

SDF1-α versus RANTES

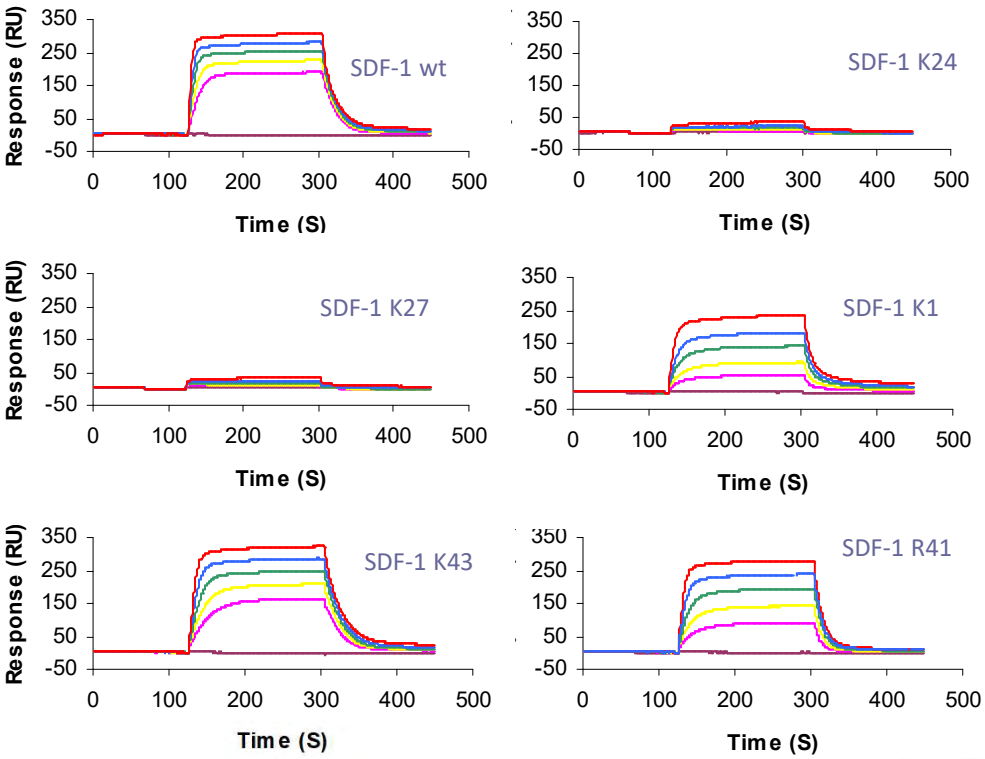


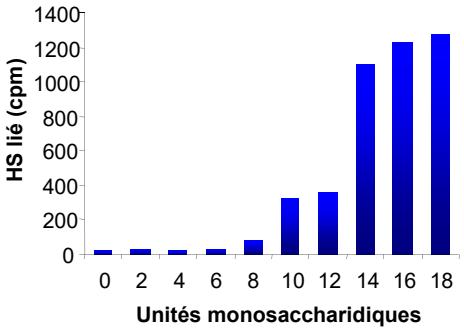


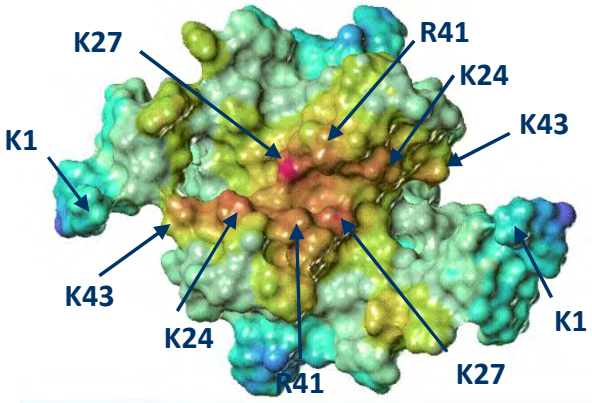
Both bind to HS

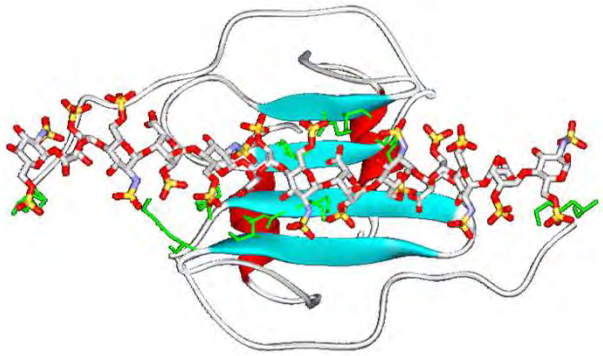
K_D ~ 220 nM

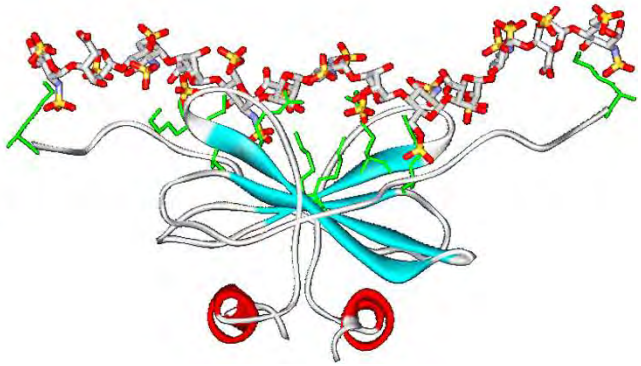
SDF1α/HS interactions







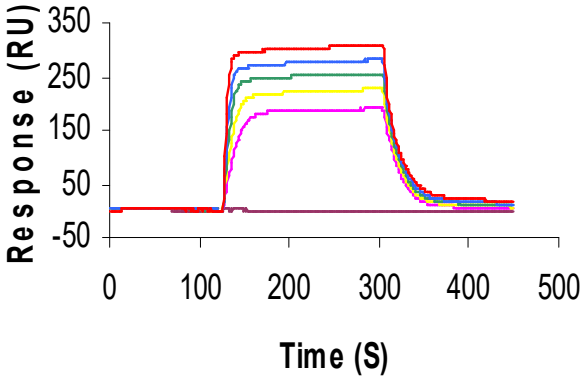


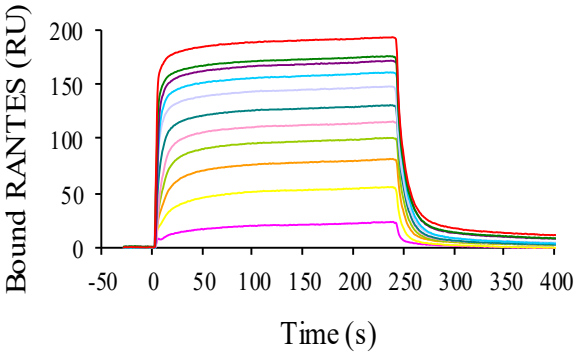


Sadir et al. *J. Biol .Chem. 2001*

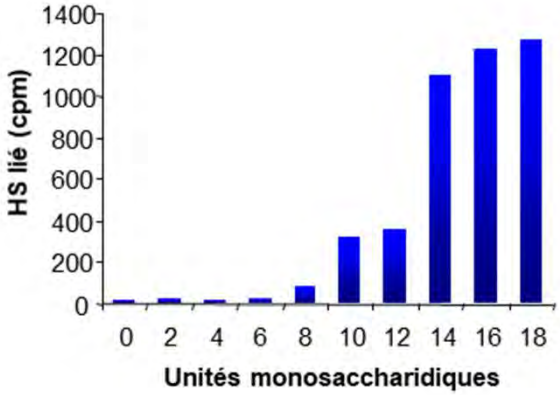
Chemokine /HS interactions

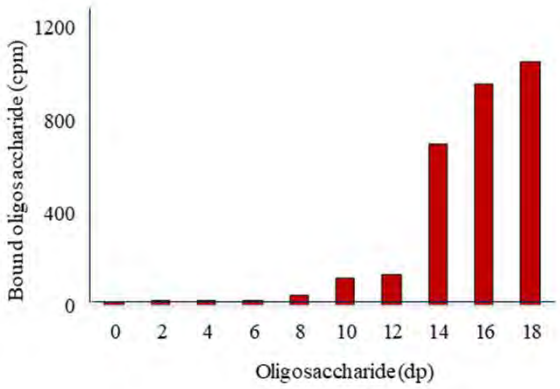
SDF1-α versus RANTES





Both bind to HS

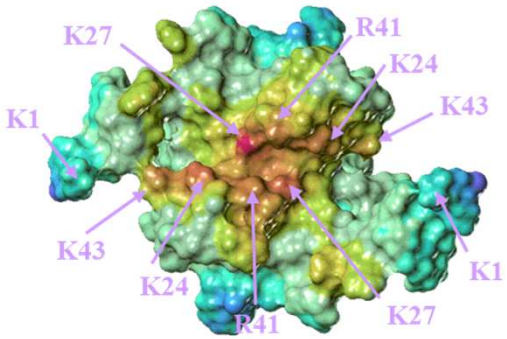


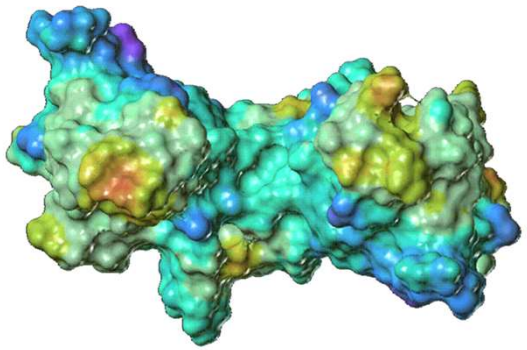


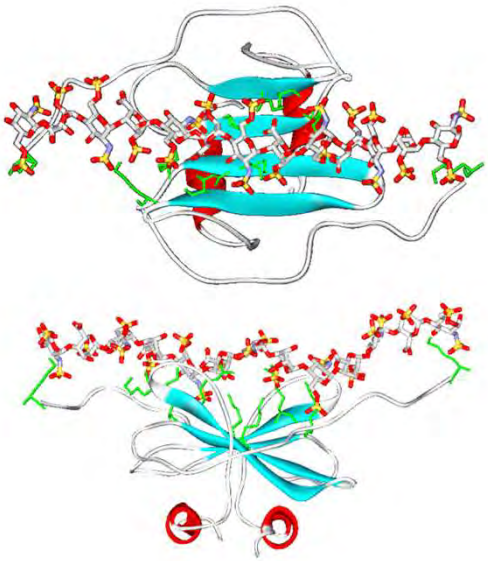
Both require a saccharide motif of ~14 saccharides

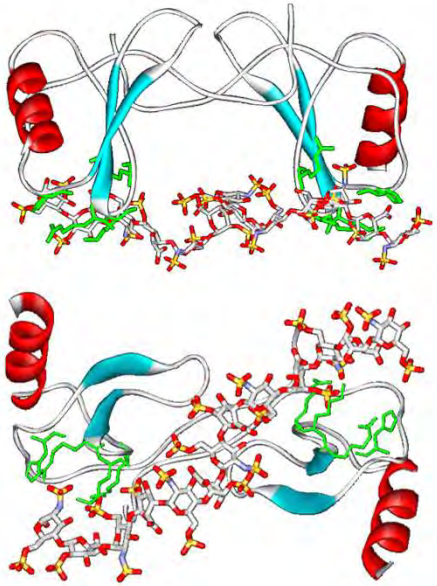
Chemokine /HS interactions

SDF1-α versus RANTES

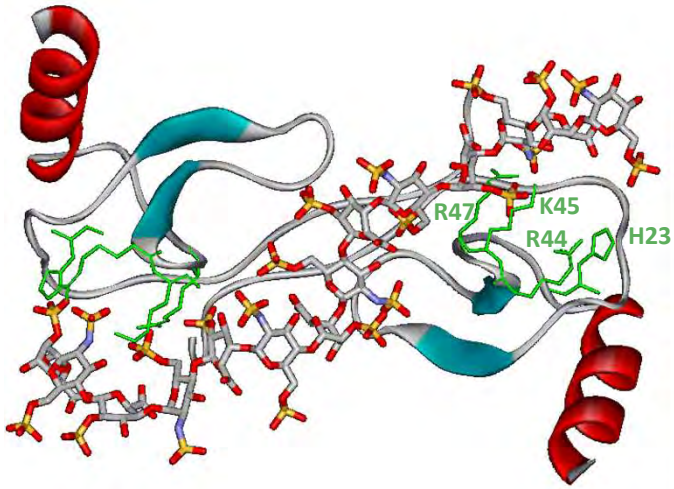


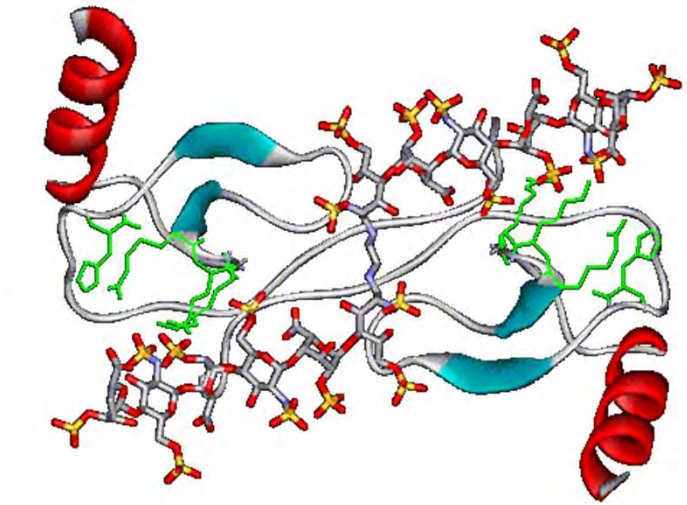




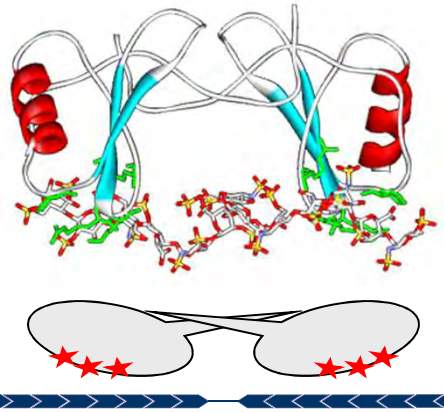


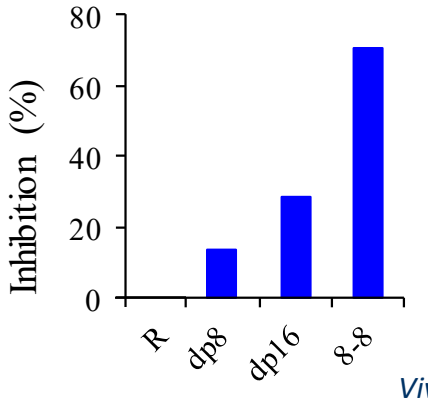
Chemokine /HS interactions





RANTES

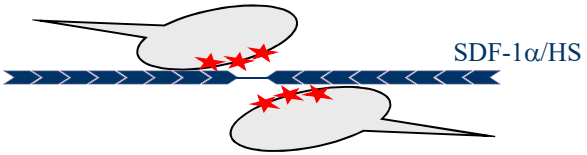


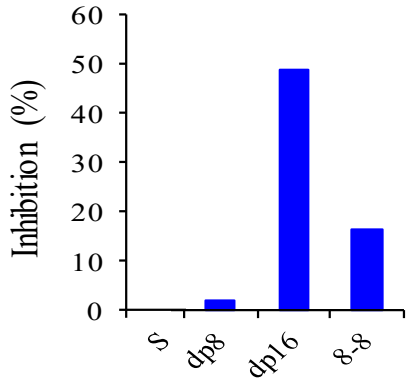


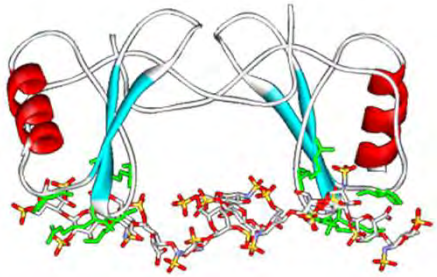
Vivès et al. Biochemistry 2002

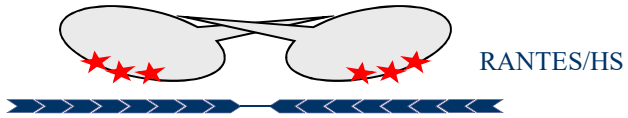
Chemokine /HS interactions

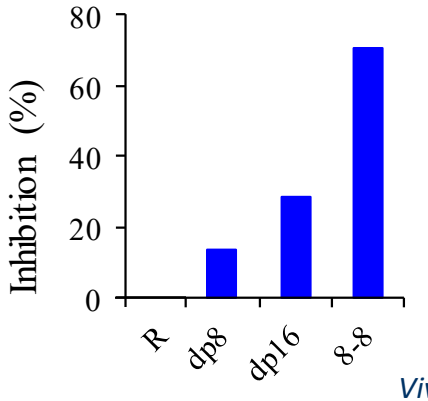
SDF1-α versus RANTES







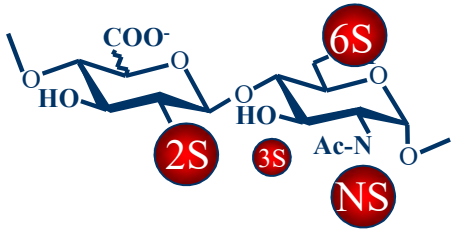


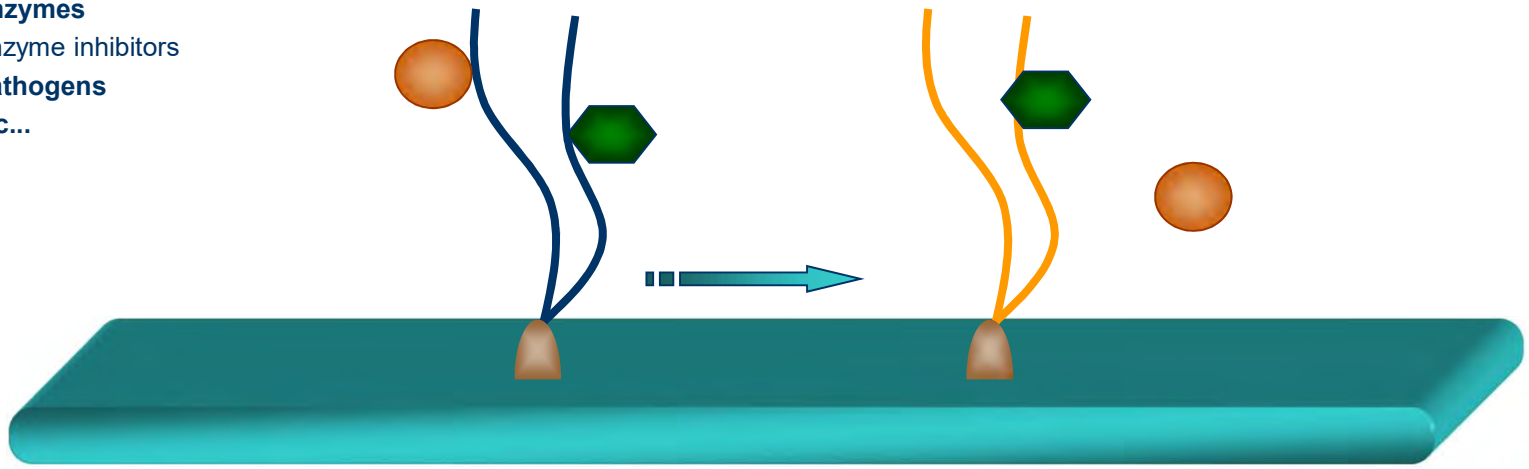


Vivès et al. Biochemistry 2002

Regulation of HS structure

- **Growth factors**
- **Cytokines**
- **Chemokines**
- Adhesion molecules Matrix proteins
- **Enzymes**
- Enzyme inhibitors
- **Pathogens**
- **etc...**

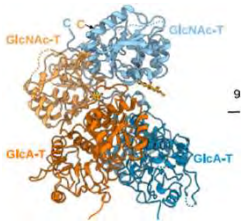




HS biosynthesis

NDSTs (4 isoforms)
C5-epimerase
2-OST
6-OSTs (3 isoforms)
3-OSTs (7 isoforms)

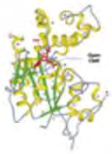
The concept of GAGosome

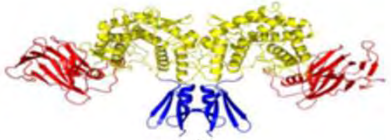


EXT1/2 (human)
Leisico et al. Nat. comm. 2022

EXT-L2 (Mouse)
Pedersen *et al.* JBC 2003

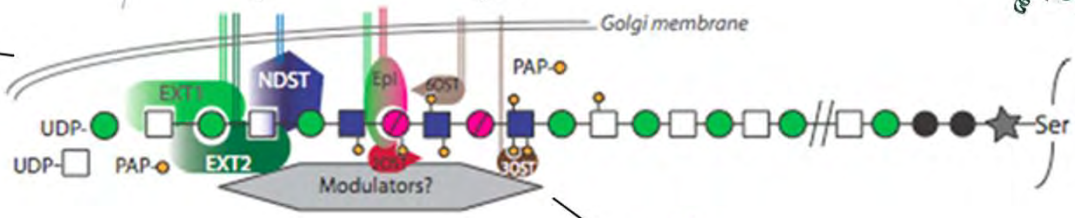
NDST (human – 4 isoforms)
Kakuta *et al.* JBC 1999



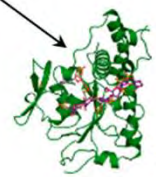


C5-epimerase (Zebrafish)
Qin et al. JBC 2015

6OST (Zebrafish – 3 isoforms)
Xu et al. ACS Chem Biol 2017

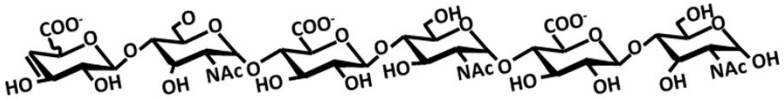


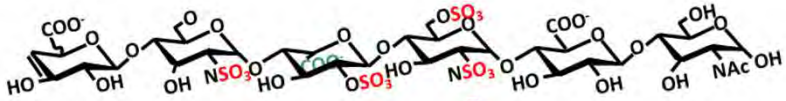
2OST (Chiken)
Bethea *et al* PNAS 2008



3OST-1 (Mouse - 7 isoforms)
Moon *et al.* PNAS 2012

Structure
organization of the complex
Stoichiometry

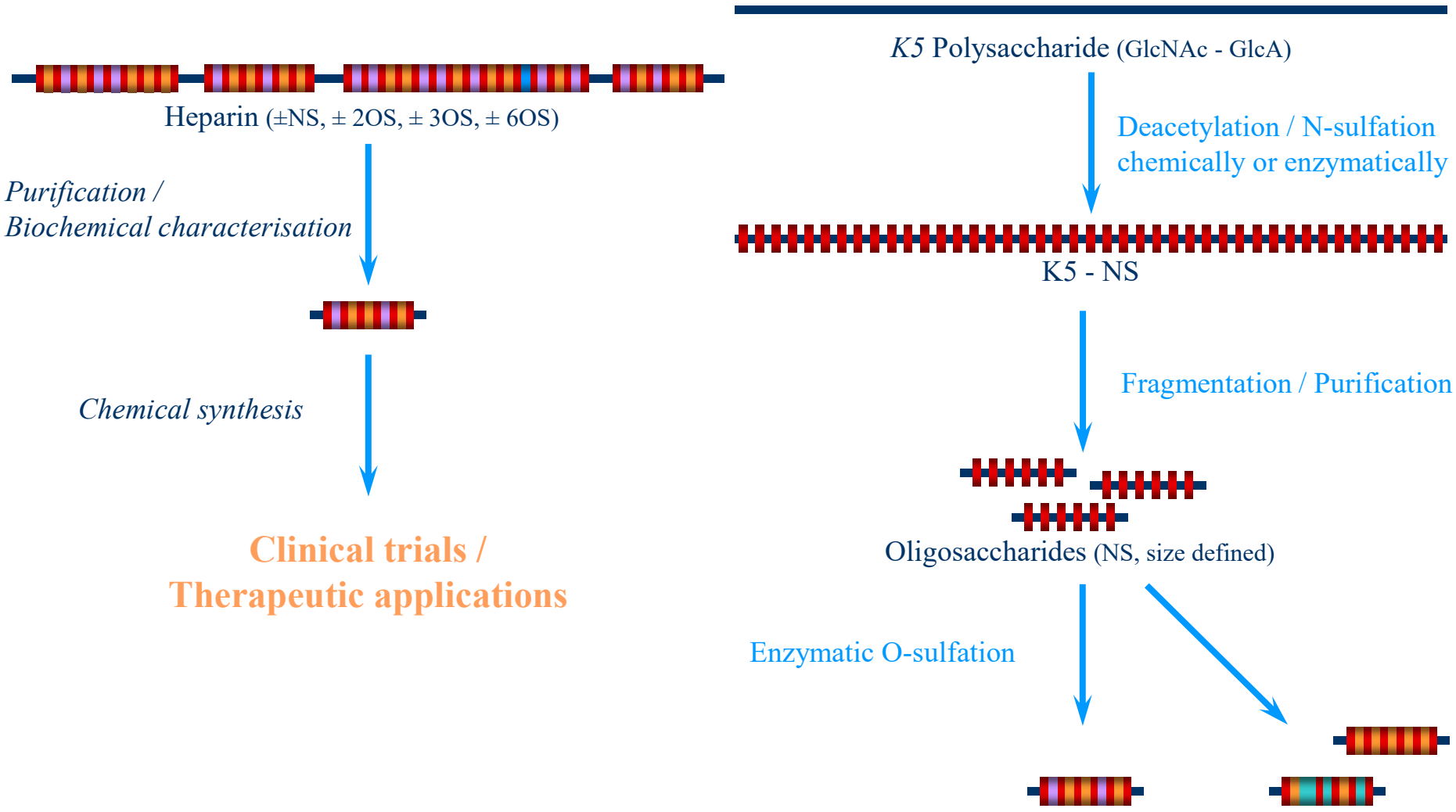




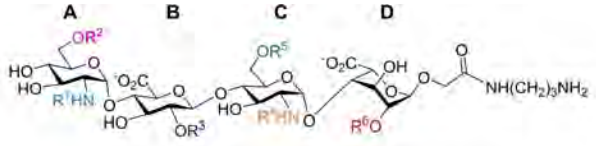
HS sequence

HS binding & function

Biotechnological applications : Use of sulfotransferases for the chemo-enzymatic synthesis of oligosaccharides

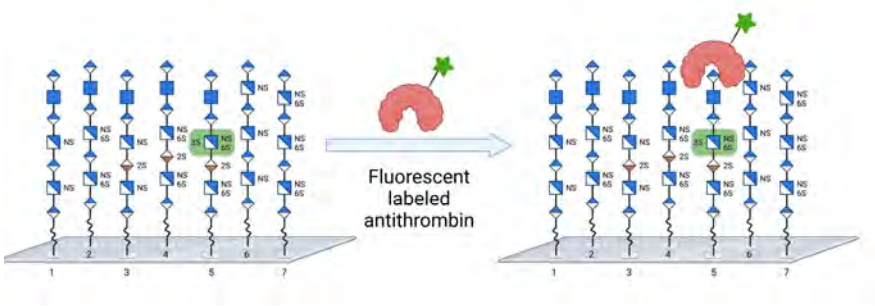


Biotechnological applications : Use of sulfotransferases for the chemo-enzymatic synthesis of oligosaccharides



72: GlcNS-GlcA2S-GlcNAc-IdoA
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = SO_3^-$, $R^4 = Ac$, $R^5 = H$, $R^6 = H$
73: GlcNS-GlcA2S-GlcNS-IdoA
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = SO_3^-$, $R^4 = SO_3^-$, $R^5 = H$, $R^6 = H$
74: GlcNS-GlcA2S-GlcNAc-IdoA2S
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = SO_3^-$, $R^4 = Ac$, $R^5 = H$, $R^6 = SO_3^-$
75: GlcNS-GlcA2S-GlcNS-IdoA2S
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = SO_3^-$, $R^4 = SO_3^-$, $R^5 = H$, $R^6 = SO_3^-$
76: GlcNS-GlcA2S-GlcNAc6S-IdoA
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = SO_3^-$, $R^4 = Ac$, $R^5 = SO_3^-$, $R^6 = H$
77: GlcNS-GlcA2S-GlcNS6S-IdoA
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = SO_3^-$, $R^4 = SO_3^-$, $R^5 = SO_3^-$, $R^6 = H$
78: GlcNS6S-GlcA2S-GlcNAc-IdoA
$R^1 = SO_3^-$, $R^2 = SO_3^-$ $R^3 = SO_3^-$, $R^4 = Ac$, $R^5 = H$, $R^6 = H$
79: GlcNS6S-GlcA2S-GlcNS-IdoA
$R^1 = SO_3^-$, $R^2 = SO_3^-$ $R^3 = SO_3^-$, $R^4 = SO_3^-$, $R^5 = H$, $R^6 = H$
80: GlcNS-GlcA2S-GlcNAc6S-IdoA2S
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = SO_3^-$, $R^4 = Ac$, $R^5 = SO_3^-$, $R^6 = SO_3^-$
81: GlcNS-GlcA2S-GlcNS6S-IdoA2S
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = SO_3^-$, $R^4 = SO_3^-$, $R^5 = SO_3^-$, $R^6 = SO_3^-$
82: GlcNS6S-GlcA2S-GlcNAc-IdoA2S
$R^1 = SO_3^-$, $R^2 = SO_3^-$, $R^3 = SO_3^-$, $R^4 = Ac$, $R^5 = H$, $R^6 = SO_3^-$
83: GlcNS6S-GlcA2S-GlcNS-IdoA2S
$R^1 = SO_3^-$, $R^2 = SO_3^-$, $R^3 = SO_3^-$, $R^4 = SO_3^-$, $R^5 = H$, $R^6 = SO_3^-$
84: GlcNS6S-GlcA2S-GlcNAc6S-IdoA2S
$R^1 = SO_3^-$, $R^2 = SO_3^-$, $R^3 = SO_3^-$, $R^4 = Ac$, $R^5 = SO_3^-$, $R^6 = SO_3^-$
85: GlcNS6S-GlcA2S-GlcNS6S-IdoA2S
$R^1 = SO_3^-$, $R^2 = SO_3^-$, $R^3 = SO_3^-$, $R^4 = SO_3^-$, $R^5 = SO_3^-$, $R^6 = SO_3^-$
86: GlcNS6S-GlcA2S-GlcNAc6S-IdoA
$R^1 = SO_3^-$, $R^2 = SO_3^-$, $R^3 = SO_3^-$, $R^4 = Ac$, $R^5 = SO_3^-$, $R^6 = H$
87: GlcNS6S-GlcA2S-GlcNS6S-IdoA
$R^1 = SO_3^-$, $R^2 = SO_3^-$, $R^3 = SO_3^-$, $R^4 = SO_3^-$, $R^5 = SO_3^-$, $R^6 = H$

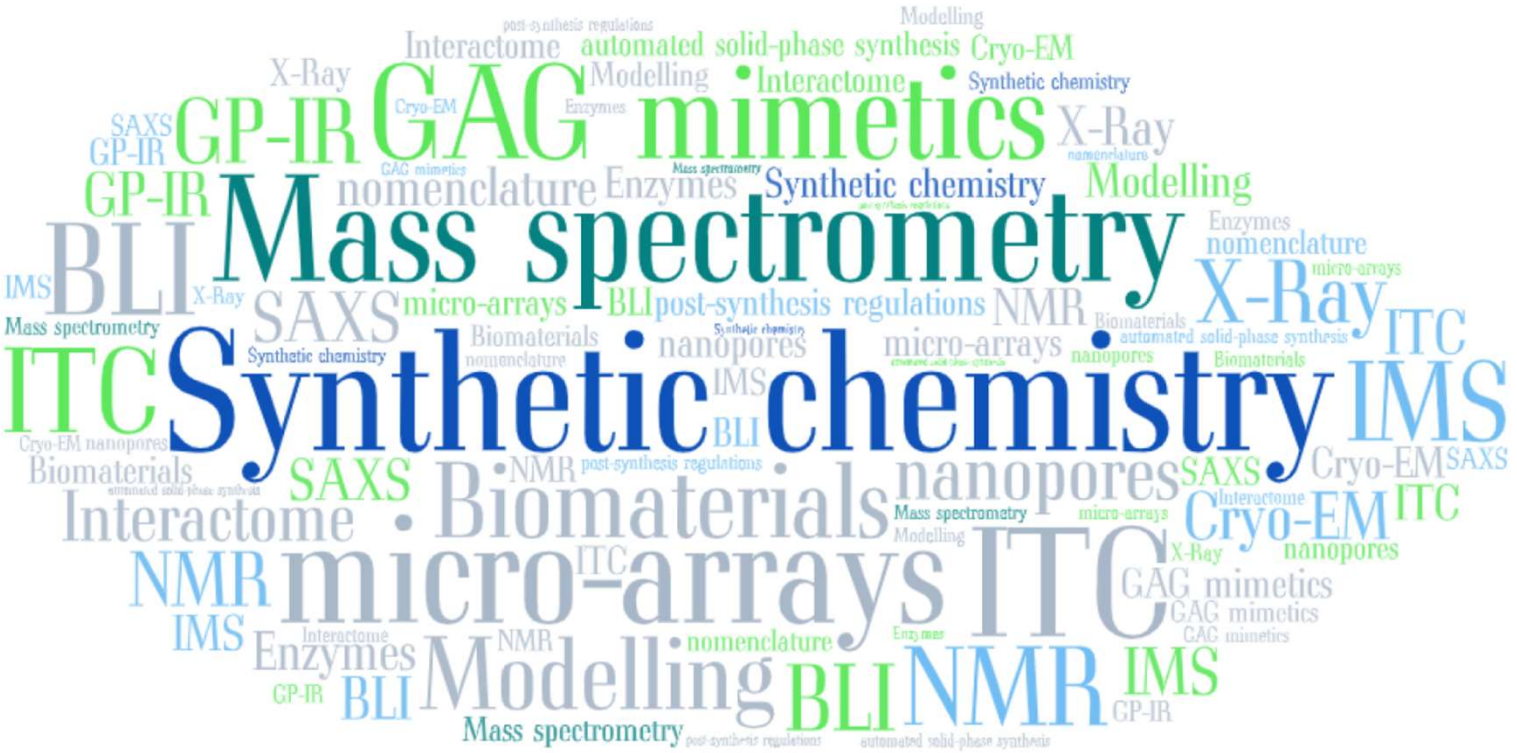
88: GlcNS-GlcA-GlcNAc-IdoA
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = H$, $R^4 = Ac$, $R^5 = H$, $R^6 = H$
89: GlcNS-GlcA-GlcNS-IdoA
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = H$, $R^4 = SO_3^-$, $R^5 = H$, $R^6 = H$
90: GlcNS-GlcA-GlcNAc-IdoA2S
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = H$, $R^4 = Ac$, $R^5 = H$, $R^6 = SO_3^-$
91: GlcNS-GlcA-GlcNS-IdoA2S
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = H$, $R^4 = SO_3^-$, $R^5 = H$, $R^6 = SO_3^-$
92: GlcNS-GlcA-GlcNAc6S-IdoA
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = H$, $R^4 = Ac$, $R^5 = SO_3^-$, $R^6 = H$
93: GlcNS-GlcA-GlcNS6S-IdoA
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = H$, $R^4 = SO_3^-$, $R^5 = SO_3^-$, $R^6 = H$
94: GlcNS6S-GlcA-GlcNAc-IdoA
$R^1 = SO_3^-$, $R^2 = SO_3^-$ $R^3 = H$, $R^4 = Ac$, $R^5 = H$, $R^6 = H$
95: GlcNS6S-GlcA-GlcNS-IdoA
$R^1 = SO_3^-$, $R^2 = SO_3^-$ $R^3 = H$, $R^4 = SO_3^-$, $R^5 = H$, $R^6 = H$
96: GlcNS-GlcA-GlcNAc6S-IdoA2S
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = H$, $R^4 = Ac$, $R^5 = SO_3^-$, $R^6 = SO_3^-$
97: GlcNS-GlcA-GlcNS6S-IdoA2S
$R^1 = SO_3^-$, $R^2 = H$, $R^3 = H$, $R^4 = SO_3^-$, $R^5 = SO_3^-$, $R^6 = SO_3^-$
98: GlcNS6S-GlcA-GlcNAc-IdoA2S
$R^1 = SO_3^-$, $R^2 = SO_3^-$, $R^3 = H$, $R^4 = Ac$, $R^5 = H$, $R^6 = SO_3^-$
99: GlcNS6S-GlcA-GlcNS-IdoA2S
$R^1 = SO_3^-$, $R^2 = SO_3^-$, $R^3 = H$, $R^4 = SO_3^-$, $R^5 = H$, $R^6 = SO_3^-$
100: GlcNS6S-GlcA-GlcNAc6S-IdoA2S
$R^1 = SO_3^-$, $R^2 = SO_3^-$, $R^3 = H$, $R^4 = Ac$, $R^5 = SO_3^-$, $R^6 = SO_3^-$
101: GlcNS6S-GlcA-GlcNS6S-IdoA2S
$R^1 = SO_3^-$, $R^2 = SO_3^-$, $R^3 = H$, $R^4 = SO_3^-$, $R^5 = SO_3^-$, $R^6 = SO_3^-$
102: GlcNS6S-GlcA-GlcNAc6S-IdoA
$R^1 = SO_3^-$, $R^2 = SO_3^-$, $R^3 = H$, $R^4 = Ac$, $R^5 = SO_3^-$, $R^6 = H$
103: GlcNS6S-GlcA-GlcNS6S-IdoA
$R^1 = SO_3^-$, $R^2 = SO_3^-$, $R^3 = H$, $R^4 = SO_3^-$, $R^5 = SO_3^-$, $R^6 = H$

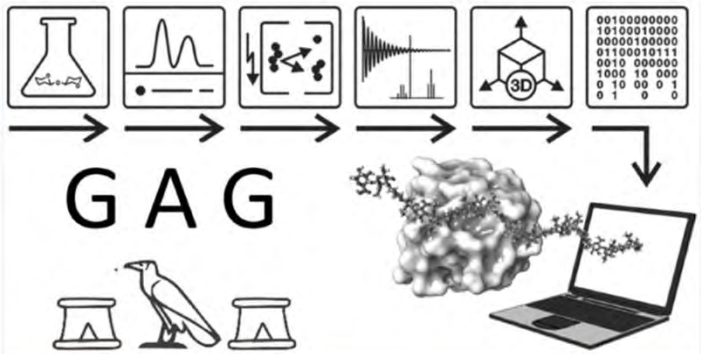


Glycan therapeutics

Baryal *et al.*, 2022 *Angew. Chem.Int.* **62**, e2022119

And so much more to talk about….





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