Heparan sulfate glycosaminoglycans and regulation of growth factor signaling

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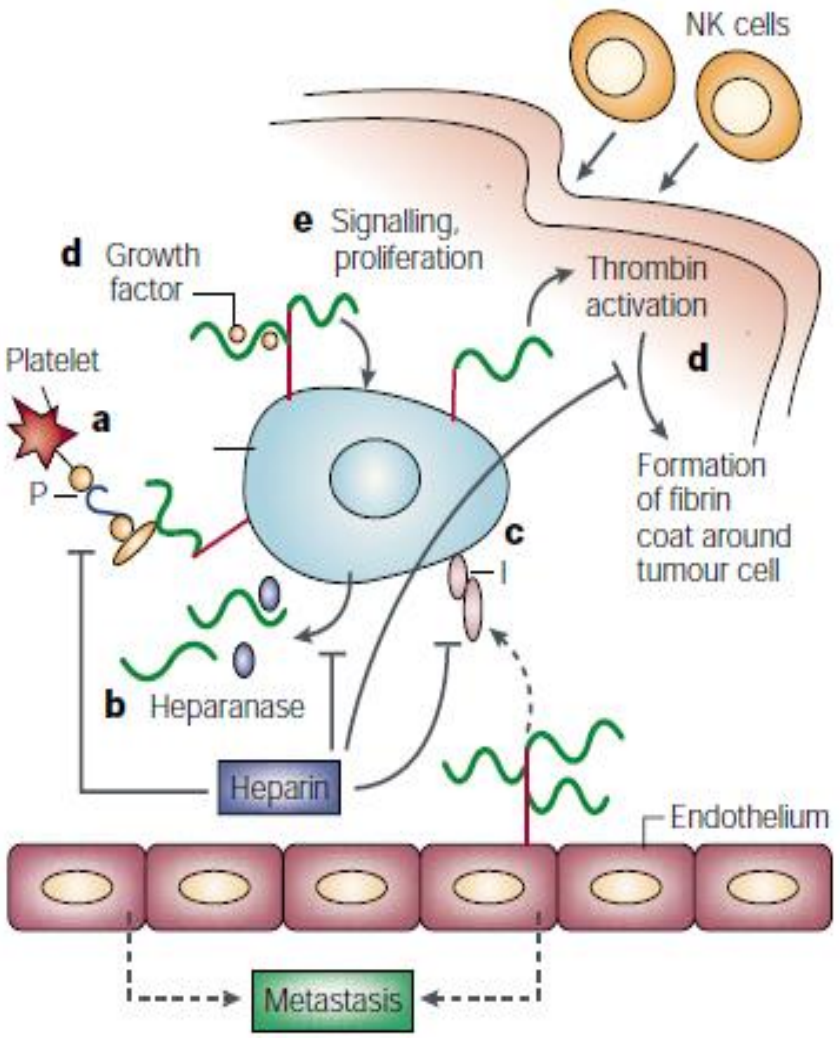
Learning objectives

- Understand the nature of Heparan sulfate (HS) interactions with growth factor (GF) ligands, receptor, or their complexes.
- Understand the role of complex, pleiotropic and yet specific interactions of Heparan sulfate in regulating a phenotype.
- Understand HS structure-activity-relationship with respect to key GF signaling.

Heparin-HS and Various Functions



Sasisekharan et al. Nature Reviews Cancer , 2002

Physiological function of HSGAGs is mediated through its interaction with ECM molecules.

- HSGAGs act at the cell–extracellular-matrix (ECM) interface to modulate cell signaling.

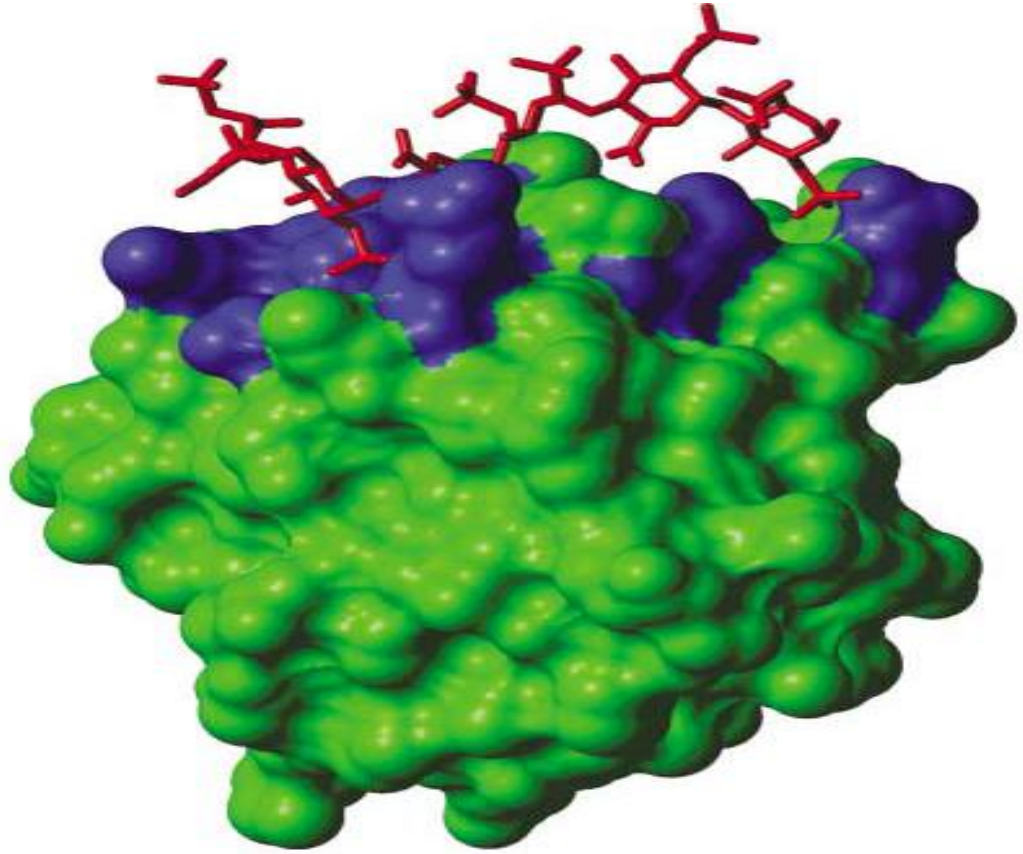
- HSGAGs interact with various extracellular signaling molecules: growth factors, morphogens, and chemokines.

Sasisekharan et al. Nature Reviews Cancer , 2002

HSGAG interacting proteins

- GF: FGF, VEGF, HGF
- CK/Chemokines: Interleukins, CXCLs (C-X-C motif ligands) and CCLs (C-C motif)
- Cell-cell interacting molecules: selectins (p-selectin)
- Cell matrix interacting molecules: Laminin, fibronectin.
- Morphogens: Wnt, Hedgehog,
- Enzymes: heparanase
- Coagulation enzymes/factors: ATIII, Thrombin, TFPI etc.

Heparan sulfates show specific binding to growth factors ligands through electrostatic interactions



Sasisekharan et al. Nature Reviews Cancer , 2002

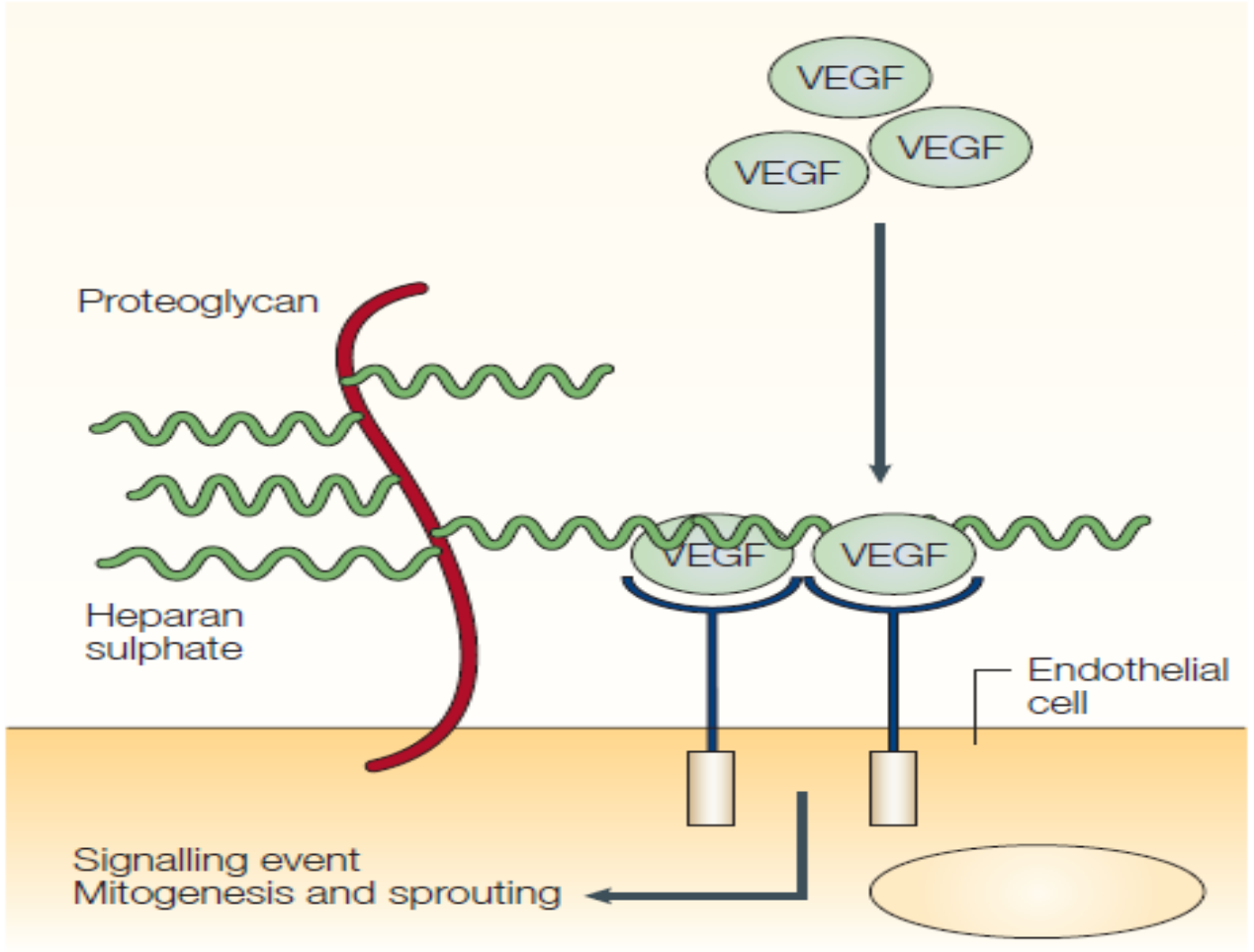
The binding of GAGs to proteins is mediated by the interaction of the negatively charged *sulfate and carboxyl groups* in the GAG and the positively charged side chains of *lysines and arginine* in the protein.

Sommer and Rifkin, 1989; Arai et al. 1994; Middleton et al. 1997; Middleton et al. 2002; Cripps et al. 2005; Yu et al. 2005; Salanga and Handel 2011.

Consequences of HSGAG-protein interactions

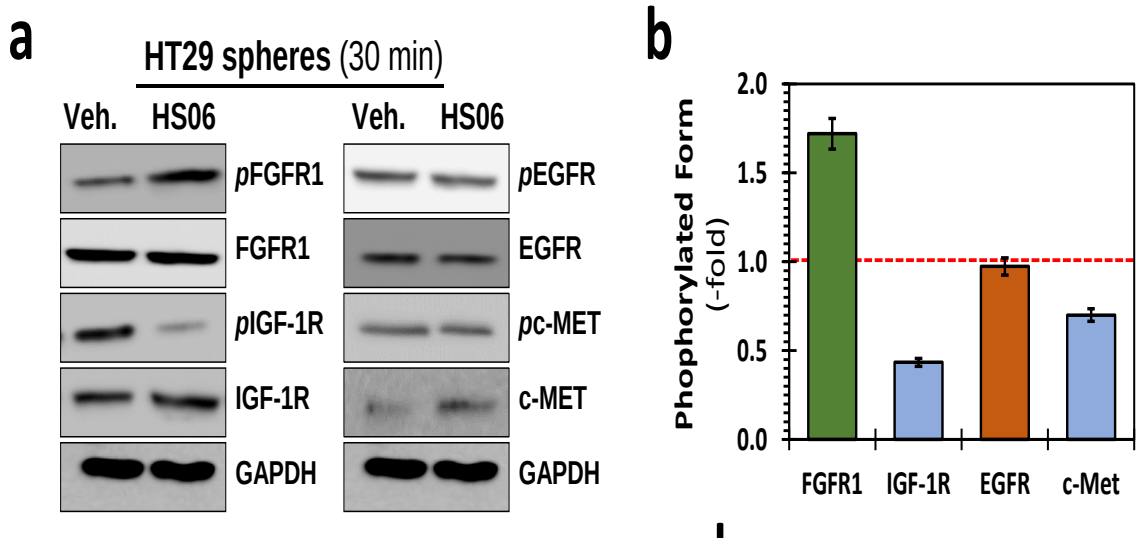
- Promotion of oligomerization of Growth factors/chemokines/cytokines leading to their active states
- Pleiotropic modulation of GF receptors,
- Mediation of intracellular signaling cascade leading to activation of key signaling hubs.
- Stabilization of protein gradients
- Presentation to cooperative binding molecules

GAG and Tyrosine Kinase Receptor Signaling

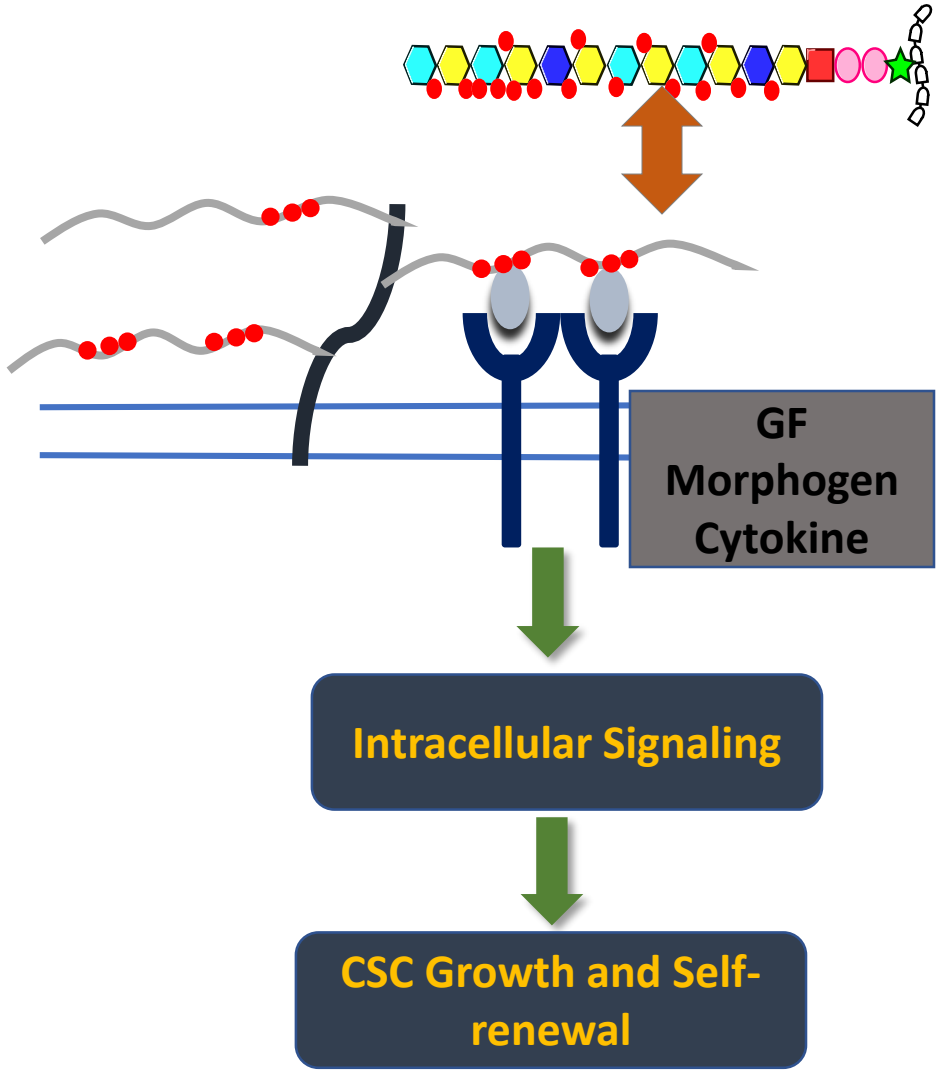


Fuster et al. Nature Reviews Cancer, 2005

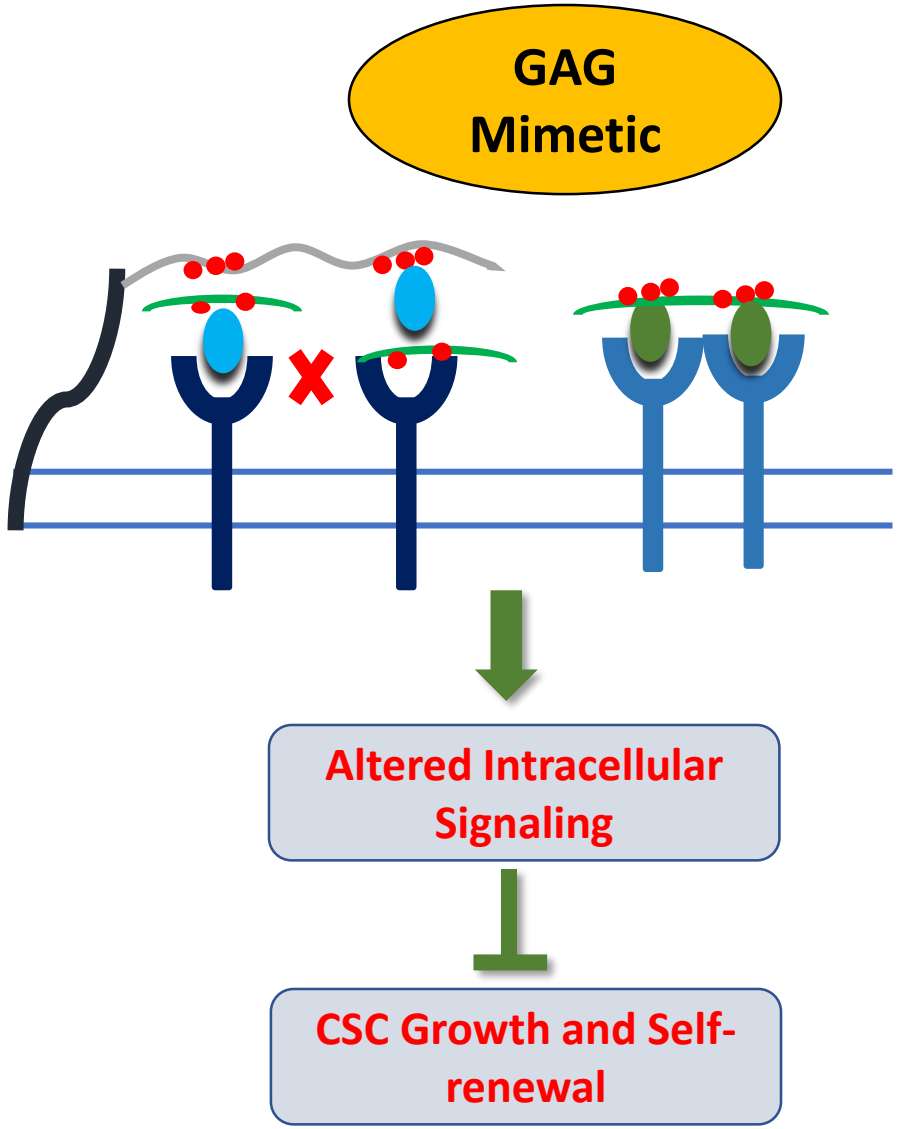
Anti-CSC HS06 induces differential but specific activation of GF receptors



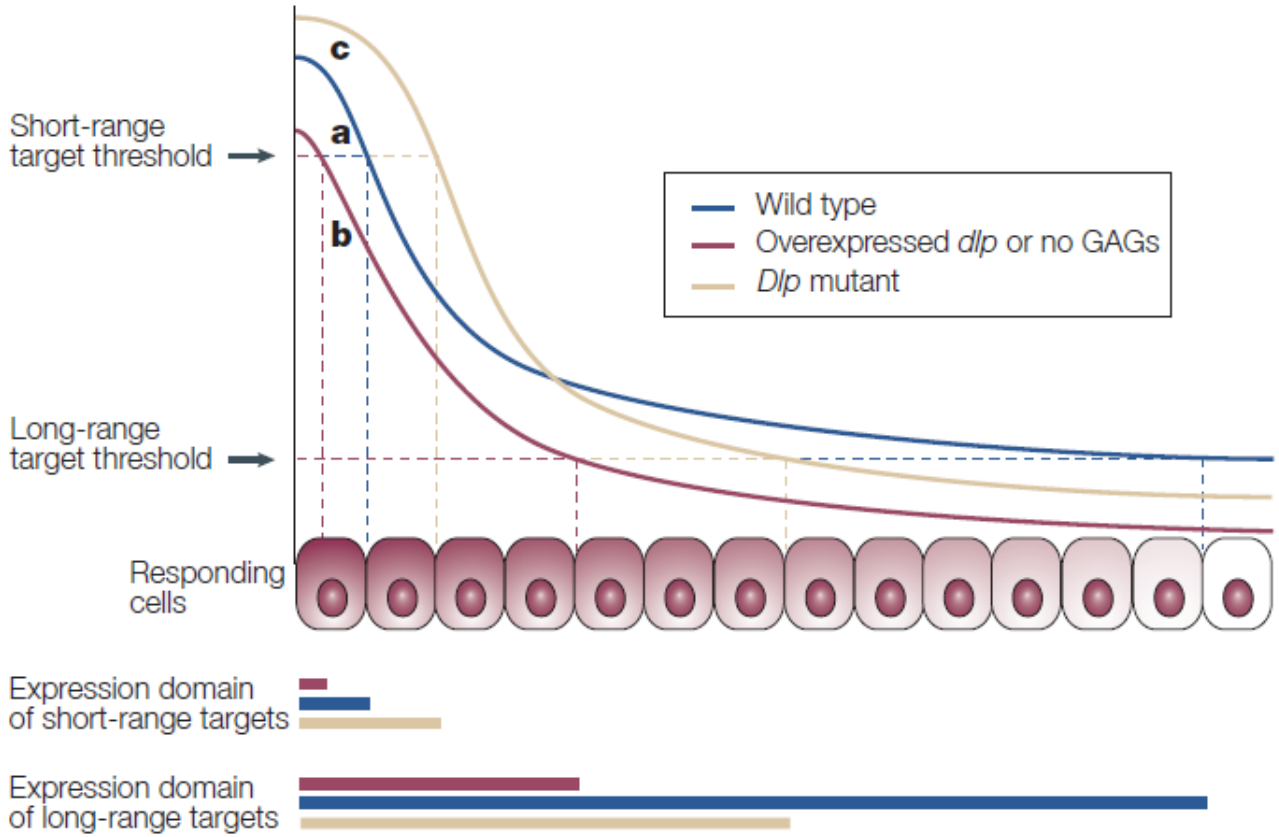
Glycosaminoglycans (GAGs)



Translational Value



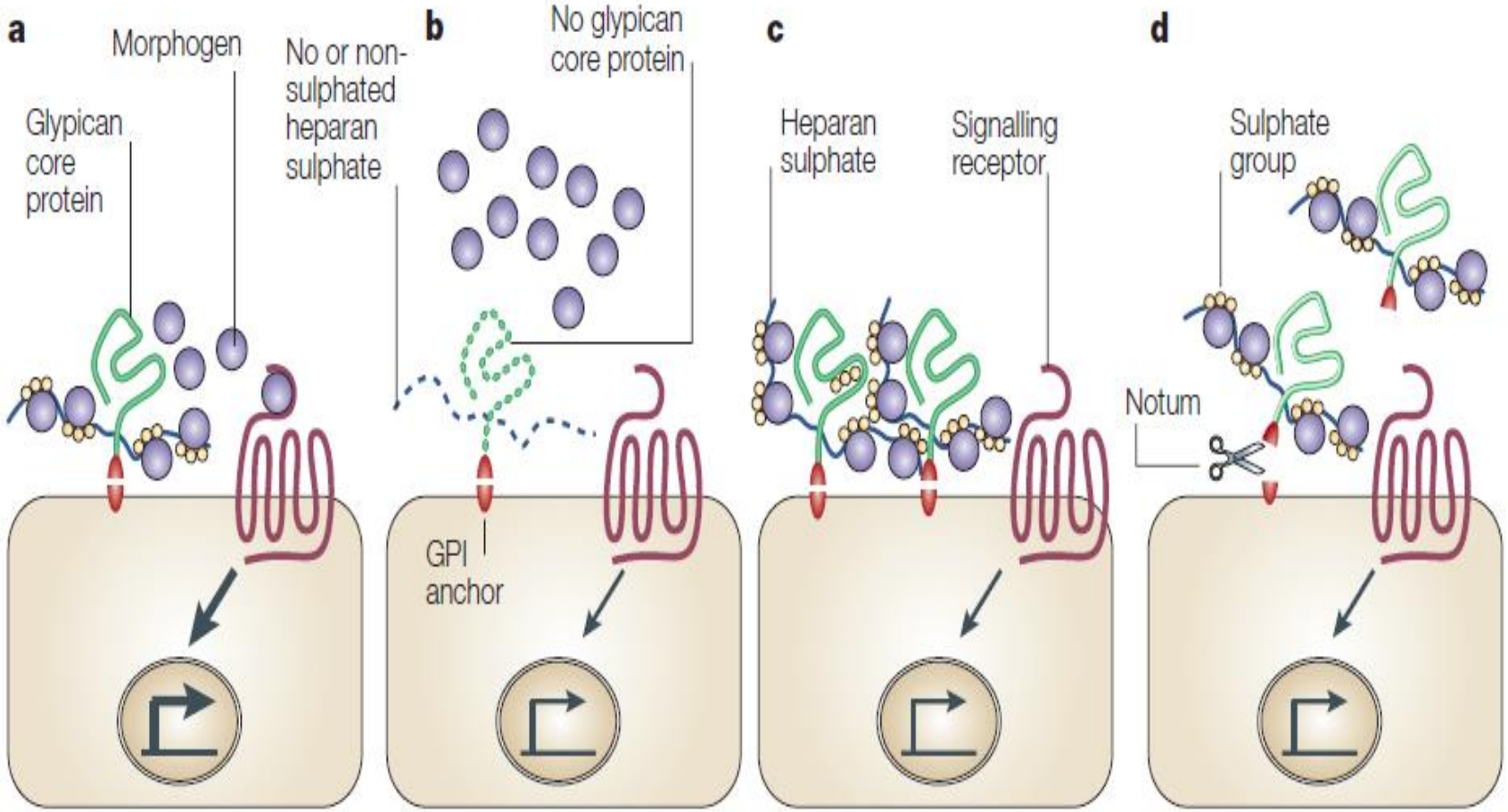
Morphogen gradient regulation by HS



GAG and Morphogen signaling

Optimum morphogen gradient is essential for signaling.

Heparan sulfate molecules regulate morphogen gradient formation.



Regulators of HSGAG-protein interactions

The specificity of HSGAG-protein interactions is dependent on:

a. **HSGAG structure:**

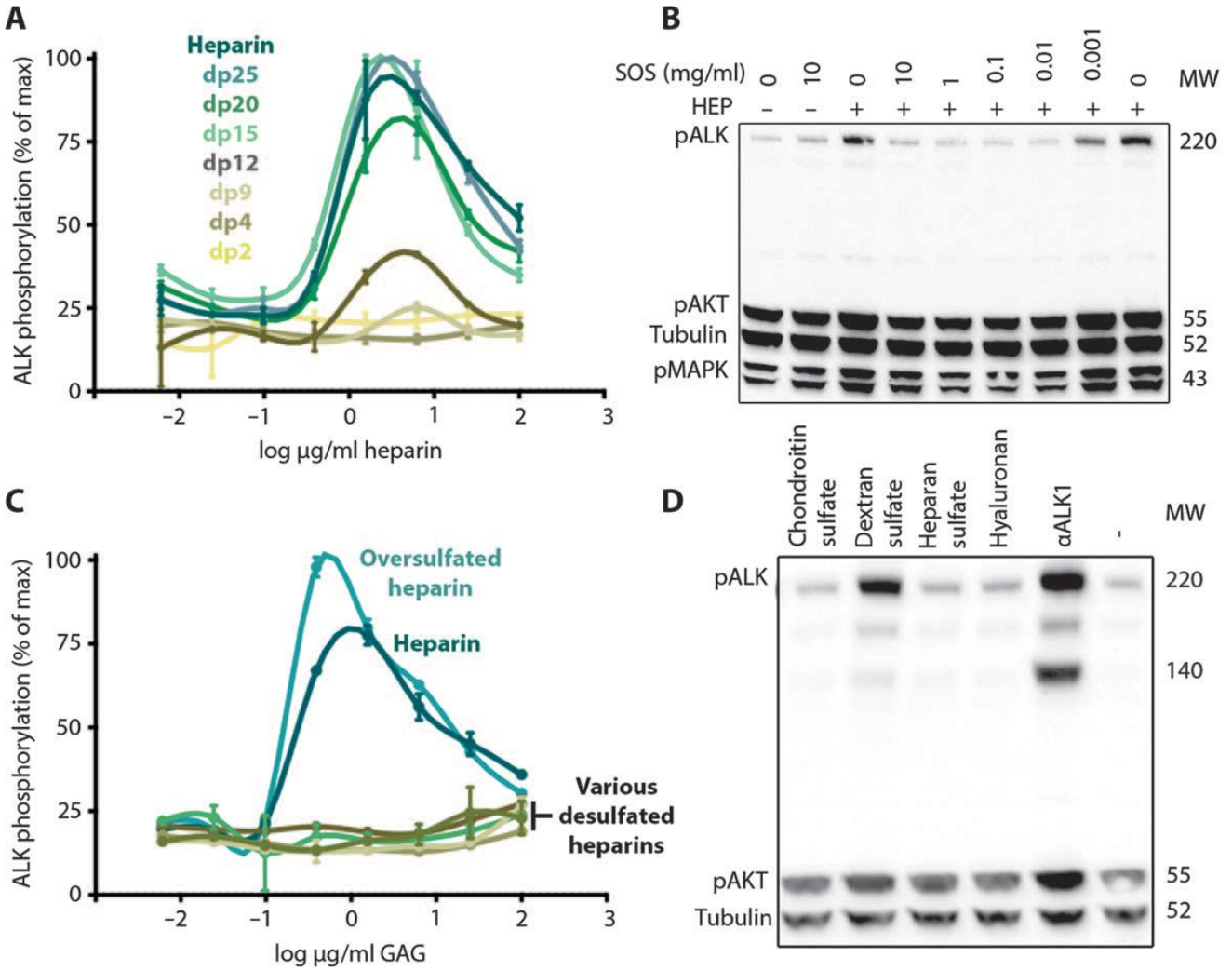
The structural complexity arises from the differential modification of individual disaccharide units within an oligosaccharide chain. There are 48 possible disaccharide units, which can form a complete HSGAG chain of 10–100 disaccharide units.

- Chain-length: number of disaccharide units
- Fine structure:
  - *Sulfation pattern*: N-, 3-O, 6-O sulfation of hexosamines and 2-O sulfation of Iduronic acid.
  - *Epimerization*: Glucoronic acid vs. iduronic acid

a. **Spacing of binding sites**

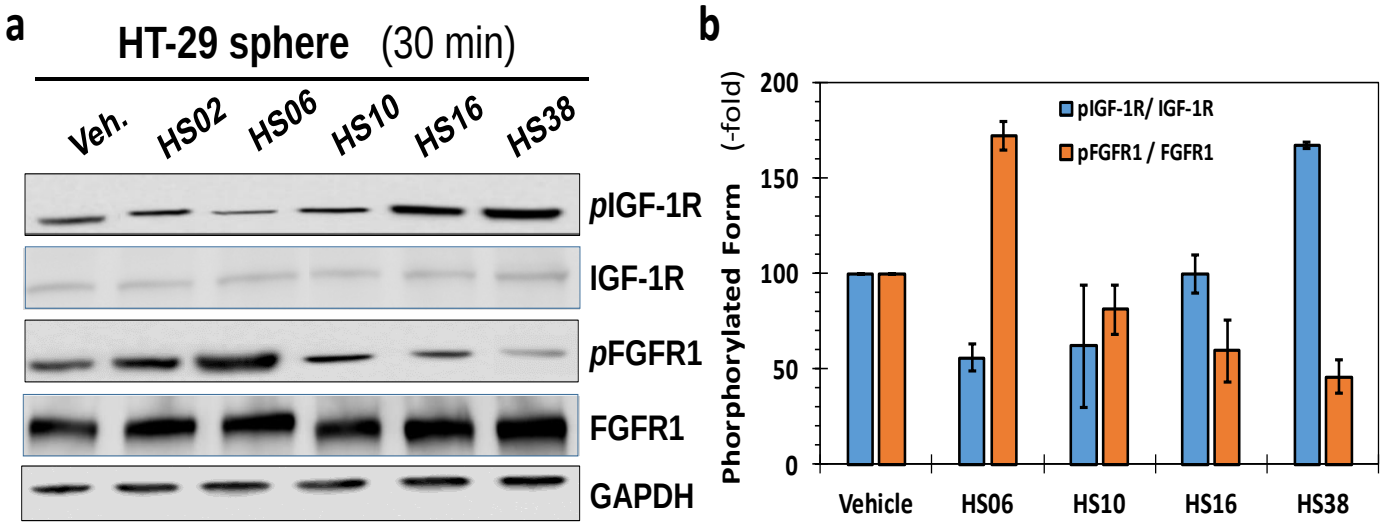
b. **3D structure of the HSGAG chain**

ALK receptor activation by HSGAGs



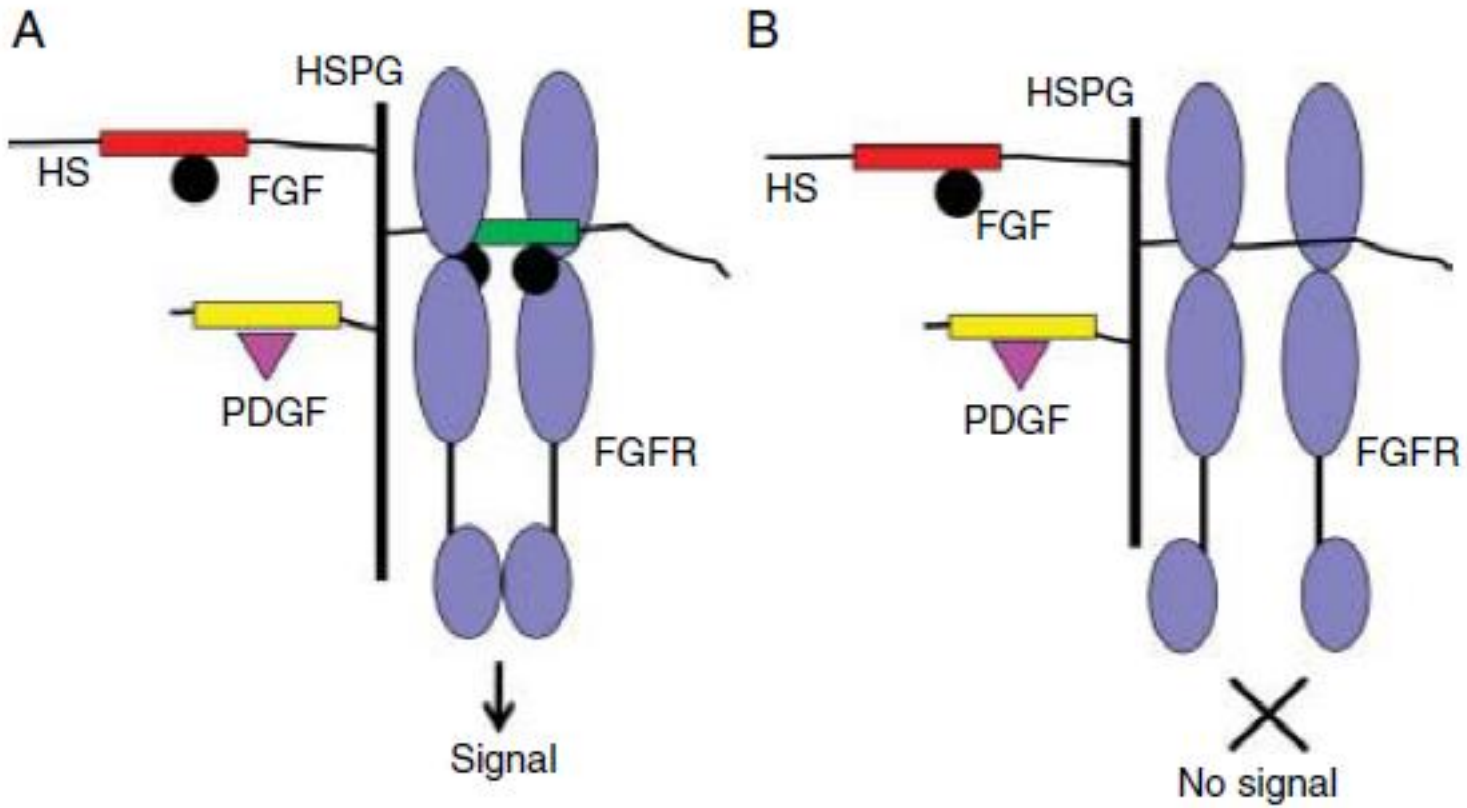
Example of increasing HSGAG chain length leading to higher activation of the receptor

Effect of GAGs of various chain length on activation of IGF-1R and FGFR



Example of the need for an optimum HSGAG chain length for the activation/inhibition of the receptor

HS fine structure and its relationship to its activity



Each HSGAG – protein interaction may have unique SAR

HSGAG binding with VEGF-A165 requires all common sulfate groups (N, 2-O, and 6-O), although with different emphasis on their relative importance.

(Ashikari-Hada et al.,2005; Robinson et al., 2006).

Hepatocyte growth factor binds a variety of glycosaminoglycan structures without any clear preference.

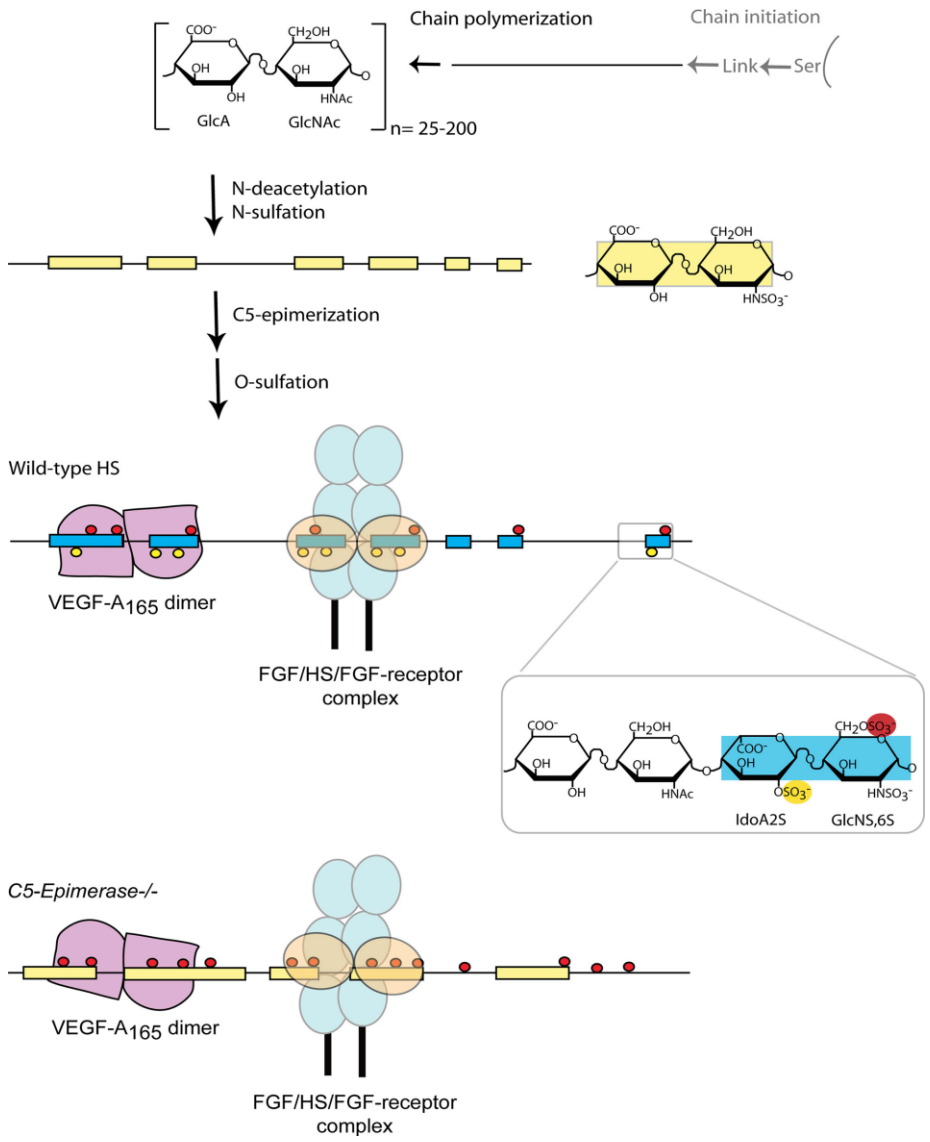
Effect of HS sulfation pattern on binding to GFs.

Equilibrium dissociation constant for the interactions of FGF-2, HGF, $VEGF_{165}$, and BMP-6 with various chemically modified heparins

The K_D (nM) value was measured from Fig. 5.

Immobilized GAG	FGF-2	HGF	$VEGF_{165}$	BMP-6
Heparin	23	12	165	6.3
2ODS-heparin	340	86	524	11
6ODS-heparin	23	58	592	15

Compensatory mechanism at play in governing HSGAG fine structure may impact its interaction with target proteins.



Effect of HS sulfation pattern on binding to GFs.

Groups	(Necessary *O*-sulfate in octasaccharide)	GF	heparin binding regions of FGFs
			Glycine box
Group 1	(2-*O*-sulfate)	FGF-2	118 L K R T G Q Y **K** L G S K T G P G **Q K A** I L
Group 2	(6-*O*-sulfate)	FGF-10	180 L N G K G A P R R G Q K T R R K N T S A H
Group 3	(2-*O*- or 6-*O*-sulfate)	FGF-18	153 F T K K G R P R K G P K T R E N Q Q D V H
		HGF	
Group 4	(2-*O*- and 6-*O*-sulfate)	FGF-4	181 L S K N G K T K K G N R V S P T M K V T H
		FGF-7	167 L N Q K G I P V R G K K T K K E Q K T A H
		(FGF-1)	111 L K **K** N G S C **K** R G P R T H Y G **Q K A** I L
Group 5		FGF-8	171 F T R K G R P R K G S K T R Q H Q R E V H
		VEGF	
		BMP-6	

Methods to Determine GAG-protein binding

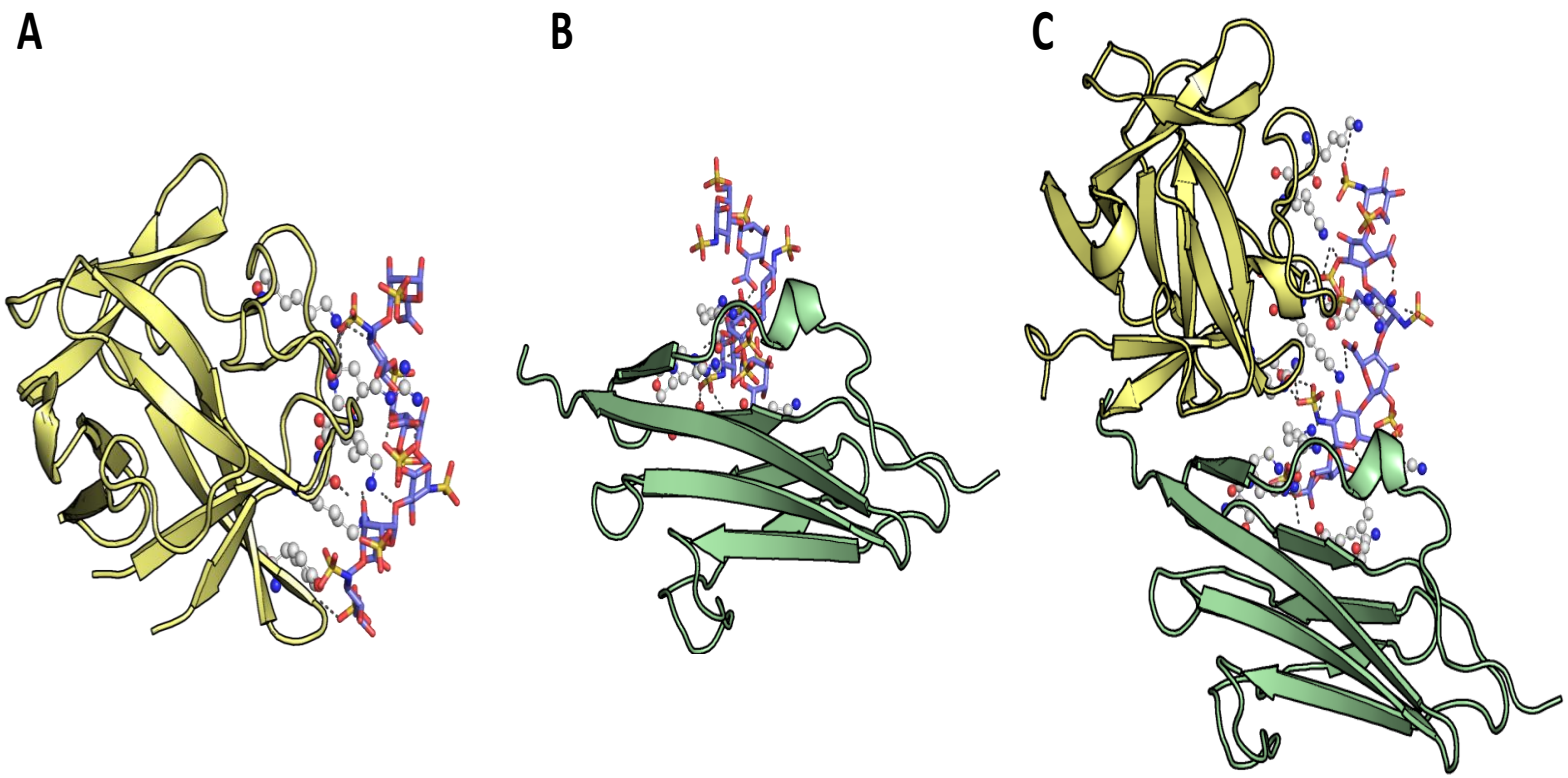
- In Silico: Including Molecular Dynamics

- In vitro:
  - Fluorescence
  - Surface Plasmon Resonance
  - NMR
  - Isothermal Titration calorimetry

- In vivo/in cell:

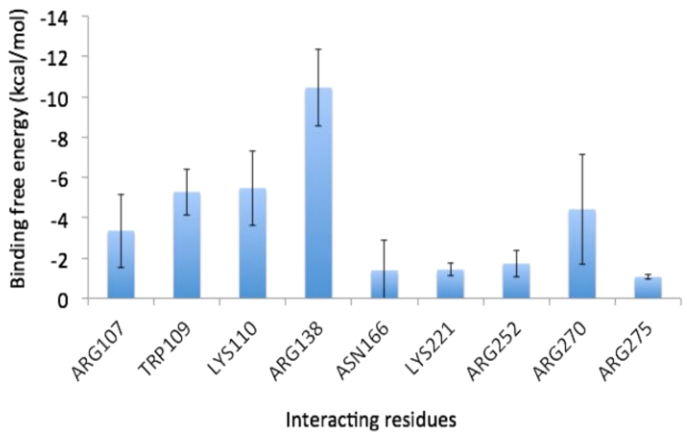
In Silico methods

HS06 interaction with FGFR



Characterization of HS06 ionic interaction with target GF

C



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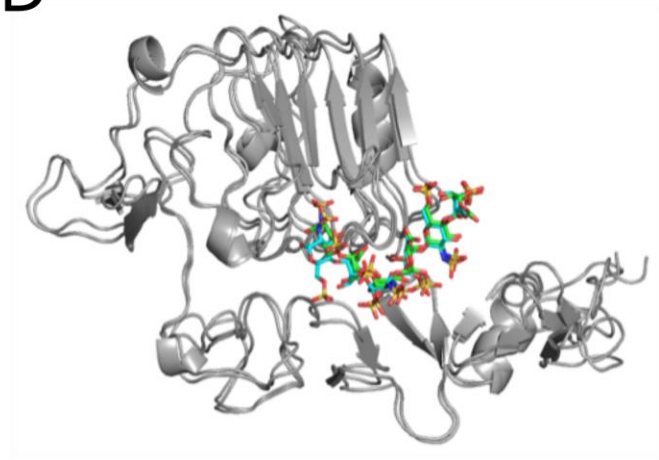
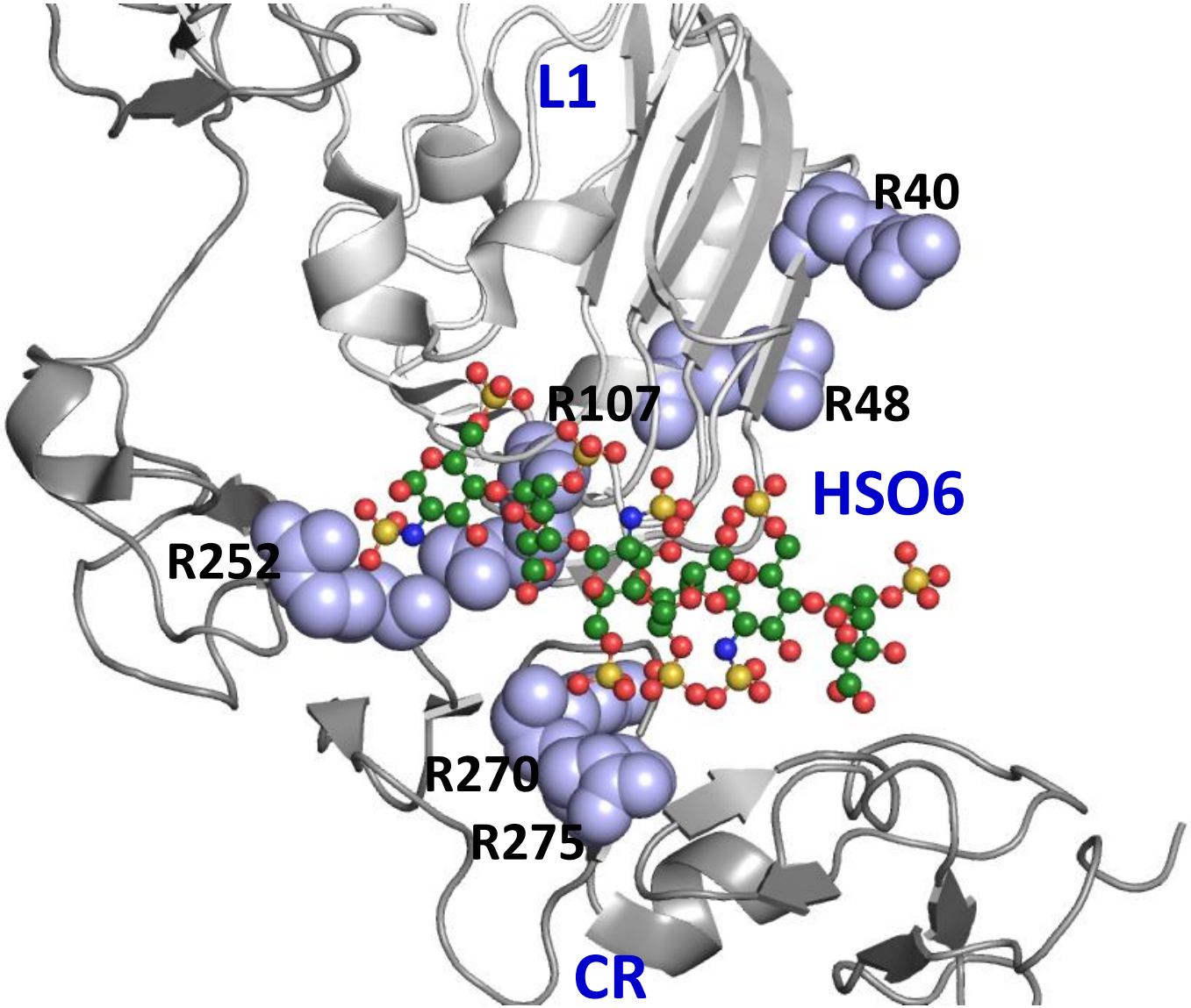


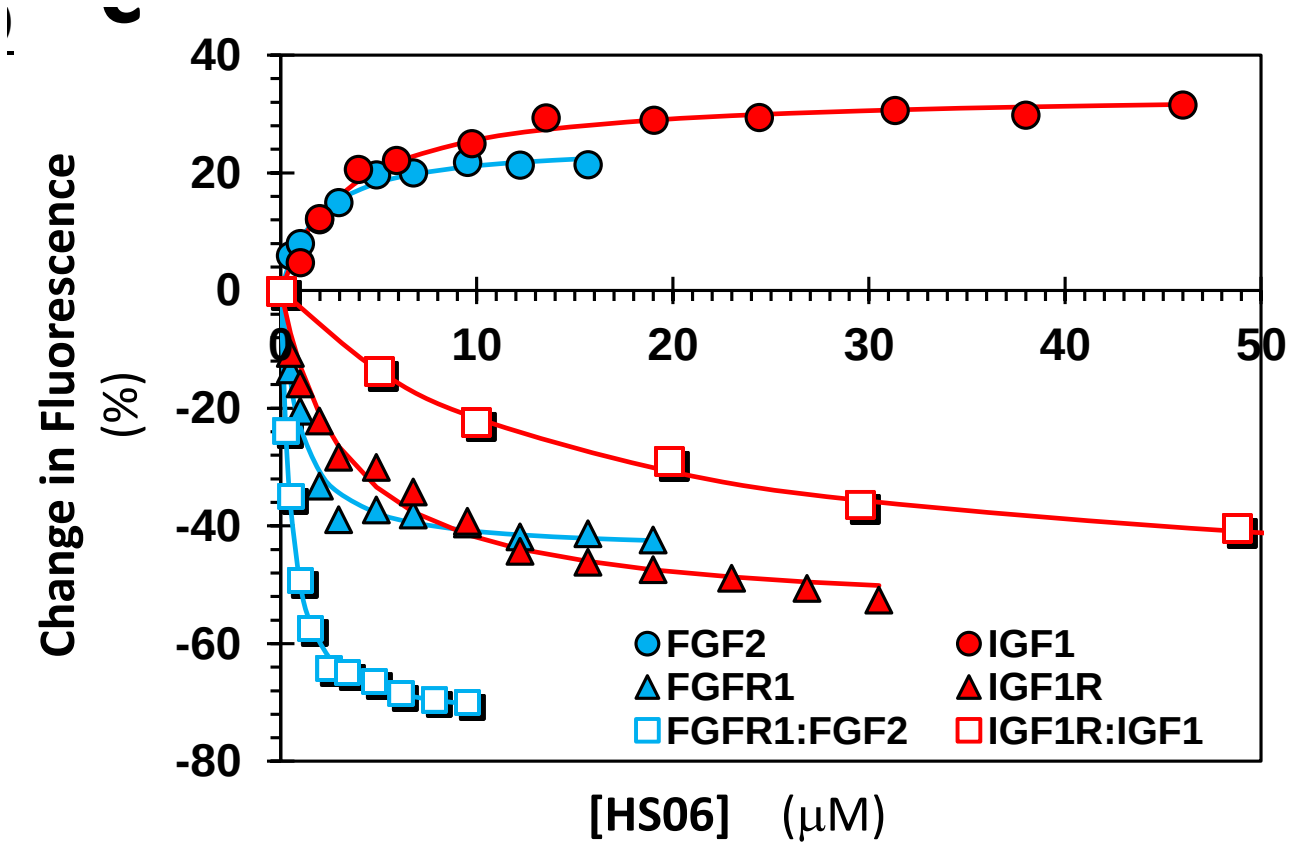
Clinical relevance of HS06-IGF1R interaction



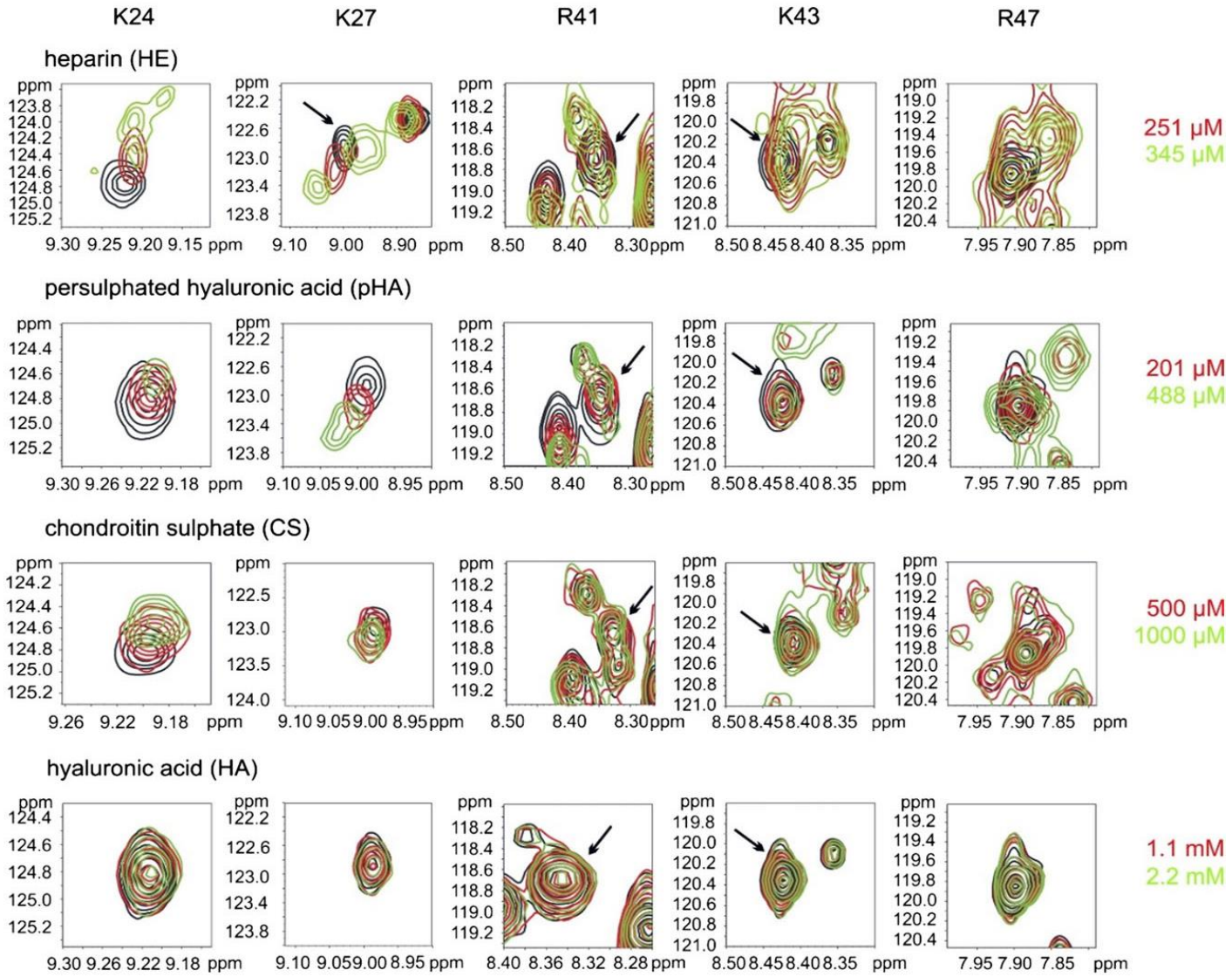
In Vitro methods

HS06 binding affinity for GFs and their receptor complexes

	K_D (μM) for the FGF family		
	FGF2	FGFR1	FGFR1–FGF2
HS05	40±6[c]	42±5	11±1
HS06	1.8±0.2	0.8±0.1	0.4±0.0
HS08	4.3±0.6	2.6±0.1	1.4±0.1
HS16	3.8±0.2	14.6±0.3	9.8±1.2

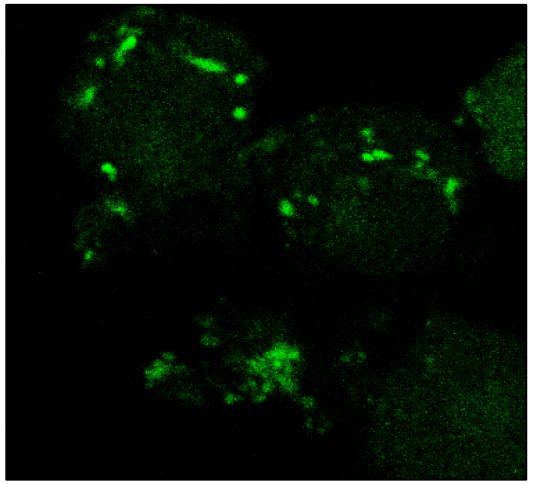


NMR study of CXCL12 and GAG interaction

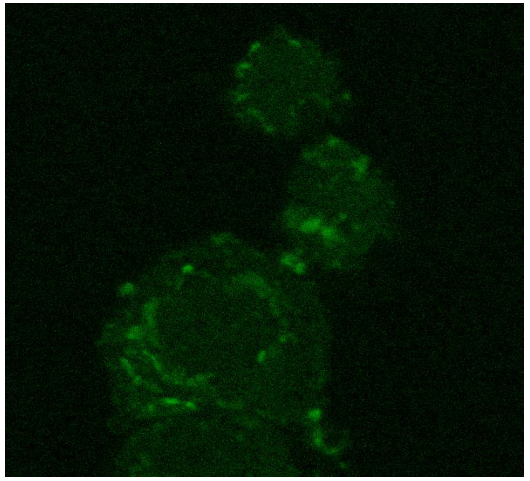


In vivo/In cell methods

HT-29 WT



HS06-AF488_S4



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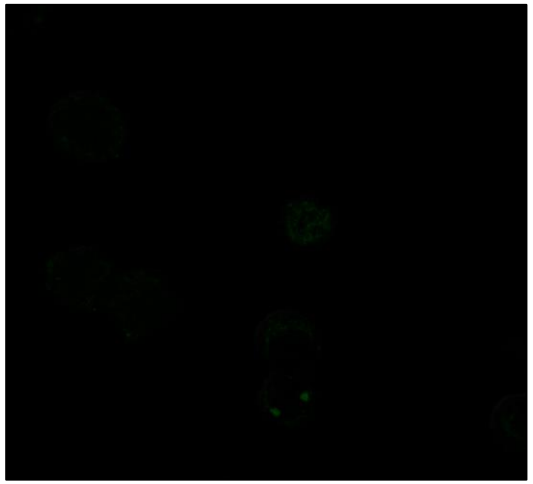
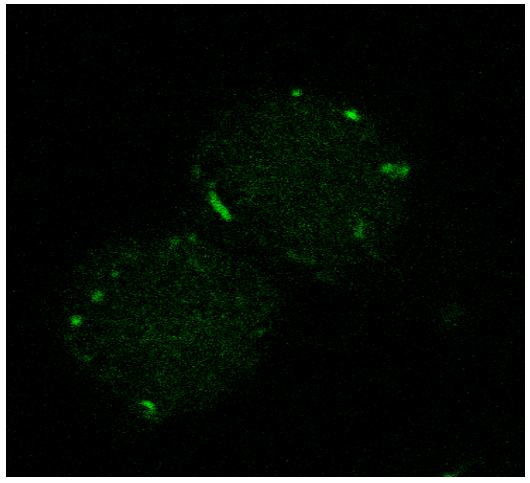


AF488

HT-29 IGF1RKD



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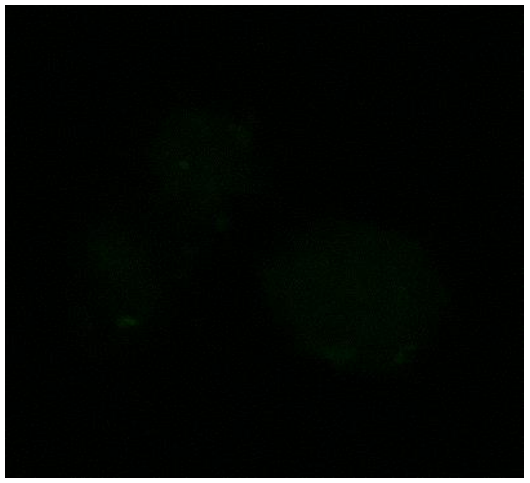

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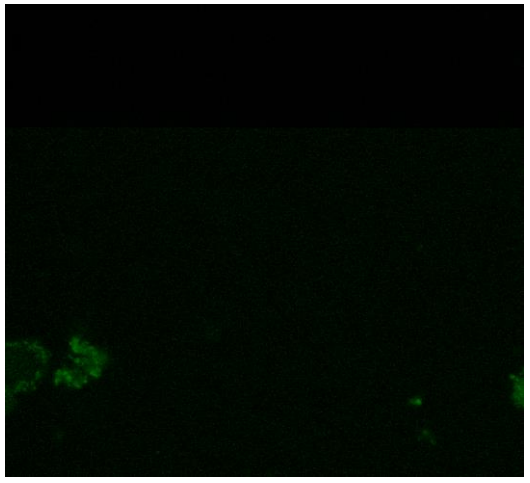

AF488